SUPPLEMENTARY DATA

Supplementary Table 1. Primer/probe sets used for TaqMan Low Density Arrays (TLDAs).

Gene	ABI Identification number
Irs2	Mm03038438_m1
Akt1	Mm01331624_m1
Akt2	Mm00545827_m1
Foxo1	Mm00490672_m1
PKCα	Mm00440858_m1
SGK1	Mm00441380_m1
Skp2	Mm00449925_m1
Pten	Mm00477210_m1
Rictor	Mm01307318_m1
Syntaxin-1	Mm00444008_m1
Snap25	Mm00456921_m1
Vamp2	Mm00494118_g1
Munc8a	Mm00436837_m1
connexin36	Mm00439121_m1
Ezh2	Mm00468449_m1
Bmi1	Mm03053308_g1
Prdm16	Mm00712556_m1
Men1	Mm00484963_m1
Cdk4	Mm00726334_s1
Ccnd2	Mm00438072_m1
Cdc25a	Mm00483162_m1
p16	Mm01257348_m1
p18	Mm00483243_m1
p21	Mm00432448_m1
p27	Mm00438168_m1
p19	Mm00486943_m1

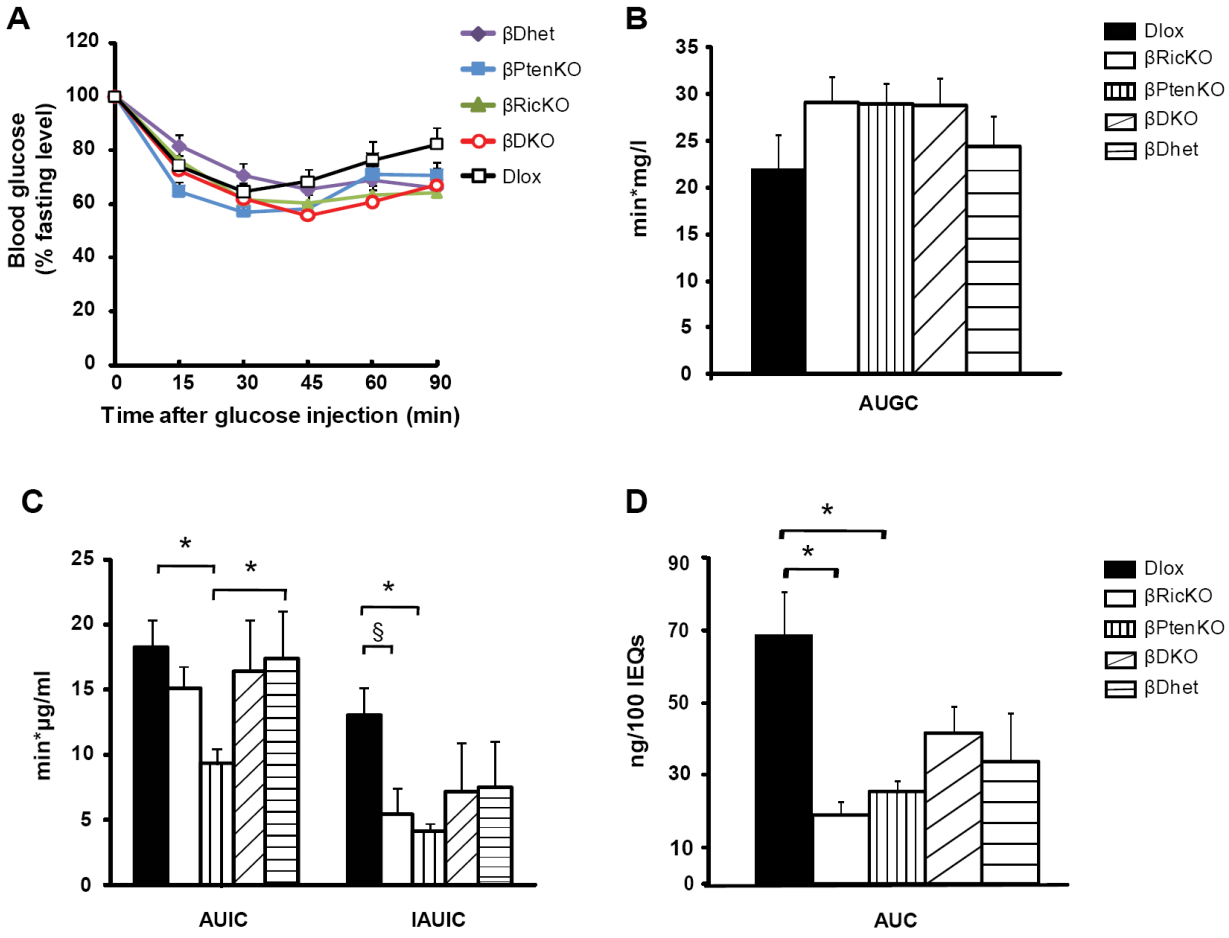
SUPPLEMENTARY DATA

Supplemental Table 2: Antibodies used.

	Antibodies	Dilution used for immunochemistry	Dilution used for Western blots	Source
Primary Antibodies	Rat anti-BrdU	1:500		Accurate Chemical
	Mouse anti-Ki67	1:400		Benton Dickenson
	Rabbit anti-Glut2	1:1000		Bernard Thorens
	Guinea pig anti-Insulin	1:1000		Linco
	Rabbit anti-Cre	1:1000		Clone-Tech
	Rabbit anti-PDX1	1:4000	1:10000	Chris Wright's Lab
	Rabbit anti-FoxO1	1:50	1:500	Cell signaling
	Rabbitanti-FoxO1	1:100		Santa-Cruz
	Rabbit anti-Rictor		1:1000	Bethyl
	Rabbit anti-Pten		1:2000	Cell Signaling
	Rabbit anti-AKT		1:4000	Cell Signaling
	Rabbit anti-pAKT-S473		1:1000	Cell Signaling
	Rabbit anti-pAKT-T308		1:1000	Cell Signaling
	Rabbit anti-S6		1:2000	Cell Signaling
	Rabbit anti-PS6		1:2000	Cell Signaling
	Mouse anti-p27		1:4000	Benton Dickenson
	Rabbit anti-GAPDH		1:6000	Santa-Cruz
	Mouse anti-PCAF		1:1000	Santa-Cruz
Secondary Antibodies	Goat anti Guinea Pig Alexa Fluor 488	1:500		Invitrogen
	Goat anti Rabbit Alexa Fluor 488	1:500		Invitrogen
	Donkey anti Rabbit Alexa Fluor 555	1:500		Invitrogen
	Donkey anti mouse cy3	1:500		Jackson ImmunoResearch
	Donkey anti Rabbit conjugated with HRP		1:10,000	Jackson ImmunoResearch
	Donkey anti Mouse conjugated with HRP		1:10,000	Jackson ImmunoResearch

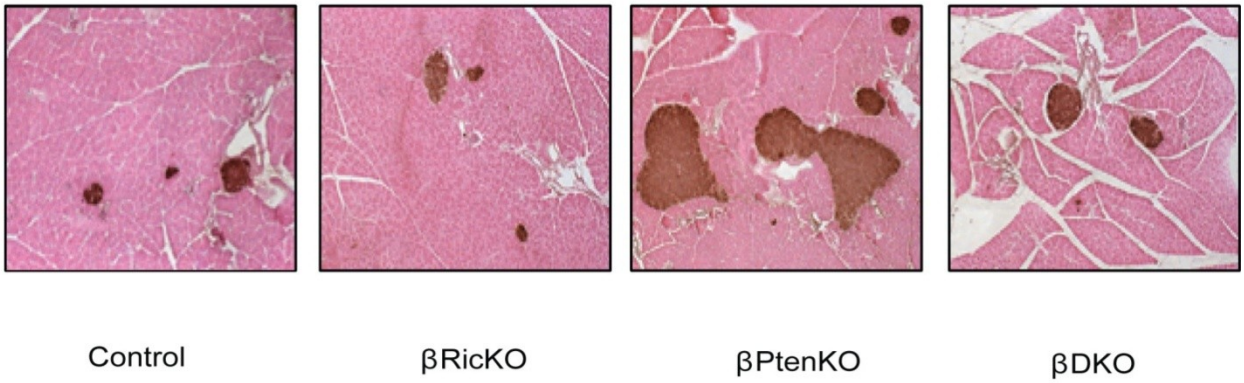
SUPPLEMENTARY DATA

Supplementary Figure 1. Analysis of insulin sensitivity and measurement of area under curve of insulin secretion. βRicKO mice were observed to have impaired glucose-stimulated insulin secretion but no change in insulin sensitivity. **A:** Intraperitoneal insulin tolerance tests were performed at 12 weeks of age. Blood glucose concentrations were tested at 0, 15, 30, 45, 60 and 90 min after insulin injection. There was no significant difference of blood glucose reduction after insulin injection at each time point was observed between groups. **B:** Area under glucose curve during insulin tolerance test showed no significance difference of total glucose reduction in respond to insulin injection in different genotypes. **C**: The area under insulin curve (AUIC) and increment of AUIC (IAUIC). β*Ric*KO mice had a lower IAUIC value than D*lox*. There was no difference in the AUIC or IAUIC between the β*Ric*KO and βDKO mice. **D**: Area under insulin secretion curve during islet perfusion test (n = 3). Islets from both the β*Ric*KO and β*Pten*KO mice showed significantly lower AUC than did islets from the Dlox control mice. $^{*}P < 0.05$, $^{\S}P = 0.07$.



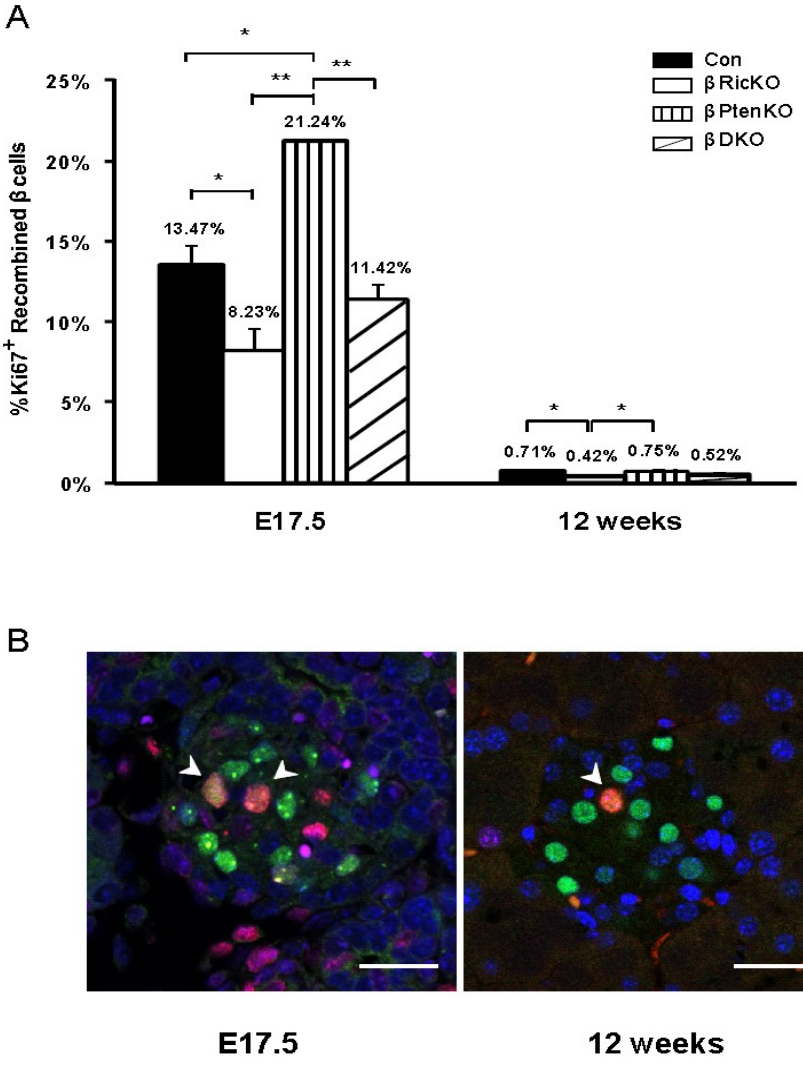
SUPPLEMENTARY DATA

Supplementary Figure 2. Insulin immunolabeling of mouse pancreas. Representative images showing immunohistochemical labeling of insulin for each the four mouse genotypes is shown. A positive insulin signal is indicated by brown color stain (DAB). Cells were counterstained with Eosin. β*Pten*KO mice had the largest β cell mass compared to other genotypes. All images were acquired at 100X magnification. The percentage of insulin-positive area to pancreatic area for all sections and β cell mass (ratio of insulin-positive area multiplied times pancreas weight) were calculated. β cell mass was calculated from 3-4 animals of each genotype, as described previously. As shown in Fig. 2A, β cell mass was reduced in the βRicKO mice and elevated in the β*Pten*KO lines compared to the control and βDKO groups.



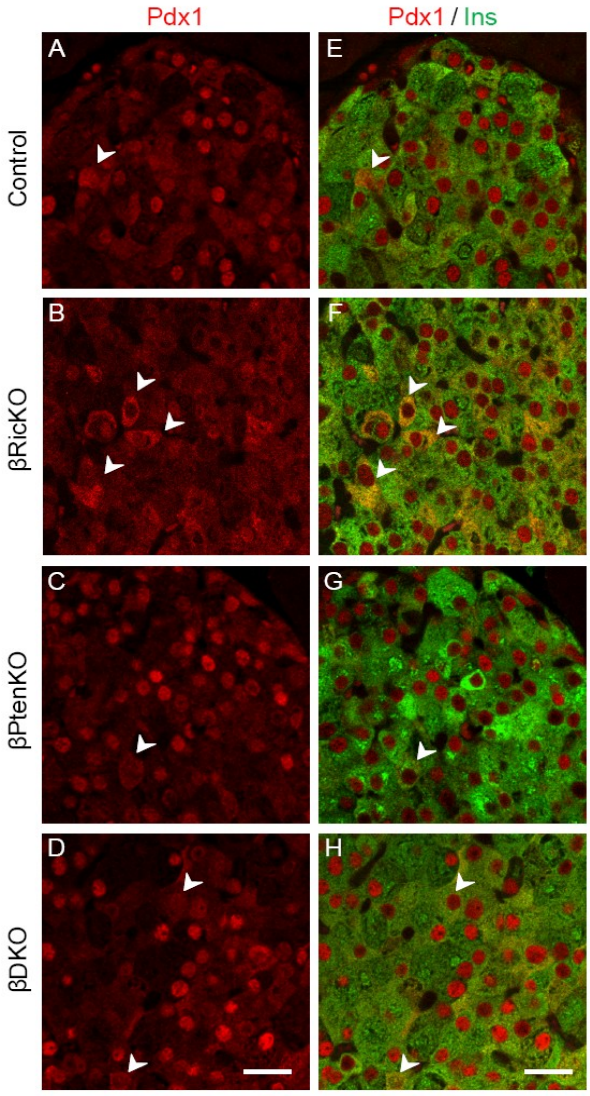
SUPPLEMENTARY DATA

Supplementary Figure 3. Analysis of cell proliferation by counting Ki67 and Cre double-positive cells. Pancreas sections from the four different genotypes were labeled by immunostaining for both Ki67 and Cre. Images were taken using a confocal microscope. **(A)** Percentage of double-positive cells at both E17.5 and 12 weeks. $^{**}P < 0.01$, $^{*}P < 0.05$. 3-4 animals in each group. For adults, more than 1,500 cells from at least 50 islets of each animal were counted. For embryos, more than 500 cells were counted. βD*het* mice served as the control. **(B)** Representative images of double-labeling of Ki67 (red) and Cre (green) in both embryonic and adult pancreas. Ki67/Cre double-positive cells are indicated by white arrows. Scale bar = 20μm.



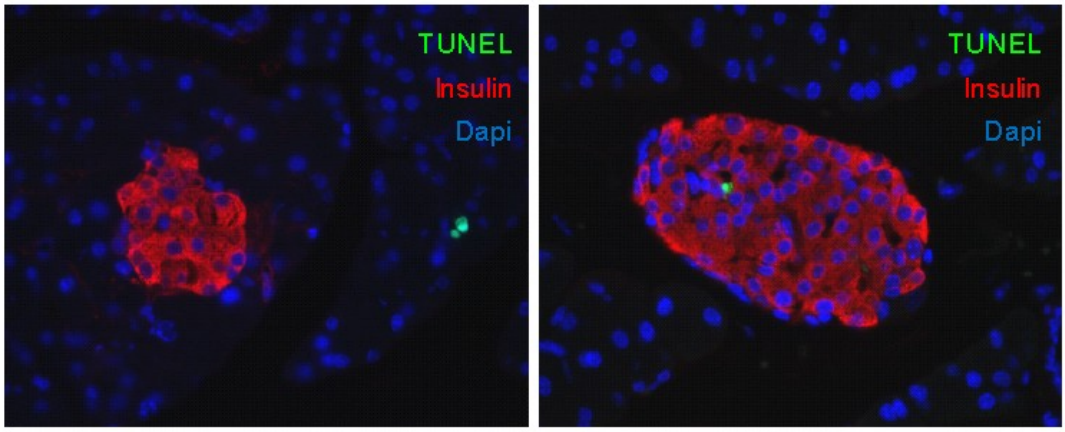
SUPPLEMENTARY DATA

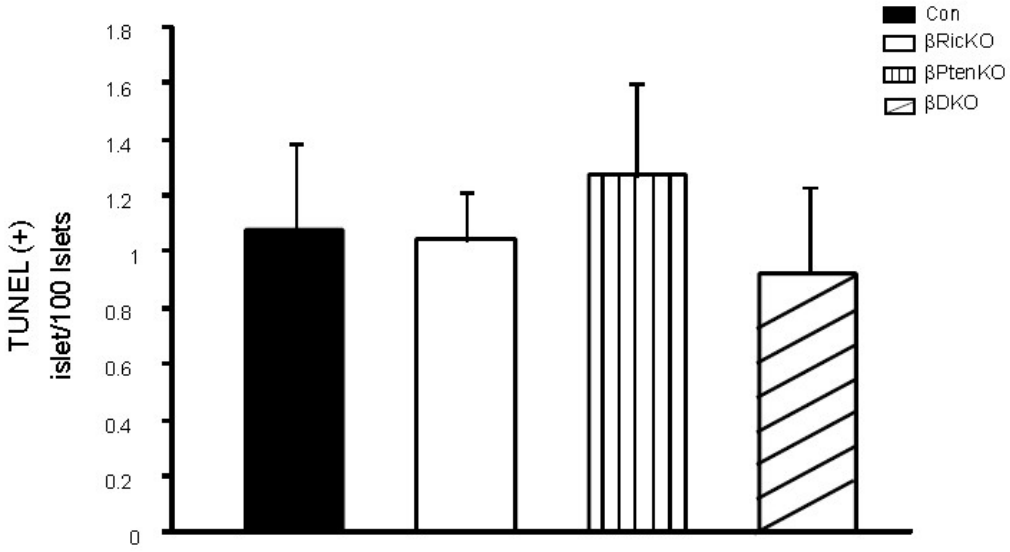
Supplementary Figure 4. Differential subcellular location of Pdx1 in pancreatic β cells. (**A, B, C, D)** Immunolabeling of Pdx1 (red) in pancreatic islets. (**E, F, G, H)** Double immunolabeling of Pdx1 (red) and insulin (green). **(A, E)** Control islets. Pdx1 was detected mainly in the nucleus; however, cytoplasmic expression was detected in a few cells, as indicated by white arrows. **(B, F)** β*Ric*KO islets. Nuclear labeling of Pdx1 was reduced compared to control group. **(C, G)** β*Pten*KO islets. More nuclear Pdx1 accumulation is seen. **(D, H)** βDKO control islets. Nuclear localization is similar to that of the control group. Scale bar = 20μm



SUPPLEMENTARY DATA

Supplementary Figure 5. β cell apoptosis is not affected in the absence of Rictor. Pancreas sections from the four different genotypes were immunolabeled for insulin and TUNEL. All images were acquired at 400X magnification. The left image in the upper panel shows TUNEL labeling of a single exocrine cells and the right image shows TUNEL labeling of a single β cell. The lower panel shows the number of islets which contained at least one TUNEL-positive cell. At least 110 islets for each genotype were examined at 12 weeks of age. No significant differences were observed.





SUPPLEMENTARY DATA

Supplementary Figure 6. Analysis of in nuclear and/cytoplasmic FoxO1. The nuclear and cytoplasmic protein extracts of islets from the four genotypes of mice were prepared and immnoblotted for Foxo1. The right four lanes show an immunoblot of cytosolic (Cyto) proteins and the left four lanes are of nuclear (Nuc) proteins. PCAF, a p300/CBP-associated factor, was used as the loading control for nuclear proteins and GAPDH was used as the loading control for cytosolic proteins. Each lane contains the islet nuclear/cyoplasmic protein from 3 animals of each genotype.