Supplemental Figure S1. A representative whole mount *Pdx1-Cre; ROSA26R* embryo at E9.5 stained with X-gal is shown. *LacZ* expression is observed in both dorsal and ventral pancreatic bud (db and vb, respectively). ß-galactosidase activity is also detected in the hypothalamus region, as it has been previously described (Wicksteed, B. et al. 2010. *Diabetes*; 59 (12):3090-3098).

Supplemental Figure S2. Glucose tolerance tests are normal in 3-4-month-old *Gata4* or *Gata6* conditional knockout mice. Mice were fasted overnight (16-18 h). The following day, mice were weighed and blood samples were collected to determine fasting glucose levels. After an intraperitoneal injection of glucose solution (2 mg/g of body weight), blood samples were collected from the tail vein at 15, 30, 60, 90, and 120 min. Blood glucose concentrations were determined by using Accu-Check Advantage glucometer (Roche). (A) control mice, n=3; *Gata4*$^{flox/flox}$*; Pdx1-Cre* mice, n=6. (B) Control mice n=4; *Gata6*$^{flox/flox;}$ *Pdx1-Cre* mice, n=5.

Supplemental Figure S3. Histological analysis of *Gata4/Gata6* double mutant mice with pancreas hypoplasia. Hematoxylin/eosin (A, A´) and immunofluorescence staining for insulin and glucagon antibodies (C, C´) or mucin and amylase antibodies (D, D´) reveal normal pancreatic architecture and differentiation in newborn double mutant mice. Staining with GATA4 antibody shows the presence of unrecombined *Gata4*$^{+}$cells in the double mutant hypoplastic pancreata (B, B´). Scale bars = 50 µm.

Supplemental Figure S4. Pancreatic epithelial morphology in *Gata4/Gata6* double mutant embryos. Control (Ctrl) or double mutant pancreata sections stained with hematoxylin/eosin (A, B, I, J), anti-GATA4 (C, E, G, K) or anti-GATA6 (D, F, H, L) antibodies at different stages of embryonic development are shown. At E11.5, the pancreatic epithelium of the control (A) and double mutant (B) embryos display normal morphology. At this stage, *Gata4* and *Gata6* expression overlap in the budding epithelial cells of control embryos (C, D). GATA4 and GATA6 proteins are not detected in the pancreatic bud of double mutant embryos (E, F). At midgestation (E13.5), *Gata4* (G) and *Gata6* (H) expression overlap at the tips of the proliferating epithelial cells in control pancreata. By E15.5, the epithelium forms the typical ductal-tree structure in control embryos (I). A cystic ductal structure in observed in the double mutant pancreata (J). Two days later at E17.5, *Gata4* expression becomes restricted to the exocrine acinar cells (K) while *Gata6* expression is restricted mostly to ductal and developing islets, with lower levels in acinar cells (L). (*): acinar cells; (d): ductal cells; (i); endocrine cells. Scale bars = 50 μm.

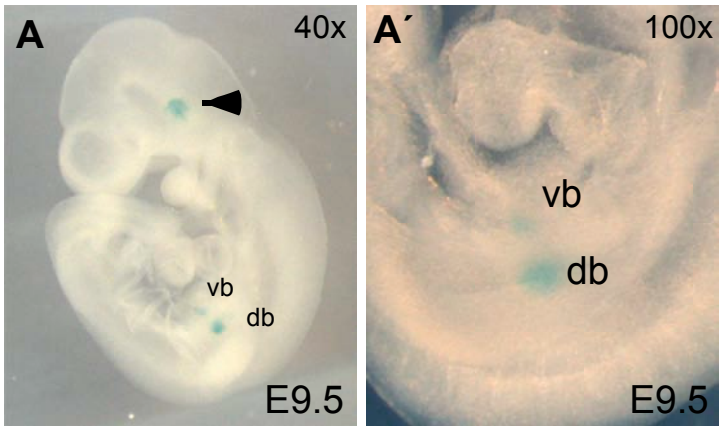
Supplemental Figure S5. Quantification of pancreatic epithelial area in double mutant embryos. Representative dissected pancreata of control (A, C) and double mutant (B, D) embryos at E13.5 were whole-mount stained for mucin, as previously described (Ahnfelt-Ronne, J. J Histochem Cytochem, 2007). Images were captured using a confocal microcospe TCS SP5 (Leica). All images were acquired using the same exposure time and magnification. Images were analyzed using Metamorph sofware and the threshold range were maintained constant throughout the experiment. Quantification of pancreatic epithelium area reveals a significant reduction of the double mutant pancreatic epithelial area compared to control littermates (E). Control mice, n=4. Double mutant mice, n=4. *$p<0.05$. Scale bars = 50 μm.

Supplemental Figure S6. Conserved GATA sites in the *Ptf1a* promoter are bound by recombinant GATA4 and GATA6. (A). Three conserved GATA sites in the *Ptf1a* promoter region are shown in blue boxes. Numbers indicate the position of the GATA sites relative to *Ptf1a* transcriptional start site. Introduced point mutations in GATA sites, G1m, G2m and G3m, are indicated in red lowercase**.** (B,C) Recombinant GATA4 and GATA6 proteins are able to bind to a radiolabeled, double-stranded oligonucleotide probe encompasing G1, G2 and G3 GATA sites in the *Ptf1a* promoter region, as shown by EMSA. Competition experiments were performed by adding excess unlabeled of G1, G2-3, control (denoted as c in competitor row) GATA site probes and the corresponding mutant versions (G1m, G2m/G3m or cm), to the binding reaction.

Supplemental Figure S7. Conserved GATA sites in *Pdx1* area I are bound by recombinant GATA4 and GATA6. (A). Two conserved GATA sites in *Pdx1* area I are shown in blue boxes. Numbers indicate the position of the GATA sites relative to *Pdx1* transcriptional start site. Introduced point mutations in GATA sites, G3m and G4m, are indicated in red lowercase. (B) Recombinant GATA4 and GATA6 proteins are able to bind to a radiolabeled, double-stranded oligonucleotide probe encompassing G3 and G4 GATA sites in area I of *Pdx1*, as shown by EMSA. Competition experiments were performed by adding excess unlabeled of G3-4 or control (denoted as c in competitor row) GATA site probes, and the corresponding the mutant versions (G3m/G4m or cm), to the binding reaction.

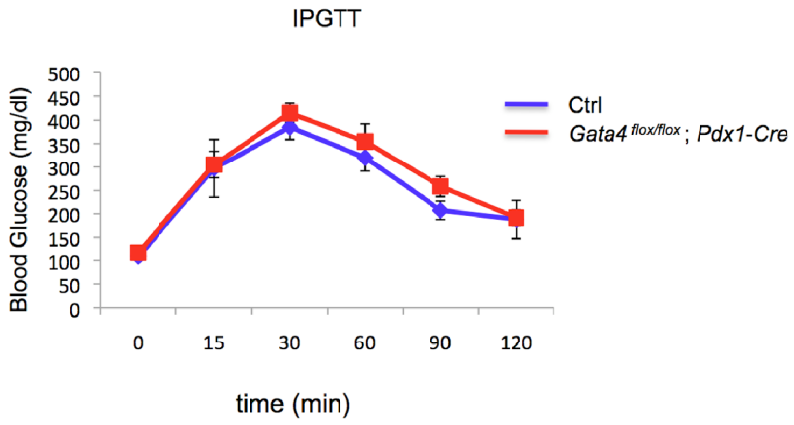
Carrasco et al., Supplemental Figure S1

Pdx1-Cre X ROSA26R



Carrasco et al., Supplemental Figure S2

A



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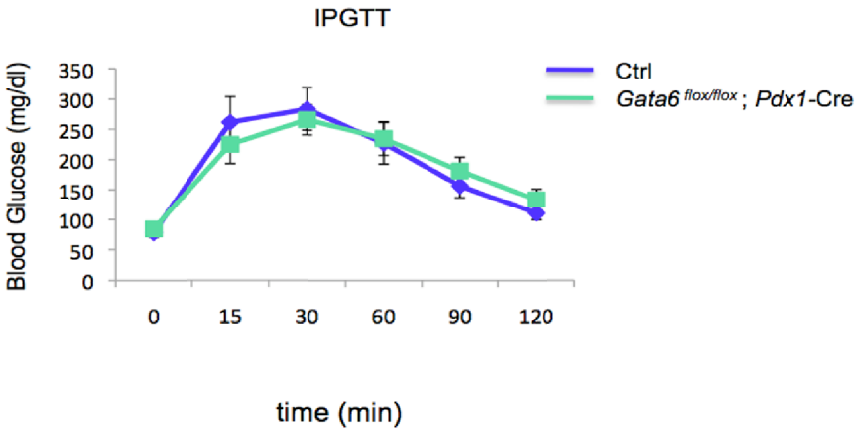
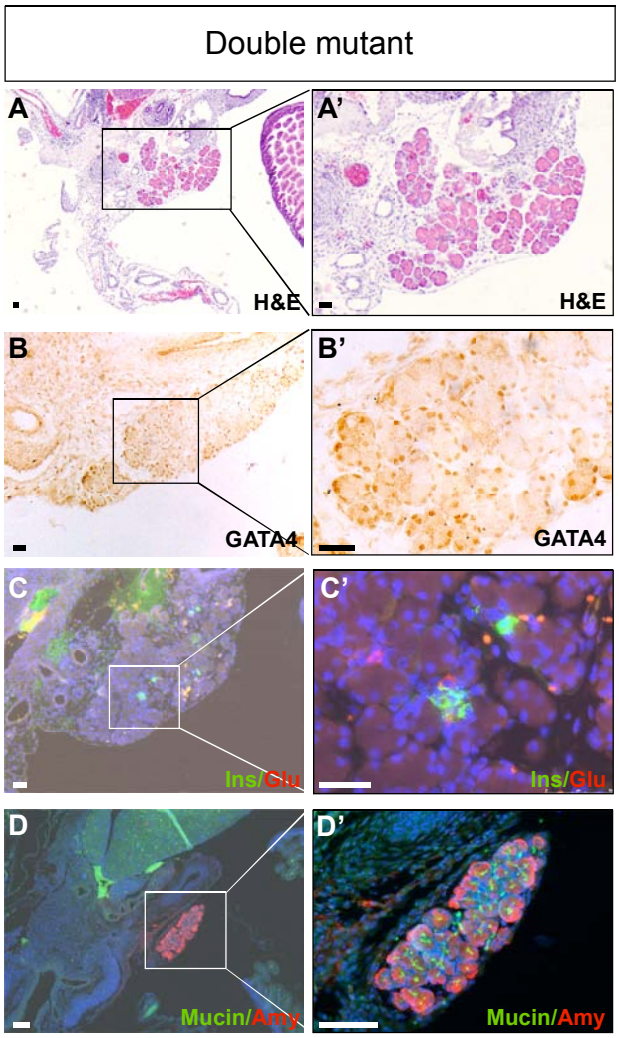
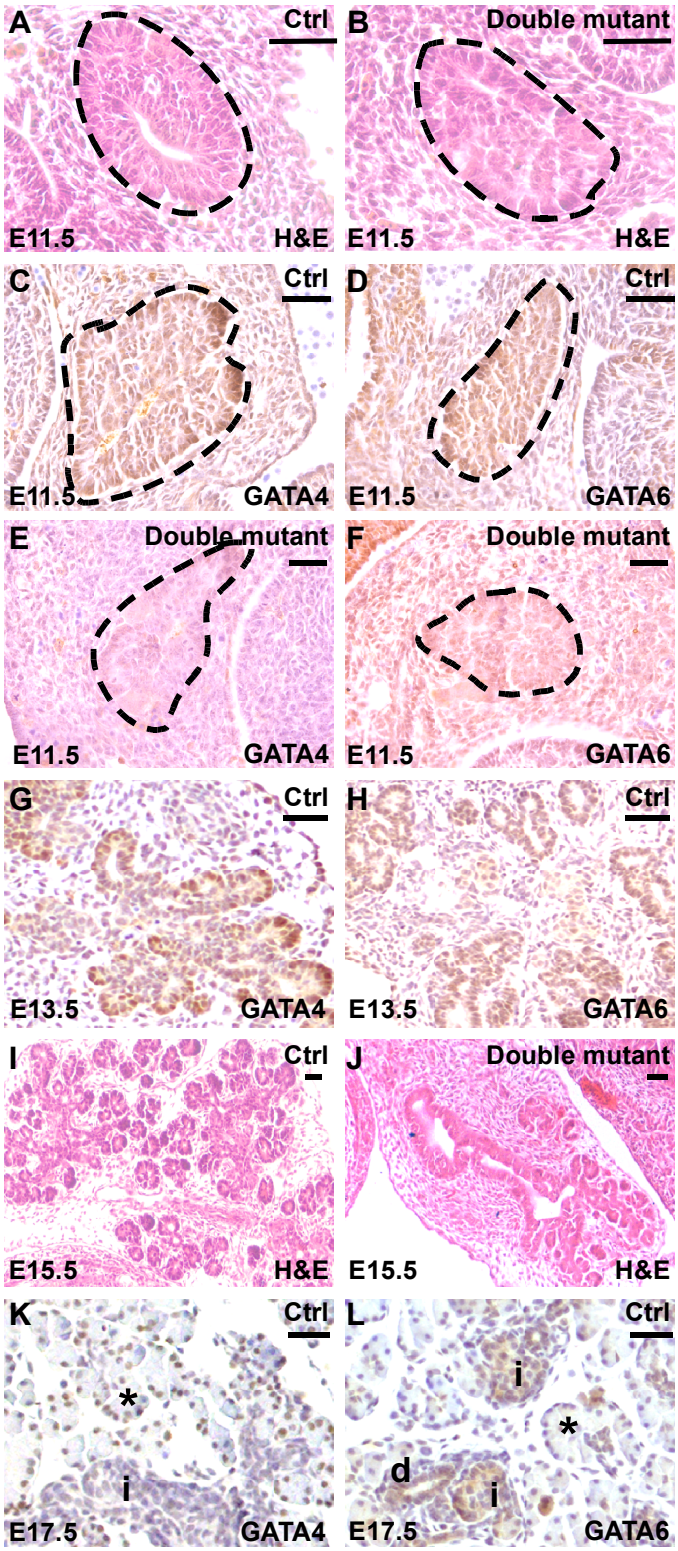


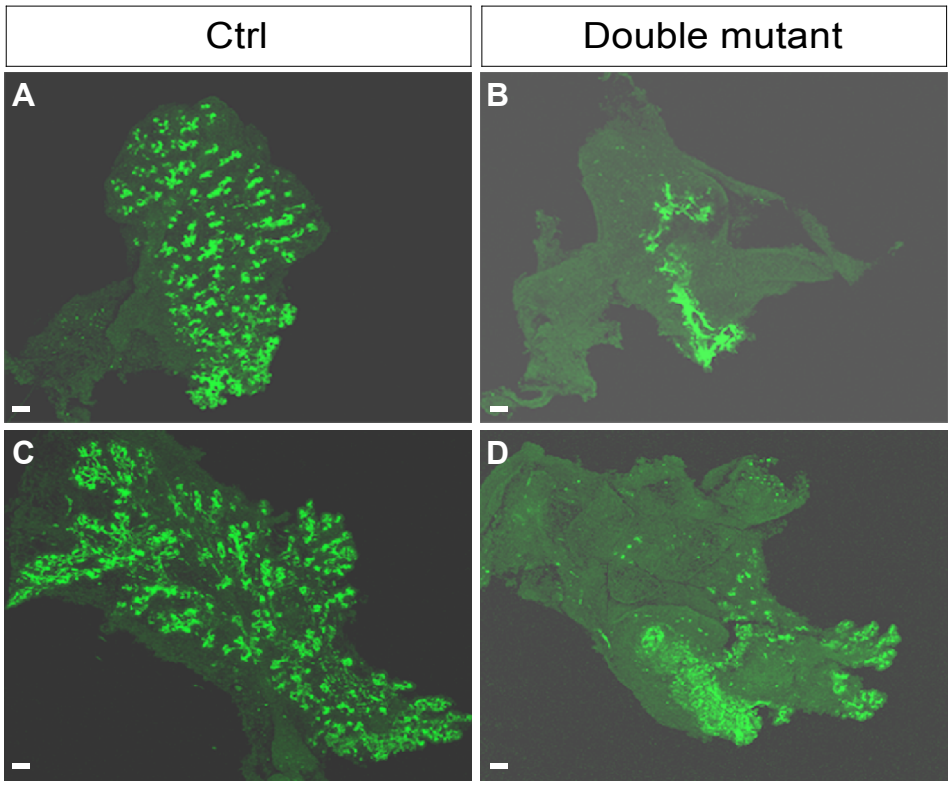
Carrasco et al., Supplemental Figure S3

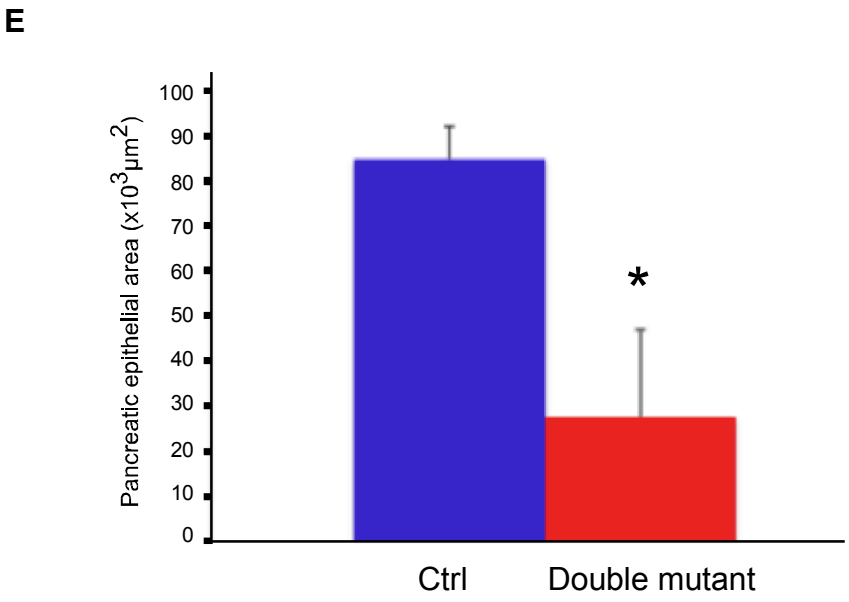


Carrasco et al., Supplemental Figure S4

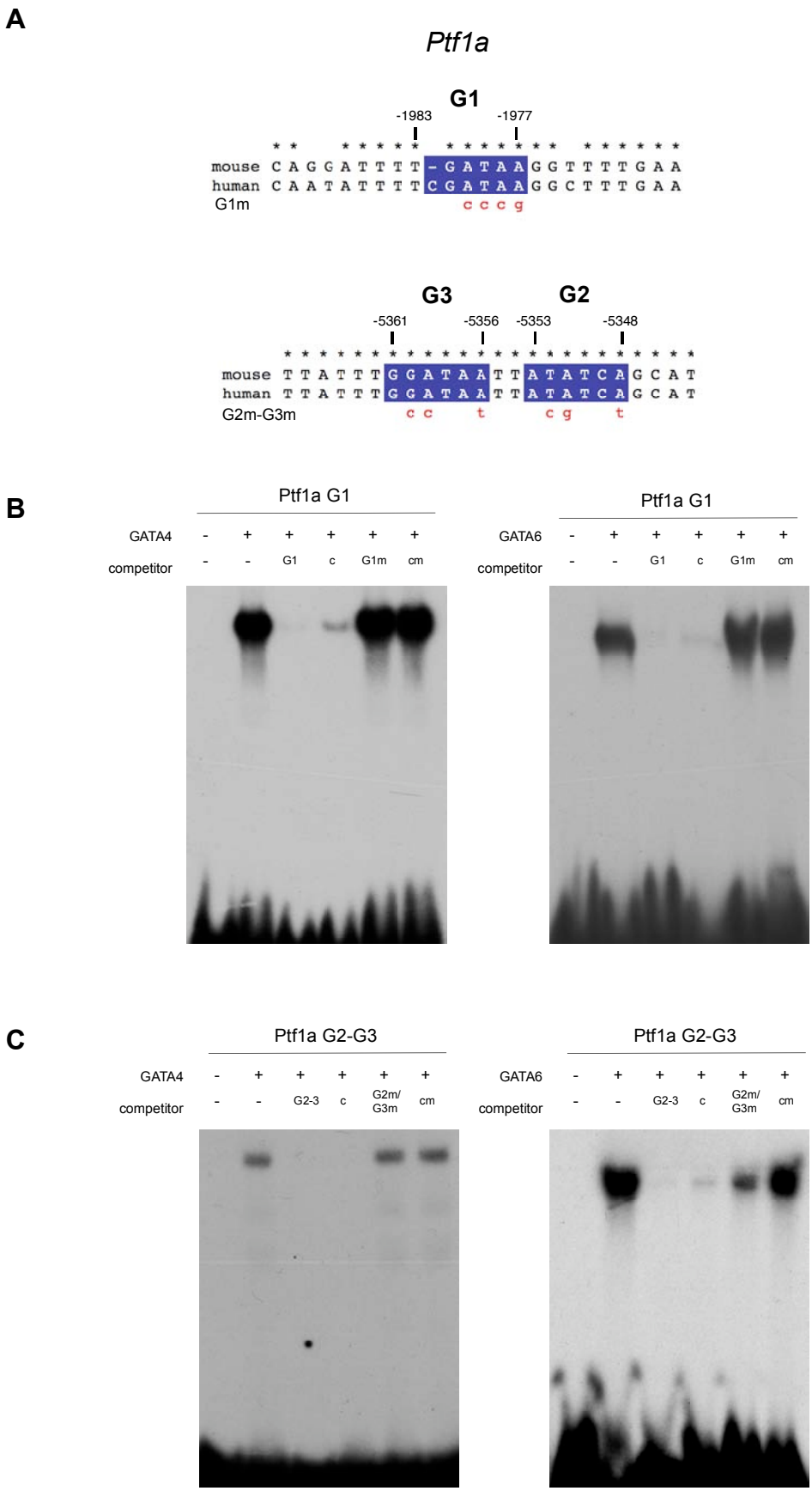


Carrasco et al., Supplemental Figure S5





Carrasco et al.,Supplemental Figure S6



Carrasco et al.,Supplemental Figure S7