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Supplementary information

A human three-dimensional neural-perivascular 'assembloid' promotes astrocytic development and enables modeling of SARS-CoV-2 neuropathology

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Supplementary Information

A Human 3D neural-perivascular 'assembloid' promotes astrocytic development and enables modeling of SARS-CoV-2 neuropathology

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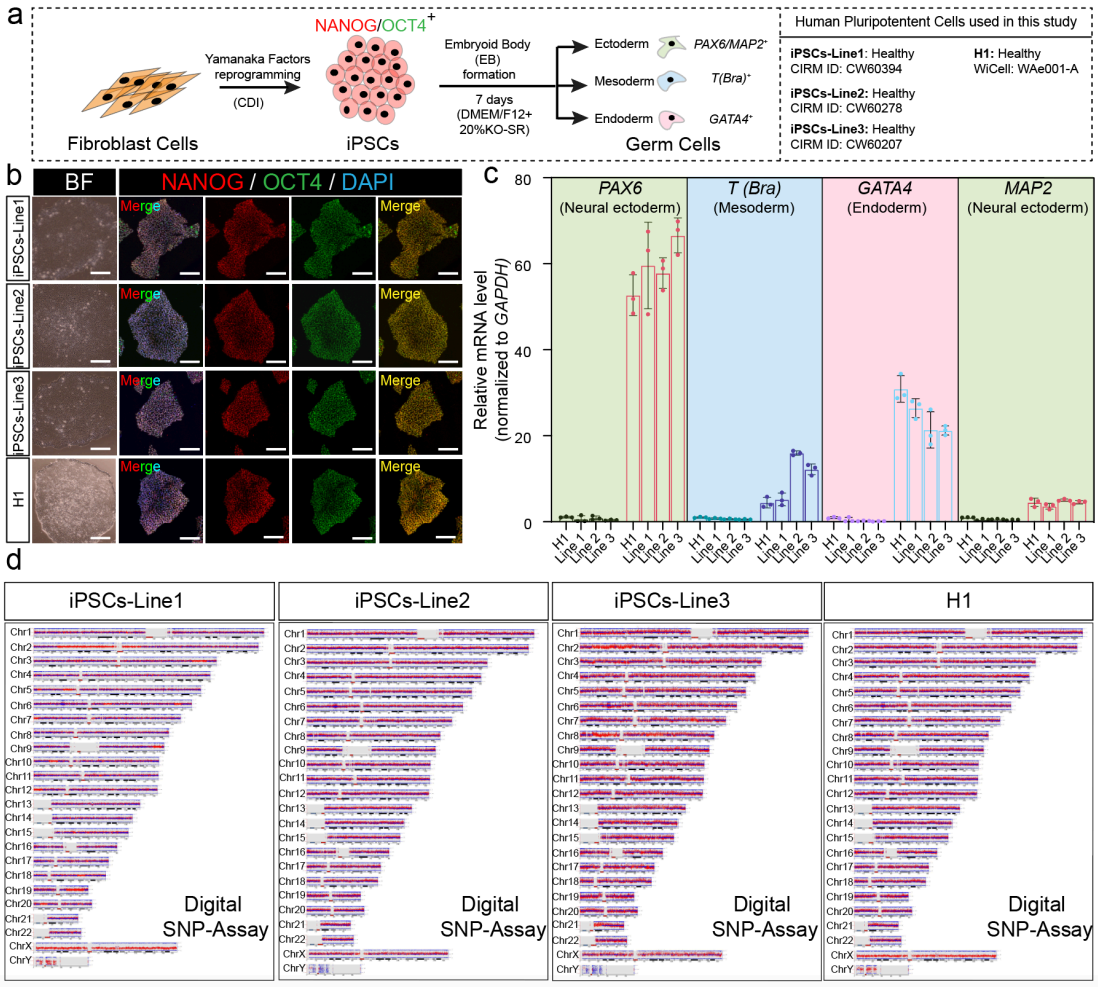
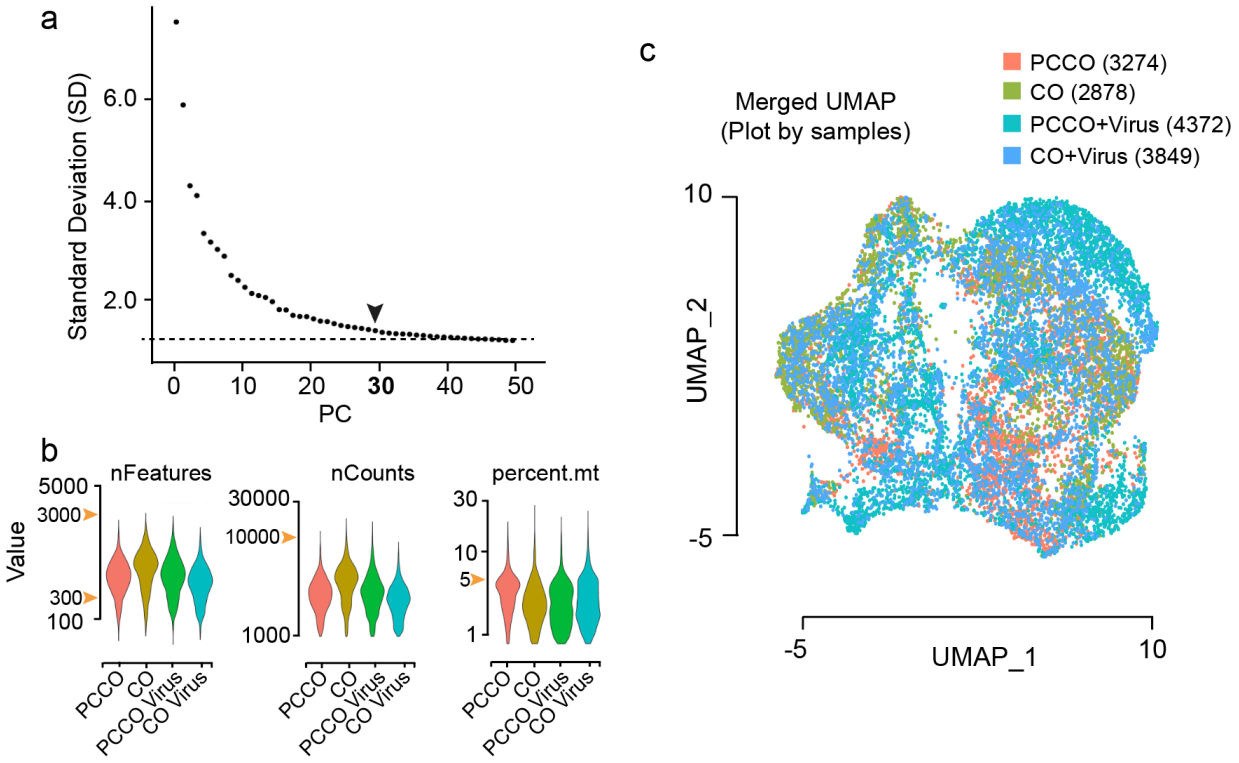
Supplementary notes

Supplementary Figure. 1 Characterization of human pluripotent stem cells (hPSCs).



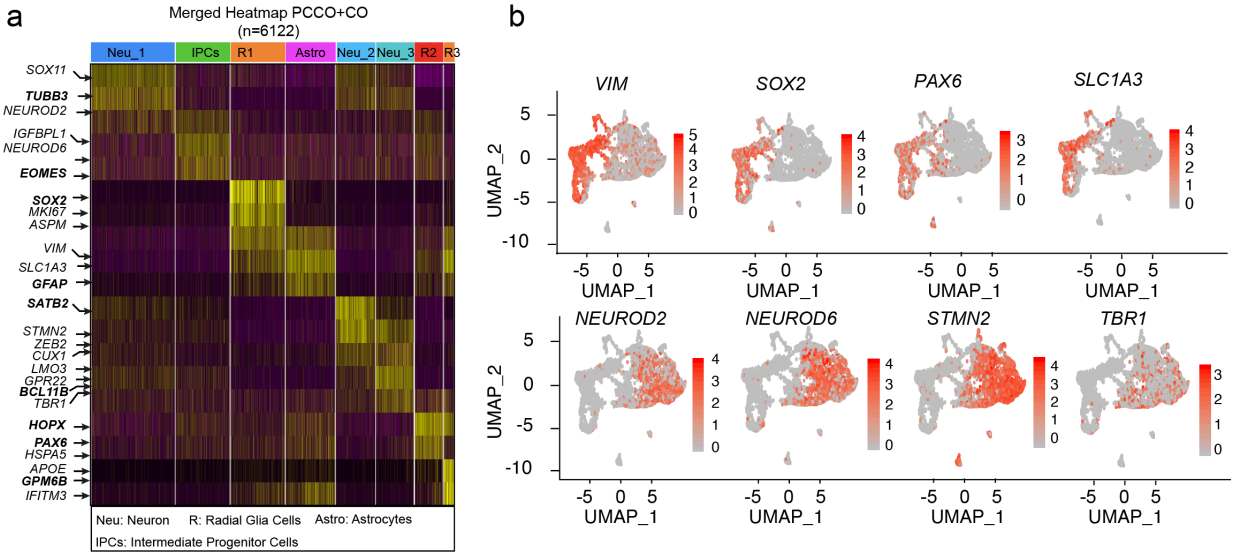
a. Protocol for generation and validation of human pluripotent stem cells (hPSCs). Fibroblast cells from three healthy individuals in three unrelated families were reprogrammed into induced pluripotent stem cells (iPSCs) at Cellular Dynamics Inc (CDI). hiPSCs were then stained against NANOG and OCT4 to show self-renewal ability. In parallel embryoid bodies (EBs) were generated from the iPSC lines to confirm their potential to differentiate into three germ layers. *PAX6/MAP2* were used to label the ectoderm (neuronal fate), *T* represents mesoderm, and *GATA4* marks endoderm. **b.** NANOG co-visualized with OCT4 for the hPSC lines used in this study. DAPI: nuclei, Bar: 400μm. Green: OCT4, Red: NANOG, pictures were taken from two biological replicates (cell lines cultured under independent researchers) and three different regions from each line. **c**. qPCR shows mRNA expression of three germ layers markers. EBs were harvested at DIV 7 for qPCR analysis. *PAX6/MAP2* were used to label the ectoderm, *T* represents the mesoderm, and *GATA4* marks the endoderm. n=3 includes 3 different biological replicates (including three differentiations done by two people at different times), error bar represents mean±1SD. *GAPDH* used as reference. **d.** Digital SNP-Assays show the normal karyotypes for the hiPSC/H1 used in this study.

Supplementary Figure. 2 Quality control for sc-RNA-seq data analysis.



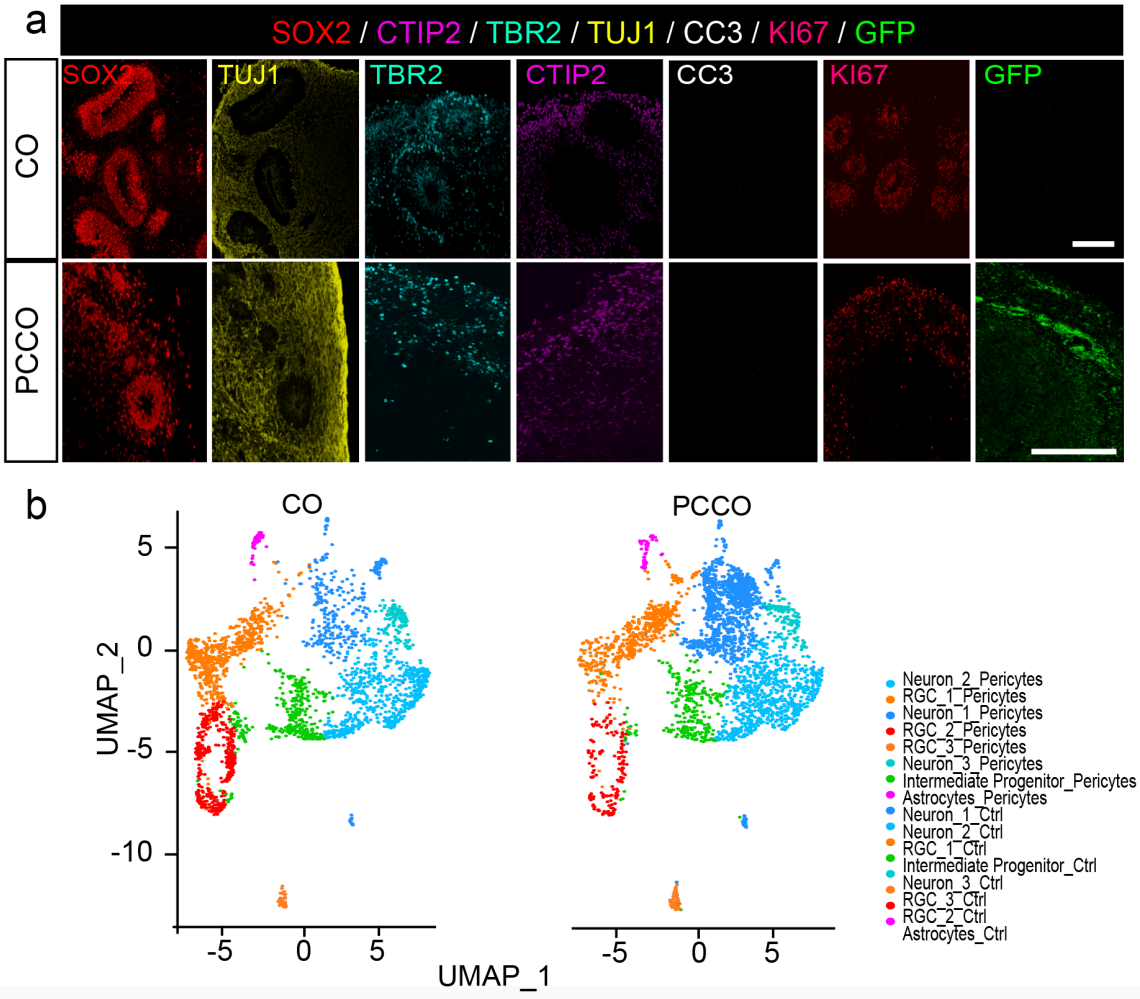
a. Plot shows the statistical standard deviation (SD) and PCs in sc-RNA-seq analysis, a total number of 50 PCs have been screened and PC=30 has been chosen for the following analysis. **b.** Violin Plot shown the total number of features, counts and percent of mitochondria DNA detected across all four samples in our sc-RNA-seq analysis. A cutoff of 300<nFeatures<3000 together with 1000<nCounts<10000 and removal of 5% mitochondria DNA for the following analysis. **c.** Merged-UMAP with different samples shown little variability. Total number of 14373 cells were captured with sc-RNA-seq.

Supplementary Figure. 3 sc-RNA-seq Heatmap shows different cell clusters.



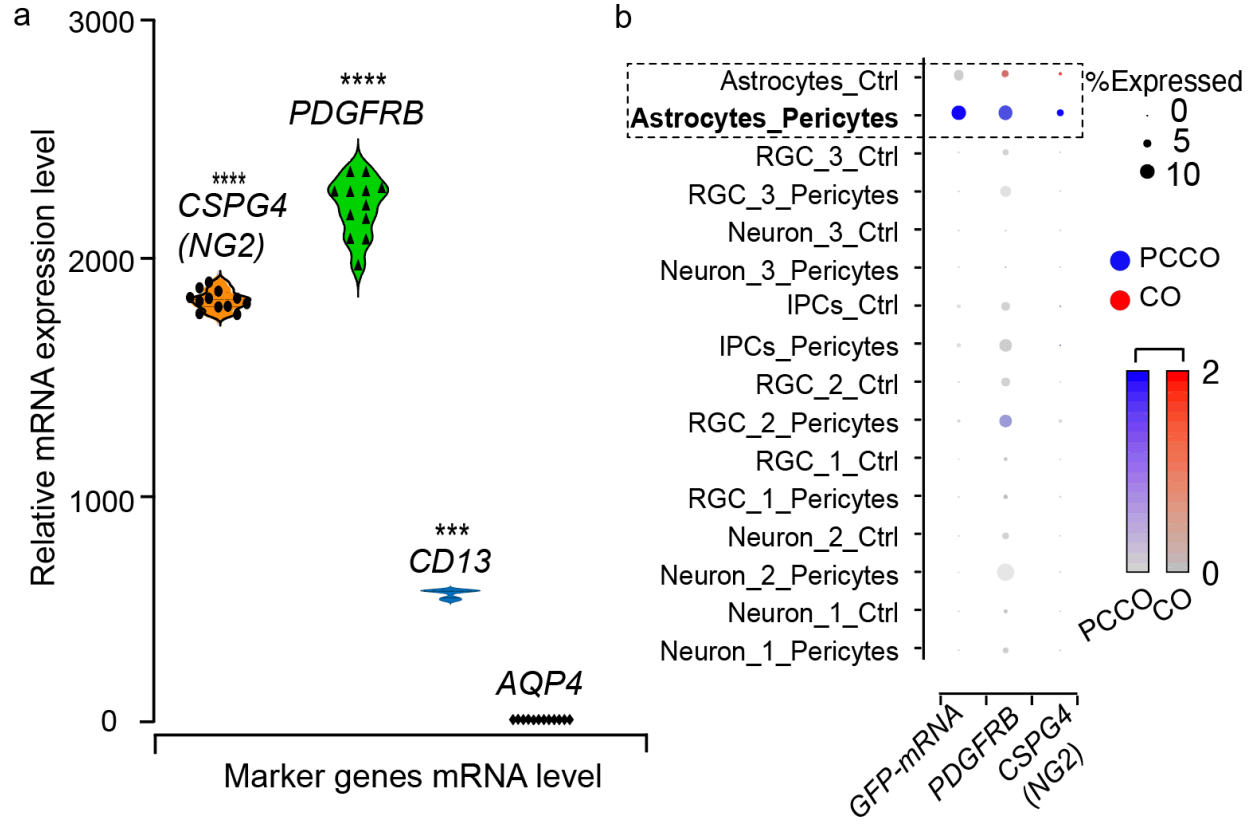
a. Merged Heatmap of COs and PCCOs shows the top genes expressed in different cell clusters. Total number of 6122 cells were captured with sc-RNA-seq. Standard marker genes were used to define different cell clusters. **b.** UMAPs show different marker expression across different cell clusters.

Supplementary Figure. 4 PCCOs retain COs primary cellular composition and structure.



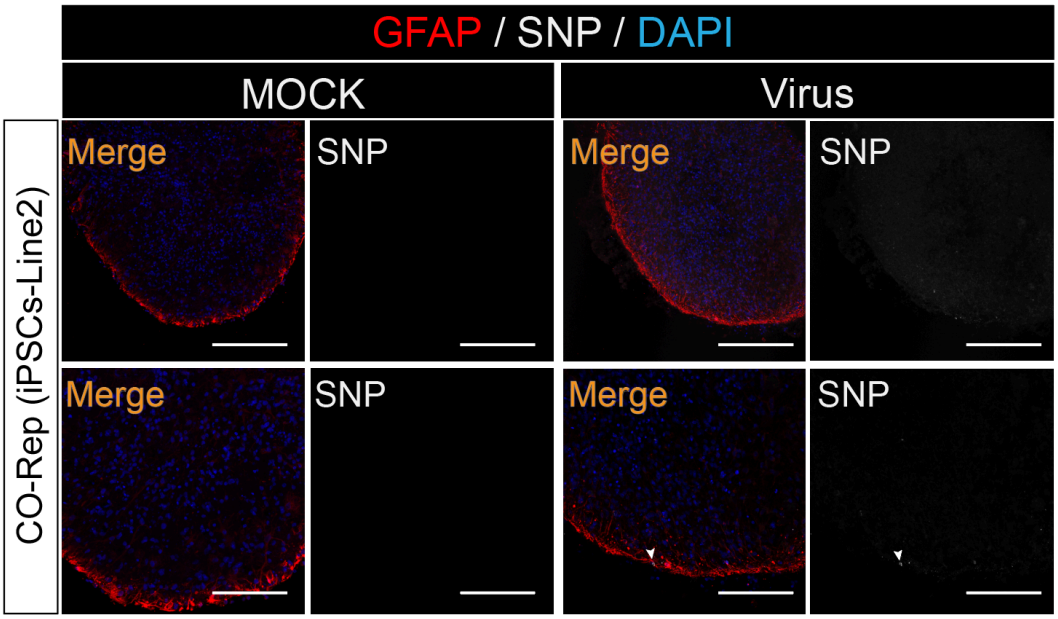
a. Split UMAPs show the cell compositions in CO and PCCO. **b.** Immunostaining against SOX2 (red), CTIP2 (magenta), TBR2 (cyan), TUJ1 (yellow), CC3 (gray) and KI67 (chocolate red) on both CO and PCCO sections. GFP shows GFP^{+} PLCs present in PCCO. Tissue from column 1-4 and columns 5-6 are serial sections from cortical organoids. n=9 includes COs generated from three independent hPSCs (iPSC-Line2, iPSC-Line3 and H1 cells), pictures were taken from three different regions. Bars: 400µm. In **b**, COs and PCCOs are derived from H1 cells. GFP-PLCs are generated from H9 cells.

Supplementary Figure. 5 Characterization of PLCs within PCCOs.



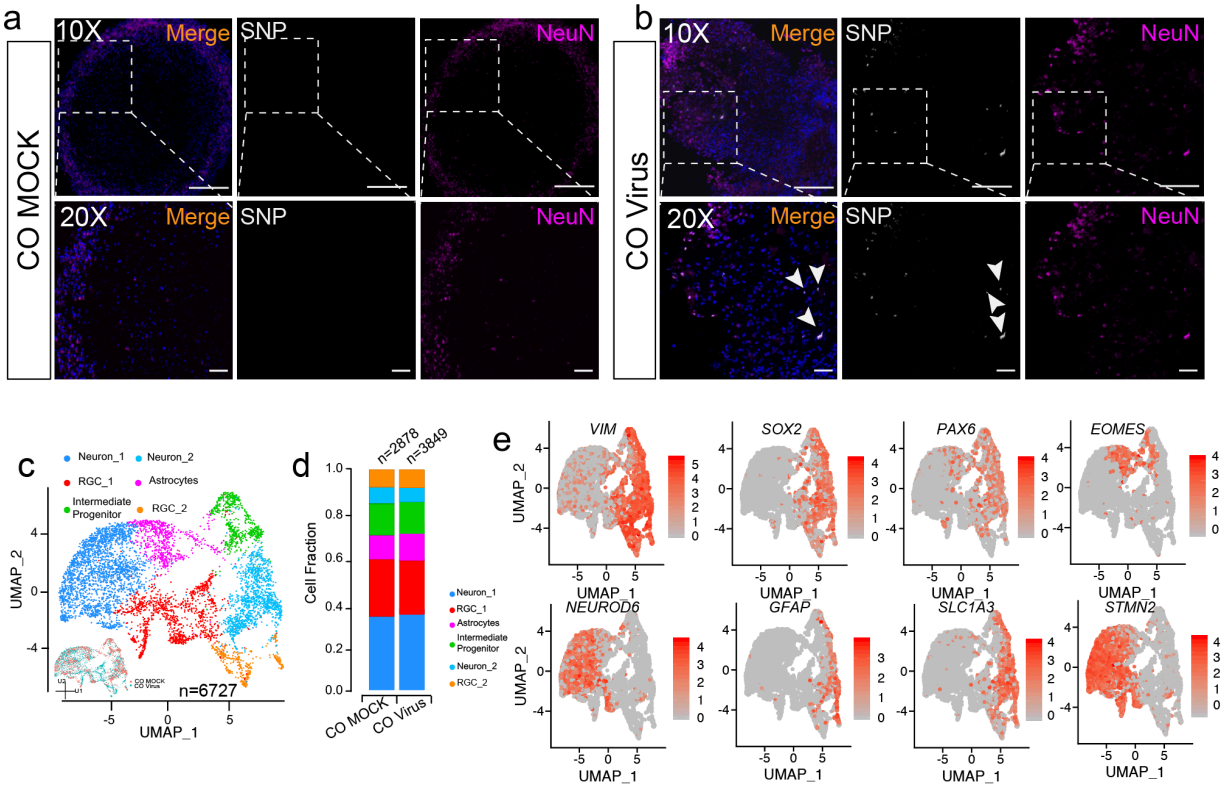
a. GFP$^+$ PLCs show high mRNA expression of pericyte marker genes (*NG2, PDGFRB, CD13*). GFP$^+$ PLCs were harvested for RNA extraction and subsequent RT-qPCR against *CSPG4* (*NG2), PDGFRB, CD13* and *AQP4. GAPDH* was used as reference control. *AQP4* used as sample control. n=12 includes 3 independent biological replicates (3 batches of PLCs cultures) and 4 technical replicates for each biological replicate. Multiple t-test was used to calculate significance followed by a Sidak multiple-comparison test correction. **** p<0.00001. **b.** Dot-plot shows *GFP-mRNA* expressed in astrocytes cluster in PCCO together with *PDGFRB* and *CSPG4 (NG2).* Dot size: % cells demonstrating expression of gene of interest. Dot shade: average expression for gene of interest in log-fold scale. For example, *GFP* is expressed only in PCCOs (blue dot vs. gray dot) whereas *PDGFRB* is expressed in both but in a much higher percentage of cells in PCCOs.

Supplementary Figure. 6 COs derived from iPSC line2 show little evidence of SARS-CoV-2 infection.



SNP and GFAP staining shows few SNP^{+} cells in COs derived from iPSC-Line2 72h after SARS-CoV-2 virus exposure. Bar: 400μm (upper panel), 100μm (lower panel). DAPI: blue, GFAP: astrocytes, and SNP: SARS-CoV-2-NP. Arrow: SNP^{+} cells. n=6 includes two biological replicates (cell line cultured under two independent researchers) and three different regions.

Supplementary Figure. 7 COs derived from iPSC line3 show little evidence of SARS-CoV-2 infection.



a-b. SARS-CoV-2-NP (SNP) and NeuN staining in CO (derived from iPSC line3) sections with (**a**) or without (**b**) SARS-CoV-2 virus exposure. DAPI: blue, bar: 400μm for 10X, 100μm for 20x, CO generated from iPSC-Line3. In **a-b**, n=6 includes two biological replicates (cell line cultured under two independent researchers) and three different regions. **c.** Merged UMAP shows the cell compositions in COs with/without SARS-CoV-2 virus exposure. 6727 cells were detected in total. **d.** Cell fractions show unchanged cellular composition in COs before and after virus exposure. 2878 cells were captured in COs without SARS-CoV-2 virus exposure, and 3849 cells were captured in COs with SARS-CoV-2 virus exposure. **e.** UMAPs show expression features from the individual cell populations.

Supplementary Table

Table 1. List of primer sequences used for qPCR.

Genes (human)	Forward sequencing (5'-3')	Reverse sequencing (5'-3')
PDGFRB	GTCATCCATCAACGTCTCTG	CGGATGTGGTAAGGCATATC
NG2	CAGGATGGCTTCCACTTTC	CTGGACCTCGTACTCAATCT
CD13	*CCACTGACGTCATCATCATC*	*TCGCTGTCCATCTCATACT*
AQP4	*CCTTTGGACCTGCAGTTATC*	*ATCAGGTCATCCGTCTCTAC*
ISG15	*GGCAGCGAACTCATCTTT*	*CCGATCTTCTGGGTGATCT*
ACE2	*GGTCTTCTGTCACCCGATTT*	*CATCCACCTCCACTTCTCTAAC*
BCL11B	*CACTCATCCGTGATCACTTC*	*GCTGGAAGGCTCATCTTTAC*
SATB2	*CAGAGCCAACCAACTCTTC*	*ATGGCCCTCAGGTTTACTA*
CUX1	*GCCCAAGCCATGGAATAA*	*TGTTACCTTCAGACCCATTTC*
TBR1	*CCCAATCACTGGAGGTTTC*	*ACCATCTGCCCATTGTTATT*
GLUT1	*CAATGCCCGACGTCTAAC*	*GAAGACACGTGCGGATG*
HIST1H2BC	*GACCAAAGCGCAGAAGAA*	*GTCGAGCGCTTGTTGTAA*
NUSAP1	*GCTGGACTCCCTCAAGT*	*GCTTCTTCCTGGTTGCTG*
DTYMK	*CCGGTTCCCGGAAAGAT*	*TCCGCCAGCTGTAACTC*
TPX2	*CCAGACCTTGCCCTACT*	*CTTGCCTCTGGGATTTGG*
CHI3L1	*GGGACCCTTGCCTACTAT*	*AGAAGGAGCCCTGGAAG*
ALDH1L1	*GGCCAGGGTTCTTCTTTG*	*CAGGGCCTTGTTGATGTC*
CD49f	*CAGCAAGGCAGATGGAATAA*	*AGAACCTGTGTTGGTTTGG*
TLR4	*CATGGCCTTCCTCTCCT*	*CCTGCAGTTCTGGGAAAC*
PAX6	CAGACACAGCCCTCACAAA	TCATAACTCCGCCCATTCAC
T(Bra)	TGCTTCCCTGAGACCCAGTT	GATCACTTCTTTCCTTTGCATCAAG
GATA4	CCTCCTCTGCCTGGTAATGACT	CGCTTCCCCTAACCAGATTG
MAP2	AGATAGAAGCCCAGGGAGAATA	CGATGGTCACAACAGACTCAA
GAPDH	ACCACAGTCCAT GCCATCAC	TCCACCACCCTGTTGCTGTA
Transcripts (SARS-CoV2)	Forward sequencing (5'-3')	Reverse sequencing (5'-3')
SARS-CoV-2-S1	GACCCCAAAATCAGCGAAAT	TCTGGTTACTGCCAGTTGAATCTG
SARS-CoV-2-S2	TTACAAACATTGGCCGCAAA	GCGCGACATTCCGAAGAA
SARS-CoV-2-S3	TCATCACGTAGTCGCAACAG	CAAAGCAAGAGCAGCATCAC
RNP	AGATTTGGACCTGCGAGCG	GAGCGGCTGTCTCCACAAGT

Supplementary notes

Methods

Human Induced Pluripotent Stem Cells, Neural Crest Stem Cells Culture

HEK293T and Hela cells (sex typed as female), and H1 ESC (sex typed as male) were obtained from ATCC (CRL-11268^{TM}), ATCC (ATCC$^{®}$CCL-2^{TM}) and WiCell (WAe001-A) and were not further authenticated. Generation of neural crest stem cells (NCSCs) was described previously[1]. Human induced pluripotent stem cells (hiPSCs) were generated from CIRM (CIRM-IT1-06611) with parents' and patients' fibroblast cells. All cells were regularly mycoplasma negative. HEK 293T and Hela cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) with 10% FBS (Gibco), 1X GlutaMax (Gibco), 1X NEAA (Gibco), 1X Sodium Pyruvate solution (Gibco), 100U/ml Penicillin/Streptomycin (Gibco). H1 cells and hiPSCs were maintained in culture with mTeSR^{TM}1 culture medium (STEMCELL Technologies) on Matrigel coated dishes (Corning). NCSCs were maintained in E6 medium (STEMCELL Technologies) with 10ng/ml FGF2 (PEPROTECH), 22.5µg/ml heparin sulfate (Sigma), 10uM SB431542 (R&D), 1µM CHIR99021 (Selleckchem) and 1µM dorsomorphin (Selleckchem) in the laboratory of Dr. Eric Shusta at Wisconsin[1]. In brief, NCSCs were derived from human pluripotent stem cells (hPSCs) with E6 basal medium in addition of 1μM CHIR99021, 10μM SB431542, 10µg/L FGF2, 1μM dorsomorphin and 22.5µg/ml heparin for 14 days. At day 15, cells were labeled with NCSC microbeads (Miltenyi Biotec; conjugated with antibody against the NCSCs markers p75-NGFR) to perform magnetic activated cell sorting (MASC) sorting to obtain a pure population (99.8%) of HNK1^{+}/p75-NGFR^{+} NCSCs. pLV-EF1a-EGFP-Puro construct was obtained from Dr. Fred Gage's lab as a gift. Lentiviral packaging plasmids pMD2.G, pPAX2 were obtained from Addgene. pcDNA3.1-hACE2 plasmid was obtained from Dr. Tom Rogers at Scripps as a gift.

Lentivirus packaging and PLCs generation

PLCs were derived from hPSC-derived NCSCs with 9 days of culture with E6 medium (STEMCELL Technologies) with 10% FBS[1]. GFP lentiviral particles were generated by co-transfection of HEK293T cells with pLV-EF1a-EGFP-Puro and pMD2.G, psPAX2 using lipo2000. Supernatant containing viral particles was harvested after 72h, 0.22µm filtered, and lentiX (Clontech) concentrated. 10µl GFP-lentiviral particles was used to infect PLCs at NCSCs differentiation day 7, after 48h infection, 10 µM puromycin was used to select GFP^{+} PLCs. After 7 days selection, GFP^{+} PLCs were maintained in E6 medium with 10% FBS for another two days for further use.

Human cortical brain organoid (CO) and PLC containing cortical organoid (PCCO) culture

H1 and hiPSCs were maintained in mTeSR1 and passaged according to manufacturer's recommendations. Cortical brain organoids were generated as previously described[2,3]. H1 cells at 36 passages and hiPSC at 12 passages were dissociated into single cells with Accutase. In total, around 9000 cells were then plated in each well of an ultra-low-attachment 96-well plate (Corning, CLS3474) in "Cortical differentiation medium" with 20 µM Rock inhibitor (Selleckchem), 5 µM SB431542 (R&D), 3 µM endo-IWR-1 (Selleckchem) for the first 4d, then cultured for an additional 13d in cortical differentiation medium with 5 µM SB431542, 3 µM endo-IWR-1, with medium changes every 2d. On 18d,

the immature organoids were transferred into ultra-low-attachment 6-well plate and cultured for another 16d in "Organoid differentiation medium." Media was changed every 3–4d. On 35d, media was changed to "Maturation medium" supplemented with 1% Matrix High Concentration (HC), Growth Factor Reduced (GFR) Matrigel (Corning), with medium replaced every 3–4d for additional 34d. HC-GFR Matrigel was added fresh at each change. On 70d, media was changed to "Long-term maintenance medium" supplemented with 2% HC-GFR-Matrigel, 50 ng/ml BDNF (R&D, 248-BD-025/CF) and 50 ng/ml GDNF (R&D, 212-GD-010/CF) for long term organoid maintenance. "Cortical differentiation medium": Glasgow's MEM (GMEM), 20% Knock-Out Serum Replacement (KO-SR), 1X Non-Essential Amino Acid (NEAA), 1X Sodium Pyruvate, 1X β-Mercaptoethanol, 1X Pen/Strep. "Organoid differentiation medium": DMEM/F12, 1X N2, 1X NEAA, 1X CD-lipid concentrate, 1XPen/Strep. "Maturation medium": DMEM/F12, 10% FBS, 1X N2, 1X CD-lipid concentrate, 1X Pen/Strep, 1%HC-GFR Matrigel, 30μg/ml Heparin. "Long term maintenance medium": DMEM/F12, 20%FBS, 1X N2, 1X B27w/o vitamin A, 1X CD-lipid concentrate, 1X Pen/Strep, 2% HC-GFR Matrigel, 30μg/ml Heparin, 50ng/ml BDNF, 50ng/ml GDNF, 50ng/ml NT3. For PCCO generation, at CO 60 div, $2x10^5$ GFP^+ PLCs were integrated into each of the CO in a low attachment 96-well plate. PLC integrated COs were maintained in Maturation medium of CO for 14 days in presence of BDNF, GDNF and NT3. At day 74, PCCOs were changed into long-term maintenance medium for long-term culture.

SARS-CoV-2 infection of PLCs, COs, and PCCOs

All work with SARS-CoV-2 was conducted in Biosafety Level-3 conditions at the University of California San Diego following the guidelines approved by the Institutional Biosafety Committee. SARS-CoV-2 isolate USA-WA1/2020 (BEI Resources) was propagated and infectious units quantified by plaque assay using Vero E6 (ATCC) cells. For PLC infection, $2x10^4$ PLCs were cultured on a coverslip as 2D in low attachment 24-well plate. SARS-CoV-2 was added at a MOI of 0.5 for one hour in 1ml of culture medium with rocking every 10-15 minutes. After one hour, the supernatant was removed, cells were washed once with media and 1ml of fresh media was added. The cells were incubated for 48h at 37°C, 5% CO2. After 48hrs, 1ml of fresh medium was added and the cells were cultured for another 24h. For infection of COs and PCCOs, SARS-CoV-2 in 1ml culture medium at an approximate MOI of 0.5 was added and cells incubated for 48h at 37°C, 5% CO2. After 48hrs, 1ml fresh medium was added into the culture for another 24h. To calculate the MOI for CO and PCCO, counts from previous single cell dissociations[2] were used to determine the cell number in the organoids. The cell numbers were approximately 1~1.5M cells per CO and approximately 1.2~1.7M cells per PCCO.

ACE2 receptor antibody blocks SARS-CoV-2 infection of PLCs

PLCs were preincubated with 20μg/ml ACE2 antibody 30min at 37°C in a final volume of 200μl per well of PLC-medium as described above. PLCs were then infected with 10^3 plaque forming units (pfu) of SARS-CoV-2 virus for 1 hour at 37°C, washed 3 times with PBS and 200μl per well of new medium was added. After 24h of infection, PLCs were harvested with 4% PFA and Trizol for analysis. Anti-SARS-CoV-2 Spike antibody (Sino Biological) preincubated with virus for 30 min at 37°C was used as positive control.

Detection of viral mRNA and replication using RT-qPCR

For viral RNA quantification, PLCs, COs or PCCOs were washed twice with PBS and lysed in TRIzol (ThermoFisher). RNA was extracted using the Qiagen-RNA extraction Kit (Qiagen). 2μg RNA was used to generate cDNA with SuperScript III First-Strand Synthesis Kit (Invitrogen). 20ng cDNA was used to perform qPCR with iTaq Universal SYBR Green Supermix and the CDC-N1/N2/N3-SARS-CoV-2 primers mix (IDT) at a final concentration of 100nM for each primer using a Bio-Rad Real-Time PCR system. Ribonucleoprotein (RNP) was used as reference. To determine viral replication, RNA was extracted from 200μl of inoculated PLC culture medium at 24h, 48h and 72h post infection by QIAmp Viral RNA mini-kit (Qiagen). Sample Cq values were converted to viral copies per reaction using the standard curve's linear regression model. Values in dot plots represent fold changes first normalized to *RNP* and then normalized to MOCK infection for each individual biological sample. All the qPCR primers were listed in Supplementary information Table1.

Plaque assay

Viral supernatants were 10-fold serially diluted in DMEM without serum. Vero E6 cells in 12-well plates were washed with PBS, and 200uL of virus dilution was added per well and incubated 1 h at 37°C with rocking every 10-15 min. The inoculum was removed and 1mL of overlay (0.6% agarose in MEM with 4% FBS) was added to each well. Overlays were prepared by mixing equal volumes of 1.2% agarose and 2xMEM supplemented with 8% FBS, 2x L-glutamine, 2x non-essential amino acids, and 2x sodium bicarbonate. Assays were incubated 48 h at 37°C and fixed by adding 2mL 10% formaldehyde/well for at least 24 h. Overlays were removed and monolayers were stained with 0.025% crystal violet in 2% EtOH.

Immunostaining and imaging for COs and PCCOs

COs and PCCOs were fixed in 4% paraformaldehyde for 72h before removal from BSL3. The fixed COs and PCCOs were washed with PBST (PBS with 0.25% Tween 20) three times for 5 min, allowed to sink in 30% sucrose at least overnight at 4 °C, embedded in 15%/15% gelatin/sucrose solution and sectioned at 20 μm. 30min antigen retrieval (Tri-sodium citrate, 0.1% Tween-20, pH 6.0) was performed on the CO/PCCO sections followed by 20min 0.5% Triton X-100 permeabilization at room temperature. The sections were then blocked with 5% BSA in PBS for 1 h at room temperature. After washing with PBST 3 times for 5 min, sections were incubated with primary antibodies in 5% BSA/ 0.5% Triton X-100 in PBS at the following dilutions: SOX2 (R&D, AF2018-SP, 1:100), TUJ1 (Biolegend, 801202, 1:1000), Cleaved Caspase 3 (Cell Signaling Technology, 9661S, 1:500), Ki67 (BD-Biosciences, 550609, 1:1000), CTIP2 (Abcam, ab28448, 1:500), TBR2 (Abcam, EPR19012, 1:250), GFAP (Abcam, ab4674, 1:250), TBR1 (Abcam, ab183032, 1:250), LAMB1 (Abcam, ab44941, 1:100), SARS-CoV-2-Nucleocapsid (SinoBiological, AB_2827977, 1:500), NeuN (Abcam, ab177487, 1:100), p53 (Abcam, ab90363, 1:300), PDGFR-*β* (R&D, AF1042, 1:100), αSMA (Invitrogen, 14-9760-82, 1:200), NG2 (ThermoFisher Scientific, PA5-92029, 1:100), ACE2 (R&D, AF933, 1:100) overnight at 4 °C, washed three times with PBST for 10 min, then incubated with secondary antibodies

(Alexa Fluor™ 488 donkey anti-mouse IgG (H + L), 1915874, 1:1000; Alexa Fluor™ 594 donkey anti-rabbit IgG (H + L), 1890862, 1:1000, Alexa Fluor™ 594 donkey anti-chicken IgG (H + L),703585155, 1:1000, Alexa Fluor™ 594 donkey anti-rat IgG (H + L), 712585153, 1:1000, Alexa Fluor™ 594 donkey anti-mouse IgG (H + L), 715585150, 1:1000, Alexa Fluor™ 647 donkey anti-mouse IgG (H + L), 715605151, 1:1000, Alexa Fluor™ 647 donkey anti-rabbit IgG (H + L), 711605152, 1:1000) together with DAPI (ThermoFisher Scientific, D1306, 1:50000) for 2 h at room temperature, washed with PBST three times for 5 min, and mounted with Fluoromount-G® (Southern Biotech, 0100-01). All the images were taken with ZEISS LSM880 Airyscan, with post-acquisition analysis done in ImageJ-6.

Light Sheet imaging of PCCO

PCCOs were harvested for clearing in PBST in a 1.5ml EP tube (1PCCO/tube). CUBIC [4] was used for clearing. In brief, PCCOs were sunk into 1.5ml R1 (25% urea, 25% Quadrol, 15% Triton-X-100)/PBST solution at RT overnight. Solution was then changed to 1.5ml fresh R1, and incubated at RT for at least 24h until PCCOs turned clear. After 24h, R1 solution was replaced with 1.5ml R2 solution (25% urea, 50% sucrose, 10% triethanolamine) along with DAPI (1:1000) for incubation overnight at RT, then embedded into 1% Agarose solution for imaging. A 5X lens was used for imaging with a light sheet microscope (Zeiss Z1) according to manufacturer recommendations. The images were further developed with IMARIS (Oxford Instruments).

CO/PCCO dissociation and dead cell removal

COs and PCCOs were dissociated using AccuMax (STEMCELL Technology) with 10μM Rock inhibitor. COs/PCCOs were washed with PBS once, before adding 500μl AccuMax, for trituration. CO/PCCO pieces were then incubated at 37°C for 20min, with gentle trituration at 10min during dissociation. After 20min, 3ml Neuronal Medium was added into the dissociation system, and gentle trituration was performed to obtain single cell suspension. The cell suspension was centrifuged at 500g, RT for 10min, supernatant removed, and cells re-suspended with 100μl of Neuronal Medium. To remove the dead cells, 1μl Dead Cell Removal cocktail (Annexin V, STEMCELL Technology) was added into the dissociated CO/PCCO single cells suspension together with 1μl Biotin selection cocktail (Annexin V, STEMCELL Technology), gently mixed and incubated at RT for 3min. After 3min incubation, RapidSpheres™ (STEMCELL Technology) was vortexed for 30s at RT and 2μl added to the above mixture. At the same time, 850μl Neuronal Medium was added and gently pipetted 2~3 times. Tubes were then placed into a magnetic holder and incubated for 3min at RT. After incubation, the supernatant was collected in a new tube for 10X GEM generation. For each of the sc-RNA-seq library, we pooled 4 individual COs or PCCOs derived from H1 cells (2 in total) and iPSC-line2 (2 in total) under different conditions for dissociation and following library preparation.

Single cell library preparation and sequencing

10X sc-RNA-seq-3'-V3.1 kit (10X Genomics) was used to generate the GEM, cDNA and library were generated according to the manufacturer's instructions (10X Genomics). Briefly, live cells were partitioned into nanoliter-scale Gel Bead-In-Emulsions (GEMs) with the 10x Chromium Controller (10X Genomics), 1000 cells were targeted.

Upon cell lysis and dissolution of the Single Cell 3′-V3.1 Gel Bead within the droplet, primers containing an Illumina P7 and R2 sequence, a 14 bp 10XBarcode, a 10 bp randomer, and a poly-dT primer sequence were released and mixed with the cell lysates and bead-derived Master Mix. Barcoded, full-length cDNA from poly-adenylated mRNA was then generated in each individual bead, then individual droplets were broken and homogenized before the remaining non-cDNA components were removed with silane magnetic beads (Invitrogen). The libraries were then size-selected, and the R2, P5 and P7 sequences were added to each selected cDNA during end repair and adapter ligation. After Illumina bridge amplification of cDNA, each library was sequenced using the Novaseq6000 with PE150bp at the IGM Core in UCSD, around 120M reads were requested for each sample.

CO/PCCO TMT4 quantitative Protein Mass Spectrometry

Three COs and three PCCOs were harvested into 1.5ml cold PBS, then centrifuged at 1500rpm at 4°C for 10min. After centrifugation, all PBS was removed and the CO/PCCO samples were flash frozen in liquid N2. The frozen cell pellets were analyzed by TMT4 quantitative mass spectrometry at the UCSD Proteomics Core.

Data processing of single-cell RNA-seq and Mass Spectrometry

Fastq files were aligned by CellRanger® (10x Genomics, 4.0) count function with default setting. GRCh38/hg38 v12 was used as the reference genome. GRChg38-2020 10x genome reference. The count matrix was generated using the count function with default settings. SARS-CoV-2 (USA-WA1/2020) genome and GFP-CDS were written into GRChg38-2020 human genome reference as a gene with mkref function in cell ranger 4.0. Data from all runs were aggregated with “aggre” function to ensure comparable read depth across runs, combined output file of all runs were loaded into R as a Seurat object[5], log normalized and scaled with a scale factor of 10,000. Cells with 2500 genes expressed (UMI count greater than 0) were removed according standard analysis principle of Seurat. The top 2000 variable genes (HVGs) were identified with Seurat FindVariableGenes, using “vst” as the method. We used PCA and UMAP as our main dimension reduction approach. PCA was performed with RunPCA function (Seurat) using HVGs. Following PCA, we conducted JACKSTRAW analysis with 100 iterations to identify statistically significant (p value < 0.01) principal components that were driving systematic variation. We used UMAP to present data in two-dimensional coordinates, generated by RunUMAP function in Seurat. Significant PCs (30) identified by JACKSTRAW analysis were used as input. Perplexity was set to 30 (default). UMAP plots were generated using R package ggplot2. Clustering was done first by establishing a shared nearest neighbor and then conducting Luvain-Jaccard analysis on the resulted graph using FindClusters function from Seurat with default setting. DEX analyses were conducted using Seurat function FindAllMarkers. Briefly, we took one group of cells and compared it with the rest of the cells, using a Wilcoxon rank sum test. For any given comparison, we only considered genes that were expressed by at least 50% of cells in either population, with log fold-change greater than 0.69. Genes that exhibit p values under 0.01 were considered statistically significant after multiple testing corrections. All violin plots were generated using ggplot2, and for Y-axis, we calculated the normalized expression level of certain genes, by naturallog transformed the feature counts for each cell and divided by the total

counts for that cell, then multiplied by the scale factor using log1p. UMAP plots were generated using UMAP plot function from R package Seurat. Except otherwise noted, all heatmaps were generated with R function heatmap.3. scTransform function was used to wrap the technical variation; Cells were considered infected if they carried the transcripts aligned to SARS-CoV-2 viral genome. DAVID-GO-Term analysis over representation tests for both up-regulated and downregulated genes in each condition shown in (Fig. 2r, Supplementary datasets 2-3). Mass Spectrometry data were generated and analyzed by the proteomic core at UCSD (Supplementary dataset 2). The protein with significance over 15 were used for GO-Term and string analysis in goprofile (https://biit.cs.ut.ee/gprofiler/gost) and String (https://string-db.org/) (Supplementary dataset 2).

Reference

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