Supplementary Methods

Total Cellular Protein - Neuro2a.HA-MC4R-eGFP cells in 24-well plates previously transfected with control, *Mgrn1*, or *Atrn* siRNA were transfected with empty-, AGRP-, or ASP-expressing plasmids for ~24 hours, rinsed with cold PBS, and lysed on ice in lysis buffer (150 mM NaCl, 20 mM Tris, 1% Igepal, and HALT protease/phosphatase inhibitor (Pierce)) for 30 minutes. Samples were centrifuged at 4°C at 12,000g for 10 minutes and the protein concentration of the supernatant determined by the bicinchoninic acid (BCA) method (Pierce).

Determination of Cell Number - Neuro2a.HA-MC4R-eGFP cells plated in 12 well plates transfected as previously described were counted using a Countess Automated Cell Counter (Invitrogen) according to the manufacturer's instructions.

Western Blotting for HA-MC4R-eGFP - Neuro2a.HA-MC4R-eGFP cells transfected with empty vector, AGRP, or ASP for 18 hours were rinsed with cold PBS and treated on ice with lysis buffer ((150 mM NaCl, 20 mM Tris, 1% Igepal, 0.1% Triton X-100, and HALT protease/phosphatase inhibitor (Pierce)) for 30 minutes. Samples were centrifuged at 4°C at 12,000g for 10 minutes and the protein concentration of the supernatant determined by the BCA method. Equal amounts of protein were subjected to SDS-PAGE electrophoresis and Western blotting using standard protocols. Band intensities were quantified using ImageJ software (NIH).

Supplementary Figure Legends

Fig S1. Transient co-transfection of *Mgrn1* siRNA and empty-, AGRP-, or ASP-expressing plasmids has no effect on Neuro2a cell total protein or cell number. (A) Neuro2a cells stably expressing HA-MC4R-eGFP previously transfected with control or *Mgrn1* siRNA were transfected with empty vector, AGRP- or ASP-expressing plasmids for ~24 hours and total protein levels were determined by the BCA method. Data are presented as mean ± S.D. of eight independent samples relative to empty vector-transfected controls. (B) Cells transfected as above were collected and counted using a Countess Automated Cell Counter according to the manufacturer's instructions. Data are presented as mean ± S.D. (N = 5-8 independent samples) relative to empty vector-transfected controls.

Fig S2. Transient co-transfection of *Atrn* siRNA and empty-, AGRP-, or ASP-expressing plasmids has no effect on Neuro2a cell total protein. (A) Neuro2a cells stably expressing HA-MC4R-eGFP previously transfected with control or *Atrn* siRNA were transfected with empty vector, AGRP- or ASP-expressing plasmids for ~24 hours and total protein levels were determined by the BCA method. Data are presented as mean ± S.D. of eight independent samples relative to empty vector-transfected controls. * $p < 0.05$.

Fig S3. Transient over-expression of AGRP increases total HA-MC4R-eGFP while ASP promotes decreased receptor levels. (A) Neuro2a.HA-MC4R-eGFP cells were transfected with empty vector, AGRP-, or ASP-expressing plasmids for 18 hours. Whole cell lysates were subjected to SDS-PAGE followed by Western blot analysis for HA-MC4R-eGFP with anti-HA-antibodies. (B) Band intensities for four independent experiments were quantified and normalized to ACTB levels using ImageJ software. All three conditions are significantly different from each other. Bars represent mean ± S.D.