

Supplementary material for “SARS-CoV-2 Spike S1 subunit triggers pericyte and microvascular dysfunction in human pancreatic islets”

Supplementary Experimental Procedures

Organ donors and slice culture

We obtained living slices (120-150 μm ; approximately 8 slices per donor) from the tail of the pancreas of organ donors processed by the Network for Pancreatic Organ Donors with Diabetes (nPOD) in Gainesville. Slices were shipped overnight from Gainesville to Miami and used within 4-36 hours. We also produced slices from local pancreatic tissue samples from the neck of the pancreas, processed at the Human Islet Cell Processing Facility at the Diabetes Research Institute, University of Miami. Once produced/arrived pancreas slices were placed on 35 mm AirHive dishes (Biorep, Miami, FL) containing BrainPhys neuronal culture medium (Stemcell Technologies, Vancouver, BC, Cat# 05790), supplemented with 2% B27 minus-insulin (Invitrogen, Carlsbad, CA, Cat# A1895601), 1% pen/strep/amphotericin B solution (Sigma Aldrich, St. Louis, MO, Cat# A5955), 1% Glutamax supplement (Invitrogen, Carlsbad, CA, Cat# 35050061), 5.5 mM D-glucose (of note, BrainPhys already contains 2.5 mM D-Glucose; Sigma Aldrich, St. Louis, MO, Cat# G8644), 100 $\mu\text{g/ml}$ trypsin inhibitor from Glycine max (Sigma Aldrich, St. Louis, MO, Cat# T6522), 10 $\mu\text{g/ml}$ aprotinin (Sigma Aldrich, St. Louis, MO, Cat# A6106), 10 $\mu\text{g/ml}$ chymostatin (solubilized initially in DMSO; Sigma Aldrich, St. Louis, MO, Cat# 11004638001) and 1% HEPES buffer (Invitrogen, Carlsbad, CA, Cat#

15630080). Dishes containing slices were placed in a humidified incubator at 30 °C and 5% CO₂ atmosphere. Culture medium was changed every 8–12 h.

In this study, we imaged slices from non-diabetic, autoantibody-negative organ donors, both male and female donors, of different ethnicities, ages ranging from 14-55 years old. A detailed description of the characteristics of donors used in this study is included in **Supplementary Table 1**.

Generation of living pancreas slices

Pancreatic tissue pieces obtained either at the University of Florida in Gainesville or produced locally at the Diabetes Research Institute (University of Miami) were collected into UW solution (Static preservation solution; SPS-1; Organ Recovery Systems). Pancreatic tissue pieces were cut into smaller pieces (~0.5 cm³) and adipose and connective tissue was carefully removed. Tissue pieces were then embedded in low-melting-point agarose (3.5%, MilliporeSigma, A9414) and sliced as previously described (1, 2). Briefly, pieces were mounted on a specimen holder using superglue (Superglue 90-120 CPS, World Precision Instruments Inc., catalog 7341). The holder was connected to the tray of a semiautomatic vibratome (Leica VT1200S, catalog 14048142066) and filled with cold HEPES buffer (125 mM NaCl, 5.9 mM KCl, 2.56 mM CaCl₂, 1 mM MgCl₂, 25 mM HEPES, 0.1% BSA [w/v], pH 7.4 supplemented with 100 µg/ml trypsin inhibitor from Glycine max (Sigma Aldrich, St. Louis, MO, Cat# T6522), 10 µg/ml aprotinin (Sigma Aldrich, St. Louis, MO, Cat# A6106), 10 µg/ml chymostatin (solubilized initially in DMSO; Sigma Aldrich, St. Louis, MO, Cat# 11004638001). Tissue slicing was performed at a step size of 120 µm, speed of (24-30 0.1mm/s, amplitude of 1 mm, and an angle of 15°. Slices

were collected into a 60 mm Petri-dish containing Dulbecco's Low Glucose Modified Eagles Medium (Thermo Fisher, Cat# 11885084) and shipped to Miami or placed in culture.

Recombinant spike protein treatment

Living human pancreas slices were incubated with a recombinant SARS-CoV-2 (2019-nCoV strain) spike protein S1 Subunit (YP_009724390.1) (Val16-Arg685), expressed with a polyhistidine tag at the C-terminus (SinoBiologicals, Cat: 40591-V08H). As control, we incubated in parallel slices from the same donors with a recombinant protein from human coronavirus (HCoV-OC43) Spike S1 Protein (AVR40344.1) (Met1-Leu794), also expressed with a polyhistidine tag at the C-terminus (SinoBiologicals, Cat: 40607-V08H1). Concentration-response assessments of binding to ACE2 in *in vitro* cell-free assays indicated that SARS-CoV-2 S1 Spike protein binds strongly to ACE2 and follows classic sigmoidal patterns corresponding to the law of mass action (3). Indeed, fitting of data gave median effective concentrations (EC_{50}) and hence binding affinity constant (K_d) estimates of 14.7 nM for SARS-CoV-2 S1 (3). For these reasons, we administered recombinant proteins at 80 nM ($\sim 5 \times EC_{50}$).

Confocal imaging of living pancreas slices

Living human pancreas slices were incubated for 1h with either 80 nM of SARS-CoV-2 spike S1 subunit (SARS spike) or with HCoV spike S1 subunit (HCoV spike). Slices were also incubated with a membrane permeant cytosolic calcium indicator ($[Ca^{2+}]_i$) Fluo4-AM (6 mM, Invitrogen, cat. #F14201) in 3 mM glucose solution prepared in HEPES buffer

(125 mM NaCl, 5.9 mM KCl, 2.56 mM CaCl₂, 1 mM MgCl₂, 25 mM HEPES, 0.1% BSA [w/v], pH 7.4), supplemented with aprotinin (25 KIU), at room temperature and in the dark. To examine pericyte calcium responses, a group of slices from each donor was incubated with fluorescent-conjugated antibody against the pericyte marker neuron-glia antigen 2 (NG2-alexa647; 1:50, R&D Systems, cat. # Fab2585R) for 2h, as previously described (4, 5). To examine vasomotive responses of islet capillaries, we incubated slices with DyLight 649 lectin from *Lycopersicon Esculentum* (3.3 mg/mL, VectorLabs, cat. #DL1178) for 1h.

After incubation, living pancreas slices were placed on a coverslip in an imaging chamber (Warner instruments, Hamden, CT, USA) and imaged under an upright confocal microscope (Leica TCS SP8 upright; Leica Microsystems). The chamber was continuously perfused with HEPES-buffered solution containing 3 mM glucose (3G) and confocal images were acquired with LAS AF software using a 40X water immersion objective (NA 0.8). We used a resonance scanner for fast image acquisition to produce time-lapse recordings spanning 50–100 μ m of the slice (z-step: 5, stack of 10–15 confocal images with a size of 512x512 pixels) at 5 seconds resolution (xyzt imaging). Fluo-4 fluorescence was excited at 488 nm and emission detected at 510–550 nm, DyLight 649 lectin or NG2-alexa 647 antibody labeling were excited at 638 nm and emission detected at 648–690 nm.

We recorded changes in islet pericyte [Ca²⁺]_i and capillary diameter induced by angiotensin II (100 nM; applied for 4 min in 3G; Tocris, cat. #1158), angiotensin 1-7 (100 nM; applied for 4 min in 3G; Tocris, cat. #1562), ACE2 inhibitor MLN4760 (at 10 μ M; applied for 5 min in 3G; Tocris cat. #3345) and norepinephrine (20 μ M; applied for 5 min

in 3G; Tocris, cat. #5169). Islets in slices were identified using the backscatter signal produced by dense-core granules present in endocrine cells. To quantify changes in pericyte $[Ca^{2+}]_i$, we used ImageJ software (<http://imagej.nih.gov/ij/>). We drew regions of interest (ROI) around all NG2-alexa647 labeled cells in different confocal images of islets and quantified changes in fluorescence in these regions. For each coverslip (islet), a ROI was drawn around the whole islet to normalize fluorescence levels in pericytes to their surroundings (to account for differences in image acquisition and dye loading). Changes in fluorescence intensity were expressed as percentage over baseline ($\Delta F/F$; %; baseline defined as the mean of the first 10 values of the recording under basal glucose concentration (3 mM) for all stimuli). We calculated the total area under the curve of these fluorescence traces to estimate the magnitude of the effect of each stimulus on cellular $[Ca^{2+}]_i$. MatLab software (<https://www.mathworks.com/>) was used to generate heatmaps showing changes in fluorescence in the whole pericyte population from different organ donors.

Vasomotion analysis

Blood vessels in living slices were labeled with DyLight-649 fluorescent lectin. We quantified changes in vessel diameter in confocal images of the islet as previously described (4). All vessel segments were quantified in each confocal image. Briefly, we drew a straight-line transversal to the vessel borders and used the “reslice” z-function in ImageJ to generate a single image showing the changes in vessel diameter over time (xt scan; temporal projection; **Figure 4C**). xt scan (resliced) images were despeckled, blurred with Gaussian filter sigma = 1, before enhancing contrast, and image sharpened using

the following kernel in order to emphasize vertical lines: -10 -5 50 -5 -10; -10 -5 75 -5 -10; -10 -5 50 -5 -10. An horizontal line was drawn on the xt scan (resliced) image (which corresponds to a single time point), and an array of pixel intensity values was sorted and first 2 maxima were considered vessel borders (tolerance = 4). Vessel diameter was calculated by subtracting these 2 values. For each stimulus, an average diameter value was calculated using the 10 last diameter values obtained during stimulus application. To determine the extent of constriction/dilation, we pooled diameter data from different capillaries from different islets for each group of donors and calculated the relative change in diameter [as % of baseline diameter (in 3G)].

Immunohistochemistry

After physiological recordings, living human pancreas slices from organ donors were fixed for 1h with 4% PFA, washed 3x in PBS, and stored at 4°C. For immunohistochemistry, slices were incubated in blocking solution (PBS-Triton X-100 0.3% and Universal Blocker Reagent; Biogenex, San Ramon, CA) for 3h. Thereafter, slices were incubated for 48-72h (20°C) with primary antibodies diluted in blocking solution. To identify pancreatic islets in slices, we immunostained either beta cells (insulin, 1:5; Dako, cat. #IR002) or delta cells (somatostatin, 1:500; Sigma-Aldrich, cat. #MAB354). We labeled pericytes using antibodies against two different pericyte markers: an anti-NG2 antibody (1:50–1:100; R&D, cat. #FAB2585R), and anti-platelet-derived growth factor receptor β (PDGFR β ; 1:100; R&D, cat.# AF385). ACE2 was labeled using anti-ACE2 (1:20; R&D, cat. #AF933), ACE with a polyclonal antibody (ThermoFischer; cat. #PA5-78711). Immunostaining was visualized using conjugated secondary antibodies (1:500 in PBS;

16h at 20°C; ThermoFischer). Cell nuclei were stained with dapi. Slides were mounted with Vectashield mounting medium (Vector Laboratories) and imaged on an inverted laser-scanning confocal microscope (Leica TCS SP5; Leica Microsystems) with LAS AF software using a 63X oil immersion objective (NA 1.4). To quantify colocalization between ACE2 and the pericyte marker NG2, we calculated Mander's coefficients in confocal images of islets from different donors using the ImageJ plugin "JACoP: Just Another Co-localization Plugin".

Measuring angII levels in the pancreas

Living human pancreas slices (n=2/donor) were incubated in 300 μ L of 3 mM glucose solution (3G) in a 24-well plate for 20 min at RT. After this incubation, the slices were transferred to a different well and incubated for 1h with either 80 nM of SARS-CoV-2 spike S1 subunit (SARS spike) or with HCoV spike S1 subunit (HCoV spike) in 3G, followed by another incubation period in only 3G for 20min. After each incubation step, the supernatant of each well was transferred to microtubes and stored at -80°C. Angiotensin II levels in the supernatant were measured using the Angiotensin II EIA kit (Catalog #RAB0010) from Sigma-Aldrich (St Louis, MO). The assays were done according to the manufacturer's instructions.

Static insulin secretion assay

Living human pancreas slices (n=2/donor) were incubated in 300 μ L of 3 mM glucose solution (3G) in a 24-well plate for 20 min at 37°C. After this incubation, the slices were transferred to a different well and incubated for 1h with either 80 nM of SARS-CoV-2 spike

S1 subunit (SARS spike) or with HCoV spike S1 subunit (HCoV spike) in 3G, followed by an incubation in 11 mM glucose (11G) for 30 min. After each incubation step, the supernatant of each well was transferred to microtubes and stored at -80°C. Slices were transferred to acid-ethanol solution for subsequent analysis of insulin content. Insulin levels in the supernatant and content were measured using an insulin elisa kit (Catalog #10-1113-01; Mercodia).

Statistical Analyses

For statistical comparisons we used Prism 9 (GraphPad software, La Jolla, CA) and performed unpaired t-tests when comparing data obtained for slices treated with either SARS-CoV-2 spike or with HCoV-OC43 spike, or paired t-tests when comparing changes in the same cell or vessel (e.g. pericyte $[Ca^{2+}]_i$ or vessel diameter) upon application of a stimulus. *p* values < 0.05 were considered statistically significant (indicated with an * in figures; the exact *p* value is written in the figure legend).

Methods references

1. Panzer JK, Hiller H, Cohrs CM, Almaça J, Enos SJ, Beery M, et al. Pancreas tissue slices from organ donors enable in situ analysis of type 1 diabetes pathogenesis. *JCI insight*. 2020;5(8).
2. Qadir MMF, Álvarez-Cubela S, Weitz J, Panzer JK, Klein D, Moreno-Hernández Y, et al. Long-term culture of human pancreatic slices as a model to study real-time islet regeneration. *Nature communications*. 2020;11(1):3265.
3. Bojadzic D, Alcazar O, Chen J, Chuang ST, Condor Capcha JM, Shehadeh LA, et al. Small-Molecule Inhibitors of the Coronavirus Spike: ACE2 Protein-Protein Interaction as Blockers of Viral Attachment and Entry for SARS-CoV-2. *ACS infectious diseases*. 2021;7(6):1519-34.

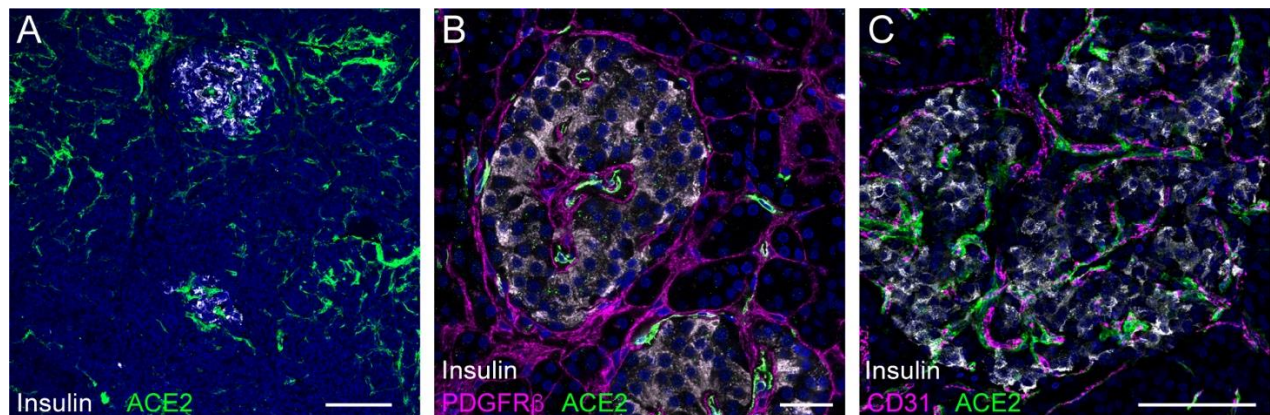
4. Mateus Gonçalves L, and Almaça J. Functional Characterization of the Human Islet Microvasculature Using Living Pancreas Slices. *Frontiers in endocrinology*. 2020;11:602519.
5. Mateus Gonçalves L, Fahd Qadir MM, Boulina M, Makhmutova M, Pereira E, and Almaça J. Pericyte dysfunction and impaired vasomotion are hallmarks of islets during the pathogenesis of type 1 diabetes. *Cell reports*. 2023;42(8):112913.

Supplementary Table 1

Characteristics of organ donors used in the study from which we produced/received living pancreas slices. Living pancreas slices were produced at nPOD (Gainesville, Florida) and shipped to Miami or locally produced by us from small pancreas pieces collected at the Diabetes Research Institute (DRI; Miami, Florida). Slices were used in physiological experiments with RAS peptides or coronaviruses spike proteins.

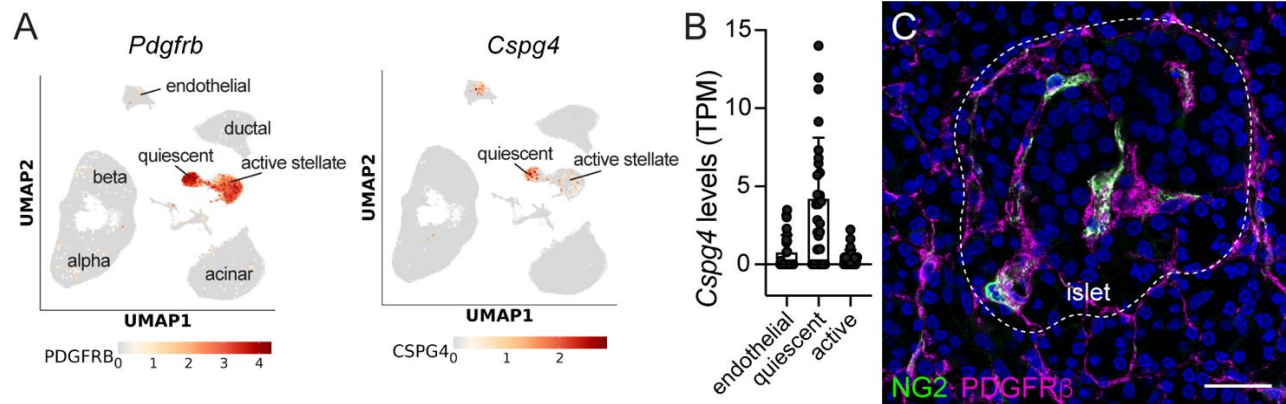
Organ donor	Source	Donor type	Ethnicity	Age	AutoAb	Gender	BMI	HbA1c (%)	C-pep (ng/mL)	Physiological experiments		
										pericyte calcium	capillary diameter	insulin secretion
6548	nPOD	No diabetes	Caucasian	20.24	negative	male	23.8	5.7	4.04	yes	no	no
6552	nPOD	No diabetes	Caucasian	33.87	negative	female	21.9	5.6	1.8	yes	yes	no
6583	nPOD	No diabetes	Caucasian	30.54	negative	male	24.8	5	10.55	no	yes	no
6584	nPOD	No diabetes	Caucasian	22.53	negative	male	21.1	5.3	6.02	no	yes	no
6590	nPOD	No diabetes	African american	16	negative	female	21.3	5.3	49.65	no	yes	no
6592	nPOD	No diabetes	Caucasian	23.84	negative	male	19.9	5.2	32.75	no	yes	no
6595	nPOD	No diabetes	Hispanic	24.94	negative	male	25.4	5	3.47	yes	yes	no
6602	nPOD	No diabetes	African american	26.55	negative	male	17.3	5.2	5.25	yes	no	no
6603	nPOD	No diabetes	African american	30.27	negative	female	30.4	6.3	4.76	yes	yes	no
6611	nPOD	No diabetes	Caucasian	14.51	negative	male	23.2	5.1	10.98	yes	no	no
6615	nPOD	Pending	Caucasian	14.44	negative	male	25.9	5.6	2.9	no	yes	no
6626	nPOD	Pending	Caucasian	15	unknown	male	28.2	5.3	NA	no	no	yes
HP2352	DRI	No diabetes	Caucasian	21	negative	male	38.1	5	NA	yes	no	no
HP2357	DRI	No diabetes	African american	55	negative	female	28.6	5.7	NA	yes	yes	no
HP2373	DRI	No Diabetes	Caucasian	39	unknown	male	23	4.9	NA	no	no	yes

Supplementary Figures



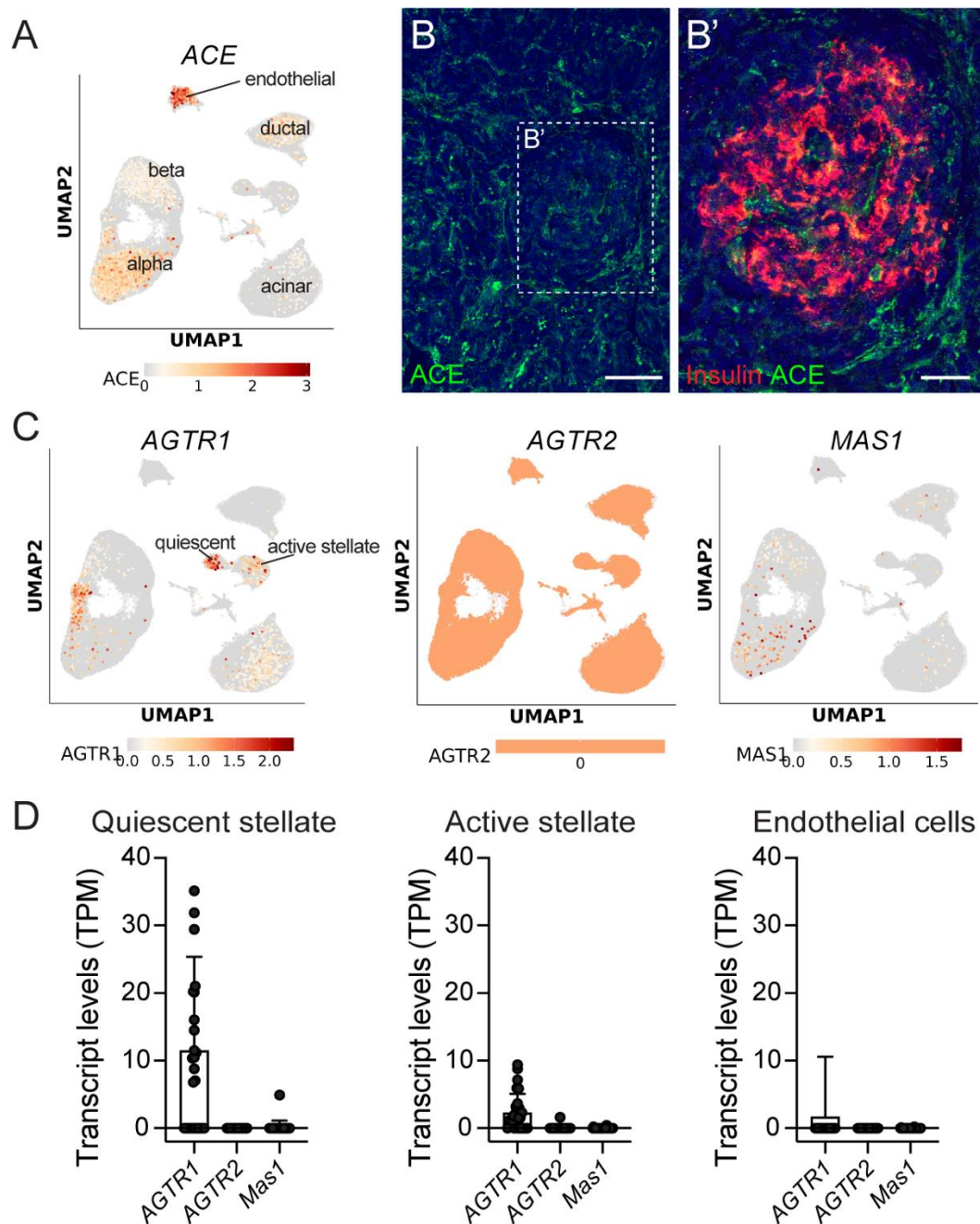
Supplementary figure 1. ACE2 is not expressed either by endocrine or endothelial cells but by peri-vascular cells in human pancreatic islets.

(A) Z-projection of confocal images of a pancreatic section from a non-diabetic organ donor 44-years old immunostained for ACE2 only (green) and insulin (gray). (B) Confocal image of pancreatic islets in a tissue section from a 44-year old donor showing ACE2 (green) and the pericyte/stellate cell marker platelet-derived growth factor receptor β (PDGFR β , magenta). (C) Z-projection of confocal images of an islet in a human pancreas immunostained for insulin (gray), endothelial cell marker CD31 (magenta) and ACE2 (green). ACE2 is expressed by cells that cover islet capillaries. Scale bar = 100 μm (A), 20 μm (B), 50 μm (C).



Supplementary figure 2. Pericytes and stellate cell clusters in human islets

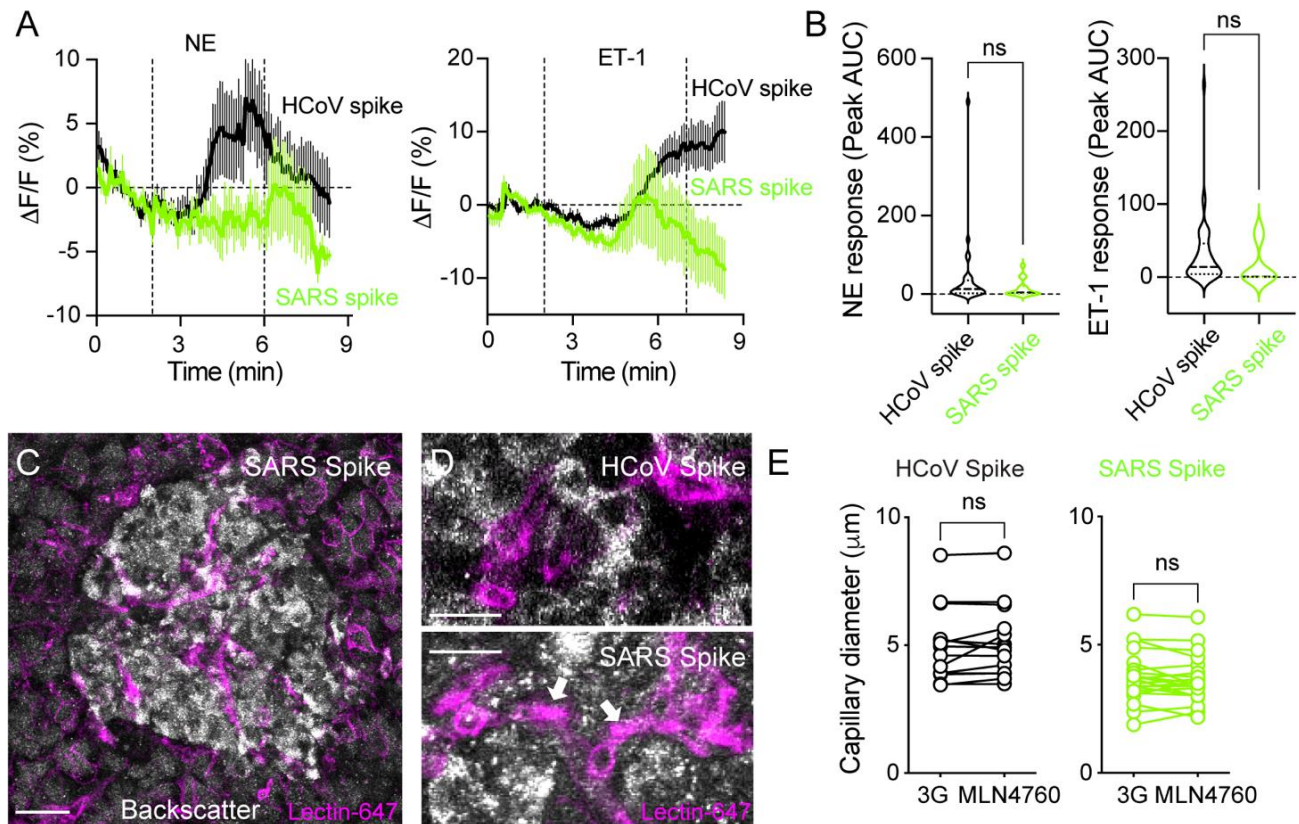
(A) UMAP plots showing the distribution of genes expressed by stellate cells (quiescent and active) such as platelet derived growth factor receptor beta (*Pdgfrb*) and chondroitin sulfate proteoglycan 4 (*Cspg4*) in single-cell RNAseq clusters obtained from isolated human islets from 27 non-diabetic donors (from nPOD and UPenn). These data were extracted from http://www.gaultonlab.org/pages/Islet_expression_HPAP.html, using *ShinyCell* application. (B) Transcript levels (TPM; transcripts per million) of *Cspg4*, the gene encoding NG2, in endothelial, quiescent and active stellate cell clusters. (C) Z-projection of confocal images of a human islet in the pancreas immunostained for NG2 (green) and PDGFRβ (magenta). Scale bar = 20 μm.



Supplementary figure 3. Expression of RAS components - ACE and receptors of angiotensin peptides - in the human pancreas.

(A) UMAP plots showing the expression of the gene encoding angiotensin converting enzyme (*ACE*) in single-cell RNAseq clusters obtained from isolated human islets from 27 non-diabetic donors (www.isletgenomics.org database). *ACE* is highly abundant in the

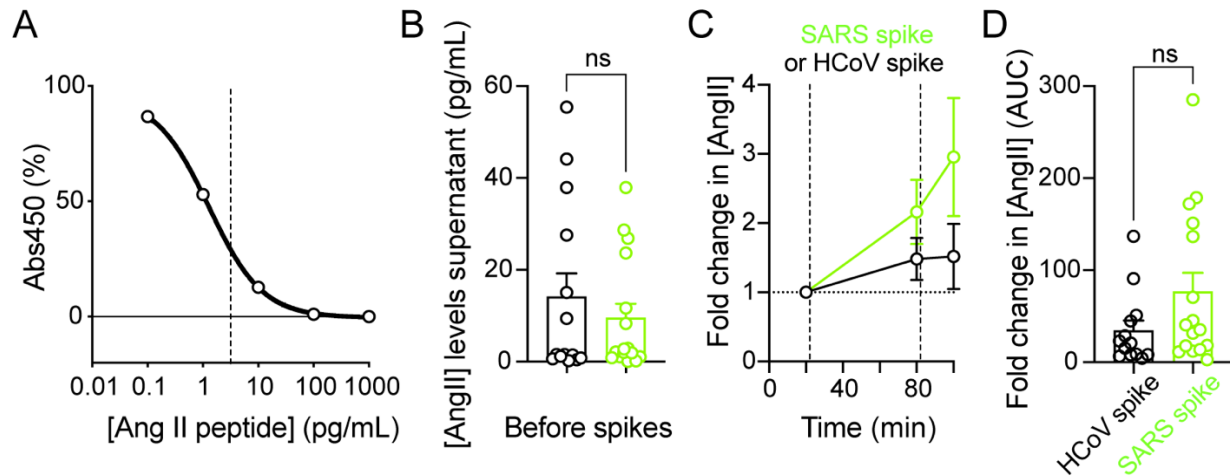
endothelial cell cluster. (B) Z-projection of confocal images of a human pancreatic section immunostained for ACE (green) and insulin (red; B'). ACE is present in both the endocrine and exocrine pancreas regions. Scale bars = 50 μm (B), 20 μm (B'). (C) UMAP plots showing the expression of genes encoding angiotensin II receptors (*AGTR1* and *AGTR2*) and angiotensin1-7 receptor (*MAS1*) in single-cell RNAseq clusters from human pancreatic islets. (D) Transcript levels (TPM) of angiotensin receptor genes *Agtr1*, *Agtr2*, *Mas1* in quiescent stellate cells (lef), active stellate (middle) and endothelial cells (right).



Supplementary figure 4. Effects of incubation with SARS-CoV-2 Spike S1 subunit on pericytes and capillaries in human islets.

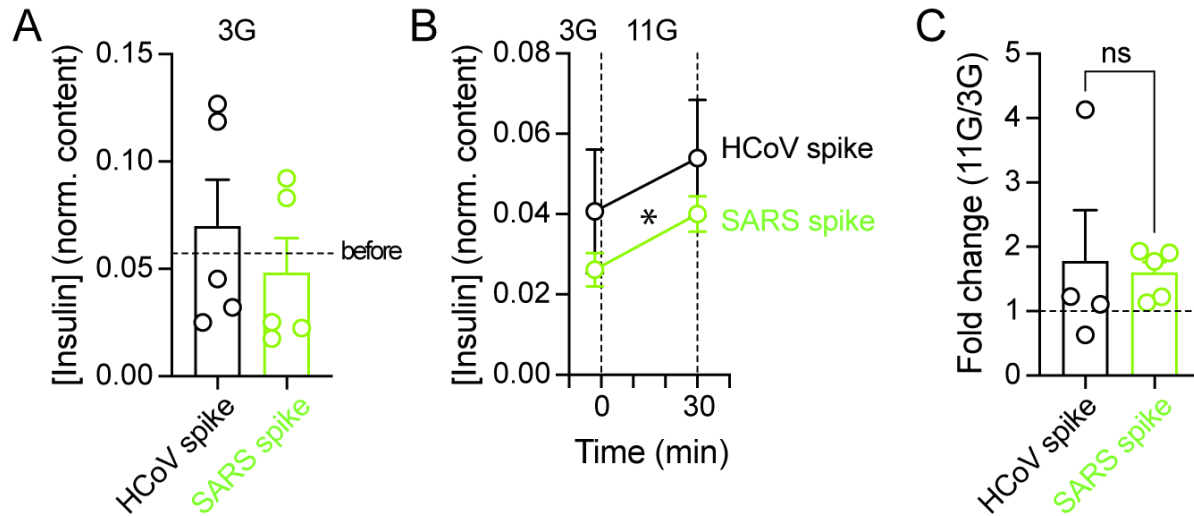
(A) Traces showing relative changes in Fluo4 fluorescence (mean \pm SEM) in islet pericytes in response to norepinephrine (NE; 20 μ M in 3G; left) or endothelin-1 (ET-1; 10 nM in 3G; right) in slices treated for 1h with HCoV spike (black) or with SARS spike (green). (B) Quantification of the area under the curve (peak AUC) of fluorescence traces of individual islet pericyte responses to NE (left; n=16-30 pericytes/2 donors) or to ET-1 (right; n=8-43 pericytes/3 donors) in slices treated with either spike (unpaired t-test; $p=ns$). (C) Z-projection of confocal images of the same islet shown in Figure 4B showing lectin-labeled capillaries (magenta) 1h after incubation with SARS spike. Islet endocrine cells are shown in white (backscatter). (D) Confocal images of islet capillaries in living human pancreas

slices after 1h incubation with either a recombinant Spike S1 subunit from HCoV-OC43 (HCoV Spike; top panel) or from SARS-CoV-2 (SARS Spike; bottom panel). Arrows indicate regions in capillaries fully constricted. Scale bars = 20 μm (C), 10 μm (D). (E) Islet capillary diameter values before (3G) and 4 min after applying the ACE2 inhibitor MLN4760 for individual vessels in slices previously treated with a recombinant spike (paired t-tests, $p=\text{ns}$).



Supplementary figure 5. Incubation with SARS-CoV-2 Spike S1 subunit increases endogenous angiotensin II levels in the pancreas.

(A) Standard curve of ELISA assay used to determine [AngII] in the supernatant of slices incubated for 1h with either recombinant spike protein. Experiments were performed in 24-well plates, each well contained 2 slices from the same donor. (B) AngII concentration in supernatant collected after 20 min incubation in 3 mM glucose. Basal [AngII] is not different. (C) Fold change in AngII concentration (normalized to levels in 3G) after 1h incubation with either recombinant spike. Dashed lines indicate period when spikes were present. (D) Quantification of the AUC of graphs shown in (C) reflecting the total change in [AngII] induced by either spike (n=13-16 wells/8 donors; unpaired t-test, $p=0.105$).



Supplementary Figure 6. SARS-CoV-2 spike does not affect beta cell responses to glucose

(A) Insulin concentration in the supernatant (normalized to total insulin content) of living pancreas slices 1h after incubation with either control (HCoV; black) or SARS spike (green). Each well contained 2 slices from the same donor, and experiments were performed at least in duplicate ($n=5$ wells/2 non-diabetic donors). Dashed line indicates average insulin concentration before incubation with either spike protein. (B) Average (\pm SEM) changes in insulin concentration upon increasing glucose concentration from 3 mM (3G) to 11 mM (11G; for 30 min) in slices pre-treated with either spike (paired t-test, $*p=0.02$). (C) Fold change in insulin levels induced by high glucose (values at 11G/3G) for each well (unpaired t-test; $p=0.8$).

Supplementary movie 1. Pericytes in human islets are activated by angiotensin II.

Movie composed of a series of projections of 3 confocal images taken every 5 sec of a human islet in a living pancreas slice incubated with calcium indicator Fluo4 (green) and a fluorescent antibody against NG2 (magenta). Islet endocrine cells are shown in white (backscatter) and pericytes in magenta. Angiotensin II (AngII, 100 nM, applied for 4 min in 3G) increases $[Ca^{2+}]_i$ in NG2-alexa647 labeled islet pericytes. Movie speed 20 frames per second (fps).

Supplementary movie 2. Capillaries in human islets constrict upon angiotensin II stimulation.

Movie composed of a series of confocal images taken every 5 sec of a capillary in a human islet in a living pancreatic slice incubated with calcium indicator Fluo4 (green) and with a fluorescent lectin (red). Reflection (backscattered light) was used to visualize endocrine cells (not shown). Angiotensin II (AngII, 100 nM, applied for 4 min in 3G) increases $[Ca^{2+}]_i$ in mural cell covering that capillary and constricts the microvessel. Static images of this movie are shown in Figure 2. Movie speed 20 frames per second (fps).

Supplementary movie 3. Incubation with a control recombinant spike (HCoV spike) does not affect islet vascular responses to angiotensin II.

Movie composed of a series of Z-projections of confocal images taken every 5 sec of a human islet in a living pancreatic slice incubated with calcium indicator Fluo4 (green), with a fluorescent lectin (magenta) and with a recombinant spike S1 subunit from HCoV-OC43 (HCoV spike). Reflection (backscattered light) was used to visualize endocrine

cells (not shown). Angiotensin II (AngII, 100 nM, applied for 4 min in 3G) increases $[Ca^{2+}]_i$ in different mural cells (2 responding cells are indicated with *), and capillaries constrict upon AngII administration.