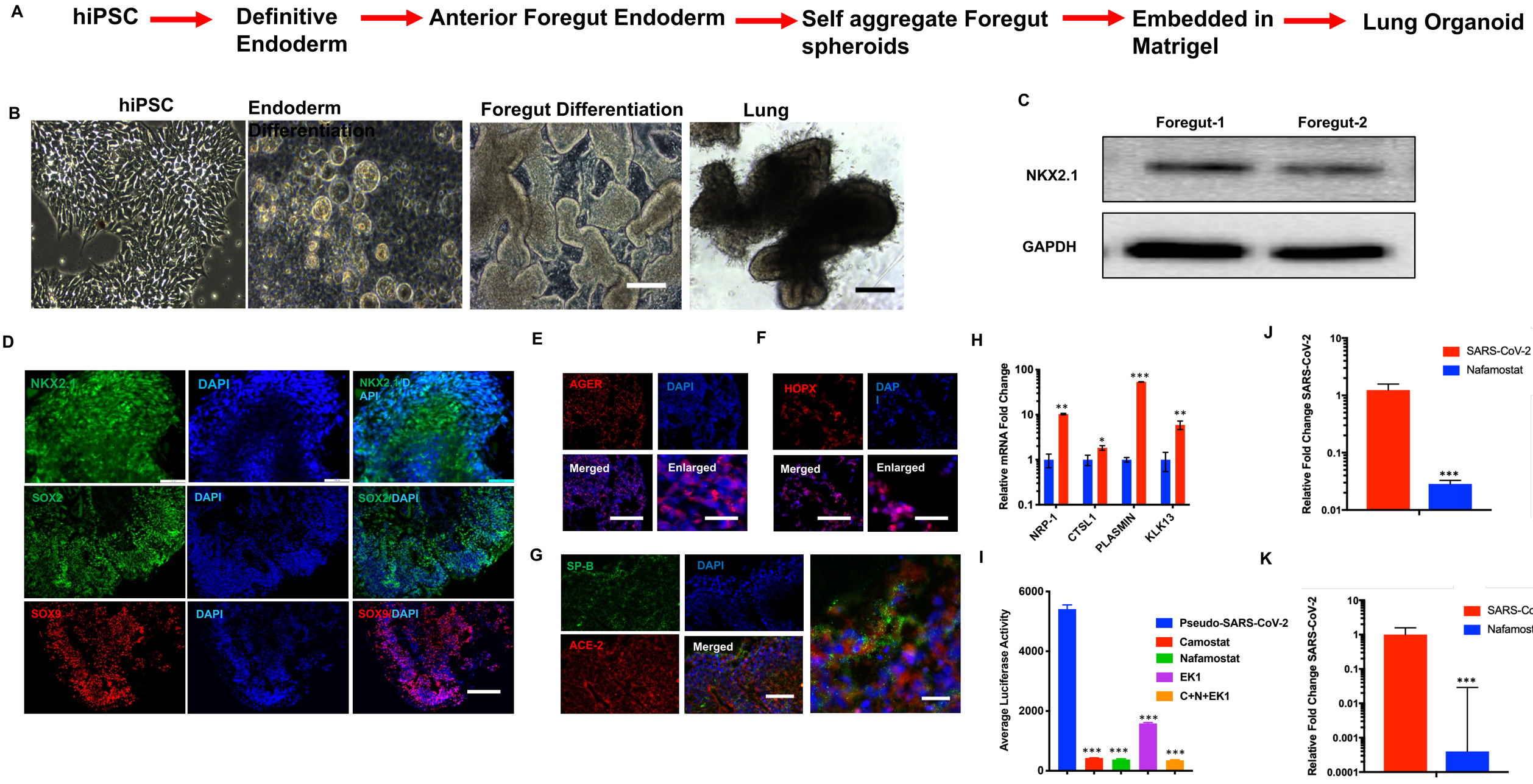
**Stem Cell Reports, Volume *16***

## Supplemental Information

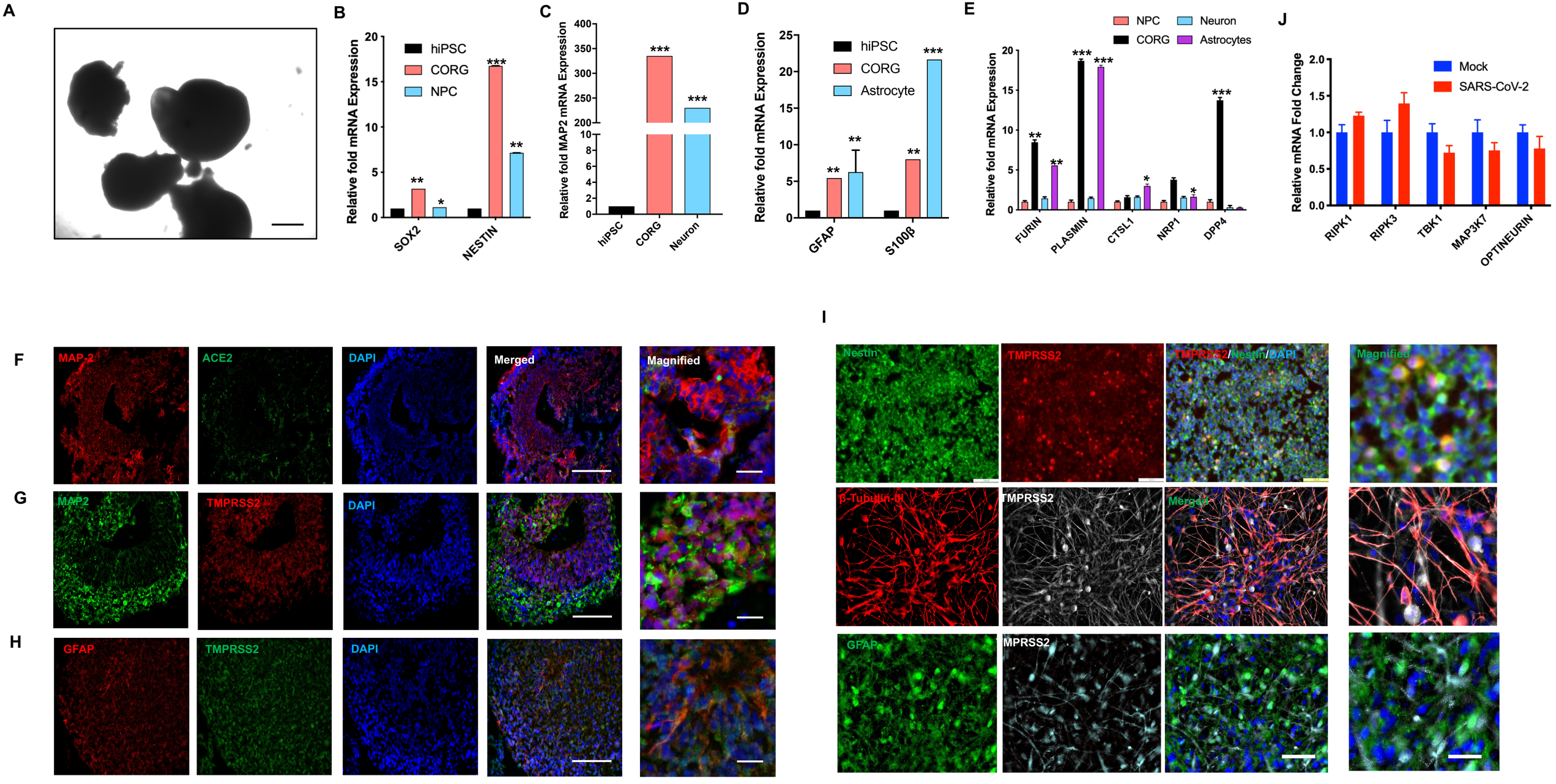
# Revealing Tissue-Specific SARS-CoV-2 Infection and Host Responses using Human Stem Cell-Derived Lung and Cerebral Organoids

**Shashi Kant Tiwari, Shaobo Wang, Davey Smith, Aaron F. Carlin, and Tariq M. Rana**

# Supplemental Figure 1



# Supplemental Figure 2



**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1: Generation and characterization of human iPSC derived lung organoids (LORGs) for modeling SARS-CoV-2 infection.**

(A) A scheme showing timeline and stepwise differentiation of human iPSC into lung organoids

(B) Brightfield phase contrast images of hiPSCs differentiation into endoderm, foregut and lung organoids. Scale bars= 100 µm

(C) Western blot for protein NKX2.1 expressed in foregut spheroids. N=2

(D) Confocal immunofluorescence images showing characteristic markers such as NKX2.1 (green), SOX2 (Red) and SOX9 (Red) in foregut spheroids and lung organoids. Scale bars= 50 µm

(E-F) Immunofluorescence images showing characteristic markers AGER and HOPX of alveolar type 1 cells in LORG. Scale bars= 200µm and for enlarged image, scale bar=50 µm

(G) Representative confocal images showing labelling of AT2 cells (SP-B, Green) co-labelled with ACE2 (Red). Scale Bars= 100µm and for enlarged image, scale bar=50µm

(H) qRT-PCR analysis shows expression of *NRP1, CTSL1, PLASMIN,* and *KLK13* in LORG. Mean ± SEM of n = 3 independent organoids, *p < 0.05, **p < 0.01 and ***p < 0.001, by Student's *t* test.

(I) Bar graph showing the luciferase activity in the presence and absence of TMPRSS2 inhibitor (Camostat and Nafamostat mesylate) and EK1 peptide to Calu 3 cells infected with pseudo-SARS-CoV-2 virus. Mean ± SEM of n = 3 independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001, by Student's *t* test.

(J, K) Inhibition of SARS-CoV-2 isolate USA-WA1/2020 infection in Calu-3 cells. Cells were infected with SARS-CoV-2 USA-WA1/2020 virus and viral RNAs from supernatant (J) and cellular (K) fractions were quantified after 48 h. Mean ± SEM of n = 3 independent experiments. ***p < 0.001, by Student's *t* test.

**Figure S2: Generation and characterization of human iPSC differentiated cerebral organoid model system for SARS-CoV-2 infection.**

(A) Phase contrast microscopic images of human iPSC differentiated 80 days old cerebral organoids (CORG). Scale bar represents 200 µm

(B) qRT-PCR for mRNA expression of neural stem/progenitor genes (Nestin and SOX-2) in cerebral organoid (Mean ± SEM of n = 3 independent organoids) and NPC (Mean ± SEM of n = 3 independent experiments). ***p < 0001, **p < 0.001, *p < 0.05, by Student's *t* test.

(C) qRT-PCR showing mRNA expression of neuronal gene MAP-2 in cerebral organoid (Mean ± SEM of n = 3 independent organoids) and 2D neurons. (Mean ± SEM of n = 3 independent experiments) ***p < 0001, by Student's *t* test.

(D) qRT-PCR for mRNA expression of astrocyte genes (GFAP and S100β) in cerebral organoid (Mean ± SEM of n = 3 independent organoids) and 2D astrocytes (Mean ± SEM of n = 3 independent experiments). ***p < 0001, **p < 0.001, by Student's *t* test.

(E) qRT-PCR analysis shows expression of host factors *FURIN, PLASMIN, CTSL1, NRP1* and *DPP4* in CORG. Mean ± SEM of n = 3 independent organoids and 2D NPC, neurons and astrocytes (Mean ± SEM of n = 3 independent experiments). *p < 0.05, **p < 0.01 and ***p < 0.001, by Student's *t* test.

(F) Confocal imaging of cerebral organoid immune stained with antibody against MAP-2 (Neuronal marker, red) co-labelled with antibody against ACE2 (SARS-CoV-2 receptor, green), and counterstained with nuclear stain DAPI (Blue). Magnified images show co-labelling of MAP-2/ACE2. Scale bar represents 100 μm and 25 μm.

(G) Confocal imaging of cerebral organoid immunostained with antibody against MAP-2 (Neuronal marker, Green) co-labelled with antibody against TMPRSS2 (SARS-CoV-2 effective protease, Red) counterstained with nuclear stain DAPI (Blue). Magnified images showing co-labelling of MAP-2/TMPRSS2. Scale bar represents 100 μm and 25 μm.

(H) Confocal imaging of cerebral organoid immunostained with antibody against GFAP (Astrocyte marker, red), co-labelled with antibody against TMPRSS2 (SARS-CoV-2 effective protease, green), and counterstained with nuclear stain DAPI (Blue). Magnified images show co-labelling of GFAP/TMPRSS2. Scale bar represents 100 μm and 25 μm.

(I) Immunofluorescence images showing expression of TMPRSS2 in various cell types: hNPC (Nestin, Green), Neurons (β-Tubulin-III, Red) and astrocytes (GFAP, Green). Scale bar represents 100 μm and 25 μm.

(J) qRT-PCR showing mRNA expression of genes regulating cell death and inflammation in neurons. Mean ± SEM of n = 3 independent experiments.

**Table S1. List of primers**

| | |
|---|---|
| hsaACE2-Fwd | TGGGACTCTGCCATTTACTTAC |
| hsaACE2-Rev | CCCAACTATCTCTCGCTTCATC |
| hsaTMPRSS2-Fwd | GGAGTGTACGGGAATGTGATG |
| hsaTMPRSS2-Rev | GGACGAAGACCATGTGGATTAG |
| hsaNKX2-1-Fwd | GCAAAGAGGACTCGCTTGTA |
| hsaNKX2-1-Rev | AGTGACAAGTGGGTTATGTTGA |
| hsaEPCAM-Fwd | GCAGTTGTTGCTGGAATTGT |
| hsaEPCAM-Rev | GCATCTCACCCATCTCCTTTAT |
| hsaKRT8-Fwd | GCAGATCAAGACCCTCAACA |
| hsaKRT8-Rev | CACTTGGTCTCCAGCATCTT |
| hsaFOXA1-Fwd | AGGGCTGGATGGTTGTATTG |
| hsaFOXA1-Rev | GTGTCTGCGTAGTAGCTGTTC |
| hsaECAD/CDH1-Fwd | GTCATTGAGCCTGGCAATTTAG |
| hsaECAD/CDH1-Rev | GTTGAGACTCCTCCATTCCTTC |
| hsaFOXA2-Fwd | GGCCAGAGTTCCACAAATCTA |
| hsaFOXA2-Rev | CCCTCCCTCCTTCTTGAAATAAT |
| hsaSOX2-Fwd | GAGAGAAAGAAGAGGAGAGAGAAAG |
| hsaSOX2-Rev | GCCGCCGATGATTGTTATTATT |
| hsaSOX9-Fwd | TGACCTATCCAAGCGCATTAC |
| hsaSOX9-Rev | GCTTGCTCTGAAGAGGGTTTA |
| hsaTP63-Fwd | AGACAAACTGCTTCCCTTACTT |
| hsaTP63-Rev | GGCCCTAGTGTTACCTGAATAG |
| hsaKRT5-Fwd | CATGGAGATTGCCTCTTCTAGG |
| hsaKRT5-Rev | TCTCCTGGGAACCAAAGAATG |
| hsaFOXJ1-Fwd | GTTTGGGTTTGGTGGTTTGG |

| | |
|---|---|
| hsaFOXJ1-Rev | CCAGTTAAGCCTCAGCTACAG |
| hsaSCGB1A1-Fwd | TGGTCACACTGGCTCTCT |
| hsaSCGB1A1-Rev | TTCCATGGCAGCCTCATAAC |
| hsaMUC5B-Fwd | GAGTGGTTTGATGAGGACTACC |
| hsaMUC5B-Rev | CTTAGGCTGCTGGCATAAGT |
| hsaSPDEF-Fwd | AGCTATGGCCGCTTCATTAG |
| hsaSPDEF-Rev | GCTCAGCTTGTCGTAGTTCA |
| hsaASCL3-Fwd | AAGGAATGAGCGGGAAAGG |
| hsaASCL3-Rev | GCTGAGTCGCTTCTCCAAATA |
| hsaPDPN-Fwd | TCCCAAAGTGCTGGGATTAC |
| hsaPDPN-Rev | CTCATCCAGCTCTTCTCATTCC |
| hsaSFTPC-Fwd | CTCCACATGAGCCAGAAACA |
| hsaSFTPC-Rev | GAGAAGGTGGCAGTGGTAAC |
| hsaSFTPB-Fwd | CCGCACCCTTAAGAAGGTATTT |
| hsaSFTPB-Rev | CGGTGGAGTTAGGAGCAATTT |
| hsaABCA3-Fwd | GAGAAGGAAAGGAGGCTGAAG |
| hsaABCA3-Rev | GGAGGAAGAGGAAGAACAAGAG |
| hsaFurin-Fwd | GACGGCTACACCAACAGTATC |
| hsaFurin-Rev | GTCACGATCTGCTTCTCATTCT |
| hsaDPP4-Fwd | TGTGAGCTGAATCCGGAAAG |
| hsaDPP4-Rev | CACGCTGCTGTGTAGAGTATAG |
| hsaANPEP-Fwd | GTTCTCCTTCTCCAACCTCATC |
| hsaANPEP-Rev | CTGTTTCCTCGTTGTCCTTCT |
| hsaOAS2-Fwd | CTGGAGCTGGTCACACAATATC |
| hsaOAS3-Rev | GAAACTTCCTCACGGTCTCATC |

| | |
|---|---|
| hsaTLR3-Fwd | CCCTGGTGGTCCCATTTATTT |
| hsaTLR3-Rev | CTCAACTGGGATCTCGTCAAAG |
| hsaTLR7-Fwd | TTTCCCAGAGCATACAGCTTAG |
| hsaTLR7--Rev | GCCTCTGATGGGACAAGTAAA |
| hsaIFIT3-Fwd | AGTGGCTCATGCCTGTAATC |
| hsaIFIT3--Rev | AGACGGGATTTCACTGTGTTAG |
| hsaHOMER1-Fwd | GGTACACTCTGGTGTTCTCTAATC |
| hsaHOMER1-Rev | CTCAGCTGCCTTCGTTAGTT |
| has-C1q-Fwd | ACAGTAGGGCTTGGTGAATG |
| has-C1q-Rev | CTGGGACACAGAGTTGAATTAGA |
| has-C3-Fwd | CAGCAGATGACCCTGAAGATAG |
| has-C3-Rev | TGCGTCAGTTTGTTCTTCTTATTC |
| hsaCAPN1-F | CCCAATTCCTCCAAGACCTATG |
| hsaCAPN1-R | GGTAGCTCCATCCACAATGAA |
| hsaCAPN2-F | GTGACTTCCTGAGGCACTATTC |
| hsaCAPN2-R | CCTCCAGTTCCCATCCATTT |
| hsaTRPC5-F | CTGGTGGAACCTGATGGATTT |
| hsaTRPC5-R | CCCTTGGACGAGAACCATTATAC |
| hsaSEMA3A-F | GGATCAGCCGTGTGTATGTATAG |
| hsaSEMA3A-R | TTGATAAGGCACCCATTGATAGT |
| hsaCDK5-F | GACCAAGCTGCCAGACTATAAG |
| hsaCDK5-R | CTCCCTGTGGCATTGAGTTT |
| hsaGRIP1-F | GCCACAGAAACTCTCTCTTCTC |
| hsaGRIP1-R | CCATCACTCTGTCTCCAATCTG |
| hsaSAP97/DLG1-F | TGCACAATATCGACCTGAAGAA |
| hsaSAP97/DLG1-R | AGAGAACCTGACCCTGAACTA |

| | |
|---|---|
| hsaKIDINS220-F | AAAGCCGAAGGGAAAGTAGAG |
| hsaKIDINS220-R | AGGAGCGCATCCGATAAATAC |
| hasPAIP2-F | GAAGAGGAGTTATGGGAAGAAGAA |
| hasPAIP2-R | GGAGATCTCGAGCTGGAATAAA |
| nCoV-N1-F | GAC CCC AAA ATC AGC GAA AT |
| nCoV-N1-R | TCT GGT TAC TGC CAG TTG AAT CTG |
| hRIPK1-F | CTGGAGAGGGAACAGGAAATG |
| hRIPK1-R | GTACACAGAGTAAACCGAGGAAG |
| hRIPK3-F | GACTCCAGAGACCTCAACTTTC |
| hRIPK3-R | CCAGTTCATGCCTTGTCTCT |
| hOptineurin-F | AGGAGAAGGAAGGAAGGAGAA |
| hOptineurin-R | GCTCGCATCAACCAAACATAAA |
| hTBK1-F | GAAGGGCCTCGTAGGAATAAAG |
| hTBK1-R | CCCGAGAAAGACTGCAAGAA |
| hMAP3K7/TAK1-F | CACGCAATGAGTTGGTGTTTAC |
| hMAP3K7/TAK1-R | GGTTTCAGGTCCCTGTGAATTA |

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Key Resources Table

| Reagent | Source | Catalogue Number |
|---|---|---|
| **Antibodies** | | |
| Mouse anti-FOXJ1 | eBioscience | 14-9965-82 |
| Mouse anti-surfactant protein B (SFTPB) | Seven Hills Bioreagents | Wmab-1B9 |
| Rabbit anti-Prosurfactant Protein C (proSP-C) | Millipore | AB3786 |
| Rabbit anti-RAGE | Abcam | Ab216329 |
| Mouse anti-Hop (E-1) | Santa Cruz | Sc-398703 |
| Rabbit anti-NKX2.1 | Abcam | ab76013 |
| Rabbit anti-SOX9 | Millipore | AB5535 |
| Rabbit anti-ECAD | Cell Signaling Technology | 3195 |
| anti-CC10 (SCGB1A1) | Santa Cruz | sc-365992 |
| Rabbit Anti-ACE2 | Novus Biologicals | NBP2-67692 |
| Rabbit anti-TMPRSS2 | Abcam | Ab92323 |
| Rabbit anti-Sox2 | Abcam | ab97959 |
| Rabbit anti-Ki67 | Millipore | AB9260 |
| GFAP (anti-mouse) | Sigma-Aldrich (USA) | G3893 |
| Sox2 (anti-rabbit) | Abcam, (USA) | ab97959 |
| β-Tubulin III (anti-mouse) | Abcam, (USA) | ab7751–100 |

| | | |
|---|---|---|
| Nestin (anti-chicken,) | Abcam, (USA). | ab134017 |
| Ki67 (anti-rabbit,), | Millipore | AB9260 |
| MAP2 (anti-chicken) | Abcam, (USA) | ab5392 |
| Alexa Fluor 488, 594, and 647 conjugated secondary antibodies | Molecular Probes (Invitrogen, USA). | A11032, A11034, A11012, A11039, A11001 |
| Antifade mounting medium with DAPI | Vectashield, Vector Laboratories, CA | H-1200 |
| **Chemicals** | | |
| 1-thioglycerol | Sigma-Aldrich | M6145 |
| L-ascorbic acid | Tocris Bioscience | 4055 |
| Bovine serum albumin (BSA) | Fischer Scientific (Gibco, Invitrogen, USA) | BP9703–100 |
| Protease and phosphatase inhibitors cocktail | Pierce (USA) | A32955 |
| Bio-Rad DC protein assay kit | Bio-Rad, USA | 5000112 |
| Chemiluminescence substrate | Thermo Scientific (USA) | 34580 |
| Optimal cutting temperature (OCT) compound | Tissue-Tech (USA) | 4583 |
| RIPA Buffer | Teknova | R3792 |
| beta-mercaptoethanol | Sigma-Aldrich (USA) | M7522 |
| Bio-Rad DC protein assay kit | Thermo Scientific (USA) | 5000112 |
| Chemiluminescence substrate | Thermo Scientific (USA) | 34580 |
| Immun-Blot PVDF Membrane | Bio-Rad Laboratories | 1620177 |

| | | |
|---|---|---|
| Optimal cutting temperature (OCT) compound | Tissue-Tech (USA). | 4583 |
| **Growth Factors** | | |
| Activin A | R&D Systems | 338-AC |
| FGF10 (recombinant human fibroblast growth factor 10) | R&D Systems | 345-FG |
| FGF4 (recombinant human fibroblast growth factor 4) | R&D Systems | 7460-F4 |
| FGF7 (recombinant human fibroblast growth factor 7) | R&D Systems | 251-KG/CF |
| NOGGIN | R&D Systems | 6057 |
| Smoothened agonist (SAG) | Enzo Life Sciences | ALX-270-426-M001 |
| Epidermal growth factor (EGF) | Sigma-Aldrich (USA) | E9644 |
| Basic fibroblast growth factor (bFGF) | Sigma-Aldrich (USA) | F0291 |
| Epidermal growth factor (EGF) | Sigma-Aldrich (USA) | E9644 |
| **Cell Lines** | | |
| Human induced pluripotent stem cell (hiPSC) | Cell Application | hiPS11-10 |
| 293FT | ATCC | |
| Calu-3 | ATCC | ATCC® HTB-55™ |
| **Culture Medium** | | |
| Advanced DMEM | Thermo Fisher Scientific | 12491015 |
| CHIR-99021 | Stemcell Technologies | 72054 |

| | | |
|---|---|---|
| GlutaMAX (100X) | Thermo Fisher Scientific | 35050061 |
| HEPES buffer (1M) | Thermo Fisher Scientific | 15630080 |
| mTeSR$^{TM1}$ | (Stemcell Technologies | 85850 |
| N-2 supplement | Thermo Fisher Scientific | 17502048 |
| Penicillin–streptomycin (100X) | Thermo Fisher Scientific | 15140122 |
| RPMI 1640 medium | Thermo Fisher Scientific | 11875119 |
| Matrigel basement membrane matrix growth factor reduced | Corning | 354230 |
| Matrigel basement membrane matrix | Corning | 354234 |
| DMEM-F12 medium | Thermo Fisher Scientific | 11320082 |
| MEM nonessential amino acids (MEM-NEAA) | Thermo Fisher Scientific (Gibco, Invitrogen, USA) | 11140050 |
| N2 supplement | Thermo Fisher Scientific (Gibco, Invitrogen, USA) | 17502048 |
| B-27 supplement without vitamin A | Thermo Fisher Scientific (Gibco, Invitrogen, USA) | 12587010 |
| B-27 supplement with vitamin A | Thermo Fisher Scientific (Gibco, Invitrogen, USA) | 17504044 |
| glutamine/glutaMAX, 200mM | Thermo Fisher Scientific (Gibco, Invitrogen, USA) | 35050061, A2916801 |
| Dispase | Thermo Fisher Scientific (Gibco, Invitrogen, USA) | 17105–0412q |
| Collagenase IV | Thermo Fisher Scientific (Gibco, Invitrogen, USA) | 17104019 |

| | | |
|---|---|---|
| Fetal bovine serum | Thermo Fisher Scientific (Gibco, Invitrogen, USA) | 10438–018 |
| Bovine serum albumin (BSA) | Fischer Scientific (Gibco, Invitrogen, USA). | BP9703–100 |

**Human induced pluripotent stem cell (hiPSC) maintenance and preparation of Lung organoids**

All studies were performed based on the approved IRB protocols by the University of California (UCSD), San Diego. Human induced pluripotent stem cell (hiPS11-10) from Cell Application (a kind gift from Frank Lab at UCSD) were cultured on basement membrane growth-factor-reduced Matrigel coated plate in mTeSR$^{TM1}$ medium following previously established protocols. These iPSC cells were differentiated into the human lung organoids by following the previously established protocol by (Leibel et al., 2019; Miller et al., 2019)) with minor changes. In brief, ~75–85% confluent undifferentiated hiPSC colonies were treated with 1 mg/mL dispase solution for 5-10 min at 37$^0$C followed by removal of dispase, washing cells gently with DMEM/F12 and scraping the colonies with a cell scraper in mTeSR$^{TM1}$ medium. Next collected iPSC colonies were dissociated into smaller colonies using serological pipette and plated 0.5mL of the smaller hiPSC clumps in 24-well Matrigel coated plate. When cells reached to 50-75% of confluency, then start stepwise differentiation into definitive endoderm, anterior foregut endoderm and lung organoid differentiation as follows:

1) Definitive Endoderm differentiation: Feed cells for 4 days with definitive endoderm differentiation medium daily by replacing existing medium. Day 1 medium consist of RPMI+100ng/mL Activin A, while Day 2 media contain RPMI+0.2% FBS+100ng/mL Activin A and Day 3 and 4 contains RPMI+2% FBS+100ng/mL Activin A.

2) Anterior foregut endoderm differentiation: At day 5 replace the medium with anterior foregut endoderm differentiation basal medium consists of Advanced DMEM/F12, 1× N-2, 1× B27, 10 mM HEPES buffer, 1× L-glutamine (2 mM), 1× penicillin–streptomycin, 0.4μM monothioglycerol, 0.25% BSA and 50 μg/mL ascorbic acid. This medium was supplemented with 10μM SB431542, 200 ng/mL Noggin, 1μM SAG, 500 ng/mL FGF4, and 2μM CHIR-99021. Change the medium every day and observe the formation of spheroid around 8 and 9 days of culture.

3) 3D Lung organoid culture: Collected foregut differentiated endoderm spheroids were mixed with Matrigel and quickly make individual droplet of 25µL on sterile parafilm and placed in incubator to solidify the Matrigel for ~10 min. To differentiate into lung organoid, each droplet were transferred into individual well of 24 well plate with basal media containing advanced DMEM/F12, 1× N-2, 1× B27, 10 mM HEPES buffer, 1× L-glutamine (2 mM), 1× penicillin–streptomycin, 0.4µM monothioglycerol, 0.25% BSA and 50 µg/mL ascorbic acid. This medium was supplemented with 3D step 1 media (FGF7 (10ng/mL, FGF10 (10ng/mL, CHIR (3µM) and EGF (10 ng/ml) added every other day for 8 days. On day 25 and onwards, the media was changed to 3D step 2 medium consisting of FGF7 (10ng/ml), FGF10 (10ng/ml), CHIR (3µM), ATRA (50nM), EGF (10ng/ml) and VEGF/PIGF (10ng/ml) and changed every 2 days for 8 days. Next step 2 media were supplemented with cAMP (100µM), IBMX (100 µM) and Dexamethasone (50nM) to continue the culture conditions. For maintenance of longer culture re-embed the organoid at every 2-3 weeks in Matrigel. We utilized 60D old lung organoids to characterize for expression of specific markers and SARS-CoV-2 infection studies.

**Cerebral organoids**

hiPS11-10 were cultured on basement membrane growth-factor-reduced Matrigel coated plate in mTeSR-TM[1] medium following previously established protocols. Feeder-dependent hESCs were detached from their feeder layer using 1 mg/ml collagenase (dissolved in DMEM-F12 medium) for 15–20 min at 37 °C in CO2 incubator and 0.5 mg/ml dispase (dissolved in DMEM-F12 medium) for an additional 15 min at 37°C in $CO_2$ incubator. Wells were washed with media to collect floating undifferentiated hESCs and colonies were dissociated using Accumax or Versene solution at 37°C for 10 min to generate a single-cell suspension. At day 0, embryoid bodies were formed using the hanging drop method with 4500 cells/drop in DMEM/F12 media supplemented with 20% knockout serum replacement (KOSR), bFGF (4 ng/ml), MEM nonessential amino acids (MEM-NEAA, 1%, vol/vol), and glutamine (200 mM, 1%, vol/vol) or grown in microwell plates. After 2 days of hanging drop culture, embryoid bodies were transferred to sterile petri dishes with refreshed media. After 6 days in culture, embryoid bodies were transferred to new petri dishes containing neural induction media consisting of DMEM/F12, N2 supplement (1%, vol/vol), MEM-NEAA (1%, vol/vol), glutamine (200 mM, 1%, vol/vol), and heparin (1µg/ml) until day 11. At day 11, organoids were transferred to Matrigel droplets (30µl) and cultured in 1:1 mixture of DMEM/F12 and Neurobasal medium supplemented with B-27 without vitamin A (1%, vol/vol), N2 (1%, vol/vol), NEAA (1%, vol/vol), insulin, beta-mercaptoethanol, and glutamine (200 mM, 1%, vol/vol). Twenty 80 days organoids were then

transferred to stir flask bioreactors (125 ml) containing magnetic shaft and stirring speed was maintained 50–60 rpm. For long term growth on day 15 in 75–100 ml of cerebral organoid differentiation media with vitamin A. Media was changed every 3 days.

**Astrocyte differentiation**

NPCs were plated on Matrigel coated plates in complete NPC medium at $2.5 \times 10^4$ cells/cm$^2$. After 2 days of NPC culture, the media was changed to astrocyte differentiation medium which consists of D-MEM supplemented with GlutaMAX-I (2 mM, 1%, vol/vol), N-2 (1%, vol/vol), FBS (1%, vol/vol), and Antibiotic-Antimycotic solution (1%, vol/vol) and then changed the media at every 3-4 days for 1 month.

**Neuronal differentiation**

To differentiate NPCs into the neurons, hNPCs were plated in polyornithine and laminin-coated culture dishes in complete NPC medium at $2.5–5 \times 10^4$ cells/cm$^2$. After 2 days, the media was changed to neural differentiation medium consisting of neurobasal medium supplemented with GlutaMAXI (200 mM, 1×, vol/vol), B-27 serum-free supplement (1×, vol/vol), and antibiotic - antimycotic (1×, vol/vol) solutions and changed medium at every 3–4 days for 2 weeks.

**Cell Lines maintenance**

293FT and Calu-3 cells were cultured and maintained under standard culture conditions at 37°C in a 5% $CO_2$ atmosphere. In brief, 293FT human embryonic kidney cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. Calu-3 cells are human lung airway epithelial cells, cultured in Eagle's Minimum Essential Medium (EMEM) with 10% FBS and 1% antibiotic-antimycotic solutions.

**Sectioning of organoids for immunofluorescence analysis**

Before sectioning, the organoids (LORG and CORG) were washed with phosphate buffer saline (PBS) to remove the residual culture medium and surrounding Matrigel was removed by incubating in cell recovery solution for 30 min. Cell recovery solution is used to recover cells or organoids from matrigel matrix for subsequent sectioning and biochemical analyses. Next after washing the organoids with PBS, fixed with 4% paraformaldehyde (PFA) for 1h followed by again washing organoid three times with PBS, stained with hematoxylin for 5 min and incubated in 30% sucrose overnight. Sucrose solution was removed, organoids were washed with PBS and embedded in OCT compound for cryosectioning. We typically cut 20-µm-thick sections of

organoid for immunohistochemical analysis using cryostat. Cryosections were blocked in 3% BSA in PBS for 1h, washed three times with PBS + 0.1% Tween-20 (PBST), and incubated at 4°C overnight with primary antibodies: ECAD (1:500, Rabbit), Sox2 (1µg/ml, Rabbit), Ki67 (1:200, Rabbit), NKX2.1 (1:500, Rabbit), Vimentin (1:200, Rabbit), SOX9 (1:200, Rabbit), FOXJ1 (1:200, Mouse), Mucin5AC (1:200, Rabbit), Surfactant protein B, SP-B (1:200, Mouse), ACE2 (1:200, Rabbit) and TMPRSS2 (1:200, Rabbit), Sox2 (1µg/ml, Rabbit, Abcam ab97959), GFAP (1:500, Mouse, Sigma, G3893), β-Tubulin III (1:500, mouse, Abcam ab7751–100), Nestin (1:1000, Chicken, Abcam ab134017). After overnight incubation in primary antibodies cryosections were washed three times with PBST to remove primary antibody and incubated 1h into secondary antibodies. We used alexa fluor conjugated secondary antibodies including anti-Mouse alexa fluor 488, 594, and anti-rabbit alexa fluor 488, 594 (Molecular Probes, USA, 1µg/ml dilution in PBS). Cryosections were washed three more times with PBST to remove secondary antibody and mounted with Vectashield hardset mounting medium with nuclear stain (DAPI) following manufacturer's instructions.

**qRT-PCR**

For RNA extraction, 100 µL cell culture supernatant was incubated with 400 µL AVL buffer (viral lysis buffer used for purifying viral nucleic acids). For cellular RNA extraction, cells were collected in Trizol reagent and RNA was extracted by Direct-zol RNA Kit (Zymo) and quantified by qRT-PCR using gene specific primers (Table S1).

**Western blotting**

Human lung organoids and foregut spheroids were lysed in 200µl of RIPA buffer containing protease inhibitor cocktail (Roche) and proteins were extracted by centrifugation at 13,000×g for 10min at 4°C. Extracted proteins concentration was determined by BioRad DC protein assay kit as per manufacturer instructions (Bio-Rad) and equal amounts of protein (25µg) were resolved by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked with 5% nonfat milk in PBST for 2h at room temperature followed by overnight incubation at 4°C with primary antibodies such as ECAD (1:1000, Rabbit), NKX2.1 (1:1000, Rabbit), Vimentin (1:1000, Rabbit), SOX9 (1:1000, Rabbit), FOXJ1 (1:1000, Mouse), Mucin5AC (1:1000, Rabbit), Surfactant protein B, SP-B (1:1000, Mouse), GAPDH (1:1000), ACE2 (1:1000, Rabbit) and TMPRSS2 (1:1000, Rabbit). On following day, membranes were then washed three times with tris-buffered saline with 0.1% Tween-20 (TBST) and incubated for 2h with secondary antibody conjugated with

horseradish peroxidase (HRP). Immunoreactive protein signals were detected with Supersignal West Pico Chemiluminescent Substrate (Pierce, USA).

**SARS-CoV-2 pseudo virus production, titration and characterization**

293FT cells were transfected with pLVX expressing SARS-CoV-2-S. Nineteen amino acids at the C terminus of SARS-CoV-2-S was deleted, given previous report that the shortened mutant has improved incorporation into the pseudovirus envelope (Fukushi et al., 2005; Hoffmann et al., 2020; Wang et al., 2020). 24h post-transfection, cells were infected with VSV pseudovirus containing Fluc or EGFP. Cells were washed four times with medium 1h post-inoculation and maintained in medium for 24h. Then supernatant containing pseudovirus was collected, centrifuged, and used or stored at -80°C.

**SARS-CoV-2 pseudovirus infection and treatment with protease inhibitor and EK-1 peptide**

These infections were performed according to published procedures (Wang et al., 2020). Briefly, lung organoids were pretreated with TMPRSS2 inhibitor Camostat mesylate (10 μM) for 2h and then infected with SARS-CoV-2 pseudovirus at MOI=2. After 2h infection, virus was removed and fresh medium containing Camostat mesylate was added. For EK-1 peptide, first SARS-CoV-2 pseudovirus was incubated with EK-1 peptide (10 μM) for 30min at $37^0$C and then infected organoids for 2h followed by changing with fresh medium. For pseudo virus with Fluc, cells were lysed at 24h post-infection and subjected for luciferase activity assay according to manufacturer's instruction. For pseudovirus with EGFP, infection was evaluated by taking bright field and fluorescent images of LORG at 24h post-infection.

**SARS-CoV-2 isolate USA-WA1/2020 infection**

SARS-CoV-2 isolate USA-WA1/2020 was obtained from BEI Resources. SARS-CoV-2 was propagated and infectious units quantified by plaque assay using Vero E6 cells. Human iPSC derived lung organoids, NPCs, neurons and astrocytes were infected with SARS-CoV-2 at MOI=2 for 2h at 37°C. Then cells were washed, and fresh medium was added. At 2h, 48 and 72h post infection supernatant and infected organoids were collected and lysed using TRIzol and RNA was extracted using a Direct-zol RNA Kit (Zymo) and quantified by RT-qPCR using SARS-CoV-2 N primers.

**Quantification and Statistical Analysis:**

Number of independent biological replicates includes 3 for most experiments unless otherwise indicated. p-values were calculated by unpaired two-tailed Student's t test. *p < 0.05, **p < 0.01 and ***p < 0.001.

**Supplemental References**

Fukushi, S., Mizutani, T., Saijo, M., Matsuyama, S., Miyajima, N., Taguchi, F., Itamura, S., Kurane, I., and Morikawa, S. (2005). Vesicular stomatitis virus pseudotyped with severe acute respiratory syndrome coronavirus spike protein. J Gen Virol *86*, 2269-2274.

Hoffmann, M., Kleine-Weber, H., and Pohlmann, S. (2020). A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. Mol Cell *78*, 779-784 e775.

Leibel, S.L., Winquist, A., Tseu, I., Wang, J., Luo, D., Shojaie, S., Nathan, N., Snyder, E., and Post, M. (2019). Reversal of Surfactant Protein B Deficiency in Patient Specific Human Induced Pluripotent Stem Cell Derived Lung Organoids by Gene Therapy. Sci Rep *9*, 13450.

Miller, A.J., Dye, B.R., Ferrer-Torres, D., Hill, D.R., Overeem, A.W., Shea, L.D., and Spence, J.R. (2019). Generation of lung organoids from human pluripotent stem cells in vitro. Nat Protoc *14*, 518-540.

Wang, S., Li, W., Hui, H., Tiwari, S.K., Zhang, Q., Croker, B.A., Rawlings, S., Smith, D., Carlin, A.F., and Rana, T.M. (2020). Cholesterol 25-Hydroxylase inhibits SARS-CoV-2 and coronaviruses by depleting membrane cholesterol. EMBO J, e2020106057.