The SARS-CoV-2 receptor ACE2 is expressed in mouse pericytes but not endothelial cells: Implications for COVID-19 vascular research

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Figure S1

A

Top 20 enriched genes in group 0/1 vs. G2
G1 = PC, vSMC, sSMC, MG, FB1, FB2, OL, EC1, EC2, EC3, vEC, capEC, aEC, AC
G2 = allSMC, MG, FB1, FB2, OL, EC1, EC2, EC3, vEC, capEC, aEC, AC

| Symbol | G1 avg | G2 avg | Fold(0/1/G2) |
|--------|--------|--------|-------------|
| Sema2  | 97.2   | 9.34   | 10.10       |
| Nfis   | 95.39  | 0.19   | 506         |
| Nras   | 104.9   | 1.16   | 88.17       |
|Tcp1   | 19     | 0.24   | 76.22       |
| Atp1b1 | 4146.56 | 120.73 | 34.67       |
| Cdk5   | 28.4   | 2.47   | 11.53       |
| Atp1b2 | 31.79   | 2.27   | 14.09       |
| Smc2   | 11,179  | 11,179 | 1.00        |
| Znhi   | 34.34   | 1.27   | 27.55       |
| Vhl    | 15921.68 | 522.46 | 305.81      |
| Ppp3r4 | 49.34   | 1.63   | 30.44       |
| Ppp3r4 | 102.97  | 9.94   | 10.37       |
| Tmem152 | 20.28 | 2.42   | 8.40        |
| Pias1  | 360.27  | 14.86  | 24.27       |
| Adsrb4 | 403.42  | 16.31  | 24.74       |
| Kcnj8  | 260.47  | 1.06   | 250.84      |
| Kcnj8  | 305.17  | 19.18  | 15.63       |
| Ppp3r4 | 1222.11 | 57.62  | 21.21       |
| Kcnj8  | 1152.9  | 52.58  | 21.21       |
| Vhl    | 146.45  | 7.17   | 20.42       |

B

C

Ace2

vSMC 187
aSMC 60

Ace2

vEC 298

Ace2

vEC 405

Ace2

vEC 397

D

E

F

Ace2 expression

Pericyte (PER3)

VSMCA

265 cell types in adult mouse brain (mousebrain.org)

G

H

Total 3416 48 3368 1.41%
EC 1280 729 551 56.95%
vSMC 275 133 142 48.36%
sSMC 377 60 317 15.82%
AC 2073 13 2060 0.63%
OPC 242 0 242 0.06%
OL 1662 15 1647 0.90%
NR 867 8 859 0.92%
MG 2307 6 2302 0.22%
PvM 95 0 95 0.06%

I

Heat map overview of the top 50 genes enriched in Ace2+ versus Ace2- endothelial cells

J

Heat map overview of the top 50 genes enriched in Ace2+ versus Ace2- astrocytes

Correlation to the Ace2+ EC

Pearson correlation

Correlation to the Ace2+ AC

Pearson correlation
Figure S1. Ace2 mRNA in brain cells other than mural cells is due to pericyte contamination. Related to Figure 1.

(A) Top enriched transcripts (red arrow indicates Ace2 at 15th place) in pericytes and venous VSMCs as compared to other vascular and perivascular cell types deduced from the brain vascular scRNA-seq database. (B) Bar plot excerpt from http://betsholtzlab.org/VascularSingleCells/database.html showing the expression of Ace2 in different vascular and perivascular cell types. (C) Magnified view of indicated part of A comparing the expression of Ace2, Cnn1 and Kncj8. Abbreviations: PC, Pericytes; SMC, Smooth muscle cells; MG, Microglia; FB, Vascular fibroblast-like cells; OL, Oligodendrocytes; EC, Endothelial cells; AC, Astrocytes; v, venous; capil, capillary; a, arterial; aa, arteriolar. (D) Excerpts from http://betsholtzlab.org/VascularSingleCells/database.html showing bar plots across VSMC subtypes of arterial VSMC-specific genes that anti-correlate with Ace2. Red arrows point at the Ace2-negative part of the cluster. Cell type abbreviations: SMC, Smooth muscle cells; v, venous; a, arterial; aa, arteriolar. (E) Expression of selected known brain pericyte specific markers in 23 Ace2-positive ECs (top) and 23 randomly selected Ace2-negative ECs (bottom). Colors indicate pericyte marker as shown, and the frequency of their expression is provided in the tables. (F) Ace2 expression in mouse brain single-cell transcriptomes from (Zeisel et al., 2018) (http://mousebrain.org/genesearch.html). Abbreviations PER - pericyte; EC - endothelial cells; VECA - vascular endothelial cells, arterial; VSMCA - vascular smooth muscle cells, arterial. (G) Heat map display of the expression of Ace2-positive, top 10 pericyte and top 10 endothelial markers, showing that PER1-2, VECA are pericyte contaminated. (H) Statistics of the number of cells, Ace2-positive cell percentage in each brain cell type. (I-J) Heat map overview of the top 50 genes enriched in Ace2-positive versus Ace2-negative ECs (I) or astrocytes (J), respectively. The pearson correlation was calculated based on the 50 genes.
Figure S2. ACE2 protein expression in the adult mouse brain cortex, spinal cord and eye. 
Related to Figure 2.

(A-D) Confocal microscopy images of sections from brain cortex (A, B upper panel) and spinal cord (B lower panel, C-D) IF stained using antibodies and the Pdgfrb^{GFP} marker as indicated (ANPEP, aka CD13 and Pdgfrb^{GFP} for pericytes, αSMA and CNN1 for VSMCs). Note the strong ACE2 staining of capillary pericytes and the co-expression with ANPEP (A) and Pdgfrb^{GFP} (B), indicated by arrows. Mural cells in terminal arterioles are weakly ACE2-positive (C, open arrowheads), whereas mural cells of larger arterioles are ACE2-negative (arrowheads in D). 

(E-F) Confocal microscopy images from the eye, IF stained using antibodies and Pdgfrb^{GFP} or Cspg4^{dsRED} markers as indicated (Pdgfrb^{GFP} and Cspg4^{dsRED} for pericytes and αSMA for VSMCs). Boxed areas are shown magnified in the right panel. Note the strong expression of ACE2 in pericytes of the retina (E, upper two panels) as well as of the choriocapillaris and of the ciliary body (E, lower two panels). Similar as to the brain and spinal cord, terminal arterioles are weakly ACE2-positive (open arrowheads), while large arterioles are negative for ACE2 expression (arrowheads). Asterisks in lower panel indicate ACE2-positive cells of the surface epithelium of the conjunctiva and cornea. 

(F) Overview image including extra-ocular skeletal muscle. Note the absence on ACE2 expression in pericytes of the extra-ocular muscle (boxed area 1), in contrast to retinal pericytes (arrows in boxed area 2). Nuclei are visualized by DAPI or Hoechst 33342. Scale bars as indicated in the figure.
Figure S3. Ace2 expression in mouse heart ECs is due to pericyte contamination.
Related to Figure 3.

(A) Ace2 expression in adult mouse heart scRNA-seq data enriched for stromal cells (Muhl et al., 2020). Bar plots show the expression in individual cells for Ace2, the VSMC marker Acta2, and the fibroblast maker Pdgfra. (B) Dot plot displaying the expression pattern of the top-50 transcripts enriched Ace2-positive versus Ace2-negative heart ECs. (C) Statistics of the number of cells, Ace2-positive cell percentage in each heart cell type. Abbreviations: EC, endothelial cells; FB, fibroblast; EDC, endocardial cells; CM, cardiomyocytes; PC, pericytes; aSMC, arterial smooth muscle cells; MAC, macrophages; ER, erythrocytes. (D) IF staining for ACE2 in adult mouse heart in combination with the indicated markers (Pdgfrb\textsuperscript{GFP} for pericytes, CD31 for the endothelium and sarcomeric alpha actinin (ACTN2) to mark cardiomyocytes). The arrows indicate ACE2-positive cells that are all in close contact with the vasculature and exhibit a positive Pdgfrb\textsuperscript{GFP} signal, indicating them as pericytes. In contrast, ACTN2-positive cardiomyocytes appear ACE2 negative. Nuclei are visualized by Hoechst 33342. The boxed area is shown magnified in the right panels. Scale bar is indicated in the figure.
Figure S4. ACE2 protein expression in the adult mouse lung.

Related to Figure 4.

(A) Ace2 expression in adult mouse lung scRNA-seq data enriched for ECs and pericytes (Vanlandewijck et al., 2018). Bar plots show the expression in individual cells for Ace2, the AT-II cell marker Sftpc, and the multiciliated cell marker Foxj1. (B-D) Confocal microscopy images of sections from adult mouse lung stained with the indicated antibodies (ACE2, CD31 for endothelium, CSPG4 (aka NG2) for mural cells, CD68 for macrophages) and the transgenic reporter PdgfrbGFP for mural cells. ACE2 IF signal is only detected in bronchial epithelium (strong) and AT-II cells (weak). Note the absence of ACE2 IF signal in ECs, in mural cells in the alveolar region and in CD68-positive macrophages (asterisk in C). (D) IF staining showing ACE2-positive pericytes (arrows) only at the capillary bed of large, primary bronchi. Nuclei are visualized by DAPI or Hoechst 33342. Scale bars as indicated in the figure. (E) Violin plot of the Ace2 mRNA expression levels in pericytes from brain, heart and lung, as indicated. The Y-axis shows the log scaled normalized expression counts. The individual dots represent single cells, and the violin shape shows the distribution density. (F) Expression of Ace2 and SARS-CoV-2 spike protein processing proteases – Tmprss2, Ctsl or Ctsb – as well as Nrp1 across meta-analysis scRNAseq datasets from the lung (upper), brain (middle) or heart (lower) presented as bar plots. Each bar represents a single cell and is colored according to the indicated data source (see Supplementary Information - Methods). Cluster annotations have been described in previous figures. Arrows indicate Ace2-positive cell clusters: in the lung AT-II cells (left) and multiciliated cells (right), in the brain from left to right, pericytes, arteriolar VSMCs, venous VSMCs and pericyte-contaminated ECs, in the heart, pericytes.
Figure S5. ACE2 expression in the developing and adult lung, brain and heart.
Related to Figure 5.

(A) IF staining for indicated proteins in mouse lung from P0.5, P10.5 and adult time-points. Note the sporadic expression of ACE2 in AT-II cells already visible at P0.5. Arrows indicate ACE2-positive bronchial epithelial cells, asterisks indicate ACE2-positive AT-II cells (also SFTPC-positive). Boxed areas are shown magnified in the right panel. (B) IF staining for the indicated proteins in the adult mouse lung, brain and heart. Note the strong expression of ACE2 in the bronchial epithelial cells (arrows, upper panel) and AT-II cells (asterisk, upper panel), or pericytes of the brain (middle panel) and heart (lower panel). Note the lack of ACE2 expression in cardiomyocytes in the heart. Nuclei are visualized by Hoechst 33342. Scale bars as indicated in the figure.
Figure S6. ACE2 protein expression in the adult gastrointestinal tract. Related to Figure 7.

IF detection of ACE2 in adult mouse gastrointestinal (GI) tract in combination with the indicated markers (Pdgfrb<sup>GFP</sup> for pericytes, CD31 for endothelial cells and cytokeratin (CK) is used for epithelial structures). (A) IF staining of adult mouse tongue, showing strong ACE2 expression, highlighted by arrows, in the surface epithelium as well as pericytes of the tongue muscle. Asterisks indicate ACE2-negative pericytes of the mucosal vasculature. (B) IF staining of adult mouse esophagus, showing strong ACE2 expression in the surface epithelium, highlighted by the arrows. The basal cell layer exhibits a weaker ACE2 signal, compared to the suprabasal cell layer. The border between basal and suprabasal cell layers is indicated by the dashed line. The asterisk indicates an ACE2-positive nerve. (C) IF staining of adult mouse stomach, showing ACE2 expression in pericytes of the mucosal vasculature, highlighted by arrows. (D) IF staining of adult mouse duodenum. The arrows highlight the ACE2-positive surface epithelium of the villi and crypts. The asterisk indicates ACE2 negative pericytes of the mucosal vasculature. (E) IF staining of adult mouse ileum. The arrows highlight the ACE2-positive surface epithelium of the villi. In the magnified box, arrows indicate the, compared to the duodenum, low signal of ACE2 at the crypt of the ileum and asterisks indicate ACE2-negative pericytes of the mucosa vasculature. (F) IF staining of adult mouse colon. The arrows indicate ACE2-positive pericytes of the muscularis vasculature. Not all pericytes of the muscularis vascular system are positive and an ACE2-negative pericyte is marked by the asterisk. Arrowheads highlight ACE2-negative arterial VSMC. Boxed areas are shown magnified in the right panel. Nuclei are visualized by Hoechst 33342. Scale bars are indicated in the figure.
Figure S7. Ace2 expression in the adult mouse gastrointestinal tract.

Related to Figure 7.

Bar plot of the Ace2 mRNA expression levels in main mouse cell types (data obtained from the Mouse Cell Atlas [http://bis.zju.edu.cn/MCA/index.html]). The X-axis shows the average normalized expression level.
Table S1. List of used antibodies.

| Primary antibody | Dilutions | Supplier               | Catalog number |
|------------------|-----------|------------------------|----------------|
| PECAM1, CD31     | 1:200     | R & D Systems          | AF3628         |
| PECAM1, CD31     | 1:100     | BD Pharmingen          | 553370         |
| PECAM1, CD31     | 1:50      | Abcam                  | ab28364        |
| ANPEP            | 1:100     | Bio-Rad                | MCA2183EL      |
| COLIV            | 1:100     | Bio-Rad                | 2150-1470      |
| ACTA2 (aSMA)-Alexa Fluor 647 | 1:200 | Santa Cruz Biotechnology, Inc. | sc-32251 |
| ACTA2 (aSMA)-FITC | 1:200 | Sigma                  | F3777          |
| ACTA2 (aSMA)-Cy3  | 1:500     | Sigma                  | C6198          |
| PDGFRb           | 1:100     | eBiosciences           | 553847         |
| ACE2             | 1:100     | R & D Systems          | AF3437         |
| CSPG4 (NG2)      | 1:200     | Abcam                  | ab40879        |
| CD68             | 1:200     | Millipore              | AB5320         |
| GLUCAGON (GCG)   | 1:200     | BioLegend              | 137001         |
| NGFR             | 1:200     | Abcam                  | ab52987        |
| THYROSIN HYDROXYLASE (TH) | 1:200 | Pel Freez Biologicals | P40101-0      |
| SFTPC            | 1:100     | Abcam                  | AB932          |
| SFTPC            | 1:100     | Abcam                  | ab137346       |
| CYTOKERATIN (CK) | 1:200     | DAKO                   | Z0622          |

Supplemental Experimental Procedures:

Fixation, sectioning and antibody incubations:

**Vibratome-sections:** Brains were removed and post-fixed in 4% buffered formaldehde for 4h at 4°C. Sagittal and coronal vibratome sections (50-75 μm) were incubated in blocking/permeabilization solution (1% bovine serum albumin, 2.5% donkey serum, 0.5% Triton X-100 in PBS) overnight at 4°C, followed by incubation in primary antibody solution for two nights at 4°C, and subsequently in secondary antibody (Jackson ImmunoResearch and Invitrogen) solution, overnight at 4°C. A list of the used primary antibodies is presented in Table S1. Sections were mounted in ProLong Gold Antifade mounting medium (cat. #P36930, Life Technologies). Micrographs were acquired with a Leica TCS SP8 confocal microscope (Leica Microsystems). All confocal images are represented as maximum intensity projections and were adjusted for brightness and contrast using Fiji v1.52p and Adobe Photoshop CC 2019.

**Cryo-sections:** Tissues were harvested from euthanized mice without perfusion and fixed by immersion in 4% formaldehyde for 4-12h at 4°C, followed by immersion in 20% sucrose/PBS solution for at least 24h at 4°C. Thereafter, tissues were embedded for cryo-sectioning and sectioned on a CryoStat NX70 (ThermoFisher Scientific) to 14 or 30 μm thick sections collected on SuperFrost Plus glass slides (Metzler Gläser) and stored at -80°C until usage. Sections were allowed to thaw at RT and thereafter blocked for > 60 min at RT with blocking-buffer (serum-free protein blocking solution, DAKO), supplemented with 0.2% Triton X-100 (Sigma Aldrich), followed by sequential incubation with primary antibodies (overnight at 4°C) (Table S1) and corresponding fluorescently conjugated secondary antibodies (1h at RT) together with 10 µg/ml Hoechst 33342 (trihydrochloride, trihydrate, ThermoFisher Scientific). Sections were mounted with ProLong Gold Antifade mounting medium, and micrographs acquired and graphically handled as described above.