Cell

EXTENDED EXPERIMENTAL PROCEDURES

Drosophila Strains

The *upd2* homozygous deletion mutant used is *upd2*$^{\Delta 3\text{-}62}$ (*upd2Δ*; Hombría et al., 2005). STAT reporter flies- 10XSTAT::GFP (Bach et al., 2007). UAS lines are: *UAS-upd2-GFP* (transgenic lines 10D4 and 10D5; Hombría et al., 2005); *UAS-slif*^{anti} (Colombani et al., 2003); *UAS-Src::GFP* (Bloomington Stock #*5432)*; *UAS-dsRed2* (Bloomington Stock #8546*); UAS-DenMark* (Nicolaï et al., 2010); *UAS-NaChBac* (Luan et al., 2006); and *UAS-EKO222* (White et al., 2001). Gal4 lines are: *cg-GAL4* (Hennig et al., 2006); *ppl-GAL4* (Zinke et al., 1999); *yolk-GAL4* (Georgel et al., 2001); *Dilp2-GAL4* (Wu et al., 2005); *Dilp3-GAL4* (Buch et al., 2008); *dome-Gal4*, *UAS-2xEYFP* (Mandal et al., 2007); *GABA-B-R2-GAL4* (Enell et al., 2010); *dVGAT-GAL4* (Fei et al., 2010); and *Dmef2-GAL4* (Ranganayakulu et al., 1996). RNAi lines from the TRiP facility at Harvard Medical School (http://www.flyrnai.org/TRiP-HOME.html) are: *Luciferase-RNAi* (JF01801); *white-RNAi* (JF01786); *GFP-RNAi* (HMS00314); *upd1-RNAi* (JF03149); *upd3-RNAi* (HMS05061); upd2-RNAi (HMS00901) [referred to as upd2-RNAi (NP)]; *STAT92E-RNAi* (HMS00035); and *dome-RNAi* (HMS00647). RNAi lines from the National Institute of Genetics, Japan are: *upd2-RNAi* (5988R-1) [referred to as *upd2(1)-RNAi*] and *upd2-RNAi* (5988R-3) [referred to as *upd2(2)-RNAi*].

Food and Temperature

Flies were raised on standard lab food containing 15 g yeast, 8.6 g soy flour, 63 g corn flour, 5g agar, 5g malt, 74 ml corn syrup per liter. Temperature regiments for specific crosses were: 18C: *dome-GAL4 > STAT-RNAi* and *upd2-RNAi, dome-GAL4; tubGAL80*^{ts} > *UAS-RNAi* and *UAS-GFP/NaChBac*. Once adults eclosed, they were shifted to 29C for 3-7 days before performing the assays. 25C: *y w* and *upd2Δ*, *cg-GAL4 > UAS-RNAi* and *UAS-overexpression*, *upd2Δ; cg-GAL4 > UAS-overexpression* rescue crosses, *upd2Δ; Dilp3-GAL4 > UAS-NaChBac/EKO222/GFP*, *ppl-GAL4 > UAS-RNAi; 10XSTAT::GFP.* 27C: *ppl-GAL4 > UAS-RNAi.* 29C: *yolk-GAL4 > UAS-RNAi.* For acute 24 hr starvation experiments (Figures 2G and 2H), 3-7 day old male flies were kept on 1% agar at 29C. For chronic starvation experiments (Figures 2A, 4C, and 4D), 3-7 day old male flies were kept on 1% sucrose agar at 25C.

Analysis of Cell Size and Number

Control and experimental flies were obtained from vials of similar population density. Wings clipped from adult male flies were imaged using differential interference contrast microscopy (Zeiss Axioskop2). Wing area was measured using ImageJ and cell number measured using Adobe photoshop CS5 by counting the number of wing hairs in 40 × 40 μm squares. Cell density was measured by dividing the number of cells by wing area.

Triglyceride Measurements

Colorimetric assay

Three to five replicates of 20 male larvae or decapitated adult flies (10 females or 15 males) of a given genotype were homogenized in 250 μl of 0.1% Triton X-100 in the presence of a protease inhibitor (EDTA-free Complete, Roche, #11836170001). Samples were centrifuged at 14,000 rpm in a refrigerated tabletop centrifuge. 10.0 μl of the supernatant was used to determine the level of TAG in the sample using the Stanbio LiquiColor triglyceride Test kit (Stanbio, #2100-225) and 100.0 μl of the supernatant was used to measure the amount of protein in the sample using the BCA assay kit (Pierce, #23221).

TLC assay

Two to three replicates of five adult males or females of a given genotype were homogenized in 100 μl of 2:1 chloroform methanol mixture. The samples were briefly centrifuged at 8,000 rpm in a refrigerated centrifuge. Coconut oil (20uL dissolved in 1 ml of 2:1 chloroform-methanol mixture) was used as standard. 2 μl of the sample and standard were loaded onto a silica gel TLC plate (Sigma Aldrich, # 70644) and run in a chamber using 4:1 hexane and ethyl ether as the mobile phase. The plate was then dipped in a general oxidizing stain for 30 s. The oxidizing ceric ammonium molybdate stain was made by dissolving 8.75 g ammonium heptamolybdate tetrahydrate (Sigma Aldrich, 431346-50G) and 3.5g Cerium(IV) sulfate hydrate (Sigma Aldrich, # 423351) in 35 ml of concentrated sulphuric acid and 315 ml of water. The plate was developed in an oven at 85–90°C for 15 min. Each experiment was run on at least three independent silica plates. The plates were scanned and analyzed using Adobe photoshop CS5. To quantify TAG levels and control for loading, the ratio of the ''gray value'' function of the TAG band to another band along the same lane was used as a measure. An average of the ratio obtained from at least three technical replicates per lane was used as a measure of the TAG level per replicate.

Hemolymph Glucose Measurements

Quantification of glucose concentration in the hemolymph was done as described in (Géminard et al., 2009). Triplicates of 10 adult females of the indicated genotypes were decapitated, placed in a perforated 0.5 ml tube, centrifuged for 6 min at 1,500 g, and the hemolymph collected in an underlying 1.5 ml tube at 4C. The hemolymph was diluted 1:10 in distilled water and the glucose concentration was determined using the Glucose Hexokinase Assay kit (Sigma #GAHK-20) after trehalose conversion into glucose with porcine trehalase (Sigma #T8778) and incubation at 37C overnight.

qPCR

Total RNA was prepared from triplicates of 15 fed (standard lab food) or starved (>72 hr on 1% sucrose agar), age-matched adult males at 25C. RNA was extracted using Trizol, treated with DNAase-I (QIAGEN #79254), and cleaned up using the RNeasy MinElute Cleanup Kit (QIAGEN # 74204). cDNA was prepared using iScript cDNA Synthesis (Bio-Rad, #1708891) and 1 μg RNA was used per reaction. qPCR was performed using iQ SYBR Green Supermix (Bio-Rad, # 1708882). *alpha-tubulin* and *rp49* were used to normalize the RNA levels. Relative quantification of mRNA levels was calculated using the comparative C_T method. List of primers:

rp49: 5′- ATCGGTTACGGATCGAACAA −3′ and 5′- GACAATCTCCTTGCGCTTCT-3′. *alpha-tubulin*: 5′-GCTGTTCCACCCCGA GCAGCTGATC-3′ and 5′-GGCGAACTCCAGCTTGGACTTCTTGC-3′.
STAT92E: 5′-CTGGGCATTCACAACAATCCAC-3′ and 5′-GTATTGCGCGTAACGAACCG-3′.
upd2: 5′- CGGAACATCACGATGAGCGAAT-3′ and 5′- TCGGCAGGAACTTGTACTCG-3′.

For Figure S2, total RNA was extracted in triplicates, each consisting of 15 larval brains dissected from male larvae which had either a knockdown of *upd2* or control (*GFP-RNAi*). The qPCR was performed as described in the main Experimental Procedures. The primers used for Dilp2 mRNA are: 5′- GAATCACGGGATTATACTCCTCG −3′ and 5′- ATGAGCAAGCCTTTGTCCTTCA −3′.

Feeding Assays

Blue dye assay

Feeding assay was adapted from (Xu et al., 2008). Briefly, three to five replicates 5 adult males or females (3-7 days old) of the indicated genotype were provided food with blue dye (15% sucrose, 1% agar and 1% brilliant blue) for 4 hr at 25°C. The flies were then decapitated and the torso was homogenized in 500 μl of PBS, the samples were then centrifuged at 13,000 rpm in a tabletop centrifuge for 20 mins. The supernatant was then used to measure absorbance at a wavelength of 625 nm.

CAFÉ assay

The CAFÉ assay was performed as described in (Ja et al., 2007). 16 hr before the assay, five replicates per genotype of 7 adult male flies (3–7 days old) were transferred from standard lab food to 1.0% agar vials and provided with a 2.5% yeast extract and 5% sucrose solution maintained in 5 ml calibrated glass micropipettes (VWR, #53432-706). At the start of the assay, the old micropipette was replaced with a new one. The amount of liquid food consumed was recorded every 2 hr and corrected on the basis of the evaporation observed in a vial without flies.

Oil Red O Staining

To prepare the 0.5% oil red O stain, 0.125 g of Oil red O (Sigma Aldrich, #0625-25G) was dissolved in 25 ml of 100% propylene glycol, heated gently in a water bath until temperature of the solution reached 95°C. It was then filtered through whatman paper (25 μM pore size) while still warm and allowed to cool to room temperature (RT) overnight. The oil red O staining was performed as previously described (Gutierrez et al., 2007; Palanker et al., 2009). Actively feeding larvae from *y w* and *upd2Δ* on standard lab food were dissected and samples fixed in 4% formaldehyde in PBS for 30 min. The tissue was then washed twice in PBS, twice in 100% propylene glycol and incubated at 65C for 10 min in 0.5% oil red O stain prewarmed to 65C. The tissue was then cleared by rinsing in 85% propylene glycol and mounted in 75% glycerol. Differential interference contrast images were then obtained using a Zeiss Axioskop2 microscope.

Immunostaining, Confocal Imaging, and Analysis

Immunostaining of larval and adult brains were performed based on protocols from (Pfeiffer et al., 2010). Primary antibodies used are: rat-anti-Dilp2 (1:200; Géminard et al., 2009), rabbit-anti-Dilp5 (1:800; Géminard et al., 2009), chicken-anti-GFP (1:2,000, Abcam, # ab13970), and mouse anti-nc82 (1:10, DSHB, # nc82 s1). Secondary antibodies used are: donkey anti-rat DyLight 649 (Jackson ImmunoResearch Labs, # 712-495-150; donkey anti-chicken Dylight 488 (Jackson ImmunoResearch Labs, # 703-485-155); and donkey anti-mouse Alexa 594 (Invitrogen, # A-21203). Additional primary antibodies used in the supplemental experiments are: Rat-Elav (1:500; O'Neill et al., 1994; DSHB #7E8A10); Rabbit- Repo (1:1,000; Halter et al., 1995; gift of G.M. Technau, University of Mainz, Germany); Mouse-Synapsin (1:10; Klagges et al., 1996; DSHB #3C11). Confocal images were analyzed using Zeiss ZenLite 2009, Leica LAS AF lite and ImageJ. To calculate the intensity of Dilp staining, mean gray value was calculated from MIPs of a similar number of confocal stacks using ImageJ.

Larval Stainings

Larval brains were dissected and fixed for 30 min in 4% formaldehyde in PBS, washed multiple times in PBS with 1% Triton X-100 (PBT) + 0.5% BSA. Tissues were then preblocked in PBT + 0.5% BSA + 5% Normal donkey serum (NDS) (referred to as block) for 1 hr and then incubated overnight at 4C with the primary antibody in block, and then washed multiple times in PBT+0.5% BSA and incubated for 30 min in block. A cocktail of secondary antibodies was then added to the block (final concentration 1:250) and the tissues were incubated in secondary for 2-4 hr at RT. The samples were then washed 3× for 15 min each time, with PBT+0.5% BSA and mounted on slides with a single scotch tape spacer in Slowfade gold antifade (Invitrogen, # S36936). Brains were imaged using Leica SP2 confocal system. Regarding the Dilp staining in larvae, when larvae are in the postfeeding state, just prior to pupariation, they already have an increase in basal levels of Dilp accumulation (Slaidina et al., 2009); hence, the sensitivity of this assay is

compromised. The difference is most prominent in actively feeding larvae. Matching the sex and population density is also crucial to detect a significant difference in Dilp accumulation.

Adult Stainings

Adult brains were dissected in PBS and fixed in cold 0.8% Para-formaldehyde (PFA) in PBS overnight at 4C. Tissues were washed the following day multiple times in 0.5% BSA and 0.5% Triton X-100 in PBS (PAT), preblocked in PAT+ 5% NDS for 2 hr at RT, and then incubated with the primary antibody overnight at 4C. The following day, the tissues were washed numerous times in PAT and then blocked again for 30 min in PAT+ 5% NDS and incubated in a cocktail of secondary antibodies in block (final concentration of 1:500) overnight at 4C. The samples were washed 3×–5× for 15 min per wash in PAT and mounted on slides with two layers of scotch tape spacers in Slowfade gold antifade (Invitrogen, #S36936) and imaged using Leica SP2 and Zeiss LSM 780 confocal systems.

Construction of Human Leptin Transgenic Flies

Leptin human cDNA clone was obtained (Origene, # SC120021, LEP [NM_000230]) and the Leptin ORF was PCR amplified using a high fidelity PCR polymerase (Bio-Rad, #172-5330). Primers used were 5′– caacatgggaATGCATTGGGGAACCCTGTG –3′ and 5′ –TCAGCATCCAGGGCTGAGGTCCAGCTG –3′. The PCR product was then cloned into the pWALIUM10-roe vector available from the TRiP facility at Harvard Medical School. The cloning was performed as per the protocol listed on the TRiP website (http://www.flyrnai.org/supplement/VALIUM10roe_Protocol.pdf). Briefly, the PCR product cloned into the gateway vector pENTR/D TOPO vector (Invitrogen, K240020). The entry plasmid pENTR-Leptin was then used in a LR clonase reaction (Invitrogen, 11791-020), with the destination vector pWALIUM10-roe (Ni et al., 2008). The plasmid was then microinjected into embryos which harbor attP2 landing sites, as per standard procedures to create transgenics. Two independent germline transformed lines were established from 50 injected embryos in the *y w* background.

Tissue-Culture and Luciferase Assays

Drosophila Kc cells were maintained in Schneider's medium (GIBCO), 10% heat-inactivated FBS (SIGMA) and 5% Pen-Strep (GIBCO) at 25°C. The day before transfection cells were passaged to 60%–80% confluency. Experiments were run in 96 well plates (in six replicates per condition), 150 ng/μl of the appropriate dsRNA (GFP (control) or *dome* (DRSC amplicon IDs– DR19583 and DR32731) prepared using the MegaScript in vitro transcription kit (Ambion) were seeded in the wells prior to the start of experiment. Each well was transfected with 0.05 ng *10XSTATLuc*, 14 ng *Act-Renilla*, 106 ng pAC-PL (used as carrier DNA), the above DNA master mix was mixed with 21.5 μl of EC buffer (QIAGEN), incubated for 2-3 min at RT, then mixed with 2.5 μl Enhancer (QIAGEN), incubated for 2–3 min at RT, then mixed with 0.75 μl Effectene transfection reagent (QIAGEN) and dispensed into the wells. The dsRNA and DNA transfection mix was incubated in the wells at RT for 5–10 min and then 100 μl Kc cells were added per well at a concentration of 3×10^4 cells. 96 hr after transfection, media supplemented with 4 nM human recombinant Leptin (Sigma Aldrich, # L4146-1MG) or control media supplemented with no Leptin was added to the wells. Luciferase activity was measured using DualGlo reagents (Promega) as per kit instructions and measured using the Analyst GT plate reader.

Calculation of the Michaelis Constant K_m of Leptin

The experimental procedure followed for the cell-based luciferase was the same as described above. The experiment was done in six replicates with four different doses of human Leptin and luciferase activity was measured 30 hr after stimulation with Leptin. The response of 10XSTATLuc reporter at different doses of human Leptin in Kc Cells was determined. The fold change in the RLU of the STAT reporter at different doses of human Leptin was plotted. A logarithmic trend-line fitted the data with a correlation coefficient of 0.93. K_m is the substrate concentration at which the reaction rate is half of V_{max}, the maximum rate achieved by the system. In this experimental data set the V_{max} was 32.8 at 40 nM of human Leptin. Solving the equation of this log curve, $y = 21.188 \ln(x) - 1.8226$, yielded a K_m for human Leptin in this assay to be 2.37 nM.

SUPPLEMENTAL REFERENCES

Bach, E.A., Ekas, L.A., Ayala-Camargo, A., Flaherty, M.S., Lee, H., Perrimon, N., and Baeg, G.H. (2007). GFP reporters detect the activation of the *Drosophila* JAK/STAT pathway in vivo. Gene Expr. Patterns *7*, 323–331.

Buch, S., Melcher, C., Bauer, M., Katzenberger, J., and Pankratz, M.J. (2008). Opposing effects of dietary protein and sugar regulate a transcriptional target of *Drosophila* insulin-like peptide signaling. Cell Metab. *7*, 321–332.

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., and Léopold, P. (2003). A nutrient sensor mechanism controls *Drosophila* growth. Cell *114*, 739–749.

Enell, L.E., Kapan, N., Söderberg, J.A., Kahsai, L., and Nässel, D.R. (2010). Insulin signaling, lifespan and stress resistance are modulated by metabotropic GABA receptors on insulin producing cells in the brain of *Drosophila*. PLoS One *5*, e15780.

Fei, H., Chow, D.M., Chen, A., Romero-Calderón, R., Ong, W.S., Ackerson, L.C., Maidment, N.T., Simpson, J.H., Frye, M.A., and Krantz, D.E. (2010). Mutation of the *Drosophila* vesicular GABA transporter disrupts visual figure detection. J. Exp. Biol. *213*, 1717–1730.

Géminard, C., Rulifson, E.J., and Léopold, P. (2009). Remote control of insulin secretion by fat cells in *Drosophila*. Cell Metab. *10*, 199–207.

Georgel, P., Naitza, S., Kappler, C., Ferrandon, D., Zachary, D., Swimmer, C., Kopczynski, C., Duyk, G., Reichhart, J.M., and Hoffmann, J.A. (2001). *Drosophila* immune deficiency (IMD) is a death domain protein that activates antibacterial defense and can promote apoptosis. Dev. Cell *1*, 503–514.

Gutierrez, E., Wiggins, D., Fielding, B., and Gould, A.P. (2007). Specialized hepatocyte-like cells regulate *Drosophila* lipid metabolism. Nature *445*, 275–280.

Halter, D.A., Urban, J., Rickert, C., Ner, S.S., Ito, K., Travers, A.A., and Technau, G.M. (1995). The homeobox gene repo is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila melanogaster*. Development *121*, 317–332.

Hennig, K.M., Colombani, J., and Neufeld, T.P. (2006). TOR coordinates bulk and targeted endocytosis in the *Drosophila melanogaster* fat body to regulate cell growth. J. Cell Biol. *173*, 963–974.

Hombría, J.C., Brown, S., Häder, S., and Zeidler, M.P. (2005). Characterisation of Upd2, a *Drosophila* JAK/STAT pathway ligand. Dev. Biol. *288*, 420–433.

Ja, W.W., Carvalho, G.B., Mak, E.M., de la Rosa, N.N., Fang, A.Y., Liong, J.C., Brummel, T., and Benzer, S. (2007). Prandiology of *Drosophila* and the CAFE assay. Proc. Natl. Acad. Sci. USA *104*, 8253–8256.

Klagges, B.R., Heimbeck, G., Godenschwege, T.A., Hofbauer, A., Pflugfelder, G.O., Reifegerste, R., Reisch, D., Schaupp, M., Buchner, S., and Buchner, E. (1996). Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila*. J. Neurosci. *16*, 3154–3165.

Luan, H., Lemon, W.C., Peabody, N.C., Pohl, J.B., Zelensky, P.K., Wang, D., Nitabach, M.N., Holmes, T.C., and White, B.H. (2006). Functional dissection of a neuronal network required for cuticle tanning and wing expansion in *Drosophila*. J. Neurosci. *26*, 573–584.

Mandal, L., Martinez-Agosto, J.A., Evans, C.J., Hartenstein, V., and Banerjee, U. (2007). A Hedgehog- and Antennapedia-dependent niche maintains *Drosophila* haematopoietic precursors. Nature *446*, 320–324.

Ni, J.Q., Markstein, M., Binari, R., Pfeiffer, B., Liu, L.P., Villalta, C., Booker, M., Perkins, L., and Perrimon, N. (2008). Vector and parameters for targeted transgenic RNA interference in *Drosophila melanogaster*. Nat. Methods *5*, 49–51.

Nicolaï, L.J., Ramaekers, A., Raemaekers, T., Drozdzecki, A., Mauss, A.S., Yan, J., Landgraf, M., Annaert, W., and Hassan, B.A. (2010). Genetically encoded dendritic marker sheds light on neuronal connectivity in *Drosophila*. Proc. Natl. Acad. Sci. USA *107*, 20553–20558.

O'Neill, E.M., Rebay, I., Tjian, R., and Rubin, G.M. (1994). The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway. Cell *78*, 137–147.

Palanker, L., Tennessen, J.M., Lam, G., and Thummel, C.S. (2009). *Drosophila* HNF4 regulates lipid mobilization and beta-oxidation. Cell Metab. *9*, 228–239.

Pfeiffer, B.D., Ngo, T.T., Hibbard, K.L., Murphy, C., Jenett, A., Truman, J.W., and Rubin, G.M. (2010). Refinement of tools for targeted gene expression in *Drosophila*. Genetics *186*, 735–755.

Ranganayakulu, G., Schulz, R.A., and Olson, E.N. (1996). Wingless signaling induces nautilus expression in the ventral mesoderm of the *Drosophila* embryo. Dev. Biol. *176*, 143–148.

Slaidina, M., Delanoue, R., Gronke, S., Partridge, L., and Léopold, P. (2009). A *Drosophila* insulin-like peptide promotes growth during nonfeeding states. Dev. Cell *17*, 874–884.

White, B.H., Osterwalder, T.P., Yoon, K.S., Joiner, W.J., Whim, M.D., Kaczmarek, L.K., and Keshishian, H. (2001). Targeted attenuation of electrical activity in *Drosophila* using a genetically modified K(+) channel. Neuron *31*, 699–711.

Wu, Q., Zhang, Y., Xu, J., and Shen, P. (2005). Regulation of hunger-driven behaviors by neural ribosomal S6 kinase in *Drosophila*. Proc. Natl. Acad. Sci. USA *102*, 13289–13294.

Xu, K., Zheng, X., and Sehgal, A. (2008). Regulation of feeding and metabolism by neuronal and peripheral clocks in *Drosophila*. Cell Metab. *8*, 289–300.

Zinke, I., Kirchner, C., Chao, L.C., Tetzlaff, M.T., and Pankratz, M.J. (1999). Suppression of food intake and growth by amino acids in *Drosophila*: the role of pumpless, a fat body expressed gene with homology to vertebrate glycine cleavage system. Development *126*, 5275–5284.

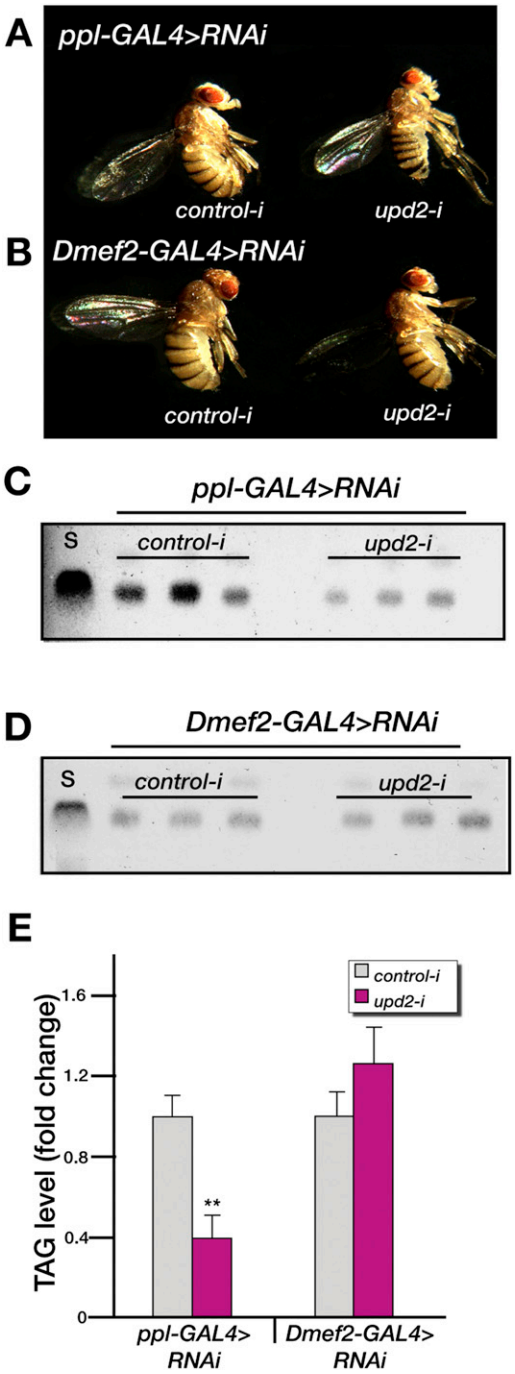

Figure S1. Upd2 Plays an FB-Specific Role in Regulating Systemic Growth and Metabolism, Related to Figure 1

(A and B) Adult female flies with FB-specific (A) and muscle-specific (B) knockdown of *upd2*. Only an *upd2* FB-specific knockdown results in growth defects (A). (C–E) TLC done on adult male flies that harbor an FB-specific (C) or muscle-specific (D) knockdown of *upd2*. Compromising *upd2* function in the FB results in reduced stored fat (C and E), whereas knocking it down in the muscle has no effect on fat storage (D and E). *control-i* refers to *GFP-RNAi*, and the *upd2(NP)-RNAi* line was used to knock down *upd2* in these experiments (*upd2-i*). The p values were calculated using Welch's t test; $^{**}p < 0.001$. S in TLC refers to the coconut oil standard in the TLC assay. Error bars indicate the SD.

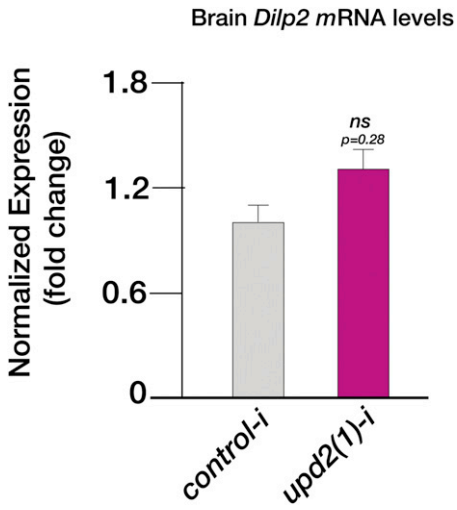

Figure S2. Dilp2 mRNA Levels in the Brain Are Not Significantly Altered in Flies with FB-Specific *upd2* RNAi, Related to Figure 3

Total RNA was extracted from brains of male larvae (see Extended Experimental Procedures), and the expression of *Dilp2* was analyzed by qPCR. The level of *Dilp2* mRNA in the brain is not significantly altered when *upd2* is knocked down in the FB. The p values were calculated using Welch's t test. Error bars indicate the SD.

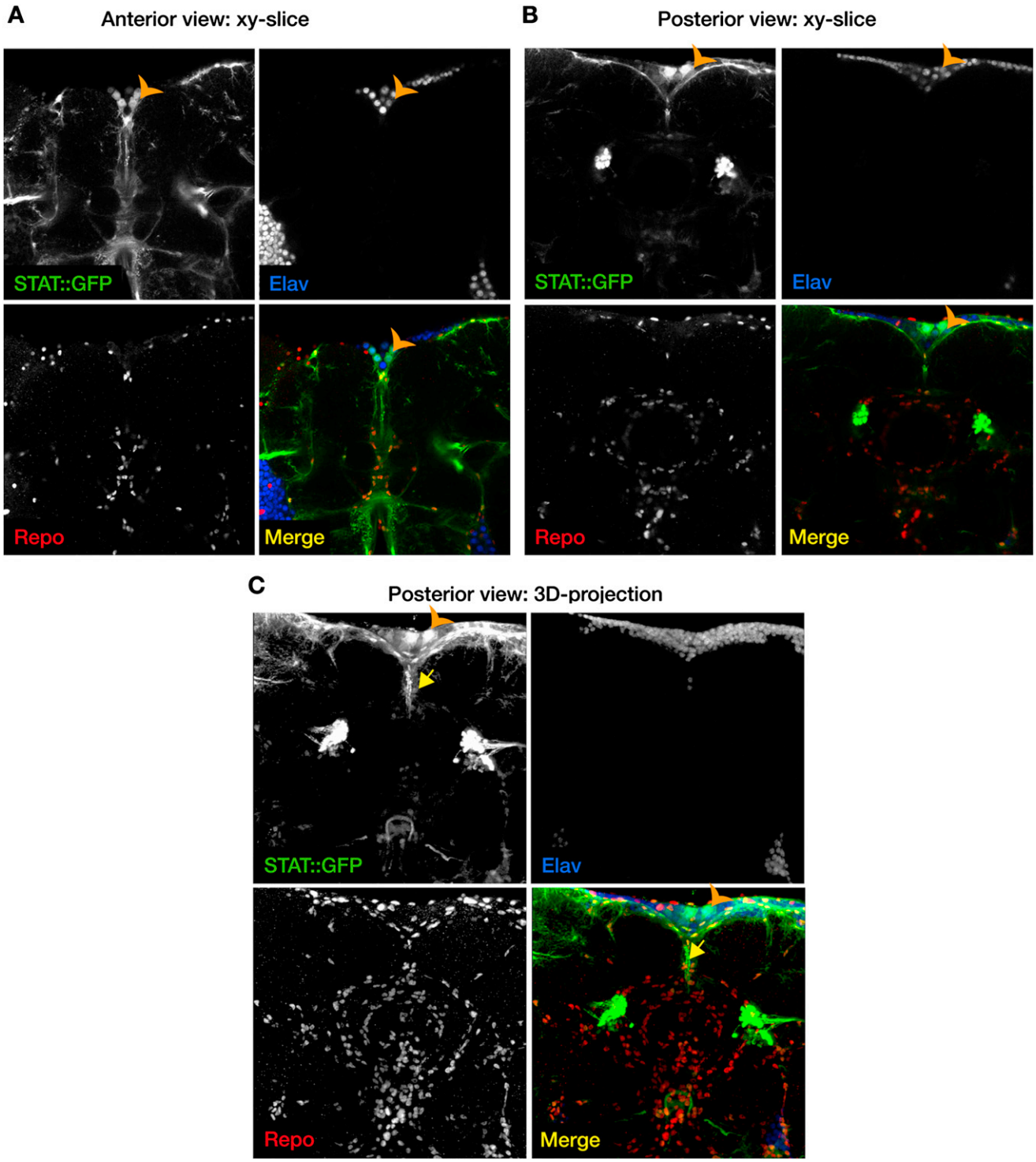

Figure S3. STAT::GFP Reporter Cells in the mNSC Region Are Expressed in Neurons, Related to Figure 4

(A–C) Single XY confocal sections of anterior (A), posterior (B), and three-dimensional XY projection (C) of adult male brains expressing the STAT::GFP reporter (green) immunostained with Elav, a neuronal nuclear marker (blue), and Repo, which is expressed in the glial cell nucleus (red). The STAT reporter expressing cells in the median neurosecretory region colocalize with the neuronal marker (orange arrowhead). The yellow arrow points to the bouton-like structure described in the Results section.

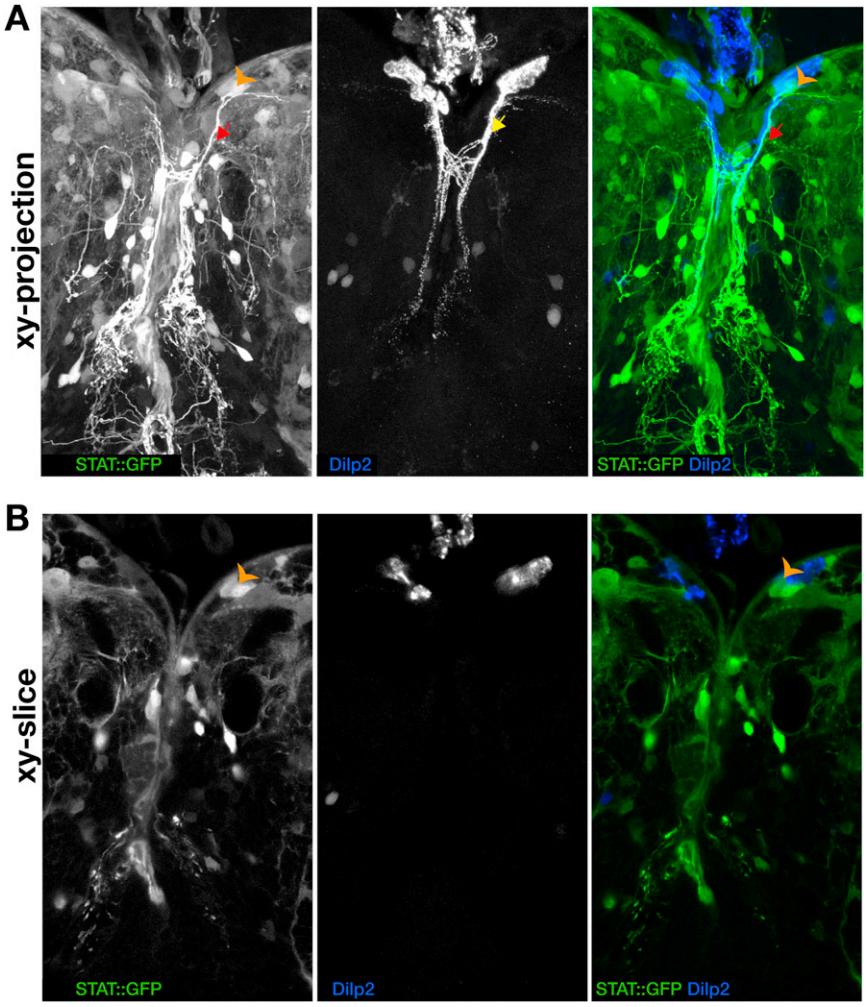

Figure S4. STAT::GFP Reporter Is Expressed in Neurons Juxtaposed with Dilp2-Producing IPCs in the Larval Brain, Related to Figure 4
(A and B) An XY projection (A) and a single confocal section (B) of larval brains expressing STAT::GFP reporter (green) immunostained for Dilp2 (blue). The STAT reporter is expressed widely, and is expressed in cells juxtaposed with Dilp-producing IPCs (orange arrowhead). The red arrow in A points to processes from the STAT reporter neurons, which run adjacent to the Dilp2 neurons.

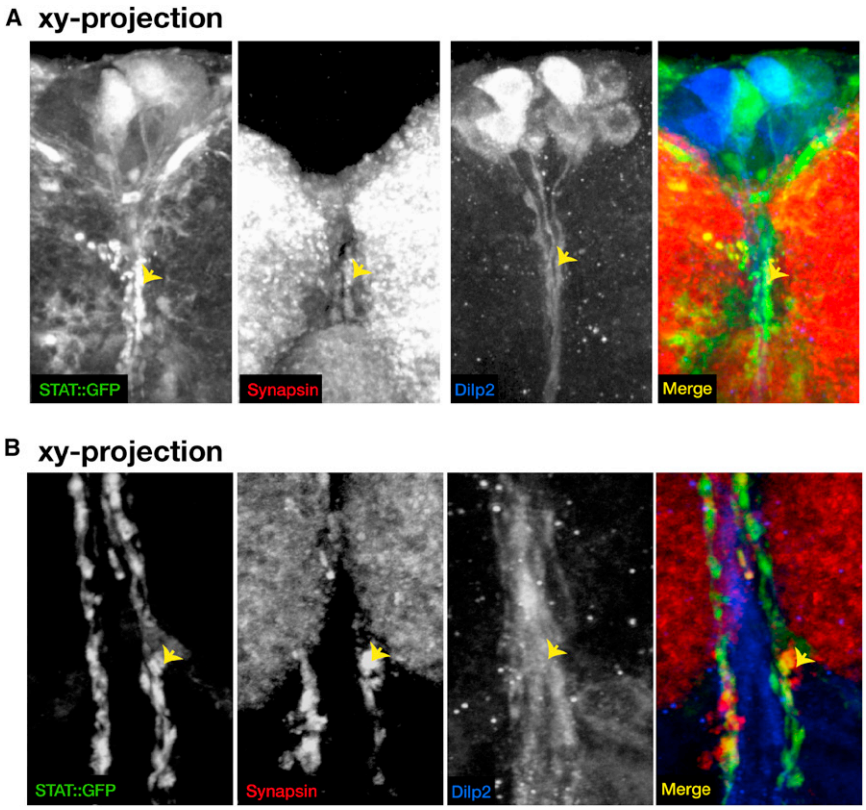

Figure S5. A Presynaptic Marker Is Colocalized with Bouton-Like Structures of the STAT::GFP Reporter, Related to Figure 4

(A and B) Images show XY projections of a few confocal slices at lower (40×, A) and higher (63×, B) magnification. Synapsin (red) is a presynaptic marker that localizes to synaptic junctions. The bouton-like structure of the STAT reporter neurons (green), which are adjacent to the Dilp2-expressing processes (blue), colocalize with synapsin (yellow arrows).

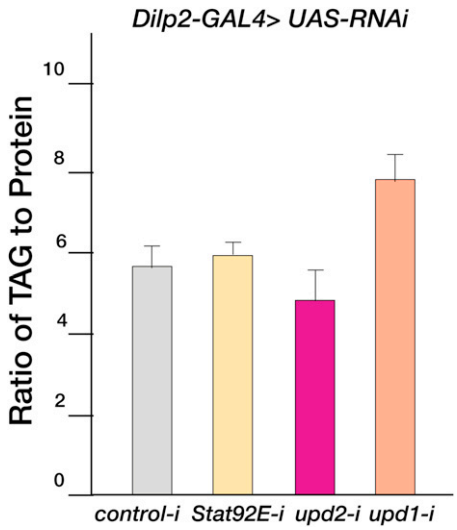

Figure S6. TAG Levels Are Unaltered when JAK/STAT Components Are Knocked Down in Dilp Neurons, Related to Figure 6

Quantification of the ratio of TAG to protein in adult males with IPC-specific knockdown of JAK/STAT pathway components. Results from two independent experiments are represented. No significant change in TAG levels is observed. *control-i* refers to *Luciferase-RNAi*. Error bars represent the SD.

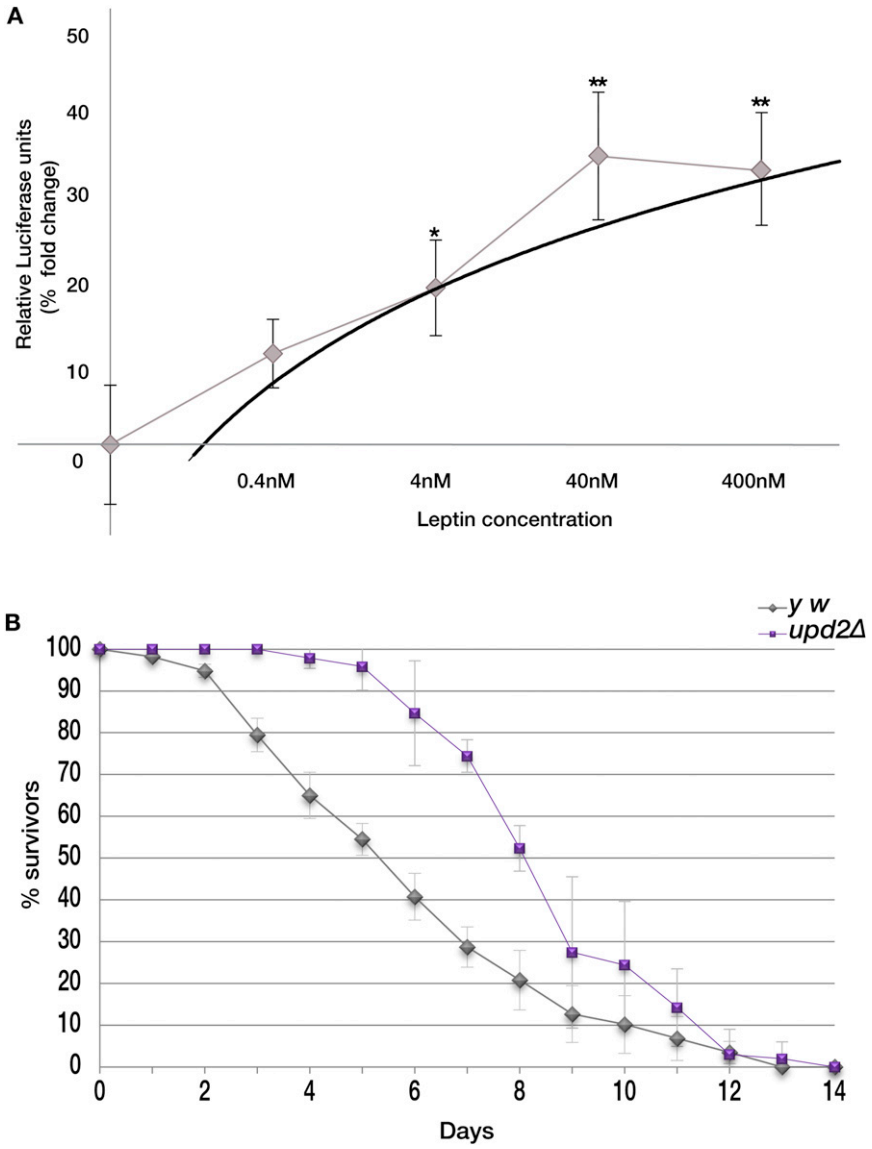

Figure S7. Human Leptin Can Signal through the *Drosophila* JAK/STAT Receptor and Upd2 Plays a Role Physiologically Similar to that of Leptin during Starvation, Related to Figure 7

(A) Affinity of human Leptin for the Dome receptor. Luciferase activity was measured after 30 hr of activation with Leptin at different concentrations. The Michaelis constant, K_m (the concentration of the substrate at which the reaction rate is half-maximum), is equal to 2.37 nM. The experiment was done in six biological replicates. *$p < 0.1$;**$p < 0.001$; p values were calculated using Welch's t test. Error bars indicate percent SD.

(B) *upd2Δ* adults are resistant to starvation. Survival of 10-day-old adult males on 1% sucrose agar at 25°C, n = 60 for *upd2Δ* (triplicates of n = 20), n = 80 for *y w* (four replicates of n = 20). Error bars represent SD.