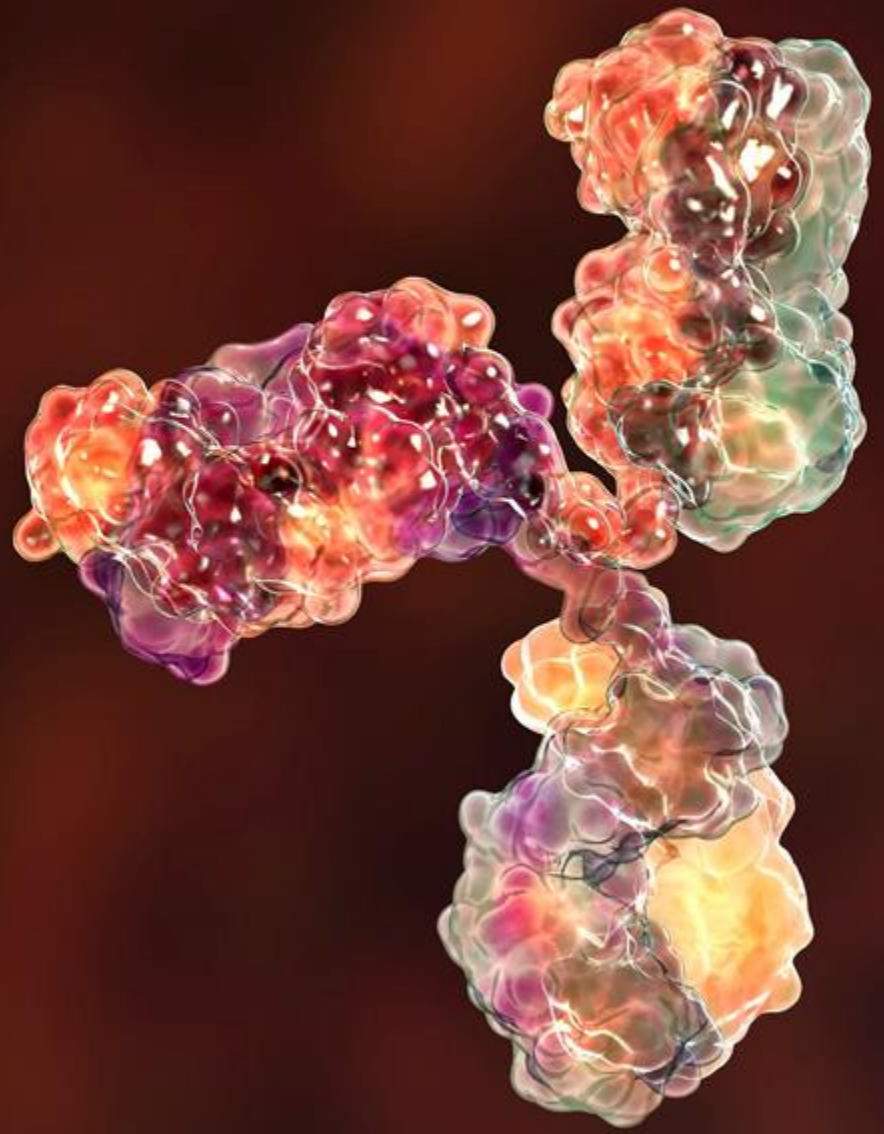
Silence is golden:
The importance of attenuating effector functions in therapeutic antibodies

Ian Wilkinson, Chief Scientific Officer



Background

- Antibodies are nature's pro-drugs, wonderfully evolved to target pathogens and activate immune systems
- Engagement of the antibody Fc domain with Fc receptors or complement results in activation of the immune system and targeted cell death
- For oncology indications this is ideal, in fact efforts have been made to enhance so-called Fc effector function
- However, approximately 50% of therapeutic antibodies block an interaction rather than kill a cell
- Activation of the immune system and inflammatory response highly undesirable in these cases

Fc silencing

- IgG subtypes vary in their binding to Fc receptors and C1q – human IgG4 naturally has lower effector function than IgG1
- IgG4 often used as the preferred subtype when ADCC/CDC not required
- Alternatively the Fc domain can be engineered
- Most commonly used backbones in order are:
  - IgG4 (wild type or S228P)
  - IgG1 LALA (L234A/L235A)
  - IgG1 aglycosylated (N297A)
  - IgG4 FALA (F234A/L235A)
  - IgG4 SPLE (S228P/L235E)

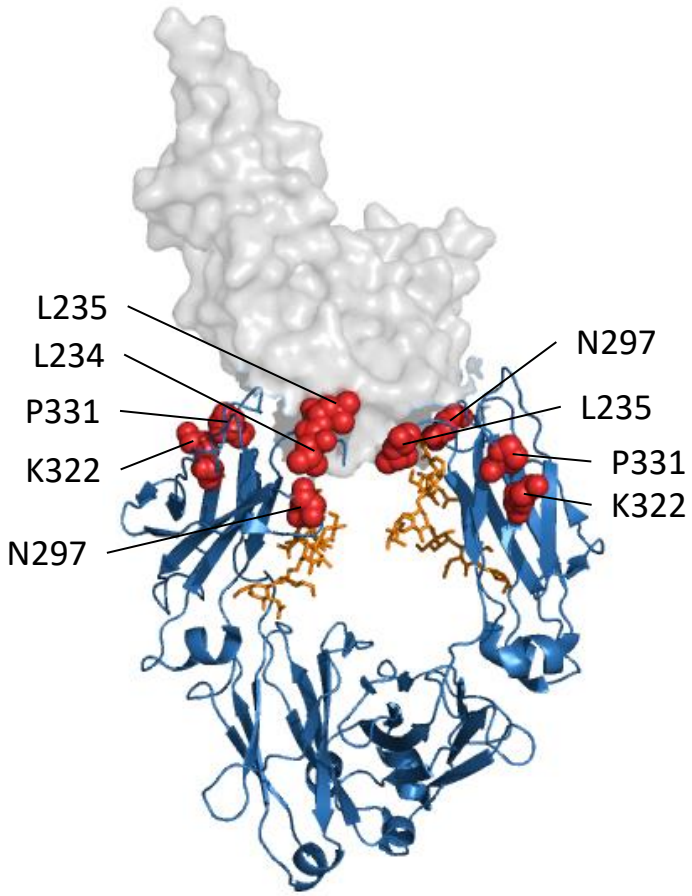


Image from Absolute Antibody website: https://absoluteantibody.com/antibody-resources/antibody-engineering/fc-engineering/

Cytokine storm

- 2006 phase 1 trial of TGN1412, anti-CD28 mAb
- Human IgG4 antibody, thought to be silent
- Caused immediate and severe cytokine release syndrome (CRS)
- All 6 patients had multiple organ failure

Fc not so silent

mAbsolve

- A few examples from the work our founders/advisors have been involved in
- These antibodies are not silent!

Aglycosylated IgG1

Keymeulen, B. et al. *The New England Journal of Medicine* 11 (2005).

- Cytokine release and adverse infusion related events
- Moderate flu-like syndrome
- EBV reactivation

IgG1-LALA

Herold, K. C. et al. *The New England Journal of Medicine* 7 (2002).

- Cytokine release
- Rash

IgG4

Isaacs, J. D. et al. *Clin Exp Immunol* **106**, 427–433 (1996).

- Target cell depletion by IgG1 and IgG4
- Cytokine release and first dose reactions with IgG1 and IgG4

Fc silencing in the clinic

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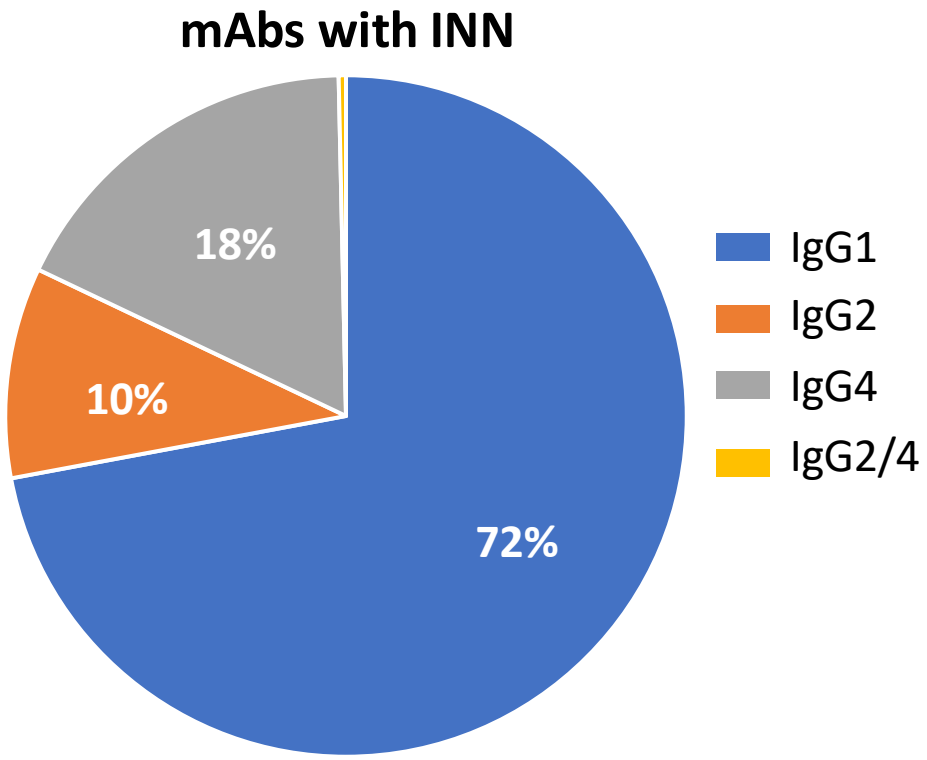
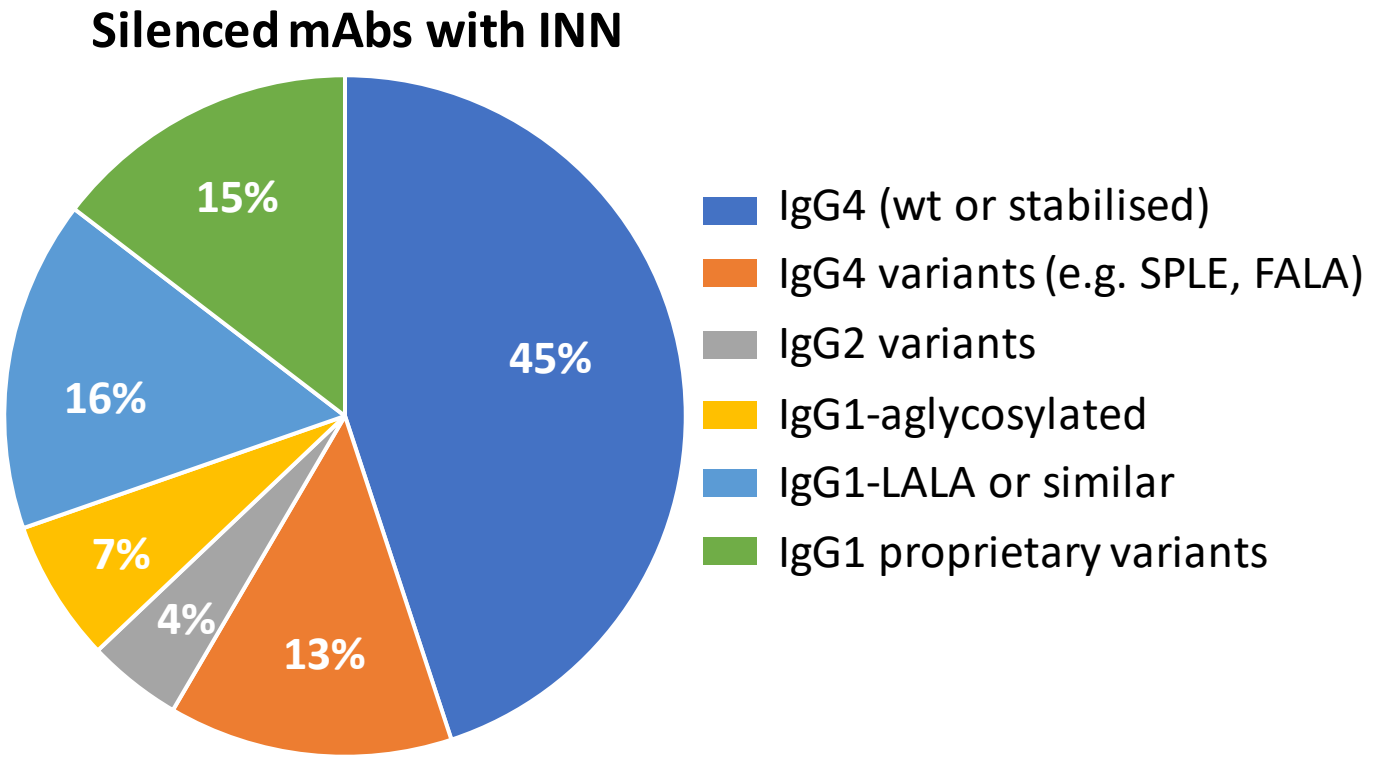
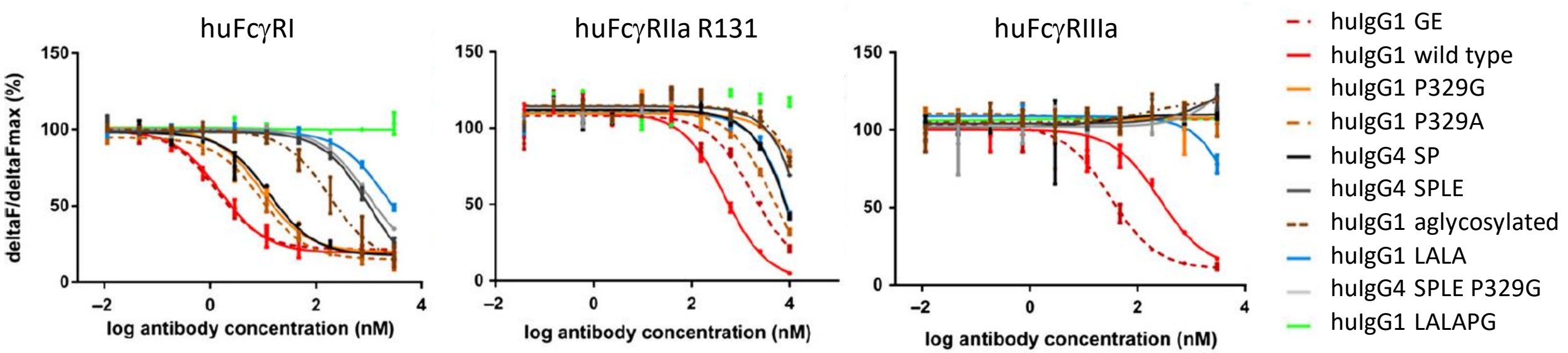




- 31% of all antibodies with a designated INN are silenced
  - 25% of approved mAbs are silenced
  - 33% of clinical phase mAbs are silenced
- A strong preference for IgG4 as well as other IP-free options (LALA and aglycosylated)

LALAPG publication

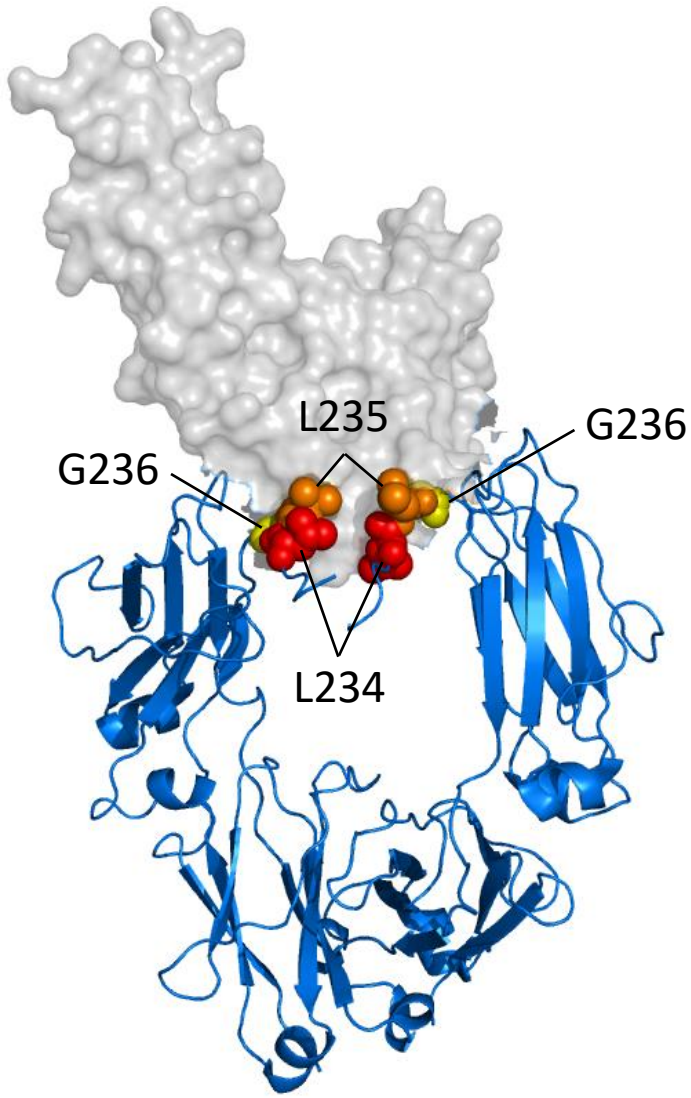


Schlothauer, T. *et al.* Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. *Protein Eng Des Sel* **29**, 457–466 (2016).

- ★First broad comparison of many of the commonly used IP-free silencing mutations
- ★Clearly demonstrated inferiority of these mutations compared to LALAPG
- ★Could we generate an alternative to LALAPG….?

Rational Fc engineering

- Positions L234, L235 and G236 known to be important for FcγR binding
- Preliminary data suggested that triple mutants substantially reduced binding and that Arginine at 236 was particularly important
- L234X/L235X/G236R gives 361 possible combinations
- Analysed all sequences in silico for liabilities including immunogenicity, resulting in 152 acceptable combinations



Binding to human FcγRI (CD64) by SPR

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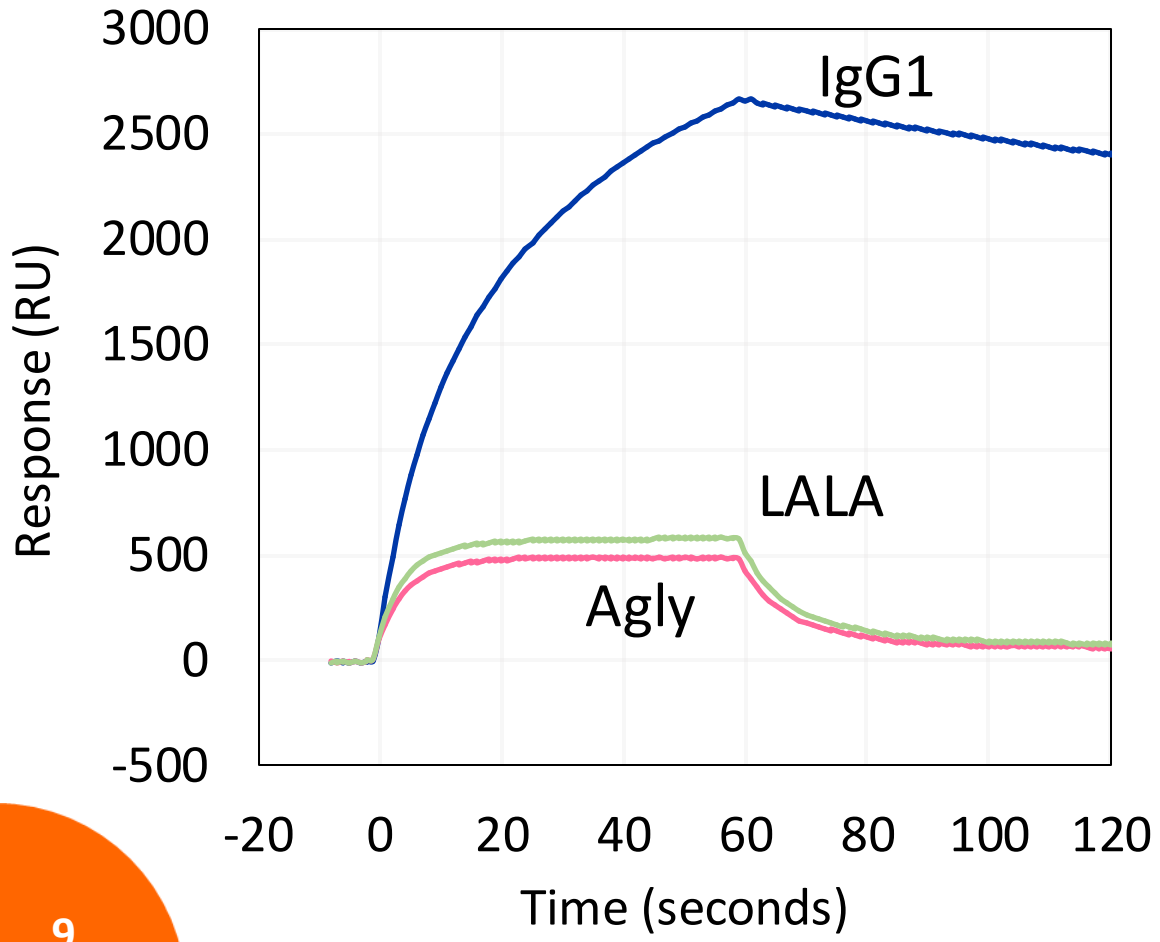
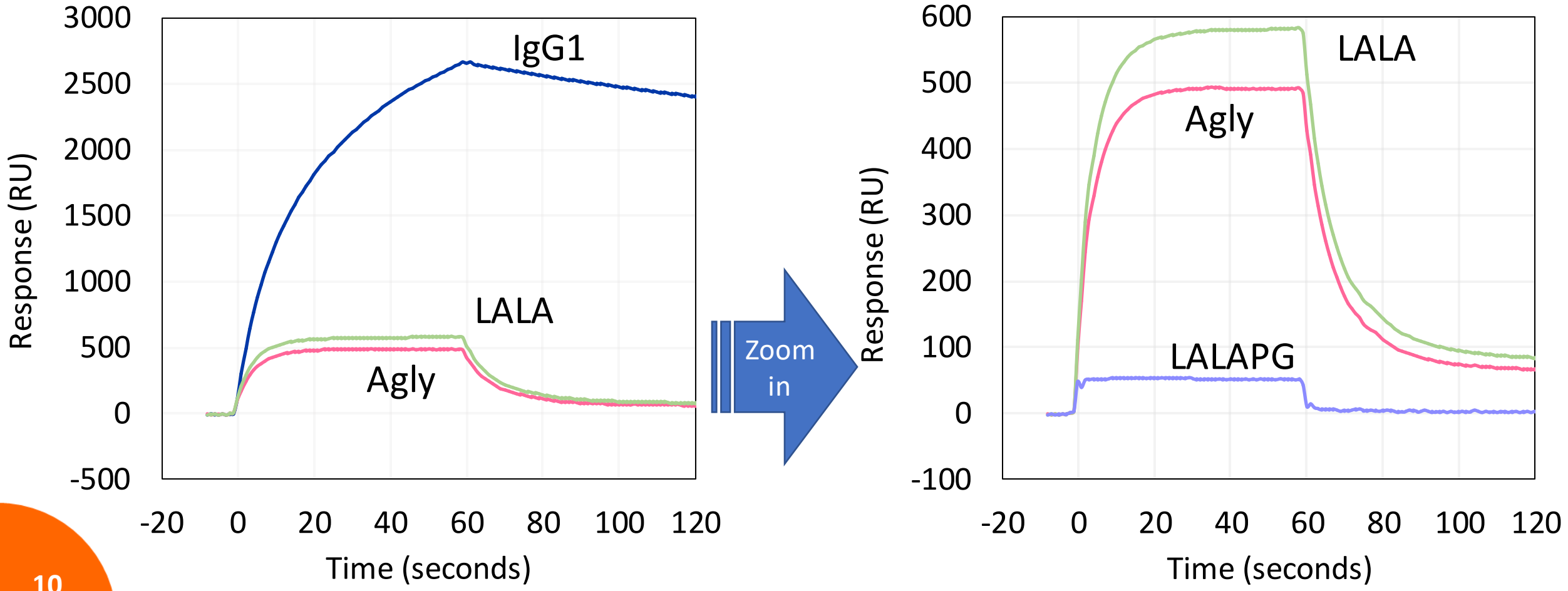
★LALA and Agly retain relatively high levels of binding



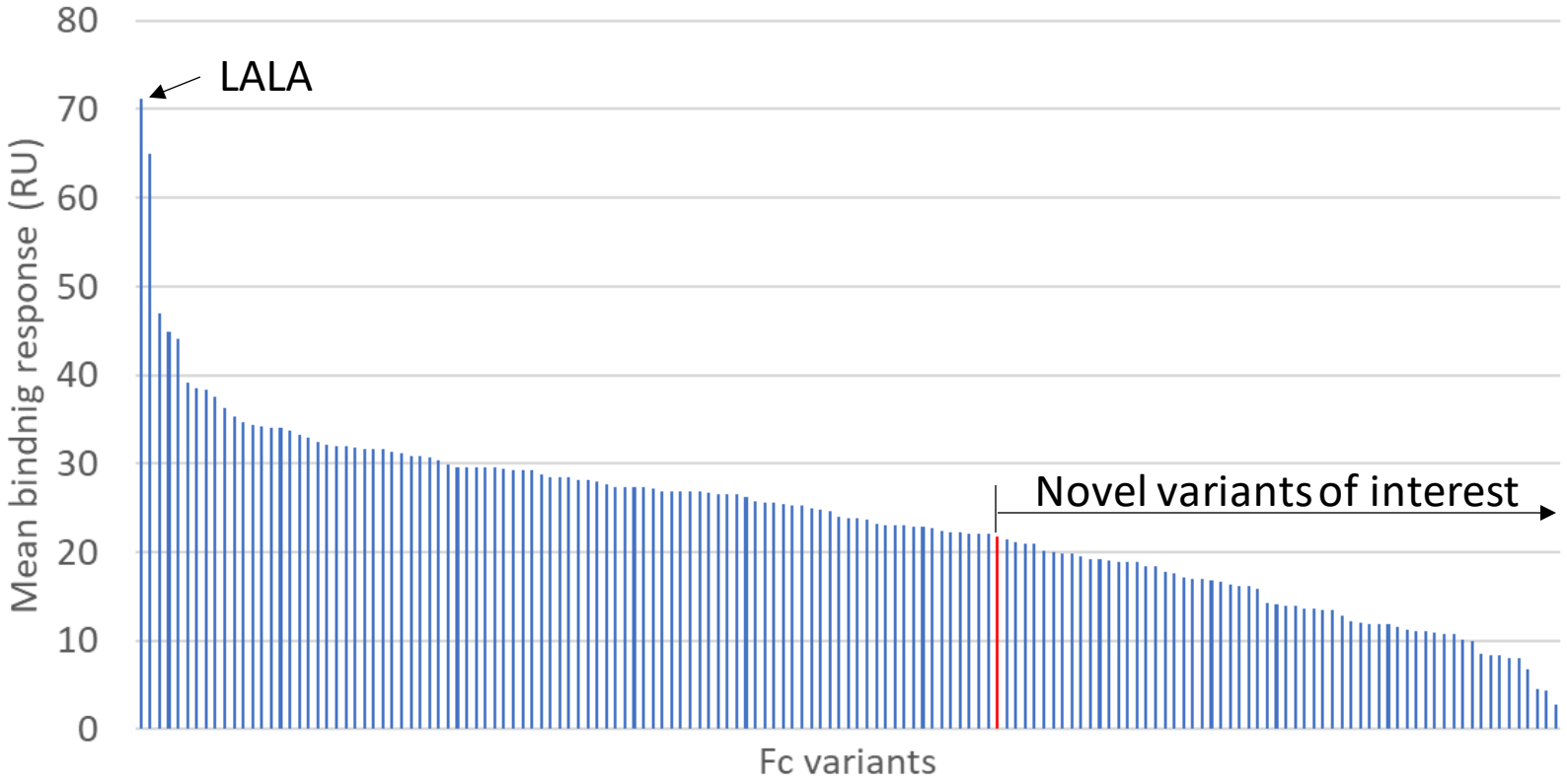
Binding to human FcγRI (CD64) by SPR

- LALA and Agly retain relatively high levels of binding
- Even the gold standard LALAPG shows residual binding



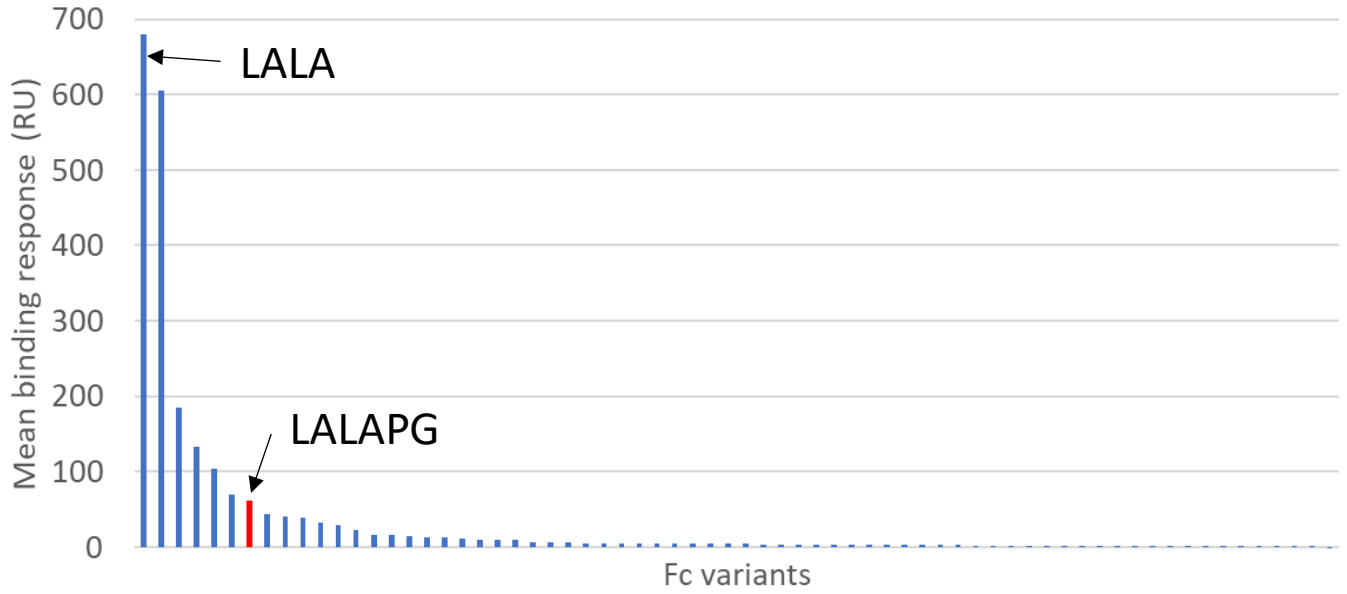
Preliminary screening

- Supernatant of 152 variant Fcs assessed for binding to human FcγRI
- All variants had lower binding than LALA
- Threshold value of 21.0 set and 61 variants carried forward to more in depth analysis



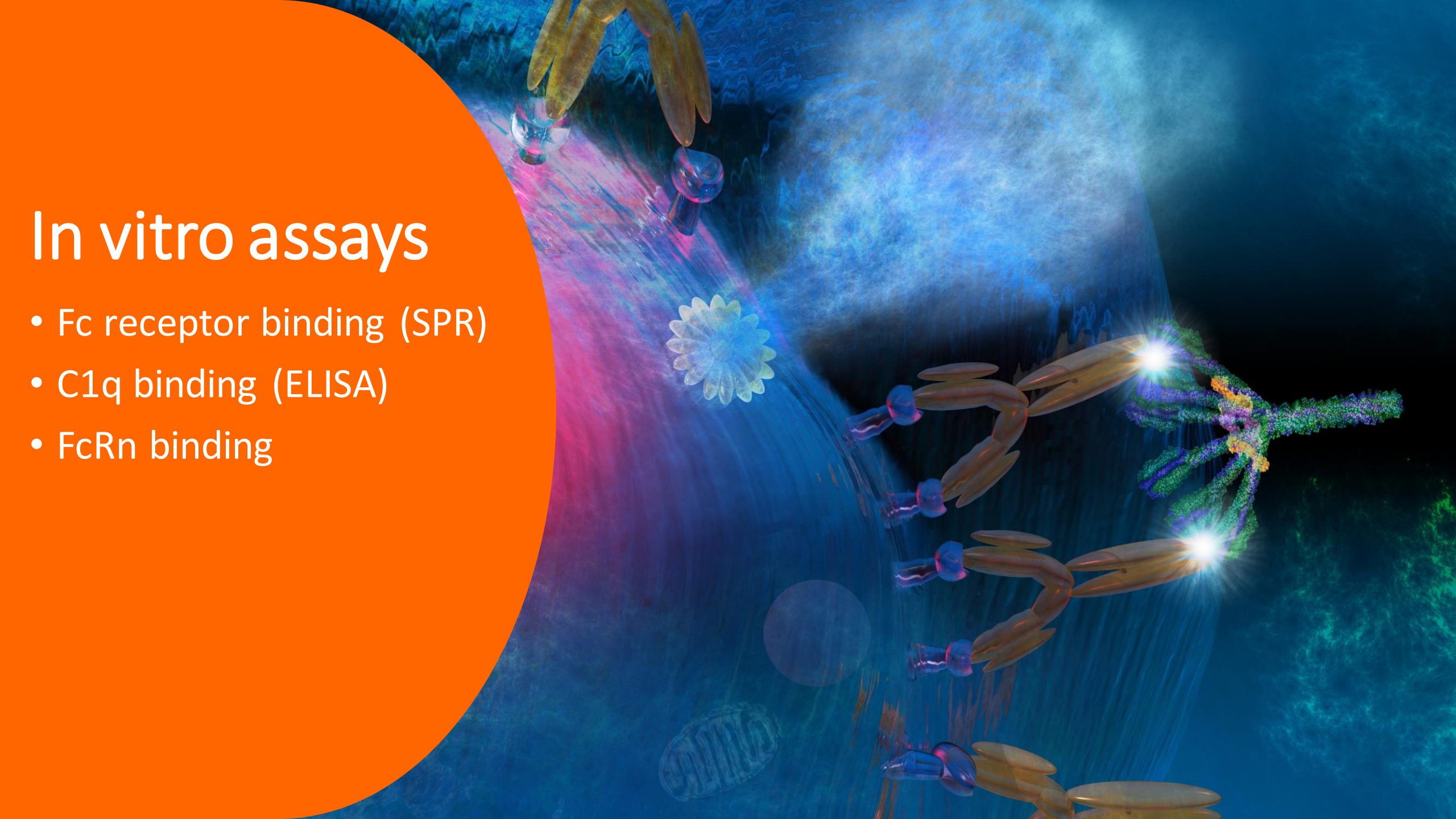
Secondary screening

- Antibodies purified and assessed for binding to human FcγRI
- 56 variants gave significantly lower binding than LALAPG
- Many variants indistinguishable from baseline noise
- A panel of variants selected for further analysis: in vitro binding to all FcRs; cell based assays; thermal stability
- L234**S**/L235**T**/G236**R** selected as optimal mutation for silencing



In vitro assays

- Fc receptor binding (SPR)
- C1q binding (ELISA)
- FcRn binding



Binding to human FcγRI (CD64) by SPR

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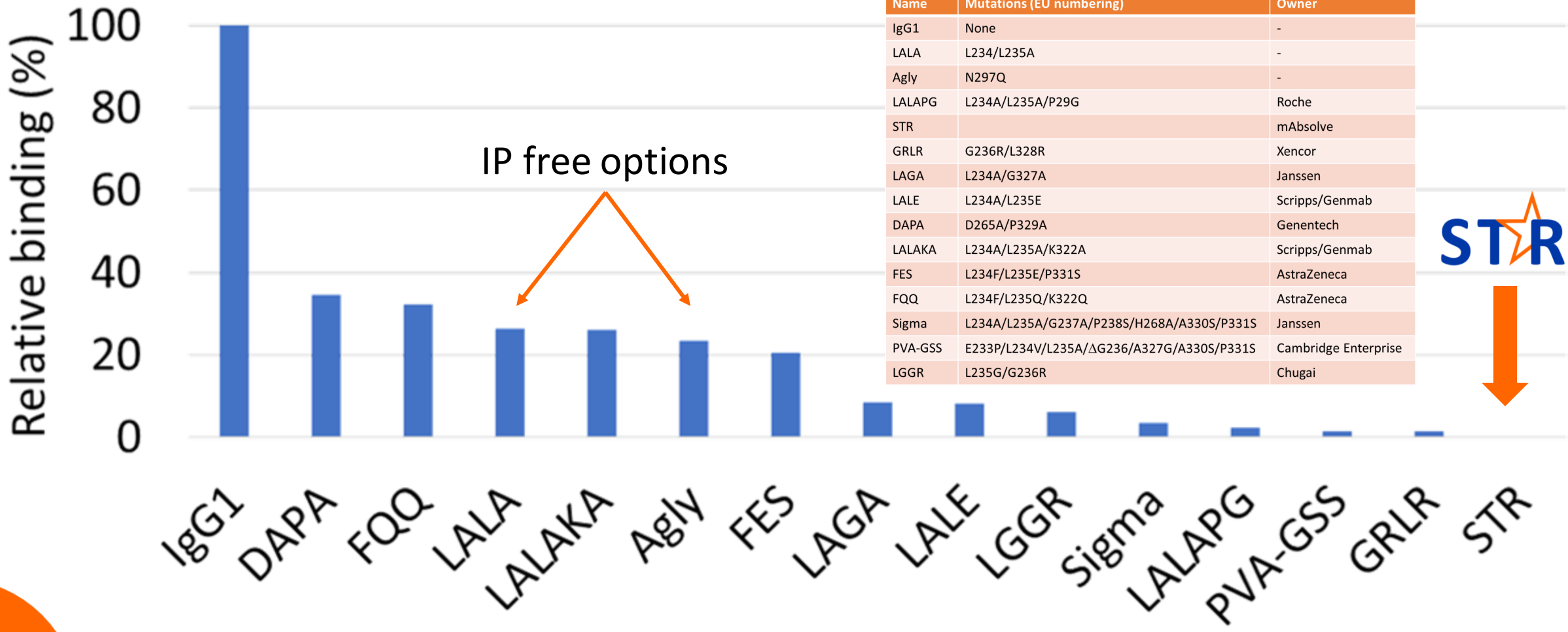
★STR is the only truly silent mutation



Name	Mutations (EU numbering)	Owner
IgG1	None	-
LALA	L234/L235A	-
Agly	N297Q	-
LALAPG	L234A/L235A/P29G	Roche
STR		mAbsolve
GRLR	G236R/L328R	Xencor
LAGA	L234A/G327A	Janssen
LALE	L234A/L235E	Scripps/Genmab
DAPA	D265A/P329A	Genentech
LALAKA	L234A/L235A/K322A	Scripps/Genmab
FES	L234F/L235E/P331S	AstraZeneca
FQQ	L234F/L235Q/K322Q	AstraZeneca
Sigma	L234A/L235A/G237A/P238S/H268A/A330S/P331S	Janssen
PVA-GSS	E233P/L234V/L235A/ΔG236/A327G/A330S/P331S	Cambridge Enterprise
LGGR	L235G/G236R	Chugai

Binding to human FcγRI (CD64) by SPR

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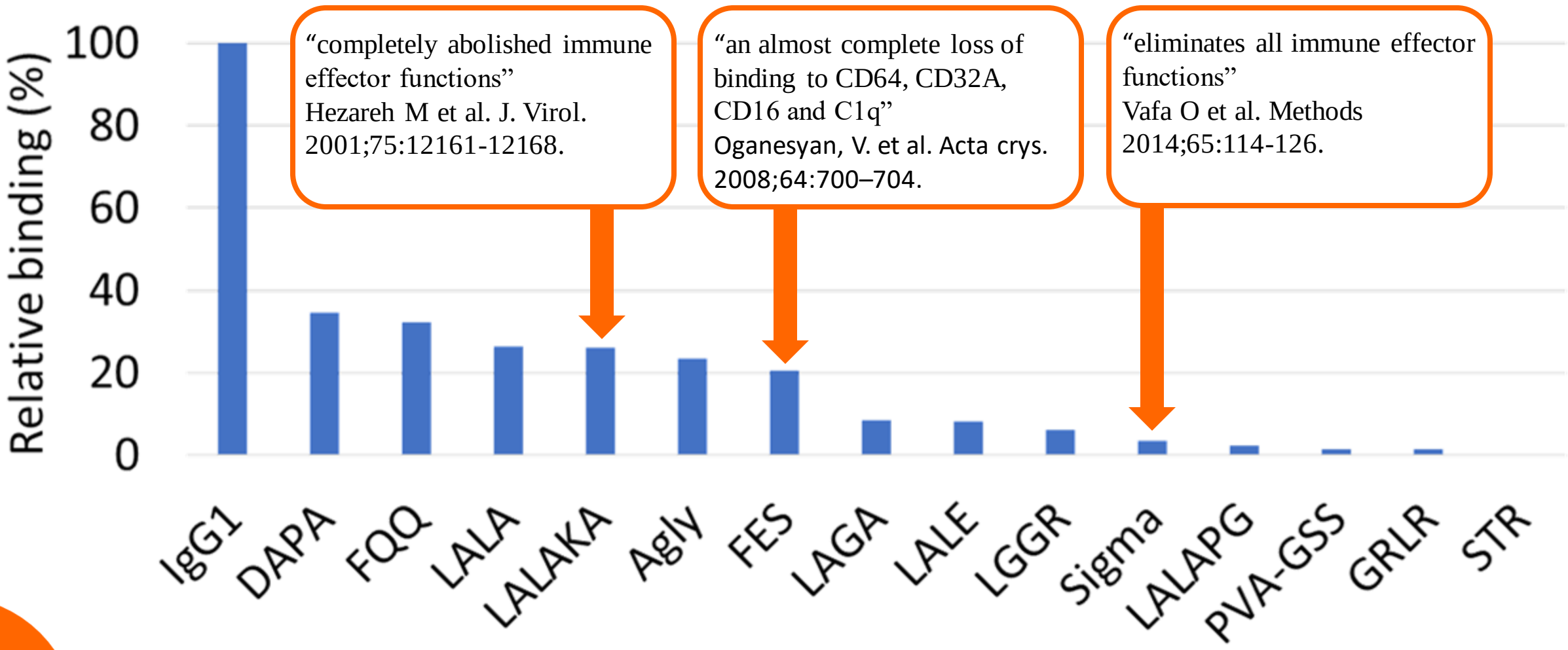
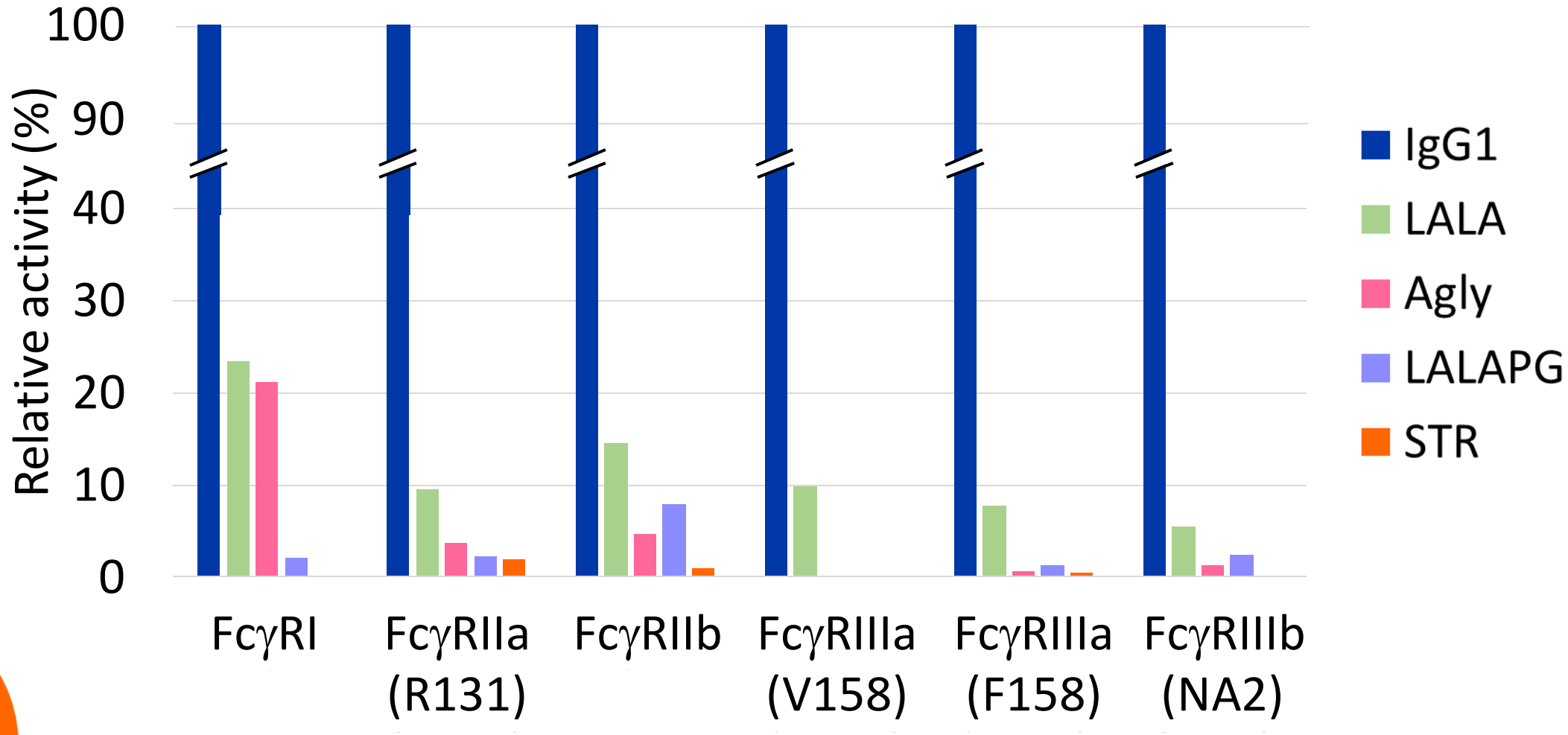


Binding to all human FcγRs by SPR

mAbsolve

- STR confirmed as silent on all FcγRs
- Data shows human FcγRs, we have also tested mouse, rat, rabbit, cyno



C1q binding ELISA

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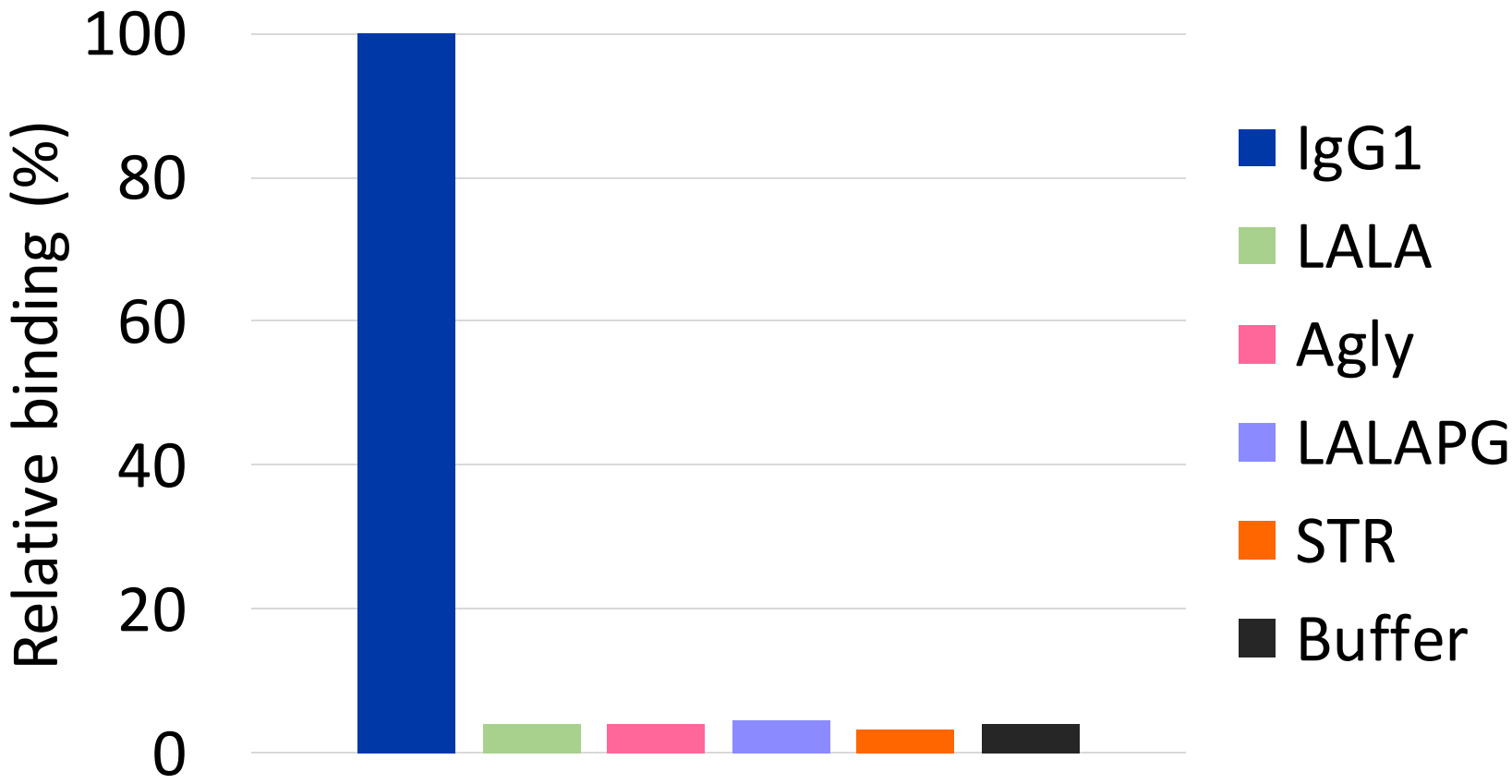
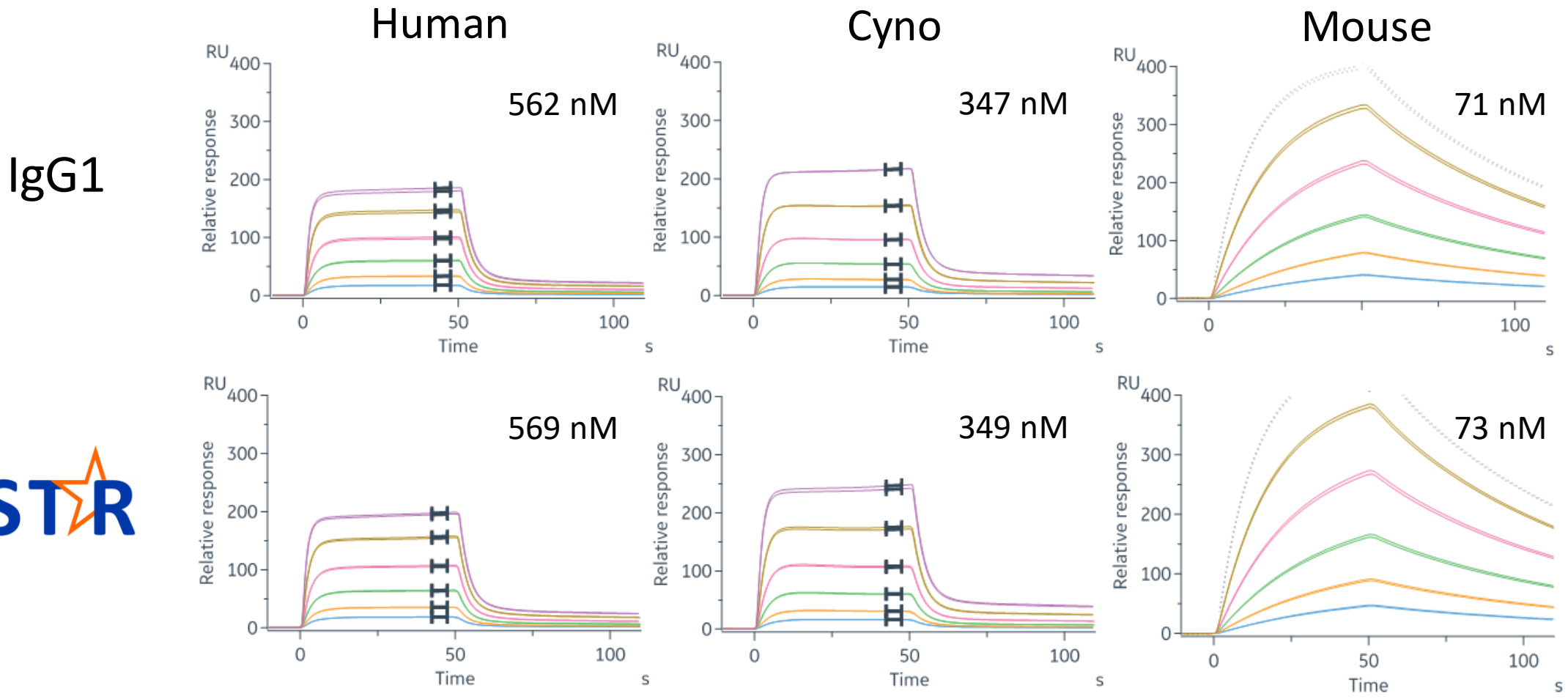
★All silencing variants show no binding to C1q



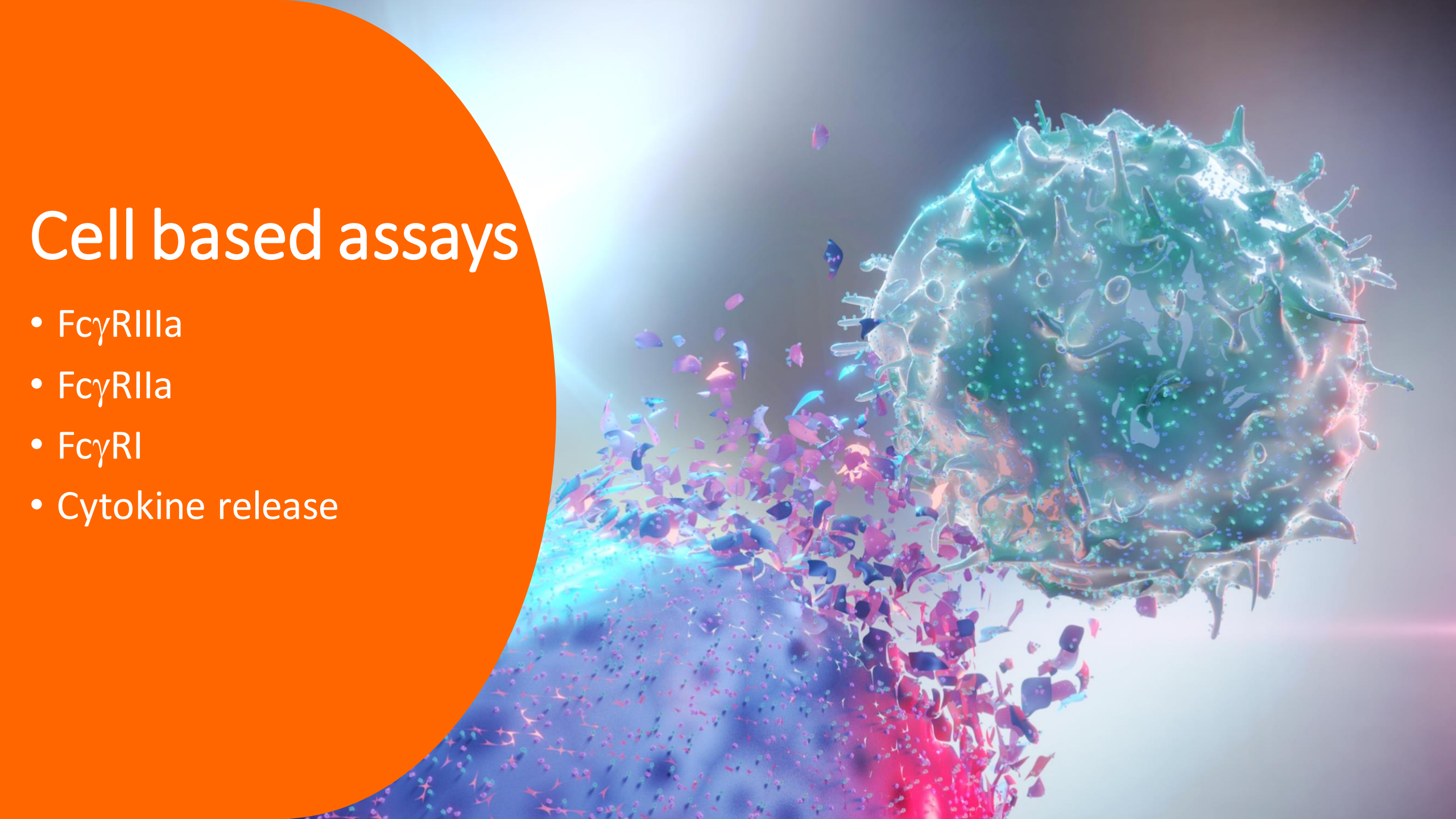
FcRn binding

- FcRn binding critical for long half-life
- STR retains binding to FcRn



Cell based assays

- FcγRIIIa
- FcγRIIa
- FcγRI
- Cytokine release



Cell based reporter assays

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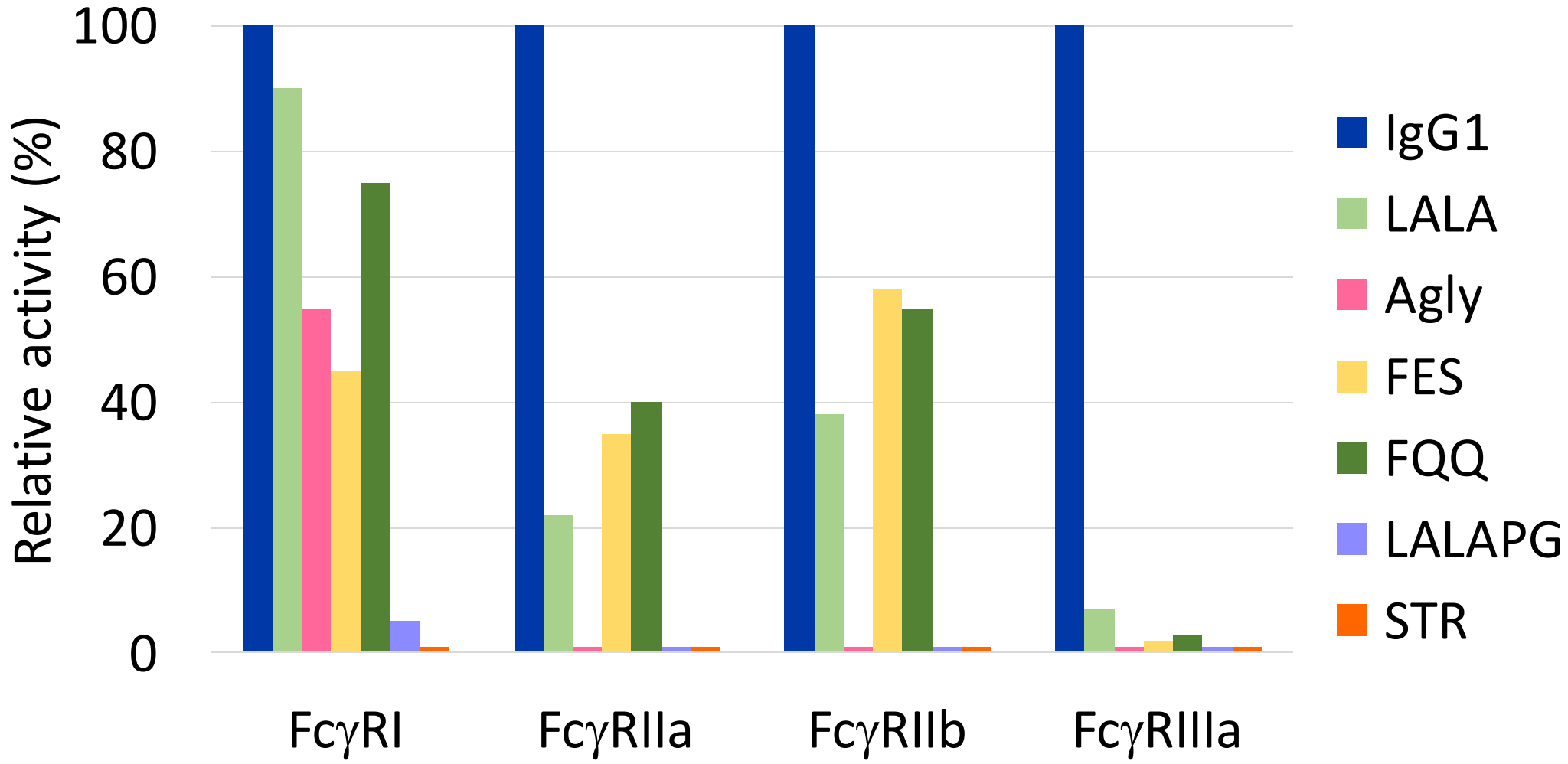
★STR shows no activity on all FcγRs



Cytokine release assay

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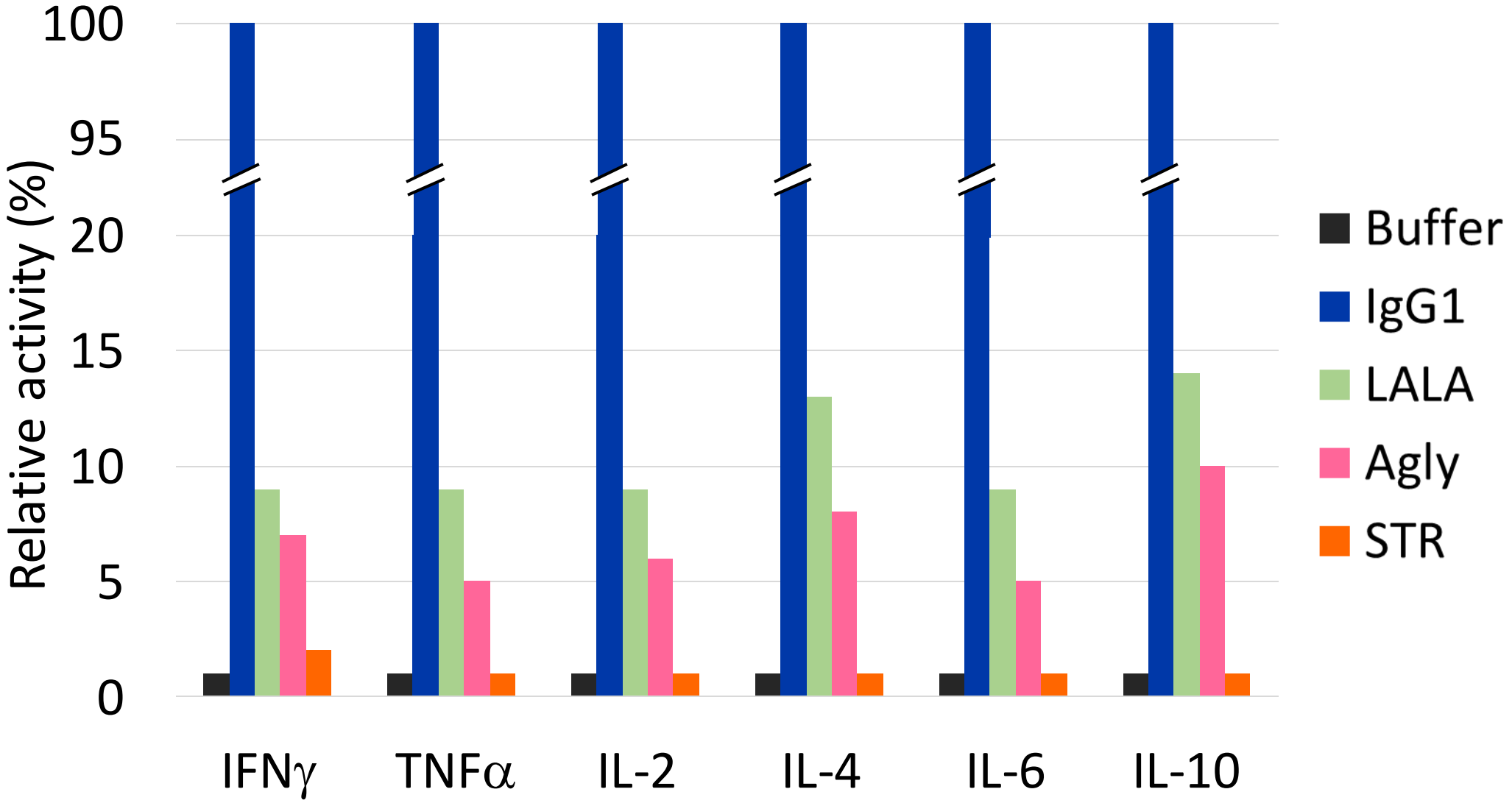
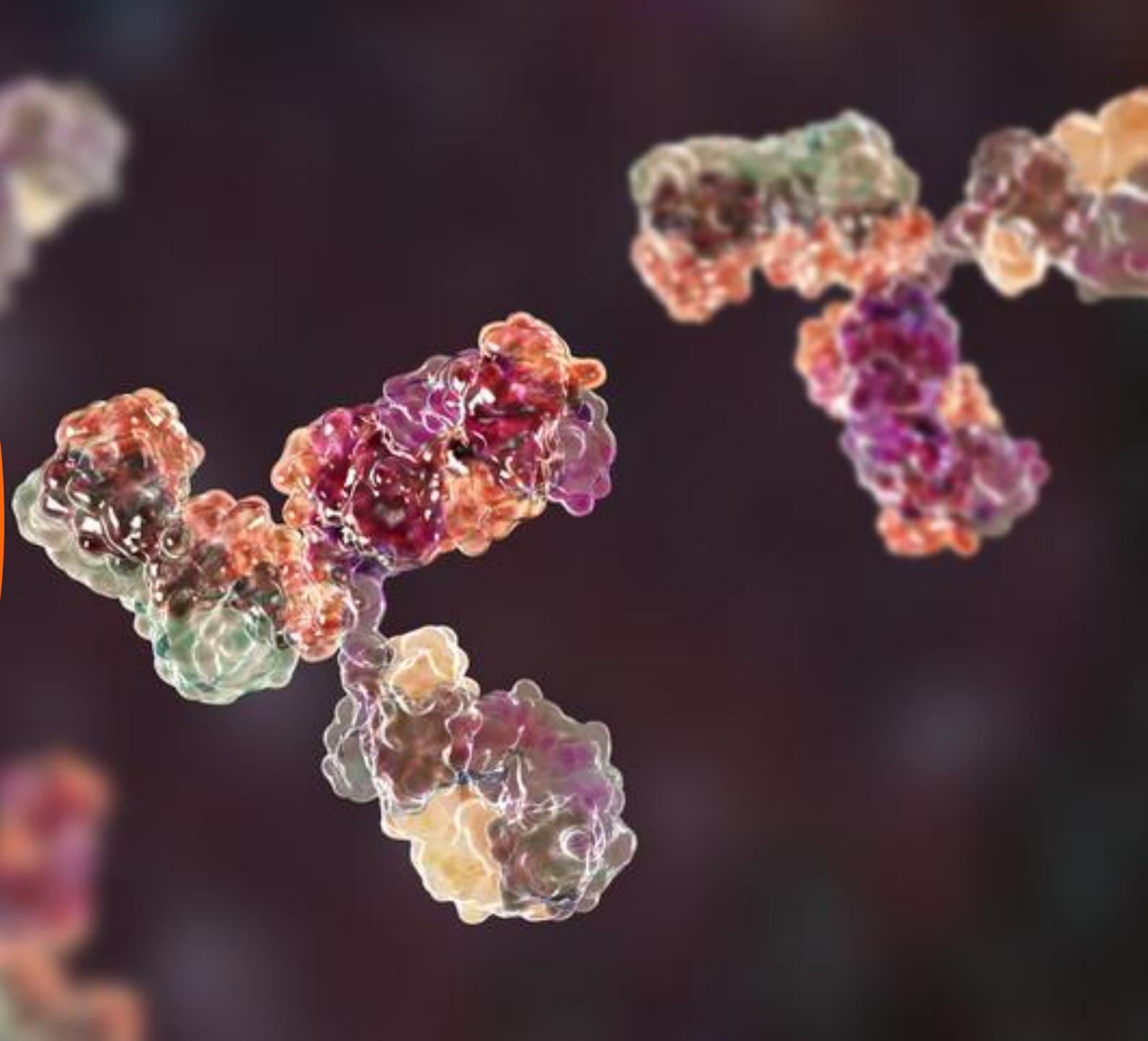
★STR shows no significant activity above buffer alone



Developability

- Immunogenicity
- Thermal stability
- Forced degradation
- Expression
- Glycosylation
- Protease sensitivity
- Pharmacokinetics



Immunogenicity

- STR mutations not immunogenic
- *In silico* analysis
  - Assessment of peptides with the theoretical capacity to bind MHC class II antigens using IEDB prediction tool
  - STR has a lower number of predicted binding peptides than wild type IgG1
- *In vitro* analysis
  - ProImmune ProMap® T-cell proliferation assay
  - Positive controls gave high level of proliferation
  - IgG1, LALA and STR gave no significant proliferation above buffer alone

Variant	Score relative to IgG1
PVA-GSS	-10
STR	**-3**
LALE	-3
Agly	-2
FES	-1
IgG1	**0**
LALA	2
LALAPG	2
LAGA	2
GRLR	3
DAPA	19
LALAKA	20
Sigma	29
FQQ	29

Decreasing in silico predicted immunogenicity

Thermal stability

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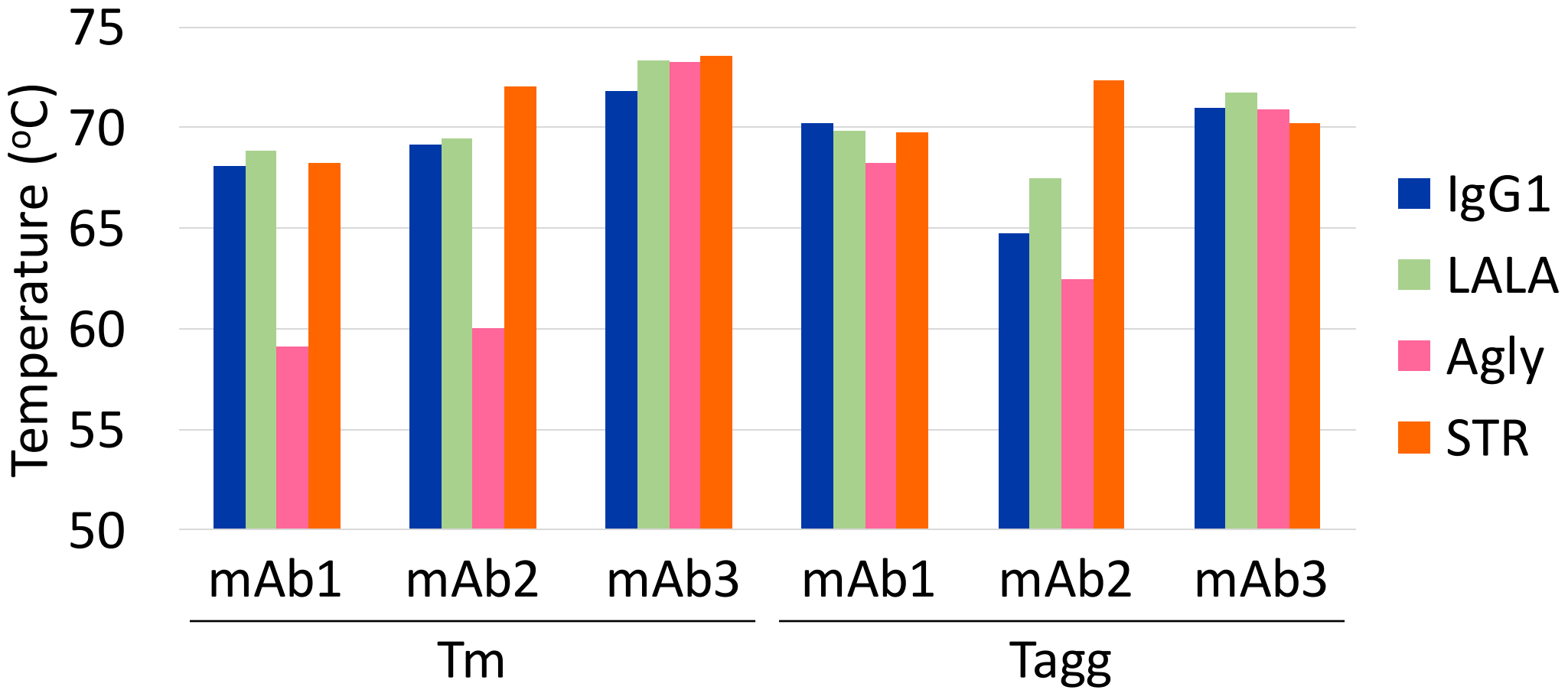
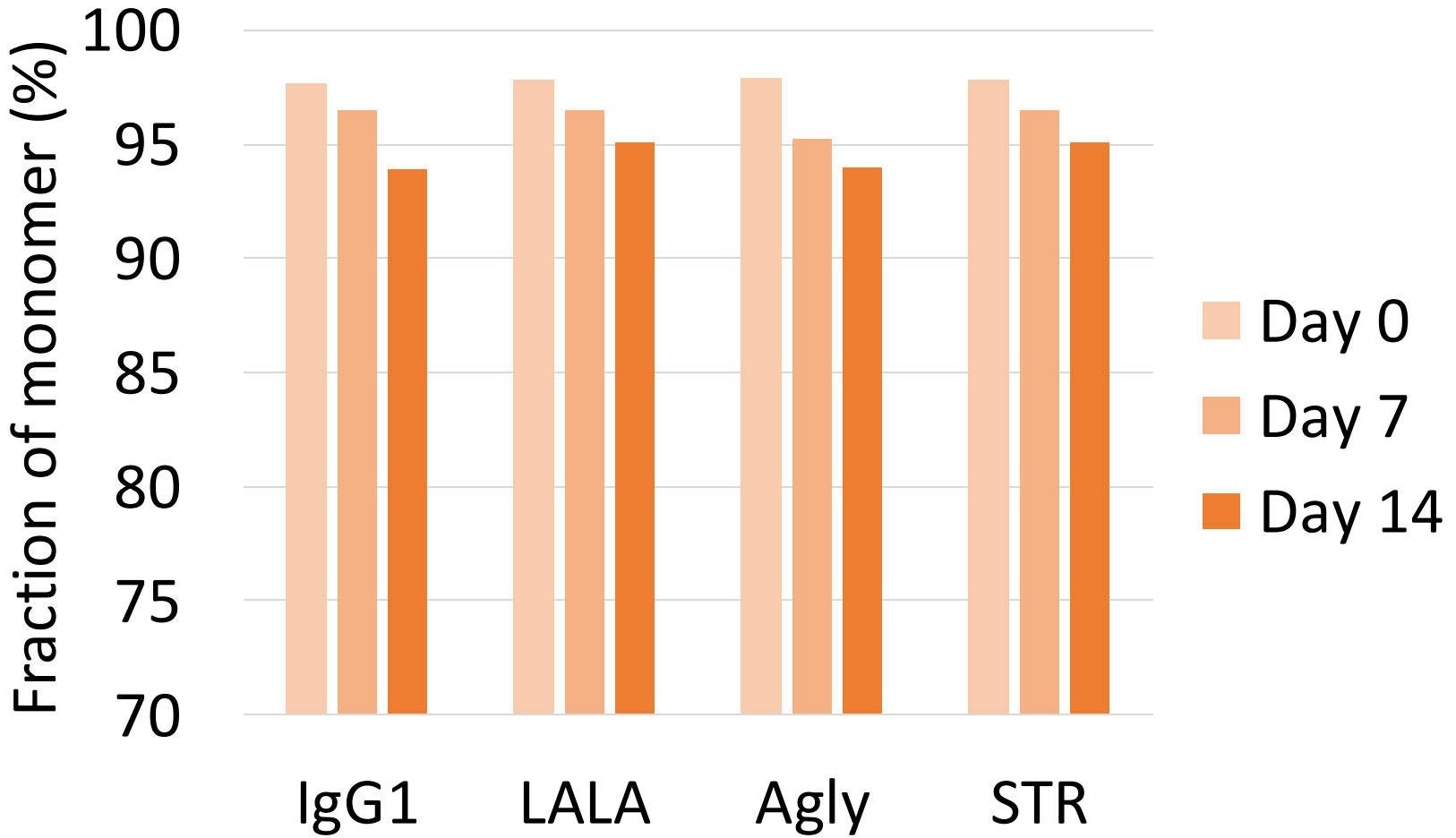
- Thermal melting (Tm) and aggregation (Tagg) measured on Uncle instrument
- STR comparable to or more stable than wild type IgG1 for 3 different mAbs



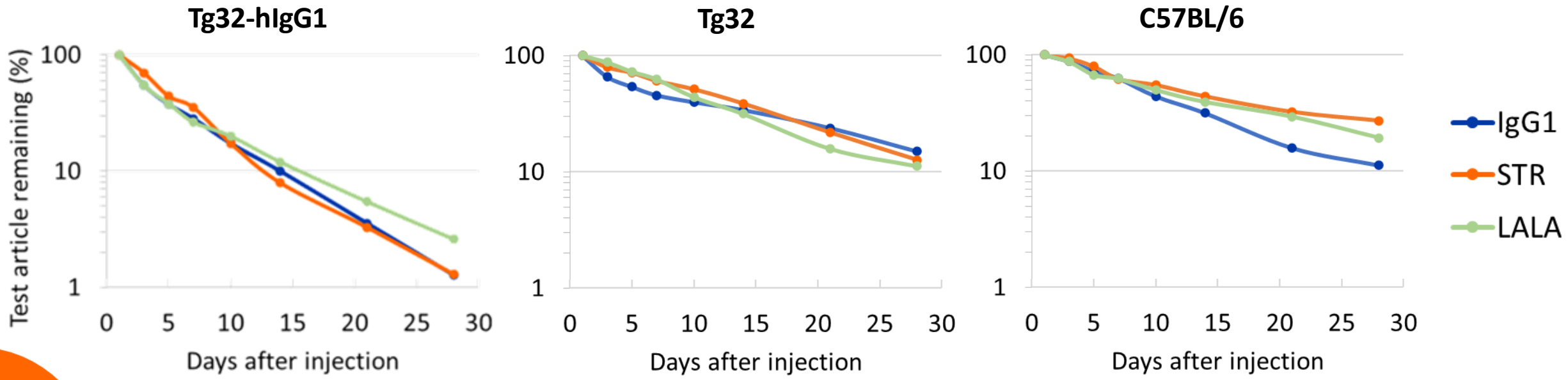
Forced degradation

- Samples incubated at 40°C for 14 days at 1 mg/ml in PBS
- STR is at least as stable as wild type IgG1



Pharmacokinetics

- PK measured in 3 strains of mice
  - Tg32-hIgG1 – human FcRn and human immunoglobulin transgenic mice
  - Tg32 – human FcRn transgenic mice
  - C57BL/6 – standard mice
- No significant difference in elimination phase half-life for STR compared to wild type IgG1 and LALA



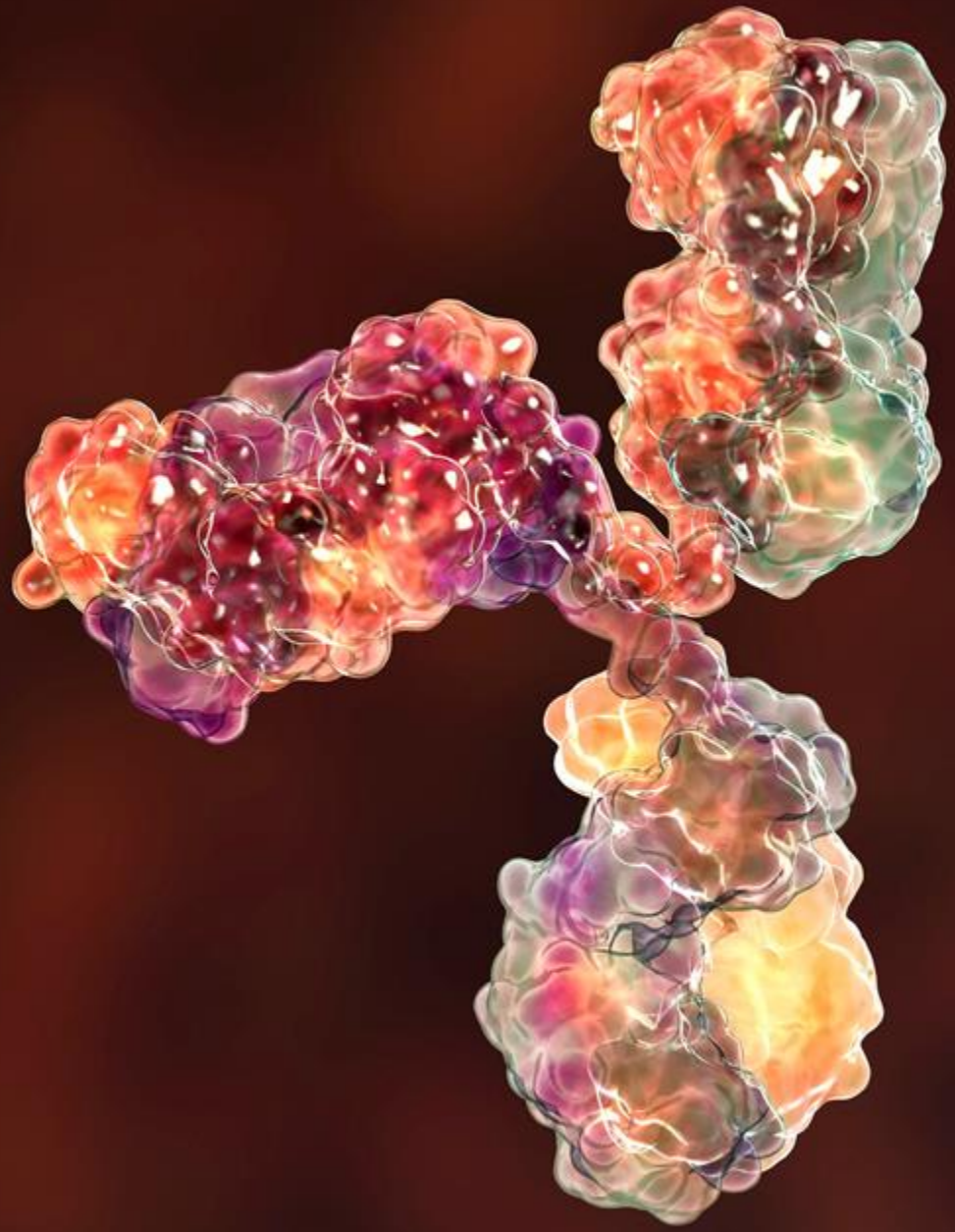
Other data

- Antigen binding
  - STR mutations do not impact antigen binding activity
- Expression titres
  - STR mutations have no detrimental impact on transient expression titres in HEK293 or CHO cells measured for >10 different antibodies
- Glycosylation
  - STR mutations do not alter glycosylation profile of HEK293 or CHO produced antibodies
  - STR mutations do not cause O-linked glycosylation
- Protease sensitivity
  - STR mutations do not increase sensitivity of antibody to metalloproteases
- Combinations of common antibody mutations
  - For example heterodimerization and half-life extension mutations

Summary

- For the first time we compared almost all reported silencing mutations
- All show residual binding to Fc receptors
- In parallel we screened >300 novel Fc mutations
- Identified a panel of truly silent variants
- STR selected as lead and shown to be highly developable
- Some of this data was recently published:
  - Wilkinson et al. (2021) Fc-engineered antibodies with immune effector functions completely abolished. PLOS ONE 16 (12).

Licensing



Licensing model

- Enthusiastic to make our STR technology available on affordable terms
- Together with patented IP, we provide detailed experimental data which may be used in a regulatory support package
- We offer a non-exclusive research license to enable pre-clinical development and evaluation of any number of compounds for a single fixed annual fee
- Commercial license for a specific drug substance based on an annual fee and a few simple success based milestone payments
- No royalties on sales

Contact

Further information available on request.

Contact Ian Wilkinson, CSO

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