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(57) Abstract

Human monoclonal antibodies against a human cytokine (such as a human interleukin, e.g., human IL-1α) and fragments of such antibodies are disclosed, together with pharmaceutical compositions and methods employing the human monoclonal antibodies and fragments, methods for screening for human monoclonal antibodies against a human protein, methods for producing a cDNA library enriched in DNA encoding V_H and/or V_L chains of a human monoclonal antibody, cell lines for making the human monoclonal antibodies, and isolated DNA for making the human monoclonal antibodies and fragments of the invention.

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Internal Application No
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y A	EP,A,0 267 611 (OTSUKA PHARMACEUTICAL CO.) 18 May 1988 see example 2 --- EP,A,0 314 402 (SCHERING BIOTECH CORP.) 3 May 1989 see page 3, line 5 - line 43 see page 4, line 18 - line 31 --- WO,A,90 06371 (COMMISSARIAT A L'ENERGIE ATOMIQUE) 14 June 1990 see page 8, line 15 - line 30 --- -/--	1-4,6, 21-23 29-32 5 1-3,15, 18,20-28 29-32 7-17 1-4 14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 434 879 (LABORATOIRES UNICET) 3 July 1991 see claims 1-9 & WO,A,91 09115 cited in the application	29-32
X	EP,A,0 364 778 (OTSUKA PHARMACEUTICAL CO.LTD.) 25 April 1990 see claims 1-9	1-3

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Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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(54) Title: USE OF ANTI-CGRP OR ANTI-CGRP-R ANTIBODIES OR ANTIBODY FRAGMENTS TO TREAT OR PREVENT CHRONIC AND ACUTE FORMS OF DIARRHEA

(57) Abstract:

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USE OF ANTI-CGRP OR ANTI-CGRP-R ANTIBODIES OR ANTIBODY FRAGMENTS TO TREAT OR PREVENT CHRONIC AND ACUTE FORMS OF DIARRHEA

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/496,873 (Atty. Docket No. 67858.770000) filed June 14, 2011, entitled "USE OF ANTI-CGRP ANTIBODIES AND ANTIBODY FRAGMENTS TO TREAT DIARRHEA IN SUBJECTS WITH DISEASES OR TREATMENTS THAT RESULT IN ELEVATED CGRP LEVELS" and U.S. Provisional Application No. 61/488,660 (Atty. Docket No. 67858.730300) filed May 20, 2011, entitled "ANTI-CGRP COMPOSITIONS AND USE THEREOF" each of which is hereby incorporated by reference in its entirety.

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 18, 2012, is named 67858o730304.txt and is 203,920 bytes in size.

BACKGROUND OF THE INVENTION

Field of the Invention

[0003] This invention pertains to the discovery that polypeptides that bind to CGRP or CGRP receptor and/or other polypeptides which inhibit the CGRP/CGRP receptor interaction such as anti-CGRP or anti-CGRP receptor antibodies and antibody fragments or fragments of CGRP or the CGRP receptor which inhibit the CGRP/CGRP receptor interaction may be used to treat or prevent diarrhea, especially diarrhea associated with conditions or treatments that result in increased levels of CGRP. Exemplary conditions and treatments involving increased CGRP are identified herein. The invention in particular relates to methods of inhibiting, preventing or treating diarrhea and/or maintaining electrolyte balance and fluid levels in the colon of a subject having a condition or treatment

associated with elevated CGRP levels that result in diarrhea and/or increased flux of electrolytes and fluids from the colon comprising administering an effective amount of an anti-CGRP antibody or anti-CGRP antibody fragment. Exemplary conditions include by way of example functional bowel disorder and inflammatory bowel diseases, bacterial or viral infections, and more specifically gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, functional abdominal pain syndrome, diverticulosis, and diverticulitis, Crohn's disease, ileitis, collagenous colitis, lymphocytic colitis, ulcerative colitis, cancers or cancer treatments associated with increased CGRP and diarrhea such as chemotherapy, radiation, medullary thyroid carcinoma, and colorectal cancer.

[0004] In addition the present invention provides methods of screening polypeptides such as anti-CGRP or anti-CGRP receptor antibodies and fragments thereof (including Fab fragments) having binding specificity to human Calcitonin Gene Related Peptide (hereinafter "CGRP") as well as fragments of CGRP or a CGRP receptor in animal models to determine the in vivo effects thereof, especially their ability to antagonize the adverse side effects of CGRP and to treat conditions involving excess CGRP, especially CGRP associated conditions or treatments associated with diarrhea. The invention also pertains to methods of screening for diseases and disorders associated with increased CGRP, which are associated with diarrhea and specific therapeutic regimens for preventing or treating diseases and disorders that involve CGRP associated diarrhea by administering said antibodies or fragments thereof, alone or in association with other actives.

Description of Related Art

[0005] Calcitonin Gene Related Peptide (CGRP) is produced as a multifunctional neuropeptide of 37 amino acids in length. Two forms of CGRP, the CGRP-alpha and CGRP-beta forms, exist in humans and have similar activities. CGRP-alpha and CGRP-beta differ by three amino acids in humans, and are derived from different genes. The CGRP family of peptides includes amylin, adrenomedullin, and calcitonin, although each has distinct receptors and biological activities. Doods, H., *Curr. Op. Invest. Drugs*, 2(9):1261-68 (2001).

[0006] CGRP is released from numerous tissues such as trigeminal nerves, which when activated release neuropeptides within the meninges, mediating neurogenic inflammation that is characterized by vasodilation, vessel leakage, and mast-cell degradation. Durham, P.L., *New Eng. J. Med.*, 350 (11):1073-75 (2004). The biological effects of CGRP are mediated via the CGRP receptor (CGRP-R), which consists of a seven-transmembrane component, in conjunction with receptor-associated membrane protein (RAMP). CGRP-R further requires the activity of the receptor component protein (RCP), which is essential for an efficient coupling to adenylate cyclase through G proteins and the production of cAMP. Doods, H., *Curr. Op. Invest. Drugs*, 2(9):1261-68 (2001).

[0007] Migraines are neurovascular disorder affecting approximately 10% of the adult population in the U.S., and are typically accompanied by intense headaches. Approximately 20-30% of migraine sufferers experience aura, comprising focal neurological phenomena that precede and/or accompany the event. CGRP is believe to play a prominent role in the development of migraines. For example, plasma concentrations of CGRP were identified elevated in jugular venous blood during the headache phase of migraines, to the exclusion of other neuropeptides. Moreover, according to Arulmozhi *et al*, the following has been identified in migraine sufferers: (1) a strong correlation between plasma CGRP concentrations and migraines; (2) the infusion of CGRP produced a migraine-like headache; (3) baseline CGRP levels were elevated; and (4) changes in plasma CGRP levels during migraine attacks significantly correlated with headache intensity. Arulmozhi, D.K., *et al.*, Vas. Pharma., 43: 176-187 (2005). In addition, in the Journal of the International Association for the Study of Pain PII:S0304-3959(11)00313-7; doi:10.1016/j.pain.2011.04.033, published online 06 June 2011, Hou et al., reported that keratinocyte expression of calcitonin gene-related peptide β has implications for neuropathic and inflammatory pain mechanisms.

[0008] One effective treatment for migraines is the administration of triptans, which are a family of tryptamine-based drugs, including sumatriptan and rizatriptan. Members of this family have an affinity for multiple serotonin receptors, including 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F}. Members of this family of drugs selectively constrict cerebral vessels, but also cause vasoconstrictive effects on coronary vessels. Durham, P.L., *New Eng. J. Med.*, 350

(11):1073-75 (2004). There is a theoretical risk of coronary spasm in patients with established heart disease following administration, and cardiac events after taking triptans may rarely occur. Noted to be contraindicated for patients with coronary vascular disease.

[0009] Similarly, pain may often be addressed through the administration of certain narcotics or non-steroidal anti-inflammatory drugs (NSAIDs). However, the administration of these treatments may occur at the cost of certain negative consequences. NSAIDs have the potential to cause kidney failure, intestinal bleeding, and liver dysfunction. Narcotics have the potential to cause nausea, vomiting, imparied mental functioning, and addiction. Therefore, it is desirable to identify alternative treatments for pain in order to avoid certain of these negative consequences.

[00010] CGRP is believed to play a role in a multitude of diseases and disorders, including but not limited to migraines, headaches, and pain. Due to the perceived involvement of CGRP in these diseases and disorders, there remains a need in the art for compositions and methods useful for preventing or treating diseases and disorders associated with CGRP, while avoiding adverse side effects. There especially remains a need in the art for compositions or methods that reduce or inhibit diseases or disorders associated with CGRP, such as migraines, headaches, and pain.

[00011] Aside from the afore-mentioned conditions there is a need for treating other conditions or adverse side effects that are associated with increased CGRP. In this regard there has been some anecdotal evidence reported in the literature which suggest that increases in CGRP levels may have a role in some diseases associated with diarrhea. For example, it was reported by Rolston et al. in Digestive Diseases and Sciences , (April 1989) 34(4):612-6, "Intravenous calcitonin gene-related peptide stimulates net water secretion in rat colon in vivo" that exogenous calcitonin gene-related peptide has an effect on net flux of water and electrolytes in the rat small and large intestine. They report that in ligated intestinal loops, intravenous calcitonin gene-related peptide (CGRP) induced colonic fluid secretion but had no effect on the small intestine. Also they report using a single-pass perfusion technique, that they observed an immediate dose-dependent secretion of water by the rat colon upon intravenous administration of CGRP and also that the net secretion of sodium, potassium, and chloride were also raised. They suggest the implications of these

observations for the possible involvement of high circulation concentrations of CGRP in the watery diarrhea syndrome accompanying medullary thyroid carcinoma.

[00012] Further, it was reported by Keates et al., Gastroenterology 114:956-64(1998), "CGRP Upregulation in dorsal root ganglia and ilea mucosa during Clostridium difficile toxin A-induced enteritis in mice" that CGRP may play a role in toxin-A mediated diarrhea and that a CGRP antagonist substantially inhibited toxin-A mediated diarrhea and inflammation.

[00013] In addition, Picard et al. reported in International Journal of Radiation Biology , (2001), Vol. 77, No. 3, pp. 349-356 , "Presence of protective role of afferent nerves in early intestinal mucosal alterations induced by abdominal irradiation in rats" that CGRP levels increase after abdominal irradiation and particularly in radiation enteritis a condition characterized by diarrhea and other inflammatory reactions.

BRIEF SUMMARY OF THE INVENTION

[00014] Aside from being uncomfortable to the afflicted individual, diarrhea, especially if chronic or severe can be life threatening especially in geriatric patients and infants and young children as well as patients with diseases such as cancer and viral infection associated with chronic diarrhea that can substantially deplete fluid and electrolyte levels. There are 2 general types of diarrhea, acute and chronic.

[00015] Diarrhea is generally classified as a condition of having three or more loose or liquid bowel movements per day. It is a common cause of death in developing countries and the second most common cause of infant deaths worldwide. The loss of fluids through diarrhea can cause dehydration and electrolyte imbalances. In 2009 diarrhea was estimated to have caused 1.1 million deaths in people aged 5 and over and 1.5 million deaths in children under the age of 5. Oral rehydration solutions (ORS) with modest amounts of electrolytes and zinc tablets are the treatment of choice and have been estimated to have saved 50 million children in the past 25 years. ORS should be begun at early as possible. Vomiting does often occurs during the first hour or two of treatment with ORS, but this seldom prevents successful rehydration as most of the fluid is still absorbed. The World Health Organization (WHO) recommends that if a child vomits, to wait five or ten minutes and then start again more slowly.

[00016] Homemade solutions recommended by WHO include salted drinks (e.g. salted rice water or a salted yoghurt drink) and vegetable or chicken soup with salt. If available, supplemental potassium, as well as supplemental zinc, can be added to or given with this homemade solution. It is s also recommended that persons with diarrhea, if able, continue or resume eating as this speeds recovery of normal intestinal function and generally leads to diarrhea of shorter duration. Clean plain water can be one of several fluids given. There are commercial solutions such as Pedialyte, and relief agencies such as UNICEF widely distribute packets of salts and sugar.

[00017] Aside from the chronic and acute designations of diarrhea, this condition is also classified into different types which classifications are based on the cause and disease manifestations. One type is "secretory diarrhea". Secretory diarrhea means that there is an increase in the active secretion, or there is an inhibition of absorption. There is little to no structural damage. The most common cause of this type of diarrhea is a cholera toxin that stimulates the secretion of anions, especially chloride ions. In this type of diarrhea intestinal fluid secretion is isotonic with plasma even during fasting.[8] <> It continues even when there is no oral food intake.

[00018] A second type is "osmotic diarrhea". Osmotic diarrhea may occur when too much water is drawn into the bowels. If a person drinks solutions with excessive sugar or excessive salt, these can draw water from the body into the bowel and cause osmotic diarrhea. Also, osmotic diarrhea can also be the result of maldigestion (e.g., pancreatic disease or Coeliac disease), in which the nutrients are left in the lumen to pull in water. Or it can be caused by osmotic laxatives (which work to alleviate constipation by drawing water into the bowels). In healthy individuals, too much magnesium or vitamin C or undigested lactose can produce osmotic diarrhea and distention of the bowel. A person who has lactose intolerance can have difficulty absorbing lactose after an extraordinarily high intake of dairy products. In persons who have fructose malabsorption, excess fructose intake can also cause diarrhea. High-fructose foods that also have a high glucose content are more absorbable and less likely to cause diarrhea. Sugar alcohols such as sorbitol (often found in sugar-free foods) are difficult for the body to absorb and, in large amounts, may lead to osmotic diarrhea.

[00019] A third type of diarrhea is exudative diarrhea". Exudative diarrhea occurs with the presence of blood and pus in the stool. This occurs with inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis, and infections such as E. coli or other forms of food poisoning.

[00020] A fourth type of diarrhea is "motility-related diarrhea". Motility-related diarrhea is caused by the rapid movement of food through the intestines (hypermotility). If the food moves too quickly through the gastrointestinal tract, there is not enough time for sufficient nutrients and water to be absorbed. This can be due to a vagotomy or diabetic neuropathy, or a complication of menstruation. Hyperthyroidism can produce hypermotility and lead to this type of diarrhea. Diarrhea can be treated with antimotility agents (such as loperamide). Hypermotility can be observed in people who have had portions of their bowel removed, allowing less total time for absorption of nutrients.

[00021] A fifth type of diarrhea is "is "inflammatory diarrhea is ". Inflammatory diarrhea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids, and a decreased ability to absorb these lost fluids. Features of all three of the other types of diarrhea can be found in this type of diarrhea. It can be caused by bacterial infections, viral infections, parasitic infections, or autoimmune problems such as inflammatory bowel diseases. It can also be caused by tuberculosis, colon cancer, and enteritis.

[00022] A related condition to diarrhea is "dysentery". Generally, if there is blood visible in the stools, it is not diarrhea, but dysentery. The blood is trace of an invasion of bowel tissue. Dysentery is a symptom of, among others, Shigella, Entamoeba histolytica, and Salmonella.

[00023] Diarrhea is most commonly due to viral gastroenteritis with rotavirus, which accounts for 40% of cases in children under five. In travelers however bacterial infections predominate. Various toxins such as mushroom poisoning and drugs can also cause acute diarrhea.

[00024] As noted above, diarrhea may be classified as chronic or acute. "Chronic diarrhea can be the part of the presentations of a number of chronic medical conditions

affecting the intestine. Common causes include ulcerative colitis, Crohn's disease, microscopic colitis, celiac disease, irritable bowel syndrome and bile acid malabsorption.

[00025] There are many causes of infectious diarrhea, which include viruses, bacteria and parasites. Norovirus is the most common cause of viral diarrhea in adults, but rotavirus is the most common cause in children under five years old. Adenovirus types 40 and 41, and astroviruses cause a significant number of infections.

[00026] The bacterium Campylobacter is a common cause of bacterial diarrhea, but infections by Salmonellae, Shigellae and some strains of Escherichia coli (E.coli) are frequent. In the elderly, particularly those who have been treated with antibiotics for unrelated infections, a toxin produced by Clostridium difficile often causes severe diarrhea.

[00027] Some parasites may cause diarrhea such as the protozoan Giardia, which can cause chronic infections if these are not diagnosed and treated with drugs such as metronidazole, and Entamoeba histolytica.

[00028] Other causes of chronic diarrhea include:enzyme deficiencies or mucosal abnormality, as in food allergy and food intolerance, e.g. celiac disease (gluten intolerance), lactose intolerance (intolerance to milk sugar, common in non-Europeans), and fructose malabsorption, pernicious anemia, or impaired bowel function due to the inability to absorb vitamin B12, loss of pancreatic secretions, which may be due to cystic fibrosis or pancreatitis, structural defects, like short bowel syndrome (surgically removed bowel) and radiation fibrosis, such as usually follows cancer treatment and other drugs, including agents used in chemotherapy; and certain drugs, like orlistat, which inhibits the absorption of fat.

[00029] Ulcerative colitis is marked by chronic bloody diarrhea and inflammation mostly affects the distal colon near the rectum. Crohn's disease typically affects fairly well demarcated segments of bowel in the colon and often affects the end of the small bowel.

[00030] Another cause of diarrhea is irritable bowel syndrome (IBS) which usually presents with abdominal discomfort relieved by defecation and unusual stool (diarrhea or constipation) for at least 3 days a week over the previous 3 months. Symptoms of diarrhea-predominant IBS can be managed through a combination of dietary changes, soluble fiber supplements, and/or medications such as loperamide or codeine. About 30% of patients

with diarrhea-predominant IBS have bile acid malabsorption diagnosed with an abnormal SeHCAT test.

[00031] Other causes of diarrhea are chronic ethanol ingestion, ichemic bowel disease, mcroscopic colitis, ble salt malabsorption (primary bile acid diarrhea) where excessive bile acids in the colon produce a secretory diarrhea, hrmone-secreting tumors, (some hormones (e.g., serotonin) can cause diarrhea if excreted in excess (usually from a tumor)).

[00032] Medications such as loperamide (Imodium) and bismuth subsalicylate may be beneficial in treating some diarrhea conditions, however they may be contraindicated in certain situations.

[00033] While antibiotics are beneficial in certain types of acute diarrhea, they are usually not used except in specific situations. In fact, antibiotics can also cause diarrhea, and antibiotic-associated diarrhea is the most common adverse effect of treatment with general antibiotics.

[00034] Bismuth compounds such as in (Pepto-Bismol) may be used to treat some diarrhea conditions. Also, anti motility agents may be used to treat some diarrhea conditions. These include loperamide. Codeine is sometimes used in the treatment of diarrhea to slow down peristalsis and the passage of fecal material through the bowels. Also, bile acid sequestrants such as cholestyramine, colestipol and colesevelam can be effective in chronic diarrhea due to bile acid malabsorption.

[00035] Zinc supplementation may be used to treat some chronic diarrhea conditions. Probiotics may sometimes be used to reduce the duration of symptoms.

[00036] As mentioned, a second type of diarrhea is acute diarrhea. The most common cause of acute diarrhea is infection--viral, bacterial, and parasitic. Bacteria also can cause acute food poisoning. A third important cause of acute diarrhea is starting a new medication.

[00037] Other specific causes of acute diarrhea include viral gastroenteritis which is the most common cause of acute diarrhea worldwide. Viral gastroenteritis can occur in a sporadic form (in a single individual) or in an epidemic form (among groups of individuals). Sporadic diarrhea probably is caused by several different viruses and is believed to be spread by person-to-person contact. The most common cause of epidemic

diarrhea (for example, on cruise ships) is infection with a family of viruses known as caliciviruses of which the genus norovirus is the most common (for example, "Norwalk agent"). The caliciviruses are transmitted by food that is contaminated by sick food-handlers or by person-to-person contact.

[00038] Another cause of acute diarrhea is food poisoning caused by toxins produced by bacteria. The toxins cause abdominal pain (cramps) and vomiting and also cause the small intestine to secrete large amounts of water that leads to diarrhea. The symptoms of food poisoning usually last less than 24 hours. With some bacteria, the toxins are produced in the food before it is eaten, while with other bacteria, the toxins are produced in the intestine after the food is eaten.

[00039] Staphylococcus aureus is an example of a bacterium that produces toxins in food before it is eaten. Typically, food contaminated with Staphylococcus (such as salad, meat or sandwiches with mayonnaise) is left un-refrigerated at room temperature overnight. The Staphylococcal bacteria multiply in the food and produce toxins. Clostridium perfringens is an example of a bacterium that multiplies in food (usually canned food), and produces toxins in the small intestine after the contaminated food is eaten.

[00040] Another cause of acute diarrhea is traveler's diarrhea usually caused by pathogenic strains of E. coli bacteria. Occasionally, other bacteria or parasites can cause diarrhea in travelers (for example, Shigella, Giardia, Campylobacter). Diarrhea caused by these other organisms usually lasts longer than 3 days.

[00041] Yet another cause of acute diarrhea is bacterial enterocolitis which occurs when disease-causing bacteria usually invade the small intestines and colon and cause enterocolitis (inflammation of the small intestine and colon). Bacterial enterocolitis is characterized by signs of inflammation (blood or pus in the stool, fever) and abdominal pain and diarrhea. Campylobacter jejuni is the most common bacterium that causes acute enterocolitis in the U.S. Other bacteria that cause enterocolitis include Shigella, Salmonella, and EPEC. These bacteria usually are acquired by drinking contaminated water or eating contaminated foods such as vegetables, poultry, and dairy products.

[00042] Enterocolitis caused by the bacterium Clostridium difficile is often is caused by antibiotic treatment. Clostridium difficile is also the most common nosocomial infection

(infection acquired while in the hospital) to cause diarrhea. Unfortunately, infection also is increasing among individuals who have neither taken antibiotics or been in the hospital.

[00043] Another cause of acute diarrhea is E. coli O157:H7 which is a strain of E. coli that produces a toxin that causes hemorrhagic enterocolitis (enterocolitis with bleeding). There was a famous outbreak of hemorrhagic enterocolitis in the U.S. traced to contaminated ground beef in hamburgers (hence it is also called hamburger colitis). Approximately 5% of patients infected with E. coli O157:H7, particularly children, can develop hemolytic uremic syndrome (HUS), a syndrome that can lead to kidney failure . Some evidence suggests that prolonged use of anti-diarrhea agents or use of antibiotics may increase the chance of developing HUS.

[00044] Still anther cause of acute diarrhea is parasite infection, more common outside of the U. S. For example, infection with Giardia lamblia occurs among individuals who hike in the mountains or travel abroad and is transmitted by contaminated drinking water. Cryptosporidium is another diarrhea-producing parasite that is typically spread by contaminated water.

[00045] Yet anther cause of acute diarrhea is drug-induced diarrhea. The medications that most frequently cause diarrhea are antacids and nutritional supplements that contain magnesium. Other classes of medication that cause diarrhea include: nonsteroidal anti-inflammatory drugs (NSAIDs), chemotherapy medications, antibiotics, medications to control irregular heartbeats (antiarrhythmics), and medications for high blood pressure. misoprostol (Cytotec), quinidine (Quinaglute, Quinidex), olsalazine (Dipentum), colchicine (Colchicine), metoclopramide (Reglan), and cisapride (Propulsid, Motilium).

[00046] Common causes of chronic diarrhea include irritable bowel syndrome. infectious diseases such as Giardia lamblia, AIDS infection, bacterial overgrowth of the small intestine, .post-infectious diarrhea wherein individuals following acute viral, bacterial or parasitic infections develop chronic diarrhea (also referred to as post-infectious IBS), inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis, and other diseases causing inflammation of the small intestine and/or colon, commonly cause chronic diarrhea, colon cancer, particularly in the distal part of the colon, can lead to thin stools. severe constipation, carbohydrate (sugar) malabsorption. such as lactase deficiency (also

known as lactose or milk intolerance), fat malabsorption pancreatitis or pancreatic cancer, diseases of the lining of the small intestine that prevent the absorption of digested fat such as celiac disease, endocrine diseases such as hyperthyroidism or an under-active pituitary or adrenal gland (Addison's disease) and laxative abuse.

[00047] Both acute and chronic diarrhea may involve adverse complications including dehydration resulting from an excessive loss of fluids and electrolytes from the body due to diarrhea. Dehydration is common among adult patients with acute diarrhea who have large amounts of stool, particularly when the intake of fluids is limited by lethargy or is associated with nausea and vomiting and is common in infants and young children who develop viral gastroenteritis or bacterial infection.

[00048] Moderate to severe dehydration may cause orthostatic hypotension with syncope (fainting upon standing due to a reduced volume of blood, which causes a drop in blood pressure upon standing), a diminished urine output, severe weakness, shock, kidney failure, confusion, acidosis (too much acid in the blood), and even coma.

[00049] Electrolytes (minerals) also are lost with water when diarrhea is prolonged or severe, and mineral or electrolyte deficiencies may occur. The most common deficiencies occur with sodium and potassium. Abnormalities of chloride and bicarbonate also may develop. Finally, there may be irritation of the anus due to the frequent passage of watery stool containing irritating substances.

[00050] Accordingly, an effective method of preventing or treating different forms of diarrhea such as are above-described, and in particular acute or chronic diarrhea would be beneficial.

[00051] Along those lines, the present invention provides methods and medicaments for treating or preventing CGRP associated diarrhea comprising the administration of at least one polypeptide that binds CGRP or the CGRP receptor and/or a polypeptide which inhibits the CGRP/CGRP receptor interaction. These polypeptides include anti-CGRP or anti-CGRP receptor antibodies and antibody fragments, and fragments or variants of CGRP or the CGRP receptor which inhibit the CGRP/CGRP receptor interaction. These therapies effectively treat or prevent diarrhea, especially diarrhea that occurs as a result of disease

conditions or treatments associated with increased levels of CGRP, e.g. increased levels in the gastrointestinal system and more particularly the colon.

[00052] The invention in particular relates to methods of inhibiting, preventing or treating diarrhea and/or maintaining electrolyte balance and fluid levels in the colon of a subject having a condition (e.g., gastrointestinal condition, cancer, viral or infectious disorder) or treatments associated resulting in elevated CGRP levels (such as radiation or chemotherapy) that result in diarrhea and/or increased flux of electrolytes and fluids from the colon comprising administering an effective amount of an anti-CGRP antibody or anti-CGRP antibody fragment. These conditions include by way of example functional bowel disorders and inflammatory bowel diseases, bacterial or viral induced diarrhea, radiation and chemotherapies and more specifically functional bowel disorders selected from the group consisting of gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, functional abdominal pain syndrome, diverticulosis, and diverticulitis, inflammatory bowel diseases selected from the group consisting of Crohn's disease, ileitis, collagenous colitis, lymphocytic colitis, and ulcerative colitis, and cancers associated with diarrhea such as medullary thyroid carcinoma, and colorectal cancer.

[00053] The invention also relates to methods of screening antibodies and fragments thereof (including Fab fragments) having binding specificity to human Calcitonin Gene Related Peptide (hereinafter "CGRP") or the CGRP receptor or which inhibit the CGRP/CGRP receptor interaction in animal models to determine the in vivo effects thereof, especially their ability to antagonize the adverse side effects of CGRP and to treat or prevent diarrhea in conditions or treatments involving excess CGRP.

[00054] Further, the invention involves a method of assessing the potential in vivo efficacy of a candidate anti-CGRP antibody or antibody fragment or another polypeptide that inhibits the CGRP/CGRP receptor interaction for treating or preventing diarrhea comprising determining whether the antibody or other polypeptide inhibits diarrhea in a rodent administered exogenous CGRP compared to a rodent administered CGRP in the absence of the candidate CGRP antibody or antibody fragment or other polypeptide inhibitor.

[00055] Also, the invention involves a method of administering an anti-CGRP or anti-CGRP receptor antibody or antibody fragment or another polypeptide that inhibits the CGRP/CGRP receptor interaction to treat neurological and pain conditions characterized by increased CGRP levels which are associated with diarrhea.

[00056] Also the invention relates to medicaments for treating a condition associated with diarrhea selected from gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, functional abdominal pain syndrome, diverticulosis, diverticulitis, Crohn's disease, ileitis, collagenous colitis, lymphocytic colitis, and ulcerative colitis, medullary thyroid carcinoma or a colorectal cancer.

[00057] Further the invention relates to methods of assessing based on results in a rodent CGRP animal model a suitable therapeutic dosage or dosage regimen of the candidate antibody or antibody fragment in humans for preventing or treating CGRP associated diarrhea.

[00058] Still further the invention relates to compositions for inhibiting, preventing or treating diarrhea and/or maintaining electrolyte balance and fluid levels in the colon of a subject having a condition associated with elevated CGRP levels that result in diarrhea and/or increased flux of electrolytes and fluids from the colon comprising an effective amount of an anti-CGRP or anti-CGRP receptor antibody or anti-CGRP or anti-CGRP receptor antibody fragment and optionally another active agent.

[00059] Related thereto the invention specifically relates to compositions for treating or preventing functional bowel disorders or an inflammatory bowel diseases, bacterial or viral induced diarrhea, cancer associated with diarrhea, such as medullary thyroid carcinoma or a colorectal cancer, and functional bowel disorders or inflammatory bowel diseases, including by way of example gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, functional abdominal pain syndrome, diverticulosis, and diverticulitis or inflammatory bowel disease is selected from the group consisting of Crohn's disease, ileitis, collagenous colitis, lymphocytic colitis, and ulcerative colitis. wherein these therapies administer an effective amount of an anti-CGRP antibody or antibody fragment which is administered as a monotherapy or in combination with another active agent. .

[00060] In preferred embodiments the present invention is directed to therapeutic usage of specific antibodies and fragments thereof for treatment or prevention of diarrhea in diseases or treatments associated with in increased levels of CGRP, said antibodies or antibody fragments having binding specificity for CGRP, in particular antibodies having desired epitopic specificity, high affinity or avidity and/or functional properties. In other preferred embodiments this invention relates to assays and usage of the antibodies described herein, comprising the sequences of the V_H, V_L and CDR polypeptides described herein, and the polynucleotides encoding them. A preferred embodiment of the invention is directed to chimeric or humanized antibodies and fragments thereof (including Fab fragments) capable of binding to CGRP and/or inhibiting the biological activities mediated by the binding of CGRP to the CGRP receptor ("CGRP-R").

[00061] In another preferred embodiment of the invention, the assays and therapies use full length antibodies and Fab fragments thereof for treatment or prevention of diarrhea in diseases or conditions resulting in increased levels of CGRP that inhibit the CGRP-alpha-, CGRP-beta-, and rat CGRP-driven production of cAMP. In a further preferred embodiment of the invention, full length and Fab fragments thereof are contemplated that reduce vasodilation in a recipient following administration.

[00062] In another embodiment of the invention, chimeric or humanized antibodies and fragments thereof (including Fab fragments) capable of binding to CGRP or the CGRP receptor are useful in methods directed to reducing, treating, or preventing diarrhea in diseases or conditions resulting in increased levels of CGRP such as migraines (with or without aura), cancer or tumors, angiogenesis associated with cancer or tumor growth, angiogenesis associated with cancer or tumor survival, weight loss, pain, hemiplagic migraines, cluster headaches, migrainous neuralgia, chronic headaches, tension headaches, general headaches, hot flushes, chronic paroxysomal hemicrania, secondary headaches due to an underlying structural problem in the head or neck, cranial neuralgia, sinus headaches (such as for example associated with sinusitis), and allergy-induced headaches or migraines.

[00063] In another embodiment of the invention, chimeric or humanized antibodies and fragments thereof (including Fab fragments) capable of binding to CGRP are useful in

methods directed to reducing, treating, or preventing diarrhea and visceral pain associated with gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, inflammatory bowel disease, Crohn's disease, ileitis, ulcerative colitis, renal colic, dysmenorrhea, cystitis, menstrual period, labor, menopause, prostatitis, or pancreatitis.

[00064] In another embodiment of the invention these antibodies and humanized versions for treatment or prevention of diarrhea in diseases or conditions resulting in increased levels of CGRP may be derived from rabbit immune cells (B lymphocytes) and may be selected based on their homology (sequence identity) to human germ line sequences. These antibodies may require minimal or no sequence modifications, thereby facilitating retention of functional properties after humanization. A further embodiment of the invention is directed to fragments from anti-CGRP antibodies encompassing V_H, V_L and CDR polypeptides, e.g., derived from rabbit immune cells and the polynucleotides encoding the same, as well as the use of these antibody fragments and the polynucleotides encoding them in the creation of novel antibodies and polypeptide compositions capable of binding to CGRP and/or CGRP/CGRP-R complexes for treatment or prevention of diarrhea in diseases or conditions resulting in increased levels of CGRP.

[00065] The invention also contemplates conjugates of anti-CGRP antibodies and binding fragments thereof conjugated to one or more functional or detectable moieties for treatment or prevention of diarrhea in diseases or conditions resulting in increased levels of CGRP. The invention also contemplates methods of making said chimeric or humanized anti-CGRP or anti-CGRP/CGRP-R complex antibodies and binding fragments thereof for treatment or prevention of diarrhea in diseases or conditions resulting in increased levels of CGRP. In one embodiment, binding fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, Fv, scFv fragments, SMIPs (small molecule immunopharmaceuticals), camelbodies, nanobodies, and IgNAR.

[00066] Embodiments of the invention pertain to the use of anti-CGRP antibodies and binding fragments thereof for the diagnosis, assessment and treatment of diseases and disorders associated with CGRP or aberrant expression thereof that result in diarrhea because of increased levels of CGRP. The invention also contemplates the use of fragments of anti-CGRP antibodies for the diagnosis, assessment and treatment of diseases

and disorders associated with CGRP or aberrant expression thereof such as diseases or conditions wherein increased levels of CGRP in the gut result in diarrhea. Other embodiments of the invention relate to the production of anti-CGRP antibodies or fragments thereof in recombinant host cells, for example mammalian cells such as CHO, NSO or HEK 293 cells, or yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[00067] Figure 1 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab1.

[00068] Figure 2 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab2.

[00069] Figure 3 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab3.

[00070] Figure 4 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab4.

[00071] Figure 5 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab5.

[00072] Figure 6 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab6.

[00073] Figure 7 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab7.

[00074] Figure 8 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab8.

[00075] Figure 9 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab9.

[00076] Figure 10 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab10.

[00077] Figure 11 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab11.

[00078] Figure 12 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab12.

[00079] Figure 13 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab13.

[00080] Figure 14 provides polynucleotide and polypeptide sequences corresponding to the full-length Antibody Ab14.

[00081] Figure 15 provides the CGRP-alpha ELISA binding data obtained following the protocol in Example 1 infra for antibodies Ab1, Ab2, Ab3, and Ab4.

[00082] Figure 16 provides the CGRP-alpha ELISA binding data obtained following the protocol in Example 1 infra for antibodies Ab5, Ab6, Ab7, and Ab8.

[00083] Figure 17 provides the CGRP-alpha ELISA binding data obtained following the protocol in Example 1 infra for antibodies Ab9, Ab10, and Ab14.

[00084] Figure 18 provides the CGRP-alpha ELISA binding data obtained following the protocol in Example 1 infra for antibodies Ab11, Ab12, and Ab13.

[00085] Figure 19 demonstrates the inhibition of CGRP-alpha-driven cAMP production by antibodies Ab1, Ab2, and Ab4, obtained following the protocol in Example 1 infra.

[00086] Figure 20 demonstrates the inhibition of CGRP-alpha-driven cAMP production by antibody Ab3, obtained following the protocol in Example 1 infra.

[00087] Figure 21 demonstrates the inhibition of CGRP-alpha-driven cAMP production by antibodies Ab5 and Ab6, obtained following the protocol in Example 1 infra.

[00088] Figure 22 demonstrates the inhibition of CGRP-alpha-driven cAMP production by antibodies Ab7, Ab8, Ab9, and Ab10, obtained following the protocol in Example 1 infra.

[00089] Figure 23 demonstrates the inhibition of CGRP-alpha-driven cAMP production by antibodies Ab11, Ab12, and Ab13, obtained following the protocol in Example 1 infra.

[00090] Figure 24 demonstrates the inhibition of CGRP-alpha-driven cAMP production by antibody Ab14, obtained following the protocol in Example 1 infra.

[00091] Figure 25 demonstrates the inhibition of CGRP-beta-driven cAMP production by antibodies Ab1, Ab2, and Ab3, obtained following the protocol in Example 1 infra.

[00092] Figure 26 demonstrates the inhibition of CGRP-beta-driven cAMP production by antibodies Ab4, Ab5, and Ab6, obtained following the protocol in Example 1 infra.

[00093] Figure 27 demonstrates the inhibition of CGRP-beta-driven cAMP production by antibodies Ab7 and Ab8, obtained following the protocol in Example 1 infra.

[00094] Figure 28 demonstrates the inhibition of CGRP-beta-driven cAMP production by antibodies Ab9, Ab10, and Ab14, obtained following the protocol in Example 1 infra.

[00095] Figure 29 demonstrates the inhibition of CGRP-beta-driven cAMP production by antibodies Ab11, Ab12, and Ab13, obtained following the protocol in Example 1 infra.

[00096] Figure 30 demonstrates the inhibition of rat CGRP-driven cAMP production by antibodies Ab1, Ab2, Ab4, and Ab5, obtained following the protocol in Example 1 infra.

[00097] Figure 31 demonstrates the inhibition of rat CGRP -driven cAMP production by antibodies Ab3 and Ab6, obtained following the protocol in Example 1 infra.

[00098] Figure 32 demonstrates the inhibition of rat CGRP-driven cAMP production by antibodies Ab7 and Ab8, obtained following the protocol in Example 1 infra.

[00099] Figure 33 demonstrates the inhibition of rat CGRP-driven cAMP production by antibody Ab9, obtained following the protocol in Example 1 infra.

[000100] Figure 34 demonstrates the inhibition of rat CGRP-driven cAMP production by antibody Ab10, obtained following the protocol in Example 1 infra.

[000101] Figure 35 demonstrates the inhibition of rat CGRP-driven cAMP production by antibodies Ab11 and Ab12, obtained following the protocol in Example 1 infra.

[000102] Figure 36 demonstrates the inhibition of rat CGRP-driven cAMP production by antibody Ab13, obtained following the protocol in Example 1 infra.

[000103] Figure 37 demonstrates the inhibition of rat CGRP-driven cAMP production by antibody Ab14, obtained following the protocol in Example 1 infra.

[000104] Figure 38 demonstrates the inhibition of binding of radiolabeled CGRP to CGRP-R by antibodies Ab1-Ab13, obtained following the protocol in Example 6 infra.

[000105] Figure 39 demonstrates a reduction in vasodilation obtained by administering antibodies Ab3 and Ab6 following capsaicin administration in a rat model, relative to a control antibody, obtained following the protocol in Example 7 infra.

[000106] Figure 40 demonstrates a reduction in vasodilation obtained by administering antibody Ab6 at differing concentrations following capsaicin administration in a rat model, relative to a control antibody, obtained following the protocol in Example 7 infra.

[000107] Figure 41 contains the results of experiments wherein the effects of CGRP in transgenic Nestin/hRamp1 mice were evaluated. The data shows that rat CGRP-alpha administration induced diarrhea in Nestin/hRAMP1 tg mice and that the intra peritoneal injection of Ab3 (30mgs/kg, ~24 hrs. prior to CGRP challenge) inhibits intra cerebroventricular (ICV) injected, rat CGRP-alpha induced diarrhea in Nestin/hRAMP1 tg mice.

[000108] Figure 42 contains the results of experiments which show that the intra cerebroventricular (ICV) injection of human CGRP-alpha induces diarrhea in a dose dependent manner in C57BL/6J mice.

[000109] Figure 43 contains the results of experiments which show that intra peritoneal injection of Ab3 (30mgs/kg ip, ~24 hrs. prior to human CGRP-alpha challenge) inhibits ICV injected human CGRP-alpha induced diarrhea in C57/BL6J mice.

[000110] Figure 44 contains the results of experiments which show that Ab3 (30mgs/kg ip injection ~24 hrs. prior to human CGRP-alpha challenge) inhibits IP injected- human CGRP-alpha induced diarrhea in C57/BL6J mice.

[000111] FIG. 45 shows prevention of CGRP-induced diarrhea by Ab3 and Ab6 (both administered at 10 mg/kg). Negative control animals (treated with a control antibody and phosphate buffered saline, left bar) did not exhibit diarrhea, and 80% of positive control animals (treated with CGRP and a negative control antibody, filled bar) exhibited diarrhea. Administration of Ab3 (diagonal striped bar) and Ab6 (cross-hatched bar) reduced incidence of diarrhea to 40% and 60%, respectively.

[000112] FIG. 46 shows gross fecal weight resulting from CGRP-induced diarrhea for the experiment shown in FIG. 45. Gross fecal weight was greatly increased by administration of CGRP (second bar) compared to negative control animals (first bar). However, Ab3- and Ab6-treated animals exhibited greatly reduced gross fecal weight (third and fourth bars, respectively). Values shown are the average of all animals in each test group plus or minus standard error of mean (SEM).

[000113] FIG. 47 confirms prevention of CGRP-induced diarrhea by Ab3 and Ab6 in a further experiment (both antibodies administered at 30 mg/kg). Diarrhea was absent in negative control animals (treated with a control antibody and phosphate buffered saline, first bar) but observed in 80% of positive control animals (second bar, filled). Diarrhea incidence was reduced to 20% and 40%, respectively by Ab3 (third bar, diagonal stripes) and Ab6 (fourth bar, crosshatch).

[000114] FIG. 48 shows gross fecal weight resulting from CGRP-induced diarrhea for the experiment shown in FIG. 47. Gross fecal weight was greatly increased by administration of CGRP (second bar, filled) compared to negative control animals (first bar, unfilled). However, Ab6-treated animals exhibited greatly reduced gross fecal weight (fourth bar, checkered), and Ab3-treated animals (third bar, crosshatch) exhibited average gross fecal weight comparable to negative control animals (left bar, unfilled). Values shown are the average of all animals in each test group plus or minus standard error of mean (SEM).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

[000115] It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise..

[000116] Calcitonin Gene Related Peptide (CGRP): As used herein, CGRP encompasses not only the following Homo sapiens CGRP-alpha and Homo sapiens CGRP-beta amino

acid sequences available from American Peptides (Sunnyvale CA) and Bachem (Torrance, CA):

[000117] CGRP-alpha: ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-NH2 (SEQ ID NO: 281), wherein the N-terminal phenylalanine is amidated;

[000118] CGRP-beta: ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF-NH2 (SEQ ID NO: 282), wherein the N-terminal phenylalanine is amidated; but also any membrane-bound forms of these CGRP amino acid sequences, as well as mutants (mutiens), splice variants, isoforms, orthologues, homologues and variants of this sequence. In particular CGRP herein encompasses rodent CGRPs and CGRP sequences of other mammals.

[000119] "CGRP receptor" herein includes all endogenous receptors that are specifically bound by CGRP, including human and rodent CGRP and other mammalian CGRP. As well CGRP receptor" includes mutants (mutiens), splice variants, isoforms, orthologues, homologues, fragments and variants of CGRP receptors that are specifically bound by CGRP. In particular CGRP receptor herein encompasses human, rat, murine and non-human primate CGRP receptors and CGRP receptor sequences of other mammals.

[000120] "CGRP/CGRP Receptor Inhibitor" herein refers to a molecule, preferably a polypeptide such as an antibody or antibody fragment that inhibits the interaction of CGRP and its receptor. Non-limiting examples thereof include antibodies and antibody fragments that specifically bind CGRP or the CGRP receptor and fragments of CGRP or the CGRP receptor.

[000121] "Diarrhea" refers to an increase in the frequency of bowel movements or a decrease in the form of stool (greater looseness of stool). Although changes in frequency of bowel movements and looseness of stools can vary independently of each other, changes often occur in both. Diarrhea needs to be distinguished from four other conditions. Although these conditions may accompany diarrhea, they often have different causes and different treatments than diarrhea. These other conditions are: incontinence of stool, which is the inability to control (delay) bowel movements until an appropriate time, for example, until one can get to the toilet , rectal urgency, which is a sudden urge to have a bowel movement that is so strong that if a toilet is not immediately available there will be

incontinence, incomplete evacuation, which is a sensation that another bowel movement is necessary soon after a bowel movement, yet there is difficulty passing further stool the second time and bowel movements immediately after eating a meal.

[000122] Diarrhea can be defined in absolute or relative terms based on either the frequency of bowel movements or the consistency (looseness) of stools.

[000123] Frequency of bowel movements. Absolute diarrhea is having more bowel movements than normal. Thus, since among healthy individuals the maximum number of daily bowel movements is approximately three, diarrhea can be defined as any number of stools greater than three. Relative diarrhea is having more bowel movements than usual. Thus, if an individual who usually has one bowel movement each day begins to have two bowel movements each day, then diarrhea is present-even though there are not more than three bowel movements a day, that is, there is not absolute diarrhea.

[000124] Consistency of stools. Absolute diarrhea is more difficult to define on the basis of the consistency of stool because the consistency of stool can vary considerably in healthy individuals depending on their diets. Thus, individuals who eat large amounts of vegetables will have looser stools than individuals who eat few vegetables. Stools that are liquid or watery are always abnormal and considered diarrheal. Relative diarrhea is easier to define based on the consistency of stool. Thus, an individual who develops looser stools than usual has diarrhea--even though the stools may be within the range of normal with respect to consistency.

[000125] Diarrhea generally is divided into two types, acute and chronic. Acute diarrhea lasts from a few days up to a week. Chronic diarrhea can be defined in several ways but almost always lasts more than three weeks. Acute and chronic diarrhea usually have different causes, require different diagnostic tests, and often involve different treatments.

[000126] "Treatment or prevention of CGRP-induced diarrhea" means that the treatment, e.g., administration of an anti-CGRP antibody or fragment effectively inhibits or treats diarrhea and/or maintains proper electrolyte and fluid levels in the colon of a subject in need thereof relative to an untreated subject.

[000127] "CGRP-induced diarrhea or CGRP-associated diarrhea" refers to a condition or treatment resulting in elevated CGRP levels, especially in the gastrointestinal system and

especially the colon that result in one or more of increased excretion of fluid from the colon, impaired electrolyte balance and one or more watery bowel movements (diarrhea).

[000128] "Treatments that result in CGRP-associated diarrhea" herein refer to any treatment for a disease condition, e.g., radiation, chemotherapy, drug therapy that result in increased CGRP levels that are associated with diarrhea.

[000129] 'CGRP associated disease or condition" is any disease or condition that is associated with increased CGRP levels relative to CGRP levels in normal individuals.

[000130] Mating competent yeast species: In the present invention this is intended to broadly encompass any diploid or tetraploid yeast which can be grown in culture. Such species of yeast may exist in a haploid, diploid, or other polyploid form. The cells of a given ploidy may, under appropriate conditions, proliferate for an indefinite number of generations in that form. Diploid cells can also sporulate to form haploid cells. Sequential mating can result in tetraploid strains through further mating or fusion of diploid strains. The present invention contemplates the use of haploid yeast, as well as diploid or other polyploid yeast cells produced, for example, by mating or spheroplast fusion.

[000131] In one embodiment of the invention, the mating competent yeast is a member of the Saccharomycetaceae family, which includes the genera Arxiozyma; Ascobotryozyma; Citeromyces; Debaryomyces; Dekkera; Eremothecium; Issatchenkia; Kazachstania; Kluyveromyces; Kodamaea; Lodderomyces; Pachysolen; Pichia; Saccharomyces; Saturnispora; Tetrapisispora; Torulaspora; Williopsis; and Zygosaccharomyces. Other types of yeast potentially useful in the invention include Yarrowia; Rhodosporidium; Candida; Hansenula; Filobasium; Sporidiobolus; Bullera; Leucosporidium and Filobasidella.

[000132] In a preferred embodiment of the invention, the mating competent yeast is a member of the genus Pichia. In a further preferred embodiment of the invention, the mating competent yeast of the genus Pichia is one of the following species: Pichia pastoris, Pichia methanolica, and Hansenula polymorpha (Pichia angusta). In a particularly preferred embodiment of the invention, the mating competent yeast of the genus Pichia is the species Pichia pastoris.

[000133] Haploid Yeast Cell: A cell having a single copy of each gene of its normal genomic (chromosomal) complement.

[000134] Polyploid Yeast Cell: A cell having more than one copy of its normal genomic (chromosomal) complement.

[000135] Diploid Yeast Cell: A cell having two copies (alleles) of essentially every gene of its normal genomic complement, typically formed by the process of fusion (mating) of two haploid cells.

[000136] Tetraploid Yeast Cell: A cell having four copies (alleles) of essentially every gene of its normal genomic complement, typically formed by the process of fusion (mating) of two haploid cells. Tetraploids may carry two, three, four or more different expression cassettes. Such tetraploids might be obtained in S. cerevisiae by selective mating homozygotic heterothallic a/a and alpha/alpha diploids and in Pichia by sequential mating of haploids to obtain auxotrophic diploids. For example, a [met his] haploid can be mated with [ade his] haploid to obtain diploid [his]; and a [met arg] haploid can be mated with [ade arg] haploid to obtain diploid [arg]; then the diploid [his] x diploid [arg] to obtain a tetraploid prototroph. It will be understood by those of skill in the art that reference to the benefits and uses of diploid cells may also apply to tetraploid cells.

[000137] Yeast Mating: The process by which two haploid yeast cells naturally fuse to form one diploid yeast cell.

[000138] Meiosis: The process by which a diploid yeast cell undergoes reductive division to form four haploid spore products. Each spore may then germinate and form a haploid vegetatively growing cell line.

[000139] Selectable Marker: A selectable marker is a gene or gene fragment that confers a growth phenotype (physical growth characteristic) on a cell receiving that gene as, for example through a transformation event. The selectable marker allows that cell to survive and grow in a selective growth medium under conditions in which cells that do not receive that selectable marker gene cannot grow. Selectable marker genes generally fall into several types, including positive selectable marker genes such as a gene that confers on a cell resistance to an antibiotic or other drug, temperature when two temperature sensitive ("ts") mutants are crossed or a ts mutant is transformed; negative selectable marker genes

such as a biosynthetic gene that confers on a cell the ability to grow in a medium without a specific nutrient needed by all cells that do not have that biosynthetic gene, or a mutagenized biosynthetic gene that confers on a cell inability to grow by cells that do not have the wild type gene; and the like. Suitable markers include but are not limited to: ZEO; G418; LYS3; MET1; MET3a; ADE1; ADE3; URA3; and the like.

[000140] Expression Vector: These DNA vectors contain elements that facilitate manipulation for the expression of a foreign protein within the target host cell. Conveniently, manipulation of sequences and production of DNA for transformation is first performed in a bacterial host, e.g. E. coli, and usually vectors will include sequences to facilitate such manipulations, including a bacterial origin of replication and appropriate bacterial selection marker. Selection markers encode proteins necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media. Exemplary vectors and methods for transformation of yeast are described, for example, in Burke, D., Dawson, D., & Stearns, T. (2000). Methods in yeast genetics: a Cold Spring Harbor Laboratory course manual. Plainview, N.Y.: Cold Spring Harbor Laboratory Press.

[000141] Expression vectors for use in the methods of the invention will further include yeast specific sequences, including a selectable auxotrophic or drug marker for identifying transformed yeast strains. A drug marker may further be used to amplify copy number of the vector in a yeast host cell.

[000142] The polypeptide coding sequence of interest is operably linked to transcriptional and translational regulatory sequences that provide for expression of the polypeptide in yeast cells. These vector components may include, but are not limited to, one or more of the following: an enhancer element, a promoter, and a transcription termination sequence. Sequences for the secretion of the polypeptide may also be included, e.g. a signal sequence, and the like. A yeast origin of replication is optional, as expression vectors are often integrated into the yeast genome. In one embodiment of the invention, the polypeptide of

interest is operably linked, or fused, to sequences providing for optimized secretion of the polypeptide from yeast diploid cells.

[000143] Nucleic acids are "operably linked" when placed into a functional relationship with another nucleic acid sequence. For example, DNA for a signal sequence is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites or alternatively via a PCR/recombination method familiar to those skilled in the art (GatewayR Technology; Invitrogen, Carlsbad California). If such sites do not exist, the synthetic oligonucleotide adapters or linkers are used in accordance with conventional practice.

[000144] Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequences to which they are operably linked. Such promoters fall into several classes: inducible, constitutive, and repressible promoters (that increase levels of transcription in response to absence of a repressor). Inducible promoters may initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature.

[000145] The yeast promoter fragment may also serve as the site for homologous recombination and integration of the expression vector into the same site in the yeast genome; alternatively a selectable marker is used as the site for homologous recombination. Pichia transformation is described in Cregg et al. (1985) Mol. Cell. Biol. 5:3376-3385.

[000146] Examples of suitable promoters from Pichia include the AOX1 and promoter (Cregg et al. (1989) Mol. Cell. Biol. 9:1316-1323); ICL1 promoter (Menendez et al. (2003) Yeast 20(13):1097-108); glyceraldehyde-3-phosphate dehydrogenase promoter (GAP) (Waterham et al. (1997) Gene 186(1):37-44); and FLD1 promoter (Shen et al. (1998) Gene

216(1):93-102). The GAP promoter is a strong constitutive promoter and the AOX and FLD1 promoters are inducible.

[000147] Other yeast promoters include ADH1, alcohol dehydrogenase II, GAL4, PHO3, PHO5, Pyk, and chimeric promoters derived therefrom. Additionally, non-yeast promoters may be used in the invention such as mammalian, insect, plant, reptile , amphibian, viral, and avian promoters. Most typically the promoter will comprise a mammalian promoter (potentially endogenous to the expressed genes) or will comprise a yeast or viral promoter that provides for efficient transcription in yeast systems.

[000148] The polypeptides of interest may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, e.g. a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the polypeptide coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed through one of the standard pathways available within the host cell. The S. cerevisiae alpha factor pre-pro signal has proven effective in the secretion of a variety of recombinant proteins from P. pastoris. Other yeast signal sequences include the alpha mating factor signal sequence, the invertase signal sequence, and signal sequences derived from other secreted yeast polypeptides. Additionally, these signal peptide sequences may be engineered to provide for enhanced secretion in diploid yeast expression systems. Other secretion signals of interest also include mammalian signal sequences, which may be heterologous to the protein being secreted, or may be a native sequence for the protein being secreted. Signal sequences include pre-peptide sequences, and in some instances may include propeptide sequences. Many such signal sequences are known in the art, including the signal sequences found on immunoglobulin chains, e.g., K28 preprotoxin sequence, PHA-E, FACE, human MCP-1, human serum albumin signal sequences, human Ig heavy chain, human Ig light chain, and the like. For example, see Hashimoto et. al. Protein Eng 11(2) 75 (1998); and Kobayashi et. al. Therapeutic Apheresis 2(4) 257 (1998).

[000149] Transcription may be increased by inserting a transcriptional activator sequence into the vector. These activators are cis-acting elements of DNA, usually about from 10 to

300 bp, which act on a promoter to increase its transcription. Transcriptional enhancers are relatively orientation and position independent, having been found 5' and 3' to the transcription unit, within an intron, as well as within the coding sequence itself. The enhancer may be spliced into the expression vector at a position 5' or 3' to the coding sequence, but is preferably located at a site 5' from the promoter.

[000150] Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from 3' to the translation termination codon, in untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA.

[000151] Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques or PCR/recombination methods. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required or via recombination methods. For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform host cells, and successful transformants selected by antibiotic resistance (e.g. ampicillin or Zeocin) where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion and/or sequenced.

[000152] As an alternative to restriction and ligation of fragments, recombination methods based on att sites and recombination enzymes may be used to insert DNA sequences into a vector. Such methods are described, for example, by Landy (1989) Ann.Rev.Biochem. 58:913-949; and are known to those of skill in the art. Such methods utilize intermolecular DNA recombination that is mediated by a mixture of lambda and E.coli –encoded recombination proteins. Recombination occurs between specific attachment (att) sites on the interacting DNA molecules. For a description of att sites see Weisberg and Landy (1983) Site-Specific Recombination in Phage Lambda, in Lambda II, Weisberg, ed.(Cold Spring Harbor, NY:Cold Spring Harbor Press), pp. 211-250. The DNA segments flanking the recombination sites are switched, such that after recombination, the att sites are hybrid

sequences comprised of sequences donated by each parental vector. The recombination can occur between DNAs of any topology.

[000153] Att sites may be introduced into a sequence of interest by ligating the sequence of interest into an appropriate vector; generating a PCR product containing att B sites through the use of specific primers; generating a cDNA library cloned into an appropriate vector containing att sites; and the like.

[000154] Folding, as used herein, refers to the three-dimensional structure of polypeptides and proteins, where interactions between amino acid residues act to stabilize the structure. While non-covalent interactions are important in determining structure, usually the proteins of interest will have intra- and/or intermolecular covalent disulfide bonds formed by two cysteine residues. For naturally occurring proteins and polypeptides or derivatives and variants thereof, the proper folding is typically the arrangement that results in optimal biological activity, and can conveniently be monitored by assays for activity, e.g. ligand binding, enzymatic activity, etc.

[000155] In some instances, for example where the desired product is of synthetic origin, assays based on biological activity will be less meaningful. The proper folding of such molecules may be determined on the basis of physical properties, energetic considerations, modeling studies, and the like.

[000156] The expression host may be further modified by the introduction of sequences encoding one or more enzymes that enhance folding and disulfide bond formation, i.e. foldases, chaperonins, etc. Such sequences may be constitutively or inducibly expressed in the yeast host cell, using vectors, markers, etc. as known in the art. Preferably the sequences, including transcriptional regulatory elements sufficient for the desired pattern of expression, are stably integrated in the yeast genome through a targeted methodology.

[000157] For example, the eukaryotic PDI is not only an efficient catalyst of protein cysteine oxidation and disulfide bond isomerization, but also exhibits chaperone activity. Co-expression of PDI can facilitate the production of active proteins having multiple disulfide bonds. Also of interest is the expression of BIP (immunoglobulin heavy chain binding protein); cyclophilin; and the like. In one embodiment of the invention, each of the

haploid parental strains expresses a distinct folding enzyme, e.g. one strain may express BIP, and the other strain may express PDI or combinations thereof.

[000158] The terms "desired protein" or "desired antibody" are used interchangeably and refer generally to a parent antibody specific to a target, i.e., CGRP or a chimeric or humanized antibody or a binding portion thereof derived therefrom as described herein. The term "antibody" is intended to include any polypeptide chain-containing molecular structure with a specific shape that fits to and recognizes an epitope, where one or more non-covalent binding interactions stabilize the complex between the molecular structure and the epitope. The archetypal antibody molecule is the immunoglobulin, and all types of immunoglobulins, IgG, IgM, IgA, IgE, IgD, etc., from all sources, e.g. human, rodent, rabbit, cow, sheep, pig, dog, other mammals, chicken, other avians, etc., are considered to be "antibodies." A preferred source for producing antibodies useful as starting material according to the invention is rabbits. Numerous antibody coding sequences have been described; and others may be raised by methods well-known in the art. Examples thereof include chimeric antibodies, human antibodies and other non-human mammalian antibodies, humanized antibodies, single chain antibodies (such as scFvs), camelbodies, nanobodies, IgNAR (single-chain antibodies derived from sharks), small-modular immunopharmaceuticals (SMIPs), and antibody fragments such as Fabs, Fab', F(ab')2 and the like. See Streltsov VA, et al., Structure of a shark IgNAR antibody variable domain and modeling of an early-developmental isotype, Protein Sci. 2005 Nov;14(11):2901-9. Epub 2005 Sep 30; Greenberg AS, et al., A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks, Nature. 1995 Mar 9;374(6518):168-73; Nuttall SD, et al., Isolation of the new antigen receptor from wobbegong sharks, and use as a scaffold for the display of protein loop libraries, Mol Immunol. 2001 Aug;38(4):313-26; Hamers-Casterman C, et al., Naturally occurring antibodies devoid of light chains, Nature. 1993 Jun 3;363(6428):446-8; Gill DS, et al., Biopharmaceutical drug discovery using novel protein scaffolds, Curr Opin Biotechnol. 2006 Dec;17(6):653-8. Epub 2006 Oct 19.

[000159] For example, antibodies or antigen binding fragments may be produced by genetic engineering. In this technique, as with other methods, antibody-producing cells are

sensitized to the desired antigen or immunogen. The messenger RNA isolated from antibody producing cells is used as a template to make cDNA using PCR amplification. A library of vectors, each containing one heavy chain gene and one light chain gene retaining the initial antigen specificity, is produced by insertion of appropriate sections of the amplified immunoglobulin cDNA into the expression vectors. A combinatorial library is constructed by combining the heavy chain gene library with the light chain gene library. This results in a library of clones which co-express a heavy and light chain (resembling the Fab fragment or antigen binding fragment of an antibody molecule). The vectors that carry these genes are co-transfected into a host cell. When antibody gene synthesis is induced in the transfected host, the heavy and light chain proteins self-assemble to produce active antibodies that can be detected by screening with the antigen or immunogen.

[000160] Antibody coding sequences of interest include those encoded by native sequences, as well as nucleic acids that, by virtue of the degeneracy of the genetic code, are not identical in sequence to the disclosed nucleic acids, and variants thereof. Variant polypeptides can include amino acid (aa) substitutions, additions or deletions. The amino acid substitutions can be conservative amino acid substitutions or substitutions to eliminate non-essential amino acids, such as to alter a glycosylation site, or to minimize misfolding by substitution or deletion of one or more cysteine residues that are not necessary for function. Variants can be designed so as to retain or have enhanced biological activity of a particular region of the protein (e.g., a functional domain, catalytic amino acid residues, etc). Variants also include fragments of the polypeptides disclosed herein, particularly biologically active fragments and/or fragments corresponding to functional domains. Techniques for in vitro mutagenesis of cloned genes are known. Also included in the subject invention are polypeptides that have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent.

[000161] Chimeric antibodies according to the invention for treatment or prevention of diarrhea in diseases or conditions resulting in increased levels of CGRP may be made by recombinant means by combining the variable light and heavy chain regions (VL and VH), obtained from antibody producing cells of one species with the constant light and heavy

chain regions from another. Typically chimeric antibodies utilize rodent or rabbit variable regions and human constant regions, in order to produce an antibody with predominantly human domains. The production of such chimeric antibodies is well known in the art, and may be achieved by standard means (as described, e.g., in U.S. Patent No. 5,624,659, incorporated herein by reference in its entirety). It is further contemplated that the human constant regions of chimeric antibodies of the invention may be selected from IgG1, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgG10, IgG11, IgG12, IgG13, IgG14, IgG15, IgG16, IgG17, IgG18 or IgG19 constant regions.

[000162] Humanized antibodies are engineered to contain even more human-like immunoglobulin domains, and incorporate only the complementarity-determining regions of the animal-derived antibody. This is accomplished by carefully examining the sequence of the hyper-variable loops of the variable regions of the monoclonal antibody, and fitting them to the structure of the human antibody chains. Although facially complex, the process is straightforward in practice. See, e.g., U.S. Patent No. 6,187,287, incorporated fully herein by reference.

[000163] In addition to entire immunoglobulins (or their recombinant counterparts), immunoglobulin fragments comprising the epitope binding site (e.g., Fab', F(ab')2, or other fragments) may be synthesized. "Fragment," or minimal immunoglobulins may be designed utilizing recombinant immunoglobulin techniques. For instance "Fv" immunoglobulins for use in the present invention may be produced by synthesizing a fused variable light chain region and a variable heavy chain region. Combinations of antibodies are also of interest, e.g. diabodies, which comprise two distinct Fv specificities. In another embodiment of the invention, SMIPs (small molecule immunopharmaceuticals), camelbodies, nanobodies, and IgNAR are encompassed by immunoglobulin fragments.

[000164] Immunoglobulins and fragments thereof may be modified post-translationally, e.g. to add effector moieties such as chemical linkers, detectable moieties, such as fluorescent dyes, enzymes, toxins, substrates, bioluminescent materials, radioactive materials, chemiluminescent moieties and the like, or specific binding moieties, such as streptavidin, avidin, or biotin, and the like may be utilized in the methods and compositions of the present invention. Examples of additional effector molecules are provided infra.

[000165] A polynucleotide sequence "corresponds" to a polypeptide sequence if translation of the polynucleotide sequence in accordance with the genetic code yields the polypeptide sequence (i.e., the polynucleotide sequence "encodes" the polypeptide sequence), one polynucleotide sequence "corresponds" to another polynucleotide sequence if the two sequences encode the same polypeptide sequence.

[000166] A "heterologous" region or domain of a DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous region is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

[000167] A "coding sequence" is an in-frame sequence of codons that (in view of the genetic code) correspond to or encode a protein or peptide sequence. Two coding sequences correspond to each other if the sequences or their complementary sequences encode the same amino acid sequences. A coding sequence in association with appropriate regulatory sequences may be transcribed and translated into a polypeptide. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence. A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. Promoter sequences typically contain additional sites for binding of regulatory molecules (e.g., transcription factors) which affect the transcription of the coding sequence. A coding sequence is "under the control" of the promoter sequence or "operatively linked" to the promoter when RNA polymerase binds the promoter sequence in a cell and transcribes the coding sequence into mRNA, which is then in turn translated into the protein encoded by the coding sequence.

[000168] Vectors are used to introduce a foreign substance, such as DNA, RNA or protein, into an organism or host cell. Typical vectors include recombinant viruses (for

polynucleotides) and liposomes (for polypeptides). A "DNA vector" is a replicon, such as plasmid, phage or cosmid, to which another polynucleotide segment may be attached so as to bring about the replication of the attached segment. An "expression vector" is a DNA vector which contains regulatory sequences which will direct polypeptide synthesis by an appropriate host cell. This usually means a promoter to bind RNA polymerase and initiate transcription of mRNA, as well as ribosome binding sites and initiation signals to direct translation of the mRNA into a polypeptide(s). Incorporation of a polynucleotide sequence into an expression vector at the proper site and in correct reading frame, followed by transformation of an appropriate host cell by the vector, enables the production of a polypepide encoded by said polynucleotide sequence.

[000169] "Amplification" of polynucleotide sequences is the in vitro production of multiple copies of a particular nucleic acid sequence. The amplified sequence is usually in the form of DNA. A variety of techniques for carrying out such amplification are described in a review article by Van Brunt (1990, Bio/Technol., 8(4):291-294). Polymerase chain reaction or PCR is a prototype of nucleic acid amplification, and use of PCR herein should be considered exemplary of other suitable amplification techniques.

[000170] The general structure of antibodies in vertebrates now is well understood (Edelman, G. M., Ann. N.Y. Acad. Sci., 190: 5 (1971)). Antibodies consist of two identical light polypeptide chains of molecular weight approximately 23,000 daltons (the "light chain"), and two identical heavy chains of molecular weight 53,000-70,000 (the "heavy chain"). The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" configuration. The "branch" portion of the "Y" configuration is designated the Fab region; the stem portion of the "Y" configuration is designated the FC region. The amino acid sequence orientation runs from the N-terminal end at the top of the "Y" configuration to the C-terminal end at the bottom of each chain. The N-terminal end possesses the variable region having specificity for the antigen that elicited it, and is approximately 100 amino acids in length, there being slight variations between light and heavy chain and from antibody to antibody.

[000171] The variable region is linked in each chain to a constant region that extends the remaining length of the chain and that within a particular class of antibody does not vary with the specificity of the antibody (i.e., the antigen eliciting it). There are five known major classes of constant regions that determine the class of the immunoglobulin molecule (IgG, IgM, IgA, IgD, and IgE corresponding to γ, μ, α, δ, and ε (gamma, mu, alpha, delta, or epsilon) heavy chain constant regions). The constant region or class determines subsequent effector function of the antibody, including activation of complement (Kabat, E. A., Structural Concepts in Immunology and Immunochemistry, 2nd Ed., p. 413-436, Holt, Rinehart, Winston (1976)), and other cellular responses (Andrews, D. W., et al., Clinical Immunobiology, pp 1-18, W. B. Sanders (1980); Kohl, S., et al., Immunology, 48: 187 (1983)); while the variable region determines the antigen with which it will react. Light chains are classified as either κ (kappa) or λ (lambda). Each heavy chain class can be prepared with either kappa or lambda light chain. The light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages when the immunoglobulins are generated either by hybridomas or by B cells.

[000172] The expression "variable region" or "VR" refers to the domains within each pair of light and heavy chains in an antibody that are involved directly in binding the antibody to the antigen. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain (VL) at one end and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain.

[000173] The expressions "complementarity determining region," "hypervariable region," or "CDR" refer to one or more of the hyper-variable or complementarity determining regions (CDRs) found in the variable regions of light or heavy chains of an antibody (See Kabat, E. A. et al., Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, Md., (1987)). These expressions include the hypervariable regions as defined by Kabat et al. ("Sequences of Proteins of Immunological Interest," Kabat E., et al., US Dept. of Health and Human Services, 1983) or the hypervariable loops in 3-

dimensional structures of antibodies (Chothia and Lesk, J Mol. Biol. 196 901-917 (1987)). The CDRs in each chain are held in close proximity by framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site. Within the CDRs there are select amino acids that have been described as the selectivity determining regions (SDRs) which represent the critical contact residues used by the CDR in the antibody-antigen interaction (Kashmiri, S., Methods, 36:25-34 (2005)).

[000174] The expressions "framework region" or "FR" refer to one or more of the framework regions within the variable regions of the light and heavy chains of an antibody (See Kabat, E. A. et al., Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, Md., (1987)). These expressions include those amino acid sequence regions interposed between the CDRs within the variable regions of the light and heavy chains of an antibody.

ANTI-CGRP ANTIBODIES AND BINDING FRAGMENTS THEREOF HAVING BINDING ACTIVITY FOR CGRP

Antibody Ab1

[000175] The present invention broadly contemplates the use of any anti-CGRP antibody or antibody fragment for the treatment or prevention of CGRP-associated diarrhea in any disease or condition resulting in increased levels of CGRP that are involved in diarrhea, and/or increased fluid or electrolyte excretion from the colon. Conditions and treatments resulting in increased CGRP which is associated with diarrhea are identified in this application.

[000176] In one preferred embodiment, the invention includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQTASPVSAAVGSTVTINCQASQSVYDNNYLAWYQQKPGQPPKQLIYSTSTL ASGVSSRFKGSGSGTQFTLTISDLECADAATYYCLGSYDCSSGDCFVFGGGTEVVV KR (SEQ ID NO: 1).

[000177] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQTASPVSAAVGSTVTINCQASQSVYDNNYLAWYQQKPGQPPKQLIYSTSTL ASGVSSRFKGSGSGTQFTLTISDLECADAATYYCLGSYDCSSGDCFVFGGGTEVVV KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 2).

[000178] The invention further includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: QSLEESGGRLVTPGTPLTLTCTVSGLDLSSYYMQWVRQAPGKGLEWIGVIGINDNT YYASWAKGRFTISRASSTTVDLKMTSLTTEDTATYFCARGDIWGPGTLVTVSS (SEQ ID NO: 3).

[000179] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: QSLEESGGRLVTPGTPLTLTCTVSGLDLSSYYMQWVRQAPGKGLEWIGVIGINDNT YYASWAKGRFTISRASSTTVDLKMTSLTTEDTATYFCARGDIWGPGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 4).

[000180] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 5; SEQ ID NO: 6; and SEQ ID NO: 7 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence

of SEQ ID NO: 1 or the light chain sequence of SEQ ID NO: 2, and/or one or more of the polypeptide sequences of SEQ ID NO: 8; SEQ ID NO: 9; and SEQ ID NO: 10 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 3 or the heavy chain sequence of SEQ ID NO: 4, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000181] The invention also contemplates fragments of the antibody for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 3 or SEQ ID NO: 4.

[000182] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 5; SEQ ID NO: 6; and SEQ ID NO: 7 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 1 or the light chain sequence of SEQ ID NO: 2.

[000183] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 8; SEQ ID NO: 9; and SEQ ID NO: 10 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 3 or the heavy chain sequence of SEQ ID NO: 4.

[000184] The invention also contemplates antibody fragments which include one or more of the antibody fragments described herein for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, fragments of the antibodies

having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 1; the variable heavy chain region of SEQ ID NO: 3; the complementarity-determining regions (SEQ ID NO: 5; SEQ ID NO: 6; and SEQ ID NO: 7) of the variable light chain region of SEQ ID NO: 1; and the complementarity-determining regions (SEQ ID NO: 8; SEQ ID NO: 9; and SEQ ID NO: 10) of the variable heavy chain region of SEQ ID NO: 3.

[000185] In a particularly preferred embodiment of the invention, the chimeric anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab1, comprising, or alternatively consisting of, SEQ ID NO: 2 and SEQ ID NO: 4, and having at least one of the biological activities set forth herein.

[000186] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab1, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 1 and the variable heavy chain sequence of SEQ ID NO: 3. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 1 and/or SEQ ID NO: 3 in said Fab while retaining binding specificity for CGRP.

[000187] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab1. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab1 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab2

[000188] In one embodiment, the invention includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSSGDCFVFGGGTKVEIK R (SEQ ID NO: 11).

[000189] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSSGDCFVFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 12).

[000190] The invention further includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGIN DNTYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT VSS (SEQ ID NO: 13).

[000191] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGIN DNTYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK

TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 14).

[000192] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 15; SEQ ID NO: 16; and SEQ ID NO: 17 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 11 or the light chain sequence of SEQ ID NO: 12, and/or one or more of the polypeptide sequences of SEQ ID NO: 18; SEQ ID NO: 19; and SEQ ID NO: 20 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 13 or the heavy chain sequence of SEQ ID NO: 14, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000193] The invention also contemplates fragments of the antibody for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 11 or SEQ ID NO: 12. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 13 or SEQ ID NO: 14.

[000194] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 15; SEQ ID NO: 16; and SEQ ID NO: 17 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 11 or the light chain sequence of SEQ ID NO: 12.

[000195] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID

NO: 18; SEQ ID NO: 19; and SEQ ID NO: 20 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 13 or the heavy chain sequence of SEQ ID NO: 14.

[000196] The invention also contemplates antibody fragments which include one or more of the antibody fragments described herein for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 11; the variable heavy chain region of SEQ ID NO: 13; the complementarity-determining regions (SEQ ID NO: 15; SEQ ID NO: 16; and SEQ ID NO: 17) of the variable light chain region of SEQ ID NO: 11; and the complementarity-determining regions (SEQ ID NO: 18; SEQ ID NO: 19; and SEQ ID NO: 20) of the variable heavy chain region of SEQ ID NO: 13.

[000197] In a particularly preferred embodiment of the invention, the humanized anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab2, comprising, or alternatively consisting of, SEQ ID NO: 12 and SEQ ID NO: 14, and having at least one of the biological activities set forth herein.

[000198] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab2, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 11 and the variable heavy chain sequence of SEQ ID NO: 13. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 11 and/or SEQ ID NO: 13 in said Fab while retaining binding specificity for CGRP.

[000199] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab2. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab2 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO

or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab3

[000200] In one embodiment, the invention includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSSGDCFVFGGGTKVEIK R (SEQ ID NO: 21).

[000201] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSSGDCFVFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 22).

[000202] The invention further includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGIN DNTYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT VSS (SEQ ID NO: 23).

[000203] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGIN DNTYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT

VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 24).

[000204] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 25; SEQ ID NO: 26; and SEQ ID NO: 27 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 21 or the light chain sequence of SEQ ID NO: 22, and/or one or more of the polypeptide sequences of SEQ ID NO: 28; SEQ ID NO: 29; and SEQ ID NO: 30 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 23 or the heavy chain sequence of SEQ ID NO: 24, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000205] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 21 or SEQ ID NO: 22. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 23 or SEQ ID NO: 24.

[000206] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 25; SEQ ID NO: 26; and SEQ ID NO: 27 which

correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 21 or the light chain sequence of SEQ ID NO: 22.

[000207] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 28; SEQ ID NO: 29; and SEQ ID NO: 30 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 23 or the heavy chain sequence of SEQ ID NO: 24.

[000208] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 21; the variable heavy chain region of SEQ ID NO: 23; the complementarity-determining regions (SEQ ID NO: 25; SEQ ID NO: 26; and SEQ ID NO: 27) of the variable light chain region of SEQ ID NO: 21; and the complementarity-determining regions (SEQ ID NO: 28; SEQ ID NO: 29; and SEQ ID NO: 30) of the variable heavy chain region of SEQ ID NO: 23.

[000209] In a particularly preferred embodiment of the invention, the chimeric anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab3, comprising, or alternatively consisting of, SEQ ID NO: 22 and SEQ ID NO: 24, and having at least one of the biological activities set forth herein.

[000210] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab3, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 21 and the variable heavy chain sequence of SEQ ID NO: 23. This embodiment of the invention further contemplates additions,

deletions, and variants of SEQ ID NO: 21 and/or SEQ ID NO: 23 in said Fab while retaining binding specificity for CGRP.

[000211] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab3. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab3 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab4

[000212] In one embodiment, the invention includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQTPSPVSAAVGSTVTINCQASQSVYHNTYLAWYQQKPGQPPKQLIYDASTL ASGVPSRFSGSGSGTQFTLTISGVQCNDAAAYYCLGSYDCTNGDCFVFGGGTEVV VKR (SEQ ID NO: 31).

[000213] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQTPSPVSAAVGSTVTINCQASQSVYHNTYLAWYQQKPGQPPKQLIYDASTL ASGVPSRFSGSGSGTQFTLTISGVQCNDAAAYYCLGSYDCTNGDCFVFGGGTEVV VKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 32).

[000214] The invention further includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: QSLEESGGRLVTPGTPLTLTCSVSGIDLSGYYMNWVRQAPGKGLEWIGVIGINGAT

YYASWAKGRFTISKTSSTTVDLKMTSLTTEDTATYFCARGDIWGPGTLVTVSS (SEQ ID NO: 33).

[000215] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: QSLEESGGRLVTPGTPLTLTCSVSGIDLSGYYMNWVRQAPGKGLEWIGVIGINGAT YYASWAKGRFTISKTSSTTVDLKMTSLTTEDTATYFCARGDIWGPGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 34).

[000216] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 35; SEQ ID NO: 36; and SEQ ID NO: 37 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 31 or the light chain sequence of SEQ ID NO: 32, and/or one or more of the polypeptide sequences of SEQ ID NO: 38; SEQ ID NO: 39; and SEQ ID NO: 40 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 33 or the heavy chain sequence of SEQ ID NO: 34, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000217] The invention also contemplates fragments of the antibody for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP. In one embodiment of the invention, antibody fragments of the invention comprise, or

alternatively consist of, the polypeptide sequence of SEQ ID NO: 31 or SEQ ID NO: 32. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

[000218] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 35; SEQ ID NO: 36; and SEQ ID NO: 37 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 31 or the light chain sequence of SEQ ID NO: 32.

[000219] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 38; SEQ ID NO: 39; and SEQ ID NO: 40 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 33 or the heavy chain sequence of SEQ ID NO: 34.

[000220] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 31; the variable heavy chain region of SEQ ID NO: 33; the complementarity-determining regions (SEQ ID NO: 35; SEQ ID NO: 36; and SEQ ID NO: 37) of the variable light chain region of SEQ ID NO: 31; and the complementarity-determining regions (SEQ ID NO: 38; SEQ ID NO: 39; and SEQ ID NO: 40) of the variable heavy chain region of SEQ ID NO: 33.

[000221] In a particularly preferred embodiment of the invention, the humanized anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab4, comprising, or alternatively consisting of, SEQ ID NO: 32 and SEQ ID NO: 34, and having at least one of the biological activities set forth herein.

[000222] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab4, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 31 and the variable heavy chain sequence of SEQ ID NO: 33. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 31 and/or SEQ ID NO: 33 in said Fab while retaining binding specificity for CGRP.

[000223] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab4. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab4 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

[000224] Antibody Ab5

[000225] In one embodiment, the invention includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIK R (SEQ ID NO: 41).

[000226] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES

VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 42).

[000227] The invention further includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGIN GATYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT VSS (SEQ ID NO: 43).

[000228] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGIN GATYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 44).

[000229] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 45; SEQ ID NO: 46; and SEQ ID NO: 47 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 41 or the light chain sequence of SEQ ID NO: 42, and/or one or more of the polypeptide sequences of SEQ ID NO: 48; SEQ ID NO: 49; and SEQ ID NO: 50 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 43 or the heavy chain sequence of SEQ ID NO: 44, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments

thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000230] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 41 or SEQ ID NO: 42. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 43 or SEQ ID NO: 44.

[000231] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 45; SEQ ID NO: 46; and SEQ ID NO: 47 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 41 or the light chain sequence of SEQ ID NO: 42.

[000232] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 48; SEQ ID NO: 49; and SEQ ID NO: 50 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 43 or the heavy chain sequence of SEQ ID NO: 44.

[000233] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 41; the variable heavy chain region of SEQ ID NO: 43; the complementarity-determining regions (SEQ ID NO: 45; SEQ ID NO: 46; and SEQ ID NO: 47) of the variable light chain region of SEQ ID NO: 41; and the complementarity-

determining regions (SEQ ID NO: 48; SEQ ID NO: 49; and SEQ ID NO: 50) of the variable heavy chain region of SEQ ID NO: 43.

[000234] In a particularly preferred embodiment of the invention, the chimeric anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab5, comprising, or alternatively consisting of, SEQ ID NO: 42 and SEQ ID NO: 44, and having at least one of the biological activities set forth herein.

[000235] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab5, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 41 and the variable heavy chain sequence of SEQ ID NO: 43. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 41 and/or SEQ ID NO: 43 in said Fab while retaining binding specificity for CGRP.

[000236] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab5. In another embodiment of the invention, anti-CGRP antibodies such as Ab5 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

[000237] Antibody Ab6

[000238] In one embodiment, the invention includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIK R (SEQ ID NO: 51).

[000239] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and

possessing a light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 52).

[000240] The invention further includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGIN GATYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT VSS (SEQ ID NO: 53).

[000241] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGIN GATYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 54).

[000242] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 52, and/or one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and

SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 54, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000243] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 51 or SEQ ID NO: 52. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 53 or SEQ ID NO: 54.

[000244] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 52.

[000245] In a further embodiment of the invention, fragments of the antibody for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 54.

[000246] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one, two, three or more, including

all of the following antibody fragments: the variable light chain region of SEQ ID NO: 51; the variable heavy chain region of SEQ ID NO: 53; the complementarity-determining regions (SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57) of the variable light chain region of SEQ ID NO: 51; and the complementarity-determining regions (SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60) of the variable heavy chain region of SEQ ID NO: 53.

[000247] In a particularly preferred embodiment of the invention, the humanized anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab6, comprising, or alternatively consisting of, SEQ ID NO: 52 and SEQ ID NO: 54, and having at least one of the biological activities set forth herein.

[000248] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab6, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 51 and the variable heavy chain sequence of SEQ ID NO: 53. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 51 and/or SEQ ID NO: 53 in said Fab while retaining binding specificity for CGRP.

[000249] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab6. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab6 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab7

[000250] In one embodiment, the invention includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and

possessing a variable light chain sequence comprising the sequence set forth below: QVLTQTASPVSAAVGSTVTINCQASQSVYNYNYLAWYQQKPGQPPKQLIYSTSTL ASGVSSRFKGSGSGTQFTLTISDVQCDDAATYYCLGSYDCSTGDCFVFGGGTEVV VKR (SEQ ID NO: 61).

[000251] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQTASPVSAAVGSTVTINCQASQSVYNYNYLAWYQQKPGQPPKQLIYSTSTL ASGVSSRFKGSGSGTQFTLTISDVQCDDAATYYCLGSYDCSTGDCFVFGGGTEVV VKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 62).

[000252] The invention further includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: QEQLKESGGRLVTPGTSLTLTCTVSGIDLSNHYMQWVRQAPGKGLEWIGVVGING RTYYASWAKGRFTISRTSSTTVDLKMTRLTTEDTATYFCARGDIWGPGTLVTVSS (SEQ ID NO: 63).

[000253] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: QEQLKESGGRLVTPGTSLTLTCTVSGIDLSNHYMQWVRQAPGKGLEWIGVVGING RTYYASWAKGRFTISRTSSTTVDLKMTRLTTEDTATYFCARGDIWGPGTLVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 64).

[000254] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 65; SEQ ID NO: 66; and SEQ ID NO: 67 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 61 or the light chain sequence of SEQ ID NO: 62, and/or one or more of the polypeptide sequences of SEQ ID NO: 68; SEQ ID NO: 69; and SEQ ID NO: 70 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 63 or the heavy chain sequence of SEQ ID NO: 64, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000255] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 61 or SEQ ID NO: 62. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 63 or SEQ ID NO: 64.

[000256] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 65; SEQ ID NO: 66; and SEQ ID NO: 67 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 61 or the light chain sequence of SEQ ID NO: 62.

[000257] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 68; SEQ ID NO: 69; and SEQ ID NO: 70 which correspond to the complementarity-

determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 63 or the heavy chain sequence of SEQ ID NO: 64.

[000258] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 61; the variable heavy chain region of SEQ ID NO: 63; the complementarity-determining regions (SEQ ID NO: 65; SEQ ID NO: 66; and SEQ ID NO: 67) of the variable light chain region of SEQ ID NO: 61; and the complementarity-determining regions (SEQ ID NO: 68; SEQ ID NO: 69; and SEQ ID NO: 70) of the variable heavy chain region of SEQ ID NO: 63.

[000259] In a particularly preferred embodiment of the invention, the chimeric anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab7, comprising, or alternatively consisting of, SEQ ID NO: 62 and SEQ ID NO: 64, and having at least one of the biological activities set forth herein.

[000260] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab7, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 61 and the variable heavy chain sequence of SEQ ID NO: 63. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 61 and/or SEQ ID NO: 63 in said Fab while retaining binding specificity for CGRP.

[000261] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab7. In another embodiment of the invention, anti-CGRP antibodies such as Ab7 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial

systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab8

[000262] In one embodiment, the invention includes chimeric or humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below:
QVLTQSPSSLSASVGDRVTINCQASQSVYNYNYLAWYQQKPGKVPKQLIYSTSTL
ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSTGDCFVFGGGTKVEIK
R (SEQ ID NO: 71).

[000263] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below:
QVLTQSPSSLSASVGDRVTINCQASQSVYNYNYLAWYQQKPGKVPKQLIYSTSTL
ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSTGDCFVFGGGTKVEIK
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES
VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 72).

[000264] The invention further includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below:
EVQLVESGGGLVQPGGSLRLSCAVSGIDLSNHYMQWVRQAPGKGLEWVGVVGIN
GRTYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT
VSS (SEQ ID NO: 73).

[000265] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below:
EVQLVESGGGLVQPGGSLRLSCAVSGIDLSNHYMQWVRQAPGKGLEWVGVVGIN
GRTYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT

VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 74).

[000266] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 75; SEQ ID NO: 76; and SEQ ID NO: 77 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 71 or the light chain sequence of SEQ ID NO: 72, and/or one or more of the polypeptide sequences of SEQ ID NO: 78; SEQ ID NO: 79; and SEQ ID NO: 80 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 73 or the heavy chain sequence of SEQ ID NO: 74, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000267] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 71 or SEQ ID NO: 72. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 73 or SEQ ID NO: 74.

[000268] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 75; SEQ ID NO: 76; and SEQ ID NO: 77 which correspond to the complementarity-

determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 71 or the light chain sequence of SEQ ID NO: 72.

[000269] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 78; SEQ ID NO: 79; and SEQ ID NO: 80 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 73 or the heavy chain sequence of SEQ ID NO: 74.

[000270] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 71; the variable heavy chain region of SEQ ID NO: 73; the complementarity-determining regions (SEQ ID NO: 75; SEQ ID NO: 76; and SEQ ID NO: 77) of the variable light chain region of SEQ ID NO: 71; and the complementarity-determining regions (SEQ ID NO: 78; SEQ ID NO: 79; and SEQ ID NO: 80) of the variable heavy chain region of SEQ ID NO: 73.

[000271] In a particularly preferred embodiment of the invention, the humanized anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab8, comprising, or alternatively consisting of, SEQ ID NO: 72 and SEQ ID NO: 74, and having at least one of the biological activities set forth herein.

[000272] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab8, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 71 and the variable heavy chain sequence of SEQ ID NO: 73. This embodiment of the invention further contemplates additions,

deletions, and variants of SEQ ID NO: 71 and/or SEQ ID NO: 73 in said Fab while retaining binding specificity for CGRP.

[000273] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab8. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab8 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab9

[000274] In one embodiment, the invention includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQTPSPVSAAVGSTVTINCQASQNVYNNNYLAWYQQKPGQPPKQLIYSTSTL ASGVSSRFRGSGSGTQFTLTISDVQCDDAATYYCLGSYDCSRGDCFVFGGGTEVV VKR (SEQ ID NO: 81).

[000275] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQTPSPVSAAVGSTVTINCQASQNVYNNNYLAWYQQKPGQPPKQLIYSTSTL ASGVSSRFRGSGSGTQFTLTISDVQCDDAATYYCLGSYDCSRGDCFVFGGGTEVV VKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 82).

[000276] The invention further includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: QSLEESGGRLVTPGTPLTLTCTVSGIGLSSYYMQWVRQSPGRGLEWIGVIGSDGKT

YYATWAKGRFTISKTSSTTVDLRMASLTTEDTATYFCTRGDIWGPGTLVTVSS (SEQ ID NO: 83).

[000277] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: QSLEESGGRLVTPGTPLTLTCTVSGIGLSSYYMQWVRQSPGRGLEWIGVIGSDGKT YYATWAKGRFTISKTSSTTVDLRMASLTTEDTATYFCTRGDIWGPGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 84).

[000278] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 85; SEQ ID NO: 86; and SEQ ID NO: 87 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 81 or the light chain sequence of SEQ ID NO: 82, and/or one or more of the polypeptide sequences of SEQ ID NO: 88; SEQ ID NO: 89; and SEQ ID NO: 90 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 83 or the heavy chain sequence of SEQ ID NO: 84, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000279] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one

embodiment of the invention, antibody fragments of the invention for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 81 or SEQ ID NO: 82. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 83 or SEQ ID NO: 84.

[000280] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 85; SEQ ID NO: 86; and SEQ ID NO: 87 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 81 or the light chain sequence of SEQ ID NO: 82.

[000281] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 88; SEQ ID NO: 89; and SEQ ID NO: 90 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 83 or the heavy chain sequence of SEQ ID NO: 84.

[000282] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 81; the variable heavy chain region of SEQ ID NO: 83; the complementarity-determining regions (SEQ ID NO: 85; SEQ ID NO: 86; and SEQ ID NO: 87) of the variable light chain region of SEQ ID NO: 81; and the complementarity-determining regions (SEQ ID NO: 88; SEQ ID NO: 89; and SEQ ID NO: 90) of the variable heavy chain region of SEQ ID NO: 83.

[000283] In a particularly preferred embodiment of the invention, the chimeric anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab9, comprising,

or alternatively consisting of, SEQ ID NO: 82 and SEQ ID NO: 84, and having at least one of the biological activities set forth herein.

[000284] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab9, the Fab fragment for the treatment or prevention of CGRP-associated diarrhea includes the variable light chain sequence of SEQ ID NO: 81 and the variable heavy chain sequence of SEQ ID NO: 83. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 81 and/or SEQ ID NO: 83 in said Fab while retaining binding specificity for CGRP.

[000285] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab9. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab9 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab10

[000286] In one embodiment, the invention includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQNVYNNNYLAWYQQKPGKVPKQLIYSTSTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSRGDCFVFGGGTKVEIK R (SEQ ID NO: 91).

[000287] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQNVYNNNYLAWYQQKPGKVPKQLIYSTSTL

ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSRGDCFVFGGGTKVEIK
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES
VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 92).

[000288] The invention further includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGIGLSSYYMQWVRQAPGKGLEWVGVIGSD
GKTYYATWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCTRGDIWGQGTLVT
VSS (SEQ ID NO: 93).

[000289] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGIGLSSYYMQWVRQAPGKGLEWVGVIGSD
GKTYYATWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCTRGDIWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 94).

[000290] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 95; SEQ ID NO: 96; and SEQ ID NO: 97 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 91 or the light chain sequence of SEQ ID NO: 92, and/or one or more of the polypeptide sequences of SEQ ID NO: 98; SEQ ID NO: 99; and SEQ ID NO: 100 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 93 or the

heavy chain sequence of SEQ ID NO: 94, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000291] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 91 or SEQ ID NO: 92. In another embodiment of the invention, antibody fragments of the invention for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 93 or SEQ ID NO: 94.

[000292] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 95; SEQ ID NO: 96; and SEQ ID NO: 97 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 91 or the light chain sequence of SEQ ID NO: 92.

[000293] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 98; SEQ ID NO: 99; and SEQ ID NO: 100 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 93 or the heavy chain sequence of SEQ ID NO: 94.

[000294] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 91; the variable heavy chain region of SEQ ID NO: 93; the

complementarity-determining regions (SEQ ID NO: 95; SEQ ID NO: 96; and SEQ ID NO: 97) of the variable light chain region of SEQ ID NO: 91; and the complementarity-determining regions (SEQ ID NO: 98; SEQ ID NO: 99; and SEQ ID NO: 100) of the variable heavy chain region of SEQ ID NO: 93.

[000295] In a particularly preferred embodiment of the invention, the humanized anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab10, comprising, or alternatively consisting of, SEQ ID NO: 92 and SEQ ID NO: 94, and having at least one of the biological activities set forth herein.

[000296] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab10, the Fab fragment for the treatment or prevention of CGRP-associated diarrhea includes the variable light chain sequence of SEQ ID NO: 91 and the variable heavy chain sequence of SEQ ID NO: 93. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 91 and/or SEQ ID NO: 93 in said Fab while retaining binding specificity for CGRP.

[000297] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab10. In another embodiment of the invention, anti-CGRP antibodies such as Ab10 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab11

[000298] In one embodiment, the invention includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQTASPVSPAVGSTVTINCRASQSVYYNNYLAWYQQKPGQPPKQLIYSTSTLA

SGVSSRFKGSGSGTQFTLTISDVQCDDAATYYCLGSYDCSNGDCFVFGGGTEVVV KR (SEQ ID NO: 101).

[000299] The invention also includes chimeric antibodies having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: QVLTQTASPVSPAVGSTVTINCRASQSVYYNNYLAWYQQKPGQPPKQLIYSTSTLA SGVSSRFKGSGSGTQFTLTISDVQCDDAATYYCLGSYDCSNGDCFVFGGGTEVVV KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 102).

[000300] The invention further includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: QSLEESGGRLVTPGGSLTLTCTVSGIDVTNYYMQWVRQAPGKGLEWIGVIGVNGK RYYASWAKGRFTISKTSSTTVDLKMTSLTTEDTATYFCARGDIWGPGTLVTVSS (SEQ ID NO: 103).

[000301] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: QSLEESGGRLVTPGGSLTLTCTVSGIDVTNYYMQWVRQAPGKGLEWIGVIGVNGK RYYASWAKGRFTISKTSSTTVDLKMTSLTTEDTATYFCARGDIWGPGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 104).

[000302] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 105; SEQ ID NO: 106; and SEQ ID NO: 107 which correspond to the

complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 101 or the light chain sequence of SEQ ID NO: 102, and/or one or more of the polypeptide sequences of SEQ ID NO: 108; SEQ ID NO: 109; and SEQ ID NO: 110 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 103 or the heavy chain sequence of SEQ ID NO: 104, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000303] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 101 or SEQ ID NO: 102. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 103 or SEQ ID NO: 104.

[000304] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 105; SEQ ID NO: 106; and SEQ ID NO: 107 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 101 or the light chain sequence of SEQ ID NO: 102.

[000305] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 108; SEQ ID NO: 109; and SEQ ID NO: 110 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 103 or the heavy chain sequence of SEQ ID NO: 104.

[000306] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 101; the variable heavy chain region of SEQ ID NO: 103; the complementarity-determining regions (SEQ ID NO: 105; SEQ ID NO: 106; and SEQ ID NO: 107) of the variable light chain region of SEQ ID NO: 101; and the complementarity-determining regions (SEQ ID NO: 108; SEQ ID NO: 109; and SEQ ID NO: 110) of the variable heavy chain region of SEQ ID NO: 103.

[000307] In a particularly preferred embodiment of the invention, the chimeric anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab11, comprising, or alternatively consisting of, SEQ ID NO: 102 and SEQ ID NO: 104, and having at least one of the biological activities set forth herein.

[000308] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab11, the Fab fragment for the treatment or prevention of CGRP-associated diarrhea includes the variable light chain sequence of SEQ ID NO: 101 and the variable heavy chain sequence of SEQ ID NO: 103. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 101 and/or SEQ ID NO: 103 in said Fab while retaining binding specificity for CGRP.

[000309] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab11. In another embodiment of the invention, anti-CGRP antibodies such as Ab11 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial

systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab12

[000310] In one embodiment, the invention includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below:
QVLTQSPSSLSASVGDRVTINCRASQSVYYNNYLAWYQQKPGKVPKQLIYSTSTL
ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSNGDCFVFGGGTKVEIK
R (SEQ ID NO: 111).

[000311] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below:
QVLTQSPSSLSASVGDRVTINCRASQSVYYNNYLAWYQQKPGKVPKQLIYSTSTL
ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSNGDCFVFGGGTKVEIK
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES
VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 112).

[000312] The invention further includes humanized antibodies having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below:
EVQLVESGGGLVQPGGSLRLSCAVSGIDVTNYYMQWVRQAPGKGLEWVGVIGVN
GKRYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT
VSS (SEQ ID NO: 113).

[000313] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below:
EVQLVESGGGLVQPGGSLRLSCAVSGIDVTNYYMQWVRQAPGKGLEWVGVIGVN
GKRYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF

PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 114).

[000314] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 115; SEQ ID NO: 116; and SEQ ID NO: 117 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 111 or the light chain sequence of SEQ ID NO: 112, and/or one or more of the polypeptide sequences of SEQ ID NO: 118; SEQ ID NO: 119; and SEQ ID NO: 120 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 113 or the heavy chain sequence of SEQ ID NO: 114, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000315] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 111 or SEQ ID NO: 112. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 113 or SEQ ID NO: 114.

[000316] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID

NO: 115; SEQ ID NO: 116; and SEQ ID NO: 117 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 111 or the light chain sequence of SEQ ID NO: 112.

[000317] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 118; SEQ ID NO: 119; and SEQ ID NO: 120 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 113 or the heavy chain sequence of SEQ ID NO: 114.

[000318] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 111; the variable heavy chain region of SEQ ID NO: 113; the complementarity-determining regions (SEQ ID NO: 115; SEQ ID NO: 116; and SEQ ID NO: 117) of the variable light chain region of SEQ ID NO: 111; and the complementarity-determining regions (SEQ ID NO: 118; SEQ ID NO: 119; and SEQ ID NO: 120) of the variable heavy chain region of SEQ ID NO: 113.

[000319] In a particularly preferred embodiment of the invention, the humanized anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab12, comprising, or alternatively consisting of, SEQ ID NO: 112 and SEQ ID NO: 114, and having at least one of the biological activities set forth herein.

[000320] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab12, the Fab fragment for the treatment or prevention of CGRP-associated diarrhea includes the variable light chain sequence of

SEQ ID NO: 111 and the variable heavy chain sequence of SEQ ID NO: 113. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 111 and/or SEQ ID NO: 113 in said Fab while retaining binding specificity for CGRP.

[000321] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab12. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab12 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab13

[000322] In one embodiment, the invention includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: AIVMTQTPSSKSVPVGDTVTINCQASESLYNNNALAWFQQKPGQPPKRLIYDASKL ASGVPSRFSGGGSGTQFTLTISGVQCDDAATYYCGGYRSDSVDGVAFAGGTEVVV KR (SEQ ID NO: 121).

[000323] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a light chain sequence comprising the sequence set forth below: AIVMTQTPSSKSVPVGDTVTINCQASESLYNNNALAWFQQKPGQPPKRLIYDASKL ASGVPSRFSGGGSGTQFTLTISGVQCDDAATYYCGGYRSDSVDGVAFAGGTEVVV KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 122).

[000324] The invention further includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and

possessing a variable heavy chain sequence comprising the sequence set forth below: QSVEESGGGLVQPEGSLTLTCTASGFDFSSNAMWWVRQAPGKGLEWIGIIYNGDG STYYASWVNGRFSISKTSSTTVTLQLNSLTVADTATYYCARDLDLWGPGTLVTVS S (SEQ ID NO: 123).

[000325] The invention also includes chimeric antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: QSVEESGGGLVQPEGSLTLTCTASGFDFSSNAMWWVRQAPGKGLEWIGCIYNGD GSTYYASWVNGRFSISKTSSTTVTLQLNSLTVADTATYYCARDLDLWGPGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 124).

[000326] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 125; SEQ ID NO: 126; and SEQ ID NO: 127 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 121 or the light chain sequence of SEQ ID NO: 122, and/or one or more of the polypeptide sequences of SEQ ID NO: 128; SEQ ID NO: 129; and SEQ ID NO: 130 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 123 or the heavy chain sequence of SEQ ID NO: 124, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000327] The invention also contemplates fragments of the antibody for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 121 or SEQ ID NO: 122. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 123 or SEQ ID NO: 124.

[000328] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 125; SEQ ID NO: 126; and SEQ ID NO: 127 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 121 or the light chain sequence of SEQ ID NO: 122.

[000329] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 128; SEQ ID NO: 129; and SEQ ID NO: 130 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 123 or the heavy chain sequence of SEQ ID NO: 124.

[000330] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 121; the variable heavy chain region of SEQ ID NO: 123; the complementarity-determining regions (SEQ ID NO: 125; SEQ ID NO: 126; and SEQ ID NO: 127) of the variable light chain region of SEQ ID NO: 121; and the complementarity-determining regions (SEQ ID NO: 128; SEQ ID NO: 129; and SEQ ID NO: 130) of the variable heavy chain region of SEQ ID NO: 123.

[000331] In a particularly preferred embodiment of the invention, the chimeric anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab13, comprising, or alternatively consisting of, SEQ ID NO: 122 and SEQ ID NO: 124, and having at least one of the biological activities set forth herein.

[000332] In a further particularly preferred embodiment of the invention, antibody fragments comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab13, the Fab fragment for the treatment or prevention of CGRP-associated diarrhea includes the variable light chain sequence of SEQ ID NO: 121 and the variable heavy chain sequence of SEQ ID NO: 123. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 121 and/or SEQ ID NO: 123 in said Fab while retaining binding specificity for CGRP.

[000333] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab13. In another embodiment of the invention, anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea such as Ab13 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab14

[000334] In one embodiment, the invention includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQNVYNNNYLAWYQQKPGKVPKQLIYSTSTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSRGDCFVFGGGTKVEIK R (SEQ ID NO: 131).

[000335] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and

possessing a light chain sequence comprising the sequence set forth below: QVLTQSPSSLSASVGDRVTINCQASQNVYNNNYLAWYQQKPGKVPKQLIYSTSTL ASGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSRGDCFVFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 132).

[000336] The invention further includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a variable heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGIGLSSYYMQWVRQAPGKGLEWVGVIGSD GKTYYATWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCTRGDIWGQGTLVT VSS (SEQ ID NO: 133).

[000337] The invention also includes humanized antibodies for the treatment or prevention of CGRP-associated diarrhea having binding specificity to CGRP and possessing a heavy chain sequence comprising the sequence set forth below: EVQLVESGGGLVQPGGSLRLSCAVSGIGLSSYYMQWVRQAPGKGLEWVGVIGSD GKTYYATWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCTRGDIWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 134).

[000338] The invention further contemplates antibodies for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the polypeptide sequences of SEQ ID NO: 135; SEQ ID NO: 136; and SEQ ID NO: 137 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 131 or the light chain sequence of SEQ ID NO: 132, and/or one or more of the polypeptide sequences of SEQ ID NO: 138; SEQ ID NO: 139;

and SEQ ID NO: 140 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 133 or the heavy chain sequence of SEQ ID NO: 134, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[000339] The invention also contemplates fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea. In one embodiment of the invention, antibody fragments of the invention for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 131 or SEQ ID NO: 132. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 133 or SEQ ID NO: 134.

[000340] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 135; SEQ ID NO: 136; and SEQ ID NO: 137 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 131 or the light chain sequence of SEQ ID NO: 132.

[000341] In a further embodiment of the invention, fragments of the antibody having binding specificity to CGRP for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 138; SEQ ID NO: 139; and SEQ ID NO: 140 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 133 or the heavy chain sequence of SEQ ID NO: 134.

[000342] The invention also contemplates antibody fragments for the treatment or prevention of CGRP-associated diarrhea which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the

antibodies having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 131; the variable heavy chain region of SEQ ID NO: 133; the complementarity-determining regions (SEQ ID NO: 135; SEQ ID NO: 136; and SEQ ID NO: 137) of the variable light chain region of SEQ ID NO: 131; and the complementarity-determining regions (SEQ ID NO: 138; SEQ ID NO: 139; and SEQ ID NO: 140) of the variable heavy chain region of SEQ ID NO: 133.

[000343] In a particularly preferred embodiment of the invention, the humanized anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is Ab14, comprising, or alternatively consisting of, SEQ ID NO: 132 and SEQ ID NO: 134, and having at least one of the biological activities set forth herein.

[000344] In a further particularly preferred embodiment of the invention, antibody fragments for the treatment or prevention of CGRP-associated diarrhea comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab14, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 131 and the variable heavy chain sequence of SEQ ID NO: 133. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 131 and/or SEQ ID NO: 133 in said Fab while retaining binding specificity for CGRP.

[000345] In one embodiment of the invention described herein (infra), Fab fragments for the treatment or prevention of CGRP-associated diarrhea may be produced by enzymatic digestion (e.g., papain) of Ab14. In another embodiment of the invention, anti-CGRP antibodies such as Ab14 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

[000346] In another embodiment, antibody fragments may be present in one or more of the following non-limiting forms: Fab, Fab', F(ab')2, Fv and single chain Fv antibody forms. In a preferred embodiment, the anti-CGRP antibodies described herein for the

treatment or prevention of CGRP-associated diarrhea further comprises the kappa constant light chain sequence comprising the sequence set forth below:

[000347] VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 283).

[000348] In another preferred embodiment, the anti-CGRP antibodies described herein for the treatment or prevention of CGRP-associated diarrhea further comprises the gamma-1 constant heavy chain polypeptide sequence comprising the sequence set forth below:

[000349] ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK (SEQ ID NO: 284).

[000350] In another embodiment, the invention contemplates an isolated anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea comprising a VH polypeptide sequence selected from: SEQ ID NO: 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, or 133, or a variant thereof; and further comprising a VL polypeptide sequence selected from: SEQ ID NO: 1, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, or 131, or a variant thereof, wherein one or more of the framework residues (FR residues) in said VH or VL polypeptide has been substituted with another amino acid residue resulting in an anti-CGRP antibody that specifically binds CGRP. The invention contemplates humanized and chimeric forms of these antibodies for the treatment or prevention of CGRP-associated diarrhea. The chimeric antibodies may include an Fc derived from IgG1, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgG10, IgG11, IgG12, IgG13, IgG14, IgG15, IgG16, IgG17, IgG18 or IgG19 constant regions.

[000351] In one embodiment of the invention, the antibodies or VH or VL polypeptides for the treatment or prevention of CGRP-associated diarrhea originate or are selected from

one or more rabbit B cell populations prior to initiation of the humanization process referenced herein.

[000352] In another embodiment of the invention, the anti-CGRP antibodies and fragments thereof for the treatment or prevention of CGRP-associated diarrhea do not have binding specificity for CGRP-R. In a further embodiment of the invention, the anti-CGRP antibodies and fragments thereof inhibit the association of CGRP with CGRP-R. In another embodiment of the invention, the anti-CGRP antibodies and fragments thereof for the treatment or prevention of CGRP-associated diarrhea inhibit the association of CGRP with CGRP-R and/or additional proteins and/or multimers thereof, and/or antagonizes the biological effects thereof.

[000353] As stated in paragraph [0127] herein, antibodies and fragments thereof for the treatment or prevention of CGRP-associated diarrhea may be modified post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent moieties, or functional moieties such as for example streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, and radioactive materials.

[000354] Antibodies or fragments thereof may also be chemically modified to provide additional advantages such as increased solubility, stability and circulating time (in vivo half-life) of the polypeptide, or decreased immunogenicity (See U.S. Pat. No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The antibodies and fragments thereof may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[000355] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired

therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa. Branched polyethylene glycols are described, for example, in U.S. Pat. No. 5,643,575; Morpurgo et al., Appl. Biochem. Biotechnol. 56:59-72 (1996); Vorobjev et al., Nucleosides Nucleotides 18:2745-2750 (1999); and Caliceti et al., Bioconjug. Chem. 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[000356] There are a number of attachment methods available to those skilled in the art, See e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), See also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[000357] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to polypeptides via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid,

glutamic acid, or cysteine) or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof).

[000358] Alternatively, antibodies or fragments thereof for the treatment or prevention of CGRP-associated diarrhea may have increased in vivo half lives via fusion with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (See, e.g., U.S. Pat. No. 5,876,969, issued Mar. 2, 1999, EP Patent 0 413 622, and U.S. Pat. No. 5,766,883, issued Jun. 16, 1998, herein incorporated by reference in their entirety)) or other circulating blood proteins such as transferrin or ferritin. In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1-585 of human serum albumin as shown in FIGS. 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

[000359] Regarding detectable moieties, further exemplary enzymes include, but are not limited to, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, beta-galactosidase and luciferase. Further exemplary fluorescent materials include, but are not limited to, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin and dansyl chloride. Further exemplary chemiluminescent moieties include, but are not limited to, luminol. Further exemplary bioluminescent materials include, but are not limited to, luciferin and aequorin. Further exemplary radioactive materials include, but are not limited to, Iodine 125 (125I), Carbon 14 (14C), Sulfur 35 (35S), Tritium (3H) and Phosphorus 32 (32P).

[000360] Regarding functional moieties, exemplary cytotoxic agents include, but are not limited to, methotrexate, aminopterin, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine; alkylating agents such as mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU), mitomycin C, lomustine (CCNU), 1-methylnitrosourea, cyclothosphamide, mechlorethamine, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin and carboplatin (paraplatin); anthracyclines include daunorubicin (formerly daunomycin), doxorubicin (adriamycin), detorubicin, carminomycin, idarubicin, epirubicin, mitoxantrone and

bisantrene; antibiotics include dactinomycin (actinomycin D), bleomycin, calicheamicin, mithramycin, and anthramycin (AMC); and antimytotic agents such as the vinca alkaloids, vincristine and vinblastine. Other cytotoxic agents include paclitaxel (taxol), ricin, pseudomonas exotoxin, gemcitabine, cytochalasin B, gramicidin D, ethidium bromide, emetine, etoposide, tenoposide, colchicin, dihydroxy anthracin dione, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons, and mixtures of these cytotoxic agents.

[000361] Further cytotoxic agents include, but are not limited to, chemotherapeutic agents such as carboplatin, cisplatin, paclitaxel, gemcitabine, calicheamicin, doxorubicin, 5-fluorouracil, mitomycin C, actinomycin D, cyclophosphamide, vincristine and bleomycin. Toxic enzymes from plants and bacteria such as ricin, diphtheria toxin and Pseudomonas toxin may be conjugated to the humanized or chimeric antibodies, or binding fragments thereof, to generate cell-type-specific-killing reagents (Youle, et al., Proc. Nat'l Acad. Sci. USA 77:5483 (1980); Gilliland, et al., Proc. Nat'l Acad. Sci. USA 77:4539 (1980); Krolick, et al., Proc. Nat'l Acad. Sci. USA 77:5419 (1980)).

[000362] Other cytotoxic agents include cytotoxic ribonucleases as described by Goldenberg in U.S. Pat. No. 6,653,104. Embodiments of the invention also relate to radioimmunoconjugates where a radionuclide that emits alpha or beta particles is stably coupled to the antibody, or binding fragments thereof, with or without the use of a complex-forming agent. Such radionuclides include beta-emitters such as Phosphorus-32 (32P), Scandium-47 (47Sc), Copper-67 (67Cu), Gallium-67 (67Ga), Yttrium-88 (88Y), Yttrium-90 (90Y), Iodine-125 (125I), Iodine-131 (131I), Samarium-153 (153Sm), Lutetium-177 (177Lu), Rhenium-186 (186Re) or Rhenium-188 (188Re), and alpha-emitters such as Astatine-211 (211At), Lead-212 (212Pb), Bismuth-212 (212Bi) or -213 (213Bi) or Actinium-225 (225Ac).

[000363] Methods are known in the art for conjugating an antibody or binding fragment thereof to a detectable moiety and the like, such as for example those methods described by Hunter et al, Nature 144:945 (1962); David et al, Biochemistry 13:1014 (1974); Pain et al,

J. Immunol. Meth. 40:219 (1981); and Nygren, J., Histochem. and Cytochem. 30:407 (1982).

[000364] Embodiments described herein further include variants and equivalents that are substantially homologous to the antibodies, antibody fragments, diabodies, SMIPs, camelbodies, nanobodies, IgNAR, polypeptides, variable regions and CDRs set forth herein. These may contain, e.g., conservative substitution mutations, (i.e., the substitution of one or more amino acids by similar amino acids). For example, conservative substitution refers to the substitution of an amino acid with another within the same general class, e.g., one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid, or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art.

[000365] In another embodiment, the invention contemplates polypeptide sequences having at least 90% or greater sequence homology to any one or more of the polypeptide sequences of antibody fragments, variable regions and CDRs set forth herein. More preferably, the invention contemplates polypeptide sequences having at least 95% or greater sequence homology, even more preferably at least 98% or greater sequence homology, and still more preferably at least 99% or greater sequence homology to any one or more of the polypeptide sequences of antibody fragments, variable regions and CDRs set forth herein. Methods for determining homology between nucleic acid and amino acid sequences are well known to those of ordinary skill in the art.

[000366] In another embodiment, the invention further contemplates the above-recited polypeptide homologs of the antibody fragments, variable regions and CDRs set forth herein further having anti-CGRP activity. Non-limiting examples of anti-CGRP activity are set forth herein, for example, in paragraphs [0329]-[0350] infra.

[000367] In another embodiment, the invention further contemplates the generation and use of anti-idiotypic antibodies that bind any of the foregoing sequences. In an exemplary embodiment, such an anti-idiotypic antibody could be administered to a subject who has received an anti-CGRP antibody to modulate, reduce, or neutralize, the effect of the anti-CGRP antibody. Such anti-idiotypic antibodies could also be useful for treatment of an autoimmune disease characterized by the presence of anti-CGRP antibodies. A further

exemplary use of such anti-idiotypic antibodies is for detection of the anti-CGRP antibodies of the present invention, for example to monitor the levels of the anti-CGRP antibodies present in a subject's blood or other bodily fluids.

[000368] The present invention also contemplates anti-CGRP antibodies comprising any of the polypeptide or polynucleotide sequences described herein substituted for any of the other polynucleotide sequences described herein. For example, without limitation thereto, the present invention contemplates antibodies comprising the combination of any of the variable light chain and variable heavy chain sequences described herein, and further contemplates antibodies resulting from substitution of any of the CDR sequences described herein for any of the other CDR sequences described herein.

Additional Exemplary Embodiments of the Invention

[000369] In another embodiment, the invention contemplates one or more anti-human CGRP antibodies or antibody fragments thereof for the treatment or prevention of CGRP-associated diarrhea which specifically bind to the same linear or conformational epitope(s) and/or competes for binding to the same linear or conformational epitope(s) on an intact human CGRP polypeptide or fragment thereof as an anti-human CGRP antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, or Ab14. In a preferred embodiment, the anti-human CGRP antibody or fragment thereof specifically binds to the same linear or conformational epitope(s) and/or competes for binding to the same linear or conformational epitope(s) on an intact human CGRP polypeptide or a fragment thereof as Ab3, Ab6, Ab13, Ab12 or Ab14.

[000370] A preferred embodiment of the invention is directed to chimeric or humanized antibodies and fragments thereof (including Fab fragments) having binding specificity for CGRP and inhibiting biological activities mediated by the binding of CGRP to the CGRP receptor for the treatment or prevention of CGRP-associated diarrhea. In a particularly preferred embodiment of the invention, the chimeric or humanized anti-CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea are selected from Ab3, Ab6, Ab10, Ab13, or Ab14.

[000371] A preferred embodiment of the invention is directed to methods of screening antibodies and fragments thereof (including Fab fragments) having binding specificity to

human Calcitonin Gene Related Peptide (hereinafter "CGRP") in animal models to determine the in vivo effects thereof, especially their ability to antagonize the adverse side effects of CGRP including CGRP-associated diarrhea and to treat conditions involving excess CGRP.

[000372] Another more specific preferred embodiment of the invention involves a method of assessing the potential in vivo efficacy of a candidate anti-CGRP antibody or antibody fragment comprising determining whether the antibody inhibits CGRP associated diarrhea compared to a rodent administered CGRP in the absence of the candidate CGRP antibody or antibody fragment.

[000373] A more specific preferred embodiment of the invention involves a method of assessing the potential in vivo efficacy of a candidate anti-CGRP antibody or antibody fragment to treat a neurological condition characterized by increased CGRP levels.

[000374] Another more specific preferred embodiment of the invention involves a method of assessing the potential in vivo efficacy of a candidate anti-CGRP antibody or antibody fragment to treat a CGRP associated disorder associated with diarrhea such as migraine or chronic migraine, (with or without aura), weight loss, cancer or tumors, angiogenesis associated with cancer or tumor growth, angiogenesis associated with cancer or tumor survival, hemiplagic migraines, cluster headaches, migrainous neuralgia, chronic headaches, tension headaches, general headaches, hot flushes, chronic paroxysomal hemicrania, secondary headaches due to an underlying structural problem in the head or neck, cranial neuralgia, sinus headaches (such as for example associated with sinusitis), allergy-induced headaches or migraines, pain, inflammatory pain, post-operative incision pain, complex regional pain syndrome, cancer pain, primary or metastatic bone cancer pain, fracture pain, chronic pain, osteoporotic fracture pain, pain resulting from burn, osteoporosis, gout joint pain, abdominal pain, pain associated with sickle cell crises, and other nociceptic pain, as well as hepatocellular carcinoma, breast cancer, liver cirrhosis, neurogenic pain, neuropathic pain, nociceptic pain, trigeminal neuralgia, post-herpetic neuralgia, phantom limb pain, fibromyalgia, menstrual pain, ovarialgia, reflex sympathetic dystrophy, neurogenic pain, osteoarthritis or rheumatoid arthritis pain, lower back pain, diabetic neuropathy, sciatica, or pain or visceral pain associated with: gastro-esophageal

reflux, dyspepsia, irritable bowel syndrome, irritable colon, spastic colon, mucous colitis, inflammatory bowel disease, Crohn's disease, ileitis, ulcerative colitis, renal colic, dysmenorrhea, cystitis, menstrual period, labor, menopause, prostatitis, pancreatitis, renal colic, dysmenorrhea, cystitis, including interstitial cystitis (IC), surgery associated with the ileus, diverticulitis, peritonitis, pericarditis, hepatitis, appendicitis, colitis, cholecystitis, endometriosis, chronic and/or acute pancreatitis, myocardial infarction, kidney pain, pleural pain, prostatitis, pelvic pain, trauma to an organ, chronic nociceptive pain, chronic neuropathic pain, chronic inflammatory pain, fibromyalgia, breakthrough pain and persistent pain.

[000375] Another more specific preferred embodiment of the invention involves a method of using an anti-CGRP antibody or antibody fragment to treat a CGRP associated disorder associated with diarrhea wherein the condition is cancer pain arising from malignancy or from cancer preferably selected from one or more of: adenocarcinoma in glandular tissue, blastoma in embryonic tissue of organs, carcinoma in epithelial tissue, leukemia in tissues that form blood cells, lymphoma in lymphatic tissue, myeloma in bone marrow, sarcoma in connective or supportive tissue, adrenal cancer, AIDS-related lymphoma, anemia, bladder cancer, bone cancer, brain cancer, breast cancer, carcinoid tumours, cervical cancer, chemotherapy, colon cancer, cytopenia, endometrial cancer, esophageal cancer, gastric cancer, head cancer, neck cancer, hepatobiliary cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, Hodgkin's disease, lymphoma, non- Hodgkin's, nervous system tumours, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, urethral cancer, bone cancer, sarcomas cancer of the connective tissue, cancer of bone tissue, cancer of blood-forming cells, cancer of bone marrow, multiple myeloma, leukaemia, primary or secondary bone cancer, tumours that metastasize to the bone, tumours infiltrating the nerve and hollow viscus, tumours near neural structures. Further preferably the cancer pain comprises visceral pain, preferably visceral pain which arises from pancreatic cancer and/or metastases in the abdomen. Further preferably the cancer pain comprises somatic pain, preferably somatic pain due to one or more of bone cancer, metastasis in the bone, postsurgical pain, sarcomas cancer of the connective tissue, cancer of bone tissue, cancer of

blood-forming cells of the bone marrow, multiple myeloma, leukaemia, primary or secondary bone cancer.

[000376] A further another preferred embodiment of the invention relates to methods of inhibiting, preventing or treating diarrhea and/or maintaining electrolyte balance and fluid levels in the intestines of a subject having a condition associated with elevated CGRP levels that result in diarrhea and/or increased flux of electrolytes and fluids from the intestines comprising administering an effective amount of an anti-CGRP antibody or anti-CGRP antibody fragment.

[000377] Related thereto another preferred embodiment of the invention specifically relates to methods of treating or preventing diarrhea in individuals with functional bowel disorders or an inflammatory bowel diseases, bacterial or viral induced diarrhea, cancer associated with diarrhea, such as medullary thyroid carcinoma or a colorectal cancer, and more specifically functional bowel disorders or inflammatory bowel diseases, including by way of example gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, functional abdominal pain syndrome, diverticulosis, and diverticulitis or inflammatory bowel disease is selected from the group consisting of Crohn's disease, ileitis, collagenous colitis, lymphocytic colitis, and ulcerative colitis wherein these therapies administer an effective amount of an anti-CGRP antibody or antibody fragment administered as a monotherapy or in combination with another active agent.

[000378] Other preferred embodiments the present invention are directed to screening assays and therapeutic usage of specific antibodies and fragments thereof having binding specificity for CGRP, in particular antibodies having desired epitopic specificity, high affinity or avidity and/or functional properties. In preferred embodiments this invention relates to assays and usage of the antibodies described herein, comprising the sequences of the VH, VL and CDR polypeptides described herein, and the polynucleotides encoding them. A preferred embodiment of the invention is directed to chimeric or humanized antibodies and fragments thereof (including Fab fragments) capable of binding to CGRP and/or inhibiting the biological activities mediated by the binding of CGRP to the CGRP receptor ("CGRP-R").

[000379] In a further embodiment of the invention is contemplated a method of reducing, treating or preventing diseases or disorders associated with CGRP which may include diarrhea as an adverse side effect by affecting those biological activities mediated via CGRP, thereby avoiding the biological activities mediated via binding of CGRP to CGRP-R. A further non-limiting listing of diseases and disorders associated with CGRP is provided herein.

[000380] In another embodiment of the invention, the anti-human CGRP antibody for the treatment or prevention of CGRP-associated diarrhea is an antibody which specifically binds to the same linear or conformational epitopes on an intact CGRP polypeptide or fragment thereof that is (are) specifically bound by Ab3, Ab6, Ab13, or Ab14 as ascertained by epitopic mapping using overlapping linear peptide fragments which span the full length of the native human CGRP polypeptide.

[000381] The invention is also directed to an anti-CGRP antibody for the treatment or prevention of CGRP-associated diarrhea that binds with the same CGRP epitope and/or competes with an anti-CGRP antibody for binding to CGRP as an antibody or antibody fragment disclosed herein, including but not limited to an anti-CGRP antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, or Ab14.

[000382] In another embodiment, the invention is also directed to an isolated anti-CGRP antibody or antibody fragment for the treatment or prevention of CGRP-associated diarrhea comprising one or more of the CDRs contained in the VH polypeptide sequences selected from: 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, or 133, or a variant thereof, and/or one or more of the CDRs contained in the VL polypeptide sequences selected from: 1, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, or 131, or a variant thereof.

[000383] In one embodiment of the invention, the anti-human CGRP antibody discussed in the two prior paragraphs comprises at least 2 complementarity determining regions (CDRs) in each the variable light and the variable heavy regions which are identical to those contained in an anti-human CGRP antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, or Ab14.

[000384] In a preferred embodiment, the anti-human CGRP antibody discussed above comprises at least 2 complementarity determining regions (CDRs) in each the variable light

and the variable heavy regions which are identical to those contained in Ab3 or Ab6. In another embodiment, all of the CDRs of the anti-human CGRP antibody discussed above are identical to the CDRs contained in an anti-human CGRP antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, or Ab14. In a preferred embodiment of the invention, all of the CDRs of the anti-human CGRP antibody discussed above are identical to the CDRs contained in an anti-human CGRP antibody selected from Ab3 or Ab6.

[000385] The invention further contemplates that the one or more anti-human CGRP antibodies discussed above for the treatment or prevention of CGRP-associated diarrhea which are aglycosylated; or minimally glycosylated, e.g., which lack N-glycosylation but may comprise some O-glycosylation, such as mannose residues, and/or that contain an Fc region that has been modified to alter effector function, half-life, proteolysis, and/or glycosylation; are human, humanized, single chain or chimeric; and are a humanized antibody derived from a rabbit (parent) anti-human CGRP antibody.

[000386] The invention further contemplates one or more anti-human CGRP antibodies for the treatment or prevention of CGRP-associated diarrhea wherein the framework regions (FRs) in the variable light region and the variable heavy regions of said antibody respectively are human FRs which are unmodified or which have been modified by the substitution of one or more human FR residues in the variable light or heavy chain region with the corresponding FR residues of the parent rabbit antibody, and wherein said human FRs have been derived from human variable heavy and light chain antibody sequences which have been selected from a library of human germline antibody sequences based on their high level of homology to the corresponding rabbit variable heavy or light chain regions relative to other human germline antibody sequences contained in the library.

[000387] In one embodiment of the invention, the anti-human CGRP antibody or fragment for the treatment or prevention of CGRP-associated diarrhea specifically binds to CGRP expressing human cells and/or to circulating soluble CGRP molecules in vivo, including CGRP expressed on or by human cells in a patient with a disease associated with cells that express CGRP.

[000388] In another embodiment, the CGRP related disease that may be associated with diarrhea is selected from migraines (with or without aura), weight loss, cancer or tumors, angiogenesis associated with cancer or tumor growth, angiogenesis associated with cancer or tumor survival, hemiplagic migraines, cluster headaches, migrainous neuralgia, chronic headaches, tension headaches, general headaches, hot flushes, chronic paroxysomal hemicrania, secondary headaches due to an underlying structural problem in the head or neck, cranial neuralgia, sinus headaches (such as for example associated with sinusitis), allergy-induced headaches or migraines, pain, inflammatory pain, post-operative incision pain, complex regional pain syndrome, cancer pain, primary or metastatic bone cancer pain, fracture pain, chronic pain, osteoporotic fracture pain, pain resulting from burn, osteoporosis, gout joint pain, abdominal pain, pain associated with sickle cell crises, and other nociceptic pain, as well as hepatocellular carcinoma, breast cancer, liver cirrhosis, neurogenic pain, neuropathic pain, nociceptic pain, trigeminal neuralgia, post-herpetic neuralgia, phantom limb pain, fibromyalgia, menstrual pain, ovarialgia, reflex sympathetic dystrophy, neurogenic pain, osteoarthritis or rheumatoid arthritis pain, lower back pain, diabetic neuropathy, sciatica, or pain or visceral pain associated with: gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, irritable colon, spastic colon, mucous colitis, inflammatory bowel disease, Crohn's disease, ileitis, ulcerative colitis, renal colic, dysmenorrhea, cystitis, menstrual period, labor, menopause, prostatitis, pancreatitis, renal colic, dysmenorrhea, cystitis, including interstitial cystitis (IC), surgery associated with the ileus, diverticulitis, peritonitis, pericarditis, hepatitis, appendicitis, colitis, cholecystitis, endometriosis, chronic and/or acute pancreatitis, myocardial infarction, kidney pain, pleural pain, prostatitis, pelvic pain, trauma to an organ, chronic nociceptive pain, chronic neuropathic pain, chronic inflammatory pain, fibromyalgia, breakthrough pain and persistent pain.

[000389] In another embodiment of the invention, the disease treated that may be associated with diarrhea is cancer pain arising from malignancy or from cancer preferably selected from one or more of: adenocarcinoma in glandular tissue, blastoma in embryonic tissue of organs, carcinoma in epithelial tissue, leukemia in tissues that form blood cells, lymphoma in lymphatic tissue, myeloma in bone marrow, sarcoma in connective or

supportive tissue, adrenal cancer, AIDS-related lymphoma, anemia, bladder cancer, bone cancer, brain cancer, breast cancer, carcinoid tumours, cervical cancer, chemotherapy, colon cancer, cytopenia, , endometrial cancer, esophageal cancer, gastric cancer, head cancer, neck cancer, hepatobiliary cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, Hodgkin's disease, lymphoma, non- Hodgkin's, nervous system tumours, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, urethral cancer, bone cancer, sarcomas cancer of the connective tissue, cancer of bone tissue, cancer of blood-forming cells, cancer of bone marrow, multiple myeloma, leukaemia, primary or secondary bone cancer, tumours that metastasize to the bone, tumours infiltrating the nerve and hollow viscus, tumours near neural structures. Further preferably the cancer pain comprises visceral pain, preferably visceral pain which arises from pancreatic cancer and/or metastases in the abdomen. Further preferably the cancer pain comprises somatic pain, preferably somatic pain due to one or more of bone cancer, metastasis in the bone, postsurgical pain, sarcomas cancer of the connective tissue, cancer of bone tissue, cancer of blood-forming cells of the bone marrow, multiple myeloma, leukaemia, primary or secondary bone cancer.

[000390] The invention further contemplates anti-human CGRPantibodies or fragments diarrhea directly or indirectly attached to a detectable label or therapeutic agent.

[000391] The invention also contemplates one or more nucleic acid sequences which result in the expression of an anti-human CGRP antibody or antibody fragment as set forth above, including those comprising, or alternatively consisting of, yeast or human preferred codons. The invention also contemplates vectors (including plasmids or recombinant viral vectors) comprising said nucleic acid sequence(s). The invention also contemplates host cells or recombinant host cells expressing at least one of the antibodies set forth above, including a mammalian, yeast, bacterial, and insect cells. In a preferred embodiment, the host cell is a yeast cell. In a further preferred embodiment, the yeast cell is a diploidal yeast cell. In a more preferred embodiment, the yeast cell is a Pichia yeast.

[000392] The invention also contemplates a method of treatment comprising administering to a patient with a disease or condition associated with CGRP expressing cells that results

in diarrhea a therapeutically effective amount of at least one anti-human CGRP antibody or fragment described herein. The invention also contemplates that the treatment method may involve the administration of two or more anti-CGRP antibodies or fragments thereof and disclosed herein. If more than one antibody is administered to the patient, the multiple antibodies may be administered simultaneously or concurrently, or may be staggered in their administration. The diseases that may be treated are presented in the non-limiting list set forth above and elsewhere herein. In a preferred embodiment, the disease is selected from migraine, headache, weight loss, pain, cancer pain or neuropathic pain. In another embodiment the treatment further includes the administration of another therapeutic agent or regimen selected from chemotherapy, radiotherapy, cytokine administration or gene therapy.

[000393] In a non-limiting embodiment of the invention, another therapeutic agent or regimen includes opioids, analgesics such as NSAIDs, Taxol (paclitaxel) or its derivatives, platinum compounds such as carboplatin or cisplatin, anthrocyclines such as doxorubicin, alkylating agents such as cyclophosphamide, anti-metabolites such as 5-fluorouracil, or etoposide.

[000394] The invention further contemplates a method of in vivo imaging which detects the presence of cells which express CGRP comprising administering a diagnostically effective amount of at least one anti-human CGRP antibody. In one embodiment, said administration further includes the administration of a radionuclide or fluorophore that facilitates detection of the antibody at CGRP expressing disease sites. In a further embodiment, the results of said in vivo imaging method are used to facilitate the design of an appropriate therapeutic regimen, including therapeutic regimens including radiotherapy, chemotherapy or a combination thereof.

[000395] The anti-CGRP activity of the anti-CGRP antibodies of the present invention, and fragments thereof having binding specificity to CGRP diarrhea, may also be described by their strength of binding or their affinity for CGRP. In one embodiment of the invention, the anti-CGRP antibodies of the present invention, and fragments thereof having binding specificity to CGRP, bind to CGRP with a dissociation constant (KD) of less than or equal to 5x10-7 M, 10-7 M, 5x10-8 M, 10-8 M, 5x10-9 M, 10-9 M, 5x10-10 M, 10-10

M, 5x10-11 M, 10-11 M, 5x10-12 M, 10-12 M, 5x10-13 M, or 10-13 M. Preferably, the anti-CGRP antibodies and fragments thereof bind CGRP with a dissociation constant of less than or equal to 10-11 M, 5x10-12 M, or 10-12 M. In another embodiment of the invention, the anti-CGRP antibodies of the present invention, and fragments thereof having binding specificity to CGRP, bind to a linear or conformational CGRP epitope.

[000396] In another embodiment of the invention, the anti-CGRP activity of the anti-CGRP antibodies of the present invention, and fragments thereof having binding specificity to CGRP, bind to CGRP with an off-rate of less than or equal to 10-4 S-1, 5x10-5 S-1, 10-5 S-1, 5x10-6 S-1, 10-6 S-1, 5x10-7 S-1, or 10-7 S-1.

[000397] In a further embodiment of the invention, the anti-CGRP activity of the anti-CGRP antibodies of the present invention, and fragments thereof having binding specificity to CGRP, exhibit anti-CGRP activity by preventing, ameliorating or reducing the symptoms of, or alternatively treating, diseases and disorders associated with CGRP especially diarrhea. Non-limiting examples of diseases and disorders associated with CGRP are set forth herein.

Polynucleotides Encoding Anti-CGRP Antibody Polypeptides

Antibody Ab1

[000398] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 1:

[000399] CAAGTGCTGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAAG
CACAGTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTATGATAACAACTACC
TAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCT
ACATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGG
GACACAGTTCACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTA

CTACTGTCTAGGCAGTTATGATTGTAGTAGTGGTGATTGTTTTGTTTTCGGCGG AGGGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 141).

[000400] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 2:

[000401] CAAGTGCTGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAAG CACAGTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTATGATAACAACTACC TAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCT ACATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGG GACACAGTTCACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTA CTACTGTCTAGGCAGTTATGATTGTAGTAGTGGTGATTGTTTTGTTTTCGGCGG AGGGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCT TCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGC TGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCC CTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAA ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 142).

[000402] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 3:

[000403] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC CCTGACACTCACCTGCACAGTCTCTGGACTCGACCTCAGTAGCTACTACATGCA ATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTA TTAATGATAACACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCC AGAGCCTCGTCGACCACGGTGGATCTGAAAATGACCAGTCTGACAACCGAGGA CACGGCCACCTATTTCTGTGCCAGAGGGGACATCTGGGGCCCAGGCACCCTCG TCACCGTCTCGAGC (SEQ ID NO: 143).

[000404] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 4:

[000405] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGACTCGACCTCAGTAGCTACTACATGCA
ATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTA
TTAATGATAACACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCC
AGAGCCTCGTCGACCACGGTGGATCTGAAAATGACCAGTCTGACAACCGAGGA
CACGGCCACCTATTTCTGTGCCAGAGGGGACATCTGGGGCCCAGGCACCCTCG
TCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCAcCCT
CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGG
CGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
CGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG
TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATC
TTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG
GACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC
GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA
GCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCG
TCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCC
CCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGA
ACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTC
CCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA
AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA
(SEQ ID NO: 144).

[000406] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 145; SEQ ID NO: 146; and SEQ ID NO: 147 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 1 or the light chain sequence of SEQ ID NO: 2.

[000407] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 148; SEQ ID NO: 149; and SEQ ID NO: 150 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 3 or the heavy chain sequence of SEQ ID NO: 4.

[000408] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 141 encoding the light chain variable sequence of SEQ ID NO: 1; the polynucleotide SEQ ID NO: 142 encoding the light chain sequence of SEQ ID NO: 2; the polynucleotide SEQ ID NO: 143 encoding the heavy chain variable sequence of SEQ ID NO: 3; the polynucleotide SEQ ID NO: 144 encoding the heavy chain sequence of SEQ ID NO: 4; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 145; SEQ ID NO: 146; and SEQ ID NO: 147) of the light chain variable sequence of SEQ ID NO: 1 or the light chain sequence of SEQ ID NO: 2; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 148; SEQ ID NO: 149; and SEQ ID NO: 150) of the heavy chain variable sequence of SEQ ID NO: 3 or the heavy chain sequence of SEQ ID NO: 4.

[000409] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab1,

the polynucleotides encoding the full length Ab1 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 142 encoding the light chain sequence of SEQ ID NO: 2 and the polynucleotide SEQ ID NO: 144 encoding the heavy chain sequence of SEQ ID NO: 4.

[000410] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab1 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab1 or Fab fragments thereof may be produced via expression of Ab1 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab2

[000411] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 11:

[000412] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTATGATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagt tcctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctg ggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTAGGCAGTTATGATT GTAGTAGTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgt (SEQ ID NO: 151).

[000413] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 12:

[000414] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTATGATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagt tcctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctg ggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTAGGCAGTTATGATT GTAGTAGTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgtACGGTGGCTG CACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCA AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGG CCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 152).

[000415] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 13:

[000416] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaCTCGACCTCagtAGCTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTATCAATGATAACACATACTACGCGAGCTGGGCGAAAGGCcg attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat TTCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGC (SEQ ID NO: 153).

[000417] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 14:

[000418] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaCTCGACCTCagtAGCTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTATCAATGATAACACATACTACGCGAGCTGGGCGAAAGGCcg

attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat
TTCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGCGCCTCCACCA
AGGGCCCATCGGTCTTCCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGCA
CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTG
TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT
ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA
GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC
ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGaTCTCCCgGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAG
CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG
AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG
CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG
TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
AGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 154).

[000419] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 155; SEQ ID NO: 156; and SEQ ID NO: 157 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 11 or the light chain sequence of SEQ ID NO: 12.

[000420] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 158; SEQ ID NO: 159; and SEQ ID

NO: 160 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 13 or the heavy chain sequence of SEQ ID NO: 14.

[000421] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 151 encoding the light chain variable sequence of SEQ ID NO: 11; the polynucleotide SEQ ID NO: 152 encoding the light chain sequence of SEQ ID NO: 12; the polynucleotide SEQ ID NO: 153 encoding the heavy chain variable sequence of SEQ ID NO: 13; the polynucleotide SEQ ID NO: 154 encoding the heavy chain sequence of SEQ ID NO: 14; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 155; SEQ ID NO: 156; and SEQ ID NO: 157) of the light chain variable sequence of SEQ ID NO: 11 or the light chain sequence of SEQ ID NO: 12; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 158; SEQ ID NO: 159; and SEQ ID NO: 160) of the heavy chain variable sequence of SEQ ID NO: 13 or the heavy chain sequence of SEQ ID NO: 14.

[000422] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab2, the polynucleotides encoding the full length Ab2 antibody comprise, or alternatively consist of,the polynucleotide SEQ ID NO: 152 encoding the light chain sequence of SEQ ID NO: 12 and the polynucleotide SEQ ID NO: 154 encoding the heavy chain sequence of SEQ ID NO: 14.

[000423] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by

enzymatic digestion (e.g., papain) of Ab2 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab2 or Fab fragments thereof may be produced via expression of Ab2 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab3

[000424] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 21:

[000425] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTATGATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagt tcctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctg ggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTAGGCAGTTATGATT GTAGTAGTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgt (SEQ ID NO: 161).

[000426] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 22:

[000427] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTATGATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagt tcctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctg ggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTAGGCAGTTATGATT GTAGTAGTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgtACGGTGGCTG CACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT

GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG
CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCA
AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGG
CCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 162).

[000428] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 23:

[000429] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt
ctggaCTCGACCTCagtAGCTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt
cGGAGTCATTGGTATCAATGATAACACATACTACGCGAGCTGGGCGAAAGGCcg
attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat
TTCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGC (SEQ ID NO: 163).

[000430] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 24:

[000431] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt
ctggaCTCGACCTCagtAGCTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt
cGGAGTCATTGGTATCAATGATAACACATACTACGCGAGCTGGGCGAAAGGCcg
attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat
TTCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGCGCCTCCACCA
AGGGCCCATCGGTCTTCCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGCA
CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTG
TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT
ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA
GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACGCGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC
ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGaTCTCCCgGACCCCTGAGGTCACATGCGTGGT

GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAG
CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG
AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG
CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG
TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
AGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 164).

[000432] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 165; SEQ ID NO: 166; and SEQ ID NO: 167 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 21 or the light chain sequence of SEQ ID NO: 22.

[000433] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 168; SEQ ID NO: 169; and SEQ ID NO: 170 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 23 or the heavy chain sequence of SEQ ID NO: 24.

[000434] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 161 encoding the light chain variable sequence of SEQ ID NO: 21; the polynucleotide SEQ ID NO: 162 encoding the light chain sequence of SEQ ID

NO: 22; the polynucleotide SEQ ID NO: 163 encoding the heavy chain variable sequence of SEQ ID NO: 23; the polynucleotide SEQ ID NO: 164 encoding the heavy chain sequence of SEQ ID NO: 24; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 165; SEQ ID NO: 166; and SEQ ID NO: 167) of the light chain variable sequence of SEQ ID NO: 21 or the light chain sequence of SEQ ID NO: 22; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 168; SEQ ID NO: 169; and SEQ ID NO: 170) of the heavy chain variable sequence of SEQ ID NO: 23 or the heavy chain sequence of SEQ ID NO: 24.

[000435] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab3, the polynucleotides encoding the full length Ab3 antibody comprise, or alternatively consist of,the polynucleotide SEQ ID NO: 162 encoding the light chain sequence of SEQ ID NO: 22 and the polynucleotide SEQ ID NO: 164 encoding the heavy chain sequence of SEQ ID NO: 24.

[000436] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab3 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab3 or Fab fragments thereof may be produced via expression of Ab3 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab4

[000437] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 31:

[000438] CAAGTGCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAAG CACAGTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTATCATAACACCTACCT GGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAACAACTGATCTATGATG CATCCACTCTGGCGTCTGGGGTCCCATCGCGGTTCAGCGGCAGTGGATCTGGG ACACAGTTCACTCTCACCATCAGCGGCGTGCAGTGTAACGATGCTGCCGCTTAC TACTGTCTGGGCAGTTATGATTGTACTAATGGTGATTGTTTTGTTTTCGGCGGA GGGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 171).

[000439] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 32:

[000440] CAAGTGCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAAG CACAGTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTATCATAACACCTACCT GGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAACAACTGATCTATGATG CATCCACTCTGGCGTCTGGGGTCCCATCGCGGTTCAGCGGCAGTGGATCTGGG ACACAGTTCACTCTCACCATCAGCGGCGTGCAGTGTAACGATGCTGCCGCTTAC TACTGTCTGGGCAGTTATGATTGTACTAATGGTGATTGTTTTGTTTTCGGCGGA GGGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCT CCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAAC ACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACA AAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 172).

[000441] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 33:

[000442] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGTTCCGTCTCTGGCATCGACCTCAGTGGCTACTACATGAA
CTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTA
TTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCC
AAAACCTCGTCGACCACGGTGGATCTGAAAATGACCAGTCTGACAACCGAGGA
CACGGCCACCTATTTCTGTGCCAGAGGGGACATCTGGGGCCCGGGCACCCTCG
TCACCGTCTCGAGC (SEQ ID NO: 173).

[000443] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 34:

[000444] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGTTCCGTCTCTGGCATCGACCTCAGTGGCTACTACATGAA
CTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTA
TTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCC
AAAACCTCGTCGACCACGGTGGATCTGAAAATGACCAGTCTGACAACCGAGGA
CACGGCCACCTATTTCTGTGCCAGAGGGGACATCTGGGGCCCGGGCACCCTCG
TCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCAcCCT
CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGG
CGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
CGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG
TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATC
TTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG
GACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC
GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA
GCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCG

TCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCC
CCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGA
ACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTC
CCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA
AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA
(SEQ ID NO: 174).

[000445] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 175; SEQ ID NO: 176; and SEQ ID NO: 177 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 31 or the light chain sequence of SEQ ID NO: 32.

[000446] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 178; SEQ ID NO: 179; and SEQ ID NO: 180 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 33 or the heavy chain sequence of SEQ ID NO: 34.

[000447] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 171 encoding the light chain variable sequence of SEQ ID NO: 31; the polynucleotide SEQ ID NO: 172 encoding the light chain sequence of SEQ ID NO: 32; the polynucleotide SEQ ID NO: 173 encoding the heavy chain variable sequence of SEQ ID NO: 33; the polynucleotide SEQ ID NO: 174 encoding the heavy chain

sequence of SEQ ID NO: 34; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 175; SEQ ID NO: 176; and SEQ ID NO: 177) of the light chain variable sequence of SEQ ID NO: 31 or the light chain sequence of SEQ ID NO: 32; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 178; SEQ ID NO: 179; and SEQ ID NO: 180) of the heavy chain variable sequence of SEQ ID NO: 33 or the heavy chain sequence of SEQ ID NO: 34.

[000448] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab4, the polynucleotides encoding the full length Ab4 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 172 encoding the light chain sequence of SEQ ID NO: 32 and the polynucleotide SEQ ID NO: 174 encoding the heavy chain sequence of SEQ ID NO: 34.

[000449] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab4 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab4 or Fab fragments thereof may be produced via expression of Ab4 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab5

[000450] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following

polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 41:

[000451] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTATCATAACACCTACCTGGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatGATGCATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctg ggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATT GTACTAATGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgt (SEQ ID NO: 181).

[000452] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 42:

[000453] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTATCATAACACCTACCTGGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatGATGCATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctg ggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATT GTACTAATGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgtACGGTGGCTG CACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCA AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGG CCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 182).

[000454] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 43:

[000455] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGACCTCagtGGCTACTACATGAACtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTATTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCcg attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat

TTCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGC (SEQ ID NO: 183).

[000456] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 44:

[000457] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGACCTCagtGGCTACTACATGAACtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTATTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCcg attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat TTCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGCGCCTCCACCA AGGGCCCATCGGTCTTCCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGCA CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTG TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGaTCTCCCgGACCCCTGAGGTCACATGCGTGGT GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAG CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 184).

[000458] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 185; SEQ ID NO: 186; and SEQ ID NO: 187 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 41 or the light chain sequence of SEQ ID NO: 42.

[000459] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 188; SEQ ID NO: 189; and SEQ ID NO: 190 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 43 or the heavy chain sequence of SEQ ID NO: 44.

[000460] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 181 encoding the light chain variable sequence of SEQ ID NO: 41; the polynucleotide SEQ ID NO: 182 encoding the light chain sequence of SEQ ID NO: 42; the polynucleotide SEQ ID NO: 183 encoding the heavy chain variable sequence of SEQ ID NO: 43; the polynucleotide SEQ ID NO: 184 encoding the heavy chain sequence of SEQ ID NO: 44; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 185; SEQ ID NO: 186; and SEQ ID NO: 187) of the light chain variable sequence of SEQ ID NO: 41 or the light chain sequence of SEQ ID NO: 42; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 188; SEQ ID NO: 189; and SEQ ID NO: 190) of the heavy chain variable sequence of SEQ ID NO: 43 or the heavy chain sequence of SEQ ID NO: 44.

[000461] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab5,

the polynucleotides encoding the full length Ab5 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 182 encoding the light chain sequence of SEQ ID NO: 42 and the polynucleotide SEQ ID NO: 184 encoding the heavy chain sequence of SEQ ID NO: 44.

[000462] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab5 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab5 or Fab fragments thereof may be produced via expression of Ab5 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

[000463] Antibody Ab6

[000464] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 51:

[000465] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTATCATAACACCTACCTGGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatGATGCATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctg ggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATT GTACTAATGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgt (SEQ ID NO: 191).

[000466] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 52:

[000467] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTATCATAACACCTACCTGGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatGATGCATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctg ggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATT GTACTAATGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgtACGGTGGCTG CACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCA AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGG CCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 192).

[000468] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 53:

[000469] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGACCTCagtGGCTACTACATGAACtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTATTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCcg attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat TTCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGC (SEQ ID NO: 193).

[000470] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 54:

[000471] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGACCTCagtGGCTACTACATGAACtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTATTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCcg

attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat
TTCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGCGCCTCCACCA
AGGGCCCATCGGTCTTCCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGCA
CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTG
TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT
ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA
GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACGCGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC
ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGaTCTCCCgGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAG
CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG
AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG
CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG
TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
AGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 194).

[000472] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 195; SEQ ID NO: 196; and SEQ ID NO: 197 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 52.

[000473] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 198; SEQ ID NO: 199; and SEQ ID

NO: 200 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 54.

[000474] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 191 encoding the light chain variable sequence of SEQ ID NO: 51; the polynucleotide SEQ ID NO: 192 encoding the light chain sequence of SEQ ID NO: 52; the polynucleotide SEQ ID NO: 193 encoding the heavy chain variable sequence of SEQ ID NO: 53; the polynucleotide SEQ ID NO: 194 encoding the heavy chain sequence of SEQ ID NO: 54; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 195; SEQ ID NO: 196; and SEQ ID NO: 197) of the light chain variable sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 52; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 198; SEQ ID NO: 199; and SEQ ID NO: 200) of the heavy chain variable sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 54.

[000475] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab6, the polynucleotides encoding the full length Ab6 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 192 encoding the light chain sequence of SEQ ID NO: 52 and the polynucleotide SEQ ID NO: 194 encoding the heavy chain sequence of SEQ ID NO: 54.

[000476] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by

enzymatic digestion (e.g., papain) of Ab6 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab6 or Fab fragments thereof may be produced via expression of Ab6 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab7

[000477] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 61:

[000478] CAAGTGCTGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAAG CACAGTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTATAATTACAACTACCT TGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTA CATCCACTCTGGCATCTGGGGTCTCATCGCGATTCAAAGGCAGTGGATCTGGG ACACAGTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTTAC TACTGTCTAGGCAGTTATGACTGTAGTACTGGTGATTGTTTTGTTTTCGGCGGA GGGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 201).

[000479] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 62:

[000480] CAAGTGCTGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAAG CACAGTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTATAATTACAACTACCT TGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTA CATCCACTCTGGCATCTGGGGTCTCATCGCGATTCAAAGGCAGTGGATCTGGG ACACAGTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTTAC TACTGTCTAGGCAGTTATGACTGTAGTACTGGTGATTGTTTTGTTTTCGGCGGA

GGGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTC
CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCT
CCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGC
ACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAAC
ACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACA
AAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 202).

[000481] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 63:

[000482] CAGGAGCAGCTGAAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGA
CATCCCTGACACTCACCTGCACCGTCTCTGGAATCGACCTCAGTAACCACTACA
TGCAATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAGTCGTT
GGTATTAATGGTCGCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCAT
CTCCAGAACCTCGTCGACCACGGTGGATCTGAAAATGACCAGGCTGACAACCG
AGGACACGGCCACCTATTTCTGTGCCAGAGGGGACATCTGGGGCCCAGGCACC
CTGGTCACCGTCTCGAGC (SEQ ID NO: 203).

[000483] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 64:

[000484] CAGGAGCAGCTGAAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGA
CATCCCTGACACTCACCTGCACCGTCTCTGGAATCGACCTCAGTAACCACTACA
TGCAATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAGTCGTT
GGTATTAATGGTCGCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCAT
CTCCAGAACCTCGTCGACCACGGTGGATCTGAAAATGACCAGGCTGACAACCG
AGGACACGGCCACCTATTTCTGTGCCAGAGGGGACATCTGGGGCCCAGGCACC
CTGGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
cCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAA
GGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCA
GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCA

GCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCA
AATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTG
GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCC
TGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGA
CAAAGCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTC
ACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTC
CAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGC
AGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC
AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACAT
CGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACG
CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA
GGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT
GA (SEQ ID NO: 204).

[000485] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 205; SEQ ID NO: 206; and SEQ ID NO: 207 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 61 or the light chain sequence of SEQ ID NO: 62.

[000486] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 208; SEQ ID NO: 209; and SEQ ID NO: 210 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 63 or the heavy chain sequence of SEQ ID NO: 64.

[000487] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In

one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 201 encoding the light chain variable sequence of SEQ ID NO: 61; the polynucleotide SEQ ID NO: 202 encoding the light chain sequence of SEQ ID NO: 62; the polynucleotide SEQ ID NO: 203 encoding the heavy chain variable sequence of SEQ ID NO: 63; the polynucleotide SEQ ID NO: 204 encoding the heavy chain sequence of SEQ ID NO: 64; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 205; SEQ ID NO: 206; and SEQ ID NO: 207) of the light chain variable sequence of SEQ ID NO: 61 or the light chain sequence of SEQ ID NO: 62; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 208; SEQ ID NO: 209; and SEQ ID NO: 210) of the heavy chain variable sequence of SEQ ID NO: 63 or the heavy chain sequence of SEQ ID NO: 64.

[000488] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab7, the polynucleotides encoding the full length Ab7 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 202 encoding the light chain sequence of SEQ ID NO: 62 and the polynucleotide SEQ ID NO: 204 encoding the heavy chain sequence of SEQ ID NO: 64.

[000489] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab7 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab7 or Fab fragments thereof may be produced via expression of Ab7 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia)

and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab8

[000490] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 71:

[000491] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTAcAATTACAACTACCTTGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctgg gacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATTG TAGTACTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgt (SEQ ID NO: 211).

[000492] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 72:

[000493] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAGTGTTTAcAATTACAACTACCTTGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctgg gacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATTG TAGTACTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgtACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGC CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGC AGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 212).

[000494] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 73:

[000495] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGACCTCagtAACCACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCGTTGGTATcAATGGTCGCACATACTACGCGAGCTGGGCGAAAGGCcga ttcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtatT TCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGC (SEQ ID NO: 213).

[000496] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 74:

[000497] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGACCTCagtAACCACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCGTTGGTATcAATGGTCGCACATACTACGCGAGCTGGGCGAAAGGCcga ttcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtatT TCtgtGCTAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGCGCCTCCACCAA GGGCCCATCGGTCTTCCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGCAC AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTA CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAA GGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCAC CGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAA AACCCAAGGACACCCTCATGaTCTCCCgGACCCCTGAGGTCACATGCGTGGTGG TGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAGCA CGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGC AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAA AACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGC

CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
GGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT
TCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC
TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAG
CCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 214).

[000498] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 215; SEQ ID NO: 216; and SEQ ID NO: 217 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 71 or the light chain sequence of SEQ ID NO: 72.

[000499] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 218; SEQ ID NO: 219; and SEQ ID NO: 220 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 73 or the heavy chain sequence of SEQ ID NO: 74.

[000500] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 211 encoding the light chain variable sequence of SEQ ID NO: 71; the polynucleotide SEQ ID NO: 212 encoding the light chain sequence of SEQ ID NO: 72; the polynucleotide SEQ ID NO: 213 encoding the heavy chain variable sequence of SEQ ID NO: 73; the polynucleotide SEQ ID NO: 214 encoding the heavy chain sequence of SEQ ID NO: 74; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 215; SEQ ID NO: 216; and SEQ ID NO: 217) of the light chain variable sequence of SEQ ID NO: 71 or the light chain sequence of SEQ ID NO: 72; and

polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 218; SEQ ID NO: 219; and SEQ ID NO: 220) of the heavy chain variable sequence of SEQ ID NO: 73 or the heavy chain sequence of SEQ ID NO: 74.

[000501] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab8, the polynucleotides encoding the full length Ab8 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 212 encoding the light chain sequence of SEQ ID NO: 72 and the polynucleotide SEQ ID NO: 214 encoding the heavy chain sequence of SEQ ID NO: 74.

[000502] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab8 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab8 or Fab fragments thereof may be produced via expression of Ab8 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab9

[000503] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 81:

[000504] CAAGTGCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAAG CACAGTCACCATCAATTGCCAGGCCAGTCAGAATGTTTATAATAACAACTACC TAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCT ACGTCCACTCTGGCATCTGGGGTCTCATCGCGATTCAGAGGCAGTGGATCTGG GACACAGTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTT ACTACTGTCTAGGCAGTTATGATTGTAGTCGTGGTGATTGTTTTGTTTTCGGCG GAGGGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 221).

[000505] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 82:

[000506] CAAGTGCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAAG CACAGTCACCATCAATTGCCAGGCCAGTCAGAATGTTTATAATAACAACTACC TAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCT ACGTCCACTCTGGCATCTGGGGTCTCATCGCGATTCAGAGGCAGTGGATCTGG GACACAGTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTT ACTACTGTCTAGGCAGTTATGATTGTAGTCGTGGTGATTGTTTTGTTTTCGGCG GAGGGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATC TTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTG CTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGC CCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGA AACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC ACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 222).

[000507] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 83:

[000508] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC CCTGACACTCACCTGCACAGTCTCTGGAATCGGCCTCAGTAGCTACTACATGCA GTGGGTCCGCCAGTCTCCAGGGAGGGGGCTGGAATGGATCGGAGTCATTGGTA GTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCC

AAGACCTCGTCGACCACGGTGGATCTGAGAATGGCCAGTCTGACAACCGAGGA
CACGGCCACCTATTTCTGTACCAGAGGGGACATCTGGGGCCCGGGGACCCTCG
TCACCGTCTCGAGC (SEQ ID NO: 223).

[000509] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 84:

[000510] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGAATCGGCCTCAGTAGCTACTACATGCA
GTGGGTCCGCCAGTCTCCAGGGAGGGGGCTGGAATGGATCGGAGTCATTGGTA
GTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCC
AAGACCTCGTCGACCACGGTGGATCTGAGAATGGCCAGTCTGACAACCGAGGA
CACGGCCACCTATTTCTGTACCAGAGGGGACATCTGGGGCCCGGGGACCCTCG
TCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCAcCCT
CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGG
CGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
CGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG
TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATC
TTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG
GACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC
GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA
GCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCG
TCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCC
CCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGA
ACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTC
CCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA
AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT

CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 224).

[000511] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 225; SEQ ID NO: 226; and SEQ ID NO: 227 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 81 or the light chain sequence of SEQ ID NO: 82.

[000512] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 228; SEQ ID NO: 229; and SEQ ID NO: 230 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 83 or the heavy chain sequence of SEQ ID NO: 84.

[000513] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 221 encoding the light chain variable sequence of SEQ ID NO: 81; the polynucleotide SEQ ID NO: 222 encoding the light chain sequence of SEQ ID NO: 82; the polynucleotide SEQ ID NO: 223 encoding the heavy chain variable sequence of SEQ ID NO: 83; the polynucleotide SEQ ID NO: 224 encoding the heavy chain sequence of SEQ ID NO: 84; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 225; SEQ ID NO: 226; and SEQ ID NO: 227) of the light chain variable sequence of SEQ ID NO: 81 or the light chain sequence of SEQ ID NO: 82; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 228; SEQ ID NO: 229; and SEQ ID NO: 230) of the heavy chain variable sequence of SEQ ID NO: 83 or the heavy chain sequence of SEQ ID NO: 84.

[000514] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab9, the polynucleotides encoding the full length Ab9 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 222 encoding the light chain sequence of SEQ ID NO: 82 and the polynucleotide SEQ ID NO: 224 encoding the heavy chain sequence of SEQ ID NO: 84.

[000515] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab9 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab9 or Fab fragments thereof may be produced via expression of Ab9 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab10

[000516] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 91:

[000517] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA
GGCCAGTCAGAATGTTTAcAATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagtt
cctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctgg
gacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATTG

TAGTCGTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgt (SEQ ID NO: 231).

[000518] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 92:

[000519] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAATGTTTAcAATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctgg gacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATTG TAGTCGTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgtACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGC CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGC AGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 232).

[000520] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 93:

[000521] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGGCCTCagtAGCTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTAGTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCcg attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat TTCtgtACCAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGC (SEQ ID NO: 233).

[000522] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 94:

[000523] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGGCCTCagtAGCTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTAGTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCcg attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat TTCtgtACCAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGCGCCTCCACCA AGGGCCCATCGGTCTTCCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGCA CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTG TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGaTCTCCCgGACCCCTGAGGTCACATGCGTGGT GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAG CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 234).

[000524] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 235; SEQ ID NO: 236; and SEQ ID NO: 237 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 91 or the light chain sequence of SEQ ID NO: 92.

[000525] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 238; SEQ ID NO: 239; and SEQ ID NO:240 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 93 or the heavy chain sequence of SEQ ID NO: 94.

[000526] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 231 encoding the light chain variable sequence of SEQ ID NO: 91; the polynucleotide SEQ ID NO: 232 encoding the light chain sequence of SEQ ID NO: 92; the polynucleotide SEQ ID NO: 233 encoding the heavy chain variable sequence of SEQ ID NO: 93; the polynucleotide SEQ ID NO: 234 encoding the heavy chain sequence of SEQ ID NO: 94; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 235; SEQ ID NO: 236; and SEQ ID NO: 237) of the light chain variable sequence of SEQ ID NO: 91 or the light chain sequence of SEQ ID NO: 92; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 238; SEQ ID NO: 239; and SEQ ID NO: 240) of the heavy chain variable sequence of SEQ ID NO: 93 or the heavy chain sequence of SEQ ID NO: 94.

[000527] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab10, the polynucleotides encoding the full length Ab10 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 232 encoding the light chain sequence of SEQ ID NO: 92 and the polynucleotide SEQ ID NO: 234 encoding the heavy chain sequence of SEQ ID NO: 94.

[000528] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO,

NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab10 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab10 or Fab fragments thereof may be produced via expression of Ab10 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab11

[000529] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 101:

[000530] CAGGTGCTGACCCAGACTGCATCCCCCGTGTCTCCAGCTGTGGGAAG CACAGTCACCATCAATTGCCGGGCCAGTCAGAGTGTTTATTATAACAACTACCT AGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTA CATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGG ACACAGTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTTAC TACTGTCTAGGCAGTTATGATTGTAGTAATGGTGATTGTTTTGTTTTCGGCGGA GGGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 241).

[000531] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 102:

[000532] CAGGTGCTGACCCAGACTGCATCCCCCGTGTCTCCAGCTGTGGGAAG CACAGTCACCATCAATTGCCGGGCCAGTCAGAGTGTTTATTATAACAACTACCT AGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTA

CATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGG
ACACAGTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTTAC
TACTGTCTAGGCAGTTATGATTGTAGTAATGGTGATTGTTTTGTTTTCGGCGGA
GGGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTC
CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCT
CCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGC
ACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAAC
ACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACA
AAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 242).

[000533] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 103:

[000534] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGAGGATC
CCTGACACTCACCTGCACAGTCTCTGGAATCGACGTCACTAACTACTATATGCA
ATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTG
TGAATGGTAAGAGATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCC
AAAACCTCGTCGACCACGGTGGATCTGAAAATGACCAGTCTGACAACCGAGGA
CACGGCCACCTATTTCTGTGCCAGAGGCGACATCTGGGGCCCGGGGACCCTCG
TCACCGTCTCGAGC (SEQ ID NO: 243).

[000535] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 104:

[000536] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGAGGATC
CCTGACACTCACCTGCACAGTCTCTGGAATCGACGTCACTAACTACTATATGCA
ATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTG
TGAATGGTAAGAGATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCC
AAAACCTCGTCGACCACGGTGGATCTGAAAATGACCAGTCTGACAACCGAGGA
CACGGCCACCTATTTCTGTGCCAGAGGCGACATCTGGGGCCCGGGGACCCTCG
TCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCAcCCT

CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGG
CGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
CGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG
TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATC
TTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGG
GACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC
GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA
GCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCG
TCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCC
CCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGA
ACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTC
CCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA
AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA
(SEQ ID NO: 244).

[000537] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 245; SEQ ID NO: 246; and SEQ ID NO: 247 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 101 or the light chain sequence of SEQ ID NO: 102.

[000538] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 248; SEQ ID NO: 249; and SEQ ID NO: 250 which correspond to polynucleotides encoding the complementarity-determining

regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 103 or the heavy chain sequence of SEQ ID NO: 104.

[000539] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 241 encoding the light chain variable sequence of SEQ ID NO: 101; the polynucleotide SEQ ID NO: 242 encoding the light chain sequence of SEQ ID NO: 102; the polynucleotide SEQ ID NO: 243 encoding the heavy chain variable sequence of SEQ ID NO: 103; the polynucleotide SEQ ID NO: 244 encoding the heavy chain sequence of SEQ ID NO: 104; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 245; SEQ ID NO: 246; and SEQ ID NO: 247) of the light chain variable sequence of SEQ ID NO: 101 or the light chain sequence of SEQ ID NO: 102; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 248; SEQ ID NO: 249; and SEQ ID NO: 250) of the heavy chain variable sequence of SEQ ID NO: 103 or the heavy chain sequence of SEQ ID NO: 104.

[000540] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab11, the polynucleotides encoding the full length Ab11 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 242 encoding the light chain sequence of SEQ ID NO: 102 and the polynucleotide SEQ ID NO: 244 encoding the heavy chain sequence of SEQ ID NO: 104.

[000541] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab11 following expression of the full-length

polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab11 or Fab fragments thereof may be produced via expression of Ab11 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab12

[000542] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 111:

[000543] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCG GGCCAGTCAGAGTGTTTAcTATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctgg gacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATTG TAGTAATGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgt (SEQ ID NO: 251).

[000544] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 112:

[000545] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCG GGCCAGTCAGAGTGTTTAcTATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctgg gacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATTG TAGTAATGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgtACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGC CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGC

AGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 252).

[000546] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 113:

[000547] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGACGTCACTAACTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgg gtcGGAGTCATTGGTGTGAATGGTAAGAGATACTACGCGAGCTGGGCGAAAGGC cgattcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgt atTTCtgtGCCAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGC (SEQ ID NO: 253).

[000548] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 114:

[000549] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGACGTCACTAACTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgg gtcGGAGTCATTGGTGTGAATGGTAAGAGATACTACGCGAGCTGGGCGAAAGGC cgattcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgt atTTCtgtGCCAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGCGCCTCCACC AAGGGCCCATCGGTCTTCCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGC ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGT GTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCC TACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGaTCTCCCgGACCCCTGAGGTCACATGCGTGGT GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG

GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAG
CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG
AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG
CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG
TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
AGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 254).

[000550] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 255; SEQ ID NO: 256; and SEQ ID NO: 257 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 111 or the light chain sequence of SEQ ID NO: 112.

[000551] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 258; SEQ ID NO: 259; and SEQ ID NO: 260 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 113 or the heavy chain sequence of SEQ ID NO: 114.

[000552] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 251 encoding the light chain variable sequence of SEQ ID NO: 111; the polynucleotide SEQ ID NO: 252 encoding the light chain sequence of SEQ ID NO: 112; the polynucleotide SEQ ID NO: 253 encoding the heavy chain variable

sequence of SEQ ID NO: 113; the polynucleotide SEQ ID NO: 254 encoding the heavy chain sequence of SEQ ID NO: 114; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 255; SEQ ID NO: 256; and SEQ ID NO: 257) of the light chain variable sequence of SEQ ID NO: 111 or the light chain sequence of SEQ ID NO: 112; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 258; SEQ ID NO: 259; and SEQ ID NO: 260) of the heavy chain variable sequence of SEQ ID NO: 113 or the heavy chain sequence of SEQ ID NO: 114.

[000553] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab12, the polynucleotides encoding the full length Ab12 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 252 encoding the light chain sequence of SEQ ID NO: 112 and the polynucleotide SEQ ID NO: 254 encoding the heavy chain sequence of SEQ ID NO: 114.

[000554] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab12 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab12 or Fab fragments thereof may be produced via expression of Ab12 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab13

[000555] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention,

polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 121:

[000556] GCCATCGTGATGACCCAGACTCCATCTTCCAAGTCTGTCCCTGTGGGA GACACAGTCACCATCAATTGCCAGGCCAGTGAGAGTCTTTATAATAACAACGC CTTGGCCTGGTTTCAGCAGAAACCAGGGCAGCCTCCCAAGCGCCTGATCTATG ATGCATCCAAACTGGCATCTGGGGTCCCATCGCGGTTCAGTGGCGGTGGGTCT GGGACACAGTTCACTCTCACCATCAGTGGCGTGCAGTGTGACGATGCTGCCAC TTACTACTGTGGAGGCTACAGAAGTGATAGTGTTGATGGTGTTGCTTTCGCCGG AGGGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 261).

[000557] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 122:

[000558] GCCATCGTGATGACCCAGACTCCATCTTCCAAGTCTGTCCCTGTGGGA GACACAGTCACCATCAATTGCCAGGCCAGTGAGAGTCTTTATAATAACAACGC CTTGGCCTGGTTTCAGCAGAAACCAGGGCAGCCTCCCAAGCGCCTGATCTATG ATGCATCCAAACTGGCATCTGGGGTCCCATCGCGGTTCAGTGGCGGTGGGTCT GGGACACAGTTCACTCTCACCATCAGTGGCGTGCAGTGTGACGATGCTGCCAC TTACTACTGTGGAGGCTACAGAAGTGATAGTGTTGATGGTGTTGCTTTCGCCGG AGGGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCT TCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGC TGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCC CTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAA ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 262).

[000559] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 123:

[000560] CAGTCGGTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGAT CCCTGACACTCACCTGCACAGCCTCTGGATTCGACTTCAGTAGCAATGCAATGT GGTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATGCATTTAc AATGGTGATGGCAGCACATACTACGCGAGCTGGGTGAATGGCCGATTCTCCAT CTCCAAAACCTCGTCGACCACGGTGACTCTGCAACTGAATAGTCTGACAGTCG CGGACACGGCCACGTATTATTGTGCGAGAGATCTTGACTTGTGGGGCCCGGGC ACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 263).

[000561] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 124:

[000562] CAGTCGGTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGAT CCCTGACACTCACCTGCACAGCCTCTGGATTCGACTTCAGTAGCAATGCAATGT GGTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATGCATTTAc AATGGTGATGGCAGCACATACTACGCGAGCTGGGTGAATGGCCGATTCTCCAT CTCCAAAACCTCGTCGACCACGGTGACTCTGCAACTGAATAGTCTGACAGTCG CGGACACGGCCACGTATTATTGTGCGAGAGATCTTGACTTGTGGGGCCCGGGC ACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG GCAcCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGAC CAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCT CAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCC CAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCC TGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATG ATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGA CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCA AGACAAAGCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGTGGTCAGCGT CCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA GGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT

GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCG
ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC
CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCAC
CGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC
ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT
AAATGA (SEQ ID NO: 264).

[000563] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 265; SEQ ID NO: 266; and SEQ ID NO: 267 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 121 or the light chain sequence of SEQ ID NO: 122.

[000564] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 268; SEQ ID NO: 269; and SEQ ID NO: 270 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 123 or the heavy chain sequence of SEQ ID NO: 124.

[000565] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 261 encoding the light chain variable sequence of SEQ ID NO: 121; the polynucleotide SEQ ID NO: 262 encoding the light chain sequence of SEQ ID NO: 122; the polynucleotide SEQ ID NO: 263 encoding the heavy chain variable sequence of SEQ ID NO: 123; the polynucleotide SEQ ID NO: 264 encoding the heavy chain sequence of SEQ ID NO: 124; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 265; SEQ ID NO: 266; and SEQ ID NO: 267) of the light chain variable sequence of SEQ ID NO: 121 or the light chain sequence of SEQ ID

NO: 122; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 268; SEQ ID NO: 269; and SEQ ID NO: 270) of the heavy chain variable sequence of SEQ ID NO: 123 or the heavy chain sequence of SEQ ID NO: 124.

[000566] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab13, the polynucleotides encoding the full length Ab13 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 262 encoding the light chain sequence of SEQ ID NO: 122 and the polynucleotide SEQ ID NO: 264 encoding the heavy chain sequence of SEQ ID NO: 124.

[000567] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab13 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab13 or Fab fragments thereof may be produced via expression of Ab13 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

Antibody Ab14

[000568] The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to CGRP. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 131:

[000569] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAATGTTTAcAATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctgg gacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATTG TAGTCGTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgt (SEQ ID NO: 271).

[000570] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 132:

[000571] CAAGTGCTGacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcAATtgcCA GGCCAGTCAGAATGTTTAcAATAACAACTACCTAGCCtggtatcagcagaaaccagggaaagtt cctaagCAActgatctatTCTACATCCACTCTGGCATCTggggtcccatctcgtttcagtggcagtggatctgg gacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttattactgtCTGGGCAGTTATGATTG TAGTCGTGGTGATTGTTTTGTTttcggcggaggaaccaaggtggaaatcaaacgtACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGC CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGC AGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 272).

[000572] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 133:

[000573] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt ctggaATCGGCCTCagtAGCTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt cGGAGTCATTGGTAGTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCcg attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat TTCtgtACCAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGC (SEQ ID NO: 273).

[000574] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 134:

[000575] gaggtgcagctTgtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcaGTCt
ctggaATCGGCCTCagtAGCTACTACATGCAAtgggtccgtcaggctccagggaaggggctggagtgggt
cGGAGTCATTGGTAGTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCcg
attcaccatctccagagacaattccaagACCACGGTGtatcttcaaatgaacagcctgagagctgaggacactgctgtgtat
TTCtgtACCAGAGGGGACATCtggggccaagggaccctcgtcaccgtcTCGAGCGCCTCCACCA
AGGGCCCATCGGTCTTCCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGCA
CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTG
TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCT
ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA
GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACGCGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC
ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGaTCTCCCgGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAG
CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG
AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG
CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG
TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
AGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 274).

[000576] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 275; SEQ ID NO: 276; and SEQ ID

NO: 277 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 131 or the light chain sequence of SEQ ID NO: 132.

[000577] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 278; SEQ ID NO: 279; and SEQ ID NO: 280 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 133 or the heavy chain sequence of SEQ ID NO: 134.

[000578] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to CGRP comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 271 encoding the light chain variable sequence of SEQ ID NO: 131; the polynucleotide SEQ ID NO: 272 encoding the light chain sequence of SEQ ID NO: 132; the polynucleotide SEQ ID NO: 273 encoding the heavy chain variable sequence of SEQ ID NO: 133; the polynucleotide SEQ ID NO: 274 encoding the heavy chain sequence of SEQ ID NO: 134; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 275; SEQ ID NO: 276; and SEQ ID NO: 277) of the light chain variable sequence of SEQ ID NO: 131 or the light chain sequence of SEQ ID NO: 132; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 278; SEQ ID NO: 279; and SEQ ID NO: 280) of the heavy chain variable sequence of SEQ ID NO: 133 or the heavy chain sequence of SEQ ID NO: 134.

[000579] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for CGRP. With respect to antibody Ab14, the polynucleotides encoding the full length Ab14 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 272 encoding the light chain sequence of SEQ

ID NO: 132 and the polynucleotide SEQ ID NO: 274 encoding the heavy chain sequence of SEQ ID NO: 134.

[000580] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, or microbial systems such as yeast cells such as the yeast Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab14 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-CGRP antibodies such as Ab14 or Fab fragments thereof may be produced via expression of Ab14 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

[000581] In one embodiment, the invention is directed to an isolated polynucleotide comprising a polynucleotide encoding an anti-CGRP VH antibody amino acid sequence selected from SEQ ID NO: 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, or 133, or encoding a variant thereof wherein at least one framework residue (FR residue) has been substituted with an amino acid present at the corresponding position in a rabbit anti-CGRP antibody VH polypeptide or a conservative amino acid substitution.

[000582] In another embodiment, the invention is directed to an isolated polynucleotide comprising the polynucleotide sequence encoding an anti-CGRP VL antibody amino acid sequence of 1, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, or 131, or encoding a variant thereof wherein at least one framework residue (FR residue) has been substituted with an amino acid present at the corresponding position in a rabbit anti-CGRP antibody VL polypeptide or a conservative amino acid substitution.

[000583] In yet another embodiment, the invention is directed to one or more heterologous polynucleotides comprising a sequence encoding the polypeptides contained in SEQ ID NO:1 and SEQ ID NO:3; SEQ ID NO:11 and SEQ ID NO:13; SEQ ID NO:21 and SEQ ID NO:23; SEQ ID NO:31 and SEQ ID NO:33; SEQ ID NO:41 and SEQ ID NO:43; SEQ ID

NO:51 and SEQ ID NO:53, SEQ ID NO:61 and SEQ ID NO:63; SEQ ID NO:71 and SEQ ID NO:73; SEQ ID NO:81 and SEQ ID NO:83; SEQ ID NO:91 and SEQ ID NO:93; SEQ ID NO:101 and SEQ ID NO:103; SEQ ID NO:111 and SEQ ID NO:113; SEQ ID NO:121 and SEQ ID NO:123; or SEQ ID NO:131 and SEQ ID NO:133.

[000584] In another embodiment, the invention is directed to an isolated polynucleotide that expresses a polypeptide containing at least one CDR polypeptide derived from an anti-CGRP antibody wherein said expressed polypeptide alone specifically binds CGRP or specifically binds CGRP when expressed in association with another polynucleotide sequence that expresses a polypeptide containing at least one CDR polypeptide derived from an anti-CGRP antibody wherein said at least one CDR is selected from those contained in the VL or VH polypeptides of SEQ ID NO: 1, 3, 11, 13, 21, 23, 31, 33, 41, 43, 51, 53, 61, 63, 71, 73, 81, 83, 91, 93, 101, 103, 111, 113, 121, 123, 131, or SEQ ID NO:133.

[000585] Host cells and vectors comprising said polynucleotides are also contemplated.

[000586] The invention further contemplates vectors comprising the polynucleotide sequences encoding the variable heavy and light chain polypeptide sequences, as well as the individual complementarity-determining regions (CDRs, or hypervariable regions), as set forth herein, as well as host cells comprising said vector sequences. In one embodiment of the invention, the host cell is a yeast cell. In another embodiment of the invention, the yeast host cell belongs to the genus Pichia.

B-cell Screening and Isolation

[000587] In one embodiment, the present invention contemplates the preparation and isolation of a clonal population of antigen-specific B cells that may be used for isolating at least one CGRP antigen-specific cell, which can be used to produce a monoclonal antibody against CGRP, which is specific to a desired CGRP antigen, or a nucleic acid sequence corresponding to such an antibody. Methods of preparing and isolating said clonal population of antigen-specific B cells are taught, for example, in U.S. patent publication no. US 2007/0269868 to Carvalho-Jensen et al., the disclosure of which is herein incorporated by reference in its entirety. Methods of preparing and isolating said clonal

population of antigen-specific B cells are also taught herein in the examples. Methods of "enriching" a cell population by size or density are known in the art. See, e.g., U.S. Patent 5,627,052. These steps can be used in addition to enriching the cell population by antigen-specificity.

Methods of Humanizing Antibodies

[000588] In another embodiment, the present invention contemplates methods for humanizing antibody heavy and light chains. Methods for humanizing antibody heavy and light chains which may be applied to anti-CGRP antibodies are taught, for example, in U.S. patent application publication no. US 2009/0022659 to Olson et al., and in U.S. patent no. 7,935,340 to Garcia-Martinez et al., the disclosures of each of which are herein incorporated by reference in their entireties.

Methods of Producing Antibodies and Fragments thereof

[000589] In another embodiment, the present invention contemplates methods for producing anti-CGRP antibodies and fragments thereof diarrhea. Methods for producing anti-CGRP antibodies and fragments thereof secreted from polyploidal, preferably diploid or tetraploid strains of mating competent yeast are taught, for example, in U.S. patent application publication no. US 2009/0022659 to Olson et al., and in U.S. patent no. 7,935,340 to Garcia-Martinez et al., the disclosures of each of which are herein incorporated by reference in their entireties.

[000590] Other methods of producing antibodies are well known to those of ordinary skill in the art. For example, methods of producing chimeric antibodies are now well known in the art (See, for example, U.S. Patent No. 4,816,567 to Cabilly et al.; Morrison et al., P.N.A.S. USA, 81:8651-55 (1984); Neuberger, M.S. et al., Nature, 314:268-270 (1985); Boulianne, G.L. et al., Nature, 312:643-46 (1984), the disclosures of each of which are herein incorporated by reference in their entireties).

[000591] Likewise, other methods of producing humanized antibodies are now well known in the art (See, for example, U.S. Patent Nos. 5,530,101, 5,585,089, 5,693,762, and

6,180,370 to Queen et al; U.S. Patent Nos. 5,225,539 and 6,548,640 to Winter; U.S. Patent Nos. 6,054,297, 6,407,213 and 6,639,055 to Carter et al; U.S. Patent No. 6,632,927 to Adair; Jones, P.T. et al, Nature, 321:522-525 (1986); Reichmann, L., et al, Nature, 332:323-327 (1988); Verhoeyen, M, et al, Science, 239:1534-36 (1988), the disclosures of each of which are herein incorporated by reference in their entireties).

[000592] Antibody polypeptides of the invention having CGRP binding specificity diarrhea may also be produced by constructing, using conventional techniques well known to those of ordinary skill in the art, an expression vector containing an operon and a DNA sequence encoding an antibody heavy chain in which the DNA sequence encoding the CDRs required for antibody specificity is derived from a non-human cell source, preferably a rabbit B-cell source, while the DNA sequence encoding the remaining parts of the antibody chain is derived from a human cell source.

[000593] A second expression vector is produced using the same conventional means well known to those of ordinary skill in the art, said expression vector containing an operon and a DNA sequence encoding an antibody light chain in which the DNA sequence encoding the CDRs required for antibody specificity is derived from a non-human cell source, preferably a rabbit B-cell source, while the DNA sequence encoding the remaining parts of the antibody chain is derived from a human cell source.

[000594] The expression vectors are transfected into a host cell by convention techniques well known to those of ordinary skill in the art to produce a transfected host cell, said transfected host cell cultured by conventional techniques well known to those of ordinary skill in the art to produce said antibody polypeptides.

[000595] The host cell may be co-transfected with the two expression vectors described above, the first expression vector containing DNA encoding an operon and a light chain-derived polypeptide and the second vector containing DNA encoding an operon and a heavy chain-derived polypeptide. The two vectors contain different selectable markers, but preferably achieve substantially equal expression of the heavy and light chain polypeptides. Alternatively, a single vector may be used, the vector including DNA encoding both the heavy and light chain polypeptides. The coding sequences for the heavy and light chains may comprise cDNA, genomic DNA, or both.

[000596] The host cells used to express the antibody polypeptides may be either a bacterial cell such as E. coli, or a eukaryotic cell such as P. pastoris. In one embodiment of the invention, a mammalian cell of a well-defined type for this purpose, such as a myeloma cell, a Chinese hamster ovary (CHO) cell line, a NSO cell line, or a HEK293 cell line may be used.

[000597] The general methods by which the vectors may be constructed, transfection methods required to produce the host cell and culturing methods required to produce the antibody polypeptides from said host cells all include conventional techniques. Although preferably the cell line used to produce the antibody is a mammalian cell line, any other suitable cell line, such as a bacterial cell line such as an E. coli-derived bacterial strain, or a yeast cell line, may alternatively be used.

[000598] Similarly, once produced the antibody polypeptides may be purified according to standard procedures in the art, such as for example cross-flow filtration, ammonium sulphate precipitation, affinity column chromatography and the like.

[000599] The antibody polypeptides described herein may also be used for the design and synthesis of either peptide or non-peptide mimetics that would be useful for the same therapeutic applications as the antibody polypeptides of the invention. See, for example, Saragobi et al, Science, 253:792-795 (1991), the contents of which is herein incorporated by reference in its entirety.

Screening Assays

[000600] The invention also includes screening assays designed to assist in the identification of diseases and disorders associated with CGRP and diarrhea in patients exhibiting symptoms of a CGRP associated disease or disorder.

[000601] In one embodiment of the invention, the anti-CGRP antibodies of the invention, or CGRP binding fragments thereof, are used to detect the presence of CGRP in a biological sample obtained from a patient exhibiting symptoms of a disease or disorder associated with CGRP and diarrhea. The presence of CGRP, or elevated levels thereof especially in the colon when compared to pre-disease levels of CGRP in a comparable

biological sample, may be beneficial in diagnosing a disease or disorder associated with CGRP associated with diarrhea.

[000602] Another embodiment of the invention provides a diagnostic or screening assay to assist in diagnosis of diseases or disorders associated with CGRP in patients exhibiting symptoms of a CGRP associated disease or disorder identified herein, comprising assaying the level of CGRP expression in a biological sample from said patient using a post-translationally modified anti-CGRP antibody or binding fragment thereof. The anti-CGRP antibody or binding fragment thereof may be post-translationally modified to include a detectable moiety such as set forth previously in the disclosure.

[000603] The CGRP level in the biological sample is determined using a modified anti-CGRP antibody or binding fragment thereof as set forth herein, and comparing the level of CGRP in the biological sample against a standard level of CGRP (e.g., the level in normal biological samples). The skilled clinician would understand that some variability may exist between normal biological samples, and would take that into consideration when evaluating results. In one embodiment of the invention, the anti-CGRP antibodies of the invention may be used to correlate CGRP expression levels with a particular stage of cancerous development. One skilled in the art would be able to measure CGRP in numerous subjects in order to establish ranges of CGRP expression that correspond to clinically defined stages of cancerous development. These ranges will allow the skilled practitioner to measure CGRP in a subject diagnosed with a cancer and correlate the levels in each subject with a range that corresponds to a stage of said cancer. One skilled in the art would understand that by measuring CGRP in the patient at different intervals, the progression of the cancer can be determined.

[000604] The above-recited assay may also be useful in monitoring a disease or disorder, where the level of CGRP obtained in a biological sample from a patient believed to have a CGRP associated disease or disorder is compared with the level of CGRP in prior biological samples from the same patient, in order to ascertain whether the CGRP level in said patient has changed with, for example, a treatment regimen.

[000605] The invention is also directed to a method of in vivo imaging which detects the presence of cells which express CGRP comprising administering a diagnostically effective

amount of a diagnostic composition. Said in vivo imaging is useful for the detection or imaging of CGRP expressing tumors or metastases, for example, and can be useful as part of a planning regimen for the design of an effective cancer treatment protocol. The treatment protocol may include, for example, one or more of radiation, chemotherapy, cytokine therapy, gene therapy, and antibody therapy, as well as an anti-CGRP antibody or fragment thereof.

[000606] The present invention further provides for a kit for detecting binding of an anti-CGRP antibody of the invention to CGRP. In particular, the kit may be used to detect the presence of a CGRP specifically reactive with an anti-CGRP antibody of the invention or an immunoreactive fragment thereof. The kit may also include an antibody bound to a substrate, a secondary antibody reactive with the antigen and a reagent for detecting a reaction of the secondary antibody with the antigen. Such a kit may be an ELISA kit and can comprise the substrate, primary and secondary antibodies when appropriate, and any other necessary reagents such as detectable moieties, enzyme substrates, and color reagents, for example as described herein. The diagnostic kit may also be in the form of an immunoblot kit. The diagnostic kit may also be in the form of a chemiluminescent kit (Meso Scale Discovery, Gaithersburg, MD). The diagnostic kit may also be a lanthanide-based detection kit (PerkinElmer, San Jose, CA).

[000607] A skilled clinician would understand that a biological sample includes, but is not limited to, sera, plasma, urine, saliva, mucous, pleural fluid, synovial fluid and spinal fluid.

[000608]

[000609] Methods of Ameliorating or Reducing Symptoms of, or Treating, or Preventing, Diseases and Disorders Associated with, CGRP

[000610] The anti-CGRP antibodies described herein, or fragments thereof, are useful for ameliorating or reducing the symptoms of, or treating, or preventing, diseases and disorders associated with CGRP especially diarrhea. In a preferred embodiment the anti-CGRP antibodies or antibody fragments will be shown to be efficacious (block adverse side effects associated with excess circulating CGRP including diarrhea in the rodent animal model disclosed in Example 8.

[000611] Anti-CGRP antibodies described herein, or fragments thereof, as well as combinations, can also be administered in a therapeutically effective amount to patients in need of treatment of diseases and disorders associated with CGRP in the form of a pharmaceutical composition as described in greater detail below.

[000612] In another embodiment of the invention, anti-CGRP antibodies described herein, or fragments thereof, are useful for ameliorating or reducing the symptoms of, or treating, or preventing diarrhea in CGRP related conditions including migraines (with or without aura), weight loss, cancer or tumors, angiogenesis associated with cancer or tumor growth, angiogenesis associated with cancer or tumor survival, pain, hemiplagic migraines, cluster headaches, migrainous neuralgia, chronic headaches, tension headaches, general headaches, hot flushes, chronic paroxysomal hemicrania, secondary headaches due to an underlying structural problem in the head or neck, cranial neuralgia, sinus headaches (such as for example associated with sinusitis), and allergy-induced headaches or migraines.

[000613] In one embodiment of the invention, anti-CGRP antibodies described herein, or fragments thereof and/or with a second agent, are useful for ameliorating or reducing the symptoms of, or treating, or preventing, diarrhea associated with the following non-limiting listing of CGRP related diseases and disorders: pain, inflammatory pain, post-operative incision pain, complex regional pain syndrome, cancer pain, primary or metastatic bone cancer pain, fracture pain, chronic pain, osteoporotic fracture pain, pain resulting from burn, osteoporosis, gout joint pain, abdominal pain, pain associated with sickle cell crises, and other nociceptic pain, as well as hepatocellular carcinoma, breast cancer, liver cirrhosis, neurogenic pain, neuropathic pain, nociceptic pain, trigeminal neuralgia, post-herpetic neuralgia, phantom limb pain, fibromyalgia, menstrual pain, ovarialgia, reflex sympathetic dystrophy, neurogenic pain, osteoarthritis or rheumatoid arthritis pain, lower back pain, diabetic neuropathy, sciatica, or pain or visceral pain associated with: gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, irritable colon, spastic colon, mucous colitis, inflammatory bowel disease, Crohn's disease, ileitis, ulcerative colitis, renal colic, dysmenorrhea, cystitis, menstrual period, labor, menopause, prostatitis, pancreatitis, renal colic, dysmenorrhea, cystitis, including interstitial cystitis (IC), surgery associated with the ileus, diverticulitis, peritonitis, pericarditis, hepatitis, appendicitis,

colitis, cholecystitis, endometriosis, chronic and/or acute pancreatitis, myocardial infarction, kidney pain, pleural pain, prostatitis, pelvic pain, trauma to an organ, chronic nociceptive pain, chronic neuropathic pain, chronic inflammatory pain, fibromyalgia, breakthrough pain and persistent pain, and cancer pain arising from malignancy or from cancer preferably selected from one or more of: adenocarcinoma in glandular tissue, blastoma in embryonic tissue of organs, carcinoma in epithelial tissue, leukemia in tissues that form blood cells, lymphoma in lymphatic tissue, myeloma in bone marrow, sarcoma in connective or supportive tissue, adrenal cancer, AIDS-related lymphoma, anemia, bladder cancer, bone cancer, brain cancer, breast cancer, carcinoid tumours, cervical cancer, chemotherapy, colon cancer, cytopenia, , endometrial cancer, esophageal cancer, gastric cancer, head cancer, neck cancer, hepatobiliary cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, Hodgkin's disease, lymphoma, non- Hodgkin's, nervous system tumours, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, urethral cancer, bone cancer, sarcomas cancer of the connective tissue, cancer of bone tissue, cancer of blood-forming cells, cancer of bone marrow, multiple myeloma, leukaemia, primary or secondary bone cancer, tumours that metastasize to the bone, tumours infiltrating the nerve and hollow viscus, tumours near neural structures. Further preferably the cancer pain comprises visceral pain, preferably visceral pain which arises from pancreatic cancer and/or metastases in the abdomen. Further preferably the cancer pain comprises somatic pain, preferably somatic pain due to one or more of bone cancer, metastasis in the bone, postsurgical pain, sarcomas cancer of the connective tissue, cancer of bone tissue, cancer of blood-forming cells of the bone marrow, multiple myeloma, leukaemia, primary or secondary bone cancer.

[000614] In another embodiment of the invention, anti-CGRP antibodies described herein, or fragments thereof and/or with a second agent, are useful for ameliorating or reducing the symptoms of, or treating, or preventing, the following non-limiting listing of diseases and disorders: cancer or tumors, angiogenesis associated with cancer or tumor growth, angiogenesis associated with cancer or tumor survival.

[000615] In another embodiment of the invention, anti-CGRP antibodies described herein, or fragments thereof and/or with a second agent, are useful for ameliorating or reducing the symptoms of, or treating, or preventing, the following non-limiting listing of diseases and disorders: neurogenic, neuropathic or nociceptic pain. Neuropathic pain may include, but is not limited to, trigeminal neuralgia, post-herpetic neuralgia, phantom limb pain, fibromyalgia, menstrual pain, ovarialgia, reflex sympathetic dystrophy and neurogenic pain. In other preferred embodiments, osteoarthritis or rheumatoid arthritis pain, lower back pain, diabetic neuropathy, sciatica, and other neuropathic pain.

[000616] In another embodiment of the invention, anti-CGRP antibodies described herein, or fragments thereof and/or with a second agent, are useful for ameliorating or reducing the symptoms of, or treating, or preventing, the following non-limiting listing of diseases and disorders: diarrhea, and visceral pain associated with gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, inflammatory bowel disease, Crohn's disease, ileitis, ulcerative colitis, renal colic, dysmenorrhea, cystitis, menstrual period, labor, menopause, prostatitis, or pancreatitis.

Administration

[000617] In one embodiment of the invention, the anti-CGRP antibodies described herein, or CGRP binding fragments thereof, as well as combinations of said antibodies or antibody fragments, are administered to a subject at a concentration of between about 0.1 and 100.0 mg/kg of body weight of recipient subject. In a preferred embodiment of the invention, the anti-CGRP antibodies described herein, or CGRP binding fragments thereof, as well as combinations of said antibodies or antibody fragments, are administered to a subject at a concentration of about 0.4 mg/kg of body weight of recipient subject. In a preferred embodiment of the invention, the anti-CGRP antibodies described herein, or CGRP binding fragments thereof, as well as combinations of said antibodies or antibody fragments, are administered to a recipient subject with a frequency of once every twenty-six weeks or less, such as once every sixteen weeks or less, once every eight weeks or less, once every four weeks or less, once every two weeks or less, once every week or less, or once daily or less.

[000618] Fab fragments may be administered every two weeks or less, every week or less, once daily or less, multiple times per day, and/or every few hours. In one embodiment of the invention, a patient receives Fab fragments of 0.1 mg/kg to 40 mg/kg per day given in divided doses of 1 to 6 times a day, or in a sustained release form, effective to obtain desired results.

[000619] It is to be understood that the concentration of the antibody or Fab administered to a given patient may be greater or lower than the exemplary administration concentrations set forth above in paragraphs [0566] and [0567].

[000620] A person of skill in the art would be able to determine an effective dosage and frequency of administration through routine experimentation, for example guided by the disclosure herein and the teachings in Goodman, L. S., Gilman, A., Brunton, L. L., Lazo, J. S., & Parker, K. L. (2006). Goodman & Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill; Howland, R. D., Mycek, M. J., Harvey, R. A., Champe, P. C., & Mycek, M. J. (2006). Pharmacology. Lippincott's illustrated reviews. Philadelphia: Lippincott Williams & Wilkins; and Golan, D. E. (2008). Principles of pharmacology: the pathophysiologic basis of drug therapy. Philadelphia, Pa., [etc.]: Lippincott Williams & Wilkins.

[000621] In another embodiment of the invention, the anti-CGRP antibodies described herein, or CGRP binding fragments thereof, as well as combinations of said antibodies or antibody fragments, are administered to a subject in a pharmaceutical formulation.

[000622] A "pharmaceutical composition" refers to a chemical or biological composition suitable for administration to a mammal. Such compositions may be specifically formulated for administration via one or more of a number of routes, including but not limited to buccal, epicutaneous, epidural, inhalation, intraarterial, intracardial, intracerebroventricular, intradermal, intramuscular, intranasal, intraocular, intraperitoneal, intraspinal, intrathecal, intravenous, oral, parenteral, rectally via an enema or suppository, subcutaneous, subdermal, sublingual, transdermal, and transmucosal. In addition, administration can occur by means of injection, powder, liquid, gel, drops, or other means of administration.

[000623] In one embodiment of the invention, the anti-CGRP antibodies described herein, or CGRP binding fragments thereof, as well as combinations of said antibodies or antibody fragments, may be optionally administered in combination with one or more active agents. Such active agents include analgesic, anti-histamine, antipyretic, anti-inflammatory, antibiotic, antiviral, and anti-cytokine agents. Active agents include agonists, antagonists, and modulators of TNF-α, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, IFN-α, IFN-γ, BAFF, CXCL13, IP-10, VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Hepcidin, including antibodies reactive against any of the foregoing, and antibodies reactive against any of their receptors. Active agents also include but are not limited to 2-Arylpropionic acids, Aceclofenac, Acemetacin, Acetylsalicylic acid (Aspirin), Alclofenac, Alminoprofen, Amoxiprin, Ampyrone, Arylalkanoic acids, Azapropazone, Benorylate/Benorilate, Benoxaprofen, Bromfenac, Carprofen, Celecoxib, Choline magnesium salicylate, Clofezone, COX-2 inhibitors, Dexibuprofen, Dexketoprofen, Diclofenac, Diflunisal, Droxicam, Ethenzamide, Etodolac, Etoricoxib, Faislamine, fenamic acids, Fenbufen, Fenoprofen, Flufenamic acid, Flunoxaprofen, Flurbiprofen, Ibuprofen, Ibuproxam, Indometacin, Indoprofen, Kebuzone, Ketoprofen, Ketorolac, Lornoxicam, Loxoprofen, Lumiracoxib, Magnesium salicylate, Meclofenamic acid, Mefenamic acid, Meloxicam, Metamizole, Methyl salicylate, Mofebutazone, Nabumetone, Naproxen, N-Arylanthranilic acids, Nerve Growth Factor (NGF), Oxametacin, Oxaprozin, Oxicams, Oxyphenbutazone, Parecoxib, Phenazone, Phenylbutazone, Phenylbutazone, Piroxicam, Pirprofen, profens, Proglumetacin, Pyrazolidine derivatives, Rofecoxib, Salicyl salicylate, Salicylamide, Salicylates, Substance P, Sulfinpyrazone, Sulindac, Suprofen, Tenoxicam, Tiaprofenic acid, Tolfenamic acid, Tolmetin, and Valdecoxib.

[000624] An anti-histamine can be any compound that opposes the action of histamine or its release from cells (e.g., mast cells). Anti-histamines include but are not limited to acrivastine, astemizole, azatadine, azelastine, betatastine, brompheniramine, buclizine, cetirizine, cetirizine analogues, chlorpheniramine, clemastine, CS 560, cyproheptadine, desloratadine, dexchlorpheniramine, ebastine, epinastine, fexofenadine, HSR 609, hydroxyzine, levocabastine, loratidine, methscopolamine, mizolastine, norastemizole, phenindamine, promethazine, pyrilamine, terfenadine, and tranilast.

[000625] Antibiotics include but are not limited to Amikacin, Aminoglycosides, Amoxicillin, Ampicillin, Ansamycins, Arsphenamine, Azithromycin, Azlocillin, Aztreonam, Bacitracin, Carbacephem, Carbapenems, Carbenicillin, Cefaclor, Cefadroxil, Cefalexin, Cefalothin, Cefalotin, Cefamandole, Cefazolin, Cefdinir, Cefditoren, Cefepime, Cefixime, Cefoperazone, Cefotaxime, Cefoxitin, Cefpodoxime, Cefprozil, Ceftazidime, Ceftibuten, Ceftizoxime, Ceftobiprole, Ceftriaxone, Cefuroxime, Cephalosporins, Chloramphenicol, Cilastatin, Ciprofloxacin, Clarithromycin, Clindamycin, Cloxacillin, Colistin, Co-trimoxazole, Dalfopristin, Demeclocycline, Dicloxacillin, Dirithromycin, Doripenem, Doxycycline, Enoxacin, Ertapenem, Erythromycin, Ethambutol, Flucloxacillin, Fosfomycin, Furazolidone, Fusidic acid, Gatifloxacin, Geldanamycin, Gentamicin, Glycopeptides, Herbimycin, Imipenem, Isoniazid, Kanamycin, Levofloxacin, Lincomycin, Linezolid, Lomefloxacin, Loracarbef, Macrolides, Mafenide, Meropenem, Meticillin, Metronidazole, Mezlocillin, Minocycline, Monobactams, Moxifloxacin, Mupirocin, Nafcillin, Neomycin, Netilmicin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Oxytetracycline, Paromomycin, Penicillin, Penicillins, Piperacillin, Platensimycin, Polymyxin B, Polypeptides, Prontosil, Pyrazinamide, Quinolones, Quinupristin, Rifampicin, Rifampin, Roxithromycin, Spectinomycin, Streptomycin, Sulfacetamide, Sulfamethizole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, Sulfonamides, Teicoplanin, Telithromycin, Tetracycline, Tetracyclines, Ticarcillin, Tinidazole, Tobramycin, Trimethoprim, Trimethoprim-Sulfamethoxazole, Troleandomycin, Trovafloxacin, and Vancomycin.

[000626] Active agents also include Aldosterone, Beclometasone, Betamethasone, Corticosteroids, Cortisol, Cortisone acetate, Deoxycorticosterone acetate, Dexamethasone, Fludrocortisone acetate, Glucocorticoids, Hydrocortisone, Methylprednisolone, Prednisolone, Prednisone, Steroids, and Triamcinolone. Any suitable combination of these active agents is also contemplated.

[000627] A "pharmaceutical excipient" or a "pharmaceutically acceptable excipient" is a carrier, usually a liquid, in which an active therapeutic agent is formulated. In one embodiment of the invention, the active therapeutic agent is a humanized antibody described herein, or one or more fragments thereof. The excipient generally does not

provide any pharmacological activity to the formulation, though it may provide chemical and/or biological stability, and release characteristics. Exemplary formulations can be found, for example, in Remington's Pharmaceutical Sciences, 19th Ed., Grennaro, A., Ed., 1995 which is incorporated by reference.

[000628] As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal, intramuscular, or sublingual administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[000629] Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The invention contemplates that the pharmaceutical composition is present in lyophilized form. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. The invention further contemplates the inclusion of a stabilizer in the pharmaceutical composition. The proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

[000630] In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and

gelatin. Moreover, the alkaline polypeptide can be formulated in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are known to those skilled in the art.

[000631] For each of the recited embodiments, the compounds can be administered by a variety of dosage forms. Any biologically-acceptable dosage form known to persons of ordinary skill in the art, and combinations thereof, are contemplated. Examples of such dosage forms include, without limitation, reconstitutable powders, elixirs, liquids, solutions, suspensions, emulsions, powders, granules, particles, microparticles, dispersible granules, cachets, inhalants, aerosol inhalants, patches, particle inhalants, implants, depot implants, injectables (including subcutaneous, intramuscular, intravenous, and intradermal), infusions, and combinations thereof.

[000632] The above description of various illustrated embodiments of the invention is not intended to be exhaustive or to limit the invention to the precise form disclosed. While specific embodiments of, and examples for, the invention are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the invention, as those skilled in the relevant art will recognize. The teachings provided herein of the invention can be applied to other purposes, other than the examples described above.

[000633] These and other changes can be made to the invention in light of the above detailed description. In general, in the following claims, the terms used should not be construed to limit the invention to the specific embodiments disclosed in the specification and the claims. Accordingly, the invention is not limited by the disclosure, but instead the scope of the invention is to be determined entirely by the following claims.

[000634] The invention may be practiced in ways other than those particularly described in the foregoing description and examples. Numerous modifications and variations of the

invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[000635] Certain teachings related to methods for obtaining a clonal population of antigen-specific B cells were disclosed in U.S. Provisional patent application no. 60/801,412, filed May 19, 2006, the disclosure of which is herein incorporated by reference in its entirety.

[000636] Certain teachings related to humanization of rabbit-derived monoclonal antibodies and preferred sequence modifications to maintain antigen binding affinity were disclosed in International Application No. PCT/US2008/064421, corresponding to International Publication No. WO/2008/144757, entitled "Novel Rabbit Antibody Humanization Methods and Humanized Rabbit Antibodies", filed May 21, 2008, the disclosure of which is herein incorporated by reference in its entirety.

[000637] Certain teachings related to producing antibodies or fragments thereof using mating competent yeast and corresponding methods were disclosed in U.S. Patent application no. 11/429,053, filed May 8, 2006, (U.S. Patent Application Publication No. US2006/0270045), the disclosure of which is herein incorporated by reference in its entirety.

[000638] Certain CGRP antibody polynucleotides and polypeptides are disclosed in the sequence listing accompanying this patent application filing, and the disclosure of said sequence listing is herein incorporated by reference in its entirety.

[000639] The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is herein incorporated by reference in their entireties.

[000640] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (e.g. amounts, temperature, concentrations, etc.) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is

average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

EXAMPLES

Example 1 *Preparation of Antibodies that Bind CGRP*

[000641] By using the antibody selection protocol described herein, one can generate an extensive panel of antibodies.

Immunization Strategy

[000642] Rabbits were immunized with human CGRP (American Peptides, Sunnyvale CA and Bachem, Torrance CA). Immunization consisted of a first subcutaneous (sc) injection of 100 μg of antigen mixed with 100 μg of KLH in complete Freund's adjuvant (CFA) (Sigma) followed by two boosts, two weeks apart each containing 50 μg antigen mixed with 50 μg in incomplete Freund's adjuvant (IFA) (Sigma). Animals were bled on day 55, and serum titers were determined by ELISA (antigen recognition) and by inhibition of CGRP driven cAMP increase in SK-N-MC.

Antibody Selection Titer Assessment

[000643] To identify and characterize antibodies that bind to human CGRP , antibody-containing solutions were tested by ELISA. Briefly, neutravidin coated plates (Thermo Scientific), were coated with N-term biotinylated human CGRP (50 L per well, 1 g/mL) diluted in ELISA buffer (0.5% fish skin gelatin in PBS pH 7.4,) either for approximately 1hr at room temperature or alternatively overnight at 4°C. The plates were then further blocked with ELISA buffer for one hour at room temperature and washed using wash buffer (PBS,0.05% tween 20). Serum samples tested were serially diluted using ELISA buffer. Fifty microliters of diluted serum samples were transferred onto the wells and incubated for one hour at room temperature for one hour. After this incubation, the plate was washed with wash buffer. For development, an anti-rabbit specific Fc-HRP (1:5000 dilution in ELISA buffer) was added onto the wells and incubated for 45 min at RT. After a 3x wash step with wash solution, the plate was developed using TMB substrate for two minutes at room temperature and the reaction was quenched using 0.5M HCl. The well absorbance was read at 450 nm.

Titer determination of serum samples by functional activity (Inhibition of CGRP driven cAMP levels)

[000644] To identify and characterize antibodies with functional activity, an inhibition of CGRP driven increase of cAMP levels assay was done using electrochemiluminescence (Meso Scale Discovery, MSD). Briefly, antibody preparations to be tested were serially diluted in MSD assay buffer (Hepes, MgCl2, pH 7.3, 1mg/mL blocker A, Meso Scale Discovery) in a 96 well round bottom polystyrene plate (Costar). To this plate, human CGRP was added (10ng/mL final concentration) diluted in MSD assay buffer and incubated for one hour at 37C. Appropriate controls were used as suggested by the assay-kit manufacturer. Human neuroepithelioma cells (SK-N-MC, ATCC) were detached using an EDTA solution (5mM in PBS) and washed using growth media (MEM, 10% FBS, antibiotics) by centrifugation. The cell number was adjusted to 2 million cells per mL in assay buffer, and IBMX (3-Isobutyl-1Methylxanthine, Sigma) was added to a final concentration of 0.2mM right before loading cells onto cAMP assay plate. After the antibody human CGRP solution was incubated for one hour 20 microliters of solution containing cells were transferred to the cAMP assay plate. All tested samples were run in duplicates with appropriate controls. Ten microliters of cells were added to the wells and the plate was incubated for 30 minutes with shaking at room temperature. While cells were being incubated with the CGRP solution, the stop solution was prepared by making a 1:200 solution of TAG labeled cAMP (MSD) in lysis buffer (MSD). To stop the cells-CGRP incubation, 20 microliters of stop solution was added to the cells and the plate was incubated for one hour with shaking at room temperature. The read buffer (MSD) was diluted four times with water and 100 microliters were added to all wells on the plate. The plate was then read using a Sector Imager 2400 (MSD) and the Prism software was used for data fit and IC50 determination.

Tissue Harvesting

[000645] Once acceptable titers were established, the rabbit(s) were sacrificed. Spleen, lymph nodes, and whole blood were harvested and processed as follows:

[000646] Spleen and lymph nodes were processed into a single cell suspension by disassociating the tissue and pushing through sterile wire mesh at 70 μm (Fisher) with a plunger of a 20 cc syringe. Cells were collected in PBS. Cells were washed twice by centrifugation. After the last wash, cell density was determined by trypan blue. Cells were centrifuged at 1500 rpm for 10 minutes; the supernatant was discarded. Cells were resuspended in the appropriate volume of 10% dimethyl sulfoxide (DMSO, Sigma) in FBS (Hyclone) and dispensed at 1 ml/vial. Vials were stored at -70°C in a slow freezing chamber for 24 hours and stored in liquid nitrogen.

[000647] Peripheral blood mononuclear cells (PBMCs) were isolated by mixing whole blood with equal parts of the low glucose medium described above without FBS. 35 ml of the whole blood mixture was carefully layered onto 8 ml of Lympholyte Rabbit (Cedarlane) into a 45 ml conical tube (Corning) and centrifuged 30 minutes at 2500 rpm at room temperature without brakes. After centrifugation, the PBMC layers were carefully removed using a glass Pasteur pipette (VWR), combined, and placed into a clean 50 ml vial. Cells were washed twice with the modified medium described above by centrifugation at 1500 rpm for 10 minutes at room temperature, and cell density was determined by trypan blue staining. After the last wash, cells were resuspended in an appropriate volume of 10% DMSO/FBS medium and frozen as described above.

B cell selection, enrichment and culture conditions

[000648] On the day of setting up B cell culture, PBMC, splenocyte, or lymph node vials were thawed for use. Vials were removed from LN2 tank and placed in a 37°C water bath until thawed. Contents of vials were transferred into 15 ml conical centrifuge tube (Corning) and 10 ml of modified RPMI described above was slowly added to the tube. Cells were centrifuged for 5 minutes at 2K RPM, and the supernatant was discarded. Cells were resuspended in 10 ml of fresh media. Cell density and viability was determined by trypan blue.

a) The following protocol was used for Ab1 and Ab13

[000649] Cells were pre-mixed with the biotinylated human CGRP as follows. Cells were washed again and resuspended at 1E07 cells/80 μL medium. Biotinylated human CGRP was added to the cell suspension at the final concentration of 5 ug/mL and

incubated for 30 minutes at 4°C. Unbound biotinylated human CGRPα was removed performing two 10 ml washes using PBF [Ca/Mg free PBS (Hyclone), 2 mM ethylenediamine tetraacetic acid (EDTA), 0.5% bovine serum albumin (BSA) (Sigma-biotin free)]. After the second wash, cells were resuspended at 1E07 cells/80 µl PBF and 20 µl of MACS® streptavidin beads (Miltenyi Biotech, Auburn CA) per 10E7 cells were added to the cell suspension. Cells and beads were incubated at 4°C for 15 minutes and washed once with 2 ml of PBF per 10E7 cells.

b) The following protocol was used for Ab4, Ab7, Ab9 and Ab11:

[000650] Biotinylated human CGRPα was pre-loaded onto the streptavidin beads as follows. Seventy five microliters of streptavidin beads (Milteny Biotec, Auburn CA) were mixed with N-terminally biotinylated huCGRPα (10ug/ml final concentration) and 300 ul PBF. This mixture was incubated at 4°C for 30 min and unbound biotinylated human CGRPα was removed using a MACS® separation column (Miltenyi Biotec, with a 1ml rinse to remove unbound material. Then material was plunged out, then used to resuspend cells from above in 100ul per 1E7 cells, the mixture was then incubated at 4°C for 30min and washed once with 10 ml of PBF.

[000651] For both a) and b) protocols the following applied: After washing, the cells were resuspended in 500µl of PBF and set aside. A MACS® MS column (Miltenyi Biotec, Auburn CA) was pre-rinsed with 500 ml of PBF on a magnetic stand (Milteni). Cell suspension was applied to the column through a pre-filter, and unbound fraction was collected. The column was washed with 2.5 ml of PBF buffer. The column was removed from the magnet stand and placed onto a clean, sterile 1.5 ml eppendorf tube. 1 ml of PBF buffer was added to the top of the column, and positive selected cells were collected. The yield and viability of positive cell fraction was determined by trypan blue staining. Positive selection yielded an average of 1% of the starting cell concentration.

[000652] A pilot cell screen was established to provide information on seeding levels for the culture. Plates were seeded at 10, 25, 50, 100, or 200 enriched B cells/well. In addition, each well contained 50K cells/well of irradiated EL-4.B5 cells (5,000 Rads) and an appropriate level of activated rabbit T cell supernatant (See U.S. Patent Application

Publication No. 20070269868)(ranging from 1-5% depending on preparation) in high glucose modified RPMI medium at a final volume of 250 µl/well. Cultures were incubated for 5 to 7 days at 37°C in 4% CO2.

B-Cell culture screening by antigen-recognition (ELISA)

[000653] To identify wells producing anti-human CGRPα antibodies, the same protocol as described for titer determination of serum samples by antigen-recognition (ELISA) was used with the following changes. Briefly, neutravidin coated plates were coated with a mixture of both N- and C- terminally biotinylated human CGRPα (50 µL per well, 1µg/mL each). B-cell supernatant samples (50µL) were tested without prior dilution.

Identification of functional activity in B-cell supernatants using CGRP driven cAMP production

[000654] To determine functional activity contained in B-cell supernatants, a similar procedure to that described for the determination of functional titer of serum samples was used with the following modifications. Briefly, B-cell supernatant (20µL) were used in place of the diluted polyclonal serum samples.

Isolation of antigen-specific B-cells

[000655] Plates containing wells of interest were removed from -70 °C, and the cells from each well were recovered using five washes of 200 microliters of medium (10% RPMI complete, 55µM BME) per well. The recovered cells were pelleted by centrifugation and the supernatant was carefully removed. Pelleted cells were resuspended in 100 µl of medium. To identify antibody expressing cells, streptavidin coated magnetic beads (M280 dynabeads, Invitrogen) were coated with a combination of both N- and C- terminal biotinylated human CGRPα . Individual biotinylated human CGRPα lots were optimized by serial dilution. One hundred microliters containing approximately 4x10E7 coated beads were then mixed with the resuspended cells. To this mixture 15 microliters of goat anti-rabbit H&L IgG-FITC (Jackson Immunoresearch) diluted 1:100 in medium were added.

[000656] Twenty microliters of cell/beads/anti-rabbit H&L suspension were removed and 5 microliter droplets were dispensed on a one-well glass slide previously treated with Sigmacote (Sigma) totaling 35 to 40 droplets per slide. An impermeable barrier of paraffin oil (JT Baker) was used to submerge the droplets, and the slide was incubated for 90 minutes at 37°C in a 4% CO2 incubator in the dark.

[000657] Specific B cells that produce antibody can be identified by the fluorescent ring around produced by the antibody secretion, recognition of the bead-associated biotinylated antigen, and subsequent detection by the fluorescent-IgG detection reagent. Once a cell of interest was identified it was recovered via a micromanipulator (Eppendorf). The single cell synthesizing and exporting the antibody was transferred into a microcentrifuge tube, frozen using dry ice and stored at -70°C.

Amplification and sequence determination of Antibody Sequences From Antigen-Specific B Cells

[000658] Antibody sequences were recovered using a combined RT-PCR based method from a single isolated B-cell. Primers containing restriction enzymes were designed to anneal in conserved and constant regions of the target immunoglobulin genes (heavy and light), such as rabbit immunoglobulin sequences, and a two-step nested PCR recovery was used to amplify the antibody sequence. Amplicons from each well were analyzed for recovery and size integrity. The resulting fragments are then digested with AluI to fingerprint the sequence clonality. Identical sequences displayed a common fragmentation pattern in their electrophoretic analysis. The original heavy and light chain amplicon fragments were then digested using the restriction enzyme sites contained within the PCR primers and cloned into an expression vector. Vector containing subcloned DNA fragments were amplified and purified. Sequence of the subcloned heavy and light chains were verified prior to expression.

Recombinant Production of Monoclonal Antibody of Desired Antigen Specificity and/or Functional Properties

[000659] To determine antigen specificity and functional properties of recovered antibodies from specific B-cells, vectors driving the expression of the desired paired heavy and light chain sequences were transfected into HEK-293 cells.

Antigen-recognition of recombinant antibodies by ELISA

[000660] To characterize recombinant expressed antibodies for their ability to bind to human-CGRPα, antibody-containing solutions were tested by ELISA. All incubations were done at room temperature. Briefly, Immulon IV plagtes (Thermo Scientific), were coated with a CGRPα containing solution (1ut/mL in PBS) for 2 hours. CGRPα-coated plates were then washed three times in wash buffer (PBS, 0.05% Tween-20). The plates were then blocked using a blocking solution (PBS, 0.5% fish skin gelatin, 0.05% Tween-20) for approximately one hour. The blocking solution was then removed and the plates were then incubated with a dilution series of the antibody being tested for approximately one hour. At the end of this incubation, the plate was washed three times with wash buffer and further incubated with a secondary antibody containing solution (Peroxidase conjugated affinipure F(ab')2 fragment goat anti-human IgG, Fc fragment specific (Jackson Immunoresearch) for approximately 45 minutes and washed three times. At that point a substrate solution (TMB peroxidase substrate, BioFx) and incubated for 3 to 5 minutes in the dark. The reaction was stopped by addition of a HCl containing solution (0.5M) and the plate was read at 450 nm in a plate-reader.

[000661] Results: Figures 15-18 demonstrate that anti-CGRP antibodies Ab1-Ab14 bind to and recognize CGRPα.

Functional characterization of recombinant antibodies by modulation of CGRP driven intracellular cAMP levels and cross reactivity to rats

[000662] To characterize recombinant expressed antibody for their ability to inhibit CGRPα mediated increased cellular levels of cAMP assay, an electrochemiluminescence assay-kit (Meso Scale Discovery, MSD) was used. Briefly, antibody preparations to be tested were serially diluted in MSD assay buffer (Hepes, MgCl2, pH 7.3, 1mg/mL blocker A ,Meso Scale Discovery) in a 96 well round bottom polystyrene plate (Costar). To this plate, human CGRPα was added (25ng/mL final concentration) diluted in MSD assay

buffer and incubated for one hour at 37°C. Appropriate controls were used as suggested by the assay-kit manufacturer. Human neuroepithelioma cells (SK-N-MC, ATCC) were detached using an EDTA solution (5mM) and washed using growth media (MEM, 10% FBS, antibiotics) by centrifugation. The cell number was adjusted to 2 million cells per mL in assay buffer, and IBMX (3-Isobutyl-1Methylxanthine, 50mM Sigma) was added to a final concentration of 0.2mM right before loading cells onto cAMP assay plate. The antibody human CGRPα solution was incubated for one hour after which 20 microliters of solution containing cells were transferred to the cAMP assay plate. All tested samples were run in duplicates with appropriate controls. Ten microliters of cells were added to the wells and the plate was incubated for 30 minutes with shaking. While cells were being incubated with the CGRP solution, the stop solution was prepared by making a 1:200 solution of TAG labeled cAMP (MSD) in lysis buffer (MSD). To stop the cells-CGRP incubation, 20 microliters of stop solution was added to the cells and the plate was incubated for one hour with shaking. The read buffer (MSD) was diluted four times with water and 100 microliters were added to all wells on the plate. The plate was then read using a Sector Imager 2400 (MSD) and the Prism software was used for data fit and IC50 determination.

[0100] To test for the ability of recombinant antibodies to antagonize human CGRPβ a similar assay was performed with the substitution of the CGRP agonist (CGRPβ 10ng/mL final concentration). Evaluation of the recombinant antibodies to recognize and inhibit rat CGRP-mediated cAMP generation was conducted using rat CGRP (5ng/mL final concentration) and the rat L6 cell line (ATCC).

[000663] Results: Figures 19-37 demonstrate that anti-CGRP antibodies Ab1-Ab14 inhibit CGRPα,.CGRPβ, and rat CGRP mediated increased cellular levels of cAMP.

Example 2: *Enzymatic Production of Fab Fragments*

[000664] Papain digestions were conducted using immobilized papain (Thermo/Pierce) as per manufacturer's instructions. Briefly, purified antibodies were incubated in a cystein/HCl-containing buffer with immobilized papain at 37°C with gentle rocking. The

digestion was monitored by taking an aliquot and analyzing using SDS-PAGE for cleavage of the heavy chain. To stop the reaction, the immobilized papain was spun out and washed using 50 mM Tris pH 7.5 and filtered. Undigested full length antibody and Fc fragments were removed by using a MabSelectSure (GE) column.

Example 3 *Yeast Cell Expression*

Construction of Pichia pastoris expression vectors for heavy and light chain.

[000665] The humanized light and heavy chain fragments were commercially synthesized and subcloned into a pGAP expression vector. The pGAP expression vector uses the GAP promoter to drive expression of the immunoglobulin chain and the human serum albumin (HSA) leader sequence for export. In addition, this vector contains common elements such as a bacterial origin of replication, and a copy of the kanamycin resistance gene which confers resistance to the antibiotic G418 in P. pastoris. G418 provides a means of selection for strains that contain the desired expression vector integrated into their genome.

Transformation of expression vectors into haploid met1 and lys3 host strains of Pichia pastoris

[000666] All methods used for transformation of haploid P. pastoris strains and manipulation of the P. pastoris sexual cycle were done as described in Pichia Protocols (Methods in Molecular Biology Higgings, DR, and Cregg, JM, Eds. 1998. Humana Press, Totowa, NJ). Prior to transformation each vector was linearized within the GAP promoter sequences to direct the integration of the vector into the GAP promoter locus of the P. pastoris genome. Haploid strains were transfected using electroporation and successful transformants were selected on YPDS (yeast extract, peptone dextrose with sorbitol) G418 agar plates. Copy numbers of heavy and light chain genes were determined for haploid strains by Southern blot analysis. Haploid strains were then mated and selected for their ability to grow in the absence of the amino acid markers (i.e., Lys and Met). Resulting diploid clones were then subjected to a final Southern blot to confirm copy numbers of heavy and light chain genes. A clone expressing the antibody of interest was selected using biolayer interferometry Protein-A biosensors to monitor expression (Octet, ForteBio).

Example 4 *Expression of Ab3, Ab6 and Ab14 in Pichia pastoris*

[000667] Three Pichia strains for expression of full-length antibody were made. For all the full length antibody expressing strains, haploids strains were created and subsequently mated. One haploid strain expressed full-length light chain sequence and another haploid strain expressed the full-length heavy chain sequence. Each diploid strain was used to generate a research cell bank and used for expression in a bioreactor.

[000668] First an inoculum was expanded using the research cell bank using medium comprised of the following nutrients (%w/v): yeast extract 3%, anhydrous dextrose 4%, YNB 1.34%, Biotin 0.004% and 100 mM potassium phosphate. To generate the inoculum for the fermenters, the cell bank was expanded for approximately 24 hours in a shaking incubator at 30°C and 300 rpm. A 10% inoculum was then added to Labfors 2.5L working volume vessels containing 1 L sterile growth medium. The growth medium was comprised of the following nutrients: potassium sulfate 18.2 g/L, ammonium phosphate monobasic 36.4 g/L, potassium phosphate dibasic 12.8 g/L, magnesium sulfate heptahydrate 3.72 g/L, sodium citrate dihydrate 10 g/L, glycerol 40 g/L, yeast extract 30 g/L, PTM1 trace metals 4.35 mL/L, and antifoam 204 1.67 mL/L. The PTM1 trace metal solution was comprised of the following components: cupric sulfate pentahydrate 6 g/L, sodium iodide 0.08 g/L, manganese sulfate hydrate 3 g/L, sodium molybdate dihyrate 0.2 g/L, boric acid 0.02 g/L, cobalt chloride 0.5 g/L, zinc chloride 20 g/L, ferrous sulfate heptahydrate 65 g/L, biotin 0.2 g/L, and sulfuric acid 5 mL/L.

[000669] The bioreactor process control parameters were set as follows: Agitation 1000 rpm, airflow 1.35 standard liter per minute, temperature 28°C and pH was controlled at six using ammonium hydroxide. No oxygen supplementation was provided.

[000670] Fermentation cultures were grown for approximately 12 to 16 hours until the initial glycerol was consumed as denoted by a dissolved oxygen spike. The cultures were starved for approximately three hours after the dissolved oxygen spike. After this starvation period, a bolus addition of ethanol was added to the reactor to reach 1% ethanol (w/v). The fermentation cultures were allowed to equilibrate for 15 to 30 minutes. Feed addition was initiated 30 minutes post-ethanol bolus and set at a constant rate of 1 mL/min

for 40 minutes, then the feed pump was controlled by an ethanol sensor keeping the concentration of ethanol at 1% for the remainder of the run using an ethanol sensing probe (Raven Biotech). The feed was comprised of the following components: yeast extract 50 g/L, dextrose 500 g/L, magnesium sulfate heptahydrate 3 g/L, and PTM1 trace metals 12 mL/L. For fermentation of the full length Ab6 and Ab14, sodium citrate dihydrate (0.5g/L) was also added to the feed. The total fermentation time was approximately 90 hours.

Example 5 *Methods of Humanizing Antibodies*

[000671] Methods of humanizing antibodies have been described previously in issued U.S. Patent No. 7935340, the disclosure of which is incorporated herein by reference in its entirety. In some instances, a determination of whether additional rabbit framework residues are required to maintain activity is necessary. In some instances the humanized antibodies still requires some critical rabbit framework residues to be retained to minimize loss of affinity or activity. In these cases, it is necessary to change single or multiple framework amino acids from human germline sequences back to the original rabbit amino acids in order to have desired activity. These changes are determined experimentally to identify which rabbit residues are necessary to preserve affinity and activity. This is now the end of the variable heavy and light chain humanized amino acid sequence.

Example 6 *Inhibition of CGRP Binding to its Cellular Receptor*

[000672] To characterize recombinantly expressed antibodies for their ability to inhibit CGRP binding to its cellular receptor, a radioligand-binding assay was performed as previously described [Elshourbagy et al, Endocrinology 139:1678 (1998); Zimmerman et al, Peptides, 16:421 (1995)]. Membrane preparations of recombinant human CGRP receptors, calcitonin receptor-like receptor and RAMP1 (Chemiscreen, Millipore) were used. Antibody dilutions were preincubated with 125I radiolabeled human CGRPα (0.03nM) for 30 minutes at room temperature. Non-specific binding was estimated in the presence of 0.1uM human CGRPα. Membranes were filtered and washed. The filters were then counted to determine 125I radiolabeled human CGRPα specifically bound.

Results: Figure 38 demonstrates that anti-CGRP antibodies Ab1-Ab13 inhibit CGRP binding to its cellular receptor.

Example 7 *Inhibition of Neurogenic Vasodilation by Anti-CGRP Antibodies in Rats*

[000673] CGRP is a potent vasodilator (Nature 313: 54-56 (1985) and Br J. Clin. Pharmacol. 26(6):691-5. (1988)). A pharmacodynamic assay to measure CGRP receptor antagonist activity non-invasively was used to characterize anti-CGRP antibodies. The model relied on changes in dermal blood flow measured using a laser Doppler imaging following the topical application of a capsaicin solution. Capsaicin activates the transient receptor potential vanilloid type 1 receptor (TRPV-1), producing neurogenic inflammation and vasodilatation via the local release of vasoactive mediators including CGRP and substance P (Br. J. Pharmacol. 110: 772-776 (1993)).

[000674] On the day prior to the vasodilatation assay, animals were dosed with the test agent or control via IP (intraperitoneal). Following dosing, the animals were shaved and depilated in the lower back region of their dorsal side, in an area approximately 2x6cm. The animals were then returned to their cages overnight. On the day of test, approximately 24 hours post dosing, animals were anesthetized with isoflurane gas and placed on a temperature controlled heating pad and fitted with a nose cone for continuous delivery of isoflurane. A laser doppler imager was used for the observation of vasodilatation. A beam of coherent red light generated by a 633 nm helium-neon laser was directed to the shaved area, a rectangle (2x6 cm), and scanned at a medium resolution mode. A baseline Doppler scan was obtained first and the location of O-ring placement predetermined by identifying two similar low flux areas. Two rubber Orings (~1cm in diameter) were placed in the selected regions and a baseline scan was performed. Immediately after completion of the scan, 1mg of capsaicin in 5 μL of an ethanol:acetone solution (1:1) was applied within each of the two O-rings Doppler scans were repeated at 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5 and 30 minutes after the application of capsaicin. Percent change from baseline mean Flux within each of the two O-rings, was plotted as the results of vasodilatation due to capsaicin.

[000675] In order to test recombinantly expressed antibodies for their ability to inhibit CGRP binding to its cellular receptor, a radioligand-binding assay was performed as previously described.

[000676] Results: Figures 39 and 40 demonstrates that anti-CGRP antibodies Ab3 and Ab6 reduced vasodilation in this model following capsaicin administration.

Example 8 Use of Anti-CGRP Antibodies to Block CGRP Induced Diarrhea in Two Strains of Mice (Nestin/ human RAMP1 Transgenic Mice and C57BL/6J Mice)

[000677] The initial discovery that CGRP antibodies can be used to prevent or treat CGRP induced diarrhea was based on studies on the effect of CGRP antibodies on CGRP induced photophobia or photoaversion. This was effected as one of the hallmarks of migraines is photophobia, or increased sensitivity to light [Mulleners et al, Headache 41: 31-39 (2001); Recober et al, J. Neuroscience 29:8798:8804 (2009)]. It is also known that migraineurs, but not non-migraineurs, are sensitive to CGRP-induced headache [reviewed in Neurology 22:241-246 (2009)]. CGRP binds to a G protein coupled receptor called CLR (calcitonin like receptor) that works concomitantly with the receptor activity modifying protein 1 (RAMP1) in mediating CGRP binding and signaling. In vitro, the activity of CGRP is strongly enhanced by overexpression of the RAMP1 subunit of the CGRP receptor [(J. Neurosci. 27:2693-2703 (2007)]. To study light aversion behavior in mice, a nestin/human-RAMP1 transgenic mouse model was developed [Recober et al, J. Neuroscience 29: 8798-8804 (2009); Russo et al, Mol. Cell. Pharmacol., 1:264-270 (2009)]. These mice when exposed to CGRP present symptoms associated with migraines in particular light aversion (ibid). This protocol is detailed below.

LIGHT AVERSION PROTOCOL

[000678] To test the ability of anti-CGRP antibodies to block CGRP-induced photophobia, mice are housed under standard conditions in groups of 2-5 per cage with a 12 hour light cycle (lights on at 0500 CST)/0600 CDT and off at 1700 CST/1800 CDT) and access to water and food ad libitum. The mice used in the studies are comprised in mice colonies of genotype Nestin/hRAMP1 that contain two transgene alleles Tg(Nes-cre)1Kln/J and Tg(RAMP1) alleles (B6;SJL-Tg(Nes-cre)1Kln Tg(RAMP1). Nes-cre was introduced in these mice by an intercross involving mice obtained from The Jackson Laboratory (stock 003771) on a B6 genetic background.

[000679] The control mice used in the protocol are littermates that are either non-transgenic, or single transgenic (not expressing hRAMP1) containing either transgene: nestin- cre or Cx1-GFP-hRAMP1. The stock colony is maintained by backcrossing CX1-GFP-hRAMP1 mice with non-transgenic littermates in the barrier facility. For behavior studies, the colony is maintained by crossing CX1-GFP-hRAMP1 single transgenic with nestin-cre mice in non-barrier facilities. All of these mice are cared for by animal care and procedures approved by the University of Iowa Animal Care and Use Committee and further are performed in accordance with the standard set by the National Institutes of Health.

[000680] The materials and equipment used in this protocol include a Light/Dark Box and testing chambers comprising a plexiglass open field (27 x 27 x 20.3 cm) containing 16 beam infrared arrays (Med Associates Inc., St. Albans, VT). The light/dark box is divided in two equally sized zones by a dark insert that is opaque to visible light. There is a opening (5.2 x 6.8 cm) in the dark insert that allows the mouse to freely move between the two zones. This testing chamber is placed inside a sound-attenuating cubicle (56 x 38 x 36 cm) with a fan for ventilation (Med Associates Inc.). There are six chambers for the overall system that integrates with a computer containing software for recording and data collection (Med Associates Inc.).

[000681] The software used to monitor results are Activity Monitor v 6.02 (Med Associates Inc.). The software settings used for recording comprise: Resolution (ms): 50, Box Size: 3, Resting Delay (ms): 500, Ambulatory Trigger: 3, Session Type: C, Session Time (min): 20, Block Interval (sec): 300, and Compressed File: DEFAULT.ZIP.

[000682] In the protocol the light source for each chamber is an LED panel which is was installed to the ceiling of the sound-attenuating cubicle. The LED panel contains 36 collimated – 1 watt LED bulbs (5500k Daylight White) (LEDwholesalers, Burlingame, CA). To control light intensity, each LED panel is connected to a dimmable LED driver (LINEARdrive; eldoLED America Inc., San Jose, CA) leading to a potential range of light intensity from ~300 to 27,000 lux. The standard light intensity is ~1000-1200 lux unless otherwise stated.

[000683] The injectors used are hand-made by inserting a stripped 30 gauge x ½" needle into non-radiopaque polyethylene tubing (inner diameter .38 mm; outer diameter 1.09 mm). Using the tubing described above, a stopper (~1cm in length) is placed over the needle leaving approximately 2.5 mm of the bevel uncovered. These injectors are connected to a 10 μL Hamilton syringe.

[000684] The mice are injected ICV with rat α-CGRP (Sigma) diluted in Dulbecco phosphate-buffered saline (D-PBS) (Hyclone). The total dose delivery is 0.5 nmol. For example, 250 or 500 μg CGRP is diluted in 250 or 500 μL sterile PBS for a final concentration of 1 μg/μL. The CGRP is stored at-20°C and aliquots are freeze-thawed at most one time. The PBS is stored at 4°C.

[000685] The mice are administered an exemplary anti-CGRP antibody disclosed herein (Ab3) which is stored at 4°C prior to administration. In this protocol prior to the administration of the antibody i.e., approximately 24 hours prior to testing, the mice are weighed and then receive a systemic (intraperitoneal (ip)) injection of either: vehicle, control antibody (anti-digoxin antibody), or CGRP-binding antibody at a dosage of 30 mg/kg. The mice are also screened to detect any abnormal physical conditions that could affect the assay such as a missing eye, cataracts, or other abnormalities such as grooming, etc. The day after antibody administration, mice are transported in cages from animal housing on a cart and then the mice are placed in the behavior room for acclimation at least 1 hour prior to any injection or testing. Any coverings required for transport are removed from the cages and normal light conditions (standard overhead fluorescent lighting) are turned on during acclimation and remain on for the remainder of the procedure. In addition, all equipment that produces sound including anesthetic devices, light/dark chambers, and LED panels are turned on during acclimation and remain until testing is complete. Typically there is minimal human presence in the room during acclimation.

[000686] After acclimation each mouse is placed in an induction chamber and administered 3.5% isoflurane. After the mouse is anesthetized, it is transferred to a nose cone maintaining 3.5% isoflurane administration, so that it remains anesthetized during injection. Thereafter drug administration is effected using the injector by direct injection

into the right lateral ventricle through the intact scalp aiming at 1 mm posterior to bregma and 1 mm right from the midline.

[000687] Typically for consistency all the injections are performed by the same person after a period of training yielding a success rate of >90% as demonstrated by injections of dye into the ventricles. The drugs injected are either 2.0 μL vehicle (D-PBS) μL or 2.0 μg CGRP in 2.0 μL vehicle (1 μg/μL) administered as a direct intracerebroventricular injection into the right lateral ventricle of the brain through the intact scalp aiming at 1 mm posterior to bregma and 1 mm right from the midline as described before [Recober et al, J. Neuroscience 29: 8798-8804 (2009)] After all 2.0 μL is delivered, the needle remains in place for 10 sec and then removed. The time of injection is then recorded.

[000688] After injection the mice are allowed to recover for 30 minutes prior to testing in an empty, uncovered cage containing a paper towel for bedding. During recovery, the following is recorded: diarrhea, excessive urination, bleeding post-injection, abnormal behavior such as lack of movement, seizures, etc. Based on these observations it is determined whether the administration of the anti-CGRP antibody has an effect (preventative or palliative) on CGRP-induced diarrhea in the transgenic mice which are administered antibody and later administered CGRP ICV relative to the transgenic mice which are only administered CGRP and the control (vehicle or control antibody in vehicle). Antibodies which inhibit CGRP induced diarrhea in this protocol are identified as being potentially useful in treating or preventing acute or chronic diarrhea, particularly diarrhea that is associated with elevated CGRP levels.

[000689] After a 30 minute recovery the light protocol testing is effected. Each mouse is placed along the back wall (furthest from the opening between the two zones) in the light zone approximately in the center. This triggers the recording to begin. Up to six mice are tested at one time (one mouse per chamber). During testing the shelf with the chamber is pushed back into the cabinet and the doors closed. The software records mouse movement for 20 minutes. After the recording is completed, each mouse is removed and placed back in home cage for transport back to animal housing.

[000690] ***Results***

[000691] Using this protocol an anti-CGRP antibody developed by Alder Biopharmaceuticals identified as Ab3 herein was demonstrated to result in the transgenic mice spending a statistically significant amount of time in the light. (These results are not shown as they relate to a different invention which is disclosed in U.S. Provisional Application No. 61/496,860 (Atty. Docket No. 67858.760000) filed June 14, 2011 and U.S. Ser. No. _____ (Attorney Docket No. 67858.730303) filed on even date as this application, and which application is incorporated by reference herein).

[000692] Of relevance to the present invention it was discovered during these experiments that the mice which were treated with the same Alder anti-CGRP antibody (Ab3) also did not exhibit CGRP-associated diarrhea. Whereas CGRP administration elicited diarrhea in the majority of the transgenic mice used in the photoaversion studies which were not administered the anti-CGRP antibody, diarrhea was not observed in the same transgenic mice who received the CGRP administration and which further were administered Ab3 systemically (intraperitoneally).

[000693] These results are shown in Figure 41. More specifically, Figure 41 contains the results of experiments wherein the effects of intracerebroventricular (ICV) injected CGRP in transgenic Nestin/hRamp1 mice. The data show that ICV injected rat CGRP induced diarrhea in Nestin/hRAMP1 tg mice and that the intraperitoneal injection of Ab3 (30mgs/kg, ~24 hrs. prior to CGRP challenge) inhibits intra cerebroventricular (ICV) injected-CGRP induced diarrhea in nestin/hRAMP1 tg mice.

[000694] It can be seen from the figure that all of the transgenic Nestin/hRamp1 mice which did not receive the CGRP (mice administered IP vehicle and ICV vehicle only) did not develop diarrhea. In these studies the RAMP1 transgenic C57/BL6J mice received rat CGRP(alpha).

[000695] By contrast, the majority of the same transgenic mice which received rat CGRP administered ICV and which further were administered controls (either the control antibody in the IP vehicle or a combination of the IP and ICV vehicle) developed diarrhea (was respectively observed in 90% or about 80% of the mice which received the CGRP and the antibody or vehicle controls) Most significantly the data in Figure 41 shows that all of

transgenic mice which received the Alder Ab3 antibody and CGRP did not develop diarrhea.

[000696] In addition, experiments were conducted in non-transgenic mice (C57BL/6J mice). In contrast to the prior studies using the Nestin/hRAMP1 mice, the C57/BL6J strain were administered human CGRP(α) These experiments resulted in similar results, i.e., the anti-CGRP antibody prevented CGRP-associated diarrhea in these animals. These results cumulatively suggest that antibodies which specifically bind CGRP (and likely other polypeptides that inhibit the CGRP/CGRP receptor interaction) may be used to inhibit CGRP associated diarrhea in different individuals, and treat different conditions or treatments involving excess CGRP levels such as those identified herein.

[000697] Figures 42-44 contain the results of these similar CGRP experiments effected in non-transgenic (C57BL/6J mice). These results show that the same anti-CGRP antibody (Ab3) prevented diarrhea in the C57BL/6J mice which received human CGRP. By contrast the majority of the C57BL/6J mice which received the human CGRP(α) and the same controls developed diarrhea.

[000698] Specifically, Figure 42 contains the results of experiments which show that the intra cerebroventricular (ICV) injection of human CGRP (similar to rat CGRP) induces diarrhea in a dose dependent manner in C57BL/6J mice. The data also shows that about 80% of the C57BL/6J mice administered 2.0 μg of human CGRP via intra cerebroventricular injection developed diarrhea whereas none of the mice who received the control or a reduced amount of human CGRP (0.4 μg) developed diarrhea.

[000699] Figure 43 contains the results of additional experiments which show that intra peritoneal injection of Ab3 (30mgs/kg ip, ~24 hrs. prior to CGRP challenge) inhibits ICV injected-CGRP induced diarrhea in C57/BL6J mice. By contrast the administration of the control antibody or vehicle had no effect on ICV injected CGRP-induced diarrhea.

[000700] Figure 44 contains the results of additional experiments which show that Ab3 (30mgs/kg ip injection ~24 hrs. prior to human CGRP challenge) inhibits IP injected-CGRP induced diarrhea in C57/BL6J mice. By contrast the administration of the control antibody contained in the same vehicle as Ab3 had no effect on CGRP-induced diarrhea.

[000701] These results obtained in different strains of mice persuasively demonstrate that the administration of an anti-CGRP antibody or antibody fragment may prevent or ameliorate diarrhea, especially in conditions which are associated with elevated CGRP levels. These results further indicate that there is a similar prophylactic effect when the CGRP is administered by 2 different means (intraperitoneal or intracerebroventricular injection) and is specific to different species. Accordingly the anti-CGRP antibody is apparently able to effectively bind the CGRP and prevent its adverse diarrhea effects irrespective of whether it was delivered systemically or locally via ICV injection.

[000702] In addition, the results show that assays in rodents which are administered CGRP may be used to assess whether a candidate anti-CGRP antibody or another CGRP/CGRP receptor polypeptide inhibitor may be used to inhibit or treat gastrointestinal disorders or other conditions characterized by excessive CGRP that involve aberrations in bowel movements, electrolyte balance and/or fluid excretion, and in particular diarrhea. These conditions include by way of example inflammatory bowel disease, bacterial or viral induced diarrhea. functional bowel disorders selected from the group consisting of gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, functional abdominal pain syndrome, diverticulosis, and diverticulitis, Crohn's disease, ileitis, collagenous colitis, lymphocytic colitis, and ulcerative colitis and cancers and cancer treatments associated with diarrhea, e.g., medullary thyroid carcinoma or colorectal cancer and other conditions previously identified.

Example 9 *Effect of CGRP Antibody Administration on Colonic Evacuation*

[000703] Experiments were conducted in C57BL/6 mice to assess the potential efficacy of CGRP antibody administration for the treatment or prevention of diarrhea and related gastrointestinal disorders. Relative to the experiments using C57BL/6J mice in Example 8, a higher dosage of CGRP and a lower dosage of antibody was utilized; nonetheless, Ab3 and Ab6 were effective in decreasing the incidence of diarrhea.

[000704] *Methods*

[000705] Male C57BL/6 mice (Harlan Laboratories) at 6-8 weeks of age were housed individually in clear polycarbonate conventional cages or clear/yellow polycarbonate microisolator cages with certified irradiated contact bedding and acclimated to the study

facility for at least 24 hours. Food and water were given *ad libitum*. Environmental controls were set to maintain temperatures of 18 to 26 degrees C (64 to 79 degrees F) with a relative humidity of 30% to 70%. A 12:12 hour light:dark cycle was maintained.

[000706] Animals were randomized into four treatment groups (ten animals each), based on body weight on the day following arrival. The mean body weights for each group was reviewed to ensure that the mean values and standard deviation satisfied the assumption of homogeneity.

[000707] On day 1, treatment groups 1 and 2 were administered the negative control antibody (of the same isotype as the anti-CGRP antibodies) and groups 3 and 4 received antibodies Ab3 and Ab6, respectively (all antibodies administered i.p. at 10 mg/kg, dose volume 2.63 mL/kg).

[000708] On day 2, treatment groups 2, 3, and 4 were administered CGRP (0.05 mg/kg, dose volume 3.33 mL/kg) i.p. and group 1 was administered an equal dose volume of phosphate buffered saline i.p.. The animals were then placed on a piece of absorbent paper inside a cage separate from their home cage immediately post dose. The piece of paper was weighed prior to placing it in the bottom of the cage and the weight was recorded. Each animal's bowel movements were monitored for 30 minutes post CGRP dose. Observations were made as incidence and total weight of diarrhea (that which sticks to the paper). A positive incidence of diarrhea was recorded if a loose stool was present. Gross fecal weight was determined by lifting the paper out of the cage by grasping the long side and lifting while holding the paper at approximately a 45 degree angle, and shaking lightly. Any stools that rolled off were considered normal, anything that stuck to the paper was considered diarrhea. The piece of paper with any stools attached was then placed on the scale and weighed.

[000709] *Results*

[000710] After CGRP administration the effects of the two different anti-CGRP antibodies on gastrointestinal distress, in this case diarrhea, were assessed during a 30 minute observation period and compared with the two control groups. As shown in FIG. 45, 80% of the positive control animals (receiving CGRP and the negative control antibody) exhibited diarrhea, compared to only 60% and 40% of the animals receiving Ab6 and Ab3.

None of the negative control animals exhibited diarrhea. In addition, the gross fecal weight in the animals which received the CGRP antibody was significantly less than the control animals (FIG. 46). Further, the fecal consistency in the animals which received the control antibody was much more fluid (watery) relative to the animals which received the anti-CGRP antibodies, another indication that the CGRP antibody had helped to restore normal gastrointestinal function and specifically normal colonic evacuation.

[000711] These results further confirm that an anti-CGRP antibody may be effective to prevent or treat diarrhea and related conditions.

Example 10 *Effect of CGRP Antibody Administration on Colonic Evacuation*

[000712] A further experiment was conducted in C57BL/6 mice to assess the potential efficacy of CGRP antibody administration for the treatment or prevention of diarrhea and related gastrointestinal disorders. A higher dosage of anti-CGRP antibodies was utilized than in Example 9 and the effect on diarrhea incidence and gross fecal weight was more pronounced.

[000713] *Methods*

[000714] Diarrhea was experimentally induced in C57BL/6 mice, with ten mice in each of four treatment groups as in Example 9, except that the dosage of each antibody was three times higher (30 mg/kg, dose volume 7.89 mL/kg), and the dose volume of CGRP was three times higher though the dosage of CGRP was the same (0.05 mg/kg, dose volume was 10 mL/kg). Group 1 (negative control) received the negative control antibody on day 1 and phosphate buffered saline on day 2. Groups 2, 3, and 4 received the control antibody, Ab3, and Ab6, respectively, on day 1, and CGRP on day 2. Antibodies and CGRP were administered i.p..

[000715] *Results*

[000716] After CGRP administration the effects of the two different anti-CGRP antibodies on gastrointestinal distress, in this case diarrhea, was assessed during a 30 minute observation period and compared with the two control groups. As shown in FIG. 47, 80% of the positive control animals (receiving CGRP and the negative control antibody) exhibited diarrhea, compared to only 40% and 20% of the animals receiving Ab6 and Ab3.

None of the negative control animals exhibited diarrhea. In addition, the average gross fecal weight in the animals which received the anti-CGRP antibodies was significantly less than the control animals (FIG. 48). Further, the fecal consistency in the animals which received the control antibody was much more fluid (watery) relative to the animals which received the anti-CGRP antibodies, another indication that the CGRP antibody had helped to restore normal gastrointestinal function and specifically normal colonic evacuation.

[000717] These results further confirm that an anti-CGRP antibody may be effective to prevent or treat diarrhea and related conditions.

CLAIMS

What is claimed is:

1). A method of inhibiting, preventing or treating diarrhea or dysentery and/or maintaining appropriate electrolyte and fluid levels in the colon of a subject having a condition associated with diarrhea or dysentery comprising administering an effective amount of an anti-CGRP antibody or anti-CGRP antibody fragment or an anti-CGRP receptor antibody or antibody fragment.

2) The method of Claim 1, wherein the diarrhea or dysentery is associated with increased CGRP.

3). The method of claim 1 or 2, wherein the diarrhea is acute diarrhea or chronic diarrhea.

4) The method of claim 1 or 2, wherein the diarrhea comprises osmotic diarrhea, secretory diarrhea, motility diarrhea, exudative diarrhea, and/or inflammatory diarrhea.

5). The method of claim 1 or 2, wherein diarrhea is caused by a chronic or acute condition selected from a functional bowel disorder, irritable bowel syndrome, coeliac disease, pancreatic disease, pancreatitis, type 1 or type 2 diabetes, cystic fibrosis, Crohn's disease, diabetic neuropathy, menstruation, hyperthyroidism, hormone imbalance, enteritis, an inflammatory bowel disease, microscopic colitis, ischemic bowel disease, ulcerative colitis, mucositis, or tuberculosis.

6). The method of claim 1 or 2, wherein the condition associated with diarrhea is a bacterial, parasitic or viral induced diarrhea.

7) The method of claim 1 or 2, wherein the condition resulting in diarrhea is a parasite selected from Entaamoeba histolytica, Giardia, or another protozoan.

8) The method of claim 1 or 2,, wherein the condition resulting in diarrhea is a bacterium selected from E coli, Shigella, Entaamoeba histolytica, Salmonella, Campylobacter, or Clostridium difficile.

9) The method of claim 1 or 2, wherein the condition resulting in diarrhea is a virus selected from rotavirus, RSV, HIV, norvovirus, adenovirus, and astrovirus.

10) The method of claim 1 or 2, wherein the condition resulting in diarrhea is food poisoning.

11). The method of claim 1 or 2, wherein the condition associated with diarrhea is a functional bowel disorder selected from the group consisting of gastro-esophageal reflux, dyspepsia, irritable bowel syndrome, functional abdominal pain syndrome, bile acid malabsorption, and diverticulitis.

12). The method according to claim 1 or 2, wherein the associated with diarrhea is an inflammatory bowel disease is selected from the group consisting of Crohn's disease, ileitis, collagenous colitis, lymphocytic colitis, and ulcerative colitis.

13). The method of claim 1 or 2, wherein the condition associated with diarrhea is a cancer or a cancer treatment associated with diarrhea.

14). The method of claim 13 wherein the cancer is medullary thyroid carcinoma, hormone secreting tumor condition, renal cancer, liver cancer, or a colorectal cancer.

15). The method of claim 13 wherein the cancer treatment associated with diarrhea is selected from chemotherapy, cytokine therapy and radiation or a combination thereof.

16) The method of claim 13 or 15, wherein said treatment results in mucositis or damage to the intestinal brush border.

17). The method of claim 15, wherein the chemotherapy includes a platinum compound.

18). The method of claim 1 or 2, wherein the condition associated with diarrhea is a drug, chemotherapy, an immunoregimen, cell therapy, and/or radiation therapy.

19). The method of Claim 18, wherein the drug associated with diarrhea is an antibiotic, analgesic agent such as an NSAID or opioid compound, antidepressant, or hormone.

20). The method of claim 1 or 2, wherein the antibody or antibody fragment is administered as a monotherapy.

21). The method of claim 1 or 2, wherein the antibody or antibody fragment is administered with another anti-diarrhea treatment agent.

22) The method of claim 21, wherein the other agent is an anti-motility agent such as loperamide, a bismuth compound, codeine, zinc compound, bile acid sequestrant such as cholestryamine, colestipol, or colesevelam, electrolyte solution or probiotic.

23). The method of any one of claims 1-22 wherein the antibody or antibody fragment is an anti-human CGRP antibody or antibody fragment which specifically binds to the same or an overlapping linear or conformational epitope(s) and/or competes for binding to the same or an overlapping linear or conformational epitope(s) on an intact CGRP polypeptide or fragment thereof as an anti-human CGRP antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13 or Ab14.

24). The method of any one of claims 1-23 wherein the antibody or antibody fragment specifically binds to the same or an overlapping linear or conformational epitope(s) and/or competes for binding to the same or an overlapping linear or conformational epitope(s) on an intact human CGRP polypeptide or a fragment thereof as Ab3, Ab6, Ab13 or Ab14.

25). The method of any one of claims 1-24 wherein the antibody fragment is selected from a Fab fragment, a Fab' fragment, or a F(ab')2 fragment.

26). The method of claim 25, wherein said fragment is a Fab fragment.

27) The method of any one of claims 1-26, wherein said anti-human CGRP antibody or antibody fragment comprises 1, 2, 3, 4, 5 or all 6 CDRs identical to those contained in an anti-human CGRP antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13 or Ab14.

28) The method of claim 27, wherein at least 2 of the CDRs are identical to those contained in an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13 or Ab14.

29) The method of claim 27, wherein at least 3 of the CDRs are identical to those contained in an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13 or Ab14.

30) The method of claim 27, wherein at least 4 of the CDRs are identical to those contained in an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13 or Ab14.

31) The method of claim 27, wherein at least 5 of the CDRs are identical to those contained in an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13 or Ab14.

32) The method of claim 27, wherein all 6 of the CDRs are identical to those contained in an antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13 or Ab14.

33). The method of any one of claims 1-32 wherein the antibody or antibody fragment comprises a variable light chain comprising the CDR 1 sequence of SEQ ID NO:25, the CDR 2 sequence of SEQ ID NO:26, and the CDR 3 sequence of SEQ ID NO:27, and/or a variable heavy chain comprising the CDR 1 sequence of SEQ ID NO:28, the CDR 2 sequence of SEQ ID NO:29, and the CDR 3 sequence of SEQ ID NO:30.

34). The method of any one of claims 1-32 wherein the antibody or antibody fragment comprises a variable light chain comprising the CDR 1 sequence of SEQ ID NO:55, the CDR 2 sequence of SEQ ID NO:56, and the CDR 3 sequence of SEQ ID NO:57, and/or a variable heavy chain comprising the CDR 1 sequence of SEQ ID NO:58, the CDR 2 sequence of SEQ ID NO:59, and the CDR 3 sequence of SEQ ID NO:60.

35). The method of any one of claims 1-32 wherein the antibody or antibody fragment comprises at least 2 complementarity determining regions (CDRs) in each of the variable light and the variable heavy regions which are identical to those contained in an anti-human CGRP antibody selected from Ab2, Ab3, Ab4, Ab5, or Ab6, Ab13 or Ab14

36). The method of any one of claims 1-32 wherein the antibody or antibody fragment comprises at least 3, 4, 5 or 6 complementarity determining regions (CDRs) in each of the variable light and the variable heavy regions which are identical to those contained in Ab3, Ab6, Ab13 or Ab14.

37). The method of any one of claims 1-36 wherein the antibody or antibody fragment is non-glycosylated or lacks N-glycosylation or if glycosylated only contains only mannose residues.

38). The method of any one of claims 1-37 wherein the antibody or antibody fragment contains an Fc region that has been modified to alter effector function, half-life, proteolysis, and/or glycosylation.

39). The method of any one of claims 1-38 wherein the antibody or antibody fragment is a humanized, single chain or chimeric antibody.

40). The method of any one of claims 1-39 wherein the antibody or antibody fragment specifically binds to CGRP expressing human cells and/or to circulating soluble CGRP molecules *in vivo*.

41). The method of any one of claims 1-40 wherein the antibody or antibody fragment comprises a V_H polypeptide sequence selected from: SEQ ID NO: 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, or 133, or a variant thereof at least 90% identical thereto; and further comprising a V_L polypeptide sequence selected from: SEQ ID NO: 1, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121 or 131, or a variant thereof at least 90% identical thereto, wherein one or more of the framework (FR) or CDR residues in said V_H or V_L polypeptide has been substituted with another amino acid residue resulting in an anti-CGRP antibody that specifically binds CGRP.

42). The method of claim 41 wherein one or more of said FR residues are substituted with an amino acid present at the corresponding site in a parent rabbit anti-CGRP antibody from which the complementarity determining regions (CDRs) contained in said V_H or V_L polypeptides have been derived or by a conservative amino acid substitution.

43). The method of any one of Claims 1-42, wherein said antibody or antibody fragment is humanized.

44). The method of any one of Claims 1-42, wherein said antibody or antibody fragment is chimeric.

45). The method of any one of Claims 1-39, wherein said antibody or antibody fragment comprises a single chain antibody.

46.). The method of claim 44, wherein said chimeric antibody comprises a human F_c.

47). The method of claim 46, wherein said human F_c is derived from IgG1, IgG2, IgG3, or IgG4.

48). The method of any one of claims 1-47 wherein the antibody or antibody fragment inhibits the association of CGRP with CGRP-R and/or multimers thereof, one or more additional proteins in a CGRP-CGRP-R complex, and/or antagonizes the biological effects thereof.

49). The method of claim 41 wherein the antibody or antibody fragment comprises a polypeptide sequence having at least 90% or greater homology to any one of the polypeptide sequences recited therein.

50). The method of claim 41 wherein the antibody or antibody fragment comprises a polypeptide sequence having at least 95% or greater homology to any one of the polypeptide sequences recited therein.

51). The method of any one of Claims 1-50, wherein the CGRP antibody or antibody fragment binds to CGRP with an off-rate (K_{off}) of less than or equal to 10^{-4} S^{-1}, $5x10^{-5}$ S^{-1}, 10^{-5} S^{-1}, $5x10^{-6}$ S^{-1}, 10^{-6} S^{-1}, $5x10^{-7}$ S^{-1}, or 10^{-7} S^{-1}.

52). The method of any one of Claims 1-51 wherein the antibody or antibody fragment inhibits the production of CGRP with CGRP-R and/or multimers thereof, and the production of CGRP with CGRP-R and one or more additional proteins in a complex.

53). The method of any one of claims 1-52 wherein the anti-CGRP antibody or antibody fragment binds to the same or an overlapping CGRP epitope as an anti-CGRP antibody selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13 or Ab14.

54). The method of any one of claims 1-53 wherein the anti-CGRP antibody or antibody fragment comprises one or more of the CDRs contained in the V_H polypeptide sequences selected from: SEQ ID NO: 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, or 133 and/or one or more of the CDRs contained in the V_L polypeptide sequences selected from: SEQ ID NO: 1, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121 or 131.

55). The method of any one of claims 1-54 wherein the antibody or antibody fragment is administered intramuscularly, subcutaneously, intravenously, rectally, by infusion, orally, transdermally or via inhalation.

56). The method of any one of claims 1-54 wherein the antibody or antibody fragment is administered intravenously.

57). The method of claim 1 or 2, wherein the CGRP-associated diarrhea is selected from diarrhea associated with irritable bowel syndrome, inflammatory bowel disease, Crohn's disease, ileitis, ulcerative colitis, cholera, pancreatic disease, lactose intolerance, fructose malabsorption, malabsorption, magnesium overdose or overingestion, vitamin C overdose or overingestion, sorbitol overdose or overingestion, food poisoning, *E. coli* infection, enzyme deficiency, mucosal abnormality, celiac disease, gluten intolerance, pernicious anemia, food allergy, food intolerance, short bowel syndrome, radiation fibrosis, diarrhea

associated with chemotherapy, orlistat treatment, cystic fibrosis, pancreatitis, chronic ethanol ingestion, ischemic bowel disease, microscopic colitis, bile salt malabsorption (primary bile acid diarrhea), elevated seratonin secretion or levels, or toddler's diarrhea.

58). The method of claim 1 or 2, wherein the treatment further includes the administration of another therapeutic agent or regimen selected from the group consisting of: antibiotics, antivirals, absorbents, anti-motility medications, bismuth compounds, bismuth subsalicylate, bile acid sequestrants, probiotics, digestive enzymes, lactase, zinc, oral rehydration therapy, and any combination thereof.

59). The method of claim 58, wherein said anti-motility agents are selected from the group consisting of loperamide (Imodium), diphenoxylate with atropine (Lomotil), opiates, paregoric tincture of opium, codeine, and morphine.

60) The method of claim 58, wherein said bile acid sequestrants are selected from the group consisting of: cholestyramine, colestipol and colesevelam.

61). The method of claim 1 or 2, wherein the anti-CGRP antibody or antibody fragment having binding specificity for CGRP comprises variable light chain CDR1, CDR2, and CDR3 polypeptide sequences and variable heavy chain CDR1, CDR2, and CDR3 polypeptide sequences selected from the following:

	V_L CDR1	V_L CDR2	V_L CDR3	V_H CDR1	V_H CDR2	V_H CDR3
A	Seq ID No: 5	Seq ID No: 6	Seq ID No: 7	Seq ID No: 8	Seq ID No: 9	Seq ID No: 10
B	Seq ID No: 15	Seq ID No: 16	Seq ID No: 17	Seq ID No: 18	Seq ID No: 19	Seq ID No: 20
C	Seq ID No: 25	Seq ID No: 26	Seq ID No: 27	Seq ID No: 28	Seq ID No: 29	Seq ID No: 30
D	Seq ID No: 35	Seq ID No: 36	Seq ID No: 37	Seq ID No: 38	Seq ID No: 39	Seq ID No: 40
E	Seq ID No: 45	Seq ID No: 46	Seq ID No: 47	Seq ID No: 48	Seq ID No: 49	Seq ID No: 50
F	Seq ID No: 55	Seq ID No: 56	Seq ID No: 57	Seq ID No: 58	Seq ID No: 59	Seq ID No: 60
G	Seq ID	Seq ID	Seq ID	Seq ID	Seq ID	Seq ID

	No: 65	No: 66	No: 67	No: 68	No: 69	No: 70
H	Seq ID No: 75	Seq ID No: 76	Seq ID No: 77	Seq ID No: 78	Seq ID No: 79	Seq ID No: 80
I	Seq ID No: 85	Seq ID No: 86	Seq ID No: 87	Seq ID No: 88	Seq ID No: 89	Seq ID No: 90
J	Seq ID No: 95	Seq ID No: 96	Seq ID No: 97	Seq ID No: 98	Seq ID No: 99	Seq ID No: 100
K	Seq ID No: 105	Seq ID No: 106	Seq ID No: 107	Seq ID No: 108	Seq ID No: 109	Seq ID No: 110
L	Seq ID No: 115	Seq ID No: 116	Seq ID No: 117	Seq ID No: 118	Seq ID No: 119	Seq ID No: 120
M	Seq ID No: 125	Seq ID No: 126	Seq ID No: 127	Seq ID No: 128	Seq ID No: 129	Seq ID No: 130
N	Seq ID No: 135	Seq ID No: 136	Seq ID No:137	Seq ID No: 138	Seq ID No: 139	Seq ID No: 140

62). The method of claim 61, wherein said antibody or antibody fragment is an scFv, camelbody, nanobody, IgNAR (single-chain antibodies derived from sharks), Fab, Fab', or F(ab')2 fragment.

63). The method of claim 61, wherein said anti-CGRP antibody or antibody fragment is a Fab fragment.

64). The method of claim 61, wherein said antibody or antibody fragment comprises a variable light chain polypeptide sequence and a variable heavy chain polypeptide sequence selected from the following:

	Variable Light Chain	Variable Heavy Chain
A	Seq ID No: 1	Seq ID No: 3
B	Seq ID No: 11	Seq ID No: 13
C	Seq ID No: 21	Seq ID No: 23
D	Seq ID No: 31	Seq ID No: 33
E	Seq ID No: 41	Seq ID No: 43
F	Seq ID No: 51	Seq ID No: 53
G	Seq ID No: 61	Seq ID No: 63
H	Seq ID No: 71	Seq ID No: 73
I	Seq ID No: 81	Seq ID No: 83
J	Seq ID No: 91	Seq ID No: 93

K	Seq ID No: 101	Seq ID No: 103
L	Seq ID No: 111	Seq ID No: 113
M	Seq ID No: 121	Seq ID No: 123
N	Seq ID No: 131	Seq ID No: 133

65). The method of claim 61, wherein said antibody or antibody fragment comprises a light chain polypeptide sequence and a heavy chain polypeptide sequence selected from the following:

	Light Chain	Heavy Chain
Ab1	Seq ID No: 2	Seq ID No: 4
Ab2	Seq ID No: 12	Seq ID No: 14
Ab3	Seq ID No: 22	Seq ID No: 24
Ab4	Seq ID No: 32	Seq ID No: 34
Ab5	Seq ID No: 42	Seq ID No: 44
Ab6	Seq ID No: 52	Seq ID No: 54
Ab7	Seq ID No: 62	Seq ID No: 64
Ab8	Seq ID No: 72	Seq ID No: 74
Ab9	Seq ID No: 82	Seq ID No: 84
Ab10	Seq ID No: 92	Seq ID No: 94
Ab11	Seq ID No: 102	Seq ID No: 104
Ab12	Seq ID No: 112	Seq ID No: 114
Ab13	Seq ID No: 122	Seq ID No: 124
Ab14	Seq ID No: 132	Seq ID No: 134

66). The method of claim 61, wherein said antibody or antibody fragment comprises a variable light chain and variable heavy chain polypeptide sequences are each at least 90% identical to one of the variable light chain polypeptide sequences of SEQ ID NOS: 1, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, or 131, and one of the variable heavy chain polypeptide sequences of SEQ ID NOS: 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123 or 133, respectively.

67). The method of claim 61, wherein said antibody or antibody fragment is chimeric or humanized.

68). The method of claim 61, wherein said anti-CGRP antibody or antibody fragment is entirely aglycosylated or lacks N-glycosylation or comprises only mannose residues.

69). The method of claim 61, wherein said anti-CGRP antibody or antibody fragment comprises a human constant domain.

70). The method of claim 61, wherein said anti-CGRP antibody or antibody fragment is an IgG1, IgG2, IgG3 or IgG4 antibody.

71). The method of claim 61, wherein said anti-CGRP antibody or antibody fragment contains an Fc region that has been modified to alter at least one of effector function, half-life, proteolysis, and/or glycosylation.

72). The method of claim 61, wherein said anti-CGRP antibody or antibody fragment has an Fc region that contains a mutation that alters or eliminates glycosylation or eliminates N-glycosylation.

73). The method of claim 61, wherein said anti-CGRP antibody or antibody fragment is directly or indirectly attached to a detectable label or therapeutic agent.

74). The method of claim 61, wherein said anti-CGRP antibody or antibody fragment further comprises an effector moiety.

75). The method of claim 74, wherein said effector moiety is a detectable moiety or a functional moiety.

76). The method of claim 75, wherein said detectable moiety is a fluorescent dye, an enzyme, a substrate, a bioluminescent material, a radioactive material, or a chemiluminescent material.

77). The method of claim 75, wherein said functional moiety is streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, or a radioactive material.

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Ab1

Ab1 Heavy chain (chimera) Full length protein sequence.

QSLEESGGRLVTPGTPLTLTCTVSGLDLSSYYMQWVRQAPGKGLEWIGVIGINDNTYYASWAKGRFTISRASSTTVDLKMTS
LTTEDTATYFCARGDIWGPGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 4)

Ab1 Variable region heavy chain (chimera) protein sequence.

QSLEESGGRLVTPGTPLTLTCTVSGLDLSSYYMQWVRQAPGKGLEWIGVIGINDNTYYASWAKGRFTISRASSTTVDLKMTS
LTTEDTATYFCARGDIWGPGTLVTVSS (SEQ ID NO: 3)

Ab1 Variable region heavy chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QSLEESGGRLVTPGTPLTLTCTVSGLDLS**SYYMQ**WVRQAPGKGLEWIGVIGINDNTYYASWAKGRFTISRASSTTVDLKMTS
LTTEDTATYFCAR*GDI*WGPGTLVTVSS (SEQ ID NOS: 8, 9, 10, respectively)

Ab1 Variable region heavy chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGACTCG
ACCTCAGT**AGCTACTACATGCAA**ATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTATTA
ATGATAACACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGCCTCGTCGACCACGGTGGATCTGA
AAATGACCAGTCTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGA*GGGGACATC*TGGGGCCCAGGCACCCTCGT
CACCGTCTCGAGC (SEQ ID NO: 143)

FIG. 1

Ab1 Heavy chain (chimera) Full length DNA sequence.

CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGACTCG
ACCTCAGTAGCTACTACATGCAATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTATTA
ATGATAACACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGCCTCGTCGACCACGGTGGATCTGA
AAATGACCAGTCTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGAGGGGACATCTGGGGCCCAGGCACCCTCG
TCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC
AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAG
CGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC
AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCC
CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAG
ACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT
CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 144)

Ab1 Light chain (chimera) Full length protein sequence.

QVLTQTASPVSAAVGSTVTINCQASQSVYDNNYLAWYQQKPGQPPKQLIYSTSTLASGVSSRFKGSGSGTQFTLTISDLECAD
AATYYCLGSYDCSSGDCFVFGGGTEVVVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 2)

Ab1 Variable region light chain (chimera) protein sequence.

QVLTQTASPVSAAVGSTVTINCQASQSVYDNNYLAWYQQKPGQPPKQLIYSTSTLASGVSSRFKGSGSGTQFTLTISDLECAD
AATYYCLGSYDCSSGDCFVFGGGTEVVVKR (SEQ ID NO: 1)

FIG. 1 (Continued)

Ab1 Variable region light chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQTASPVSAAVGSTVTINC**QASQSVYDNNYLA**WYQQKPGQPPKQLIYSTSTLASGVSSRFKGSGSGTQFTLTISDLECA
DAATYYC*LGSYDCSSGDCFV*FGGGTEVVVKR (SEQ ID NOS: 5, 6, 7, respectively)

Ab1 Variable region light chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAAGCACAGTCACCATCAATTGC**CAGGCCAGTCAG**
AGTGTTTATGATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTAC
ATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGAC
CTGGAGTGTGCCGATGCTGCCACTTACTACTGT*CTAGGCAGTTATGATTGTAGTAGTGGTGATTGTTTTGTT*TTCGGCGGAG
GGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 141)

Ab1 Light chain (chimera) Full length DNA sequence.

CAAGTGCTGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAAGCACAGTCACCATCAATTGCCAGGCCAGTCAG
AGTGTTTATGATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTACAT
CCACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGACCT
GGAGTGTGCCGATGCTGCCACTTACTACTGTCTAGGCAGTTATGATTGTAGTAGTGGTGATTGTTTTGTTTTCGGCGGAG
GGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
TGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCC
CTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 142)

FIG. 1 (Continued)

Ab2

Ab2 Heavy chain (humanized) Full length protein sequence – mammalian produced.

EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGINDNTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 14)

Ab2 Variable region heavy chain (humanized) protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGINDNTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSS (SEQ ID NO: 13)

Ab2 Variable region heavy chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

EVQLVESGGGLVQPGGSLRLSCAVSGLDLS**SYYMQ**WVRQAPGKGLEWVGVIGINDNTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCAR*GDI*WGQGTLVTVSS (SEQ ID NOS: 18, 19, 20, respectively)

Ab2 Variable region heavy chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAC
TCGACCTCAGT**AGCTACTACATGCAA**TGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
TCAATGATAACACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCTAGA*GGGGACATC*TGGGGCCAAGGGAC
CCTCGTCACCGTCTCGAGC (SEQ ID NO: 153)

FIG. 2

Ab2 Heavy chain (humanized) Full length DNA sequence – mammalian produced.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAC
TCGACCTCAGTAGCTACTACATGCAATGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
TCAATGATAACACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCTAGAGGGGACATCTGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 154)

Ab2 Light chain (humanized) Full length protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSSGDCFVFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 12)

Ab2 Variable region Light chain (humanized) protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSSGDCFVFGGGTKVEIKR (SEQ ID NO: 11)

FIG. 2 (Continued)

Ab2 Variable region Light chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQSPSSLSASVGDRVTINC**QASQSVYDNNYLA**WYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYC*LGSYDCSSGDCFV*FGGGTKVEIKR (SEQ ID NOS: 15, 16, 17, respectively)

Ab2 Variable region Light chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGC**CAGGCCAGTCAG**
AGTGTTTATGATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTAC
ATCCACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATGTTGCAACTTATTACTGT*CTAGGCAGTTATGATTGTAGTAGTGGTGATTGTTTTGTT*TTCGGCGGAG
GAACCAAGGTGGAAATCAAACGT (SEQ ID NO: 151)

Ab2 Light chain (humanized) Full length DNA sequence.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAGGCCAGTCAGA
GTGTTTATGATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTACATC
CACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTG
CAGCCTGAAGATGTTGCAACTTATTACTGTCTAGGCAGTTATGATTGTAGTAGTGGTGATTGTTTTGTTTTCGGCGGAGG
AACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 152)

FIG. 2 (Continued)

Ab3

Ab3 Heavy chain (humanized) Full length protein sequence – yeast produced.

EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGINDNTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 24)

Ab3 Variable region heavy chain (humanized) protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGINDNTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSS (SEQ ID NO: 23)

Ab3 Variable region heavy chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

EVQLVESGGGLVQPGGSLRLSCAVSGLDLS**SYYMQ**WVRQAPGKGLEWVGVIGINDNTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCAR*GDI*WGQGTLVTVSS (SEQ ID NOS: 28, 29, 30, respectively)

Ab3 Variable region heavy chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAC
TCGACCTCAGT**AGCTACTACATGCAA**TGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
TCAATGATAACACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCTAGA*GGGGACATC*TGGGGCCAAGGGAC
CCTCGTCACCGTCTCGAGC (SEQ ID NO: 163)

FIG. 3

Ab3 Heavy chain (humanized) Full length DNA sequence – yeast produced.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAC
TCGACCTCAGTAGCTACTACATGCAATGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
TCAATGATAACACACATACTACGCCGAGCTGGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGGCTGAGGACACTGCTGTGTATTTCTGTGCTAGAGGGGACATCTGGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGCGGCCTCCACCAAGGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACGCGAGAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGACGTGAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 164)

Ab3 Light chain (humanized) Full length protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSSGDCFVFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 22)

Ab3 Variable region Light chain (humanized) protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSSGDCFVFGGGTKVEIKR (SEQ ID NO: 21)

FIG. 3 (Continued)

Ab3 Variable region Light chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQSPSSLSASVGDRVTINC**QASQSVYDNNYLA**WYQQKPGKVPKQLIY**STSTLAS**GVPSRFSGSGSGTDFTLTISSLQPED
VATYYC*LGSYDCSSGDCFV*FGGGTKVEIKR (SEQ ID NOS: 25, 26, 27, respectively)

Ab3 Variable region Light chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGC**CAGGCCAGTCAG**
AGTGTTTATGATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTAC
ATCCACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATGTTGCAACTTATTACTGT*CTAGGCAGTTATGATTGTAGTAGTGGTGATTGTTTTGTTTCGGCGGAG*
GAACCAAGGTGGAAATCAAACGT (SEQ ID NO: 161)

Ab3 Light chain (humanized) Full length DNA sequence.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAGGCCAGTCAGA
GTGTTTATGATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTACATC
CACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTG
CAGCCTGAAGATGTTGCAACTTATTACTGTCTAGGCAGTTATGATTGTAGTAGTGGTGATTGTTTTGTTTCGGCGGAGG
AACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 162)

FIG. 3 (Continued)

Ab4

Ab4 Heavy chain (chimera) Full length protein sequence.

QSLEESGGRLVTPGTPLTLTCSVSGIDLSGYYMNWVRQAPGKGLEWIGVIGINGATYYASWAKGRFTISKTSSTTVDLKMTS
LTTEDTATYFCARGDIWGPGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 34)

Ab4 Variable region heavy chain (chimera) protein sequence.

QSLEESGGRLVTPGTPLTLTCSVSGIDLSGYYMNWVRQAPGKGLEWIGVIGINGATYYASWAKGRFTISKTSSTTVDLKMTS
LTTEDTATYFCARGDIWGPGTLVTVSS (SEQ ID NO: 33)

Ab4 Variable region heavy chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QSLEESGGRLVTPGTPLTLTCSVSGIDLS**GYYMN**WVRQAPGKGLEWIGVIGINGATYYASWAKGRFTISKTSSTTVDLKMTS
LTTEDTATYFCAR*GDI*WGPGTLVTVSS (SEQ ID NOS: 38, 39, 40, respectively)

Ab4 Variable region heavy chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGTTCCGTCTCTGGCATCG
ACCTCAGT**GGCTACTACATGAAC**TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTATT
AATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGGTGGATCTG
AAAATGACCAGTCTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGA*GGGGACATC*TGGGGCCCGGGCACCCTC
GTCACCGTCTCGAGC (SEQ ID NO: 173)

FIG. 4

Ab4 Heavy chain (chimera) Full length DNA sequence.

CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGTTCCGTCTCTGGCATCG
ACCTCAGTGGCTACTACATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTATTA
ATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGGTGGATCTGA
AAATGACCAGTCTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGAGGGGACATCTGGGGCCCGGGCACCCTCG
TCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC
AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAG
CGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC
AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCC
CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAG
ACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT
CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 174)

Ab4 Light chain (chimera) Full length protein sequence.

QVLTQTPSPVSAAVGSTVTINCQASQSVYHNTYLAWYQQKPGQPPKQLIYDASTLASGVPSRFSGSGSGTQFTLTISGVQCND
AAAYYCLGSYDCTNGDCFVFGGGTEVVVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 32)

Ab4 Variable region light chain (chimera) protein sequence.

QVLTQTPSPVSAAVGSTVTINCQASQSVYHNTYLAWYQQKPGQPPKQLIYDASTLASGVPSRFSGSGSGTQFTLTISGVQCND
AAAYYCLGSYDCTNGDCFVFGGGTEVVVKR (SEQ ID NO: 31)

FIG. 4 (Continued)

Ab4 Variable region light chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQTPSPVSAAVGSTVTINC**QASQSVYHNTYLA**WYQQKPGQPPKQLIY<u>DASTLAS</u>GVPSRFSGSGSGTQFTLTISGVQCN
DAAAYYC*LGSYDCTNGDCFV*FGGGTEVVVKR (SEQ ID NOS: 35, 36, 37, respectively)

Ab4 Variable region light chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAAGCACAGTCACCATCAATTGC**CAGGCCAGTCAG**
AGTGTTTATCATAACACCTACCTGGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAACAACTGATCTATG<u>ATGC</u>
<u>ATCCACTCTGGCGTCT</u>GGGGTCCCATCGCGGTTCAGCGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGGC
GTGCAGTGTAACGATGCTGCCGCTTACTACTGT*CTGGGCAGTTATGATTGTACTAATGGTGATTGTTTTGTTTTCGGCGGAG*
GGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 171)

Ab4 Light chain (chimera) Full length DNA sequence.

CAAGTGCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAAGCACAGTCACCATCAATTGCCAGGCCAGTCAGA
GTGTTTATCATAACACCTACCTGGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAACAACTGATCTATGATGCATC
CACTCTGGCGTCTGGGGTCCCATCGCGGTTCAGCGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGGCGTG
CAGTGTAACGATGCTGCCGCTTACTACTGTCTGGGCAGTTATGATTGTACTAATGGTGATTGTTTTGTTTTCGGCGGAGG
GACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 172)

FIG. 4 (Continued)

Ab5

Ab5 Heavy chain (humanized) Full length protein sequence – mammalian produced.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGATYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 44)

Ab5 Variable region heavy chain (humanized) protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGATYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSS (SEQ ID NO: 43)

Ab5 Variable region heavy chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLS**GYYMN**WVRQAPGKGLEWVGVIGINGATYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCAR*GDI*WGQGTLVTVSS (SEQ ID NOS: 48, 49, 50, respectively)

Ab5 Variable region heavy chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGACCTCAGT**GGCTACTACATGAAC**TGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGT
ATTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTG
TATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCTAGA*GGGGACATC*TGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGC (SEQ ID NO: 183)

FIG. 5

Ab5 Heavy chain (humanized) Full length DNA sequence – mammalian produced.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGACCTCAGTGGCTACTACATGAACTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
TTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCTAGAGGGGACATCTGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 184)

Ab5 Light chain (humanized) Full length protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCTNGDCFVFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 42)

Ab5 Variable region Light chain (humanized) protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCTNGDCFVFGGGTKVEIKR (SEQ ID NO: 41)

FIG. 5 (Continued)

Ab5 Variable region Light chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQSPSSLSASVGDRVTINC**QASQSVYHNTYLA**WYQQKPGKVPKQLIYDASTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYC*LGSYDCTNGDCFV*FGGGTKVEIKR (SEQ ID NOS: 45, 46, 47, respectively)

Ab5 Variable region Light chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGC**CAGGCCAGTCAG**
AGTGTTTATCATAACACCTACCTGGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATGATGC
ATCCACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATGTTGCAACTTATTACTGT*CTGGGCAGTTATGATTGTACTAATGGTGATTGTTTTGTT*TTCGGCGGAG
GAACCAAGGTGGAAATCAAACGT (SEQ ID NO: 181)

Ab5 Light chain (humanized) Full length DNA sequence.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAGGCCAGTCAGA
GTGTTTATCATAACACCTACCTGGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATGATGCATC
CACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTG
CAGCCTGAAGATGTTGCAACTTATTACTGTCTGGGCAGTTATGATTGTACTAATGGTGATTGTTTTGTTTTCGGCGGAGG
AACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 182)

FIG. 5 (Continued)

Ab6

Ab6 Heavy chain (humanized) Full length protein sequence – yeast produced.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGATYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 54)

Ab6 Variable region heavy chain (humanized) protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGATYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSS (SEQ ID NO: 53)

Ab6 Variable region heavy chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLS**GYYMN**WVRQAPGKGLEWVGVIGINGATYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCAR*GDI*WGQGTLVTVSS (SEQ ID NOS: 58, 59, 60, respectively)

Ab6 Variable region heavy chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGACCTCAGT**GGCTACTACATGAAC**TGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGT
ATTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTG
TATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCTAGA*GGGGACATC*TGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGC (SEQ ID NO: 193)

FIG. 6

Ab6 Heavy chain (humanized) Full length DNA sequence – yeast produced.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGACCTCAGTGGCTACTACATGAACTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
TTAATGGTGCCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGGCTGAGGACACTGCTGTGTATTTCTGTGCTAGAGGGGGACATCTGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGCGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAGCGAGAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGACGTGAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 194)

Ab6 Light chain (humanized) Full length protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCTNGDCFVFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 52)

Ab6 Variable region Light chain (humanized) protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCTNGDCFVFGGGTKVEIKR (SEQ ID NO: 51)

FIG. 6 (Continued)

Ab6 Variable region Light chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQSPSSLSASVGDRVTINC**QASQSVYHNTYLA**WYQQKPGKVPKQLIYDASTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYC*LGSYDCTNGDCFV*FGGGTKVEIKR (SEQ ID NOS: 55, 56, 57, respectively)

Ab6 Variable region Light chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGC**CAGGCCAGTCAG**
AGTGTTTATCATAACACCTACCTGGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATGATGC
ATCCACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATGTTGCAACTTATTACTGT*CTGGGCAGTTATGATTGTACTAATGGTGATTGTTTTGTT*TTCGGCGGAG
GAACCAAGGTGGAAATCAAACGT (SEQ ID NO: 191)

Ab6 Light chain (humanized) Full length DNA sequence.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAGGCCAGTCAGA
GTGTTTATCATAACACCTACCTGGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATGATGCATC
CACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTG
CAGCCTGAAGATGTTGCAACTTATTACTGTCTGGGCAGTTATGATTGTACTAATGGTGATTGTTTTGTTTTCGGCGGAGG
AACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 192)

FIG. 6 (Continued)

Ab7

Ab7 Heavy chain (chimera) Full length protein sequence.

QEQLKESGGRLVTPGTSLTLTCTVSGIDLSNHYMQWVRQAPGKGLEWIGVVGINGRTYYASWAKGRFTISRTSSTTVDLKM
TRLTTEDTATYFCARGDIWGPGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 64)

Ab7 Variable region heavy chain (chimera) protein sequence.

QEQLKESGGRLVTPGTSLTLTCTVSGIDLSNHYMQWVRQAPGKGLEWIGVVGINGRTYYASWAKGRFTISRTSSTTVDLKM
TRLTTEDTATYFCARGDIWGPGTLVTVSS (SEQ ID NO: 63)

Ab7 Variable region heavy chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QEQLKESGGRLVTPGTSLTLTCTVSGIDLS**NHYMQ**WVRQAPGKGLEWIGVVGINGRTYYASWAKGRFTISRTSSTTVDLKM
TRLTTEDTATYFCAR*GDI*WGPGTLVTVSS (SEQ ID NOS: 68, 69, 70, respectively)

Ab7 Variable region heavy chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAGGAGCAGCTGAAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACATCCCTGACACTCACCTGCACCGTCTCTGGA
ATCGACCTCAGT**AACCACTACATGCAA**TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAGTCGTTGG
TATTAATGGTCGCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAACCTCGTCGACCACGGTGGAT
CTGAAAATGACCAGGCTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGA*GGGGACATC*TGGGGCCCAGGCACC
CTGGTCACCGTCTCGAGC (SEQ ID NO: 203)

FIG. 7

Ab7 Heavy chain (chimera) Full length DNA sequence.

CAGGAGCAGCTGAAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACATCCCTGACACTCACCTGCACCGTCTCTGGA
ATCGACCTCAGTAACCACTACATGCAATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAGTCGTTGGT
ATTAATGGTCGCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAACCTCGTCGACCACGGTGGAT
CTGAAAATGACCAGGCTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGAGGGGGACATCTGGGGCCCAGGCACC
CTGGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGA
CCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTC
CAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGA
GCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACG
AAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG
CAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG
TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA
CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTG
GACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCAT
GCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID
NO: 204)

Ab7 Light chain (chimera) Full length protein sequence.

QVLTQTASPVSAAVGSTVTINCQASQSVYNYNYLAWYQQKPGQPPKQLIYSTSTLASGVSSRFKGSGSGTQFTLTISDVQCD
DAATYYCLGSYDCSTGDCFVFGGGTEVVVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 62)

Ab7 Variable region light chain (chimera) protein sequence.

QVLTQTASPVSAAVGSTVTINCQASQSVYNYNYLAWYQQKPGQPPKQLIYSTSTLASGVSSRFKGSGSGTQFTLTISDVQCD
DAATYYCLGSYDCSTGDCFVFGGGTEVVVKR (SEQ ID NO: 61)

FIG. 7 (Continued)

Ab7 Variable region light chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQTASPVSAAVGSTVTINC**QASQSVYNYNYLA**WYQQKPGQPPKQLIY<u>STSTLAS</u>GVSSRFKGSGSGTQFTLTISDVQCD
DAATYYC*LGSYDCSTGDCFV*FGGGTEVVVKR (SEQ ID NOS: 65, 66, 67, respectively)

Ab7 Variable region light chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAAGCACAGTCACCAATTGC**CAGGCCAGTCAG**
AGTGTTTATAATTACAACTACCTTGCCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATT<u>CTACA</u>
<u>TCCACTCTGGCATCT</u>GGGGTCTCATCGCGATTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGACG
TGCAGTGTGACGATGCTGCCACTTACTACTGT*CTAGGCAGTTATGACTGTAGTACTGGTGATTGTTTTGTTTTCGGCGGAGG*
GACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 201)

Ab7 Light chain (chimera) Full length DNA sequence.

CAAGTGCTGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAAGCACAGTCACCAATTGCCAGGCCAGTCAG
AGTGTTTATAATTACAACTACCTTGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTACAT
CCACTCTGGCATCTGGGGTCTCATCGCGATTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGACGT
GCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCAGTTATGACTGTAGTACTGGTGATTGTTTTGTTTTCGGCGGAG
GGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
TGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCC
CTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 202)

FIG. 7 (Continued)

Ab8

Ab8 Heavy chain (humanized) Full length protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSNHYMQWVRQAPGKGLEWVGVVGINGRTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 74)

Ab8 Variable region heavy chain (humanized) protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSNHYMQWVRQAPGKGLEWVGVVGINGRTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSS (SEQ ID NO: 73)

Ab8 Variable region heavy chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

EVQLVESGGGLVQPGGSLRLSCAVSGIDLS**NHYMQ**WVRQAPGKGLEWVGVVGINGRTYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCAR*GDI*WGQGTLVTVSS (SEQ ID NOS: 78, 79, 80, respectively)

Ab8 Variable region heavy chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGACCTCAGT**AACCACTACATGCAA**TGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCGTTGGTA
TCAATGGTCGCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCTAGA*GGGGACATC*TGGGGCCAAGGGAC
CCTCGTCACCGTCTCGAGC (SEQ ID NO: 213)

FIG. 8

Ab8 Heavy chain (humanized) Full length DNA sequence.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGACCTCAGTAACCACTACATGCAATGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCGTTGGTA
TCAATGGTCGCACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGCTGTGTATTTCTGTGCTAGAGGGGACATCTGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 214)

Ab8 Light chain (humanized) Full length protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYNYNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSTGDCFVFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 72)

Ab8 Variable region Light chain (humanized) protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQSVYNYNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSTGDCFVFGGGTKVEIKR (SEQ ID NO: 71)

FIG. 8 (Continued)

Ab8 Variable region Light chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQSPSSLSASVGDRVTINC**QASQSVYNYNYLA**WYQQKPGKVPKQLIY**STSTLAS**GVPSRFSGSGSGTDFTLTISSLQPED
VATYYC*LGSYDCSTGDCFV*FGGGTKVEIKR (SEQ ID NOS: 75, 76, 77, respectively)

Ab8 Variable region Light chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGC**CAGGCCAGTCAG**
AGTGTTTACAATTACAACTACCTTGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATT**CTAC**
ATCCACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATGTTGCAACTTATTACTGT*CTGGGCAGTTATAGATTGATTATGTTTTGTTTCGGCGGAG*
GAACCAAGGTGGAAATCAAACGT (SEQ ID NO: 211)

Ab8 Light chain (humanized) Full length DNA sequence.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAGGCCAGTCAGA
GTGTTTACAATTACAACTACCTTGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTACATC
CACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTG
CAGCCTGAAGATGTTGCAACTTATTACTGTCTGGGCAGTTATAGTACTGGTGATTATTTTGTTTTCGGCGGAGGG
AACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 212)

FIG. 8 (Continued)

Ab9

Ab9 Heavy chain (chimera) Full length protein sequence.

QSLEESGGRLVTPGTPLTLTCTVSGIGLSSYYMQWVRQSPGRGLEWIGVIGSDGKTYYATWAKGRFTISKTSSTTVDLRMAS
LTTEDTATYFCTRGDIWGPGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 84)

Ab9 Variable region heavy chain (chimera) protein sequence.

QSLEESGGRLVTPGTPLTLTCTVSGIGLSSYYMQWVRQSPGRGLEWIGVIGSDGKTYYATWAKGRFTISKTSSTTVDLRMAS
LTTEDTATYFCTRGDIWGPGTLVTVSS (SEQ ID NO: 83)

Ab9 Variable region heavy chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QSLEESGGRLVTPGTPLTLTCTVSGIGLS**SYYMQ**WVRQSPGRGLEWIGVIGSDGKTYYATWAKGRFTISKTSSTTVDLRMAS
LTTEDTATYFCTR*GDI*WGPGTLVTVSS (SEQ ID NOS: 88, 89, 90, respectively)

Ab9 Variable region heavy chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGAATCG
GCCTCAGT**AGCTACTACATGCAG**TGGGTCCGCCAGTCTCCAGGGAGGGGGCTGGAATGGATCGGAGTCATTGGTAGT
GATGGTAAGACATACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCCAAGACCTCGTCGACCACGGTGGATCTG
AGAATGGCCAGTCTGACAACCGAGGACACGGCCACCTATTTCTGTACCAGA*GGGGACATC*TGGGGCCCGGGGACCCTC
GTCACCGTCTCGAGC (SEQ ID NO: 223)

FIG. 9

Ab9 Heavy chain (chimera) Full length DNA sequence.

CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGAATCG
GCCTCAGTAGCTACTACATGCAGTGGGTCCGCCAGTCTCCAGGGAGGGGGCTGGAATGGATCGGAGTCATTGGTAGTG
ATGGTAAGACATACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCCAAGACCTCGTCGACCACGGTGGATCTGA
GAATGGCCAGTCTGACAACCGAGGACACGGCCACCTATTTCTGTACCAGAGGGGACATCTGGGGCCCGGGGACCCTCG
TCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC
AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAG
CGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC
AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCC
CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAG
ACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT
CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 224)

Ab9 Light chain (chimera) Full length protein sequence.

QVLTQTPSPVSAAVGSTVTINCQASQNVYNNNYLAWYQQKPGQPPKQLIYSTSTLASGVSSRFRGSGSGTQFTLTISDVQCD
DAATYYCLGSYDCSRGDCFVFGGGTEVVVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 82)

Ab9 Variable region light chain (chimera) protein sequence.

QVLTQTPSPVSAAVGSTVTINCQASQNVYNNNYLAWYQQKPGQPPKQLIYSTSTLASGVSSRFRGSGSGTQFTLTISDVQCD
DAATYYCLGSYDCSRGDCFVFGGGTEVVVKR (SEQ ID NO: 81)

FIG. 9 (Continued)

Ab9 Variable region light chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQTPSPVSAAVGSTVTINC**QASQNVYNNNYLA**WYQQKPGQPPKQLIY<u>STSTLAS</u>GVSSRFRGSGSGTQFTLTISDVQCD
DAATYYC*LGSYDCSRGDCFV*FGGGTEVVVKR (SEQ ID NOS: 85, 86, 87, respectively)

Ab9 Variable region light chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAAGCACAGTCACCATCAATTGC**CAGGCCAGTCAG**
AATGTTTATAATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATT<u>CTAC</u>
<u>GTCCACTCTGGCATCTGGGGTCTCATCGCGATTCAGAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGAC</u>
GTGCAGTGTGACGATGCTGCCACTTACTACTGT*CTAGGCAGTTATGATTGTAGTCGTGGTGATTGTTTTGTTTTCGGCGGAG*
GGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 221)

Ab9 Light chain (chimera) Full length DNA sequence.

CAAGTGCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAAGCACAGTCACCATCAATTGCCAGGCCAGTCAGA
ATGTTTATAATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTACGTC
CACTCTGGCATCTGGGGTCTCATCGCGATTCAGAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGACGTG
CAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCAGTTATGATTGTAGTCGTGGTGATTGTTTTGTTTTCGGCGGAGG
GACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGGACAGCAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 222)

FIG. 9 (Continued)

Ab10

Ab10 Heavy chain (humanized) Full length protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIGLSSYYMQWVRQAPGKGLEWVGVIGSDGKTYYATWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCTRGDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 94)

Ab10 Variable region heavy chain (humanized) protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIGLSSYYMQWVRQAPGKGLEWVGVIGSDGKTYYATWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCTRGDIWGQGTLVTVSS (SEQ ID NO: 93)

Ab10 Variable region heavy chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

EVQLVESGGGLVQPGGSLRLSCAVSGIGLS**SYYMQ**WVRQAPGKGLEWVGVIGSDGKTYYATWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCTR*GDI*WGQGTLVTVSS (SEQ ID NOS: 98, 99, 100, respectively)

Ab10 Variable region heavy chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGGCCTCAGT**AGCTACTACATGCAA**ATGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
GTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTACCAGA*GGGGACATC*TGGGGCCAAGGGAC
CCTCGTCACCGTCTCGAGC (SEQ ID NO: 233)

FIG. 10

Ab10 Heavy chain (humanized) Full length DNA sequence.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGGCCTCAGTAGCTACTACATGCAATGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
GTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTACCAGAGGGGACATCTGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGACGTGAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 234)

Ab10 Light chain (humanized) Full length protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQNVYNNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSRGDCFVFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 92)

Ab10 Variable region Light chain (humanized) protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQNVYNNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSRGDCFVFGGGTKVEIKR (SEQ ID NO: 91)

FIG. 10 (Continued)

Ab10 Variable region Light chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQSPSSLSASVGDRVTINC**QASQNVYNNNYLA**WYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYC*LGSYDCSRGDCFV*FGGGTKVEIKR (SEQ ID NOS: 95, 96, 97, respectively)

Ab10 Variable region Light chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGC**CAGGCCAGTCAG**
AATGTTTACAATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTAC
ATCCACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATGTTGCAACTTATTACTGT*CTGGGCAGTTATGATTGTAGTCGTGGTGATTGTTTTGTT*TTCGGCGGAG
GAACCAAGGTGGAAATCAAACGT (SEQ ID NO: 231)

Ab10 Light chain (humanized) Full length DNA sequence.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAGGCCAGTCAGA
ATGTTTACAATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTACATC
CACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTG
CAGCCTGAAGATGTTGCAACTTATTACTGTCTGGGCAGTTATGATTGTAGTCGTGGTGATTGTTTTGTTTTCGGCGGAGG
AACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 232)

FIG. 10 (Continued)

Ab11

Ab11 Heavy chain (chimera) Full length protein sequence.

QSLEESGGRLVTPGGSLTLTCTVSGIDVTNYYMQWVRQAPGKGLEWIGVIGVNGKRYYASWAKGRFTISKTSSTTVDLKMT
SLTTEDTATYFCARGDIWGPGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 104)

Ab11 Variable region heavy chain (chimera) protein sequence.

QSLEESGGRLVTPGGSLTLTCTVSGIDVTNYYMQWVRQAPGKGLEWIGVIGVNGKRYYASWAKGRFTISKTSSTTVDLKMT
SLTTEDTATYFCARGDIWGPGTLVTVSS (SEQ ID NO: 103)

Ab11 Variable region heavy chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QSLEESGGRLVTPGGSLTLTCTVSGIDVT**NYYMQ**WVRQAPGKGLEWIGVIGVNGKRYYASWAKGRFTISKTSSTTVDLKMT
SLTTEDTATYFCAR*GDI*WGPGTLVTVSS (SEQ ID NOS: 108, 109, 110, respectively)

Ab11 Variable region heavy chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGAGGATCCCTGACACTCACCTGCACAGTCTCTGGAATCG
ACGTCACT**AACTACTATATGCAA**TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTGTGA
ATGGTAAGAGATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGGTGGATCTGA
AAATGACCAGTCTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGA*GGCGACATC*TGGGGCCCGGGGACCCTCGT
CACCGTCTCGAGC (SEQ ID NO: 243)

FIG. 11

Ab11 Heavy chain (chimera) Full length DNA sequence.

CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGAGGATCCCTGACACTCACCTGCACAGTCTCTGGAATCG
ACGTCACTAACTACTATATGCAATGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTGGTGTGA
ATGGTAAGAGATACTACGCCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGGTGGATCTGA
AAATGACCAGTCTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGAGGCGACATCTGGGGCCCGGGGACCCTCG
TCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC
AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAG
CGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC
AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCC
CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGACGTGAGCCACGAAG
ACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT
CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 244)

Ab11 Light chain (chimera) Full length protein sequence.

QVLTQTASPVSPAVGSTVTINCRASQSVYYNNYLAWYQQKPGQPPKQLIYSTSTLASGVSSRFKGSGSGTQFTLTISDVQCDD
AATYYCLGSYDCSNGDCFVFGGGTEVVVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 102)

Ab11 Variable region light chain (chimera) protein sequence.

QVLTQTASPVSPAVGSTVTINCRASQSVYYNNYLAWYQQKPGQPPKQLIYSTSTLASGVSSRFKGSGSGTQFTLTISDVQCDD
AATYYCLGSYDCSNGDCFVFGGGTEVVVKR (SEQ ID NO: 101)

FIG. 11 (Continued)

Ab11 Variable region light chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQTASPVSPAVGSTVTINC**RASQSVYYNNYLA**WYQQKPGQPPKQLIYSTSTLASGVSSRFKGSGSGTQFTLTISDVQCD
DAATYYC*LGSYDCSNGDCFV*FGGGTEVVVKR (SEQ ID NOS: 105, 106, 107, respectively)

Ab11 Variable region light chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAGGTGCTGACCCAGACTGCATCCCCCGTGTCTCCAGCTGTGGGAAGCACAGTCACCATCAATTGC**CGGGCCAGTCAG**
AGTGTTTATTATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTAC
ATCCACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGAC
GTGCAGTGTGACGATGCTGCCACTTACTACTGT*CTAGGCAGTTATGATTGTAGTAATGGTGATTGTTTTGTT*TTCGGCGGAG
GGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 241)

Ab11 Light chain (chimera) Full length DNA sequence.

CAGGTGCTGACCCAGACTGCATCCCCCGTGTCTCCAGCTGTGGGAAGCACAGTCACCATCAATTGCCGGGCCAGTCAGA
GTGTTTATTATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCAACTGATCTATTCTACATC
CACTCTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGACGTG
CAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCAGTTATGATTGTAGTAATGGTGATTGTTTTGTTTTCGGCGGAGG
GACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 242)

FIG. 11 (Continued)

Ab12

Ab12 Heavy chain (humanized) Full length protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIDVTNYYMQWVRQAPGKGLEWVGVIGVNGKRYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 114)

Ab12 Variable region heavy chain (humanized) protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIDVTNYYMQWVRQAPGKGLEWVGVIGVNGKRYYASWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCARGDIWGQGTLVTVSS (SEQ ID NO: 113)

Ab12 Variable region heavy chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

EVQLVESGGGLVQPGGSLRLSCAVSGIDVT**NYYMQ**WVRQAPGKGLEWVGVIGVNGKRYYASWAKGRFTISRDNSKTTVY
LQMNSLRAEDTAVYFCAR*GDI*WGQGTLVTVSS (SEQ ID NOS: 118, 119, 120, respectively)

Ab12 Variable region heavy chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGACGTCACT**AACTACTACATGCAA**TGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTG
TGAATGGTAAGAGATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCCAGA*GGGGACATC*TGGGGCCAAGGGAC
CCTCGTCACCGTCTCGAGC (SEQ ID NO: 253)

FIG. 12

Ab12 Heavy chain (humanized) Full length DNA sequence.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGACGTCACTAACTACTACATGCAATGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTG
TGAATGGTAAGAGATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTGCCAGAGGGGACATCTGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 254)

Ab12 Light chain (humanized) Full length protein sequence.

QVLTQSPSSLSASVGDRVTINCRASQSVYYNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPEDV
ATYYCLGSYDCSNGDCFVFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 112)

Ab12 Variable region Light chain (humanized) protein sequence.

QVLTQSPSSLSASVGDRVTINCRASQSVYYNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPEDV
ATYYCLGSYDCSNGDCFVFGGGTKVEIKR (SEQ ID NO: 111)

FIG. 12 (Continued)

Ab12 Variable region Light chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQSPSSLSASVGDRVTINC**RASQSVYYNNYLA**WYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYC*LGSYDCSNGDCFV*FGGGTKVEIKR (SEQ ID NOS: 115, 116, 117, respectively)

Ab12 Variable region Light chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGC**CGGGCCAGTCAG**
AGTGTTTACTATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTAC
ATCCACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATGTTGCAACTTATTACTGT*CTGGGCAGTTATGATTGTAGTAATGGTGATTGTTTTGTT*TTCGGCGGAG
GAACCAAGGTGGAAATCAAACGT (SEQ ID NO: 251)

Ab12 Light chain (humanized) Full length DNA sequence.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCGGGCCAGTCAGA
GTGTTTACTATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTACATC
CACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTG
CAGCCTGAAGATGTTGCAACTTATTACTGTCTGGGCAGTTATGATTGTAGTAATGGTGATTGTTTTGTTTTCGGCGGAGG
AACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 252)

FIG. 12 (Continued)

Ab13

Ab13 Heavy chain (chimera) Full length protein sequence.

QSVEESGGGLVQPEGSLTLTCTASGFDFSSNAMWWVRQAPGKGLEWIGCIYNGDGSTYYASWVNGRFSISKTSSTTVTLQL
NSLTVADTATYYCARDLDLWGPGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 124)

Ab13 Variable region heavy chain (chimera) protein sequence.

QSVEESGGGLVQPEGSLTLTCTASGFDFSSNAMWWVRQAPGKGLEWIGCIYNGDGSTYYASWVNGRFSISKTSSTTVTLQL
NSLTVADTATYYCARDLDLWGPGTLVTVSS (SEQ ID NO: 123)

Ab13 Variable region heavy chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QSVEESGGGLVQPEGSLTLTCTASGFDFS**SNAMW**WVRQAPGKGLEWIGCIYNGDGSTYYASWVNGRFSISKTSSTTVTLQL
NSLTVADTATYYCAR*DLDL*WGPGTLVTVSS (SEQ ID NOS: 128, 129, 130, respectively)

Ab13 Variable region heavy chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAGTCGGTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGATCCCTGACACTCACCTGCACAGCCTCTGGATTC
GACTTCAGT**AGCAATGCAATGTGG**TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATGCATTTACAA
TGGTGATGGCAGCACATACTACGCGAGCTGGGTGAATGGCCGATTCTCCATCTCCAAAACCTCGTCGACCACGGTGACT
CTGCAACTGAATAGTCTGACAGTCGCGGACACGGCCACGTATTATTGTGCGAGA*GATCTTGACTTG*TGGGGCCCGGGCA
CCCTCGTCACCGTCTCGAGC (SEQ ID NO: 263)

FIG. 13

Ab13 Heavy chain (chimera) Full length DNA sequence.

CAGTCGGTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGATCCCTGACACTCACCTGCACAGCCTCTGGATTC
GACTTCAGTAGCAATGCAATGTGGTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATGCATTTACAAT
GGTGATGGCAGCACATACTACGCGAGCTGGGTGAATGGCCGATTCTCCATCTCCAAAACCTCGTCGACCACGGTGACTC
TGCAACTGAATAGTCTGACAGTCGCGGACACGGCCACGTATTATTGTGCGAGAGATCTTGACTTGTGGGGCCCGGGCAC
CCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG
GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG
ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCT
CCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTG
AGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCT
CTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC
GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGA
GCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACC
ACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT
GGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA
TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 264)

Ab13 Light chain (chimera) Full length protein sequence.

AIVMTQTPSSKSVPVGDTVTINCQASESLYNNNALAWFQQKPGQPPKRLIYDASKLASGVPSRFSGGGSGTQFTLTISGVQCD
DAATYYCGGYRSDSVDGVAFAGGTEVVVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 122)

Ab13 Variable region light chain (chimera) protein sequence.

AIVMTQTPSSKSVPVGDTVTINCQASESLYNNNALAWFQQKPGQPPKRLIYDASKLASGVPSRFSGGGSGTQFTLTISGVQCD
DAATYYCGGYRSDSVDGVAFAGGTEVVVKR (SEQ ID NO: 121)

FIG. 13 (Continued)

Ab13 Variable region light chain (chimera) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

AIVMTQTPSSKSVPVGDTVTINC**QASESLYNNNALA**WFQQKPGQPPKRLIYDASKLASGVPSRFSGGGSGTQFTLTISGVQCD
DAATYYC*GGYRSDSVDGVA*FAGGTEVVVKR (SEQ ID NOS: 125, 126, 127, respectively)

Ab13 Variable region light chain (chimera) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GCCATCGTGATGACCCAGACTCCATCTTCCAAGTCTGTCCCTGTGGGAGACACAGTCACCATCAATTGC**CAGGCCAGT**
GAGAGTCTTTATAATAACAACGCCTTGGCCTGGTTTCAGCAGAAACCAGGGCAGCCTCCCAAGCGCCTGATCTATGA
TGCATCCAAACTGGCATCTGGGGTCCCATCGCGGTTCAGTGGCGGTGGGTCTGGGACACAGTTCACTCTCACCATCAGT
GGCGTGCAGTGTGACGATGCTGCCACTTACTACTGT*GGAGGCTACAGAAGTGATAGTGTTGATGGTGTTGCT*TTCGCCGGA
GGGACCGAGGTGGTGGTCAAACGT (SEQ ID NO: 261)

Ab13 Light chain (chimera) Full length DNA sequence.

GCCATCGTGATGACCCAGACTCCATCTTCCAAGTCTGTCCCTGTGGGAGACACAGTCACCATCAATTGCCAGGCCAGTG
AGAGTCTTTATAATAACAACGCCTTGGCCTGGTTTCAGCAGAAACCAGGGCAGCCTCCCAAGCGCCTGATCTATGATGC
ATCCAAACTGGCATCTGGGGTCCCATCGCGGTTCAGTGGCGGTGGGTCTGGGACACAGTTCACTCTCACCATCAGTGGC
GTGCAGTGTGACGATGCTGCCACTTACTACTGTGGAGGCTACAGAAGTGATAGTGTTGATGGTGTTGCTTTCGCCGGAG
GGACCGAGGTGGTGGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
TGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCC
CTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 262)

FIG. 13 (Continued)

Ab14

Ab14 Heavy chain (humanized) Full length protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIGLSSYYMQWVRQAPGKGLEWVGVIGSDGKTYYATWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCTRGDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 134)

Ab14 Variable region heavy chain (humanized) protein sequence.

EVQLVESGGGLVQPGGSLRLSCAVSGIGLSSYYMQWVRQAPGKGLEWVGVIGSDGKTYYATWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCTRGDIWGQGTLVTVSS (SEQ ID NO: 133)

Ab14 Variable region heavy chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

EVQLVESGGGLVQPGGSLRLSCAVSGIGLS**SYYMQ**WVRQAPGKGLEWVGVIGSDGKTYYATWAKGRFTISRDNSKTTVYL
QMNSLRAEDTAVYFCTR*GDI*WGQGTLVTVSS (SEQ ID NOS: 138, 139, 140, respectively)

Ab14 Variable region heavy chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGGCCTCAGT**AGCTACTACATGCAA**ATGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
GTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTACCAGA*GGGGACATC*TGGGGCCAAGGGAC
CCTCGTCACCGTCTCGAGC (SEQ ID NO: 273)

FIG. 14

Ab14 Heavy chain (humanized) Full length DNA sequence.

GAGGTGCAGCTTGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGTCTCTGGAA
TCGGCCTCAGTAGCTACTACATGCAATGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGGAGTCATTGGTA
GTGATGGTAAGACATACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCCAGAGACAATTCCAAGACCACGGTGT
ATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTGTATTTCTGTACCAGAGGGGACATCTGGGGCCAAGGGA
CCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACGCGAGAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 274)

Ab14 Light chain (humanized) Full length protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQNVYNNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSRGDCFVFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 132)

Ab14 Variable region Light chain (humanized) protein sequence.

QVLTQSPSSLSASVGDRVTINCQASQNVYNNNYLAWYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCLGSYDCSRGDCFVFGGGTKVEIKR (SEQ ID NO: 131)

FIG. 14 (Continued)

Ab14 Variable region Light chain (humanized) protein sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

QVLTQSPSSLSASVGDRVTINC**QASQNVYNNNYLA**WYQQKPGKVPKQLIYSTSTLASGVPSRFSGSGSGTDFTLTISSLQPED
VATYYC*LGSYDCSRGDCFV*FGGGTKVEIKR (SEQ ID NOS: 135, 136, 137, respectively)

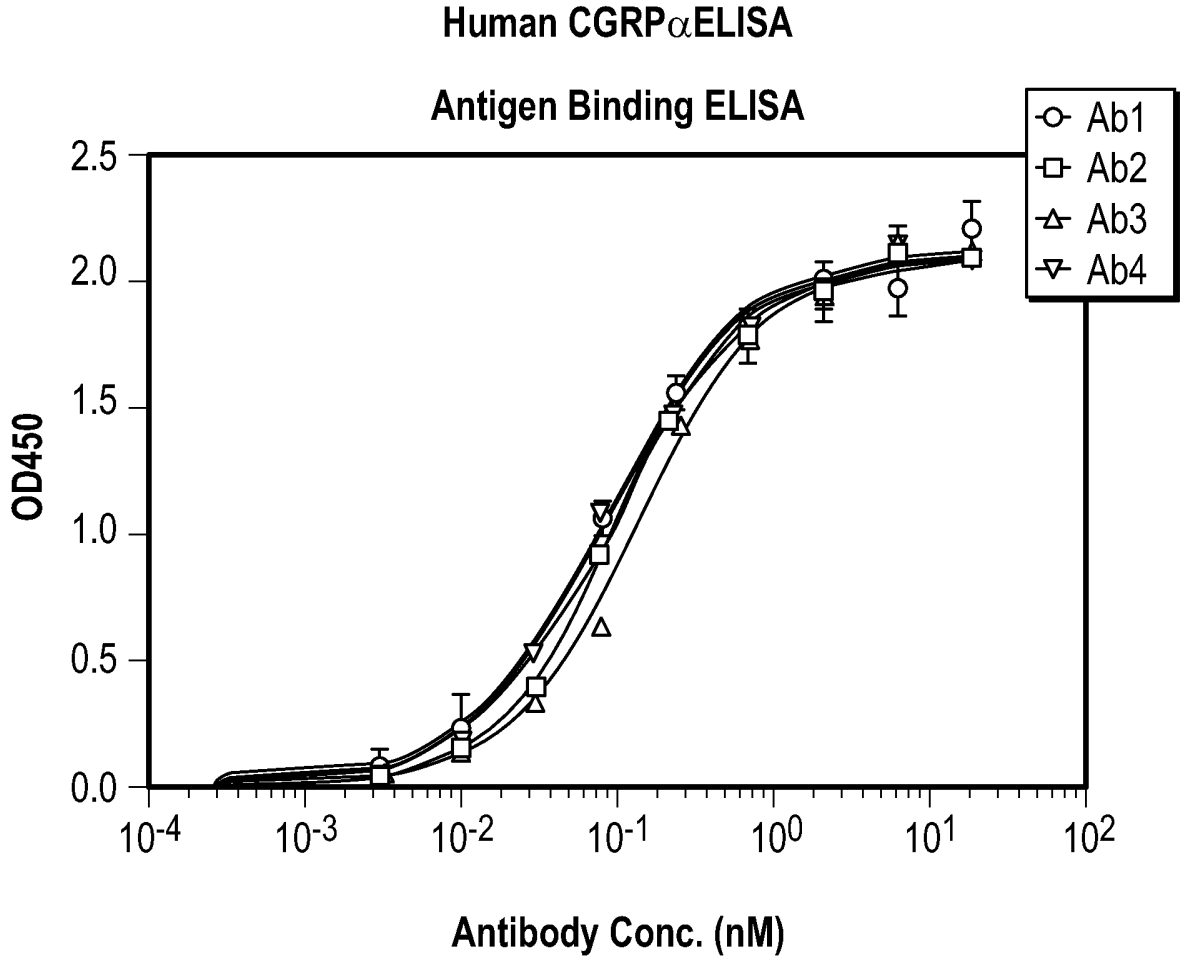
Ab14 Variable region Light chain (humanized) DNA sequence. CDR1: Bold; CDR2: Underlined; CDR3: Italics.

CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGC**CAGGCCAGTCAG**
AATGTTTACAATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTAC
ATCCACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATGTTGCAACTTATTACTGT*CTGGGCAGTTATGATTGTAGTCGTGGTGATTGTTTTGTT*TTCGGCGGAG
GAACCAAGGTGGAAATCAAACGT (SEQ ID NO: 271)

Ab14 Light chain (humanized) Full length DNA sequence.

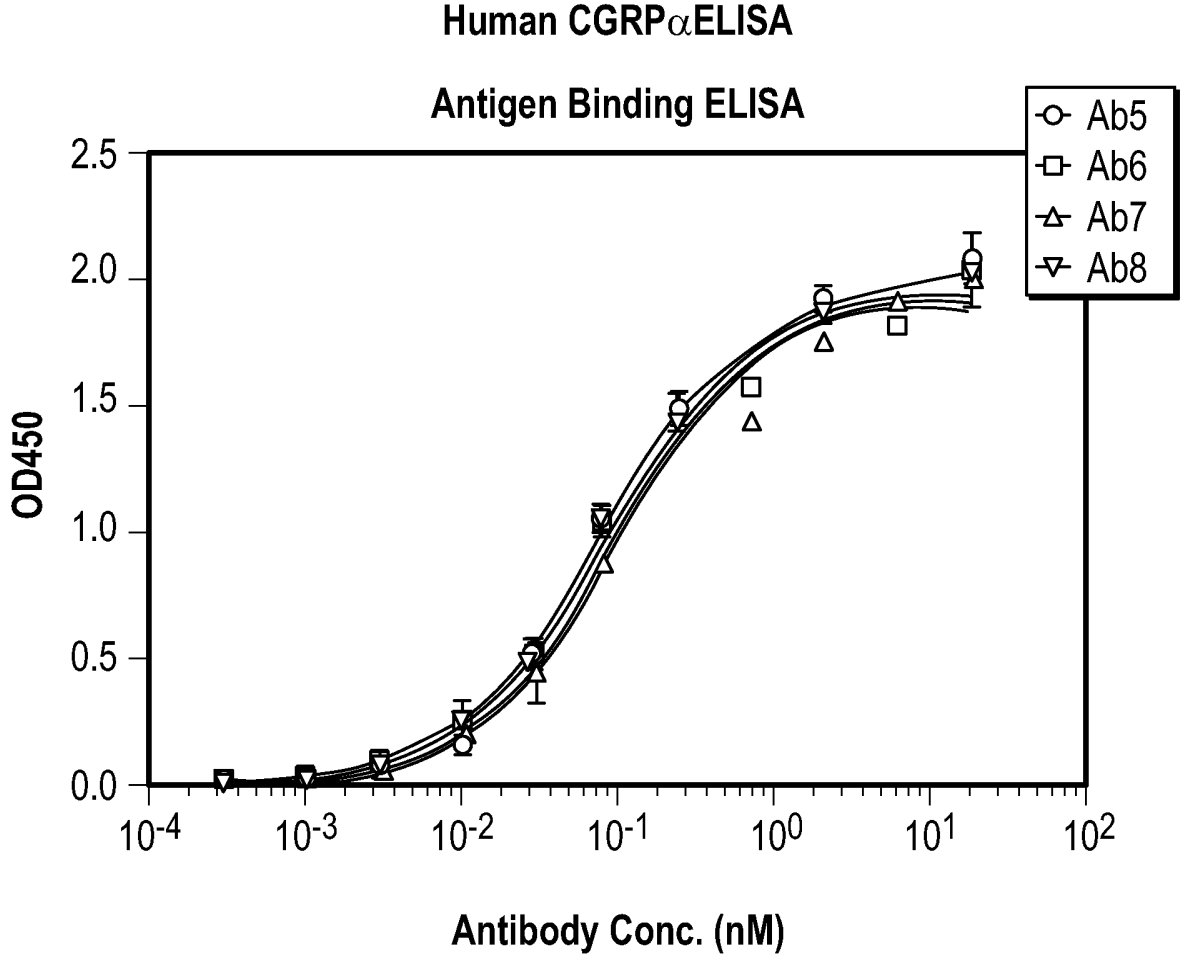
CAAGTGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAGGCCAGTCAGA
ATGTTTACAATAACAACTACCTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCAACTGATCTATTCTACATC
CACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTG
CAGCCTGAAGATGTTGCAACTTATTACTGTCTGGGCAGTTATGATTGTAGTCGTGGTGATTGTTTTGTTTTCGGCGGAGG
AACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 272)

FIG. 14 (Continued)



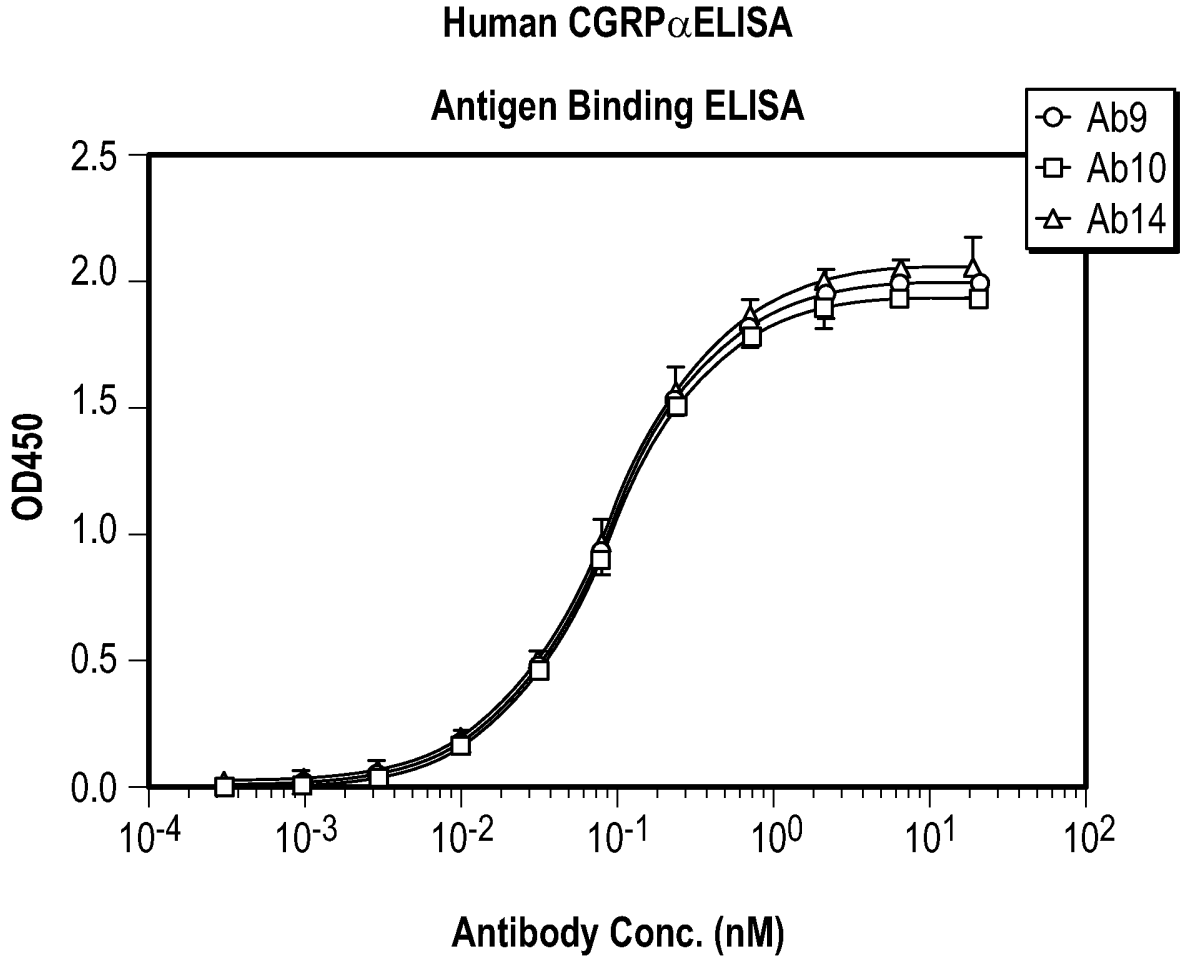
	EC50 (pM)
Ab1	103
Ab2	83
Ab3	154
Ab4	88

FIG. 15



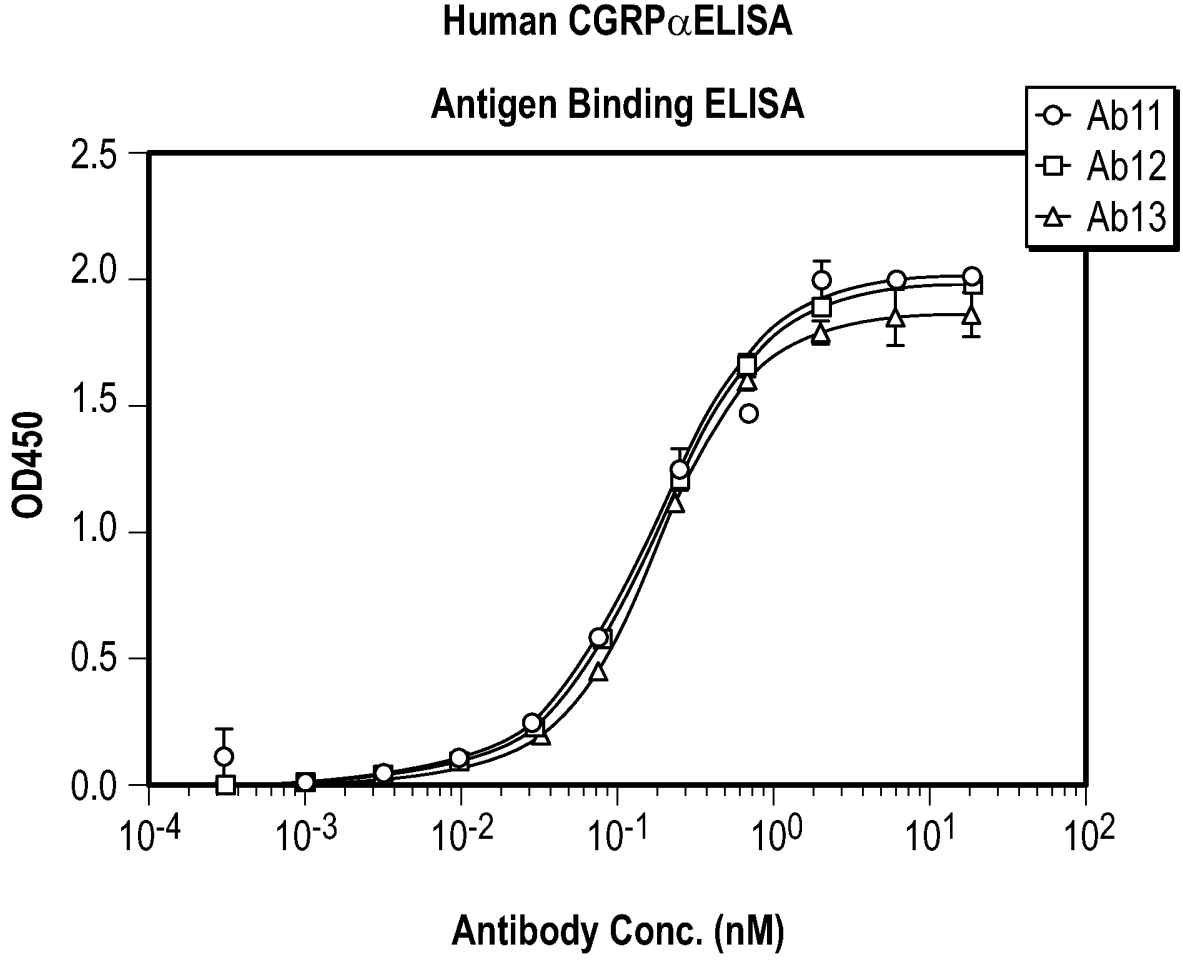
	EC50 (pM)
Ab5	103
Ab6	95
Ab7	70
Ab8	74

FIG. 16



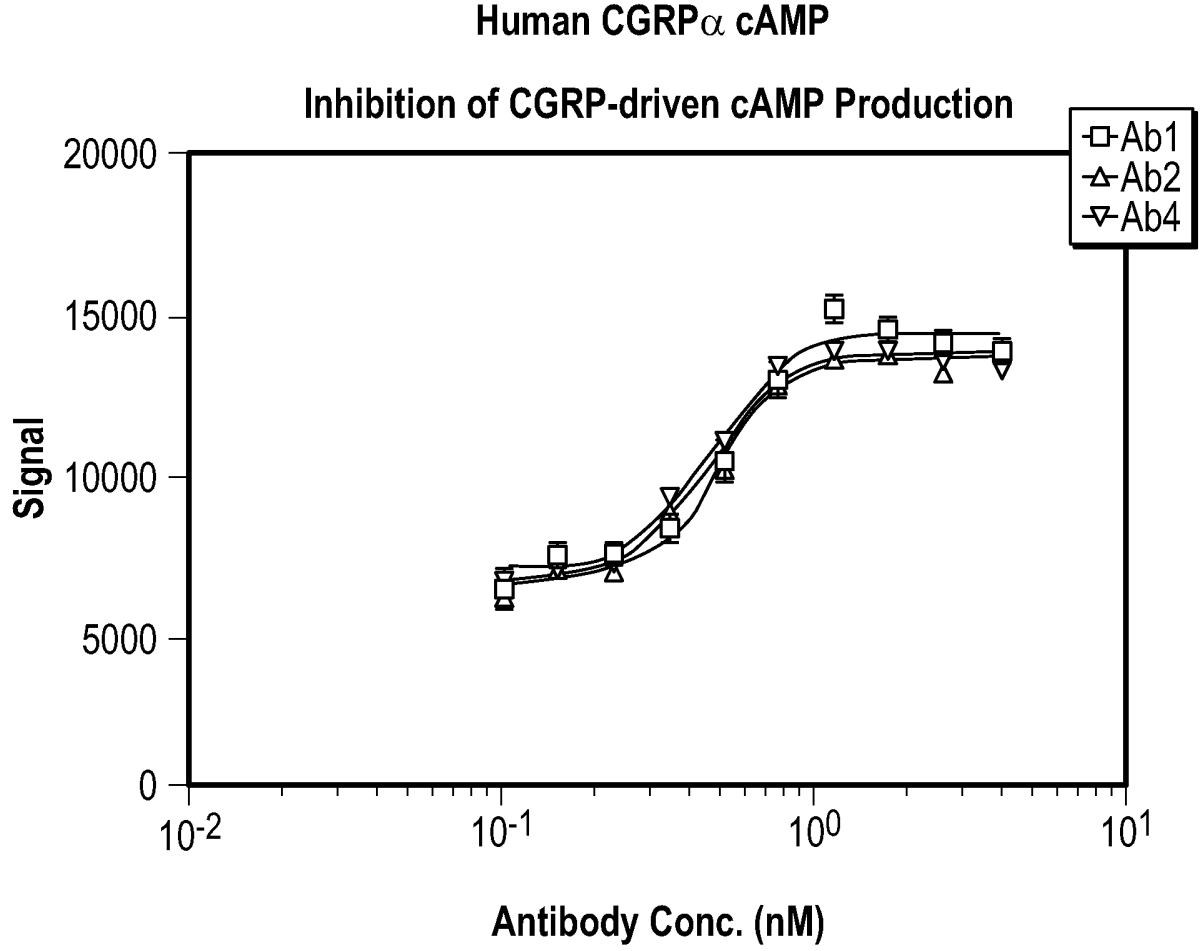
	EC50 (pM)
Ab9	79
Ab10	92
Ab14	89

FIG. 17



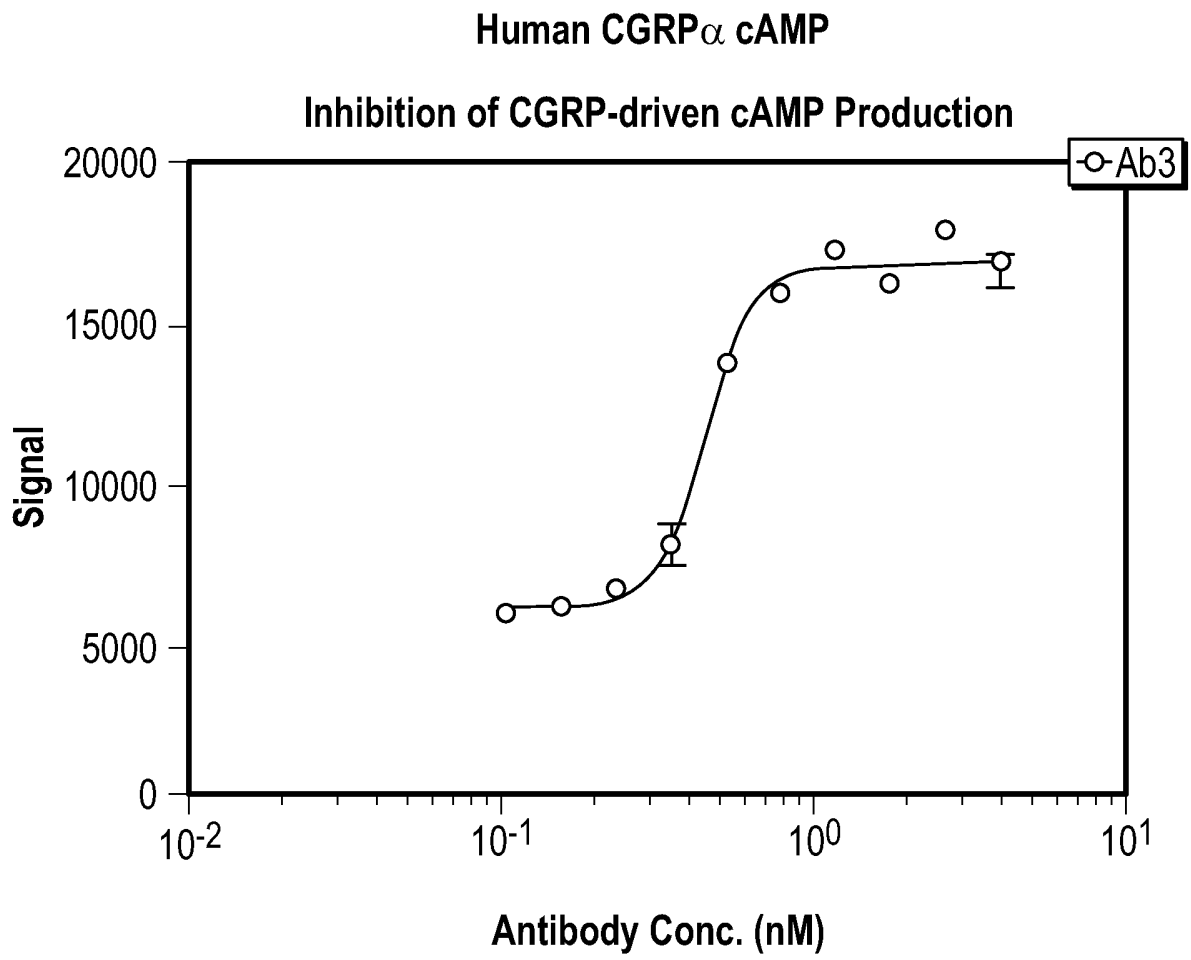
	EC50 (pM)
Ab11	184
Ab12	171
Ab13	188

FIG. 18



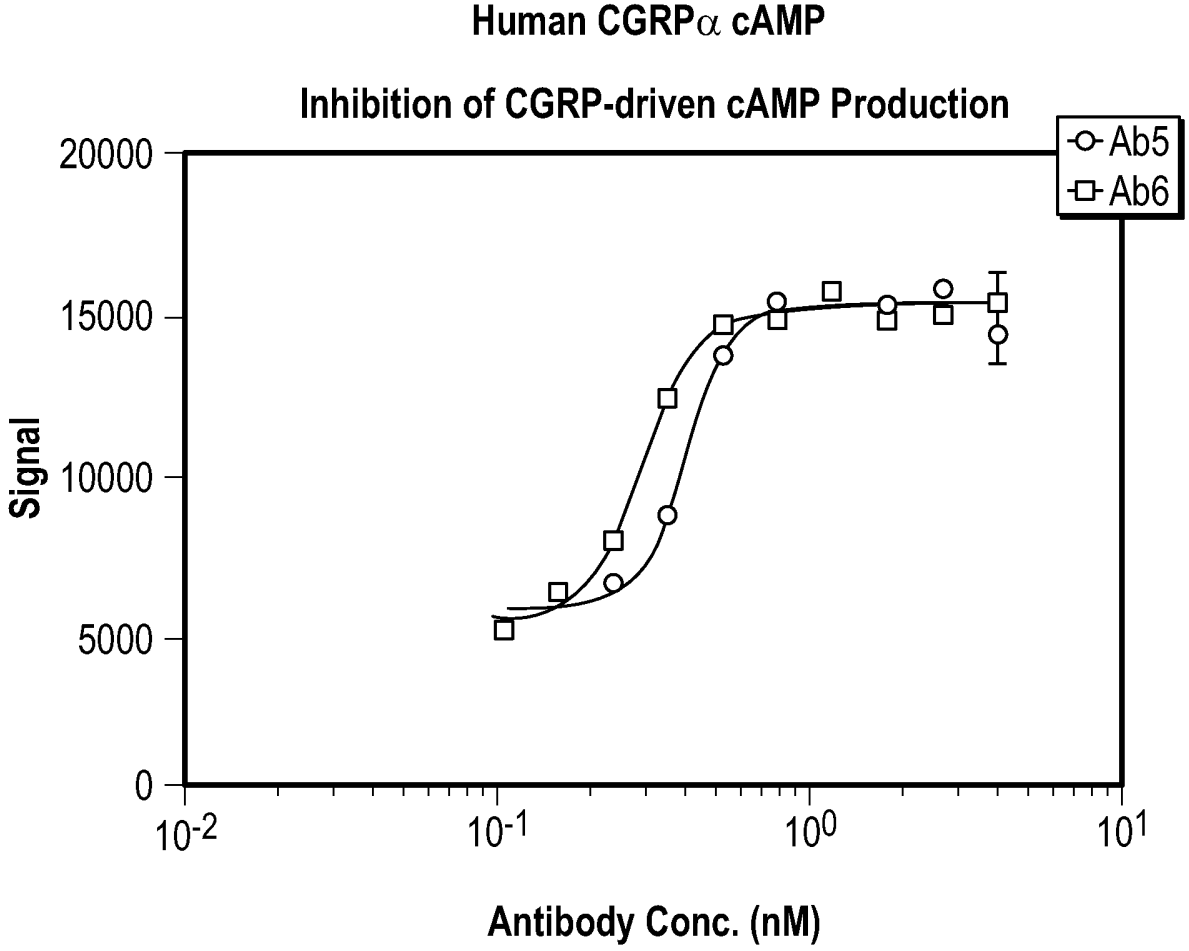
	IC50 (pM)
Ab1	531
Ab2	452
Ab4	429

FIG. 19



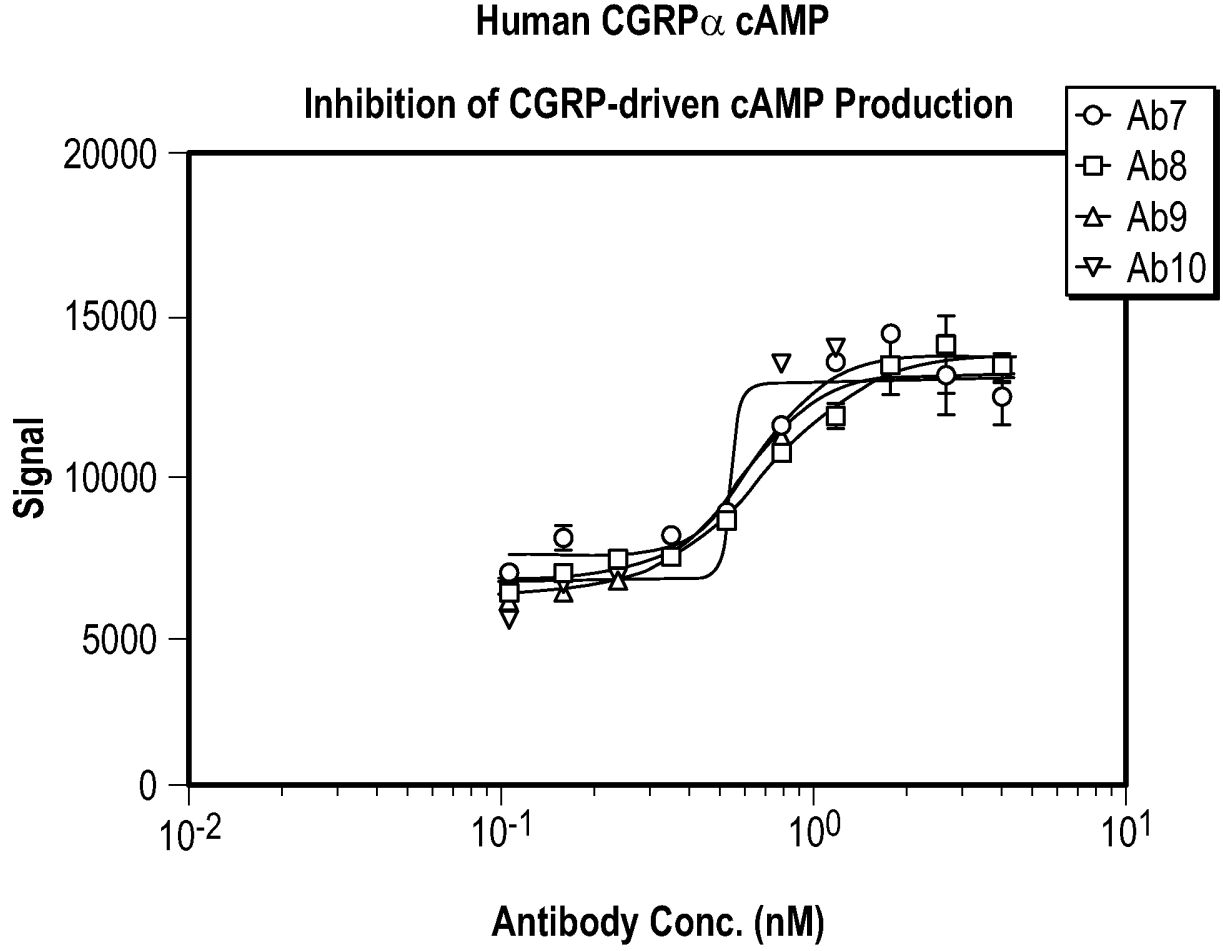
	IC50 (pM)
Ab3	452

FIG. 20



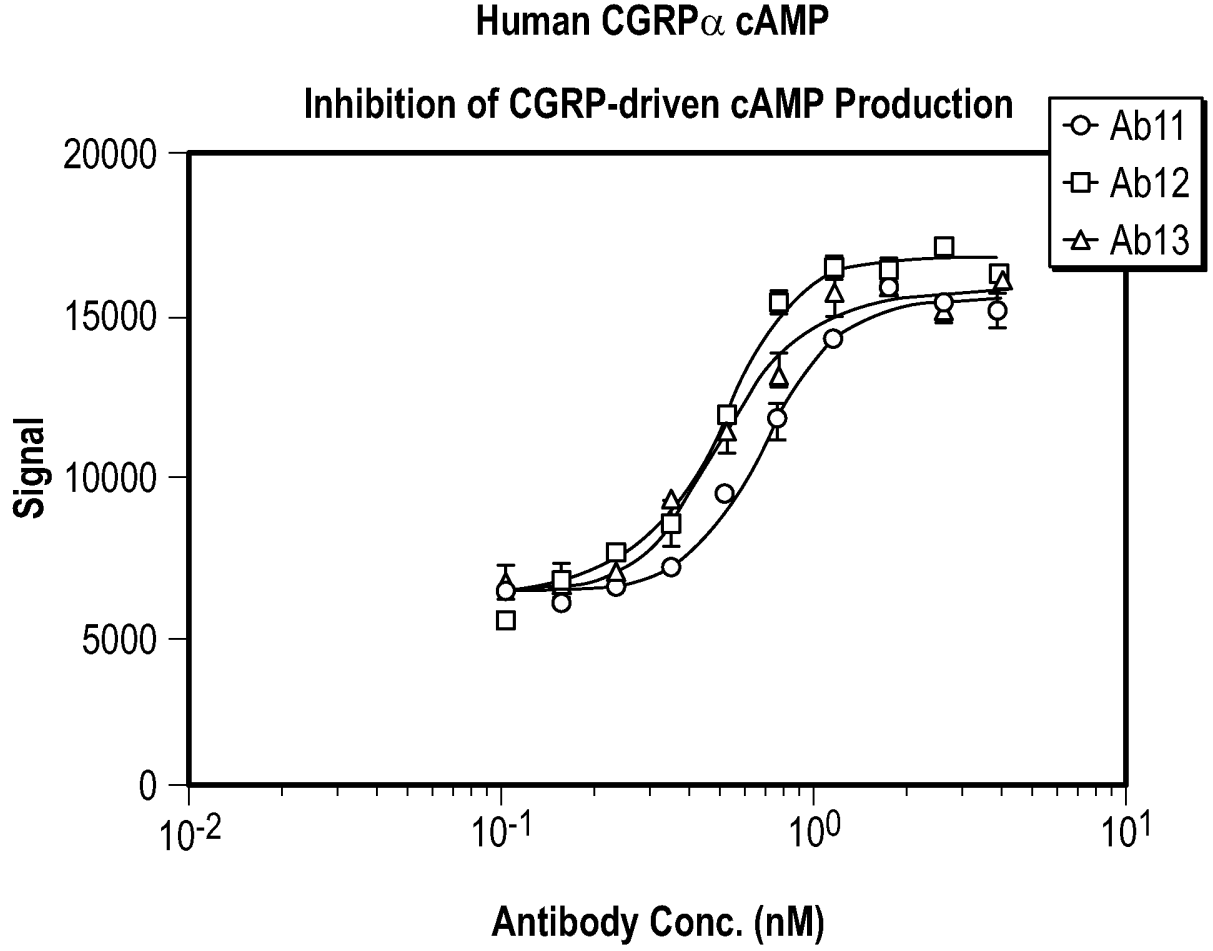
	IC50 (pM)
Ab5	400
Ab6	288

FIG. 21



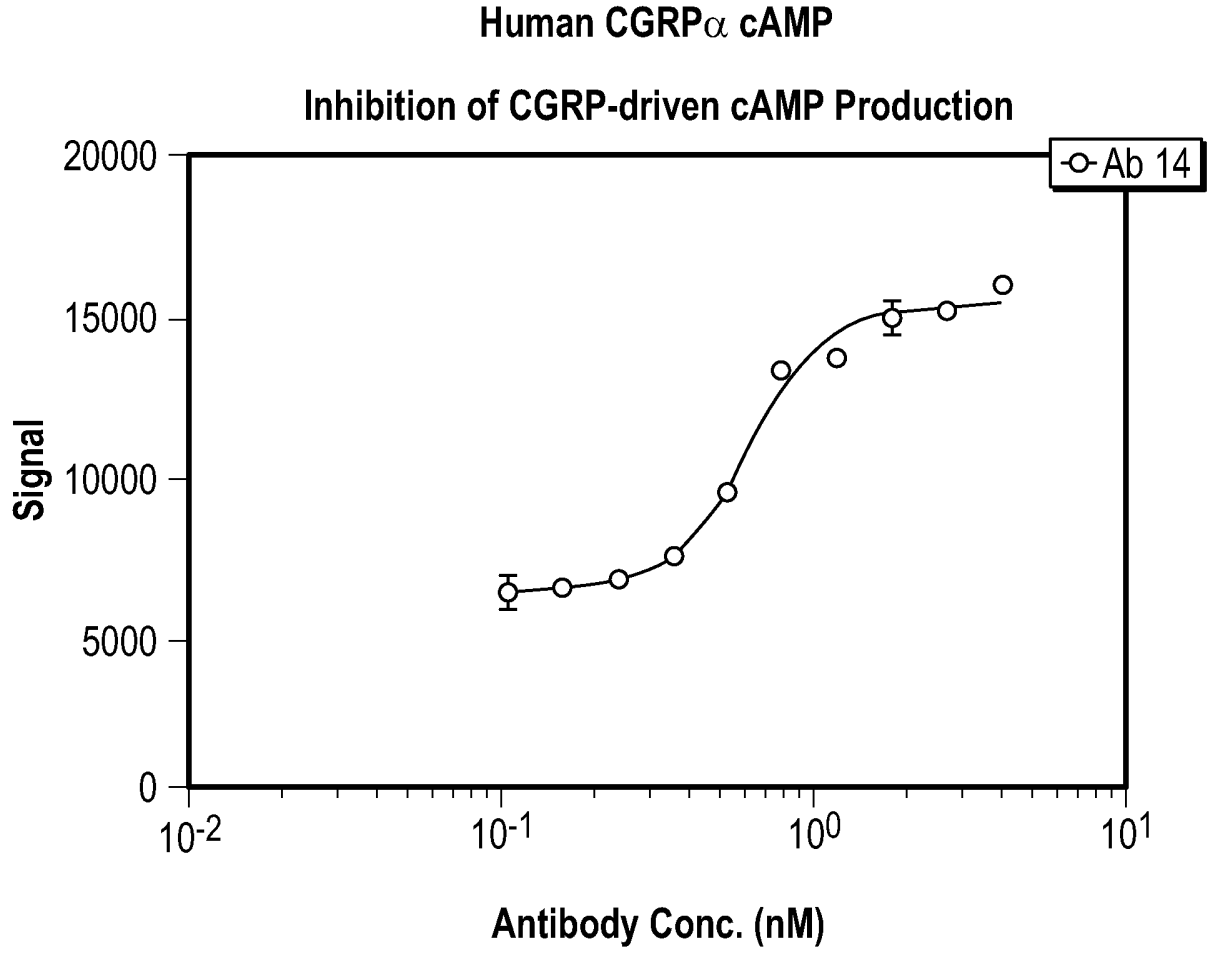
	IC50 (pM)
Ab7	743
Ab8	734
Ab9	568
Ab10	542

FIG. 22



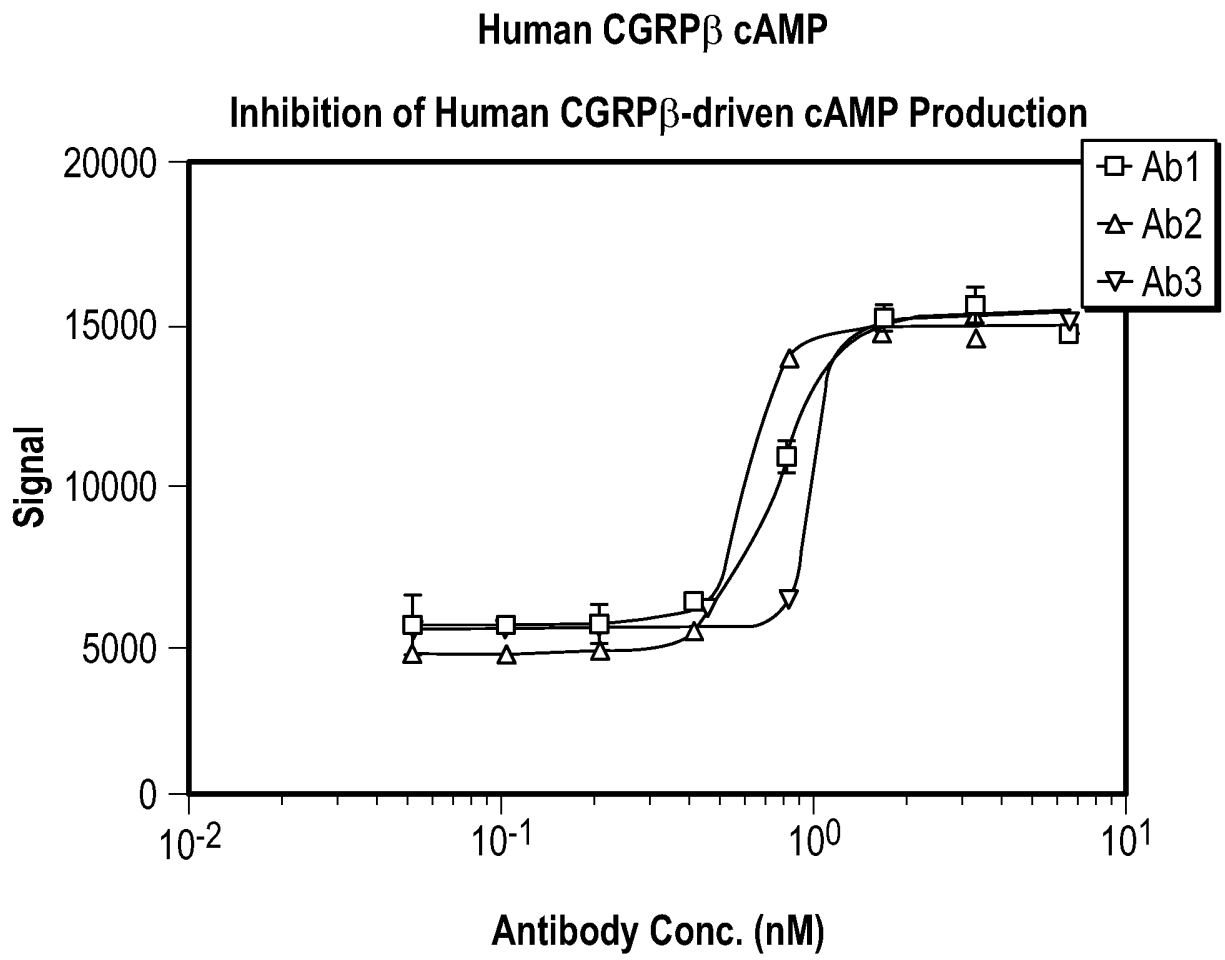
	IC50 (pM)
Ab11	698
Ab12	511
Ab13	498

FIG. 23



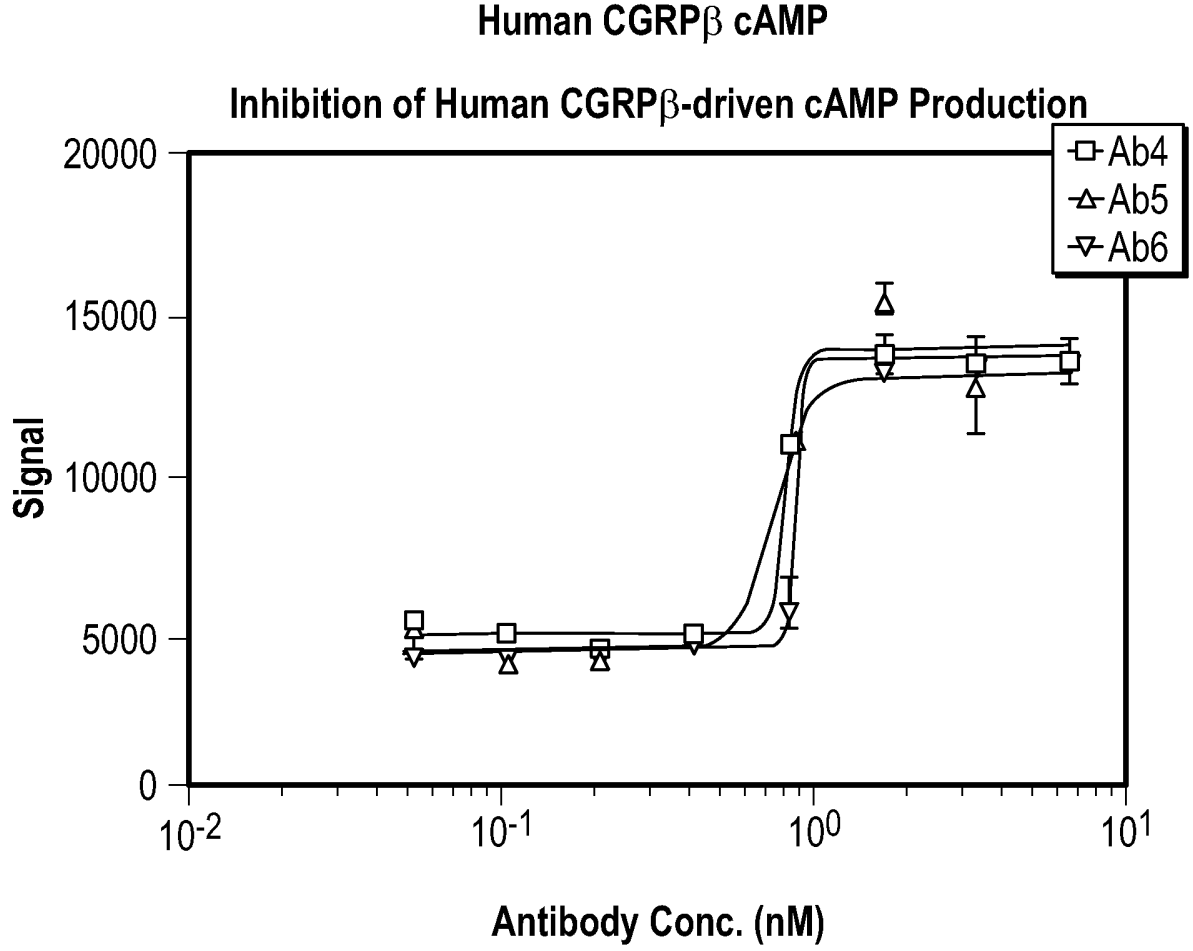
	IC50 (pM)
Ab14	631

FIG. 24



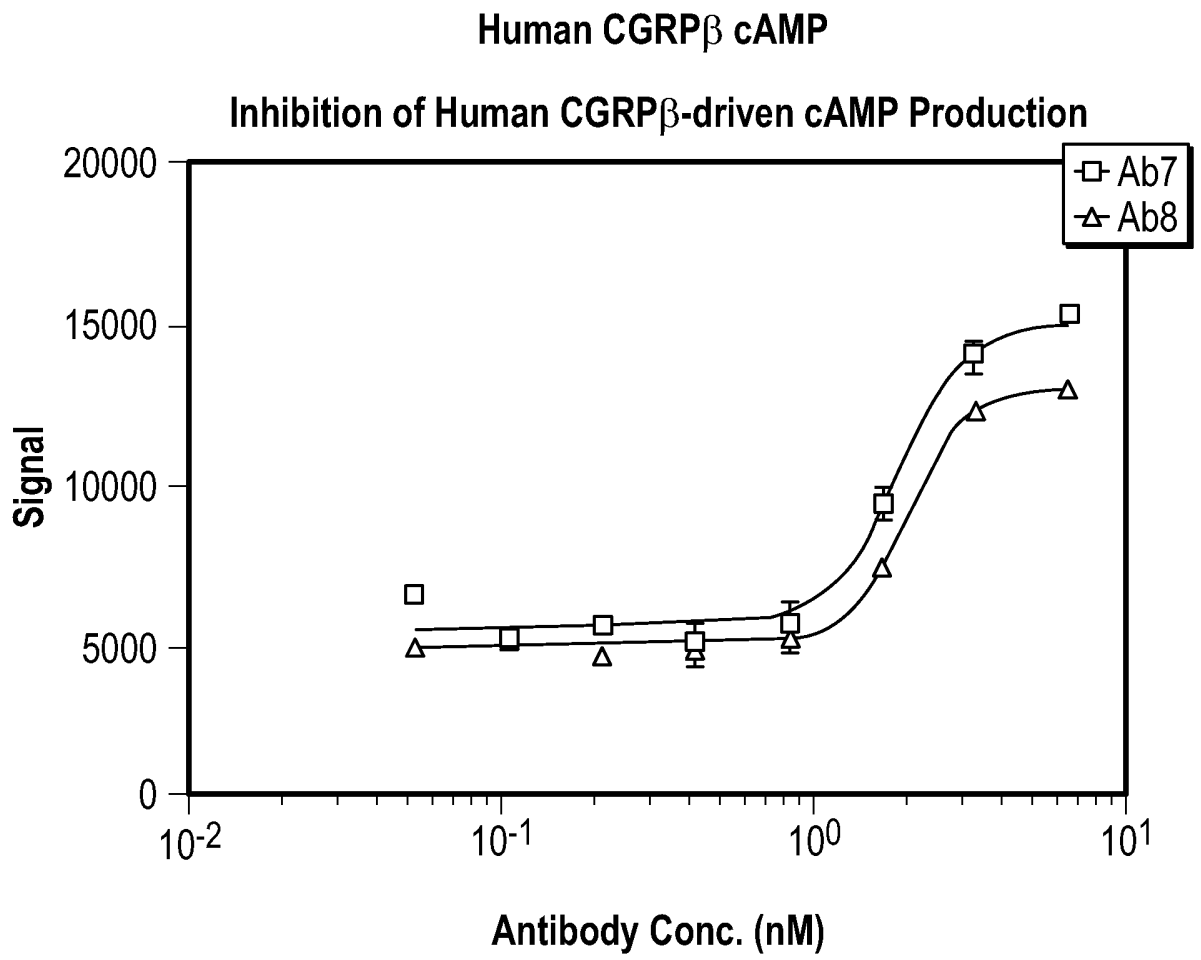
	IC50 (pM)
Ab1	801
Ab2	601
Ab3	989

FIG. 25



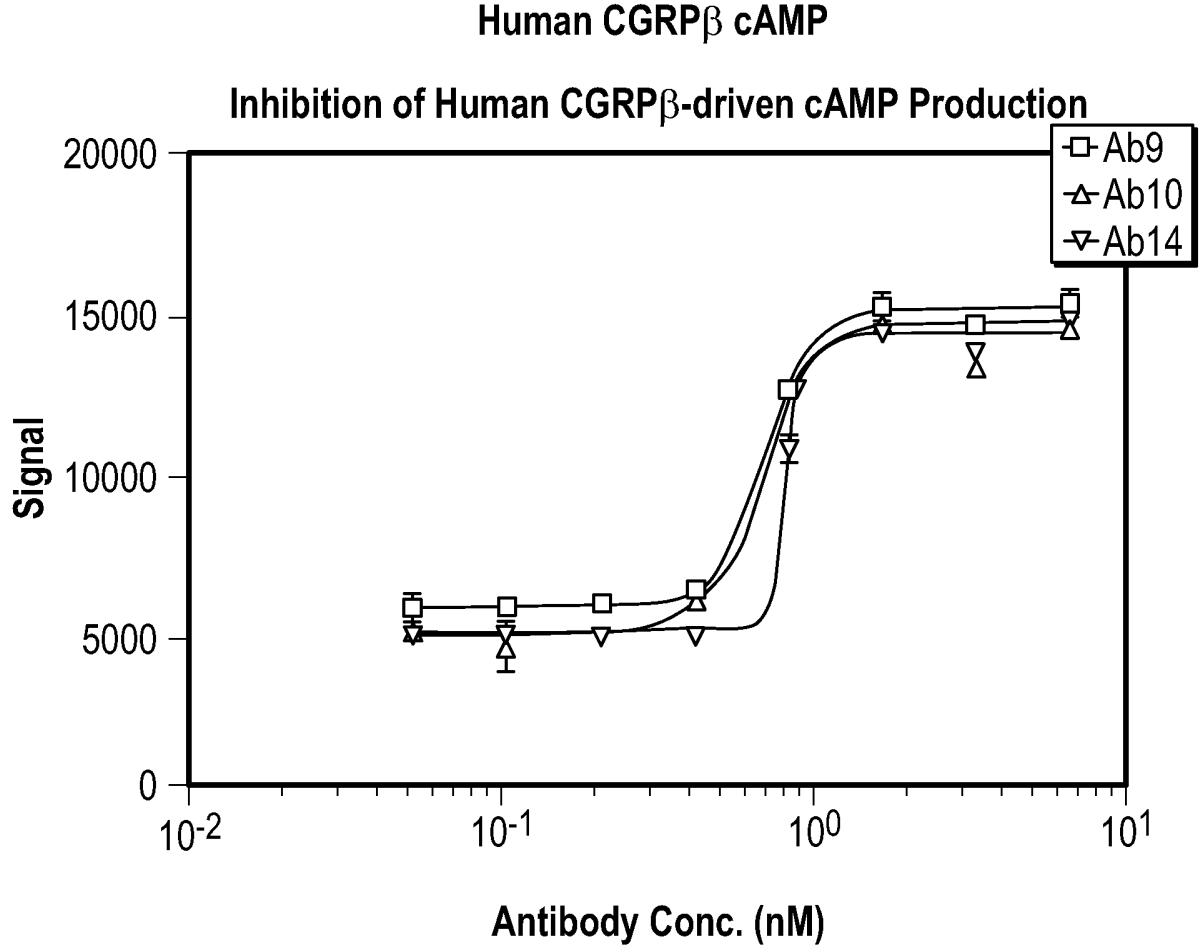
	IC50 (pM)
Ab4	805
Ab5	875
Ab6	740

FIG. 26



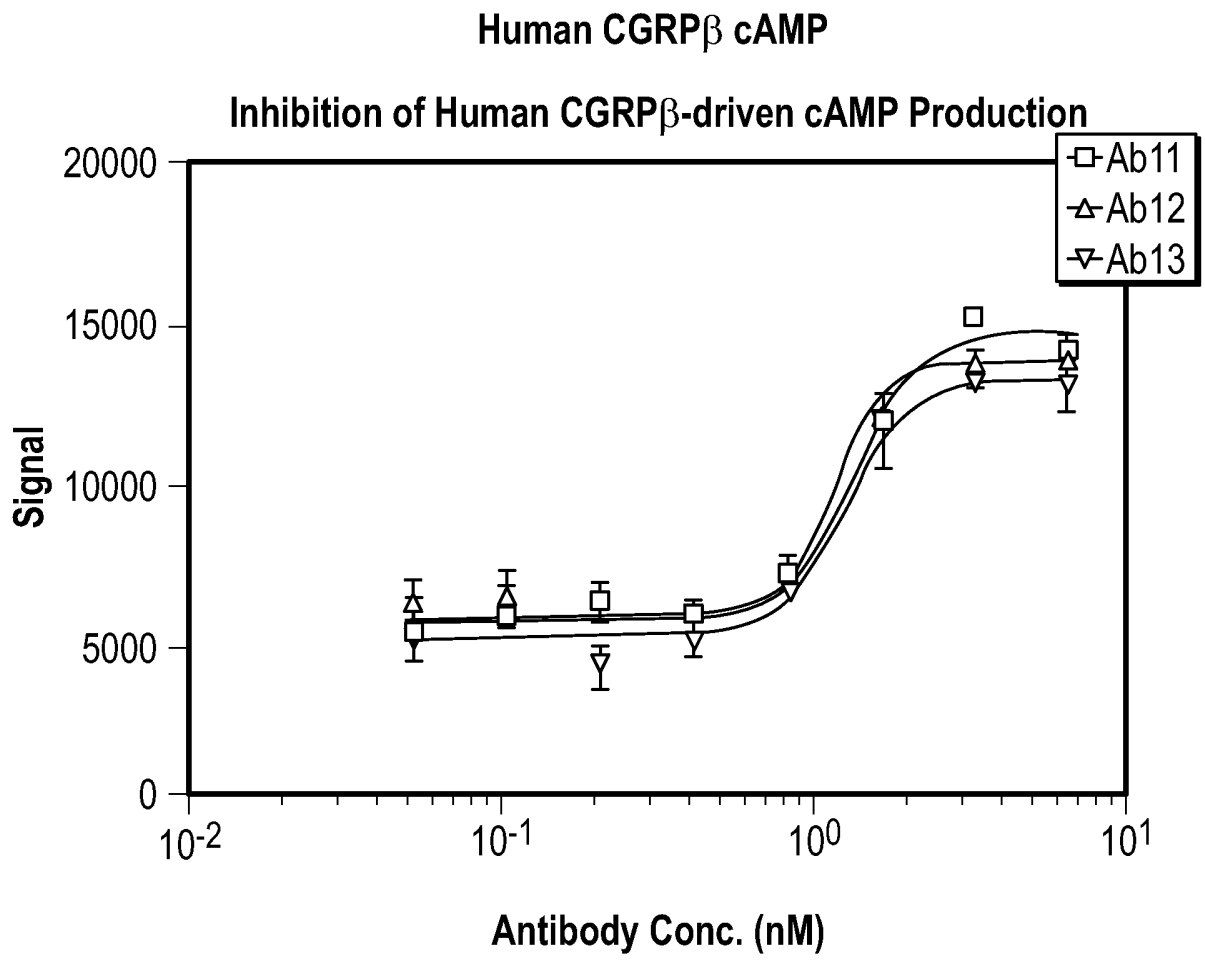
	IC50 (pM)
Ab7	1858
Ab8	1981

FIG. 27



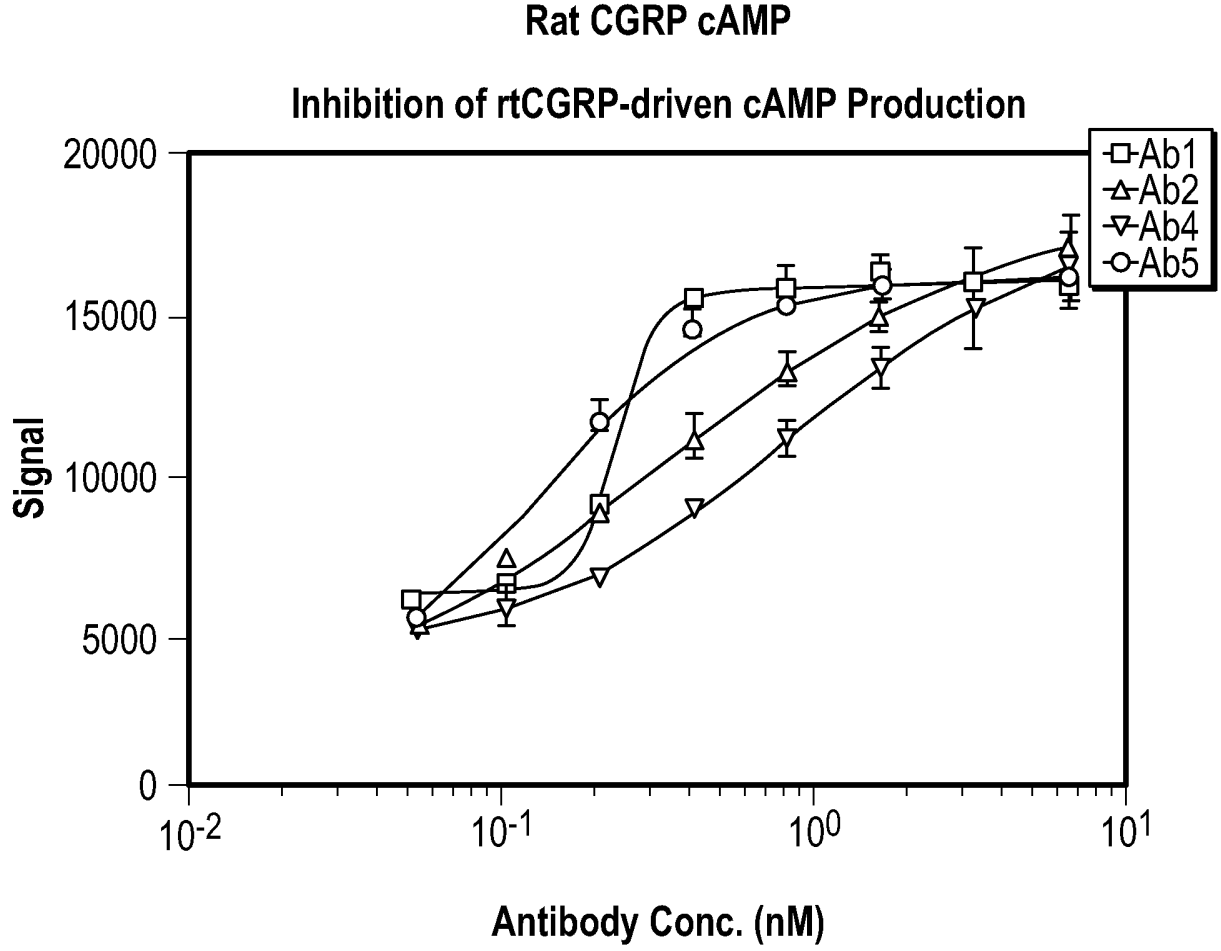
	IC50 (pM)
Ab9	716
Ab10	641
Ab14	812

FIG. 28



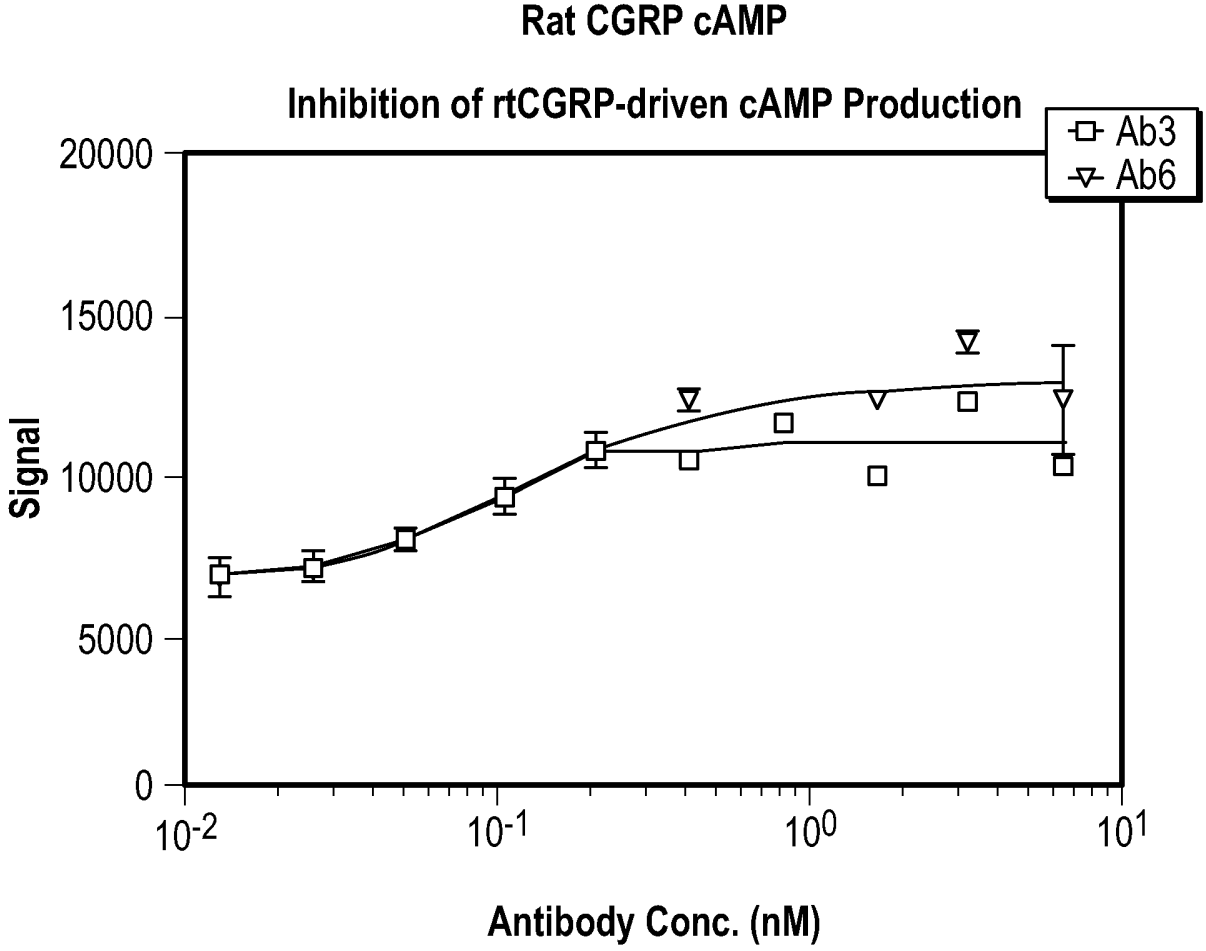
	IC50 (pM)
Ab11	1344
Ab12	1181
Ab13	1276

FIG. 29



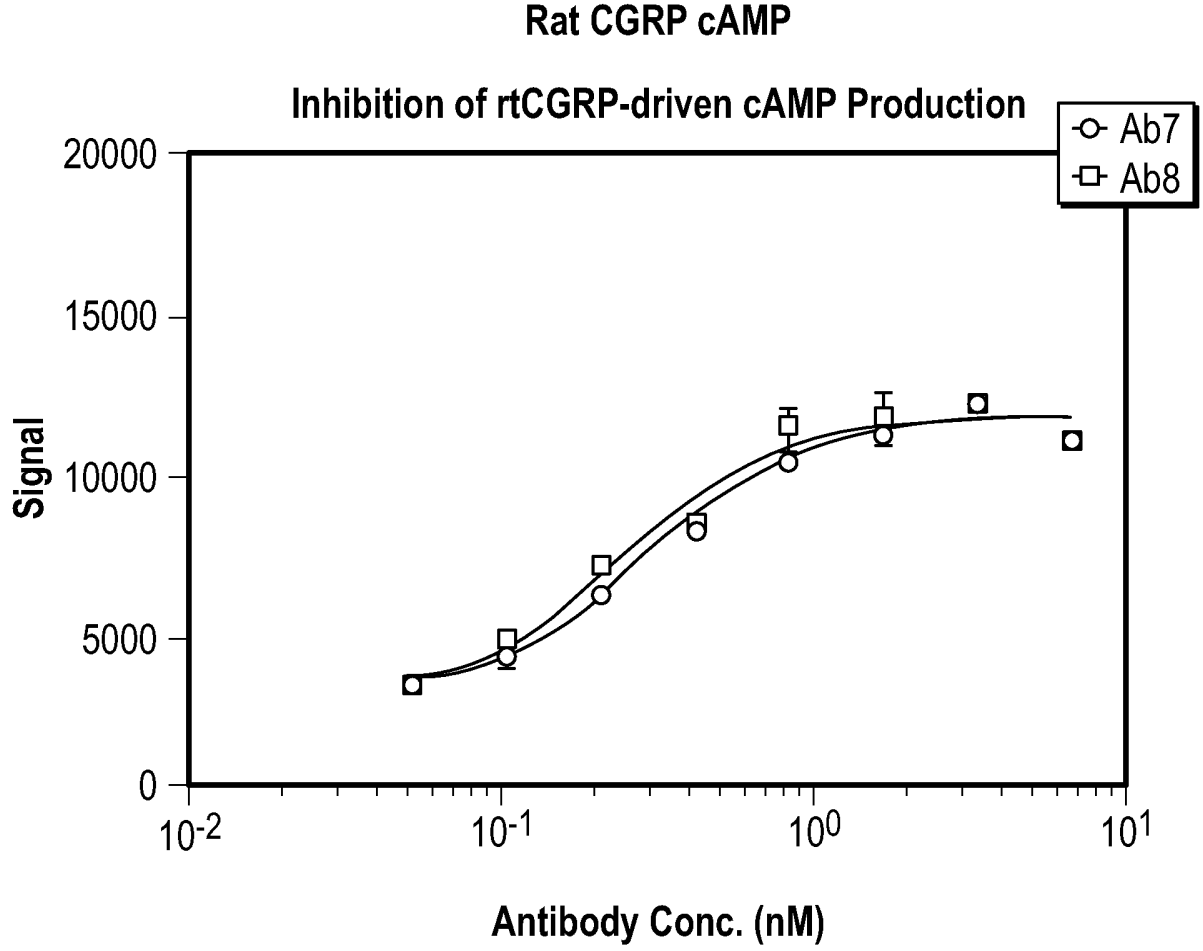
	IC50 (pM)
Ab1	239
Ab2	142
Ab4	868
Ab5	334

FIG. 30



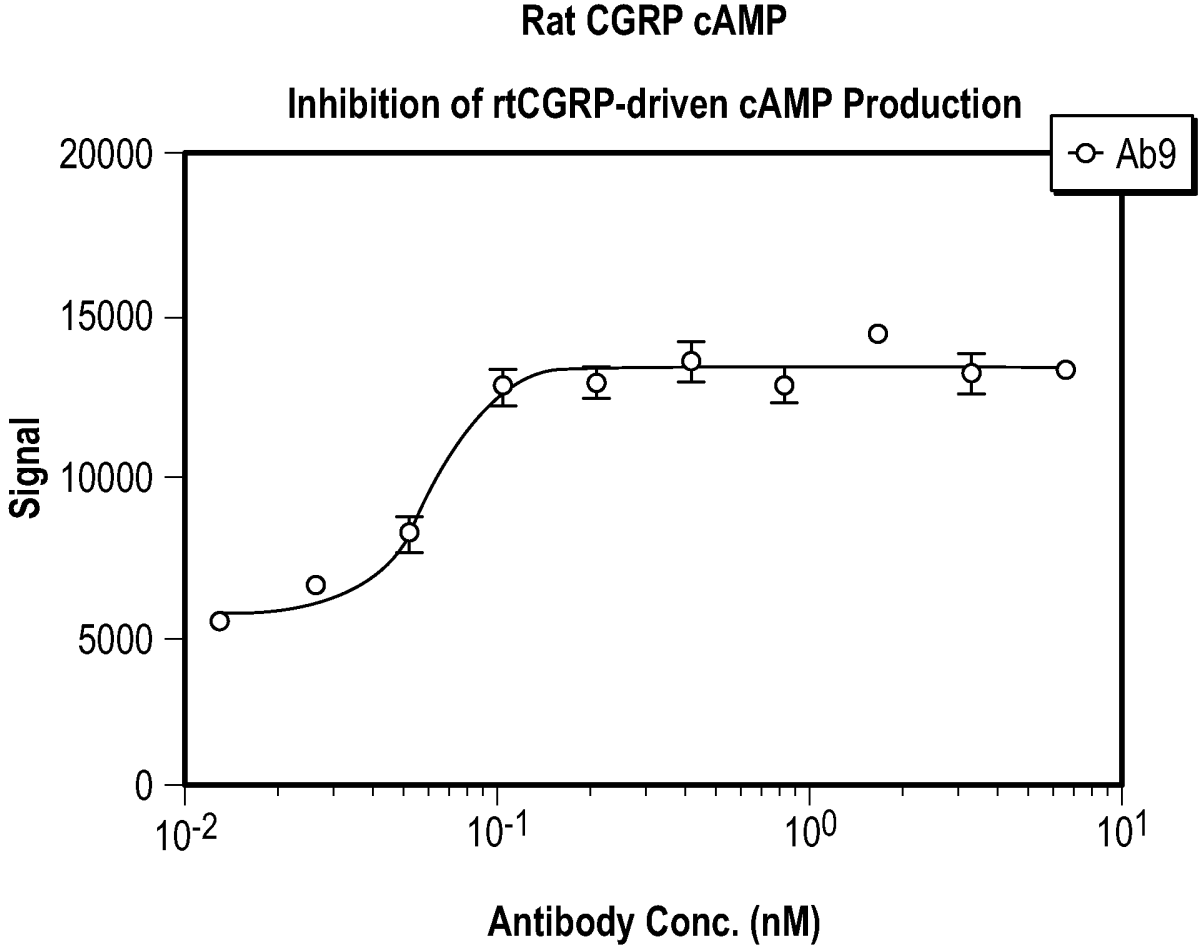
	IC50 (pM)
Ab3	85
Ab6	111

FIG. 31



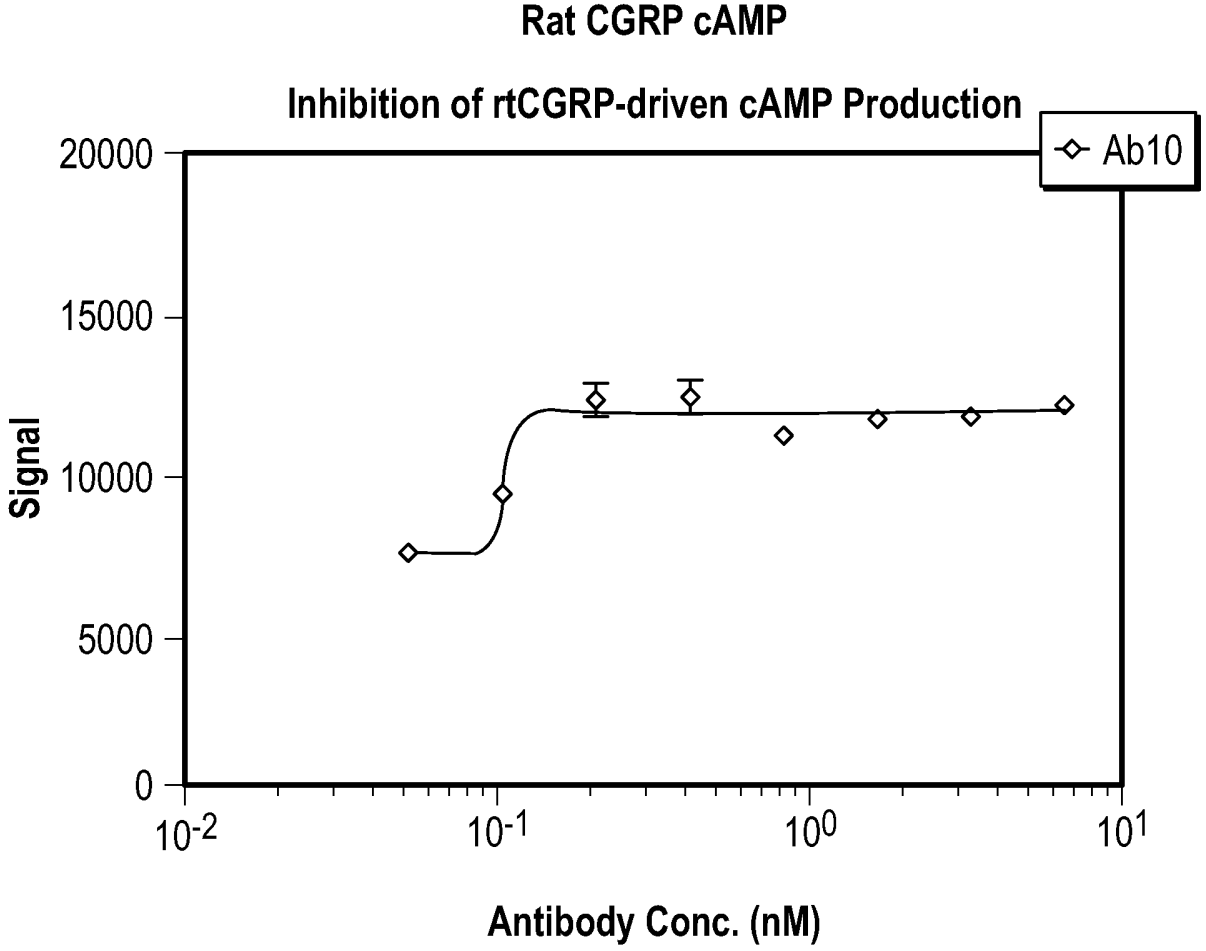
	IC50 (pM)
Ab7	297
Ab8	243

FIG. 32



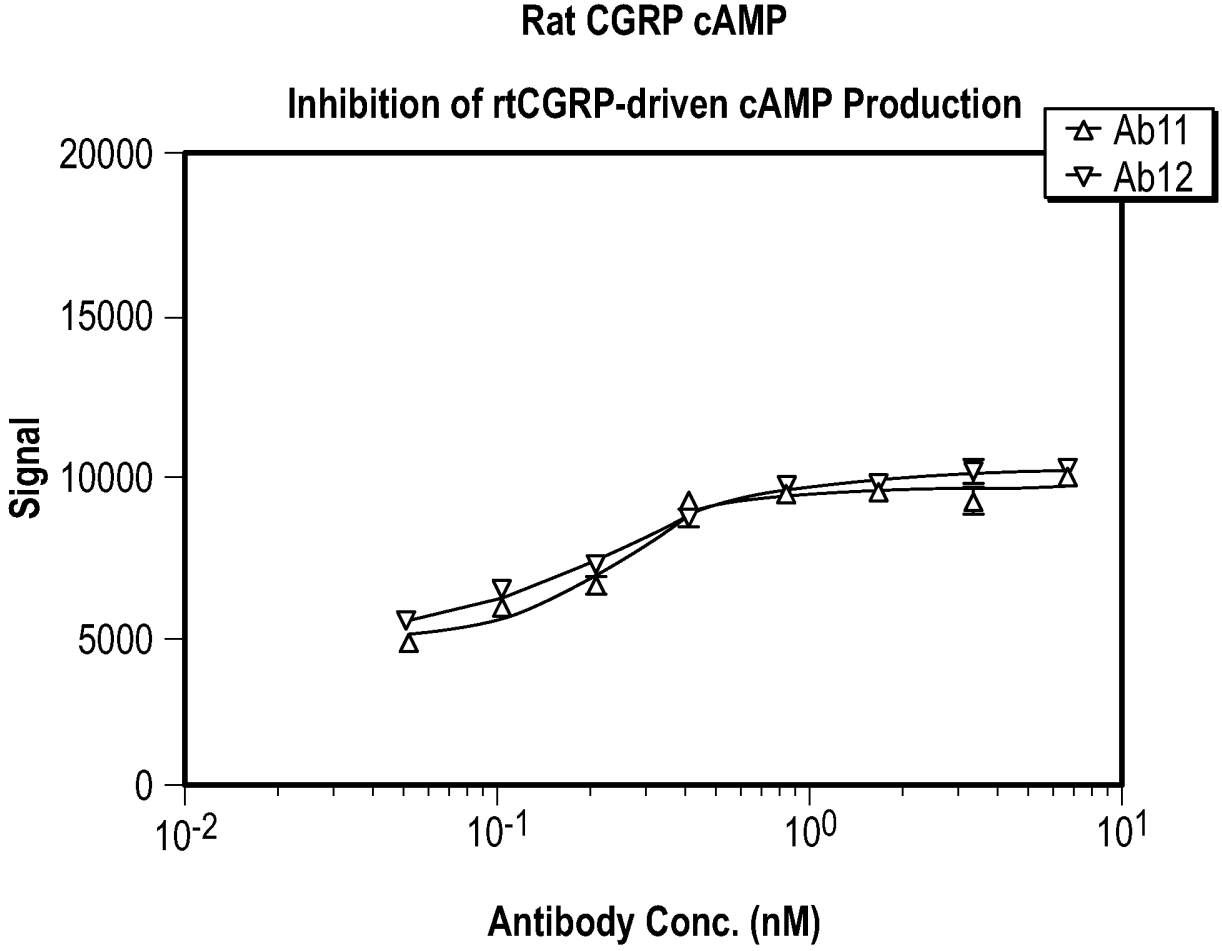
	IC50 (pM)
Ab9	62

FIG. 33



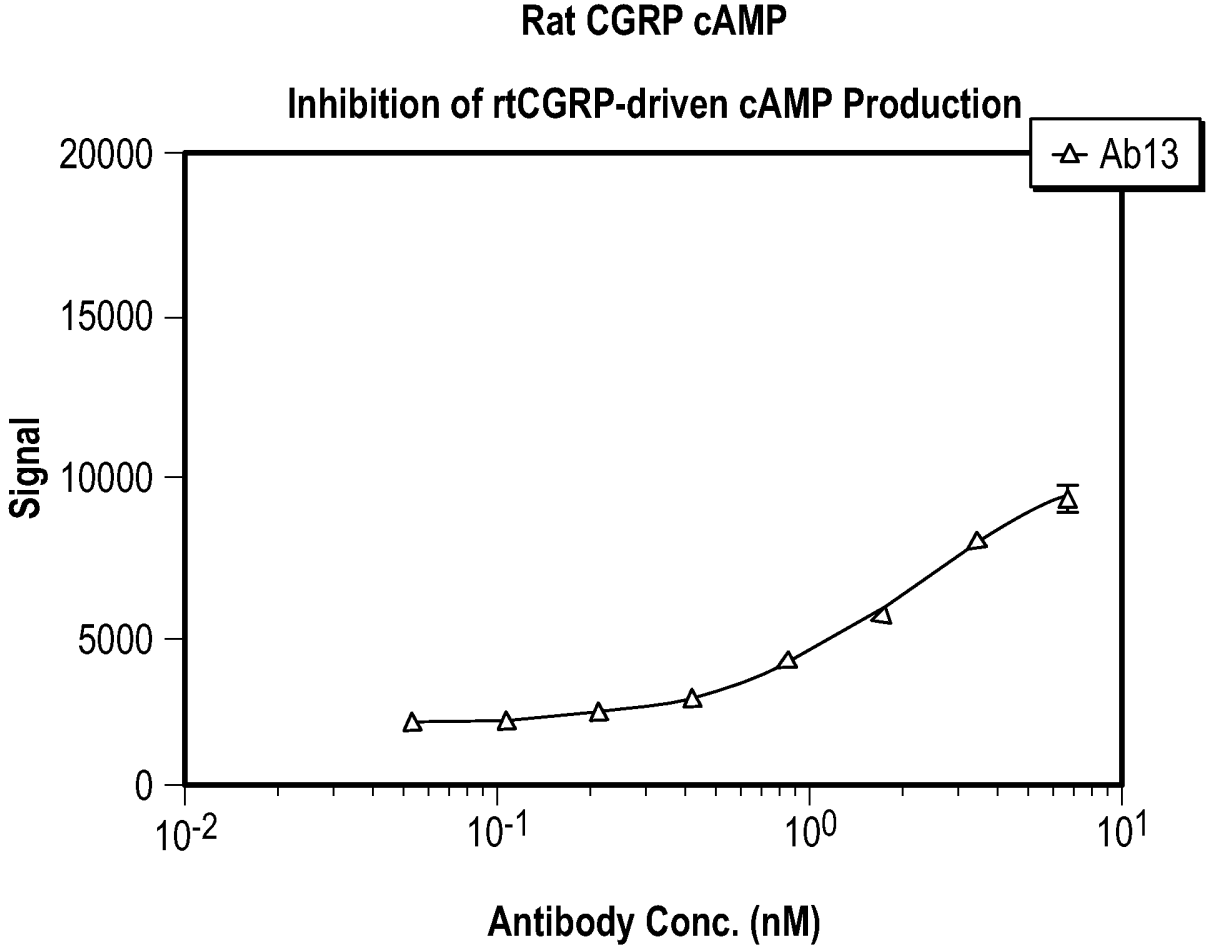
	IC50 (pM)
Ab10	105

FIG. 34



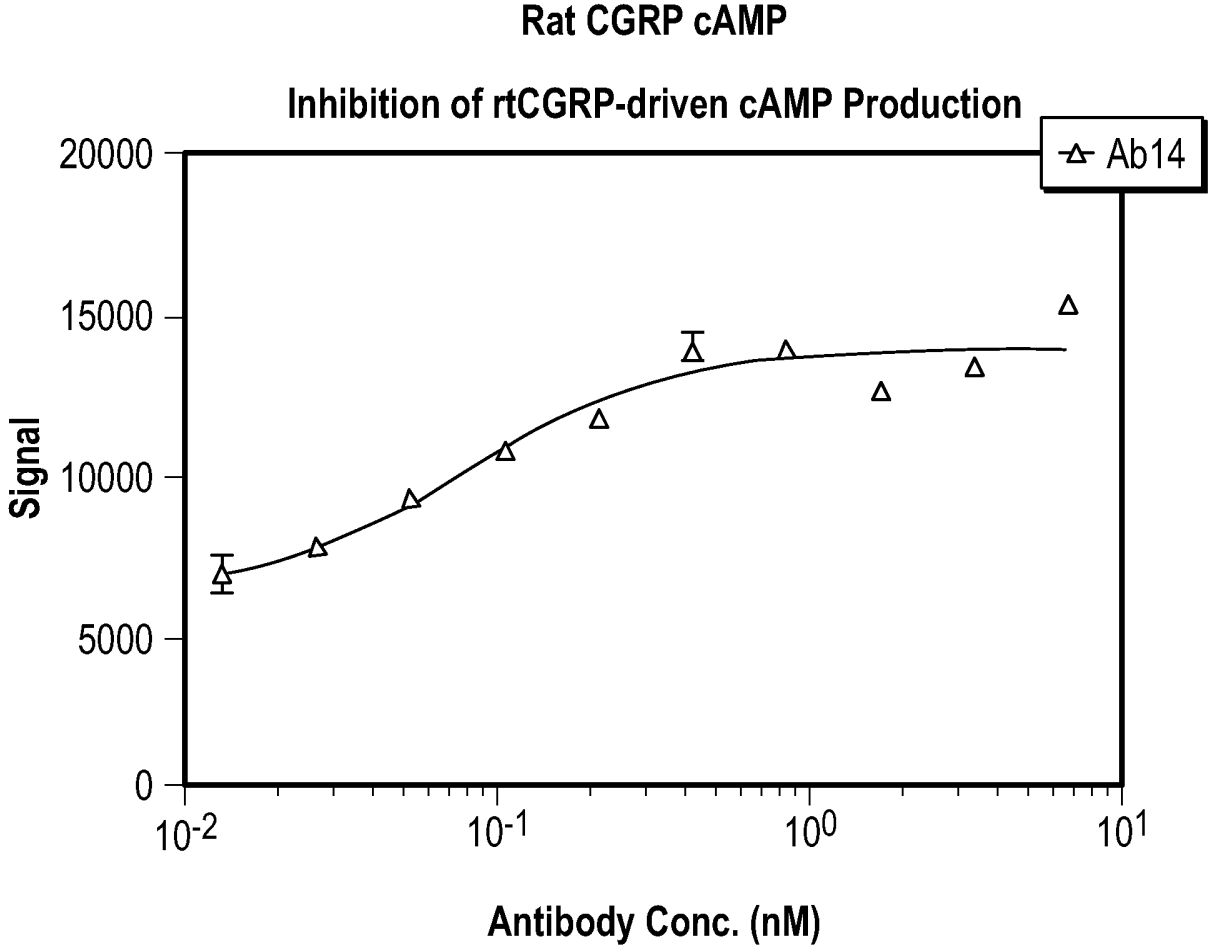
	IC50 (pM)
Ab11	239
Ab12	236

FIG. 35



	IC50 (pM)
Ab13	2036

FIG. 36



	IC50 (pM)
Ab14	81

FIG. 37

Inhibition of Radioligand Binding

	IC_{50}(nM)	K_I(nM)
Ab1	0.585	0.46
Ab2	0.482	0.378
Ab3	2.49	10.96
Ab4	0.579	0.455
Ab5	0.586	0.461
Ab6	2.46	1.94
Ab7	4.53	3.56
Ab8	0.936	0.736
Ab9	2.03	1.6
Ab10	0.28	0.22
Ab11	2.26	1.78
Ab12	0.315	0.248
Ab13	0.335	0.264

FIG. 38

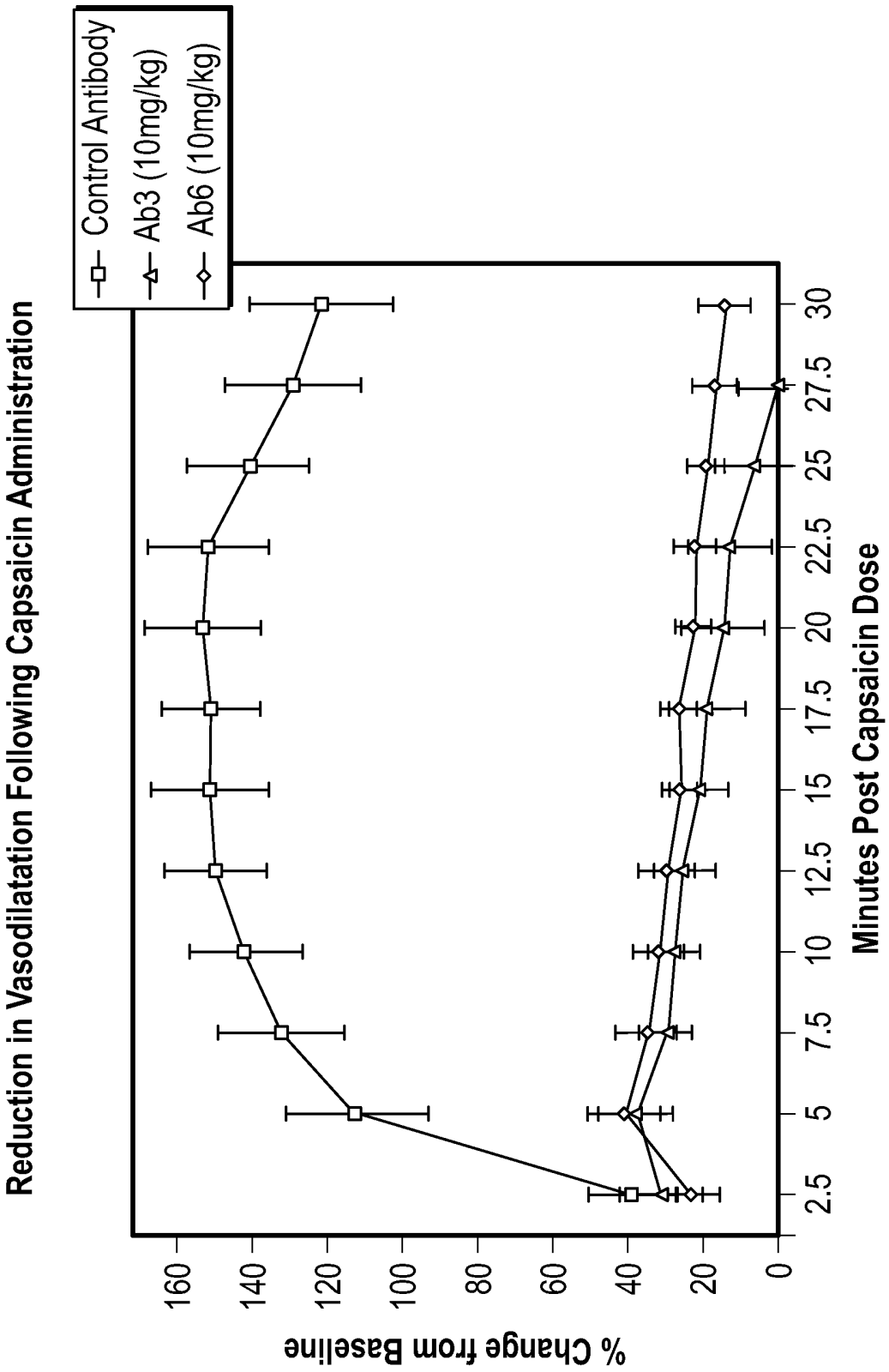

Reduction in Vasodilatation Following Capsaicin Administration
Control Antibody
Ab3 (10mg/kg)
Ab6 (10mg/kg)
% Change from Baseline
0
20
40
60
80
100
120
140
160
2.5
5
7.5
10
12.5
15
17.5
20
22.5
25
27.5
30
Minutes Post Capsaicin Dose

FIG. 39

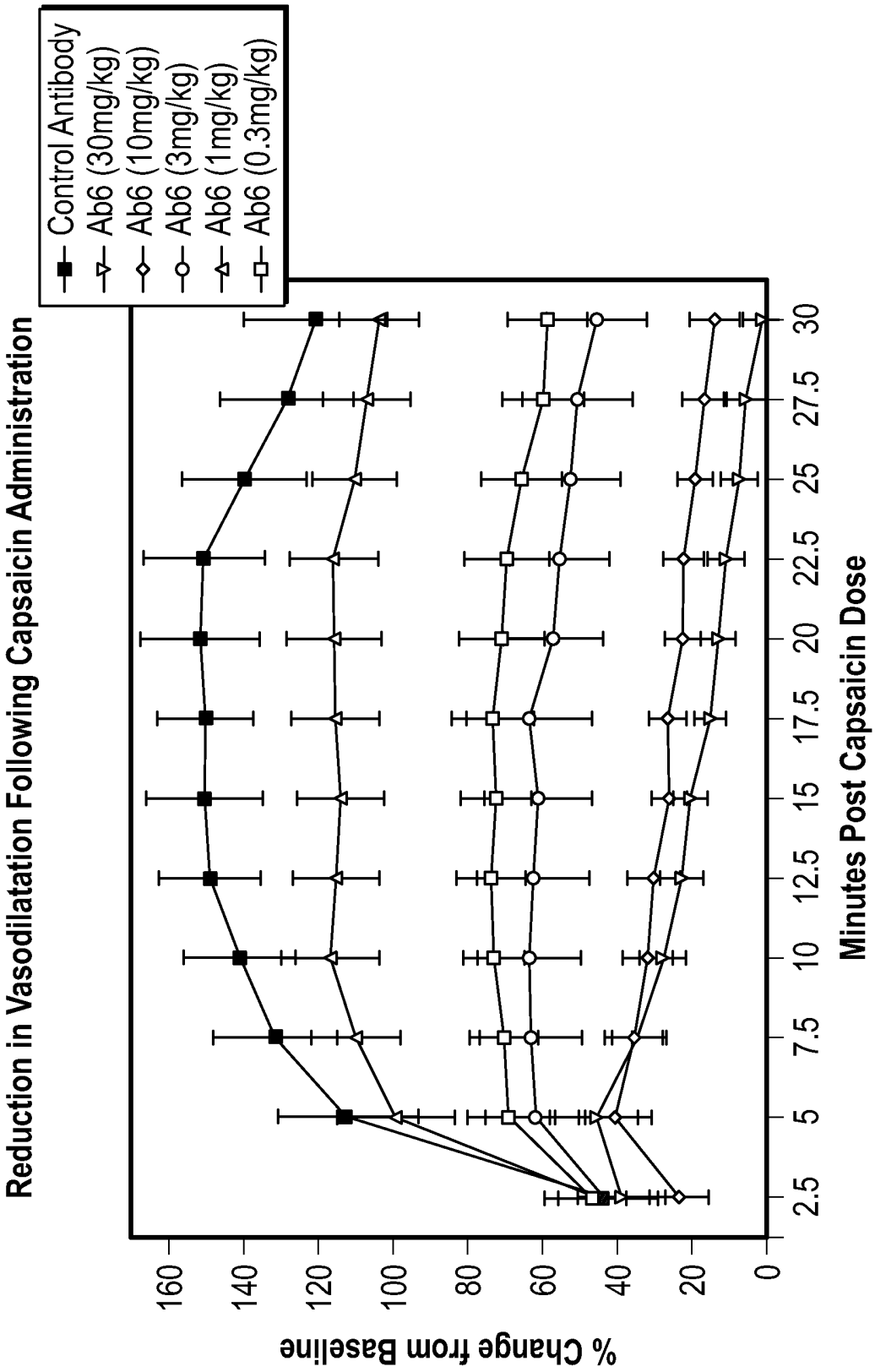

Reduction in Vasodilatation Following Capsaicin Administration
Control Antibody
Ab6 (30mg/kg)
Ab6 (10mg/kg)
Ab6 (3mg/kg)
Ab6 (1mg/kg)
Ab6 (0.3mg/kg)
% Change from Baseline
0
20
40
60
80
100
120
140
160
2.5
5
7.5
10
12.5
15
17.5
20
22.5
25
27.5
30
Minutes Post Capsaicin Dose

FIG. 40

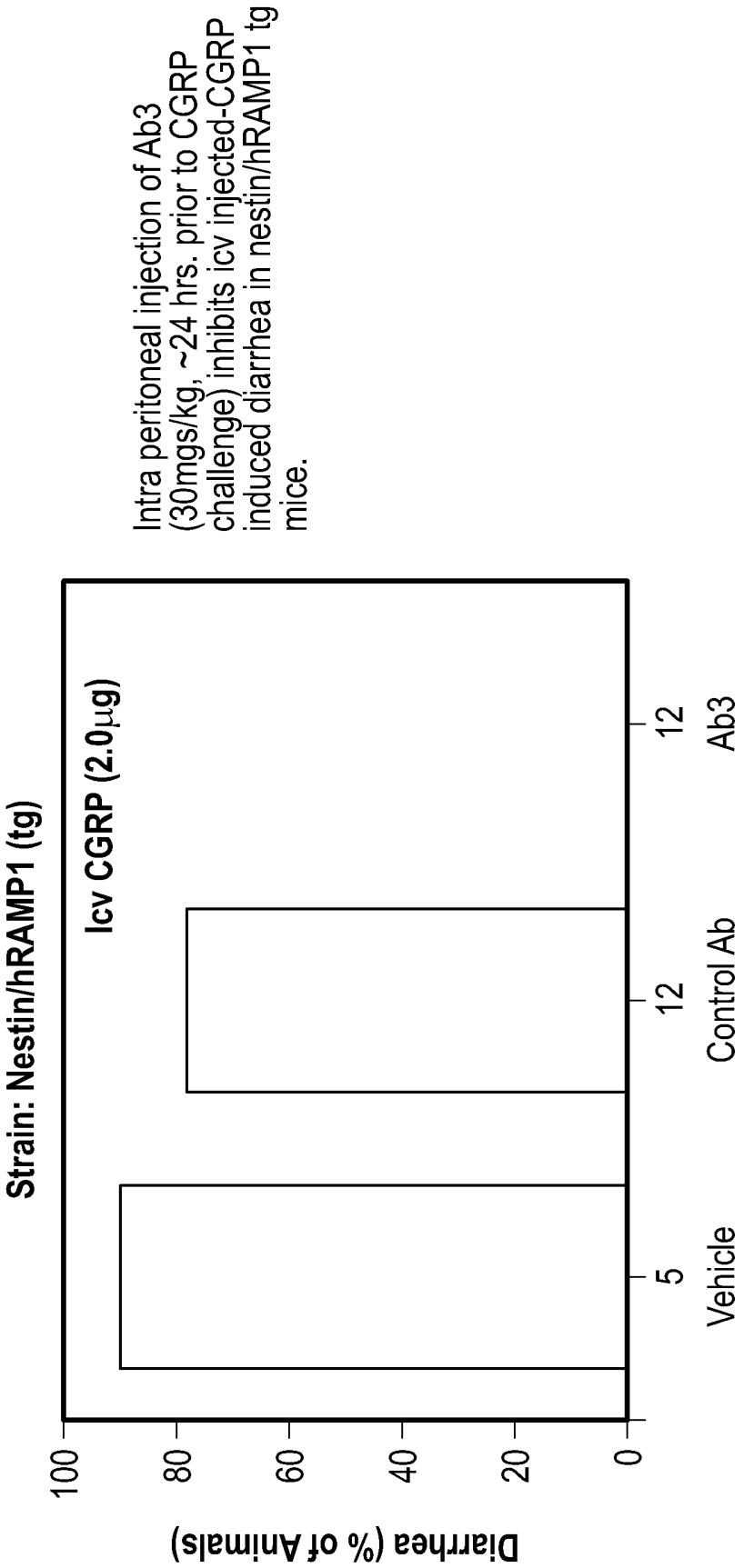

Intra peritoneal injection of Ab3 (30mgs/kg, ~24 hrs. prior to CGRP challenge) inhibits icv injected-CGRP induced diarrhea in nestin/hRAMP1 tg mice.
Strain: Nestin/hRAMP1 (tg)
Icv CGRP (2.0µg)
Diarrhea (% of Animals)
100
80
60
40
20
0
5
12
12
Vehicle
Control Ab
Ab3

FIG. 41

Intra cerebroventricular injection of CGRP induces diarrhea in a dose dependent manner in C57BL/6J mice.

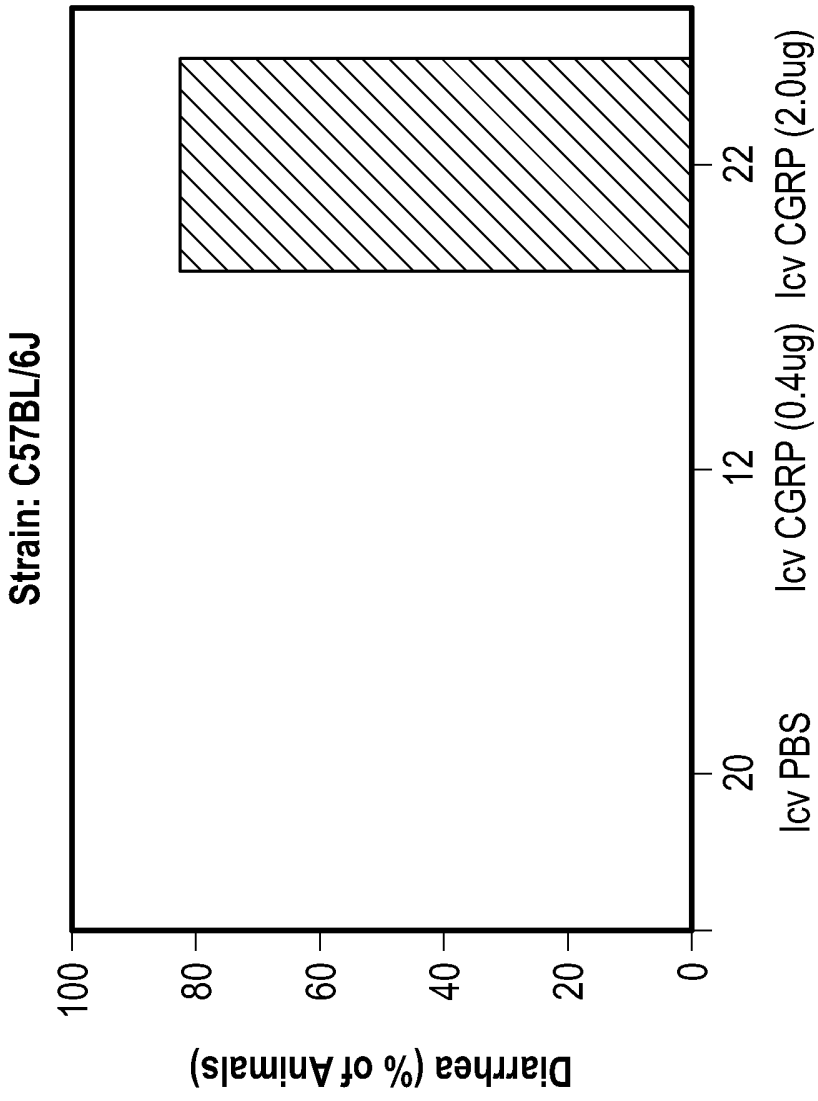


FIG. 42

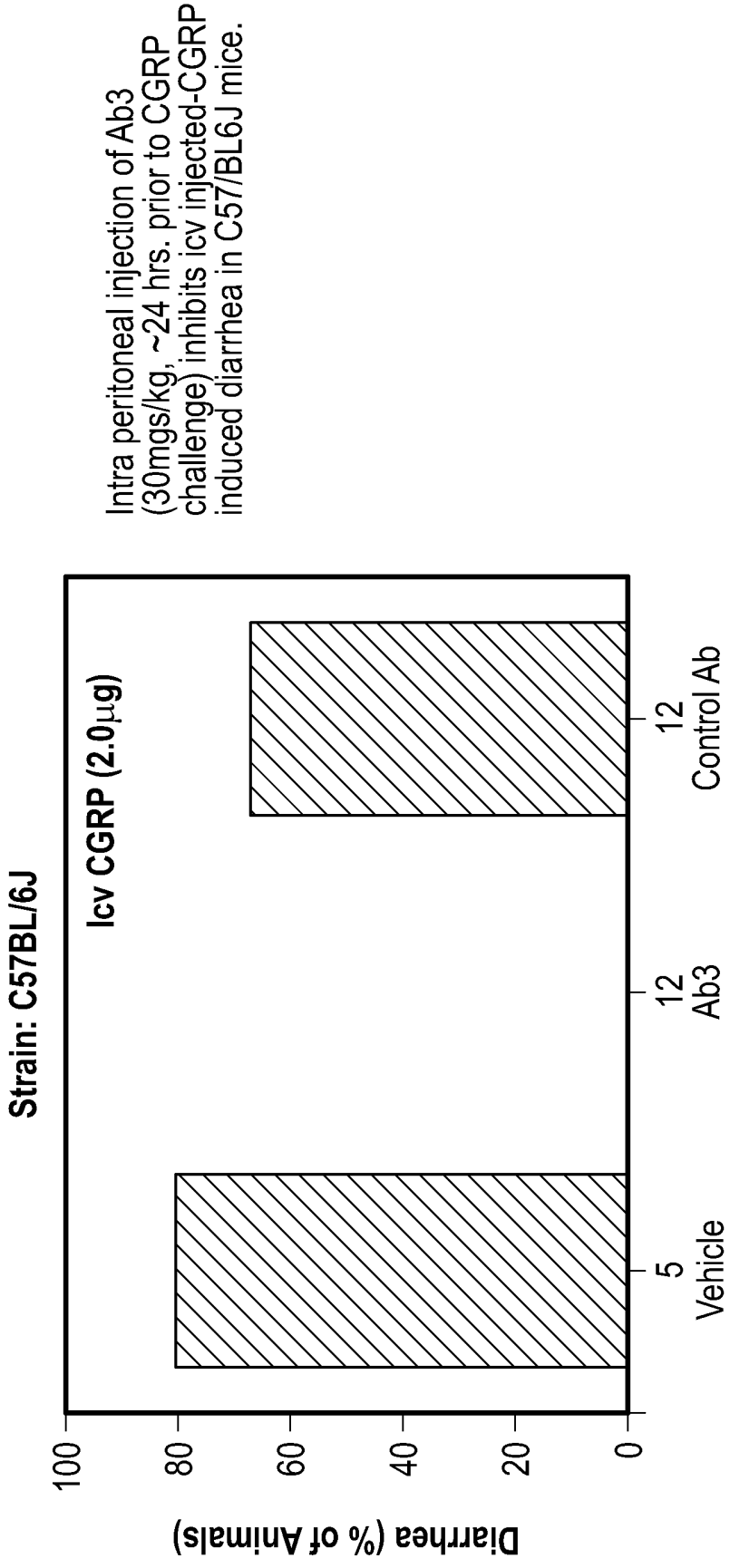

Intra peritoneal injection of Ab3 (30mgs/kg, ~24 hrs. prior to CGRP challenge) inhibits icv injected-CGRP induced diarrhea in C57/BL6J mice.
Strain: C57BL/6J
Icv CGRP (2.0μg)
Diarrhea (% of Animals)
100
80
60
40
20
0
5
Vehicle
12
Ab3
12
Control Ab

FIG. 43

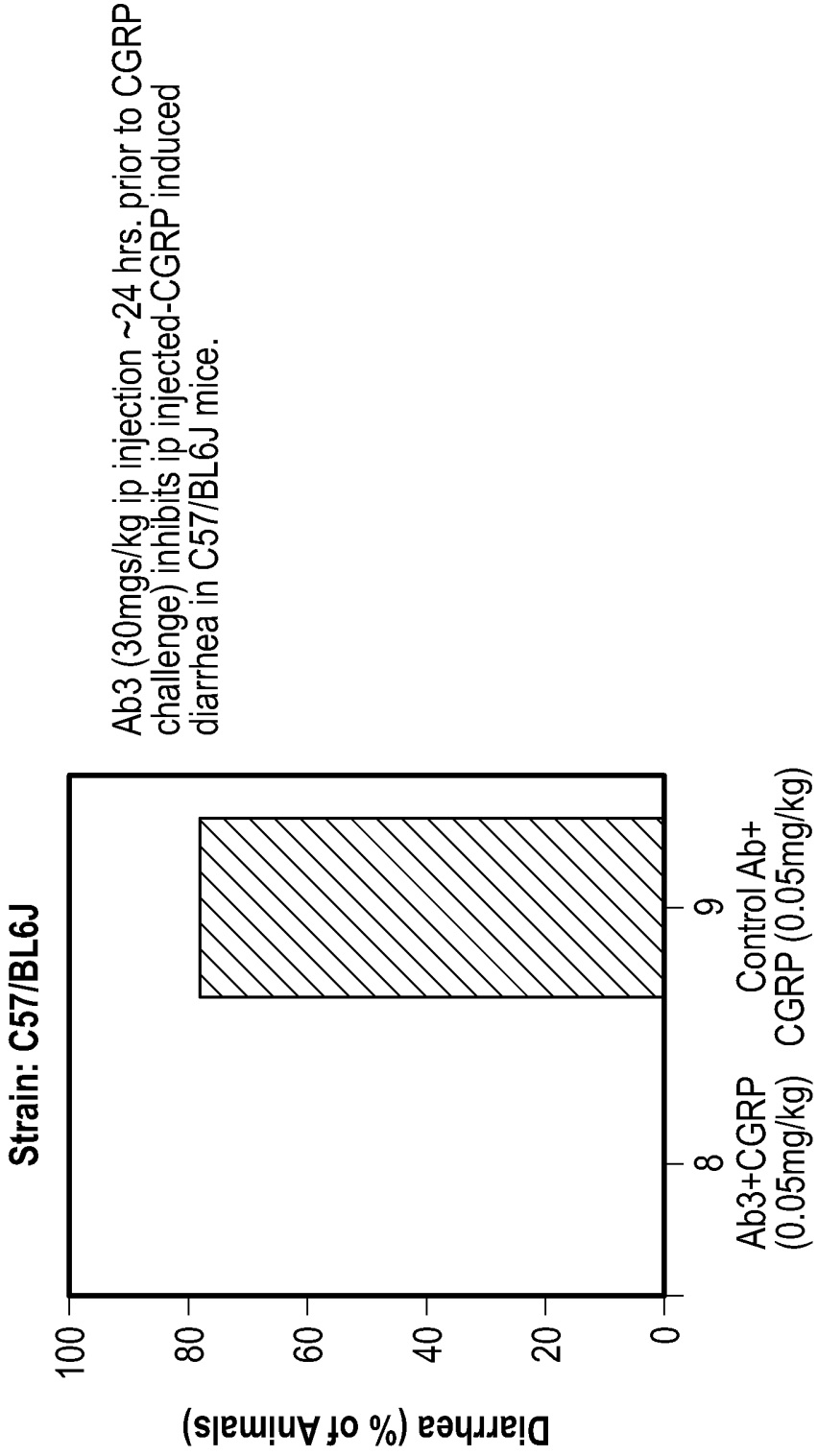

Ab3 (30mgs/kg ip injection ~24 hrs. prior to CGRP challenge) inhibits ip injected-CGRP induced diarrhea in C57/BL6J mice.
Strain: C57/BL6J
Diarrhea (% of Animals)
100
80
60
40
20
0
8
9
Ab3+CGRP (0.05mg/kg)
Control Ab+ CGRP (0.05mg/kg)

FIG. 44

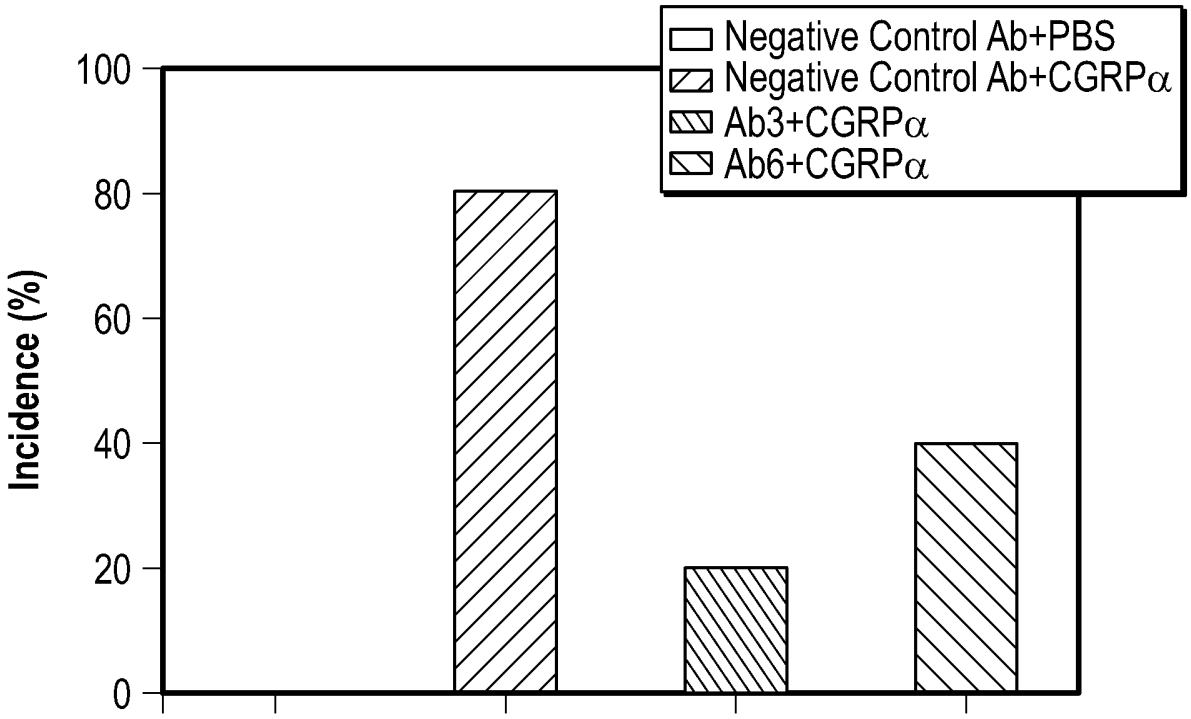

Negative Control Ab+PBS
Negative Control Ab+CGRPα
Ab3+CGRPα
Ab6+CGRPα
Incidence (%)
100
80
60
40
20
0

FIG. 45

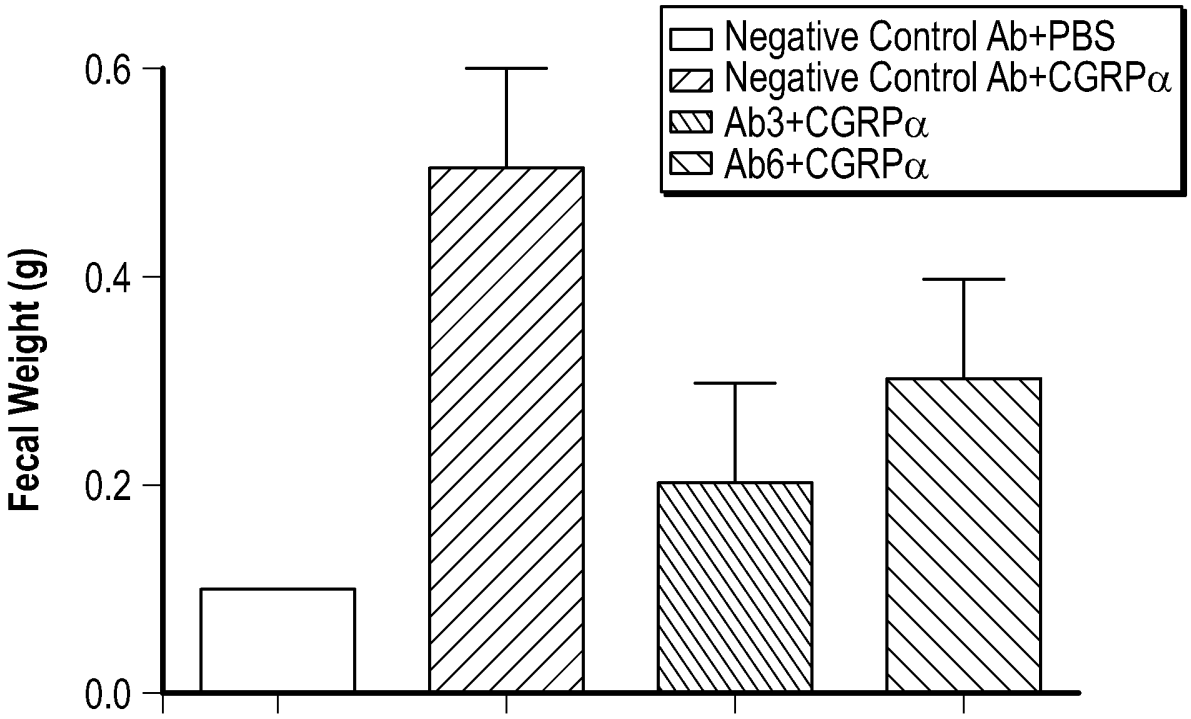

Negative Control Ab+PBS
Negative Control Ab+CGRPα
Ab3+CGRPα
Ab6+CGRPα
Fecal Weight (g)
0.6
0.4
0.2
0.0

FIG. 46

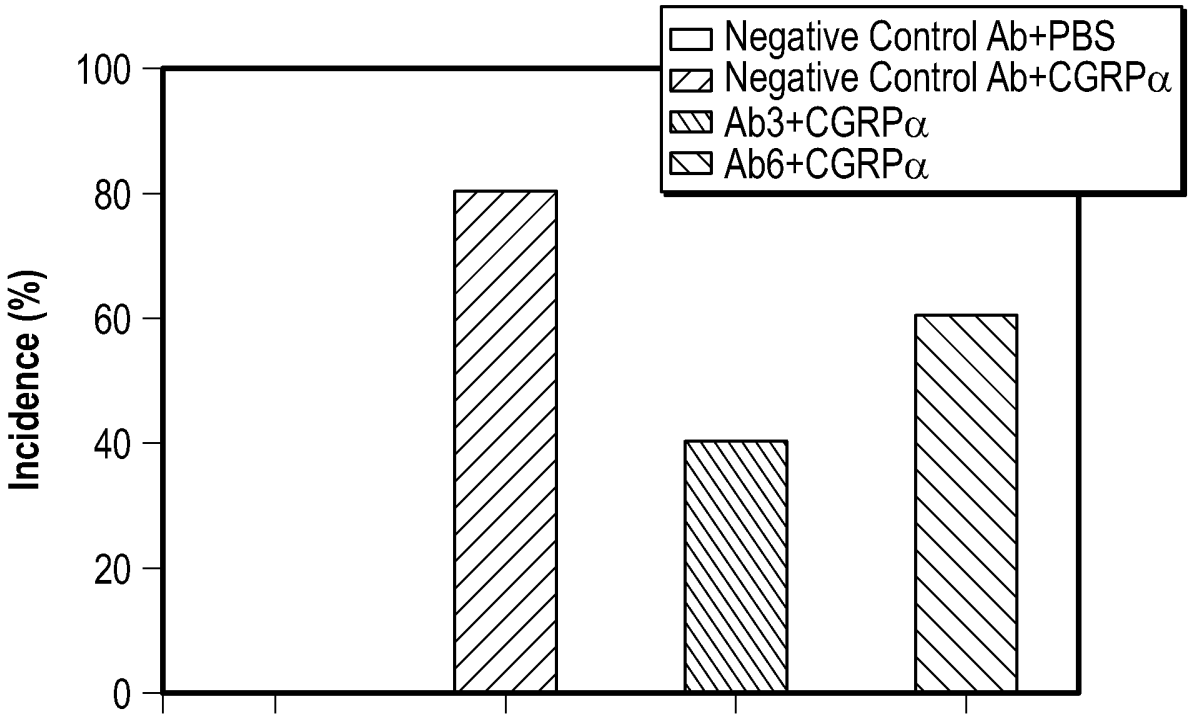

Negative Control Ab+PBS
Negative Control Ab+CGRPα
Ab3+CGRPα
Ab6+CGRPα
Incidence (%)
100
80
60
40
20
0

FIG. 47

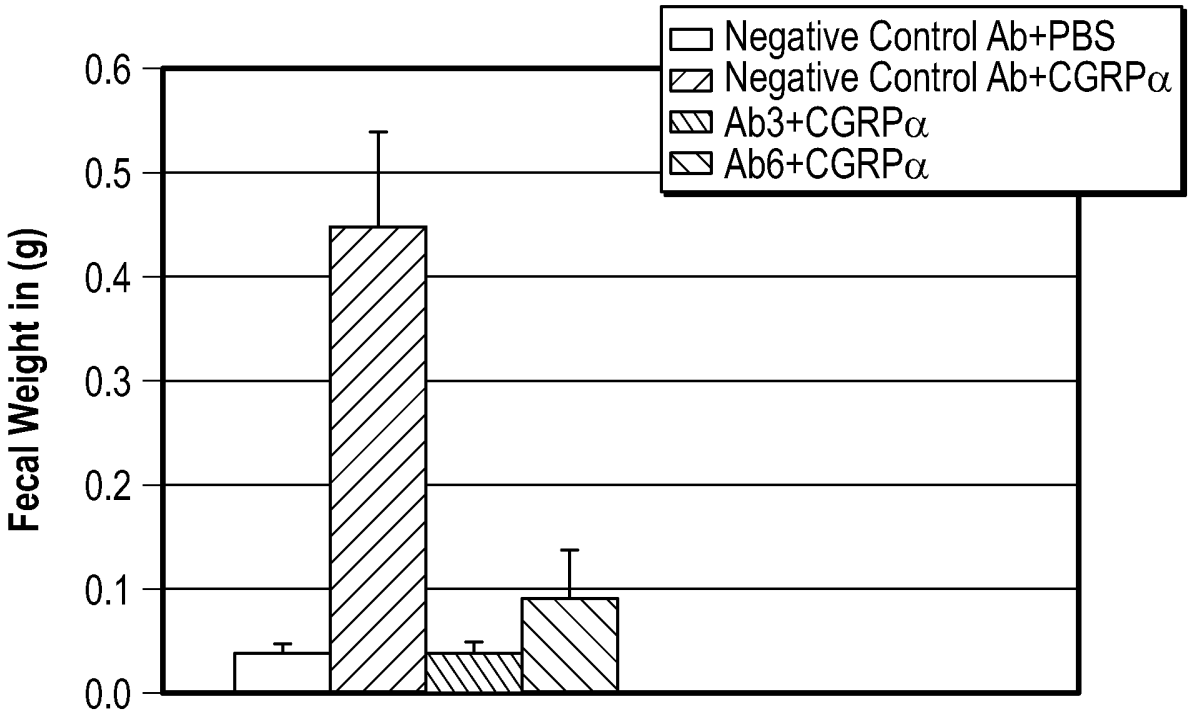

Negative Control Ab+PBS
Negative Control Ab+CGRPα
Ab3+CGRPα
Ab6+CGRPα
Fecal Weight in (g)
0.6
0.5
0.4
0.3
0.2
0.1
0.0

FIG. 48

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13*ter*.1(c) and (d) and 39)

Applicant's or agent's file reference	**IMPORTANT DECLARATION**	Date of mailing (*day/month/year*)
67858-730306		31 OCTOBER 2012 (31.10.2012)
International application No.	International filing date (*day/month/year*)	(Earlist) Priority date *(day/month/year)*
PCT/US2012/038869	**21 MAY 2012 (21.05.2012)**	20 MAY 2011 (20.05.2011)

International Patent Classification (IPC) or both national classification and IPC

A61K 39/395(2006.01)i, A61K 48/00(2006.01)i, A61K 38/16(2006.01)i, A61K 31/7088(2006.01)i, A61P 1/12(2006.01)i

Applicant

ALDERBIO HOLDINGS LLC et al

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☒ The subject matter of the international application relates to:
   - a. ☐ scientific theories.
   - b. ☐ mathematical theories.
   - c. ☐ plant varieties.
   - d. ☐ animal varieties.
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
   - f. ☐ schemes, rules or methods of doing business.
   - g. ☐ schemes, rules or methods of performing purely mental acts.
   - h. ☐ schemes, rules or methods of playing games.
   - i. ☒ methods for treatment of the human body by surgery or therapy.
   - j. ☐ methods for treatment of the animal body by surgery or therapy.
   - k. ☐ diagnostic methods practised on the human or animal body.
   - l. ☐ mere presentation of information.
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.
2. ☒ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

   ☐ the description ☒ the claims ☐ the drawings

3. ☐ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:
   - ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
   - ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
   - ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b)

4. Further coments:

Name and mailing address of ISA/KR

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Facsimile No. 82-42-472-7140

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61/789,156	15 March 2013 (15.03.2013)	US
61/826,747	23 May 2013 (23.05.2013)	US

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(74) Agents: **CALVO, Paul A.** et al.; Sterne, Kessler, Goldstein & Fox, PLLC, 1100 New York Ave., NW, Washington, DC 20005 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: RSPO3 BINDING AGENTS AND USES THEREOF

(57) **Abstract**: The present invention relates to RSPO-binding agents, particularly RSPO3-binding agents and methods of using the agents for treating diseases such as cancer. The present invention provides antibodies that specifically bind human RSPO3 proteins and modulate ?-catenin activity. The present invention further provides methods of using agents that modulate the activity of RSPO3 proteins and inhibit tumor growth. Also described are methods of treating cancer comprising administering a therapeutically effect amount of an agent or antibody of the present invention to a patient having a tumor or cancer.

WO 2014/012007 A2

RSPO3 BINDING AGENTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATONS

[001] This application claims priority benefit of U.S. Provisional Application No. 61/671,421, filed July 13, 2012, U.S. Provisional Application No. 61/753,184, filed January 16, 2013, U.S. Provisional Application No. 61/789,156, filed March 15, 2013, and U.S. Provisional Application No. 61/826,747, filed May 23, 2013, each of which is hereby incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[002] The field of this invention generally relates to antibodies and other agents that bind R-Spondin proteins (RSPO), particularly human R-Spondin protein RSPO3, as well as to methods of using the antibodies or other agents for the treatment of diseases such as cancer.

BACKGROUND OF THE INVENTION

[003] The R-Spondin (RSPO) family of proteins is conserved among vertebrates and comprises four members, RSPO1, RSPO2, RSPO3, and RSPO4. These proteins have been referred to by a variety of names, including roof plate-specific spondins, hPWTSR (hRSPO3), THS2D (RSPO3), Cristin 1-4, and Futrin 1-4. The RSPOs are small secreted proteins that overall share approximately 40-60% sequence homology and domain organization. All RSPO proteins contain two furin-like cysteine-rich domains at the N-terminus followed by a thrombospondin domain and a basic charged C-terminal tail (Kim et al., 2006, *Cell Cycle*, 5:23-26).

[004] Studies have shown that RSPO proteins have a role during vertebrate development (Kamata et al., 2004, *Biochim. Biophys Acta*, 1676:51-62) and in *Xenopus* myogenesis (Kazanskaya et al., 2004, *Dev. Cell*, 7:525-534). RSPO1 has also been shown to function as a potent mitogen for gastrointestinal epithelial cells (Kim et al., 2005, *Science*, 309:1256-1259). It has been reported that RSPO3 is prominently expressed in or close by endothelial cells and their cellular precursors in *Xenopus* and mouse. Furthermore, it has been suggested that RSPO3 may act as an angiogenic factor in embryogenesis (Kazanskaya et al., 2008, *Development*, 135:3655-3664). RSPO proteins are known to activate β-catenin signaling similar to Wnt signaling, however the relationship between RSPO proteins and Wnt signaling is still being investigated. It has been reported that RSPO proteins possess a positive modulatory activity on Wnt ligands (Nam et al., 2006, *JBC* 281:13247-57). This study also reported that RSPO proteins could function as Frizzled8 and LRP6 receptor ligands and induce β-catenin signaling (Nam et al., 2006, *JBC* 281:13247-57). Recent studies have identified an interaction between RSPO proteins and LGR (leucine-rich repeat containing, G protein-coupler receptor) proteins, such as LGR5 (U.S. Patent Publication Nos.

2009/0074782 and 2009/0191205), and these data present an alternative pathway for the activation of β-catenin signaling.

[005] The Wnt signaling pathway has been identified as a potential target for cancer therapy. The Wnt signaling pathway is one of several critical regulators of embryonic pattern formation, post-embryonic tissue maintenance, and stem cell biology. More specifically, Wnt signaling plays an important role in the generation of cell polarity and cell fate specification including self-renewal by stem cell populations. Unregulated activation of the Wnt pathway is associated with numerous human cancers where it is believed the activation can alter the developmental fate of cells. The activation of the Wnt pathway may maintain tumor cells in an undifferentiated state and/or lead to uncontrolled proliferation. Thus carcinogenesis can proceed by overtaking homeostatic mechanisms which control normal development and tissue repair (reviewed in Reya & Clevers, 2005, *Nature*, 434:843-50; Beachy et al., 2004, *Nature*, 432:324-31).

[006] The Wnt signaling pathway was first elucidated in the Drosophila developmental mutant wingless (wg) and from the murine proto-oncogene int-1, now Wnt1 (Nusse & Varmus, 1982, *Cell*, 31:99-109; Van Ooyen & Nusse, 1984, *Cell*, 39:233-40; Cabrera et al., 1987, *Cell*, 50:659-63; Rijsewijk et al., 1987, *Cell*, 50:649-57). Wnt genes encode secreted lipid-modified glycoproteins of which 19 have been identified in mammals. These secreted ligands activate a receptor complex consisting of a Frizzled (FZD) receptor family member and low-density lipoprotein (LDL) receptor-related protein 5 or 6 (LRP5/6). The FZD receptors are seven transmembrane domain proteins of the G-protein coupled receptor (GPCR) superfamily and contain a large extracellular N-terminal ligand binding domain with 10 conserved cysteines, known as a cysteine-rich domain (CRD) or Fri domain. There are ten human FZD receptors, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. Different FZD CRDs have different binding affinities for specific Wnt proteins (Wu & Nusse, 2002, *J. Biol. Chem.*, 277:41762-9), and FZD receptors have been grouped into those that activate the canonical β-catenin pathway and those that activate non-canonical pathways (Miller et al., 1999, *Oncogene*, 18:7860-72).

[007] A role for Wnt signaling in cancer was first uncovered with the identification of Wnt1 (originally int1) as an oncogene in mammary tumors transformed by the nearby insertion of a murine virus (Nusse & Varmus, 1982, *Cell*, 31:99-109). Additional evidence for the role of Wnt signaling in breast cancer has since accumulated. For instance, transgenic over-expression of β-catenin in the mammary glands results in hyperplasias and adenocarcinomas (Imbert et al., 2001, *J. Cell Biol.*, 153:555-68; Michaelson & Leder, 2001, *Oncogene*, 20:5093-9) whereas loss of Wnt signaling disrupts normal mammary gland development (Tepera et al., 2003, *J. Cell Sci.*, 116:1137-49; Hatsell et al., 2003, *J. Mammary Gland Biol. Neoplasia*, 8:145-58). In human breast cancer, β-catenin accumulation implicates activated Wnt signaling in over 50% of carcinomas, and though specific mutations have not been identified, up-regulation of Frizzled

receptor expression has been observed (Brennan & Brown, 2004, *J. Mammary Gland Biol. Neoplasia*, 9:119-31; Malovanovic et al., 2004, *Int. J. Oncol.*, 25:1337-42).

[008] Activation of the Wnt pathway is also associated with colorectal cancer. Approximately 5-10% of all colorectal cancers are hereditary with one of the main forms being familial adenomatous polyposis (FAP), an autosomal dominant disease in which about 80% of affected individuals contain a germline mutation in the adenomatous polyposis coli (APC) gene. Mutations have also been identified in other Wnt pathway components including Axin and β-catenin. Individual adenomas are clonal outgrowths of epithelial cells containing a second inactivated allele, and the large number of FAP adenomas inevitably results in the development of adenocarcinomas through additional mutations in oncogenes and/or tumor suppressor genes. Furthermore, activation of the Wnt signaling pathway, including loss-of-function mutations in APC and stabilizing mutations in β-catenin, can induce hyperplastic development and tumor growth in mouse models (Oshima et al., 1997, *Cancer Res.*, 57:1644-9; Harada et al., 1999, *EMBO J.*, 18:5931-42).

[009] Similar to breast cancer and colon cancer, melanoma often has constitutive activation of the Wnt pathway, as indicated by the nuclear accumulation of β-catenin. Activation of the Wnt/β-catenin pathway in some melanoma tumors and cell lines is due to modifications in pathway components, such as APC, ICAT, LEF1 and β-catenin (see e.g., Larue et al. 2006, *Frontiers Biosci.*, 11:733-742). However, there are conflicting reports in the literature as to the exact role of Wnt/β-catenin signaling in melanoma. For example, one study found that elevated levels of nuclear β-catenin correlated with improved survival from melanoma, and that activated Wnt/β-catenin signaling was associated with decreased cell proliferation (Chien et al., 2009, *PNAS*, 106:1193-1198).

[010] The focus of cancer drug research is shifting toward targeted therapies aimed at genes, proteins, and pathways involved in human cancer. There is a need for new agents targeting signaling pathways and new combinations of agents that target multiple pathways that could provide therapeutic benefit for cancer patients. Thus, biomolecules (e.g., anti-RSPO3 antibodies) that disrupt β-catenin signaling are a potential source of new therapeutic agents for cancer, as well as other β-catenin-associated diseases.

BRIEF SUMMARY OF THE INVENTION

[011] The present invention provides binding agents, such as antibodies, that bind RSPO3 proteins, as well as compositions, such as pharmaceutical compositions, comprising the binding agents. Binding agents that bind RSPO3 as well as at least one additional antigen or target, and pharmaceutical compositions of such binding agents, are also provided. In certain embodiments, the RSPO3-binding agents are novel polypeptides, such as antibodies, antibody fragments, and other polypeptides related to such antibodies. The invention further provides methods of inhibiting the growth of a tumor by administering the RSPO3-binding agents to a subject with a tumor. The invention further provides

methods of treating cancer by administering the RSPO3-binding agents to a subject in need thereof. In some embodiments, the methods of treating cancer or inhibiting tumor growth comprise targeting cancer stem cells with the RSPO3-binding agents. In some embodiments, the methods comprise disrupting β-catenin signaling. In some embodiments, the methods comprise modulating (e.g., inhibiting) angiogenesis. In certain embodiments, the methods comprise reducing the frequency of cancer stem cells in a tumor, reducing the number of cancer stem cells in a tumor, reducing the tumorigenicity of a tumor, and/or reducing the tumorigenicity of a tumor by reducing the number or frequency of cancer stem cells in the tumor.

[012] In one aspect, the invention provides a binding agent, such as an antibody, that specifically binds human RSPO3. The sequence of human RSPO3 is known in the art and is included herein as SEQ ID NO:3. In certain embodiments, the RSPO3-binding agent binds within amino acids 22-272 of human RSPO3. In certain embodiments, the RSPO3-binding agent binds within amino acids 22-207 of human RSPO3. In certain embodiments, the RSPO3-binding agent binds within amino acids 35-135 of human RSPO3. In certain embodiments, the RSPO3-binding agent binds within amino acids 35-86 of human RSPO3. In certain embodiments, the RSPO3-binding agent binds within amino acids 92-135 of human RSPO3. In some embodiments, the RSPO3-binding agent (e.g., an antibody) specifically binds at least one other human RSPO selected from the group consisting of RSPO1, RSPO2, and RSPO4. In some embodiments, the RSPO3-binding agent or antibody modulates β-catenin activity, is an antagonist of β-catenin signaling, inhibits β-catenin signaling, and/or inhibits activation of β-catenin. In some embodiments, the RSPO3-binding agent inhibits RSPO3 signaling. In some embodiments, the RSPO3-binding agent inhibits, interferes with, and/or disrupts binding of RSPO3 to one or more LGR proteins (e.g., LGR4, LGR5, and/or LGR6). In some embodiments, the RSPO3-binding agent inhibits binding of RSPO3 to LGR5.

[013] In certain embodiments, the RSPO3-binding agent is an antibody which binds human RSPO3. In some embodiments, the antibody binds human RSPO3 and mouse RSPO3. In certain embodiments, the antibody comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), or TYFANNFD (SEQ ID NO:80). In some embodiments, the antibody further comprises a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83). In some embodiments, the antibody comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY

(SEQ ID NO:35), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the antibody comprises a heavy chain CDR1 comprising DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLTF (SEQ ID NO:83). In some embodiments, the antibody comprises a heavy chain CDR1 comprising DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the antibody comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14).

[014] In certain embodiments, the RSPO3-binding agent is an antibody which comprises: (a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), DYSIH (SEQ ID NO:78), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), YIYPSNGDSGYNQKFK (SEQ ID NO:79), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (c) a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), TYFANNFD (SEQ ID NO:80), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (d) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), KASQSVDYDGDSYMN (SEQ ID NO:81), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (e) a light chain CDR2 comprising AAS (SEQ ID NO:13), AASNLES (SEQ ID NO:82), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and (f) a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14), QQSNEDPLTF (SEQ ID NO:83), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In some embodiments, the amino acid substitutions are conservative amino acid substitutions. In some embodiments, the substitutions are made as part of a germline humanization process.

[015] In certain embodiments, the RSPO3-binding agent is an antibody which comprises: (a) a heavy chain variable region having at least 80% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62; and/or (b) a light chain variable region having at least 80% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID

NO:86. In certain embodiments, the RSPO3-binding agent is an antibody that comprises: (a) a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62; and/or (b) a light chain variable region having at least 90% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86.

[016] In some embodiments, the RSPO3-binding agent is a monoclonal antibody. In some embodiments, the monoclonal antibody is an IgG1 antibody. In some embodiments, the monoclonal antibody is an IgG2 antibody. In some embodiments, the RSPO3-binding agent is monoclonal antibody 131R002 or monoclonal antibody 131R003. In some embodiments, the RSPO3-binding agent is an affinity-matured variant of monoclonal antibody 131R002 or monoclonal antibody 131R003. In some embodiments, the RSPO3-binding agent is a chimeric antibody comprising the antigen-binding sites from antibody 131R002 or antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized form of antibody 131R002 or antibody 131R003. In some embodiments, the RSPO3-binding agent is antibody h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011.

[017] In another aspect, the invention provides a binding agent (e.g., an antibody) that competes for specific binding to human RSPO3 with an antibody of the invention. In some embodiments, the binding agent (e.g., an antibody) competes for specific binding to human RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62, and a light chain variable region comprising SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In some embodiments, the antibody with which the RSPO3-binding agent competes is antibody 131R002 or antibody 131R003. In some embodiments, the antibody with which the RSPO3-binding agent competes is a humanized form of antibody 131R002 or antibody 131R003. In some embodiments, the antibody with which the RSPO3-binding agent competes is antibody h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011. In some embodiments, the binding agent competes for specific binding to RSPO3 with an antibody of the invention in an *in vitro* competitive binding assay.

[018] In certain embodiments, the binding agent is an antibody that binds the same epitope, or essentially the same epitope, on RSPO3 as an antibody of the invention (e.g., 131R002, 131R003, or humanized forms/variants thereof). In certain embodiments, the binding agent is an antibody that antibody binds the same epitope, or essentially the same epitope, on RSPO3 as antibody h131R005/131R007, h131R008, h131R010, or h131R011.

[019] In still another aspect, the binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by an antibody of the invention (e.g., 131R002, 131R003, or humanized forms/variants thereof). In some embodiments, the binding agent is an antibody that binds an

epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by antibody h131R005/131R007, h131R008, h131R010, or h131R011.

[020] In certain embodiments of each of the aforementioned aspects or embodiments, as well as other aspects and/or embodiments described elsewhere herein, the binding agent is a bispecific antibody. In some embodiments, the bispecific antibody specifically binds human RSPO3 and a second target. In some embodiments, the bispecific antibody specifically binds human RSPO3 and human RSPO1. In some embodiments, the bispecific antibody specifically binds human RSPO3 and human RSPO2. In some embodiments, the bispecific antibody specifically binds human RSPO3 and human RSPO4. In some embodiments, the bispecific antibody modulates β-catenin activity. In certain embodiments, the bispecific antibody inhibits β-catenin activity. In certain embodiments, the bispecific antibody inhibits β-catenin signaling. In certain embodiments, the bispecific antibody inhibits activation of β-catenin. In some embodiments, the bispecific antibody reduces the number of frequency of cancer stem cells. In certain embodiments, the bispecific antibody comprises two identical light chains. In certain embodiments, the bispecific antibody is an IgG antibody. In certain embodiments, the bispecific antibody is an IgG1 antibody. In certain embodiments, the bispecific antibody is an IgG2 antibody.

[021] In some embodiments, the bispecific antibody comprises: a first antigen-binding site that specifically binds human RSPO3, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), or TYFANNFD (SEQ ID NO:80). In some embodiments, the first antigen-binding site comprises a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83). In some embodiments, the bispecific antibody further comprises a second antigen-binding site that specifically binds human RSPO1. In some embodiments, the bispecific antibody further comprises a second antigen-binding site that specifically binds human RSPO2. Non-limiting examples of antibodies to RSPO1 or antibodies to RSPO2 have been described in, for example, International Patent Application Pub. No. WO 2013/012747. In some embodiments, the first and second binding sites comprise a common (e.g., identical) light chain.

[022] In some embodiments, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human RSPO3, and b) a second antigen-binding site that specifically binds human RSPO1, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID

NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), or TYFANNFD (SEQ ID NO:80). In some embodiments, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human RSPO3, and b) a second antigen-binding site that specifically binds human RSPO2, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), or TYFANNFD (SEQ ID NO:80). In some embodiments, the first antigen-binding site comprises a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83).

[023] In some embodiments, the bispecific antibody specifically binds human RSPO3 and comprises: a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62. In some embodiments, the bispecific antibody specifically binds human RSPO3 and comprises: a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62. In some embodiments, the bispecific antibody comprises a first and second binding site, wherein the first and second binding sites comprise a common (e.g., identical) light chain. In some embodiments, the bispecific antibody comprises a light chain variable region having at least 95% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86.

[024] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the RSPO3-binding agent or antibody is isolated. In some embodiments, the RSPO3-binding agent or antibody is substantially pure.

[025] In another aspect, the invention provides polypeptides. In some embodiments, the polypeptide comprises a sequence selected from the group consisting of: SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:86, SEQ ID NO:87, and SEQ ID NO:88. In some embodiments, the polypeptide comprises SEQ ID NO:15 and/or SEQ ID NO:17. In some embodiments, the polypeptide comprises SEQ ID NO:16 and/or SEQ ID NO:17. In some embodiments, the polypeptide comprises SEQ ID NO:36 and/or SEQ ID NO:17. In some embodiments, the polypeptide comprises SEQ

ID NO:37 and/or SEQ ID NO:17. In some embodiments, the polypeptide comprises SEQ ID NO:44 and/or SEQ ID NO:17. In some embodiments, the polypeptide comprises SEQ ID NO:45 and/or SEQ ID NO:17. In some embodiments, the polypeptide comprises SEQ ID NO:62 and/or SEQ ID NO:17. In some embodiments, the polypeptide comprises SEQ ID NO:44 and/or SEQ ID NO:72. In some embodiments, the polypeptide comprises SEQ ID NO:45 and/or SEQ ID NO:72. In some embodiments, the polypeptide comprises SEQ ID NO:62 and/or SEQ ID NO:72. In some embodiments, the polypeptide comprises SEQ ID NO:44 and/or SEQ ID NO:86. In some embodiments, the polypeptide comprises SEQ ID NO:45 and/or SEQ ID NO:86. In some embodiments, the polypeptide comprises SEQ ID NO:62 and/or SEQ ID NO:86.

[026] In some embodiments, the polypeptide comprises SEQ ID NO:21 and/or SEQ ID NO:23. In some embodiments, the polypeptide comprises SEQ ID NO:22 and/or SEQ ID NO:23. In some embodiments, the polypeptide comprises SEQ ID NO:38 and/or SEQ ID NO:23. In some embodiments, the polypeptide comprises SEQ ID NO:41 and/or SEQ ID NO:23. In some embodiments, the polypeptide comprises SEQ ID NO:46 and/or SEQ ID NO:23. In some embodiments, the polypeptide comprises SEQ ID NO:47 and/or SEQ ID NO:23. In some embodiments, the polypeptide comprises SEQ ID NO:63 and/or SEQ ID NO:23. In some embodiments, the polypeptide comprises SEQ ID NO:68 and/or SEQ ID NO:23. In some embodiments, the polypeptide comprises SEQ ID NO:46 and/or SEQ ID NO:73. In some embodiments, the polypeptide comprises SEQ ID NO:47 and/or SEQ ID NO:73. In some embodiments, the polypeptide comprises SEQ ID NO:63 and/or SEQ ID NO:73. In some embodiments, the polypeptide comprises SEQ ID NO:68 and/or SEQ ID NO:73. In some embodiments, the polypeptide comprises SEQ ID NO:46 and/or SEQ ID NO:87. In some embodiments, the polypeptide comprises SEQ ID NO:47 and/or SEQ ID NO:87. In some embodiments, the polypeptide comprises SEQ ID NO:63 and/or SEQ ID NO:87. In some embodiments, the polypeptide comprises SEQ ID NO:68 and/or SEQ ID NO:87.

[027] In some embodiments, the polypeptide comprises SEQ ID NO:27 and/or SEQ ID NO:29. In some embodiments, the polypeptide comprises SEQ ID NO:28 and/or SEQ ID NO:29. In some embodiments, the polypeptide comprises SEQ ID NO:39 and/or SEQ ID NO:29. In some embodiments, the polypeptide comprises SEQ ID NO:42 and/or SEQ ID NO:29. In some embodiments, the polypeptide comprises SEQ ID NO:48 and/or SEQ ID NO:29. In some embodiments, the polypeptide comprises SEQ ID NO:49 and/or SEQ ID NO:29. In some embodiments, the polypeptide comprises SEQ ID NO:64 and/or SEQ ID NO:29. In some embodiments, the polypeptide comprises SEQ ID NO:69 and/or SEQ ID NO:29. In some embodiments, the polypeptide comprises SEQ ID NO:48 and/or SEQ ID NO:74. In some embodiments, the polypeptide comprises SEQ ID NO:49 and/or SEQ ID NO:74. In some embodiments, the polypeptide comprises SEQ ID NO:64 and/or SEQ ID NO:74. In some embodiments, the polypeptide comprises SEQ ID NO:69 and/or SEQ ID NO:74. In some embodiments, the polypeptide

comprises SEQ ID NO:48 and/or SEQ ID NO:88. In some embodiments, the polypeptide comprises SEQ ID NO:49 and/or SEQ ID NO:88. In some embodiments, the polypeptide comprises SEQ ID NO:64 and/or SEQ ID NO:88. In some embodiments, the polypeptide comprises SEQ ID NO:69 and/or SEQ ID NO:88.

[028] In some embodiments, the polypeptide is isolated. In certain embodiments, the polypeptide is substantially pure. In certain embodiments, the polypeptide is an antibody or part of any antibody, such as an antibody fragment.

[029] In another aspect, the invention provides isolated polynucleotide molecules comprising a polynucleotide that encodes the antibodies and/or polypeptides of each of the aforementioned aspects, as well as other aspects and/or embodiments described herein. In some embodiments, the polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, and SEQ ID NO:95. In some embodiments, the polynucleotide comprises a polynucleotide that encodes a polypeptide selected from the group consisting of: SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:86, SEQ ID NO:87, and SEQ ID NO:88.

[030] The invention further provides expression vectors that comprise the polynucleotides, as well as cells that comprise the expression vectors and/or the polynucleotides. In some embodiments, the cell is a hybridoma cell line. In some embodiments, the cell is a monoclonal cell line. In some embodiments, the cell is a prokaryotic cell. In some embodiments, the cell is an eukaryotic cell.

[031] In other aspects, the invention provides methods of inhibiting growth of a tumor, comprising contacting the tumor with an effective amount of a RSPO3-binding agent or antibody, including each of those described herein.

[032] In another aspect, the invention provides a method of inhibiting the growth of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of a RSPO3-binding agent or antibody, including each of those described herein.

[033] In another aspect, the invention provides a method of inhibiting β-catenin signaling in a cell, comprising contacting the cell with an effective amount of a RSPO3-binding agent or antibody, including

each of those described herein. In some embodiments, the cell is a tumor cell. In some embodiments, the tumor is a colorectal tumor. In some embodiments, the tumor is an ovarian tumor. In some embodiments, the tumor is a pancreatic tumor. In some embodiments, the tumor is a lung tumor. In some embodiments, the tumor is a breast tumor. In some embodiments, the tumor expresses elevated levels of at least one RSPO protein. In some embodiments, the tumor expresses elevated levels of RSPO1. In some embodiments, the tumor expresses elevated levels of RSPO2. In some embodiments, the tumor expresses elevated levels of RSPO3. In some embodiments, the tumor expresses a high level of at least one RSPO protein. In some embodiments, the tumor expresses a high level of RSPO1. In some embodiments, the tumor expresses a high level of RSPO2. In some embodiments, the tumor expresses a high level of RSPO3. In certain embodiments, the RSPO3-binding agent inhibits growth of the tumor, for example, by reducing the number and/or frequency of cancer stem cells in the tumor. In some embodiments, the tumor contains a RSPO gene fusion. In some embodiments, the tumor contains a RSPO2 gene fusion. In some embodiments, the tumor contains a RSPO3 gene fusion.

[034] In another aspect, the invention provides methods of treating cancer in a subject. In some embodiments, the method comprises administering to a subject a therapeutically effective amount of any of the RSPO3-binding agents or antibodies described above, as well as those described elsewhere herein. In some embodiments, the cancer is pancreatic cancer. In some embodiments, the cancer is colorectal cancer. In some embodiments, the colorectal cancer comprises an inactivating mutation in the adenomatous polyposis coli (APC) gene. In some embodiments, the colorectal cancer does not comprise an inactivating mutation in the APC gene. In some embodiments, the colorectal cancer comprises a wild-type APC gene. In some embodiments, the colorectal cancer comprises a RSPO gene fusion. In some embodiments, the colorectal cancer comprises a RSPO2 gene fusion. In some embodiments, the colorectal cancer comprises a RSPO3 gene fusion. In some embodiments, the cancer is ovarian cancer. In some embodiments, the cancer is lung cancer. In some embodiments, the cancer is breast cancer. In some embodiments, the cancer expresses elevated levels of at least one RSPO protein. In some embodiments, the cancer is an ovarian cancer that expresses elevated levels of RSPO3. In some embodiments, the cancer is lung cancer that expresses elevated levels of RSPO3. In some embodiments, the cancer is breast cancer that expresses elevated levels of RSPO3. In some embodiments, the cancer is pancreatic cancer that expresses elevated levels of RSPO3.

[035] In another aspect, the invention provides methods of treating a disease in a subject wherein the disease is associated with activation of β-catenin, increased β-catenin signaling, and/or aberrant β-catenin signaling, wherein the method comprises administering to the subject a therapeutically effective amount of a RSPO3-binding agent or antibody, including each of those described herein.

[036] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the treatment methods further comprise a step of determining the expression level of at least one RSPO protein in the tumor or cancer.

[037] In another aspect, the invention provides a method of identifying a human subject or selecting a human subject for treatment with a RSPO3-binding agent or antibody, including but not limited to, each of those described herein. In some embodiments, the method comprises determining if the subject has a tumor that has an elevated expression level of a specific RSPO (e.g., RSPO3) as compared to the expression of the same RSPO protein in normal tissue or to a pre-determined level of the same RPSO protein. In some embodiments, the method comprises identifying a subject for treatment or selecting a subject for treatment if the tumor has an elevated level of RSPO expression. In some embodiments, the method comprises determining if the subject has a tumor that comprises an inactivating mutation in the APC gene. In some embodiments, the method comprises identifying a subject for treatment or selecting a subject for treatment if the tumor comprises an inactivating mutation in the APC gene. In some embodiments, the method comprises determining if the subject has a tumor that comprises a RSPO gene fusion (e.g., a RSPO3 gene fusion). In some embodiments, the method comprises identifying a subject for treatment or selecting a subject for treatment if the tumor comprises a RSPO gene fusion (e.g., a RSPO3 gene fusion).

[038] In certain embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the treatment methods comprise administering to the subject the RSPO3-binding agent and at least one additional therapeutic agent.

[039] Pharmaceutical compositions comprising a RSPO3-binding agent or antibody described herein and a pharmaceutically acceptable carrier are further provided, as are cell lines that produce the RSPO3-binding agents. Methods of treating cancer and/or inhibiting tumor growth in a subject (e.g., a human) comprising administering to the subject an effective amount of a pharmaceutical composition comprising the RSPO3-binding agents are also provided.

[040] Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but also each member of the group individually and all possible subgroups of the main group, and also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

BRIEF DESCRIPTION OF THE FIGURES

[041] Figure 1. RSPO expression in tumors and normal tissues. Shown is a summary of microarray data from normal, benign, and malignant tissue human samples. Individual tick marks indicate the expression level of RSPO mRNA. (A) RSPO1 (B) RSPO2 (C) RSPO3

[042] Figure 2. Binding studies of RSPO proteins and LGR5. FACS analysis of HEK-293 cells expressing LGR5. HEK-293 cells were transiently transfected with a cDNA expression vector encoding FLAG-LGR5-CD4TM-GFP and then subsequently mixed with soluble RSPO1-Fc, RSPO2-Fc, RSPO3-Fc, or RSPO4-Fc fusion proteins. An anti-FLAG antibody was used as a positive control, and soluble FZD8-Fc was used as a negative control. Specific binding is indicated by the presence of signal within the dark lined box overlay on each FACS plot.

[043] Figure 3. Anti-RSPO3 antibodies inhibit β-catenin signaling induced by RSPO3 and WNT3A. A TOPflash luciferase reporter assay was used to measure β-catenin signaling in HEK-293 cells after exposure to a combination of WNT3a (5ng/ml) and RSPO3 (10ng/ml) and in the presence of increasing concentrations of anti-RSPO3 antibodies (131R002 or 131R003). Antibodies were used as 4-fold serial dilutions from 20μg/ml to 0.02μg/ml. Controls included exposure to control medium (no WNT3a and no RSPO), WNT3a alone, or a combination of WNT3a and RSPO3 in the absence of antibody.

[044] Figure 4. Affinity-matured 131R003 antibody variants inhibit β-catenin signaling induced by RSPO3 and WNT3A. A TOPflash luciferase reporter assay was used to measure β-catenin signaling in HEK-293 cells after exposure to a combination of WNT3a and RSPO3 and in the presence of increasing concentrations of anti-RSPO3 antibodies (131R003 (-▲-), 131R003 CDR1 variant (-■-), or 131R003 CDR3 variant (-●-)). Antibodies were used as 5-fold serial dilutions from 20μg/ml to 0.006μg/ml. Controls included exposure to control medium (no WNT3a and no RSPO)/cells only (-Δ-), a control antibody (-▼-), WNT3a alone (-♦-), or a combination of WNT3a and RSPO3 in the absence of antibody (-□-).

[045] Figure 5. Inhibition of tumor growth with anti-RSPO antibodies. OV38 ovarian tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with a combination of anti-RSPO1 antibody 89M5 and anti-RSPO3 antibody 131R003 (-●-), taxol (-▲-), a combination of anti-RSPO1 antibody 89M5, anti-RSPO3 antibody 131R003, and taxol (-▼-), or a control antibody (-■-). Data is shown as tumor volume (mm^3) over days post-treatment.

[046] Figure 6. Inhibition of tumor growth with anti-RSPO antibodies. OV38 ovarian tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with a combination of anti-RSPO1 antibody 89M5 and anti-RSPO3 antibody 131R002 (-▲-), a combination of anti-RSPO1 antibody 89M5 and taxol (-○-), a combination of anti-RSPO3 antibody 131R002 and taxol (-□-), a combination of anti-

RSPO1 antibody 89M5, anti-RSPO3 antibody 131R002, and taxol (-Δ-), taxol alone (-▼-), or a control antibody (-■-). Data is shown as tumor volume (mm^3) over days post-treatment.

[047] Figure 7. Inhibition of tumor growth with anti-RSPO3 antibodies. (A) LU45 lung tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with anti-RSPO3 antibody 131R002 (-○-) or a control antibody (-■-). (B) LU25 lung tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with anti-RSPO3 antibody 131R002 (-○-) or a control antibody (-■-). Data is shown as tumor volume (mm^3) over days post-treatment.

[048] Figure 8. Affinity-matured antibody variants inhibit β-catenin signaling induced by RSPO3 and WNT3A. A TOPflash luciferase reporter assay was used to measure β-catenin signaling in HEK-293T cells after exposure to a combination of WNT3a and human RSPO3 and in the presence of increasing concentrations of anti-RSPO3 antibody 131R002 (-▲-), 131R006 (-●-), or 131R007 (-■-). Antibodies were used as 5-fold serial dilutions from 20μg/ml to 0.0064μg/ml. Controls included exposure to control medium (no WNT3a and no RSPO/cells (-○-)), WNT3a alone (-▼-), or a combination of WNT3a and human RSPO3 in the absence of antibody (-◆-).

[049] Figure 9. Inhibition of RSPO3 and LGR5 interaction by anti-RSPO3 antibodies. FACS analysis of HEK-293T cells expressing LGR5. HEK-293T cells were transiently transfected with a cDNA expression vector encoding the extracellular domain of human LGR5 (FLAG-LGR5-CD4TM-GFP) and then subsequently mixed with RSPO3-biotin fusion protein in combination with anti-RSPO3 antibodies 131R006 or 131R007. Binding was detected with PE-conjugated streptavidin. Relative RSPO3-biotin binding is shown on the y-axis and expression of the FLAG-LGR5-CD4TM-GFP fusion protein is indicated on the x-axis. Positive binding is indicated by the presence of signal within the dark lined box overlay on each FACS plot.

[050] Figure 10. Inhibition of tumor growth with anti-RSPO antibodies. NCI-H2030 cells were injected subcutaneously into NOD/SCID mice. Mice were treated with anti-RSPO3 antibody 131R002 (-❋-), carboplatin alone (-Δ-), a combination of anti-RSPO3 antibody 131R002 and carboplatin (-○-), or a control antibody (-■-). Data is shown as tumor volume (mm^3) over days post-treatment.

[051] Figure 11. Inhibition of tumor growth with anti-RSPO antibodies. LU102 lung tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with anti-RSPO3 antibody 131R002 (-●-), carboplatin alone (-Δ-), a combination of anti-RSPO3 antibody 131R002 and carboplatin (-○-), or a control antibody (-■-). (A) Data is shown as tumor volume (mm^3) over days post-treatment. (B) Gene set enrichment analysis results.

[052] Figure 12. Inhibition of tumor growth with anti-RSPO antibodies. PN35 pancreatic tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with anti-RSPO3 antibody 131R002 (-●-), a combination of gemcitabine and nab-paclitaxel (ABRAXANE) (-Δ-), a combination of anti-RSPO3 antibody 131R002 and gemcitabine and nab-paclitaxel (ABRAXANE) (-○-), or a control

antibody (-■-). Data is shown as tumor volume (mm^3) over days post-treatment. (A) All four treatment groups; (B) the gemcitabine and nab-paclitaxel treatment group and the anti-RSPO3 antibody gemcitabine and nab-paclitaxel treatment on an expanded scale.

[053] Figure 13. Inhibition of β-catenin signaling induced by RSPO3 and WNT3A. A TOPflash luciferase reporter assay was used to measure β-catenin signaling in HEK-293T cells after exposure to a combination of WNT3a and human RSPO3 and in the presence of increasing concentrations of anti-RSPO3 antibody 131R007 (-□-) or 131R010 (-●-). Antibodies were used as 5-fold serial dilutions from 20μg/ml to 0.0064μg/ml. Controls included exposure to control medium (no WNT3a and no RSPO/cells (-▲-)), WNT3a alone (-▼-), or a combination of WNT3a and human RSPO3 in the absence of antibody (-◆-).

[054] Figure 14. Inhibition of tumor growth with anti-RSPO antibodies. LU25 NSCLC lung tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with anti-RSPO3 antibody 131R008 (-▲-), paclitaxel alone (-○-), a combination of anti-RSPO3 antibody 131R008 and paclitaxel (-●-), or a control antibody (-■-). Data is shown as tumor volume (mm^3) over days post-treatment.

DETAILED DESCRIPTION OF THE INVENTION

[055] The present invention provides novel agents, including, but not limited to polypeptides such as antibodies, that bind RSPO proteins, particularly human RSPO3. The RSPO3-binding agents include, but are not limited to, antagonists of β-catenin signaling. The RSPO3-binding agents include, but are not limited to, inhibitors of RSPO3 and LGR protein interactions. Related polypeptides and polynucleotides, compositions comprising the RSPO3-binding agents, and methods of making the RSPO3-binding agents are also provided. Methods of using the novel RSPO3-binding agents, such as methods of inhibiting tumor growth, methods of treating cancer, methods of modulating angiogenesis, methods of reducing the frequency of cancer stem cells in a tumor, methods of inhibiting β-catenin signaling, and/or methods of identifying and/or selecting subjects for treatment, are further provided.

[056] Monoclonal antibodies that specifically bind human RSPO3 have been identified - monoclonal antibodies 131R002 and 131R003 (Example 3). Anti-RSPO3 antibodies 131R002 and 131R003 have binding affinities for human RSPO3 of less than 10 nM (Example 3). Anti-RSPO3 antibodies 131R002 and 131R003 inhibit RSPO3-induced β-catenin signaling (Example 4, Fig. 3). Affinity-matured variants of 131R003 inhibit RSPO3-induced β-catenin signaling and have greater activity than parental 131R003 (Example 5, Fig. 4). Anti-RSPO3 antibodies inhibit tumor growth as single agents, in combination with anti-RSPO1 antibodies, and in combination with one or more chemotherapeutic agents (Examples 6, 7, 11 12 and 14; Figs. 5-7, 10-12 and 14). Humanized anti-RSPO3 antibodies h131R006 and h131R007 are stronger inhibitors of β-catenin activity than antibody 131R002 (Example 8, Fig. 8). Anti-RSPO3

antibodies h131R006 and h131R007 block binding of RSPO3 to LGR5 (Example 9, Fig. 9). Humanized anti-RSPO3 antibody h131R010 isotype IgG1 inhibits β-catenin activity similar to the IgG2 isotype antibody h131R007 (Example 13, Fig. 13).

I. Definitions

[057] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[058] The terms "antagonist" and "antagonistic" as used herein refer to any molecule that partially or fully blocks, inhibits, reduces, or neutralizes a biological activity of a target and/or signaling pathway (e.g., the β-catenin signaling). The term "antagonist" is used herein to include any molecule that partially or fully blocks, inhibits, reduces, or neutralizes the activity of a protein (e.g., a RSPO protein). Suitable antagonist molecules specifically include, but are not limited to, antagonist antibodies or antibody fragments.

[059] The terms "modulation" and "modulate" as used herein refer to a change or an alteration in a biological activity. Modulation includes, but is not limited to, stimulating or inhibiting an activity. Modulation may be an increase or a decrease in activity (e.g., a decrease in RSPO signaling; a decrease in β-catenin signaling), a change in binding characteristics, or any other change in the biological, functional, or immunological properties associated with the activity of a protein, pathway, or other biological point of interest.

[060] The term "antibody" as used herein refers to an immunoglobulin molecule that recognizes and specifically binds a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing, through at least one antigen-binding site within the variable region(s) of the immunoglobulin molecule. As used herein, the term encompasses intact polyclonal antibodies, intact monoclonal antibodies, single chain antibodies, antibody fragments (such as Fab, Fab', F(ab')2, and Fv fragments), single chain Fv (scFv) antibodies, multispecific antibodies such as bispecific antibodies, monospecific antibodies, monovalent antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen-binding site of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site (i.e., antigen-binding site) as long as the antibodies exhibit the desired biological activity. An antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well-known subunit structures and three-dimensional configurations. Antibodies can be naked or conjugated to other molecules, including but not limited to, toxins and radioisotopes.

[061] The term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments. "Antibody fragment" as used herein comprises an antigen-binding site or epitope-binding site.

[062] The term "variable region" of an antibody refers to the variable region of an antibody light chain, or the variable region of an antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chains each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs), also known as "hypervariable regions". The CDRs in each chain are held together in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of the antibody. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al., 1991, *Sequences of Proteins of Immunological Interest, 5th Edition*, National Institutes of Health, Bethesda, MD), and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-Lazikani et al., 1997, *J. Mol. Biol.*, 273:927-948). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

[063] The term "monoclonal antibody" as used herein refers to a homogeneous antibody population involved in the highly specific recognition and binding of a single antigenic determinant or epitope. This is in contrast to polyclonal antibodies that typically include a mixture of different antibodies directed against a variety of different antigenic determinants. The term "monoclonal antibody" encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (e.g., Fab, Fab', F(ab')2, Fv), single chain (scFv) antibodies, bispecific antibodies, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site (antigen-binding site). Furthermore, "monoclonal antibody" refers to such antibodies made by any number of techniques, including but not limited to, hybridoma production, phage selection, recombinant expression, and transgenic animals.

[064] The term "humanized antibody" as used herein refers to forms of non-human (e.g., murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Typically, humanized antibodies are human immunoglobulins in which residues of the CDRs are replaced by residues from the CDRs of a non-human species (e.g., mouse, rat, rabbit, or hamster) that have the desired specificity, affinity, and/or binding capability (Jones et al., 1986, *Nature*, 321:522-525; Riechmann et al., 1988, *Nature*, 332:323-327; Verhoeyen et al., 1988, *Science*, 239:1534-1536). In some instances, the Fv framework region residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species that has the desired specificity, affinity, structural, and/or binding capability. The humanized antibody

can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, structural, and/or binding capability. In general, the humanized antibody will comprise substantially all of at least one, and typically two or three of the CDRs that correspond to the non-human immunoglobulin whereas all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Examples of methods used to generate humanized antibodies are described in, for example, U.S. Pat. 5,225,539.

[065] The term "human antibody" as used herein refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human. A human antibody may be made using any of the techniques known in the art. This definition of a human antibody specifically excludes a humanized antibody comprising non-human CDRs.

[066] The term "chimeric antibody" as used herein refers to an antibody wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. Typically, the variable region of both light and heavy chains corresponds to the variable region of antibodies derived from one species of mammal (e.g., mouse, rat, rabbit, etc.) with the desired specificity, affinity, and/or binding capability, while the constant regions correspond to sequences in antibodies derived from another species (usually human).

[067] The phrase "affinity-matured antibody" as used herein refers to an antibody with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for an antigen, compared to a parent antibody that does not possess those alterations(s). The definition also includes alterations in non-CDR residues made in conjunction with alterations to CDR residues. Preferred affinity-matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al., 1992, *Bio/Technology* 10:779-783, describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al., 1994, *PNAS*, 91:3809-3813; Schier et al., 1995, *Gene*, 169:147-155; Yelton et al., 1995, *J. Immunol.* 155:1994-2004; Jackson et al., 1995, *J. Immunol.*, 154:3310-9; and Hawkins et al., 1992, *J. Mol. Biol.*, 226:889-896. Site-directed mutagenesis may also be used to obtain affinity-matured antibodies.

[068] The terms "epitope" and "antigenic determinant" are used interchangeably herein and refer to that portion of an antigen capable of being recognized and specifically bound by a particular antibody. When the antigen is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids (also referred to as linear epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding (also referred to as conformational epitopes) are typically lost upon protein

denaturing. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[069] The terms "heteromultimeric molecule" or "heteromultimer" or "heteromultimeric complex" or "heteromultimeric polypeptide" are used interchangeably herein to refer to a molecule comprising at least a first polypeptide and a second polypeptide, wherein the second polypeptide differs in amino acid sequence from the first polypeptide by at least one amino acid residue. The heteromultimeric molecule can comprise a "heterodimer" formed by the first and second polypeptide or can form higher order tertiary structures where additional polypeptides are present.

[070] The terms "selectively binds" or "specifically binds" mean that a binding agent or an antibody reacts or associates more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the epitope, protein or target molecule than with alternative substances, including unrelated proteins. In certain embodiments "specifically binds" means, for instance, that an antibody binds a protein with a K_D of about 0.1mM or less, but more usually less than about 1μM. In certain embodiments, "specifically binds" means that an antibody binds a target at times with a K_D of at least about 0.1μM or less, at other times at least about 0.01μM or less, and at other times at least about 1nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include an antibody that recognizes a protein in more than one species (e.g., human RSPO3 and mouse RSPO3). Likewise, because of homology within certain regions of polypeptide sequences of different proteins, specific binding can include an antibody (or other polypeptide or binding agent) that recognizes more than one protein (e.g., human RSPO3 and human RSPO1). It is understood that, in certain embodiments, an antibody or binding moiety that specifically binds a first target may or may not specifically bind a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding, i.e. binding to a single target. Thus, an antibody may, in certain embodiments, specifically bind more than one target. In certain embodiments, multiple targets may be bound by the same antigen-binding site on the antibody. For example, an antibody may, in certain instances, comprise two identical antigen-binding sites, each of which specifically binds the same epitope on two or more proteins (e.g., RSPO3 and RSPO1). In certain alternative embodiments, an antibody may be multispecific and comprise at least two antigen-binding sites with differing specificities. By way of non-limiting example, a bispecific antibody may comprise one antigen-binding site that recognizes an epitope on one protein (e.g., human RSPO3) and further comprise a second, different antigen-binding site that recognizes a different epitope on a second protein (e.g., human RSPO2). Generally, but not necessarily, reference to binding means specific binding.

[071] The terms "polypeptide" and "peptide" and "protein" are used interchangeably herein and refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino

acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art. It is understood that, because the polypeptides of this invention may be based upon antibodies, in certain embodiments, the polypeptides can occur as single chains or associated chains.

[072] The terms "polynucleotide" and "nucleic acid" are used interchangeably herein and refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase.

[073] "Conditions of high stringency" may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 15mM NaCl/1.5mM sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 in 5x SSC (0.75M NaCl, 75mM sodium citrate) at 42°C; or (3) employ during hybridization 50% formamide in 5x SSC, 50mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2x SSC and 50% formamide, followed by a high-stringency wash consisting of 0.1x SSC containing EDTA at 55°C.

[074] The terms "identical" or percent "identity" in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity may be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that may be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variations thereof. In some embodiments, two nucleic acids or polypeptides of the invention are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the sequences that is at least about 10, at least about 20, at least about 40-60 residues, at least about 60-80 residues in length or any integral value therebetween. In some embodiments, identity exists over a longer

region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a nucleotide sequence.

[075] A "conservative amino acid substitution" is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Preferably, conservative substitutions in the sequences of the polypeptides and antibodies of the invention do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen(s), i.e., the one or more RSPO protein(s) to which the polypeptide or antibody binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen binding are well-known in the art.

[076] The term "vector" as used herein means a construct, which is capable of delivering, and usually expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid, or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, and DNA or RNA expression vectors encapsulated in liposomes.

[077] A polypeptide, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, antibodies, polynucleotides, vectors, cells, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure.

[078] The term "substantially pure" as used herein refers to material which is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[079] The terms "cancer" and "cancerous" as used herein refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, blastoma, sarcoma, and hematologic cancers such as lymphoma and leukemia.

[080] The terms "tumor" and "neoplasm" as used herein refer to any mass of tissue that results from excessive cell growth or proliferation, either benign (noncancerous) or malignant (cancerous) including pre-cancerous lesions.

[081] The term "metastasis" as used herein refers to the process by which a cancer spreads or transfers from the site of origin to other regions of the body with the development of a similar cancerous lesion at a new location. A "metastatic" or "metastasizing" cell is one that loses adhesive contacts with neighboring cells and migrates via the bloodstream or lymph from the primary site of disease to invade neighboring body structures.

[082] The terms "cancer stem cell" and "CSC" and "tumor stem cell" and "tumor initiating cell" are used interchangeably herein and refer to cells from a cancer or tumor that: (1) have extensive proliferative capacity; 2) are capable of asymmetric cell division to generate one or more types of differentiated cell progeny wherein the differentiated cells have reduced proliferative or developmental potential; and (3) are capable of symmetric cell divisions for self-renewal or self-maintenance. These properties confer on the cancer stem cells the ability to form or establish a tumor or cancer upon serial transplantation into an immunocompromised host (e.g., a mouse) compared to the majority of tumor cells that fail to form tumors. Cancer stem cells undergo self-renewal versus differentiation in a chaotic manner to form tumors with abnormal cell types that can change over time as mutations occur.

[083] The terms "cancer cell" and "tumor cell" refer to the total population of cells derived from a cancer or tumor or pre-cancerous lesion, including both non-tumorigenic cells, which comprise the bulk of the cancer cell population, and tumorigenic stem cells (cancer stem cells). As used herein, the terms "cancer cell" or "tumor cell" will be modified by the term "non-tumorigenic" when referring solely to those cells lacking the capacity to renew and differentiate to distinguish those tumor cells from cancer stem cells.

[084] The term "tumorigenic" as used herein refers to the functional features of a cancer stem cell including the properties of self-renewal (giving rise to additional tumorigenic cancer stem cells) and proliferation to generate all other tumor cells (giving rise to differentiated and thus non-tumorigenic tumor cells).

[085] The term "tumorigenicity" as used herein refers to the ability of a random sample of cells from the tumor to form palpable tumors upon serial transplantation into immunocompromised hosts (e.g., mice). This definition also includes enriched and/or isolated populations of cancer stem cells that form palpable tumors upon serial transplantation into immunocompromised hosts (e.g., mice).

[086] The term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, canines, felines, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[087] The term "pharmaceutically acceptable" refers to a product or compound approved (or approvable) by a regulatory agency of the Federal government or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans.

[088] The terms "pharmaceutically acceptable excipient, carrier or adjuvant" or "acceptable pharmaceutical carrier" refer to an excipient, carrier or adjuvant that can be administered to a subject, together with at least one binding agent (e.g., an antibody) of the present disclosure, and which does not destroy the activity of the binding agent. The excipient, carrier, or adjuvant should be non-toxic when administered with a binding agent in doses sufficient to deliver a therapeutic effect.

[089] The terms "effective amount" or "therapeutically effective amount" or "therapeutic effect" refer to an amount of a binding agent, an antibody, polypeptide, polynucleotide, small organic molecule, or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of a drug (e.g., an antibody) has a therapeutic effect and as such can reduce the number of cancer cells; decrease tumorigenicity, tumorigenic frequency or tumorigenic capacity; reduce the number or frequency of cancer stem cells; reduce the tumor size; reduce the cancer cell population; inhibit and/or stop cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibit and/or stop tumor or cancer cell metastasis; inhibit and/or stop tumor or cancer cell growth; relieve to some extent one or more of the symptoms associated with the cancer; reduce morbidity and mortality; improve quality of life; or a combination of such effects. To the extent the agent, for example an antibody, prevents growth and/or kills existing cancer cells, it can be referred to as cytostatic and/or cytotoxic.

[090] The terms "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both 1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. In some embodiments, a subject is successfully "treated" according to the methods of the present invention if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer cells into soft tissue and bone; inhibition of or an absence of tumor or cancer cell metastasis; inhibition or an absence of cancer growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity; reduction in the number or frequency of cancer stem cells; or some combination of effects.

[091] As used in the present disclosure and claims, the singular forms "a", "an" and "the" include plural forms unless the context clearly dictates otherwise.

[092] It is understood that wherever embodiments are described herein with the language "comprising" otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided. It is also understood that wherever embodiments are described herein with the language "consisting essentially of" otherwise analogous embodiments described in terms of "consisting of" are also provided.

[093] The term "and/or" as used in a phrase such as "A and/or B" herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

II. RSPO-binding agents

[094] The present invention provides agents that specifically bind human RSPO proteins. These agents are referred to herein as "RSPO-binding agents". In some embodiments, the RSPO-binding agent is an antibody. In some embodiments, the RSPO-binding agent is a polypeptide. In certain embodiments, the RSPO-binding agent binds RSPO3 ("RSPO3-binding agents"). In certain embodiments, the RSPO3-binding agent specifically binds at least one other human RSPO. In some embodiments, the at least one other human RSPO bound by a RSPO3-binding agent is selected from the group consisting of RSPO1, RSPO2, and RSPO4. In some embodiments, the RSPO3-binding agent is an antibody that binds a common epitope on RSPO1, RSPO2, and/or RSPO4. In some embodiments, the RSPO3-binding agent is a bispecific antibody that binds a first epitope on RSPO3 and binds a second, different epitope on RSPO1, RSPO2, and/or RSPO4. The full-length amino acid (aa) sequences for human RSPO1, RSPO2, RSPO3, and RSPO4 are known in the art and are provided herein as SEQ ID NO:1 (RSPO1), SEQ ID NO:2 (RSPO2), SEQ ID NO:3 (RSPO3), and SEQ ID NO:4 (RSPO4).

[095] In certain embodiments, the antigen-binding site of a RSPO-binding agent (e.g., an antibody or a bispecific antibody) described herein is capable of binding (or binds) one, two, three, or four RSPOs. In certain embodiments, the antigen-binding site of a RSPO-binding agent (e.g., an antibody or a bispecific antibody) described herein is capable of binding (or binds) RSPO3 as well as one, two, or three other RSPOs. For example, in certain embodiments, the antigen-binding site of a RSPO3-binding agent is capable of specifically binding RSPO3 as well as at least one other RSPO selected from the group consisting of RSPO1, RSPO2, and RSPO4. In certain embodiments, the RSPO3-binding agent specifically binds RSPO3 and RSPO1. In certain embodiments, the RSPO3-binding agent specifically binds RSPO3 and RSPO2. In certain embodiments, the RSPO3-binding agent specifically binds RSPO3 and RSPO4. In certain embodiments, the RSPO3-binding agent specifically binds RSPO3, RSPO1, and RSPO2. In certain embodiments, the RSPO3-binding agent specifically binds RSPO3, RSPO1, and RSPO4. In certain embodiments, the RSPO3-binding agent specifically binds RSPO3, RSPO2, and

RSPO4. In some embodiments, the RSPO3-binding agent specifically binds human RSPO3. In some embodiments, the RSPO3-binding agent (e.g., antibody) specifically binds both human RSPO3 and mouse RSPO3.

[096] In certain embodiments, the agent-binding agent is an antibody that specifically binds within amino acids 22-272 of human RSPO3. In certain embodiments, the agent-binding agent is an antibody that specifically binds within amino acids 22-207 of human RSPO3. In certain embodiments, the antigen-binding agent is an antibody that specifically binds within amino acids 35-135 of human RSPO3. In certain embodiments, the antigen-binding agent is an antibody that specifically binds within amino acids 35-86 of human RSPO3. In certain embodiments, the antigen-binding agent is an antibody that specifically binds within amino acids 92-135 of human RSPO3. In certain embodiments, the RSPO3-binding agent binds within SEQ ID NO:5. In certain embodiments, the RSPO3-binding agent or antibody binds a furin-like cysteine-rich domain of RSPO3. In some embodiments, the agent or antibody binds at least one amino acid within a furin-like cysteine-rich domain of RSPO3. In certain embodiments, the RSPO3-binding agent or antibody binds within sequence SEQ ID NO:6 or SEQ ID NO:7. In certain embodiments, the RSPO3-binding agent or antibody binds within sequence SEQ ID NO:6 and SEQ ID NO:7. In some embodiments, the RSPO3-binding agent binds the thrombospondin domain of RSPO3. In some embodiments, the RSPO3-binding agent or antibody binds at least one amino acid within the thrombospondin domain of RSPO3. In some embodiments, the RSPO3-binding agent or antibody binds within SEQ ID NO:8.

[097] In certain embodiments, the RSPO-binding agent or antibody binds at least one RSPO protein with a dissociation constant (K_D) of about 1μM or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In certain embodiments, a RSPO3-binding agent or antibody binds RSPO3 with a dissociation constant (K_D) of about 1μM or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In some embodiments, a RSPO3-binding agent or antibody binds RSPO3 with a K_D of about 20nM or less. In some embodiments, a RSPO3-binding agent or antibody binds RSPO3 with a K_D of about 10nM or less. In some embodiments, a RSPO3-binding agent or antibody binds RSPO3 with a K_D of about 1nM or less. In some embodiments, a RSPO3-binding agent or antibody binds RSPO3 with a K_D of about 0.5nM or less. In some embodiments, a RSPO3-binding agent or antibody binds RSPO3 with a K_D of about 0.1nM or less. In certain embodiments, a RSPO3-binding agent or antibody described herein binds at least one other RSPO. In certain embodiments, a RSPO3-binding agent or antibody described herein that binds at least one other RSPO, binds at least one other RSPO with a K_D of about 100nM or less, about 20nM or less, about 10nM or less, about 1nM or less or about 0.1nM or less. For example, in some embodiments, a RSPO3-binding agent or antibody also binds RSPO1, RSPO2, and/or RSPO4 with a K_D of about 10nM or less. In some embodiments, the RSPO-binding agent binds both

human RSPO and mouse RSPO with a K_D of about 10nM or less. In some embodiments, a RSPO3-binding agent binds both human RSPO3 and mouse RSPO3 with a K_D of about 1nM or less. In some embodiments, a RSPO3-binding agent binds both human RSPO3 and mouse RSPO3 with a K_D of about 0.1nM or less. In some embodiments, the dissociation constant of the binding agent (e.g., an antibody) to a RSPO3 protein is the dissociation constant determined using a RSPO3 fusion protein comprising at least a portion of the RSPO3 protein immobilized on a Biacore chip. In some embodiments, the dissociation constant of the binding agent (e.g., an antibody) to a RSPO3 protein is the dissociation constant determined using the binding agent captured by an anti-human IgG antibody on a Biacore chip and a RSPO3 protein.

[098] In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a first antigen-binding site that specifically binds RSPO3 and a second antigen-binding site that specifically binds a second target. In some embodiments, a RSPO3-binding agent or antibody binds both RSPO3 and the second target with a K_D of about 100nM or less. In some embodiments, a RSPO3-binding agent or antibody binds both RSPO3 and the second target with a K_D of about 50nM or less. In some embodiments, a RSPO3-binding agent or antibody binds both RSPO3 and the second target with a K_D of about 20nM or less. In some embodiments, a RSPO3-binding agent or antibody binds both RSPO3 and the second target with a K_D of about 10nM or less. In some embodiments, a RSPO3-binding agent or antibody binds both RSPO3 and the second target with a K_D of about 1nM or less. In some embodiments, the affinity of one of the antigen-binding sites may be weaker than the affinity of the other antigen-binding site. For example, the K_D of one antigen binding site may be about 1nM and the K_D of the second antigen-binding site may be about 10nM. In some embodiments, the difference in affinity between the two antigen-binding sites may be about 2-fold or more, about 3-fold or more, about 5-fold or more, about 8-fold or more, about 10-fold or more, about 15-fold or more, about 20-fold or more, about 30-fold or more, about 50-fold or more, or about 100-fold or more. Modulation of the affinities of the two antigen-binding sites may affect the biological activity of the bispecific antibody. For example, decreasing the affinity of the antigen-binding site for RSPO3 or the second target, may have a desirable effect, for example decreased toxicity of the binding agent and/or increased therapeutic index.

[099] By way of non-limiting example, the bispecific antibody may comprise (a) a first antigen-binding site that binds human RSPO3 with a K_D between about 0.1nM and about 10nM, and (b) a second antigen-binding site that specifically binds a second target (e.g., human RSPO2) with a K_D between about 0.1nM and about 20nM, between about 0.5nM and about 20nM, or between about 1.0nM and 10nM.

[0100] In certain embodiments, the RSPO-binding agent (e.g., an antibody) binds to at least one human RSPO protein with a half maximal effective concentration (EC_{50}) of about 1μM or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds to human RSPO3 with a

half maximal effective concentration (EC_{50}) of about 1μM or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) also binds to human RSPO1, RSPO2, and/or RSPO4 with an EC_{50} of about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less or about 0.1nM or less.

[0101] In certain embodiments, the RSPO3-binding agent is an antibody. In some embodiments, the antibody is a recombinant antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody. In some embodiments, the antibody is an IgA, IgD, IgE, IgG, or IgM antibody. In certain embodiments, the antibody is an IgG1 antibody. In certain embodiments, the antibody is an IgG2 antibody. In certain embodiments, the antibody is an antibody fragment comprising an antigen-binding site. In some embodiments, the antibody is a bispecific antibody or a multispecific antibody. In some embodiments, the antibody is a monovalent antibody. In some embodiments, the antibody is a monospecific antibody. In some embodiments, the antibody is a bivalent antibody. In some embodiments, the antibody is conjugated to a cytotoxic moiety. In some embodiments, the antibody is isolated. In some embodiments, the antibody is substantially pure.

[0102] The RSPO3-binding agents (e.g., antibodies) of the present invention can be assayed for specific binding by any method known in the art. The immunoassays which can be used include, but are not limited to, competitive and non-competitive assay systems using techniques such as Biacore analysis, FACS analysis, immunofluorescence, immunocytochemistry, Western blot analysis, radioimmunoassays, ELISA, "sandwich" immunoassays, immunoprecipitation assays, precipitation reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. Such assays are routine and well-known in the art (see, e.g., Ausubel et al., Editors, 1994-present, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York, NY).

[0103] For example, the specific binding of an antibody to human RSPO3 may be determined using ELISA. An ELISA assay comprises preparing antigen, coating wells of a 96 well microtiter plate with antigen, adding the RSPO3-binding antibody or other RSPO3-binding agent conjugated to a detectable compound such as an enzymatic substrate (e.g. horseradish peroxidase or alkaline phosphatase) to the well, incubating for a period of time and detecting the presence of the antibody bound to the antigen. In some embodiments, the RSPO3-binding antibody or agent is not conjugated to a detectable compound, but instead a second conjugated antibody that recognizes the RSPO3-binding agent or antibody (e.g., an anti-Fc antibody) and is conjugated to a detectable compound is added to the well. In some embodiments, instead of coating the well with the antigen, the RSPO3-binding agent or antibody can be coated to the well and a second antibody conjugated to a detectable compound can be added following the addition of

the antigen to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art.

[0104] In another example, the specific binding of an antibody to human RSPO3 may be determined using FACS. A FACS screening assay may comprise generating a cDNA construct that expresses an antigen as a fusion protein (e.g., RSPO3-Fc or RSPO3-CD4TM), transfecting the construct into cells, expressing the antigen on the surface of the cells, mixing the RSPO3-binding agent with the transfected cells, and incubating for a period of time. The cells bound by the RSPO3-binding agent may be identified using a secondary antibody conjugated to a detectable compound (e.g., PE-conjugated anti-Fc antibody) and a flow cytometer. One of skill in the art would be knowledgeable as to the parameters that can be modified to optimize the signal detected as well as other variations of FACS that may enhance screening (e.g., screening for blocking antibodies).

[0105] The binding affinity of an antibody or other binding-agent to an antigen (e.g., RSPO3) and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^{3}H or ^{125}I), or fragment or variant thereof, with the antibody of interest in the presence of increasing amounts of unlabeled antigen followed by the detection of the antibody bound to the labeled antigen. The affinity of the antibody for the antigen and the binding off-rates can be determined from the data by Scatchard plot analysis. In some embodiments, Biacore kinetic analysis is used to determine the binding on and off rates of antibodies or agents that bind an antigen (e.g., RSPO3). In some embodiments, Biacore kinetic analysis comprises analyzing the binding and dissociation of antibodies from chips with immobilized antigen (e.g., RSPO3) on their surface. In some embodiments, Biacore kinetic analysis comprises analyzing the binding and dissociation of antigen (e.g., RSPO3) from chips with immobilized antibody (e.g., anti-RSPO3 antibody) on their surface.

[0106] In certain embodiments, the invention provides a RSPO3-binding agent (e.g., an antibody) that specifically binds human RSPO3, wherein the RSPO3-binding agent (e.g., an antibody) comprises one, two, three, four, five, and/or six of the CDRs of antibody 131R002, antibody 131R003, or the humanized variants thereof, including h131R005/131R007, h131R006A, h131R006B, h131R008, h131R010, or h131R011 (see Table 1). In some embodiments, the RSPO3-binding agent comprises one or more of the CDRs of 131R002, 131R003, or the humanized variants thereof, including h131R005/131R007, h131R006A, h131R006B, h131R008, h131R010, or h131R011; two or more of the CDRs of 131R002, 131R003, or the humanized variants thereof, including h131R005/131R007, h131R006A, h131R006B, h131R008, h131R010, or h131R011; three or more of the CDRs of 131R002, 131R003, or the humanized variants thereof, including h131R005/131R007, h131R006A, h131R006B, h131R008, h131R010, or h131R011; four or more of the CDRs of 131R002, 131R003, or the humanized variants thereof, including h131R005/131R007, h131R006A, h131R006B, h131R008, h131R010, or h131R011; five or more of the

CDRs of 131R002, 131R003, or the humanized variants thereof, including 131R005/131R007, h131R006A, h131R006B, or h131R008, h131R010, or h131R011; or all six of the CDRs of 131R002, 131R003, or the humanized variants thereof, including h131R005/131R007, h131R006A, h131R006B, h131R008, h131R010, or h131R011.

Table 1

	131R002/131R003 and Humanized Variants
HC CDR1	KASGYTFTDYS (SEQ ID NO:9) or KASGYTFTSYTF (SEQ ID NO:34) or DYSIH (SEQ ID NO:78)
HC CDR2	IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79)
HC CDR3	ATYFANYFDY (SEQ ID NO:11) or ATYFANNFDY (SEQ ID NO:35) or TYFANNFD (SEQ ID NO:80
LC CDR1	QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81)
LC CDR2	AAS (SEQ ID NO: 13) or AASNLES (SEQ ID NO:82)
LC CDR3	QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83)

[0107] In certain embodiments, the invention provides a RSPO3-binding agent (e.g., an antibody) that specifically binds human RSPO3, wherein the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), or TYFANNFD (SEQ ID NO:80). In some embodiments, the RSPO3-binding agent further comprises a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83). In some embodiments, the RSPO3-binding agent comprises a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83). In certain embodiments, the RSPO3-binding agent comprises: (a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), and (b) a light chain CDR1 comprising

QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In certain embodiments, the RSPO3-binding agent comprises: (a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY (SEQ ID NO:35), and (b) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In certain embodiments, the RSPO3-binding agent comprises: (a) a heavy chain CDR1 comprising KASGYTFTSYTF (SEQ ID NO:34), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), and (b) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In certain embodiments, the RSPO3-binding agent comprises: (a) a heavy chain CDR1 comprising KASGYTFTSYTF (SEQ ID NO:34), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY (SEQ ID NO:35), and (b) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In certain embodiments, the RSPO3-binding agent comprises: (a) a heavy chain CDR1 comprising DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and (b) a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLTF (SEQ ID NO:83). In certain embodiments, the RSPO3-binding agent comprises: (a) a heavy chain CDR1 comprising DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and (b) a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In certain embodiments, the RSPO3-binding agent comprises: (a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and (b) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14).

[0108] In certain embodiments, the invention provides a RSPO3-binding agent (e.g., an antibody or bispecific antibody) that specifically binds human RSPO3, wherein the RSPO3-binding agent comprises: (a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), DYSIH (SEQ ID NO:78), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions;

(b) a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), YIYPSNGDSGYNQKFK (SEQ ID NO:79), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (c) a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), TYFANNFD (SEQ ID NO:80), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (d) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), KASQSVDYDGDSYMN (SEQ ID NO:81), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (e) a light chain CDR2 comprising AAS (SEQ ID NO:13), AASNLES (SEQ ID NO:82), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and (f) a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14), QQSNEDPLTF (SEQ ID NO:83), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain embodiments, the amino acid substitutions are conservative substitutions. In some embodiments, the substitutions are made as part of a germline humanization process.

[0109] In certain embodiments, the invention provides a RSPO3-binding agent (e.g., an antibody) that specifically binds RSPO3, wherein the RSPO3-binding agent comprises a heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62 and/or a light chain variable region having at least 80% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:15. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:16. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:36. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:37. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:44. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:45. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:62. In certain embodiments, the RSPO3-binding agent comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a light chain

variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62 and/or a light chain variable region having at least about 95% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62, and/or a light chain variable region comprising SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62, and a light chain variable region consisting essentially of SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62, and a light chain variable region consisting of SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86.

[0110] In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:45 and a light chain variable region comprising SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:44 and a light chain variable region consisting essentially of SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:45 and a light chain variable region consisting essentially of SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:62 and a light chain variable region consisting essentially of SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:44 and a light chain variable region consisting of SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable

region consisting of SEQ ID NO:45 and a light chain variable region consisting of SEQ ID NO:17. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:62 and a light chain variable region consisting of SEQ ID NO:17.

[0111] In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:45 and a light chain variable region comprising SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:44 and a light chain variable region consisting essentially of SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:45 and a light chain variable region consisting essentially of SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:62 and a light chain variable region consisting essentially of SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:44 and a light chain variable region consisting of SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:45 and a light chain variable region consisting of SEQ ID NO:72. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:62 and a light chain variable region consisting of SEQ ID NO:72.

[0112] In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:45 and a light chain variable region comprising SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:44 and a light chain variable region consisting essentially of SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:45 and a light chain variable region consisting essentially of SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:62 and a light chain variable region consisting essentially of SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:44 and a light chain variable region consisting of SEQ ID NO:86. In certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:45 and a light chain variable region consisting of SEQ ID NO:86. In

certain embodiments, the RSPO3-binding agent comprises a heavy chain variable region consisting of SEQ ID NO:62 and a light chain variable region consisting of SEQ ID NO:86.

[0113] In certain embodiments, the invention provides a RSPO3-binding agent (e.g., an antibody) that specifically binds RSPO3, wherein the RSPO3-binding agent comprises: (a) a heavy chain having at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69; and/or (b) a light chain having at least 90% sequence identity to SEQ ID NO:29, SEQ ID NO:74, or SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises: (a) a heavy chain having at least 95% sequence identity to SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69; and/or (b) a light chain having at least 95% sequence identity to SEQ ID NO:29, SEQ ID NO:74, or SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:27 and/or a light chain comprising SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:28 and/or a light chain comprising SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:39 and/or a light chain comprising SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:42 and/or a light chain comprising SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:48 and/or a light chain comprising SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:49 and/or a light chain comprising SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:64 and/or a light chain comprising SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:69 and/or a light chain comprising SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:48 and/or a light chain comprising SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:49 and/or a light chain comprising SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:64 and/or a light chain comprising SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:69 and/or a light chain comprising SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69; and a light chain consisting essentially of SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:28, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69 and a light chain consisting of SEQ ID NO:29. In some embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:28, SEQ ID

NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69 and a light chain consisting of SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:27 and/or a light chain comprising SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:28 and/or a light chain comprising SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:39 and/or a light chain comprising SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:42 and/or a light chain comprising SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:48 and/or a light chain comprising SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:49 and/or a light chain comprising SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:64 and/or a light chain comprising SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:69 and/or a light chain comprising SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:48 and/or a light chain comprising SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:49 and/or a light chain comprising SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:64 and/or a light chain comprising SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:69 and/or a light chain comprising SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69; and a light chain consisting essentially of SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69 and a light chain consisting of SEQ ID NO:74. In some embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69; and a light chain consisting essentially of SEQ ID NO:88. In some embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69 and a light chain consisting of SEQ ID NO:88.

[0114] In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:48 and a light chain comprising SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:49 and a light chain variable region comprising SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:64 and a light chain variable region comprising SEQ ID NO:29. In certain embodiments, the RSPO3-

binding agent comprises a heavy chain comprising SEQ ID NO:69 and a light chain variable region comprising SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:48 and a light chain consisting essentially of SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:49 and a light chain consisting essentially of SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:64 and a light chain consisting essentially of SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:69 and a light chain consisting essentially of SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:48 and a light chain consisting of SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:49 and a light chain consisting of SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:64 and a light chain consisting of SEQ ID NO:29. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:69 and a light chain consisting of SEQ ID NO:29.

[0115] In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:48 and a light chain comprising SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:49 and a light chain variable region comprising SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:64 and a light chain variable region comprising SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:69 and a light chain variable region comprising SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:48 and a light chain consisting essentially of SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:49 and a light chain consisting essentially of SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:64 and a light chain consisting essentially of SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:69 and a light chain consisting essentially of SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:48 and a light chain consisting of SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:49 and a light chain consisting of SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:64 and a light chain consisting of SEQ ID NO:74. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:69 and a light chain consisting of SEQ ID NO:74.

[0116] In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:48 and a light chain comprising SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent

comprises a heavy chain comprising SEQ ID NO:49 and a light chain variable region comprising SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:64 and a light chain variable region comprising SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain comprising SEQ ID NO:69 and a light chain variable region comprising SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:48 and a light chain consisting essentially of SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:49 and a light chain consisting essentially of SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:64 and a light chain consisting essentially of SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting essentially of SEQ ID NO:69 and a light chain consisting essentially of SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:48 and a light chain consisting of SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:49 and a light chain consisting of SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:64 and a light chain consisting of SEQ ID NO:88. In certain embodiments, the RSPO3-binding agent comprises a heavy chain consisting of SEQ ID NO:69 and a light chain consisting of SEQ ID NO:88.

[0117] In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and light chain variable region of the 131R002 antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain and light chain of the 131R002 antibody (with or without the leader sequence). In certain embodiments, a RSPO3-binding agent is the 131R002 antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain variable region of the 131R002 antibody in a chimeric form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain variable region of the 131R002 antibody in a humanized form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain CDRs and/or light chain CDRs of the 131R002 antibody in a humanized form of the antibody. In some embodiments, the humanized version of 131R002 is an IgG1 antibody. In some embodiments, the humanized version of 131R002 is an IgG2 antibody.

[0118] In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, the antibody 131R002.

[0119] In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and light chain variable region of the 131R003 antibody. In some embodiments, the RSPO3-binding agent comprises the heavy chain variable region of the 131R003 antibody wherein the heavy chain variable region from 131R003 has been affinity-matured. In some embodiments, the RSPO3-binding agent comprises the heavy chain variable region of the 131R003 antibody wherein the heavy chain variable

region comprises at least one modified or altered CDR as compared to the parent 131R003 antibody. In some embodiments, the RSPO-binding agent comprises the heavy chain variable region of the 131R003 antibody wherein the heavy chain variable region comprises a modified CDR1 as compared to the parent 131R003 antibody. In some embodiments, the RSPO-binding agent comprises the heavy chain variable region of the 131R003 antibody wherein the heavy chain variable region comprises a modified CDR2 as compared to the parent 131R003 antibody. In some embodiments, the RSPO-binding agent comprises the heavy chain variable region of the 131R003 antibody wherein the heavy chain variable region comprises a modified CDR3 as compared to the parent 131R003 antibody. In some embodiments, the RSPO-binding agent comprises the heavy chain variable region of the 131R003 antibody wherein the heavy chain variable region comprises a modified CDR1 and CDR3 as compared to the parent 131R003 antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain and light chain of the 131R003 antibody (with or without the leader sequence). In certain embodiments, a RSPO3-binding agent is the 131R003 antibody. In certain embodiments, a RSPO3-binding agent is a variant of the 131R003 antibody that comprises a different heavy chain CDR1 as compared to the parent 131R003 antibody. In certain embodiments, a RSPO3-binding agent is a variant of the 131R003 antibody that comprises a different heavy chain CDR3 as compared to the parent 131R003 antibody. In certain embodiments, a RSPO3-binding agent is a variant of the 131R003 antibody that comprises a different heavy chain CDR1 and a different heavy chain CDR3 as compared to the parent 131R003 antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain variable region of the 131R003 antibody or of any of the variants of 131R003 in a chimeric form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain variable region of the 131R003 antibody or of any of the variants of 131R003 in a humanized form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain CDRs and/or light chain CDRs of the 131R003 antibody or of any of the variants of 131R003 in a humanized form of the antibody. In some embodiments, the humanized version of 131R003 or of 131R003 variants is an IgG1 antibody. In some embodiments, the humanized version of 131R003 or of 131R003 variants is an IgG2 antibody.

[0120] In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, the antibody 131R003. In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, a variant of the antibody 131R003.

[0121] In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and light chain variable region of the 131R006B antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain and light chain of the 131R006B antibody (with or without the leader sequence). In certain embodiments, a RSPO3-binding agent is the 131R006B antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain

variable region of the 131R006B antibody in a chimeric form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain variable region of the 131R006B antibody in a humanized form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain CDRs and/or light chain CDRs of the 131R006B antibody in a humanized form of the antibody. In some embodiments, the humanized version of 131R006B is an IgG1 antibody. In some embodiments, the humanized version of 131R006B is an IgG2 antibody.

[0122] In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, the antibody 131R006B. In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, a variant of the antibody 131R006B.

[0123] In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and light chain variable region of the 131R005/131R007 antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain and light chain of the 131R005/131R007 antibody (with or without the leader sequence). In certain embodiments, a RSPO3-binding agent is the 131R005/131R007 antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain variable region of the 131R005/131R007 antibody in a chimeric form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain variable region of the 131R005/131R007 antibody in a humanized form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain CDRs and/or light chain CDRs of the 131R005/131R007 antibody in a humanized form of the antibody. In some embodiments, the humanized version of 131R005/131R007 is an IgG1 antibody. In some embodiments, the humanized version of 131R005/131R007 is an IgG2 antibody. In some embodiments, the anti-RSPO3 antibody is 131R008.

[0124] In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, the antibody 131R005/131R007. In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, a variant of the antibody 131R005/131R007.

[0125] In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, the antibody 131R008. In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, a variant of the antibody 131R008.

[0126] In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and light chain variable region of the h131R010 or h131R011 antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain and light chain of the h131R010 or 131R011 antibody (with or without the leader sequence). In certain embodiments, a RSPO3-binding agent is the h131R010 antibody. In certain embodiments, a RSPO3-binding agent is the h131R011 antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain variable region and/or light chain variable region of the h131R010 or h131R011 antibody in a chimeric form of the antibody. In certain embodiments, a RSPO3-binding agent comprises the heavy chain CDRs and/or light chain CDRs of the h131R010 or h131R011

antibody. In some embodiments, the anti-RSPO3 antibody is h131R010. In some embodiments, the anti-RSPO3 antibody is h131R011.

[0127] In some embodiments, the RSPO3-binding agent comprises a heavy chain variable region encoded by the plasmid deposited with American Type Culture Collection (ATCC), and designated PTA-_____. In some embodiments, the RSPO3-binding agent comprises a light chain variable region encoded by the plasmid deposited with ATCC and designated PTA-_____. In some embodiments, the RSPO3-binding agent comprises a heavy chain variable region encoded by the plasmid deposited with ATCC and designated PTA-_____, and a light chain variable region encoded by the plasmid deposited with ATCC and designated PTA-_____. In some embodiments, the RSPO3-binding agent comprises a heavy chain encoded by the plasmid deposited with ATCC and designated PTA-_____. In some embodiments, the RSPO3-binding agent comprises a light chain encoded by the plasmid deposited with ATCC and designated PTA-_____. In some embodiments, the RSPO3-binding agent comprises a heavy chain encoded by the plasmid deposited with ATCC and designated PTA-_____, and a light chain encoded by the plasmid deposited with ATCC and designated PTA-_____.

[0128] In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, the antibody h131R010. In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, a variant of the antibody h131R010.

[0129] In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, the antibody h131R011. In certain embodiments, a RSPO3-binding agent comprises, consists essentially of, or consists of, a variant of the antibody h131R011.

[0130] In certain embodiments, the invention provides a RSPO3-binding agent that is a bispecific antibody. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first antigen-binding site that specifically binds human RSPO3. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first antigen-binding site that specifically binds human RSPO3 and a second antigen-binding site that binds a second target. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human RSPO3, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), OR TYFANNFD (SEQ ID NO:80). In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human RSPO3, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY (SEQ ID NO:35). In some embodiments, the RSPO3-binding agent is a

bispecific antibody comprising: a first antigen-binding site that specifically binds human RSPO3, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTSYTF (SEQ ID NO:34), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY (SEQ ID NO:35). In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human RSPO3, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTSYTF (SEQ ID NO:34), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11). In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human RSPO3, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising DYSIH (SEQ ID NO:80), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80). In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human RSPO3, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising DYSIH (SEQ ID NO:80) or KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80). In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human RSPO3, wherein the first antigen-binding site comprises (a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35) or TYFANNFD (SEQ ID NO:80), and a second antigen-binding site, wherein the first antigen-binding site and the second antigen-binding site comprise a common (i.e., identical) light chain. In some embodiments, the bispecific antibody comprises a first antigen-binding site comprising a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83).

[0131] In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62. In certain embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62. In some embodiments, the bispecific antibody comprises a light chain variable region at least about 85%, at least about 90%, at least about 95%, at least about 97%,

or at least about 99% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region comprising SEQ ID NO:44. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region comprising SEQ ID NO:45. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region comprising SEQ ID NO:62. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first light chain variable region comprising SEQ ID NO:17. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first light chain variable region comprising SEQ ID NO:72. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first light chain variable region comprising SEQ ID NO:86.

[0132] In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region comprising SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62 and a first heavy chain constant region comprising SEQ ID NO:60 or SEQ ID NO:61. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region comprising SEQ ID NO:44 and a first heavy chain constant region comprising SEQ ID NO:60 or SEQ ID NO:61. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region comprising SEQ ID NO:45 and a first heavy chain constant region comprising SEQ ID NO:60 or SEQ ID NO:61. In some embodiments, the RSPO3-binding agent is a bispecific antibody comprising a first heavy chain variable region comprising SEQ ID NO:62 and a first heavy chain constant region comprising SEQ ID NO:60 or SEQ ID NO:61.

[0133] In certain embodiments, the RSPO3-binding agent is a bispecific antibody that specifically binds human RSPO3 and a second target. In some embodiments, the RSPO3-binding agent is a bispecific antibody that specifically binds human RSPO3 and a second human RSPO. In some embodiments, the RSPO3-binding agent is a bispecific antibody that specifically binds human RSPO3 and a second human RSPO selected from the group consisting of RSPO1, RSPO2, and RSPO4. Non-limiting examples of antibodies to human RSPO have been described in, for example, International Patent Pub. No. WO 2013/012747.

[0134] In some embodiments, the RSPO3-binding agent is a bispecific antibody that specifically binds human RSPO3 and human RSPO1. In some embodiments, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human RSPO3, and b) a second antigen-binding site that specifically binds human RSPO1, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11),

ATYFANNFDY (SEQ ID NO:35) or TYFANNFD (SEQ ID NO:80); and wherein both the first and second antigen-binding sites comprise a common light chain.

[0135] In some embodiments, the RSPO3-binding agent is a bispecific antibody that specifically binds human RSPO3 and human RSPO2. In some embodiments, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human RSPO3, and b) a second antigen-binding site that specifically binds human RSPO2, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35) or TYFANNFD (SEQ ID NO:80); and wherein both the first and second antigen-binding sites comprise a common light chain.

[0136] In some embodiments, the RSPO3-binding agent is a bispecific antibody that comprises a heavy chain variable region from the anti-RSPO3 antibody 131R003. In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a heavy chain variable region from a variant of the anti-RSPO3 antibody 131R003. In some embodiments, the RSPO3-binding agent is a bispecific antibody that comprises a heavy chain variable region from the anti-RSPO3 antibody 131R006B. In some embodiments, the RSPO3-binding agent is a bispecific antibody that comprises a heavy chain variable region from the anti-RSPO3 antibody h131R005/131R007. In some embodiments, the RSPO3-binding agent is a bispecific antibody that comprises a heavy chain variable region from the anti-RSPO3 antibody h131R010 or h131R011.

[0137] In some embodiments, the RSPO3-binding agent is a bispecific antibody that comprises a first CH3 domain and a second CH3 domain, each of which is modified to promote formation of heteromultimers. In some embodiments, the first and second CH3 domains are modified using a knobs-into-holes technique. In some embodiments, the first and second CH3 domains comprise changes in amino acids that result in altered electrostatic interactions. In some embodiments, the first and second CH3 domains comprise changes in amino acids that result in altered hydrophobic/hydrophilic interactions.

[0138] In some embodiments, the RSPO3-binding agent is a bispecific antibody that comprises heavy chain constant regions selected from the group consisting of: (a) a first human IgG1 constant region, wherein the amino acids corresponding to positions 253 and 292 of IgG1 (SEQ ID NO:56) are replaced with glutamate or aspartate, and a second human IgG1 constant region, wherein the amino acids corresponding to positions 240 and 282 of IgG1 (SEQ ID NO:56) are replaced with lysine; (b) a first human IgG2 constant region, wherein the amino acids corresponding to positions 249 and 288 of IgG2 (SEQ ID NO:57) are replaced with glutamate or aspartate, and a second human IgG2 constant region wherein the amino acids corresponding to positions 236 and 278 of IgG2 (SEQ ID NO:57) are replaced with lysine; (c) a first human IgG3 constant region, wherein the amino acids corresponding to positions

300 and 339 of IgG3 (SEQ ID NO:58) are replaced with glutamate or aspartate, and a second human IgG3 constant region wherein the amino acids corresponding to positions 287 and 329 of IgG3 (SEQ ID NO:58) are replaced with lysine; and (d) a first human IgG4 constant region, wherein the amino acids corresponding to positions 250 and 289 of IgG4 (SEQ ID NO:59) are replaced with glutamate or aspartate, and a second IgG4 constant region wherein the amino acids corresponding to positions 237 and 279 of IgG4 (SEQ ID NO:59) are replaced with lysine.

[0139] In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a first human IgG1 constant region with amino acid substitutions at positions corresponding to positions 253 and 292 of IgG1 (SEQ ID NO:56), wherein the amino acids at positions corresponding to positions 253 and 292 of IgG1 (SEQ ID NO:56) are replaced with glutamate or aspartate, and a second human IgG1 constant region with amino acid substitutions at positions corresponding to positions 240 and 282 of IgG1 (SEQ ID NO:56), wherein the amino acids at positions corresponding to positions 240 and 282 of IgG1 (SEQ ID NO:56) are replaced with lysine. In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a first human IgG2 constant region with amino acid substitutions at positions corresponding to positions 249 and 288 of IgG2 (SEQ ID NO:57), wherein the amino acids at positions corresponding to positions 249 and 288 of IgG2 (SEQ ID NO:57) are replaced with glutamate or aspartate, and a second human IgG2 constant region with amino acid substitutions at positions corresponding to positions 236 and 278 of IgG2 (SEQ ID NO:57), wherein the amino acids at positions corresponding to positions 236 and 278 of IgG2 (SEQ ID NO:57) are replaced with lysine. In some embodiments, the RSPO-binding agent is a bispecific antibody which comprises a first human IgG3 constant region with amino acid substitutions at positions corresponding to positions 300 and 339 of IgG3 (SEQ ID NO:58), wherein the amino acids at positions corresponding to positions 300 and 339 of IgG3 (SEQ ID NO:58) are replaced with glutamate or aspartate, and a second human IgG3 constant region with amino acid substitutions at positions corresponding to positions 287 and 329 of IgG3 (SEQ ID NO:58), wherein the amino acids at positions corresponding to positions 287 and 329 of IgG3 (SEQ ID NO:58) are replaced with lysine. In some embodiments, the RSPO-binding agent is a bispecific antibody which comprises a first human IgG4 constant region with amino acid substitutions at positions corresponding to positions 250 and 289 of IgG4 (SEQ ID NO:59), wherein the amino acids at positions corresponding to positions 250 and 289 of IgG4 (SEQ ID NO:59) are replaced with glutamate or aspartate, and a second human IgG4 constant region with amino acid substitutions at positions corresponding to positions 237 and 279 of IgG4 (SEQ ID NO:59), wherein the amino acids at positions corresponding to positions 237 and 279 of IgG4 (SEQ ID NO:59) are replaced with lysine.

[0140] In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a first human IgG1 constant region with amino acid substitutions at positions corresponding to positions 253 and 292 of IgG1 (SEQ ID NO:56), wherein the amino acids are replaced with glutamate, and a second human

IgG1 constant region with amino acid substitutions at positions corresponding to positions 240 and 282 of IgG1 (SEQ ID NO:56), wherein the amino acids are replaced with lysine. In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a first human IgG1 constant region with amino acid substitutions at positions corresponding to positions 253 and 292 of IgG1 (SEQ ID NO:56), wherein the amino acids are replaced with aspartate, and a second human IgG1 constant region with amino acid substitutions at positions corresponding to positions 240 and 282 of IgG1 (SEQ ID NO:56), wherein the amino acids are replaced with lysine.

[0141] In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a first human IgG2 constant region with amino acid substitutions at positions corresponding to positions 249 and 288 of IgG2 (SEQ ID NO:57), wherein the amino acids are replaced with glutamate, and a second human IgG2 constant region with amino acid substitutions at positions corresponding to positions 236 and 278 of IgG2 (SEQ ID NO:57), wherein the amino acids are replaced with lysine. In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a first human IgG2 constant region with amino acid substitutions at positions corresponding to positions 249 and 288 of IgG2 (SEQ ID NO:57), wherein the amino acids are replaced with aspartate, and a second human IgG2 constant region with amino acid substitutions at positions corresponding to positions 236 and 278 of IgG2 (SEQ ID NO:57), wherein the amino acids are replaced with lysine.

[0142] In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a heavy chain constant region of SEQ ID NO:60. In some embodiments, the RSPO-binding agent is a bispecific antibody which comprises a heavy chain constant region of SEQ ID NO:61. In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises a first heavy chain constant region of SEQ ID NO:60 and a second heavy chain constant region of SEQ ID NO:61.

[0143] In some embodiments, the RSPO3-binding agent is a bispecific antibody which binds RSPO3 with a K_D of about 50nM or less, about 25nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In some embodiments, the RSPO3-binding agent is a bispecific antibody which binds a second target (e.g., RSPO2) with a K_D of about 50nM or less, about 25nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In some embodiments, the RSPO3-binding agent is a bispecific antibody which binds RSPO3 with a K_D of about 50nM or less and binds a second target (e.g., RSPO2) with a K_D of about 50nM or less. In some embodiments, the RSPO3-binding agent is a bispecific antibody which binds RSPO3 with a K_D of about 25nM or less and binds a second target (e.g., RSPO2) with a K_D of about 25nM or less. In some embodiments, the RSPO3-binding agent is a bispecific antibody which binds RSPO3 with a K_D of about 10nM or less and binds a second target (e.g., RSPO2) with a K_D of about 10nM or less. In some embodiments, the RSPO3-binding agent is a bispecific antibody which binds RSPO3 with a K_D of about 1nM or less and binds a second target (e.g., RSPO2) with a K_D of about 1nM or less.

[0144] In some embodiments, the RSPO3-binding agent is a bispecific antibody which comprises one antigen-binding site with a binding affinity that is weaker than the binding affinity of the second antigen-binding site. For example, in some embodiments, the bispecific antibody may bind RSPO3 with a K_D ranging from about 0.1nM to 1nM and may bind a second target (e.g., RSPO2) with a K_D ranging from about 1nM to 10nM. Or the bispecific antibody may bind RSPO3 with a K_D ranging from about 1nM to 10nM and may bind a second target (e.g., RSPO2) with a K_D ranging from about 0.1nM to 1nM. In some embodiments, the bispecific antibody may bind RSPO3 with a K_D ranging from about 0.1nM to 1nM and may bind a second target (e.g., RSPO2) with a K_D ranging from about 1nM to 10nM. Or the bispecific antibody may bind RSPO3 with a K_D ranging from about 1nM to 10nM and may bind a second target (e.g., RSPO2) with a K_D ranging from about 0.1nM to 1nM. In some embodiments, the difference in affinity between the two antigen-binding sites may be about 2-fold or more, about 3-fold or more, about 5-fold or more, about 8-fold or more, about 10-fold or more, about 15-fold or more, about 30-fold or more, about 50-fold or more, or about 100-fold or more. In some embodiments, at least one amino acid residue in at least one CDR of the antigen-binding site for RSPO3 is substituted with a different amino acid so that the affinity of the RSPO3-binding site is altered. In some embodiments, the affinity of the RSPO3-binding site is increased. In some embodiments, the affinity of the RSPO3-binding site is decreased. In some embodiments, at least one amino acid residue in at least one CDR of the antigen-binding site for the second target (e.g., RSPO2) is substituted with a different amino acid so that the affinity of the second antigen-binding site is altered. In some embodiments, the affinity of the second antigen-binding site is increased. In some embodiments, the affinity of the second antigen-binding site is decreased. In some embodiments, the affinities of both the RSPO3 and the second antigen-binding sites are altered.

[0145] The invention provides polypeptides, including, but not limited to, antibodies that specifically bind human RSPO proteins. In some embodiments, the polypeptides bind human RSPO3. In some embodiments, the polypeptides bind human RSPO3 and at least one additional human RSPO selected from the group consisting of RSPO1, RSPO2, and RSPO4.

[0146] In certain embodiments, the polypeptide comprises one, two, three, four, five, and/or six of the CDRs of antibody 131R002, 131R003, or variants of 131R003 including h131R005/131R007, h131R006A, h131R006B, h131R010, and h131R011 (see Table 1 herein). In some embodiments, the polypeptide comprises CDRs with up to four (i.e., 0, 1, 2, 3, or 4) amino acid substitutions per CDR. In certain embodiments, the heavy chain CDR(s) are contained within a heavy chain variable region. In certain embodiments, the light chain CDR(s) are contained within a light chain variable region.

[0147] In some embodiments, the invention provides a polypeptide that specifically binds human RSPO3, wherein the polypeptide comprises an amino acid sequence having at least about 80% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID

NO:45, or SEQ ID NO:62, and/or an amino acid sequence having at least about 80% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:17,SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62, and/or an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:15 and/or an amino acid sequence comprising SEQ ID NO:17. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:16 and/or an amino acid sequence comprising SEQ ID NO:17. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:36 and/or an amino acid sequence comprising SEQ ID NO:17. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:37 and/or an amino acid sequence comprising SEQ ID NO:17. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:44 and/or an amino acid sequence comprising SEQ ID NO:17. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:45 and/or an amino acid sequence comprising SEQ ID NO:17. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:62 and/or an amino acid sequence comprising SEQ ID NO:17. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:15 and/or an amino acid sequence comprising SEQ ID NO:72. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:16 and/or an amino acid sequence comprising SEQ ID NO:72. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:36 and/or an amino acid sequence comprising SEQ ID NO:72. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:37 and/or an amino acid sequence comprising SEQ ID NO:72. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:44 and/or an amino acid sequence comprising SEQ ID NO:72. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:45 and/or an amino acid sequence comprising SEQ ID NO:72. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:62 and/or an amino acid sequence comprising SEQ ID NO:72. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:44 and/or an amino acid sequence comprising SEQ ID NO:86. In certain embodiments, the

polypeptide comprises an amino acid sequence comprising SEQ ID NO:45 and/or an amino acid sequence comprising SEQ ID NO:86. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:62 and/or an amino acid sequence comprising SEQ ID NO:86.

[0148] In some embodiments, the invention provides a polypeptide that specifically binds human RSPO3, wherein the polypeptide comprises an amino acid sequence having at least about 80% sequence identity to SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:63, or SEQ ID NO:68, and/or an amino acid sequence having at least about 80% sequence identity to SEQ ID NO:23, SEQ ID NO:73, or SEQ ID NO:87. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:63, or SEQ ID NO:68. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:23, SEQ ID NO:73, or SEQ ID NO:87. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:63, or SEQ ID NO:68, and/or an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:23, SEQ ID NO:73, or SEQ ID NO:87. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:63, or SEQ ID NO:68, and/or an amino acid sequence comprising SEQ ID NO:23, SEQ ID NO:73, or SEQ ID NO:87. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:63, or SEQ ID NO:68, and/or SEQ ID NO:23, SEQ ID NO:73, or SEQ ID NO:87.

[0149] In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69, and/or an amino acid sequence comprising SEQ ID NO:29, SEQ ID NO:74, or SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:48 and/or an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:49 and/or an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:64 and/or an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:69 and/or an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:48 and/or an amino acid sequence comprising SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID

NO:49 and/or an amino acid sequence comprising SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:64 and/or an amino acid sequence comprising SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:69 and/or an amino acid sequence comprising SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:48 and/or an amino acid sequence comprising SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:49 and/or an amino acid sequence comprising SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:64 and/or an amino acid sequence comprising SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:69 and/or an amino acid sequence comprising SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69 and/or an amino acid sequence consisting essentially of SEQ ID NO:29, SEQ ID NO:74, SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:48 and/or an amino acid sequence consisting essentially of SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:49 and/or an amino acid sequence consisting essentially of SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:64 and/or an amino acid sequence consisting essentially of SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:69 and/or an amino acid sequence consisting essentially of SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:48 and/or an amino acid sequence consisting essentially of SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:49 and/or an amino acid sequence consisting essentially of SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:64 and/or an amino acid sequence consisting essentially of SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:69 and/or an amino acid sequence consisting essentially of SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:48 and/or an amino acid sequence consisting essentially of SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:49 and/or an amino acid sequence consisting essentially of SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:64 and/or an amino acid sequence consisting essentially of SEQ ID NO:88. In certain embodiments,

the polypeptide comprises an amino acid sequence consisting essentially of SEQ ID NO:69 and/or an amino acid sequence consisting essentially of SEQ ID NO:88.

[0150] In some embodiments, the invention provides a polypeptide that specifically binds human RSPO3, wherein the polypeptide comprises an amino acid sequence having at least about 80% sequence identity to SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69, and/or an amino acid sequence having at least about 80% sequence identity to SEQ ID NO:29, SEQ ID NO:74, or SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:29, SEQ ID NO:74, or SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69, and/or an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:29, SEQ ID NO:74, SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69, and/or an amino acid sequence comprising SEQ ID NO:29, SEQ ID NO:74, or SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:48 and/or an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:49 and/or an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:64 and/or an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:69 and/or an amino acid sequence comprising SEQ ID NO:29. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:27 and/or SEQ ID NO:29. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:28 and/or SEQ ID NO:29. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:48 and/or SEQ ID NO:29. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:49 and/or SEQ ID NO:29. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:64 and/or SEQ ID NO:29. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:69 and/or SEQ ID NO:29. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:48 and/or an amino acid sequence comprising SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:49 and/or an amino acid sequence comprising SEQ ID NO:74. In certain

embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:64 and/or an amino acid sequence comprising SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:69 and/or an amino acid sequence comprising SEQ ID NO:74. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:27 and/or SEQ ID NO:74. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:28 and/or SEQ ID NO:74. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:48 and/or SEQ ID NO:74. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:49 and/or SEQ ID NO:74. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:64 and/or SEQ ID NO:74. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:69 and/or SEQ ID NO:74. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:48 and/or an amino acid sequence comprising SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:49 and/or an amino acid sequence comprising SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:64 and/or an amino acid sequence comprising SEQ ID NO:88. In certain embodiments, the polypeptide comprises an amino acid sequence comprising SEQ ID NO:69 and/or an amino acid sequence comprising SEQ ID NO:88. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:48 and/or SEQ ID NO:88. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:49 and/or SEQ ID NO:88. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:64 and/or SEQ ID NO:88. In certain embodiments, the polypeptide consists essentially of SEQ ID NO:69 and/or SEQ ID NO:88.

[0151] In some embodiments, a RSPO3-binding agent comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:86, SEQ ID NO:87, and SEQ ID NO:88.

[0152] Many proteins, including antibodies, contain a signal sequence that directs the transport of the proteins to various locations. Signal sequences (also referred to as signal peptides or leader sequences) are located at the N-terminus of nascent polypeptides. They target the polypeptide to the endoplasmic reticulum and the proteins are sorted to their destinations, for example, to the inner space of an organelle, to an interior membrane, to the cell's outer membrane, or to the cell exterior via secretion. Most signal sequences are cleaved from the protein by a signal peptidase after the proteins are transported to the endoplasmic reticulum. The cleavage of the signal sequence from the polypeptide usually occurs at a specific site in the amino acid sequence and is dependent upon amino acid residues within the signal

sequence. Although there is usually one specific cleavage site, more than one cleavage site may be recognized and/or may be used by a signal peptidase resulting in a non-homogenous N-terminus of the polypeptide. For example, the use of different cleavage sites within a signal sequence can result in a polypeptide expressed with different N-terminal amino acids. Accordingly, in some embodiments, the polypeptides as described herein may comprise a mixture of polypeptides with different N-termini. In some embodiments, the N-termini differ in length by 1, 2, 3, 4, or 5 amino acids. In some embodiments, the polypeptide is substantially homogeneous, i.e., the polypeptides have the same N-terminus. In some embodiments, the signal sequence of the polypeptide comprises one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, etc.) amino acid substitutions and/or deletions as compared to a "native" or "parental" signal sequence. In some embodiments, the signal sequence of the polypeptide comprises amino acid substitutions and/or deletions that allow one cleavage site to be dominant, thereby resulting in a substantially homogeneous polypeptide with one N-terminus. In some embodiments, a signal sequence of the polypeptide affects the expression level of the polypeptide, e.g., increased expression or decreased expression.

[0153] In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62, and a light chain variable region comprising SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:17. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:45 and a light chain variable region comprising SEQ ID NO:17. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:17. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:72. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:45 and a light chain variable region comprising SEQ ID NO:72. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:72. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain

variable region comprising SEQ ID NO:86. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:45 and a light chain variable region comprising SEQ ID NO:86. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:86.

[0154] In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69 and a light chain comprising SEQ ID NO:29, SEQ ID NO:74, or SEQ ID NO:88. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:48 and a light chain comprising SEQ ID NO:29. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:49 and a light chain comprising SEQ ID NO:29. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:64 and a light chain comprising SEQ ID NO:29. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:69 and a light chain comprising SEQ ID NO:29. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:48 and a light chain comprising SEQ ID NO:74. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:49 and a light chain comprising SEQ ID NO:74. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:64 and a light chain comprising SEQ ID NO:74. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:69 and a light chain comprising SEQ ID NO:74. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:48 and a light chain comprising SEQ ID NO:88. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:49 and a light chain comprising SEQ ID NO:88. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3 with an antibody that comprises a heavy chain comprising SEQ ID NO:64 and a light chain comprising SEQ ID NO:88. In certain embodiments, a RSPO3-binding agent (e.g., antibody) competes for specific binding to RSPO3

with an antibody that comprises a heavy chain comprising SEQ ID NO:69 and a light chain comprising SEQ ID NO:88.

[0155] In certain embodiments, a RSPO3-binding agent competes with antibody 131R002 or antibody 131R003 for specific binding to human RSPO3. In certain embodiments, a RSPO3-binding agent competes with a variant of antibody 131R003 for specific binding to human RSPO3. In certain embodiments, a RSPO3-binding agent competes with a humanized version of antibody 131R003 for specific binding to human RSPO3. In certain embodiments, a RSPO3-binding agent competes with antibody h131R005/131R007 for specific binding to human RSPO3. In certain embodiments, a RSPO3-binding agent competes with antibody h131R008 for specific binding to human RSPO3. In certain embodiments, a RSPO3-binding agent competes with antibody h131R006A or antibody h131R006B for specific binding to human RSPO3. In certain embodiments, a RSPO3-binding agent competes with antibody h131R010 for specific binding to human RSPO3. In certain embodiments, a RSPO3-binding agent competes with antibody h131R011 for specific binding to human RSPO3. In some embodiments, a RSPO3-binding agent or antibody competes for specific binding to RSPO3 in an *in vitro* competitive binding assay. In some embodiments, the RSPO3 is human RSPO3. In some embodiments, the RSPO3 is mouse RSPO3.

[0156] In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as an antibody of the invention. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as antibody 131R002 or antibody 131R003. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as a variant of antibody 131R003. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as a humanized version of antibody 131R003. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as antibody h131R006A or antibody h131R006B. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as antibody h131R005/131R007. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as antibody h131R008. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as antibody h131R010. In certain embodiments, a RSPO3-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on RSPO3 as antibody h131R011.

[0157] In another embodiment, a RSPO3-binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by an antibody of the invention. In some embodiments, the RSPO3-binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by antibody 131R002 or antibody 131R003. In another embodiment, the RSPO3-binding

agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by a humanized version of antibody 131R003. In certain embodiments, the RSPO3-binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by antibody h131R006A or antibody h131R006B. In certain embodiments, the RSPO3-binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by antibody h131R005/131R007. In certain embodiments, the RSPO3-binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by antibody h131R008. In certain embodiments, the RSPO3-binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by antibody h131R010. In certain embodiments, the RSPO3-binding agent is an antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by antibody h131R011.

[0158] In certain embodiments, the RSPO-binding agent (e.g., an antibody) described herein binds at least one human RSPO protein and modulates RSPO activity. In some embodiments, the RSPO-binding agent is a RSPO antagonist and decreases RSPO activity. In some embodiments, the RSPO-binding agent is a RSPO antagonist and decreases β-catenin activity.

[0159] In certain embodiments, a RSPO3-binding agent (e.g., an antibody) described herein binds human RSPO3 and modulates RSPO3 activity. In some embodiments, a RSPO3-binding agent is a RSPO3 antagonist and decreases RSPO3 activity. In some embodiments, a RSPO3-binding agent is a RSPO3 antagonist and decreases β-catenin activity.

[0160] In certain embodiments, the RSPO-binding agent (e.g., an antibody) is an antagonist of at least one human RSPO protein. In some embodiments, the RSPO-binding agent is an antagonist of at least one RSPO and inhibits RSPO activity. In certain embodiments, the RSPO-binding agent inhibits RSPO activity by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In some embodiments, the RSPO-binding agent inhibits activity of one, two, three, or four RSPO proteins. In some embodiments, the RSPO-binding agent inhibits activity of human RSPO1, RSPO2, RSPO3, and/or RSPO4. In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent inhibits RSPO3 activity. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 activity is antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 activity is a humanized version of antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 activity is antibody h131R006A or antibody h131R006B. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 activity is antibody h131R005/131R007. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 activity is antibody h131R008. In certain embodiments, a

RSPO3-binding agent that inhibits human RSPO3 activity is antibody h131R010. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 activity is antibody h131R011.

[0161] In certain embodiments, the RSPO-binding agent (e.g., antibody) is an antagonist of at least one human RSPO protein. In certain embodiments, the RSPO-binding agent inhibits RSPO signaling by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In some embodiments, the RSPO-binding agent inhibits signaling by one, two, three, or four RSPO proteins. In some embodiments, the RSPO-binding agent inhibits signaling of human RSPO1, RSPO2, RSPO3, and/or RSPO4. In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent inhibits human RSPO3 signaling. In certain embodiments, a RSPO3-binding agent that inhibits RSPO3 signaling is antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that inhibits RSPO3 signaling is a humanized version of antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 signaling is antibody h131R006A or antibody h131R006B. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 signaling is antibody h131R005/131R007. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 signaling is antibody h131R008. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 signaling is antibody h131R010. In certain embodiments, a RSPO3-binding agent that inhibits human RSPO3 signaling is antibody h131R011.

[0162] In certain embodiments, the RSPO-binding agent (e.g., antibody) is an antagonist of β-catenin signaling. In certain embodiments, the RSPO-binding agent inhibits β-catenin signaling by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In some embodiments, the RSPO-binding agent that inhibits β-catenin signaling is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent inhibits β-catenin signaling. In certain embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is a humanized version of antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R006A or antibody h131R006B. In certain embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R005/131R007. In certain embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R008. In certain embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R010. In certain embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R011.

[0163] In certain embodiments, the RSPO-binding agent (e.g., antibody) inhibits binding of at least one RSPO protein to a receptor. In certain embodiments, the RSPO-binding agent inhibits binding of a human

RSPO protein to one or more of its receptors. In some embodiments, the RSPO-binding agent inhibits binding of a RSPO protein to at least one LGR protein. In some embodiments, the RSPO-binding agent inhibits binding of a RSPO protein to LGR4, LGR5, and/or LGR6. In some embodiments, a RSPO3-binding agent inhibits binding of RSPO3 to LGR4. In some embodiments, a RSPO3-binding agent inhibits binding of RSPO3 to LGR5. In some embodiments, a RSPO3-binding agent inhibits binding of RSPO3 to LGR6. In certain embodiments, the inhibition of binding of a RSPO-binding agent to at least one LGR protein is at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain embodiments, a RSPO-binding agent that inhibits binding of at least one RSPO to at least one LGR protein further inhibits β-catenin signaling. In certain embodiments, a RSPO3-binding agent that inhibits binding of human RSPO3 to at least one LGR protein is antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that inhibits binding of human RSPO3 to at least one LGR protein is a humanized version of antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that inhibits binding of human RSPO3 to at least one LGR protein is antibody h131R006A or antibody h131R006B. In certain embodiments, a RSPO3-binding agent that inhibits binding of human RSPO3 to at least one LGR protein is antibody h131R005/131R007. In certain embodiments, a RSPO3-binding agent that inhibits binding of human RSPO3 to at least one LGR protein is antibody h131R008. In certain embodiments, a RSPO3-binding agent that inhibits binding of human RSPO3 to at least one LGR protein is antibody h131R010. In certain embodiments, a RSPO3-binding agent that inhibits binding of human RSPO3 to at least one LGR protein is antibody h131R011.

[0164] In certain embodiments, the RSPO-binding agent (e.g., antibody) blocks binding of at least one RSPO to a receptor. In certain embodiments, the RSPO-binding agent blocks binding of a human RSPO protein to one or more of its receptors. In some embodiments, the RSPO-binding agent blocks binding of a RSPO to at least one LGR protein. In some embodiments, the RSPO-binding agent blocks binding of at least one RSPO protein to LGR4, LGR5, and/or LGR6. In some embodiments, a RSPO3-binding agent blocks binding of RSPO3 to LGR4. In some embodiments, a RSPO3-binding agent blocks binding of RSPO3 to LGR5. In some embodiments, a RSPO3-binding agent blocks binding of RSPO3 to LGR6. In certain embodiments, the blocking of binding of a RSPO-binding agent to at least one LGR protein is at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain embodiments, a RSPO-binding agent that blocks binding of at least one RSPO protein to at least one LGR protein further inhibits β-catenin signaling. In certain embodiments, a RSPO3-binding agent that blocks binding of human RSPO3 to at least one LGR protein is antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent that blocks binding of human RSPO3 to at least one LGR protein is a humanized version of antibody 131R002, antibody 131R003, or a variant of 131R003. In certain embodiments, a RSPO3-binding agent

that blocks binding of human RSPO3 to at least one LGR protein is antibody h131R006A or antibody h131R006B. In certain embodiments, a RSPO3-binding agent that blocks binding of human RSPO3 to at least one LGR protein is antibody h131R005/131R007. In certain embodiments, a RSPO3-binding agent that blocks binding of human RSPO3 to at least one LGR protein is antibody h131R008. In certain embodiments, a RSPO3-binding agent that blocks binding of human RSPO3 to at least one LGR protein is antibody h131R010. In certain embodiments, a RSPO3-binding agent that blocks binding of human RSPO3 to at least one LGR protein is antibody h131R011.

[0165] In certain embodiments, the RSPO-binding agent (e.g., an antibody) inhibits β-catenin signaling. It is understood that a RSPO-binding agent that inhibits β-catenin signaling may, in certain embodiments, inhibit signaling by one or more receptors in the β-catenin signaling pathway but not necessarily inhibit signaling by all receptors. In certain alternative embodiments, β-catenin signaling by all human receptors may be inhibited. In certain embodiments, β-catenin signaling by one or more receptors selected from the group consisting of LGR4, LGR5, and LGR6 is inhibited. In certain embodiments, the inhibition of β-catenin signaling by a RSPO-binding agent is a reduction in the level of β-catenin signaling of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In some embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody 131R002, antibody 131R003, or a variant of 131R003. In some embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is a humanized version of antibody 131R002, antibody 131R003, or a variant of 131R003. In some embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R006A or antibody h131R006B. In some embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R005/131R007. In some embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R008. In some embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R010. In some embodiments, a RSPO3-binding agent that inhibits β-catenin signaling is antibody h131R011.

[0166] In certain embodiments, the RSPO-binding agent (e.g., an antibody) inhibits activation of β-catenin. It is understood that a RSPO-binding agent that inhibits activation of β-catenin may, in certain embodiments, inhibit activation of β-catenin by one or more receptors, but not necessarily inhibit activation of β-catenin by all receptors. In certain alternative embodiments, activation of β-catenin by all human receptors may be inhibited. In certain embodiments, activation of β-catenin by one or more receptors selected from the group consisting of LGR4, LGR5, and LGR6 is inhibited. In certain embodiments, the inhibition of activation of β-catenin by a RSPO-binding agent is a reduction in the level of activation of β-catenin of at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In some embodiments, a RSPO3-binding agent that inhibits activation of β-catenin is antibody 131R002, antibody 131R003, or a variant of 131R003. In some embodiments, a RSPO3-binding agent that inhibits activation of β-catenin is a humanized version of

antibody 131R002, antibody 131R003, or a variant of 131R003. In some embodiments, a RSPO3-binding agent that inhibits activation of β-catenin is antibody h131R006A or antibody h131R006B. In some embodiments, a RSPO3-binding agent that inhibits activation of β-catenin is antibody h131R005/131R007. In some embodiments, a RSPO3-binding agent that inhibits activation of β-catenin is antibody h131R008. In some embodiments, a RSPO3-binding agent that inhibits activation of β-catenin is antibody h131R010. In some embodiments, a RSPO3-binding agent that inhibits activation of β-catenin is antibody h131R011.

[0167] *In vivo* and *in vitro* assays for determining whether a RSPO-binding agent (or candidate RSPO-binding agent) inhibits β-catenin signaling are known in the art. For example, cell-based, luciferase reporter assays utilizing a TCF/Luc reporter vector containing multiple copies of the TCF-binding domain upstream of a firefly luciferase reporter gene may be used to measure β-catenin signaling levels *in vitro* (Gazit et al., 1999, *Oncogene,* 18; 5959-66; TOPflash, Millipore, Billerica MA). The level of β-catenin signaling in the presence of one or more Wnts (e.g., Wnt(s) expressed by transfected cells or provided by Wnt-conditioned media) with or without a RSPO protein or RSPO-conditioned media in the presence of a RSPO-binding agent is compared to the level of signaling without the RSPO-binding agent present. In addition to the TCF/Luc reporter assay, the effect of a RSPO-binding agent (or candidate agent) on β-catenin signaling may be measured *in vitro* or *in vivo* by measuring the effect of the agent on the level of expression of β-catenin-regulated genes, such as c-myc (He et al., 1998, *Science*, 281:1509-12), cyclin D1 (Tetsu et al., 1999, *Nature*, 398:422-6) and/or fibronectin (Gradl et al. 1999, *Mol. Cell Biol.,* 19:5576-87). In certain embodiments, the effect of a RSPO-binding agent on β-catenin signaling may also be assessed by measuring the effect of the agent on the phosphorylation state of Dishevelled-1, Dishevelled-2, Dishevelled-3, LRP5, LRP6, and/or β-catenin.

[0168] In certain embodiments, the RSPO3-binding agents have one or more of the following effects: inhibit proliferation of tumor cells, inhibit tumor growth, reduce the tumorigenicity of a tumor, reduce the tumorigenicity of a tumor by reducing the frequency of cancer stem cells in the tumor, inhibit tumor growth, trigger cell death of tumor cells, induce cells in a tumor to differentiate, differentiate tumorigenic cells to a non-tumorigenic state, induce expression of differentiation markers in the tumor cells, prevent metastasis of tumor cells, decrease survival of tumor cells, or modulate angiogenesis.

[0169] In certain embodiments, the RSPO3-binding agents are capable of inhibiting tumor growth. In certain embodiments, the RSPO3-binding agents are capable of inhibiting tumor growth *in vivo* (e.g., in a xenograft mouse model, and/or in a human having cancer). In certain embodiments, tumor growth is inhibited at least about two-fold, about three-fold, about five-fold, about ten-fold, about 50-fold, about 100-fold, or about 1000-fold as compared to a untreated tumor.

[0170] In certain embodiments, the RSPO3-binding agents are capable of reducing the tumorigenicity of a tumor. In certain embodiments, the RSPO3-binding agent or antibody is capable of reducing the

tumorigenicity of a tumor comprising cancer stem cells in an animal model, such as a mouse xenograft model. In certain embodiments, the RSPO3-binding agent or antibody is capable of reducing the tumorigenicity of a tumor by decreasing the number or frequency of cancer stem cells in the tumor. In certain embodiments, the number or frequency of cancer stem cells in a tumor is reduced by at least about two-fold, about three-fold, about five-fold, about ten-fold, about 50-fold, about 100-fold, or about 1000-fold. In certain embodiments, the reduction in the number or frequency of cancer stem cells is determined by limiting dilution assay using an animal model. Additional examples and guidance regarding the use of limiting dilution assays to determine a reduction in the number or frequency of cancer stem cells in a tumor can be found, e.g., in International Publication Number WO 2008/042236, U.S. Patent Publication No. 2008/0064049, and U.S. Patent Publication No. 2008/0178305.

[0171] In certain embodiments, the RSPO3-binding agents described herein have a circulating half-life in mice, cynomolgus monkeys, or humans of at least about 2 hours, at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the RSPO3-binding agent is an IgG (e.g., IgG1 or IgG2) antibody that has a circulating half-life in mice, cynomolgus monkeys, or humans of at least about 2 hours, at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. Methods of increasing (or decreasing) the half-life of agents such as polypeptides and antibodies are known in the art. For example, known methods of increasing the circulating half-life of IgG antibodies include the introduction of mutations in the Fc region which increase the pH-dependent binding of the antibody to the neonatal Fc receptor (FcRn) at pH 6.0 (see, e.g., U.S. Patent Publication Nos. 2005/0276799, 2007/0148164, and 2007/0122403). Known methods of increasing the circulating half-life of antibody fragments lacking the Fc region include such techniques as PEGylation.

[0172] In some embodiments, the RSPO3-binding agents are polyclonal antibodies. Polyclonal antibodies can be prepared by any known method. In some embodiments, polyclonal antibodies are produced by immunizing an animal (e.g., a rabbit, rat, mouse, goat, donkey) with an antigen of interest (e.g., a purified peptide fragment, full-length recombinant protein, or fusion protein) using multiple subcutaneous or intraperitoneal injections. The antigen can be optionally conjugated to a carrier such as keyhole limpet hemocyanin (KLH) or serum albumin. The antigen (with or without a carrier protein) is diluted in sterile saline and usually combined with an adjuvant (e.g., Complete or Incomplete Freund's Adjuvant) to form a stable emulsion. After a sufficient period of time, polyclonal antibodies are recovered from the immunized animal, usually from blood or ascites. The polyclonal antibodies can be purified from serum or ascites according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

[0173] In some embodiments, the RSPO3-binding agents are monoclonal antibodies. Monoclonal antibodies can be prepared using hybridoma methods known to one of skill in the art (see e.g., Kohler and

Milstein, 1975, *Nature*, 256:495-497). In some embodiments, using the hybridoma method, a mouse, hamster, or other appropriate host animal, is immunized as described above to elicit from lymphocytes the production of antibodies that specifically bind the immunizing antigen. In some embodiments, lymphocytes can be immunized *in vitro*. In some embodiments, the immunizing antigen can be a human protein or a portion thereof. In some embodiments, the immunizing antigen can be a mouse protein or a portion thereof.

[0174] Following immunization, lymphocytes are isolated and fused with a suitable myeloma cell line using, for example, polyethylene glycol. The hybridoma cells are selected using specialized media as known in the art and unfused lymphocytes and myeloma cells do not survive the selection process. Hybridomas that produce monoclonal antibodies directed specifically against a chosen antigen may be identified by a variety of methods including, but not limited to, immunoprecipitation, immunoblotting, and *in vitro* binding assays (e.g., flow cytometry, FACS, ELISA, and radioimmunoassay). The hybridomas can be propagated either in *in vitro* culture using standard methods (J.W. Goding, 1996, *Monoclonal Antibodies: Principles and Practice*, 3rd *Edition*, Academic Press, San Diego, CA) or *in vivo* as ascites tumors in an animal. The monoclonal antibodies can be purified from the culture medium or ascites fluid according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

[0175] In certain embodiments, monoclonal antibodies can be made using recombinant DNA techniques as known to one skilled in the art. The polynucleotides encoding a monoclonal antibody are isolated from mature B-cells or hybridoma cells, such as by RT-PCR using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using standard techniques. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors which produce the monoclonal antibodies when transfected into host cells such as E. coli, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin proteins.

[0176] In certain other embodiments, recombinant monoclonal antibodies, or fragments thereof, can be isolated from phage display libraries expressing variable domains or CDRs of a desired species (see e.g., McCafferty et al., 1990, *Nature*, 348:552-554; Clackson et al., 1991, *Nature*, 352:624-628; and Marks et al., 1991, *J. Mol. Biol.*, 222:581-597).

[0177] The polynucleotide(s) encoding a monoclonal antibody can be modified, for example, by using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light and heavy chains of, for example, a mouse monoclonal antibody can be substituted for those regions of, for example, a human antibody to generate a chimeric antibody, or for a non-immunoglobulin polypeptide to generate a fusion antibody. In some embodiments, the constant regions are truncated or removed to generate the desired antibody fragment of a monoclonal antibody. Site-

directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of a monoclonal antibody.

[0178] In some embodiments, a monoclonal antibody against human RSPO3 is a humanized antibody. Typically, humanized antibodies are human immunoglobulins in which residues from the CDRs are replaced by residues from a CDR of a non-human species (e.g., mouse, rat, rabbit, hamster, etc.) that have the desired specificity, affinity, and/or binding capability using methods known to one skilled in the art. In some embodiments, the Fv framework region residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species that has the desired specificity, affinity, and/or binding capability. In some embodiments, a humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or capability. In general, a humanized antibody will comprise substantially all of at least one, and typically two or three, variable domain regions containing all, or substantially all, of the CDRs that correspond to the non-human immunoglobulin whereas all, or substantially all, of the framework regions are those of a human immunoglobulin consensus sequence. In some embodiments, a humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. In certain embodiments, such humanized antibodies are used therapeutically because they may reduce antigenicity and HAMA (human anti-mouse antibody) responses when administered to a human subject. One skilled in the art would be able to obtain a functional humanized antibody with reduced immunogenicity following known techniques (see e.g., U.S. Patent Nos. 5,225,539; 5,585,089; 5,693,761; and 5,693,762).

[0179] In certain embodiments, the RSPO3-binding agent is a human antibody. Human antibodies can be directly prepared using various techniques known in the art. In some embodiments, human antibodies may be generated from immortalized human B lymphocytes immunized *in vitro* or from lymphocytes isolated from an immunized individual. In either case, cells that produce an antibody directed against a target antigen can be generated and isolated (see, e.g., Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77; Boemer et al., 1991, *J. Immunol.*, 147:86-95; and U.S. Patent Nos. 5,750,373; 5,567,610 and 5,229,275). In some embodiments, the human antibody can be selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., 1996, *Nature Biotechnology*, 14:309-314; Sheets et al., 1998, *PNAS*, 95:6157-6162; Hoogenboom and Winter, 1991, *J. Mol. Biol.*, 227:381; Marks et al., 1991, *J. Mol. Biol.*, 222:581). Alternatively, phage display technology can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable domain gene repertoires from unimmunized donors. Techniques for the generation and use of antibody phage libraries are also described in U.S. Patent Nos. 5,969,108; 6,172,197; 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915; 6,593,081; 6,300,064; 6,653,068; 6,706,484; and 7,264,963; and Rothe

et al., 2008, *J. Mol. Bio.*, 376:1182-1200. Once antibodies are identified, affinity maturation strategies known in the art, including but not limited to, chain shuffling (Marks et al., 1992, *Bio/Technology*, 10:779-783) and site-directed mutagenesis, may be employed to generate high affinity human antibodies.

[0180] In some embodiments, human antibodies can be made in transgenic mice that contain human immunoglobulin loci. Upon immunization these mice are capable of producing the full repertoire of human antibodies in the absence of endogenous immunoglobulin production. This approach is described in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016.

[0181] This invention also encompasses bispecific antibodies that specifically recognize at least one human RSPO protein. Bispecific antibodies are capable of specifically recognizing and binding at least two different antigens or epitopes. The different epitopes can either be within the same molecule (e.g., two epitopes on human RSPO3) or on different molecules (e.g., one epitope on RSPO3 and one epitope on RSPO2). In some embodiments, a bispecific antibody has enhanced potency as compared to an individual antibody or to a combination of more than one antibody. In some embodiments, a bispecific antibody has reduced toxicity as compared to an individual antibody or to a combination of more than one antibody. It is known to those of skill in the art that any binding agent (e.g., antibody) may have unique pharmacokinetics (PK) (e.g., circulating half-life). In some embodiments, a bispecific antibody has the ability to synchronize the PK of two active binding agents wherein the two individual binding agents have different PK profiles. In some embodiments, a bispecific antibody has the ability to concentrate the actions of two binding agents (e.g., antibodies) in a common area (e.g., a tumor and/or tumor microenvironment). In some embodiments, a bispecific antibody has the ability to concentrate the actions of two binding agents (e.g., antibodies) to a common target (e.g., a tumor or a tumor cell). In some embodiments, a bispecific antibody has the ability to target the actions of two binding agents (e.g., antibodies) to more than one biological pathway or function.

[0182] In certain embodiments, the bispecific antibody specifically binds RSPO3 and a second target. In certain embodiments, the bispecific antibody specifically binds RSPO3 and a second human RSPO (e.g., RSPO1, RSPO2, or RSPO4). In certain embodiments, the bispecific antibody specifically binds RSPO3 and RSPO2. In some embodiments, the bispecific antibody is a monoclonal antibody. In some embodiments, the bispecific antibody is a humanized antibody. In some embodiments, the bispecific antibody is a human antibody. In some embodiments, the bispecific antibody is an IgG1 antibody. In some embodiments, the bispecific antibody is an IgG2 antibody. In some embodiments, the bispecific antibody has decreased toxicity and/or side effects. In some embodiments, the bispecific antibody has decreased toxicity and/or side effects as compared to a mixture of the two individual antibodies or the antibodies as single agents. In some embodiments, the bispecific antibody has an increased therapeutic index. In some embodiments, the bispecific antibody has an increased therapeutic index as compared to a mixture of the two individual antibodies or the antibodies as single agents.

[0183] In some embodiments, the antibodies can specifically recognize and bind a first antigen target, (e.g., RSPO3) as well as a second antigen target, such as an effector molecule on a leukocyte (e.g., CD2, CD3, CD28, CTLA-4, CD80, or CD86) or a Fc receptor (e.g., CD64, CD32, or CD16) so as to focus cellular defense mechanisms to the cell expressing and/or producing the first antigen target. In some embodiments, the antibodies can be used to direct cytotoxic agents to cells which express a particular target antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA.

[0184] Techniques for making bispecific antibodies are known by those skilled in the art, see for example, Millstein et al., 1983, *Nature*, 305:537-539; Brennan et al., 1985, *Science*, 229:81; Suresh et al., 1986, *Methods in Enzymol.*, 121:120; Traunecker et al., 1991, *EMBO J.*, 10:3655-3659; Shalaby et al., 1992, *J. Exp. Med.*, 175:217-225; Kostelny et al., 1992, *J. Immunol.*, 148:1547-1553; Gruber et al., 1994, *J. Immunol.*, 152:5368; U.S. Patent No. 5,731,168; International Publication No. WO 2009/089004; and U.S. Patent Publication No. 2011/0123532. In some embodiments, the bispecific antibodies comprise heavy chain constant regions with modifications in the amino acids which are part of the interface between the two heavy chains. In some embodiments, the bispecific antibodies can be generated using a "knobs-into-holes" strategy (see, e.g., U.S. Patent No. 5,731,168; Ridgway et. al., 1996, *Prot. Engin.*, 9:617-621). In some cases, the "knobs" and "holes" terminology is replaced with the terms "protuberances" and "cavities". In some embodiments, the bispecific antibodies may comprise variant hinge regions incapable of forming disulfide linkages between the heavy chains (see, e.g., WO 2006/028936). In some embodiments, the modifications may comprise changes in amino acids that result in altered electrostatic interactions. In some embodiments, the modifications may comprise changes in amino acids that result in altered hydrophobic/hydrophilic interactions.

[0185] Bispecific antibodies can be intact antibodies or antibody fragments comprising antigen-binding sites. Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared (Tutt et al., 1991, *J. Immunol.*, 147:60). Thus, in certain embodiments the antibodies to RSPO3 are multispecific.

[0186] In certain embodiments, the antibodies (or other polypeptides) described herein may be monospecific. In certain embodiments, each of the one or more antigen-binding sites that an antibody contains is capable of binding (or binds) a homologous epitope on RSPO proteins. In certain embodiments, an antigen-binding site of a monospecific antibody described herein is capable of binding (or binds), for example, RSPO3 and RSPO2 (i.e., the same epitope is found on both RSPO3 and RSPO2 proteins).

[0187] In certain embodiments, the RSPO3-binding agent is an antibody fragment. Antibody fragments may have different functions or capabilities than intact antibodies; for example, antibody fragments can have increased tumor penetration. Various techniques are known for the production of antibody

fragments including, but not limited to, proteolytic digestion of intact antibodies. In some embodiments, antibody fragments include a F(ab')2 fragment produced by pepsin digestion of an antibody molecule. In some embodiments, antibody fragments include a Fab fragment generated by reducing the disulfide bridges of an F(ab')2 fragment. In other embodiments, antibody fragments include a Fab fragment generated by the treatment of the antibody molecule with papain and a reducing agent. In certain embodiments, antibody fragments are produced recombinantly. In some embodiments, antibody fragments include Fv or single chain Fv (scFv) fragments. Fab, Fv, and scFv antibody fragments can be expressed in and secreted from E. coli or other host cells, allowing for the production of large amounts of these fragments. In some embodiments, antibody fragments are isolated from antibody phage libraries as discussed herein. For example, methods can be used for the construction of Fab expression libraries (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a RSPO protein or derivatives, fragments, analogs or homologs thereof. In some embodiments, antibody fragments are linear antibody fragments. In certain embodiments, antibody fragments are monospecific or bispecific. In certain embodiments, the RSPO3-binding agent is a scFv. Various techniques can be used for the production of single-chain antibodies specific to one or more human RSPOs (see, e.g., U.S. Patent No. 4,946,778).

[0188] It can further be desirable, especially in the case of antibody fragments, to modify an antibody in order to alter (e.g., increase or decrease) its serum half-life. This can be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle (e.g., by DNA or peptide synthesis).

[0189] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune cells to unwanted cells (see, e.g., U.S. Patent No. 4,676,980). It is also contemplated that the heteroconjugate antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[0190] For the purposes of the present invention, it should be appreciated that modified antibodies can comprise any type of variable region that provides for the association of the antibody with the target (i.e., human RSPO3). In this regard, the variable region may comprise or be derived from any type of mammal that can be induced to mount a humoral response and generate immunoglobulins against the desired antigen. As such, the variable region of the modified antibodies can be, for example, of human, murine, non-human primate (e.g. cynomolgus monkeys, macaques, etc.) or rabbit origin. In some embodiments, both the variable and constant regions of the modified immunoglobulins are human. In other

embodiments, the variable regions of compatible antibodies (usually derived from a non-human source) can be engineered or specifically tailored to improve the binding properties or reduce the immunogenicity of the molecule. In this respect, variable regions useful in the present invention can be humanized or otherwise altered through the inclusion of imported amino acid sequences.

[0191] In certain embodiments, the variable domains in both the heavy and light chains are altered by at least partial replacement of one or more CDRs and, if necessary, by partial framework region replacement and sequence modification and/or alteration. Although the CDRs may be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs may be derived from an antibody of different class and often from an antibody from a different species. It may not be necessary to replace all of the CDRs with all of the CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are required to maintain the activity of the antigen-binding site.

[0192] Alterations to the variable region notwithstanding, those skilled in the art will appreciate that the modified antibodies of this invention will comprise antibodies (e.g., full-length antibodies or immunoreactive fragments thereof) in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical characteristics such as increased tumor localization or increased serum half-life when compared with an antibody of approximately the same immunogenicity comprising a native or unaltered constant region. In some embodiments, the constant region of the modified antibodies will comprise a human constant region. Modifications to the constant region compatible with this invention comprise additions, deletions or substitutions of one or more amino acids in one or more domains. The modified antibodies disclosed herein may comprise alterations or modifications to one or more of the three heavy chain constant domains (CH1, CH2 or CH3) and/or to the light chain constant domain (CL). In some embodiments, one or more domains are partially or entirely deleted from the constant regions of the modified antibodies. In some embodiments, the modified antibodies will comprise domain deleted constructs or variants wherein the entire CH2 domain has been removed (ΔCH2 constructs). In some embodiments, the omitted constant region domain is replaced by a short amino acid spacer (e.g., 10 amino acid residues) that provides some of the molecular flexibility typically imparted by the absent constant region.

[0193] In some embodiments, the modified antibodies are engineered to fuse the CH3 domain directly to the hinge region of the antibody. In other embodiments, a peptide spacer is inserted between the hinge region and the modified CH2 and/or CH3 domains. For example, constructs may be expressed wherein the CH2 domain has been deleted and the remaining CH3 domain (modified or unmodified) is joined to the hinge region with a 5-20 amino acid spacer. Such a spacer may be added to ensure that the regulatory elements of the constant domain remain free and accessible or that the hinge region remains flexible.

However, it should be noted that amino acid spacers may, in some cases, prove to be immunogenic and elicit an unwanted immune response against the construct. Accordingly, in certain embodiments, any spacer added to the construct will be relatively non-immunogenic so as to maintain the desired biological qualities of the modified antibodies.

[0194] In some embodiments, the modified antibodies may have only a partial deletion of a constant domain or substitution of a few or even a single amino acid. For example, the mutation of a single amino acid in selected areas of the CH2 domain may be enough to substantially reduce Fc binding and thereby increase cancer cell localization and/or tumor penetration. Similarly, it may be desirable to simply delete the part of one or more constant region domains that control a specific effector function (e.g. complement C1q binding) to be modulated. Such partial deletions of the constant regions may improve selected characteristics of the antibody (serum half-life) while leaving other desirable functions associated with the subject constant region domain intact. Moreover, as alluded to above, the constant regions of the disclosed antibodies may be modified through the mutation or substitution of one or more amino acids that enhances the profile of the resulting construct. In this respect it may be possible to disrupt the activity provided by a conserved binding site (e.g., Fc binding) while substantially maintaining the configuration and immunogenic profile of the modified antibody. In certain embodiments, the modified antibodies comprise the addition of one or more amino acids to the constant region to enhance desirable characteristics such as decreasing or increasing effector function or provide for more cytotoxin or carbohydrate attachment sites.

[0195] It is known in the art that the constant region mediates several effector functions. For example, binding of the C1 component of complement to the Fc region of IgG or IgM antibodies (bound to antigen) activates the complement system. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can also be involved in autoimmune hypersensitivity. In addition, the Fc region of an antibody can bind a cell expressing a Fc receptor (FcR). There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell cytotoxicity or ADCC), release of inflammatory mediators, placental transfer, and control of immunoglobulin production.

[0196] In certain embodiments, the modified antibodies provide for altered effector functions that, in turn, affect the biological profile of the administered antibody. For example, in some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating modified antibody thereby increasing cancer cell localization and/or

tumor penetration. In other embodiments, the constant region modifications increase the serum half-life of the antibody. In other embodiments, the constant region modifications reduce the serum half-life of the antibody. In some embodiments, the constant region is modified to eliminate disulfide linkages or oligosaccharide moieties. Modifications to the constant region in accordance with this invention may easily be made using well known biochemical or molecular engineering techniques.

[0197] In certain embodiments, a RSPO3-binding agent that is an antibody does not have one or more effector functions. For instance, in some embodiments, the antibody has no ADCC activity, and/or no complement-dependent cytotoxicity (CDC) activity. In certain embodiments, the antibody does not bind an Fc receptor, and/or complement factors. In certain embodiments, the antibody has no effector function.

[0198] The present invention further embraces variants and equivalents which are substantially homologous to the chimeric, humanized, and human antibodies, or antibody fragments thereof, set forth herein. These can contain, for example, conservative substitution mutations, i.e. the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of an amino acid with another amino acid within the same general class such as, for example, one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art and described herein.

[0199] Thus, the present invention provides methods for producing an antibody that binds RSPO3, including bispecific antibodies that specifically bind both RSPO3 and a second target (e.g., a human RSPO). In some embodiments, the method for producing an antibody that binds RSPO3 comprises using hybridoma techniques. In some embodiments, a method for producing an antibody that binds human RSPO3 is provided. In some embodiments, the method comprises using amino acids 22-272 of human RSPO3. In some embodiments, the method comprises using amino acids 22-272 of SEQ ID NO:3. In some embodiments, the method of generating an antibody that binds RSPO3 comprises screening a human phage library. The present invention further provides methods of identifying an antibody that binds RSPO3. In some embodiments, the antibody is identified by FACS screening for binding to RSPO3 or a portion thereof. In some embodiments, the antibody is identified by FACS screening for binding to RSPO3 and a second RSPO or a portion thereof. In some embodiments, the antibody is identified by FACS screening for binding to both RSPO3 and RSPO2 or a portion thereof. In some embodiments, the antibody is identified by screening using ELISA for binding to RSPO3. In some embodiments, the antibody is identified by screening using ELISA for binding to RSPO3 and a second RSPO. In some embodiments, the antibody is identified by screening using ELISA for binding to both RSPO3 and RSPO2. In some embodiments, the antibody is identified by screening by FACS for blocking of binding of RSPO3 to a human LGR protein. In some embodiments, the antibody is identified by screening for inhibition or blocking of β-catenin signaling.

[0200] In some embodiments, a method of generating an antibody to human RSPO3 protein comprises immunizing a mammal with a polypeptide comprising amino acids 22-272 of human RSPO3. In some embodiments, a method of generating an antibody to human RSPO3 protein comprises immunizing a mammal with a polypeptide comprising at least a portion of amino acids 22-272 of human RSPO3. In some embodiments, the method further comprises isolating antibodies or antibody-producing cells from the mammal. In some embodiments, a method of generating a monoclonal antibody which binds RSPO3 protein comprises: (a) immunizing a mammal with a polypeptide comprising at least a portion of amino acids 22-272 of human RSPO3; (b) isolating antibody producing cells from the immunized mammal; (c) fusing the antibody-producing cells with cells of a myeloma cell line to form hybridoma cells. In some embodiments, the method further comprises (d) selecting a hybridoma cell expressing an antibody that binds RSPO3 protein. In some embodiments, the at least a portion of amino acids 22-272 of human RSPO3 is selected from the group consisting of SEQ ID NOs:5-8. In some embodiments, the at least a portion of amino acids 22-272 of human RSPO3 is SEQ ID NO:5. In some embodiments, the at least a portion of amino acids 22-272 of human RSPO3 is SEQ ID NO:6 or SEQ ID NO:7. In some embodiments, the at least a portion of amino acids 22-272 of human RSPO3 is SEQ ID NO:6 and SEQ ID NO:7. In certain embodiments, the mammal is a mouse. In some embodiments, the antibody is selected using a polypeptide comprising at least a portion of amino acid 22-272 of human RSPO3. In certain embodiments, the polypeptide used for selection comprising at least a portion of amino acids 22-272 of human RSPO3 is selected from the group consisting of SEQ ID NOs:5-8. In some embodiments, the antibody binds RSPO3 and at least one other RSPO protein. In certain embodiments, the at least one other RSPO protein is selected from the group consisting of RSPO1, RSPO2, and RSPO4. In certain embodiments, the antibody binds RSPO3 and RSPO1. In certain embodiments, the antibody binds RSPO3 and RSPO2. In certain embodiments, the antibody binds RSPO3 and RSPO4. In certain embodiments, the antibody binds RSPO3, RSPO1, and RSPO2. In certain embodiments, the antibody binds RSPO3, RSPO1, and RSPO4. In certain embodiments, the antibody binds RSPO3, RSPO2, and RSPO4. In some embodiments, the antibody binds both human RSPO3 and mouse RSPO3.

[0201] In some embodiments, the antibody generated by the methods described herein is a RSPO antagonist, particularly a RSPO3 antagonist. In some embodiments, the antibody generated by the methods described herein inhibits β-catenin signaling.

[0202] In some embodiments, a method of producing an antibody to at least one human RSPO protein comprises identifying an antibody using a membrane-bound heterodimeric molecule comprising a single antigen-binding site. In some non-limiting embodiments, the antibody is identified using methods and polypeptides described in International Publication WO 2011/100566.

[0203] In some embodiments, a method of producing an antibody to at least one human RSPO protein comprises screening an antibody-expressing library for antibodies that bind a human RSPO protein. In

some embodiments, the antibody-expressing library is a phage library. In some embodiments, the screening comprises panning. In some embodiments, the antibody-expressing library is a phage library. In some embodiments, the antibody-expressing library is a mammalian cell library. In some embodiments, the antibody-expressing library is screened using at least a portion of amino acids 22-272 of human RSPO3. In some embodiments, antibodies identified in the first screening, are screened again using a different RSPO protein thereby identifying an antibody that binds RSPO3 and a second RSPO protein. In certain embodiments, the polypeptide used for screening comprises at least a portion of amino acids 22-272 of human RSPO3 selected from the group consisting of SEQ ID NOs:5-8. In some embodiments, the antibody identified in the screening binds RSPO3 and at least one other RSPO protein. In certain embodiments, the at least one other RSPO protein is selected from the group consisting of RSPO1, RSPO2, and RSPO4. In certain embodiments, the antibody identified in the screening binds RSPO3 and RSPO1. In certain embodiments, the antibody identified in the screening binds RSPO3 and RSPO2. In certain embodiments, the antibody identified in the screening binds RSPO3 and RSPO4. In some embodiments, the antibody identified in the screening binds both human RSPO3 and mouse RSPO3. In some embodiments, the antibody identified in the screening is a RSPO3 antagonist. In some embodiments, the antibody identified in the screening inhibits β-catenin signaling induced by RSPO3.

[0204] In certain embodiments, the antibodies described herein are isolated. In certain embodiments, the antibodies described herein are substantially pure.

[0205] In some embodiments of the present invention, the RSPO3-binding agents are polypeptides. The polypeptides can be recombinant polypeptides, natural polypeptides, or synthetic polypeptides comprising an antibody, or fragment thereof, that bind RSPO3. It will be recognized in the art that some amino acid sequences of the invention can be varied without significant effect of the structure or function of the protein. Thus, the invention further includes variations of the polypeptides which show substantial activity or which include regions of an antibody, or fragment thereof, against human RSPO3. In some embodiments, amino acid sequence variations of RSPO-binding polypeptides include deletions, insertions, inversions, repeats, and/or other types of substitutions.

[0206] In certain embodiments, the polypeptides described herein are isolated. In certain embodiments, the polypeptides described herein are substantially pure.

[0207] The polypeptides, analogs and variants thereof, can be further modified to contain additional chemical moieties not normally part of the polypeptide. The derivatized moieties can improve or otherwise modulate the solubility, the biological half-life, and/or absorption of the polypeptide. The moieties can also reduce or eliminate undesirable side effects of the polypeptides and variants. An overview for chemical moieties can be found in *Remington: The Science and Practice of Pharmacy*, 22st *Edition*, 2012, Pharmaceutical Press, London.

[0208] The polypeptides described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods to constructing a DNA sequence encoding polypeptide sequences and expressing those sequences in a suitable host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional analogs thereof. See, e.g., Zoeller et al., 1984, *PNAS,* 81:5662-5066 and U.S. Patent No. 4,588,585.

[0209] In some embodiments, a DNA sequence encoding a polypeptide of interest may be constructed by chemical synthesis using an oligonucleotide synthesizer. Oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize a polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[0210] Once assembled (by synthesis, site-directed mutagenesis, or another method), the polynucleotide sequences encoding a particular polypeptide of interest can be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction enzyme mapping, and/or expression of a biologically active polypeptide in a suitable host. As is well-known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[0211] In certain embodiments, recombinant expression vectors are used to amplify and express DNA encoding antibodies, or fragments thereof, against human RSPO3. For example, recombinant expression vectors can be replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of a RSPO-binding agent, such as an anti-RSPO antibody, or fragment thereof, operatively linked to suitable transcriptional and/or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Regulatory elements can include an operator sequence to control transcription. The ability to

replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are "operatively linked" when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. In some embodiments, structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. In other embodiments, in situations where recombinant protein is expressed without a leader or transport sequence, it can include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

[0212] The choice of an expression control sequence and an expression vector depends upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from E. coli, including pCR1, pBR322, pMB9 and their derivatives, and wider host range plasmids, such as M13 and other filamentous single-stranded DNA phages.

[0213] The RSPO-binding agents (e.g., polypeptides or antibodies) of the present invention can be expressed from one or more vectors. For example, in some embodiments, one heavy chain polypeptide is expressed by one vector, a second heavy chain polypeptide is expressed by a second vector and a light chain polypeptide is expressed by a third vector. In some embodiments, a first heavy chain polypeptide and a light chain polypeptide is expressed by one vector and a second heavy chain polypeptide is expressed by a second vector. In some embodiments, two heavy chain polypeptides are expressed by one vector and a light chain polypeptide is expressed by a second vector. In some embodiments, three polypeptides are expressed from one vector. Thus, in some embodiments, a first heavy chain polypeptide, a second heavy chain polypeptide, and a light chain polypeptide are expressed by a single vector.

[0214] Suitable host cells for expression of a RSPO3-binding polypeptide or antibody (or a RSPO protein to use as an antigen) include prokaryotes, yeast cells, insect cells, or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram-negative or gram-positive organisms, for example E. coli or Bacillus. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems may also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described in Pouwels et al., 1985, *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, NY. Additional information regarding methods of protein production, including antibody production, can be found, e.g.,

in U.S. Patent Publication No. 2008/0187954, U.S. Patent Nos. 6,413,746, 6,660,501; and International Patent Publication No. WO 04/009823.

[0215] Various mammalian culture systems may be used to express recombinant polypeptides. Expression of recombinant proteins in mammalian cells may be desirable because these proteins are generally correctly folded, appropriately modified, and biologically functional. Examples of suitable mammalian host cell lines include, but are not limited to, COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants thereof. Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

[0216] Expression of recombinant proteins in insect cell culture systems (e.g., baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art (see, e.g., Luckow and Summers, 1988, *Bio/Technology*, 6:47).

[0217] Thus, the present invention provides cells comprising the RSPO3-binding agents described herein. In some embodiments, the cells produce the RSPO3-binding agents described herein. In certain embodiments, the cells produce an antibody. In some embodiments, the cells produce an antibody that binds human RSPO3. In certain embodiments, the cells produce antibody 131R002. In certain embodiments, the cells produce antibody 131R003. In certain embodiments, the cells produce variants of antibody 131R003. In certain embodiments, the cells produce a humanized version of antibody 131R002, antibody 131R003, or variants of antibody 131R003. In some embodiments, the cells produce a chimeric version of antibody 131R002, antibody 131R003, or variants of antibody 131R003. In some embodiments, the cells produce antibody h131R006A or antibody h131R006B. In some embodiments, the cells produce antibody h131R005/131R007. In some embodiments, the cells produce antibody h131R008. In some embodiments, the cells produce antibody h131R010. In some embodiments, the cells produce antibody h131R011. In some embodiments, the cells produce a bispecific antibody that binds RSPO3. In some embodiments, the cells produce a bispecific antibody that binds RSPO3 and RSPO2. In some embodiments, the cell is a hybridoma cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a prokaryotic cell. In some embodiments, the cell is an eukaryotic cell.

[0218] The proteins produced by a transformed host can be purified according to any suitable method. Standard methods include chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein

purification. Affinity tags such as hexa-histidine, maltose binding domain, influenza coat sequence, and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Affinity chromatography used for purifying immunoglobulins can include Protein A, Protein G, and Protein L chromatography. Isolated proteins can be physically characterized using such techniques as proteolysis, size exclusion chromatography (SEC), mass spectrometry (MS), nuclear magnetic resonance (NMR), isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and x-ray crystallography. The purity of isolated proteins can be determined using techniques known to those of skill in the art, including but not limited to, SDS-PAGE, SEC, capillary gel electrophoresis, IEF, and capillary isoelectric focusing (cIEF).

[0219] In some embodiments, supernatants from expression systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. In some embodiments, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. In some embodiments, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In some embodiments, a hydroxyapatite media can be employed, including but not limited to, ceramic hydroxyapatite (CHT). In certain embodiments, one or more reverse-phase HPLC steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a recombinant protein (e.g., a RSPO3-binding agent). Some or all of the foregoing purification steps, in various combinations, can be employed to provide a homogeneous recombinant protein.

[0220] In some embodiments, heterodimeric proteins such as bispecific antibodies are purified according the any of the methods described herein. In some embodiments, anti-RSPO bispecific antibodies are isolated and/or purified using at least one chromatography step. In some embodiments, the at least one chromatography step comprises affinity chromatography. In some embodiments, the at least one chromatography step further comprises anion exchange chromatography. In some embodiments, the isolated and/or purified antibody product comprises at least 90% heterodimeric antibody. In some embodiments, the isolated and/or purified antibody product comprises at least 95%, 96%, 97%, 98% or 99% heterodimeric antibody. In some embodiments, the isolated and/or purified antibody product comprises about 100% heterodimeric antibody.

[0221] In some embodiments, recombinant protein produced in bacterial culture can be isolated, for example, by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange, or size exclusion chromatography steps. HPLC can be employed for final

purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

[0222] Methods known in the art for purifying antibodies and other proteins also include, for example, those described in U.S. Patent Publication Nos. 2008/0312425, 2008/0177048, and 2009/0187005.

[0223] In certain embodiments, the RSPO3-binding agent is a polypeptide that is not an antibody. A variety of methods for identifying and producing non-antibody polypeptides that bind with high affinity to a protein target are known in the art. See, e.g., Skerra, 2007, *Curr. Opin. Biotechnol.*, 18:295-304; Hosse et al., 2006, *Protein Science*, 15:14-27; Gill et al., 2006, *Curr. Opin. Biotechnol.*, 17:653-658; Nygren, 2008, *FEBS J.*, 275:2668-76; and Skerra, 2008, *FEBS J.*, 275:2677-83. In certain embodiments, phage or mammalian display technology may be used to produce and/or identify a RSPO3-binding polypeptide. In certain embodiments, the polypeptide comprises a protein scaffold of a type selected from the group consisting of protein A, protein G, a lipocalin, a fibronectin domain, an ankyrin consensus repeat domain, and thioredoxin.

[0224] In certain embodiments, the RSPO3-binding agents or antibodies can be used in any one of a number of conjugated (i.e. an immunoconjugate or radioconjugate) or non-conjugated forms. In certain embodiments, the antibodies can be used in a non-conjugated form to harness the subject's natural defense mechanisms including complement-dependent cytotoxicity and antibody dependent cellular toxicity to eliminate malignant or cancer cells.

[0225] In some embodiments, the RSPO3-binding agent (e.g., an antibody or polypeptide) is conjugated to a cytotoxic agent. In some embodiments, the cytotoxic agent is a chemotherapeutic agent including, but not limited to, methotrexate, adriamicin, doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents. In some embodiments, the cytotoxic agent is an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof, including, but not limited to, diphtheria A chain, non-binding active fragments of diphtheria toxin, exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, Sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. In some embodiments, the cytotoxic agent is a radioisotope to produce a radioconjugate or a radioconjugated antibody. A variety of radionuclides are available for the production of radioconjugated antibodies including, but not limited to, ^{90}Y, ^{125}I, ^{131}I, ^{123}I, ^{111}In, ^{131}In, ^{105}Rh, ^{153}Sm, ^{67}Cu, ^{67}Ga, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re and ^{212}Bi. Conjugates of an antibody and one or more small molecule toxins, such as calicheamicins, maytansinoids, trichothenes, and CC1065, and the derivatives of these toxins that have toxin activity, can also be used. Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyidithiol) propionate

(SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

III. Polynucleotides

[0226] In certain embodiments, the invention encompasses polynucleotides comprising polynucleotides that encode a polypeptide (or a fragment of a polypeptide) that specifically binds RSPO3. The term "polynucleotides that encode a polypeptide" encompasses a polynucleotide which includes only coding sequences for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequences. For example, in some embodiments, the invention provides a polynucleotide comprising a polynucleotide sequence that encodes an antibody to human RSPO3 or encodes a fragment of such an antibody (e.g., a fragment comprising the antigen-binding site). The polynucleotides of the invention can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or single-stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand.

[0227] In certain embodiments, the polynucleotide comprises a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:86, SEQ ID NO:87, and SEQ ID NO:88. In some embodiments, the polynucleotide comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, and SEQ ID NO:95.

[0228] In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:18. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:19. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:20. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:24. In some embodiments, a plasmid

comprises a polynucleotide comprising SEQ ID NO:25. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:26. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:30. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:31. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:32. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:40. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:43. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:50. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:51. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:52. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:53. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:54. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:55. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:65. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:66. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:67. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:70. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:71. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:75. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:76. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:77. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:84. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:85. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:89. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:90. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:91. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:92. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:93. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:94. In some embodiments, a plasmid comprises a polynucleotide comprising SEQ ID NO:95.

[0229] In certain embodiments, the polynucleotide comprises a polynucleotide having a nucleotide sequence at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, and in some embodiments, at least about 96%, 97%, 98% or 99% identical to a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID

NO:84, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, and SEQ ID NO:95. Also provided is a polynucleotide that comprises a polynucleotide that hybridizes to SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:32, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:990, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, or SEQ ID NO:95. Also provided is a polynucleotide that comprises a polynucleotide that hybridizes to the complement of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:32, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, or SEQ ID NO:95. In certain embodiments, the hybridization is under conditions of high stringency.

[0230] In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:18 and SEQ ID NO:20. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:19 and SEQ ID NO:20. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:50 and SEQ ID NO:20. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:51 and SEQ ID NO:20. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:65 and SEQ ID NO:20. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:18 and SEQ ID NO:75. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:19 and SEQ ID NO:75. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:50 and SEQ ID NO:75. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:51 and SEQ ID NO:75. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:65 and SEQ ID NO:75. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:95 and SEQ ID NO:89. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:92 and SEQ ID NO:89.

[0231] In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:24 and SEQ ID NO:26. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:25 and SEQ ID NO:26. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:40 and SEQ ID NO:26. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:43 and SEQ ID NO:26. In some embodiments, an antibody is

encoded by a polynucleotide comprising SEQ ID NO:52 and SEQ ID NO:26. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:53 and SEQ ID NO:26. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:66 and SEQ ID NO:26. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:70 and SEQ ID NO:26. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:24 and SEQ ID NO:76. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:25 and SEQ ID NO:76. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:40 and SEQ ID NO:76. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:43 and SEQ ID NO:76. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:52 and SEQ ID NO:76. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:53 and SEQ ID NO:76. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:66 and SEQ ID NO:76. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:70 and SEQ ID NO:76. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:84 and SEQ ID NO:90. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:93 and SEQ ID NO:90.

[0232] In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:30 and SEQ ID NO:32. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:31 and SEQ ID NO:32. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:54 and SEQ ID NO:32. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:55 and SEQ ID NO:32. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:67 and SEQ ID NO:32. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:71 and SEQ ID NO:32. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:30 and SEQ ID NO:77. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:31 and SEQ ID NO:77. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:54 and SEQ ID NO:77. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:55 and SEQ ID NO:77. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:67 and SEQ ID NO:77. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:71 and SEQ ID NO:77. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:85 and SEQ ID NO:91. In some embodiments, an antibody is encoded by a polynucleotide comprising SEQ ID NO:94 and SEQ ID NO:91.

[0233] In certain embodiments, the polynucleotides comprise the coding sequence for the mature polypeptide fused in the same reading frame to a polynucleotide which aids, for example, in expression

and secretion of a polypeptide from a host cell (e.g., a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell). The polypeptide having a leader sequence is a preprotein and can have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides can also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

[0234] In certain embodiments, the polynucleotides comprise the coding sequence for the mature polypeptide fused in the same reading frame to a marker sequence that allows, for example, for purification of the encoded polypeptide. For example, the marker sequence can be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or the marker sequence can be a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (e.g., COS-7 cells) is used. In some embodiments, the marker sequence is a FLAG-tag, a peptide of sequence DYKDDDDK (SEQ ID NO:33) which can be used in conjunction with other affinity tags.

[0235] The present invention further relates to variants of the hereinabove described polynucleotides encoding, for example, fragments, analogs, and/or derivatives.

[0236] In certain embodiments, the present invention provides polynucleotides comprising polynucleotides having a nucleotide sequence at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, and in some embodiments, at least about 96%, 97%, 98% or 99% identical to a polynucleotide encoding a polypeptide comprising a RSPO3-binding agent (e.g., an antibody), or fragment thereof, described herein.

[0237] As used herein, the phrase a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence is intended to mean that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence can include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence can be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence can be inserted into the reference sequence. These mutations of the reference sequence can occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[0238] The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some embodiments, a polynucleotide variant contains alterations which produce silent substitutions, additions, or deletions, but does not alter the properties or activities of the encoded

polypeptide. In some embodiments, a polynucleotide variant comprises silent substitutions that result in no change to the amino acid sequence of the polypeptide (due to the degeneracy of the genetic code). In some embodiments, nucleotide variants comprise nucleotide sequences which result in expression differences (e.g., increased or decreased expression) at the transcript level. Polynucleotide variants can be produced for a variety of reasons, for example, to optimize codon expression for a particular host (i.e., change codons in the human mRNA to those preferred by a bacterial host such as E. coli). In some embodiments, a polynucleotide variant comprises at least one silent mutation in a non-coding or a coding region of the sequence.

[0239] In some embodiments, a polynucleotide variant is produced to modulate or alter expression (or expression levels) of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to increase expression of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to decrease expression of the encoded polypeptide. In some embodiments, a polynucleotide variant has increased expression of the encoded polypeptide as compared to a parental polynucleotide sequence. In some embodiments, a polynucleotide variant has decreased expression of the encoded polypeptide as compared to a parental polynucleotide sequence.

[0240] In some embodiments, at least one polynucleotide variant is produced (without changing the amino acid sequence of the encoded polypeptide) to increase production of a heteromultimeric molecule. In some embodiments, at least one polynucleotide variant is produced (without changing the amino acid sequence of the encoded polypeptide) to increase production of a bispecific antibody.

[0241] In certain embodiments, the polynucleotides are isolated. In certain embodiments, the polynucleotides are substantially pure.

[0242] Vectors comprising the polynucleotides described herein are also provided. Cells comprising the polynucleotides described herein are also provided. In some embodiments, an expression vector comprises a polynucleotide molecule. In some embodiments, a host cell comprises an expression vector comprising the polynucleotide molecule. In some embodiments, a host cell comprises a polynucleotide molecule.

IV. Methods of use and pharmaceutical compositions

[0243] The RSPO3-binding agents (including polypeptides and antibodies) of the invention are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as the treatment of cancer. In certain embodiments, the agents are useful for inhibiting β-catenin signaling, inhibiting tumor growth, modulating angiogenesis, inhibiting angiogenesis, inducing differentiation, reducing tumor volume, reducing the frequency of cancer stem cells in a tumor, and/or reducing the tumorigenicity of a tumor. The methods of use may be *in vitro*, *ex vivo*, or *in vivo* methods. In certain embodiments, a RSPO3-binding agent or polypeptide or antibody is an antagonist of human RSPO3.

[0244] In certain embodiments, the RSPO3-binding agents are used in the treatment of a disease associated with activation of β-catenin, increased β-catenin signaling, and/or aberrant β-catenin signaling. In certain embodiments, the disease is a disease dependent upon β-catenin signaling. In certain embodiments, the RSPO3-binding agents are used in the treatment of disorders characterized by increased angiogenesis. In certain embodiments, the RSPO3-binding agents are used in the treatment of disorders characterized by increased levels of stem cells and/or progenitor cells. In some embodiments, the methods comprise administering a therapeutically effective amount of a RSPO3-binding agent (e.g., antibody) to a subject. In some embodiments, the subject is human.

[0245] The present invention provides methods for inhibiting growth of a tumor using the RSPO3-binding agents or antibodies described herein. In certain embodiments, the method of inhibiting growth of a tumor comprises contacting a cell with a RSPO3-binding agent (e.g., an antibody) *in vitro*. For example, an immortalized cell line or a cancer cell line is cultured in medium to which is added an anti-RSPO3 antibody or other agent to inhibit tumor growth. In some embodiments, tumor cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and cultured in medium to which is added a RSPO3-binding agent to inhibit tumor growth.

[0246] In some embodiments, the method of inhibiting growth of a tumor comprises contacting the tumor or tumor cells with a RSPO3-binding agent (e.g., an antibody) *in vivo*. In certain embodiments, contacting a tumor or tumor cell with a RSPO3-binding agent is undertaken in an animal model. For example, a RSPO3-binding agent may be administered to immunocompromised mice (e.g. NOD/SCID mice) which have xenografts. In some embodiments, cancer cells or cancer stem cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and injected into immunocompromised mice that are then administered a RSPO3-binding agent to inhibit tumor cell growth. In some embodiments, a RSPO3-binding agent is administered to the animal. In some embodiments, the RSPO3-binding agent is administered at the same time or shortly after introduction of tumorigenic cells into the animal to prevent tumor growth ("preventative model"). In some embodiments, the RSPO3-binding agent is administered as a therapeutic after tumors have grown to a specified size ("therapeutic model"). In some embodiments, the RSPO3-binding agent is an antibody. In some embodiments, the RSPO3-binding agent is an anti-RSPO3 antibody. In some embodiments, the anti-RSPO3 antibody is antibody 131R002. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R002. In some embodiments, the anti-RSPO3 antibody is antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is antibody h131R006A or antibody h131R006B. In some embodiments, the anti-RSPO3 antibody is antibody h131R005/131R007. In some embodiments, the anti-RSPO3 antibody is

antibody h131R008. In some embodiments, the anti-RSPO3 antibody is antibody h131R010. In some embodiments, the anti-RSPO3 antibody is antibody h131R011.

[0247] In certain embodiments, the method of inhibiting growth of a tumor comprises administering to a subject a therapeutically effective amount of a RSPO3-binding agent, wherein the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), or TYFANNFD (SEQ ID NO:80). In some embodiments of the method, the RSPO3-binding agent further comprises a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY (SEQ ID NO:35), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTSYTF (SEQ ID NO:34), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY (SEQ ID NO:35), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLTF (SEQ ID NO:83). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ

ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14).

[0248] In certain embodiments, the method of inhibiting growth of a tumor comprises administering to a subject a therapeutically effective amount of a RSPO3-binding agent. In certain embodiments, the subject is a human. In certain embodiments, the subject has a tumor or has had a tumor which was removed. In some embodiments, the subject has a tumor with an elevated expression level of at least one RSPO protein (e.g., RSPO1, RSPO2, RSPO3, or RSPO4). In some embodiments, the subject has a tumor with a high expression level of at least one RSPO protein (e.g., RSPO1, RSPO2, RSPO3, or RSPO4). In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent is an antibody. In some embodiments, the RSPO3-binding agent is antibody 131R002. In some embodiments, the anti-RSPO3 antibody is antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is antibody h131R006A or antibody h131R006B. In some embodiments, the RSPO3-binding agent is antibody h131R005/131R007. In some embodiments, the RSPO3-binding agent is antibody h131R008. In some embodiments, the RSPO3-binding agent is antibody h131R010. In some embodiments, the RSPO3-binding agent is antibody h131R01.

[0249] In certain embodiments, the tumor is a tumor in which β-catenin signaling is active. In some embodiments, the tumor is a tumor in which β-catenin signaling is aberrant. In certain embodiments, the tumor comprises an inactivating mutation (e.g., a truncating mutation) in the APC tumor suppressor gene. In certain embodiments, the tumor does not comprise an inactivating mutation in the APC tumor suppressor gene. In some embodiments, the tumor comprises a wild-type APC gene. In some embodiments, the tumor does not comprise an activating mutation in the β-catenin gene. In certain embodiments, a cancer for which a subject is being treated involves such a tumor.

[0250] In some embodiments, the tumor comprises a RSPO gene fusion. In some embodiments, the tumor comprises a RSPO2 gene fusion. In some embodiments, the tumor comprises a RSPO3 gene fusion.

[0251] In certain embodiments, the tumor expresses RSPO3 to which a RSPO3-binding agent or antibody binds. In certain embodiments, the tumor has elevated expression levels of RSPO1 or over-expresses RSPO1. In certain embodiments, the tumor has elevated expression levels of RSPO2 or over-

expresses RSPO2. In certain embodiments, the tumor has elevated expression levels of RSPO3 or over-expresses RSPO3. The phrase "a tumor has elevated expression levels of" may refer to expression levels of a protein or expression levels of a nucleic acid. In general, the phrase "a tumor has elevated expression levels of" a protein or a gene (or similar phrases) refers to expression levels of a protein or a gene in a tumor as compared to expression levels of the same protein or the same gene in a reference sample or to a pre-determined expression level. In some embodiments, the reference sample is normal tissue of the same tissue type. In some embodiments, the reference sample is normal tissue of a group of tissue types. In some embodiments, the reference sample is a tumor or group of tumors of the same tissue type. In some embodiments, the reference sample is a tumor or group of tumors of a different tissue type. Thus in some embodiments, the expression levels of a protein or a gene in a tumor are "elevated" or "high" as compared to the average expression level of the protein or the gene within a group of tissue types. In some embodiments, the expression levels of a protein or a gene in a tumor are "elevated" or "high" as compared to the expression level of the protein or the gene in other tumors of the same tissue type or a different tissue type. In some embodiments, the tumor expresses "elevated" or "high" levels of RSPO1, RSPO2, RSPO3, and/or RSPO4 as compared to the RSPO levels expressed in normal tissue of the same tissue type. In some embodiments, the tumor expresses "elevated" or "high" levels of RSPO1, RSPO2, RSPO3, and/or RSPO4 as compared to a predetermined level.

[0252] In addition, the invention provides a method of inhibiting growth of a tumor in a subject, comprising administering a therapeutically effective amount of a RSPO3-binding agent to the subject. In certain embodiments, the tumor comprises cancer stem cells. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of the RSPO3-binding agent. The invention also provides a method of reducing the frequency of cancer stem cells in a tumor, comprising contacting the tumor with an effective amount of a RSPO3-binding agent (e.g., an anti-RSPO3 antibody). In some embodiments, a method of reducing the frequency of cancer stem cells in a tumor in a subject, comprising administering to the subject a therapeutically effective amount of a RSPO3-binding agent (e.g., an anti-RSPO3 antibody) is provided. In some embodiments, the RSPO3-binding agent is an antibody. In some embodiments, the RSPO3-binding agent is an anti-RSPO3 antibody. In some embodiments, the anti-RSPO3 antibody is 131R002. In some embodiments, the anti-RSPO3 antibody is antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is antibody h131R006A or antibody h131R006B. In some embodiments, the anti-RSPO3 antibody is antibody h131R005/131R007. In some embodiments, the anti-RSPO3 antibody is antibody h131R008. In some embodiments, the anti-RSPO3 antibody is antibody h131R010. In some embodiments, the anti-RSPO3 antibody is antibody h131R011.

[0253] In some embodiments, the tumor is a solid tumor. In certain embodiments, the tumor is a tumor selected from the group consisting of colorectal tumor, pancreatic tumor, lung tumor, ovarian tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor. As used herein, "lung cancer" includes but is not limited to, small cell lung carcinoma and non-small cell lung carcinoma (NSCLC). In certain embodiments, the tumor is a colorectal tumor. In certain embodiments, the tumor is an ovarian tumor. In some embodiments, the tumor is a lung tumor. In certain embodiments, the tumor is a pancreatic tumor. In some embodiments, the tumor is a colorectal tumor that comprises an inactivating mutation in the APC gene. In some embodiments, the tumor is a colorectal tumor that does not comprise an inactivating mutation in the APC gene. In some embodiments, the tumor is a colorectal tumor that contains a RSPO gene fusion. In some embodiments, the tumor is a colorectal tumor that contains a RSPO2 gene fusion. In some embodiments, the tumor is a colorectal tumor that contains a RSPO3 gene fusion. In some embodiments, the tumor is an ovarian tumor with an elevated expression level of RSPO1. In some embodiments, the tumor is a pancreatic tumor with an elevated expression level of RSPO2. In some embodiments, the tumor is a colon tumor with an elevated expression level of RSPO2. In some embodiments, the tumor is a lung tumor with an elevated expression level of RSPO2. In some embodiments, the tumor is a lung tumor with an elevated expression level of RSPO3. In some embodiments, the tumor is an ovarian tumor with an elevated expression level of RSPO3. In some embodiments, the tumor is a breast tumor with an elevated expression level of RSPO3. In some embodiments, the tumor is a colorectal tumor with an elevated expression level of RSPO3.

[0254] The present invention further provides methods for treating cancer comprising administering a therapeutically effective amount of a RSPO3-binding agent to a subject. In certain embodiments, the cancer is characterized by cells expressing elevated levels of at least one RSPO protein as compared to expression levels of the same RSPO protein in a reference sample. As used herein, a "reference sample" includes but is not limited to, normal tissue, non-cancerous tissue of the same tissue type, tumor tissue of the same tissue type, and tumor tissue of a different tissue type. In certain embodiments, the cancer is characterized by cells expressing elevated levels of at least one RSPO protein as compared to a pre-determined level of the same RSPO protein. In some embodiments, determining the expression level of at least one RSPO is done prior to treatment. In some embodiments, determining the expression level of at least one RSPO is by immunohistochemistry. Thus, in certain embodiments, the cancer is characterized by cells expressing elevated levels of at least one RSPO protein as compared to expression levels of the same RSPO protein in normal tissue. In certain embodiments, the cancer is characterized by cells over-expressing RSPO1. In certain embodiments, the cancer is characterized by cells over-expressing RSPO2. In certain embodiments, the cancer is characterized by cells over-expressing RSPO3. In certain embodiments, the cancer over-expresses at least one RSPO protein selected from the group consisting of

RSPO1, RSPO2, RSPO3, and/or RSPO4. In certain embodiments, the cancer is characterized by cells expressing β-catenin, wherein the RSPO3-binding agent (e.g., an antibody) interferes with RSPO3-induced β-catenin signaling and/or activation.

[0255] In some embodiments, the RSPO-binding agent binds RSPO3, and inhibits or reduces growth of the cancer. In some embodiments, the RSPO-binding agent binds RSPO3, interferes with RSPO3/LGR interactions, and inhibits or reduces growth of the cancer. In some embodiments, the RSPO-binding agent binds RSPO3, inhibits β-catenin activation, and inhibits or reduces growth of the cancer. In some embodiments, the RSPO-binding agent binds RSPO3, and reduces the frequency of cancer stem cells in the cancer. In some embodiments, the RSPO-binding agent is an antibody. In some embodiments, the RSPO-binding agent is an anti-RSPO3 antibody. In some embodiments, the anti-RSPO3 antibody is antibody 131R002. In some embodiments, the anti-RSPO3 antibody is antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a variant of antibody 131R003. In some embodiments, the anti-RSPO antibody is a humanized version of antibody 131R002. In some embodiments, the anti-RSPO antibody is a humanized version of antibody 131R003. In some embodiments, the anti-RSPO antibody is a humanized version of a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is antibody h131R006A or antibody h131R006B. In some embodiments, the anti-RSPO3 antibody is antibody h131R005/131R007. In some embodiments, the anti-RSPO3 antibody is antibody h131R008. In some embodiments, the anti-RSPO3 antibody is antibody h131R010. In some embodiments, the anti-RSPO3 antibody is antibody h131R011.

[0256] The present invention provides for methods of treating cancer comprising administering a therapeutically effective amount of a RSPO3-binding agent to a subject (e.g., a subject in need of treatment). In certain embodiments, the method of treating cancer comprises administering to a subject a therapeutically effective amount of a RSPO3-binding agent, wherein the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), or TYFANNFD (SEQ ID NO:80). In some embodiments of the method, the RSPO3-binding agent further comprises a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising

QQSNEDPLT (SEQ ID NO:14). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY (SEQ ID NO:35), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTSYTF (SEQ ID NO:34), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANNFDY (SEQ ID NO:35), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLTF (SEQ ID NO:83). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SEQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In some embodiments, the RSPO3-binding agent comprises a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80), and/or a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14). In certain embodiments, the subject is a human. In certain embodiments, the subject has a cancerous tumor. In certain embodiments, the subject has had a tumor removed. In some embodiments, a method of treating cancer comprises administering a therapeutically effective amount of a RSPO3-binding agent to a subject, wherein the subject has a tumor that has elevated expression of at least one RSPO protein as compared to a reference sample or a pre-determined level. In some embodiments, the subject has a lung tumor that has elevated expression of RSPO3 and is administered an anti-RSPO3 antibody.

[0257] The invention also provides a RSPO3-binding agent for use in a method of treating cancer, wherein the RSPO3-binding agent is an antibody described herein. The invention also provides the use of an RSPO3-binding agent (e.g., an antibody) described herein for the manufacture of a medicament for the treatment of cancer.

[0258] In certain embodiments, the cancer is a cancer selected from the group consisting of colorectal cancer, pancreatic cancer, lung cancer, ovarian cancer, liver cancer, breast cancer, kidney cancer, prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, bladder cancer, glioblastoma, and head and neck cancer. In certain embodiments, the cancer is pancreatic cancer. In certain embodiments, the cancer is ovarian cancer. In certain embodiments, the cancer is colorectal cancer. In certain embodiments, the cancer is breast cancer. In certain embodiments, the cancer is prostate cancer. In certain embodiments, the cancer is lung cancer.

[0259] In addition, the invention provides a method of reducing the tumorigenicity of a tumor in a subject, comprising administering to a subject a therapeutically effective amount of a RSPO3-binding agent. In certain embodiments, the tumor comprises cancer stem cells. In some embodiments, the tumorigenicity of a tumor is reduced by reducing the frequency of cancer stem cells in the tumor. In some embodiments, the methods comprise using the RSPO3-binding agents described herein. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of a RSPO3-binding agent.

[0260] In certain embodiments, the methods further comprise a step of determining the expression level of at least one RSPO (i.e., protein or nucleic acid) in the tumor or cancer. In some embodiments, the step of determining the expression level of a RSPO in the tumor or cancer comprises determining the expression level of RSPO1, RSPO2, RSPO3, and/or RSPO4. In some embodiments, the expression level of RSPO1, RSPO2, RSPO3, and/or RSPO4 in a tumor or cancer is compared to the expression level of RSPO1, RSPO2, RSPO3, and/or RSPO4 in a reference sample. In some embodiments, the expression level of RSPO1, RSPO2, RSPO3, and/or RSPO4 in a tumor or cancer is compared to the expression level of RSPO1, RSPO2, RSPO3, and/or RSPO4 in normal tissue. In some embodiments, the level of expression of RSPO1, RSPO2, RSPO3, and/or RSPO4 in a tumor or cancer is compared to a pre-determined level of expression of RSPO1, RSPO2, RSPO3, and/or RSPO4. In some embodiments, the level of expression of RSPO1, RSPO2, RSPO3, and/or RSPO4 in a tumor or cancer is compared to a pre-determined level of expression of RSPO1, RSPO2, RSPO3, and/or RSPO4 in normal tissue. In some embodiments, the tumor has a high expression level of RSPO1. In some embodiments, the tumor has a high expression level of RSPO3. In general, the expression level of a RSPO (i.e., protein or nucleic acid) is compared to the expression level of the RSPO (i.e., protein or nucleic acid) in normal tissue of the same tissue type. However, in some embodiments, the expression level of a RSPO (i.e., protein or nucleic acid) is compared to the average expression level of the RSPO (i.e., protein or nucleic acid) within a group of tissue types. In some embodiments, the expression levels of a RSPO (i.e., protein or nucleic acid) in a tumor is compared to the expression level of the RSPO (i.e., protein or nucleic acid) in other tumors of the same tissue type or a different tissue type.

[0261] In some embodiments, determining the level of RSPO expression is done prior to treatment. In some embodiments, the subject is administered a RSPO3-binding agent or antibody describe herein if the tumor or cancer has an elevated expression level of RSPO as compared to the expression level of the same RSPO in a reference sample (e.g., normal tissue) or a pre-determined level. For example, in some embodiments, the subject is administered a RSPO3-binding agent (e.g., anti-RSPO3 antibody) if the tumor or cancer has an elevated expression level of RSPO3 (i.e., protein or nucleic acid) as compared to the expression level of RSPO3 in normal or control tissue.

[0262] In certain embodiments, the methods further comprise a step of determining if the tumor or cancer has an inactivating mutation in the APC gene. In some embodiments, the methods further comprise a step of determining if the tumor or cancer has an activating mutation in the β-catenin gene. In some embodiments, the methods further comprise a step of determining if the tumor or cancer has a RSPO gene fusion.

[0263] In addition, the invention provides a method of modulating angiogenesis, comprising administering to a subject a therapeutically effective amount of a RSPO3-binding agent. In some embodiments, the modulating angiogenesis comprises inhibiting angiogenesis. In some embodiments, the methods comprise using the RSPO3-binding agents described herein. In certain embodiments, the RSPO3-binding agent binds RSPO3 and inhibits or reduces angiogenesis. In certain embodiments, the inhibition and/or reduction of angiogenesis inhibits or reduces growth of a tumor or cancer. In some embodiments, the RSPO3-binding agent binds RSPO3 and promotes aberrant angiogenesis. In some embodiments, the RSPO3-binding agent binds RSPO3 and promotes unproductive angiogenesis. In certain embodiments, the aberrant angiogenesis or the unproductive angiogenesis inhibits or reduces growth of a tumor or cancer.

[0264] In addition, the present invention provides methods of identifying a human subject for treatment with a RSPO-binding agent, comprising determining if the subject has a tumor that has an elevated expression level of RSPO (i.e., protein or nucleic acid) as compared to expression of the same RSPO (i.e., protein or nucleic acid) in normal tissue, in a reference sample, or to a pre-determined level of the RSPO protein. In some embodiments, a method of identifying a human subject for treatment with a RSPO3-binding agent comprises determining if the subject has a tumor that has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3. In some embodiments, a method of identifying a human subject for treatment with a RSPO3-binding agent comprises: obtaining a tumor sample from the subject, and determining if the tumor has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3. In some embodiments, if the tumor has an elevated expression level of RSPO3, the subject is selected for treatment with an antibody that specifically binds RSPO3. In some embodiments, if selected for treatment, the subject is administered a RSPO3-binding agent or antibody describe herein. In some embodiments, if the tumor has

an elevated expression level of more than one RSPO (i.e., protein or nucleic acid), the subject is administered a RSPO-binding agent that binds the RSPO with the highest level of expression. In certain embodiments, the subject has had a tumor removed. For example, in some embodiments, the expression level of RSPO1, RSPO2, RSPO3, and/or RSPO4 in a tumor is determined, if the tumor has an elevated level of RSPO3 expression as compared to the level of RSPO3 in normal tissue, the subject is selected for treatment with an antibody that specifically binds RSPO3. If selected for treatment, the subject is administered an anti-RSPO3 antibody describe herein. In some embodiments, the RSPO3-binding agent is antibody 131R002. In some embodiments, the RSPO3-binding agent is antibody 131R003. In some embodiments, the RSPO3-binding agent is a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized form of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized form of a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is antibody h131R006A or antibody h131R006B. In some embodiments, the RSPO3-binding agent is antibody h131R005/131R007. In some embodiments, the RSPO3-binding agent is antibody h131R008. In some embodiments, the RSPO3-binding agent is antibody h131R010. In some embodiments, the RSPO3-binding agent is antibody h131R011.

[0265] The present invention provides methods of selecting a human subject for treatment with a RSPO-binding agent, comprising determining if the subject has a tumor that has an elevated expression level of at least one RSPO (i.e., protein or nucleic acid), as compared to expression of the same RSPO in normal tissue or as compared to a predetermined level, wherein if the tumor has an elevated expression level of at least one RSPO, the subject is selected for treatment with an antibody that specifically binds the RSPO with the elevated expression level. In some embodiments, if selected for treatment, the subject is administered a RSPO-binding agent or antibody describe herein. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3 comprises: determining if the subject has a tumor that has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3 comprises obtaining a tumor sample from the subject, and determining if the tumor has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprises determining if the subject has a tumor that has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3, wherein if the tumor has an elevated expression level of RSPO3 the subject is selected for treatment with the antibody. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprises obtaining a tumor sample from the subject, and determining if the tumor has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3, wherein if the tumor

has an elevated expression level of RSPO3 the subject is selected for treatment with the antibody. In certain embodiments, the subject has had a tumor removed. In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent is an anti-RSPO3 antibody. In some embodiments, the anti-RSPO3 antibody is antibody 131R002. In some embodiments, the anti-RSPO3 antibody is antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R002. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is antibody h131R006A or h131R006B. In some embodiments, the anti-RSPO3 antibody is antibody h131R005/131R007. In some embodiments, the anti-RSPO3 antibody is antibody h131R008. In some embodiments, the anti-RSPO3 antibody is antibody h131R010. In some embodiments, the anti-RSPO3 antibody is antibody h131R011.

[0266] The present invention also provides methods of treating cancer in a human subject, comprising: (a) selecting a subject for treatment based, at least in part, on the subject having a cancer that has an elevated level of a RSPO, and (b) administering to the subject a therapeutically effective amount of a RSPO3-binding agent described herein. In some embodiments, the RSPO3-binding agent is antibody 131R002. In some embodiments, the RSPO3-binding agent is antibody 131R003. In some embodiments, the RSPO3-binding agent is a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R002. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is antibody h131R006A or h131R006B. In some embodiments, the anti-RSPO3 antibody is antibody h131R005/131R007. In some embodiments, the anti-RSPO3 antibody is antibody h131R008. In some embodiments, the anti-RSPO3 antibody is antibody h131R010. In some embodiments, the anti-RSPO3 antibody is antibody h131R011.

[0267] Methods for determining the level of RSPO expression in a cell, tumor or cancer are known by those of skill in the art. For nucleic acid expression these methods include, but are not limited to, PCR-based assays, microarray analyses and nucleotide sequencing (e.g., NextGen sequencing). For protein expression these methods include, but are not limited to, Western blot analysis, protein arrays, ELISAs, immunohistochemistry (IHC) assays, and FACS.

[0268] The present invention provides methods of identifying a human subject for treatment with a RSPO3-binding agent, comprising obtaining a tumor sample from the subject, and determining if the tumor has a RSPO gene fusion. In some embodiments, a method of identifying a human subject for treatment with a RSPO3-binding agent comprises: determining if the subject has a tumor that has a RSPO gene fusion, wherein if the tumor has a RSPO gene fusion, then the subject is selected for treatment with

the antibody. In some embodiments, a method of identifying a human subject for treatment with a RSPO3-binding agent comprises: (a) obtaining a tumor sample from the subject, and (b) determining if the tumor has a RSPO gene fusion, wherein if the tumor has a RSPO gene fusion, then the subject is selected for treatment with the antibody. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprises determining if the subject has a tumor that has a RSPO gene fusion.

[0269] The present invention also provides methods of selecting a human subject for treatment with a RSPO-binding agent, comprising determining if the subject has a tumor that has a RSPO gene fusion, wherein if the tumor has a RSPO gene fusion, the subject is selected for treatment with an antibody that specifically binds a RSPO protein. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3 comprises determining if the subject has a tumor that has a RSPO gene fusion. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprises obtaining a tumor sample from the subject, and determining if the tumor has a RSPO gene fusion. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprises determining if the subject has a tumor that has a RSPO gene fusion, wherein if the tumor has a RSPO gene fusion the subject is selected for treatment with the antibody. In some embodiments, a method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprises obtaining a tumor sample from the subject, and determining if the tumor has a RSPO gene fusion, wherein if the tumor has a RSPO gene fusion the subject is selected for treatment with the antibody. In some embodiments, the RSPO gene fusion is a RSPO2 gene fusion. In some embodiments, the RSPO gene fusion is a RSPO3 gene fusion. In some embodiments, if selected for treatment, the subject is administered a RSPO-binding agent or antibody describe herein. In certain embodiments, the subject has had a tumor removed. In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent is an anti-RSPO3 antibody. In some embodiments, the anti-RSPO3 antibody is antibody 131R002. In some embodiments, the anti-RSPO3 antibody is antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R002. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is antibody h131R006A or h131R006B. In some embodiments, the anti-RSPO3 antibody is antibody h131R005/131R007. In some embodiments, the anti-RSPO3 antibody is antibody h131R008. In some embodiments, the anti-RSPO3 antibody is antibody h131R010. In some embodiments, the anti-RSPO3 antibody is antibody h131R011.

[0270] The present invention also provides methods of treating cancer in a human subject, comprising: (a) selecting a subject for treatment based, at least in part, on the subject having a cancer that has a RSPO gene fusion, and (b) administering to the subject a therapeutically effective amount of a RSPO3-binding agent described herein. In some embodiments, the RSPO3-binding agent is antibody 131R002. In some embodiments, the RSPO3-binding agent is antibody 131R003. In some embodiments, the RSPO3-binding agent is a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R002. In some embodiments, the anti-RSPO3 antibody is a humanized version of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is a humanized version of a variant of antibody 131R003. In some embodiments, the anti-RSPO3 antibody is antibody h131R006A or h131R006B. In some embodiments, the anti-RSPO3 antibody is antibody h131R005/131R007. In some embodiments, the anti-RSPO3 antibody is antibody h131R008. In some embodiments, the anti-RSPO3 antibody is antibody h131R010. In some embodiments, the anti-RSPO3 antibody is antibody h131R011.

[0271] Methods for determining whether a tumor has a RSPO gene fusion are known by those of skill in the art. Methods may include but are not limited to, PCR-based assays, microarray analyses, and nucleotide sequencing (e.g., NextGen sequencing, whole-genome sequencing (WGS)).

[0272] Methods for determining whether a tumor or cancer has an elevated level of RSPO expression or has a RSPO gene fusion can use a variety of samples. In some embodiments, the sample is taken from a subject having a tumor or cancer. In some embodiments, the sample is a fresh tumor/cancer sample. In some embodiments, the sample is a frozen tumor/cancer sample. In some embodiments, the sample is a formalin-fixed paraffin-embedded sample. In some embodiments, the sample is processed to a cell lysate. In some embodiments, the sample is processed to DNA or RNA.

[0273] Methods of treating a disease or disorder in a subject, wherein the disease or disorder is associated with aberrant (e.g., increased levels) β-catenin signaling are further provided. Methods of treating a disease or disorder in a subject, wherein the disease or disorder is characterized by an increased level of stem cells and/or progenitor cells are further provided. In some embodiments, the treatment methods comprise administering a therapeutically effective amount of a RSPO-binding agent, polypeptide, or antibody to the subject. In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent is an antibody. In some embodiments, the RSPO3-binding agent is antibody 131R002. In some embodiments, the RSPO3-binding agent is antibody 131R003. In some embodiments, the RSPO3-binding agent is a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized version of antibody 131R002. In some embodiments, the RSPO3-binding agent is a humanized version of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized version of a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is antibody h131R006A or antibody h131R006B. In some embodiments, the

RSPO3-binding agent is antibody h131R005/131R007. In some embodiments, the RSPO3-binding agent is antibody h131R008. In some embodiments, the RSPO3-binding agent is antibody h131R010. In some embodiments, the RSPO3-binding agent is antibody h131R011.

[0274] The invention also provides a method of inhibiting β-catenin signaling in a cell comprising contacting the cell with an effective amount of a RSPO-binding agent. In certain embodiments, the cell is a tumor cell. In certain embodiments, the method is an *in vivo* method wherein the step of contacting the cell with the RSPO3-binding agent comprises administering a therapeutically effective amount of the RSPO3-binding agent to the subject. In some embodiments, the method is an *in vitro* or *ex vivo* method. In certain embodiments, the RSPO-binding agent inhibits β-catenin signaling. In some embodiments, the RSPO-binding agent inhibits activation of β-catenin. In certain embodiments, the RSPO-binding agent interferes with a RSPO/LGR interaction. In certain embodiments, the LGR is LGR4, LGR5, and/or LGR6. In certain embodiments, the LGR is LGR4. In certain embodiments, the LGR is LGR5. In certain embodiments, the LGR is LGR6. In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent is an antibody. In some embodiments, the RSPO3-binding agent is antibody 131R002. In some embodiments, the RSPO3-binding agent is antibody 131R003. In some embodiments, the RSPO3-binding agent is a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized version of antibody 131R002. In some embodiments, the RSPO3-binding agent is a humanized version of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized version of a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is antibody h131R006A or antibody h131R006B. In some embodiments, the RSPO3-binding agent is antibody h131R005/131R007. In some embodiments, the RSPO3-binding agent is antibody h131R008. In some embodiments, the RSPO3-binding agent is antibody h131R010. In some embodiments, the RSPO3-binding agent is antibody h131R011.

[0275] The use of the RSPO-binding agents, polypeptides, or antibodies described herein to induce the differentiation of cells, including, but not limited to tumor cells, is also provided. In some embodiments, methods of inducing cells to differentiate comprise contacting the cells with an effective amount of a RSPO-binding agent (e.g., an anti-RSPO antibody) described herein. In certain embodiments, methods of inducing cells in a tumor in a subject to differentiate comprise administering a therapeutically effective amount of a RSPO-binding agent, polypeptide, or antibody to the subject. In some embodiments, methods for inducing differentiation markers on tumor cells comprise administering a therapeutically effective amount of a RSPO-binding agent, polypeptide, or antibody. In some embodiments, the tumor is a solid tumor. In some embodiments, the tumor is selected from the group consisting of colorectal tumor, pancreatic tumor, lung tumor, ovarian tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor. In certain embodiments, the tumor is an ovarian tumor. In certain other embodiments, the tumor is a

colon tumor. In some embodiments, the tumor is a lung tumor. In certain embodiments, the method is an *in vivo* method. In certain embodiments, the method is an *in vitro* method. In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent is an antibody. In some embodiments, the RSPO3-binding agent is antibody 131R002. In some embodiments, the RSPO3-binding agent is antibody 131R003. In some embodiments, the RSPO3-binding agent is a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized version of antibody 131R002. In some embodiments, the RSPO3-binding agent is a humanized version of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized version of a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is antibody h131R006A or antibody h131R006B. In some embodiments, the RSPO3-binding agent is antibody h131R005/131R007. In some embodiments, the RSPO3-binding agent is antibody h131R008. In some embodiments, the RSPO3-binding agent is antibody h131R010. In some embodiments, the RSPO3-binding agent is antibody h131R011.

[0276] The invention further provides methods of differentiating tumorigenic cells into non-tumorigenic cells comprising contacting the tumorigenic cells with a RSPO-binding agent. In some embodiments, the method comprises administering the RSPO-binding agent to a subject that has a tumor comprising tumorigenic cells or that has had such a tumor removed. In certain embodiments, the tumorigenic cells are ovarian tumor cells. In certain embodiments, the tumorigenic cells are colon tumor cells. In some embodiments, the tumorigenic cells are lung tumor cells. In some embodiments, the RSPO-binding agent is a RSPO3-binding agent. In some embodiments, the RSPO3-binding agent is an antibody. In some embodiments, the RSPO3-binding agent is antibody 131R002. In some embodiments, the RSPO3-binding agent is antibody 131R003. In some embodiments, the RSPO3-binding agent is a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized version of antibody 131R002. In some embodiments, the RSPO3-binding agent is a humanized version of antibody 131R003. In some embodiments, the RSPO3-binding agent is a humanized version of a variant of antibody 131R003. In some embodiments, the RSPO3-binding agent is antibody h131R006A or antibody h131R006B. In some embodiments, the RSPO3-binding agent is antibody h131R005/131R007. In some embodiments, the RSPO3-binding agent is antibody h131R008. In some embodiments, the RSPO3-binding agent is antibody h131R010. In some embodiments, the RSPO3-binding agent is antibody h131R011.

[0277] In certain embodiments, the disease treated with the RSPO3-binding agents described herein is not a cancer. For example, the disease may be a metabolic disorder such as obesity or diabetes (e.g., type II diabetes) (Jin T., 2008, *Diabetologia*, 51:1771-80). Alternatively, the disease may be a bone disorder such as osteoporosis, osteoarthritis, or rheumatoid arthritis (Corr M., 2008, *Nat. Clin. Pract. Rheumatol.*, 4:550-6; Day et al., 2008, *Bone Joint Surg. Am.*, 90 Suppl 1:19-24). The disease may also be a kidney

disorder, such as a polycystic kidney disease (Harris et al., 2009, *Ann. Rev. Med.*, 60:321-337; Schmidt-Ott et al., 2008, *Kidney Int.*, 74:1004-8; Benzing et al., 2007, *J. Am. Soc. Nephrol.*, 18:1389-98). Alternatively, eye disorders including, but not limited to, macular degeneration and familial exudative vitreoretinopathy may be treated (Lad et al., 2009, *Stem Cells Dev.*, 18:7-16). Cardiovascular disorders, including myocardial infarction, atherosclerosis, and valve disorders, may also be treated (Al-Aly Z., 2008, *Transl. Res.*, 151:233-9; Kobayashi et al., 2009, *Nat. Cell Biol.*, 11:46-55; van Gijn et al., 2002, *Cardiovasc. Res.*, 55:16-24; Christman et al., 2008, *Am. J. Physiol. Heart Circ. Physiol.*, 294:H2864-70). In some embodiments, the disease is a pulmonary disorder such as idiopathic pulmonary arterial hypertension or pulmonary fibrosis (Laumanns et al., 2008, *Am. J. Respir. Cell Mol. Biol.*, 2009, 40:683-691; Königshoff et al., 2008, *PLoS ONE*, 3:e2142). In some embodiments, the disease treated with the RSPO3-binding agent is a liver disease, such as cirrhosis or liver fibrosis (Cheng et al., 2008, *Am. J. Physiol. Gastrointest. Liver Physiol.*, 294:G39-49).

[0278] The present invention further provides pharmaceutical compositions comprising the RSPO3-binding agents described herein. In certain embodiments, the pharmaceutical compositions further comprise a pharmaceutically acceptable vehicle. In some embodiments, these pharmaceutical compositions find use in inhibiting tumor growth and treating cancer in a subject (e.g., a human patient).

[0279] In certain embodiments, formulations are prepared for storage and use by combining a purified antibody or agent of the present invention with a pharmaceutically acceptable vehicle (e.g., a carrier or excipient). Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (e.g., less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes; and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG). (*Remington: The Science and Practice of Pharmacy, 22nd Edition*, 2012, Pharmaceutical Press, London.)

[0280] The pharmaceutical compositions of the present invention can be administered in any number of ways for either local or systemic treatment. Administration can be topical by epidermal or transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, and intranasal;

oral; or parenteral including intravenous, intraarterial, intratumoral, subcutaneous, intraperitoneal, intramuscular (e.g., injection or infusion), or intracranial (e.g., intrathecal or intraventricular).

[0281] The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and diluents (e.g., water). These can be used to form a solid pre-formulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. The solid pre-formulation composition is then subdivided into unit dosage forms of a type described above. The tablets, pills, etc. of the formulation or composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

[0282] The RSPO3-binding agents or antibodies described herein can also be entrapped in microcapsules. Such microcapsules are prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions as described in *Remington: The Science and Practice of Pharmacy, 22st Edition*, 2012, Pharmaceutical Press, London.

[0283] In certain embodiments, pharmaceutical formulations include a RSPO3-binding agent (e.g., an antibody) of the present invention complexed with liposomes. Methods to produce liposomes are known to those of skill in the art. For example, some liposomes can be generated by reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes can be extruded through filters of defined pore size to yield liposomes with the desired diameter.

[0284] In certain embodiments, sustained-release preparations can be produced. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing a RSPO3-binding agent (e.g., an antibody), where the matrices are in the form of shaped articles (e.g., films or microcapsules). Examples of sustained-release matrices include polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(vinyl alcohol), polylactides, copolymers of L-glutamic acid

and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[0285] In certain embodiments, in addition to administering a RSPO3-binding agent (e.g., an antibody), the method or treatment further comprises administering at least one additional therapeutic agent. An additional therapeutic agent can be administered prior to, concurrently with, and/or subsequently to, administration of the RSPO3-binding agent. Pharmaceutical compositions comprising a RSPO3-binding agent and the additional therapeutic agent(s) are also provided. In some embodiments, the at least one additional therapeutic agent comprises 1, 2, 3, or more additional therapeutic agents.

[0286] Combination therapy with two or more therapeutic agents often uses agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action may result in additive or synergetic effects. Combination therapy may allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of the agent(s). Combination therapy may decrease the likelihood that resistant cancer cells will develop. In some embodiments, combination therapy comprises a therapeutic agent that affects (e.g., inhibits or kills) non-tumorigenic cells and a therapeutic agent that affects (e.g., inhibits or kills) tumorigenic CSCs.

[0287] In some embodiments, the combination of a RSPO3-binding agent and at least one additional therapeutic agent results in additive or synergistic results. In some embodiments, the combination therapy results in an increase in the therapeutic index of the RSPO3-binding agent. In some embodiments, the combination therapy results in an increase in the therapeutic index of the additional agent(s). In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the RSPO3-binding agent. In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the additional agent(s).

[0288] Useful classes of therapeutic agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain embodiments, the second therapeutic agent is an alkylating agent, an antimetabolite, an antimitotic, a topoisomerase inhibitor, or an angiogenesis inhibitor. In some embodiments, the second therapeutic agent is a platinum complex such as carboplatin or cisplatin. In some embodiments, the additional therapeutic agent is a platinum complex in combination with a taxane.

[0289] Therapeutic agents that may be administered in combination with the RSPO3-binding agents include chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the administration of a RSPO3-binding agent or antibody of the present invention in combination with a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents. Treatment with a RSPO3-binding agent (e.g, an antibody) can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *The Chemotherapy Source Book, 4th Edition*, 2008, M. C. Perry, Editor, Lippincott, Williams & Wilkins, Philadelphia, PA.

[0290] Chemotherapeutic agents useful in the instant invention include, but are not limited to, alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamime; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytosine arabinoside, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane;

sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); taxoids, e.g. paclitaxel (TAXOL) and docetaxel (TAXOTERE); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine (XELODA); and pharmaceutically acceptable salts, acids or derivatives of any of the above. Chemotherapeutic agents also include anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (FARESTON); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In certain embodiments, the additional therapeutic agent is cisplatin. In certain embodiments, the additional therapeutic agent is carboplatin. In certain embodiments, the additional therapeutic agent is paclitaxel (taxol). In some embodiments, a method comprises administering anti-RSPO3 antibody 131R002, 131R003, a variant of 131R003, 131R006A, 131R006B, 131R005/131R007, or 131R008 in combination with cisplatin.

[0291] In certain embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapy agents that interfere with the action of a topoisomerase enzyme (e.g., topoisomerase I or II). Topoisomerase inhibitors include, but are not limited to, doxorubicin HCl, daunorubicin citrate, mitoxantrone HCl, actinomycin D, etoposide, topotecan HCl, teniposide (VM-26), and irinotecan, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In some embodiments, the additional therapeutic agent is irinotecan. Thus, in some embodiments, a method comprises administering a RSPO3-binding agent in combination with a topoisomerase inhibitor. In some embodiments, a method comprises administering anti-RSPO3 antibody 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with irinotecan.

[0292] In certain embodiments, the chemotherapeutic agent is an anti-metabolite. An anti-metabolite is a chemical with a structure that is similar to a metabolite required for normal biochemical reactions, yet different enough to interfere with one or more normal functions of cells, such as cell division. Anti-metabolites include, but are not limited to, gemcitabine, fluorouracil, capecitabine, methotrexate sodium, ralitrexed, pemetrexed, tegafur, cytosine arabinoside, thioguanine, 5-azacytidine, 6-mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In certain embodiments, the additional therapeutic agent is gemcitabine. Thus, in some embodiments, a method comprises

administering a RSPO3-binding agent in combination with an anti-metabolite. In some embodiments, a method comprises administering anti-RSPO3 antibody 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with gemcitabine. In some embodiments, a method comprises administering anti-RSPO3 antibody 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with pemetrexed.

[0293] In certain embodiments, the chemotherapeutic agent is an antimitotic agent, including, but not limited to, agents that bind tubulin. In some embodiments, the agent is a taxane. In certain embodiments, the agent is paclitaxel or docetaxel, or a pharmaceutically acceptable salt, acid, or derivative of paclitaxel or docetaxel. In certain embodiments, the agent is paclitaxel (TAXOL), docetaxel (TAXOTERE), albumin-bound paclitaxel (nab-paclitaxel; ABRAXANE), DHA-paclitaxel, or PG-paclitaxel. In certain alternative embodiments, the antimitotic agent comprises a vinca alkaloid, such as vincristine, binblastine, vinorelbine, or vindesine, or pharmaceutically acceptable salts, acids, or derivatives thereof. In some embodiments, the antimitotic agent is an inhibitor of kinesin Eg5 or an inhibitor of a mitotic kinase such as Aurora A or Plk1. In certain embodiments, where the chemotherapeutic agent administered in combination with a RSPO-binding agent is an anti-mitotic agent, the cancer or tumor being treated is breast cancer or a breast tumor. In some embodiments, a method comprises administering anti-RSPO3 antibody 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with paclitaxel. In some embodiments, a method comprises administering anti-RSPO3 antibody 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with nab-paclitaxel (ABRAXANE). In some embodiments, a method comprises administering anti-RSPO3 antibody 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with gemcitabine and nab-paclitaxel (ABRAXANE).

[0294] In some embodiments, an additional therapeutic agent comprises an agent such as a small molecule. For example, treatment can involve the combined administration of a RSPO3-binding agent (e.g. an antibody) of the present invention with a small molecule that acts as an inhibitor against additional tumor-associated antigens including, but not limited to, EGFR, ErbB2, HER2, and/or VEGF. In certain embodiments, the additional therapeutic agent is a small molecule that inhibits a cancer stem cell pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Notch pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Wnt pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the BMP pathway. In some embodiments, the additional therapeutic agent is a molecule that inhibits β-catenin signaling.

[0295] In some embodiments, an additional therapeutic agent comprises a biological molecule, such as an antibody. For example, treatment can involve the combined administration of a RSPO3-binding agent (e.g. an antibody) of the present invention with other antibodies against additional tumor-associated antigens including, but not limited to, antibodies that bind EGFR, ErbB2, HER2, and/or VEGF. In some embodiments, the additional therapeutic agent is an antibody that binds a second RSPO, e.g., RSPO1, RSPO2, and/or RSPO4. In some embodiments, the additional therapeutic agent is an anti-RSPO2 antibody. In some embodiments, the additional therapeutic agent is an anti-RSPO1 antibody. In certain embodiments, the additional therapeutic agent is an antibody specific for an anti-cancer stem cell marker. In some embodiments, the additional therapeutic agent is an antibody that binds a component of the Notch pathway. In some embodiments, the additional therapeutic agent is an antibody that binds a component of the Wnt pathway. In certain embodiments, the additional therapeutic agent is an antibody that inhibits a cancer stem cell pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Notch pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the Wnt pathway. In some embodiments, the additional therapeutic agent is an inhibitor of the BMP pathway. In some embodiments, the additional therapeutic agent is an antibody that inhibits β-catenin signaling. In certain embodiments, the additional therapeutic agent is an antibody that is an angiogenesis inhibitor (e.g., an anti-VEGF or VEGF receptor antibody). In certain embodiments, the additional therapeutic agent is bevacizumab (AVASTIN), trastuzumab (HERCEPTIN), panitumumab (VECTIBIX), or cetuximab (ERBITUX).

[0296] In some embodiments, the methods described herein comprise administering a therapeutically effective amount of a RSPO3-binding agent in combination with Wnt pathway inhibitors. In some embodiments, the Wnt pathway inhibitors are frizzled (FZD) protein binding agents, "FZD-binding agents". Non-limiting examples of FZD-binding agents can be found in U.S. Patent No. 7,982,013, which is incorporated by reference herein in its entirety. FZD-binding agents may include, but are not limited to, anti-FZD antibodies. In some embodiments, a method comprises administering a RSPO-binding agent in combination with an anti-FZD antibody. In some embodiments, a method comprises administering a RSPO-binding agent in combination with the anti-FZD antibody 18R5. In some embodiments, the Wnt pathway inhibitors are Wnt protein binding agents, "Wnt-binding agents". Nonlimiting examples of Wnt-binding agents can be found in U.S. Patent Nos. 7,723,477 and 7,947,277; and International Publications WO 2011/088127 and WO 2011/088123, which are incorporated by reference herein in their entirety. Wnt-binding agents may include, but are not limited to, anti-Wnt antibodies and FZD-Fc soluble receptors. In some embodiments, a method comprises administering a RSPO3-binding agent in combination with a FZD-Fc soluble receptor. In some embodiments, a method comprises administering a RSPO3-binding agent in combination with a FZD8-Fc soluble receptor. In some embodiments, a method comprises administering a RSPO3-binding agent in combination with an anti-FZD antibody. In some

embodiments, a method comprises administering anti-RSPO3 antibodies 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with an anti-FZD antibody. In some embodiments, a method comprises administering anti-RSPO3 antibodies 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with anti-FZD antibody 18R5. In some embodiments, a method comprises administering anti-RSPO3 antibodies 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with a FZD-Fc soluble receptor. In some embodiments, a method comprises administering anti-RSPO3 antibodies 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with a FZD8-Fc soluble receptor.

[0297] In some embodiments, the methods described herein comprise administering a therapeutically effective amount of a RSPO-binding agent in combination with more than one additional therapeutic agent. Thus, in some embodiments, a method comprises administering a RSPO-binding agent in combination with a chemotherapeutic agent and a Wnt pathway inhibitor. In some embodiments, a method comprises administering a RSPO3-binding agent in combination with a chemotherapeutic agent and a Wnt pathway inhibitor. In some embodiments, a method comprises administering a RSPO3-binding agent in combination with a chemotherapeutic agent and anti-FZD antibody 18R5. In some embodiments, a method comprises administering a RSPO3-binding agent in combination with a chemotherapeutic agent and a FZD8-Fc soluble receptor. In some embodiments, a method comprises administering a RSPO3-binding agent in combination with gemcitabine and a Wnt pathway inhibitor. In some embodiments, a method comprises administering anti-RSPO3 antibodies 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with gemcitabine and anti-FZD antibody 18R5. In some embodiments, a method comprises administering anti-RSPO3 antibodies 131R002, 131R003, a variant of 131R003, a humanized version of 131R003, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, or h131R011 in combination with gemcitabine and FZD8-Fc soluble receptor.

[0298] Furthermore, treatment with a RSPO3-binding agent described herein can include combination treatment with other biologic molecules, such as one or more cytokines (e.g., lymphokines, interleukins, tumor necrosis factors, and/or growth factors) or can be accompanied by surgical removal of tumors, cancer cells or any other therapy deemed necessary by a treating physician.

[0299] In certain embodiments, the treatment involves the administration of a RSPO3-binding agent (e.g. an antibody) of the present invention in combination with radiation therapy. Treatment with a RSPO3-

binding agent can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Dosing schedules for such radiation therapy can be determined by the skilled medical practitioner.

[0300] Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously.

[0301] It will be appreciated that the combination of a RSPO3-binding agent and at least one additional therapeutic agent may be administered in any order or concurrently. In some embodiments, the RSPO3-binding agent will be administered to patients that have previously undergone treatment with a second therapeutic agent. In certain other embodiments, the RSPO3-binding agent and a second therapeutic agent will be administered substantially simultaneously or concurrently. For example, a subject may be given a RSPO3-binding agent (e.g., an antibody) while undergoing a course of treatment with a second therapeutic agent (e.g., chemotherapy). In certain embodiments, a RSPO3-binding agent will be administered within 1 year of the treatment with a second therapeutic agent. In certain alternative embodiments, a RSPO3-binding agent will be administered within 10, 8, 6, 4, or 2 months of any treatment with a second therapeutic agent. In certain other embodiments, a RSPO3-binding agent will be administered within 4, 3, 2, or 1 weeks of any treatment with a second therapeutic agent. In some embodiments, a RSPO3-binding agent will be administered within 5, 4, 3, 2, or 1 days of any treatment with a second therapeutic agent. It will further be appreciated that the two (or more) agents or treatments may be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[0302] For the treatment of a disease, the appropriate dosage of an RSPO3-binding agent (e.g., an antibody) of the present invention depends on the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, whether the RSPO3-binding agent or antibody is administered for therapeutic or preventative purposes, previous therapy, the patient's clinical history, and so on, all at the discretion of the treating physician. The RSPO3-binding agent or antibody can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (e.g., reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual antibody or agent. The administering physician can easily determine optimum dosages, dosing methodologies, and repetition rates. In certain embodiments, dosage is from 0.01μg to 100mg/kg of body weight, from 0.1μg to 100mg/kg of body weight, from 1μg to 100mg/kg of body weight, from 1mg to 100mg/kg of body weight, 1mg to 80mg/kg of body weight from 10mg to 100mg/kg of body weight, from 10mg to 75mg/kg of body weight, or from 10mg to 50mg/kg of body weight. In certain embodiments, the dosage of the antibody or other RSPO3-binding agent is from about 0.1mg to about 20mg/kg of body weight. In certain embodiments, dosage can

be given once or more daily, weekly, monthly, or yearly. In certain embodiments, the antibody or other RSPO3-binding agent is given once every week, once every two weeks or once every three weeks.

[0303] In some embodiments, a RSPO3-binding agent (e.g., an antibody) may be administered at an initial higher "loading" dose, followed by one or more lower doses. In some embodiments, the frequency of administration may also change. In some embodiments, a dosing regimen may comprise administering an initial dose, followed by additional doses (or "maintenance" doses) once a week, once every two weeks, once every three weeks, or once every month. For example, a dosing regimen may comprise administering an initial loading dose, followed by a weekly maintenance dose of, for example, one-half of the initial dose. Or a dosing regimen may comprise administering an initial loading dose, followed by maintenance doses of, for example one-half of the initial dose every other week. Or a dosing regimen may comprise administering three initial doses for 3 weeks, followed by maintenance doses of, for example, the same amount every other week.

[0304] As is known to those of skill in the art, administration of any therapeutic agent may lead to side effects and/or toxicities. In some cases, the side effects and/or toxicities are so severe as to preclude administration of the particular agent at a therapeutically effective dose. In some cases, drug therapy must be discontinued, and other agents may be tried. However, many agents in the same therapeutic class often display similar side effects and/or toxicities, meaning that the patient either has to stop therapy, or if possible, suffer from the unpleasant side effects associated with the therapeutic agent.

[0305] Thus, the present invention provides methods of treating cancer in a subject comprising using an intermittent dosing strategy for administering one or more agents, which may reduce side effects and/or toxicities associated with administration of a RSPO3-binding agent, chemotherapeutic agent, etc. In some embodiments, a method for treating cancer in a human subject comprises administering to the subject a therapeutically effective dose of a RSPO3-binding agent in combination with a therapeutically effective dose of a chemotherapeutic agent, wherein one or both of the agents are administered according to an intermittent dosing strategy. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a RSPO3-binding agent to the subject, and administering subsequent doses of the RSPO3-binding agent about once every 2 weeks. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a RSPO3-binding agent to the subject, and administering subsequent doses of the RSPO3-binding agent about once every 3 weeks. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a RSPO3-binding agent to the subject, and administering subsequent doses of the RSPO3-binding agent about once every 4 weeks. In some embodiments, the RSPO3-binding agent is administered using an intermittent dosing strategy and the chemotherapeutic agent is administered weekly.

V. Kits comprising RSPO-binding agents

[0306] The present invention provides kits that comprise the RSPO3-binding agents (e.g., antibodies) described herein and that can be used to perform the methods described herein. In certain embodiments, a kit comprises at least one purified antibody against at least one human RSPO protein in one or more containers. In some embodiments, the kits contain all of the components necessary and/or sufficient to perform a detection assay, including all controls, directions for performing assays, and any necessary software for analysis and presentation of results. One skilled in the art will readily recognize that the disclosed RSPO3-binding agents of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

[0307] Further provided are kits comprising a RSPO3-binding agent (e.g., an anti-RSPO3 antibody), as well as at least one additional therapeutic agent. In certain embodiments, the second (or more) therapeutic agent is a chemotherapeutic agent. In certain embodiments, the second (or more) therapeutic agent is a Wnt pathway inhibitor. In certain embodiments, the second (or more) therapeutic agent is an angiogenesis inhibitor.

[0308] Embodiments of the present disclosure can be further defined by reference to the following non-limiting examples, which describe in detail preparation of certain antibodies of the present disclosure and methods for using antibodies of the present disclosure. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the present disclosure.

EXAMPLES

Example 1

Expression of RSPO and LGR in human tumors

[0309] mRNA from normal tissue, benign tumor and malignant tumor samples of a large number of human patients was analyzed by microarray analysis (Genelogic BioExpress Datasuite). This data revealed elevated expression levels of RSPO1 in malignant tissue relative to normal tissue in several tumor types including kidney, endometrial, and ovarian. RSPO1 was noted to be frequently over-expressed in ovarian cancer (Fig. 1A). In addition, this data suggested elevated expression levels of RSPO3 in malignant tissue relative to normal tissue in several tumor types including ovarian, pancreas, and lung (Fig. 1C). In addition, it was found that LGR5 and LGR6 were over-expressed in malignant breast tumors, colon tumors, lung tumors, and ovarian tumors relative to normal tissue, while LGR4 was over-expressed in lung tumors. LGR5 and LGR6 over-expression appeared to be restricted to triple-negative ($ER^{neg}PR^{neg}HER2^{neg}$) breast tumors relative to other breast tumor subtypes.

[0310] RNA was isolated from a series of human tumors grown in murine xenografts. The RNA samples were prepared and processed using established Affymetrix protocols for the generation of labeled cRNA. The processed RNA was hybridized to Affymetrix HG-U133 plus 2.0 microarrays (Affymetrix, Santa Clara, CA) as outlined in the manufacturer's technical manuals. After hybridization, the microarrays were washed, scanned, and analyzed. Scanned array background adjustment and signal intensity normalization were performed using the GCRMA algorithm (Bioconductor, www.bioconductor.org).

[0311] Particular human RSPOs and human LGRs were evaluated – RSPO1 (241450_at), RSPO2 (1554012_at), RSPO3 (228186_s_at), RSPO4 (237423_at), LGR4 (218326_s_at), LGR5 (210393_at) and LGR6 (227819_at). Microarray analysis showed that, while LGR4 and LGR6 were broadly expressed in almost all tumors, many tumors were found to greatly over-express only particular RSPO family members and LGR5 (Table 2), although these expression levels were not compared to expression levels in normal tissue. Generally there is only a single RSPO family member that is highly expressed in a given tumor, suggesting that there may be functional redundancy within the RSPO family.

Table 2

Tumor	RSPO1	RSPO2	RSPO3	RSPO4	LGR4	LGR5	LGR6
Breast tumor							
B34	4.79	4.93	**303.31**	4.41			
B39	20.59	**588.88**	22.60	4.40			
B60	4.60	4.92	10.89	**64.79**			
B02	4.60	4.92	**692.34**	4.41	2678.95	4.28	50.88
B03	5.56	4.89	**1870.42**	4.41	686.47	**30.78**	73.49
B06	4.60	4.91	4.51	**120.72**	274.54	4.26	20.77
B59	4.60	4.91	4.53	**1158.11**	200.48	4.26	6467.15
Colon tumors							
C11	4.63	4.98	4.56	4.43	3852.26	6.22	11.31
C17	4.64	5.00	4.57	4.44	2822.46	**62.34**	43.94
C18	4.63	4.95	**13.83**	4.42	2454.15	4.29	723.15
C27	6.66	**980.49**	4.75	4.40	5083.84	4.30	20.82
Lung tumors							
LU02	4.62	**15190.40**	4.55	4.43	13.95	4.29	14.56
LU11	4.60	4.92	**4.53**	4.41	999.55	4.27	146.67
LU25	4.64	5.56	**11123.06**	4.44	1208.92	4.29	41089
LU33	4.64	5.01	12.02	**62.98**	329.62	4.30	20.96
LU45	4.64	4.99	4.62	4.44	3877.47	4.29	4.86
Melanoma tumors							

M06	4.73	**21.80**	4.65	4.50	1077.93	4.34	3.90
Ovarian tumors							
OV12	4.72	5.12	4.64	**460.40**	5383.63	**1152.73**	115.04
OV19	**960.19**	4.74	69.77	20.90	494.67	5.72	4302.78
OV22	4.66	5.10	**132.85**	37.43	3743.91	**482.33**	812.05
OV27	4.55	4.86	**125.78**	4.92			
OV38	9.19	4.83	**3439.88**	16.35	1528.12	4.24	19.49
Pancreatic tumors							
PN07	4.58	**689.52**	4.51	4.40	6777.41	4.28	746.38
PN18	4.72	**2508.47**	4.65	4.50	6750.73	**51.15**	564.94

Example 2

Binding of RSPO proteins to LGR5

[0312] A cell surface LGR5 protein was generated by ligating amino acids 22-564 of human LGR5 to an N-terminal FLAG tag and to the transmembrane domain of CD4 and a C-terminal GFP protein tag using standard recombinant DNA techniques (FLAG-LGR5-CD4TM-GFP). RSPO-Fc constructs were generated using standard recombinant DNA techniques. Specifically, full-length human RSPO1, RSPO2, RSPO3 and RSPO4 were ligated in-frame to a human Fc region and the recombinant RSPO-Fc proteins were expressed in insect cells using baculovirus. The fusion proteins were purified from the insect medium using protein A chromatography.

[0313] HEK-293 cells were transiently transfected with the FLAG-LGR5-CD4TM-GFP construct. After 48 hours, transfected cells were suspended in ice cold PBS containing 2% FBS and heparin and incubated on ice in the presence of 10μg/ml RSPO1-Fc, RSPO2-Fc, RSPO3-Fc, RSPO4-Fc, or FZD8-Fc fusion proteins for 15 minutes. A second incubation with 100μl PE-conjugated anti-human Fc secondary antibody was performed to detect cells bound by the Fc fusion proteins. Cells were incubated with an anti-FLAG antibody (Sigma-Aldrich, St. Louis, MO) as a positive control and with an anti-PE antibody as a negative control. The cells were analyzed on a FACSCalibur instrument (BD Biosciences, San Jose, CA) and the data was processed using FlowJo software.

[0314] As shown in Figure 2, RSPO1, RSPO2, RSPO3 and RSPO4 all bound to LGR5 expressed on the surface of the HEK-293 cells, while FZD8, the negative control, did not bind LGR5.

[0315] Binding affinities between RSPO proteins and LGR5 were analyzed by surface plasmon resonance. A soluble LGR5-Fc construct was generated using standard recombinant DNA techniques. Specifically, amino acids 1-564 of human LGR5 were ligated in frame to human Fc and the recombinant LGR5-Fc fusion protein was expressed in insect cells using baculovirus. The LGR5-Fc fusion protein was purified from the insect medium using protein A chromatography. Cleavage of the LGR5 signal

sequence results in a mature LGR5-Fc fusion protein containing amino acids 22-564 of LGR5. Recombinant RSPO1-Fc, RSPO2-Fc, RSPO3-Fc and RSPO4-Fc fusion proteins were immobilized on CM5 chips using standard amine-based chemistry (NHS/EDC). Two-fold dilutions of soluble LGR5-Fc were injected over the chip surface (100nM to 0.78nM). Kinetic data were collected over time using a Biacore 2000 system from Biacore Life Sciences (GE Healthcare) and the data were fit using the simultaneous global fit equation to yield affinity constants (K_D values) for each RSPO protein (Table 3).

Table 3

	LGR5 (nM)
RSPO1	110
RSPO2	14
RSPO3	<1.0
RSPO4	73

[0316] Human RSPO1, RSPO2, RSPO3 and RSPO4 all bound to LGR5, demonstrating that RSPO proteins may be ligands for LGR proteins.

Example 3

Identification of anti-RSPO3 antibodies

[0317] A mammalian cell antibody library was screened and two anti-RSPO3 antibodies, 131R002 and 131R003, were identified. Sequence data subsequently demonstrated that antibodies 131R002 and 131R003 have the same light chain sequence but different heavy chain sequences.

[0318] The K_Ds of antibodies 131R002 and 131R003 were determined using a Biacore 2000 system from Biacore LifeSciences (GE Healthcare). Recombinant human RSPO3 protein was biotinylated and captured on streptavidin-coated chips (GE Healthcare) with coating densities of 400-700ru. The antibodies were serially diluted 2-fold from 100nM to 0.78nM in HBS-P (0.01M HEPES pH 7.4, 0.15M NaCl, 0.005% v/v Surfactant P20) and were injected over the chip surface. Kinetic data were collected over time and were fit using the simultaneous global fit equation to yield affinity constants (K_D values) for each antibody.

[0319] Antibody 131R002 had an affinity constant (K_D) for human RSPO3 of 8.2nM and antibody 131R003 had a K_D for human RSPO3 of 7.3nM.

Example 4

In vitro testing for inhibition of β-catenin activity by anti-RSPO3 antibodies

[0320] HEK-293 cells were transfected with a 6xTCF-luciferase reporter vector (TOPflash, Millipore, Billerica, MA). After 24-48 hrs, the transfected HEK-293 cells were incubated with a combination of WNT3a (5ng/ml) and human RSPO3 (10ng/ml, R&D BioSystems, Minneapolis, MN) in the presence of anti-RSPO3 antibodies 131R002 and 131R003. Antibodies 131R002 and 131R003 were added to the cells in 4-fold serial dilutions from 20μg/ml to 0.02μg/ml. As controls, cells were incubated with a combination of WNT3a and RSPO3, WNT3a only, RSPO3 only, or with no addition. The cells were incubated for 16 hours and luciferase activity was measured using Steady-Glo® Luciferase Assay System according to the manufacturer's instructions (Promega, Madison, WI).

[0321] As shown in Figure 3, anti-RSPO3 antibodies 131R002 and 131R003 each reduced RSPO3-induced β-catenin signaling in a dose-dependent manner. These results demonstrated that antibodies 131R002 and 131R003 are specific inhibitors of RSPO3 and are capable of reducing and/or blocking RSPO3-induced β-catenin signaling.

Example 5

Affinity maturation and humanization of RSPO3 antibodies

[0322] Anti-RSPO3 antibody 131R003 was affinity matured and several variants were identified. One 131R003 variant had an altered heavy chain CDR1 (SEQ ID NO:34) as compared to parental 131R003 antibody. A second variant had an altered heavy chain CDR3 (SEQ ID NO:35) as compared to parental 131R003. An additional variant was generated that comprised both the altered heavy chain CDR1 and CDR3 as compared to parental 131R003.

[0323] HEK-293 cells were transfected with a 6xTCF-luciferase reporter vector (TOPflash, Millipore, Billerica, MA). After 24-48 hrs, the transfected HEK-293 cells were incubated with a combination of WNT3a and human RSPO3 in the presence of anti-RSPO3 antibodies 131R003, 131R003 CDR1 variant and 131R003 CDR3 variant. 131R003, 131R003 CDR1 variant, and 131R003 CDR3 variant were added to the cells in 5-fold serial dilutions from 20μg/ml to 0.006μg/ml. As controls, cells were incubated with a combination of WNT3a and RSPO3, WNT3a only, RSPO3 only, a control antibody, or with no addition. The cells were incubated for 16 hours and luciferase activity was measured using Steady-Glo® Luciferase Assay System according to the manufacturer's instructions (Promega, Madison, WI).

[0324] As shown in Figure 4, anti-RSPO3 antibodies 131R003 CDR1 variant and 131R003 CDR3 variant each reduced RSPO3-induced β-catenin signaling in a dose-dependent manner and at lower concentrations than parental 131R003. These results demonstrated that the 131R003 variants retained the characteristics of parental 131R003, i.e., they were specific inhibitors of RSPO3 and were capable of reducing and/or blocking RSPO3-induced β-catenin signaling. In addition, these results demonstrated that the 131R003 variants had better activity than parental 131R003.

[0325] Humanized forms of 131R003 variants were generated using standard techniques. Humanized antibodies h131R005, h131R007, h131R008, h131R010, h131R011 comprise an altered heavy chain CDR3 as compared to parental 131R003 antibody. Humanized 131R006B comprises an altered heavy chain CDR3 as compared to parental 131R003 antibody. Antibodies h131R005/131R007, h131R010, and h131R011 comprise several amino acid substitutions in framework region 3 as compared to antibody 131R006B. Antibodies h131R005/131R007, h131R006, and h131R011 are IgG2 antibodies. Antibodies h131R008 and h131R010 are IgG1 antibodies. Antibodies h131R005/131R007, h131R010, and h131R011 comprise the same heavy chain variable region. Antibodies h131R010 and h131R011 comprise the same light chain variable region, which is different than the light chain variable region of h131R005/131R007.

[0326] A plasmid encoding the heavy chain of the 131R010 antibody was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA, USA, under the conditions of the Budapest Treaty on June 18, 2013, and assigned ATCC deposit designation number PTA-_____. A plasmid encoding the light chain of the 131R010 antibody was deposited with ATCC, 10801 University Boulevard, Manassas, VA, USA, under the conditions of the Budapest Treaty on June 18, 2013, and assigned ATCC deposit designation number PTA-_____.

Example 6

Inhibition of ovarian tumor growth *in vivo* by anti-RSPO antibodies

[0327] Dissociated OMP-OV38 ovarian tumor cells (1 x 10^5cells) were injected in to 6-8 week old NOD/SCID mice. Tumors were allowed to grow for 39 days until they reached an average volume of 150 mm^3. The mice were randomized (n = 8 per group) and treated with a combination of anti-RSPO1 antibody 89M5 and anti-RSPO3 antibody 131R003, a combination of anti-RSPO1 antibody 89M5, anti-RSPO3 antibody 131R003, and taxol, taxol as a single agent, or a control antibody. Antibodies were dosed at 20 mg/kg once a week, and taxol was dosed at 15 mg/ml once a week. Administration of the antibodies and taxol was performed via injection into the intraperitoneal cavity. Tumor growth was monitored and tumor volumes were measured with electronic calipers at the indicated time points. Data are expressed as mean ± S.E.M.

[0328] As shown in Figure 5, a combination of anti-RSPO1 and anti-RSPO3 antibodies inhibited OMP-OV38 ovarian tumor growth. Surprisingly, a combination of anti-RSPO1 antibody 89M5, anti-RSPO3 antibody 131R002, and taxol inhibited tumor growth to a significantly greater level than taxol alone or the antibody combination alone.

[0329] Dissociated OMP-OV38 ovarian tumor cells (1 x 10^5cells) were injected in to 6-8 week old NOD/SCID mice. Tumors were allowed to grow for 35 days until they reached an average volume of 140 mm^3. The mice were randomized (n = 10 per group) and treated with anti-RSPO3 antibody 131R002,

anti-RSPO1 antibody 89M5, taxol, a combination of 89M5 and taxol, a combination of 131R002 and taxol, a combination of 89M5 and 131R002, a combination of 89M5, 131R002 and taxol, or a control antibody. Antibodies were dosed at 20 mg/kg once a week, and taxol was dosed at 15 mg/ml once a week through day 46 and subsequently dosed at 7.5 mg/kg. Administration of the antibodies and taxol was performed via injection into the intraperitoneal cavity. Tumor growth was monitored and tumor volumes were measured with electronic calipers at the indicated time points. Data are expressed as mean ± S.E.M.

[0330] As shown in Figure 6, a combination of anti-RSPO1 antibody 89M5 and anti-RSPO3 antibody 131R002 inhibited OMP-OV38 ovarian tumor growth as compared to control antibody. Combinations of anti-RSPO1 antibody 89M5 and taxol or anti-RSPO3 antibody 131R002 and taxol had no effect relative to taxol alone. However, surprisingly a combination of anti-RSPO1 89M5, anti-RSPO3 antibody 131R002, and taxol showed activity that was greater than taxol alone.

Example 7

Inhibition of lung tumor growth *in vivo* by anti-RSPO3 antibodies

[0331] In OMP-LU45 non-small cell lung tumors, it has been observed that $CD201^+$ cells are more tumorigenic than $CD201^-$ cells. Furthermore, RSPO3 was found to be highly expressed in the $CD201^+$ cell population. Dissociated and sorted OMP-LU45 $CD44^+CD201^+$ lung tumor cells (5×10^4 cells) were injected into 6-8 week old NOD/SCID mice. Tumors were allowed to grow for 38 days until they reached an average volume of 140 mm^3. The mice were randomized (n = 10 per group) and treated with anti-RSPO3 antibody 131R002 or a control antibody. Antibodies were dosed at 25 mg/kg once a week and administration of the antibodies was performed via injection into the intraperitoneal cavity. Tumor growth was monitored and tumor volumes were measured with electronic calipers at the indicated time points. Data are expressed as mean ± S.E.M.

[0332] In a study with a second lung tumor, dissociated OMP-LU25 lung tumor cells (5×10^4 cells) were injected into 6-8 week old NOD/SCID mice. Tumors were allowed to grow for 48 days until they reached an average volume of 110 mm^3. The mice were randomized (n = 9 per group) and treated with anti-RSPO3 antibody 131R002 or a control antibody. Antibodies were dosed at 25 mg/kg once a week and administration of the antibodies was performed via injection into the intraperitoneal cavity. Tumor growth was monitored and tumor volumes were measured with electronic calipers at the indicated time points. Data are expressed as mean ± S.E.M.

[0333] As shown in Figure 7A and 7B, anti-RSPO antibody 131R002 inhibited growth of both lung tumors OMP-LU45 and OMP-LU25 as compared to a control antibody.

Example 8

Inhibition of β-catenin activity by anti-RSPO3 antibodies

[0334] HEK-293 cells were transfected with a 6xTCF-luciferase reporter vector (TOPflash, Millipore, Billerica, MA). After 24-48 hrs, the transfected HEK-293 cells were incubated with a combination of WNT3a conditioned medium (5ng/ml) and human RSPO3 (10ng/ml, R&D BioSystems) in the presence of anti-RSPO3 antibodies 131R002, 131R006B, or 131R007. Antibodies 131R002, 131R006 or 131R007 were added to the cells in 5-fold serial dilutions from 20μg/ml to 0.0064μg/ml. As controls, cells were incubated with WNT3a conditioned medium alone, a combination of WNT3a conditioned medium and human RSPO3, or with no addition to cells. The cells were incubated for 16 hours and luciferase activity was measured using Steady-Glo® Luciferase Assay System according to the manufacturer's instructions (Promega, Madison, WI).

[0335] As shown in Figure 8, all three anti-RSPO3 antibodies reduced WNT3a/RSPO3-induced β-catenin signaling in a dose-dependent manner. The humanized antibodies 131R006B and 131R007 appeared to have a greater ability to inhibit β-catenin activity than antibody 131R002. These results demonstrated that humanized antibodies 131R006B and 131R007 are stronger inhibitors of RSPO3 than 131R002 and are capable of reducing and/or blocking WNT3a/RSPO3-induced β-catenin signaling.

Example 9

Inhibition of RSPO3 binding to LGR5

[0336] HEK-293T cells were transfected with a cDNA expression vector that encoded the extracellular domain of human LGR5 (FLAG-LGR5-CD4TM-GFP). Transfected cells were incubated with recombinant RSPO3-biotin fusion protein in the presence of anti-RSPO3 antibodies 131R006B or 131R007. Cells were incubated without antibody as a control. Cells were washed in PBS and binding of RSPO3 to LGR5-expressing transfected cells was determined by addition of PE-conjugated streptavidin and analysis by flow cytometry.

[0337] As shown in Figure 9, anti-RSPO3 antibodies 131R006B and 131R007 were highly effective in blocking binding of RSPO3 to LGR5-expressing cells.

Example 10

Binding affinities of RSPO3 antibodies

[0338] The K_D of RSPO3 antibodies 131R002, 131R003, 131R003 CDR3 variant, h131R007, h131R008, and h131R011 were determined using a Biacore 2000 system from Biacore LifeSciences (GE Healthcare). The method used was different than described in Example 3. A goat anti-human IgG antibody was coupled to a carboxymethyl-dextran (CM5) SPR chip using standard amine-based chemistry (NHS/EDC) and blocked with ethanolamine. Antibodies (purified antibody or culture supernatant) were diluted to a concentration of 10μg/ml in HBS-P-BSA (0.01M HEPES pH7.4, 0.15M NaCl, 0.005% v/v Polysorbate 20, 100ug/ml BSA) and captured onto the chip via the anti-human IgG antibody. Human RSPO3 (R&D

Systems) was serially diluted 2-fold from 300nM to 37.5nM in HBS-P-BSA and injected sequentially over the captured anti-RSPO3 antibodies. RSPO3 association and dissociation was measured at each concentration. After each antigen injection 5µl of 100mM H_3P0_4 was injected to remove the antigen-antibody complex and a subsequent injection performed. Kinetic data were collected over time and were fit using the simultaneous global fit equation to yield affinity constants (K_D values) for each antibody (Table 4).

Table 4

RSPO3 Antibody	K_D
131R002 (IgG2)	1.3nM
131R003 (IgG2)	1.9nM
131R003 CDR3 variant (IgG2)	1.7nM
h131R007 (IgG2)	654pM
h131R008 (IgG1)	876pM
h131R010 (IgG1)	ND
h131R011 (IgG2)	686pM

[0339] In additional experiments, antibody h131R008 was shown to have a K_D as low as 448pM for human RSPO3, no detectable binding to human RSPO1 or RSPO2, and weak binding to human RSPO4. Antibody h131R008 was shown to have a K_D of 248pM for murine RSPO3, no detectable binding to murine RSPO1 or RSPO2 and weak binding to murine RSPO4.

Example 11

Inhibition of lung tumor growth *in vivo* by anti-RSPO3 antibodies

[0340] The non-small cell lung cancer (NSCLC) cell line NCI-H2030 was selected for testing based on a high level of RPSO3 expression in microarray data. NCI-H2030 cells (1×10^6) were injected into NOD-SCID mice. Tumors were allowed to grow for approximately 60 days until they reached an average volume of 100 mm^3. Tumor-bearing mice were randomized into 4 groups (n = 7-9 per group). Tumor-bearing mice were treated with anti-RSPO3 antibody 131R002, carboplatin, a combination of anti-RSPO3 antibody 131R002 and carboplatin, or a control antibody. Antibodies were dosed at 25 mg/kg once a week. Carboplatin was dosed at 50 mg/kg once a week. Tumor growth was monitored and tumor volumes were measured with electronic calipers on the indicated days post-treatment.

[0341] As shown in Figure 10, treatment with anti-RSPO3 antibody in combination with carboplatin inhibited NCI-H2030 tumor growth better than carboplatin alone or the antibody alone.

[0342] OMP-LU102 is a patient-derived non-small cell lung cancer (NSCLC) xenograft that was selected for testing based on a high level of RPSO3 expression in microarray data. OMP-LU102 lung tumor cells

(1×10^5) were injected into NOD-SCID mice. Tumors were allowed to grow for 22 days until they reached an average volume of 90 mm^3. Tumor-bearing mice were randomized into 4 groups (n = 10 per group). Tumor-bearing mice were treated with anti-RSPO3 antibody 131R002, carboplatin, a combination of anti-RSPO3 antibody 131R002 and carboplatin, or a control antibody. Antibodies were dosed at 25 mg/kg once a week. Carboplatin was dosed at 50 mg/kg once a week. Tumor growth was monitored and tumor volumes were measured with electronic calipers on the indicated days post-treatment.

[0343] As shown in Figure 11A, treatment with anti-RSPO3 antibody inhibited OMP-LU102 lung tumor growth as a single agent but had much greater effect in combination with carboplatin.

[0344] RNA was prepared from tumors from each of the four experimental groups following the treatment. Gene expression was characterized by microarray analysis. Gene set enrichment analysis indicated that anti-RSPO3 antibody treatment (either as a single agent or in combination with carboplatin) inhibited the expression of various gene sets characteristic of normal stem cells or cancer stem cells as shown in Figure 11B. Treatment with carboplatin alone did not have this effect on gene expression.

Example 12

Inhibition of pancreatic tumor growth *in vivo* by anti-RSPO3 antibodies

[0345] OMP-PN35 is patient-derived pancreatic ductal adenocaricoma (PDAC) xenograft that was selected for testing based on high level of RPSO3 expression in microarray data. OMP-PN35 (1×10^5) tumor cells were injected into NOD-SCID mice. Tumors were allowed to grow for 30 days until they reached an average volume of 90 mm^3. Tumor-bearing mice were randomized into 4 groups (n = 10 per group). Tumor-bearing mice were treated with anti-RSPO3 antibody 131R002, gemcitabine plus nab-paclitaxel (ABRAXANE), a combination of anti-RSPO3 antibody and gemcitabine and nab-paclitaxel (ABRAXANE). Antibodies were dosed at 25 mg/kg once a week. Gemcitabine was dosed at 20 mg/kg once a week and nab-paclitaxel (ABRAXANE) was dosed at 30 mg/kg once a week. Tumor growth was monitored and tumor volumes were measured with electronic calipers on the indicated days post-treatment.

[0346] In Figure 12A the results from all four treatment groups are shown and in Figure 12B only the combination treatments are shown on an expanded scale. Figure 12A and 12B show that anti-RSPO3 antibody in combination with gemcitabine and nab-paclitaxel (ABRAXANE) inhibited OMP-PN35 pancreatic tumor growth better than gemcitabine and nab-paclitaxel (ABRAXANE) alone.

Example 13

Inhibition of β-catenin activity by anti-RSPO3 antibodies

[0347] HEK-293 cells were transfected with a 6xTCF-luciferase reporter vector (TOPflash, Millipore, Billerica, MA). After 24-48 hrs, the transfected HEK-293 cells were incubated with a combination of WNT3a conditioned medium (5ng/ml) and human RSPO3 (2ng/ml, R&D BioSystems) in the presence of anti-RSPO3 antibodies h131R007 or h131R010. Antibodies h131R007 or h131R010 were added to the cells in 5-fold serial dilutions from 20μg/ml to 0.0064μg/ml. As controls, cells were incubated with WNT3a conditioned medium alone, a combination of WNT3a conditioned medium and human RSPO3, or with no addition to cells. The cells were incubated for 16 hours and luciferase activity was measured using Steady-Glo® Luciferase Assay System according to the manufacturer's instructions (Promega, Madison, WI).

[0348] As shown in Figure 13, antibody h131R010 reduced WNT3a/RSPO3-induced β-catenin signaling in a dose-dependent manner and to a similar extent as h131R007. Since h131R010 inhibited β-catenin signaling to the same extent as h131R007, it is clear that activity of the anti-RSPO3 antibody was not affected by conversion to an IgG1 isotype.

Example 14

Inhibition of lung tumor growth *in vivo* by anti-RSPO3 antibodies

[0349] OMP-LU25 is a patient-derived non small cell lung cancer (NSCLC) xenograft that was selected for testing based on high level of RPSO3 expression in microarray data. OMP-LU25 tumor cells (5×10^4) were injected into NOD-SCID mice. Tumors were allowed to grow for 33 days until they reached an average volume of 120 mm^3. Tumor-bearing mice were randomized into 4 groups (n = 9 per group). Tumor-bearing mice were treated with either control antibody, anti-RSPO3 antibody 131R008, paclitaxel, or the combination of anti-RSPO3 antibody 131R008 and paclitaxel. Antibodies were dosed weekly at 20mg/kg. Paclitaxel was dosed weekly at 15mg/kg. Tumor growth was monitored and tumor volumes were measured with electronic calipers on the indicated days post-treatment.

[0350] As shown in Figure 14, anti-RSPO3 antibody 131R008 inhibited OMP-LU25 tumor growth as a single agent and in combination with chemotherapy. Furthermore, the combination of anti-RSPO3 antibody 131R008 with paclitaxel led to tumor regression.

[0351] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to person skilled in the art and are to be included within the spirit and purview of this application.

[0352] All publications, patents, patent applications, internet sites, and accession numbers/database sequences including both polynucleotide and polypeptide sequences cited herein are hereby incorporated by reference herein in their entirety for all purposes to the same extent as if each individual publication,

patent, patent application, internet site, or accession number/database sequence were specifically and individually indicated to be so incorporated by reference.

[0353] The sequences disclosed in the application are:

Human RSPO1 protein sequence with signal sequence (SEQ ID NO:1)
MRLGLCVVALVLSWTHLTISSRGIKGKRQRRISAEGSQACAKGCELCSEVNGCLKCSPKL
FILLERNDIRQVGVCLPSCPPGYFDARNPDMNKCIKCKIEHCEACFSHNFCTKCKEGLYL
HKGRCYPACPEGSSAANGTMECSSPAQCEMSEWSPWGPCSKKQQLCGFRRGSEERTRRVL
HAPVGDHAACSDTKETRRCTVRRVPCPEGQKRRKGGQGRRENANRNLARKESKEAGAGSR
RRKGQQQQQQQGTVGPLTSAGPA

Human RSPO2 protein sequence with signal sequence (SEQ ID NO:2)
MQFRLFSFALIILNCMDYSHCQGNRWRRSKRASYVSNPICKGCLSCSKDNGCSRCQQKLF
FFLRREGMRQYGECLHSCPSGYYGHRAPDMNRCARCRIENCDSCFSKDFCTKCKVGFYLH
RGRCFDECPDGFAPLEETMECVEGCEVGHWSEWGTCSRNNRTCGFKWGLETRTRQIVKKP
VKDTIPCPTIAESRRCKMTMRHCPGGKRTPKAKEKRNKKKRKLIERAQEQHSVFLATDR
ANQ

Human RSPO3 protein sequence with signal sequence (SEQ ID NO:3)
MHLRLISWLFIILNFMEYIGSQNASRGRRQRRMHPNVSQGCQGGCATCSDYNGCLSCKPR
LFFALERIGMKQIGVCLSSCPSGYYGTRYPDINKCTKCKADCDTCFNKNFCTKCKSGFYL
HLGKCLDNCPEGLEANNHTMECVSIVHCEVSEWNPWSPCTKKGKTCGFKRGTETRVREII
QHPSAKGNLCPPTNETRKCTVQRKKCQKGERGKKGRERKRKKPNKGESKEAIPDSKSLES
SKEIPEQRENKQQQKKRKVQDKQKSVSVSTVH

Human RSPO4 protein sequence with signal sequence (SEQ ID NO:4)
MRAPLCLLLLVAHAVDMLALNRRKKQVGTGLGGNCTGCIICSEENGCSTCQQRLFLFIRR
EGIRQYGKCLHDCPPGYFGIRGQEVNRCKKCGATCESCFSQDFCIRCKRQFYLYKGKCLP
TCPPGTLAHQNTRECQGECELGPWGGWSPCTHNGKTCGSAWGLESRVREAGRAGHEEAAT
CQVLSESRKCPIQRPCPGERSPGQKKGRKDRRPRKDRKLDRRLDVRPRQPGLQP

Human RSPO3 protein sequence without predicted signal sequence (SEQ ID NO:5)
QNASRGRRQRRMHPNVSQGCQGGCATCSDYNGCLSCKPRLFFALERIGMKQIGVCLSSCP
SGYYGTRYPDINKCTKCKADCDTCFNKNFCTKCKSGFYLHLGKCLDNCPEGLEANNHTME
CVSIVHCEVSEWNPWSPCTKKGKTCGFKRGTETRVREIIQHPSAKGNLCPPTNETRKCTV
QRKKCQKGERGKKGRERKRKKPNKGESKEAIPDSKSLESSKEIPEQRENKQQQKKRKVQD
KQKSVSVSTVH

Human RSPO3 furin-like domain 1 (SEQ ID NO:6)
PNVSQGCQGGCATCSDYNGCLSCKPRLFFALERIGMKQIGVCLSSCPSGYYG

Human RSPO3 furin-like domain 2 (SEQ ID NO:7)
INKCTKCKADCDTCFNKNFCTKCKSGFYLHLGKCLDNCPEGLEA

Human RSPO3 thrombospondin domain (SEQ ID NO:8)
HCEVSEWNPWSPCTKKGKTCGFKRGTETRVREIIQHPSAKGNLCPPTNETRKCTVQRKKCQ

131R002/131R003 Heavy chain CDR1 (SEQ ID NO:9)
KASGYTFTDYS

131R002/131R003 Heavy chain CDR2 (SEQ ID NO:10)

IYPSNGDS

131R002/131R003 Heavy chain CDR3 (SEQ ID NO:11)
ATYFANYFDY

131R002/131R003 Light chain CDR1 (SEQ ID NO:12)
QSVDYDGDSYM

131R002/131R003 Light chain CDR2 (SEQ ID NO:13)
AAS

131R002/131R003 Light chain CDR3 (SEQ ID NO:14)
QQSNEDPLT

131R002 Heavy chain variable region (SEQ ID NO:15)
QVQLQESGPELVKPGASVKISCKASGYTFTDYSIHWVKQNHGKSLDWIGYIYPSNGDSGYN
QKFKNRATLTVDTSSSTAYLEVRRLTFEDSAVYYCATYFANYFDYWGQGTTLTVSSAST

131R003 Heavy chain variable region (SEQ ID NO:16)
QVQLKQSGPELVKPGASVKISCKASGYTFTDYSIHWVKQNHGKSLDWIGYIYPSNGDSGYN
QKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFANYFDYWGQGTTLTVSSAST

131R002/131R003 Light chain variable region (SEQ ID NO:17)
DIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYAASNLESG
IPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPLTFGAGTKLELKR

131R002 Heavy chain variable region nucleotide sequence (SEQ ID NO:18)
CAGGTACAATTGCAAGAATCCGGACCCGAACTTGTGAAGCCCGGAGCGTCAGTCAAGATC
TCGTGTAAGGCCAGCGGGTACACCTTTACGGATTATTCGATCCATTGGGTAAAACAGAAT
CACGGGAAGTCGCTCGACTGGATTGGTTATATCTACCCGTCCAACGGTGATTCGGGATAC
AACCAGAAGTTCAAAAATCGGGCCACACTTACAGTGGACACATCGTCGTCAACTGCATAT
CTCGAGGTCCGCAGACTGACGTTTGAGGACTCAGCTGTCTACTATTGCGCGACTTATTTC
GCCAACTACTTCGATTACTGGGGCCAGGGGACGACACTGACGGTCAGCTCCGCGAGCACC

131R003 Heavy chain variable region nucleotide sequence (SEQ ID NO:19)
CAGGTGCAACTTAAACAGTCGGGGCCTGAGTTGGTCAAACCAGGAGCCTCAGTAAAGATT
AGCTGCAAAGCATCAGGTTATACCTTTACGGATTACTCGATCCACTGGGTGAAGCAGAAC
CACGGAAAGTCACTGGATTGGATCGGGTACATCTACCCCTCGAATGGAGATTCGGGGTAT
AACCAAAAGTTCAAAAACCGGGCCACGCTGACTGTGGACACGTCGTATTCCACCGCATAT
TTGGAAGTCCGCAGACTCACGTTCGAGGACTCCGCGGTATACTATTGTGCCACATACTTT
GCGAATTACTTTGACTACTGGGGTCAGGGCACAACGCTTACTGTCTCCAGCGCGTCAACA

131R002/131R003 Light chain variable region nucleotide sequence (SEQ ID NO:20)
GACATCGTGCTCACACAGAGCCCTGCATCGCTCGCAGTATCGCTTGGTCAGCGAGCGACC
ATTTCATGCAAAGCGTCACAATCGGTAGATTACGACGGAGACTCCTACATGAACTGGTAT
CAGCAGAAACCAGGGCAGCCCCCGAAGTTGCTCATCTACGCCGCGTCCAATCTGGAGTCA
GGCATTCCCGCCAGATTCAGCGGGAGCGGGTCAGGAACGGATTTTACCCTCAATATCCAT
CCGGTAGAGGAGGAAGATGCGGCGACTTACTATTGTCAGCAGTCGAATGAGGACCCACTC
ACGTTCGGGGCTGGAACAAAACTGGAACTTAAACGG

131R002 Heavy chain amino acid sequence with predicted signal sequence underlined (SEQ ID NO:21)
MKHLWFFLLLVAAPRWVLSQVQLQESGPELVKPGASVKISCKASGYTFTDYSIHWVKQNH
GKSLDWIGYIYPSNGDSGYNQKFKNRATLTVDTSSSTAYLEVRRLTFEDSAVYYCATYFA

NYFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

131R003 Heavy chain amino acid sequence with predicted signal sequence underlined (SEQ ID NO:22)
MKHLWFFLLLVAAPRWVLSQVQLKQSGPELVKPGASVKISCKASGYTFTDYSIHWVKQNH
GKSLDWIGYIYPSNGDSGYNQKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFA
NYFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

131R002/131R003 Light chain amino acid sequence with predicted signal sequence underlined (SEQ ID NO:23)
MKHLWFFLLLVAAPRWVLSDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNWYQ
QKPGQPPKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPLT
FGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

131R002 Heavy chain nucleotide sequence with predicted signal sequence (SEQ ID NO:24)
ATGAAACACTTGTGGTTCTTTCTTTTGCTGGTGGCAGCGCCTAGGTGGGTGCTCAGCCAG
GTACAATTGCAAGAATCCGGACCCGAACTTGTGAAGCCCGGAGCGTCAGTCAAGATCTCG
TGTAAGGCCAGCGGGTACACCTTTACGGATTATTCGATCCATTGGGTAAAACAGAATCAC
GGGAAGTCGCTCGACTGGATTGGTTATATCTACCCGTCCAACGGTGATTCGGGATACAAC
CAGAAGTTCAAAAATCGGGCCACACTTACAGTGGACACATCGTCGTCAACTGCATATCTC
GAGGTCCGCAGACTGACGTTTGAGGACTCAGCTGTCTACTATTGCGCGACTTATTTCGCC
AACTACTTCGATTACTGGGGCCAGGGGACGACACTGACGGTCAGCTCCGCGAGCACCAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAAC
GTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTG
GAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCT
AAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC
AACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTG
CTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAG
CCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTG
ACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGC
CAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTC
CTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGC
TCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCT
GGCAAGTGA

131R003 Heavy chain nucleotide sequence with predicted signal sequence (SEQ ID NO:25)
ATGAAGCATCTTTGGTTCTTCCTGCTCTTGGTGGCTGCGCCGAGGTGGGTGCTCAGCCAG
GTGCAACTTAAACAGTCGGGGCCTGAGTTGGTCAAACCAGGAGCCTCAGTAAAGATTAGC

TGCAAAGCATCAGGTTATACCTTTACGGATTACTCGATCCACTGGGTGAAGCAGAACCAC
GGAAAGTCACTGGATTGGATCGGGTACATCTACCCCTCGAATGGAGATTCGGGGTATAAC
CAAAAGTTCAAAAACCGGGCCACGCTGACTGTGGACACGTCGTATTCCACCGCATATTTG
GAAGTCCGCAGACTCACGTTCGAGGACTCCGCGGTATACTATTGTGCCACATACTTTGCG
AATTACTTTGACTACTGGGGTCAGGGCACAACGCTTACTGTCTCCAGCGCGTCAACAAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAAC
GTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTG
GAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCT
AAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC
AACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTG
CTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAG
CCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTG
ACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGC
CAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTC
CTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGC
TCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCT
GGCAAGTGA

131R002/131R003 Light chain nucleotide sequence with predicted signal sequence (SEQ ID NO:26)
ATGAAGCACCTCTGGTTCTTTCTTCTTCTGGTCGCAGCGCCGAGATGGGTACTTAGCGAC
ATCGTGCTCACACAGAGCCCTGCATCGCTCGCAGTATCGCTTGGTCAGCGAGCGACCATT
TCATGCAAAGCGTCACAATCGGTAGATTACGACGGAGACTCCTACATGAACTGGTATCAG
CAGAAACCAGGGCAGCCCCCGAAGTTGCTCATCTACGCCGCGTCCAATCTGGAGTCAGGC
ATTCCCGCCAGATTCAGCGGGAGCGGGTCAGGAACGGATTTTACCCTCAATATCCATCCG
GTAGAGGAGGAAGATGCGGCGACTTACTATTGTCAGCAGTCGAATGAGGACCCACTCACG
TTCGGGGCTGGAACAAAACTGGAACTTAAACGGACTGTGGCGGCTCCCTCAGTGTTCATC
TTCCCTCCCTCCGACGAACAATTGAAGTCGGGTACTGCCTCCGTCGTCTGTTTGTTGAAC
AACTTTTATCCGAGGGAAGCCAAGGTGCAGTGGAAGGTGGATAATGCGCTGCAGAGCGGT
AACTCGCAAGAGTCAGTCACAGAGCAAGACTCGAAGGATTCGACGTATTCGCTCAGCAGC
ACATTGACGCTGTCGAAGGCAGATTACGAGAAACACAAGGTGTACGCGTGCGAGGTCACC
CATCAGGGATTGTCGTCACCCGTGACGAAATCCTTTAACCGCGGAGAATGCTGA

131R002 Heavy chain amino acid sequence without predicted signal sequence (SEQ ID NO:27)
QVQLQESGPELVKPGASVKISCKASGYTFTDYSIHWVKQNHGKSLDWIGYIYPSNGDSGY
NQKFKNRATLTVDTSSSTAYLEVRRLTFEDSAVYYCATYFANYFDYWGQGTTLTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

131R003 Heavy chain amino acid sequence without predicted signal sequence (SEQ ID NO:28)
QVQLKQSGPELVKPGASVKISCKASGYTFTDYSIHWVKQNHGKSLDWIGYIYPSNGDSGY
NQKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFANYFDYWGQGTTLTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS

LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

131R002/131R003 Light chain amino acid sequence without predicted signal sequence (SEQ ID NO:29)
DIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYAASNLES
GIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPLTFGAGTKLELKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

131R002 Heavy chain amino acid sequence without predicted signal sequence (SEQ ID NO:30)
CAGGTACAATTGCAAGAATCCGGACCCGAACTTGTGAAGCCCGGAGCGTCAGTCAAGATC
TCGTGTAAGGCCAGCGGGTACACCTTTACGGATTATTCGATCCATTGGGTAAAACAGAAT
CACGGGAAGTCGCTCGACTGGATTGGTTATATCTACCCGTCCAACGGTGATTCGGGATAC
AACCAGAAGTTCAAAAATCGGGCCACACTTACAGTGGACACATCGTCGTCAACTGCATAT
CTCGAGGTCCGCAGACTGACGTTTGAGGACTCAGCTGTCTACTATTGCGCGACTTATTTC
GCCAACTACTTCGATTACTGGGGCCAGGGGACGACACTGACGGTCAGCTCCGCGAGCACC
AAGGGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCC
GCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCT
GGCGCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTAC
TCCCTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGC
AACGTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGC
GTGGAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCT
CCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTG
GACGTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTG
CACAACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCT
GTGCTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCC
AACAAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGC
GAGCCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCC
CTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAAC
GGCCAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTC
TTCCTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCC
TGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCT
CCTGGCAAGTGA

131R003 Heavy chain amino acid sequence without predicted signal sequence (SEQ ID NO:31)
CAGGTGCAACTTAAACAGTCGGGGCCTGAGTTGGTCAAACCAGGAGCCTCAGTAAAGATT
AGCTGCAAAGCATCAGGTTATACCTTTACGGATTACTCGATCCACTGGGTGAAGCAGAAC
CACGGAAAGTCACTGGATTGGATCGGGTACATCTACCCCTCGAATGGAGATTCGGGGTAT
AACCAAAAGTTCAAAAACCGGGCCACGCTGACTGTGGACACGTCGTATTCCACCGCATAT
TTGGAAGTCCGCAGACTCACGTTCGAGGACTCCGCGGTATACTATTGTGCCACATACTTT
GCGAATTACTTTGACTACTGGGGTCAGGGCACAACGCTTACTGTCTCCAGCGCGTCAACA
AAGGGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCC
GCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCT
GGCGCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTAC
TCCCTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGC
AACGTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGC
GTGGAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCT
CCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTG
GACGTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTG
CACAACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCT
GTGCTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCC
AACAAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGC
GAGCCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCC

CTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAAC
GGCCAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTC
TTCCTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCC
TGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCT
CCTGGCAAGTGA

131R002/131R003 Light chain amino acid sequence without predicted signal sequence (SEQ ID NO:32)
GACATCGTGCTCACACAGAGCCCTGCATCGCTCGCAGTATCGCTTGGTCAGCGAGCGACC
ATTTCATGCAAAGCGTCACAATCGGTAGATTACGACGGAGACTCCTACATGAACTGGTAT
CAGCAGAAACCAGGGCAGCCCCCGAAGTTGCTCATCTACGCCGCGTCCAATCTGGAGTCA
GGCATTCCCGCCAGATTCAGCGGGAGCGGGTCAGGAACGGATTTTACCCTCAATATCCAT
CCGGTAGAGGAGGAAGATGCGGCGACTTACTATTGTCAGCAGTCGAATGAGGACCCACTC
ACGTTCGGGGCTGGAACAAAACTGGAACTTAAACGGACTGTGGCGGCTCCCTCAGTGTTC
ATCTTCCCTCCCTCCGACGAACAATTGAAGTCGGGTACTGCCTCCGTCGTCTGTTTGTTG
AACAACTTTTATCCGAGGGAAGCCAAGGTGCAGTGGAAGGTGGATAATGCGCTGCAGAGC
GGTAACTCGCAAGAGTCAGTCACAGAGCAAGACTCGAAGGATTCGACGTATTCGCTCAGC
AGCACATTGACGCTGTCGAAGGCAGATTACGAGAAACACAAGGTGTACGCGTGCGAGGTC
ACCCATCAGGGATTGTCGTCACCCGTGACGAAATCCTTTAACCGCGGAGAATGCTGA

FLAG Tag (SEQ ID NO:33)
DYKDDDDK

131R003 Heavy chain CDR1 variant (SEQ ID NO:34)
KASGYTFTSYTF

131R003 Heavy chain CDR3 variant (SEQ ID NO:35)
ATYFANNFDY

131R003 Heavy chain variable region - Variant 1 (SEQ ID NO:36)
QVQLKQSGPELVKPGASVKISCKASGYTFTDYSIHWVKQNHGKSLDWIGYIYPSNGDSGY
NQKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFANNFDYWGQGTTLTVSS

131R003 Heavy chain variable region - Variant 2 (SEQ ID NO:37)
QVQLKQSGPELVKPGASVKISCKASGYTFTSYTFHWVKQNHGKSLDWIGYIYPSNGDSGY
NQKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFANNFDYWGQGTTLTVSS

131R003 Heavy chain - Variant 1 with predicted signal sequence underlined (SEQ ID NO:38)
MKHLWFFLLLVAAPRWVLSQVQLKQSGPELVKPGASVKISCKASGYTFTDYSIHWVKQNH
GKSLDWIGYIYPSNGDSGYNQKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFA
NNFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

131R003 Heavy chain - Variant 1 without predicted signal sequence (SEQ ID NO:39)
QVQLKQSGPELVKPGASVKISCKASGYTFTDYSIHWVKQNHGKSLDWIGYIYPSNGDSGY
NQKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFANNFDYWGQGTTLTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS

LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

131R003 Heavy chain - Variant 1 nucleic acid with predicted signal sequence (SEQ ID NO:40)
ATGAAGCATCTTTGGTTCTTCCTGCTCTTGGTGGCTGCGCCGAGGTGGGTGCTCAGCCAG
GTGCAACTTAAACAGTCGGGGCCTGAGTTGGTCAAACCAGGAGCCTCAGTAAAGATTAGC
TGCAAAGCATCAGGTTATACCTTTACGGATTACTCGATCCACTGGGTGAAGCAGAACCAC
GGAAAGTCACTGGATTGGATCGGGTACATCTACCCCTCGAATGGAGATTCGGGGTATAAC
CAAAAGTTCAAAAACCGGGCCACGCTGACTGTGGACACGTCGTATTCCACCGCATATTTG
GAAGTCCGCAGACTCACGTTCGAGGACTCCGCGGTATACTATTGTGCCACATACTTTGCG
AATAACTTTGACTACTGGGGTCAGGGCACAACGCTTACTGTCTCCAGCGCGTCAACAAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAAC
GTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTG
GAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCT
AAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC
AACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTG
CTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAG
CCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTG
ACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGC
CAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTC
CTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGC
TCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCT
GGCAAGTGA

131R003 Heavy chain - Variant 2 with predicted signal sequence underlined (SEQ ID NO:41)
MKHLWFFLLLVAAPRWVLSQVQLKQSGPELVKPGASVKISCKASGYTFTSYTFHWVKQNH
GKSLDWIGYIYPSNGDSGYNQKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFA
NNFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

131R003 Heavy chain - Variant 2 without predicted signal sequence (SEQ ID NO:42)
QVQLKQSGPELVKPGASVKISCKASGYTFTSYTFHWVKQNHGKSLDWIGYIYPSNGDSGY
NQKFKNRATLTVDTSYSTAYLEVRRLTFEDSAVYYCATYFANNFDYWGQGTTLTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

131R003 Heavy chain - Variant 2 nucleic acid with predicted signal sequence (SEQ ID NO:43)
ATGAAGCATCTTTGGTTCTTCCTGCTCTTGGTGGCTGCGCCGAGGTGGGTGCTCAGCCAG
GTGCAACTTAAACAGTCGGGGCCTGAGTTGGTCAAACCAGGAGCCTCAGTAAAGATTAGC
TGCAAAGCATCAGGATACACCTTCACTAGCTATACATTCCACTGGGTGAAGCAGAACCAC

GGAAAGTCACTGGATTGGATCGGGTACATCTACCCCTCGAATGGAGATTCGGGGTATAAC
CAAAAGTTCAAAAACCGGGCCACGCTGACTGTGGACACGTCGTATTCCACCGCATATTTG
GAAGTCCGCAGACTCACGTTCGAGGACTCCGCGGTATACTATTGTGCCACATACTTTGCG
AATAACTTTGACTACTGGGGTCAGGGCACAACGCTTACTGTCTCCAGCGCGTCAACAAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAAC
GTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTG
GAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCT
AAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC
AACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTG
CTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAG
CCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTG
ACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGC
CAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTC
CTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGC
TCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCT
GGCAAGTGA

Humanized 131R003 Antibodies

Humanized 131R005/131R007/131R008/131R010/131R011 Heavy chain variable region (SEQ ID NO:44)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYSIHWVRQAPGQGLEWIGYIYPSNGDSGY
NQKFKNRVTMTRDTSTSTAYMELSRLRSEDTAVYYCATYFANNFDYWGQGTTLTVSS

Humanized 131R006A Heavy chain variable region (SEQ ID NO:45)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYTFHWVRQAPGQGLEWIGYIYPSNGDSGY
NQKFKNRVTMTRDTSTSTAYMELSRLRSEDTAVYYCATYFANNFDYWGQGTTLTVSS

Humanized 131R005/131R007/131R011 Heavy chain (IgG2) with predicted signal sequence underlined (SEQ ID NO:46)

MKHLWFFLLLVAAPRWVLSQVQLVQSGAEVKKPGASVKVSCKASGYTFTDYSIHWVRQAP
GQGLEWIGYIYPSNGDSGYNQKFKNRVTMTRDTSTSTAYMELSRLRSEDTAVYYCATYFA
NNFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Humanized 131R006A Heavy chain with predicted signal sequence underlined (SEQ ID NO:47)

MKHLWFFLLLVAAPRWVLSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYTFHWVRQAP
GQGLEWIGYIYPSNGDSGYNQKFKNRVTMTRDTSTSTAYMELSRLRSEDTAVYYCATYFA
NNFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Humanized 131R005/131R007/131R011 Heavy chain (IgG2) without predicted signal sequence (SEQ ID NO:48)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYSIHWVRQAPGQGLEWIGYIYPSNGDSGY
NQKFKNRVTMTRDTSTSTAYMELSRLRSEDTAVYYCATYFANNFDYWGQGTTLTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

Humanized 131R006A Heavy chain without predicted signal sequence (SEQ ID NO:49)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYTFHWVRQAPGQGLEWIGYIYPSNGDSGY
NQKFKNRVTMTRDTSTSTAYMELSRLRSEDTAVYYCATYFANNFDYWGQGTTLTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

Humanized 131R005/131R007 Heavy chain variable region nucleic acid (SEQ ID NO:50)

CAAGTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTG
AGCTGCAAGGCTTCTGGATACACCTTCACTGACTATTCAATCCACTGGGTGAGACAGGCA
CCTGGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTAC
AACCAAAAGTTCAAGAACCGGGTGACTATGACCAGAGATACCTCAACATCTACTGCCTAC
ATGGAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTC
GCTAATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCA

Humanized 131R006A Heavy chain variable region nucleic acid (SEQ ID NO:51)

CAAGTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTG
AGCTGCAAGGCTTCTGGATACACCTTCACTAGCTATACATTCCACTGGGTGAGACAGGCA
CCTGGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTAC
AACCAAAAGTTCAAGAACCGGGTGACTATGACCAGAGATACCTCAACATCTACTGCCTAC
ATGGAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTC
GCTAATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCA

Humanized 131R005/131R007 Heavy chain nucleic acid with predicted signal sequence (SEQ ID NO:52)

ATGAAGCATCTGTGGTTTTTCCTCCTCCTTGTCGCCGCTCCACGCTGGGTGCTTTCCCAA
GTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTGAGC
TGCAAGGCTTCTGGATACACCTTCACTGACTATTCAATCCACTGGGTGAGACAGGCACCT
GGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTACAAC
CAAAAGTTCAAGAACCGGGTGACTATGACCAGAGATACCTCAACATCTACTGCCTACATG
GAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTCGCT
AATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCAGCCTCAACCAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAAC

GTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTG
GAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCT
AAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC
AACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTG
CTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAG
CCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTG
ACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGC
CAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTC
CTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGC
TCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCT
GGCAAGTGA

Humanized 131R006A Heavy chain nucleic acid with predicted signal sequence (SEQ ID NO:53)
ATGAAGCATCTGTGGTTTTTCCTCCTCCTTGTCGCCGCTCCACGCTGGGTGCTTTCCCAA
GTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTGAGC
TGCAAGGCTTCTGGATACACCTTCACTAGCTATACATTCCACTGGGTGAGACAGGCACCT
GGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTACAAC
CAAAAGTTCAAGAACCGGGTGACTATGACCAGAGATACCTCAACATCTACTGCCTACATG
GAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTCGCT
AATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCAGCCTCAACCAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAAC
GTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTG
GAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCT
AAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC
AACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTG
CTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAG
CCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTG
ACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGC
CAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTC
CTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGC
TCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCT
GGCAAGTGA

Humanized 131R005/131R007 Heavy chain nucleic acid without predicted signal sequence (SEQ ID NO:54)
CAAGTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTG
AGCTGCAAGGCTTCTGGATACACCTTCACTGACTATTCAATCCACTGGGTGAGACAGGCA
CCTGGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTAC
AACCAAAAGTTCAAGAACCGGGTGACTATGACCAGAGATACCTCAACATCTACTGCCTAC
ATGGAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTC
GCTAATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCAGCCTCAACC
AAGGGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCC
GCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCT
GGCGCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTAC
TCCCTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGC
AACGTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGC
GTGGAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCT

CCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTG
GACGTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTG
CACAACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCT
GTGCTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCC
AACAAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGC
GAGCCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCC
CTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAAC
GGCCAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTC
TTCCTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCC
TGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCT
CCTGGCAAGTGA

Humanized 131R006A Heavy chain - nucleic acid without predicted signal sequence (SEQ ID NO:55)
CAAGTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTG
AGCTGCAAGGCTTCTGGATACACCTTCACTAGCTATACATTCCACTGGGTGAGACAGGCA
CCTGGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTAC
AACCAAAAGTTCAAGAACCGGGTGACTATGACCAGAGATACCTCAACATCTACTGCCTAC
ATGGAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTC
GCTAATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCAGCCTCAACC
AAGGGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCC
GCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCT
GGCGCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTAC
TCCCTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGC
AACGTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGC
GTGGAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCT
CCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTG
GACGTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTG
CACAACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCT
GTGCTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCC
AACAAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGC
GAGCCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCC
CTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAAC
GGCCAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTC
TTCCTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCC
TGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCT
CCTGGCAAGTGA

Human IgG1 Heavy chain constant region (SEQ ID NO:56)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG2 Heavy chain constant region (SEQ ID NO:57)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR
VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PMLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG3 Heavy chain constant region (SEQ ID NO:58)
ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSC
DTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHE
ALHNRFTQKSLSLSPGK

Human IgG4 Heavy chain constant region (SEQ ID NO:59)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG
NVFSCSVMHEALHNHYTQKSLSLSLGK

Human IgG2 Heavy chain constant region (13A Chain variant) (SEQ ID NO:60)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR
VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREKMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLKSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

Human IgG2 Heavy chain constant region (13B Chain variant) (SEQ ID NO:61)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR
VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVEGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSELTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

Humanized 131R006B Heavy chain variable region (SEQ ID NO:62)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYSIHWVRQAPGQGLEWIGYIYPSNGDSGY
NQKFKNRVTMTVDTSYSTAYMELSRLRSEDTAVYYCATYFANNFDYWGQGTTLTVSS

Humanized 131R006B Heavy chain with predicted signal sequence underlined (SEQ ID NO:63)
MKHLWFFLLLVAAPRWVLSQVQLVQSGAEVKKPGASVKVSCKASGYTFTDYSIHWVRQAP
GQGLEWIGYIYPSNGDSGYNQKFKNRVTMTVDTSYSTAYMELSRLRSEDTAVYYCATYFA
NNFDYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Humanized 131R006B Heavy chain without predicted signal sequence (SEQ ID NO:64)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYSIHWVRQAPGQGLEWIGYIYPSNGDSGY
NQKFKNRVTMTVDTSYSTAYMELSRLRSEDTAVYYCATYFANNFDYWGQGTTLTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS

VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

Humanized 131R006B Heavy chain variable region nucleic acid (SEQ ID NO:65)
CAAGTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTG
AGCTGCAAGGCTTCTGGATACACCTTCACTGACTATTCAATCCACTGGGTGAGACAGGCA
CCTGGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTAC
AACCAAAAGTTCAAGAACCGGGTGACTATGACCGTGGATACCTCATACTCTACTGCCTAC
ATGGAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTC
GCTAATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCA

Humanized 131R006B Heavy chain nucleic acid with sequence signal (SEQ ID NO:66)
ATGAAGCATCTGTGGTTTTTCCTCCTCCTTGTCGCCGCTCCACGCTGGGTGCTTTCCCAA
GTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTGAGC
TGCAAGGCTTCTGGATACACCTTCACTGACTATTCAATCCACTGGGTGAGACAGGCACCT
GGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTACAAC
CAAAAGTTCAAGAACCGGGTGACTATGACCGTGGATACCTCATACTCTACTGCCTACATG
GAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTCGCT
AATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCAGCCTCAACCAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAAC
GTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTG
GAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCT
AAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC
AACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTG
CTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAG
CCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTG
ACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGC
CAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTC
CTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGC
TCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCT
GGCAAGTGA

Humanized 131R006B Heavy chain nucleic acid without predicted sequence signal (SEQ ID NO:67)
CAAGTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTG
AGCTGCAAGGCTTCTGGATACACCTTCACTGACTATTCAATCCACTGGGTGAGACAGGCA
CCTGGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTAC
AACCAAAAGTTCAAGAACCGGGTGACTATGACCGTGGATACCTCATACTCTACTGCCTAC
ATGGAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTC
GCTAATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCAGCCTCAACC
AAGGGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCC
GCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCT
GGCGCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTAC
TCCCTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGC
AACGTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGC
GTGGAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCT
CCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTG
GACGTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTG
CACAACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCT

GTGCTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCC
AACAAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGC
GAGCCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCC
CTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAAC
GGCCAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTC
TTCCTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCC
TGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCT
CCTGGCAAGTGA

Humanized 131R008/131R010 Heavy chain (IgG1) with predicted signal sequence underlined (SEQ ID NO:68)
MKHLWFFLLLVAAPRWVLSQVQLVQSGAEVKKPGASVKVSCKASGYTFTDYSIHWVRQAP
GQGLEWIGYIYPSNGDSGYNQKFKNRVTMTRDTSTSTAYMELSRLRSEDTAVYYCATYFA
NNFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Humanized 131R008/131R010 Heavy chain (IgG1) without predicted signal sequence (SEQ ID NO:69)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYSIHWVRQAPGQGLEWIGYIYPSNGDSGY
NQKFKNRVTMTRDTSTSTAYMELSRLRSEDTAVYYCATYFANNFDYWGQGTTLTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

Humanized 131R008 Heavy chain (IgG1) with signal sequence nucleic acid (SEQ ID NO:70)
ATGAAGCATCTGTGGTTTTTCCTCCTCCTTGTCGCCGCTCCACGCTGGGTGCTTTCCCAA
GTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTGAGC
TGCAAGGCTTCTGGATACACCTTCACTGACTATTCAATCCACTGGGTGAGACAGGCACCT
GGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTACAAC
CAAAAGTTCAAGAACCGGGTGACTATGACCAGAGATACCTCAACATCTACTGCCTACATG
GAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTCGCT
AATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCAGCCTCAACCAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTCCTCCAAGTCCACCTCCGGCGGCACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCAGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCTCCCTGGGCACCCAGACCTACATCTGCAAC
GTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGCGGGTGGAGCCTAAGTCCTGCGAC
AAGACCCACACCTGCCCTCCCTGCCCTGCCCCTGAGCTGCTGGGCGGACCTTCCGTGTTC
CTGTTCCCTCCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAGGTGACCTGC
GTGGTGGTGGACGTGTCCCACGAGGATCCTGAGGTGAAGTTCAATTGGTACGTGGACGGC
GTGGAGGTGCACAACGCTAAGACCAAGCCAAGGGAGGAGCAGTACAACTCCACCTACCGG
GTGGTGTCTGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGC
AAGGTCTCCAACAAGGCCCTGCCCGCTCCCATCGAGAAAACCATCTCCAAGGCCAAGGGC
CAGCCTCGCGAGCCTCAGGTGTACACCCTGCCACCCAGCCGGGAGGAGATGACCAAGAAC
CAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGG
GAGTCTAACGGCCAGCCCGAGAACAACTACAAGACCACCCCTCCTGTGCTGGACTCCGAC
GGCTCCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAAC
GTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTG

TCTCTGTCTCCTGGCAAGTGA

Humanized 131R008 Heavy chain (IgG1) without predicted signal sequence nucleic acid (SEQ ID NO:71)
CAAGTCCAATTGGTCCAGAGCGGTGCCGAAGTGAAGAAACCGGGAGCTTCCGTGAAAGTG
AGCTGCAAGGCTTCTGGATACACCTTCACTGACTATTCAATCCACTGGGTGAGACAGGCA
CCTGGTCAGGGACTGGAGTGGATTGGATACATCTACCCCTCAAATGGGGACTCTGGCTAC
AACCAAAAGTTCAAGAACCGGGTGACTATGACCAGAGATACCTCAACATCTACTGCCTAC
ATGGAACTCAGCAGGCTGCGCTCAGAGGACACCGCAGTGTATTACTGTGCCACCTACTTC
GCTAATAACTTCGACTATTGGGGGCAGGGCACCACCCTGACTGTCAGCTCAGCCTCAACC
AAGGGCCCCTCCGTGTTCCCTCTGGCCCCTTCCTCCAAGTCCACCTCCGGCGGCACCGCC
GCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCT
GGCGCCCTGACCTCTGGCGTGCACACCTTCCCAGCCGTGCTGCAGTCCTCCGGCCTGTAC
TCCCTGTCCTCCGTGGTGACCGTGCCTTCCTCCTCCCTGGGCACCCAGACCTACATCTGC
AACGTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGCGGGTGGAGCCTAAGTCCTGC
GACAAGACCCACACCTGCCCTCCCTGCCCTGCCCCTGAGCTGCTGGGCGGACCTTCCGTG
TTCCTGTTCCCTCCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAGGTGACC
TGCGTGGTGGTGGACGTGTCCCACGAGGATCCTGAGGTGAAGTTCAATTGGTACGTGGAC
GGCGTGGAGGTGCACAACGCTAAGACCAAGCCAAGGGAGGAGCAGTACAACTCCACCTAC
CGGGTGGTGTCTGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAG
TGCAAGGTCTCCAACAAGGCCCTGCCCGCTCCCATCGAGAAAACCATCTCCAAGGCCAAG
GGCCAGCCTCGCGAGCCTCAGGTGTACACCCTGCCACCCAGCCGGGAGGAGATGACCAAG
AACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAG
TGGGAGTCTAACGGCCAGCCCGAGAACAACTACAAGACCACCCCTCCTGTGCTGGACTCC
GACGGCTCCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGC
AACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGC
CTGTCTCTGTCTCCTGGCAAGTGA

Humanized 131R005/131R007/131R008 Light chain variable region (SEQ ID NO:72)
DIVLTQSPASLAVSLGQRATITCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYAASNLES
GIPARFSGSGSGTDFTLTINPVEAEDVATYYCQQSNEDPLTFGAGTKLELKR

Humanized 131R005/131R007/131R008 Light chain with predicted signal sequence underlined (SEQ ID NO:73)
MKHLWFFLLLVAAPRWVLSDIVLTQSPASLAVSLGQRATITCKASQSVDYDGDSYMNWYQ
QKPGQPPKLLIYAASNLESGIPARFSGSGSGTDFTLTINPVEAEDVATYYCQQSNEDPLT
FGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Humanized 131R005/131R007/131R008 Light chain without predicted signal sequence underlined (SEQ ID NO:74)
DIVLTQSPASLAVSLGQRATITCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYAASNLES
GIPARFSGSGSGTDFTLTINPVEAEDVATYYCQQSNEDPLTFGAGTKLELKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Humanized 131R005/131R007/131R008 Light chain variable region nucleic acid (SEQ ID NO:75)
GATATCGTCCTGACCCAAAGCCCTGCTTCACTTGCTGTGAGCCTGGGGCAACGCGCCACC
ATCACTTGCAAGGCATCTCAGAGCGTGGACTATGATGGAGACTCTTACATGAATTGGTAT
CAACAGAAGCCAGGTCAACCTCCCAAACTGCTGATCTACGCCGCATCTAATCTTGAAAGC
GGCATCCCGGCTCGGTTCTCTGGTTCTGGATCAGGAACCGACTTCACCCTCACCATTAAC
CCAGTGGAGGCCGAGGACGTGGCTACTTACTACTGCCAGCAGTCAAACGAGGACCCCCTG
ACTTTCGGAGCCGGGACCAAGCTGGAGCTTAAGCGT

Humanized 131R005/131R007/131R008 Light chain with signal sequence nucleic acid (SEQ ID NO:76)
ATGAAACATCTTTGGTTCTTCCTTCTGCTGGTCGCTGCTCCTCGGTGGGTGCTTAGCGAT
ATCGTCCTGACCCAAAGCCCTGCTTCACTTGCTGTGAGCCTGGGGCAACGCGCCACCATC
ACTTGCAAGGCATCTCAGAGCGTGGACTATGATGGAGACTCTTACATGAATTGGTATCAA
CAGAAGCCAGGTCAACCTCCCAAACTGCTGATCTACGCCGCATCTAATCTTGAAAGCGGC
ATCCCGGCTCGGTTCTCTGGTTCTGGATCAGGAACCGACTTCACCCTCACCATTAACCCA
GTGGAGGCCGAGGACGTGGCTACTTACTACTGCCAGCAGTCAAACGAGGACCCCCTGACT
TTCGGAGCCGGGACCAAGCTGGAGCTTAAGCGTACGGTGGCCGCACCGTCAGTCTTTATC
TTTCCACCCTCCGACGAACAGCTTAAGTCAGGCACTGCCTCAGTCGTGTGTCTCCTCAAT
AACTTCTACCCCAGGGAGGCCAAGGTGCAGTGGAAAGTGGACAACGCCCTCCAGTCCGGG
AACTCTCAAGAAAGCGTCACCGAGCAGGACAGCAAGGACTCCACCTACTCACTGTCAAGC
ACTCTCACCCTCTCAAAGGCCGATTATGAGAAGCACAAGGTGTACGCATGCGAAGTGACC
CATCAGGGTCTGTCCTCTCCTGTCACCAAGTCCTTCAATAGAGGAGAATGTTGA

Humanized 131R005/131R007/131R008 Light chain without predicted signal sequence nucleic acid (SEQ ID NO:77)
GATATCGTCCTGACCCAAAGCCCTGCTTCACTTGCTGTGAGCCTGGGGCAACGCGCCACC
ATCACTTGCAAGGCATCTCAGAGCGTGGACTATGATGGAGACTCTTACATGAATTGGTAT
CAACAGAAGCCAGGTCAACCTCCCAAACTGCTGATCTACGCCGCATCTAATCTTGAAAGC
GGCATCCCGGCTCGGTTCTCTGGTTCTGGATCAGGAACCGACTTCACCCTCACCATTAAC
CCAGTGGAGGCCGAGGACGTGGCTACTTACTACTGCCAGCAGTCAAACGAGGACCCCCTG
ACTTTCGGAGCCGGGACCAAGCTGGAGCTTAAGCGTACGGTGGCCGCACCGTCAGTCTTT
ATCTTTCCACCCTCCGACGAACAGCTTAAGTCAGGCACTGCCTCAGTCGTGTGTCTCCTC
AATAACTTCTACCCCAGGGAGGCCAAGGTGCAGTGGAAAGTGGACAACGCCCTCCAGTCC
GGGAACTCTCAAGAAAGCGTCACCGAGCAGGACAGCAAGGACTCCACCTACTCACTGTCA
AGCACTCTCACCCTCTCAAAGGCCGATTATGAGAAGCACAAGGTGTACGCATGCGAAGTG
ACCCATCAGGGTCTGTCCTCTCCTGTCACCAAGTCCTTCAATAGAGGAGAATGTTGA

Variant Heavy chain CDR1 (SEQ ID NO:78)
DYSIH

Variant Heavy chain CDR2 (SEQ ID NO:79)
YIYPSNGDSGYNQKFK

Variant Heavy chain CDR3 (SEQ ID NO:80)
TYFANNFD

Variant Light chain CDR1 (SEQ ID NO:81)
KASQSVDYDGDSYMN

Variant Light chain CDR2 (SEQ ID NO:82)
AASNLES

Variant Light chain CDR3 (SEQ ID NO:83)
QQSNEDPLTF

Humanized 131R010 Heavy chain (IgG1) with signal sequence nucleic acid (SEQ ID NO:84)
ATGAAACACTTGTGGTTCTTTCTGCTCCTTGTCGCAGCACCACGGTGGGTGCTGTCGCAA
GTGCAATTGGTGCAGTCCGGAGCGGAAGTGAAGAAGCCTGGTGCCTCGGTCAAAGTCTCA
TGCAAGGCCAGCGGATACACTTTCACCGACTACTCCATCCATTGGGTGAGGCAGGCTCCG
GGCCAGGGCCTGGAGTGGATTGGGTACATCTACCCGTCGAACGGAGATTCGGGGTACAAT
CAGAAGTTCAAGAACCGCGTGACCATGACTCGGGACACCTCAACTTCCACGGCTTATATG

GAACTGAGCCGCCTGAGATCCGAGGACACTGCGGTGTACTACTGTGCCACCTACTTTGCG
AACAATTTCGATTACTGGGGACAAGGAACCACGCTCACTGTCAGCTCAGCCAGCACCAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTCCTCCAAGTCCACCTCCGGCGGCACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCAGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCTCCCTGGGCACCCAGACCTACATCTGCAAC
GTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGCGGGTGGAGCCTAAGTCCTGCGAC
AAGACCCACACCTGCCCTCCCTGCCCTGCCCCTGAGCTGCTGGGCGGACCTTCCGTGTTC
CTGTTCCCTCCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAGGTGACCTGC
GTGGTGGTGGACGTGTCCCACGAGGATCCTGAGGTGAAGTTCAATTGGTACGTGGACGGC
GTGGAGGTGCACAACGCTAAGACCAAGCCAAGGGAGGAGCAGTACAACTCCACCTACCGG
GTGGTGTCTGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGC
AAGGTCTCCAACAAGGCCCTGCCCGCTCCCATCGAGAAAACCATCTCCAAGGCCAAGGGC
CAGCCTCGCGAGCCTCAGGTGTACACCCTGCCACCCAGCCGGGAGGAGATGACCAAGAAC
CAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGG
GAGTCTAACGGCCAGCCCGAGAACAACTACAAGACCACCCCTCCTGTGCTGGACTCCGAC
GGCTCCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAAC
GTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTG
TCTCTGTCTCCTGGCAAGTGATAA

Humanized 131R010 Heavy chain (IgG1) without signal sequence nucleic acid (SEQ ID NO:85)
CAAGTGCAATTGGTGCAGTCCGGAGCGGAAGTGAAGAAGCCTGGTGCCTCGGTCAAAGTC
TCATGCAAGGCCAGCGGATACACTTTCACCGACTACTCCATCCATTGGGTGAGGCAGGCT
CCGGGCCAGGGCCTGGAGTGGATTGGGTACATCTACCCGTCGAACGGAGATTCGGGGTAC
AATCAGAAGTTCAAGAACCGCGTGACCATGACTCGGGACACCTCAACTTCCACGGCTTAT
ATGGAACTGAGCCGCCTGAGATCCGAGGACACTGCGGTGTACTACTGTGCCACCTACTTT
GCGAACAATTTCGATTACTGGGGACAAGGAACCACGCTCACTGTCAGCTCAGCCAGCACC
AAGGGCCCCTCCGTGTTCCCTCTGGCCCCTTCCTCCAAGTCCACCTCCGGCGGCACCGCC
GCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCT
GGCGCCCTGACCTCTGGCGTGCACACCTTCCCAGCCGTGCTGCAGTCCTCCGGCCTGTAC
TCCCTGTCCTCCGTGGTGACCGTGCCTTCCTCCTCCCTGGGCACCCAGACCTACATCTGC
AACGTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGCGGGTGGAGCCTAAGTCCTGC
GACAAGACCCACACCTGCCCTCCCTGCCCTGCCCCTGAGCTGCTGGGCGGACCTTCCGTG
TTCCTGTTCCCTCCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAGGTGACC
TGCGTGGTGGTGGACGTGTCCCACGAGGATCCTGAGGTGAAGTTCAATTGGTACGTGGAC
GGCGTGGAGGTGCACAACGCTAAGACCAAGCCAAGGGAGGAGCAGTACAACTCCACCTAC
CGGGTGGTGTCTGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAG
TGCAAGGTCTCCAACAAGGCCCTGCCCGCTCCCATCGAGAAAACCATCTCCAAGGCCAAG
GGCCAGCCTCGCGAGCCTCAGGTGTACACCCTGCCACCCAGCCGGGAGGAGATGACCAAG
AACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAG
TGGGAGTCTAACGGCCAGCCCGAGAACAACTACAAGACCACCCCTCCTGTGCTGGACTCC
GACGGCTCCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGC
AACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGC
CTGTCTCTGTCTCCTGGCAAGTGATAA

Humanized 131R010/131R011 Light chain variable region (SEQ ID NO:86)
DIQMTQSPSSLSASVGDRVTITCKASQSVDYDGDSYMNWYQQKPGKAPKLLIYAASNLES
GVPSRFSGSGSGTDFTLTISPVQAEDFATYYCQQSNEDPLTFGAGTKLELKR

Humanized 131R010/131R011 Light chain with predicted signal sequence underlined (SEQ ID NO:87)
MKHLWFFLLLVAAPRWVLSDIQMTQSPSSLSASVGDRVTITCKASQSVDYDGDSYMNWYQ
QKPGKAPKLLIYAASNLESGVPSRFSGSGSGTDFTLTISPVQAEDFATYYCQQSNEDPLT
FGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Humanized 131R010/131R011 Light chain without predicted signal sequence (SEQ ID NO:88)
DIQMTQSPSSLSASVGDRVTITCKASQSVDYDGDSYMNWYQQKPGKAPKLLIYAASNLES
GVPSRFSGSGSGTDFTLTISPVQAEDFATYYCQQSNEDPLTFGAGTKLELKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Humanized 131R010/131R011 Light chain variable region nucleic acid (SEQ ID NO:89)
GATATCCAGATGACTCAGTCGCCCTCATCGTTGAGCGCCTCGGTCGGGGATCGCGTGACT
ATTACTTGTAAAGCGTCCCAGAGCGTGGACTACGACGGAGATTCCTACATGAACTGGTAT
CAGCAAAAACCGGGAAAGGCTCCTAAACTTCTCATCTACGCAGCCTCGAATCTGGAATCA
GGAGTCCCGAGCCGGTTCAGCGGATCAGGCTCCGGTACTGATTTTACCCTCACGATCTCG
CCAGTGCAAGCCGAGGACTTCGCGACCTACTACTGCCAACAGTCCAACGAGGACCCGCTG
ACCTTCGGCGCAGGGACCAAGCTGGAACTGAAGCGT

Humanized 131R010/131R011 Light chain with signal sequence nucleic acid (SEQ ID NO:90)
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTCCTGTCCGAT
ATCCAGATGACTCAGTCGCCCTCATCGTTGAGCGCCTCGGTCGGGGATCGCGTGACTATT
ACTTGTAAAGCGTCCCAGAGCGTGGACTACGACGGAGATTCCTACATGAACTGGTATCAG
CAAAAACCGGGAAAGGCTCCTAAACTTCTCATCTACGCAGCCTCGAATCTGGAATCAGGA
GTCCCGAGCCGGTTCAGCGGATCAGGCTCCGGTACTGATTTTACCCTCACGATCTCGCCA
GTGCAAGCCGAGGACTTCGCGACCTACTACTGCCAACAGTCCAACGAGGACCCGCTGACC
TTCGGCGCAGGGACCAAGCTGGAACTGAAGCGTACGGTGGCCGCTCCATCCGTGTTTATC
TTTCCGCCGTCCGATGAGCAGCTCAAGTCGGGCACTGCCAGCGTGGTCTGCCTGCTTAAC
AATTTCTACCCTAGGGAAGCCAAGGTGCAGTGGAAGGTGGATAACGCGCTCCAATCCGGT
AACTCGCAAGAGAGCGTGACCGAACAGGACTCAAAGGACTCGACGTACAGCCTGTCATCG
ACCTTGACTCTCTCAAAGGCCGACTACGAAAAGCACAAGGTCTACGCGTGCGAAGTCACC
CATCAGGGACTGTCCTCGCCTGTGACCAAGAGCTTCAATCGCGGAGAGTGCTGA

Humanized 131R010/131R011 Light chain without signal sequence nucleic acid (SEQ ID NO:91)
GATATCCAGATGACTCAGTCGCCCTCATCGTTGAGCGCCTCGGTCGGGGATCGCGTGACT
ATTACTTGTAAAGCGTCCCAGAGCGTGGACTACGACGGAGATTCCTACATGAACTGGTAT
CAGCAAAAACCGGGAAAGGCTCCTAAACTTCTCATCTACGCAGCCTCGAATCTGGAATCA
GGAGTCCCGAGCCGGTTCAGCGGATCAGGCTCCGGTACTGATTTTACCCTCACGATCTCG
CCAGTGCAAGCCGAGGACTTCGCGACCTACTACTGCCAACAGTCCAACGAGGACCCGCTG
ACCTTCGGCGCAGGGACCAAGCTGGAACTGAAGCGTACGGTGGCCGCTCCATCCGTGTTT
ATCTTTCCGCCGTCCGATGAGCAGCTCAAGTCGGGCACTGCCAGCGTGGTCTGCCTGCTT
AACAATTTCTACCCTAGGGAAGCCAAGGTGCAGTGGAAGGTGGATAACGCGCTCCAATCC
GGTAACTCGCAAGAGAGCGTGACCGAACAGGACTCAAAGGACTCGACGTACAGCCTGTCA
TCGACCTTGACTCTCTCAAAGGCCGACTACGAAAAGCACAAGGTCTACGCGTGCGAAGTC
ACCCATCAGGGACTGTCCTCGCCTGTGACCAAGAGCTTCAATCGCGGAGAGTGCTGA

Humanized 131R011 Heavy chain variable region nucleic acid (SEQ ID NO:92)
CAAGTGCAATTGGTGCAGTCCGGAGCGGAAGTGAAGAAGCCTGGTGCCTCGGTCAAAGTC
TCATGCAAGGCCAGCGGATACACTTTCACCGACTACTCCATCCATTGGGTGAGGCAGGCT
CCGGGCCAGGGCCTGGAGTGGATTGGGTACATCTACCCGTCGAACGGAGATTCGGGGTAC
AATCAGAAGTTCAAGAACCGCGTGACCATGACTCGGGACACCTCAACTTCCACGGCTTAT
ATGGAACTGAGCCGCCTGAGATCCGAGGACACTGCGGTGTACTACTGTGCCACCTACTTT
GCGAACAATTTCGATTACTGGGGACAAGGAACCACGCTCACTGTCAGCTCA

Humanized 131R011 Heavy chain (IgG2) with signal sequence nucleic acid (SEQ ID NO:93)
ATGAAACACTTGTGGTTCTTTCTGCTCCTTGTCGCAGCACCACGGTGGGTGCTGTCGCAA
GTGCAATTGGTGCAGTCCGGAGCGGAAGTGAAGAAGCCTGGTGCCTCGGTCAAAGTCTCA

TGCAAGGCCAGCGGATACACTTTCACCGACTACTCCATCCATTGGGTGAGGCAGGCTCCG
GGCCAGGGCCTGGAGTGGATTGGGTACATCTACCCGTCGAACGGAGATTCGGGGTACAAT
CAGAAGTTCAAGAACCGCGTGACCATGACTCGGGACACCTCAACTTCCACGGCTTATATG
GAACTGAGCCGCCTGAGATCCGAGGACACTGCGGTGTACTACTGTGCCACCTACTTTGCG
AACAATTTCGATTACTGGGGACAAGGAACCACGCTCACTGTCAGCTCAGCCAGCACCAAG
GGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCCGCT
CTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCTGGC
GCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTACTCC
CTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGCAAC
GTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGCGTG
GAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCTCCT
AAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC
AACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCTGTG
CTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGCGAG
CCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTG
ACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAACGGC
CAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTCTTC
CTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGC
TCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCT
GGCAAGTGATAA

Humanized 131R011 Heavy chain (IgG2) without signal sequence nucleic acid (SEQ ID NO:94)
CAAGTGCAATTGGTGCAGTCCGGAGCGGAAGTGAAGAAGCCTGGTGCCTCGGTCAAAGTC
TCATGCAAGGCCAGCGGATACACTTTCACCGACTACTCCATCCATTGGGTGAGGCAGGCT
CCGGGCCAGGGCCTGGAGTGGATTGGGTACATCTACCCGTCGAACGGAGATTCGGGGTAC
AATCAGAAGTTCAAGAACCGCGTGACCATGACTCGGGACACCTCAACTTCCACGGCTTAT
ATGGAACTGAGCCGCCTGAGATCCGAGGACACTGCGGTGTACTACTGTGCCACCTACTTT
GCGAACAATTTCGATTACTGGGGACAAGGAACCACGCTCACTGTCAGCTCAGCCAGCACC
AAGGGCCCCTCCGTGTTCCCTCTGGCCCCTTGCTCCCGGTCCACCTCTGAGTCTACCGCC
GCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCCTGGAACTCT
GGCGCCCTGACCTCTGGCGTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCCTGTAC
TCCCTGTCCTCCGTGGTGACCGTGCCTTCCTCCAACTTCGGCACCCAGACCTACACCTGC
AACGTGGACCACAAGCCTTCCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGCTGC
GTGGAGTGCCCTCCTTGTCCTGCTCCTCCTGTGGCTGGCCCTTCTGTGTTCCTGTTCCCT
CCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAAGTGACCTGCGTGGTGGTG
GACGTGTCCCACGAGGACCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAGGTG
CACAACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTGTCT
GTGCTGACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCC
AACAAGGGCCTGCCTGCCCCTATCGAAAAGACCATCTCTAAGACCAAGGGCCAGCCTCGC
GAGCCTCAGGTCTACACCCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCC
CTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCTAAC
GGCCAGCCTGAGAACAACTACAAGACCACCCCTCCTATGCTGGACTCCGACGGCTCCTTC
TTCCTGTACTCCAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCC
TGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCT
CCTGGCAAGTGATAA

Humanized 131R010 Heavy chain variable region (SEQ ID NO:95)
CAAGTGCAATTGGTGCAGTCCGGAGCGGAAGTGAAGAAGCCTGGTGCCTCGGTCAAAGTC
TCATGCAAGGCCAGCGGATACACTTTCACCGACTACTCCATCCATTGGGTGAGGCAGGCT
CCGGGCCAGGGCCTGGAGTGGATTGGGTACATCTACCCGTCGAACGGAGATTCGGGGTAC
AATCAGAAGTTCAAGAACCGCGTGACCATGACTCGGGACACCTCAACTTCCACGGCTTAT
ATGGAACTGAGCCGCCTGAGATCCGAGGACACTGCGGTGTACTACTGTGCCACCTACTTT

GCGAACAATTTCGATTACTGGGGACAAGGAACCACGCTCACTGTCAGCTC

WHAT IS CLAIMED IS:

1. An isolated antibody that specifically binds human R-spondin 3 (RSPO3), which comprises:
(a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9), KASGYTFTSYTF (SEQ ID NO:34), or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10) or YIYPSNGDSGYNQKFK (SQ ID NO:79), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11), ATYFANNFDY (SEQ ID NO:35), or TYFANNFD (SEQ ID NO:80); and/or
(b) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12) or KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AAS (SEQ ID NO:13) or AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14) or QQSNEDPLTF (SEQ ID NO:83).

2. The antibody of claim 1, which comprises:
(a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or KASGYTFTSYTF (SEQ ID NO:34), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising ATYFANYFDY (SEQ ID NO:11) or ATYFANNFDY (SEQ ID NO:35), and/or
(b) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2 comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14).

3. The antibody of claim 1, which comprises:
(a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising YIYPSNGDSGYNQKFK (SQ ID NO:79), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80); and/or
(b) a light chain CDR1 comprising KASQSVDYDGDSYMN (SEQ ID NO:81), a light chain CDR2 comprising AASNLES (SEQ ID NO:82), and a light chain CDR3 comprising QQSNEDPLTF (SEQ ID NO:83).

4. The antibody of claim 1, which comprises:
(a) a heavy chain CDR1 comprising KASGYTFTDYS (SEQ ID NO:9) or DYSIH (SEQ ID NO:78), a heavy chain CDR2 comprising IYPSNGDS (SEQ ID NO:10), and a heavy chain CDR3 comprising TYFANNFD (SEQ ID NO:80); and/or
(b) a light chain CDR1 comprising QSVDYDGDSYM (SEQ ID NO:12), a light chain CDR2

comprising AAS (SEQ ID NO:13), and a light chain CDR3 comprising QQSNEDPLT (SEQ ID NO:14).

5. An isolated antibody that specifically binds human RSPO3, which comprises:
(a) a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, OR SEQ ID NO:62; and/or
(b) a light chain variable region having at least 90% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86.

6. The antibody of any one of claims 1-5, which comprises:
(a) a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:62; and/or
(b) a light chain variable region having at least 95% sequence identity to SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86.

7. The antibody of claim 6, which comprises:
(a) a heavy chain variable region comprising SEQ ID NO:15; and
(b) a light chain variable region comprising SEQ ID NO:17 or SEQ ID NO:72.

8. The antibody of claim 6, which comprises:
(a) a heavy chain variable region comprising SEQ ID NO:16; and
(b) a light chain variable region comprising SEQ ID NO:17 or SEQ ID NO:72.

9. The antibody of claim 6, which comprises:
(a) a heavy chain variable region comprising SEQ ID NO:36; and
(b) a light chain variable region comprising SEQ ID NO:17 or SEQ ID NO:72.

10. The antibody of claim 6, which comprises:
(a) a heavy chain variable region comprising SEQ ID NO:37; and
(b) a light chain variable region comprising SEQ ID NO:17 or SEQ ID NO:72.

11. The antibody of claim 6, which comprises:
(a) a heavy chain variable region comprising SEQ ID NO:44; and
(b) a light chain variable region comprising SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86.

12. The antibody of claim 6, which comprises:
(a) a heavy chain variable region comprising SEQ ID NO:45; and
(b) a light chain variable region comprising SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86.

13. The antibody of claim 6, which comprises:
(a) a heavy chain variable region comprising SEQ ID NO:62; and
(b) a light chain variable region comprising SEQ ID NO:17, SEQ ID NO:72, or SEQ ID NO:86.

14. An isolated antibody that competes with the antibody according to any one of claims 1-13 for specific binding to RSPO3.

15. An isolated antibody that binds the same epitope on RSPO3 as the antibody according to any one of claims 1-13.

16. An isolated antibody that binds an epitope on RSPO3 that overlaps with the epitope on RSPO3 bound by the antibody according to any one of claims 1-13.

17. The antibody according to any one of claims 1-16, which is a recombinant antibody, a monoclonal antibody, a chimeric antibody, or a bispecific antibody.

18. The antibody according to any one of claims 1-17, which is a humanized antibody.

19. The antibody according to any one of claims 1-17, which is a human antibody.

20. The antibody according to any one of claims 1-19, which is an IgG1 antibody.

21. The antibody according to any one of claims 1-19, which is an IgG2 antibody.

22. The antibody according to any one of claims 1-19, which is an antibody fragment comprising an antigen binding site.

23. An antibody comprising:
(a) a heavy chain sequence of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:64, or SEQ ID NO:69; and
(b) a light chain sequence of SEQ ID NO:29, SEQ ID NO:74, or SEQ ID NO:88.

24. The antibody of claim 23, which comprises a heavy chain sequence of SEQ ID NO:48 and a light chain sequence of SEQ ID NO:74 or SEQ ID NO:88.

25. The antibody of claim 23, which comprises a heavy chain sequence of SEQ ID NO:64 and a light chain sequence of SEQ ID NO:74 or SEQ ID NO:88.

26. The antibody of claim 23, which comprises a heavy chain sequence of SEQ ID NO:69 and a light chain sequence of SEQ ID NO:74 or SEQ ID NO:88.

27. An antibody comprising the heavy chain variable region and the light chain variable region from an antibody selected from the group consisting of: 131R102, 131R103, a variant of 131R103, a humanized version of 131R103, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, and h131R011.

28. An antibody selected from the group consisting of: 131R102, 131R103, a variant of 131R103, a humanized version of 131R103, h131R006A, h131R006B, h131R005/131R007, h131R008, h131R010, and h131R011.

29. An antibody comprising the heavy chain variable region encoded by the plasmid deposited with ATCC as PTA-_____.

30. An antibody comprising the heavy chain encoded by the plasmid deposited with ATCC as PTA-_____.

31. An antibody comprising the light chain variable region encoded by the plasmid deposited with ATCC as PTA-_____.

32. An antibody comprising the light chain encoded by the plasmid deposited with ATCC as PTA-_____.

33. An antibody comprising the heavy chain variable region encoded by the plasmid deposited with ATCC as PTA-_____ and the light chain variable region encoded by the plasmid deposited with ATCC as PTA-_____.

34. An antibody comprising the heavy chain encoded by the plasmid deposited with ATCC as PTA-_____ and the light chain encoded by the plasmid deposited with ATCC as PTA-_____.

35. The antibody according to any one of claims 1-34, which inhibits binding of RSPO3 to at least one leucine-rich repeat containing G protein coupled receptor (LGR).

36. The antibody of claim 35, wherein the LGR is selected from the group consisting of LGR4, LGR5, and LGR6.

37. The antibody of claim 36, wherein the LGR is LGR5.

38. The antibody according to any one of claims 1-37, which inhibits RSPO3 signaling.

39. The antibody according to any one of claims 1-38, which inhibits activation of β-catenin.

40. The antibody according to any one of claims 1-39, which inhibits β-catenin signaling.

41. The antibody according to any one of claims 1-40, which inhibits tumor growth.

42. The antibody according to any one of claims 1-41, which induces expression of differentiation markers in a tumor.

43. The antibody according to any one of claims 1-42, which induces cells in a tumor to differentiate.

44. The antibody according to any one of claims 1-41, which reduces the frequency of cancer stem cells in a tumor.

45. A polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ

ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64 SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:86, SEQ ID NO:87, and SEQ ID NO:88.

46. The polypeptide of claim 45, which is an antibody.

47. A cell comprising or producing the antibody or polypeptide according to any one of claims 1-46.

48. An isolated polynucleotide molecule comprising a polynucleotide that encodes an antibody or polypeptide according to any one of claims 1-46.

49. An isolated polynucleotide molecule comprising a polynucleotide sequence selected from the group consisting of: SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, and SEQ ID NO:95.

50. A vector comprising the polynucleotide of claim 48 or claim 49.

51. A cell comprising the polynucleotide of claim 48 or claim 49 or the vector of claim 50.

52. A pharmaceutical composition comprising the antibody or polypeptide according to any one of claims 1-46 and a pharmaceutically acceptable carrier.

53. A method of inhibiting growth of a tumor, wherein the method comprises contacting the tumor with an effective amount of an antibody according to any of claims 1-44.

54. A method of inhibiting growth of a tumor in a subject, wherein the method comprises administering to the subject a therapeutically effective amount of an antibody according to any of claims 1-44.

55. A method of inducing differentiation of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an antibody according to any one of claims 1-44.

56. A method of reducing the frequency of cancer stem cells in a tumor in a subject, comprising administering to the subject a therapeutically effective amount of an antibody according to any one of claims 1-44.

57. A method of inhibiting β-catenin signaling in a cell, comprising contacting the cell with an effective amount of an antibody according to any one of claims 1-44.

58. The method of claim 57, wherein the cell is a tumor cell.

59. The method according to any one of claims 53-56 or 58, wherein the tumor is selected from the group consisting of colorectal tumor, ovarian tumor, pancreatic tumor, lung tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor.

60. The method of claim 59, wherein the tumor is a pancreatic tumor.

61. The method of claim 59, wherein the tumor is a lung tumor.

62. The method of claim 59, wherein the tumor is a colorectal tumor.

63. The method of claim 59, wherein the tumor is an ovarian tumor.

64. A method of treating cancer in a subject, wherein the method comprises administering to the subject a therapeutically effective amount of an antibody according to any of claims 1-44.

65. The method of claim 64, wherein the cancer is selected from the group consisting of colorectal cancer, ovarian cancer, pancreatic cancer, lung cancer, liver cancer, breast cancer, kidney cancer, prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, bladder cancer, glioblastoma, and head and neck cancer.

66. The method of claim 65, wherein the cancer is colorectal cancer.

67. The method of claim 65, wherein the cancer is pancreatic cancer.

68. The method of claim 65, wherein the cancer is lung cancer.

69. The method of claim 65, wherein the cancer is ovarian cancer.

70. The method according to any one of claims 53-56 or 58-69, wherein the tumor or the cancer expresses elevated levels of RSPO3 as compared to levels of RSPO3 in a reference sample or to a pre-determined level of RSPO3.

71. The method according to any one of claims 53-56 or 58-70, further comprising a step of determining the level of RSPO3 expression in the tumor or cancer.

72. The method of claim 71, wherein determining the level of RSPO3 expression is done prior to treatment or contact with the antibody.

73. The method of claim 71 or claim 72, wherein if the tumor or cancer has an elevated expression level of RSPO3, the antibody is:
(a) administered to the subject; or
(b) contacted with the tumor or tumor cell.

74. The method according to any one of claims 53-56 or 58-69, wherein the tumor or the cancer has a RSPO gene fusion.

75. The method according to any one of claims 53-56 or 58-69, further comprising a step of determining if the tumor or cancer has a RSPO gene fusion.

76. The method of claim 74 or claim 75, wherein the RSPO gene fusion is a RSPO2 gene fusion.

77. The method of claim 74 or claim 75, wherein the RSPO gene fusion is a RSPO3 gene fusion.

78. The method according to any one of claims 53-56 or 58-77, wherein the subject has had a tumor or a cancer removed.

79. A method of treating a disease in a subject wherein the disease is associated with activation of β-catenin, comprising administering a therapeutically effective amount of an antibody according to any one of claims 1-44.

80. The method according to any one of claims 53-56 or 58-79, which further comprises administering at least one additional therapeutic agent.

81. The method of claim 80, wherein the additional therapeutic agent is a chemotherapeutic agent.

82. The method of claim 80, wherein the additional therapeutic agent is an angiogenesis inhibitor.

83. The method of claim 80, wherein the additional therapeutic agent is an antibody.

84. The method of claim 83, wherein the antibody is an anti-RSPO1 antibody.

85. The method of claim 83, wherein the antibody is an anti-RSPO2 antibody.

86. The method according to any one of claims 53-56 or 58-85, wherein the subject is human.

87. A method of identifying a human subject for treatment with an antibody that specifically binds RSPO3 comprising: determining if the subject has a tumor that has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3.

88. A method of identifying a human subject for treatment with an antibody that specifically binds RSPO3 comprising:
(a) obtaining a tumor sample from the subject, and
(b) determining if the tumor has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3.

89. A method of identifying a human subject for treatment with an antibody that specifically binds RSPO3 comprising: determining if the subject has a tumor that has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3, wherein if the tumor has an elevated expression level of RSPO3 the subject is selected for treatment with the antibody.

90. A method of identifying a human subject for treatment with an antibody that specifically binds RSPO3 comprising:
(a) obtaining a tumor sample from the subject, and
(b) determining if the tumor has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3,
wherein if the tumor has an elevated expression level of RSPO3 the subject is selected for treatment with the antibody.

91. A method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprising: determining if the subject has a tumor that has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3.

92. A method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprising:
(a) obtaining a tumor sample from the subject, and
(b) determining if the tumor has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3.

93. A method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprising: determining if the subject has a tumor that has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3, wherein if the tumor has an elevated expression level of RSPO3 the subject is selected for treatment with the antibody.

94. A method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprising:
(a) obtaining a tumor sample from the subject, and
(b) determining if the tumor has an elevated expression level of RSPO3 as compared to a reference sample or a pre-determined level of RSPO3,
wherein if the tumor has an elevated expression level of RSPO3 the subject is selected for treatment with the antibody.

95. A method of identifying a human subject for treatment with an antibody that specifically binds RSPO3 comprising: determining if the subject has a tumor that has a RSPO gene fusion.

96. A method of identifying a human subject for treatment with an antibody that specifically binds RSPO3 comprising:
(a) obtaining a tumor sample from the subject, and
(b) determining if the tumor has a RSPO gene fusion.

97. A method of identifying a human subject for treatment with an antibody that specifically binds RSPO3 comprising: determining if the subject has a tumor that has a RSPO gene fusion, wherein if the tumor has a RSPO gene fusion, then the subject is selected for treatment with the antibody.

98. A method of identifying a human subject for treatment with an antibody that specifically binds RSPO3 comprising:
(a) obtaining a tumor sample from the subject, and
(b) determining if the tumor has a RSPO gene fusion,
wherein if the tumor has a RSPO gene fusion, then the subject is selected for treatment with the antibody.

99. A method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprising: determining if the subject has a tumor that has a RSPO gene fusion.

100. A method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprising:
(a) obtaining a tumor sample from the subject, and
(b) determining if the tumor has a RSPO gene fusion.

101. A method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprising: determining if the subject has a tumor that has a RSPO gene fusion, wherein if the tumor has a RSPO gene fusion, then the subject is selected for treatment with the antibody.

102. A method of selecting a human subject for treatment with an antibody that specifically binds RSPO3, comprising:
(a) obtaining a tumor sample from the subject, and
(b) determining if the tumor has a RSPO gene fusion,
wherein if the tumor has a RSPO gene fusion, then the subject is selected for treatment with the antibody.

103. The method of any one of claims 95-102, wherein the RSPO gene fusion is a RSPO2 gene fusion.

104. The method of any one of claims 95-102, wherein the RSPO gene fusion is a RSPO3 gene fusion.

105. The method of any one of claims 87-104, wherein the antibody is the antibody of any one of claims 1-44.

106. The method of any one of claims 87-105, wherein the tumor is selected from the group consisting of colorectal tumor, colon tumor, ovarian tumor, pancreatic tumor, lung tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor.

107. The method of claim 106, wherein the tumor is a lung tumor.

108. The method of claim 106, wherein the tumor is a pancreatic tumor.

109. The method of claim 106, wherein the tumor is an ovarian tumor.

110. The method any one of claims 87-94 or 105-109, wherein the expression level of RSPO3 is determined by a PCR-based assay, microarray analysis, or immunohistochemistry.

111. The method of any one of claims 95-109, wherein the RSPO gene fusion is determined by a PCR-based assay, microarray analysis, or nucleotide sequencing.

112. The method of claim 111, wherein the nucleotide sequencing is NextGen sequencing or whole-genome sequencing.

113. The method of claim 88, 90, 92, 94, 96, 98, 100, or 102-109, wherein the sample is a fresh tumor sample, a frozen tumor sample, or a formalin-fixed paraffin-embedded sample.

Figure 1A

RSPO1

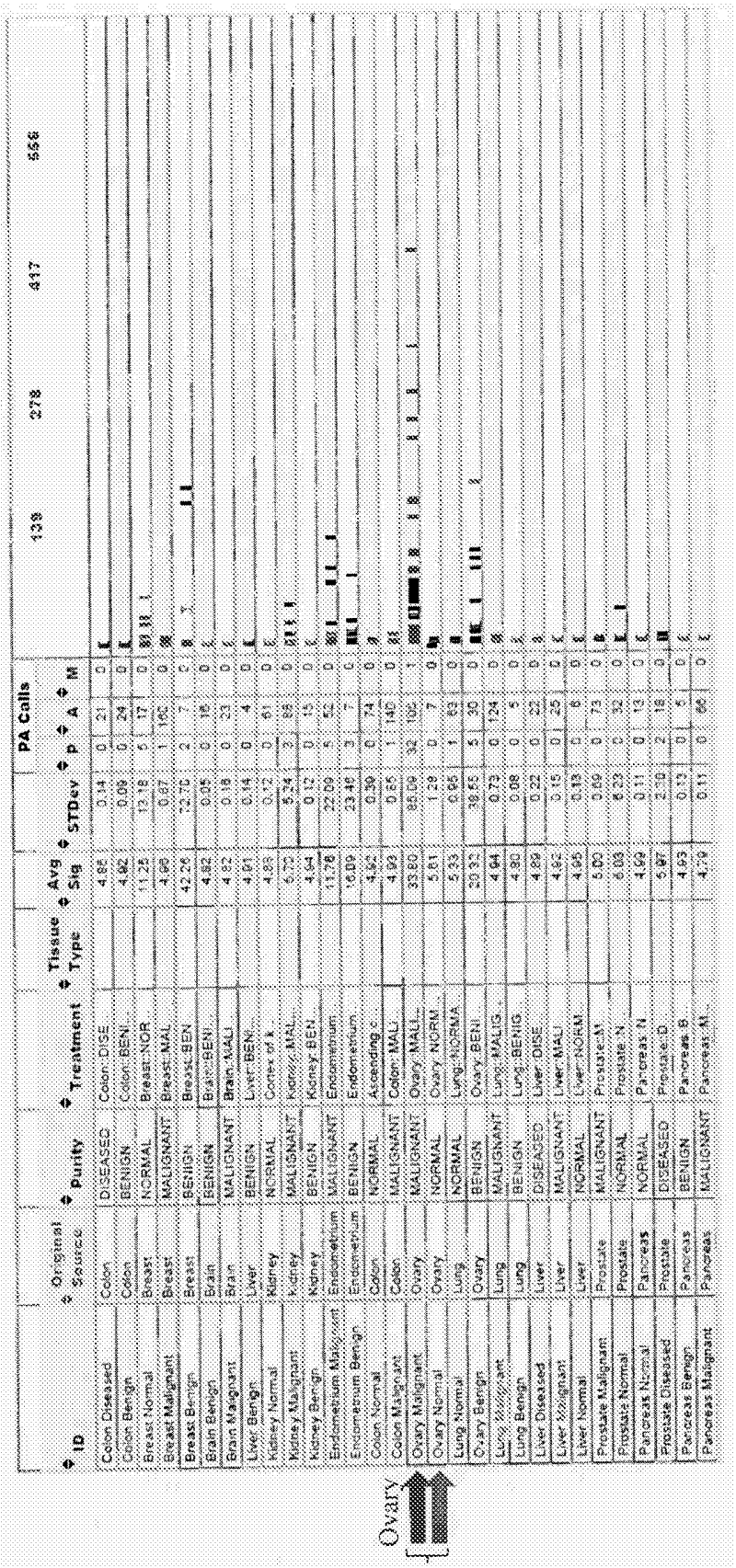


Figure 1B

RSPO2

ID	Original Source	Purity	Treatment	Tissue Type	Avg Sig	STDev	PA Calls P	A	M	152 304 456 609
Colon Diseased	Colon	DISEASED	Colon::DISE...		28.82	51.62	16	5	0	
Colon Benign	Colon	BENIGN	Colon::BENI...		7.40	6.58	3	21	0	
Breast Normal	Breast	NORMAL	Breast::NOR...		7.04	7.00	1	21	0	
Breast Malignant	Breast	MALIGNANT	Breast::MAL...		5.82	2.16	5	156	0	
Breast Benign	Breast	BENIGN	Breast::BEN...		5.59	0.12	1	8	0	
Brain Benign	Brain	BENIGN	Brain::BENI...		5.66	0.15	0	16	0	
Brain Malignant	Brain	MALIGNANT	Brain::MALI...		16.70	30.11	11	12	0	
Liver Benign	Liver	BENIGN	Liver::BENI...		6.00	0.43	0	4	0	
Kidney Normal	Kidney	NORMAL	Cortex of k...		5.76	0.22	0	61	0	
Kidney Malignant	Kidney	MALIGNANT	Kidney::MAL...		5.61	0.20	1	90	0	
Kidney Benign	Kidney	BENIGN	Kidney::BEN...		18.30	34.71	5	10	0	
Endometrium Malignant	Endometrium	MALIGNANT	Endometrium...		6.31	3.17	7	49	1	
Endometrium Benign	Endometrium	BENIGN	Endometrium...		5.54	0.10	0	10	0	
Colon Normal	Colon	NORMAL	Ascending c...		14.65	25.57	43	28	3	
Colon Malignant	Colon	MALIGNANT	Colon::MALI...		6.67	11.61	10	130	1	
Ovary Malignant	Ovary	MALIGNANT	Ovary::MALI...		10.33	50.15	7	131	0	
Ovary Normal	Ovary	NORMAL	Ovary::NORM...		5.64	0.14	0	7	0	
Lung Normal	Lung	NORMAL	Lung::NORMA...		16.18	14.17	53	11	0	
Ovary Benign	Ovary	BENIGN	Ovary::BENI...		5.66	0.41	4	21	0	
Lung Malignant	Lung	MALIGNANT	Lung::MALIG...		7.00	5.27	26	95	2	
Lung Benign	Lung	BENIGN	Lung::BENIG...		5.61	0.15	0	5	0	
Liver Diseased	Liver	DISEASED	Liver::DISE...		6.30	2.71	1	21	0	
Liver Malignant	Liver	MALIGNANT	Liver::MALI...		5.91	0.42	0	24	1	
Liver Normal	Liver	NORMAL	Liver::NORM...		5.96	0.27	0	6	0	
Prostate Malignant	Prostate	MALIGNANT	Prostate::M...		18.11	61.02	40	31	2	
Prostate Normal	Prostate	NORMAL	Prostate::N...		18.65	23.89	22	9	1	
Pancreas Normal	Pancreas	NORMAL	Pancreas::N...		5.98	0.23	0	13	0	
Prostate Diseased	Prostate	DISEASED	Prostate::D...		23.67	18.90	17	3	0	
Pancreas Benign	Pancreas	BENIGN	Pancreas::B...		156.95	337.57	1	4	0	
Pancreas Malignant	Pancreas	MALIGNANT	Pancreas::M...		5.57	0.17	1	65	0	

Figure 1C

RSPO3

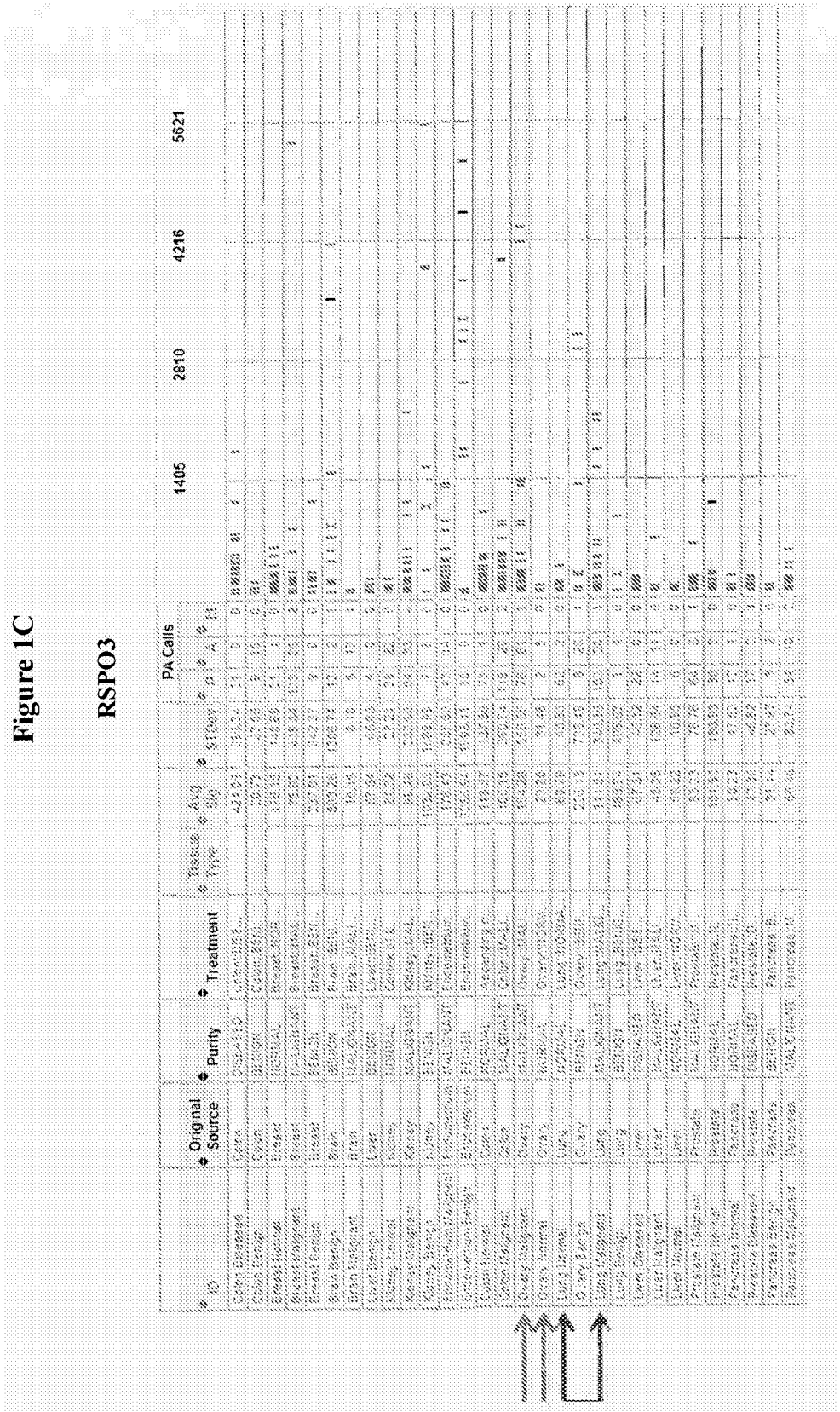


Figure 2

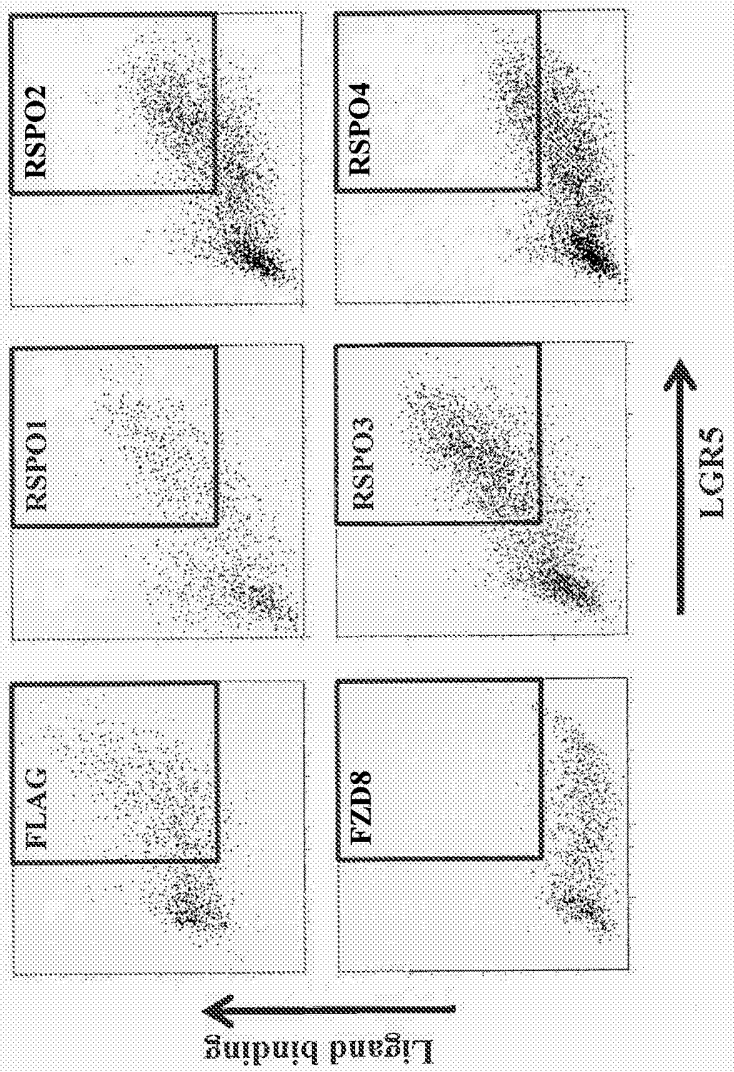


Figure 3

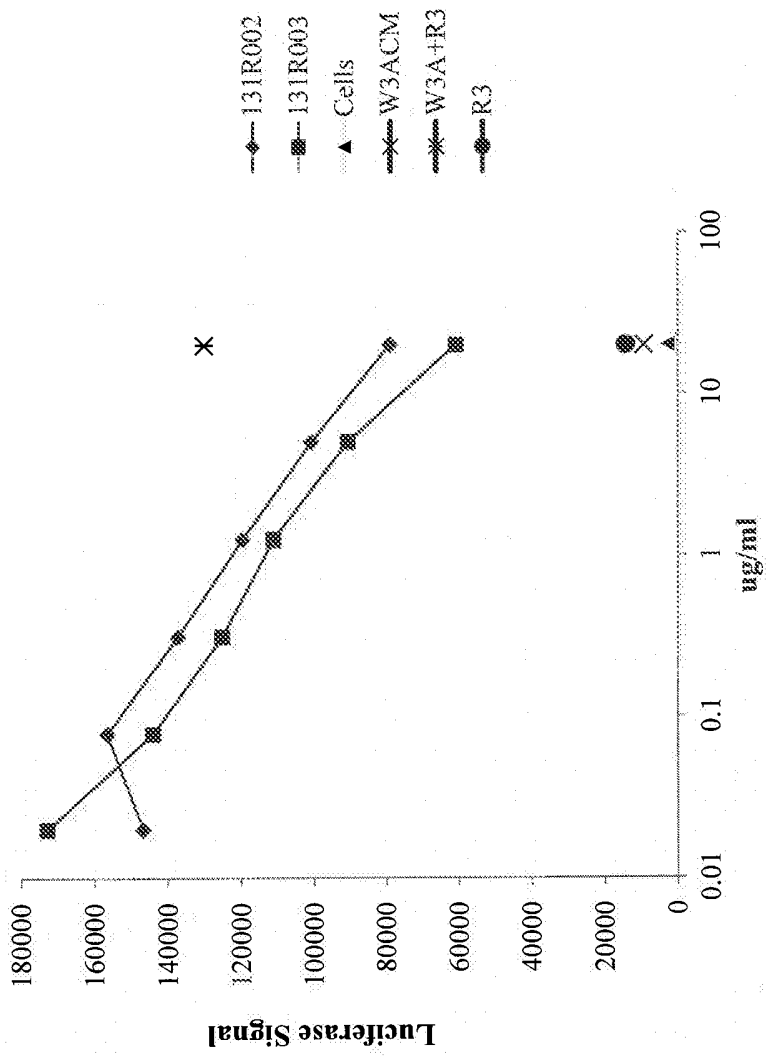

131R002
131R003
Cells
W3ACM
W3A+R3
R3
Luciferase Signal
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0.01
0.1
1
10
100
ug/ml

Figure 4

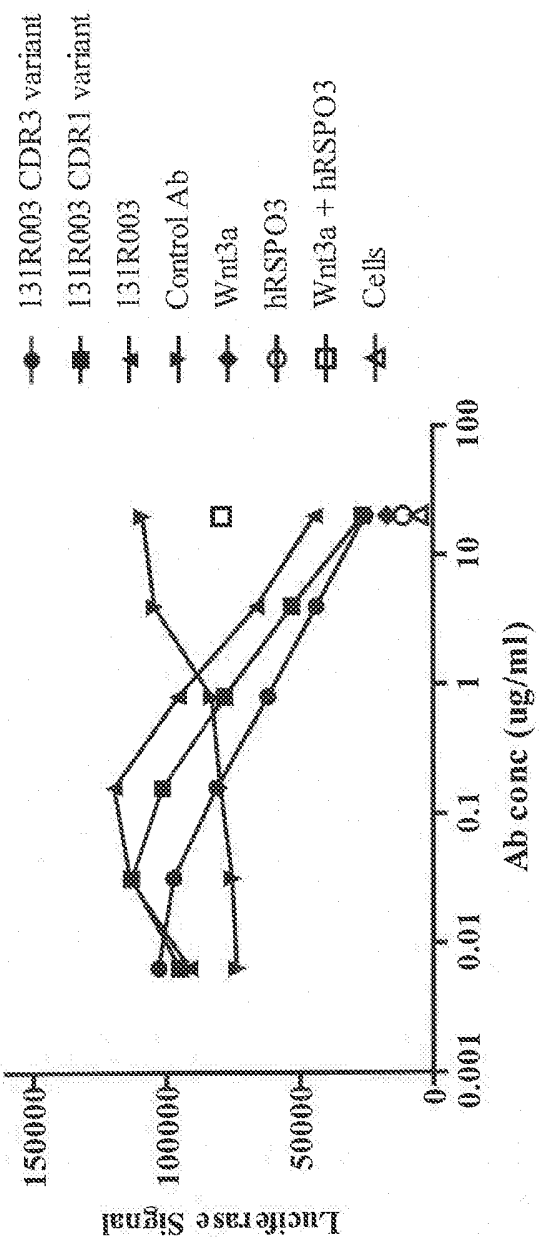

131R003 CDR3 variant
131R003 CDR1 variant
131R003
Control Ab
Wnt3a
hRSPO3
Wnt3a + hRSPO3
Cells
Luciferase Signal
150000
100000
50000
0
0.001
0.01
0.1
1
10
100
Ab conc (ug/ml)

Figure 5

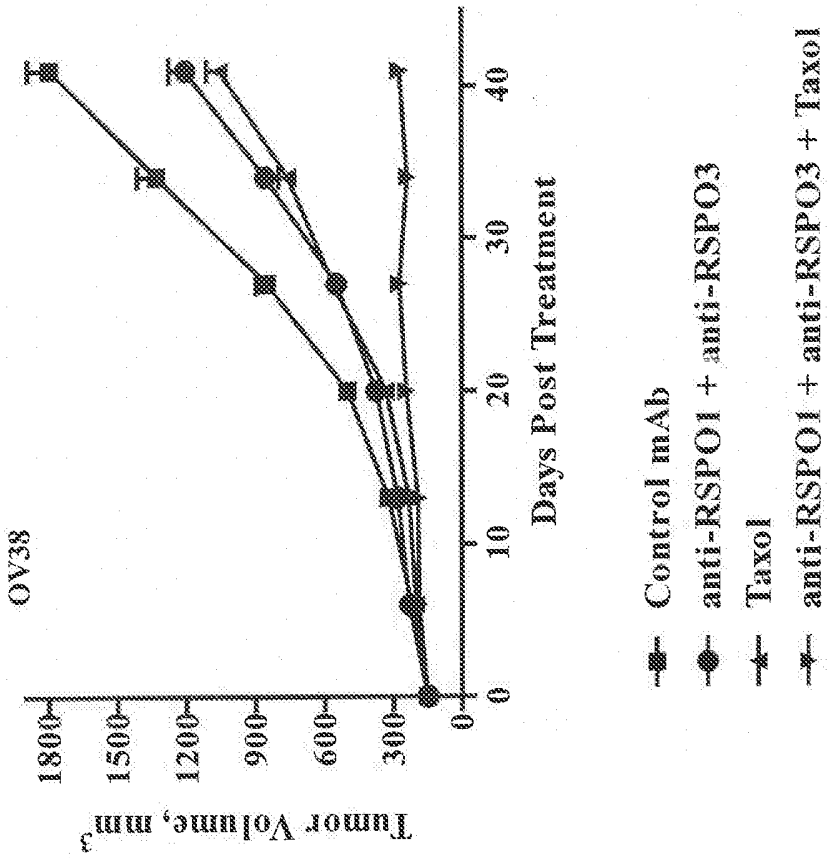

OV38
Tumor Volume, mm3
1800
1500
1200
900
600
300
0
0
10
20
30
40
Days Post Treatment
Control mAb
anti-RSPO1 + anti-RSPO3
Taxol
anti-RSPO1 + anti-RSPO3 + Taxol

Figure 6

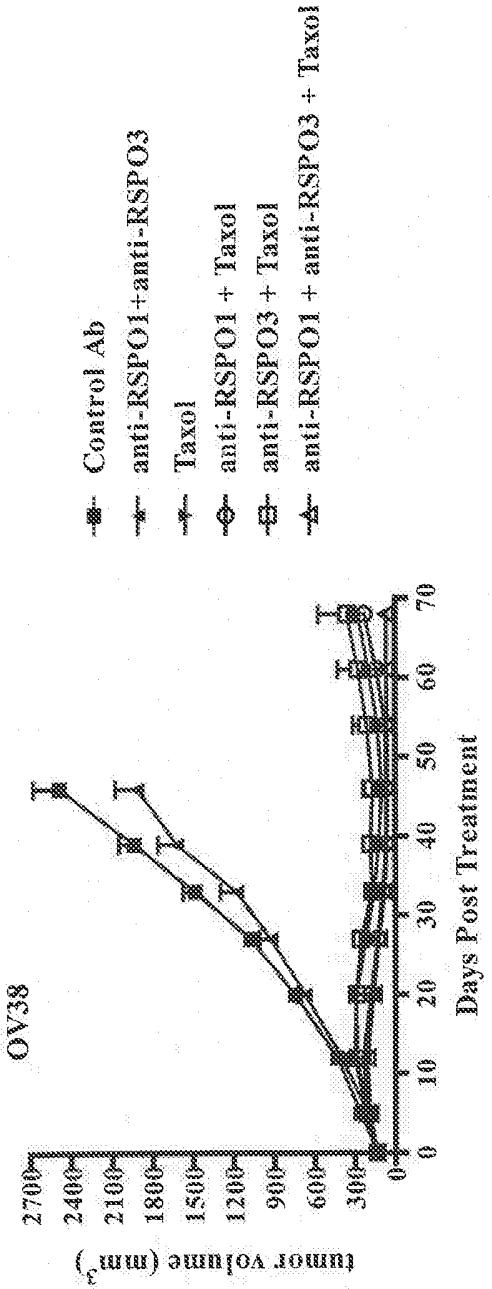

OV38
tumor volume (mm3)
2700
2400
2100
1800
1500
1200
900
600
300
0
0
10
20
30
40
50
60
70
Days Post Treatment
Control Ab
anti-RSPO1+anti-RSPO3
Taxol
anti-RSPO1 + Taxol
anti-RSPO3 + Taxol
anti-RSPO1 + anti-RSPO3 + Taxol

Figure 7

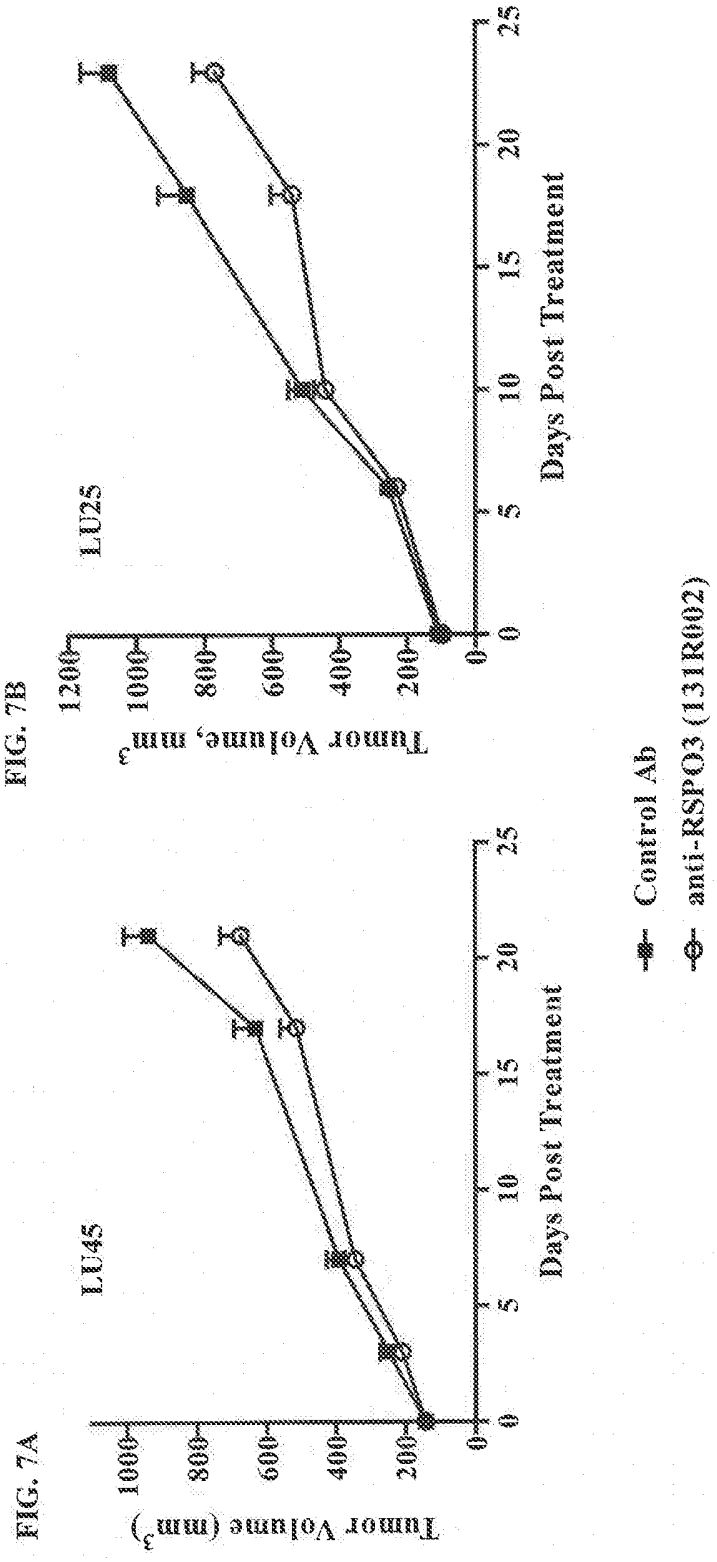


FIG. 7A
LU45
Tumor Volume (mm³)
1000
800
600
400
200
0
0
5
10
15
20
25
Days Post Treatment
FIG. 7B
LU25
Tumor Volume, mm³
1200
1000
800
600
400
200
0
0
5
10
15
20
25
Days Post Treatment
Control Ab
anti-RSPO3 (131R002)

Figure 8

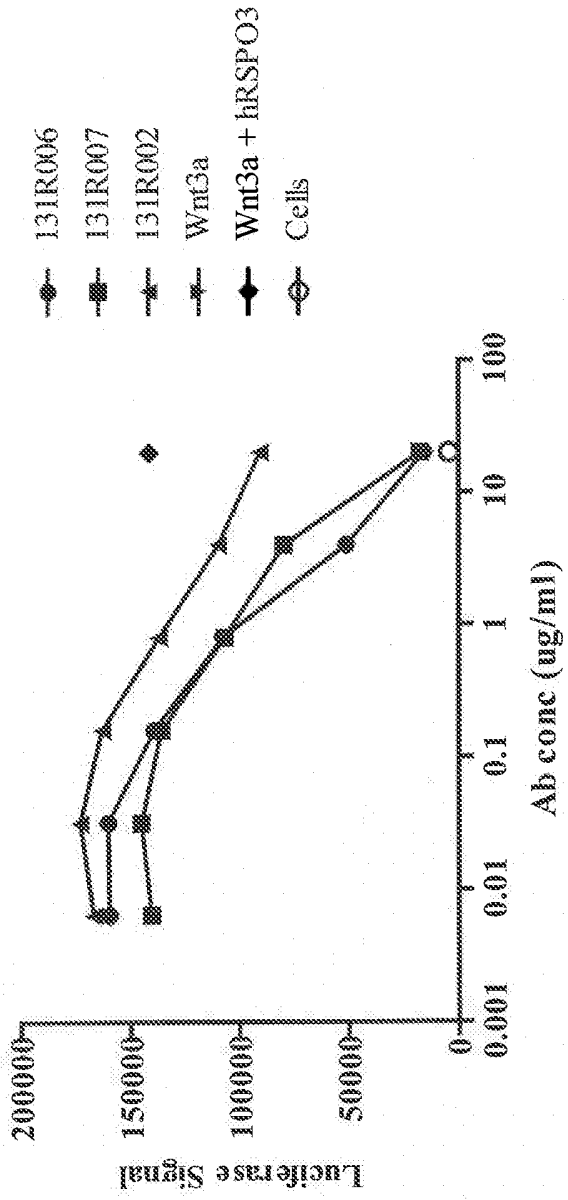

131R006
131R007
131R002
Wnt3a
Wnt3a + hRSPO3
Cells
Luciferase Signal
200000
150000
100000
50000
0
0.001
0.01
0.1
1
10
100
Ab conc (ug/ml)

Figure 9

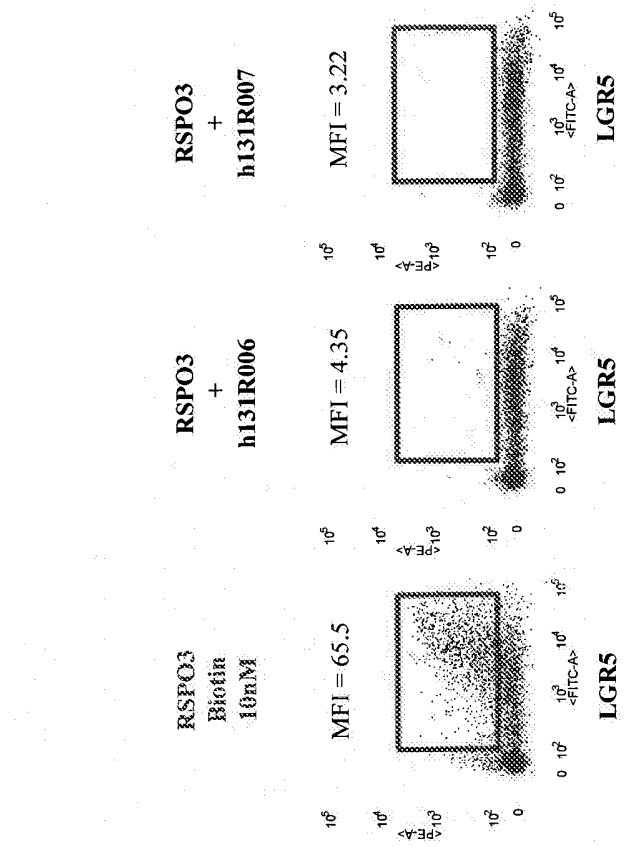

RSPO3
Biotin
10nM
MFI = 65.5
LGR5
RSPO3
+
h131R006
MFI = 4.35
LGR5
RSPO3
+
h131R007
MFI = 3.22
LGR5
<PE-A>
<FITC-A>

Figure 10

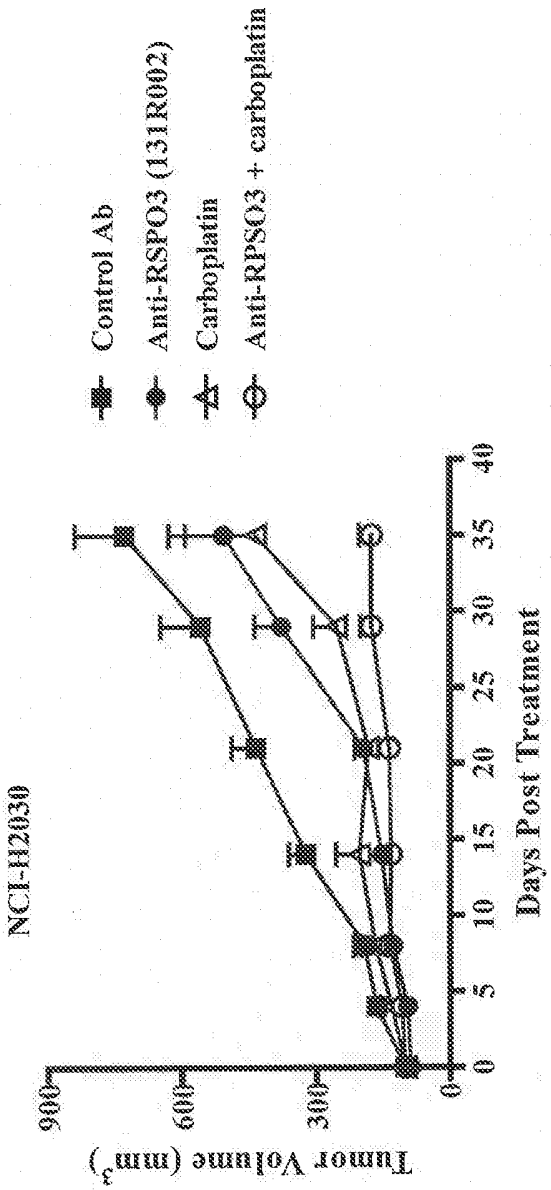

NCI-H2030
Control Ab
Anti-RSPO3 (131R002)
Carboplatin
Anti-RPSO3 + carboplatin
Tumor Volume (mm3)
900
600
300
0
0
5
10
15
20
25
30
35
40
Days Post Treatment

Figure 11

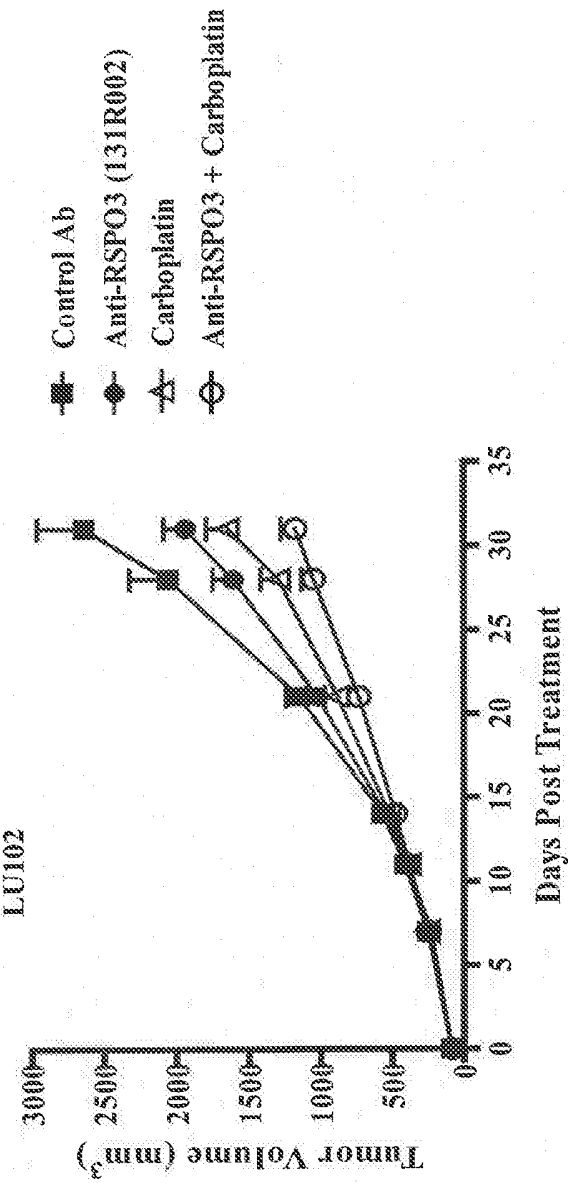


FIG. 11A

Figure 11

FIG. 11B

Anti-RSPO3 antibody as single agent

Geneset	SIZE	p-val
OMP_DLL4_UP	88	0.00E+00
WEINBERG_ES_1	371	7.30E-03
OMP_NEWCSC_UP	66	8.32E-03
CURATED_TGFB	200	2.23E-02
CURATED_STEMCELL	280	2.48E-02

Carboplatin as single agent

Geneset	SIZE	p-val
ASSOU_ESC_DN	69	1.43E-03

Anti-RSPO3 antibody + Carboplatin

Geneset	SIZE	p-val
BATLLE_HU_PROLIFERATION	184	0.00E+00
TIAN_GBM_CD133_UP	83	0.00E+00
NEVINS_CSR	85	0.00E+00
OMP_CD201+_HIGH	240	0.00E+00
WONG_EMBRYONIC_STEM_CELL_CORE	329	0.00E+00
WEINBERG_PROLIFERATION	147	0.00E+00
WEINBERG_ES_1	371	0.00E+00
RICKMAN_TUMOR_DIFFERENTIATED_W	106	0.00E+00
WEINBERG_OCT4_TARGETS	286	1.33E-02
MILANO_GSI_RAT_DN	57	1.66E-02
WEINBERG_ES_2	35	2.34E-02
PN_CD201_LOGIT18	18	4.71E-02

Figure 12

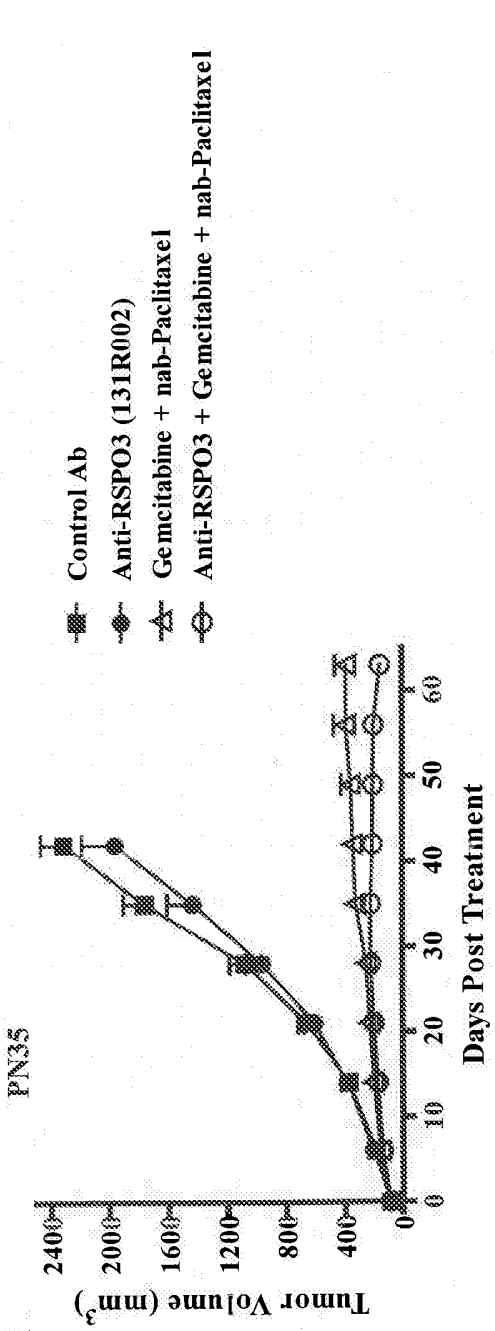


FIG. 12A

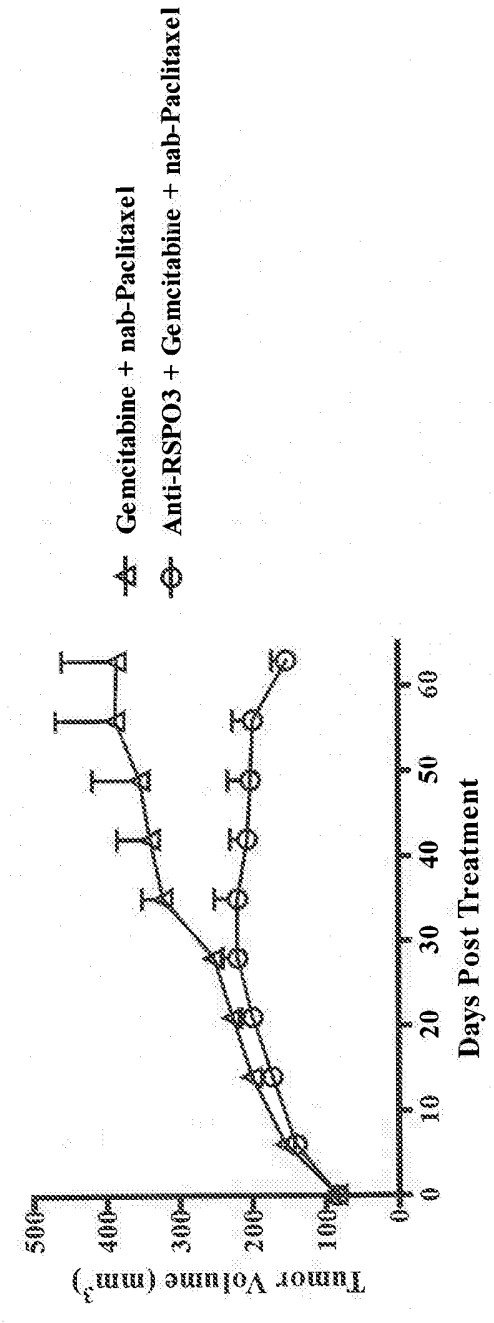


FIG. 12B

Figure 13

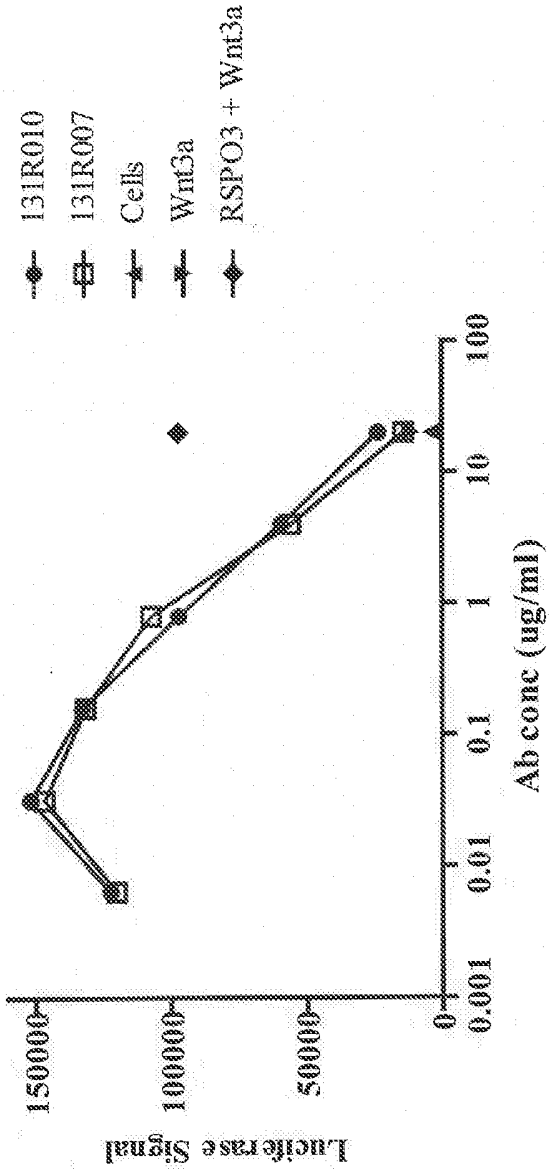
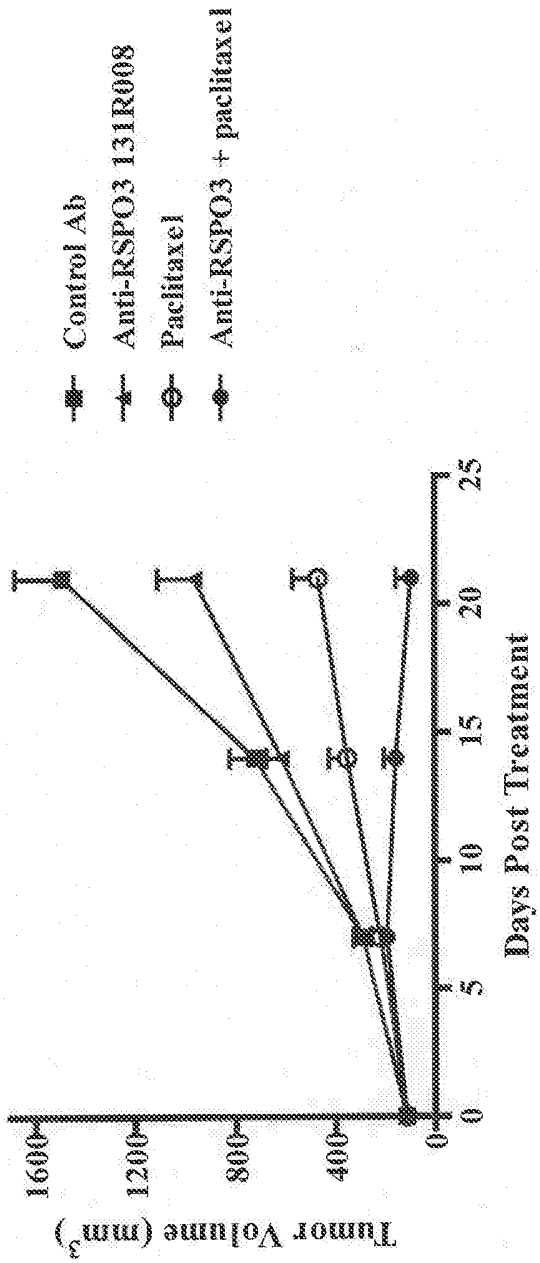

131R010
131R007
Cells
Wnt3a
RSPO3 + Wnt3a
Luciferase Signal
150000
100000
50000
0
0.001
0.01
0.1
1
10
100
Ab conc (ug/ml)

Figure 14


Control Ab
Anti-RSPO3 131R008
Paclitaxel
Anti-RSPO3 + paclitaxel
Tumor Volume (mm3)
1600
1200
800
400
0
0
5
10
15
20
25
Days Post Treatment