Cell Metabolism, Volume *32*

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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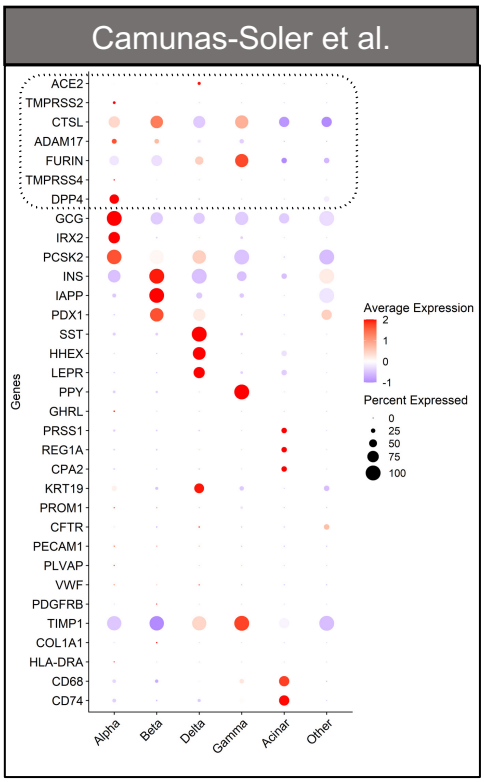
A


Camunas-Soler et al.
ACE2
TMPRSS2
CTSL
ADAM17
FURIN
TMPRSS4
DPP4
GCG
IRX2
PCSK2
INS
IAPP
PDX1
SST
HHEX
LEPR
PPY
GHRL
PRSS1
REG1A
CPA2
KRT19
PROM1
CFTR
PECAM1
PLVAP
VWF
PDGFRB
TIMP1
COL1A1
HLA-DRA
CD68
CD74
Genes
Alpha
Beta
Delta
Gamma
Acinar
Other
Average Expression
2
1
0
-1
Percent Expressed
0
25
50
75
100

HPAP

B

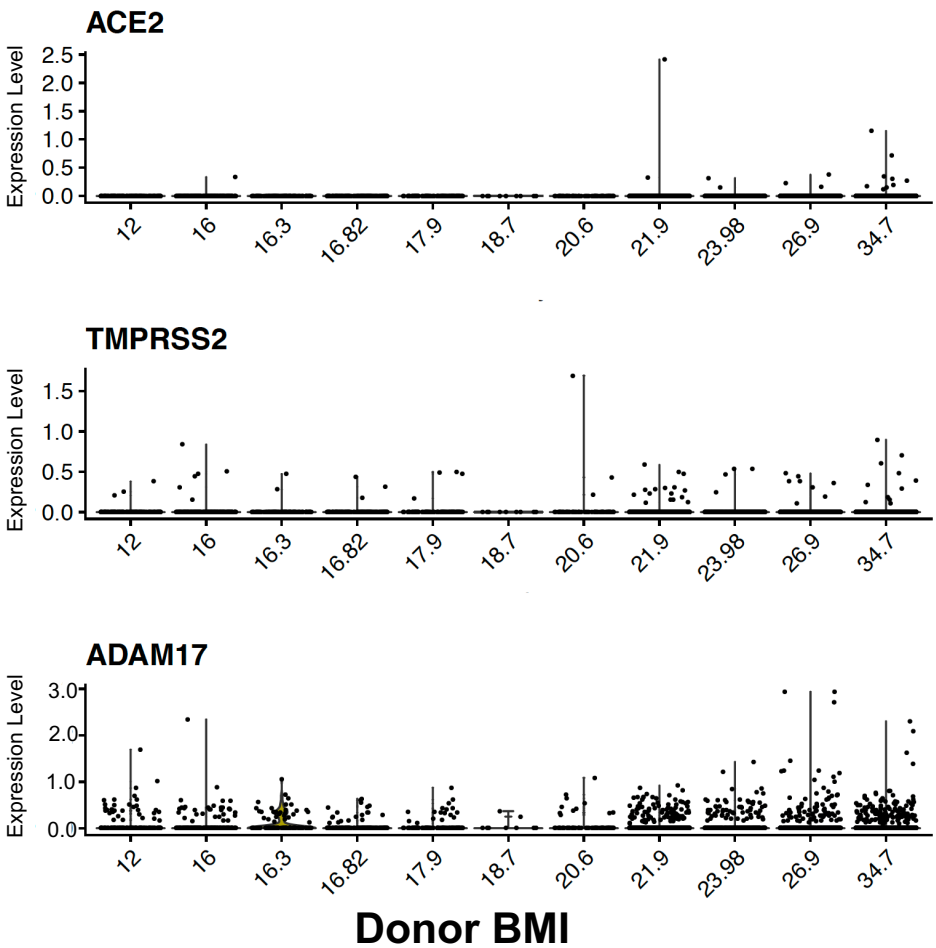

ACE2
TMPRSS2
ADAM17
Expression Level
12
16
16.3
16.82
17.9
18.7
20.6
21.9
23.98
26.9
34.7
Donor BMI

Figure S2

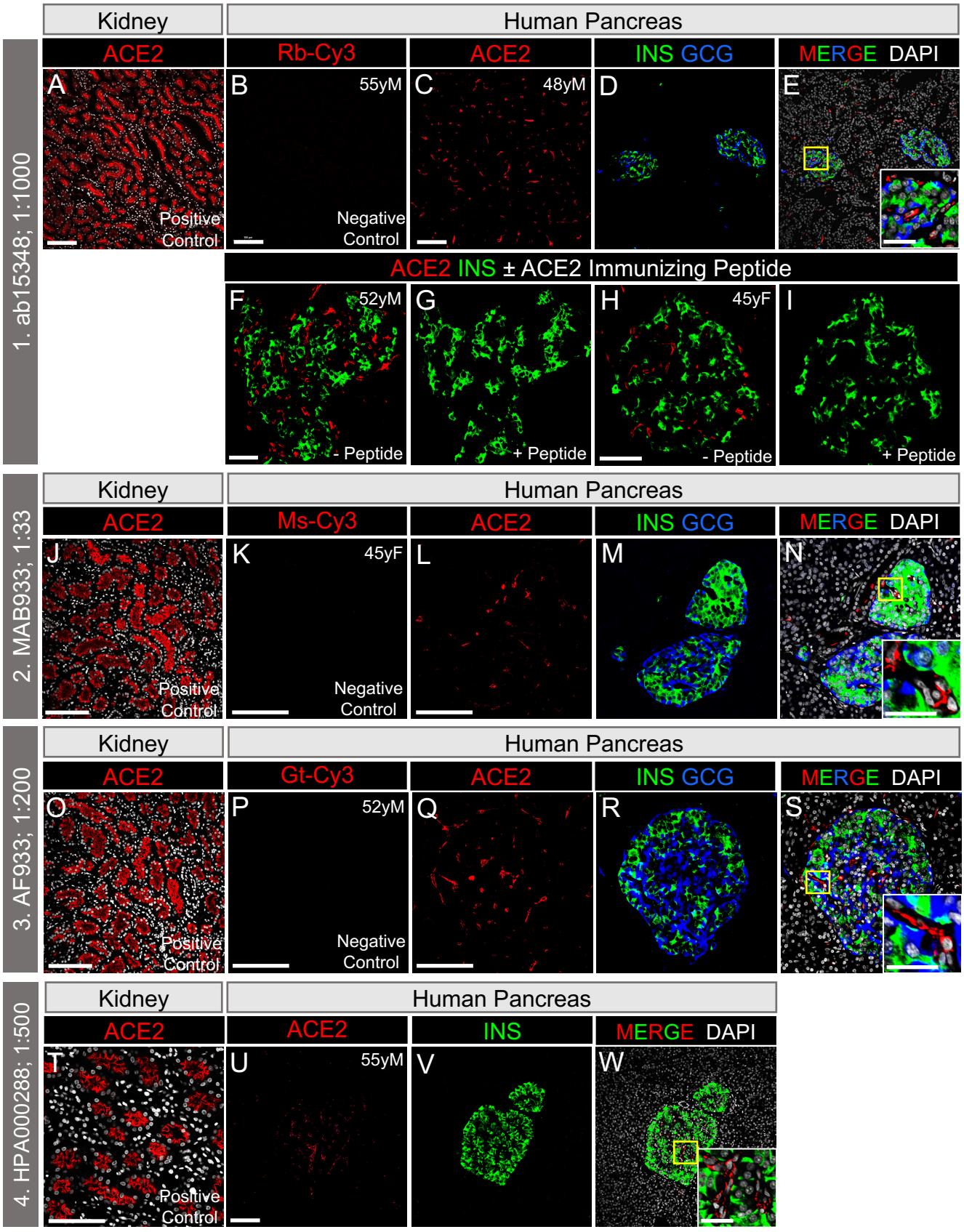

1. ab15348; 1:1000
Kidney
Human Pancreas
ACE2
Rb-Cy3
ACE2
INS GCG
MERGE DAPI
A
Positive Control
B
55yM
Negative Control
C
48yM
D
E
ACE2 INS ± ACE2 Immunizing Peptide
F
52yM
- Peptide
G
+ Peptide
H
45yF
- Peptide
I
+ Peptide
2. MAB933; 1:33
Kidney
Human Pancreas
ACE2
Ms-Cy3
ACE2
INS GCG
MERGE DAPI
J
Positive Control
K
45yF
Negative Control
L
M
N
3. AF933; 1:200
Kidney
Human Pancreas
ACE2
Gt-Cy3
ACE2
INS GCG
MERGE DAPI
O
Positive Control
P
52yM
Negative Control
Q
R
S
4. HPA000288; 1:500
Kidney
Human Pancreas
ACE2
ACE2
INS
MERGE DAPI
T
Positive Control
U
55yM
V
W

Figure S3

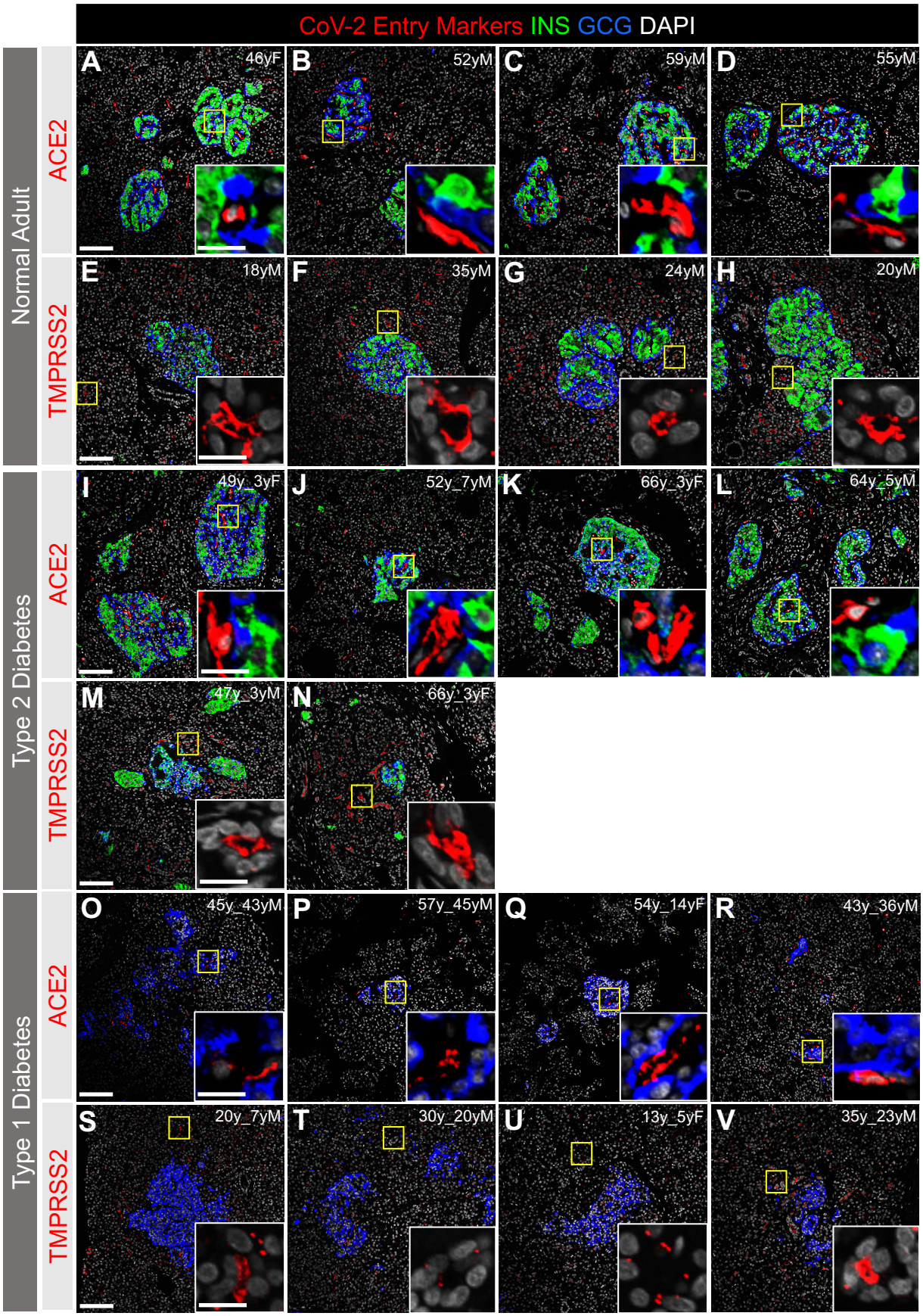

CoV-2 Entry Markers INS GCG DAPI
Normal Adult
Type 2 Diabetes
Type 1 Diabetes
ACE2
TMPRSS2
A 46yF
B 52yM
C 59yM
D 55yM
E 18yM
F 35yM
G 24yM
H 20yM
I 49y_3yF
J 52y_7yM
K 66y_3yF
L 64y_5yM
M 47y_3yM
N 66y_3yF
O 45y_43yM
P 57y_45yM
Q 54y_14yF
R 43y_36yM
S 20y_7yM
T 30y_20yM
U 13y_5yF
V 35y_23yM

Figure S4

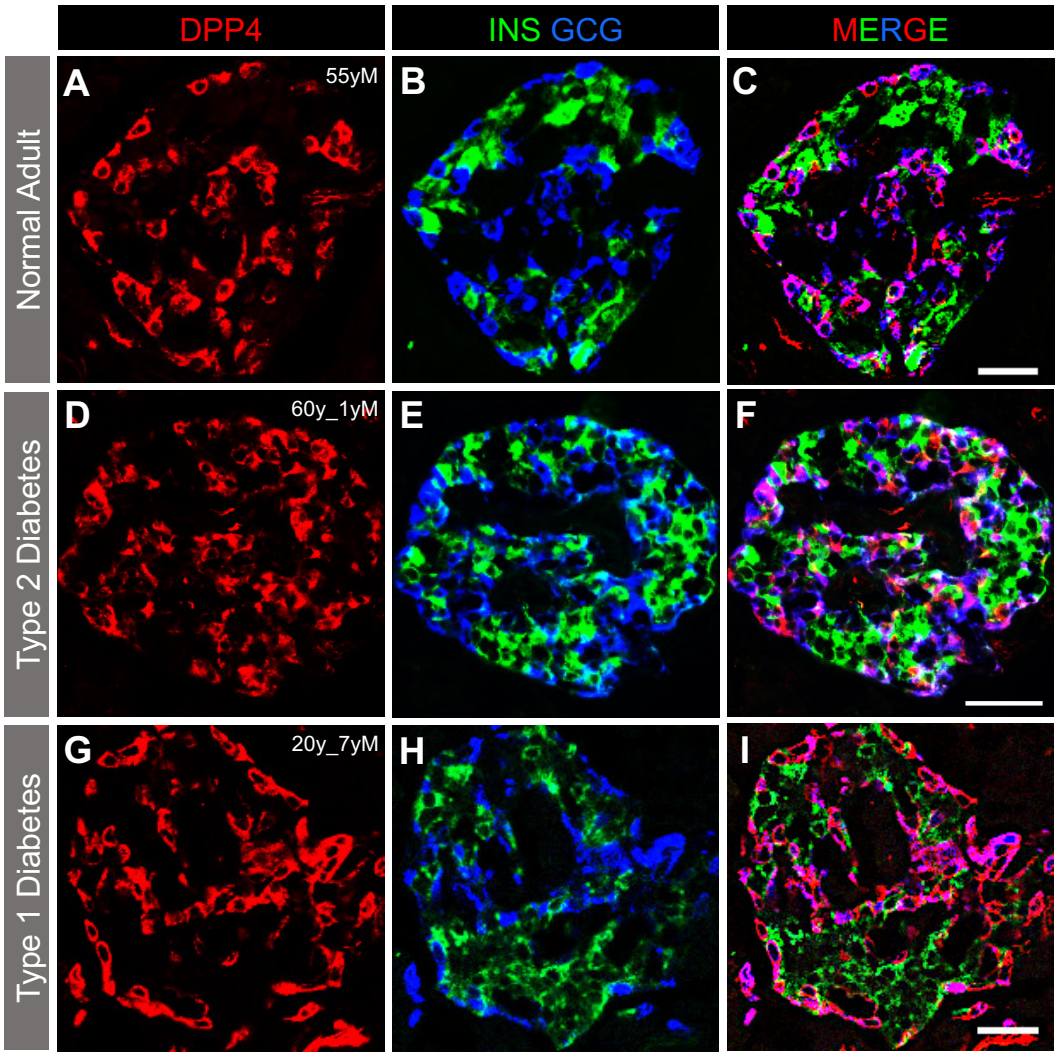

DPP4
INS GCG
MERGE
Normal Adult
Type 2 Diabetes
Type 1 Diabetes
A
55yM
B
C
D
60y_1yM
E
F
G
20y_7yM
H
I

Figure S5

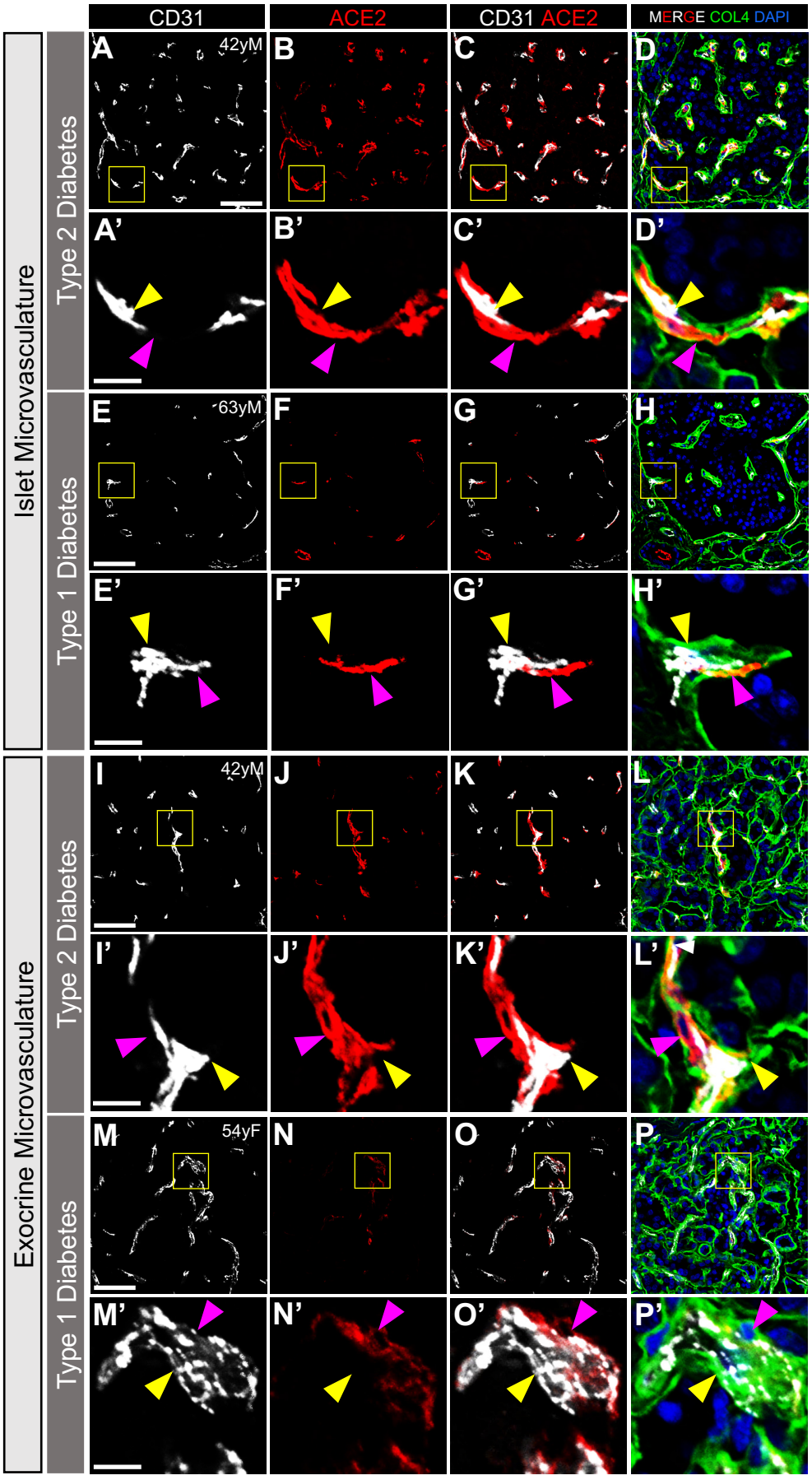

CD31
ACE2
CD31 ACE2
MERGE COL4 DAPI
Islet Microvasculature
Exocrine Microvasculature
Type 2 Diabetes
Type 1 Diabetes
Type 2 Diabetes
Type 1 Diabetes
A
42yM
B
C
D
A'
B'
C'
D'
E
63yM
F
G
H
E'
F'
G'
H'
I
42yM
J
K
L
I'
J'
K'
L'
M
54yF
N
O
P
M'
N'
O'
P'

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 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 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 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 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 total images analyzed).

Human islet and pancreatic donor information is available in Table S2 (A-C, donor N8; D-F,

donor 2K; G-I, donor 1H). Scale bars are 50 μm (A-I).

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[a] (β cell total, *n* = 2828)		Baron et al.[b] (β cell total, *n* = 2525)		Segerstolpe et al.[c] (β cell total, *n* = 157)		Camunas-Soler et al.[c] (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
CTSL	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,TMPRSS4	0	0.0	0	0.0	0	0.0	0	0.0
ACE2, CTSL	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

[a]10x genomics; [b]InDrop (Klein et al., 2015); [c]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
Juvenile (Histology)	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
Normal Adult (Histology)	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
Adult T1D (Histology)	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
Adult T2D (Histology)	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.