Pax6 maintains pancreatic beta-cell identity by repressing alternative islet cell genes

Legends to Supplemental Figures and Tables

Supplementary Figure S1: Expression of β cell transcription factors in islets of db/db mice.

Expression level of Nkx6.1, MafA and Pdx1 mRNA as a function of blood glucose levels in db/db and control mice. The mice used for this analysis are the same as in figure 1E. Note the early and deeper drop in MafA expression suggesting greater sensitivity to high blood glucose levels as compared with the other factors.

Supplementary Figure S2: Pax6 deletion in adult β cells.

A. Representative images of islets from $Pax6^{lox/lox}$ (lox/lox) and MIP-CreER; $Pax6^{lox/lox}$ (βPAX6) mice stained for PAX6 (green) and insulin (blue). Mice are 3 months old, 1 week after injection of tamoxifen or vehicle. Original magnification 400x.
B. Number of Pax6 positive β cells in $Pax6^{lox/lox}$ mice (no Cre), MIP-CreER; $Pax6^{lox/lox}$; Rosa26-LSL-YFP mice without tamoxifen (no TM), and MIP-CreER; $Pax6^{lox/lox}$; Rosa26-LSL-YFP mice 1 week after tamoxifen injection (βPAX6). n=3 for each group of mice.
C. Pax6 mRNA in sorted YFP+ β cells from MIP-CreER; Rosa26-LSL-YFP (YFP, n=5) and MIP-CreER; $Pax6^{lox/lox}$; Rosa26-LSL-YFP (βPAX6, n=3) mice. Mice were 6 weeks old, 1 week after tamoxifen injection.
D. Measurements of blood glucose following tamoxifen (TM) injection in an additional cohort of βPAX6 mice showing development of severe hyperglycemia. n=24 lox/lox and 23 βPAX6 mice.
***$P < 0.001$ as determined by 2-tailed Student's *t*-test.

Supplemental Figure S3: Correlation between blood ketones and insulin or glucagon in βPAX6 mice.

Blood ketones in βPAX6 mice are plotted against plasma insulin (A) and plasma glucagon (B). Note that the highest levels of ketone bodies were observed in hyperglucagonemic mice.

Supplementary Figure S4: TUNEL staining for detection of cell death in βPAX6 islets.

Pancreatic sections from lox/lox and βPAX6 mice at different time points after tamoxifen injection as indicated, stained for insulin (red), TUNEL (green) and DNA (blue). Original magnification 400x.

Supplemental Figure S5: Immunostaining of islet hormones following Pax6 deletion.

Immunohistochemical staining of pancreatic sections from control and βPAX6 mice for chromogranin A, glucagon, somatostatin, pancreatic polypeptide and ghrelin. βPAX6 mice were 1 week or 1 month after tamoxifen. Ghrelin cells appeared shortly after Pax6 deletion, while expansion of glucagon and somatostatin cells was seen only 1 month after deletion. Original magnification 400x.

Supplemental Figure S6: Ultrastructural resemblance of granules in βPAX6 islet cells and in gastric ghrelin cells.

Left panel, EM images of cells from a βPAX6 islet, 2 months after tamoxifen administration. Original magnification 1000x or 3000x (indicated). Right panel, EM images of gastric ghrelin cells, from Kojima et al. (with permission). Image C is presented in main figure 3E and is shown here again for comparison with the gastric ghrelin cells.

Supplemental Figure S7: Origins of somatostatin and pancreatic polypeptide-expressing cells in βPAX6 mice.

Pax6-deleted β cells do not express detectable somatostatin or pancreatic polypeptide. Staining for somatostatin or pancreatic polypeptide (red), YFP (green) and insulin (blue). Original magnification 400x.

Supplemental Figure S8: Changes in protein level of key β cell factors following Pax6 ablation.

A. Representative islets stained for insulin and for β cell specific markers that were differentially expressed in βPAX6 as observed by RNA-seq. Maf-A and Glut2 were most dramatically reduced in βPAX6 islets. Original magnification 400x.
B. Elevation of Ngn3 protein in Pax6-deficient β cells. Immunohistochemistry of Ngn3 in lox/lox and βPAX6 islets, 1 week after tamoxifen. The pancreas of an embryonic day 14.5 mouse serves as a positive control. Original magnification 400x.

Supplemental Table S1

Expression levels of β cell genes following Pax6 deletion, divided into functional categories. Upregulated genes are highlighted in red and downregulated are in green. FC=fold change. We considered FC of 1.5 and FDR<0.05 as significant change in expression.

Supplemental Table S2

Expression levels of specific and general transcription factors in β cells, obtained from RNA-seq analysis of sorted beta cells from βPAX6 mice as described in Figure 5. We considered FC of 1.5 and FDR<0.05 as significant change in expression.

Supplemental Table S3

List of genes significantly changed during maturation of mouse beta cells between post-natal day 18 and p35. RNA was extracted from sorted beta cells.

Supplemental Table S4

A combined list of all Pax6 bound sites obtained from ChIP-seq analysis on min6 cells, and their association with histone marks (H3K27ac, H3K4me1, H3K27me3, H3K9ac) and TF maps (Nkx6-1, Foxa2, Pdx1, MafA, NeuroD1) as well as expression data from RNAseq analysis on βPAX6 mice (1-3). Pax6 ChIP-seq was done in three replicates and all peaks were combined for our analyses. For full description of integrated analysis see second and third tab in the S4 file.

Supplemental Table S5

A short list obtained from table S4, showing central islet genes that are directly regulated by Pax6 including details of nearby Pax6 binding sites, additional previously published TFs binding sites and histone marks as well as differential expression data from βPAX6 RNA-seq.

Supplemental Table S6

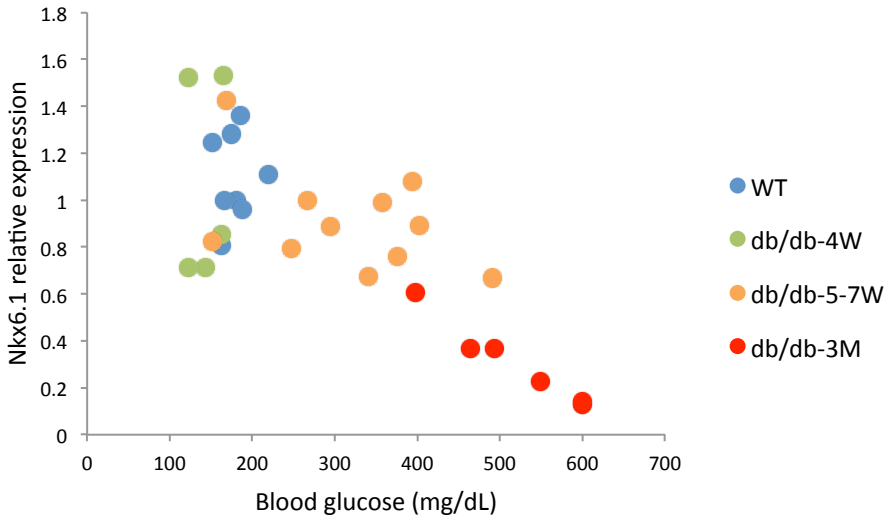
A combined list of all PAX6 bound sites obtained from ChIP-seq analysis on EndoC-betaH2 cells, and their association with histone marks (H3K27ac, H3K4me1, H3K4me3, H3K27me3) and TF maps (PDX1, NKX2-2, NKX6-1, FOXA2, MAFB) performed on human islets or sorted beta cells (4, 5). Pax6 ChIP-seq was done in two replicates and all peaks were combined for our analyses. For full description of integrated analysis see 2nd and 3rd tab in the S6 file. For a list of common genes bound by Pax6 in Min6 and EndoC cells, see 4th tab.

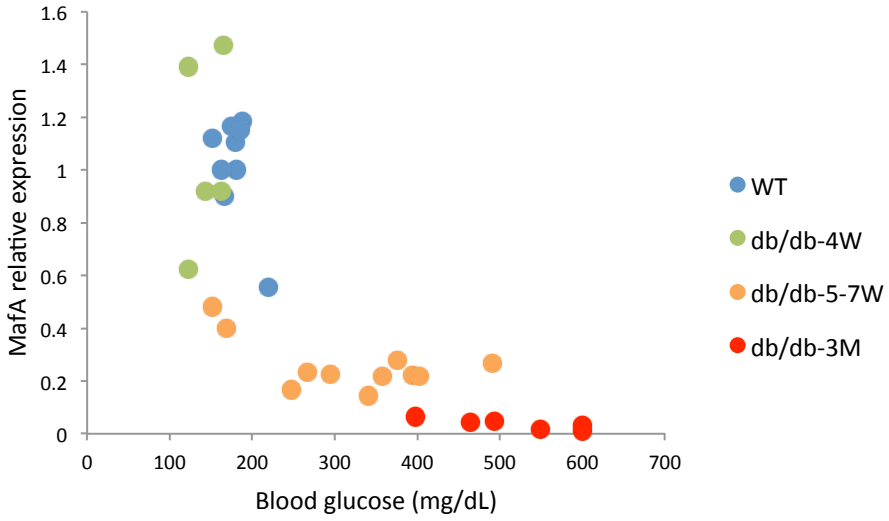
Supplemental Table S7

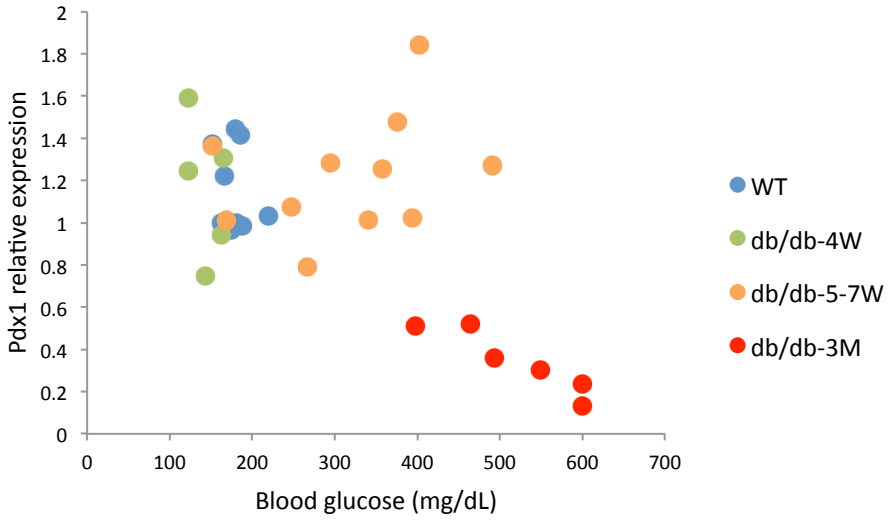
Primary antibodies used for immunohistochemistry, including their dilutions and commercial details, as well as sequence of primers used for qPCR in this study and for amplifying mouse genomic regions cloned into the luciferase reporter vector.

Supplemental References

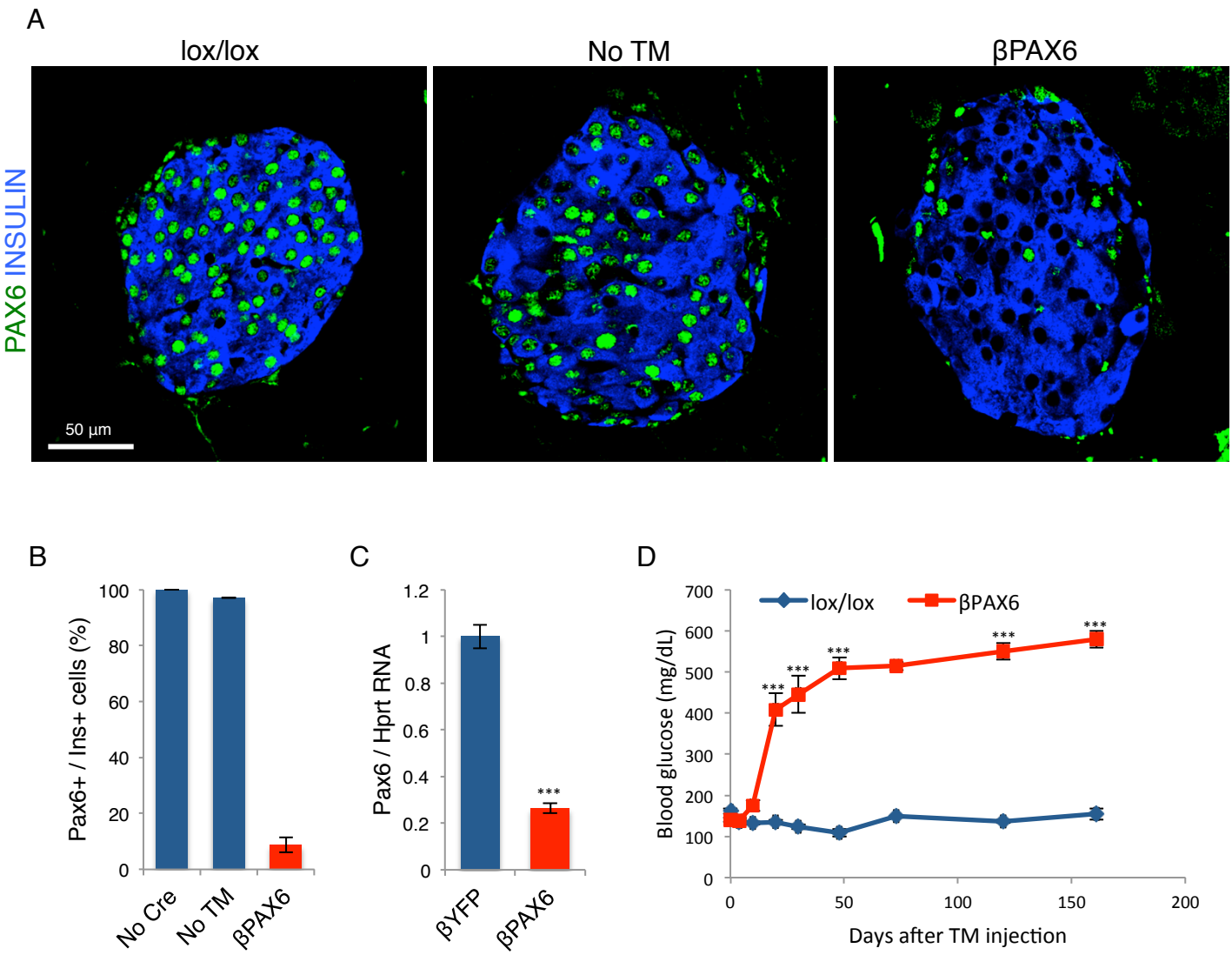
1. Avrahami D, Li C, Zhang J, Schug J, Avrahami R, Rao S, Stadler MB, Burger L, Schubeler D, Glaser B, et al. Aging-Dependent Demethylation of Regulatory Elements Correlates with Chromatin State and Improved beta Cell Function. *Cell Metab.* 2015;22(4):619-32.
2. Taylor BL, Liu FF, and Sander M. Nkx6.1 is essential for maintaining the functional state of pancreatic beta cells. *Cell Rep.* 2013;4(6):1262-75.
3. Tennant BR, Robertson AG, Kramer M, Li L, Zhang X, Beach M, Thiessen N, Chiu R, Mungall K, Whiting CJ, et al. Identification and analysis of murine pancreatic islet enhancers. *Diabetologia.* 2013;56(3):542-52.
4. Bramswig NC, Everett LJ, Schug J, Dorrell C, Liu C, Luo Y, Streeter PR, Naji A, Grompe M, and Kaestner KH. Epigenomic plasticity enables human pancreatic alpha to beta cell reprogramming. *J Clin Invest.* 2013;123(3):1275-84.
5. Pasquali L, Gaulton KJ, Rodriguez-Segui SA, Mularoni L, Miguel-Escalada I, Akerman I, Tena JJ, Moran I, Gomez-Marin C, van de Bunt M, et al. Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. *Nature genetics.* 2014;46(2):136-43.





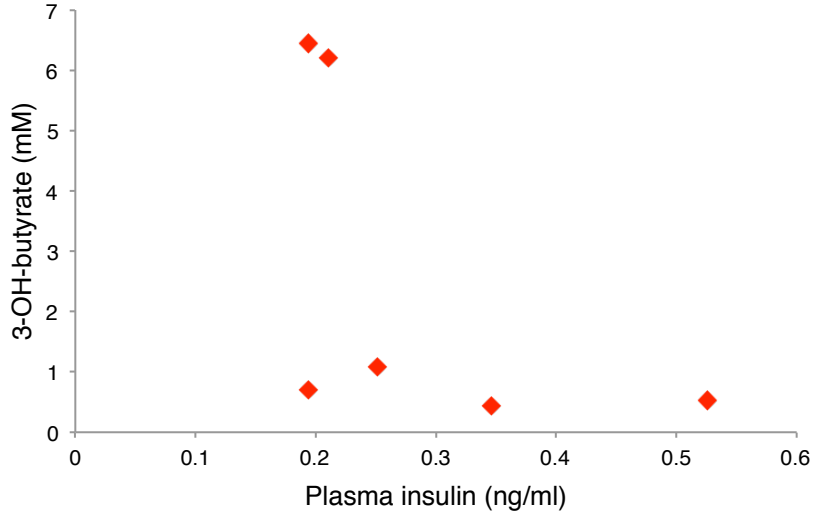


Supplemental Figure S1

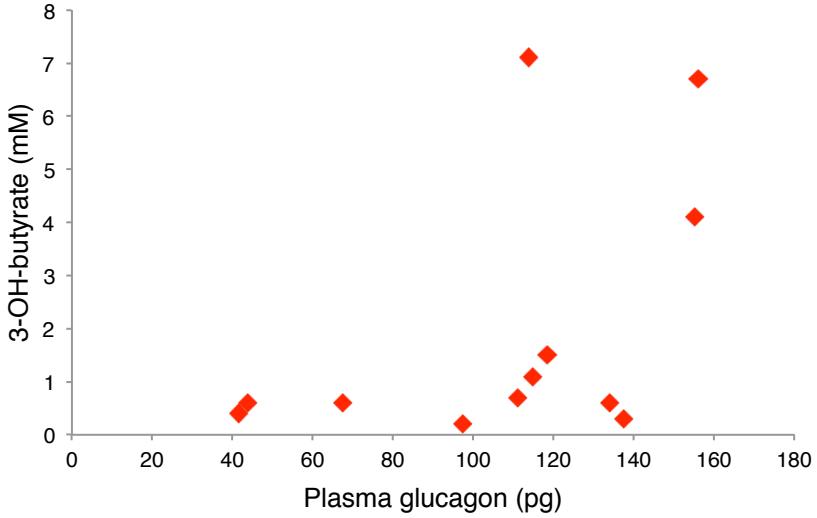


Supplemental Figure S2

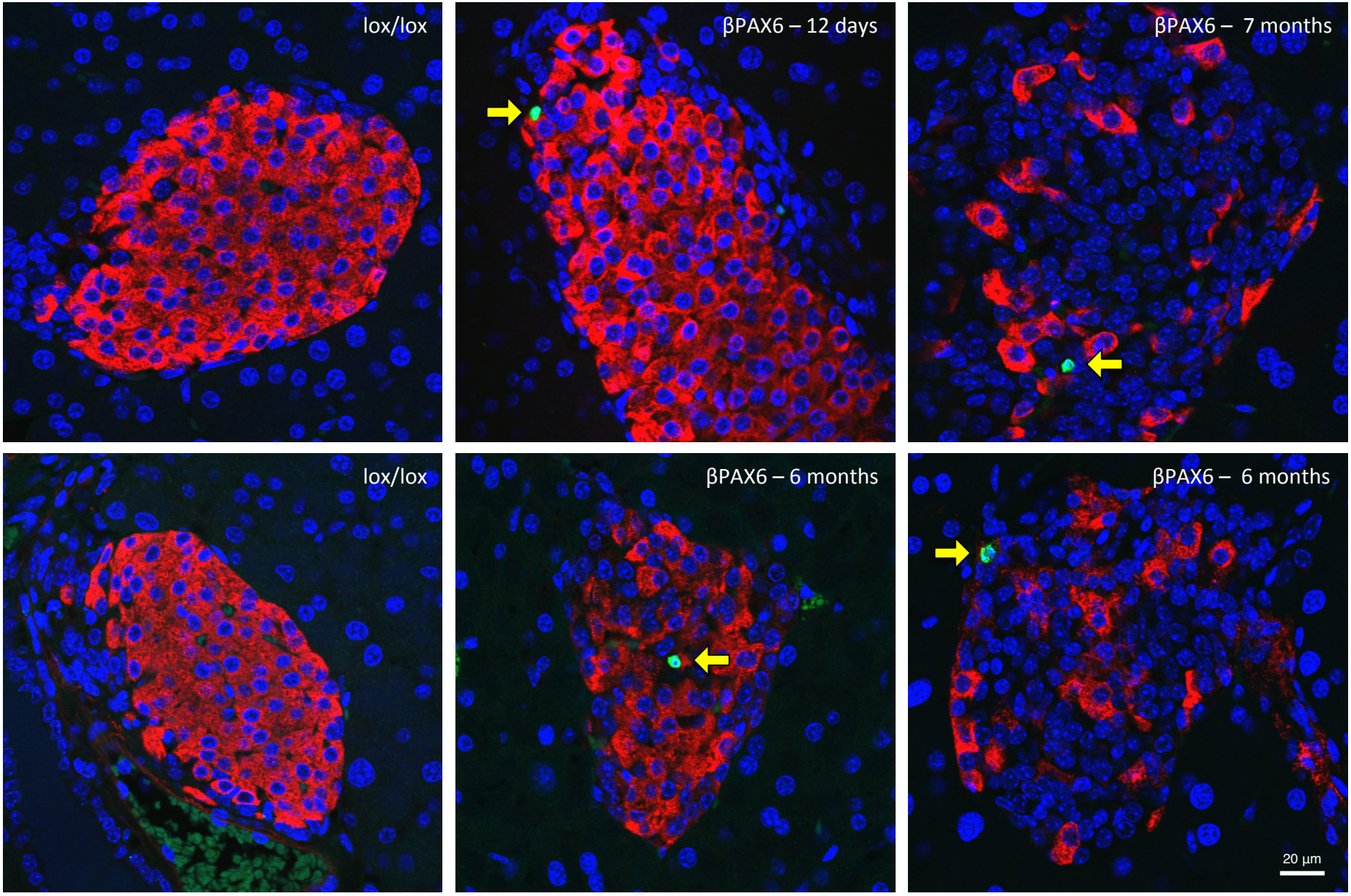
A



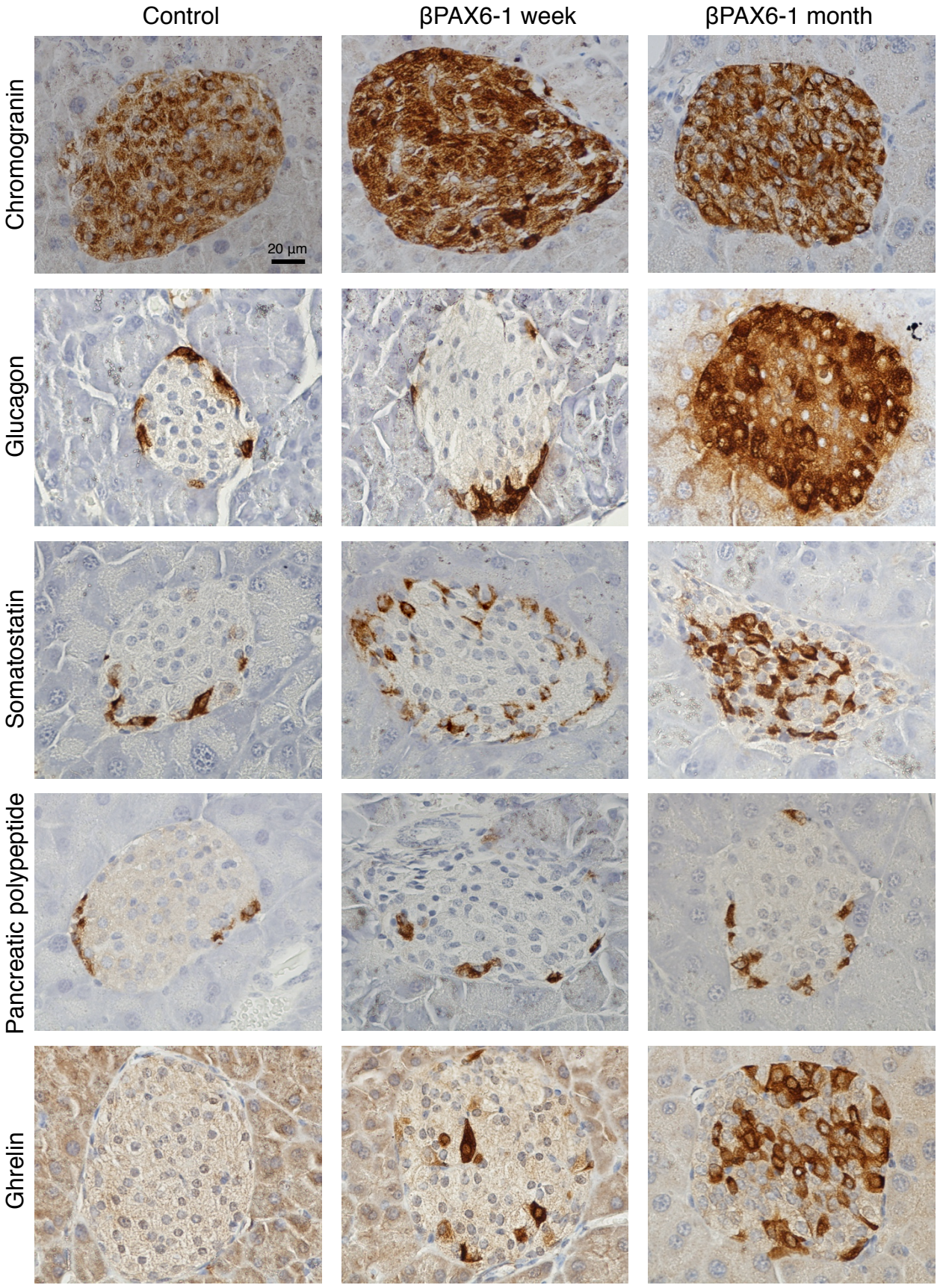
B



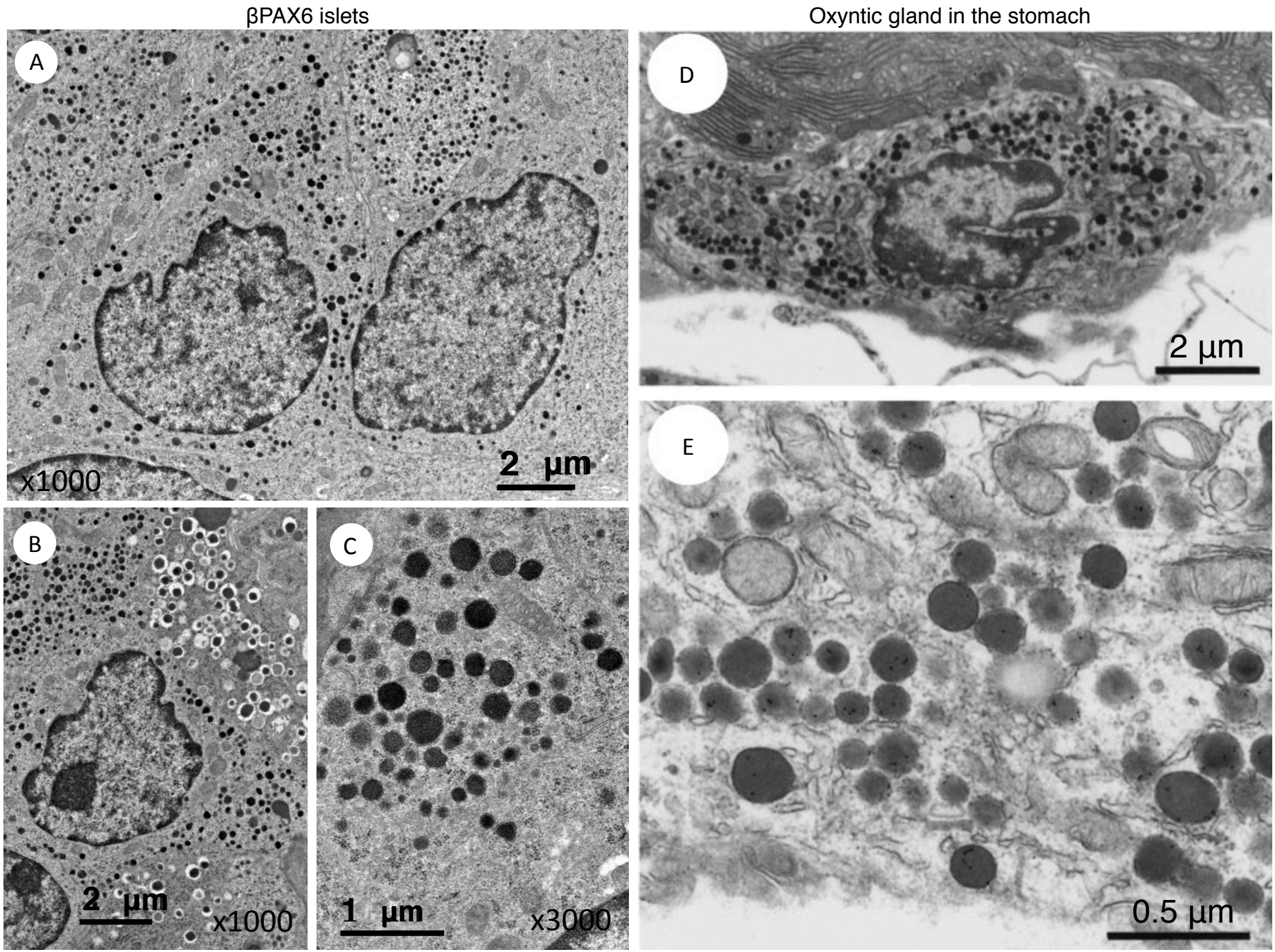
Supplemental Figure S3



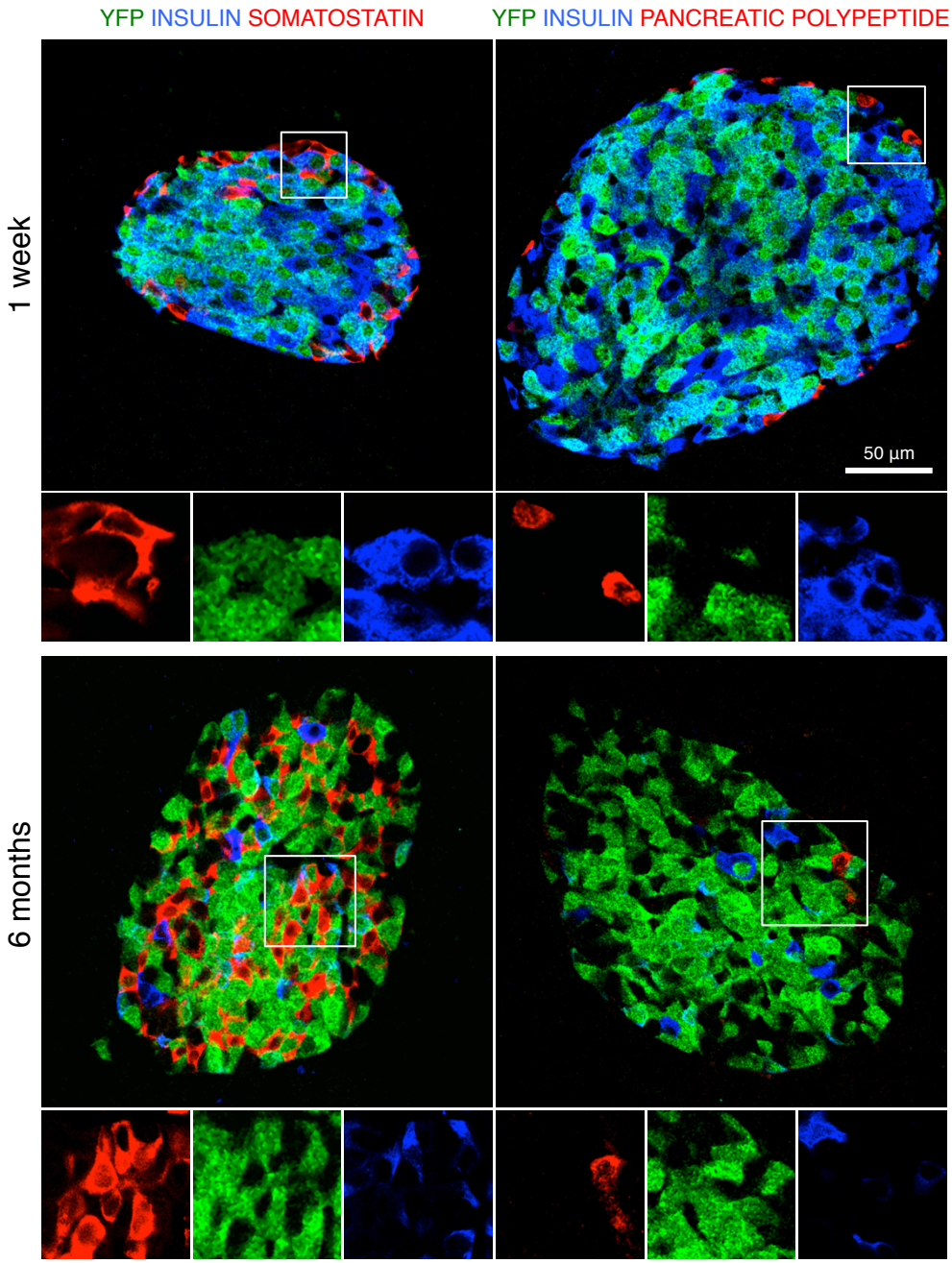
Supplemental Figure S4



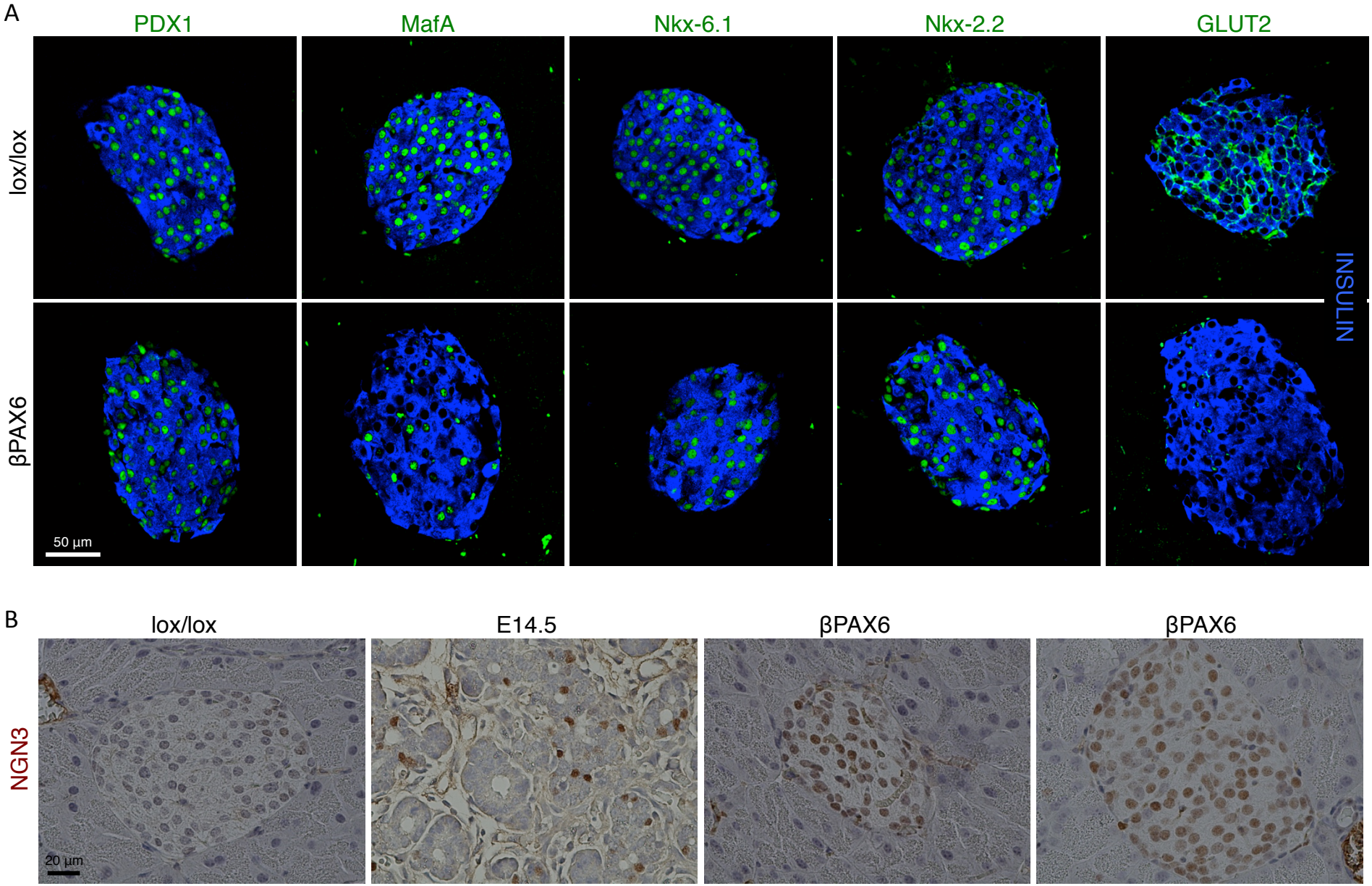
Supplemental Figure S5



Supplemental Figure S6



Supplemental Figure S7



Supplemental Figure S8

Supplementary table S1

Insulin Secretion

Gene	Full name	FC	FDR	Control	βPAX6
Acox2	acyl-Coenzyme A oxidase 2, branched chain	2.43	0.839	1.00	1.90
Acss1	acyl-CoA synthetase short-chain family member 1	3.31	0.612	1.21	2.13
Acss3	acyl-CoA synthetase short-chain family member 3	2.73	0.710	1.21	2.42
Aldoa	aldolase A, fructose-bisphosphate	1.16	0.822	1944.54	2064.87
Atp5G3	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C3 (subunit 9)	1.39	0.312	778.06	1031.06
Cox4I1	cytochrome c oxidase subunit IV isoform 1	1.46	0.167	611.77	823.55
Gpi1	glucose phosphate isomerase 1	1.37	0.450	412.57	496.70
Nnt	nicotinamide nucleotide transhydrogenase	-1.93	0.009	952.05	456.20
Pcsk1	proprotein convertase subtilisin/kexin type 1	-1.13	0.879	8552.70	7533.76
Pcsk2	proprotein convertase subtilisin/kexin type 2	-1.70	0.020	36860.26	19898.46
Pdia6	protein disulfide isomerase associated 6	-1.09	0.879	3301.01	2864.53
Pdk3	pyruvate dehydrogenase kinase, isoenzyme 3	1.49	0.574	120.15	157.41
Pgap1	post-GPI attachment to proteins 1	-1.12	0.879	2066.23	1732.33
Prkcsh	protein kinase C substrate 80K-H	1.04	0.928	800.72	759.07
Rims1	regulating synaptic membrane exocytosis 1	7.81	0.027	1.63	9.35
Rims2	regulating synaptic membrane exocytosis 2	1.04	0.926	963.48	915.72
Rims3	regulating synaptic membrane exocytosis 3	-1.54	0.578	182.72	93.17
Rims4	regulating synaptic membrane exocytosis 4	3.61	0.560	1.00	2.09
Slc30A8	solute carrier family 30 (zinc transporter), member 8	-6.50	0.000	7328.85	1071.16
Snap25	synaptosomal-associated protein 25	1.52	0.041	5420.61	8056.10
Stx1A	syntaxin 1A (brain)	2.45	0.226	35.76	74.56
Stxbp1	syntaxin binding protein 1	-1.41	0.169	3922.67	2569.96
Syp	synaptophysin	-1.41	0.225	5788.16	4059.99
Syt4	synaptotagmin IV	-1.48	0.076	2928.13	1786.89
Syt7	synaptotagmin VII	1.55	0.026	3643.04	5446.87
Syt8	synaptotagmin VIII	-1.30	0.879	1.00	1.00
Vamp2	vesicle-associated membrane protein 2	-1.01	1.000	3743.33	3501.68
Vamp3	vesicle-associated membrane protein 3	1.21	0.687	1466.83	1599.38

G6Pc2	glucose-6-phosphatase, catalytic, 2	-41.12	0.000	41206.87	932.29
Gck	glucokinase	1.74	0.013	658.57	1031.50
Slc2a1	solute carrier family 2 (facilitated glucose transporter), member 1	-2.24	0.070	251.53	100.93
Slc2a2	solute carrier family 2 (facilitated glucose transporter), member 2	-9.46	0.000	7945.63	769.89
Slc2a3	solute carrier family 2 (facilitated glucose transporter), member 3	-4.05	0.445	1.93	1.00

Granules

Gene	Full name	FC	FDR	Control	βPAX6
Acly	ATP citrate lyase	-1.41	0.152	14225.58	9203.69
Aco2	aconitase 2, mitochondrial	1.06	0.879	1726.69	1691.29
Acta1	actin, alpha 1, skeletal muscle	3.84	0.080	14.70	44.81
Actb	actin, beta	-1.10	0.879	8589.09	7662.14
Aldoa	aldolase A, fructose-bisphosphate	1.16	0.822	1944.54	2064.87
Atp1a1	ATPase, Na+/K+ transporting, alpha 1 polypeptide	1.73	0.005	9454.26	15702.58
Atp5a1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1	1.10	0.879	4261.30	4632.55
Atp5b	ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit	-1.01	0.996	8062.58	7922.67
Atp6ap2	ATPase, H+ transporting, lysosomal accessory protein 2	-1.09	0.879	1567.93	1345.15
Calr	calreticulin	-1.45	0.144	7247.46	4878.98
Chga	chromogranin A	-1.18	0.765	63506.02	56358.82
Chgb	chromogranin B	-1.17	0.788	88946.12	92158.57
Clu	clusterin	1.53	0.136	589.96	827.76
Cox5b	cytochrome c oxidase subunit Vb	1.88	0.471	40.75	71.22
Cpe	carboxypeptidase E	1.75	0.003	23762.23	37355.85
Cpn1	carboxypeptidase N, polypeptide 1	1.16	0.879	399.52	415.26
Ctsd	cathepsin D	-1.11	0.879	2593.07	2123.72
Ctsl	cathepsin L	1.33	0.354	1697.13	2094.89
Dnajc5	DnaJ (Hsp40) homolog, subfamily C, member 5	-1.25	0.620	1307.49	955.70
Eef1a1	eukaryotic translation elongation factor 1 alpha 1	1.20	0.690	8659.13	9845.81
Eef2	eukaryotic translation elongation factor 2	1.23	0.623	7088.22	8564.37
Eno1	enolase 1, alpha non-neuron	2.78	0.010	148.53	299.77
Fn1	fibronectin 1	3.14	0.598	1.00	2.05
Gapdh	glyceraldehyde-3-phosphate dehydrogenase	1.14	0.896	32.01	26.89
Gc	group specific component	13.15	0.000	99.34	1595.39
Gnal	guanine nucleotide binding protein, alpha stimulating, olfactory type	1.77	0.305	93.41	142.27
Gnao1	guanine nucleotide binding protein, alpha O	1.03	0.895	7719.09	8220.17
Gnb2	guanine nucleotide binding protein (G protein), beta 2	1.37	0.496	294.36	375.42
Gnb4	guanine nucleotide binding protein (G protein), beta 4	2.52	0.544	5.67	9.96
Got2	glutamatic-oxaloacetic transaminase 2, mitochondrial	-1.50	0.295	424.57	260.51
Hnrnpk	heterogeneous nuclear ribonucleoprotein K [Mus musculus	1.15	0.842	1422.23	1506.19

Hsp90ab1	heat shock protein 90 alpha (cytosolic), class B member 1	1.53	0.036	11361.04	15661.49
Hspa5	heat shock protein 5	-1.06	0.879	37445.54	35586.00
Hspe1	heat shock protein 1 (chaperonin 10)	-1.05	0.987	117.69	99.09
Hyou1	hypoxia up-regulated 1	-1.30	0.456	14037.00	9677.91
Ins1	insulin 1	-1.45	0.126	4913677.92	4913677.92
Ins2	insulin 2	-1.70	0.007	1867622.71	1867622.71
Mdh2	malate dehydrogenase 2, NAD (mitochondrial)	1.18	0.786	1229.82	1314.69
Npm1	nucleophosmin 1	1.98	0.001	701.58	1281.99
Nptx1	neuronal pentraxin 1	3.78	0.002	64.95	241.60
Nucb2	nucleobindin 2	1.11	0.879	6858.46	7711.71
P4hb	prolyl 4-hydroxylase, beta polypeptide	-1.26	0.559	10390.76	8370.10
Pam	peptidylglycine alpha-amidating monooxygenase	3.18	0.000	4661.44	13797.20
Pcsk1	proprotein convertase subtilisin/kexin type 1	-1.13	0.879	8552.70	7533.76
Pcsk1n	proprotein convertase subtilisin/kexin type 1 inhibitor	1.41	0.226	1833.80	2345.13
Pcsk2	proprotein convertase subtilisin/kexin type 2	-1.70	0.020	36860.26	19898.46
Pdia3	protein disulfide isomerase associated 3	-1.04	0.883	15107.31	13349.17
Pkm2	pyruvate kinase, muscle	-1.09	0.879	2129.49	1833.83
Prdx4	peroxiredoxin 4	1.91	0.255	88.31	146.80
Prkcsh	protein kinase C substrate 80K-H	1.04	0.928	800.72	759.07
Rab2a	RAB2A, member RAS oncogene family	1.06	0.879	4395.79	4434.55
Rab3a	RAB3A, member RAS oncogene family	1.06	0.879	668.97	673.41
Rpl18	ribosomal protein L18	1.59	0.567	80.79	111.18
Rpl23a	ribosomal protein L23A	1.13	0.906	28.35	27.99
Ptprn	protein tyrosine phosphatase, receptor type, N	1.76	0.006	6264.05	10544.18
Scg2	secretogranin II	-1.77	0.004	51692.89	29594.53
Scg3	secretogranin III	1.22	0.553	21057.03	24162.81
Scg5	secretogranin V	-1.01	1.000	4791.13	4691.24
Sod1	superoxide dismutase 1, soluble	1.53	0.068	1414.92	1965.13
Stc1	stanniocalcin 1	37.30	0.000	4.44	318.67
Syt5	synaptotagmin V	1.50	0.259	505.68	660.97
Sytl4	synaptotagmin-like 4	-1.27	0.552	4454.07	3390.75
Tcn2	transcobalamin 2	-1.18	0.833	1247.05	974.06
Tmed10	transmembrane emp24-like trafficking protein 10	1.21	0.623	2448.95	2788.60
Tuba1a	tubulin, alpha 1A	1.13	0.879	4194.25	4788.15

Vamp2	vesicle-associated membrane protein 2	-1.01	1.000	3743.33	3501.68
Vamp3	vesicle-associated membrane protein 3	1.21	0.687	1466.83	1599.38
Vamp7	vesicle-associated membrane protein 7	1.46	0.302	324.55	421.42
Vamp8	vesicle-associated membrane protein 8	1.18	0.822	498.14	548.12
Vdac1	voltage-dependent anion channel 1	1.10	0.879	1131.27	1127.57
Vgf	VGF nerve growth factor inducible	-1.16	0.879	115.56	82.20
Wif1	Wnt inhibitory factor 1	8.35	0.131	1.00	2.87
Ywhag	yrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	-1.01	0.967	2506.42	2246.05
Ywhaq	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	-1.44	0.260	692.07	439.59
Ywhaz	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	-1.07	0.879	9002.03	8361.28

Hormones

Gene	Full name	FC	FDR	Control	βPAX6
Calca	Calcitonin gene-related peptide 1	1.01	1.000	1.00	1.07
Calcb	Calcitonin gene-related peptide 2	-1.30	0.879	1.00	1.00
Cck	Cholecystokinin	5.74	0.158	1.00	4.88
Chga	Chromogranin-A	-1.18	0.765	63506.02	56358.82
Chgb	Chromogranin-B	-1.17	0.788	88946.12	92158.57
Gal	Galanin	2.19	0.879	1.00	1.60
Gast	Gastrin	17.82	0.001	1.00	17.19
Gcg	Glucagon	2.37	0.138	10729.16	28287.35
Ghrh	Somatoliberin/Growth hormone releasing hormone	2.43	0.855	1.00	1.75
Ghrl	Ghrelin	772.06	0.000	23.71	15178.35
Gip	Gastric inhibitory polypeptide	0.77	0.879	1.00	1.00
Grp	Gastrin-releasing peptide	0.77	0.879	1.00	1.00
Ins1	Insulin 1	-1.45	0.126	4913677.92	4913677.92
Ins2	Insulin 2	-1.70	0.007	1867622.71	1867622.71
Lep	Leptin	0.77	0.879	1.00	1.00
Muc6	Gastric mucin-like protein	1.15	0.936	30.67	14.28
Nmb	Neuromedin-B	1.95	0.739	12.11	22.18
Npy	Pro-neuropeptide Y	-68.57	0.000	76.55	1.07
Nts	Neurotensin	0.77	0.879	1.00	1.00
Pdyn	Proenkephalin-B	-21.90	0.000	265.24	9.73
Penk	Proenkephalin-A	-2.99	0.357	35.46	5.39
Ppy	Pancreatic polypeptide	4.00	0.000	387.99	1757.14
Pyy	Peptide YY	2.29	0.042	570.02	1501.79
Sct	Secretin	0.77	0.879	1.00	1.00
Sst	Somatostatin	4.01	0.000	2293.25	12584.82
Vip	Vasoactive intestinal polypeptide	0.77	0.879	1.00	1.00

Hormone receptors

Gene	Full name	FC	FDR	Control	βPAX6
Calcr	Calcitonin receptor	-5.89	0.286	2.15	1.00
Cckar	Cholecystokinin A receptor	-1.30	0.879	1.00	1.00
Cckbr	Cholecystokinin B receptor	-1.30	0.879	1.00	1.00
Galr1	galanin receptor 1	1.02	0.972	538.65	492.25
Galr2	galanin receptor 2	1.32	0.879	29.60	27.28
Galr3	galanin receptor 3	-2.85	0.001	282.73	86.28
Gcgr	Glucagon receptor	3.59	0.000	101.91	350.48
Ghrhr	growth hormone releasing hormone receptor	-1.30	0.879	1.00	1.00
Ghsr	growth hormone secretagogue receptor	1.02	1.000	5.61	13.65
Gipr	Gastric inhibitory polypeptide receptor	-4.17	0.000	249.42	52.05
Glp1r	GLP1 receptor	-5.08	0.000	15002.04	2603.02
Grpr	Gastrin releasing peptide receptor	1.95	0.879	1.00	1.51
Igf1r	Insulin-like growth factor 1 receptor	-2.28	0.000	4121.75	1686.68
Igf2r	Insulin-like growth factor 2 receptor	-1.41	0.153	2929.19	1919.10
Insr	Insulin receptor	1.11	0.879	1388.43	1361.57
Lepr	Leptin receptor	0.81	0.955	1.21	1.07
Nmbr	Neuromedin B receptor	2.90	0.774	1.00	1.98
Npy1r	Neuropeptide Y receptor Y1	1.01	1.000	1.00	1.07
Npy2r	Neuropeptide Y receptor Y2	-1.30	0.879	1.00	1.00
Npy5r	Neuropeptide Y receptor Y5	-1.30	0.879	1.00	1.00
Npy6r	Neuropeptide Y receptor Y6	-1.30	0.879	1.00	1.00
Ntsr1	neurotensin receptor 1	-4.05	0.416	1.99	1.00
Ntsr2	neurotensin receptor 2	-1.30	0.879	1.00	1.00
Oprd1	opioid receptor, delta 1	-1.30	0.879	1.00	1.00
Sctr	Secretin receptor	10.00	0.016	1.00	9.73
Sstr1	Somatostatin receptor 1	-1.70	0.879	1.58	1.07
Sstr2	Somatostatin receptor 2	-13.23	0.038	5.64	1.00
Sstr3	Somatostatin receptor 3	-1.91	0.004	2606.95	1301.58
Sstr4	Somatostatin receptor 4	-1.30	0.879	1.00	1.00
Sstr5	Somatostatin receptor 5	-1.30	0.879	1.00	1.00
Vipr1	Vasoactive intestinal peptide receptor 1	-22.22	0.000	222.44	6.31
Vipr2	Vasoactive intestinal peptide receptor 2	3.31	0.294	3.79	14.46

Supplementary table S2

Islet transcription factors

Gene	FC	FDR	Control	βPAX6
Alx3	-1.30	8.79E-01	1.00	1.00
Arx	1.25	8.79E-01	5.50	6.64
Cmip	1.03	9.67E-01	4142.30	3933.12
En1	-1.30	8.79E-01	1.00	1.00
Esr1	-1.87	9.98E-03	12934.03	6871.19
Foxa1	-4.66	4.10E-01	1.92	1.00
Foxa2	1.94	5.79E-02	193.40	375.05
Foxa3	1.27	8.79E-01	88.11	99.77
Foxo1	-1.35	3.59E-01	1855.47	1256.91
Gata4	-1.32	9.28E-01	4.42	2.51
Gata6	-1.73	5.31E-01	127.43	73.54
Glis3	-1.08	8.79E-01	1800.07	1511.17
Hes1	1.36	8.79E-01	22.45	28.24
Hhex	8.19	4.93E-03	4.63	38.15
Hnf1A	1.24	8.78E-01	254.88	280.31
Hnf1B	-1.04	9.36E-01	582.33	508.00
Hnf4A	-1.05	8.79E-01	667.78	583.68
Insm1	1.80	1.10E-02	1653.43	3019.07
Irx2	3.60	3.25E-01	2.31	6.71
Isl1	2.89	6.35E-10	740.77	1995.76
Lhx6	3.70	2.26E-01	4.87	22.79
Lmx1A	-1.30	8.79E-01	1.00	1.00
Lmx1B	1.90	7.68E-01	4.63	11.98
Maf	1.48	9.72E-01	1.00	1.31
Mafa	-18.54	3.48E-04	40.33	1.41
Mafb	-3.60	2.72E-05	581.66	146.11
Mlxipl	-2.29	1.37E-05	2819.03	1123.31
Mnx1	1.56	8.53E-01	25.75	33.00
Myt1	2.05	1.07E-04	882.25	1636.46
Nanog	0.62	8.79E-01	1.28	1.00
Neurod1	1.05	8.79E-01	2897.94	2843.51
Neurog3	18.72	8.33E-05	1.35	28.08
Nkx2-2	1.88	2.03E-03	1283.91	2284.78
Nkx6-1	-2.17	1.69E-04	1615.21	686.19
Nkx6-2	1.26	8.79E-01	35.27	35.15
Nkx6-3	1.72	8.79E-01	1.00	1.41

Notch1	-1.04	9.29E-01	450.10	383.97
Onecut1	-1.30	8.79E-01	1.00	1.00
Onecut2	5.33	6.52E-03	13.39	72.35
Onecut3	-1.30	8.79E-01	1.00	1.00
Pax2	-1.30	8.79E-01	1.00	1.00
Pax4	-1.30	8.79E-01	1.00	1.00
Pax6	2.31	2.19E-07	2614.12	5766.85
Pbx1	1.15	8.78E-01	2808.21	2943.46
Pdx1	-2.26	2.42E-04	1714.94	703.50
Pou3F4	-2.51	8.35E-01	1.83	1.31
Pou5F1	-1.30	8.79E-01	1.00	1.00
Prox1	2.75	5.80E-10	1875.07	4810.82
Rfx2	1.01	9.58E-01	357.27	347.11
Rfx3	1.24	5.19E-01	1820.16	2103.52
Rfx6	4.39	2.03E-21	1375.85	5777.94
Snai1	2.16	8.79E-01	1.21	1.84
Snai2	1.93	8.34E-01	9.85	21.67
Sox4	4.60	1.40E-05	65.69	278.56
Sox9	1.27	1.00E+00	3.53	3.64

All changed transcription factors

Gene	FC	FDR	Control	βPAX6
Glis1	30.33	1.91E-06	1.35	38.88
Pdlim4	26.58	3.90E-05	1.00	25.69
Gli2	21.37	6.82E-04	1.00	14.60
Neurog3	18.72	8.33E-05	1.35	28.08
Sox11	11.42	3.68E-02	1.00	5.13
Mef2c	9.12	3.86E-03	3.08	34.68
Zfp41	9.00	1.28E-03	3.64	34.64
Hhex	8.19	4.93E-03	4.63	38.15
Arnt2	7.77	2.59E-12	48.81	337.02
Egr2	7.62	1.71E-10	57.19	372.02
Ank1	7.17	2.89E-02	4.46	45.83
Rorc	6.36	1.55E-14	110.80	629.19
Rorb	5.60	3.90E-02	4.47	21.75
Asb4	4.63	7.49E-03	34.45	157.19
Sox4	4.60	1.40E-05	65.69	278.56
Fhl2	4.40	4.18E-02	26.02	159.99
Nfatc1	3.76	5.03E-07	119.71	399.99

Supt3h	3.73	4.26E-02	19.42	70.27
Lass6	3.17	1.62E-10	775.35	2294.10
Arid3a	3.09	2.44E-04	107.54	305.00
Sin3b	3.01	2.62E-06	295.61	850.68
Isl1	2.89	6.35E-10	740.77	1995.76
Arid5b	2.83	2.58E-04	199.07	540.51
Tle4	2.79	3.01E-04	155.80	370.18
Aip	2.79	2.14E-04	231.17	692.59
Prox1	2.75	5.80E-10	1875.07	4810.82
Ank2	2.70	9.45E-05	681.22	1582.16
Mycbp	2.65	1.08E-06	389.23	932.51
Pla2g6	2.62	1.22E-07	563.51	1317.98
Cited2	2.60	6.55E-06	988.59	2546.31
Mkl1	2.49	3.27E-03	145.02	332.85
Zfp386	2.35	2.50E-05	381.46	821.03
Sox13	2.34	5.06E-02	104.95	223.12
Thrb	2.32	1.07E-04	365.17	785.22
Ifnar2	2.31	1.80E-03	281.04	587.58
Pax6	2.31	2.19E-07	2614.12	5766.85
Sall4	2.16	2.45E-02	155.36	322.84
Foxj2	2.12	1.19E-05	1109.04	2152.75
Ppp1r10	2.08	1.69E-04	1769.79	3591.23
Myt1	2.05	1.07E-04	882.25	1636.46
Fem1c	2.04	2.49E-02	204.70	412.34
Hdac1	2.00	4.50E-02	176.68	322.23
Asxl1	1.98	9.38E-04	444.59	806.43
Cbfb	1.96	3.70E-03	389.57	679.13
Mtpn	1.88	1.13E-03	1821.04	3226.57
Nkx2-2	1.88	2.03E-03	1283.91	2284.78
Insm1	1.80	1.10E-02	1653.43	3019.07
Zhx3	1.78	3.65E-03	1000.60	1597.71
Irf6	1.78	4.53E-03	805.02	1286.81
St18	1.69	7.87E-03	2829.35	4437.89
Tead1	1.69	6.11E-03	2919.50	4572.73
Dek	1.67	2.20E-02	606.72	923.59
Ldb1	1.64	4.56E-02	472.99	711.78
Nptxr	1.60	3.89E-02	1594.51	2327.91
Wasl	1.54	4.50E-02	1159.86	1654.41
Hbp1	1.53	4.64E-02	1327.58	1841.69
Nr1d1	-1.70	4.54E-02	4861.46	2766.11
Mbd2	-1.73	5.38E-03	2504.99	1309.83

Bpnt1	-1.77	3.59E-02	1503.24	719.55
Esr1	-1.87	9.98E-03	12934.03	6871.19
Zfp36	-1.87	1.97E-02	13163.10	7225.49
Klf4	-1.88	6.38E-03	1295.21	646.32
Atf4	-1.88	6.31E-04	5622.96	2822.81
Klf13	-1.93	3.86E-03	749.43	347.01
Srebf1	-1.95	1.55E-03	1132.78	525.18
Mafg	-2.11	1.71E-02	371.12	160.09
Nkx6-1	-2.17	1.69E-04	1615.21	686.19
Tnfaip3	-2.25	2.01E-04	1237.19	501.16
Vdr	-2.48	3.24E-05	4046.74	1579.99
Cebpd	-2.50	1.44E-02	239.86	80.35
Scrt1	-2.75	8.04E-03	235.33	78.09
Rara	-2.85	2.45E-03	259.31	81.11
Nr4a2	-2.86	4.41E-02	373.80	168.19
Rel	-3.05	2.91E-02	237.83	93.94
Meox1	-3.29	1.55E-04	399.47	95.48
Mafb	-3.60	2.72E-05	581.66	146.11
Ehf	-3.63	4.73E-06	551.66	123.40
Taf4b	-3.76	3.21E-03	196.05	40.97
Ddit3	-3.85	2.29E-13	1420.39	332.98
Tle6	-5.67	2.68E-02	44.64	6.23
Trib3	-8.93	3.51E-06	157.81	14.20
Uhrf1	-14.79	2.67E-02	7.05	1.23
Foxf2	-16.59	5.20E-02	2.80	1.00
Runx1	-17.20	4.46E-03	15.31	1.00
Mafa	-18.54	3.48E-04	40.33	1.41
Spic	-31.85	7.90E-06	50.18	1.31

Supplementary table S5

Group	Gene	Histon Marks	Other TFs bind	Distance from TSS	Fold change	p-value	FDR
Hormones	Ins1	H3K27ac,H3K9ac	Nkx6-1	-169	-1.45	1.33E-02	1.26E-01
		H3K27ac	MafA,Nkx6-1,pdx1	-11780			
	Ins2	H3K27ac,H3K9ac	NA	-363	-1.70	2.87E-04	6.56E-03
	Gcg	H3K27me3	NA	-80	2.37	1.52E-02	1.38E-01
		H3K27me3	NA	-19036			
	Sst	H3K27me3	Foxa2,Nkx6-1,pdx1	48055	4.01	9.77E-06	3.63E-04
		H3K27me3,H3K4me1	Foxa2,Nkx6-1,pdx1	56871			
	Ghrl	NA	Nkx6-1,pdx1	-79596	772.06	1.30E-178	3.73E-174
β cell function	G6pc2	H3K27ac,H3K9ac	Foxa2,MafA,NeuroD1,Nkx61, pdx1	-121	-41.12	5.41E-71	2.58E-67
		H3K27ac	NeuroD1,Nkx6-1,pdx1	2987			
		H3K27ac,H3K4me1	NA	28359			
		NA	NA	-4067			
	Slc30a8	NA	Foxa2,MafA,Nkx6-1,pdx1	-78032	-6.50	3.26E-24	1.56E-21
		H3K4me1	MafA,NeuroD1,Nkx6-1,pdx1	74634			
		H3K27ac	Foxa2,pdx1	-16885			
		NA	NA	20228			
		H3K27ac	MafA	-68			
	Glp1r	H3K27ac	pdx1	-24050	-5.08	6.25E-23	2.67E-20
		NA	NA	-10125			
		NA	NA	-16240			
		H3K27ac	Foxa2,NeuroD1,Nkx6-1,pdx1	-13869			
	Gcgr	H3K27ac	Foxa2,NeuroD1,pdx1	8730	3.59	3.54E-07	1.88E-05
	Cpe	H3K27ac	Foxa2,NeuroD1,Nkx6-1,pdx1	63436	1.75	1.14E-04	3.08E-03
		H3K27ac	Foxa2,NeuroD1,pdx1	28002			
		NA	Nkx6-1	74205			
		H3K27ac,H3K4me1, H3K9ac	pdx1	-46			
		H3K27ac	NeuroD1,pdx1	68009			
		H3K27ac	NA	22789			
Islet TFs	Mafa	H3K27ac	MafA,Nkx6-1,pdx1	-30456	-18.54	9.31E-06	3.48E-04
		H3K27ac	Nkx6-1,pdx1	-8309			
		H3K27ac	NeuroD1,pdx1	-34093			
		H3K27ac	NeuroD1	-8556			
	Nkx6-1	H3K27ac	NA	8385	-2.17	4.10E-06	1.69E-04
		NA	Foxa2,Nkx6-1,pdx1	19302			
		H3K27ac	NeuroD1,Nkx6-1,pdx1	85238			
		H3K27ac	Nkx6-1	84724			
		H3K27ac	NeuroD1,Nkx6-1,pdx1	73641			
		NA	Nkx6-1	61368			
		NA	NA	33938			
	Pdx1	H3K27ac	Foxa2	-5869	-2.26	6.23E-06	2.42E-04
		H3K27ac	Nkx6-1,pdx1	-2568			
		H3K27ac	NeuroD1	-1097			

Pax6	H3K27ac	Foxa2,Nkx6-1,pdx1	62882	2.31	2.63E-09	2.19E-07
	H3K27ac	Foxa2,Nkx6-1,pdx1	63498			
	H3K27ac	Foxa2,Nkx6-1,pdx1	70115			
	H3K27ac	NA	-6950			
	H3K27ac	NA	-1896			
	H3K27ac,H3K9ac	NA	-24			
	NA	pdx1	51076			
	NA	pdx1	44459			
	NA	pdx1	43843			
Nkx2-2	H3K27ac	Foxa2,MafA,Nkx6-1,pdx1	-61795	1.88	7.05E-05	2.03E-03
	H3K27ac,H3K9ac	NA	-6933			
	H3K27ac,H3K9ac	NA	-7871			
	H3K27ac,H3K4me1	NA	-60776			
Isl1	H3K4me1	NA	50080	2.89	4.57E-12	6.35E-10
	NA	NA	-70501			
	H3K4me1	NA	49517			
	NA	NA	-51418			
Foxa2	H3K27ac	Foxa2,NeuroD1,pdx1	62110	1.94	4.56E-03	5.79E-02
Sox4	H3K27ac,H3K4me1	Foxa2,Nkx6-1	-21036	4.60	2.55E-07	1.40E-05
Rfx6	NA	Foxa2	10591	4.39	4.53E-24	2.03E-21
	NA	Foxa2	18349			
	NA	Nkx6-1,pdx1	7667			
	NA	Nkx6-1,pdx1	-91			
	H3K4me1	NA	17772			
	H3K4me1	NA	10014			
Myt1	H3K27ac,H3K9ac	Foxa2,Nkx6-1,pdx1	52	2.05	2.42E-06	1.07E-04
	H3K27ac,H3K9ac	Foxa2,Nkx6-1,pdx1	3749			
	NA	NA	-7754			
	NA	NA	21524			
	NA	NA	25221			
	H3K27ac	NeuroD1,pdx1	-4035			
	H3K27ac	NA	703			
	NA	NA	38384			
	NA	NA	34687			
	NA	NA	5409			
Insm1	H3K27ac	NA	1	1.80	5.43E-04	1.10E-02

Supplementary Table S7

Primary antibodies used for immunohistochemistry

Antigen	Source	Dilution	Company	Catalog #
Insulin	Guinea-pig	1:400	Abcam	ab7842
Pax6	Mouse	1:200	Hybridoma bank	PAX6
GFP	Goat	1:500	Abcam	ab6673
GFP	Rabbit	1:100	Invitrogen	A6455
Chromogranin-A	Rabbit	1:200	Novus Biologicals	NB120-15160
Glucagon	Mouse	1:800	Abcam	AB1984
Somatostatin	Mouse	1:400	Beta Cell Biology Consortium	AB1985
Pancreatic polypeptide	Rabbit	1:200	Zymed	18-0043
Ghrelin	Goat	1:100	Santa cruz	sc-10368
Neurogenin3	Mouse	1:400	Hybridoma bank	F25A1B3
Prohormone convertase 1/3	Rabbit	1:100	Chemicon	AB1260
Pro-insulin	Mouse	1:400	Beta cell biology consortium	AB2009
MafA	Rabbit	1:1000	Bethyl Laboratories	IHC-00352
Nkx6.1	Rabbit	1:800	Beta Cell Biology Consortium	AB1069
Pdx1	Goat	1:2500	A gift from Christopher Wright, Vanderbilt University Medical Center, Nashville, TN, USA	
Glut2	Rabbit	1:150	Santa Cruz	sc-9117

RT-qPCR primer sequences

Mouse		
	Forward Primer	**Reversed Primer**
Ins1	5’-AGACCATCAGCAAGCAGGTCA-3’	5’-AAGTGCACCAACAGGGCC-3’
Ins1-pre mRNA	5’-CCCTGCCTATCTTTCAGGTCAT-3’	5’-AAGTGCACCAACAGGGCC-3’
Cre	5’-GATTTCGACCAGGTTCGTTC-3’	5’-GCTAACCAGCGTTTTCGTTC-3’
Pax6	5'-CCCATGCAGATGCAAAAGTC-3'	5'-GCCAGTCTCGTAATACCTGC-3'
MafA	5’-CAAGGAGGAGGTCATCCGAC-3’	5’-TCTCCAGAATGTGCCGCTG-3’
Nkx6-1	5'-CCCGGAGTGATGCAGAGTC-3'	5'-AGAACGTGGGTCTGGTGTGT-3'
Pdx1	5'-AGGCCAGTGGGCAGGAG-3'	5'-CTCTTGTTTTCCTCGGGTTCC-3'
Actb	5'-CACAGCTTCTTTGCAGCTCCT-3'	5'-GTCATCCATGGCGAACTGG-3'

Human		
	Forward Primer	**Reversed Primer**
INS	5'-CCCGTCCTGTCTCCCAGAT-3'	5'-CACCTGCCCCACCTGC-3'
GCG	5'-GGAAGGCGAGATTTCCCAGAA-3'	5'-TGGCATGCAAAGCAATGTGG-3'
GHRL	5'-GTCAGGGGTTCAGTACCAGC-3'	5'-GAGTAAGGCTTGTGGGCGAT-3'
PAX6	5'-GGTTGGTATCCGGGGACTTC-3'	5'-TCCGTTGGAACTGATGGAGT-3'
MAFA	5'-GAGTCACAGCATGAGTCGCT-3'	5'-GGCTTCGACCTCCAAGGAAC-3'
NKX6-1	5'-GCCTGTACCCCTCATCAAGGA-3'	5'-TGCTGTCCGGAAAAAGTGGG-3'
G6PC2	5'-ACCTTACTGGTGGGTCCAAG-3'	5'-CATGGCCAGATGGACTTCCT-3'
FOXA2	5'-AGAGCCCGAGGGCTACTCC-3'	5'-TCATGTACGTGTTCATGCCGT-3'
NEUROG3	5'-TCACCAAGATCGAGACGCTG-3'	5'-GTACAAGCTGTGGTCCGCTA-3'
PDX1	5'-GATTGGCGTTGTTTGTGGCT-5'	5'-GCCGGCTTCTCTAAACAGGT-3'
ACTB	5'-CCTTTGCCGATCCGCCG-3'	5'-GATATCATCATCCATGGTGAGCTGG-3'

Primers for Luciferase assay

	Forward Primer	**Reverse Primer**
Pax6 (63K)	5'-ATATATGGTACCACACACAAGCGCTAAGGAAA-3'	5-'ATATATAAGCTTCAGCAGCTGTAACCATGGG-3'
Pax6 (-1.9K)	5'-ATATATGGTACCGGCCCTAACGAGCCCTTTAT-3'	5'-ATATATCTCGAGTTTTCACCCACCCCTTGTTC-3'
Nkx2-2 (-62K)	5'-ATATATGGTACCCCAGGGTGAAGAAGAAGATGC-3'	5'-ATATATAAGCTTGTGTTTGCTTATGGGCTCTGT-3'
Sox4 (-21K)	5'-ATATATGGTACCCCTTTGGGCAGATAAGAAAAGG-3'	5'-ATATATAAGCTTGGAGAAAACACACAGCTGAACT-3'
Pdx1 (-2.5K)	5'-ATATATGGTACCTCACTGTCCACAGTATAATTGGTT-3'	5'-ATATATAAGCTTGGTGATACGCATTTCCGCTC-3'