Qazvin University of Medical Sciences

# Type I and Type III Interferons Restrict SARS-CoV-2 Infection of Human Airway Epithelial Cultures


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# Type I and Type III Interferons Restrict SARS-CoV-2 Infection of Human Airway Epithelial Cultures

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**ISI Rankings:**

| Subject | Rank | Quartile | Percentile |
|---|---|---|---|
| Virology (SCIE) | 9/36 | Q1 | N/A |

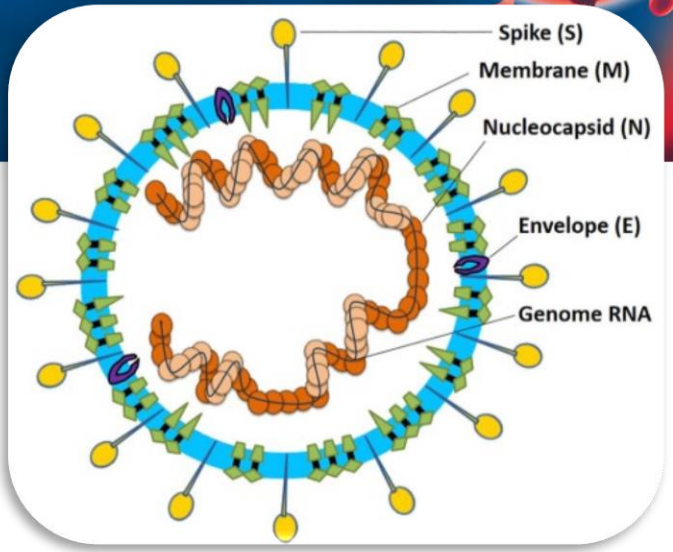
**Scopus Rankings:**

| Subject | Rank | Quartile | Percentile |
|---|---|---|---|
| Insect Science | 2/153 | Q1 | 99% |
| Microbiology | 21/150 | Q1 | 86% |
| Virology | 13/69 | Q1 | 81% |
| Immunology | 41/202 | Q1 | 79% |

# Abbreviations

| | |
|---|---|
| **SARS-CoV-2** | **severe acute respiratory syndrome coronavirus-2** |
| **IL-6** | interleukin 6 |
| **TNF-α** | tumor necrosis factor alpha |
| **pHAE** | primary human airway epithelial |
| **IFN** | interferon |
| **ISG** | interferon-stimulated gene |
| **p.i.** | postinfection |

# Introduction




- COVID-19 caused by SARS-CoV-2
- Emerged in Wuhan, China, in December 2019
- Has caused a pandemic of respiratory illness
- More than millions of cases and thousands of deaths were declared so far
- β-coronavirus genus
- Single strands of RNA (29.8 kb in length)

# Introduction

- Manifests as an upper and lower respiratory disease
- Infects airway and lung cells
- Causing fever, dry cough, and shortness of breath

# Introduction

Severe Infection

- dysregulated immune response
- high levels of proinflammatory cytokines (IL-6, TNF-α,…)
- lung tissue destruction
- low level of lymphocytes in the blood
- low blood oxygen levels
- respiratory failure
- even death

# Introduction

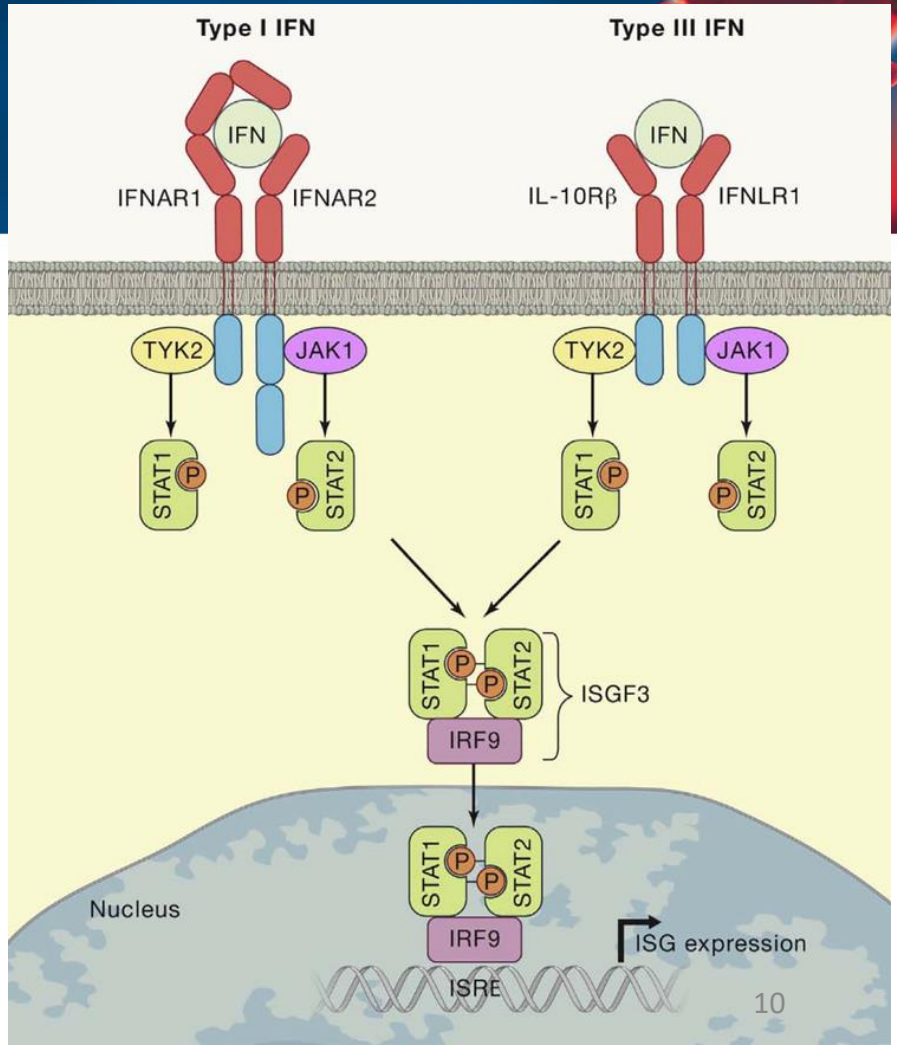
- To gain entry to target cells uses ACE-2 & cellular protease TMPRSS2
- Expressed in epithelial tissue (lung & gut), ↑ciliated cells (nasal cavity)
- pHAE cultures (bronchial region & nasal cavity) are ↑susceptible to SARS-CoV-2
- Replication occurs primarily in ciliated cells (ACE-2 localized expression )

# Introduction

- Type I IFNs: first line of defense against viruses
- Critical for blocking early virus replication, spread & tropism as well as promoting the adaptive immune response
- Induces a systemic response that impacts nearly every cell in the host
- Binds IFNAR1 & IFNAR2 (expressed ubiquitously)

# Introduction

- type III IFNs: restricted to anatomic barriers & selected immune cells
- binds IFNLR1 & IL-10-Rβ (expressed preferentially on epithelial cells)
- induces lower levels of ISG expression
- produces a less inflammatory & localized response



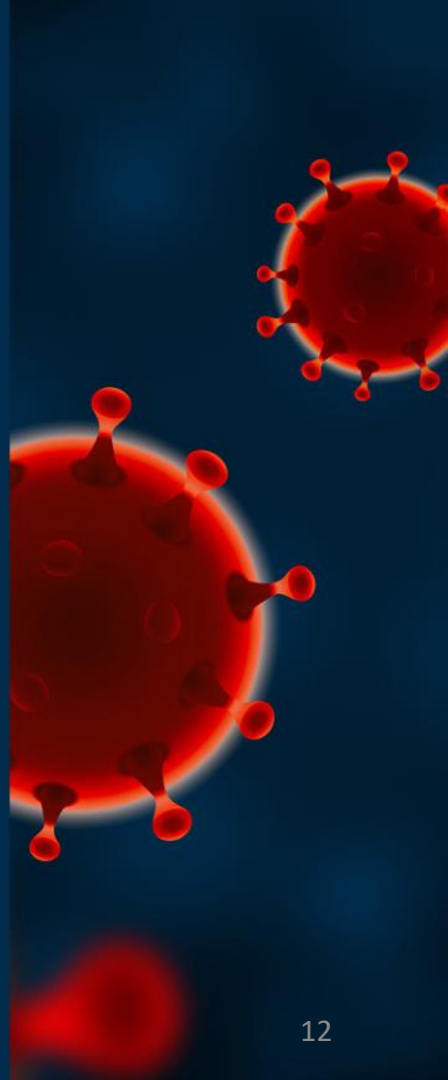
# Introduction

1. SARS-CoV2 is released directionally from the apical but not basolateral surface of (pHAE) cells

2. Transcriptional profiling of infected pHAE cells suggests NF-κB (pro-inflammatory) & ATF4 (cellular stress pathway) as the dominating transcription factors in SARS-CoV-2 infection

3. Identify type I and III IFNs as potential therapeutics to restrict infection in the airways of COVID-19 patients

# Materials & Methods



# Viruses and cells

1. SARS-CoV-2 (2019-nCoV/WA1) → isolate → first reported case
2. VeroE6 → cultured → complete DMEM
3. GFP-tagged SARS-CoV-2 (icSARS-CoV-2-mNG) → propagated
4. Influenza virus (H1N1) → MDCK → cultured → complete DMEM
5. Viral titers → determined → **plaque assay**

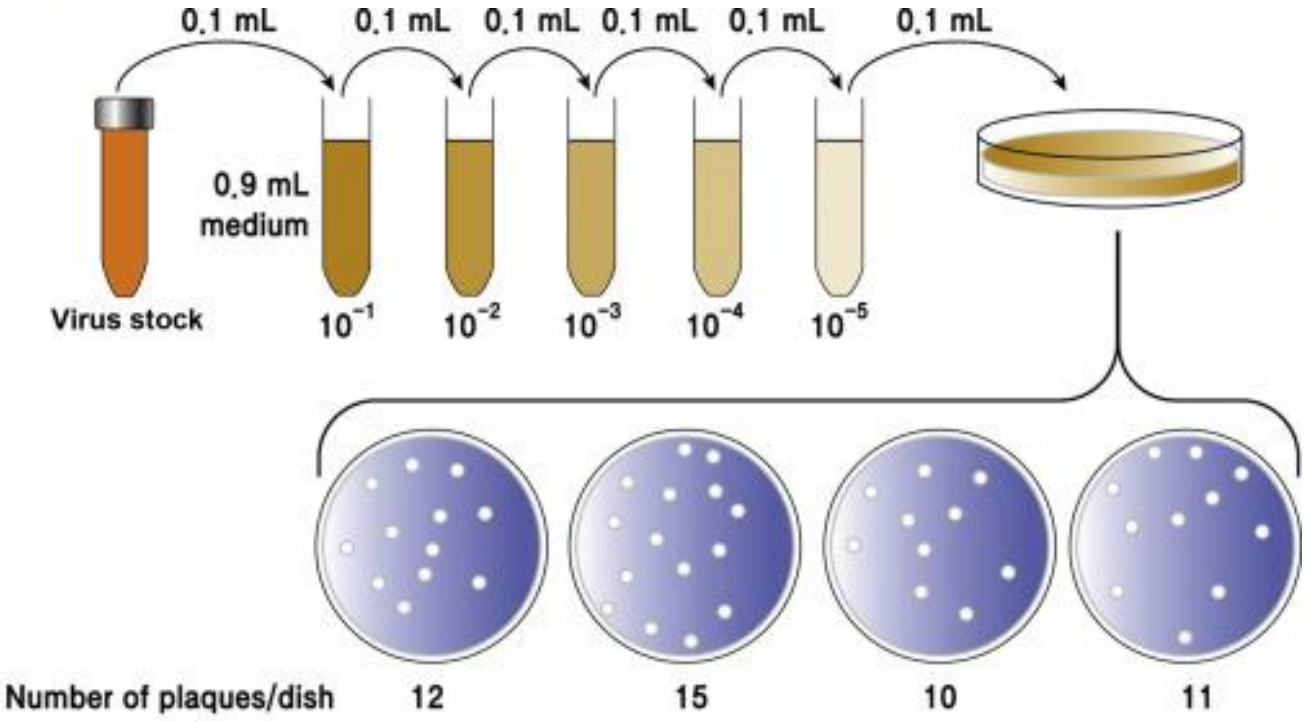
# Quantification of infectious virus

**plaque assay**

1. 10-fold dilutions of viral supernatant → serum-free DMEM → overlaid → VeroE6 cell monolayers
2. After adsorption → Oxoid agarose+ DMEM+ FBS+ sodium bicarbonate → overlaid → incubated (72h)
3. Plaques → crystal violet staining

# Quantification of infectious virus

plaque assay



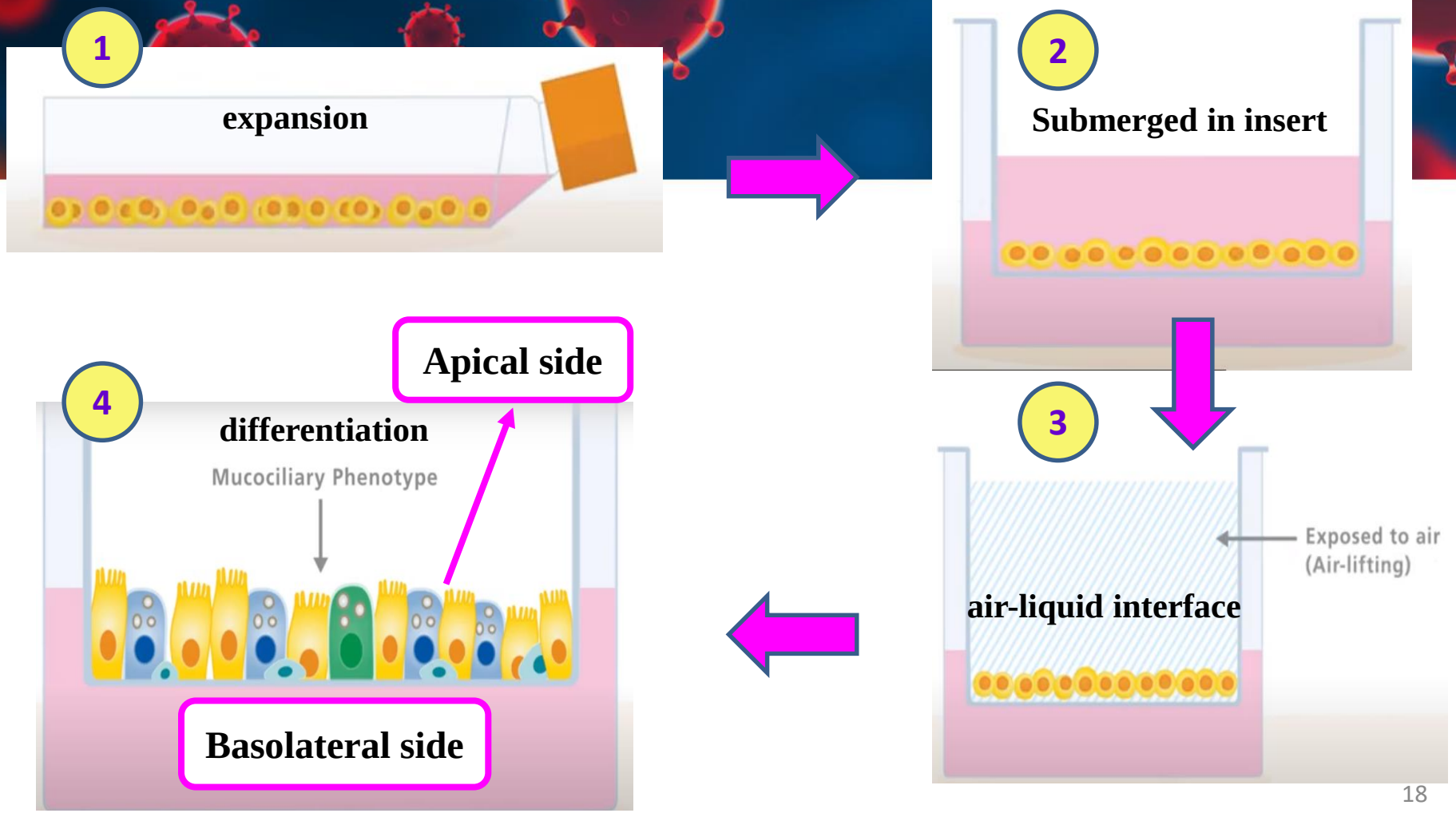
# Quantification of infectious virus

**focus-forming assay (FFA)**

1. 10-fold dilutions of viral supernatant → VeroE6 → + methylcellulose → incubated (24h)
1. Methylcellulose removed → Cells fixation → PFA
2. Cells permeabilization → BSA+PBS
3. Cells incubation → anti-SARS-CoV-2 spike protein primary antibody +biotin (2h) → avidin-horseradish peroxidase (HRP)-conjugated secondary antibody (1h)
4. Foci → visualized → True Blue HRP substrate
5. Imaged on → ELISPOT reader

# Generation of pHAE cultures

1. Bronchial or tracheal lung specimens (pHAE cells) → expanded F medium+ ROCK inhibitor
2. Seeded → Transwell permeable support inserts + cultured until confluent
3. transferred → air-liquid interface
4. Cultures → differentiated + maintained → DMEM/Ham's F-12+Ultroser G
5. TEER (>1,000) → ready for use


1
expansion
2
Submerged in insert
3
Exposed to air
(Air-lifting)
air-liquid interface
4
differentiation
Mucociliary Phenotype
Apical side
Basolateral side

# Generation of pHAE cultures

**result**

- create a polarized, pseudostratified epithelial layer
- It has unique features of the human respiratory tract

(mucus production and coordinated cilium movement)

# SARS-CoV-2 infection of pHAE cultures

1. Apical side of the pHAE culture → PBS
2. Virus → diluted (MOI of 0.1 & 0.25) → PBS
3. Adsorb → for 1 h at 37°C
4. Apical side → PBS → remove excess virus
5. Collect viral supernatant → PBS+ apical side → incubated → plaque assay

1. pHAE cultures+ type I or type III IFN (human IFN-β/IFN-λ1)
2. Cytokine levels → measured → cytokine and chemokine kit

# RNA sequencing and bioinformatics

1. pHAE cultures → infected (MOI 0.5 for 48h)
2. RNA harvested → mock-infected & Infected pHAE cultures (n = 3)
3. Total RNA extraction → Zymo Quick-RNA miniprep kit
4. Libraries generation → Clontech SMART-Seq v4 kit
5. Adding barcoding & sequencing primers → NexteraXT library prep kit
6. Sequencing → Illumina NovaSeq 6000 in 100-base single-read reactions
7. Demultiplexing → Illumina bcl2fastq v2.17.1.14

# RNA sequencing and bioinformatics

8. Mapping → hg38 human reference genome & FDAARGOS_983 strain of the 2019-nCoV/USA-WA1/2020 SARS-CoV2 isolate → STAR v2.7.3a
9. Reads were normalized
10. Differentially expressed genes were analyzed → DESeq2
11. Gene set enrichment analysis → MSigDB database
12. Pathway analysis → Cytoscape software

# Quantitative reverse transcription-PCR (qRT-PCR)

1. RNA extracted → pHAE cultures
2. RNA → Purified + transcribed → cDNA
3. RNA levels → quantified → master mix+ TaqMan gene expression Primer/Probe sets
4. qPCR performed → 384-well plates + QuantStudio5 qPCR system
5. quantify SARS-CoV-2 & influenza RNA & GAPDH/ IFIT2/ IFIT3/ DDX58/ IFIH1/ OAS1/ IRF1/ IRF7/MX1

# Confocal imaging

1. pHAE cultures → fixed → PFA +DPBS
2. PFA → removed → glycine
3. Cultures → washed → DPBS
4. Cells → permeabilized & blocked → Triton X-100 & PBS-BGT
5. Phalloidin -AF647 → diluted → PBS-BGT → incubated on cells
6. Samples → PBS → counterstained Hoechst 33342
7. PBS → mounted on a glass slide
8. Cultures → imaged → confocal microscope
9. Analyzed → ImageJ and Imaris software

# Statistical analysis

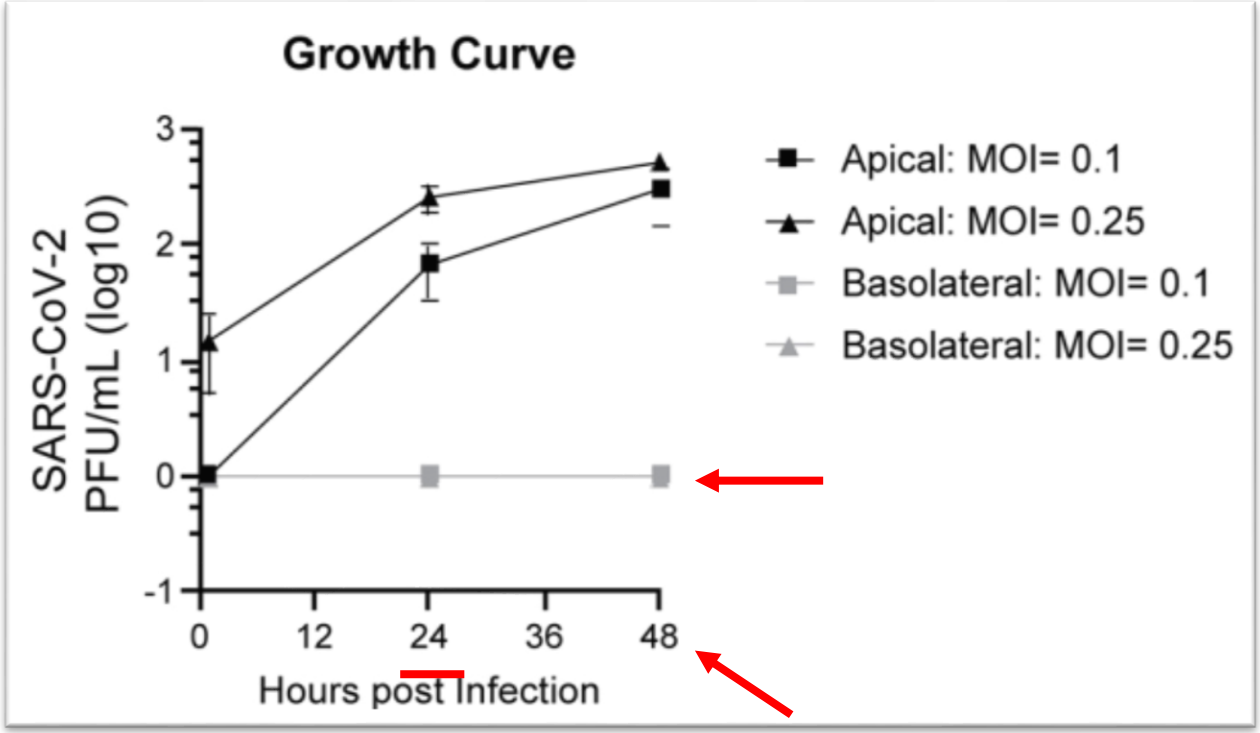
1. Statistical analyses

   GraphPad Prism 8, ggplot2 R package, and GSEA software

2. Statistical significance

   Student’s t test &  ANOVA

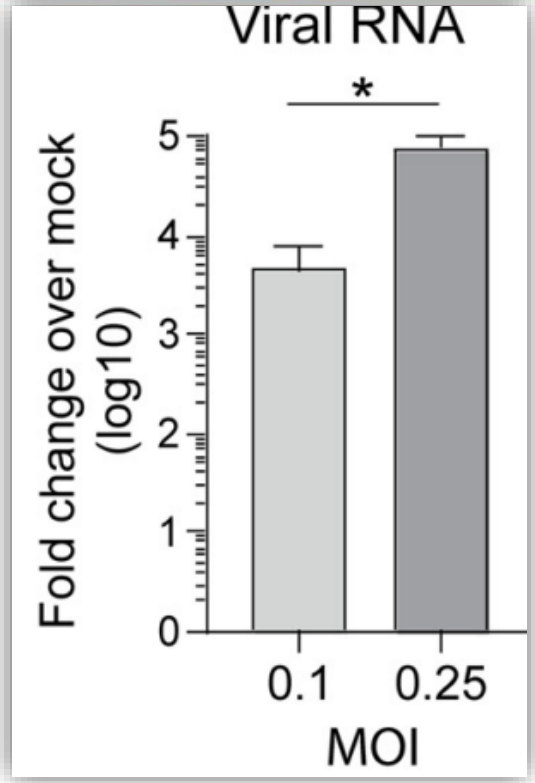
# Human bronchial airway epithelial cells are permissive to SARS-CoV-2 infection




**plaque assay**

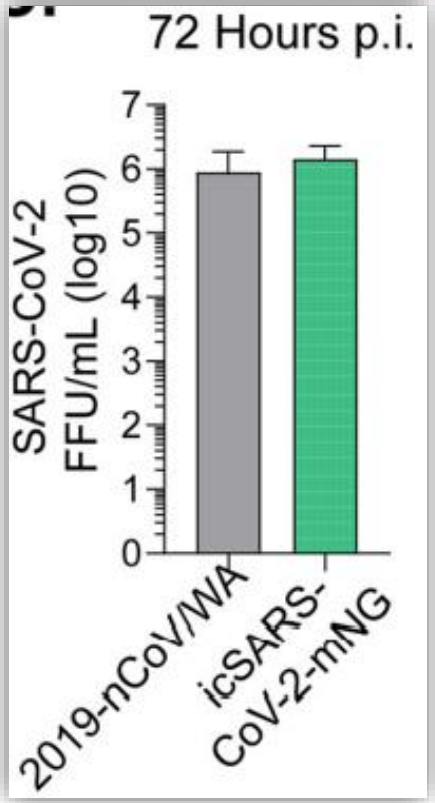
- SARS-CoV-2 beginning 24 h p.i
- through 48 h p.i
- directional release of the virus (apical side)

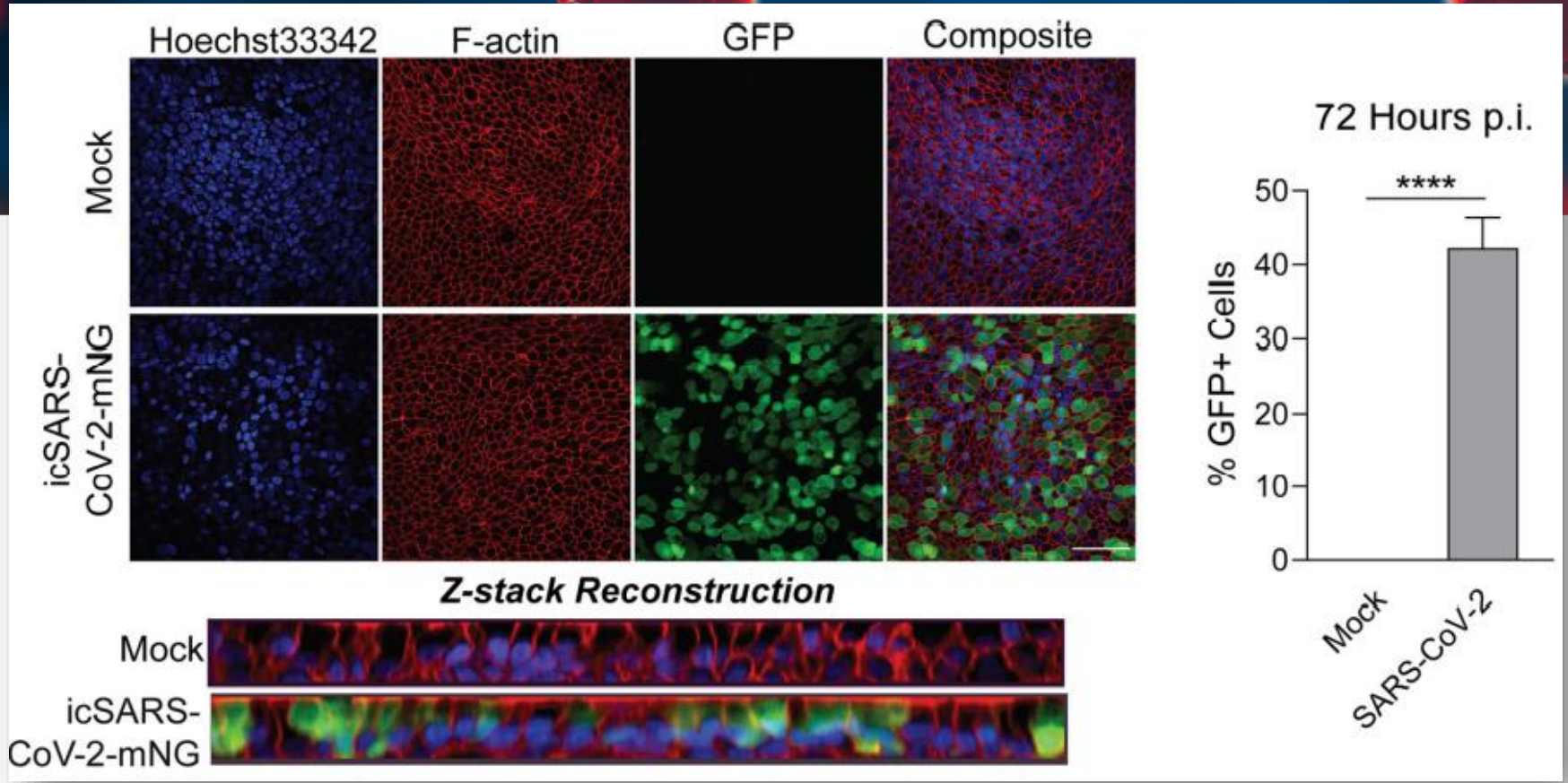
# Human bronchial airway epithelial cells are permissive to SARS-CoV-2 infection




**qRT-PCR:** increase in viral RNA at 48 h p.i.

**FFA:** viral burden at 72 h p.i.

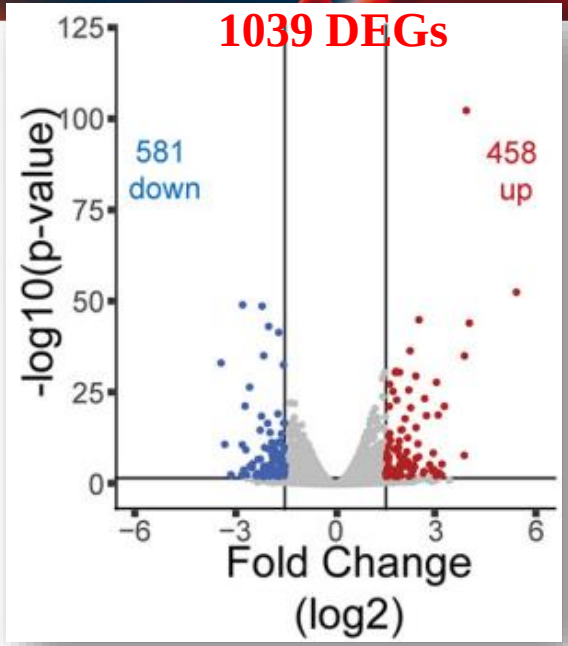


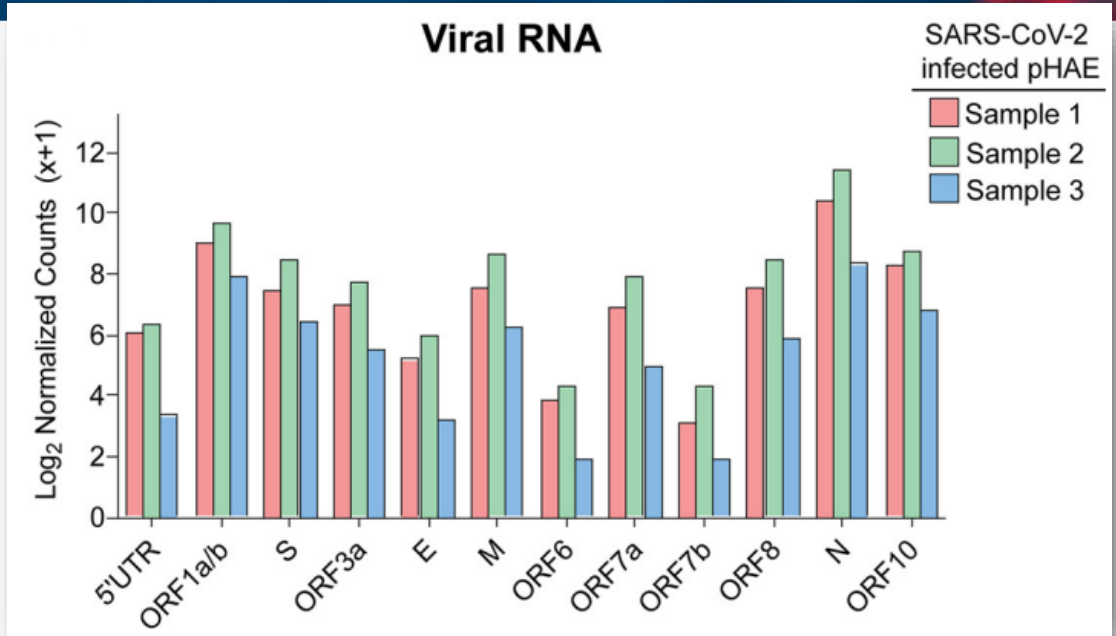



**confocal microscope**: 40% GFP+ cells

z-stack: GFP expression localized to the apical side

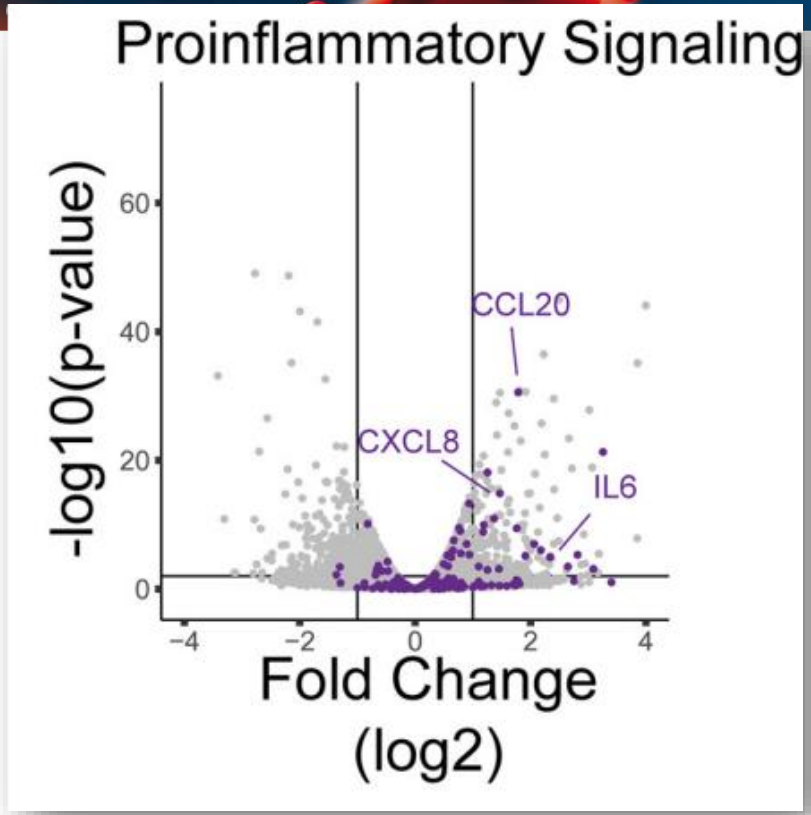
# SARS-CoV-2 infection prompts a proinflammatory response in pHAE cultures

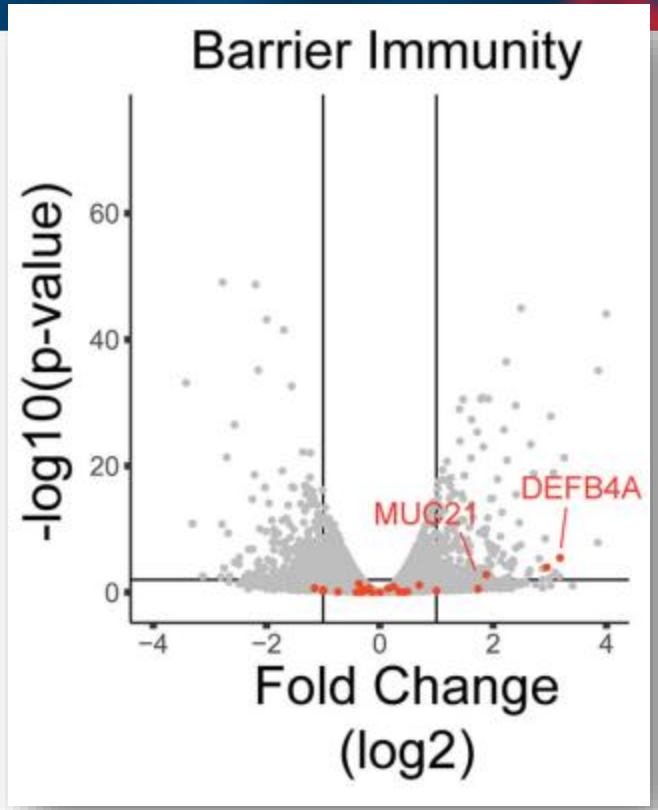


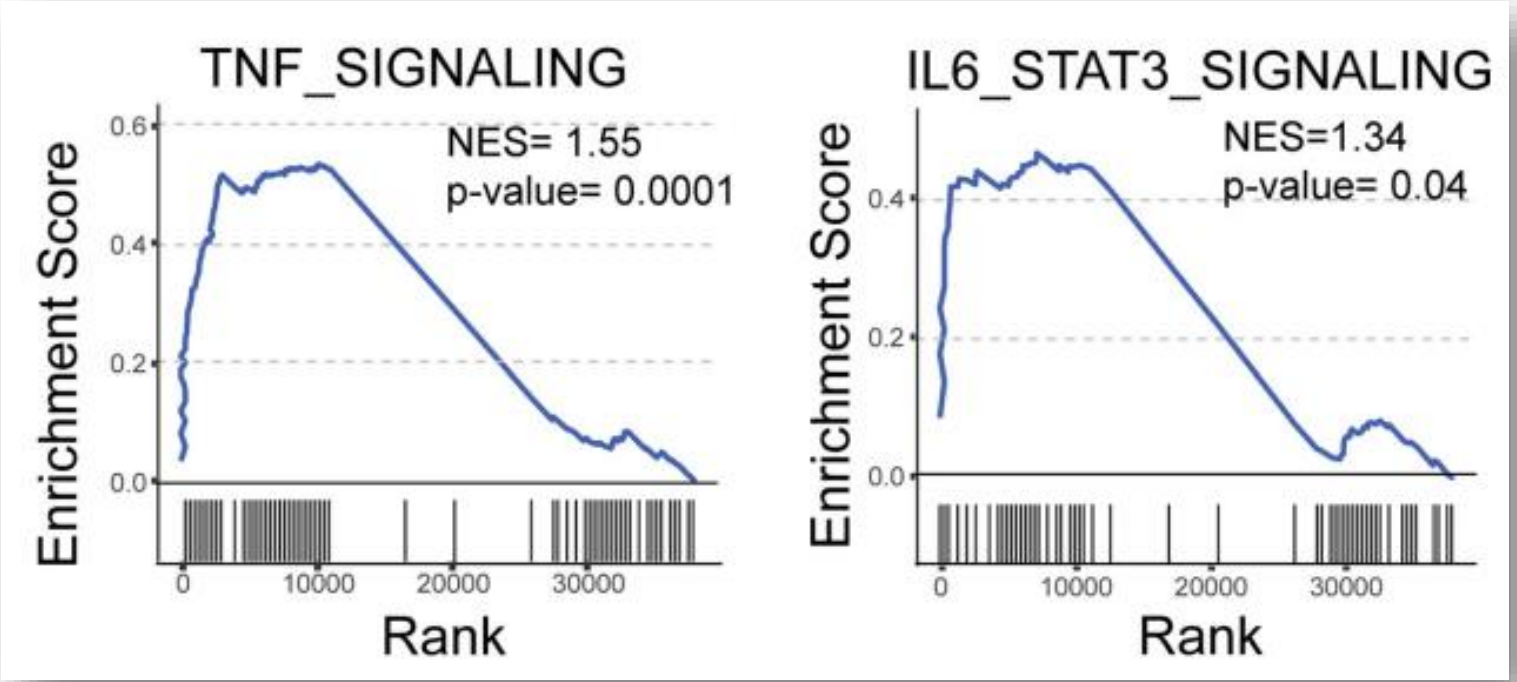

pHAE cultures were infected apically with SARS-CoV-2 (MOI = 0.25) for 48 h
Bulk RNA-Seq analysis of mock & SARS-CoV-2-infected (n=3) samples

# SARS-CoV-2 infection prompts a proinflammatory response in pHAE cultures



# SARS-CoV-2 infection prompts a proinflammatory response in pHAE cultures

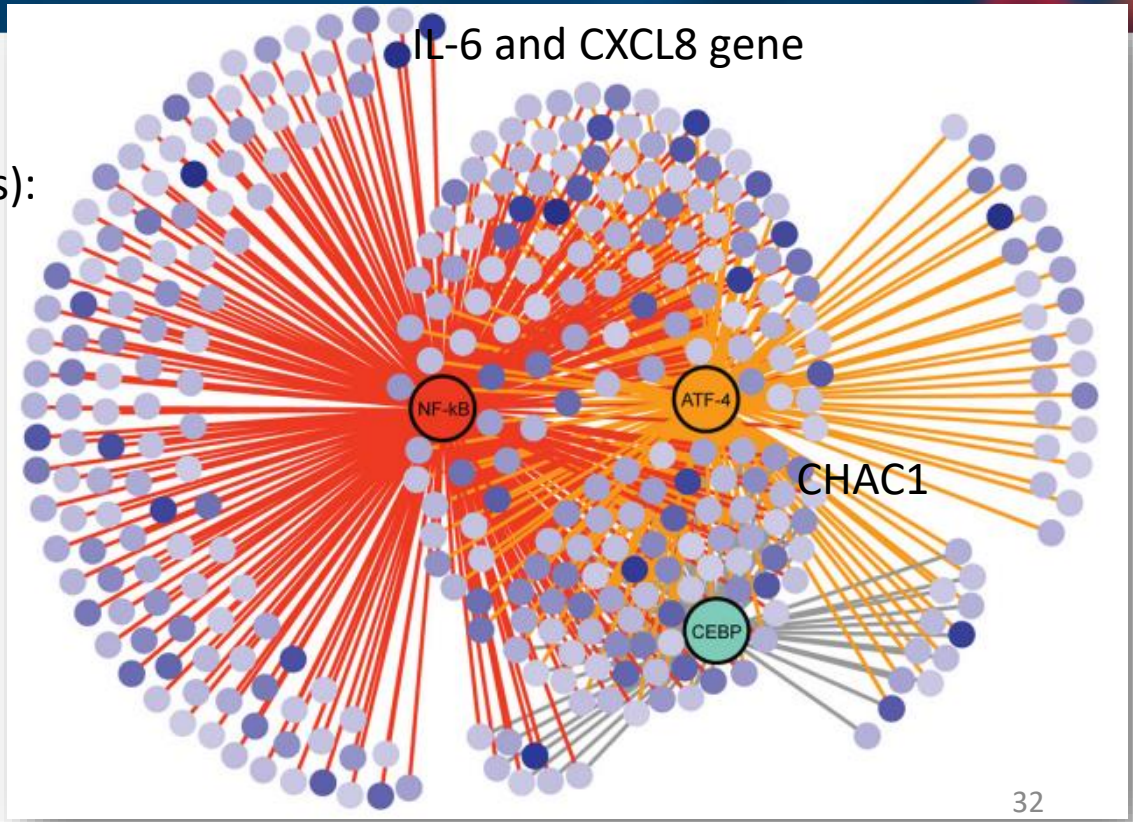


# SARS-CoV-2 infection prompts a proinflammatory response in pHAE cultures

To determine the transcriptional regulatory network(regulatory nodes): performed cis-regulatory sequence analysis using iRegulon

identified NF-B and ATF-4 as key drivers of this proinflammatory cytokine response. transcriptional regulators following SARS-CoV-2 infection

NF-B regulates a substantial portion of the DEGs



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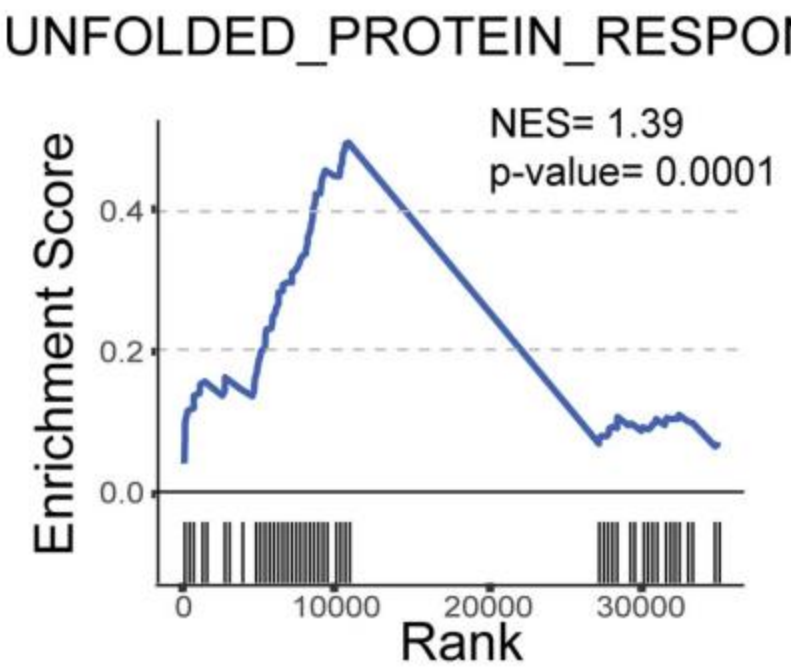
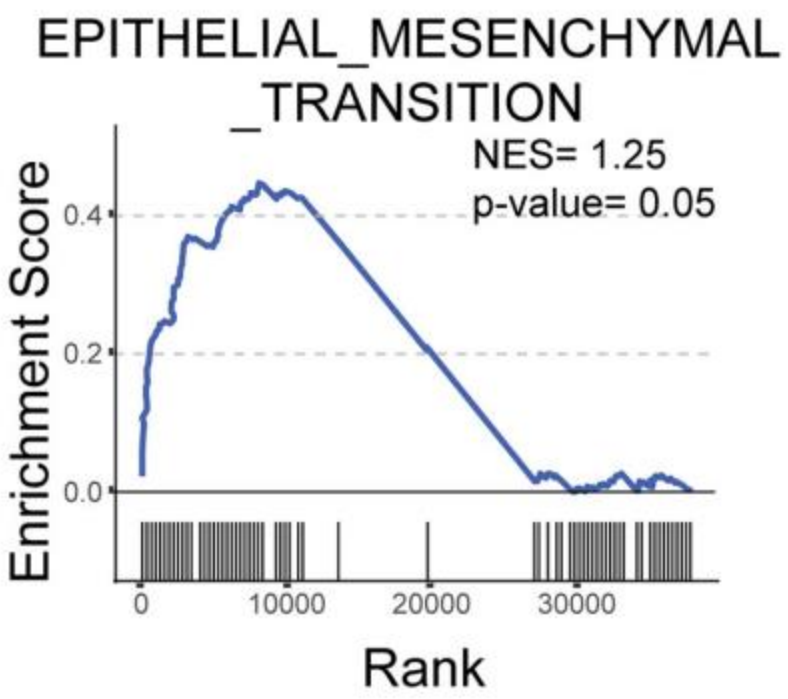
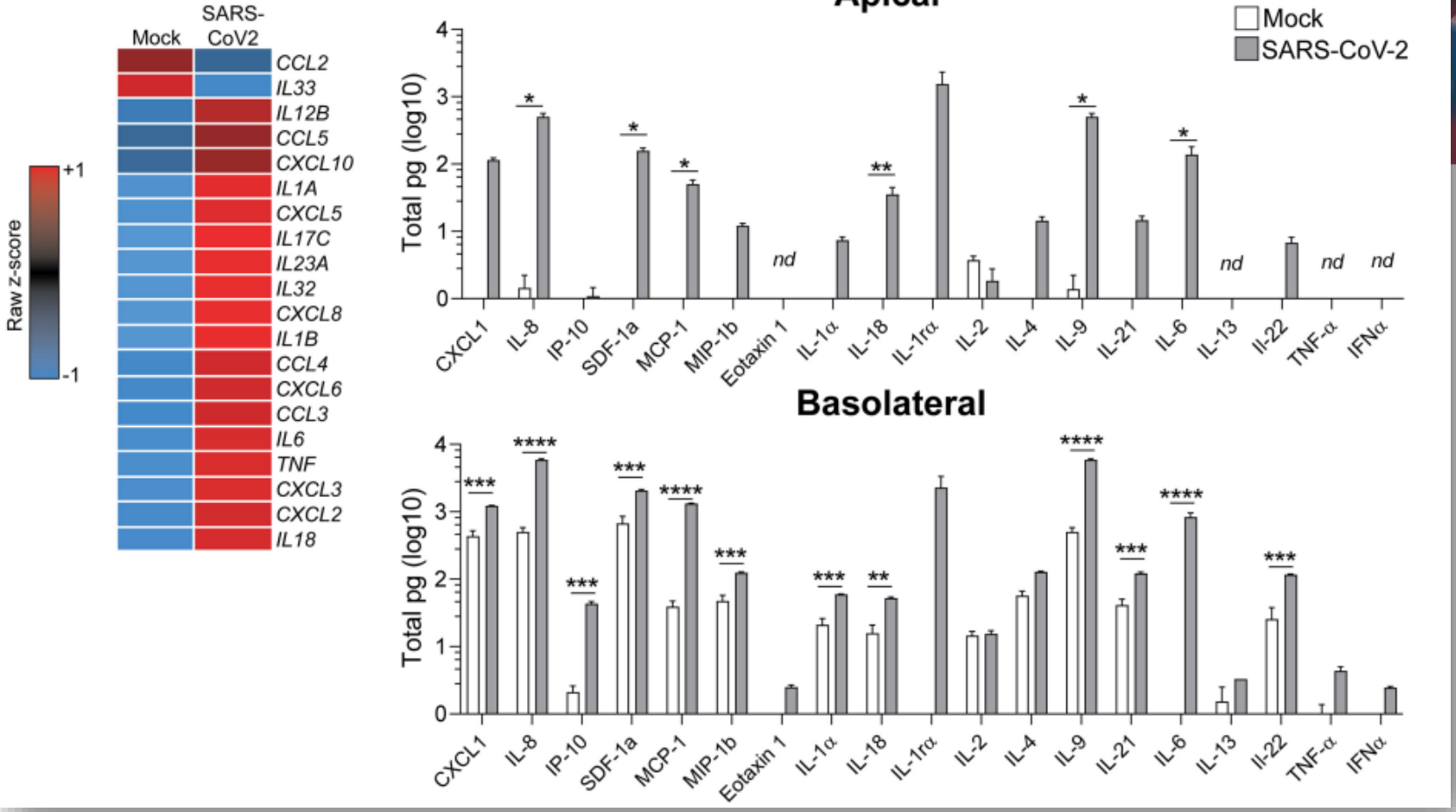
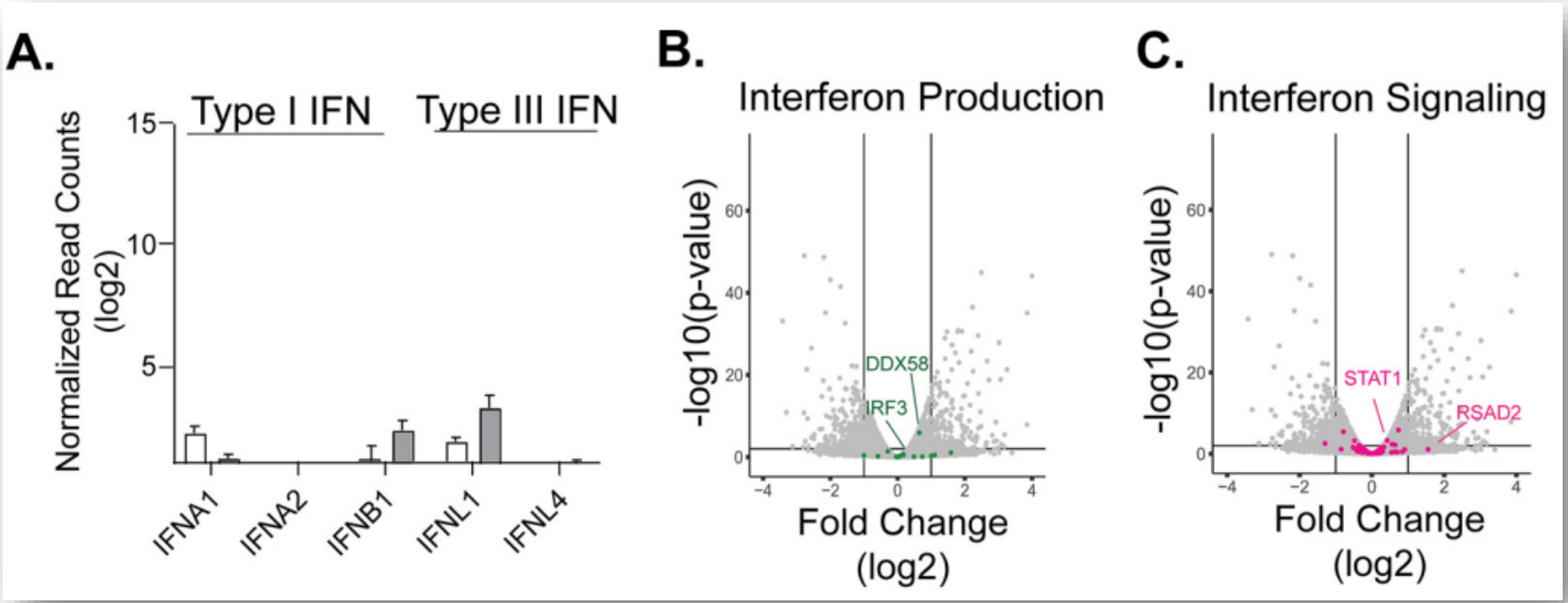



Mock
SARS-CoV2
CCL2
IL33
IL12B
CCL5
CXCL10
IL1A
CXCL5
IL17C
IL23A
IL32
CXCL8
IL1B
CCL4
CXCL6
CCL3
IL6
TNF
CXCL3
CXCL2
IL18
Raw z-score
+1
-1
Apical
Mock
SARS-CoV-2
Total pg (log10)
nd
CXCL1
IL-8
IP-10
SDF-1a
MCP-1
MIP-1b
Eotaxin 1
IL-1α
IL-18
IL-1rα
IL-2
IL-4
IL-9
IL-21
IL-6
IL-13
Il-22
TNF-α
IFNα
Basolateral

# SARS-CoV-2 does not induce IFN production or signaling in pHAE cultures



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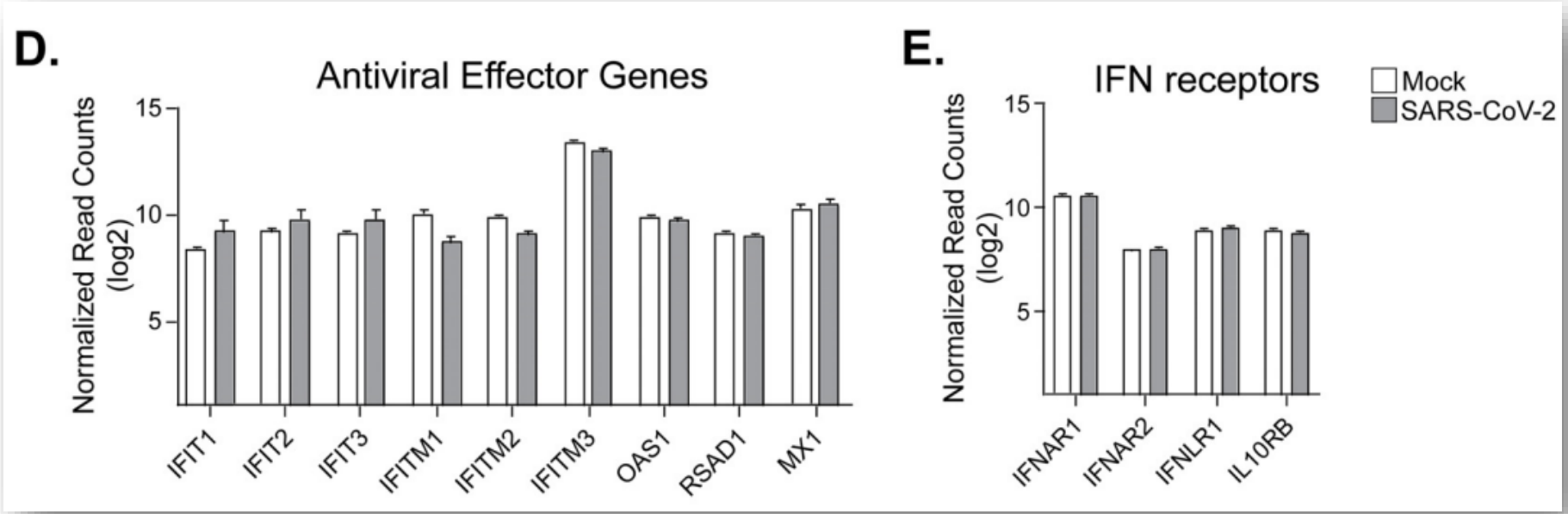
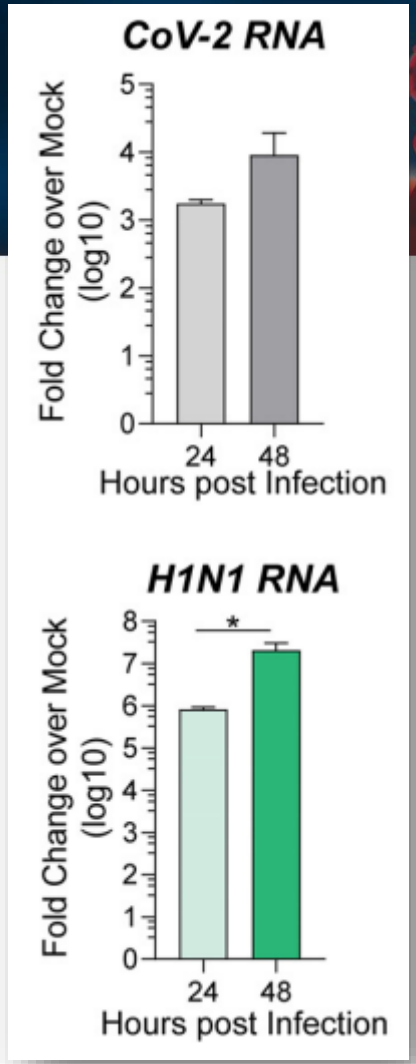
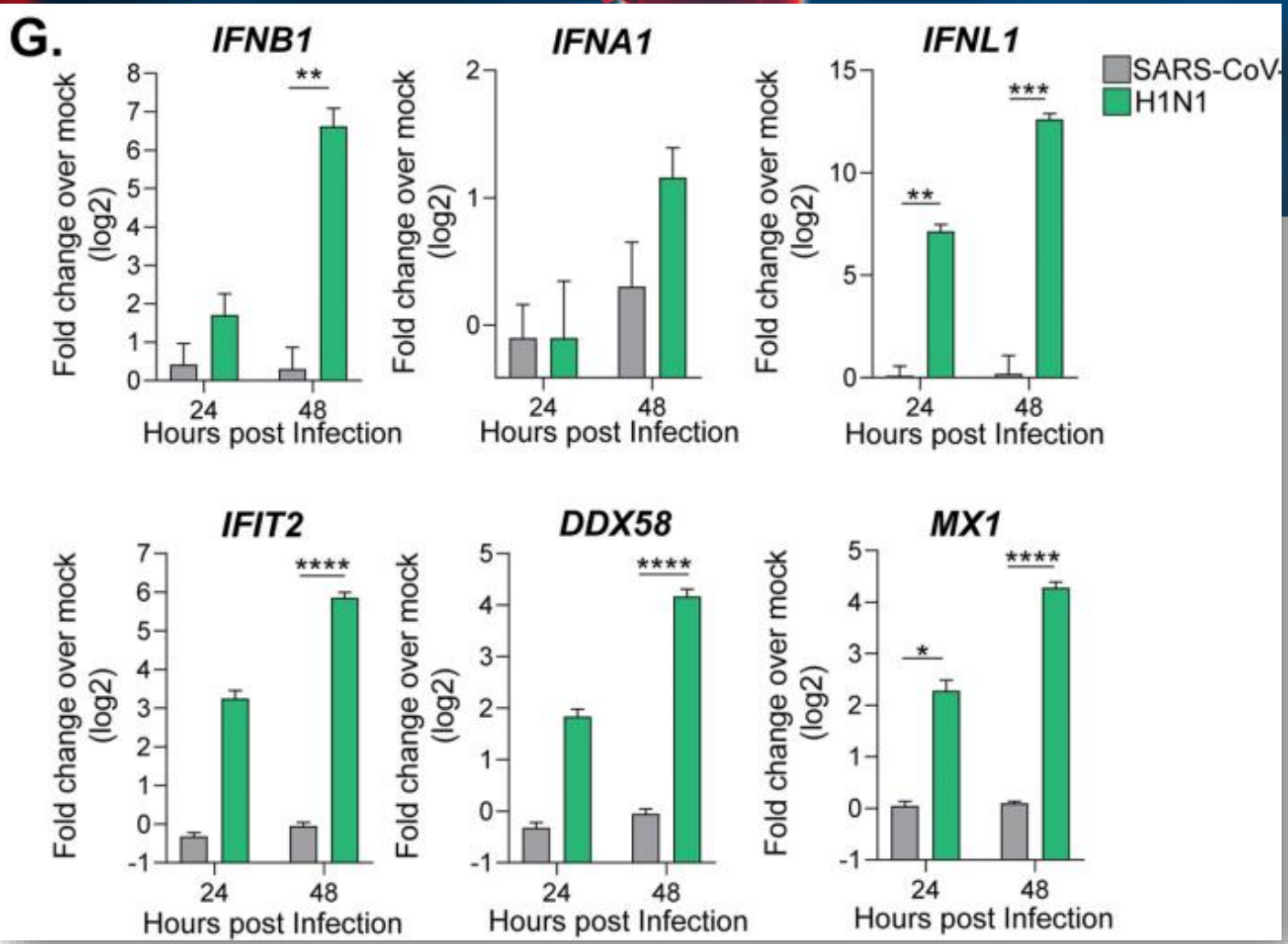
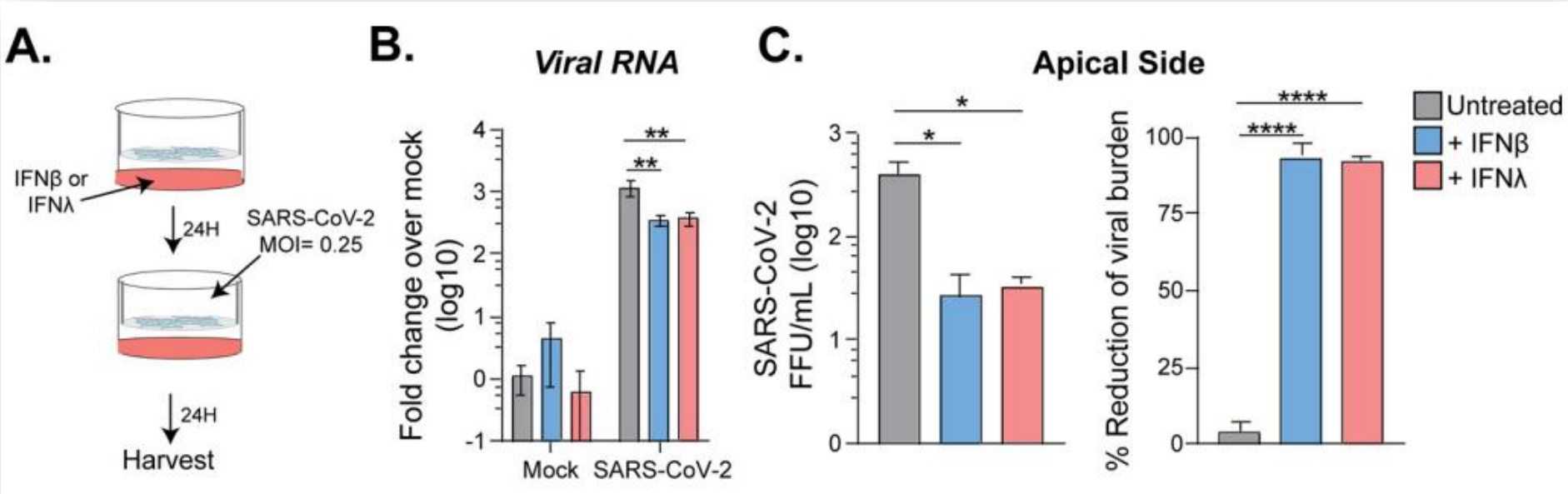
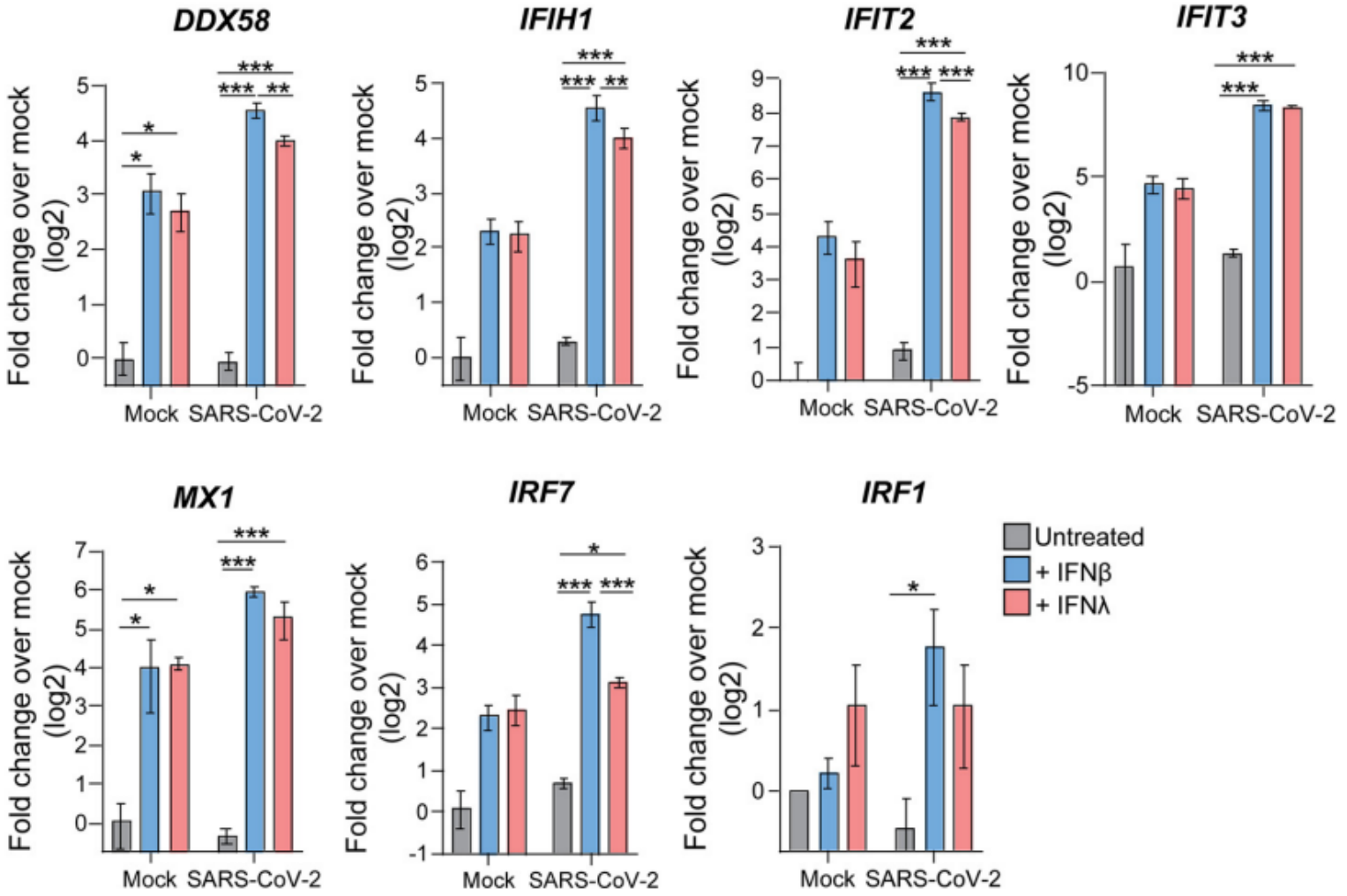



CoV-2 RNA
Fold Change over Mock (log10)
0
1
2
3
4
5
24
48
Hours post Infection
H1N1 RNA
Fold Change over Mock (log10)
*
0
1
2
3
4
5
6
7
8
24
48
Hours post Infection

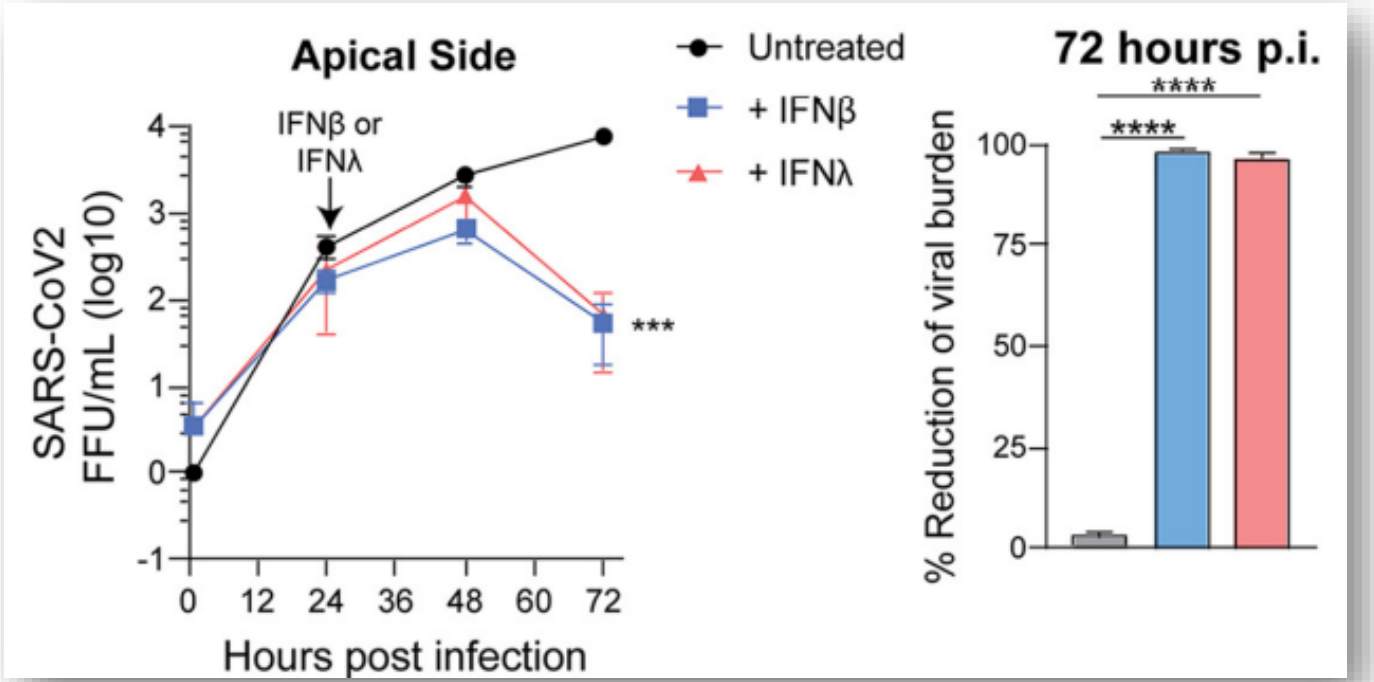

G.
IFNB1
IFNA1
IFNL1
SARS-CoV-
H1N1
Fold change over mock (log2)
Hours post Infection
24
48
**
**
***
IFIT2
DDX58
MX1
****
****
*

# Pretreatment with type I and type III IFN restricts SARS-CoV-2 replication




DDX58
IFIH1
IFIT2
IFIT3
MX1
IRF7
IRF1
Fold change over mock (log2)
Mock
SARS-CoV-2
Untreated
+ IFNβ
+ IFNλ

# Treatment of SARS-CoV-2-infected pHAE cultures with type I and III IFN reduces viral burden



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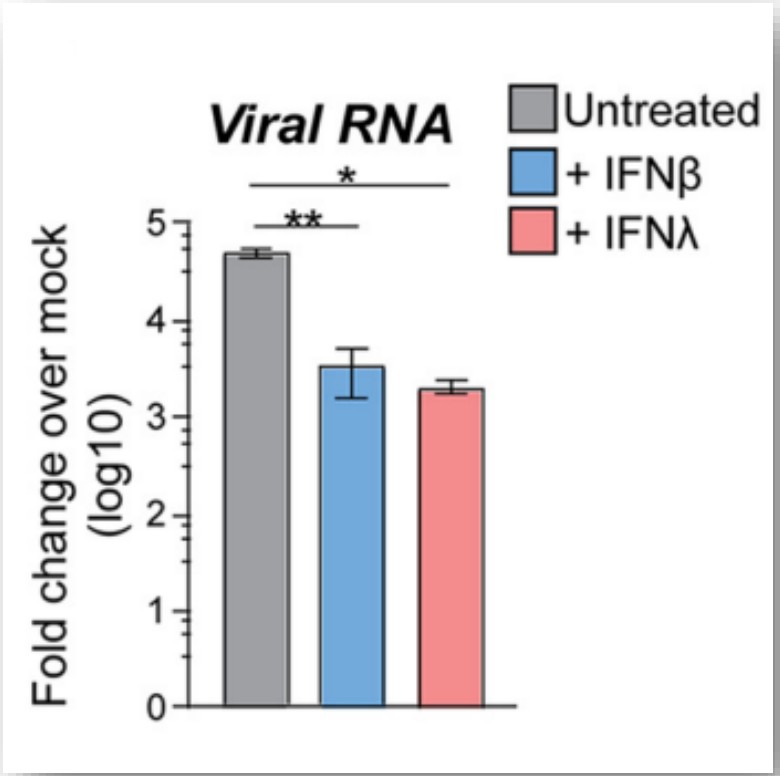
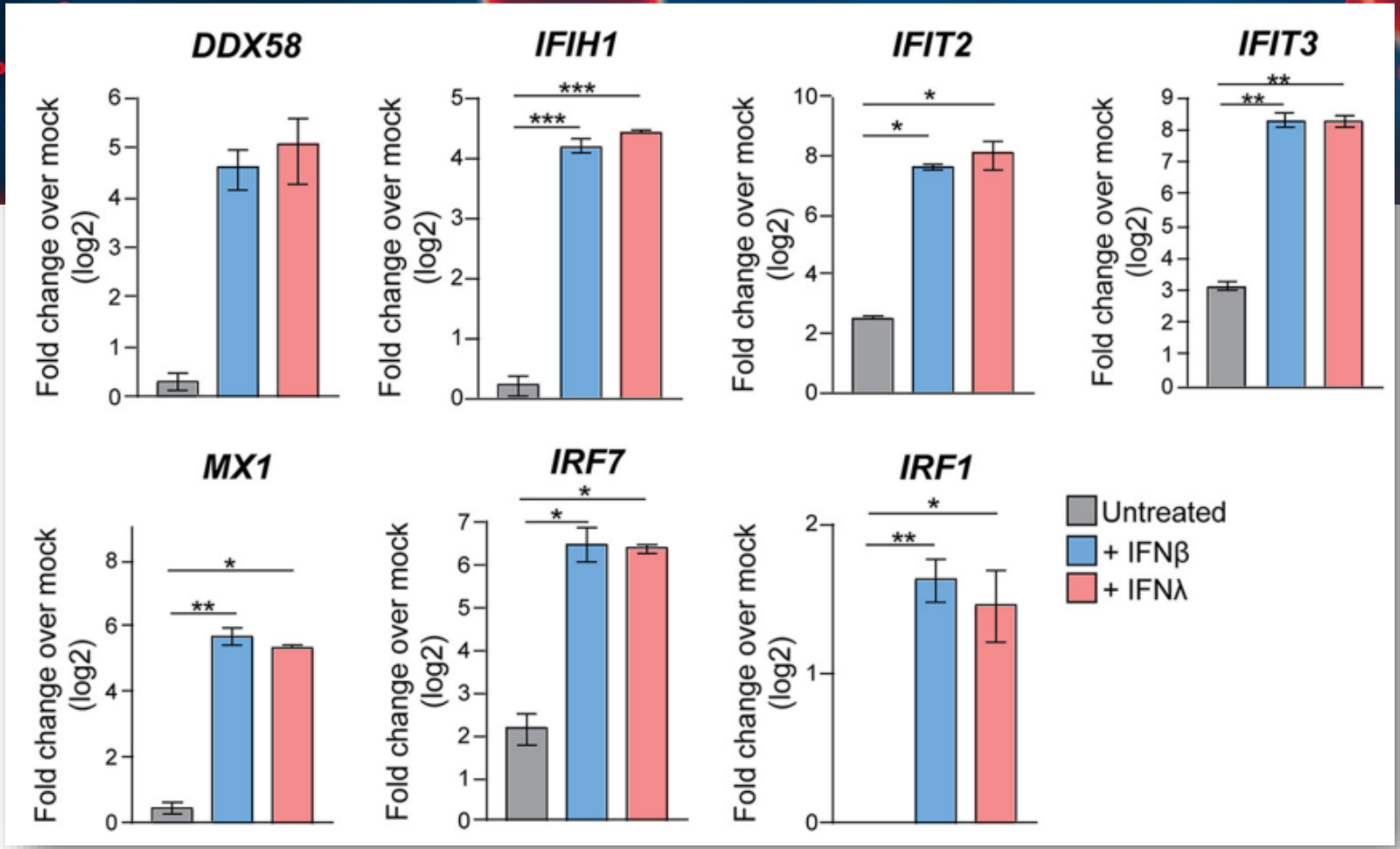
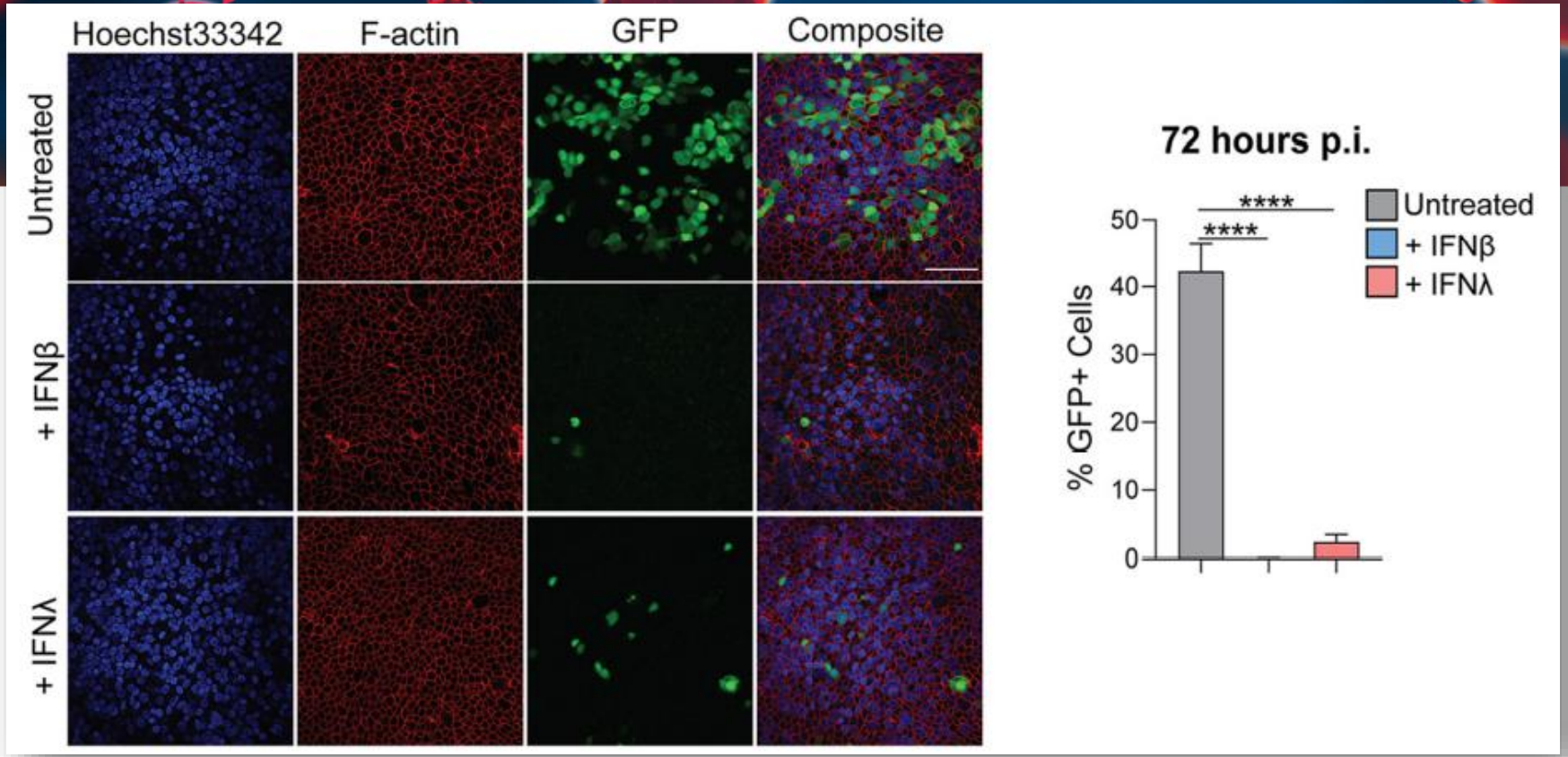



DDX58
IFIH1
IFIT2
IFIT3
MX1
IRF7
IRF1
Fold change over mock (log2)
Untreated
+ IFNβ
+ IFNλ


Hoechst33342
F-actin
GFP
Composite
Untreated
+ IFNβ
+ IFNλ
72 hours p.i.
% GFP+ Cells
50
40
30
20
10
0
****
****
Untreated
+ IFNβ
+ IFNλ

# Discussion

- ✓ pHAE cultures → permissive to SARS-CoV-2 infection
- ✓ Virus → unilaterally released from the apical surface
- ✓ Transcriptional profiling: SARS-CoV-2- infected pHAE cultures trigger a proinflammatory cytokines (by ATF-4 & NF-κB)
- ✓ Promote localized edema, fever & recruitment of immune cells into the respiratory tract
- ✓ Dysregulated immune response + ↑ ER stress may promote the formation of fibrotic epithelial tissue during SARS-CoV-2 infection

# Discussion

- ✓ Enrichment of ER stress pathways: SARS-CoV-2 infection disrupted normal cellular functions of pHAE cultures
- ✓ pHAE cultures did not produce type I or III IFN in response to SARS-CoV-2 infection (capable of producing type I and III IFNs)
- ✓ Pretreatment & posttreatment with exogenous IFNs: significantly ↓ viral burden in pHAE cultures & ↑ ISGs
- ✓ Airway epithelial cells → highly polarized, often have differential receptors, such as ACE-2, on the apical side

# Discussion

- ✓ A recent clinical study → type I IFN may be an effective COVID-19 treatment when applied apically (aerosolized)

- ➢ pHAE cultures mount a misdirected innate immune response to SARS-CoV-2 infection
- ➢ But the early administration of type I or III IFN could potentially decrease virus replication and disease