Supplemental Information

SARS-CoV-2 Cell Entry Factors ACE2 and TMPRSS2
Are Expressed in the Microvasculature and Ducts
of Human Pancreas but Are Not Enriched in β Cells

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Figure S1

A: Camunas-Soler et al. 

B: 

**ACE2**

Donor BMI

**TMPRSS2**

**ADAM17**

Donor BMI

HPAP
Figure S4

Normal Adult

DPP4

Type 2 Diabetes

INS GCG

Type 1 Diabetes

MERGE

A 55yM

B 60y_1yM

C 20y_7yM
Figure S1. Related to Figure 1. Stratification of ACE2, TMPRSS2, and ADAM17 Expression in β cells by BMI.

(A) Dot plots of ACE2, TMPRSS2, CTSL, ADAM17, FURIN, TMPRSS4, and DPP4 mRNA expression compared with cell type-enriched genes from a previously published single cell (sc) RNA-seq datasets (Camunas-Soler et al., 2020). Dot size indicates percentage of cells in a given population expressing the gene; dot color represents scaled average expression. Dotted line highlights ACE2, TMPRSS2, CTSL, ADAM17, FURIN, TMPRSS4, and DPP4 expression.

(B) ACE2, TMPRSS2, and ADAM17 mRNA expression in single β cells according to BMI. β cell gene expression from eleven donors (ages 1-39 years) from the HPAP scRNA-seq dataset (Kaestner et al., 2019) are displayed from lowest to highest BMI. Only one donor had a BMI in the obese range in this dataset. Human pancreatic donor information is available in Table S2.

Figure S2. Related to Figure 1 and 2. Testing and Characterization of Four ACE2-directed Antibodies on Human Pancreatic Tissue.

(A-E) Characterization of ACE2 antibody (red; ab15348) used by Yang et al. (Yang et al., 2020) and Fignani et al. (Fignani, 2020). Antibody epitope encompasses the ACE2 C-terminal domain (human aa 788-805). Mouse kidney tissue served as a positive control for ACE2 (A), while normal adult human pancreatic tissue incubated with anti-rabbit-Cy3 secondary antibody only served as a negative control (B). Normal adult human pancreas labeled for ACE2 (red), INS (green, β cells) and GCG (blue, α cells) (C-E). Inset area is marked by a yellow box in MERGE column (n = 14 total images analyzed).

(F-I) ACE2 neutralization with immunizing peptide. Scale bars are 100 µm (A-E) and 50 µm (Inset, E and F-I) (n = 8 total images analyzed).

(J-N) Characterization of ACE2 antibody (red; R&D MAB933) at same dilution (1:33) reported by Fignani et al. (Fignani, 2020). Antibody epitope encompasses the ACE2 extracellular domain (human aa 18-740). Human kidney tissue served as a positive control for ACE2 (J), while normal adult human pancreatic tissue incubated with anti-mouse-Cy3 secondary antibody only
served as a negative control (K). Normal adult human pancreas labeled for ACE2 (red), INS (green, β cells) and GCG (blue, α cells) (L-N). Inset area is marked by a yellow box in MERGE column. Scale bars are 50 µm (J-N) and 25 µm (Inset, N) (n = 18 total images analyzed).

(O-S) Characterization of ACE2 antibody (red; R&D AF933) at same dilution (1:200) reported by Yang et al. (Yang et al., 2020). Antibody epitope encompasses the ACE2 extracellular domain (human aa 18-740). Human kidney served as a positive control for ACE2 (O), while normal adult human pancreatic tissue incubated with anti-goat-Cy3 secondary antibody only served as a negative control (P). Normal adult human pancreas labeled for ACE2 (red), INS (green, β cells) and GCG (blue, α cells) (Q-S). Inset area is marked by a yellow box in MERGE column. Scale bars are 50 µm (O-R) and 25 µm (Inset, S) (n = 13 total images analyzed).

(T-W) Characterization of ACE2 antibody (red; HPA000288) used by the Human Protein Atlas (Uhlen et al., 2015) and Hikmet et al. (Hikmet et al., 2020). Antibody epitope encompasses the ACE2 extracellular domain (human aa 1-111). Human kidney tissue served as a positive control for ACE2 (T). Normal adult human pancreas labeled for ACE2 (red) and INS (green, β cells) (U-W). Inset area is marked by a white dashed box in MERGE column. Scale bars are 100 µm (T-V) and 50 µm (Inset, W). DAPI (white) (n = 6 total images analyzed).

Human pancreatic donor information is available in Table S2 (B, donor N8; C-E, donor N4; F-I, donors N6 and N2; J-N, donor N2; O-S, donor N7; T-W, donor N8).

**Figure S3. Related to Figures 2 and 5. ACE2 and TMPRSS2 Protein in Human Islets and Exocrine Tissue from Adult Donors With and Without Diabetes.**

(A-H) Immunostaining of SARS-CoV-2 cell entry markers ACE2 (antibody ab15348) and TMPRSS2, both shown in red, in islet α cells (GCG, blue) or β cells (INS, green) in pancreatic sections from adult donors without diabetes. Insets are depicted by a yellow box. DAPI (white) (n = 14 total images analyzed).
(I-N) Immunostaining of SARS-CoV-2 cell entry markers ACE2 (antibody ab15348) and TMPRSS2, both shown in red, in islet α cells (GCG, blue) or β cells (INS, green) in pancreatic sections from adult donors with type 2 diabetes. Insets are depicted by a yellow box. DAPI (white) \( (n = 12 \text{ total images analyzed}) \).

(O-V) Immunostaining of SARS-CoV-2 cell entry markers ACE2 (antibody ab15348) and TMPRSS2, both shown in red, in islet α cells (GCG, blue) or β cells (INS, green) in pancreatic sections from adult donors with type 1 diabetes. Insets are depicted by a yellow box. DAPI (white) \( (n = 11 \text{ total images analyzed}) \).

Human islet and pancreatic donor information is available in Table S2 (A-D, donors N3, N7, N9, N8; E-H, donors N14, N12, N11, N10; I-L, donors 2L, 2B, 2G, 2I; M-N, donors 2H, 2G; O-R, donors 1B, 1D, 1C, 1A; S-V, donors 1H, 1K, 1J, 1G). Scale bars are 100 µm (A-V) and 25 µm (Insets).

**Figure S4. Related to Figures 1 and 2. DPP4 Protein in Human Islets from Adult Donors With and Without Diabetes.**

(A-C) Immunostaining of DPP4 (red) in human pancreatic islet α cells (GCG, blue; merged, magenta) and β cells (INS, green) in pancreatic sections from adult donors without diabetes \( (n = 2 \text{ total images analyzed}) \).

(D-F) Immunostaining of DPP4 (red) in human pancreatic islet α cells (GCG, blue; merged, magenta) and β cells (INS, green) in pancreatic sections from adult donors with type 2 diabetes \( (n = 2 \text{ total images analyzed}) \).

(G-I) Immunostaining of DPP4 (red) in human pancreatic islet α cells (GCG, blue; merged, magenta) and β cells (INS, green) in pancreatic sections from adult donors with type 1 diabetes \( (n = 2 \text{ total images analyzed}) \).

Human islet and pancreatic donor information is available in Table S2 (A-C, donor N8; D-F,
Figure S5. Related to Figures 3 and 4. ACE2 Protein Localization with Islet and Exocrine Capillaries in Adult Human Pancreas of Individuals with Diabetes.

(A-H’) Representative images of endothelial cells (CD31, white) and ACE2-positive perivascular cells (red; antibody ab15348) in the islet microvasculature of individuals with type 2 (A-D’) or type 1 diabetes (E-H’). DAPI (blue). ACE2-positive perivascular cells (red; antibody ab15348) and the extracellular matrix marker collagen-IV (COL4, green) within the vascular basement membrane are shown (D, D’, H and H’); DAPI counterstain (blue) (n = 23 total images analyzed).

(I-P’) Representative images of endothelial cells (CD31, white) and ACE2-positive perivascular cells (red; antibody ab15348) in the exocrine tissue microvasculature of individuals with type 2 (I-L’) or type 1 diabetes (M-P’). DAPI (blue). ACE2-positive perivascular cells (red; antibody ab15348) and the extracellular matrix marker collagen-IV (COL4, green) within the vascular basement membrane are shown (L, L’, P and P’); DAPI counterstain (blue) (n = 23 total images analyzed).

Human pancreatic donor information is available in Table S2 (A-D’, donor 2E; E-H’, donor 1F; I-L’, donor 2E; M-P’, donor 1C). Yellow arrowheads point to CD31-positive endothelial cells, while magenta arrowheads point to perivascular ACE2-positive cells. Insets (A’-P’) are depicted by yellow boxes in A-P. Scale bars are 50 µm (A-P) and 10 µm (Insets, A’-P’).
Table S1. Related to Figure 1. Number and Percentage of β cells that Express and Co-express Putative SARS-CoV-2 Cell Entry Genes Across Four Independent scRNA-seq Datasets.

| Genes          | Droplet-based scRNA-seq |                      | SMART-seq |                      |
|----------------|-------------------------|----------------------|-----------|----------------------|
|                | HPAP<sup>a</sup>        | Baron et al.<sup>b</sup> | Segerstolpe et al.<sup>c</sup> | Camunas-Soler et al.<sup>c</sup> |
|                | (β cell total, n = 2828) | (β cell total, n = 2525) | (β cell total, n = 157) | (β cell total, n = 194) |
| # β cell | % β cells | # β cell | % β cells | # β cell | % β cells | # β cell | % β cells |
| ACE2       | 17        | 0.6      | 4         | 0.2      | 3        | 1.9      | 7         | 3.6       |
| TMPRSS2    | 60        | 2.1      | 7         | 0.3      | 4        | 2.5      | 2         | 1.0       |
| TMPRSS4    | 0         | 0.0      | 0         | 0.0      | 0        | 0.0      | 3         | 1.5       |
| CTSK       | 1421      | 50.2     | 977       | 38.7     | 132      | 84.1     | 161       | 83.0      |
| FURIN      | 779       | 27.5     | 942       | 37.3     | 91       | 58.0     | 138       | 71.1      |
| ADAM17     | 494       | 17.5     | 251       | 9.9      | 78       | 49.7     | 52        | 26.8      |
| ACE2, TMPRSS2 | 0      | 0.0      | 0         | 0.0      | 0        | 0.0      | 0         | 0.0       |
| ACE2, TMPSRSS4 | 0     | 0.0      | 0         | 0.0      | 0        | 0.0      | 0         | 0.0       |
| ACE2, CTSK | 0         | 0.0      | 2         | 0.1      | 3        | 1.9      | 6         | 3.1       |
| ACE2, FURIN | 0        | 0.0      | 1         | 0.0      | 1        | 0.6      | 3         | 1.5       |
| ACE2, ADAM17 | 0      | 0.0      | 0         | 0.0      | 1        | 0.6      | 2         | 1.0       |

<sup>a</sup>10x genomics; <sup>b</sup>InDrop (Klein et al., 2015); <sup>c</sup>SMART-seq2 (Picelli et al., 2014)
Table S2. Related to STAR Methods. Demographic Information of Donors.

| Donor ID | Age       | Ethnicity / Race | Diabetes Duration | Sex | BMI | Cause of Death | Tissue/Islet Source |
|----------|-----------|------------------|-------------------|-----|-----|----------------|---------------------|
| J1       | 5 days    | Caucasian        | --                | F   | 14.9| Anoxia         | IIAM                |
| J2       | 3 months  | Caucasian        | --                | M   | 16.8| Anoxia         | NDRI                |
| J3       | 10 months | Caucasian        | --                | F   | 15.4| CVA            | NDRI                |
| J4       | 20 months | Caucasian        | --                | F   | 23.5| Anoxia         | IIAM                |
| J5       | 5 years   | Caucasian        | --                | M   | 16.2| Anoxia         | IIAM                |
| N1       | 42 years  | Caucasian        | --                | M   | 32.2| Overdose       | TNDS                |
| N2       | 45 years  | Caucasian        | --                | F   | 29.7| Anoxia         | OPO                 |
| N3       | 46 years  | Caucasian        | --                | F   | 32.9| CVA            | IIAM                |
| N4       | 48 years  | Caucasian        | --                | M   | 24.6| Anoxia         | OPO                 |
| N5       | 51 years  | Caucasian        | --                | M   | 20.4| Anoxia         | OPO                 |
| N6       | 52 years  | Black            | --                | M   | 29.2| ICH            | TNDS                |
| N7       | 52 years  | Caucasian        | --                | M   | 28.1| Head Trauma    | OPO                 |
| N8       | 55 years  | Black            | --                | M   | 35.6| CVA            | IIAM                |
| N9       | 59 years  | Caucasian        | --                | M   | 32.7| Head Trauma    | IIAM                |
| N10      | 20 years  | Hispanic         | --                | M   | 19.4| Head Trauma    | IIAM                |
| N11      | 24 years  | Caucasian        | --                | M   | 35.5| ICH            | IIAM                |
| N12      | 35 years  | Caucasian        | --                | M   | 26.8| Head Trauma    | IIAM                |
| N13      | 20 years  | Caucasian        | --                | M   | 27.8| Head Trauma    | NDRI                |
| N14      | 18 years  | Caucasian        | --                | M   | 25.1| Head Trauma    | IIAM                |
| HP1754   | 15 years  | N/A              | --                | M   | 22.6| Head Trauma    | IIAM                |
| HP2041   | 29 years  | N/A              | --                | M   | 22.3| Head Trauma    | IIAM                |
| HP2091   | 44 years  | N/A              | --                | F   | 23.7| CVA            | IIAM                |
| 1A       | 43 years  | N/A              | 36 years          | M   | 31.2| CVA            | NDRI                |
| 1B       | 45 years  | Caucasian        | 43 years          | M   | 25.0| Anoxia         | IIAM                |
| 1C       | 54 years  | Caucasian        | 14 years          | F   | 24.9| Anoxia         | IIAM                |
| 1D       | 57 years  | Black            | 45 years          | M   | 33.3| CVA            | IIAM                |
| 1E       | 58 years  | Caucasian        | 31 years          | M   | 21.8| Anoxia         | NDRI                |
| 1F       | 63 years  | Caucasian        | 44 years          | M   | 24.1| Anoxia         | IIAM                |
| 1G       | 35 years  | Caucasian        | 23 years          | M   | 26.9| Anoxia         | NDRI                |
| 1H       | 20 years  | Caucasian        | 7 years           | M   | 25.5| Anoxia         | NDRI                |
| 1I       | 27 years  | Caucasian        | 17 years          | M   | 18.4| Anoxia         | NDRI                |
| 1J       | 13 years  | Caucasian        | 5 years           | M   | 19.1| Anoxia         | IIAM                |
| 1K       | 30 years  | Caucasian        | 20 years          | M   | 29.8| Anoxia         | NDRI                |
| 2A       | 44 years  | Caucasian        | 7 years           | M   | 44.4| CVA            | IIAM                |
| 2B       | 52 years  | Caucasian        | 7 years           | M   | 33.6| CVA            | IIAM                |
| 2C       | 52 years  | Asian            | 10 years          | F   | 21.9| CVA            | NDRI                |
| 2D       | 52 years  | Caucasian        | < 1 year          | F   | 29.2| CVA            | IIAM                |
| 2E       | 42 years  | Black            | < 1 year          | M   | 42.0| CVA            | IIAM                |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 2F | 43 years | Black | 1 year | M | 36.0 | Head Trauma | IIAM |
| 2G | 66 years | Caucasian | 3 years | F | 32.8 | CVA | IIAM |
| 2H | 47 years | Caucasian | 3 years | M | 31.3 | CVA | IIAM |
| 2I | 64 years | Caucasian | 5 years | M | 33.2 | ICH | IIAM |
| 2J | 59 years | Caucasian | 6 years | F | 27.5 | CVA | IIAM |
| 2K | 60 years | Caucasian | 1 year | M | 38.3 | CVA | IIAM |
| 2L | 49 years | Caucasian | 3 years | F | 33.8 | CVA | IIAM |

**Normal Adult Islets (Gels and scRNA-Seq)**

|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| I1 | 40 years | Caucasian | -- | F | 30.8 | Head Trauma | IIDP |
| I2 | 41 years | N/A | -- | M | 20.3 | N/A | IIDP |
| I3 | 42 years | Caucasian | -- | M | 32.2 | Overdose | IIDP |
| HPAP022 | 39 years | Caucasian | -- | F | 34.7 | Anoxia | HPAP |
| HPAP026 | 24 years | Caucasian | -- | M | 20.8 | Anoxia | HPAP |
| HPAP034 | 13 years | Caucasian | -- | M | 18.6 | Head Trauma | HPAP |
| HPAP035 | 35 years | Caucasian | -- | M | 26.9 | Anoxia | HPAP |
| HPAP036 | 23 years | Caucasian | -- | F | 16 | Head Trauma | HPAP |
| HPAP037 | 35 years | Caucasian | -- | F | 21.9 | CVA | HPAP |
| HPAP039 | 5 years | Caucasian | -- | F | 16.3 | Anoxia | HPAP |
| HPAP040 | 35 years | Caucasian | -- | M | 23.9 | CVA | HPAP |
| HPAP042 | 1 year | Caucasian | -- | M | 17.9 | Anoxia | HPAP |
| HPAP044 | 3 years | Caucasian | -- | F | 12 | Anoxia | HPAP |
| HPAP047 | 8 years | Caucasian | -- | M | 16.8 | CVA | HPAP |

**COVID-19 Patient Autopsy Samples (Histology)**

|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 1 | 82 years | Caucasian | -- | M | 26.8 | ALI | VUMC Autopsy |
| 2 | 97 years | Caucasian | -- | F | 19.7 | ALI | VUMC Autopsy |
| 3 | 81 years | Caucasian | >10 years\(^a\) | M | 23.3 | ALI | VUMC Autopsy |
| 4 | 60 years | Hispanic | -- | M | 36.7 | ALI | VUMC Autopsy |
| 5 | 51 years | Hispanic | 23 years | M | 29.4 | ALI | VUMC Autopsy |
| 6 | 60 years | Caucasian | -- | F | 38.4 | PE | VUMC Autopsy |
| 7 | 71 years | Black | Pre-existing\(^b\) | M | 31.5 | ALI | VUMC Autopsy |

ALI – acute lung injury; CVA, cerebrovascular accident; HPAP – Human Pancreas Analysis Program (Human Islet Research Network); ICH, intracerebral hemorrhage; IIAM – International Institute for the Advancement of Medicine; IIDP – Integrated Islet Distribution Program; N/A – not available; NDRI – National Disease Research Interchange; OPO – Organ Procurement Organization; PE – pulmonary embolism; T1D = type 1 diabetes; T2D – type 2 diabetes; TNDS – Tennessee Donor Services, Nashville; VUMC Autopsy – Vanderbilt University Medical Center Autopsy Pathology

\(^a\)Oldest clinical patient note including diagnosis of diabetes mellitus was signed in 2010, suggesting disease duration of at least 10 years.

\(^b\)Patient was prescribed an oral anti-diabetic medication confirming pre-existing diabetes diagnosis of unknown duration prior to admission with COVID-19.