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Supplemental Information

MDA5 Governs the Innate Immune Response to SARS-CoV-2 in Lung Epithelial Cells

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Fig S1

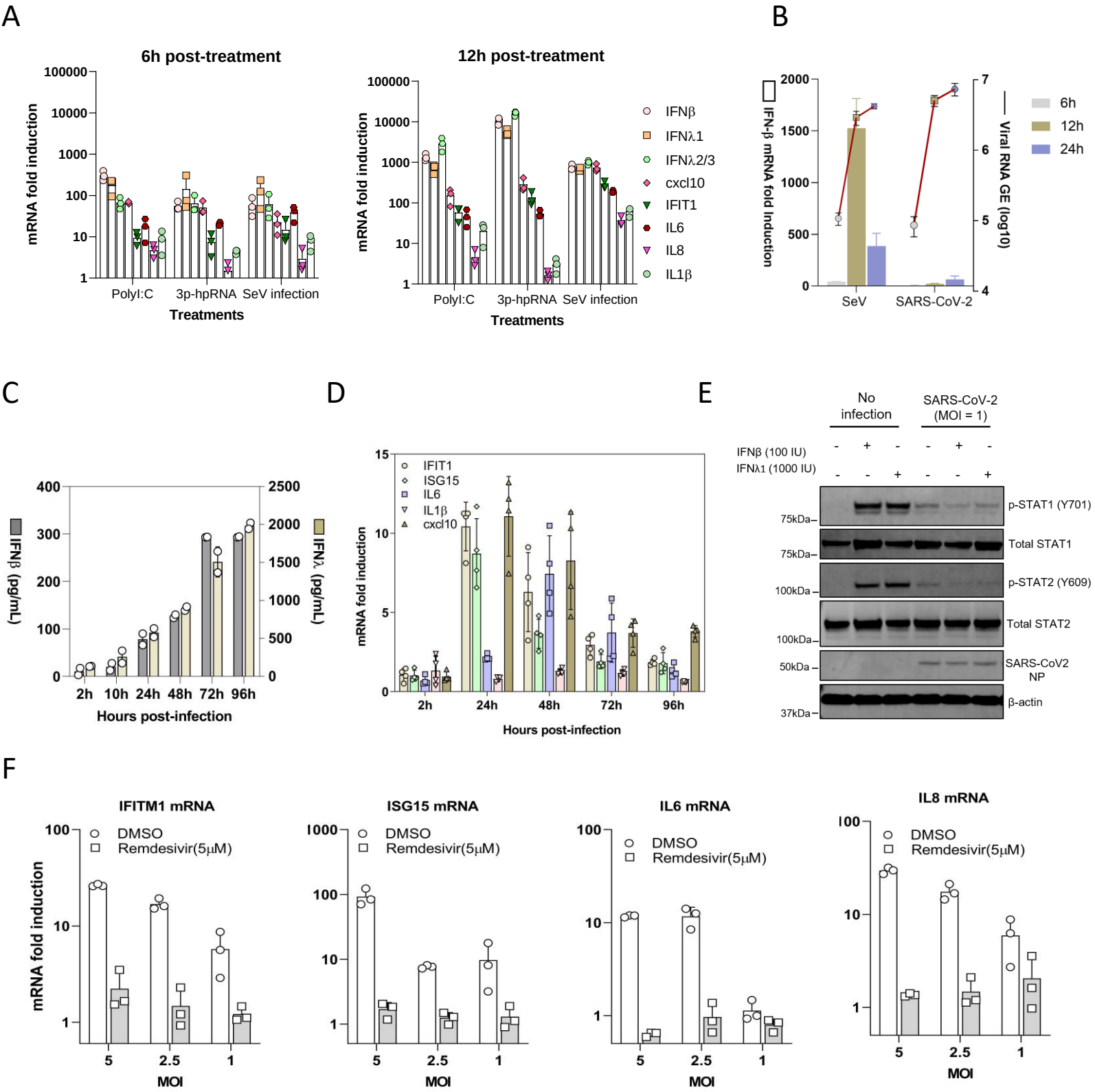
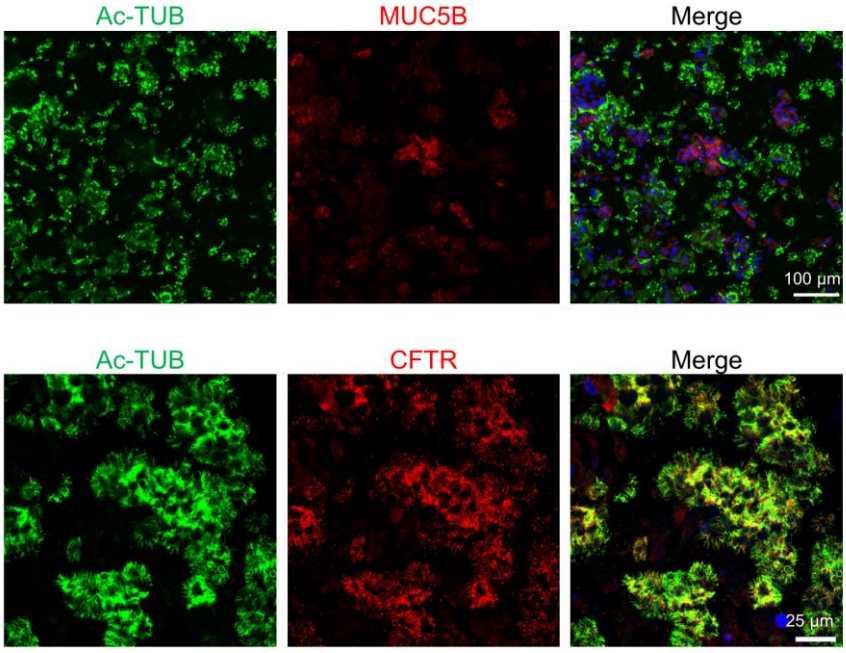

A
6h post-treatment
12h post-treatment
mRNA fold induction
PolyI:C
3p-hpRNA
SeV infection
Treatments
IFNβ
IFNλ1
IFNλ2/3
cxcl10
IFIT1
IL6
IL8
IL1β
B
IFN-β mRNA fold Induction
Viral RNA GE (log10)
6h
12h
24h
SeV
SARS-CoV-2
C
IFNβ (pg/mL)
IFNλ (pg/mL)
2h 10h 24h 48h 72h 96h
Hours post-infection
D
IFIT1
ISG15
IL6
IL1β
cxcl10
mRNA fold induction
2h 24h 48h 72h 96h
Hours post-infection
E
No infection
SARS-CoV-2 (MOI = 1)
IFNβ (100 IU)
IFNλ1 (1000 IU)
p-STAT1 (Y701)
Total STAT1
p-STAT2 (Y609)
Total STAT2
SARS-CoV2 NP
β-actin
75kDa
100kDa
50kDa
37kDa
F
IFITM1 mRNA
ISG15 mRNA
IL6 mRNA
IL8 mRNA
DMSO
Remdesivir(5μM)
mRNA fold induction
MOI

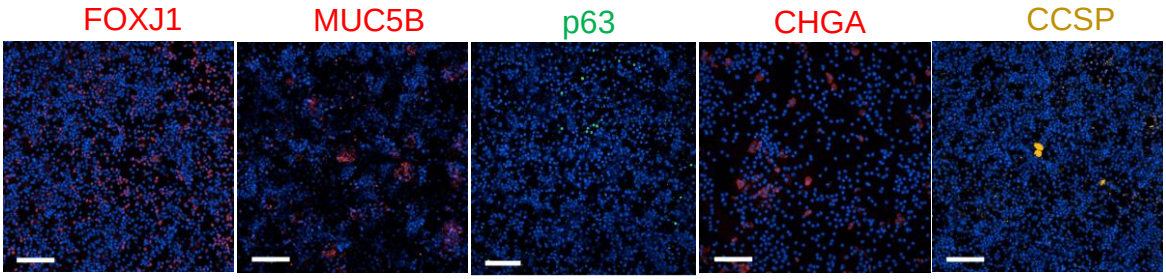
Figure S1: The innate immune response in Calu-3 cells upon stimulation (related to Figure 1). (A) Calu-3 cells were either transfected with poly IC (5 µg/mL) or 3p-hpRNA (2.5 µg/mL) or were infected with 100 hemagglutinin units/ml of Sendai virus (SeV) for activation of MDA5 and RIG-I signaling. After 6h or 12 h, the intracellular mRNA was extracted for quantification of mRNA levels of IFNs, ISGs, and proinflammatory cytokines by qRT-PCR. Data are expressed as fold changes relative to mock transfected cells and the results show the mean ± SEM of three independent experiments. (B) Calu3 cells were infected with equal amount (100 GE/cell) of Sendai virus and SARS-CoV-2, the cells were collected for quantification of IFN-β and viral mRNAs by qRT-PCR at the indicated time points. (C) Kinetics of IFN secretion responses in infected cells. Concentrations of IFN-β and IFN-λ in the culture supernatants were measured by specific ELISA, respectively. The results show the mean ± SEM of the average of the duplicates in each of 2 independent experiments. (D) The kinetics for the expression of mRNAs encoding different ISGs upon SARS-COV-2 infection of Calu-3 cells are shown. Data are expressed as fold change relative to mock-infected cells. (E) Calu-3 cells infected with SARS-CoV-2 at an MOI of 1.0 for 48 hours were treated with either IFNβ (100 IU) or IFNλ1 (1000 IU) for 3 hours prior to detection of protein expression with specific antibodies. (F) Calu-3 cells ($1x10^5$) were infected with SARS-CoV-2 at the indicated MOI in the presence of remdesivir for 48 hours. The induction of ISGs and proinflammatory cytokines was quantified by qRT-PCR. Data are expressed as fold change relative to mock-infected cells.

Fig S2

A


Ac-TUB
MUC5B
Merge
100 μm
Ac-TUB
CFTR
Merge
25 μm

B


FOXJ1
MUC5B
p63
CHGA
CCSP

C

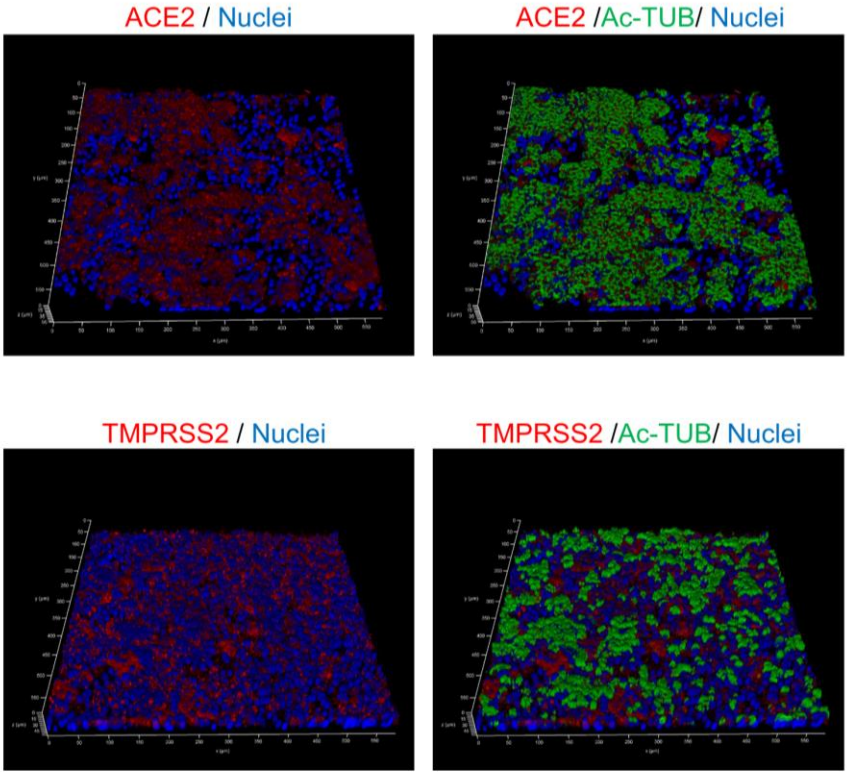

ACE2 / Nuclei
ACE2 /Ac-TUB/ Nuclei
TMPRSS2 / Nuclei
TMPRSS2 /Ac-TUB/ Nuclei

Figure S2: Characterization of iPSC-derived airway epithelial cell model (related to Figure 2). (A) Immunofluorescent analysis of iPSC-derived airway epithelial cells by confocal microscopy. Nuclei were counterstained with DAPI. Acetylated tubulin (Ac-TUB) + ciliated cells and MUC5B+ mucus producing cells were observed. Expression of cystic fibrosis transmembrane conductance regulator (CFTR) was recognized on the apical side of ciliated cells. (B) Representative immunofluorescent images of iPSC-derived airway epithelial cells for quantification of each markers. FoxJ1(Red), a marker for ciliated cells, MUC5B (Red, a marker for goblet cells), p63 (Green, a marker for basal cells), CHGA (Red, a marker for neuroendocrine cells). CCSP (Orange, a marker for club cells). Scale bars, 100 μm. (C) 3D imaging analysis showed enriched expression of ACE2 and TMPRSS2 on iPSC-derived airway epithelial cells. Expression of ACE2 was localized on the apical side of cells, whereas localization of TMPRSS2 on both apical and lateral side of cells.

Fig S3

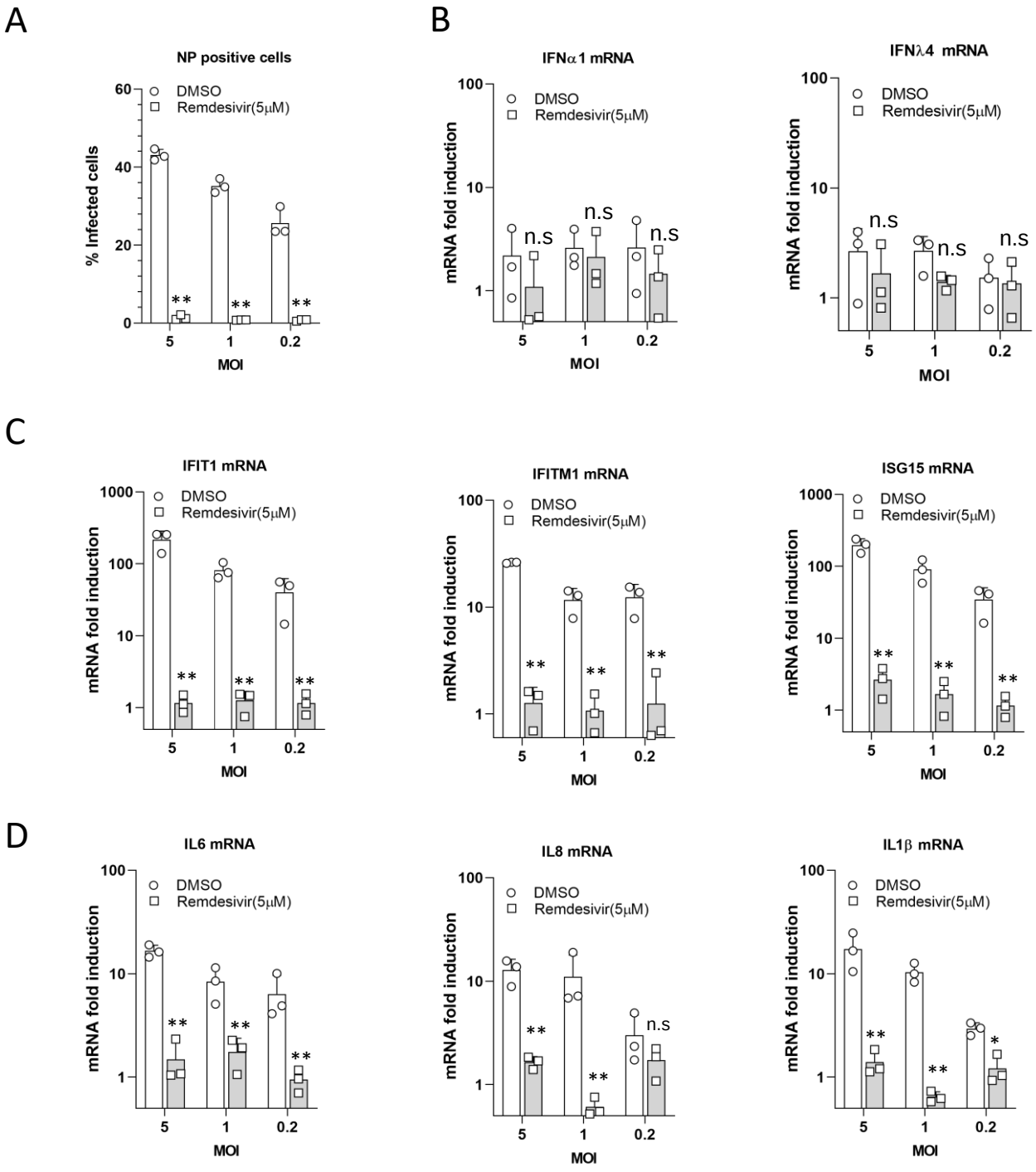
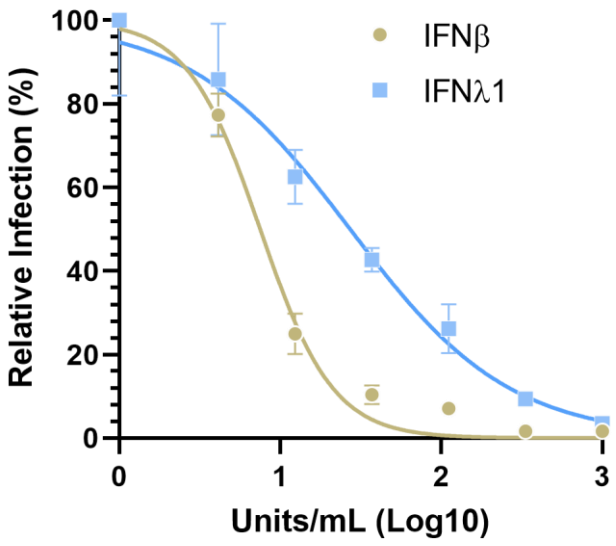

A
NP positive cells
% Infected cells
DMSO
Remdesivir(5μM)
MOI
B
IFNα1 mRNA
IFNλ4 mRNA
mRNA fold induction
n.s
C
IFIT1 mRNA
IFITM1 mRNA
ISG15 mRNA
D
IL6 mRNA
IL8 mRNA
IL1β mRNA

Figure S3: The IFN response in iPSC-derived airway epithelial cells infected with SARS-CoV-2 (related to Figure 2).

(A) The percentage of infection was calculated as the ratio between the number of infected cells stained for SARS-CoV-2 NP and the total amount of cells stained with DAPI. Data are from three technical replicates. (B-D) The induction of IFN, ISG and proinflammatory cytokines was quantified by qRT-PCR in SARS-CoV-2 infected iPS cell–derived airway epithelium. Data are expressed as fold change relative to mock-infected cells from three independent experiments with two technical replicates.

Fig S4

A


Relative Infection (%)
100
80
60
40
20
0
0
1
2
3
Units/mL (Log10)
IFNβ
IFNλ1

B

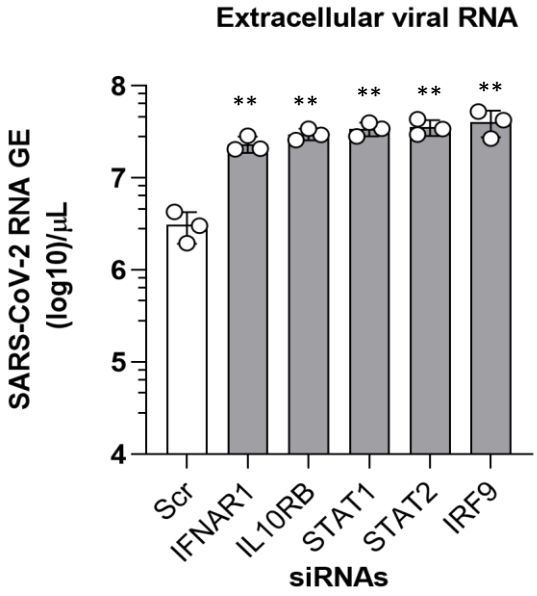
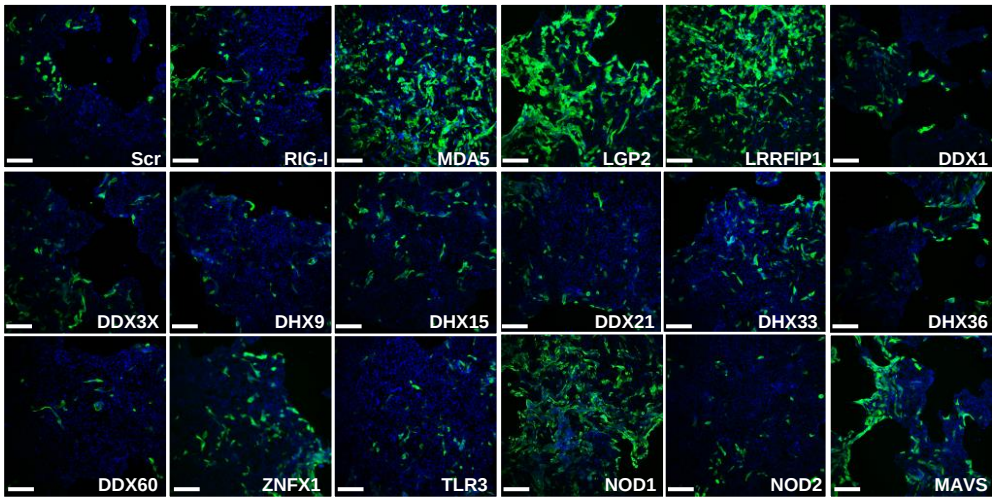

Extracellular viral RNA
SARS-CoV-2 RNA GE (log10)/μL
8
7
6
5
4
**
**
**
**
**
Scr
IFNAR1
IL10RB
STAT1
STAT2
IRF9
siRNAs

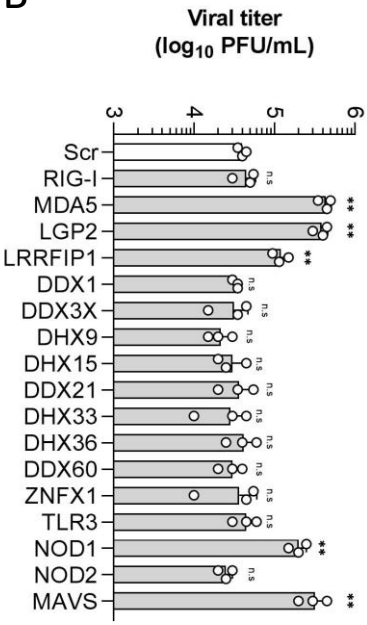
Figure S4: The antiviral effect of exogenous and endogenous IFNs (related to Figure 3). (A) Inhibition of SARS-CoV-2 replication in Calu-3 cells by IFNβ and IFNλ. Calu-3 cells were pretreated with IFNβ or IFNλ at indicated concentration for 2 hours prior to infection. After treatment, the cells were infected with SARS-CoV-2 at an MOI of 0.625 for 48 hours. Cells were fixed, and immunostained with rabbit-anti-SARS-CoV-2 NP antibody, and imaged with the Celigo high content imager. Results from duplicate experiments are presented with bars representing standard deviations. (B) The viral RNA in supernatants collected from the infected cells was quantified by qRT-PCR using primers targeting the N2 regions. Data are from three independent experiments with two technical replicates.

Fig S5

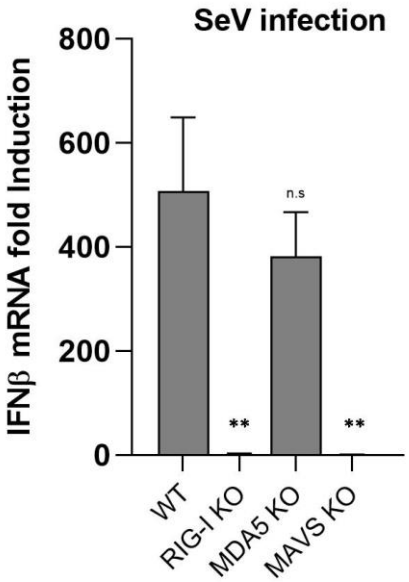
A


Scr
RIG-I
MDA5
LGP2
LRRFIP1
DDX1
DDX3X
DHX9
DHX15
DDX21
DHX33
DHX36
DDX60
ZNFX1
TLR3
NOD1
NOD2
MAVS

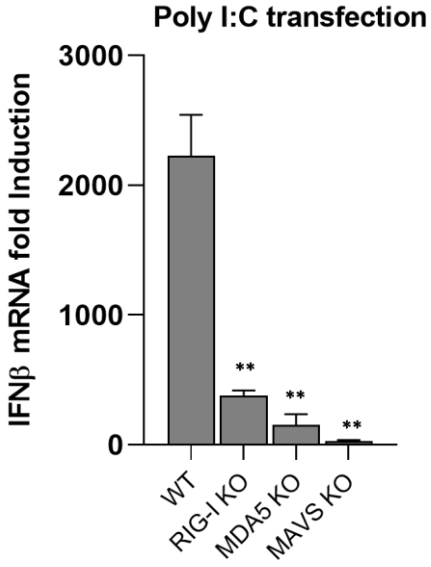
B


Viral titer
(log10 PFU/mL)
3
4
5
6
Scr
RIG-I
MDA5
LGP2
LRRFIP1
DDX1
DDX3X
DHX9
DHX15
DDX21
DHX33
DHX36
DDX60
ZNFX1
TLR3
NOD1
NOD2
MAVS
n.s
**

C


SeV infection
IFNβ mRNA fold Induction
800
600
400
200
0
n.s
**
WT
RIG-I KO
MDA5 KO
MAVS KO

D


Poly I:C transfection
IFNβ mRNA fold Induction
3000
2000
1000
0
**
WT
RIG-I KO
MDA5 KO
MAVS KO

E

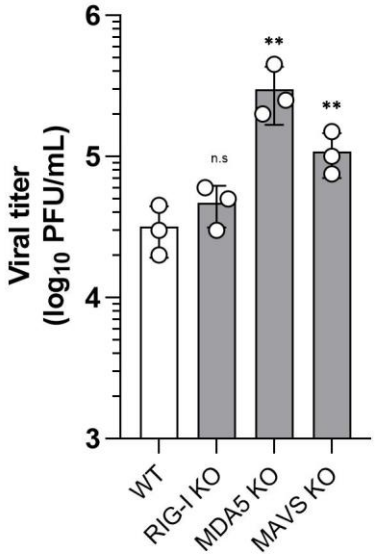
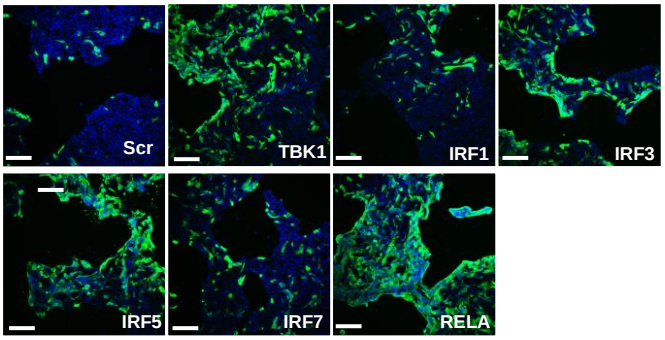

Viral titer
(log10 PFU/mL)
6
5
4
3
n.s
**
WT
RIG-I KO
MDA5 KO
MAVS KO

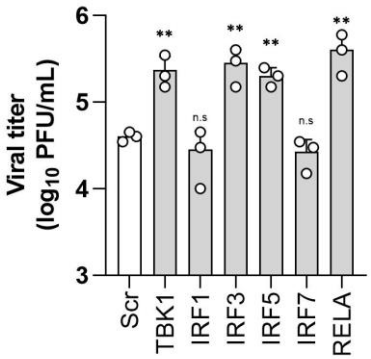
Figure S5: MDA5/LGP2 are the dominant RNA sensors responsible for innate immune induction in Calu-3 cells infected with SARS-CoV-2 (related to Figure 4).
(A) Representative immunofluorescence images from Figure 4C are shown. Scale bar, 100 μm. (B) Viral titer in the supernatants collected from infected cells (Figure 4C) were determined by plaque assay in vero E6 cells. Data show mean ± SD from one representative experiment in triplicate (n=3). (C-D) The cells were either infected with SeV or transfected with poly IC (5μg/mL). Cells were lysed 20 h after SeV infection or 8 h after poly IC transfection for IFN-β mRNA quantification. Data are presented as fold changes relative to non-treated cells. (E) The supernatants were collected from the infected cells at 48 h.p.i. Viral titer in the collected supernatants were determined by plaque assay in vero E6 cells. Data show mean ± SD from one representative experiment in triplicate (n=3).

Fig S6

A


Scr
TBK1
IRF1
IRF3
IRF5
IRF7
RELA

B


Viral titer
(log10 PFU/mL)
6
5
4
3
**
n.s
**
**
n.s
**
Scr
TBK1
IRF1
IRF3
IRF5
IRF7
RELA

C

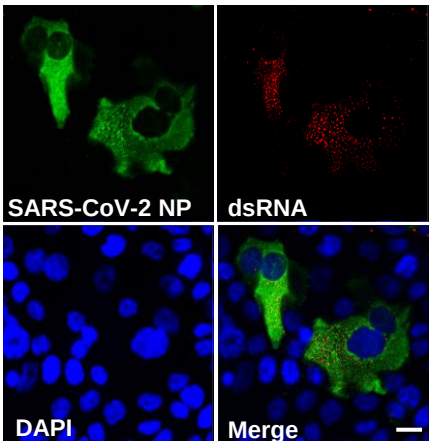

SARS-CoV-2 NP
dsRNA
DAPI
Merge

Figure S6: IRF3, IRF5, and p65 are required for the IFN signaling transduction in response to SARS-CoV-2 infection (related to Figure 5). (A) Representative immunofluorescence images from Figure 5C are shown. Scale bar, 100 μm. (B) Viral titer in the collected supernatants were determined by plaque assay in vero E6 cells. 100μL of virus-containing supernatants that were serially diluted 1:10 for inoculation in vero E6 cells. Plaques were quantified and recorded as PFU/mL. Data show mean ± SD from one representative experiment in triplicate (n=3). (C) Confocal images showing viral NP and dsRNA in Calu-3 cells infected with SARS-CoV-2 at MOI of 1. Cells were stained 48 hours after infection with a rabbit anti-SARS-CoV-2 NP antibody, and anti-dsRNA [rJ2] antibody, DAPI was used to stain the nuclei. Scale bar, 10 μm.

Table S1. The primers used for qPCR in this study. Related to STAR Methods

Gene name	Sequence (5'-3')
RIG-I	F: CACCTCAGTTGCTGATGAAGGC
	R: GTCAGAAGGAAGCACTTGCTACC
MDA5	F: GCTGAAGTAGGAGTCAAAGCCC
	R: CCACTGTGGTAGCGATAAGCAG
LGP2	F: ATGACCACCTGGAGATGCCTGA
	R: CATTGTAGCGCCTCAGGTGAAG
LRRFIP1	F: GAGAGACTTCCGACACCCTCAA
	R: CACCTCCACTTCACTGGCTCTT
DDX1	F: AAACCAAGCCCTCTTTCCTGCC
	R: GCCTTGGAAAGAGCAACAAAGCC
DDX3	F: ACTATGCCTCCAAAGGGTGTCC
	R: AGAGCCAACTCTTCCTACAGCC
DHX9	F: AGCTGTGGCTACAGCGTTCGAT
	R: CTGATTCCTCGAATGCCTGCTTC
DHX15	F: CACTGCTGAACGTCTACCATGC
	R: CATTGTCTGCGGACATCAGGGA
DDX21	F: TGGACTCAGAGGGCAGCAGTTA
	R: TGTCTCCATGCAAGGACTGAGC
DHX33	F: GAGAGGACAGTGGCATCTGCTA
	R: GAAGCTGAAGCATCACACTGGC
DHX36	F: CCCACCATCAAATGAGGCAGTG
	R: TGTGGCTCAACGGGTAATCGTG
DDX60	F: GGTGTTTTCACCAGGGAGTATCG
	R: CCAGTTTTGGCGATGAGGAGCA
ZNFX1	F: TGAGACTCCAGGAAGACCTGCA
	R: ACAATCCTCGGCTCCACCTTCT
TLR3	F: GCGCTAAAAAGTGAAGAACTGGAT
	R: GCTGGACATTGTTCAGAAAGAGG
NOD1	F: CAACGGCATCTCCACAGAAGGA
	R: CCAAACTCTCTGCCACTTCATCG
NOD2	F: GCACTGATGCTGGCAAAGAACG
	R: CTTCAGTCCTTCTGCGAGAGAAC
MAVS	F: ATGGTGCTCACCAAGGTGTCTG
	R: TCTCAGAGCTGCTGTCTAGCCA
TBK1	F: AGATGTGGTGGGTGGAATGA
	R: ACACAGACTGTCCATCTTCCC

IRF1	F: GAGGAGGTGAAAGACCAGAGCA
	R: TAGCATCTCGGCTGGACTTCGA
IRF3	F: TCTGCCCTCAACCGCAAAGAAG
	R: TACTGCCTCCACCATTGGTGTC
IRF5	F: TATGCCATCCGCCTGTGTCAGT
	R: GCCCTTTTGGAACAGGATGAGC
IRF7	F: CCACGCTATACCATCTACCTGG
	R: GCTGCTATCCAGGGAAGACACA
RELA/p65	F: TGAACCGAAACTCTGGCAGCTG
	R: CATCAGCTTGCGAAAAGGAGCC
IFNA1	F: GCCTCGCCCTTTGCTTTACT
	R: CTGTGGGTCTCAGGGAGATCA
IFNB1	F: ATGACCAACAAGTGTCTCCTCC
	R: GGAATCCAAGCAAGTTGTAGCTC
IFNL1	F: GTGACTTTGGTGCTAGGCTTG
	R: GCCTCAGGTCCCAATTCCC
IFNL2/3	F: AGTTCCGGGCCTGTATCCAG
	R: GAGCCGGTACAGCCAATGGT
IFNL4	F: CGATCCTGGAGCTGCTG
	R: TTTGTGACGCCTCTTCTGG
CXCL10	F: GTGGCATTCAAGGAGTACCTC
	R: GCCTTCGATTCTGGATTCAGACA
ISG15	F: GGCTGGGAGCTGACGGTGAAG
	R: GCTCCGCCCGCCAGGCTCTGT
IFIT1	F: AAGCTTGAGCCTCCTTGGGTTCGT
	R: TCAAAGTCAGCAGCCAGTCTCAGG
IFIT2	F: CAG CTGAGAATTGCACTGCAA
	R: GTAGGCTGCTCTCCAAGG AA
IFITM1	F: CCAAGGTCCACCGTGATTAAC
	R: ACCAGTTCAAGAAGAGGGTGTT
IL6	F: AGACAGCCACTCACCTCTTCAG
	R: TTCTGCCAGTGCCTCTTTGCTG
IL-1β	F: AAGCTGATGGCCCTAAACAG
	R: AGGTGCATCGTGCACATAAG
IL8	F: GAGAGTGATTGAGAGTGGACCAC
	R: CACAACCCTCTGCACCCAGTTT
GAPDH	F: CATGAGAAGTATGACAACAGCCT
	R: AGTCCTTCCACGATACCAAAGT