



Supplementary Materials for

Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity

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Other Supplementary Materials for this manuscript includes the following:
(available at science.sciencemag.org/cgi/content/full/science.abd2985/DC1)

MDAR Reproducibility Checklist (.pdf)

Supplementary Materials

Material and Methods

Animals

All animal studies were performed in compliance with the animal policies of the German Center for Neurodegenerative Diseases (Munich), and were approved by the district government of upper Bavaria. Animals were group-housed (3-5 mice) with 12-hour dark/light cycle and had access to food and water *ad libitum*. All experiments were conducted in adult male and female C57BL/6N mice (8 to 10 weeks of age). Male mice were subjected to intranasal delivery of the particles. BALB/c mice were purchased from Jackson Laboratories.

Plasmids

pQCXIBL-hTMPRSS2 and pCG1-SARS-2-S-HA were a kind gift from S. Pöhlmann (German Primate Center, Göttingen, Germany) (27). Human ACE2 (hACE2) was a gift from H. Choe (Addgene plasmid # 1786). pCG1-MCS, pCG1-ACE2, pCG1-NRP1 were cloned by PCR amplification of vector and insert, followed by 2-fragment Gibson assemblies. PCRs were performed using Q5 polymerase (New England Biolabs Inc) according to the manufacturer's protocol. Fragments were generated with the following primers (template plasmids indicated in brackets): pCG1-MCS: 5' fragment (pCG1-SARS-2-S-HA): 5'-gctcagtggaaacgaaa-3' (fwd) and 5'-aacagtcgactctagaggatccgaattcgcc-3' (rev), 3' fragment: (pCG1-SARS-2-S-HA): 5'-gcgaattcggatcctctagagtcgactgtttaaac-3' (fwd) and 5'-ccttaacgtgagtttcgt-3' (rev), pCG1-ACE2: vector (pCG1-SARS-2-S-HA): 5'-gtcgactgtttaaacctgc-3' (fwd) and 5'-aacgctagccagcttccgaattcgccctatag-3' (rev), insert (hACE2): 5'-atagggcgaaattcggaagctggctagcgt-3' (fwd) and 5'-cttgcatgcctgcaggtttaaacagtcgacggatccaagcttaagcg-3' (rev), pCG1-NRP1: vector pCG1-SARS-2-S-HA): 5'-agagtcgacctgcag-3' (fwd) and 5'-gatcgctccttactaccgaattcgccctat-3' (rev), insert (NRP1, native human ORF in pF1K, FXC23812, Promega): 5'-atagggcgaaattcggttagaaggagcgatcg-3' (fwd) and 5'-gatcagcttgcatgcc-3' (rev). Gibson assembly was performed using NEBuilder® HiFi DNA Assembly Cloning Kit (New England Biolabs Inc.) according to the manufacturer's recommendations. All constructs were verified by Sanger sequencing. The experiments described in Figure 1A, B and C, and in Figure 2 were performed

using the following plasmids: pLenti-6.3-hNRP1, pLenti-6.3-hNRP1_L9_mCherry, pLenti-7.3-hACE2, pLenti-6.3 hTMPRSS2. These plasmids were cloned by DNA-Dream-Lab local facility with the cDNAs obtained from the Genome Biology Unit core facility of the University of Helsinki (<https://www.helsinki.fi/en/researchgroups/genome-biology-unit/clones-and-cloning>).
Genome Biology Unit is supported by HiLIFE and the Faculty of Medicine, University of Helsinki, and Biocenter Finland. The genes and the vectors were PCR linearized, purified, and assembled using NEBuilder® HiFi (New England Biolabs Inc.) Primers used to generate fragments for pLenti-6.3-hNRP1_L9_mCherry: 5'-atggacgagctgtacaagggccgcgggtcgaaggaag-3' (fwd) and 5'-tgcagaacctgaagaaccagatgctgcctccgaataagtactctgtgtattcagtttg-3' (rev) for vector with hNRP1 gene, 5'- atctgggtcttcaggttctgcaggtatggtgagcaagggcgagg -3' (fwd) and 5'- ccttgtagctcgtccatgccg -3'(rev) for mCherry; pLenti-7.3-hACE2: 5'- gtggttgatatccagcacagtggc-3' (fwd) and 5'- cttgttgatatctgcagaattccaccacac-3' (rev) for the vector, 5'- gcagatatcaacaagttgtacaaaaagcaggctaccatgtcaagctcttctggctcc -3' (fwd) 5'- ctgtgctggatatcaaccactttgtacaagaaagctgggtaagcgggcgccacctgggag -3' (rev) for the hACE2 gene; pLenti-6.3 hTMPRSS2: 5'- agacggctaaccgaagcttacgcgtaccagc -3'(fwd) and 5'- ttcaaagccataccgggtaccagcctgctttttgtac -3'(rev) for the vector, 5'- tggtagcggtagtggttgaactcagggtcaccac -3'(fwd) and 5'- gtaagcttgggttagccgtctgccctcattgtcgataaatc -3'(rev) for the hTMPRSS2 gene. All constructs were verified by Sanger sequencing. pLenti-6.3-hNRP1-L9-mCherry was used for generation of stable HEK 293T cell lines expressing TMPRSS2, hAce2 and NRP1 (see below for stable cell line generation methods).

Cell culture

HEK-293T and Caco-2 (ATCC) cells were grown in DMEM media supplemented with 10% fetal calf serum (FCS), pen/strep, L-Glutamine, and passaged 1:8 (HEK-293 T) or 1:5 (Caco-2) every three days. CHO parental and CHO FD11 (kind gift of Dr. Stephen Leppla, NIH/NIAID, Bethesda, USA) where grown in DMEM media supplemented with 10% fetal calf serum (FCS), pen/strep, and L-Glutamine. Media for CHO FD11 cells were supplemented with 40mg/L L-Proline. PPC-1 human primary prostate cells were obtained from Erkki Ruoslahti's laboratory at the Cancer Research Center, Sanford-Burnham-Prebys Medical Discovery Institute. Cells were cultivated as an attached culture in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Lonza) containing 100 IU/ml of streptomycin and penicillin (both Thermo Fischer Scientific),

and 10% fetal bovine serum (FBS; Thermo Scientific) in a 37°C incubator with 5% CO₂. For the transfection of NRP1, ACE2 and TMPRSS1, 8 mm glass coverslips were coated with 0.01% poly-L-lysine for 1 hour at 37°C and 5×10⁵ cells were plated in each well of 24-well plates. Cells were also plated on 96-well imaging plates (PerkinElmer) at a dilution of 1×10⁴ cells per well in 100 µl of full growth medium. 24 hours after plating, cells were transfected in Opti-MEM supplemented with GlutaMAX (Gibco) and 5% FCS with Lipofectamine 3000 reagent (Thermo Fisher Scientific) or Transit LT-1 (Mirus) according to the manufacturer's instructions. Briefly, 0.25 µg (24-well plates) or 0-10 µg (96-well plates) of DNA per well was prepared in Opti-MEM and P3000 reagent (for Lipofectamine 3000) or Opti-MEM (for Transit LT-1). The DNA was then mixed with the transfection reagent and left at room temperature for 5 minutes (for Lipofectamine) or 15 minutes (for Transit LT-1). Cells were then incubated with the mix for 4 hours for Lipofectamine) or 8 h (for Transit LT-1) before the transfection reagent was replaced with fresh growth media. The viral particles were added 24 hours after transfection. After treatment, the cells were fixed in 4% paraformaldehyde (PFA) for 20 min. After washes with PBS, the cell nuclei were counterstained with Hoechst 33342 DNA stain solution (Thermo Fisher Scientific) and mounted with ProLong Gold Antifade (8 mm glass coverslips) or left in PBS (96-well plates) before imaging at SP5 confocal microscope or Molecular Device Image Xpress Nano high content imaging microscope, respectively. For confocal imaging of NRP1 expressing PPC-1 cells, 30,000 cells/well were cultured at 37°C in 5% CO₂ as a monolayer on coverslips (d = 12 mm; Paul Marienfeld GmbH & Co. KG) in 24-well plates (#08-772-1H, Thermo Fisher Scientific) with DMEM. In all experiments where anti-NRP1 monoclonal Ab were used, the attached cells were pre-incubated at 37°C for 30 min with mAbs (30 µg/ml), which were dialyzed prior to use overnight against 1X PBS using 10 kDa dialysis cassettes (#66380, Thermo Fischer Scientific). Next, RPARPAR-targeted or non-targeted CF555-labeled or CF488-labeled AgNPs (1.5 nM final concentration) in supplemented DMEM were added to the wells, and the cells were further incubated at 37°C for 35 min (fig. S1D) or 1 h (fig. S2D). Next, the cells were washed 3 times with PBS, incubated for 4 min in etching solution to remove non internalized particles or with PBS (as indicated in the figure legends), and fixed with -20°C methanol (MeOH; Naxo; Fig.2) for 5 min, of with 20% PFA for 20 min (Fig S1 D), and counterstained with DAPI (500 µl, 1 µg/ml; Thermo Fischer Scientific). Coverslips on microscopy slides (76 × 26 mm, Glaswarenfabrik Karl Hecht GmbH & Co. KG) were mounted using Fluoromount-G

aqueous mounting medium (Electron Microscopy Sciences). For fig. S1D, PerkinElmer 96-well imaging plates were imaged with a Molecular Device Image Xpress Nano high content imaging microscope. For fig. S2, confocal imaging was performed using an Olympus FV1200MPE confocal microscope (Olympus Europa SE & Co. KG) with an UPlanSApo 60x/1.35na objective (Olympus Europa SE & Co. KG), and images were analyzed with FluoView FV10-ASW 4.0 software (Olympus Europa SE & Co. KG).

HEK-293T and CHO cells stably expressing indicated entry factors were generated by transduction with the respective lentivirus constructs. Infections were performed at an MOI of 0.3, in MEM medium supplemented with 10% FCS, 0.5% BSA, 1xGlutaMax and 20 mM HEPES pH 7.2. The virus inoculum was removed after 6-8 hours and, 48 h later the culture was passaged in media containing selection antibiotics (1mg/ml Blasticidine in the case of viruses generated using pLenti-6.3 expression plasmids). The selection antibiotic was removed from the media one week before using the cells for SARS-CoV-2 experiments. FACS was used to sort cells expressing fluorescent reporter proteins (EGFP in the case of viruses generated using pLenti-7.3 expression plasmids). To generate HEK-293T cells expressing combinations of one, two or three entry factors, cells were first infected with lentiviruses expressing TMPRSS2 in pLenti 6.3. After selection with blasticidine, cells were infected either with viruses expressing hACE2 and EGFP (pLenti 7.3) or a combination of viruses expressing ACE2 and GFP (pLenti 7.3) and NRP1-L8-mCherry. FACS was used to sort EGFP-positive or EGFP- and mCherry-double positive cells. All stable cell lines generated with lentivirus expression vectors were checked for the presence of residual infectious lentiviral particles and were found negative after three passages.

Virus Infections

All experiments with wild-type or mutant SARS-CoV-2 were performed in BSL3 facilities of the University of Helsinki with appropriate institutional permits. Infection of cell cultures was performed in DMEM, supplemented with 2% FBS, 0.2% BSA, 1xGlutaMax, 20 mM HEPES pH 7.2. For the inhibition of infection by soluble recombinant hACE2 in Caco-2 cells, viruses (MOI 10) and human protein (25 µg/ml), or PBS control, were pre-incubated for 30 min at room temperature and then used to infect cells in 96-well imaging plates.

For analysis of S protein cleavage in Vero-E6 and CHO parental or furin deficient (FD11), cells were seeded in 6-well plates (2×10^5 cells per well) and infected on the next day for 16-24 h with SARS-CoV-2 produced in Caco-2 cells passage 3, or with a SARS-CoV-2 mutant (SARS2-mut), produced in Vero-E6 cells, passage 7. Virus infections were carried out in DMEM, 2% FBS, 0.2% BSA, 1xGlutaMax, 20 mM HEPES pH 7.2, at a MOI of 1. Vero-E6 cells were infected with MOI 1. CHO stably expressing hACE2 were infected with MOI 10. For infection of Caco-2 cells, the amount of wild-type and mutant virus was adjusted to obtain a similar percentage of infected cells in PBS treated cells. For this experiment, the mutant virus was used at MOI 50, the wt virus at MOI 10. In this work, all viral titers were determined in Vero-E6 cells.

Production of SARS-CoV-2 S-pseudotyped lentiviral particles

HEK293T cells were cultured in complete growth media (10% FCS, Pen/strep, L-Glutamine in DMEM) until 60 to 70% confluent on a 10 cm dish. The medium was then replaced with Opti-MEM (supplemented with L-Glutamine and 5% FCS, no antibiotics) for two hours. Cells were then transfected with Lipofectamine 3000 according to the manufacturer's instructions. Briefly, a solution with Lipofectamine 3000 and a solution with a mix of 18 μ g of the following plasmids were separately prepared in Opti-MEM: pMDL g/p RRE, pRSV-REV, pLenti-GFP and a plasmid encoding the spike proteins of SARS-CoV-2 or VSV-G. Plasmid ratio for pMDL g/p RRE, pRSV-REV, the spike protein plasmid and pLenti-GFP is 1:1:1:2, respectively. The P3000 reagent was then added to the mix. After 5 minutes, the DNA mix was added to Lipofectamine solution and the mix was left for 20 minutes at room temperature. The mix was then added to the cells for 6 hours. Cell medium was then changed to normal growth medium (without antibiotics). 24 and 48 hours after transfection, the medium was collected, centrifuged at $1000 \times g$ for 5 minutes, and filtered through a 0.45μ m filter. The medium was centrifuged at $65,000 \times g$ with a SW28 rotor for 3 hours over a cushion of 10% sucrose prepared in PBS. The pellet was finally resuspended in TBS buffer containing 5% bovine serum albumin overnight at 4°C , aliquoted and frozen at -80°C .

Recombinant NRP1 b1b2 soluble protein expression and purification

Wild type and triple mutant human NRP1 b1b2 domain (residues 274-584) were expressed in *Escherichia coli* strain Rosetta-gami-2 (Novagen, Madison, WI) as a His-tag fusion in pET28b

(Novagen). Cells were grown in Terrific-Broth at 37°C to an OD₆₀₀ = 1.2 and, after 15 min at 4°C, induced with 1 mM isopropyl β-d-thiogalactoside. After growth at 16°C for 16 h, cells were harvested by centrifugation, lysed, and centrifuged, and proteins were purified over HIS-Select (Sigma–Aldrich, St. Louis, MO) nickel affinity resin in 20 mM Tris (pH 8.0) and 400 mM NaCl with an imidazole gradient from 25-500 mM. Further purification was performed on 5 ml HiTrap heparin HP column (GE). Protein was loaded in 20mM Tris pH =8.0, 100 mM NaCl and eluted using a linear gradient 100-800 M NaCl. Final purification was performed using Superdex 75 16/100 (Amersham Pharmacia) column equilibrated in 20 mM Tris pH 8.0, 150mM NaCl.

Generation of monoclonal antibodies against NRP1 b1b2

Female BALB/c and C57BL/6 mice, 8–9 weeks old, were immunized intraperitoneally with 17 µg of recombinant NRP1 b1b2 mixed with an equal volume of complete Freund's adjuvant (Sigma-Aldrich Chemie, Steinheim, Germany), followed by a booster immunization four weeks later of the same dose mixed with incomplete Freund's adjuvant (Sigma–Aldrich). Mice received three boosts of the same amount of antigen in PBS on days –3, –2, and –1 prior to fusion. Spleens were excised and the splenocytes were fused with myeloma cells (P3X63Ag8.653) as previously described (28). On day 10 after fusion, hybridoma supernatants were screened for specific antibodies. Before experiments, the hybridoma supernatants were centrifuged at 300 x g for 5 min at room temperature and 500 µl dialyzed against 2 L of PBS over night at 4°C prior use.

Synthesis and functionalization of AgNPs

The silver nanoparticles (AgNPs) were synthesized and functionalized as described previously (29), wherein biotin-Ahx-RPARPAR-OH (RPARPAR; TAG Copenhagen A/S) was used as the targeting moiety and CF555 dye (#92130, Biotium) or CF647 (#92135, Biotium) as the fluorophore. Briefly, AgNO₃ (360 mg; #209139, Sigma-Aldrich) was added to ultrapure Milli-Q (MQ) water (2 L; resistivity 18 MΩ cm⁻¹) in a flask cleaned with a piranha solution (H₂SO₄/H₂O₂). Next, trisodium citrate hydrate (400 mg; #25114, Sigma-Aldrich) was dissolved in MQ water (40 ml) and added to the vessel. The solution was boiled for 30 min in the dark. The resulting Ag-citrate was used directly in the next step. Next, NeutrAvidin (NA; #31055, Thermo Scientific) was modified with a OPSS-PEG(5K)-SCM linker (OPSS; JenKem Technology USA)

as previously described (29). Subsequently, NeutrAvidin-OPSS (3.9 ml, 2.9 mg/ml) was added to Ag-citrate (500 ml). After 2 min, 4-morpholineethanesulfonic acid hemisodium salt (5 ml, 0.5 M in MQ water; #M0164, Sigma-Aldrich) was added. The pH of the solution was adjusted to 6.0, and the solution was kept at 37°C for 24 h. The solution was brought to RT and 10X PBS (50 ml; Naxo) was added, followed by Tween® 20 (250 µl; #P9416, Sigma-Aldrich). The solution was centrifuged at $12,200 \times g$ for 1 h (4°C), the supernatant was removed, and the particles were resuspended in PBST (0.005% Tween® 20 in PBS). Next, tris(2-carboxyethyl)phosphine hydrochloride solution (TCEP; #646547, Sigma-Aldrich) was added to a final concentration of 1 mM, followed by a 30 min incubation at RT. Then lipoic acid-PEG(1k)-NH₂ (#PG2-AMLA-1k, Nanocs) was added to a final concentration of 5 µM, and the mixture was incubated at RT for 2.5 h. The solution was centrifuged at $17,200 \times g$ for 20 min (4°C), the supernatant was removed and the particles were resuspended in PBST to half of the initial volume. The AgNP solution was filtered through a 0.45 µm filter and stored at 4°C in dark. NHS-functionalized CF555 or CF647 dye was coupled to the NH₂ groups of the linker on the AgNPs. For this, NHS-CF555 (5 µl, 2 mM) in dimethyl sulfoxide (DMSO; Sigma-Aldrich Co., LLC) was added to AgNP (500 µl), followed by an overnight incubation at 4°C. The particles were washed 3 times by centrifugation at $3,500 \times g$ for 10 min at 4°C, followed by resuspension of the particles in PBST by sonication. Next, biotinylated peptides were coupled to the particles by adding peptide (10 µl, 2 mM in MQ water) to AgNPs (500 µl), followed by incubation at RT for 30 min. The AgNPs were washed three times, 0.2 µm filtered and stored at 4°C in dark for a maximum of 2 weeks.

Antibody labeling and binding assay with b1b2 domain of NRP-1

Anti-NRP1 monoclonal antibodies were labeled with DyLight™ 488 NHS Ester (DL488; #46402, Thermo Fisher Scientific). First, the mAbs were purified of glycerol by dialysis in 3L of 1X PBS for 2 days at 4 °C, using 3.5K MWCO Slide-A-Lyzer™ Dialysis Cassettes (#66330, Thermo Fisher Scientific). Next, 500 µl of 0.6 mg/ml mAb1, mAb2 or mAb3 was incubated with 7 µl of 2 mM dye in DMF for 2 h at RT. Excess of dye was removed using 3K MWCO Protein Concentrators PES (#88512, Thermo Fisher Scientific) by centrifugation at $14,000 \times g$ at RT for 30 min and topping off with PBS; the washes were repeated 3 times.

For cell-free binding studies, hexahistidine-tagged (6xHis-tagged) recombinant b1b2 domain of NRP-1, or the mutant NRP-1 b1b2 with an inactive CendR-binding pocket, (0.5 mg/ml; in-house) was coated onto Ni-NTA magnetic agarose beads (5% solution; #36113, Qiagen GmbH) by incubating with end-over-end mixing at RT for 1 h. PBS containing 0.05% of Triton X-100 (Sigma Aldrich) and 0.2% BSA (GE Healthcare) was used as the washing buffer. Protein-coated magnetic beads were incubated with DL488-labeled mAb1, mAb2 or mAb3 (40 µg/ml) with end-over-end mixing at RT for 1 h, followed by 3 washes with the washing buffer, and the release of protein-mAb complexes with imidazole elution buffer [washing buffer + 400 mM imidazole (Sigma Aldrich)]. A Victor X5 Multilabel Plate Reader (PerkinElmer) with a 488/515 nm filter set was used for the fluorescence measurements, and all measurements were done in triplicates.

Mouse immunohistochemistry and immunofluorescence

The mouse olfactory epithelium, the olfactory bulbs, and the brain were isolated and left in 30% sucrose until sunk to the bottom of the tube. After embedding in Tissue Tek OCT, the tissues were sectioned with a thickness of 14 µm. For the multiplex fluorescence labeling of NRP1 mRNA (RNAScope Probe-Mm-Nrp1, Advanced Cell Diagnostics, 471621), the RNAScope® Multiplex Fluorescent v2 Assay (Advanced Cell Diagnostics, 323100) was used according to the manufacturer's instructions. For the immunohistochemistry, the sections were air dried overnight at room temperature, washed in PBS and permeabilized with 0.1% Triton-X-100 for 15 min at room temperature. The blocking performed with 1% fetal calf serum, 1% fish gelatin and 1% bovine serum albumin in PBS for one hour at room temperature, followed by a second blocking step for endogenous mouse Ig with AffiniPure Fab fragment Donkey anti-mouse IgG (Biozol, JIM-715-007-003) diluted 1:100 in blocking solution for one hour at room temperature. Primary antibodies were diluted in 10% blocking solution: 1:250 NRP1 (monoclonal rabbit, ab81321, Abcam); 1:1000 TuJ1 (monoclonal mouse, G712A, Promega); 1:250 NeuN (polyclonal chicken, ABN91, Milipore); 1:2000 GFP (polyclonal rabbit, A-6455, Thermo Fisher Scientific), 1:250 ColIV (polyclonal goat, 1340-01, Southern Biotech), 1:250 CD31 (monoclonal mouse, sc-376764, Santa Cruz Biotechnology), 1:500 AQP4 (polyclonal rabbit, AQP-004, TMH-Medizinhandel) and incubated overnight at 4°C. After three washes in PBS, the sections were incubated in secondary antibody: Alexa Fluor 488 donkey anti-mouse (R37114, Thermo Fischer Scientific); Alexa Fluor 488 goat anti-rabbit (A21428, Thermo Fischer

Scientific); Alexa Fluor 488 goat anti-chicken (A11039, Thermo Fischer Scientific); Alexa Fluor 488 donkey anti-goat (A11055, Thermo Fischer Scientific); Alexa Fluor 555 donkey anti-goat (A21432, Thermo Fischer Scientific); Alexa Fluor 555 goat anti-mouse (A21422, Thermo Fischer Scientific); Alexa Fluor 647 donkey anti-rabbit (A31573, Thermo Fischer Scientific),
 5 Alexa Fluor 488 Phalloidin (A12379 Thermo Fischer Scientific), for two hours at room temperature. After three washes in PBS, the sections were counterstained with Hoechst 33342 stain solution (Thermo Fisher Scientific) and mounted with Prolong Gold Antifade Mountant (ThermoFisher, P36930). For immunofluorescence, the cells were permeabilized on ice for 10 minutes in 0.1% Triton-X-100 and 0.1% Sodium citrate in PBS, blocked for one hour in 1% fetal
 10 calf serum, 1% fish gelatin and 1% bovine serum albumin in PBS at room temperature and left overnight at 4°C in primary antibody diluted in 10% blocking solution. After three washes in PBS, the cells were incubated with secondary antibodies (diluted 1:500 in 10% blocking solution) for one hour at room temperature. After three washes in PBS, the cells were stained with Hoechst 33342 (Thermo Fisher Scientific) and mounted with ProLong Gold Antifade. The
 15 stained tissues and cells were imaged on a Leica SP5 confocal microscope.

Human immunohistochemistry

All experiments including human tissue were approved by the ethics committee of the University Medical Center Göttingen and performed in accordance with the respective national, federal and
 20 institutional regulations. Human tissue blocks were fixed in 3.7% formalin and embedded in paraffin. 3 µm deparaffinized tissue sections were pre-treated for epitope retrieval as indicated. Sections were treated with 3% H₂O₂, permeabilized with 1% Triton-X-100 and then blocked with PBS containing 10% goat serum. Primary antibodies were applied over night at a dilution of 1:100 for SARS-CoV S protein (monoclonal mouse, ab272420, Abcam; microwave, citric acid
 25 buffer, 10 mM, pH 6.0), 1:250 for NRP1 (monoclonal rabbit, ab81321, Abcam; microwave, Tris-EDTA, pH 8.0), 1:50 for NRP1 (monoclonal mouse, sc-5307, Santa Cruz, microwave, Tris-EDTA, pH 8.0 and citric acid buffer, 10 mM, pH 6.0, 1 % Triton-X-100 for 15 min), 1:50 for ACE2 (polyclonal goat, AF933, R&D Systems, microwave, Tris-EDTA, pH 8.0) and 1:150 for OLIG2 (polyclonal rabbit, 18953, IBL; microwave, Tris-EDTA, pH 8.0). Secondary antibodies
 30 were added as follows: biotinylated anti-goat/sheep 1:200 (Amersham RPN1025), biotinylated anti-mouse 1:200 (GE Healthcare RPN 1001) followed by Tyramide Super Boost with Alexa

Fluor 488 1:500 (Thermo Fisher Scientific) and Alexa Fluor 555 anti rabbit 1:500 for 2 h, at room temperature. Coverslips were mounted with ProLong Diamond Antifade with DAPI (Thermo Fisher Scientific). Antibody binding for immunohistochemistry was visualized using peroxidase-conjugated avidin and DAB (Sigma-Aldrich), the Envision+ System for mouse and rabbit (Dako) and alkaline phosphatase-conjugated anti-rabbit or anti-mouse antibodies with FastBlue (Sigma Aldrich). Images were taken using a light and fluorescence microscope (Olympus BX-63 with a colour camera (Olympus DP80). Image visualization was performed using Omero Server software 5.6, Omero figure 4.0.2 (<https://github.com/ome/omero-figure>) and InkScape 0.92. To evaluate the percentage of OLIG2 positive cells in the olfactory epithelium positive for S protein, sections were scanned (magnification 200x) using the Virtual Slide Microscope VS120 and visualized using the VS-ASW software (Olympus, Germany). Immunopositive cells were counted manually using Olympus cellSens dimension 1.16 (Olympus life science).

Western blotting

Vero-E6 cells were seeded in 6-well plates (2×10^5 cells per well) and on next day infected for 16 h with WT (SARS2-wt produced in Caco-2 cells passage 3) or deletion mutant (SARS2-mut, produced in Vero-E6 cells, passage 7) viruses, in DMEM, 2% FBS, 0.2% BSA, 1xGlutaMax, 20 mM HEPES pH 7.2, at a MOI of 1. Cells were lysed in 1x Laemmli sample buffer (Bio-Rad cat. #161-0737), boiled for 10 min and the DNA sheered by aspirating up and down 20 times through a 25-G needle and 1ml syringe. Before SDS-PAGE, 2-mercaptoethanol was added to a final concentration of 5% and 15 μ l of this mixture was loaded in each lane of a stain-free polyacrylamide 5-12% gel (Bio-Rad cat. #4568096). Samples were subjected to electrophoresis for 40 min at 200 V, and the gel blotted on nitrocellulose membrane (Bio-Rad cat. #170-4270) using a Trans-blot Turbo system (Bio-Rad) set at 'standard' 30 min program. After blotting, membranes were washed three times for 10 min with tris-buffer saline solution containing 0.1% Tween-20 (TBST) and 5% milk powder, pH 7.4, at room temperature. This buffer was also used to incubate the membrane with a dilution 1:500 of the antibodies against SARS-CoV-2 S (2019-nCoV spike, Rabbit anti-RBD, Sino Biological, 40592-T62, HD14AP2102), 1:250 of mouse anti-TMPRSS2 (Santa Cruz), and 1:1000 of rabbit anti-tubulin (Abcam, ab 15246), over-night at 4°C with slow agitation. After three washes for 10 min in the same buffer, the membranes were

incubated for 30 min with secondary IR dye 680 RD Goat anti-Rabbit (cat. 926-68071) antibodies conjugated with different fluorophores and the membrane imaged with an Odyssey (model no. 9120, S/N ODY 2146) detection system.

Quantitative PCR

Total RNA was isolated from HEK-293T and Caco-2 cells with the RNeasy mini kit (QIAGEN) after cell lysis with the QIAshredder columns (QIAGEN) and removal of genomic DNA with the gDNA eliminator columns (QIAGEN), according to the manufacturer's instructions. Reverse transcription of 5 ug of RNA was carried out with the Superscript III cDNA synthesis kit, according to the manufacturer's instructions (Invitrogen). Quantitative PCR was performed with the PowerUp™ SYBR™ Green Master Mix (Thermo Scientific) on a Applied Biosystems 7500 fast real-time PCR system. The following primers were used: ACE2, forward 5'-gggatcagagatcggaagaagaaa-3', reverse 5'-aggaggtctgaacatcatcagt-3'; NRP1, forward 5'-gccacagtgaacaggtgat-3', reverse 5'-atgaccgtgggcttttctgt-3'; CYC1 forward 5'-gaggaggccaagatggcg-3', reverse 5'-gctgacagcatcactttccg-3'.

The threshold cycle (Ct) for each gene was normalized to the housekeeping gene (Delta Ct) and expressed as power of two.

Intranasal delivery of silver nanoparticles in mice

Intranasal delivery was performed as previously described (30). Mice were anesthetized with a mix of medetomidine, midazolam and fentanyl (MMF) and positioned in a head down and forward position. 3 µl of the solution containing the silver nanoparticles was then positioned close to the nostril of the animals until inhaled. After 3 minutes, 3 µl of the solution containing the viral particles was delivered to the other nostril. This procedure was repeated until 30 µl of solution were delivered to each animal. The animals were then woken up with a mix of atipamezole, flumazenil, and naloxone (AFN). Analgesia was achieved with daily injection of buprenorphine. At the end of the experiment, the animals were anesthetized with ketamine and xylazine and perfused with PBS and 4% PFA.

Image analysis

Image visualization was performed using Fiji software (31). The numbers of NRP1⁺ positive neurons in the mouse main olfactory epithelium (MOE) and brain were manually counted from three representative sections of three independent animals. All other measurements were performed using Fiji software (31). To measure the infectivity of cultured cells, three randomly selected areas per coverslip were imaged. The percentage of GFP positive cells was determined by dividing the number of GFP⁺ nuclei by the number of Hoechst⁺ nuclei. The same threshold value was applied to all the coverslips of an independent experiment. To measure the AgNP uptake in cultured HEK293-T cells, three randomly selected areas per coverslip were imaged. AgNP positive cells were determined the following way: each cell was identified by the positivity for the nuclear staining Hoechst 33342 and the actin-binding toxin phalloidin. Hoechst⁺ nuclei were used to define the cell ROI. Within each ROI, the area occupied by the signal of AgNPs was measured and expressed as area fraction. ROIs with an area fraction higher than 30% were considered AgNP⁺ cells. The percentage of AgNP⁺ cells was determined by dividing the number of AgNP⁺ cells by the number of Phalloidin⁺ cells. All samples were analyzed at the same threshold values. For quantification of AgNPs internalization in Caco-2 cells (fig. S1 D), after automated imaging of 96-well plates, the fraction of AgNP-positive cells was determined using Cell Profiler-3 software. Nuclei and cell cytoplasm were identified using the fluorescent signal of nuclei stained with DNA staining Hoechst and whole cells stained with actin marker phalloidin-A647. A threshold of fluorescence was determined such that no positive cells were identified in samples that did not receive the AgNPs. The accuracy of detection was visually inspected in representative images from each experiment before analyzing the whole data set. Nuclei were detected using the Otsu algorithm of CellProfiler (www.Cellprofiler.org). For the in vivo analysis three representative sections from four independent animals were stained with the specific antibodies. For each tissue a representative area was selected and the area occupied by AgNP particles with a size higher than 1.8 μm^2 was measured. The same threshold value was applied to the images of each area.

Isolation of WT SARS-CoV-2 from COVID-19 patient and virus propagation

Samples were obtained under the Helsinki University Hospital laboratory research permit HUS/32/2018 § 16. 500 μl of nasopharyngeal swab in Copan UTM® Universal Transport Medium was inoculated on Calu-3 cells and incubated for 1 h in +37°C, after which the

inoculum was removed and replaced with Minimum Essential Medium supplemented with 2% FBS, L-glutamine, penicillin and streptomycin. Virus replication was determined by RT-PCR for SARS-CoV-2 RdRP (32), and the infectious virus titer was determined by plaque assay in Vero-E6 cells. Viruses used in Fig. 2 were propagated in Vero-E6 cells (SARS_{mutFC}) or Caco-2 cells up to passage 7 or passage 3, respectively.

Sequencing of virus isolates

Viral RNA was extracted from cell culture supernatants using QIAamp Viral RNA Mini Kit (Qiagen) and reverse-transcribed to cDNA using LunaScript RT SuperMix kit (New England Biolabs) following manufacturer's instructions. Primer pools targeting SARS-CoV-2 were designed using PrimalScheme tool <http://primal.zibraproject.org> (33) and PCR was conducted using PhusionFlash PCR master mix (ThermoFisher). Sequencing libraries were prepared using NEBNext ultra II FS DNA library kit (New England Biolabs) according to the manufacturer's instructions, and sequenced using Illumina Miseq with v3 sequencing kit. Raw sequence reads were trimmed and low quality (quality score <30) and short (<25 nt) sequences removed using Trimmomatic (34). The trimmed sequence reads were assembled to the reference sequence (NC_045512.2) using BWA-MEM algorithm implemented in SAMTools version 1.8 (35). The minority variants and insertion/deletion sites were called using LoFreq* version 2.1.4 (36).

scRNA-seq analysis

Analyses of the scRNA-seq datasets including filtering, normalization and clustering were conducted using Seurat 3.1 (37). Human lung data from Han *et al.* (17) was downloaded from https://figshare.com/articles/HCL_DGE_Data/7235471, in the form of batch-corrected digital gene expression matrices and cell annotation csv files. Cell annotation included cell types, tissue of origin and age. Gene expressions were log-normalized with a scale factor of 10 000 using the NormalizeData function. Next, data was scaled using the ScaleData function and the number of UMI and the percentage of mitochondrial gene content were regressed out as described by the authors (17). The first 40 principal components were considered for the UMAP (Uniform Manifold Approximation and Projection). Human olfactory neuroepithelium raw data from Durante *et al.* (18) was downloaded from Gene Expression Omnibus under accession code GSE139522. Data was quality controlled, pre-processed, filtered, normalized, scaled and

clustered as described (18). Data from 4 patients were integrated using the Seurat 3 standard integration (37) using the parameters described (18). Since cell type annotations were not provided publicly, we used the cell type specific markers indicated by the authors to identify cell type clusters. Organotypic human bronchial epithelial cells (HBECs) datasets from Ravindra *et al.* (15) were kindly provided by the authors upon request. Differentially expressed genes table from Ravindra *et al.* (15) was obtained from the GitHub page maintained by the authors (https://github.com/vandijklab/HBEC_SARS-CoV-2_scRNA-seq). Datasets from different time points were already integrated and batch-corrected as previously described (15). Bronchoalveolar lavage fluid (BALF) data was acquired from GSE145926 and only the scRNA-seq data was used (15). Datasets from 12 samples were quality controlled, filtered and integrated using the Seurat 3 standard integration workflow as described by the authors. Differentially expressed genes were identified comparing SARS-CoV-2 positive and negative cells with the FindMarkers function in Seurat. Cell annotation of infection status, cell type, etc. was provided by the authors on their GitHub page (https://github.com/zhangzlab/covid_balf). For all datasets except the DEG tables (15), DotPlot and DimPlot functions within Seurat were used for visualization purposes.

Statistics

All measurements were derived from distinct samples. Statistics were performed in GraphPad Prism. Statistical significance was determined by one-way ANOVA and two-ANOVA followed by pairwise Student's *t*-test with Tukey's or Sidak's correction. Two-tailed unpaired Student's *t* test was performed if only two conditions were compared. Data in the text is presented as mean $\pm$ s.d. Adjusted P values are reported in the figures.

Supplemental Figures

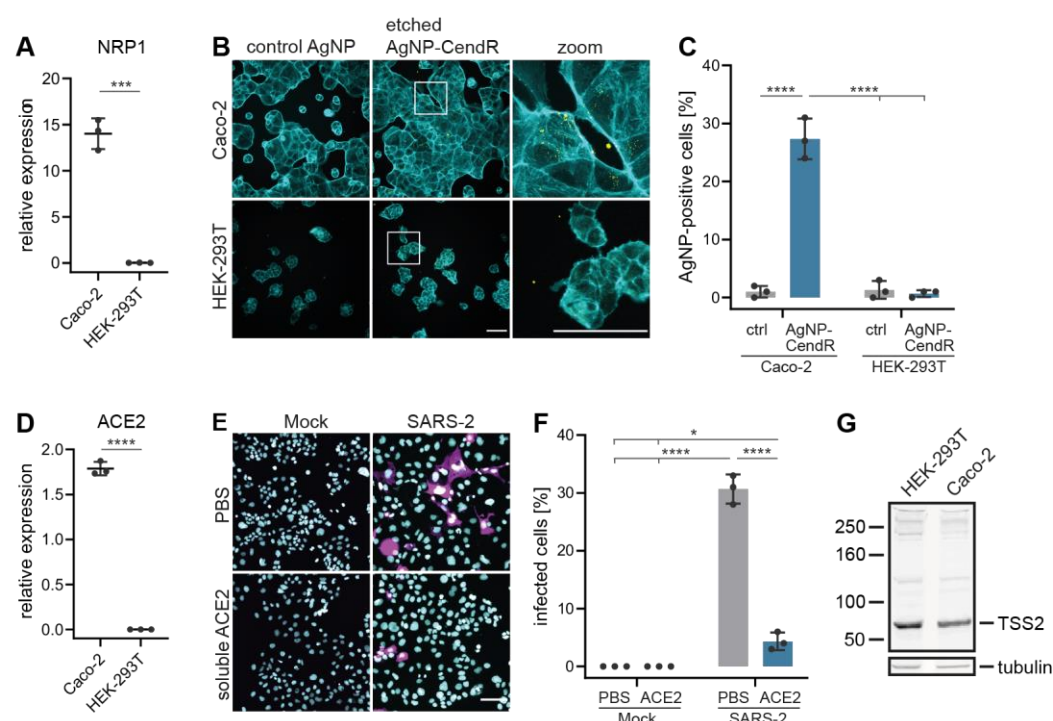


Fig. S1. Detection and functional activity of SARS-CoV-2 entry factors in HEK-293T and Caco-2 cells. (A) Quantitative PCR analysis of human NRP1 in Caco-2 and HEK-293T cells. Data represent the cycle threshold (Ct) of each indicated gene normalized to the Ct of the housekeeping gene (*CYC1*) and expressed by the power of 2. Two-tailed unpaired Student's t test. (B and C) Representative images (B) and quantification (C) of NRP1-dependent endocytic uptake of fluorescent AgNP-RPARPAR_{OH} (AgNP-CendR) occurs in non-transfected Caco-2 cells (upper panels in B), but not in HEK-293T (lower panels in B). Incubation with fluorescent AgNP-CendR or control AgNP-biotin (ctrl) was for 35 min. Actin=cyan; AgNP=yellow. Two-way ANOVA with Tukey's correction for multiple comparisons. (D) Quantitative PCR analysis of human ACE2 in Caco-2 and HEK-293T cells. Data represent the cycle threshold (Ct) of each indicated gene normalized to the Ct of the housekeeping gene (*CYC1*) and expressed by the power of 2. Two-tailed unpaired Student's t test. (E and F) SARS-CoV-2 infection of Caco-2 cells is blocked by soluble recombinant human ACE2 protein (ACE2). Representative fluorescence images (E) and quantification of infection (F) of Caco-2 cells pre-incubated for 30 min with PBS (PBS) or with 25 µg/ml ACE2 and infected for 16 h with SARS-CoV-2 (SARS-2). Nuclei=cyan; SARS-CoV-2 N=magenta. Two-way ANOVA with Tukey's correction for multiple comparisons. (G) Western blot analysis indicates TMPRSS2 expression in Caco-2 and HEK-293T cells. Tubulin was used as a loading control. Data are means of three independent experiments ± s.d. *p<0.05, ***p<0.001, ****p<0.0001. Scale bars are 100 µm.

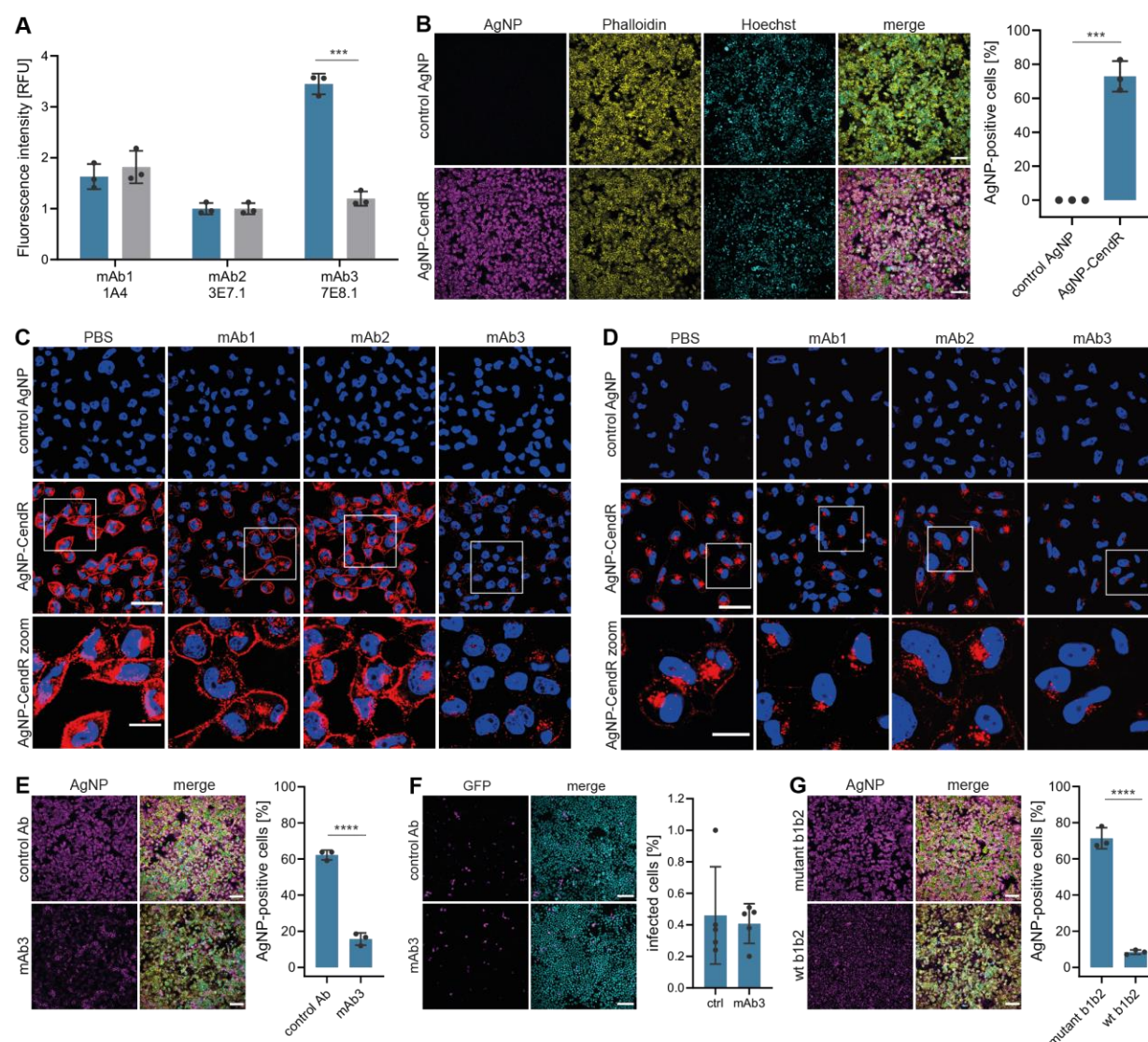


Fig. S2. Functional validation of monoclonal blocking antibodies against NRP1 and characterization of nanoparticles. (A) Recombinant wild-type (blue bars) and mutant (grey bars) b1b2 domains of NRP-1 with a 6xHis-tag were coupled to Ni-NTA magnetic agarose beads. After 1-h incubation with DL488-labeled mAb1, mAb2 or mAb3 (40 μ g/ml) at RT, the unbound mAbs were removed by washing, and bound mAbs in complex with b1b2 were released from the beads with imidazole, and the fluorescence of the eluate at 515 nm was measured with a Victor X5 plate reader. Two-way ANOVA with Sidak's correction for multiple comparisons. (B) Representative images and quantification showing the uptake of CF647-labeled AgNP conjugated with biotin-X-RPARPAR (AgNP-CendR, lower panels) or biotin alone (control AgNP, upper panels) after 30 minutes in HEK-293T cells expressing NRP1. AgNPs (magenta) were visible inside the cells, counterstained with Alexa-Fluor488-phalloidin (yellow) and Hoechst (cyan). Two-tailed unpaired Student's t test. (C) Representative images of primary Prostate Carcinoma-1 (PPC-1) cells incubated for 1h with CF555-labeled silver nanoparticles (AgNP, red signal) conjugated with biotin (control AgNP) or biotin-X-RPARPAR (AgNP-CendR). Cells were pre-treated with a vehicle control (PBS) or the indicated mAb against NRP1

showing the corresponding inhibition of binding and internalizing of the AgNP-CendR nanoparticles. **(D)** PPC-1 cells treated as in (C) were incubated with an etching solution 5 min before fixation to solubilize the non-internalized nanoparticles bound to the external side of the cells. AgNP (red), DAPI (blue). **(E)** Representative images and quantification of NRP1-expressing HEK-293T cells treated for 30 minutes with the blocking mAb3 antibody (lower row) or control mAb2 (upper row) and AgNP-CendR. Two-tailed unpaired Student's t test. **(F)** Representative images and quantification of SARS-CoV-2 S virus pseudotypes (SARS-2) infectivity of HEK-293T cells expressing ACE2 and TMPRSS2 and treated with either mAb3 (lower panel) or control antibody mAb2 (upper panel). Two-tailed unpaired Student's t test was not significant. Images show GFP-positive, infected cells (magenta) and Hoechst (cyan). Two-tailed unpaired Student's t test. **(G)** Representative images and quantification of NRP1-expressing HEK-293T cells incubated for 30 minutes with AgNPs-CendR, together with wild-type b1b2 domain (wt b1b2, lower row) or the mutant b1b2 domain of NRP-1 (mutant b1b2, upper row). Two-tailed unpaired Student's t test. All data is represented as mean $\pm$ s.d. *** $p < 0.001$, **** $p < 0.0001$. Scale bars, 100 μ m (B, E to G), 50 μ m (C and D) and 20 μ m (C and D, zoom).

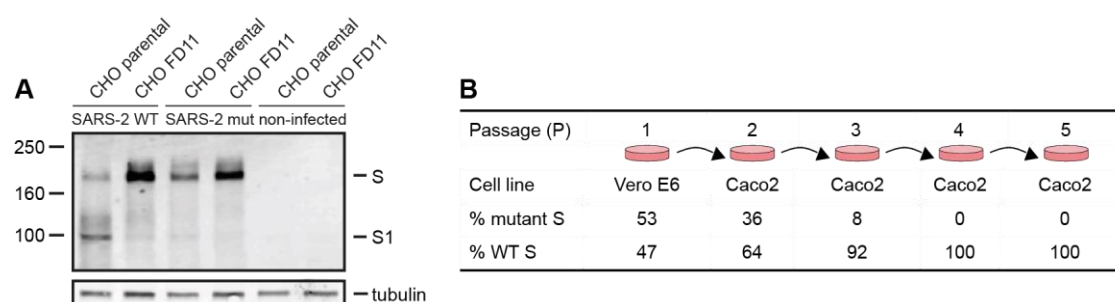


Fig. S3. SARS-CoV-2 S protein is cleaved by furin. **(A)** Western blot analysis of SARS-CoV-2 S protein cleavage in CHO cells with functional (parental) or deficient (FD11) furin enzyme. The Western blot analysis shows the lysates from cells infected with wt SARS-CoV-2 (SARS2-wt) or the mutant with a deletion adjacent to the furin cleavage site (SARS2-mut). **(B)** Sequencing of a virus population isolated from Vero-E6 demonstrates that a deletion around the furin cleavage site has accumulated in 53% of the viral genomes already after one passage (P1). When this mixed-virus isolate was sequentially passaged in Caco-2 cells, the viral sequences with the wt furin-cleavage site became predominant in the population, and the mutant virus became undetectable within three passages in these cells (Caco-2, P4).

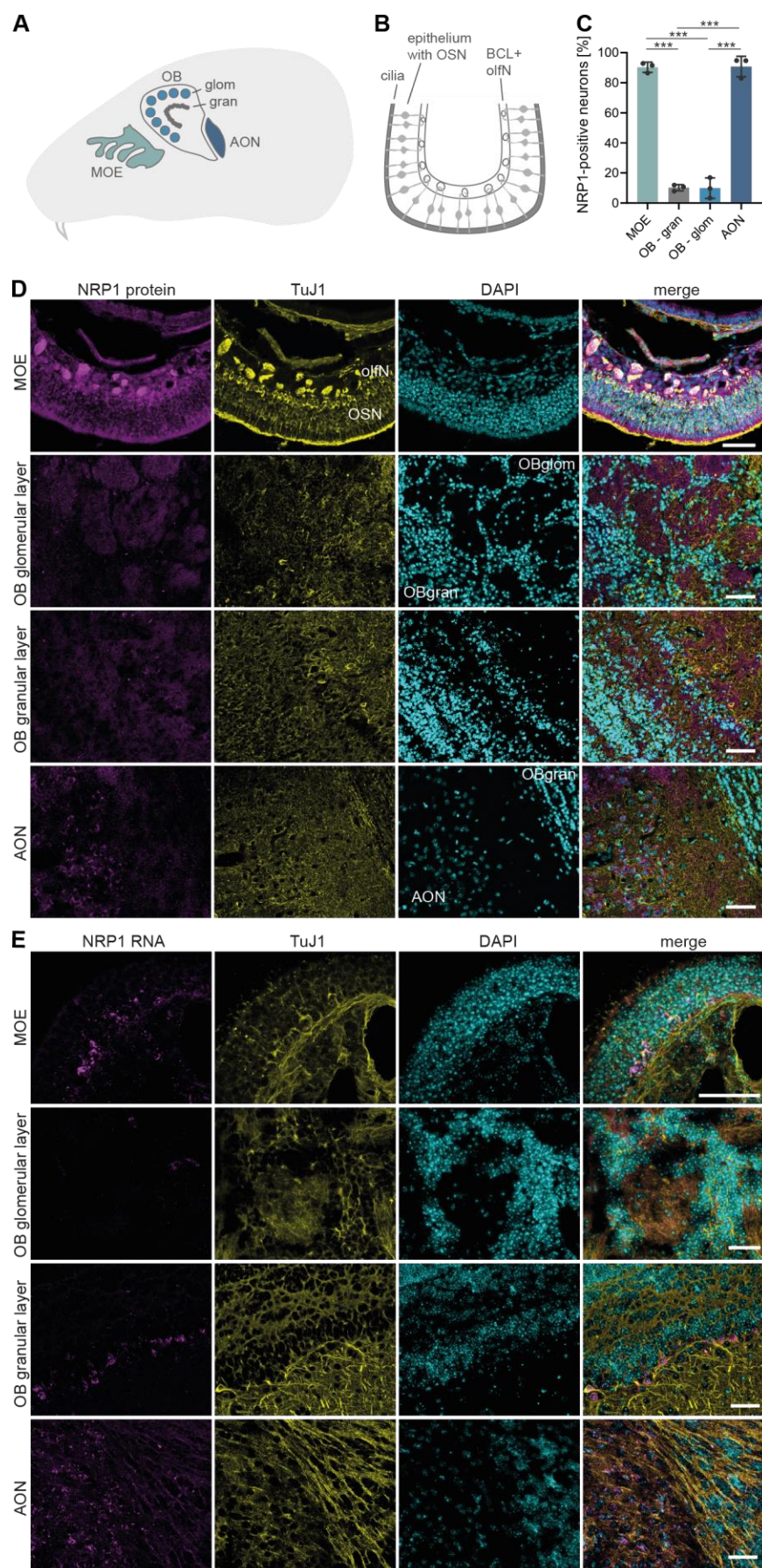


Fig. S4. NRP1 is expressed in the mouse main olfactory epithelium (MOE). (A) Schematic representation of the mouse head showing the position of the main olfactory epithelium (MOE), the glomerular (OBglom) and granular (OBgran) layers of the olfactory bulb (OB), and the anterior olfactory nucleus (AON). (B) Schematic representation of the MOE, depicting olfactory sensory neurons (OSN) within the epithelium, their apical cilia, and olfactory nerves (olfN) within the basal cell layer (BCL). (C) Quantification of NRP1⁺ neurons in the mouse MOE, OB and AON. Data are means $\pm$ s.d ($n = 3$ mice). One-way ANOVA with Tukey's correction for multiple comparisons. *** $p < 0.001$. (D and E) Confocal images of NRP1 protein (D) and NRP1 RNA (E) in the mouse olfactory system, showing co-localization of NRP1 with the neuronal marker TuJ1. Pseudocolored images, NRP1 protein and NRP1 RNA (magenta), the neuronal marker TuJ1 (yellow), Hoechst (cyan). Scale bars, 50 μ m.

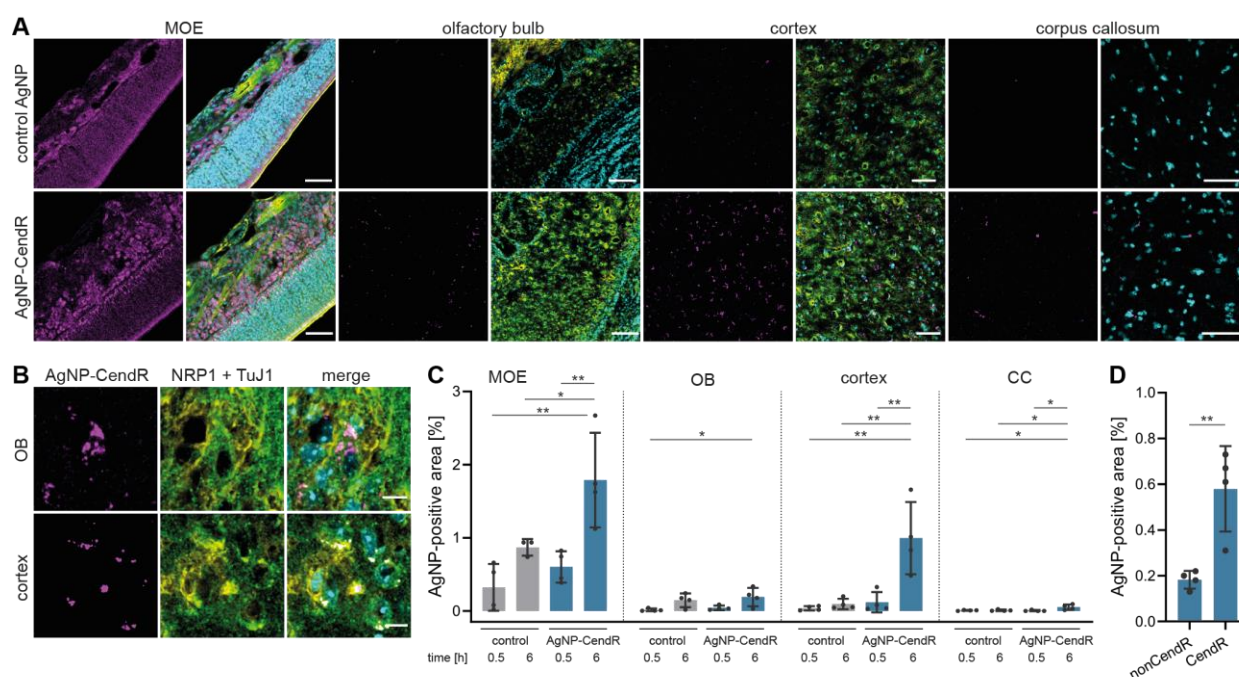


Fig. S5. NRP1-dependent delivery of nanoparticles to the central nervous system. (A) Representative images of the main olfactory epithelium (MOE), olfactory bulb (OB), cortex and corpus callosum (CC) of animals treated for 0.5 or 6 hours with CF647-labeled silver nanoparticles (AgNP) conjugated with biotin (control AgNP, top row) or biotin-X-RPARPAR (AgNP-CendR, bottom row). Scale bars, 50 μ m. (B) Confocal images of OB and cortex of AgNP-CendR treated mice, showing that several AgNP-CendR⁺ cells (magenta) were positive for NRP1 (green) and the neuronal marker TuJ1 (yellow). Scale bars, 10 μ m. (C) Quantification of the area of tissue occupied by AgNPs, showing a significant accumulation of AgNP-CendR in the MOE, cortex and CC. (D) Quantification of the area of tissue occupied by AgNPs conjugated with biotin-X-RPARPAR-OH (CendR) or its amide form biotin-X-RPARPAR-NH₂ (nonCendR), showing a significant accumulation of AgNP-CendR in cortex $n = 4$ mice. Pseudocolored images (A and B), AgNP (magenta), NRP1 (green), TuJ1 (yellow), Hoechst

(cyan). Data are means $\pm$ s.d. One-way ANOVA with Tukey's correction for multiple comparisons within each group; * $p < 0.05$, ** $p < 0.01$.

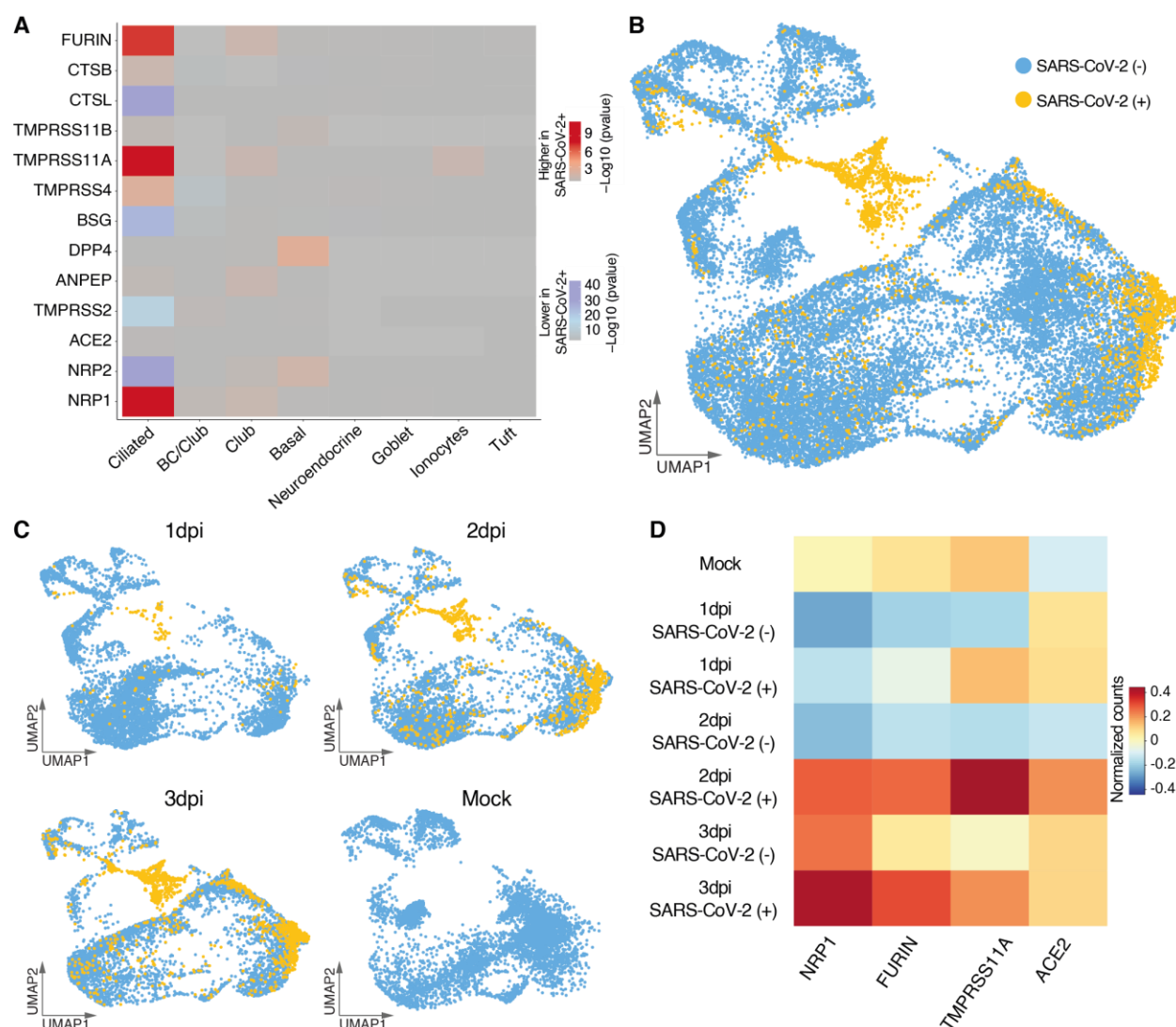


Fig. S6. Comparing known SARS-CoV-2 entry factors across infected versus non-infected bronchial epithelial cells. Ravindra *et al.* (15) infected human bronchial epithelial cells (HBECS) with SARS-CoV-2 and used scRNA-seq to identify ciliated cells as the major target of infection. **(A)** Among the proposed cell entry and amplification factors, only NRP1, FURIN, and TMPRSS11A were highly enriched in SARS-CoV-2 positive ciliated cells. **(B)** Uniform Manifold Approximation and Projection (UMAP) visualization of HBECS is colored according to the detection of the viral Open Reading Frame (SARS-CoV-2 (+)). Each dot represents a cell ($n = 77650$ cells). **(C)** UMAP plots were color annotated by infection status as in (B) and split by days of infection (dpi). **(D)** Heatmap shows the average expression of NRP1, FURIN, TMPRSS11A, and ACE2 in the ciliated cells of mock-infected and SARS-CoV-2 infected

cultures. The heat map shows gene expression for both SARS-CoV-2 infected (+) and non-infected (-) cells during a time course of infection in a SARS-CoV-2 infected culture.

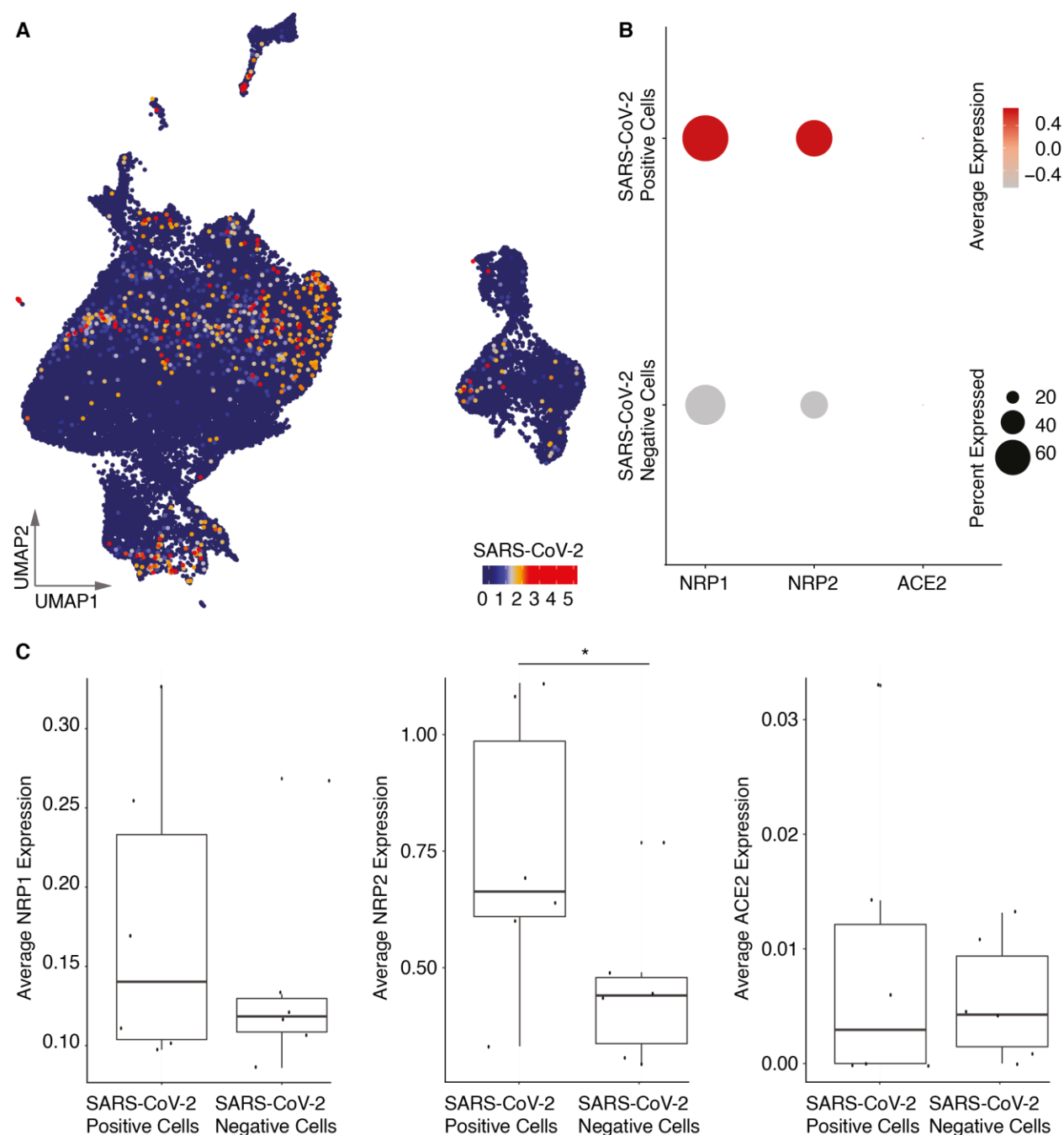


Fig. S7. RNA expression of NRP1, NRP2 and ACE2 in SARS-CoV-2 positive cells isolated from patient bronchoalveolar lavage fluids (BALF). (A) UMAP dimensionality reduction plot of all 63,103 cells with the normalized counts of SARS-CoV-2 reads obtained from Liao *et al.* (17). (B) Dot plot visualization of the expression of NRP1, NRP2 and ACE2 in BALF cells. Average expression is the scaled value. (C) Box plots comparing the average expression levels

of NRP1, NRP2 and ACE2 between SARS-CoV-2 positive and negative (bystander) cells from severely affected patients. Each dot represents a severely affected patient, Bonferroni-corrected p-value for NRP2 = 3.42×10^{-25} (Wilcoxon rank-sum test).

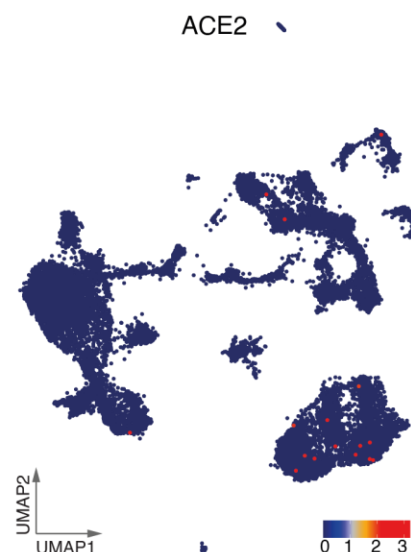
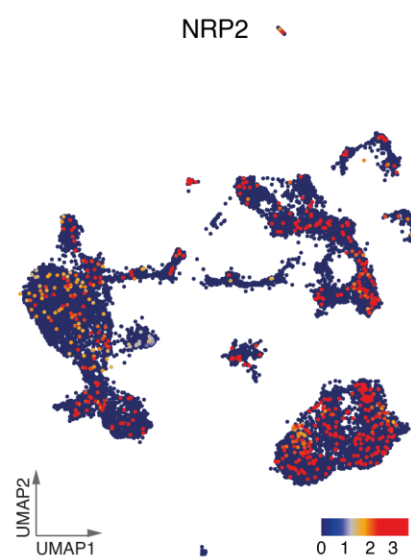
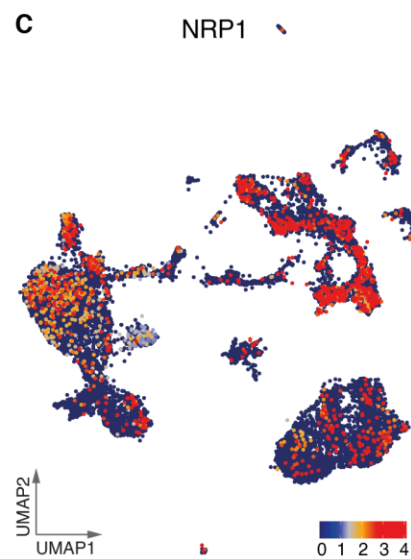
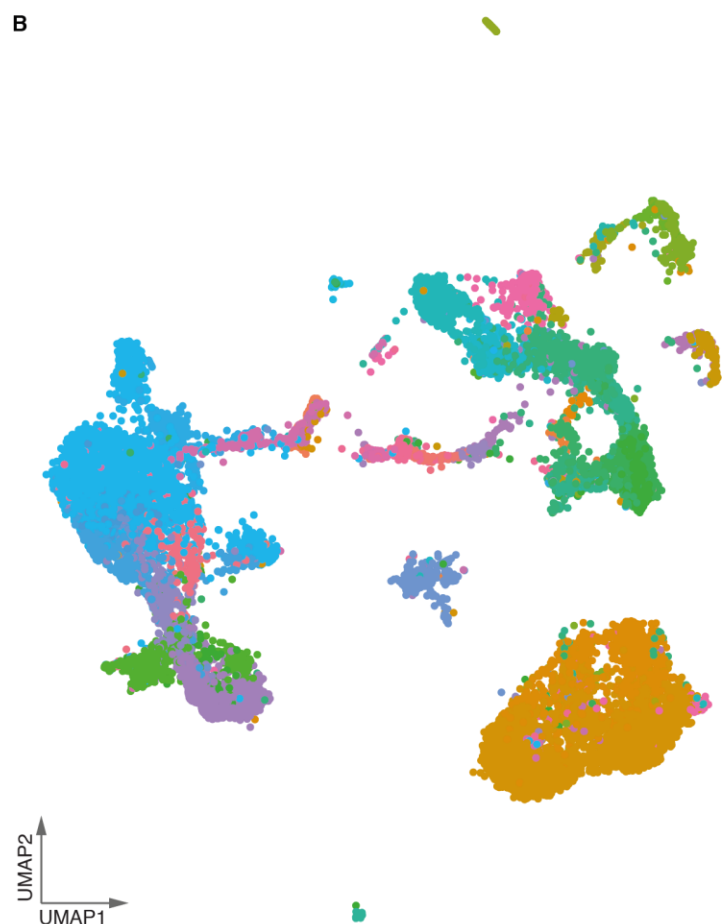
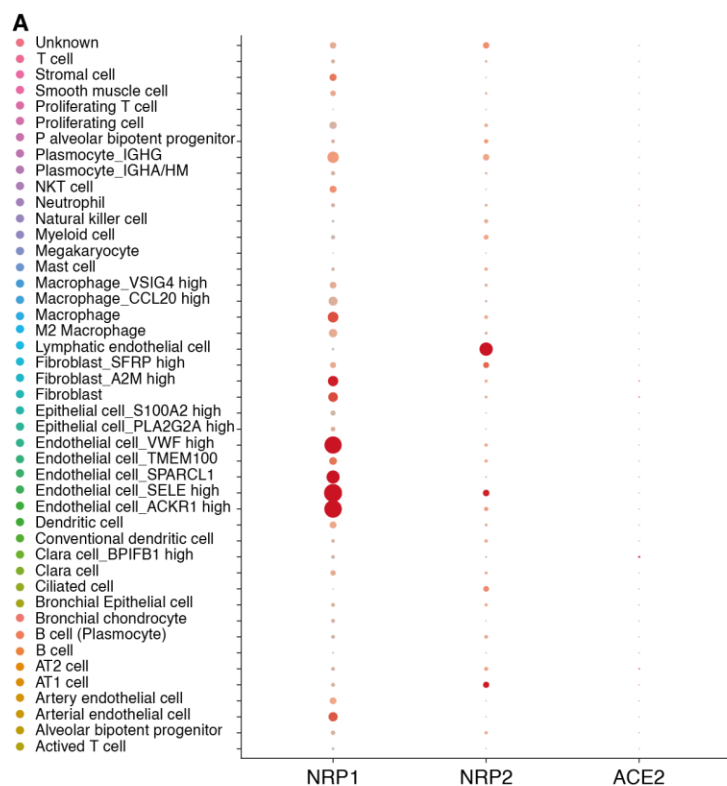


Fig. S8. RNA expression of NRP1, NRP2 and ACE2 in adult human lung cells. (A) Dot plot visualization of the expression of NRP1, NRP2 and ACE2 in lung cell subtypes. The size of the dots indicates the proportion of cells in the respective cell type having greater-than-zero expression of NRP1 (first column), NRP2 (second column) and ACE2 (third column) while the color indicates the mean expression of aforementioned genes. Average expression is the scaled value. **(B)** UMAPs are showing the lung cell subtypes and expression levels of NRP1, NRP2 and ACE2. Each dot represents a single cell (cell number n = 17,438). The cell cluster identity is noted on the color key legend and labels based on Han *et al.* (18). AT1 and AT2 cells are alveolar type I and II cells, respectively.

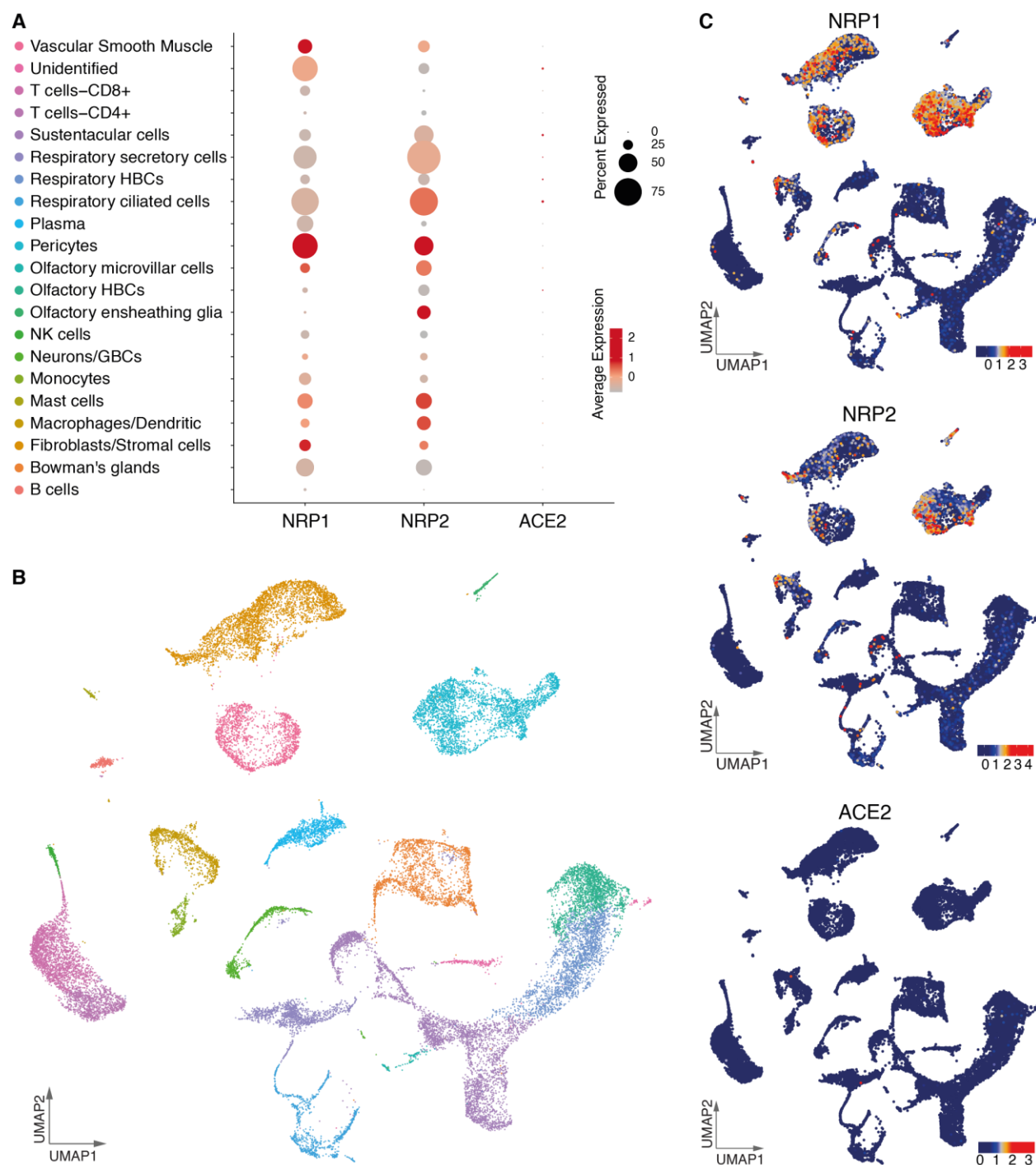


Fig. S9. RNA expression of NRP1, NRP2 and ACE2 in the adult human olfactory neuroepithelium. (A) Dot plot visualization of the expression of NRP1, NRP2 and ACE2 in olfactory neuroepithelia. The size of the dots indicates the proportion of cells in the respective cell type having greater-than-zero expression of NRP1 (first column), NRP2 (second column) and ACE2 (third column) while the color indicates the mean expression of aforementioned genes. Average expression is the scaled value. (B) UMAPs depicting the olfactory neuroepithelial cell types (cell number n = 28,622) and expression levels of NRP1, NRP2 and

ACE2. The cell cluster identity is noted on the color key legend and labels based on Durante *et al.* (19).

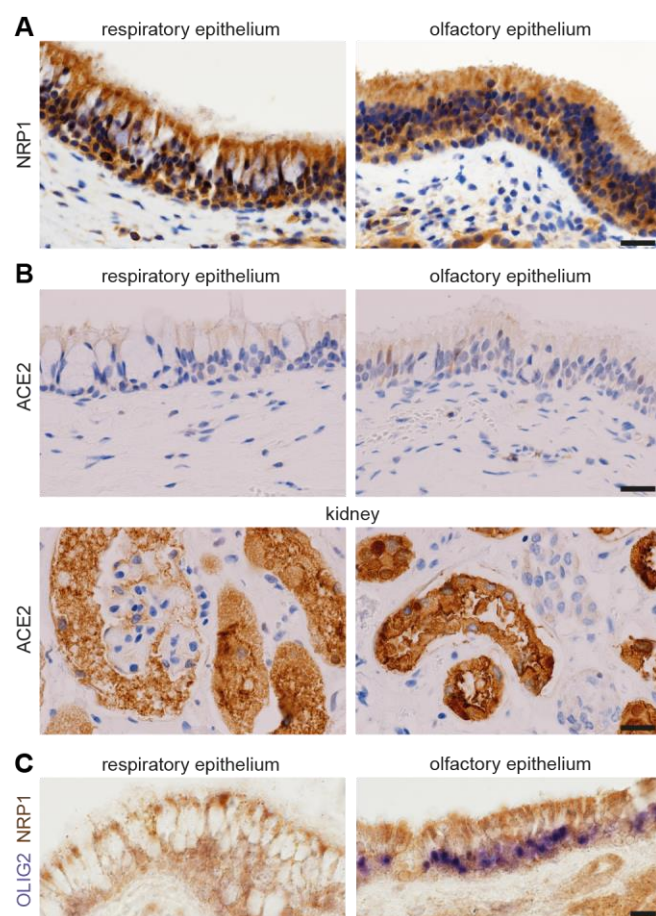


Fig. S10. NRP1 and ACE2 immunohistochemistry in human nasal respiratory and olfactory epithelium. (A) NRP1 immunohistochemistry (brown) counterstained with hematoxylin (blue) showing NRP1 immunoreactivity in the respiratory and olfactory epithelium. (B) ACE2 immunohistochemistry (brown) counterstained with hematoxylin (blue) showing weak but detectable immunoreactivity in the respiratory and olfactory epithelium, and stronger ACE2 immunoreactivity in the kidney. Scale bar, 25 μ m. (C) Co-staining of OLIG2 with NRP1 showing colocalization of OLIG2 and NRP1 in the olfactory epithelium. Scale bar, 20 μ m.

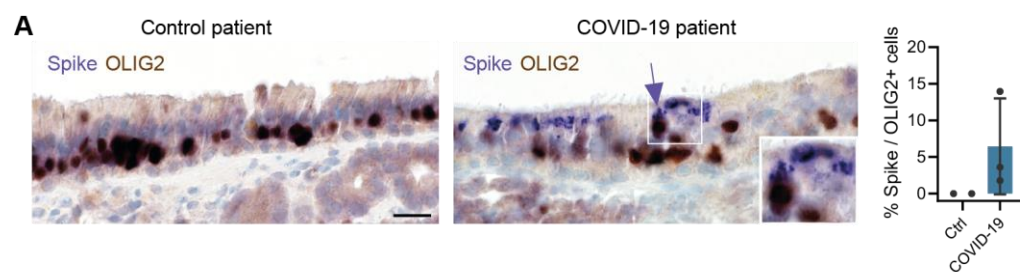


Fig. S11. SARS-CoV-2 infected OLIG2 positive cells in the olfactory epithelium. (A) Co-staining of OLIG2 with Spike counterstained with hematoxylin (blue) and percentage of OLIG2 positive cells that show co-localization with Spike. Data are means $\pm$ s.d. Scale bar, 20 μ m.

Table. S1 | Patient characteristics of COVID-19 (1 to 6) and control patients (7 to 14).

ID	Gender	Age	Clinical history	Spike Protein	SARS-CoV-2
				OE	PCR (NP)
1	m	71	aHT, Parkinson's disease, prostate hyperplasia	+	+
2	m	80	aHT, Afib, endocarditis (2014), stage 4 CKD, anemia	+	+
3	f	72	-	+	+
4	f	98	aHT, Afib, bradycardia	+	+
5	m	68	-	-	+
6	m	68	aHT, Afib, CHD, MI, s/p BAO with MT, T2DM, COPD, stage 2 CKD, AP, bullous pemphigoid	+	+
7	f	45	HCC	-	-
8	f	78	aHT, Afib, stage 3 CKD, MI	-	-
9	f	27	bM, osteoid osteoma (biopsy)	-	n/t
10	f	68	pituitary adenoma (biopsy)	-	-
11	m	78	CHD, Parkinson's disease	-	n/t
12	m	45	MI	-	n/t
13	f	67	Lewy-Body dementia, anemia	-	n/t
14	m	61	Alzheimer disease, cerebral amyloid angiopathy	-	n/t

Abbreviations: Afib = atrial fibrillation, aHT = arterial hypertension, AP = acute pancreatitis, BAO = basilar artery occlusion, CHD = chronic heart disease, CKD = chronic kidney disease, COPD = chronic obstructive pulmonary disease, GPA = granulomatosis with polyangiitis, HCC=hepatocellular carcinoma, MI = myocardial infarction, MT = mechanical thrombectomy, NP = nasopharyngeal swab, n/t = not tested, OE = olfactory epithelium, s/p = status post, T2DM= type 2 diabetes mellitus.

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