Intertissue Control of the Nucleolus

via a Myokine-Dependent Longevity Pathway

Fabio Demontis, Vishal K. Patel, William R. Swindell, and Norbert Perrimon,

SUPPLEMENTAL FIGURES AND LEGENDS
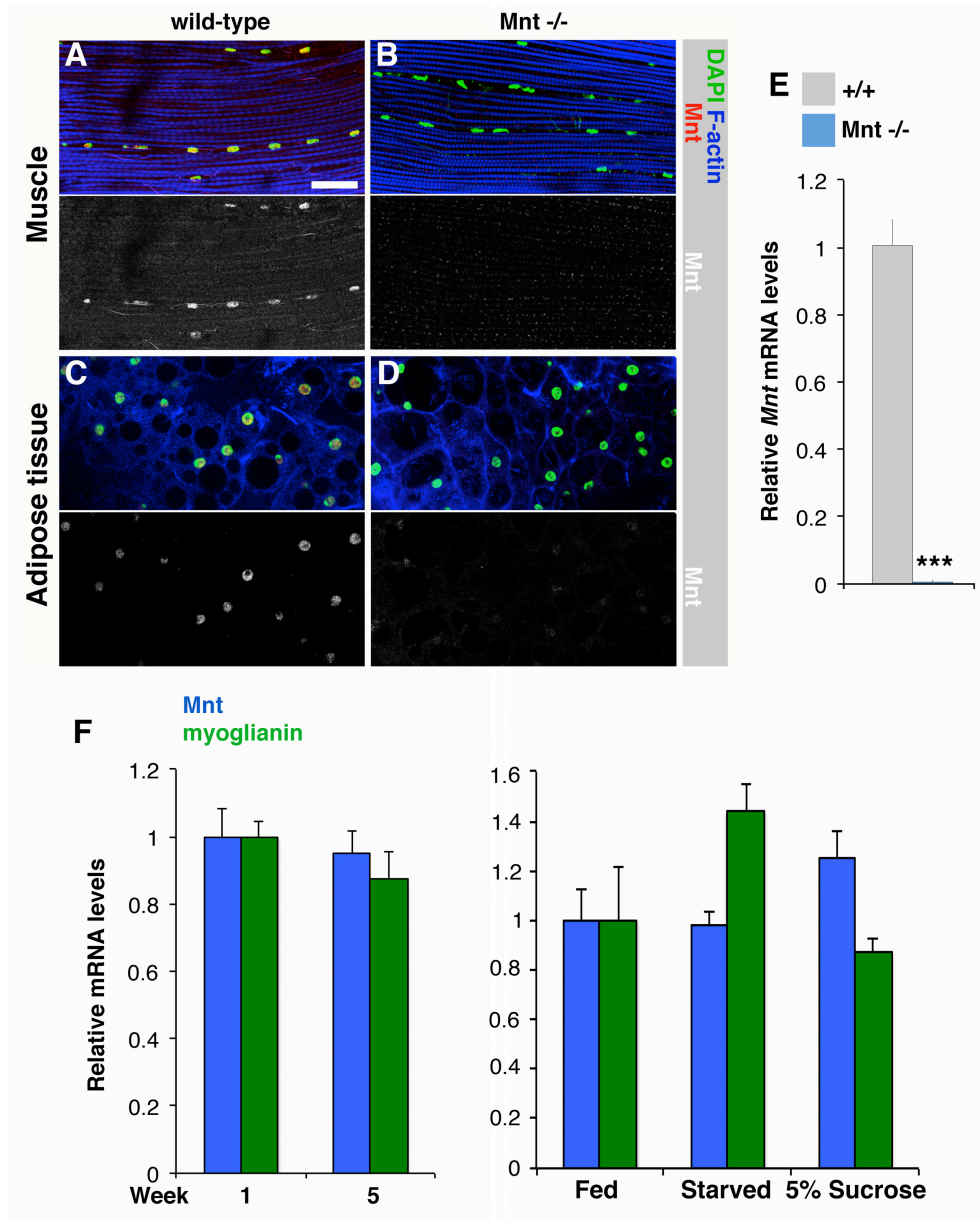
Figure S1. ***Drosophila* Mnt is Detected in Skeletal Muscle and Adipose Tissue, Related to Figure 1.** (A-D) Immunostaining of indirect flight muscle with anti-Mnt antibodies (red), F-actin (phalloidin, blue), and nuclei (DAPI, green). Mnt is detected in nuclei of adult skeletal muscle (A) and adipose tissue (C) from wild-type flies but not from *Mnt-/-* null flies (B, D, E). Scale bar is 20 μm. In (E), relative *Mnt* mRNA levels are shown with SEM indicated and n=4. ***$P<0.001$, unpaired two-tailed Student's *t*-test. (F) No apparent changes in *Mnt* and *myoglianin* mRNA levels are detected in muscle during aging and in response to starvation and a 5% sucrose diet. Mnt activity and Myoglianin signaling may be modulated via post-transcriptional mechanisms during aging independently from gene expression.
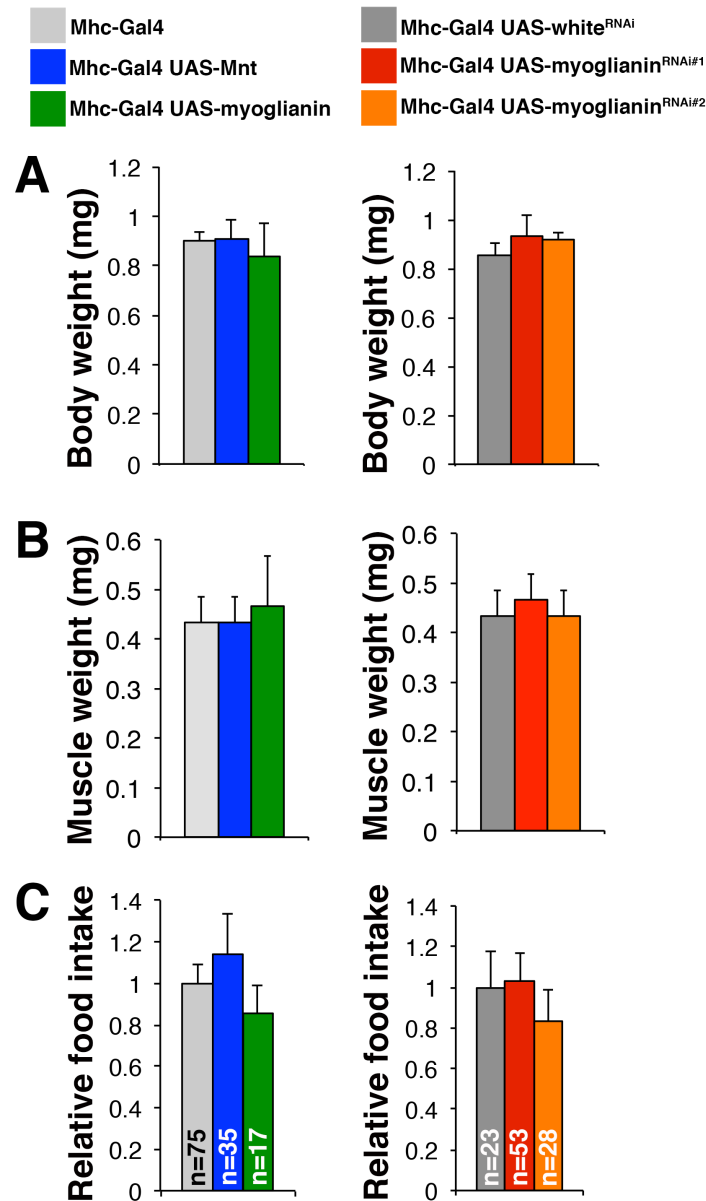
Figure S2. No Differences in Body Weight, Muscle Mass, and Relative Food Intake in Response to Mnt/Myoglianin Levels, Related to Figure 1 and 4. (A) Average body weight and (B) thoracic weight (consisting mostly of skeletal muscle and hence indicative of muscle mass) of flies overexpressing *Mnt* (blue) or *myoglianin* (green), and with *myoglianin*[RNAi#1] and *myoglianin*[RNAi#2] (red and orange) in muscle in comparison with controls (grey). SEM is indicated and n(batches of 10 flies)=3. No significant changes were detected by two-way ANOVA and Tukey's post test. (C) Relative food intake estimated with the capillary feeding (CAFÉ) assay reveals no substantial differences in the food intake of the genotypes analyzed. SEM and n(measurements) are indicated.
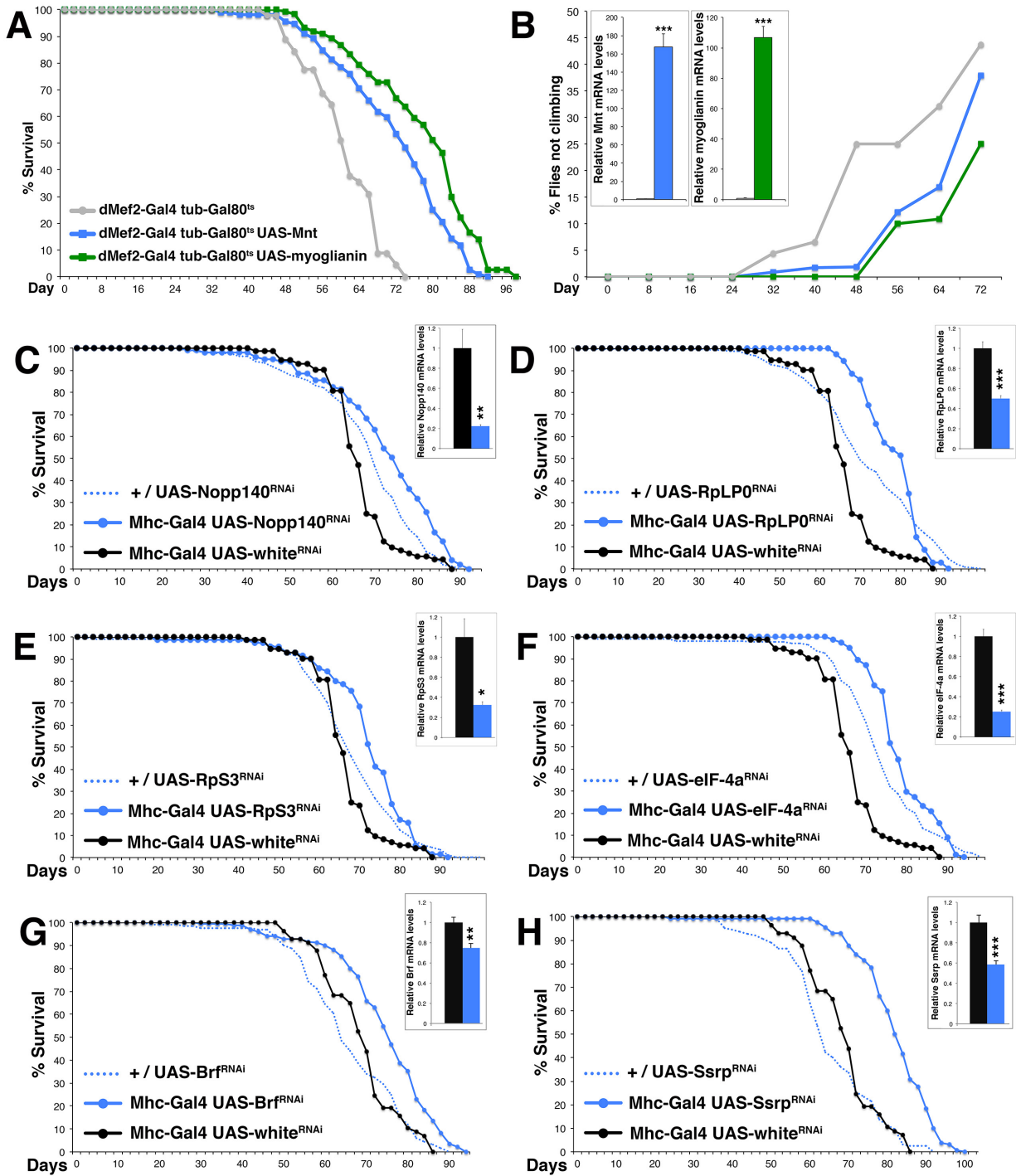
Figure S3. Lifespan Extension in Response to Mnt/Myoglianin Overexpression and RNAi-mediated knockdown of Genes Involved in Ribosome Biogenesis and Function, Related to Figure 1 and 4.

(A-B) Overexpression of *Mnt* and *myoglianin* in adult skeletal muscle with the *dMef2-Gal4* driver under the temporal control of Gal80[ts]. Flies were raised at 18°C to avoid transgene expression during development and shifted to 25°C three days after eclosion to inactivate Gal80[ts] and induce *Mnt* and *myoglianin*

expression in muscle of adult flies. Overexpression of *Mnt* (blue; *dMef2-Gal4/UAS-Mnt;* n=112; median/maximum lifespan=73.3/92 days) and *myoglianin* (green; *dMef2-Gal4/UAS-myoglianin;* n=121; median/maximum lifespan=80.5/98 days) in adult skeletal muscle extends lifespan (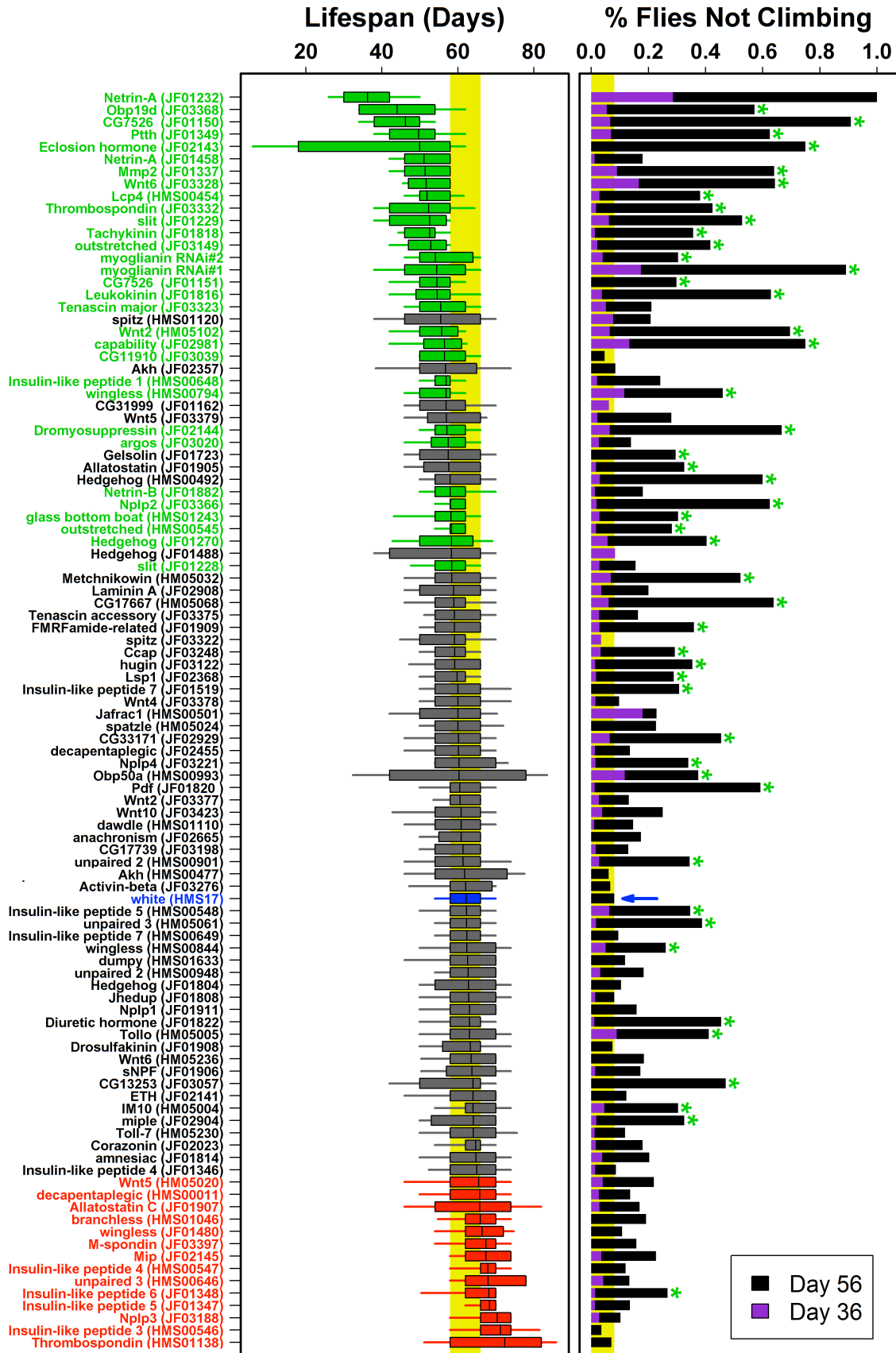*P*<0.001; Log-rank tests) and decreases age-related climbing defects (*P*<0.01; Log-rank tests) in comparison to control flies (grey; *dMef2-Gal4/+;* n=45; median/maximum lifespan=60.2/74 days). Relative mRNA levels of *Mnt* and *myoglianin* are shown with SEM indicated and n=4. ****P*<0.001, unpaired two-tailed Student's *t*-test.

(C-H) RNAi-mediated knockdown (blue) in muscle of *Nopp140* (C; n=97; median/maximum lifespan=74.1/92 days), *RpLP0* (D; n=35; median/maximum lifespan=80.2/92 days), *RpS3* (E; n=70; median/maximum lifespan=72.3/92 days), *eIF-4a* (F; n=77; median/maximum lifespan=76.5/94 days), *Brf* (G; n=170; median/maximum lifespan=75.2/94 days), and *Ssrp* (H; n=130; median/maximum lifespan=82/100) extends the median lifespan in comparison with UAS-RNAi alone controls (dashed blue line; respectively in C to H: n=166; n=271; n=396; n=271; n=132; n=125; having respectively median/maximum lifespan: 69.3/90; 69.8/100; 66.8/100; 72/98; 63.8/90; 62.6/92 days) and *white* RNAi (black; n=72 and median/maximum lifespan=65.3/88 days in C-F; n=57 and median/maximum lifespan=68.6/86 days in G-H; *P*<0.001; Log-rank tests).

Relative mRNA levels of *Nopp140*, *RpLP0*, *RpS3*, *eIF-4a*, *Brf*, and *Ssrp* are respectively shown in boxes in C-H**,** with SEM indicated and n=4. **P*<0.05; ***P*<0.01; ****P*<0.001, unpaired two-tailed Student's *t*-test.
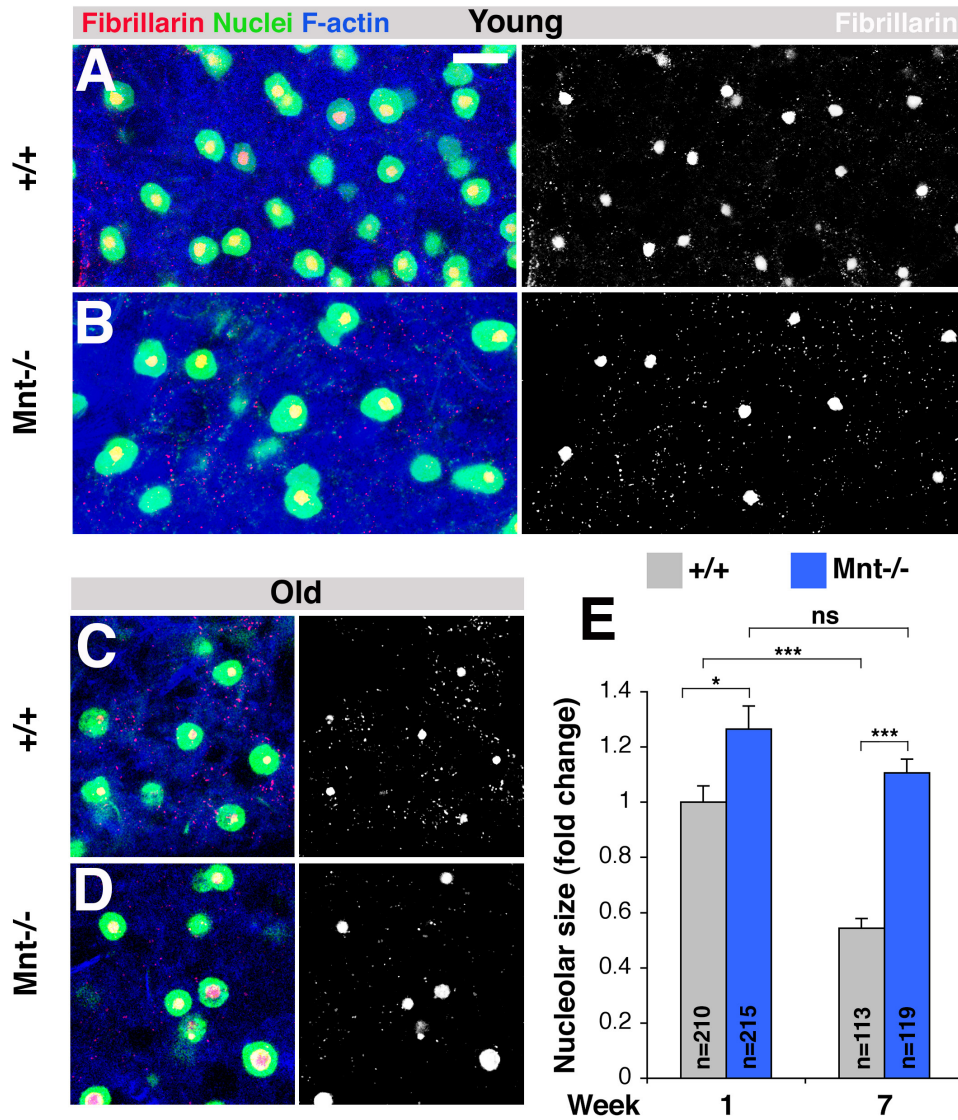
Figure S4. Regulation of Nucleolar Size in *Mnt* Null Adipocytes during Aging, Related to Figure 2. Immunostaining of adipose tissues (fat body) from control (+/+) and *Mnt*$^{-/-}$ null flies in young (1 week) and old age (7 weeks). Although nucleolar size decreases during aging in wild-type adipocytes (A-C), it does not in the absence of *Mnt* (B-D; *Mnt*$^{-/-}$). In A-D, scale bar is 10 μm. Nucleolar size quantification is shown in (E) with SEM and n indicated; ns, not significant; *P<0.05; ***P<0.001; two-way ANOVA followed by Tukey's post test.

Lifespan (Days)   % Flies Not Climbing

Netrin-A (JF01232)
Obp19d (JF03368)
CG7526 (JF01150)
Ptth (JF01349)
Eclosion hormone (JF02143)
Netrin-A (JF01458)
Mmp2 (JF01337)
Wnt6 (JF03328)
Lcp4 (HMS00454)
Thrombospondin (JF03332)
slit (JF01229)
Tachykinin (JF01818)
outstretched (JF03149)
myoglianin RNAi#2
myoglianin RNAi#1
CG7526 (JF01151)
Leukokinin (JF01816)
Tenascin major (JF03323)
spitz (HMS01120)
Wnt2 (HM05102)
capability (JF02981)
CG11910 (JF03039)
Akh (JF02357)
Insulin-like peptide 1 (HMS00648)
wingless (HMS00794)
CG31999 (JF01162)
Wnt5 (JF03379)
Dromyosuppressin (JF02144)
argos (JF03020)
Gelsolin (JF01723)
Allatostatin (JF01905)
Hedgehog (HMS00492)
Netrin-B (JF01882)
Nplp2 (JF03366)
glass bottom boat (HMS01243)
outstretched (HMS00545)
Hedgehog (JF01270)
Hedgehog (JF01488)
slit (JF01228)
Metchnikowin (HM05032)
Laminin A (JF02908)
CG17667 (HM05068)
Tenascin accessory (JF03375)
FMRFamide-related (JF01909)
spitz (JF03322)
Ccap (JF03248)
hugin (JF03122)
Lsp1 (JF02368)
Insulin-like peptide 7 (JF01519)
Wnt4 (JF03378)
Jafrac1 (HMS00501)
spatzle (HMS05024)
CG33171 (JF02929)
decapentaplegic (JF02455)
Nplp4 (JF03221)
Obp50a (HMS00993)
Pdf (JF01820)
Wnt2 (JF03377)
Wnt10 (JF03423)
dawdle (HMS01110)
anachronism (JF02665)
CG17739 (JF03198)
unpaired 2 (HMS00901)
Akh (HMS00477)
Activin-beta (JF03276)
white (HMS17)
Insulin-like peptide 5 (HMS00548)
unpaired 3 (HM05061)
Insulin-like peptide 7 (HMS00649)
wingless (HMS00844)
dumpy (HMS01633)
unpaired 2 (HMS00948)
Hedgehog (JF01804)
Jhedup (JF01808)
Nplp1 (JF01911)
Diuretic hormone (JF01822)
Tollo (HM05005)
Drosulfakinin (JF01908)
Wnt6 (HM05236)
sNPF (JF01906)
CG13253 (JF03057)
ETH (JF02141)
IM10 (HM05004)
miple (JF02904)
Toll-7 (HM05230)
Corazonin (JF02023)
amnesiac (JF01814)
Insulin-like peptide 4 (JF01346)
Wnt5 (HM05020)
decapentaplegic (HMS00011)
Allatostatin C (JF01907)
branchless (HMS01046)
wingless (JF01480)
M-spondin (JF03397)
Mip (JF02145)
Insulin-like peptide 4 (HMS00547)
unpaired 3 (HMS00646)
Insulin-like peptide 6 (JF01348)
Insulin-like peptide 5 (JF01347)
Nplp3 (JF03188)
Insulin-like peptide 3 (HMS00546)
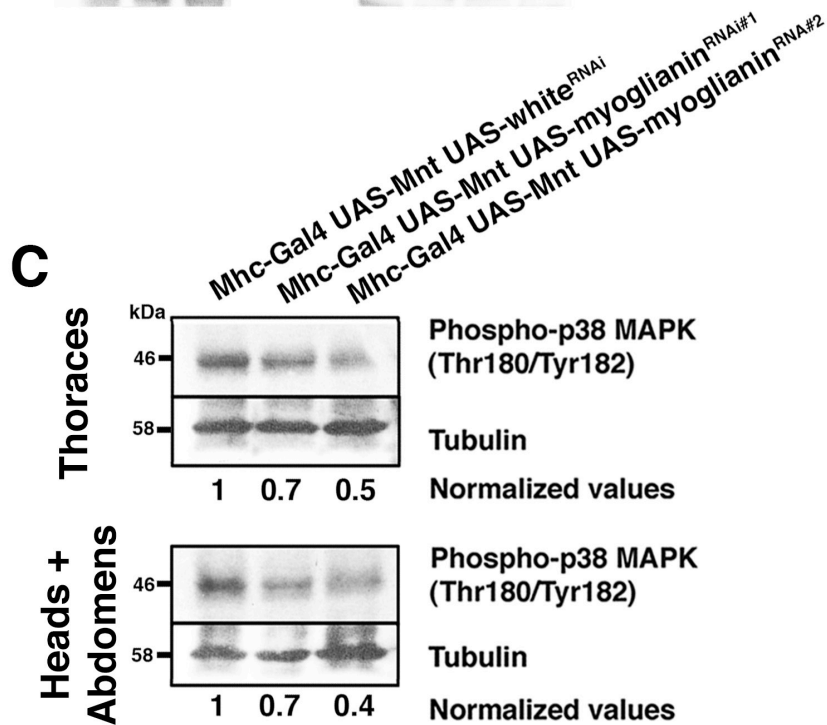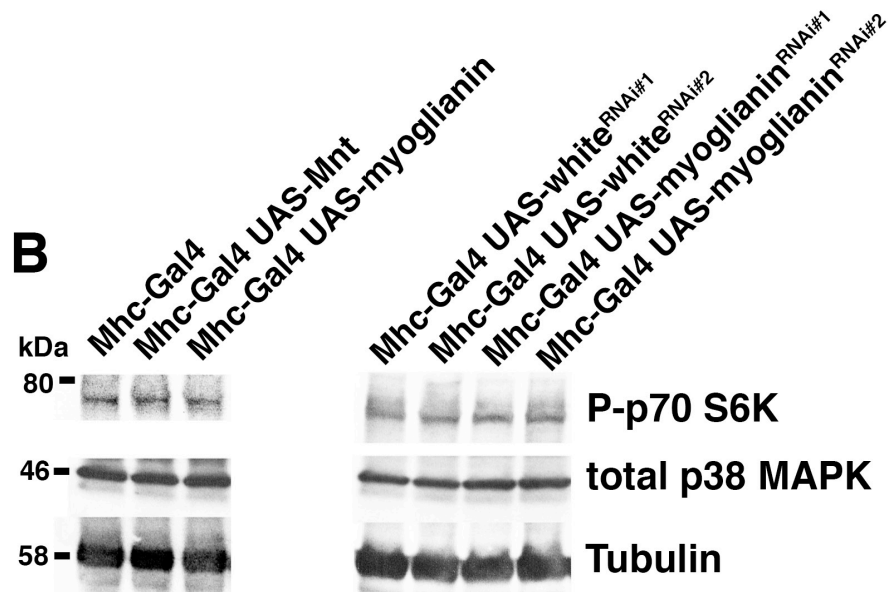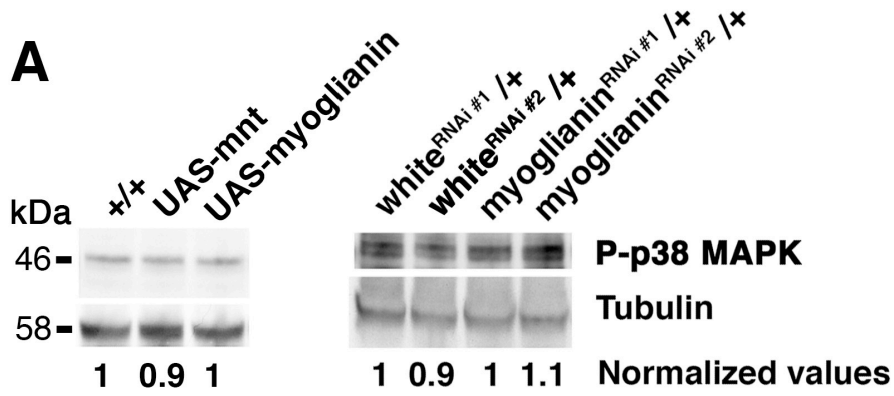Thrombospondin (HMS01138)

Day 56
Day 36

Figure S5. Statistical Analysis of the Myokine RNAi Screen (Ordered by Ascending Median Lifespan), Related to Figure 3. Left panel: Results of the RNAi screen (Figure 3) are ordered by ascending median lifespan with each box outlining the middle 50% of survival times for flies in each RNAi treatment, and whiskers outlining the middle 80% of survival times. RNAi treatments significantly increasing lifespan relative to controls (RNAi against the *white*; HMS17) are represented by red boxes, and treatments significantly decreasing lifespan are represented by green boxes (log-rank test; FDR-adjusted $P<0.05$ with at least a 5% change in median lifespan). The vertical yellow region outlines the middle 50% of survival times in the control treatment (HSM17). Two different RNAi treatments in skeletal muscles against the TGF-β ligand *myoglianin* significantly shortened the median lifespan as compared with RNAi against *white* (HMS17).

Right panel: Percentage of flies with climbing defects at days 36 (violet) and 56 (black). A green asterisk is used to denote cohorts for which the percentage of flies with climbing defects at day 56 is significantly higher than in controls at day 56 (chi-square test; FDR-adjusted $P<0.05$). *Myoglianin* RNAi significantly increases the percentage of flies with climbing defects in old age. None of the RNAi treatments resulted in climbing defects in young age (day 8; Figure 3).

A — % Survival vs Days (0–100)

B — % Flies not climbing vs Days (0–72)

- △ Mhc-Gal4 UAS-white$^{RNAi\#1}$
- ▲ Mhc-Gal4 UAS-myoglianin$^{RNAi\#1}$
- ▲ Mhc-Gal4 UAS-myoglianin$^{RNAi\#2}$
- ● Mhc-Gal4 UAS-Mnt UAS-white$^{RNAi\#1}$
- ● Mhc-Gal4 UAS-Mnt UAS-white$^{RNAi\#2}$
- ■ Mhc-Gal4 UAS-Mnt UAS-myoglianin$^{RNAi\#1}$
- ■ Mhc-Gal4 UAS-Mnt UAS-myoglianin$^{RNAi\#2}$

C — myoglianin mRNA levels (fold change)

Thoraces: Mhc-Gal4 UAS-Mnt UAS-white^{RNAi} (1.0); Mhc-Gal4 UAS-Mnt UAS-myoglianin$^{RNAi\#1}$ (~0.38, **); Mhc-Gal4 UAS-Mnt UAS-myoglianin$^{RNAi\#2}$ (~0.4, **)

Heads + Abdomens: Mhc-Gal4 UAS-Mnt UAS-white^{RNAi} (1.0); Mhc-Gal4 UAS-Mnt UAS-myoglianin$^{RNAi\#1}$ (~0.98); Mhc-Gal4 UAS-Mnt UAS-myoglianin$^{RNAi\#2}$ (~1.05)

Figure S6. Myoglianin RNAi Leads to Partial Suppression of the Lifespan Extension Induced by Mnt Overexpression in Muscle, Related to Figure 4.
(A-B) Overlay of the data shown in Figure 4C-F reporting lifespan and climbing assays. Myoglianin RNAi in Mnt overexpressing muscle is statistically different from Myoglianin RNAi alone, indicating that myoglianin is required only in part for the preservation of climbing ability and lifespan extension induced by Mnt (P<0.001, Log-rank tests). See the legend of Figure 4 for additional information on the statistical analysis of these data. (C) Myoglianin RNAi in muscle overexpressing Mnt results in lower myoglianin mRNA levels in thoraces, which consist mostly of muscle, but not in heads and abdomens, which are enriched in non-muscle tissues. SEM is indicated and n=4. **P<0.01, unpaired two-tailed Student's t-test.

A

kDa | +/+ | UAS-mnt | UAS-myoglianin | white^RNAi #1 /+ | white^RNAi #2 /+ | myoglianin^RNAi #1 /+ | myoglianin^RNAi #2 /+

46 — P-p38 MAPK

58 — Tubulin

1   0.9   1   1   0.9   1   1.1   Normalized values

B

Mhc-Gal4   Mhc-Gal4 UAS-Mnt   Mhc-Gal4 UAS-myoglianin   Mhc-Gal4 UAS-white^RNAi#1   Mhc-Gal4 UAS-white^RNAi#2   Mhc-Gal4 UAS-myoglianin^RNAi#1   Mhc-Gal4 UAS-myoglianin^RNAi#2

kDa

80 —   P-p70 S6K

46 —   total p38 MAPK

58 —   Tubulin

C

Mhc-Gal4 UAS-Mnt UAS-white^RNAi   Mhc-Gal4 UAS-Mnt UAS-myoglianin^RNAi#1   Mhc-Gal4 UAS-Mnt UAS-myoglianin^RNA#2

Thoraces

kDa

46 — Phospho-p38 MAPK (Thr180/Tyr182)

58 — Tubulin

1   0.7   0.5   Normalized values

Heads + Abdomens

46 — Phospho-p38 MAPK (Thr180/Tyr182)

58 — Tubulin

1   0.7   0.4   Normalized values

Figure S7. Additional Analysis of P-p38 MAPK, total p38 MAPK, and P-p70 S6K levels in Response to Mnt/Myoglianin Signaling, Related to Figure 7.

(A) Absence of changes in P-p38 MAPK levels in UAS-transgene alone controls. Western blot analysis of phospho-p38 (P-p38) MAPK levels in thoraces from heterozygous flies carrying UAS- transgenes but no Mhc-Gal4. Normalization of P-p38 MAPK levels with α-tubulin levels (loading control) indicates the lack of changes in P-p38 MAPK levels in the absence of Gal4-driven transgene expression.

(B) Western blot analysis of phospho-p70 S6K and total p38 MAPK levels in response to *Mnt* and *myoglianin* overexpression and RNAi in muscle. No substantial changes were detected, indicating that Mnt/Myoglianin signaling regulates phosphorylation of p38 MAPK (Figure 7) but not total p38 MAPK protein levels and phospho-p70 S6K levels.

(C) Myoglianin RNAi in muscle overexpressing Mnt results in lower P-p38 MAPK levels in thoraces and heads and abdomens when compared to white RNAi (control). See also Figure 7.

EXTENDED EXPERIMENTAL PROCEDURES

Fly Stocks

The following fly stocks were used: *ppl-Gal4* (Colombani et al., 2003); *Mhc-Gal4* (Schuster et al., 1996); *dMef2-Gal4* (Ranganayakulu et al., 1996), *UAS-Mnt* and *Mnt$^{1/1}$* (Loo et al., 2005); *UAS-white^{RNAi} #1* and *#2* (TRiP ID: HMS0017 and JF01786), *UAS-myoglianin^{RNAi} #1* and *#2* (Awasaki et al., 2011), *UAS-RpS3^{RNAi}* (JF01410), *UAS-RpLP0^{RNAi}* (JF01335), *UAS-Nopp140^{RNAi}* (HMS00564), *UAS-eIF-4a^{RNAi}* (HMS00927), *UAS-Brf^{RNAi}* (JF02482), *UAS-Ssrp^{RNAi}* (JF02120), *UAS-CG5033^{RNAi}* (HMS01433), *UAS-p38MAPK WT* (wild-type) and *UAS-p38MAPK KD^3* (kinase dead; (Vrailas-Mortimer et al., 2011)). Additional transgenic RNAi stocks used in the screen were from the DRSC/TRiP (http://www.flyrnai.org/TRiP-HOME.html; (Ni et al., 2009; Ni et al., 2011)) and are indicated in Figures S5.

Construction of *UAS-myoglianin*

To generate *UAS-myoglianin*, the *Drosophila melanogaster myoglianin (CG1838)* coding sequence, corresponding to nucleotides 844-2640 of sequence AY061367, was amplified by PCR using the full-length cDNA clone LD29161 as template with primers 5'-gcc<u>agatct</u>atgctggccaactcatgctgcggttgg-3' and 5'-gccc<u>tcgag</u>ctaagaacag ctgcaaccctcgacagac-3' (underlined are *Bgl*II and *Xho*I restriction sites, respectively). The PCR product was cut with *Bgl*II and *Xho*I for cloning into the pUAST vector, followed by sequencing and injection into *y^{1}w^{1118}* embryos.

Capillary Feeding (CAFÉ) Assays

For capillary feeding (CAFÉ) assays (Ja et al., 2007), groups of 10 flies were transferred twelve hours before the assay from standard food to 1.5% agar vials and fed a 5% sucrose solution in PBS provided via a 5 μl calibrated glass micropipette (VWR, #53432-706). At the start of the assay, the old micropipette was replaced with a new one and the amount of liquid food consumed was recorded every 2 hours and corrected on the basis of the evaporation observed in a vial without flies.

Muscle and Body Weight Measurements

For measurement of body weight, 3 groups of 10 flies were weighted on a precision balance and the average body weight calculated. For measurement of thoracic weight (indicative of muscle mass), 3 groups of 10 thoraces were weighted and the average weight calculated.

Immunostaining, Laser Scanning Confocal Microscopy, and Image Analysis

Dissected tissues were fixed for 20 minutes in PBS with 4% paraformaldehyde and 0.1% Triton X-100, washed, and incubated overnight with 1:100 dilution of anti-Fibrillarin (Nop1p; Encore Biotechnology #MCA-38F3) or mouse anti-Mnt (P5D6) (Loo et al., 2005). The following day, samples were washed and

incubated overnight with DAPI (Invitrogen, 1μg/mL), Alexa555-conjugated secondary antibodies (Molecular Probes, 1:200), and Alexa635-conjugated Phalloidin (Molecular Probes, 1:100). Samples were imaged with a Leica SP2 confocal microscope. Image analysis was done with the "Analyze particle" function of ImageJ.

Luciferase Assays

Luciferase assays were done as before (Demontis and Perrimon, 2010). 15×10^4 S2R+ cells/cm^2 were seeded in Schneider's medium (Gibco) containing 10% FCS and transfected one day later using the Effectene Transfection Kit (Qiagen). The following day the medium was changed and, after an additional 3 days in culture, the luciferase assay was performed with the Dual-Glo Luciferase Assay (Promega) according to manufacturer's instructions.

The *CG4364-* and *CG5033-firefly* luciferase reporters were previously described (Hulf et al., 2005). To generate the *myoglianin-Renilla* luciferase reporter, the proximal promoter region (>4:718244,716674) of the *myoglianin* gene (*CG1838*) was amplified by PCR and cloned into the pRL-null vector (Promega) with *Xho*I and *EcoR*I. *UAS-Mnt* and *UAS-YFP* plasmids for overexpression of either *Mnt* (*CG13316*) or *yellow fluorescent protein* (*YFP*) were provided by the DRSC at Harvard Medical School.

In experiments with the *myoglianin-Renilla* luciferase reporter, an *actin-firefly* luciferase reporter was co-transfected as normalization control and luciferase activity refers to ratio of *Renilla* to firefly luciferase activity. In experiments with the *CG4364- and CG5033-firefly* luciferase reporters (Hulf et al., 2005), an *actin-Renilla* luciferase reporter was co-transfected as normalization control and luciferase activity refers to ratio of firefly to *Renilla* luciferase activity. S2R+ cells were also co-transfected with either *UAS-YFP* (control) or *UAS-Mnt* as indicated.

Western Blots

Samples were prepared by homogenizing in a bullet blender (NextAdvance) either fly thoraces (consisting mainly of muscles) or heads and abdomens (consisting mainly of non-muscle tissues) from at least 15 male flies per group in RIPA buffer with protease and phosphatase inhibitors. SDS-PAGE was done as previously (Demontis and Perrimon, 2010). Primary antibodies used are anti-P-p38 MAPK (Cell Signaling Technologies #9215), anti-*Drosophila* P-p70 S6K (Cell Signaling Technologies #9209), anti-total p38 MAPK (Cell Signaling Technologies #9212), and anti-α-Tubulin (Cell Signaling Technologies #2125). For densitometry of Western blots, band intensity was quantified with the Histogram function of Photoshop.

Bioinformatic Analysis

Multiple sequence alignment and phylogenetic analysis were done with ClustalW, available at http://www.ebi.ac.uk/Tools/msa/clustalw2/, and orthology prediction with DIOPT, available at http://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl.

Statistical Analysis

Statistical analysis was performed with Excel and the GraphPad Prism software. Unpaired two-tailed Student's *t*-test was used to compare means of two independent groups to each other, while two-way ANOVA followed by Tukey's post test was used to compare the means of more than two independent groups of normally distributed data. Log-rank tests were employed for the statistical analysis of lifespan data and negative geotaxis assays.

Statistical Analysis of the RNAi Screen

Differences in median lifespan were analyzed using the log-rank test as implemented in the "survdiff" function included within the R package "survival" (Therneau, 2012). Survival times for each RNAi treatment were compared with the control (white[RNAi]; HMS00017). To control the false discovery rate, *P*-values generated from each log-rank test were adjusted by using the Benjamini-Hochberg method (Benjamini Y, 1995). To qualify as a statistically significant difference compared with white[RNAi], an FDR-adjusted *P*-value of less than 0.05 (log-rank test) was required in addition to at least a 5% difference in median lifespan. Similarly, differences in the percentage of flies with climbing defects at day 56 were analyzed using a chi-square test, as implemented in the function "prop.test" included within the R statistical software, with *P*-values from each test adjusted by using the Benjamini-Hochberg method. For a statistically significant difference in climbing ability compared with white[RNAi], an FDR-adjusted *P*-value of less than 0.05 (chi-square test) was required.

Statistical Analysis of Microarrays

Genome-wide expression levels were evaluated using the Affymetrix *Drosophila* Genome 2.0 array platform (18,952 probe sets representing transcripts associated with 12,440 unique fly genes). Array hybridizations were performed following standard Affymetrix protocols, yielding CEL files in standard format containing probe-level intensity estimates. Quality control metrics were calculated by using CEL files generated for each array, including average background, scale factor, percent present, RNA degradation score, RLE median, RLE IQR, NUSE median and NUSE IQR (Bolstad BM, 2005). There was no indication of problematic array hybridizations based upon these quality-control metrics. Normalized expression scores were calculated for each array by using robust multichip average (RMA) (Bolstad BM, 2003). Presence and absence calls were calculated by using the Wilcoxon rank sum test, as implemented in the MAS 5.0 algorithm (Liu WM, 2002). Of 18,952 probe sets, 6,353 were identified for which signal intensities were not significantly above background with respect to any of the arrays. These 6,353 probe sets were excluded, leaving a total of 12,599 probe sets for further analyses. Differential expression between treatments was analyzed by using linear models with variance estimates moderated using empirical Bayes methods (Smyth, 2005). Raw *P*-values were generated based upon moderated T statistics generated for each gene and each treatment comparison (Smyth, 2005). To assess significance given the total number of hypotheses evaluated, raw *P*-values were adjusted to control the false discovery

using the Benjamini-Hochberg method (Benjamini Y, 1995). Probe set annotation for the Affymetrix *Drosophila* Genome 2.0 array was obtained from the R Bioconductor package "drosophila2.db" (Carlson M, 2012a). Additional annotation for the *Drosophila* genome was obtained using the package "org.Dm.eg.db" (Carlson M, 2012b).

Overrepresentation of gene ontology (GO) terms among differentially expressed genes was evaluated using a conditional version of the Fisher's exact test (Bolstad BM, 2005; Falcon S, 2007). In this approach, the significance of a GO term is evaluated based on a filtered set of associated genes, which excludes any genes associated with more specific GO terms already known to be overrepresented. This approach serves to reduce redundancy among GO terms identified as significantly overrepresented with respect to a set of differentially expressed genes (Bolstad BM, 2005; Falcon S, 2007). In these analyses, enrichment of GO terms among differentially expressed genes was evaluated relative to a reference set of fly genes, which was filtered to include only those genes associated with the 12,599 probe sets expressed above background levels.

SUPPLEMENTAL REFERENCES

Awasaki, T., Huang, Y., O'Connor, M.B., and Lee, T. (2011). Glia instruct developmental neuronal remodeling through TGF-beta signaling. Nat Neurosci *14*, 821-823.

Benjamini Y, H.Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series, 289-300.

Bolstad BM, C.F., Brettschneider J, Simpson K, Cope L, et al. (2005). Quality assessment of Affymetrix GeneChip Data (New York: Springer).

Bolstad BM, I.R., Astrand M, Speed TP. (2003). A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics, 185-193.

Carlson M, F.S., Pages H, Li N. (2012a). drosophila2.db: Affymetrix Drosophila Genome 2.0 Array annotation data (chip drosophila2).

Carlson M, F.S., Pages H, Li N. (2012b). org.Dm.eg.db: Genome wide annotation for Fly.

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

Demontis, F., and Perrimon, N. (2010). FOXO/4E-BP signaling in Drosophila muscles regulates organism-wide proteostasis during aging. Cell *143*, 813-825.

Falcon S, G.R. (2007). Using GOstats to test gene lists for GO term association. Bioinformatics, 257-258.

Hulf, T., Bellosta, P., Furrer, M., Steiger, D., Svensson, D., Barbour, A., and Gallant, P. (2005). Whole-genome analysis reveals a strong positional bias of conserved dMyc-dependent E-boxes. Mol Cell Biol *25*, 3401-3410.

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.

Liu WM, M.R., Di X, Ryder TB, Hubbell E, Dee S, Webster TA, Harrington CA, Ho MH, Baid J, Smeekens SP (2002). Analysis of high density expression microarrays with signed-rank call algorithms. Bioinformatics *18(12)*, 1593-1599.

Loo, L.W., Secombe, J., Little, J.T., Carlos, L.S., Yost, C., Cheng, P.F., Flynn, E.M., Edgar, B.A., and Eisenman, R.N. (2005). The transcriptional repressor dMnt is a regulator of growth in Drosophila melanogaster. Mol Cell Biol *25*, 7078-7091.

Ni, J.Q., Liu, L.P., Binari, R., Hardy, R., Shim, H.S., Cavallaro, A., Booker, M., Pfeiffer, B.D., Markstein, M., Wang, H*., et al.* (2009). A Drosophila Resource of Transgenic RNAi Lines for Neurogenetics. Genetics *182*, 1089-1100.

Ni, J.Q., Zhou, R., Czech, B., Liu, L.P., Holderbaum, L., Yang-Zhou, D., Shim, H.S., Tao, R., Handler, D., Karpowicz, P*., et al.* (2011). A genome-scale shRNA resource for transgenic RNAi in Drosophila. Nat Methods *8*, 405-407.

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.

Schuster, C.M., Davis, G.W., Fetter, R.D., and Goodman, C.S. (1996). Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin II controls synaptic stabilization and growth. Neuron *17*, 641-654.

Smyth, G. (2005). Limma: linear models for microarray data. (New York: Springer).

Therneau, T. (2012). A Package for Survival Analysis in S.

Vrailas-Mortimer, A., del Rivero, T., Mukherjee, S., Nag, S., Gaitanidis, A., Kadas, D., Consoulas, C., Duttaroy, A., and Sanyal, S. (2011). A muscle-specific

p38 MAPK/Mef2/MnSOD pathway regulates stress, motor function, and life span in Drosophila. Dev Cell *21*, 783-795.