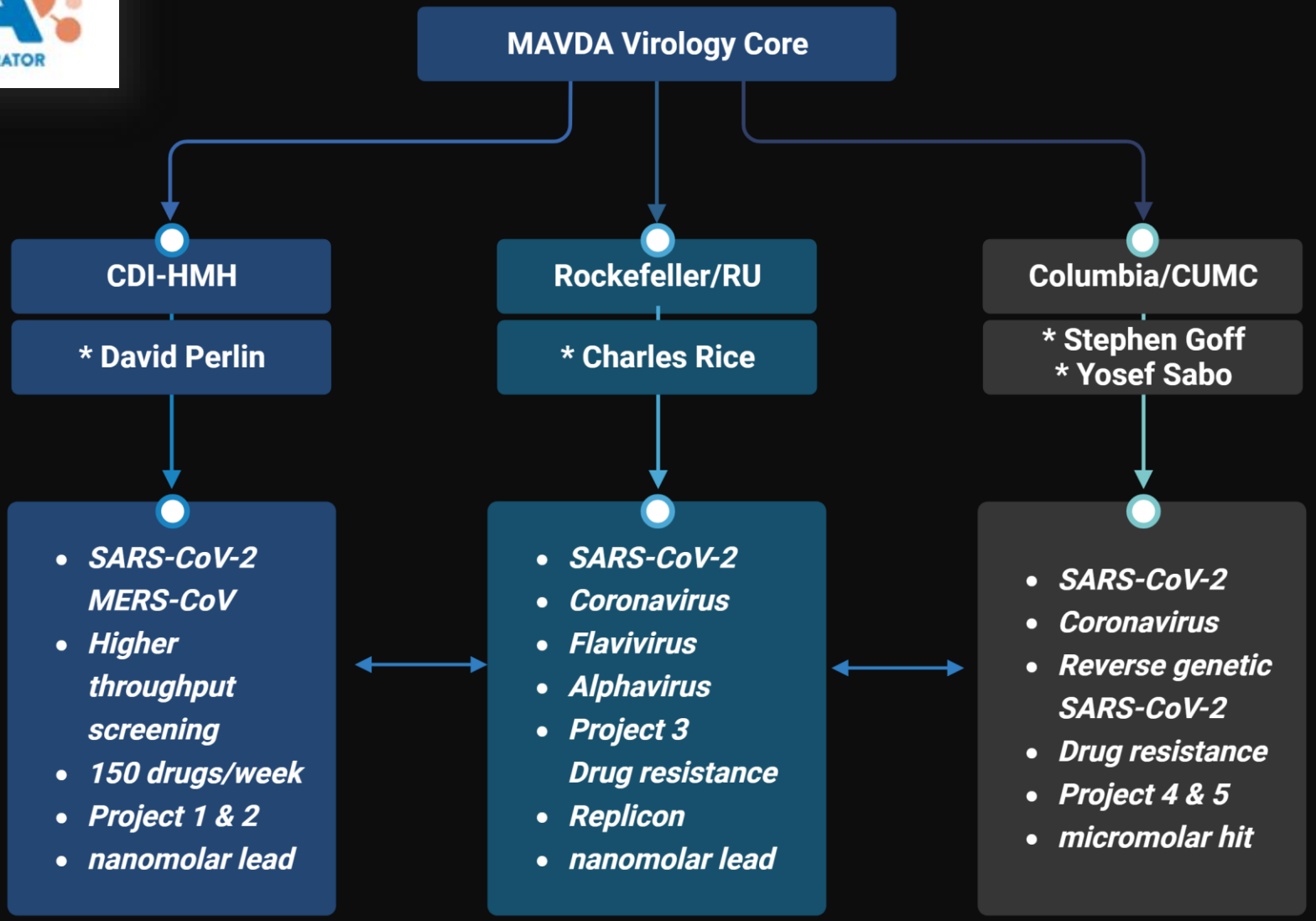
Center for Discovery & Innovation
Member of Hackensack Meridian *Health*

"MAVDA- Developing Novel Cellular Tools for Antiviral Drug Discovery"

Virology Core
for Antiviral Drug Screening

Ching-Wen Chang PhD
MAVDA Virology Core coordinator
David Perlin Lab
AViDD Open Science Forum, Oct 18, 2023



* Core director

Table: Available flavivirus, alphavirus & coronavirus strains and constructs at the MVC

Virus	Isolates	Infectious clones	Replicons
DENV-1	WP Thailand 1964 160067 Jamaica CV1636/77 Puerto Rico/94 PUO-359 852679 RRH		
DENV-2	Columbia 362981 CAREC 860435	16681 16681-GFP 16681-Luc	16681-GFP 16681-Luc
DENV-3	PR6 Thailand TVP D83-144		
DENV-4	Philip Ryder BE Ar612288		
LGTV		TP21 E5	
POWV	Byers	DTV	
WNV	NY99 68856 India, TVP-2365 Egypt 101, 30461 An 4766, Ethiopia, M8818 An 4767, Ethiopia, M9250 Dak B310, TVP-2548 Dak M G 798, TVP-2567 B956 Uganda, TVP-3040	TX02 TX02-GFP NY99	TX02-GFP
YFV		17D Asibi	17D-Venus 17D-Luc replicon
ZIKV	PRVABC59 Brazilian isolate Mouse-adapted Dakar strain	MR766 Honduras strain ZIKV 2013 FSS13025	FSS13025-Luc

Virus	Infectious clones	Reporter version	Replicons
CHIKV	LR2006-OPY1 AF15561 181/25	LR2006-OPY1-GFP 181/25-mKate2	SL15649-based (-/+ trans-packaging constructs)
EEEV			Florida 91-based
MAYV	CH		
ONNV	SG650	SG650-GFP	
RRV	T48	T48-GFP	
SFV	SFV4		
SINV	Toto1101 Toto1106 SVN SVNI S.A.AR86 GirdwoodS.A.	Tot1101-Luc, Toto1101-nGreen TE/5'2J-GFP, TE/3'2J-GFP SVN-nanoLuc SVNI-nanoLuc GirdwoodS.A.-GFP	Toto1101-based (-/+ trans-packaging constructs)
VEEV	TC83	TC83-GFP	TC83-based (-/+ trans-packaging constructs)

NanoLuc Reporter strains for coronavirus:

WA1/2020 (UNC)
WA1/2020 (plasmid-based)
MERS-CoV (UNC)
Delta (Yale)
Omicron-BA1 (Yale)

CDI Virology Core Capabilities
Higher throughput cell-based assays for antiviral activity evaluation

High throughput screening of compound using 96- and 384-well plate assays.

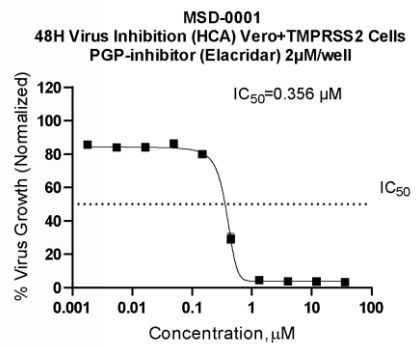
Cell seeding, 24hr attachment
(EL406 liquid handling)

Compounds dispensing
2hr cell-treatment with compounds
(Cellink I.DOT or Tecan D300e)

Cell infection
Virus dispensing process
(HamiltonStarlet or Integra Viaflow 384)

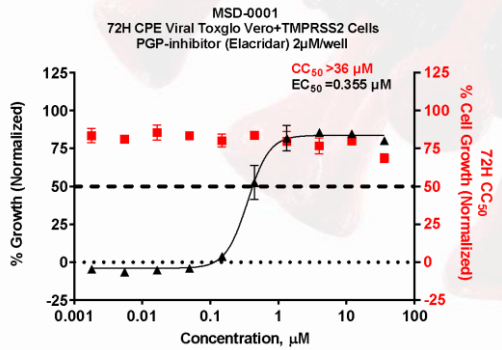
Evaluation of inhibitory concentration_48hr assay
(Cytation Confocal Imaging Reader 10 or
Celigo Nexelom Imaging Reader)

Detection of virus growth by using reporter virus or antibody staining.
Readout:
- Fluorescence detection.
- High content imaging



Evaluation of Effective concentration / Cytotoxicity effect_72hr assays
(Tecan plate reader)

Detection of cell viability by intracellular ATP detection
Readout:
Luminescence detection (Celltiter Glo)



By Nadine Alvaraz

RU Virology Core

Low/Med throughput screening of compounds related to **Project 3** in 96-well plate assays

cell seeding ---> 24 h attachment

compounds dispensing ---> 2 h incubation

virus infection ---> low and high MOI

fixation at 24 - 48 hpi

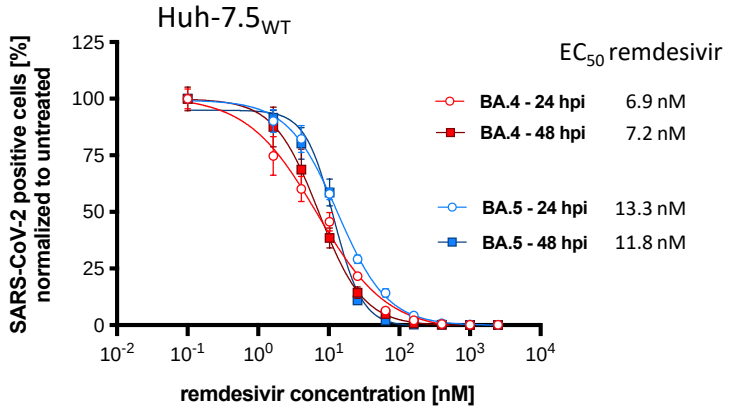
staining cells for viral antigen and nuclei

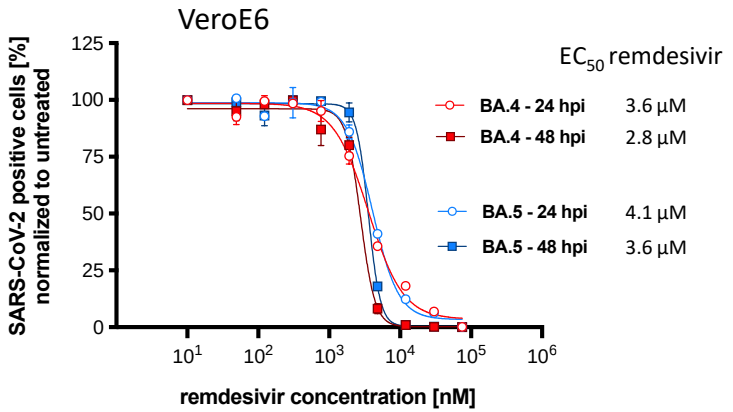
EC_{50} and CC_{50}

- ImageXpress Micro
- Cytation7
- Operetta CLS

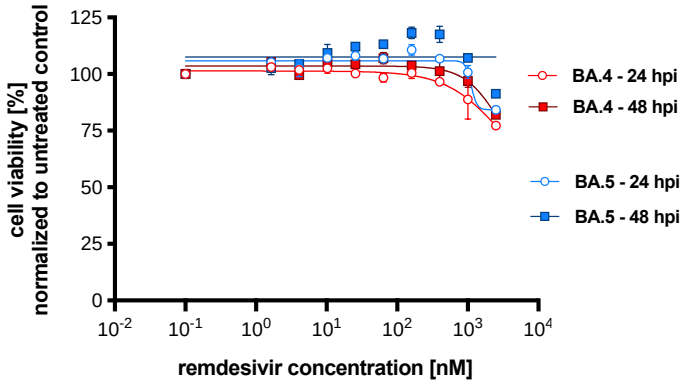
Example: Optimizing infection assay for SARS-CoV-2 variants using remdesivir as known antiviral

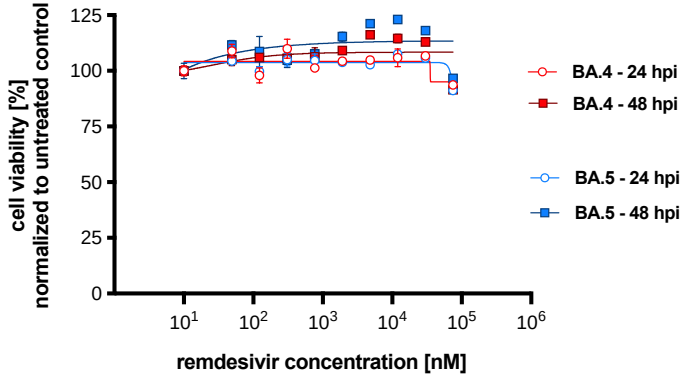
virus infection





cell viability





Generation of SARS-CoV-2 stocks and virus assay optimization

- original (WT): WA1/2020
- variants: beta, delta, omicron

Generation of SARS-CoV-2 variants resistant to antiviral compounds

Low/Med throughput screening of compound using 96-well plate assays.

Cell seeding, 24hr attachment

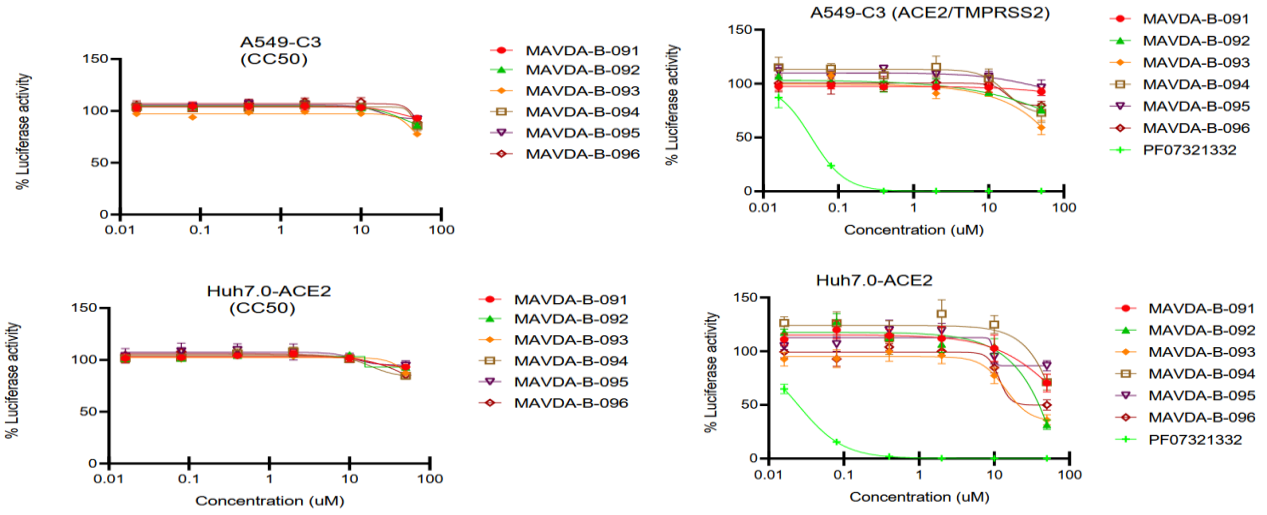
Compounds dispensing

Reporter Virus infection

IC50 24hr

CC50 48hr

Luminescence / fluorescence signal readout



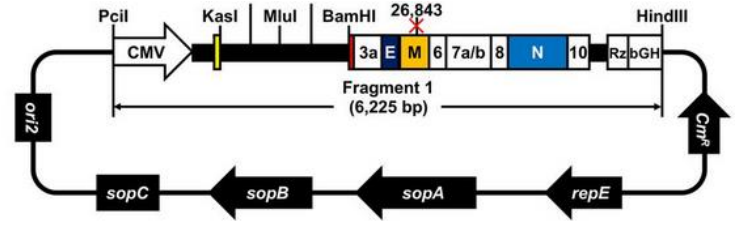
SCoV-2 Reverse genetic system

SCOV-2 USA-WA1/2020

- **Del ORF7b/NanoLuc**
- Del ORF7b/Venus
- Del ORF7b/mCherry
- ORF7b-P2A-NanoLuc

Seasonal coronavirus

- HCoV-OC43 assay (MRC5)
- HCoV-229E assay (MRC5)



Chengjin Ye., et al., mBio 2020

Human coronaviruses

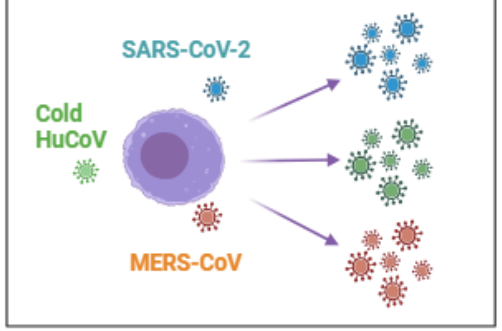
7 strains known

Enveloped, Positive-sense, Single-stranded, RNA viruses

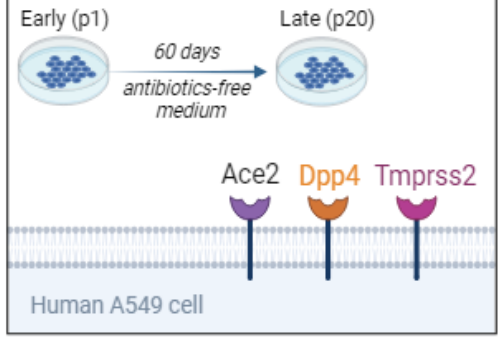
- Alpha-CoVs
  - **HCoV-229E**
  - **HCoV-NL63**
- Beta-CoVs
  - **HCoV-OC43/HKU1**
  - **MERS-CoV**
  - **SARS-CoV**
  - **SARS-CoV-2**

Lethal coronaviruses

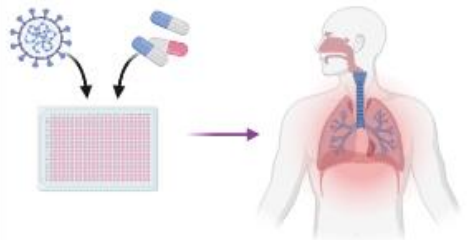
① A highly permissive human cell model for coronavirus replication



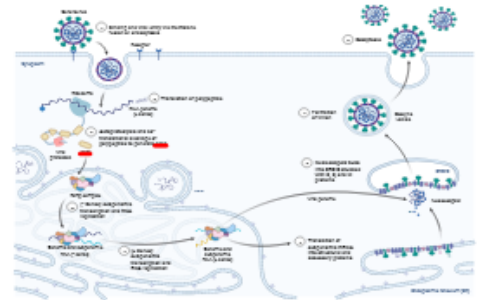
② This cell line can maintain consistent level of viral receptors over passage number



③ Support antiviral drug discovery



④ Support viral pathogenesis study



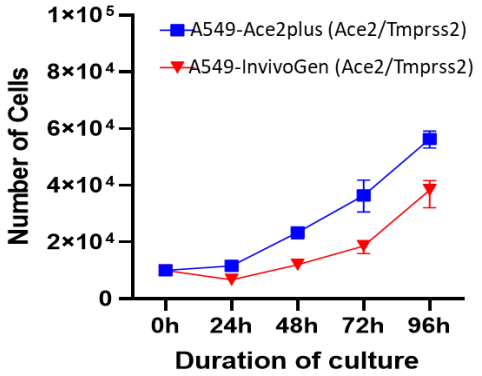
The most commonly Cell lines for SARS-CoV2 study and drug screen

	ACE2	TMPRSS2	Syncytia-CPE	Species	Tissues	Drug screen & Validation
A549^{Ace2plus}	✓	✓	✓	*Human*	*Lung*	*MAVDA project & PNAS, 2023, 120 (11): e2219523120*
A549$^{Ace2\ GenScript}$	✓	-	-			*(GenScript)*
A549$^{Ace2\ InvivoGen}$	✓	-	-			*(InvivoGen)*
A549$^{AT\ InvivoGen}$	✓	✓	✓*			*(InvivoGen)*
Calu-3	✓	✓	✓*	*Human*	*Lung*	*Cell Rep, 2021, 35(1): 10895*
Caco-2	✓	✓	-	*Human*	*Colon*	*Biomedicine & Pharmacotherapy, 2022, 151:113104*
Huh7.5	✓	✓	✓	*Human*	*Liver*	*Cell Rep, 2021, 35(1): 10895*
Huh7^{ACE2}	✓	✓	✓	*Human*	*Liver*	*Nature, 2020, 586:113-119*
VeroE6^{TMPRSS2}	✓	✓	✓	*Monkey*	*Kidney*	*Cell Rep, 2021, 35(1): 10895*

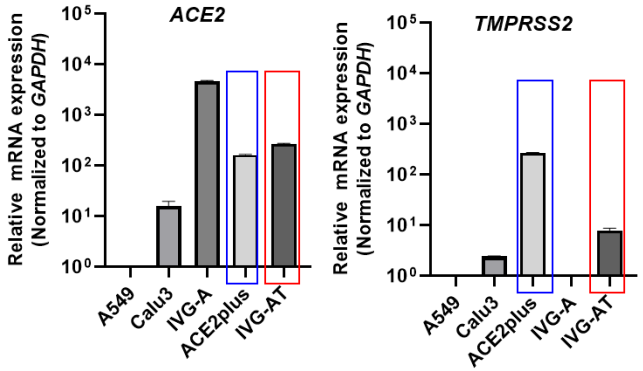
* Slow cell growth rate

Criteria

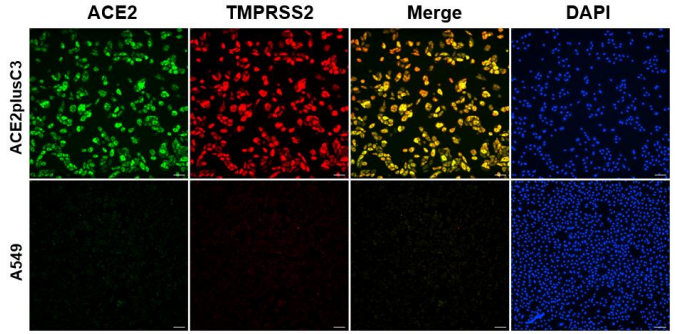
1. cell growth rate



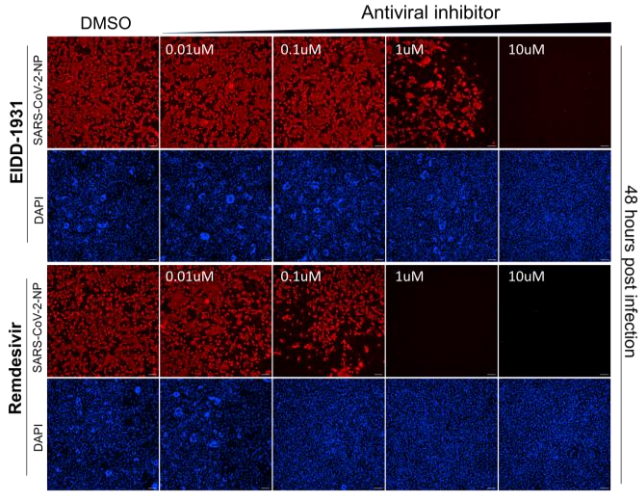
2. viral receptor expression



3. homogenous cell population

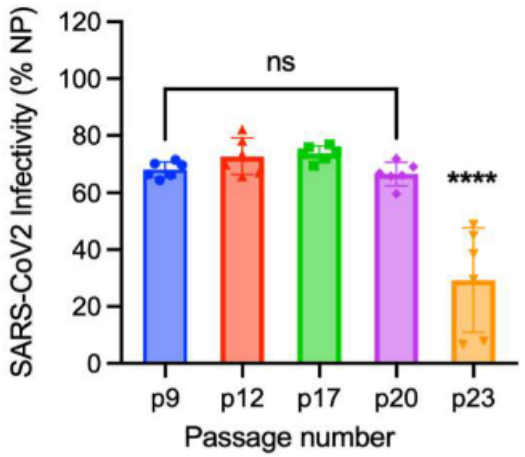


4. drug screening

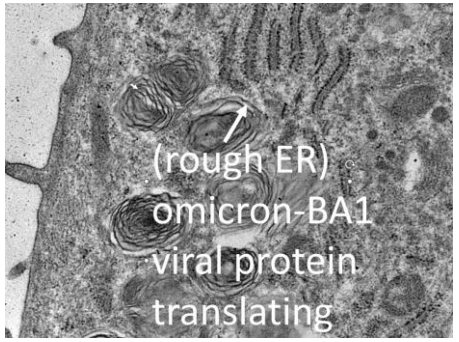


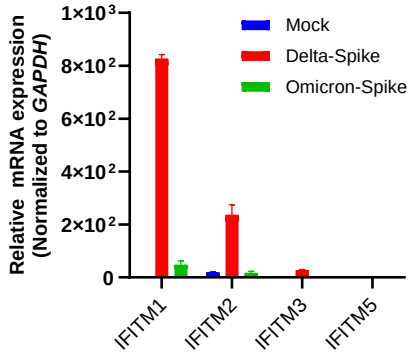
5. stable infectivity

p9-p20: 1.5~2 months



6. support viral replication and host cell response





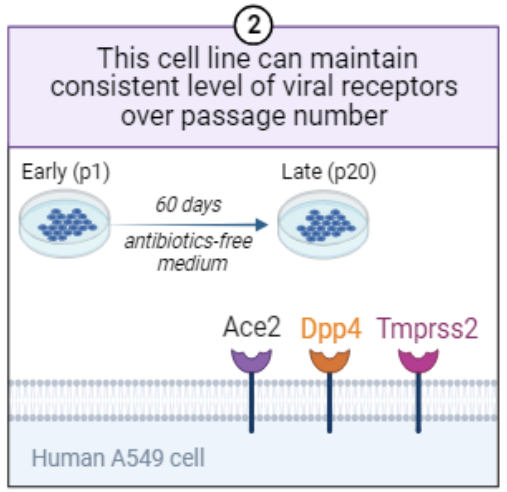
*data generated from A549-Ace2plus

Establish A549-based cell model for MERS-CoV and SARS-CoV2 study

	ACE2	TMPRSS2	Syncytia-CPE	Species	Tissues	Drug screen & Validation
A549^{Ace2plus}	✓	✓	✓			*MAVDA project & PNAS, 2023, 120 (11): e2219523120*
A549$^{Ace2\ GenScript}$	✓	-	-	*Human*	*Lung*	*(GenScript)*
A549$^{Ace2\ InvivoGen}$	✓	-	-			*(InvivoGen)*
A549$^{AT\ InvivoGen}$	✓	✓	✓*			*(InvivoGen)*

A549-Ace2plus (Ace2/Tmprss) → Clone selection → Transduce hDpp4

Ace2 : Tmprss2 : Dpp4

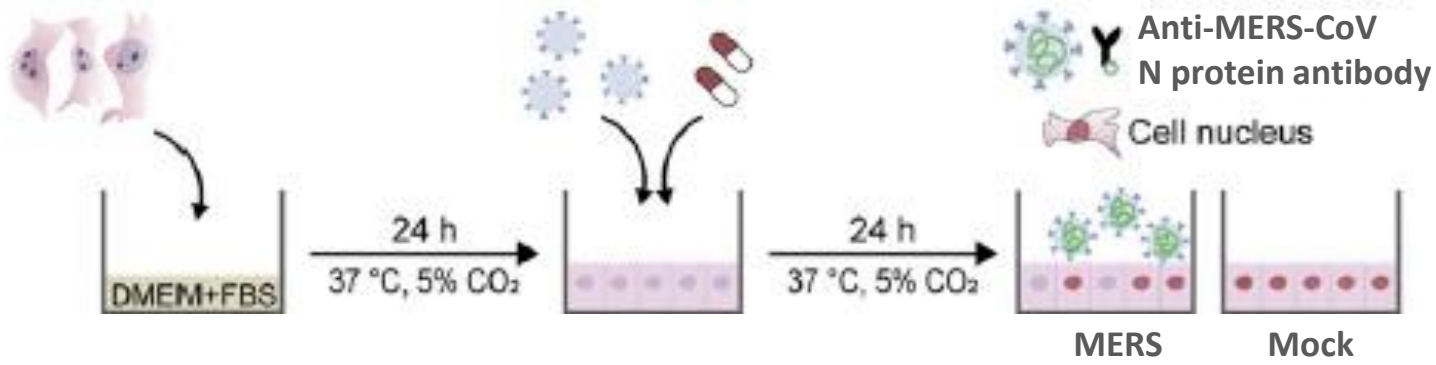


A schematic of the immunofluorescence-based assay to examine anti-MERS-CoV activity in engineered A549 cells

1. A549$^{Ace2+Dpp4+Tmprss2+}$ cell seeding (1.5x10^4/well)

2. Drug treatment and Virus infection (MOI 0.1)

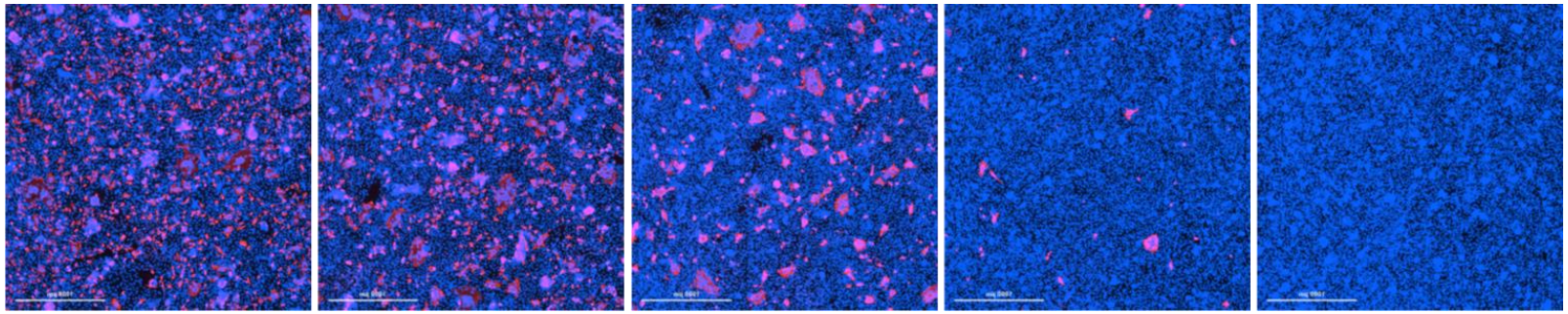
3. Cell fixation and Immunofluorescence

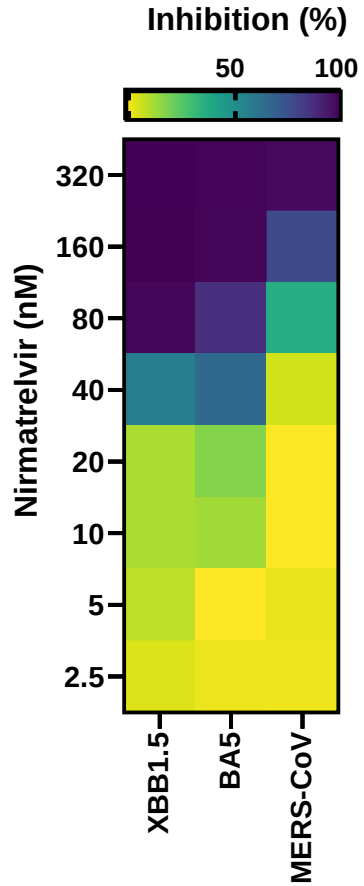


4. Dose-response curve analysis by Cytation C10

Cell nucleus (blue)
MERS-CoV N protein (red)

Nirmatrelvir concentration [nM]



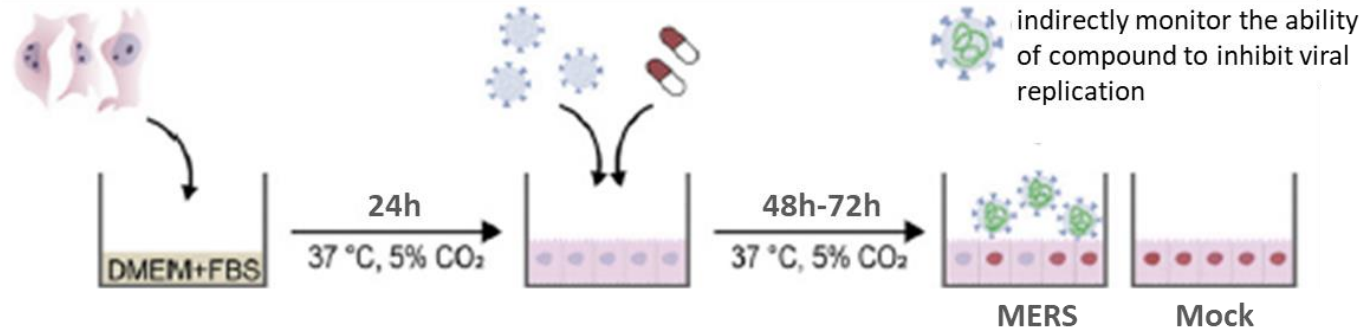


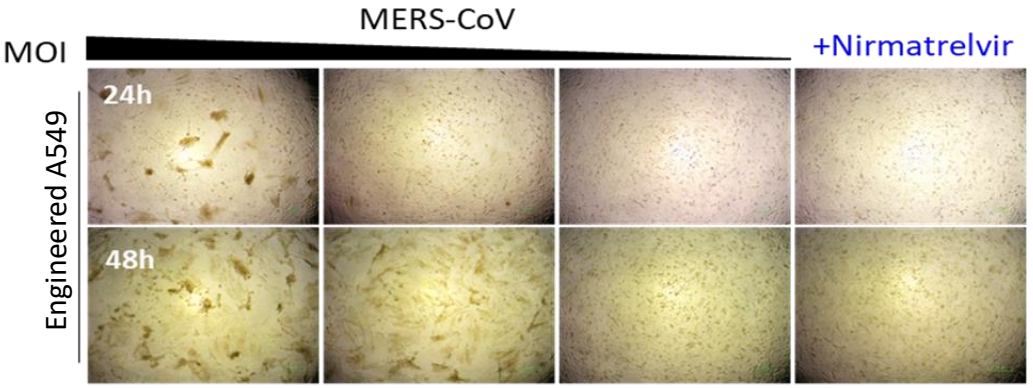
Measuring the ability of compound to reverse the viral induced cytopathic effect (CPE) in engineered A549 cells

1. A549$^{Ace2+Dpp4+Tmprss2+}$ cell seeding (3000/well)

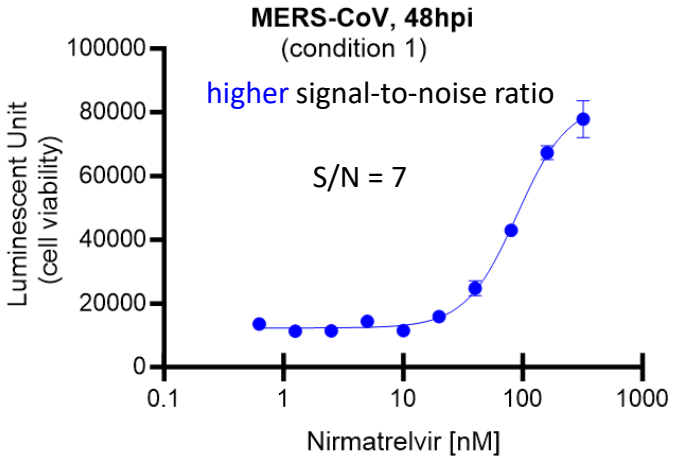
2. Drug treatment and Virus infection

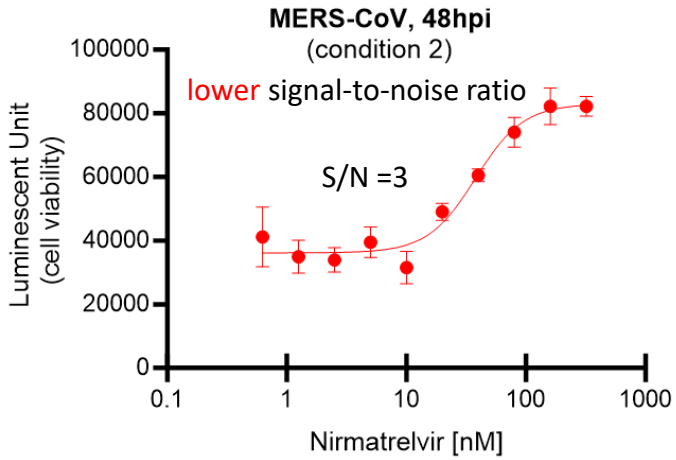
3. Monitor cell viability by CellTiter-Glo (CPE reduction)

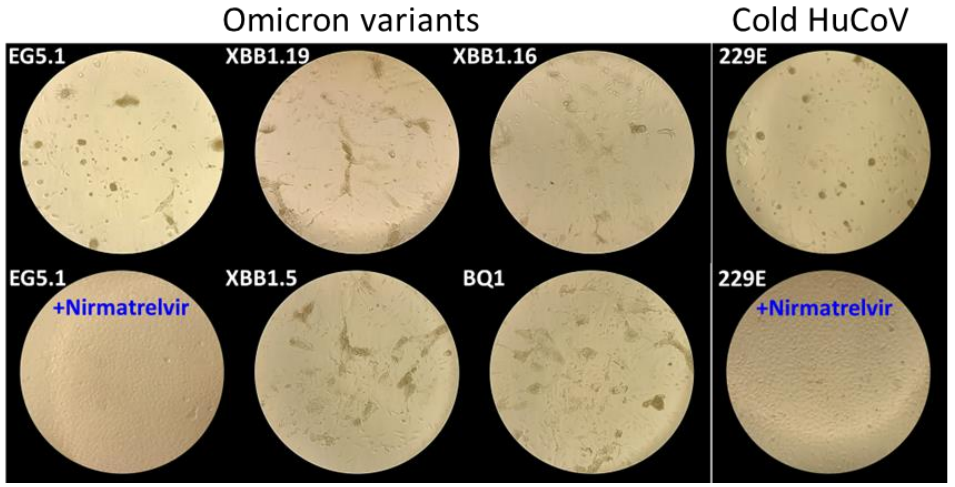




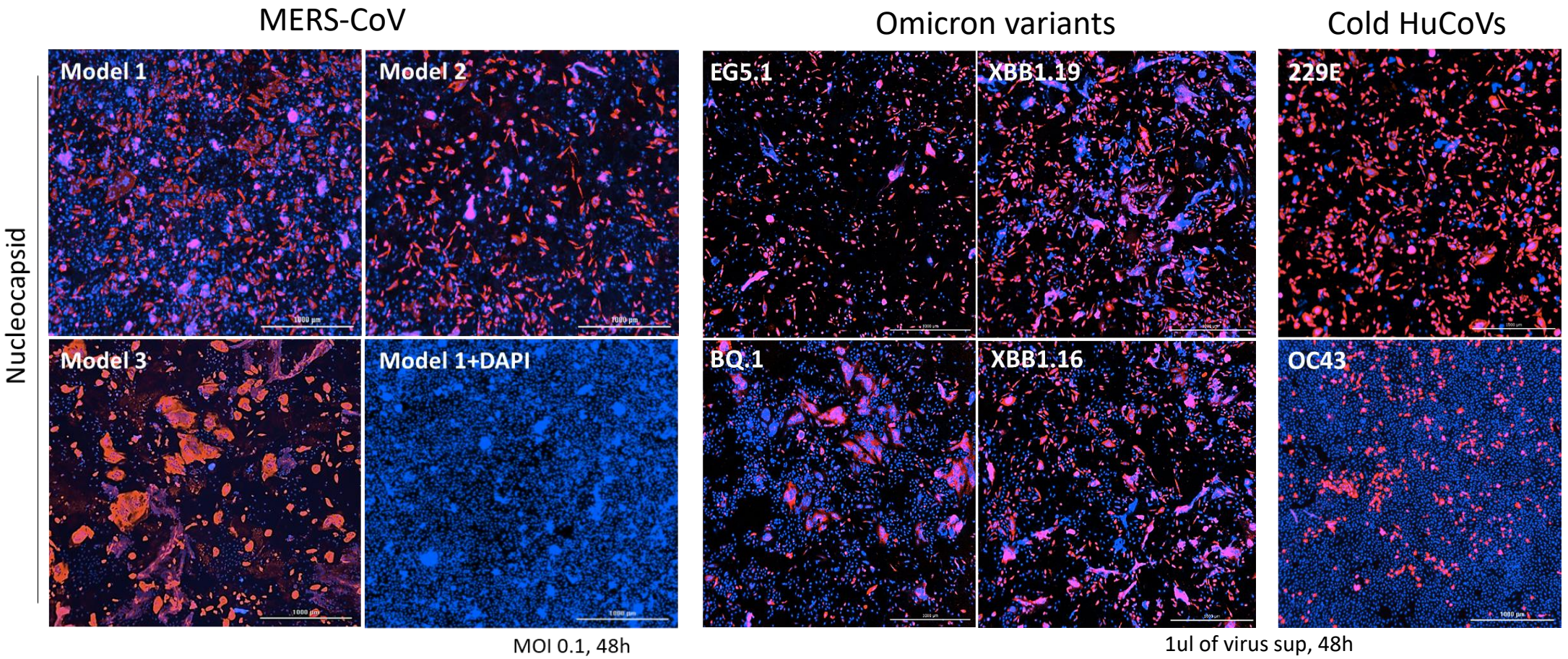
4. Readout & non-linear regression curve







Establishing a highly permissive A549 cell line for MERS-CoV and SARS-CoV-2 infection



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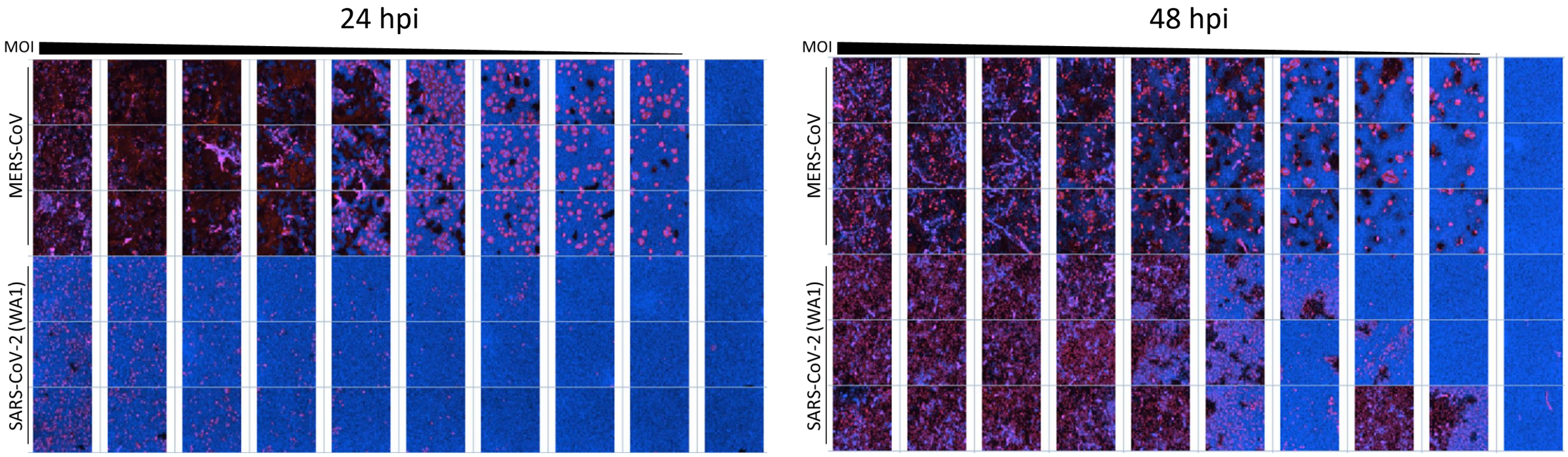
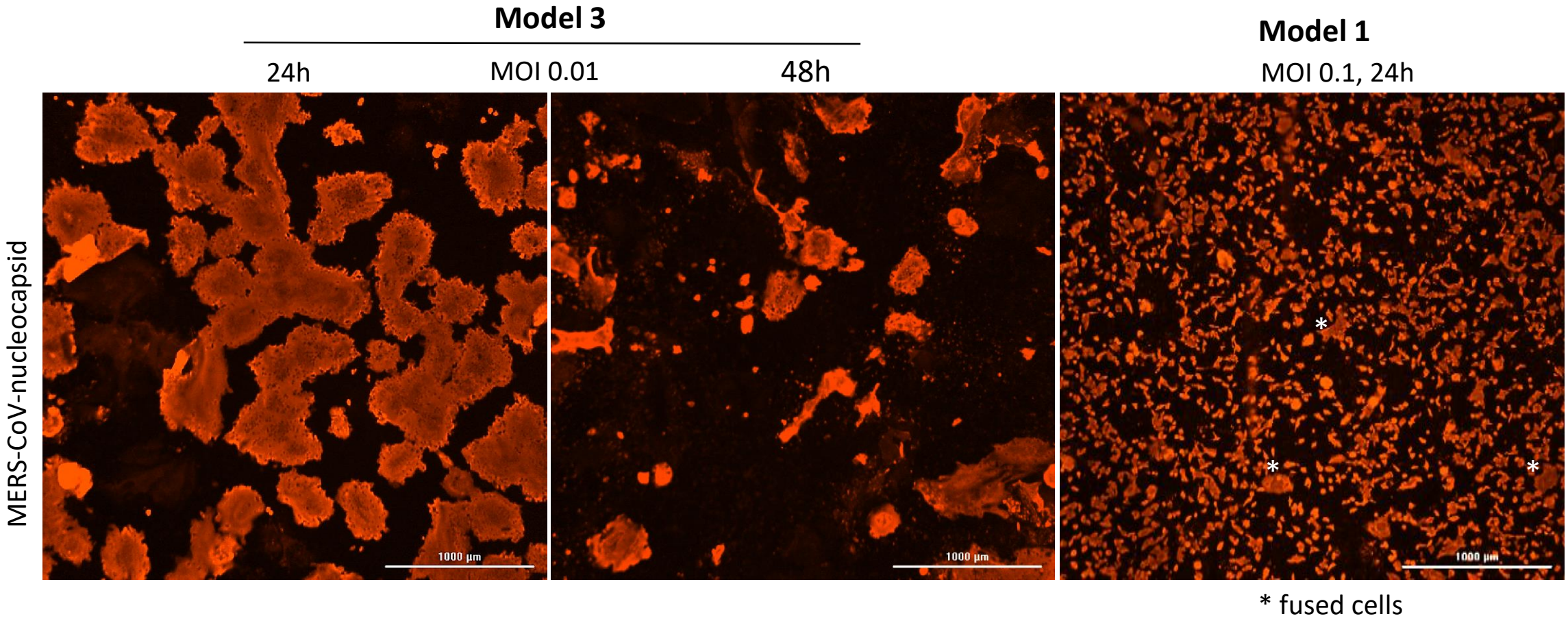
Immunofluorescence staining of the nucleocapsid protein of MERS-CoV & SARS-CoV-2 at 24h, 48h post-infection



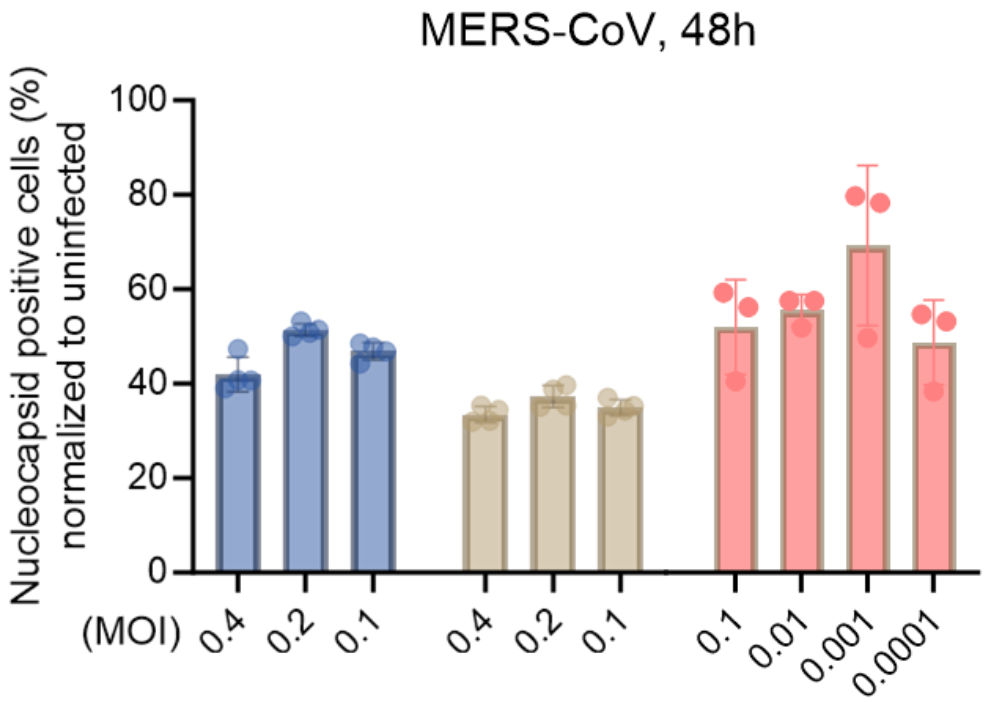
Model 3

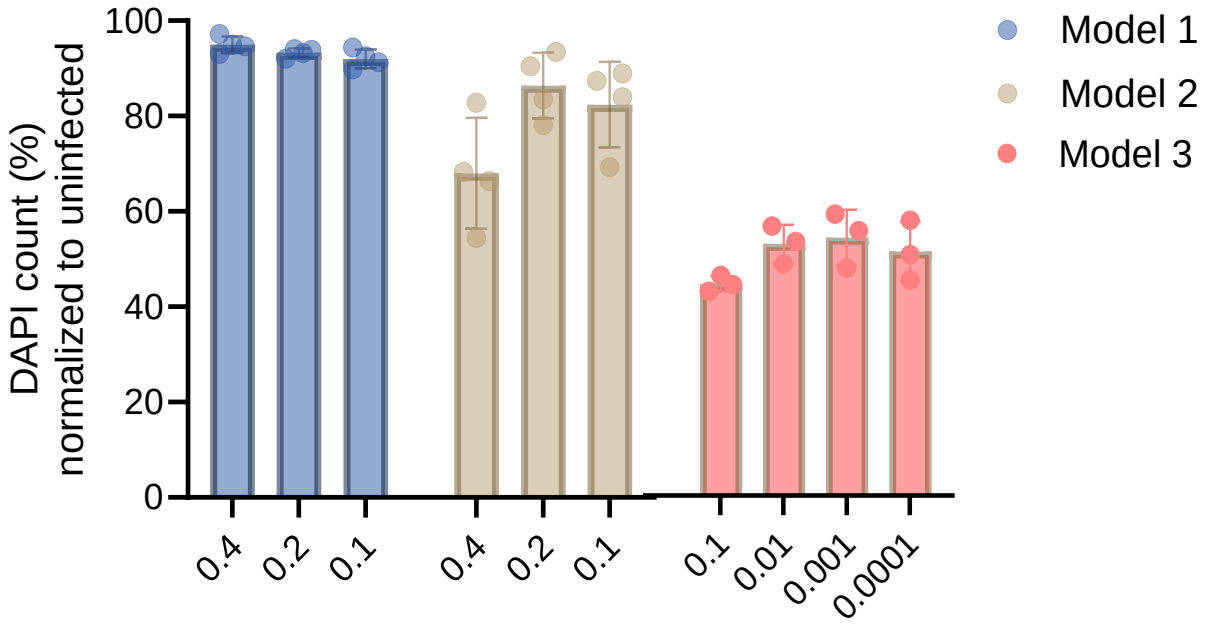
Observing MERS-CoV Spike-induced cell fusion after infection



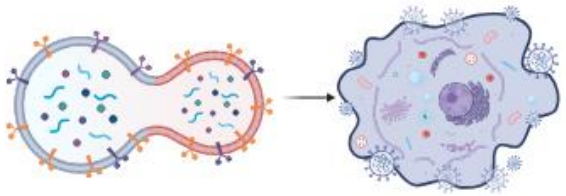
* fused cells

Establishing a highly permissive A549 cell line for MERS-CoV infection

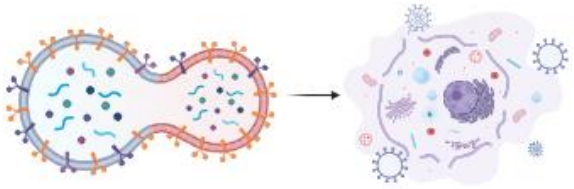




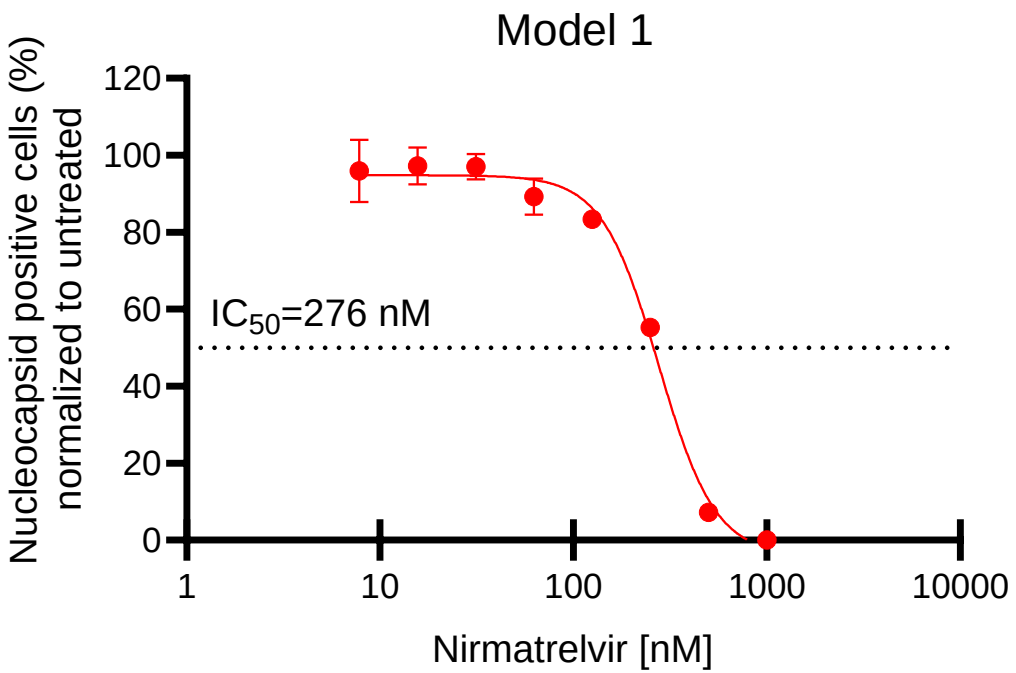
Model 1

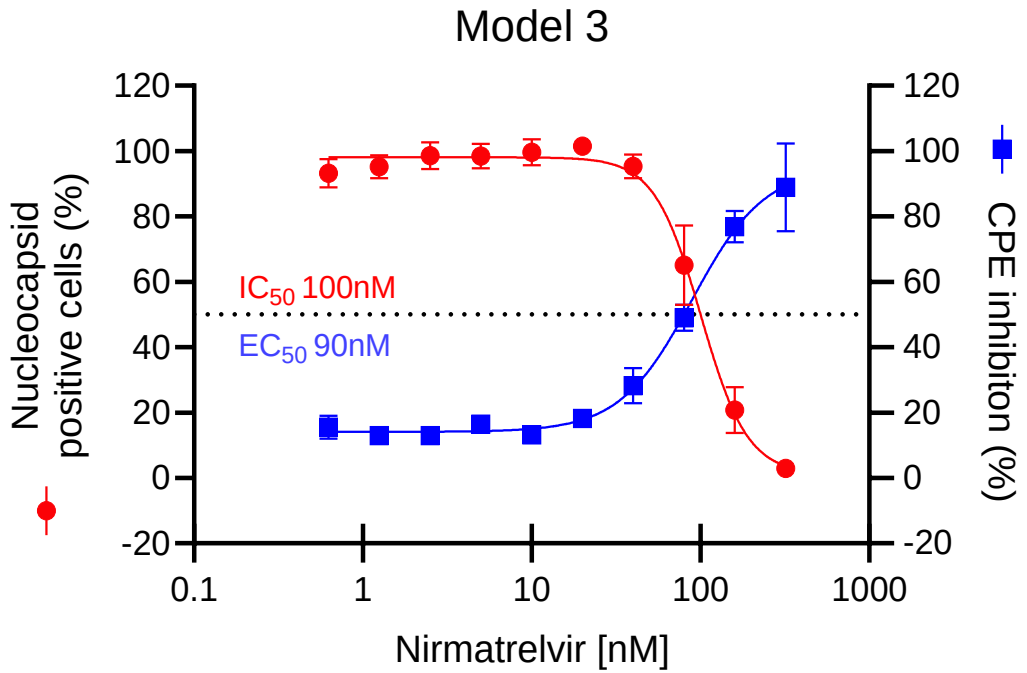


Model 3



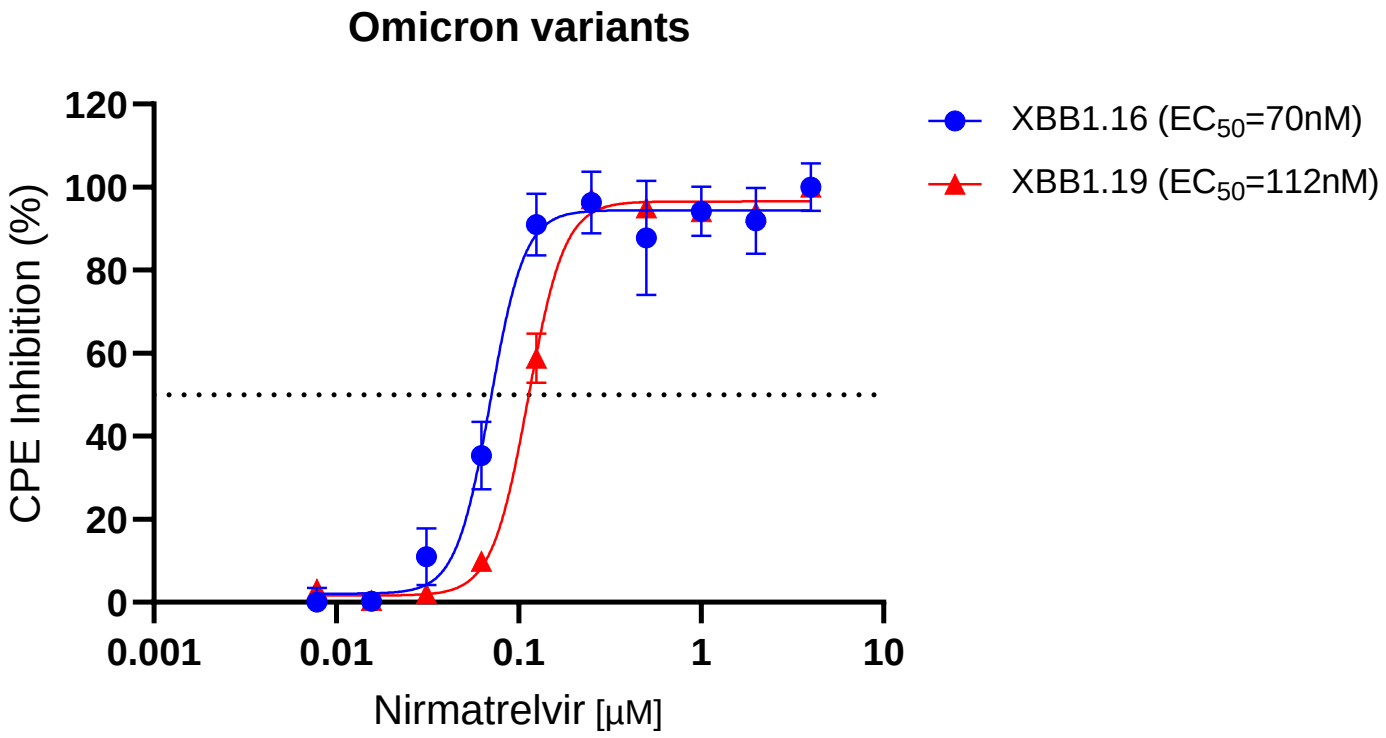
Establishing a highly permissive A549 cell line for MERS-CoV infection





MERS-CoV, Moi 0.1, 48 hpi

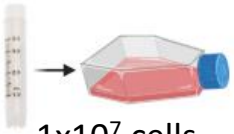
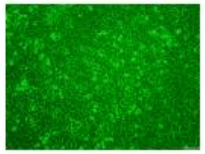
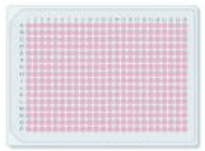
Measuring the ability of compound to reverse the viral induced cytopathic effect (CPE) in engineered A549 model 1



A549-C3D4 Model	Viral-induced CPE	Nirmatrelvir (reverse CPE)
MERS-CoV	√	√
SARS2-CoV	√	√
*EG5.1	√	√
*XBB1.19	√	√
*XBB1.16	√	√
*XBB1.5	√	√
*BQ.1	√	√
CoV-229E	√	√
CoV-OC43	TBD	TBD

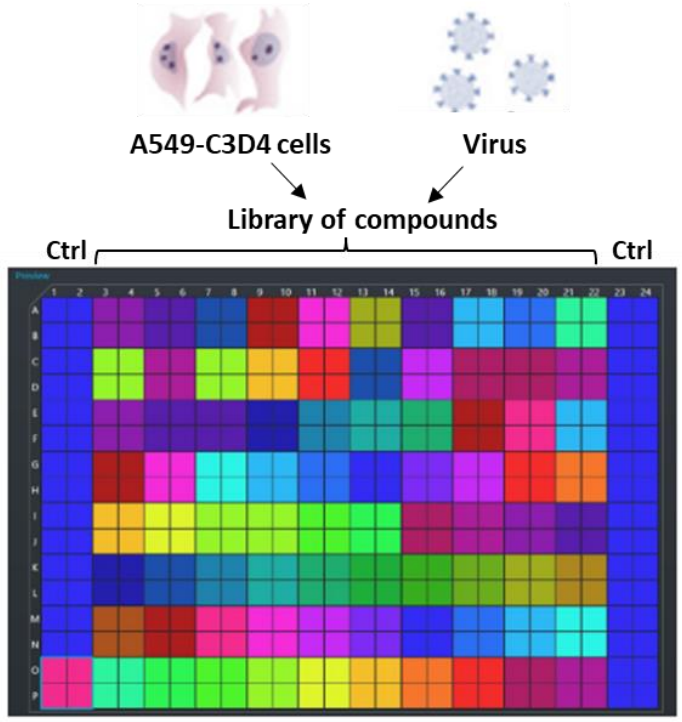
* Omicron variants
TBD: to be determined

Workflow of High Throughput Screening of Antiviral Drug

Step 1	Step 2	Step 3	Step 4
72h	**24h**	**2h**	**24h**
 1x10^7 cells 	 	 	
Cell recovery & growth • > 90% cell viability after thawing • SOP	**Seeding cells in 384w plate** • EL-406 • SOP	**Dispensing compounds** • Celllink I.DOT • SOP	**BSL-3 virus infection:** • Hamilton Microlab STAR • Integra Viaflo 384 • SOP

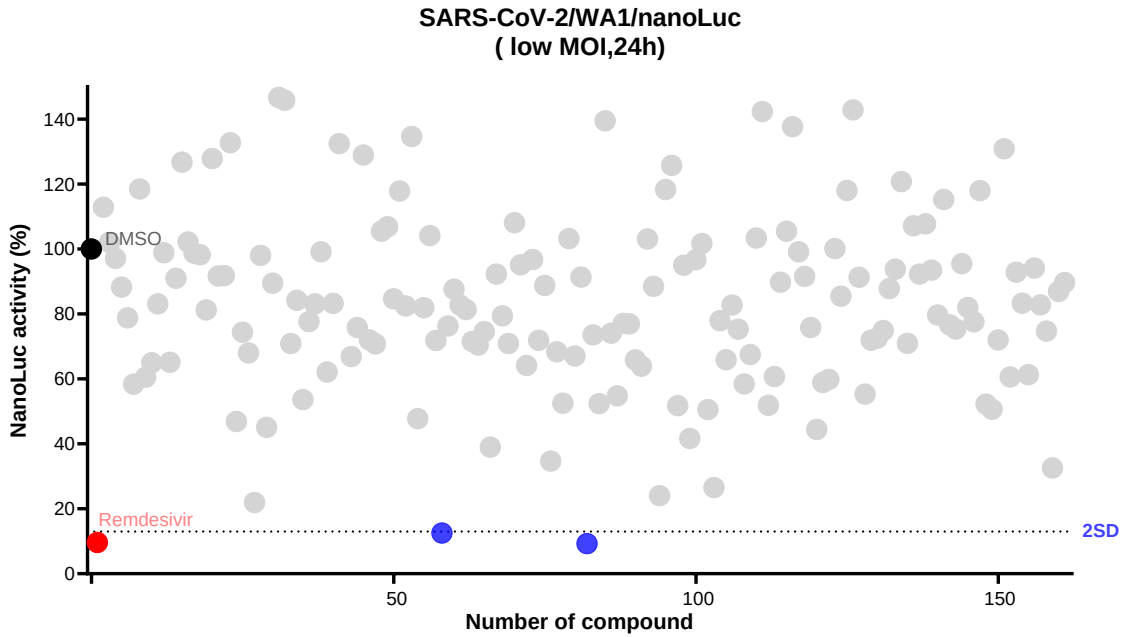
Credit by Jalil

Schematic Overview of the Higher-Throughput assay



Hamilton automated liquid handler

(This line also can be applied to antiviral drug screening)



- In each plate we can run **80** compounds at a single dose having **4 replicates**
- Or **106** compounds having **3 replicates**

Credit by Jalil/Nadine

Summary and Next Steps

- Newly engineered A549 models are highly permissive to MERS-CoV, SARS-CoV-2 infection, including emerging omicron variants EG5.1, BQ1, XBB1.19 and XBB1.16.
- This engineered A549 cell model shows significant cytopathic effects (CPE) and virus-induced CPE can be rescued by adding Niamatrelvir during virus infection.
- This novel A549 cell model shows its potential for antiviral drug discovery, further condition optimization for integrating this model into our current HTS assay is ongoing.
- MERS-CoV shows faster virus replication kinetics than SARS-CoV-2 in the engineered A549 cell model, suggesting that this model can be used for studying coronavirus pathogenesis.
- Further characterization of this novel A549 cell model is ongoing.

Acknowledgements

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Special Thanks!

David Perlin, PhD
Charles Rice, PhD
Stephen Goff, PhD
Yosef Sabo, PhD
Susan Weiss, PhD
Nicholas Parenti, MScSho

Sho Iketani, PhD
Alejandro Chavez, MD/PhD

Funding Support

NIH National Institute of Allergy and Infectious Diseases