Supporting Information

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SI Methods

Mice. To generate cohorts, females homozygous for the *Ift88*^{flox} conditional allele (*Ift88*^{tm1Bky}) were crossed with CAGG-CreER transgenic; homozygous *Ift88*^{flox}[B6.Cg-Tg(CAGG-cre/Esr1*)5Amc/J] mice to yield either experimental *Ift88*$^{flox/flox}$; CAGG-CreER mice or sex-matched control *Ift88*$^{flox/flox}$ (1, 2). *Bbs4*$^{-/-}$ congenital mutant mice were obtained from Jackson Labs (*Bbs4*^{tmVcs}). All mice in this study were maintained on an inbred C57BL/6 genetic background in accordance with Institutional Animal Care and Use Committee (IACUC) regulations at the University of Alabama at Birmingham.

Induction of Cilia Loss. Tamoxifen (Sigma-Aldrich) was administered to 8-wk-old mice by i.p. injection for 5 consecutive d at a dose of 6.0 mg/40 g body weight as described previously (1). Initial induction of the *Ift88*^{flox} allele was confirmed through genotyping, followed by Western blotting and immunofluorescence.

Fixation and Tissue Processing. Animals were anesthetized by a 0.1 mL/10 g of body weight i.p. injection of 2.5% (vol/vol) tribromoethanol (Sigma-Aldrich), killed by cardiac puncture, and perfused with PBS followed by 4% (wt/vol) paraformaldehyde (Amresco). The brains were then immersion fixed in 4% (wt/vol) paraformaldehyde overnight at 4 °C followed by cryoprotection in 30% (wt/vol) sucrose in PBS overnight at 4 °C. Cryoprotected brains were embedded in Optimal Cutting Temperature compound (Fisher Scientific) and cryosectioned at 20 μm.

Immunoblotting. Whole hypothalami were dissected and isolated into ice-cold lysis buffer (137 mM NaCl, 20 mM Tris, pH 8.0, 1% Triton X-100, 10% (vol/vol) glycerol, and complete EDTA-free protease inhibitor mixture; Roche Diagnostics). After a 5-s sonication, the tissue was incubated on ice for 30 min and then vortexed briefly before centrifugation at 10,000 × *g* at 4 °C for 10 min. Protein concentrations were determined by the DC Protein assay (Bio-Rad Laboratories). Protein samples were resolved on a denaturing 10% Tris-HCL gel (Bio-Rad Laboratories) and transferred to a PVDF transfer membrane (Bio-Rad Laboratories). Membranes were blocked in TBS-T (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20) with 5% (wt/vol) milk for 1 h and incubated with primary antibody diluted in TBS-T with 2% (wt/vol) BSA for 16–24 h at 4 °C. Membranes were probed with horseradish peroxidase (HRP)-conjugated secondary antibodies diluted in TBS-T with 1% milk for 1 h at room temperature. Secondary antibodies were detected using SuperSignal West Pico Chemiluminescent Substrate (Pierce/Thermo Scientific), and bands were visualized using Blue Ultra Autorad Film (Bioexpress ISC). The following primary antibodies and dilutions were used: anti-actin (1:1,000, A2066; Sigma-Aldrich), anti-Adenylate cyclase III (1:500, sc-588; Santa Cruz Biotechnology), anti-Ift88 rabbit polyclonal [1:500; Yoder Laboratory(3)], and anti-Bbs4 rabbit polyclonal (1:500; gift of Kirk Mykytyn, The Ohio State University, Columbus, OH). Secondary antibodies were HRP-conjugated anti-rabbit (1:5,000, #31460) and HRP-conjugated anti-mouse (1:10,000, #31430; Pierce/Thermo Scientific).

Immunofluorescence. Sections were permeabilized and blocked in PBS with 1% BSA, 0.3% Triton X-100, 2% (vol/vol) donkey serum, and 0.02% sodium azide. Sections were incubated in primary antibody overnight at 4 °C, and secondary antibody incubations were carried out for 1 h at room temperature. All incubations were performed in PBS with 1% BSA, 0.3% Triton X-100, 2% donkey serum, and 0.02% sodium azide. Primary antibodies included anti-Adenylate cyclase III (1:500, sc-588; Santa Cruz Biotechnology), anti-cFos (1:200, #2250; Cell Signaling), and anti-pSTAT3 (1:200, MA5-11189; Pierce/Thermo Scientific). Secondary antibodies included the following: Alexa Fluor-546–conjugated donkey anti-rabbit IgG (1:1,000; Invitrogen). Nuclei were visualized by Hoechst nuclear stain (Sigma-Aldrich). Coverslips were mounted using DABCO (Sigma-Aldrich).

Confocal Microscopy. All fluorescence images were captured on a Perkin-Elmer ERS 6FE spinning disk confocal microscope, and images were processed and analyzed in Volocity version 6.1.1 software (Perkin-Elmer).

cFos and pSTAT3 Image Analysis. Confocal image stacks of arcuate nuclei sections were analyzed for cFos and pSTAT3 staining using Volocity and Microsoft Excel 2010. The volume of pSTAT3 and cFos staining was determined as staining intensity greater than the mean intensity of the images + 3 SD. The percent of positive cells was calculated by comparing cFos and pSTAT3 volumes to the volume of all Hoechst stained nuclei in the section.

Serum Leptin Analysis. Mice were handled daily to minimize effects of stress on serum leptin measurements. Individual mice were transported into a procedure room for blood collection from a tail clip. Samples were allowed to coagulate for 30 min at room temperature and then were centrifuged at 2,500 × *g* for 15 min at 4 °C. Serum was collected and stored at −20 °C for future analysis. To calculate leptin levels, a mouse leptin ELISA kit was used (Millipore) according to the manufacturer's protocol. Briefly, a reference curve was plotted using data from standards provided by the manufacturer, and the validity of the assay was confirmed using two separate quality control samples per assay. The measurements of unknown samples were then calculated using a four-parameter logistic function in Microsoft Excel.

Feeding Studies. All body weights were measured with a Mettler-Toledo digital scale. All food intake measurements were conducted on individually housed mice with the BioDAQ episodic intake monitor. To induce weight loss in experimental animals for time point III analysis, food consumption was measured and averaged for each sex. Mice were then given a daily amount 10% less than what they would eat on an ad libitum diet for ∼1 wk. Further 10% reductions from the previous week's consumptions were applied until body weights did not differ from their control counterparts. All animals were monitored daily for signs of distress. Once normal body weight had been achieved through calorie restriction, mice were given the daily amount of food that controls consumed for 10 d. Finally, experimental mice were fed ad libitum until entrained behavior was lost. Time point III leptin sensitivity experiments were then performed as described below.

Leptin Sensitivity Assays. Intraperitoneal injections of 50 μg of recombinant mouse leptin (Phoenix Pharmaceuticals) in 20 mM Tris, pH 8.0 were given just before initiation of the dark cycle. Food intake was assessed with a BioDAQ episodic intake monitor. Leptin sensitivity was assessed by comparing cumulative food intake for the dark cycle period after leptin injection with the cumulative food intake from the previous night when a control injection of 20 mM Tris, pH 8.0, was administered. The leptin sensitivity at each time point was carried out in three separate cohorts of mice.

Activity Measurements. For long-term locomotive activity, independent cages with a camera system (detecting infrared) recorded animal activity over a 24-h period. The system consists of four home cages (30 × 30 cm) with a camera in the center of the top of each cage. The animal is put in the arena, is acclimated with the home cage for 24 h, and is then observed for 24 h with a camera-driven tracker system (Phenotyper; Noldus). The test measures the circadian activity pattern of the mice. The activity is measured on a 12:12-h light/dark cycle, in which the lights turn on at 0600 hours and turn off at 1800 hours. Animals are provided a small black box home in the corner so that they can sleep where movement is undetected by infrared light.

Quantitative Magnetic Resonance Body Composition Measurements. Body composition was measured using the EchoMRI 3-in-1 composition analyzer Quantitative Magnetic Resonance (QMR) instrument (Echo Medical Systems) as previously described (4).

Thermoregulation. Rectal temperatures were measured using a Thermalert TH-5 instrument (Physitemp). Mice were acclimated to the procedure by taking measurements once a day for 3 d before the cold temperature challenge. Baseline temperatures were recorded the morning of the experiment. Thermoregulation of mice was then tested as previously described (5). Briefly, mice were housed without bedding or enrichment at 4 °C for 4 h, during which measurements were taken at 30 min, 2 h, and 4 h. Mice were then moved to 24 °C, and measurements were taken at 30 min, 2 h, and 4 h. All mice had unlimited access to food and water during the course of the experiment. Cold challenge was terminated for an entire group when a single mouse body temperature fell below 32 °C.

Statistical Methods and Analysis. The difference between mice strains and time points of QMR data and serum leptin levels, the difference in rectal temperature in cold exposure between IFT and *ob*/*ob* mice, and the activity chamber data for *intraflagellar transport* (*IFT*) and *ob*/*ob* mice were determined using one-way ANOVA, followed by Tukey's honestly significant difference test. The Student *t* test was used for assessing the effects of vehicle and leptin treatment on food intake and rectal temperature during cold exposure in *Bbs* mice. Statistics were analyzed using Microsoft excel and R 2.14.1 (the R Foundation for Statistical Computing).

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2. Bishop GA, Berbari NF, Lewis J, Mykytyn K (2007) Type III adenylyl cyclase localizes to primary cilia throughout the adult mouse brain. *J Comp Neurol* 505(5):562–571.
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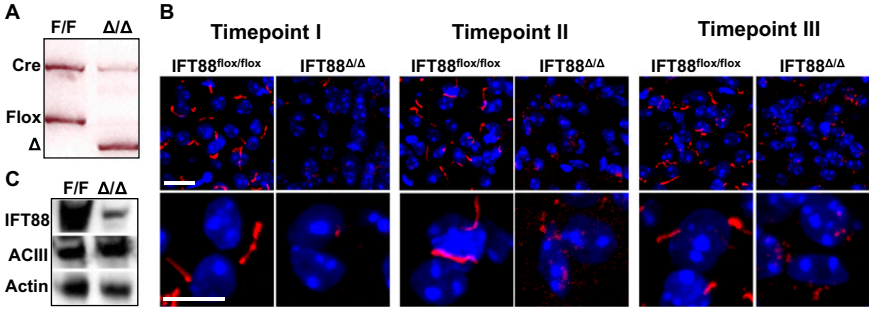

Fig. S1. Genotyping, Western blot, and immunofluorescence in *Ift88* conditional mice. (*A*) Genotyping PCR on nontreated (F/F) and tamoxifen-treated (Δ/Δ) mice, cre transgene (Cre), floxed conditional (Flox), or mutant (Δ) alleles. (*B*) Immunofluorescence for neuronal cilia marker ACIII in arcuate nucleus at each time point. (Scale bars: *Upper*, 21 μm; *Lower*, 10 μm.) (*C*) Western blot on hypothalamic protein 10 d after cre induction in control (F/F) and mutant (Δ/Δ) mice for Ift88, ACIII, and actin loading control.

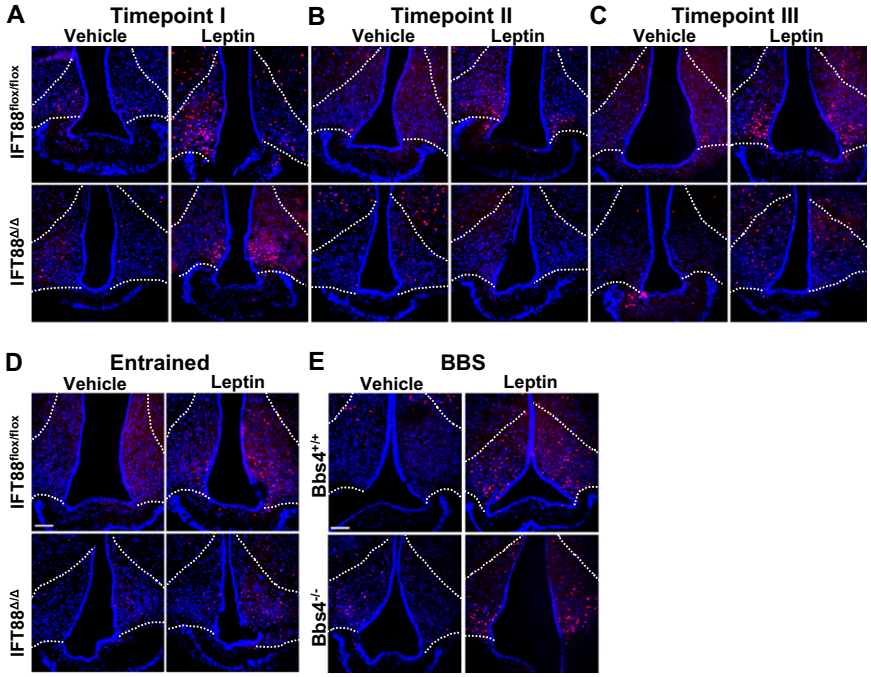

Fig. S2. Acute nuclear cFos induction in the arcuate nucleus in *Ift88* conditional and *Bbs4* mutant mice after leptin injection. (*A*–*C*) Acute induction of cFos (red) in the arcuate nucleus of the hypothalamus 90 min after i.p. leptin or vehicle injection in *Ift88*$^{\Delta/\Delta}$ mutant and *Ift88*$^{flox/flox}$ control mice at time points I, II, and III. (*D*) Leptin induced cFos in *Ift88*$^{flox/flox}$ control and *Ift88*$^{\Delta/\Delta}$ mutant mice during the food anticipatory activity period (Entrained). (*E*) Leptin induced cFos (red) in *Bbs4*$^{+/+}$ and *Bbs4*$^{-/-}$ preobese mice. Dotted lines indicate approximate border of arcuate nucleus based on morphology and nuclear density. (Scale bar, 86 μm.) Hoechst nuclear stain is blue.

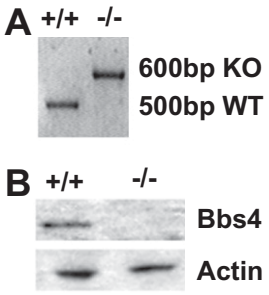

Fig. S3. Verification of *Bbs4* KO mice. (*A*) Genotyping PCR on *Bbs4*$^{+/+}$ and *Bbs4*$^{-/-}$ mice. (*B*) Western blot on hypothalamic protein of *Bbs4*$^{+/+}$ and *Bbs4*$^{-/-}$ for Bbs4 and actin loading control.