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Supplemental Information

Human Pluripotent Stem Cell-Derived Intestinal Organoids Model SARS-CoV-2 Infection Revealing a Common Epithelial Inflammatory Response

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SUPPLEMENTAL INFORMATION

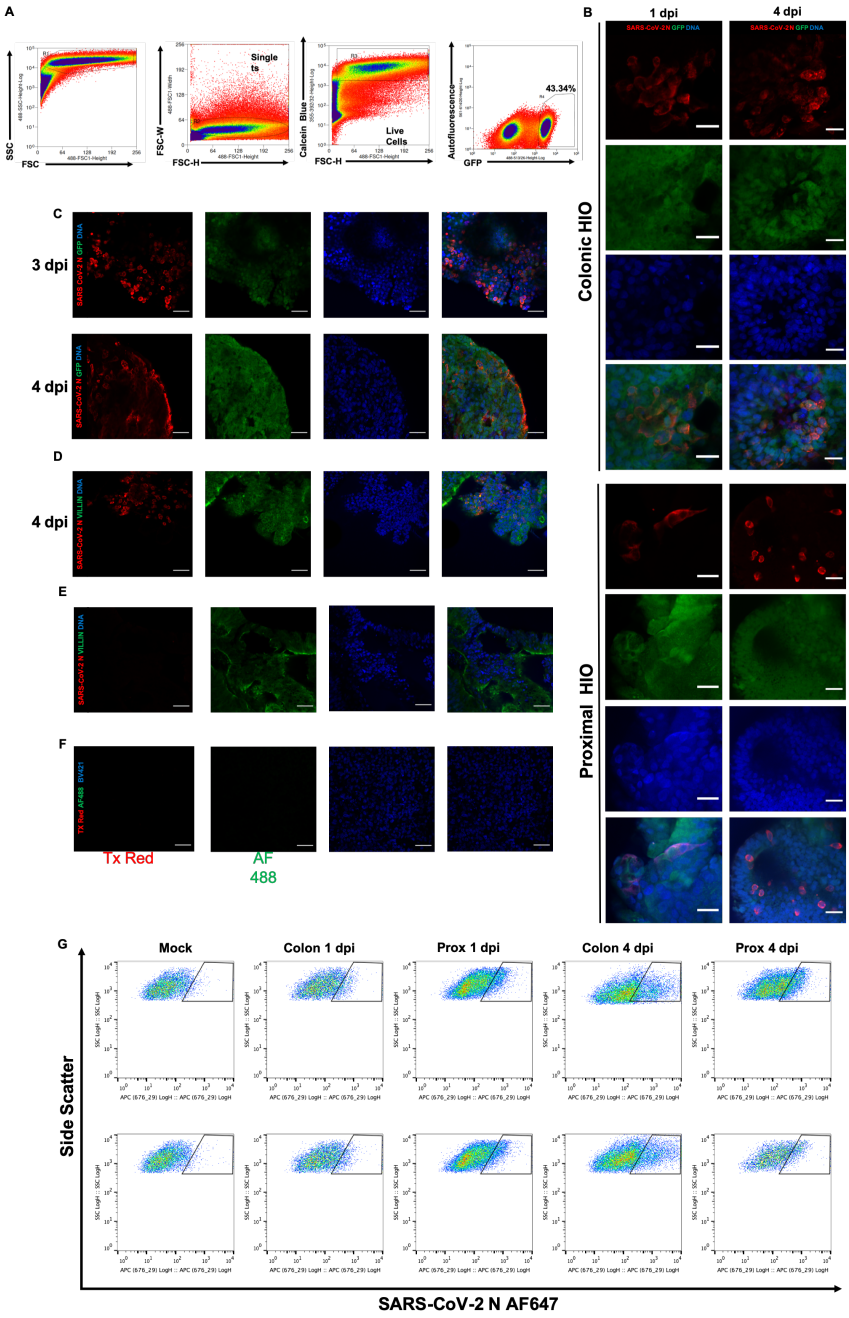

Figure S1. Further characterization of regionally patterned HIOs and subsequent SARS-CoV-2 infection. (A) FACS gating strategy for sorting D15 CDX2-eGFP^{+} intestinal progenitors during directed differentiation of mesenchyme free HIOs from BU1CG iPSCs. (B-F) Immunofluorescence micrographs of SARS-CoV-2 infected HIOs (B) Higher magnification confocal micrographs of whole mount colonic (top) and proximal (bottom) HIOs stained for SARS-CoV-2 N protein and GFP (scale bar = 25 µm) (C) Immunofluorescent confocal micrographs of whole mount HIOs stained for SARS-CoV-2 N protein and GFP (scale bar = 50 µm) at 3- and 4-days post infection demonstrates robust and widespread SARS-CoV-2 infection of CDX2-GFP+ intestinal epithelial cells. (D) Immunofluorescent confocal micrographs of whole mount HIOs stained for SARS-CoV-2 N protein and enterocyte cytoskeletal component Villin at 4 dpi. (E) Immunofluorescent micrographs of whole mounted mock infected day 36 HIOs stained for SARS-CoV-2 N protein and Villin. (F) Whole mounted SARS-CoV-2 4 dpi HIOs demonstrating minimal background signal from staining with only secondary antibodies. (scale bar = 50 µm) (G) Flow Cytometry for SARS-CoV-2 N protein expression in HIOs at 1 and 4 dpi, gated on single cells by forward and side scatter (n=2 replicates per condition), Related to Figures 1-2.

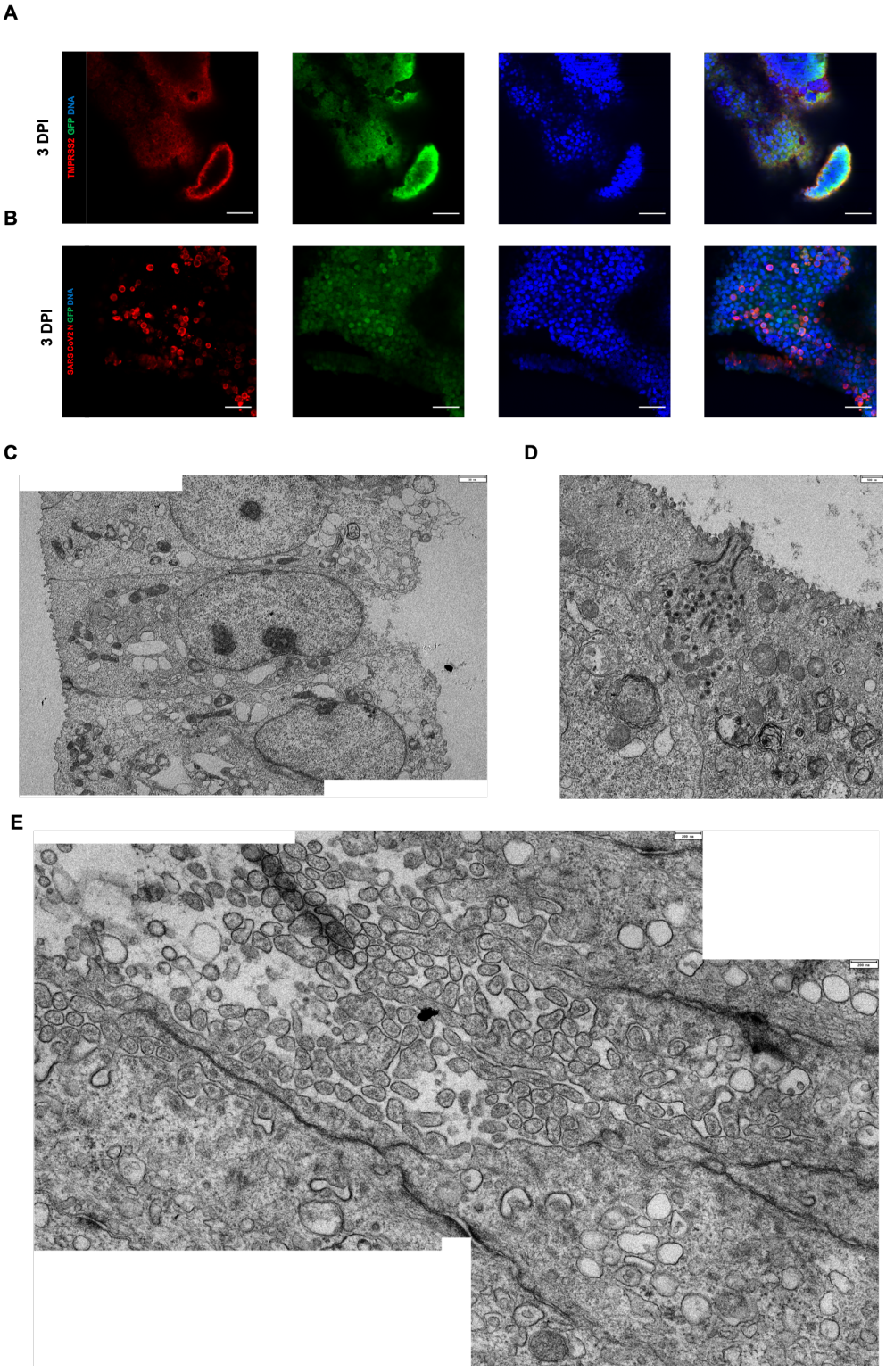

Figure S2: Further characterization of infected and mock HIOs. (A) Differentiated iPSC-derived colonic HIOs express TMPRSS2 and (B) SARS-CoV-2 Nucleocapsid at 3 dpi. (Scale bar = 50 µm). (C-E) Electron microscopy of mock colonic and proximal HIOs showing putative enterocytes with characteristic brush border and polarized nuclei (C) and putative secretory cells containing high density secretory granules (D-E) (scale bars noted in each image), Related to Figure 2-3.

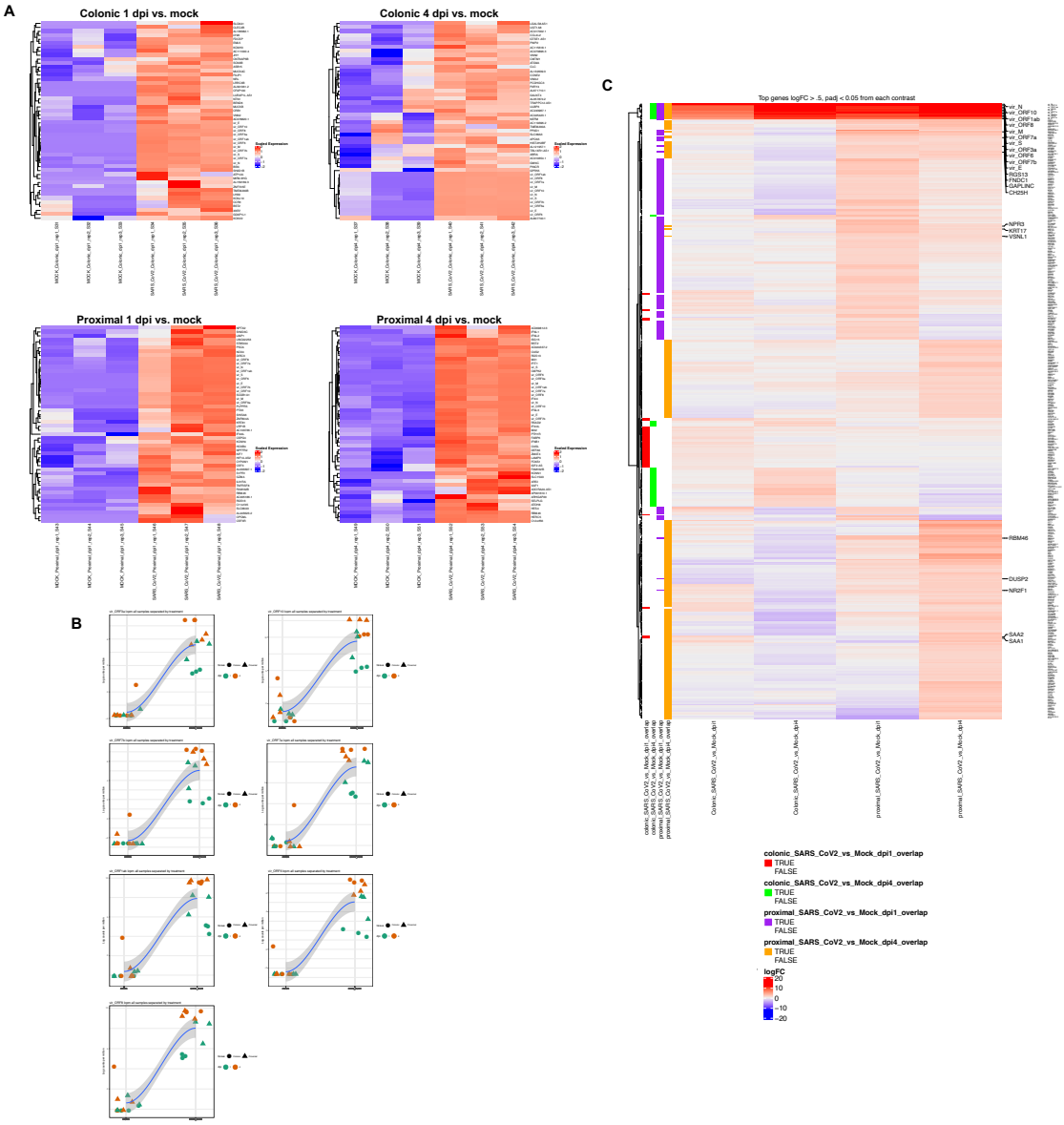

Figure S3: Additional bulk RNA sequencing analysis of SARS-CoV-2 infected HIOs. (A) Unsupervised hierarchical clustering heat maps of the top 50 differentially expressed genes with each replicate independently represented. (B) LOESS plots of additional viral genes in mock (left) and infected (right) HIOs in both tissue types at 1 and 4 dpi. (C) Unsupervised hierarchical clustering heatmap of differentially expressed genes in infected vs. mock samples at 1 and 4 dpi (LogFc>0.5, FDR <0.05), Related to Figure 4.

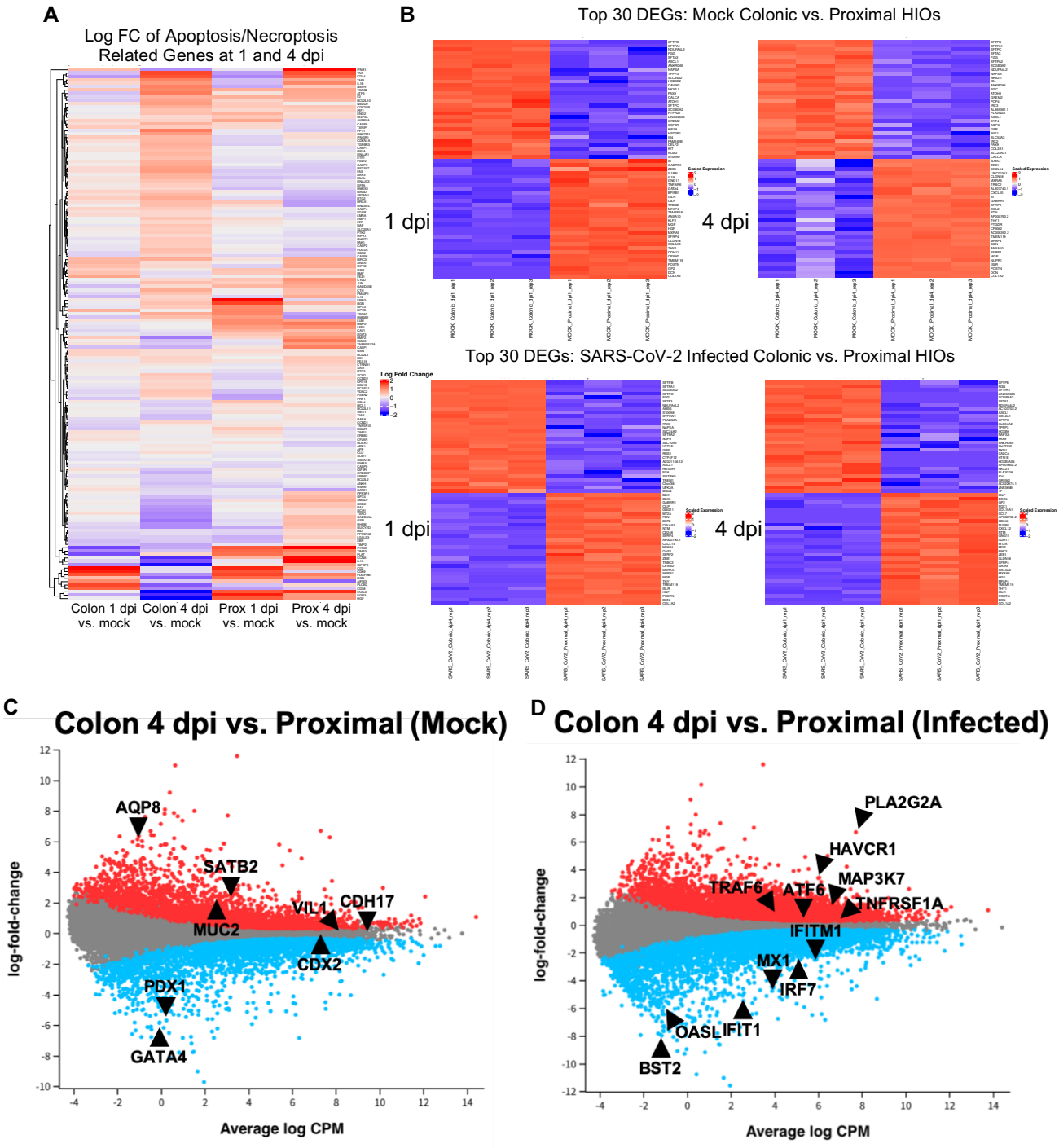

Figure S4: Differential baseline and host response to infection between colonic and proximal HIOs. Unsupervised hierarchical clustering heat maps analyzing expression of (A) a panel of genes associated with apoptosis and necroptosis by log fold change and (B) of the top 30 differentially expressed genes comparing colonic HIOs to proximal HIOs at 1 and 4 dpi, separating the mock and infected conditions, with each replicate independently represented. (C) Mean average plots comparing the transcriptional state of mock infected colonic vs. proximal HIOs at 4 dpi, with key phenotypic markers of proximal and distal intestinal epithelium highlighted by the black arrowheads. (D) Mean average plots comparing the transcriptional state of SARS-CoV-2 infected colonic vs. proximal HIOs at 4 dpi, with key viral host response markers highlighted by the black arrowheads. Related to Figure 4.

Table S1: Media Recipes/Composition, Related to Figures 1 - 4			
Proximal (IM+CK)	DMEM F/12	Noggin	500ng/mL
	Primocin (100ng/mL)	R-Spondin	100ng/mL
	B27	EGF	100ng/mL
	HEPES	CHIR99021	3 μM
	Glutamax (100x)	KGF/FGF7	10ng/mL
	N2		
Complete Serum Free Differentiation Medium (csFDM)	IMDM (75%)		
	Ham's F/12 (25%)		
	B27 (with RA)		
	N2		
	0.05% BSA		
	Primocin (100ng/mL)		
	Glutamax (100x)		
	Ascorbic Acid (50ug/mL)		
	MTG (0.45mM)		
Colonic (CK+DCI)	cSFDM	CHIR99021	3μM
		KGF/FGF7	10ng/mL
		Dexamethasone	50nM
		cAMP	0.1mM
		3-isobutyl-1-methyxanthine [IBMX]	0.1mM

Table S2: Comprehensive List of Reagents, Related to Figures 1 - 4		
REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Antibodies		
Calcein Blue	Life Technologies	C1429
Donkey serum	Jackson Immunoresearch Labs	017-000-121
13 mm cover slips	ThermoFisher Scientific	174950
Fluoromont -G	Southern Biotech	0100-01
Rabbit anti-SARS-CoV-2 N	Rockland Immunochemicals	Cat# 200-401-A50
Mouse anti-Villin	Millipore	MAB1671
Hoechst 33342	ThermoFisher Scientific	62249
Chicken Anti- GFP IgY	ThermoFisher Scientific	A10262
Donkey anti-chicken AF488	Jackson Immunoresearch Labs	703-545-155
Anti-TMPRSS2	Abcam	Ab 92323
Bacterial and Virus Strains		
SARS-CoV-2 IsolateUSA_WA1/2020	Kindly provided by CDC's Principal Investigator Natalie Thornburg and the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA	N/A
Chemicals, Peptides, and Recombinant Proteins		
Growth Factor Reduced Matrigel	Corning	356230
Matrigel Basement Membrane Matrix	Corning	354234
SB431542	Tocris	1614
Dorsomorphin	Stemgent	04-0024
CHIR99021	Tocris	4423
Recombinant human KGF	R&D Systems	251-KG-010
Recombinant human BMP4	R&D Systems	314-BP
Retinoic acid	Sigma	R2625
Y-27632 dihydrochloride	Tocris	1254
Dexamethasone	Sigma	D4902
8-bromoadenosine 3′,5′-cyclic monophosphate sodium salt (cAMP)	Sigma	B7880
3-Isobutyl-1-methylxanthine (IBMX)	Sigma	I5879
0.05% trypsin-EDTA	Invitrogen	25300-120
Defined Fetal Bovine Serum	Thermo Fisher	NC0652331
Recombinant human Noggin	R&D Systems	6057NG025
Recombinant human EGF	R&D Systems	236EG200

Recombinant Human R-Spondin 1 Protein	R&D Systems	4645-RS-025
Ascorbic Acid	Sigma	A4403
Critical Commercial Assays		
RNeasy Mini Kit	QIAGEN	79306
NEBNext Low Input RNA Kit	New England Biolabs	E6420
SuperScript™ III First-Strand Synthesis System	Invitrogen	18080093
Deposited Data		
Bulk RNA-seq	This paper	GEO: GSE159201
Experimental Models: Cell Lines		
Human: Normal donor iPSC line targeted with CDX2-eGFP (BU1CG)	Mostoslavsky Lab (Mithal et al, 2020)	http://stemcellbank.bu.edu
Oligonucleotides		
Taqman Gene Expression Assay Primer/Probe	N/A	N/A
CDH17	Thermo Fisher	Hs00900408_m1
GAPDH	Thermo Fisher	Hs99999905_m1
CDX2	Thermo Fisher	Hs01078080_m1
LYZ	Thermo Fisher	Hs00426232_m1
VIL1	Thermo Fisher	Hs00200229_m1
ACE2	Thermo Fisher	Hs01085333_m1
TMPRSS2	Thermo Fisher	Hs01122322_m1
SARS-CoV-2 N	IDT	10006606
Software and Algorithms		
FlowJo	TreeStar, Inc	https://www.flowjow.com
Prism 8.0	Graphpad, Inc	https://www.graphpad.com
ImageJ	National Institutes of Health	https://imagej.nih.gov/ij
Other		
Cell Recovery Solution	Corning	354253
Paraformaldehyde	Electron Microscopy Sciences	19208
ReLeSR	StemCell Technologies	05873
StemDiff Definitive Endoderm Kit	StemCell Technologies	05110
N2 Supplement	Invitrogen	17502-048
B27 Supplement	Invitrogen	12587-010
GlutaMAX™	Thermo Fisher	35050061
Gentle Cell Dissociation Reagent	StemCell Technologies	07174