

**Supplemental Information**

**Dysfunction of Persisting  $\beta$  Cells Is a Key  
Feature of Early Type 2 Diabetes Pathogenesis**

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## SUPPLEMENTAL MATERIAL

### **Dysfunction of Persisting $\beta$ -Cells is a Key Feature of Early Type 2 Diabetes Pathogenesis**

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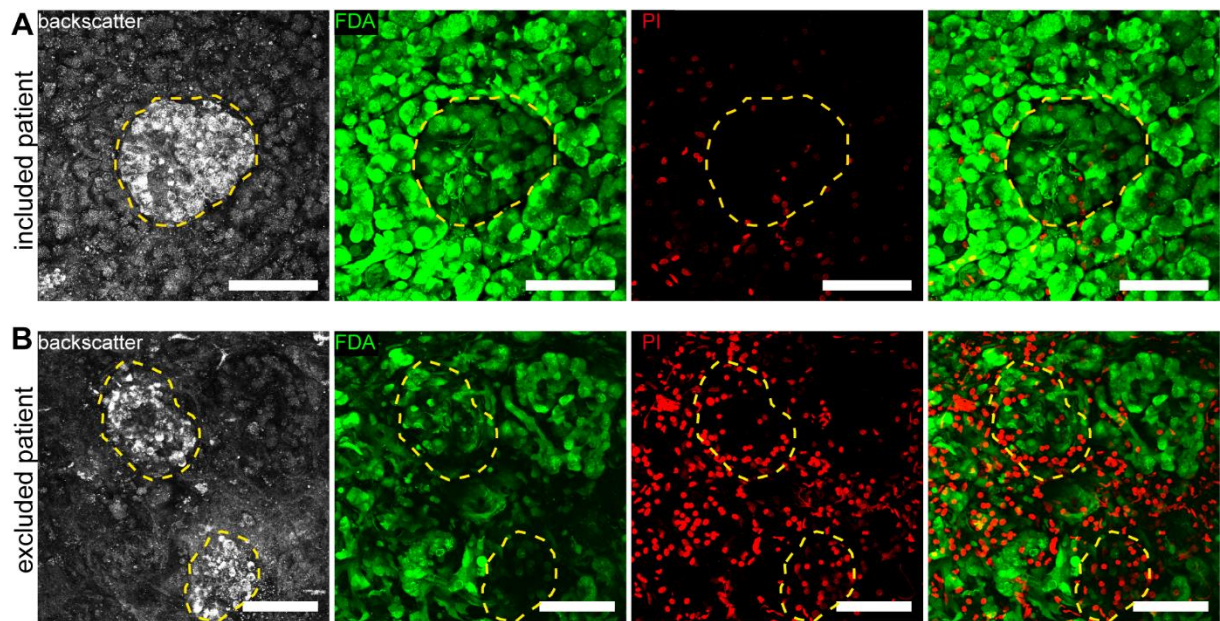
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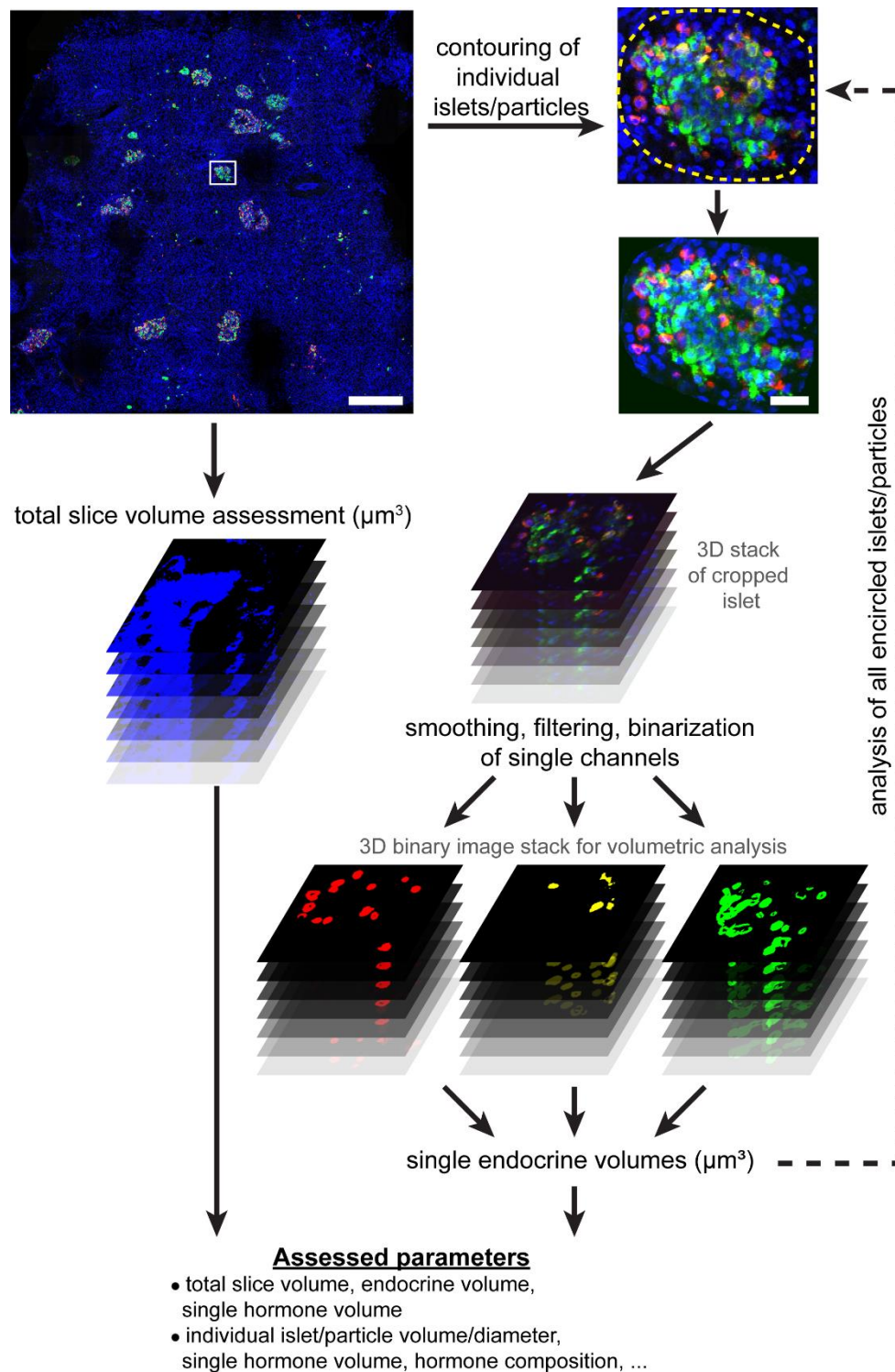
<sup>9</sup>These authors contributed equally to this work.

No.	gender	age	BMI	diabetic status	tumor type	surgical method	pancreas region	diabetes duration (yrs)	medication	insulin supplement	HbA1c (%)	fasting BG (mmol/L)	2h BG during oGTT (mmol/L)	fasting insulin (pmol/L)	fasting C-peptide (nmol/L)	fasting proinsulin (pmol/L)	insulin : proinsulin ratio	HOMA2-IR	HOMA2-%B
1	M	69	31.2	ND	pseudocyst	PL	tail	-	-	-	5.1	5.76	7.20	110	1.16	1.8	61.11	2.67	140.00
2	M	64	24.4	ND	adenocarcinoma	PPPD	head	-	-	-	4.8	5.29	6.75	120	1.27	2.7	44.44	2.84	174.90
3	M	62	24.7	ND	adenocarcinoma	PPPD	head	-	-	-	5	4.73	5.50	30	0.41	1.7	17.65	0.89	98.90
4	M	52	32	ND	metastasis renal cancer	PL	tail	-	-	-	5.3	5.84	5.50	60	0.59	1.5	40.00	1.37	84.50
5	F	72	39.4	IGT	pancreatic dysplasia	total pancreatectomy	tail	-	-	-	5.3	5.98	9.54	170	1.74	1.5	113.33	4.03	175.70
6	M	67	22.4	IGT	adenocarcinoma	Whipple	head	-	-	-	4.9	5.49	10.35	70	1.14	2.8	25.00	2.58	151.10
7	M	67	31.7	IGT	IPMN	PPPD	head	-	-	-	5.2	5.30	8.97	30	0.46	1.6	18.75	1.03	85.70
8	F	79	28.1	IGT	adeno-neuroendocrine carcinoma	PPPD	head	-	-	-	5.8	4.90	10.91	90	1.28	2.8	32.14	2.79	203.00
9	M	74	36.0	T2D	IPMN	Whipple	head	15	-	X	7.4	14.00	-	50	0.66	2.2	22.73	2.16	20.30
10	M	76	31.8	T2D	solitary fibrous tumor	PPPD	head	17	Metformin	X	6.6	9.45	-	40	0.81	1.4	28.57	2.18	44.60
11	F	63	22.8	T2D	adenocarcinoma	total pancreatectomy	head	3	diet	-	7.2	7.72	-	90	0.96	3.7	24.32	2.42	71.90
12	F	80	23.3	T2D	adenocarcinoma	total pancreatectomy	head	10	Metformin	X	7.3	9.02	-	30	0.95	3.1	9.68	2.52	54.50
13	F	66	27.8	T2D	adenocarcinoma	PPPD	head	20	EMPA, Metformin	-	7.7	10.11	-	30	0.87	4.4	6.82	2.40	42.10
14	F	71	34.9	T2D	carcinoma of distal bile duct	total pancreatectomy	head	6	SGLT-2i, Metformin	X	6.7	9.61	-	30	0.52	3.3	9.09	1.41	30.90

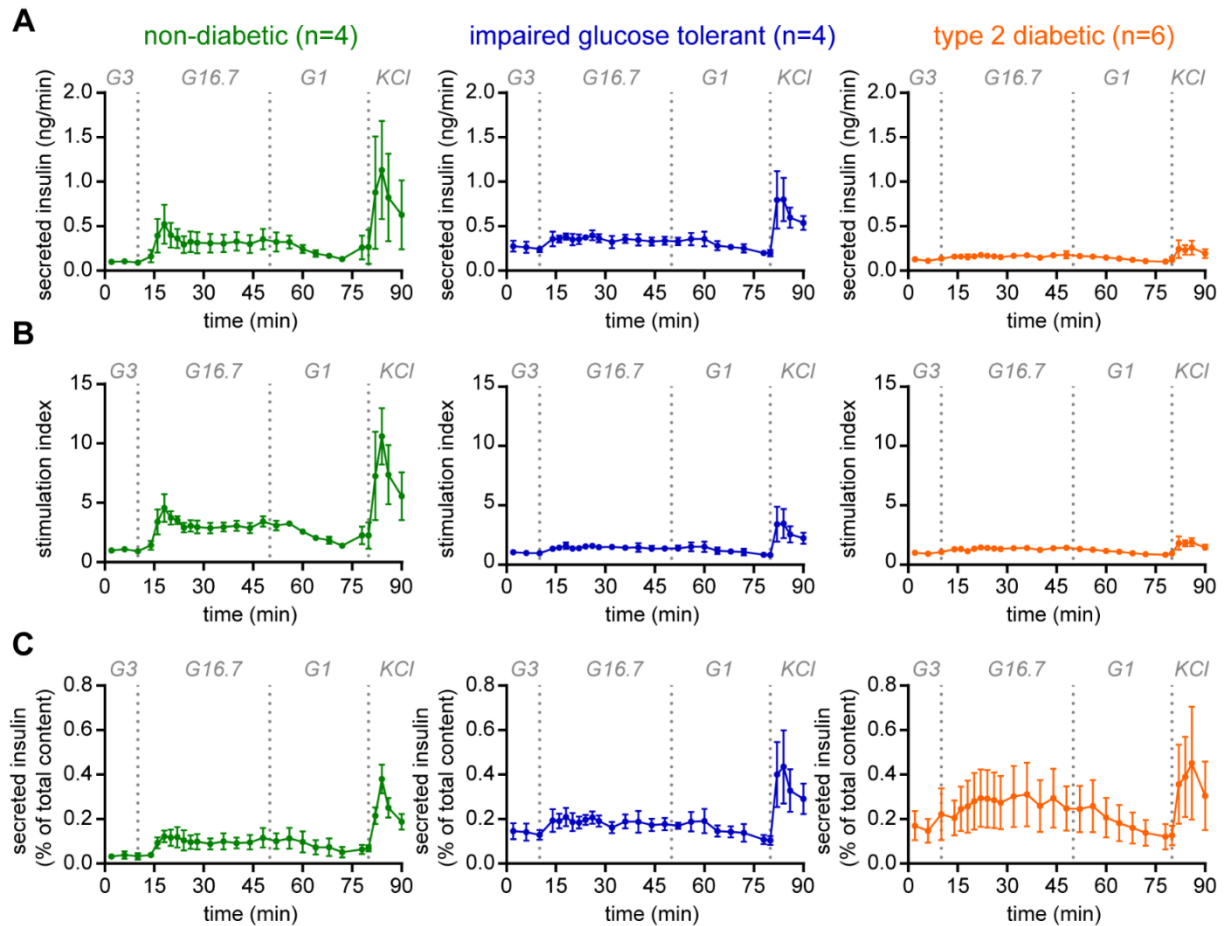
**Table S1: Related to Figure 1.** Clinical parameters of donor patient used for tissue slice study



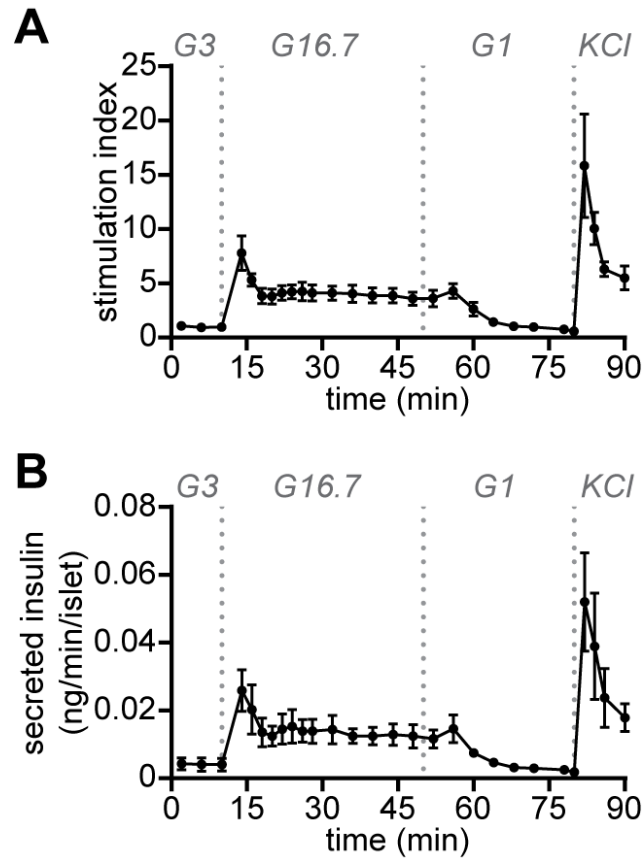
**Figure S1. Viability assessment of human pancreas tissue slices.** Related to STAR methods. Maximum intensity projections of the slice backscatter light signal (grey), FDA (green) and PI (red) as well as the merged FDA/PI signal from *A*: viable slices included in the study and *B*: from primarily dead slices where tissue of the respective patient was excluded for subsequent assays. Scale bar, 100  $\mu\text{m}$ .



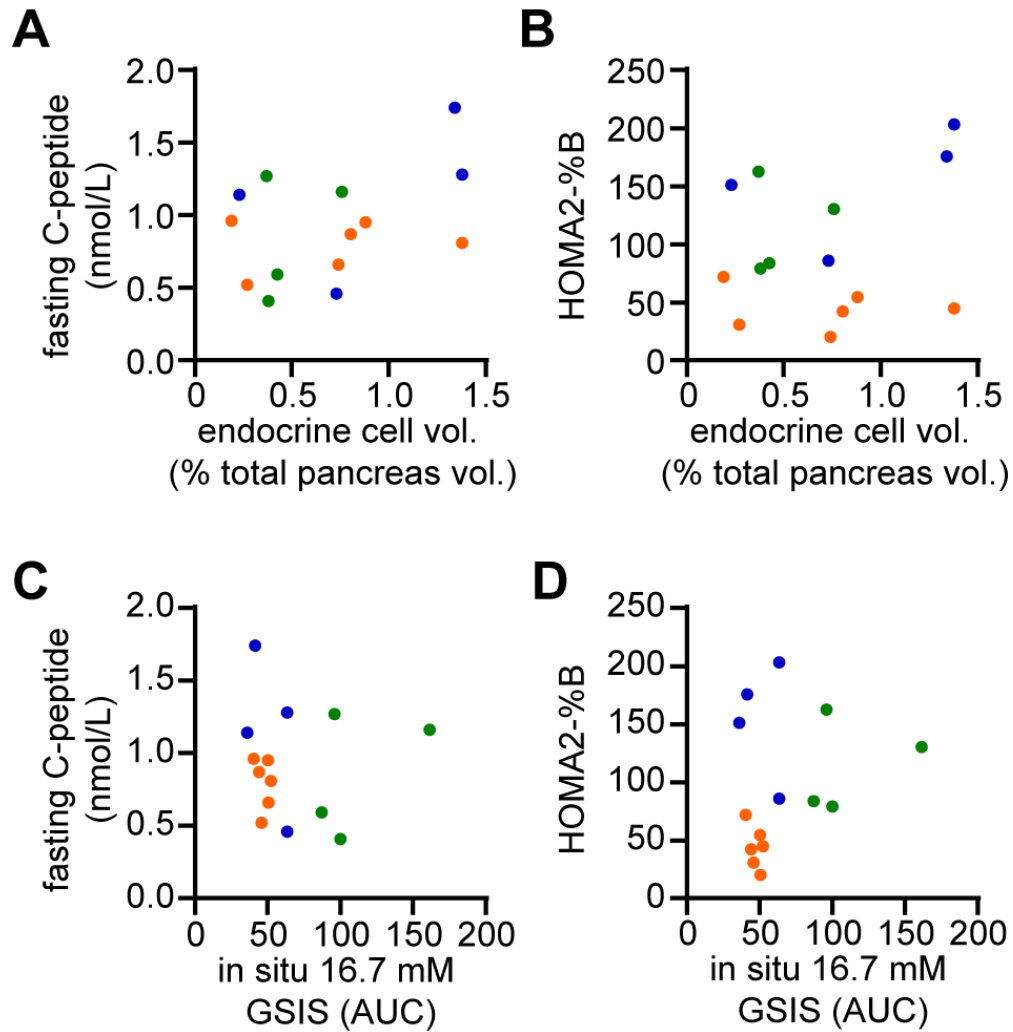
**Figure S2. Analysis pipeline for 3D histomorphometry.** Related to Figure 2. The maximum intensity projection of a stitched whole tissue slice scan will be used to contour all individual endocrine objects. These regions are then cropped out and the entire z-stack (from the original file) will be used for pre-processing (smoothing, filtering and binarization) of the single channels and then subjected to volumetric analysis to resulting in individual endocrine volumes that can be used for further analyses. In addition, the whole tissue volume of the slice is assessed volumetrically (using all fluorophores imaged). Scale bars = 500  $\mu\text{m}$  (overview image), 30  $\mu\text{m}$  (single islet image)



**Figure S3. Separate traces of insulin secretory pattern.** Related to Figure 3. Separate traces of ND (green), IGT (blue) and T2D (orange) pancreas tissue slices expressed as *A*: as absolute secretion in ng/min, *B*: fold increase to basal secretion within the first 10 minutes and *C*: % of total content. G3 = KRBH buffer containing 3mM glucose, G16.7mM = KRBH buffer with 16.7mM glucose, G1 = KRBH buffer containing 1mM glucose, KCl = KRBH buffer with 16.7mM glucose and 60mM KCl (f.c.). ND n=4, IGT n=4, T2D n=6 with data presented as mean  $\pm$  SEM.

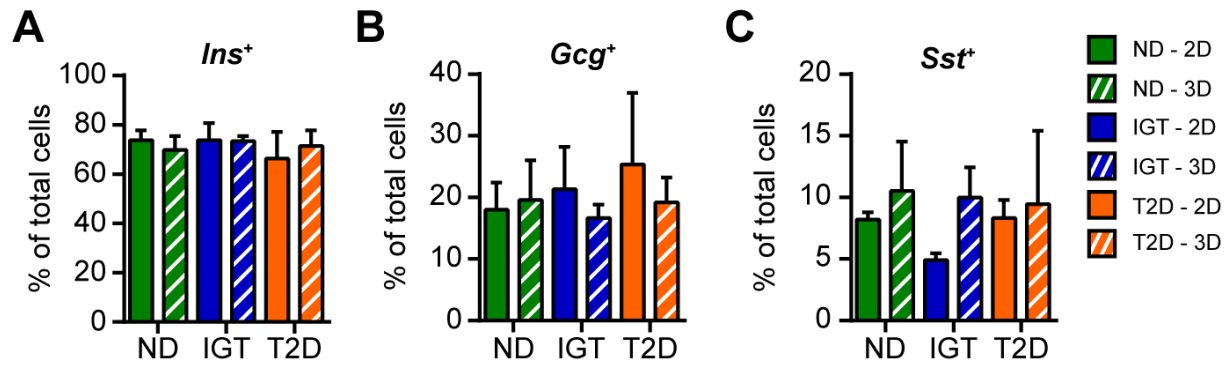


**Figure S4.  $\beta$ -cell function in isolated human islets.** Related to Figure 3. Insulin secretory pattern during perfusion of isolated human islets provided by King's College London after shipment and overnight rest from ND donors ( $n=4$ ) expressed as A: fold increase to basal secretion within the first 10 minutes (3mM glucose) and B: as absolute secreted insulin per minute for one islet. G3 = KRBH buffer containing 3mM glucose, G16.7 = KRBH buffer with 16.7mM glucose, G1 = KRBH buffer containing 1mM glucose, KCl = KRBH buffer with 16.7mM glucose and 60mM KCl (f.c.). Data is presented as mean  $\pm$  SEM. Donor information: male, age: 24-49 years, BMI: 23.55 – 34.02



**Figure S5. Association of clinical parameters with  $\beta$ -cell function and mass.** Related to Figure 4. *A-B*: Correlation of endocrine cell volume (see Fig. 2D) with *A*: fasting C-peptide and *B*: HOMA2-%B. *C-D*: Correlation of AUC values from insulin secretion (stimulation index) during stimulating conditions with 16.7mM glucose (see Fig. 3) with *C*: fasting C-peptide and *D*: HOMA2-%B. ND (green)  $n=4$ , IGT (blue)  $n=4$ , T2D (orange)  $n=6$ .





**Figure S6. Comparison of 2D and 3D islet morphometry.** Related to STAR methods. A-C: Total endocrine cell fraction for A: insulin<sup>+</sup>, B: glucagon<sup>+</sup> and C: somatostatin<sup>+</sup> cells separated by disease type and analysis method. 2D analysis was conducted by manual counting of 3 individual planes within a stack with at least 15  $\mu\text{m}$  separation (2D). A total of 10-20 islets per patient were analyzed with islet diameter ranging between 35 and 265  $\mu\text{m}$ . For islet compositional analysis from the automated whole slice 3D analysis an islet size threshold of >35  $\mu\text{m}$  was used to be comparable to the manual 2D analysis. ND n=3, IGT n=4, T2D n=5 with data presented as mean  $\pm$  SEM; statistical analysis was performed between 2D and 3D values by unpaired two-tailed t-tests at 95% CI.