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Supplemental Data

Disruption of Intraflagellar Transport in Adult Mice Leads to Obesity and Slow-Onset Cystic Kidney Disease

James R. Davenport, Amanda J. Watts, Venus C. Roper, Mandy J. Croyle, Thomas van Groen, J. Michael Wyss, Tim R. Nagy, Robert A. Kesterson, and Bradley K. Yoder

Supplemental Experimental Procedures

Mice

The *Kif3a* mice containing loxP or null alleles were obtained from Dr. L. Goldstein and were maintained on a C57BL/6 background [S1].

The *Tg737* conditional mutant allele was generated such that exons four through six would be deleted upon Cre-recombinase-mediated excision, resulting in a null allele [S2]. The *Tg737* null allele used was generated by the germline deletion of the *Tg737* conditional allele. For all the studies in this manuscript, the conditional mutants were generated with the $Tg737^{flox/\Delta}$ or $Kif3a^{flox/\Delta}$ mice because this would require only a single Cre excision event for the generation of the mutants. Identically treated $Tg737^{flox/WT}$ or $Kif3a^{flox/WT}$ mice carrying the Cre transgene were used as the control animals unless stated otherwise. Nearly identical results were obtained with either the *Tg737* or *Kif3a* conditional mutant mice when crossed with the same Cre line, and data are not always presented for both lines.

The *CAGG-creER*^{TM} mice were obtained from Jackson Laboratories and were generated by Dr. A. McMahon [S3]. They were maintained on a mixed C57BL/6 and FVB background. *POMC-cre* mice were obtained from Dr. G. Barsh and were maintained on a mixed C57BL/6 and FVB background [S4]. All mice were maintained in accordance with Institutional Animal Care and Use Committee (IACUC) regulations at the University of Alabama at Birmingham. Genotyping of mice was performed as described previously [S5].

For the induction of Cre activity in the *CAGG-creER*^{TM} line, tamoxifen administration was performed for five consecutive days when *CAGG-creER*^{TM} mice were between 8 and 12 weeks of age. Tamoxifen (Sigma [St. Louis, MO]) dissolved in corn oil (Sigma) was administered IP (interperitineal injection) at a dose of 6.0 mg/40 g body weight as described previously [S3]. For the induction of Cre activity in the *CAGG-creER*^{TM} line in embryonic animals, one injection of tamoxifen was administered IP to the mother at 9.0 mg/40 g body weight.

Western-Blot Analysis

In brief, pancreas and brain tissues of *Kif3a* or *Tg737* mice were dounce homogenized in a 50 mM Tris (pH 7.2) buffer as described previously [S6]. Kidney tissues from *Kif3a* or *Tg737* were homogenized with a PowerGen700 tissue homogenizer (Fisher Scientific [Pittsburgh, PA]) in a 50 mM Tris (pH 7.5) buffer (adapted from [S7]). Protein concentrations were determined with a DC protein assay kit (Bio-Rad [Hercules, CA]) according to the manufacturer's instructions. Equivalent amounts of protein lysate (20 μg) were separated by electrophoresis on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to nitrocellulose (Nitrobind; Micron Separations [Westboro, MA]). Western-blot analysis was performed with either mouse monoclonal antibody against KIF3A (diluted 1:1000, Covance [Berkeley, CA]) or TG737/Polaris (GN1700, diluted 1:2500, [S8]). The filters were washed and incubated with horseradish peroxidase (HRP)-conjugated secondary anti-rabbit or anti-mouse antibodies (diluted 1:1500, Bio-Rad). Filters were washed four times in a 1× phosphate-buffered saline (PBS)/0.2% Tween-20 solution and HRP signal was detected with a Super-signal West Dura Chemiluminescence Kit (Pierce Biotechnology [Rockford, IL]).

Immunofluorescence Analysis

Tissue from kidney and pancreas was isolated from Tg737-cWT and Tg737-cKO mice and snap frozen in Tissue Tek O.C.T. compound (Sakura Finetek USA [Torrance, CA]) as described previously. Forty micron sections from tissues were fixed in 3% paraformaldehyde for 1 hr at 20°C and permeablized in 100% methanol for 30 min at −20°C. Sections were then blocked for 30 min and incubated for 3 hr at 20°C with an antibody raised against acetylated alpha-tubulin (Sigma, T6793) that was directly conjugated to fluorescein isothiocyanate (FITC) with the Aminolink Kit (Pierce Biotechnology) and then stained with Hoechst (1:1000, Sigma #33258) so that the nucleus could be visualized. Slides were mounted and analyzed by confocal immunofluorescence microscopy with a Leica confocal microscope TCS SP unit (Leica Microsystems [Wetzlar, Germany]) and the Leica imaging software.

Tissue from the hypothalamus in Kif3a-pomcWT and Kif3a-pomcKO mice on the *Z/EG* reporter background was isolated after perfusion of a 2% paraformaldehyde in PBS solution and then cryoprotected with a 2% paraformaldehyde (PFA) in 30% sucrose solution diluted in PBS. Free-floating sections (30 μm) were blocked and stained with TAP952 (1:100,000), a mouse monoclonal antibody directed against monoglycylated tubulin, and visualized with a rhodamine-red-conjugated secondary antibody (1:5000, Jackson Labs [Bar Harbor, ME]) [S9]. The rest of the process is described above.

Body-Weight and Feeding Studies

All body weights from mice were measured between 5:00 p.m. and 6:00 p.m. weekly with a Mettler-Toledo digital weight scale (Columbus, OH). All animals undergoing food intake analysis studies were individually caged, and food intake measurements were conducted weekly between 5:00 and 6:00 p.m. by the measurement of the food left in the food compartment of the cage and any noticeable pieces of food within the cage. Animals were fed 11% fat breeders diet throughout the study (Harlin Foods).

For pair-feeding studies, food consumption of the tamoxifen-induced Kif3a-cWT controls was measured daily, and this amount plus 0.05 g of food was given to pair-fed mice so that any error resulting in starvation could be prevented. For the diet-restriction studies, 4.00 ± 0.10 g of food were given to both Kif3a-cWT and Kif3a-cKO mice after the initial tamoxifen administration. At eight weeks, the animals were then given access to unlimited food. Weights were measured as described above.

Serum Hormone Analysis

All animals were handled for several days prior to collection of serum so that the effects caused by stress could be reduced. Each individual animal was transported into an isolated room, decapitated without anesthesia, and trunk blood was collected. Serum was isolated by centrifugation (24 min at 2500 rpm at 4°C, followed by an additional 5 min at 2500 rpm at 4°C) and stored at −80°C until analysis. Serum leptin and corticosterone analysis was performed as described previously [S10]. Insulin analysis was performed with 100 μl aliquots of serum with materials purchased from Linco Research (St. Charles, MO) and analyzed with an Ektachem DT II System (Johnson and Johnson Clinical Diagnostics [Rochester, NY]) with the assistance of the Energy Metabolism/Body Composition Core in the UAB Clinical Nutrition Research Center.

Body-Composition Analysis

All mice were analyzed for body composition between 22 and 25 weeks of age with the assistance of the Small Animal Phenotyping Core in the UAB Clinical Nutrition Research Center. Animals were briefly anesthetized and placed under a PIXImus Dual-energy X-Ray absorptiometer (DXA, GE-Lunar [Madison, WI]) so that fat and lean mass could be evaluated. One week after DXA analysis, animals

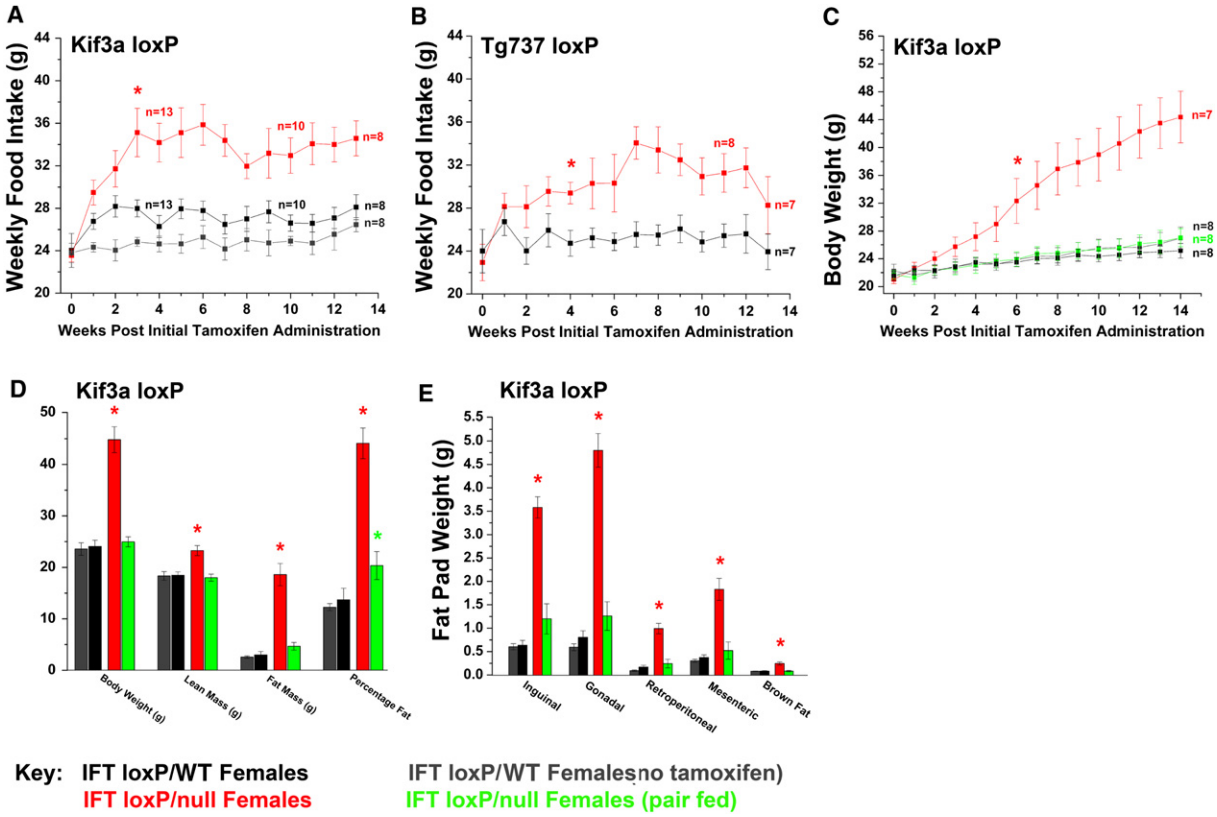

Figure S1. Conditional Disruption of Either *Kif3a* or *Tg737* from Adult Female Mice Results in the Development of Hyperphagia-Induced Obesity

(A and B) Analysis of the weekly food intake of female Kif3a-cKO and Kif3a-cWT (A) and Tg737-cKO and Tg737-cWT (B) adult mice after tamoxifen administration (week 0). The weekly food intake of non-tamoxifen-injected Kif3a-cWT females is also presented.

(C) Body-weight comparison between non-tamoxifen- and tamoxifen-induced Kif3a-cWT males and ad libitum- and pair-fed tamoxifen-induced Kif3a-cKO females.

(D) DXA analysis at 14 weeks after the initial tamoxifen administration in Kif3a-cWT and Kif3a-cKO females reveals significantly increased fat mass and percentage fat. Pair-fed Kif3a-cKO females also have significantly higher body fat than controls, but are significantly lower than ad libitum-fed Kif3a-cKO female littermates.

(E) Carcass analysis of various fat pads and brown fat in Kif3a-cWT and Kif3a-cKO females 16 weeks after the initial tamoxifen administration. "*" indicates $p \leq 0.05$ and shows the initial point of significant deviation between controls and mutants in body-weight graphs. Error bars indicate the SD.

were killed, and individual tissues were isolated and weighed as described previously.

Serum Glucose Measurement

Kif3a-cWT and cKO mice were fasted for 4 hr at 16 weeks after the initial tamoxifen administration (age 21–25 weeks). An initial measurement of fasting blood glucose was acquired from the tail vein. Blood glucose levels were then measured with an OneTouch Ultra Glucometer and OneTouch Ultra blood glucose test strips (Lifescan [Milpitas, CA]). All animals were handled several days prior to the taking of the serum glucose sample, and all blood samples were measured in a separate room so that the stress placed on the tested animals could be minimized.

Histological Analysis

For all hematoxylin and eosin staining, pancreas and kidney tissues from mutant and wild-type mice were isolated and fixed in formalin overnight at 4°C. The tissues were then sectioned and stained with hematoxylin and eosin (H&E) as described previously [S6]. Hepatic tissues used for oil red O sections were isolated and snap frozen in Tissue Tek O.C.T. compound (Sakura Finetek USA) as described above. Sections of the liver were then stained as described previously [S11]. Images of tissues were captured on a Nikon TE200S microscope (Melville, NY) with a Micropublisher 3.3RTV camera (Burnaby, British Columbia, Canada) and Q-imaging software.

Body-Length Measurement

Immediately after DXA analysis, when the animal was still anesthetized, ventral measurements from the tip of the nose to the middle of the anus (to the nearest 0.1 cm) were taken by two independent observers unaware of the genotypes. These measurements were averaged, and this value was then used in statistical analysis for the calculation of the body length.

Activity Measurements

For long-term locomotive activity, independent cages with a camera system (detecting infrared) recorded animal activity over a 24 hr 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 acquainted with the home cage for 24 hr, and is then observed for 24 hr with a camera-driven tracker system (Phenotyper, Noldus [The Netherlands]). The test measures the circadian activity pattern of the mice. The activity is measured on a 12:12 hr light/dark cycle, in which the lights turn on at 6:00 and turn off at 18:00. Animals are provided a small black box home in the corner so that they can sleep where movement is undetected by infrared light.

An open field maze was used for measuring short-term locomotive behavior and fear responses. The maze consists of an arena of 42 × 42 cm square with clear plexiglass sides (20 cm high). The animal is put in the arena and observed for 5 min with a camera-driven tracker system known as Ethovision (Noldus [The Netherlands]). The arena is subdivided into three areas: the open center area, the sides, and

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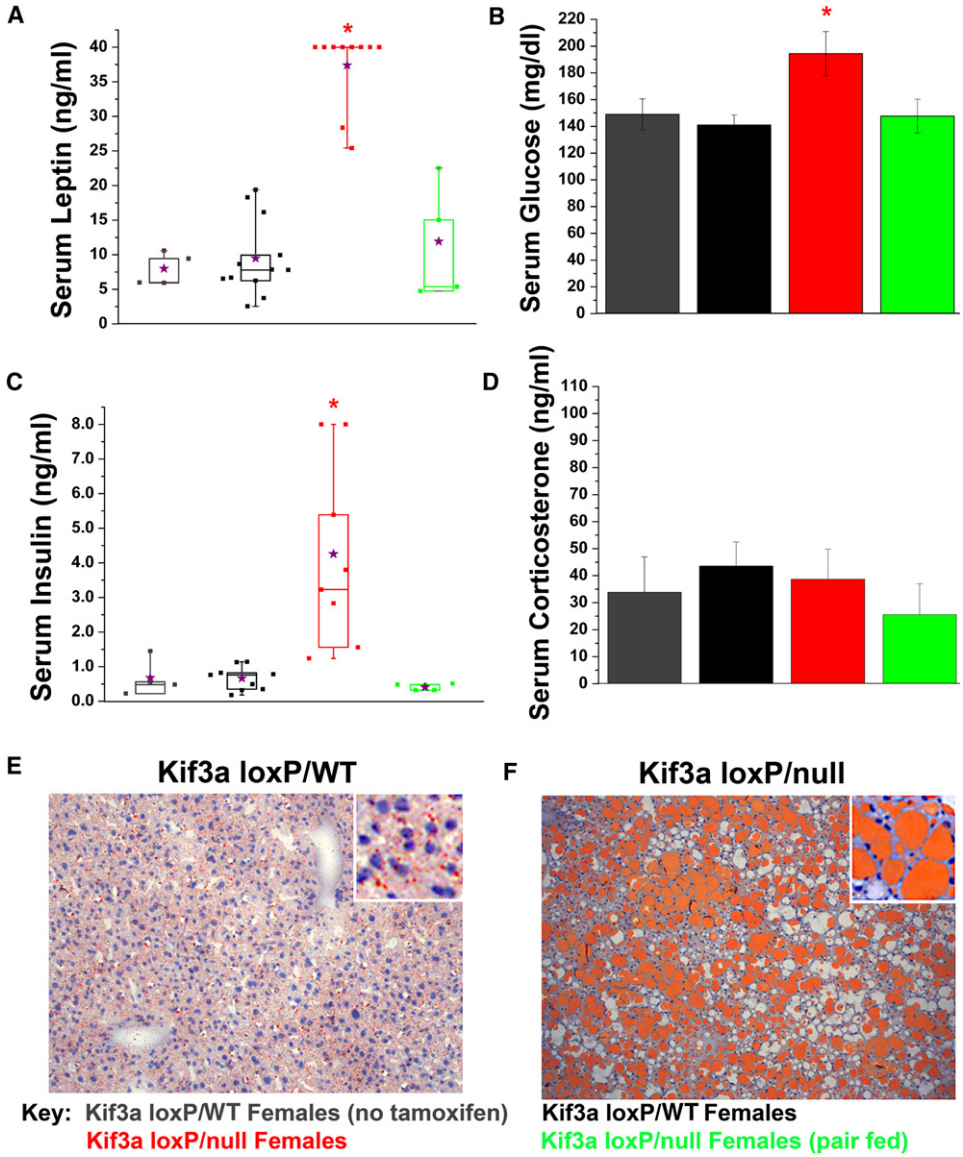

Figure S2. Serum Analysis and Hepatic Pathologies Associated with the Obesity Phenotype in Kif3a-cKO Females

(A) Serum leptin levels in Kif3a-cWT and KO females 16 weeks after the initial tamoxifen administration. Eight out of ten Kif3a-cKO ad libitum-fed mice were above the maximum threshold of detection (40 ng/ml) and were assigned a value of 40 ng/ml. Mean serum leptin levels are indicated by purple stars.

(B) Serum glucose analysis of Kif3a-cWT and Kif3a-cKO females measured after 4 hr of fasting conditions.

(C) Nonfasting serum insulin analysis of Kif3a-cWT and Kif3a-cKO females. Two Kif3a-cKO ad libitum-fed females reached the maximum threshold of detection (8 ng/ml) and were assigned that value. Mean serum insulin levels are indicated by purple stars.

(D) Serum corticosterone analysis shows no significant differences between Kif3a-cWT and Kif3a-cKO females.

(E and F) Oil Red O staining of Kif3a-cWT liver (E) and Kif3a-cKO liver (F) shows extensive fat infiltration (red) 16 weeks after tamoxifen administration. Inserts show higher magnification of the liver. This phenotype is also seen in male Kif3a-cKO livers and male and female Tg737-cKO livers.

Error bars indicate the SD.

the wall. The system records the position of the animal in the arena at 5 frames/s.

Statistical Analysis

All scientific graphs and statistical analysis were completed with OriginLab Software (Northhampton, MA). Either independent two-tailed Student's t tests or one-way analysis of variance (ANOVA) analyses were used for the calculation of significant differences. In all cases, a value was deemed significant if $p < 0.05$.

Sample Sizes and Statistical Analyses

Figure 2C

The systemic loss of *Kif3a* in adult mice results increased weight gain. Kif3a-cKO males (n = 10) had a significantly higher body weight than did Kif3a-cWT males (n = 14) 4 weeks after tamoxifen administration (Kif3a-cKO = 30.82 ± 1.16 g versus Kif3a-cWT = 27.89 ± 0.64 g, t = 2.36, p = 0.03). Kif3a-cKO females (n = 5 at 6 weeks, n = 2 at 12 weeks after tamoxifen administration) significantly deviated in weight from their control Kif3a-cWT littermates (n = 5 at 6 weeks, n = 2 at 12 weeks) 4 weeks after the initial tamoxifen administration

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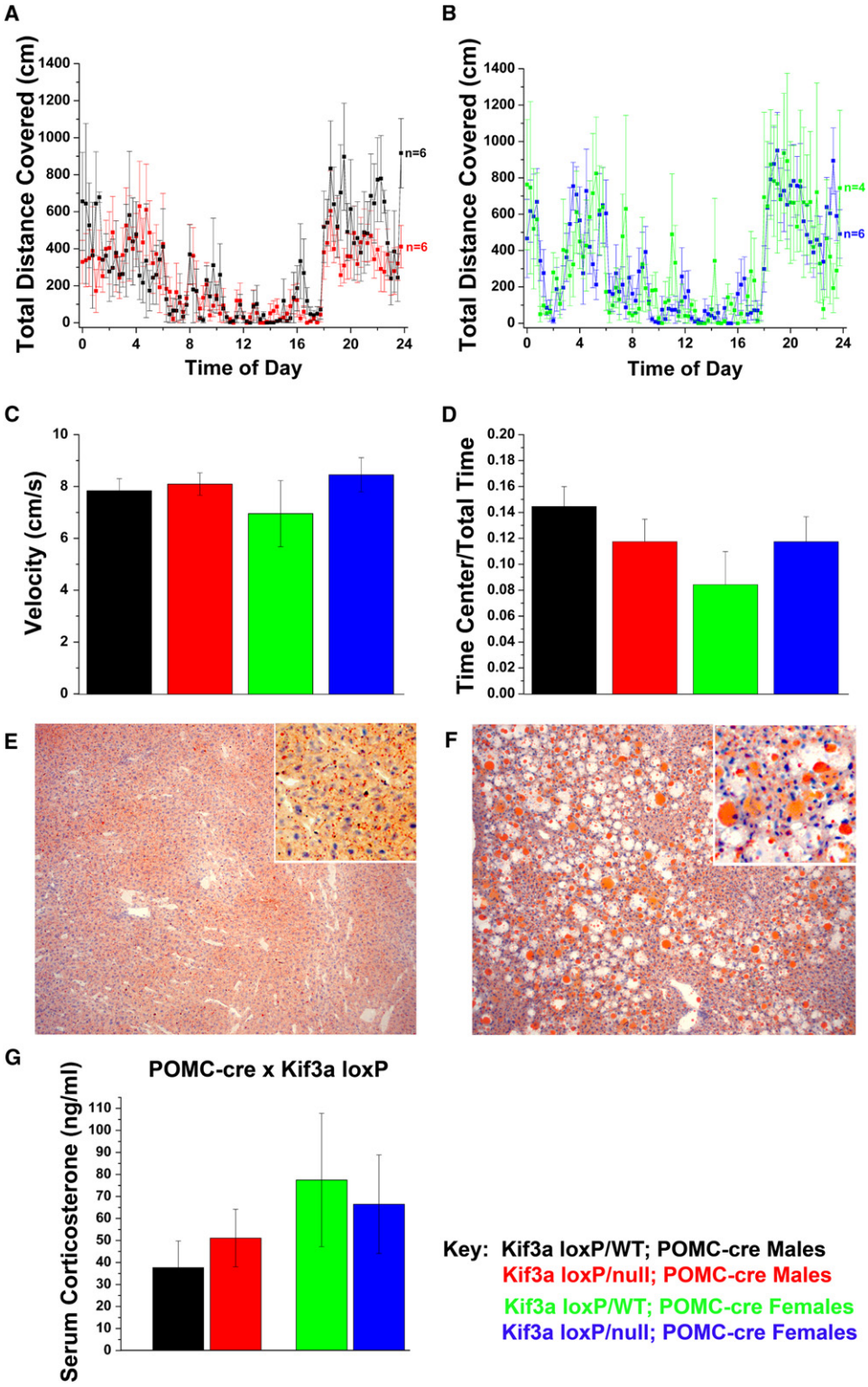

Figure S3. Disruption of Kif3a in POMC-Expressing Cells Results in a Hyperphagia-Induced Obese Phenotype Associated with Hepatic Steatosis with No Overt Changes in Activity

(A and B) Analysis over a 24 hr period showed no overt disruption in day-night movement patterns or any major activity changes in Kif3a-pomcKO males (A) or Kif3a-pomcKO females (B) compared to littermate controls. Lower movement was noted in Kif3a-pomcKO males during the start of the dark cycle for short periods of time. Movement was analyzed in 15 min intervals.

(C and D) Open-field activity tests also confirmed that Kif3a-pomcKO males and females had no significant differences in velocity (C) or percentage of time spent in the center of the field (D) compared to littermate controls.

(E and F) Oil red O staining of Kif3a-pomcWT liver (E) and Kif3a-pomcKO liver (F) at 26 weeks of age. Fat deposits (red) are visible in the Kif3a-pomcKO mice but not the Kif3a-pomcWT mice. Inserts show higher magnification of the liver.

(Kif3a-cKO = 30.62 ± 2.09 g, Kif3a-cWT = 24.50 ± 1.08 g, t = 2.60, p = 0.03). The number of Kif3a-cKO and Kif3a-cWT females analyzed in this study changed at week 6, when several of the mice were used to evaluate fat pad and organ weight masses.

Figure 2D

Systemic loss of *Tg737* in adult mice results in increased weight gain. Tg737-cKO males (n = 6) had significantly higher body weight than did Tg737-cWT males (n = 6) at 4 weeks after tamoxifen administration (Tg737-cKO = 31.78 ± 1.70 g versus Tg737-cWT = 26.69 ± 1.23 g, t = 2.42, p = 0.04). Tg737-cKO females (n = 8) significantly deviated in weight from their control Tg737-cWT littermates (n = 8) at 4 weeks after tamoxifen administration (Tg737-cKO = 24.2 ± 1.16 g, Tg737-cWT = 21.0 ± 0.60 g, t = 2.39, p = 0.03).

Figure E

Weekly food intake in Kif3a-cKO ad libitum-fed males is significantly higher by 3 weeks after tamoxifen administration, compared to Kif3a-cWT male littermates. Three weeks after tamoxifen administration, one-way ANOVA analysis indicates that Kif3a-cKO males (n = 12) consume significantly more food weekly (34.27 ± 1.74 g) compared to tamoxifen-induced Kif3a-cWT controls (29.27 ± 0.78 g [n = 14]). It is also significantly increased compared to non-tamoxifen-induced Kif3a-cWT control males (27.38 ± 1.11 g [n = 6], F = 6.54, p < 0.01). Several animals near the end of the study were sacrificed for histological analysis before the completion of this experiment.

Figure 2F

Weekly food intake in Tg737-cKO ad libitum-fed males is significantly higher 2 weeks after tamoxifen administration compared to Tg737-cWT male littermates. At 2 weeks after tamoxifen administration, Tg737-cKO males (n = 6) eat 32.06 ± 1.92 g, whereas controls eat 26.73 ± 0.48 g (n = 6). Independent t tests confirm that these values are significantly different (t = 2.69, p = 0.02).

Figure 2G

Pair-feeding analysis shows a significant increase in weight gain in ad libitum-fed Kif3a-cKO mutant males that is virtually eliminated with pair-feeding mutant males. One-way ANOVA analysis measured a significant difference between ad libitum-fed mutants compared to the other three groups at 7 weeks after the initial tamoxifen administration (n = 6, F = 3.20, p < 0.05). The significant difference was only the ad libitum-fed mutants, and this was confirmed by determining there were no differences in weight at 7 weeks after the initial tamoxifen administration between controls and pair-fed males (F = 0.09, p = 0.92).

Figure 2H

The changes in body weight for the diet-restriction experiments were analyzed by independent two-sample t tests comparing the weights of Kif3a-cKO (n = 3) and Kif3a-cWT males (n = 3). The data indicate that there is a significant difference between the two weights 2 weeks after the release of diet restriction and 10 weeks after the initial tamoxifen administration (t = 3.99, p = 0.02).

Figure 3A

DXA analysis at 14 weeks after the initial tamoxifen administration indicates that there are significant increases in fat mass and percentage fat in ad libitum-fed Kif3a-cKO males compared to controls and pair-fed mutant males. Body weight in ad libitum-fed mutants (45.10 ± 4.81 g, n = 3) is significantly higher than either nontamoxifen (31.05 ± 2.12 g, n = 3) or tamoxifen controls (30.04 ± 1.33 g, n = 3) and is also significantly higher than pair-fed mutants (31.72 ± 2.59 g, n = 3, F = 5.61, p = 0.02). This was confirmed by one-way ANOVA analyzing only the two control groups and pair-fed mice, which showed no differences (F = 0.17, p = 0.85). Lean mass measurements showed no significant differences between the groups (F = 0.83, p = 0.52). Fat mass was significantly different between groups (F = 14.19, p = 0.001). This fat mass difference was equated to be due to excess fat in ad libitum-fed mutants (16.67 ± 2.54 g). One-way ANOVA confirmed no difference in fat masses between controls and pair-fed males (F = 0.38, p = 0.70). The percent fat of the males was also significantly higher in ad libitum-fed mutants (38.77 ± 2.28% fat) compared to controls (F = 18.74, p = 0.001). There was no difference in percentage fat between controls and pair-fed mutants (F = 0.33, p = 0.73).

Figure 3B

Fat pad mass in ad libitum-fed Kif3a-cKO males is uniformly higher throughout the body. One-way ANOVA analysis of the four groups showed a significant difference between the inguinal fat pads of ad libitum-fed Kif3a-cKO males (2.86 ± 0.22 g, n = 13) compared to Kif3a-cWT controls (nontamoxifen Kif3a-cWT: 0.73 ± 0.13 g [n = 4], tamoxifen Kif3a-cWT: 0.66 ± 0.06 g [n = 12]) and pair-fed Kif3a-cKO males (1.16 ± 0.21 g, n = 4, F = 38.48, p < 0.001). In addition, the pair-fed mutant males had a higher fat pad mass than did the controls; one-way ANOVA analysis between the three groups showed a significant difference (F = 5.29, p = 0.02). The ad libitum-fed Kif3a-cKO males carried significantly more fat than did the pair-fed Kif3a-cKO males, however (t = 4.03, p = 0.001).

Gonadal fat pads also showed significant differences between ad libitum-fed mutant males (2.63 ± 0.14 g) and the three other groups (nontamoxifen Kif3a-cWT: 0.91 ± 0.22 g, tamoxifen Kif3a-cWT: 0.79 ± 0.10 g, pair-fed Kif3a-cKO: 1.28 ± 0.23 g, F = 40.95, p < 0.001). This difference was seen in the ad libitum-fed Kif3a-cKO mice; the other three groups did not differ (F = 2.40, p = 0.12).

Retroperitoneal fat pads were significantly heavier in ad libitum-fed Kif3a-cKO males (1.09 ± 0.09 g) compared to other groups (nontamoxifen Kif3a-cWT: 0.30 ± 0.06 g, tamoxifen Kif3a-cWT: 0.31 ± 0.04 g, pair-fed Kif3a-cKO: 0.49 ± 0.08 g, F = 25.48, p < 0.001). The pair-fed mutants did not significantly differ from controls when comparing retroperitoneal fat pad weight (F = 2.61, p = 0.10).

One-way ANOVA analysis of the four groups showed a significant difference between the mesenteric fat pads of ad libitum-fed Kif3a-cKO males (1.65 ± 0.16 g) compared to Kif3a-cWT controls (nontamoxifen Kif3a-cWT: 0.49 ± 0.12 g, tamoxifen Kif3a-cWT: 0.49 ± 0.04 g) and pair-fed Kif3a-cKO males (0.89 ± 0.07 g, n = 4, F = 21.51, p < 0.001). In addition, the pair-fed mutant males had a higher fat pad mass than did the controls; one-way ANOVA analysis between the three groups showed a significant difference (F = 9.61, p = 0.001). The ad libitum-fed Kif3a-cKO males carried significantly more fat than did the pair fed Kif3a-cKO males, however (t = 2.58, p = 0.02).

Brown fat masses were also significantly different from each other: Ad libitum-fed mutant males (0.32 ± 0.02 g, n = 8) were significantly different from the other three groups (nontamoxifen Kif3a-cWT: 0.14 ± 0.03 g [n = 4], tamoxifen Kif3a-cWT: 0.12 ± 0.01 g [n = 7], pair-fed Kif3a-cKO: 0.14 ± 0.03 g [n = 4], F = 22.37, p < 0.001).

Figure 3C

Serum leptin levels in Kif3a-cKO males are significantly elevated in ad libitum-fed obese mice, but are not different from controls in pair-fed mutant males. One-way ANOVA analysis of the four groups showed a significant difference between the ad libitum-fed Kif3a-cKO serum leptin levels (29.99 ± 3.25 ng/ml, n = 15) and both control and pair-fed serum leptin levels (nontamoxifen Kif3a-cWT = 9.30 ± 3.54 ng/ml [n = 4], tamoxifen Kif3a-cWT: 9.85 ± 1.60 ng/ml [n = 14], pair-fed Kif3a-cKO: 12.03 ± 2.44 ng/ml [n = 4], F = 12.96, p < 0.001). It must be noted that five out of 15 samples of ad libitum-fed Kif3a-cKO males reached the maximum detectable limit of serum leptin at 40 ng/ml and were assigned that value in the statistical analysis. One-way ANOVA analysis of the two control groups and the pair-fed mutant males showed no difference in leptin levels (F = 0.25, p = 0.78).

Figure 3D

Serum glucose levels in ad libitum-fed Kif3a-cKO males after 4 hr of fasting are significantly higher than in controls or pair-fed mutants. One-way ANOVA analysis between four groups showed a significant difference in means between ad libitum-fed mutants (186.60 ± 7.48 mg/dl, n = 10) and the other three groups (non-tamoxifen-induced Kif3a-cWT [159.80 ± 5.65, n = 5], tamoxifen-induced Kif3a-cWT [164.89 ± 9.97, n = 9], pair-fed Kif3a-cKO [146.00 ± 5.33, n = 5], F = 3.91, p = 0.02). One-way ANOVA analysis confirmed that it was only the ad libitum null males that differed; the other three groups had no significant difference in means (F = 1.11, p = 0.36).

(G) Serum corticosterone levels in Kif3a-pomcKO and Kif3a-pomcWT males and females show no significant differences. Error bars indicate the SD.

Figure 3E
Serum nonfasting insulin levels in ad libitum-fed Kif3a-cKO males 16 weeks after tamoxifen administration are significantly higher compared to controls or pair-fed Kif3a-cKO males. Kif3a-cKO males fed ad libitum displayed an average sensitive rat insulin level equal to 4.60 ± 0.66 ng/ml (n = 15). It must be noted that this mean and standard error is not entirely accurate because there was a maximum detectable limit of 8.0 ng/ml for insulin, and four out of 15 samples exceeded this limit. This was significantly different than non-tamoxifen- and tamoxifen-induced Kif3a-cWT controls (1.07 ± 0.67 ng/ml [n = 2], and 1.18 ± 0.14 ng/ml [n = 14], respectively) and pair-fed Kif3a-cKO males (1.35 ± 0.48 ng/ml, n = 3). This test was performed with one-way ANOVA analysis ($F = 10.10$, $p < 0.001$). Further comparisons show that there is no significant difference between controls and pair-fed mutant females with concern to serum insulin levels ($F = 0.15$, $p = 0.86$).

Figure 3F
Serum corticosterone levels showed no significant differences between Kif3a-cKO and Kif3a-cWT males. One-way ANOVA analysis showed no significant differences between any group tested (Kif3a-cWT [no tamoxifen, n = 4] = 13.12 ± 2.91 ng/ml; Kif3a-cWT [tamoxifen, n = 14] = 26.26 ± 4.55 ng/ml; Kif3a-cKO [ad libitum fed, n = 15] = 39.66 ± 8.54 ng/ml; Kif3a-cKO [pair fed, n = 4] = 13.34 ± 2.59 ng/ml, $F = 2.17$, $p = 0.11$).

Table 1
Organ weight differences are significant in ad libitum-fed Kif3a-cKO females compared to Kif3a-cWT controls and pair-fed Kif3a-cKO females. In relation to liver weight, there was a significant difference in weight between Kif3a-cKO ad libitum-fed females (n = 7) compared to non-tamoxifen- (n = 4) and tamoxifen-induced (n = 7) controls, as well as pair-fed mutants (n = 4). This was done with one-way ANOVA analysis ($F = 8.87$, $p < 0.001$). We found significant differences between the kidney weights of ad libitum-fed Kif3a-cKO females compared to non-tamoxifen- and tamoxifen-induced controls and pair-fed mutants with one-way ANOVA analysis ($F = 8.90$, $p < 0.001$). Independent t test comparisons showed that there were no significant differences between controls and pair-fed mutants.

Figure 4
Body-weight analysis in Tg737-syn1WT and Tg737-syn1KO males and females shows significant increases in body weight with conditional loss of Tg737 from all neurons. At 27 weeks of age, Tg737-syn1KO males (38.86 ± 2.24 g, n = 10) initially deviate from their control Tg737-syn1WT littermate males in body weight at a significant level (33.02 ± 0.96 g, n = 8, $t = 2.20$, $p = 0.04$). Tg737-syn1KO females start to significantly deviate in weight 25 weeks after birth, when Tg737-syn1KO females (30.32 ± 1.41 g, n = 10) were heavier than their Tg737-syn1WT control littermates (25.68 ± 1.19 g, n = 8, $t = 2.43$, $p = 0.03$). These values are indicated by red and blue stars above the respective Tg737-syn1KO weights.

Figure 5C
Body-weight analysis in Kif3a-pomcWT and Kif3a-pomcKO males and females shows significant increases in body weight with loss of Kif3a from POMC-expressing cells. At 6 weeks of age, Kif3a-pomcKO males (21.91 ± 0.39 g, n = 15) initially deviate from their control Kif3a-pomcWT littermate males in body weight at a significant level (20.03 ± 0.47 g, n = 18, $t = 3.00$, $p < 0.01$). Kif3a-pomcKO females start to significantly deviate in weight 5 weeks after birth, when Kif3a-pomcKO mice (16.99 ± 0.36 g, n = 20) were heavier than their Kif3a-pomcWT control littermates (15.48 ± 0.57 g, n = 13, $t = 2.36$, $p = 0.03$). These values are indicated by red and blue stars above the respective Kif3a-pomcKO weights.

Figure 5D
Analysis of the weekly food intake of Kif3a-pomcWT and Kif3a-pomcKO females shows increases in weekly food intake after disruption of Kif3a in POMC-expressing cells, but this is not initially significant until 17 weeks of age. At week 17, Kif3a-pomcKO females (29.85 ± 1.13 g, n = 7) eat significantly more food than do their Kif3a-pomcWT counterparts (24.77 ± 1.74 g, n = 4, $t = 2.58$, $p = 0.03$).

Figure 5E
DXA analysis of 22–25-week-old Kif3a-pomcWT and Kif3a-pomcKO mice shows increased adiposity and lean mass. Body weight was measured separately, and there measurements showed a higher body weight in Kif3a-pomcKO males than in controls (40.54 ± 1.60 g versus 31.27 ± 1.71 g, n = 6, $t = 3.96$, $p < 0.01$). Kif3a-pomcKO females also showed significant increases in body weight at this age (36.62 ± 1.42 [n = 8] versus 26.33 ± 1.17 [n = 7], $t = 5.47$, $p = 0.0001$). There was a significant increase in lean mass in Kif3a-pomcKO males (26.07 ± 0.47 g) compared to Kif3a-pomcWT controls (22.80 ± 0.82 g, $t = 3.45$, $p < 0.01$). Female mice showed no significant differences in lean mass (19.43 ± 0.71 g versus 20.68 ± 0.80 g, $t = 1.15$, $p = 0.27$). Kif3a-pomcKO males had a higher fat mass (11.37 ± 1.21 g) than did Kif3a-pomcWT males (5.78 ± 0.85 g, $t = 3.76$, $p < 0.01$). Kif3a-pomcKO females also has a significantly higher fat mass (13.33 ± 0.68 g) than did controls (4.39 ± 0.64 g, $t = 9.47$, $p < 0.001$). Both male and female POMC mutants had a significantly elevated percentage fat content in their bodies. Kif3a-pomcKO males had a 29.95 ± 2.33% fat content, whereas Kif3a-pomcWT males had 19.75 ± 1.93% fat content (n = 6, $t = 3.37$, $p < 0.01$). In females, Kif3a-pomcKO mice had a 35.58 ± 0.95% fat, whereas controls had a 17.29 ± 1.75% fat (n = 7, $t = 9.20$, $p < 0.001$).

Figure 5F
Nose-to-anal-length was determined in 22–25-week-old Kif3a-pomcKO and Kif3a-pomcWT animals. Independent two-sample t tests compared the averaged length of two independent measurements from the tip of the nose to the anus in both male and female Kif3a-pomcWT mice and Kif3a-pomcKO mice. Kif3a-pomcKO males were significantly longer (10.23 ± 0.05 cm, n = 11) than were controls (9.68 ± 0.08 cm, n = 10, $t = 5.62$, $p < 0.001$). In females, there was a slight increase in mutant body length in comparing Kif3a-pomcKO (9.80 ± 0.09 cm, n = 14) mice and Kif3a-pomcWT mice (9.71 ± 0.09 cm, n = 11), but it was not significant ($t = 0.71$, $p = 0.49$).

Figure 5G
Serum leptin analysis in Kif3a-pomcKO males showed increases compared to Kif3a-pomcWT littermates, but these were not significant, and no values reached the maximum level of 40 ng/ml used in the analysis (Kif3a-pomcKO [n = 6]: 19.93 ± 5.60 ng/ml versus Kif3a-pomcWT [n = 6]: 11.10 ± 2.15 ng/ml, $t = 1.47$, $p = 0.17$). However, serum leptin levels were significantly increased in females (Kif3a-pomcKO [n = 7]: 36.79 ± 2.15 ng/ml versus Kif3a-pomcWT [n = 7]: 11.49 ± 2.55 ng/ml, $t = 7.59$, $p < 0.001$). This statistical analysis does not account for the likelihood that five of the seven Kif3a-pomcKO samples were greater than the maximum of 40 ng/ml detected in this analysis; instead, all maximum samples were assigned the value of 40 ng/ml.

Figures 5H–5I
Serum analysis of 25-week-old Kif3a-pomcKO mice detects significant increases in insulin levels in mutant females but no significant changes in serum fasting glucose between control and mutant males or females. Male Kif3a-pomcKO mice showed increases in serum insulin levels (3.44 ± 1.02 ng/ml [n = 6]) compared with controls (1.55 ± 0.42 ng/ml [n = 6]), but this data was not significant ($t = 1.72$, $p = 0.12$). Serum insulin levels in females, however, were significantly different from controls (Kif3a-pomcKO [n = 8]: 3.07 ± 0.53 ng/ml versus Kif3a-pomcWT [n = 7]: 0.78 ± 0.22 ng/ml; $t = 3.79$, $p < 0.01$). Serum glucose levels after 4 hr of fasting were not significantly different in either males (n = 6, $t = 0.82$, $p = 0.43$) or females (n = 6 [Kif3a-pomcWT], n = 8 [Kif3a-pomcKO], $t = 0.62$, $p = 0.55$).

Figure S1A
Weekly food intake in Kif3a-cKO ad libitum-fed females is significantly higher 3 weeks after tamoxifen administration as compared to Kif3a-cWT female littermates. At 3 weeks after tamoxifen administration, one-way ANOVA analysis shows that Kif3a-cKO females (n = 13) eat significantly more weekly food (35.13 ± 2.28 g) compared to tamoxifen-induced Kif3a-cWT controls (27.99 ± 0.80 g [n = 13]). It is also significantly increased compared to non-tamoxifen-induced Kif3a-cWT control males (24.38 ± 0.43 g [n = 8], $F = 10.25$, $p < 0.01$). Independent t tests confirm that Kif3a-cKO females eat more food 3 weeks after tamoxifen administration than do both groups of control females.

Figure S1B
Weekly food intake in Tg737-cKO ad libitum-fed females is significantly higher 4 weeks after tamoxifen administration compared to Tg737-cWT female littermates. At the fourth week after tamoxifen administration, Tg737-cKO females (n = 7) eat 29.04 ± 1.08 g, whereas controls eat 24.72 ± 1.20 g (n = 7). Independent t tests confirm that these amounts are significantly different ($t = 2.67$, $p = 0.02$). With the exception of week 13, food intake was significantly increased in the mutants compared to controls after the fourth week.

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Figure S1C

The body weights of pair-fed of Kif3a-cKO females were analyzed by one-way ANOVA with two control groups (n = 8 for both) and two experimental groups (ad libitum-fed Kif3a-cKO [n = 7] and pair-fed Kif3a-cKO [n = 8]). There was a significant difference in body weights at 6 weeks after tamoxifen administration ($F = 4.11$, $p = 0.02$). One-way ANOVA analysis of the three nonobese phenotypes showed no significant difference in body weight at any time point throughout the 14 week study.

Figure S1D

DXA analysis shows that ad libitum-fed Kif3a-cKO females display significantly higher lean and fat mass compared to controls and pair-fed mutants. With regards to lean mass, one-way ANOVA statistical analysis determined that ad libitum-fed Kif3a-cKO females were significantly higher (23.25 ± 0.97 g) than were either nontamoxifen or tamoxifen controls (18.33 ± 0.84 g and 18.48 ± 0.66 g, respectively) and pair-fed Kif3a-cKO mutants (18.00 ± 0.68, n = 4 for all groups, $F = 5.02$, $p = 0.02$). Fat mass was also significantly higher: One-way ANOVA analysis showed that there is a significant difference between the mean of the ad libitum-fed Kif3a-cKO fat mass (18.60 ± 2.16 g) to controls (nontamoxifen Kif3a-cWT: 2.583 ± 0.2 g, tamoxifen Kif3a-cWT: 3.00 ± 0.65 g) and pair-fed Kif3a-cKO mutants (4.68 ± 0.75 g) ($F = 40.74$, $p < 0.001$). One-way ANOVA of only pair-fed mutants versus controls showed no significant differences between these groups ($F = 3.54$, $p = 0.07$). The percentage fat of the females was measured and shown to be significantly different between controls and both ad libitum- and pair-fed mutants. With all four mice in the analysis, there was a significant difference in the percentage fat ($F = 40.08$, $p < 0.001$). One-way ANOVA analysis of pair-fed Kif3a-cKO mutants compared to the two control groups showed a significant increase (20.35 ± 2.72% versus 12.28 ± 0.69% (nontamoxifen Kif3a-cWT) and 13.73 ± 2.21% (tamoxifen Kif3a-cWT)) in their percentage fat ($F = 4.35$, $p < 0.05$).

Figure S1E

Fat pad mass in ad libitum-fed Kif3a-cKO females is uniformly higher throughout the body. One-way ANOVA analysis of the four groups showed a significant difference between the inguinal fat pads of ad libitum-fed Kif3a-cKO females (3.58 ± 0.23 g, n = 10) compared to Kif3a-cWT controls (nontamoxifen Kif3a-cWT: 0.60 ± 0.07 g [n = 4], tamoxifen Kif3a-cWT: 0.64 ± 0.10 g [n = 10]) and pair-fed Kif3a-cKO females (1.20 ± 0.32 g, n = 4, $F = 60.61$, $p < 0.001$). However, even though the pair-fed mutant females had a higher fat pad mass than did the controls, one-way ANOVA analysis between the three groups showed no significant differences ($F = 3.38$, $p = 0.06$).

Gonadal fat pads also showed significant differences between ad libitum-fed mutant females (4.80 ± 0.36 g) and the three other groups (nontamoxifen Kif3a-cWT: 0.59 ± 0.07 g, tamoxifen Kif3a-cWT: 0.81 ± 0.14 g, pair-fed Kif3a-cKO: 1.26 ± 0.31 g, $F = 54.97$, $p < 0.001$). This difference was seen in the ad libitum-fed Kif3a-cKO mice; the other three groups did not differ ($F = 2.39$, $p = 0.13$).

Retroperitoneal fat pads were significantly heavier in ad libitum-fed Kif3a-cKO females (0.99 ± 0.11 g) compared to other groups (nontamoxifen Kif3a-cWT: 0.09 ± 0.02 g, tamoxifen Kif3a-cWT: 0.17 ± 0.04 g, pair-fed Kif3a-cKO: 0.24 ± 0.09 g, $F = 26.11$, $p < 0.001$). The pair-fed mutants did not significantly differ from controls when comparisons were made between the retroperitoneal fat pad weights ($F = 1.53$, $p = 0.25$).

Mesenteric fat pad masses were significantly different from each other (one-way ANOVA analysis: $F = 18.75$, $p < 0.001$). It was determined that only ad libitum-fed Kif3a-cKO mesenteric fat pads (1.83 ± 0.24 g) were significantly different from the other groups (nontamoxifen loxP/wt: 0.31 ± 0.03 g, tamoxifen loxP/wt: 0.39 ± 0.06 g, pair-fed loxP/null: 0.52 ± 0.19 g) after comparison of the two control groups and pair-fed groups showed no difference ($F = 1.04$, $p = 0.38$).

Brown fat masses were also significantly different from each other: Ad libitum-fed mutant females (0.25 ± 0.03 g, n = 4) were significantly different from the other three groups (nontamoxifen Kif3a-cWT: 0.08 ± 0.01 g [n = 4], tamoxifen Kif3a-cWT: 0.08 ± 0.01 g [n = 4], pair-fed Kif3a-cKO: 0.08 ± 0.02 g [n = 4], $F = 16.79$, $p = 0.0001$).

Figure S2A

Serum leptin levels in Kif3a-cKO females are significantly elevated in ad libitum-fed obese mice, but are not different in pair-fed Kif3a-cKO females. One-way ANOVA analysis of the four samples showed a significant difference between ad libitum-fed Kif3a-cKO female serum leptin levels (37.38 ± 1.76 ng/ml, n = 10) and both control and pair-fed serum leptin levels (nontamoxifen Kif3a-cWT = 7.99 ± 1.20 ng/ml [n = 4], tamoxifen Kif3a-cWT: 9.48 ± 1.59 ng/ml [n = 12], Kif3a-cKO pair fed: 11.92 ± 4.25 ng/ml [n = 4], $F = 52.64$, $p < 0.001$). It must be noted that eight out of ten samples of ad libitum-fed Kif3a-cKO females reached the maximum detectable limit in this assay for serum leptin (40 ng/ml) and were assigned that value in the analysis. One-way ANOVA analysis of the two control groups and the pair-fed females showed no difference in leptin levels, confirming that it was only the ad libitum-fed mutants with elevated levels ($F = 0.49$, $p = 0.63$).

Figure S2B

Serum glucose levels in ad libitum-fed Kif3a-cKO females after 4 hr of fasting are significantly higher than those of controls or pair-fed mutants. One-way ANOVA analysis between four groups (n = 6 for all groups) showed a significant difference in means between ad libitum-fed Kif3a-cKO females (194.33 ± 16.60 mg/dl) and the other three groups ($F = 3.83$, $p = 0.03$). One-way ANOVA analysis confirmed that it was only the ad libitum mutant mice that differed; the other three groups had no significant difference in means ($F = 0.16$, $p = 0.86$).

Figure S2C

Serum nonfasting insulin levels in Kif3a-cKO females 16 weeks after tamoxifen administration are significantly higher in ad libitum-fed obese mice compared to controls or pair-fed Kif3a-cKO females. Kif3a-cKO females fed ad libitum displayed an average sensitive rat insulin level equal to 4.27 ± 0.95 ng/ml (n = 9). This was significantly different than non-tamoxifen- and tamoxifen-induced Kif3a-cWT controls (0.68 ± 0.27 ng/ml (n = 4), and 0.66 ± 0.12 ng/ml [n = 8], respectively) and pair-fed Kif3a-cKO females (0.41 ± 0.06 ng/ml, n = 4). This test was done with one-way ANOVA analysis ($F = 9.89$, $p < 0.001$). One-way ANOVA analysis shows there is no significant difference between controls and pair-fed mutant females with concern to serum insulin levels ($F = 0.78$, $p = 0.48$).

Figure S2D

Serum corticosterone levels showed no significant differences between Kif3a-cKO and Kif3a-cWT females. One-way ANOVA analysis indicates that there are no significant differences between any group tested (Kif3a-cWT [no tamoxifen, n = 4] = 33.86 ± 13.11 ng/ml; Kif3a-cWT [tamoxifen, n = 12] = 43.63 ± 8.83 ng/ml; Kif3a-cKO [ad libitum fed, n = 11] = 36.86 ± 11.11 ng/ml; Kif3a-cKO [pair fed, n = 3] = 19.79 ± 11.43 ng/ml, $F = 0.29$, $p = 0.83$).

Figures S3C–S3D

One-way ANOVA analysis of the four groups showed no difference in velocity between any sex or conditional mutation in *Kif3a* in POMC-expressing cells (Male Kif3a-pomcWT: 7.83 ± 0.47 cm/s [n = 8], Male Kif3a pomcKO: 8.10 ± 0.43 [n = 8], Female Kif3a-pomcWT: $F = 0.43$, $p = 0.73$). One-way ANOVA analysis of the four groups showed no difference in the ratio of the percentage of time spent in the center versus the total time in the open field between any sex or conditional mutation in Kif3a in POMC-expressing cells (Male Kif3a-pomcWT: 0.14 ± 0.02 cm/s [n = 8], Male Kif3a pomcKO: 0.12 ± 0.02 [n = 8], Female Kif3a-pomcWT: 0.08 ± 0.03 [n = 5], Female Kif3a-pomcKO: 0.12 ± 0.02 [n = 8], $F = 1.47$, $p = 0.25$).

Figure S3G

Serum corticosterone levels showed no significant difference in either Kif3a-pomcKO (51.12 ± 13.07 ng/ml, n = 6) and Kif3a-pomcWT males (37.69 ± 12.08, n = 6, $t = 0.75$, $p = 0.47$) or Kif3a-pomcKO females (66.49 ± 22.38, n = 8) and Kif3a-pomcWT females (77.46 ± 30.30, n = 7, $t = 0.30$, $p = 0.77$).

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