Expanded View Figures

Figure EV1. Active RET leads to increased ROS and decreased NAD^+/NADH ratio in aged flies.

A, B Mito-Sox staining (A) and data quantification (B) showing mitochondrial ROS level in different aged fly brains with or without treatment with the indicated RET inhibitors ($n = 5$ per group).
C Quantification of NAD^+/NADH ratio in young flies, old flies, and old flies treated with the indicated chemicals.
D Western blot analysis comparing NDUFS3 level in young and aged flies. $^{**}P < 0.01$ in Student's *t*-test.
E Quantification of FET-ROS in isolated mitochondria purified from aged wild-type flies and assayed under FET condition without or with treatment at the indicated concentrations of CPT ($n = 4$).
F Quantification of NAD^+/NADH ratio in isolated mitochondria after induction of FET and with or without CPT (2.5 μM) treatment ($n = 4$).
G Co-immunoprecipitation assay showing the effect of aging and CPT treatment on the various protein–protein interactions between C-I proteins involved in RET.
H Quantification of ROS level from brain samples of DES and DES + CPT-treated flies ($n = 4$).
I Quantification of NAD^+/NADH ratio from brain samples of DES and DES + CPT-treated flies ($n = 5$ sets, 20 flies per set).
J Quantification of NAD^+ and NADH levels measured with SoNar in different aged fly brains with or without treatment with the indicated chemicals as shown in Fig 1D ($n = 4$ sets, 5 flies per set).
K Quantification of NAD^+/NADH ratio in wild-type flies treated with CPT or co-treated with CPT and NADH ($n = 4$ sets, 20 flies per set).
L Mitochondrial C-I activity assay using mitochondria purified from different aged flies ($n = 3$).
M In-gel activity assays of respiratory complexes using mitochondria purified from different aged flies.

Data information: Data are representative of at least three repeats. Data are shown as mean ± SEM. Asterisks indicate statistical significance ($^{**}P < 0.01$) in single-factor ANOVA with Scheffe's analysis as a *post hoc* test.

© 2023 The Authors

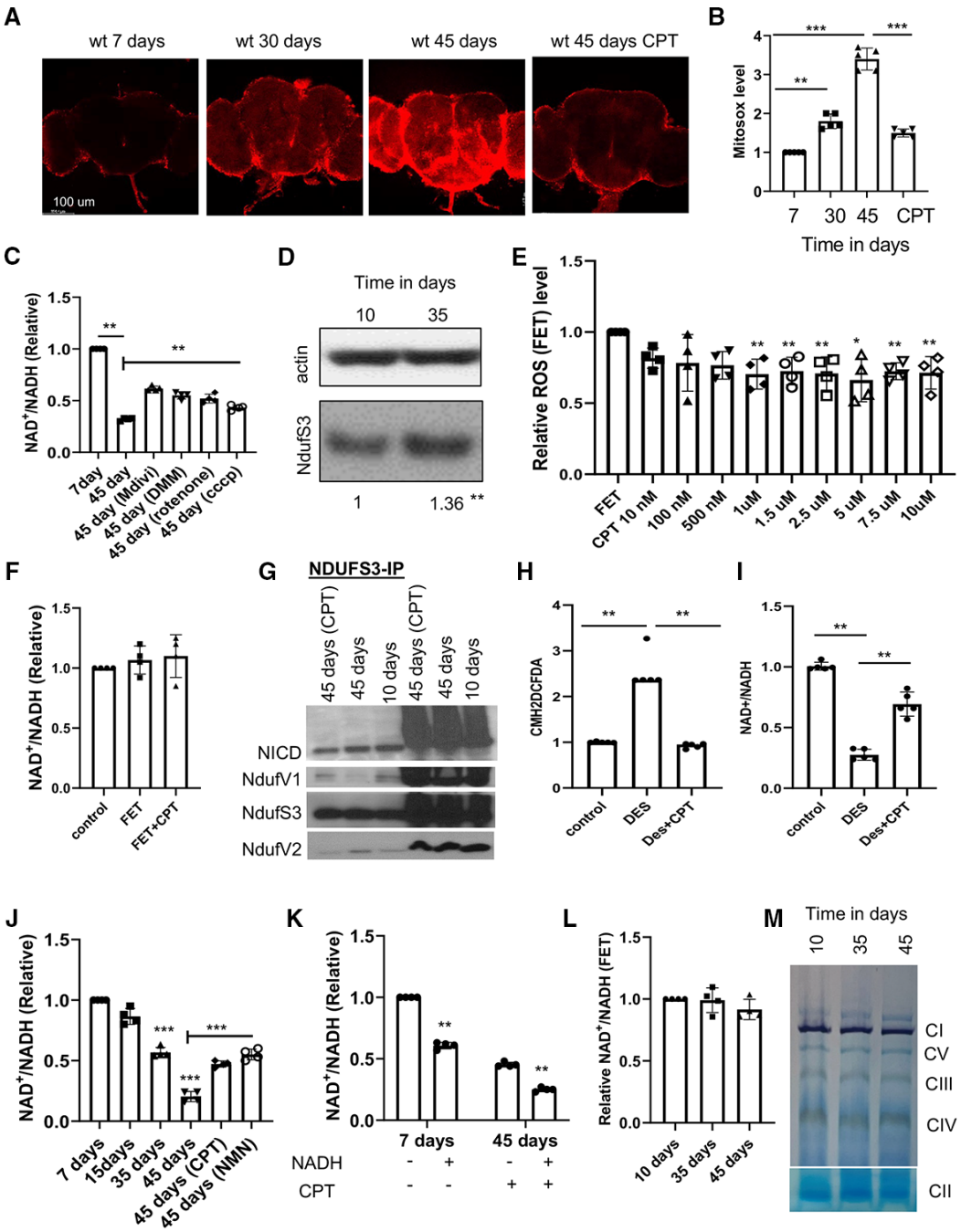
A
wt 7 days
wt 30 days
wt 45 days
wt 45 days CPT
100 um
B
Mitosox level
Time in days
7
30
45
CPT
C
NAD+/NADH (Relative)
7day
45 day
45 day (Mdivi)
45 day (DMM)
45 day (rotenone)
45 day (cccp)
D
Time in days
10
35
actin
NdufS3
1
1.36
E
Relative ROS (FET) level
FET
CPT 10 nM
100 nM
500 nM
1uM
1.5 uM
2.5 uM
5 uM
7.5 uM
10uM
F
NAD+/NADH (Relative)
control
FET
FET+CPT
G
NDUFS3-IP
45 days (CPT)
45 days
10 days
45 days (CPT)
45 days
10 days
NICD
NdufV1
NdufS3
NdufV2
H
CMH2DCFDA
control
DES
Des+CPT
I
NAD+/NADH
control
DES
Des+CPT
J
NAD+/NADH (Relative)
7 days
15days
35 days
45 days
45 days (CPT)
45 days (NMN)
K
NAD+/NADH (Relative)
7 days
45 days
NADH
CPT
L
Relative NAD+/NADH (FET)
10 days
35 days
45 days
M
Time in days
10
35
45
CI
CV
CIII
CIV
CII

Figure EV1.

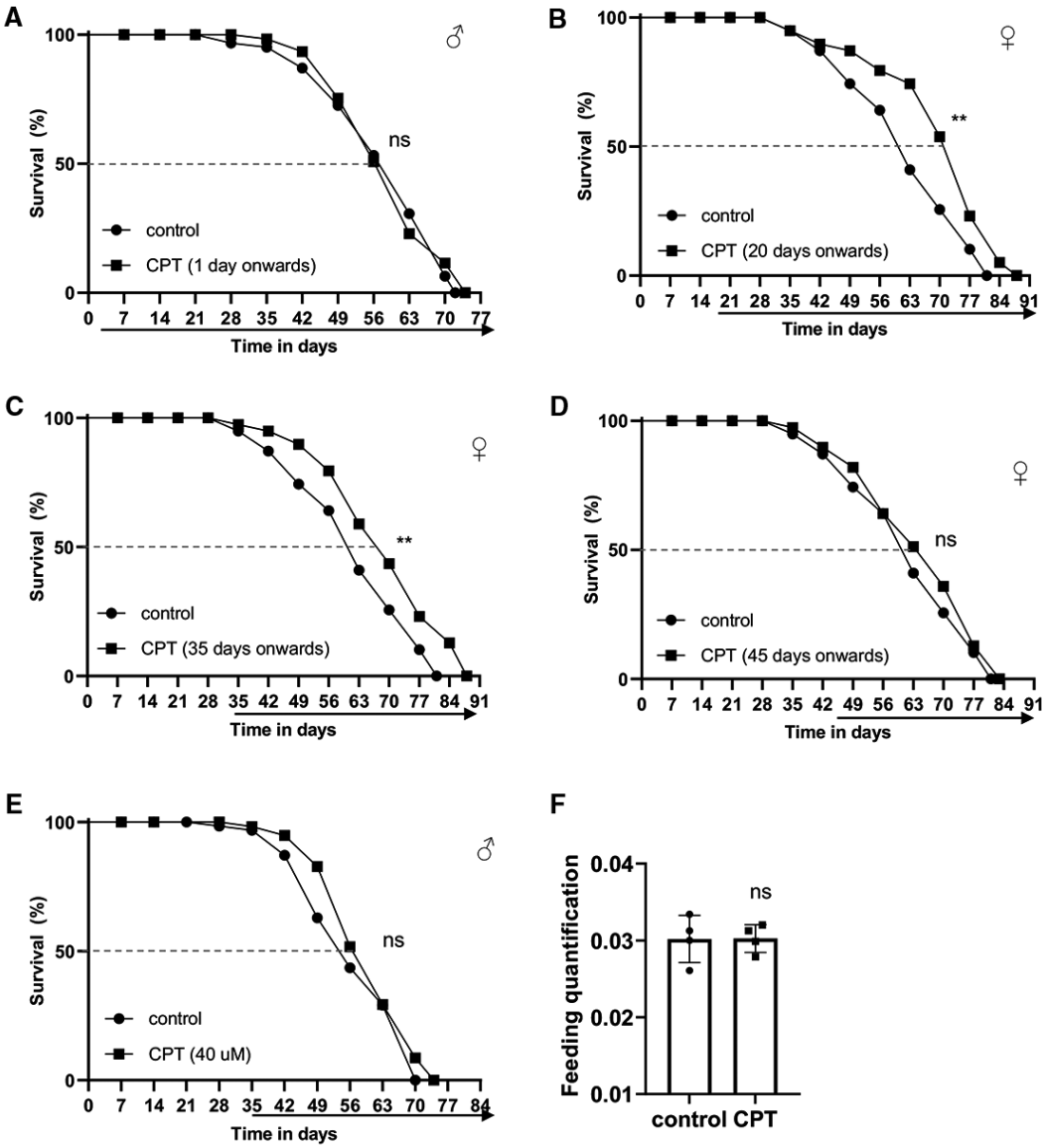

Figure EV2. Inhibition of RET by CPT treatment extends *Drosophila* lifespan.

A Survival curves comparing control flies and flies fed with food containing 10 μM CPT from day 1 onwards.

B–D Survival curves comparing overall survival (by logrank test) and maximum lifespan (by Wang–Allison test) of control flies and flies fed with food containing 10 μM CPT from 20 days (**P < 0.001 logrank, **P < 0.001 Wang–Allison) (B), 35 days (**P < 0.001 logrank, **P < 0.001 Wang–Allison) (C), and 45 days (ns) (D) onwards (n = 3 groups, 20–25 flies per group).

E Survival curves comparing control flies and flies fed with food containing 40 μM CPT from 35 days onwards (ns; n = 3 groups, 20–25 flies per group).

F Quantification of food consumption in control and CPT-fed flies (n = 4 groups, 20 per group).

Data information: Data are representative of at least three repeats. Data are shown as mean ± SEM in (F). Survival curves were analyzed with logrank test along with Wang–Allison test for maximum lifespan. Dashed lines mark time points of 50% survival. Asterisks indicate statistical significance (**P < 0.01, ns: not significant) in single-factor ANOVA with Scheffe's analysis as a *post hoc* test.

© 2023 The Authors

Figure EV3. Inhibition of RET by genetic manipulation of C-I subunits extends fly lifespan.

A, B Survival curves of control flies and flies with NDUFS2 (A), or NDUFS3 (B) knocked down in neurons ($n = 3$ groups, 20–25 flies per group).

C Quantification of food consumption in flies treated with CPT or NMN.

D Quantification of Smurf assay data in young flies, old flies, and old flies treated with CPT and NMN ($n = 3$ groups, 20 flies per group).

E–I Survival curves showing the lifespan effect of CPT in combination with NMN (E) (control vs NMN: $^{**}P < 0.01$ logrank, $^{**}P < 0.01$ Wang Alisson; CPT vs CPT + NMN, ns), mito-Tempo (F) (control vs mito-Tempo: $^{**}P < 0.01$ logrank, $^{**}P < 0.01$ Wang Alisson; CPT vs CPT + mito-Tempo: ns), melatonin (G) (control vs melatonin: $^{**}P < 0.01$ logrank, $^{**}P < 0.01$ Wang Alisson; CPT vs CPT+ melatonin: ns), FK866 (H) (control vs FK866: $^{**}P < 0.01$ logrank, $^{**}P < 0.01$ Wang Alisson; FK866 vs FK866 + CPT: ns), and paraquat (I) (control vs paraquat: $^{**}P < 0.01$ logrank, $^{**}P < 0.01$ Wang Alisson; paraquat vs paraquat+CPT: ns) ($n = 3$ groups, 20 flies per group).

Data information: Data are representative of at least three repeats. Data are shown as mean ± SEM in (C, D). Survival curves were analyzed with logrank test along with Wang–Allison test for maximum lifespan. Dashed lines mark time points of 50% survival. Asterisks indicate statistical significance ($^{**}P < 0.01$) in single-factor ANOVA with Scheffe's analysis as a *post hoc* test.

© 2023 The Authors

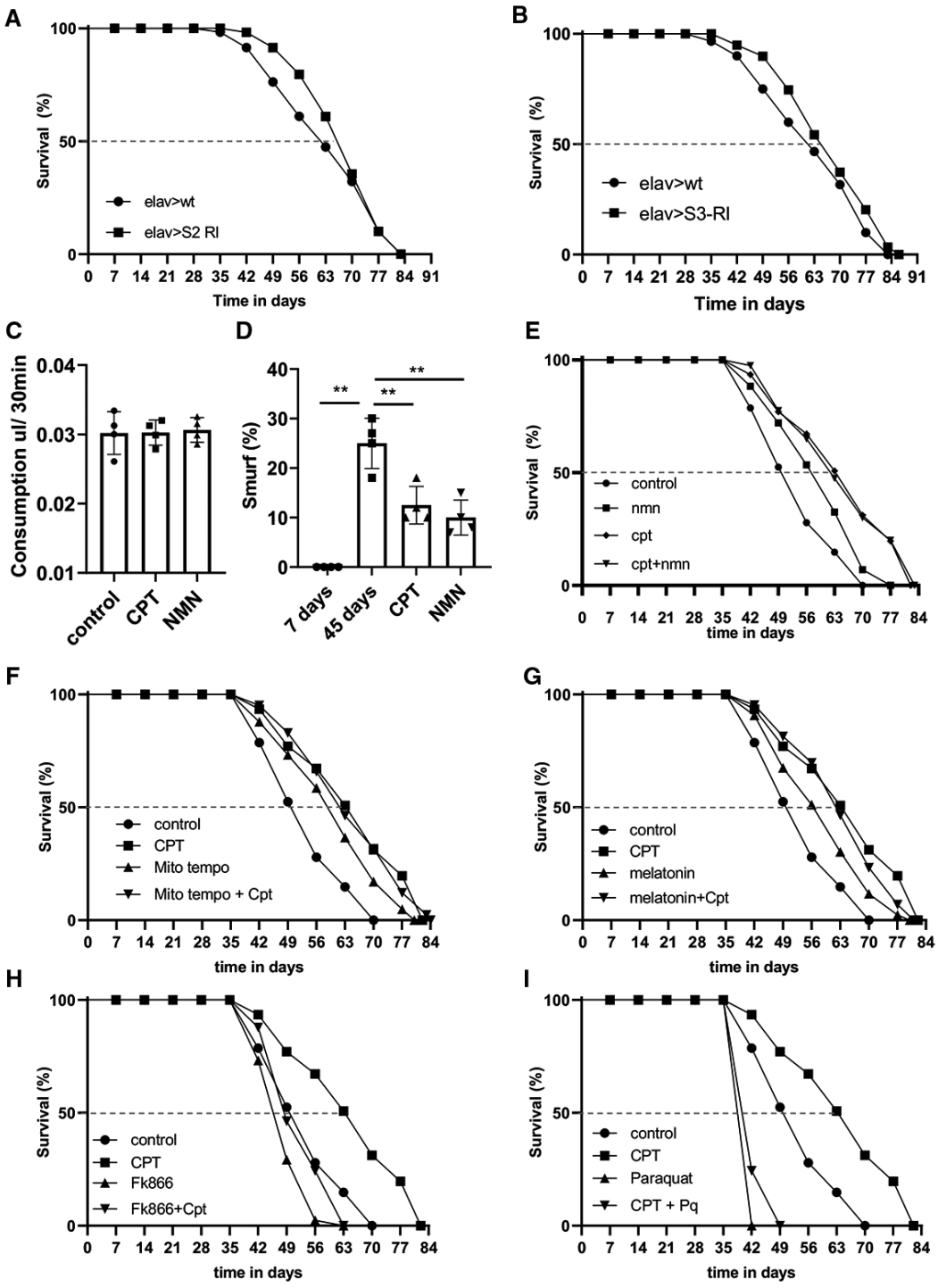

Figure EV3.

© 2023 The Authors

Figure EV4. Lifespan effect of RET inhibition by CPT is mediated by Sirtuin, Foxo, and autophagy pathways.

A, B Immunoblots showing the effect of aging on dFoxo acetylation (A), and effect of CPT treatment on dFoxo acetylation (B). dFoxo was immunoprecipitated from whole fly extracts and detected with anti-acetyl-K antibody.

C RT–PCR analysis of levels of the indicated transcripts in aged flies relative to young flies.

D Images and data quantification showing p62 staining in the brain of old flies and old flies treated with CPT ($n = 5$ per group).

E–I Survival curve showing the effect of NMN on lifespan in control (*Mhc* > *wt*) flies (**$P < 0.01$, logrank) (E), and NDUFS3-RNAi (F), Foxo-RNAi (**$P < 0.01$, logrank) (G), Sirt-2-RNAi (H), and ATG1-RNAi (**$P < 0.01$, logrank) (I) flies ($n = 3$ groups, 20 flies per group).

Data information: Data are representative of at least three repeats. Data are shown as mean ± SEM in (C, D). Survival curves were analyzed with logrank test along with Wang–Allison test for maximum lifespan. Dashed lines mark time points of 50% survival. Asterisks indicate statistical significance (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$) in single-factor ANOVA with Scheffe's analysis as a *post hoc* test.

© 2023 The Authors

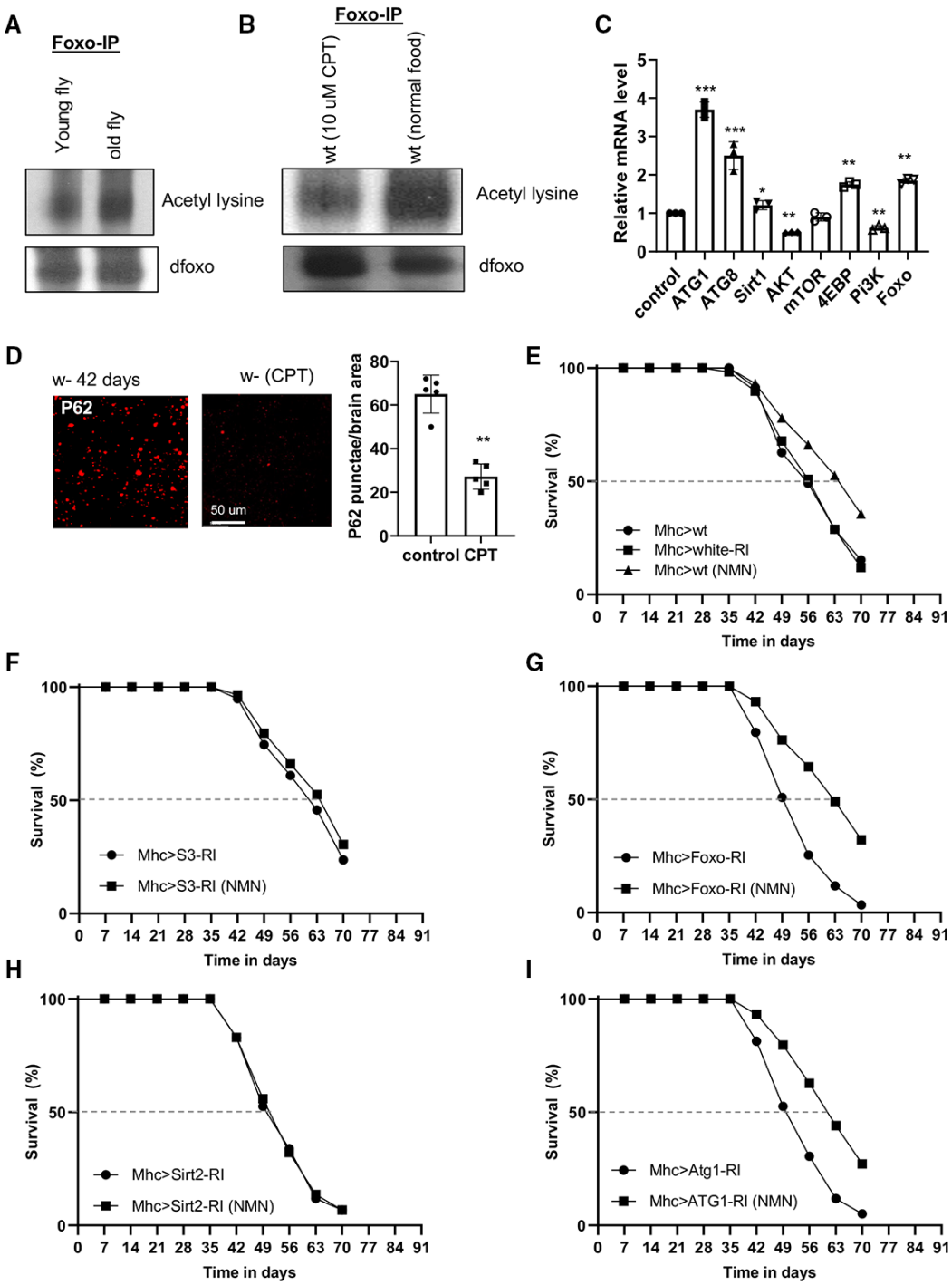

Figure EV4.

© 2023 The Authors

Figure EV5. Inhibition of RET rescues disease phenotypes of fly AD models.

A Staining images and data quantification showing the effect of CPT on ROS level in the brain of *elav* > *APP.BACE* flies ($n = 3$ set, 5 brains per set).
B Immunofluorescence images and data quantification showing the effect of CPT on mitochondrial morphology in DA neurons of *TH* > *APP.C99* flies co-expressing a mito-GFP reporter ($n = 5$ brains per group).
C Immunofluorescence images showing the effect of CPT treatment on lysosomal morphology in the muscle of *Mhc* > *APP.C99* flies.
D Immunofluorescence images showing the effect of CPT treatment on DA neuron number in the PPL1 cluster of *elav* > *APP.BACE* fly brain.
E, F Immunostaining showing the effect of NDUFS3-RNAi on 6E10-positive amyloid (E) and ubiquitin- and p62-positive protein aggregates (F) in the muscle of *Mhc* > *APP.C99* flies.
G Quantification of the effect of AOX or NMNAT overexpression on wing posture in *Mhc* > *APP.C99* flies ($n = 4$ groups, 20 flies per group).
H Immunoblots showing the effect of AOX or NMNAT overexpression on aberrant APP.C99 translation products in *Mhc* > *APP.C99* flies.
I Quantification of the effect of dFoxo overexpression on wing posture in *Mhc* > *APP.C99* flies ($n = 4$ groups, 20 flies per group).

Data information: Data are representative of at least three repeats. Data are shown as mean ± SEM. Asterisks indicate statistical significance (***$P < 0.001$,**$P < 0.01$) using single=factor ANOVA with Scheffe's analysis as a *post hoc* test.

© 2023 The Authors

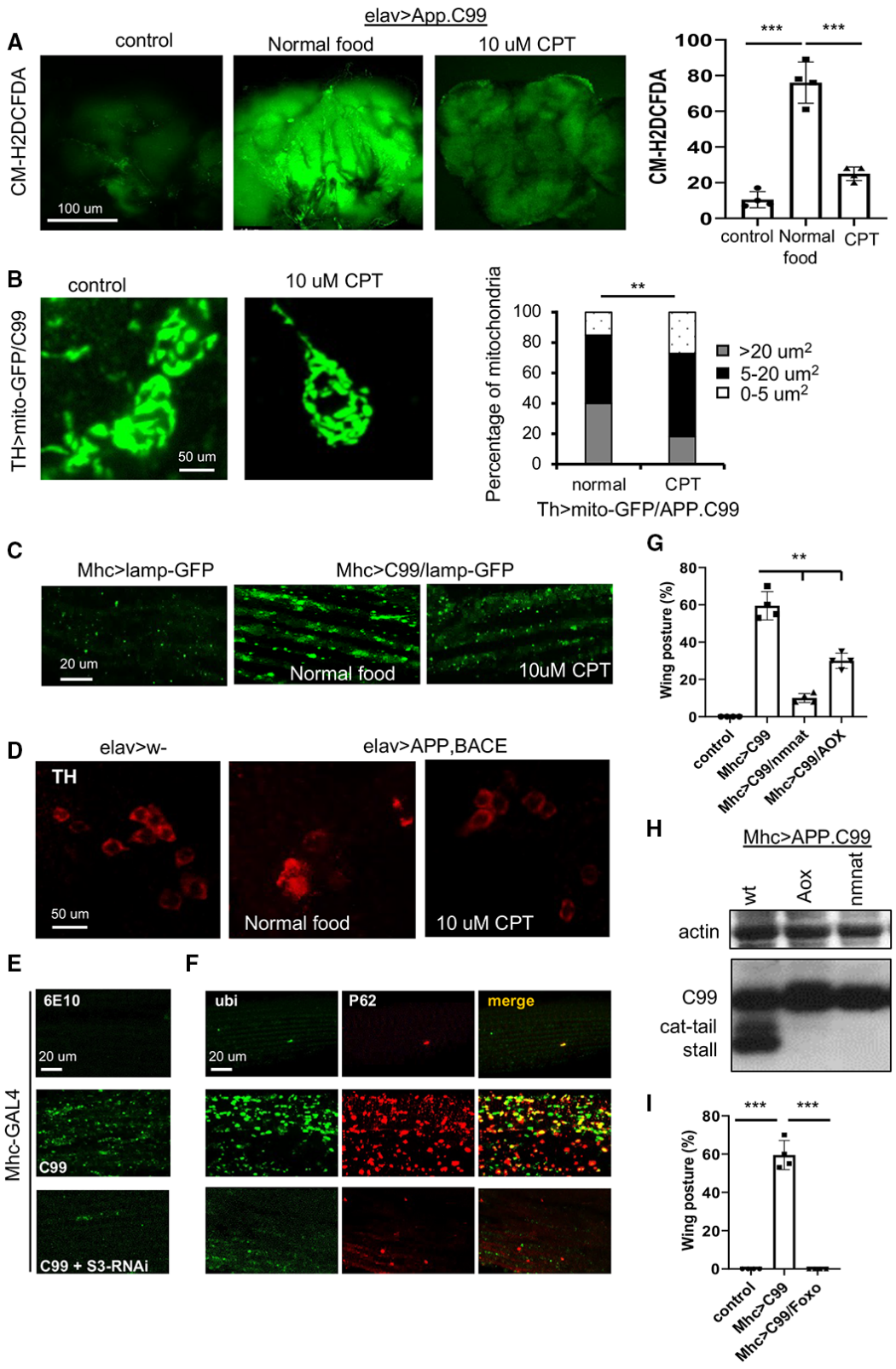

Figure EV5.

© 2023 The Authors