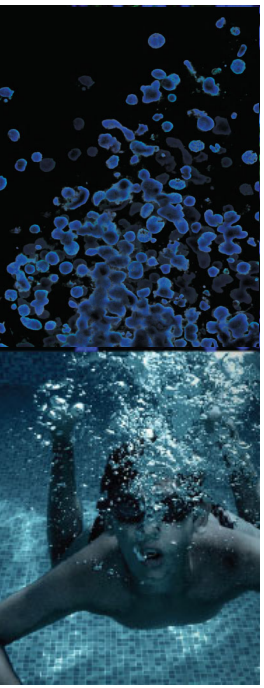
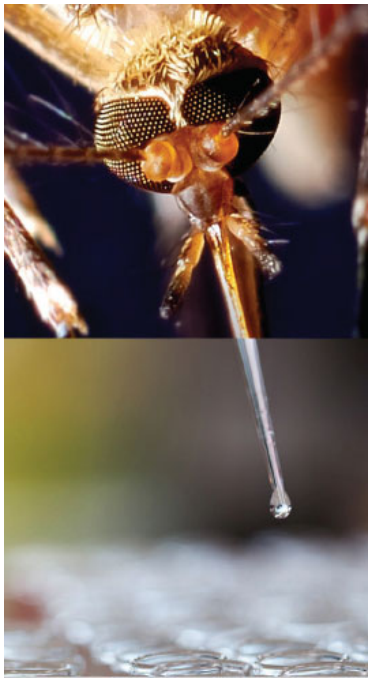
Track THP-1 Monocyte Signal Transduction with a Bigger, Better, Brighter Signal

Brian Della Fera, BS
Biologist, ATCC

Credible Leads to Incredible™





About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell biology – the "*gold standard*"
- Innovative R&D company featuring gene editing, microbiome, advanced cell models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viruses, and microbial standards
- Sales and distribution in 150 countries, 18 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

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Luciferase-expressing Monocyte Reporter Cell Lines as a Predictive Human Cell-Based Model for In Vitro Immune Activation Studies

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Poster #1018

Abstract

Cancer immunotherapy has emerged as an exciting new approach for cancer treatment, and immuno-oncology is one of the fastest growing fields in oncology. The development of immunomodulatory drugs and biologics dictate a clear need for human cell-based models to evaluate immune activation. THP-1 cell line is one of the best surrogate models for in vitro human monocytes. Additionally, luciferase reporters provide a relatively simple, robust, and highly sensitive means to measure biological processes through *in vitro* bioluminescence measurements. Here we report the generation of a panel of THP-1 luciferase reporter cell lines that have been transduced with a Luc2P plasmid containing the response element (RE) of various transcription factors, which include NF-κB, GAS, NFAT, ISRE, AP-1, and CRE. After introduction of the pLenti-RE-LUC2P plasmid into the parental THP-1, single cell cloning was performed to isolate stable clones with the best signal-to-noise ratio of luciferase signals upon stimulation. THP-1 NF-κB reporter cells showed greater than 30-fold increase in bioluminescence signals while stimulated by TNFα or LPS. THP-1 GAS reporter cells responded with high sensitivity to IFN-γ which allowed signal fold change to be greater than 100 folds. We also found that IFN-α can stimulate GAS through cross-talk between STAT1 and STAT2 signaling which showed a greater than 10 folds change in bioluminescence signal. In addition, these cell lines were characterized and authenticated using cell morphology, growth kinetics, and STR analysis. The growth of these clones was comparable to that of parental THP-1, and the STR analysis showed that the derived luciferase reporter cell clones were identical to the parental THP-1 cell line STR profile. The selected transcription factors play critical roles in regulating immune reactions, antiviral responses, and inflammation. In addition to allowing the study of specific signaling pathway activity, these THP-1 reporter cell lines can be used to examine various immune response conditions and monocyte activation during immuno-oncology drug discovery. For example, THP-1 NF-κB reporter cells were not only highly sensitive to the stimulation of toll-like receptors and pro-inflammatory cytokine such as TNF-α in a dose-dependent manner, but also responded to the simulation of common damage-associated molecular pattern (DAMP) such as HMGB1, which is released during cancer cell death. Meanwhile, the activation of THP-1 GAS reporter cells were examined not only by cytokine IFN-γ simulation alone, but also were used in co-culture system with activated T cells to evaluate IFN-γ release during various immune-oncology drug treatments. In summary, luciferase-expressing monocytes are valuable tools for studying signal transduction pathway activation, screening of compounds to find activators of signal transduction pathways, and efficacy evaluation of inflammatory effect of new drugs and chemicals.

Background

Background and applications

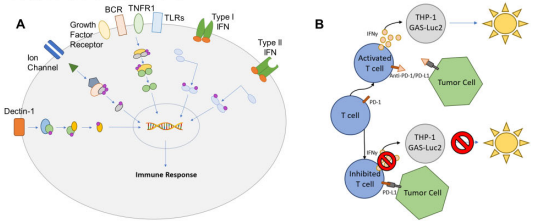

Figure 1. Background of THP-1 Luciferase lines. A) There are many pathways used by the immune system to trigger an immune response. These responses result in the release of a variety of proteins and cytokines that create a cascade of stimulation in other immune cells. B) Tumor cells evade detection by immune cells through a variety of mechanisms. Cancer immunotherapies stimulate immune cells to detect the cancer cells and induce an immune response. The THP-1 Luc2 cell lines can be used to evaluate the efficiency of these therapeutics.

Generation of Luciferase-expressing cell lines

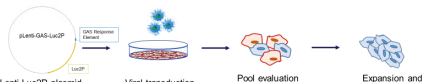

Figure 2. Scheme of developing a stable cell line containing the Luc2 gene.

Results

Characterization of THP-1 Luc2 cell lines in vitro

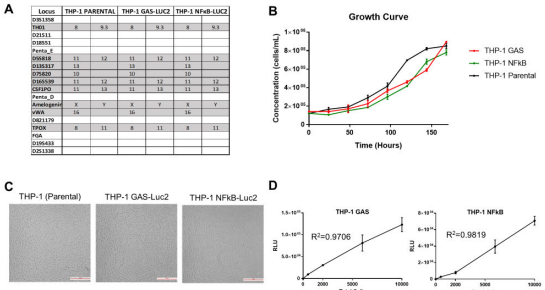

Figure 3. Characterization of luciferase-expressing cell lines. A) STR Profiling of THP-1 GAS-Luc2 and THP-1 NFκB-Luc2 showed identical alleles as the parental THP-1. B) Cell growth kinetics are similar to the kinetics of parental THP-1. C) Cell morphology of THP-1 GAS-Luc2 and THP-1 NFκB-Luc2 cell lines are similar to parental THP-1. Red size bar represents 1000 µm. D) THP-1 GAS-Luc2 and THP-1 NF-κB-Luc2 cell lines were stimulated overnight with IFN-γ (10ng/mL; 285-IF-100, R&D Systems®) and LPS (1 µg/mL; tlrl-pb5ps, InvivoGen) respectively. Luciferase assay was performed by using Bright-Glo™ (Promega®) Luciferase Assay System and GloMax™ Luminometer (Promega®). Data showed a linear correlation between bioluminescence intensity and cell number.

GAS and NF-κB signaling pathway activation following exogenous stimulation

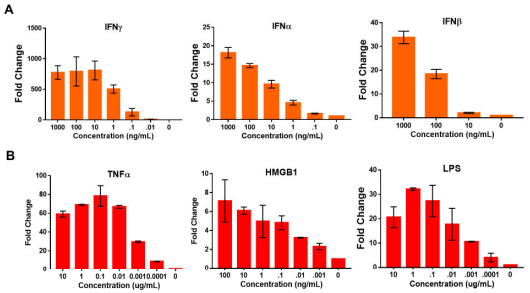

Figure 4. Activation of GAS and NF-κB signaling pathways by various cytokines and TLR ligands in a dose dependent manner. A) THP-1 GAS-Luc2 cells were stimulated overnight with stimulus associated with the JAK-STAT pathway. IFNγ, IFNα (130-093-874, Miltenyi Biotec), IFNβ (130-094-619, Miltenyi Biotec). B) THP-1 NFκB-Luc2 cells were stimulated overnight with stimulus associated with the NF-κB pathway. TNFα (210-TA-100, R&D Systems®), HMGB1 (1690-HMB-050, R&D Systems®), LPS.

CD8⁺ T cell stimulation results in expression of IFNγ and activation of THP-1 GAS-Luc2 cell line

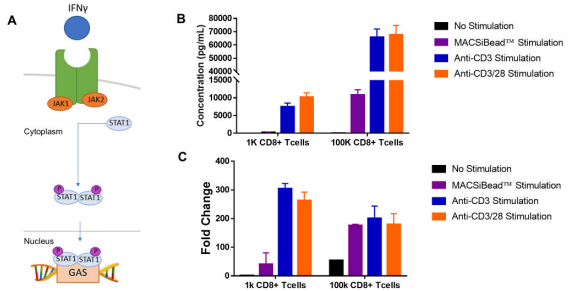

Figure 5. *In vitro* detection of IFNγ expression using THP-1 GAS-Luc2. (A) Schematic of the IFNγ-JAK-STAT pathway. B) CD8⁺ T cells were stimulated using multiple reagents for 7 days and IFNγ expression was measured using FlexMap3D® (Luminex®). MACSiBead™ (Miltenyi Biotec) is a T cell stimulation kit using anti-CD2, CD3, and CD28 antibodies. C) Supernatant from stimulated plates were incubated with THP-1 GAS-Luc2 overnight. Luciferase activity was measured using Bright-Glo™. The results of the luciferase analysis are due to the IFNγ inhibitory effect that is seen once concentrations reach 10 ng/mL (refer to Figure 4A).

Additional THP-1 Luc2 cell lines respond appropriately to immuno-signaling pathway agonists

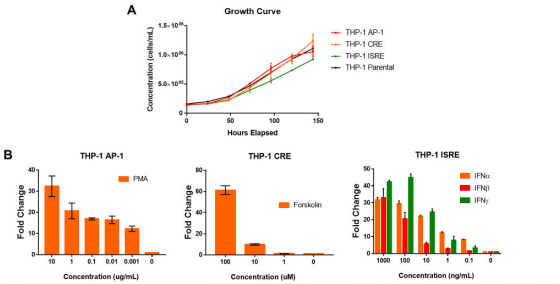

Figure 6. THP-1 Luc2 cell lines serve as a useful tool to study various arms of the innate immune response. Additional THP-1 Luc2 cell lines responding to various signal transduction pathways have been developed. Examples here show growth kinetics A) and appropriate stimulation B) of AP-1, CRE, and ISRE response elements. Luciferase activity was measured using Bright-Glo™.

Conclusion

We have created several human monocytic luciferase reporting cell lines that provides a simple and robust means to measure immune activation through in vitro bioluminescence measurements. The cell lines show reliable performance in dose titration experiments and demonstrate versatility in more complex experiments. This panel of THP-1 Luc2 cells provides an authentic evaluation tool that can be used in the development of immunomodulatory drugs and biologics, studying signaling pathways, and be a safety evaluation tool for new chemicals and drugs.

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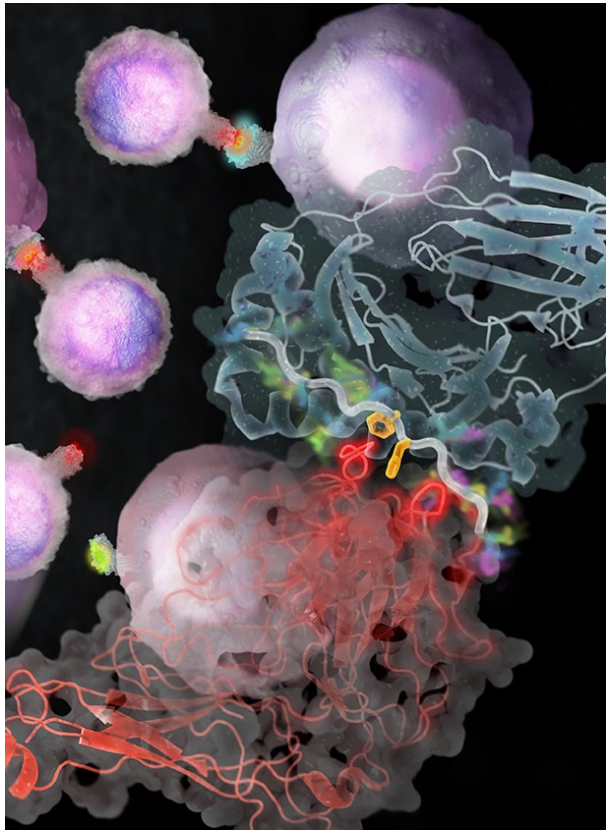
Outline: THP-1 Reporter Cell Lines



https://www.hhmi.org/news/hunting-immune-cells-cancer-targets

- Introduction to THP-1 Monocytes and Luciferase Technology
- Development of THP-1 Reporter Cell Lines
- Application of Cell Lines

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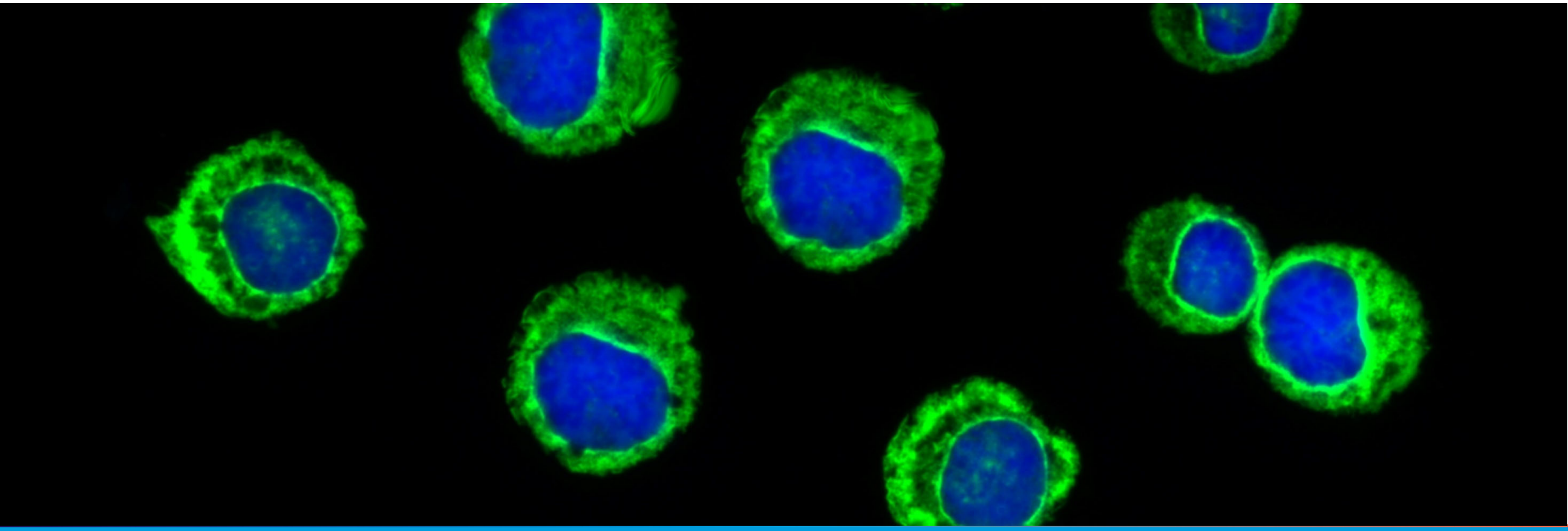
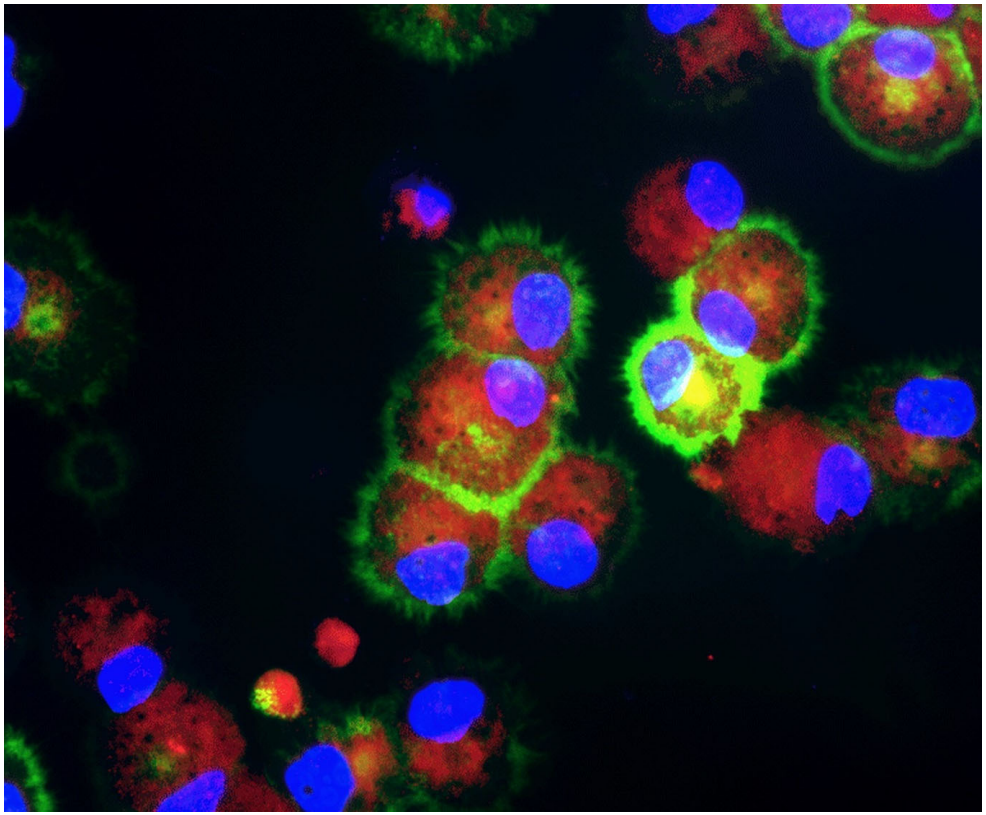


Introduction Of THP-1 Monocytes And Luciferase Technology

Introduction

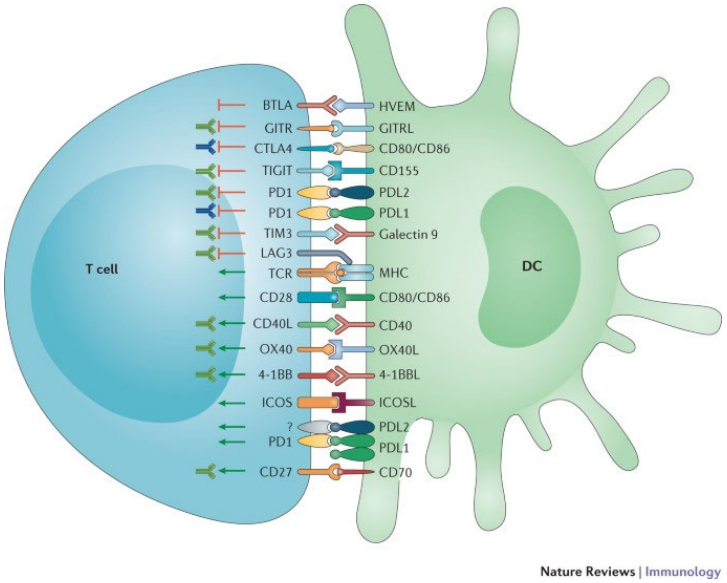
Overview

- Background
- THP-1 Project
- Cell Line
- Luciferase
- Response Elements used



Introduction

Product Background



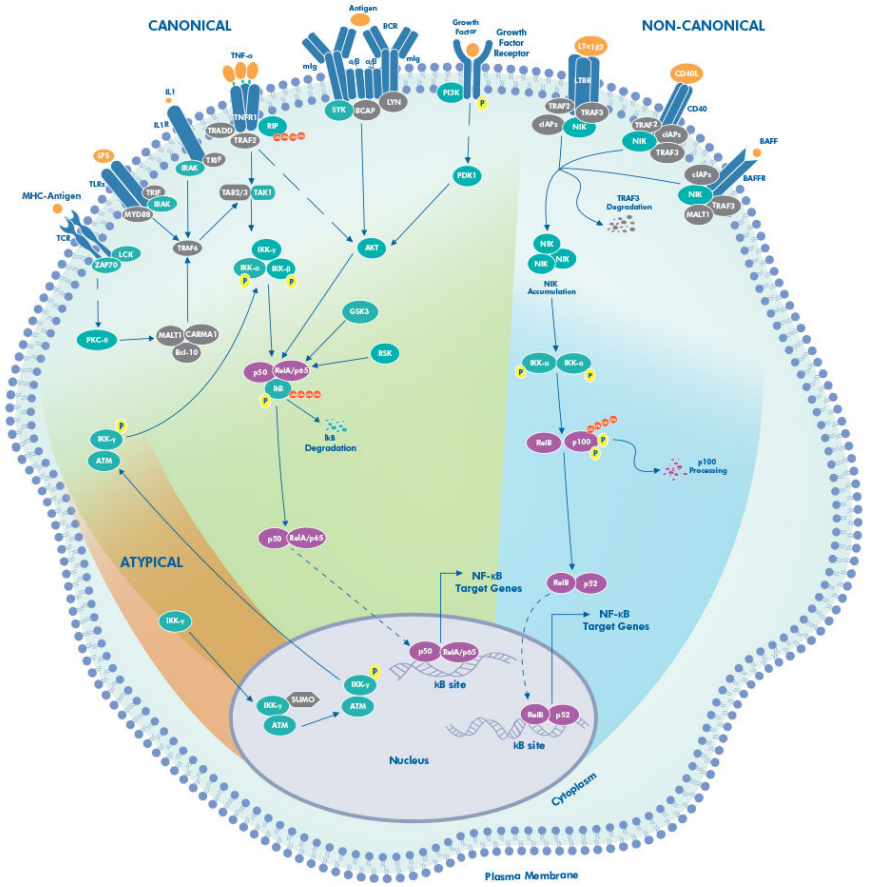
https://www.nature.com/collections/gqznlfngkz

- Immunotherapy has emerged as an exciting new approach for cancer treatment
- Current methods are time consuming, labor intensive, or expensive
- Clear need for a straightforward, human cell-based model that can be implemented as a evaluation tool

Introduction

Scientific Background

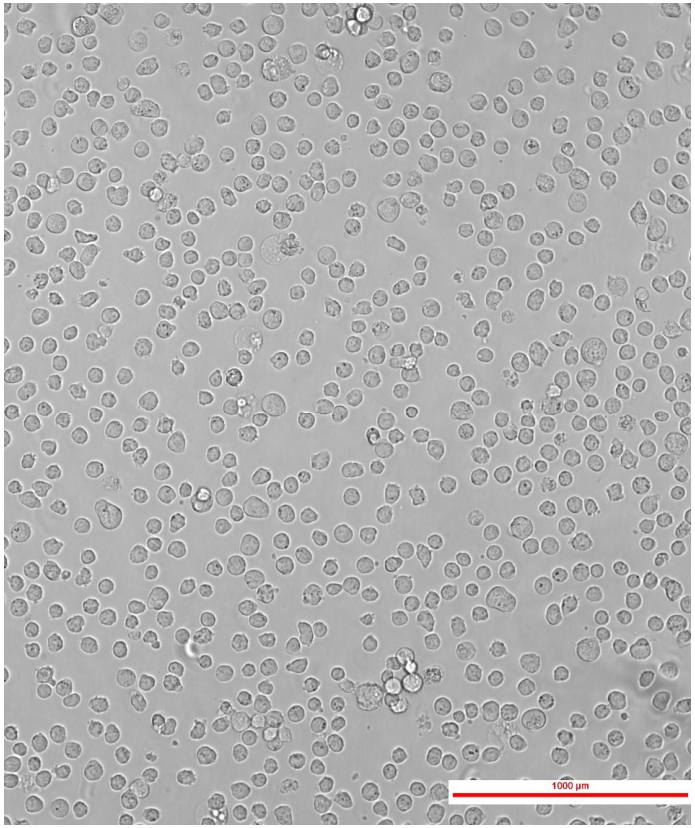
- Applicable in academic research and pharmaceutical R&D
  - Signaling pathways
  - New drug development
  - Safety evaluation tool
- THP-1
  - Established cell line
- Luciferase
  - Well characterized reporter gene system



https://www.novusbio.com/nfkbpathway

Introduction

THP-1 (TIB-202™) Cell Line

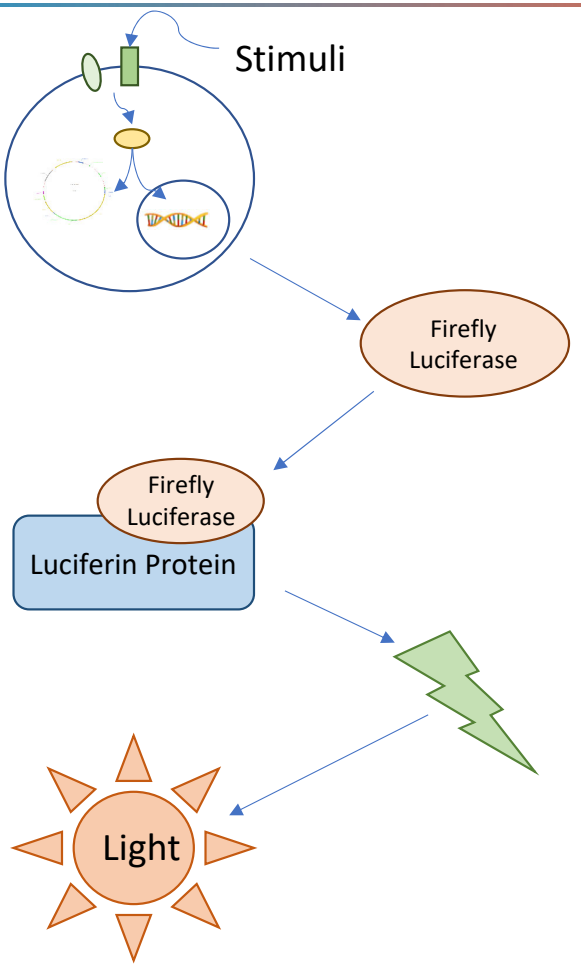


- Best surrogate model for studying *in vitro* human monocytes
- Originated from the blood of a leukemia patient
- Differentiate into macrophages
- Homogenous genetic background minimizes variability

Introduction

Luciferase

- Derived from fireflies, *Photinus pyralis*
- Higher expression and quicker protein transcription
- Quantified by measuring bioluminescence
- High-throughput, sensitive readings



Introduction

Response elements

Transcription Factor	Signaling Pathway	Function
AP-1	MAPK/ERK	Regulates innate and adaptive immune response
CRE	cAMP/PKA	Inflammatory mediator and phagocytosis modulator
GAS	JAK-STAT (Type II)	Initiates immune cell activation and recruitment
ISRE	JAK-STAT (Type I)	Initiates immune cell activation and recruitment
NFAT	Calcineurin-NFAT	Mediates adaptive T cell activation
NF-κB	NF-κB	Pivotal mediator of inflammatory response

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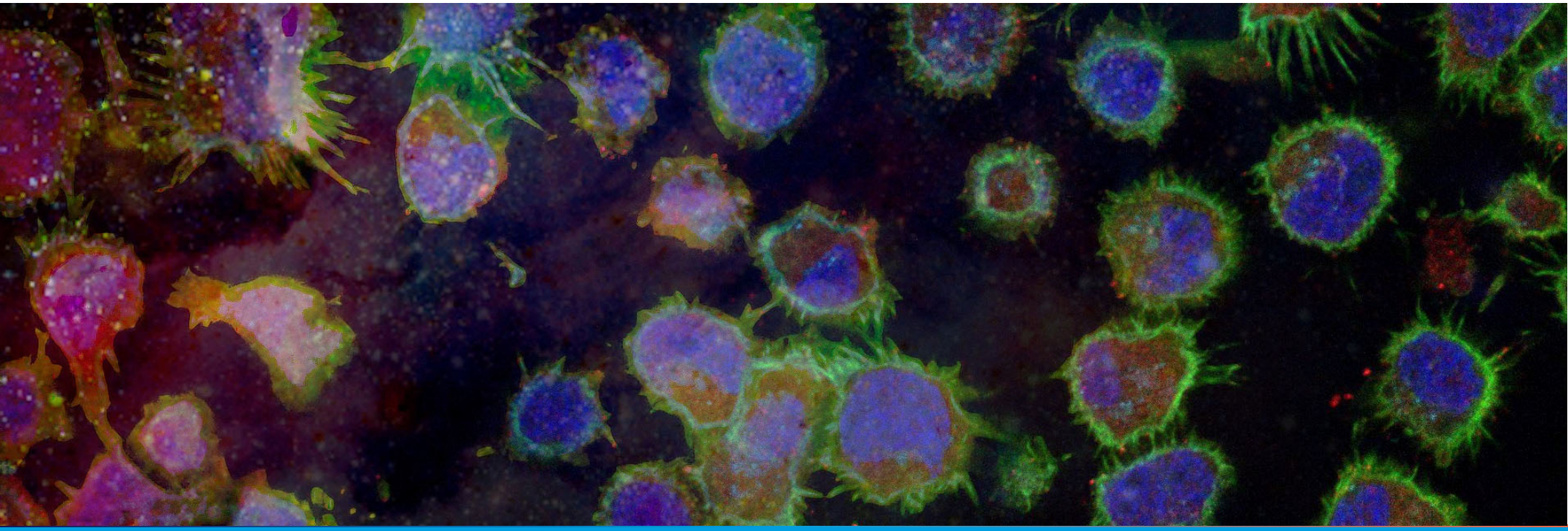

Development of THP-1 Reporter Cell Lines

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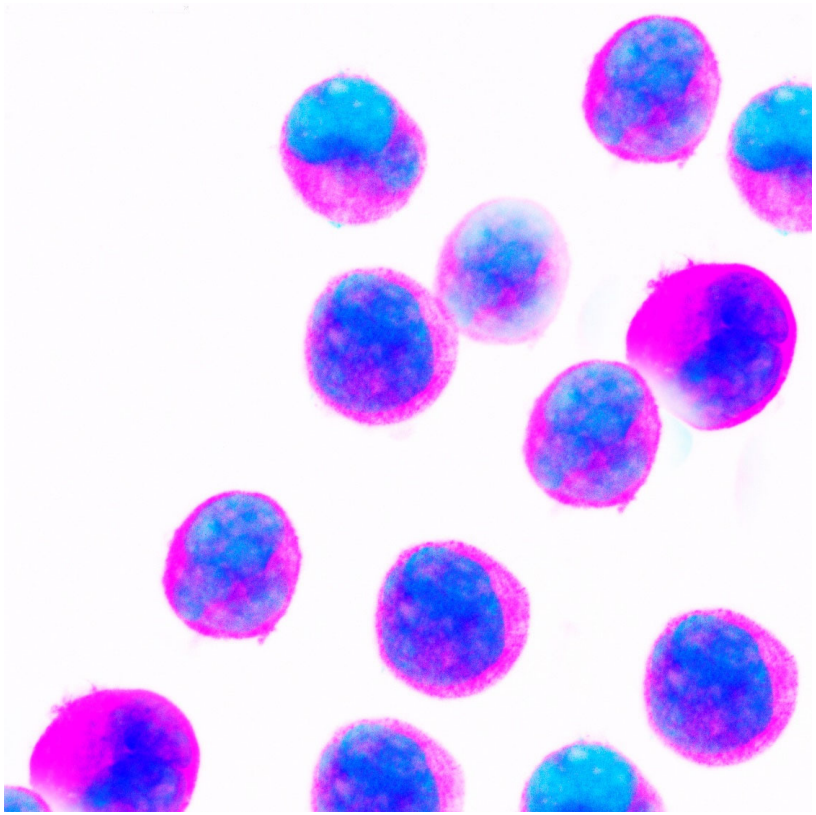
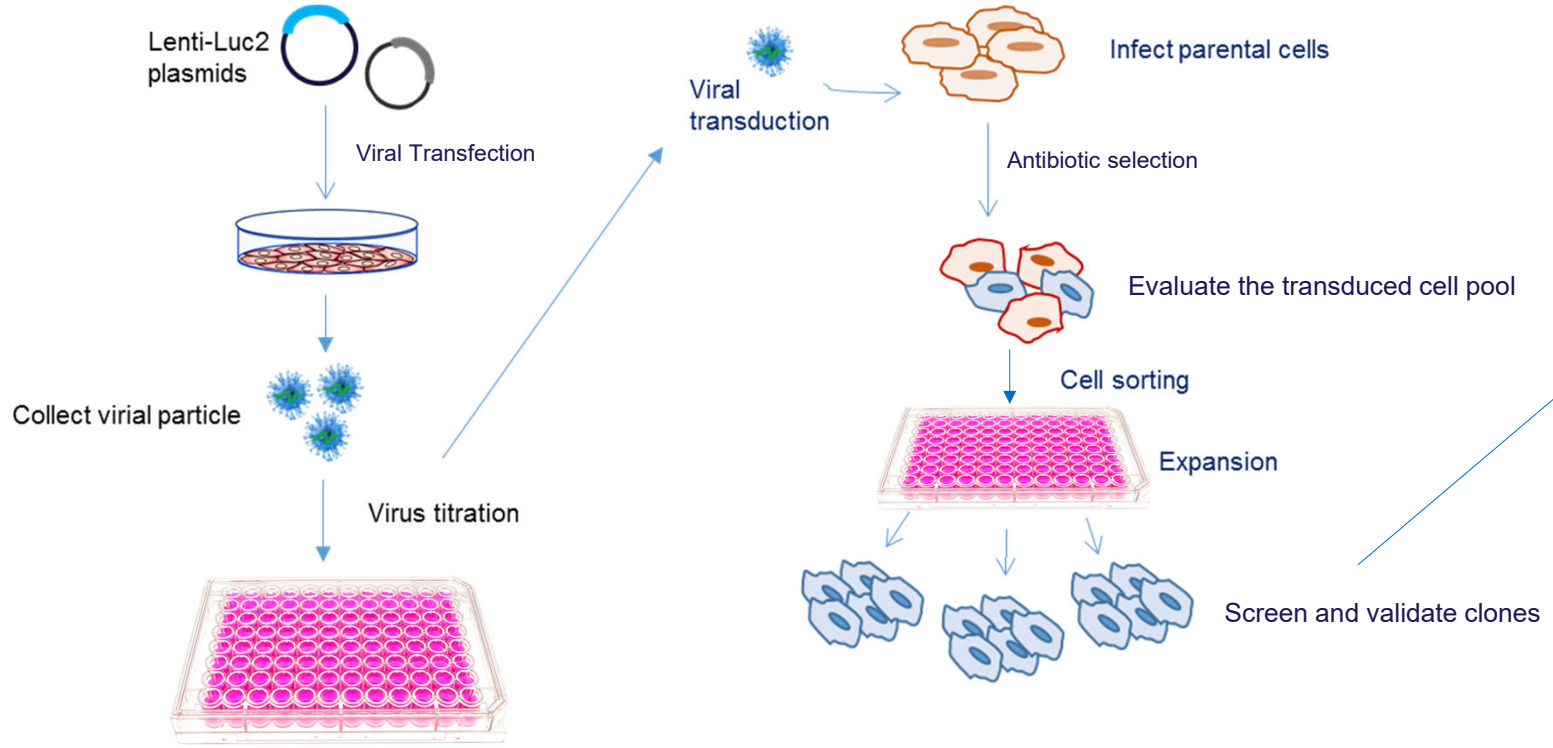
Development

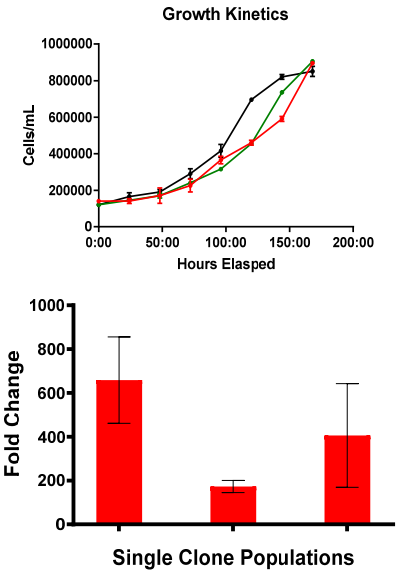
- Workflow
- Authentication
- Verification testing



Workflow for Developing Cell Lines

Viral transduction → Recovery post-selection → Single clone isolation → Screen luc activity/clone selection → Clonal expansion/ functional testing





Authentication – STR Profiling

Locus	THP-1 PARENTAL		THP-1 GAS-LUC2		THP-1 NFκB-LUC2		THP-1 AP-1-LUC2		THP-1 CRE-LUC2		THP-1 ISRE-LUC2		THP-1 NFAT-LUC2	
D3S1358														
TH01	8	9.3	8	9.3	8	9.3	8	9.3	8	9.3	8	9.3	8	9.3
D21S11														
D18S51														
Penta_E														
D5S818	11	12	11	12	11	12	11	12	11	12	11	12	11	12
D13S317	13		13		13		13		13		13		13	
D7S820	10		10		10		10		10		10		10	
D16S539	11	12	11	12	11	12	11	12	11	12	11	12	11	12
CSF1PO	11	13	11	13	11	13	11	13	11	13	11	13	11	13
Penta_D														
Amelogenin	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y
vWA	16		16		16		16		16		16		16	
D821179														
TPOX	8	11	8	11	8	11	8	11	8	11	8	11	8	11
FGA														
D19S433														
D2S1338														

No change in STR markers

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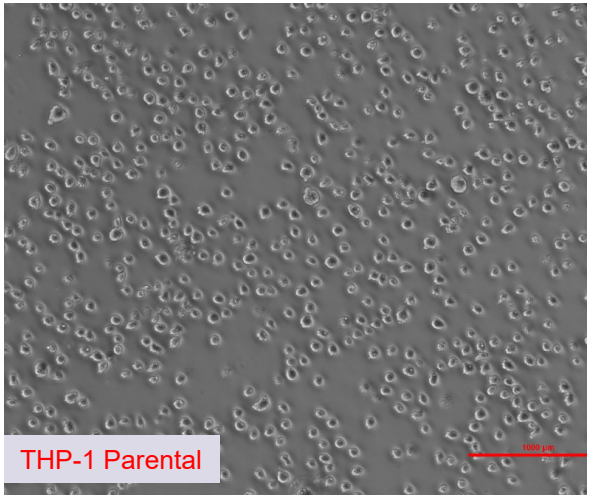
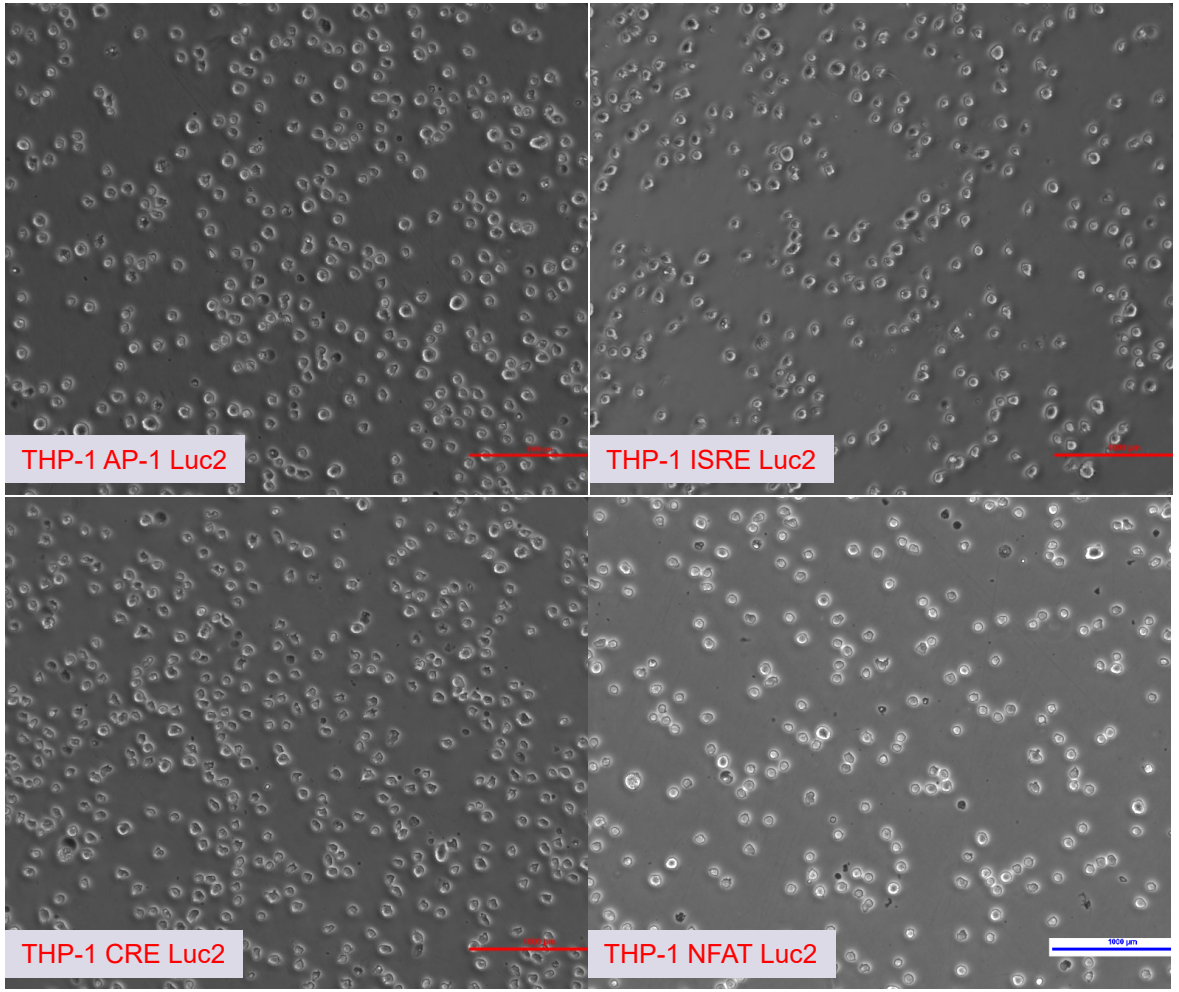
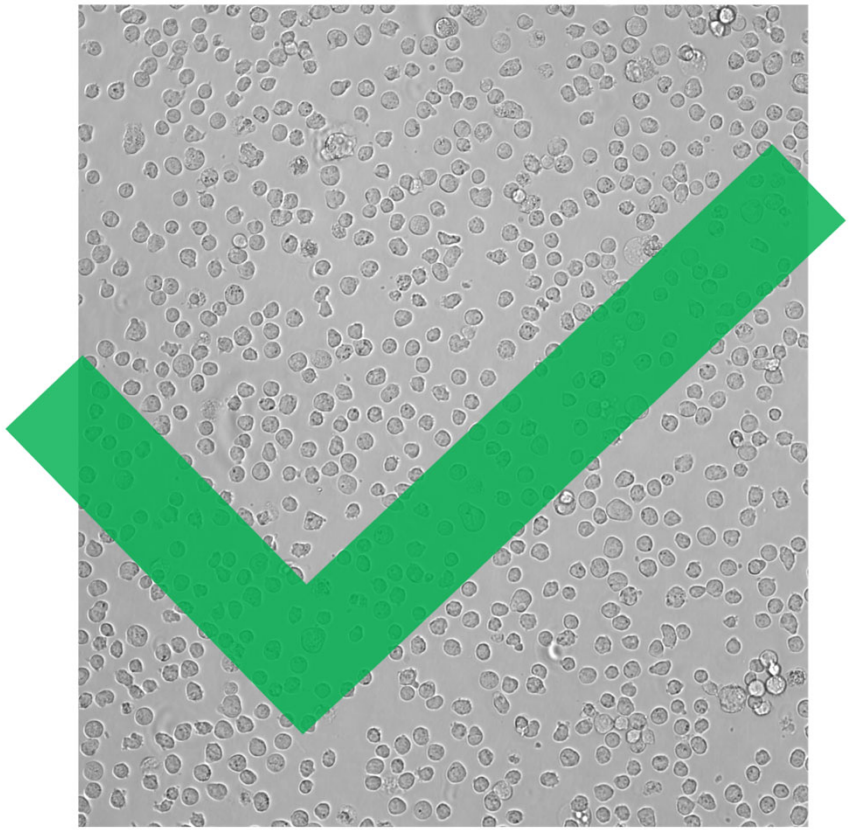
Authentication – Morphology



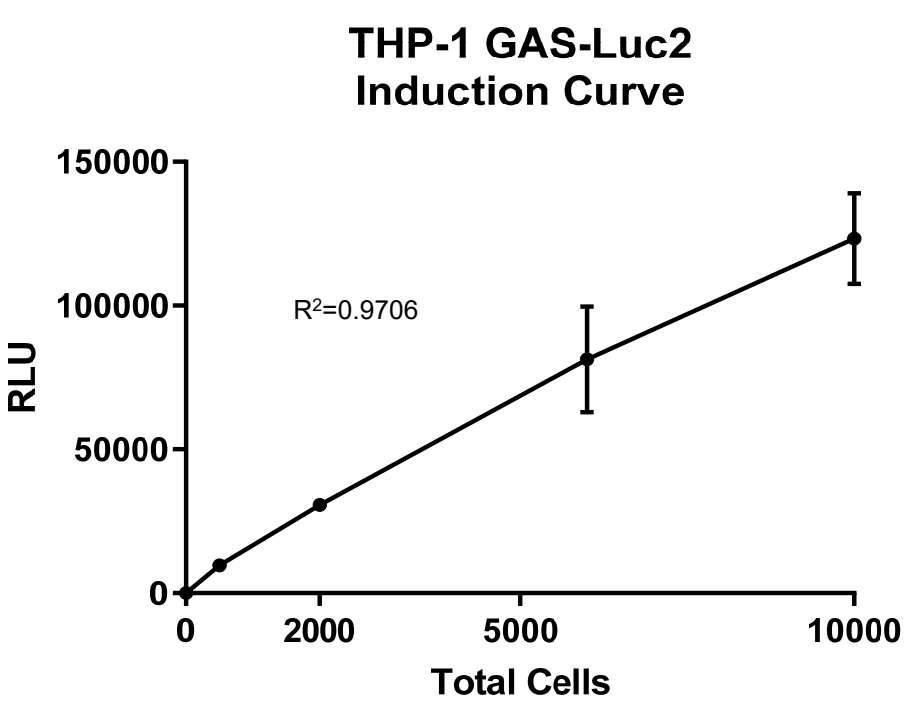


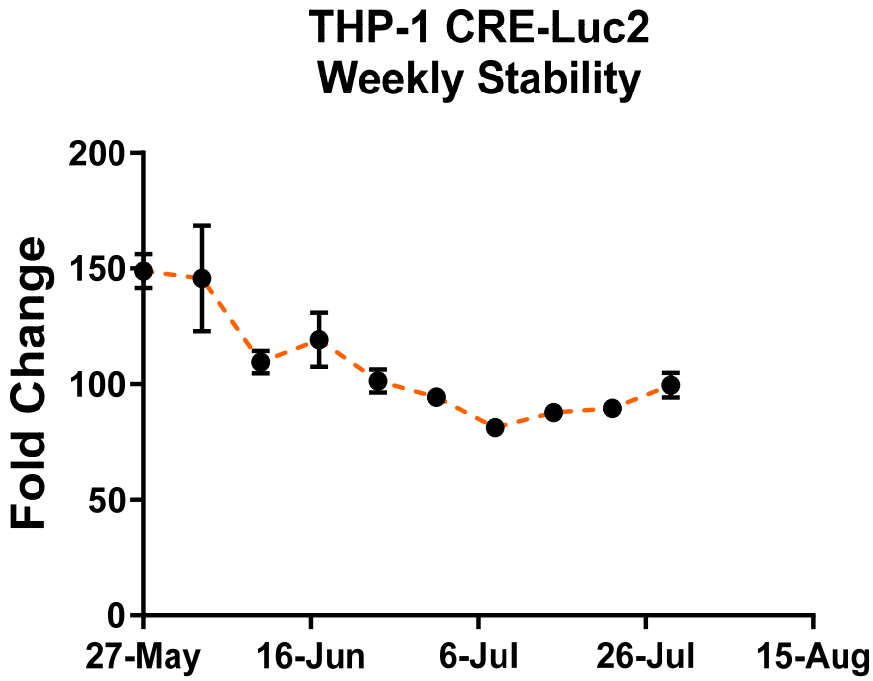
Authentication – Contaminants and CO1 Barcode

- Mycoplasma
  - Negative
- Bacterial Contamination
  - Negative
- CO1 Barcode
  - Human
  - No cross contamination

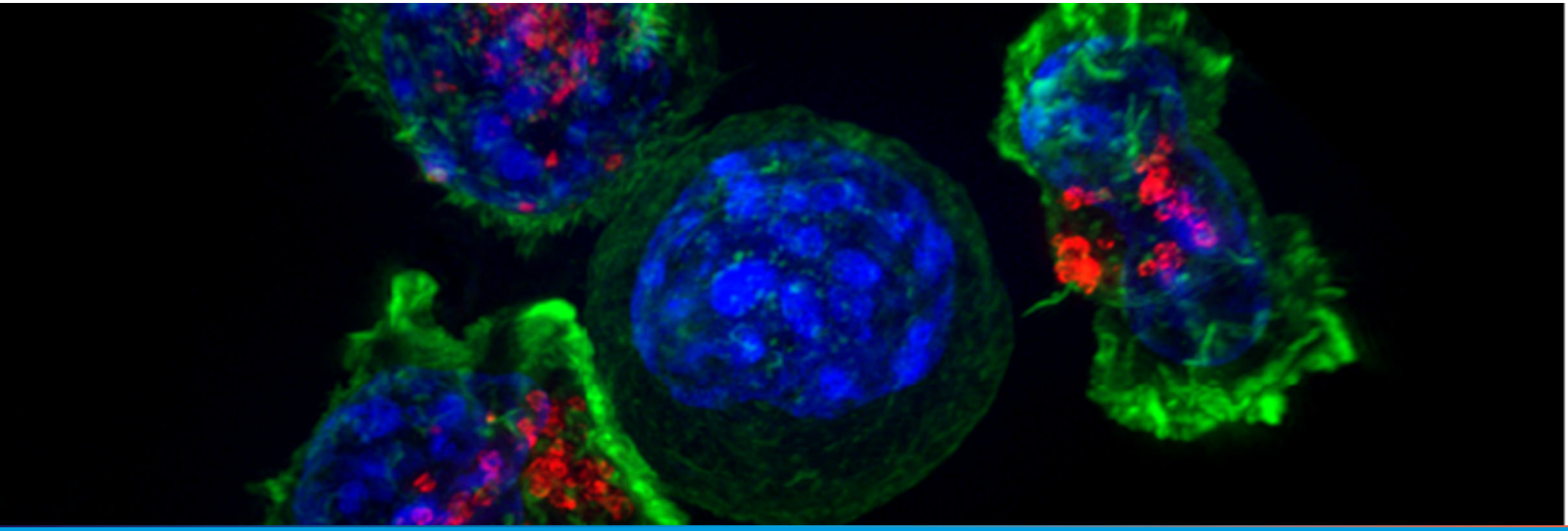


Verification Testing





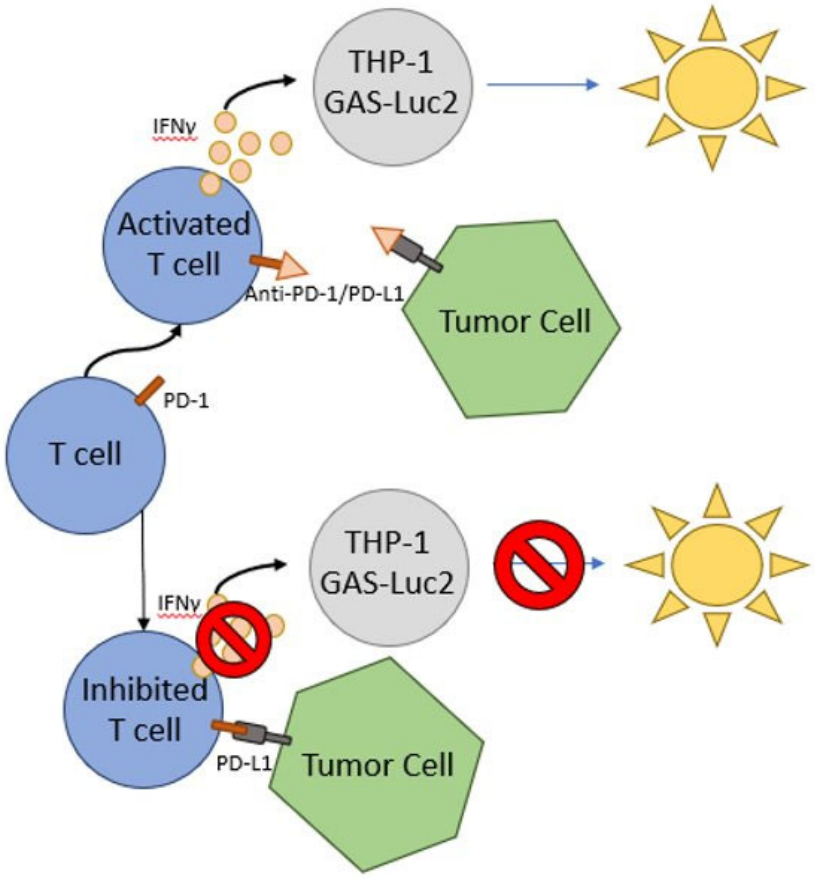
- Induction curve validates the linear correlation between bioluminescence and cell number
- Weekly stability demonstrates the consistent expression of luciferase



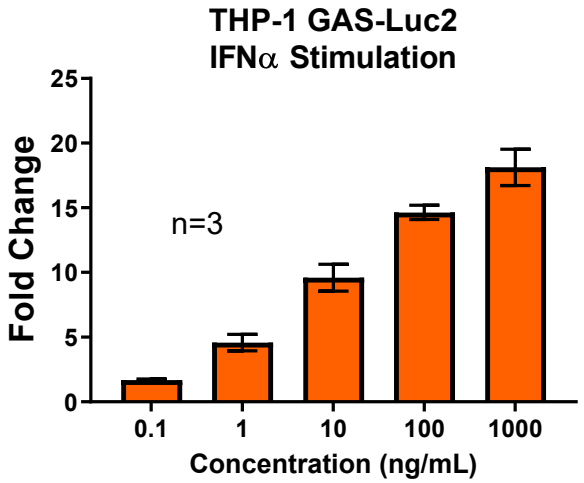
Application Data

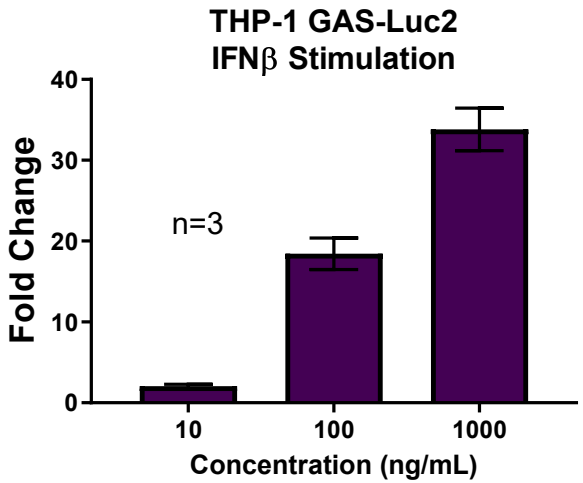
Application Data

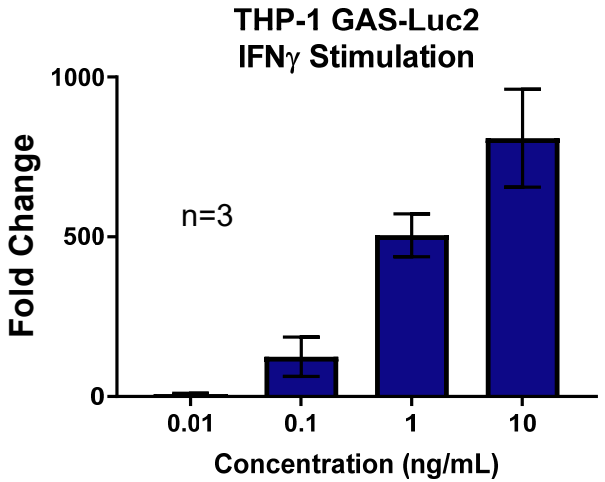
- Exogenous Stimulation
- Small Molecule Inhibitors
- T Cell Proliferation Analysis

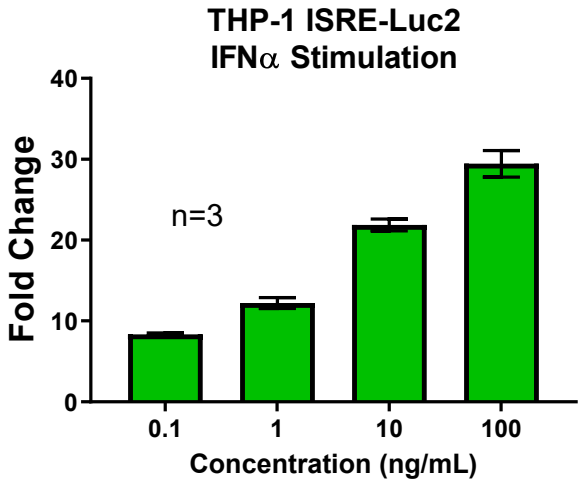


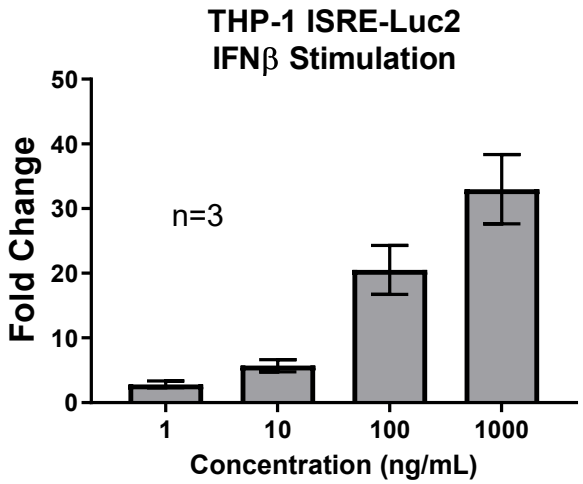
Exogenous Stimulation

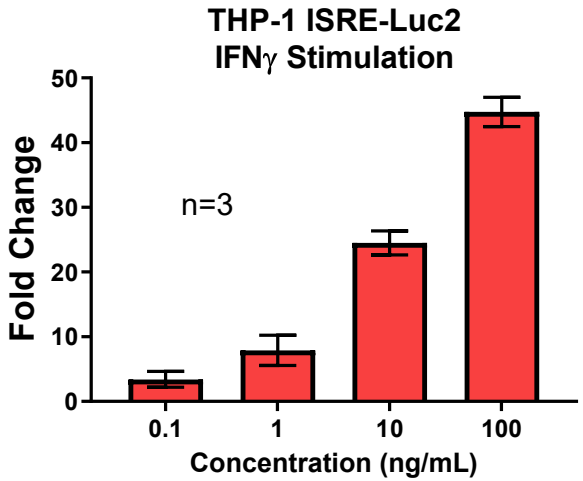




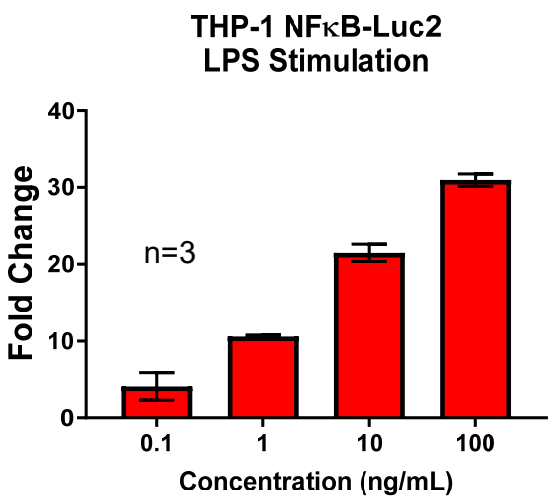


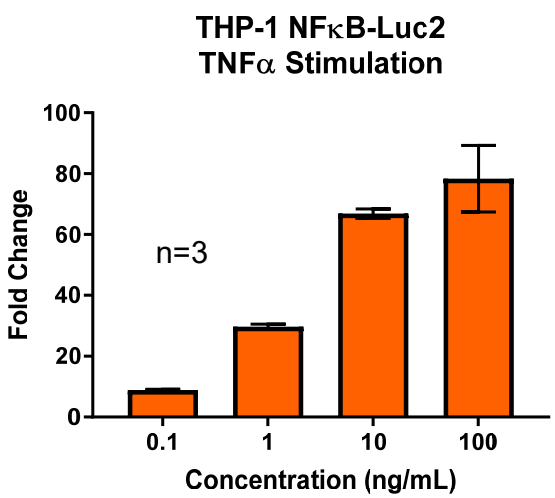


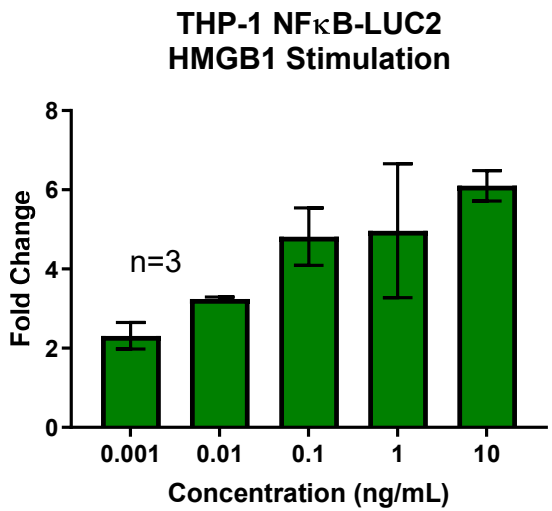


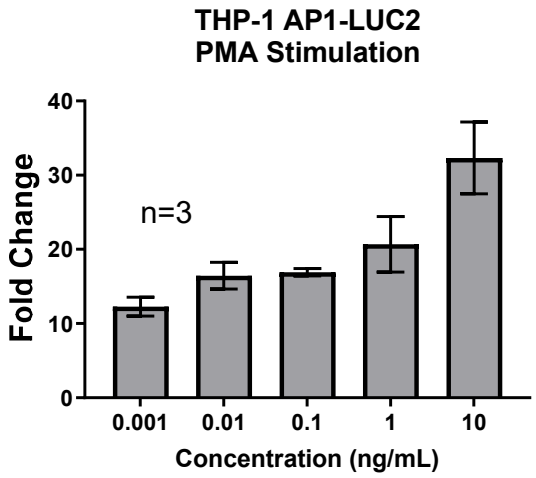


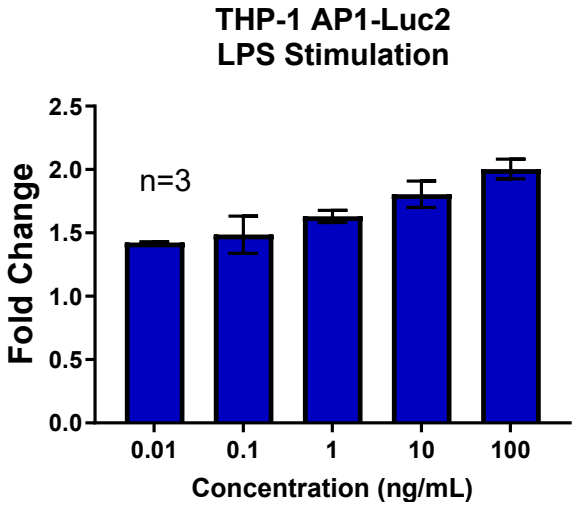
Exogenous Stimulation



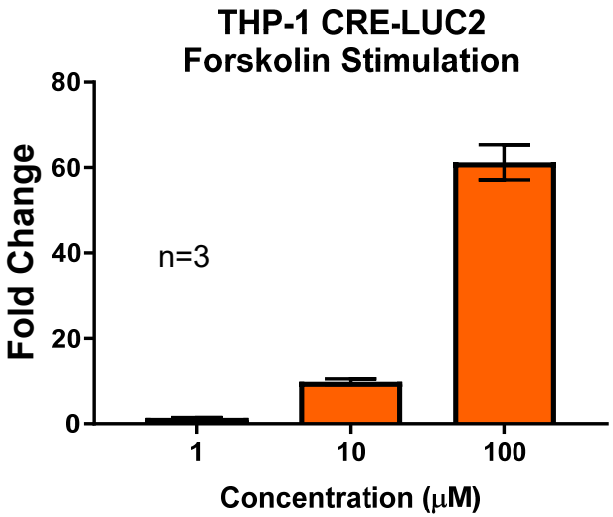


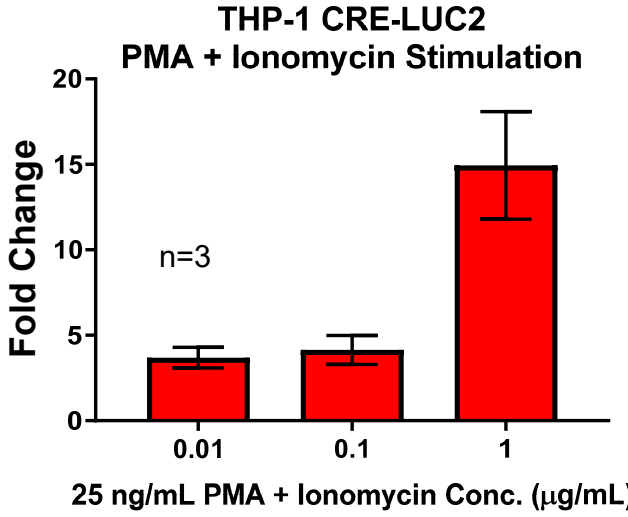


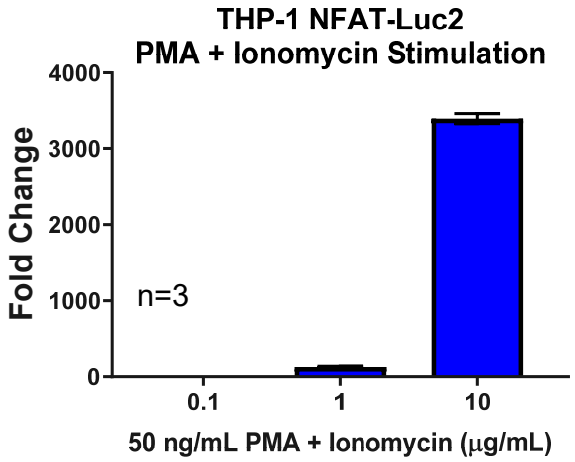


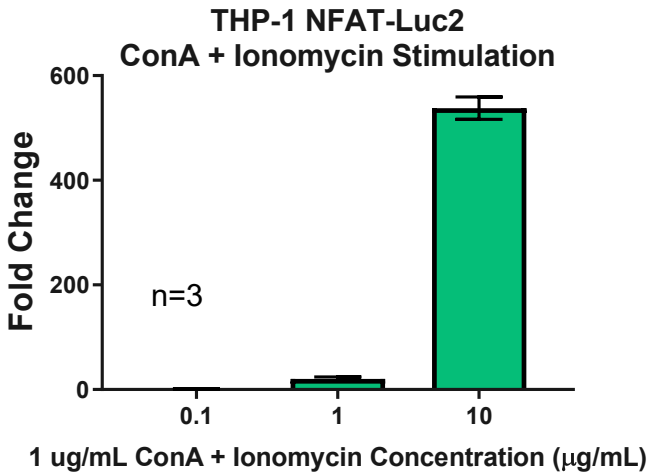


Exogenous Stimulation

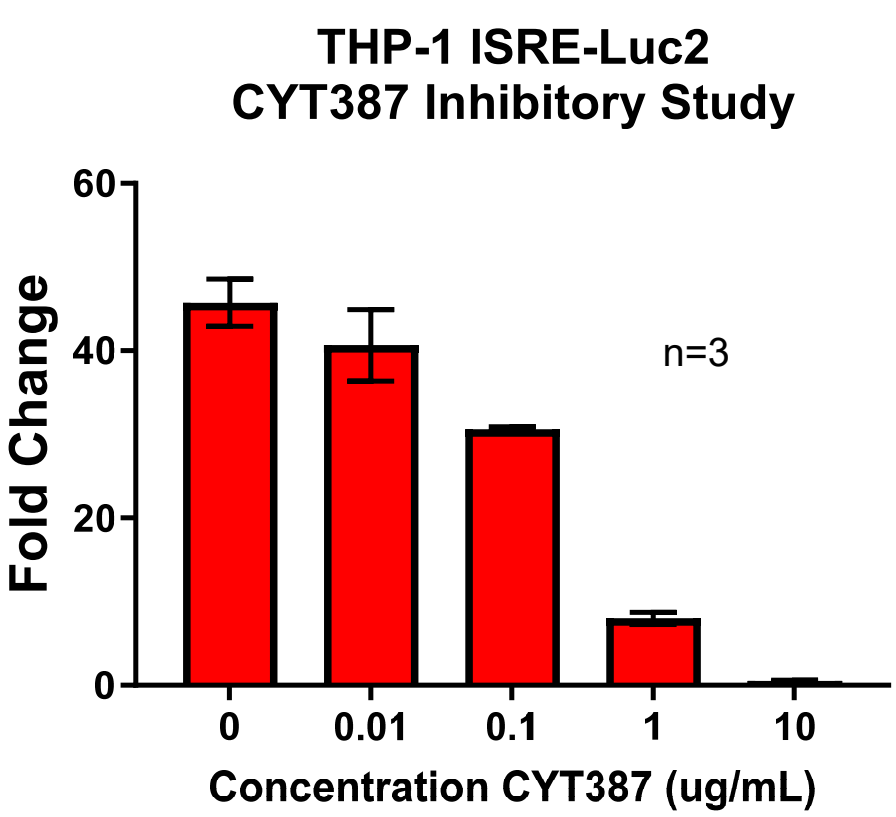


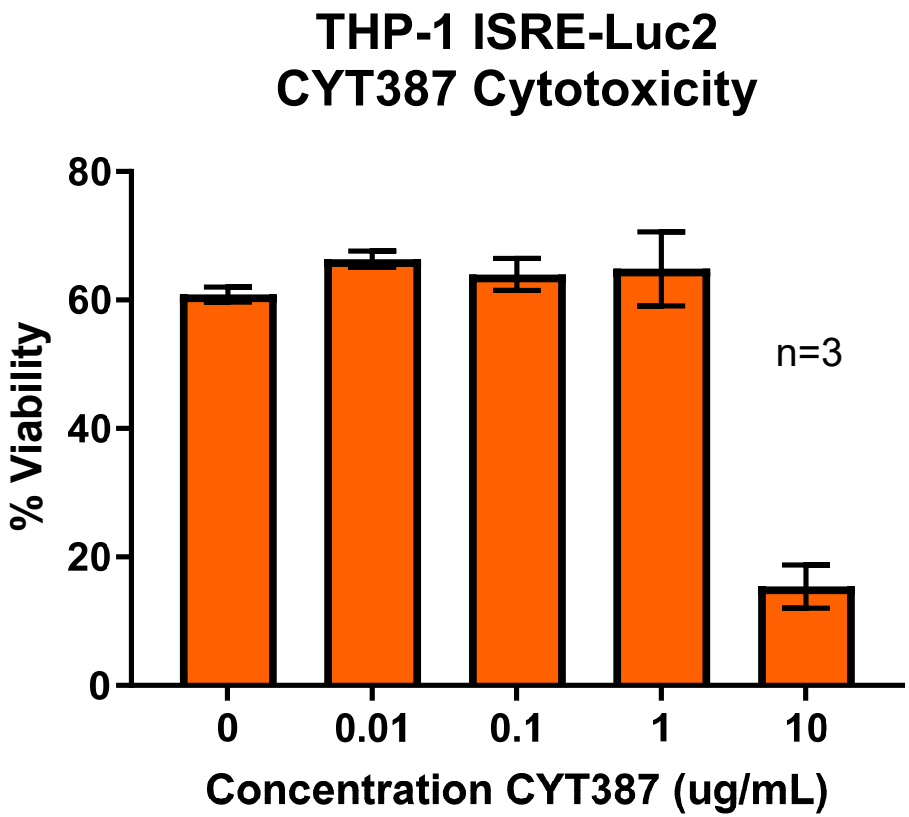




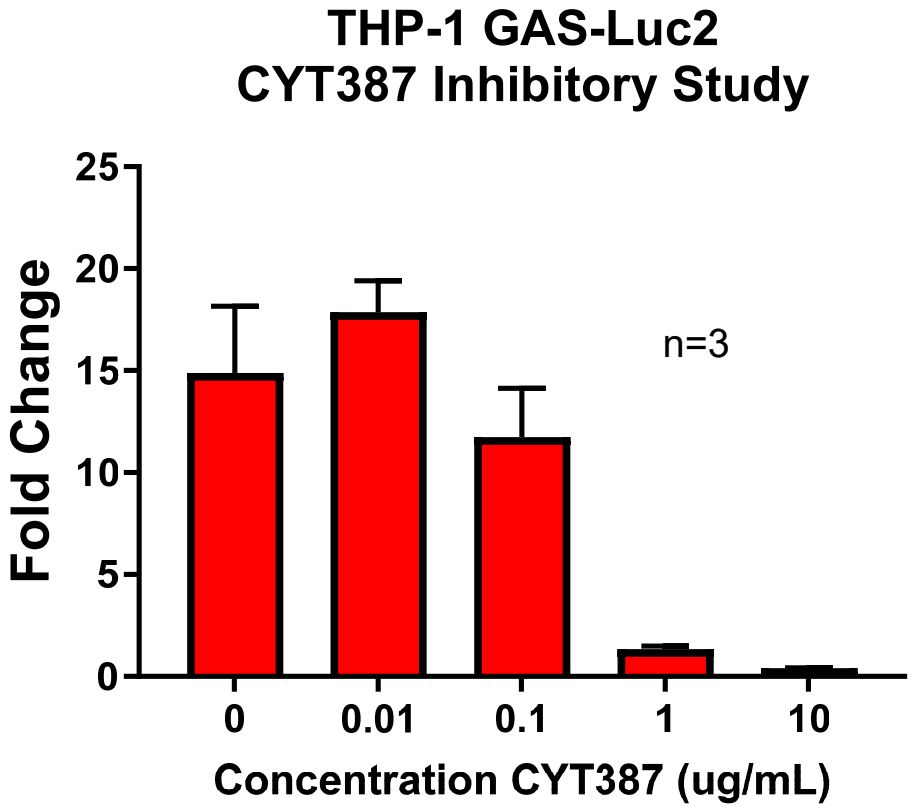


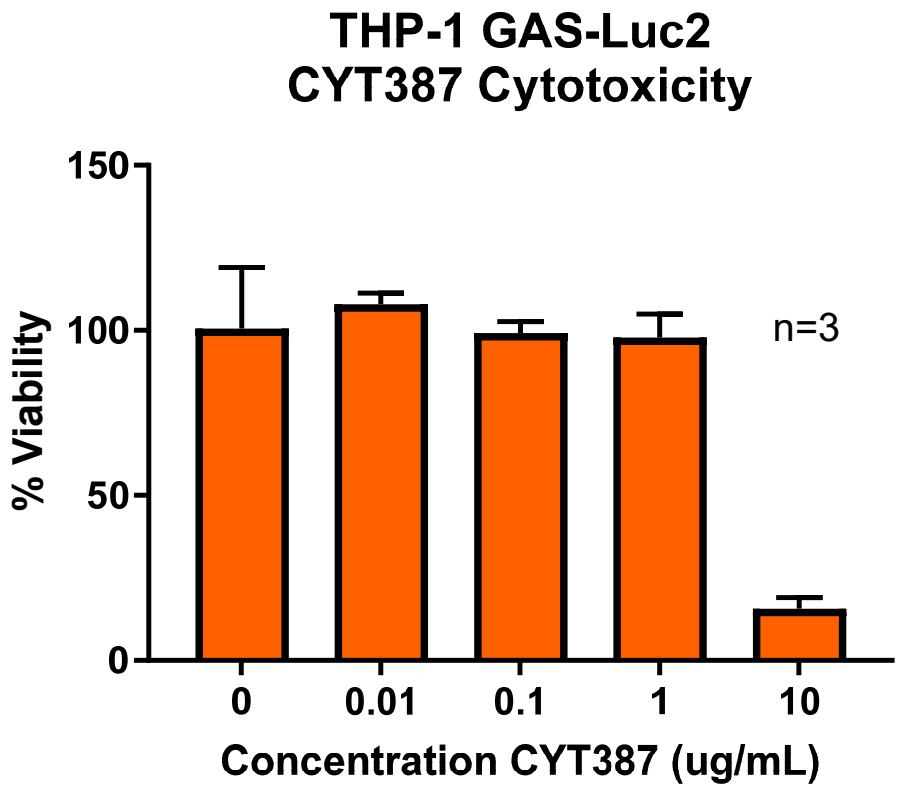
Small Molecule Inhibitor Effects on Expression



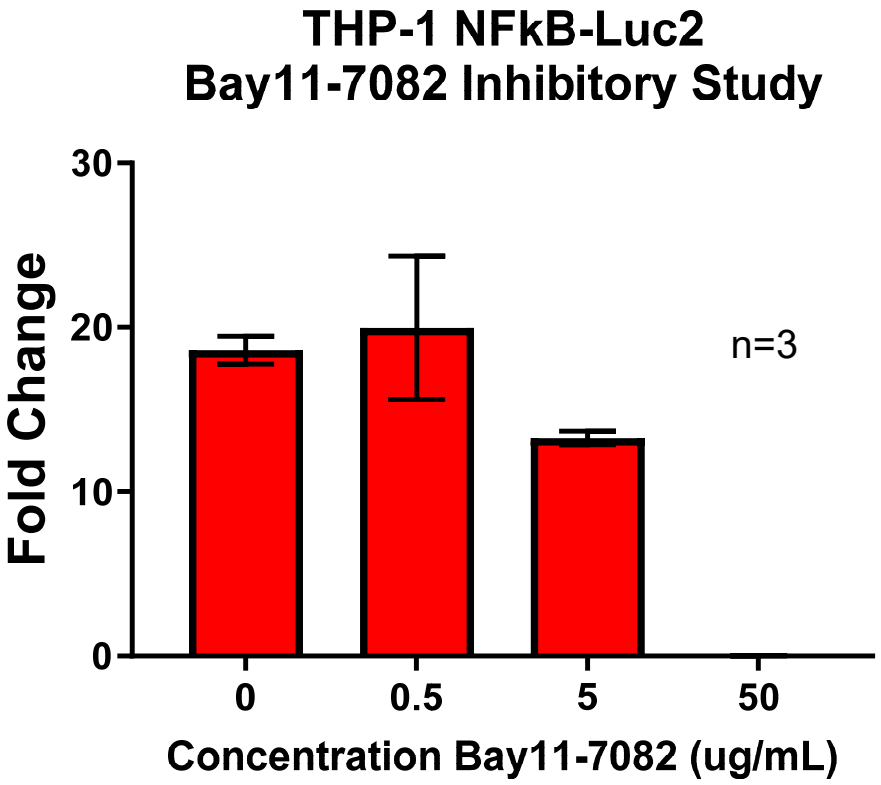


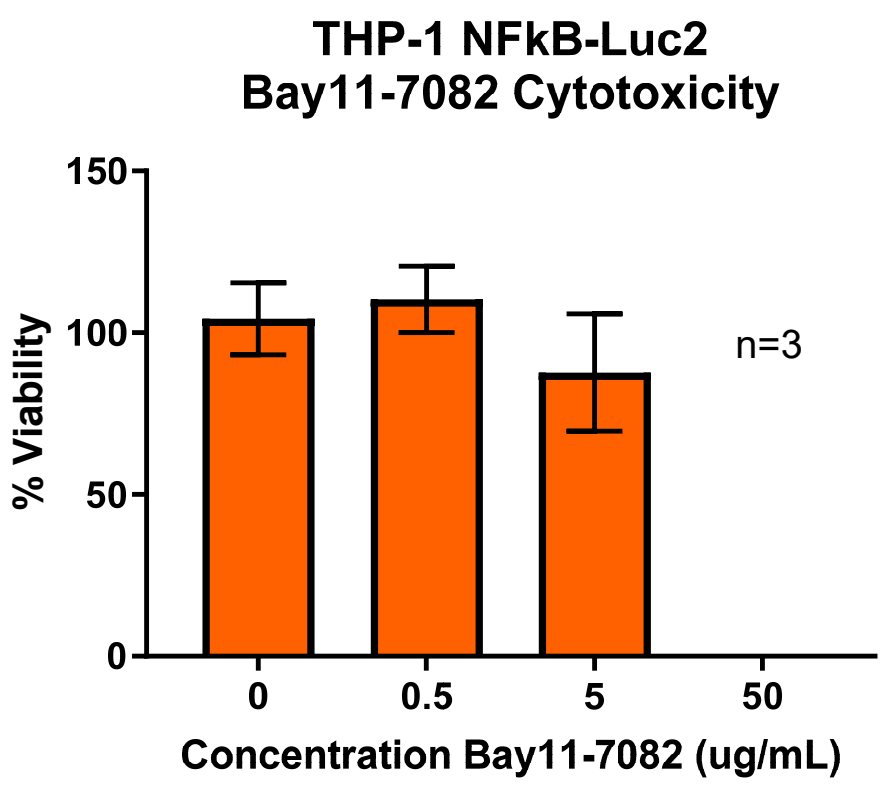
Small Molecule Inhibitor Effects on Expression

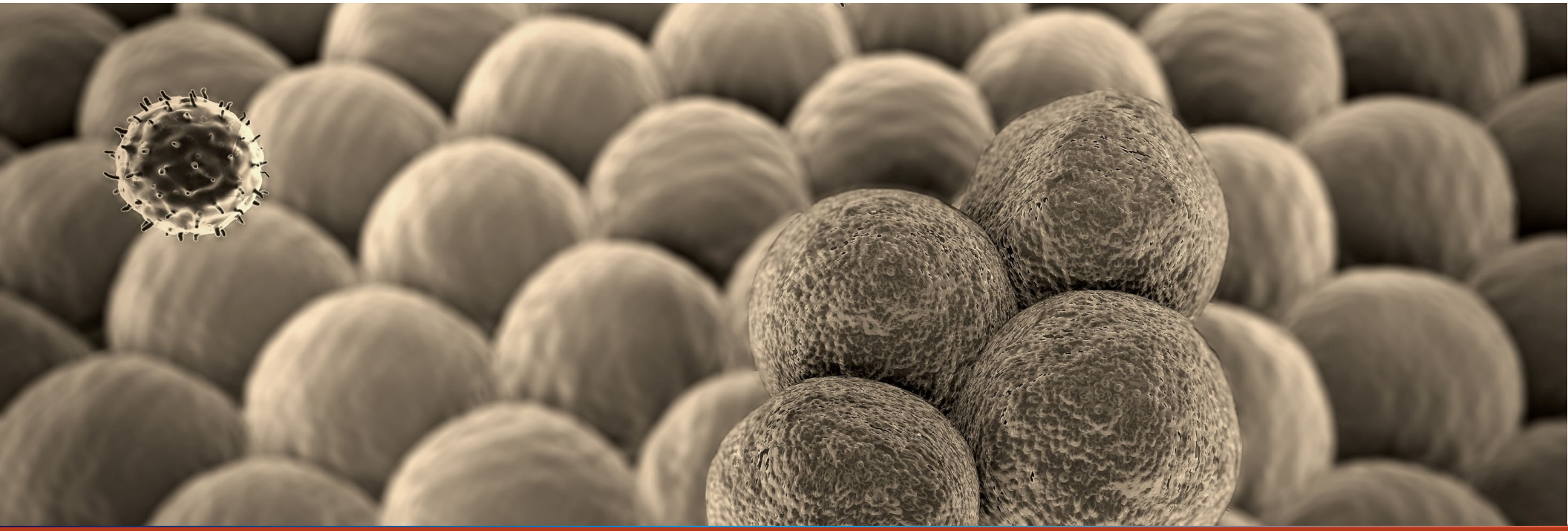




Small Molecule Inhibitor Effects on Expression







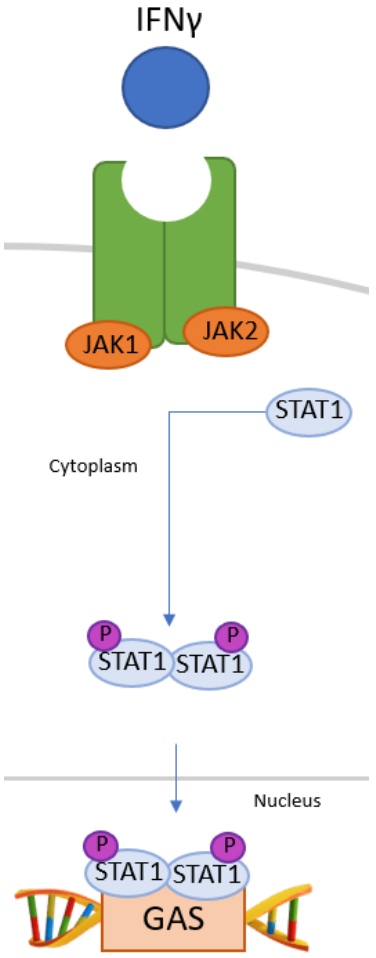
PBMC T Cell Proliferation Study

Using stimulated T cell supernatant to study IFN expression with THP-1 GAS-Luc2

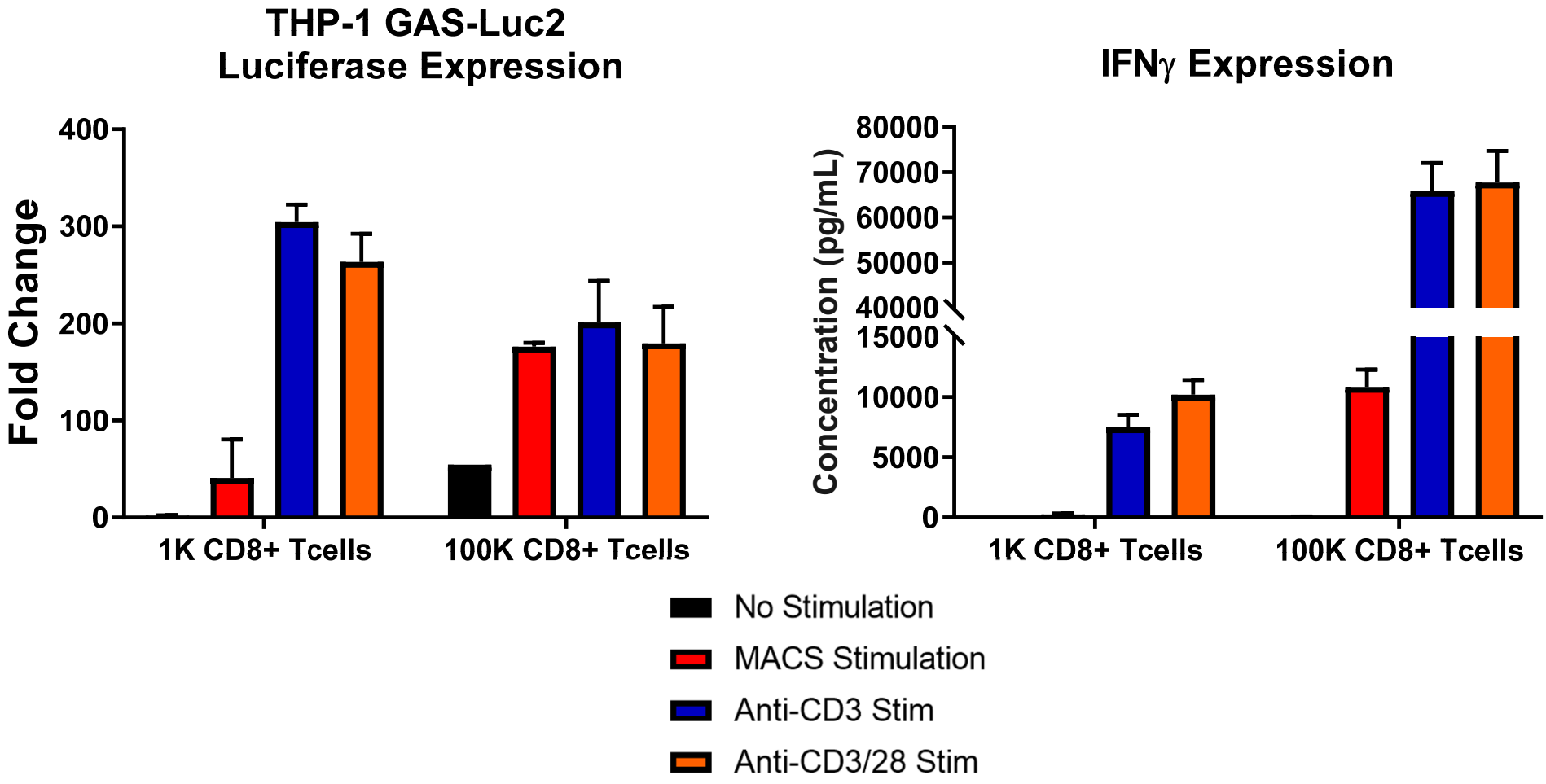
ATCC

CD8+ T Cell Proliferation Protocol

- Stimulation Reagents
  - MACS™ – Miltenyi Biotec© – Antibody based reagent that mimics a superantigen binding to TCR
  - Anti-CD3 coated well
  - Anti-CD3/CD28 coated well
- Supernatant Removed
  - IFNγ concentration quantified by immunoassay
  - Cultured with THP-1 GAS-Luc2 to measure expression



CD8+ T Cell Cytokine Expression Quantification



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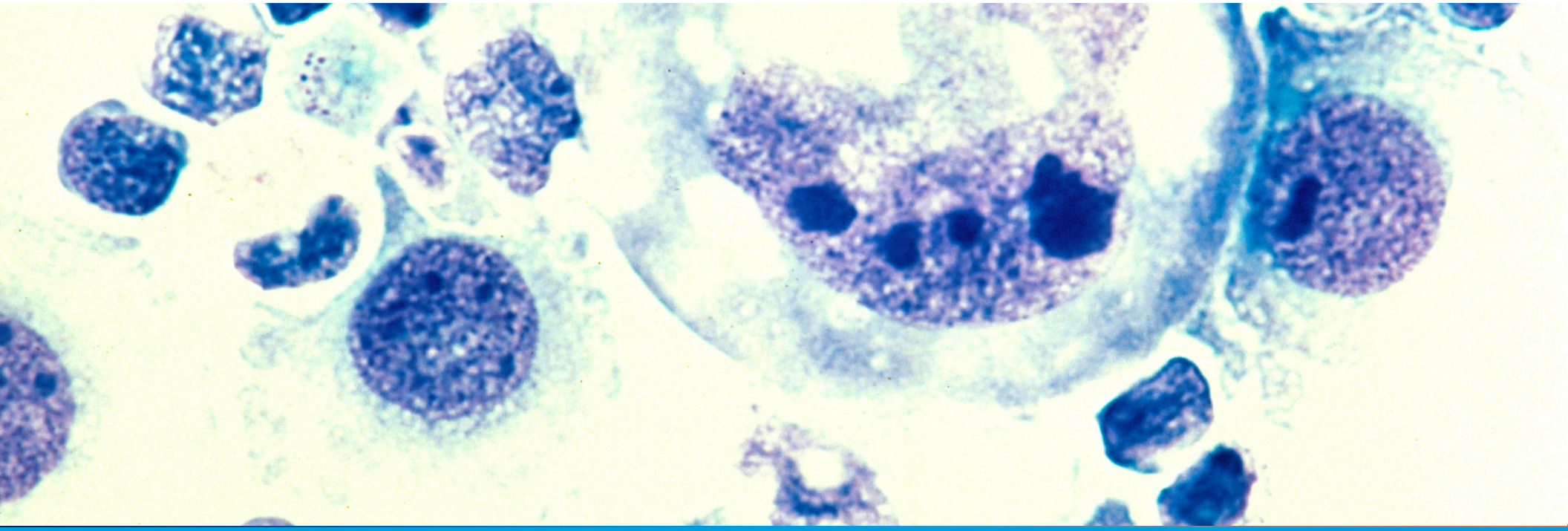


Conclusion

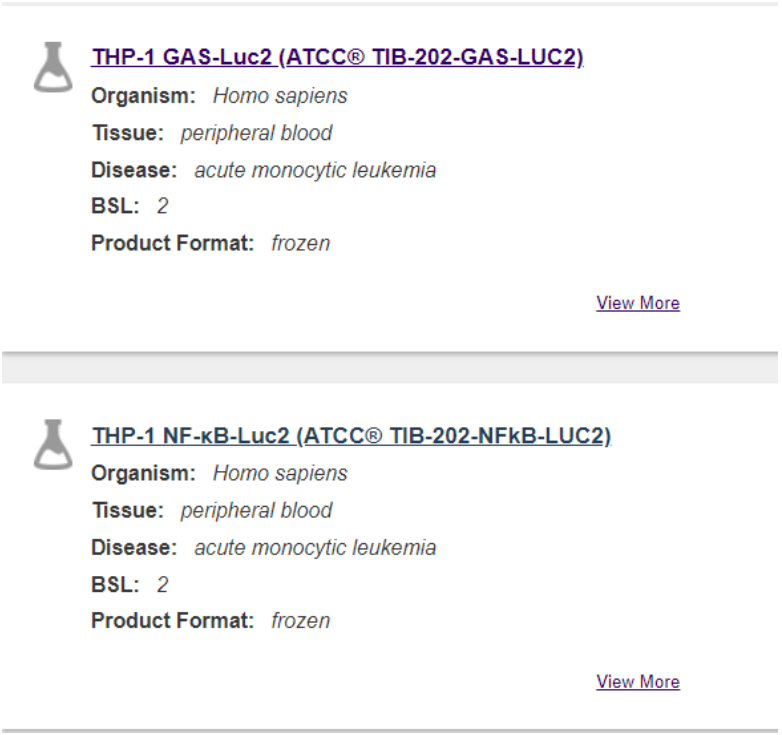
ATCC®

Conclusion

Available Cell Lines

- All 6 cell lines are available for purchase at www.ATCC.org

Designation	ATCC® No.
THP-1 NF-kB-Luc2	TIB-202-NF-kB-Luc2™
THP-1 GAS-Luc2	TIB-202-GAS-Luc2™
THP-1 AP-1-Luc2	TIB-202-AP-1-Luc2™
THP-1 CRE-Luc2	TIB-202-CRE-Luc2™
THP-1 ISRE-Luc2	TIB-202-ISRE-Luc2™
THP-1 NFAT-Luc2	TIB-202-NFAT-Luc2™



Conclusion

Summary

- THP-1 reporter cell line will save you time and money
  - No need to undergo the development process
  - Performance already tested

- Completed verification and QC testing
  - Tested activation against appropriate stimuli
  - Cells are well-authenticated and contaminant free

- The reporter cell lines were created to give the scientific community a simple, robust evaluation tool
  - Signaling pathway identification
  - Immunomodulatory drug screening
  - Safety assessment

www.atcc.org/immuno-oncology

ATCC

Conclusion

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www.atcc.org/immuno-oncology

ATCC

Learn more: **www.atcc.org/advancedimmunology**

Going to the Society for Immunotherapy of Cancer Conference (SITC) 2022?

Stop by our booth #313, or visit our scientific posters:

A PD-L1 reporter cell line based on the immune checkpoint protein profiling of ATCC cell lines facilitates cancer immunotherapy drug screening
Hyeyoun Chang, PhD, Scientist, ATCC
November 10, 2022 – November 11, 2022
9:00 AM - 9:00 PM
Abstract Number: 510

Luciferase reporter cancer cell lines facilitate CAR-T development
John Foulke, MS, Lead Biologist, ATCC
November 10, 2022 – November 11, 2022
9:00 AM - 9:00 PM
Abstract Number: 220