A New Approach Method for T Cell Immunotoxicity Using CD3/CD28 Co-stimulation and IL-2 NanoLuc Luciferase Reporter

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United States Environmental Protection Agency

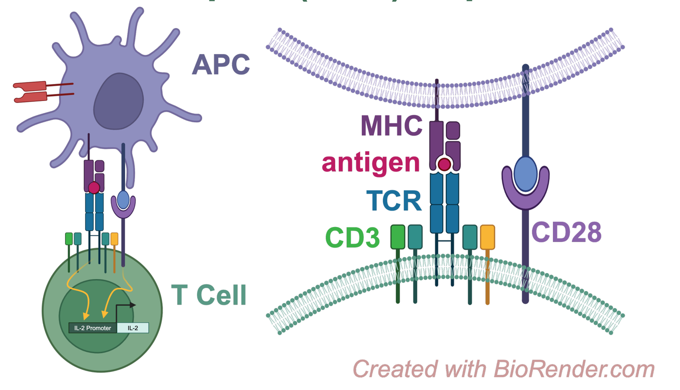
Abstract: 5084
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Background

Scope: Develop approach to screen chemicals for potential to dysregulate immune function.

T Cell Receptor (TCR)-dependent T Cell Activation



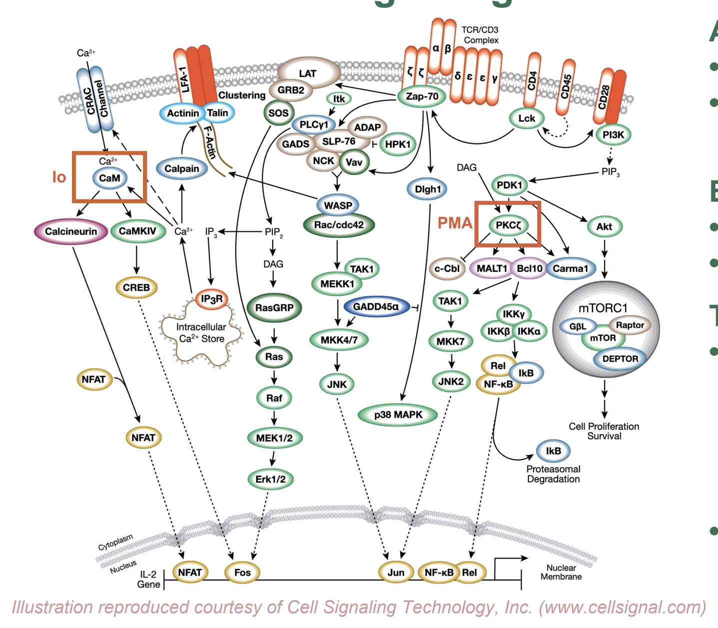
APC
MHC
antigen
TCR
CD3
CD28
T Cell

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Dictates immune specificity and establishes immunological memory.

Is initiated by two signals from antigen presenting cells (APCs):
- **Signal 1: TCR engagement by major histocompatibility complex (MHC) + antigen.**
- **Signal 2: co-engagement of costimulatory receptors (e.g., CD28).**

T Cell Activation Signaling



Io
PMA
Calcineurin
CaMKIV
CaM
Calpain
CREB
NFAT
LAT
TCR/CD3 Complex
Zap-70
PKCζ
Akt
PDK1
PI3K
Lck
mTORC1
p38 MAPK
JNK
NF-κB
Rel
IκB
Fos
Jun

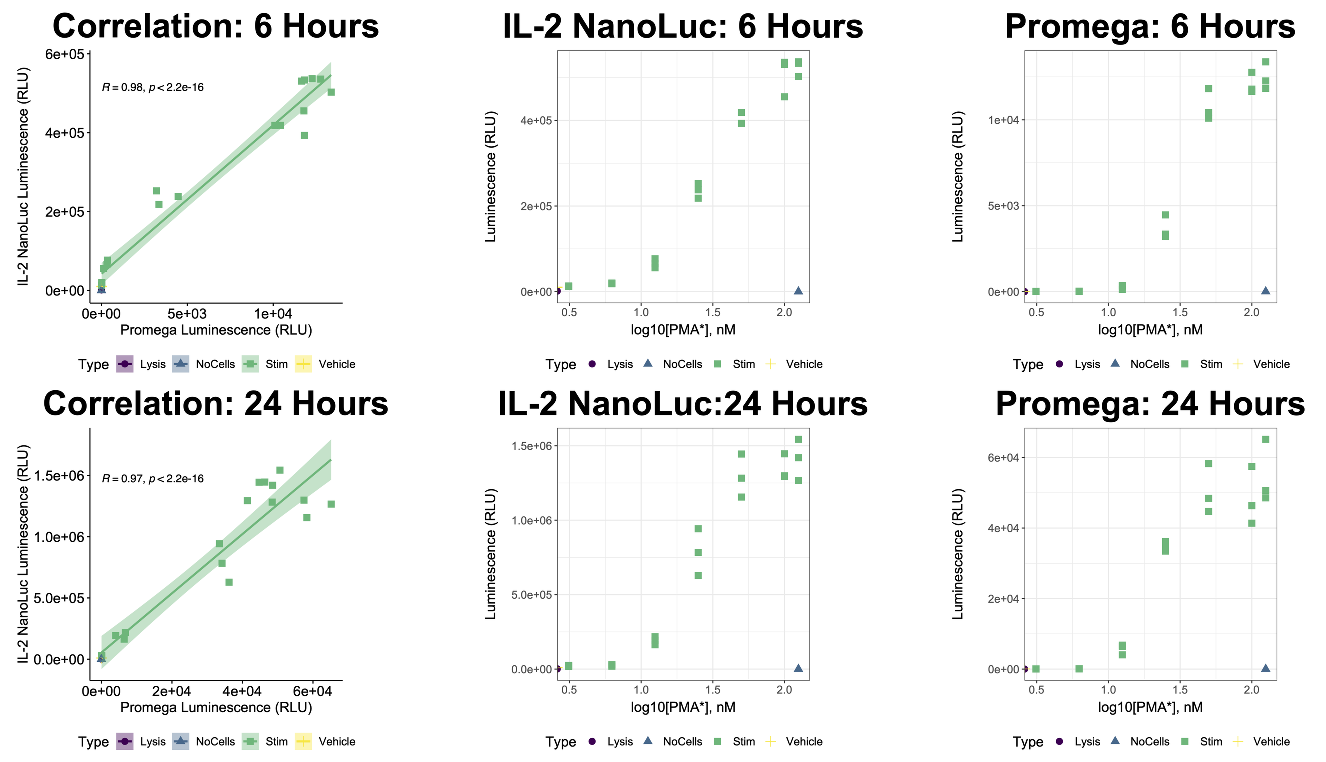
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Activation induces expression of
- **Genes for proliferation and differentiation.**
- **IL-2, a critical cytokine necessary for primary and secondary T-cell responses.**

Established assays use IL-2 promoter with
- **Firefly luciferase (Promega).**
- **Green luciferase (OECD Test 444A).**

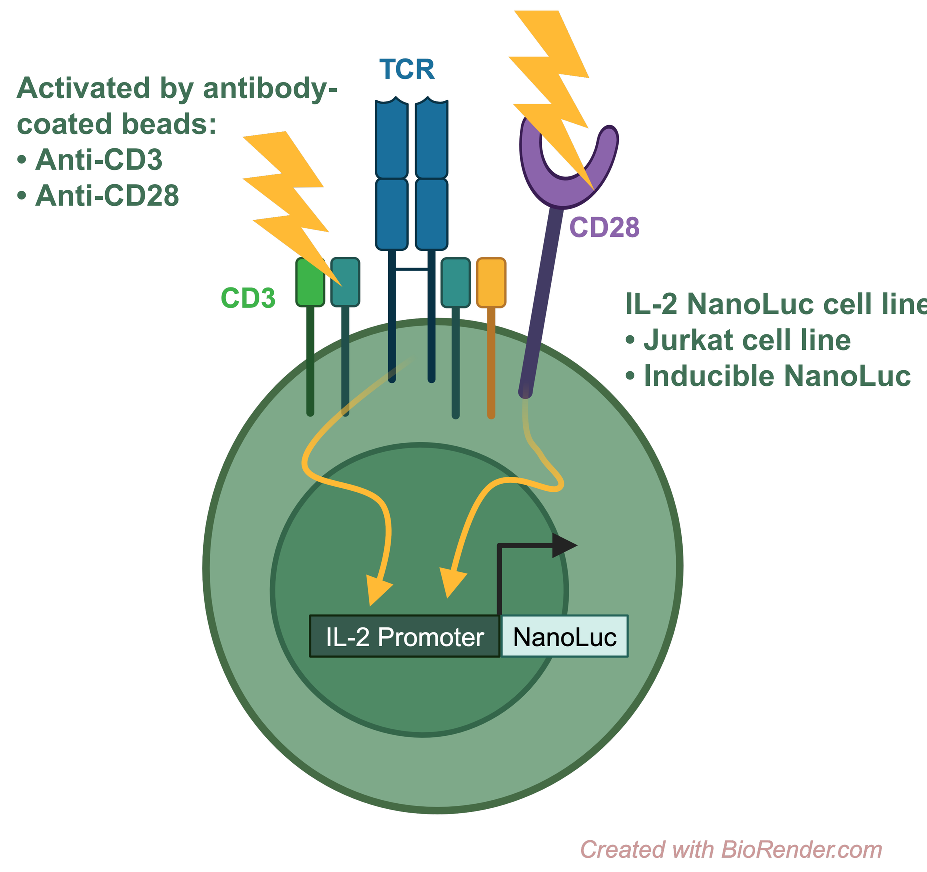
T activation without APCs:
- **Approach 1: phorbol 12-myristate 13-acetate (PMA) and ionomycin (Io) stimulation, which bypasses TCR/CD3 complex and CD28 signaling upstream of calmodulin (CaM) and protein kinase C zeta (PKCζ).**
- **Approach 2: Co-stimuation with anti-CD3 and anti-CD28 antibodies.**

Approach 1: IL-2 NanoLuc and Promega Cells Respond to PMA/Io Stimulation



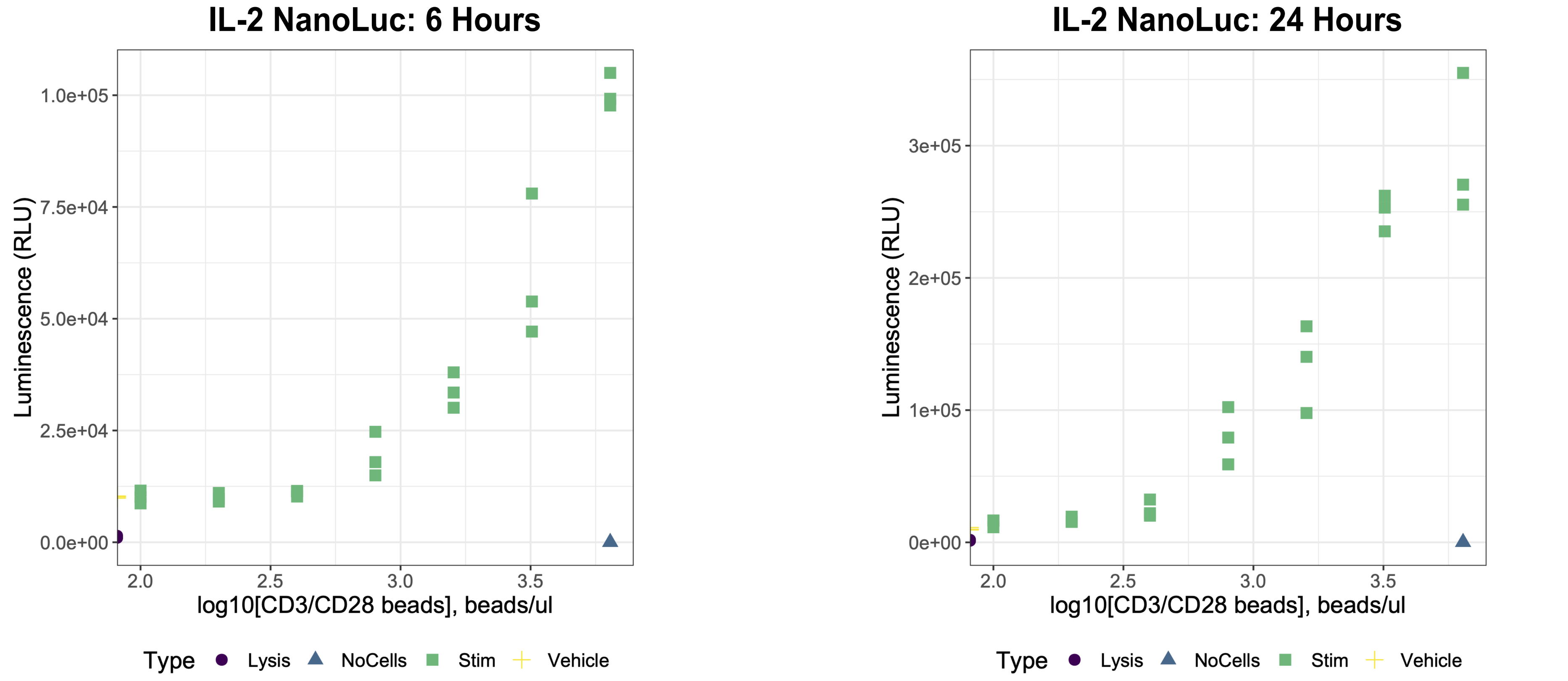
Correlation: 6 Hours
IL-2 NanoLuc: 6 Hours
Promega: 6 Hours
Correlation: 24 Hours
IL-2 NanoLuc:24 Hours
Promega: 24 Hours
R = 0.98, p < 2.2e-16
R = 0.97, p < 2.2e-16
IL-2 NanoLuc Luminescence (RLU)
Promega Luminescence (RLU)
Luminescence (RLU)
log10[PMA*], nM
Type Lysis NoCells Stim Vehicle

Approach 2: IL-2 NanoLuc Cell Line Responds to CD3/CD28 Co-stimulation



Activated by antibody-coated beads:
• Anti-CD3
• Anti-CD28
TCR
CD3
CD28
IL-2 NanoLuc cell line:
• Jurkat cell line
• Inducible NanoLuc
IL-2 Promoter
NanoLuc

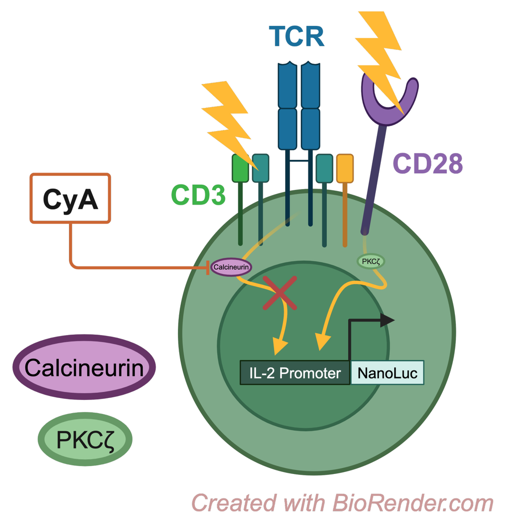
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IL-2 NanoLuc: 6 Hours
IL-2 NanoLuc: 24 Hours
Luminescence (RLU)
log10[CD3/CD28 beads], beads/ul
Type Lysis NoCells Stim Vehicle

Lysed cell, no cell, and unstimulated vehicle controls exhibit minimal luminescence relative to stimulated (stim) cells.

Activation of IL-2 NanoLuc by CD3/CD28 Co-stimulation is Inhibited by Cyclosporin A (CyA) and Bisindolylmaleimide I (Bis I)

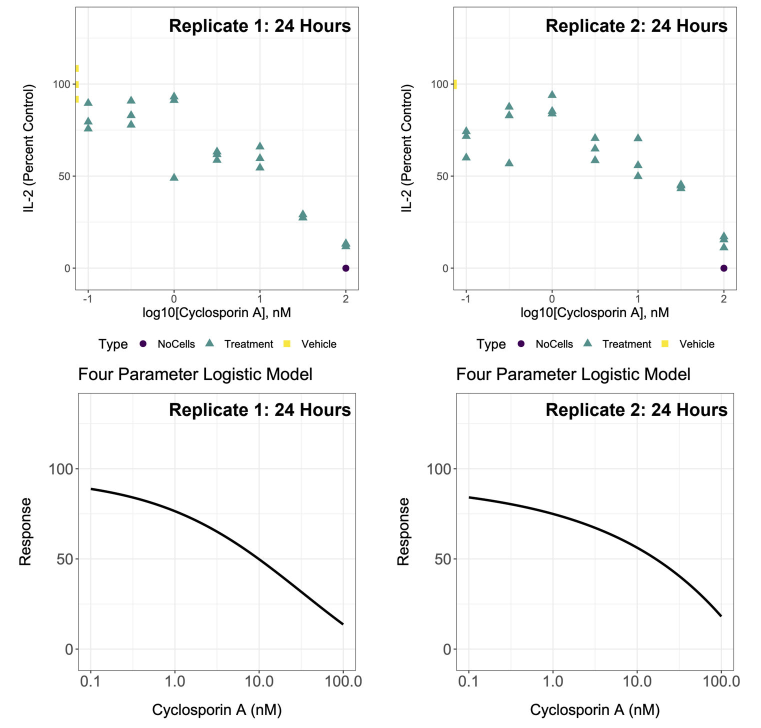


TCR
CD3
CD28
CyA
Calcineurin
PKCζ
IL-2 Promoter
NanoLuc

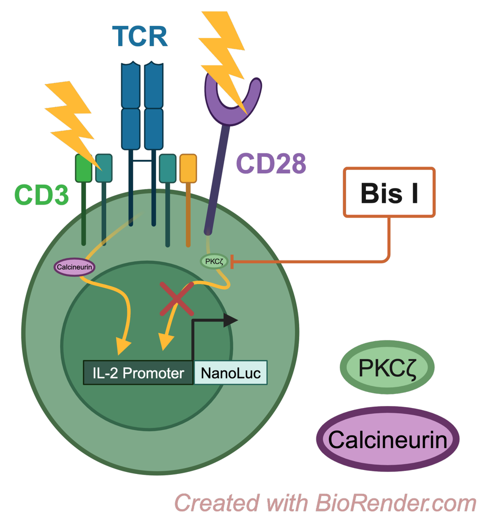
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CyA is a well-known inhibitor of Calcineurin.

Treatment with Cyclosporin A



Replicate 1: 24 Hours
Replicate 2: 24 Hours
IL-2 (Percent Control)
log10[Cyclosporin A], nM
Type NoCells Treatment Vehicle
Four Parameter Logistic Model
Response
Cyclosporin A (nM)

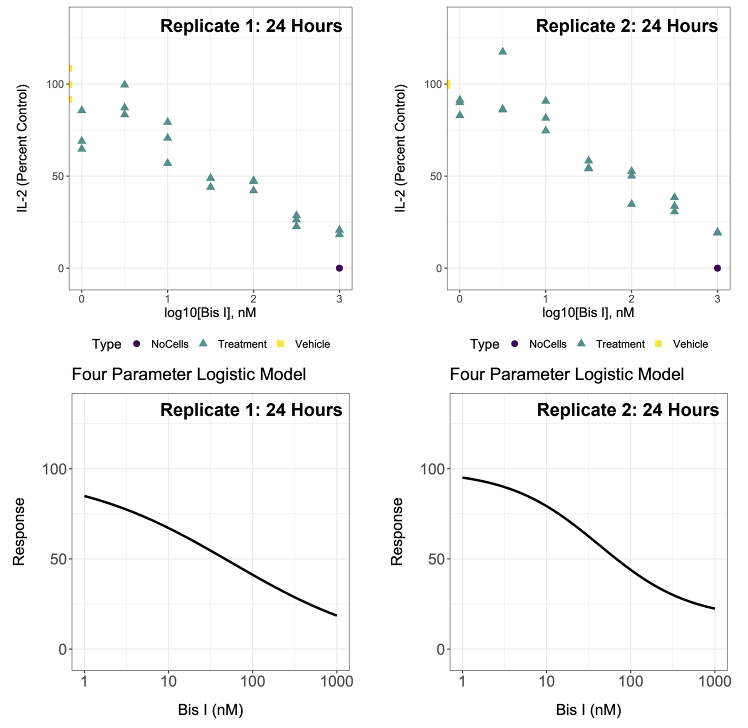


TCR
CD3
CD28
Bis I
PKCζ
Calcineurin
IL-2 Promoter
NanoLuc

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Bis I is a well-known inhibitor of Protein Kinase C (PKC).

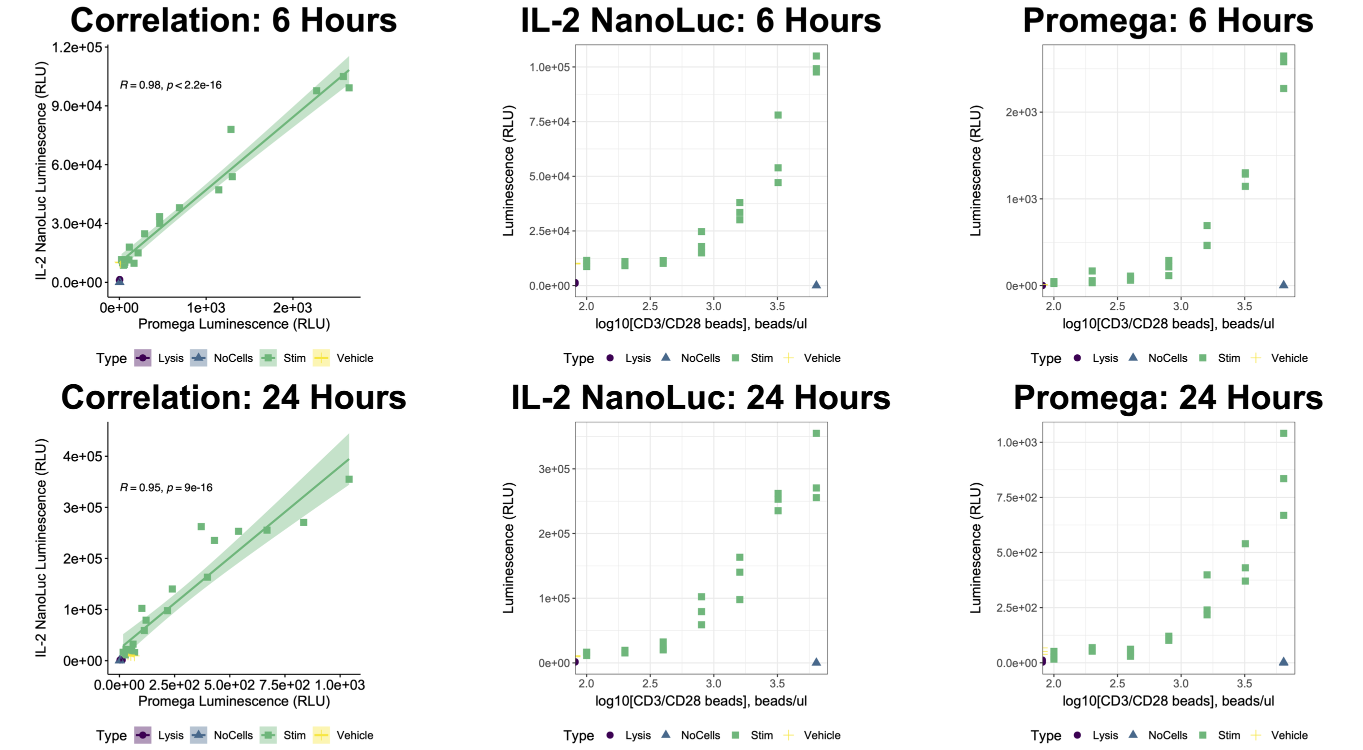
Treament with Bisindolylmaleimide I



Replicate 1: 24 Hours
Replicate 2: 24 Hours
IL-2 (Percent Control)
log10[Bis I], nM
Type NoCells Treatment Vehicle
Four Parameter Logistic Model
Response
Bis I (nM)

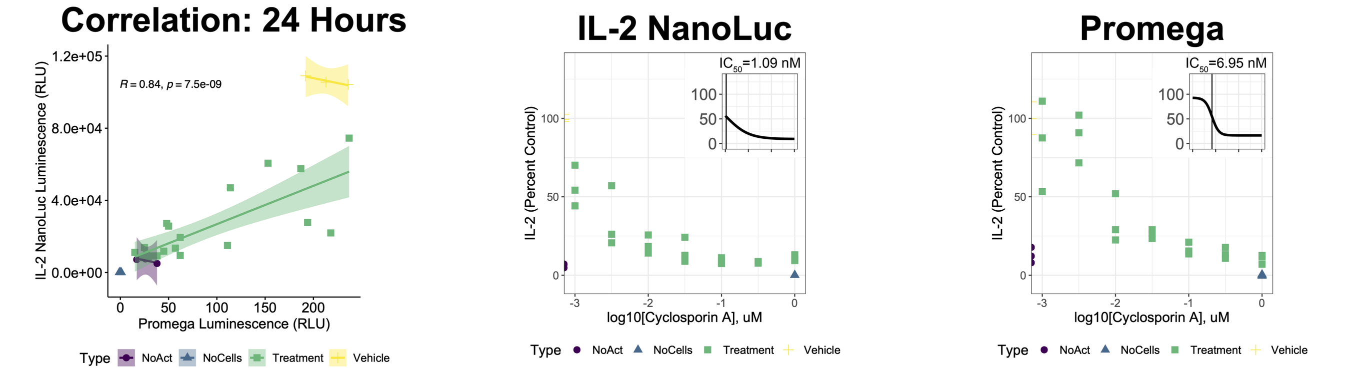
Comparison with Promega IL-2 Assay

CD3/CD28 Co-stimulation (Approach 2)



Correlation: 6 Hours
IL-2 NanoLuc: 6 Hours
Promega: 6 Hours
Correlation: 24 Hours
IL-2 NanoLuc: 24 Hours
Promega: 24 Hours
R = 0.98, p < 2.2e-16
R = 0.95, p = 9e-16
IL-2 NanoLuc Luminescence (RLU)
Promega Luminescence (RLU)
Luminescence (RLU)
log10[CD3/CD28 beads], beads/ul
Type Lysis NoCells Stim Vehicle

Inhibition by Cyclosporin A (CyA)



Correlation: 24 Hours
IL-2 NanoLuc
Promega
R = 0.84, p = 7.5e-09
IC50=1.09 nM
IC50=6.95 nM
IL-2 (Percent Control)
log10[Cyclosporin A], uM
Type NoAct NoCells Treatment Vehicle

Conclusions

IL-2 NanoLuc cell line:
- **Responds to PMA/Io activation and CD3/C28 co-stimulation.**
- **Generates higher background-subtracted luminescence compared to Promega IL-2 T Cell Activation Bioassay.**

Activation of IL-2 NanoLuc cells by CD3/CD28 co-stimulation:
- **Is inhibited by calcineurin inhibitor, Cyclosporin A, confirming the role of calcineurin in IL-2 induction.**
- **Is inhibited by PKC inhibitor, Bisindolylmaleimide I, confirming the role of PKC in IL-2 induction.**

New IL-2 NanoLuc cell line, combined with receptor complex-mediated co-stimulation represents a biologically-relevant approach for detecting T cell activation and suppression.

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