

Expanded View Figures

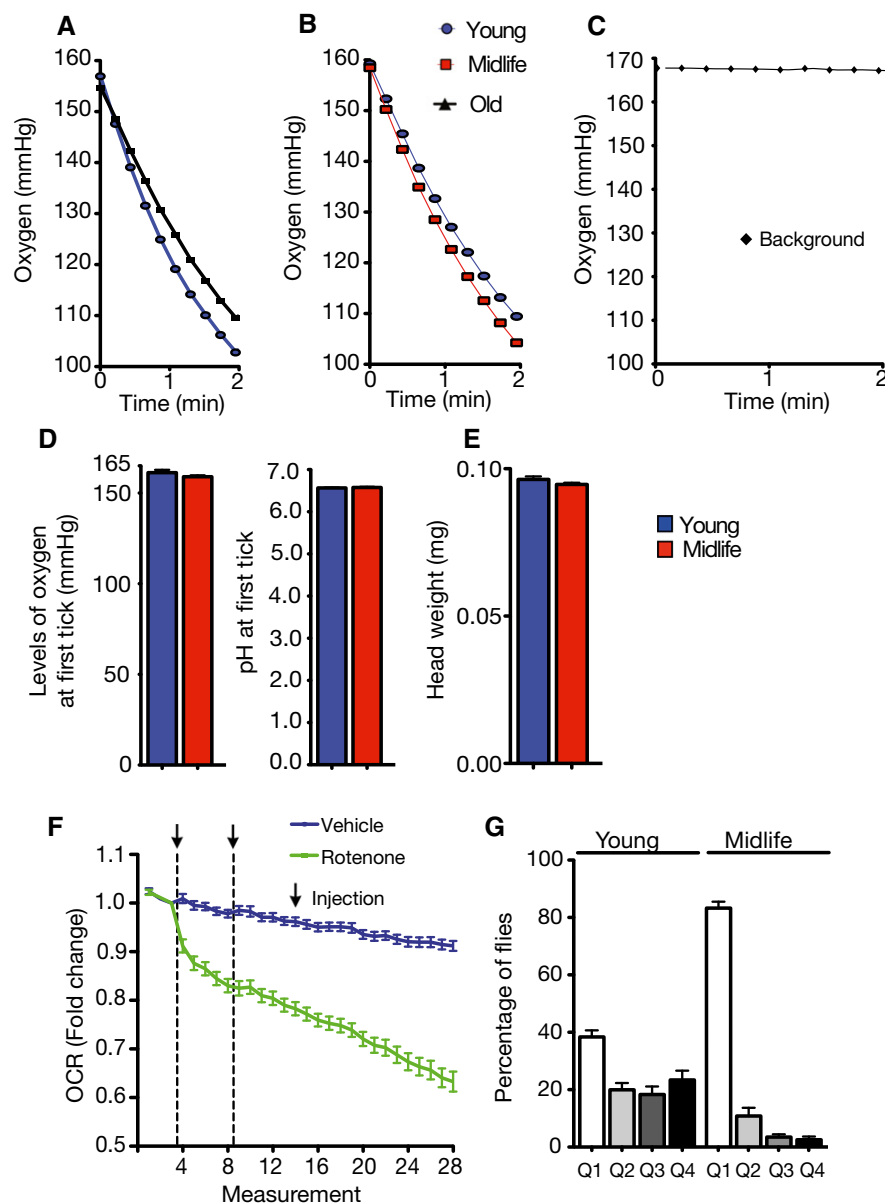


Figure EV1. Technical parameters in young and midlife flies.

- A Representative oxygen consumption rate profiles in young (blue circles) and old flies (black squares), showing lower consumption in the heads of 7-week-old flies. During an assay lasting 2 min, a series of 10 oxygen submeasurements (ticks) is taken. The slope of these 10 ticks is calculated to generate the oxygen consumption rate (OCR) measurement.
- B Representative oxygen consumption rate profiles in young (blue circles) and midlife flies (red squares), showing higher consumption in the heads of 4-week-old flies.
- C Negative control of wells containing buffer without any tissue. Background wells showed no changes in oxygen levels and served as background for all other measurements.
- D Oxygen levels during the first tick of each measurement series were similar in wells containing young and midlife male fly heads. The pH during the first tick was similar in the wells of the two groups. $N = 21$ young and $N = 24$ midlife.
- E Measurements of fly male heads show similar weight in young and midlife males. $N = 6-7$ per group.
- F Addition of 5 μM rotenone, a complex I inhibitor, significantly decreases oxygen consumption. This result demonstrates that the rate of oxygen consumption being measured relates to oxygen consumed by the respiratory complexes. Data were normalized to the data point before the addition of rotenone. Dashed line indicates addition of the drug.
- G Fly activity assay after 3 s. Distribution of flies across four sections of a vial following 3 s of flipping (Q4 is the section at the top of the vial). $N = 7$ young and $N = 5$ midlife.

Data information: Error bars in each graph indicate the SEM.

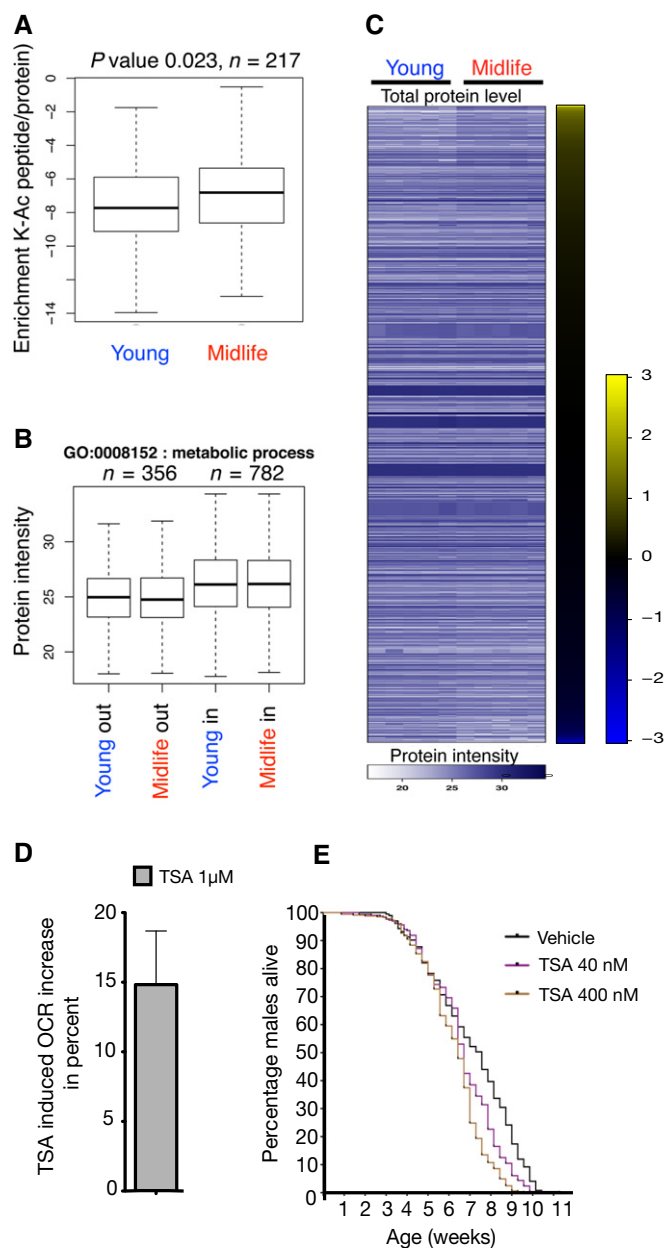


Figure EV2. Proteomic analysis indicates similar profile of metabolic proteins in young and midlife flies and the KDAC inhibitor TSA increases OCR and has negative impact on life span in *Drosophila*.

- A** Quantification of lysine acetylation levels by mass spectrometry (MS) reveals a general increase in protein acetylation in midlife flies. The box represents the interval that contains the central 50% of the data with the line indicating the median. The length of the whiskers is 1.5 times the interquartile distance (IQD).
- B** Proteome analysis of the input samples by MS shows similar absolute levels for proteins involved in metabolic (in) and non-metabolic (out) processes in 1-week-old (young) and 4-week-old (midlife) flies. The box represents the interval that contains the central 50% of the data with the line indicating the median. The length of the whiskers is 1.5 times the interquartile distance (IQD).
- C** Proteome heat map comparing the protein intensities (see Materials and Methods) of the input. $N = 5$ per group. fc , fold change.
- D** Trichostatin A (TSA) induces an increase in oxygen consumption in the heads of young flies. Data were normalized to the measurement prior to addition of TSA. $N = 5$ per group. Error bar indicates the SEM
- E** 400-nM TSA-treated male flies in mixed male/female population reach the end of the premortality plateau phase at a similar age of 4 weeks. However, 400-nM TSA-treated flies show reduced median and maximal life span. Survival for control = 53 days, 40 nM TSA = 47 days, 400 nM TSA = 45, $N = 368$ vehicle, 296 (40 nM TSA) and 326 (400 nM TSA). Log-rank test of TSA40 nM, $\chi^2 = 24.33$, $P < 0.0001$. Log-rank test of TSA400 nM, $\chi^2 = 79.55$, $P < 0.0001$.

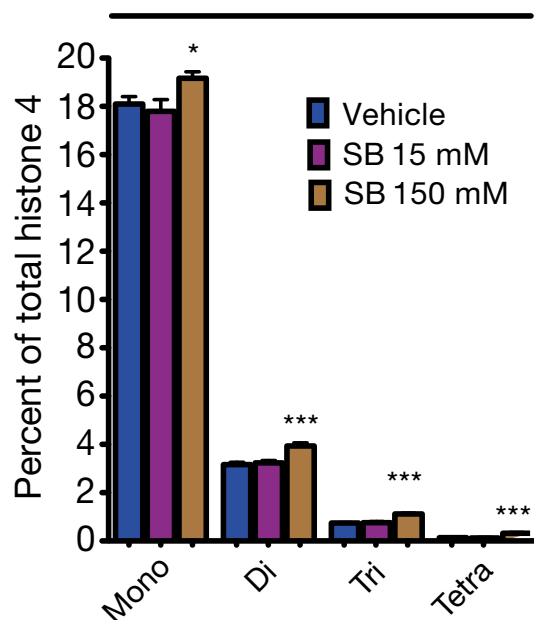


Figure EV3. Daily consumption of the KDAC inhibitor sodium butyrate (SB) increases histone acetylation in 11-day-old flies.

Quantification of a mono-, di-, tri-, and tetra-acetylation states of histone H4 in vehicle and SB-treated flies, showing an increase in all states in high SB treatment. Unpaired two-tailed *t*-tests were used for calculating the *P*-values. **P* < 0.05, ****P* < 0.001. Error bars indicate the SEM.

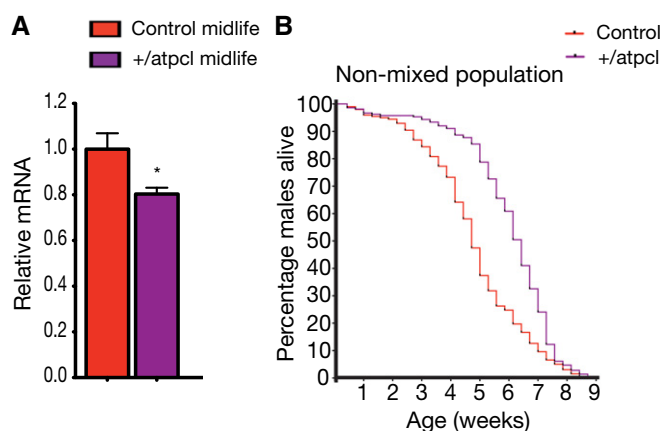


Figure EV4. Reduced ATPCL prolongs life span of male flies living without females.

A The mutant *atpcl* allele we used (Bloomington 11055) consists of a P-element insertion between the 5' UTR of isoforms E/D and the 5' UTR of isoforms F/G. RT-PCR reveals a 20% decrease in midlife *atpcl* heterozygote mutants (+/*atpcl*) compared to wild-type control. *N* = 5–6 per group. Unpaired two-tailed *t*-tests were used for calculating the *P*-values. **P* < 0.05. Error bars indicate the SEM.

B +/*atpcl* male flies in homogenous male population show increased mean life span and latency at arrival to accelerated population decline. Survival 90% control = 18 days, *atpcl* = 28 days. Median survival for control = 33 days, *atpcl* = 45 days, *N* = 198 (control) and 212 (*Atpcl*). Log-rank test, $\chi^2 = 43.99$, *P* < 0.0001. Unpaired two-tailed *t*-tests were used for calculating the *P*-values.

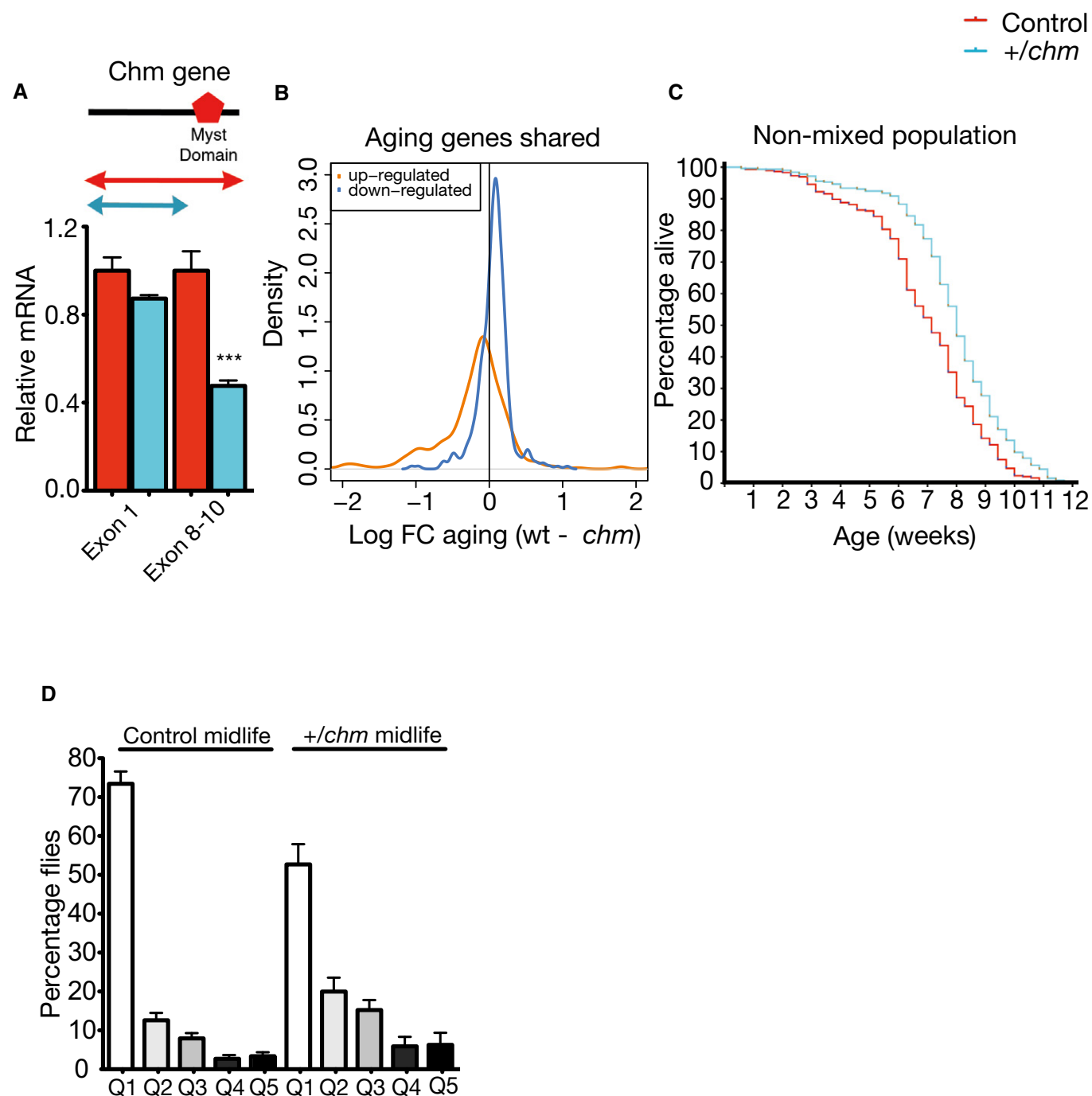


Figure EV5. Reduced Chameau enzymatic activity prolongs life span of male flies living without females.

A The mutant *chm* allele we used removes the catalytic, histone acetyltransferase MYST domain spanning exons 8–10. $N = 6$ –8 per group. *** $P < 0.001$. Unpaired two-tailed t-tests were used for calculating the P -values.

B Density plot showing decreased fold change of shared upregulated genes in *chm* mutants.

C *Chm* male flies in homogenous male population show increased mean life span and latency at arrival to accelerated population decline. Survival 90% control = 25 days, *chm* = 41 days. Median survival for control = 50 days, *chm* = 56 days, $N = 296$ (control) and 318 (*chm*). Log-rank test, $\chi^2 = 36.83$, $P < 0.0001$.

D Fly activity assay after 8 s. Distribution of flies across five sections of a vial following 8 s of flipping (Q5 is the section at the top of the vial). $N = 7$.

Data information: Error bars in each graph indicate the SEM.