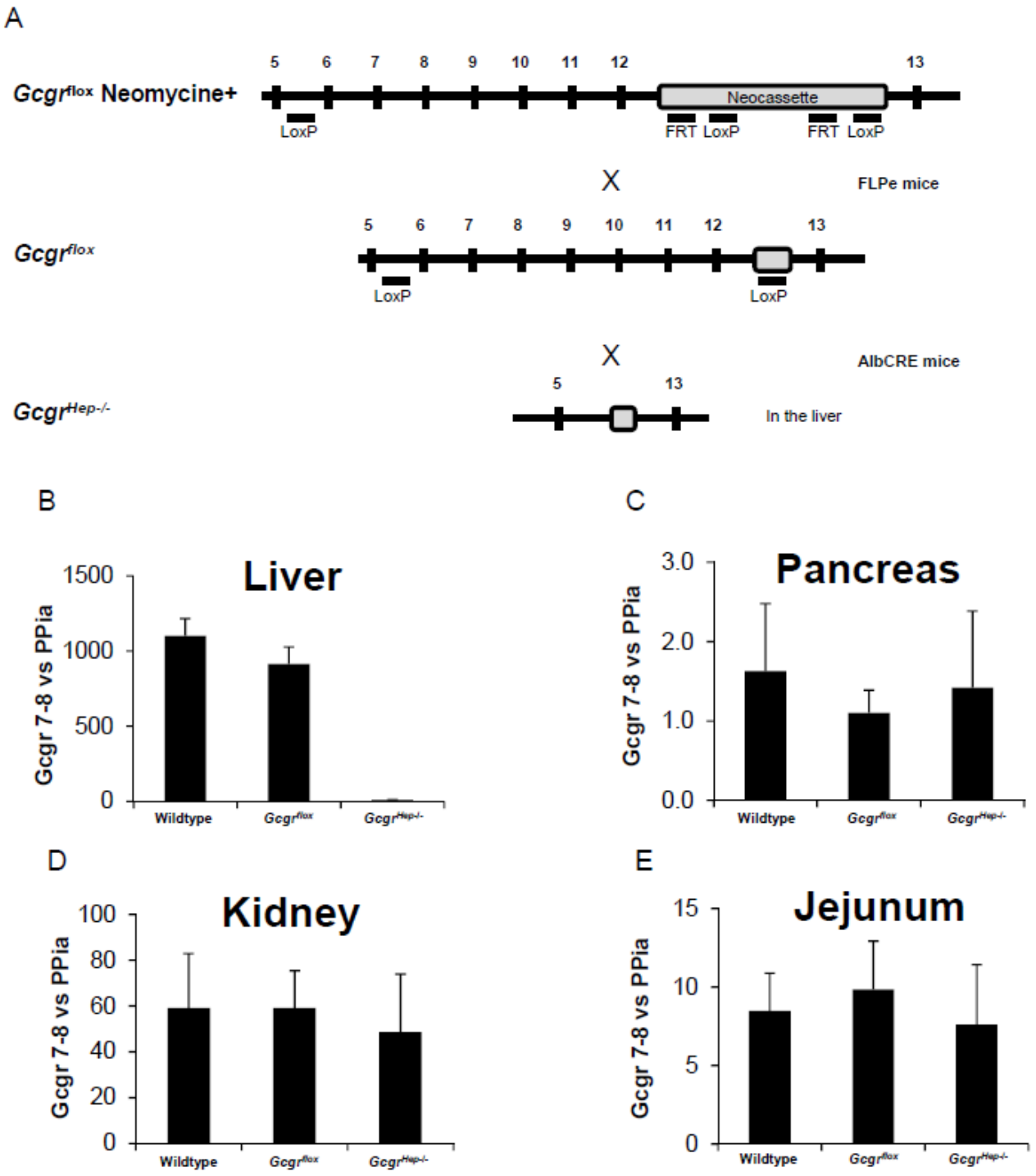
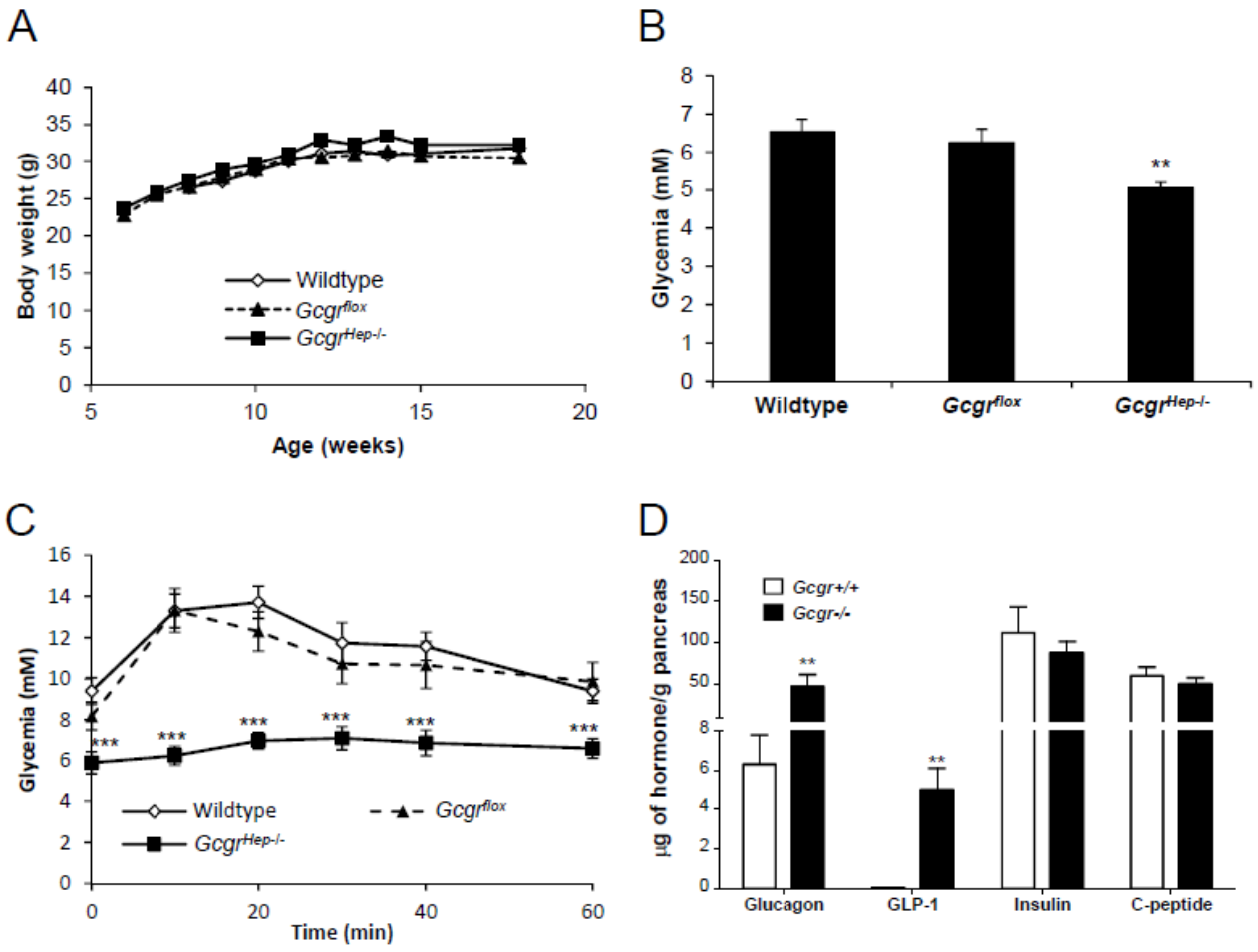
SUPPLEMENTARY DATA

Supplementary Figure 1. Generation of *Gcgr*^{Hep}-/- mice and *Gcgr* expression. (A) *Gcgr*^{flox} mice positive for the neomycin cassette were mated with FLPe mice to remove the neomycin cassette. The resulting *Gcgr*^{flox} mice were then mated to AlbCRE mice to generate *Gcgr*^{Hep}-/- mice. (B-E) Relative *Gcgr* expression assessed in liver (B), pancreas (C), kidney (D) and jejunum (E) of *Gcgr*^{Hep}-/- males and littermate controls using real time PCR amplifying a region spanning exon 7 and 8 of the *Gcgr* mRNA. *Ppia* was used as an internal control.



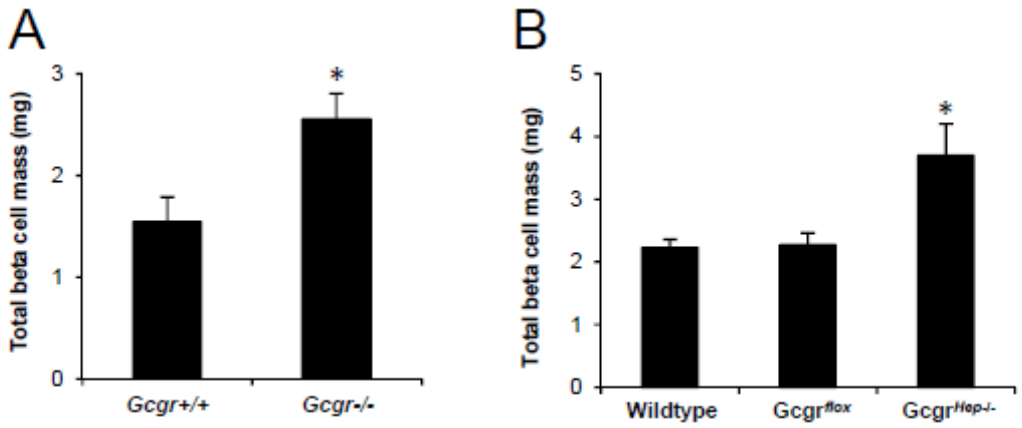
SUPPLEMENTARY DATA

Supplementary Figure 2. Ablation of the hepatic Gcgr does not alter body weight, but leads to mild hypoglycemia and glucagon unresponsiveness. (A) Body weight measured in ad-libitum fed *Gcgr*^{Hep}-/- males and littermate controls (n=8-10 mice). (B) Glycemia in random fed 20 week old *Gcgr*^{Hep}-/- males and littermate controls (n=10-12 mice per group). (C) Intraperitoneal glucagon challenge in 12 week old *Gcgr*^{Hep}-/- males and littermate controls (n=10-12 mice per group). (D) Pancreatic hormone content from 22 week old Gcgr-/-and littermate control mice corrected for pancreas mass (n=3-6 per group). Data are mean + SEM. ** p<0.01 and *** p<0.001 vs. WT mice.

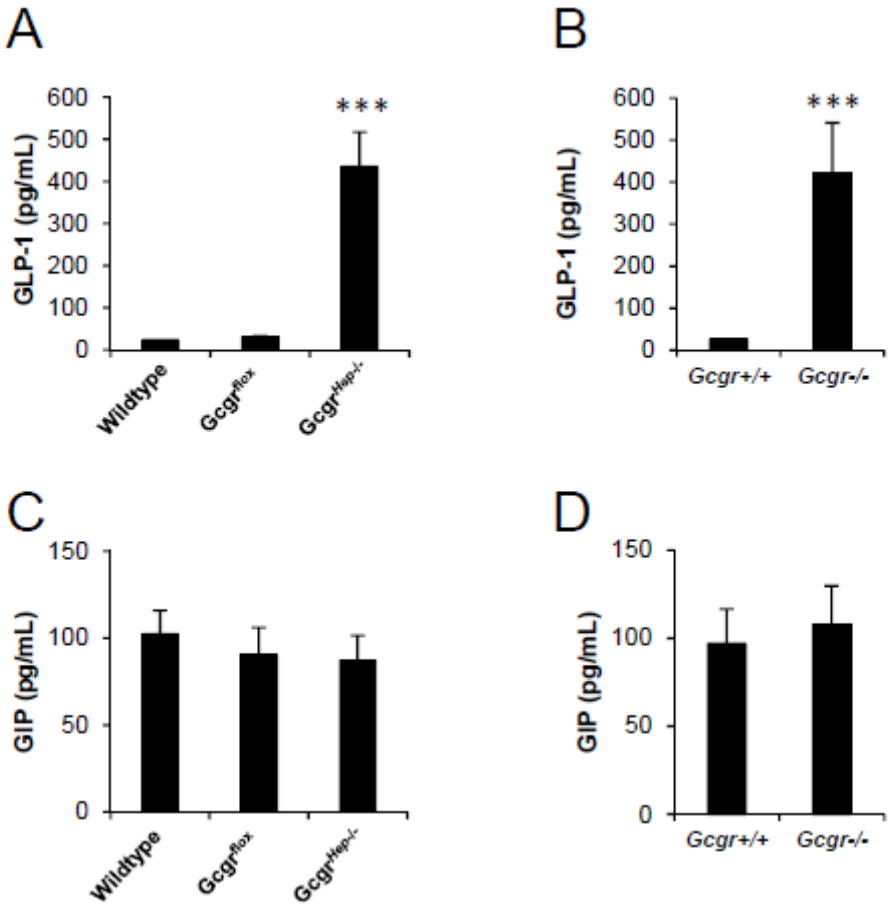


SUPPLEMENTARY DATA

Supplementary Figure 3. Absolute ß-cell mass is increased in Gcgr-/-and *Gcgr*^{Hep}-/- mice vs littermate controls. ß-cell mass per pancreas of 20 week-old Gcgr-/-males (A) and *Gcgr*^{Hep}-/- (B) and littermate controls (n=8-10 mice). Data are mean + SEM. * p<0.05 vs. WT.

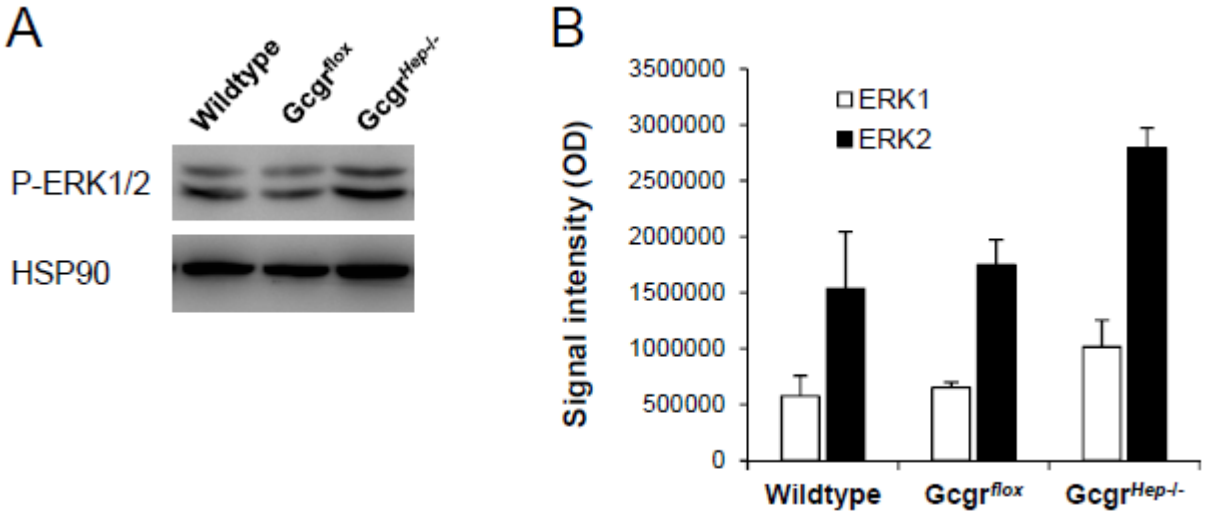


Supplementary Figure 4. Incretin plasma levels in Gcgr-/-and *Gcgr*^{Hep}-/- mice. Plasma GLP-1 levels (A and B) and GIP (C and D) were measured in random fed 20 weeks old *Gcgr*^{Hep}-/- (A and C) and Gcgr-/-(B and D) males and littermate controls. (n=8-10 mice). Data are mean + SEM. *** p<0.001 vs. WT.



SUPPLEMENTARY DATA

Supplementary Figure 5. ERK1/2 activation in *Gcgr*^{Hep}-/- mice. (A) Active forms of phosphorylated ERK1/2 (top panel) and HSP90 used as a loading control (bottom panel) were detected in liver from *Gcgr*^{Hep}-/- mice and littermate controls fasted overnight. Total liver protein lysates were subjected to western blot analysis as described in methods. A representative blot of 4 independent experiments is shown (A) and densitometry for all 4 experiments is shown in (B). Signal intensity of active ERK1/2 was measured as described in methods.



SUPPLEMENTARY DATA

Supplementary Figure 6. IL6, SDF-1 and cholic acid are not responsible for alpha cell hyperplasia. (A-B) IL6 plasma levels in random fed 20 weeks old GcgrHep-/-(A) and Gcgr-/-(B). (C-D) SDF-1 plasma levels in random fed 20 weeks old GcgrHep-/- (C) and Gcgr-/-(D) and littermate controls. (E-F) Total plasma bile acid levels in random fed 20 weeks old GcgrHep-/-(E) and Gcgr-/-(F) and and littermate controls. (G-M) 12 weeks old wildtype males fed for 4.5 weeks a diet enriched with 1% cholic acid. Mice were euthanized random fed and total plasma bile acid levels (G), glycemia (H), insulin (I), pancreas weight (J), and glucagon (K) were measured. Pancreas were collected for immunostaining for insulin and glucagon (L-M). (n=8-10 mice). Data are mean + SEM. *, p<0.05, **, p<0.01, *** p<0.001 vs. WT or control diet.