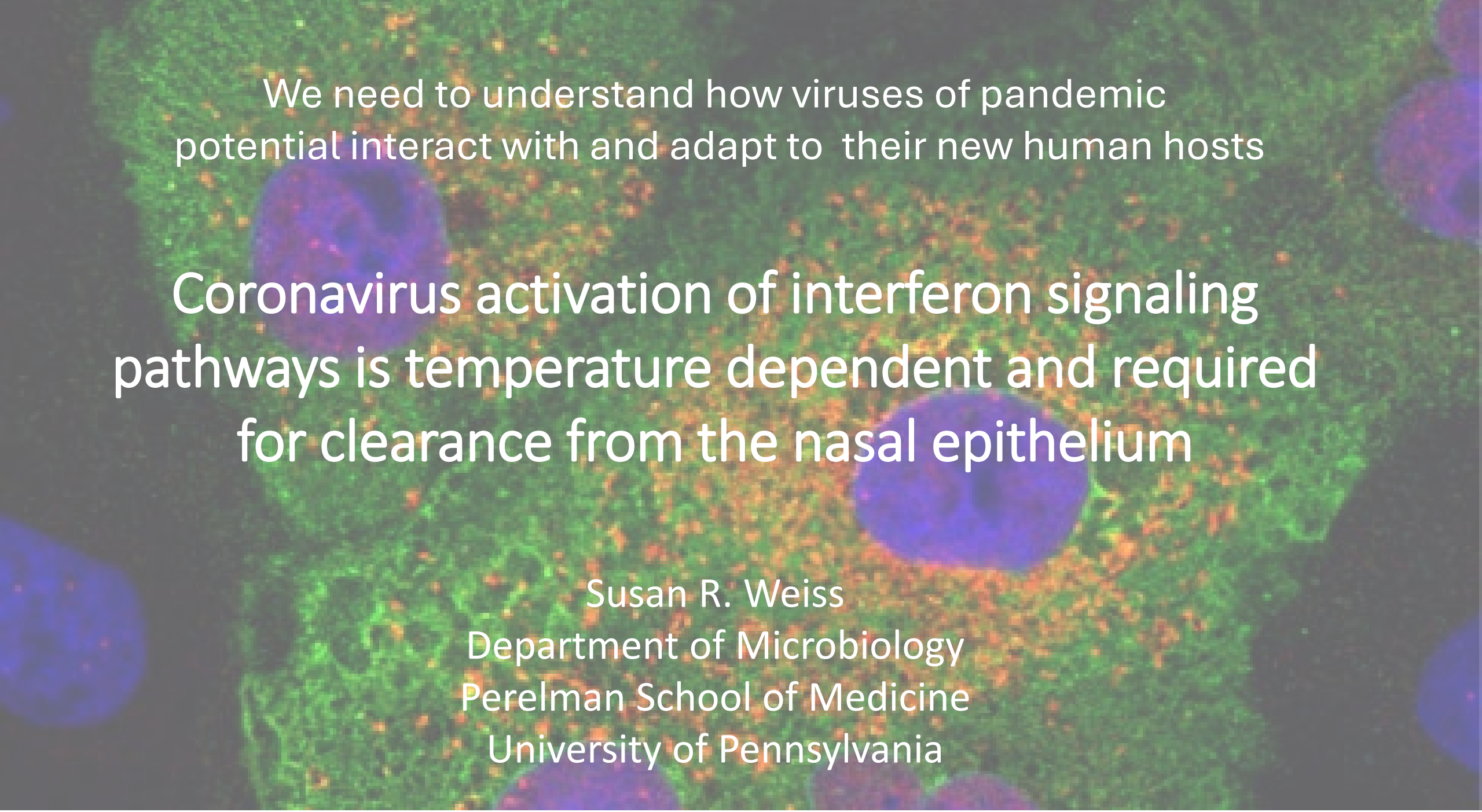
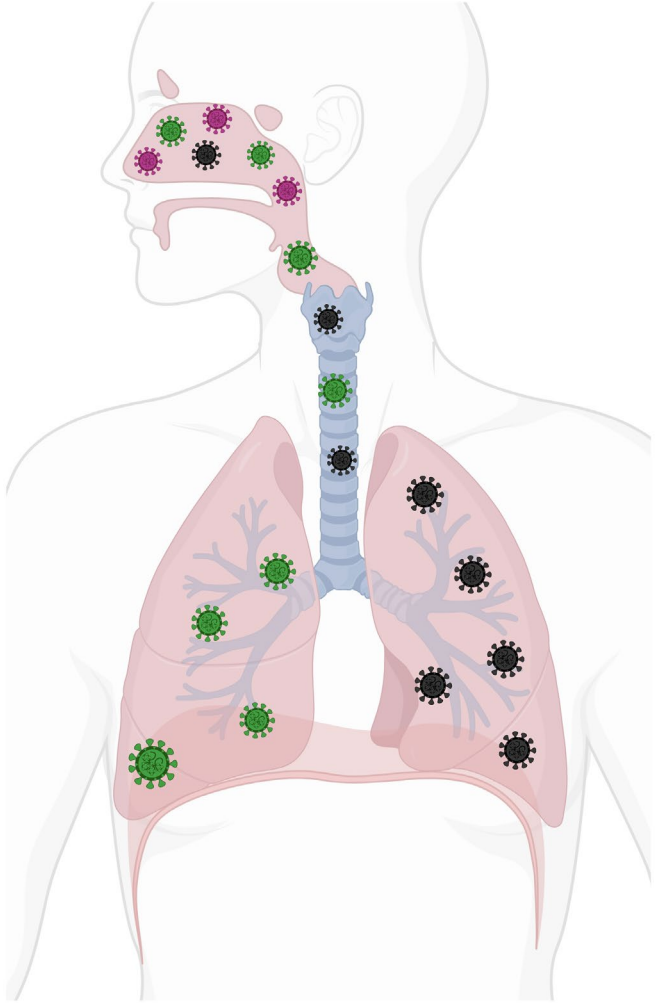


We need to understand how viruses of pandemic potential interact with and adapt to their new human hosts

Coronavirus activation of interferon signaling pathways is temperature dependent and required for clearance from the nasal epithelium

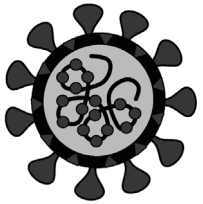
Susan R. Weiss
Department of Microbiology
Perelman School of Medicine
University of Pennsylvania

Human respiratory coronaviruses display a range of pathologies



Upper respiratory tract infection
common cold

229E	1965-67	(B814)
OC43	~1969	(OC38)
NL63	2004	
HKU-1	2004	



Lower respiratory tract infection
Severe pneumonia

SARS-CoV	2002
MERS-CoV	2012

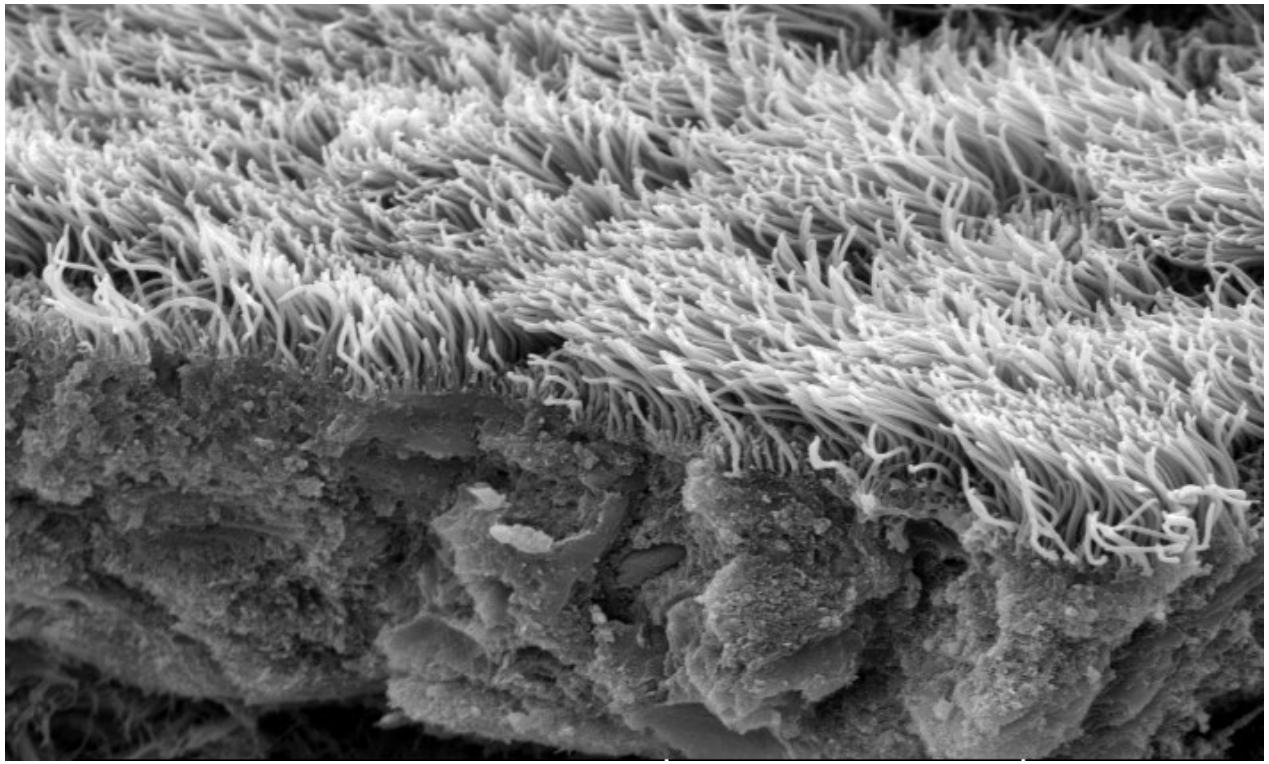
Upper and lower respiratory tract infection
Asymptomatic to severe pneumonia

SARS-CoV-2	2019

A. Z. Kapikian, The coronaviruses. *Dev Biol Stand* **28**, 42-64 (1975).

Alejandra Fausto
Biorender

How does interferon response influence human coronavirus infection in the nasal epithelium?



MERS-CoV
SARS-CoV-2

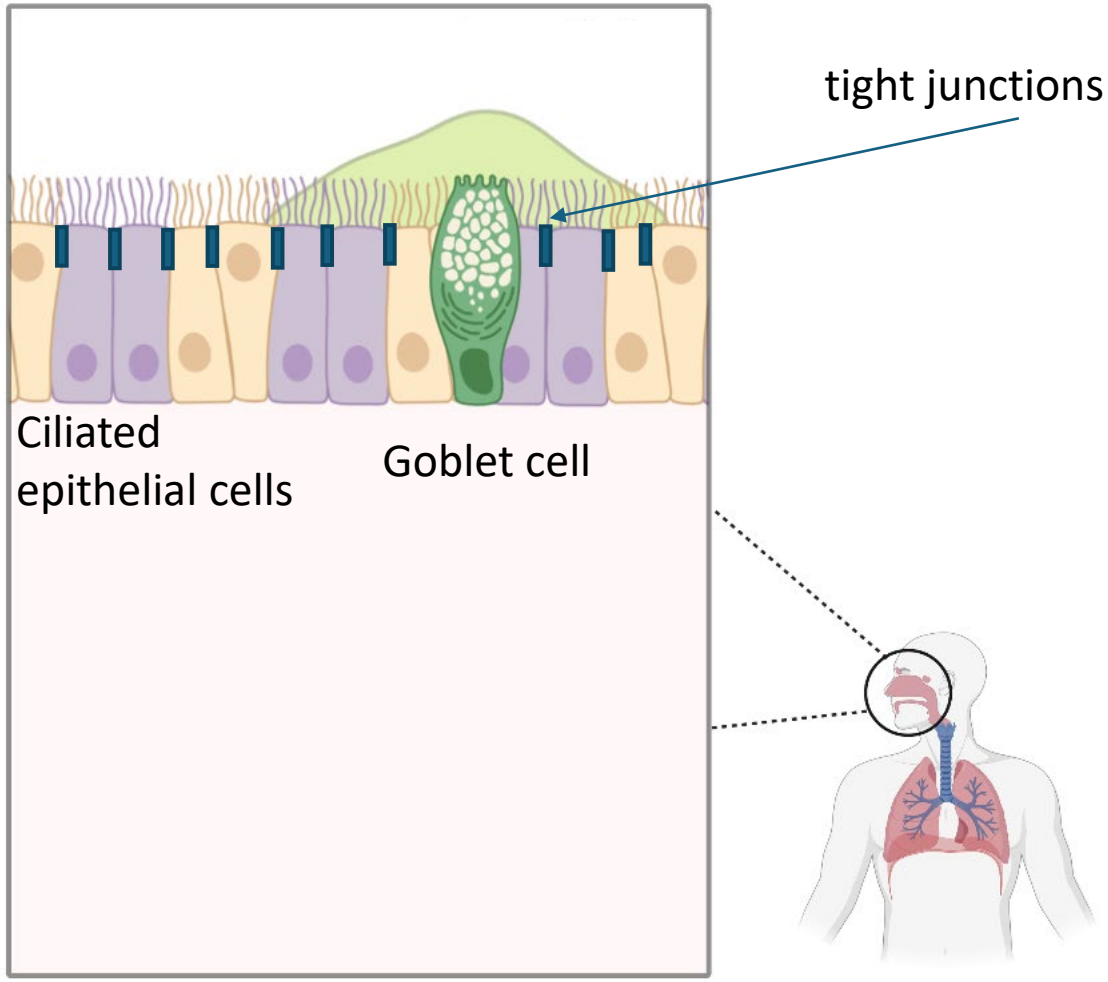
HCoV-NL63, HCoV-229E

Human rhinovirus (HRV)-16

\

Importance of the nasal epithelium

- First line of defense against respiratory pathogens
- Site of initial viral replication
- Primarily composed of ciliated cells and goblet cells (produce mucus)
  - Mucociliary clearance mechanism to prevent spread to lower airway
- High basal expression levels of interferon and other antiviral genes – nasal cells may be “primed” to defend against invading viruses



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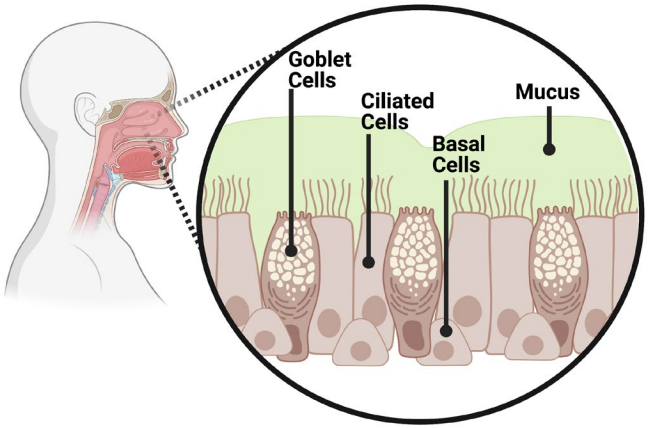
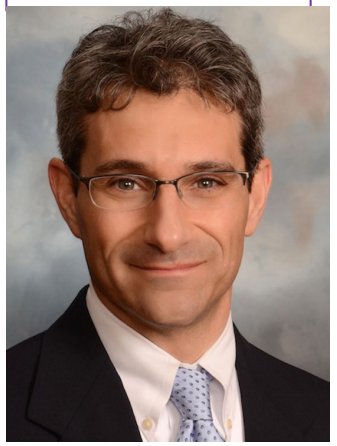
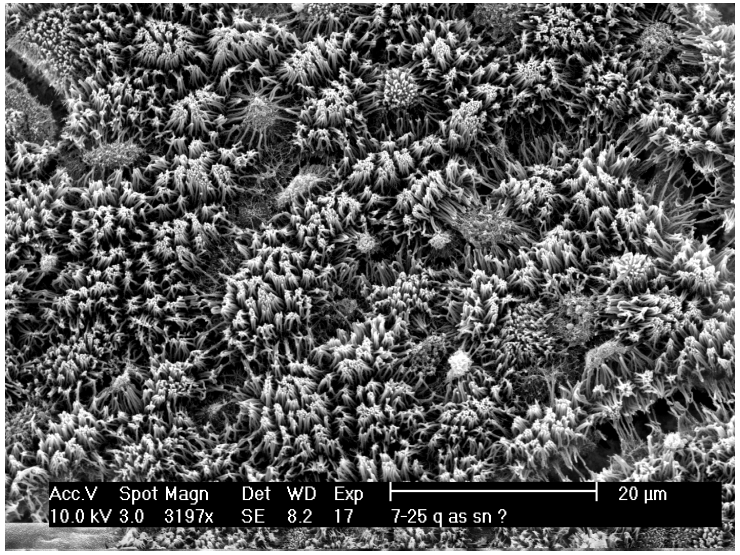


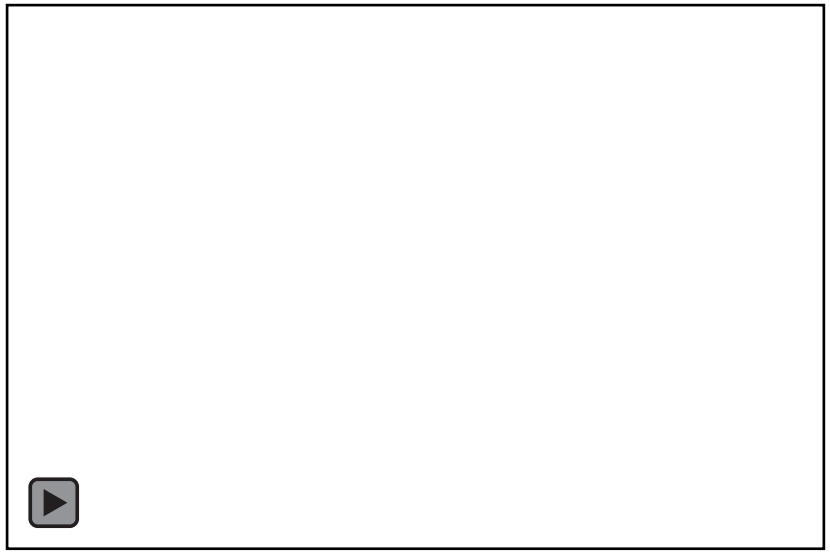
Nasal air liquid interface (ALI) cultures recapitulate in vivo airway

Noam Cohen
ENT, PENN

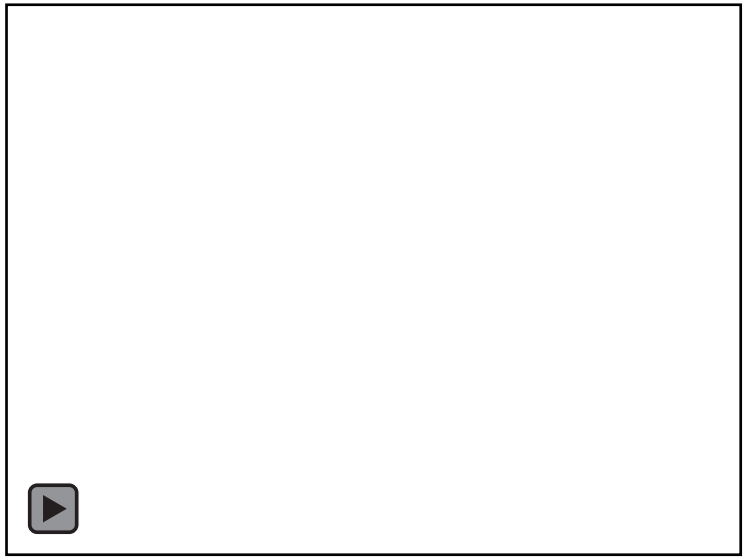




scanning EM image of culture

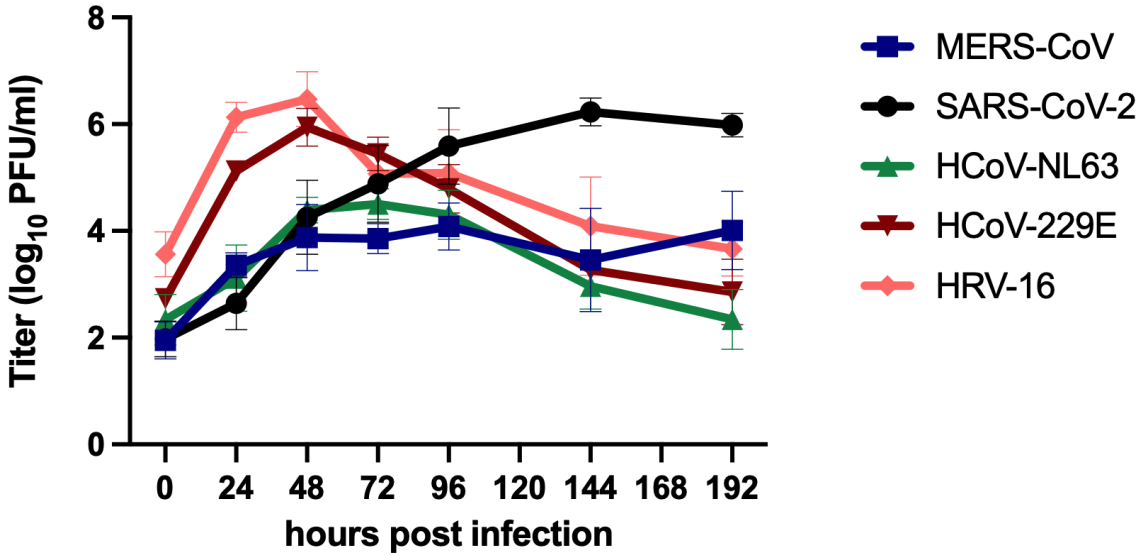


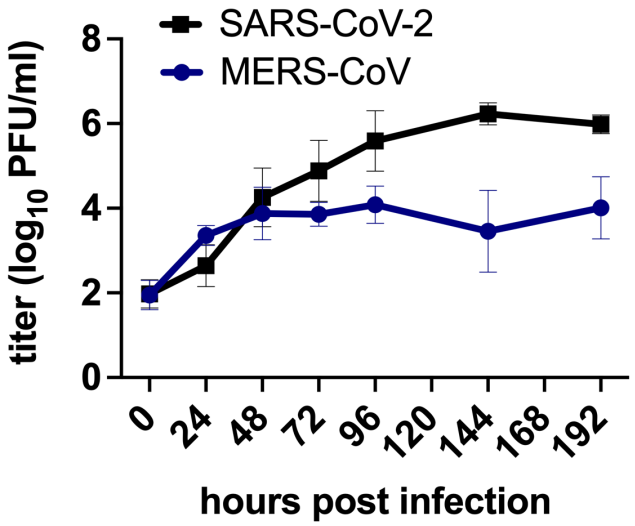
ciliary beating

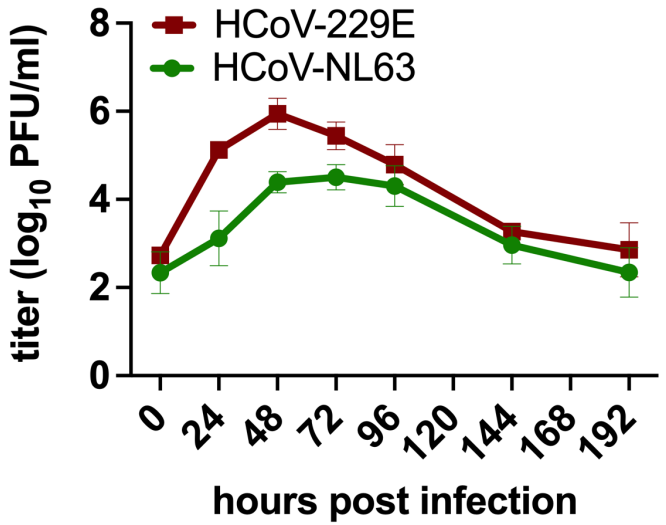


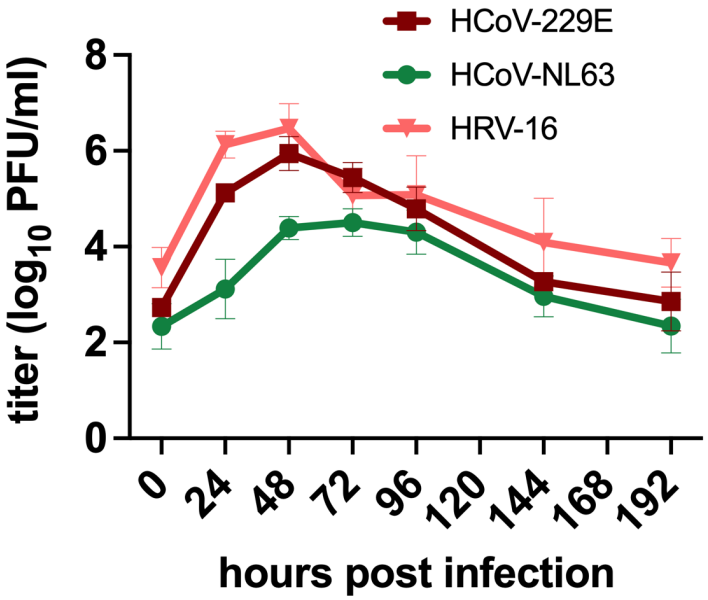
mucociliary clearance
of cellular debris

Kinetics of virus replication in primary nasal cell ALI cultures





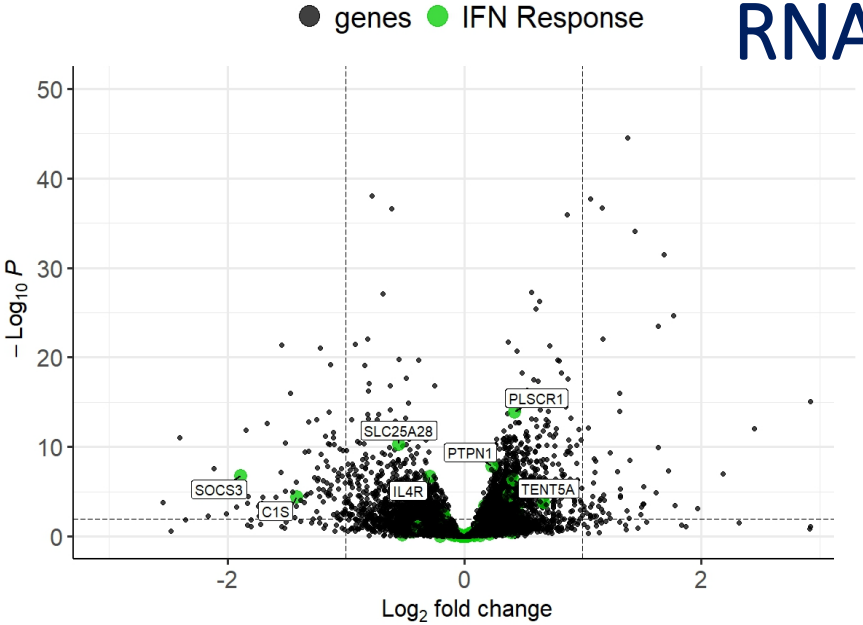




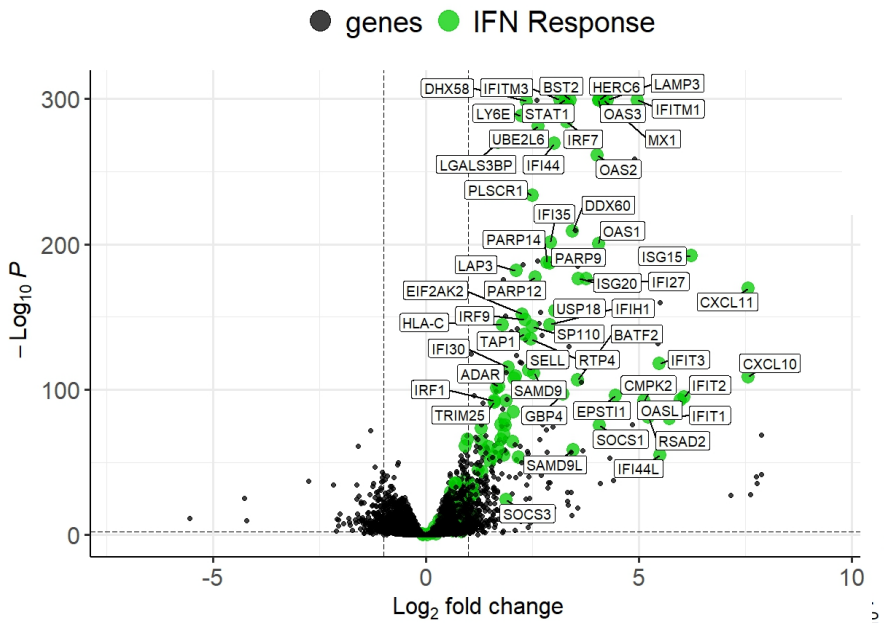
Otter et al., PNAS, 2024

RNAseq:IFN signaling response

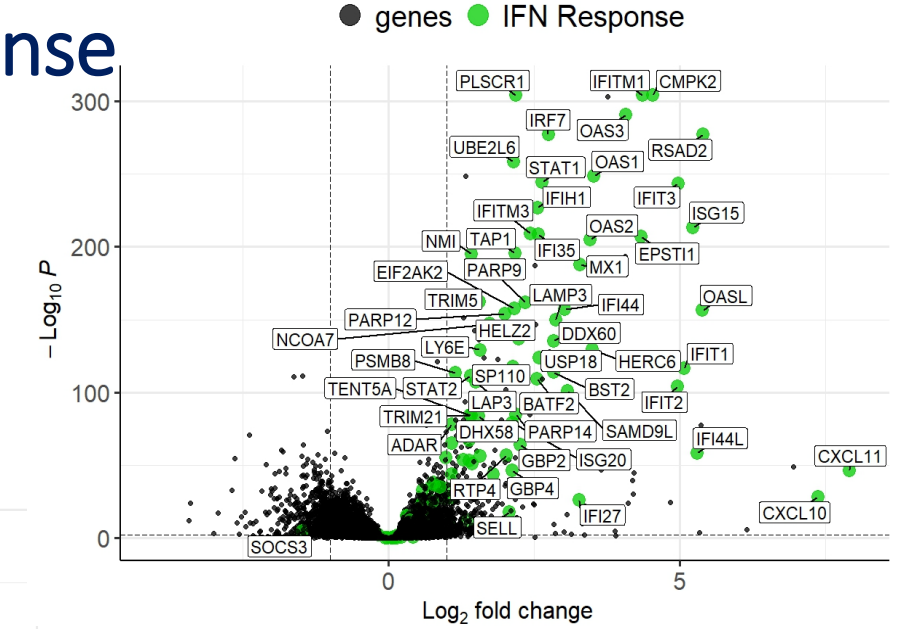
MERS – almost no IFN signaling



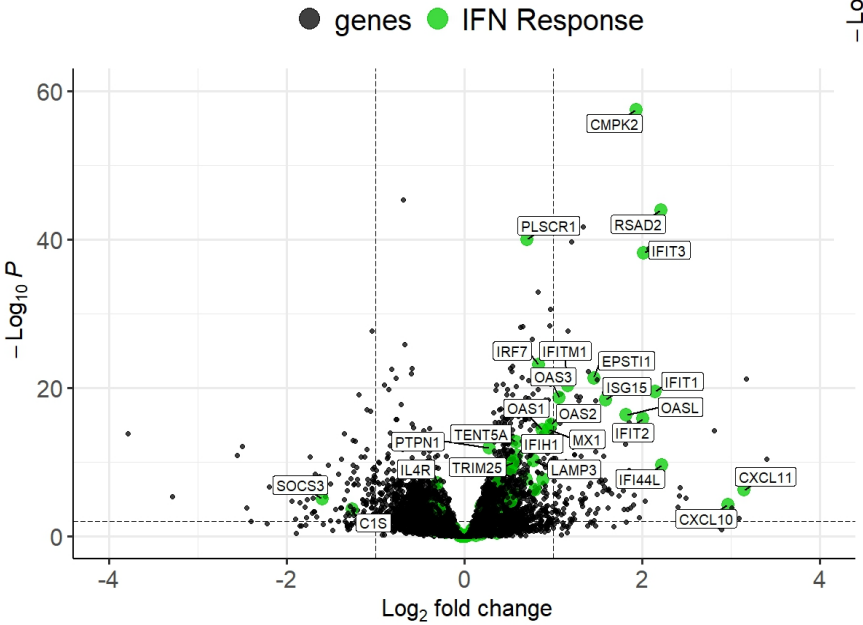
Human Rhinovirus-16
similar to common HCoVs



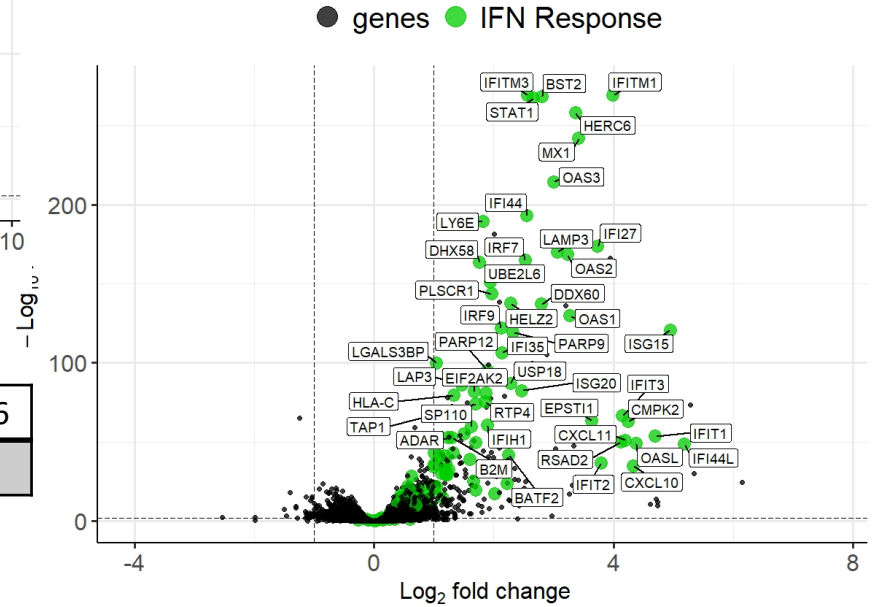
NL63 – strong IFN signaling



SARS-2 – moderate IFN signaling



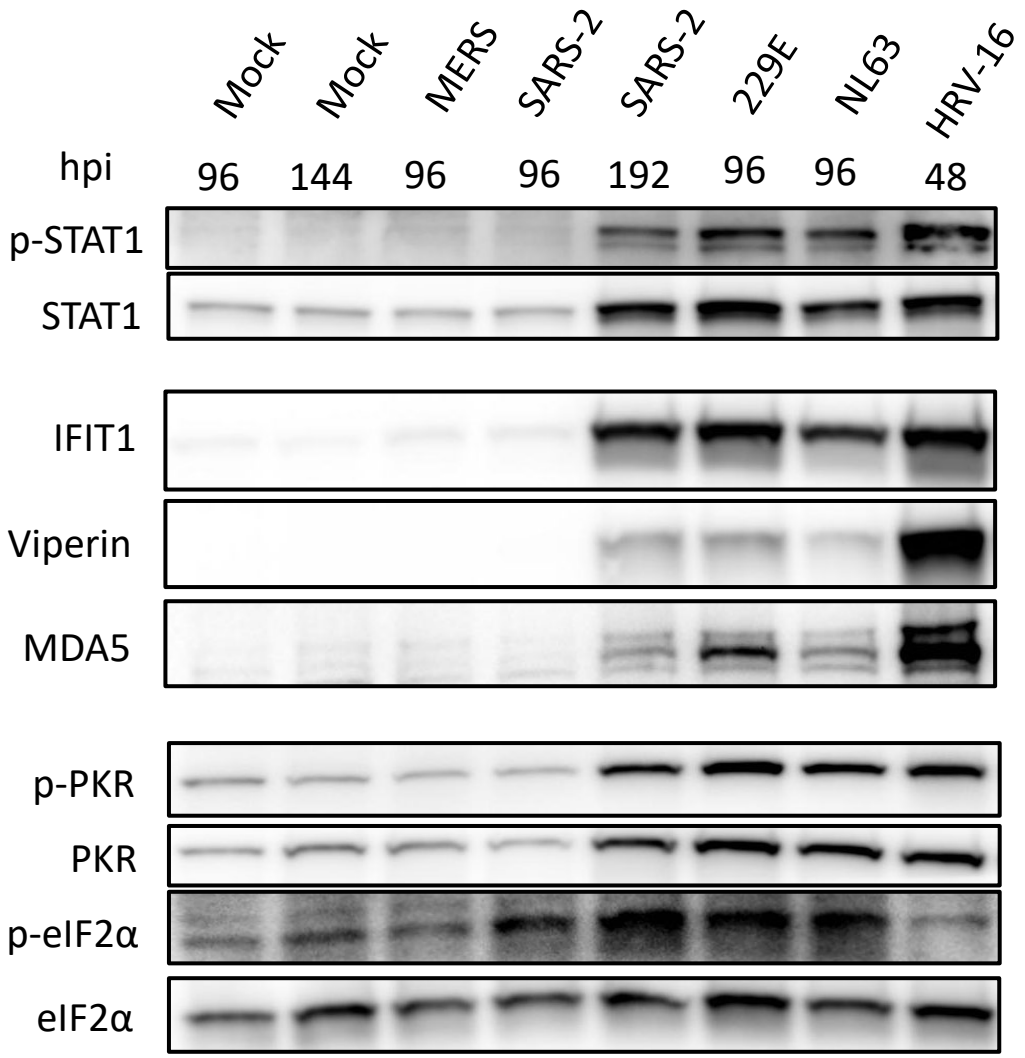
229E – strong IFN signaling



Virus	MERS	SARS-2	NL63	229E	HRV-16
# ISGs	2	15	70	70	82

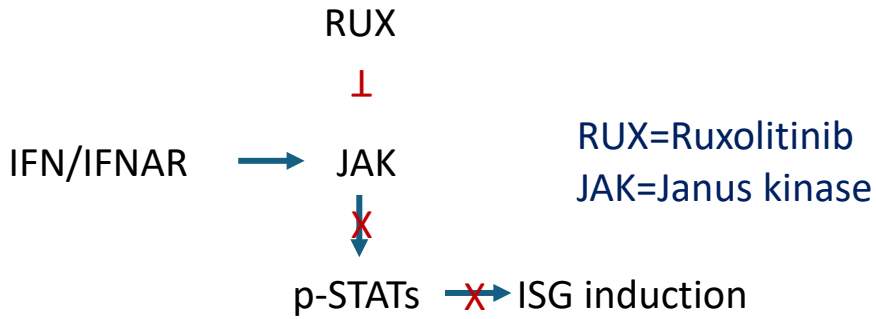
Otter et al., PNAS, 2024

ISG protein expression and PKR pathway activation follow similar patterns to mRNA expression



IFN signaling and PKR response

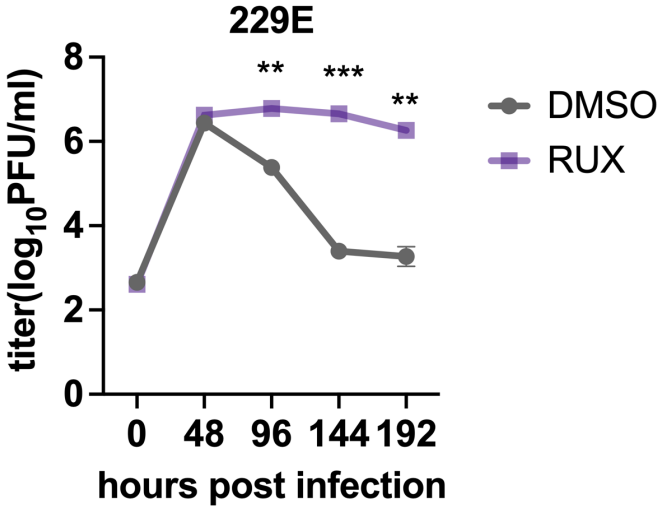
MERS- none detectable
SARS-2- delayed responses
NL63/229E/HRV16- early and robust responses

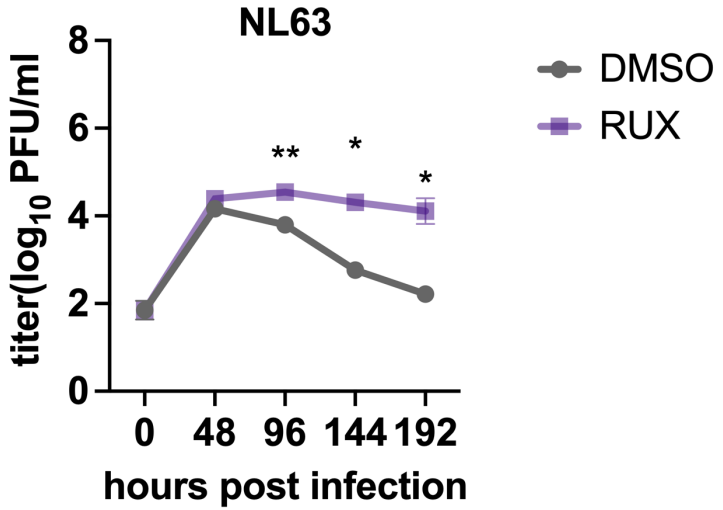


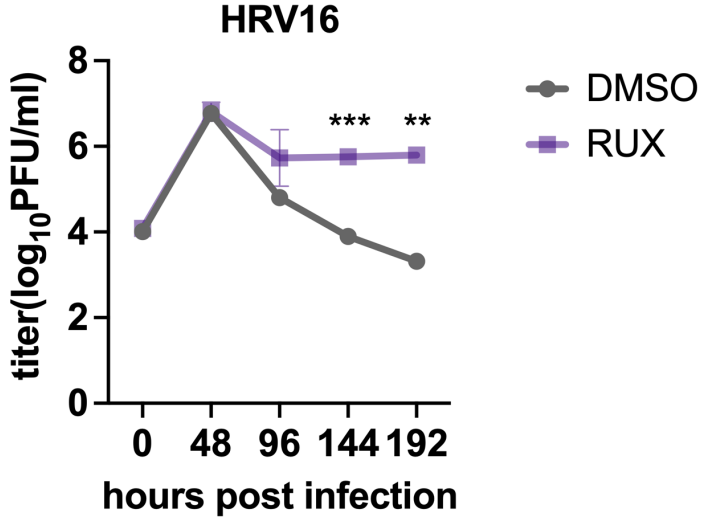
RUX=Ruxolitinib
JAK=Janus kinase

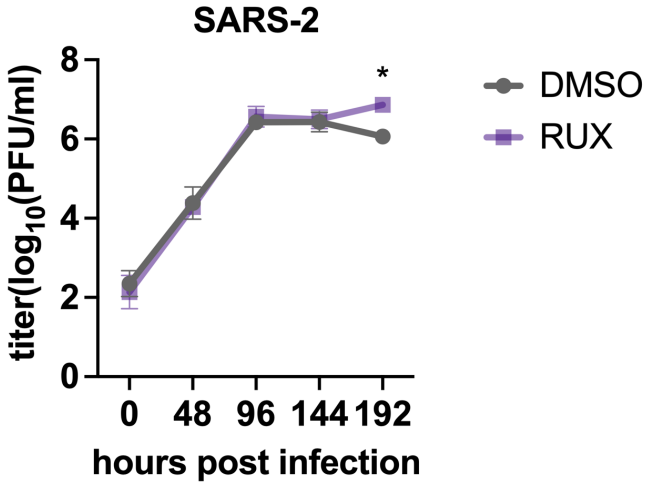
Otter et al., PNAS, 2024

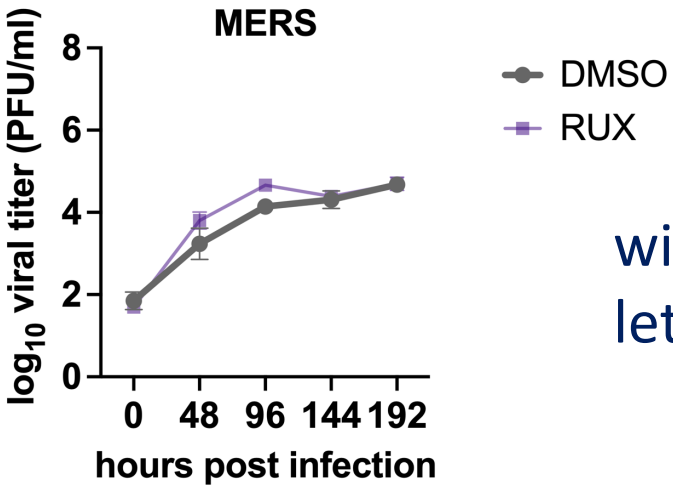
Inhibition of IFN signaling leads to delayed clearance of common cold viruses









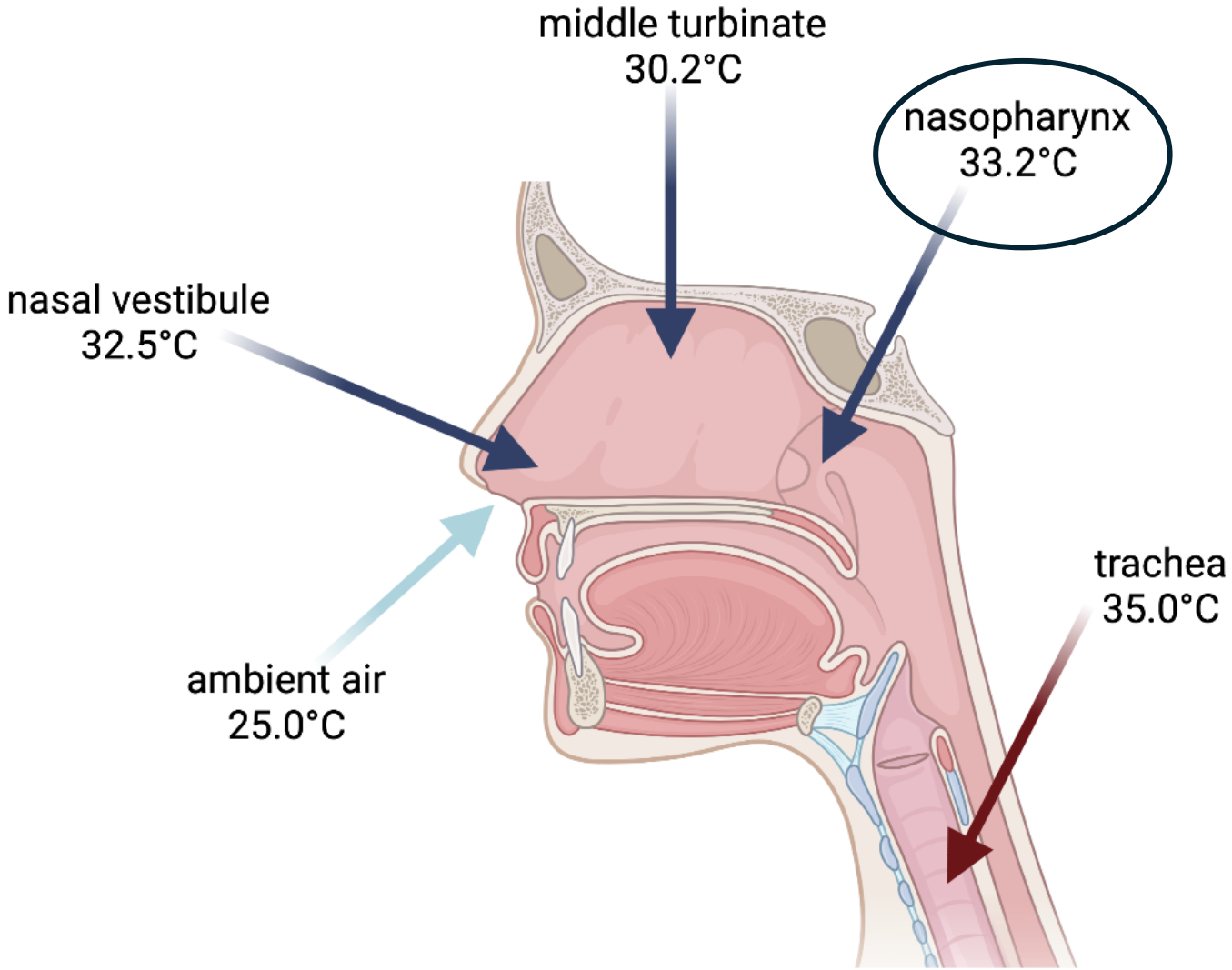


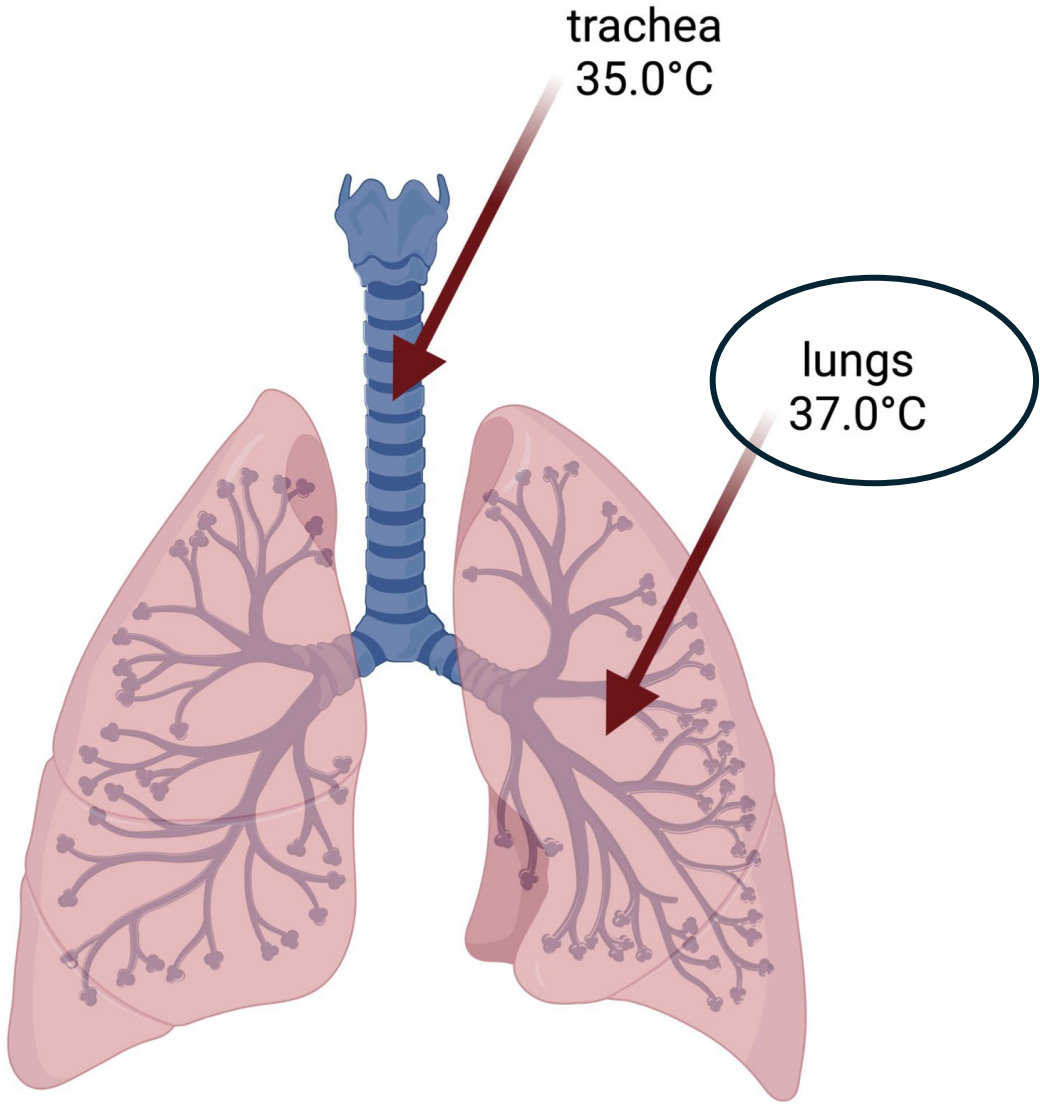
with little effect on lethal viruses

10uM RUX treatment;
pre-treat 48hr pre infection

Otter et al., PNAS, 2024

Temperatures in the upper and lower respiratory tracts





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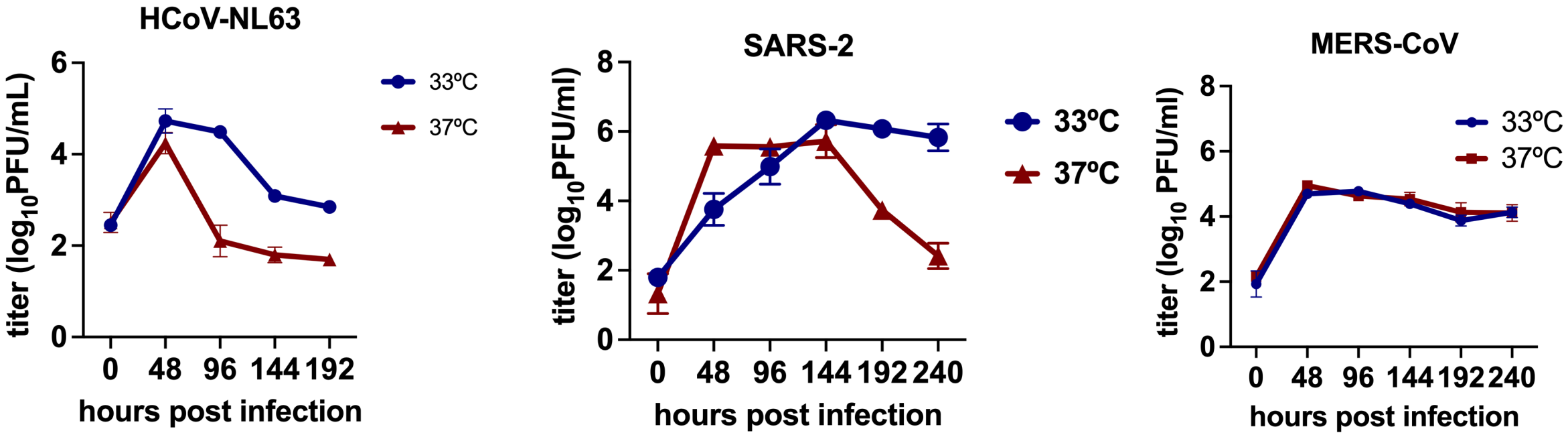
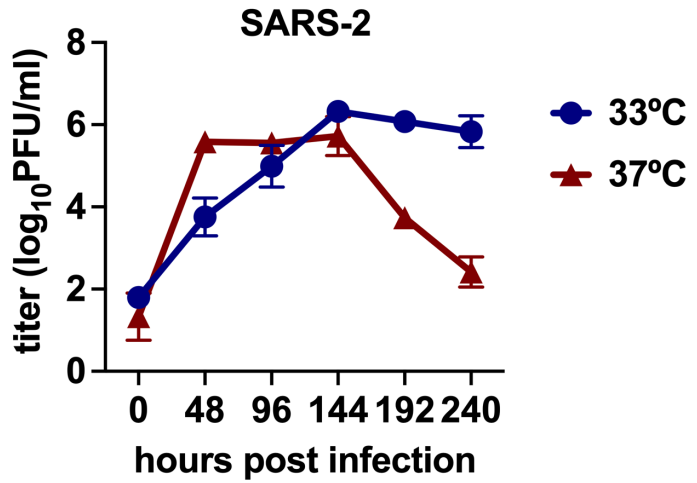
In addition to inducing different patterns of IFN signaling, HCoVs require different temperatures for optimal replication

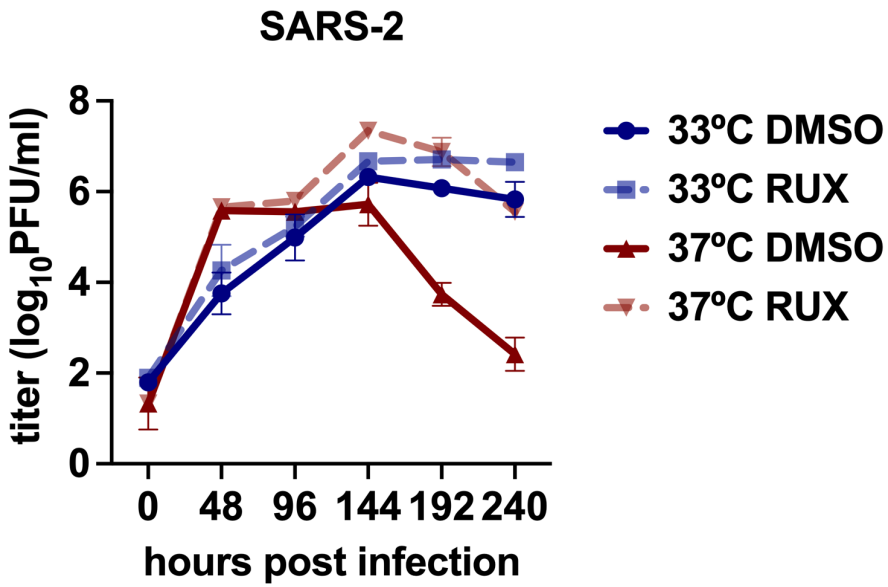


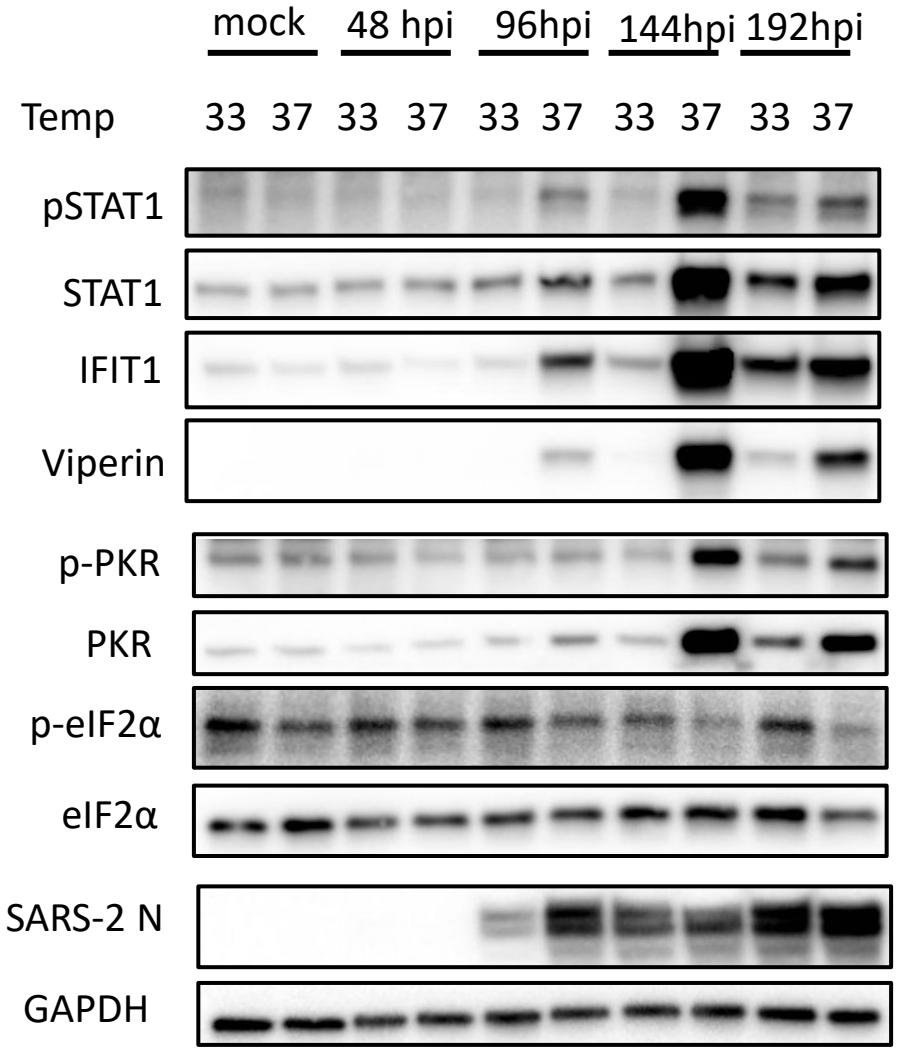
Does IFN signaling response contribute to temperature dependence of replication?

Otter et al., PNAS, 2024

IFN signaling response to SARS-2 is more robust at 37C



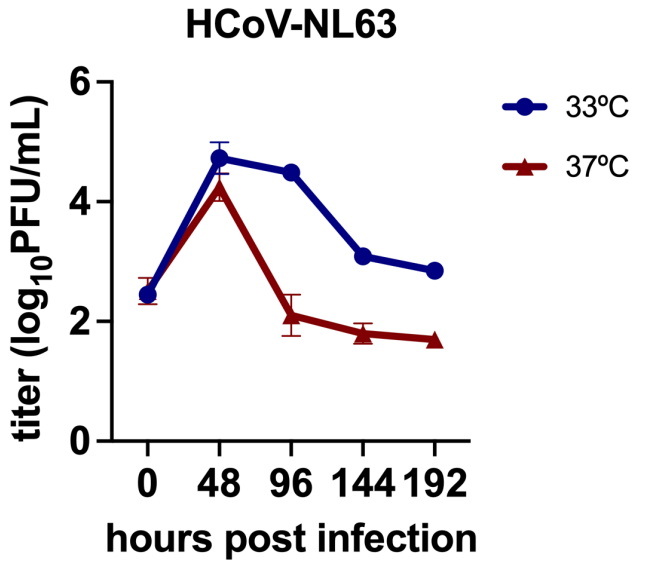


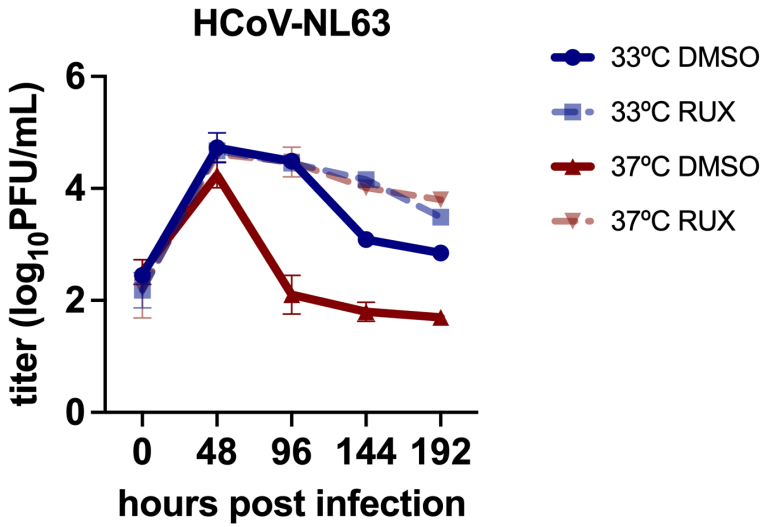


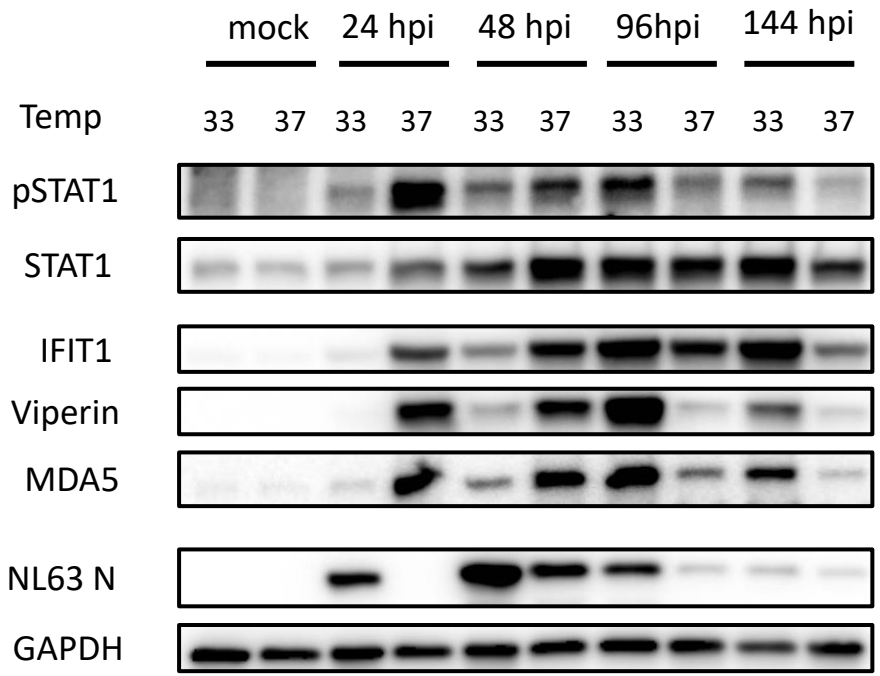
Inhibition of IFN signaling rescues replication at 37C

Otter et al., PNAS, 2024

IFN signaling response to NL63 is more robust at 37C



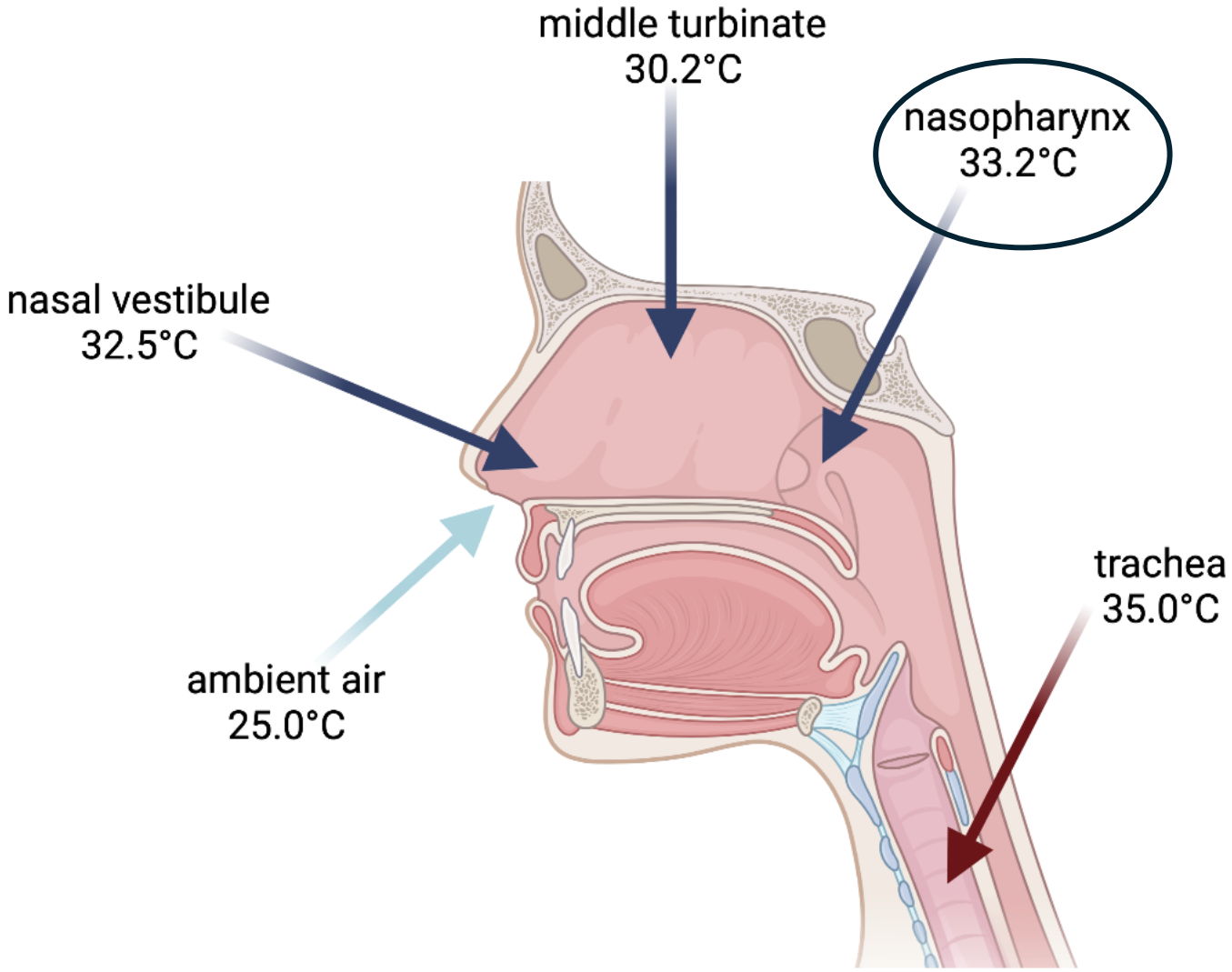


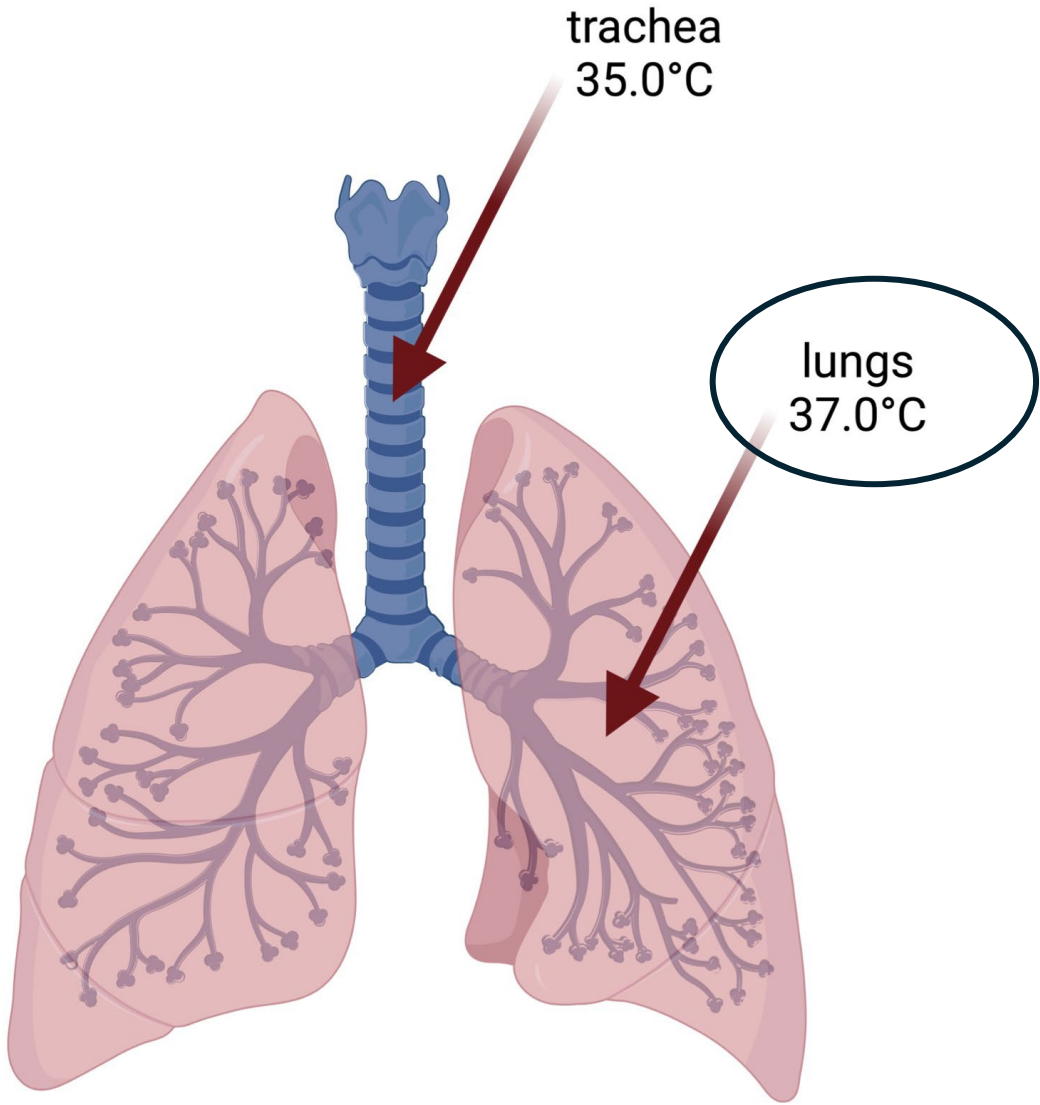


Inhibition of IFN signaling rescues replication at 37C

Otter et al., PNAS, 2024

Temperatures in the upper and lower respiratory tracts





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What happens at 39C, simulating fever ?

Decreased replication at 39C is largely but not completely due to increase IFN signaling at higher temperature

Evolution of SARS-Cov-2 variants- temperature optima

Omicron variants evolve to replicate most efficiently at the lower temperature of the upper respiratory tract

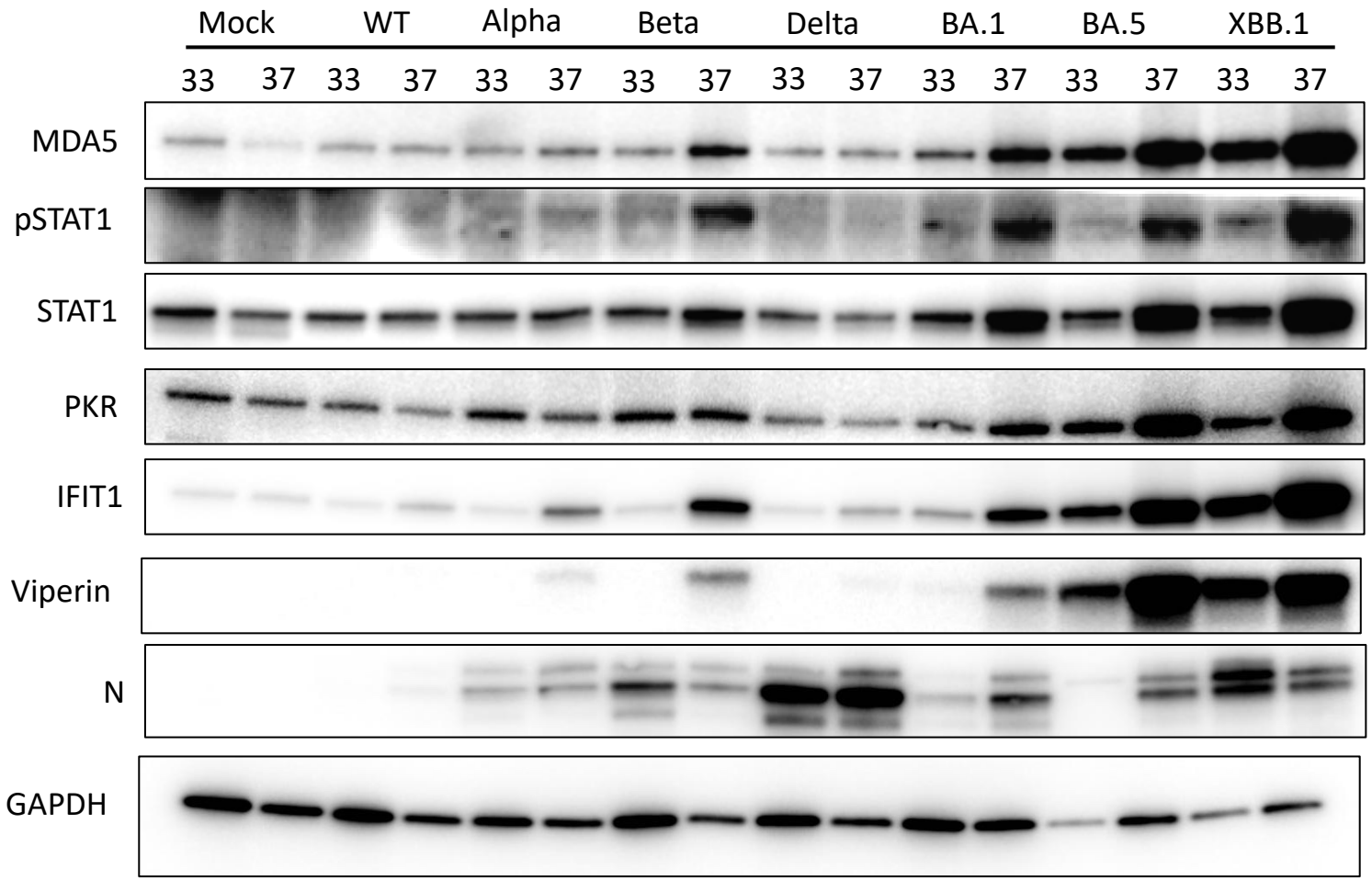
The Delta variants replicates more efficiently than other variants at the higher temperature of the lower respiratory tract

Omicron variants induce more ISGs than previous variants

Omicron variants are evolving to induce increased IFN signaling

Interferon signaling is more intense at 37C (temperature of lung) then at 33C (temperature of nasal compartment)

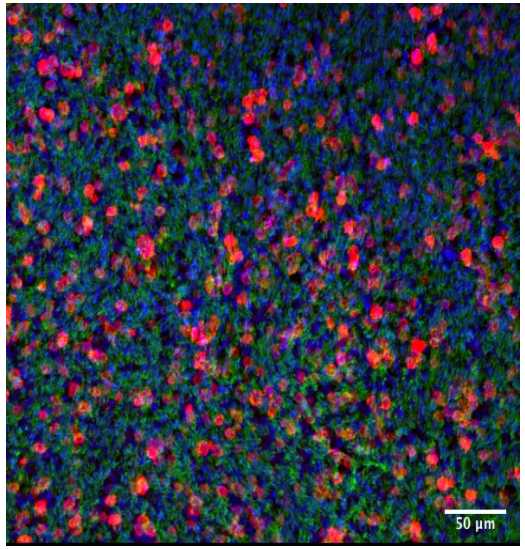
More intense Interferon signaling in the upper respiratory tract leads to clearance and lack of aspiration into the lung



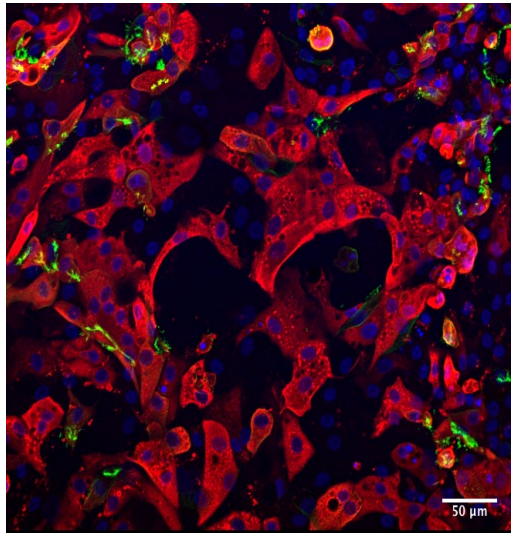
96hpi
MOI 1

Delta variant is more cytopathic than parental SARS-2 or omicron variant in primary nasal ALI cultures

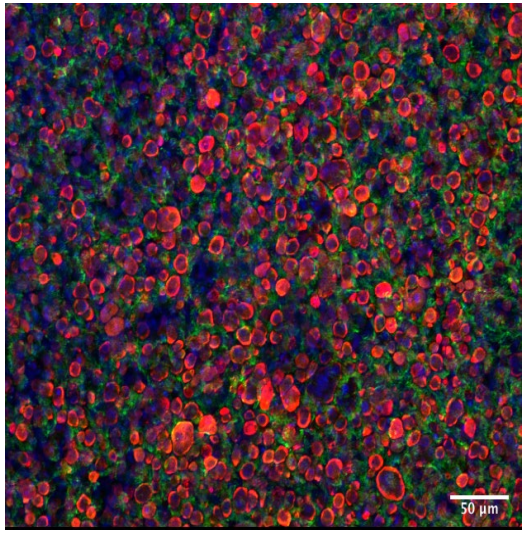
parental SARS-2



Delta



Omicron (BA1)



cilia
nucleocapsid
Nuclei

96 hpi, 37C

deciliation

moi=1pfu/cell, 3 donors

What we have learned ….

- In primary nasal ALI cultures, common cold coronaviruses, like HRV-16, prefer lower temperature and induce IFN signaling which leads to viral clearance

- Lethal MERS and SARS-2 induce little if any IFN signaling and persist in nasal ALI cultures; MERS-CoV and SARS-2 mutants like common cold CoVs induce IFN signaling and are cleared in the nasal ALIs; IFN signaling may convert a lethal coronavirus into a common cold like virus

- IFN signaling response is more robust at 37C which contributes to 33C temperature optima for common cold viruses and for SARS-2 in late infection; common cold viruses cannot replicate at 39C mimicking fever, at least in part due to extreme innate response. In contrast, MERS and SARS delta variant can replicate at 39C

- SARS variants have evolved to behave more like common cold viruses in replicating preferentially at 33C and inducing a robust IFN signaling response

Could IFN induction be used as a therapeutic approach?

Why is the innate immune response more robust at 37C?

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