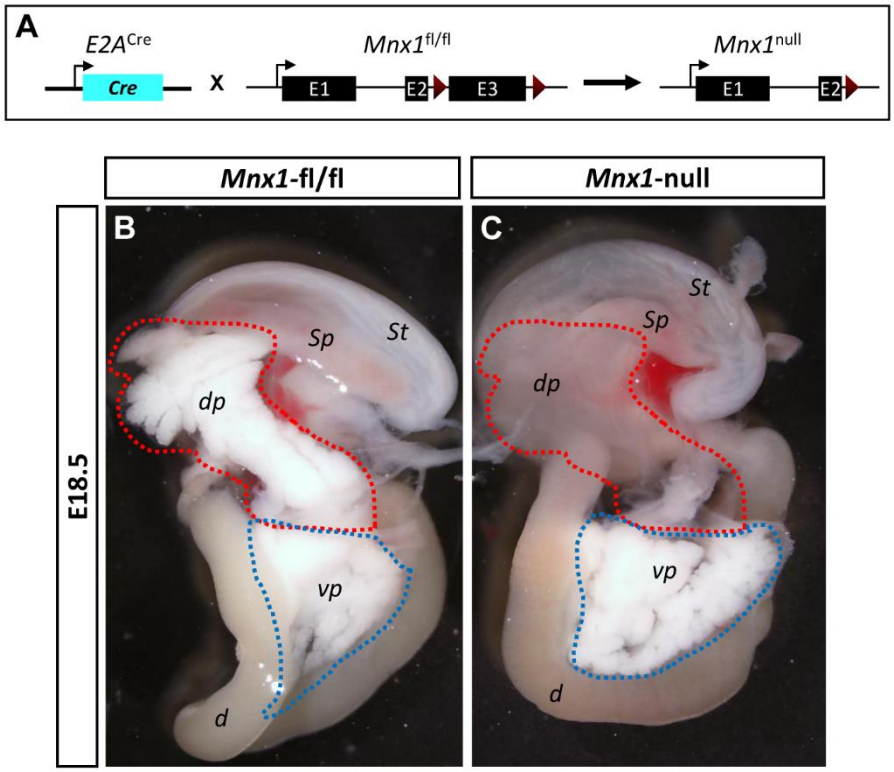
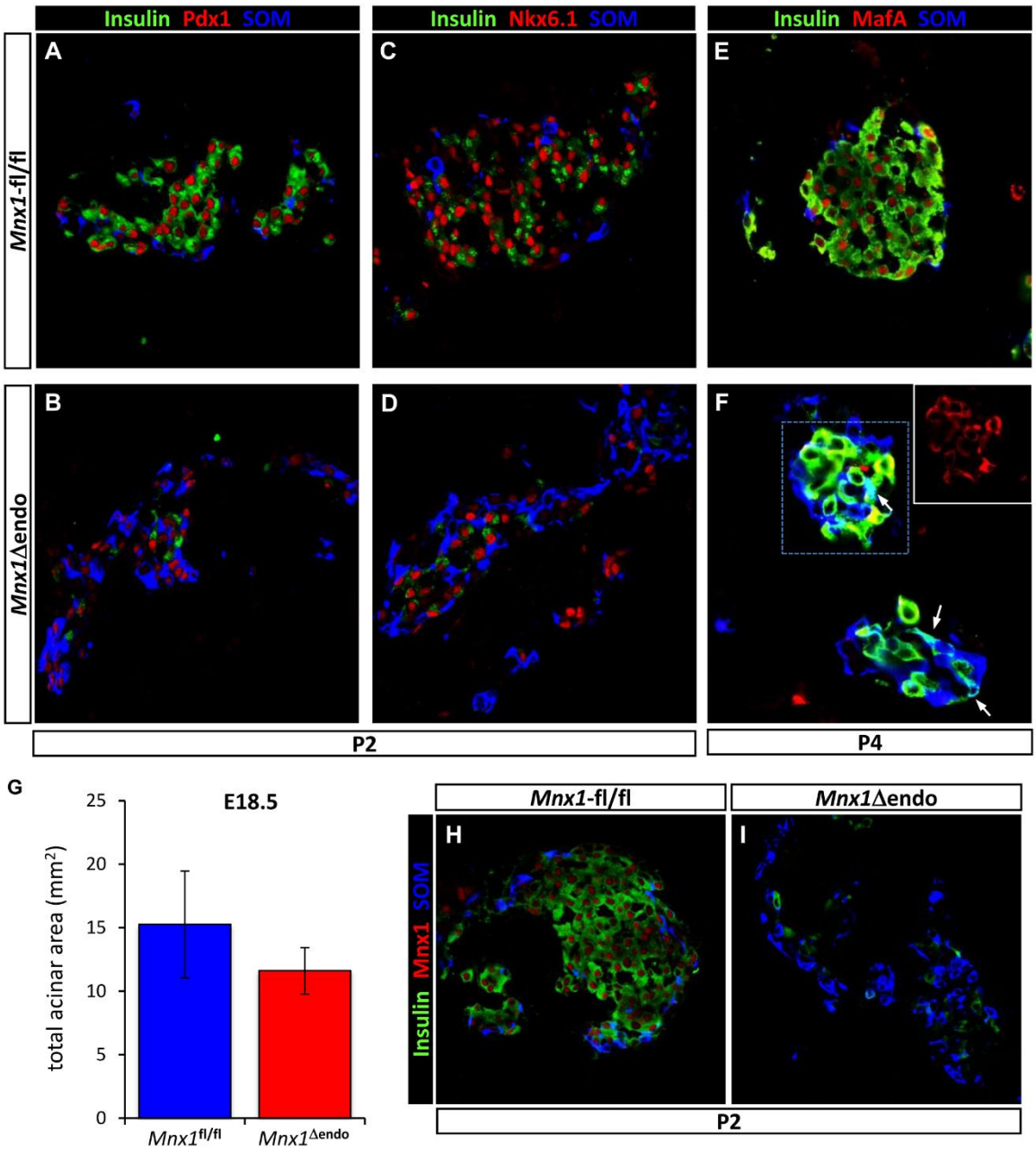
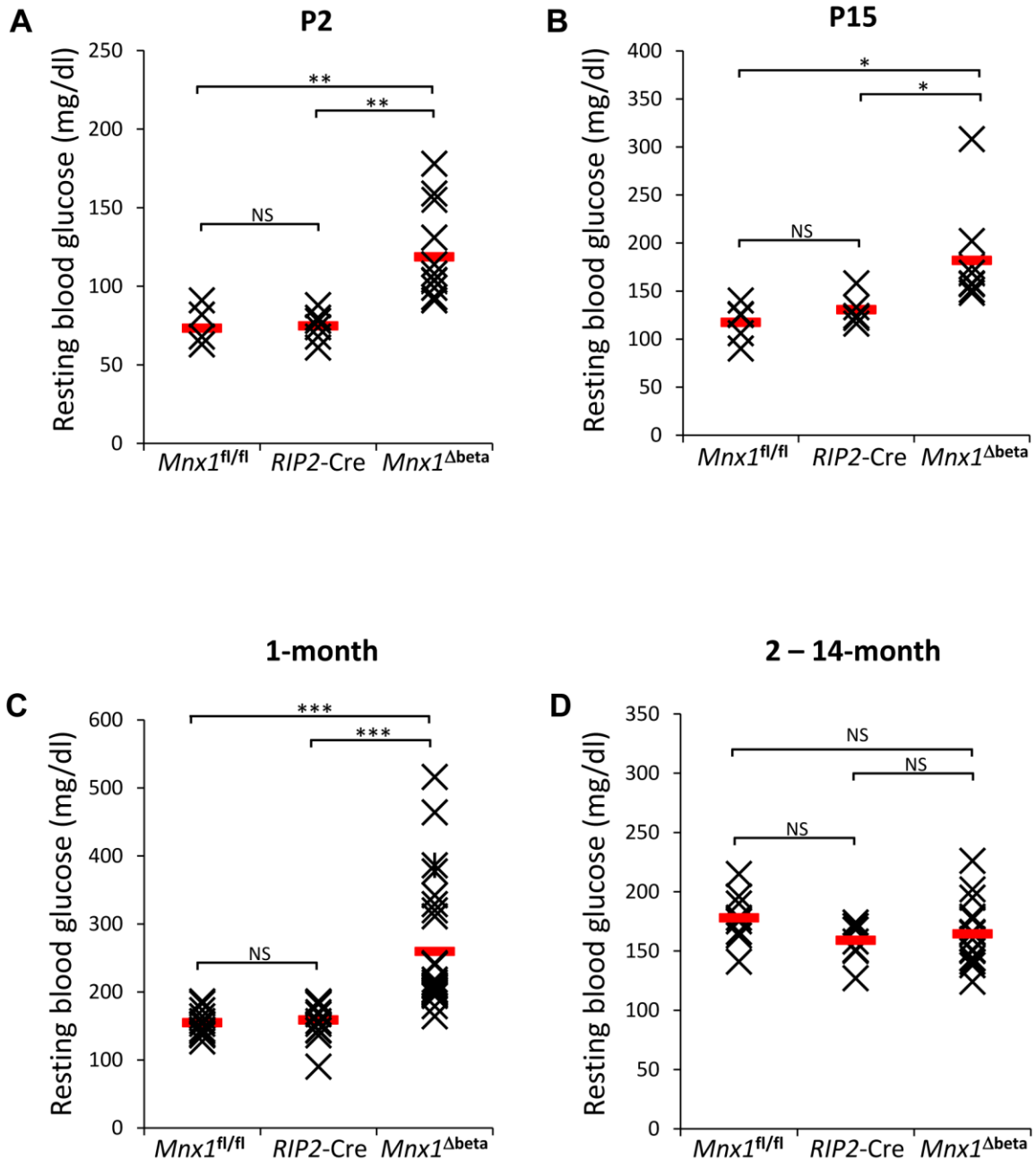


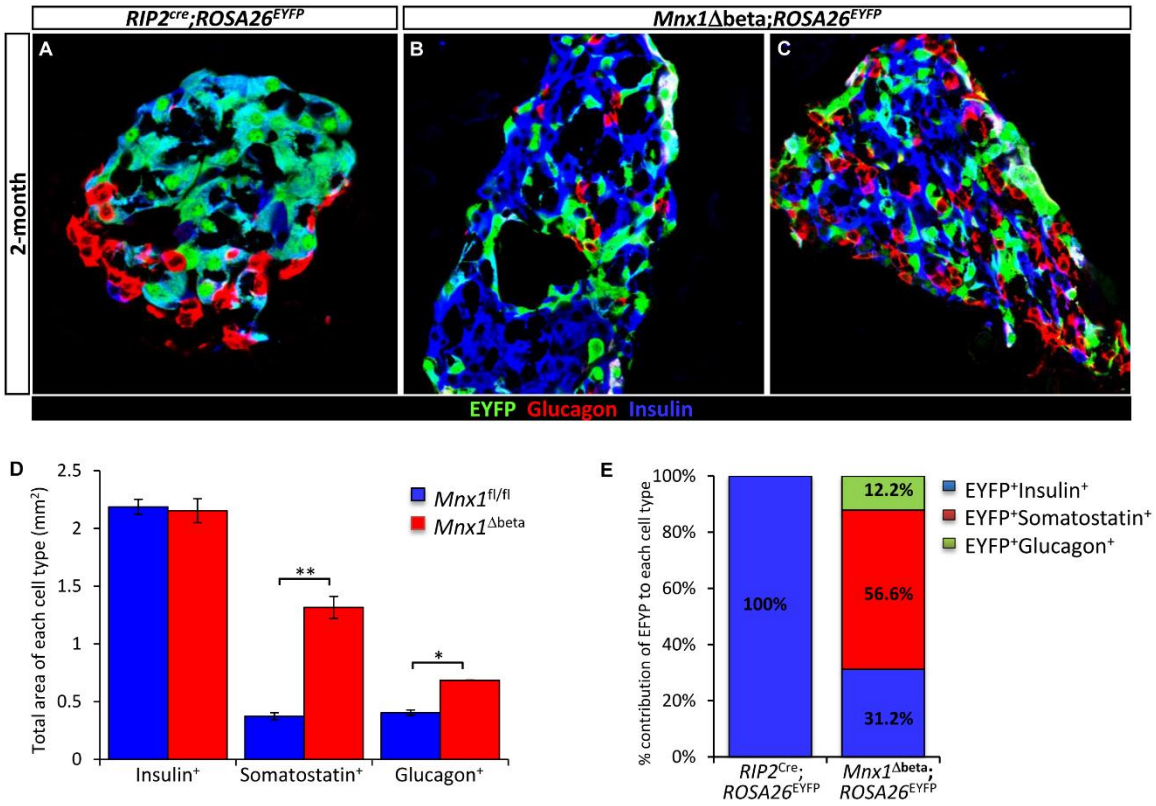
Supplementary Fig. 1 Verification of *Mnx1* floxed allele using germ-line activated Cre line (*EIIA*^{Cre}). (A) Schematic showing generation of *Mnx1* global null mice by crossing *Mnx1*$^{fl/fl}$ mice to the germ line deleter *EIIa*^{Cre}. (B, C) Gross morphology of E18.5 gut show the absence of dorsal pancreas (demarcated in red dotted line), whereas ventral pancreas (delineated by blue dotted line) develop normally in the *Mnx1*^{null} mice. We are not able to test for the presence of the truncated protein with the currently available specific Mnx1 antibody using immunofluorescence, because this antibody was raised against the C-terminal region of Mnx1, which is absent when exon 3 is deleted. Nevertheless, the *EIIa*^{Cre};*Mnx1*$^{FL/+}$, *Ngn3*-Cre;*Mnx1*$^{FL/+}$ and *RIP2*-Cre;*Mnx1*$^{FL/+}$ mice are normal and do not exhibit any defects in motor neuron function, pancreatic endocrine differentiation and β-cell function, suggesting that the truncated protein is either not made or is degraded, not exhibiting any dominant negative effects.



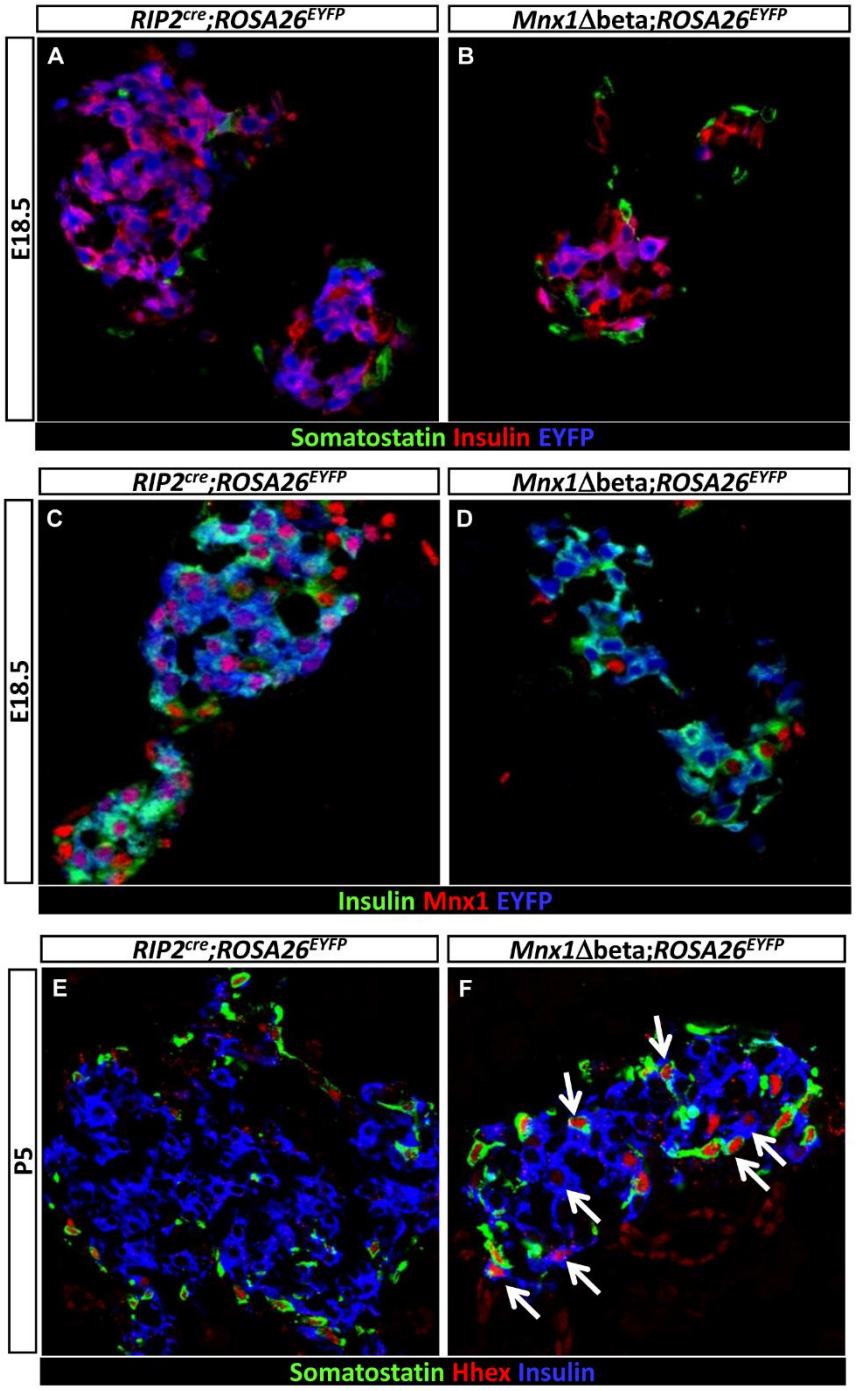
Supplementary Fig. 2 **The remaining β cells in *Mnx1*$^{\Delta endo}$ are not mature.** Immunofluorescence analysis show that the remaining β cells in the *Mnx1*$^{\Delta endo}$ expressed (A, B) Pdx1, and (C, D) Nkx6.1 as in control. (E, F) But MafA protein become localized to the cytoplasm compared to the nucleus localization in control β cell, indicating that these mutant β cells are immature. Insulin^{+}somatostatin^{+} cells (arrow) were MafA^{-} indicating the departure of this cell type from β cells. (G) Total acinar area were not changed significantly in *Mnx1*$^{\Delta endo}$. (H, I) The remaining β cells in the *Mnx1*$^{\Delta endo}$ mutants do not express Mnx1.



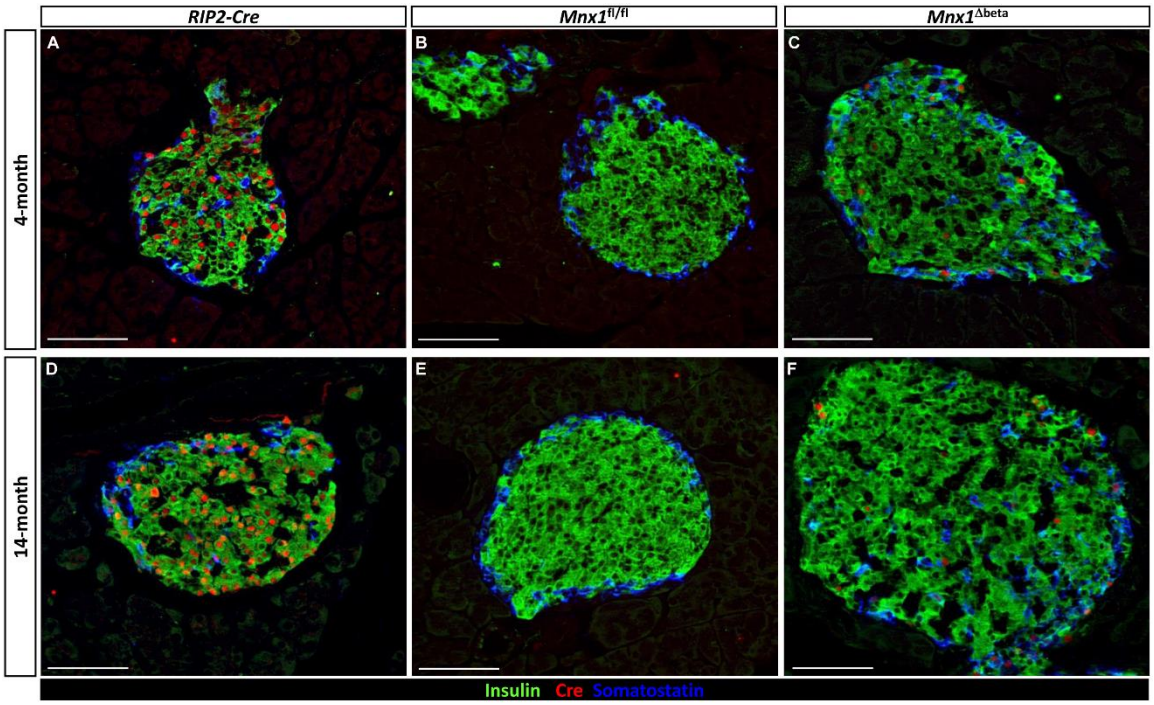
Supplementary Fig. 3 **Resting blood glucose of *Mnx1*$^{\Delta beta}$ mutants improved with age.** Measurement of resting blood glucose of *Mnx1*$^{fl/fl}$, *RIP2*-Cre and *Mnx1*$^{\Delta beta}$ mice at (A) P2, (B) P15, (C) 1-month old, and (D) 2 – 14-month old show a slightly elevated blood glucose at early age but blood glucose level improved with age. *p<0.05, **p<0.005, ***p<0.001



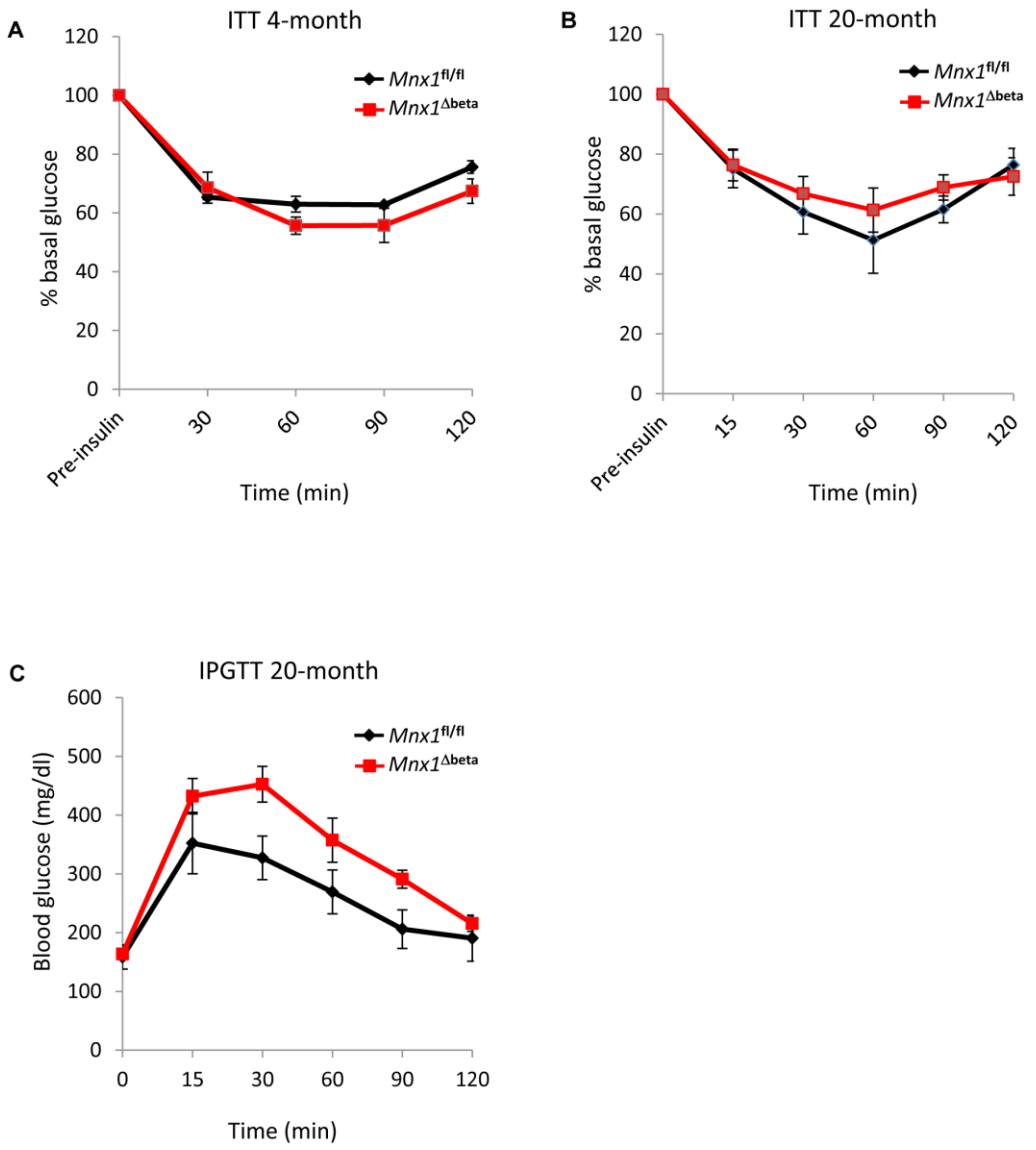
Supplementary Fig. 4 β-to-α transdifferentiation was also observed in Mnx1$^{\Delta beta}$ mutants, albeit at lower frequency. (A, B, C) The presence of EYFP^{+}Glucagon^{+} cells in *Mnx1*$^{\Delta beta}$ islets indicate β-to-α transdifferentiation also occurred when Mnx1 function is deleted in β cells. (D) Quantitative analysis of total area of each hormone^{+} cell types show that β-cell numbers were restored at 4-month old *Mnx1*$^{\Delta beta}$, concomitant with increased of δ and α-cell numbers; (E) Quantitative analysis show that the percentage contribution of EYFP^{+} cell in each of the β, δ, and α cell compartments in *RIP2*^{Cre} and *Mnx1*$^{\Delta beta}$ mutants.



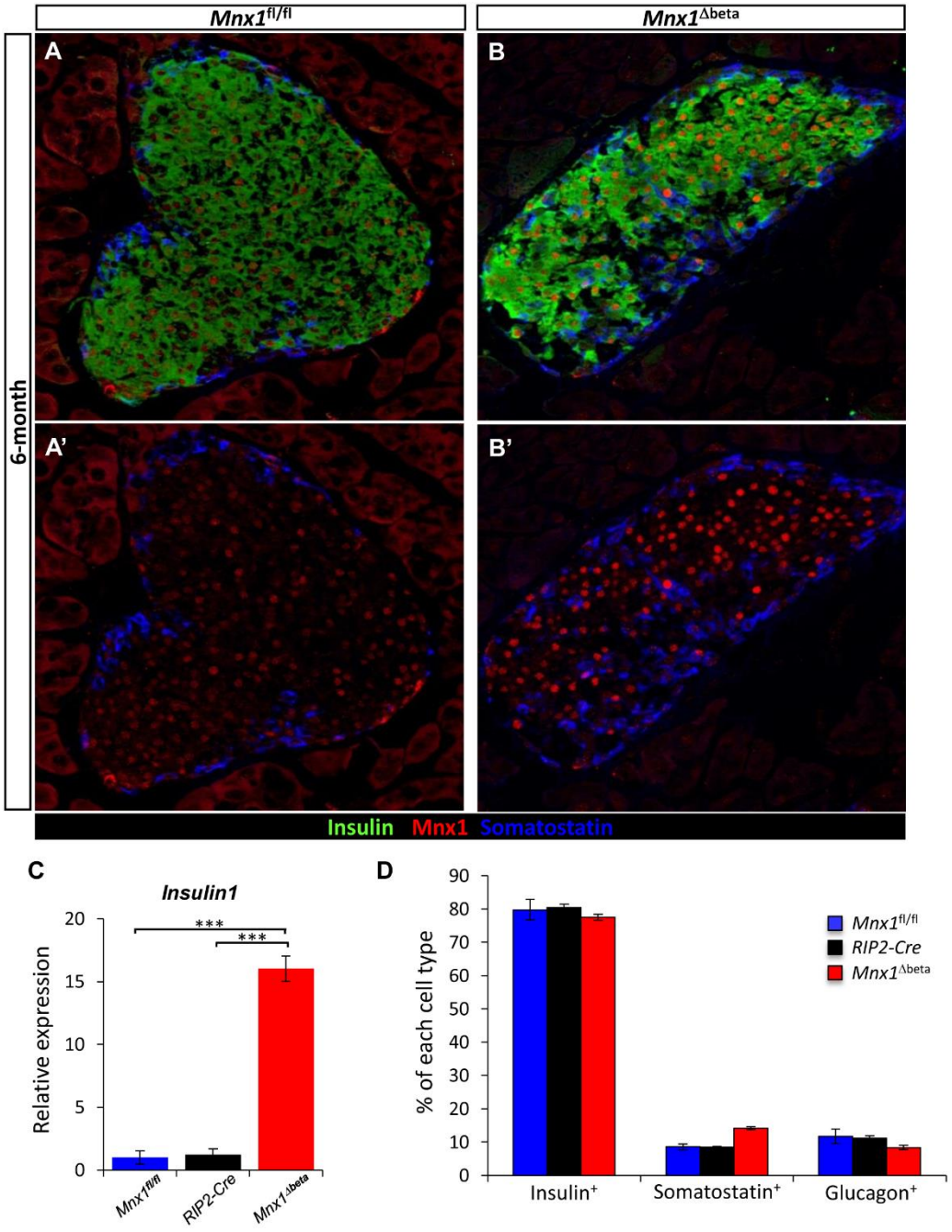
Supplementary Fig. 5 Initiation of β-to-δ cell transdifferentiation in *Mnx1*$^{\Delta beta}$ mutants was observed at P5 but not at E18.5. (A, B) Immunofluorescence analysis with EYFP, insulin and somatostatin show the absence of EYFP^{+}somatostatin^{+} cells at E18.5 in *Mnx1*$^{\Delta beta}$ mice, indicating no β-to-δ transdifferentiation at this stage. (C, D) All EYFP+ cells are Mnx1^{-}, indicating *Mnx1* is efficiently deleted in the *Mnx1*$^{\Delta beta}$ β cells at E18.5. (E, F) The presence of Hhex+insulin+ and Hhex^{+}insulin^{+}somatostatin^{+} (white arrows) cells in the *Mnx1*$^{\Delta beta}$ mutants at P5 indicate the initiation of β-to-δ transdifferentiation.



Supplementary Fig. 6 Escaper β cells in *Mnx1*$^{\Delta beta}$ mutants were devoided of Cre. Immunolabeling of Cre showed that majority of the escaper β cells that repopulated the islet in *Mnx1*$^{\Delta beta}$ do not produce Cre recombinase at (A, B, C) 4 months and, (D, E, F) 14 months, compared to the RIP2-Cre control islets. It is noteworthy that even in RIP2-Cre islets, 15-20% of insulin^{+} β cells do not produce Cre recombinase, consistent with the 85% recombination efficiency in the islet in this allele. Scale bar, 25 μm.



Supplementary Fig. 7 Glucose clearance defects in *Mnx1*$^{\Delta beta}$ mutants were not caused by peripheral insulin resistance. (A) Insulin tolerance test on *Mnx1*$^{fl/fl}$ and *Mnx1*$^{\Delta beta}$ indicate that *Mnx1*$^{\Delta beta}$ mice do not have peripheral insulin resistance at 6-month old, but (B) developed mild insulin resistance and (C) glucose intolerance at 20-month old. ITT, Insulin tolerance test; IPGTT, intraperitoneal glucose tolerance test.



Supplementary Fig. 8 Mnx1 protein level and insulin mRNA expression were upregulated in the β cells of *Mnx1*$^{\Delta beta}$ mutants. (A, A', B, B') Immunolabeling of Mnx1 show that Mnx1 protein was significantly induced in *Mnx1*$^{\Delta beta}$ β cells at 6-month old. (C) qRT-PCR data showed that *Insulin1* mRNA expression is highly upregulated in the remaining Cre^{-} β cells at 6-month old. (D) Quantitative analysis of β, δ, and α cell fraction showing the percentage of β cells within islets of *Mnx1*$^{\Delta beta}$ is comparable to control *Mnx1*$^{fl/fl}$ and *RIP2*-Cre at 14-month old.

Supplementary Table S1: List of antibodies used

Primary antibodies				
Antigen	**Species**	**Dilution**	**Staining type**	**Source**
Mnx1	Rabbit	1:5000	IF	Samuel Pfaff (Salk Institute)
Mnx1	Mouse	1:500	TSA	DSHB
Ptf1a	Goat	1:1000	TSA	Chris Wright
Sox9	Rabbit	1:1000	IF	Chemicon
Pax6	Rabbit	1:800	TSA	Covance
GFP	Rabbit	1:500 1:1000	IF TSA	Clontech
Menin	Goat	1:500	IF	Bethyl
Insulin	Guinea Pig	1:1000	IF	Linco
Insulin-A	Goat	1:250	IF	Santa Cruz
Glucagon	Guinea Pig	1:1000	IF	Linco
Glucagon	Rabbit	1:1000	IF	Linco
Somatostatin	Goat	1:1000	IF	Santa Cruz
Pancreatic Polypeptide	Guinea Pig	1:1000	IF	Linco
CpaI	Goat	1:250	IF	BD Bioscience
E-cadherin	Mouse	1:500	IF	BD Bioscience
Nkx6.1	Rabbit	1:1000	IF	BCBC
MafA	Rabbit	1:1000	TSA	Bethyl
GLUT2	Rat	1:200	IF	Alpha Diagnostic
Pdx1	Rabbit	1:1000	IF	Chris Wright (Vanderbilt)
Ki67	Rabbit	1:500	IF	Sigma
Ngn3	Guinea Pig	1:2000	TSA	Maike Sander (UCSD)
Hhex	Rabbit	1:300	IF	Clifford Bogue (Yale University)
Cre	Rabbit	1:500	IF	Novagen

Secondary antibodies			
Antigen	**Conjugation**	**Dilution**	**Source**
Rabbit/Guinea pig/ Goat/Mouse/Chicken	Cy3	1:300	Jackson ImmunoResearch
Rabbit/Guinea pig/ Goat/Mouse/Chicken	Cy2	1:300	Jackson ImmunoResearch
Rabbit/Guinea pig/Goat/Mouse	Cy5	1:300	Jackson ImmunoResearch
Rabbit/Guinea pig/ Goat/Mouse/Chicken	Biotinylated	1:1000	Vector Laboratories

Supplementary Table S2: Primers used in qRT-PCRs

Primer name	Sequence
GAPDH	Forward: AACTTTGGCATTGTGGAAGG
	Reverse: GGATGCAGGGATGATGTTCT
Insulin1	Forward: CAGCAAGCAGGTCATTGTTT
	Reverse: GGGACCACAAAGATGCTGTT
Mnx1	Forward: AAGCGTTTTGAGGTGGCTAC
	Reverse: CCATTTCATTCGGCGGTTCT
Cdkn2a	Forward: GGGATGATGGACTTTTGAGG
	Reverse: TCTGGCTTCTAAGAGAAGATCTAA
Bmi1	Forward: AAACCAGACCACTCCTGAACA
	Reverse: TCTTCTTCTTCTTCATCTCATTTTTGA
Cdkn1a	Forward: GCTTGGATGTCAGCGGGA
	Reverse: CAGAGTTTGCCTGAGACCCA
CDK4	Forward: CGAGCGTAAGATCCCCTGCT
	Reverse: CGAGCGTAAGATCCCCTGCT
CDK6	Forward: TGCGAGTGCAGACCAGTGG
	Reverse: AGGTCTCCAGGTGCCTCAGC
CyclinD1	Forward: CCCTCGGTGTCCTACTTCAA
	Reverse: GGGGATGGTCTCCTTCATCT
Menin	Forward: ACCCACTCACCCTTTATCACA
	Reverse: ACATTTCGGTTGCGACAGT
Insulin2	Forward: GGCTTCTTCTACACACCCAT
	Reverse: CCAAGGTCTGAAGGTCACCT