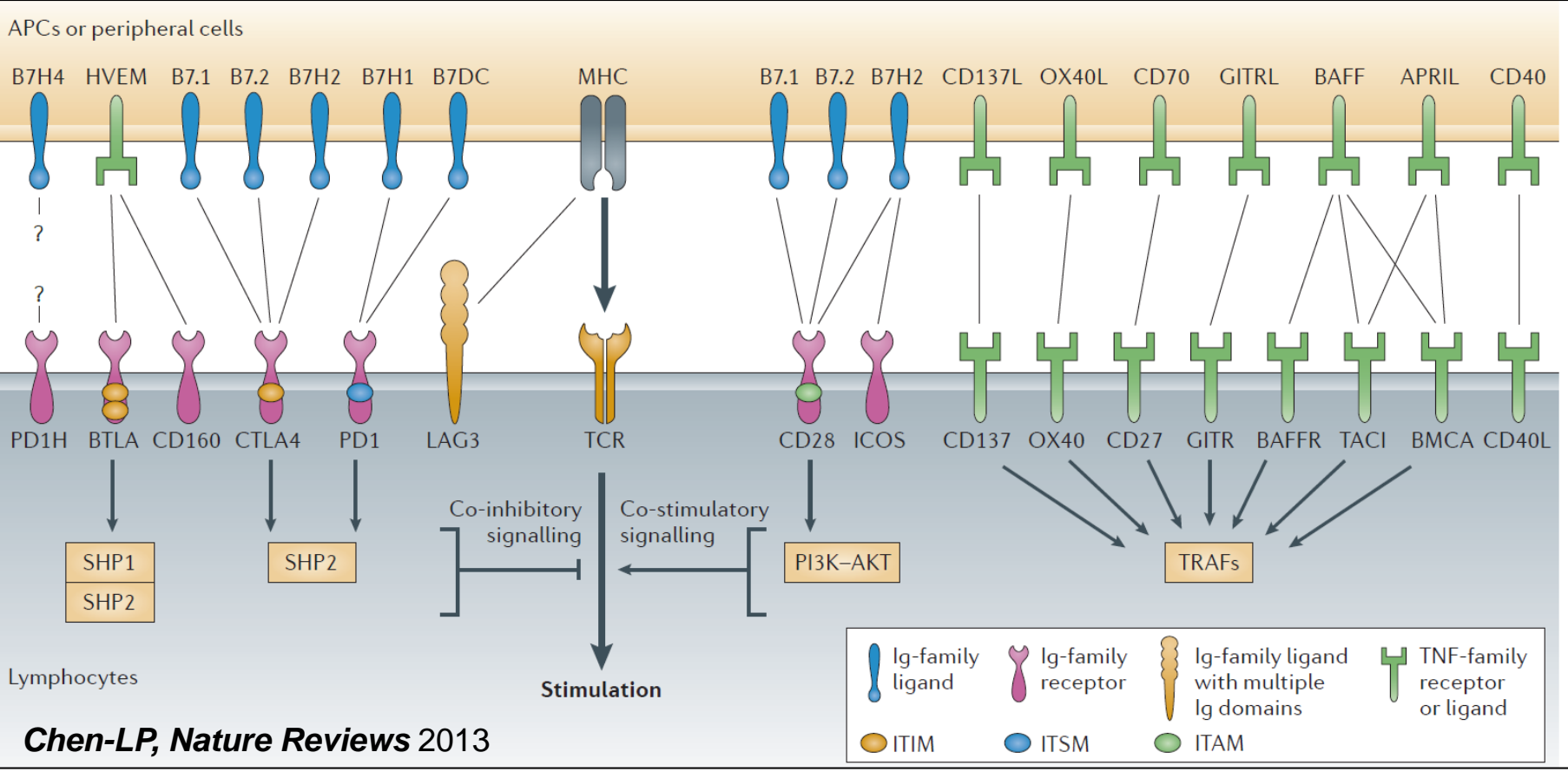


Better Cell-Based Assays for Anti-CTLA-4, Anti-PD-1/PD-L1, and Bispecific Immunotherapy Drug Studies

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1. Drug Targets in Immunotherapy



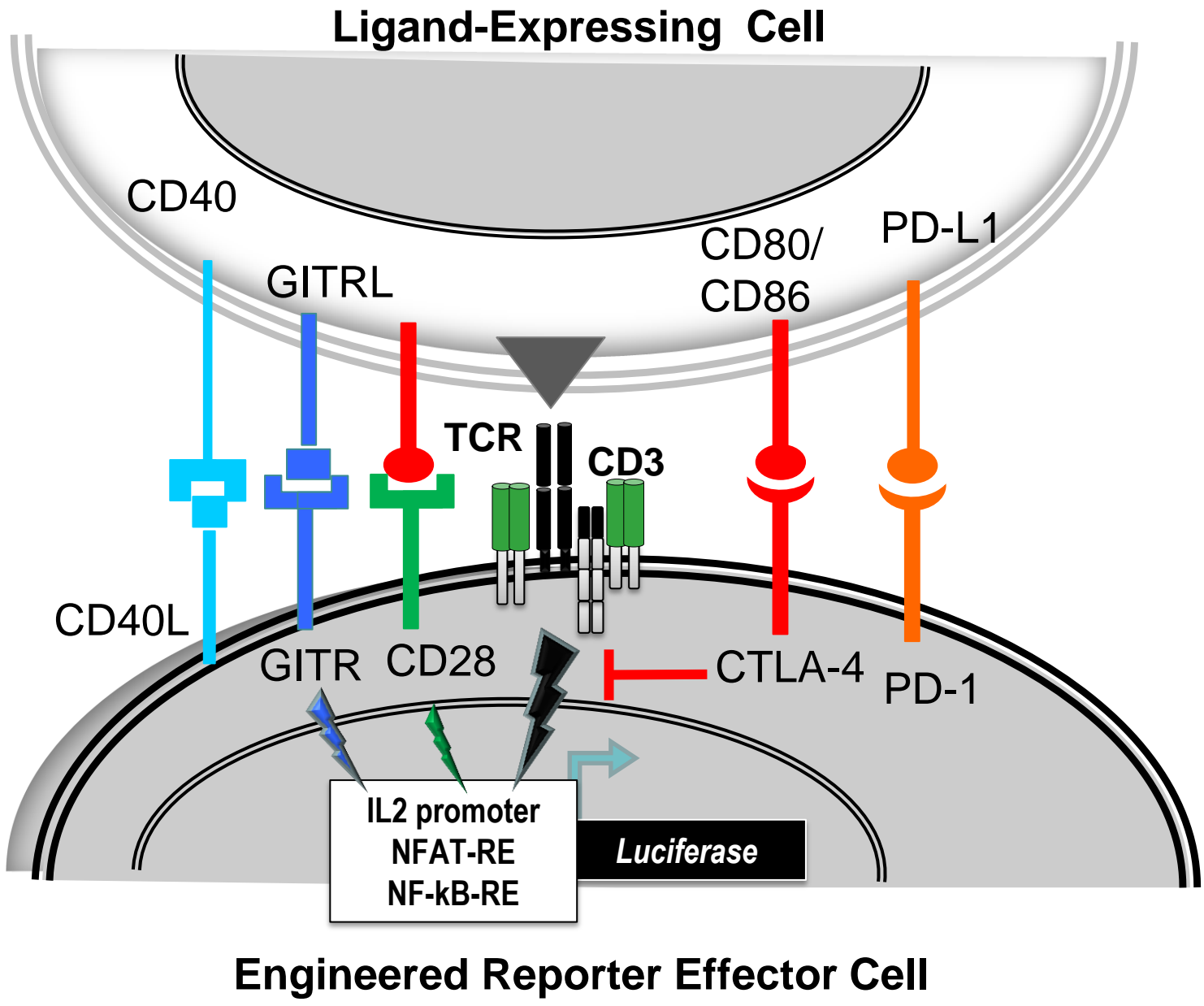
Immunotherapy is also called *biologic therapy* or *biotherapy*. It stimulates certain parts of the immune system to fight diseases such as cancer.

Example drug targets in immunotherapy:

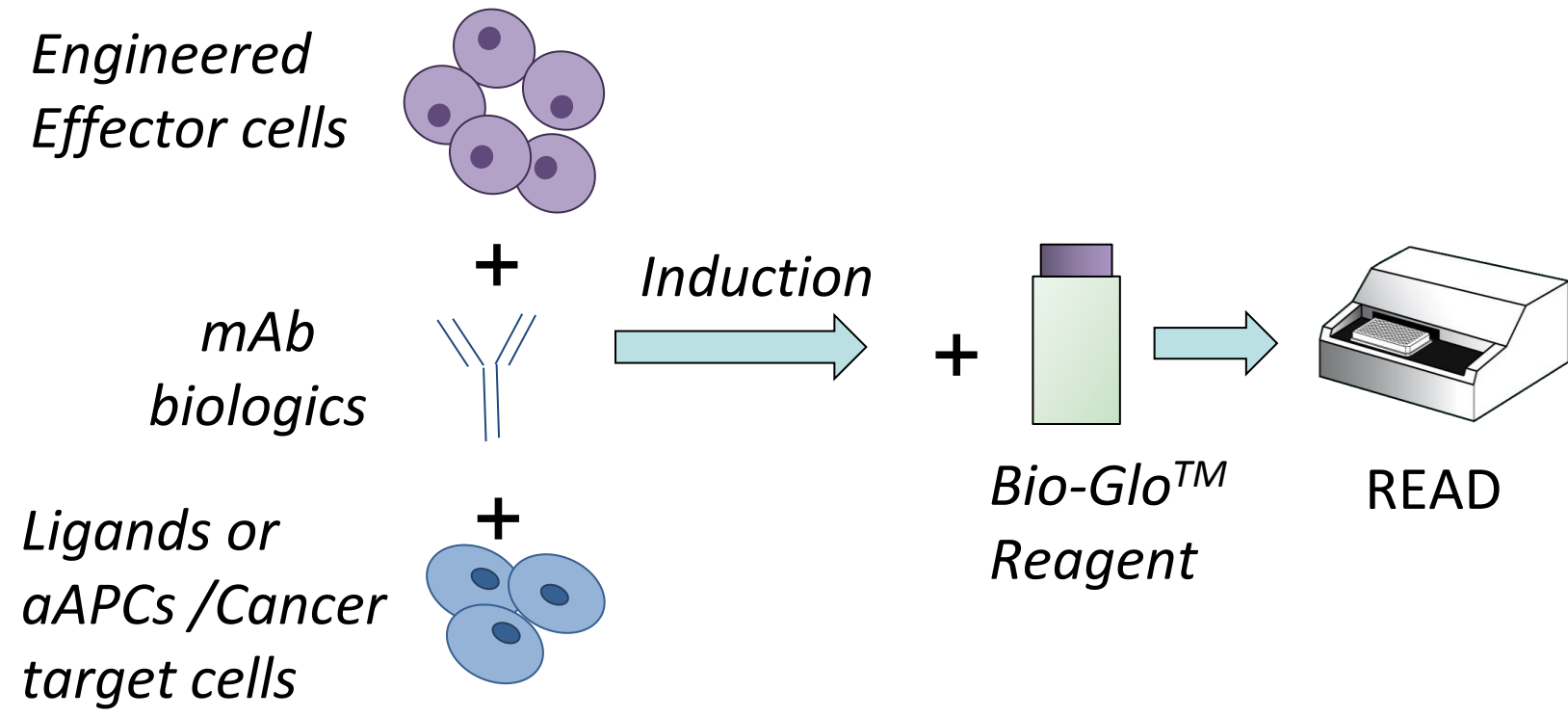
- Modulation of T cell activation: TCR, CD3, CD28
- Co-inhibitory receptors: PD-1/PD-L1, CTLA-4
- Co-stimulatory receptors: GITR, CD40, OX40

2. Reporter Assay Principles to Measure the Potency of the Biologics for Immunotherapy

A. Schematic Design

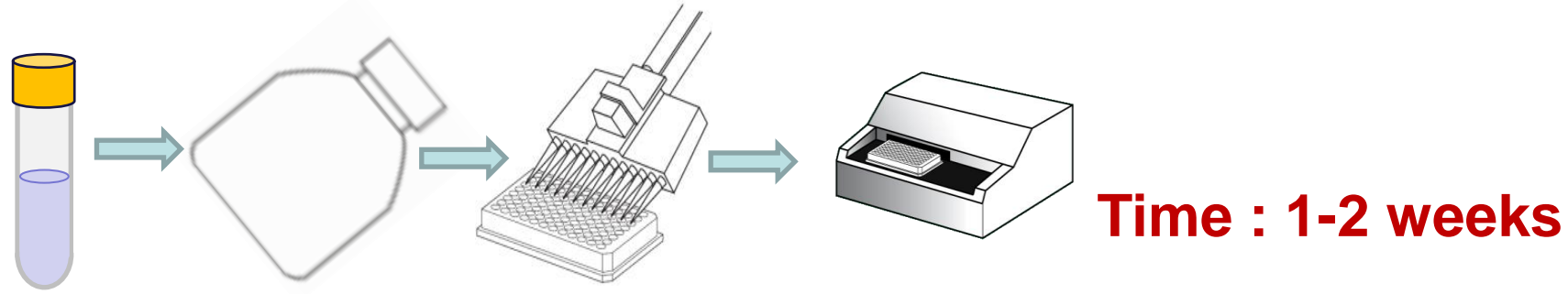


B. General Reporter Assay Procedure

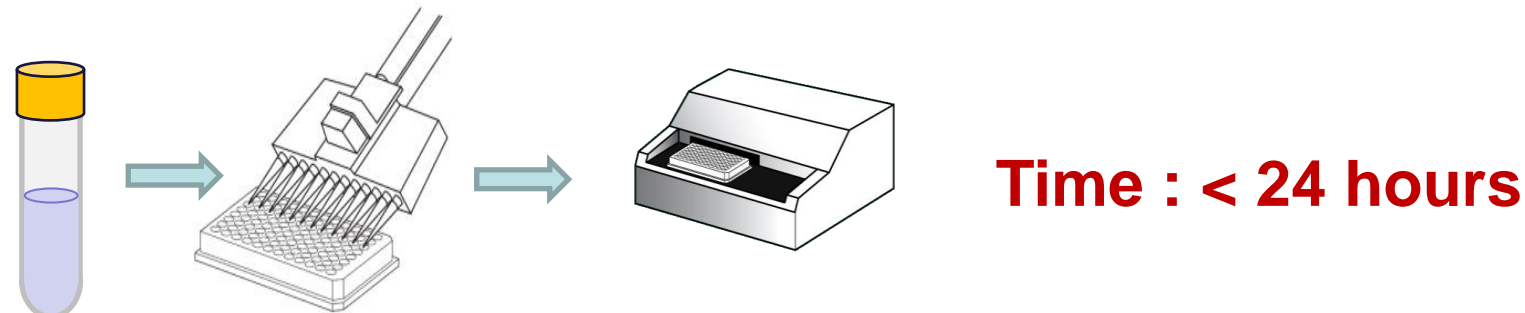


3. Thaw-and-Use Cells Improve Assay Consistency

A. Traditional cell-based assay using fresh cells from cell culture

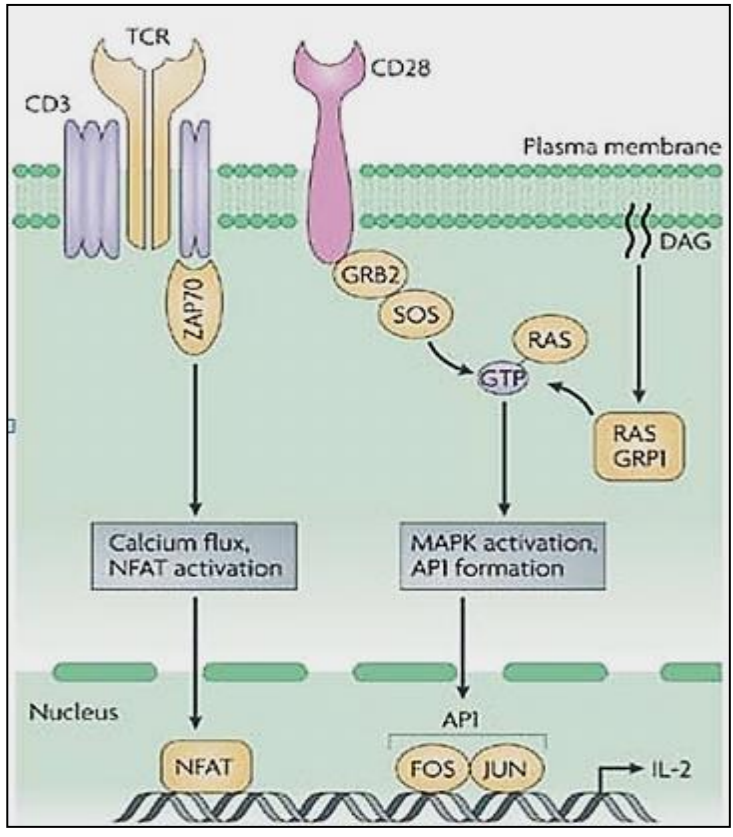


B. New assay format using Thaw-and-Use effector cells



- Advantages of using thaw-and-use cells:
- Cultureless: no cell culture needed
 - Convenient: Ready to use cells
 - Time saving: Complete within a working day
 - Low variability and high reproducibility
 - Equivalent biology

4. General T Cell Activation Assays



Lineberry-N et al.
Nature Reviews Immunology 2007

Assay Principle

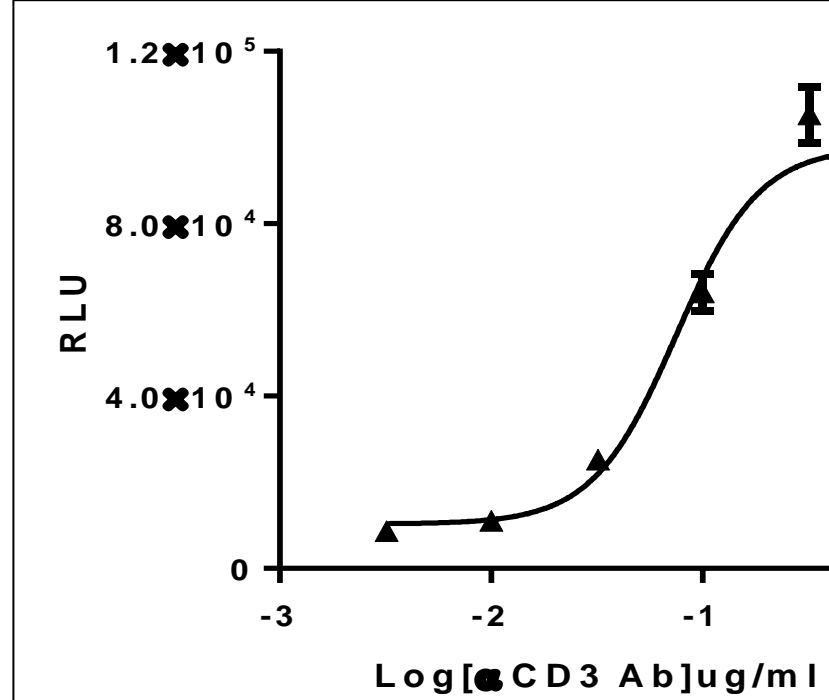
1. Jurkat / IL-2 Reporter Cell Line: TCR/CD3 activation plus CD28 co-stimulation

IL-2 promoter luc2P

2. Jurkat / NFAT-RE Reporter Cell Line: TCR/CD3 activation

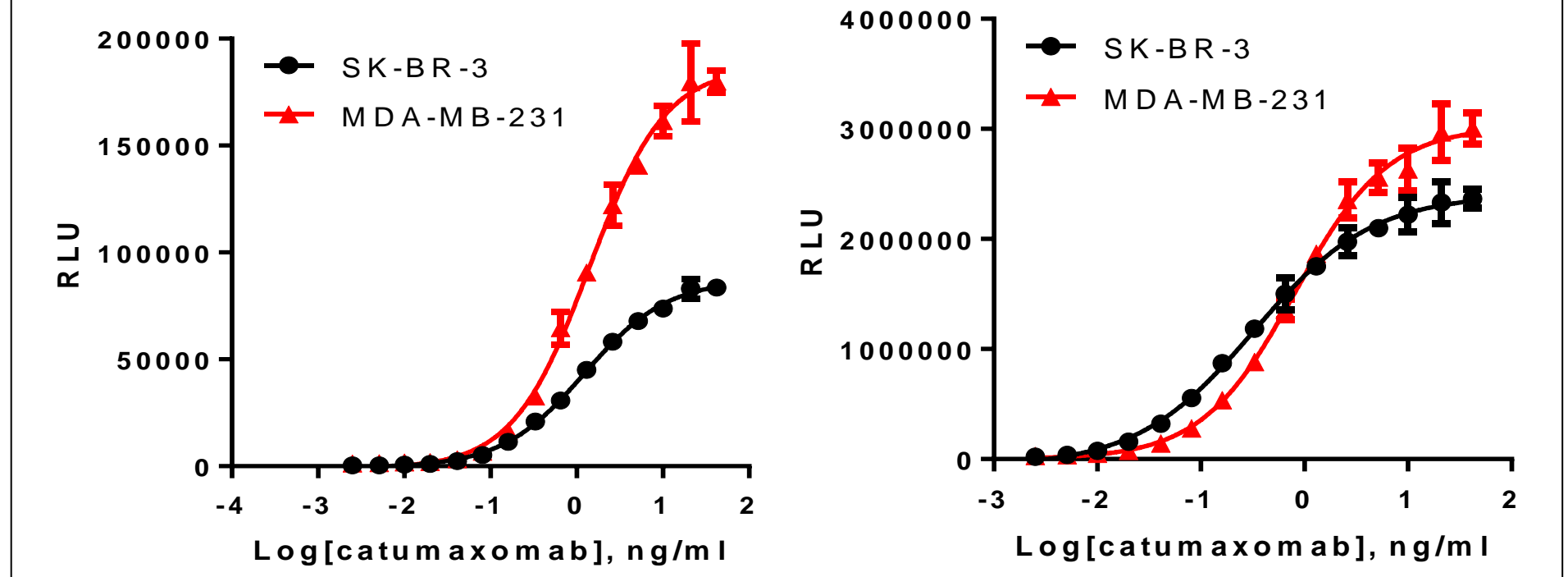
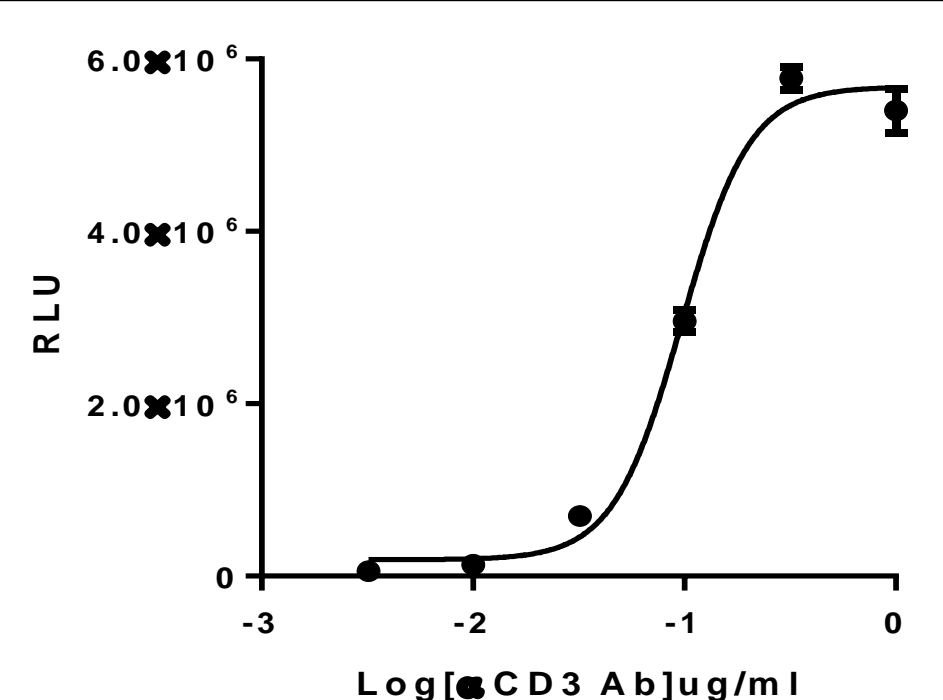
NFAT-RE luc2

Jurkat / IL-2 reporter cells

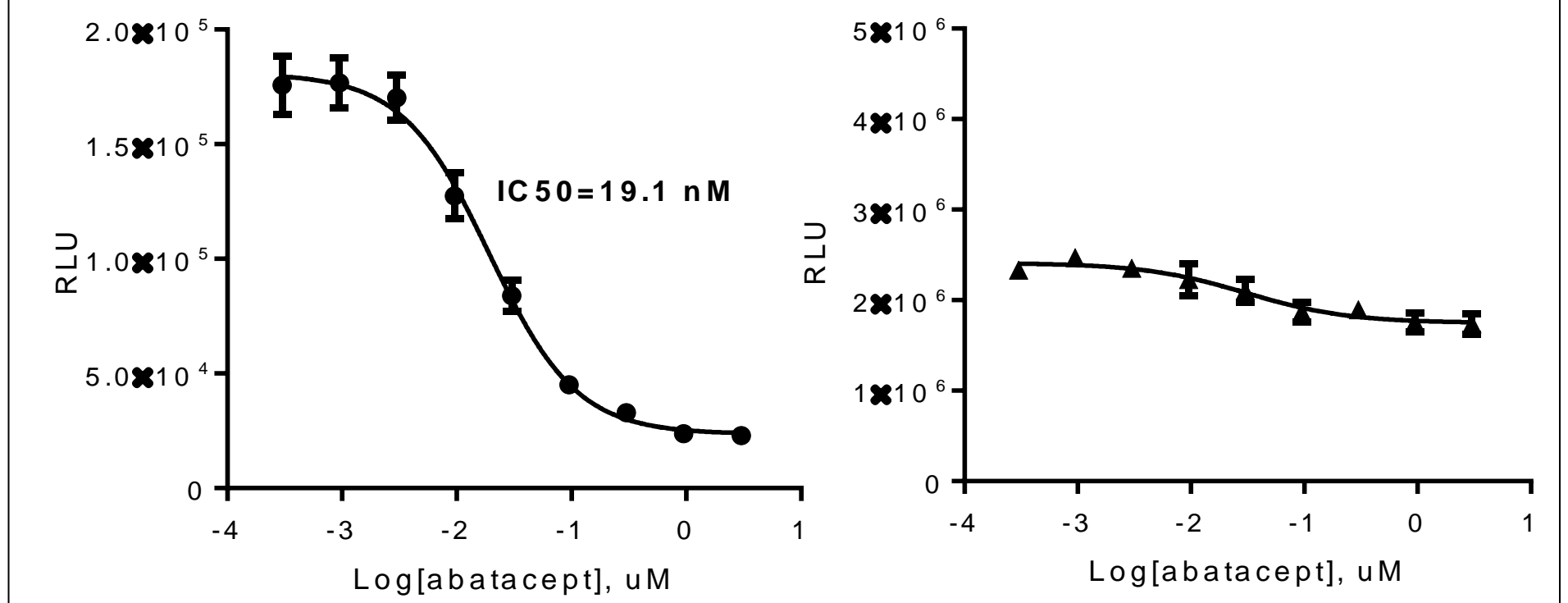


Dose-dependent reporter response to mouse anti-human CD3 Ab in the presence of anti-mouse secondary Ab in Jurkat reporter cells. Similar results seen with PMA or PHA stimulation (data not shown).

Jurkat / NFAT-RE reporter cells

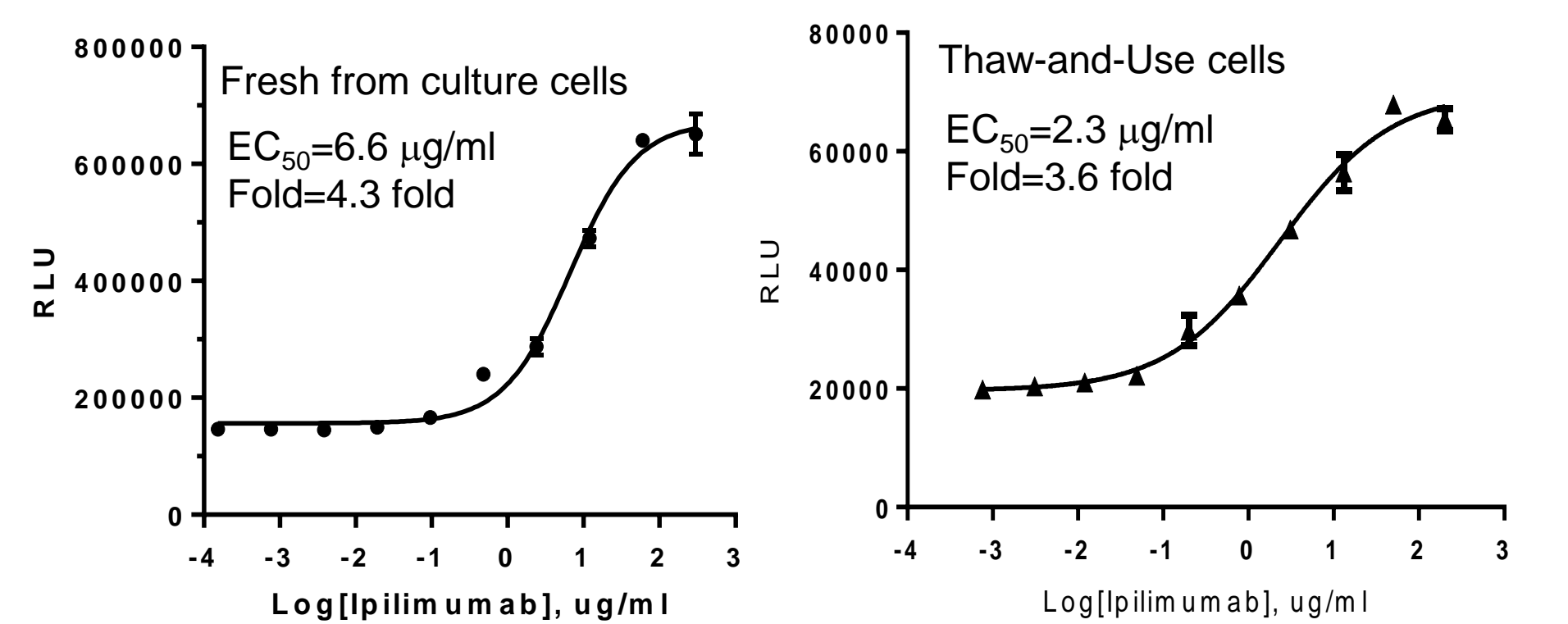
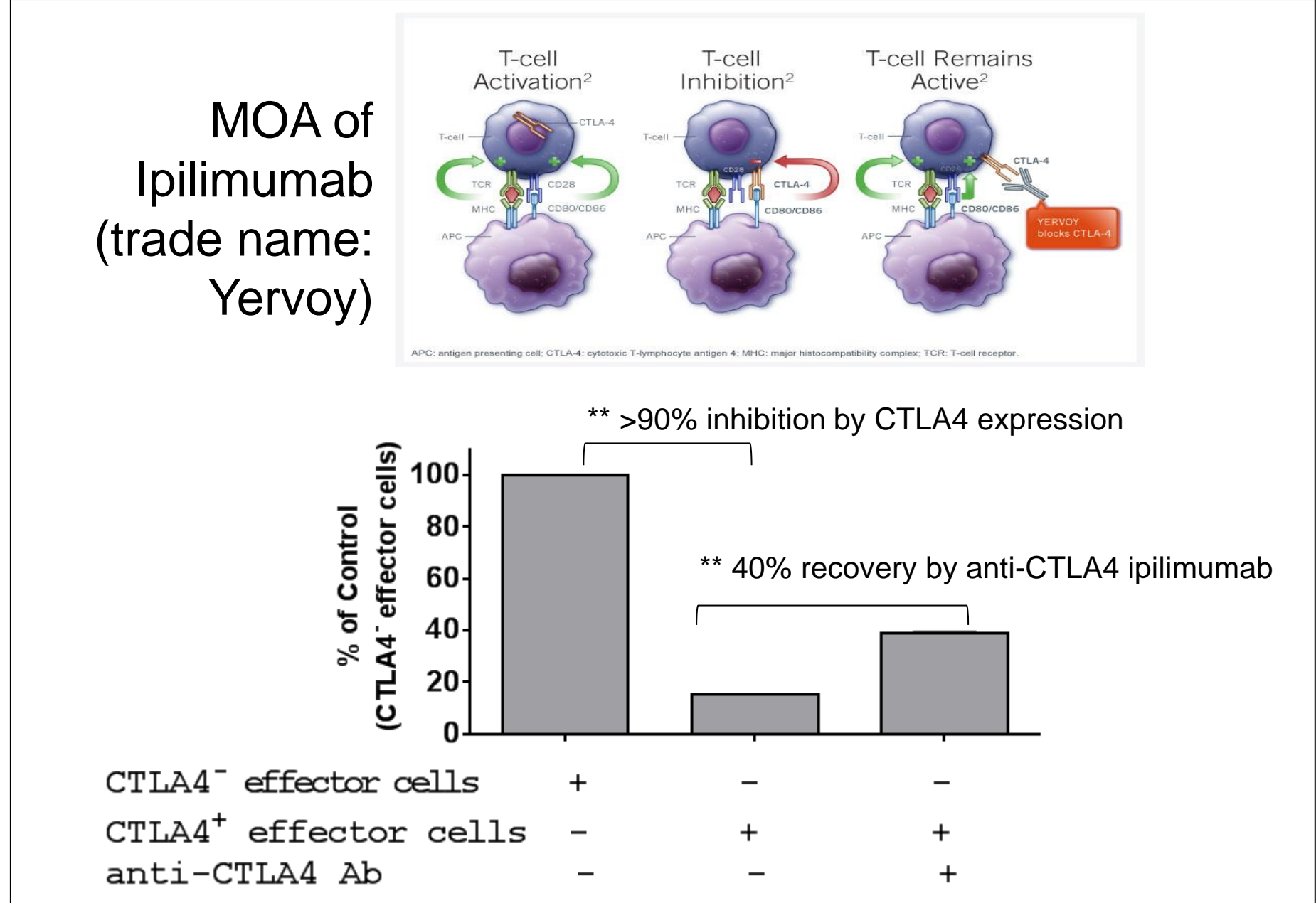


Dose-dependent reporter response to a bispecific antibody (CD3 and EpCAM) catumaxomab (Trade name: Removab) in Jurkat reporter cells using EpCAM⁺ target cells SK-BR-3 and MDA-MB-231.



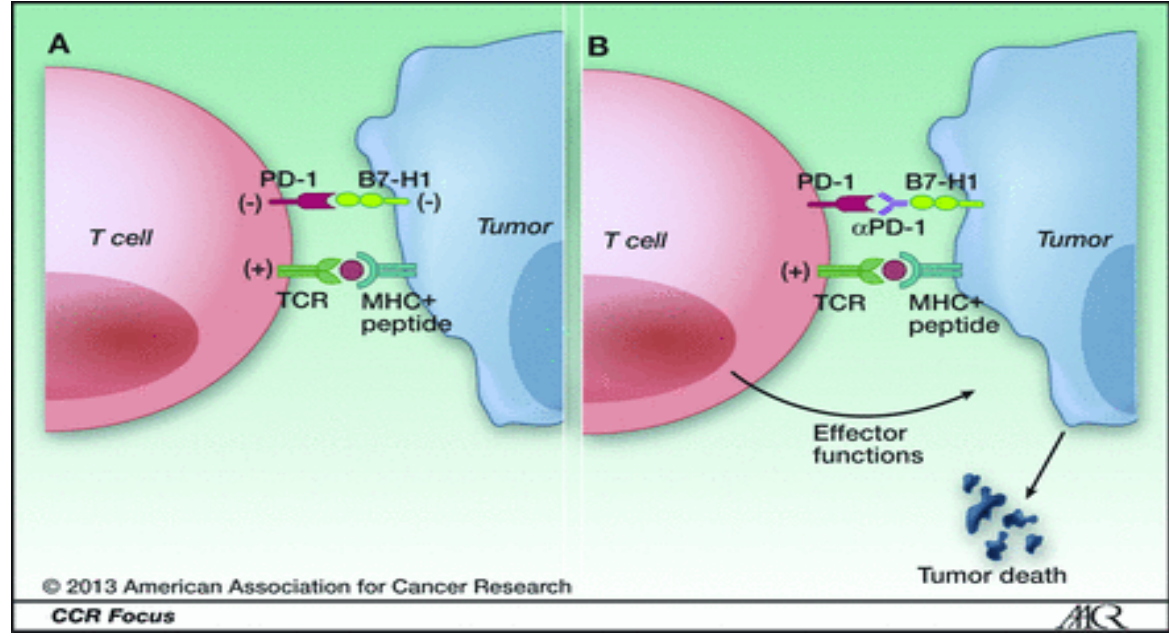
Specific inhibition by abatacept (Trade name: Orencia), a CTLA-4-Fc fusion, in Jurkat reporter cells after stimulation with cross-linked CD3 antibody and Raji cells which express CD28 / CTLA-4 ligand, B7.

5. CTLA-4 Assay

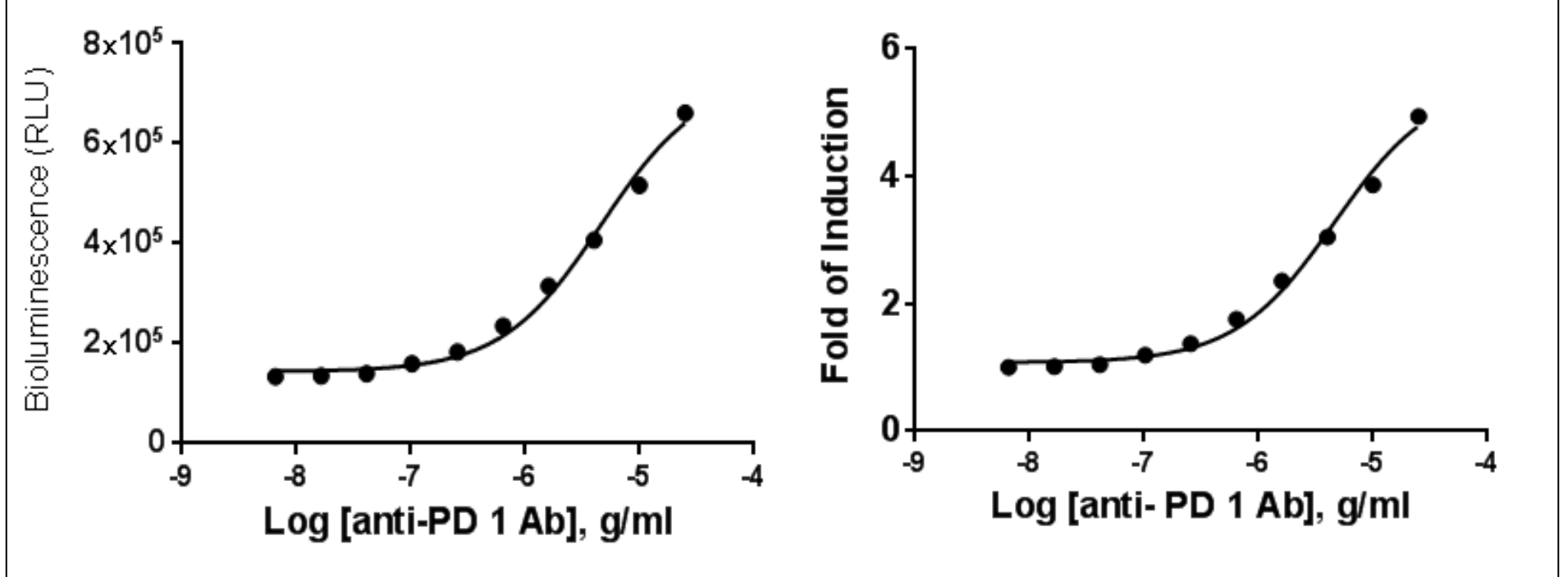
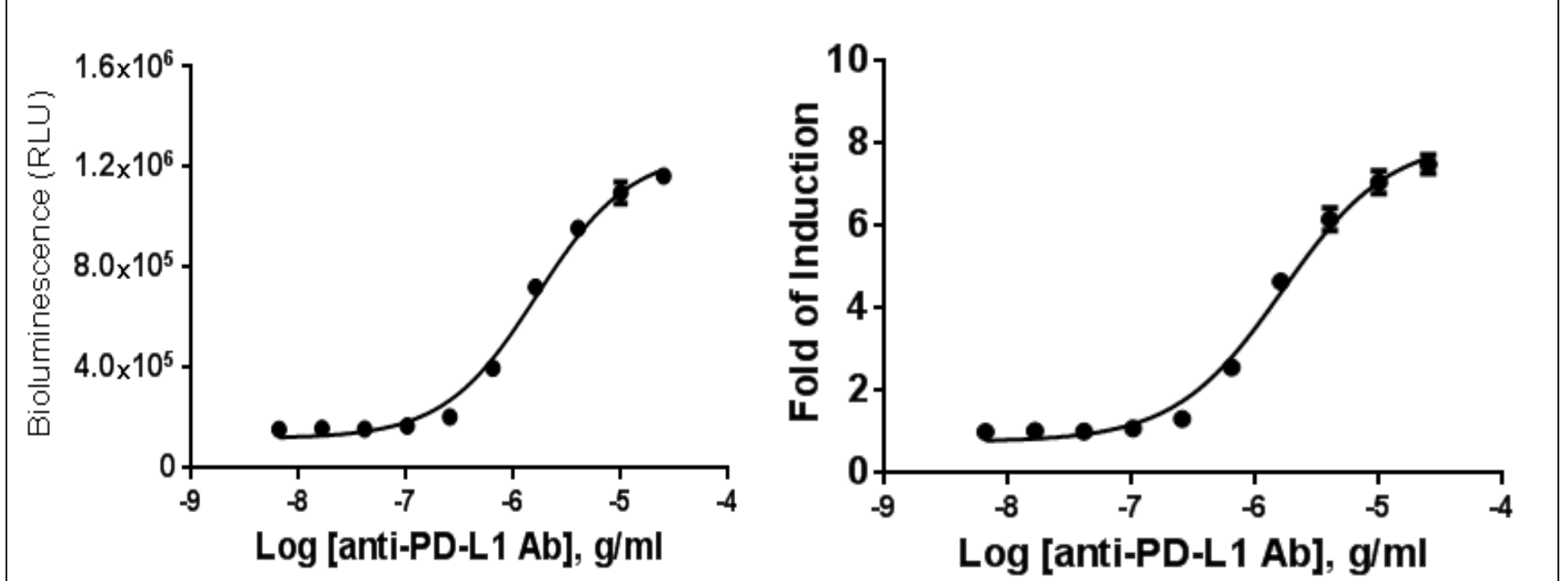
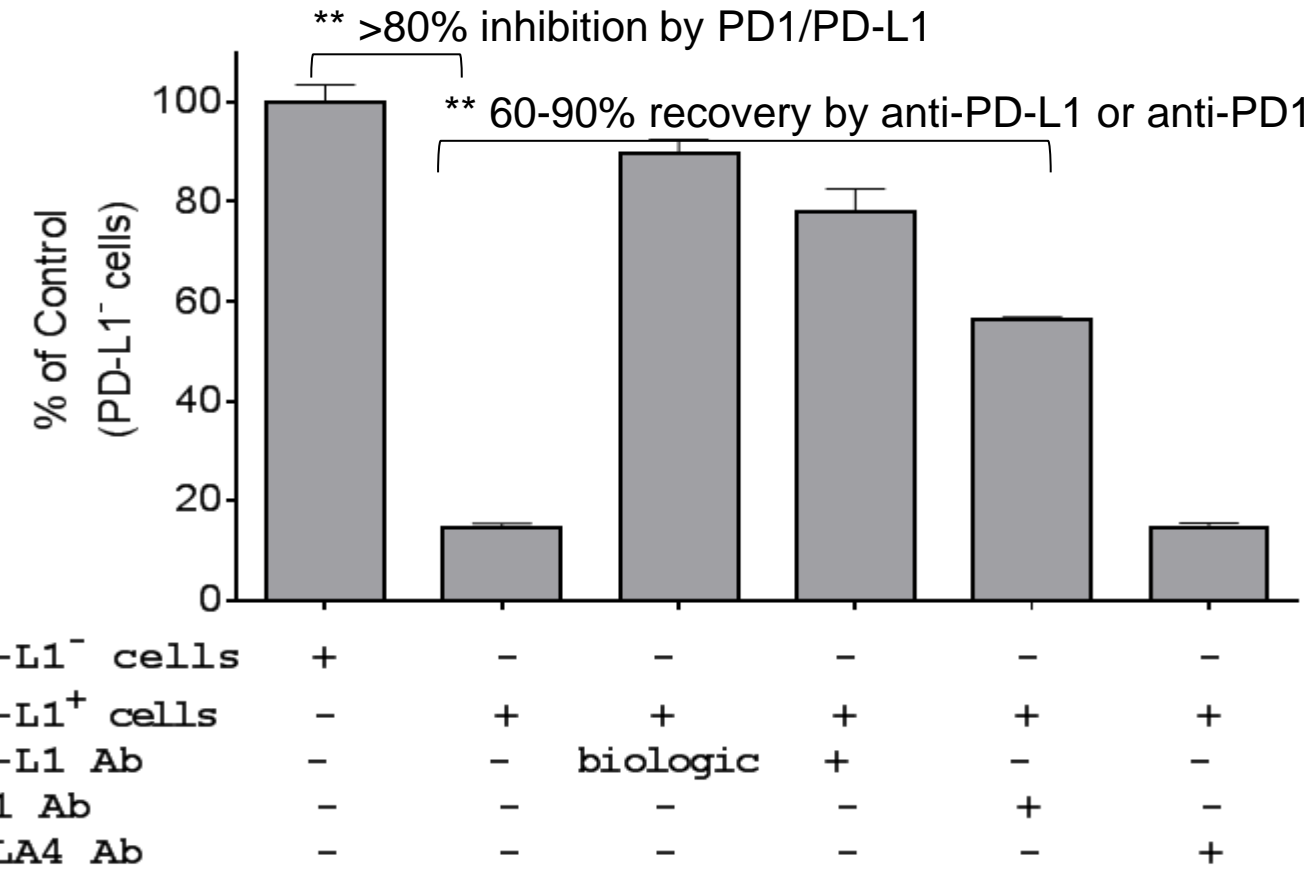


Equivalent response by ipilimumab in Jurkat / CTLA-4 / IL-2 reporter effector cells, fresh-from-culture or thaw-and-use, after incubation with Raji cells in the presence of cross-linked CD3 Ab.

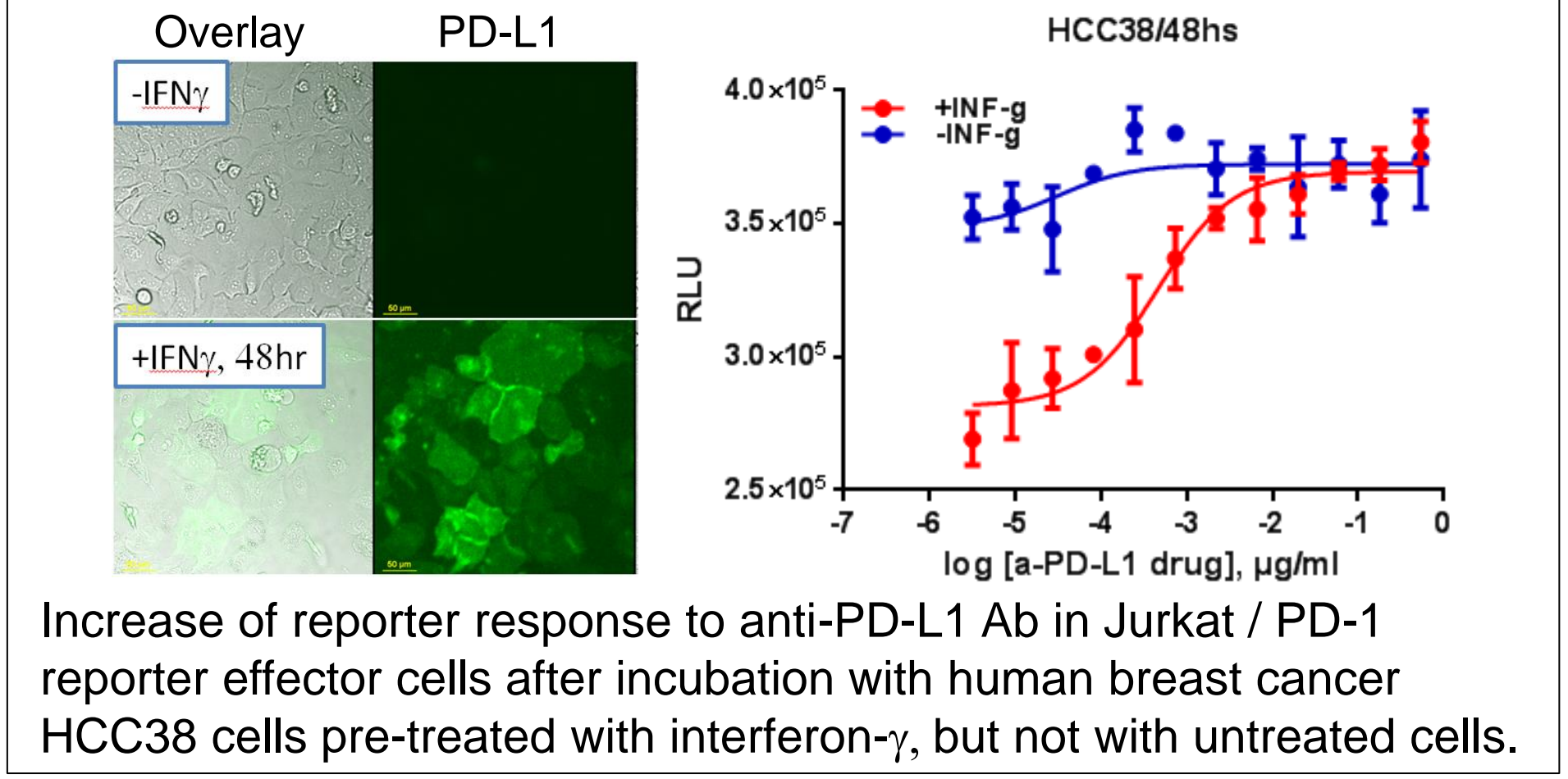
6. PD-1 Assay



MOA of PD-1 or PD-L1 blocking antibody

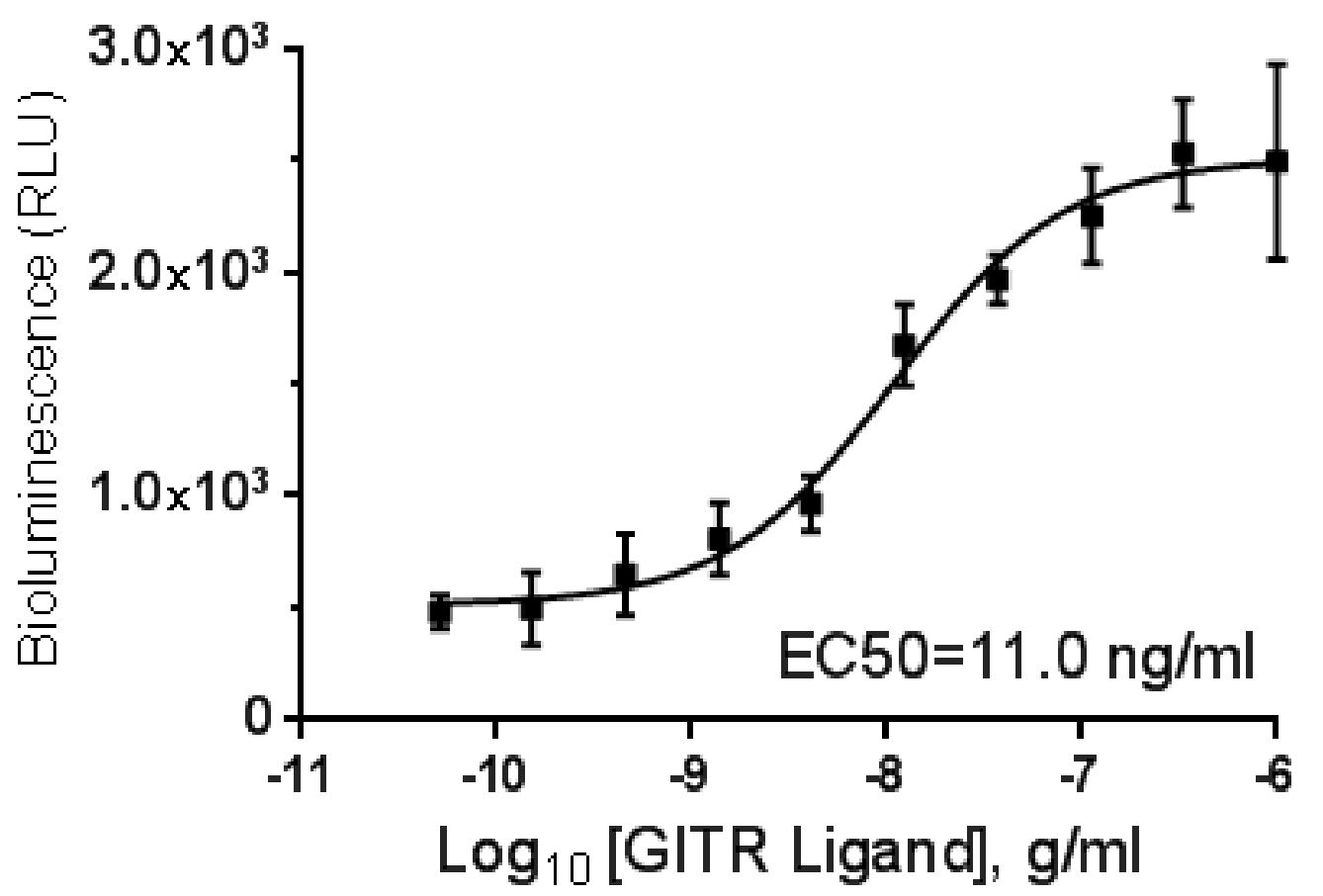


Dose-dependent increase of reporter response to anti-PD-L1 or anti-PD-1 Ab (research grade) in Jurkat / PD-1 effector cells after incubation with PD-L1⁺ cells.



7. Immune Co-Stimulatory Receptor Assays

For the co-stimulatory receptors GITR, CD40, OX40, we are developing a stable Jurkat / NF-kB reporter cell line.



Dose-dependent response to GITR Ligand in transient Jurkat / GITR / NF-kB reporter cells.

8. Conclusions

- Here we report the development of a panel of robust reporter assays to measure the potencies for biologics in immunotherapy.
- These assays reflect mode of action and can serve as valuable tools in immunotherapy drug development and discovery.