Supplemental Information Inventory

A Mitochondrial ATP synthase Subunit Interacts with TOR Signaling to Modulate Protein Homeostasis and Lifespan in Drosophila

Xiaoping Sun[1], Charles T. Wheeler[1], Jason Yolitz[1], Mara Laslo[1], Thomas Alberico[1], Yaning Sun[1], Qisheng Song[2] and Sige Zou[1,*]

[1]Translational Gerontology Branch, National Institute on Aging, Baltimore, MD 21224, USA; [2]Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA

This manuscript includes the following supplemental materials:

1. Supplemental Materials and Methods, related to the materials and methods in the main text.
2. Figure S1. The Effect of ATPsyn-d Knockdown and Overexpression on Lifespan and Stress Response, Related to Figure 1 and 2.
3. Figure S2. The Effect of RU486 Alone on the Lifespan of Flies Fed SY1:1, SY1:9, SY9:1 or Cornmeal Diet, Related to Figure 1 and 2.
4. Figure S3. The Effect of ATPsyn-d Knockdown on Food Intake, Locomotor Activity and Reproduction, Related to Figure 1, 2 and 6.
5. Figure S4. The Effect of RU486 Feeding on Gene Expression, Related to Figure 3 and 4.
6. Figure S5. The Effect of ATPsyn-d Knockdown or RU486 Feeding Alone on Stress Response, Mitochondrial and Metabolism Function in Females, Related to Figure 4.

Supplemental Information

A Mitochondrial ATP synthase Subunit Interacts with TOR Signaling to Modulate Protein Homeostasis and Lifespan in Drosophila

Xiaoping Sun[1], Charles T. Wheeler[1], Jason Yolitz[1], Mara Laslo[1], Thomas Alberico[1], Yaning Sun[1], Qisheng Song[2] and Sige Zou[1,*]

[1]Translational Gerontology Branch, National Institute on Aging, Baltimore, MD 21224, USA; [2]Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA

*Corresponding author: Sige Zou, Ph.D., Translational Gerontology Branch, National Institute on Aging, Baltimore, MD 21224, USA; Phone: 410-558-8461; Fax: 410-558-8302; Email: zous@mail.nih.gov

Supplemental Materials and Methods

Fly media and stocks

Fly stocks were maintained on standard cornmeal medium at 25±1 ^{o}C, 60±5% humidity and a 12:12h light/dark cycle (Ashburner et al., 2005). *Canton S*, w^{1118}, and da-Gal4 (w^{1118}; P{w[+mW.hs]=GAL4-da.G32},3) were obtained from Bloomington *Drosophila* Stock Center (Bloomington, Indiana). UAS-ATPsyn-d-RNAi lines, w^{1118}; P{GD9915}v21018 and w^{1118}; P{KK107800}v104353, were from Vienna *Drosophila* RNAi center (Vienna, Austria), which are labeled as UAS-ATPsyn-d-RNAi(v21018) and UAS-ATPsyn-d-RNAi(v104353) in the main text, respectively. UAS-ATPsyn-d transgenic lines were generated by injecting w^{1118} with a full-length ATPsyn-d cDNA cloned into the pUAST vector. actin-GSG, da-GSG and UAS-Tsc2 were kindly provided by J. Tower, V. Monnier and D. Pan, respectively. The SY1:9 diet contained 2% sugar and 18% autolyzed yeast (Cat. #103304, MP Biomedicals); SY1:1 had 10% sugar and 10% yeast; SY9:1 had 18% sugar and 2% yeast. All sugar-yeast (SY) diets contained 1.5% agar. The cornmeal food contained 5% glucose, 2.5% sucrose, 6% cornmeal, 1.5 % dry yeast and 0.6% agar. The ratio of the total amount of glucose, sucrose and cornmeal to yeast is 9:1. Therefore, the C:P ratio was at the similar level between the cornmeal and SY9:1 diet. For RU486 and rapamycin diets, final concentrations of RU486 and rapamycin were 200 μM, while control diets contained equal amounts of ethanol. RU486 was added to the surface of the food as previously described (Poirier et al., 2008). RU486 or rapamycin feeding was initiated in 3-d old flies.

Lifespan, lifetime reproduction and food intake assays

da-Gal4, cad-Gal4, UAS-ATPsyn-d-RNAi, UAS-ATPsyn-d and UAS-Tsc2 lines were backcrossed with w^{1118} for >5 generations before being used in lifespan assays. To obtain ATPsyn-d knockdown or overexpression flies by RU486 treatment, UAS-ATPsyn-d-RNAi or UAS-ATPsyn-d flies were mated with actin-GSG or da-GSG driver flies on the cornmeal food. Half of the progeny was cultured on RU486-containing diets to induce ATPsyn-d knockdown or overexpression. The other half was cultured on SY-matched control diets without RU486. The GSG driver only and/or UAS transgene only flies were collected as controls for evaluating the effect of RU486 feeding alone. Flies eclosed within 24 h were collected and allowed to mate for 24 h in bottles with SY1:1. Males and females were sorted out, placed in vials and fed SY1:1 for another 24 h. Flies were then transferred to a cornmeal or SY diet supplemented with or without 200 μM RU486 and/or 200 μM rapamycin and subsequently to fresh diets once every 2–3 days. For the lifespan assay, each vial contained approximately 20 males or females. The number of dead flies was recorded at the time of transfer until all the flies were dead. Lifespan of 100-200 flies in 6-10 vials was measured for each strain and was repeated at least twice. Maximum lifespan was calculated as mean lifespan of the 10% longest survived flies.

For lifetime reproduction assay, each vial contained 5 once-mated females. The number of eggs laid in each vial was counted at each time when flies were transferred to fresh food. Lifetime reproduction was calculated by dividing the lifetime egg production in each vial by the total number of females at the initiation of the experiment. The reproduction assay was repeated six times in six separate vials.

Daily food intake was measured using capillary feeder method (CAFE) previously described by Ja *et al.* with minor modifications (Ja et al., 2007). Briefly, sixteen 14-d old flies were housed in eight capillary feeding chambers with two flies per chamber, which therefore resulted in eight replicates per dietary condition. Each feeding capillary was filled with a specified liquid SY diet without agar. Two capillaries for each dietary condition were separately inserted into two feeding chambers without flies in order to account for evaporation. Food intake was measured once every 24 h for three consecutive days. The average daily food intake for each dietary condition was calculated based on eight independent replicate measurements of food intake in three days.

Quantitative real-time PCR (qPCR)

Total RNA was isolated from tissues of 14-d old flies using Trizol reagent (Cat #15596-018, Invitrogen, Grand Island, NY) following the manufacture's instruction. cDNA was synthesized with reverse transcriptase SuperScript II (Cat. # 18064-014, Invitrogen) and qPCR was performed with the Step-One plus system (Applied Biosystems, Grand Island, NY) using SYBR green Rox (Cat. # 330523, Qiagen, Valencia, CA) according to the manufacturers' suggested protocols. The transcript level of each target gene was normalized with rp49. Each assay was repeated with at least three biological replicates. The sequences of a pair of primers for each gene tested in this study are as follows (5' to 3'): *ATPsyn-d*, tcaacaagcccaccttctg and tgctccttggacttgtagcc; *CG19794*, agcctgaaccactggcata and agatcgaatccttgctttgg; *CG8175*, tcttggagcgatttttctgg and tctgccagcactgatgtagc; *CG16844*, cctatcactcgccttcgttt and gatgacattgccaggattca;

CG9119, cccattgtcagagttgcaga and attcacattggcgaagttgg; *CG18673*, gcagcactacggactcaacaag and cggttatcgtggcattgga; *CG5387*, tggttgaagactcgaaggaaa and cacttagcttgttcacaatgacg; *CG3533*, tcattggatgacaaactaacgtg and tttgtgcccaaaagaggtg; *CG11821*, catggcgggagtggatacta and gcctgcttctccggattc; *CG9511*, cagtcacccggcttcaac and ggtgcggtagaagtgcaag; *CG6027*, gcgcagatagtgaaatcaacg and gagcaagtggctctgctgtt; *CG4501*, agcactacatggtgagtcaagg and gtaaggacgcgatcgaagc; *Ucp5*, catgaccgatgcctttgtaa and ccataggtcgcttgtctcaaa.

Western blot analysis

Proteins were extracted from 14-d old flies using tissue extraction reagent 1 (Cat. # FNN0071, Invitrogen) with protease inhibitor cocktail set III (Cat. # 539134, Calbiochem, Billerica, MA) for all assays except for polyubiquitinated protein detection. Protein concentration was measured using the BCA^{TM} protein Assay kit (Cat. # 23225, Thermo Fisher, Waltham, MA) according to the manufacture's instruction. Protein was separated by electrophoresis with the NuPAGE gel and was transferred to the PVDF membrane with the iBot Dry blotting system (Invitrogen). For measuring polyubiquitinated protein, detergent-insoluble pellet was re-suspended in 3x NuPAGE LDS sample buffer (Cat. # NP 0007, Invitrogen) and subjected to SDS-PAGE electrophoresis as previously described (Rana et al., 2013). In Western blot analyses, proteins of interest were probed with specific primary antibodies against ATPsyn-d (custom made with a part of ATPsyn-d coding sequence amplified from a cDNA clone (RH59211) using primers (forward-GGAATTCCAGAGCGAAATCGATGCCTAC and reverse-CGGGATCCGAACAGACGACCAACGAAGC).), phosphorylated S6K (Cat # sc-11759,

Santa cruz biotechnology, Santa Cruz, CA), S6K (provided by T. P. Neufeld), phosphorylated ERK (Cat. #9101, Cell signaling, Danvers, MA), ERK (Cat. # 4695, Cell signaling), 4-HNE-protein adducts (Cat. # 393206, CalBiochem), porin (Cat # MSA03, Mitoscience, Eugene, OR), AKT (Cat. # 9272 and # 4691, Cell signaling), ubiquitin (Cat. # 3936s, Cell Signaling), NDUFS3 (Cat. # ab14711, Abcam) and βActin (Cat. #8224, Abcam) at an appropriate dilution from 1000-5000. Secondary antibodies were Horseradish peroxidase (HRP) conjugated, including HRP goat anti-rabbit IgG (Cat. # ab6721, Abcam), HRP goat anti-mouse IgG (Cat. # ab6789, Abcam), and HRP donkey anti-goat IgG (Cat. # sc-2020, Santa cruz) at 5000-20,000 of dilution. The signals were detected with Amersham ECL Plus (Cat. # RPN2132, GE healthcare life sciences, Piscataway, NJ). Protein bands were scanned by using the Epson perfection V500 scanner and quantified by using the Image Quant TL software in the Typhoon TRIO+ Variable Mode Imager (GE Healthcare life sciences). For 4-HNE protein adducts and ubiquitinated protein, the whole smear was used for quantification.

Microarray studies

Customized oligonucleotide arrays (Microarrays Inc, Huntsville, AL) representing approximately 13,000 predicted or known *Drosophila* genes were used for gene expression studies. The 3DNA array kit was used for microarray detection (Cat. # W300130 for the Cy3™ kit and W300140 for the Cy5™ kit, Genisphere, Hatfield, PA). Total RNA was extracted from 14-day old female heads of experimental flies fed SY1:9 using Trizol reagent from Invitrogen. Experimental flies were actin-GSG>UAS-ATPsyn-d RNAi(v21018) females treated with or without 200 μM RU486. RNA from whole body of

14-day old *Canton S* females fed SY1:1 was used as the common reference sample to normalize the expression of all experimental flies. RNA was quantified with Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, DE) and qualified with Experion automated electrophoresis system (Cat. # 701-7001, Bio-Rad) before performing cDNA synthesis. Equal amount (approximately 3.5 µg) of total RNA from experimental and reference samples were used to generate cDNA for each microarray hybridization. cDNA synthesis, hybridization and wash, and signal detection were performed according to the 3DNA Array Detection protocol suggested by the manufacturer (Genisphere). Experimental cDNA was labeled with Cy3 (green signal) and the common reference was labeled with Cy5 (red signal). Hybridized slides were scanned with a GenePix 4100A Scanner (Axon Instruments, Sunnyvale, CA) and images were analyzed with GenePix Pro 6 software. BRB-ArrayTools (http://linus.nci.nih.gov/BRB-ArrayTools.html, Biological research Branch, NCI) and DAVID v6.7 software (http://david.abcc.ncifcrf.gov) were used to analyze microarray data and determine the significance of individual genes and biological processes, respectively, between ATPsyn-d RNAi and its control flies. Genes with $p<0.01$ and false discovery rate (FDR) <0.08 and biological processes with $p<0.05$ were considered having significant changes. Expression patterns of a selected list of genes were confirmed by qPCR with aforementioned sets of primers. Each assay was repeated with 4-6 biological independent samples.

Generation of transgenic flies

UAS-ATPsyn-d transgenic flies were generated by P element mediated transformation. Genomic DNA isolated from *Canton S* flies was used to amplify the full-length coding sequence of *ATPsyn-d* by polymerase chain reaction (PCR) with forward primer-CTCGAG CCCGCTTTTGTTGTTGTCTT and reverse primer-TCTAGA GTGTCAGAGTGCTCCTCGTG**.** The resulting fragment (602 bps) was confirmed by sequencing and was cloned into the XhoI and XbaI sites of the pUAST vector. The resulting pUAST-ATPsyn-d construct was injected to w^{1118} flies to make transgenic flies by Rainbow Transgenic Flies Inc. (Camarillo, CA, USA). A transformant with the transgene inserted on chromosome II was mapped and backcrossed to w^{1118} for more than 5 generations before used in subsequent studies.

Generation of ATPsyn-d antibody

The ATPsyn-d polyclonal antibody was raised in rabbit against maltose-binding protein (MBP) fusion protein from Josman, LLC (Napa, CA). The pMAL^{TM}-c2 vector was used to clone and express MBP-ATPsyn-d fusion protein (New England Biolabs (NEB), Ipswich, MA). To generate the clone with the fusion protein, a part of ATPsyn-d coding sequence was amplified from an ATPsyn-d cDNA clone (RH59211) using PCR primers (forward -GGAATTCCAGAGCGAAATCGATGCCTAC and reverse -CGGGATCCGAACAGACGACCAACGAAGC). The PCR product was inserted into the pMAL^{TM}-c2 vector at EcoR1 and BamH1 sites. The inserted sequence was verified by sequencing using malE primers (Cat. #s1237s, NEB) by GENEWIZ Inc. (South Plainfield, NJ). The MBP-ATPsyn-d fusion protein was induced in *E. coli* DH5α cells by IPTG and verified by SDS-PAGE. Affinity chromatography was applied to purify the

fusion protein for antibody production according to the manufacturer's instruction (Cat. #E8000, NEB) with minor modifications. Briefly, one liter of LB medium containing 2 g of glucose and 0.1 g of ampicillin was inoculated with 50 ml of overnight culture of DH5α cells containing the MBP-ATPsyn-d fusion plasmid. When the cell density reached approximately $2x10^6$ cells/ml (A_{600}~0.5), 400 µl of 1M IPTG was added to the culture to induce the expression of the fusion protein. After 2 h incubation at 37^oC, the cells were harvested, frozen, and suspended in 20 ml column buffer (20 mM Tirs-HCl, 200 mM NaCl, 1 mM EDTA, and 10 mM β- mercaptoethanol) followed by 4-min sonication on ice (10' tones and 5' pauses). The crude mixture was centrifuged at 17,000 rpm for 30 min. The supernatant was incubated with ~5 ml pre-washed amylose resin (Cat. # E8021L, Bio-Rad) for 1 h at 4^oC with shaking to bind MBP fusion protein. The crude mixture was filtered with an Econo disposable column (Cat. # 7321010, Bio-Rad) and washed with 2-3 column volumes of the column buffer. The MBP fusion protein was eluted with 10-20 ml of 30 mM maltose in the column buffer. The eluted fusion protein was concentrated to ~1 mg/ml using Amicon ultra-15 (Cat. # UFC901024, Millipore, Billerica, MA) for antibody production (Josman LLC, Napa, CA). ATPsyn-d polyclonal rabbit antibody was confirmed by Western blot analysis using S2 cell treated with ATPsyn-d double stranded RNA (dsRNA), which knocked down the expression of ATPsyn-d. ATPsyn-d dsRNA was generated by using the MEGASCRIPT T7 transcription kit (Cat. # AM1333, Ambion, Grand Island, NY) from a ~ 300bp coding sequence of ATPsyn-d. This fragment was PCR amplified from the ATPsyn-d cDNA clone using gene specific primers containing T7 promoter sequence at 5' end of each primer (forward-TAATACGACTCACTATAGGGAGAATCGATTGGGCCAACTACAA and reverse-

TAATACGACTCACTATAGGGAGACTTGTTGAGGGGATCGAGTG). GFP dsRNA was generated as the control using a GFP specific DNA fragment as the template. The GFP fragment was amplified using a plasmid DNA containing the full length GFP by PCR primers (forward-TAATACGACTCACTATAGGGAGACAAGATACCCAGATCATATGA and reverse-TAATACGACTCACTATAGGGAGAGCCATTCTTTGGTTTGTCTCC). The dsRNA products were purified using the Ambion NucAway spin column (Ambion Cat # AM10070) before added to cell culture.

Mitochondria purification

Approximately fifty female flies were homogenized in the mitochondrial isolation medium (MIM: 250 mM sucrose, 10 mM Tris pH 7.4, 0.15 mM MgCl2) with 10-12 strokes by a glass dounce homogenizer. The homogenate was centrifuged twice at 1,000x g for 5min at 4°C to remove debris. The supernatant was collected and then spun at 13,000 × g for 5 min at 4°C to separate the cytosolic and mitochondrial portion. The pellet was washed with 1 ml MIM and resuspended in 30-50 µl MIM. Fresh mitochondria were immediately used for blue native polyacrylamide gel electrophoresis (BN-PAGE) or other mitochondria activity assays. The purity of cytosolic and mitochondrial fractions was assessed by Western blot analysis using antibodies against AKT and porin, which are cytosol and mitochondria specific proteins, respectively.

Blue native polyacrylamide gel electrophoresis (BN-PAGE)

BN-PAGE was performed using Novex native PAGE bis-Tris gel system according to the manufacturer's instruction (Invitrogen). All reagents were from Invitrogen. In brief, 60

μg of purified mitochondria was centrifuged at 13,000× g for 5 min at 4°C. The mitochondria pellet was resuspended in 20 μl of 1X Native PAGE Sample buffer (Cat. # BN2003) with 2% digitonin (Cat. # BN2006) and incubated on ice for 15 min. After centrifugation at 20,000x g for 30 min at 4°C, the supernatant was collected and mixed with 1 μl of 5% G250 sample additive (Cat. # BN2004) for electrophoresis on 3-12% Bis-Tris Native PAGE gels (Cat. # BN1001BOX). The native unstained protein maker (Mark™, Cat. # LC0725) was used as the protein standard. After electrophoresis, BN-PAGE gel was stained with the colloidal blue staining kit (Ca.t # LC6025) according to manufacturer's suggested protocol.

Measurement of ATP level

ATP level was determined from whole-body lysates using the ATP Bioluminescence Assay HS II kit (Cat. # 11699709001 Roche Applied Science, Indianapolis, IN). Specifically, the ATP level was measured as the intensity of green fluorescence emitted due to the oxidation of luciferin catalyzed by ATP-dependent luciferase. Approximately 20 frozen females were homogenized in 150 μl ATP cell lysis buffer. The homogenate was sonicated on ice for 4 min (30 sec tones, 30 sec pauses), and then centrifuged at 10,000x g for 5 min to collect the supernatant. The supernatant containing approximately 50 μg total protein was used for each ATP assay. The resulting green fluorescence was measured at absorbance 560 nm in a black plate by using a plate reader (2030 Victor X3, PerkinElmer, Waltham, MA). A standard curve was generated with a series of standard solutions containing ATP at 10^{-5} to 10^{-11} M. The liner regression between the ATP level and bioluminescence values was plotted using the

Prism 3 program (GraphPad Software, La Jolla, CA). ATP levels were calculated based on the standard curve and normalized to total protein content. Each assay was repeated with 5-6 biological replicates.

Stress resistance

Oxidative stress and starvation assays were performed using female adults of 14 days old after fed the SY diets. For the oxidative stress assay, flies were first starved for 3 h in vials with Kimwipe tissue wetted with water only and then transferred to vials containing 3M filter circles (Whatman, Piscataway, NJ) wetted with 350 µl of 20 mM paraquat (Cat. # 856117, Sigma, St. Louis, MO) in 5% sucrose solution. Flies were kept in dark and the number of the dead flies was recorded once every 12 hrs. Flies were transferred to new vial with fresh made paraquat sugar solution once every 48 hrs. For the starvation assay, flies were transferred to vials contained only 1.5% agar and the number of dead flies was recorded once every 24 hrs. Each assay was repeated with 6-10 vials each with approximately 20 flies.

Aconitase activity assay

The cytosolic (c-Acon) and mitochondrial Aconitase (m-Acon) activities were measured in female flies using the Aconitase assay kit (Cat. # 705502, Cayman, Ann Arbor, MI) according to the manufacturer's suggested protocol. The Aconitase activity is reflected by the rate of NADPH formation due to conversion of citrate to isocitrate by Aconitase and conversion of isocitrate to α-ketoglutarate in the presence of isocitric dehydrogenase, which can be measured at the absorbance at 340 nm. Specially,

approximately 20 females were homogenized in 300 µl Aconitase homogenization buffer and centrifuged at 800x g for 10 min at 4^{o}C. The supernatant was centrifuged again at 20,000x g for 10 min at 4^{o}C to separate cytosolic and mitochondrial protein. The supernatant was collected as cytosolic portion, while the pellet contained mitochondrial fragment and was resuspended in 60 µl cold homogenization buffer. To measure the Aconitase activity, 40 µg cytosolic protein or 30 µg mitochondria protein was added to one well of a 96 well plate per reaction. The absorbance at 340 nm was measured for 10-15 min at 37^{o}C using a plate reader (PerkinElmer 2030 Victor X3). The Aconitase activity was normalized with total protein content. Each assay was repeated with 5-6 biological replicates.

Citrate synthase (CS) activity assay

CS activity was measured in female flies using the Citrate synthase assay kit according to the manufacturer's instruction (Cat. # CS0720, Sigma). The CS activity was reflected by the increase in absorbance at 412 nm due to the reduction of DTNB [(5,5'-dithiobis-(2-nitrobenzoic acid)] induced by the reaction between acetyl CoA and oxaloacetate catalyzed by CS. The CS activity was measured in both total protein and mitochondrial fractions. Mitochondrial biogenesis was calculated as the ratio of the CS activity per total protein to the CS activity per mitochondria protein. Specifically, Mitochondria and total protein was isolated from approximately 20 flies using the MITOSO1 kit (Cat. # MITOSO1, Sigma) and CelLytic MT cell lysis buffer (Cat. # C3228, Sigma), respectively, according to the manufacturer's instructions. Isolated mitochondria were resuspended in CelLytic MT cell lysis. 20 µg total protein or 4 µg mitochondrial protein was added to a

1.5-ml cuvette containing the CS reaction mixture. Absorbance was measured at 412 nm on the kinetic program (20 sec delay, 10 sec interval and 90 sec duration) at room temperature using a UV/VIS spectrometer (PerkinElmer Lambda 25). The CS activity was normalized with total or mitochondrial protein content. Each assay was repeated with 5-6 biological replicates.

Complex III activity (Cytochrome c oxidoreductase) assay

Complex III activity was measured as the increase of absorbance at 550nm due to the reduction of cytochrome c (ferri- Fe^{3+} to ferro- Fe^{2+}) from ubiquinol (coenzyme Q_{10}) electron transfer by Cytochrome c oxidoreductase. The specific complex III activity is sensitive to antimycin A inhibition, and was determined by subtracting the antimycin A insensitive activity from the total Cytochrome c oxidoreductase activity. Fresh isolated mitochondria contain 5 µg protein was added to the reaction medium (200 µM coenzyme Q10, 50 µM cytochrome C, 100 mM K phosphate pH 7.5, 250 µM EDTA and 1mM KCN) with or without antimycin inhibitor (5 µl of 2.5 mg/ml) in a 1.5-ml cuvette. Absorbance was measured at 550 nm at 37^{o}C using the kinetic program (5 sec delay, 10 sec interval and 90 sec duration) in the Lambda 25 UV/VIS spectrometer. Enzymatic activities were normalized with mitochondrial protein content. Each assay was repeated with 5-6 biological replicates.

Complex IV (Cytochrome c oxidase) activity assay

Complex IV activity was performed using the cytochrome c oxidase assay kit (Cat. # CYOCOX1, Sigma) according to the manufacturer's instruction. Complex IV activity was

measured as the decrease rate in absorbance at 550 nm of ferrocytochrome c, which is oxidized to ferricytochrome c by cytochrome c oxidase. Mitochondria were extracted from 20 female flies using the MITOISO1 kit. 5 µg fresh mitochondrial protein was resuspended in 10 µl assay buffer and added to the reaction mixture containing 10 mM Tris-HCl, pH 7.0, 120 mM KCl, 12.5 mM sucrose and 110 nM ferrocytochrome c to the final volume of 1.1 ml in a 1.5-ml cuvette. Absorbance was measured at 550nm at room temperature using the kinetic program (5 sec delay, 10 sec interval and 80 sec duration) in the Lambda 25 UV/VIS spectrometer. Enzymatic activities were normalized with mitochondrial protein content. Each assay was repeated with 5-6 biological replicates.

Hydrogen peroxide (H_2O_2) production assay

H_2O_2 mainly produced in mitochondria is measured using the Amplex Red Hydrogen peroxide/peroxidase assay kit (Cat. # A22188, Invitrogen) by measuring the red fluorescence signal at excitation ~530 nm and emission ~590 nm, generated from the oxidation of the Amplex Red reagent (10-aceyl-3, 7-dihydroxyphenoxazine) by H_2O_2 in the presence of horseradish peroxidase (HRP). Mitochondrial solution containing approximately 30 µg protein was suspended in 50 µl assay buffer (50 mM sodium phosphate, pH 7.4), and added to the reaction mixture containing 100 µM Amplex red reagent and 0.2 unit/ml HRP to the final volume of 100 µl per microplate well. H_2O_2 standard curve was generated with a series of assay buffers containing H_2O_2 at 0 to 10 µM. The reaction was incubated at room temperature in dark for 30 min, and the fluorescence was measured at excitation 525 nm and emission 580-640 nm using the Glomax Multi Detection System (Promega, Madison, WI). H_2O_2 production was

normalized to mitochondrial protein content. Each assay was repeated with 5-6 biological replicates.

Mitochondrial membrane potential ($\Delta\Psi_m$) assay

The mitochondrial membrane potential ΔΨm (i.e. electrical gradient) is a major driving force for ATP production. Loss of mitochondria ΔΨm results in the depletion of ATP production. The ΔΨm was measured by using the cationic carbocyanine lipophilic dye JC-1 (5,5′,6,6’-tetrachloro-1,1’,3,3′- tetraethylbenzimidazolyl carbocyanine iodide), which fluoresces in red at 590 nm when formed into aggregates in mitochondria. More polarized mitochondria accumulate more dye and produce higher relative fluorescence units (RFU) of red signal. On the other hand, depolarized mitochondria accumulate less dye and exhibit a lower red RFU reading. Therefore, mitochondrial $\Delta\Psi_m$ and ATP production can be assessed by the changes in the red RFU. Specifically, to measure mitochondrial ΔΨm, fresh isolated mitochondria containing 3 µg protein was suspended in 10 µl MITOSO1 storage buffer and added to the JC-1 assay buffer containing 4 mM MOPs, pH 7.5, 22 mM KCl, 2 mM ATP, 2 mM MgCl2, 2 mM sodium succinate, and 0.2 mM EGTA to the final volume of 200 µl per well in a 96-well plate. The reaction was initiated by adding 10 µl JC-1 (4 µg/ml) in the MITOSO1 kit and incubated for 7 min at room temperature in the dark to allow the uptake of the dye into the mitochondria. Absorbance was measured using the kinetic program (5 sec delay, 1 min interval and 30 min duration) at excitation/emission 490nm/590nm in a plate reader (PerkinElmer 2030 Victor X3). The mitochondrial $\Delta\Psi_m$ values were normalized with mitochondrial protein content. Each assay was repeated with 5-6 biological replicates.

Measurement of whole body glucose and trehalose levels

Glucose and trehalose levels were measured in 2-week old females fed SY1:1 and SY1:9 with and without RU486 treatment following the protocols as previously described (Broughton et al., 2008). Briefly, fly bodies without heads were grinded in the lysis buffer containing 1xPBS and 0.05% Triton X-100. Supernatant were collected after centrifugation at 4 ºC for 10 min at 10,000 rpm. The glucose concentration was measured shortly after sample preparation using the Infinity Glucose kit according to the manufacturer's instruction (Cat. # TR 15221, Thermo Fisher, Waltham, MA). Total protein concentration in each sample was measured using the BCA^{TM} protein Assay kit as described above. The glucose level was calculated based on the glucose standards and normalized to total protein level in each sample. Then 20 ul of supernatant from each sample was digested with trehalase at 37 ºC for overnight. The glucose concentrations in the digested samples were measured with the Infinity Glucose kit as described above. The trehalose level was calculated by subtracting the original glucose present in the sample from the amount of glucose after trehalase digestion, and normalized to total protein level in each sample. Seven biologically replicate samples were measured for gluose and trehalose levels in ATPsyn-d RNAi and control female flies.

Measurement of polyubiquitinated protein aggregates

Indirect flight muscle was dissected in ice-cold PBS and subsequently fixed in 3.7% paraformaldehyde PBS solution containing 0.2% Triton X-100 for 20 min at room temperature. Samples were then washed briefly with 1xPBS and were immunostained

with anti-ubiquitin antibody (Cat. # BML-PW8810, Enzo) at 1: 200 dilution, followed by probed with secondary anti-mouse (Alexa-568) (Cat. # A11004, Invitrogen) at 1:400 dilution and phalloidin (FITC) (Cat. # ALX-350-268-MC01, Enzo) at 1: 200 dilution for F-actin as previously described (Rana et al., 2013). Fluorescent images were visualized and recorded with Zeiss LSM 710 confocal microscopy. Total number of protein aggregates was quantified from a selected area of 150x150 μm^2 using “Find Maxima” function in Image J.

References

Ashburner, M., Golic, K.G., and Hawley, R.S., eds. (2005). *Drosophila:* A Laboratory Handbook, 2nd edn (Woodbury, NY: Cold Spring Harbor Laboratory Press).

Broughton, S., Alic, N., Slack, C., Bass, T., Ikeya, T., Vinti, G., Tommasi, A.M., Driege, Y., Hafen, E., and Partridge, L. (2008). Reduction of DILP2 in Drosophila triages a metabolic phenotype from lifespan revealing redundancy and compensation among DILPs. PLoS One *3*, e3721.

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 U S A *104*, 8253-8256.

Poirier, L., Shane, A., Zheng, J., and Seroude, L. (2008). Characterization of the Drosophila gene-switch system in aging studies: a cautionary tale. Aging Cell *7*, 758-770.

Rana, A., Rera, M., and Walker, D.W. (2013). Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. Proc Natl Acad Sci U S A *110*, 8638-8643.

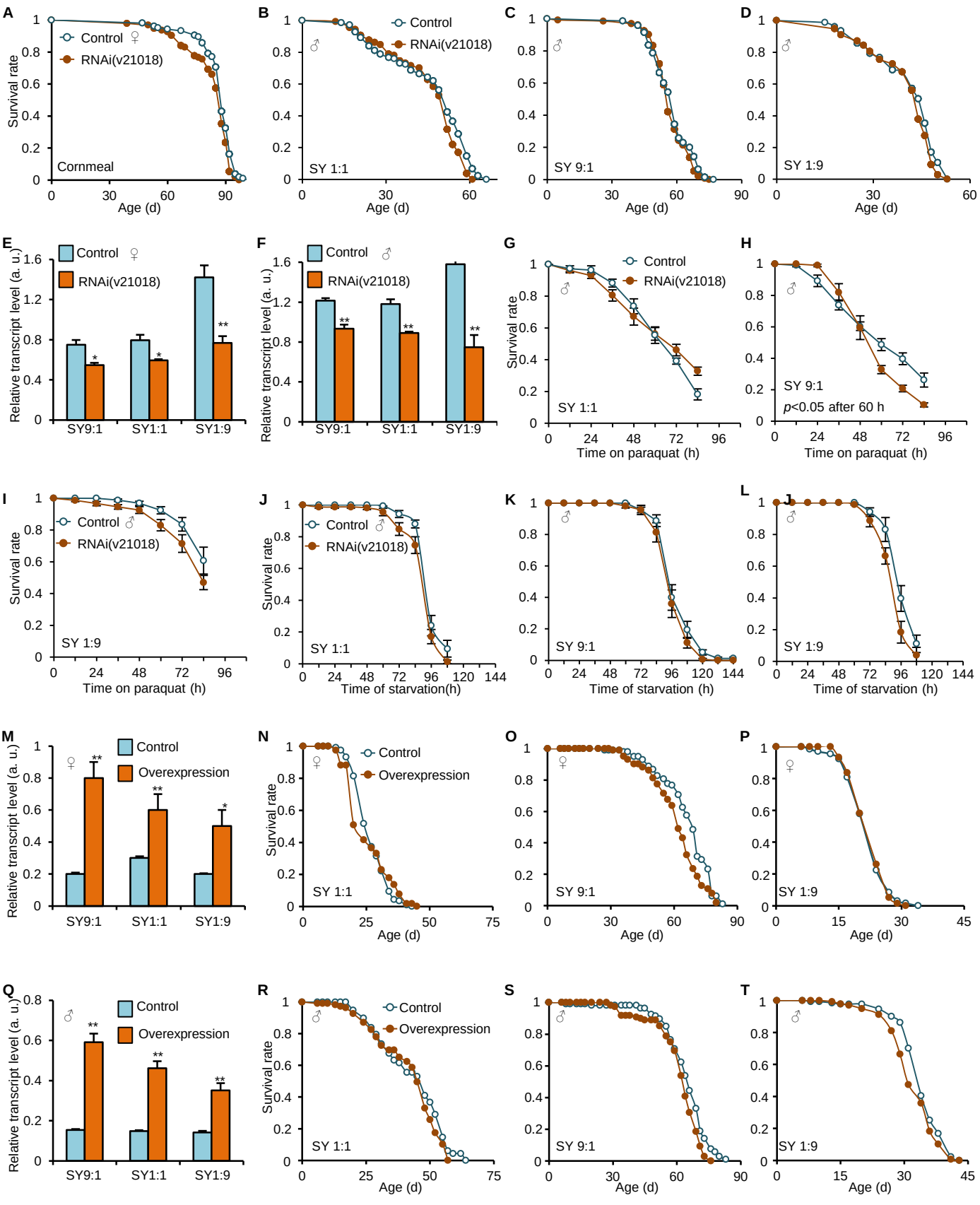
A
Control ♀
RNAi(v21018)
Survival rate
Cornmeal
Age (d)
B
Control
RNAi(v21018)
♂
SY 1:1
Age (d)
C
♂
SY 9:1
Age (d)
D
♂
SY 1:9
Age (d)
E
Control ♀
RNAi(v21018)
Relative transcript level (a. u.)
SY9:1
SY1:1
SY1:9
F
Control ♂
RNAi(v21018)
Relative transcript level (a. u.)
SY9:1
SY1:1
SY1:9
G
Control
RNAi(v21018)
♂
Survival rate
SY 1:1
Time on paraquat (h)
H
♂
SY 9:1
p<0.05 after 60 h
Time on paraquat (h)
I
Control ♂
RNAi(v21018)
Survival rate
SY 1:9
Time on paraquat (h)
J
Control ♂
RNAi(v21018)
Survival rate
SY 1:1
Time of starvation(h)
K
♂
SY 9:1
Time of starvation (h)
L
♂
SY 1:9
Time of starvation (h)
M
♀
Control
Overexpression
Relative transcript level (a. u.)
SY9:1
SY1:1
SY1:9
N
♀
Control
Overexpression
Survival rate
SY 1:1
Age (d)
O
♀
SY 9:1
Age (d)
P
♀
SY 1:9
Age (d)
Q
♂
Control
Overexpression
Relative transcript level (a. u.)
SY9:1
SY1:1
SY1:9
R
♂
Control
Overexpression
Survival rate
SY 1:1
Age (d)
S
♂
SY 9:1
Age (d)
T
♂
SY 1:9
Age (d)

Figure S1

Figure S1. The Effect of ATPsyn-d Knockdown and Overexpression on Lifespan and Stress Response, Related to Figure 1 and 2. (A) Lifespan of actin-GSG>UAS-ATPsyn-d-RNAi(v21018) females on cornmeal. ATPsyn-d knockdown does not significantly affect lifespan of females. (B to D) Lifespan of actin-GSG/UAS-ATPsyn-d-RNAi(v21018) males fed SY 1:1, SY 9:1 or SY 1:9 diet with and without 200 µM RU486. ATPsyn-d knockdown does not significantly affect lifespan of males. (E and F) RU 486 decreases the mRNA level of ATPsyn-d in actin-GSG>UAS-ATPsyn-d-RNAi(v21018) females and males, respectively, fed SY 9:1, SY 1:1 and SY 1:9 diets, when compared to gender- and diet-matched un-induced controls. The transcript level was normalized to rp49. (G to I) The survival of 14-day old actin-GSG/UAS-ATPsyn-d-RNAi(v21018) males on 20 mM paraquat after being fed SY 1:1, SY 9:1 or SY 1:9 diet with and without 200 µM RU486. (J to L) The survival of 14-day old actin-GSG/UAS-ATPsyn-d-RNAi(v21018) males under starvation after being fed SY 1:1, SY 9:1 or SY 1:9 diet with and without 200 µM RU486. (M) Transcript levels of ATPsyn-d in actin-GSG/UAS-ATPsyn-d females flies fed SY 1:1, SY 9:1 or SY 1:9 diet with and without 200 µM RU486 . RU486 significantly increases the mRNA level of ATPsyn-d in actin-GSG/UAS-ATPsyn-d females. (N to P) Lifespan of actin-GSG/UAS-ATPsyn-d females fed SY 1:1, SY 9:1 or SY 1:9 diets with and without 200 µM RU486. ATPsyn-d overexpression does not significantly affect lifespan of females under any of the three SY dietary conditions. (Q) Transcript levels of ATPsyn-d in actin-GSG/UAS-ATPsyn-d(v21018) male flies fed SY 1:1, SY 9:1 or SY 1:9 diet with and without 200 µM RU486 . RU486 significantly increases the mRNA level of ATPsyn-d in actin-GSG/UAS-ATPsyn-d males. (R to T) Lifespan of actin-GSG/UAS-ATPsyn-d males fed SY 1:1, SY 9:1 or SY 1:9 diet with and without 200 µM RU486. ATPsyn-d overexpression does not significantly affect lifespan of males under any of the three SY dietary conditions. ATPsyn-d knockdown flies with line v21018 are labeled as "RNAi". Overexpression of ATPsyn-d is labeled as "Overexpression". Control flies of the same genotype were treated without RU486, labeled as "Control". a.u., arbitrary unit. The transcript level of ATPsyn-d was measured with three biological independent samples and normalized to *rp49*. **p*<0.05; ***p*<0.01 by Student's *t*-test.

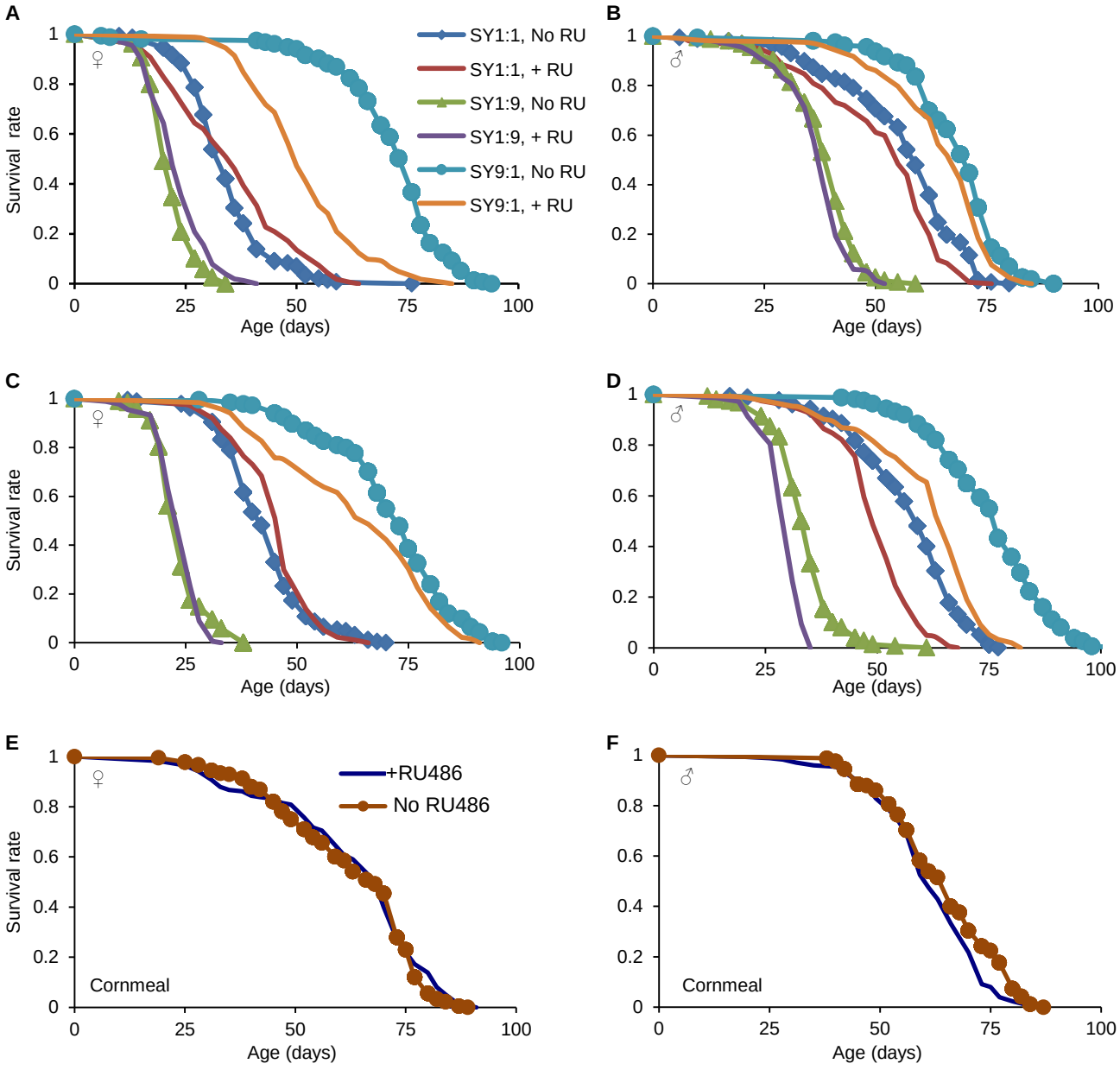

Figure S2. The Effect of RU486 Alone on the Lifespan of Flies Fed SY1:1, SY1:9, SY9:1 or Cornmeal Diet, Related to Figure 1 and 2. (A and B) Lifespan of actin-GSG/UAS-GFP-RNAi females and males fed SY1:1, SY1:9 or SY9:1 diet. (C and D) Lifespan of da-GSG/UAS-GFP-RNAi females and males fed SY1:1, SY1:9 or SY9:1 diet. (E and F) Lifespan of w^{1118}; actin-GSG/+ females and males fed the cornmeal food. Lifespan curves with markers represent flies treated with 200 μM RU486 (+RU486 or +RU). Plain lifespan curves represent flies without RU486 treatment (no RU or no RU486). RU486 treatment does not significantly increase lifespan in flies under any dietary condition tested here.

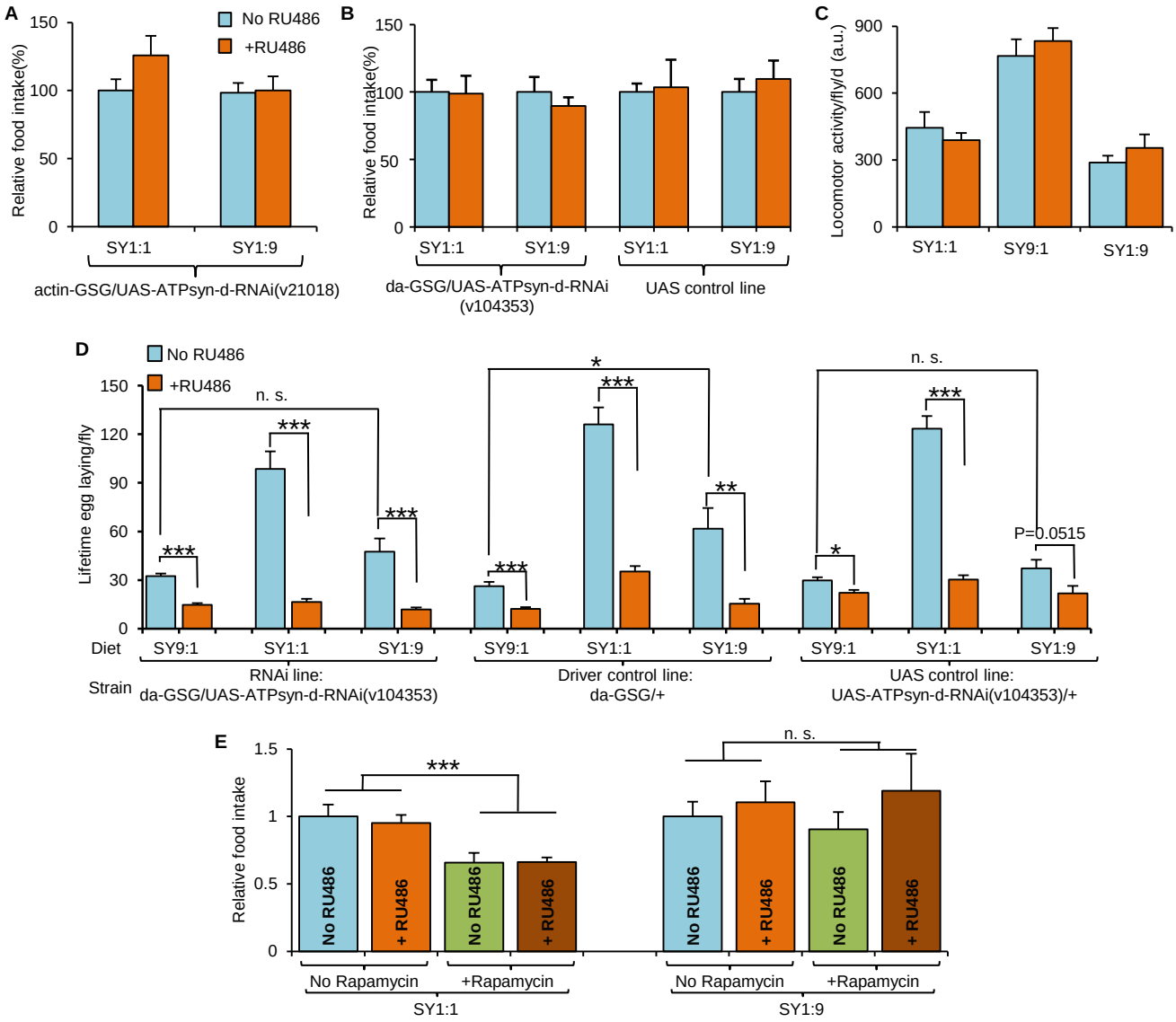

Figure S3. The Effect of ATPsyn-d Knockdown on Food Intake, Locomotor Activity and Reproduction, Related to Figure 1, 2 and 6. (A) Food intake of actin-GSG/UAS-ATPsyn-d-RNAi (line v21018) females fed SY1:1 or SY1:9. ATPsyn-d knockdown (+RU486) does not significantly affect food intake in females when compared to diet-matched controls (No RU486). Food intake in the no RU486 fed flies (no RU486) is set at 100% for comparion. (B) Food intake of da-GSG/UAS-ATPsyn-d-RNAi(v104353) and UAS-ATPsyn-d-RNAi/+ (UAS control line) females fed SY1:1 or SY1:9. ATPsyn-d knockdown (+RU486) does not significantly affect food intake in females when compared to diet-matched controls (No RU486). RU486 feeding alone does not affect food intake (UAS control line). Food intake in No RU486 flies is set at 100%. (C) Locomotor activity of actin-GSG/UAS-ATPsyn-d-RNAi(v21018) females fed SY1:1, SY9:1 or SY1:9. ATPsyn-d knockdown does not affect locomotor activity in females when compared to diet-matched controls. (D) The effect of ATPsyn-d knockdown and RU486 feeding on lifetime reproductive output. Life-time egg production was measured in three fly strains, drive control line da-GSG/+, UAS control line UAS-ATPsyn-d-RNAi(v104353)/+ and knockdown line da-GSG/UAS-ATPsyn-d-RNAi(v104353) with and without RU486 feeding. The number of egg laid and the number of dead flies were counted every time when flies were transferred to fresh food once every 2-3 days. Lifetime egg production was calculated by dividing lifetime egg production in each vial by the initial number of females, which were 5 per vial. Each egg laying assay was repeated six times with six separate vials. (E) The effect of ATPsyn-d knockdown and rapamycin feeding on food intake of da-GSG/UAS-ATPsyn-d-RNAi(v104353) females fed SY1:1 or SY1:9 with and without rapamycin. ATPsyn-d knockdown (+RU486) does not affect food intake in females when compared to diet-matched controls (No RU486). Rapamycin by itself decreases food intake of flies on SY1:1 diet but does not significantly affect food intake of flies on SY1:9 when compared to flies without rapamycin feeding. Food intake of "no RU486 and no rapamycin" fed flies (No RU486-No Rapamycin) on SY1:1 or SY1:9 is set at 1 for comparison among SY diet-matched flies. ATPsyn-d knockdown was induced by 200 μM RU486 (+RU486). The concentration of rapamycin in the food was 200 μM. d, day; a. u., arbitrary unit. **p*<0.05; ***p*<0.01; ****p*<0.001; n. s., not statistically significant by Student's *t*-test.

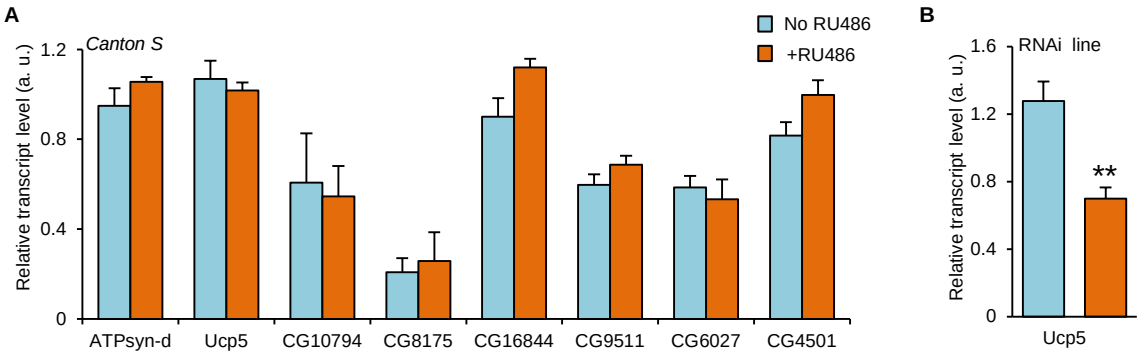

Figure S4. The Effect of RU486 Feeding on Gene Expression, Related to Figure 3 and 4. (A) The effect of RU486 feeding alone on transcript levels of ATPsyn-d and Ucp5 and candidate genes from microarray experiments. The transcript levels of these genes were measured by qPCR in the heads of 14-day old wild type *Canton S* females fed SY1:9 supplemented with and without 200 µM RU486. (B) The effect of ATPsyn-d knockdown on the transcript level of Ucp5. The transcript level of Ucp5 was measured by qPCR in the heads of 14-d old knockdown line da-GSG/UAS-ATPsyn-d-RNAi(v104353) females fed SY1:9 with and without 200 µM RU486. The transcript levels of all the genes were normalized to the transcript level of rp49 . Error bars represent standard errors. n=5-6 biologically independent samples for each measurement. ***p*<0.01 by Student's *t*-test between genotype matched '+RU486" and "no RU486" groups.

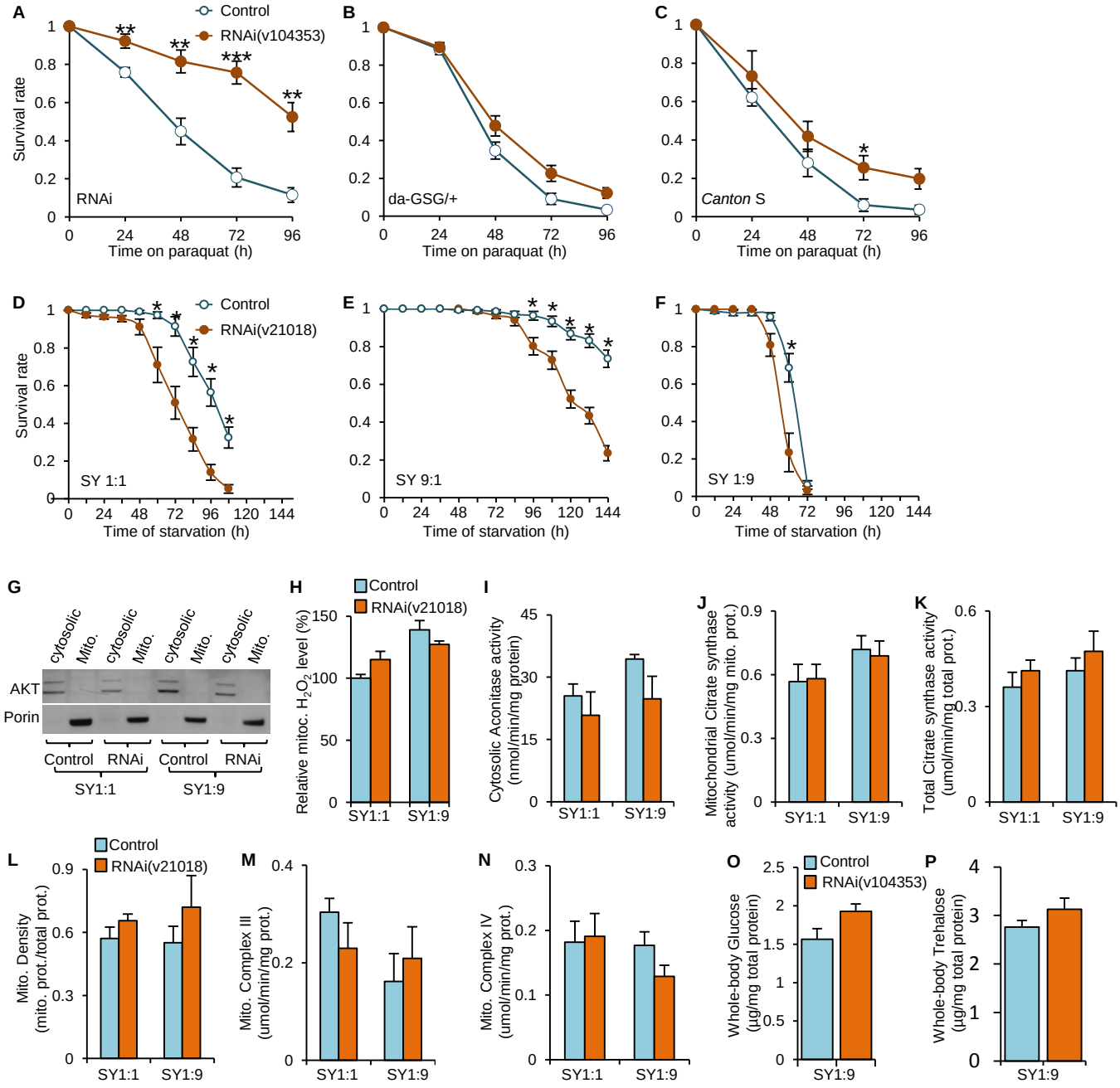
A
Control
RNAi(v104353)
Survival rate
RNAi
Time on paraquat (h)
B
da-GSG/+
Time on paraquat (h)
C
Canton S
Time on paraquat (h)
D
Control
RNAi(v21018)
Survival rate
SY 1:1
Time of starvation (h)
E
SY 9:1
Time of starvation (h)
F
SY 1:9
Time of starvation (h)
G
cytosolic
Mito.
AKT
Porin
Control
RNAi
SY1:1
SY1:9
H
Relative mitoc. H2O2 level (%)
I
Cytosolic Aconitase activity (nmol/min/mg protein)
J
Mitochondrial Citrate synthase activity (umol/min/mg mito. prot.)
K
Total Citrate synthase activity (umol/min/mg total prot.)
L
Mito. Density (mito. prot./total prot.)
M
Mito. Complex III (umol/min/mg prot.)
N
Mito. Complex IV (umol/min/mg prot.)
O
Whole-body Glucose (µg/mg total protein)
P
Whole-body Trehalose (µg/mg total protein)

Figure S5

Figure S5. The Effect of ATPsyn-d Knockdown or RU486 Feeding Alone on Stress Response, Mitochondrial and Metabolism Function in Females, Related to Figure 4. (A) The survival of da-GSG/UAS-ATPsyn-d-RNAi(v104353) (RNAi) females fed SY1:9 on paraquat treatment. ATPsyn-d knockdown increases flies' resistance to paraquat-induced oxidative stress. (B) The survival of the driver control (da-GSG/+) females fed SY1:9 on paraquat treatment. RU486 feeding alone does not increase flies' resistance to paraquat under SY 1:9. (C) The survival of wild-type *Canton* S females fed SY1:9 on paraquat treatment. RU486 feeding alone does not increase flies' resistance to paraquat under SY 1:9. All flies were fed with 20 mM paraquat starting at the age of 14 days and dead flies were recorded daily for four consecutive days. (D to F) The effect of ATPsyn-d knockdown on starvation resistance in actin-GSG/UAS-ATPsyn-d-RNAi(v21018) females on SY1:1, SY9:1 or SY1:9. ATPsyn-d knockdown (RNAi) decreases resistance to starvation in females, regardless of diet composition, when compared to diet-matched controls (Control). (G) Verification of the purity of cytosolic and mitochondrial protein fractions for physiological and biochemical assays by Western blot. Cytosolic and mitochondrial proteins were isolated from actin-GSG/UAS-ATPsyn-dRNAi(v21018) females fed SY1:1 or SY1:9, and were probed with antibodies against cytosolic protein AKT and mitochondrial protein Porin, respectively. Western blot analyses indicate no or undetectable cross contamination between cytosolic and mitochondrial fractions in the study. (H) Relative amount of mitochondrial H_2O_2 levels in da-GSG/UAS-ATPsyn-d RNAi(v104353) female flies. The H_2O_2 value at "Control" on SY1:1 was set at 100% to normalize the rest of H_2O_2 values. (I) The activity of cytosolic Aconitase in actin-GSG/UAS-ATPsyn-dRNAi(v21018) females fed SY1:1 or SY1:9. Enzymatic activities were normalized to total protein concentration. No significantly difference in Aconitase activity was observed between diet-matched RNAi and control groups. (J) Citrate synthase activity in mitochondrial fraction of actin-GSG/UAS-ATPsyn-d-RNAi (v21018) females treated with or without RU486. (K) Citrate synthase activity in total protein of actin-GSG/UAS-ATPsyn-dRNAi(v21018) females treated with or without RU486. (L) The ratio of mitochondrial protein to total protein was calculated based on values in Fig. S5J and K, which reflects mitochondrial content and hence mitochondrial biogenesis. (M) The activity of mitochondrial Complex III in mitochondrial fraction of actin-GSG/UAS-ATPsyn-d-RNAi (v21018) females treated with or without RU486. (N) The activity of mitochondrial Complex IV in mitochondrial fraction of actin-GSG/UAS-ATPsyn-d-RNAi(v21018) females treated with or without RU486. (O) The whole-body glucose level of da-GSG/UAS-ATPsyn-d-RNAi(v104353) females treated with or without RU486 (n=7 replicates) on SY1:9. No significantly difference in the glucose level was observed between ATPsyn-d knockdown and control groups. (P) The whole-body trehalose level of da-GSG/UAS-ATPsyn-d-RNAi(v104353) females treated with or without RU486 (n=7 replicates) on SY1:9. No significantly difference in the trehalose was observed between ATPsyn-d knockdown and control groups. All enzymatic activities were normalized to total protein concentration. No significant difference in enzymatic activity and mitochondrial content was observed between diet-matched ATPsyn-d knockdown and control groups in the assays shown here. Each assay was repeated at least three times with at least three biologically independent samples and 2-week old flies. Flies were fed with and without 200 μM RU486, labeled as "RNAi" and "Control", respectively. Error bars represent standard errors. *p<0.05; **p<0.01; ***p<0.001 between "+RU486" and "no RU486" groups by Student's t-test.

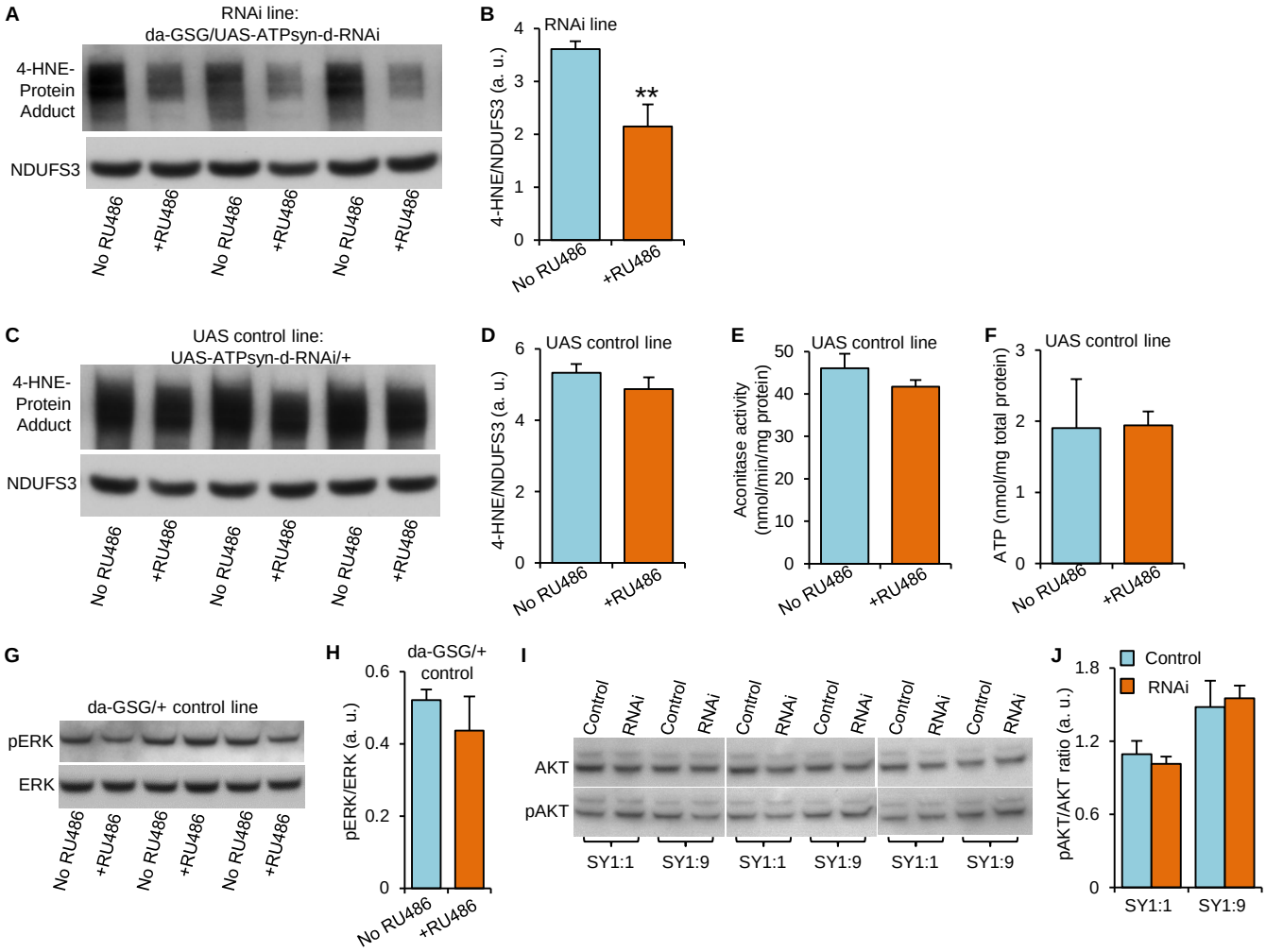

Figure S6. The Effect of ATPsyn-d Knockdown and RU486 Feeding alone on Oxidative Damage, ATP Level, pERK/ERK and pAKT/AKT in Females, Related to Figure 4 and 5. (A and B) The effect of ATPsyn-d knockdown induced by RU486 on the level of 4-HNE-protein adducts in the mitochondria of da-GSG/UAS-ATPsyn-d RNAi(v104353) females fed SY1:9. (C and D) The effect of RU486 feeding alone on the level of 4-HNE-protein adducts in the mitochondria of UAS-ATPsyn-d RNAi/+ (UAS control) females fed SY1:9. The level of 4-HNE-protein adducts is normalized to NDUFS3.(E) The effect of RU486 feeding alone on the level of mitochondrial Aconitase activity in UAS-ATPsyn-d RNAi/+ (UAS control) females on SY1:9. (F) The effect of RU486 feeding alone on the ATP level of UAS-ATPsyn-d RNAi/+ (UAS control) flies fed SY1:9. (G and H) The effect of RU486 feeding alone on the ratio of pERK/ERK. The driver control line da-GSG/+ females were fed SY1:9 without RU486 (no RU486 group) or with 200 μM RU486 (+RU486 group). The protein levels of pERK and ERK were measured by densitometry to calculate the ratio of phosphorylated ERK to total ERK (pERK/ERK). (I and J) The effect of ATPsyn-d knockdown (RNAi) on the phosphorylation of AKT in da-GSG/UAS-ATPsyn-d RNAi(v104353) females fed SY1:1 or SY1:9. The protein levels of pAKT and AKT were measured by densitometry to calculate the ratio of phosphorylated AKT to total AKT (pAKT/AKT). Each assay was repeated ≥4 times with ≥4 biologically independent samples. Three representative samples are shown. "+RU486" represents feeding with 200 μM RU486. Error bars represent standard errors. a. u., arbitrary unit; ***p*<0.01 by Student's *t*-test between genotype matched '+RU486" and "no RU486" groups.

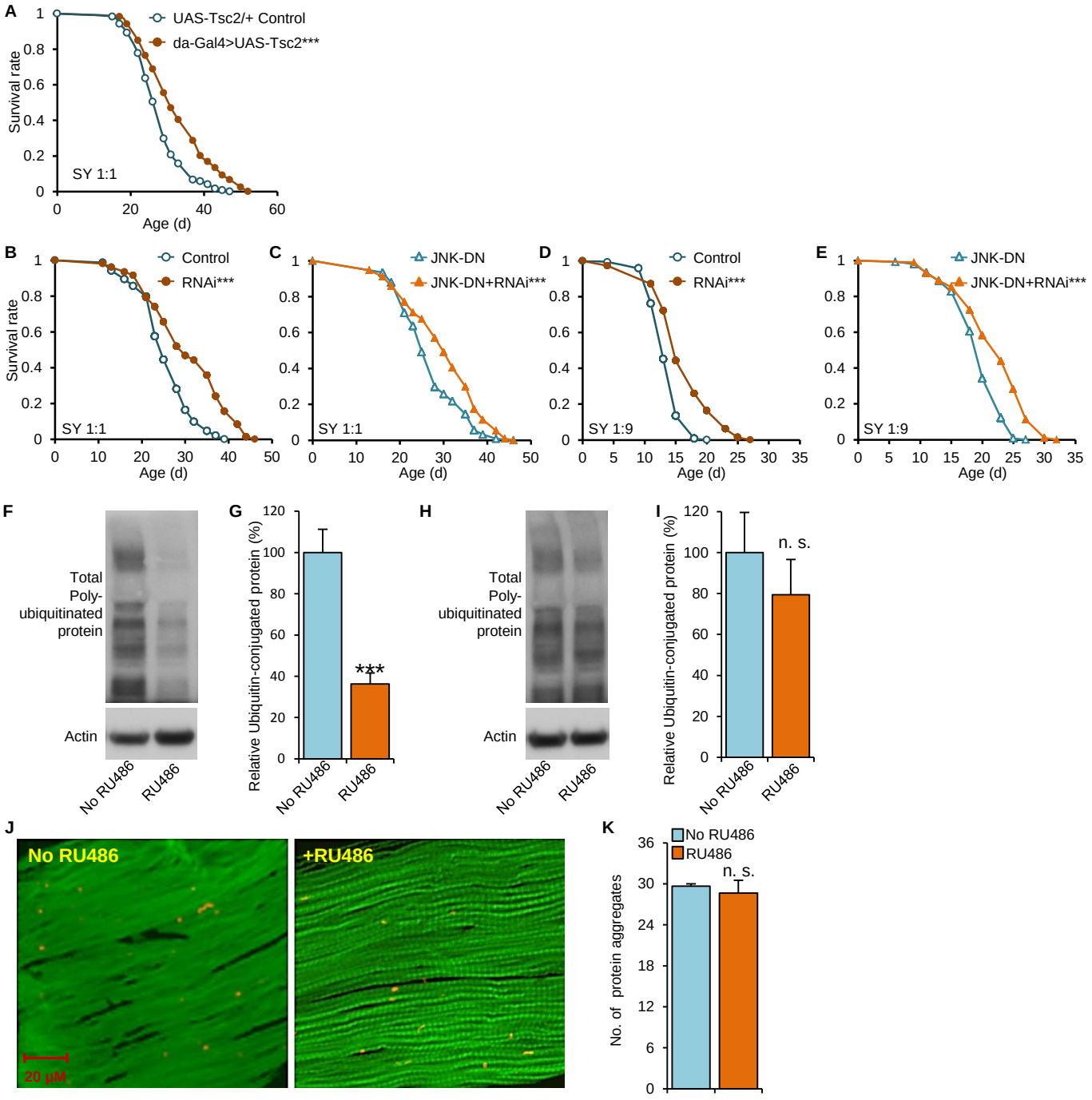

Figure S7. The Genetic Interaction of ATPsyn-d with Tsc2 and JNK in Modulating Lifespan and the Impact of ATPsyn-d Knockdown and RU486 Feeding Alone on Proteostasis, Related to Figure 5 and 6. (A) Lifespan of da-Gal4>UAS-Tsc2 and its control Tsc2/+ females fed SY1:1. (B and E) Lifespan of ATPsyn-d knockdown female flies with or without the expression of dominant negative JNK fed SY1:1 or SY1:9. "RNAi" indicates ATPsyn-d knockdown by 200 μM RU486. "Control" represents genotype- and diet-matched controls without RU486 treatment. "JNK-DN" represents expression of dominant negative JNK. Note that Fig. S7B and S7D are the same as Fig. 5H and 5J in the main text, respectively, and are presented for the purpose of comparison to Fig. S7C and S7E. (F and G) The effect of ATPsyn-d knockdown on the level of total polyubiquitinated protein normalized with Actin in da-GSG/UAS-ATPsyn-d RNAi(v104353) females. Relative polyubiquitinated protein level in "No RU486" is set at 100% for comparison. (H and I) The effect of RU486 feeding alone on the level of total polyunbiquitinated protein normalized with Actin in +/UAS-ATPsyn-d RNAi(v104353) (UAS control line) females. Relative polyubiquitinated protein level in "No RU486" is set at 100% for comparison. (J and K) The effect of RU486 feeding alone on the accumulation of polyubiquitinated protein aggregates in fly flight muscle. The scale bar is shown in the image of J. "RU486" indicates feeding of 200 μM RU486. "No RU486" represents flies without RU486 treatment. Error bars represent standard errors. ***p<0.001 by Logrank test in Fig. 7A to 7E; ***p<0.001 by Student's t-test in Fig. 7G, 7I and 7K; n. s., not statistically significant.

Table S1. Lifespan of ATPsyn-d Knockdown Flies Fed Sugar-Yeast Diets, Related to Figure 1 and 2

Strain	Sex	Replicate	SY diet[a]	Presence of RU486[b]	# of flies per trial	Mean lifespan±SE (d)[c]	Change of mean lifespan[d]	p value[e]	Maxi lifespan±SE (d)[f]	Change of maxi lifespan[d]
actin-GSG/UAS-ATPsyn-dRNAi (line v21018)	♀	#1	SY9:1	No RU486	114	64.5±0.9			80.4±0.8	
				RU486	143	64.7±0.9	0.3%	0.6973	78.1±0.9	-2.86%
			SY1:1	No RU486	142	30.4±0.8			50.2±1.5	
				RU486	138	34.3±1.1	12.8%	0.0012	57.4±1.3	14.34%
			SY1:9	No RU486	139	21.9±0.5			31.6±0.3	
				RU486	136	26.1±0.6	19.2%	<.0001	37.5±0.8	18.67%
	♀	#2	SY9:1	No RU486	134	65.2±0.9			81.1±0.6	
				RU486	136	63.3±1.1	-2.9%	0.9416	80.9±0.5	-0.25%
			SY1:1	No RU486	137	30.9±0.7			45.9±1.0	
				RU486	138	35.6±1.0	15.2%	<.0001	55.4±1.4	20.70%
			SY1:9	No RU486	140	21.1±0.4			29.3±0.4	
				RU486	140	24.1±0.5	14.2%	<.0001	32.3±0.2	10.24%
	♂	#1	SY9:1	No RU486	140	57.6±0.8			72.5±0.6	
				RU486	131	56.7±0.8	-1.6%	0.1356	70.0±0.7	-3.45%
			SY1:1	No RU486	137	46.2±1.3			62.9±0.5	
				RU486	137	45.5±1.1	-1.5%	0.0099	59.7±0.3	-5.09%
			SY1:9	No RU486	137	30.7±0.6			40.5±0.4	
				RU486	133	35.2±0.5	14.7%	<.0001	44.9±0.3	10.86%
	♂	#2	SY9:1	No RU486	132	61.9±0.9			79.5±1.5	
				RU486	135	56.6±0.9	-8.6%	<.0001	69.9±1.0	-12.08%
			SY1:1	No RU486	132	47.4±1.3			63.1±0.4	
				RU486	136	43.1±1.2	-9.1%	<.0001	58.7±0.3	-6.97%
			SY1:9	No RU486	132	31.6±0.6			42.7±0.4	
				RU486	140	31.8±0.6	0.6%	0.625	43.3±0.3	1.41%
da-GSG/UAS-ATPsyn-dRNAi (line v104353)	♀		SY9:1	No RU486	121	60.6±1.0			80.5±0.4	
				RU486	125	54.5±1.5	-10.0%	0.003	78.1±0.7	-3.0%
			SY1:1	No RU486	121	29.4±0.6			41.7±0.9	
				RU486	119	43.2±1.0	46.9%	<.0001	58.8±0.4	41.0%
			SY1:9	No RU486	117	15.8±0.3			20.1±0.6	
				RU486	123	20.7±0.4	31.0%	<.0001	26.7±0.5	32.8%

[a]SY, sugar-yeast diet. The ratio of sugar to yeast is listed after "SY".

[b]The presence of RU486 induces ATPsyn-d RNAi.

[c]Lifespan is expressed as mean±Standard error (SE) in days (d).

[d]Percentage changes were referenced to SY diet-matched control flies without RU486 treatment.

[e]P values were based on comparing diet-matched flies with and without RU486 by logrank test.

[e]Maxi refers to maximum lifespan calculated as mean lifespan of the top 10% longest-live flies.

Table S2. The Effect of RU486 Feeding Alone on Lifespan of Flies, Related to Figure 1 and 2

Genotype	Sex	SY or cornmeal diet[a]	Presence of RU486[b]	# of flies per trial	Mean lifespan±SE (d)[c]	Change of mean lifespan[d]	p value (logrank)[e]
actin-GSG; UAS-GFP-RNAi	♀	SY9:1	No RU486	153	71.5±1.1		
			RU486	163	51.8±0.9	-27.6%	<.0001
		SY1:1	No RU486	145	34.4±0.8		
			RU486	163	35.1±1.0	2.0%	0.0514
		SY1:9	No RU486	159	21.8±0.4		
			RU486	154	23.3±0.5	6.9%	0.0017
	♂	SY9:1	No RU486	159	68.4±0.9		
			RU486	158	64.4±1.0	-5.8%	0.0018
		SY1:1	No RU486	157	56.1±1.1		
			RU486	157	51.3±1.2	-8.6%	<.0001
		SY1:9	No RU486	156	38.5±0.6		
			RU486	156	37.0±0.6	-3.9%	0.0218
da-GSG; UAS-GFP-RNAi	♀	SY9:1	No RU486	185	70.9±1.1		
			RU486	186	62.9±1.3	-11.3%	<.0001
		SY1:1	No RU486	186	42.6±0.7		
			RU486	175	44.6±0.7	4.7%	0.0717
		SY1:9	No RU486	190	23.7±0.4		
			RU486	180	23.9±0.4	0.8%	0.2782
	♂	SY9:1	No RU486	163	75.1±1.1		
			RU486	154	60.9±1.1	-18.9%	<.0001
		SY1:1	No RU486	176	56.9±0.9		
			RU486	164	49.2±0.8	-13.5%	<.0001
		SY1:9	No RU486	151	33.8±0.6		
			RU486	150	29.2±0.4	-13.6%	<.0001
w^{1118}; actin-GSG/+	♀	Cornmeal	No RU486	183	62.7 ± 1.2		
			RU486	173	63.2 ± 1.3	0.8%	0.3239
	♂	Cornmeal	No RU486	165	64.1 ± 1.0		
			RU486	175	61.1 ± 0.9	-4.7%	0.0055

[a]SY, sugar-yeast diet. The ratio of sugar to yeast is listed after "SY".

[b]The presence of RU486 refers to 200 μM RU486.

[c]Lifespan is expressed as mean±Standard error (SE) in days (d).

[d]Percentage changes were referenced to SY diet-matched control flies without RU486 treatment.

[e]P values were based on comparing diet-matched flies with and without RU486 by logrank test.

Table S3. Lifespan of actin-GSG/UAS-ATPsyn-d Overexpression Flies Fed Sugar-Yeast Diets, Related to Figure 1 and 2

Sex	Replicate	SY diet[a]	Presence of RU486[b]	# of flies per trial	Mean lifespan±SE (d)[c]	Change of mean lifespan[d]	p value[e]	Maxi lifespan±SE (d)[f]	Change of maxi lifespan[d]
♀	#1	SY9:1	No RU486	99	66.5±1.2			81.2±0.9	
			RU486	102	61.7±1.2	-7.2%	0.0019	80.2±0.5	-1.2%
		SY1:1	No RU486	118	27.1±0.6			37.8±0.8	
			RU486	118	25.6±0.8	-5.5%	0.9732	40.9±0.7	8.2%
		SY1:9	No RU486	130	22.3±0.4			29.8±0.6	
			RU486	134	22.6±0.3	1.3%	0.9559	28.4±0.4	-4.7%
♀	#2	SY9:1	No RU486	144	64.3±1.1			81.1±0.4	
			RU486	137	57.8±1.0	-10.1%	<.0001	76.9±0.9	-5.2%
		SY1:1	No RU486	138	24.3±0.5			33.0±0.7	
			RU486	148	28.3±0.7	16.5%	<.0001	42.3±0.8	28.2%
		SY1:9	No RU486	120	20.6±0.4			27.3±0.3	
			RU486	119	22.4±0.4	8.7%	0.0007	28.8±0.6	5.5%
♂	#1	SY9:1	No RU486	106	64.5±1.1			79.5±0.9	
			RU486	108	61.3±1.1	-5.0%	0.0033	73.6±0.5	-7.4%
		SY1:1	No RU486	117	43.3±1.2			60.3±1.0	
			RU486	109	42.0±1.2	-3.0%	0.0927	57.0±0	-5.5%
		SY1:9	No RU486	131	34.1±0.5			41.5±0.2	
			RU486	137	32.2±0.5	-5.9%	0.0081	41.1±0.1	-1.0%
♂	#2	SY9:1	No RU486	152	60.1±1.0			77.5±0.8	
			RU486	146	58.9±0.9	-2.0%	0.0677	73.5±0.9	-5.2%
		SY1:1	No RU486	139	46.5±1.1			62.0±0	
			RU486	142	44.0±1.0	-5.4%	0.0005	57.0±0.6	-8.1%
		SY1:9	No RU486	141	32.7±0.4			39.6±0.9	
			RU486	130	29.5±0.5	-9.8%	<.0001	36.8±0.3	-7.1%

[a]SY, sugar-yeast diet. The ratio of sugar to yeast is listed after "SY".

[b]The presence of RU486 induces ATPsyn-d overexpression.

[c]Lifespan is expressed as mean±Standard error (SE) in days (d).

[d]Percentage changes were referenced to SY diet-matched control flies without RU486 treatment.

[e]P values were based on comparing diet-matched flies with and without RU486 by logrank test.

[f]Maxi refers to maximum lifespan calculated as mean lifespan of the top 10% longest-live flies.

Table S4. Lifetime Egg Laying of Flies Fed the Sugar-Yeast Diets, Related to Figure 1 and 2

Genotype	SY diet[a]	Presence of RU486[b]	No. of replicates	Mean lifetime egg laying±SE [c]	Percentage of reproduction decrease[d]	p value[e]
da-GSG/UAS-ATPsyn-dRNAi	SY9:1	No RU486	6	32.5±1.5		
(v104353)		RU486	6	14.7±1.0	-54.8%	<.0001
	SY1:1	No RU486	6	98.7±10.7		
		RU486	6	16.4±2.0	-83.4%	<.0001
	SY1:9	No RU486	6	47.6±8.0		
		RU486	6	11.8±1.2	-75.2%	<.0001
da-GSG/+	SY9:1	No RU486	6	26.3±2.6		
(driver only line)		RU486	6	12.2±1.1	-53.6%	0.0005
	SY1:1	No RU486	6	126.0±10.6		
		RU486	6	35.2±3.4	-72.1%	<.0001
	SY1:9	No RU486	6	61.6±12.8		
		RU486	6	15.4±3.0	-75.0%	0.0056
UAS-ATPsyn-dRNAi/+	SY9:1	No RU486	6	29.8±2.0		
(UAS (v104353) only line)		RU486	6	22.2±1.7	-25.3%	0.0149
	SY1:1	No RU486	6	123.4±7.9		
		RU486	6	30.3±2.6	-75.4%	<.0001
	SY1:9	No RU486	6	37.3±5.3		
		RU486	6	21.8±4.6	-41.5%	0.0515

[a]SY, sugar-yeast diet. The ratio of sugar to yeast is listed after "SY".

[b]The presence of RU486 refers to 200 μM RU486.

[c]Lifetime egg laying is expressed as mean±Standard error (SE) per fly.

[d]Percentage changes were referenced to SY diet-matched flies without RU486 treatment.

[e]P values were based on comparing diet-matched flies with and without RU486 by Student's *t* -test.

Table S5. The List of Genes that are Significantly Changed by ATPsyn-d Knockdown in Flies Fed SY1:9, Related to Figure 3

Unique array id	Gene symbol[a]	Gene name[b]	Fold-change	Parametric p-value	FDR[c]
03: F-12	CG5472	peptidyl-α-hydroxyglycine-α-amidating lyase	5.84	0.00002	0.00326
14: J-23	CG40306	stoned A	5.54	0.00012	0.00664
24: J-21	CG33245	CG33245	4.86	0.00009	0.00623
38: B-22	CG3257	CG3257	4.75	0.00004	0.00418
21: M-24	CG1168	7B2	4.59	0.00058	0.01350
26: G-24	CG4779	homogentisate 1,2-dioxygenase	4.55	0.00011	0.00653
07: J-12	CG6656	CG6656	4.54	0.00010	0.00637
22: H-24	CG13928	CG13928	4.2	0.00401	0.04180
32: I-11	CG31778	CG31778	4.13	0.00002	0.00326
27: E-22	CG3533	unzipped	4.05	0.00000	0.00114
13: I-11	CG13335	CG13335	4	0.00001	0.00281
33: F-22	CG12660	retinal degeneration A	3.91	0.00036	0.01130
18: J-10	CG7135	CG7135	3.83	0.00001	0.00281
19: O-11	CG8539	CG8539	3.77	0.00008	0.00623
24: G-22	CG14994	glutamic acid decarboxylase 1	3.75	0.00119	0.02000
13: F-21	CG13086	CG13086	3.68	0.00003	0.00418
14: J-10	CG16777	CG16777	3.64	0.00018	0.00779
06: B-12	CG9372	CG9372	3.64	0.00050	0.01280
07: B-12	CG6571	retinal degeneration C	3.57	0.00074	0.01540
38: N-23	CG7722	serpin 47C	3.56	0.00009	0.00627
04: F-23	CG3159	excitatory amino acid transporter 2	3.52	0.00005	0.00426
20: J-22	CG18214	trio	3.49	0.00001	0.00281
05: G-23	CG11206	liprin-γ	3.47	0.00039	0.01180
28: N-20	CG30492	CG30492	3.43	0.00270	0.03200
24: F-23	CG33203	CG33203	3.43	0.00131	0.02080
30: F-10	CG12023	GV1	3.39	0.00001	0.00282
32: A-11	CG31706	CG31706	3.39	0.00001	0.00281
39: N-09	CG32169	RNA-binding protein 6	3.38	0.00002	0.00326
33: F-23	CG11387	cut	3.36	0.00077	0.01560
31: C-23	CG1299	CG1299	3.36	0.00043	0.01230
34: I-23	CG31217	modular serine protease	3.35	0.00002	0.00326
16: B-11	CG12269	CG12269	3.32	0.00020	0.00793
09: N-23	CG14275	CG14275	3.32	0.00016	0.00779
16: F-24	CG32800	CG32800	3.3	0.00028	0.00961
26: F-22	CG8086	CG8086	3.29	0.00008	0.00623
11: F-08	CG10047	synaptotagmin 4	3.27	0.00288	0.03320
36: I-11	CG18598	CG18598	3.26	0.00038	0.01170
29: J-24	CR32646	CR32646	3.26	0.00066	0.01460
40: K-11	CG4409	CG4409	3.24	0.00051	0.01280
06: N-12	CG9511	CG9511	3.24	0.00013	0.00708
06: H-12	CG9441	punch	3.21	0.00866	0.06610
03: C-11	CG17117	homothorax	3.2	0.00152	0.02310
11: N-21	CG9631	CG9631	3.19	0.00047	0.01260
04: I-12	CG30019	CG30019	3.18	0.00245	0.03070
12: G-23	CG7921	Mgat2	3.16	0.00051	0.01280
12: F-20	CG30151	CG30151	3.15	0.00011	0.00653

36: K-10	CG30052	odorant-binding protein 49a	3.1	0.00164	0.02460
13: A-10	CG14528	CG14528	3.08	0.00011	0.00653
09: B-10	CG5387	Cdk5α	3.08	0.00031	0.01050
19: J-21	CG6329	CG6329	3.04	0.00024	0.00872
05: K-24	CG31160	CG31160	3.02	0.00086	0.01660
29: E-22	CG6438	amontillado	3	0.00183	0.02600
17: K-09	CG7203	CG7203	3	0.00028	0.00961
29: K-11	CG14032	Cyp4ac1	2.98	0.00855	0.06600
24: M-11	CG2381	Syt7	2.98	0.00002	0.00329
26: I-23	CG6282	oxidoreductase activity	2.97	0.00080	0.01600
10: F-24	CG6698	NtR	2.96	0.00339	0.03660
15: L-12	CG1889	CG1889	2.95	0.00846	0.06560
24: G-09	CG2239	jdp	2.94	0.00455	0.04470
40: B-10	CG14132	CG14132	2.91	0.00048	0.01270
03: F-11	CG3966	neither inactivation nor afterpotential A	2.91	0.00130	0.02080
36: K-11	CG18673	CG18673	2.88	0.00070	0.01490
28: O-10	CG14904	Sarcoplasmic calcium-binding protein 2	2.86	0.00259	0.03180
30: I-23	CG15203	CG15203	2.86	0.00004	0.00426
27: G-22	CG3566	CG3566	2.85	0.00319	0.03570
08: G-11	CG8295	myelodysplasia/myeloid leukemia factor	2.85	0.00273	0.03200
05: D-22	CG5073	CG5073	2.82	0.00430	0.04320
08: N-22	CG32019	bent	2.8	0.00006	0.00525
26: I-06	CG4783	CG4783	2.8	0.00019	0.00779
39: M-22	CG7052	thiolester containing protein II	2.8	0.00069	0.01480
15: I-23	CG9347	neither inactivation nor afterpotential B	2.8	0.00015	0.00759
22: F-08	CG13833	CG13833	2.77	0.00245	0.03070
24: C-23	CG2194	suppressor of rudimentary	2.77	0.00283	0.03290
18: E-23	CG3244	C-type lectin 27kD	2.76	0.00004	0.00418
29: C-09	CG13908	CG13908	2.74	0.00335	0.03640
34: B-24	CG4356	muscarinic Acetylcholine Receptor 60C	2.74	0.00502	0.04630
27: F-23	CG9813	CG9813	2.74	0.00303	0.03420
18: M-21	CG32523	CG32523	2.72	0.00966	0.07060
03: P-10	CG5577	CG5577	2.72	0.00794	0.06260
40: H-07	CG9629	CG9629	2.71	0.00115	0.01970
08: N-23	CG16978	CG16978	2.7	0.00413	0.04240
22: C-11	CG14567	CG14567	2.69	0.00042	0.01230
12: A-22	CG10205	CG10205	2.61	0.00394	0.04170
41: K-09	CG4660	CG4660	2.59	0.00200	0.02710
16: A-23	CG4894	Ca-α1D	2.59	0.00139	0.02150
05: F-09	CG6027	center divider	2.59	0.00004	0.00426
35: J-08	CG8896	18 wheeler,immune response	2.59	0.00016	0.00779
23: N-08	CG5249	blimp-1	2.58	0.00972	0.07060
24: N-24	CG9338	CG9338	2.56	0.00189	0.02620
32: B-24	CG11147	CG11147	2.54	0.00675	0.05530
31: H-12	CG4501	bubblegum	2.51	0.00323	0.03570
11: B-22	CG10011	CG10011	2.49	0.00003	0.00418
10: K-11	CG11062	activin-β	2.48	0.00322	0.03570
40: I-07	CG4325	CG4325	2.48	0.00047	0.01260
21: F-23	CG4432	Peptidoglycan recognition protein LC	2.48	0.00098	0.01810
10: B-23	CG1969	CG1969	2.47	0.00103	0.01850

24: A-07	CG2155	vermilion	2.47	0.00479	0.04490
27: G-23	CG3772	cryptochrome	2.47	0.00823	0.06410
38: K-10	CG4259	CG4259	2.46	0.00264	0.03200
08: M-22	CG5744	frequenin 1	2.46	0.00143	0.02190
15: J-09	CG7365	CG7365	2.46	0.00056	0.01340
27: C-22	CG3505	CG3505	2.44	0.00561	0.04890
32: H-20	CG11241	CG11241	2.42	0.00062	0.01390
06: J-11	CG11641	pou domain motif 3	2.42	0.00005	0.00426
38: F-21	CG6356	CG6356	2.42	0.00066	0.01460
06: F-12	CG9427	CG9427	2.4	0.00228	0.02950
32: N-23	CG1632	CG1632	2.39	0.00185	0.02600
12: J-22	CG30190	CG30190	2.39	0.00053	0.01290
11: N-20	CG10137	CG10137	2.37	0.00270	0.03200
34: M-12	CG13607	CG13607	2.37	0.00652	0.05440
41: G-23	CG4629	CG4629	2.37	0.00572	0.04920
34: B-09	CG1448	innexin 3, gap junction channel	2.35	0.00086	0.01660
20: I-22	CG1092	CG1092	2.33	0.00128	0.02060
32: F-21	CG15855	ecdysone-induced protein 63F 1	2.33	0.00139	0.02150
08: C-09	CG8245	CG8245	2.31	0.00619	0.05230
11: A-23	CG12187	CG8245	2.29	0.00019	0.00779
33: F-12	CG12643	CG8245	2.29	0.00396	0.04170
15: I-22	CG13845	CG8245	2.29	0.00173	0.02570
14: C-23	CG8733	Cyp305a1	2.29	0.00435	0.04350
38: G-22	CG4220	elbow B	2.28	0.00091	0.01700
30: F-08	CG12023	GV1	2.27	0.00463	0.04480
21: J-20	CG40041	glycoprotein hormone beta 5	2.27	0.00036	0.01130
05: A-23	CG10361	CG10361	2.26	0.00203	0.02720
38: C-11	CG17594	scarecrow	2.25	0.00405	0.04200
31: H-10	CG4500	CG4500	2.25	0.00953	0.07040
23: O-23	CG6912	CG6912	2.25	0.00109	0.01920
36: C-09	CG1819	CG1819	2.23	0.00469	0.04480
30: B-21	CG1998	CG1998	2.23	0.00477	0.04490
35: F-21	CG11821	Cyp12a5	2.2	0.00053	0.01290
33: K-11	CG15658	CG15658	2.2	0.00012	0.00669
10: G-09	CG11010	equilibrative nucleoside transporter 3	2.18	0.00473	0.04480
28: H-12	CG30418	nord	2.18	0.00184	0.02600
24: L-11	CG33261	CG33261	2.18	0.00552	0.04890
03: N-22	CG5562	glass bottom boat	2.16	0.00060	0.01380
12: J-21	CG15661	CG15661	2.15	0.00320	0.03570
10: C-24	CG18375	CG18375	2.15	0.00179	0.02590
12: N-24	CG30289	CG30289	2.15	0.00125	0.02040
34: A-23	CG31145	CG31145	2.15	0.00558	0.04890
13: I-08	CG14630	CG14630	2.13	0.00072	0.01520
33: C-21	CG15553	CG15553	2.12	0.00078	0.01580
38: O-22	CG4330	CG4330	2.11	0.00560	0.04890
19: J-10	CG9170	CG9170	2.11	0.00222	0.02890
11: H-24	CG10079	EGFR	2.1	0.00573	0.04920
37: L-15	CG9119	CG9119	2.1	0.00121	0.02000
25: B-11	CG10353	CG10353	2.09	0.00873	0.06610
28: J-11	CG12090	CG12090	2.08	0.00418	0.04240

08: E-10	CG5646	CG5646	2.08	0.00035	0.01120
12: G-24	CG11066	scarface	2.07	0.00473	0.04480
10: K-10	CG18507	CG18507	2.07	0.00544	0.04880
07: O-11	CG5939	paramyosin	2.07	0.00273	0.03200
30: P-18	CG12139	CG12139	2.05	0.00456	0.04470
12: G-22	CG1102	melanization Protease 1	2.04	0.00905	0.06820
21: E-22	CG11388	CG11388	2.04	0.00041	0.01230
30: E-22	CG4139	karl	2.04	0.00872	0.06610
03: J-12	CG5501	myosin 95E	2.04	0.00259	0.03180
24: C-11	CG2177	CG2177	2.03	0.00050	0.01280
38: L-11	CG7152	syntrophin-like 1	2.03	0.00659	0.05450
31: N-21	CG3556	CG3556	2.02	0.00707	0.05720
25: N-22	CG10698	GRHRII	2.01	0.00103	0.01850
38: K-09	CG1770	HDAC4	2	0.00211	0.02770
19: C-07	CG8193	CG8193	2	0.00108	0.01910
27: L-10	CG8547	CG8547	2	0.00536	0.04830
11: F-10	CG1004	rhomboid	1.98	0.00017	0.00779
06: N-08	CG9508	CG9508	1.98	0.00603	0.05120
32: D-12	CG11158	CG11158	1.97	0.00656	0.05440
18: D-10	CG6124	eater	1.96	0.00052	0.01290
26: F-20	CG8086	CG8086	1.95	0.00557	0.04890
06: B-24	CG9384	CG9384	1.94	0.00705	0.05720
37: A-11	CG1689	lozenge	1.93	0.00998	0.07120
24: M-23	CG2457	inaF-D	1.93	0.00044	0.01230
20: B-23	CG30084	Z band alternatively spliced PDZ-motif protein 52	1.93	0.00245	0.03070
17: H-10	CG12529	zwischenferment	1.92	0.00294	0.03340
27: F-22	CG8486	CG8486	1.91	0.00270	0.03200
04: F-11	CG31555	CG31555	1.9	0.00087	0.01660
08: E-24	CG5661	semaphorin-5c	1.9	0.00947	0.07040
37: I-11	CG16987	dawdle	1.89	0.00397	0.04170
23: N-21	CG3413	windpipe	1.89	0.00239	0.03060
05: N-21	CG6139	CG6139	1.89	0.00749	0.05960
08: M-23	CG8384	groucho	1.89	0.00112	0.01960
28: J-10	CG30438	CG30438	1.88	0.00328	0.03600
27: E-24	CG3534	CG3534	1.88	0.00175	0.02570
24: K-22	CG15029	CG15029	1.87	0.00474	0.04480
21: F-11	CG4276	arouser	1.87	0.00208	0.02760
24: P-06	CG9342	microsomal triacylglycerol transfer protein	1.87	0.00451	0.04460
24: D-09	CG33181	CG33181	1.84	0.00442	0.04400
04: N-11	CG31664	CG31664	1.83	0.00872	0.06610
36: D-08	CG7084	CG7084	1.81	0.00921	0.06920
17: L-20	CG1271	CG1271	1.79	0.00710	0.05720
40: N-12	CG14430	boudin (bou)	1.79	0.00533	0.04820
26: K-21	CG6304	CG6304	1.77	0.00973	0.07060
25: N-23	CG10534	Lcp65Ag2	1.76	0.00934	0.06990
19: M-11	CG8461	CG8461	0.57	0.00788	0.06240
11: L-24	CG10120	malic enzyme	0.56	0.00996	0.07120
17: G-23	CG7175	CG7175	0.56	0.00653	0.05440
14: A-09	CG8597	lark	0.56	0.00951	0.07040
41: G-21	CG4626	frizzled 4	0.55	0.00493	0.04570

36: D-14	CG7130	heat shock protein binding	0.55	0.00378	0.04050
38: E-22	CG4206	minichromosome maintenance 3	0.54	0.00512	0.04700
04: L-24	CG12505	Arc1	0.53	0.00624	0.05250
36: A-04	CG17295	reduction in Cnn dots 4	0.53	0.00471	0.04480
20: F-08	CG18107	CG18107	0.53	0.00489	0.04550
32: M-10	CG17054	CG17054	0.52	0.00523	0.04770
08: M-11	CG8369	CG8369	0.52	0.00864	0.06610
23: O-01	CG6891	CG6891	0.51	0.00186	0.02600
10: F-21	CG2023	CG2023	0.48	0.00046	0.01260
22: I-22	CG31938	Rrp40	0.48	0.00176	0.02570
22: K-22	CG32023	CG32023	0.48	0.00821	0.06410
05: B-10	CG5041	Tfb4	0.48	0.00558	0.04890
24: D-14	CG9211	interference Hedgehog	0.48	0.00419	0.04240
25: K-11	CG10387	tosca	0.47	0.00981	0.07090
20: E-22	CG1078	Fip1	0.47	0.00183	0.02600
17: M-10	CG3319	cyclin-dependent kinase 7	0.47	0.00116	0.01970
12: G-10	CG10965	companion of reaper	0.46	0.00573	0.04920
04: E-11	CG1675	CG1675	0.46	0.00020	0.00793
20: F-10	CG18108	immune induced molecule 1	0.46	0.00417	0.04240
39: N-21	CG3217	CKII-alpha subunit interactor-3	0.46	0.00380	0.04060
20: B-24	CG18067	CG18067	0.45	0.00710	0.05720
20: N-10	CG1825	microtubule-associated protein 60	0.45	0.00467	0.04480
26: C-16	CG4740	attacin-C	0.45	0.00414	0.04240
20: M-22	CG11100	Mes2	0.44	0.00197	0.02710
16: N-09	CG14125	CG14125	0.44	0.00089	0.01670
40: I-03	CG4312	metallothionein B	0.44	0.00294	0.03340
20: I-12	CG10911	CG10911	0.43	0.00579	0.04950
25: E-24	CG14375	CCHamide-2	0.43	0.00154	0.02320
17: M-22	CG33250	AlkB	0.43	0.00035	0.01120
11: J-09	CG9494	tetraspanin 29Fa	0.43	0.00589	0.05020
34: I-22	CG13570	spaghetti	0.42	0.00017	0.00779
14: N-20	CG16844	immune induced molecule 3	0.42	0.00019	0.00779
33: I-24	CG32796	brother of iHog	0.42	0.00201	0.02710
06: M-22	CG5399	CG5399	0.42	0.00724	0.05810
23: I-11	CG6833	CG6833	0.42	0.00334	0.03640
39: B-07	CG32041	Hsp22	0.41	0.00212	0.02770
19: O-21	CG8577	PGRP-SC1b	0.41	0.00268	0.03200
05: I-21	CG11381	CG11381	0.4	0.00967	0.07060
32: F-11	CG15835	histone demethylase 4A	0.4	0.00043	0.01230
15: B-12	CG18628	CG18628	0.4	0.00525	0.04770
08: I-11	CG8319	CG8319	0.4	0.00024	0.00872
36: N-23	CG8767	mos	0.4	0.00128	0.02060
19: F-09	CG6137	aubergine	0.39	0.00018	0.00779
22: I-23	CG14781	meiotic 38	0.37	0.00102	0.01850
04: B-11	CG31517	CG31517	0.37	0.00244	0.03070
40: B-21	CG9576	CG9576	0.37	0.00957	0.07050
35: I-11	CG9135	CG9135	0.35	0.00200	0.02710
27: O-11	CG3868	CG3868	0.34	0.00133	0.02080
27: E-11	CG3738	cyclin-dependent kinase subunit 30A	0.32	0.00057	0.01350
24: F-10	CG9241	sensitized chromosome inheritance modifier 19	0.32	0.00081	0.01610

24: B-11	CG33166	stem cell tumor	0.3	0.00022	0.00827
39: E-11	CG32282	drosomycin-4	0.29	0.00799	0.06270
28: O-11	CG10336	response to DNA damage stimulus	0.27	0.00017	0.00779
14: J-08	CG16775	CG16775	0.27	0.00010	0.00627
26: C-12	CG4734	CG4734	0.27	0.00665	0.05470
12: C-12	CG10308	Cyclin J	0.26	0.00087	0.01660
21: J-10	CG3994	ZnT35C	0.25	0.00022	0.00827
13: E-11	CG13299	CG13299	0.24	0.00029	0.00970
27: B-10	CG8420	CG8420	0.23	0.00068	0.01480
36: F-24	CG7242	Spc25	0.22	0.00260	0.03180
33: B-09	CG11330	cortex	0.21	0.00004	0.00418
07: F-22	CG6620	IpII-aurora-like kinase	0.2	0.00115	0.01970
24: B-12	CG9188	septin interacting protein 2	0.2	0.00121	0.02000
37: J-22	CG13083	CG13083	0.19	0.00009	0.00627
20: A-23	CG7670	WRN exonuclease	0.16	0.00007	0.00566
23: E-14	CG10794	diptericin B	0.12	0.00074	0.01540
23: G-10	CG10810	drosomycin	0.11	0.00745	0.05940
39: E-09	CG32274	drosomycin-like	0.078	0.00285	0.03300
19: C-01	CG8175	metchnikowin	0.073	0.00001	0.00326

[a,b]Gene symbol and name are based on the information in the flybase, www.flybase.org.

[d]FDR, false discovery rate.

Table S6. The List of GO Categories Significantly Changed by ATPsyn-d Knockdown in Flies Fed SY1:9, Related to Figure 3

Gene ontology category	Unique array id	Gene symbol[a]	Gene nameb	Fold-change	Parametric p-value	FDR[c]
GO 23052	11: F-10	CG1004	rhomboid	1.98	0.00017	0.00779
Signaling	11: F-08	CG10047	synaptotagmin 4	3.27	0.00288	0.03320
	11: H-24	CG10079	EGFR	2.10	0.00573	0.04920
	25: N-22	CG10698	GRHRII	2.01	0.00103	0.01850
	14: N-20	CG16844	immune induced molecule 3	0.42	0.00019	0.00779
	37: A-11	CG1689	lozenge	1.93	0.00998	0.07120
	37: I-11	CG16987	dawdle	1.89	0.00397	0.04170
	20: B-24	CG18067	CG18067	0.45	0.00710	0.05720
	20: J-22	CG18214	Trio	3.49	0.00001	0.00281
	15: L-12	CG1889	CG1889	2.95	0.00846	0.06560
	24: M-11	CG2381	Syt7	2.98	0.00002	0.00329
	24: M-23	CG2457	inaF-D	1.93	0.00044	0.01230
	33: I-24	CG32796	brother of iHog	0.42	0.00201	0.02710
	24: B-11	CG33166	stem cell tumor	0.30	0.00022	0.00827
	27: G-23	CG3772	cryptochrome	2.47	0.00823	0.06410
	03: F-11	CG3966	neither inactivation nor afterpotential A	2.91	0.00130	0.02080
	14: J-23	CG40306	stoned A	5.54	0.00012	0.00664
	38: G-22	CG4220	elbow B	2.28	0.00091	0.01700
	21: F-11	CG4276	arouser	1.87	0.00208	0.02760
	34: B-24	CG4356	muscarinic Acetylcholine Receptor 60C	2.74	0.00502	0.04630
	41: G-21	CG4626	frizzled 4	0.55	0.00493	0.04570
	03: N-22	CG5562	glass bottom boat	2.16	0.00060	0.01380
	08: M-22	CG5744	frequenin 1	2.46	0.00143	0.02190
	07: B-12	CG6571	retinal degeneration C	3.57	0.00074	0.01540
	35: J-08	CG8896	18 wheeler	2.59	0.00016	0.00779
	24: D-14	CG9211	interference Hedgehog	0.48	0.00419	0.04240
	15: I-23	CG9347	neither inactivation nor afterpotential B	2.80	0.00015	0.00759
	12: G-10	CG10965	companion of reaper	0.46	0.00573	0.04920
	21: M-24	CG1168	7B2	4.59	0.00058	0.01350
	24: G-22	CG14994	glutamic acid decarboxylase 1	3.75	0.00119	0.02000
GO 16491	11: L-24	CG10120	malic enzyme	0.56	0.00996	0.07120
Oxidoreductase	35: F-21	CG11821	Cyp12a5	2.20	0.00053	0.01290
	17: H-10	CG12529	Zwischenferment	1.92	0.00294	0.03340
	22: F-08	CG13833	CG13833	2.77	0.00245	0.03070
	29: K-11	CG14032	Cyp4ac1	2.98	0.00855	0.06600
	13: I-08	CG14630	CG14630	2.13	0.00072	0.01520
	32: F-11	CG15835	histone demethylase 4A	0.40	0.00043	0.01230
	30: B-21	CG1998	CG1998	2.23	0.00477	0.04490
	24: A-07	CG2155	vermilion	2.47	0.00479	0.04490
	24: C-23	CG2194	suppressor of rudimentary	2.77	0.00283	0.03290
	04: I-12	CG30019	CG30019	3.18	0.00245	0.03070
	17: M-22	CG33250	AlkB	0.43	0.00035	0.01120
	26: G-24	CG4779	homogentisate 1,2-dioxygenase	4.55	0.00011	0.00653
	26: I-23	CG6282	CG6282	2.97	0.00080	0.01600

	19: C-07	CG8193	CG8193	2.00	0.00108	0.01910
	14: C-23	CG8733	Cyp305a1	2.29	0.00435	0.04350
	15: I-23	CG9347	neither inactivation nor afterpotential B	2.80	0.00015	0.00759
	40: H-07	CG9629	CG9629	2.71	0.00115	0.01970
GO 6508	11: N-21	CG9631	CG9631	3.19	0.00047	0.01260
Proteolysis	27: C-22	CG3505	no homolog	2.44	0.00561	0.04890
	32: N-23	CG1632	CG1632	2.39	0.00185	0.02600
	32: I-11	CG31778	CG31778	4.13	0.00002	0.00326
	29: E-22	CG6438	amontillado	3.00	0.00183	0.02600
	06: B-12	CG9372	CG9372	3.64	0.00050	0.01280
	12: G-24	CG11066	scarface	2.07	0.00473	0.04480
	31: C-23	CG1299	CG1299	3.36	0.00043	0.01230
	13: A-10	CG14528	CG14528	3.08	0.00011	0.00653
	12: G-22	CG1102	melanization Protease 1	2.04	0.00905	0.06820
	34: I-23	CG31217	modular serine protease	3.35	0.00002	0.00326
	18: M-21	CG32523	CG32523	2.72	0.00966	0.07060
	19: O-11	CG8539	CG8539	3.77	0.00008	0.00623
	12: N-24	CG30289	CG30289	2.15	0.00125	0.02040
	38: K-10	CG4259	CG4259	2.46	0.00264	0.03200
	11: F-10	CG1004	rhomboid	1.98	0.00017	0.00779
	33: B-09	CG11330	cortex	0.21	0.00004	0.00418
	27: E-11	CG3738	Cyclin-dependent kinase subunit 30A	0.32	0.00057	0.01350
GO 6952	14: N-20	CG16844	immune induced molecule 3	0.42	0.00019	0.00779
Defense	39: M-22	CG7052	thiolester containing protein II	2.80	0.00069	0.01480
response	26: C-16	CG4740	attacin-C	0.45	0.00414	0.04240
	20: F-10	CG18108	immune induced molecule 1	0.46	0.00417	0.04240
	23: E-14	CG10794	diptericin B	0.12	0.00074	0.01540
	19: C-01	CG8175	metchnikowin	0.07	0.00001	0.00326
	23: G-10	CG10810	Drosomycin	0.11	0.00745	0.05940
	35: J-08	CG8896	18 wheeler	2.59	0.00016	0.00779
	21: F-23	CG4432	peptidoglycan recognition protein LC	2.48	0.00098	0.01810
	34: I-23	CG31217	modular serine protease	3.35	0.00002	0.00326
	12: G-22	CG1102	melanization Protease 1	2.04	0.00905	0.06820
	19: O-21	CG8577	PGRP-SC1b	0.41	0.00268	0.03200
	39: E-11	CG32282	drosomycin-4	0.29	0.00799	0.06270
	39: E-09	CG32274	drosomycin-like	0.08	0.00285	0.03300
	37: A-11	CG1689	lozenge	1.93	0.00998	0.07120
GO 9628	24: G-22	CG14994	glutamic acid decarboxylase 1	3.75	0.00119	0.02000
Response to	24: M-23	CG2457	inaF-D	1.93	0.00044	0.01230
abiotic stimulus	39: B-07	CG32041	Hsp22	0.41	0.00212	0.02770
	27: G-23	CG3772	cryptochrome	2.47	0.00823	0.06410
	03: F-11	CG3966	neither inactivation nor afterpotential A	2.91	0.00130	0.02080
	14: J-23	CG40306	stoned A	5.54	0.00012	0.00664
	07: B-12	CG6571	retinal degeneration C	3.57	0.00074	0.01540
	36: D-14	CG7130	heat shock protein binding	0.55	0.00378	0.04050
	15: I-23	CG9347	neither inactivation nor afterpotential B	2.80	0.00015	0.00759

GO 6259	28: O-11	CG10336	response to DNA damage stimulus	0.27	0.00017	0.00779
DNA metabolic	25: K-11	CG10387	tosca	0.47	0.00981	0.07090
process	22: I-23	CG14781	meiotic 38	0.37	0.00102	0.01850
	27: G-23	CG3772	cryptochrome	2.47	0.00823	0.06410
	38: E-22	CG4206	minichromosome maintenance 3	0.54	0.00512	0.04700
	05: B-10	CG5041	Tfb4	0.48	0.00558	0.04890
	20: A-23	CG7670	WRN exonuclease	0.16	0.00007	0.00566
	15: J-09	CG7365	phospholipase activity	2.46	0.00056	0.01340
GO 8307	38: L-11	CG7152	syntrophin-like 1	2.03	0.00659	0.05450
Muscle	08: N-22	CG32019	bent	2.80	0.00006	0.00525
structure	07: O-11	CG5939	paramyosin	2.07	0.00273	0.03200

[a,b]Gene symbol and name are based on the information in the flybase, www.flybase.org.

[c]FDR, false discovery rate.

Table S7. The Interaction between ATPsyn-d and TOR Signaling on Lifespan of Females, Related to Figure 5 and 6

Strain[a]	SY diet[b]	Presence of RU486[c]	Rapamycin feeding[d]	# of flies per trial	Mean lifespan±SE (d)[e]	Change of mean lifespan	p value[l]
da-GSG/v104353;+/+	SY1:1	No RU486	–	153	25.5 ± 0.5		
da-GSG/v104353;+/+		RU486	–	154	30.6 ± 0.7	20.0%[f]	<.0001
da-GSG/+;UAS-Tsc2 /+		RU486	–	93	26.7 ± 0.8		
da-GSG/v104353;UAS-Tsc2/+		RU486	–	148	26.0 ± 0.6	- 2.6%[g]	0.5017
da-GSG/v104353;+/+	SY1:9	No RU486	–	142	13.7 ± 0.2		
da-GSG/v104353;+/+		RU486	–	147	16.5 ± 0.4	20.4%[f]	<.0001
da-GSG/+;UAS-Tsc2 /+		RU486	–	127	20.0 ± 0.4		
da-GSG/v104353;UAS-Tsc2/+		RU486	–	179	19.9 ± 0.3	0.5%[g]	0.8675
da-GSG/v104353;+/+	SY1:1	No RU486	No Rapa	253	29.0±0.5		
		RU486	No Rapa	268	39.9±0.6	37.6%[h]	<0.0001
		No RU486	+Rapa	258	41.3±0.9	42.5%[i]	<0.0001
		RU486	+Rapa	257	30.8±0.7	-25.5%[h]	<0.0001
da-GSG/v104353;+/+	SY1:9	No RU486	No Rapa	242	20.1±0.3		
		RU486	No Rapa	230	22.5±0.4	11.6%[h]	<0.0001
		No RU486	+Rapa	219	28.9±0.7	43.6%[i]	<0.0001
		RU486	+Rapa	233	27.0±0.6	-6.7%[h]	0.00380

[a]"v104353" represents UAS-ATPsyn-d RNAi (line v104353).

[b]SY, sugar-yeast diet. The ratio of sugar to yeast is listed after "SY".

[c]The presence of RU486 refers to 200 μM RU486.

[d]The concentration of rapamycin was 200 μM.

[e]Lifespan is expressed as mean±Standard error (SE) in days (d).

[f]Percentage change was referenced to diet-matched da-GSG/v104353;+/+ flies without RU486 treatment.

[g]Percentage change was referenced to diet-matched da-GSG/+;UAS-Tsc2 /+ flies.

[h]Percentage changes were referenced to SY diet- and rapamycin feeding-matched reference flies without RU486 treatment.

[i]Percentage changes were referenced to SY diet- and RU486-matched flies without rapamycin feeding.

[l]P values were based on logrank test.