Supplemental Figure Legends

Figure S1 (related to Figure 1)

(A) Triple staining of an islet from a *RIPCreER; Pdx1$^{fl/+}$, Rosa^{YFP}* mouse showing that Pdx1^{+}Somatostatin^{+} cells do not exhibit YFP staining, demonstrating the specificity of the *RIPCreER* strain for β-cells.

(B) Glucose tolerance test results of 2-months-old wild-type (CD1 control, n=6), *RIP-CreER; Pdx1$^{fl/+}$, Rosa^{YFP}* (Het control, n=6), and *RIP-CreER; Pdx1$^{fl/fl}$, Rosa^{YFP}* (PKO, n=4) mice showing that, compared to PKO, both CD1 and Het Control groups are euglycemic.

(C) Staining of sections from lineage-traced control (*RIP-CreER; Pdx1$^{fl/+}$, Rosa^{YFP}*) or PKO pancreata with YFP and either somatostatin (Som; δ-cells) or pancreatic polypeptide (PP-cells) revealed no significant overlap.

(D) Immunostaining for Ki67 in PKO pancreata 1 mo after TAM administration. A low rate of turnover was observed in both control (*RIP-CreER; Pdx1$^{fl/+}$, Rosa^{YFP}*) and PKO islets. A section of pancreas from a mouse with a pancreas-specific deletion of *Mst1/2*, a pancreatitis model (Gao et al., 2013) was applied as positive control.

(E) Immunostaining for cleaved caspase3 (CAP3) in PKO pancreata different time points after TAM administration. A low rate of turnover was observed in both control (*RIP-CreER; Pdx1$^{fl/+}$, Rosa^{YFP}*) and PKO islets. A section of pancreas from a mouse with a pancreas-specific deletion of *Mst1/2*, a pancreatitis model (Gao et al., 2013) was applied as positive control.

Figure S2 (related to Figure 2)

Sections from pancreata of control animals or PKO animals either 5 days or 30 days after tamoxifen treatment (5D or 30D post TAM) were co-stained for YFP, Pdx1, and Ngn3. As expected, strong nuclear Ngn3 staining was observed in E15.5 pancreata. In contrast, there was no nuclear Ngn3 staining in YFP positive cells of control, 5D post-TAM, and 30D post-TAM pancreata.

Figure S3 (related to Figure 2)

(A-B) Representative images showing co-staining of YFP and Glu (A) or Ins and Glu (B) either 0, 2, 3, or 5 days following TAM administration to PKO animals. Arrows show YFP cells that co-express glucagon (A) or insulin and glucagon (B).

(C) Pancreata co-staining for YFP/Glucagon/Insulin of 5 month-old animals (termed 5 months PKO, N=4) that were treated with TAM one month earlier. Robust YFP^+/Glu^+ double staining cells were observed (a, b). In addition, a fraction of YFP^+ cells are Glu^-/Ins^- (c).

(D) *Insulin 1* (Ins 1) mRNA levels were measured in YFP^+ cells sorted from islets of control or PKO animals 5 days or 5 weeks post-TAM, showing a further reduction in transcript levels over time. The qPCR data for the 5 day time-points are the same as those presented in Fig. 5C.

Figure S4. Comparing insulin and glucagon secretion responses in wild type and PKO islets (related to Figure 4)

(A-B) Experiment 1. Measurement of insulin and glucagon secretion from pooled islets obtained from either control (*RIP-CreER; Pdx1*$^{fl/+}$, *Rosa*^{YFP}) animals, PKO animals 5 days post-TAM (n=3), or PKO animals 5 weeks post-TAM (n=3). Islets were exposed either to basal medium

conditions (G0), 4 mM amino acid mixture (AAM), or 4 mM AAM plus 10 mM glucose. Hormone levels are plotted as ng insulin protein (A) or pg glucagon protein (B) per 35 islets per hour.

(C-D) Experiment 2. Measurement of insulin and glucagon secretion from pooled control (*RIP-CreER; Pdx1*$^{fl/+}$, *Rosa*^{YFP}) animals, PKO 5 days post-TAM (n=3), or PKO 5 weeks post-TAM (n=3). Islets were exposed either to basal medium conditions (G0) or 10 mM glucose (G10). Hormone levels are plotted as ng insulin protein (C) or pg glucagon protein (D) per 35 islets per hour.

Figure S5. Isolation of cells for microarray studies (related to Figure 5)

(A) Islets were prepared from mice of the indicated genotype 5 days after TAM administration and prepared for fluorescence-activated cell sorting (FACS). Images show YFP/Glu staining of pancreatic sections, revealing that YFP expression is specific for β-cells (left panel), α-cells (right panel), and lineage-traced *Pdx1*-deleted cells (middle panel) in each of these genotypes. FACS plots shows that YFP^{+} β-cells (*RIP-CreER; Pdx1*$^{fl/+}$*; Rosa*^{YFP}) and YFP^{+} α-cells (*glucagon-Cre; Pdx1*$^{fl/+}$*; Rosa*^{YFP}) have different YFP-side scatter profiles (red outlines). Notably, YFP^{+} reprogrammed cells (*RIP-CreER; Pdx1*$^{fl/fl}$*; Rosa*^{YFP}) have the same side-scatter profile as α-cells. YFP^{+} cells were not detected in islets isolated from mice that lack Cre (*Pdx1*$^{fl/+}$*; Rosa*^{YFP}).

(B) Triple staining of Glu^{+}/Arx^{+}/YFP* cells shows that YFP staining was strictly limited to the α-cells of *glucagon-Cre; Rosa*^{YFP} pancreata.

Supplemental Tables

Supplemental Table S1 (related to Figure 5)

Data from microarray examining the expression of 28,350 transcripts in sorted α-cells, β-cells and PKO cells 5d after TAM administration.

Supplemental Table S2 (related to Figure 5)

List of genes differentially expressed between α-cells, β-cells, and PKO cells using a cutoff of FDR<0.1.

Supplemental Table S3 (related to Figure 5)

List of genes differentially expressed between α-cells and β-cells using a cutoff of FDR<0.1 and >2-fold difference in expression.

Supplemental Table S4 (related to Figure 5)

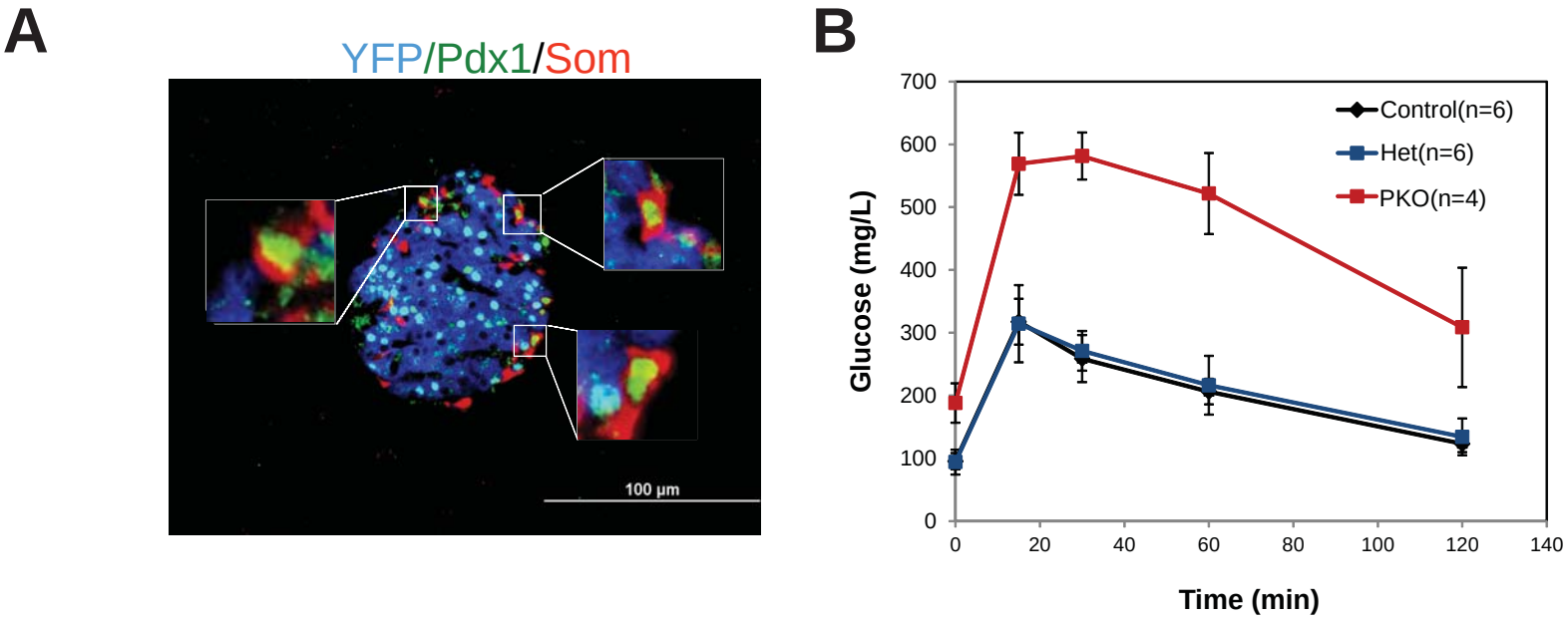
List of genes differentially expressed between PKO cells and β-cells using a cutoff of FDR<0.1 and >2-fold difference in expression.

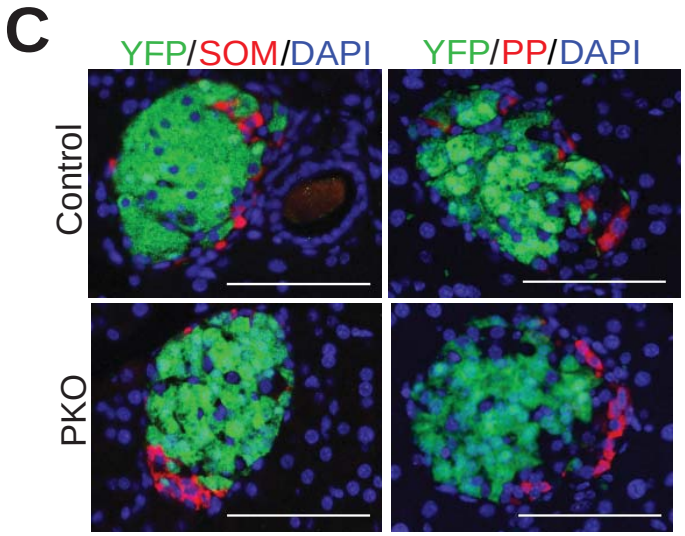
Supplemental Table S5 (related to Figure 5)

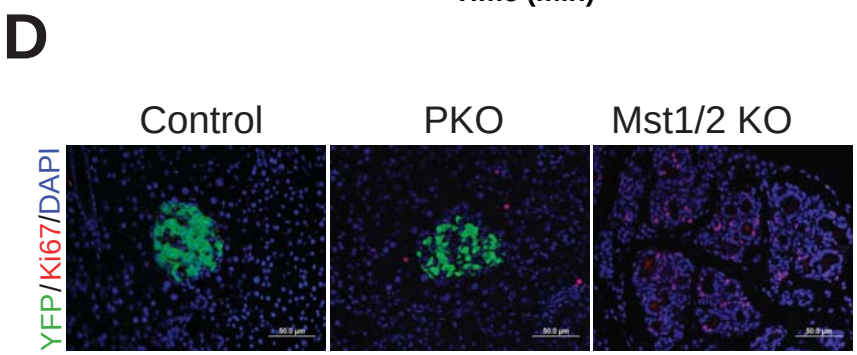
List of differentially expressed shared in the comparison of α-cells vs β-cells and PKO vs. β-cells using a cutoff of FDR<0.1 and >2-fold difference in expression.

Supplemental Table S6 (related to Figures 1, 5, and 6)

List of primers for used for quantitative real time PCR.


A
YFP/Pdx1/Som
100 μm
B
Control(n=6)
Het(n=6)
PKO(n=4)
Glucose (mg/L)
Time (min)
0
100
200
300
400
500
600
700
0
20
40
60
80
100
120
140


C
YFP/SOM/DAPI
YFP/PP/DAPI
Control
PKO


D
Control
PKO
Mst1/2 KO
YFP/Ki67/DAPI

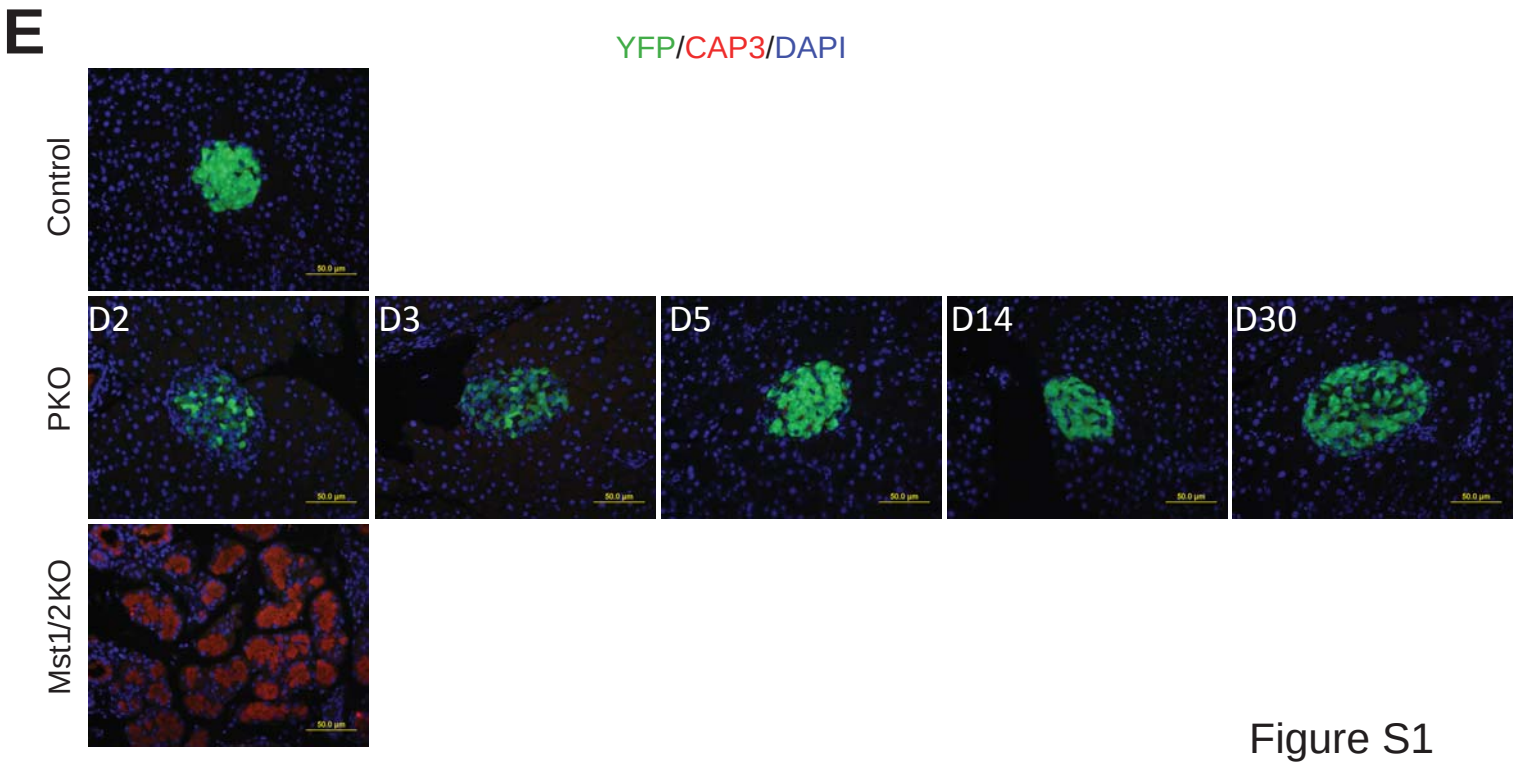
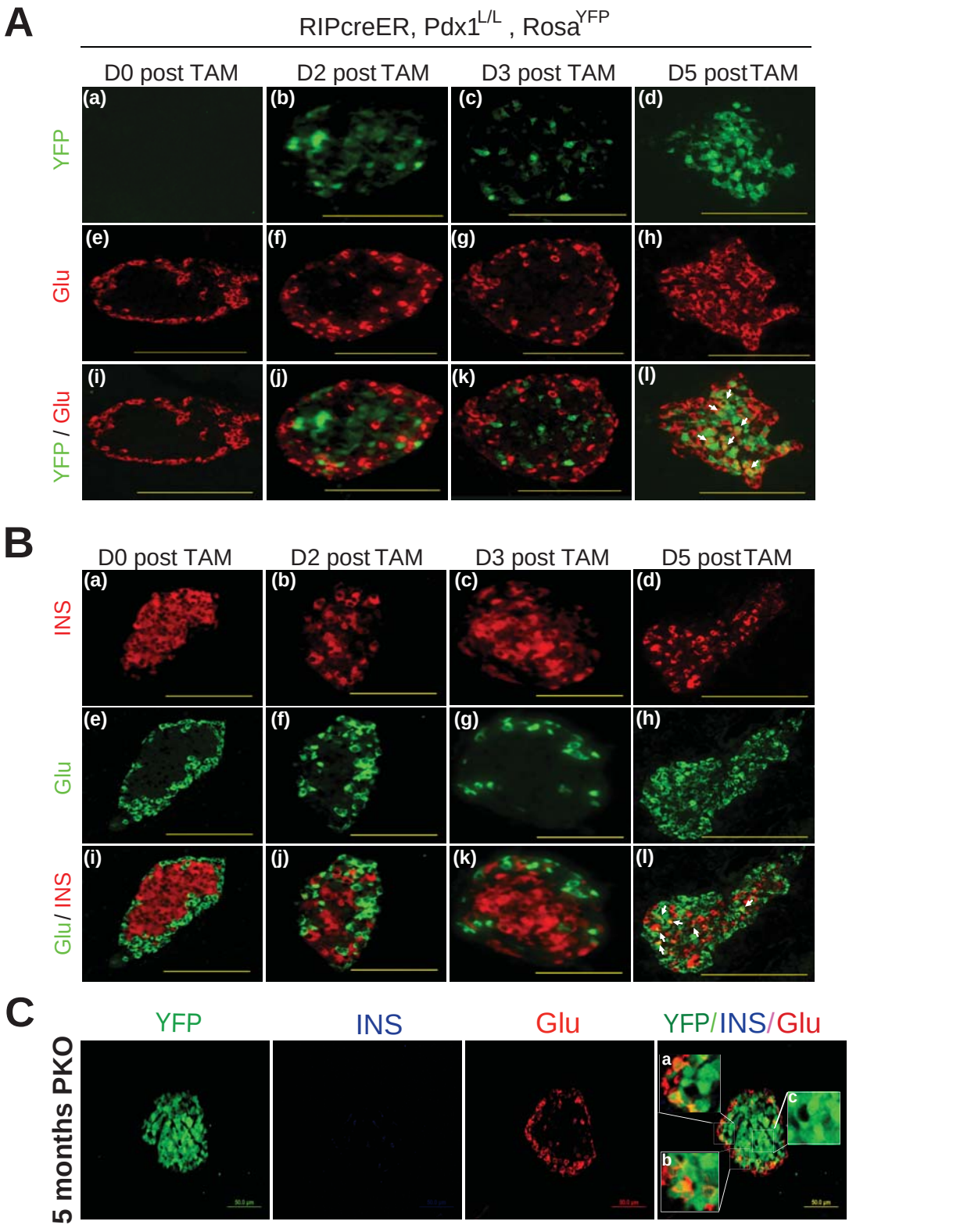

E
YFP/CAP3/DAPI
Control
PKO
Mst1/2KO
D2
D3
D5
D14
D30

Figure S1


A
RIPcreER, Pdx1L/L , RosaYFP
D0 post TAM
D2 post TAM
D3 post TAM
D5 post TAM
YFP
Glu
YFP / Glu
B
INS
Glu
Glu/ INS
C
YFP
INS
Glu
YFP/INS/Glu
5 months PKO
D
Beta (n=3)
PKO 5 days (n=3)
PKO 30 days (n=3)
Relative mRNA (normalized to GAPDH)
INS1
INS2

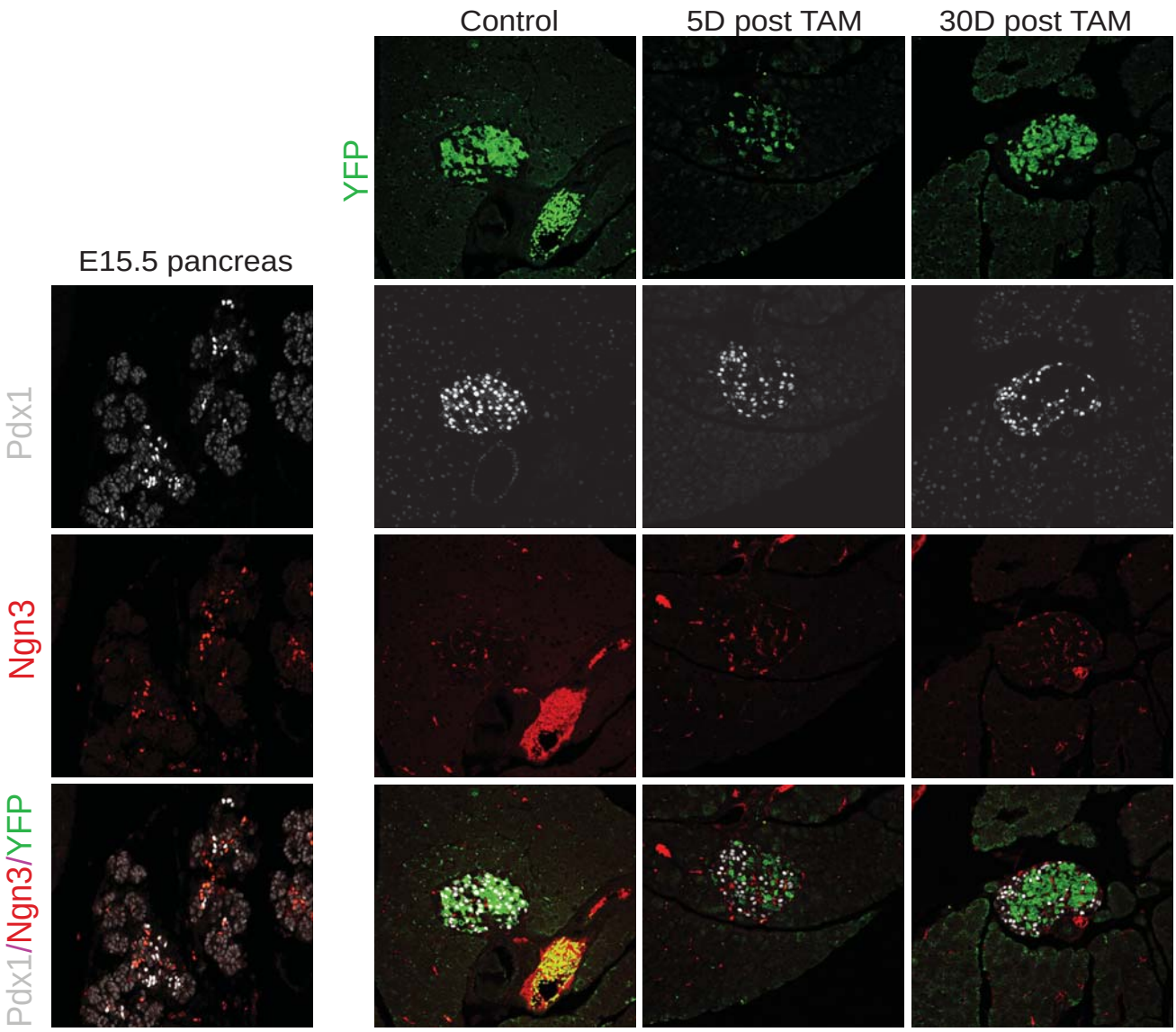
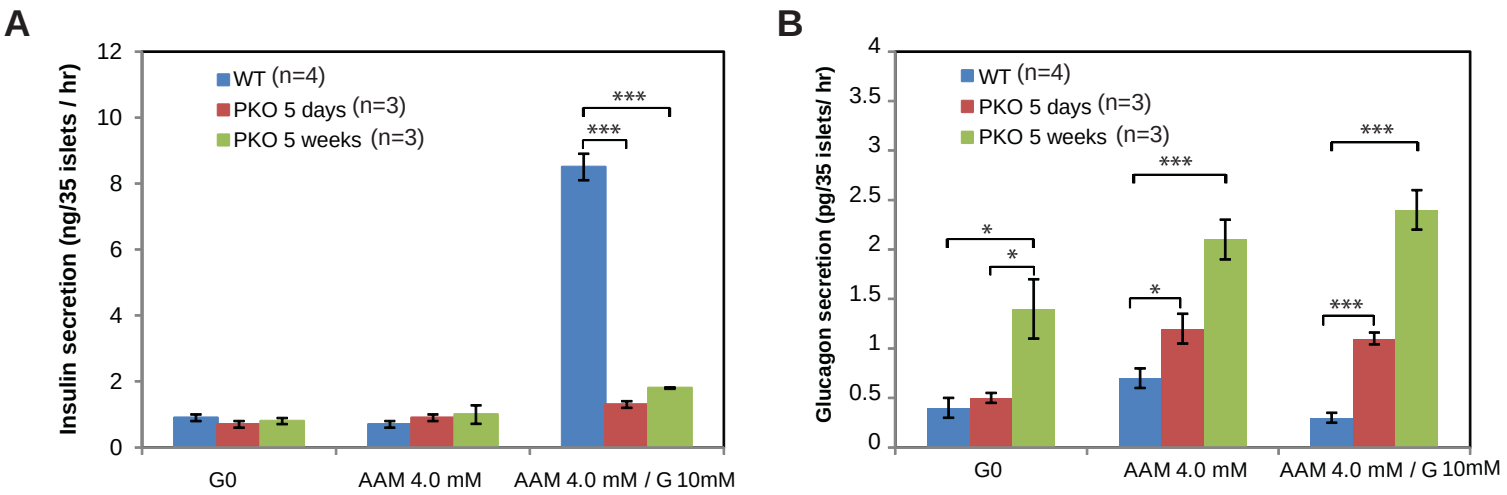

Control
5D post TAM
30D post TAM
YFP
E15.5 pancreas
Pdx1
Ngn3
Pdx1/Ngn3/YFP

Figure S3

Experiment 1



Experiment 2

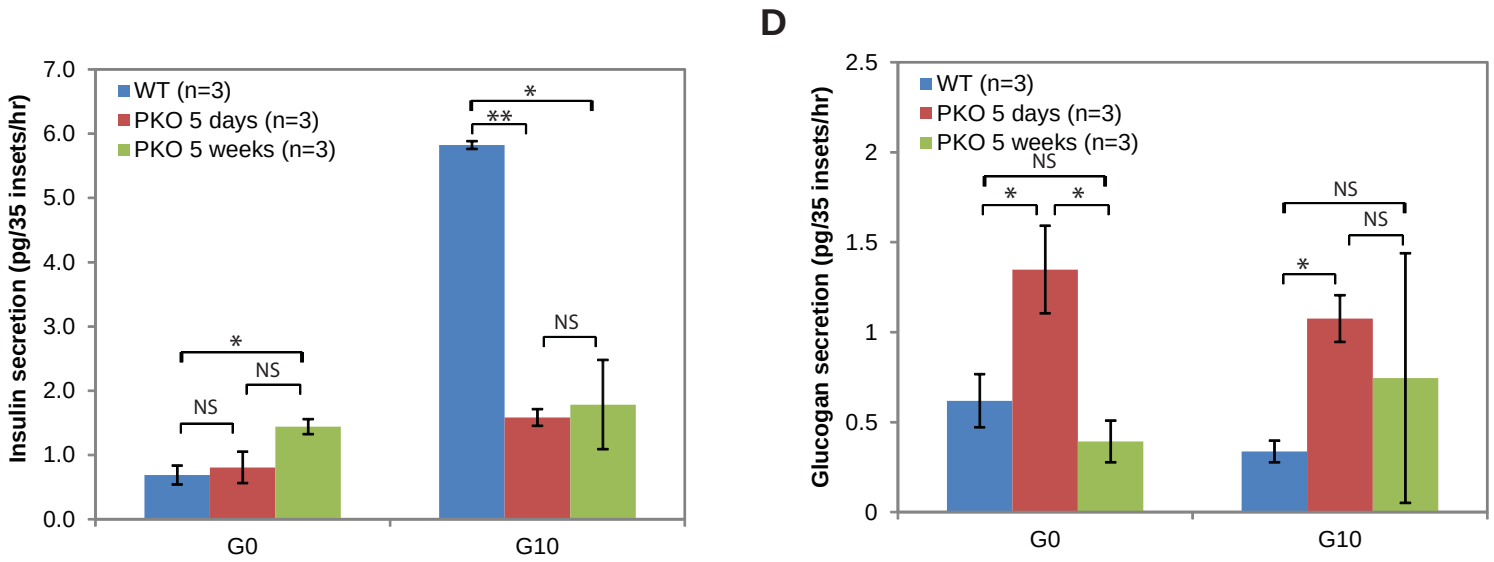
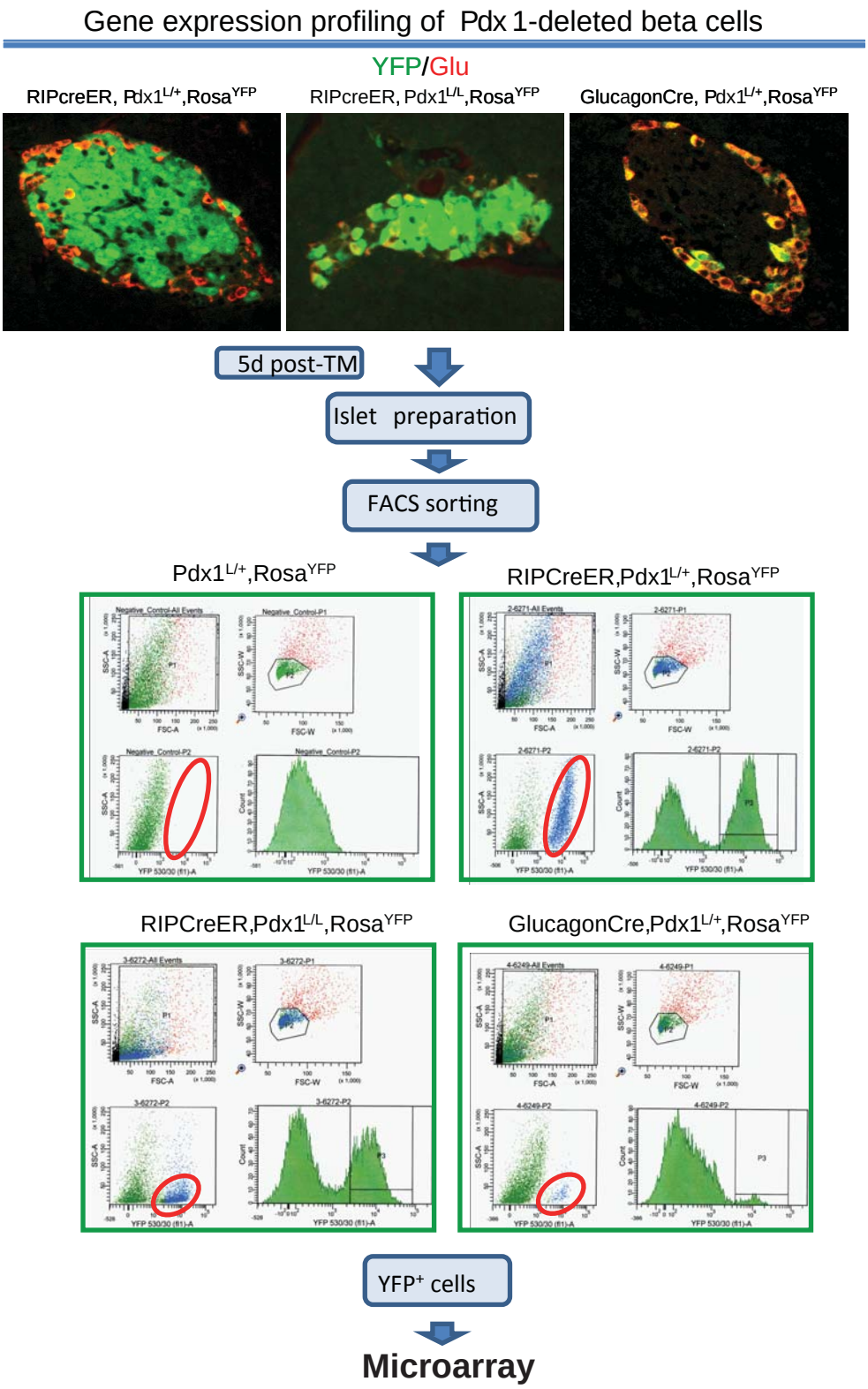


Figure S4

A


Gene expression profiling of Pdx 1-deleted beta cells
YFP/Glu
RIPcreER, Pdx1L/+,RosaYFP
RIPcreER, Pdx1L/L,RosaYFP
GlucagonCre, Pdx1L/+,RosaYFP
5d post-TM
Islet preparation
FACS sorting
Pdx1L/+,RosaYFP
RIPCreER,Pdx1L/+,RosaYFP
RIPCreER,Pdx1L/L,RosaYFP
GlucagonCre,Pdx1L/+,RosaYFP
YFP+ cells
Microarray

B

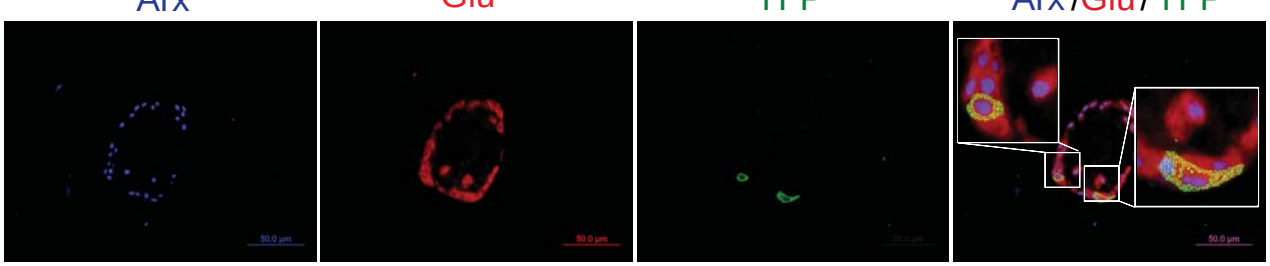

Arx
Glu
YFP
Arx/Glu/YFP

Figure S5

Supplemental Reference

Gao, T., Zhou, D., Yang, C., Singh, T., Penzo-Mendez, A., Maddipati, R., Tzatsos, A., Bardeesy, N., Avruch, J., and Stanger, B.Z. (2013). Hippo signaling regulates differentiation and maintenance in the exocrine pancreas. Gastroenterology *144*, 1543-1553, 1553 e1541.