**Supplementary Figures for**

# The neuronal receptor tyrosine kinase Alk is a target for longevity

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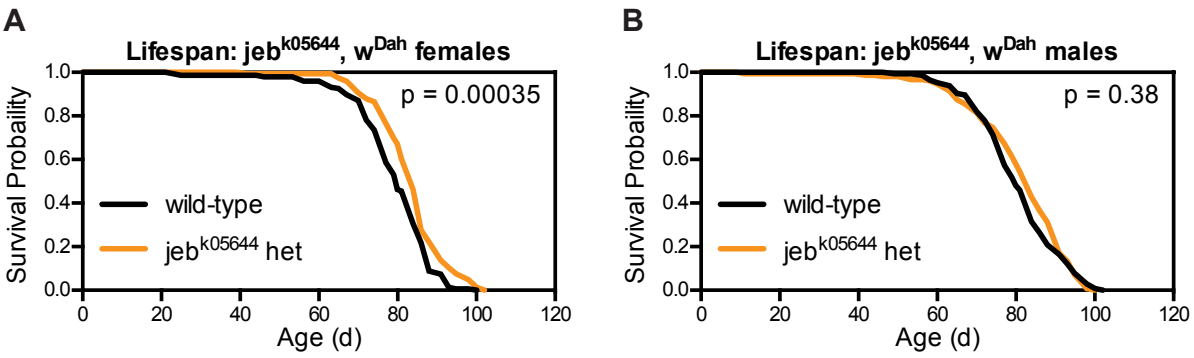
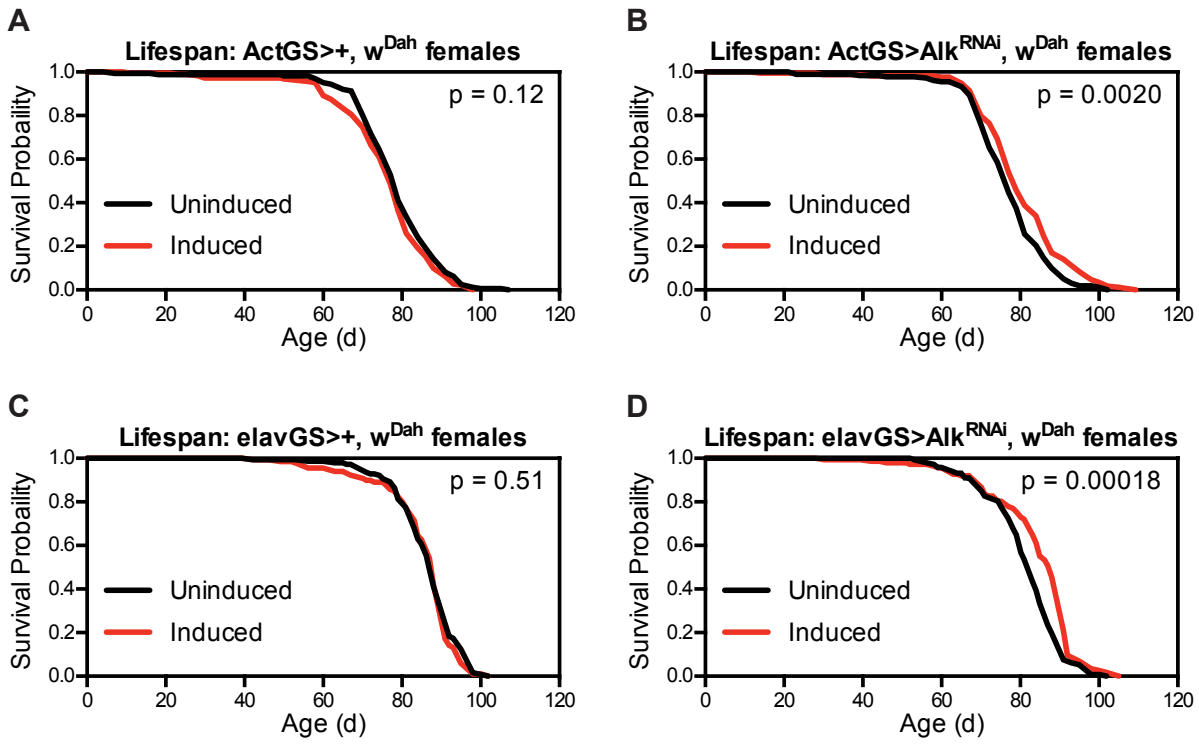
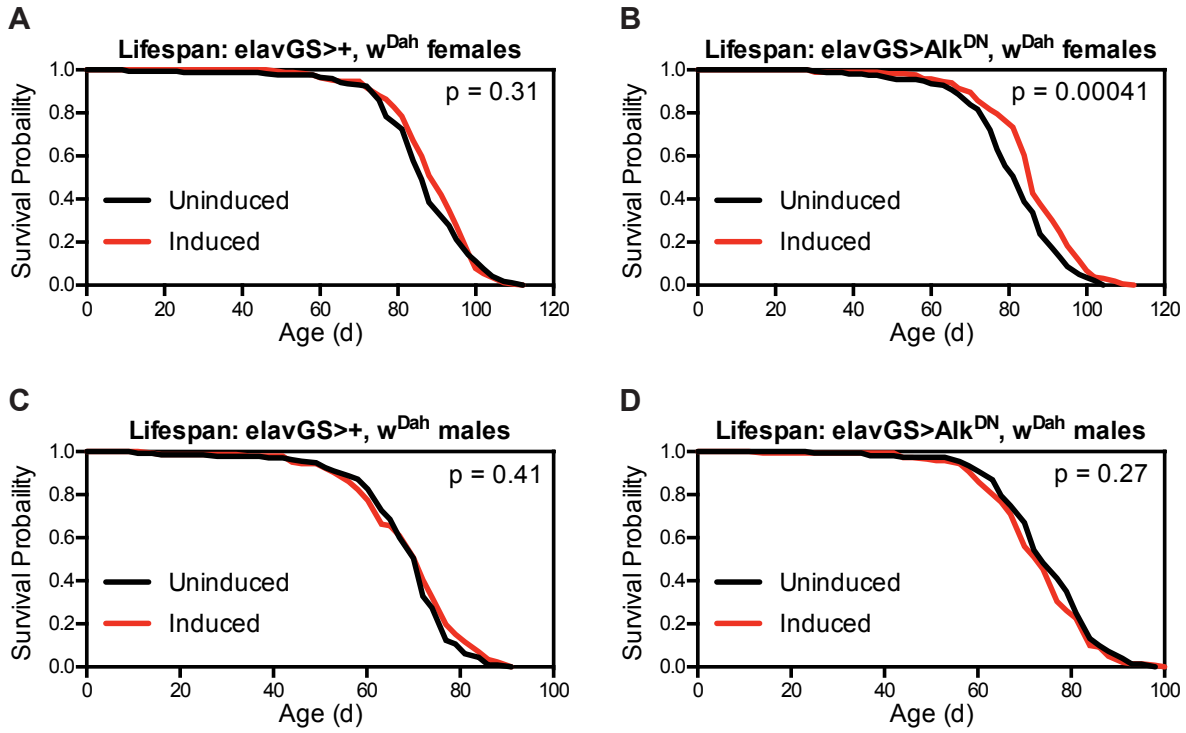


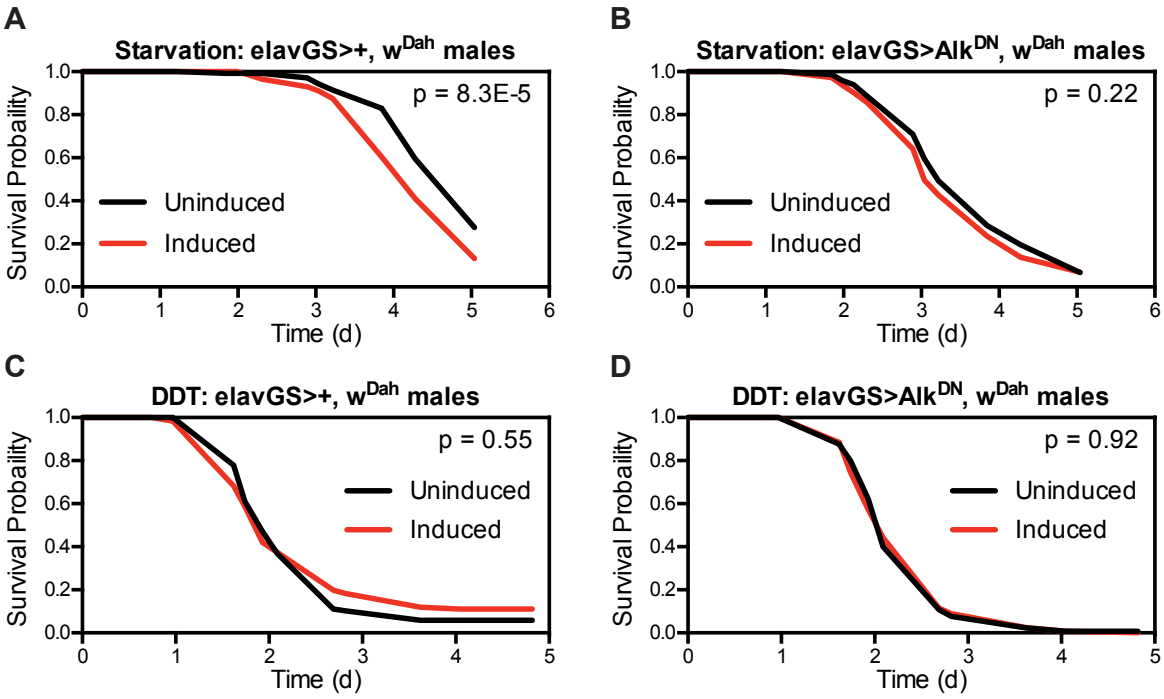

**Supplementary Figure 1. Heterozygous loss of *jeb* reproducibly extends lifespan in female but not male flies**. (**A**) Survival curves showed extended lifespan for female $w^{Dah}$;$jeb^{k05644}$/+; flies compared to their $w^{Dah}$;+/+; siblings. (**B**) Survival curves showed no significant change in lifespan for male $w^{Dah}$;$jeb^{k05644}$/+; flies compared to their $w^{Dah}$;+/+; siblings. For all survival experiments, n>145 deaths counted per condition; p values are from log-rank tests versus the wild-type condition.



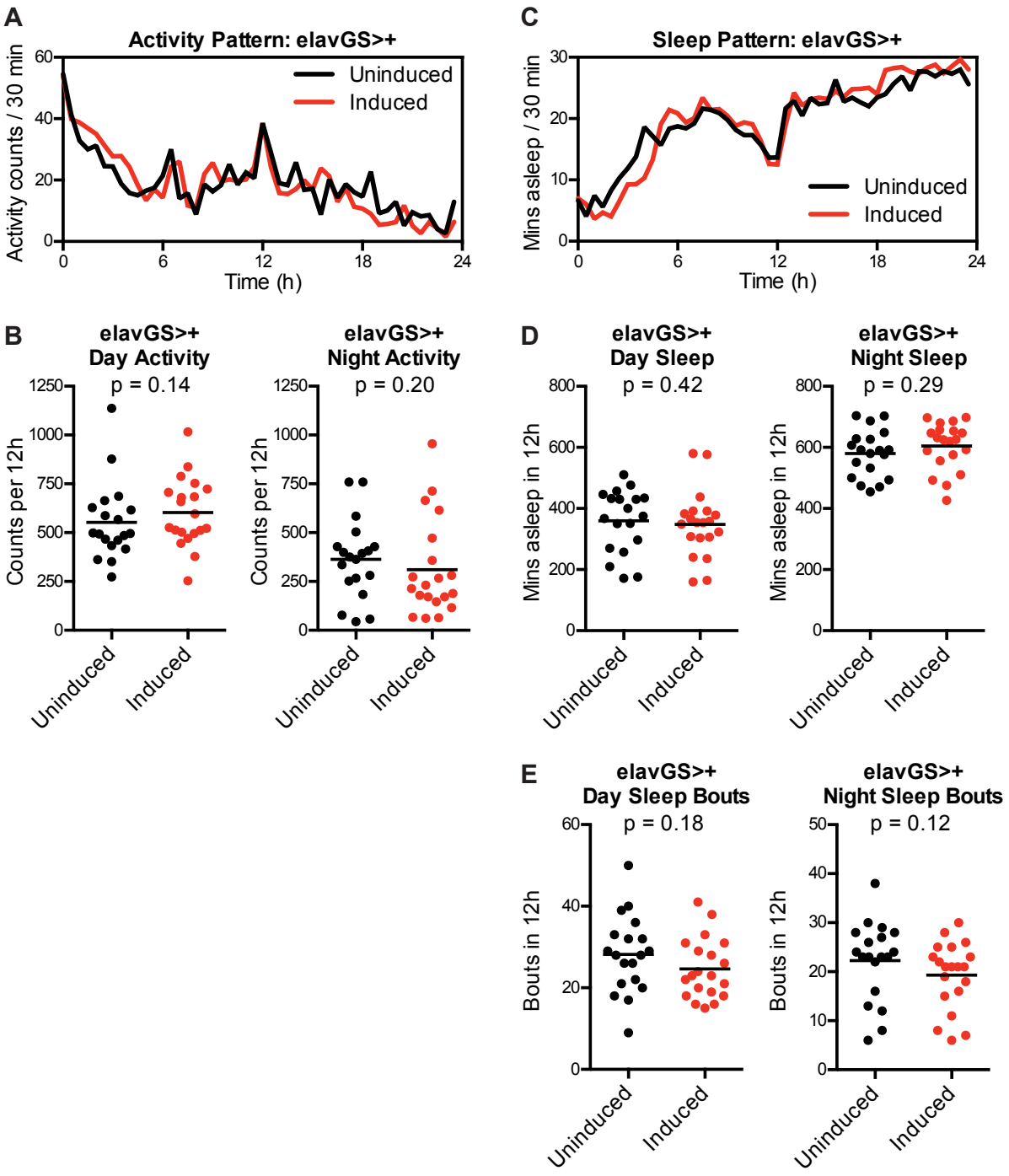

**Supplementary Figure 2. RNAi knock-down of *Alk* ubiquitously or in neurons reproducibly extends lifespan**. (**A**) Survival curves showed no significant change in lifespan for female $w^{Dah}$;Act-GS/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**B**) Survival curves showed extended lifespan for female $w^{Dah}$,UAS-$Alk^{RNAi}$/$w^{Dah}$;Act-GS/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**C**) Survival curves showed no significant change in lifespan for female $w^{Dah}$;;elav-$GS^{Tricoire}$/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**D**) Survival curves showed extended lifespan for female $w^{Dah}$,UAS-$Alk^{RNAi}$/$w^{Dah}$;;elav-$GS^{Tricoire}$/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. For all survival experiments, n>110 deaths counted per condition; p values are from log-rank tests versus the vehicle-treated (uninduced) group.



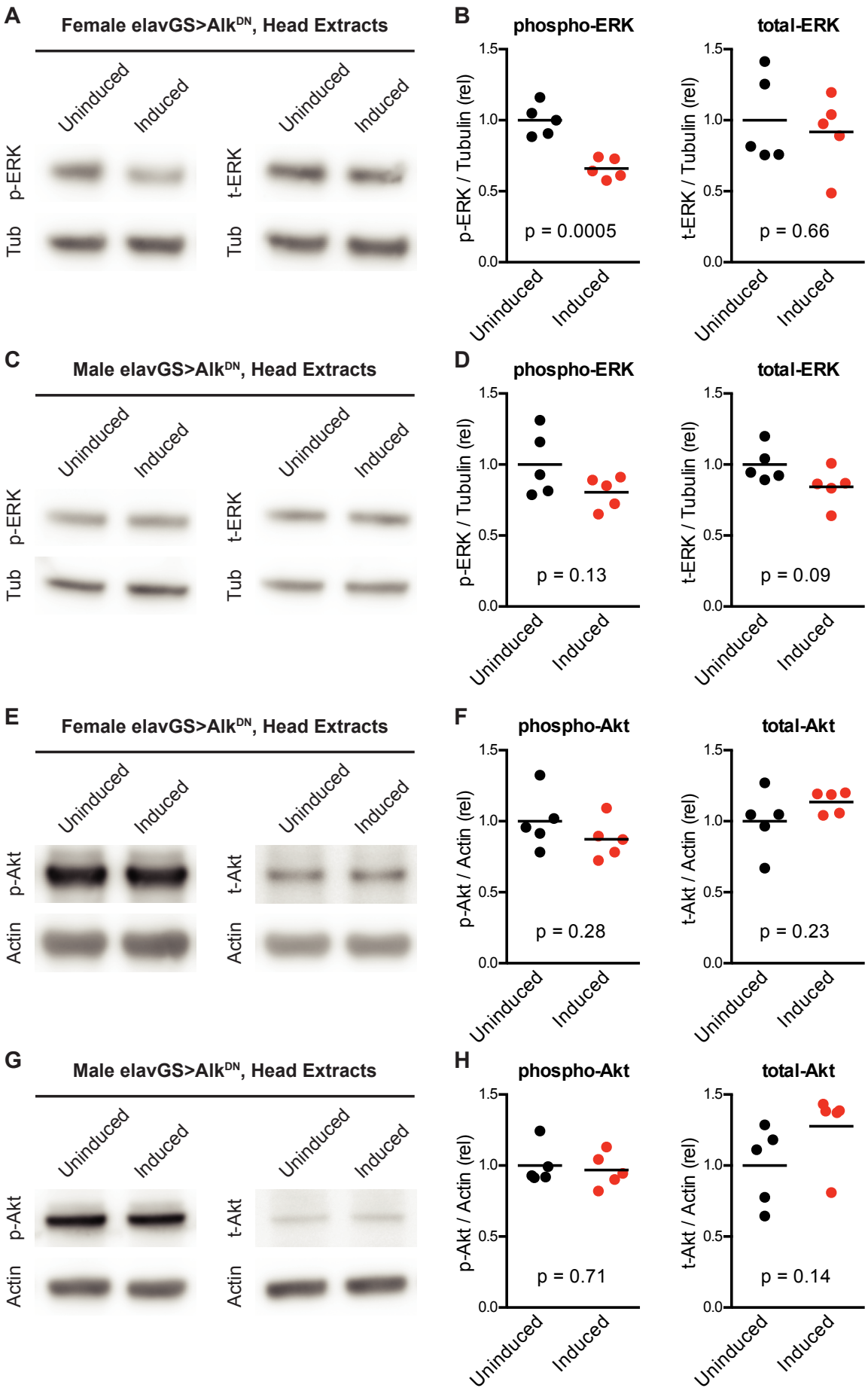

**Supplementary Figure 3. Expression of dominant-negative *Alk* in neurons reproducibly extends lifespan in female but not male flies**. (**A**) Survival curves showed no significant change in lifespan for female $w^{Dah}$;;elav-$GS^{301}$/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**B**) Survival curves showed extended lifespan for female $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**C**) Survival curves showed no significant change in lifespan for male $w^{Dah}$;;elav-$GS^{301}$/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**D**) Survival curves showed no significant change in lifespan for male $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. For all survival experiments, n>115 deaths counted per condition; p values are from log-rank tests versus the vehicle-treated (uninduced) group.



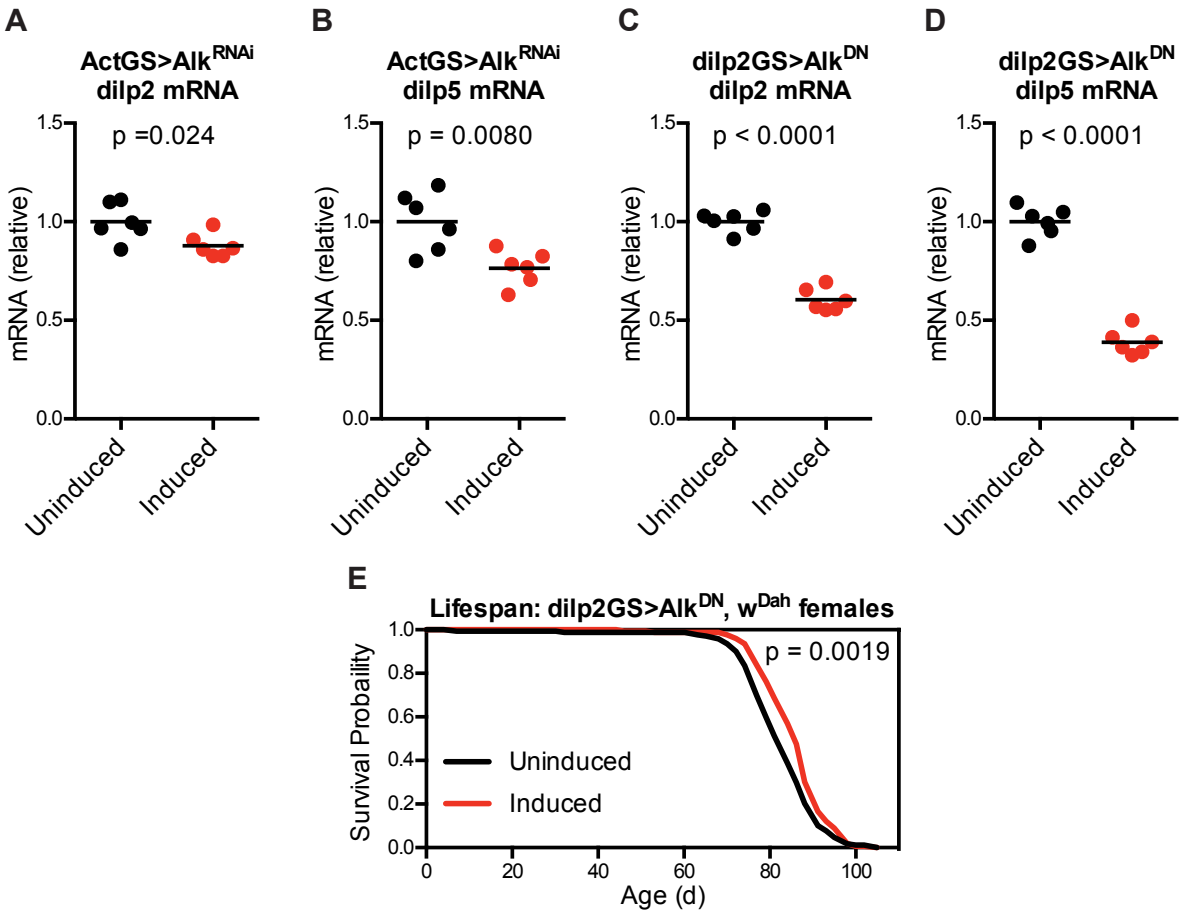

**Supplementary Figure 4. Expression of dominant-negative *Alk* in neurons does not improve resistance to starvation or resistance to the xenobiotic toxin DDT in male flies**. (**A-B**) Survival curves in starvation experiments started at 14 days of age showed (**A**) decreased survival for male $w^{Dah}$;;elav-$GS^{301}$/+ flies and (**B**) no significant change in survival for male $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies treated with RU-486 (200μM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**C-D**) Survival curves in DDT (0.03%) experiments started at 14 days of age showed (**C**) no significant change in survival for male $w^{Dah}$;;elav-$GS^{301}$/+ flies and (**D**) no significant change in survival for male $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies treated with RU-486 (200μM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. For all survival experiments, n>100 deaths counted per condition; p values are from log-rank tests versus the vehicle-treated (uninduced) group.



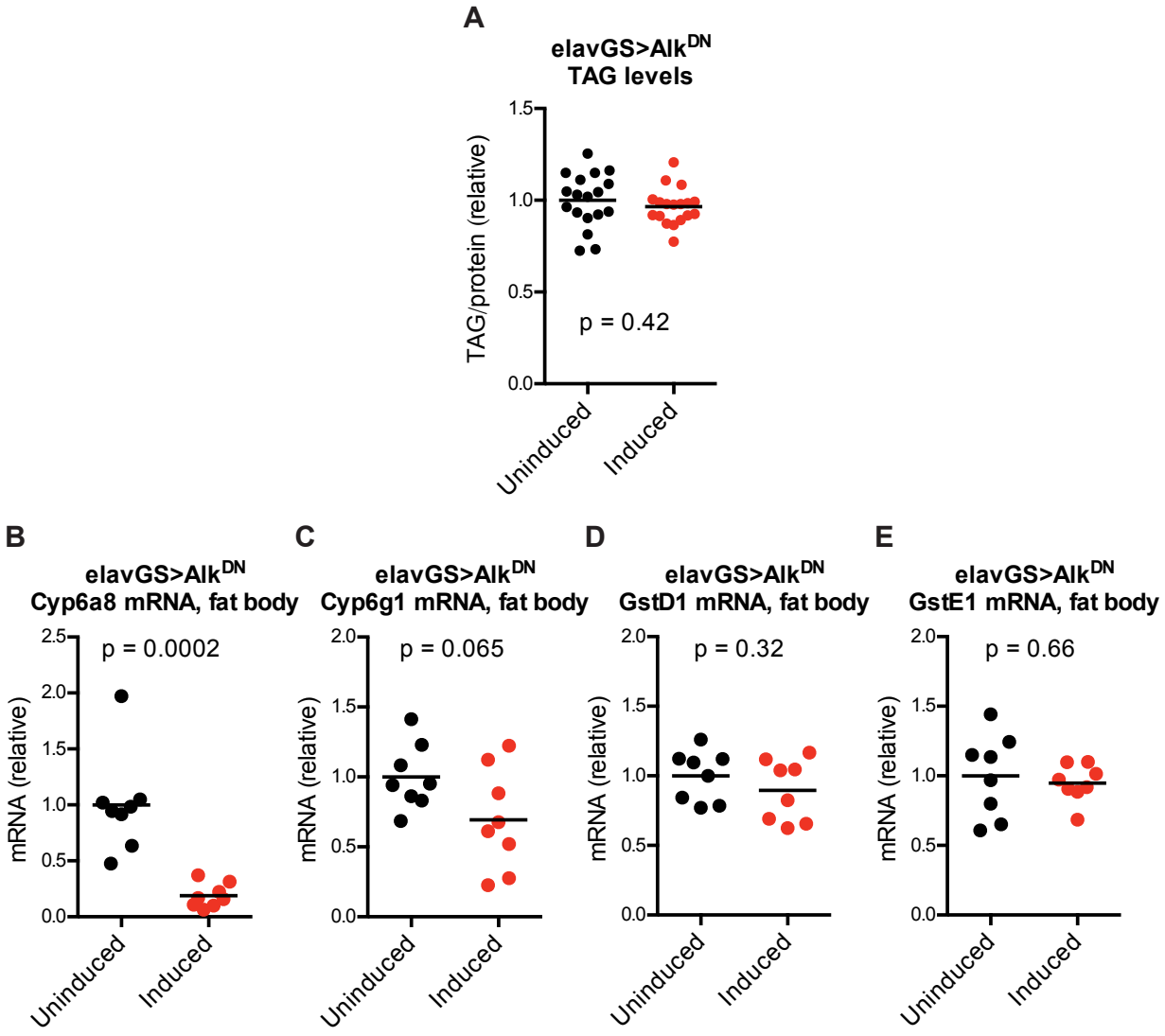

**Supplementary Figure 5. Driver-alone elavGS/+ flies show no change in activity or sleep patterns with RU-486 treatment**. (**A**) Activity traces and (**B**) quantification of day and night activity counts from mated female flies at 15 days of age showed no significant change in activity for $w^{Dah}$;;elav-$GS^{301}$/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**C**) Sleep traces, (**D**) quantification of day and night sleep, and (**E**) quantification of the number of day and night sleep bouts from mated female flies at 15 days of age showed no significant change in activity for $w^{Dah}$;;elav-$GS^{301}$/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. n=19-20 individual flies per condition; p values are from Mann-Whitney tests versus the vehicle-treated (uninduced) group.


A
Female elavGS>Alk^DN, Head Extracts
Uninduced
Induced
Uninduced
Induced
p-ERK
t-ERK
Tub
Tub
B
phospho-ERK
total-ERK
p-ERK / Tubulin (rel)
t-ERK / Tubulin (rel)
1.5
1.0
0.5
0.0
p = 0.0005
p = 0.66
Uninduced
Induced
C
Male elavGS>Alk^DN, Head Extracts
D
phospho-ERK
total-ERK
p = 0.13
p = 0.09
E
Female elavGS>Alk^DN, Head Extracts
p-Akt
t-Akt
Actin
Actin
F
phospho-Akt
total-Akt
p-Akt / Actin (rel)
t-Akt / Actin (rel)
p = 0.28
p = 0.23
G
Male elavGS>Alk^DN, Head Extracts
H
phospho-Akt
total-Akt
p = 0.71
p = 0.14

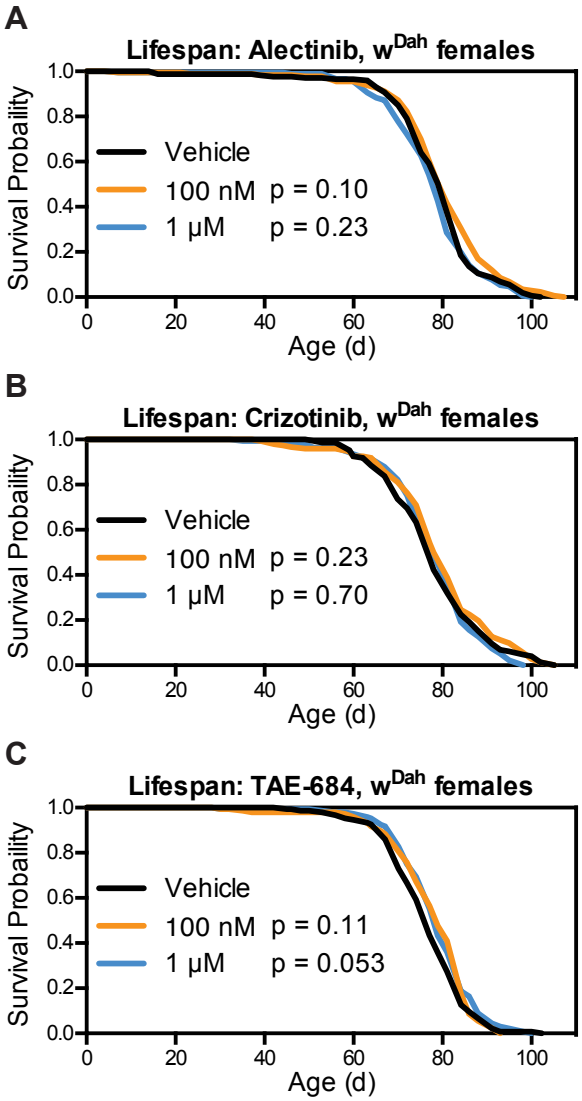
**Supplementary Figure 6 (previous page). Expression of dominant-negative *Alk* in neurons reduces Erk phosphorylation in females but not males, while not significantly affecting Akt phosphorylation**. (**A-B**) Western blots from head extracts showed decreased levels of phosphorylated Erk and no significant change in the level of total Erk in female $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies given food containing the inducing drug RU-486 (200μM) from 2 to 10 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**C-D**) Western blots from head extracts showed no significant change in the levels of phosphorylated Erk or total Erk in male $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies given food containing the inducing drug RU-486 (200μM) from 2 to 10 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**E-F**) Western blots from head extracts showed no significant change in the levels of phosphorylated Akt (Thr342) or total Akt in female $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies given food containing the inducing drug RU-486 (200μM) from 2 to 10 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**G-H**) Western blots from head extracts showed no significant change in the levels of phosphorylated Akt (Thr342) or total Akt in male $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies given food containing the inducing drug RU-486 (200μM) from 2 to 10 days of age compared to sibling flies of the same genotype treated with vehicle control food. n=5 biological replicates of 8 heads per replicate for each condition; p values are from unpaired t-tests between groups.



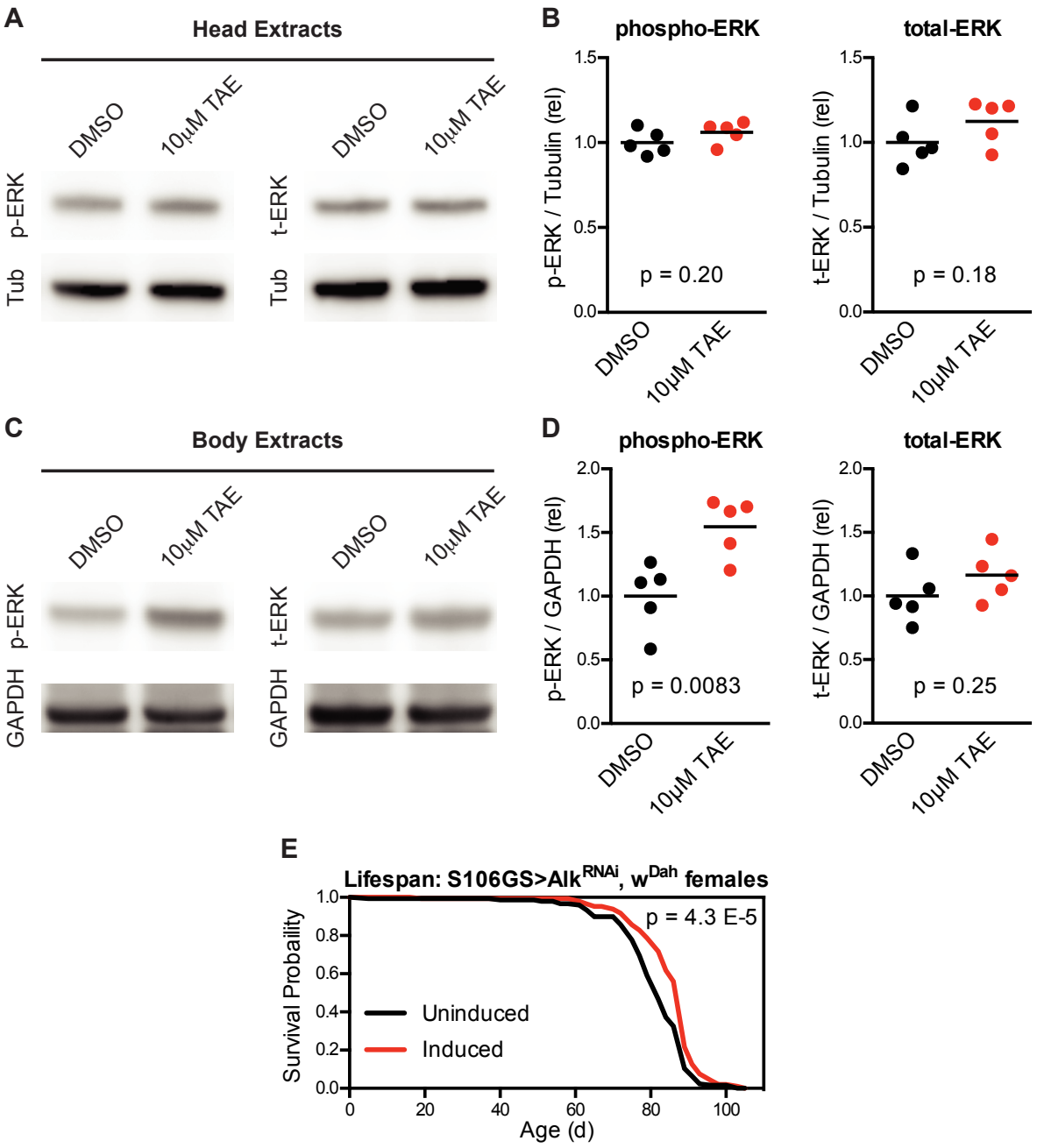

**Supplementary Figure 7. RNAi knock-down of *Alk* or dilp-neuron-specific expression of dominant-negative *Alk* reduces mRNA levels for *dilp2* and *dilp5* and extends lifespan.** (**A-B**) qPCR from head RNA showed decreased mRNA levels for *dilp2* and *dilp5* in female $w^{Dah}$,UAS-$Alk^{RNAi}$/$w^{Dah}$;Act-GS/+; flies given food containing the inducing drug RU-486 (200µM) from 2 to 11 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**C-D**) qPCR from head RNA showed decreased mRNA levels for *dilp2* and *dilp5* in female $w^{Dah}$;dilp2-GS/+;UAS-$Alk^{DN}$/+ flies given food containing the inducing drug RU-486 (200µM) from 2 to 11 days of age compared to sibling flies of the same genotype treated with vehicle control food. n=6 biological replicates of 6 heads per replicate for each condition; p values are from unpaired t-tests between groups. (**E**) Survival curves showed extended lifespan for female $w^{Dah}$;dilp2-GS/+;UAS-$Alk^{DN}$/+ flies treated with RU-486 (200µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. n>165 deaths counted per condition; p value is from log-rank tests versus the vehicle-treated (uninduced) group.




**Supplementary Figure 8. Expression of dominant-negative *Alk* in neurons does not alter TAG levels but reduces mRNA levels for *Cyp6a8* in abdominal fat bodies.** (**A**) Triglyceride (TAG) quantification from whole flies showed no change in TAG levels for female $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies given food containing the inducing drug RU-486 (200μM) from 2 to 17 days of age compared to sibling flies of the same genotype treated with vehicle control food. n=18 individual flies for each condition; p values are from unpaired t-tests between groups. (**B-E**) qPCR from abdominal fat body RNA showed decreased mRNA levels for *Cyp6a8*, a trend towards decreased mRNA levels for *Cyp6g1*, and no change in mRNA levels for *GstD1* and *GstE1* in female $w^{Dah}$;;UAS-$Alk^{DN}$/elav-$GS^{301}$ flies given food containing the inducing drug RU-486 (200μM) from 2 to 18 days of age compared to sibling flies of the same genotype treated with vehicle control food. n=8 biological replicates of 4 abdominal carcasses per replicate for each condition; p values are from unpaired t-tests between groups.




**Supplementary Figure 9. The Alk inhibitors Alectinib, Crizotinib, and TAE-684 do not significantly extend lifespan at doses of 100nM or 1µM.** Survival curves showed no significant change in lifespan for female $w^{Dah}$ flies treated with (**A**) Alectinib (100nM or 1µM), (**B**) Crizotinib (100nM or 1µM), or (**C**) TAE-684 (100nM or 1µM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. Note that the vehicle control lifespan curves are the same as in Figure 7A-C, as these experiments were run in parallel. For all survival experiments, n>130 deaths counted per condition; p values are from log-rank tests versus the vehicle-treated group.




**Supplementary Figure 10. Treatment with the Alk inhibitor TAE-684 alters Erk phosphorylation in body extracts, and RNAi knock-down of *Alk* in the fat body and gut extends lifespan**. (**A-B**) Western blots from head extracts showed no significant change in the levels of phosphorylated Erk and total Erk in female $w^{Dah}$ flies treated with TAE-684 (10μM) from 2 to 10 days of age compared to sibling flies of the same genotype treated with vehicle control food. (**C-D**) Western blots from headless body extracts showed increased levels of phosphorylated Erk and no significant change in the level of total Erk in female $w^{Dah}$ flies treated with TAE-684 (10μM) from 2 to 10 days of age compared to sibling flies of the same genotype treated with vehicle control food. n=5 biological replicates for each condition; p values are from unpaired t-tests between groups. (**E**) Survival curves showed extended lifespan for female $w^{Dah}$,UAS-$Alk^{RNAi}$/$w^{Dah}$;$S_1$106-GS/+; flies treated with RU-486 (200μM) from 2 days of age compared to sibling flies of the same genotype treated with vehicle control food. n>130 deaths counted per condition; p value is from log-rank tests versus the vehicle-treated (uninduced) group.