iScience, Volume *25*

Supplemental information

Pro-α-cell-derived β-cells contribute to β-cell neogenesis induced by antagonistic glucagon receptor antibody in type 2 diabetic mice

Xiaona Cui, Jin Feng, Tianjiao Wei, Liangbiao Gu, Dandan Wang, Shan Lang, Kun Yang, Jin Yang, Hai Yan, Rui Wei, and Tianpei Hong

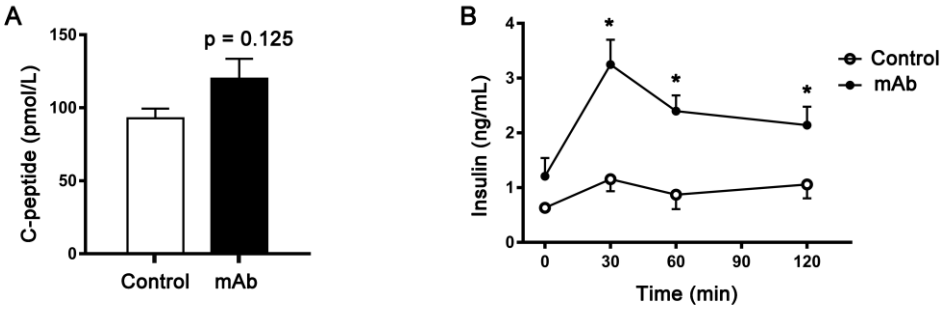

Figure S1. Plasma C-peptide and insulin levels in HFD + STZ-induced T2D mice treated with GCGR mAb or IgG control for 4 weeks, related to Figure 1.

(A) Fasting plasma C-peptide.

(B) Plasma insulin levels during the intraperitoneal glucose tolerance test.

n = 6 in control group and n = 9 in GCGR mAb group. Data represent the mean ± SEM. Statistical analysis was conducted by Student's *t*-test. *p < 0.05 *vs*. control.

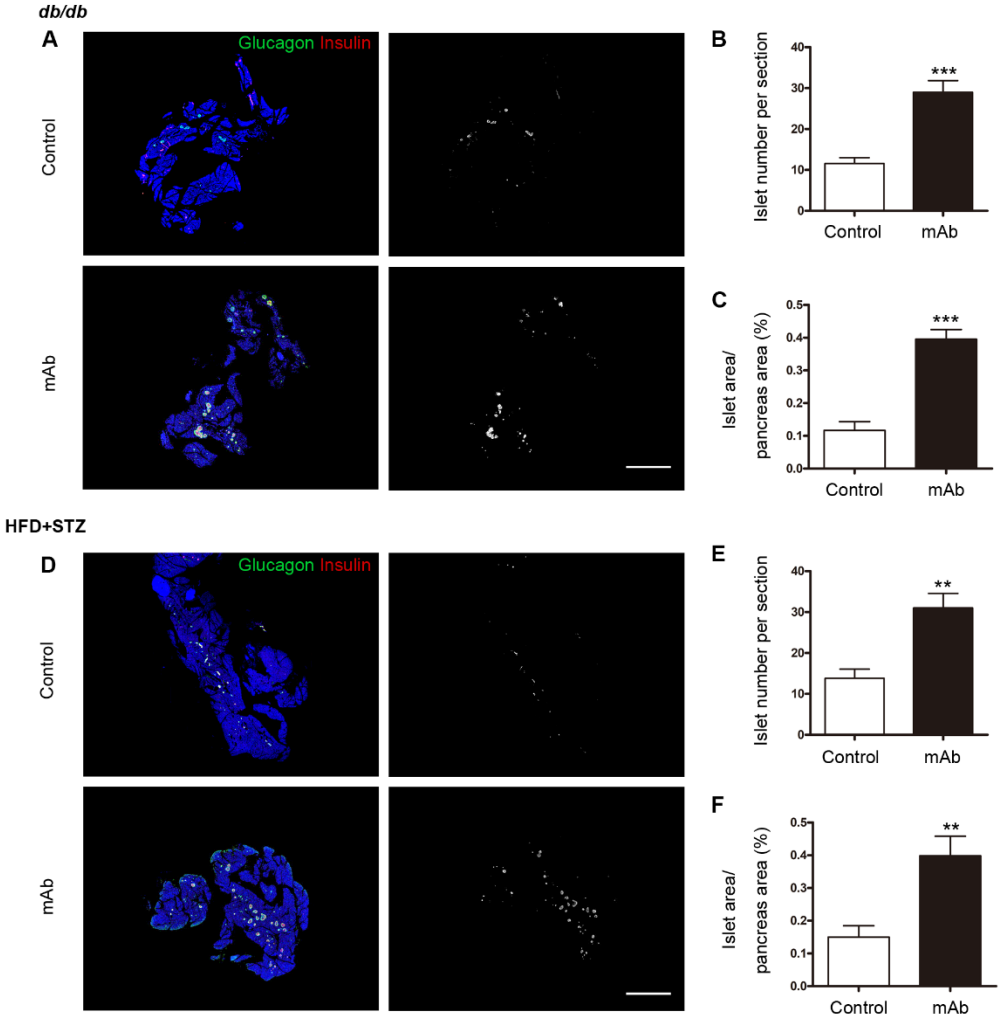

Figure S2. Histological analysis of the entire pancreata of two T2D mouse models treated with GCGR mAb or IgG control for 4 weeks, related to Figure 2.

(A, D) Left panels: representative images of the whole pancreata immunostained for glucagon (green) and insulin (red) in *db/db* mice (A) and HFD + STZ-induced T2D mice (D). Right panels: immunostaining in the same tissues as the left panels was transformed into monochrome images, and the cells immunolabeled positively for either glucagon or insulin are displayed in white. Scale bar = 2,000 μm.

(B, C) Quantification of the islet number (B) and islet area (C) per pancreatic section in *db/db* mice. n = 3 sections/mouse multiplied by 6 mice/group.

(E, F) Quantification of the islet number (E) and islet area (F) per pancreatic section in HFD + STZ-induced T2D mice. n = 3 sections/mouse multiplied by 9 mice/group.

Data represent the mean ± SEM. Statistical analysis was conducted by Student's *t*-test. **p < 0.01, ***p < 0.001 *vs.* control.

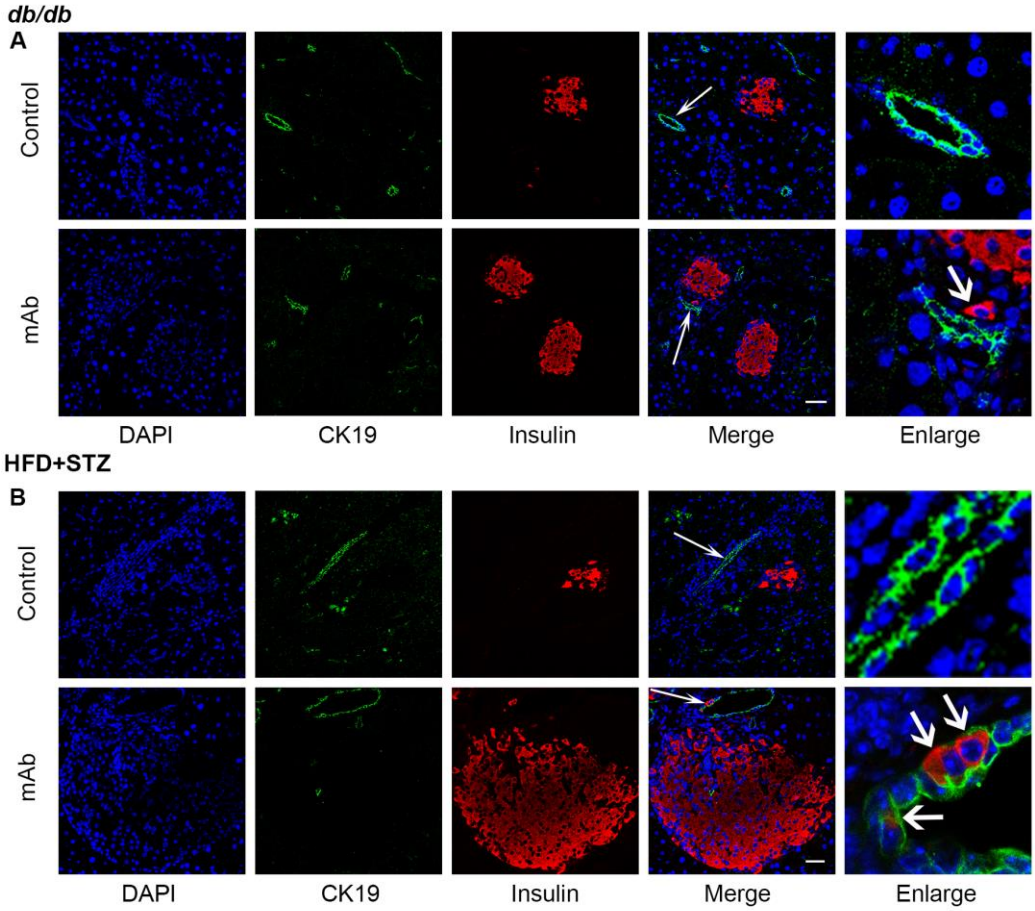

Figure S3. Immunofluorescent analysis of duct-derived β-cell neogenesis in the pancreatic tissues of two T2D mouse models treated with GCGR mAb or IgG control, related to Figure 3.

(A, B) Representative image of islets immunostained with cytokeratin 19 (CK19, a marker of mature duct cells) and insulin in the ductal region of *db/db* mice (A) and HFD + STZ-induced T2D mice (B). The cells indicated by the arrows are enlarged at right of the image. Scale bar = 50 μm.

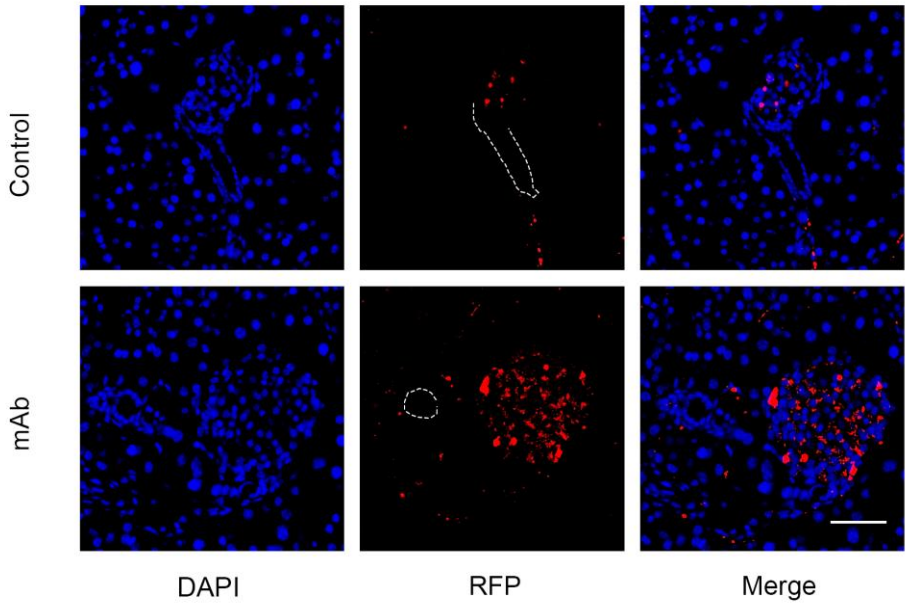

Figure S4. Immunofluorescent analysis of RFP (progenitor lineage-tracing marker) positive cells located within or near pancreatic ducts in the Ngn3^{+} cell lineage-tracing T2D mice treated with GCGR mAb or IgG control for 4 weeks, related to Figure 3.

The ductal lumen is outlined with dashed lines. Scale bar = 50 μm.

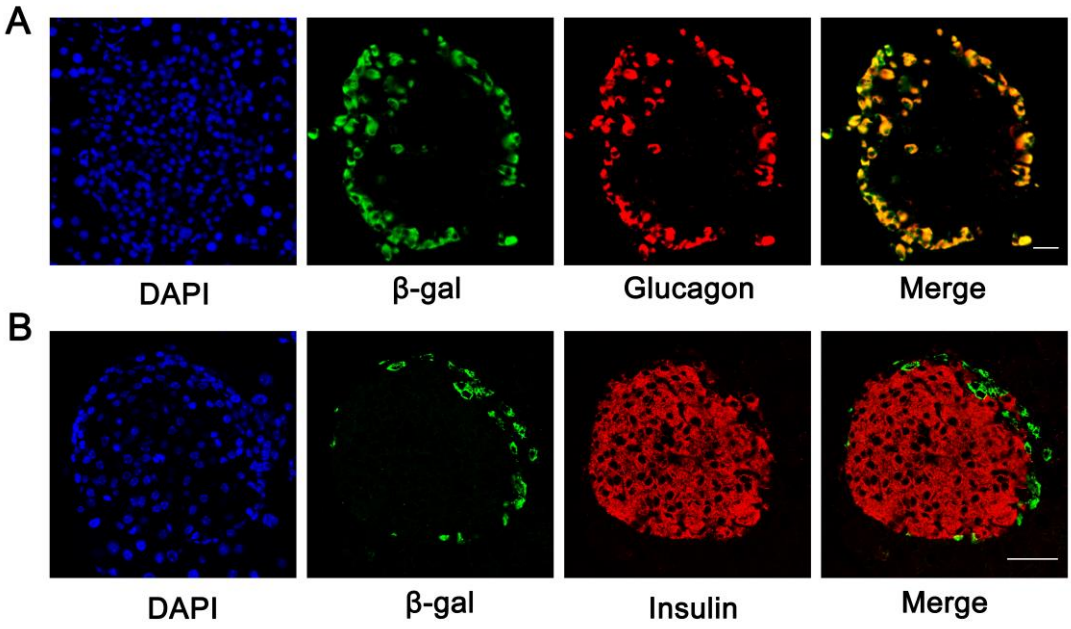

Figure S5. Identification of the tracing efficiency in the 3-month-old pancreatic α-cell lineage-tracing (*glucagon-cre-β-gal*) mice fed on normal diet, related to Figure 4 and 5.

(A) Representative image of an islet immunostained with β-gal (α-cell lineage-tracing marker) and glucagon.

(B) Representative image of an islet immunostained with β-gal and insulin.

Scale bar = 50 μm.

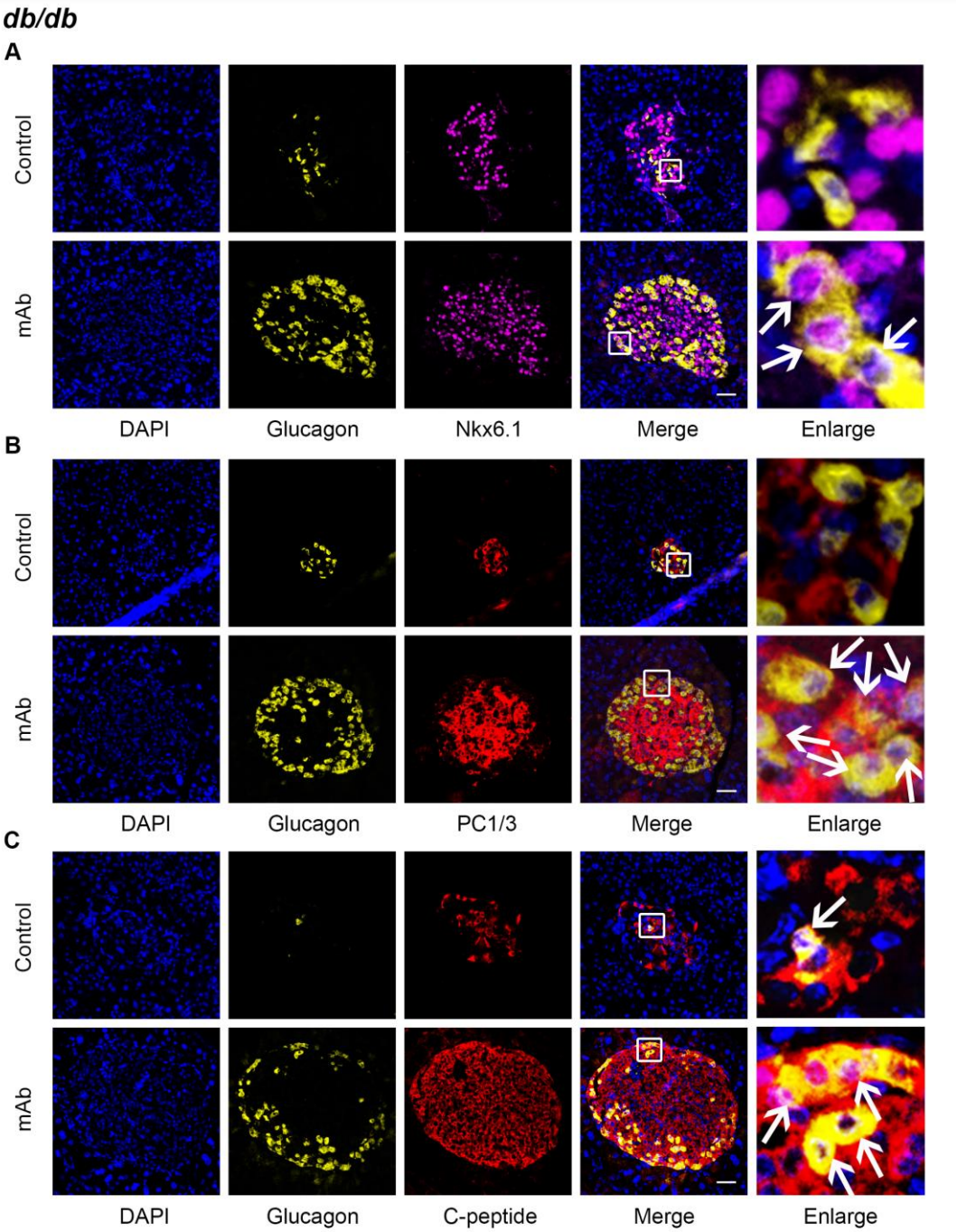

Figure S6. Immunofluorescent analysis of α-to-β cell conversion in the pancreatic tissues of *db/db* mice treated with GCGR mAb or IgG control for 4 weeks, related to Figure 5.

(A) Representative photograph of an islet immunolabeled with glucagon and Nkx6.1, a transcription factor that participates in β-cell development and function.

(B) Representative photograph of an islet immunolabeled with glucagon and PC1/3, an enzyme essential for processing proinsulin to insulin in β-cells.

(C) Representative photograph of an islet immunolabeled with glucagon and C-peptide.

The arrows indicate co-labeled cells. The cells in the small box are enlarged at right of the image. Scale bar = 50 μm.

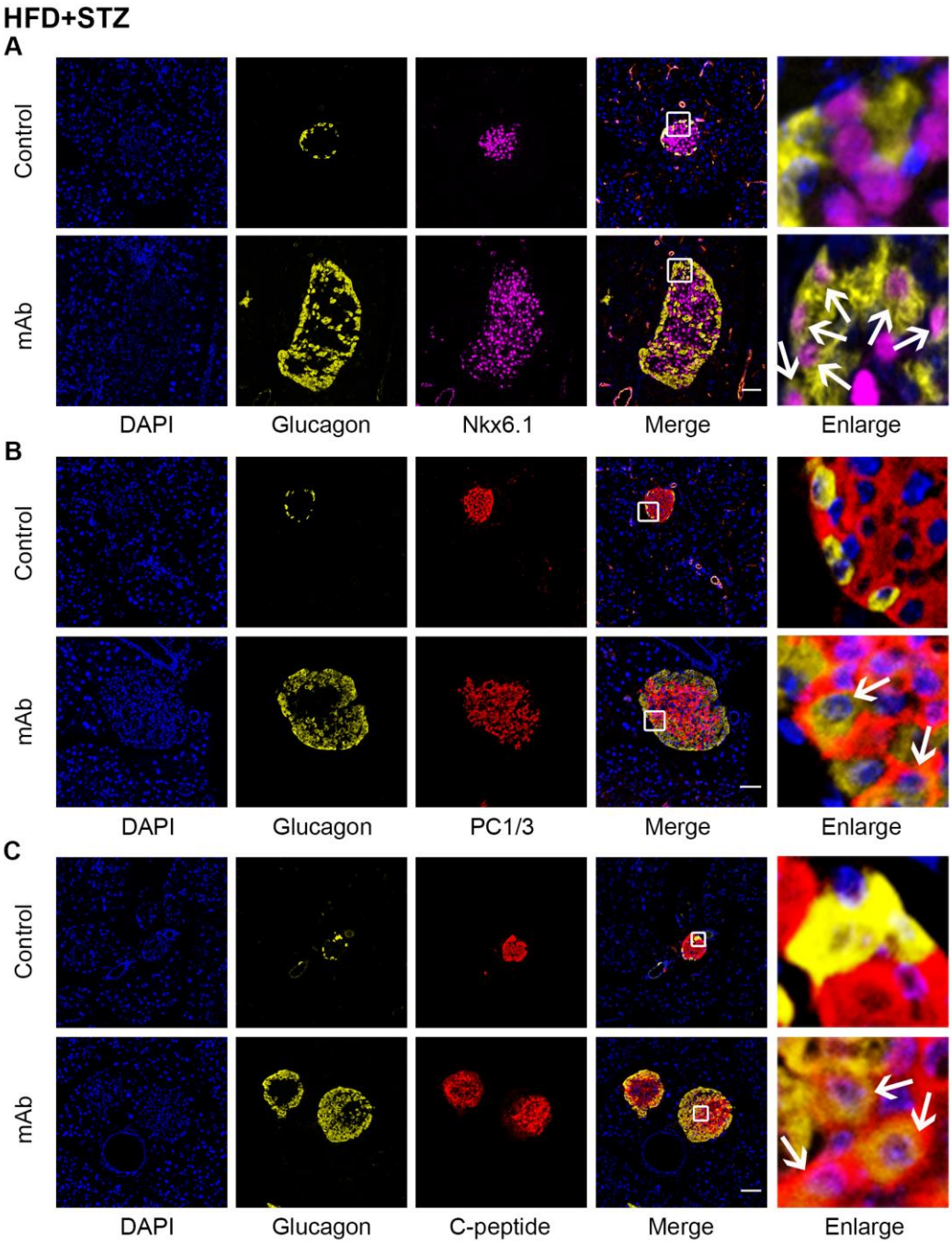

Figure S7. Immunofluorescent analysis of α-to-β cell conversion in the pancreatic tissues of HFD + STZ-induced T2D mice treated with GCGR mAb or IgG control for 4 weeks, related to Figure 5.

(A) Representative photograph of an islet immunolabeled with glucagon and Nkx6.1.

(B) Representative photograph of an islet immunolabeled with glucagon and PC1/3.

(C) Representative photograph of an islet immunolabeled with glucagon and C-peptide.

The arrows indicate co-labeled cells. The cells in the small box are enlarged at right of the image. Scale bar = 50 μm.

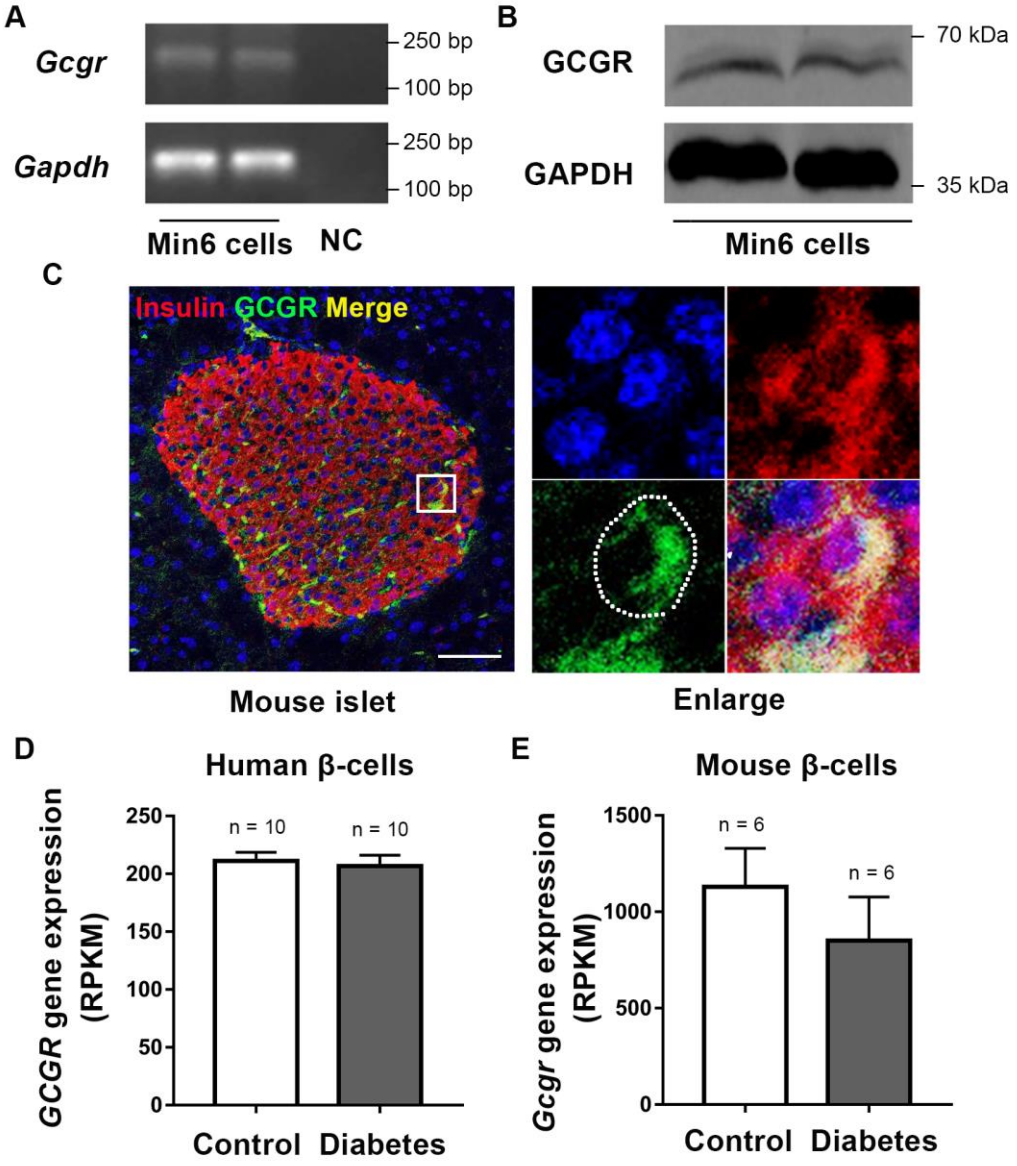

Figure S8. GCGR expression in pancreatic β-cells, related to Figure 6.

(A) The mRNA expression of *Gcgr* in the mouse pancreatic β-cell line Min6 cells was detected by RT-PCR. NC, negative control.

(B) The protein expression of GCGR in Min6 cells was detected by western blot.

(C) Representative image of an islet immunostained for GCGR and insulin in the normal control *db/m* mice. The cells in the small box are enlarged at right of the image. One co-labeled cell is outlined with dashed line. Scale bar = 50 μm.

(D, E) *GCGR* expression in human (D) and mouse (E) β-cells isolated from T2D and control pancreata. The original data were from the datasets GSE20966 (D) and GSE168743 (E). Data represent the mean ± SEM. Statistical analysis was conducted by Student's *t*-test.

Table S1. Quantitative RT-PCR primers used for mRNA expression analysis, related to STAR Methods.

Gene symbol	Gene name	Species	Gene ID	Primer sequences (5'-3')	Tm (˚C)	Product length (bp)
Gcg	glucagon	Mouse	14526	F: TTCATCTCATCAGGGTCCTC R: GCTTATAATGCTGGTGCAAG	60	114
Pcsk1	proprotein convertase subtilisin/kexin type 1	Mouse	18548	F: AGTTGGAGGCATAAGAATGCTG R: GCCTTCTGGGCTAGTCTGC	60	159
Pcsk2	proprotein convertase subtilisin/kexin type 2	Mouse	18549	F: GTGTGATGGTTTTTGCGTCTG R: GGGAGCTTTCGGACTCCAA	59	130
Ins1	insulin I	Mouse	16333	F: TAGTGACCAGCTATAATCAGAG R: ACGCCAAGGTCTGAAGGTCC	62	289
Ins2	insulin II	Mouse	16334	F: CCCTGCTGGCCCTGCTCTT R: AGGTCTGAAGGTCACCTGCT	60	213
Pdx1	pancreatic and duodenal homeobox 1	Mouse	18609	F: AGGAAAACAAGAGGACCCGT R: CTTCATGCGACGGTTTTGGA	59	170
Ngn3	neurogenin 3	Mouse	11925	F: GTCGGGAGAACTAGGATGGC R: GGAGCAGTCCCTAGGTATG	56	156
Actb	actin, beta	Mouse	11461	F: TGTACCCAGGCATTGCTGAC R: CTGCTGGAAGGTGGACAGTG	60	149
Gcgr	glucagon receptor	Mouse	14527	F: ATTGGCGATGACCTCAGTGTGA R: GCAATAGTTGGCTATGATGCCG	60	105
Gapdh	glyceraldehyde-3-phosphate dehydrogenase	Mouse	14433	F: TGCACCACCAACTGCTTAGC R: GGCATGGACTGTGGTCATGAG	61	87

Abbreviations: Tm, temperature; bp, base pair.