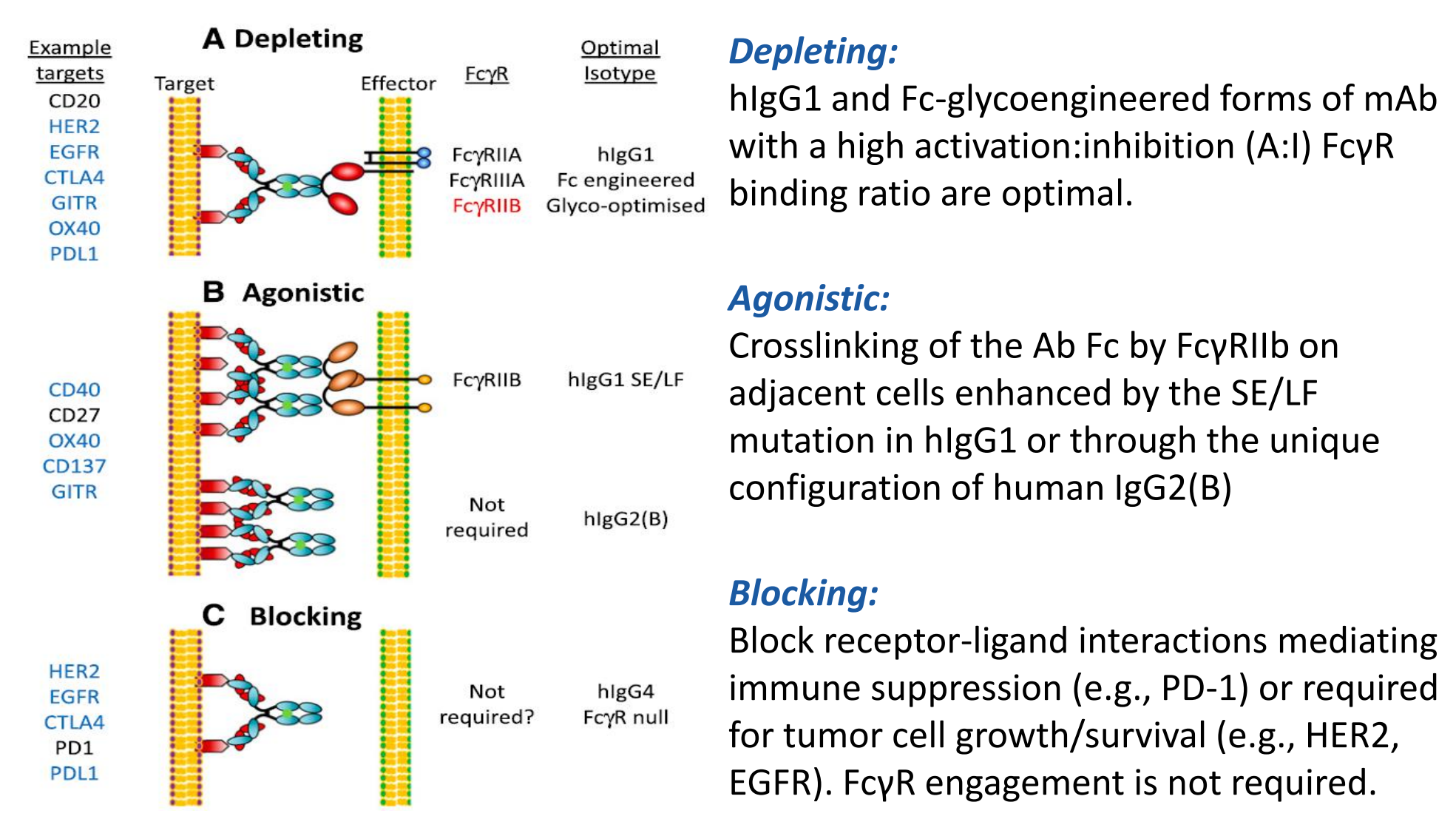


# Novel reporter-based bioassays for evaluating FcγR-dependent functions of therapeutic antibodies

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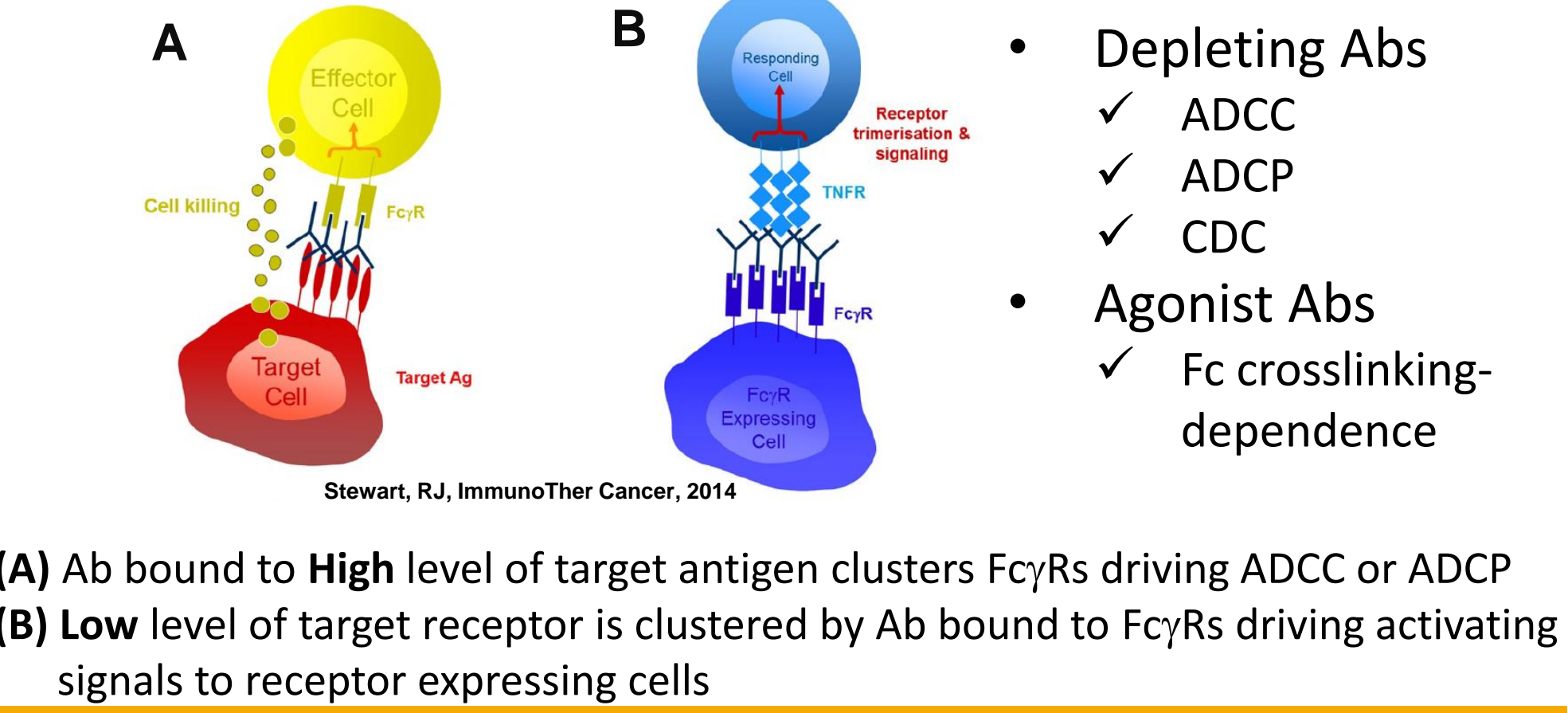


## 1. Antibody constant regions dictate functional activity

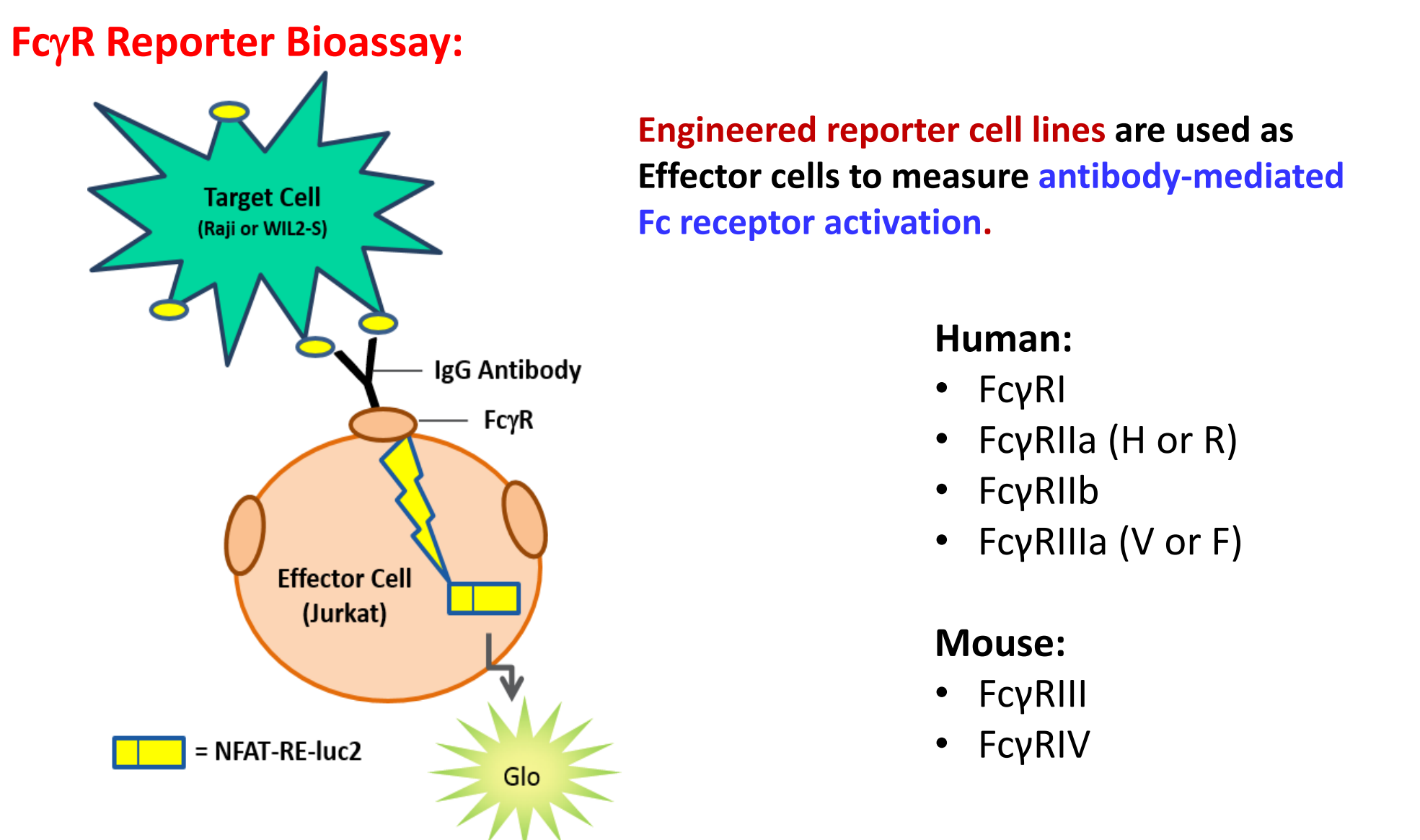
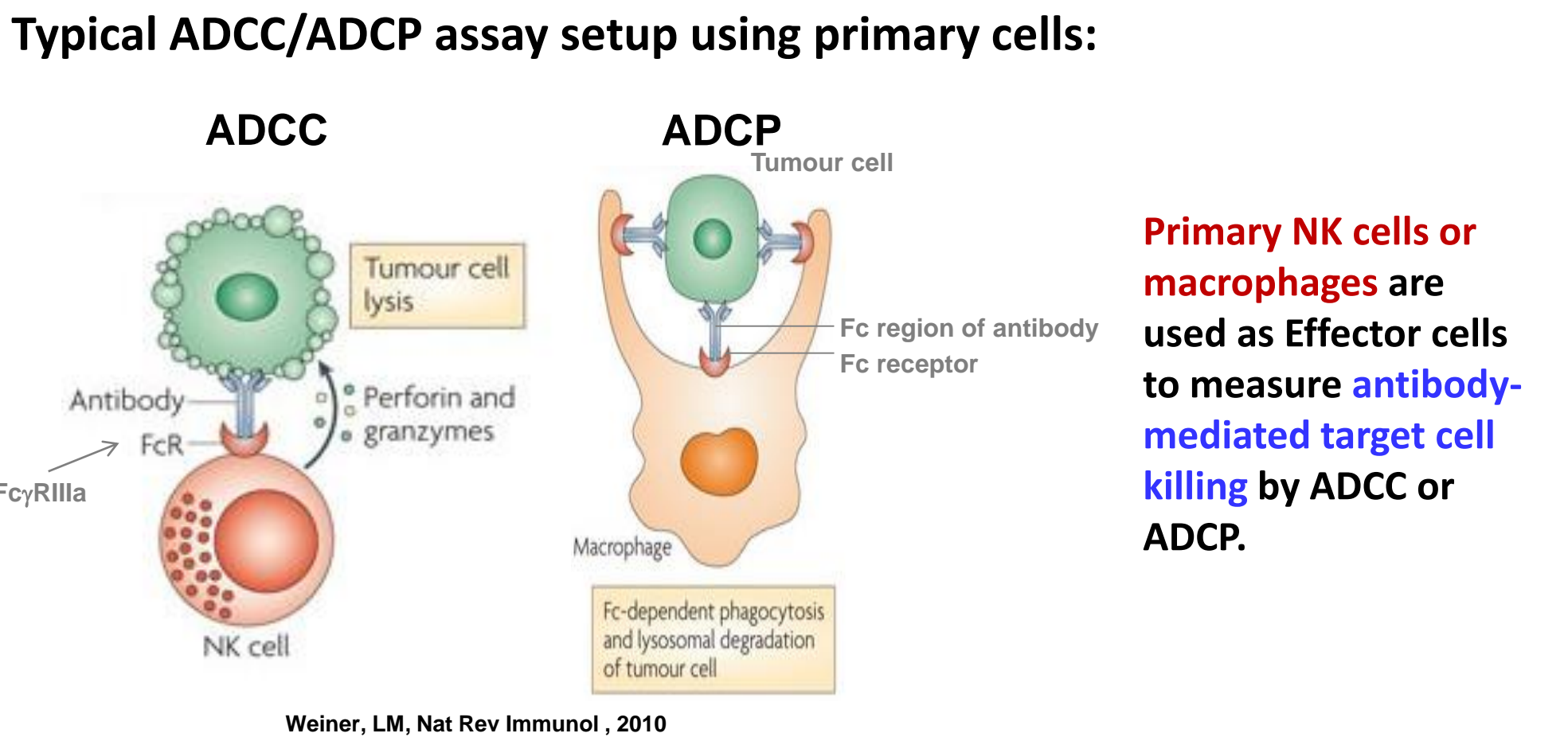


## Engineering of antibody constant regions can alter function

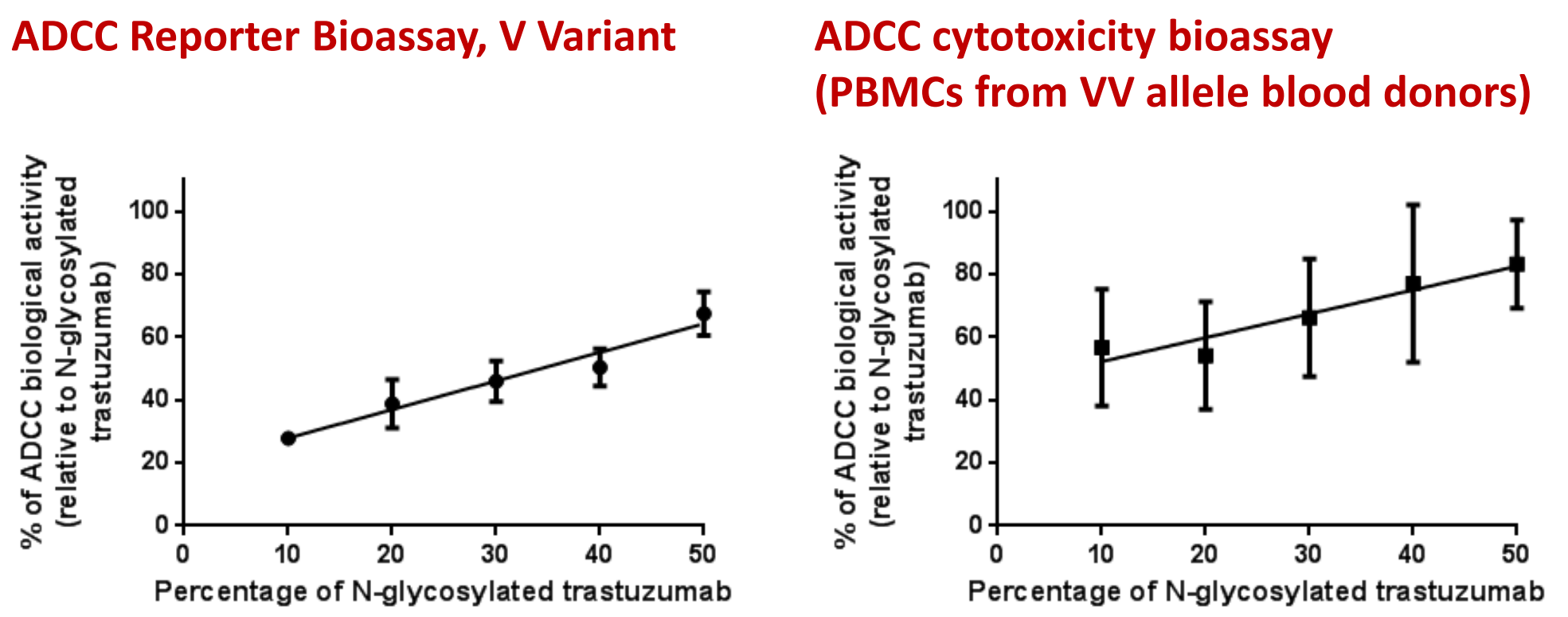
### Comprehensive suite of bioassays to measure Ab Fc function



## 2. ADCC and ADCP bioassay designs



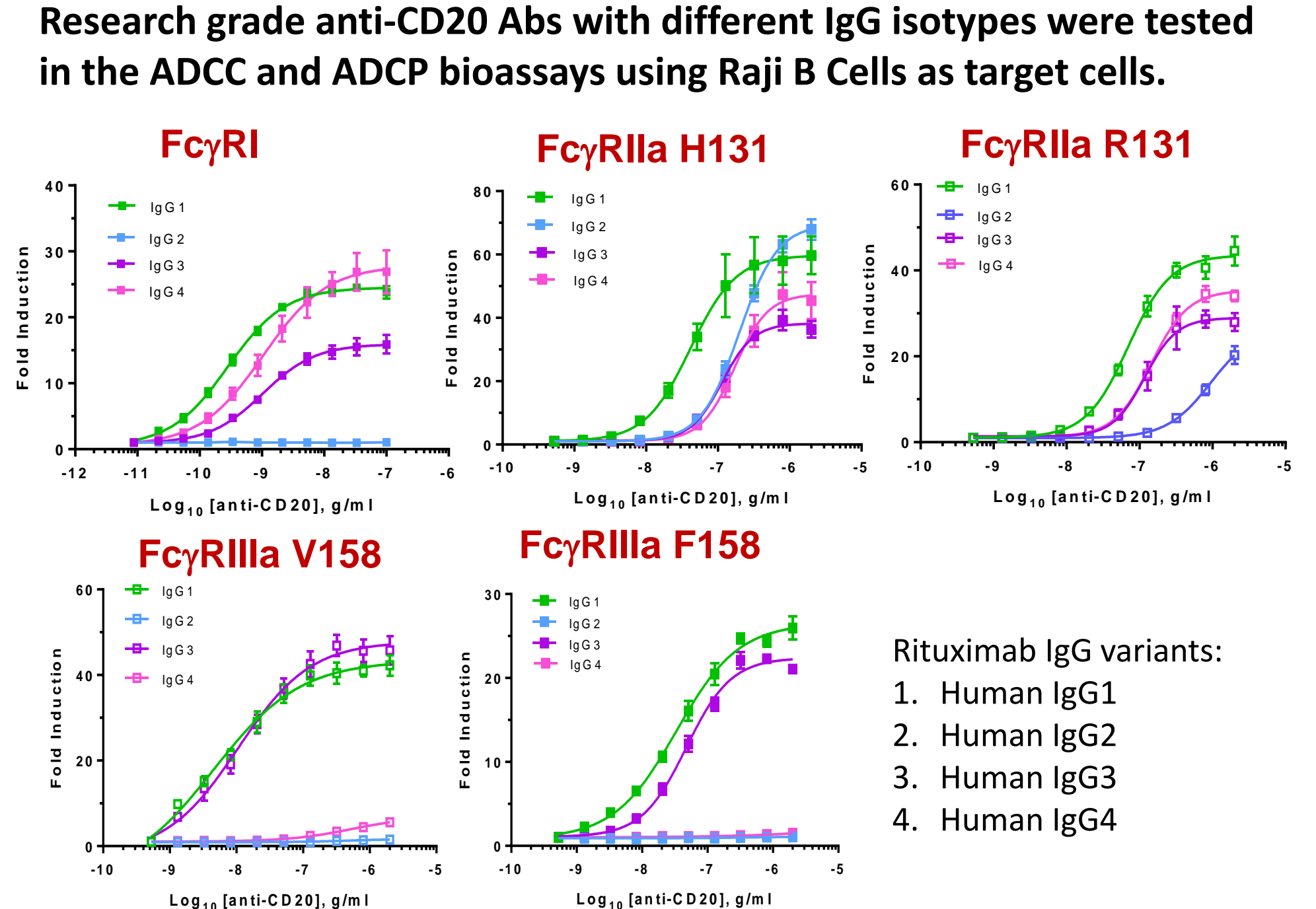
## 3. Reporter-based ADCC bioassay correlates with primary cell killing assay



- A series of trastuzumab glycosylation blend mixtures were prepared by blending PNGase F-treated (deglycosylated) and untreated trastuzumab (N-glycosylated).
- The antibody samples were tested with SK-BR-3 target cells, using untreated antibody as 100% reference.
- Assay responses were correlated between reporter-based and cytotoxicity ADCC bioassays
- Reporter-based ADCC bioassay exhibited much lower variability

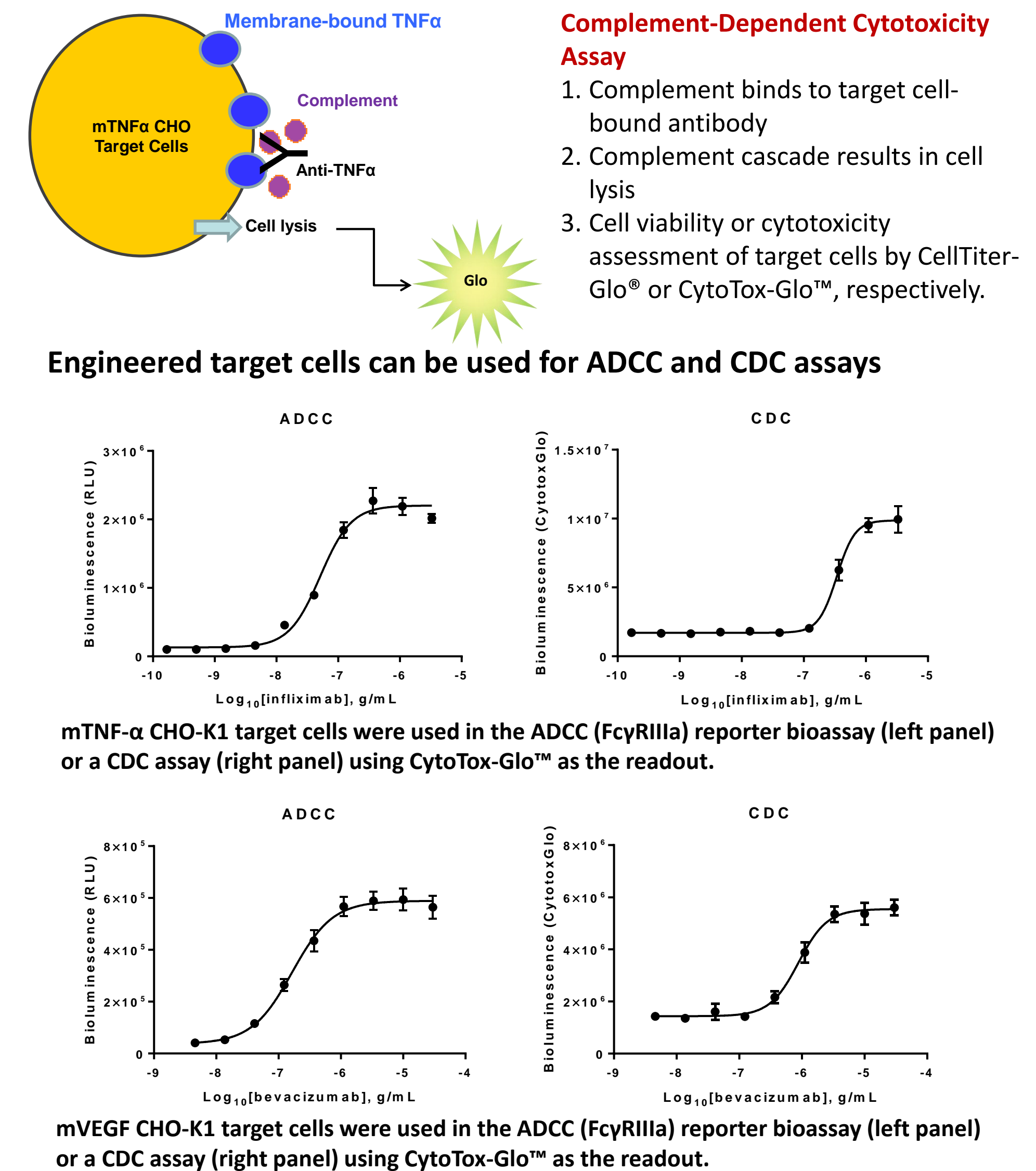
Cheng-ZJ et al., J of Immunol Methods (2014)

## 4. Bioassays demonstrate isotype selectivity for human FcγRs

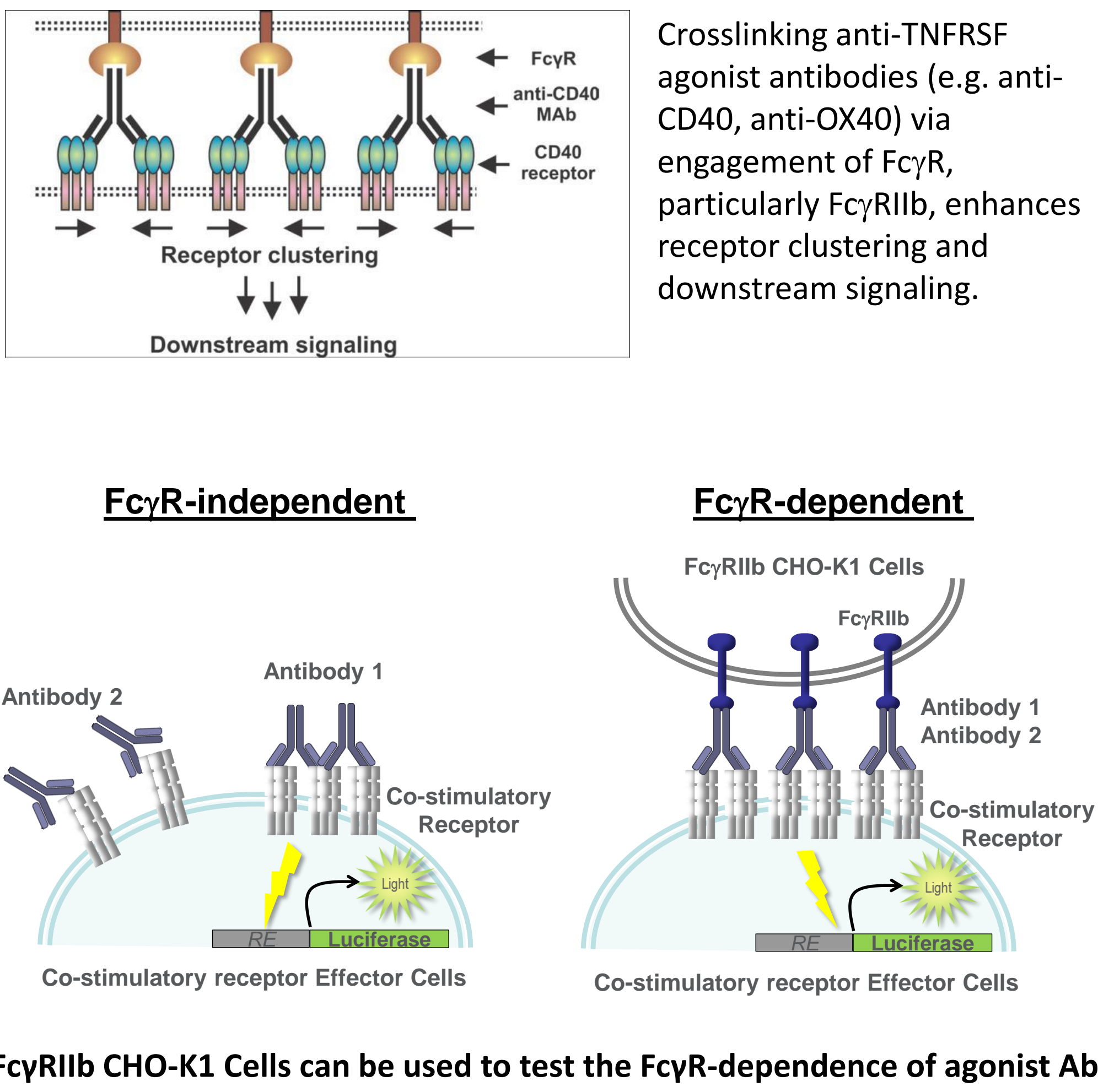


EC <sub>50</sub> (g/mL)	FcγRI	FcγRIIa H131	FcγRIIa R131	FcγRIIa V158	FcγRIIa V158
IgG1	2.8x10 <sup>-10</sup>	4.15x10 <sup>-8</sup>	7.03x10 <sup>-8</sup>	3.34x10 <sup>-8</sup>	4.50x10 <sup>-9</sup>
IgG2	NB	1.98x10 <sup>-7</sup>	>9.60x10 <sup>-7</sup>	3.55x10 <sup>-7</sup>	4.87x10 <sup>-7</sup>
IgG3	1.0x10 <sup>-9</sup>	1.14x10 <sup>-7</sup>	1.18x10 <sup>-7</sup>	NB	NB
IgG4	~ 1.0x10 <sup>-9</sup>	1.71x10 <sup>-7</sup>	1.45x10 <sup>-7</sup>	NB	NB

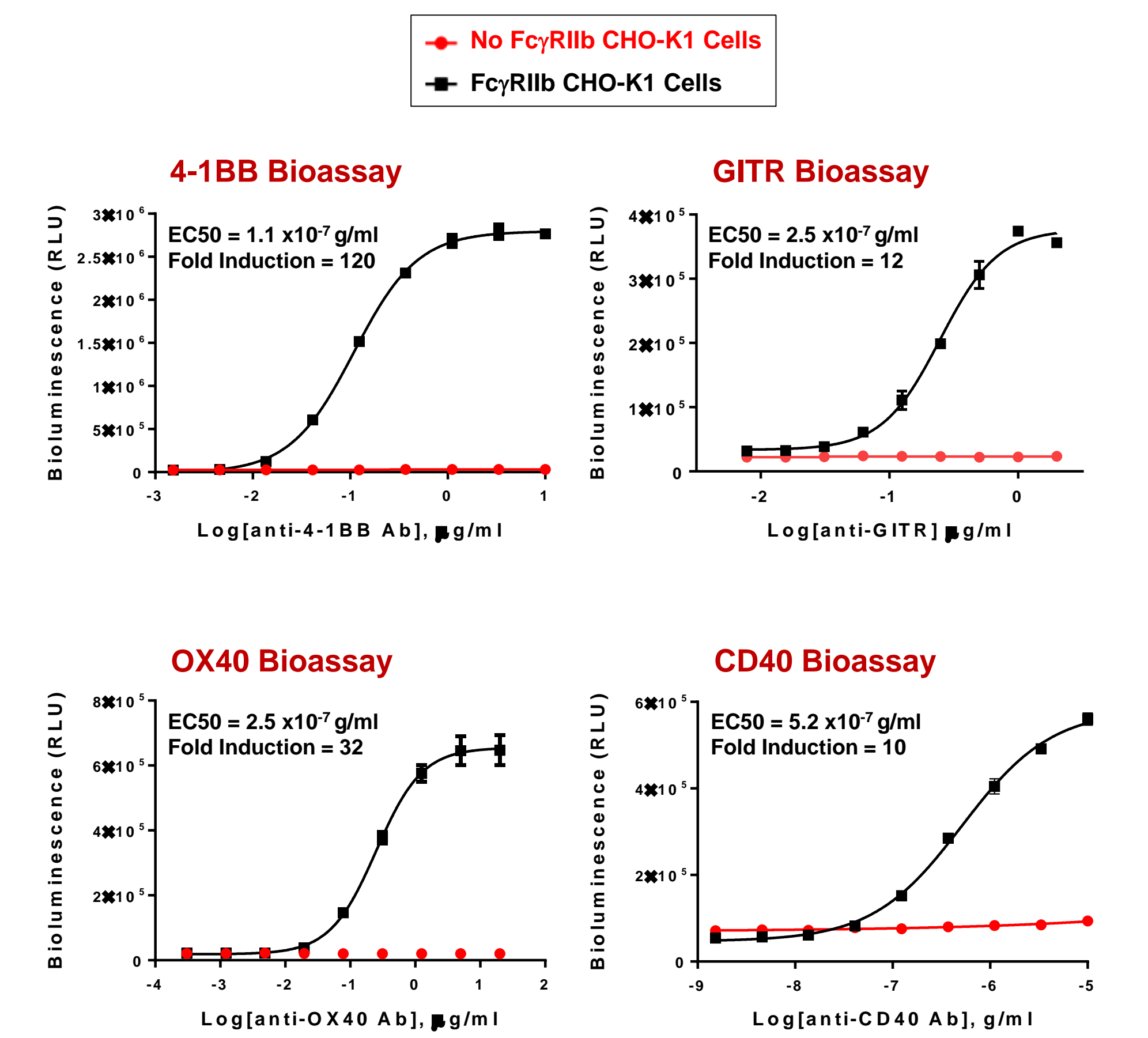
## 5. Cytotoxicity assay reagents and engineered target cells enable CDC assays



## 6. Fc crosslinking by FcγRs can enhance agonist antibody functions

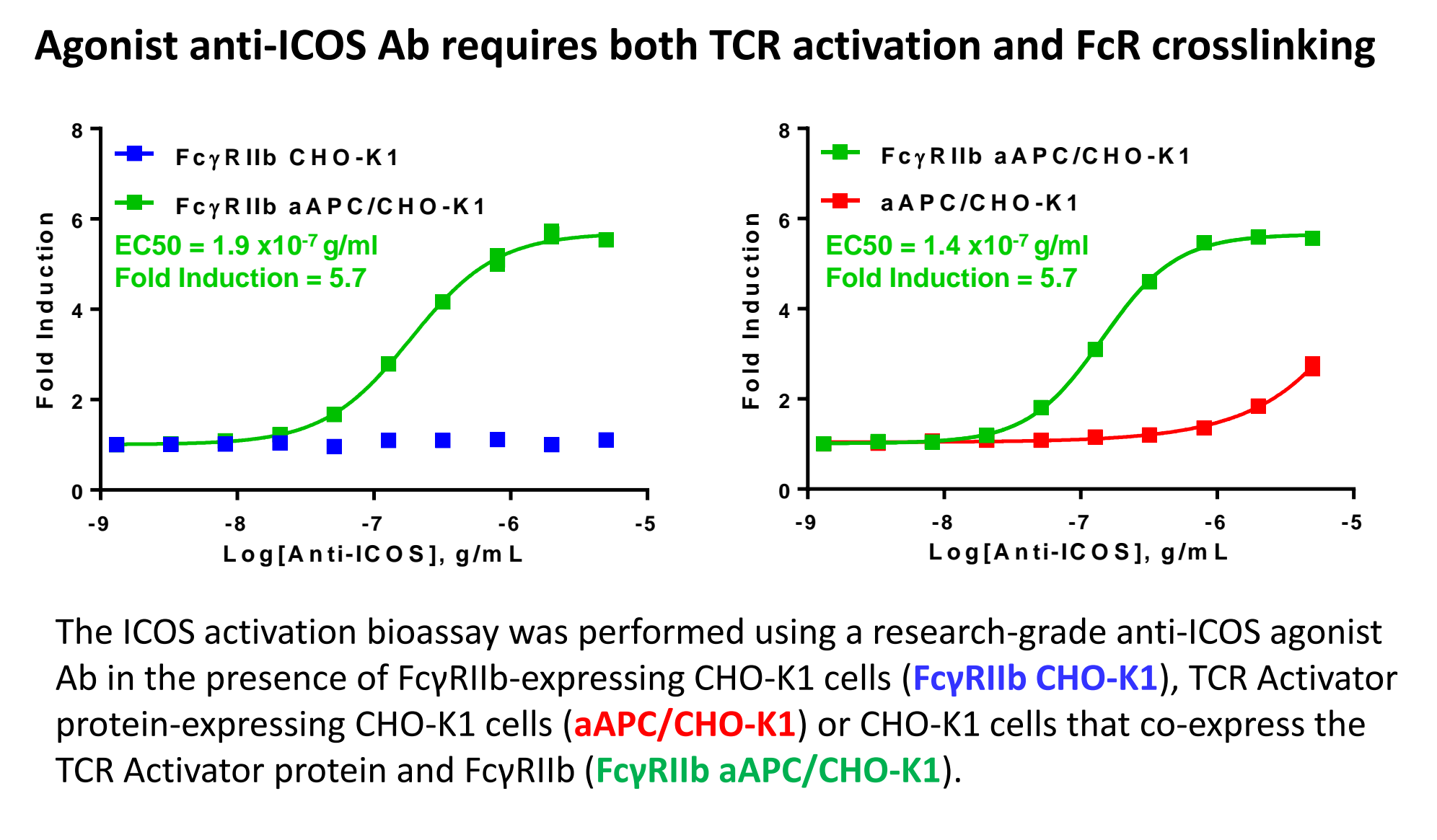
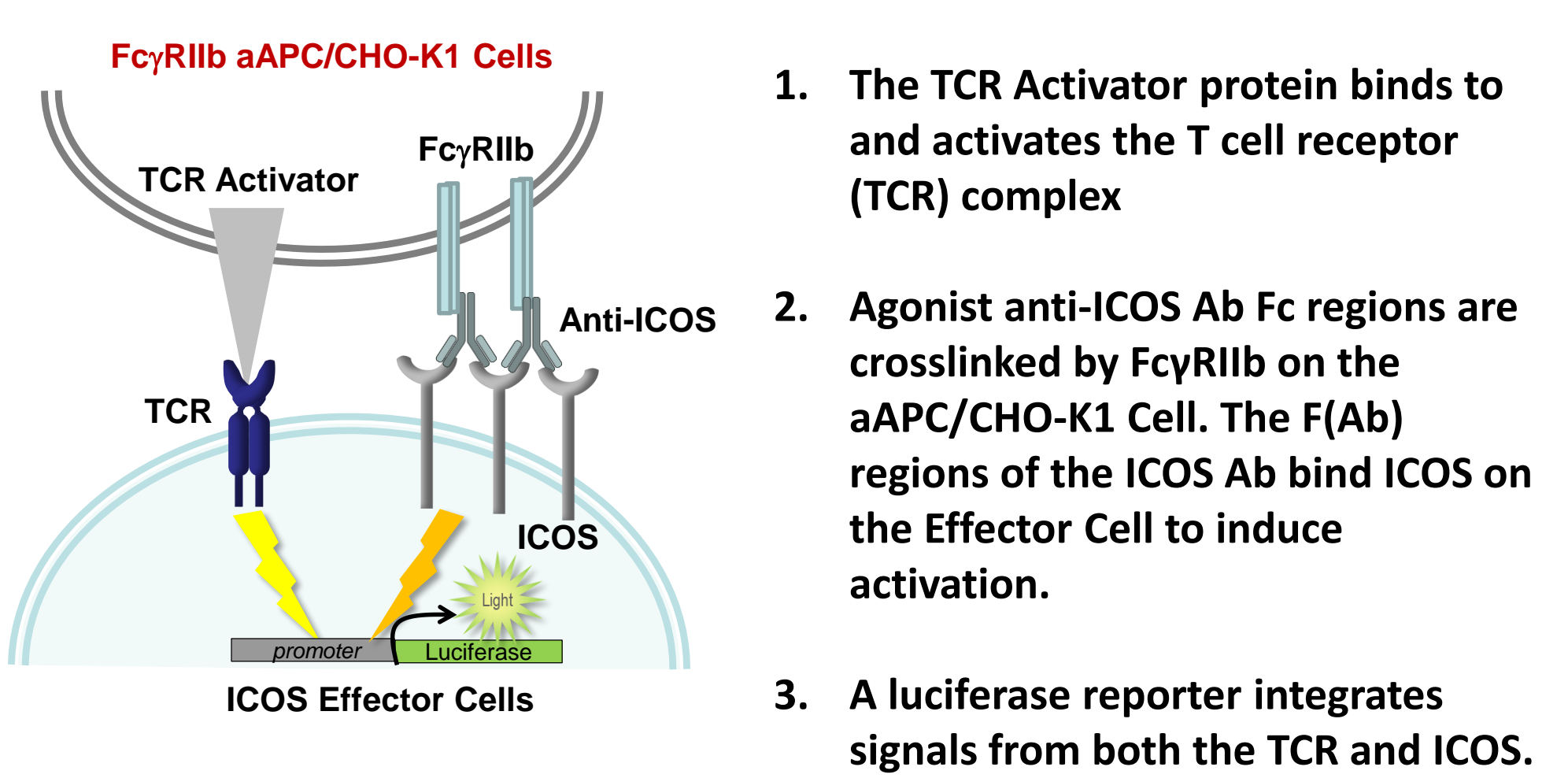


## 7. FcγRIIb CHO-K1 cells demonstrate Fc crosslinking dependence of agonist Abs



Functional analysis of research grade TNFRSF agonist antibodies in the context of FcγR crosslinking. 4-1BB, G1TR, OX40, or CD40 reporter cells were activated with their respective agonist antibodies in the presence or absence of FcγRIIb CHO-K1 Cells. Fc crosslinking by FcγRIIb CHO-K1 cells enabled agonist activity of these antibodies.

## 8. FcγRIIb aAPC/CHO-K1 cells are used with agonist Abs that require TCR co-stimulation



## 9. Conclusions

- We have developed a platform of cell-based bioassays for characterizing the Fc function of therapeutic antibodies.
- A comprehensive suite of FcγR-specific bioassays have been developed for determining ADCC and ADCP activity of Ab
  - The bioassays are homogeneous, easy to use, sensitive, and robust
  - Reagents and Target cells to enable CDC assays
  - FcγRIIb CHO-K1 cells enable easy determination of agonist antibody dependence on FcγR crosslinking of Fc regions
  - Integration of a TCR Activator into FcγRIIb CHO-K1 cells enables analysis of co-stimulatory targets that require TCR activation
- Together, these bioassay tools enable the comprehensive characterization of antibody Fc effector function