

**Supplemental Figure 1: Foxd3 is expressed at normal levels in heterozygous mice.**

Expression of *Foxd3* in islets isolated from *Foxd3<sup>fl/-</sup>* and *Foxd3<sup>fl/+</sup>*; *Pdx1-Cre* animals was similar to *Foxd3* expression in *Foxd3<sup>fl/+</sup>* controls. The expression in heterozygous animals was normalized to the expression in *Foxd3<sup>fl/+</sup>* controls and this value was arbitrarily set to 1 (red line). n=3 animals per group.

**Supplemental Figure 2: Foxd3 expression was not detected in the hypothalamus.** RT-PCR

specific for *Foxd3* mRNA indicated that *Foxd3* was not expressed in the hypothalamus but was expressed in islets as expected. *HPRT* is a housekeeping gene that serves as a loading control. Note: 30 PCR cycles were needed to detect *Foxd3* while 25 PCR cycles were used to detect *HPRT* expression from the same cDNA samples.

**Supplemental Figure 3: Aged mutant mice maintain glucose tolerance.** IPGTTs on one-year

old mice indicate mutant mice ( $\Delta$ ) had glucose tolerance curves similar to control littermates ( $\bullet$ ). n= 7-10 mice in each group. Error bars indicate SEM.

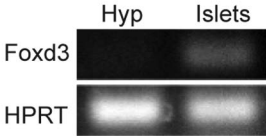
**Supplemental Figure 4: Foxd3 heterozygous animals are phenotypically indistinguishable**

**from control littermates.** A-B. IPGTTs on *Foxd3<sup>fl/-</sup>* ( $\bullet$ ) and *Foxd3<sup>fl/+</sup>*; *Pdx1-Cre* ( $\blacksquare$ ) animals each carrying one intact *Foxd3* locus in the pancreas demonstrated that mice heterozygous for *Foxd3* were euglycemic prior to pregnancy (A) and at 15.5 days gestation (B). n=8-12 animals per group. C. The  $\beta$ -cell mass in pregnant heterozygous females at 15.5 days gestation was not statistically different from *Foxd3<sup>fl/+</sup>* controls at the same time (compared to 1.53 mg for controls as shown in Fig. 5 of the manuscript). n=3 animals in each group. Error bars indicate SEM.

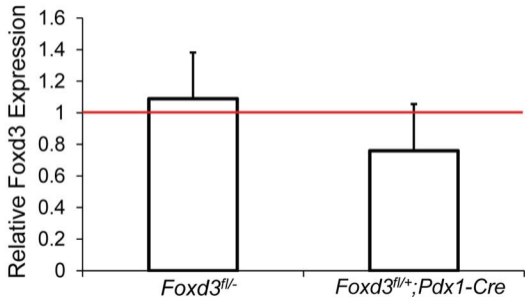
**Supplemental Figure 5: Foxd3 is required for  $\beta$  cell proliferation but not  $\beta$  cell survival. A-**

B. During pregnancy, fewer  $\beta$  cells from mutant females (B) incorporate BrdU (red) compared to control littermates (A).  $\beta$  cells are indicated by insulin immunofluorescence (green). n = 4-6 mice in each group. Arrows indicate BrdU-positive  $\beta$  cells. Images were taken at 400x magnification. C-D. Using TUNEL assay together with insulin immunofluorescence, we were unable to detect TUNEL positive  $\beta$  cells in control (C) or mutant (D) mice. n= 4 animals in each group. Arrows indicate rare TUNEL-positive acinar cells. Images were taken at 200x magnification.

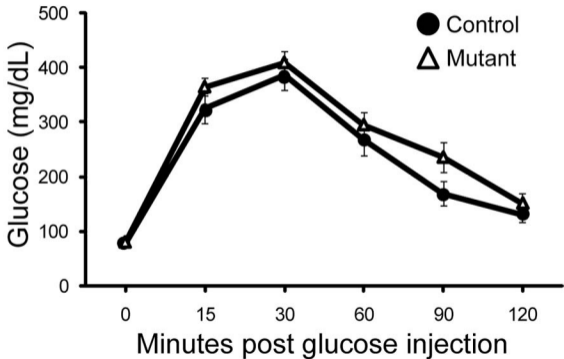
# Supplemental Figure 1



## Supplemental Figure 2

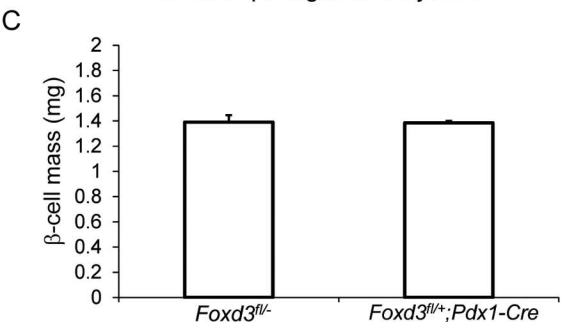
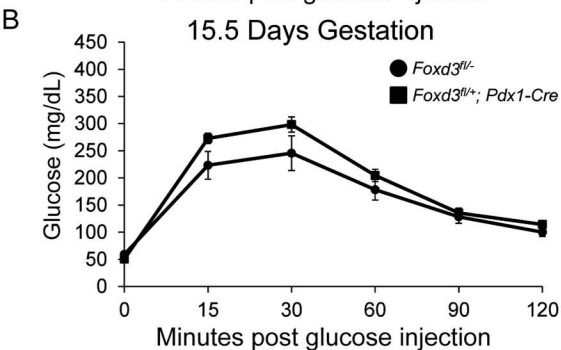
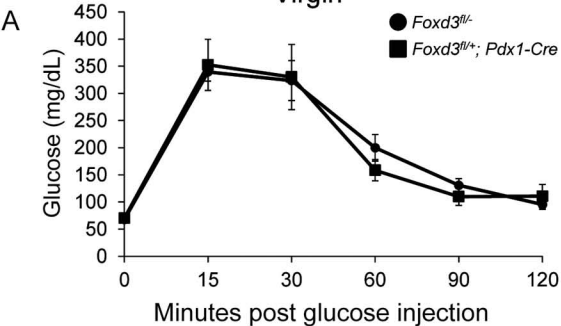


Supplemental Figure 3



## Supplemental Figure 4

Virgin



Control

Mutant

