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Supplementary Materials for

A 3D atlas of the dynamic and regional variation of pancreatic innervation in diabetes

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/41/eaaz9124/DC1)

Movies S1 to S21

Supplementary Figures

Figure S1. β -cell distribution and innervation in C57BL/6 mice.

A) Distribution of insulin+ islets at increasing distance from the nearest nerve (left axis) and median distance from the nearest nerve (right axis) in C57BL/6 mice. $P < 0.0001$, Mann-Whitney test. $N=5$, islets/group: 25310, 10030 and 15280.

B) Mean volume of insulin+ islets located $< 1.6 \mu\text{m}$ or $> 1.6 \mu\text{m}$ from the nearest nerve across the entire pancreas (T), in the duodenal (D) and splenic (S) regions. Kruskal-Wallis test: $p < 0.0001$. Dunn's test: Total $< 1.6 \mu\text{m}$ vs. $> 1.6 \mu\text{m}$: $p < 0.0001$; D $< 1.6 \mu\text{m}$ vs. $> 1.6 \mu\text{m}$: $p < 0.0001$; S $< 1.6 \mu\text{m}$ vs. $> 1.6 \mu\text{m}$: $p < 0.0001$; D $> 1.6 \mu\text{m}$ vs. S $> 1.6 \mu\text{m}$: $p < 0.0001$; $N=5$ samples, islets/group: 770, 11885, 325, 4690, 445, 7195.

C) Proportion of innervated (NF200+) insulin+ islets with volumes $< 10,000 \mu\text{m}^3$, $10,000\text{--}49,999 \mu\text{m}^3$, $50,000\text{--}500,000 \mu\text{m}^3$ or $> 50,000 \mu\text{m}^3$.

D) Number of β -cells contacting NF200+ nerves per insulin+ islet based on size. Small: $< 10 \beta$ -cells, medium: 10-100 β -cells, large $> 100 \beta$ -cells. Kruskal-Wallis test: $p = 0.0175$. Dunn's test: $p = 0.0103$, small vs. large.

E) Percentage of β -cells contacting NF200+ nerves per insulin+ islet based on size. Small: $< 10 \beta$ -cells, medium: 10-100 β -cells, large $> 100 \beta$ -cells. Kruskal-Wallis test: $p = 0.3041$.

F) Large and thin nerve fibers expressing NF200 (magenta).

G) Analysis of innervation (yellow) and β -cells (red) (left panel) and the detection of nerve/ β -cell contacts by distance transformation (left panel). β -cell clusters in cyan are touching the nerve surface (distance: 0).

Data are shown as mean \pm SEM, or as median \pm 95% confidence interval where indicated. T: Total; D: Duodenal; S: Splenic.

Figure S2. β -cell distribution and innervation in nondiabetic and diabetic NOD mice.

A) Correlation between insulin+ islet number (left axis) and β -cell volume (right axis) and blood glucose in NOD mice. Islet number, T: $p=0.0009$; D: $p=0.015$; S: $p=0.0034$; Islet V, T: $p=0.0021$; D: $p=0.0010$; S: $p=0.0137$, linear regression. N=14.

B) Correlation between blood glucose and exocrine nerve volume. T: $p=0.9520$, D: $p=0.7351$, S: $p=0.5316$, linear regression. N=15.

C) Correlation between blood glucose and endocrine nerve volume corrected for islet volume (right axis) and endocrine nerve volume per insulin+ islet (left axis). Nerve V/Islet V: T: $p=0.1540$; D: $p=0.2217$; S: $p=0.0436$; Nerve V per islet, T: $p=0.1784$; D: $p=0.4104$; S: $p=0.2396$, linear regression. N=15.

D) Distribution of insulin+ islets at increasing distance from the nearest nerve (left axis) and median distance from the nearest nerve (right axis) in NOD mice. Kruskal-Wallis test: $p<0.001$; Dunn's test: nondiabetic S vs. diabetic S: $p<0.001$; nondiabetic D vs. S: $p<0.001$; diabetic D vs. S: $p<0.001$. N=8 nondiabetic, 7 diabetic. Islets/group: 9616, 5486, 4130, 4739, 3473, 1293.

E) Mean volume of insulin+ islets $<1.6 \mu\text{m}$ and $>1.6 \mu\text{m}$ from the nearest nerve in NOD mice. Kruskal-Wallis test: $p<0.001$, Dunn's test, $<1.6 \mu\text{m}$: nondiabetic T vs. diabetic T: $p<0.001$; nondiabetic D vs. nondiabetic S: $p<0.001$; nondiabetic S vs. diabetic S: $p<0.001$; $>1.6 \mu\text{m}$: nondiabetic T vs. diabetic T: $p<0.001$; nondiabetic D vs. S: $p<0.001$; nondiabetic D vs. diabetic D: $p<0.001$; diabetic D vs. S: $p=0.0096$. N=8 nondiabetic, 7 diabetic. Islets/group: 901, 4585, 500, 3605, 312, 3163, 199, 1187, 1401, 8190, 511, 4350.

F) Proportion of innervated (NF200+) insulin+ islets with volumes $<10,000 \mu\text{m}^3$, $10,000\text{--}49,999 \mu\text{m}^3$, $50,000\text{--}500,000 \mu\text{m}^3$ or $>50,000 \mu\text{m}^3$ in NOD mice.

Data are shown as mean \pm SEM, or as median \pm 95% confidence interval where indicated. T: Total; D: Duodenal; S: Splenic.

Figure S3. β -cell distribution and innervation in STZ-treated mice.

- A) Correlation between insulin+ islet number (left axis), β -cell volume (right axis) and blood glucose in control or STZ-treated mice. Islet volume, total: $p=0.0055$, duodenal: $p=0.0012$, splenic: $p=0.0187$; Islets per mm^3 , total: $p=0.0258$, duodenal: $p=0.0091$, splenic: $p=0.823$, linear regression. $N=18$.
- B) Correlation between exocrine nerve volume and blood glucose across in control or STZ-treated mice. Total: $p=0.0719$, duodenal: $p=0.3879$, splenic: $p=0.4624$, linear regression. $N=15$.
- C) Correlation between blood glucose and endocrine nerve volume corrected for islet volume (left axis) or endocrine nerve volume per insulin+ islet (right axis) in control or STZ-treated mice. Nerve V / Islet V, total: $p=0.0149$, duodenal: $p=0.6467$, splenic: $p=0.1547$; Nerve V per islet, total: $p=0.0396$, duodenal: $p=0.3790$, splenic: $p=0.200$, linear regression. $N=15$.
- D) Insulin+ islet volume distribution (left axis) and median insulin+ islet volume (right axis) in control or STZ-treated mice. Kruskal-Wallis test: $p<0.001$; Dunn's test: Control T vs. STZ day 15 T: $p<0.001$; Control D vs. STZ day 5 D, $p=0.0027$; Control D vs. STZ day 15 D, $p<0.001$; STZ day 5 T vs. STZ day 15 T, $p<0.001$; STZ day 5 D vs. STZ day 5 S, $p<0.0020$; STZ day 5 S vs. STZ day 15 S, $p<0.001$; STZ day 15 D vs. STZ day 15 S, $p<0.001$. Islets/group: 10479, 4682, 5797, 10091, 5162, 4929, 14380, 7543 and 6837.
- E) Mean volume of insulin+ islets located $<1.6 \mu\text{m}$ or $>1.6 \mu\text{m}$ from the nearest nerve in control or STZ-treated mice. Kruskal-Wallis test: $p<0.001$. For islets $<1.6 \mu\text{m}$ from innervation: Dunn's test: Control T vs. STZ day 15 T; $p<0.001$; Control D vs. Control S, STZ day 5 D, STZ day 15 D, $p<0.001$; STZ day 5 T vs. STZ day 15 T, $p<0.001$; STZ day 5 S vs. STZ day 15 S, $p<0.001$; STZ day 15 D vs. STZ day 15 S, $p<0.001$. For islets $>1.6 \mu\text{m}$ from innervation: Control T vs. STZ day 5 T, $p<0.001$; Control D vs. STZ day 5 D, $p<0.001$; STZ day 5 T vs. STZ day 15 T, $p<0.001$; STZ day 5 D vs. STZ day 5 S, $p<0.001$; STZ day 5 S vs. STZ day 15 S, $p<0.001$; STZ day 15 D vs. STZ day 15 S, $p<0.001$. Between STZ day 5 T, D and S: $p<0.001$. Between STZ day 15 T, D and S: $p<0.001$. Control T vs. STZ day 5 T, between islets $<1.6 \mu\text{m}$ and $>1.6 \mu\text{m}$: $p<0.001$ for Control, STZ day 5 and STZ day 15 respectively, denoted by *** on bars. Islets/group: 237, 6384, 271, 4355, 508, 5680, 217, 3979, 145, 2614, 362, 3789, 307, 7233, 512, 5548, 819, 5395.
- F) Proportion of innervated (NF200+) insulin+ islets with volumes $<10,000 \mu\text{m}^3$, $10,000\text{--}49,999 \mu\text{m}^3$, $50,000\text{--}500,000 \mu\text{m}^3$ or $>50,000 \mu\text{m}^3$ in control or STZ-treated mice.

Data are shown as mean \pm SEM, or as median \pm 95% confidence interval where indicated. T: Total; D: Duodenal; S: Splenic.

Figure S4. Neuronal, endocrine and vascular markers in cleared pancreatic mouse tissue.

Maximum projections of small pancreatic samples cleared with iDISCO+ and immunolabeled for insulin with

A) NF200

B) Sympathetic marker, tyrosine hydroxylase (TH)

C) Parasympathetic marker, vesicular acetylcholine transporter (VACht)

D) Sensory marker, Transient receptor potential cation channel subfamily V member 1 (TRPV1)

E) Pan-neuronal marker, synapsin

F) Glucagon

G) Somatostatin (SST)

H) Exocrine pancreas marker, mucin 1 (Muc1)

Maximum projections of small pancreatic samples cleared with a modified protocol for ECI and immunolabeled for insulin and vasculature using:

I) Alexa-488 dextran

J) Alexa-647 CD31

Scale bars: 50 μ m.

Figure S1

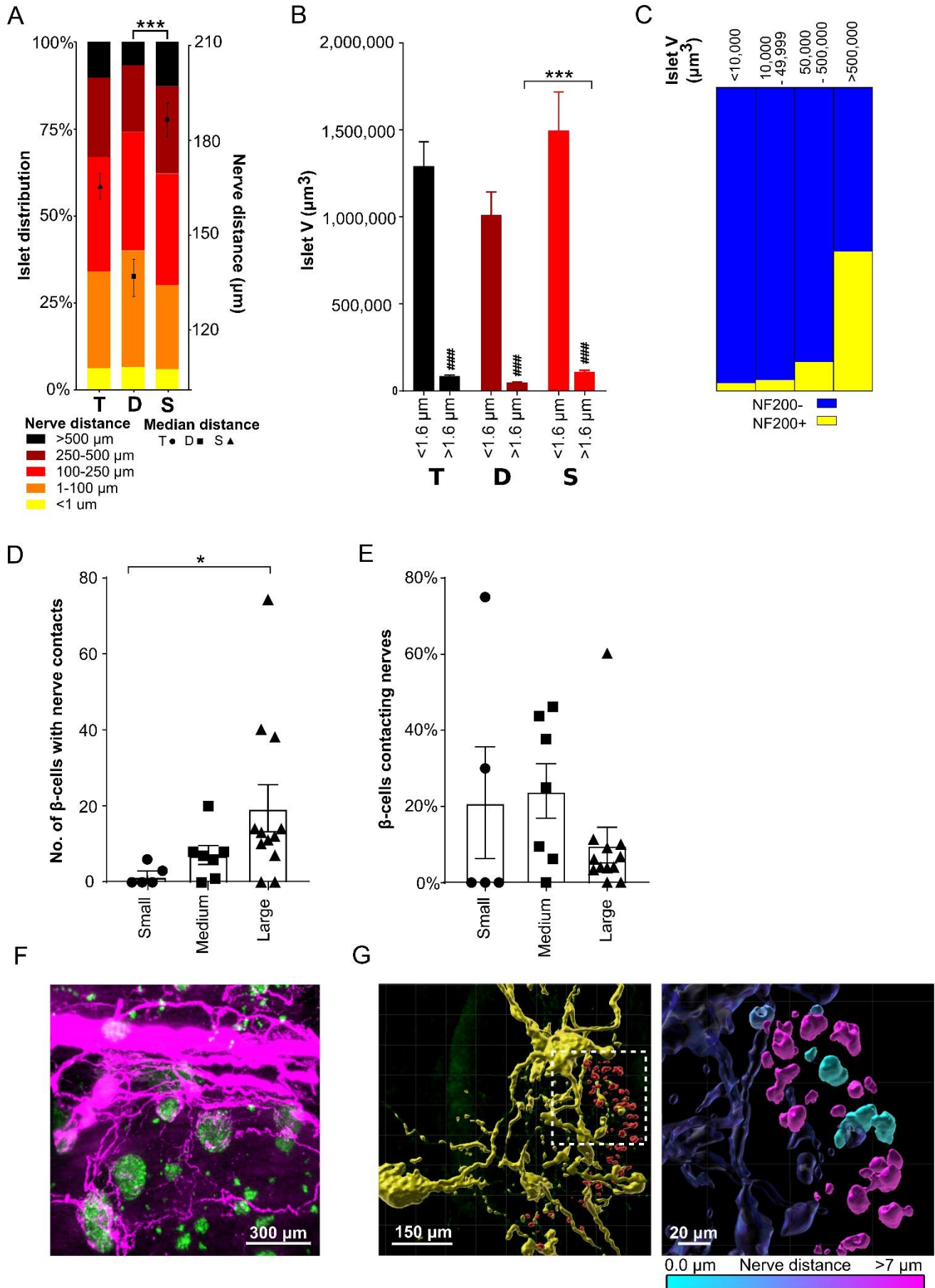


Figure S2

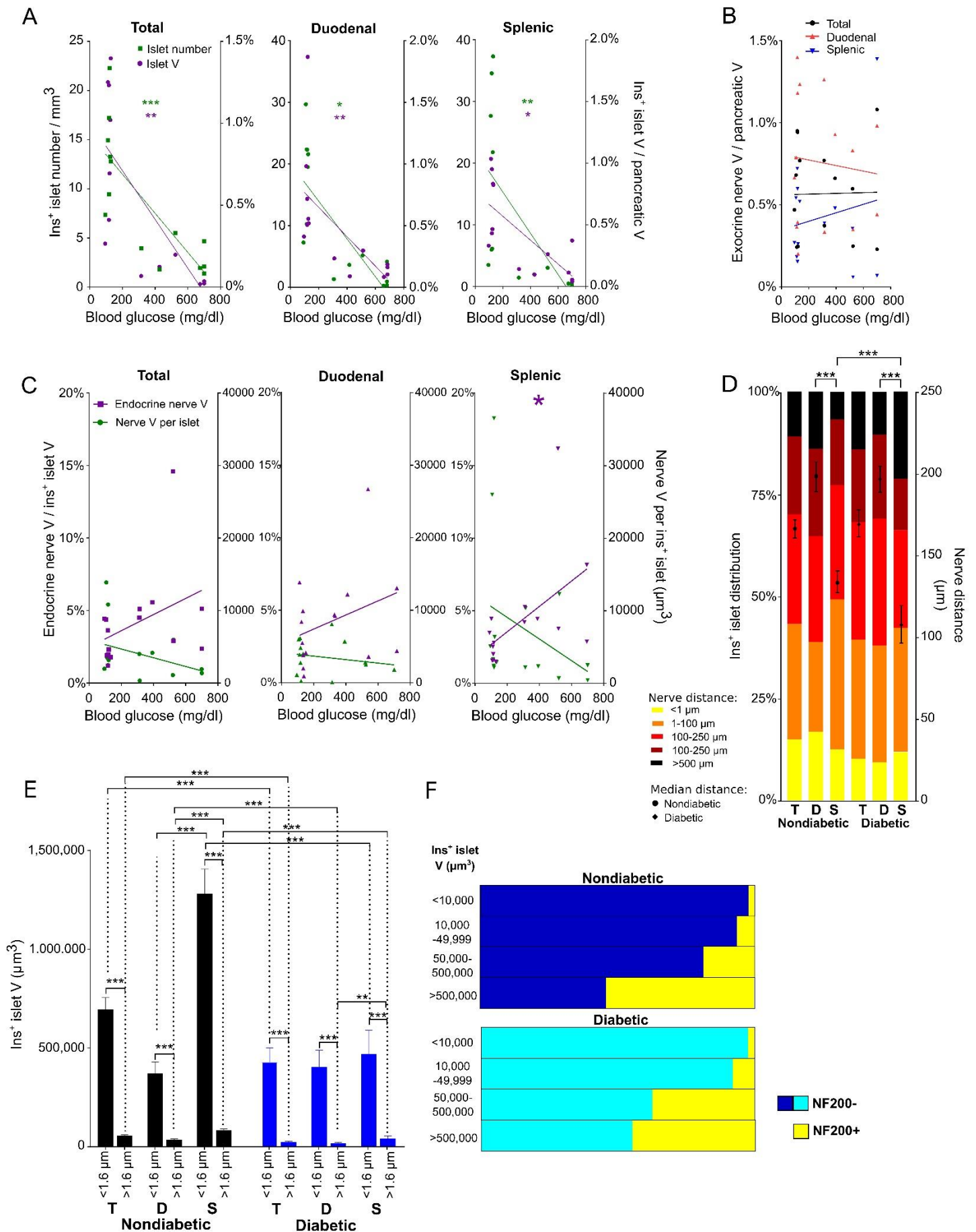


Figure S3

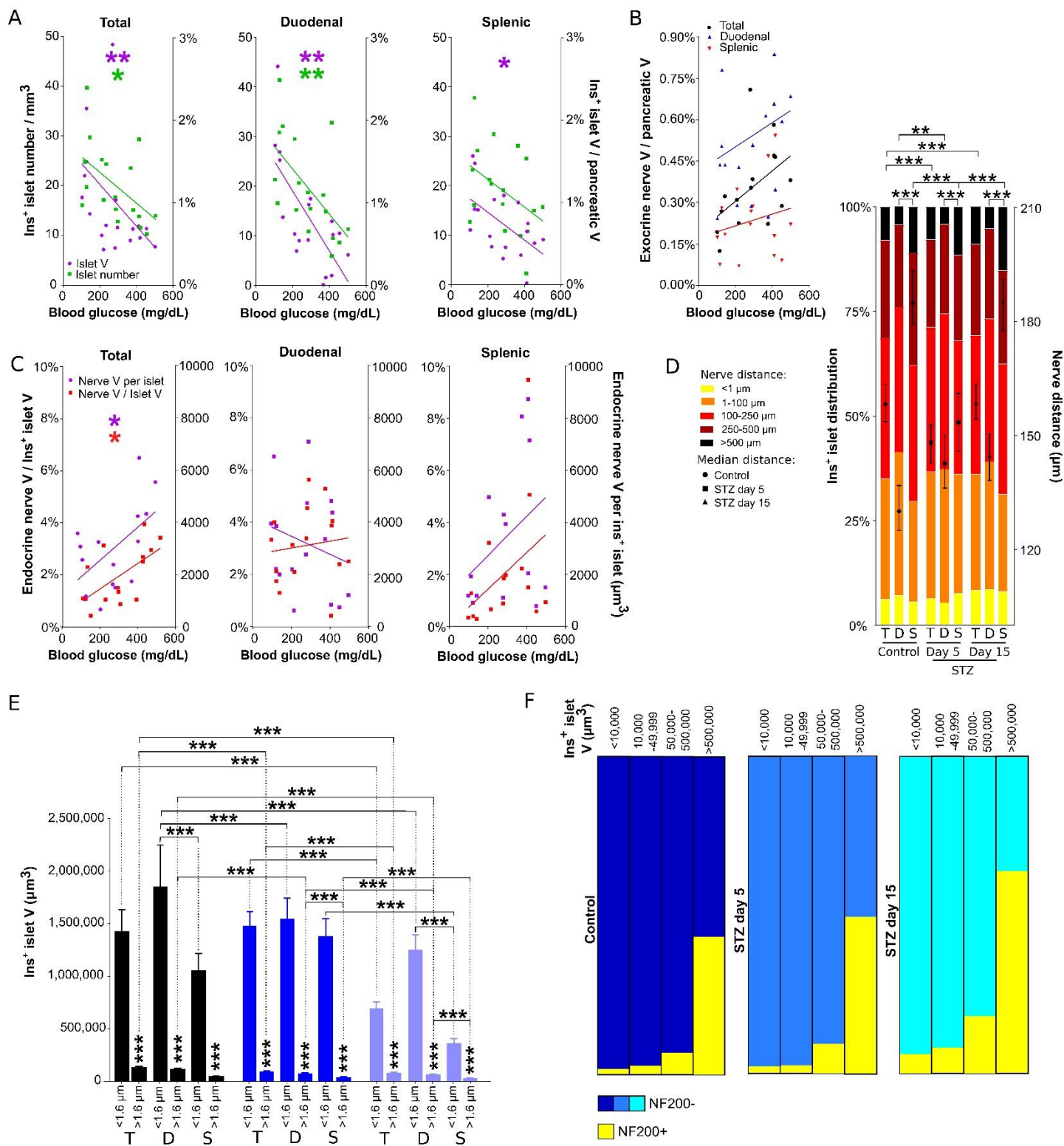


Figure S4

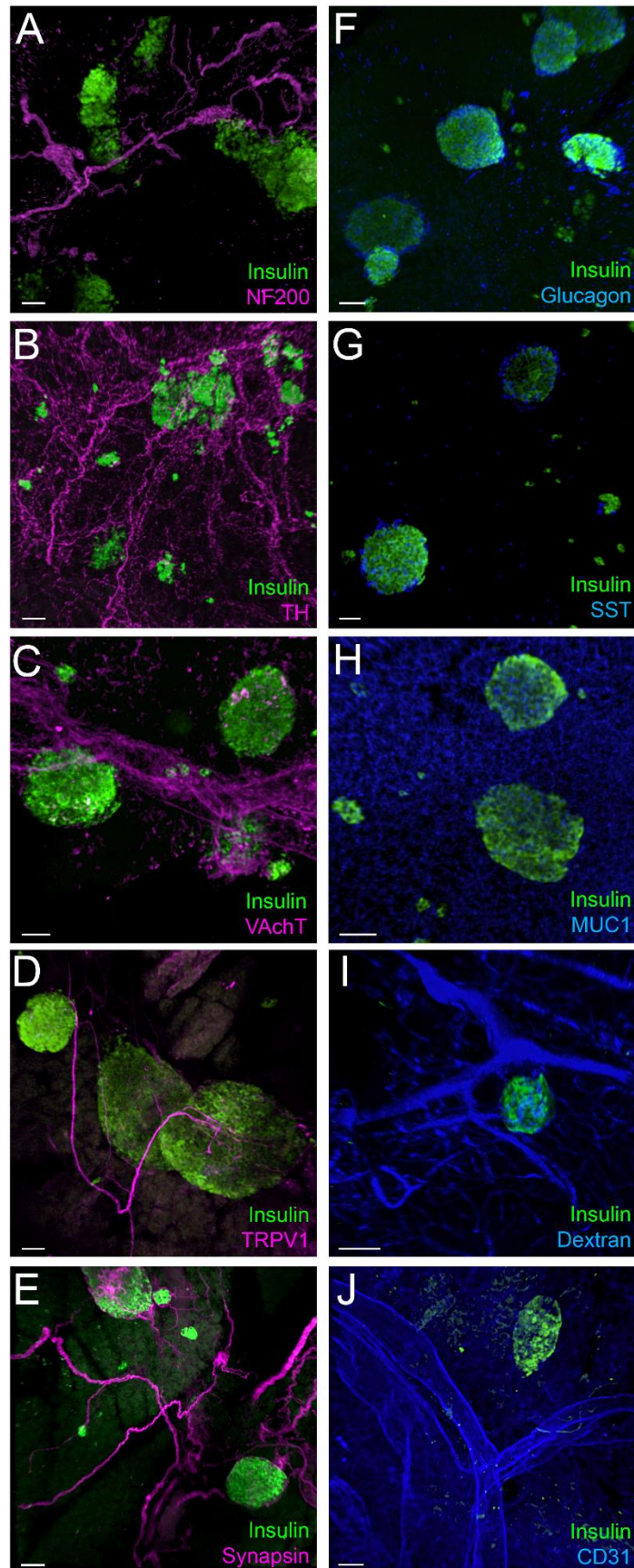


Table S1. List of evaluated antibodies.

Primary antibodies						
Target	Dilution	Host species	Manufacturer	Product number	RRID number	
Insulin	1:1000	Guinea pig	Dako / Agilent	A0564	AB_10013624	
Neurofilament 200 kDA	1:500	Rabbit	Sigma-Aldrich	N4142	AB_477272	
Tyrosine Hydroxylase	1:500	Rabbit	Millipore	AB152	AB_390204	
Vesicular Acetylcholine Transporter	1:500	Rabbit	Synaptic Systems	139 103	AB_887864	
Glucagon	1:200	Rabbit	Cell Signaling Technology	2760	AB_659831	
Glucagon	1:2000	Mouse	Sigma-Aldrich	G2654	AB_259852	
Somatostatin	1:1000	Goat	Santa Cruz	SC7819	AB_2302603	
Mucin 1	1:200	Armenian hamster	Thermo Fisher Scientific	MA5-11202	AB_11000874	
Transient receptor potential cation channel subfamily V member 1	1:500	Rabbit	Alomone labs	ACC-030	AB_2313819	
Synapsin	1:500	Rabbit	Cell Signaling Technology	5297	AB_2616578	
Insulin	1:500	Rat	R&D Systems	MAB1417	AB_2126533	
CD31	50 mg/ml	Rat	Biologend	102515	AB_2161030	
Secondary antibodies						
Target	Conjugate	Dilution	Host species	Manufacturer	Product number	RRID number
Guinea pig	Alexa Fluor® 647	1:500	Donkey	Jackson ImmunoResearch	706-605-148	AB_2340476
Rabbit	Alexa Fluor® 647	1:500	Donkey	Jackson ImmunoResearch	711-605-152	AB_2492288
Rabbit	Alexa Fluor® 594	1:500	Donkey	Invitrogen	A-21207	AB_141637
Rabbit	Alexa Fluor® 546	1:500	Donkey	Thermo Fisher Scientific	A10040	AB_2534016
Mouse	Alexa Fluor® Plus 647	1:500	Donkey	Invitrogen	A32787	AB_2762830
Goat	Alexa Fluor® 546	1:500	Donkey	Thermo Fisher Scientific	A-11056	AB_2534103
Armenian Hamster	Alexa Fluor® 594	1:500	Goat	Jackson ImmunoResearch	127-585-099	AB_2338998
Rat	DyLight® 550	1:500	Donkey	Thermo Fisher Scientific	SA5-10027	AB_2556607
Rat	Alexa Fluor® 488	1:500	Donkey	Thermo Fisher Scientific	A-21208	AB_25357794