**Supplemental Information:**

**Supplemental Methods:**

**Generation of mice with global $\beta_1$AR deletion:** Zona Pellucida 3 (ZP3)-Cre mice (1), which express Cre recombinase controlled by regulatory factors of the *Zp3* gene – a gene that is normally expressed exclusively in the oocyte prior to the first meiotic division – were crossed with heterozygous $\beta_1AR^{w/fl}$ mice to generate female breeders with one copy of the *loxP*-flanked *Adrb1* allele and one copy of the ZP3-Cre transgene (Z-$Cre^{Tg+}$). These breeders were crossed for two generations with C57BL/6N males to generate mice with one copy of the deleted *Adrb1* allele globally, one copy of the wild-type *Adrb1* allele and no ZP3-Cre transgene. Finally, these latter mice were crossed to each other to obtain mice with global knock-out of the *Adrb1* gene. We were able to generate three viable mice (1 male and 2 females) containing two copies of the deleted *Adrb1* allele, and we crossed the male to the two females once they reached 8 wks of age in order to ascertain the viability of the resulting progeny. Over a period of 5 mo, during which time the three breeding mice remained housed together, the two females were noted to be pregnant 3 and 4 times, respectively, but only yielded 6 pups that lived into adulthood. This yield represented approximately 10-15% of the expected number of pups normally observed in our C57BL/6N breeding colony, suggesting a possible developmental abnormality in mice with global $\beta_1$AR deletion, as had been reported previously in mice with global deletion of $\beta_1$AR generated by germline substitution of a neomycin resistance gene cassette for *Adrb1* (2).

**Generation of ghrelin-Cre-dependent tdTomato fluorescence reporter mice and characterization of ghrelin-Cre activity:** Our ghrelin-Cre line previously has been used to express the pertussis toxin catalytic S1 subunit selectively in ghrelin cells, resulting in high plasma ghrelin (3), and to express diphtheria toxin receptor in ghrelin cells, permitting selective toxin-mediated ablation of ghrelin cells and an 80-95% reduction in plasma ghrelin within adult mice administered diphtheria toxin (4). For this ghrelin-Cre transgenic (G-$Cre^{Tg+}$) line, we previously (3) showed a high degree of Cre activity – as assessed in tdTomato

reporter mice − co-localized with ghrelin-immunoreactivity within the gastric mucosa (>95% of gastric ghrelin cells) as well as Cre activity within the majority (69%) of the highly dispersed and far less numerous population of ghrelin-immunoreactive cells in the duodenum. We also previously used this method to assess Cre activity in pancreatic islets, since ghrelin cells have been repeatedly described in islets of mice in the fetal period, persisting into the neonatal period, although becoming markedly less numerous or disappearing completely by 2 wks of age (5-9). Our original validation studies demonstrated lack of Cre activity in representative 10 μm-thick sections of adult and fetal pancreata (3). Neither was Cre activity observed in 25 μm-thick coronal sections (every 5$^{th}$ section was examined) extending from the level of the anterior olfactory nucleus to the cervical spinal cord of the adult brain, where ghrelin expression has been inconsistently localized by others and remains highly controversial (3, 10-18).

Here, we more comprehensively surveyed Cre expression and activity within the G-Cre$^{Tg+}$ line by again crossing it to a Rosa26-lox-STOP-lox-tdTomato reporter line (B6.Cg-Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$/J; The Jackson Laboratory) (19). Cre recombinase mRNA expression was highest in tdTomato+ FACS-separated gastric mucosal cells (Supplemental Figure 1). From a series of ten other tissues, the only organ besides the stomach (tdTomato+ gastric mucosal cells) with significant Cre recombinase mRNA expression was testis (Supplemental Figure 1). Despite qPCR confirmation of testicular expression of Cre, no reduction in $\beta_1$AR expression was observed in testis from $\beta_1$AR$^{fl/fl}$/G-Cre$^{Tg+}$ mice, suggesting the possibility of non-overlapping patterns of Cre and $\beta_1$AR expression within testis (Supplemental Table 1).

We also further examined tdTomato fluorescence in cryostat-sectioned, 8 μm-thick representative sections of various peripheral organs and in sliding microtome-sectioned, 25 μm-thick brain sections (every 5$^{th}$ section extending from the level of the anterior olfactory nucleus to the cervical spinal cord) from 18 wk-old male mice carrying both the G-Cre$^{Tg+}$ and the Rosa26-lox-STOP-lox-tdTomato reporter. The sections were coverslipped with Vectashield Hardset Mounting medium with DAPI (Vector Laboratories, Burlingame, CA) to visualize tdTomato fluorescence. For histologic context, adjacent or nearby sections

were submitted to hematoxylin and eosin staining. As had been confirmed previously, Cre activity was demonstrated in scattered cells within the gastric mucosa, in a pattern reminiscent of ghrelin cell distribution (15). We newly demonstrated Cre activity in testis, in a distribution consistent with Leydig cell expression (Supplemental Figure 2), paralleling immunohistochemical detection of ghrelin reported previously in human and rat testis (20-23) and Cre mRNA expression in testis (Supplemental Figure 1). While exocrine pancreatic tissue was devoid of tdTomato fluorescence, tdTomato fluorescence was observed in islets, unlike our previous report (3), albeit only occasionally (Supplemental Figure 2). In particular, out of 111 different islets that were evaluated from a total of 3 animals, only one out of nine demonstrated cells with tdTomato fluorescence. Furthermore, within individual cross-sections, at most only one to three tdTomato-expressing cells were observed, nearly all of which were located in the islet periphery. Brain sections revealed no tdTomato fluorescence (Supplemental Figure 2). Epithelial cells within the epididymis also demonstrated tdTomato fluorescence (not shown), corresponding to the pattern of ghrelin-immunoreactive cells reported previously in human epididymis (20).

**Generation of mice with ghrelin cell-specific deletion of $\beta_1$ARs on a ghrelin cell hrGFP reporter background:** Ghrelin-Cre$^{Tg}$ (G-Cre$^{Tg+}$) mice were crossed with the heterozygous $\beta_1AR^{w/fl}$ to generate breeders harboring one copy of the "floxed"-*Adrb1* allele and one copy of the ghrelin-Cre transgene. Separately, ghrelin-hrGFP reporter mice (15) were also bred to the heterozygous $\beta_1AR^{w/fl}$ to generate heterozygous $\beta_1AR^{w/fl}$ mice containing the ghrelin-hrGFP transgene. The resulting two groups of mice were used as breeders to yield $\beta_1AR^{fl/fl}$/G-Cre$^{Tg+}$ mice and $\beta_1AR^{fl/fl}$/G-Cre$^{Tg-}$ control mice all containing the ghrelin-hrGFP transgene. Gastric ghrelin cells from these 2 mice groups were FACS-purified and *Adrb1* mRNA levels quantitated by qPCR to validate ghrelin cell-specific deletion of the *Adrb1* gene in $\beta_1AR^{fl/fl}$/G-Cre$^{Tg+}$ mice. Procedures for FACS and qPCR are described below.

**Twenty four hour fast:** To perform the 24h fast, food was removed from cages between 10 and 11 AM. Blood was collected from animals 24h later, between 10 and 11 AM the next day.

**Long-term feeding studies:** Female mice were chosen for the food intake and body weight studies as we had previously demonstrated GHSR-dependent body weight and adiposity phenotypes that were more marked in females compared to males (24).

**Alzet osmotic pump implantation:** For ghrelin infusion experiments, mice were anesthetized with a ketamine-xylazine cocktail (120 and 16 mg/kg BW respectively) and then were implanted with pre-filled Alzet osmotic pumps (Model 1002) at 8 wks of age to deliver continuous infusions of saline or rat acyl-ghrelin (SP-GHRL-1; Innovagen, Lund, Sweden) at a rate of 0.25 μL/h in order to achieve a cumulative daily dose of 4 mg/kg body weight, as described previously (25). Mice were given access to one Carprofen wafer (5g/wafer) daily for 2 days following the osmotic pump implantation. The mice were subjected to the 60% caloric restriction protocol 5 days after implantation of the pump. Food consumption in mice implanted with ghrelin-filled pumps, but not vehicle-filled pumps, increased (by about 20%) during the 3d before starting the caloric restriction. During the caloric restriction regimen, these mice were, however, fed 40% of their daily caloric intake as determined prior to the ghrelin infusion regimen, so as to better match the control groups. Plasma ghrelin levels achieved with ghrelin infusion were confirmed at the start of the caloric restriction protocol (Figure 4D).

**Fat composition analysis:** Percentage body fat mass was calculated by dividing the fat mass as determined by the EchoMRI-100 by body weight as measured by a scale.

**Isolation of gastric mucosal cells and fluorescence-activated cell sorting (FACS)**: FACS was performed as described previously (15, 26). Briefly, 3 to 4 fed male or female mice of the same genotype were deeply sedated with chloral hydrate anesthesia (700 mg/kg i.p), followed by laparotomy. The stomachs were tied off at both ends with surgical sutures, excised and placed in ice-cold PBS and then incised at the non-glandular portion to clear the digesta. The stomach was then inverted inside-out by passing the distal part

of the stomach through the incision and inflated with and briefly placed in ice-cold DMEM medium with no glucose (Life Technologies, Grand Island, NY) until all stomachs in the group were isolated. The residual digesta adhering to the stomach mucosa was cleared off gently by using soft paper pads. The stomachs were then incubated for 1.5 hrs at 37°C in 35 U dispase II/3 mL PBS (Roche Diagnostics, Indianapolis, IN) in a 50 mL centrifuge tube, and then the mucosa was scraped off using a transfer pipette into sterile DMEM/F-12 (1:1) medium (Mediatech Inc. Manassas, VA) containing 10% (vol/vol) FBS (Atlanta Biologicals, Lawrenceville, GA), supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin sulfate. The cells were centrifuged at 310 g for 3 min, and the media was removed and subjected to digestion with 0.25% trypsin EDTA (Mediatech Inc. Manassas, VA). Trypsin was inactivated after 5 min with addition of FBS containing DMEM/F-12 medium and the mucosal cells were tritutrated to disperse them. The suspension was then passed through a 100 µM filter and then centrifuged at 310 g for 3 min to remove the supernatant medium. The resulting cell pellets were suspended in 1 mL FACS buffer and passed through a 30 µM filter. The cells were next subjected to FACS analyses using MOFLO$^{TM}$ high speed cell sorter (Beckman Coulter, Brea, CA) at the UT Southwestern Flow Cytometry Core to separate enriched populations of tdTomato- or hrGFP-positive ghrelin cells as well as tdTomato- or hrGFP-negative control cells, respectively. The cells were collected directly into RNA-STAT 60 (for cells from mice with the tdTomato reporter) or buffer provided with the Arcturus PicoPure RNA Isolation Kit [Applied Biosystems by Thermo Fisher Scientific] (for cells from mice with hrGFP reporter) and were stored at -80°C until qPCR analysis.

***Ex vivo* ghrelin secretion studies using a primary gastric mucosal cell culture system:** Primary gastric mucosal cells were isolated from 3 stomachs of the same genotype and dispersed enzymatically and mechanically as described above for FACS analysis, with the following changes: For primary cultures, the cells were suspended in FBS containing DMEM/F-12 medium containing instead of FACS buffer and plated at a density of 1 X $10^5$ cells/mL/well supplemented with sodium octanoate-bovine serum albumin (BSA) to achieve a final concentration of 50 µM in poly-D-lysine pre-coated 24-well plates, as described

previously (27). The cells were placed overnight in a 37°C incubator with 5% $CO_2$. The next day, the cells were treated with 10 μM norepinephrine (Sigma-Aldrich, St. Louis, MO) or vehicle (saline) for 6 h in serum free DMEM (Life Technologies, Grand Island, NY) containing 5 mM glucose and 50 μM sodium octanoate-BSA. At the end of 6 h incubation period, the medium was collected, placed on ice and immediately centrifuged at 4°C at 800 g X 5 min. Hydrochloric acid was added to the supernatant to achieve a final concentration of 0.1 N (for stabilization of acyl-ghrelin) and stored at -80°C until analysis.

**Quantitative reverse-transcriptase PCR:**

Total RNA was isolated using one of the two following RNA isolation methods. For FACS-separated cells from tdTomato mice or homogenized tissues, RNA was isolated using the guanidium thiocyanate-phenol-chloroform extraction method by addition of RNA STAT-60. For FACS-seperated cells from hrGFP mice, RNA was isolated using the Arcturus PicoPure RNA Isolation Kit Cell Pellet protocol (Applied Biosystems by Thermo Fisher Scientific). The isolated RNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Sceientific, Wilmington, DE). Total RNA (2 μg for organs and 25 or 50 ng for sorted cells) was treated with ribonuclease-free deoxyribonuclease (Roche, Indianapolis, IN), and complementary DNA was synthesized by reverse transcription using SuperScript III (Invitrogen, Carlsbad, CA). Our gene-specific primers were as used in previous publications or as designed *de novo* using Primer Express Software (PerkinElmer Life Sciences, Boston, MA), and are listed in Supplemental Table 3. All primers were validated by analysis of template titration and dissociation curves. Quantitative PCR was performed using an Applied Biosystems 7900HT fast real-time system (Applied Systems, Foster City, CA) and SYBR green chemistry. The reaction mixture for qPCR contained the reverse-transcribed RNA, 150 nM of each primer and 5 μl of 2X iTaq Universal SYBR Green PCR master mix (Bio-Rad, Herculus, CA). The mRNA levels are represented relative to the invariant control gene, cyclophilin B and were calculated by the comparative threshold cycle (ΔΔCt) method. Data are presented as a percentage of the expression of the gene in wild-type control mice or as indicated.

**Plasma hormone and catecholamine analysis:**

Plasma insulin was analyzed using an ultrasensitive mouse Insulin ELISA kit (Crystal Chem, Downers Grove, IL, Catalog # 90080) and GH was analyzed using a Rat/mouse growth hormone ELISA kit (EMD Millipore Corporation, St. Charles, MO, Catalog # EZRMGH-45K). For glucagon estimation, blood was collected in tubes containing aprotinin to achieve a final concentration of 250 KIU/ml. Glucagon was estimated in the plasma samples by using radioimmunoassay (EMD Millipore Corporation, Catalog # GL-32K) as per manufacturer's instructions. For norepinephrine and epinephrine estimation, blood was collected in tubes with EGTA-glutathione solution, and levels were determined by an HPLC method at the Hormone Assay and Analytical Services Core at Vanderbilt University Medical Center.

**Supplemental Tables:**

**Supplemental Table 1: Ghrelin cell-selective $\beta_1$AR deletion does not alter $\beta_1$AR expression in organs outside the gastrointestinal tract.**

| Organs | Genotype | | | | |
|---|---|---|---|---|---|
| | $\beta_1AR^{w/w}$/ G-$Cre^{Tg-}$ | $\beta_1AR^{w/w}$/ G-$Cre^{Tg+}$ | $\beta_1AR^{fl/fl}$/ G-$Cre^{Tg-}$ | $\beta_1AR^{fl/fl}$/ G-$Cre^{Tg+}$ | $\beta_1AR^{fl/fl}$/ Z-$cre^{wt/Tg}$ |
| **Heart** | 1.15 ±0.25 | 0.91±0.09 | 1.13±0.12 | 0.94±0.10 | Undetectable |
| **Anterior cortex (Brain)** | 1.02±0.08 | 1.22±0.38 | 1.17±0.15 | 0.85±0.12 | Undetectable |
| **Basal ganglia (Brain)** | 1.16 ± 0.23 | 1.17 ±0.049 | 2.01±0.26 | 1.17±0.13 | Undetectable |
| **Kidney** | 1.17± 0.24 | 1.02±0.12 | 0.99±0.24 | 0.80±0.11 | Undetectable |
| **Liver** | 1.09 ±0.15 | 1.08±0.21 | 0.6±0.06 | 0.69±0.15 | Undetectable |
| **Gastrocnemius Muscle** | 0.88±0.08 | 1.37±0.14 | 1.12±0.11 | 0.69±0.10 | Undetectable |
| **Testis** | 1.15±0.34 | 1.51±0.18 | 0.87±0.49 | 1.16±0.29 | Undetectable |

Data are expressed as relative expression levels comparing the individual qPCR ΔCt values to the mean ΔCt values of wild type ($\beta_1AR^{w/w}$/G-$Cre^{Tg-}$) control mice for each organ. No significant difference in the expression levels of $\beta_1$AR was observed among the four genotype groups. Tissues from global $\beta_1$AR-knockout mice ($\beta_1AR^{fl/fl}$/Z-$cre^{wt/Tg}$; obtained by crossing $\beta_1AR^{fl/fl}$ mice with ZP3-$Cre^{Tg+}$ mice) served as negative controls, and demonstrated no $\beta_1$AR mRNA transcripts, as expected.

**Supplemental Table 2: Genotyping primers used**

| Genotype | Sequence | Product Size (bp) and notes | Reference/ Source |
|---|---|---|---|
| $\beta_1AR^{fl/fl}$ | F: 5′-CAGCAGATAGGCTGTCCAAG-3′<br>R: 5′-GCTTCTTCCAGAGTCTAGTCT-3′<br>R: 5′-ATATGCACAGAGTGAGGTAGAGGA-3′ | 246: Indicates presence of WT *Adrb1* allele.<br>358: Indicates presence of *loxP*-flanked recombinant *Adrb1*allele.<br>508: Indicates deleted *Adrb1*, with only one *loxP* site. | This work |
| Ghrelin Cre (G-$Cre^{Tg+}$) | F: 5′-GATCTCCAGCTCCTCCTCTGTCT-3′<br>R: 5′-TGCGAACCTCATCACTCGTTGCAT-3′<br>R: 5′-GGTCAGCCTAATTAGCTCTGTCAT-3′ | 619: Indicates presence of ghrelin, but not ghrelin specific Cre Tg.<br>396: Indicates presence of ghrelin specific Cre Tg. | (3) |
| tdTomato (tdTM) | F: 5′-AAGGGAGCTGCAGTGGAGTA-3′<br>R: 5′-CCGAAAATCTGTGGGAAGTC-3′<br>F: 5′-CTGTTCCTGTACGGCATGG-3′<br>R: 5′-GGCATTAAAGCAGCGTATCC-3′ | 297: Indicates Rosa *loxP*-STOP-*loxP* tdTM present.<br>196: Indicates Rosa-*loxP* tdTM present. | Jackson Labs |
| Zona Pellucida 3 Cre | F: 5′-GGTCAGCCTAATTAGCTCTGTCAT-3′<br>R: 5′-GATCTCCAGCTCCTCCTCTGTCT-3′<br>F: 5′-GCCACAGGTCTAATAATGTGTTGAT-3′<br>R: 5′-TGCGAACCTCATCACTCGTTGCAT-3′ | 620: Indicates presence of WT *ghrelin* gene (internal control)<br>290: Indicates presence of *Zp3*-specific Cre Tg. | This work |

**Supplemental Table 3: Primer sequences used for real-time quantitative polymerase chain reaction**

| Gene | Primer sequence | Product size (bps) | Reference/ Source |
|---|---|---|---|
| *Adrb1* ($\beta_1$ Adrenergic receptor) | F: 5′-CGTGCCCCTGTGCATCA-3′<br>R: 5′-GTCGATCTTCTTTACCTGTTTTTGG-3′ | 76 | This work |
| Cre recombinase | F: 5′-GCGGTCTGGCAGTAAAAACTATC-3′<br>R: 5′-GTGAAACAGCATTGCTGTCACTT-3′ | 100 | (28) |
| *Ghrl*<br>Ghrelin | F: 5′-GTCCTCACCACCAAGACCAT-3′<br>R: 5′-TGGCTTCTTGGATTCCTTTC-3′ | 150 | (15) |
| *Ppid*<br>(Peptidylprolyl isomerase B; Cyclophilin) | F: 5′-TGGAGAGCACCAAGACAGACA-3′<br>R: 5′-TGCCGGAGTCGACAATGAT-3′ | 66 | (29) |
| PGC1α - peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (*Ppargc1a*) | F: 5’-TATGGAGTGACATAGAGTGTGCT-3’<br>R: 5’-CCACTTCAATCCACCCAGAAAG-3’ | 134 | (30) |
| PEPCK - phosphoenolpyruvate carboxykinase 1 (*Pck1*) | F: 5’-TGACAACTGTTGGCTGGCTC-3’<br>R: 5’-GACATACATGGTGCGGCCTT-3’ | 208 | (31) |
| G6P - Glucose-6-phosphatase (*G6pc*) | F: 5’-GGCGCAGCAGGTGTATACTA-3’<br>R: 5’-ATGCCTGACAAGACTCCAGC-3’ | 203 | (31) |
| HNF4α - Hepatic nuclear factor 4, alpha (*Hnf4a*) | F: 5’-GCATGGATATGGCCGACTAC-3’<br>R: 5’-TGTGGTTCTTCCTCACGCTC-3’ | 252 | (31) |
| Pyruvate carboxylase (*Pcx)* | F: 5’-GTGAGATTGCCATCCGAGTG-3’<br>R: 5’-TCTGCTCGCTCTGAGAGGAA-3’ | 241 | (31) |
| Liver glycogen phosphorylase *(Pygl)* | F: 5’-CAAGTGTCCCAAGAGGGTGTAT-3’<br>R: 5’-CTGTAATGTTCGGCCCATGT-3’ | 61 | (31) |
| IGF-1 - Insulin-like growth factor 1 (*Igf1*) | F: 5’-GGCATTGTGGATGAGTGTTG-3’<br>R: 5’-TCTCCTTTGCAGCTTCGTTT-3’ | 180 | (32) |
| IGFBP-1 - Insulin-like growth factor binding protein 1 (*Igfbp1*) | F: 5’-GGAGATCGCCGACCTCAAG-3’<br>R:5’-<br>CTGCAGCTAATCTCTCTAGCACTTTATAG-3’ | 71 | (29) |

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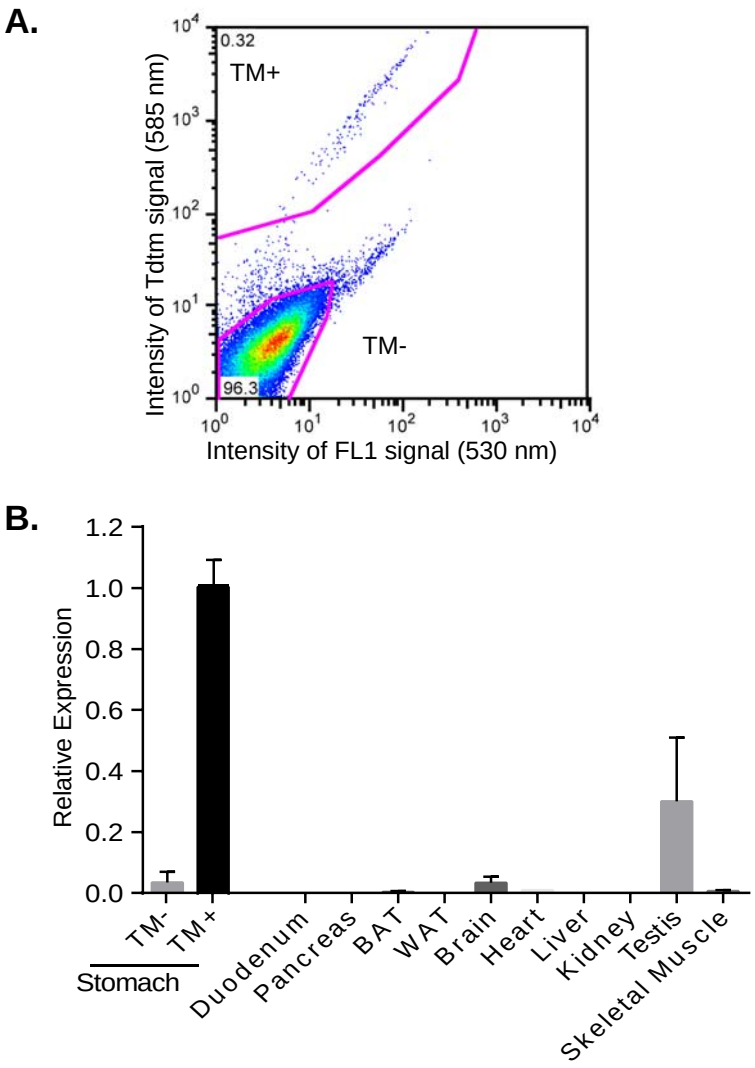
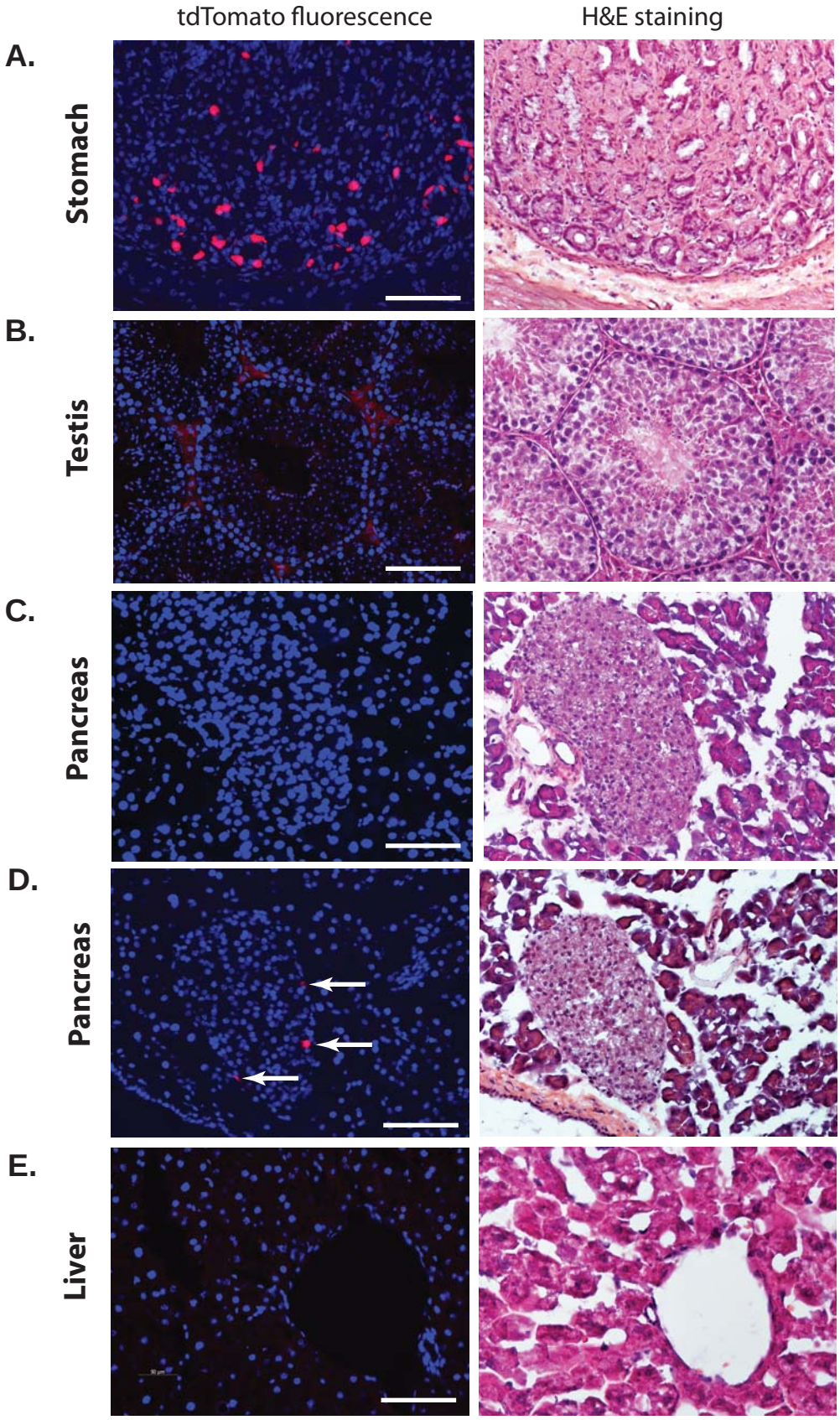
# Supplemental Figure 1

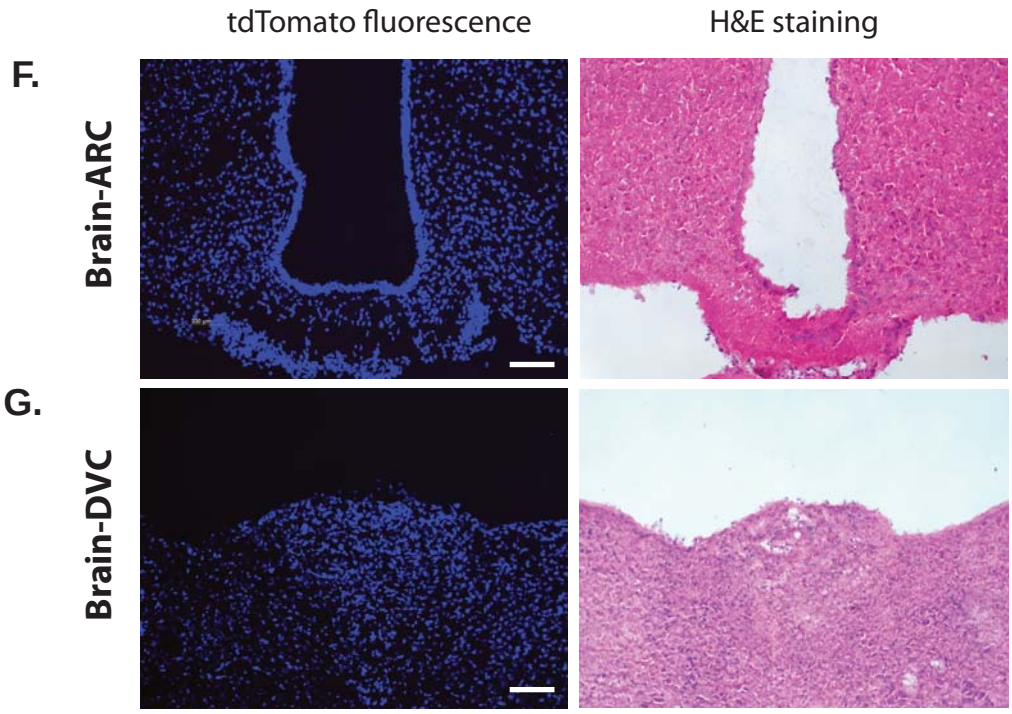



**Expression pattern of Cre recombinase in Ghrelin-Cre$^{Tg+}$/tdTm mice.** The expression profile of Cre recombinase mRNA in Ghrelin-Cre$^{Tg+}$ mice was assessed by crossing the G-Cre$^{Tg+}$ mice to a Rosa26-lox-STOP-lox-tdTomato reporter line. (A) Representative fluorescence activated cell sorting (FACS) scatter plot of the gastric mucosal cells indicating separation into a tdTomato positive (TM+) pool (0.32% of the sorted live single cells) and a tdTomato negative (TM-) pool (96.3% of the sorted live single cells). (B) Relative Cre recombinase mRNA expression was estimated by qPCR in the FACS-purified TM+ gastric mucosal cells, control TM- gastric mucosal cells and a panel of other organs, as indicated. TM+ cells expressed high levels of preproghrelin mRNA, whereas none was detected in TM- FACS-separated gastric mucosal cells. Data are ΔΔCt values relative to Cre expression in TM+ cells. The mRNA levels were estimated in organs collected from 3 mice and from sorted cells collected from 2 independent FACS-sorts with 3 or 4 mice for each sort. Values are expressed as mean ± SEM.

# Supplemental Figure 2



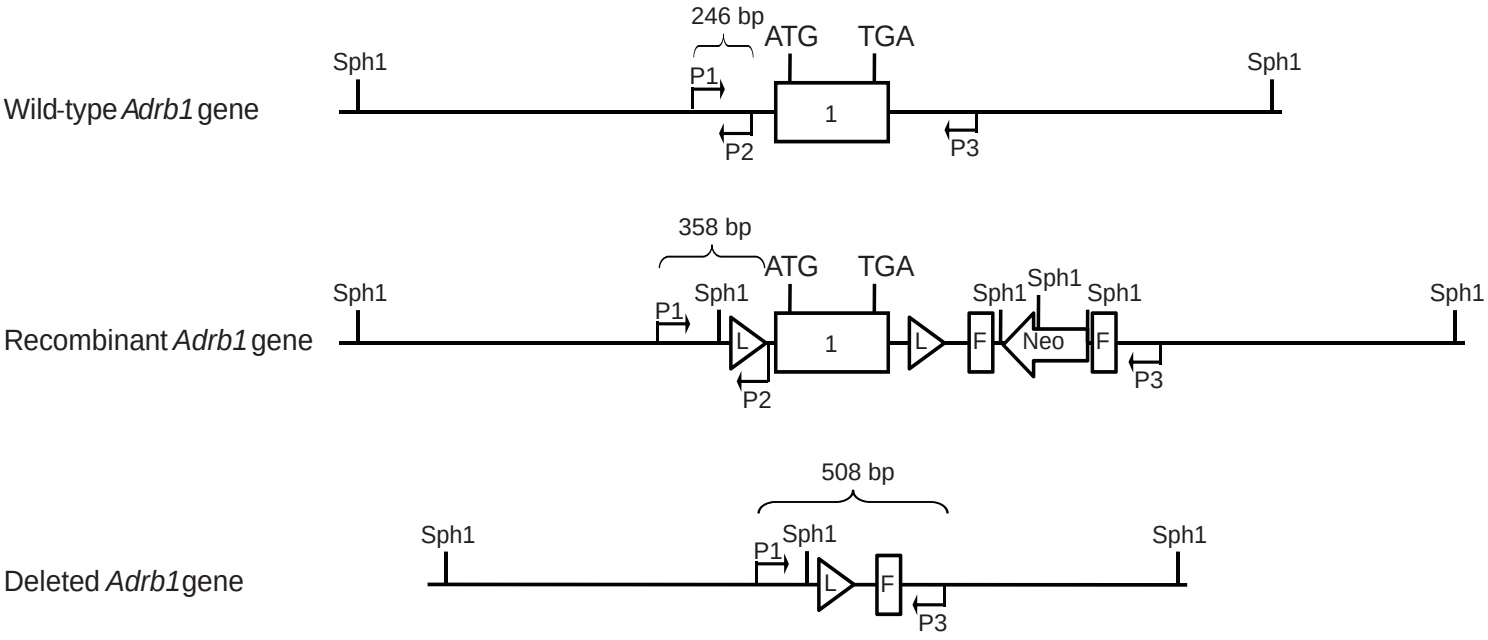
# Supplemental Figure 2



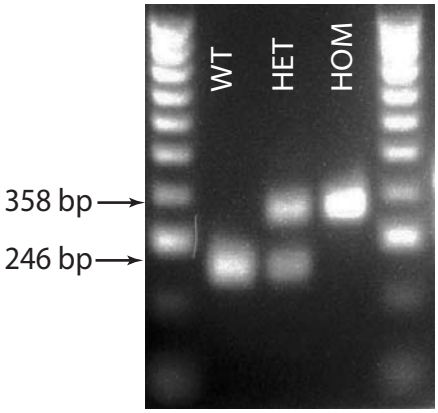

**Histological assessment of Cre recombinase activity in Ghrelin-Cre$^{Tg+}$ mice.** Cre recombinase activity in Ghrelin-Cre$^{Tg+}$ mice was assessed by crossing the G-Cre$^{Tg+}$ mice to a Rosa26-lox-STOP-lox-tdTomato reporter line. Left panels show tdTomato expression (red) with DAPI nuclear counterstain (Blue). Right panels detail the histology by hematoxylin and eosin staining on corresponding adjacent sections or on nearby sections within a few μm of the corresponding left panel. (A) Stomach section shows tdTomato expression in a pattern reminiscent of ghrelin expression. (B) Cross section of testis shows tdTomato expression in Leydig cells of the testis. (C and D) Pancreatic sections show a representative islet with no tdTomato expression and a representative islet with tdTomato expression, marked with arrows. (E) No expression of tdTomato was observed in the liver or brain [representative sections of the (F) hypothalamic arcuate nucleus (ARC) region approximately -1.82 mm from bregma and the (G) dorsal vagal complex (DVC) approximately -7.56 mm from bregma are shown]. Scale bar = 100 μM.

# Supplemental Figure 3

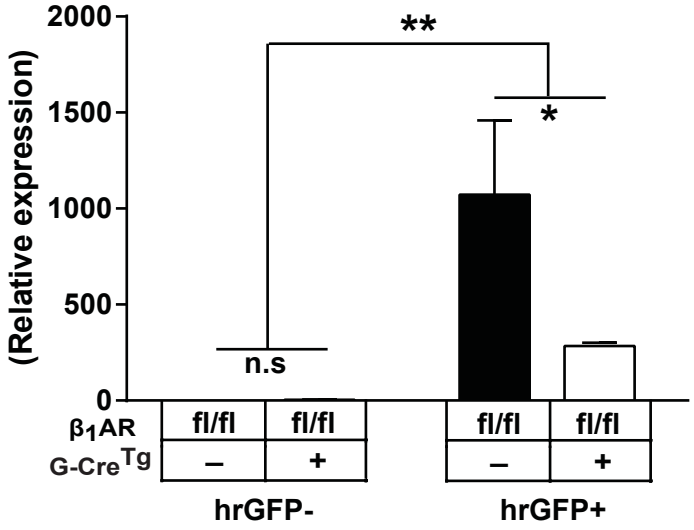
**A.**




**B.**



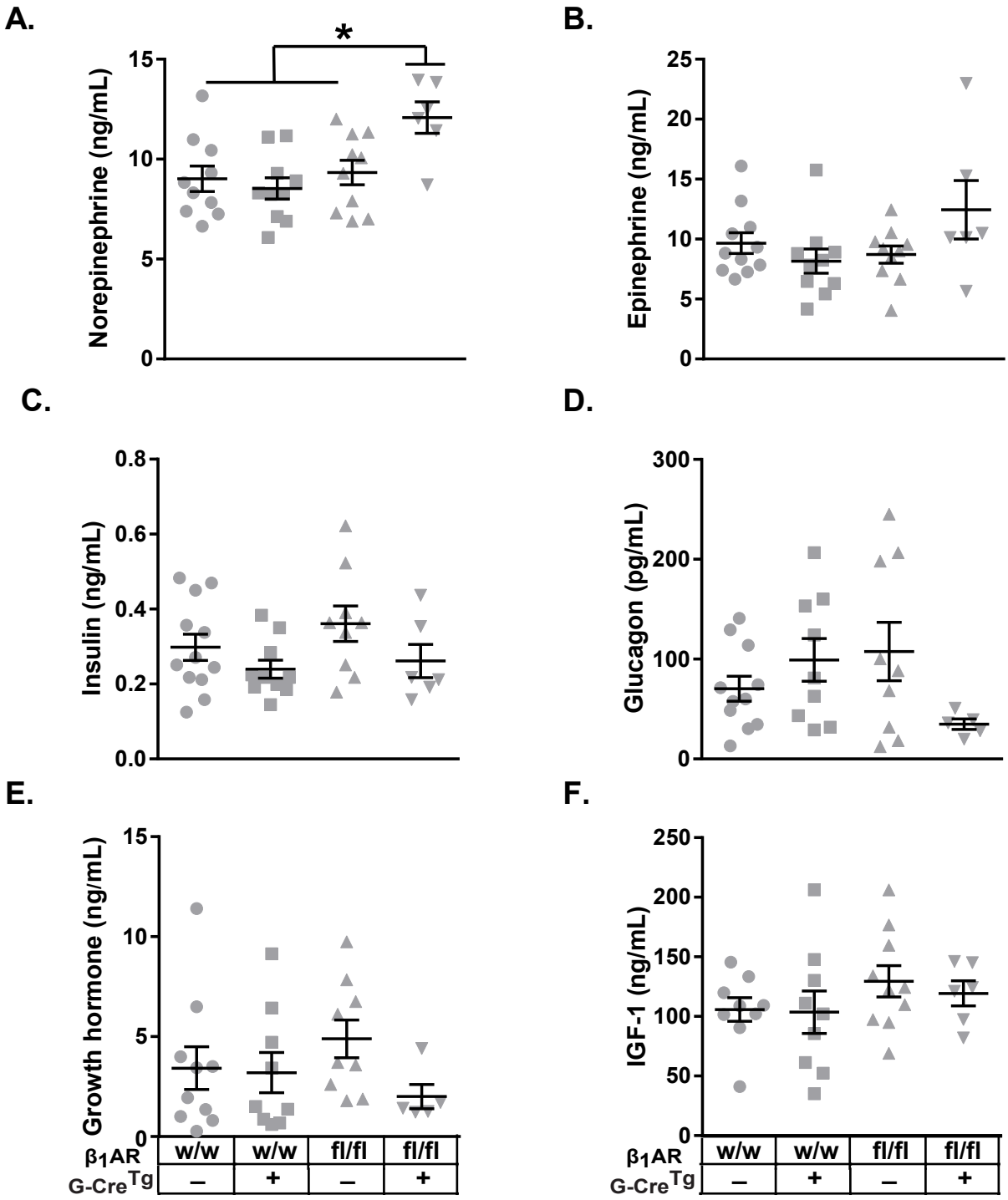

**C.**



# Supplemental Figure 3

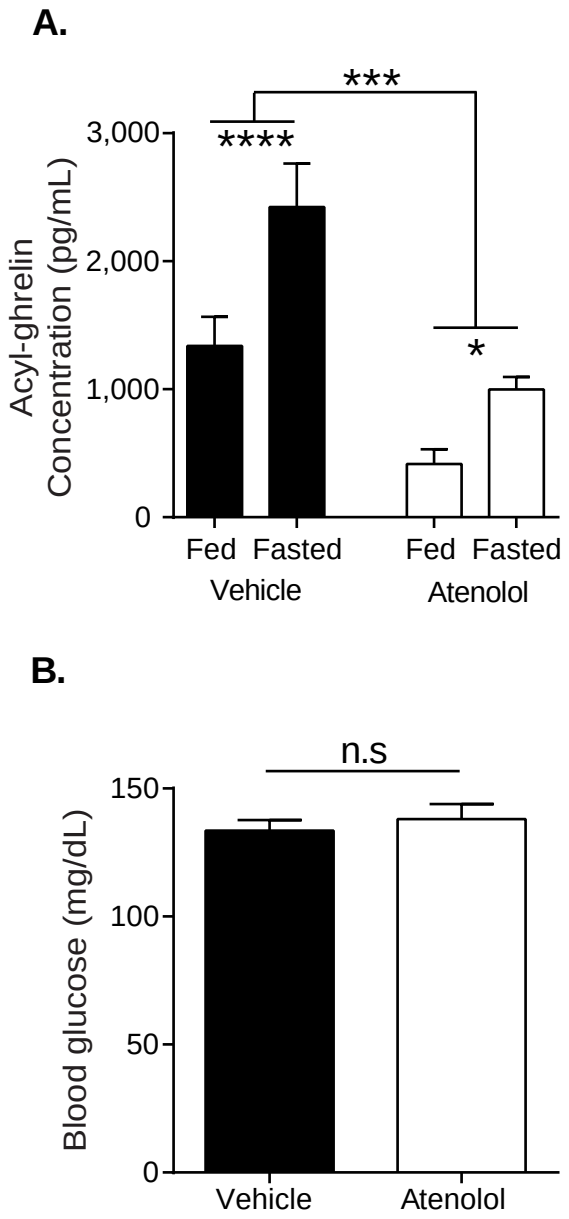
**Validation of germline transmission of the *loxP*-flanked *Adrb1* allele and ghrelin-Cre-mediated deletion of *Adrb1* gene expression.** (A) Schematic diagram shows binding sites of the genotyping primers (P1 = forward primer, P2 and P3 = reverse primers) in the genomic DNA to detect the wild-type *Adrb1* allele (246 bp), the *loxP*-flanked *Adrb1* allele (358 bp), and Cre recombinase-mediated removal of the $\beta_1$AR coding sequence (508 bp). *Sph*1-indicates the sites for restriction enzyme digestions used in Southern blot analysis. (B) PCR analysis of genomic DNA obtained by tail biopsies of representative pups derived from crosses of mice heterozygous for the *loxP*-flanked *Adrb1* allele, demonstrating identification of mice homozygous for the $\beta_1AR^{fl}$ allele ("HOM"), as well as mice with two copies of the wild-type *Adrb1* allele ("WT"), and heterozygotes ("HET"). (C) Quantitative estimation of $\beta_1$AR (*Adrb1*) mRNA expression in FACS-purified ghrelin cells obtained from $\beta_1AR^{fl/fl}$/G-$Cre^{Tg+}$ mice and $\beta_1AR^{fl/fl}$/G-$Cre^{Tg-}$ control mice generated by genetic crosses with ghrelin-hrGFP transgene reporter mice. Data are expressed as $\Delta\Delta$Ct values relative to $\beta_1$AR mRNA expression in hrGFP- cells from $\beta_1AR^{fl/fl}$/G-$Cre^{Tg-}$ control mice. *$p<0.05$, **$p<0.01$, significant difference in *Adrb1* mRNA expression analyzed by one-way ANOVA followed by Holm-Sidak's post hoc multiple comparison test. n.s.= not significant. The mRNA was estimated from sorted cells collected from 2 independent FACS-sorts with 3 mice for each sort. Values are expressed as mean ± SEM.

# Supplemental Figure 4



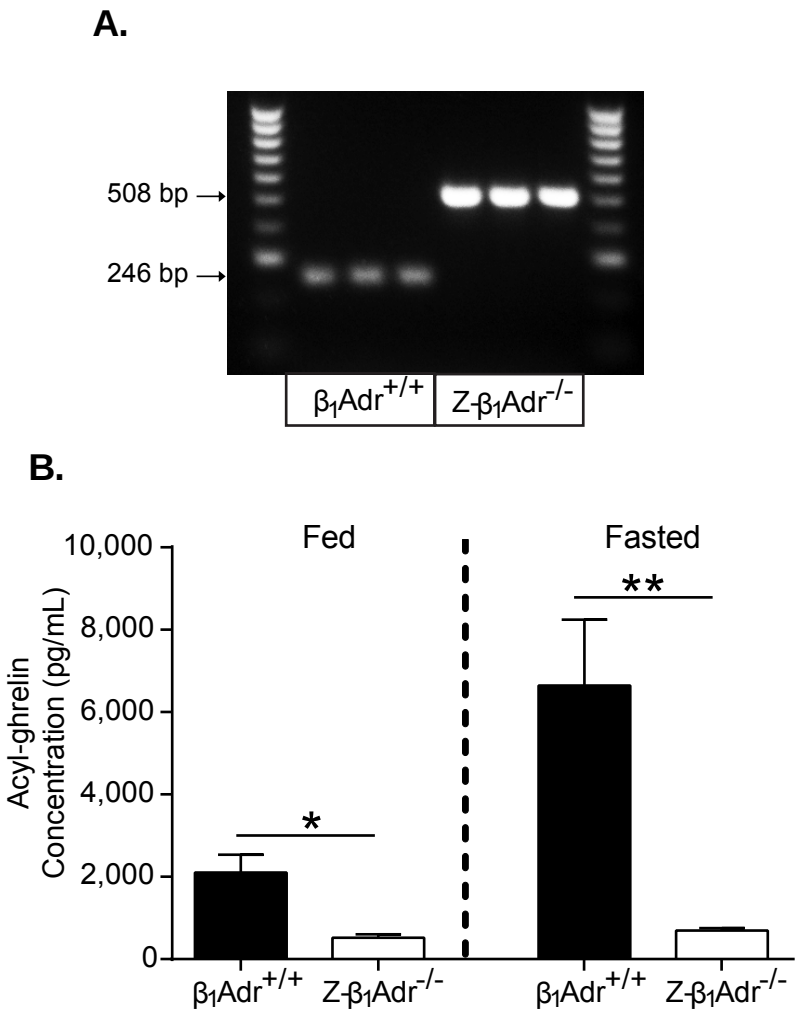

**Ghrelin cell-selective $\beta_1$AR deletion-induced changes to glucoregulatory hormones after a 24h fast.** Plasma levels of norepinephrine (A), epinephrine (B), insulin (C), glucagon (D), GH (E) and IGF-1 (F). n= 6-11, *$p<0.05$, significant change in GC-$\beta_1AR^{-/-}$ mice vs. control groups by one-way ANOVA followed by Holm-Sidak's post hoc multiple comparison test.

# Supplemental Figure 5



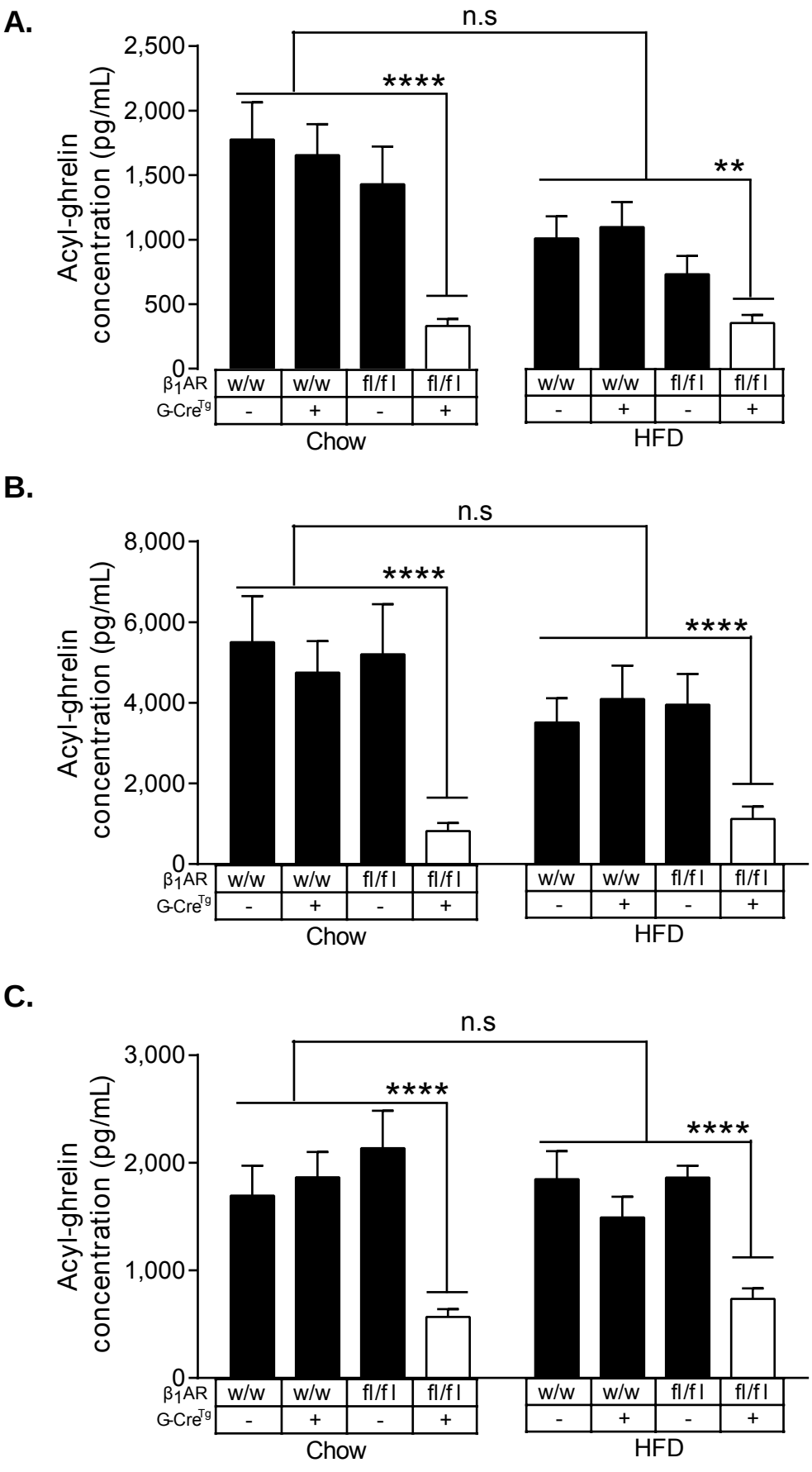

**Administration of atenolol to 6 wk-old male mice reduces plasma ghrelin without affecting blood glucose.** A. Plasma acyl-ghrelin measured in 6 wk-old male C57BL/6N mice treated with atenolol (10 mg/kg BW i.p.) twice daily for 3 days or vehicle before (Fed state; measured on Day 2) and after a 24h fast (measured on Day 3). n=8 in each group. *p<0.05, ***p<0.005, ****p<0.001 data analyzed by two-way repeated measures ANOVA followed by Holm-Sidak's post hoc multiple comparison test. B. Fasted blood glucose levels in mice treated with atenolol or vehicle. n.s.= not significant. Values are expressed as mean ± SEM.

# Supplemental Figure 6




**Plasma acyl-ghrelin levels are lower in mice with global $\beta_1$AR deletion.** Global $\beta_1$AR knockout mice were generated by ZP3-Cre$^{Tg+}$-mediated deletion of *Adrb1* in $\beta_1$AR$^{fl/fl}$ mice. (A) PCR of genomic DNA from tail snips show deletion of the portion of genome containing the $\beta_1$AR gene in three representative mice identified as having global *Adrb1* deletion (508-bp band), but preservation of that region in three wild-type mice (246-bp band). (B) Plasma acyl-ghrelin levels in *ad lib*-fed and 24h-fasted states in 8-12 wk-old mice with ZP3-Cre$^{Tg+}$-mediated global $\beta_1$AR deletion (Z-$\beta_1$AR$^{-/-}$) and age-matched wild-type mice ($\beta_1$AR$^{+/+}$). n=6-9. *p<0.05, **p<0.01, data analyzed by unpaired students "t" test. Values are expressed as mean ± SEM.

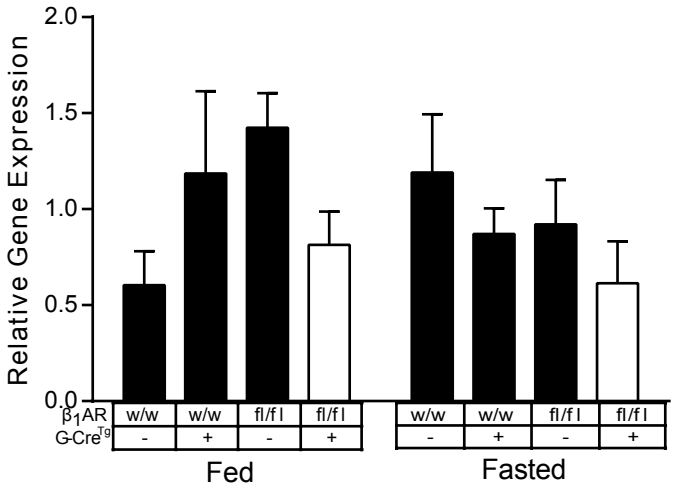
# Supplemental Figure 7



# Supplemental Figure 7

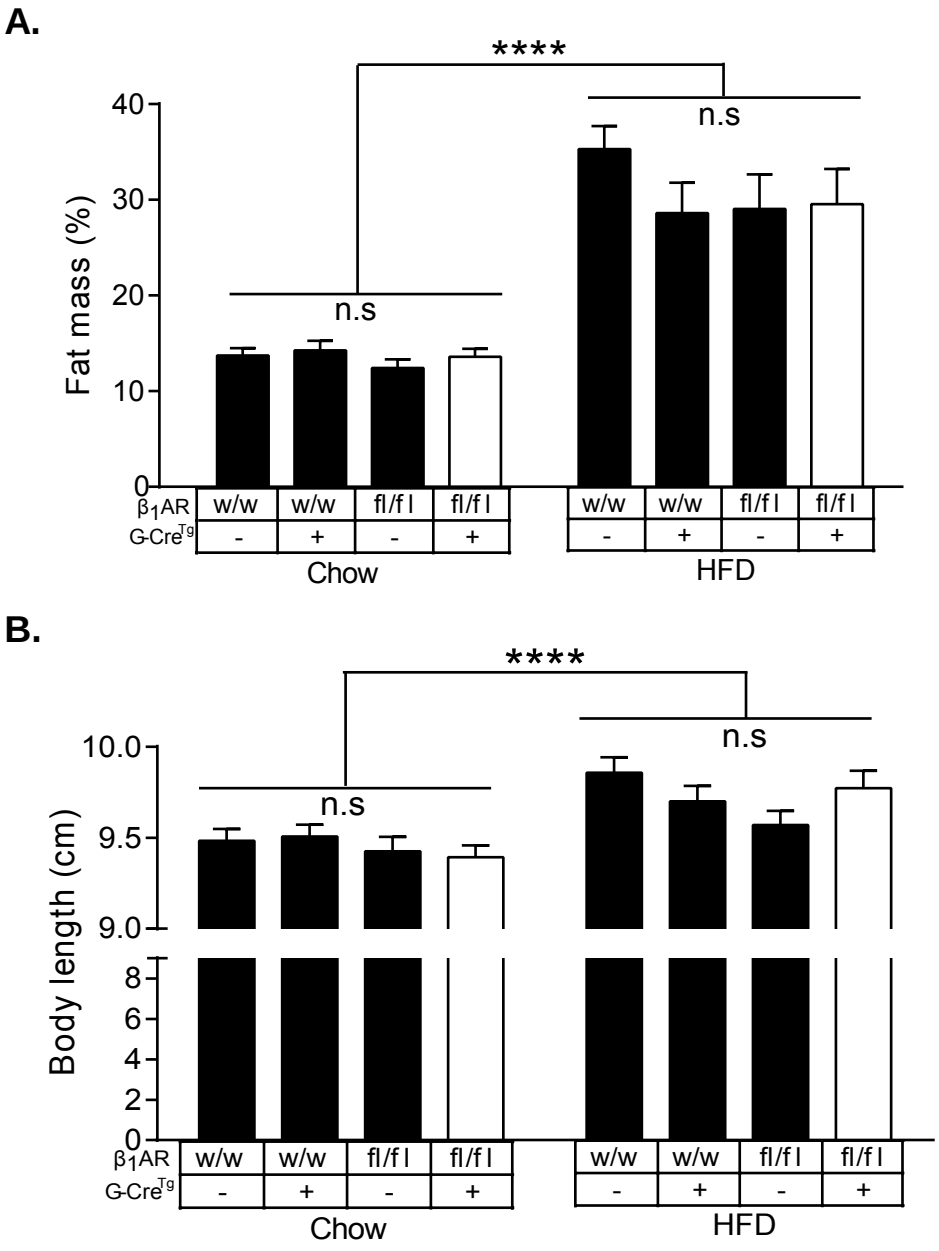
**Plasma acyl-ghrelin levels in female GC-$\beta_1$AR$^{-/-}$ mice during the course of a 16 wk-long food intake study**. Plasma acyl-ghrelin in female mice measured in the *ad lib*-fed condition after 4 wks (A), in the 24h fasted condition after 8 wks (B) and again in the *ad lib*-fed condition after 12 wks exposure to standard chow or HFD. n=11-16; **p<0.01, ****p<0.001, significant difference in plasma ghrelin concentrations in GC-$\beta_1$AR$^{-/-}$ mice compared to control genotypes within mice fed same diet. n.s., no significant change in plasma acyl-ghrelin in mice fed HFD compared to mice fed standard chow. Data analyzed by two-way ANOVA followed by Holm-Sidak's post hoc multiple comparison test. Values are expressed as mean ± SEM.

# Supplemental Figure 8



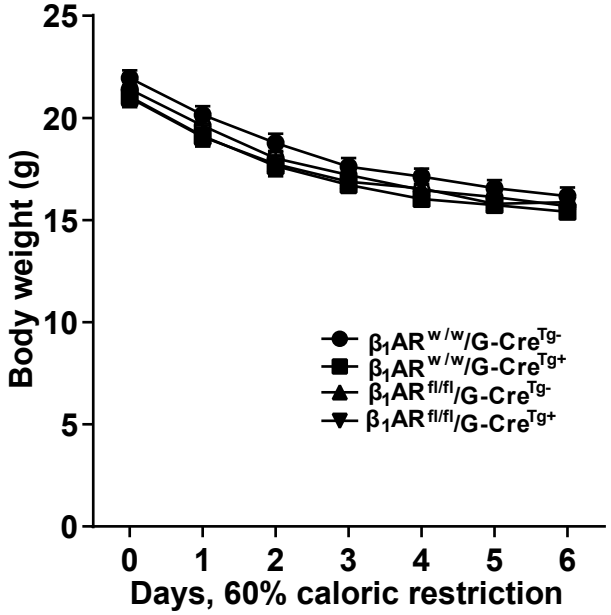

**Preproghrelin mRNA in female GC-$\beta_1$AR$^{-/-}$ mice.** Quantitative estimation of relative preproghrelin mRNA levels in gastric mucosal cells isolated after 16 wks exposure to HFD, in *ad lib*-fed vs. 24h-fasted mice. Data are expressed as ΔΔCt values relative to levels in $\beta_1$AR$^{w/w}$/G-Cre$^{Tg-}$ control mice given 16 wks *ad lib* access to standard chow. n=5-7; n.s., no significant change in mRNA levels among genotypes at either HFD *ad lib*-fed or 24h-fasted conditions. Data analyzed by two-way ANOVA test. Values are expressed as mean ± SEM.

# Supplemental Figure 9



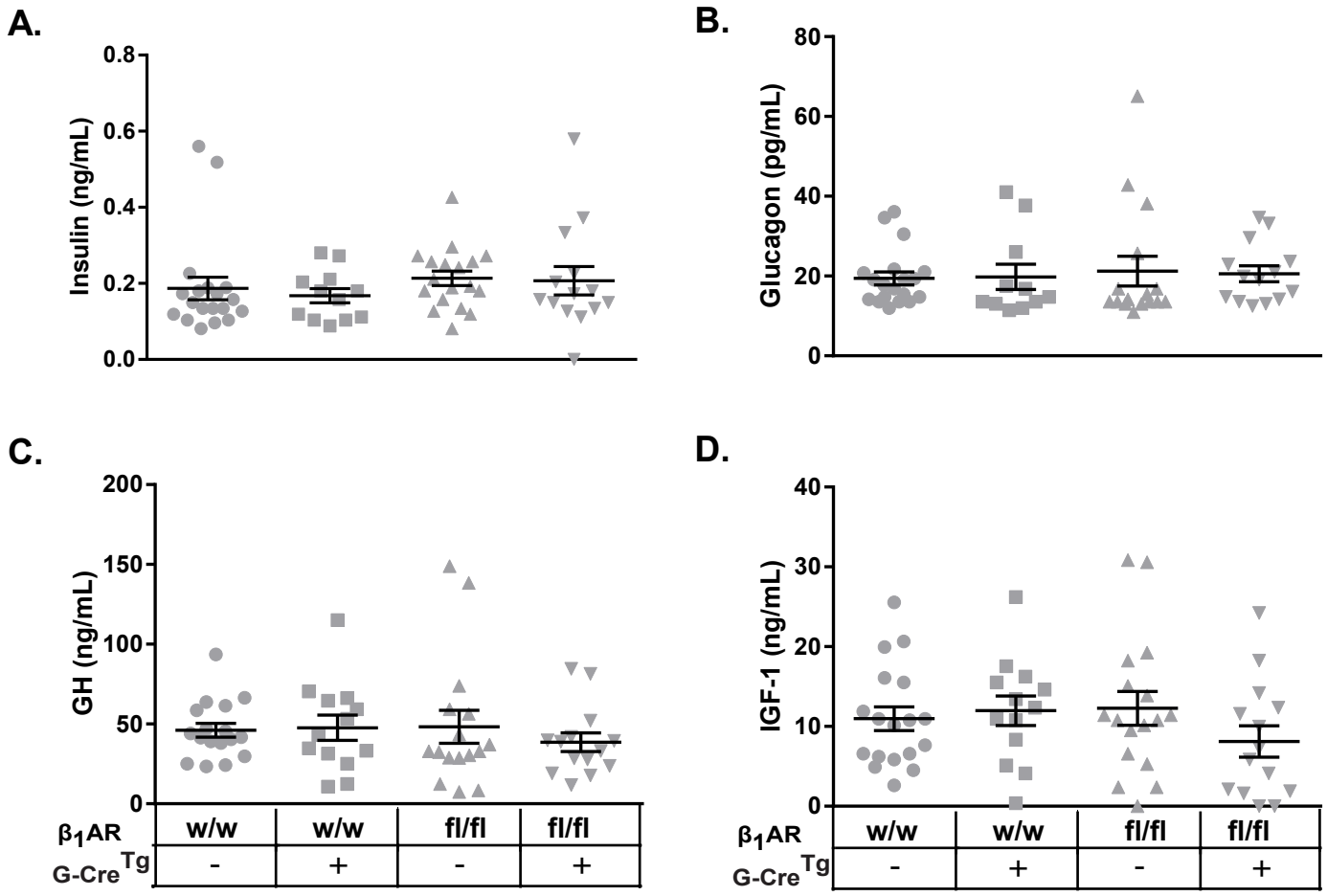

**Body fat mass content and body length in female GC-$\beta_1$AR$^{-/-}$ mice.** (A) Body fat mass content in female GC-$\beta_1$AR$^{-/-}$ mice after 16 wks *ad lib*-access to standard chow or HFD. Fat mass content is expressed as percentage of fat mass to body weight. Data analyzed by two-way ANOVA followed by Sidak post hoc multiple comparison test. ****$p<0.001$, significant difference in body fat mass between mice groups fed standard chow diet compared to mice groups exposed to HFD. n.s., no significant change in body length among genotypes fed the same diet. (B) Body (nose-to-anus) length measured at the end of the study. ****$p<0.001$, significant difference in body length between mice groups fed chow diet compared to mice groups exposed to HFD. n.s., no significant change in body length among genotypes fed the same diet. Values are expressed as mean ± SEM.

# Supplemental Figure 10



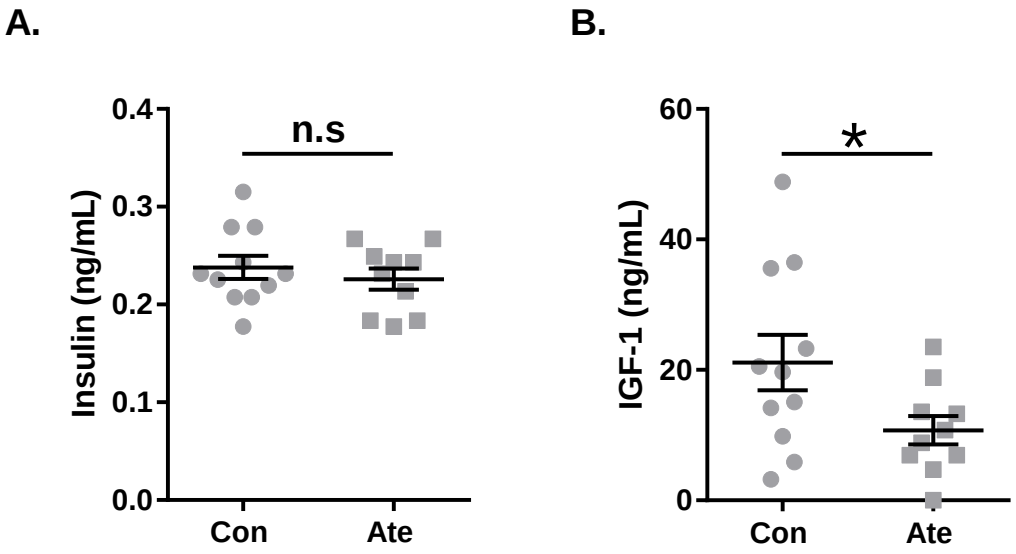

**Body weight of mice subjected to 60% chronic caloric restriction.** Body weights of singly-housed male mice subjected to 60% chronic caloric restriction. No significant change in body weight was observed among the different genotypes when analyzed by two-way ANOVA test. Values are expressed as mean ± SEM.

# Supplemental Figure 11



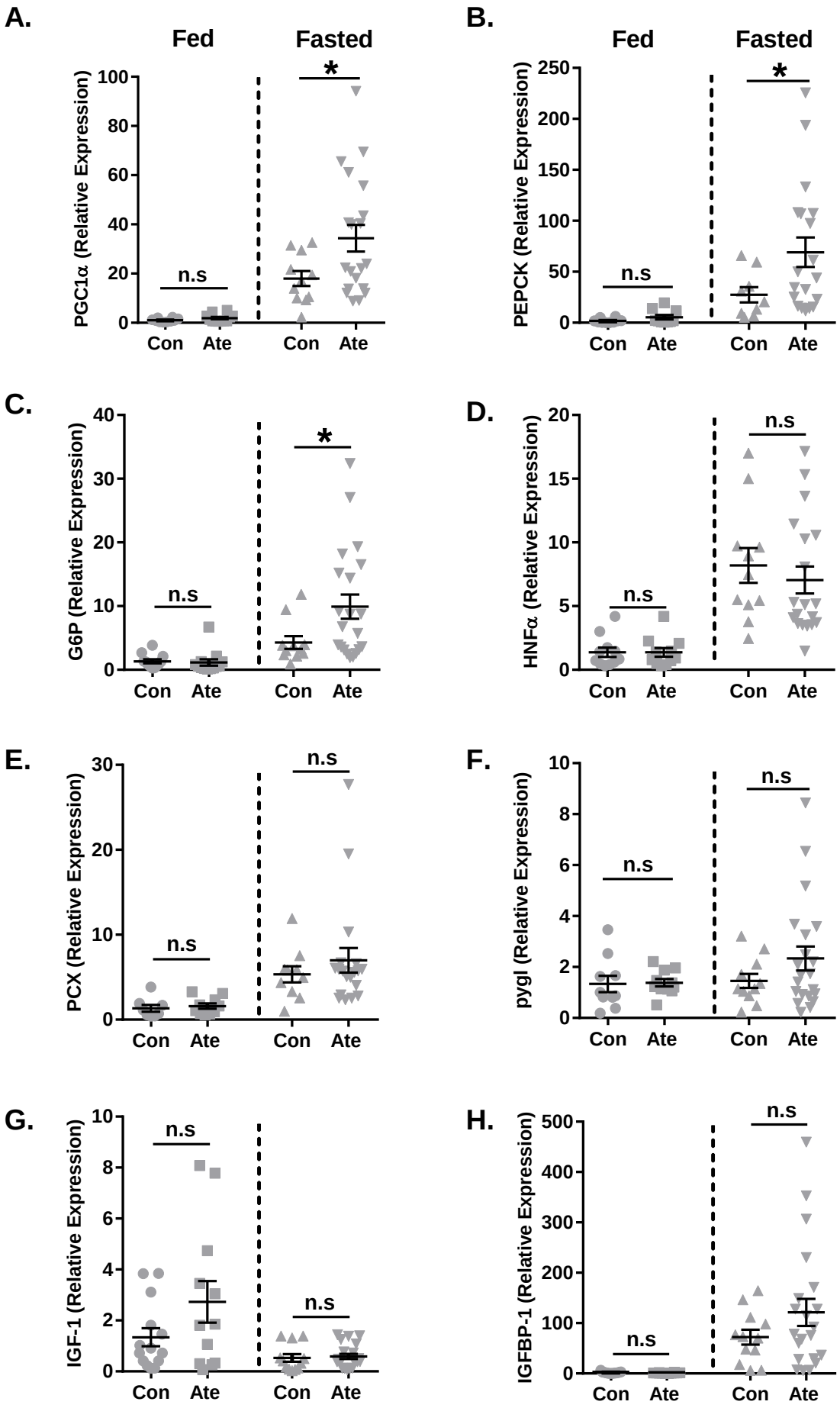

**Hormone levels in mice subjected to 60% chronic caloric restriction.** Plasma insulin (A), glucagon (B) GH (C) and IGF-1 levels in mice after 6d of access to a 60% caloric restriction protocol. No significant difference in the plasma hormone levels were observed. Values are expressed as mean ± SEM. Note that the hormone levels were estimated in the plasma of mice that survived the caloric restriction protocol.

# Supplemental Figure 12




**Insulin and IGF-1 levels in juvenile mice treated with atenolol.** Plasma insulin (A) and IGF-1 (B) levels in 3-wk old male wild-type mice fasted for 24h, which were treated with either vehicle or atenolol (10mg/kg) twice daily for 3d. *$p<0.05$, data analyzed by unpaired Students "t" test. . n.s- no significant difference. Values are expressed as mean ± SEM.

# Supplemental Figure 13



# Supplemental Figure 13

**Effect of atenolol treatment on expression of hepatic glucoregulatory genes in juvenile mice.** Messenger RNA expression of hepatic genes involved in gluconeogenesis: (A) peroxisome proliferative activated receptor, gamma coactivator 1 alpha (PGC1α; *Ppargc1a*), (B) phosphoenolpyruvate carboxykinase 1 (PEPCK; *Pck1*), (C) glucose-6-phosphatase (G6P; *G6pc*), (D) hepatic nuclear factor 4, alpha (HNFα; *Hnf4a*), and (E) pyruvate carboxylase *(Pcx*); involved in glycogeneolysis: (F) liver glycogen phosphorylase *(Pygl);* and linked to the GH pathway*:* (G) insulin-like growth factor 1 (IGF-1; *Igf1*) and (H) insulin-like growth factor binding protein-1 (IGFBP-1; *Igfbp1*) in 3-wk old wild-type mice treated for 3d with atenolol (Ate) or vehicle (Control, Con) under *ad lib* fed or 24h fasted conditions are depicted. *$p<0.05$, data analyzed for each condition by unpaired Students "t" test. n.s- no significant difference. Values are expressed as mean ± SEM.