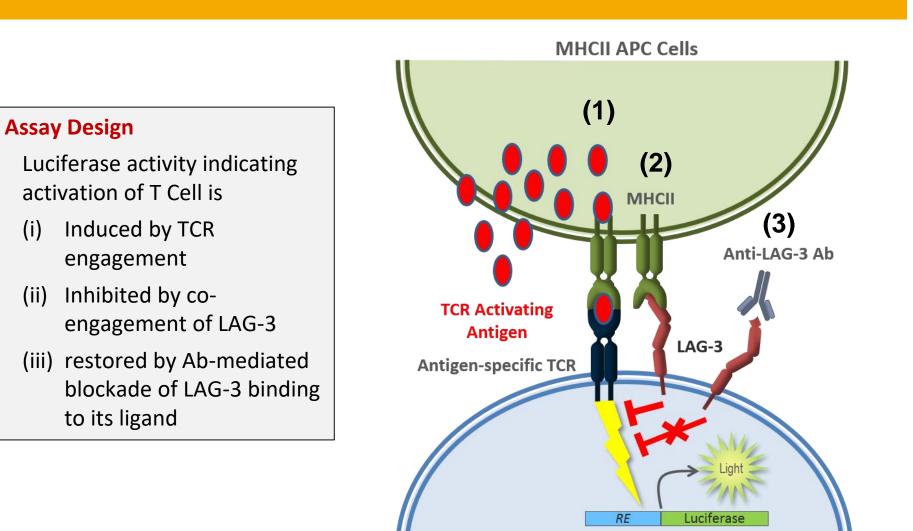
# **Quantitative Cell-Based Reporter Gene Bioassays to Advance Individual or Combination Cancer Immunotherapy**

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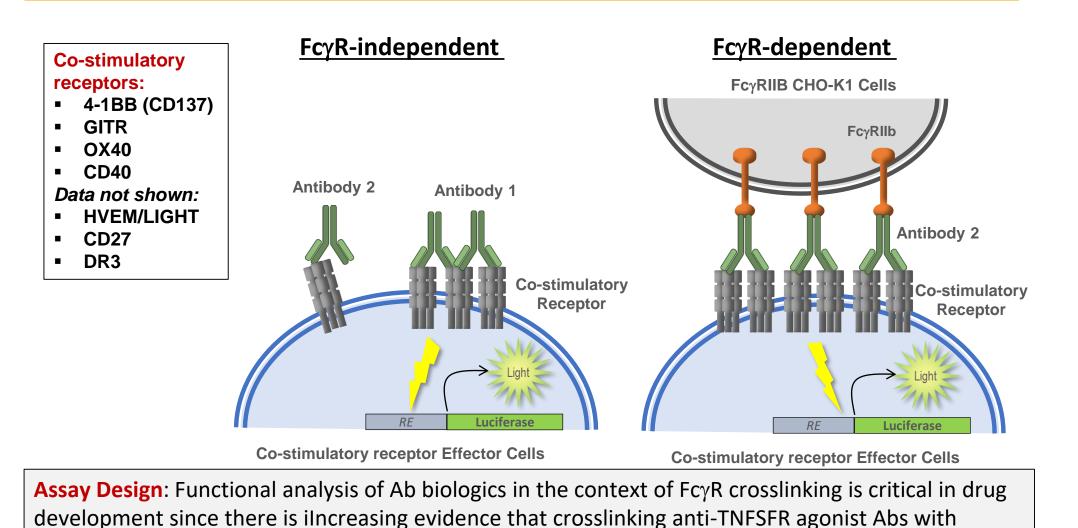
## **1. Introduction**

A major challenge in the development of antibody-based biologics drugs is access to quantitative and reproducible functional bioassays. In contrast to the cumbersome, variable methods currently used that rely on primary cells, we have developed a portfolio of functional cell-based reporter bioassays to easily measure the activity of biologics drugs designed to target immune checkpoint receptors including **co-inhibitory** (e.g. PD-1, CTLA-4, LAG-3) and **co-stimulatory** (e.g. 4-1BB, GITR, OX40) receptors. These bioassays consist of stable cell lines that express luciferase under the precise control of receptor-mediated intracellular signals. Here we describe the application of these MOA-based bioassays for biologics drug discovery, development, potency and stability studies.

## 4. LAG-3/MHCII Blockade Bioassay



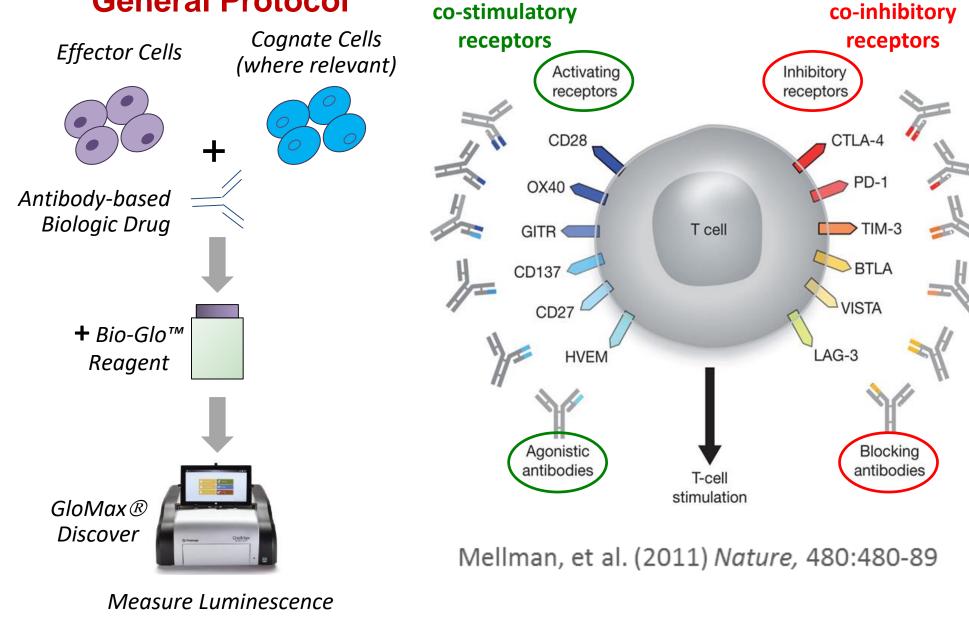
## **7.** FcγR-independent and –dependent **Co-stimulatory Bioassays**



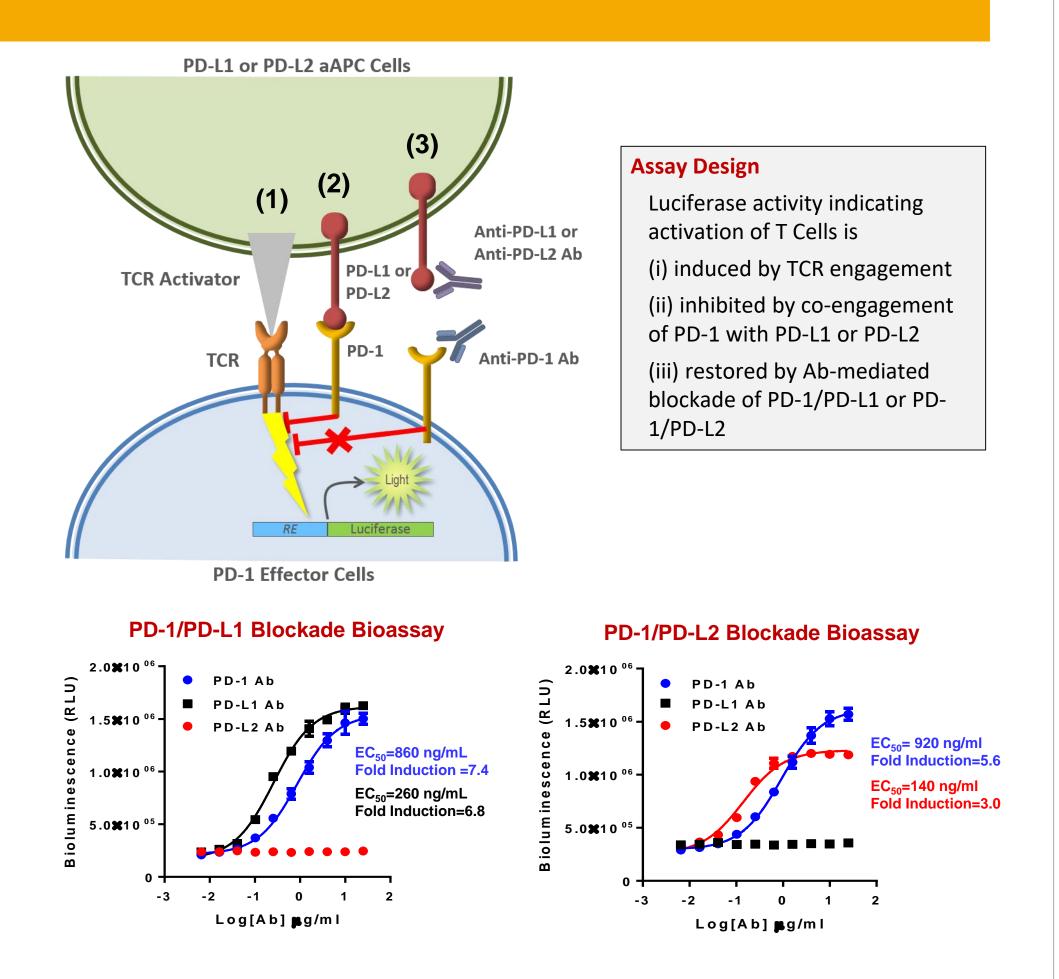
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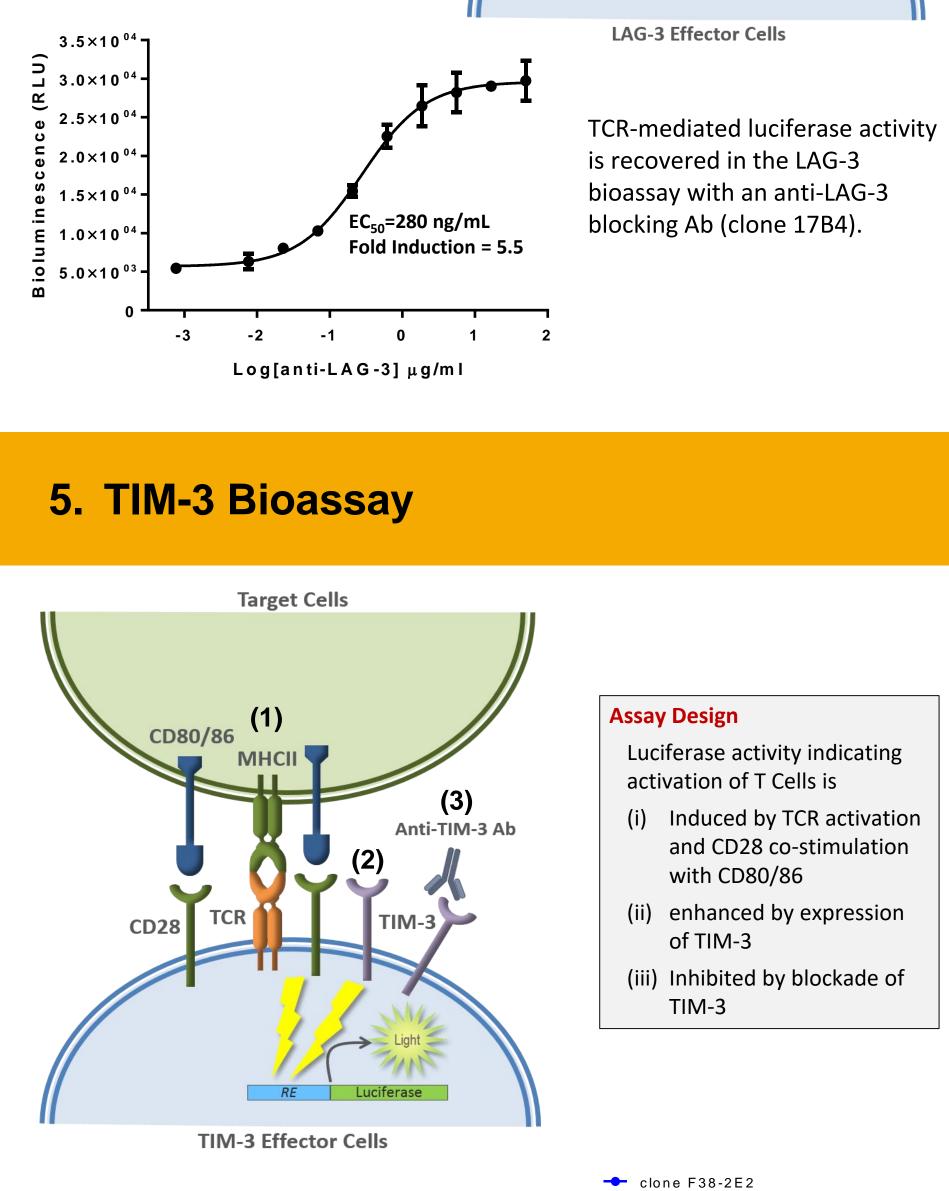
#### **General Protocol**

co-inhibitory

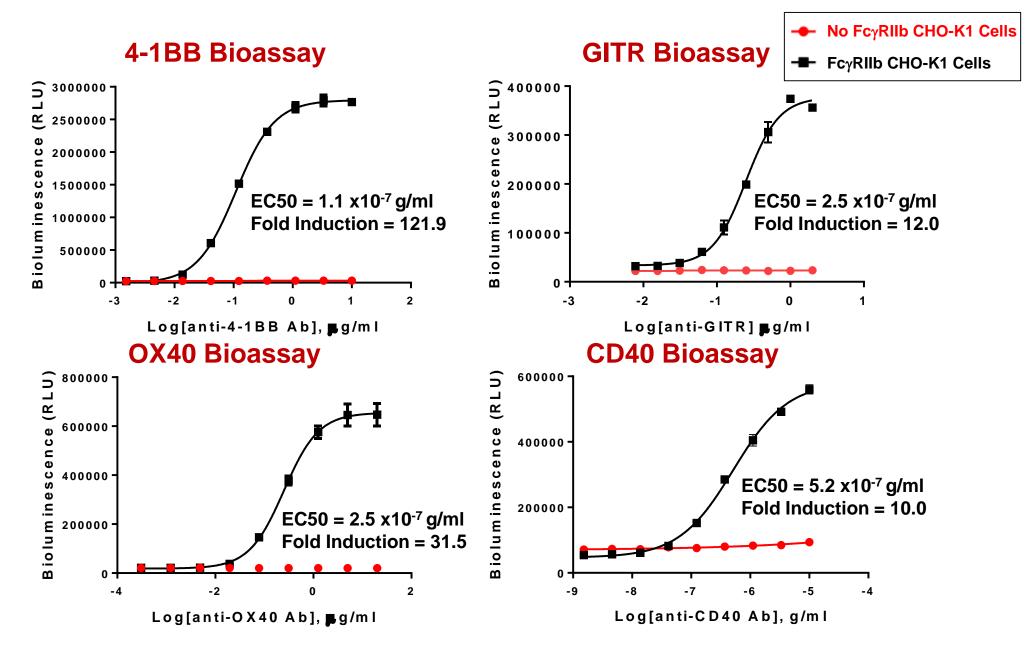


## 2. PD-1 Blockade Bioassays



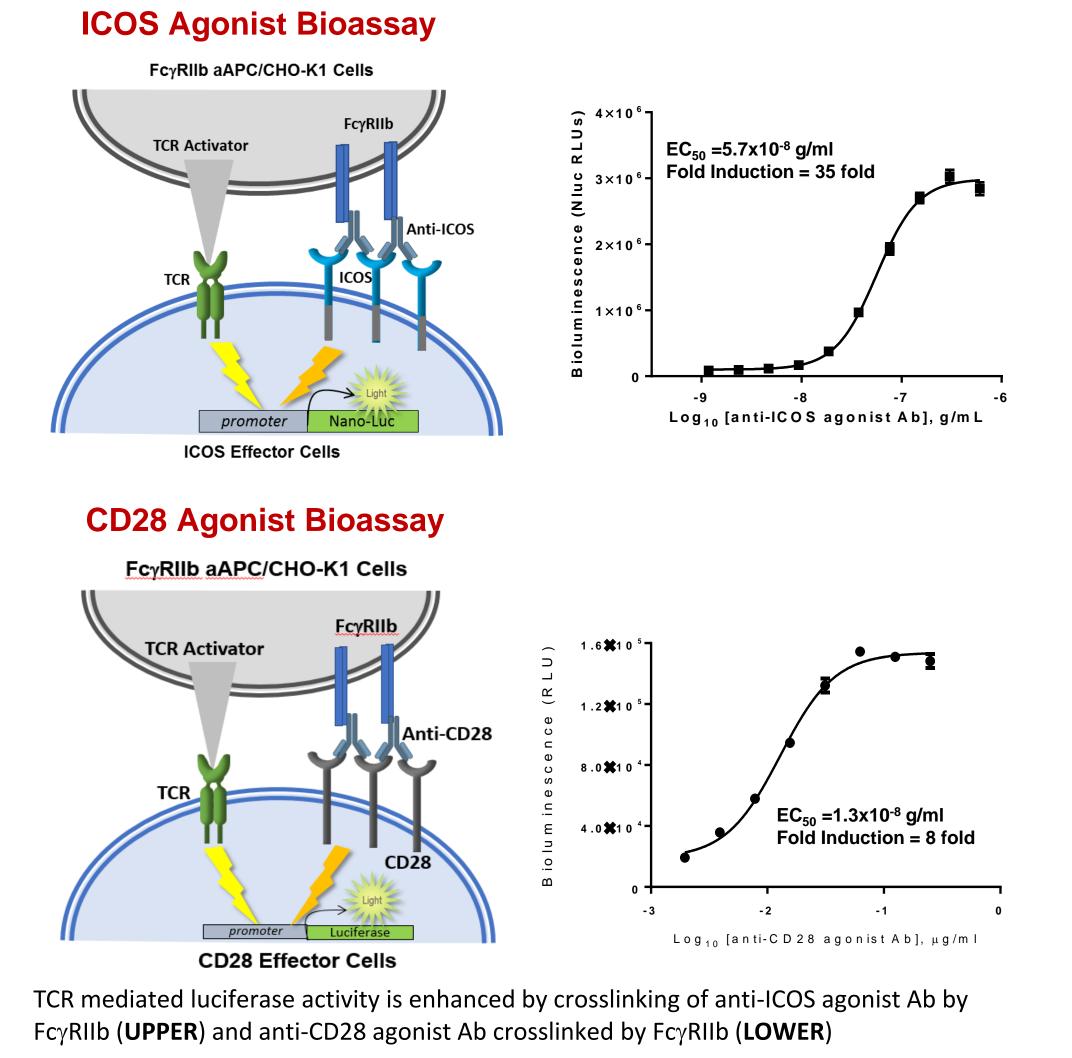


FcγRIIb enhances receptor clustering and downstream signaling (**RIGHT**) whereas such cross-linking may not be always necessary (LEFT) to induce luciferase activity indicating activation of T Cells.



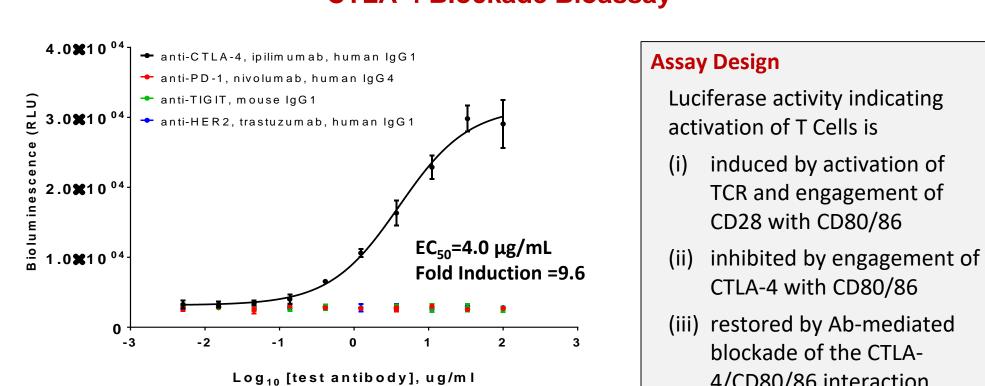
Similar agonist activity is observed using **4-1BB, GITR, OX40 and CD40 ligands** (data not shown).

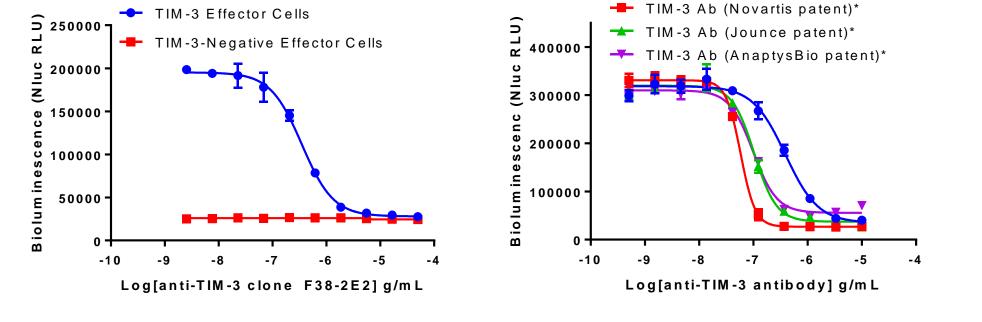
## 8. ICOS and CD28 Bioassays



TCR mediated luciferase activity is recovered in the PD-1 Blockade Bioassay with (LEFT) anti-PD-1 and PD-L1 blocking Abs and anti-PD-1 and PD-L2 blocking Abs (**RIGHT**), but not with unrelated control antibodies.

## 3. CTLA-4 and TIGIT Blockade Bioassays

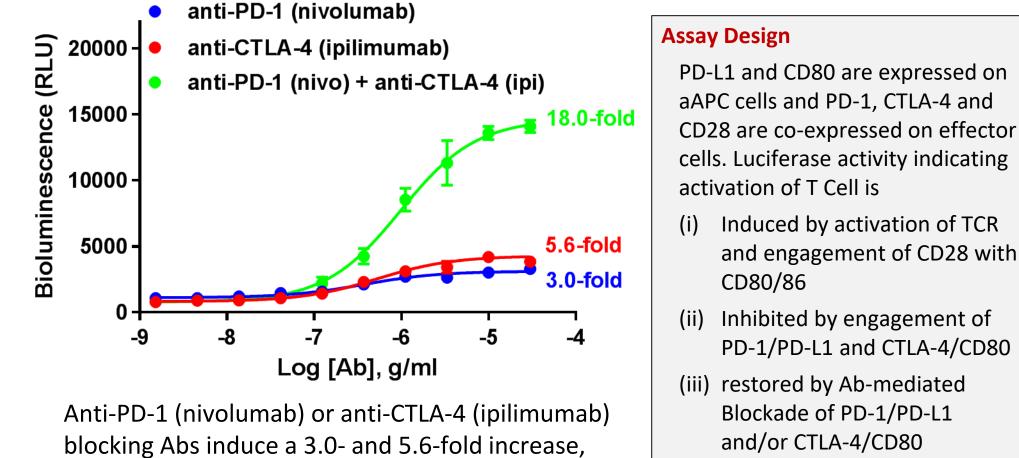




TCR/CD28-mediated luciferase activity is enhanced with expression of (LEFT) TIM-3 in effector cells, which is inhibited in a dose-dependent manner with the anti-TIM-3 blocking Ab. TIM-3-mediated luciferase activity is inhibited (**RIGHT**) in a dose-dependent manner with clone F38-2E2 and TIM-3 blocking antibodies from Novartis, Jounce, and AnaptysBio.

## 6. Combination Bioassays

#### **PD-1+CTLA-4 Combination Bioassay**



**ICOS and CD28 Bioassays** for blocking antibodies are also available (data not shown).

## 9. Conclusions

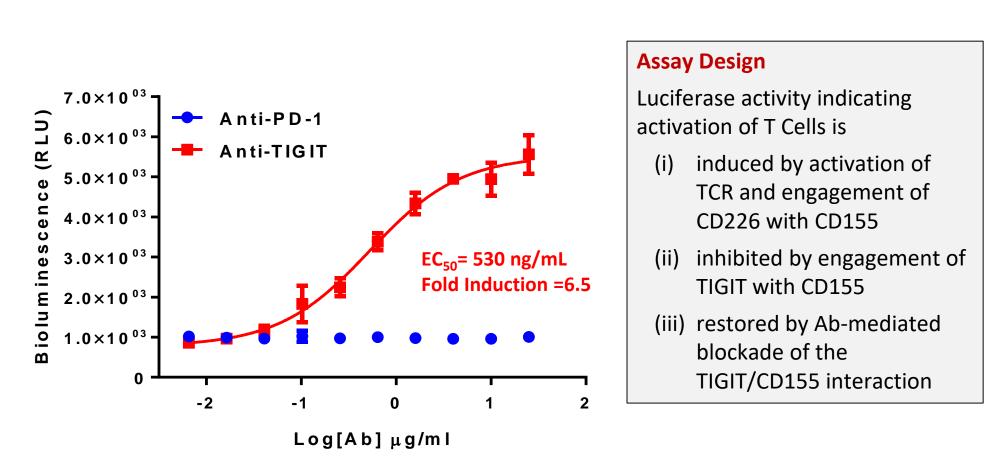
Cell-based reporter bioassays overcome the limitations of primary cellbased assays for functional characterization of antibody and other biologics drugs targeting individual or combination immune checkpoint receptors. Here we show a portfolio of MOA-based bioassays for coinhibitory and co-stimulatory immune checkpoint receptors that can be used for antibody screening, characterization, potency and stability studies. These bioassays provide the following:

#### **CTLA-4 Blockade Bioassay**

4/CD80/86 interaction

TCR and CD28-mediated luciferase activity is recovered in the CTLA-4 bioassay with an anti-CTLA-4 blocking Ab (ipilimumab), but not with unrelated control antibodies.

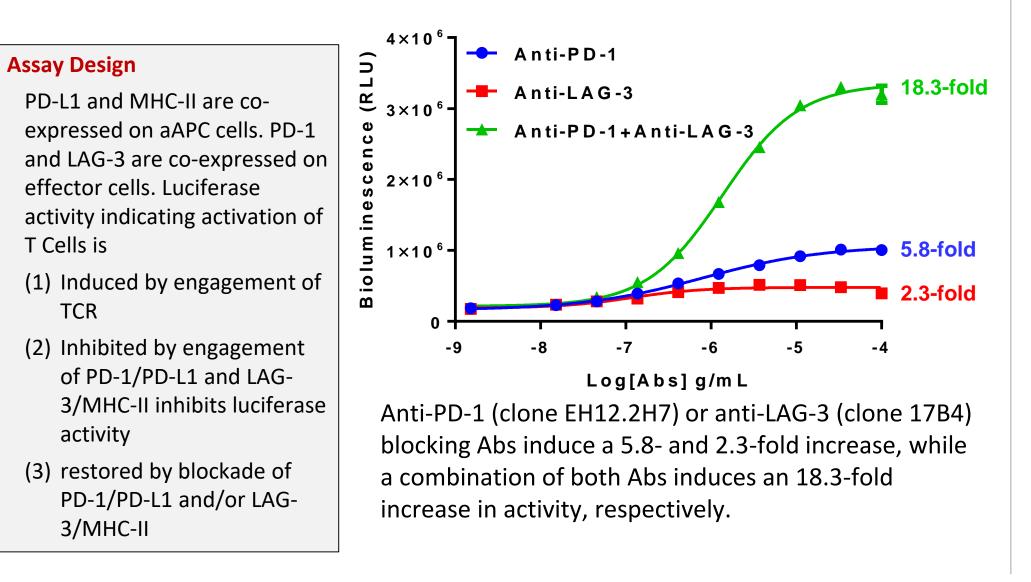
#### **TIGIT/CD155 Blockade Bioassay**



TCR- and CD226-mediated luciferase activity is recovered in the TIGIT/CD155 bioassay with an anti-TIGIT blocking Ab (clone MBSA-43), but not with an anti-PD-1 blocking Ab (nivolumab).

while a combination of both Abs induces an 18-fold increase in luciferase activity, respectively.

#### **PD-1+LAG-3** Combination Bioassay



Similar results were achieved using the **PD-1+TIGIT Combination Bioassay** (data not shown).

#### **Biologically relevant measurement of antibody MOA**

- Specific immune checkpoint regulated expression of luciferase that reflects the native biology of T cell activation.
- Demonstrated ability to measure the potencies of immune checkpointtargeted antibodies

### **Consistent and reliable measure of antibody activity**

- Demonstrated precision, accuracy, reproducibility, robustness
- All assays can be used as "Thaw-and-use" cell format, no cell culture required
- Functional performance suitable for development into potency, stability, and NAb assays

#### **Easy-to-implement**

- Rapid and convenient workflow
- Amenable to standard 96-well and 384-well plate formats

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