Supplemental Information.

AMPK modulates tissue and organismal aging in a cell-non-autonomous manner

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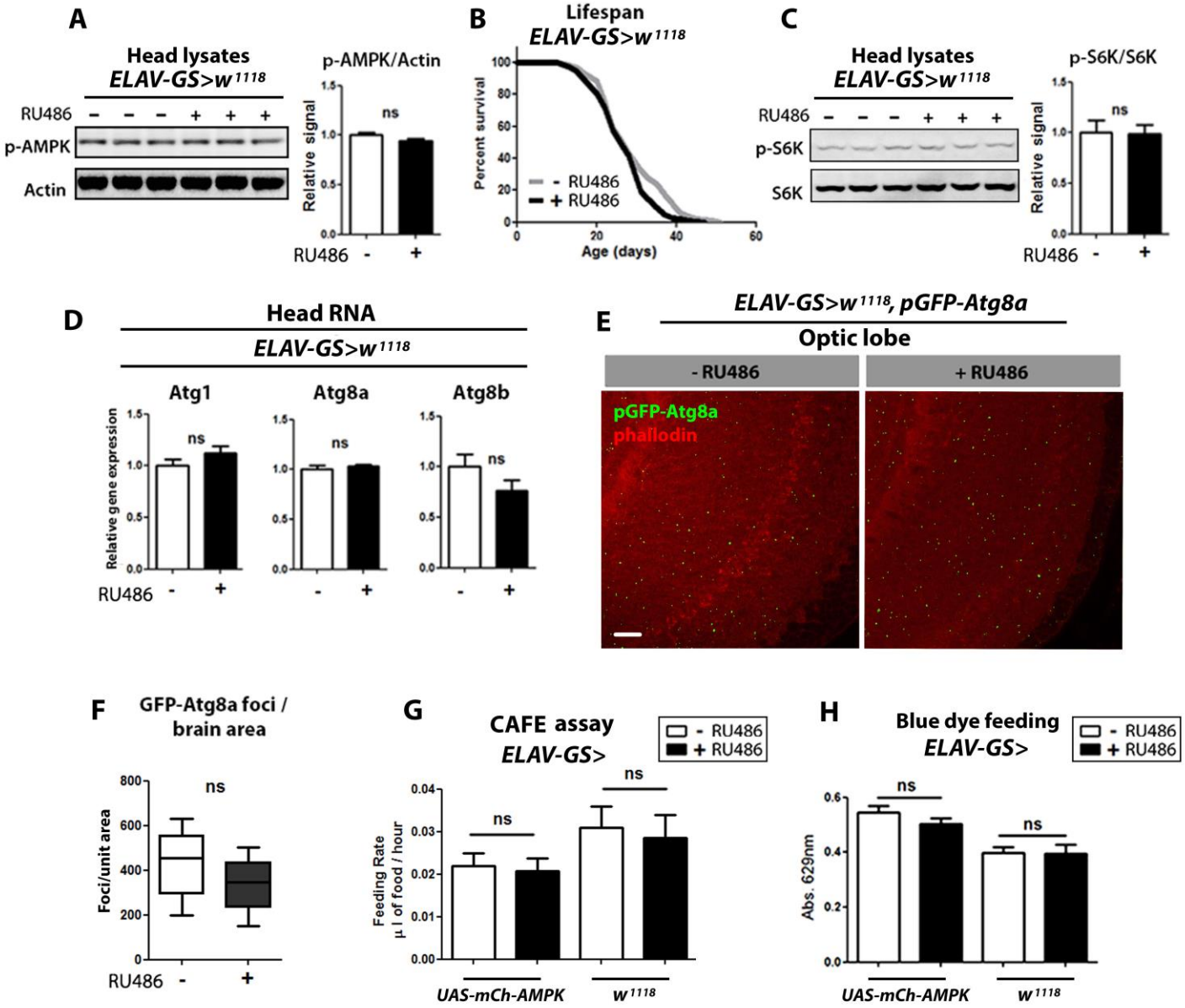

Figure S1. Neuronal AMPK induction does not alter feeding behavior, fecundity, spontaneous activity, extends lifespan under hyperoxia and heat stress, and effects of RU486 feeding on control flies, associated with main text Figure 1.

(A) Western blot analysis of AMPK phosphorylated on T184 (p-AMPK) and loading control (actin) from head lysates of 10 day old *ELAV-GS>W^{1118}* female flies. Densitometry quantification (right) showed no significant increase in phosphorylation of AMPK upon RU486 feeding (*p>0.05*; *t-test; n=3 replicates; 10 heads/replicate*).

(B) Survival curves of *ELAV-GS>W^{1118}* flies with or without RU486 feeding. There was no significant effect on the lifespan of control flies fed RU486 (*p>0.05*; *log-rank test; n>171 flies*).

(C) Western blot analysis of S6K phosphorylated at T398 and total S6K from head lysates from *ELAV-GS>W^{1118}* female flies at 10 days of adulthood. Densitometry (right) showed no significant change in S6K phosphorylation upon RU486 feeding (*p>0.05*; *t-test; n=3 replicates; 10 heads/replicate*).

(D) Expression of autophagy genes in head tissue of *ELAV-GS>W^{1118}* female control flies at 10 days of adulthood. RU486 feeding had no significant effect on Atg1, Atg8a, and Atg8b RNA levels (*p>0.05*; *t-test; n=3 of RNA extracted from 10 heads/replicate*).

(E) Brain GFP-atg8a staining. Representative images from optic lobes of 10 day old female *ELAV-GS>W^{1118}*, *pGFP-Atg8a* flies (*red channel-phalloidin, green channel-GFP-Atg8a, scale bar represents 10μm*).

(F) Quantification of brain GFP-Atg8a foci in *ELAV-GS>W^{1118}*, *pGFP-Atg8a* control flies. RU486 feeding had no influence on the number of GFP-Atg8a foci (*p>0.05*; *t-test; n>10 confocal stacks from brains/condition, one brain per replicate stack*).

(G) Capillary feeding assay (CAFE) of 10 day old *ELAV-GS>UAS-mCh-AMPK*, and control *ELAV-GS>W^{1118}* flies. RU486 treatment had no effect on feeding rate of flies *(p>0.05*; *t-test, n>7 vials of 10 flies/condition*).

(H) Blue dye feeding assay of 10 day old *ELAV-GS>UAS-mCh-AMPK,* and *ELAV-GS>W^{1118}* control flies. Induction of AMPK in neurons had no effect on the amount of blue food consumed by *ELAV-GS>UAS-mCh-AMPK*, and RU486 treatment had no effect on the feeding control flies (*p>0.05*; *t-test; n>37 flies/condition*).

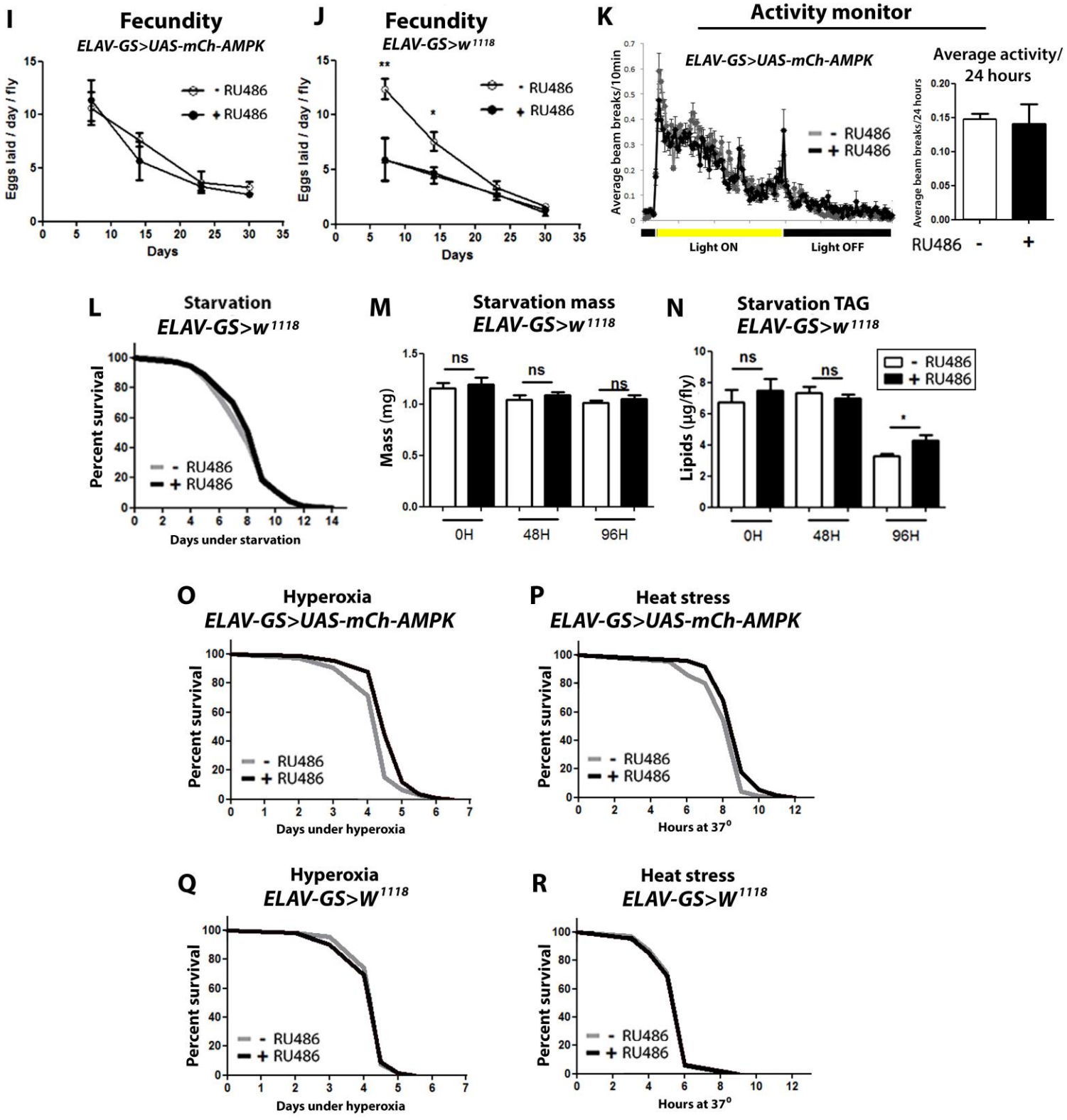

Figure S1 continued.

(I) Fecundity timecourse of *ELAV-GS>UAS-mCh-AMPK* flies. Induction of AMPK in the nervous system had no effect on the number of eggs laid over lifespan of the organisms (*p>0.05*; *t-test; n>7 vials of 10 flies/condition*).

(J) Fecundity timecourse of control *ELAV-GS>W^{1118}* flies. RU486 feeding significantly reduced the number of eggs laid at early timepoints (*p<0.05*; *t-test; n>7 vials of 10 flies/condition*).

(K) Spontaneous activity of 10 day old female *ELAV-GS>UAS-mCh-AMPK* flies measured by DAM system beam breaks. Neuronal upregulation of AMPK had no effect on spontaneous activity measured over a 24 hour period (*p>0.05*; *t-test; n>3 vials of 10 flies/condition*).

(L) Starvation survival curves of *ELAV-GS>W^{1118}* controls. Female control flies fed RU486 for 10 days had no significant effect on starvation lifespan (*p>0.05*; *log-rank; n>203 flies*).

(M) Body mass during starvation of *ELAV-GS>W^{1118}* controls. RU486 feeding had no significant influence over mass of control flies (*p>0.05*; *t-test; n>6 samples/condition from 10 flies weighed/sample*)

(N) Whole body lipid stores during starvation of *ELAV-GS>W^{1118}* controls. RU486 feeding of control flies moderately increased the amount of lipids at 96 hours of starvation compared to ethanol fed flies (*p<0.05; t-test; n>3 samples /condition; lipids extracted from 5 flies/sample*).

(O) Hyperoxia survival curves of 10 day old female *ELAV-GS>UAS-mCh-AMPK* flies. Neuronal upregulation of AMPK moderately increased survival under 80% atmospheric oxygen (*p<0.0001; log-rank; n>173 flies/condition*).

(P) Heat stress survival curves of 10 day old female *ELAV-GS>UAS-mCh-AMPK* flies. Neuronal upregulation of AMPK moderately increased survival at 37° C. (*p=0.0001; log-rank; n>136 flies/condition*).

(Q) Hyperoxia survival curves of 10 day old female *ELAV-GS>W^{1118}* controls. RU486 feeding had no effect on survival under 80% atmospheric oxygen (*p>0.05; log-rank; n>130 flies/condition*).

(R) Heat stress survival curves of 10 day old female *ELAV-GS>W^{1118}* controls. RU486 feeding had no effect on survival at 37° C. (*p>0.05; log-rank; n>133 flies/condition*).

Data are represented as mean ± SEM (*ns = p>0.05, * = p<0.05, ** = p<0.01, *** = p<.001).* RU486 was provided in the media after eclosion at a concentration of 50μg/ml (A, B) and 25μg/ml for all other figures.

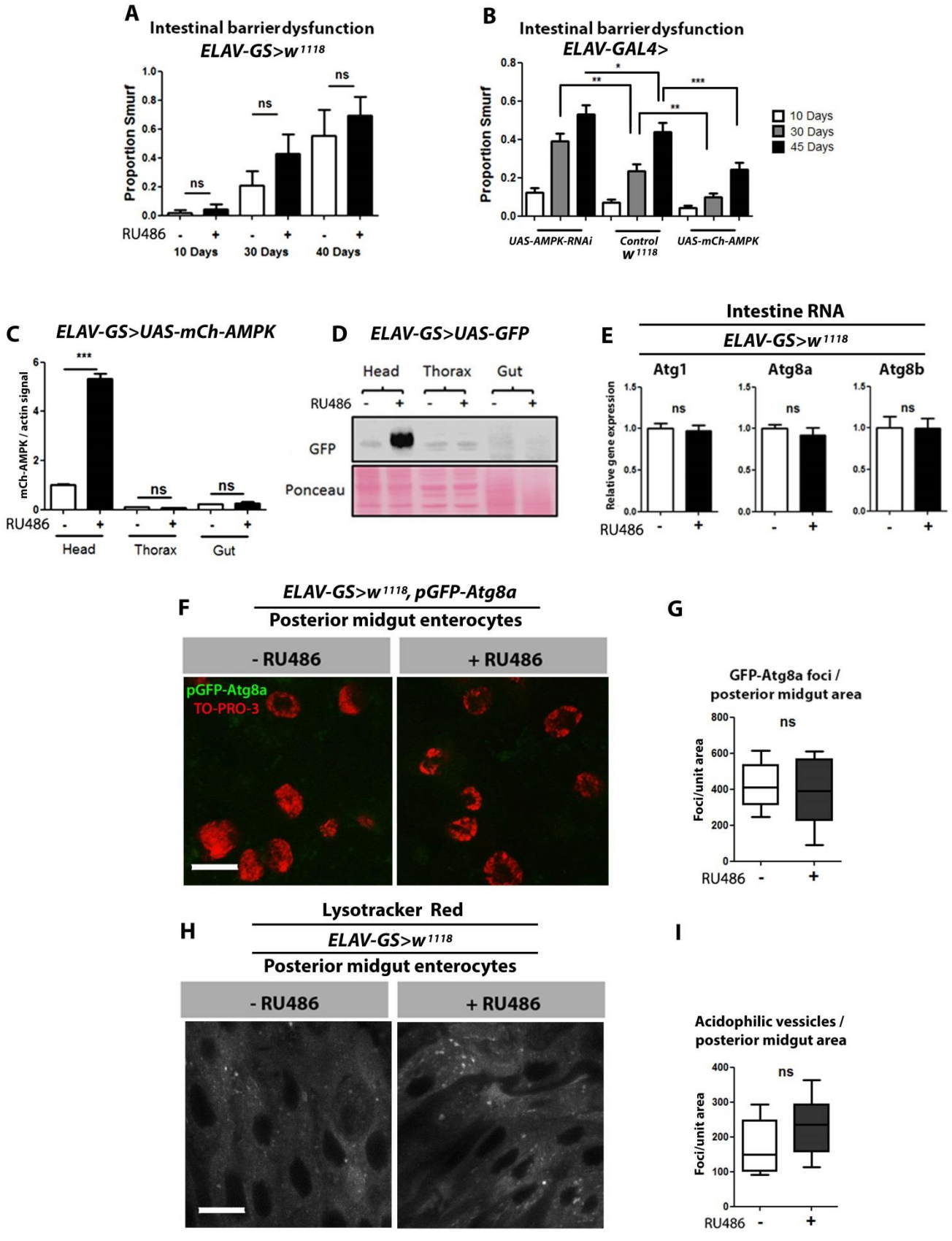

Figure S2. Constitutive neuronal expression of AMPK maintains intestinal integrity during aging, *ELAV-GS* tissue specificity, and effects of RU486 on control flies, related to Figure 2.

(A) Intestinal integrity during aging in *ELAV-GS>W^{1118}* controls. RU486 has no effect on the proportion of flies exhibiting intestinal barrier dysfunction during aging. (*p>0.05*; *binomial test; n>40 flies/condition*)

(B) Intestinal integrity during aging with constitutive neuronal expression of *AMPK-RNAi, mCh-AMPK,* and *W^{1118}* controls. *ELAV-GAL4>AMPK-RNAi* had significantly increased numbers of 'Smurf flies' at 30 and 45 days adulthood compared to *ELAV-GAL4> W^{1118}* controls. Flies constitutively overexpressing mCh-AMPK showed reduced numbers of 'Smurfs' compared to *ELAV-GAL4> W^{1118}* controls (*binomial test; n>96 flies/condition*).

(C) Exogenous mCherry-tagged AMPK expression levels of RNA extracted from body parts of *ELAV-GS>UAS-mCh-AMPK* female flies at 10 days of age. Statistically significant increase in exogenous mCherry-AMPK is only detectable in the head tissue of RU486 fed flies compared to uninduced controls. (*p<0.001; t-test; n=3 of RNA extracted from 10 body parts/replicate*)

(D) Western blot analysis for GFP and membrane stain for loading (Poncea S.) of protein extracted from body parts of *ELAV-GS>UAS-eGFP* flies at 10 days of age. An RU486-dependent increase of GFP-signal was only observed in the head tissue flies.

(E) Expression of autophagy genes in the intestines of female *ELAV-GS>W^{1118}* control flies at 10 days of adulthood. RU486 feeding had no significant effect on Atg1, Atg8a, and Atg8b RNA levels (*p>0.05*; *t-test; n=3 of RNA extracted from 15 intestines/replicate*)

(F) GFP-Atg8a staining. Representative images of enterocytes from the posterior midgut of 10 day old female *ELAV-GS>W^{1118}, pGFP-Atg8a* flies fed RU486 and vehicle (*red channel-TO-PRO-3 DNA stain, green channel-GFP-Atg8a, scale bar represents 10μm*).

(G) Quantification of posterior midgut Atg8a foci. Control flies fed RU486 showed no difference in foci number (*p>0.05*; *t-test; n>10 confocal stacks from posterior midgut/condition, one fly per replicate stack*).

(H) Lysotracker Red staining. Representative images of posterior midgut enterocytes from 10 day old female *ELAV-GS>W^{1118}* flies stained with the acidophilic dye.

(I) Quantification of acidophilic vesicles. Feeding control flies RU486 had no effect on the number of acidophilic vesicles (*t-test*; *n>9 confocal stacks from posterior midgut/condition, one fly per replicate stack*).

Data are represented as mean ± SEM (*ns = p>0.05, * = p<0.05, ** = p<0.01, *** = p<.001).* RU486 was provided in the media after eclosion at a concentration of 50μg/ml (H, I) and 25μg/ml for all other figures.

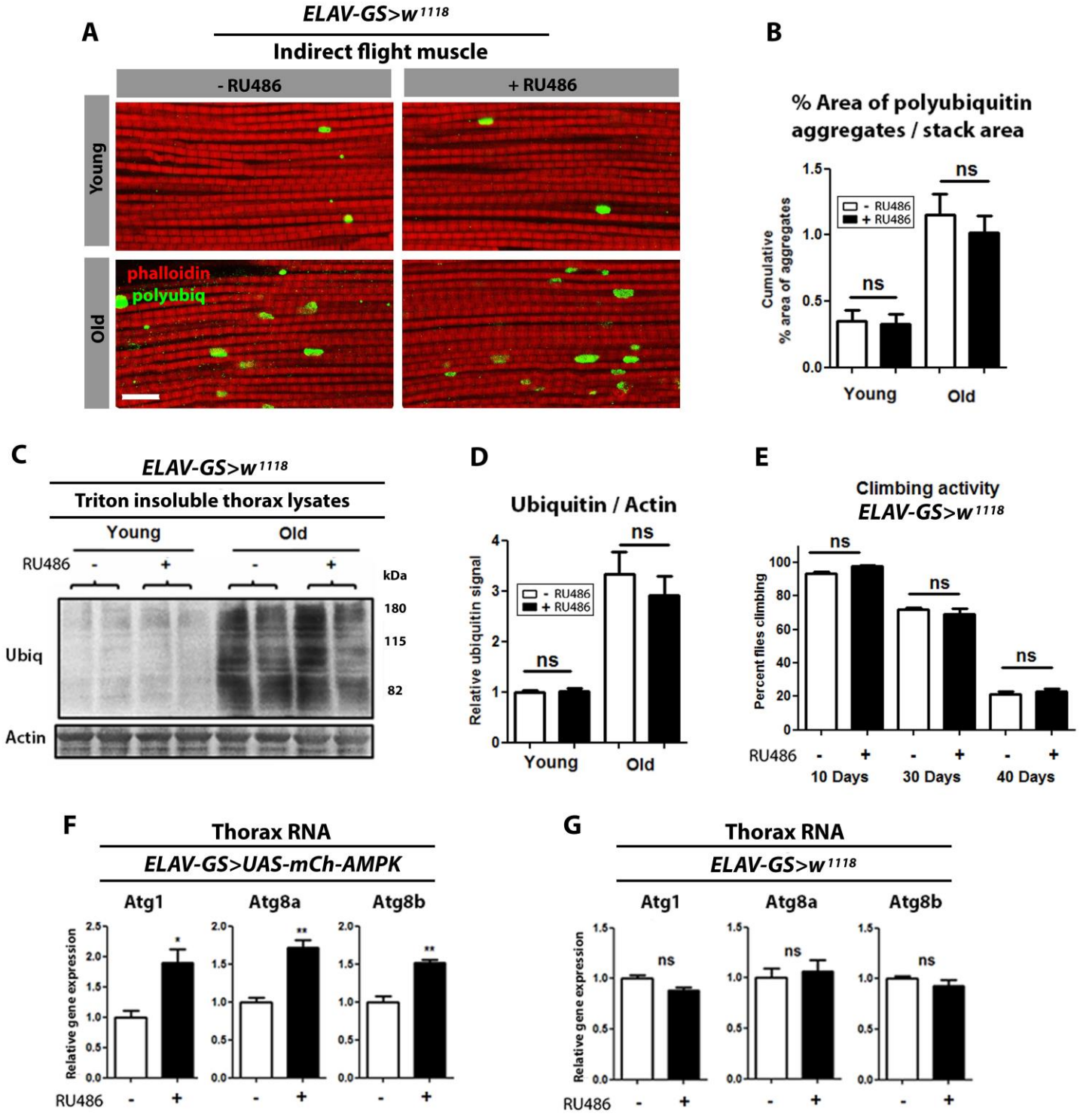

Figure S3. Neuronal expression of AMPK increases autophagy gene expression in the thorax, and effects of RU486 on control flies, related to Figure 3.

(A) Confocal imaging of adult female *ELAV-GS>W*^{1118} flight muscle showing protein polyubiquitinated aggregates at young (10 days), and old (30 days) timepoints (*red channel-phalloidin/F-actin, green channel- anti-polyubiquitin, scale bar represents 10μm*).

(B) Quantification of polyubiquitin aggregates in the muscle of *ELAV-GS>W^{1118}* flies. Feeding control flies RU486 had no effect on protein aggregate accumulation with age (*p>0.05*; *t-test, n>10, one fly / replicate stack*).

(C) Western blot detection of total ubiquitin-conjugated proteins from thorax detergent-insoluble extracts of young (10 days) and aged (30 days) *ELAV-GS>W^{1118}* female flies.

(D) Densitometry of ubiquitin blots from thoraces of flies. RU486 feeding had no effect on amount of thoracic detergent-insoluble ubiquitin-conjugated proteins, normalized to actin, in aged flies (*p>0.05*; *t-test; n=4 samples/condition; 10 thoraces/sample).*

(E) Climbing activity of *ELAV-GS>W^{1118}* controls. Female control flies fed RU486 for 10 days had no significant effect on climbing activity with age (*p>0.05*; *t-test; n=6 vials/condition; 30 flies/vial).*

(F) Expression of autophagy genes in dissected thoraces of female *ELAV-GS>UAS-mCh-AMPK* flies at 10 days of adulthood. Upon AMPK induction in the nervous system significantly increased Atg1, Atg8a, and Atg8b gene expression levels are observed (*t-test; n>3 of RNA extracted from 10 thoraces/replicate*).

(G) Expression of autophagy genes in dissected thoraces of female *ELAV-GS>W^{1118}* control flies at 10 days of adulthood. RU486 feeding showed no significant changes in Atg1, Atg8a, and Atg8b gene expression levels (*p>0.05*; *t-test; n=3 of RNA extracted from 10 thoraces /replicate*)

Data are represented as mean ± SEM (*ns = p>0.05, * = p<0.05, ** = p<0.01, *** = p<.001).* RU486 was provided in the media after eclosion at a concentration of 25μg/ml.

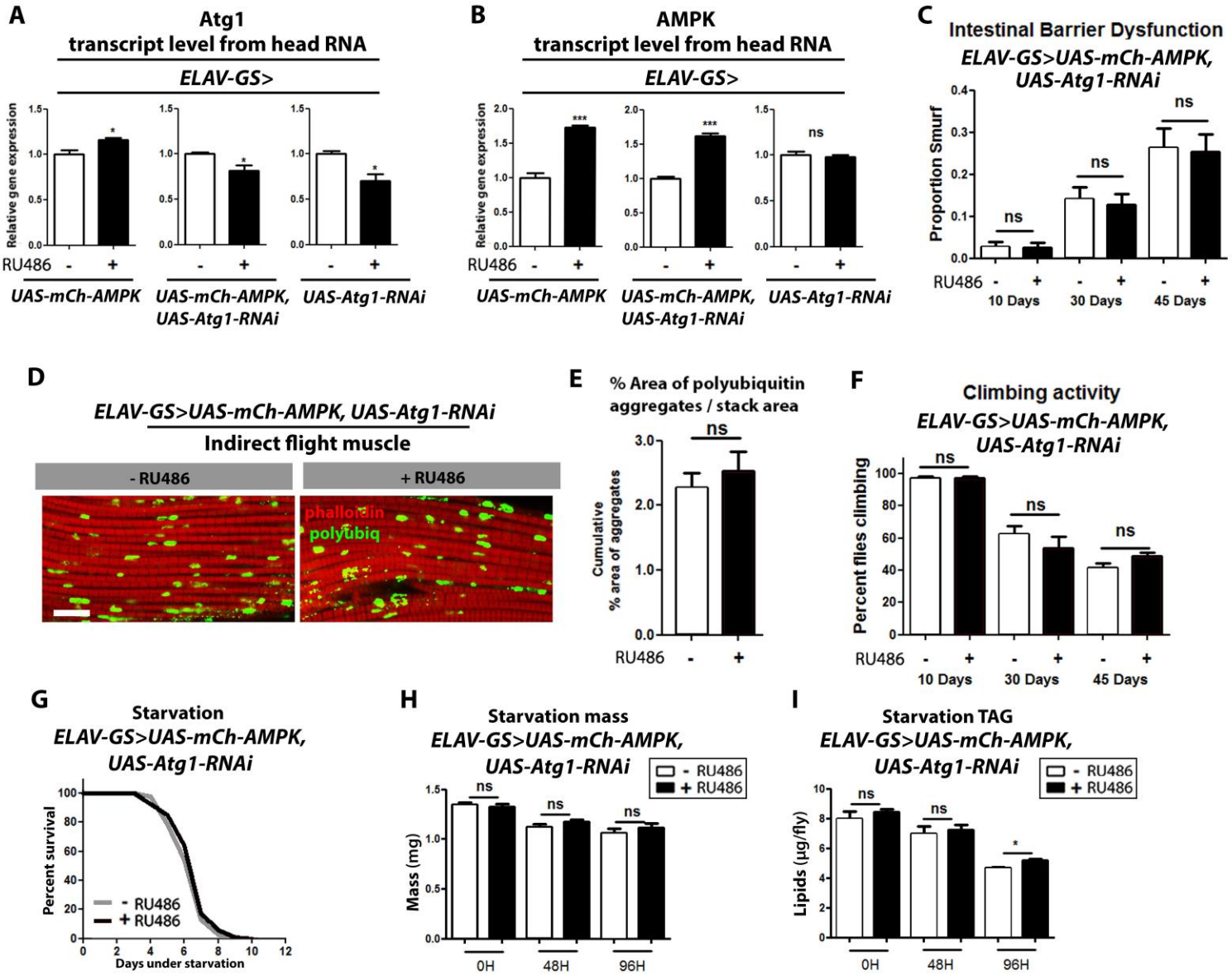

Figure S4. Atg1 mediates the systemic effects of neuronal AMPK upregulation, associated with main text Figure 4.

(A) Expression level of Atg1 in dissected heads of indicated genotypes of female flies at 10 days of adulthood. AMPK induction in the nervous system significantly increased Atg1 transcript levels. Simultaneous overexpression of AMPK, and knockdown of Atg1 significantly reduced Atg1 transcript levels upon RU486 induction. Atg1 knockdown alone significantly decreased Atg1 transcript in heads of female flies. (*t-test; n>3 of RNA extracted from 10 heads /replicate*).

(B) Expression level of AMPK in dissected heads of indicated genotypes of female flies at 10 days of adulthood. AMPK induction in the nervous system significantly increased AMPK transcript levels. Flies with simultaneous overexpression of AMPK, and knockdown of Atg1significantly maintain increased AMPK transcript levels upon RU 486 induction. Atg1 knockdown alone does not influence AMPK transcript levels in heads of female flies (*t-test; n>3 of RNA extracted from 10 heads /replicate*).

(C) Intestinal integrity during aging of *ELAV-GS>UAS-mCh-AMPK, UAS-Atg1-RNAi*. Simultaneous upregulation of AMPK, and reduction in Atg1 expression has no effect on the proportion of flies exhibiting intestinal barrier dysfunction (*p>0.05*; *binomial test; n>100 flies/condition*).

(D) Confocal imaging of adult female *ELAV-GS>UAS-mCh-AMPK, UAS-Atg1-RNAi* flight muscle showing protein polyubiquitinated aggregates at old (30 days) timepoint with or without RU486 feeding (*red channel-phalloidin/F-actin, green channel- anti-polyubiquitin, scale bar represents 10μm*).

(E) Quantification of polyubiquitin aggregates in the muscle of *ELAV-GS>UAS-mCh-AMPK, UAS-Atg1-RNAi* flies. Simultaneous upregulation of AMPK, and reduction in Atg1 expression has no effect on protein aggregate accumulation with age (*p>0.05*; *t-test, n>10, one fly / replicate stack*).

(F) Climbing activity of *ELAV-GS>UAS-mCh-AMPK, UAS-Atg1-RNAi* flies with age. Simultaneous upregulation of AMPK, and reduction in Atg1 expression had no effect on climbing ability with age (*p>0.05*; *t-test, n=6 vials, 30 flies / vial*).

(G) Starvation survival curves of *ELAV-GS>UAS-mCh-AMPK, UAS-Atg1-RNAi* female flies. Simultaneous upregulation of AMPK, and reduction in Atg1 expression exhibits no significant effect on starvation lifespan (*p>0.05*; *log-rank; n>206 flies*).

(H) Body mass during starvation of *ELAV-GS>UAS-mCh-AMPK, UAS-Atg1-RNAi* female flies. Simultaneous upregulation of AMPK, and reduction of Atg1 expression had no significant effect on mass of control flies compared to ethanol controls (*p>0.05*; *t-test; n>6 samples/condition from 10 flies weighed/sample*).

(I) Whole body lipid stores during starvation of *ELAV-GS>UAS-mCh-AMPK, UAS-Atg1-RNAi* female flies. Simultaneous upregulation of AMPK, and reduction of Atg1 expression moderately increased the amount of lipids at 96 hours of starvation compared to ethanol fed flies (*p<0.05; t-test; n>3 samples /condition; lipids extracted from 5 flies/sample*).

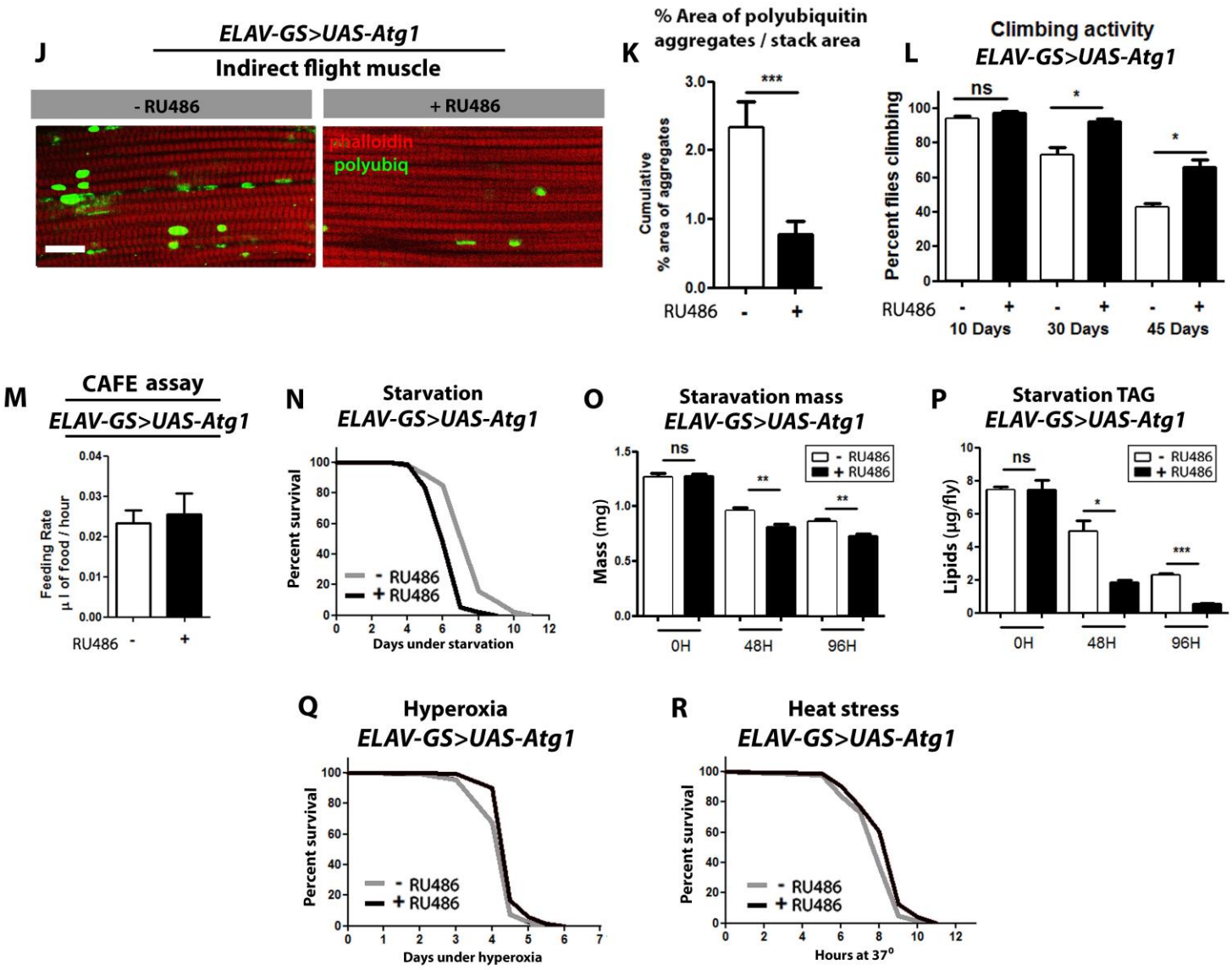

Figure S4 continued.

(J) Confocal imaging of adult female *ELAV-GS>UAS-Atg1* flight muscle showing polyubiquitinated aggregates at old (30 days) timepoint with or without RU486 feeding (*red channel-phalloidin/F-actin, green channel- anti-polyubiquitin, scale bar represents 10µm*).

(K) Quantification of polyubiquitin aggregates in the muscle of *ELAV-GS>UAS-Atg1*female flies at 30 days of age. Upregulation of Atg1 in neurons significantly decreased the accumulation of muscle protein aggregates with age ($p<0.001$; *t-test, n>10, one fly / replicate stack*).

(L) Climbing activity of *ELAV-GS>UAS-Atg1* flies with age. Neuronal upregulation of Atg1 increased climbing ability of aged flies compared to uninduced controls (*p<0.05 at 30 and 45 days*, *t-test, n>6 vials, 30 flies / vial*).

(M) Capillary feeding assay (CAFE) of 10 day old female *ELAV-GS>UAS-Atg1* flies with or without RU486-mediated transgene induction. No significant difference in feeding rate was observed (*p>0.05; t-test; n>8 vials of 10 flies/condition*).

(N) Survival curves without food of *ELAV-GS>UAS-Atg1* females with or without RU486-mediated transgene induction. Upregulation of Atg1 in neurons significantly decreased survival under wet starvation (*p<0.0001; log-rank; n>220 flies/condition*).

(O) Body mass during starvation of *ELAV-GS>UAS-Atg1* females with or without RU486-mediated transgene induction. Neuronal upregulation of Atg1 significantly reduced body mass at 48 and 96 hours of starvation (*p<0.01, at 48hours and 96 hours of starvation; t-test; n>6 samples/condition; 10 flies weighed/sample).*

(P) Whole body lipid stores during starvation of *ELAV-GS>UAS-Atg1* females with or without RU486-mediated transgene induction. Neuronal upregulation of Atg1 significantly decreased lipid stores at 48 and 96 hours of starvation (*p<0.05 at 48 hours, and p<0.001 at 96 hours of starvation; t-test; n>3 samples/condition/timepoint; lipids extracted from 5 flies/sample).*

(Q) Survival curves of 10 day old female *ELAV-GS>UAS-Atg1*flies under hyperoxia. Neuronal upregulation of Atg1 moderately increased survival under 80% atmospheric oxygen (*p<0.0001; log-rank; n>172 flies/condition*).

(R) Survival curves of 10 day old female *ELAV-GS>UAS-Atg1*flies under heat stress. Neuronal upregulation of Atg1 moderately increased survival at 37° C. (*p=0.0097; log-rank; n>75 flies/condition*).

Data are represented as mean ± SEM (*ns = p>0.05, * = p<0.05, ** = p<0.01, *** = p<.001)*. RU486 was provided in the media after eclosion at a concentration of 50μg/ml.

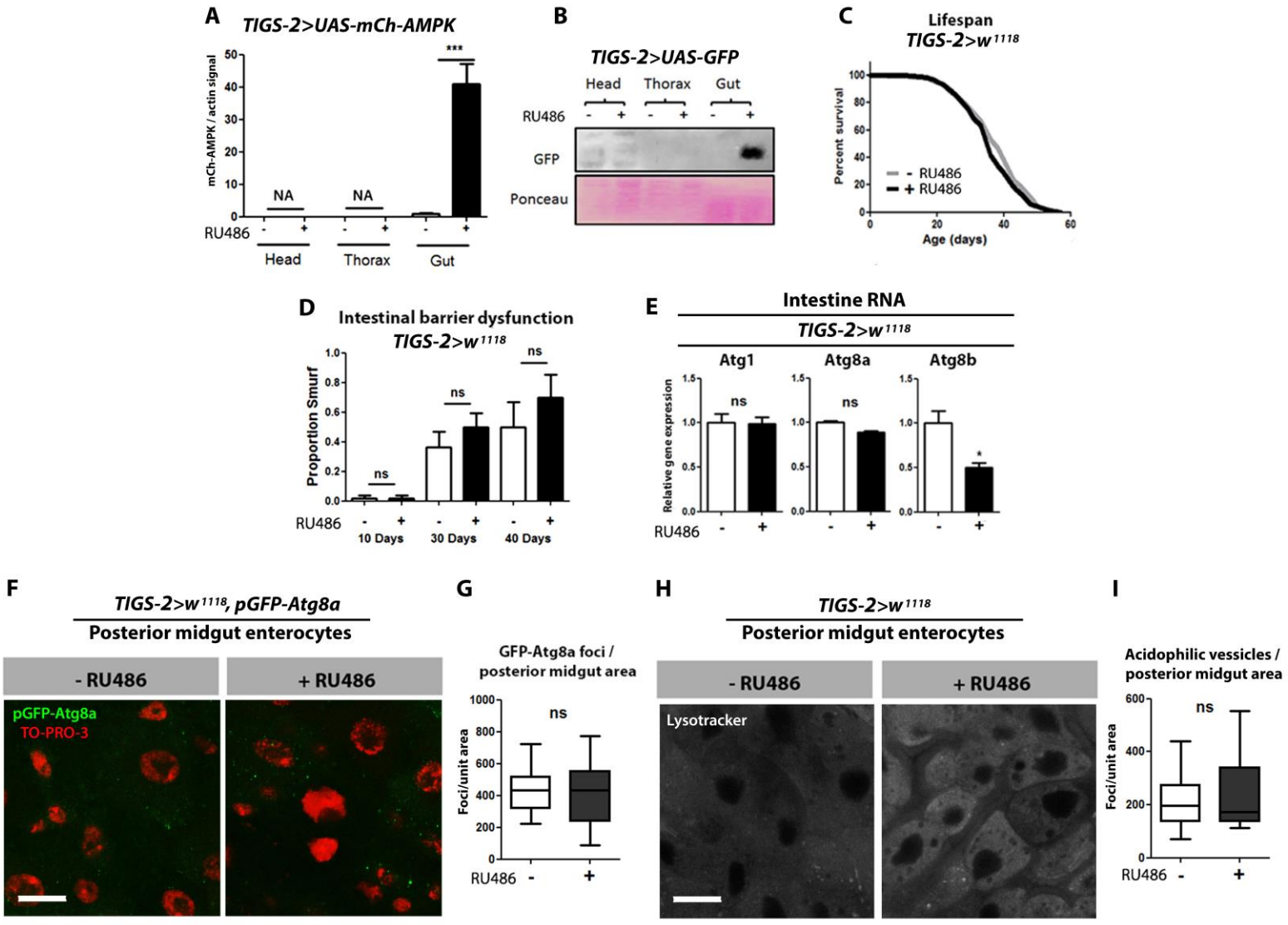

Figure S5. *TIGS-2* tissue specificity, intestine-specific AMPK overexpression extends lifespan under hyperoxia and heat stress, does not alter feeding behavior, fecundity, and effects of RU486 feeding on control flies, related to Figure 5.

(A) Exogenous mCherry-tagged AMPK expression levels of RNA extracted from body parts of *TIGS-2>UAS-mCh-AMPK* female flies at 10 days of age. Statistically significant increase in exogenous mCherry-AMPK is only detectable in the intestines of RU486 fed flies compared to uninduced controls. (*$p<0.001$; t-test; NA indicates "no amplification detectable after 45 cycles;" n=3 of RNA extracted from 10 body parts/replicate*)

(B) Western blot analysis for GFP and membrane stain for loading (Poncea S.) of protein extracted from body parts of *TIGS-2>UAS-eGFP* flies at 10 days of age. An RU486-dependent increase of GFP-signal was only observed in the intestines flies.

(C) Survival curves of *TIGS-2>W^{1118}* flies with or without RU486 feeding. There was no significant effect on the lifespan of control flies fed RU486 (*p>0.05*; *log-rank test; n>171 flies*).

(D) Intestinal integrity during aging in *TIGS-2>W^{1118}* controls. RU486 has no effect on the proportion of flies exhibiting intestinal barrier dysfunction (*p>0.05*; *binomial test; n>30 flies/condition*)

(E) Expression levels of autophagy genes from intestines of *TIGS-2>W^{1118}* control flies at 10 days of adulthood. RU486 feeding showed no significant increase Atg1, Atg8a, and Atg8b gene expression levels (*t-test; n=3 of RNA extracted from 15 intestines/replicate*)

(F) GFP-atg8a foci staining. Representative images of enterocytes from the posterior midgut of 10 day old female *TIGS-2>W^{1118}, pGFP-Atg8a* flies fed RU486 and vehicle (*red channel-TO-PRO-3 DNA stain, green channel-GFP-Atg8a, scale bar represents 10μm*).

(G) Quantification of posterior midgut GFP-Atg8a foci. Control flies fed RU486 showed no difference in foci number (*p>0.05*; *t-test; n>10 confocal stacks from posterior midgut/condition, one fly per replicate stack*).

(H) Lysotracker Red staining. Representative images of posterior midgut enterocytes from 10 day old female *TIGS-2>W^{1118}* flies stained with the acidophilic dye.

(I) Quantification of acidophilic vesicles. Feeding control flies RU486 had no effect on the number of acidophilic vesicles (*p>0.05*; *t-test*; *n>10 confocal stacks from posterior midgut/condition, one fly per replicate stack*).

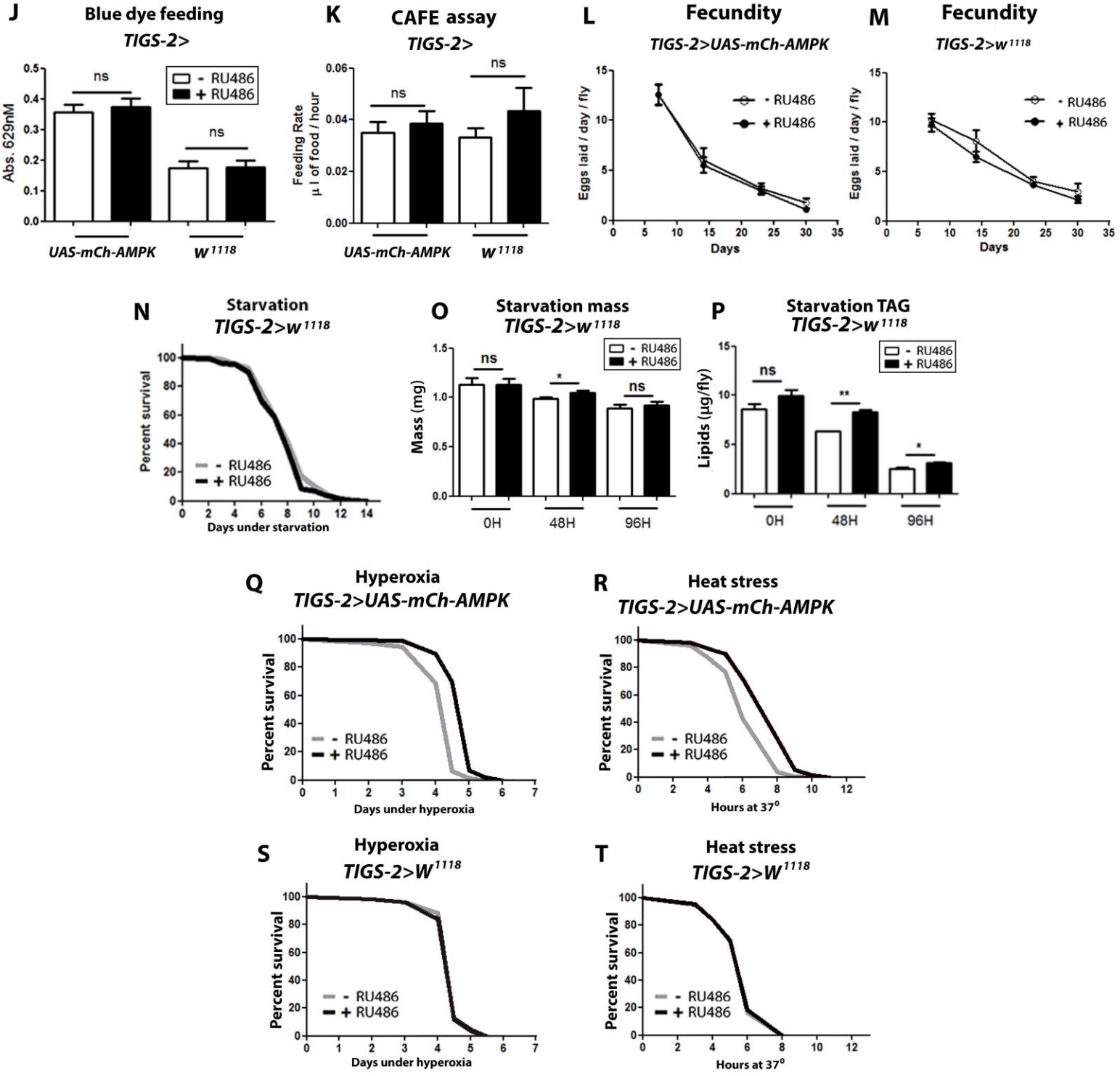

Figure S5 continued.

(J) Blue dye feeding assay of 10 day old *TIGS-2>UAS-mCh-AMPK,* and *TIGS-2>W^{1118}* control flies. Induction of AMPK in the gut had no effect on the amount of blue food consumed by *TIGS-2>UAS-mCh-AMPK*, and RU486 treatment had no effect on the feeding of control flies (*p>0.05*; *t-test; n>37 flies/condition*).

(K) Capillary feeding assay (CAFE) of 10 day old *TIGS-2>UAS-mCh-AMPK* flies and *TIGS-2>W^{1118}*controls. RU486 treatment had no effect on feeding rate of flies overexpressing AMPK in the intestine or control flies (*p>0.05*; *t-test, n>7 vials of 10 flies/condition*).

(L) Fecundity timecourse of *TIGS-2>UAS-mCh-AMPK* flies. Induction of AMPK in the intestine had no effect on the number of eggs laid over lifespan of the organisms (*p>0.05*; *t-test; n>7 vials of 10 flies/condition*).

(M) Fecundity timecourse of control *TIGS-2>W^{1118}* flies. RU486 feeding had no effect on the number of eggs laid over lifespan of the organisms (*p>0.05*; *t-test; n>7 vials of 10 flies/condition*).

(N) Starvation survival curves of *TIGS-2>W^{1118}* controls. Female control flies fed RU486 for 10 days had no significant effect on starvation lifespan (*p>0.05*; *log-rank; n>203 flies*).

(O) Body mass during starvation of *TIGS-2>W^{1118}* controls. RU486 fed flies showed a minimal increase in body mass at 48 hours compared to control flies (*p<0.05*; *t-test; n>6 samples/condition from 10 flies weighed/sample*)

(P) Whole body lipid stores during starvation of *TIGS-2>W^{1118}* controls. RU486 feeding of control flies moderately increased the amount of lipids at 48 and 96 hours of starvation compared to ethanol fed flies (*p<0.01 at 48 hours; p<0.05 at 96 hours; t-test; n>3 samples /condition; lipids extracted from 5 flies/sample*).

(Q) Hyperoxia survival curves of 10 day old female *TIGS-2>UAS-mCh-AMPK* flies. Intestine-specific upregulation of AMPK significantly increased survival under 80% atmospheric oxygen (*p<0.0001; log-rank; n>154 flies/condition*).

(R) Heat stress survival curves of 10 day old female *TIGS-2>UAS-mCh-AMPK* flies. Intestine-specific upregulation of AMPK significantly increased survival at 37° C. (*p<0.0001; log-rank; n>118 flies/condition*).

(S) Hyperoxia survival curves of 10 day old female *TIGS-2>W^{1118}* controls. RU486 feeding had no effect on survival under 80% atmospheric oxygen (*p>0.05; log-rank; n>125 flies/condition*).

(T) Heat stress survival curves of 10 day old female *TIGS-2>W^{1118}* controls. RU486 feeding had no effect on survival at 37° C. (*p>0.05; log-rank; n>139 flies/condition*).

Data are represented as mean ± SEM (*ns = p>0.05, * = p<0.05, ** = p<0.01, *** = p<.001).* RU486 was provided in the media after eclosion at a concentration of 25μg/ml (I, J) and 100μg/ml for all other figures.

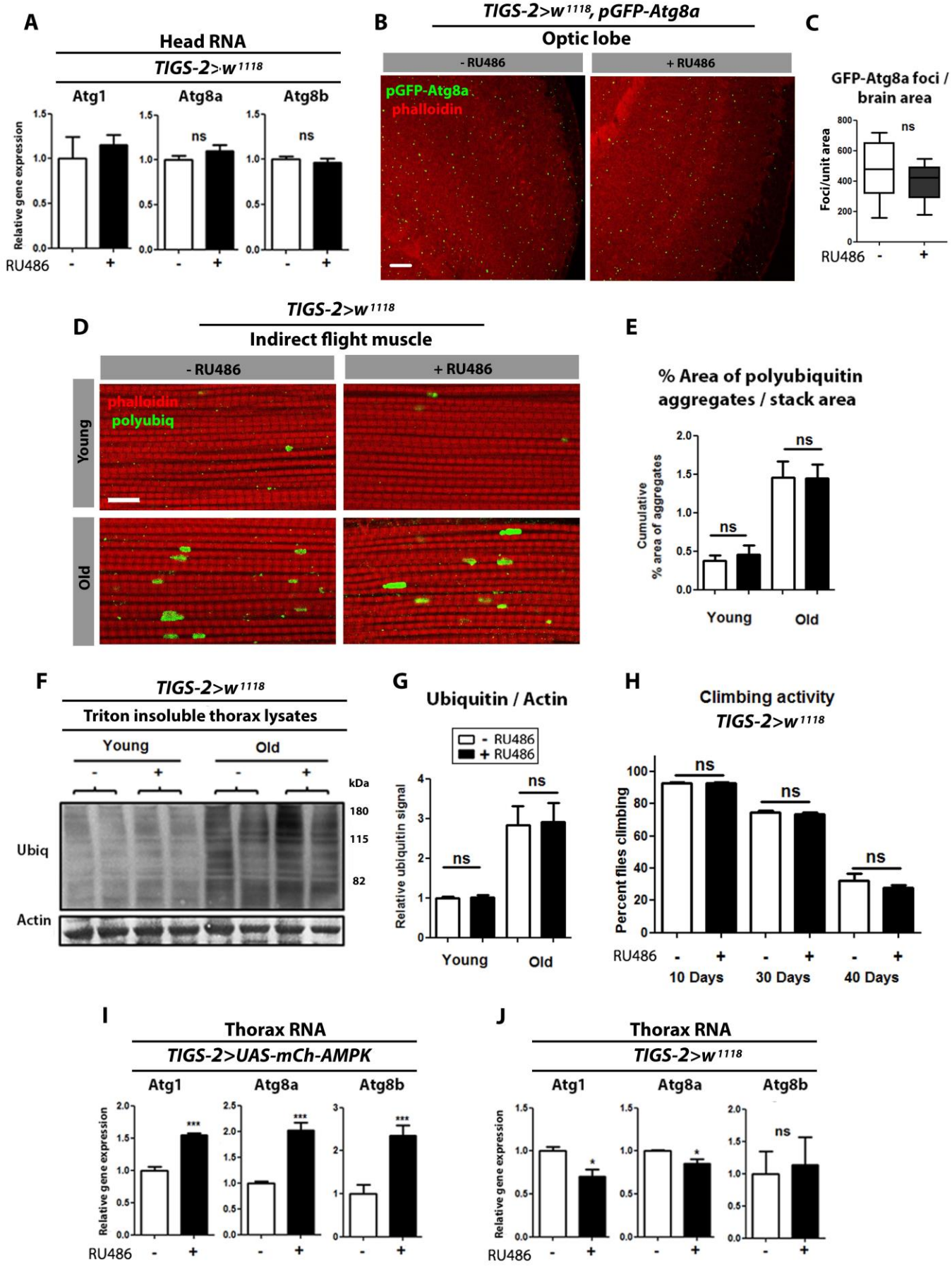

Figure S6. Intestine-specific AMPK induction increases expression of autophagy genes in the thorax, and effects of RU486 feeding on control flies, related to Figure 6.

(A) Expression levels of autophagy genes in heads *TIGS-2>W^{1118}* control flies at 10 days of adulthood. RU486 feeding had no significant effect on Atg1, Atg8a, and Atg8b gene expression levels *($p>0.05$*; *t-test; n=3 of RNA extracted from 10 heads/replicate*).

(B) Brain GFP-Atg8a staining. Representative images from optic lobes of 10 day old female *TIGS-2>W^{1118}*, *pGFP-Atg8a* flies (*red channel-phalloidin, green channel-GFP-Atg8a, scale bar represents 10μm*).

(C) Quantification of brain GFP-Atg8a foci in *TIGS-2>W^{1118}*, *pGFP-Atg8a* control flies. RU486 feeding had no influence on the number of foci marked with GFP-Atg8a *($p>0.05$*; *t-test; n>10 confocal stacks from brains/condition, one brain per replicate stack*).

(D) Confocal imaging of adult female *TIGS-2>W^{1118}* flight muscle showing protein polyubiquitinated aggregates at young (10 days), and old (30 days) timepoints (*red channel-phalloidin/F-actin, green channel- anti-polyubiquitin, scale bar represents 10μm*).

(E) Quantification of polyubiquitin aggregates in the muscle of *TIGS-2>W^{1118}* flies. RU486 feeding had no effect on protein aggregate accumulation with age. *($p>0.05$*; *t-test, n>10, one fly / replicate stack*).

(F) Western blot detection of total ubiquitin-conjugated proteins from thorax detergent-insoluble extracts of young (10 days) and aged (30 days) *TIGS-2>W^{1118}* female flies.

(G) Densitometry of ubiquitin blots from thoraces of flies. RU486 feeding had no effect on amount of thoracic detergent-insoluble ubiquitin-conjugated proteins, normalized to actin, in aged flies *($p>0.05$*; *t-test; n=4 samples/condition; 10 thoraces/sample*).

(H) Climbing activity of *TIGS-2>W^{1118}* female flies with age. RU486 feeding had no effect on climbing activity of control flies *($p>0.05$; t-test; n=6 vials; 30 flies/vial).*

(I) Expression levels of autophagy genes from dissected thoraces of *TIGS-2>UAS-mCh-AMPK* female flies at 10 days of adulthood. Upon AMPK induction in the intestine we see significantly increased Atg1, Atg8a, and Atg8b gene expression levels in the thorax (*$p<0.001$*; *t-test; n>3 of RNA extracted from 10 thoraces/replicate*).

(J) Expression level of autophagy genes from dissected thoraces of *TIGS-2>W^{1118}* female control flies at 10 days of adulthood. RU486 feeding showed a moderate reduction in the levels of Atg1 and Atg8a gene expression levels (*t-test; n=3 of RNA extracted from 10 thoraces /replicate*).

Data are represented as mean ± SEM (ns = *$p>0.05$, * = $p<0.05$, ** = $p<0.01$, *** = $p<.001$).* RU486 was provided in the media after eclosion at a concentration of 100μg/ml.

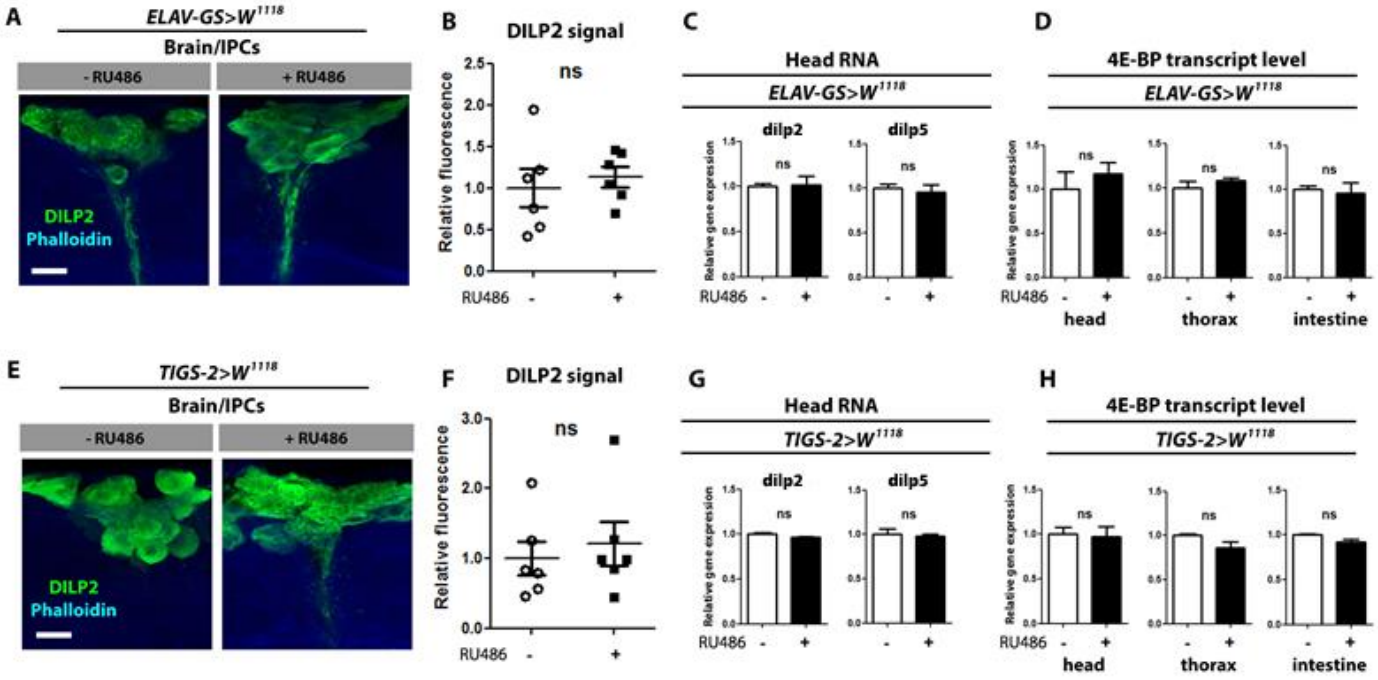

Figure S7. Effects of RU486 feeding in control flies, related to main text Figure 7.

(A) Representative images of DILP2 antibody stained insulin producing cells (IPCs) from 10 day old female *ELAV-GS>W^{1118}* flies *(green channel – Dilp2 antibody, blue channel – phalloidin, scale bars represent 10 μm).*

(B) Quantification of DILP2 signal from IPCs of 10 day old female *ELAV-GS>W^{1118}* flies. RU486 feeding had no effect on the relative fluorescence of DILP2 in IPCs *(p>0.05; t-test; n>6 brains/condition).*

(C) Expression level of *dilp* genes from dissected heads of *ELAV-GS>W^{1118}* female control flies at 10 days of adulthood. RU486 feeding showed no difference in the levels of *dilp2*, or *dilp5* RNA (*p>0.05; t-test; n=3 of RNA extracted from 10 heads /replicate*).

(D) Expression level of *4E-BP* from dissected body parts of *ELAV-GS>W^{1118}* female control flies at 10 days of adulthood. RU486 feeding showed no difference in the levels *4E-BP* RNA in the indicated tissues (*p>0.05; t-test; n=3 of RNA extracted from 10 body parts/replicate*).

(E) Representative images of DILP2 antibody stained insulin producing cells from 10 day old female *TIGS-2>W^{1118}* flies *(green channel – Dilp2 antibody, blue channel – phalloidin, scale bars represent 10μm).*

(F) Quantification of DILP2 signal from IPCs of 10 day old female *TIGS-2>W^{1118}* flies. RU486 feeding had no effect on the relative fluorescence of DILP2 in IPCs *(p>0.05; t-test; n>6 brains/condition).*

(G) Expression level of *dilp* genes from dissected heads of *TIGS-2>W^{1118}* female control flies at 10 days of adulthood. RU486 feeding showed no difference in the levels of *dilp2*, or *dilp5* RNA (*p>0.05; t-test; n=3 of RNA extracted from 10 heads /replicate*).

(H) Expression level of 4E-BP from dissected body parts of *TIGS-2>W*^{1118} female control flies at 10 days of adulthood. RU486 feeding showed no difference in the levels *4E-BP* RNA in the indicated tissues (*p>0.05; t-test; n=3 of RNA extracted from 10 body parts/replicate*).

Repeat	Genotype	RU-486 dose µg / ml of food	Sex	Median lifespan days	Percent change median lifespan to "0 RU"	Sample size	Log Rank p-value
1	ELAV-GS>UAS-AMPK	0	F	63		119	
	ELAV-GS>UAS-AMPK	25	F	65	**+3.17**	188	0.0112
	ELAV-GS>UAS-AMPK	50	F	71.5	**+13.49**	106	< 0.0001
controls	ELAV-GS>W^{1118}	0	F	47		121	
	ELAV-GS>W^{1118}	25	F	42	-10.6	122	< 0.0001
	ELAV-GS>W^{1118}	50	F	45	-4.2	118	0.037
2	ELAV-GS>UAS-AMPK	0	F	51		239	
	ELAV-GS>UAS-AMPK	50	F	56	**+9.8**	226	< 0.0001
controls	ELAV-GS>W^{1118}	0	F	48		202	
	ELAV-GS>W^{1118}	50	F	46	-4.1	168	0.033
3	ELAV-GS>UAS-AMPK	0	F	43		281	
	ELAV-GS>UAS-AMPK	50	F	48	**+11.62**	284	< 0.0001
controls	ELAV-GS>W^{1118}	0	F	35		218	
	ELAV-GS>W^{1118}	50	F	35	NS	171	NS
1	ELAV-GS>UAS-AMPK	0	M	43		162	
	ELAV-GS>UAS-AMPK	50	M	48	NS	120	NS
controls	ELAV-GS>W^{1118}	0	M	30		140	
	ELAV-GS>W^{1118}	50	M	28	NS	172	NS
1	ELAV-GS>UAS-mCh-AMPK	0	F	46		120	
	ELAV-GS>UAS-mCh-AMPK	25	F	64	**+39.13**	105	< 0.0001
controls	ELAV-GS>W^{1118}	0	F	28		153	
	ELAV-GS>W^{1118}	25	F	26	-7.14	108	0.0146
2	ELAV-GS>UAS-mCh-AMPK	0	F	49		180	
	ELAV-GS>UAS-mCh-AMPK	25	F	55	**+12.24**	165	0.0034
controls	ELAV-GS>W^{1118}	0	F	37		116	
	ELAV-GS>W^{1118}	25	F	29	-21.6	92	< 0.0001
3	ELAV-GS>UAS-mCh-AMPK	0	F	49		205	
	ELAV-GS>UAS-mCh-AMPK	25	F	59	**+20.4**	159	< 0.0001
controls	ELAV-GS>W^{1118}	0	F	40		187	
	ELAV-GS>W^{1118}	25	F	35	-12.5	176	0.0003
1	ELAV-GS>UAS-mCh-AMPK	0	M	42		87	
	ELAV-GS>UAS-mCh-AMPK	25	M	40	NS	85	NS
	ELAV-GS>UAS-mCh-AMPK	100	M	49	**+16.6**	94	< 0.0001
controls	ELAV-GS>W^{1118}	0	M	31		98	
	ELAV-GS>W^{1118}	25	M	24	-22.5	93	0.01564
	ELAV-GS>W^{1118}	100	M	24	-22.5	88	< 0.0001

Table S1. Lifespan information and controls associated with Figure 1. Statistically significant changes in median lifespan are listed as percentage and NS describes no significant change. Increased lifespan percentage in bold font, and decreased percentages in standard font. Lifespan experiments highlighted in red font are shown in main figure.

Repeat	Genotype	RU-486 dose µg / ml of food	Sex	Median lifespan days	Percent change median lifespan to "0 RU"	Sample size	Log Rank p-value
1	ELAV-GS>UAS-mCh-AMPK	0	F	42		356	
	ELAV-GS>UAS-mCh-AMPK	50	F	46	**+9.53**	344	< 0.0001
2	ELAV-GS>UAS-mCh-AMPK	0	F	47		197	
	ELAV-GS>UAS-mCh-AMPK	50	F	55.5	**+15.31**	138	< 0.0001
1	ELAV-GS>UAS-Atg1-RNAi	0	F	48		204	
	ELAV-GS>UAS-Atg1-RNAi	50	F	48	**NS**	212	0.32
2	ELAV-GS>UAS-Atg1-RNAi	0	F	47		111	
	ELAV-GS>UAS-Atg1-RNAi	50	F	50	**NS**	116	0.48
1	ELAV-GS>UAS-mCh-AMPK, Atg1-RNAi	0	F	52		276	
	ELAV-GS>UAS-mCh-AMPK, Atg1-RNAi	50	F	50	**NS**	286	0.497
2	ELAV-GS>UAS-mCh-AMPK, Atg1-RNAi	0	F	50		210	
	ELAV-GS>UAS-mCh-AMPK, Atg1-RNAi	50	F	52	**+4.0**	217	0.0284
1	ELAV-GS>UAS-Atg1	0	F	40		716	
	ELAV-GS>UAS-Atg1	25	F	47	**+17.5**	386	< 0.0001
	ELAV-GS>UAS-Atg1	50	F	46	**+15.0**	494	< 0.0001
controls	ELAV-GS>W^{1118}	0	F	38		200	
	ELAV-GS>W^{1118}	25	F	38	**NS**	196	NS
	ELAV-GS>W^{1118}	50	F	36	**NS**	126	NS
2	ELAV-GS>UAS-Atg1	0	F	39		274	
	ELAV-GS>UAS-Atg1	25	F	42	**+7.69**	137	< 0.0001
	ELAV-GS>UAS-Atg1	50	F	49	**+25.64**	209	< 0.0001
3	ELAV-GS>UAS-Atg1	0	F	40		151	
	ELAV-GS>UAS-Atg1	25	F	47	**+17.5**	179	< 0.0001
	ELAV-GS>UAS-Atg1	50	F	50	**+25.0**	186	< 0.0001
4	ELAV-GS>UAS-Atg1	0	F	42		328	
	ELAV-GS>UAS-Atg1	50	F	47	**+11.90**	332	< 0.0001

Table S2. Lifespan information and controls associated with Figure 4. Statistically significant changes in median lifespan are listed as percentage and NS describes no significant change. Increased lifespan percentage in bold font, and decreased percentages in standard font. Lifespan experiments highlighted in red font are shown in main figure.

Repeat	Genotype	RU-486 dose µg / ml of food	Sex	Median lifespan days	Percent change median lifespan to "0 RU"	Sample size	Log Rank p-value
1	TIGS-2>UAS-AMPK	0	F	36		266	
	TIGS-2>UAS-AMPK	25	F	43	**+19.4**	244	<0.0001
controls	TIGS-2>W^{1118}	0	F	38		291	
	TIGS-2>W^{1118}	25	F	36	-5.26	292	0.0143
2	TIGS-2>UAS-AMPK	0	F	40		258	
	TIGS-2>UAS-AMPK	25	F	43	**+7.5**	284	0.0135
controls	TIGS-2>W^{1118}	0	F	41		252	
	TIGS-2>W^{1118}	25	F	43	NS	325	NS
1	TIGS-2>UAS-AMPK	0	M	49		295	
	TIGS-2>UAS-AMPK	25	M	46	NS	292	NS
	TIGS-2>UAS-AMPK	50	M	49	NS	228	NS
controls	TIGS-2>W^{1118}	0	M	46		287	
	TIGS-2>W^{1118}	25	M	42	-8.6	288	< 0.0001
	TIGS-2>W^{1118}	50	M	42	-8.6	276	< 0.0001
1	TIGS-2>UAS-mCh-AMPK	0	F	25		117	
	TIGS-2>UAS-mCh-AMPK	100	F	50	**+100**	81	< 0.0001
controls	TIGS-2>W^{1118}	0	F	32		145	
	TIGS-2>W^{1118}	100	F	29	-9.3	159	0.0048
2	TIGS-2>UAS-mCh-AMPK	0	F	38		116	
	TIGS-2>UAS-mCh-AMPK	100	F	53	**+39.4**	104	< 0.0001
controls	TIGS-2>W1118	0	F	28		131	
	TIGS-2>W1118	100	F	28	NS	123	NS
3	TIGS-2>UAS-mCh-AMPK	0	F	45		106	
	TIGS-2>UAS-mCh-AMPK	100	F	51	**+13.3**	120	< 0.0001
controls	TIGS-2>W^{1118}	0	F	41		146	
	TIGS-2>W^{1118}	100	F	39	NS	152	NS
1	TIGS-2>UAS-mCh-AMPK	0	M	41		95	
	TIGS-2>UAS-mCh-AMPK	100	M	45	**+9.75**	110	0.002
controls	TIGS-2>W^{1118}	0	M	41		104	
	TIGS-2>W^{1118}	100	M	39	-4.8	107	0.0006

Table S3. Lifespan information and controls associated with Figure 5. Statistically significant changes in median lifespan are listed as percentage and NS describes no significant change. Increased lifespan percentage in bold font, and decreased percentages in standard font. Lifespan experiments highlighted in red font are shown in main figure.