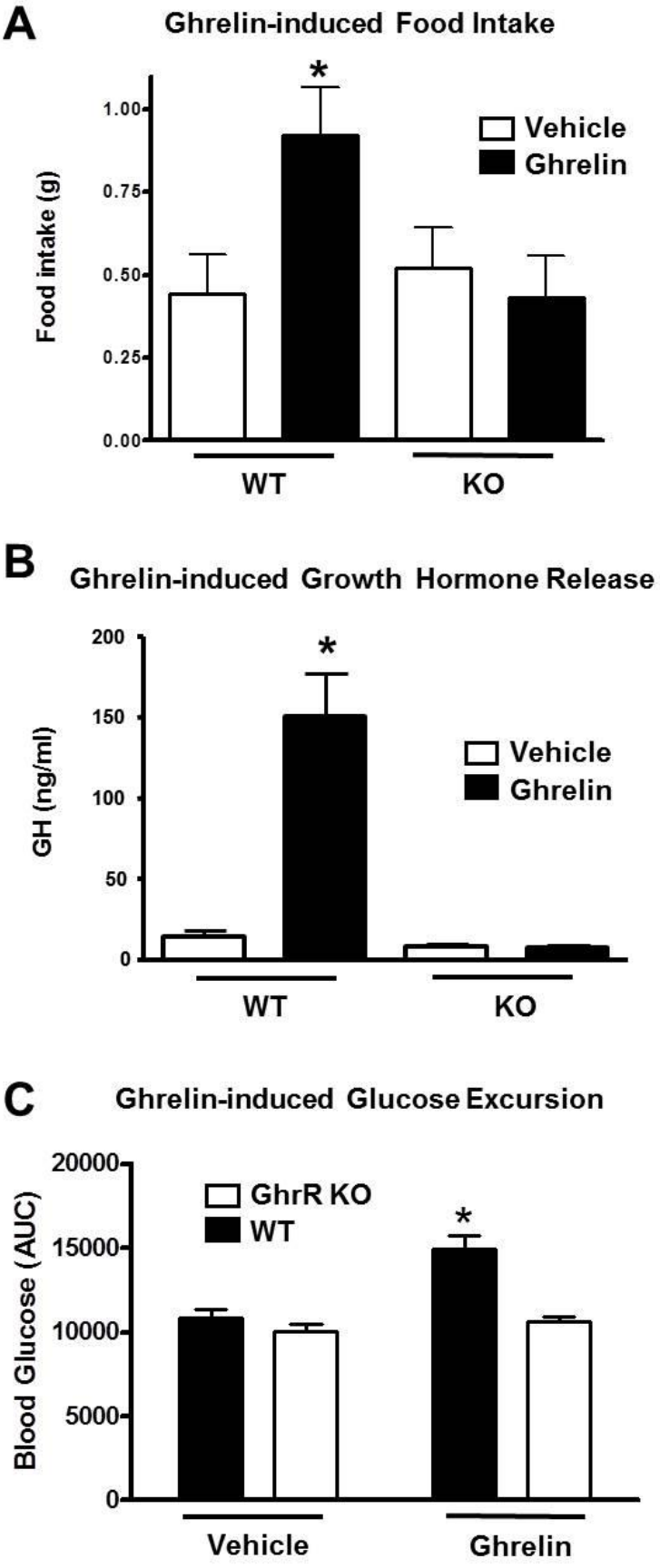
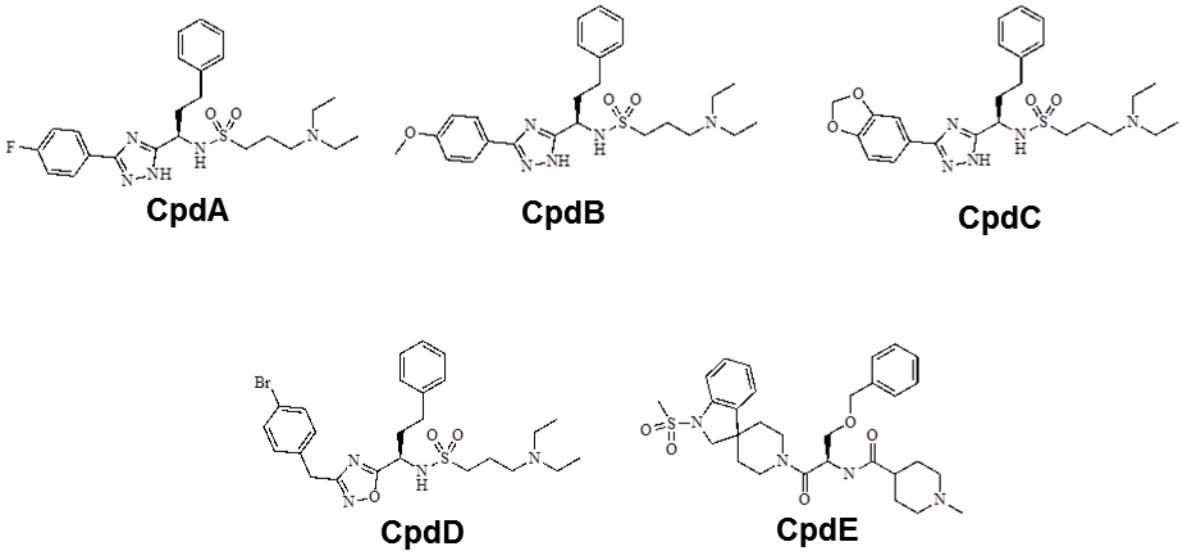
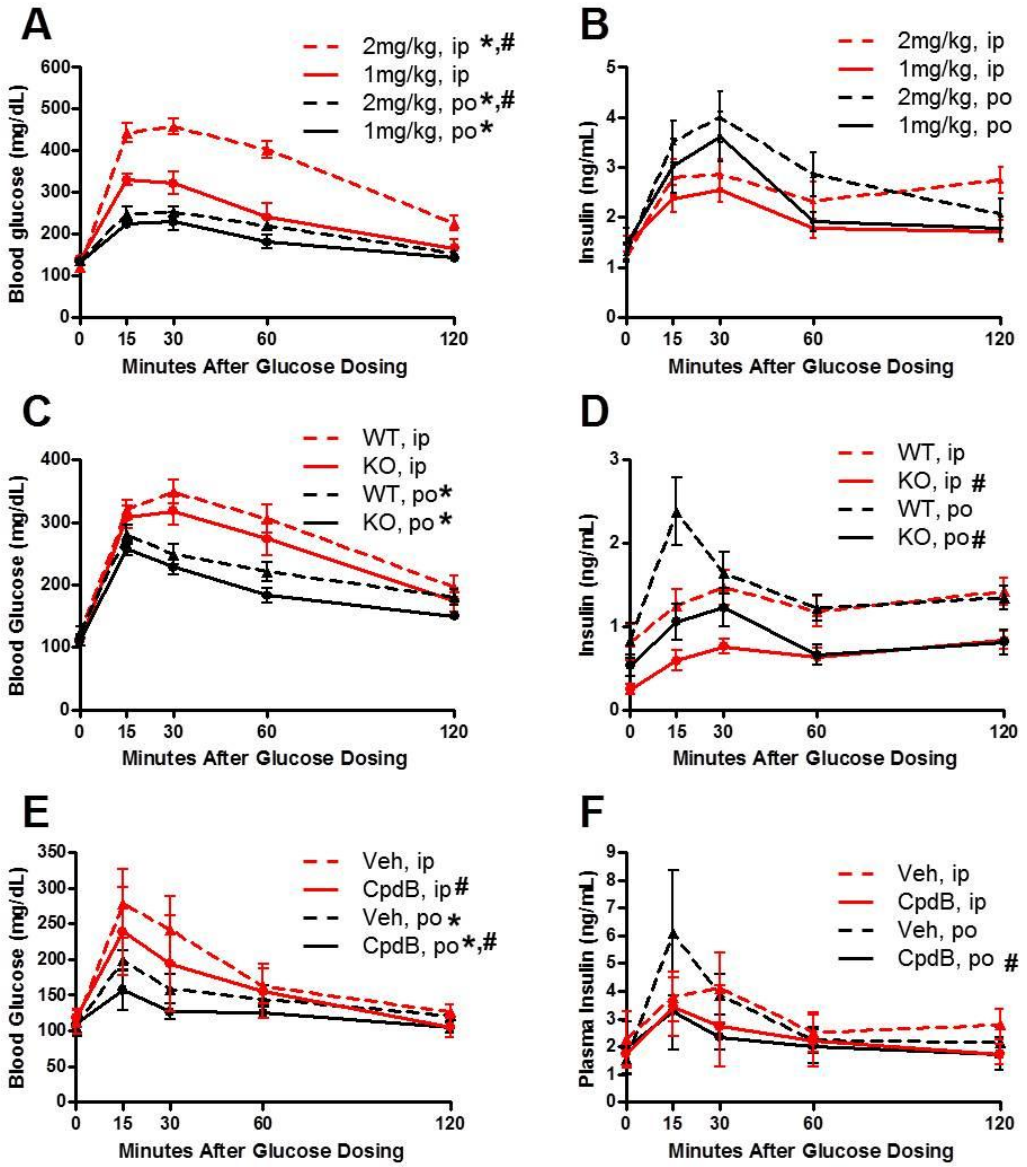


Supplemental Figure 1. Ghrelin is unable to stimulate FI, growth hormone release or insulin resistance in GhrR KO mice. GhrR KO and WT mice (n=12/group, **A**; n=6/group, **B**; n=8/group, **C**) were dosed with either vehicle (PBS) or ghrelin (5 mg/Kg, ip). **(A)** Mice were dosed at 09:00 and FI was measured at 4 hr. Ghrelin stimulated a significant (*, $p<0.05$) increase in FI in WT, but not GhrR KO mice. **(B)** Mice were anesthetized by ip injection of ketamine/xylazine and maintained at 37^{o}C on an electric warming blanket. Five min after complete anesthesia was verified a baseline blood sample was drawn from a tail nick followed by an iv injection of ghrelin. Tail vein blood was drawn 30 min post injection and samples were analyzed for growth hormone content by ELISA. Ghrelin stimulated a significant (*, $p<0.05$) increase in GH release in WT, but not GhrR KO mice. **(C)** HFD-fed GhrR KO and WT mice were fasted overnight (16 hr) prior to the experiment. The morning of the experiment a baseline blood draw was removed by tail nick. Thirty min later mice received vehicle or ghrelin (ip), followed 1 min later with an oral dose of glucose (1.5 mg/Kg). Blood glucose data are presented as an AUC generated from samples obtained at 15, 30, and 60 min after the oral glucose load. Ghrelin treatment resulted in a significant (*, $p<0.05$) impairment of glucose disposal in WT, but not GhrR KO mice.



Supplemental Figure 2: Chemical structures of ghrelin receptor antagonists.



Supplemental Figure 3: Investigation of the incretin effect in animals with genetic or pharmacologic blockade of GhrR signaling. In these studies, the 'incretin effect' is defined as the observed relative increase in glucose-stimulated insulin release following oral vs systemic (eg, ip or iv) glucose load. This is thought to result from the ability of oral, and not systemic, glucose to stimulate secretion of GLP-1. GLP-1 release leads to enhanced insulin secretion and thus improved glucose disposal (reviewed in Pratley & Gilbert, 2008). In all graphs the data are presented as absolute levels over time; for statistical comparison AUC data were analyzed with a two-way analysis of variance (ANOVA) and Bonferroni post-hoc test.

A,B) Preliminary assessment of the incretin effect in HFD-fed C57Bl/6 mice. Effects of intraperitoneal and oral glucose loads of either 1 g/Kg or 2 g/Kg on blood glucose and plasma insulin levels (n=8/group). In this study we found that oral glucose dosing

improved glucose disposal (A). However, although there was also a slight increase in insulin levels in oral-dosed glucose groups relative to their ip-dosed comparison groups, the increases were not statistically significant. (*, significant effect of dose route, $p<0.05$; #, significant effect of dose, $p<0.05$)

C,D) Assessment of incretin effect in HFD-fed GhrR KO and WT mice. Effects of 2 g/Kg intraperitoneal and oral glucose loads on blood glucose and plasma insulin levels (n=8/group). In this study we found that in both KO and WT mice, oral dosing of glucose resulted in lower blood glucose exposures (C). The relative improvement in glucose disposal in GhrRKO mice in both dose groups was not statistically significant. Interestingly, in both ip and oral glucose dosed groups, GhrR KO mice had reduced insulin excursions relative to their WT controls (D). (*, significant effect of dose route, $p<0.05$; #, significant effect of genotype, $p<0.05$)

E,F) Assessment of the incretin effect in C57Bl/6 mice treated for 28 days with CpdB (60mg/Kg, po, bid). In mice (n=8/group) treated with either vehicle or CpdB, the glucose excursion was significantly reduced in the oral glucose compared with the ip glucose group. Additionally, treatment of mice with CpdB significantly reduced the glucose excursion, regardless of how glucose was delivered. As seen in the GhrR KO mice, we were not able to detect significant changes in the insulin levels following oral vs ip dosed glucose challenge, although there was a trend observed for the vehicle-treated mice. As observed in other studies, treatment of mice with CpdB significantly reduced the insulin required for glucose disposal following in response to an oral glucose challenge. (*, significant effect of dose route, $p<0.05$; #, significant effect of treatment, $p<0.05$).

Reference:

Pratley RE, Gilbert M. Targeting Incretins in Type 2 Diabetes: Role of GLP-1 Receptor Agonists and DPP-4 Inhibitors, Rev Diabet Stud. 2008 Summer;5(2):73-94.

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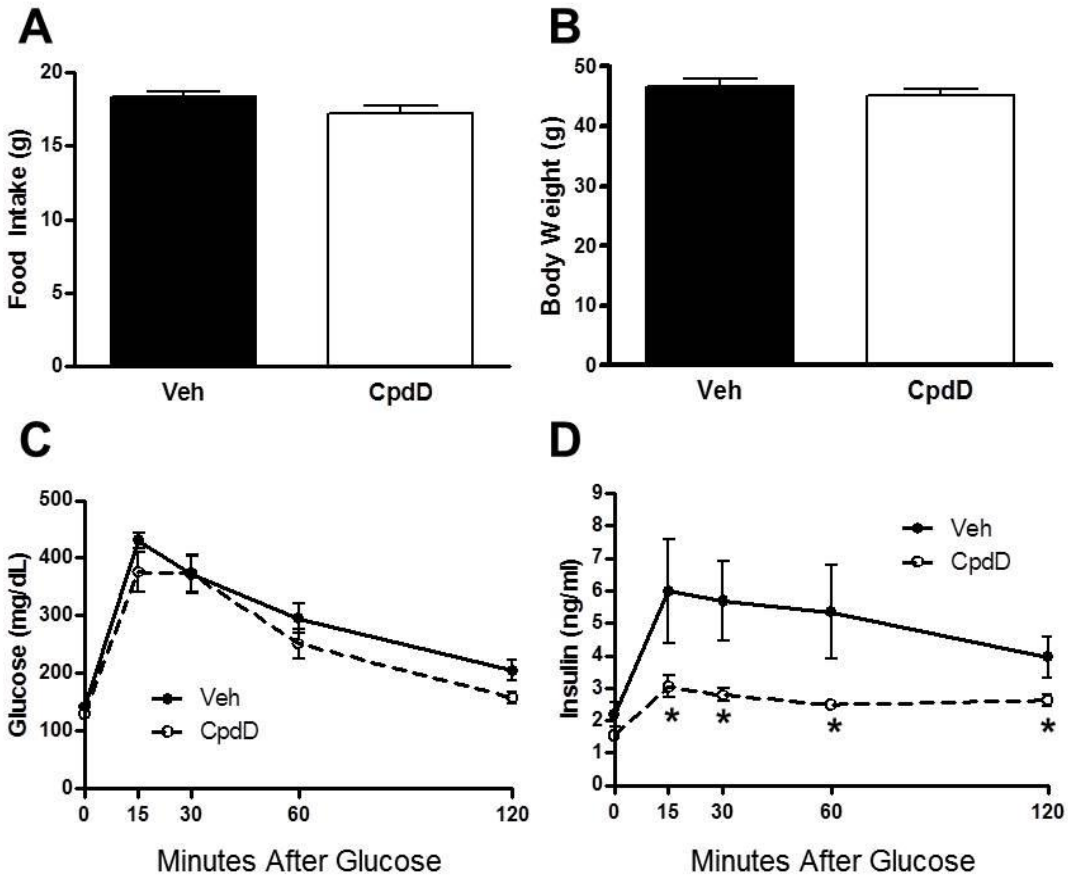
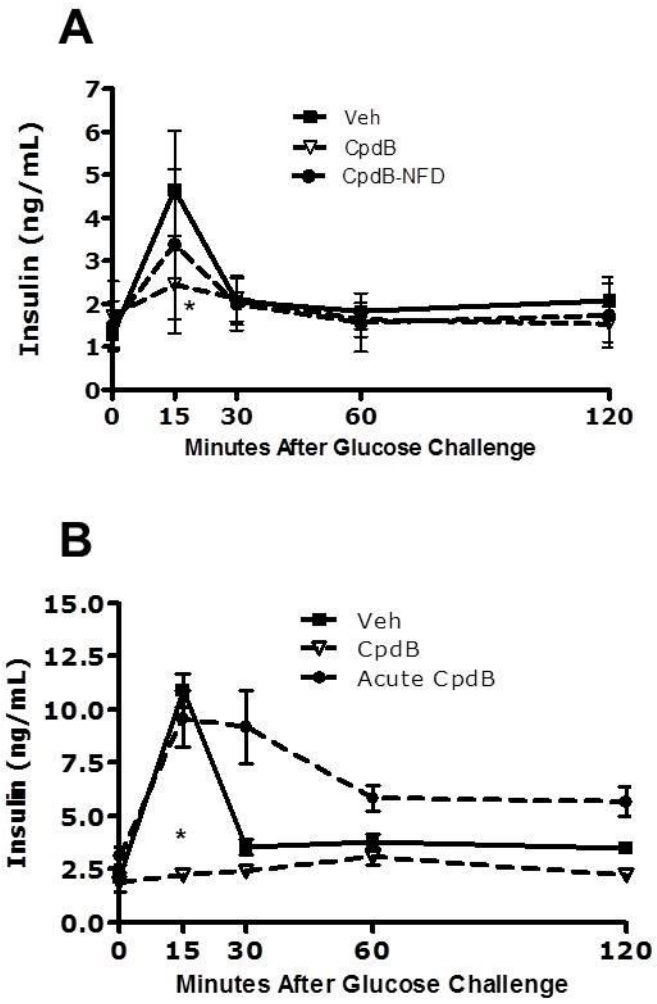
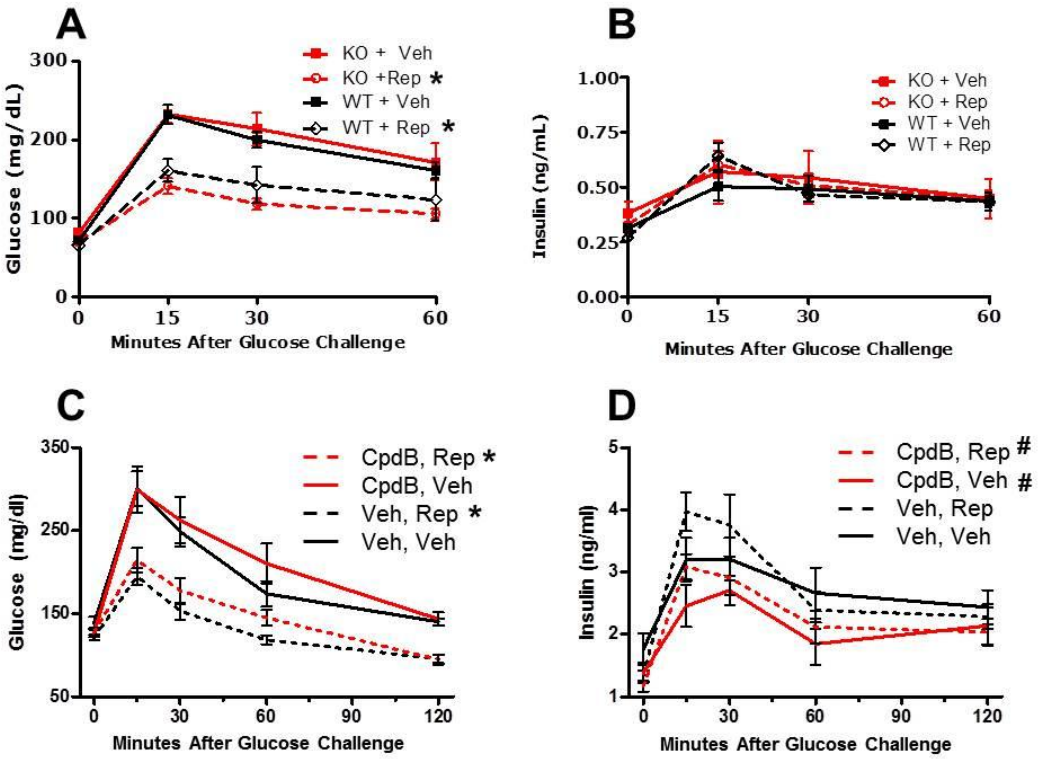


Supplemental Figure 4: BW-independent improvement in insulin sensitivity by GhrR antagonism. HFD-fed C7bl6/j mice were dosed orally for 1 week with either vehicle or CpdD (bid, 30 mg/Kg; n=8/group). (A, B) There were no differences in either cumulative FI or BW in mice dosed with vehicle or CpdD. Furthermore, in a GTT there was no improvement in glucose disposal in CpdD-treated mice relative to controls (C). However, the striking observation is that CpdD treatment caused a significant reduction in the insulin required for glucose disposal. Data analyzed with a repeated measures two-way analysis of variance (ANOVA) and Bonferroni post-hoc tests. (GTT, Glucose Tolerance Test; *$p<0.05$).



Supplemental Figure 5: Repeated dosing with the GhrR antagonist CpdB is required in order to achieve improvements in insulin sensitivity. **A)** Plasma insulin measurements from an oral GTT performed after 7 days of oral dosing with CpdB (60 mg/Kg, bid) with and without the final dose (no final dose; NFD) on the morning of the GTT (1hr prior to the GTT). Only mice receiving all doses plus the final morning dose of CpdB showed a significant decrease in insulin release (and reduced glucose excursion, data not shown) at the 15 min time point. B) In another study, mice receiving only a single oral dose of CpdB (60 mg/Kg) on the morning of a GTT were compared alongside mice receiving CpdB for 56 days (60 mg/Kg, bid, po). A single dose of CpdB was unable to improve the glucose excursion (data not shown) or the insulin required for that glucose disposal. Data are the means ± SEM, n=8/group, and are analyzed with a repeated measures two-way analysis of variance (ANOVA) and Bonferroni post-hoc tests. (GTT, Glucose Tolerance Test; *$p<0.05$ *vs.* vehicle-treated control mice).

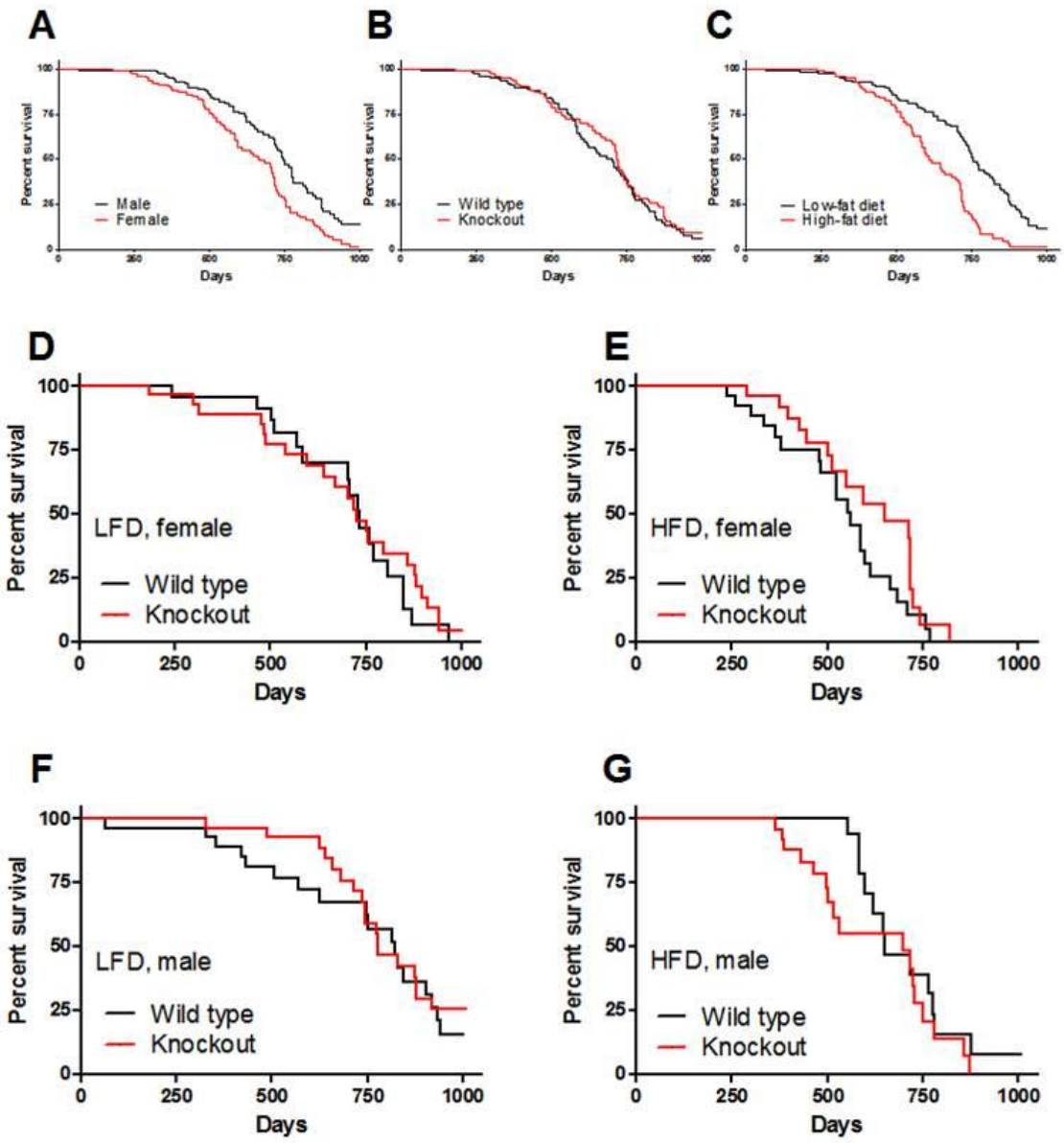


Supplemental Figure 6. Effect of genetic or pharmacologic blockade of GhrR signaling on the ability of the insulin secretagogue repaglinide to stimulate insulin release and improve glucose disposal. In all graphs the data are presented as absolute levels over time and AUC data were analyzed with a two-way analysis of variance (ANOVA) and Bonferroni post-hoc test.

A,B) HFD-fed GhrR KO and WT mice were treated with repaglinide 1 min prior to an oral GTT (2 g/Kg) and blood glucose and plasma insulin were evaluated at 15, 30 and 60 min after the glucose load. In this study we found that in both KO and WT mice, repaglinide treatment resulted in lower blood glucose exposures **(A)**. However, there were no significant differences in the insulin secretion between the groups **(B)**.

C,D) HFD-fed C57Bl/6 mice were treated orally for 7 days with vehicle or CpdB (60 mg/Kg, bid). On day 7 an oral GTT was performed in which either vehicle or repaglinide was dosed 1 min prior to the oral glucose load (2 g/Kg glucose). Blood glucose and plasma insulin were evaluated at 15, 30 and 60 min after the glucose load.

In mice treated with either vehicle or CpdB, the glucose excursion was significantly reduced in mice treated with repaglinide **(C)**. Additionally, in both vehicle-treated and CpdB-treated mice there was a trend to increased insulin following repaglinide treatment. Interestingly, CpdB treatment significantly reduced the insulin levels in both repaglinide and non-repaglinide treated mice **(D)**. Data are presented as mean ± SEM, n=8/group. *, significant effect of repaglinide, $p<0.05$; #, significant effect of CpdB, $p<0.05$.



Supplemental Figure 7: Longevity of male and female GhrR KO and WT mice. The longevity of all mice on study was compared in a number of ways. Statistical comparisons are described in Supplemental Table 3 below.
Global effects of the main factors are presented in A-C. A) comparison of all females versus all males (both LFD and HFD groups are combined for each gender) indicates male mice lived significantly longer than female mice in our studies. B) There were no significant differences in GhrR KO versus WT mice (male and female, all diets, combined). C) HFD feeding significantly shortened lifespan versus LFD feeding (all genders and genotypes combined).
Specific comparisons are illustrated in D-G. There were no significant differences in longevity of GhrR KO vs WT female mice on either a LFD (D) or HFD (E). Similar results were obtained for male mice (F, G).

Methods:
All mice were weaned at three weeks of age onto a chow diet (Purina), and then individually housed beginning at six weeks of age. Mice were randomly sorted into eight groups, partitioned by the factors of gender (male or female), genotype (wild type or GhrR knockout), and diet (low-fat or high-fat). At eight weeks of age, all mice were started on LFD or HFD (D12450B or D12492, respectively; Research Diets). Fresh water was given once a week; hoppers were maintained with ~60 g of food, with average replenishment of 15-20 g/week. Mice were monitored daily for general health; in cases of obvious illness and distress, mice were euthanized and censored from the study. Dates of death were recorded within 24 hours of the event.