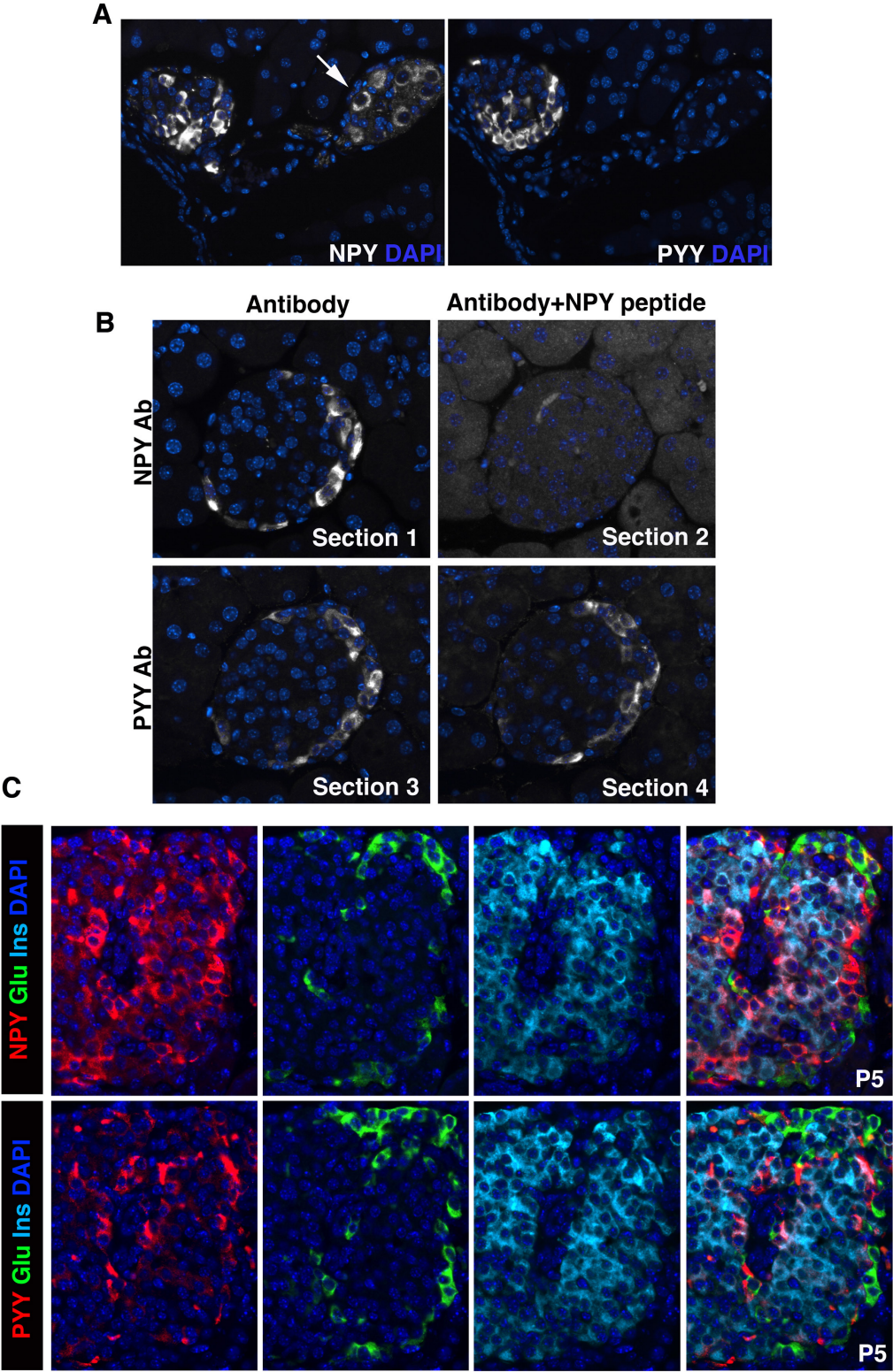
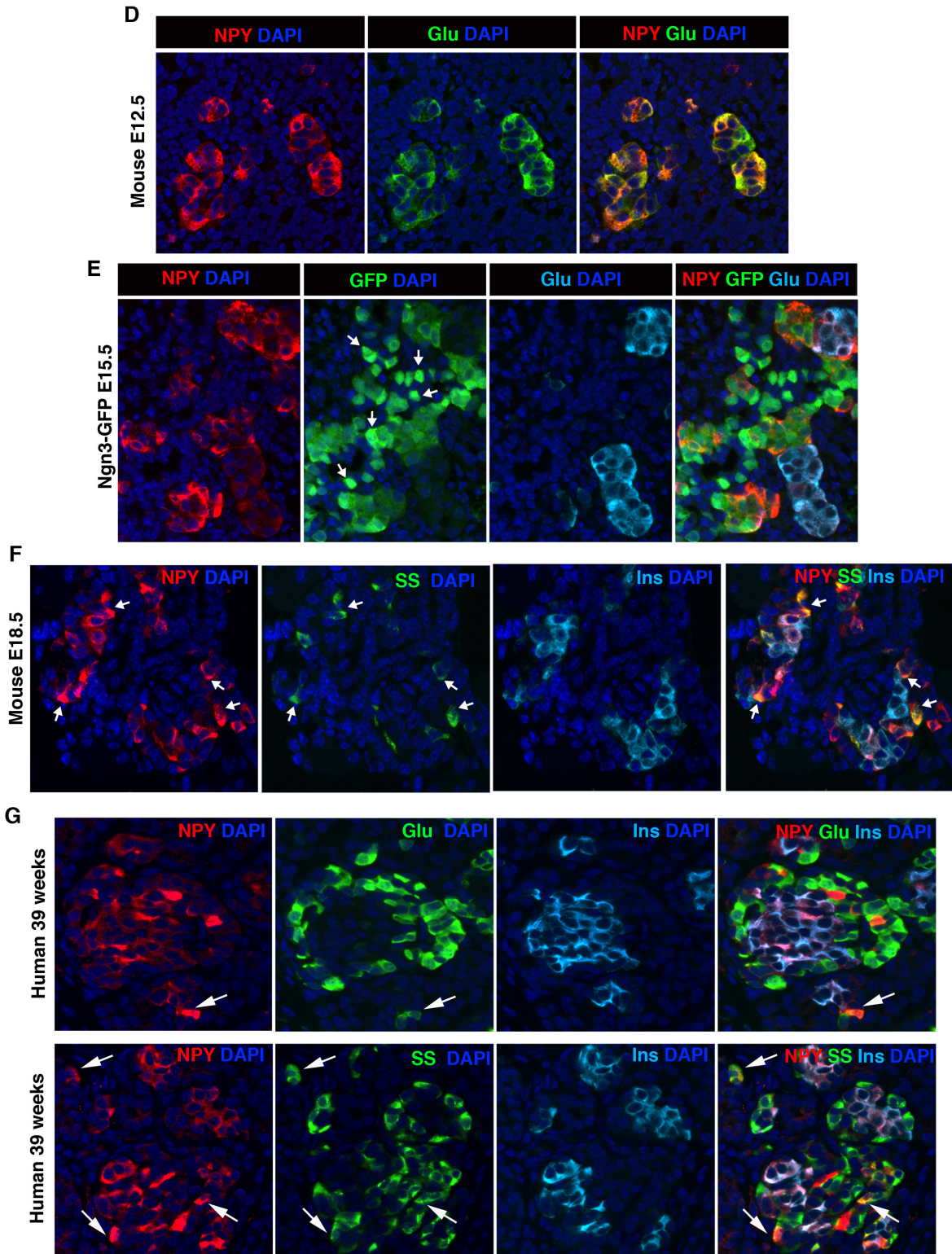


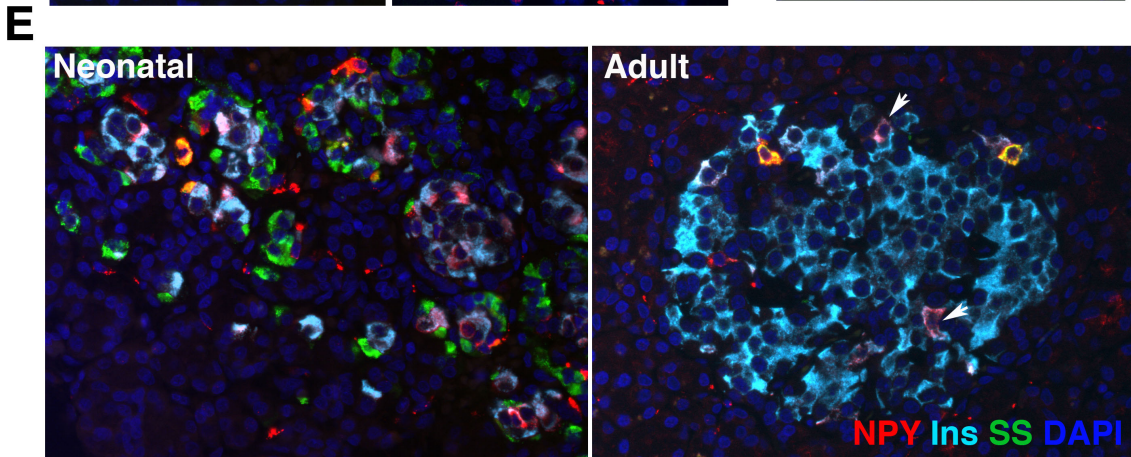
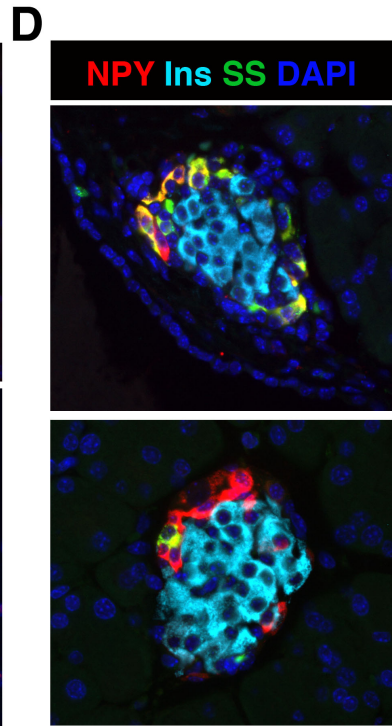
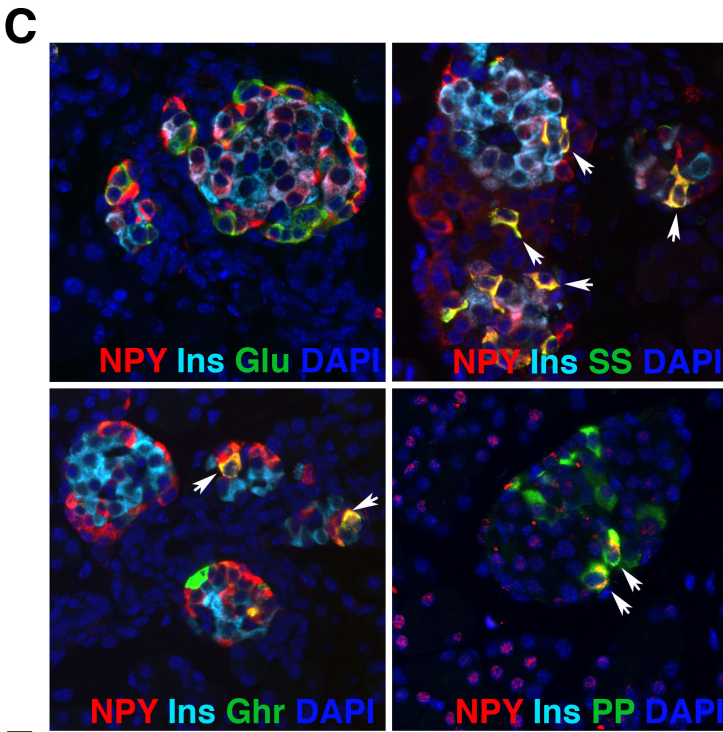
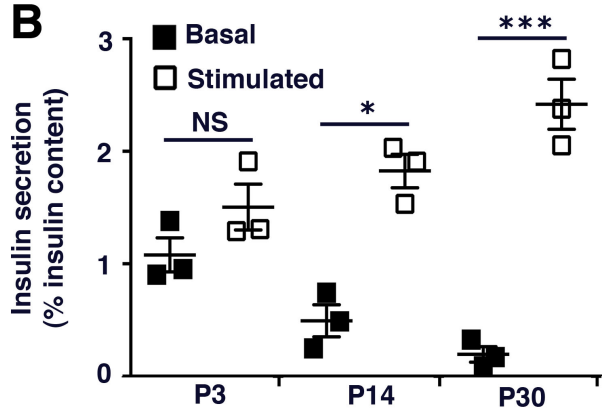
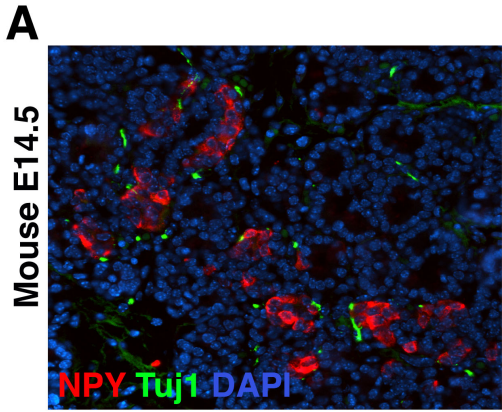
Supplemental Figures



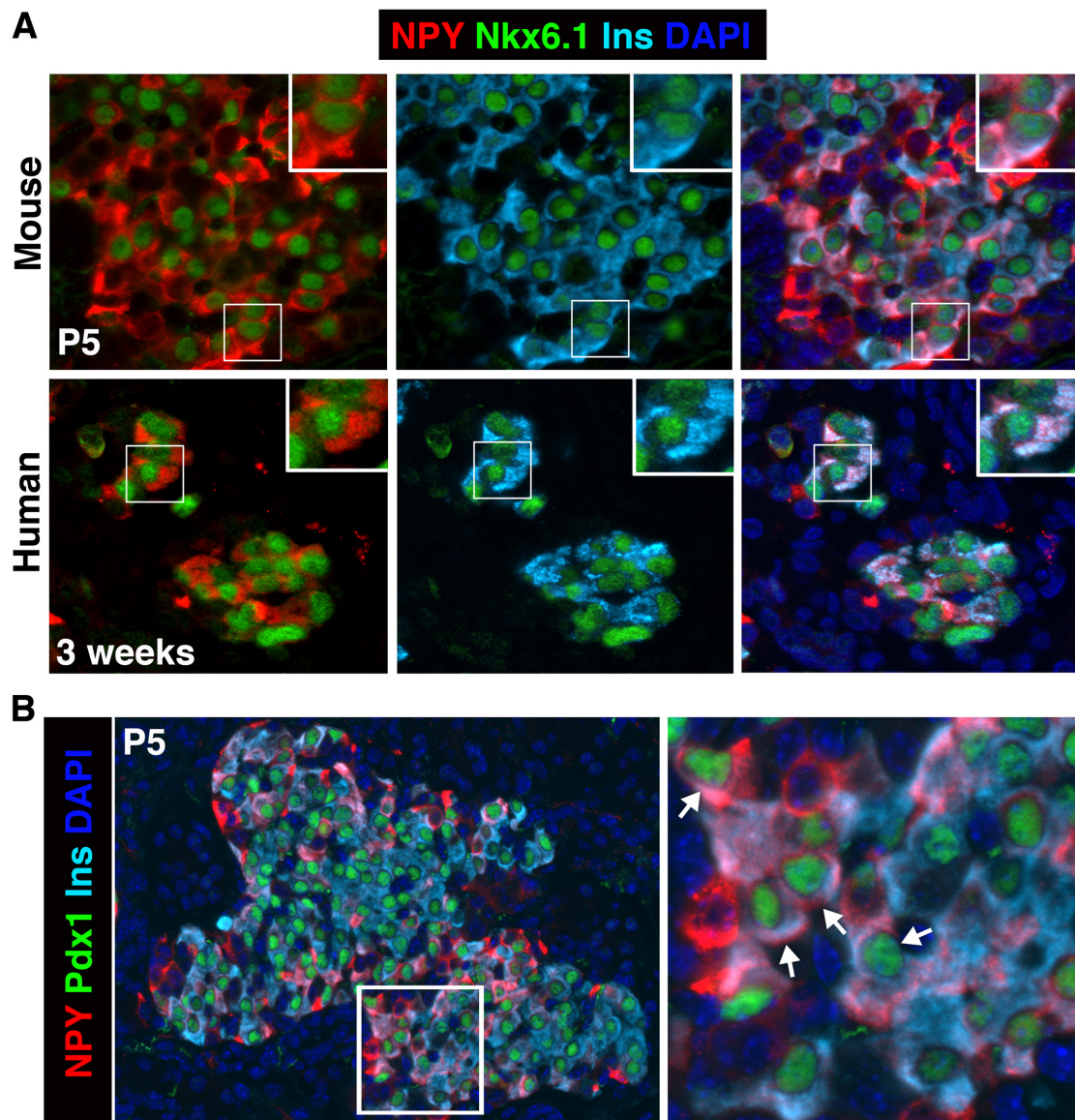


Supplemental Figure 1. (A) Representative serial sections from wildtype, adult (2.5 months old) mouse pancreata, immunostained with anti-NPY (left), or anti-PYY (right) antibodies,

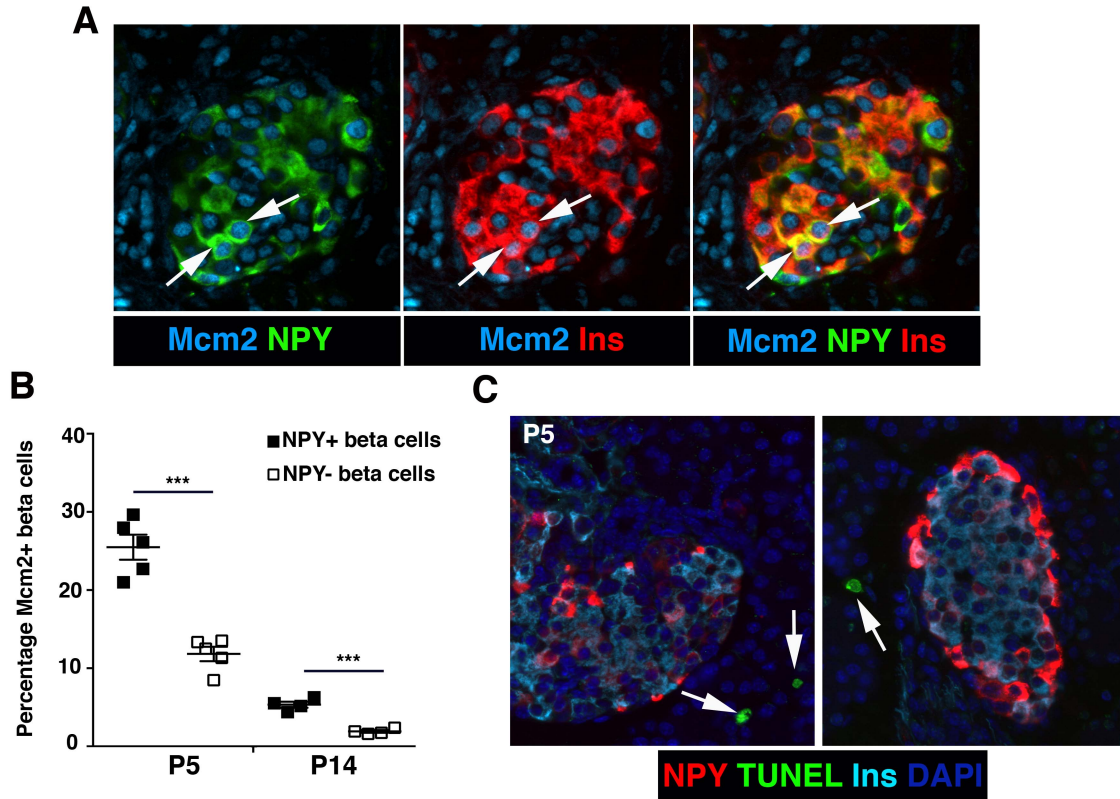
shown in gray. DAPI is shown in blue. Arrow marks a pancreatic ganglion. **(B)** Representative serial sections from wildtype, adult (2.5 months old) mice, immunostained with anti-NPY (top panels), or anti-PYY (bottom panels) antibodies, with, or without blocking by NPY peptide. Representative pancreatic sections from wild-type mouse embryos at embryonic day E15.5 and 18.5 immunostained for NPY (red), somatostatin (SS; green), and insulin (Ins; cyan), with DAPI to mark the nuclei. **(C)** Immunostaining of representative, serial pancreatic sections from wildtype, neonatal (P5) mice for NPY or PYY (red), with glucagon (Glu; green), and insulin (Ins; cyan), along with DAPI in blue. **(D)** Immunostaining for NPY (red), and glucagon (green), in a representative pancreatic section from E12.5 mouse embryos. **(E)** A representative pancreatic section from Ngn3-EGFP embryos at E15.5 showing NPY expression (red), along with GFP (green), glucagon (Glu; cyan) and overlay with DAPI in blue. Arrows mark cells with high GFP levels, indicating endocrine progenitors. **(F)** Immunostaining of embryonic (E18.5) mouse pancreatic section for NPY (red), somatostatin (SS; green), insulin (Ins; cyan), along with DAPI in blue. Arrows indicate cells with NPY and SS co-localization, **(G)** Immunostaining of fetal human pancreatic section, 39 weeks gestation (nPOD 6349) for NPY (red), glucagon or somatostatin (Glu or SS; green), and insulin (Ins; cyan), with DAPI shown in blue. Arrows mark cells co-expressing NPY with glucagon (top panels) or somatostatin (bottom panels). For **(A)**, **(B)**, and **(C)** N=4 animals.



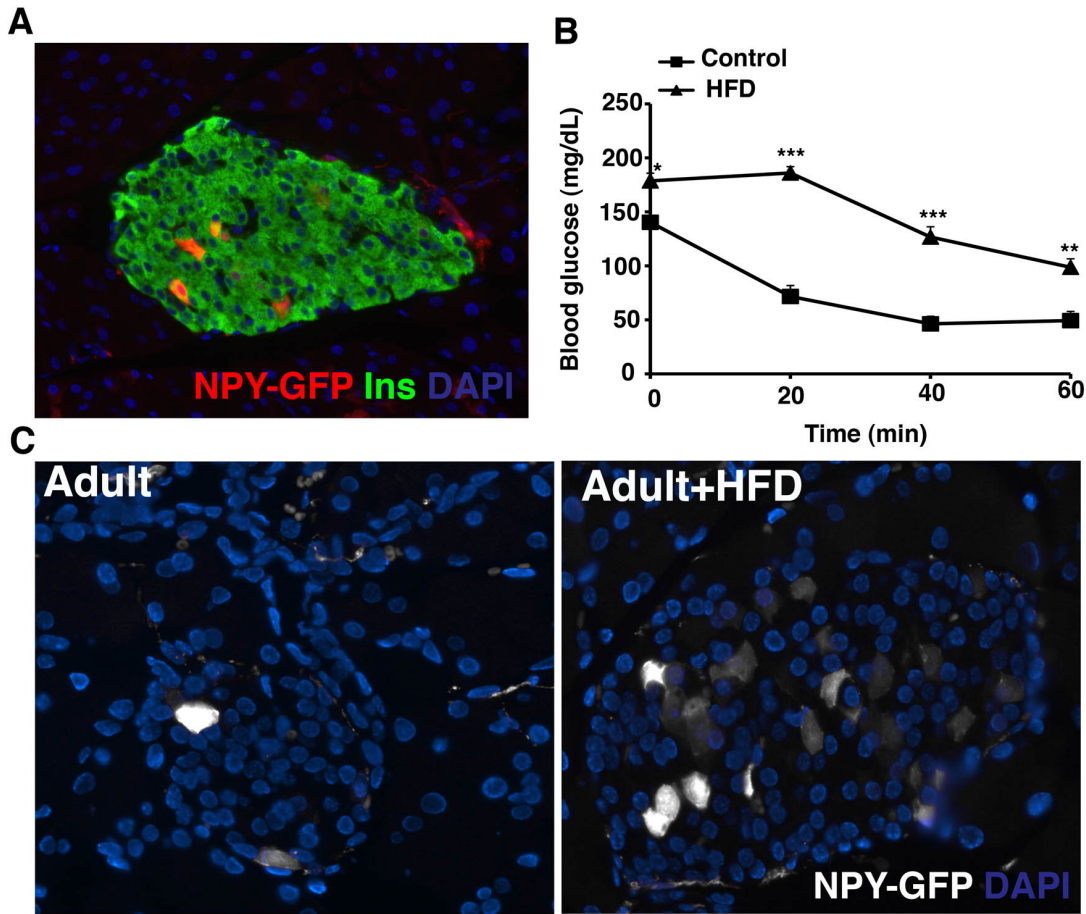
Supplemental Figure 2. (A) Representative mouse embryonic (E14.5) pancreatic section stained for NPY (red) and Tuj1 (green), with DAPI to mark the nuclei. (B) A static incubation GSIS assay in islets from wildtype mice at indicated stages in postnatal life. (C) Immunostaining of representative pancreatic sections from neonatal wildtype mice (P5) showing immunostaining for NPY (red), insulin (Ins; cyan), and glucagon (Glu), or somatostatin (SS), or ghrelin (Ghr), or pancreatic polypeptide (PP) in green, with DAPI in blue. Arrowheads mark NPY positive cells co-expressing the indicated hormone. (D) Immunostaining for NPY (red), somatostatin (SS; green), insulin (Ins; cyan) with DAPI in blue, in representative pancreatic sections from wild-type adult (2.5 months old) mice. (E) Immunostaining of neonatal (2 days postnatal; Mayo repository; left) and adult (nPOD 6003; right) human pancreatic section for NPY (red), glucagon or somatostatin (Glu or SS; green), and insulin (Ins; cyan), with DAPI shown in blue. Arrowheads mark the rare NPY+Ins+ double positive cells found in adult human islets. For (A), N=4 animals. For (B), N=3 independent experiments, with each replicate representing a pool of islets from 6-8 pups. The error bars represent standard error (SEM) of the mean. * $P < 0.05$, *** $P < 0.005$; 1-way ANOVA followed by Bonferroni's post-hoc test.



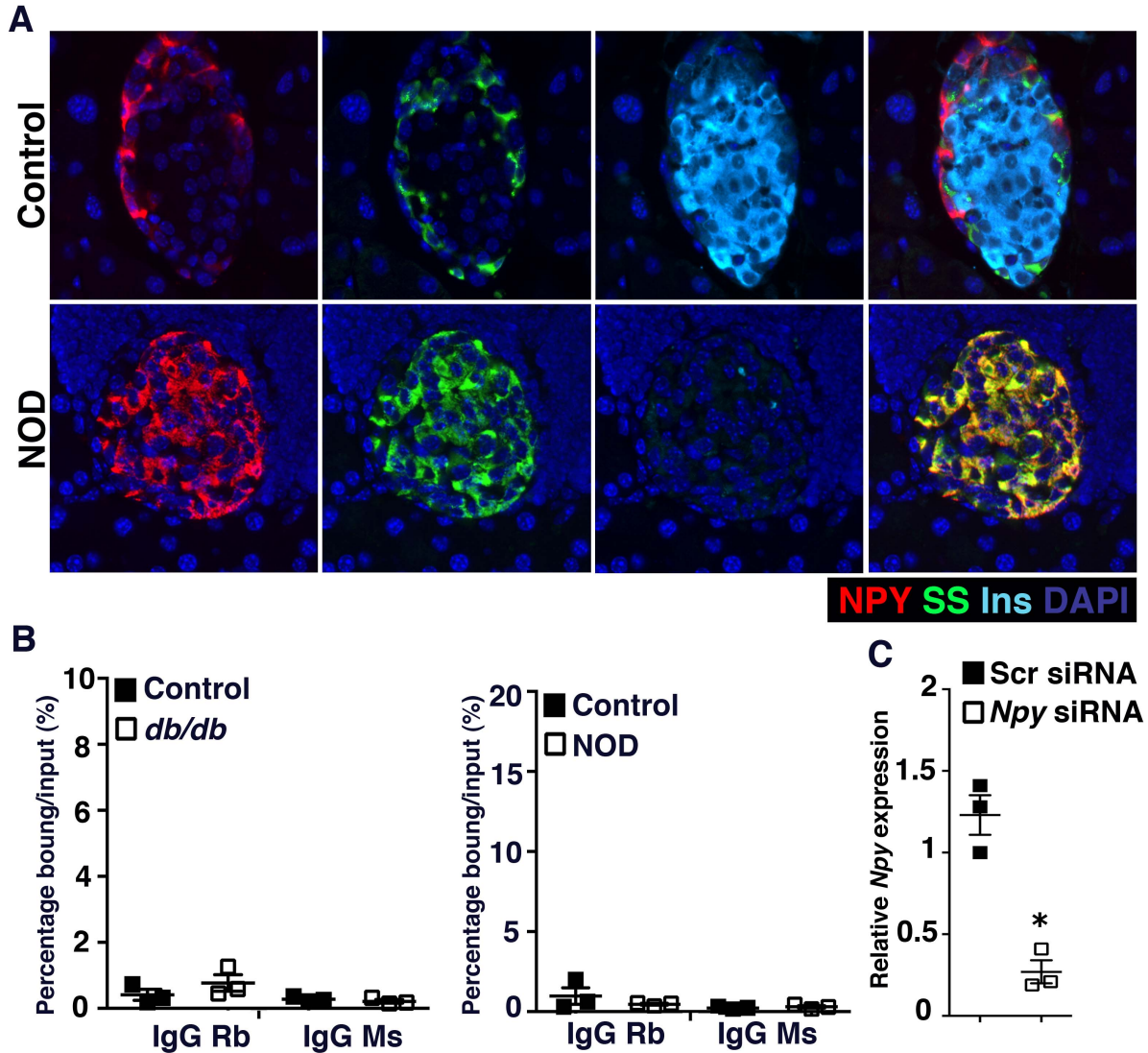
Supplemental Figure 3. (A) Immunostaining of representative pancreatic sections from neonatal mice (upper panels) and humans (lower panels) at indicated developmental stages for NPY (red), Nkx6.1 (green), and insulin (Ins; cyan). DAPI (blue) marks the nuclei. Insets show a zoomed in view of areas highlighted with a box. (B) Immunostaining of a representative pancreatic section from wild-type neonatal (P5) mouse for NPY (red), Pdx1 (green), and insulin (Ins; cyan), with DAPI in blue. For mouse experiments in (A) and (B) N=4 animals.



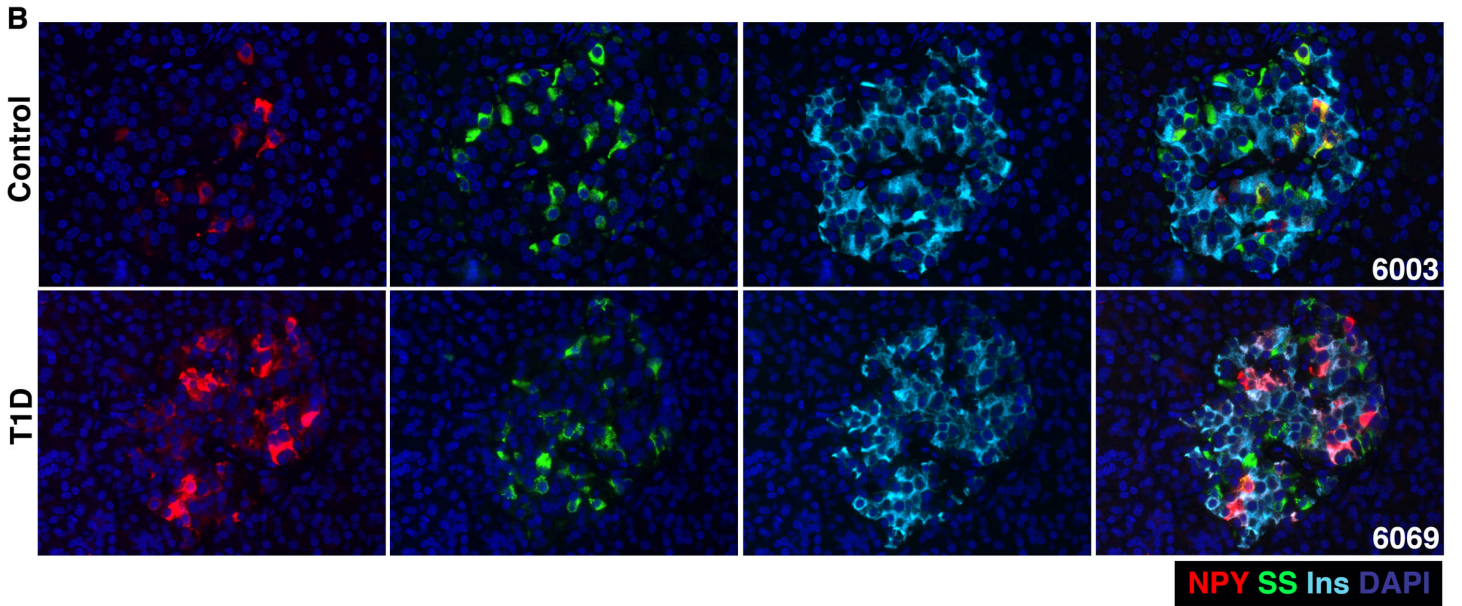
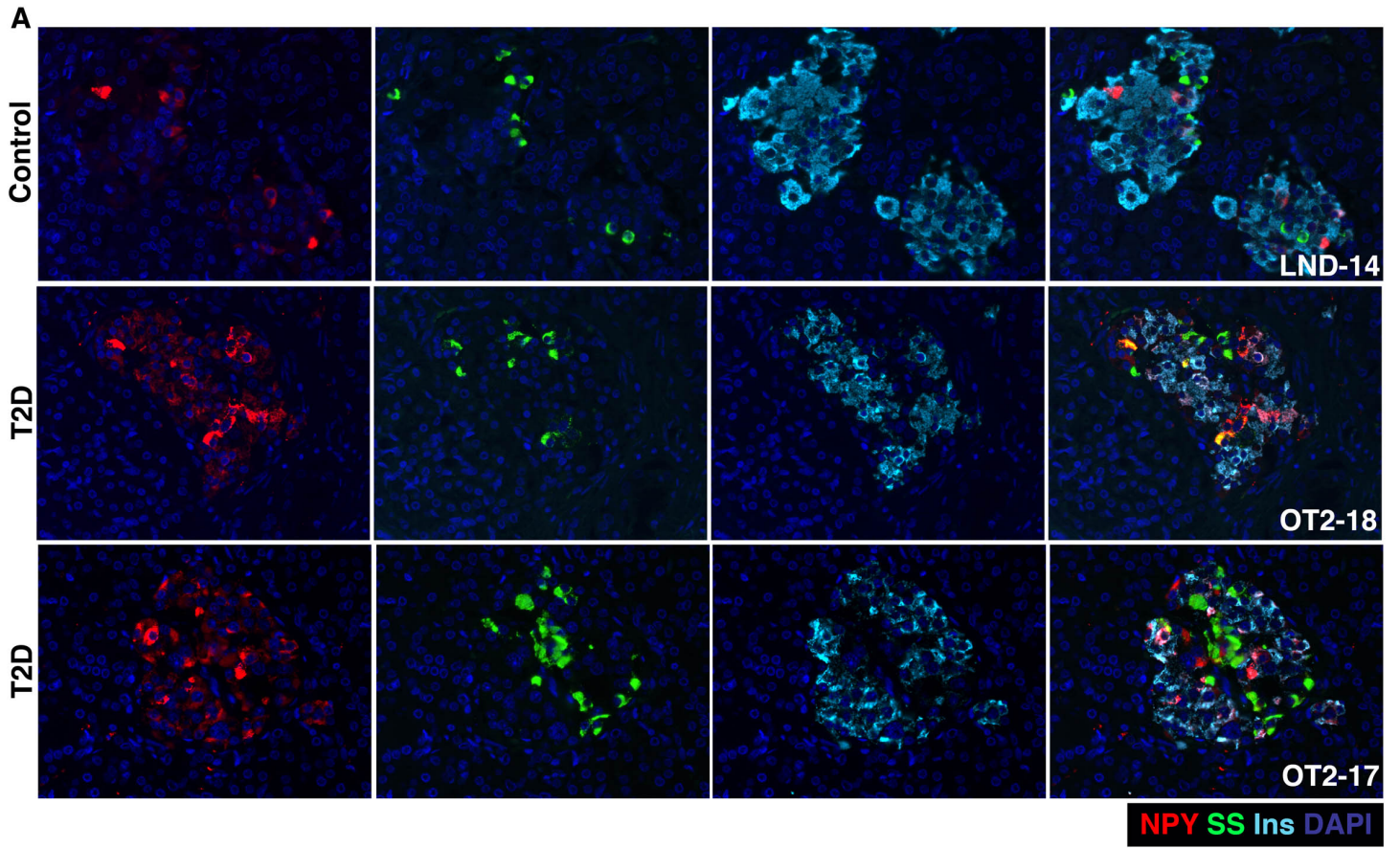
Supplemental Figure 4. (A) A representative pancreatic section from wildtype, neonatal (P5) mouse showing immunostaining for NPY (green), Insulin (Ins; red), and Mcm2 (cyan). Arrows mark replicating NPY-positive beta-cells. (B) Quantification of Mcm2 in NPY-positive and negative subpopulations of beta-cells in wildtype neonatal mice at P5 and P14. (C) Representative pancreatic sections from wild-type, neonatal (P5) mice showing immunostaining for NPY (red), TUNEL (marker of apoptosis; green), and insulin (Ins; cyan). DAPI (blue) labels the nuclei. For (A-C), N=4 animals. The error bars represent standard error (SEM) of the mean. *** $P < 0.005$; 1-way ANOVA followed by Bonferroni's post-hoc test.

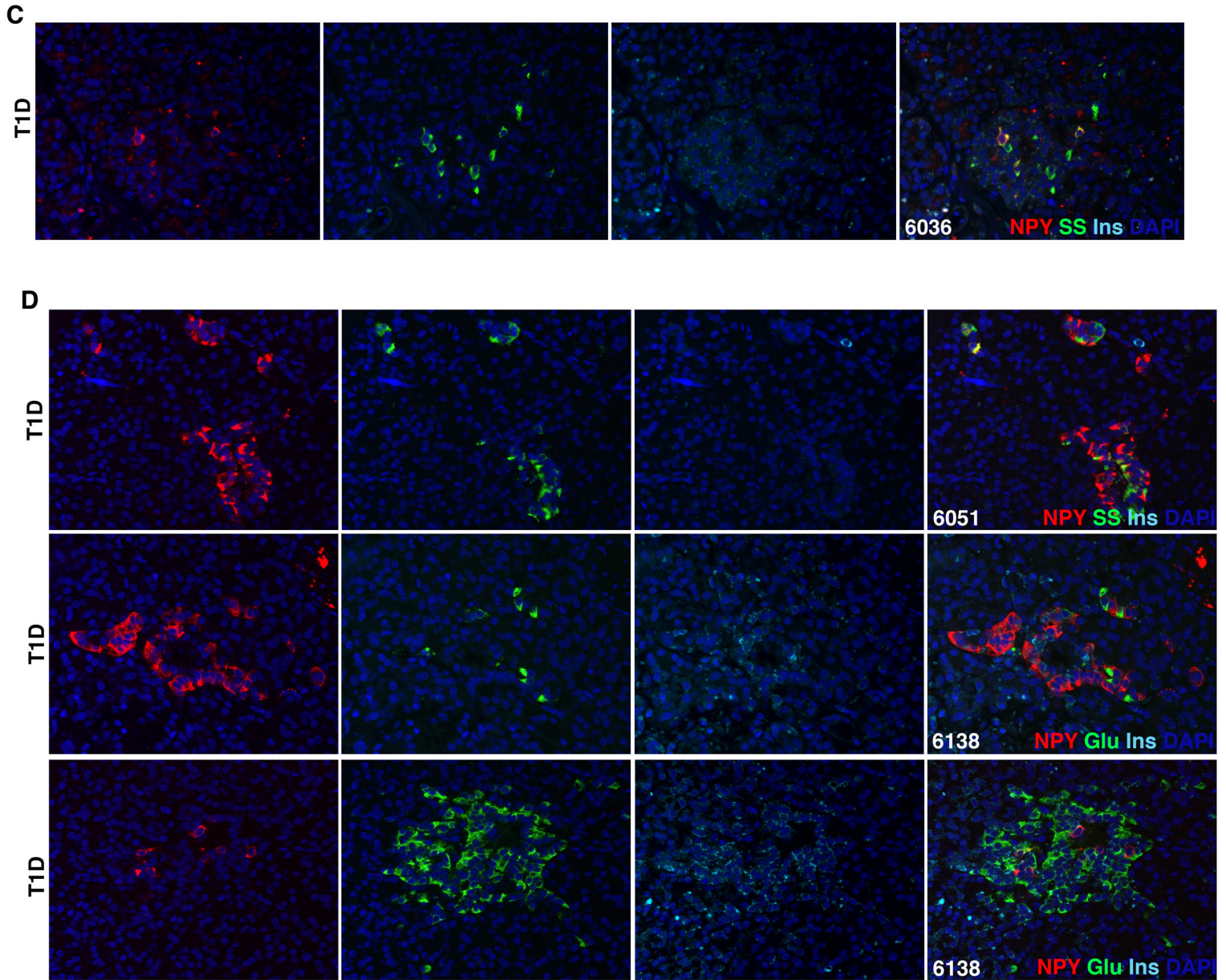


Supplemental Figure 5. (A) Immunostaining of a representative pancreatic section from adult (2.5 months old) NPY-GFP mice for insulin (Ins; green). hrGFP fluorescence driven by NPY promoter is shown in red, along with DAPI in blue. (B) Intra-peritoneal insulin tolerance test (IP-ITT) in adult (2 months old) mice, fed with control diet, or high fat diet (HFD). (C) NPY promoter driven hrGFP (shown in gray) expression in pancreatic sections from adult (2 months old) NPY-GFP mice, fed with control diet (Adult), or a high fat diet (Adult+HFD). For (A-C), N=5 animals. The error bars represent standard error (SEM) of mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$; 1-way ANOVA followed by Fisher's LSD post-hoc test.



Supplemental Figure 6. (A) Immunostaining of representative pancreatic sections from NOD (age=8 weeks; bottom panels) with age-matched controls, for NPY (red), somatostatin (SS; green), and insulin (Ins; cyan), with DAPI marking nuclei in blue. Islets in NOD mice are surrounded by infiltrate. (B) Chromatin immunoprecipitation (ChIP) showing background controls with control rabbit and mouse IgGs for H3K9me3 and H3K9Ac, respectively, at the *Npy* promoter region in islets isolated from *db/db* (6 weeks old) and NOD (8 weeks old) mice, along with age-matched controls. (C) *Npy* mRNA levels in islets from 6 weeks old *b/db* mice, treated either with an siRNA targeting *Npy*, or a control, scrambled (Scr) siRNA. For (B, C), N=3 independent experiments, with each replicate representing a pool of islets from 3 mice. The error bars represent standard error (SEM) of the mean. $P > 0.05$, Not significant for (B), $*P < 0.05$, 2-tailed Student's *t*-test.





Supplemental Figure 7. (A) Pancreatic sections were immunostained for NPY (red), somatostatin (SS; green), and insulin (Ins; cyan), with DAPI in blue in the following samples: human donors with type2 diabetes (T2D; OT2-18; Mayo; ~48% beta-cells positive for NPY. OT2-17; Mayo; ~53% beta-cells positive for NPY) and control (LND-14, Mayo; ~23% beta-cells positive for NPY). (B) Immunostaining of pancreatic sections from human donor with type1 diabetes (T1D; 6069, nPOD; ~70% beta-cells positive for NPY), and control (6003, nPOD; ~10% beta-cells positive for NPY) with NPY (red), somatostatin (SS; green), and insulin (Ins; cyan), with DAPI in blue. (C) Pancreatic sections from a human donor subject with type1 diabetes (T1D; 6036 nPOD) displaying residual beta cells, were immunostained for NPY (red), somatostatin (SS; green), and insulin (Ins; cyan), along with nuclear counterstaining with DAPI (blue). (D) Pancreatic sections from human donor subjects with type1 diabetes (T1D; 6051 nPOD, top panel, and 6138 nPOD, middle and

bottom panels) were immunostained for NPY (red), glucagon or somatostatin (Glu or SS; green), and insulin (Ins; cyan), with DAPI in blue.

Supplemental Table 1

Real-time RT-PCR primers used for mouse mRNA expression analysis.

Gene	Forward	Reverse
<i>Npy</i>	5'- CCGCTCTGCGACACTACAT-3'	5'-TGTCTCAGGGCTGGATCTCT-3'
<i>Ucn3</i>	5'-GACCTGAGCATTTCCTCC-3'	5'-CAGAAGTGGCAGCAGGAAGT-3'
<i>MafA</i>	5'-CTCCAGAGCCAGGTGGAG-3'	5'-GTACAGGTCCCCTCCTTG-3'
<i>MafB</i>	5'-GAAGCCCGCAGGCATAT-3'	5'-GGCCCTGGCACTCACAAA-3'
<i>Cyclophilin</i>	5'-GTCTCCTTCGAGCTGTTTGC-3'	5'-AGCCAAATCCTTTCTCTCCA-3'

Supplemental Table 2

Primers used for ChIP analysis in mice.

	Location	Forward	Reverse
<i>Npy</i>	Promoter	5'-GTCCCGCTTCATCTCCCTA -3'	5'-GTGACTCCCACAGCCACTTC -3'
<i>HoxC13B</i> (negative control)	Exon 2	5' -CATTTTCACTGATTCCTAAG CA-3'	5' -CAATGATGTCACCCCTCCTC- 3'