

From Curiosity to Breakthroughs: Accelerate Your Drug Development with Assay Ready Cells

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Heather Branscome, PhD, Senior Scientist, ATCC



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 culture – the “gold standard”
- Innovative R&D company featuring new product formats that support drug development, safety testing, and high-throughput screening
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 20 international distributors
- Talented team of 550+ employees, over one-third with advanced degrees



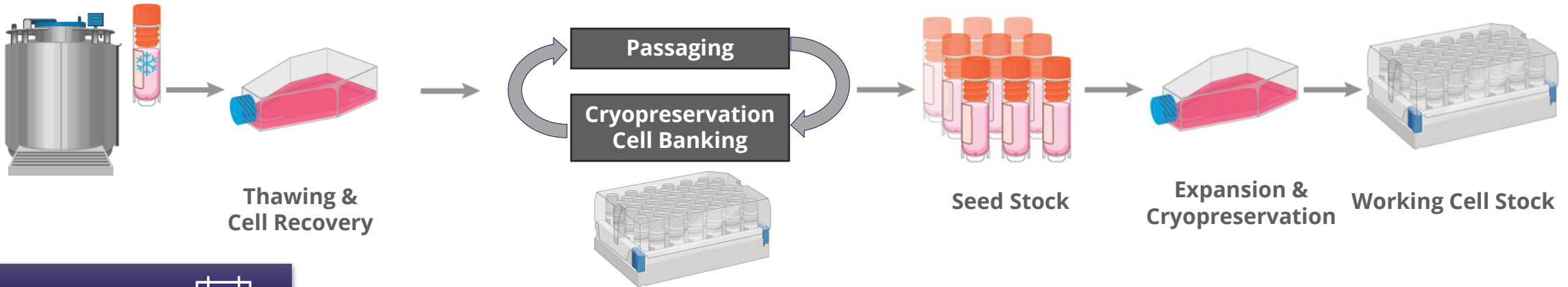
Outline

- ThawReady™ introduction
- ThawReady™ solution offerings
- Speaker introduction
- ThawReady™ applications

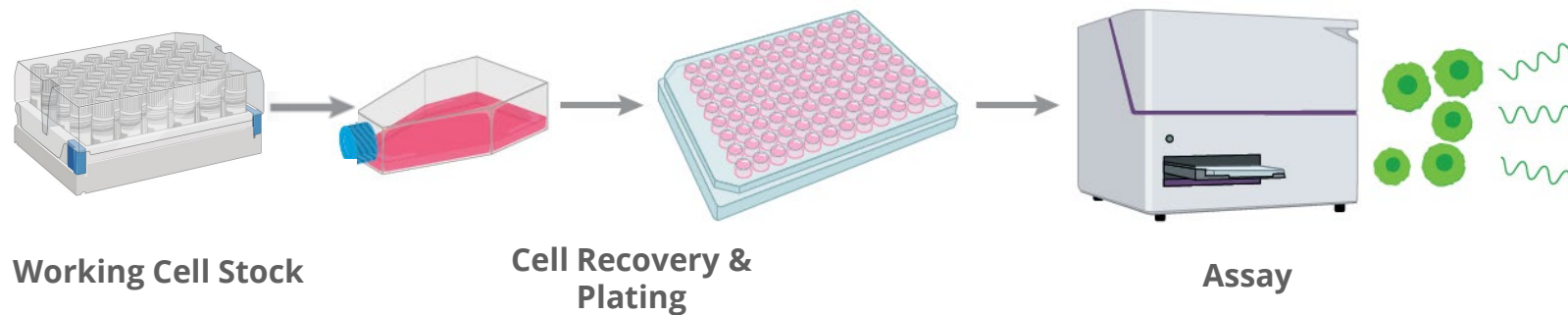



The Problem

Phase 1 Cell Banking



Phase 2 Assay Workflow



	Lengthy
	Risky
	Costly

ThawReady[™]

by ATCC



ThawReady™ Solutions



ATCC® ThawReady™ Assay Ready Cells – From frozen to data in one day

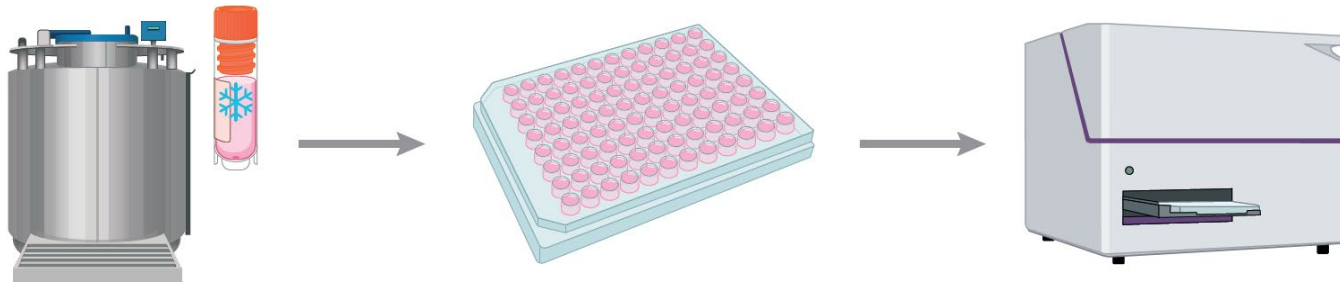


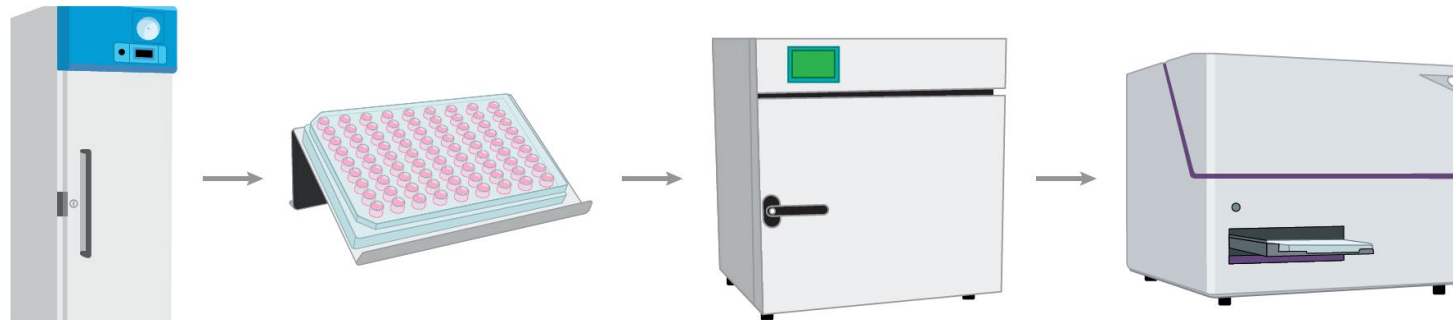
Plate Directly

Assay Ready Immediately

Product Format : Vials; Parental and reporter cell lines

ATCC® No.	Product Designation
TIB-202-NFkB-LUC2-AR™	ThawReady™ THP-1 NF-kB-Luc2
TIB-202-AR™	ThawReady™ THP-1

ATCC® ThawReady™ 3-D Spheroid Kits – A uniform spheroid in every well



Thaw Plate

Assay Ready Spheroids in 5 Days

Product Format : Pre-plated cells; 96-well plates

ATCC® No.	Product Designation
SCM-CCL-185-2PLT™	ThawReady™ A549 Spheroid Kit
SCM-CCL-247-2PLT™	ThawReady™ HCT 116 Spheroid Kit
SCM-HTB-133-2PLT™	ThawReady™ T47D Spheroid Kit

Our Speakers



Fatah Kashanchi, PhD,
Professor, Laboratory of Molecular
Virology, George Mason University



Heather Branscome, PhD,
Senior Scientist, ATCC

Application Data

Heather Branscome, PhD



Overview

- Challenges with bioassays
- ThawReady™ overview
- Application data
 - Post-thaw recovery
 - Functionality testing using extracellular vesicles (EVs)
 - HIV-1 infection and drugs of abuse

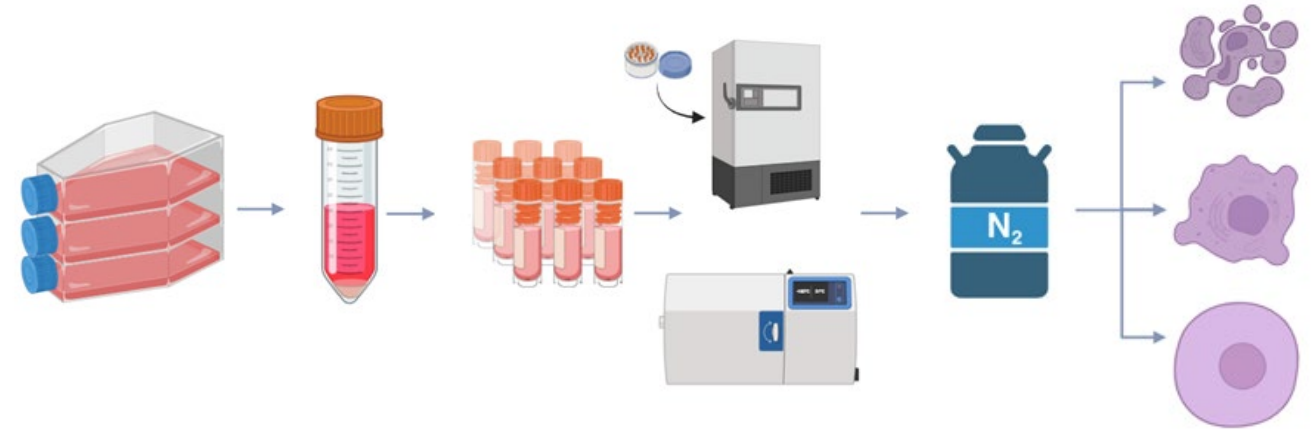


Challenges with Bioassays



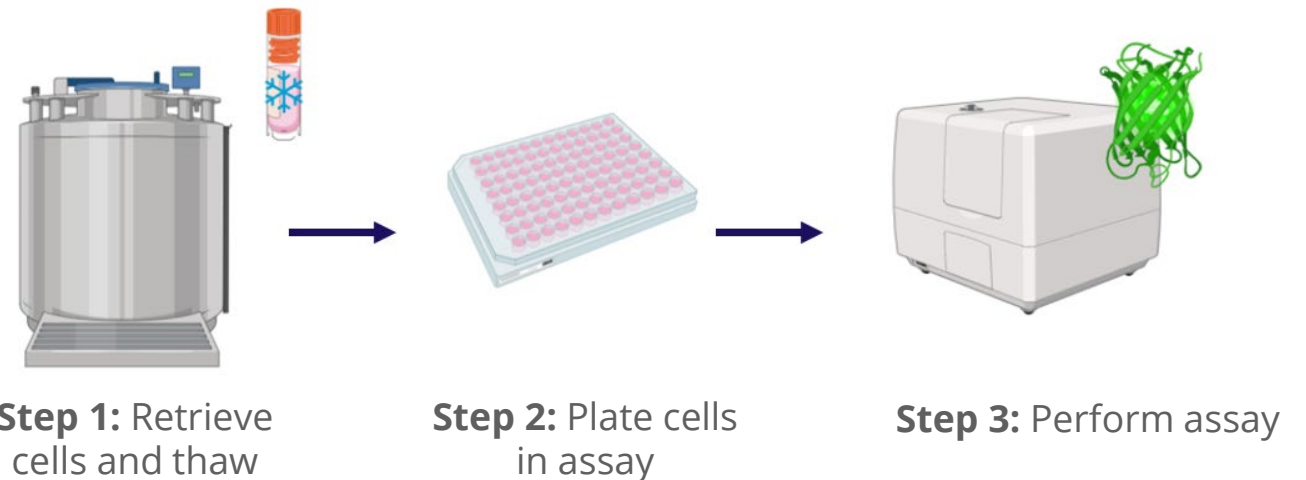
Upstream/downstream challenges

- Time consuming
- Cost and resource dependent
- Introduces variability and genetic drift
- Increased risk of contamination
- Post-thaw recovery period is required



ThawReady™ Solution

- Consumable format
- High reproducibility
- High functionality
- Unique cryobiology



ThawReady™ Overview



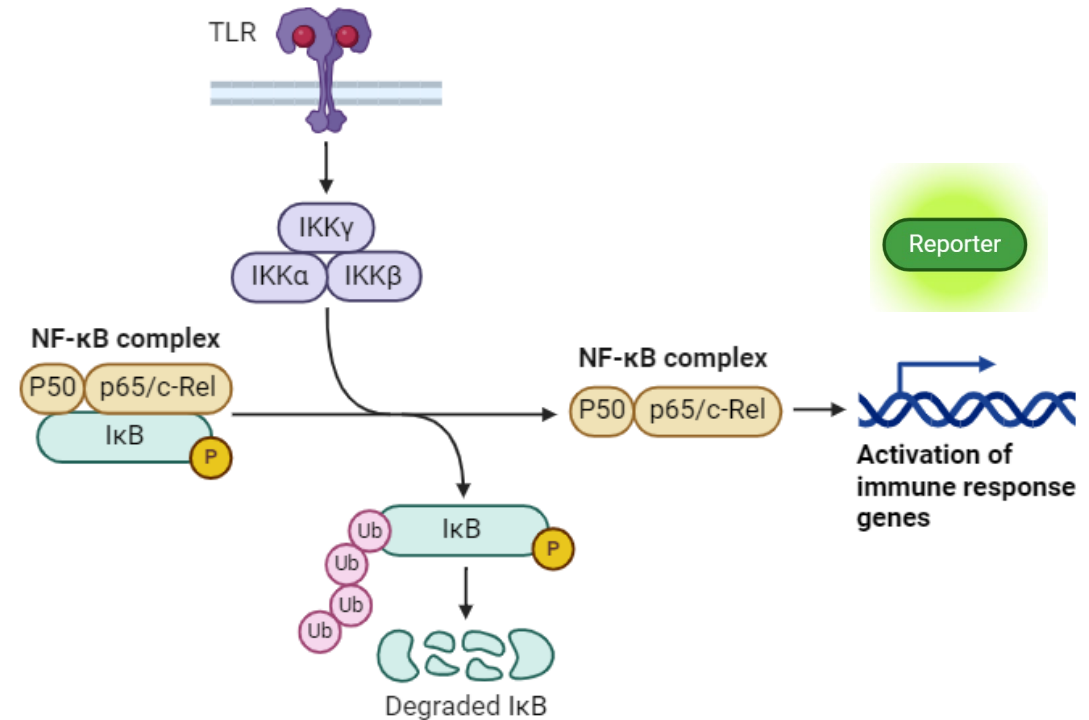
ThawReady[™]
by ATCC

THP-1 (ATCC® TIB-202™)

- Monocytic cells derived from the peripheral blood of an acute monocytic leukemia patient
- Versatile and essential for the biomedical research community
- Routinely used in research related to immune system, toxicology/drug development, and cell therapy

ThawReady™ THP-1 NF-κB-Luc2 (ATCC® TIB-202-NFκB-LUC2-AR™)

- Assay-ready, luciferase reporter cell line derived from the THP-1 parental cell line
- Animal by-product free
- Expresses the firefly luciferase gene (*luc2*) under the control of a NFκB promoter
- Useful for monitoring the activity of NFκB and cellular immune responses in vitro



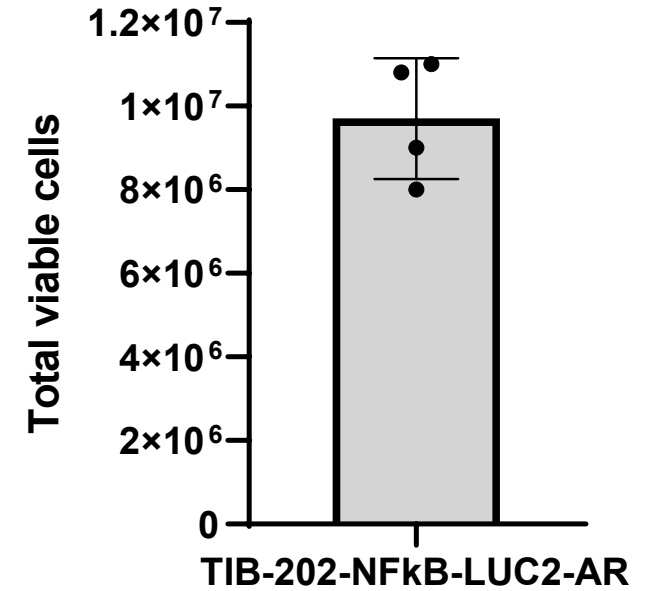
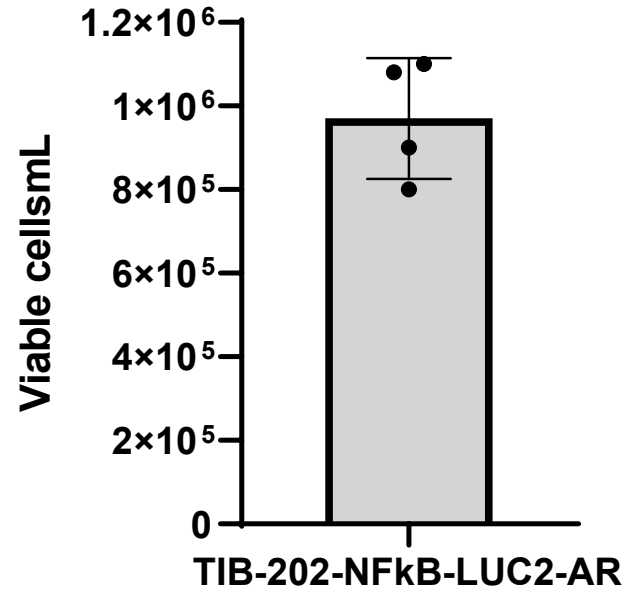
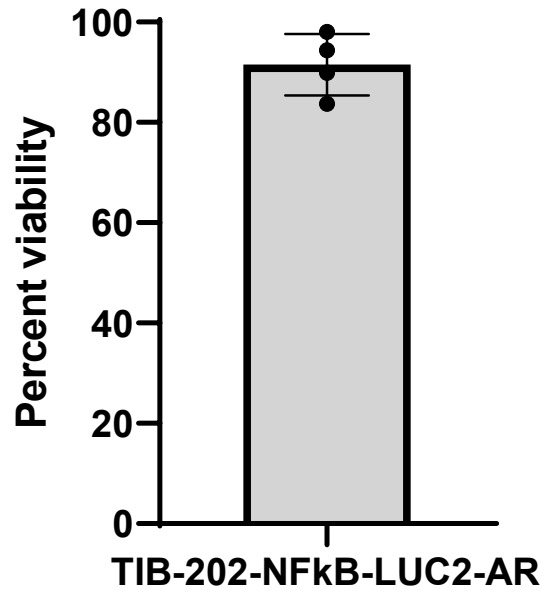
ThawReady™ THP-1 NF-kB-Luc2

ATCC® TIB-202-NFkB-LUC2-AR™



ThawReady™
by ATCC

- Reproducible and consistent viability and cell yield
- Measure immediately post-thaw



ThawReady™ THP-1 NF-kB-Luc2

ATCC® TIB-202-NFkB-LUC2-AR™

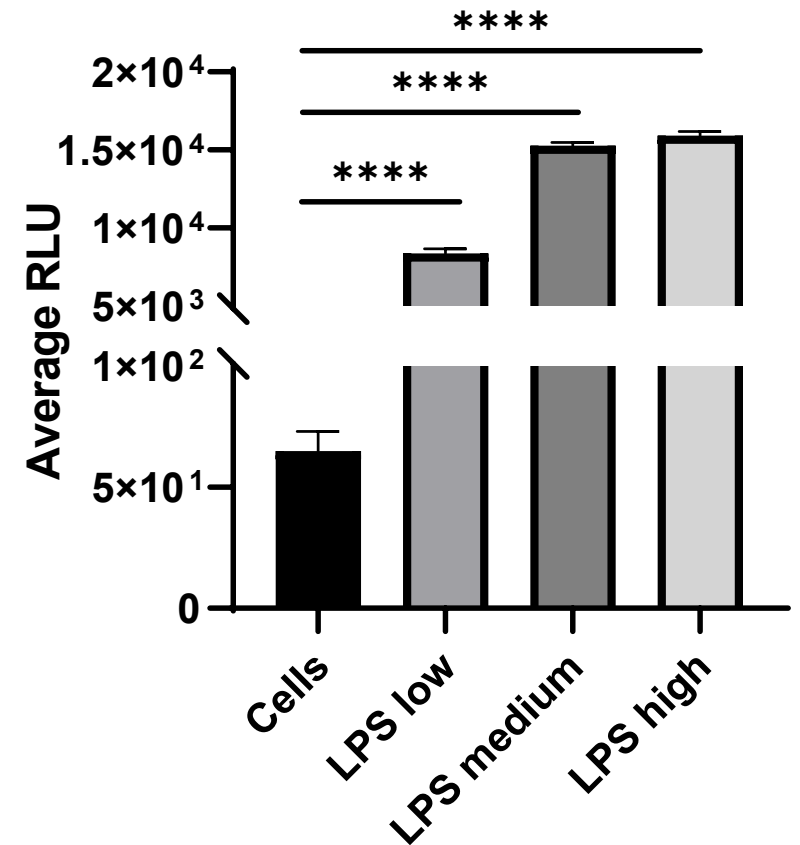


ThawReady™
by ATCC

LPS Challenge Assay

- **Average post-thaw viability:** 89.9%
- **Seeding:** Immediate post-thaw
- **Seeding density:** 50,000 viable cells
- **Treatment:**
 - LPS (*E. coli* O111:B4)
 - Stimulation period: 3 hours
 - Low dose: 1 µg/mL
 - Medium dose: 10 µg/mL
 - High dose: 25 µg/mL
- **Assay/Instrument:** Bright-Glo™ assay on BioTek Cytation®

Assayed
Directly
from
Frozen



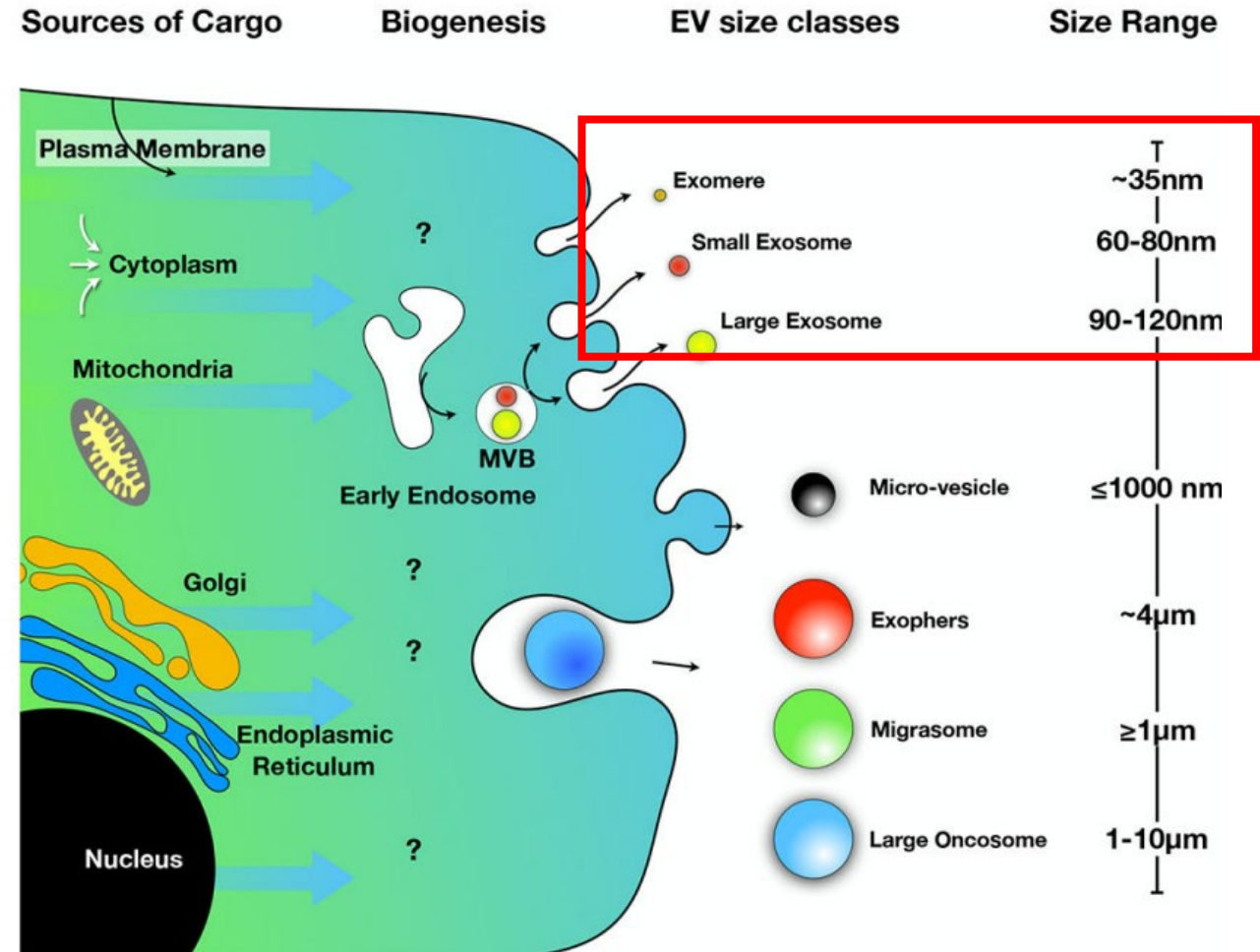
Application Data: Extracellular Vesical (EV) Functionality



Application Data: EV Functionality

Extracellular Vesicles:

- Nano-sized particles (30 nm-1000 nm) released by all cell types
- Carry various components of the cytoplasm and cell membrane
- Mediate intercellular communication (physiological and pathological)
- Can be utilized as **diagnostic markers** (e.g., cancer EVs) or **therapeutic tools** (e.g., stem cell EVs)

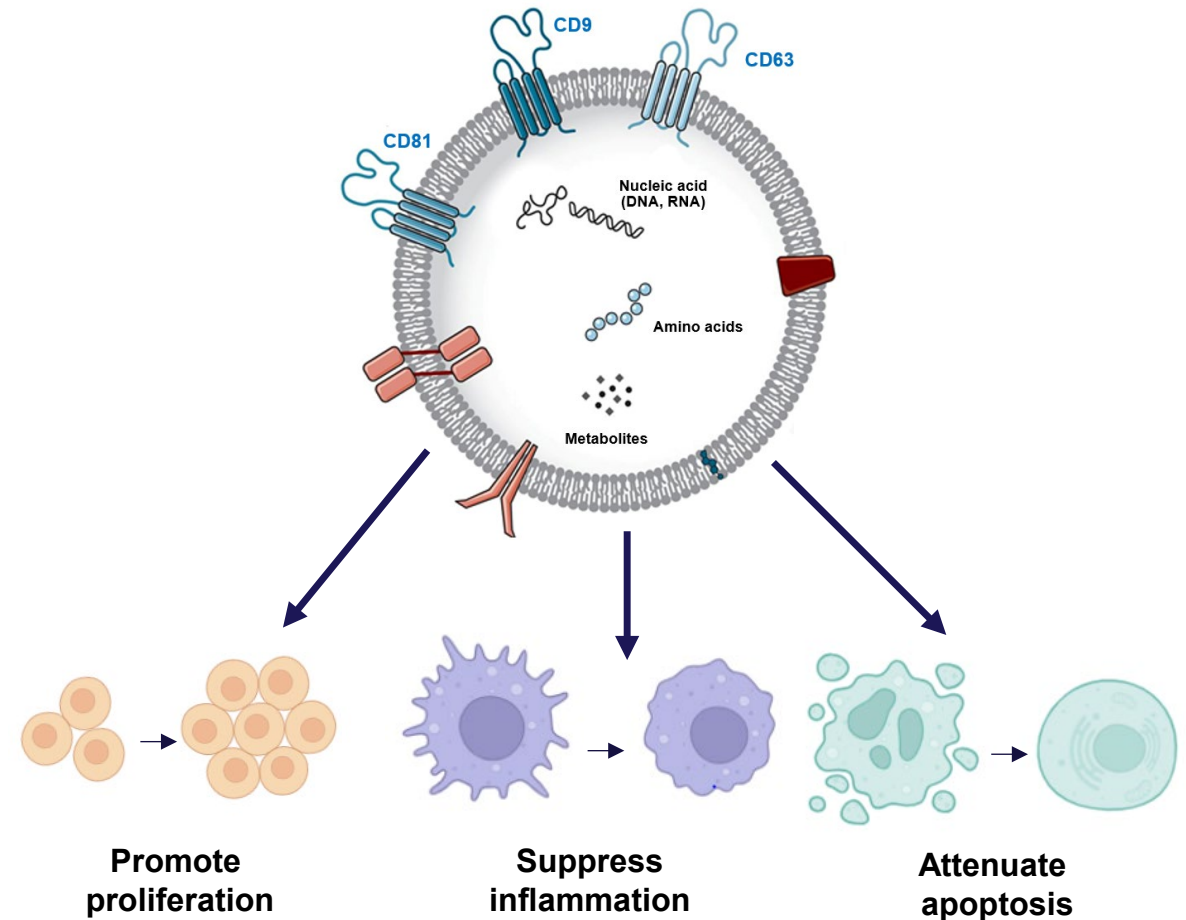


Zijlstra A, Di Vizio D. Nat Cell Biol 20(3): 228-230, 2018. PubMed: 29476154

Application Data: EV Functionality

Stem cell EVs:

- Contribute to tissue repair and regeneration
- Have reduced immunogenicity compared to parental stem cells
- Can be engineered to carry additional therapeutic agents to target damaged cells
- Can be engineered to carry additional therapeutic agents to target cells



Application Data: EV Functionality

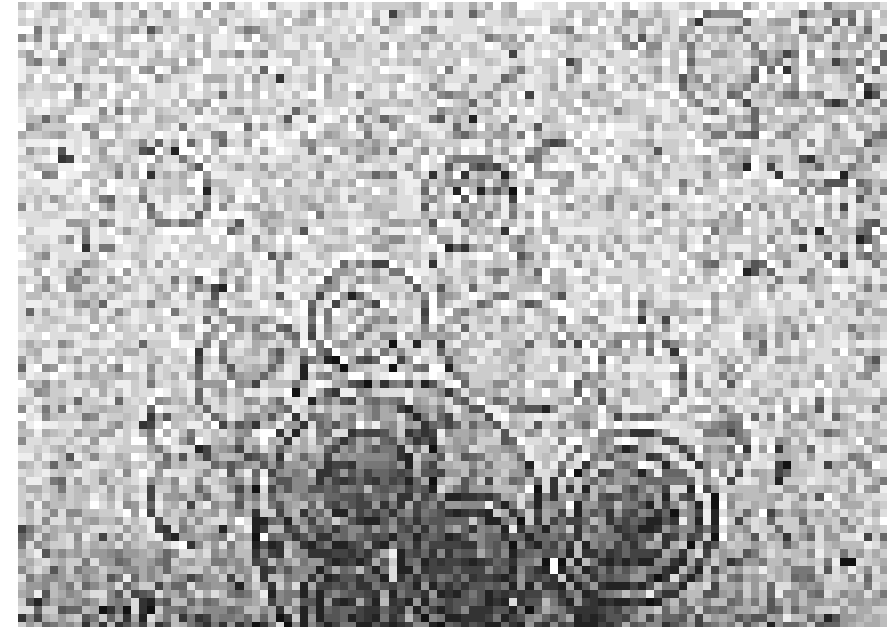


Donor cells:

- Mesenchymal Stem Cells (MSCs) (ATCC[®] PCS-500-012[™])
- Induced Pluripotent Stem Cells (iPSCs) (ATCC[®] ACS-1019[™])
- hTERT-immortalized MSCs (ATCC[®] SCRC-4000[™])

In vitro functionality:

- Pro-migratory
 - Epithelial cells, endothelial cells, fibroblasts
- Pro-angiogenic
 - Mesenchymal stem cells and aortic endothelial cells (co-culture)
- Anti-apoptotic
 - Neurons (ATCC[®] CRL-2266[™])
 - Astrocytes (ATCC[®] CRL-1718[™])
 - Monocyte-derived macrophages (ATCC[®] TIB-202[™])
 - Retinal epithelium (ATCC[®] CRL-2302[™])
- Anti-inflammatory
 - Neurospheres (ATCC[®] ACS-5003[™])



doi.org/10.1007/s11481-019-09865-y



doi.org/10.1038/s41598-022-05848-x



doi.org/10.3390/cells13100861

Application Data: EV Functionality



ATCC® No.	Parental Cell Designation	Model	Applications (pre-clinical)
SCRC-4000-EXM™	hTERT-immortalized adipose-derived mesenchymal stem cell (MSC)	Stem cell (non-cancer)	Therapeutics/ Drug delivery
CRL-1740-EXM™	LNCaP	Carcinoma, prostate	Diagnostics/biomarker discovery
CRL-1435-EXM™	PC-3	Adenocarcinoma, prostate	Diagnostics/biomarker discovery
CCL-185-EXM™	A549	Carcinoma, lung	Diagnostics/biomarker discovery
CCL-247-EXM™	HCT 116	Carcinoma, colorectal	Diagnostics/biomarker discovery

Application Data: ATCC® Stem Cell EVs

TIB-202-NFkB-LUC2-AR™ EV Function Assay



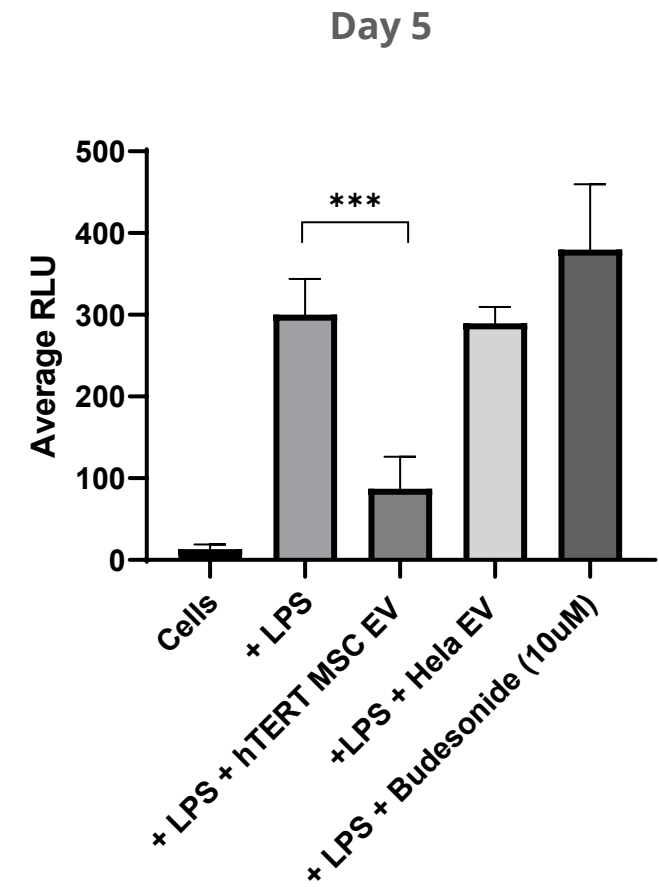
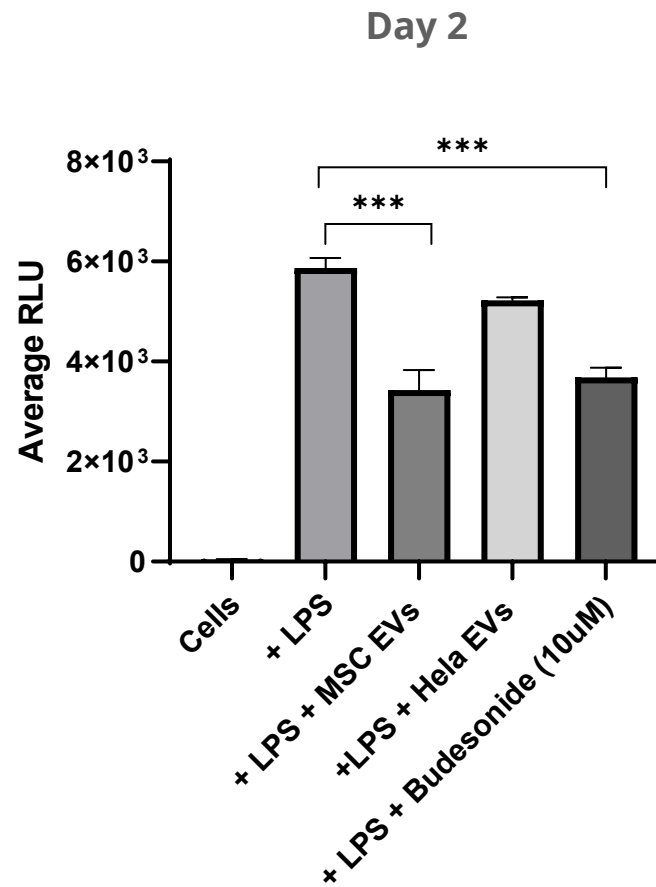
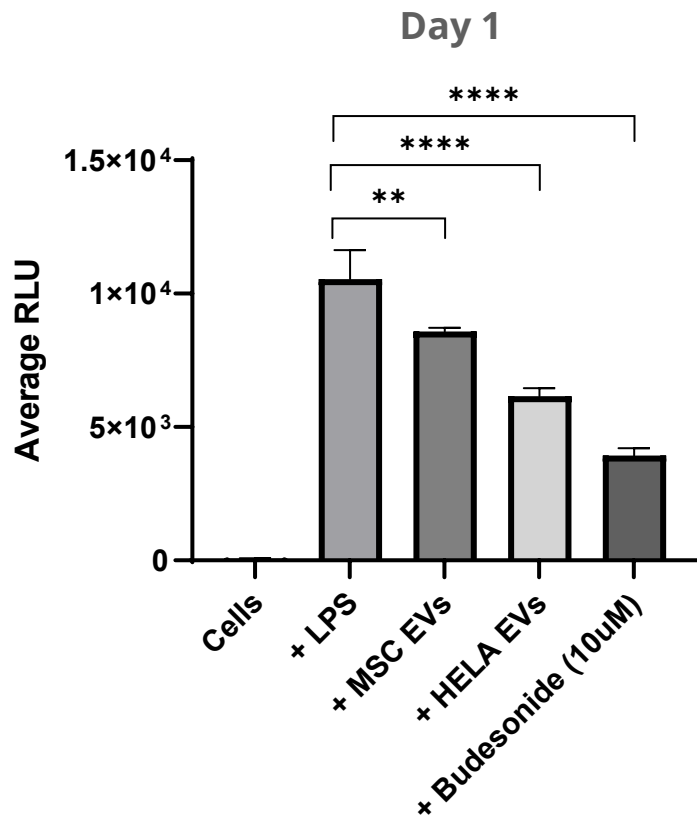
ThawReady™
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- **Seeding:** Immediate post-thaw
- **Seeding density:** 50,000 viable cells
- **Treatment:**
 - LPS: *E. coli* O111:B4
 - 3 hour stimulation; 10 µg/mL
 - LPS + stem cell EVs
 - LPS + cancer cell EVs
 - Positive control: Budesonide (10µm)
- **Assay/Instrument:** Bright-Glo™ assay/CellTiter-Glo® on BioTek Cytation®

Application Data: ATCC® Stem Cell EVs



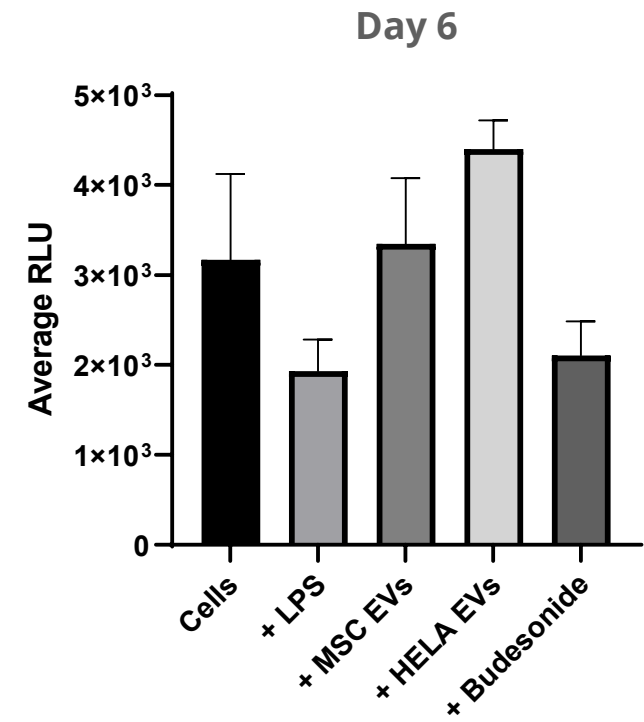
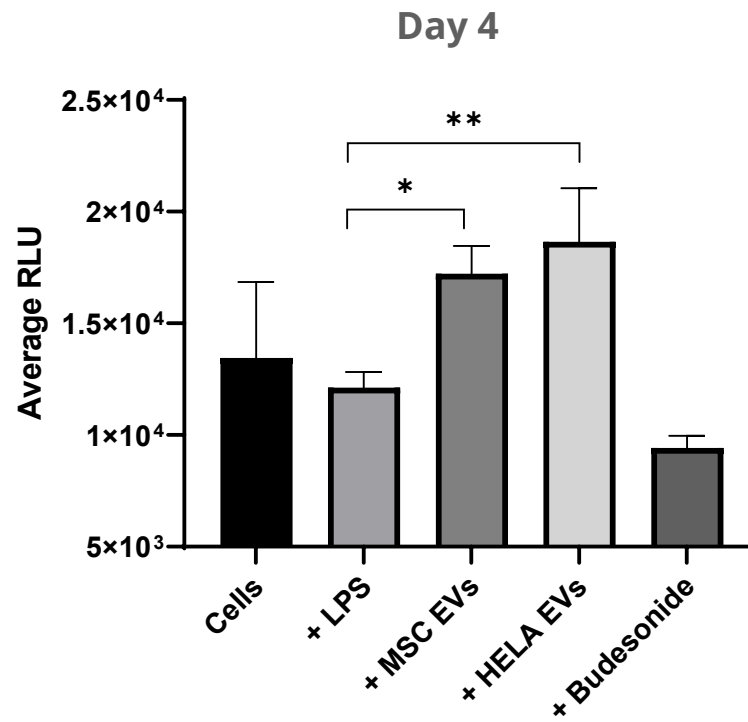
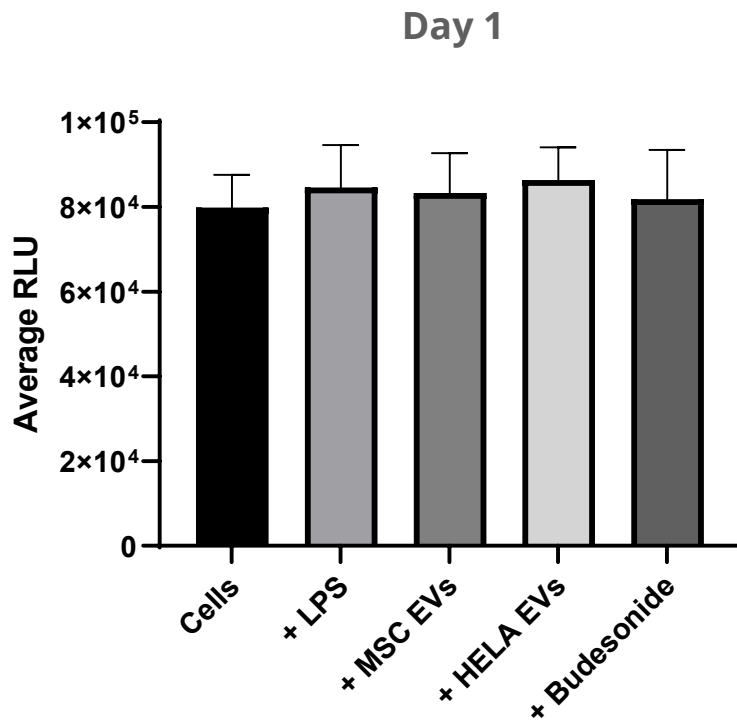
ThawReady™
by ATCC



Application Data: ATCC[®] Stem Cell EVs



ThawReady[™]
by ATCC



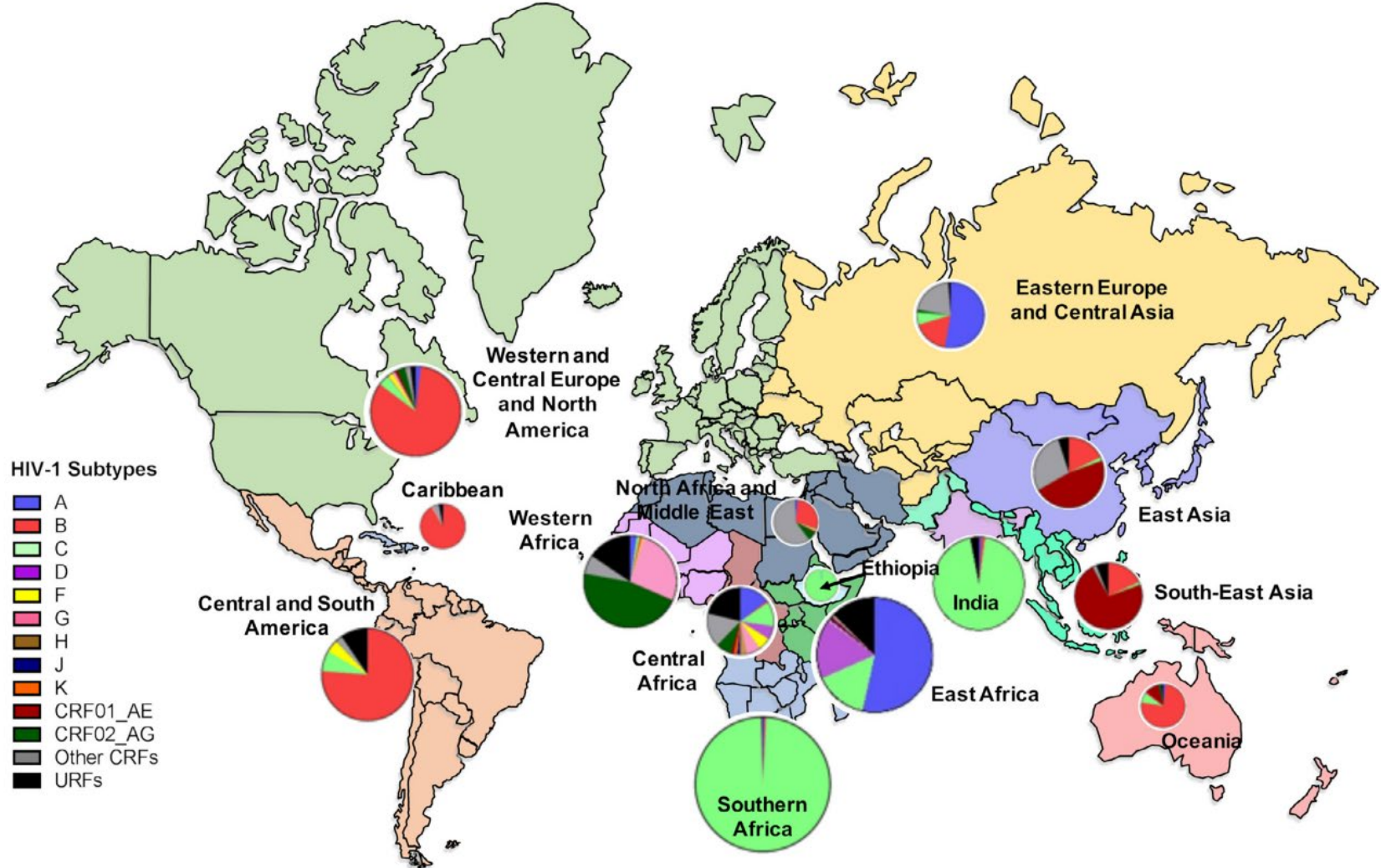


Application Data

Fatah Kashanchi, PhD

Application Data: HIV-1 Infection

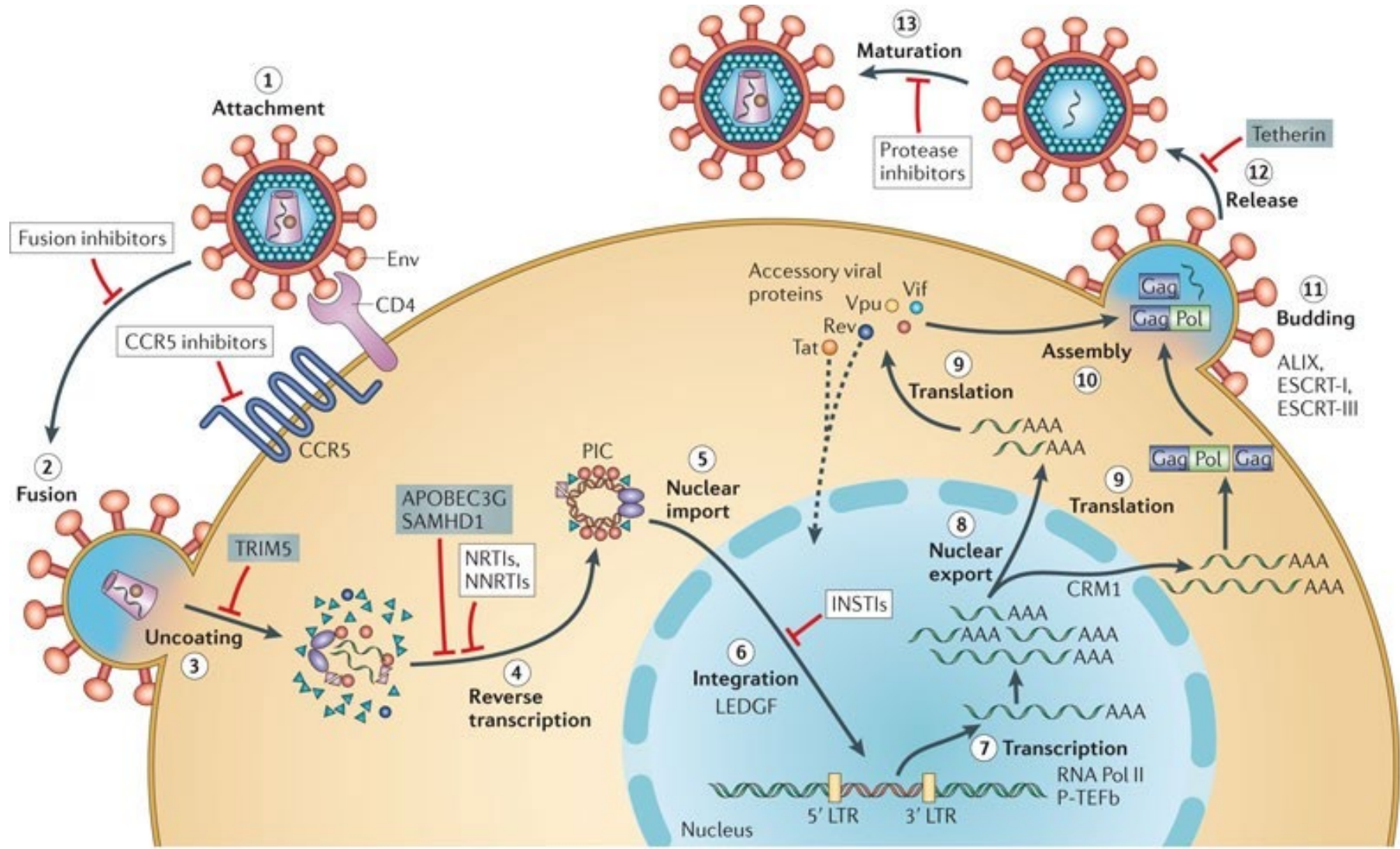
Global prevalence of HIV-1



Gartner MJ, et al. EBioMedicine 53: 102682, 2020. PubMed: 32114391



Application Data: HIV-1 Infection



Engelman A, Cherepanov P. Nature Rev Microbiol 10: 279-290, 2012.

Application Data: HIV-1 Infection

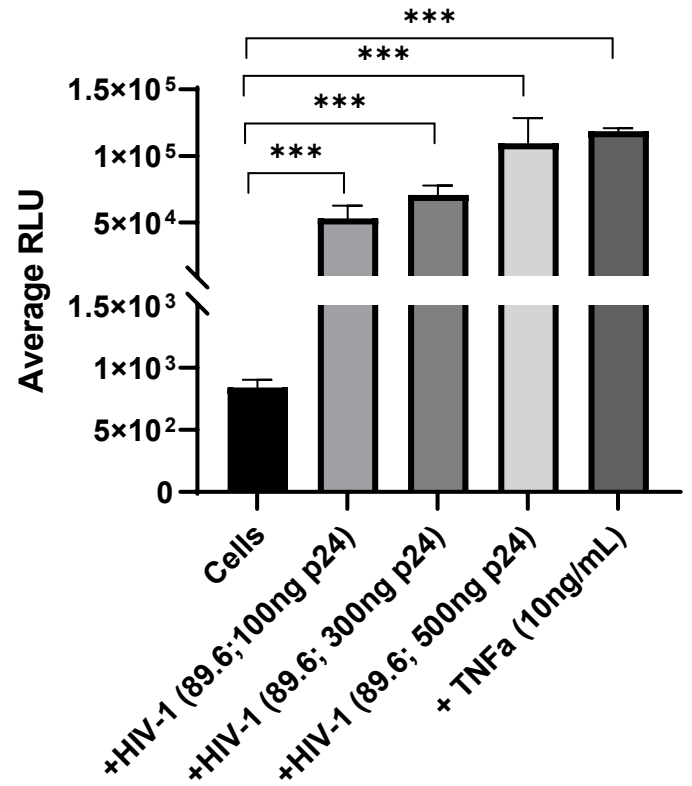
ATCC® TIB-202-NFkB-LUC2-AR™ HIV-1 infection assay

- **Seeding:** Immediate post-thaw
- **Seeding density:** 50,000 viable cells
- **Treatment:**
 - PMA (100 ng/mL)
 - HIV-1 dual tropic 89.6 (100, 300, 500 ng p24)
 - cART (5 mM)
 - TNFα (10 ng/mL)
 - CBD (5 μM)
 - HU308 (5 μM)
- **Assay/Instrument:** Bright-Glo™ assay/GloMax® (multiple reads over 10 minutes)

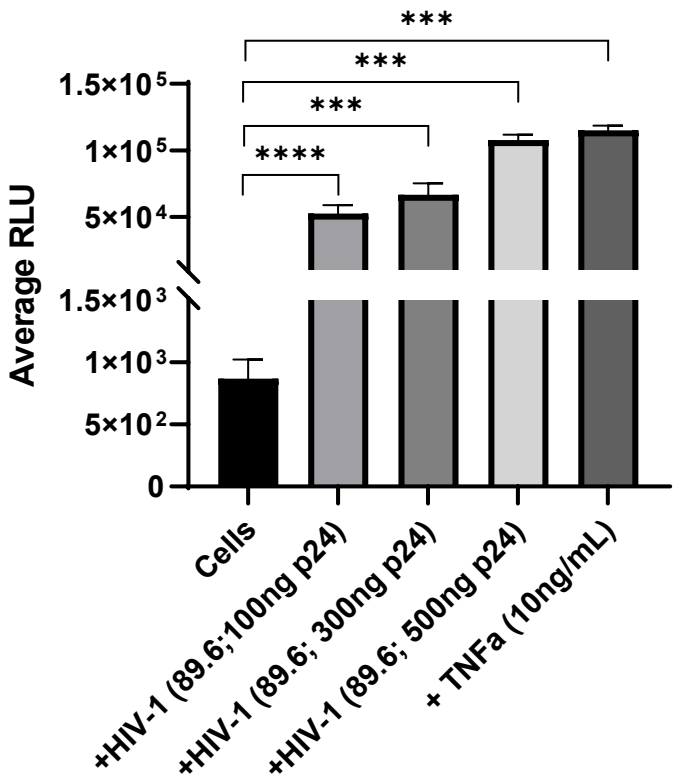
Application Data: HIV-1 Infection



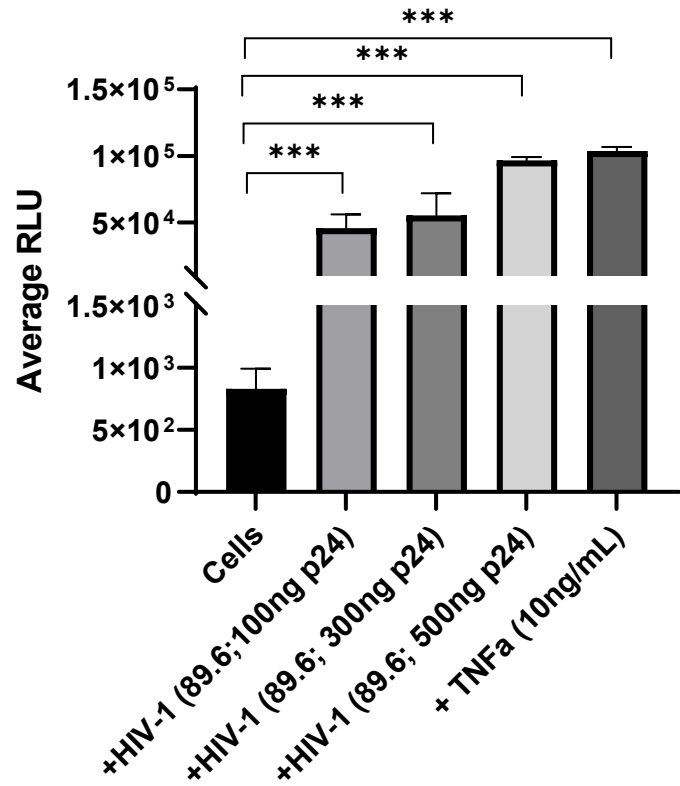
Assay 1



Assay 2

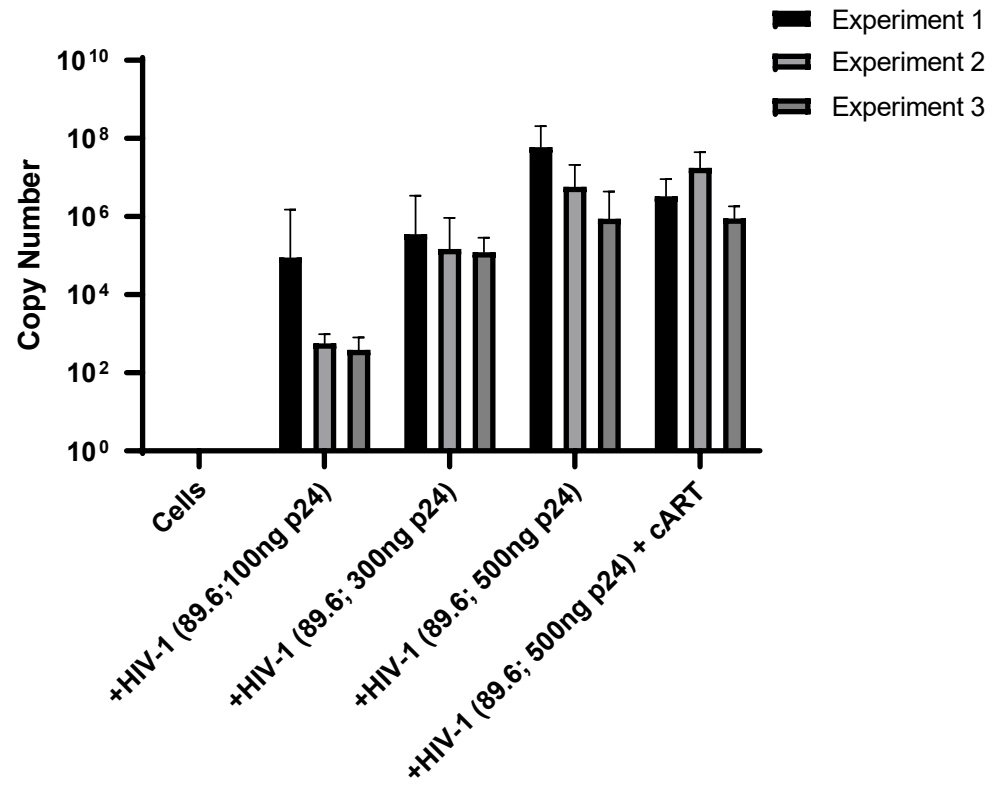


Assay 3

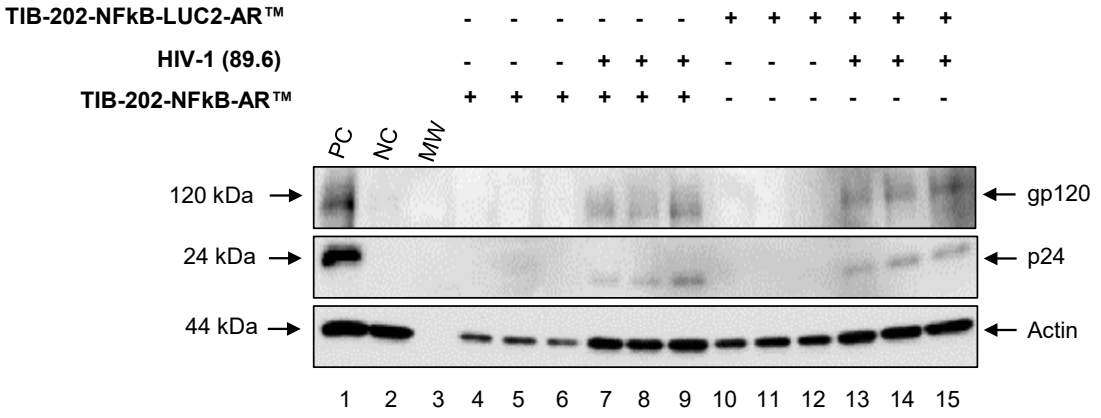


Application Data: HIV-1 Infection

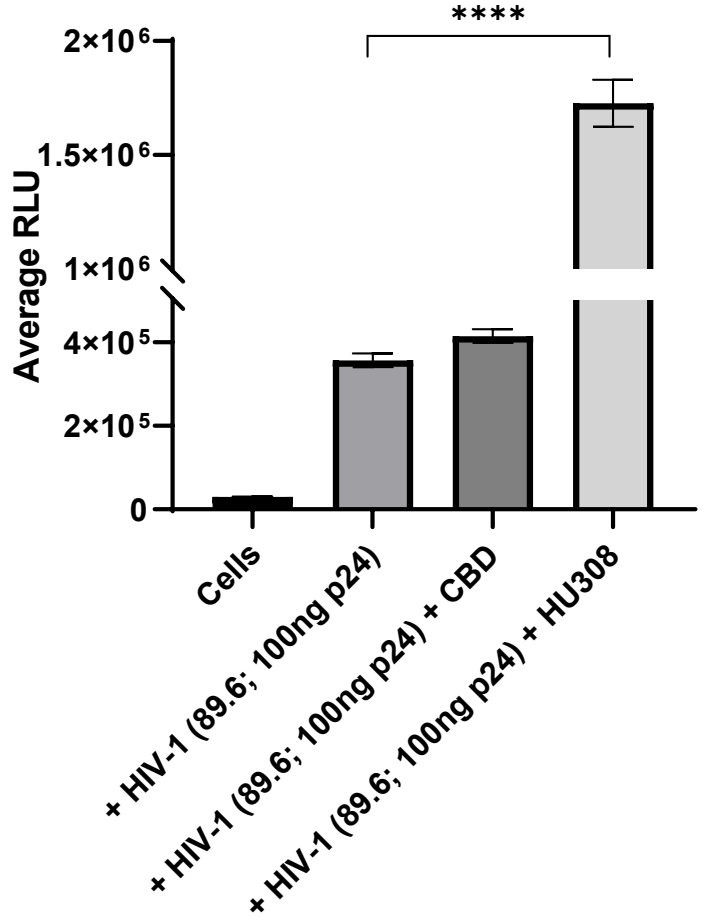
a)



b)

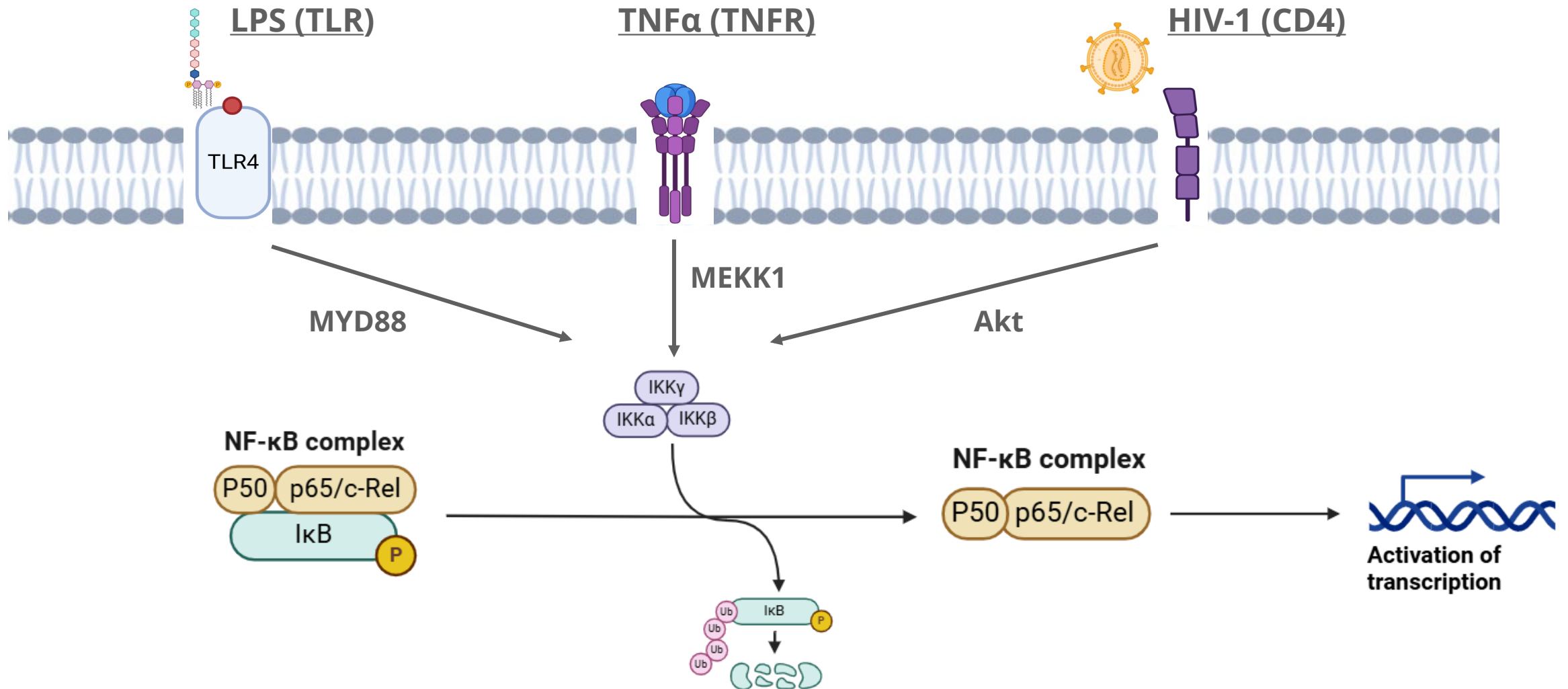


c)



Mechanism

Activation of NFκB signaling via multiple stimulants/receptors



Summary



- ThawReady™ cells overcome many of the challenges associated with traditional bioassays
- ThawReady™ cells offer high viability, high cell yields, and high functionality in a reproducible manner
- ThawReady™ cells can be used in assays relating to cellular damage (e.g., cancer EVs, viral infection) or cellular repair (e.g., stem cell EVs, drug screening)
- ThawReady™ cells can be used to study various mechanisms of NFκB activation, including TLR4, TNFR, and viral attachment

Learn more about ThawReady™ cells



Check out our poster presentations

Accelerate Cell-based Assays with the ThawReady™ THP-1 NF-κB-Luc2 Reporter Line

Presenter: Rajnee Kanwal, PhD, Scientist, ATCC

Poster Number: 1256-A

Date: Monday, January 27, 2025

Time: 12:00 PM - 1:00 PM

Development of the ThawReady™ THP-1 Product for Cell-based Assays

Presenter: Diana Douglas, PhD, Lead Biologist, ATCC

Poster Number: 1242-C

Date: Tuesday, January 28, 2025

Time: 12:00 PM - 1:00 PM

Visit us online at
www.atcc.org/thawready-cells



Acknowledgements



ATCC

- Dr. Joseph Leonelli
- Becky Bradford
- Dr. Heather Couch
- Dr. Sujata Choudhury
- Dr. Fang Tian
- Zach Cuba
- Sarah Swineford
- Jessica Moore
- Dr. Utsav Sharma
- Dr. Brian Shapiro
- Dr. Nilay Chakraborty
- Tiffany Cato

GMU

- Dr. Yuriy Kim
- Kajal Patil
- Anastasia Williams
- Gwen Cox





CREDIBLE LEADS TO INCREDIBLE

Questions

For more information, visit us
at **Booth #2652**

Contact info: Utsav Sharma: usharma@atcc.org



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