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Supplementary Materials for

The healthy exocrine pancreas contains preproinsulin-specific CD8 T cells that attack islets in type 1 diabetes

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Supplementary Methods:

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were thawed in RPMI (11835-030 Life Technologies) (supplemented with 15 U Benzonase) at 37°C. After counting, 100 µl of 2 x 10^6 cells/mL were added per well to a sterile 96-well round bottom plate and stained with a live/dead aqua dye (L34957, Life Technologies) for 30 minutes at room temperature in the dark, followed by washing with PBS. PBMCs were incubated with the protein kinase inhibitor dasatinib (D3307, LC laboratories) at a final concentration of 50 nM for 30 minutes at 37°C. After washing with PBS, PBMCs were stained with 1µg APC-labeled tetramer (PPI_{15-24} and $CMV_{495-503}$) for 10 minutes at 37°C. Subsequently, PBMCs were stained with biotinylated anti-APC antibody (1/1000, clone APC003, IgG1, BioLegend) for 30 minutes at 4°C. After washing with PBS, AF647, or BV421-labeled streptavidin (1/1000, S32357 Life technologies, 405225 Bio Legend) was added to the cells. Stained PBMCs were kept on ice and in the dark until the next step or acquisition with LSR II (BD Biosciences). Finally, mouse CD8-AF488 (1/100, clone RFT8, Sothern Biotech 9536-30 and mouse CD3-PE (1/100, clone OKT3e, Bioscience 12-0037-42) monoclonal antibodies were added to the cells for 30 minutes at 4°C to stain T lymphocytes.

Immunofluorescence staining of PBMCs

PBMCs (10^6) were washed in PBS and blocked with 1% BSA. Afterward, cells were prepared on Superfrost plus slides using the Shandon Cytospin 4 (Fisher Scientific). After air-drying overnight in a desiccation chamber, cells were incubated with 1µg APC labeled PPI_{15-24} tetramer overnight at 4°C. The next day, cells were fixed with 1% paraformaldehyde (PFA) for 15 minutes at room temperature. After blocking with TBS/Protease inhibitor/2% Goat serum for 30 minutes at room temperature, cells were incubated with purified mouse anti-APC antibody (1/50, Biolegend) for 12 hours at room temperature. Subsequently, the goat anti-mouse AF647 antibody was added for 1h. Slides were incubated with anti-CD8-AF488 (1/100, clone RFT8, Sothern Biotech 9536-30) for 1 hour at room temperature. Nuclei were counterstained with Hoechst (1/5000, Life Technologies), and coverslips were mounted using Prolong Gold antifade mounting medium (Invitrogen). Slides were examined using a laser scanning Olympus FV10i confocal microscope. Images were captured with a 60x objective.

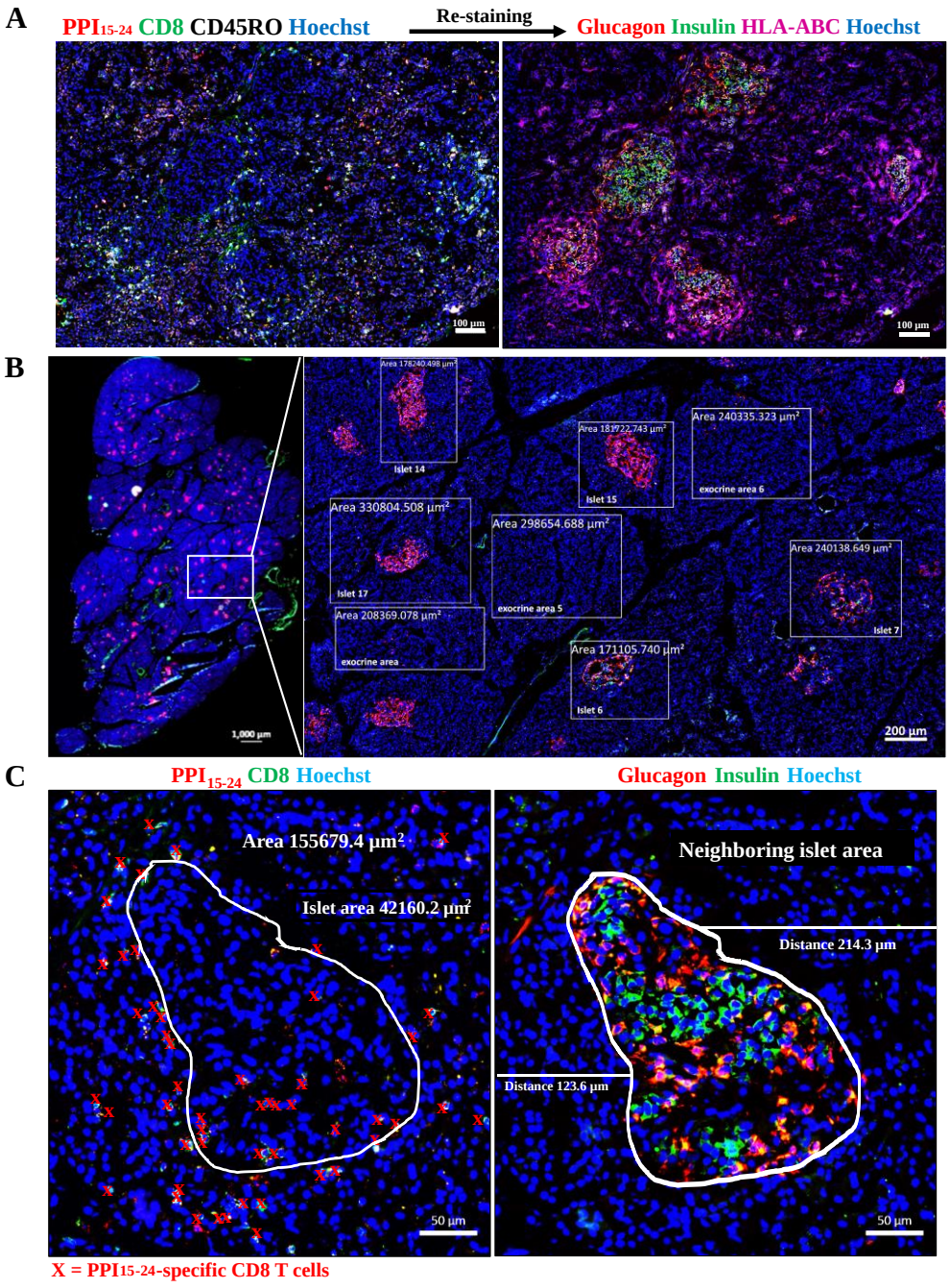


Fig. S1. **Representative immunofluorescence images of a pancreas section from a donor with T1D** (#6052, one year of disease duration). **A)** Re-staining strategy: Left image represents the tetramer staining before chemical fluorophore inactivation with H_2O_2. The right image represents the same tissue sections re-stained for insulin (green), glucagon (red), and MHC class I (magenta). **B)** Different exocrine regions and islets (white squares) across the pancreas section were randomly picked to quantify PPI_{15-24}-specific CD8+ T cells. **C)** PPI_{15-24}–specific CD8+ T cells were counted manually, and the density was calculated per mm^2. Scale bar 50 µm. Images were taken with the AxioScan.Z1 slide scanner (Zeiss, 20x magnification).

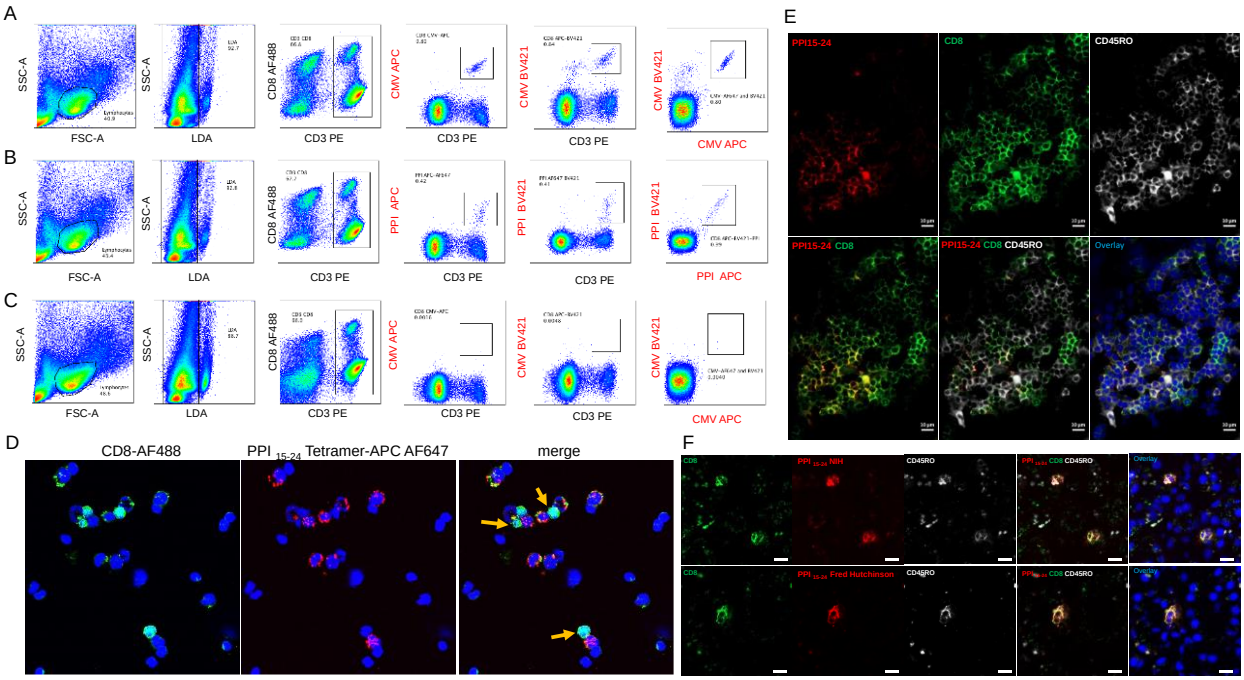
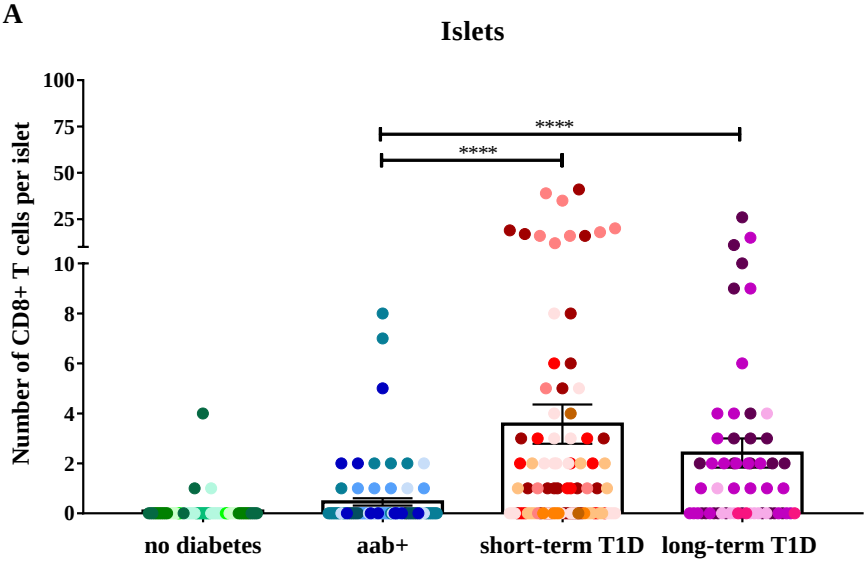


Fig. S2. Tetramer quality control. A) PBMCs spiked with $CMV_{495-503}$ specific T cell clones were stained for Aqua Live-Dead (LDA), CD3, CD8, and CMV tetramer-APC. Purified mouse anti-APC antibody plus goat anti-mouse BV421 were added for double fluorescent detection of antigen-specific cells. **B)** A similar strategy was applied for PPI_{15-24}-specific cells. **C)** CMV tetramer staining was performed in PBMCs from a negative donor. **D)** PBMCs were spun onto a microscopy slide with Cytospin. Immunofluorescence staining of human PBMCs with AF647-labeled PPI_{15-24} tetramer in combination with anti-CD8-AF488. Yellow arrows indicate PPI-negative CD8-positive T cells (merge image). Nuclei were counterstained with Hoechst. The image was captured with a 40x objective. **E)** Representative immunofluorescence staining of PPI_{15-24} specific CD8+ CD45RO+ T cells on a PLN section from a donor with T1D (#6046, eight years of disease duration). **F)** PPI_{15-24} tetramers labeled with APC from NIH and Fred Hutchinson were compared by immunofluorescence staining in combination with CD8-AF488 (green) and CD45RO-AF594 (white) on a pancreas section from a donor with T1D (#6224, disease duration 1.5 years). Scale bar 10 µm.

A



B

	no diabetes	aab+	short-term T1D	long-term T1D
Islets *n*	**63**	**83**	**98**	**58**
CD8+ islets *n* (%)	**3 (5.8)**	**15 (18.1)**	**48 (49.0)**	**31 (53.4)**
1 CD8 T cell per islet *n* (%)	**2 (3.9)**	**6 (7.3)**	**12 (12.2)**	**5 (8.6)**
≥ 2 CD8 T cells per islet *n* (%)	**1 (1.9)**	**9 (10.8)**	**36 (36.8)**	**26 (44.8)**
CD8 negative islets *n* (%)	**60 (94.2)**	**68 (81.9)**	**50 (51.0)**	**27 (46.6)**

Fig. S3. Higher total numbers of CD8+ T cells in islets from donors with T1D irrespective of disease duration compared with donors without diabetes. Islets were randomly selected from pancreas sections of donors without diabetes (n=63), aab+ (n=83), and T1D (n=156). **A)** Higher numbers of CD8+ T cells were found in donors with T1D ($p<0.0001$) compared to donors with aab+ irrespective of disease duration. The total number of CD8+ T cells is displayed per islet. Bars represent the mean ± SEM values. Every color represents a donor. For statistical analysis, non-parametric Kruskal-Wallis test followed by Dunn multiple comparison test was used to determine significance: **** $p<0.0001$. **B)** The table summarizes the numbers of islets infiltrated with CD8 T cells per group. The percentages of islets positive for 1 CD8+ T cell or ≥ 2 CD8+ T cells were calculated.

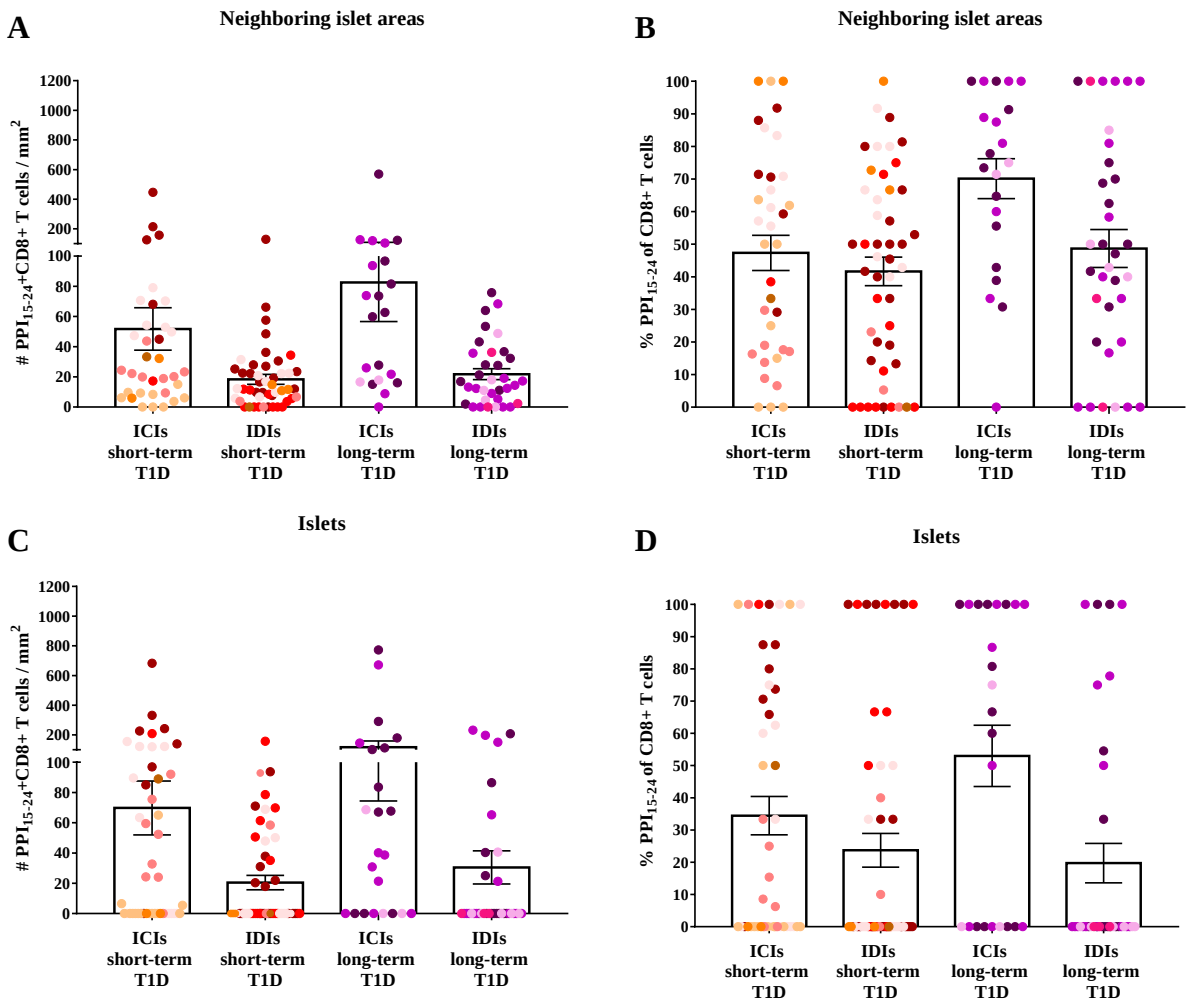


Fig. S4. PPI_{15-24}-specific CD8+ T cell infiltration or retention in the islets depends on the presence of insulin irrespective of disease duration. ICIs (n=69) and IDIs (n=87) from donors with short disease duration (n=7) and long-term T1D (n=4) were analyzed for the presence of PPI_{15-24}-specific CD8+ T cells. Every dot represents **A+B),** a neighboring islet area to ICIs (n=56), and IDIs (n=81) or **C+D)** an islet. Bars represent the mean ± SEM numbers **(A+C)** and frequencies **(B+D)** of PPI_{15-24}–specific CD8 T cells/mm^2 in donors with short-term or long-term disease duration. Every color represents a donor. For statistical analysis, non-parametric Kruskal-Wallis test followed by Dunn multiple comparison test was used.

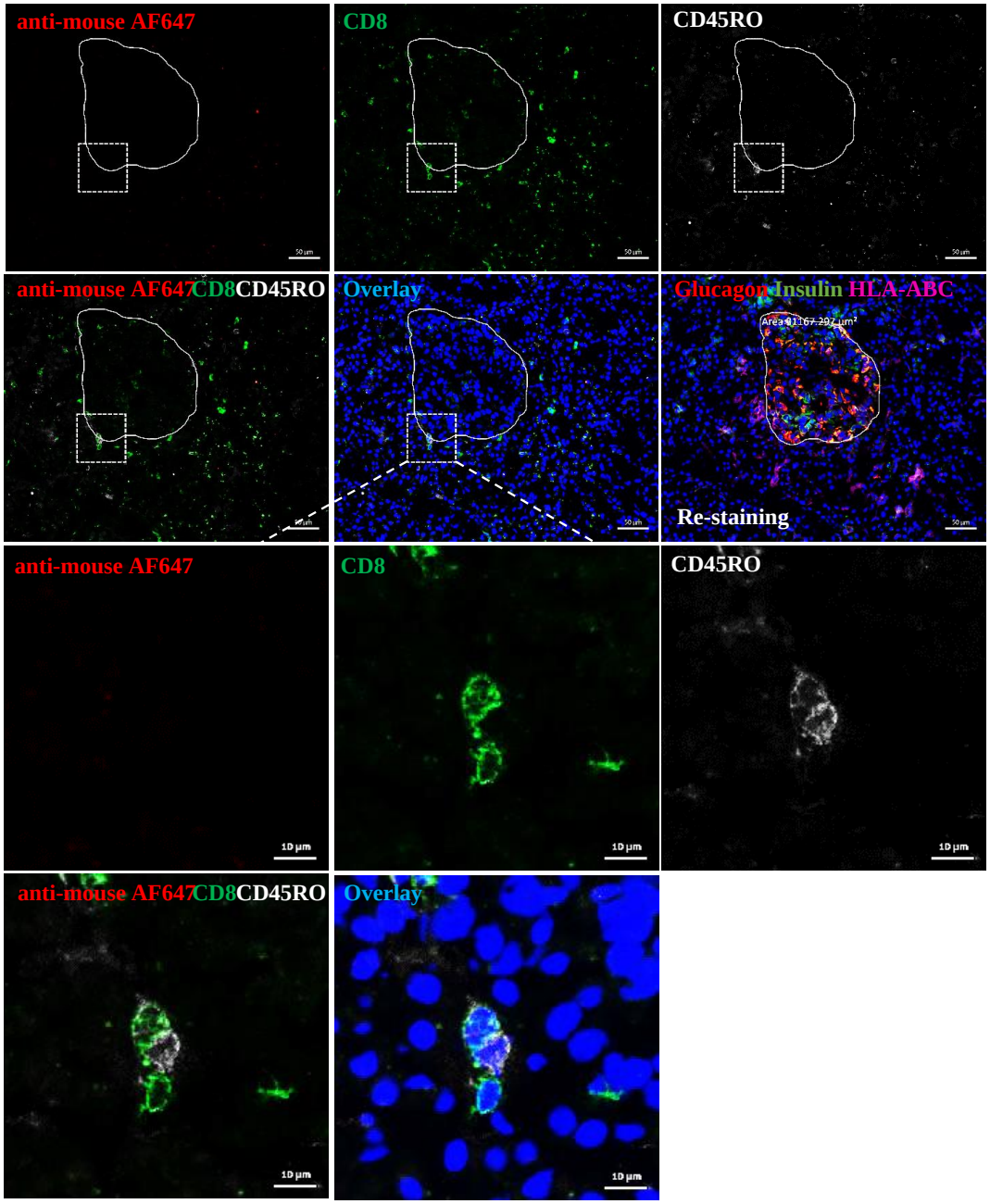


Fig. S5. PPI_{15-24} staining control. Complimentary to Fig. S1. Immunofluorescence staining on a consecutive section without tetramer, including CD8-AF488 (green), CD45RO-AF594 (white), and secondary antibody anti-mouse AF647 as a staining control for the tetramer**.**

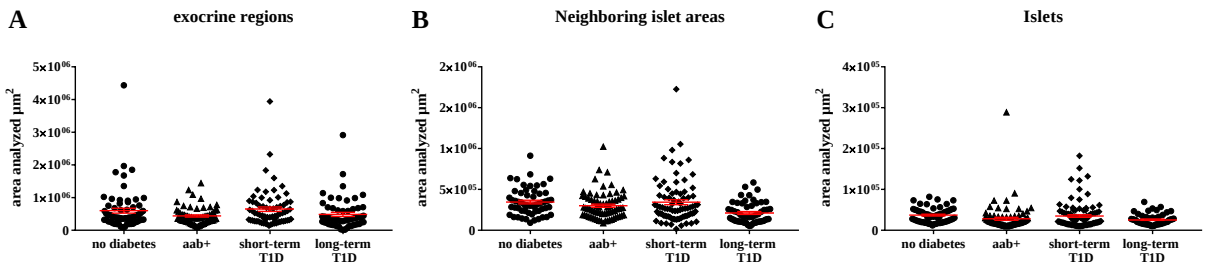


Fig. S6. Analyzed area size. The analyzed area size is shown in µm^2 for each group: no diabetes (donors without diabetes), aab+ (autoantibody-positive donors), and T1D donors with short and long disease duration. Every dot represents an **A)** exocrine region, **B)** neighboring islet area or, **C)** islet area (≥10 000 µm^2). Red lines represent the mean size values ± SEM for different groups.

NO DIABETES	islets (n)	Neighboring area (n)	exocrine regions (n)
6232	10	9	10
6271	8	7	7
6295	16	16	21
6335	15	12	13
6377	4	4	3
6386	10	9	15
Total	**63**	**57**	**69**

AAB+	islets (n)	Neighboring area (n)	exocrine regions (n)
6123	12	12	8
6151	24	15	12
6154	26	25	27
6171	11	11	9
6429	10	10	7
Total	**83**	**73**	**63**

T1D	islets (n)		Neighboring area (n)	exocrine regions (n)
	ICIs	IDIs		
6046	9	14	23	22
6052	8	20	25	20
6070	11	12	22	24
6088	0	3	3	7
6113	1	13	13	15
6209	8	3	11	7
6224	12	12	16	12
6268	3	6	7	5
6456	14	0	10	9
6367	2	3	5	6
6342	1	1	2	2
Total	**69**	**87**	**137**	**129**
	156			

Total	302	267	261

Table S1. Numbers of analyzed areas. Numbers are presented for each donor and donor group. A total of 302 islets, 267 neighboring areas, and 261 exocrine regions were analyzed. ICIs =insulin-containing islets, IDIs= insulin-deficient islets. IDIs were not present in tissue samples from non-diabetic and aab+ donors.

	no diabetes	aab+	T1D short-term	T1D long-term
Total Islets (n)	**63**	**83**	**156**	
CD8+ islets (%)	6232 (3.9)	6123 (0)	6052 (9.6)	6046 (10.3)
	6271 (0)	6151 (7.2)	6113 (5.1)	6070 (8.3)
	6295 (1.9)	6154 (4.9)	6209 (6.4)	6088 (0)
	6335 (0)	6171 (2.4)	6224 (5.8)	6268 (1.3)
	6377 (0)	6429 (3.6)	6342 (0.6)	
	6386 (0)		6367 (0)	
			6456 (3.2)	
Total CD8+ islets (%)	5.8	18.1	50.6	

Table S2. Percentage of islets infiltrating with CD8+ T cells. Islets were randomly selected from pancreas sections of donors without diabetes (n=63), aab+ (n=83), and T1D (n=156). In the T1D group, 50.6 % of all selected islets were infiltrated with CD8+ T cells. In comparison, we detected CD8+ T cells in 18.1% of all analyzed islets from aab+, and 5.8% from non-diabetic donors. Every color represents a donor. aab+= autoantibody-positive, T1D short-term (2≤years), T1D long-term (2-8 years).

Donor Type	nPOD case ID	Gender	Age (y)	Race	BMI	T1D Duration (y)	Time in ICU (days)	C-peptide (ng/ml)	Clinical History	Regions analyzed
No Diabetes	6232	Female	14	Caucasian	20.8	-	3.03	19.5	Negative	Body
No Diabetes	6271	Male	17	Caucasian	24.4	-	0.48	11.47	Negative	Tail
No Diabetes	6295	Female	47	African American	30.4	-	4.1	10.91	Negative	Tail
No Diabetes	6335	Male	18.8	Multiracial	23.6	-	4.11	8.85	Negative	Body
No Diabetes	6377	Male	9.2	African American	16.6		8.17	1.5	Negative	Body
No Diabetes	6386	Male	14	Caucasian	23.9		2.34	1.12	Negative	Body
Autoantibody-positive	6123	Female	23.2	Caucasian	17.6	-	3.55	2.01	GADA	Tail
Autoantibody-positive	6151	Male	30	Caucasian	24.2	-	2.16	5.49	GADA	Head, Tail
Autoantibody-positive	6154	Female	48.5	Caucasian	24.5	-	3.73	<0.05	GADA	Head, Body, Tail
Autoantibody-positive	6171	Female	4.4	Caucasian	14.8	-	3.98	8.95	GADA	Body
Autoantibody-positive	6429	Male	22.1	African American	19.6	-	6.9	2.25	GADA, mIAA	Tail, Body
T1D	6046	Female	18.8	Caucasian	25.2	8	2.3	<0.05	IA2A, ZnT8A	Head, Body, Tail
T1D	6052	Male	12	African American	20.3	1	1.84	0.18	IA2A, mIAA	Body, Tail
T1D	6070	Female	22.6	Caucasian	21.6	7	4.85	<0.05	IA2A, mIAA	Body, Tail
T1D	6088	Male	31.2	Caucasian	27.0	5	0.93	<0.05	GADA, IA2A, mIAA, ZnT8A	Tail
T1D	6113	Female	13.1	Caucasian	24.8	1.58	5.1	<0.05	mIAA,	Head, Body
T1D	6209	Female	5	Caucasian	15.9	0.25	-	0.1	IA2A, mIAA, ZnT8A	Tail
T1D	6224	Female	21	Caucasian	22.8	1.5	3.34	<0.05	Negative	Head, Body, Tail
T1D	6268	Feamle	12	Caucasian	26.6	3	3.38	0.05	mIAA	Tail
T1D	6342	Female	14	Caucasian	24.3	2	3.13	0.26	IA2A, mIAA	Head
T1D	6367	Male	24	Caucasian	25.7	2	4.62	0.39	Negative	Head
T1D	6456	Female	30.49	African American	30.1	0	1.91	10.33	GADA, ZnT8A	Tail, Body

Table S3. Detailed donor information. Age and duration of disease are expressed in years. BMI, body mass index. Time in the intensive care unit (ICU). mIAA, micro assay for insulin autoantibodies; GADA, glutamic acid decarboxylase 65 autoantibodies; IA-2A, the intracytoplasmic domain of the tyrosine phosphatase (IA-2) autoantibodies. ZnT8A, zinc transporter 8 autoantibodies.