Supplemental Experimental Methods

Evaluating the glucose responsiveness of hypothalamic POMC Neurons

We first performed the proof-of-principle experiment to carry out the electrophysiological recording to measure the glucose responsiveness of hypothalamic POMC neurons. We conducted electrophysiological analyses on coronal brain slices (300 um) taken through the hypothalamus of two female POMC-GFP mice (6 weeks). Detailed experimental procedures regarding brain slice preparation and whole-cell patch recording are described in the Material and Methods part of the main text.

Validating insulin receptor knockdown using short hairpin (shRNA) expressing adeno-associated viral (AAV) vector.

AAV Validation: C57BL6 mice were anesthetized with isoflurane and the VMH was located using the co-ordinates (from bregma: -1.46mm AP, ±0.4mm ML and -5.2mm DV at an angle of 0°). A microinjection needle was inserted and 0.5ul of the AAV was bilaterally microinjected at a rate of 0.05ul/min over the course of 10 minutes. Following microinjection, the needles were left in place for an additional 5 minutes before being removed. Four weeks after viral inoculation, the animals were sacrificed and processed for immunohistocchemistry or their brains were collected and frozen in dry ice for western blot analysis.

Immunohistochemistry: Mice were deeply anesthetized with sodium pentobarbital, the mice were perfused with 0.01M PBS followed by 10% neutral buffered formalin. The brains were then collected and sunk in 30% sucrose. The brains were subsequently cryosectioned into 30um sections and stored in an antifreeze solution at -20°C. The sections were then washed in PBS, and incubated overnight in mouse anti-GFP antibody overnight. The next day, the sections were
washed in PBS and incubated with the Alexafluor 488 conjugated donkey anti-mouse secondary antibody. The sections were mounted on glass slides and imaged on an Olympus IX81 confocal microscope.

**Western Blot:** Frozen coronal sections were made through the brain and the VMH was located and micropunched. The tissue sections were homogenized in brain lysis buffer, protein concentration of the homogenate was quantified using the Bradford assay. 20ug of protein was loaded on a 4-20% gradient gel and run at 85V for 1-1.5hrs. The protein was then transferred onto a nitrocellulose membrane using the BioRad Turboblot. The membrane was then blocked in PBS-T containing 5% milk and incubated in the primary rabbit anti-inulin receptor antibody (Abcam #ab5500) overnight at 4°C. The next day, the membrane was washed and incubated in the secondary antibody (Perkin Elmer #NEF812001EA) for 1hr at room temperature. The membranes were then washed and incubated in chemiluminescent reagent before being exposed to film and developed. Quantification was performed using ImageLab software from BioRad.
Supplemental Figure 1. POMC neurons respond to glucopenic condition.

(A) POMC neurons in ARC were identified using a fluorescent microscope. (B) Spontaneous action potentials (sAPs) were recorded in the whole-cell current-clamp mode in POMC neurons perfused sequentially with aCSF containing 5 mM glucose (baseline), no glucose and 5 mM glucose (washout). Representative 30 s sections of a current-clamp recording from the same neuron in aCSF containing different extracellular glucose concentration were showed in right side. (C) Mean changes in membrane potential with changes in extracellular glucose concentration. (D) Mean fold changes in sAP frequency with changes in extracellular glucose concentration. Data show means ± SEM (n=6) (**= p<0.01, paired t-test).

Supplemental Figure 2. In vivo validation of the insulin receptor shRNA expressing adeno-associated viral vector. A) Immunohistochemical staining for the green fluorescent protein reporter expression in the ventromedial nucleus of the hypothalamus (VMH) four weeks after viral inoculation. Image shown taken at 60x magnification. B) Representative image and densitometric analysis of insulin receptor (IR) protein levels in VMH micropunches collected from mice four weeks following microinjection of AAV expressing either the scrambled RNA (Scr; white bar) or the insulin receptor shRNA (IR shRNA; black bar). The IR shRNA reduced insulin receptor expression by ~45% in the VMH (* P<0.03 vs Scr).
Supplemental Figure 2