Supplementary Figure 1. Ghrelin-secreting endocrine cell numbers are unaltered, but ghrelin mRNA expression is higher within the gastric mucosa of Gcgr\textsuperscript{-/-} mice. (A) Representative images demonstrate ghrelin immunoreactivity in the corpus region of the stomach in Gcgr\textsuperscript{+/+} (top) and Gcgr\textsuperscript{-/-} (bottom) mice. Scale bar = 100 µm. Ghrelin immunoreactivity was detected with goat polyclonal anti-ghrelin primary antibody (Santa Cruz Biotechnology, Dallas, TX; 1:1000) and Alexa Fluor 594\textsuperscript{®} donkey anti-Goat IgG secondary antibody (Jackson ImmunoResearch, West Grove, PA; 1:300). B. Mean ghrelin-immunoreactive cell numbers in the corpus and antrum regions of the stomachs of Gcgr\textsuperscript{+/+} and Gcgr\textsuperscript{-/-} mice (n = 3 mice each). For each mouse, the average numbers were obtained by counting the immunopositive cells in 3 sections along the stomach region at 10X magnification (3 fields for each section). No significant changes in ghrelin cell numbers were observed between the two groups either in the corpus or antrum region of the stomach. Quantitative estimation of relative preproglucagon (C; Gcg), preproghrelin (D; Ghrl), Insulin I (E; Ins1) and Insulin II (F; Ins2) mRNA levels in gastric mucosal cell lysates from Gcgr\textsuperscript{+/+} and Gcgr\textsuperscript{-/-} mice (n=6-11). Numbers within each bar denote the respective threshold cycle (Ct) values. †p=0.06, significant statistical trend, when analyzed by Student’s unpaired “t” test. n.s = no statistical difference. n=6-12. Values are represented as mean ± SEM.
Supplementary Figure 2. Ghrelin mRNA expression is unaltered in pancreas of Gcgr⁻/⁻ mice. Quantitative estimation of relative preproglucagon (A; Gcg), preproghrelin (B; Ghrl), Insulin I (C; Ins1) and Insulin II (D; Ins2) mRNA levels in whole pancreas from Gcgr⁺/⁺ or Gcgr⁻/⁻ mice (n=3–8). Numbers within or above each bar denote the respective threshold cycle (Ct) values. ***p<0.005, significant difference, when analyzed by Student’s unpaired “t” test. n.s = no statistical difference. n=3–8. Values are represented as mean ± SEM.

Supplementary Figure 3. Glucagon does not alter acyl-ghrelin secretion in primary gastric mucosal cell cultures. Acyl-ghrelin concentrations in cell culture media after 6h treatment with different concentrations of glucagon, all in the presence of with 5mM glucose. Data were normalized to mean acyl-ghrelin levels after 6h treatment with vehicle. Results were obtained from n=6 wells, from 2 independent experiments. Glucagon treatment did not induce significant change in ghrelin secretion, when analyzed by one-way ANOVA test. Values are represented as mean ± SEM.
Supplementary Figure 4. Streptozotocin treatment reduces pancreatic islet β-cell populations and plasma insulin in both Gcgr<sup>−/−</sup> and Gcgr<sup>+/+</sup> mice. (A) Representative images demonstrate insulin (red) and glucagon (green) immunoreactivity in the pancreatic islets of Gcgr<sup>−/−</sup> (left panels) and Gcgr<sup>+/+</sup> (right panels) mice without STZ treatment (-STZ; top panels) or 18 days after STZ treatment (+STZ; bottom panels). Although the islet sizes of STZ-treated Gcgr<sup>+/+</sup>mice were mostly smaller than those of STZ-treated Gcgr<sup>−/−</sup>mice, reduced numbers of insulin-immunoreactive cells were observed in both groups. Glucagon and insulin immunoreactivity was detected with rabbit anti-Glucagon (Millipore, Temecula, CA; 1:300) and guinea pig anti-Insulin (DakoCytomation, Carpinteria, CA; 1:300) primary antibodies and TRITC-conjugated goat anti-Guinea pig and FITC-conjugated donkey anti-Rabbit secondary antibodies (both from Jackson ImmunoResearch; 1:300). Nuclei were stained blue by using Vectashield mounting medium containing DAPI (Vector Laboratories, Burlingame, CA). Scale bar = 50 µm. B. Plasma insulin in Gcgr<sup>−/−</sup> mice and Gcgr<sup>+/+</sup> mice before and 7 days after STZ administration. Data analyzed by repeated measures 2-way ANOVA followed by Tukey’s post hoc analysis. ****p<0.001, significant fall in plasma insulin after STZ treatment in both the genotypes. No significant difference was observed in the magnitude of falls in plasma insulin levels between the two genotypes after STZ treatment. n=11-22. Values are represented as mean ± SEM.
Supplementary Figure 5. Plasma insulin, body weight and food intake in db/db mice after GcgR blockade with GcgR mAb. (A) Plasma insulin in db/db mice measured 5 days after single administration of GcgR mAb B or vehicle (control). Body weights (B) and 24h food intake (C) measured 8 days after a single administration of GcgR mAb B. The 24h food intake normalized to body weight presented in D. *p<0.05, ***p<0.005, significant difference in body weight and food intake with GcgR mAb B as compared with vehicle. Data analyzed by Student’s unpaired “t” test. n.s = no statistical difference. n=6 in each group. Values are expressed as mean ± SEM.
Supplementary Figure 6. Pair-feeding does not influence blood glucose or plasma acyl-ghrelin in GcgR mAb B-treated db/db mice, but restricts body weight gain. Blood glucose (A), plasma acyl-ghrelin (B), body weight (C), and food intake (D) measured in pair-fed 9-wk old db/db mice 5 days after last treatment with vehicle as compared to that in ad lib-fed 9 wk-old db/db mice 5 days after last treatment with mAb B to block GcgR function. ***p<0.005, ****p<0.001, significant difference in blood glucose and plasma acyl-ghrelin in pair fed db/db mice administered vehicle vs. ad lib-fed db/db mice administered mAb B, when analyzed by Student’s unpaired “t” test. n.s- no significant difference. n= 6 in each group. Values are expressed as mean ± SEM.
SUPPLEMENTARY DATA

Supplementary Figure 7. Pharmacological blockade of GHSR receptors in non-STZ-treated Gcgr<sup>-/-</sup> mice increases susceptibility to hypoglycemia during fasting. Blood glucose levels measured from tail vein in the Gcgr<sup>-/-</sup> mice 2h following administration of the last dose of [D-Lys<sup>3</sup>]-GHRP-6 or vehicle (saline), by which time the animals had been fasted 14h. n=6 in each group. **p<0.01 significant lower blood glucose levels with ([D-Lys<sup>3</sup>]-GHRP-6 treatment compared to vehicle treatment. Data analyzed by Student’s unpaired “t” test. Values are expressed as mean ± SEM.

Supplementary Figure 8. Treatment with GcgR mAb B reduces blood glucose in both wild-type and Ghsr-null mice; blood glucose falls further in fasted Ghsr-null mice. Fed blood glucose (A) in STZ-administered wild-type and Ghsr-null mice treated with GcgR mAb B or vehicle, measured on day 4 after treatment. Corresponding body weights (B) and 24h food intake (C). *p<0.05, ****p<0.001, Significant differences in parameters due to treatment or genotype. No differences in body weight was observed among the treatment groups. n.s- no significant difference. Data analyzed by repeated measures 2-way ANOVA followed by Tukey’s post hoc analysis. n= 5-6. Values are expressed as mean ± SEM.
Supplementary Figure 9. Primary gastric mucosal cell cultures from STZ-treated mice are sensitive to insulin and 2-deoxy-D-glucose but not growth hormone; STZ does not influence ghrelin secretion in primary gastric mucosal cell cultures from wild-type mice. (A) Effect of insulin on acyl-ghrelin secretion from primary gastric mucosal cells derived from STZ-treated wild-type mice in different concentrations of glucose. Effect of 10 mM 2-deoxy-D-glucose (B) and 10 μM growth hormone (C) on acyl-ghrelin secretion in 5 mM glucose concentration in primary cultures derived from STZ-treated wild-type mice. D. Effect of 100 μM STZ on acyl-ghrelin secretion in primary cultures derived from wild-type mice. Data analyzed by repeated measures 2-way ANOVA followed by Tukey’s post hoc analysis or Student’s unpaired “t” test. n.s- no significant difference. n= 6-9. Values are expressed as mean ± SEM.
Supplementary Table 1. Primers used in quantitative RT-PCR assays.

| Gene                              | Amplicon Length (bps) | Probe ID               |
|-----------------------------------|-----------------------|------------------------|
| Preproglucagon (Gcg)              | 85                    | Mm00801714_m1          |
| Preproghrelin (Ghrl),             | 104                   | Mm00612524_m1          |
| Insulin I (Ins1)                  | 80                    | Mm01950294_s1          |
| Insulin II (Ins2)                 | 99                    | Mm00731595_gH          |
| 18S ribosomal RNA (Rn18s/Rn45s)  | 115                   | Mm04277571_s1          |