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Supplemental information

Pericyte dysfunction and impaired vasomotion are hallmarks of islets during the pathogenesis of type 1 diabetes

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Supplementary Tables

Table S1. Characteristic of organ donors used in the study. Living pancreas slices were obtained from nPOD (Gainesville, University of Florida) and used for physiology and/or for immunohistochemistry.

Case ID	Donor Type	Gender	Age	Race	BMI	AutoAb	T1D (yrs)	HbA1c	C-peptide	Physiology	IHC	scRNAseq
6516	ND	M	20	Caucasian	28.8			5.5	8.91	x	x	
6531	ND	F	19	Hispanic	30			5.5	26.53	x	x	
6535	ND	F	31	Caucasian	29.6				6.59	x	x	
6537	ND	M	33	Caucasian	20.8			5.6	0.32	x	x	
6539	ND	M	24	Hispanic	19.3			5.7	39.23	x	x	
6546	ND	M	22	Asian	23.7			5.6	11	x	x	
6548	ND	M	20	Caucasian	23.8			5.7	4.04	x	x	
6552	ND	F	33	Caucasian	21.9			5.6	1.8	x	x	
6555	ND	F	18	Caucasian	18.4			5.2	3.6	x	x	
6468	ND	M	16	Caucasian	15.9			4.4	5.27		x	
6470	ND	M	30	Caucasian	22.5			5.5	6.03		x	
6471	ND	M	16	African American	18.4			5.5	1.99		x	
HPAP026	ND	M	24	Caucasian	20.8			4.9	0.25			x
HPAP034	ND	M	13	Caucasian	18.6			5.2	12.7			x
HPAP035	ND	M	35	Caucasian	26.9			5.2	15.9			x
HPAP036	ND	F	23	Caucasian	16			5.2	1.12			x
HPAP037	ND	F	35	Caucasian	21.9			5.3	4.75			x
HPAP040	ND	M	35	Caucasian	23.9			5.4	7.01			x
HPAP047	ND	M	8	Caucasian	16.8			N/A	1.24			x
HPAP082	ND	M	25	Caucasian	23.9			5.6	2.7			x
HPAP099	ND	F	28	Hispanic	24.7			5	6.62			x
6532	AAB+	M	20	Hispanic	23.9	GADA+		5.9	22.12	x	x	
6538	AAB+	M	19	Caucasian	32.9	GADA+		5.8	11.33	x	x	
6558	AAB+	F	21	African American	27.8	GADA+		4.4	8.03	x	x	
6562	AAB+	F	29	Caucasian	18.2	GADA+		5.6	13.19	x	x	
6569	AAB+	F	20	Hispanic	24.2	GADA+		6	2.44	x	x	
6573	AAB+	F	24	Caucasian	35.5	GADA+		5.1	1.85	x	x	
6575	AAB+	M	23	Caucasian	26.9	GADA+		5.5	5.21	x		
HPAP024	AAB+	M	18	Caucasian	24.4	GADA+		5.5	5.6			x
HPAP029	AAB+	M	23	Caucasian	28.6	GADA+		5.3	3.83			x
HPAP038	AAB+	M	13	Caucasian	18.3	GADA+		5.7	8.29			x
HPAP045	AAB+	F	27	Caucasian	26.2	GADA+		5.2	1.7			x
HPAP050	AAB+	F	21	Hispanic	28.9	GADA+		5.1	3.79			x
HPAP072	AAB+	M	19	Hispanic	23.1	GADA+		5.6	4.37			x
HPAP092	AAB+	M	21	Hispanic	25.6	GADA+		5.6	15.35			x
6523	T1D	F	12	African American	22.5	GADA+ mIAA*+	3	11.1	0.04	x	x	
6536	T1D	F	20	Caucasian	25.4	GADA+	4	12.7	0.04	x	x	
6550	T1D	M	25	Caucasian	16.4	GADA+ ZnT8A+	0	14	<0.02		x	
6551	T1D	M	20	Caucasian	23.1	GADA+IA2A+ mIAA+* ZnT8A+	0.6	6.4	0.11	x	x	
6563	T1D	F	14	Caucasian	25.5	IA2A+	0	9.6	1.04	x	x	
6566	T1D	M	15	Caucasian	21.8	GADA+IA2A+ mIAA+* ZnT8A+	2	10.3	0.05	x	x	
6578	T1D	F	11	Caucasian	22.5	IA2A+ ZnT8A+	0	13.6	0.35	x		
6579	T1D	F	13	NA	18.4	GADA+mIAA+	1	15	0.31	x		
6456	T1D	F	30	African American	30.1	GADA+ ZnT8A+	0	6.8	10.33		x	
6469	T1D	F	27	Caucasian	26.9	GADA+	1.5	7.4	0.66		x	
6472	T1D	F	10	Caucasian	16.6	mIAA*+	4	9.7	0.02		x	
6480	T1D	M	17	Caucasian	27.1	IA2A+ mIAA*+	2.5	10.2	0.13		x	
HPAP032	T1D	F	10	Caucasian	16.3	IA2A+ mIAA+	3	9	0.02			x
HPAP064	T1D	M	24	African American	16.9	ZnT8A+	N/A	13	0.25			x
HPAP071	T1D	F	12	African American	15.4	IA2A+ mIAA+	3	9.8	0.06			x
HPAP084	T1D	F	12	African American	18.5	GADA+IA2A+ mIAA+	N/A	13.3	2.2			x

Supplementary Figures

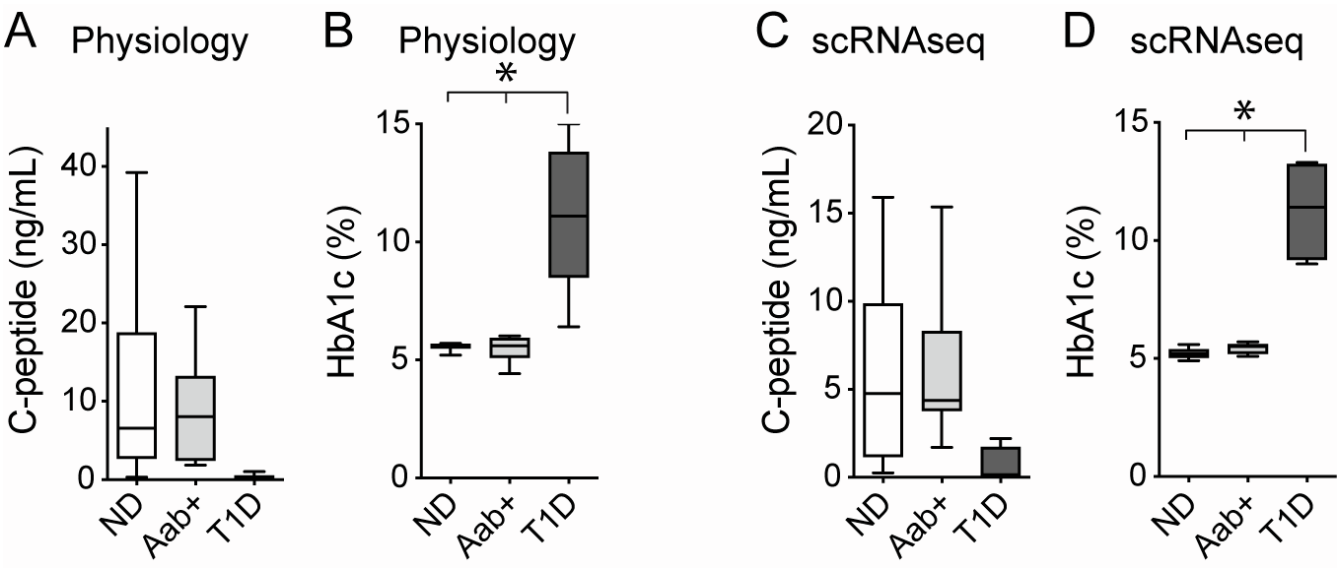

Figure S1. C-peptide and HbA1C levels of organ donors used in this study. (A,B) Plasma C-peptide (A) and HbA1C levels (B) of organ donors whose pancreas slices were used for physiology in this study (non-diabetic donors (ND), GADA+ donors (Aab+) and T1D donors). * indicates $p < 0.05$ when compared with Hba1C levels in ND or Aab+ donors (one-way ANOVA followed by Tukey's multiple comparisons test). (C,D) Plasma C-peptide (C) and HbA1C levels (D) of organ donors chosen from HPAP database to analyze changes in gene expression in pancreatic islet endocrine alpha and beta cells, endothelial and stellate cells.

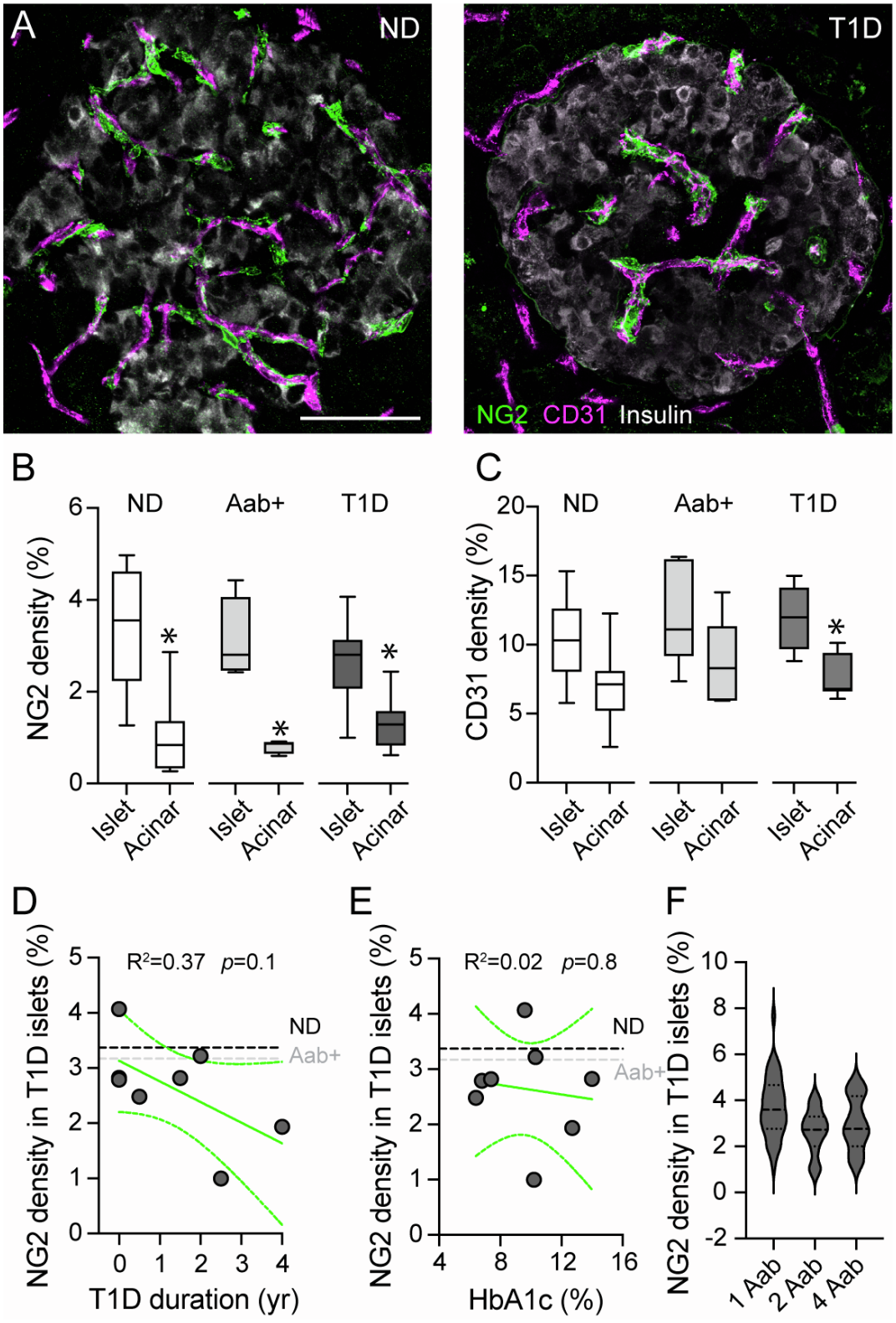

Figure S2. Changes in pericyte and endothelial cell densities in islets from Aab+ and T1D organ donors (related to Figure 1). (A) Maximal Projections of confocal images of islets

in fixed pancreatic slices from a non-diabetic (ND; nPOD6531) and a T1D donor (T1D duration 1.5y; nPOD6469) immunostained with antibodies against the endothelial cell marker CD31 (magenta), the pericyte marker neuron-glial antigen 2 (NG2; green) and insulin (gray). Note that in T1D islets several capillaries lack pericyte coverage. Scale bar = 50 μm. (B,C) Quantification of the % of tissue area (islet or acinar) immunostained with NG2 (B) or CD31 (C) in pancreas slices from non-diabetic (ND, n=9 donors), GADA+ (Aab+, n=7 donors) and T1D donors (T1D, n=8). For each donor, around 5-7 islets were imaged, and an average value was calculated. * indicates $p < 0.05$ when compared with islet densities (one-way ANOVA followed by Tukey's multiple comparisons test. (D,E) Correlation between the density of pericytes in T1D islets and the duration of T1D (D) or the HbA1C levels (E). For each donor, an average of 5-7 islets is shown. Linear regressions are not significant (*p*-values shown in graphs). 95% confidence intervals are shown in green. Dashed lines indicate average NG2 densities obtained for non-diabetic (ND; black) and Aab+ donors (gray). (F) Density of pericytes in islets from T1D donors depending on the number of circulating autoantibodies. Data represent individual islets (n=37 (1 Aab+), 12 (2 Aab+) and 20 (4 Aab+)).

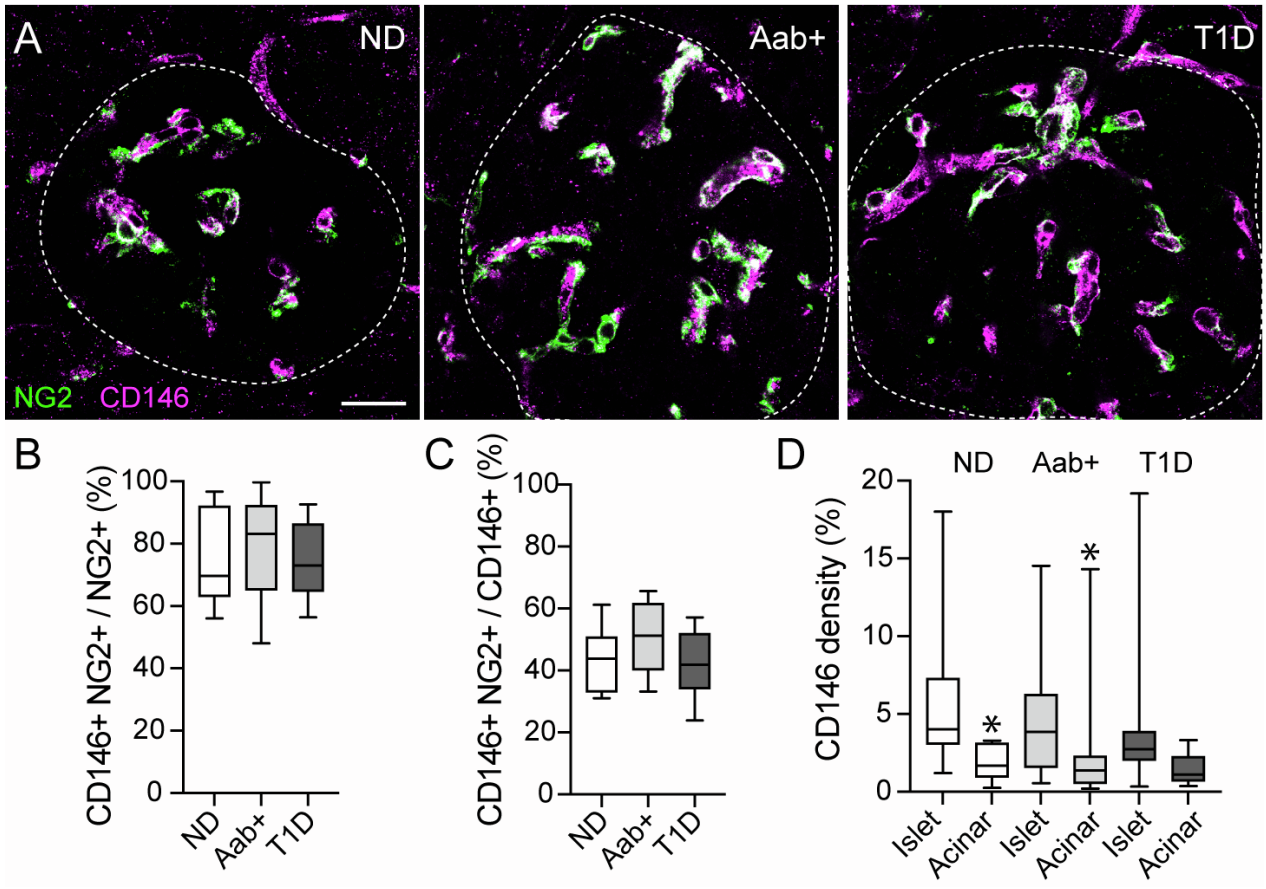

Figure S3. Colocalization between pericyte markers NG2 and CD146 at different stages of T1D (related to Figure 1). (A) Confocal images of islets in fixed pancreatic slices from a non-diabetic (ND; nPOD6590), a Aab+ donor (nPOD6573) and a T1D donor (T1D duration 0y; nPOD6563) immunostained with antibodies against pericyte markers NG2 (green) and CD146 (magenta). Scale bar = 20 μm. (B,C) Mander's correlation coefficients estimating colocalization between NG2 and CD146 in confocal images of islets from ND, Aab+ and T1D donors (n = 5-15 islets/3-5 donors each group). (D) Quantification of the % of tissue area (islet or acinar) immunostained with CD146 in pancreas slices from non-diabetic (ND, n=3 donors), GADA+ (Aab+, n=4 donors) and T1D donors (T1D, n=3). For each donor, around 5-15 islets were imaged, and an average value was calculated. * indicates $p < 0.05$ when compared with islet densities (one-way ANOVA followed by Tukey's multiple comparisons test.

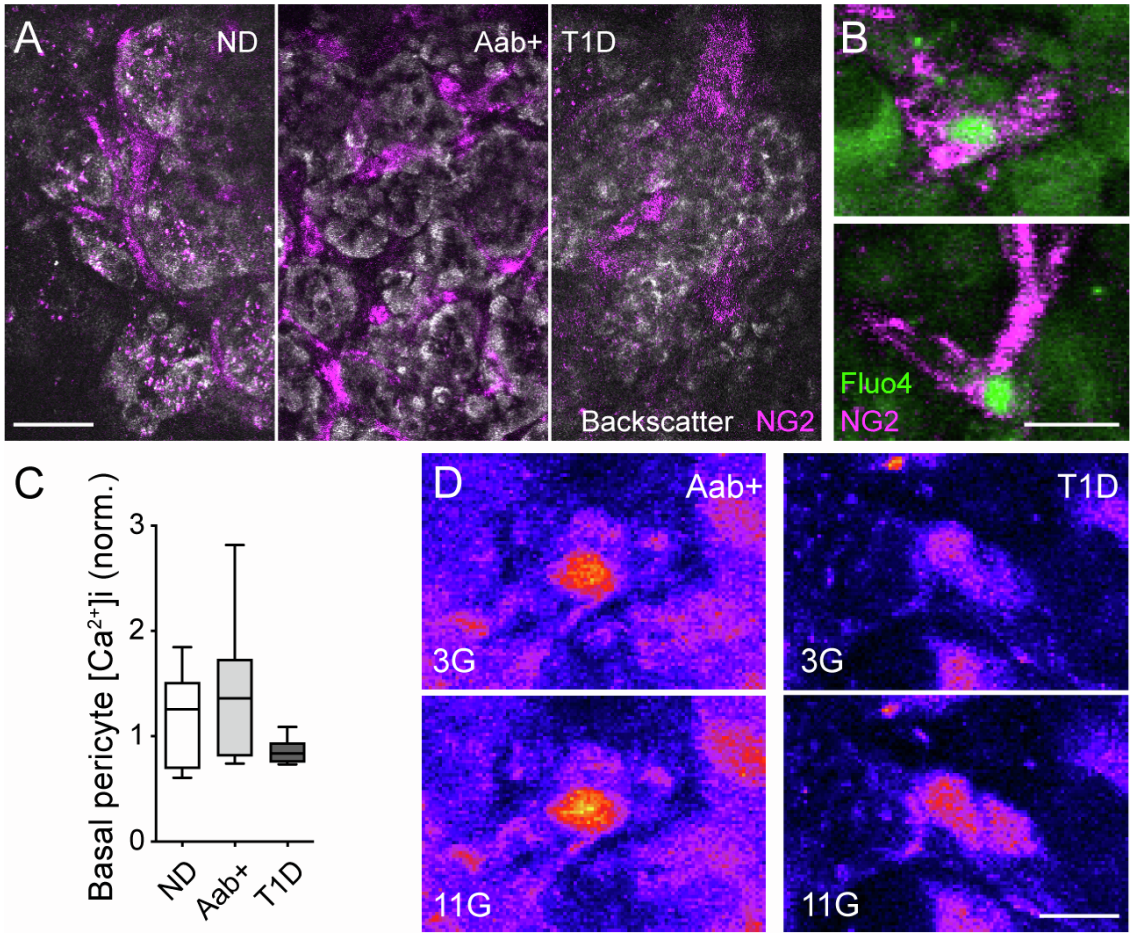

Figure S4. Using living pancreas slices to measure pericyte $[Ca^{2+}]i$ responses to high glucose (related to Figure 2). (A) Confocal images of islets (backscatter; gray) in living pancreas slices from a non-diabetic (ND; nPOD6546), a Aab+ (nPOD6538) and a T1D donor (T1D duration 2y; nPOD6566) showing NG2-alexa647-labeled pericytes (magenta). (B) Slices were loaded with the calcium indicator Fluo4 (green). Different cells incorporate the indicator but pericytes can be distinguished as they are labeled with NG2-alexa647. (C) Quantification of basal $[Ca^{2+}]_i$ levels in islet pericytes normalized to fluorescence levels in the whole islet (n = 9 ND donors; 6 Aab+ donors; 6 T1D donors). 8-12 pericytes were analyzed per islet, 3-5 islets per donor and an average per donor was calculated. (D) Confocal images of pericytes in islets from Aab+ or T1D donors in low glucose (3G) or upon high glucose stimulation (11G). These pericytes are not inhibited by high glucose application. Scale bars = 20 μm (A), 10 μm (B,C).

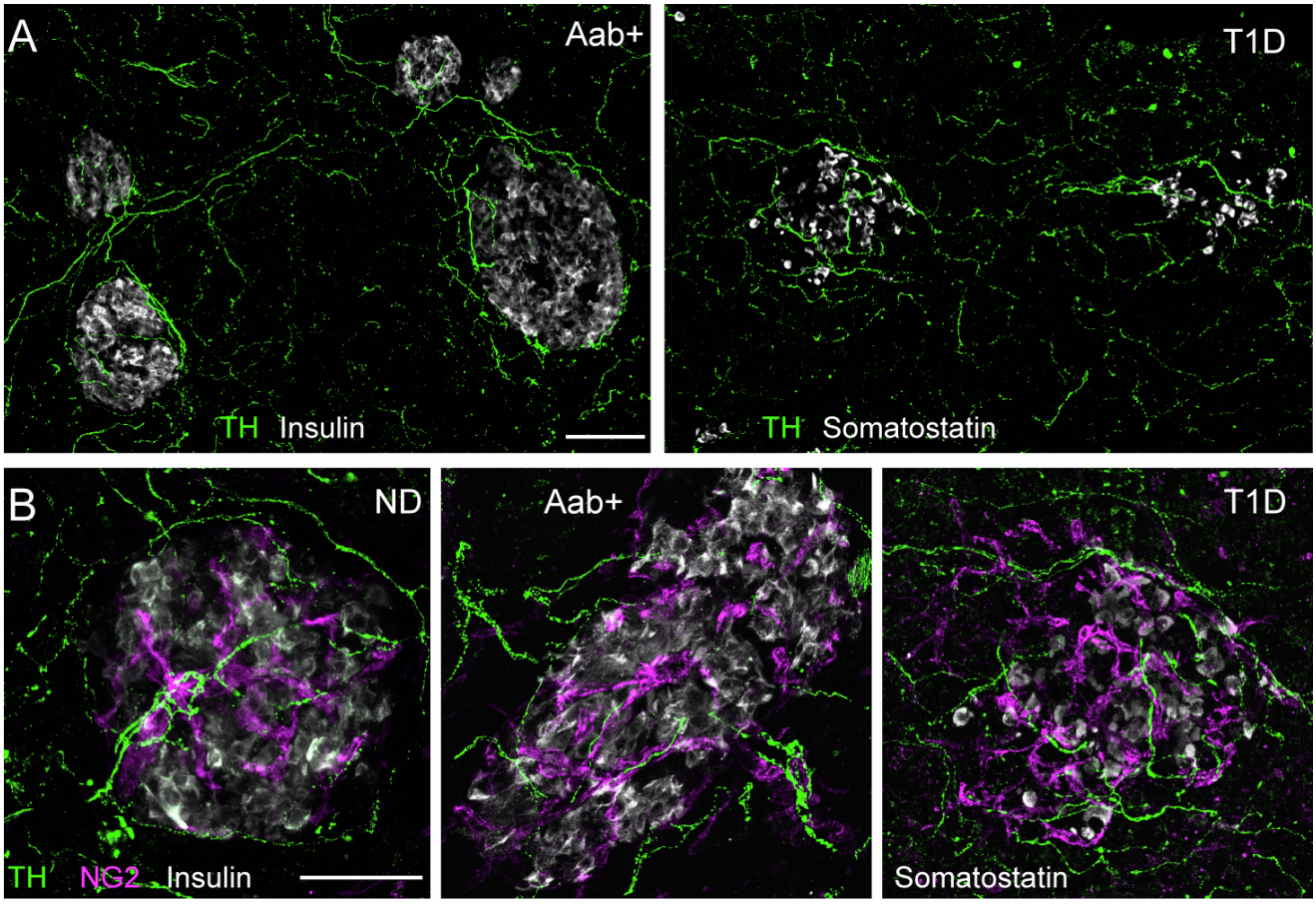

Figure S5. Sympathetic innervation patterns of islets at different stages of T1D (related to Figure 4). (A) Maximal projections of confocal images of fixed pancreas slices from an Aab+ donor (nPOD6573) and a T1D donor (T1D duration 2 years; nPOD6566) immunostained with the sympathetic nerve marker tyrosine hydroxylase (TH; green). Islets were identified with insulin or somatostatin immunostainings (gray). Sympathetic nerves are present in both endocrine and exocrine compartments of the pancreas. (B) Projections of confocal images of fixed pancreas slices from a ND (nPOD6546), an Aab+ donor (nPOD6573) and a T1D donor (nPOD6566) immunostained for TH (green), insulin/somatostatin (gray) and the pericyte marker NG2 (magenta). Sympathetic nerves reach the islet and contact a subset of islet pericytes (~20% of NG2-expressing pericytes) within the islet parenchyma. Scale bars = 50 μm (A,B).

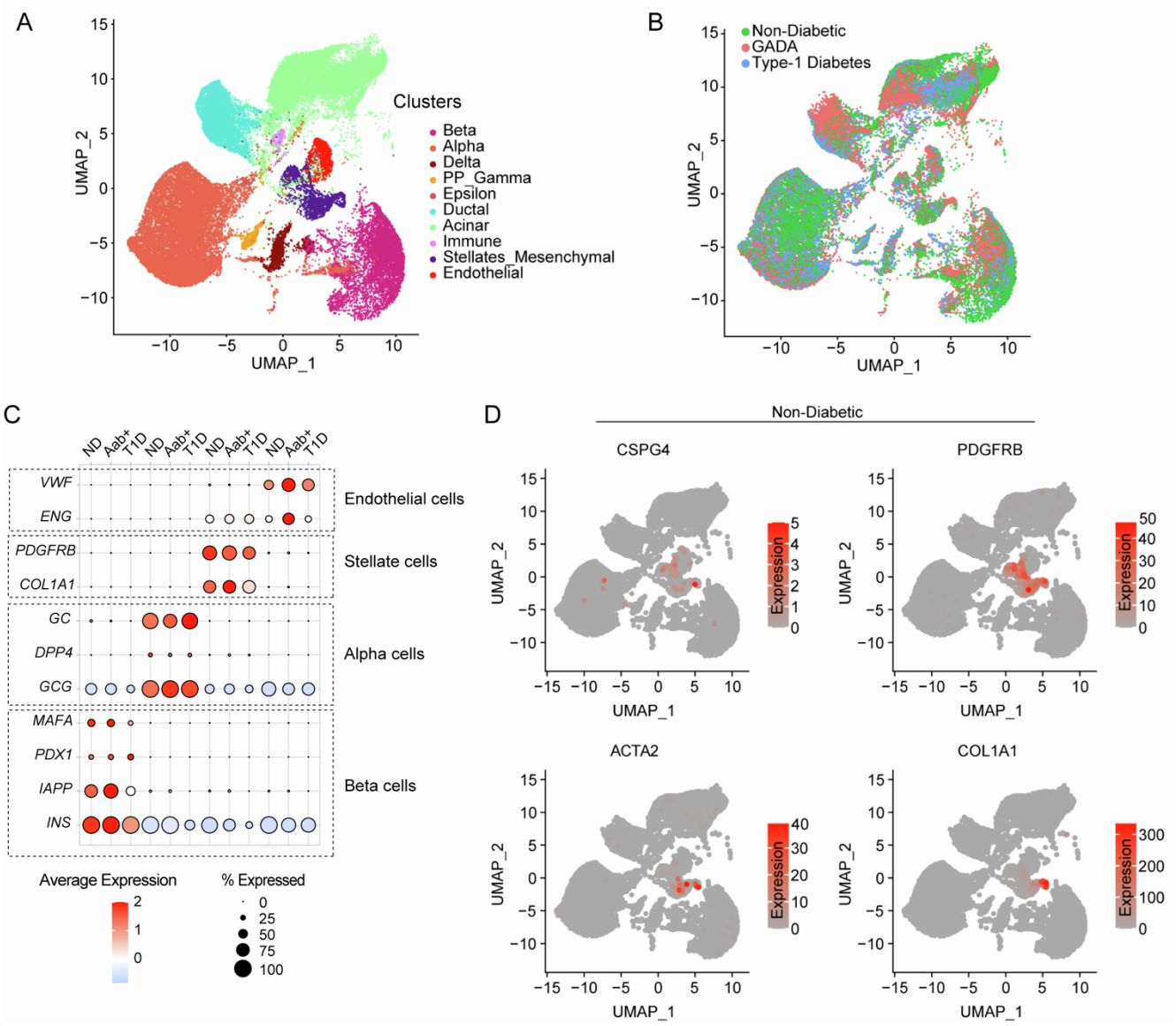

Figure S6. Identification of different cell populations in the human pancreatic islets by scRNAseq (related to Figure 6). (A) Uniform Manifold Approximation Projection (UMAP) of human pancreatic cells taken from the HPAP database and clustered based on cell type. Each dot represents the unique transcriptional profile of a single cell. (B) UMAP plot showing the cluster distribution of cells across three disease states: non-diabetic, single Aab+ (GADA+) and T1D. (C) Dotplot showing the expression of key genes specific to beta, alpha, stellate, and endothelial cell populations. Size of the circle denotes percentage of cells within a cluster expressing a gene and the intensity of the color denotes the magnitude of normalized average

expression. (D) UMAP plots of gene expression in non-diabetic donors for genes *CSPG4, PDGFRB, ACTA2* and *COL1A1*.

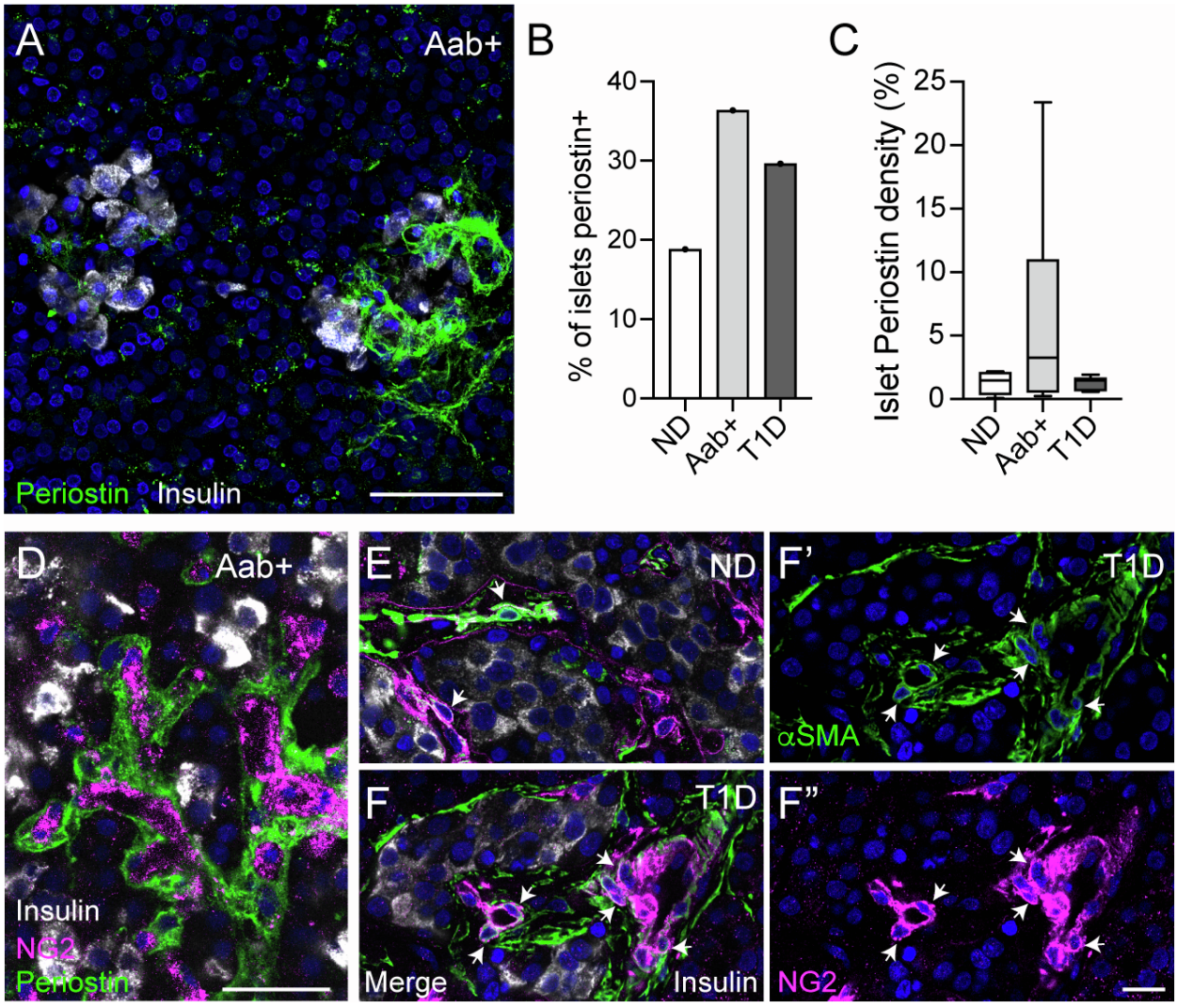

Figure S7. Expression of myofibroblast markers periostin and αSMA in islets at different stages of T1D (related to Figure 6). (A) Maximal projection of confocal images of islets in a fixed pancreatic slice from an Aab+ donor (nPOD6538) immunostained for insulin (gray) and the ECM and myofibroblast marker periostin (green). Periostin accumulates in spaces between beta cells but its presence in islets is heterogenous. (B) Quantification of the proportion of islets for each group of donors that contains periostin. (C) Quantification of the % of islet area immunostained with periostin in pancreas slices from non-diabetic, Aab+ and T1D donors (n = 6-16 islets/group). (D) Projection of confocal images of an islet in a fixed pancreas slice from an Aab+ donor (nPOD6538) immunostained for insulin (gray), periostin (green) and NG2

(magenta). (E,F-F”) Confocal images of regions within islets in sections from a ND donor (E) and a T1D donor (T1D duration 1.5 years; nPOD6469; F-F”) showing NG2-expressing cells (magenta) and αSMA (green). Beta cells are shown in gray (insulin). Note the increase in the proportion of mural cells that expresses both NG2 and αSMA (white arrows). Scale bars = 50 μm (A), 20 μm (D) and 10 μm (E,F-F”).