



Cryopreservation Impact on Functional Recovery of Luciferase Reporter Cells

Quinn Osgood, BS
Lead Biologist, ATCC

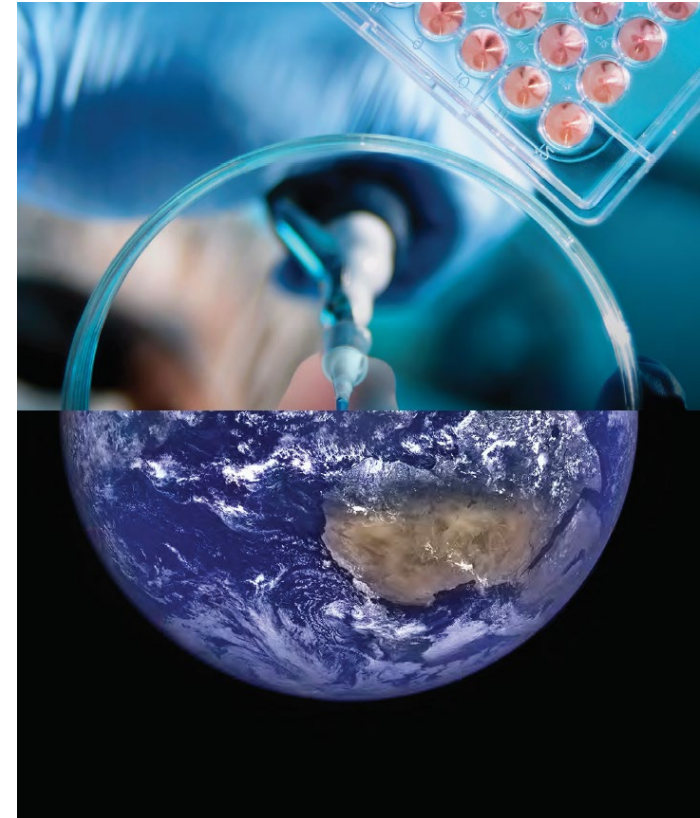
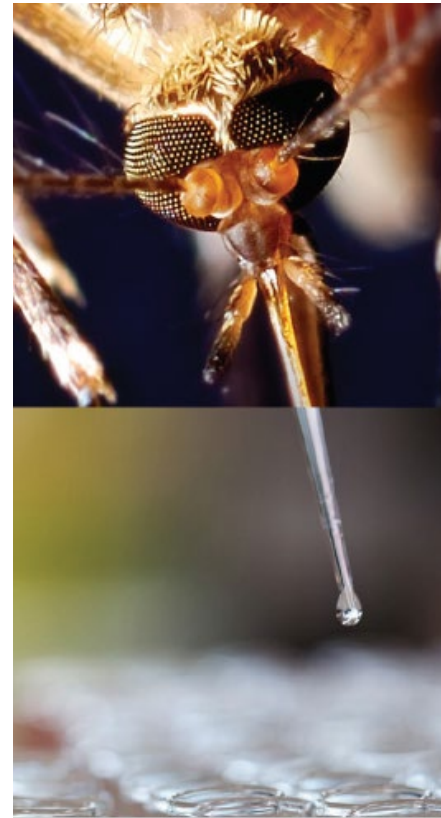
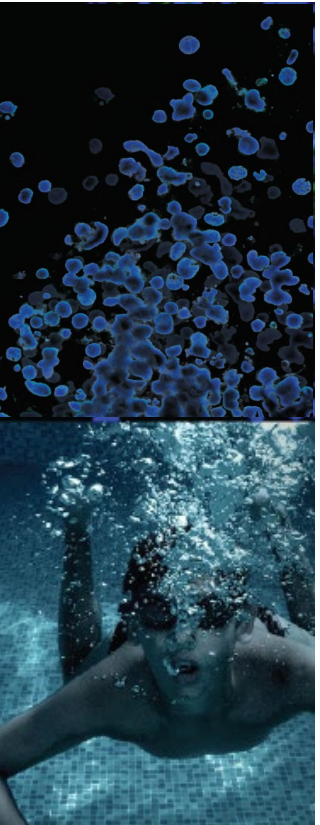
Lukas Underwood, PhD
Scientist, ATCC

Jacqueline Mikhaylov, BS
Biologist, ATCC

Nilay Chakraborty, PhD MBA
BioNexus Foundation Principal Scientist,
Cryobiology, ATCC

CRYO2023
July 27, 2023

Credible Leads to Incredible®

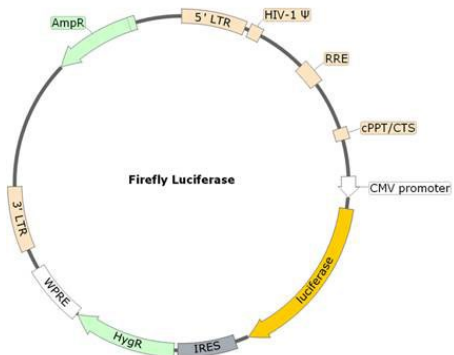


About ATCC®

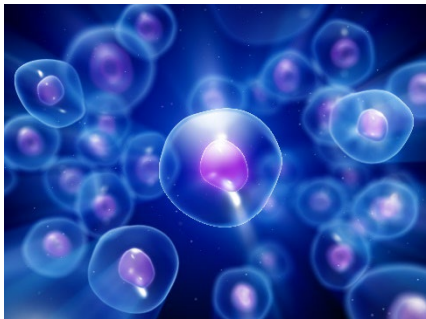
- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
 - 5,000 cell lines
 - 80,000 microorganisms
 - Genomic & synthetic nucleic acids
 - Media/reagents
- ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 500+ employees, over one-third with advanced degrees

Luciferase Reporter Cells

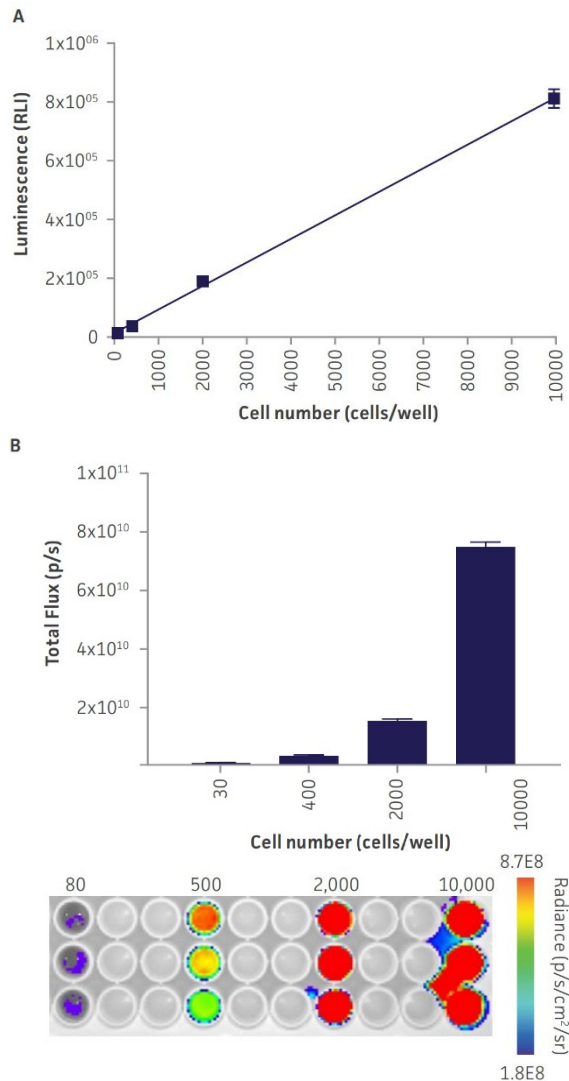
Lentiviral transduction



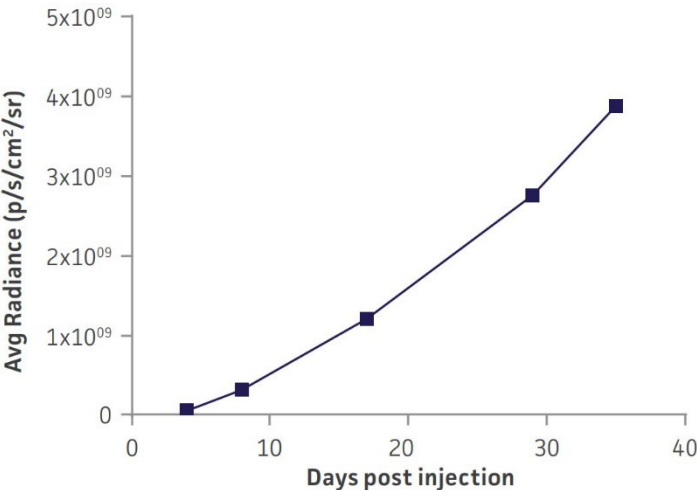
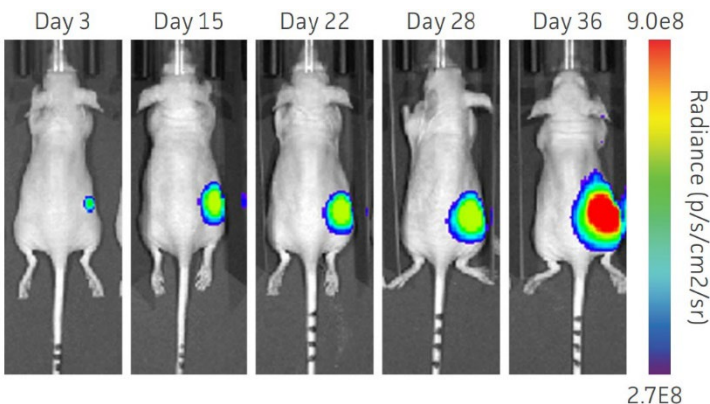
Single cell cloning
and expansion



In vitro assays



In vivo modeling



THP-1 Reporter Cells

Let's start with the parent cell line (ATCC® TIB-202™)

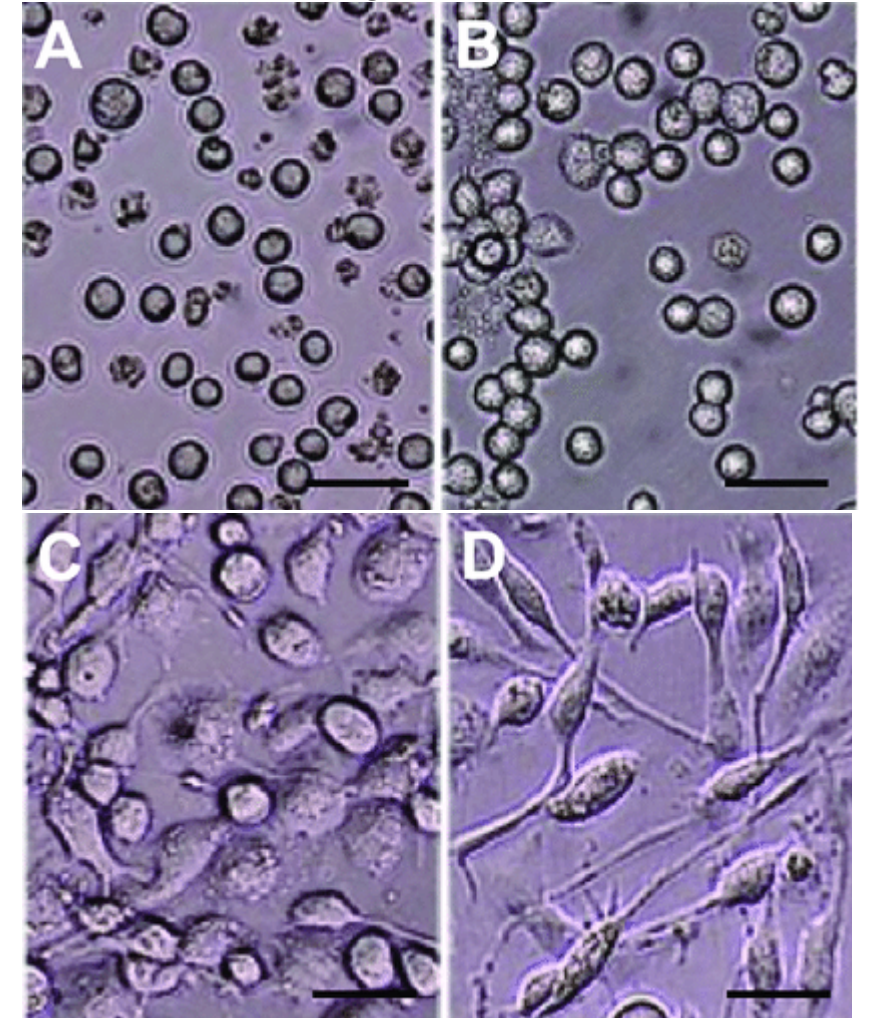
Characteristics

- Human monocytic cell line
- Acute monocytic leukemia
- Analogous to peripheral blood mononuclear cells (PBMCs)

Applications

- In vitro cancer modelling
- Monocyte-macrophage differentiation
- Dendritic cell differentiation and modelling

Macrophage Differentiation



Wheeler KC, et al. VEGF may contribute to macrophage recruitment and M2 polarization in the decidua. PLoS One 13(1): e0191040, 2018. PubMed: 29324807

THP-1 Reporter Cells

ATCC products and advantages

Response Element	ATCC No.	Signaling Pathway	Function
NFκB	TIB-202-NFκB-LUC2™	NFκB	Pivotal mediator of inflammatory response
GAS	TIB-202-GAS-LUC2™	JAK-STAT (Type II)	Initiates immune cell activation and recruitment
CRE	TIB-202-CRE-LUC2™	cAMP/PKA	Inflammatory mediator and phagocytosis modulator
ISRE	TIB-202-ISRE-LUC2™	JAK-STAT (Type I)	Initiates immune cell activation and recruitment
AP1	TIB-202-AP1-LUC2™	MAPK/ERK	Regulates innate and adaptive immune response
NFAT	TIB-202-NFAT-LUC2™	Calcineurin-NFAT	Mediates adaptive T and B cell activation

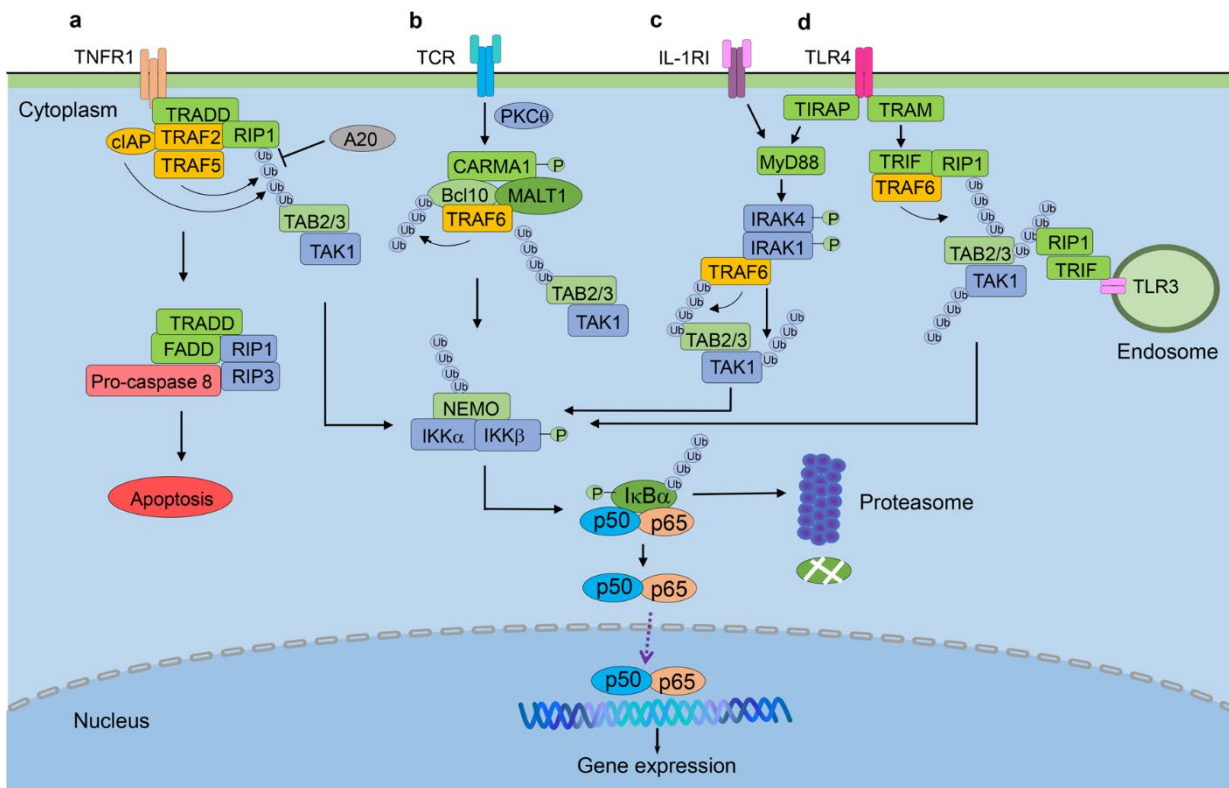
Key Features	Key Benefits
Fully authenticated parental THP-1 cell line	No concerns about cross-contamination and misidentification, saves time and money
High signal-to-noise ratio	Clear and more intense results, straightforward data analysis
Verified, characterized stable expression	Reduced variability, reproducible results
Easy to culture, robust, and highly sensitive	Ease of use, customer convenience
Amenable to complex experimentation (combinatorial stimulation, co-culture)	Versatile and compatible with multiple platforms
High density cryopreservation	More viable cells post-thaw



THP-1-NFκB-Luc2 (ATCC® TIB-202-NFκB-LUC2™)

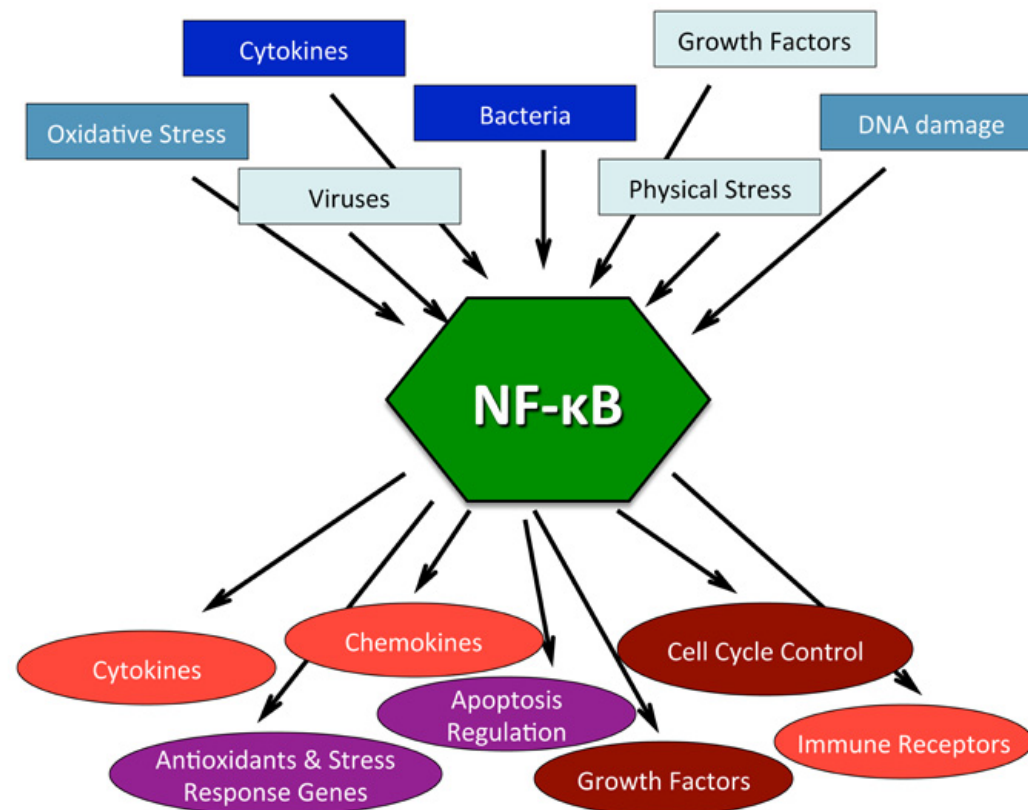
NF-κB signaling

Complete NF-κB Signaling



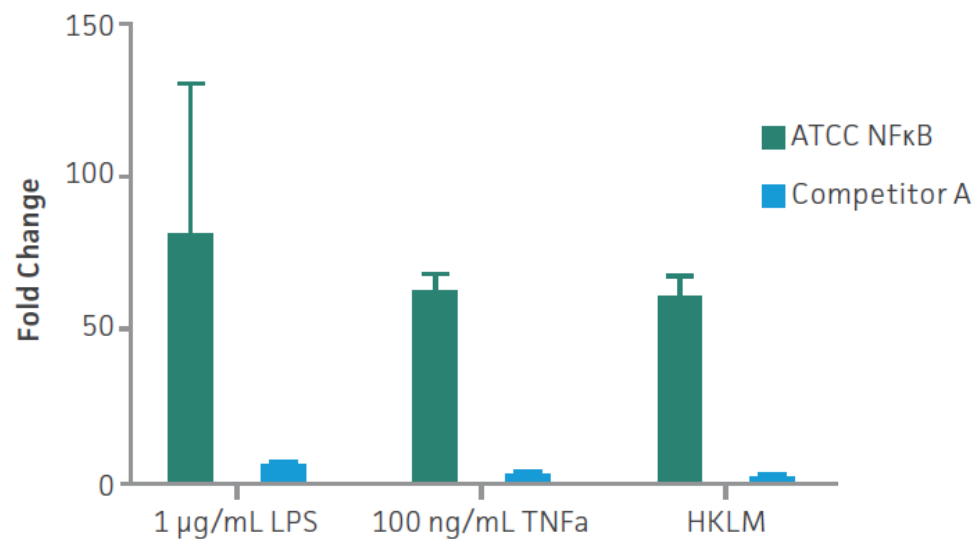
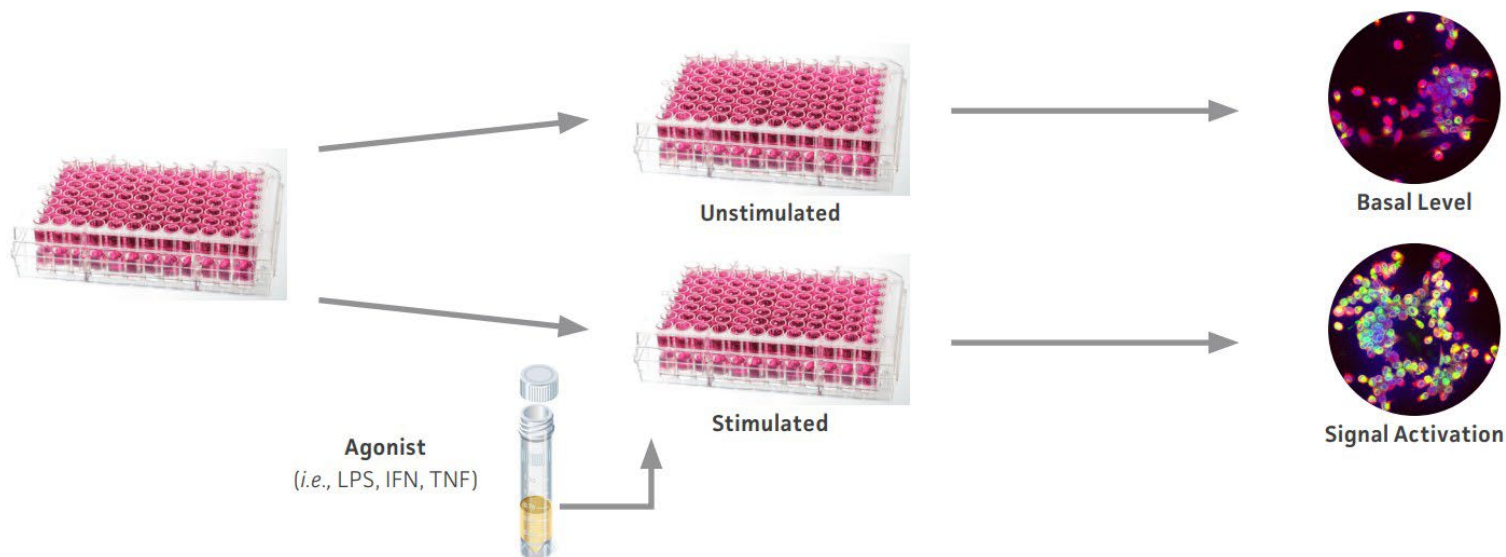
Yu H, et al. Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. Signal Transduct Target Ther 5(1): 209, 2020. PubMed: 32958760

Simplified NF-κB Signaling

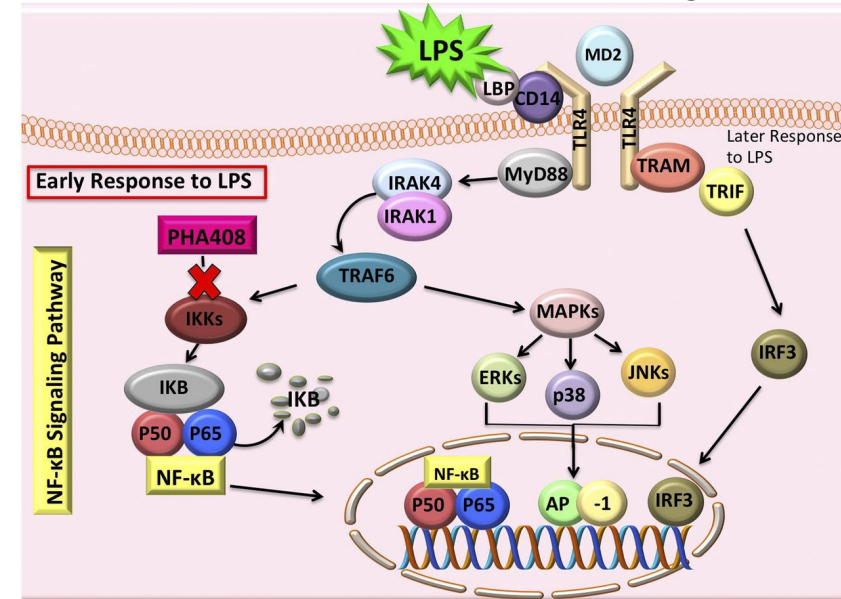


Prof. David Wallach's Lab, Weizmann Institute of Science, Department of Biomolecular Sciences.
https://www.weizmann.ac.il/Biomolecular_Sciences/Wallach/

THP-1-NFκB-Luc2 (ATCC® TIB-202-NFκB-LUC2™)



LPS Stimulated Pathway



Abualsunun WA, Piquette-Miller M. Involvement of Nuclear Factor κ B, not Pregnane X Receptor, in Inflammation-Mediated Regulation of Hepatic Transporters. Drug Metab Dispos 45(10): 1077-1083, 2017. PubMed: 28778997

Cryopreservation of Reporter Cells

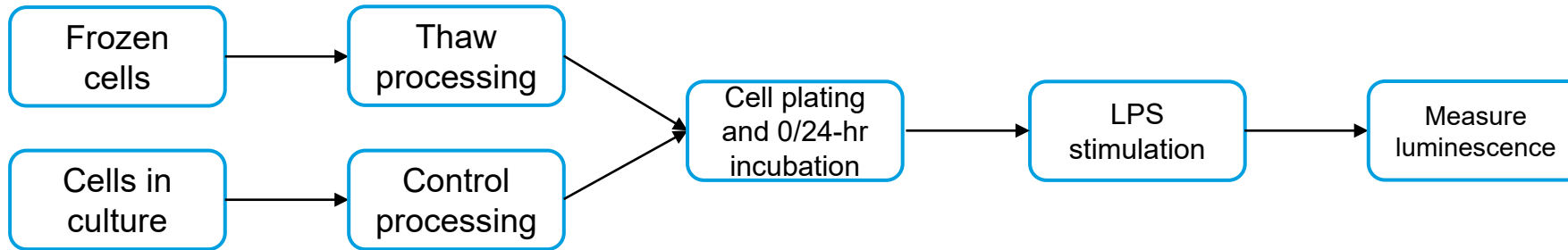
Overarching goals:

- Reporter cells must be preserved for long-term storage and transport. Robust recovery and return to normal culture state are essential.

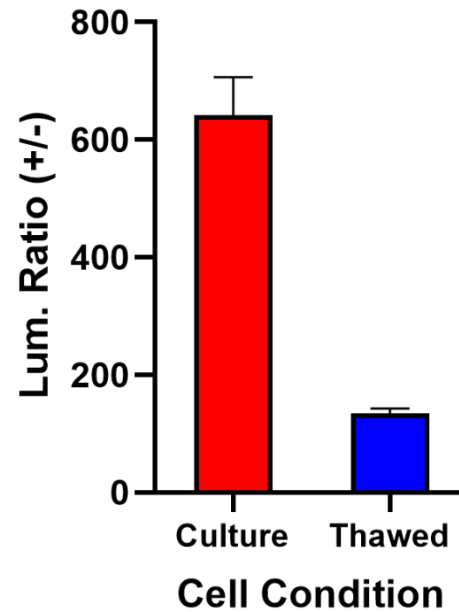
Open questions and potential challenges:

- Are reporter cells impacted by cryopreservation?
- If there is a difference in response, what is the lag time before acceptable responses are possible? Can this be impacted by improved preservation processing?
- Do the modifications needed to create the reporter functionality impact overall cell functionality?

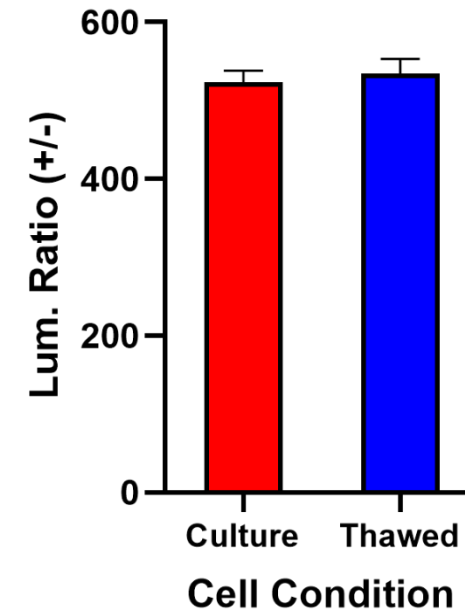
Initial Freeze Experiment



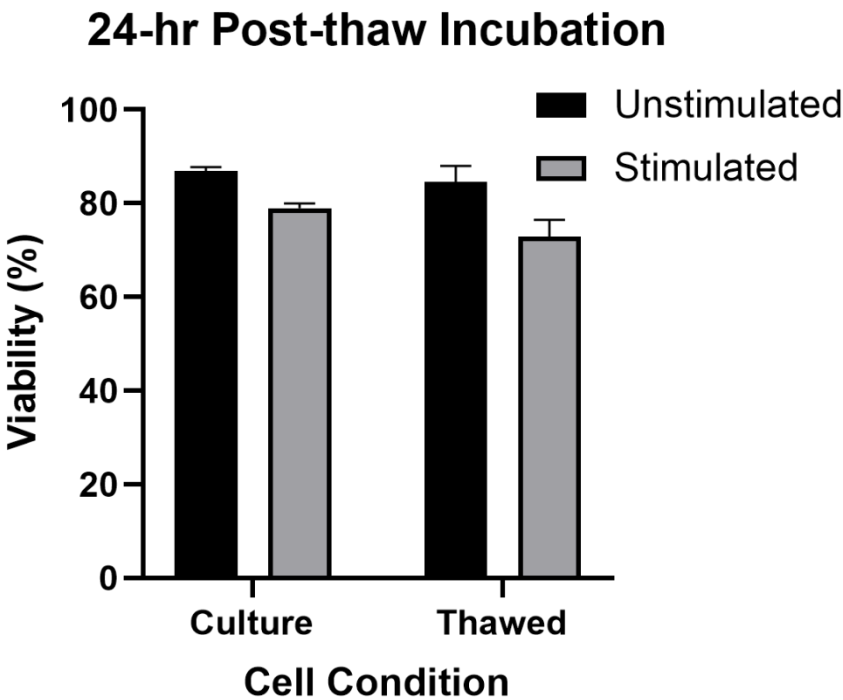
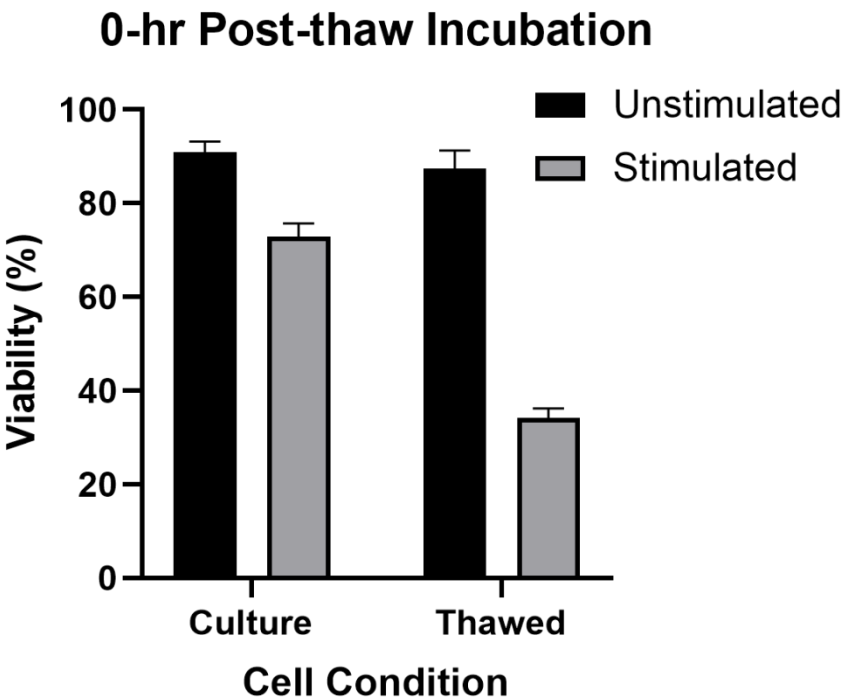
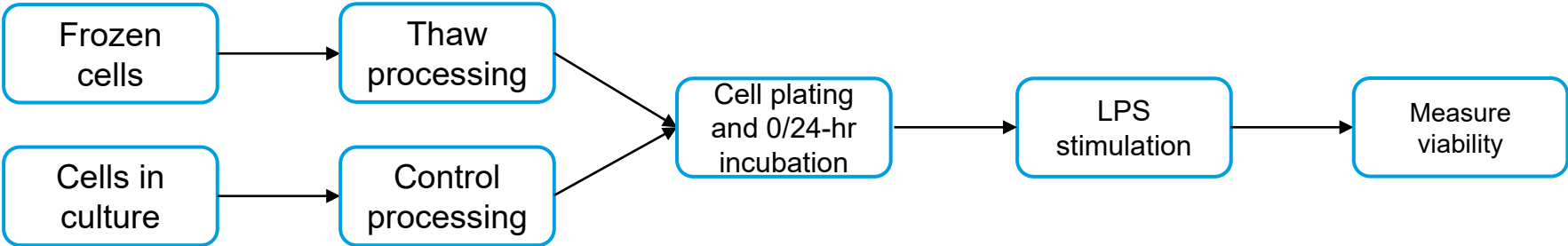
0-hr Post-thaw Incubation



24-hr Post-thaw Incubation



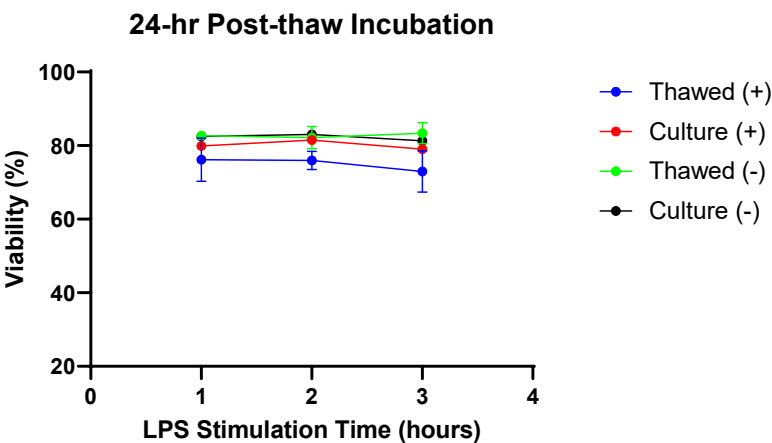
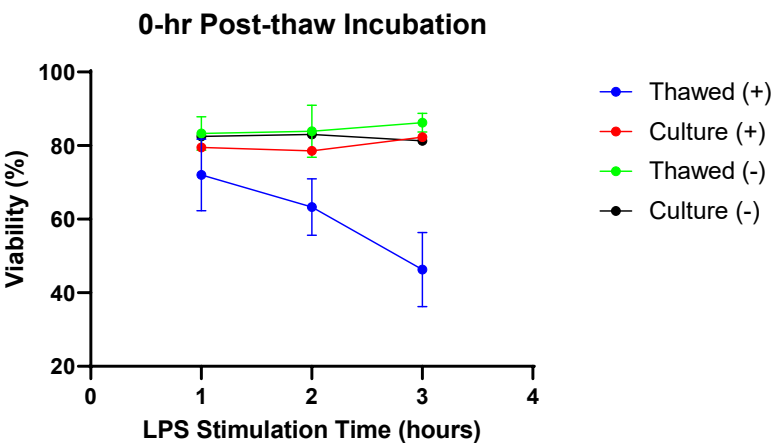
LPS-Induced Viability Loss



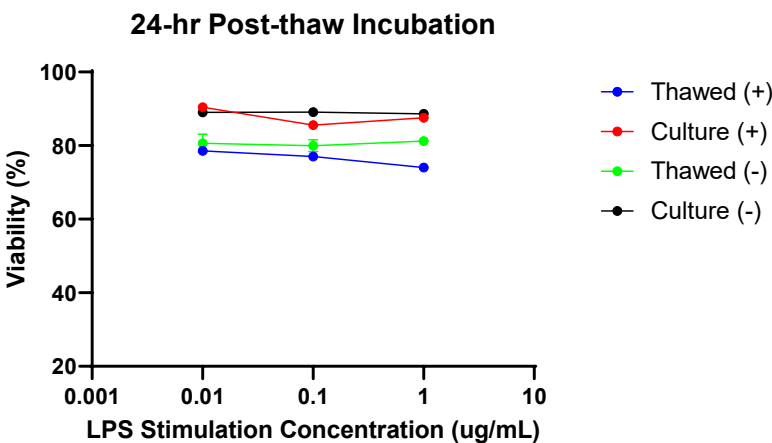
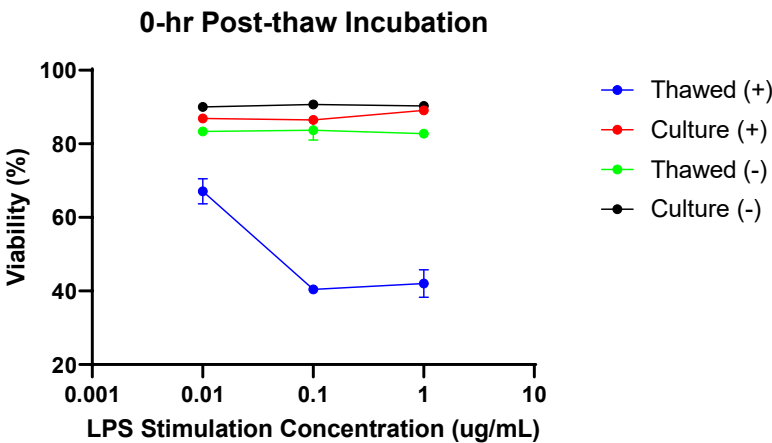
Changing LPS Dosage and Time Reduces Viability Loss

LPS stimulation

LPS Stimulation Time



LPS Dosage



LPS-Induced Cell Death

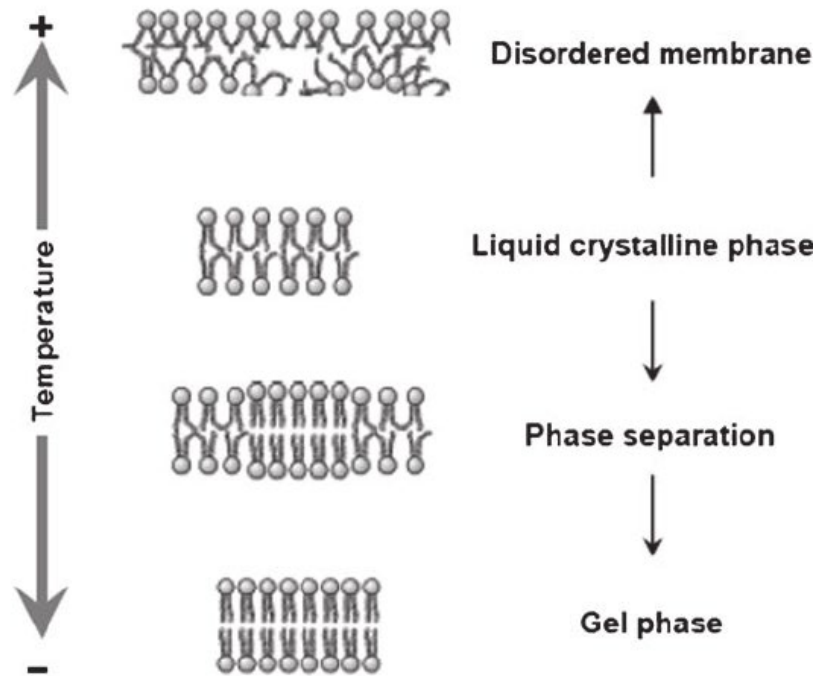


Video showing the uptake of ethidium bromide by THP-1-NFkB-LUC2 cells during a 60-minute exposure to LPS

Possible biophysical mechanism

Cryopreservation-induced leaky membrane

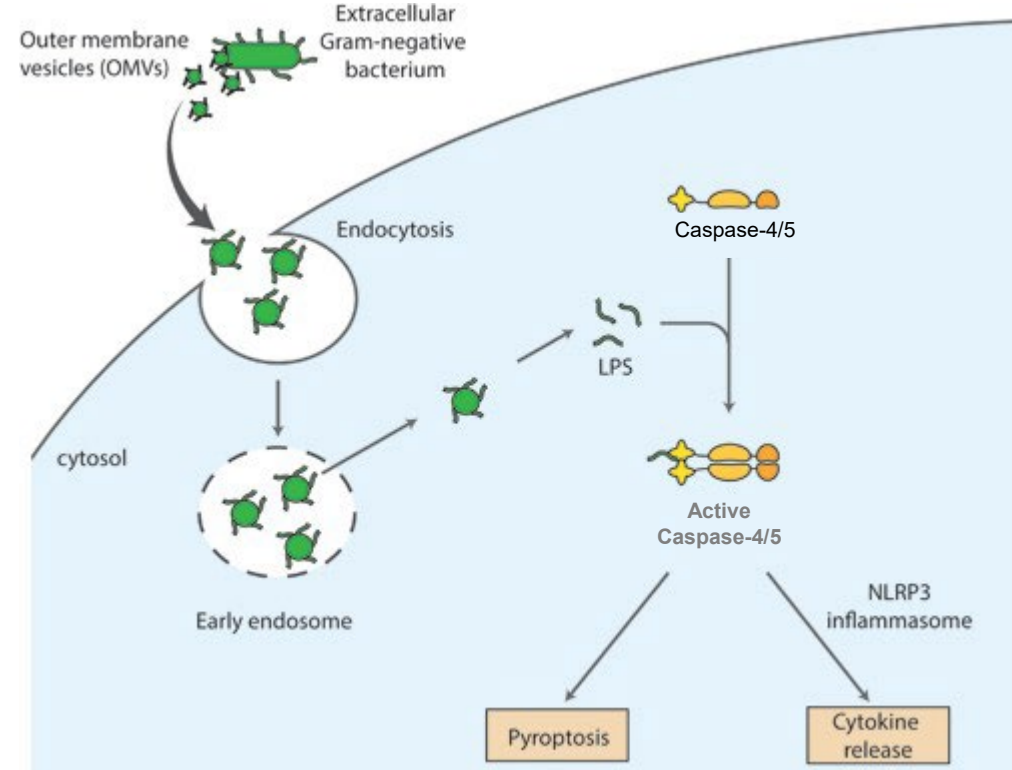
- Membrane phase transitions
- Uptake of impermeable solutes



Los DA., Zinchenko VV. Regulatory role of membrane fluidity in gene expression. Lipids in Photosynthesis: Essential and Regulatory Functions 329-348, 2009.

Intracellular LPS-induced pyroptosis

- Faster response than other cell-programmed death responses



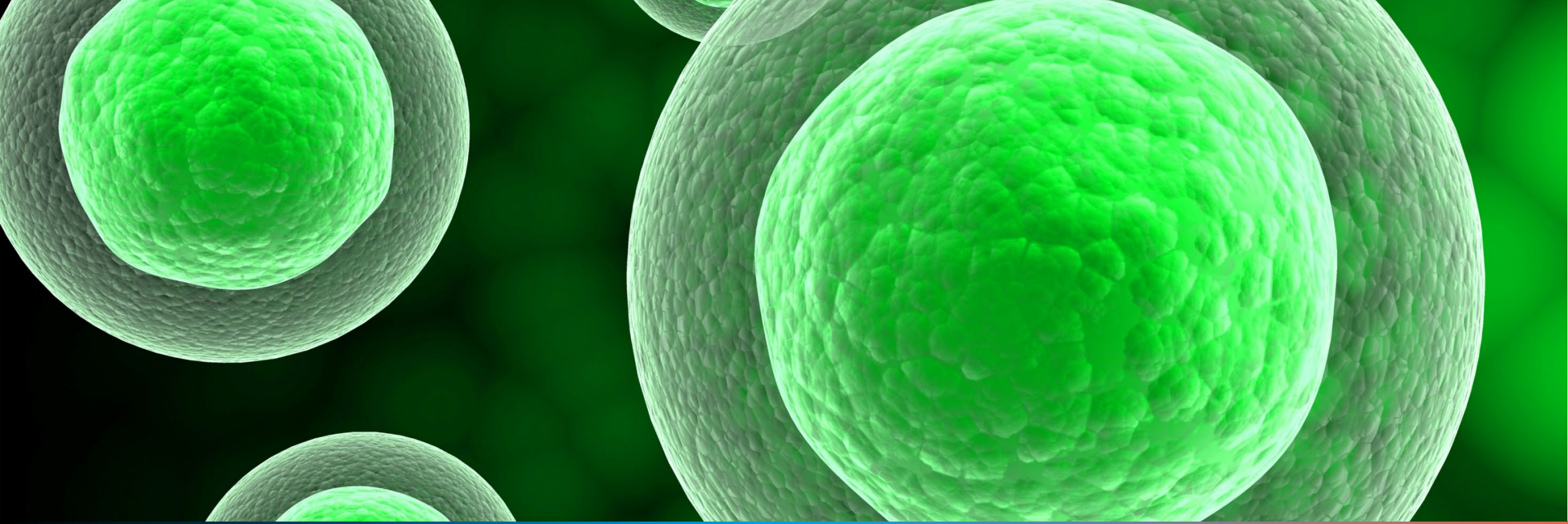
Modified from: Broz P. Intracellular detection of extracellular bacteria. Cell Res 26(8): 859–860, 2016. PubMed: 27283800

Closing Remarks

- We have identified a response of THP-1-NFκB-LUC2 cells to LPS immediately after thaw that directly impacts assay outcomes.
- THP-1-NFκB-LUC2 reporter cells can produce expected outcomes to LPS exposure within 24 hours post thaw.

Future Work

- Understand the mechanisms causing cell death after LPS exposure to develop targeted cryopreservation strategies to improve post thaw outcomes.



Questions?

Special thanks to Lukas Underwood, who performed the bulk of the work presented.