Supplemental Information

Common Genetic Variation in Humans Impacts \textit{In Vitro} Susceptibility to SARS-CoV-2 Infection

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Supplemental Figures

Supplemental Figure 1 (related to Figure 1). ACE2 in situ hybridization RNA staining over post-mortem human ventral midbrain cell with neuronal nucleus-like morphology in DAPI stain. Scale bar: 20μm.

Supplemental Figure 2 (related to Figure 3). Plaque assay showing effective viral production from the alveolospheres 24hpi at an MOI of 0.1.
Supplemental Table 1. Excess of nominally significant GWAS variants within the SARS-CoV-2 host gene cis-regions when comparing individuals with COVID-19 to the general population.

| Gene  | Study name                  | Phenotype                                                                 | Case number | Control number | N SNPs in cis-region (+/- 1e06) | N nominal (p<0.05) | % reaching nominal significance | binomial p value |
|-------|-----------------------------|---------------------------------------------------------------------------|-------------|----------------|-------------------------------|-------------------|---------------------------------|------------------|
| ACE2  | COVID19_HGI_ANA_B2          | hospitalized covid vs. population                                          | 3199        | 897488         | 4839                         | 480               | 9.919405                       | 3.28E-44         |
| ACE2  | COVID19_HGI_ANA_C2          | covid vs. population                                                       | 6696        | 1073072        | 5281                         | 497               | 9.411096                       | 1.02E-39         |
| BSG   | COVID19_HGI_ANA_D1          | predicted covid from self-reported symptoms vs. predicted or self-reported non-covid | 1865        | 29174          | 6456                         | 584               | 9.045849                       | 2.54E-41         |
| FURIN | COVID19_HGI_ANA_B2          | hospitalized covid vs. population                                          | 3199        | 897488         | 11556                        | 869               | 7.519903                       | 3.57E-31         |
| TMPRSS2 | COVID19_HGI_ANA_B2         | hospitalized covid vs. population                                         | 3199        | 897488         | 14047                        | 919               | 6.542322                       | 1.00E-15         |
| TMPRSS2 | COVID19_HGI_ANA_C1        | covid vs. lab/self-reported negative                                      | 3523        | 36634          | 20141                        | 1104              | 5.481356                       | 0.00201          |
| FURIN | COVID19_HGI_ANA_C1          | covid vs. lab/self-reported negative                                      | 3523        | 36634          | 17346                        | 921               | 5.309581                       | 6.00E-02         |
| TMPRSS2 | COVID19_HGI_ANA_C2        | covid vs. population                                                       | 6696        | 1073072        | 21814                        | 21814             | 4.776749                       |                  |
| TMPRSS2 | COVID19_HGI_ANA_D1        | predicted covid from self-reported symptoms vs. predicted or self-reported non-covid | 1865        | 29174          | 12030                        | 12030             | 4.763092                       |                  |
| BSG   | COVID19_HGI_ANA_C2          | covid vs. population                                                       | 6696        | 1073072        | 14419                        | 14419             | 4.722935                       |                  |
| BSG   | COVID19_HGI_ANA_B2          | hospitalized covid vs. population                                         | 3199        | 897488         | 9554                         | 9554              | 4.406531                       |                  |
| BSG   | COVID19_HGI_ANA_B2          | hospitalized covid vs. population                                         | 3199        | 897488         | 4.406531                     |                   |                                |                  |
| BSG   | COVID19_HGI_ANA_C1          | covid vs. lab/self-reported negative                                      | 3523        | 36634          | 13358                        | 13358             | 3.960174                       |                  |
| FURIN | COVID19_HGI_ANA_C2          | covid vs. population                                                       | 6696        | 1073072        | 9432                         | 9432              | 3.042833                       |                  |
| ACE2  | COVID19_HGI_ANA_D1          | predicted covid from self-reported symptoms vs. predicted or self-reported non-covid | 1865        | 29174          | 532                          | 532               | 3.383459                       |                  |
| ACE2  | COVID19_HGI_ANA_C1          | covid vs. lab/self-reported negative                                      | 3523        | 36634          | 4098                         | 4098              | 3.29429                        |                  |
| FURIN | COVID19_HGI_ANA_D1          | predicted covid from self-reported symptoms vs. predicted or self-reported non-covid | 1865        | 29174          | 9432                         | 9432              | 3.042833                       |                  |
Supplemental Experimental Procedures

Cell culture and differentiations:

**hiPSC-NPCs:**

hiPSC-NPCs were cultured in hNPC media (DMEM/F12 (Life Technologies #10565), 1x N-2 (Life Technologies #17502-048), 1x B-27-RA (Life Technologies #12587-010), 20 ng/mL FGF2 (Life Technologies)) on Matrigel (Corning, #354230). hiPSC-NPCs at full confluence (1-1.5x10^7 cells / well of a 6-well plate) were dissociated with Accutase (Innovative Cell Technologies) for 5 mins, spun down (5 mins X 1000g), resuspended and seeded onto Matrigel-coated plates at 3-5x10^6 cells / well. Media was replaced every 24 days for 4 to 7 days until next split.

**NGN2-glutamatergic neuron induction** (Ho et al., 2016; Zhang et al., 2013):

1) Neurons d21 (Fig.1B,C): hiPSCs were dissociated with Accutase Cell Detachment Solution (Innovative Cell Technologies, # AT-104), counted and transduced with rtTA (Addgene 20342) and NGN2 (Addgene 99378) lentiviruses in StemFlex media containing 10 μM Thiazovivin (Millipore, #S1459). They were subsequently seeded at 1x10^6 cells/well in the prepared 6-well plate. On day 1, medium was switched to non-viral induction medium (DMEM/F12 (Thermofisher, #10565018), 1% N-2 (Thermofisher, #17502048), 2% B-27-RA (Thermofisher, #12587010)) and doxycycline (dox) was added to each well at a final concentration of 1 μg/mL. At day 2, transduced hiPSCs were treated with 500 μg/mL G418 (Thermofisher, #10131035). At day 4, medium was replaced including 1 μg/mL dox and 4 μM cytosine arabinoside (Ara-C) to reduce the proliferation of non-neuronal cells. On day 5, young neurons were dissociated with Accutase Cell Detachment Solution (Innovative Cell Technologies, # AT-104), counted and seeded at a density of 1x10^6 per well of a Matrigel-coated 12-well plate. Medium was switched to Brainphys neuron medium (Brainphys (STEMCELL, # 05790), 1% N-2, 2% B-27-RA, 1 μg/mL Natural Mouse Laminin (Thermofisher, # 23017015), 20 ng/mL BDNF (R&D, #248), 20 ng/mL GDNF (R&D, #212), 250 μg/mL Dibutyril cyclic-AMP (Sigma, #D0627), 200 μM L-ascorbic acid (Sigma, # A4403)). For seeding, 10 μM Thiazovivin (Millipore, #S1459), 500 μg/mL G418 and 4 μM Ara-C and 1 μg/mL dox were added. At day 6, medium was replaced with Brainphys neuron medium with 4 μM Ara-C and 1 μg/mL dox. Subsequently, 50% of the medium was replaced with fresh neuronal medium (lacking dox and Ara-C) once every other day until the neurons were fixed or harvested at day 21.

2) shRNA treated neurons (Fig.1H,I): On day -1 NPCs were dissociated with Accutase Cell Detachment Solution for 5min at 37°C, counted and seeded at a density of 5x10^5 cells/well on Matrigel coated 24-well plates in hNPC media (DMEM/F12 (Life Technologies #10565), 1x N-2 (Life Technologies #17502-048), 1x B-27-RA, 20 ng/mL FGF2 (Life Technologies)) on Matrigel (Corning, #354230). On day 0, cells were transduced with rtTA and NGN2 lentiviruses as well as desired shRNA viruses in NPC media containing 10 μM Thiazovivin and spinfected (centrifuged for 1 hour at 1000g). On day 1, media was replaced and dox was added with 1μg/mL working concentration. On day 2, transduced hNPCs were treated with corresponding antibiotics to the lentiviruses (1 μg/mL puromycin for shRNA, 1 mg/mL G-418 for NGN2-Neo). On day 4, medium was
switched to Brainphys neuron medium plus 1 μg/mL dox. Medium was replaced every second day until SARS-CoV-2 infection on day 7.

**iii) Neurons d7 (Fig. 5):** On day 0, hiPSCs were transduced with rtTA (Addgene 20342) and NGN2 (Addgene 99378) lentiviruses. hiPSCs were infected in a conical tube in low volume StemFlex media and high virus concentration and subsequently seeded onto 6-wells at 1x10^5 cells/well and spininfected. On day 1, Medium was switched to non-viral neurobasal neuron medium (Neurobasal (Thermofisher Scientific, #21103049), 1x N-2 (Life Technologies #17502-048), 1x B-27-RA (Life Technologies #12587-010), 1 μg/mL Natural Mouse Laminin (Life Technologies), 20 ng/mL BDNF, 20 ng/mL GDNF, 250 μg/mL Dibutyryl cyclic-AMP, 200 μM L-ascorbic acid) and Doxycycline (dox) was added to each well at a final concentration of 1 μg/mL. On day 2, transduced hiPSCs were treated with corresponding antibiotics to the lentiviruses (1 mg/mL G-418). On day 4, medium was replaced including 1 μg/mL dox and 4 μM cytosine arabinoside (Ara-C) to reduce the proliferation of non-neuronal cells along with antibiotic withdrawal. On day 5, young neurons were dissociated with Accutase Cell Detachment Solution, counted and seeded at a density of 1x10^6 per well of a Matrigel-coated 12-well plate. Medium was switched to Brainphys neuron medium. On day 6 medium was replaced (including 1 μg/mL dox). At day 7, neurons were infected.

**Differentiation of lung alveolospheres** (Jacob et al., 2019): Alveolospheres, composed of distal lung epithelial cells, were generated from hiPSCs cultured in mTeSR Plus medium (STEMCELL Technologies, #05825) through a sequential directed differentiation protocol (Jacob et al., 2019). Developmental lung epithelial lineage differentiation was initiated by dissociating hiPSCs with Accutase Cell Detachment Solution (Innovative Cell Technologies, # AT-104) and seeding 2x10^6 cells onto one well of a matrigel-coated 6-well plate in mTeSR Plus medium with 10 μM THX. For the next three days, cells were patterned into definitive endoderm using the StemDiff Definitive Endoderm kit (STEMCELL Technologies, #05110). On day 3, cells were dissociated with Gentle Cell Dissociation Reagent (STEMCELL Technologies 07174 and passed 1:4 (C1) or 1:6 (C2) and seeded in DS/SB Media (cSFDM Base (Jacob et al., 2019), 10 μM SB43152 (Tocris 1614), 2 μM Dorsomorphin (Stemgent 04-0024)) plus 10 μM THX. Followed by patterning into anterior foregut endoderm (until day 6) using inhibition of BMP/TGFB signaling. Specification of lung lineage was achieved by using three factors (CHIR99021, BMP4, and retinoic acid) to produce primordial lung progenitors which were enriched by sorting using the cell-surface markers CD47hi/CD26lo (Jacob et al., 2019; McCauley et al., 2018) and then plated in Matrigel for 3D culture on day 15. These progenitors were then differentiated into distal lung epithelium using CHIR99021, keratinocyte growth factor, dexamethasone, cyclic AMP, and 3-isobutyl-1-methyloxanthine). After day 25, monolayered epithelial spheres (“alveolospheres”) emerged, and were infected and harvested as specified, typically ~day 30.

For shRNA experiments, lung progenitors were collected, dissociated and seeded as single cells in the presence of ROCK inhibitor THX at day 14. The following day, these cells were infected with lentiviral shRNAs against SARS-CoV-2 host genes, followed by 2 days of puromycin selection. One day after withdrawal of antibiotic selection for dissociated alveolar cells (or media change for control 3D alveolospheres), cells were infected with SARS-CoV-2 (MOI 0.1-0.5) on day 33 and harvested 24 hours later.
Differentiation of intestinal organoids (Koike et al., 2019; Zhang et al., 2018): Human posterior gut endoderm can be generated from hiPSCs through a stepwise differentiation protocol adapted from (Koike et al., 2019; Zhang et al., 2018). The first step is patterning to definitive endoderm by initial treatment with activin A and Wnt3a (2 days), followed by BMP4, FGF2, VEGF, and activin A treatment (through day 6). Definitive endoderm is replated and matured with FGF2, A83-01 and CHIR99021 into CDX2+ posterior gut endoderm progenitor cells. Gut progenitors can be expanded (FGF2, VEGF, EGF, A83-01 and CHIR99021) and differentiated into gut organoids, simply by treating with CHIR99021 and FGF4 for 3 days until dissociated intestinal cell sheets started budding off and formed floating hindgut spheroids. After 7 additional days embedded in Matrigel, spheroids differentiate and protrude into the lumen. Hindgut organoids comprise a polarized epithelium that includes absorptive enterocytes (VILLIN+) and hindgut epithelial cells (E-CAD+). On day 20, intestinal organoids were collected and dissociated and seeded as single cells. The next day, cells were infected with shRNA viruses against SARS-CoV-2 receptor targets, followed by 2 days of puromycin selection. One day after selection withdrawal for dissociated intestinal cells and regular feeding for 3D intestinal organoids, cells were infected with SARS-CoV-2 (MOI 0.05-1) on day 25 or day 50 and harvested 24 or 48 hours later.

RNA-seq of SARS-CoV-2 infected hamster brains (GSE161200): 3-5-week-old male Golden Syrian hamsters (Mesocricetus auratus) were obtained from Jackson Laboratories. Hamsters were acclimated to the CDC/USDA-approved BSL-3 facility of the Global Health and Emerging Pathogens Institute at the Icahn School of Medicine at Mount Sinai for 2-4 days. Before intranasal infection, hamsters were anesthetized by intraperitoneal injection with 200 µl of a ketamine HCl/xyalzine (4:1) solution. Inoculum at the referenced doses (high and low, 100pfu and 10,000 pfu, respectively) were resuspended in PBS to a total volume of 100 µL. All hamsters analyzed by RNA-seq were perfused with PBS before brains were harvested. The brain was cut in half longitudinally after removing hindbrain and half was used for RNA extraction and analysis. Brain tissues were homogenized for 40 seconds at 6.00 m/s for 2 cycles (MP Biomedicals, Cat#: SKU 116005500) in Lysing Matrix A homogenization tubes (MP Biomedicals, Cat#6910-100). All tissues for RNA-seq analysis were homogenized directly in TRIzol (Invitrogen, Cat#15596026). RNA was isolated by phenol/chloroform extraction according to manufacturer’s instructions.

RNA-seq libraries were prepared using the TruSeq Stranded mRNA Library Prep kit (Illumina) according to the manufacturer’s instructions. cDNA libraries were sequenced and single-end sequencing reads were aligned using an Illumina NextSeq 500 platform. Reads were aligned to Mesocricetus auratus Ensembl MesAur1.0 using STAR aligner, and mappid using CustomDESeq2.

RNA sequencing analysis was performed in R v.3.6.0. Differential expression analysis was performed using edgeR (v3.26.8) (Robinson et al., 2010), limma (v3.40.6) (Ritchie et al., 2015) and Glimma (v1.12.0) (Su et al., 2017) packages. Raw read counts were transformed into log2 CPM using the edgeR package. The limma voom function was used to compute the weights for heteroscedasticity adjustment by estimating the mean variance trend for log2 counts. Linear models were fitted to the expression values of each
gene using the lmFit function. Empirical Bayesian moderation was applied using the eBayes function to obtain more precise estimates of gene-wise variability. \( P \) values were adjusted for multiple hypotheses testing using FDR estimation; DEGs were determined as those with an estimated FDR \( \leq 5\% \). Enrichment analysis was performed using WebGestaltR (0.4.4) using custom neural gene sets of interest (Schrode et al., 2019) and interferon gene sets (Blanco-Melo et al., 2020). Genes of interest were ranked by \(-\log_{10}(P)\) and enrichment was performed against a background of all expressed genes.

**Molecular and biochemical analysis**

i. *Real time-quantitative PCR:* Cell were harvested with TRIzol and total RNA extraction was carried out following the manufacturer’s instructions. Quantitative transcript analysis was performed using a QuantStudio 7 Flex Real-Time PCR System with the Power SYBR Green RNA-to-Ct Real-Time qPCR Kit (all ThermoFisher). Total RNA template (25 ng per reaction) was added to the PCR mix, including primers. qPCR conditions were as follows; 48°C for 15 min, 95°C for 10 min followed by 45 cycles (95°C for 15 s, 60°C for 60 s). All qPCR data is collected from at least 3 independent biological replicates of one experiment. If not otherwise stated, obtained Ct values were normalized against 18S one super-control (C1 alveolosphere MOI 0.1) to ensure comparability across plates, experiments and cell types (except for shRNA experiments, which were normalized to either infected or uninfected scrambled control). Data analyses were performed using GraphPad PRISM 8 software.

Primers were used as follows:

| Oligo name               | Oligo sequence (5' to 3')                   |
|-------------------------|---------------------------------------------|
| FURIN qPCR fw           | ACAGTGTGGCAGGGAAGCATGG                     |
| FURIN qPCR rev          | AGGGACCGCTTCGTCACTCCTC                     |
| ACE2_qPCR_1-fw          | TAACCACGAAGCGAAGACC                       |
| ACE2_qPCR_1-rev         | CAGACCATTTGTCCTCCAGCA                      |
| CD147_BSG_qPCR_1-fw     | CTGACTGGCCTGCTGACAGA                      |
| CD147_BSG_qPCR_1-rev    | AATGTGTAGCTGCTGACAGC                      |
| 18S fw                  | ACACGGACAGGAATTGACAGA                     |
| 18S rev                 | GGACATCTAAGGGCATCAGA                      |
| TMPRSS2_F1              | AGGTGAAAGCGGGGTGAG                        |
| TMPRSS2_R1              | ATAGCTGGTGTTACCAG                         |
| DPP4_F2                 | GGAATGCCAGGGAAGGAAGA                      |
| DPP4_R2                 | CAGGACCGGAGAATCTCAGC                      |
| SARS-CoV-2_TRS-L        | CTCTTTGTAGATCTGTCTCTAAACAGA               |
| SARS-CoV-2_TRS-N        | GTGCCACCAACGTATGCG                       |
| h_IFNB1_QF              | GTCAGAGTGAAATCTCAGA                      |
| h_IFNB1_QR              | ACACGATCTGCTGGTTGAAG                     |

ii. *Immunostaining and microscopy:* Cells were washed with ice-cold PBS and fixed with 4% PFA solution at pH 7.4 overnight at 4°C. Then, fixative solution was replaced with PBS (Ca/Mg++). Alveolar and intestinal cells were permeabilized and blocked with 0.1%
Triton-X in PBS (Ca/Mg++) + 2% donkey serum for 1 hour at room temperature. Neurons were permeabilized and blocked with 0.05% Tween in PBS (Ca/Mg++) + 2% donkey serum for 1 hour at room temperature. The blocking solution was aspirated and replaced with the same solution with primary antibodies. Mouse monoclonal anti-SARS-CoV-2 Nucleocapsid [1C7C7] protein (1:1000, a kind gift by Dr. T. Moran, Center for Therapeutic Antibody Discovery at the Icahn School of Medicine at Mount Sinai) and rabbit anti-β-III-tubulin (Convence, PRB-435P, 1:2000) or goat anti-EpCAM, (R&D Biosystems #AF960, 1:500) overnight at 4°C. Cells were washed 3x for 5 min with PBS (Ca/Mg++) and subsequently incubated with secondary antibodies, prepared in blocking solution, for 1 hour at room temperature, followed by 300 nM DAPI staining for 3-4 min and washed 2x for 5 min with PBS (Ca/Mg++).

Organoids were fixed with 4% PFA and incubated in 20% sucrose overnight, then frozen in OCT. Frozen organoids were cryosectioned to 10um sections and mounted on VWR Superfrost Plus slides. Slides were washed and permeabilized with 0.01% Triton-X PBS and incubated with blocking solution (2% donkey serum in PBS) for 1 hour. Then slides were incubated with primary antibody (EpCAM, 1:50, R&D Biosystems #AF960; Villin, Abcam, ab130751, COV2-NP 1:100, ACE2 1:100) overnight. Slides were then incubated with corresponding anti-mouse, anti-rabbit, and anti-goat secondary antibodies (1:200, 2 hrs) and DAPI (1:2000, 10 mins) and mounted with Immumount.

Cells and organoids were imaged with a Zeiss LSM 780, Nikon C2 confocal microscope or ThermoFisher HCS CX7 microscope. The images were quantified using the CellProfiler software. Data points represent at least 100 cells or one 96-well each.

iii. Fluorescent in situ hybridization (FISH): RNA FISH was performed on post-mortem brain (74-year-old male donor, post-mortem interval 87 hours) using the RNAscope Multiplex Fluorescent v2 protocol (Advanced Cell Diagnostics) after fixation with 4% formaldehyde for 6 hours at 4°C, followed by incubation in 30% sucrose overnight. Immediately after the in situ labeling was completed, endogenous background was reduced using TrueBlack Lipofuscin Autofluorescence Quencher (Biotium). Sections were Mounted with Vectashield antifade medium and images were acquired on a Zeiss LSM780 inverted confocal microscope.

iii. Immunoblot: For the Western Blot Analysis of COVID-19 receptors in human brain, 100 mg (1-volume) of brain tissue was Dounce homogenized in 1 mL (10 volumes) of ice-cold protein extraction buffer (50 mM-Tris-HCl, pH 8; 20 mM-NaCl; 2 mM-MgCl2; 4 M-Urea, 0.35% Triton-X100; 0.35% Sodium deoxycholate; 0.05% SDS; supplemented with protease and phosphatase inhibitors). Benzonase was added to eliminate viscosity of the sample for 10 min on ice, then sonicated and centrifuged at 20,817 x g for 15 minutes at 4°C. Clarified total lysates were quantified using a BCA Protein Assay kit (Pierce). Western blot analysis was performed using 100 μg of total protein using anti-ACE2 (Abcam/ab239924/EPR4435(2)), anti-CD147 (Abcam/ab666/MEM-M6/1), anti-FURIN (Abcam/ab183495/EPR14674) or anti-β-ACTIN (Cell Signaling/#4970S/13E5) primary antibodies (1:5000, 1-hr) followed by autoradiographic detection using corresponding anti-mouse (Cell Signaling/#7076S) or anti-rabbit (Cell Signaling/#7074S) secondary antibodies conjugated with HRP (1:5000, 1-hr). For cell
cultures, weight of the harvested cell pellet was used to prepare the extracts and followed the rest of the protocol as described above. Several antibodies against host SARS-CoV-2 host proteins were tested to identify the most suitable ones. The results are shared as technical resource on zenodo (Peter et al., 2020).

iv. Lentivirus generation: Third-generation VSV.G pseudotyped HIV-1 lentiviruses were produced by polyethyleneimine (PEI, Polysciences #23966-2)-transfection of HEK293T cells and packaged with VSVG-coats using established methods. Used shRNA against ACE2 (SHCLNG-NM_021804), CD147 (SHCLNG-NM_001728), TMPRSS2 (SHCLNG-NM_005656) were obtained from Sigma.

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