Supplemental figure legends

Supplementary Figure 1. (A, B) Heat map showing the metabolites significantly affected in both yw and OreR females between 1 and 7 weeks of age.

Supplementary Figure 2. Venn diagrams for commonly changed metabolites between OreR and yw males (A), OreR and yw females (B), OreR females and males (C), yw females and males (D), OreR and yw males and females (E). (F) Principal component analysis of 1, 4 and 7 week yw and OreR males.

Supplementary Figure 3. (A) Relative mRNA levels of dAhcyL1 from wandering third instar larval stage expressing white (w) RNAi (control), dAhcyL1 RNAi-1 and dAhcyL1 RNAi-2 under constitutive Actin5c-Gal4 driver. Means ± SD. (B) Relative mRNA levels of dAhcyL2 from wandering third instar larval stage expressing w RNAi, dAhcyL2 RNAi-1 or dAhcyL2 RNAi-2 under constitutive Actin5c-Gal4 driver. Means ± SD. (C) Relative mRNA levels of Ahcy13, dAhcyL1 and dAhcyL2 in 1 week old control (B3) and long-lived (O1, O3) males. Means ± SD. (D) Relative mRNA levels of Itp-r83A from wandering third instar larval stage expressing Itp-r83A RNAi under constitutive Actin5c-Gal4 driver. **p<0.01. Means ± SD. (E) Relative mRNA levels of RnrL from wandering third instar larval stage expressing RnrL RNAi under constitutive Actin5c-Gal4 driver. **p<0.01. Means ± SD. (F) Relative mRNA levels of Ahcy13 from wandering third instar larval stage expressing w RNAi (control) or Ahcy13 RNAi-1 under the constitutive Actin5c-Gal4 driver. *p < 0.05. Means ± SD.

Supplementary Figure 4. Ubiquitous adult-onset expression of dAhcyL1 RNAi does not affect lifespan in males under oxidative stress.

Supplementary Figure 5. Coimmunoprecipitation of overexpressed HA- and FLAG-tagged dAHCYL1 and Ahcy13 in Drosophila S2R cells. To test whether dAHCYL1 and Ahcy13 can form a heteromultimeric complex, we overexpressed HA- and FLAG-tagged dAHCYL1 and Ahcy13 in
Drosophila S2R cells. As expected, FLAG-tagged dAHCYL1 and Ahcy13 were coimmunoprecipitated with their HA-tagged forms confirming that they form a homomultimeric complexes. Moreover, FLAG-tagged dAHCYL1 was coimmunoprecipitated with HA-tagged Ahcy13 suggesting that they form a heteromultimeric complexes as well.

**Supplemental List 1. 21 common metabolites in "OreR Females 1-7w", "OreR Males 1-7w", "yw Females 1-7w" and "yw Males 1-7w":**

1-Methyl-Histidine, betaine, Methionine sulfoxide, Carbamoyl phosphate, 2-Aminooctanoic acid, methionine, Cystathionine, allantoin, valine, UDP-N-acetyl-glucosamine, glutathione, UDP-D-glucose, glutathione-nega, Spermidine, cytidine, O8P-O1P, D-sedoheptulose-1-7-phosphate, tryptophan, L-arginino-succinate, SBP, acetoacetate

**Supplemental_Methods**

**qRT-PCR.** Total RNA was extracted with the TRIzol reagent (Life Technologies), followed by DNase digestion using RQ1 RNase-Free DNase (Promega). Total RNA was reverse transcribed with the iScript cDNA synthesis kit (Bio-Rad). qRT-PCR was performed with the iQ SYBR Green Supermix (Bio-Rad) and a CFX96 Real-Time PCR Detection System (Bio-Rad). RpL32 and alpha-Tubulin 84B were used as a normalization reference. Relative quantitations of mRNA levels were determined with the comparative C_T method.

**Antibodies and Immunoblot Analyses.** Total histone antibody and H3K4Me3 antibody were obtained from Abcam, and the tubulin antibody from Sigma-Aldrich. For immunoblot analyses, 10 flies were grinded in bead beader in RIPA (Cell Signaling) lysis buffer. Whole-cell lysates were resolved by electrophoresis, and proteins were transferred onto PVDF membranes
(Immobilon P; Millipore), blocked in Tris-buffered saline Tween-20 buffer (Cell Signaling Technology) containing 2.5% dry milk, and probed with the indicated antibodies in this buffer.

**Climbing Assays.** Negative geotaxis assays were performed as previously described (Rhodenizer et al., 2008). Flies were gently tapped to the bottom of an empty plastic vial, and the number of flies that climbed to the top of the vial after 10s was scored.

**Oxidative stress.** For measuring resistance to oxidative stress induced by methyl viologen (Sigma), 40d old males were exposed to 10 mM methyl viologen in 5% sucrose solution. Dead flies were scored periodically.

**Targeted Mass Spectrometry and data analyses.** 20 flies per sample (5 biological replicates) were collected and intracellular metabolites were extracted using 1.6 mL of cold (-80°C) 80% (v/v) aqueous methanol. Flies were homogenized using 0.5 mm zirconium beads and insoluble material in lysates was centrifuged at 5,000g for 5 min, and the resulting supernatant was evaporated using a speed vac. Samples were re-suspended using 20 μL HPLC grade water for mass spectrometry. 10 μL were injected and analyzed using a 5500 QTRAP triple quadrupole mass spectrometer (AB/SCIEX) coupled to a Prominence UFLC HPLC system (Shimadzu) via selected reaction monitoring (SRM) of a total of 254 endogenous water soluble metabolites for steady-state analyses of samples. Some metabolites were targeted in both positive and negative ion mode for a total of 287 SRM transitions using positive/negative polarity switching. ESI voltage was +4900V in positive ion mode and −4500V in negative ion mode. The dwell time was 3 ms per SRM transition and the total cycle time was 1.55 seconds. Approximately 10-14 data points were acquired per detected metabolite. Samples were delivered to the mass spectrometer via normal phase chromatography using a 4.6 mm i.d x 10 cm Amide Xbridge HILIC column (Waters Corp.) at 350 μL/min. Gradients were run starting from 85% buffer B (HPLC grade acetonitrile) to 42% B from 0-5 minutes; 42% B to 0% B from 5-16 minutes; 0% B
was held from 16-24 minutes; 0% B to 85% B from 24-25 minutes; 85% B was held for 7 minutes to re-equilibrate the column. Buffer A was comprised of 20 mM ammonium hydroxide/20 mM ammonium acetate (pH=9.0) in 95:5 water:acetonitrile. Peak areas from the total ion current for each metabolite SRM transition were integrated using MultiQuant v2.0 software (AB/SCIEX).

The resulting raw data from the MultiQuant software were uploaded in metaboanalyst (http://www.metaboanalyst.ca/MetaboAnalyst/) and subsequent data processing and analyses were performed using this tool. Metabolites that were not detected in 40% of the samples were excluded from the analysis. Data were normalized to the median (per sample) and processed through log transformation. Heat map and hierarchical clustering was generated using Pearson correlations and Ward’s method. Metabolite Set Enrichment Analysis (MSEA) was performed using Metaboanalyst which uses the KEGG (http://www.genome.jp/kegg/pathway.html) pathway database. Metabolite sets containing at least 5 compounds were employed in the analysis. MSEA calculates hypergeometrics test scores based on cumulative binominal distribution.

**Primers for qRT-PCR**

Primers for qRT-PCR were designed in accordance to (Hu et al., 2013).

| Gene    | FlyPrimerBank ID | Forward Primer                     | Reverse Primer                     |
|---------|------------------|------------------------------------|------------------------------------|
| CG9977  | PP26084          | GGCGAGACGGAAGAGGACT                | AGAGAGCTGATAGAGACG                 |
| (dAhcyL1)|                 |                                    | GGTG                               |
| Ahcy89E | PP37398          | AAGAGTGCATTTGGAAGGCG               | ACCCTTATGGGGCTTCT                  |
| (dAhcyL2)|                 |                                    | CATC                               |
| Ahcy13  | PP36991          | AGCCCTGAACATGATCCTG                | CGACCCTCCTTTGAACAT                 |
| #  | CG#     | RNAi or ORF line ID | CTTGT  | CGGTTTCTTGAGCACACTCG  |
|----|---------|---------------------|--------|-----------------------|
| 1  | CG10160 | HMS00039            | CTGAACTTCTTAGGCCG | ACAG      |
| 2  | CG10621 | HMS02412            | CCTGTTACTATCACCCTGCAAG | AGGATGGCGTAGTCGGG |
|    | CG10621 | FL4205              | AT      | GACAGTATCGGCTCAGG    |
| 3  | CG10623 | HMC02683            | GAA     | GAGAATCCGACGGGTTTGA  |
| 4  | CG10692 | HMS10667            |         | GACAGTATCGGCTCAGG    |
| 5  | CG10692 | HMS02599            |         | GACAGTATCGGCTCAGG    |
|    | CG10692 | HMC03318            |         | GACAGTATCGGCTCAGG    |
| 6  | CG10903 | 10903R-1 (II)       |         | GACAGTATCGGCTCAGG    |
| 7  | CG11134 | JF03052             |         | GACAGTATCGGCTCAGG    |
|    | CG11334 | v109672             |         | GACAGTATCGGCTCAGG    |
|    | CG11334 | FL0052              |         | GACAGTATCGGCTCAGG    |
| 8  | CG11654 | HMC03222            |         | GACAGTATCGGCTCAGG    |
| 9  | CG12173 | HMJ21083            |         | GACAGTATCGGCTCAGG    |

Supplemental Table 1. List of lines tested for the effect on lifespan:
|   |       |         |
|---|-------|---------|
| 10| CG13598 | HMC03239 |
| 11| CG1461 | HMC03212 |
| 12| CG14882 | HMC04302 |
| 13| CG15100 | ORF-3xHA |
|    |         | F000999  |
|    | CG15100 | HMC04303 |
| 14| CG17230 | 17230R-2 |
|    | CG17230 | HMC04543 |
| 15| CG1753 | HMS03028 |
| 16| CG2674 | JF03351  |
| 17| CG2759 | HMS00017 |
| 18| CG31322 | HMC04301 |
| 19| CG32068 | v106831  |
|    | CG32068 | HMC04304 |
| 20| CG4067 | HMS02643 |
| 21| CG4210 | HMC02982 |
| 22| CG4300 | v105402  |
|    | CG4300 | FL2564   |
|    | CG4300 | CG4300   |
|    | CG4300 | HMC03665 |
| 23| CG4743 | HMC04321 |
| 24| CG4802 | 4802R-2 (II) |
|    | CG4802 | FL3987   |
|    | CG4802 | CG4802   |
| 25| CG5029 | v101753/KK |
|    |     |        |
|----|-----|--------|
| 26 | CG5345 | HMS03027 |
| 27 | CG5493 | v50637 |
| 28 | CG6188 | HMC03498 |
|    | CG6188 | FL2157 |
|    | CG6188 | CG6188 |
|    | CG6188 | HMS02473 |
| 29 | CG6584 | HMC03659 |
| 30 | CG7266 | HMS02570 |
| 31 | CG7560 | 7560R-1 (II) |
| 32 | CG8290 | JF02410 |
| 33 | CG8327 | 8327R-2 (III) |
|    | CG8327 | FL1085 |
|    | CG8327 | CG8327 |
| 34 | CG8956 | 8956R-1 |
|    | CG8956 | HMJ23469 |
| 35 | CG9977 | HM05009 |
|    | CG9977 | G4143 |
|    | CG9977 | HMC04803 |
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A

OreR ♂ 7/1 w  yw ♂ 7/1 w

56 (33.3%)
51 (30.4%)
61 (36.3%)

B

OreR ♀ 7/1 w  yw ♀ 7/1 w

67 (37.2%)
54 (30.0%)
59 (32.8%)

C

OreR ♀ 7/1 w  OreR ♂ 7/1 w

61 (36.3%)
60 (35.7%)
47 (28.0%)

D

yw ♀ 7/1 w  yw ♂ 7/1 w

43 (27.7%)
70 (45.2%)
42 (27.1%)

E

OreR ♂ 7/1 w  yw ♀ 7/1 w

19 (9.5%)
6 (3.1%)
17 (8.7%) 22 (10.9%)

F

Score plot

1w yw ♂
4w yw ♂
7w yw ♂

1w OreR ♂
4w OreR ♂
7w OreR ♂
