Reverse electron transfer is activated during aging and contributes to aging and age-related disease

APPENDIX

Appendix Figure S1. Effect of RET inhibition by CPT on healthspan in *Drosophila.*

(A) Images of Smurf assay of intestinal integrity in aged flies with or without CPT treatment. Scale bar: 1 mm.

(B) Images and data quantification showing accumulation of protein aggregates in the muscle of aged flies with or without CPT treatment (n=5 per group). Scale bar: 20 μm.

(C) Western blot analysis of ubiquitination level of young and aged muscle tissue and aged muscle tissue with CPT treatment.

(D) Images and data quantification showing muscle mitochondrial morphology in aged flies with or without CPT treatment (n=5 per group). Scale bar: 20 μm.

(E) Western blot analysis to show the mitochondrial mass in the thoracic muscle of NDUFS2, NDUFS3, and NDUFV1 knockdown flies.
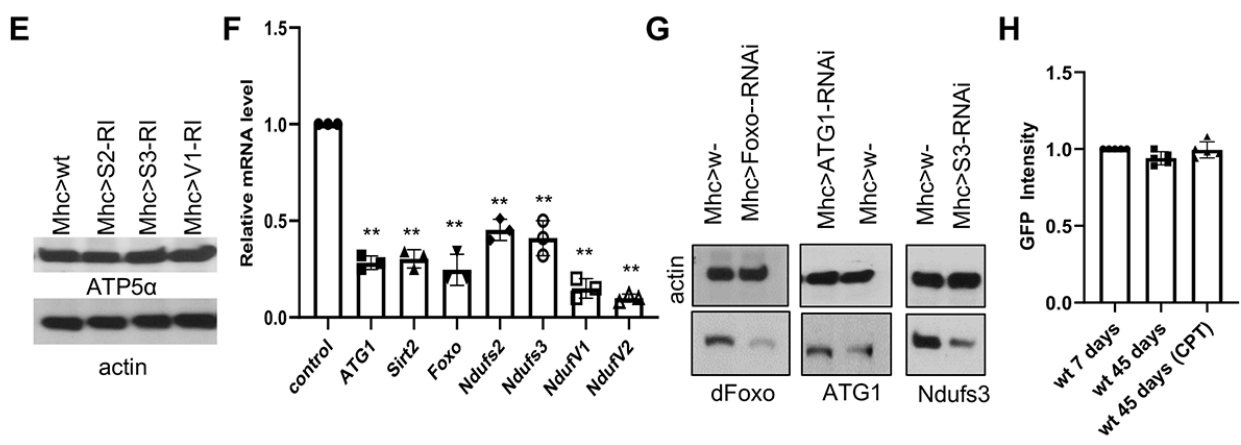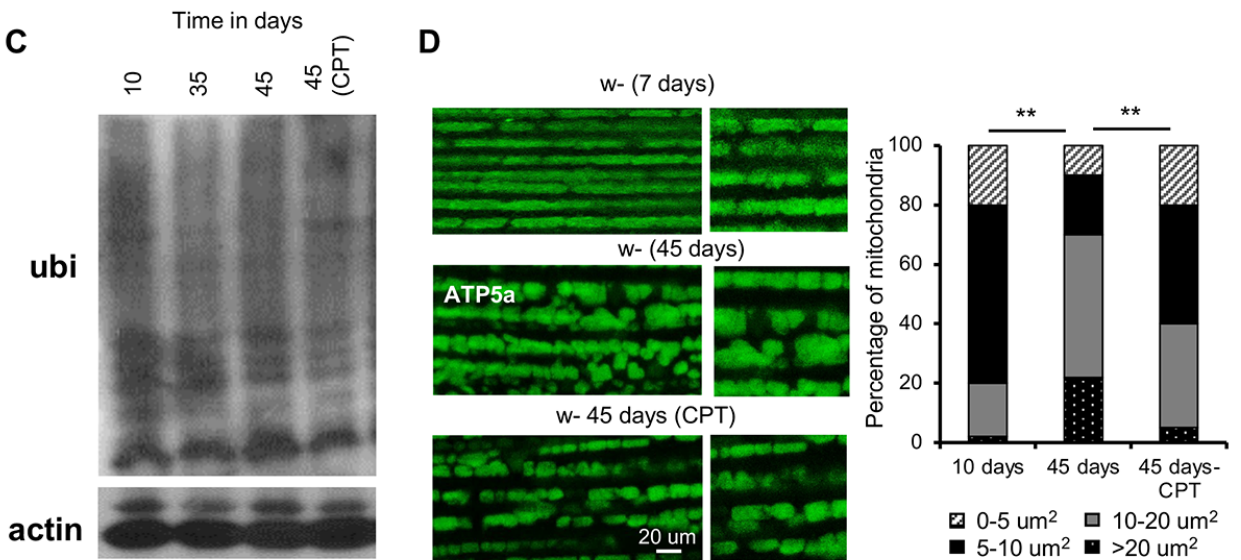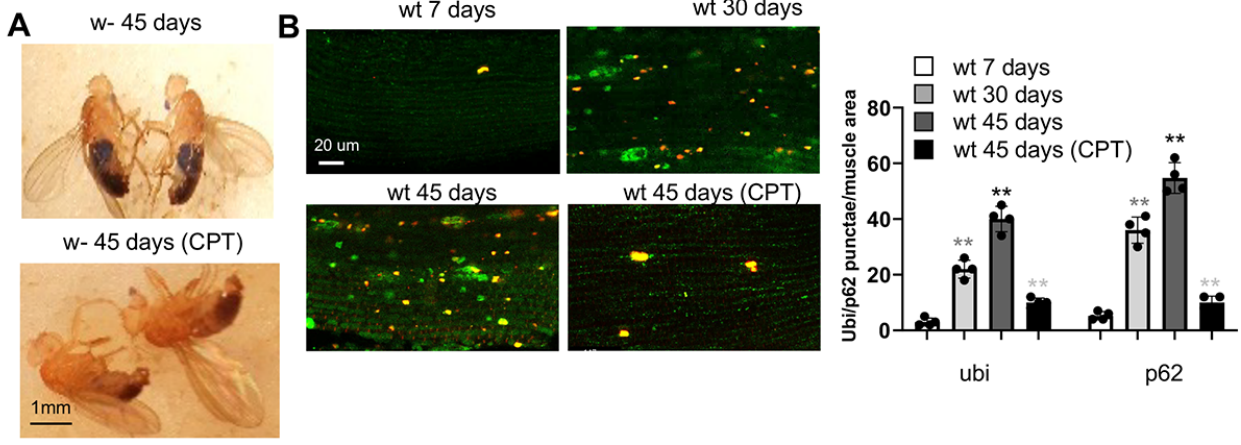
(F) Quantification of mRNA level by qRT-PCR to assess the knockdown efficiency of the various RNAi lines used (n=3 biological repeats).

(G) Western blot analysis showing protein knockdown efficiency of select RNAi lines.

(H) Quantification of mito-GFP signal intensity in DA neurons of young flies, old flies, and old flies treated with CPT (n=5 sets, 5 brain samples per set).

Data Information: Data are representative of at least three repeats. (B, D, F, H) 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.

Appendix Figure S1

A w- 45 days

w- 45 days (CPT)

1mm

B wt 7 days   wt 30 days

20 um

wt 45 days   wt 45 days (CPT)

□ wt 7 days
▨ wt 30 days
■ wt 45 days
■ wt 45 days (CPT)

Ubi/p62 punctae/muscle area

ubi   p62

C Time in days

10   35   45   45 (CPT)

ubi

actin

D w- (7 days)

ATP5a   w- (45 days)

w- 45 days (CPT)

20 um

Percentage of mitochondria

10 days   45 days   45 days-CPT

▨ 0-5 um^2 ■ 10-20 um^2
■ 5-10 um^2 ■ >20 um^2

E

Mhc>wt
Mhc>S2-RI
Mhc>S3-RI
Mhc>V1-RI

ATP5α

actin

F

Relative mRNA level

control ATG1 Sirt2 Foxo Ndufs2 Ndufs3 NdufV1 NdufV2

G

Mhc>w-
Mhc>Foxo--RNAi
Mhc>ATG1-RNAi
Mhc>w-
Mhc>w-
Mhc>S3-RNAi

actin

dFoxo   ATG1   Ndufs3

H

GFP Intensity

wt 7 days   wt 45 days   wt 45 days (CPT)

3

Appendix Figure S2. RET inhibition by CPT rescues disease phenotypes in mammalian AD models.

(A) Representative immunostaining of control, FAD, and DS iPSC-derived neurons with anti-MAP2 antibody.

(B) Images of CMH2DCFDA staining of H_2O_2 in control, FAD, and DS iPSC-derived neurons. Cells were treated with vehicle or 1 μM CPT for 72-96 hrs.

(C, D) Graphs of ROS tracing (C) and data quantification (D) showing effect of CPT on DES-induced RET-ROS production in BV-2 cells. (n=4 biological repeats).

(E) Quantification of effect of CPT treatment on NAD^+/NADH ratio in BV-2 cells (n=3 biological repeats).

Data Information: Data are shown as mean ± SEM. Asterisks indicate statistical significance (**$p < 0.01$, ***$p < 0.001$) in single factor ANOVA with Scheffe's analysis as a *post hoc* test (D) or Student's t-test (E). Scale bars: 5 μm in A and B.

Appendix Figure S2