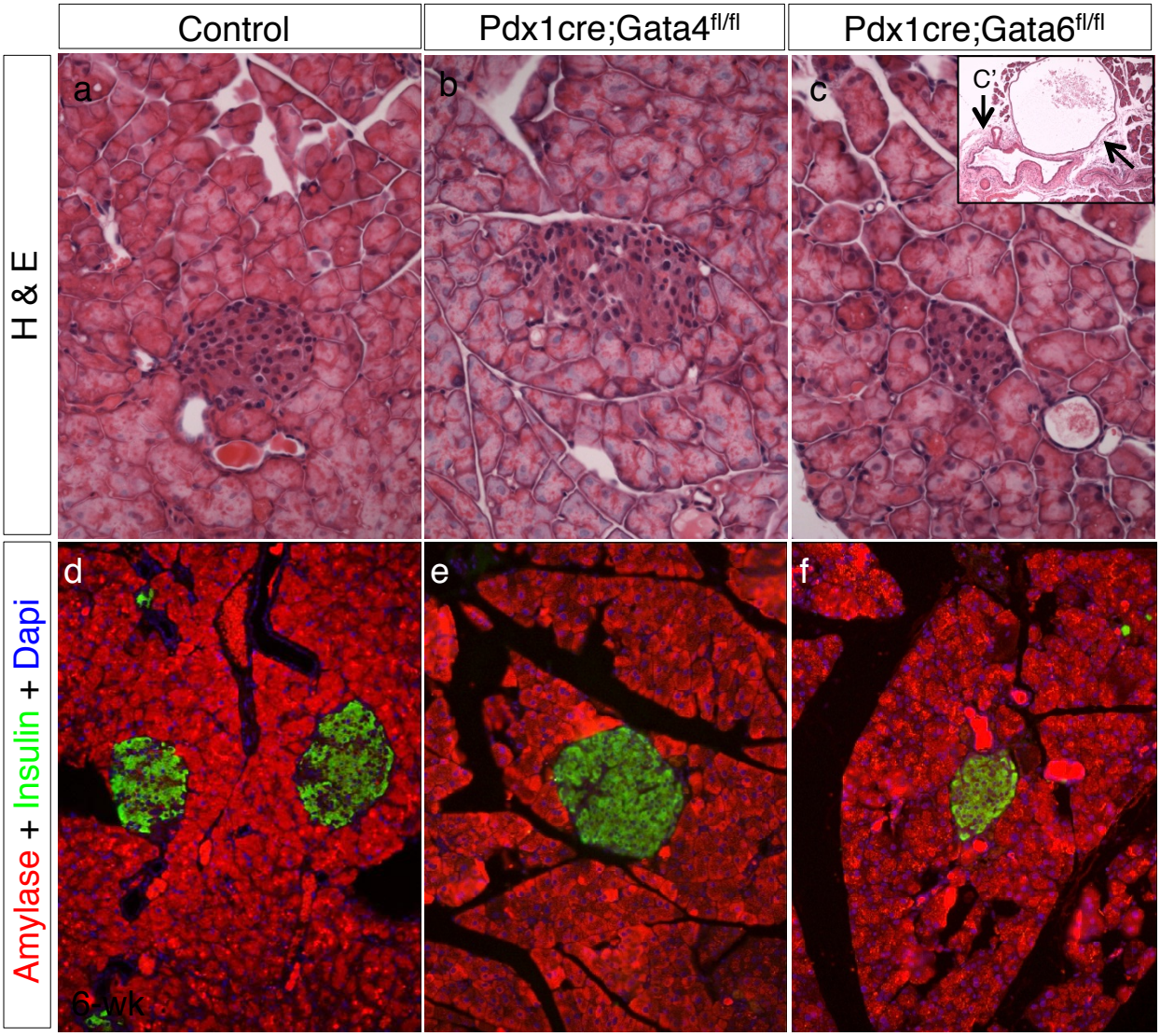
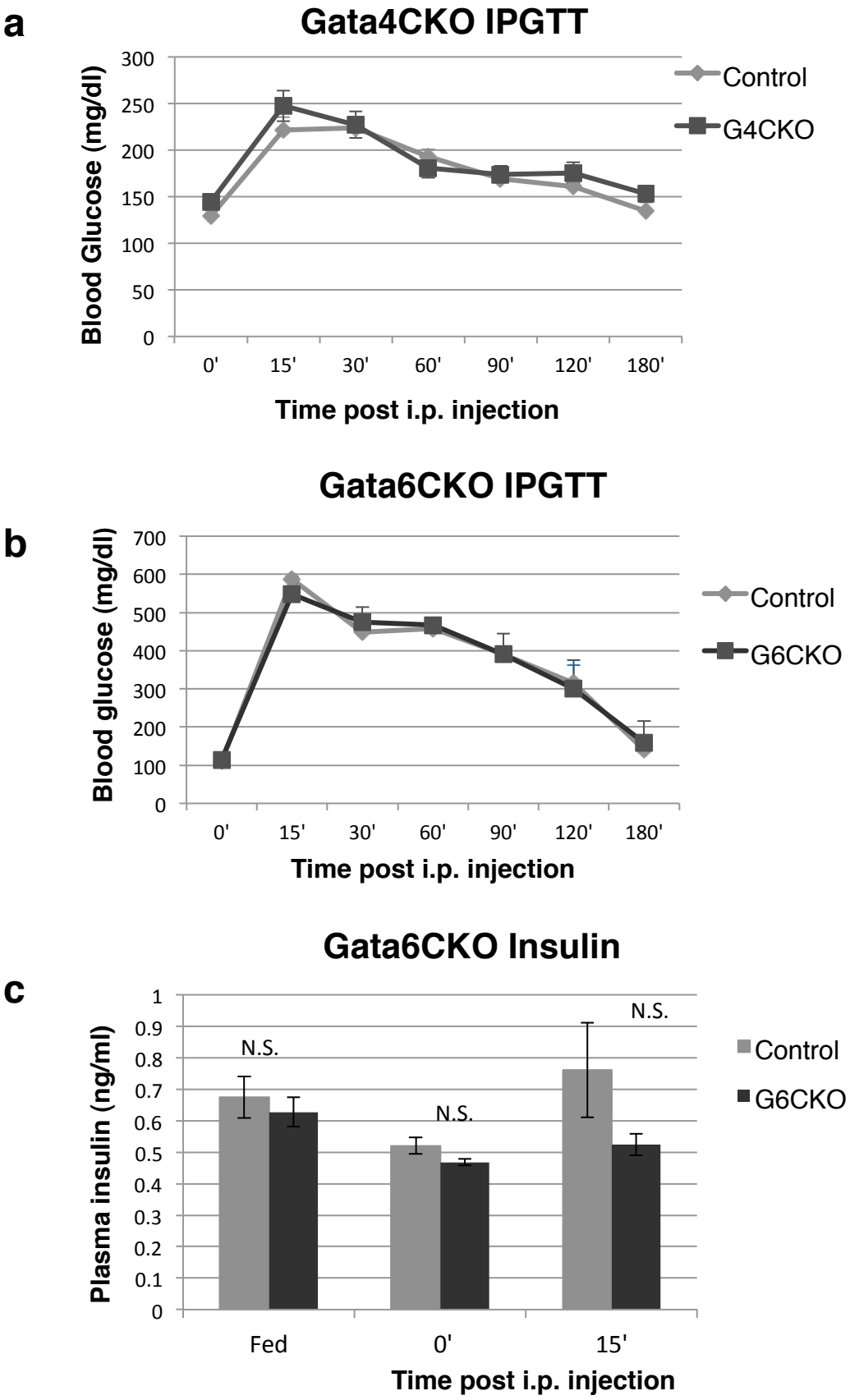
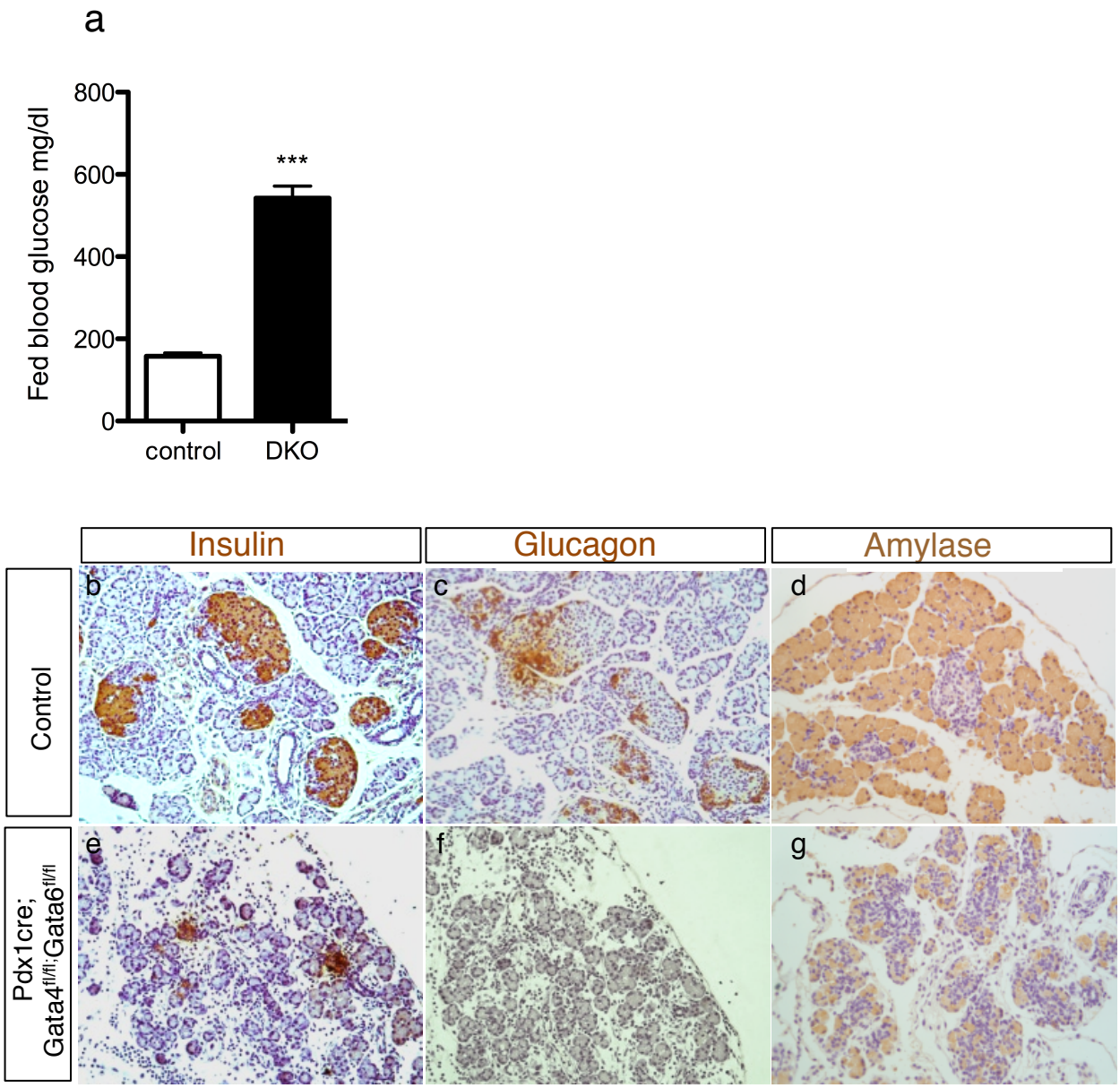
Xuan et al., Supplemental Figure 1



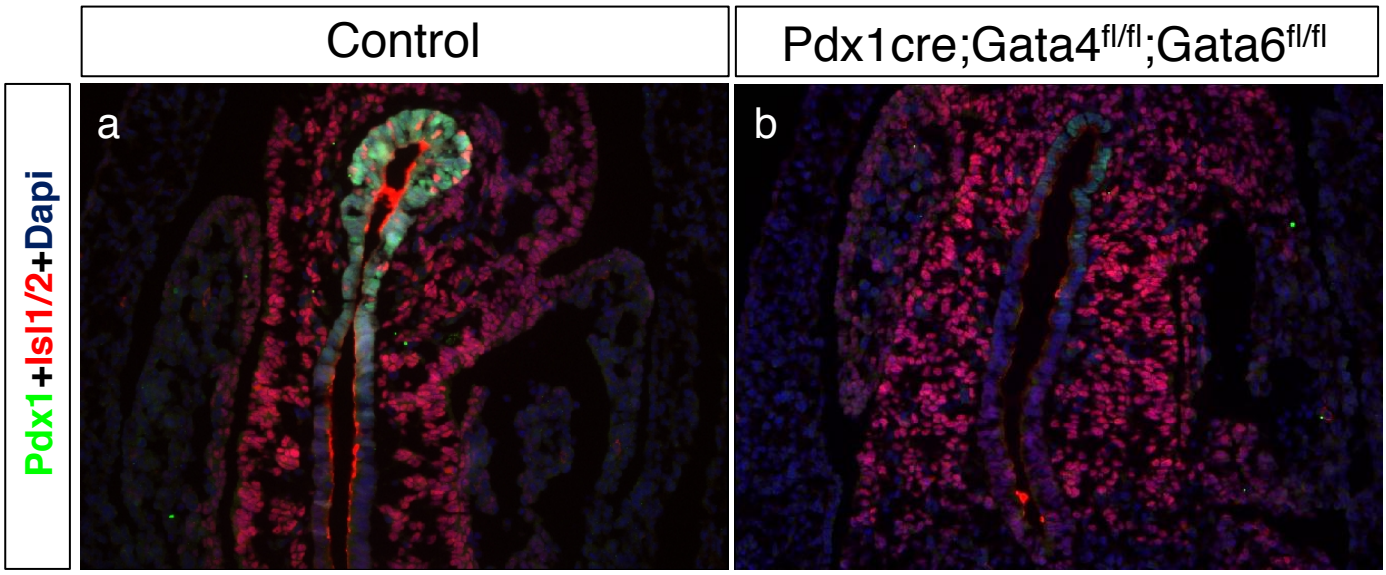
Xuan et al., Supplemental Figure 2



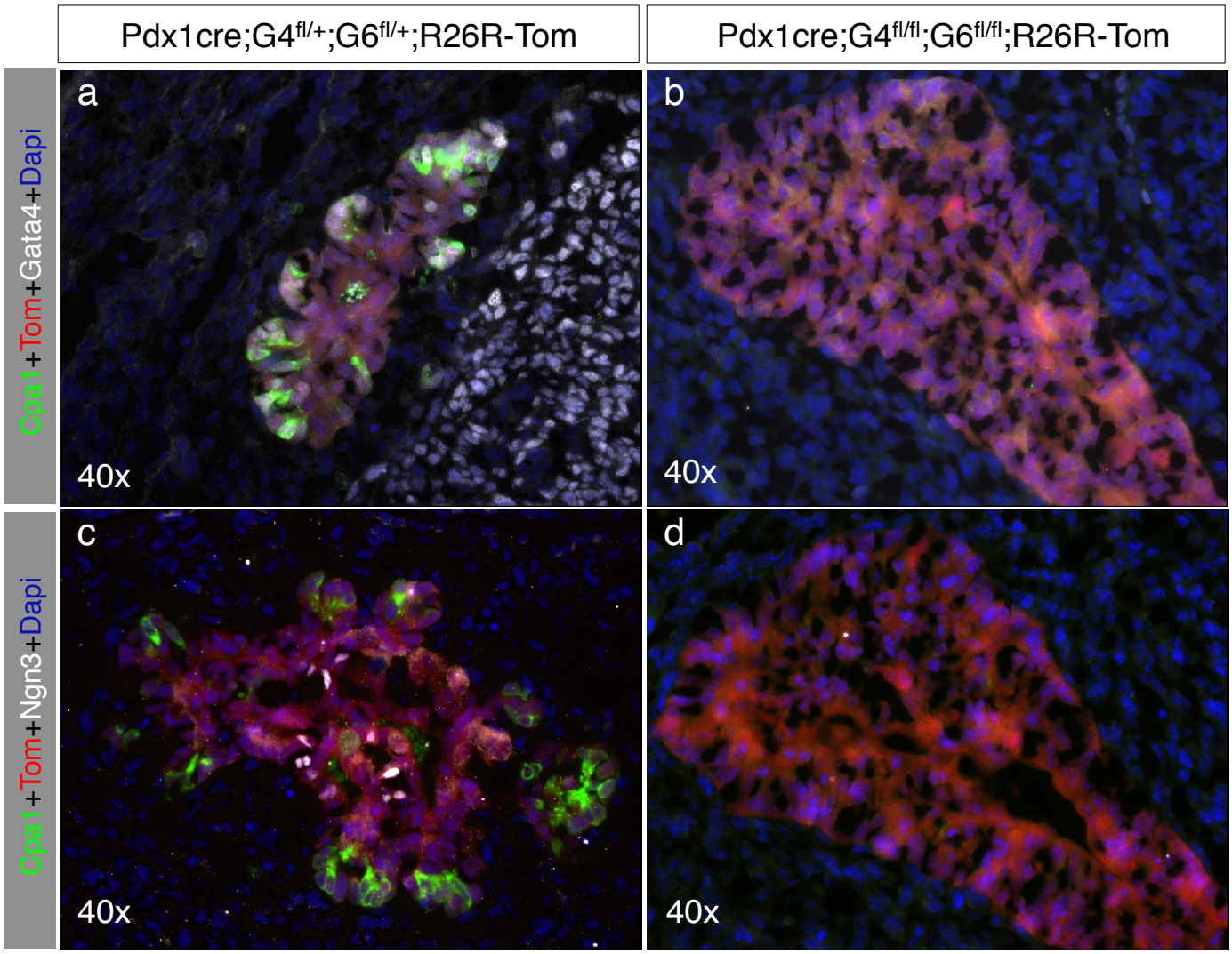
Xuan et al., Supplemental Figure 3



Xuan et al., Supplemental Figure 4



Xuan et al., Supplemental Figure 5



e

Cpa1 promoter (-183)

mouse TGGCTTATCTCTCCACCTGCCTTGTTCCCTGATACTTTATCAGG

human TGGCTTATCTCTCCAGCTGCCCAGTTCCCTGCCACTTTATCATG

Ngn3 promoter (-395)

mouse GGCAGAGCAGATAAAGCGTGCCAGG

human GGCGAAGCAGATAAAGCGTGCCAAG

Pdx1 promoter (Area III; -1833)

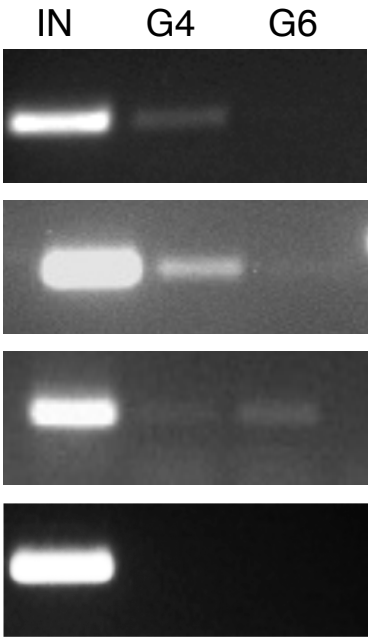
mouse GCCGAGGAGATAGCATCCGAGTCCC

human GCCAGGGTGATAGGGTACGAGTTCT

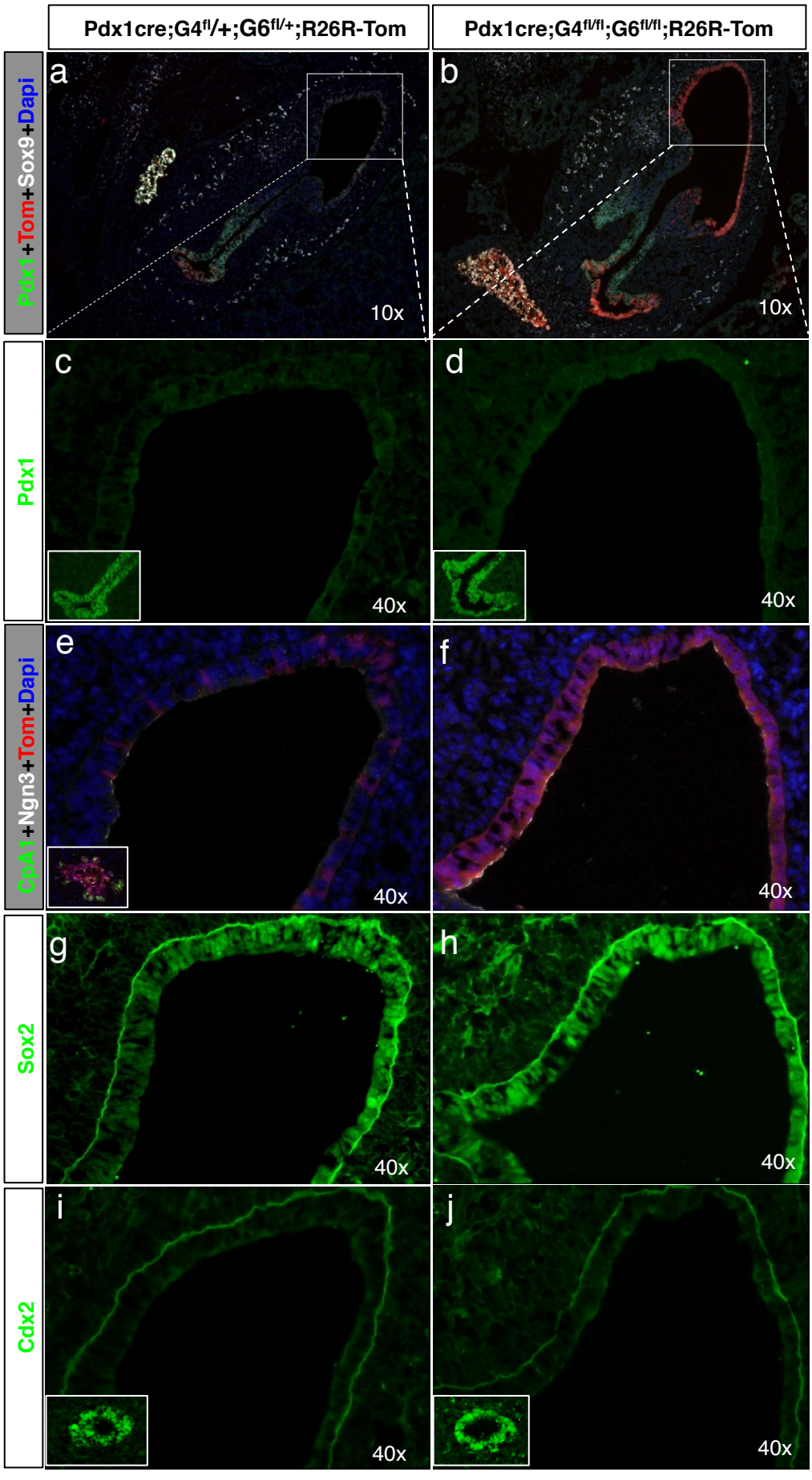
Cpa1 exon (+3561)

mouse CCCCTATGGCTACACATCAGAACCAGCT

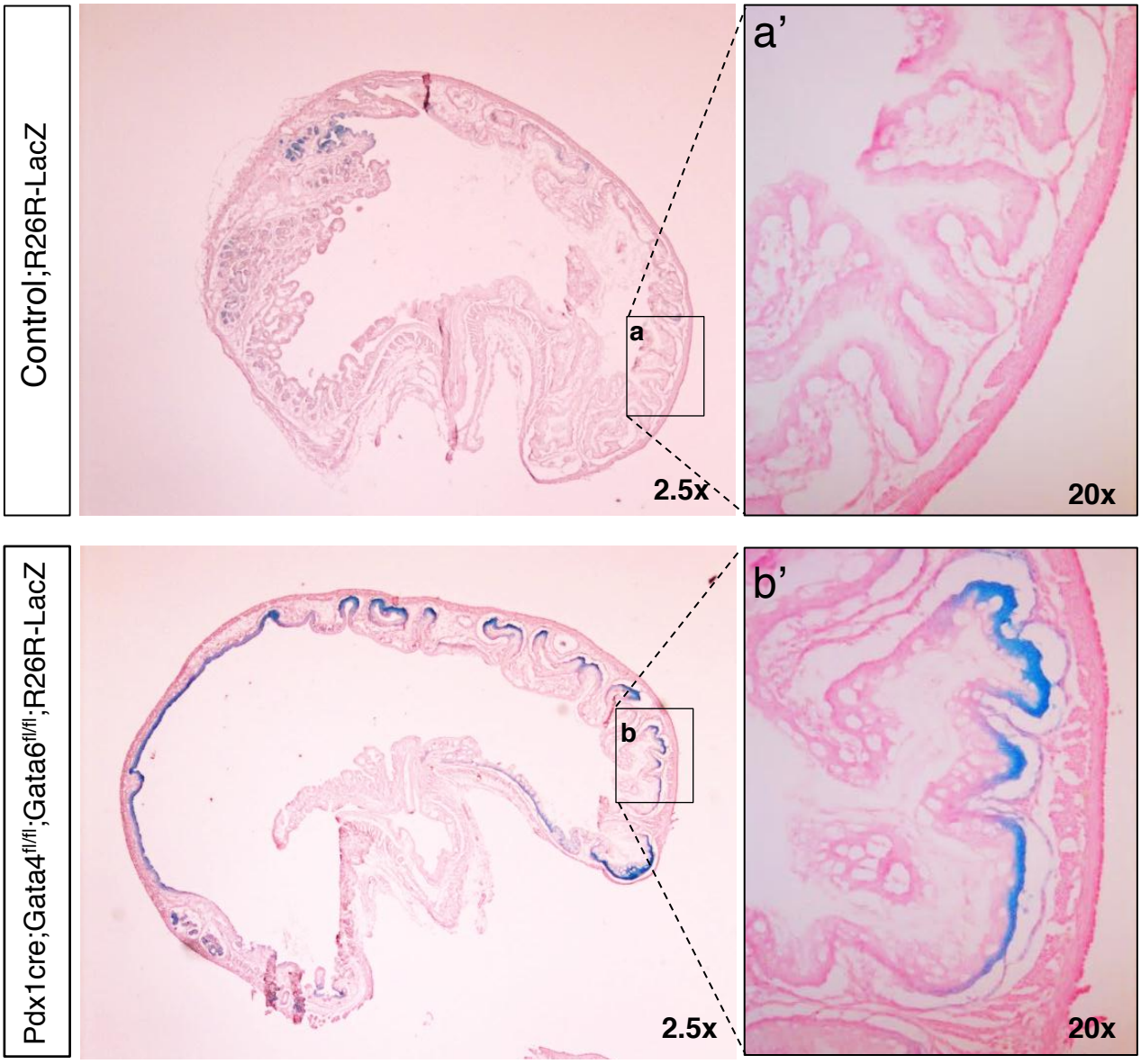
human TCCCTATGGCTACAAAACAGAACCAGTC



Xuan et al., Supplemental Figure 6



Xuan et al., Supplemental Figure 7



Supplemental Figure 1. Adult pancreata from Gata4 CKO and Gata6 CKO mice display minor morphological defects. (A-C) H&E staining of pancreatic sections from 6-week old mice. When compared with the control (a), neither single Gata4CKO (b) nor Gata6CKO (c) pancreata show significant morphological changes in the exocrine and endocrine compartments. A small number of cysts can be found in Gata6CKO pancreas (inset g, arrows). (D-F) Amylase and insulin co-immunofluorescent labeling of pancreatic sections. In comparison to the control (d), neither Gata4CKO (e) nor Gata6CKO (f) show detectable abnormalities in expression of exocrine enzymes or endocrine hormone. Original magifications: 200x (a-f), 400x (g).

Supplemental Figure 2. Single *Pdx1cre; Gata4$^{fl/fl}$* and *Pdx1cre;Gata6$^{fl/fl}$* mice have normal glucose tolerance at 6-8 weeks old. (A) 8-week old *Pdx1cre; Gata4$^{fl/fl}$* mice have normal glucose tolerance; n=10 for both control and *Pdx1cre;Gata4$^{fl/fl}$* mice. (B) 6-week old *Pdx1cre;Gata6$^{fl/fl}$* mice have normal glucose tolerance; n=4 for both control and *Pdx1cre;Gata6$^{fl/fl}$* animals; (C) Normal plasma insulin levels in *Pdx1cre;Gata6$^{fl/fl}$* mice at 6 weeks old. The insulin levels were measured in mice that were fed and fasted, and at 15 minutes post glucose injection. N=4 for both control and *Pdx1cre;Gata6$^{fl/fl}$* mice.

Supplemental Figure 3. A small number of DKO animals survive postnatally, but are severely diabetic. (A) The few number of DKO mice that are able survive postnatally (presumably due to incomplete *Gata4* and/or *Gata6* deletion) are severely hyperglycemic at one month of age. Control: n = 12, DKO: n = 6. (B-G) Immunohistochemical analysis of representative sections through the pancreas from one month old control (b-d) versus DKO animals (e-g) indicate that the DKO mice have smaller islets with fewer insulin-producing cells (b and e), almost no detectable

glucagon-producing cells (c and f). Exocrine tissue is significantly reduced with fewer amylase-producing cells (d and g). Original magnifications: 200x (b-g).

Supplemental Figure 4. Mesenchymal tissues appear normal in the *Pdx1cre;Gata4$^{fl/fl}$;Gata6$^{fl/fl}$* DKO embryos. E10.5 embryos were stained with mesenchymal marker Isl1/2 (red). Representative transverse sections from (A) control; (B) DKO. Pdx1 staining (green) indicates pancreatic epithelium/bud. Original magnifications: 200x (a and b).

Supplemental Figure 5. Gata proteins regulate key molecular events during the secondary transition of pancreas development. (A) Cpa1 (green) is expressed in a large fraction of Gata4 (white) expressing cells in the control pancreas (B) Loss of Cpa1 expression in the e12.5 DKO pancreatic remnant (B). (C) Neurog3 (white) is expressed in the pancreatic epithelial cells in the control but not in the DKO embryos (D). Tomato (red) positive cells indicate Pdx1 lineage cells. (E) ChIP assays demonstrate Gata binding to conserved regions within the Cpa1, Neurog3, and Pdx1 promoters respectively. Input was used a positive control, and a sequence within a Cpa1 coding exon was used as a negative control. IN: input, G4: anti-Gata4 antibody, G6: anti-Gata6 antibody. Original magnifications: 400x (a-d).

Supplemental Figure 6. Pdx1-derived cells in the anterior stomach adopt local stomach cell fates.

Pdx1 derived cells (marked by red tomato positive cells) are found in the anterior stomach in e12.5 DKO embryos (b) but not in that of the control embryo (a). These cells do not express pancreatic markers such as Pdx1 (green in c and d), Cpa1(green), Neurog3 (white) (e and f). Instead, they express Sox2 (green), which marks anterior

region of the stomach (g and h). These cells do not appear to be duodenal-derived cells as they do not express Cdx2 (green,i and j). Original magnifications: 100x (a and b) 400x (c-j, and insets). Insets indicate positive staining of the corresponding markers in control tissue.

Supplemental Figure 7. At e17.5, Pdx1 derived cells show morphological features of squamous stomach epithelium. Sections of LacZ stained whole mount stomach from control and DKO embryos (a and b respectively). Boxed areas represent a segment of the squamous stomach from control and DKO embryos (c and d respectively). Original magnifications: 25x (a and b), 200x (c and d).