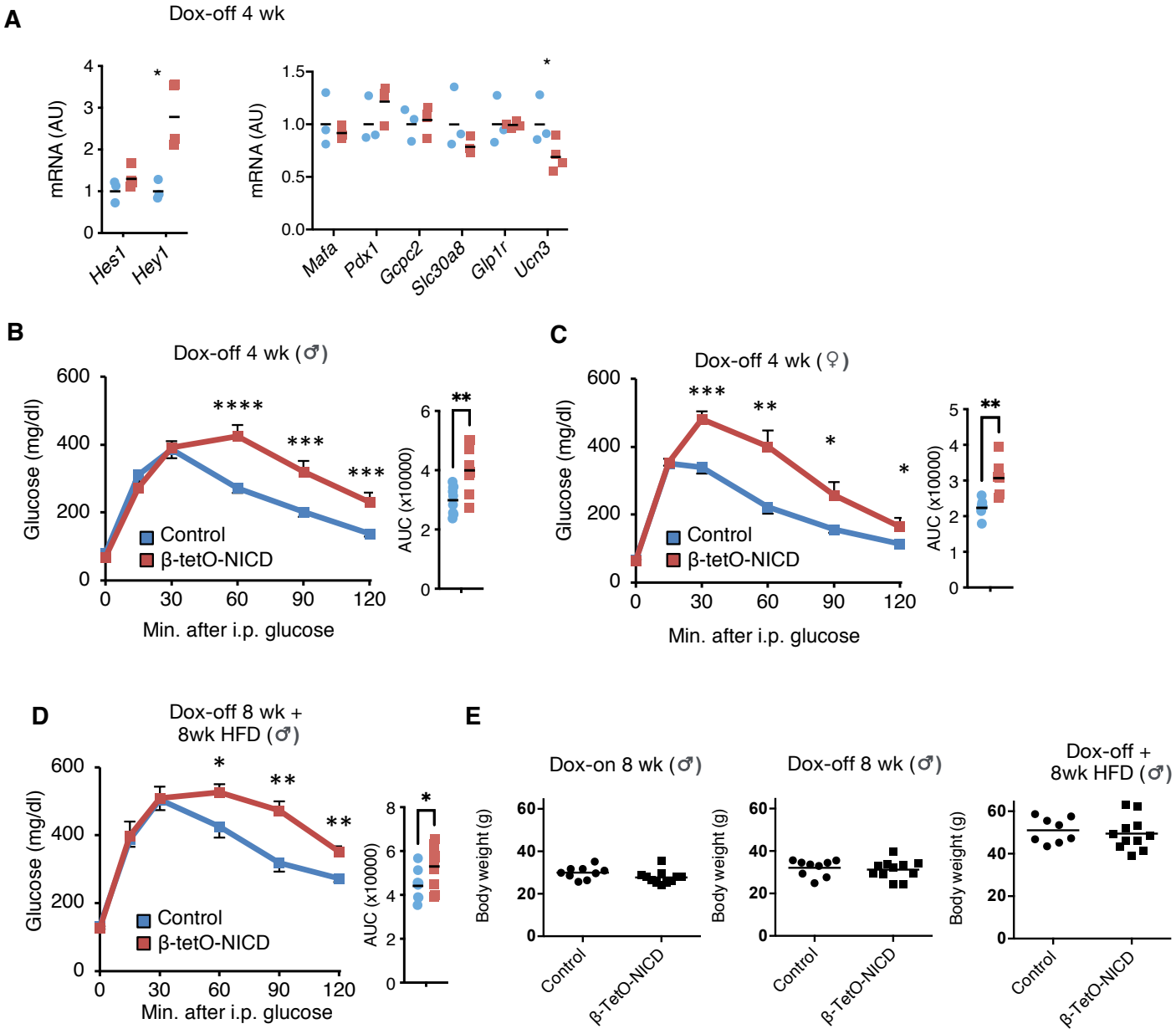
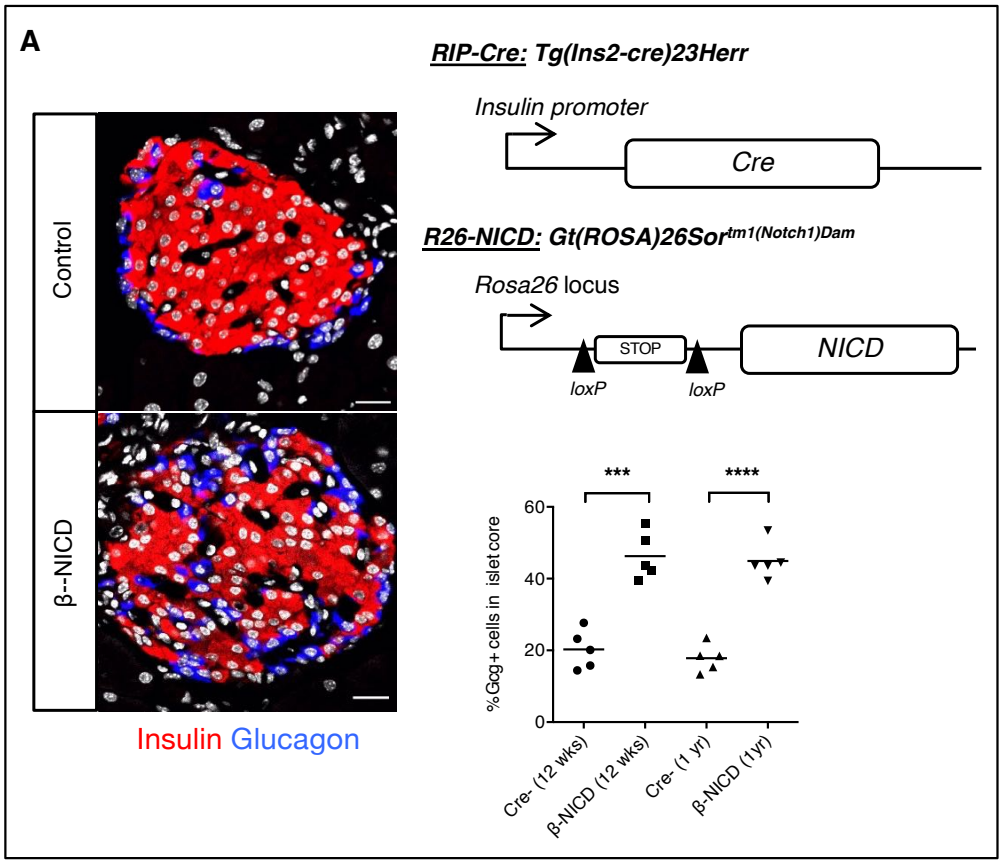
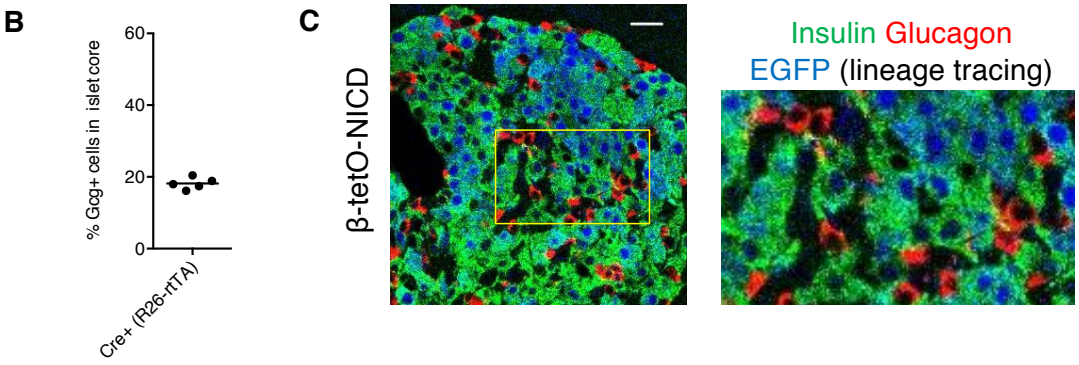
Supplemental Figure S1



Supplemental Figure S1. Further characterization of β-tetO-NICD mice.
(A) Notch target (left) and β cell maturity (right) gene expression in islets isolated from β-tetO-NICD and *Cre*$^{-}$ mice after 4 weeks of Dox withdrawal (N=3-4 mice/group).
(B) GTT in males and (C) females after 4 weeks of Dox withdrawal. (N=7-9 mice/group). AUC: area under curve (mg × dl^{-1} × min).
(D) GTT in Dox-off β-tetO-NICD and control male mice, followed by 8 weeks HFD (total 16 weeks Dox withdrawal, N=9-11 mice/group).
(E) Body weight in Dox 8 weeks (left), Dox-off 8 weeks (middle), 8 weeks Dox-off followed by 8 weeks HFD (right) male mice (N=9-11 mice/group).
All data are shown with group means; *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$ by two-tailed *t* test.

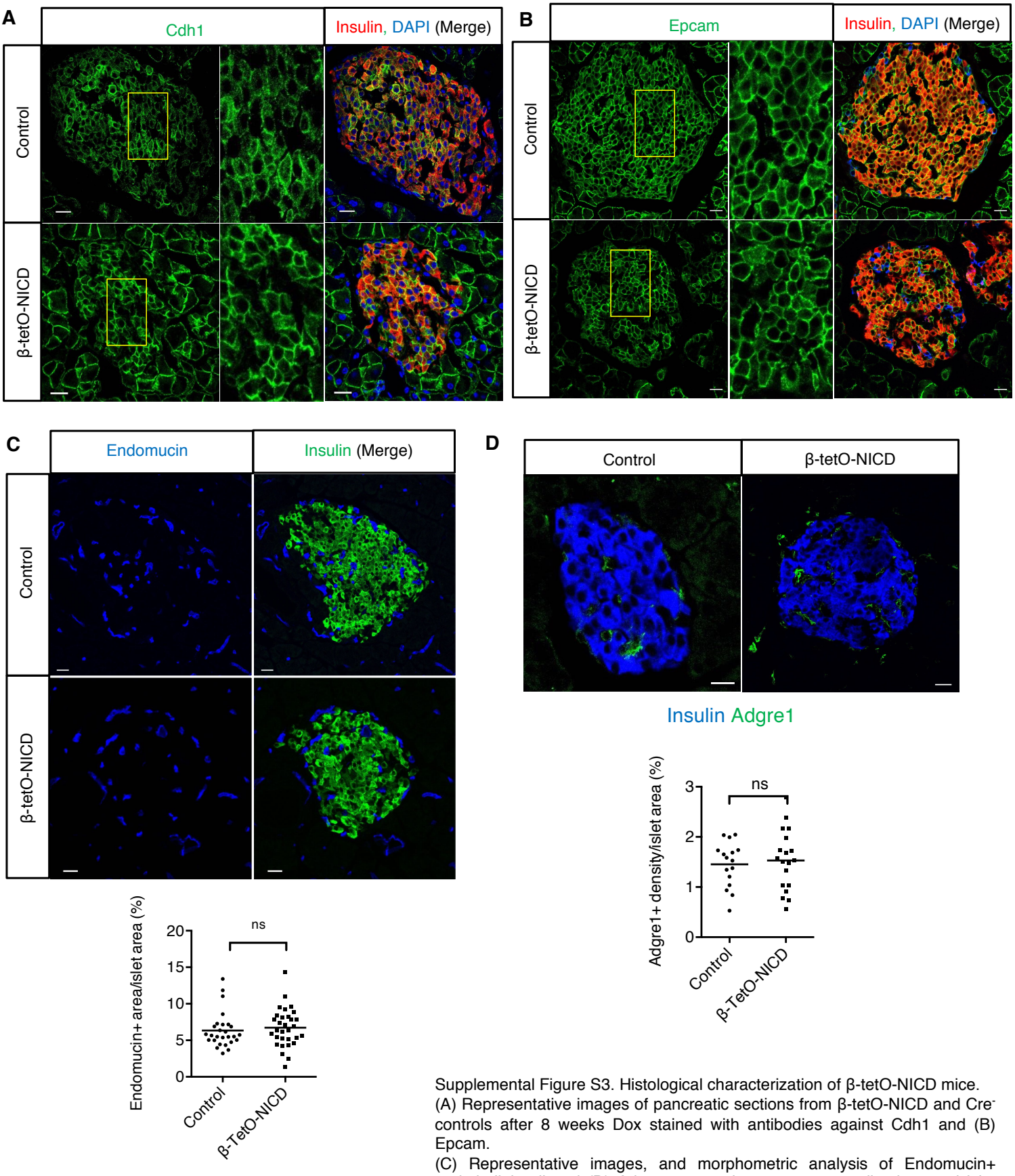
Supplemental Figure S2





Supplemental Figure S2. Islet architectural abnormalities in Notch gain-of-function mice.
(A) Generation of β-NICD mice, and representative images of pancreatic sections from β-NICD and *Cre*$^{-}$ control mice, with quantification of Gcg+ cells in the islet core in 12 week and 1 year old mice (N=5 mice/group).
(B) Quantification of Gcg+ cells in the islet core of 8 week old *Cre*$^{+}$*; Rosa26-rtTA*$^{+}$*; tetO-NICD*$^{-}$ control mice.
(C) Representative image of pancreatic sections from Dox-off β-tetO-NICD mice. EGFP is expressed in all cells that underwent Cre-mediated recombination in the Rosa26 locus due to NICD-EGFP bicistronic expression). EGFP staining is only observed in Ins+ cells. Scale bars: 20 μm

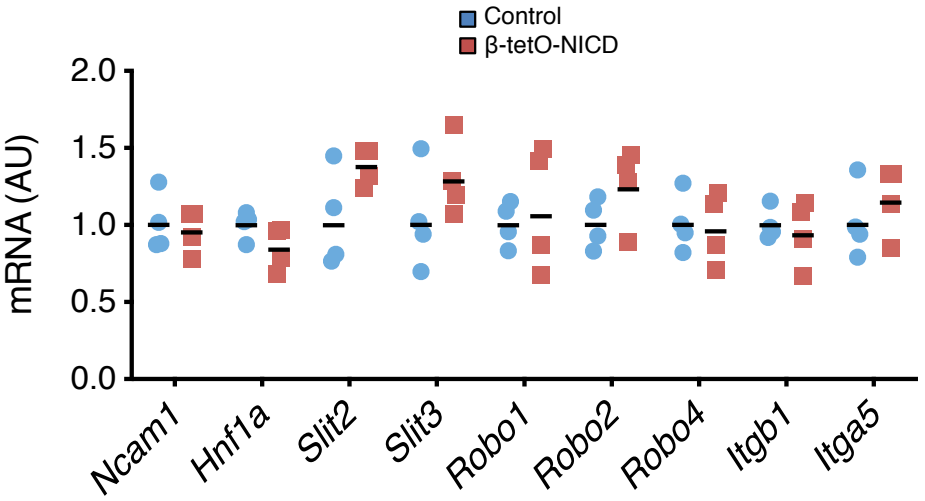
Supplemental Figure S3



Supplemental Figure S3. Histological characterization of β-tetO-NICD mice. (A) Representative images of pancreatic sections from β-tetO-NICD and Cre$^-$ controls after 8 weeks Dox stained with antibodies against Cdh1 and (B) Epcam.

(C) Representative images, and morphometric analysis of Endomucin+ endothelial cell and (D) Adgre+ macrophage area, normalized to total islet area in β-tetO-NICD and *Cre*$^-$ controls after 8 weeks Dox. Individual islets from at least 4 mice/group are plotted. Scale bars: 20 μm

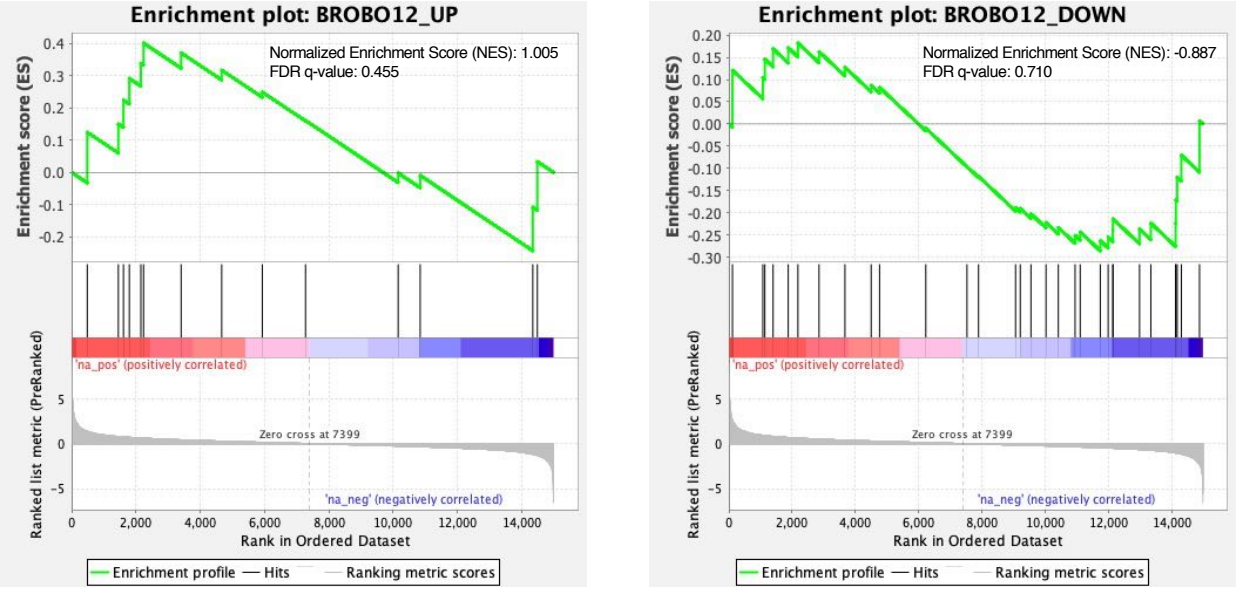
Supplemental Figure S4



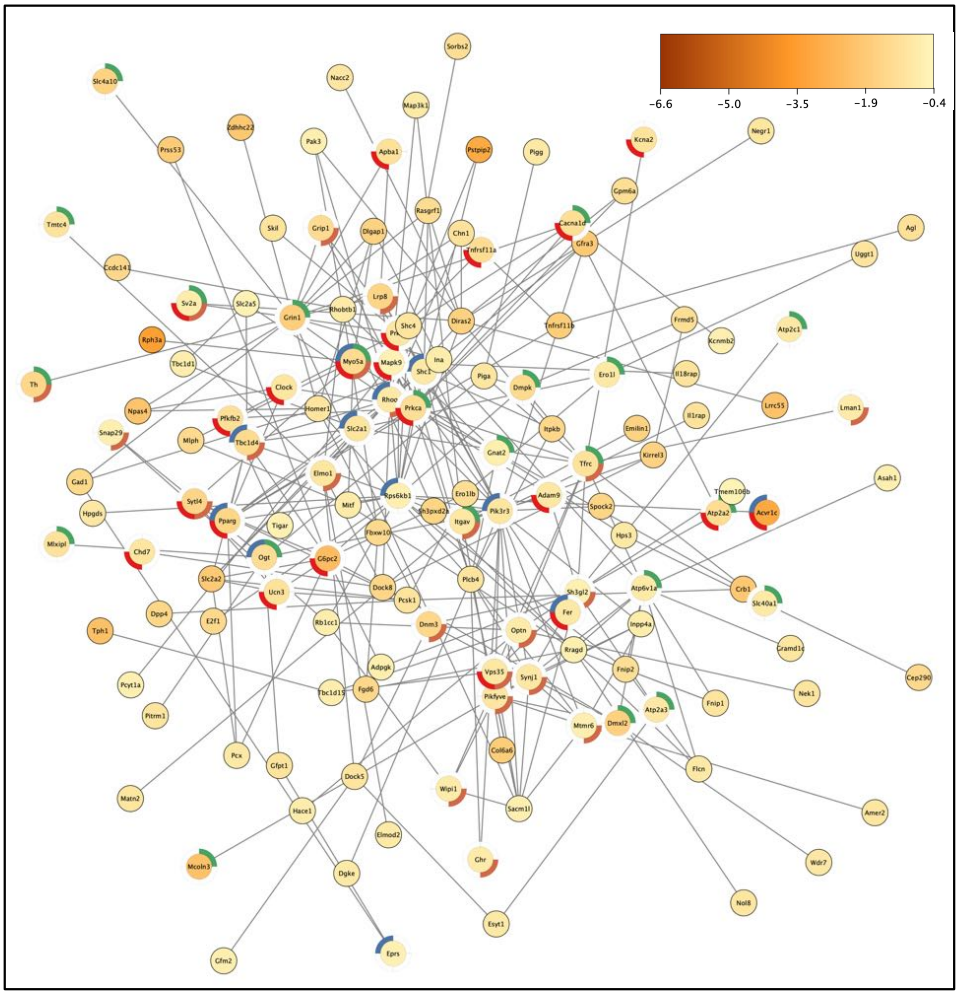
Supplemental Figure S4. Unchanged known morphogenetic pathways in Notch-active islets.
Gene expression of other described islet morphogenetic effectors in islets from β-tetO-NICD and *Cre*$^{-}$ controls after 8 weeks Dox. N=4 mice/group. All data are shown with group means.

Supplemental Figure S5

A



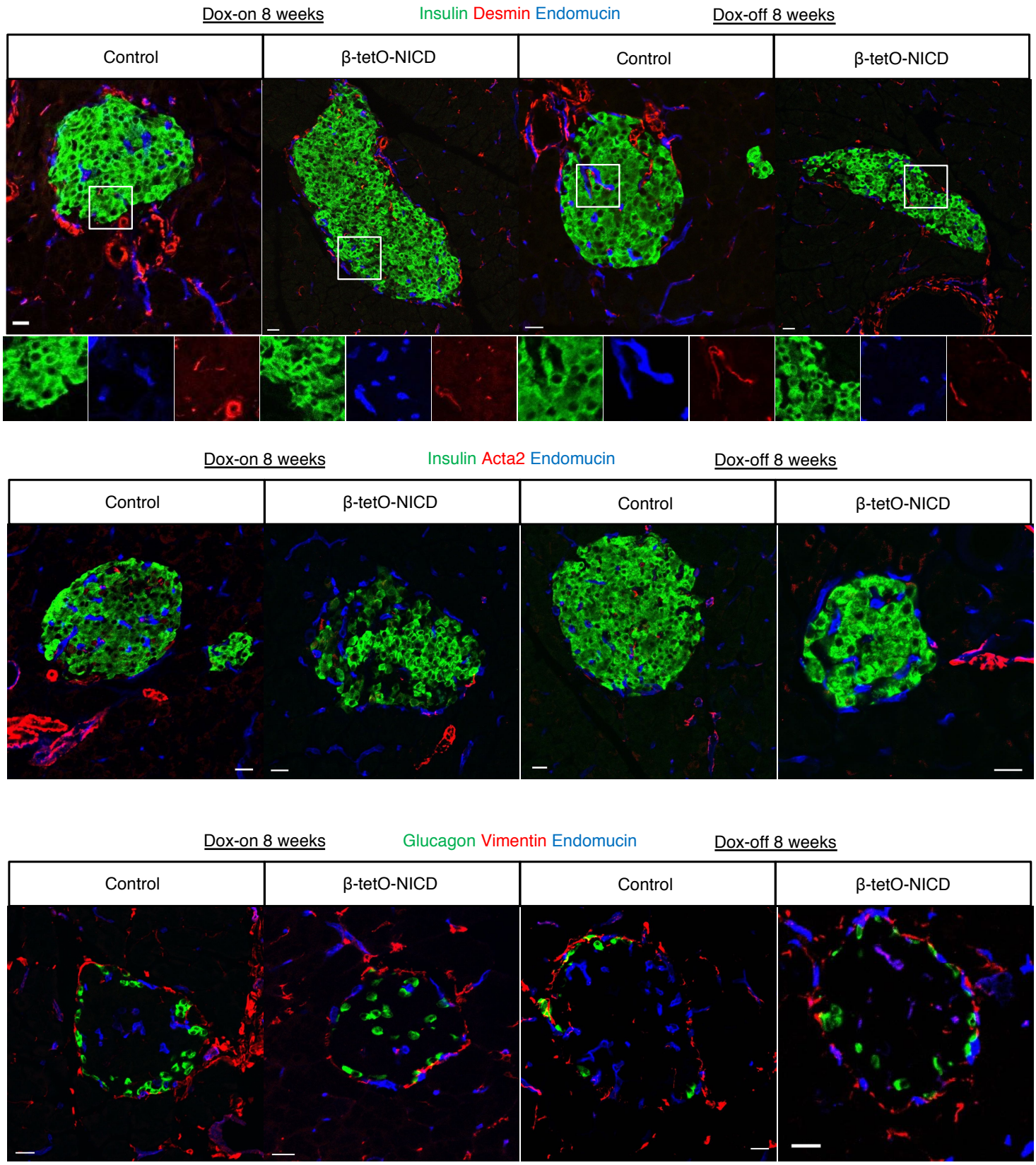
B



- Vesicle-mediated transport
- Cellular chemical homeostasis
- Regulation of secretion
- Response to insulin

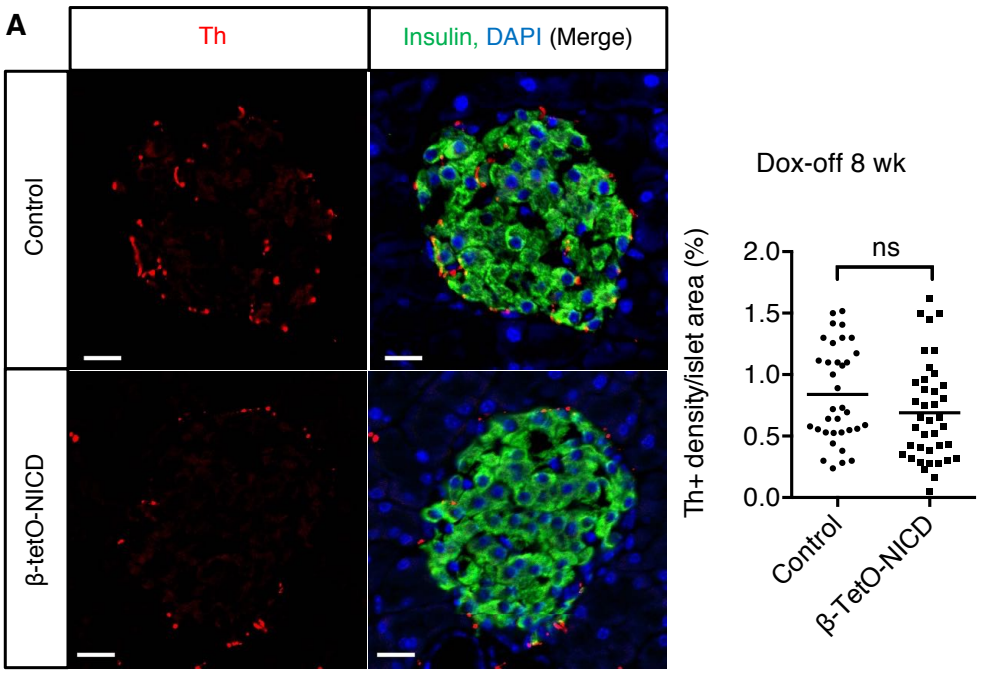
Supplemental Figure S5. RNA-seq characterization of Notch-active islets
(A) Gene set enrichment analysis, comparing upregulated (left) and downregulated (right) genes in Robo1/2 dKO β cells (Adams et al, 2021) with β-tetO-NICD islets.
(B) STRING network from downregulated DEGs in β-tetO-NICD islets. Enrichment analysis of processes associated with the network, highlighting processes associated with β cell secretory function.

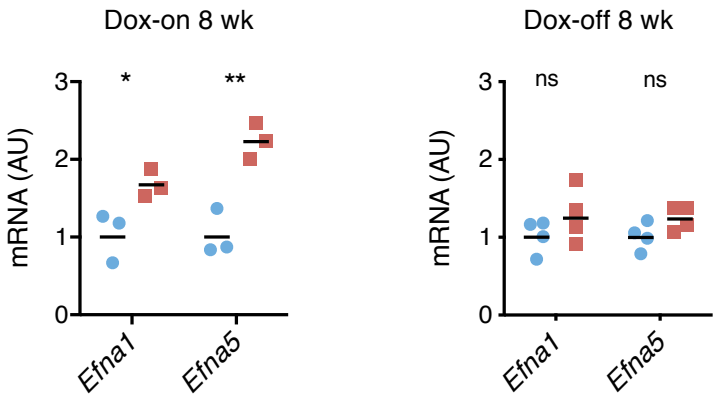
Supplemental Figure S6



Supplemental Figure S6. Unchanged fibrosis markers in Notch-active islets.
Representative image of pancreatic sections from β-tetO-NICD and control mice, either after 8 weeks Dox or 8 weeks Dox-off, stained with fibrosis markers Desmin (top), Acta2 (middle) and Vimentin (bottom). Scale bars: 20 µm

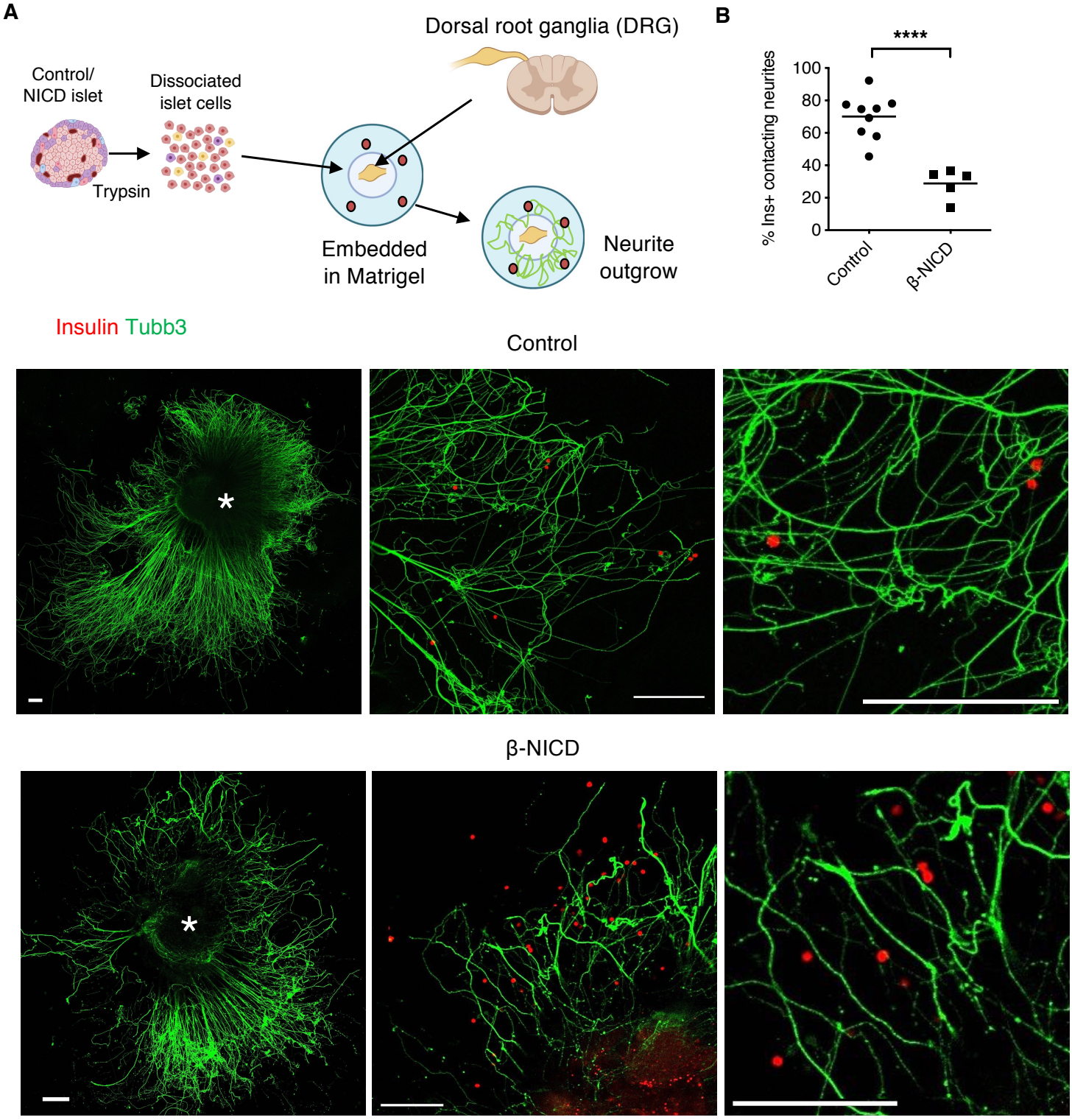
Supplemental Figure S7





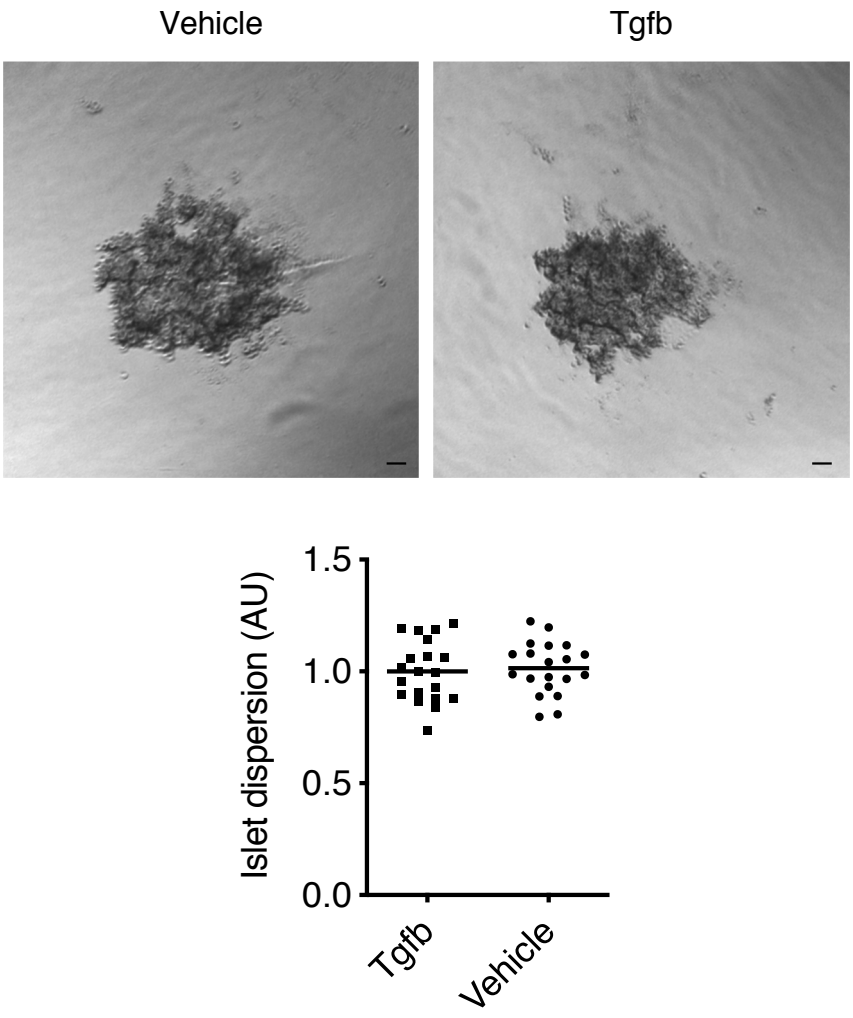
Supplemental Figure S7. Efna5 expression and islet innervation is dependent on continued Notch activity.
(A) Representative images and morphometric analysis of tyrosine hydroxylase (Th) positive area in Dox-off β-tetO-NICD and *Cre*$^{-}$ controls. Individual islets from at least 5 mice/group are plotted. Scale bars: 20 μm
(B) Ephrin ligand expression in islets isolated from β-tetO-NICD and *Cre*$^{-}$ mice after 8 weeks Dox (left) or 8 weeks Dox-off (right, N=4 mice/group).
All data are shown with group means; *, $P < 0.05$, **, $P < 0.01$ by two-tailed *t* test.

Supplemental Figure S8



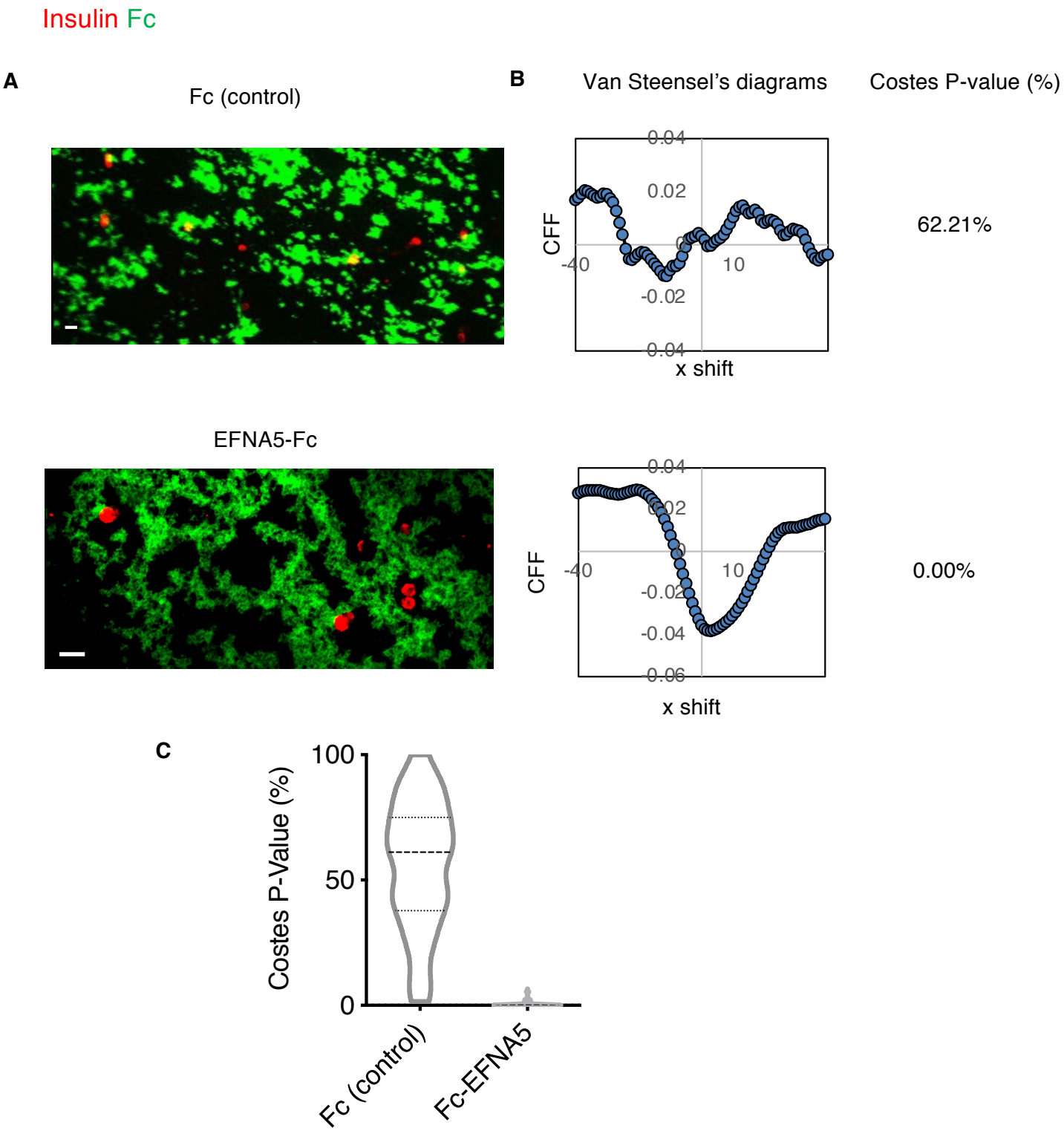
Supplemental Figure S8. Reduced neurite-β cell contact in Notch active β cells.
(A) Dorsal root ganglia (DRG) from WT mice were embedded in a Matrigel drop. Islet cells were dispersed and seeded in a Matrigel disk around the DRG explant, then cultured with nerve growth factor for one week.
(B) Representative images in cells fixed and stained with insulin (Ins) and Tubb3, and quantification on Ins+ cells contacting neurites. Scale bars: 200 μm.
(C) Three-dimensional reconstruction from z-stacks of control and β-NICD mice.
All data are shown with group means, ****, $P < 0.0001$ by two-tailed *t* test.

Supplemental Figure S9



Supplemental Figure S9. TGFβ does not affect islet dispersion. Representative image and quantitation of pseudoislet formation dispersed islet cells from WT mice, in the presence of recombinant 10 ng/ml TGFβ or vehicle. Data plotted from individual pseudoislets, pooled from 3 independent experiments. All data are shown with group means. Scale bars: 20 μm

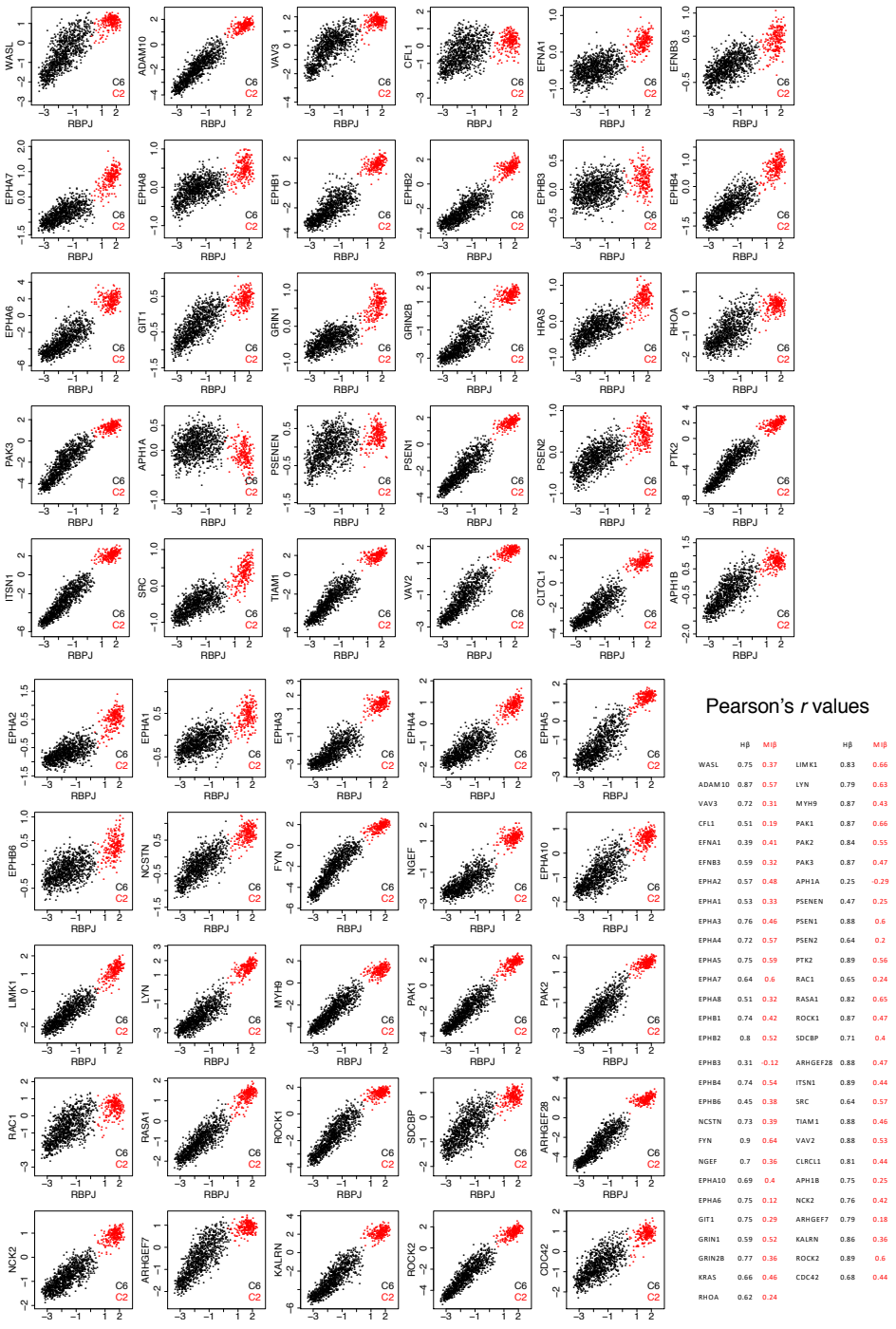
Supplemental Figure S10



Supplemental Figure S10. Image analysis algorithms used for cell adhesion assays.
(A) Representative images of dispersed β cells seeded onto Fc (top) or EFNA5-Fc-coated surfaces (bottom), showing insulin and Fc staining. Scale bars: 20 µm.
(B) Van Steensel's cross correlation function (CFF) for cell adhesion assays. The diagram represents the variation in the correlation of green/red pixels as a function of the lateral shift of the green channel. A dip in x=0 indicates perfect anti-correlation with EFNA5-Fc (bottom graph), while cells adhered to Fc show random distribution.
(C) Distribution of Costes P-value (%) for all images analyzed.

Supplemental Figure S11

A



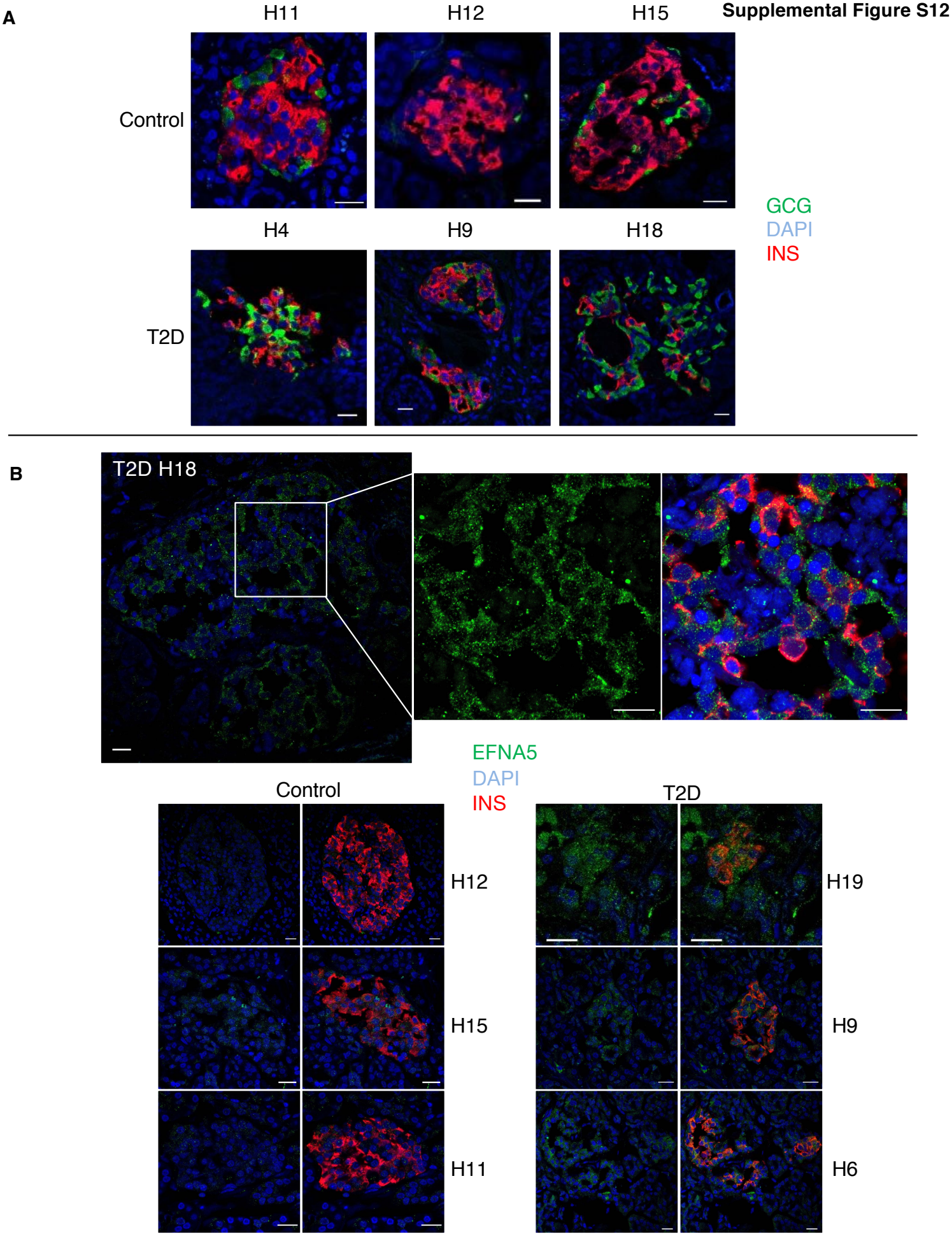
Pearson's *r* values

	Hβ	MIβ		Hβ	MIβ
WASL	0.75	0.37	LIMK1	0.83	0.66
ADAM10	0.87	0.57	LYN	0.79	0.63
VAV3	0.72	0.31	MYH9	0.87	0.43
CFL1	0.51	0.19	PAK1	0.87	0.66
EFNA1	0.39	0.41	PAK2	0.84	0.55
EFNB3	0.59	0.32	PAK3	0.87	0.47
EPHA2	0.57	0.48	APH1A	0.25	-0.29
EPHA1	0.53	0.33	PSENEN	0.47	0.25
EPHA3	0.76	0.46	PSEN1	0.88	0.6
EPHA4	0.72	0.57	PSEN2	0.64	0.2
EPHA5	0.75	0.59	PTK2	0.89	0.56
EPHA7	0.64	0.6	RAC1	0.65	0.24
EPHA8	0.51	0.32	RASA1	0.82	0.65
EPHB1	0.74	0.42	ROCK1	0.87	0.47
EPHB2	0.8	0.52	SDCBP	0.71	0.4
EPHB3	0.31	-0.12	ARHGEF28	0.88	0.47
EPHB4	0.74	0.54	ITSN1	0.89	0.44
EPHB6	0.45	0.38	SRC	0.64	0.57
NCSTN	0.73	0.39	TIAM1	0.88	0.46
FYN	0.9	0.64	VAV2	0.88	0.53
NGEF	0.7	0.36	CLRCL1	0.81	0.44
EPHA10	0.69	0.4	APH1B	0.75	0.25
EPHA6	0.75	0.12	NCK2	0.76	0.42
GIT1	0.75	0.29	ARHGEF7	0.79	0.18
GRIN1	0.59	0.52	KALRN	0.86	0.36
GRIN2B	0.77	0.36	ROCK2	0.89	0.6
KRAS	0.66	0.46	CDC42	0.68	0.44
RHOA	0.62	0.24			

B

SYMBOL	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
EFNA5	39	0.441	0.0614	Yes
EPHA6	356	0.346	0.0973	Yes
EPHA3	378	0.343	0.1455	Yes
VAV2	393	0.341	0.1937	Yes
TIAM1	731	0.307	0.223	Yes
AP2A1	1084	0.281	0.248	Yes
CLTCL1	1100	0.28	0.2874	Yes
AP2B1	1348	0.267	0.315	Yes
PSEN1	1467	0.262	0.3473	Yes
EPHB2	1630	0.254	0.3767	Yes
AP2A2	1839	0.246	0.4029	Yes
FYN	2274	0.23	0.4171	Yes
LYN	2388	0.227	0.4447	Yes
EPHB4	2400	0.227	0.4768	Yes
DNM1	2692	0.219	0.4955	Yes
ADAM10	4596	0.178	0.4384	No
EPHB1	4702	0.176	0.459	No
CLTC	5163	0.167	0.463	No
YES1	6180	0.152	0.4407	No
EPHA4	6414	0.148	0.4518	No
EPHA7	6584	0.146	0.4654	No
EFNA2	7938	0.127	0.4248	No
VAV3	9632	0.105	0.3664	No
EPHB6	10161	0.099	0.3578	No
SRC	10714	0.093	0.3472	No
PSEN2	11618	0.084	0.32	No
EPHA10	11789	0.082	0.3244	No
EPHA2	12414	0.076	0.3082	No
NCSTN	12816	0.072	0.301	No
RAC1	13638	0.063	0.2744	No
EPHA5	13644	0.063	0.2832	No
EFNA1	13652	0.063	0.2919	No
APH1A	13809	0.061	0.2939	No
EPHA1	13932	0.06	0.2972	No
ACTG1	14369	0.056	0.2863	No
EFNB2	14503	0.054	0.2882	No
CLTB	15452	0.045	0.2535	No
MMP2	15555	0.044	0.2553	No
EPHB3	15952	0.04	0.2438	No
EFNA3	16059	0.039	0.2447	No
APH1B	16167	0.038	0.2455	No
CLTA	16211	0.037	0.2489	No
EFNB3	16308	0.036	0.25	No
AP2S1	20228	-0.01	0.0814	No
EFNB1	20468	-0.013	0.073	No
EFNA4	20953	-0.02	0.0548	No
MMP9	21038	-0.021	0.0542	No
ACTB	21877	-0.042	0.024	No
AP2M1	21935	-0.045	0.0279	No
EPHA8	22178	-0.057	0.0256	No
PSENEN	22671	-0.1	0.0186	No

Supplemental Figure S11. Notch-Ephrin signaling in scRNA-seq experiments.
(A) RBPJ activity as compared to EPH-Ephrin signaling components, with corresponding Pearson's *r* values. Each dot represents a single Hβ (black) and MIβ (red) cell.
(B) List of all EPH-Ephrin gene-set components enriched in ranked MIβ vs Hβ DEGs, corresponding to GSEA plot shown in Figure 7C.



Supplemental Figure S12. Further characterization of nondiabetic and T2D patient islets.
(A) Representative Glucagon (GCG), and (B) EFNA staining (with insulin, INS) of human pancreas in nondiabetic and T2D patient islets. Scale bars: 20 μm