ESM Methods

IBAT temperature measures

Mice were anesthetized with isoflurane (Baxter, Deerfield, IL) and surgically implanted with a temperature transponder probe (IPPT-300, Bio Medic Data Systems, Seaford, DE), placed directly below the iBAT pad. This transponder is a passive, battery-free, glass-encapsulated microchip device that transmits temperature and identification data to a hand-held transponder reader located in close proximity. The probe was modified by removing the tissue-adherent "anti-migration" capsule, then sliding a ~3mm-long segment of silastic tubing over it to provide a means of suture attachment. The probe was inserted underneath the iBAT through an incision caudal to the pad and sutured to the underlying muscle tissue. Since the length of the probe (~14 mm) exceeds the anterior-posterior length of the iBAT pad, the temperature-sensing tip of the probe was positioned under the iBAT pads, with the other end slightly protruding. After recovery, baseline iBAT temperatures were collected for 2 days, at times 11am, 12pm, 1pm, 1:30pm, 2:00pm, 2:30pm, 30pm, 3:30pm, 4:00pm (beginning of dark cycle) 4:30, pm 5:00pm, 6:00pm, 7:00pm. Then, mice were divided in 2 groups (n=6) receiving daily i.p. injections of vehicle or SR at 12pm and IBAT temperatures were recorded for 12 days. Correct placement of the temperature probe was verified post mortem.

Quantitative PCR

Total RNA from liver, skeletal muscle, BAT, and WAT was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) and purified using the RNEasy Kit (Qiagen, Valencia, CA). cDNA was synthesized from 1-2 µg of total RNA, using the Superscript III First-Strand Synthesis System kit (Invitrogen, Carlsbad, CA). The cDNA and no-RT controls were diluted, and 10ng of cDNA from each sample was used for PCR reactions in duplicate. QPCR was performed with iQ Sybr Green Supermix (BioRad, Hercules, CA) in a MyIQ PCR instrument (Biorad). Relative gene expression was normalized to reference genes (36B4 for liver, soleus, EDL; α- tubulin for BAT, WAT). See supplementary data for primer information.

Histology & IHC

Tissue was removed immediately after the animals were euthanized, and fixed with 4% paraformaldehyde (PFA) for 24hrs, imbedded in paraffin, cut to 10µm sections and adhered to slides in a warm water bath. Staining was performed with Harris hematoxalyn and eosin (H&E). For IHC, tissue was deparaffinized in xylenes and rehydrated through an alcohol series. Tissue was blocked with normal goat serum 1:50 for 1hr. Primary antibody was against TH (Millipore Billerica, MA, catalog # ab152) and was used at a 1:300 dilution; secondary antibody was goat anti-rabbit conjugated to cy5 and diluted 1:500. Tissues were cover-slipped using Gelvitol.