Supplemental Materials and Procedures.

Fly Strains and media

UAS-AMPK flies were obtained from Jongkyeong Chung. *pGFP-Atg8a* flies were provided by Eric Baehrecke. The following lines were obtained from the Bloomington Stock Center; *UAS-mCh-AMPK* (32109), *AMPK-RNAi* (35137), *UAS-Atg1-6B* (51655). *UAS-Atg1-RNAi* line was received from the Vienna Drosophila RNAi (VDRC) Center (stock no. 16133). *ELAV-GAL4* constitutive (Bloomington) expression experiments were performed on flies that have undergone 12 rounds of backcrossing into a W^{1118} background. All epistasis experiments were performed on flies that have undergone 12 rounds of backcrossing into a W^{1118} background. *Drosophila* were reared in vials containing cornmeal medium (1% agar, 3% yeast,1.9% sucrose, 3.8% dextrose, 9.1% cornmeal, 1% acid mix, and 1.5% methylparaben, all concentrations given in wt/vol) throughout all experiments.

Lifespan analysis and starvation.

Flies that eclosed over a 36 hour period were collected and allowed to mate for approximately 60 hours. Female or male flies were collected under light nitrogen-induced anesthesia and maintained at a density of 30-35 flies per vial in a humidified, temperature-controlled (25° C.) incubator with a 12 hour light-dark cycle. Flies were transferred to new vials every 2-3 days and scored for death. For starvation analysis female flies were aged for 10 days in vials containing cornmeal medium and then transferred to 1% agar solution in a humidified, temperature-controlled incubator with 12 h on/off light cycle at 25° C. Percentage of survival was measured every day, with survivors transferred to fresh vials every 2 days.

Immunostaining procedure for DILP2 or GFP-Atg8a in brain/intestinal tissues.

Female flies were anesthetized on ice and intestines/brains were dissected in cold PBS. Samples were then fixed in 4% formaldehyde in PBS at room temp for 30 minutes, rinsed three times in PBS + 0.2% Triton X-100 for 10 minutes at room temperature. Blocking was performed in 5% BSA in PBS + 0.2% triton X-100 for one hour at room temperature. Primary antibody mouse anti-DILP2 (a generous gift from Dr. Seung Kim), or rabbit anti-GFP (Cell Signaling, D5.1, XP), was added 1:250 in 5% BSA in PBS + 0.2% triton X-100 and incubated overnight at 4 degrees Celsius. Samples were then rinsed three times in PBS + 0.2% Triton X-100 for 10 minutes at room temperature, and secondary antibody incubation, anti-rabbit AlexaFluor-488, or anti-mouse AlexaFluor-488 (Invitrogen) was added 1:200, and To-Pro-3 DNA stain (Invitrogen) 1:200, or phalloidin AlexaFlour-633 (Invitrogen) 1:200, in 5% BSA in PBS + 0.2% triton X-100 for 4 hours at room temperature. Samples were rinsed three times in PBS + 0.2% triton X-100 for 10 minutes at room temperature. Intestines/brains were then mounted in Vectashield mounting medium (Vector Labs) and Imaged using Zeiss single point LSM 5 exciter confocal microscope.

Quantification of DILP2 signal was performed similarly to (Broughton et al., 2010). Briefly, confocal Z stacks were taken to capture all IPCs of the brain, with identical excitation parameters for all conditions. Stacks were merged as projections using Axiovision v. 4.8 and average fluorescent intensity was measured using Image J. Average fluorescent intensity of IPCs of a given area per brain was analyzed via student's t-test. A minimum of 6 brains were used for all conditions.

For brain GFP-Atg8a foci quantifications, Z stacks of the optic lobe were taken using identical settings with the 63X objective. For Intestinal quantification of GFP-atg8a foci, Z stacks of enterocytes in regions 200–500 μm anterior to the pylorus were imaged. The average number of foci in either the gut or brain was counted using the local maxima tool in Image J. Statistical analysis was conducted on mean autophagosome counts averaged from a set area of 120 μm^2 for each midgut or brain using a two-tailed, unpaired Student's t test (n>9 brains or guts were used per condition).

Lysotracker Red.

Female flies were anesthetized on ice and intestines were dissected in cold PBS**.** Intestines were washed once in PBS, followed by three 30s rinses in freshly prepared 1 μM Lysotracker Red (Invitrogen) in PBS at room temperature. Intestines were washed five times for 30s in PBS at room temperature, then mounted in Vectashield, and imaged immediately. Imaging should not proceed for very long as apoptosis can be observed after approximately 60 min. Quantification of acidophilic vesicles was performed similarly to above GFP-atg8a quantification.

Immunostaining procedure for dissected indirect flight muscle.

Staining was performed similarly to (Rana et al., 2013). Hemithoraces were dissected and fixed for 20 minutes in PBS with 4% paraformaldehyde and 0.2% Triton X-100. After washing, samples were incubated overnight at 4 °C with an antibody detecting polyubiquitinated proteins at 1:250 mouse mAb FK2 (Enzo). Washed thoroughly and incubated with secondary anti-mouse AlexaFlour-568 (1:250), with phalloidin AlexaFlour-633. Samples were rinsed three times in PBS + 0.2% triton X-100 for 10 minutes at room temperature, then mounted in Vectashield mounting medium (Vector Labs) and Imaged. For quantification of protein aggregates in hemithoraces, single-channel images were converted into grayscale and the area and total number of protein aggregates was measured using ImageJ. Statistical analysis was conducted the area of protein aggregates/indirect flight muscle region averaged using a two-tailed, unpaired Student's t test (n>10 thoraces were used per condition).

Western blot.

Heads of 10 day old female flies were dissected and lysates were separated by SDS page using standard procedures. Membranes were probed with antibodies against AMPK phospho-T184 at 1:1000 (Cell Signaling, 40H9), anti-phospho-S6K T398 (Cell Signaling, 9209), anti-total S6K 1:300 (Santa Cruz, C-18, SC-230), anti-Ubiquitin added at 1:1000 (Cell Signaling, P4D1), and with horseradish peroxidase-conjugated monoclonal mouse antibody against Actin diluted 1:5000 (Sigma). The rabbit antibodies were detected using horseradish peroxidase-conjugated anti-rabbit IgG antibodies at 1:2000 (Sigma). The mouse antibodies were detected using horseradish peroxidase-conjugated anti-mouse IgG antibodies 1:2000 dilution (Sigma). ECL 2 chemiluminescent/chemifluorescent reagent (Pierce) was used to visualize horse radish peroxidase activity, and the chemifluorescence was detected using a Typhoon scanner (GE Healthcare).

Triton-Insoluble protein extracts.

Dissected thoraces were homogenized in ice-cold PBS with 1% Triton X-100 and protease inhibitors. The mixture was spun for 10 min at 4 °C, and the pellet and supernatant were collected. The Triton X-100–insoluble pellet was washed and resuspended in lysis solution with 5% lithium dodecyl sulfate (LDS) (NuPAGE LDS Sample Buffer; Invitrogen). N>4 independent samples of 5 thoraces were used for densitometry post-blotting.

Quantitative real-time PCR.

Head, thorax, or intestines were dissected on ice, and then total RNA was extracted using TRIzol reagent (Invitrogen) following manufacturer protocols. Samples were treated with DNase, and then cDNA was synthesized using the standard kit (Fermentas). Equalized amplicons of Actin5C were used as a reference to normalize exogenous AMPK—mCherry RFP fwd-CCACAACGAGGACTACACCA and AMPK rev-GGTAGTGCCCAATCTTGACC, total AMPK— fwd-CAAGATCCGGCGTGAGAT and rev-GCTTGCCGTGCTTCACAATA, Atg1— fwd- GCTTCTTTGTTCACCGCTTC and rev-GCTTGACCAGCTTCAGTTCC, Atg8a— fwd-AGTCCCAAAAGCAAACGAAG and rev-TTGTCCAAATCACCGATGC, Atg8b— fwd-AATGTGATCCCACCGACATC and rev-TTGAGCGAGTAGTGCCAATG, Actin5C—fwd-TTGTCTGGGCAAGAGGATCAG and rev-ACCACTCGCACTTGCACTTTC. dilp2—fwd-GCTTTAATACGCTGCCAAGG and rev-CGGATCCGTACAGATTGGTT. dilp5—fwd-AGAGAACTTTGGACCCCGTGA and rev-TGAACCGAACTATCACTCAAC. 4E-BP—fwd- TACACGTCCAGCGGAAAGTT and rev-CCTCCAGGAGTGGTGGAGTA.

Mass/weight.

Flies were weighed in groups of 10 in preweighed microcentrifuge tubes, using an analytical scale (Torbal, Clifton, NJ, USA).

Quantification of Triglycerides.

Lipids were extracted from five whole female flies in a chloroform:ethanol solution (2:1 vol/vol), and nonpolar lipids (fatty acid, triacylglycerol) were separated by thin-layer chromatography with a n-hexane/diethylether/glacial acetic acid solution (70:30:1, vol/vol/vol). Plates were air-dried and stained (with 0.2% Amido Black 10B in 1 M NaCl), and lipid bands were quantified by photo densitometry using ImageJ.

Intestinal barrier dysfunction assay.

The 'Smurf fly'/intestinal barrier dysfunction assay was performed similarly to (Rera et al., 2012). Flies were aged on standard medium until the day of the Smurf assay. Dyed medium was prepared using standard medium with blue dye no. 1 added at a concentration of (2.5% wt/vol). The blue no. 1 was purchased from SPS Alfachem. A fly was counted as a Smurf when dye coloration was observed outside the digestive tract. Comparisons of Smurf proportion per time point were carried out using binomial tests to calculate the probability of having as many Smurfs in population A as in population B. The binomial tests were performed using R version 2.14.2.

Feeding assays.

Quantification of food intake was assayed at 10 d of age using two different approaches. Firstly, food intake was assayed based upon the uptake of a blue food dye (FD&C Blue Dye No. 1; SPS Alfachem) as described in (Hur et al., 2013; Rana et al., 2013; Wong et al., 2009). Three vials of female flies of each genotype were transferred onto fresh medium containing blue dye 2.5% wt/vol at 9:00 AM hours for 2

hours. Flies were frozen, decapitated, and homogenized separately in 250µl H2O. Cell debris was pelleted via centrifugation, and absorbance (629nm) of a 1:2.5 dilution of the supernatant was used to determine whether the fly ate (flies were categorized as having eaten if they had A629nm greater than 110% of the absorbance outside of the dye absorption range, at 800nm), and if so, the relative meal size (A629-A800) and absorbance was measured at 630 nm using an Epoch spectrophotometer (BioTek).

Analysis of capillary feeding ("the CAFE assay") was performed similarly to (Hur et al., 2013; Ja et al., 2007) with minor modifications. Briefly, 10 flies were placed in vials with wet tissue paper as a water source and a capillary food source (5% sucrose, 5% yeast extract, 2.5% FD&C Blue No. 1) Feeding was monitored from 1 h after lights on until lights off, with capillaries being replaced and feeding amounts recorded every hour, for 11-12 hours.

Fecundity.

Eight vials of mated female flies (10 flies per vial) were collected and kept in a humidified, temperature-controlled incubator with 12 h on/off light cycle at 25 °C. Eggs laid per fly in 24 h were counted on days 7, 14, 23, and 30 post-eclosion.

Spontaneous Activity Assay.

Spontaneous activity was measured via the Drosophila Activity Monitor (DAM) system as in (Rana et al., 2013). Each experiment was performed on 3 vials of 10 flies each, with average number of beam breaks condensed into 10 minute intervals over 24 hours of a 12 hour light/dark cycle.

Climbing Assay.

Assessment of climbing ability was performed similarly to (Copeland et al., 2009) with minor modifications. Briefly, 30 flies were placed in a standard 23mmX95mm plastic vial and gently tapped to the bottom. The number of flies that reached the top 1/4 of the vial within 20 seconds were then scored as climbing. Each experiment was performed on a minimum of 6 vials of 30 flies.

Hyperoxia lifespan.

Vials of 10 day old flies were maintained on food with or without RU486, kept in a humidified Plexiglas chamber where oxygen was constantly passed through at a rate sufficient to maintain an atmospheric concentration of 80%. Flies were scored for death 1-2 times daily, and media was changed every 2 days.

Heat stress.

Vials of 10 day old flies were maintained on food with or without RU486, kept in a humidified incubator at 37°C. Flies were scored for death every hour to ½ hour.

Supplemental References.

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