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Accelerating Immunotherapy Drug Development with Simple Cell-Based Assays for Immune Checkpoint Receptors

Jennifer Lin-Jones, Ph.D.

Senior Group Leader, Assay Development

October 2021

Eurofins DiscoverX
Strong Foundation | Technical Expertise | End-to-End Solutions

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Dedicated Operations

Supporting programs from Research, Discovery to Lot Release

- **Products Division headquartered in Fremont, CA**
- Additional sites in Missouri, USA and Poitiers, France
- **800+ off-the-shelf assays for in-house development**
- Over 10,000 customers in NA, APAC and EMEA

Deep Domain Expertise

Over 45 years of cumulative technical experience in

- **Cell line engineering & characterization**
- **Bioassay development, optimization & qualification**
- Analytical Cell Banks
- Membrane Preps and Frozen Assay Ready Cells
- Bulk Enzyme Production

Established Brand

Successfully implemented at global Pharma, Biotech & CRO

- **Products implemented in discovery & development**
- Over 50 billion data points screened
- **2,000+ publications**
- Several active Biotech/CRO-partnered programs
- **Implemented in lot release of several marketed biologics**

Enzyme Fragment Complementation (EFC)
Robust Platform for Cell-Based Assays

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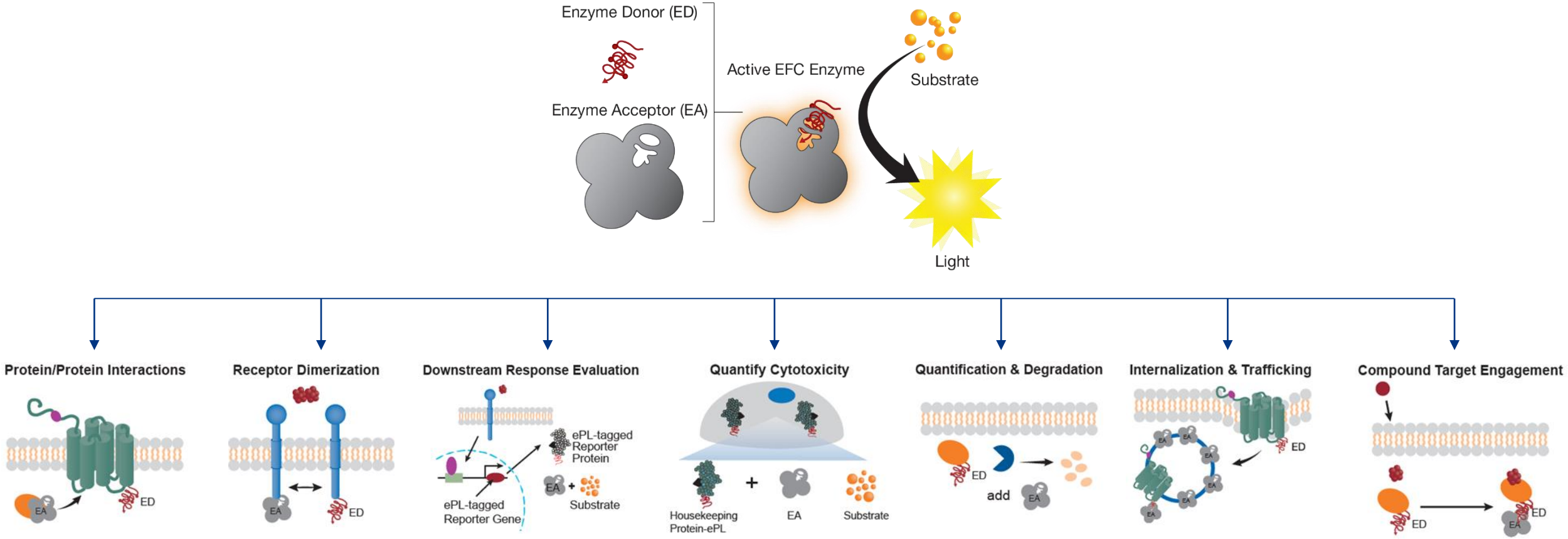
Split β-galactosidase reporter system can be engineered to generate target-specific, homogeneous cell-based assays for immuno-oncology therapeutics



Targeting T-Cell Co-Stimulatory and Inhibitory Checkpoint Receptors

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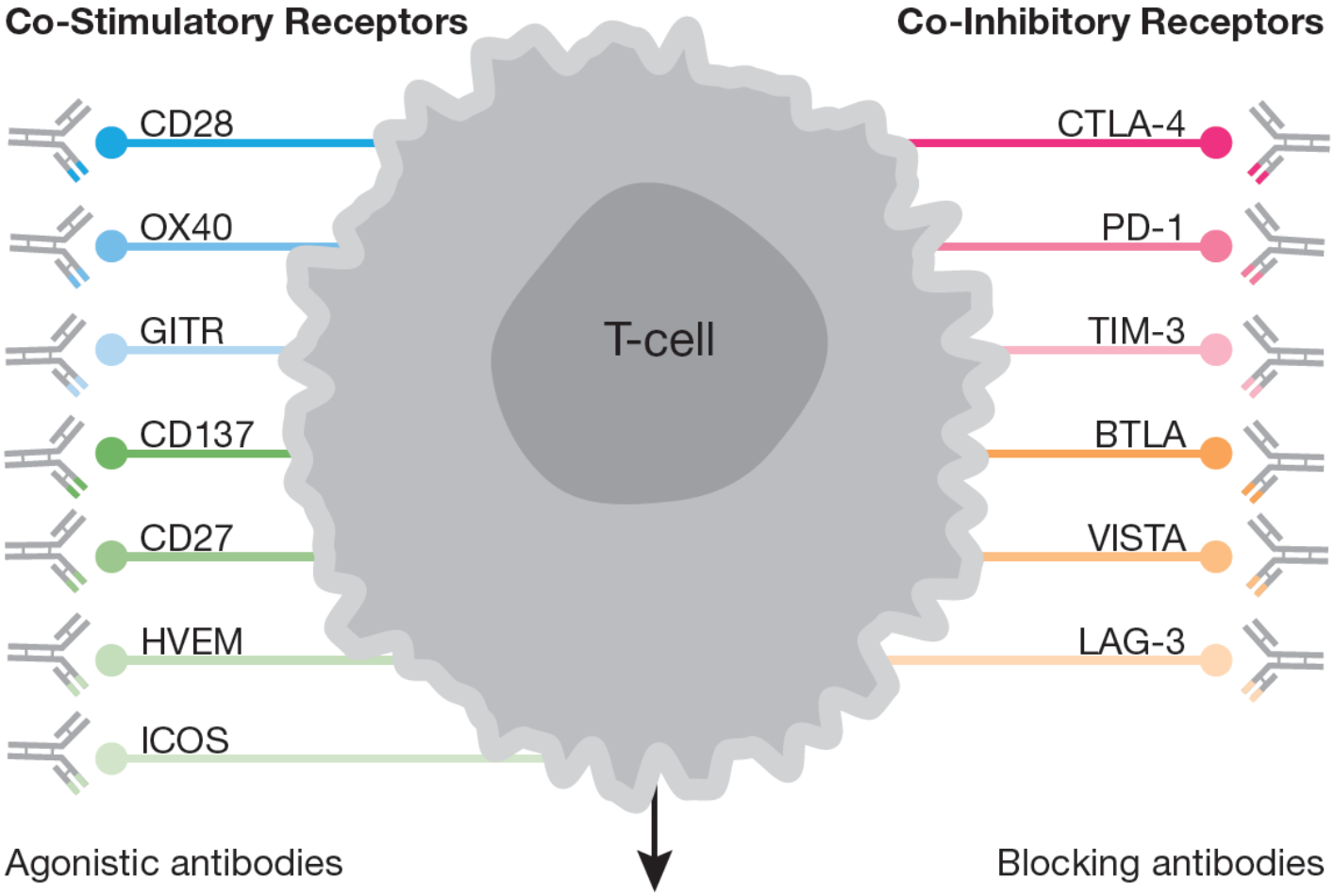
Tools are needed to screen for and develop new therapeutics

Push the gas pedal on T cell activation to stimulate the immune system



Remove the brakes inhibiting T cell activation to stimulate the immune system

Modulate immune response to destroy cancer cells

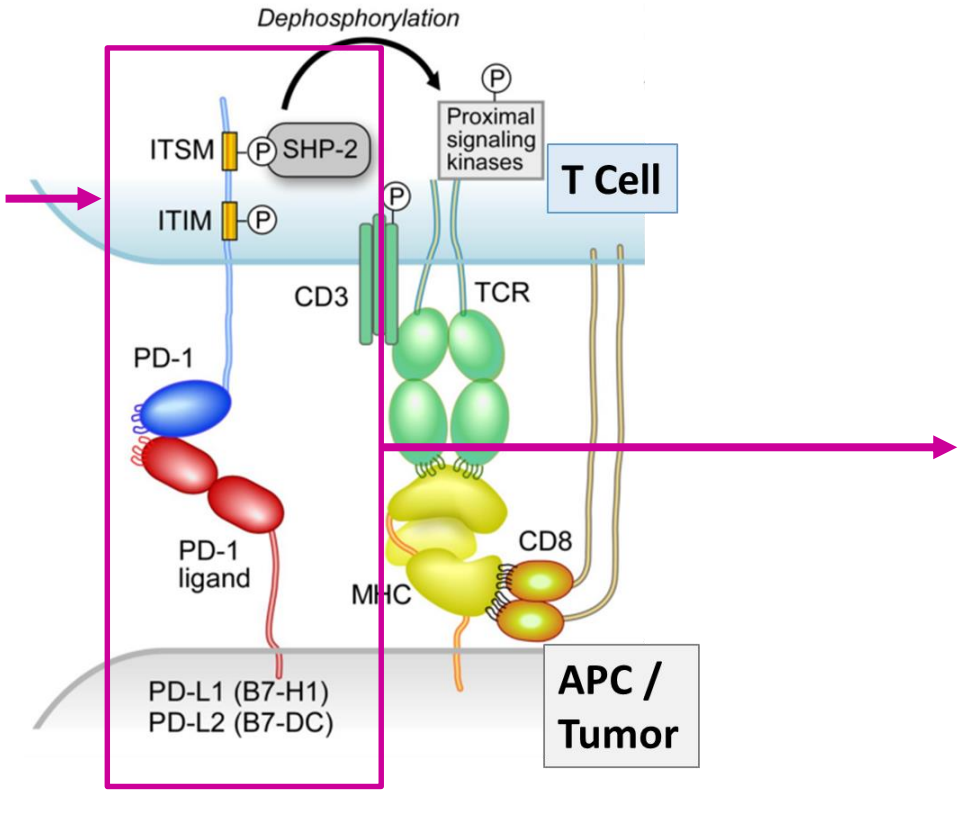
PD-1 Signaling Assay Concept

Quantify early step in PD-1 mediated inhibition of T cell activation: SHP recruitment

Mechanism of Action

Assay measures this early event:

SHP recruitment



Assay Design

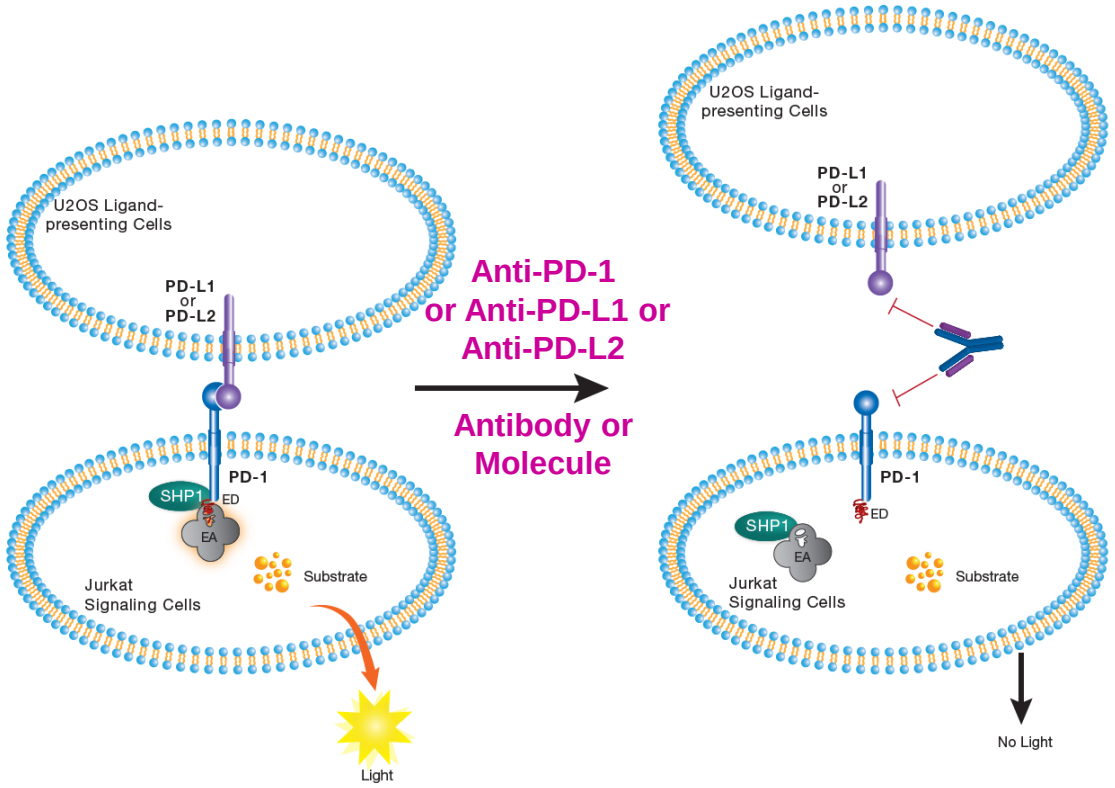
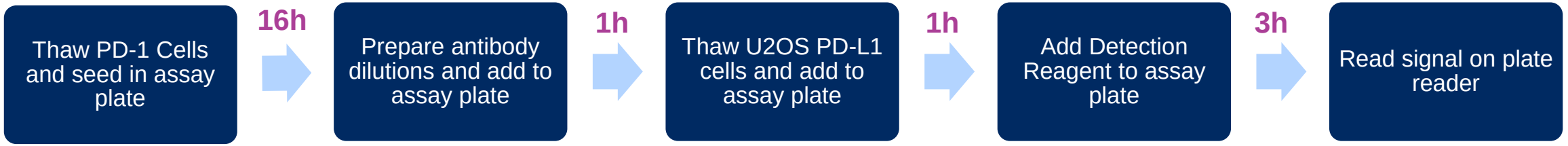


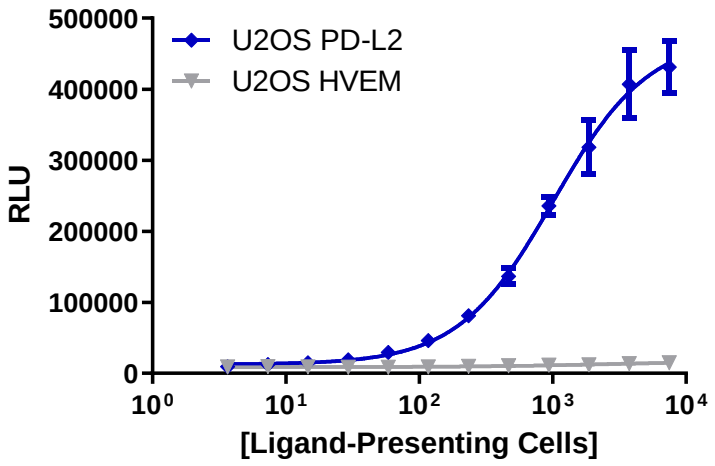
Figure from Science Webinar Series, Part 5: Gordon J. Freeman, Ph.D.

PD-1 Signaling Assay is Highly Specific and Stability-Indicating

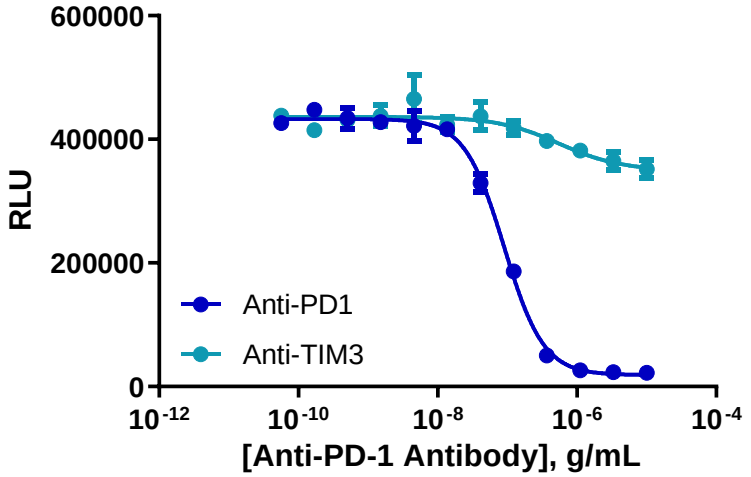
Assay-Ready (RTU) Protocol



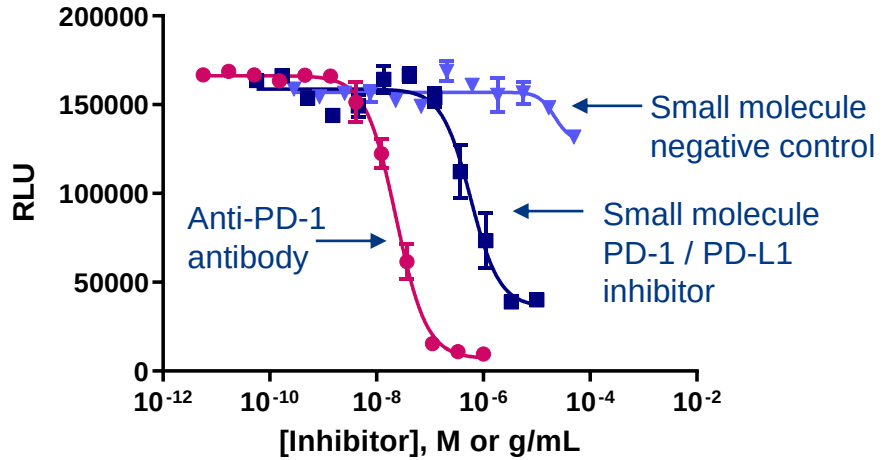
Specificity (Agonist)



Specificity (Antagonist)



Suitable for Small Molecules or Biologics



PD-1 Signaling Assay Suitable for Therapeutic Development of Both PD-1 Agonists and Antagonists

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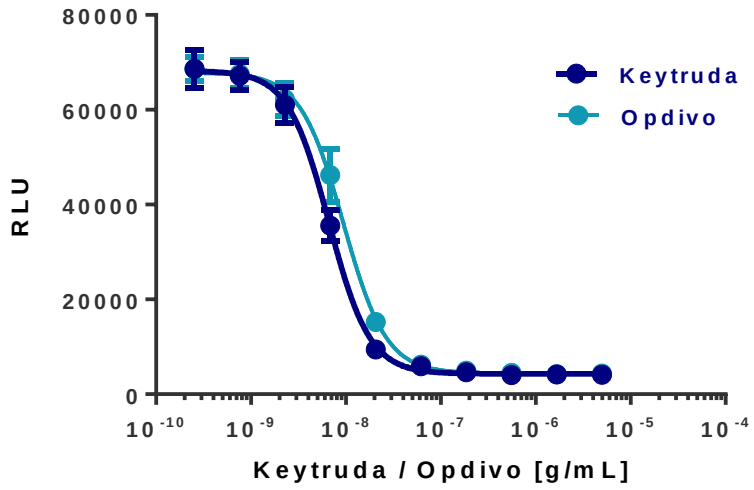
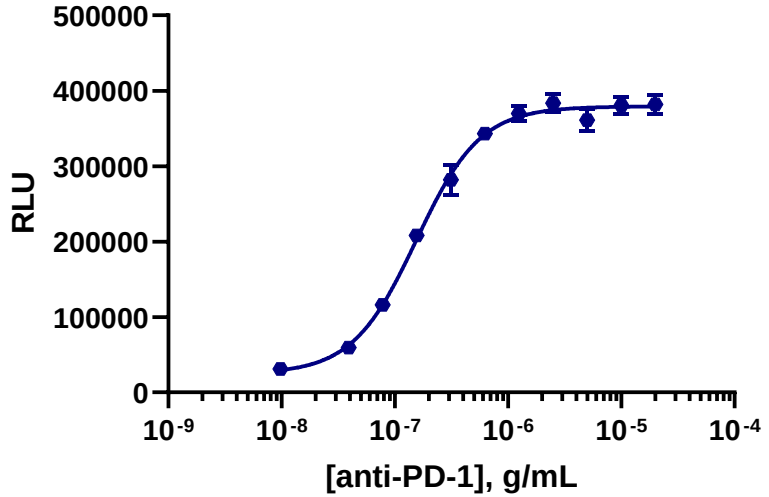
Immuno-oncology

PD-1 Antagonist



Auto-immunity

PD-1 Agonist

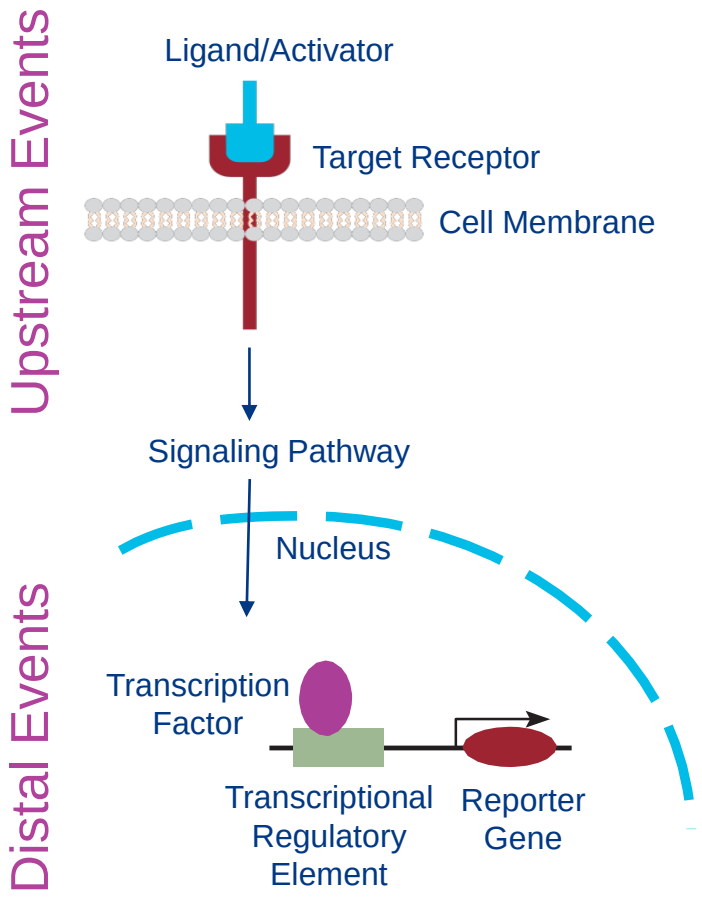


KEYTRUDA® (pembrolizumab) Injection 100 mg

OPDIVO® (nivolumab)
INJECTION FOR INTRAVENOUS USE 10 mg/mL

PD-1 Signaling assay is a versatile screening tool for PD-1 antagonists (I/O) or for PD-1 agonists (novel therapies for autoimmunity). The latter data were generated in co-culture with Fcγ receptor expressing cells.

Complementary Cell-Based Assays for Comprehensive Understanding of a Drug Molecule's MOA



Cell-based Signaling Pathway Assays	
Upstream (Receptor-Proximal) Readout (Non-Reporter Gene Assays)	**Downstream (Distal) Readout** (Reporter Gene Pathway Assays)
Represent a readout more proximal to receptor activation and upstream of signal transduction and transcriptional changes.	Represent a distal pathway readout reflective of a phenotypic endpoint (e.g. proliferation)
Measure status of a specific functional event upstream of transcription	Measure changes in transcription that affect reporter protein expression
Results may more directly reflect drug MOA: measures a specific functional event	Results can integrate effects from multiple inputs on a signaling pathway: sum of downstream signaling events
Produce modest to large assay windows, with short assay times and low variability	

Monitor PD-1 Inhibitor Activity Downstream of TCR Activation: Assay Principle

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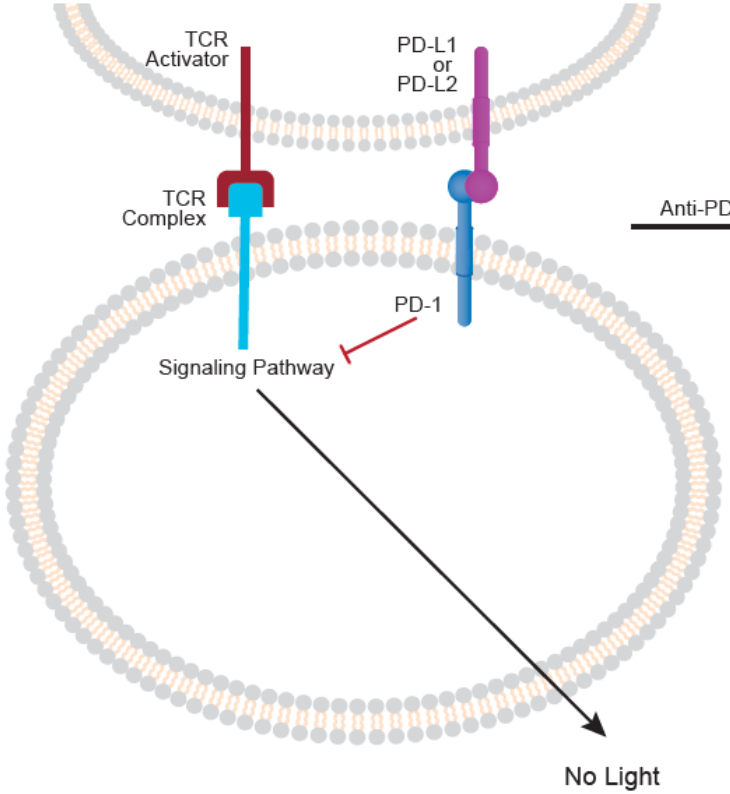
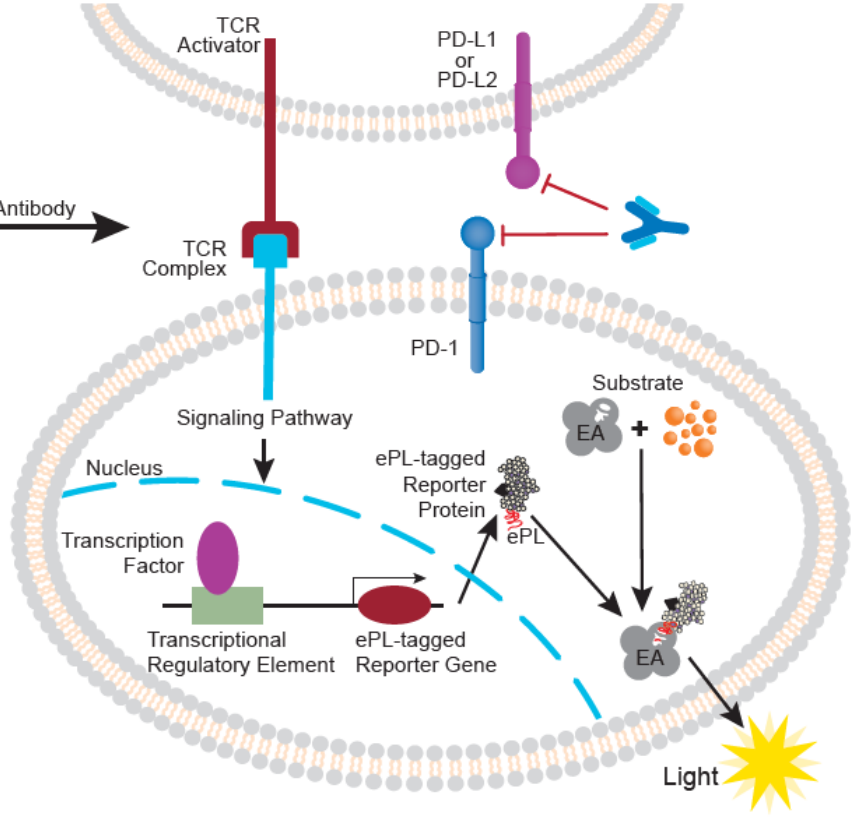
U2OS PD-L1/TCR Activator cells + Jurkat PD-1-NFAT



- TCR activation inhibited by PD-1 activation
- Reduction in reporter protein expression

U2OS PD-L1/TCR Activator cells + Jurkat PD-1-NFAT + anti-PD-1 Ab



- Antibody inhibits PD-1 activation
- PD-1 inhibition of T cell activation released
- Increase in reporter protein expression

TCR = T cell receptor

PathHunter® Jurkat PD-1-NFAT Pathway Reporter Assay:
Evaluate PD-1 Checkpoint Receptor Mediated Inhibition of T Cell Activation

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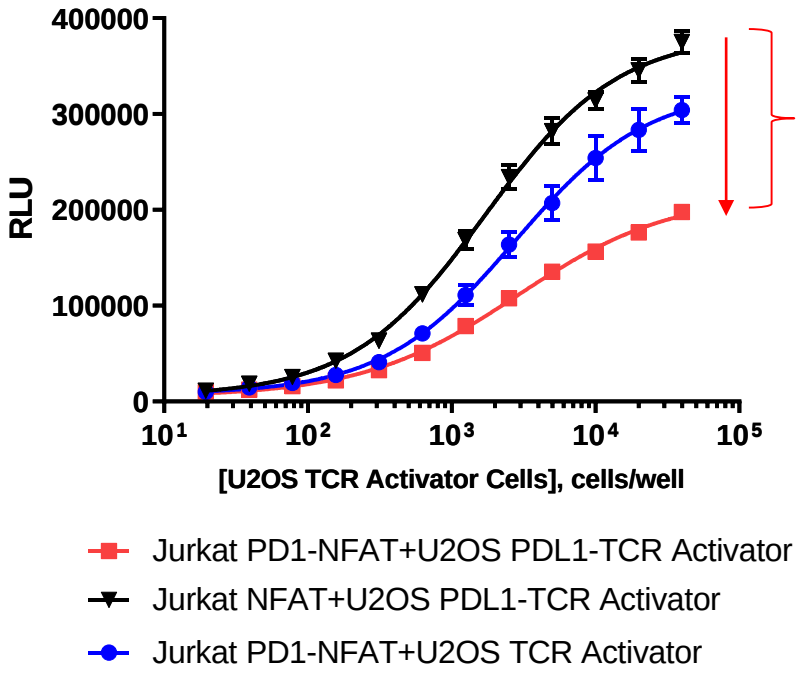
- PD-1 inhibitory receptor introduced into PH Jurkat NFAT cells attenuates T cell activation
- PD-1 checkpoint receptor inhibition of T cell activation can be monitored by PD-1 receptor co-expression in Jurkat NFAT reporter cells in co-cultures with U2OS TCR activator cells co-expressing PD-L1 ligand

Jurkat NFAT / U2OS TCR Activator Co-Cultures



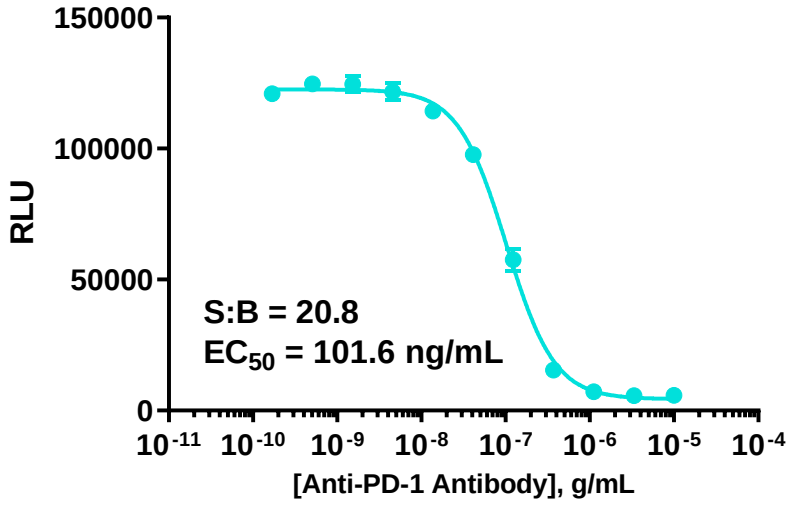
Decreased T cell activation when PD-1 is co-expressed in Jurkat NFAT cells and PD-L1 co-expressed with TCR activator in U2OS

	U2OS TCR activator	
Jurkat NFAT reporter	-PD-L1	+PD-L1
-PD1	(+++)	+++
+PD-1	++	+

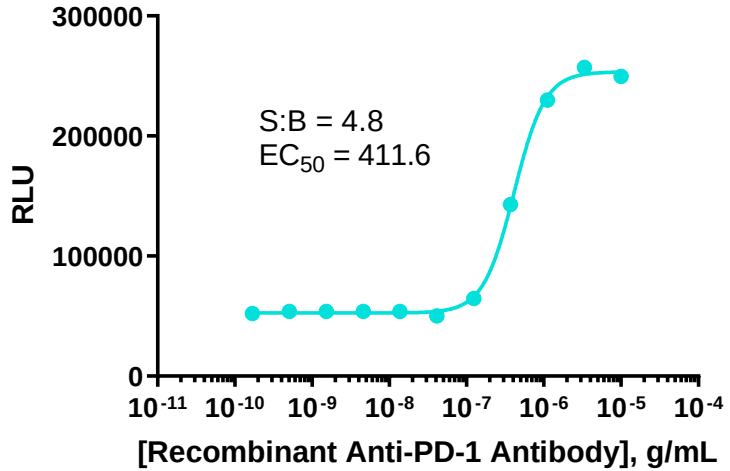
Measure Proximal & Distal Events in the PD-1 Signaling Pathway

- Both PathHunter® PD-1 assays types provide a comprehensive understanding of the PD-1 pathway and the MOA of the anti-PD-1 Antibody
- Assays are robust and measure PD-1 inhibition with sensitive responses from an event either **proximal** (PD-1 SH2 recruitment, Jurkat PD-1 Signaling) to receptor activation, or **distal** (NFAT-regulated reporter, Jurkat PD-1-NFAT)

PathHunter Jurkat PD-1 Signaling Assay
(93-1106C19)



PathHunter Jurkat PD-1 NFAT Reporter Assay
(93-1141C19)



Targeting T-Cell Co-Stimulatory and Inhibitory Checkpoint Receptors

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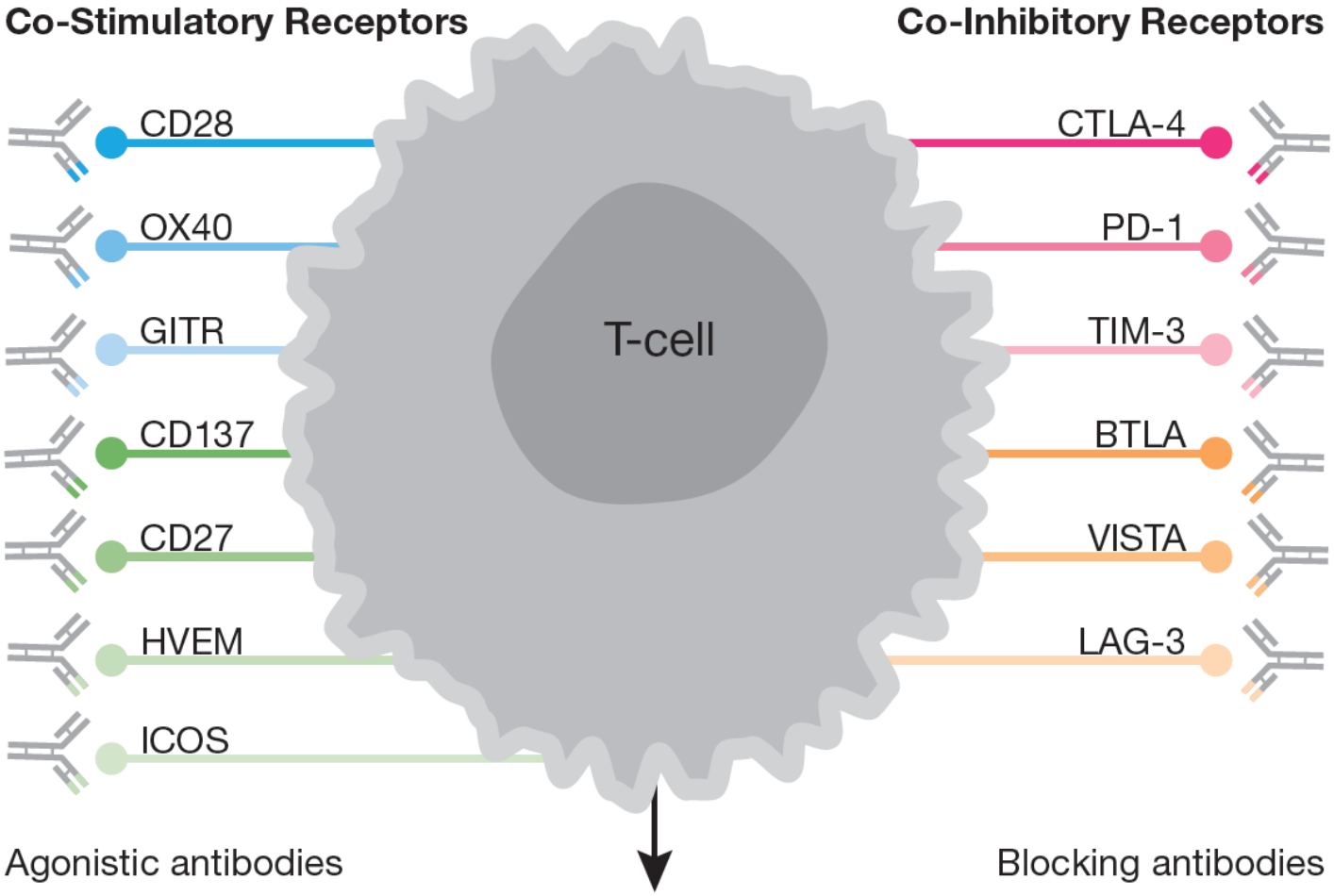
Tools are needed to screen for and develop new therapeutics

Push the gas pedal on T cell activation to stimulate the immune system



Remove the brakes inhibiting T cell activation to stimulate the immune system

Modulate immune response to destroy cancer cells

CD28 and ICOS Co-Stimulatory Receptors Also Signal Through SH2- Domain Containing Proteins

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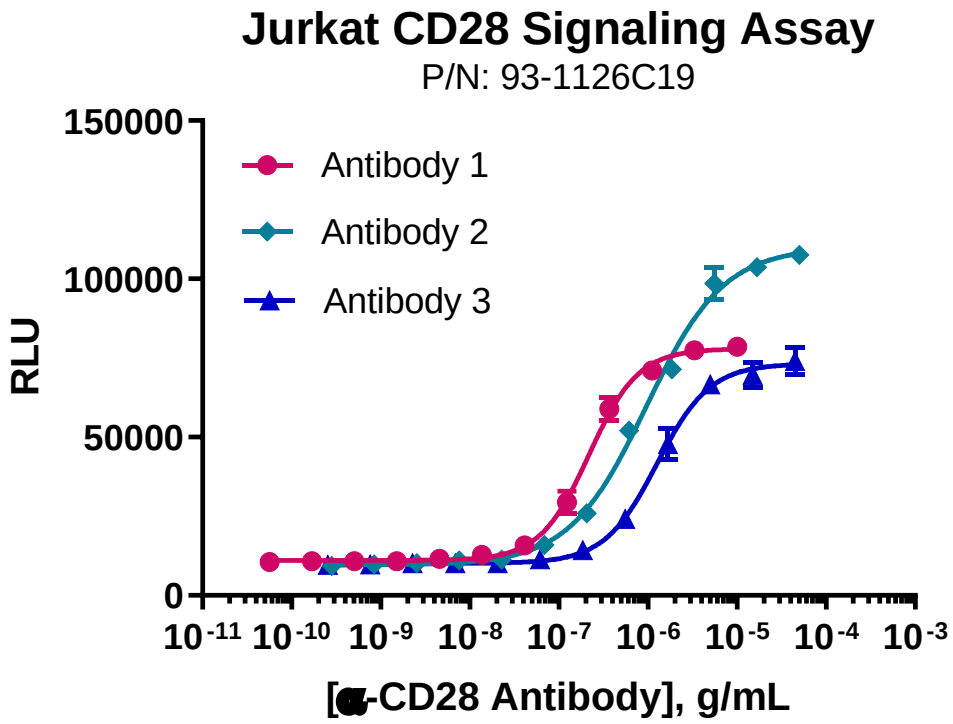
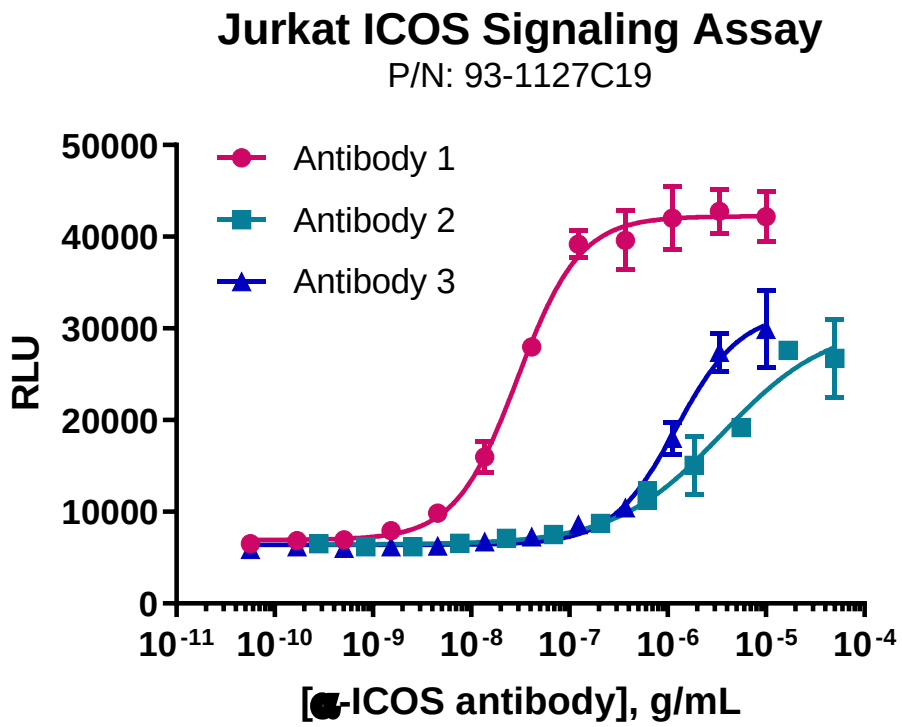
Assays measure ligand-mediated recruitment of Grb2 (CD28) or p85 (ICOS)





Robust Assays for CD28 and ICOS Co-Stimulatory Receptors Enable Rank Ordering of Agonist Antibodies

PathHunter® OX40 Assay – Quantifies NIK Stabilization in Response to Pathway Activation

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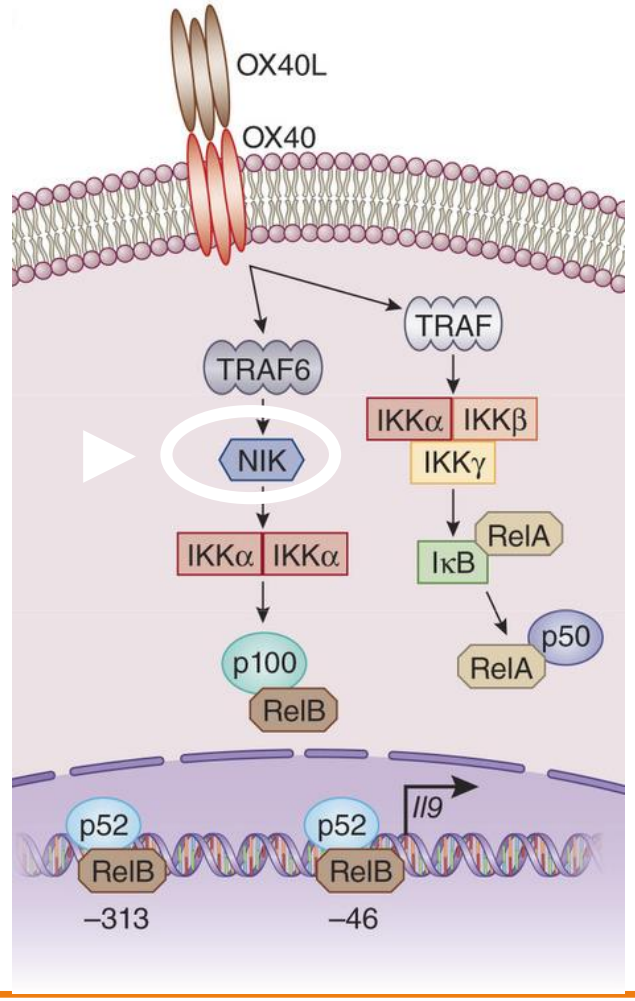
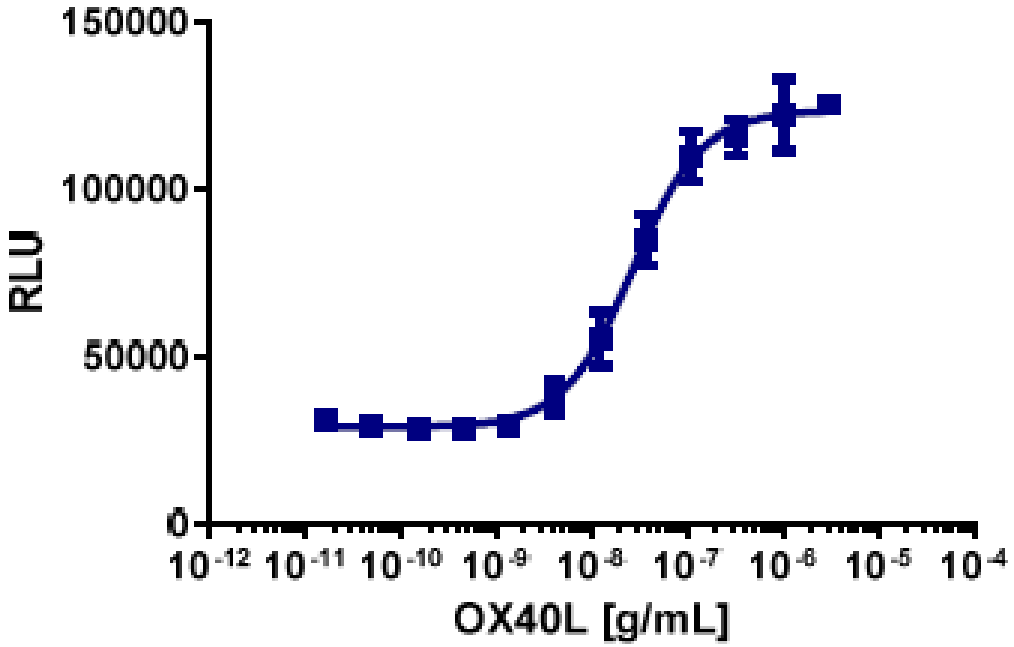
Mechanism of Action



OX40 Signaling Assay (93-1080C3)



EC_{50}	27.4 ng/mL
S:B	4
Assay Time	5 h

PathHunter® Pathway Reporter Assays – Measure Pathway activation using Endogenous or Heterologously-Expressed Receptors

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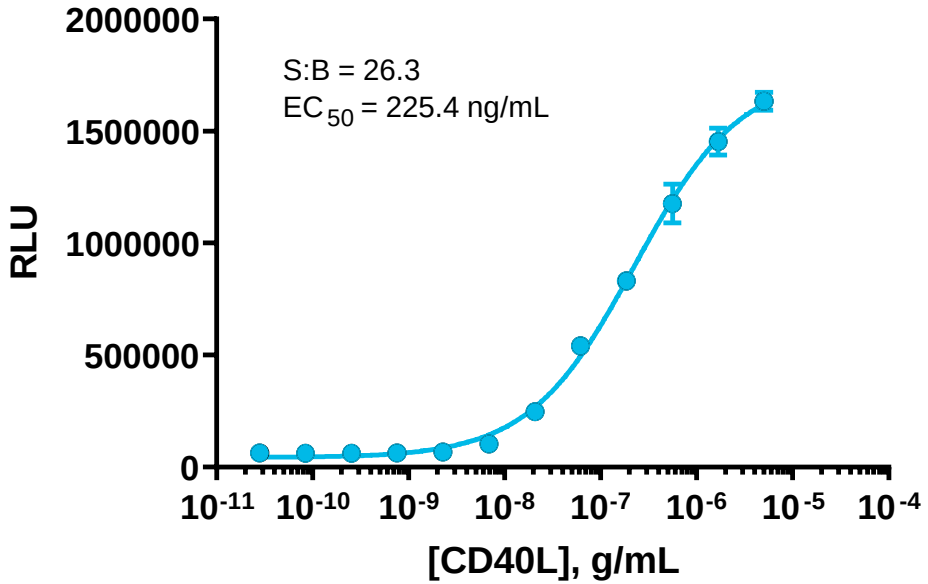
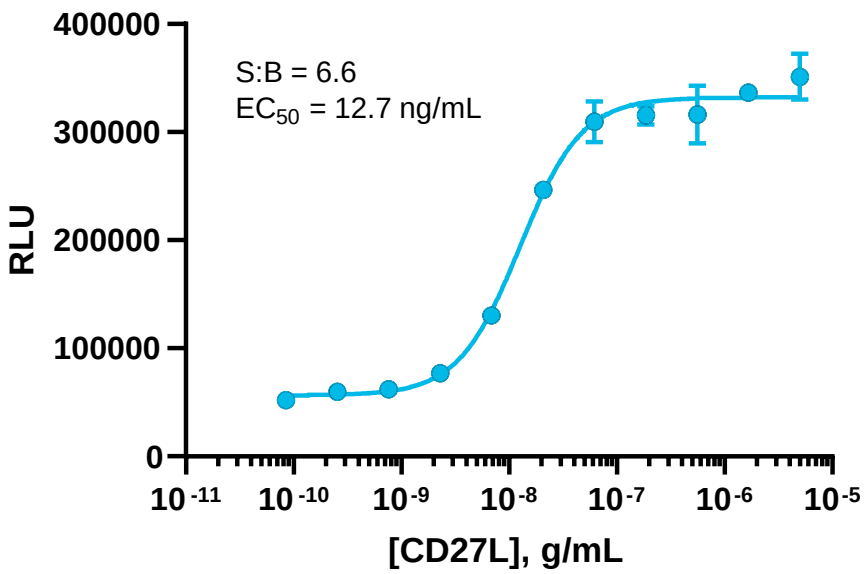
Assays for Co-stimulatory TNFR superfamily receptors

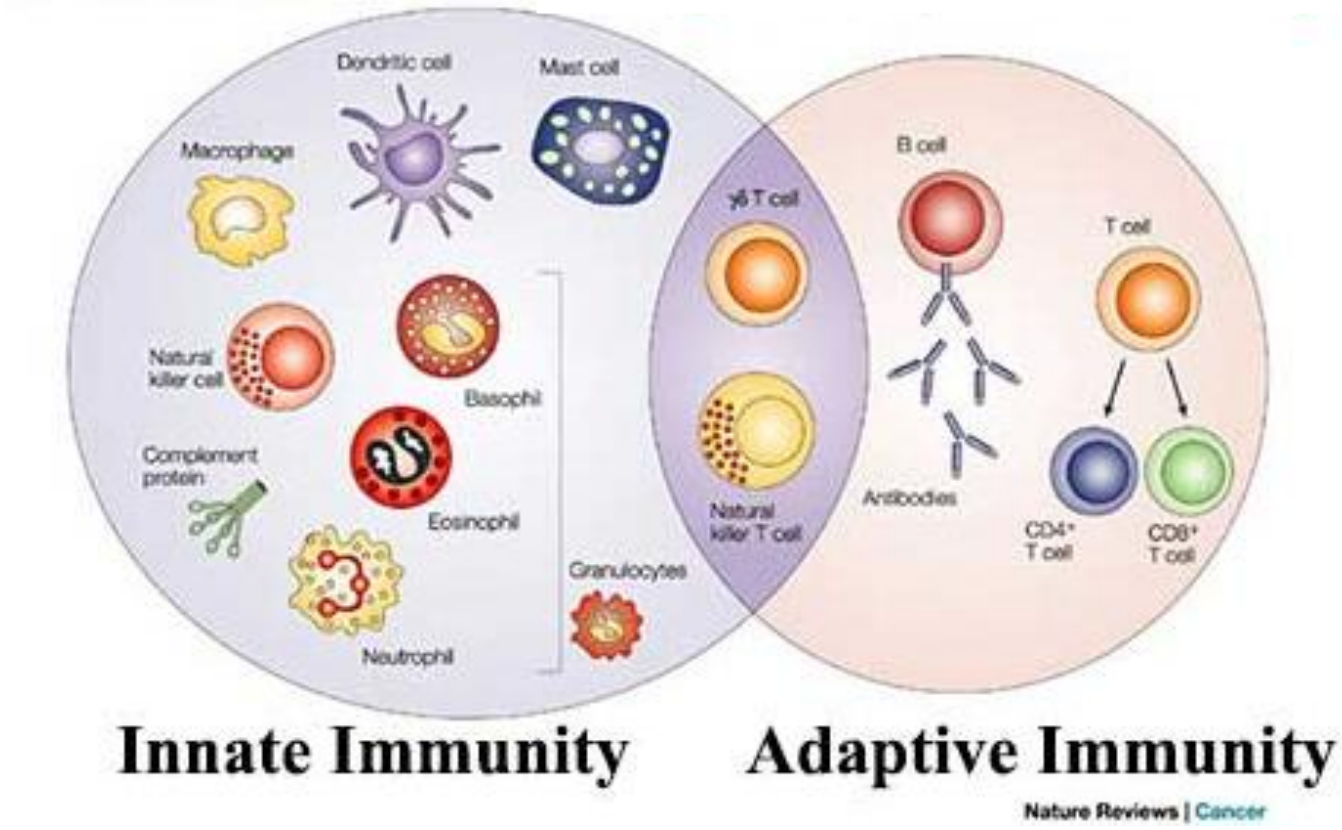
PathHunter U2OS NF-κB Pathway Reporter Assay

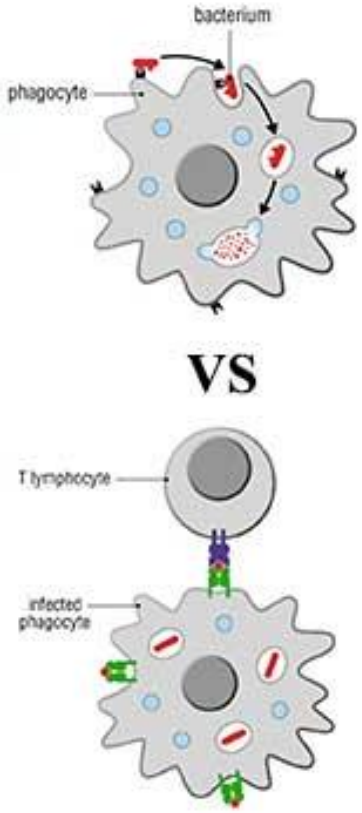


PathHunter HEK293 CD27-NF-κB Pathway Reporter Assay



Immunotherapy Agents:
Targeting Innate vs Adaptive Immunity





Therapeutic Modalities

TLR Agonists
STING Agonists
SIRPα / CD47

Checkpoint Inhibitors
(**anti-PD-1/PD-L1**)

Checkpoint agonists
(OX40, CD137, ICOS)

BiTEs, TRIKEs, etc

The SIRPα / CD47 Axis
An Innate Immune Checkpoint

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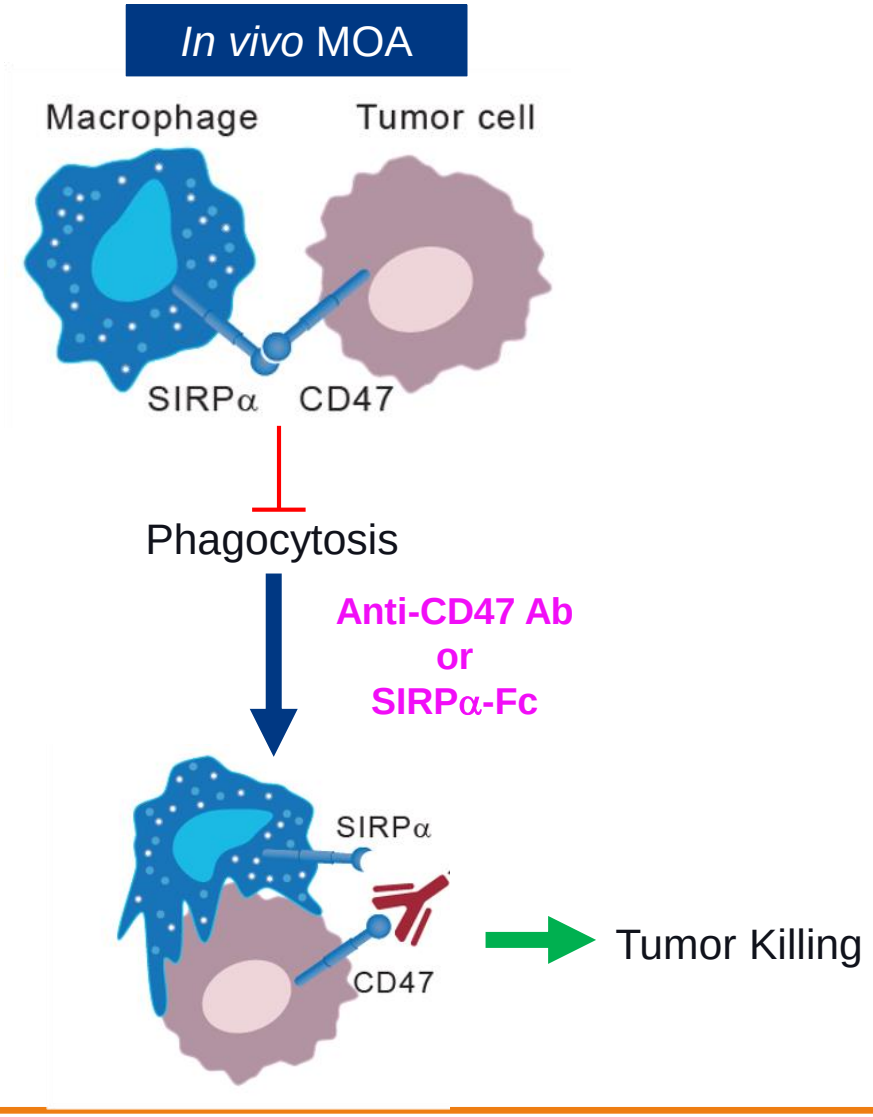


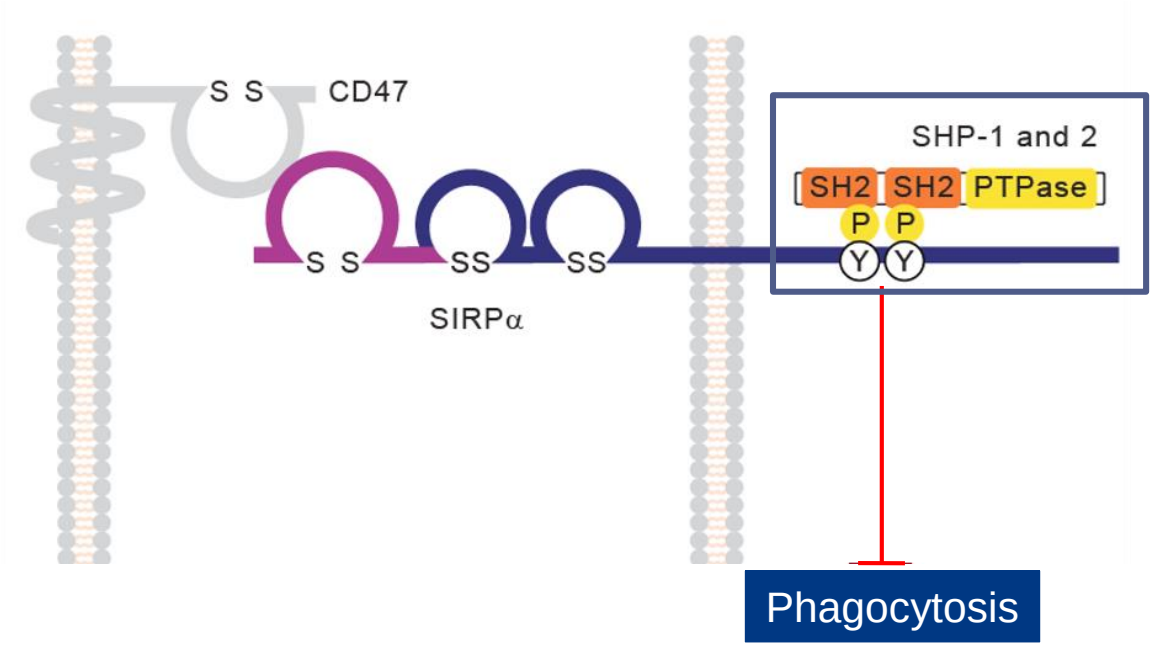
- SIRPα is an inhibitory receptor expressed on macrophages and dendritic cells that promotes phagocytosis of foreign objects
- CD47, the ligand for SIRPα, is expressed on nearly all cells, but is significantly up-regulated in many tumor types, especially hematological malignancies such as AML and MDS
  - 'Don't eat me' signal that represses signaling via SIRPα, preventing myosin-IIA accumulation at the phagocytic synapse, leading to inhibition of phagocytosis
- Blocking the CD47 / SIRPα axis (e.g. with anti-CD47 antibodies, engineered receptor decoys, anti-SIRPα antibodies and bispecific agents) promotes tumor killing
  - phagocytosis of the tumor
  - Anti-CD47 blockade has also been shown to enhance adaptive immunity (e.g. prime an anti-tumor cytotoxic T cell response)

PathHunter® SIRPα Signaling Assay: Assay Concept

Co-culture SHP recruitment model based on β-galactosidase enzyme fragment complementation

Molecular MOA

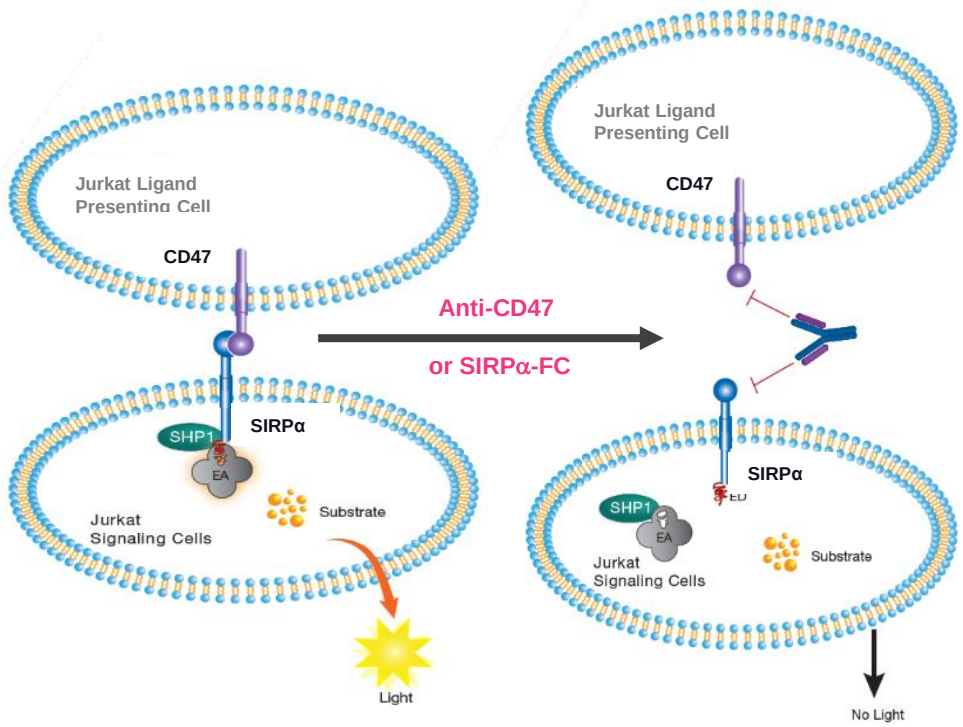
Tumor Cell **Macrophage**



Phagocytosis

Adapted from Trends in Cell Biology, 2008. Vol 19, No. 2

Co-Culture SHP Recruitment Model



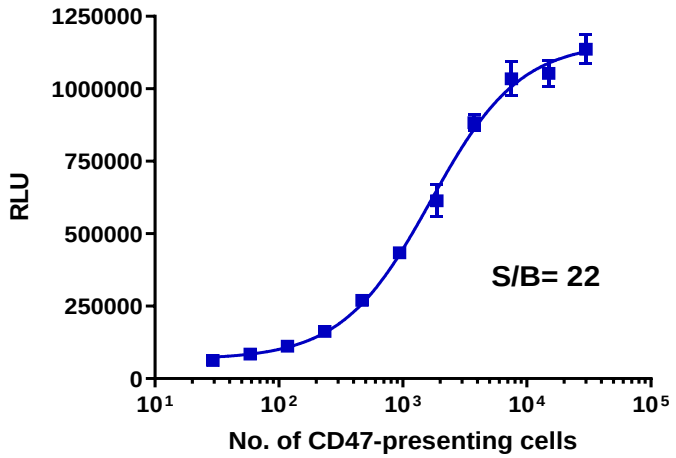
Assay quantifies ligand-induced recruitment of SHP-1 to ITIM motifs in C-terminal tail of SIRPα in response to phosphorylation

PathHunter® SIRPα (CD47) Signaling Assay

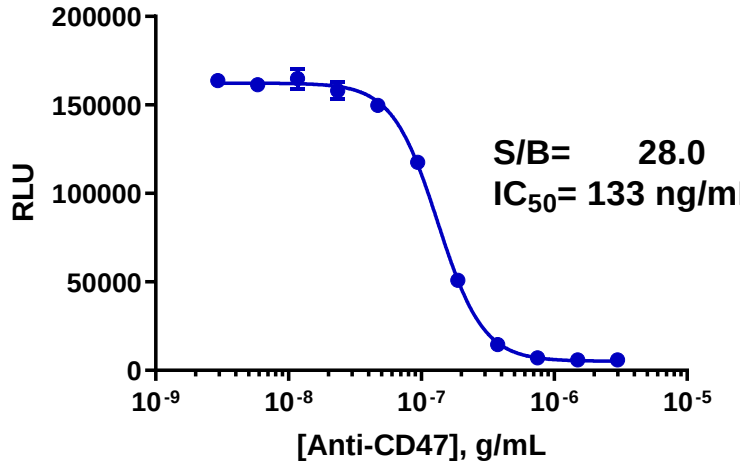
Co-culture model with stable surface expression of SIRPα and a stable functional response over 45+ passages

Cell-Presented Ligand

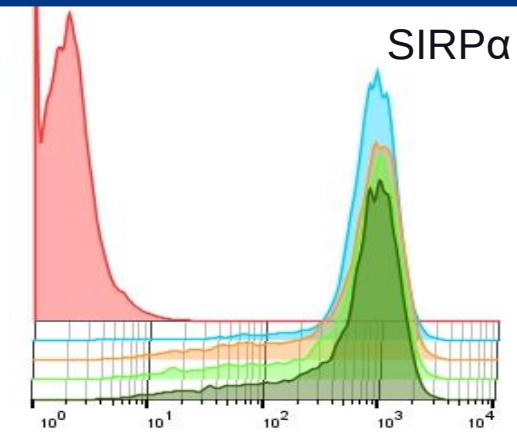
Agonist



Antagonist

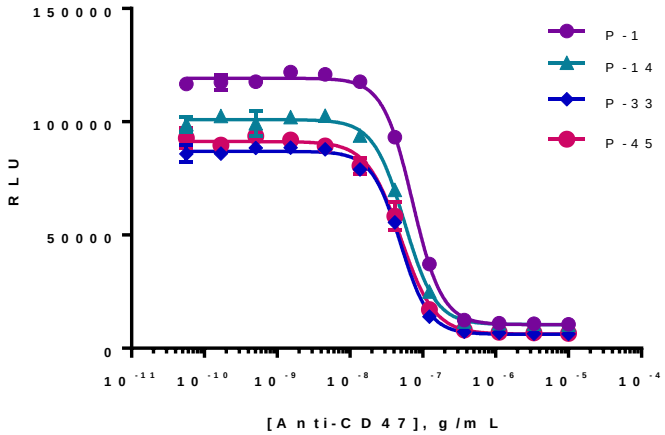


Stable SIRPα Surface Expression



SIRPα expression varies by <20% RSD over 45 passages

Stable Functional Response



S/B : 17% RSD over 45 passages

IC_{50} : <19% RSD over 45 passages

PathHunter SIRPα Signaling Assay: Excellent Specificity and Stability-Indicating Properties

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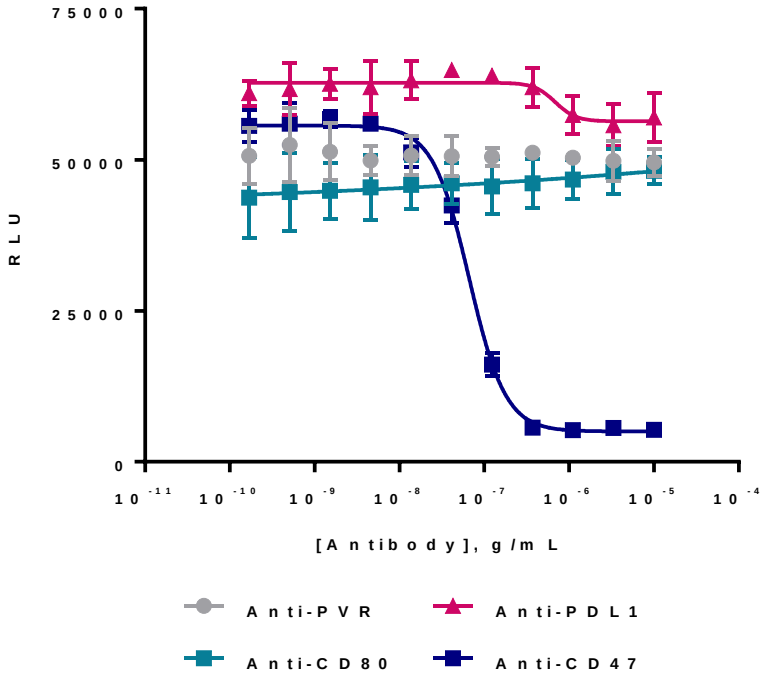
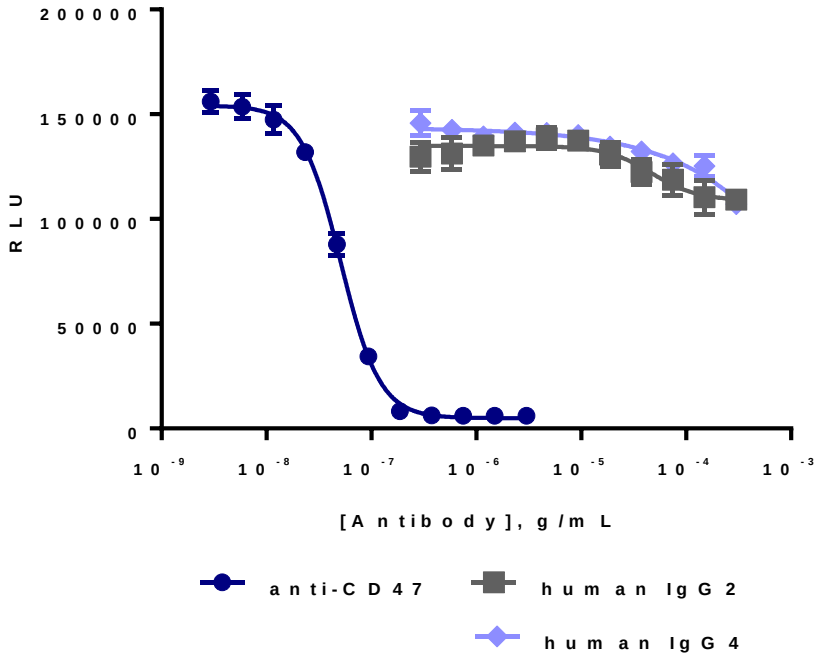
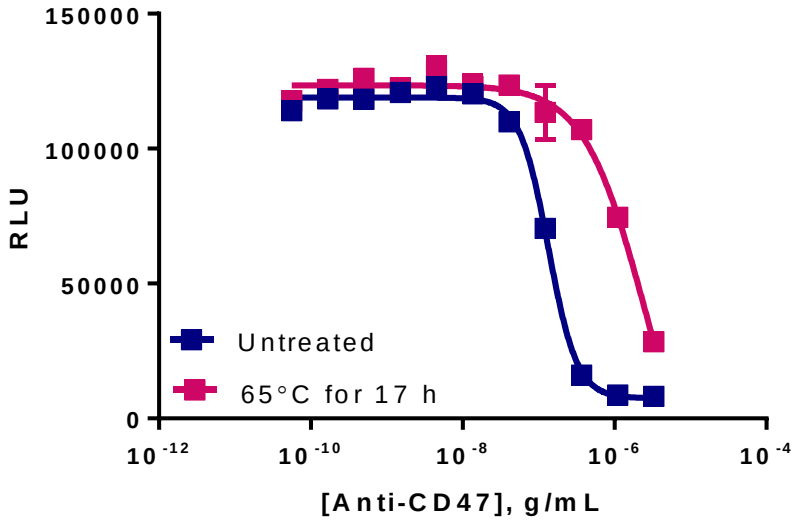
Assay Specificity



Assay Specificity

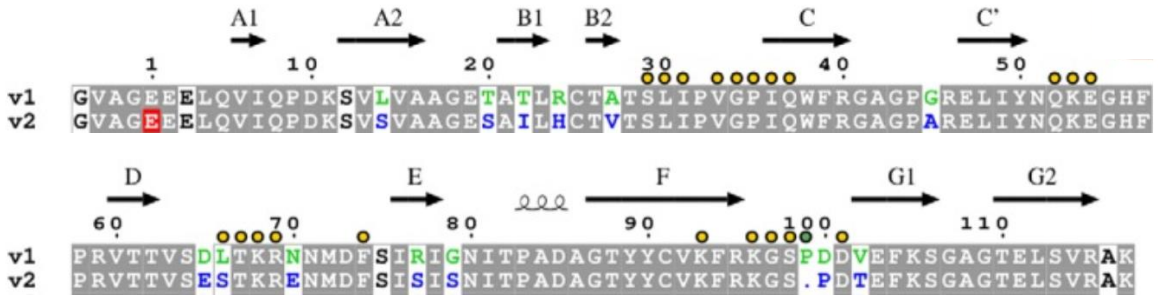


Stability-Indicating



Signaling Assays for Most Common SIRPα Variants: V1 and V2

- At least 10 SIRPα variants identified
- SIRPα variants 1 and 2 (V1, V2) are most prevalent; differ by 15 amino acids



JBC JOURNAL OF BIOLOGICAL CHEMISTRY
Published by the American Society for Biochemistry and Molecular Biology

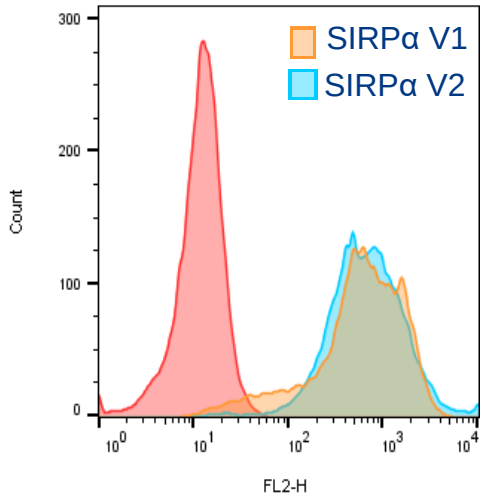
J Biol Chem. 2014 Apr 4; 289(14): 10024–10028. PMCID: PMC3974974
Published online 2014 Feb 18. doi: 10.1074/jbc.M114.550558 PMID: 24550402

Polymorphisms in the Human Inhibitory Signal-regulatory Protein α Do Not Affect Binding to Its Ligand CD47*

Deborah Hatherley, Susan M. Lea, Steven Johnson, and A. Neil Barclay[1]

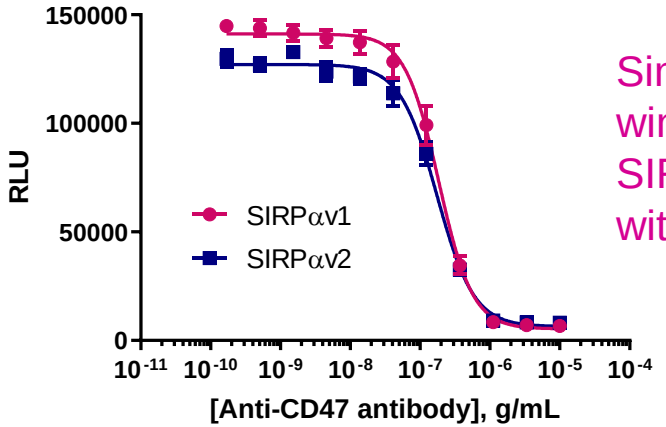
* = *This work was supported by Medical Research Council Grants G9826026 and G0900888 and by Welcome Trust Senior Investigator Award 100298 (to the S. M. L. group).

SIRPα Surface Expression



Comparable surface expression for SIRPα V1 and V2

SIRPα Functional Response



Similar assay window and IC_{50} for SIRPα V1 and V2 with anti-CD47

Mutation of SIRPα ITIM Motifs Disrupts CD47-Mediated Signaling

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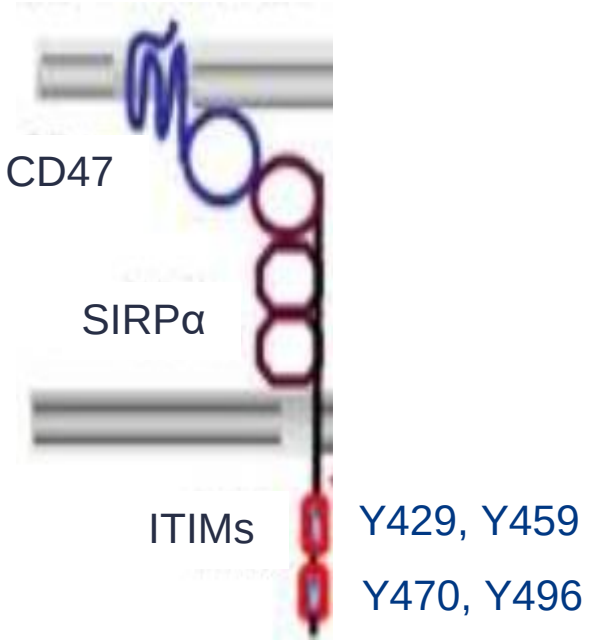
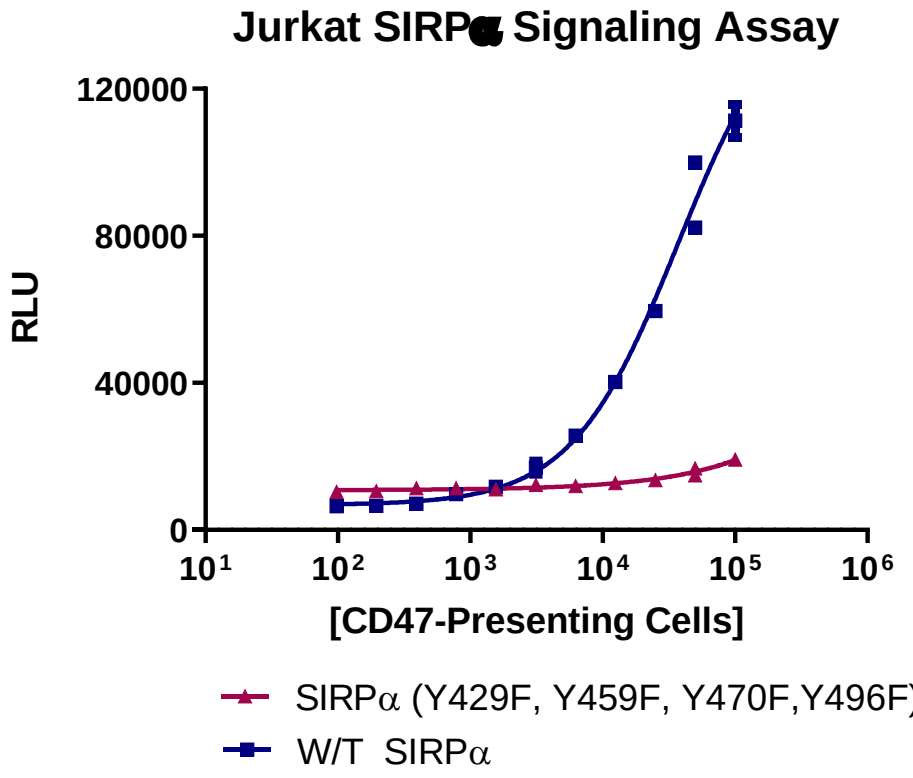
Evaluation of impact of mutations in the 4 tyrosine residues that are potential sites for phosphorylation: Y429, Y459, Y470 and Y496

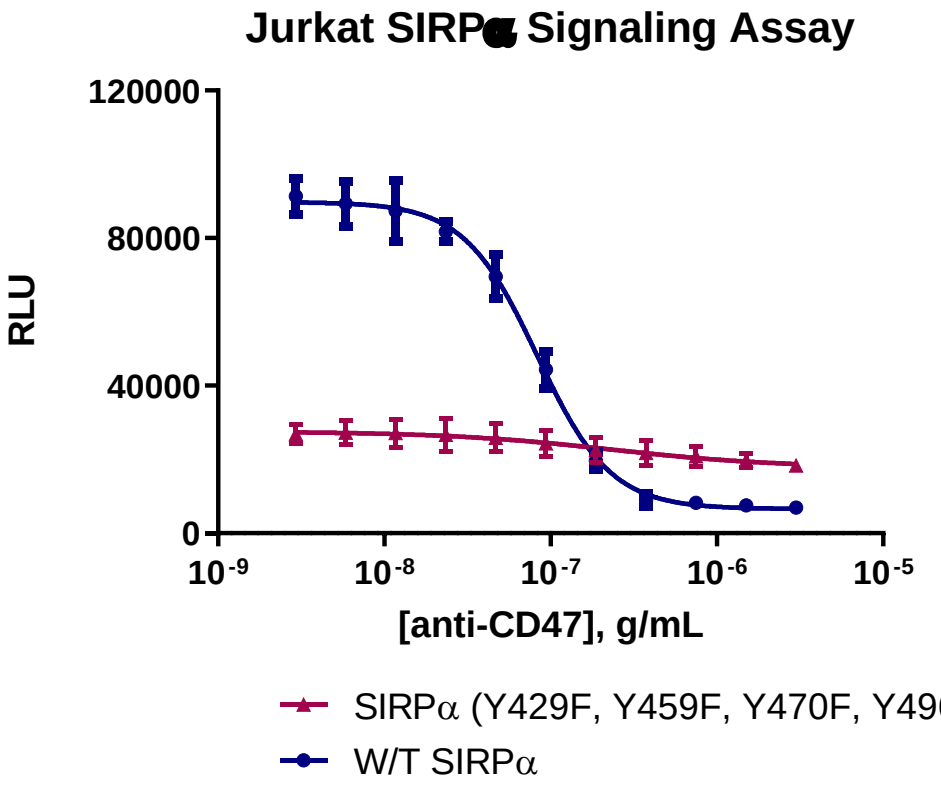


Adapted from Zen, K. et al. Nat Commun 2013; 4: 2436

Agonist



Antagonist



Single mutations disrupted signaling to different degrees (data not shown), but mutation of 3 or more tyrosine residues completely abrogated CD47-mediated SHP recruitment

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CD28		OX40
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PD1 (SHP2)	PD-L2	
BTLA	HVEM	
CTLA4	CD86	
mPD1	mPD-L1	
mCTLA4	mCD80	
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PD-1/CEACAM1	TIM3/CEACAM1
PD-1/CD28	TIGIT/LAG3
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mPD-1/mCTLA4	

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