SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Immunohistochemistry

Free-floating sections were cut at 30 mm from perfused brains using a sliding microtome (Leica Microsystems, Bannockburn, IL). Four sets of sections were generated from the hypothalamus of each brain. Hypothalamic sections were collected from the diagonal band of Broca (Bregma 1.0 mm) caudally through the mammillary bodies (Bregma -3.00 mm). Hindbrain sections were collected from the facial nucleus (Bregma -5.75 mm) caudally through the spinal cord. For the cFos and EGFP IHC, the sections were incubated for 1 h at room temperature in blocking reagent (5% normal donkey serum in 0.01 M PBS and 0.3% Triton X-100). After the initial blocking step, the sections were incubated in rabbit anti-cFos antibody (PC38; EMD Biosciences, Inc., San Diego, CA) diluted 1:75,000 in blocking reagent for 48 h at 4°C, followed by incubation in 1:500 donkey anti-rabbit Alexa 594 (Molecular Probes, Inc., Eugene, OR) for 1 h at room temperature. Hindbrain sections were then incubated in rabbit anti-GFP antibody directly conjugated to Alexa 488 (Molecular Probes) diluted 1:4000 in blocking reagent for 1 h at room temperature. Hypothalamic sections did not require this step. For the pSTAT3 and ACTH IHC, sections were pretreated for 20 min in 1% NaOH, 1% H_2O_2 in 0.01 M PBS; followed by 10 min in 0.3% glycine in 0.01 M PBS; then incubated 10 min in 0.3% SDS in 0.01 M PBS. Sections were then incubated for 1 h in blocking reagent (5% normal donkey serum in 0.01M PBS and 0.3% Triton X-100). The sections were then incubated in rabbit anti-pSTAT3 antibody (Cell Signaling Technology, Danvers, MA) diluted 1:5000 in blocking reagent for 48 h at 4°C, followed by incubation in 1:1000 donkey anti-rabbit Alexa 594 (Molecular Probes, Inc.) for 1 h at room temperature. Sections were then incubated in a second blocking reagent (5% normal goat serum in 0.01M PBS and 0.3% Triton X-100) for 3 h at room temperature. Following this second blocking step, guinea pig anti-ACTH antibody (Peninsula Laboratories, San Carlos, CA) was applied diluted 1:500 in blocking reagent and incubated 72 h at 4°C, followed by incubation in 1:200 FITC-conjugated

goat anti-guinea pig secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Between each stage, the sections were washed thoroughly with 0.01 M PBS. Incubating the sections in the absence of primary antisera was used to ensure specificity of the secondary antibodies. Sections were mounted onto gelatin-coated slides, coverslipped using Vectashield mounting media (Vector Laboratories, Burlingame, CA), and viewed under a fluorescence microscope (Leica 4000 DM).

Cell counting

Results are expressed as the number of cells per section as well as the percentage that are double-labeled. Each set of ARC sections contained seven to nine sections expressing immunopositive cells. POMC immunopositive sections were matched to stereotaxic coordinates (52). Five sections from each animal corresponding to -0.96 mm, -1.22 mm, -1.58 mm, -1.92 mm, and -2.34 mm (from Bregma) were counted and used in the analysis. A cell was determined to be single-labeled when visible only under the fluorescence filter corresponding to the emission wavelength of the primary/secondary antibody complex used. Cells were examined at multiple focal planes within the section and at multiple magnifications to ensure that the cell was indeed representative of a single-labeled cell. When the cell was visible at both 594- and 488-nm filters, it was deemed to be double-labeled. Double-labeled cells were examined at multiple focal planes within the section and at multiple magnifications to ensure that the cell was indeed representative of a single cell labeled with both antibody complexes and not two single-labeled cells in close proximity within different levels of the optical section. The cells were also examined under a third wavelength (350 nm) not corresponding to the emission wavelength of either of the secondary antibodies to ensure that the immunoreactivity was specific.

Double-label in situ hybridization histochemistry

Coronal sections (20 mm) were cut on a cryostat and thaw-mounted onto Superfrost Plus slides (VWR Scientific, West Chester, PA). Hypothalamic sections were collected in a 1:6 series from the diagonal band of Broca (bregma 0.50 mm) caudally through the mammillary bodies (bregma -5.00 mm). Antisense ^{33}P-labeled rat LIF-R riboprobe (corresponding to bases 785-1645 of rat LIF-R; GenBank accession no. NM_031048) (0.2 pmol/ml) and antisense digoxigenin-labeled rat POMC riboprobe (corresponding to bases 49–644 of rat POMC; GenBank accession no. AF_510391) (concentration determined empirically) were denatured, dissolved in hybridization buffer along with tRNA (1.7 mg/ ml), and applied to slides. Controls used to establish the specificity of the LIF-R riboprobe included slides incubated with an equivalent concentration of radiolabeled sense LIF-R riboprobe or radiolabeled antisense probe in the presence of excess (1000X) unlabeled antisense probe. Slides were covered with glass coverslips, placed in a humid chamber, and incubated overnight at 55°C. The following day, slides were treated with RNase A and washed under conditions of increasing stringency. The sections were incubated in blocking buffer and then in Tris buffer containing antidigoxigenin fragments conjugated to alkaline phosphatase (Roche Molecular Biochemicals, Indianapolis, IN), diluted 1:250, for 3 h at room temperature. POMC cells were visualized with Vector Red substrate (SK-5100; Vector Laboratories) according to the manufacturer's protocol. Slides were dipped in 100% ethanol, air dried, and then dipped in NTB-2 liquid emulsion (Eastman Kodak Co., Rochester, NY). Slides were developed 6 d later and coverslipped. POMC-mRNA-containing cells were identified under fluorescent illumination, and Grains 2.0.b (University of Washington, Seattle, WA) was used to count the silver grains (corresponding to radiolabeled LIF-R mRNA) over each cell. Signal-to-background ratios for individual cells were calculated; an individual cell was considered to be double-labeled if it had a signal-to-background ratio of 2.5 or more. For each animal, the amount of double-labeling was calculated as a percentage of the total number of POMC-mRNA-expressing cells and then averaged across animals to produce mean ± SEM.

α-MSH RIA

α-MSH immunoreactivity was measured with a rabbit anti- α-MSH specific for α-MSH (Phoenix Pharmaceuticals, Inc., Belmont, CA). The antibody cross-reacts fully with the mature α-MSH (*N*-acetylated α-MSH), and partially (46%) with desacetylated α-MSH, but not with NPY or AgRP. ^{125}I-labeled α-MSH was prepared by the iodogen method and purified by high-pressure liquid chromatography (University of Mississippi Peptide Radioiodination Service Center, University, MS). All samples were assayed in duplicate. The assay was performed in a total volume of 350 ml 0.06 M phosphate buffer (pH 7.3) containing 1% BSA. The sample was incubated for 3 d at 4°C before the separation of free and antibody-bound label by goat anti-rabbit IgG serum (Phoenix Pharmaceuticals).

200 ml of supernatant were assayed.

Metabolic Phenotyping of PomcCre-gp130$^{flox/flox}$ mice

Food intake and body weight of PomcCre-gp130$^{flox/flox}$ mice (n=10) and littermate controls (n=9) were measured daily from 6 wks to 8 wks after birth to confirm there were no differences in either parameter during the ages used in reported experiments. Refeeding behavior was assessed by removing food from the cages of 6 week-old male, individually-housed PomcCre-gp130$^{flox/flox}$ mice (n=8) and littermate controls (n=8) at lights off (1800 h). Food was returned at 0900 h and food intake was measured at 1, 2, 4, 8, 12, and 24 h. Body weight was also measured daily at 0900 h beginning the morning before food was removed from the cages. Feeding behavior and voluntary activity were measured using metabolic cages equipped with a running wheel and an infrared feeding monitor (Mini-Mitter, Sunriver, OR). Feeding frequency and duration were quantified by measuring the number and duration of infrared beam breaks caused by the animals' heads while feeding. Activity was recorded as the number of wheel revolutions. of Seven week-

old male PomcCre-gp130$^{flox/flox}$ mice (n=10) and littermate controls (n=9) were individually housed in these cages for 5 days. Data from the first two days of adaptation were discarded and data from the remaining three days were averaged across the 24 h period to compare feeding and activity patterns. Metabolic rate was evaluated by indirect calorimetry (Oxymax, Columbus Instruments, Columbus, OH). Mice were housed in separate chambers at 24 ± 1°C. Eight week-old male PomcCre-gp130$^{flox/flox}$ mice (n=10) and littermate controls (n=9) were first acclimatized to the chambers for two days. VO_2 and VCO_2 were simultaneously recorded for 4–5 h during the middle of the light cycle (1100–1600 h). Samples were recorded every 3 min with the room air reference taken every 30 min and the air flow to chambers at 500 ml/min. Basal oxygen consumption was determined for individual curves as the average of the lowest two intervals corresponding to resting periods. Total oxygen consumption was the average of all measurements during periods of activity and inactivity.

Peripheral LPS injections in WT and PomcCre-gp130$^{flox/flox}$ mice for feeding study

On the day of the experiment at 0900 h, 7 week old PomcCre-gp130$^{flox/flox}$ mice (n=4 per treatment) or littermate controls (n=4 per treatment) received i.p. injections of LPS [100 μg/kg (Sigma-Aldrich Corp., St. Louis, MO) dissolved in 0.5% BSA (Sigma-Aldrich) in 0.9% saline] or 0.5% BSA in 0.9% saline alone, and were placed in clean cages with weighed quantities of food. Food intake was measured at 1, 2, 4, 8, 12, and 24 h post-treatment by experimenters blinded to condition and genotype.

Central LIF injection for LIF mRNA study

Eight week old male C57BL/6J mice had stainless steel cannulae placed in their lateral ventricle as previously described (Scarlett et al., 2007). After one week of recovery and restraint handling, animals received i.c.v. injections of LIF (50 ng, n=4) or vehicle (n=6) twice daily at 600 h and again at 1800 h for 5 days. On the morning of the sixth day, animals again received an i.c.v.

injection at 600 h and were sacrificed 90 min later. Hypothalami were isolated and RNA extracted for quantitative RT-PCR analysis as described above.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Hypothalamic CNTF and IL-6 expression in response to LPS and IL-1b.

(A) Relative quantity of hypothalamic CNTF mRNA following i.p. LPS (100 μg/kg). LPS suppresses CNTF expression at 1 h following injection, though CNTF expression is not significantly altered at 4 or 8 h. (B) Central (i.c.v.) IL-1β (10 ng) does not significantly alter CNTF expression 8 h after administration. (C) LPS induces IL-6 expression 4 h following i.p. injection, but IL-6 expression is not significantly altered at 1 or 8 h. (D) IL-1β potently induces IL-6 expression 8 h after i.c.v. administration. Data normalized to vehicle at each time point. Results expressed as mean ± SEM. Statistics calculated by two-way ANOVA followed by a *post hoc* analysis using a Bonferroni corrected *t* test (A, C) or by two-tailed Student's *t* test (B, D)(*$p<0.05$, ***$p<0.001$ vs. vehicle).

Figure S2. LIF does not activate brainstem POMC-EGFP neurons.

(A, D) POMC-EGFP expression in the NTS. (B) Low cFos IR (*red*) in the NTS from vehicle-treated mice (n=4). (C) Few POMC neurons exhibit nuclear cFos IR following vehicle treatment. (E, G) Central (i.c.v.) LIF treatment (10 ng) increases total cFos expression (n=5). (F, H) NTS POMC neurons do not exhibit increased nuclear cFos IR following LIF administration. Results expressed as mean ± SEM. Statistics calculated by two-tailed Student's *t* test (***$p<0.001$ vs. vehicle). *Scale bars,* 100 mm. CC, central canal.

Figure S3. Diagram of PomcCre-gp130$^{flox/flox}$ mouse genetics.

gp130$^{lox/lox}$ mice were generated with *loxP* sites flanking exon 16, which encodes the transmembrane region of the receptor. These mice were crossed with PomcCre mice, which express the viral *Cre* recombinase under the control of the POMC promoter. In POMC neurons

from PomcCre-gp130$^{flox/flox}$ mice, exon 16 is removed from gp130, specifically inactivating the receptor.

Figure S4. Metabolic phenotype of PomcCre-gp130$^{flox/flox}$ mice.

(A) Average daily food intake did not differ between PomcCre-gp130$^{flox/flox}$ mice (n=10) and control littermates (n=9). (B) No changes in body weight were observed between PomcCre-gp130$^{flox/flox}$ mice (n=10) and control littermates (n=9) during the ages of animals used in the experiments described in this manuscript. (C) No changes in refeeding following an overnight fast, measured as cumulative food intake, were observed between transgenic (n=8) and control (n=8) mice. (D) Changes in body weight due to fasting and refeeding did not differ between genotypes. No differences in feeding frequency (E) or duration (F) were observed between PomcCre-gp130$^{flox/flox}$ mice (n=10) and control littermates (n=9). (G) Voluntary activity, measured by running wheel turns, did not differ between PomcCre-gp130$^{flox/flox}$ mice (n=10) and control littermates (n=9). (E, F, G) Measurements were taken for three days and averaged. Cumulative values for each hour were then averaged across animals in each group. (H) No significant differences in basal or total metabolic rate were observed between transgenic (n=10) and control (n=9) animals. Data are expressed as mean ± SEM. Statistics calculated by Student's *t* test (A, H) or two-way ANOVA (B-G).

Figure S5. PomcCre-gp130$^{flox/flox}$ mice show a normal anorectic response to LPS-induced global inflammation. Animals were injected i.p. with LPS (100μg/kg, n=4 per genotype) or vehicle (n=4 per genotype) and food intake was measured at 1, 2, 4, 8, 12, and 24 h post-injection. LPS reduced food intake significantly in both groups, though no effect of genotype was observed at any time point. Data are expressed as mean ± SEM. Statistics calculated by two-way ANOVA.

Figure S6. Hypothalamic LIF expression induced by chronic central LIF administration. Animals were injected i.c.v. twice daily with LIF (50 ng/injection, n=4) or vehicle (n=6) for 5 days. Chronic LIF exposure induced LIF mRNA expression in the hypothalamus compared to

vehicle. Data expressed as mean ± SEM. Statistics calculated by Student's *t* test (*$p<0.05$ vs. vehicle).

Figure S1

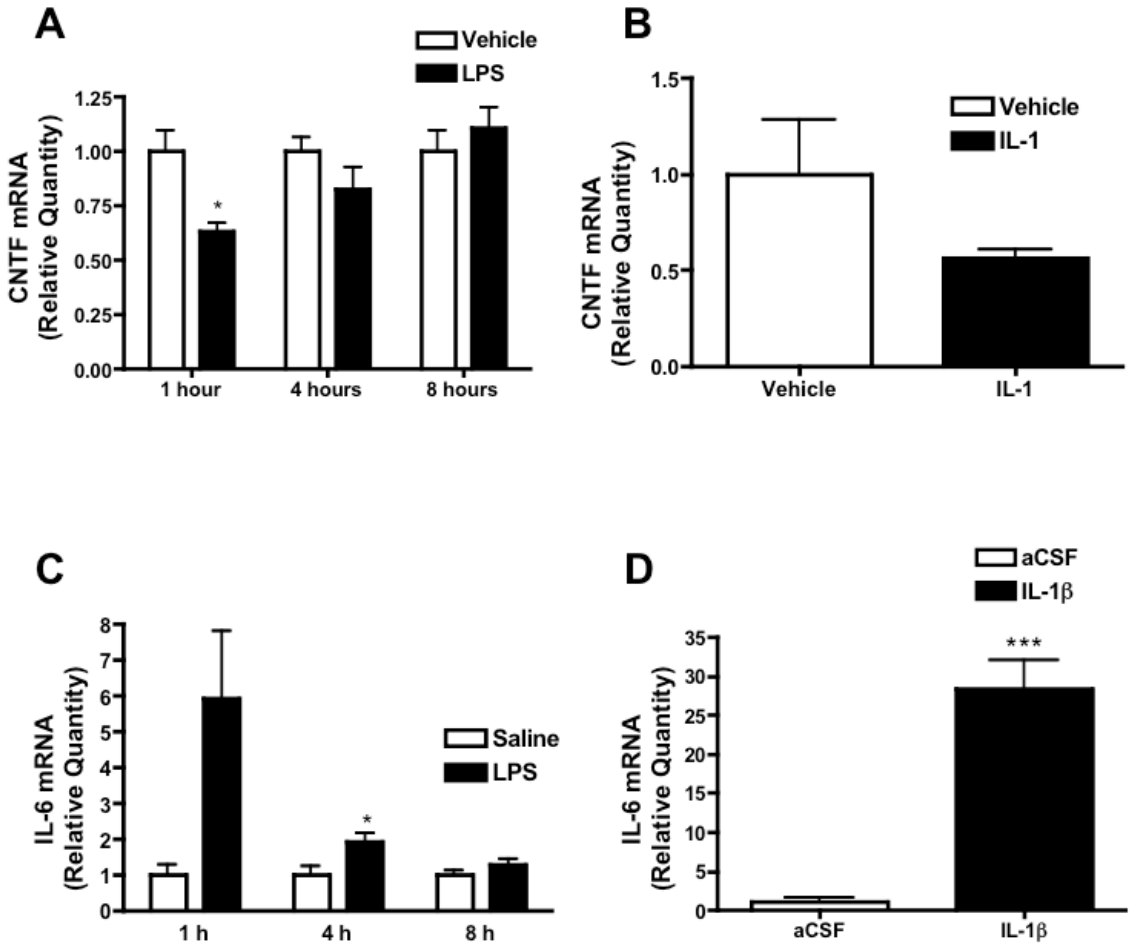
A
Vehicle
LPS
CNTF mRNA (Relative Quantity)
1.25
1.00
0.75
0.50
0.25
0.00
*
1 hour
4 hours
8 hours
B
Vehicle
IL-1
CNTF mRNA (Relative Quantity)
1.5
1.0
0.5
0.0
Vehicle
IL-1
C
Saline
LPS
IL-6 mRNA (Relative Quantity)
8
7
6
5
4
3
2
1
0
*
1 h
4 h
8 h
D
aCSF
IL-1β
IL-6 mRNA (Relative Quantity)
35
30
25
20
15
10
5
0
***
aCSF
IL-1β

Figure S2

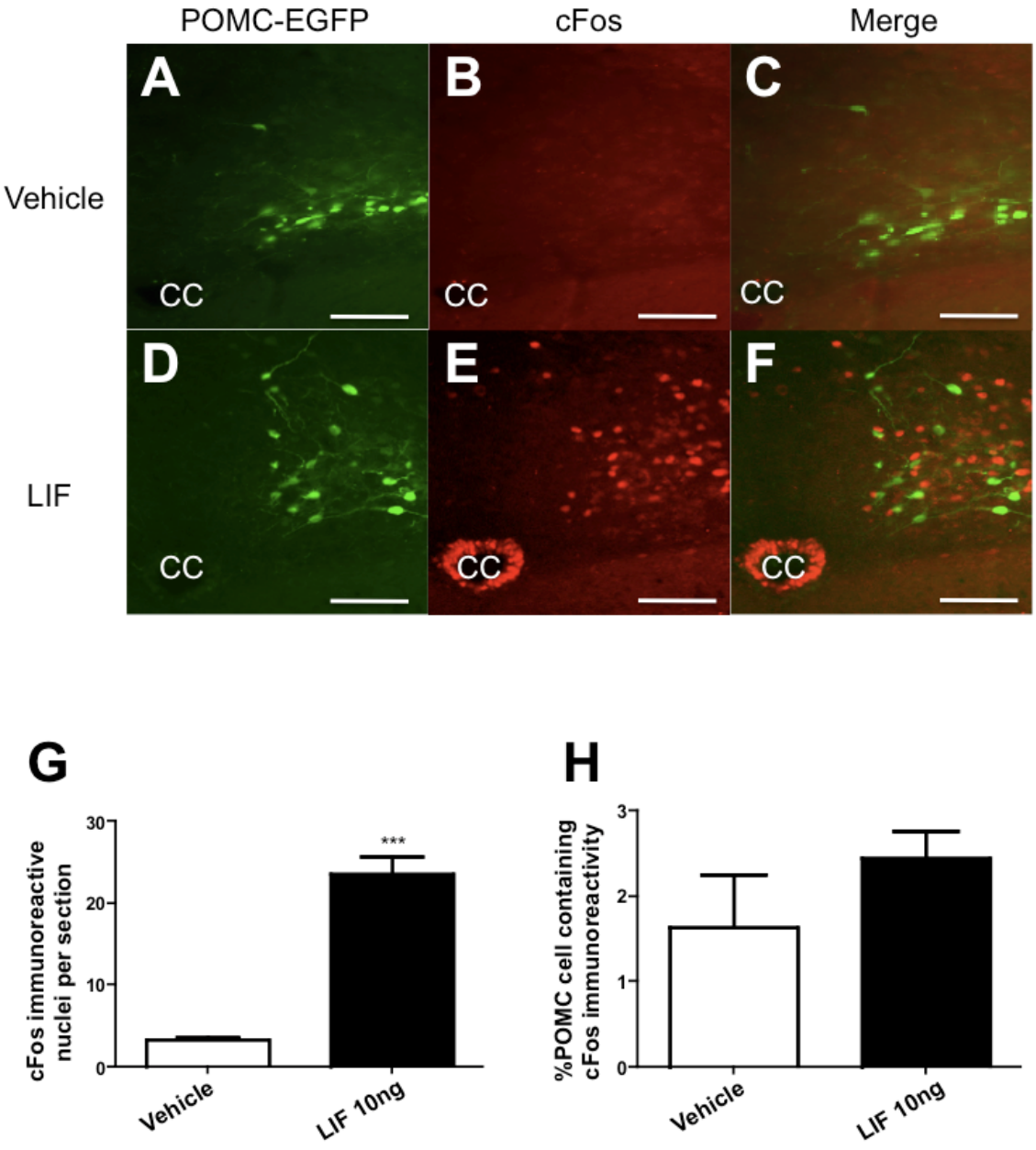
POMC-EGFP
cFos
Merge
Vehicle
LIF
A
B
C
D
E
F
CC
G
cFos immunoreactive nuclei per section
30
20
10
0
***
Vehicle
LIF 10ng
H
%POMC cell containing cFos immunoreactivity
3
2
1
0
Vehicle
LIF 10ng

Figure S3

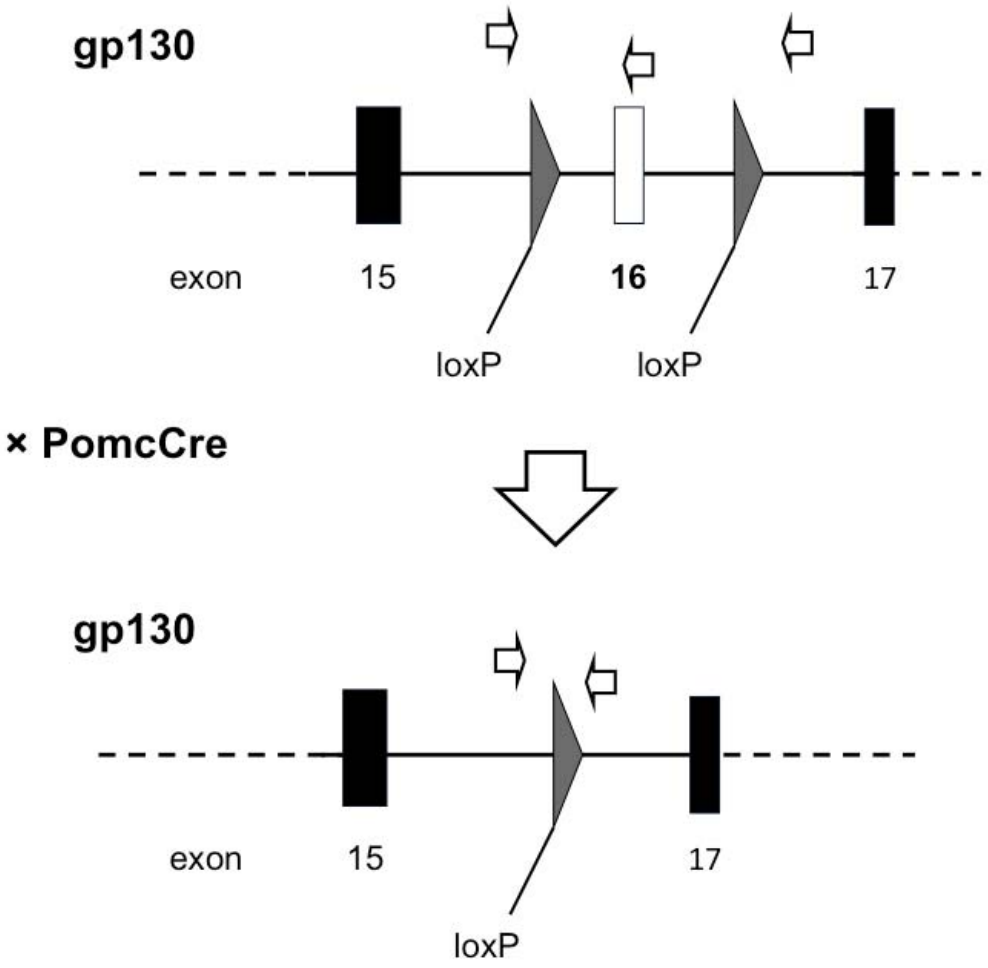

Figure S4

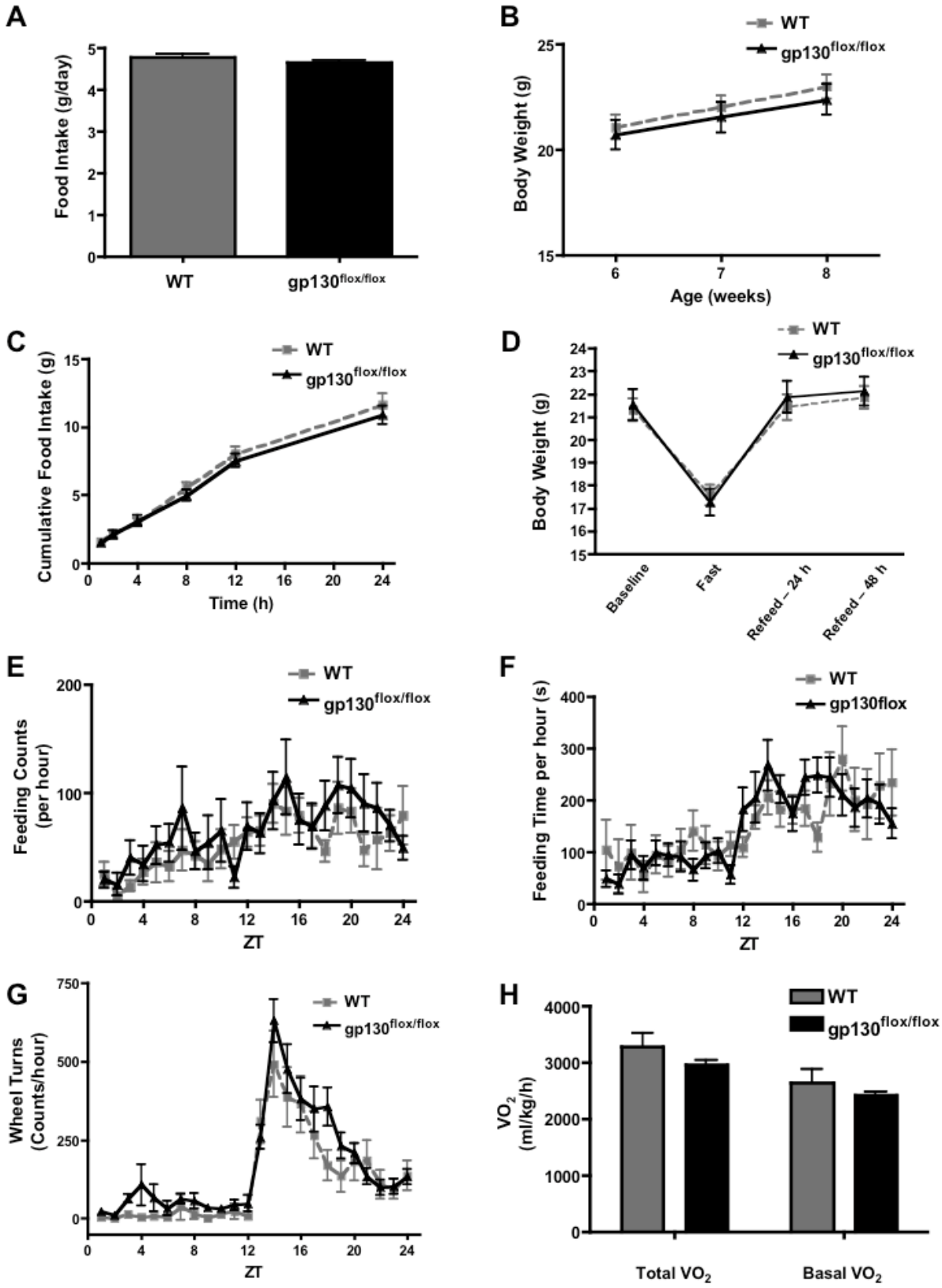
A
Food Intake (g/day)
WT
gp130flox/flox
B
Body Weight (g)
Age (weeks)
C
Cumulative Food Intake (g)
Time (h)
D
Body Weight (g)
Baseline
Fast
Refeed – 24 h
Refeed – 48 h
E
Feeding Counts (per hour)
ZT
F
Feeding Time per hour (s)
gp130flox
G
Wheel Turns (Counts/hour)
H
VO2 (ml/kg/h)
Total VO2
Basal VO2

Figure S5

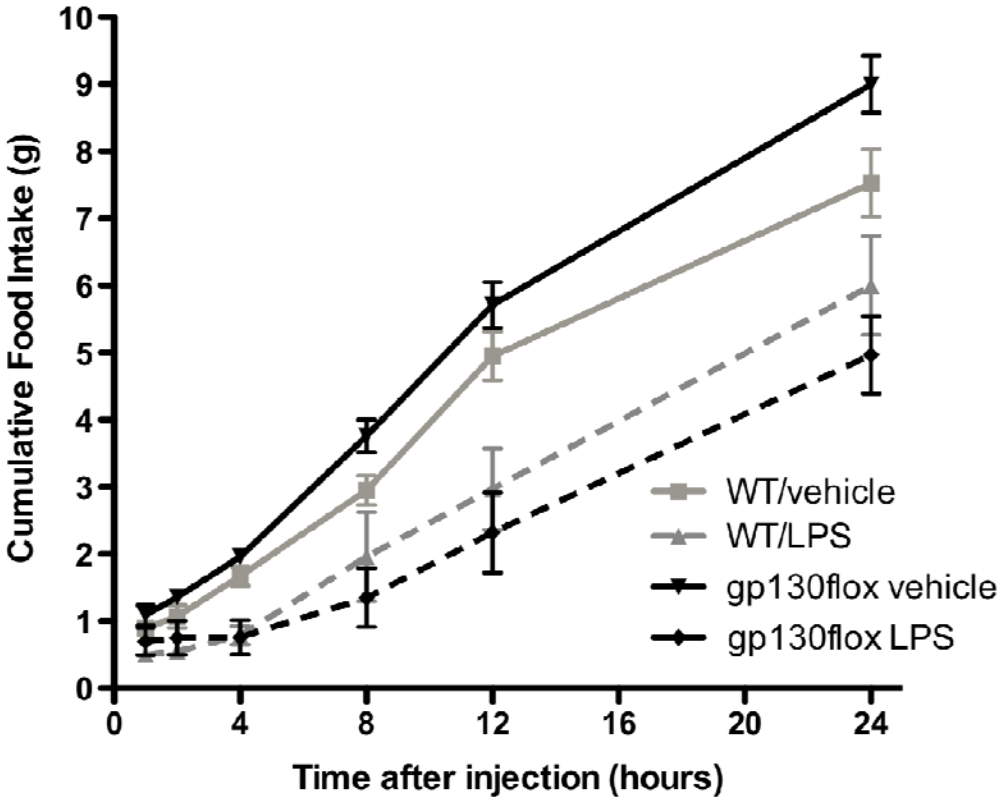
Cumulative Food Intake (g)
10
9
8
7
6
5
4
3
2
1
0
0
4
8
12
16
20
24
Time after injection (hours)
WT/vehicle
WT/LPS
gp130flox vehicle
gp130flox LPS

Figure S6