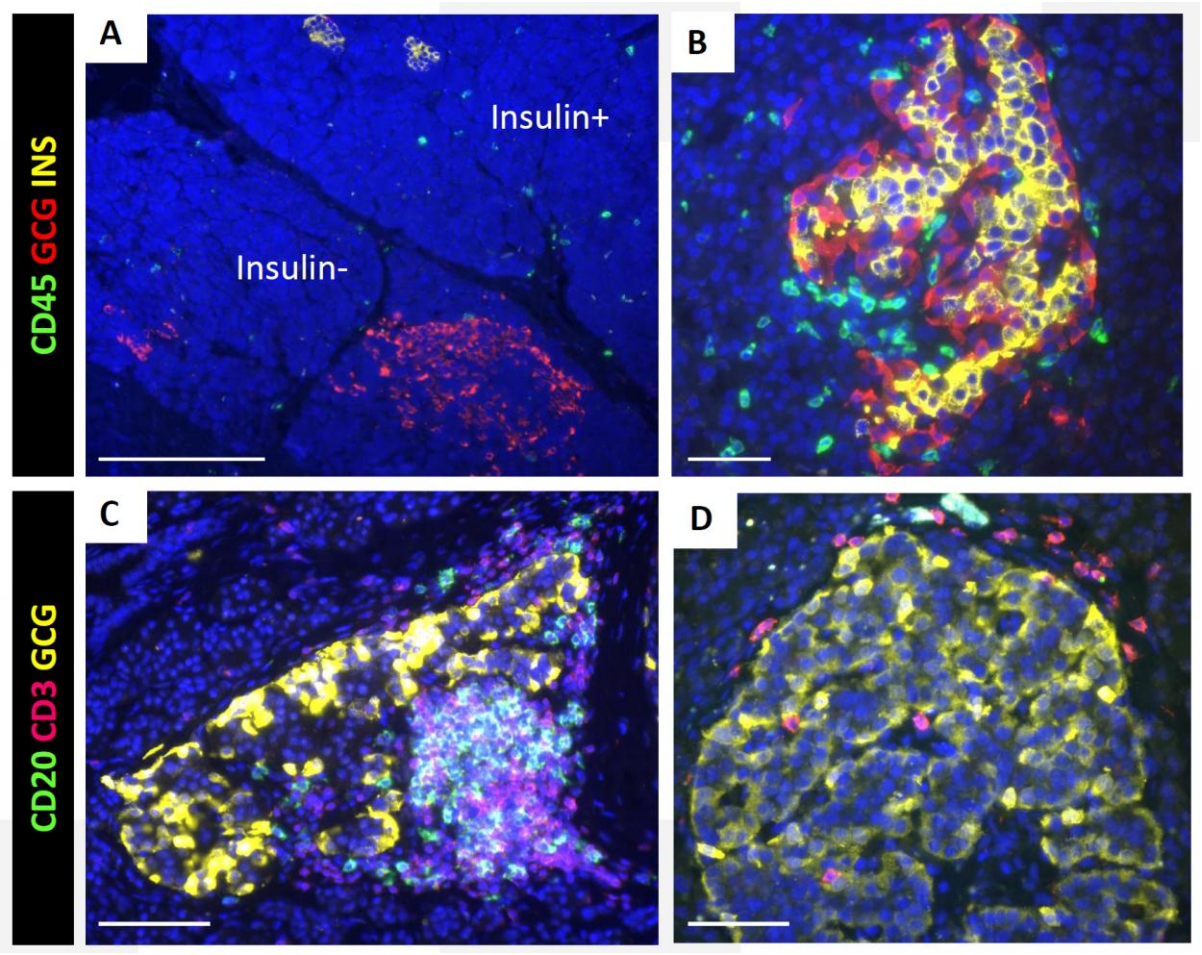
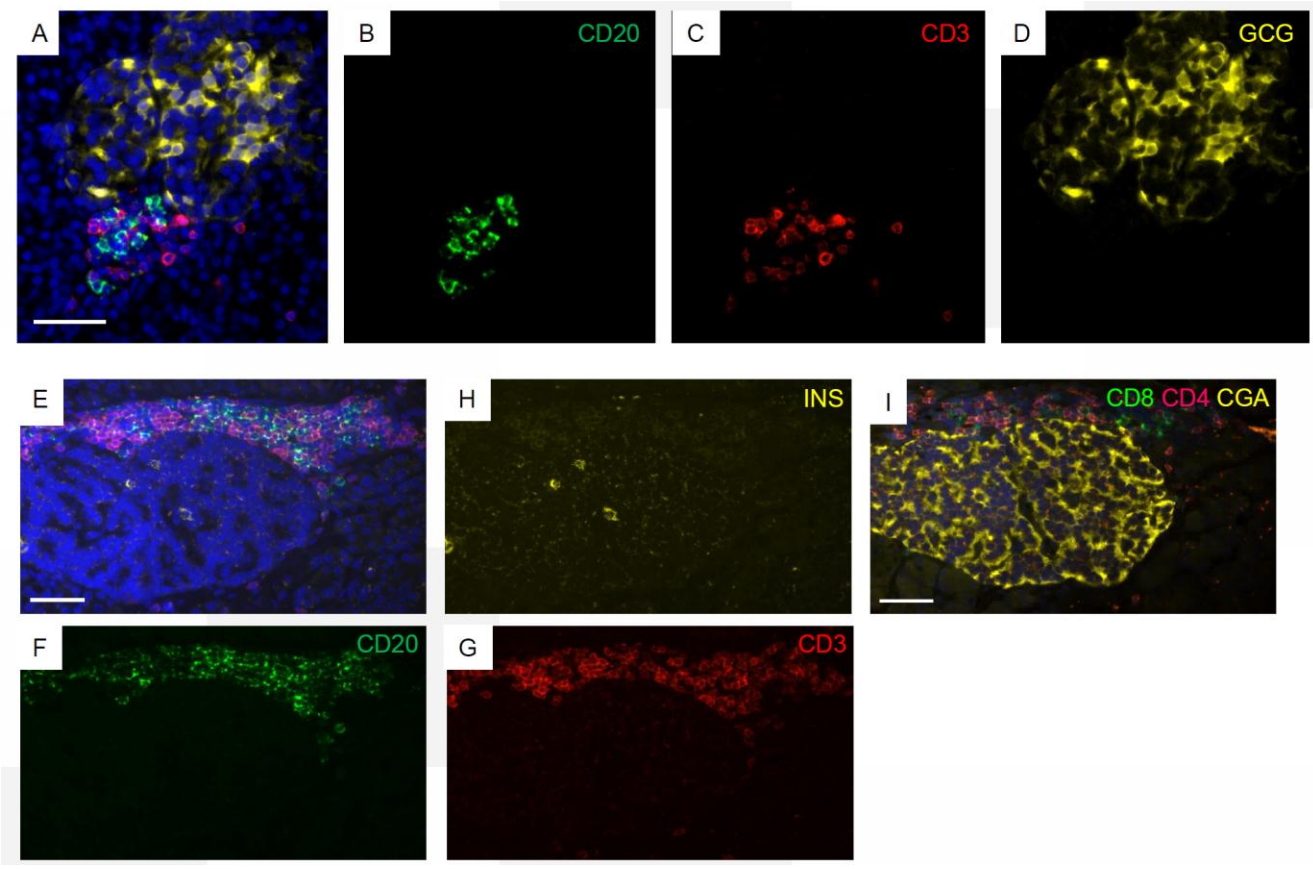
SUPPLEMENTARY DATA

Supplementary Figure 1. - Insulitic islets in AAb+ donors without diabetes. Islets were imaged from a 22-year-old donor (6197; *A-B*) and a 23-year-old donor (6267; *C-D*). Cells in the insulitic islets were immunotyped using multiple immunofluorescence as described in Methods. Islet endocrine cells were identified using glucagon (GCG) and insulin (INS) stains. Heterogeneous distribution of Ins+ and Ins- islets was observed between lobules (*A*). An insulitic islet is shown with diffuse infiltration by CD45+ cells (*B*). A large insulitic aggregate is shown composed of similar numbers of CD20+ and CD3+ lymphocytes (*C*). Another large islet with fewer CD20+ and CD3+ cells demonstrates the wide variability between leukocyte numbers/islet within a given donor (*D*). Scale bars: 200μm *A*, 50 μm *B*, *D*, 100μm *C*.



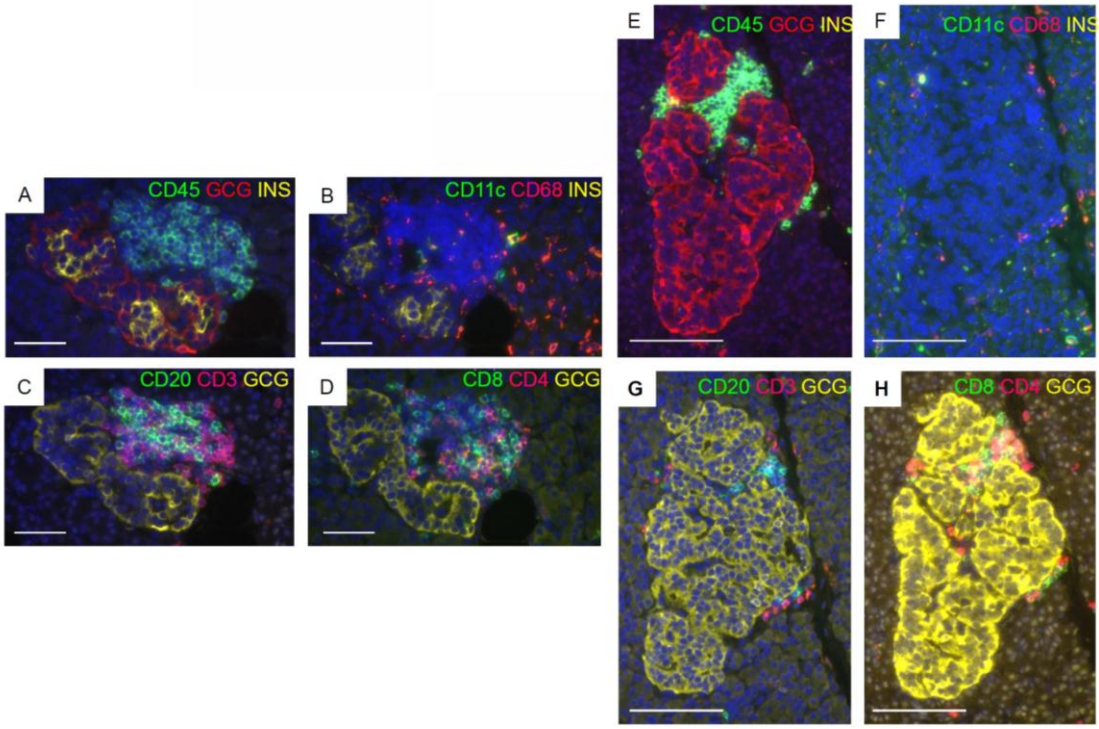
SUPPLEMENTARY DATA

Supplementary Figure 2. B and T lymphocytes in insulitic islets from donors with longstanding T1D. Focal aggregates in islets were imaged from a 12-year-old donor with T1D for 3 years (6268) (*A-D*) and a 22.6-year-old patient with T1D for 7 years (6070) (*E-I*). Sections were stained for CD20 (*B, F*), CD3 (*C, G*), CD8 and CD4 (*I*), glucagon (GCG, *D*), insulin (INS, H), or chromogranin A (CGA, *I*) as described in Methods. Both CD20+ and CD3+ lymphocytes were found in insulitic aggregates though in varying numbers. DAPI-stained DNA to delineate nuclei is shown in merged images (*A, E, I*). Scale bars: 50μm.



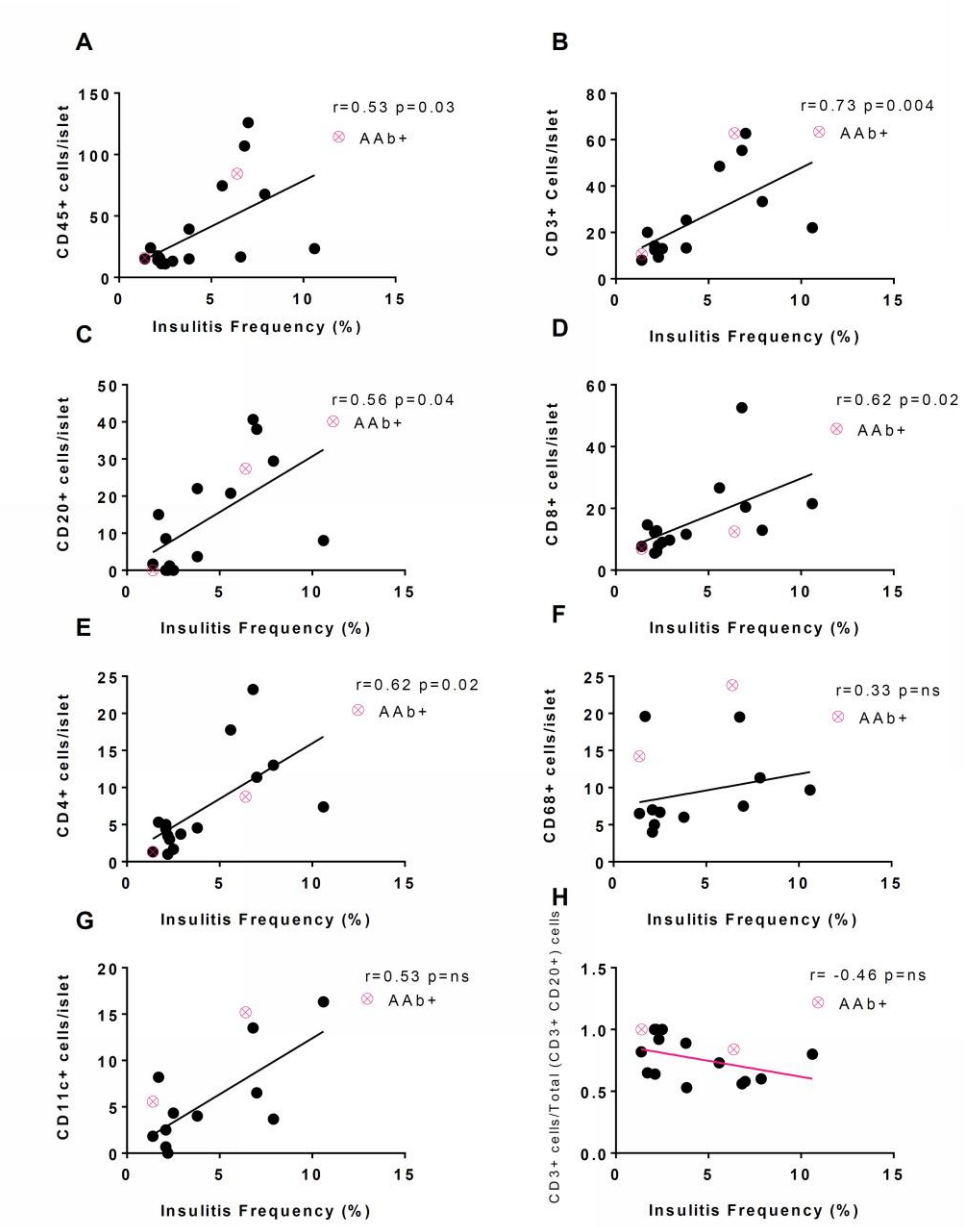
SUPPLEMENTARY DATA

Supplementary Figure 3. Leukocytes in insulitic islets from insulin+ and insulin- islets. Two islets in a pancreas section were imaged from a 13-year-old donor with T1D for 5 years (6243) (insulin+ islet (*A-D*); insulin- islet (*E-H*)). Serial sections were stained as described in Methods with endocrine cells identified using glucagon (GCG) and insulin (INS) stains. Aggregates in both islets were comprised of both CD20+ and CD3+ cells (*C, G*) as well as CD8+ and CD4+ T cells (*D, H*). Dendritic cells (CD11c+) and CD68+ macrophages were observed adjacent to mononuclear aggregates and endocrine cells (*B, F*). A large number of macrophages (CD68) are also seen in the adjacent exocrine region in panel *B*. Scale bars: *A-D* 50μm; *E-H 100*μm.



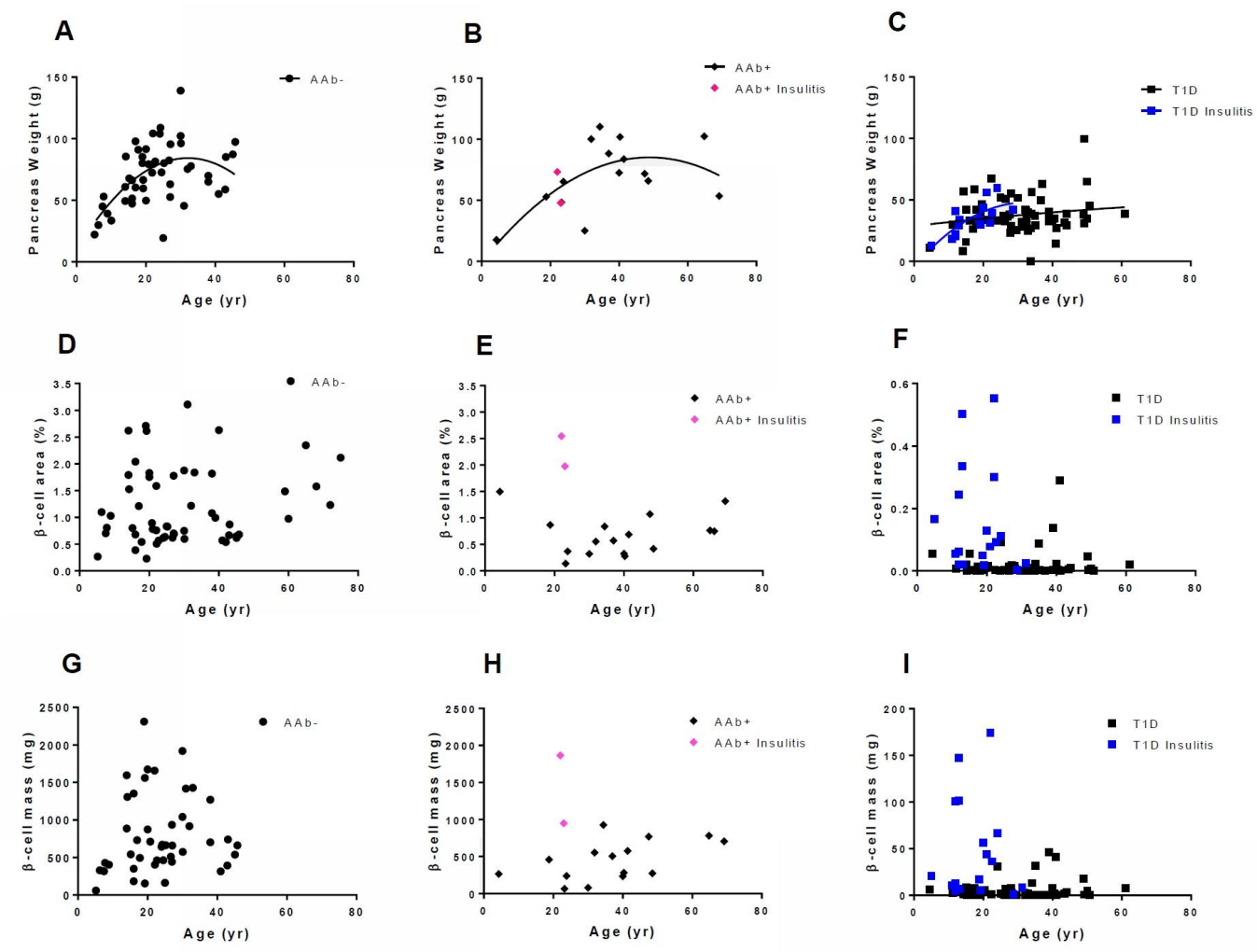
SUPPLEMENTARY DATA

Supplementary Figure 4. Heterogeneity in leukocyte markers in insulitic islets and correlations with insulitis frequency. Mean numbers of leukocyte subtypes / insulitic islets from each donor with T1D were plotted in comparison to insulitis frequency (%) (*A*-*G*). Data were displayed pink) for the two AAb+ donors with insulitis and were excluded from statistical analyses due to small sample size. The ratio of CD3/(CD3+CD20) was also calculated and plotted by insulitis frequency (*H*). Numbers of CD45+, CD3+, CD20+, CD8+, and CD4+ cells/islet had variable significant correlations with insulitis frequency but numbers of CD68+ and CD11c+ cells/islet or the ratio of CD3+/(CD3+CD20) did not. Linear regression lines with Spearmen r and p values are shown.



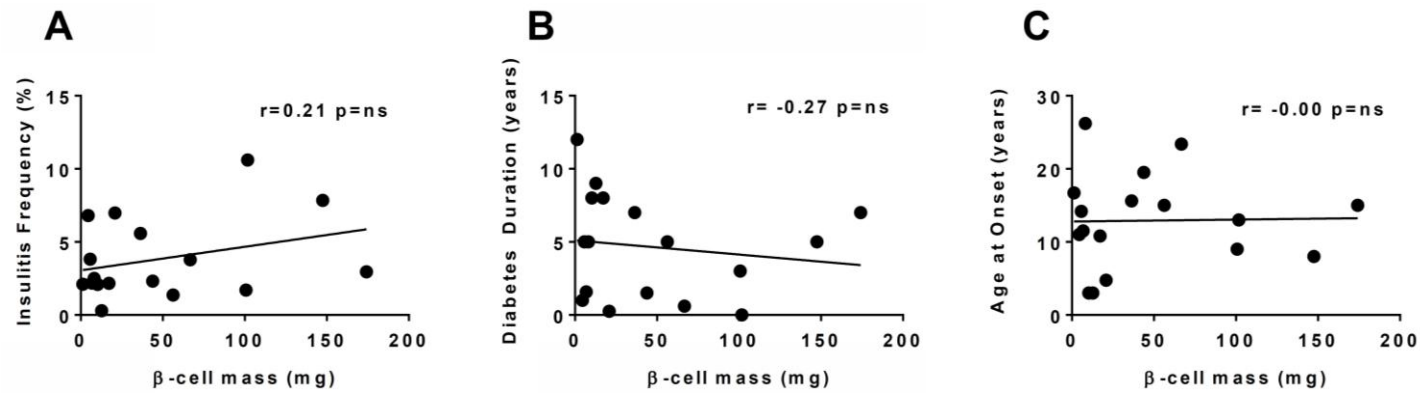
SUPPLEMENTARY DATA

Supplementary Figure 5. Pancreas weight and β-cell area and mass by donor age and group. Data from donors with no diabetes (AAb- (*A, D, G*), AAb+ (*B, E, H*)) or T1D (*C, F, I*) were depicted with data for donors with insulitis indicated in color (AAb+, pink; T1D, blue) within each respective group. An x-axis of 0-80 years was used in all plots; although, some older donors did not have pancreas weight data due to receipt of partial pancreas sample (n=3) or no recording n=13). Smaller Y axis scales were used for donors with T1D in panels for β-cell area (*F*) and mass (*I*) to allow better visualization of the significant differences, as shown in Fig. 6, for these two variables by insulitis status within this group. Pancreas weights were plotted with regression lines from best-fit second order polynomial equations (*A-C*). Variations in β-cell area (%) were seen by age in donors without diabetes (AAb-, AAb+) (*D-E*) and in donors with T1D (*F*). The β- cell mass (mg) was calculated from β-cell area (%) multiplied by pancreas weight (gram) (*G-I*) and similar variations were observed as for β-cell areas.



SUPPLEMENTARY DATA

Supplementary Figure 6. β-cell mass from donors with T1D and insulitis in comparison to insulitis frequency, diabetes duration and age at onset. The β-cell mass in donors with T1D and insulitis was not significantly correlated with insulitis frequency *(A*), diabetes duration (*B*) or age at onset (*C*) due to heterogeneity in residual β-cell distribution independent of age. Linear regression lines with Spearmen r and p values are shown.



SUPPLEMENTARY DATA

Supplementary Table 1. Clinical characteristics of 159 organ donors from study.

Characteristic	No Diabetes (AAb-)		No Diabetes Autoantibody Positive (AAb+)				T1D			
			No Insulitis		Insulitis		No Insulitis		Insulitis	
	n	Value*	n	Value*	n	Value*	n	Value*	n	Value*
Age at Death (yr)	61	28.6 (±16.4)	16	38.8 (±17.8)	2	22.5 (±0.7)	62	30.6 (±11.9)	18	17.8 (±6.8)
Age at Diabetes Onset (yr) †	-	-	-	-	-	-	61	11.4 (±8.2)	18	13.3 (±6.5)
Diabetes Duration (yr)	-	-	-	-	-	-	61	19.1 (±11.5)	18	4.6 (±3.4)
Ethnicity										
African American	5	8%	1	6%	1	50%	7	11%	1	6%
White	49	82%	13	81%	1	50%	53	86%	16	88%
Hispanic/Latino	6	10%	2	13%	0	0%	2	3%	1	6%
Sex										
Female	23	38%	7	44%	1	50%	26	42%	9	50%
Male	38	62%	9	56%	1	50%	36	58%	9	50%
Death‡ or Admit Course§										
DKA Related	0	0%	0	0%	0	0%	16	26%	8	44%
Other	61	100%	15	100%	2	100%	46	74%	10	56%
Whole Body Weight (kg)	61	73.7 (±21.9)	16	75.3 (±27.0)	2	76.5 (±17.7)	62	71.4 (±16.7)	18	59.5 (±19.8)
BMI (kg/m^2)	61	25.4 (±5.2)	16	25.8 (±6.5)	2	25.9 (±3.3)	62	25.0 (±4.1)	18	22.2 (±4.4)
C-peptide (ng/mL)‖	61	4.6 (0.4, 22.9)	14	3.9 (0.001, 26.2)	2	17.0 (16.6, 17.5)	61	0.001 (0.001, 0.140)	18	0.001 (0.001, 0.470)
HbA1c (%)	21	5.4 (±1.3)	4	5.8 (±0.8)	2	5.3 (±0.4)	19	9.8 (±2.3)	5	10.3 (±2.9)
(mmol/mol)		35.1 (±14.4)		39.6 (±8.4)		33.9 (±3.9)		83.6 (±24.6)		89.1 (±32.0)
Autoantibodies Detected¶										
0	61	100%	0	0%	0	0%	30	49%	7	39%
1	0	0%	13	89%	0	0%	23	38%	3	17%
≥2	0	0%	3	19%	2	100%	8	13%	8	44%
Hospitalization Stay (days)	57	3.2 (0.3, 23.6)	13	3.2 (1.1, 6.8)	2	3.7 (3.0, 4.5)	60	3.3 (0.9, 11.3)		3.4 (0.9, 21.7)

*% reported for all categorical variables; use of mean or median (minimum, maximum) based on evaluation of normal distribution
† Calculated as age at death minus diabetes duration
‡ Cause or mechanism of death
§ Clinical records were examined for mention of DKA
‖ A fill value of 0.001 was used when assay results <0.05 (lower limit of detection)
¶ All RIA data values were converted to NIDDK units and defined as positive if one or more of the following applied: GADA if ≥20, IA2A if ≥5, ZnT8A if ≥0.020, or insulin if ≥0.010. Insulin autoantibodies excluded from counts for donors with T1D as described in Methods. Three AAb+ donors with no insulitis had positive autoantibody results from the screening serum samples by ELISA and/or RIA in lieu of positive results from the serum sample obtained at pancreas recovery.

SUPPLEMENTARY DATA

Supplementary Table 2. Primary and secondary antibodies used in immunolocalization assays.

Antigen	Species	Clone	Company	Catalogue Number
CD11c	Rabbit	EP1347Y	Abcam	ab52632
CD3	Rabbit	Polyclonal	DAKO	A0452
CD4	Mouse	4B12	DAKO	M7310
CD8	Rabbit	Sp16	Abcam	ab27605
CD20cy	Mouse	L26	DAKO	M0755
CD45	Mouse	2B11 + PD7/26	DAKO	M0701
CD68	Mouse	PG-M1	DAKO	M0876
Chromogranin A	Rabbit	DAK-A3	DAKO	M0869
Glucagon	Mouse	K79bB10	Abcam	ab10988
Insulin	Guinea Pig	Polyclonal	DAKO	A0564
Ki67	Mouse	MIB-1	DAKO	M7240
Mouse IgG	Goat	AF488 conjugate	Invitrogen	A-11029
Rabbit IgG	Goat	AF555 conjugate	Invitrogen	A-21429
Guinea pig IgG	Goat	AF647 conjugate	Invitrogen	A-21450

SUPPLEMENTARY DATA

Supplementary Table 3. Clinical and analytical characteristics of matched donors with T1D with and without insulitis.

Name	**(%)[*], Mean (± 1 SD)[*], or Median (min, max)[*]**				***P*-value**
	n	**No Insulitis**	**n**	**Insulitis**	
Age at Demise (yrs)	15	27.6 (4.4, 61.0)	15	20 (12.0, 31.2)	0.005
Age at Diabetes Onset (yrs) †, ‡	14	11.7 (±8.9)	15	14.5 (±6.0)	0.319
Diabetes Duration (yrs)	14	14.5 (3.0, 52.0)	15	5 (0.6, 12.0)	<0.001
Ethnicity †					0.791
Caucasian	11	73%	13	86%	
African American	3	20%	1	7%	
Hispanic/Latino	1	7%	1	7%	
Sex †					0.264
Female	4	27%	8	53%	
Male	11	73%	7	47%	
Death[§] or Admit Course[‖]					0.035
DKA related	1	7%	7	47%	
Other	14	93%	8	53%	
Height (cm)	15	172.7 (81.3, 193.0)	15	165.1 (124.5, 182.8)	0.134
Whole Body Weight (kg)	15	69.9 (±19.8)	15	65.7 (±14.1)	0.508
BMI (kg/m^2)[†]	15	23.9 (±2.5)	15	23.9 (±2.3)	0.984
C-peptide (ng/mL) [¶]	15	0.001 (0.001, 0.060)	15	0.001 (0.001, 0.470)	0.142
HbA1c (%) (mmol/mol)	5	11.5 (±1.5) 102.0 (±16.8)	4	9.6 (±2.8) 80.9 (±30.3)	0.223
Autoantibody Results[#]					0.403
0	8	53%	7	47%	
1	4	27%	2	13%	
≥2	3	20%	6	40%	
Hospitalization Stay (days)	14	3.2 (0.9, 11.3)	14	3.2 (0.9, 21.7)	0.801
Transport Duration (hrs)	13	16.8 (±4.6)	15	14.4 (±5.7)	0.235
Cytomegalovirus Present					0.999
No	8	62%	9	64%	
Yes	5	38%	5	36%	
History of Hypertension					0.999
No	8	62%	9	64%	
Yes	5	38%	5	36%	
Heavy Alcohol Use					
No	14	100%	13	100%	
History of Cigarette Use					0.482
No	12	93%	14	100%	
Yes	1	7%	0	0%	
Cocaine Use					0.222
No	13	100%	11	79%	
Yes	0	0%	3	21%	
Other Drug Use					0.648
No	10	71%	11	85%	
Yes	4	29%	2	15%	
HLA Haplotypes					
DRB1*04-DQB1*03:02 alone					0.390
Absent	13	87%	10	67%	
Present	2	13%	5	33%	
DRB1*03:01-DQB1*02:01 alone					0.999

Absent	10	67%	11	73%	
Present	5	33%	4	27%	
Both of Above					0.427
Absent	9	60%	12	80%	
Present	6	40%	3	20%	
DRB1*15:01-DQB1*06:02					0.999
Absent	15	100%	14	93%	
Present	0	0%	1	7%	
Pancreas Weight (grams)	14	39.2 (11.1, 117.4)	14	33.8 (20.4, 59.8)	0.6625
Fractional Insulin Area (%)	14	0.00 (0.00, 0.14)	15	0.08 (0.00, 0.55)	0.0016
Insulin Mass (mg)	13	1.73 (0.11, 30.80)	14	26.77 (1.27, 174.18)	0.0028

[*] % reported for all categorical variables; use of mean or median based on evaluation of normal distribution.
[†] Factors used for matching (see Table 1 for characteristics of all donors in study).
[‡] Calculated as age at death minus diabetes duration.
[§] Cause or mechanism of death.
[‖] Clinical records were examined for DKA in relation to cause or mechanism of death or during the terminal admission history.
[¶] A fill value of 0.001 was used when assay results <0.05 (lower limit of detection).
[#] All data values were converted to NIDDK units and defined as positive if one or more of the following applied: GADA if ≥20, IA2A if ≥5, or ZnT8A if ≥0.020. Insulin autoantibodies were excluded from counts for donors with T1D as described in Methods.

SUPPLEMENTARY DATA

Supplementary Table 4. Correlation analyses between numbers of leukocytes/islet and insulitis frequency, diabetes duration, and age of onset. Mean numbers of leukocytes/islet for each donor with T1D and insulitis were analyzed for correlations to other CD markers and insulitis frequency (%), diabetes duration, and age of onset. Spearman r values are shown with grey shading indicating no significant correlations. Numbers of CD3+, CD20+, CD8+ and CD4+ cells/islet showed significant correlations to each other, total leukocytes (CD45), and insulitis frequency (%). Mean numbers of CD11c+ or CD68 cells/islet were significantly correlated with each other and numbers of CD3+ cells/islet but not to other leukocytes or other donor variables. * P<0.05 ** P<0.01 *** P <0.001.

	CD45	CD3	CD20	CD8	CD4	CD68	CD11c	Insulitis Frequency (%)	Diabetes Duration (Years)	Age onset (Years)
CD45	1.00	0.95***	0.96***	0.90***	0.85***	0.58	0.51	0.67*	-0.42	-0.26
CD3		1.00	0.89***	0.87***	0.90***	0.63*	0.68*	0.78***	-0.24	-0.38
CD20			1.00	0.78***	0.78***	0.33	0.49	0.56*	-0.38	-0.07
CD8				1.00	0.85	0.22	0.54	0.62*	-0.55	0.08
CD4					1.00	0.33	0.55	0.62*	-0.42	-0.15
CD68						1.00	0.75	0.33	-0.32	-0.47
CD11c							1.00	0.53	0.55	-0.17
Insulitis Frequency (%)								1.00	-0.51	-0.05

SUPPLEMENTARY DATA

Supplementary Table 5. 43 nPOD cases reported herein were previously examined by Coppieters et al. (N = 14 donors without diabetes, N = 4 AAb+ donors without diabetes, and N = 25 donors with T1D).

nPOD ID	Diabetes Status
6009	No diabetes
6012	No diabetes
6013	No diabetes
6017	No diabetes
6019	No diabetes
6020	No diabetes
6021	No diabetes
6024	No diabetes
6030	No diabetes
6034	No diabetes
6047	No diabetes
6075	No diabetes
6098	No diabetes
6099	No diabetes
6027	AAb+
6044	AAb+
6080	AAb+
6101	AAb+
6025	T1D
6026	T1D
6031	T1D
6032	T1D
6035	T1D
6039	T1D
6041	T1D
6045	T1D
6049	T1D
6051	T1D
6054	T1D
6061	T1D
6062	T1D
6063	T1D
6064	T1D
6067	T1D
6076	T1D
6077	T1D
6079	T1D
6083	T1D
6087	T1D
6088	T1D
6089	T1D
6113	T1D
6180	T1D