ESM Methods

Primary hypothalamic neurons isolation and treatments. Primary cultures of hypothalamic neurons were prepared as described previously [1]. On day 5, primary cultured hypothalamic neurons were infected with Ad-shPRLR (108pfu/60 cm2 cells), or Ad-Scramble, Ad-PRLR or Ad-GFP for 12 h, and incubated in fresh medium for another 36 h. Cells were incubated in serum-free medium overnight followed by addition of insulin (100nM) for 10 min as previously described [2]. Cells were collected and lysed and proteins were extracted for western blot.

Intracerebroventricular (icv) administration of insulin experiments. Icv administration experiments were conducted as previously described [3]. 1 μl 4x108 pfu/mice of Ad-PRLR, or Ad-GFP were injected into the third ventricle (at the midline coordinates of 1.8 mm posterior to the bregma and 5.0 mm below the bregma) using a micro syringe. For insulin administration experiments, mice were icv injected with adenovirus and implanted with cannula, then allowed to recover for 5 days. After the recovery, mice were fasting overnight and injected with 2mU insulin for 20 min as previously described [3].

References
2. Choi SJ, Kim F, Schwartz MW, Wisse BE. Cultured hypothalamic neurons are resistant to inflammation and insulin resistance induced by saturated fatty acids. Am J Physiol Endocrinol Metab 2010;298:E1122-1130