Supplementary table 1 (related to Figure 7). Acute effects of leptin and insulin in identified *Pomc* neurons from *PLT* mice.

Table 1. Acute effects of leptin and insulin in POMC neurons from PLT mice

| | POMC-hrGFP/ LepR-Positive | | POMC-hrGFP/ LepR-Negative | |
|---|---|---|---|---|
| | leptin | insulin | leptin | insulin |
| Depolarized | 15 (75%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Hyperpolarized | 0 (0%) | 0 (0%) | 0 (0%) | 8 (40%) |
| No response | 5 (25%) | 15 (100%) | 14 (100%) | 12 (60%) |
| total recorded | 20 | 15 | 14 | 20 |

# Supplemental Table 1

## Supplementary Experimental Methods

### Hypothalamic organotypic slice culture

The hypothalamic slices were made essentially as described before(Fukuda et al., 2008; Fukuda et al., 2011). Briefly, C57BL/6 mice pups, 8–11 days old, were decapitated, and the brains were quickly removed. Hypothalamic tissues were blocked and sectioned in depth of 250 μm on a vibratome (VT1000 S, Leica) in chilled Gey's Balanced Salt Solution (Invitrogen) enriched with glucose (0.5%) and KCl (30 mM). The coronal slices containing the arcuate nucleus were then placed on Millicell-CM filters (Millipore, pore size 0.4 μm, diameter 30 mm), and then maintained at an air-media interface in MEM (Invitrogen) supplemented with heat-inactivated horse serum (25%, Invitrogen), Glucose (32 mM) and GlutaMAX (2 mM, Invitrogen). Cultures were typically maintained for 10 days in standard medium, which was replaced three times a week. After overnight incubation in low-serum (2.5%) MEM supplemented with GlutaMAX (2 mM), slices were used for experiments.

### Immunohistochmistry (IHC)

Organotypic slices that were cut out from the membrane were rinsed 3 times for 5 minutes each in PBS, pH 7.4 and then for 20 minutes in 1.0% hydrogen peroxide and 1% Sodium hydroxide in PBS to quench endogenous peroxidase activity. Following a series of PBS washes, slices was incubated for 48-72 hours at 4 °C in pSTAT3 antibodies (Cell Signaling Technology) diluted to 1:3,000 in 3% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA) with 0.25% Triton X-100 in PBS (PBT) with 0.02% sodium azide. After washing in PBS, slices were incubated in a biotinylated donkey anti-rabbit antibody (Jackson ImmunoResearch Laboratories) diluted to 1:1,000 in 3% donkey serum in PBT for 1 hour at room temperature. Slices were then incubated in Avidin, Alexa Fluor 488 conjugated (Invoitrogen) diluted to 1:500

in PBT for 1 hour at room temperature. Slices were then rinsed in PBS and mounted on slides followed by coverslipped using Vectashield (Vector Labs).

**Image analysis**

All fluorescence images were acquired as z-stacks comprising sequential x-y sections taken at 1.0 µm z-intervals by using an ApoTome imaging system (Imager Z1; Zeiss) with a x10/1.4 objective (Zeiss), avoiding saturation of maximum pixel value. The micrographs were generated by compiling Z-stacks of images taken at 1.0 µm z-intervals. Photomicrographs were produced with a Zeiss digital camera attached to the microscope and a Dell desktop computer.

**Arcuate nucleus RNA isolation and cDNA preparation.**

A coronal slice between bregma –1.22 mm and –2.70 mm was made, and then the arcuate nucleus was microdissected with a scalpel under a microscope. RNA was extracted using an RNeasy kit (Qiagen) according to the manufacturer's directions. Genomic DNA contamination was eliminated by DNase treatment. Preparation of cDNA for quantitative PCR assays was performed according to published protocols (Mao et al., 2001) with the following changes: 2.4 µg of RNA was first treated with 2U DNase I and 4.2 mM MgCl2 in a final volume of 40 µl. The reverse transcription reaction was carried out in 100 µl final volume. Following cDNA synthesis, DEPC-H2O was added to increase the sample volume to 300 µl.

**Fluorescence-activated cell sorting (FACS)**

Fluorescence-activated cell sorting collection and analysis of neurons were performed as described elsewhere(Cravo et al., 2011). RNA extraction was performed using the PicoPure RNA Isolation kit (Arcturis, St Louis, Missouri). Before quantitative PCR (QPCR), cDNA was preamplified using 2× TaqMan PreAmp Master Mix (Applied Biosystems, Foster City, California).

**Quantitative PCR.**

Tissue mRNA levels were measured with an ABI 7900HT Sequence Detection System. Analysis of neuropeptide expression was performed using the TaqMan-based efficiency-corrected ΔCt assay with 10 ng cDNA per reaction for 50 cycles(Bookout and Mangelsdorf, 2003). mRNAs with cycle times of 34 or greater were determined to be below detection. We used predeveloped/validated assays purchased from Applied Biosystems for Ptp1b (Mm00448426_m1), Socs3 (Mm00545913_s1), Bip (Mm00517691 m1), and Chop (Mm00492097 A1). qPCR for *Xbp1s* and *GalE* was performed as previously described (Deng et al., 2013). Quantitative PCR data were analyzed using ABI instrument software SDS2.1. Baseline values of amplification plots were set automatically, and threshold values were kept constant to obtain normalized cycle times and linear regression data. PCR efficiencies were calculated from the slope of the resulting standard curves. Normalized mRNA levels in arbitrary units were obtained by dividing the averaged, efficiency-corrected values for sample mRNA expression by that for 18S mRNA expression for each sample. The resulting values were expressed as fold change above control levels.

**Basal glucose and insulin levels**

Fed and fasted glucose and insulin were measured in mice at 3 months of age. Food was removed from the home cages overnight (fasted condition), and blood was collected from the tails. Blood glucose was analyzed using an AlphaTRAK meter (Abbott Laboratories) designed for use with rodents. Blood samples were centrifuged and serum samples were collected from the supernatants. Insulin levels were measured using an ELISA kit (Crystal Chem Inc. Downers Grove, IL) according to manufacturer's instruction.

***Hyperinsulinemic-euglycemic clamps.*** Hyperinsulinemic-euglycemic clamps were performed in the UTSW Mouse Phenotyping Core. Experiments were done in conscious, chronically catheterized mice using previously described techniques (Hill et al., 2010). After a 5 d recovery, food was removed on day of experiment at 0900 h to begin a 4 h fast. [3-$^{3}$H]glucose (PerkinElmer) was infused beginning at $t$ = –120 minutes to calculate glucose turnover. Humulin R (2.5 mU/kg/min; Eli Lilly) was then infused at t = 0 min to induce hyperinsulinemia. Blood samples from the cut tail were taken every 10 min and dextrose (50%) was infused as needed to maintain target blood glucose levels (150 mg/dL).