Supplemental Online Material To:

Generation Of Functional Insulin-Producing Cells In The Gut By Foxo1 Ablation

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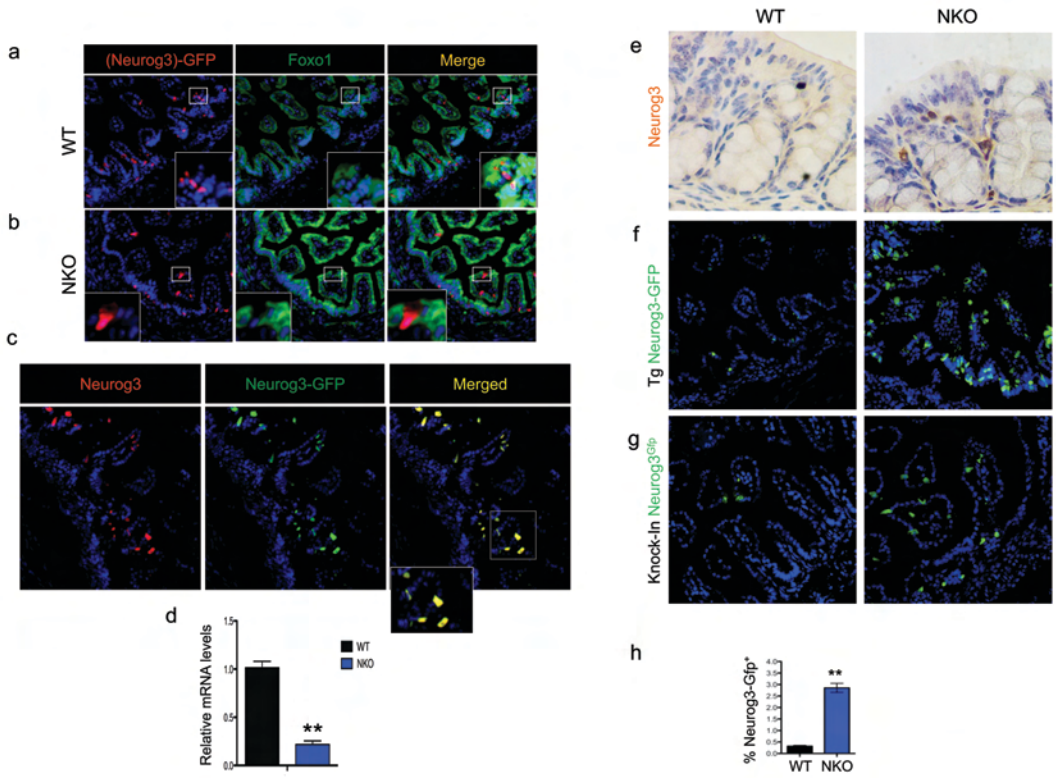
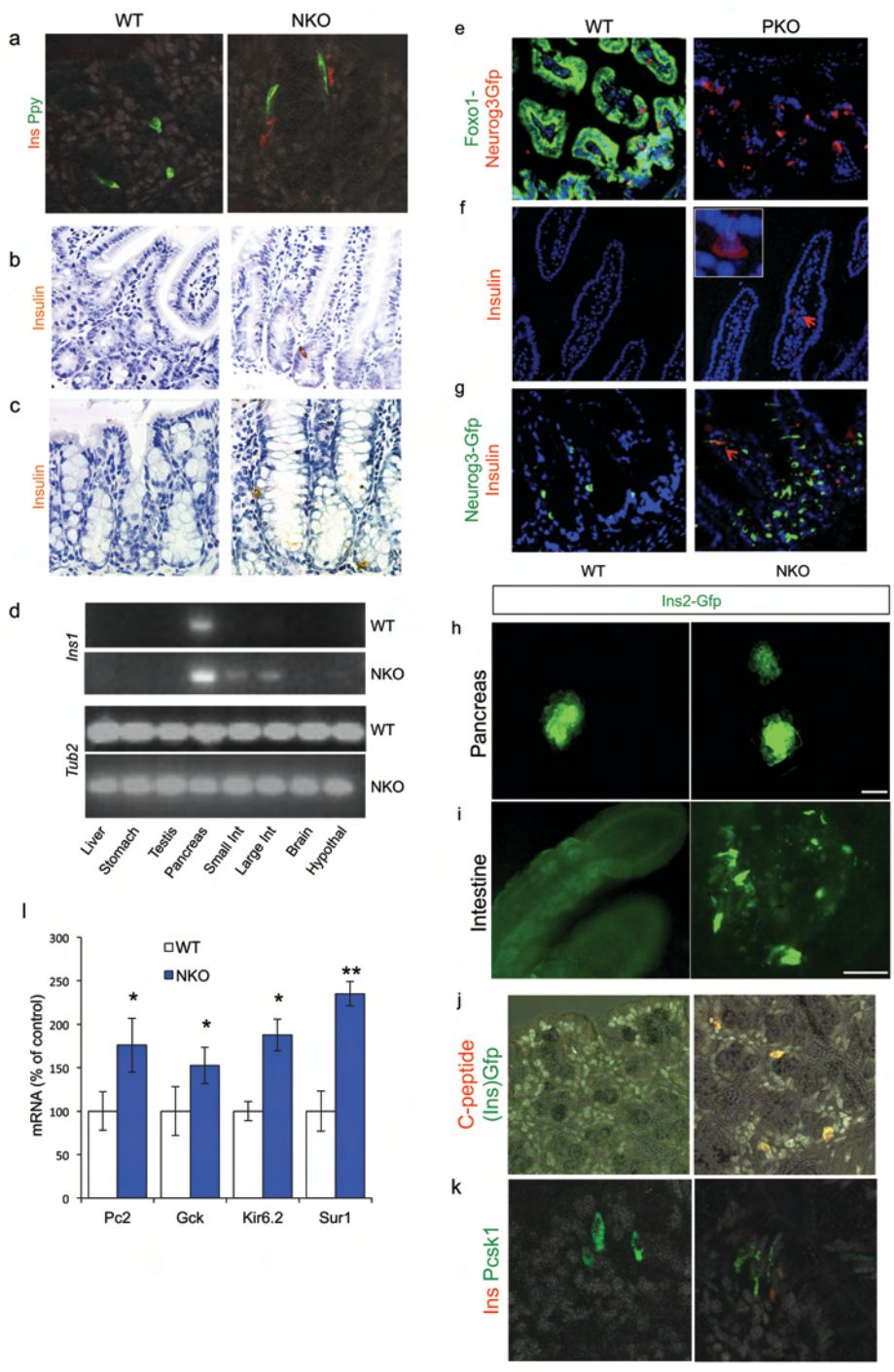
Contains 7 supplemental figures with figure legends

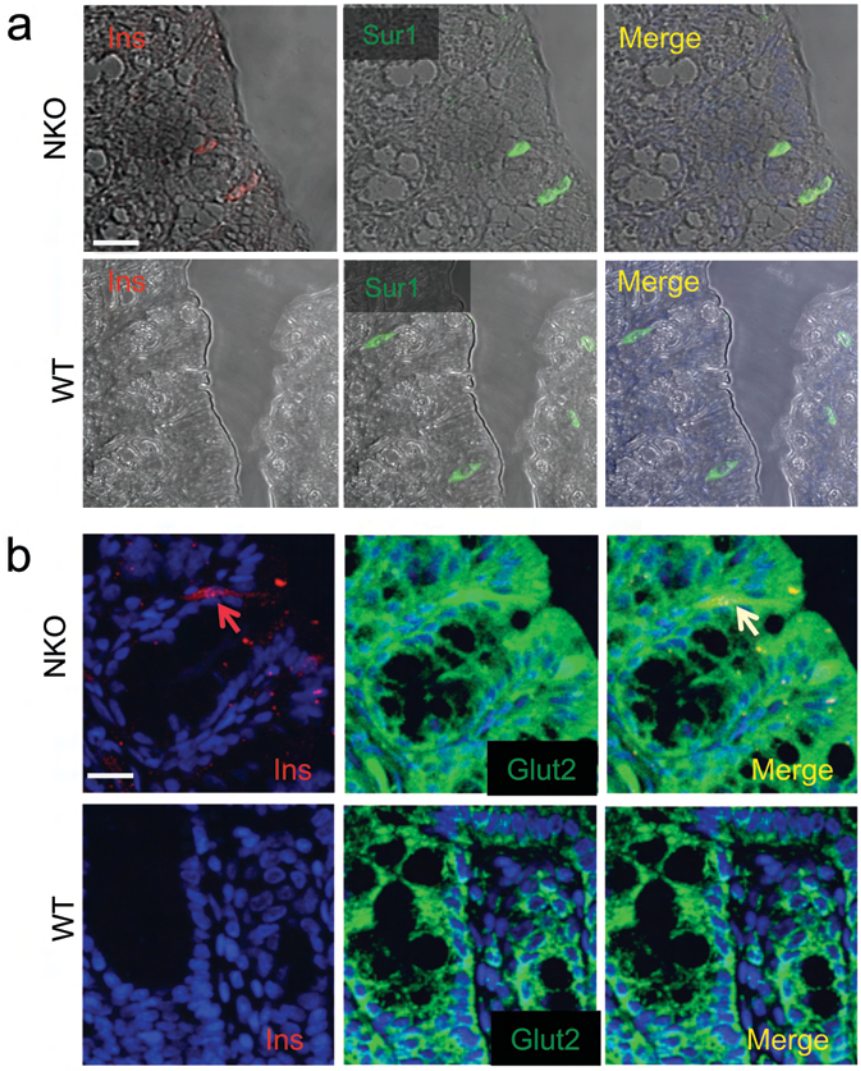


Supplementary Figure 1 Neurog3 localization and expression. (**a**) Co-localization of Gfp (red) and Foxo1 (green) in intestines of control and *NKO* mice crossed with *Neurog3-Gfp* reporter mice. (**b**) Lack of co-localization of Neurog-Gfp with Foxo1 in *NKO* mice. (**c**) Immunofluorescence with anti-Neurog3 (red) and anti-Gfp (green) in neonatal gut from *Neurog3-Gfp* transgenic mice, indicating complete overlap between the two detection methods. (**d**) qPCR analysis of *Foxo1* mRNA expression from Neurog3-Gfp sorted cells. (**e**) Immunohistochemistry with anti-Neurog3 (brown) in adult large intestines. (**f-g**) Immunofluorescence with anti-Gfp (green) in adult gut from *Neurog3-Gfp* transgenic mice **(f)** and *Neurog3-Gfp* knock-in mice (**g**). (**h**) Quantification of Neurog3-Gfp^{+} cells using anti-Gfp by flow cytometry. Original magnification: 20X . n=3 for histology and n= 4 for qPCR and flow cytometry. * = $P < 0.05$, ** = $P < 0.01$

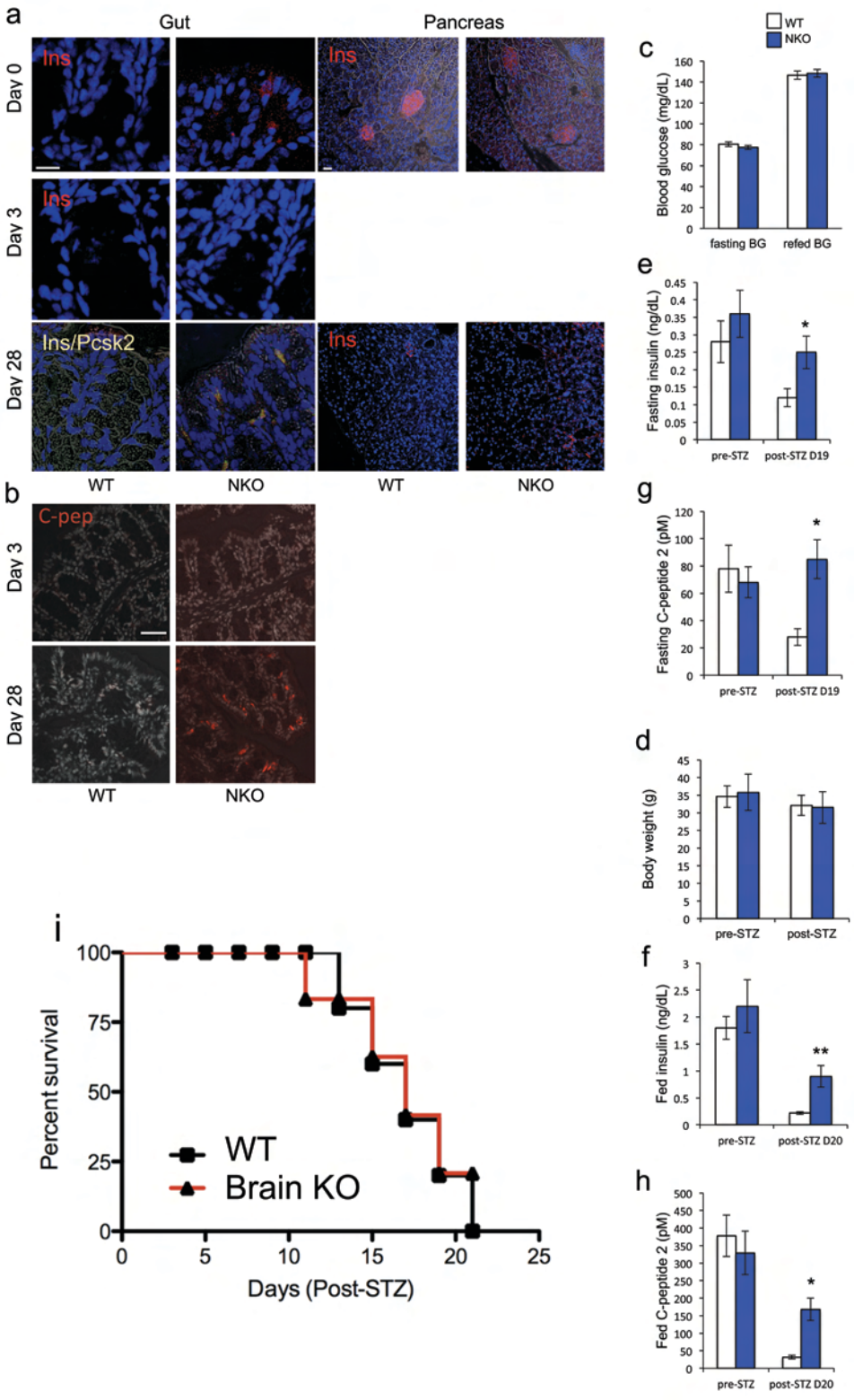


Supplementary Figure 2 Gut immunohistochemistry. (**a**) Immunofluorescence with anti-Pancreatic polypeptide antibody (green) and insulin (red) in large intestines of adult WT and *NKO* mice. (**b-c**) Immunohistochemistry with anti-insulin (brown) in adult distal ileum (**b**) or colon (**c**). RT-qPCR of *insulin1* and *tubulin2* expression in various tissues of

WT and *NKO* mice. (**e-g**) Generation and analysis of *PKO* mice. (**e**) Foxo1 (green) and Gfp immunochemistry (red) in 1 day-old *PKO*:*Neurog3-Gfp* and control mice. (**f**) Insulin immunostaining (red) in the duodenum of 9-month-old *PKO* mice. (**g**) Insulin (red) and Gfp immunostaining (green) in the duodenum of 1 day-old *PKO:Neurog3-Gfp* and *Neurog3-Gfp* control mice. (**h-i**) Direct fluorescence in pancreata (**h**) and villi (**i**) of control *Ins2-Gfp* and *NKO*:*Ins2-Gfp* mice. (**j**) Immunofluorescence with C-peptide (red) and Gfp (green) in *NKO:Ins2-Gfp* mice. (**k**) Co-localization of insulin (red) and Prohormone convertase 1 (Pc1) (green). (**l**) qPCR analysis of mRNA expression of markers of β-cell differentiation in isolated gut epithelial cell preparations from DTZ-enriched segments in *NKO* mice (blue bars) and from anatomically matched segments in control mice (black bars) (n = 8). * = $P < 0.05$, ** = $P < 0.01$.

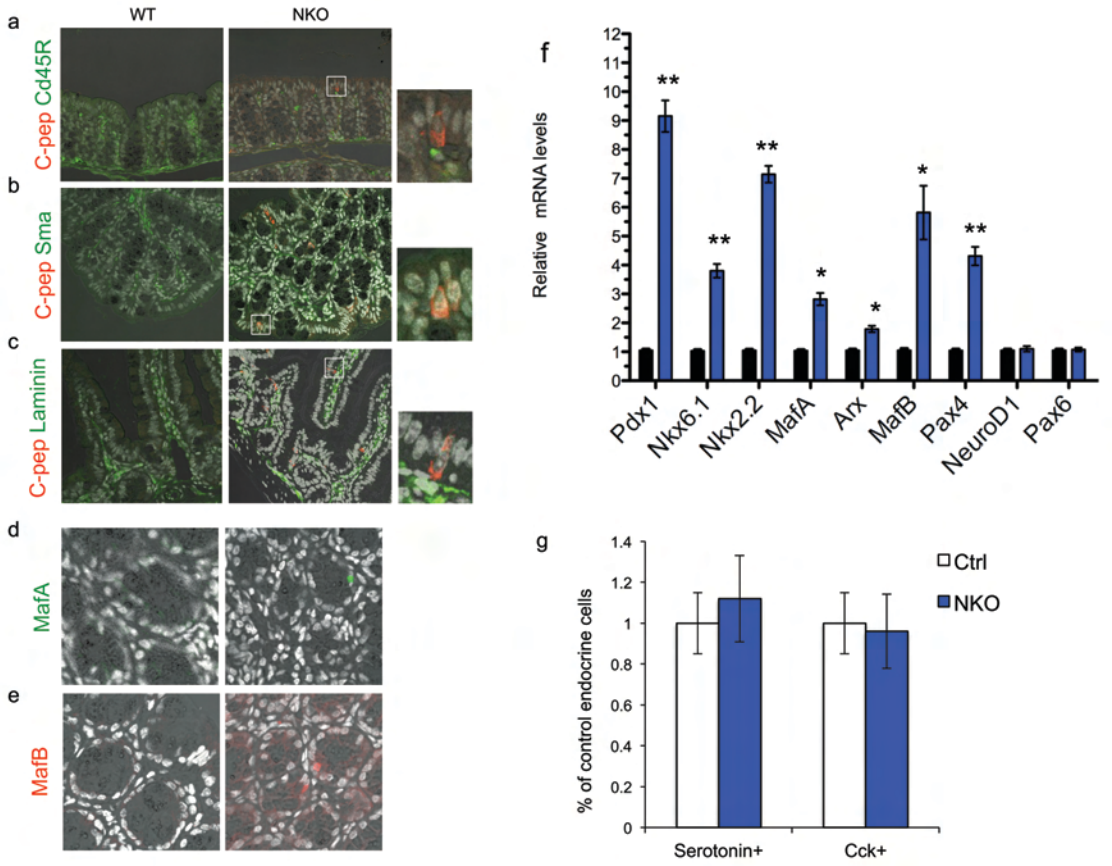


Supplementary Figure 3 Marker analysis of gut Ins^+ cells by immunohistochemistry. (**a**) Insulin (red), Sulfonylurea Receptor 1 (Sur1), or (**b**) glucose transporter 2 (Glut2) antibodies (green). Scale bars: 40 μm (**a**), and 30 μm (**b**).

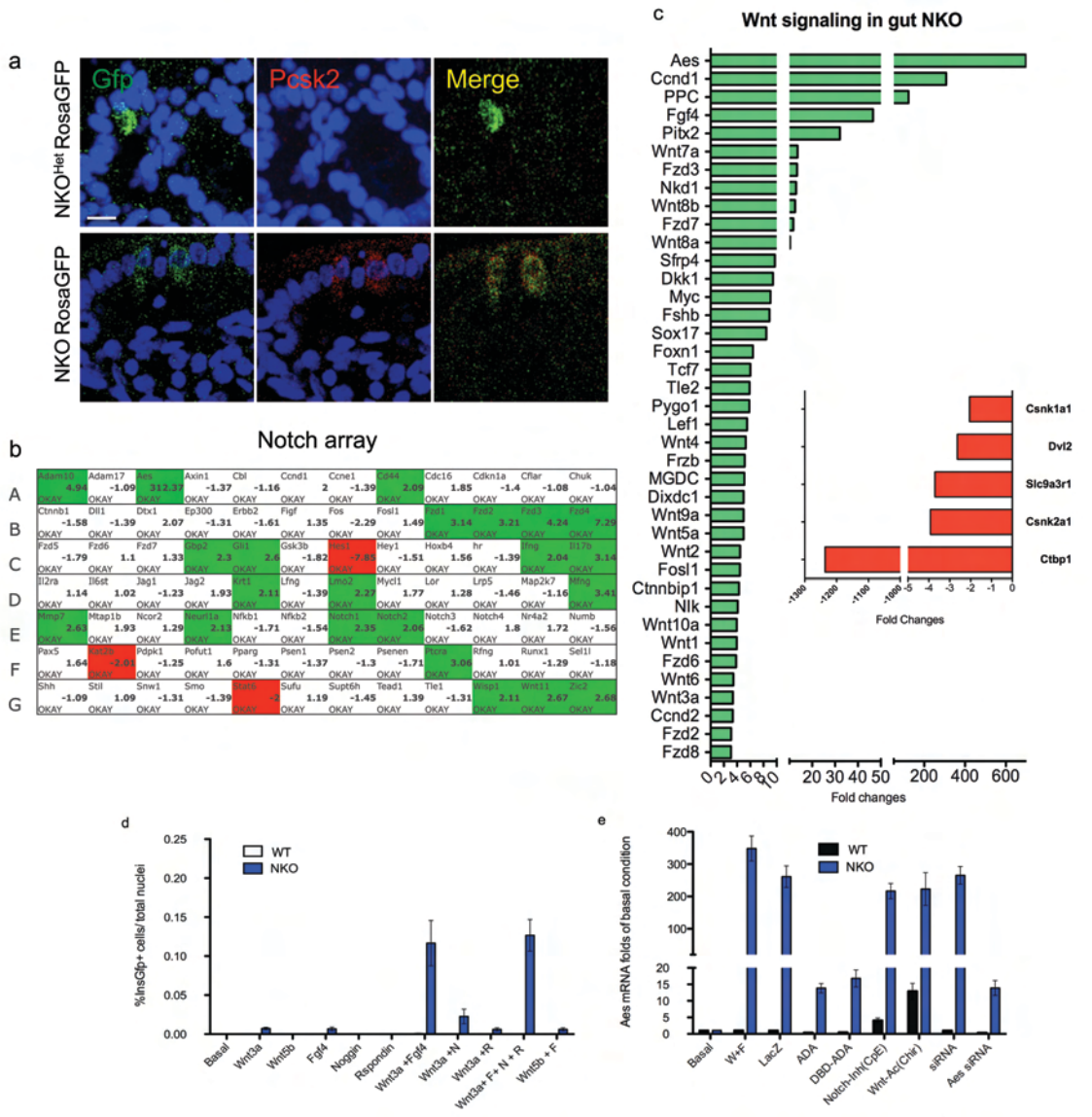


Supplementary Figure 4 Insulin, C-peptide, and Pcsk2 immunohistochemistry. **(a)**

Insulin immunohistochemistry in gut (left two panels) and islet (right two panels) of *NKO* intestine before (D0), and after STZ injection (D3 and D28) with anti-insulin (red) (D0 and D3) and anti-insulin and anti-Pc2 antibodies (merged yellow flourescence) (D28). (**b**) Immunofluorescence of anti C-peptide (red) after STZ injection day 3 (top) and day 28 (bottom). (**c-h**) metabolic profiles of WT (white bars) and NKO (blue bars) mice pre- and post-STZ treatment (**c**) fast and refed blood glucose levels, (**d**) body weight, (**e**) 12-hr-fasted serum insulin (**f**) fed serum insulin, (**g**) 12-hr-fasted serum C-peptide 2 (**h**) fed serum C-peptide 2. (**i**) survival plots of WT and *Foxo1* brain knock-out after STZ treatment (n=5). Scale bars: 30 μm (**a**), and 40 μm (**b**).

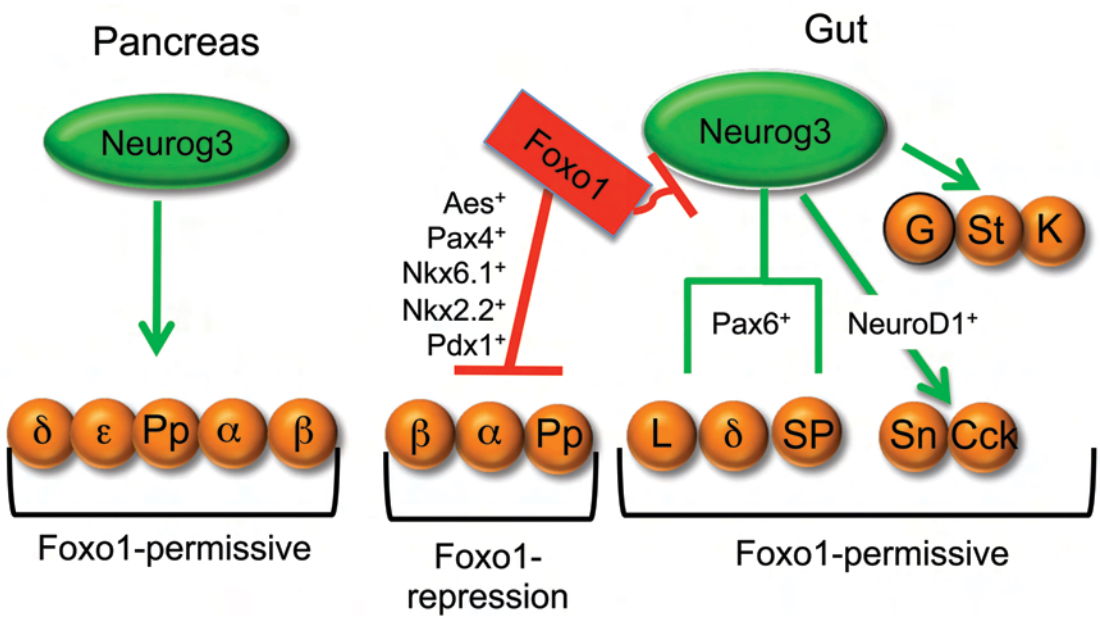


Supplementary Figure 5 Analysis of epithelial and mesenchymal markers. **(a-c)** Immunohistochemical analysis of expression of C-peptide (red) and mesenchymal markers Cd45R (**a**), smooth muscle actin (SMA)(**b**), or laminin (**c**) (green) in adult colon and distal ileum of *NKO* mice and WT controls. (**d-e**). Immunostaining with antibodies to pancreatic islet transcription factors MafA (**d**), and MafB (**e**). **(f)** qPCR analysis of mRNA expression of markers of β-cell differentiation in isolated gut epithelial cell preparations from DTZ-enriched segments in *NKO* mice (blue bars) and from anatomically matched segments in control mice (black bars) (n = 8). (**g**) Morphometric quantitation of gut serotonin$^+$ cells and cholecystokinin$^+$ cells from small intestines normalized by total number of ChgA$^+$ cells (n=3). * = $P < 0.05$, ** = $P < 0.01$.



Supplementary Figure 6 Lineage tracing and low-density array profiling. (**a**) Lineage tracing of gut Ins^{+} cells post-STZ in *NKO:Rosa26eGfp* mice using Gfp (green) or Pc2 (red). Scale bars: 20 μm. Gene transcript profiles of Notch signaling (**b**) and (**c**) Wnt signaling using low-density arrays with mRNA isolated from epithelial cells from DTZ-enriched gut segments. A green color indicates transcript levels increased > 2-fold in *NKO* compared to WT. A red color indicates transcript levels decreased > 2-fold in *NKO* compared to WT (p-value < 0.01). (**d**) Quantification of *Ins2-Gfp* differentiation assay of

primary gut culture: basal condition is without adding recombinant proteins, while other treatments include Wnt3a, Wnt5b, Fgf4, Noggin, R-spondin. (**e**) qPCR analysis of *Aes* mRNA isolated from *ex vivo* gut culture in Fig. 6f (n = 6).



Supplementary Figure 7 Model of Foxo1 function in endocrine differentiation. In the pancreas, Foxo1 is permissive for terminal differentiation of the endocrine lineage (α, β, δ, ε, and Ppy cells). In the gut, Foxo1 actively prevents generation of pancreas-like endocrine cells (β, α, and Ppy), but is permissive for the generation of enteroendocrine cells. G: gastrin, K: K cells, L: L cells, SP: substance P, Sn: secretin, St: serotonin, Cck: colecystokinin.