

## **Supplementary Materials and Methods**

**Strains:** *P{PZ}rpd3<sup>04556</sup> ry<sup>506</sup>/TM3, ry<sup>RK</sup> Sb<sup>1</sup> Ser<sup>1</sup>* (Bloomington Reference 11633) and *y<sup>1</sup> w<sup>67c23</sup>*; *P{lacW}Tor<sup>k17004</sup>/CyO* (Bloomington Reference 11218) were obtained from the *Drosophila* stock centre (Bloomington, Indiana). *cn EcR<sup>V559fs</sup> bw/SM6G* was a kind gift from Dr Anne Simon [12]. *mth* and *w<sup>1118</sup>* strains were graciously provided by the late Dr Seymour Benzer [9]. The *CyO/Sp;Dk/Ubx* quad-balancer was made in our laboratory.

**Crossing into *w<sup>1118</sup>* control background:** *mth* was backcrossed to *w<sup>1118</sup>* four times. The mutation was tracked using the linked miniwhite marker. *EcR*, *Tor*, and *rpd3* were placed in the *w<sup>1118</sup>* background except for the foreign chromosome they were on using the *CyO/Sp;Dk/Ubx* quad-balancer strain. This involved a total of three crosses. (not shown)

**Crossing mutants:** Males and females of the four mutants and the *w<sup>1118</sup>* control strain were crossed to each other to generate single mutants and double mutants. Mutations were tracked either by their selective marker (i.e. *mth* had a miniwhite), or by selecting against the balancer chromosome from the quad-balancer.

**Longevity assay:** Progeny were collected from the mutant crosses less than one day after they eclosed. Male and female adults of the desired genotype were placed into separate 50mL cylindrical vials, with a maximum of 25 flies in each vial. Flies were kept in sealed plastic bags to protect against mites, and placed in a humidified, temperature-controlled incubator at 25°C ± 1°C. The medium was changed and the number of dead flies was recorded every two days. The longevity assay was carried out for 80 days and there were a total of 42 assays carried out (one for each possible gender, genotype, and maternal genotype). Starting on day 12 of the assay, pieces of paper towel were added to the media to absorb any moisture that accumulated during the two days between changes.

**Fly food:** Standard fly media was obtained from the Lipschitz lab at the University of Toronto. 10mL of media was used per vial.

**Statistical Analysis:** Kaplan-Meier survival plots were created using GraphPad Prism (Graphpad software, 2007). Statistical significance was determined by applying the Log-rank test taking into account multiple comparisons (i.e. Single comparison p-values were multiplied by 42 to account for the 42 different assays).

**Test for Longevity Gene Synergism:** If double mutants had significantly increased lifespan relative to both of their corresponding single mutants (by the Log-rank test above), then their % lifespan increase relative to control was compared to both of their single mutants' % increase relative to control. If double mutant % lifespan increase was greater than the product of the single mutant % increases, then the mutations were deemed synergistic. Note that statistical significance could not be evaluated as only one trial was done for each double mutant.

### **Supplementary Discussion**

#### ***Assay Design and Possible Improvements***

Since time constraints prevented thorough testing of the assay before application to the longevity mutants, there were a number of confounding factors present that were only realized after the experiment was completed. As noted above, changing fly density as flies died may have been a confounding factor. At decreased density, flies have less competition for food and space and the media is cleaner. In the *Drosophila* ageing literature, the space/fly in longevity assays is not mentioned, so empirical evidence of a significant effect has not been noted. Ways to control for this include giving each fly its own small vial, perhaps using centrifuge tubes.

Another problem was the wetness of the media, which caused flies to become trapped. In particular, the less active flies became trapped, so this introduced a selection against lethargic flies. The addition of paper towels did not change this situation. It is possible that having a humidified incubator led to the wet media, so it would probably be better to use a drier incubator.

Other problems include that since mutants were collected up to one day after eclosing, many of the females became pregnant. Although pregnant females were present in all mutants, pregnancy and egg laying may effect the mutants differently. Therefore, it would be advisable to collect virgin females (which would lead to lower number of flies to assay) or no females at all (which has been done in a number of studies, but prevents assaying gender-specific effects).