

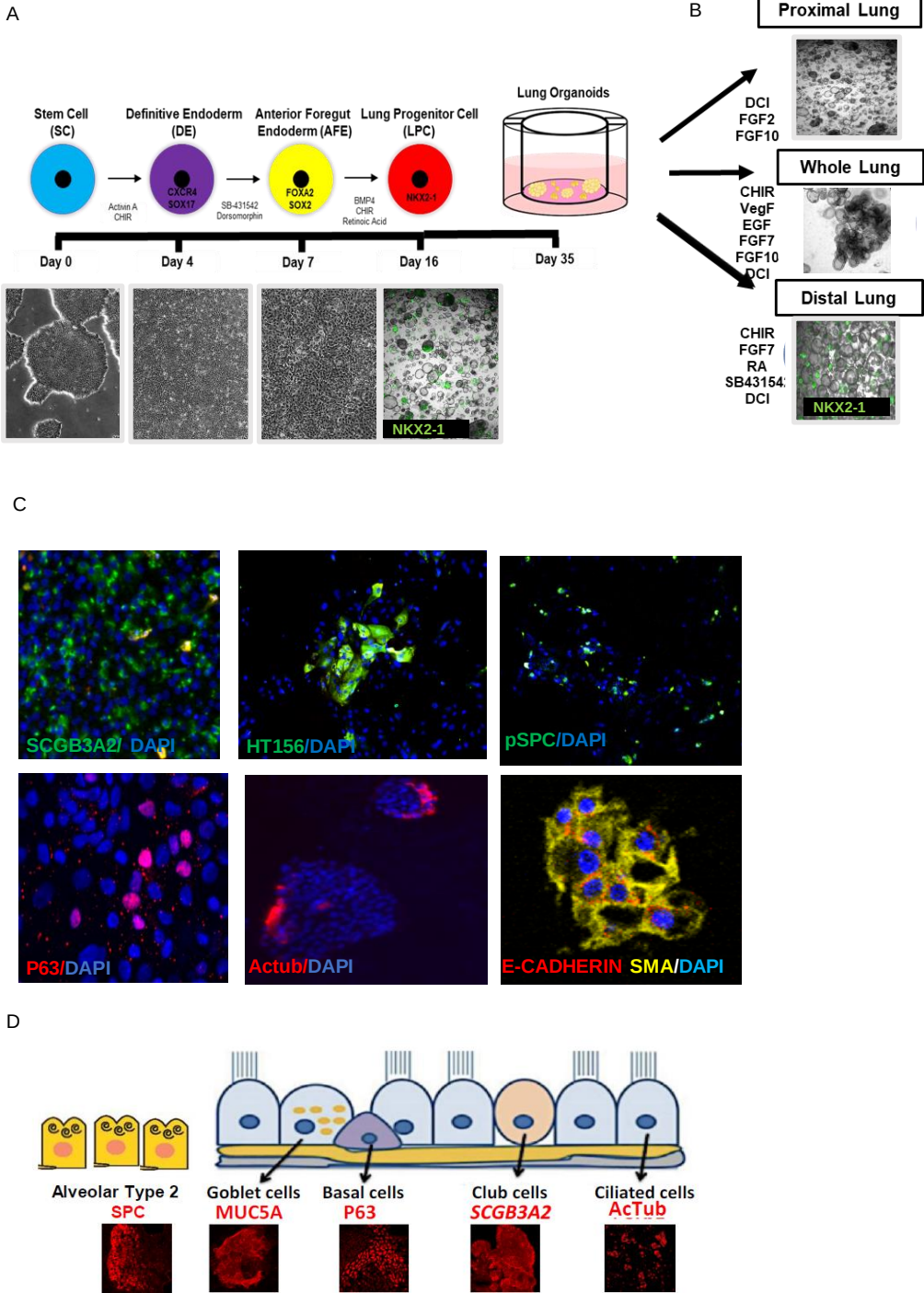


FIGURE S1: Differentiation of human pluripotent stem cells (hPSC) into multi-cell type 3D lung organoids (LOs). (A) Schematic for directed differentiation of hPSCs into 3D LOs with corresponding phase contrast images of each step, including undifferentiated hPSCs, definitive endoderm, anterior foregut endoderm, and lung progenitor cells (LPC). Image of 3D LPCs shows *NKX2-1* via GFP expression. (B) Live cell phase contrast images of the representative morphology of 3 different 3D lung organoid types: proximal (PLO), distal (DLO) and whole lung organoids (WLO). (C) Immunostaining of dissociated 3D WLOs with some representative lung epithelial and mesenchymal markers. Their respective cell types are shown in the schematic in (D). (D) Schematic of the epithelial cells in the PLOs and DLOs with representative immunofluorescent images of the epithelial markers found in the DLOs (alveolar type 2 cells) and PLOs (airway cells).

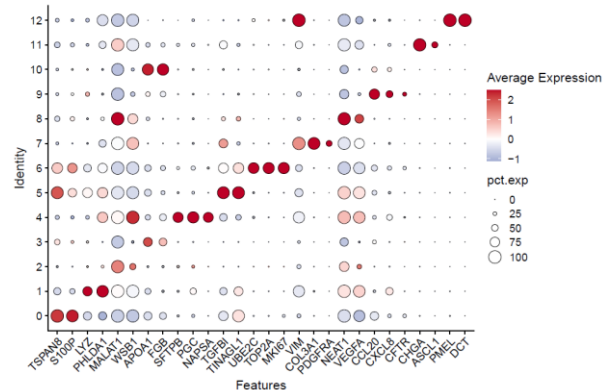
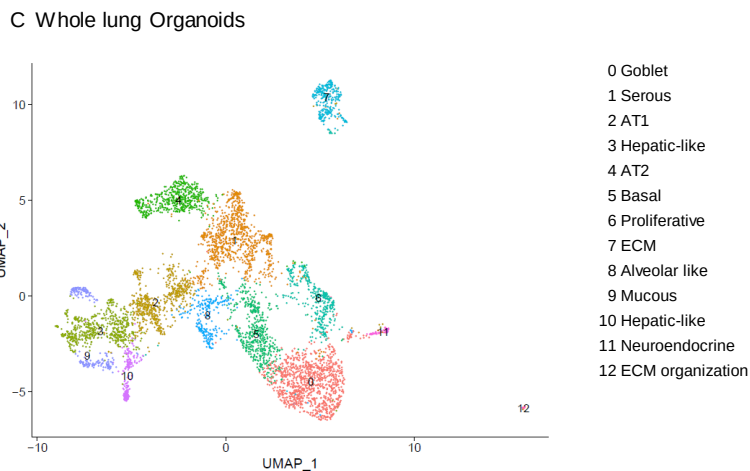
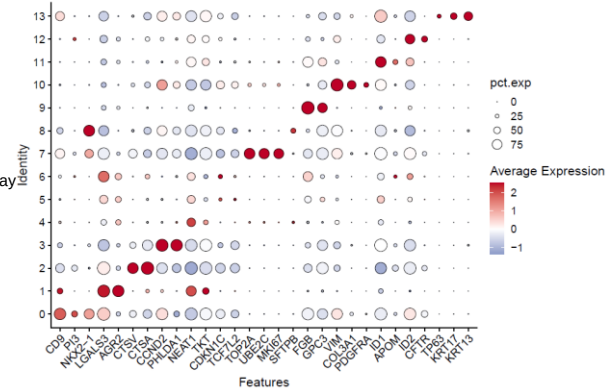
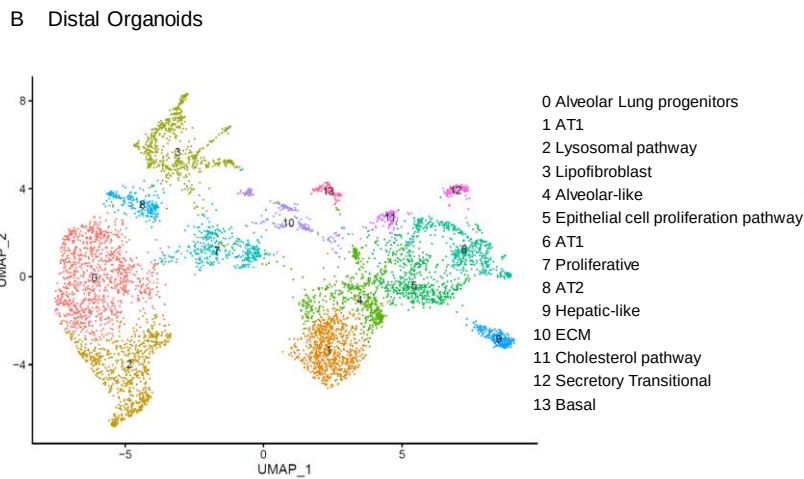
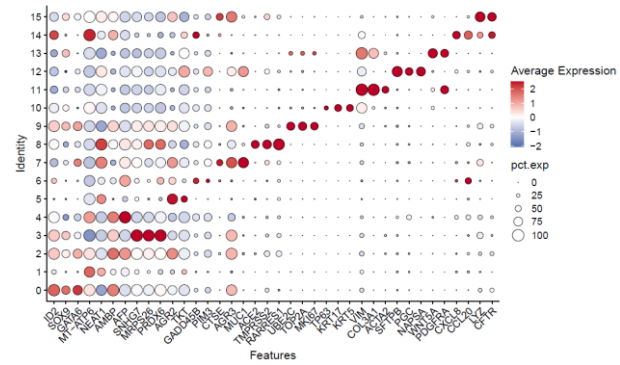
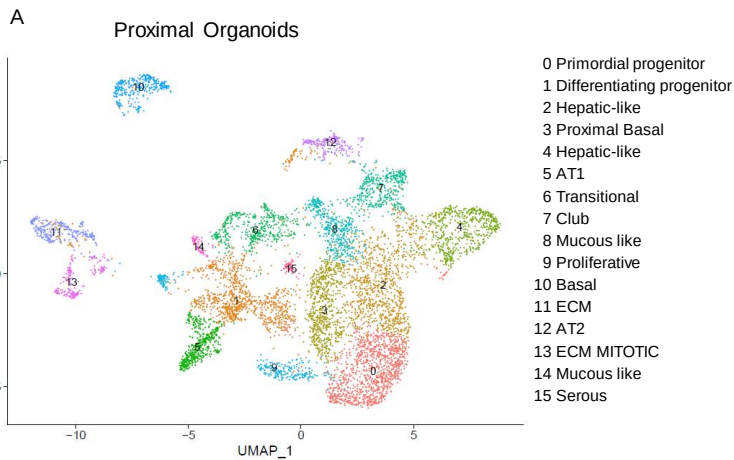


FIGURE S2: Transcriptional characterization of LOs. (A) Uniform manifold approximation and projection (UMAP) of hPSC-PLOs and characteristic genes per cluster. (B) UMAP of hPSC-DLOs and characteristic genes per cluster. (C) UMAP of hPSC-WLOs and characteristic genes per cluster.

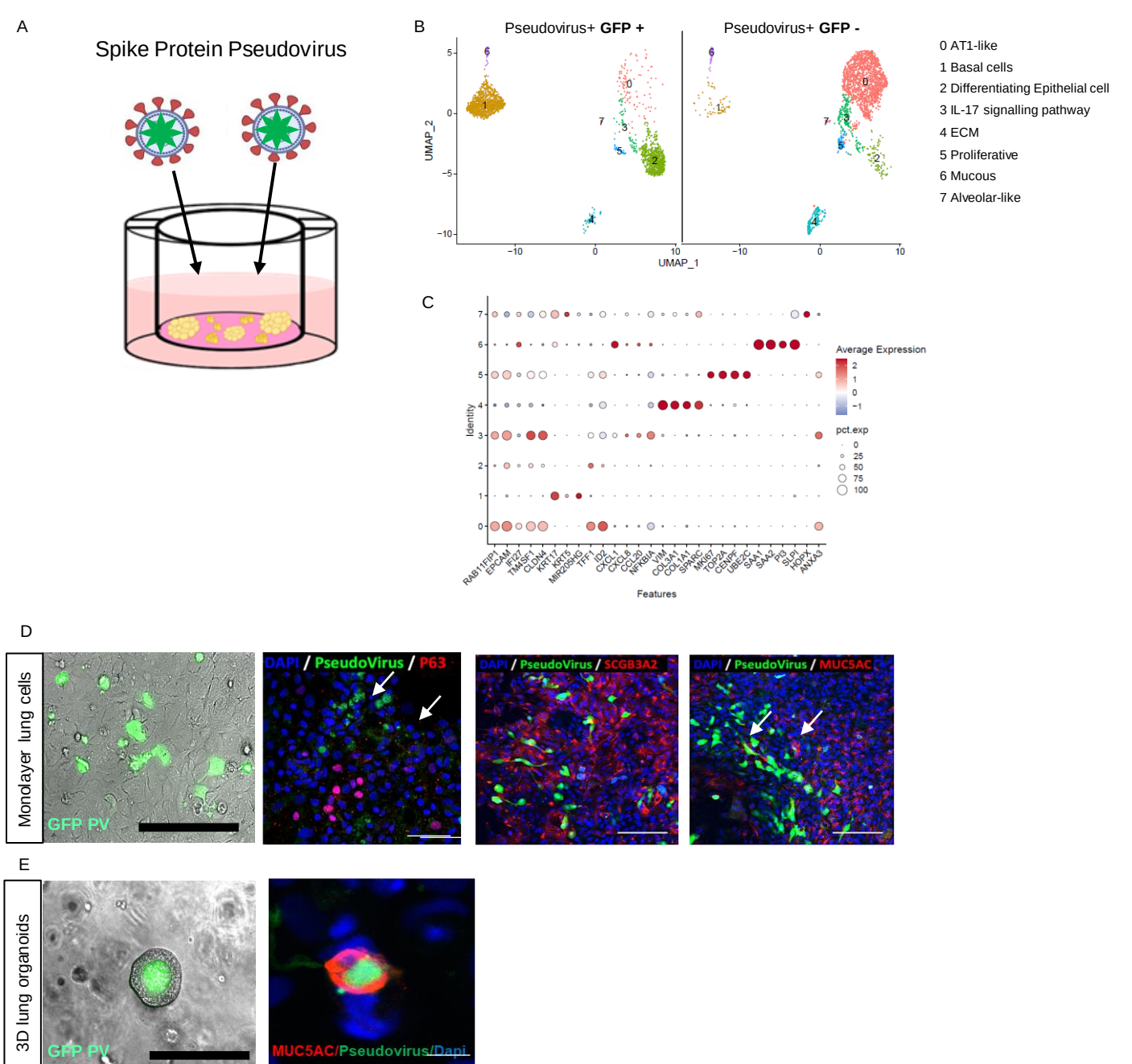
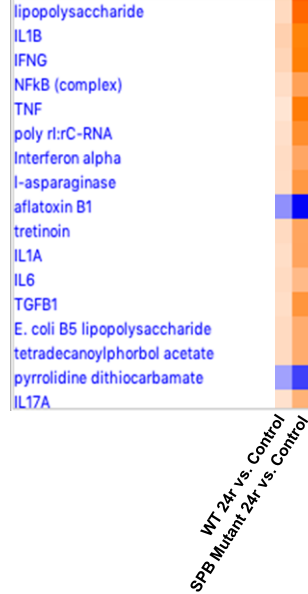


FIGURE S3: Infection of hPSC-derived lung organoids (LOs) with SARS-CoV-2 pseudovirus. (A) Schematic for SARS-CoV-2 pseudovirus infection in human lung organoids (LO). LOs were cultured in serum free basal medium and infected with SARS-CoV-2 pseudovirus for 24 hours. (B) Uniform manifold approximation and projection (UMAP) of 3D hPSC-PLOs infected with pseudovirus conjugated to a GFP reporter. The clusters show the tropism of the SARS-CoV-2 spike protein. The UMAP cluster on the left represents LOs that were exposed to pseudovirus and infected. The UMAP cluster on the right represents LOs that were exposed to the pseudovirus but were not infected. (C) Characteristic genes identifying each cluster. (D) Dual immunostaining of 3D PLOs (acutely dissociated to enable visualization of individual cells in monolayer without extensive overlapping of cells) for both identifying lung epithelial markers and pseudovirus-GFP. (E) Representative immunostaining of live, intact 3D PLOs with goblet cell marker MUC5AC and pseudovirus-GFP.

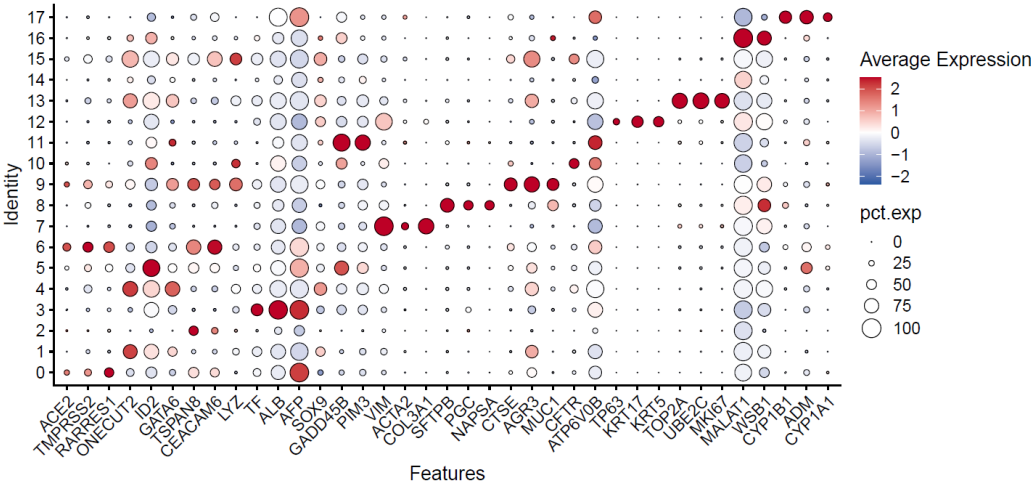
A IPA canonical pathways



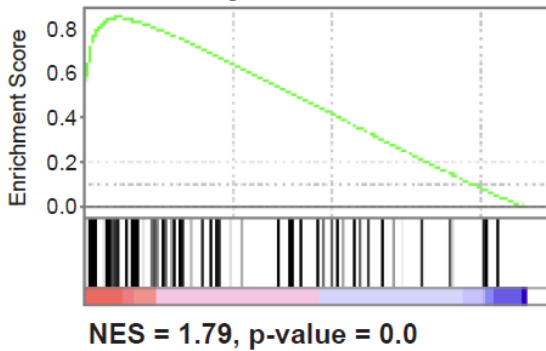
B Upstream Regulators



C Cluster identification of PLO data



D SPB Mutant, 24h Infected vs. Control IFNome90 gene set



WT, 24h Infected vs. Control IFNome90 gene set

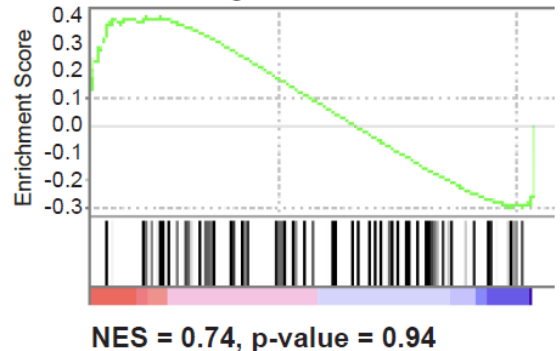
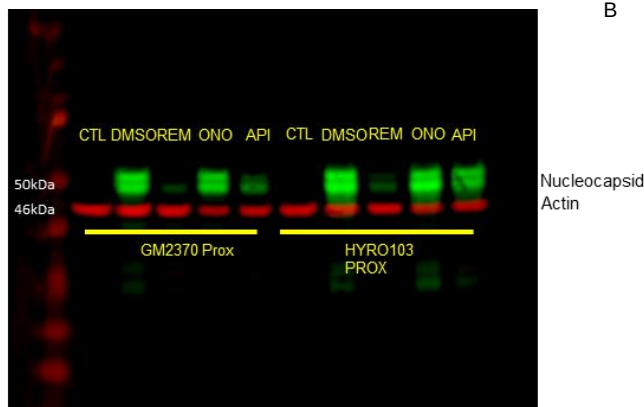


FIGURE S4: Gene enrichment pathways of infected WT and surfactant protein B (SP-B)-deficient LO cells (by virtue of a deletion mutation in a patient) vs mock infected controls. (A) Ingenuity pathway analysis (IPA) canonical pathways comparing the gene expression patterns of WT vs. SP-B deficient PLOs 24 hours post infection (hpi) with a replication-competent SARS-CoV-2 virus vs. mock-infected controls (B) IPA upstream regulators of the 24 hpi and mock infected wt and SP-B deficient PLOs. (C) Characteristic genes per cluster for the PLO single cell RNA sequencing data. (D) IFNome90 gene set enrichment scores of 24 hpi vs mock infected wild type and SP-B deficient PLOs. Positive NES defines interferon enrichment.

A



B

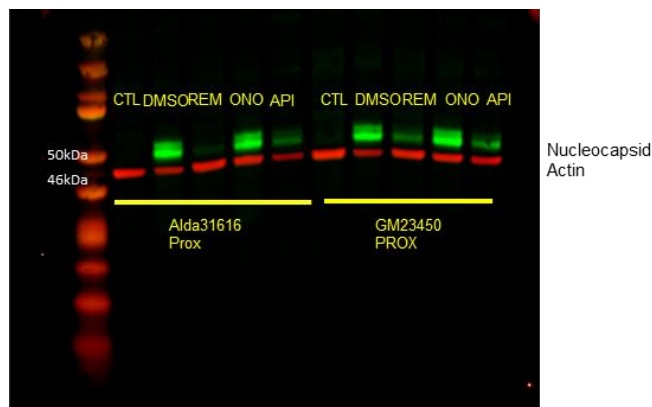
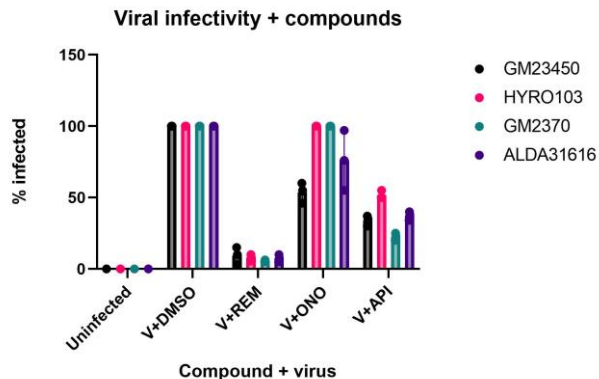
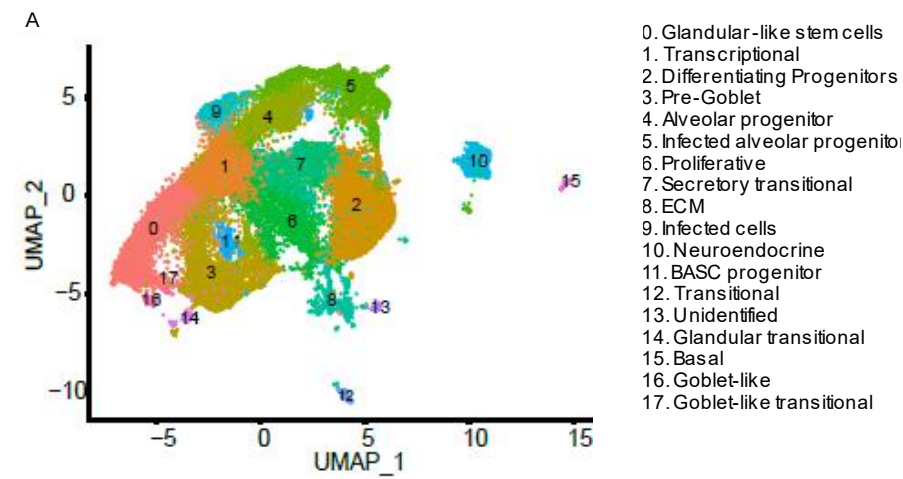


FIGURE S5: Compounds blocking various routes of viral entry and processing show different efficacies in reducing viral infections. (A) Western blot of PLOs from different racial groups, pre-treated with the endocytic blocking agent (apilimod ["API"]), the cathepsin B blocking agent (ONO5334 ["ONO"]), or an agent that blocks viral replication (Remdesivir ["REM"]) 24 hpi with SARS-CoV-2 ("V"). (B) FACS results of infected cells after exposure to the same compounds. The results are normalized to virus + DMSO. The data are representative of three independent experiments. PLO GM23450 was derived from hiPSCs generated from an African-American female, HYRO103 from a Hispanic male, GM2370 from a Caucasian female, and ALDA31616 from a Caucasian male (Supplementary Table 1).



B Cluster identification of mixed LO data

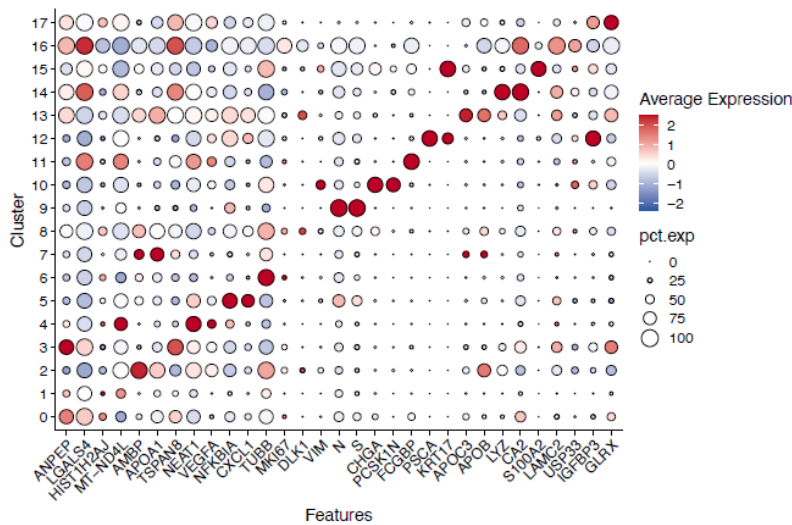


FIGURE S6: Single cell transcriptional characteristics of the hPSC-LOs. (A) Uniform manifold approximation and projection (UMAP) of hPSC-PLOs 16 hpi and (B) characteristic genes per cluster.

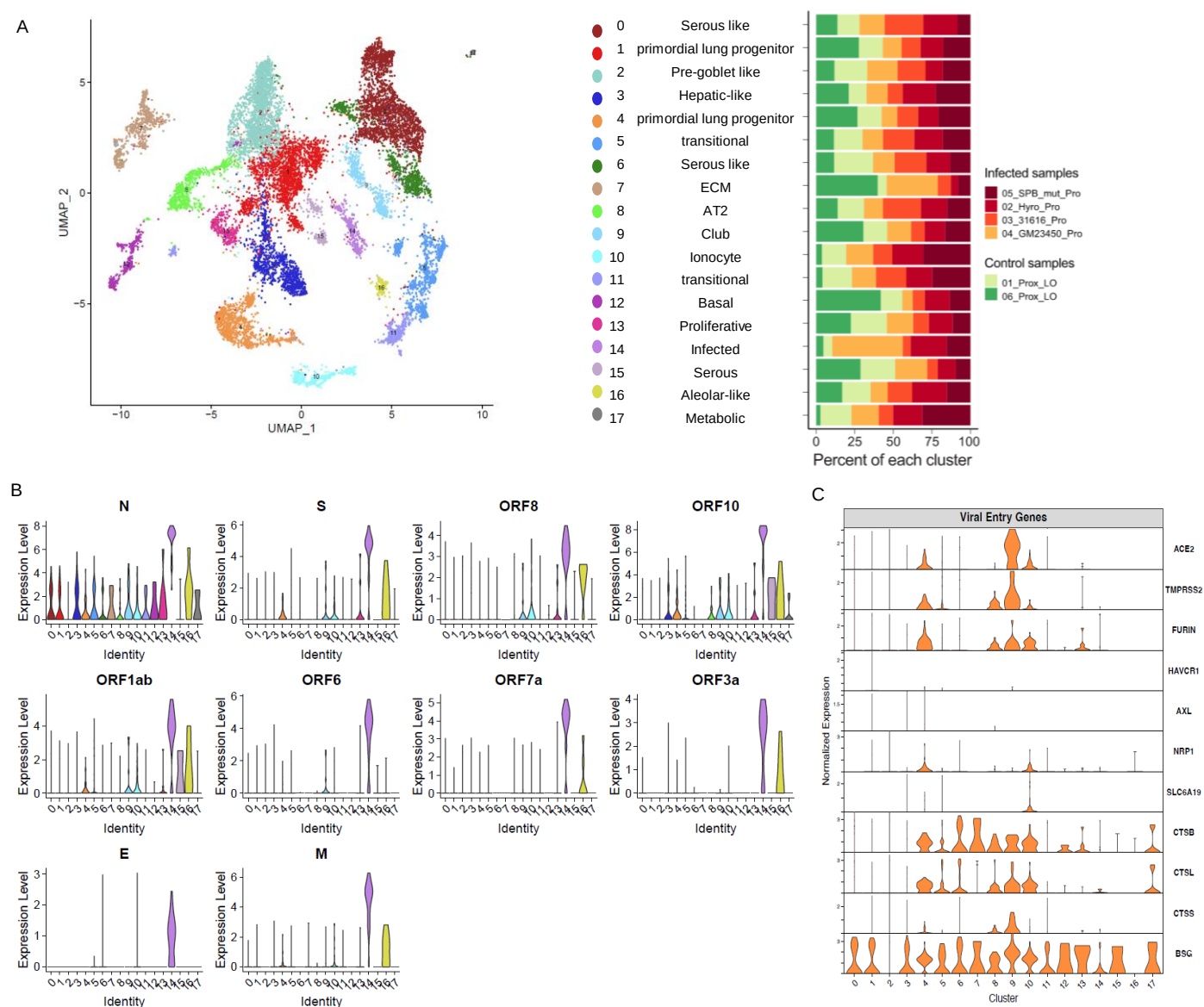


FIGURE S7: Transcriptional changes and tropism in SP-B deficient hiPSC-LOs 16 hpi with SARS-CoV-2 (A) UMAP of 3D SP-B deficient PLOs, 24 hpi with the Alpha variant. Clusters were generated via Harmony Integration of 1 SP-B deficient PLO and 3 wild type PLO independent samples. ECM = extracellular matrix (B) SARS-CoV-2 viral genes in each cluster as violin plots. The vertical bars represent the expression level. (C) Violin plots of SARS-CoV-2 entry genes in each cluster in the PLOs. Most cells in the LOs get infected by SARS-CoV-2 with viral entry either via a canonical route (ACE2 and TMPRSS2) or a non-canonical route (e.g., endocytosis/macropinocytosis).

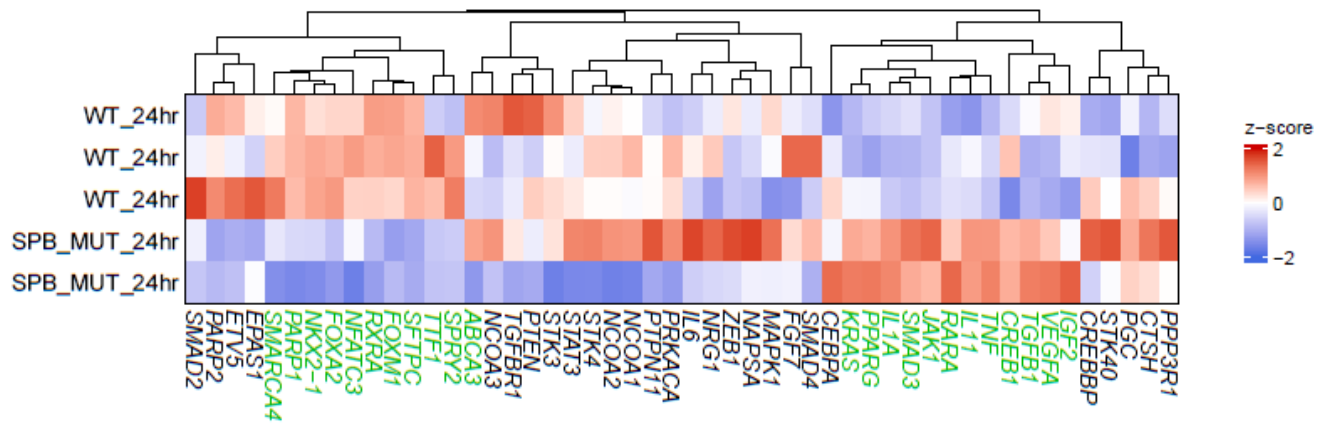


FIGURE S8: Transcriptional changes in wild type (WT) and SP-B deficient PLOs 24 hpi. Heat map showing average expression (represented as z-score) of key regulatory and transcriptional genes known to interact with surfactant protein B in WT and SP-B deficient 3D PLOs at 24 hpi with SARS-CoV-2. The genes differentially expressed between the infected wild type and SP-B deficient 3D PLOs are shown in green.

Supplemental Information

Supplementary Tables

Supplementary Table 1: Induced pluripotent stem cell lines

Cell Name	Ethnicity	Sex	Age	Tissue	Produced Method	Type	Passage	Source
HYRO103	Hispanic	Male	31	Hepatic fibroblast	Retroviral	iPSC	Not specified	ATCC
GM23450	Black/African American	Female	20	Skin	Retroviral	iPSC	29	Coriell
GM23720	White	Female	22	Peripheral vein	Episomal	iPSC	44	Coriell
Alda-31616	White	Male	25	Lymphoblastoid Cell line	Episomal	iPSC	10	NIH
hiPro133	White	Female	n/a	Skin	Retroviral	iPSC	12	CCRM
SP212-1-Cr3 Corr R11	White	Female	n/a	Skin	Retroviral	iPSC	20	CRcM

iPSC= induced pluripotent stem cell

Supplementary Table 2. Profile of poractant alfa

	Portactant Alfa
Source	Porcine
Phospholipid concentration (mg/mL)	76
Dipalmitoylphosphatidylcholine (mg/mL)	30
SP-B (mg/mL)	0.45
SP-C (μg / protein/ $\mu\text{mol L}^{-1}$ phospholipid)	5.0-11.6
Additives	No
Manufacturing	
Organic solvent extraction	Yes
Liquid-gel chromatography	Yes

Adapted from <https://curosurf.com/why-curosurf/surfactant-profiles/>

Supplementary Table 3: Materials used in lung differentiations

Name of Material/ Equipment	Company	Catalog Number
Cell Culture		
12 well plates	Corning	3512
12-well inserts, 0.4um, translucent	VWR	10769-208
2-mercaptoethanol	Sigma-Aldrich	M3148
Accutase	Innovative Cell Tech	AT104
ascorbic acid	Sigma	A4544
B27 without retinoic acid	ThermoFisher	12587010
Bovine serum albumin (BSA) Fraction V, 7.5% solution	Gibco	15260-037
Dispase	StemCellTech	7913
DMEM/F12	Gibco	10565042
FBS	Gibco	10082139
Glutamax	Life Technologies	35050061
Ham's F12	Invitrogen	11765-054
HEPES	Gibco	15630-080
Iscove's Modified Dulbecco's Medium (IMDM) + Glutamax	Invitrogen	31980030
Knockout Serum Replacement (KSR)	Life Technologies	10828028
Matrigel	Corning	354230
Monothioglycerol	Sigma	M6145
mTeSR plus Kit (10/case)	Stem Cell Tech	5825
N2	ThermoFisher	17502048
NEAA	Life Technologies	11140050
Pen/strep	Lonza	17-602F
ReleSR	Stem Cell Tech	5872
RPMI1640 + Glutamax	Life Technologies	12633012
TrypLE	Gibco	12605-028
Y-27632 (Rock Inhibitor)	R&D Systems	1254/1
Growth Factors/Small Molecules		
Activin A	R&D Systems	338-AC
All-trans retinoic acid (RA)	Sigma-Aldrich	R2625

BMP4	R&D Systems	314-BP/CF
Br-cAMP	Sigma-Aldrich	B5386
CHIR99021	Abcam	ab120890
Dexamethasone	Sigma-Aldrich	D4902
Dorsomorphin	R&D Systems	3093
EGF	R&D Systems	236-EG
FGF10	R&D Systems	345-FG/CF
FGF7	R&D Systems	251-KG/CF
IBMX (3-Isobutyl-1-methylxanthine)	Sigma-Aldrich	I5879
SB431542	R&D Systems	1614
VEGF/PIGF	R&D Systems	297-VP/CF

Supplementary Table 4: Recipes of Medias used in lung differentiations

3D organoid induction medium (day 17-22)

Serum-free basal medium (see recipe) supplemented with:

FGF7 (10 ng/mL)

FGF10 (10 ng/mL)

CHIR99021 (3 μ M)

EGF (10 ng/mL)

3D organoid branching medium (day 23-28)

3D organoid induction medium (see recipe) supplemented with:

All-trans retinoic acid (0.1 μ M)

VEGF/PIGF (10 ng/mL)

3D organoid maturation medium (day 29-34)

3D organoid branching medium (see recipe) supplemented with:

Dexamethasone (50 nM)

Br-cAMP (100 μ M)

IBMX (100 μ M)

3D Proximal lung organoid medium (day17-30)

Serum-free basal medium (SFBM) supplemented with:

FGF10 (100ng/ml)

bFGF (250ng/ml)

Y27632 (10 μ M)

Dexamethasone (50 nM)

Br-cAMP (100uM)

IBMX (100uM)

3D Distal lung organoid medium (day17-30)

Distal- Serum-free basal medium (SFBM) supplemented with:

CHIR99021 (3 μ M)

SB431542 (10 μ M)

FGF7 (10ng/ml)

RA (0.1uM)

Dexamethasone (50 nM)

Br-cAMP (100uM)

IBMX (100uM)

APE induction medium (day 4-6)

Serum-free basal medium (see recipe) supplemented with:

SB431542 (10 μ M)

Dorsomorphin (2 μ M)

DE induction medium (day 1-3)

48.5 mL RPMI1640 + Glutamax

1 mL B27 without retinoic acid

500 μ l HEPES (1%)

500 μ l pen/strep

Human activin A (100 ng/mL)

CHIR99021 (5 μ M) - only in the first 24 hours

LPC induction medium (day 7-16)

Serum-free basal medium (see recipe) supplemented with:

BMP4 (10 ng/mL)

All-trans retinoic acid (RA) (0.1 μ M)

CHIR99021 (3 μ M)

Quenching medium 50ml

49 mL DMEM/F12
1 mL FBS
500ul Pen/Strep

Serum-free basal medium (SFBM) 500ml

375 mL Iscove's Modified Dulbecco's Medium (IMDM) + Glutamax
125 mL Ham's F12
5 mL B27 without retinoic acid
2.5 mL N2
500 µl ascorbic acid, 50 mg/mL
19.5 µl monothioglycerol, 500 µg/mL
3.75 mL bovine serum albumin (BSA) Fraction V, 7.5% solution
5ml pen/strep

Distal Lung Organoid Serum-free base medium (DLO-SFBM) 500ml

475mL F12
16.65mL BSA
7.5mL HEPES
400µL CaCl₂
500µL ITS
5mL Pen/Strep
5ml B27 without retinoic acid

Stem cell passaging medium (day 0) 50ml

50 mL DMEM/F12 with Glutamax
13 mL Knockout serum replacement (KSR)
650 mL NEAA
130uL 2-mercaptoethanol
650uL pen/strep

Supplementary Table 5. SARS-CoV-2 strains

Strain Name	Pango Lineage	Greek Name	Original sample sequencing links
USA/WA1-2020	A	none "WA1"	GenBank: MN985325.1
USA/NY-PV08410/2020	B.1	none "D614G"	GenBank: MT370900.1 and GISAID: EPI_ISL_421374
hCoV-19/USA/CA_UCSD_5574/2020	B.1.1.7	Alpha	GISAID: EPI_ISL_751801
hCoV-19/South Africa/KRISP-K005325/2020	B.1.351	Beta	GISAID: EPI_ISL_678615 with changes during passage at BEI listed in BEI datasheet https://www.beiresources.org/Catalog/animalviruses/NR-54009.aspx
hCoV-19/Japan/TY7-503/2021	P.1	Gamma	GISAID: EPI_ISL_792683
hCoV-19/USA/PHC658/2021	B.1.617.2	Delta	none, changes from reference listed in BEI datasheet (https://www.beiresources.org/Catalog/animalviruses/NR-55611.aspx) and supplement to original publication (https://www.nejm.org/doi/suppl/10.1056/NEJMc2107799/suppl_file/nejmc2107799_appendix.pdf)
hCoV-19/USA/CA-SEARCH-59467/2021	BA.1.20	Omicron	GISAID: EPI_ISL_8186377

Supplementary Table 6. Antibodies

Primary antibodies	Company	Catalogue #	Dilution rate
Nucleocapsid	GeneTex	GTX135357	1:1000
Spike antibody	GeneTex	GTX632604	1:1000
ACE2	Cell signaling	4355S	1:500
MUC5AC	Millipore	MAB2011	1:200
SOX9	R&D Systems	AF3075	1:200
SCGB3A2	abcam	ab181853	1:300
HOPX	Santa Cruz Biotech	sc-398703	1:200
proSPC	abcam	ab40871	1:250
AcTub			1:600
SPC (mature)	LS Bio	LS-B9161	1:300
SPB (mature)	7 Hills	48604	1: 500 (I) 1:500 (W) ^a
SPA			
SPD			
HTI-56	Terrace Biotech	TB-29AHT1-56	1:150
p63	Boster	PB9152	1:250
E-cadherin			
SMA			

Secondary Antibodies	Company	Catalogue #	Dilution rate
Donkey anti-mouse IgG (Alexa 488)	Abcam	Ab150105	1:500
Donkey anti-mouse IgG (Alexa 555)	Abcam	Ab150106	1:500
Donkey anti-mouse IgG (Alexa 647)	Abcam	Ab150107	1:500
Donkey anti-rabbit IgG (Alexa 488)	Abcam	Ab150073	1:500
Donkey anti-rabbit IgG (Alexa 555)	Abcam	Ab150074	1:500
Donkey anti-rabbit IgG (Alexa 647)	Abcam	Ab150075	1:500
Donkey anti-goat IgG (Alexa 488)	Abcam	Ab150129	1:500
Donkey anti-goat IgG (Alexa 555)	Abcam	Ab150130	1:500
Donkey anti-goat IgG (Alexa 647)	Abcam	Ab150131	1:500

SUPPLEMENTARY VIDEOS

Supplementary Video #1: <https://giphy.com/gifs/jTwljBoVJgmz0rFArn>

Supplementary Video #2: <https://giphy.com/gifs/eHEUP2X6fn4cPC5dp2>

Supplementary Video #3: [<https://giphy.com/gifs/ZBhrTM9f1j9FQqgXWq>]