**Supplemental Methods**

***Animals***

Ten to eleven weeks−old male C57BL/6 (B6), ob/ob and db/db mice were obtained from Japan SLC (Hamamatsu, Japan). Mice were fed a standard chow diet (Samyang, Seoul, Korea) *ad libitum*, unless otherwise indicated, and were housed in a controlled temperature environment (22 ± 1℃) under a 12 h light-dark cycle (light from 07:00 to 19:00 h). Either saline or leptin (10 μg/day, R&D systems) was infused to the cerebroventricle of ob/ob mice for 7 days using an Alzet osmotic mini-pump (Alzet, Cupertino, CA). Leptin treatment significantly reduced body weights of ob/ob mice (saline 51.7 ± 0.6 g vs. leptin 44.9 ± 1.0 g, $P < 0.05$). DIO mice were obtained by feeding mice a high fat and high sucrose diet (58% kcal% fat with sucrose 175 g/kg food wt, Cat no. D12331, Research Diet Co. New Brunswick, NJ) for 14 weeks. Lean controls were fed a normal chow diet for the same period. Before sacrifice, the DIO mice were significantly heavier than lean controls (46.5 ± 1.1 g vs. 33.2 ± 0.8 g). In parallel with cilia analysis, lean and DIO mice were implanted with a permanent cannulae into the 3rd ventricle (ICV: 1.8 mm caudal to the bregma and 5.0 mm ventral to the sagittal sinus) and received saline to leptin (3 μg) via ICV-implanted cannulae to test leptin sensitivity.

Mice were sacrificed following 5 h fasting in the afternoon unless indicated otherwise. For the feeding manipulation study, freely-fed B6 mice were either starved for 36 h (fasted group) or starved for 36 h followed by refeeding for 6 h (refed group) before being sacrificed. All procedures were approved by the Institutional Animal Care and Use Committee of the Asan Institute for Life Sciences.

***Cell culture***

N1 murine hypothalamic neuron cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, penicillin and streptomycin (100 units/ ml each). Primary cultures of hypothalamic neurons were prepared as previously described (Ref 15). For induction of ciliogenesis, culture medium was replaced with medium without supplements when the cells were ~90 % confluent and the cells were cultured in serum free medium for 48 h before cilia analysis. Cells were treated with leptin (R&D Systems, 10−1000 nM) with or without LY294002 (1 nM, Sigma), wortmannin (10 nM, Sigma) and AG490 (1 μM, Sigma) or insulin (10−50 nM, Sigma) dissolved in serum-free medium for indicated time periods before fixing for cilia staining.

***Staining of cilia***

For the staining of neuron cilia in mice, whole brain was collected following a 15 min-cardiac perfusion with 4% paraformaldehyde (PFA), post-fixed overnight and then cryoprotected with 30% sucrose for 48 h at 4℃. Coronal brain slices, including the hypothalamus and hippocampus (20 or 150 μm thickness), were obtained using a cryostat (Leica, Wetzlar, Germany). Tissue sections were incubated with primary antibodies against type 3 adenylyl cyclase (AC3, 1:1000, rabbit, Santa Cruz) or somatostatin receptor 3 (SSTR3, 1:500, goat, Santa Cruz) at 4℃ for 48 h. Thick (150 μm) brain slices were incubated with anti-AC3 antibody (1:500, Santa Cruz) at 4℃ for 6 days and then overnight at 37℃. For double staining of AC3 with α-melanocyte stimulating hormone (αMSH), microtubule-associated protein (MAP2) and glial fibrillary acidic protein (GFAP), slices were simultaneously incubated with anti-AC3 and anti-αMSH (Chemicon, sheep, 1:15,000), anti-MAP2 (1:1000, mouse, Sigma) or anti-GFAP antibodies (1:1000, mouse, Sigma) for 48 h at 4℃. After washing, slides were incubated with Alexa-Fluor-555-conjugated donkey anti-rabbit antibody, Alexa Fluor-488 donkey anti-mouse antibody or anti-sheep antibody (1:1000, Invitrogen) at room temperature for 1 h. For nuclear staining, slides were treated with DAPI (1:10,000, Invitrogen) for 10 min before mounting.

For cilia staining in neuron cell lines, cells were fixed with 4% PFA for 15 min at room temperature and incubated with primary antibodies against AC3 (1:1,000, rabbit, Santa Cruz) or acetylated α-tubulin (1:1000, mouse, Sigma) at 4℃ for 48 h. For the staining of basal body, cells were fixed with 100% methanol for 4 min at −20℃ and incubated with anti-γ-tubulin antibody (1:400, goat, Santa Cruz) at room temperature for 1 h.

***Cilia analysis***

Immunofluorescence was examined using a laser scanning confocal microscope (Carl Zeiss or Leica). Cilia images were taken in three brain slices per animal (10 fields per brain region per slice) using confocal microscopy through a Z-axis stack, and images were merged using maximum intensity projection. For cilia analysis of thick brain slices, about 100 confocal images per field were taken by Z-stacking (1.5 μm thickness) and reconstructed into 3D projections using IMARIS software (Bitplane AG, Zurich, Switzerland). Measurements of cilia length, area, volume, and number were conducted using the IMARIS program. The cilia

of neuron cell lines were analyzed in randomly selected fields (at least 100 cilia per well and three wells per condition). Cilia analysis of cultured cells was conducted using the Image-Pro Plus program (Media Cybernetics, Silver Spring, MD). The total cell number was determined by DAPI staining of cell nuclei, and data are presented as the percentage of ciliated cells. Cell experiments were repeated at least three times.

***Immunoblotting***

Tissue or cell lysates (50 μg proteins) were separated by 8% SDS-PAGE and transferred to a PVDF membrane (GE Healthcare, Bur). Following incubation in blocking buffer, membranes were incubated overnight at 4℃ with antibodies directed against total- and phospho-PTEN (1:1000, rabbit), total- and phospho-AKT (1:1000, rabbit), total and phospho-GSK3ß (1:1000, rabbit), or total- and phospho-STAT3 (1:1000, rabbit) (all antibodies from Cell Signaling). Blots were developed using horseradish peroxidase (HRP)-linked secondary antibody and a chemiluminescent detection system (New England Biolabs, Beverly, MA).

***Small inhibitory RNA***

The small inhibitory RNAs (siRNA) specific to murine *Ift88*, *Kif3a*, *Pten* and *Gsk3b* were purchased from Dharmacon (Chicago, IL) and *Pi3k p85a* siRNA was obtained from Sigma. For the cell study, N1 cells were transfected with non-targeting scrambled control siRNA (from Dharmacon), *Ift88* siRNA, *Kif3a* siRNA or *p85a* siRNA (50 nM each) using Lipofectamine. For the animal study, siRNA was resuspended in RNase-free water and mixed with Lipofectamine (9:1 v/v) to a final concentration of 1 mM. *Ift88* and *Kif3a* siRNA (0.5 μl each side) were injected bilaterally into the mediobasal hypothalamus over a 5-min period as previously described. The same amount of control siRNA was administered to the control group. Food intake, energy expenditure, locomotor activity, and body weight were monitored for 48 h post-siRNA injection using a comprehensive animal metabolic monitoring system (Columbus Instruments). In a separate experiment, mice injected with siRNAs received leptin (3 μg), insulin (Humulin-R, 3 mU, Eli Lilly), glucose (0.75 mg), or saline through ICV-implanted cannulae after an overnight fast, and food intake was monitored for 24 h following the ICV injection. Some of the mice were sacrificed 45-60 min after ICV injection of saline or leptin to analyze hypothalamic STAT3 phosphorylation or c-

fos activation. At the end of the study, mediobasal hypothalami were collected to confirm the successful knockdown of *Kif3a* and *Ift88*. Results from animals showing successful knockdown of the target gene (defined as less than 70% of the average value of the control siRNA group) were included in analyses.

***Transfection***

N1 cells were cultured in 12-well plates and were transfected with mock plasmid or plasmids expressing *Flag-Pten* or *Gsk3b* (300 ng) using Lipofectamine (Invitrogen). After transfection, cells were cultured in serum-free medium for 30 h and then treated with PBS or leptin (100 M) for 18 h before cilia analysis. Data are shown as fold changes above those of mock vector-transfected controls. Transfections were performed in quadruplicate and the experiments were repeated at least twice. N1 cells were transfected with plasmid expressing *Lepr-eGFP* (300 ng). At 48 h after transfection, cells were subjected to immunostaining of AC-3 or γ-tubulin.

***Real time PCR***

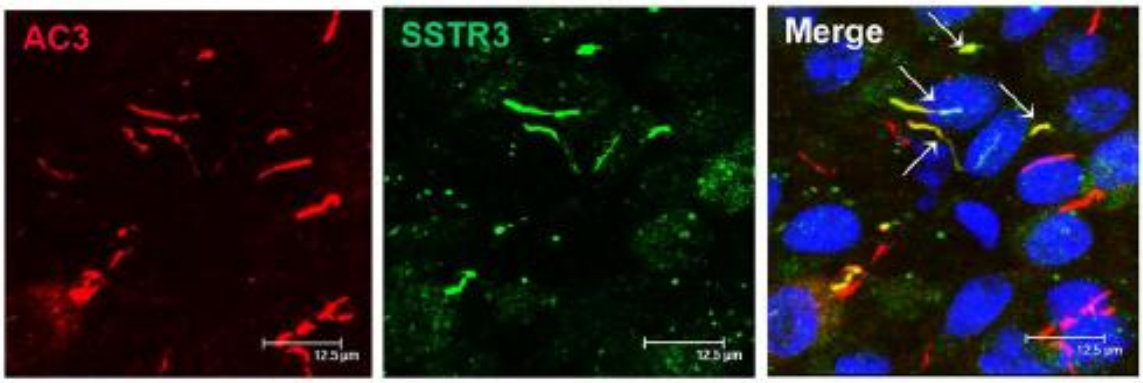
Total RNA was extracted from the N1 cells and hypothalamic tissue block using Trizol reagent (Invitrogen, Carlsbad, CA) and quantified by spectrophotometry. The mRNA expression levels of *Kif3a*, *Ift88*, *Pten*, *Gsk3b*, and *Pi3k p85a* were determined by real time PCR analysis using the primer sets in the following table. The expression level of each mRNA was normalized to that of *Gapdh* mRNA.

| Gene | | **Primer (5' to 3')** |
|---|---|---|
| *Pten* | Forward | GCAAACAAAGACAAGGCCAA |
| | Reverse | AGCCTCTGGATTTGATGGCT |
| *Gsk3b* | Forward | ATGGTCTGCAGGCTGTGTGT |
| | Reverse | TCCACCAACTGATCCACACC |
| *Kif3a* | Forward | AGCTGCGATAATGTGAAGGTG |

| | | |
|---|---|---|
| | Reverse | GTTCCCCTCATTTCATCCACG |
| *Ift88* | Forward | GCAGTGACAGTGGCCAGAAC |
| | Reverse | AAGGTTCATCTGTCCCAGGC |
| *Pi3k p85a* | Forward | GCCCAAGAACTACAGAGCCC |
| | Reverse | GTCCCATCAGCAGTGTCTCG |
| *Gapdh* | Forward | ACTCTTCCACCTTCGATGC |
| | Reverse | CCTGTTGCTGTAGCCGTAT |

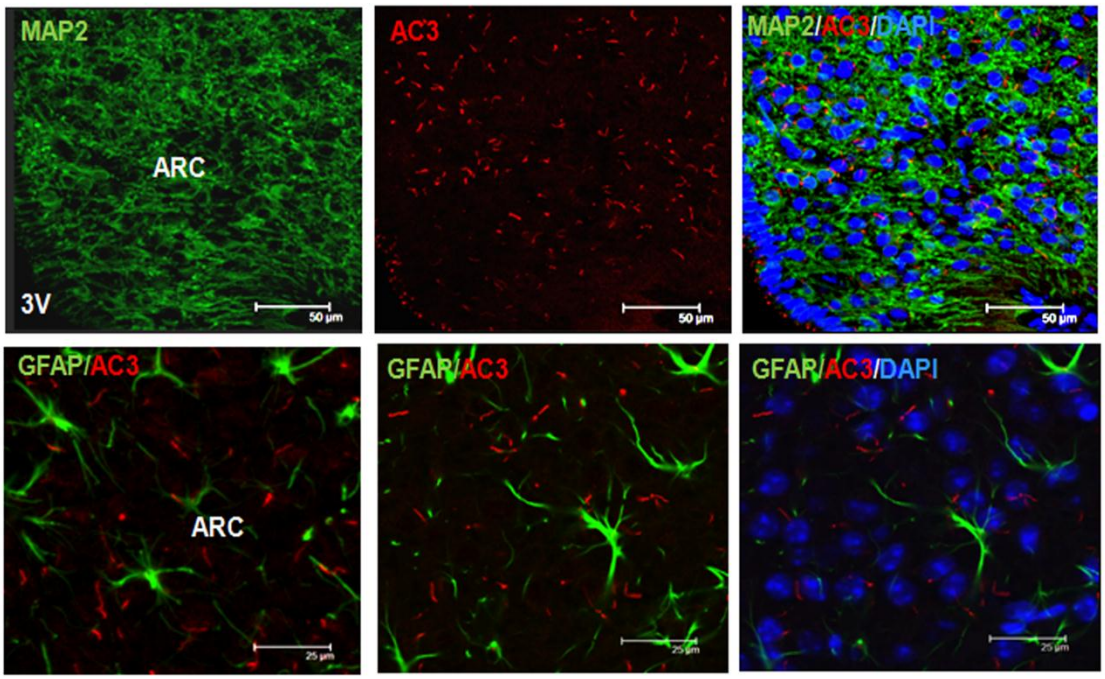
***Statistical analysis***

Data are presented as mean ± SEM. Statistical analysis was performed using SPSS-PC13 (Chicago, IL). Statistical significance among the groups was tested using one-way analysis of variance (ANOVA) followed by a *post-hoc* LSD test. Repeated ANOVA (a test for sphericity followed by the Greenhouse-Geisser test) was used to compare cilia frequency between groups according to cilia length. Significance was defined as $P < 0.05$.



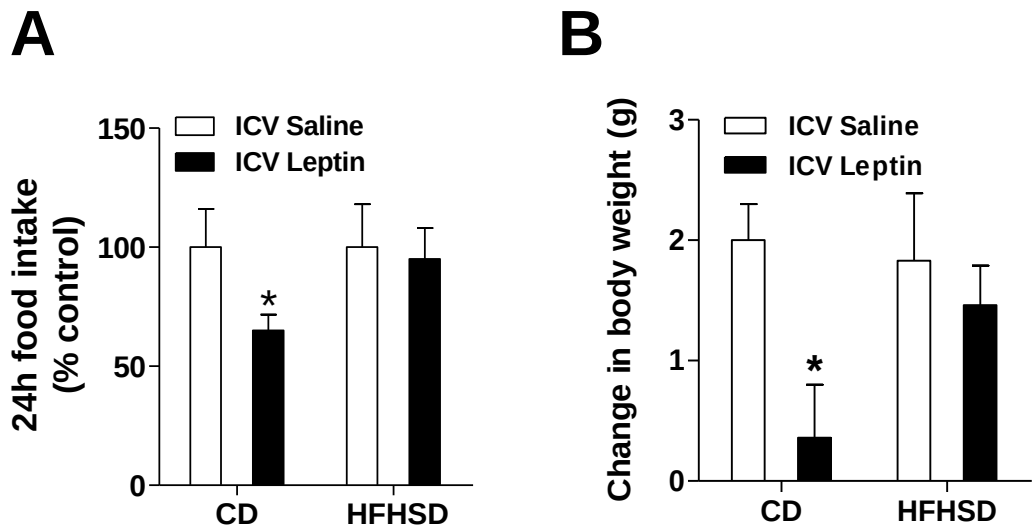

**Supplemental Figure 1. Coexpression of type 3 adenylyl cyclase (AC3) and somatostatin receptor 3 (SSTR3) in hypothalamic neuron cilia.**

Double immunofluorescence staining of AC3 and SSTR3 in hypothalami of normal mice. Some cilia express AC3 but not SSTR3. Arrows indicate hypothalamic neuron cilia coexpressing AC3 and SSTR3. Scale bars, 12.5 μm



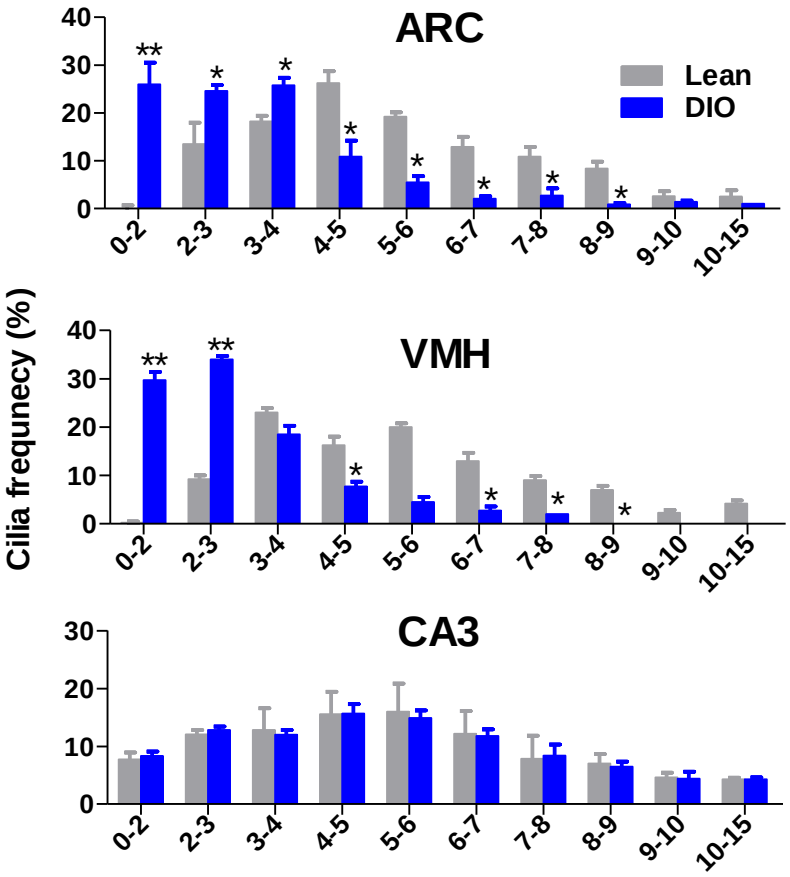

**Supplemental Figure 2. Dual staining of the cilia marker AC3 and the neuronal marker MAP2 or the glial marker GFAP in the hypothalamic ARC of normal mice.**

Brain slices that included the hypothalamic arcuate nucleus (ARC), obtained from normal mice, were simultaneously incubated with anti-AC3 and anti-MAP2 or anti-GFAP antibodies for 48 h at 4℃. Most hypothalamic cilia are found on MAP2-expressing neurons. Scale bars, 50 μm (upper panel), 25 μm (lower panel).



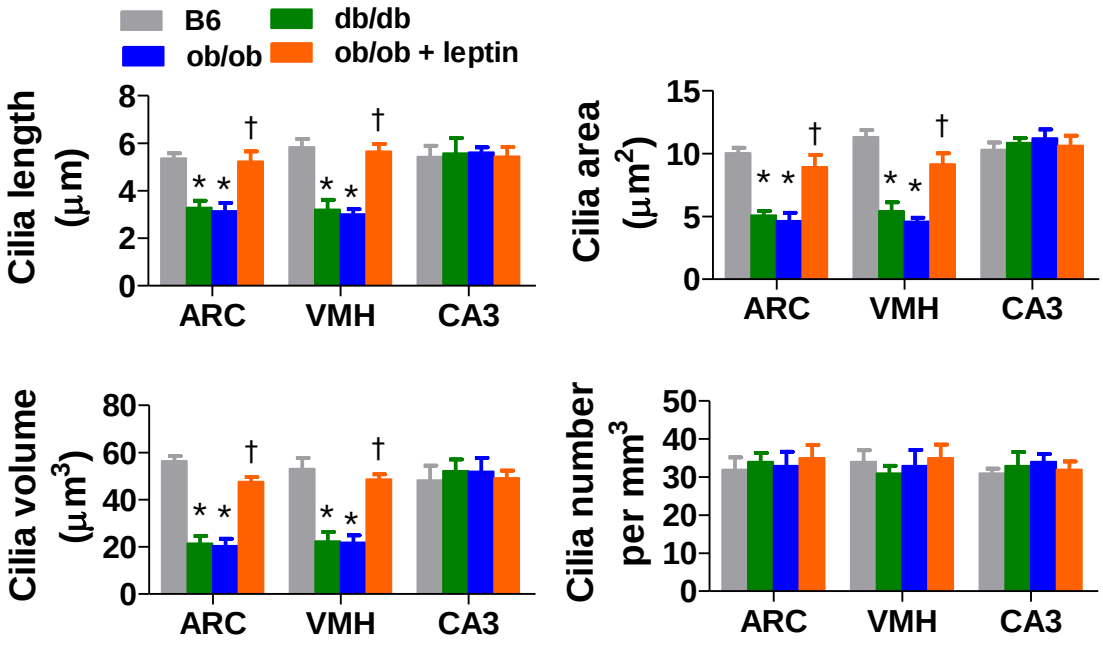

**Supplemental Figure 3. Development of central leptin resistance in diet- induced obese (DIO) mice fed a high-fat, high-sucrose diet (HFHSD).**

Mice were fed either a chow diet (CD) or a HFHSD for 14 weeks until the feeding study. One week before the feeding study, permanent cannulae were implanted into the 3rd ventricle of B6 mice. Either saline or leptin (3 μg) was injected via ICV-implanted cannulae following an overnight fast ($n$ = 5 per group). Food intake and body weight were monitored before and at 24 h after ICV injection. Data are expressed as a % of the saline-injected control for each diet group. *$P < 0.05$ *vs.* saline-injected CD group.



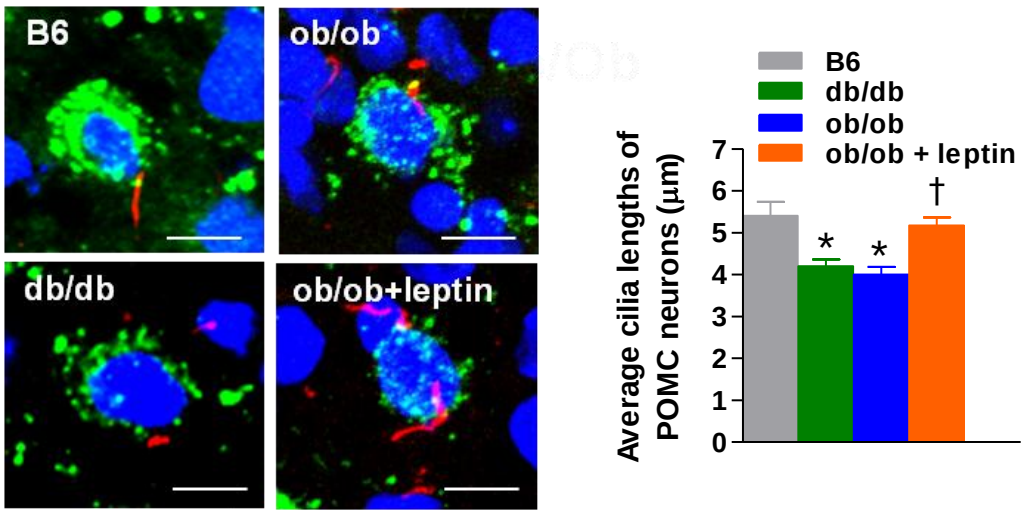

**Supplemental Figure 4. Changes in cilia length distribution in hypothalamus and hippocampus of DIO mice.**

The cilia length distribution was analyzed in the hypothalamic ARC, VMH and hippocampus region CA3 (CA3) of DIO mice. ($n$ = 5 per group). Neuronal cilia were stained with AC3 and analyzed in the hypothalamic ARC, VMH and hippocampus region CA3 (CA3). Data represent mean ± SEM. *$P$ < 0.05, **$P$ < 0.005 *vs.* lean B6 mice.



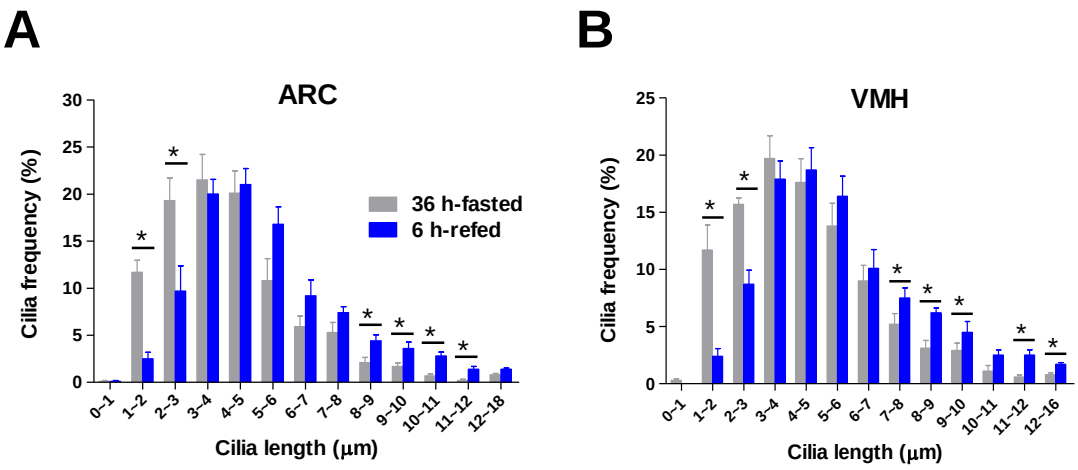

**Supplemental Figure 5. Cilia analysis in hypothalamus and hippocampus in ob/ob mice with or without leptin treatment and db/db mice.**

Cilia analysis was performed in the ARC, VMH, and CA3 in 12-week-old lean (B6) or obese (ob/ob and db/db) mice, and in ob/ob mice that were treated with leptin (10 μg/day) for 7 days ($n$ = 3–4 mice/group). Data represent mean ± SEM. *$P < 0.05$ *vs.* lean B6 mice; $^{\dagger}P < 0.05$ *vs.* untreated ob/ob mice.



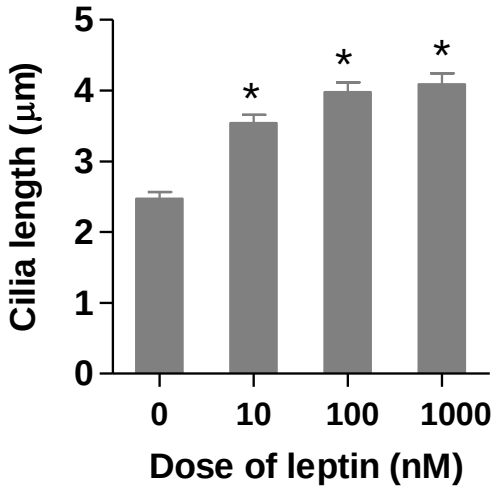

**Supplemental Figure 6. Alterations in POMC neuron cilia in db/db mice and ob/ob mice with or without leptin treatment.**

Brains were collected from 12-week-old lean B6 mice, db/db mice, ob/ob mice, and ob/ob mice after leptin treatment (10 μg/day for 7 days) ($n$ = 5-6). For double staining of AC3 and α-melanocyte-stimulating hormone (αMSH), slices were simultaneously incubated with anti-AC3 and anti-αMSH antibodies for 48 h at 4℃. About 100 POMC neuron cilia were measured per animal. Scale bars represent 12.5 μm. Data are expressed as the mean ± SEM. *$P$ < 0.01 *vs.* B6 mice; $^{\dagger}P$ < 0.05 *vs.* leptin-untreated ob/ob mice.



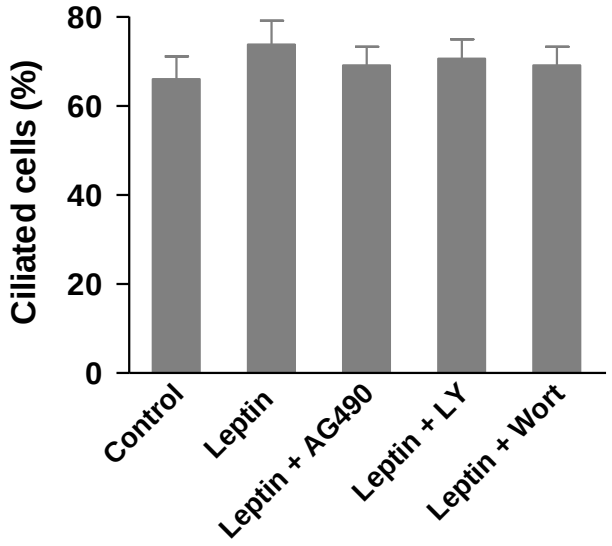

**Supplemental Figure 7. Changes in hypothalamic neuron cilia according to feeding conditions.**

The cilia length distribution in the hypothalamic ARC and VMH was analyzed in the brains of B6 mice that were either subjected to a 36 h fast **(A)** or a 36 h fast followed by 6 h of refeeding prior to sacrifice **(B)** ($n$ = 6 per group). Neuronal cilia were stained with AC3 and analyzed in the hypothalamic ARC and VMH (10 fields from each brain region per section, three sections per animal) using the Image-Pro Plus program (Media Cybernetics). *$P < 0.05$ between the indicated groups.



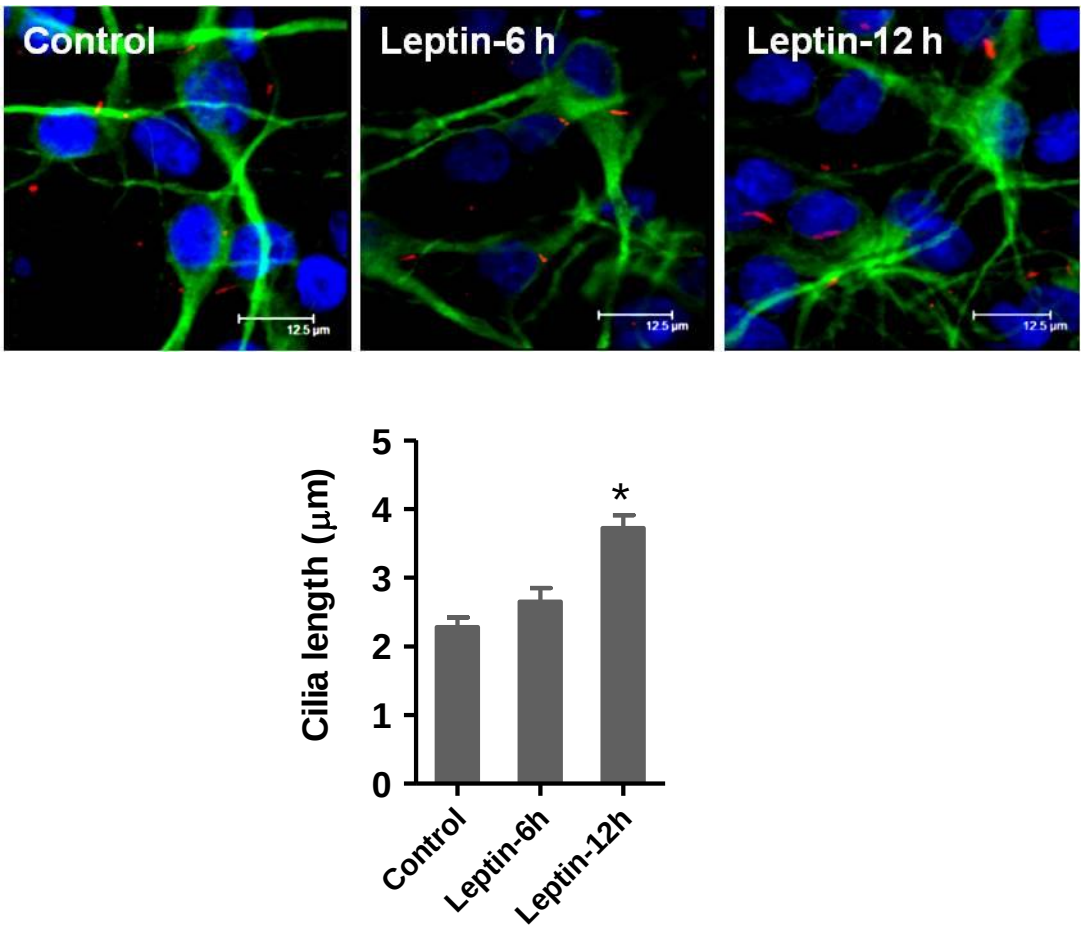

**Supplemental Figure 8. Dose-response study of leptin treatment on cilia length in N1 cells.**

N1 neuronal cells were cultured in serum-free media for 30 h and then treated with leptin (10-1000 nM) for 18 h before cilia staining. For cilia staining, cells were fixed with 4% PFA for 15 min at room temperature and stained with an anti-AC3 antibody. The percentage of ciliated cells was determined based on DAPI staining of cell nuclei. Experiments were conducted in triplicate wells and repeated at least three times.



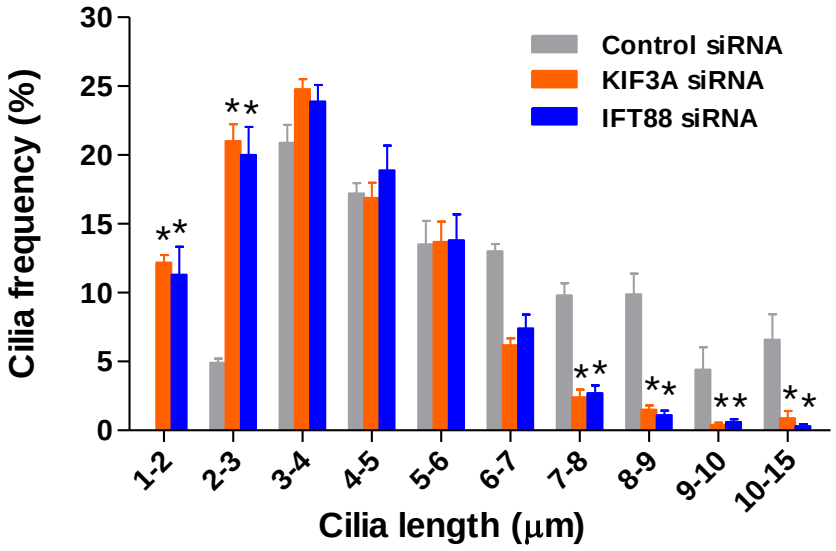

**Supplemental Figure 9. Effect of leptin on the percentage of ciliated cells in N1 hypothalamic neuron cells**.

N1 neuronal cells were cultured in serum-free media for 30 h and then treated with leptin (100 nM) with or without a JAK2 inhibitor (1 μM AG490) or PI3K inhibitors (1 nM LY294002 or 10 nM wortmannin) for 18 h before cilia staining. For cilia staining, cells were fixed with 4% PFA for 15 min at room temperature and stained with an anti-AC3 antibody. The percentage of ciliated cells was determined based on DAPI staining of cell nuclei. Experiments were conducted in triplicate wells and repeated at least three times.



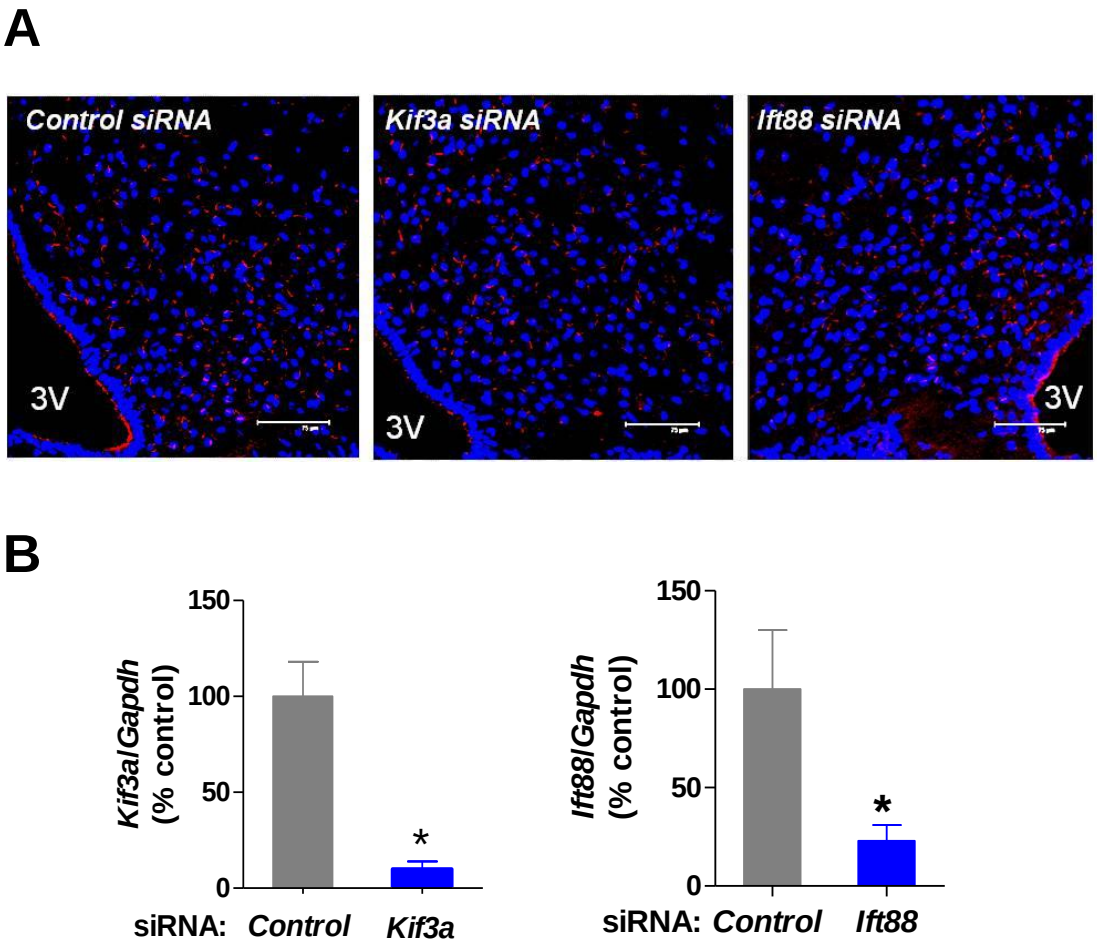

**Supplemental Figure 10. Effect of leptin treatment on cilia length in primary cultured hypothalamic neurons.**

Primary cultured hypothalamic neurons were treated with leptin treatment (100 nM) for 6-12 h before cilia analysis using AC-3 immunostaining (shown in red). Neuronal soma and processes were stained with MAP2 (shown in green). Data represent mean ± SEM. **P* < 0.01 *vs.* control. Scale bars, 15 μm.



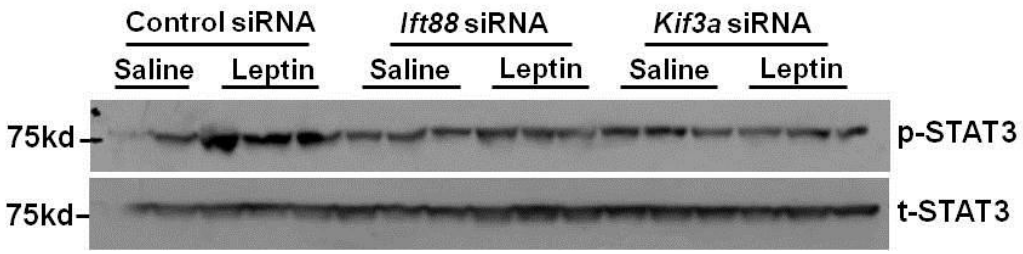

**Supplemental Figure 11. Intrahypothalamic injection of Kif3a or Ift88 siRNA induced a short cilia phenotype.**

A non-targeting scrambled control siRNA or siRNAs specific for murine *Ift88* or *Kif3a* (Dharmacon) were injected bilaterally into the mediobasal hypothalamus (0.5 μl of 1 mM siRNA solution per hemisphere). At the end of the study (about 48 h post-injection), whole brains were collected for AC3 immunostaining. Neuronal cilia were stained with AC3 and analyzed in the hypothalamic ARC (10 fields from each brain region per section, three sections per animal) using the Image-Pro Plus program (Media Cybernetics). *$P < 0.05$ vs. control siRNA groups corresponding to each cilia length.



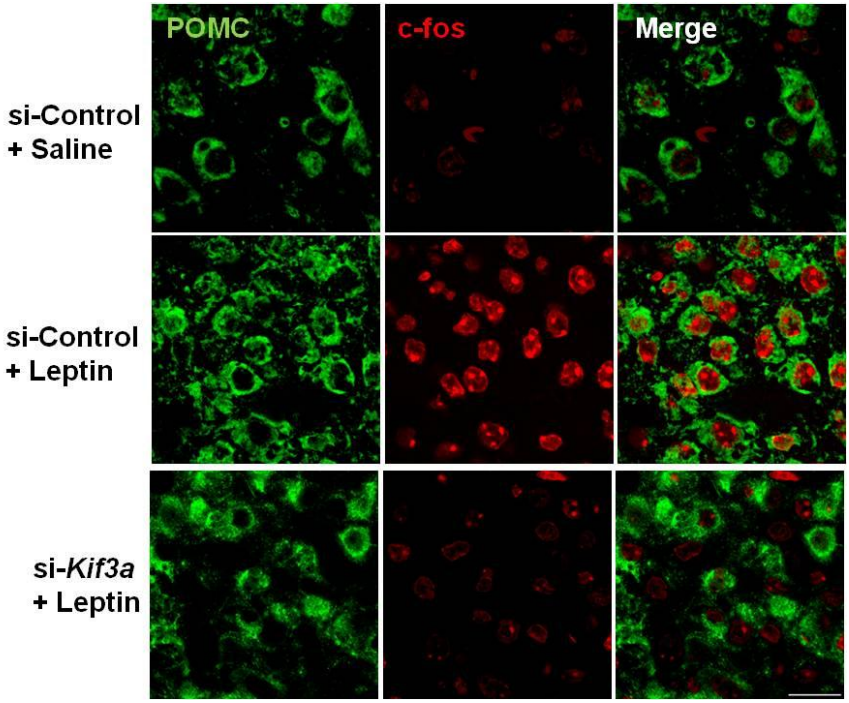

**Supplemental Figure 12. Successful knockdown of *Kif3a* and *Ift88* in the mediobasal hypothalamus.**

A non-targeting scrambled control siRNA or siRNAs specific for murine *Ift88* or *Kif3a* (Dharmacon) were injected bilaterally into the mediobasal hypothalamus (0.5 μl of 1 mM siRNA solution per hemisphere). At the end of the study (about 48 h post-injection), whole brains were collected for AC3 immunostaining **(A)** or mediobasal hypothalamic blocks were collected for measurement of *Kif3a* and *Ift88* mRNA expression **(B)**. *Kif3a* and *Ift88* mRNA expression was determined by real-time PCR. The expression level of each mRNA was normalized to that of *Gapdh*. Data are expressed as a percentage of the average values of control siRNA-injected mice. **P* < 0.01 *vs.* control. Scale bars, 50 μm.




**Supplemental Figure 13. Immunoblot images of hypothalamic total and phospho-STAT3 following leptin treatment.**

Mice were injected with control siRNA, *Kif3a* siRNA, or *Ift88* siRNA in bilateral mediobasal hypothalamus. At 24 h after siRNA injection, mice received an ICV injection of saline or leptin (3 μg). Mediobasal hypothalamic blocks were collected 1 h after leptin injection and subjected to immunoblot analysis ($n$ = 3-5 per group).




**Supplemental Figure 14. C-fos staining in hypothalamic POMC neurons following IP leptin injection.**

*Kif3a* siRNA or non-targeting control siRNA was injected into the bilateral mediobasal hypothalamus of B6 mice. About 24 h after siRNA injection, mice received intraperitoneal injection of saline or leptin (3 mg/kg). Mice were perfused transcardially with 4% PFA 45 min following IP injection. Dual immunostaining was performed by using anti-αMSH (POMC) antibody (Chemicon, sheep, 1:15,000) and anti-c-fos antibody (1:2000, rabbit, Millipore). Scale bars, 20 μm.