SUPPLEMENTAL MATERIAL (Cernea et al., 2016):

Table 1: Primary antibody information

Peptide/protein target	Antigen sequence (if known)	Name of Antibody	Manufacturer, catalog #, and/or name of individual providing the antibody	Species raised in; monoclonal or polyclonal	Dilution used
Agouti-Related Peptide	82-131-NH_2	Agouti-related protein (AgRP)	Antibodies Australia, catalog# GPAAGRP.1	Guinea Pig, polyclonal	1: 800
Pro-opiomelanocortin (POMC)	AA 27-52	Pro-opiomelanocortin Precursor (POMC)	Phoenix Pharmaceuticals, catalog #H-029-30	Rabbit, polyclonal	1: 4000
Kisspeptin	YNWNSFGLR Y (AA 43-52)	Kisspeptin Antibody	gift from Alain Caraty, catalog# 564	Rabbit, polyclonal	1:200,000
Insulin Receptor beta (β) subunit	AA 1332-1382	Insulin Receptor β Antibody (C-19): sc-711	Santa Cruz Biotechnology, catalog#sc-711	Rabbit, polyclonal	1: 300
Gonadotropin releasing hormone	N/A	Gonadotropin-Releasing Hormone (LHRH)	Covance, catalog #SMI-41R	Mouse, monoclonal	1: 400

Figure 1: IRβ antibody controls

The specificity of the antibody used for IRβ was verified by preabsorption of the diluted primary antibody with immunizing peptide (Santa Cruz) at a concentration of 10μg/ml for 24 hours at 4°C. This pre-treatment eliminated all specific immunolabeling (Fig. 1B). We also ran controls for potential cross-reactivity between the antigen layers for the dual-label fluorescent procedure (Fig. 1C-H). Omission of the second primary antibody (POMC) completely eliminated labelling for that antigen without altering detection of the other (IRβ).

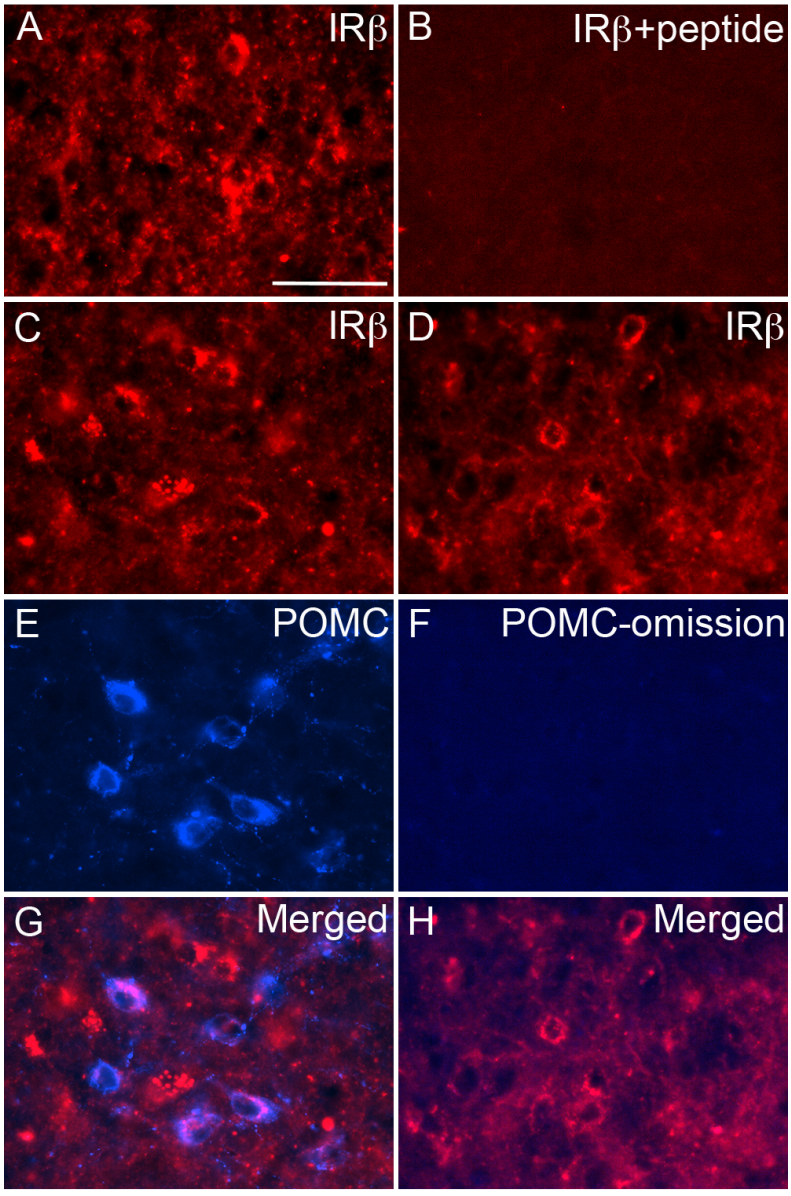

Figure 1: Antibody controls. **Images using fluorescent microscope (DM500B, Leica Microsystems, Wetzlar, Germany) demonstrate complete elimination of IRβ staining by peptide pre-absorption and lack of cross-reactivity with POMC antibody.** **A**-B. Sections processed simultaneous for IRβ immunofluorescence using IRβ antibody (A) or IRβ antibody pre-absorpted with the corresponding peptide (B). C-H) Sections processed simultaneously for dual fluorescence for IRβ and POMC (C, E, G) or for IRβ and POMC with omission of the POMC primary antibody (D, F, H) show that omission of POMC primary antibody completely prevented staining in the appropriate wavelength, demonstrating the lack of cross-reactivity of donkey-anti rabit-CY5 in the absence of rabbit-anti POMC. All images were captured with identical camera settings. **Brightness was increased in B and F.** Scale bar indicates 50 µm.

Figure 2: Triple-label detection of AgRP, POMC, and IRβ

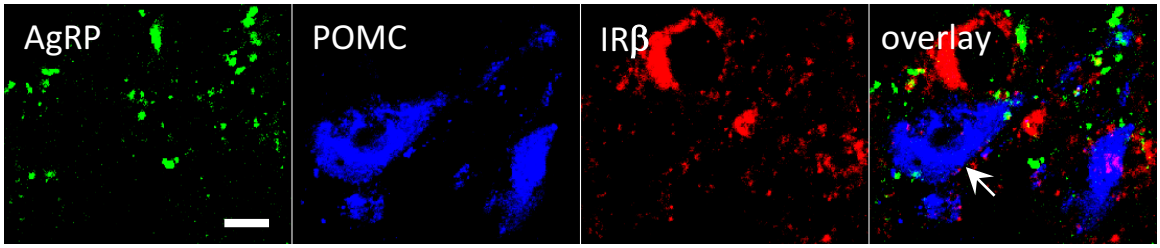

Figure 2: Triple-label immunofluorescent section showing single-labeled POMC (blue cell indicated by arrow) and IRβ (red) positive neurons in the arcuate nucleus of a control ewe. A portion of a dual-labeled POMC/IRβ neuron is also seen at the right edge of the panel.