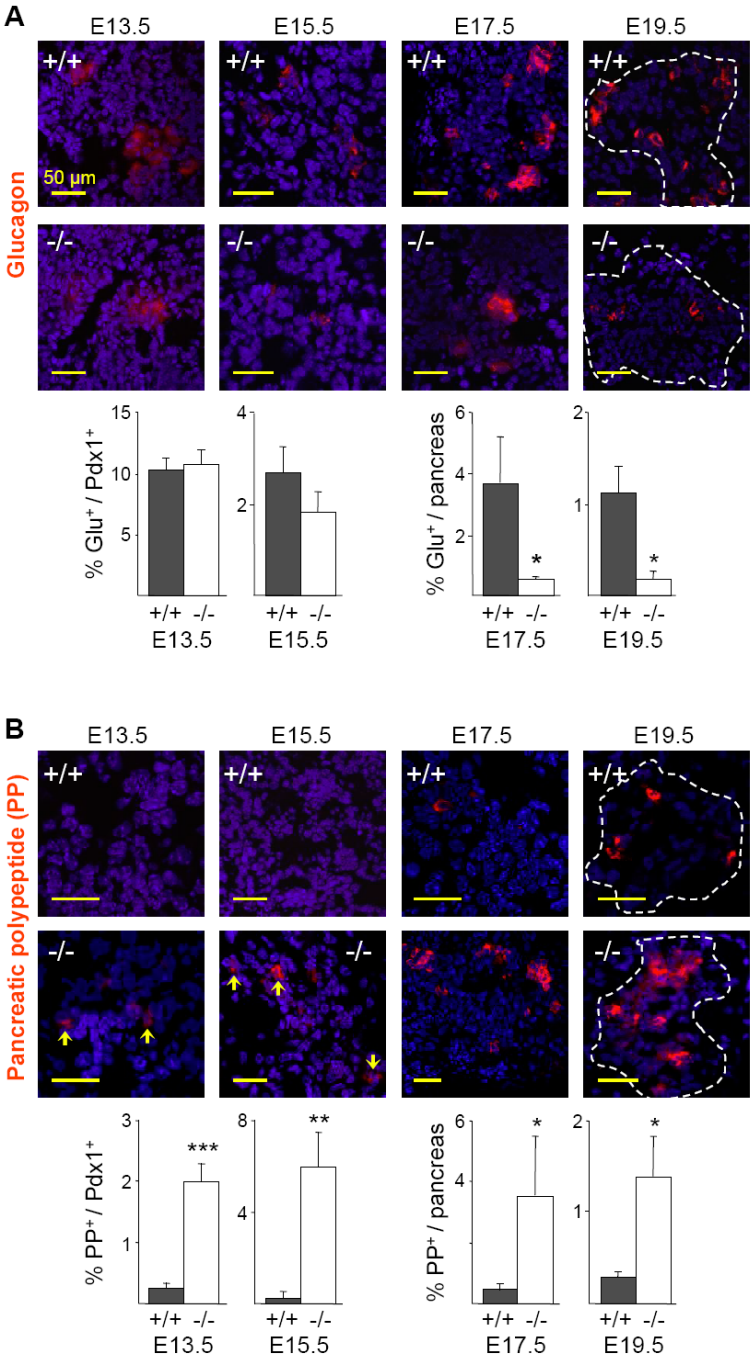
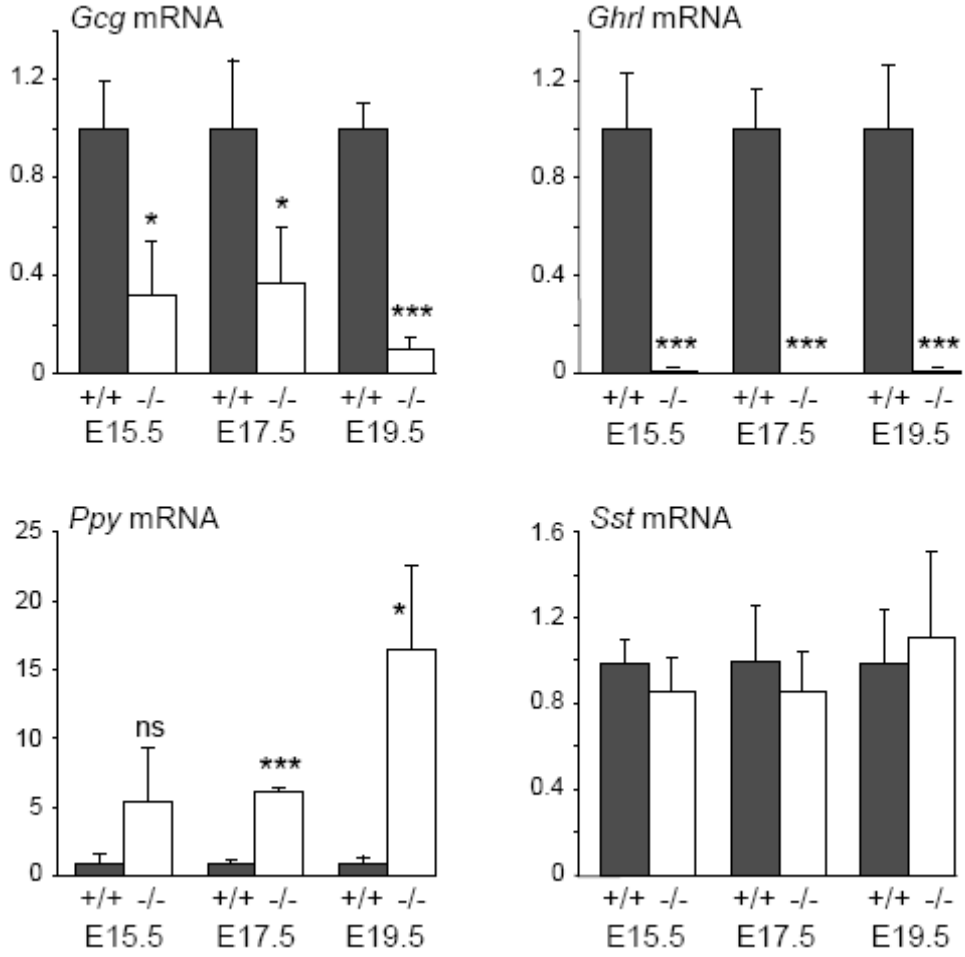
ONLINE APPENDIX

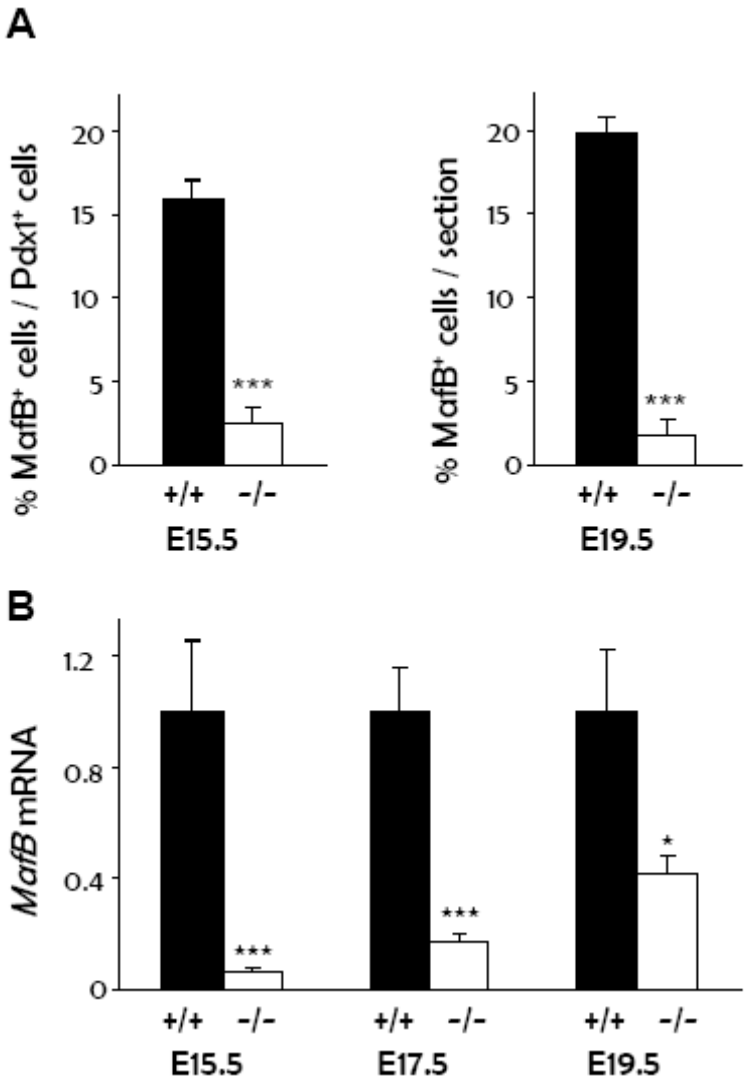
Supplemental Figure 1. Altered development of glucagon^{+} and PP^{+} cells in *Rfx3*$^{-/-}$ embryos. (A) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E13.5, E15.5, E17.5 and E19.5 were stained with DAPI and antibodies against glucagon. Representative sections are shown (top). The percentages of glucagon^{+} cells were quantified in total Pdx1^{+} cells (E13.5 and E15.5) or in the entire pancreas (E17.5 and E19.5) (bottom). Pdx1^{+} cells were counted on adjacent sections. (B) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E13.5, E15.5, E17.5 and E19.5 were stained with DAPI and antibodies against PP. Representative sections are shown (top). The percentages of PP^{+} cells were quantified in total Pdx1^{+} cells (E13.5 and E15.5) or in the entire pancreas (E17.5 and E19.5) (bottom). Pdx1^{+} cells were counted on adjacent sections.



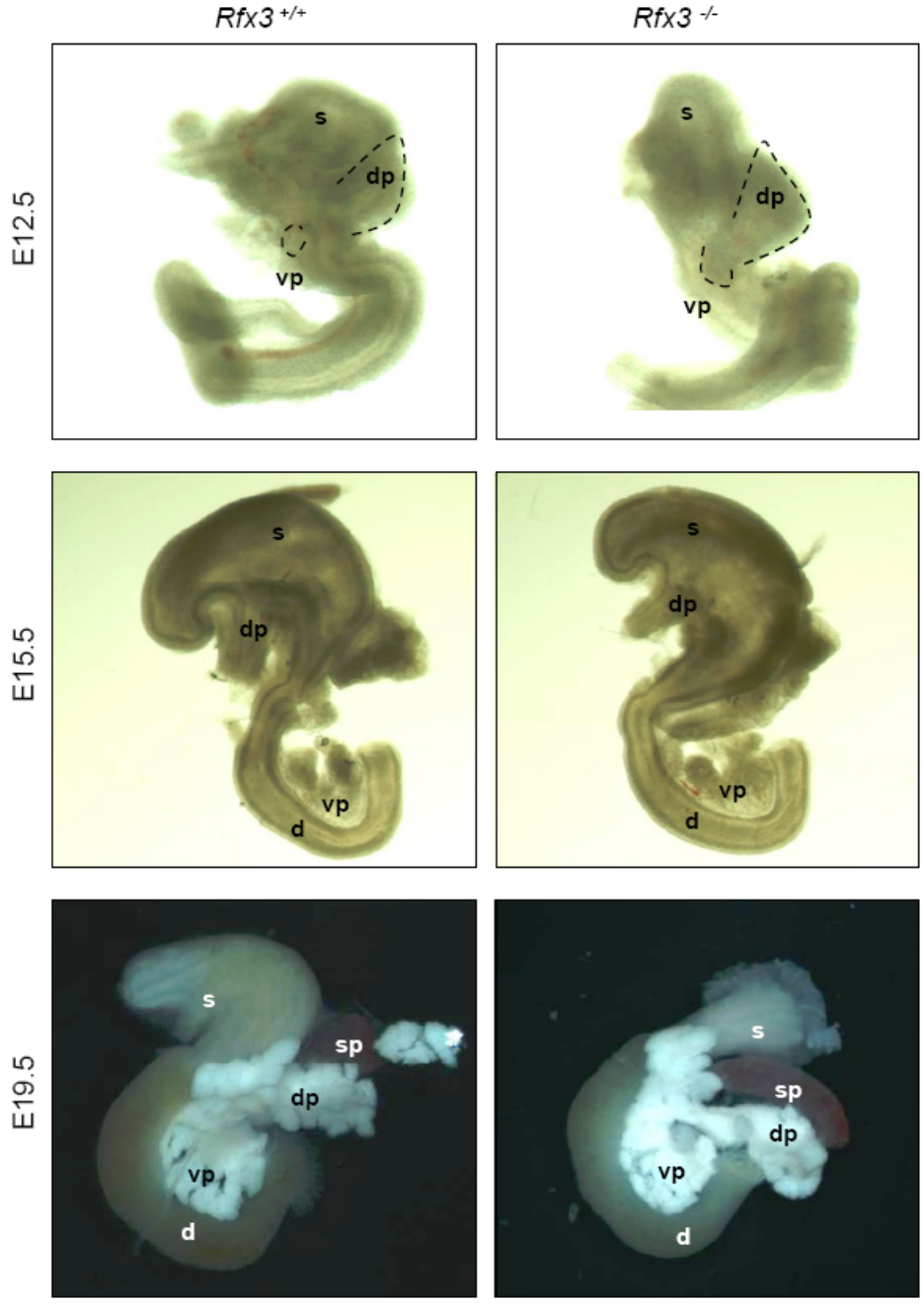
Supplemental Figure 2. Altered expression of Glucagon, ghrelin and PP in *Rfx3*$^{-/-}$ embryos. Glucagon (*Gcg*), Ghrelin (*Ghrl*), PP (*PPy*) and somatostatin (*Sst*) mRNA levels were quantified in the pancreases of WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E15.5, E17.5 and E19.5. The results were normalized using *Tbp* mRNA and are expressed relative to the levels found in WT embryos. The mean ± s.e.m. derived from 5 embryos is shown for each stage and genotype; ***, $P < 0.0001$; *, $P < 0.05$; ns, not significant.



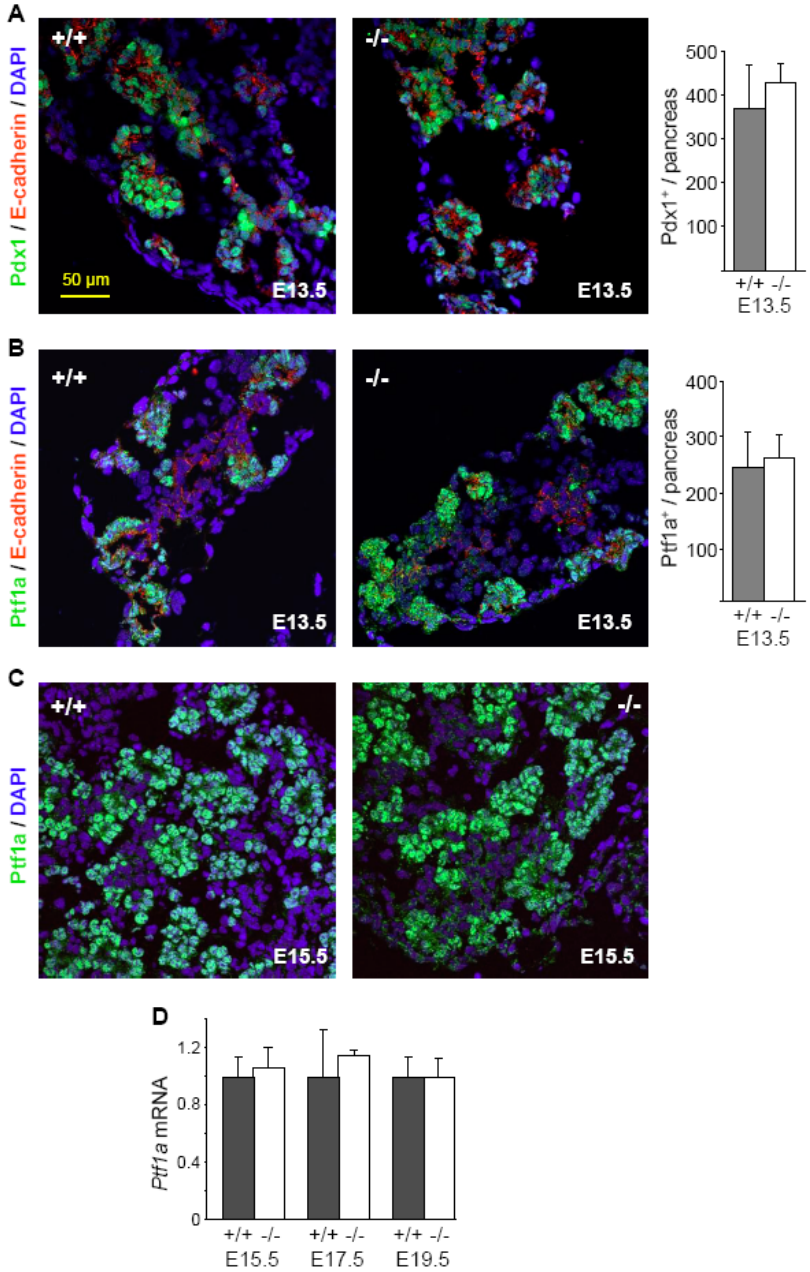
Supplemental Figure 3. MafB expression is reduced in *Rfx3*$^{-/-}$ mice. (A) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E15.5 and E19.5 were stained with antibodies against MafB and Pdx1. The percentages of MafB^{+} cells were quantified in Pdx1^{+} cells (E15.5) or per section (E19.5). *MafB* mRNA levels were quantified in the pancreases of WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E15.5, E17.5 and E19.5. The results were normalized using *Tbp* mRNA and are expressed relative to the levels found in WT embryos. The mean ± s.e.m. derived from 5 embryos is shown for each stage and genotype; ***, P < 0.0001; *, P < 0.05.



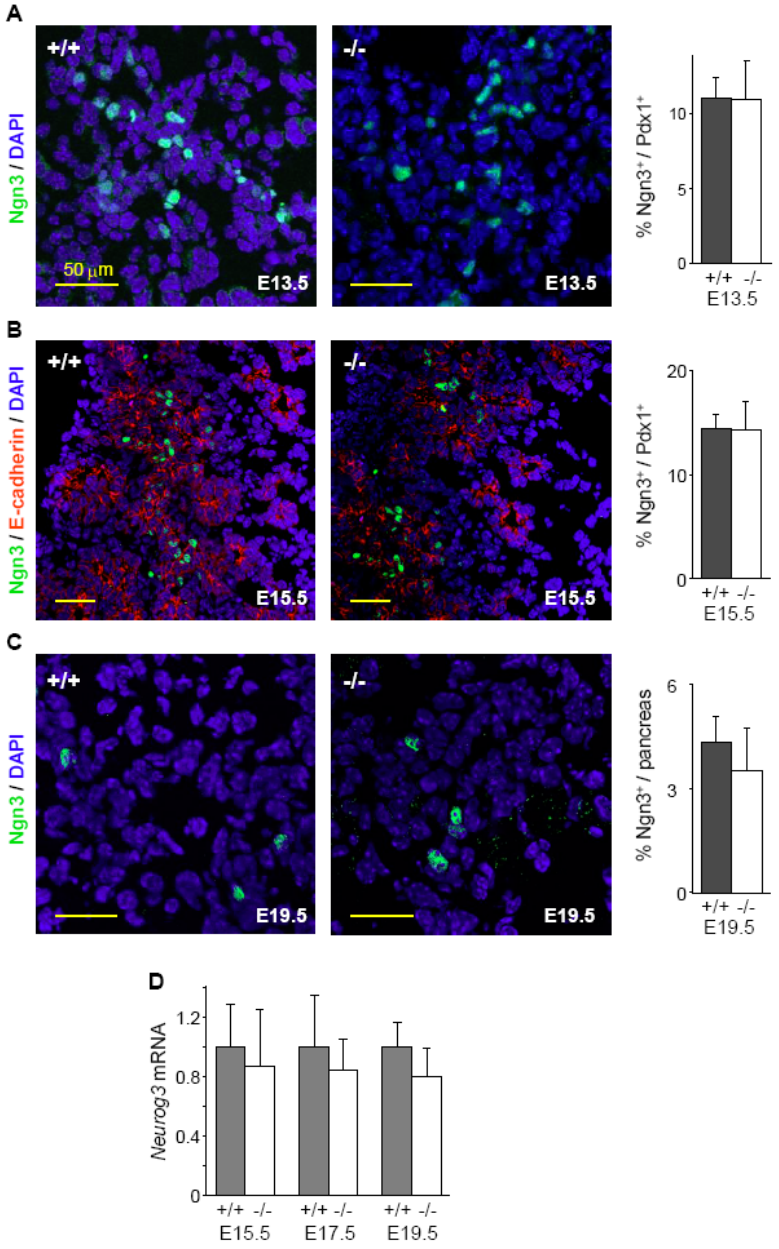
Supplemental Figure 4. Development of the pancreas is normal in *Rfx3*$^{-/-}$ embryos. The developing pancreas was examined in WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E12.5, E15.5 and E19.5: vp and dp, ventral and dorsal pancreatic primordia; s, stomach; d, duodenum; sp, spleen.



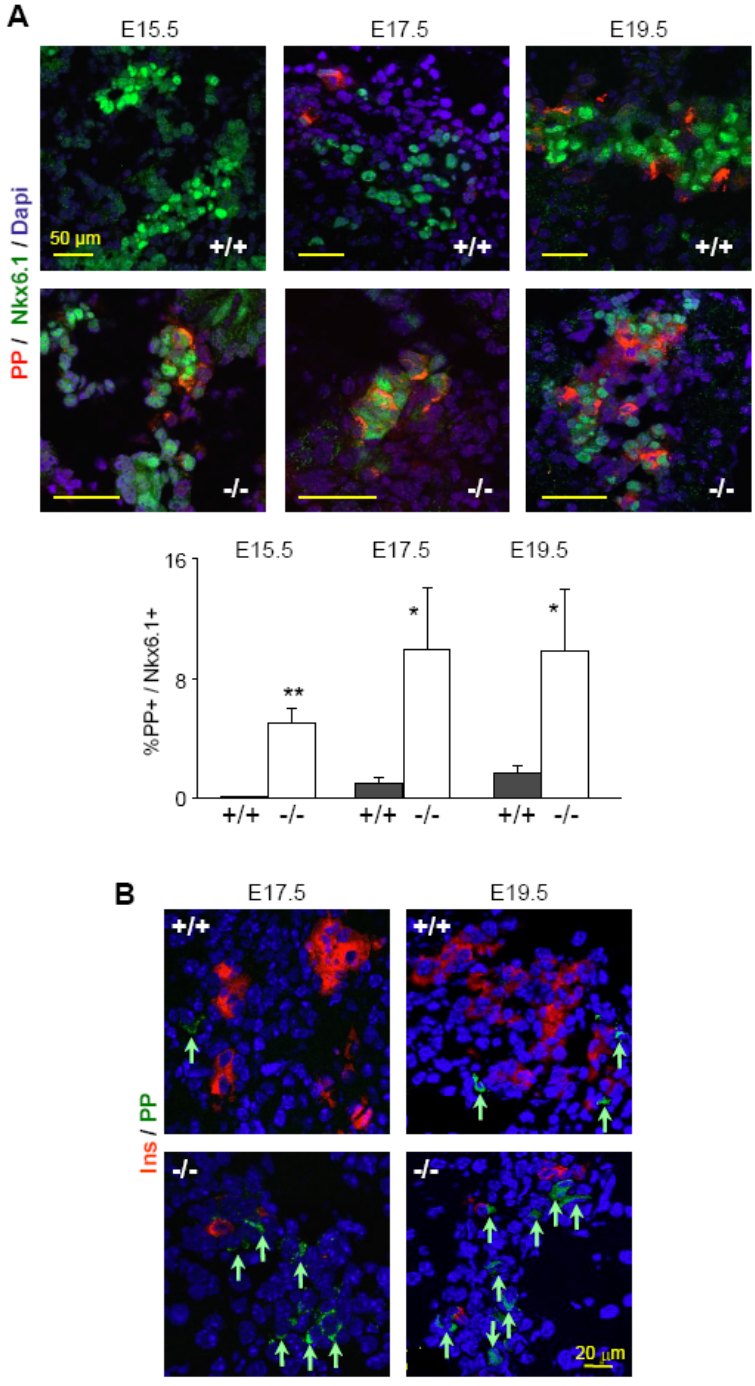
Supplemental Figure 5. The development of pancreatic and exocrine progenitor cells is not affected in *Rfx3*$^{-/-}$ embryos. A) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stage E13.5 were stained with DAPI and antibodies against Pdx1 and E-cadherin. The latter was used to identify the pancreatic epithelium. Representative sections are shown (left). Pdx1^{+} cells were quantified (right). (B) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$(-/-) embryos at stage E13.5 were stained with DAPI and antibodies against Ptf1a and E-cadherin. Representative sections are shown (left). Ptf1a^{+} cells were quantified (right). (C) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$(-/-) embryos at stage E15.5 were stained with DAPI and antibodies against Ptf1a. Representative sections are shown (left). (D) *Ptf1a* mRNA levels were quantified in the pancreases of WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E15.5, E17.5 and E19.5. The results were normalized using *Tbp* mRNA and are expressed relative to the levels found in WT embryos. (A, B and D) The mean ± s.e.m. derived from 5 embryos is shown for each stage and genotype.



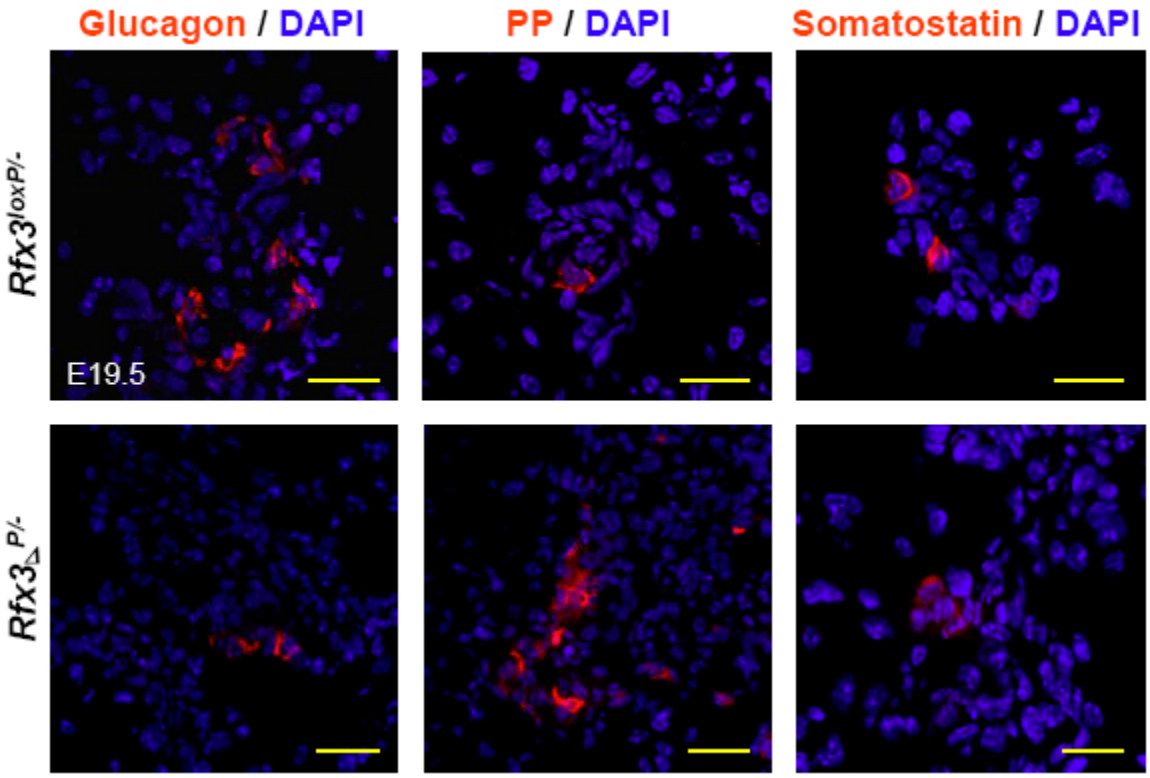
Supplemental Figure 6. The development of Ngn3^{+} endocrine progenitor cells is not affected in *Rfx3*$^{-/-}$ embryos. (A) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stage E13.5 were stained with DAPI and antibodies against Ngn3. Representative sections are shown (left). Ngn3^{+} cells were quantified as percentage of Pdx1^{+} cells (right). Pdx1^{+} cells were counted on adjacent sections. (B) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stage E15.5 were stained with DAPI and antibodies against Ngn3 and E-cadherin. Representative sections are shown (left). Ngn3^{+} cells were quantified as percentage of Pdx1^{+} cells (right). Pdx1^{+} cells were counted on adjacent sections. (C) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stage E19.5 were stained with DAPI and antibodies against Ngn3. Representative sections are shown (left). Ngn3^{+} cells were quantified (right). (D) *Ngn3* mRNA abundance was measured by qRT-PCR in total pancreas RNA from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E15.5, E17.5 and E19.5. The results were normalized using *Tbp* mRNA and are expressed relative to the levels found in WT embryos. (A-D) The mean ± s.e.m. derived from 5 embryos is shown for each stage and genotype.



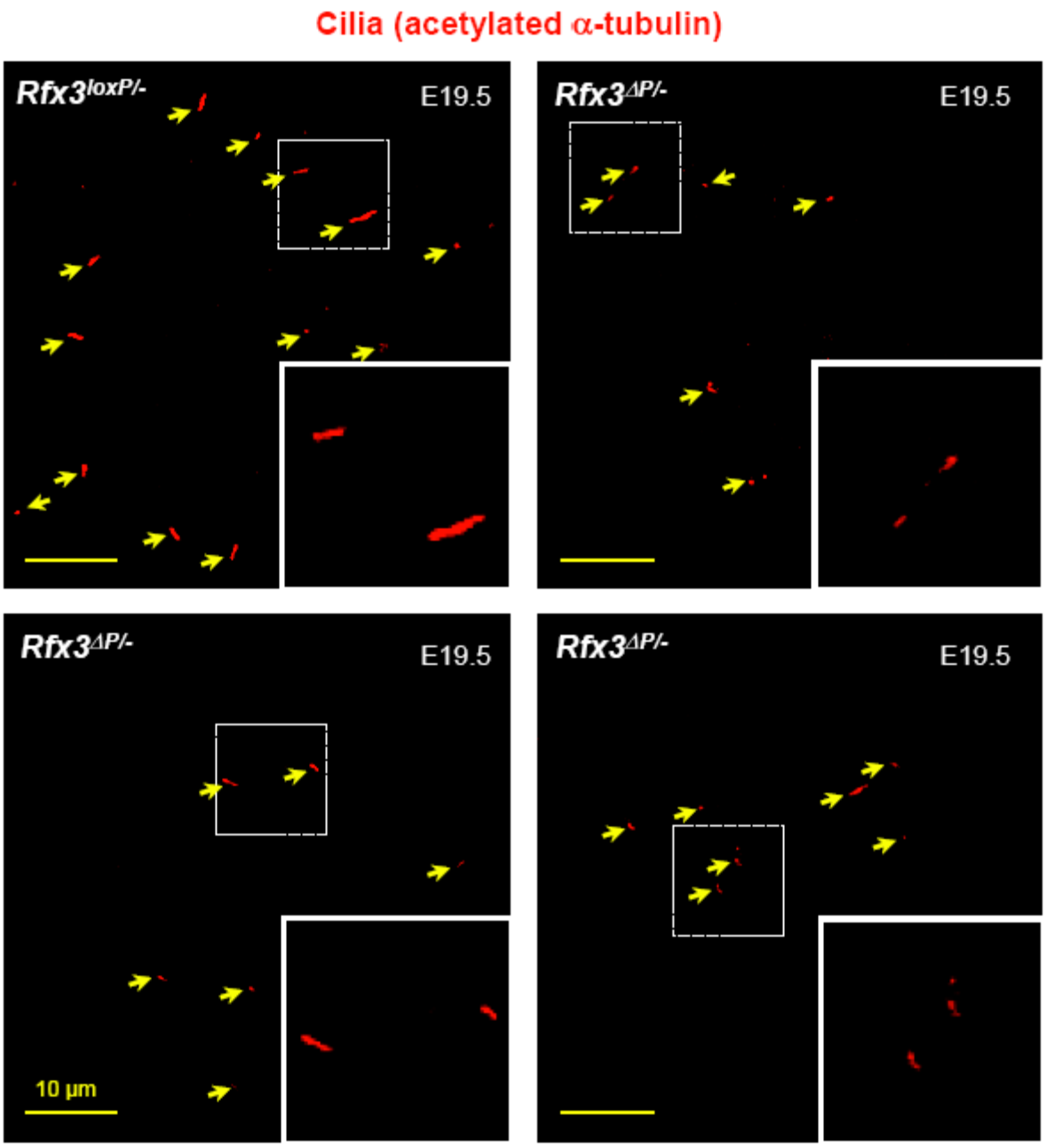
Supplemental Figure 7. A significant fraction of Nkx6.1^{+} insulin^{-} cells in *Rfx3*$^{-/-}$ embryos exhibit ectopic PP expression. (A) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E15.5, E17.5 and E19.5 were co-stained with DAPI and antibodies against PP and Nkx6.1. Representative sections are shown (top). The percentages of PP^{+} cells were quantified in total Nkx6.1^{+} cells (bottom). The mean ± s.e.m. derived from 5 embryos is shown for each stage and genotype; **, P < 0.001; *, P < 0.05. (B) Pancreas sections from WT (+/+) and *Rfx3*$^{-/-}$ (-/-) embryos at stages E17.5 and E19.5 were co-stained with DAPI and antibodies against PP and Nkx6.1. Representative sections are shown. Most PP^{+} cells (green arrows) do not co-express insulin.



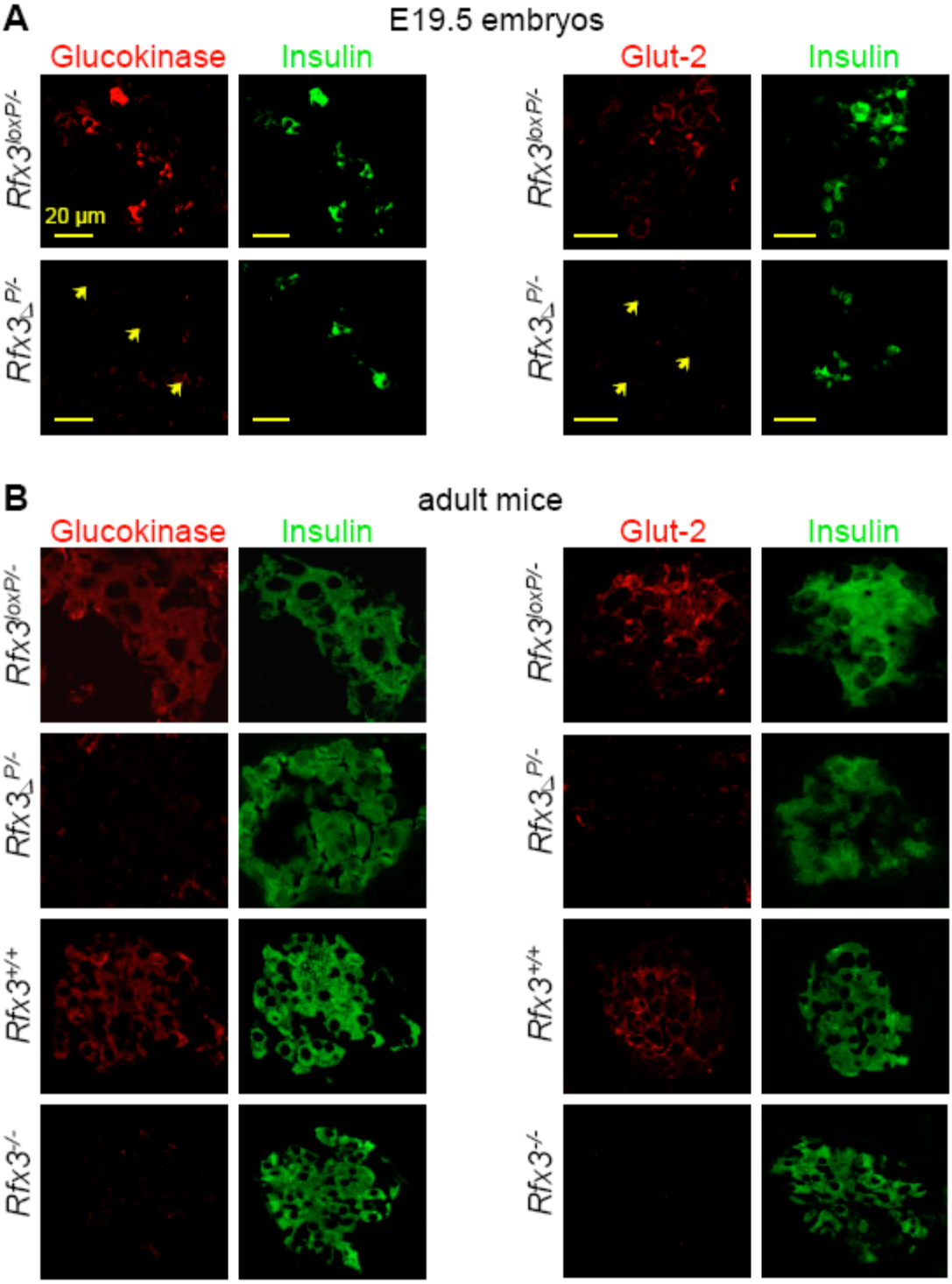
Supplemental Figure 8. Altered development of glucagon^{+} and PP^{+} cells in pancreas-specfic Rfx3-deficient embryos. Pancreas sections from *Rfx3*$^{loxP/-}$ and *Rfx3*$^{\Delta P/-}$ E19.5 embryos were stained with DAPI and antibodies against glucagon, PP or somatostatin. Representative sections are shown.



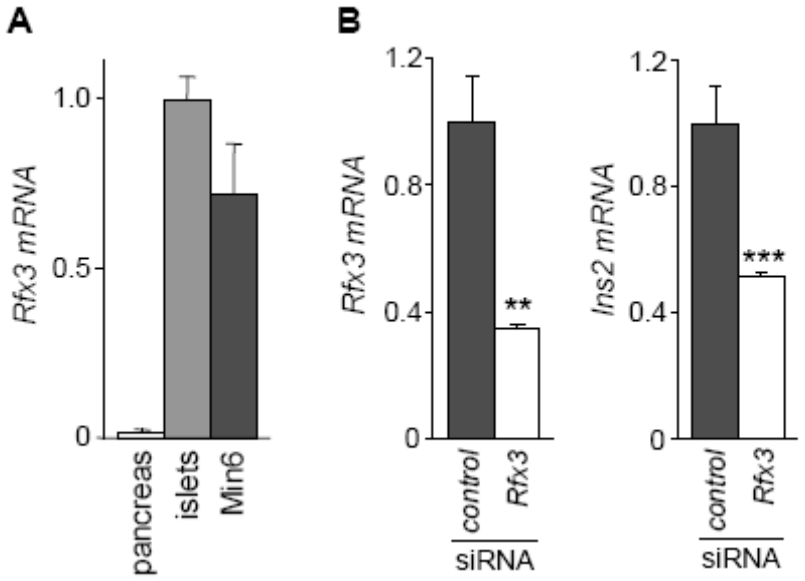
Supplemental Figure 9. Altered development of cilia in the islets of pancreas-specfic Rfx3-deficient embryos. Pancreas sections from *Rfx3*$^{loxP/-}$ and *Rfx3*$^{\Delta P/-}$ E19.5 embryos were stained with antibodies against acetylated α-tubulin. Representative sections are shown (one for *Rfx3*$^{loxP/-}$ and three for *Rfx3*$^{\Delta P/-}$). Cilia (arrows) are reduced in numbers and are on the average shorter (insets) in *Rfx3*$^{\Delta P/-}$ embryos. The insets show higher magnifications of the cilia enclosed by the dashed boxes.



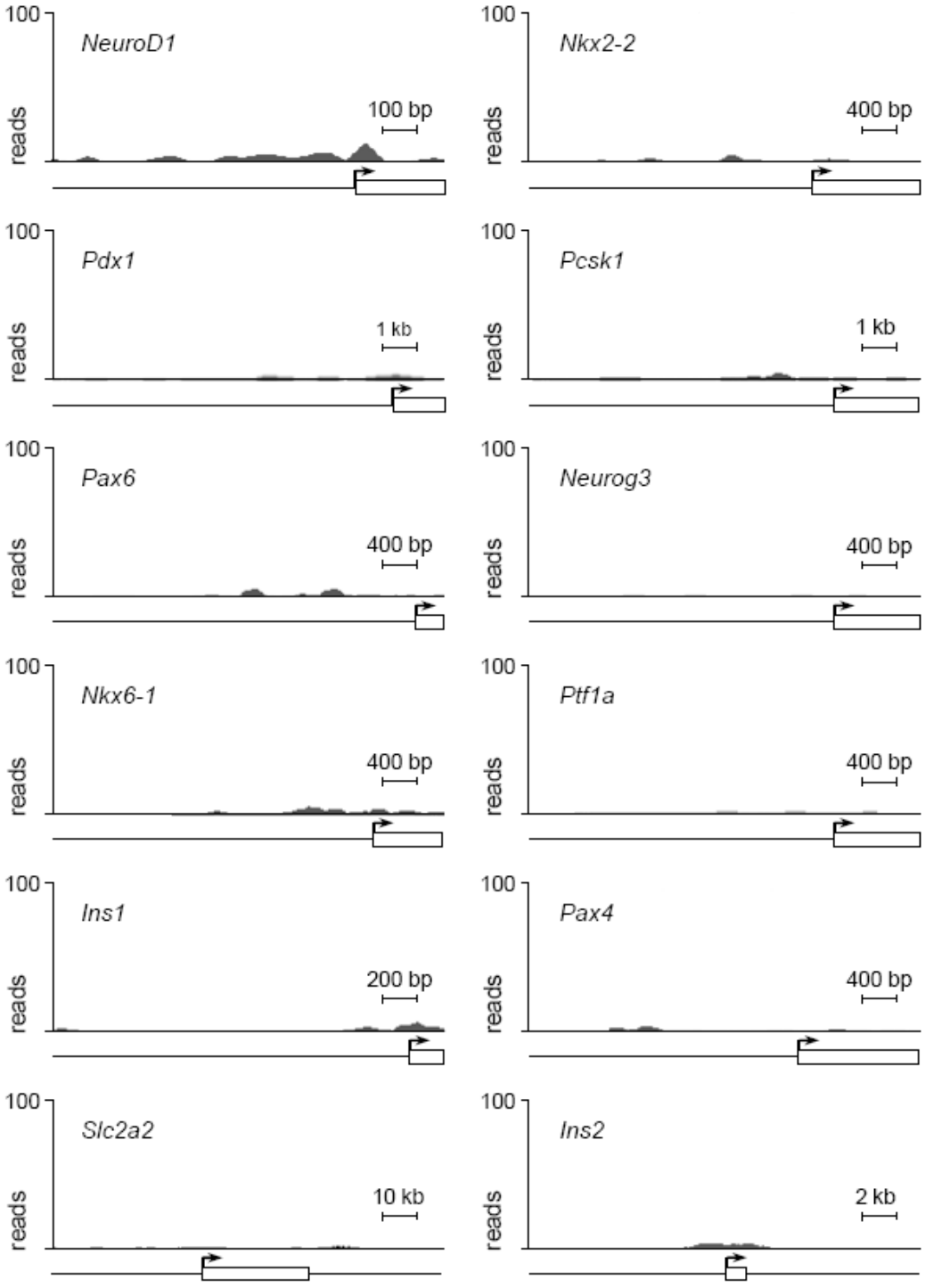
Supplemental Figure 10. β-cells in pancreas-specific Rfx3-deficient mice exhibit strongly decreased Glut-2 and glucokinase expression. Pancreas sections from *Rfx3*$^{loxP/-}$ and *Rfx3*$^{\Delta P/-}$ embryos at stage E19.5 (A) and adult *Rfx3*$^{loxP/-}$, *Rfx3*$^{\Delta P/-}$, *Rfx3*$^{+/+}$ and *Rfx3*$^{-/-}$ mice (B) were co-stained with antibodies against insulin and glucokinase (left) or insulin and Glut-2 (right). Representative sections are shown. Paraffin fixed sections are shown for E19.5 embryos whereas frozen sections are shown for adult mice. Residual insulin^{+} cells in *Rfx3*$^{\Delta P/-}$ embryos showing little or no Glut-2 and glucokinase expression are indicated by arrows.



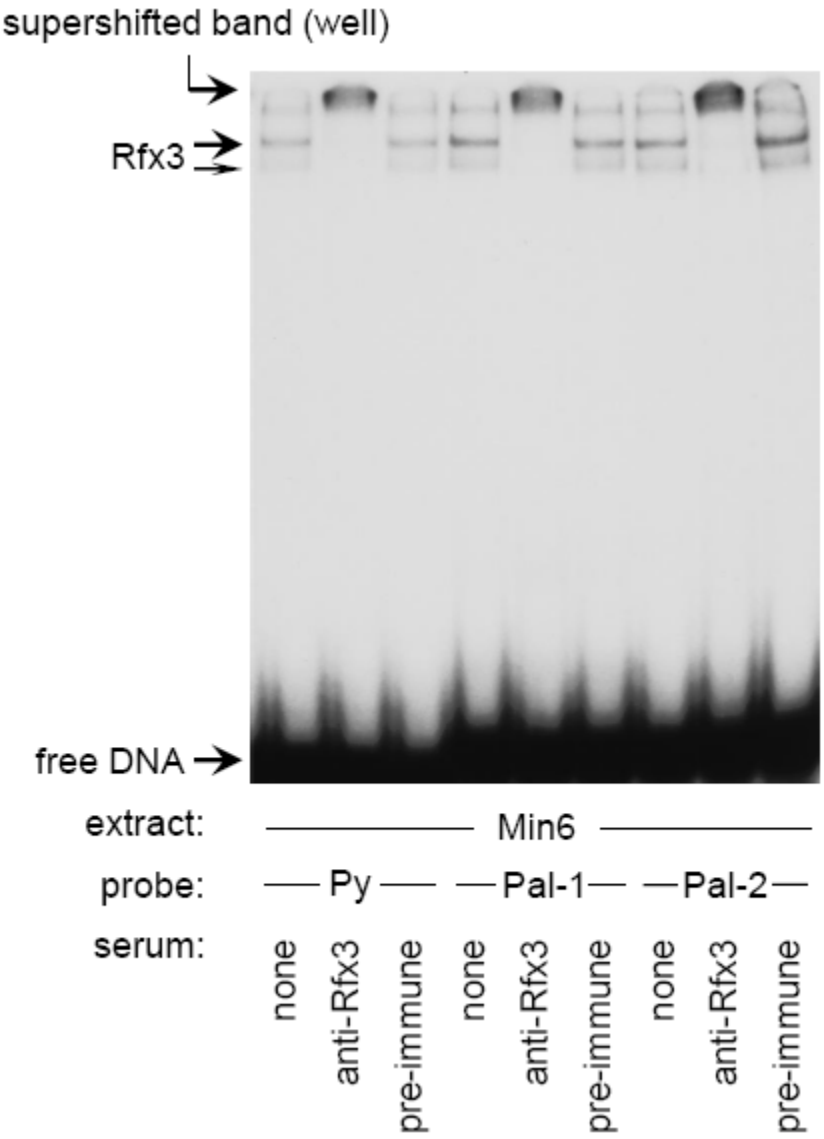
Supplemental Figure 11. The inhibition of Rfx3 expression by RNA interference in Min6 cells leads to reduced insulin expression. (A) *Rfx3* mRNA levels were measured in total pancreas, purified islets and Min6 cells. The results were normalized using *Tbp* mRNA and are expressed relative to the islets. (B) *Rfx3* and *Ins2* mRNAs were quantified in Min6 cells transduced with control and *Rfx3*-specific siRNA vectors. The results were normalized using *Tbp* mRNA, are expressed relative to cells transduced with the control vector, and show the mean ± s.e.m. derived from 3 independent experiments; **, P < 0.001; *** P < 0.0001.



Supplemental Figure 12. ChIP-seq results are shown for the *Neurod1*, *Pdx1*, *Pax6*, *Nkx6-1*, *Ins1*, *Slc2a2*, *Nkx2-2*, *Pcsk1*, *Neurog3*, *Ptf1a*, *Pax4* and *Ins2* genes. No significant signals above background (greater than 10-20 reads) are observed at any of these genes. Transcription start sites (arrows), transcribed regions (open boxes) and scales in base pairs (bp) or kilobases (kb) are indicated.



Supplemental Figure 13. Rfx3 binds to the Pal-1 and Pal-2 sites of the *Gck* promoter in Min6 cells. Bandshift experiments were performed with Min6 extracts using double-standed oligonucleotide probes containing Pal-1, Pal-2 or the Rfx binding site (Py) from polyoma-virus. Binding reactions were supplemented as indicated with Rfx3 antiserum or the control pre-immune serum. Positions of the wells, free DNA and the major band corresponding to binding of Rfx3 are indicated. The major Rfx3 complex is supershifted specifically by the Rfx3 antiserum. The weaker band below the major Rfx3 complex also contains Rfx3, as it is supershifted by the Rfx3 antiserum. This lower Rfx3 band varies in intensity in between different extracts and experiments. The minor band above the Rfx3 complexes corresponds to binding of Rfx1 (data not shown).



Supplementary Table 1: Reagents used for immunofluorescence studies

	Origin	Dilution[2]	
Primary antibodies			
Acetylated-alpha-tubulin (mouse)	Sigma, Missouri, USA	1:1000	F
phospho-histone H3 (rabbit)	Upstate, Lake Placid, NY, USA	1:500	P
E-cadherin (mouse)	BD, Biosciences, Allschwil, Switzerland	1:500	F
Glut2 (rabbit)	Chemicon, USA	1:1000	P
Glucokinase (rabbit)	Sigma, Missouri, USA	1:50	P
Glucagon (mouse)	Sigma, Missouri, USA	1:4000	P
Pancreatic polypeptide (guinea pig)	Linco Research, Missouri, USA	1:1000	F
Insulin (mouse)	Sigma, Missouri, USA	1:2000	F
Neurogenin-3 (rabbit)	Antibody Core, BCBC 2010	1:500	F
Nkx6-1 (rabbit)	Antibody Core, BCBC	1:1000	F
Nkx2-2 (mouse)	Developmental Studies Hybridoma Bank, Iowa, USA	1:200	F
Pax4 (rabbit)	gift from Beatriz Sosa-Pineda	1:500	F
Pdx1 (rabbit)	Antibody Core, BCBC	1:500	F
PC1/3 (rabbit	gift from Don Steiner	1/500	F
Ptf1a (rabbit)	gift from Bernadette Bréant	1/500	F
RFX3 (rabbit)	Reith et al. 1994[1]	1:100	F
Secondary reagents			
Biotin-xx-goat anti-rabbit IgG (H+L)	Invitrogen, Basel, Switzerland	1:200	
FITC-labeled Goat anti-Mouse IgG_1	Southern biotechnology, Birmingham, USA	1:100	
FITC-labeled Goat anti-Mouse IgG_{2b}	Southern biotechnology, Birmingham, USA	1:100	
FITC-labeled Mouse anti-Rabbit	Invitrogen, Basel, Switzerland	1:500	
TRITC-labeled Goat anti-Mouse IgG_{2a}	Southern biotechnology, Birmingham, USA	1:200	
TRITC-labeled Goat anti-Mouse IgG_{2b}	Southern biotechnology, Birmingham, USA	1:100	
Streptavidin, Alexa Fluor 488 conjugate	Invitrogen, Basel, Switzerland	1:300	

[1]Reith W, Ucla C, Barras E, Gaud A, Durand B, Herrero-Sanchez C, Kobr M, Mach B: RFX1, a transactivator of hepatitis B virus enhancer I, belongs to a novel family of homodimeric and heterodimeric DNA-binding proteins. *Mol Cell Biol* 14:1230-1244, 1994.

[2]Antibodies were used at the indicated dilutions to label frozen (F) or paraffin (P) sections.

Supplementary Table 2: primers used for real-time RT-PCR

mRNA	*orientation	sequence
Gck	F	5'-AGCTGCACCCGAGCTTCA-3'
	R	5'-GATTTCGCAGTTGGGTGTCA-3'
Slc2a2	F	5'-GTCACACCAGCATACACAAC-3'
	R	5'-AAAGCTGGACACAGACAGAG-3'
Ins 2	F	5'-AACATGGCCCTGTGGATGCG-3'
	R	5'-ACCAGGTGGGAACCACAAAG-3'
Mafa	F	5'-CAGCAGCGGCACATTCTG-3'
	R	5'-GCCCGCCAACTTCTCGTAT-3'
Neurod1	F	5'-ATAGAGACACTGCGCTTGGC-3'
	R	5'-AGAGCGTCTGTACGAAGGAG-3'
Neurog3	F	5'-CAAGAAGGCCAATGATCGG-3'
	R	5'-GTGCCCAGATGTAGTTGTG-3'
Nkx2-2	F	5'-TCGCTCTCCCCTTTGAACTTT-3'
	R	5'-GTTAACGTTGGGATGGTTTGG-3'
Nkx6-1	F	5'-AGAGAGCACGCTTGGCCTATTC-3'
	R	5'-GTCGTCAGAGTTCGGGTCCAG-3'
Pax4	F	5'-ACTACCGCACAGGTGTCTTGG-3'
	R	5'-GTCCTGGGTACAAAGCCCTTC-3'
Pcsk1	F	5'-TGGAGTTGCATATAATTCCAAAGTT-3'
	R	5'-CTAGCCTCAATGGCATCAGTT -3'
Pdx1	F	5'-CCGAGAGACACACAAAATCTGG-3'
	R	5'-CCCGCTACTACGTTTCTTATCTTCC-3'
Ptf1a	F	5'-ATCGAGGCACCCGTTCAC-3'
	R	5'-GAAAGAGAGTGCCCTGCAAGA-3'
Tbp	F	5'-ATGCTGAATATAATCCCAAGCGA-3'
	R	5'-GAAAATCAACGCAGTTGTCCG-3'

*F, forward; R, reverse

Supplementary Table 3: primers used for ChIP

Gene (position)	*orientation	sequence
Mouse *Dync2li1* promoter	F	5'-GCCGAAGGTGGAGAACTAC-3'
	R	5'-AGTTGGGCCAGAGAATGC-3'
Mouse *Dync2li1* downstream (background)	F	5'-AACTCCGGCTACTCTTCC-3'
	R	5'-AGGGCTTCTGATCCCTTG-3'
Mouse *Gck* promoter	F	5'-CGCCTCAGCTTTCTTCTCTGG-3'
	R	5'-GCCCTGACAGGAGACATCTAC-3'
Mouse *Slc2a2* promoter	F	5'-CAGGTCAGTGTACGAGAATC-3'
	R	5'-TGCGCTTACAAGTTCTCC-3'
Mouse *Ins2* promoter	F	5'-CAGGTACCCAGAGTGTGAATG-3'
	R	5'-TCCGCAAGGCTTTACAGAC-3'
Human *GCK* promoter	F	5'-GCTGACCATCCTGTCATTAG-3'
	R	5'-CAGGTGGCAAAGGCTTAAC-3'
Human *DYNC2LI* promoter	F	5'-AAGGCTTGAGGCCGAGAATGAG-3'
	R	5'-CGGGCATCATGGGAAATGTA-3'
Human *TBP* promoter (background)	F	5'-CAGTGACCCAGGTAACAG-3'
	R	5'-GCGCCAATTCCTAGTCTC-3'

*F, forward; R, reverse

Supplementary Table 4: Double stranded oligonucleotides used for bandshift experiments

oligonucleotide	[1]strand	sequence
Mouse Pal-1 (wild type)	U	5'-GGGAGTGGTCACCATGGTGACAGGAG-3'
	L	5'-GGCTCCTGTCACCATGGTGACCACTC-3'
Mouse Pal-2 (wild type)	U	5'-GGCAATGGTCACCATAGAAACCACAG-3'
	L	5'-GGCTGTGGTTTCTATGGTGACCATTG-3'
Mouse Pal-1m (mutated[2])	U	5'-GGGAGTGtgCAaaATttTGcaAGGAG-3'
	L	5'-GGCTCCTtgCAaaATttTGcaCACTC-3'
Mouse Pal-2m (mutated[2])	U	5'-GGCAATGtgCAaaATttttcaCACAG-3'
	L	5'-GGCTGTGtgaaaaATttTGcaCATTG-3'

[1] U, upper strand; L, lower strand
[2] Mutated nucleotides are indicated in underlined lower case