SUPPLEMENTARY DATA

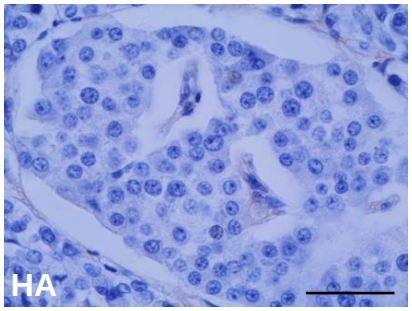
Supplementary Table 1. Clinical characteristics of tissue donors.

Case ID	Diabetes duration (years)	Age (years)	Gender	C-peptide (ng/ml)	BMI (units)	Autoantibodies
T1D						
H1204	onset	29	Male	NA	24.2	GADA+
H911	onset	40	Male	NA	27.2	NA
6209	0.3	5	Female	0.1	NA	IA-2A+ mIAA+ ZnT8+
6052	1	12	Male	0.2	20	IA-2A+ mIAA+
6113	1	13	Female	<0.05	25	mIAA+
6063	3	4	Male	<0.05	24	mIAA+
6084	4	14	Male	<0.05	26	mIAA+
6087	4	18	Male	<0.05	22	ZnT8+ mIAA+ GADA+ IA-2A+
6088	5	31	Male	<0.05	27	ZnT8A+ mIAA+
6062	6	11	Male	NA	22	NA
6079	6	11	Female	<0.05	19	Negative
6070	7	23	Female	<0.05	22	IA-2A+ mIAA+
6069	7	23	Male	NA	29	No serum available
6089	8	14	Male	<0.05	26	mIAA+
6064	9	20	Female	<0.05	23	GADA+ IA-2A+ ZnT8+ GADA+ IA-2A+ ZnT8+
6141	28	37	Male	<0.05	26	mIAA+
6155	43	50	Female	<0.05	26	mIAA+
6042	59	60	Female	<0.05	23	GAD+ mIAA+
6074	66	73	Female	0.2	20	GADA+
Mean ± SD		**26 ± 19**		**0.1 ± 0.1**	**24 ± 3**	
healthy						
6103		1.5	Male	6.1	17	negative
6107		2	Male	5.2	16	negative
6106		3	Male	7.4	18	negative
6005		5	Female	NA	16	negative
6047		8	Male	0.7	24	negative
6137		9	Female	12.1	24	negative
6153		15	Male	8.4	21	negative
6075		16	Male	2.9	15	negative
6096		16	Female	2.9	NA	negative
6098		18	Male	1.4	23	negative
6160		22	Male	0.4	24	negative
6058		27	Male	9.1	19	negative
6055		27	Male	0.6	23	negative
6129		43	Female	0.5	23	negative
6008		50	Female	NA	24	negative
6013		65	Male	2.8	24	negative
6021		72	Female	22.9	25	negative
Mean ± SD		**23 ± 22**		**6 ± 6**	**21 ± 4**	

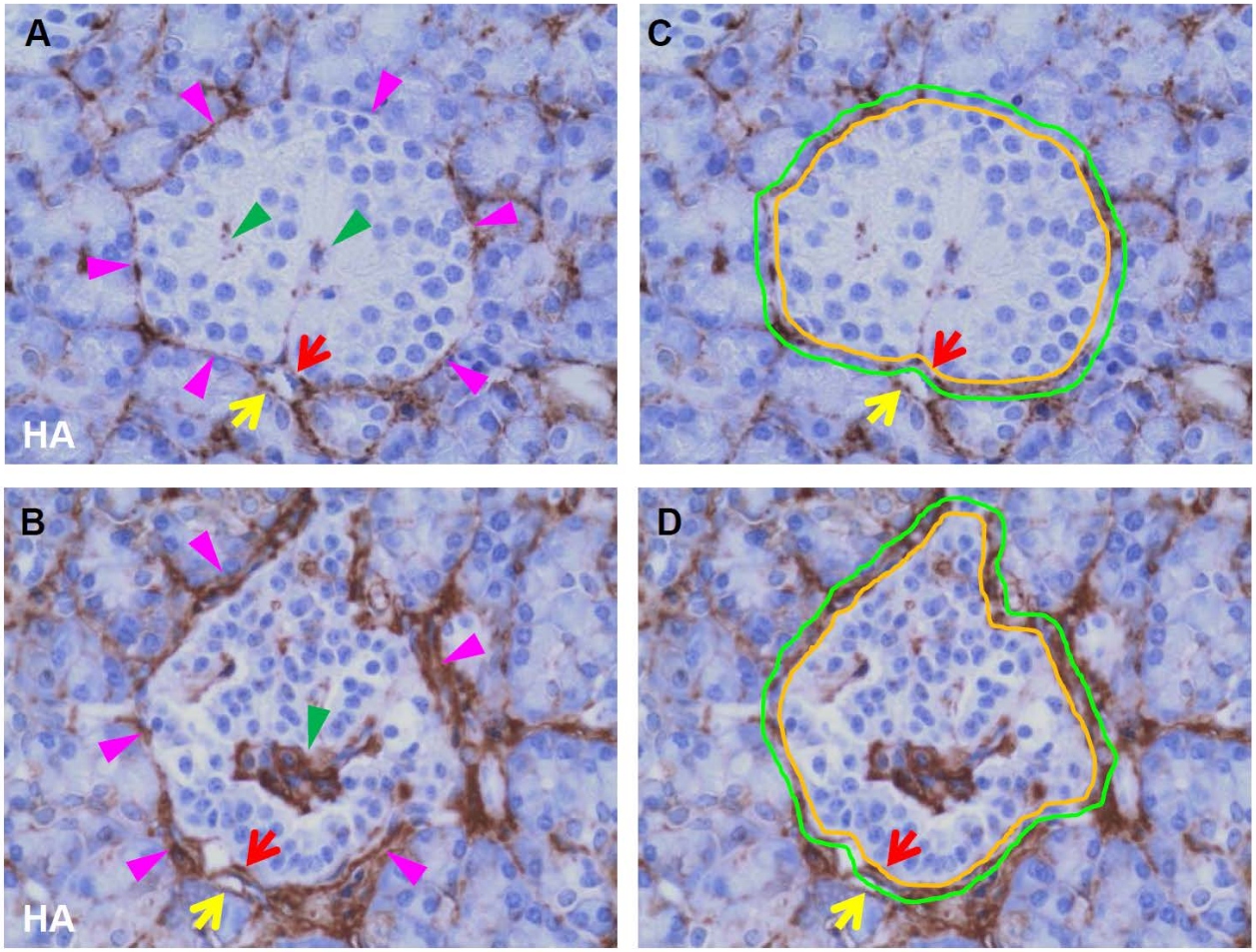
NA, not available

SUPPLEMENTARY DATA

Supplementary Figure 1. related to *Histochemistry* in Research Design and Methods. Treatment of the pancreatic sections with hyaluronidase before applying the b-HABP probe abolishes hyaluronan staining. Scale bar, 50 µm. HA, hyaluronan.

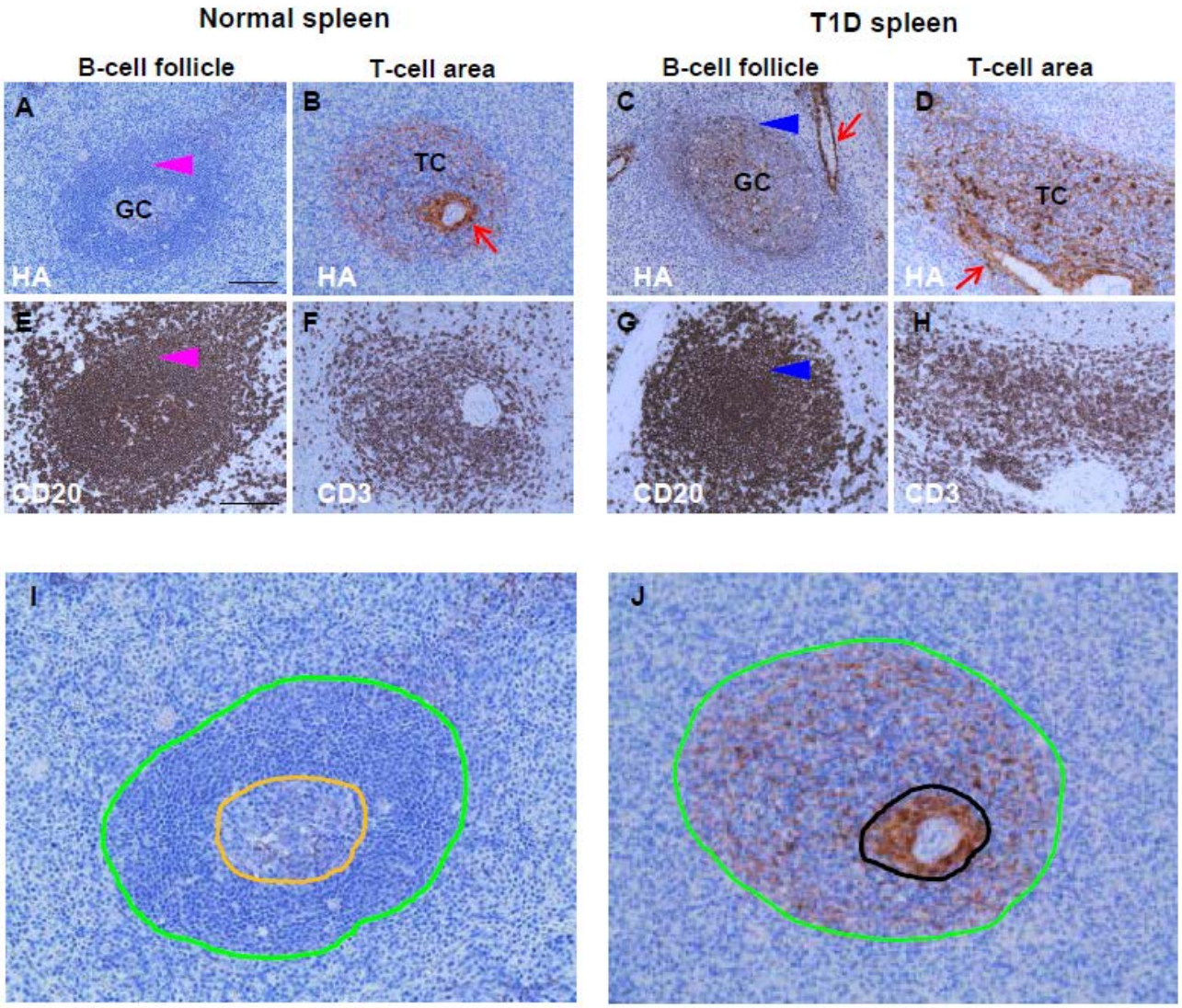


Supplementary Figure 2. related to *Morphometric analysis and quantitation* in Research Design and Methods. Schematic representation of the intra- and peri-islet stained areas. Hyaluronan (HA) staining of a normal (*A*) and a diabetic (*B*) islet shows HA located extracellularly juxtaposed to peri-islet (magenta arrowheads) and intra-islet (green arrowheads) microvessels. The arrows point to the endocrine (red arrow) and exocrine (yellow arrow) sides of a peri-islet capillary, respectively. (*C* and *D*), Demarcation of the intra- and peri-islet areas. The orange line delineates the endocrine side of the peri-islet capillaries. The green line delineates the outer border of the peri-islet area. The intra-islet stained area is the brown area within the orange line. The peri-islet stained area is the brown area located between the orange and green lines.



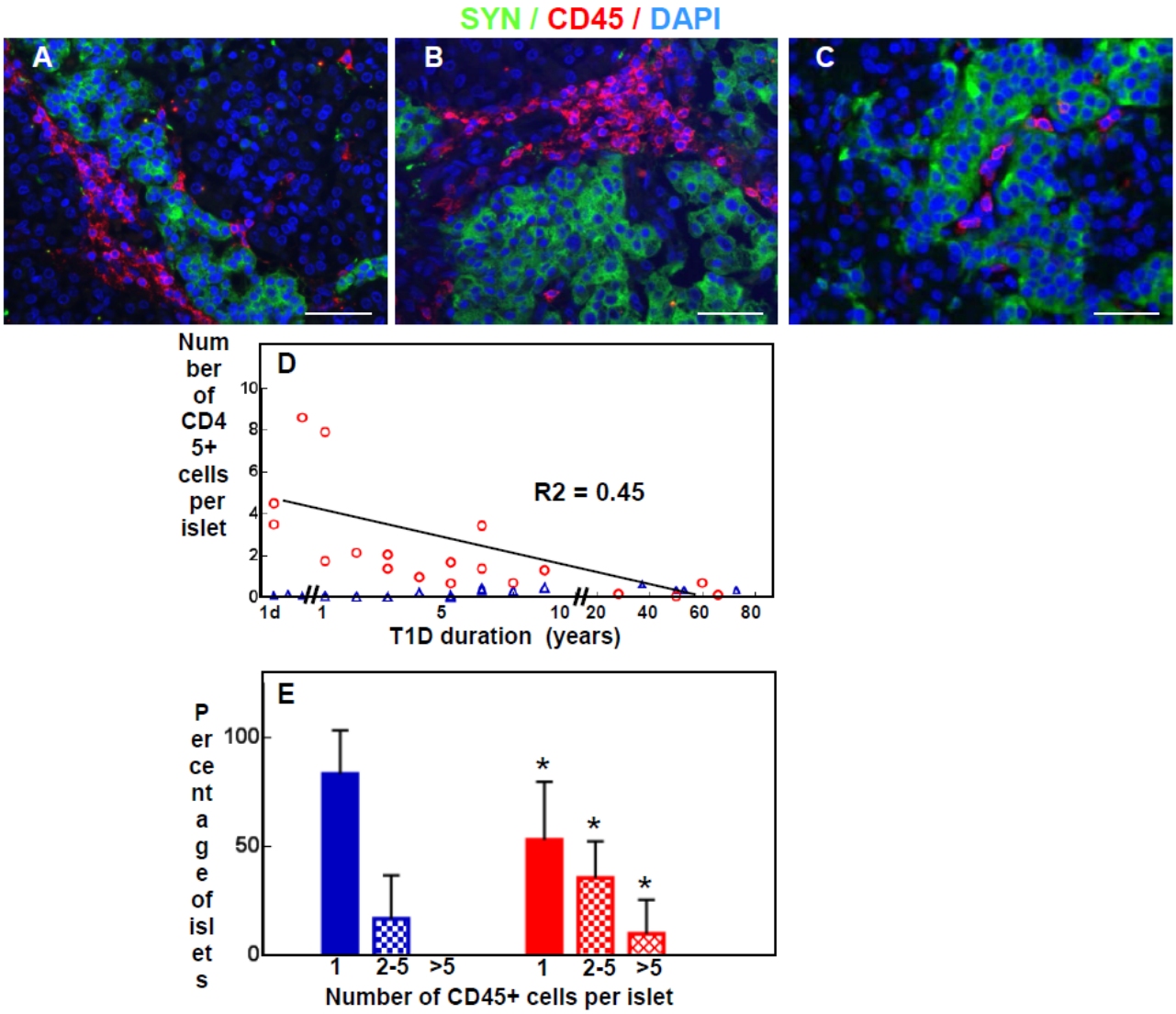
SUPPLEMENTARY DATA

Supplementary Figure 3. related to *Morphometric analysis and quantitation* and *Spleen and pancreatic lymph node*, in Research Design and Methods. (*A-D*), Histochemistry for hyaluronan (HA) in normal (*A* and *B*) and diabetic (*C* and *D*) spleen tissue sections. (*E-H*), Immunohistochemistry of adjacent spleen sections for CD20 (*E* and *G*) or CD3 (*F* and *H*). Magenta and blue arrowheads point to follicular germinal centers (GC). Red arrows in *B-D* point to splenic vessels. Scale bars, 200µm. TC, T-cell area. (*I* and *J*), Schematic representation of follicular B-cell and T-cell stained areas. (*I*), Delineation of the B-cell follicle (green line) and GC (orange line) borders. (*J*), Delineation of the T-cell area (green line). The HA-positive vessels (delineated with a black line), when present, were excluded from the measurements.



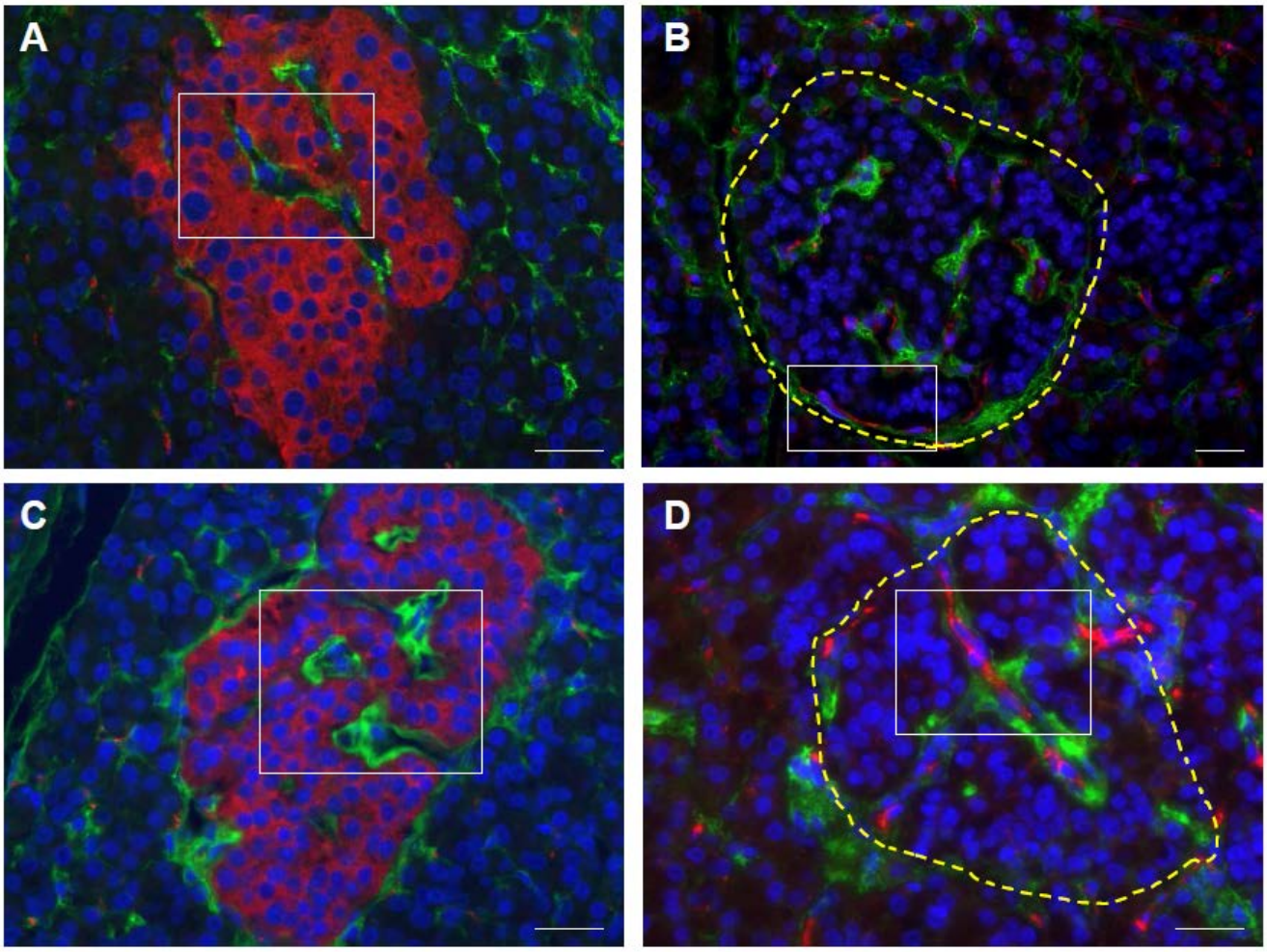
SUPPLEMENTARY DATA

Supplementary Figure 4. related to *Evaluation of insulitis* in Research Design and Methods and Figure 4. (*A-C*), Immunohistochemistry for synaptophysin (green) and CD45 (red) shows diabetic islets with insulitis. Images were prepared from nPOD cases 6209 (*A*), 6052 (*B*), and 6084 (*C*). Scale bar, 50µm. (*D*), The number of CD45-positive inflammatory cells in close contact with synaptophysin-positive endocrine cells plotted as a function of T1D duration. Red circles, diabetic islets; each blue triangle represents an age-matched control tissue. Spearman correlation coefficient is shown. (*E*), Proportion of islets containing the indicated number of CD45-positive cells in close contact with synaptophysin-positive endocrine cells. Blue bars, normal islets; red bars, diabetic islets. Asterisks indicate significance vs. normal tissues, $P < 0.0001$. Data represent (*D*) mean and (*E*) mean ± SD of the measurements obtained examining 2030 normal and 930 T1D islets of 17 healthy and 19 T1D donors.



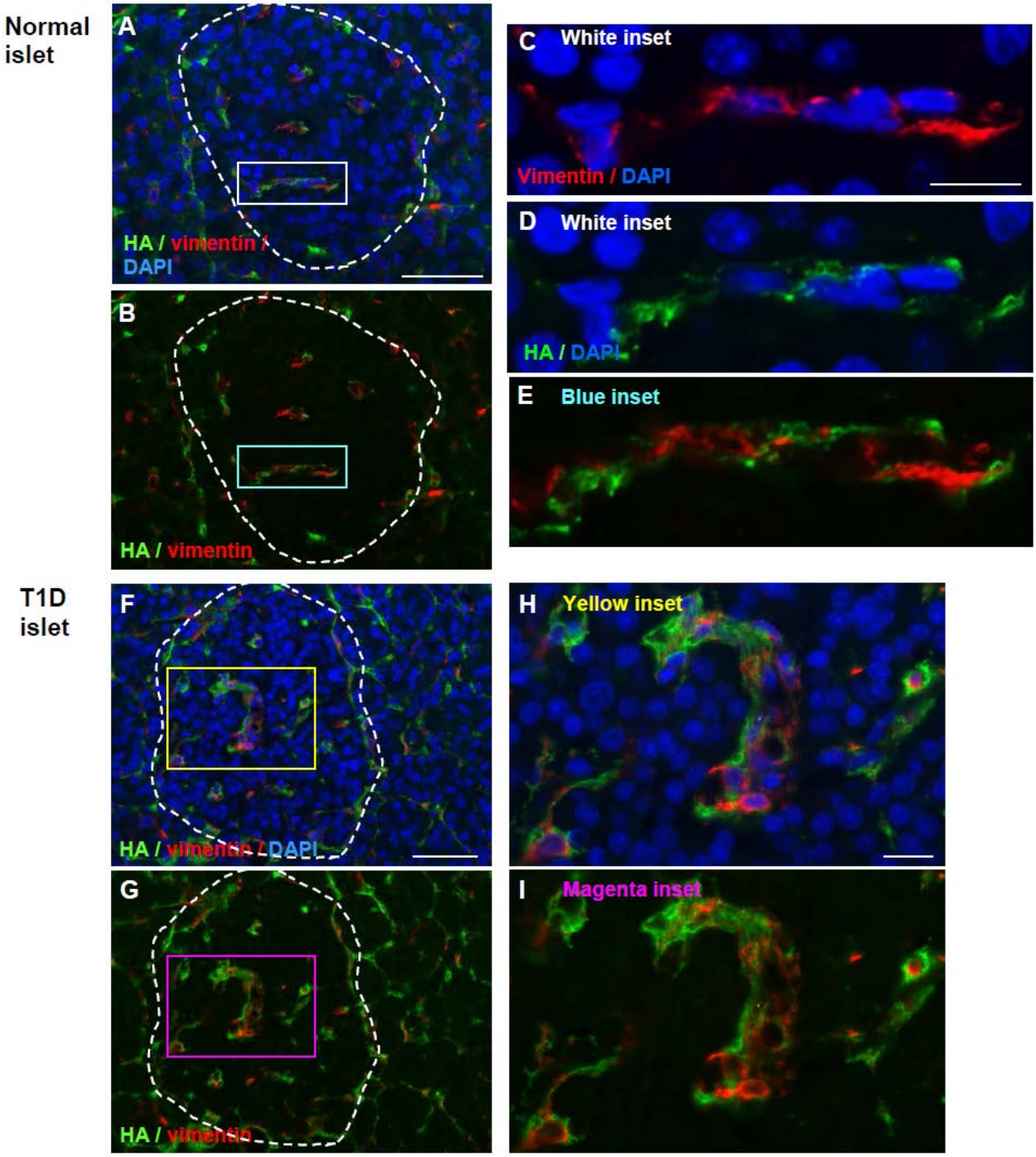
SUPPLEMENTARY DATA

Supplementary Figure 5. related to Figure 1. HA accumulation along the microvasculature of human islets in T1D. (*A*-*B*), Images of normal human islets prepared from nPOD case 6153. (*A*), Co-labeling of HA (green) with the pan-endocrine marker synaptophysin (red) shows HA outside the endocrine cells. (*B*), Co-labeling of HA (green) with the endothelial cell marker CD31 (red) reveals HA juxtaposed to the islet microvessels. (*C*-*D*), Images of human diabetic islets prepared from nPOD case 6052. (*C*), Co-labeling of HA (green) with the pan-endocrine marker synaptophysin (red) reveals abundant HA located outside the endocrine cells. (*D*), Co-labeling of HA (green) with the endothelial cell marker CD31 (red) shows HA accumulated along the islet microvessels. Higher magnification of the boxed areas in *A*, *B*, *C*, and *D* are shown in Figure 1 *C*, *D*, *G*, and *H*, respectively. Yellow lines delineate the islet border. Scale bars, 25 µm.



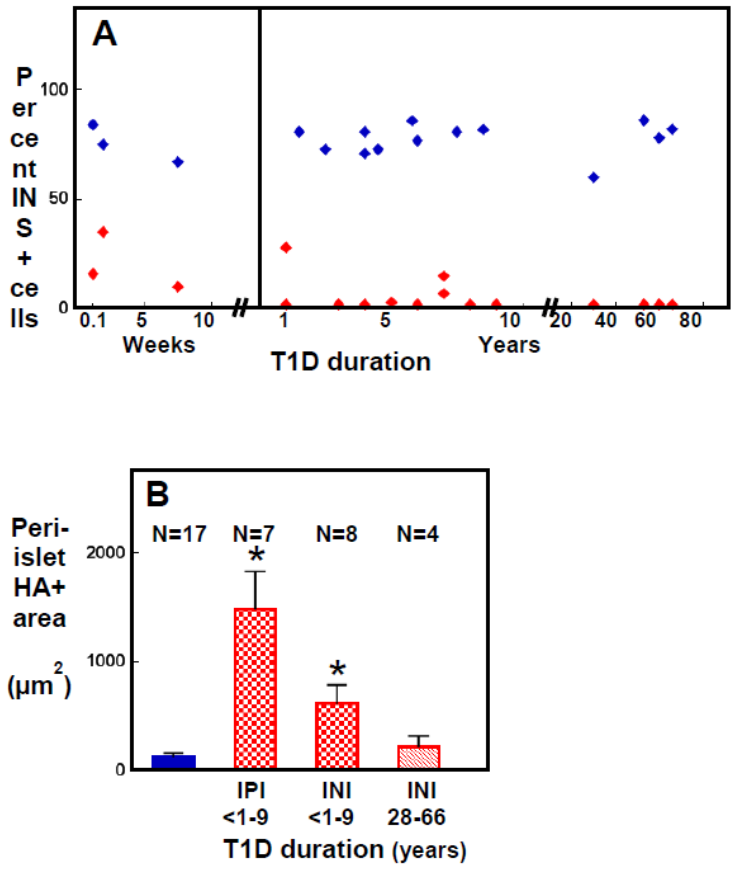
SUPPLEMENTARY DATA

Supplementary Figure 6. related to Figure 1. Co-labeling hyaluronan (HA, green) with vimentin (red) shows HA juxtaposed to microvessels in both normal (*A*-*E*) and diabetic (*F*-*I*) islets. Islets are delineated by white dashed lines. (*B* and *G*), 2-color images of the islets shown in (*A* and *F*), respectively. Higher magnification of the tissue areas within the insets in (*A* and *B*) and (*F* and *G*) is shown in (*C*-*E*) and (*H* and *I*), respectively. The cell nuclei are visualized with DAPI (blue). Images were prepared from nPOD cases 6153 (healthy) and 6064 (T1D). Scale bars, 50µm (*A*, *B*, *F*, *G*) and 20µm (*C*-*E*, *H*, *I*).



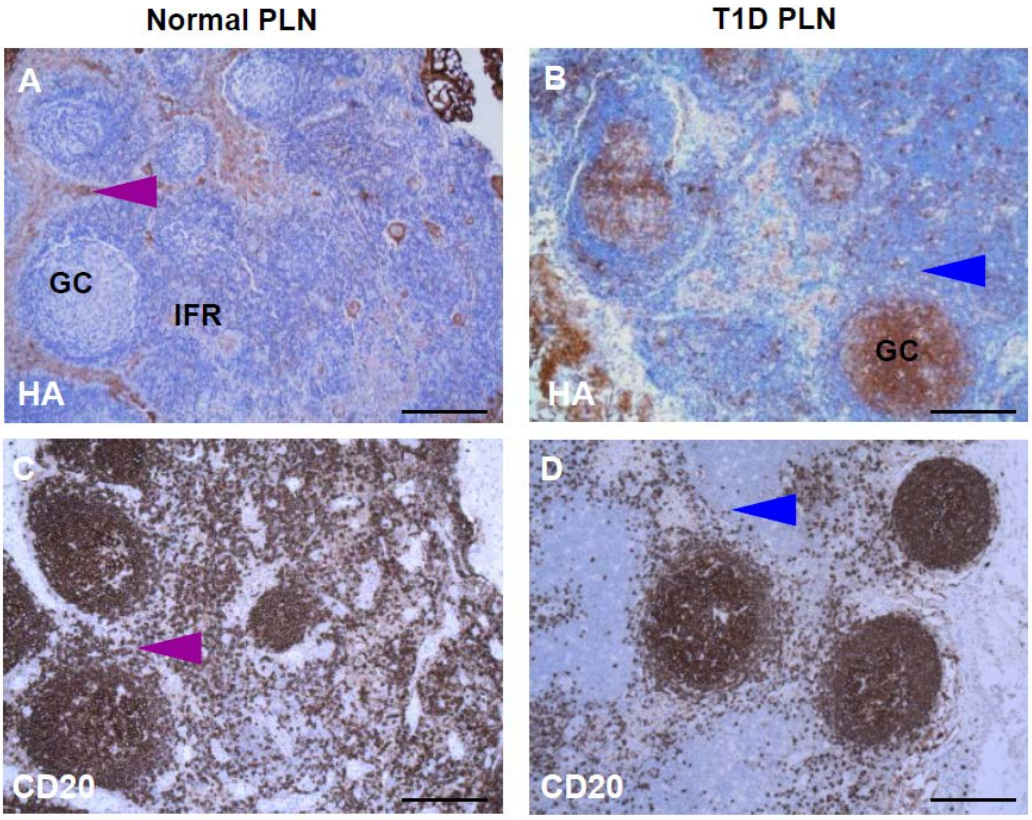
SUPPLEMENTARY DATA

Supplementary Figure 7. related to Figure 3. (*A*), Quantitative analysis of the relative proportion of insulin-positive cells in human pancreas. Red diamonds represent diabetic tissues; blue diamonds, age-matched normal tissues. Data represent mean values of measurements obtained for each individual donor. (*B*), Quantitative analysis of the peri-islet HA-positive area. Blue bar represents normal tissues; red bars, diabetic tissues. Data are mean ± SD of measurements obtained from tissues of 17 healthy and 19 T1D donors. The long-standing diabetic tissues did not contain insulin-positive cells. Asterisks indicate significance vs. normal tissues, $P < 0.0001$. There was no significant difference in peri-islet HA-positive area between the long-standing diabetic tissues and the age-matched normal tissues, $P = 0.14$. IPI, insulin-positive islets; INI, insulin-negative islets.



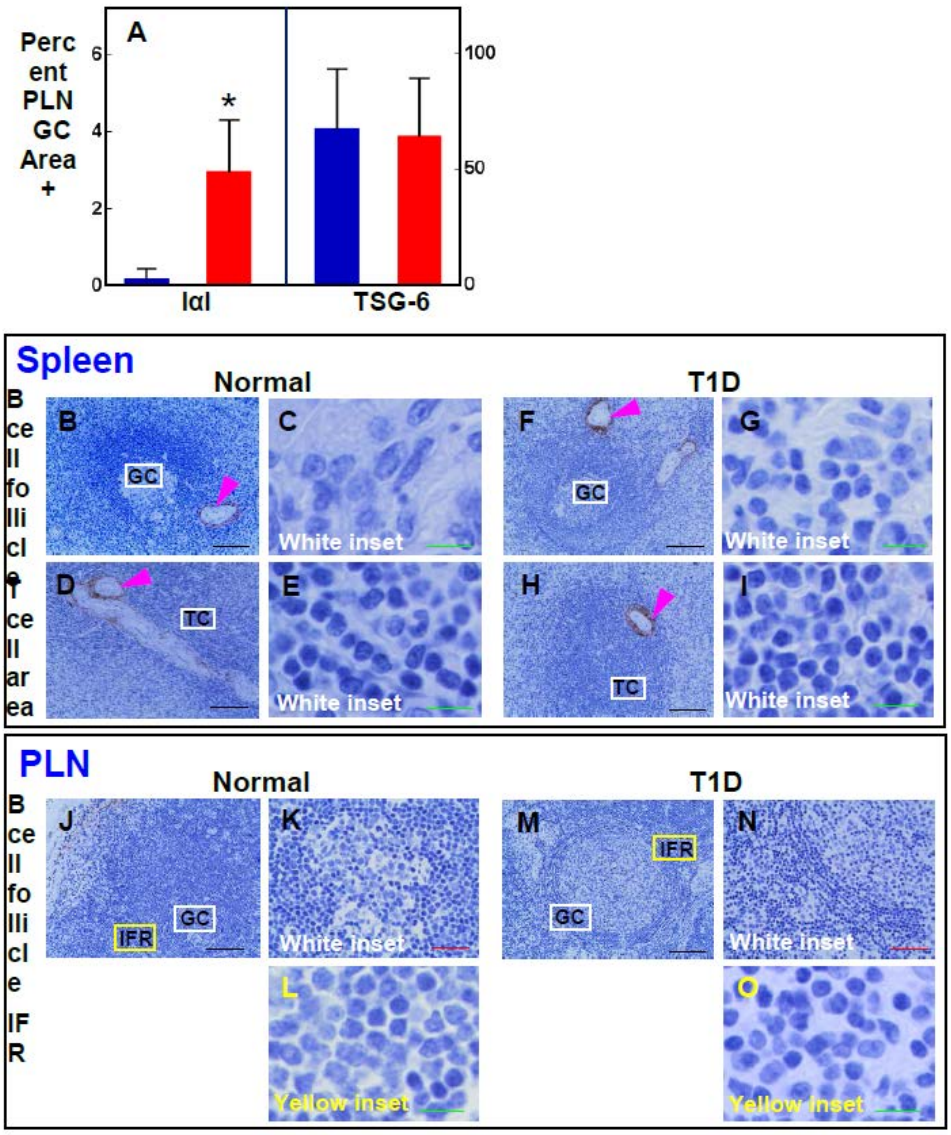
SUPPLEMENTARY DATA

Supplementary Figure 8. related to Figure 6. Hyaluronan accumulates in pancreatic lymph nodes (PLN) in T1D. *(A* and *B*), Hyaluronan (HA) staining in normal (*A*) and diabetic (*B*) PLN tissue sections. (*C* and *D*), Immunohistochemistry of adjacent PLN sections for CD20. Magenta arrowheads indicate a normal PLN B-cell follicle stained for HA in (*A*) and CD20 in (*C*). Blue arrowheads indicate a diabetic PLN B-cell follicle stained for HA in (*B*) and for CD20 in (*D*). Images were prepared from nPOD cases 6117 (*A* and *C*) and 6052 (*B* and *D)*. GC, germinal center. Scale bar, 200µm.



Supplementary Figure 9. related to Figure 7. (*A*), Quantitative analysis of IαI-positive and TSG-6-positive areas in pancreatic lymph node (PLN) follicular germinal centers (GC). Blue bars, normal tissues; red bars, diabetic tissues. The asterisk indicates significance vs. normal tissues; $P = 0.0012$. TSG-6-positive tissue areas in normal and diabetic tissues were comparable. $P = 0.9090$. (*B-O*), Versican immunostaining (brown) in normal and diabetic spleen and PLN. For the spleen, representative images of versican staining in normal and diabetic tissues are shown in (*B-E*) and (*F-I*), respectively. Higher magnification of the boxed areas within the follicular GCs in (*B*) and (*F*) is shown in (*C*) and (*G*), respectively. Higher magnification of the boxed areas within the T-cell areas (TC) in (*D*) and (*H*) is shown in (*E*) and (*I*), respectively. Magenta arrowheads point to splenic vessels. For the PLN, representative images of versican staining in normal and diabetic tissues are shown in (*J-L*) and (*M-O*), respectively. Higher magnification of the tissue areas within the white insets in the follicular GCs in (*J*) and (*M*) is shown in (*K*) and (*N*), respectively. Higher magnification of the tissue areas within the yellow insets in interfollicular region (IFR) in (*J*) and (*M*) is shown in (*L*) and (*O*), respectively. Scale bars, 100µm (black), 50µm (red), 10µm (green).

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 10. Quantification of the relative HA-positive tissue area in the indicated tissues. *P* = 0.6 (*A*), 0.4 (*B*) and 0.3 (*C*). Blue bars represent normal tissues; red bars, diabetic tissues. Data are mean ± SD of measurements obtained from tissues of 17 healthy and 19 T1D donors (*A*), 4 healthy and 3 T1D donors (*B*) and 7 healthy and 7 T1D donors (*C*) .