

# Supplementary Materials for

## **Social Perception of Young Adults Prolongs the Lifespan of Aged *Drosophila***

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### **This PDF file includes:**

Supplemental Figures 1 to 7

Raw images of Western blots (Ref(2)P, ubH2A, Atg8a-I/II, and GAPDH)

## Supplemental Figures

### Supplemental Fig. 1. The youth impacts can be observed in $w^{1118}$ and Oregon-R flies

(a) Survival curves of the Canton-S flies. Flies were kept at the density of 20 flies per vial and their survival was recorded every two days. The median lifespan of Canton-S was about 62 days in male flies (n=189) and 60 days in female flies (n=184). (b) 40d-old Oregon-R or (c)  $w^{1118}$  flies were Co-housed with 1d-old young flies at the ratio of 1:3. The lifespan of individual flies was recorded and analyzed. In both WT lines, the mean lifespan of aged flies was notably increased to different levels, except the case of Oregon-R male flies. Results were expressed as means  $\pm$ SEM. Each column contains results of 50 flies from 10 independent assays. (d) Summary of results shown in (Supplementary Fig. 1b-1c). (e) Survival analysis of 40d-old Canton-S flies co-housed with 1d-old young flies at the ratio of 1:3 (8 aged vs. 24 young). Columns were expressed as means  $\pm$ SEM. (f) Summary of results shown in Figs. 1b-1f. (g) Summary of results shown in Figs. 1h-1i. P values: ns>0.1234; <0.0021 \*\*; <0.0002 \*\*\*; \*\*\*\* <0.0001. p values of the columns were annotated by the Mann-Whitney test. p values of the survival curves were annotated by the Log-rank (Mantel-Cox) test.

### Supplemental Fig. 2. Survival analyses of 30d- and 50d-old flies that are co-housed with young flies

Summary of results shown in Figs. 2a-2d.

### Supplemental Fig. 3. The lifespan of experimental co-housed young flies is not affected by the aged flies

(a) Survival curves of the experimental 1d-old young flies (Exp) (three WT species were shown: Canton-S, Oregon-R, and  $w^{1118}$ ) that were co-housed with 40d-old aged flies at the 1:3 ratio. There is no significant differences between the Exp flies and the control flies (Cont; 20 flies/vial). p values were annotated by the Log-rank (Mantel-Cox) test. In all cases, p values were greater than 0.05. (b)

Summary of results shown in (a). **(c and d)** Survival curves of Canton-S 1d-old young flies that were co-housed with 40d-old aged flies at the ratio of 1:9, 1:3, 1:1, and 3:1. Columns were expressed as means  $\pm$ SEM. P values: ns>0.1234; <0.0332 \*; <0.0021 \*\*; <0.0002 \*\*\*. P values of the columns were annotated by the Kruskal-Wallis test and followed by Dunn's multiple comparison test. P values of the survival curves were annotated by the Log-rank (Mantel-Cox) test. **(e)** Summary of results shown in (c and d).

**Supplemental Fig. 4. The lifespan extension phenotype is observed in the aged flies that are co-housed with**

**flies of selected chronological ages**

Summary of results shown in Figs. 3b-3d.

**Supplemental Fig. 5. Results of co-housing experiments using aged *or83b* and *ppk23* mutant flies**

**(a)** Summary of results shown in Fig. 5A. **(b)** Summary of results shown in Figs. 5b-5g.

**Supplemental Fig. 6. Explore the lifespan modulatory effects of cuticular extracts collected from young flies**

**(a)** The experimental illustration describes the processes of retrieving cuticular extracts from 1~2d-old Canton-S flies and the subsequent culturing conditions. **(b)** To explore the potential source of youth impacts conferred by the young flies, a single Canton-S fly was cultured in a vial containing the regular cornmeal food and a piece of filter paper rinsed with cuticular extracts isolated from three 1d to 2d-old same gender flies (+ Cuticular extracts). The fly was transferred to another vial containing cornmeal food and the freshly prepared cuticular extract-rinsed filter paper every two days. For the hexane control experiments, a single fly was cultured with the filter paper rinsed with hexane (+ Hexane). Another control experiment included only a single Canton-S fly without any

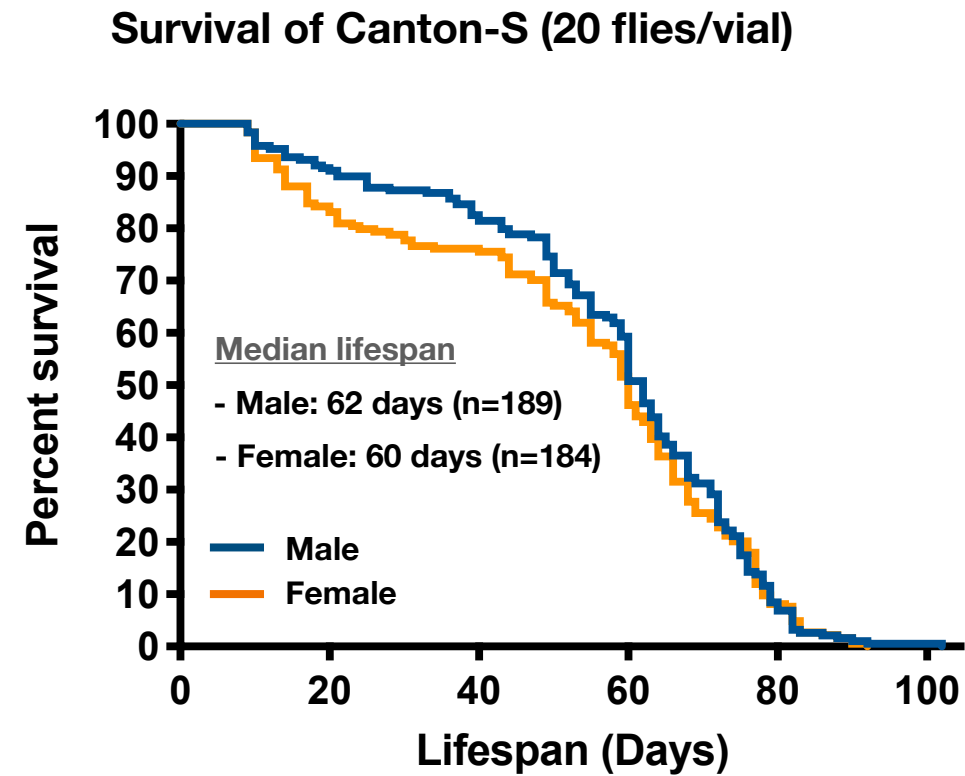
treatment (Control). Results were expressed as means  $\pm$ SEM. P values were annotated by the Kruskal-Wallis test and followed by Dunn's multiple comparison test (each column, n=10). P values: ns>0.1234; <0.0332 \*; <0.0002 \*\*\*.

**Supplemental Fig. 7. Co-housing experiments using the young CHC-deficient flies**

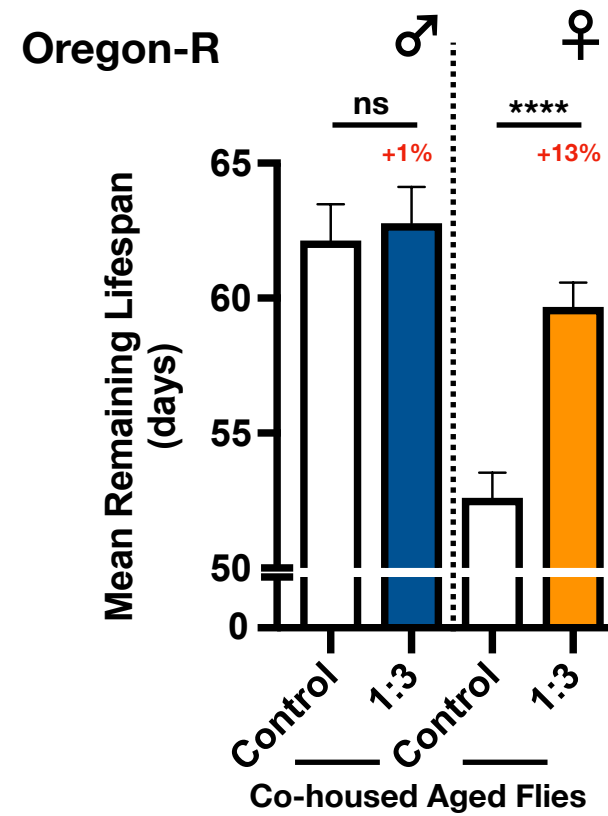
Summary of results shown in Figs. 6a-6d.

# Supplementary Figure 1

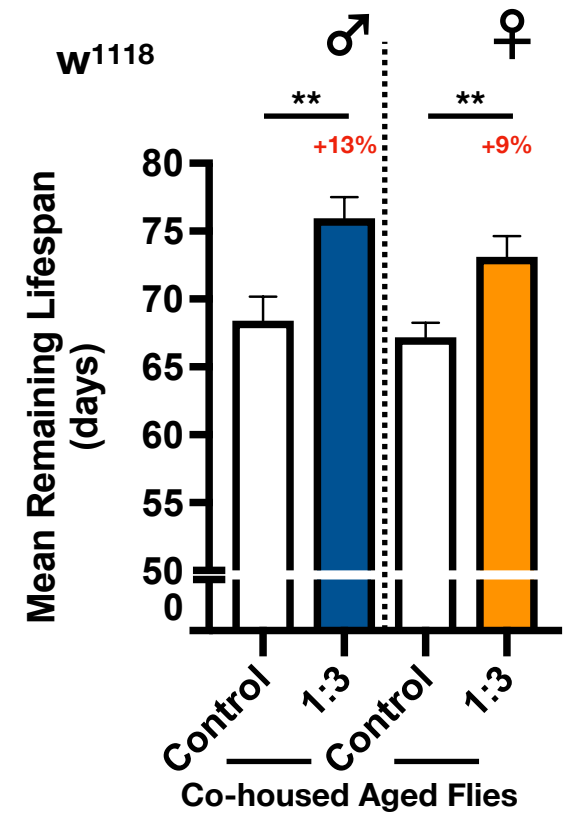
**a**



**b**



**c**

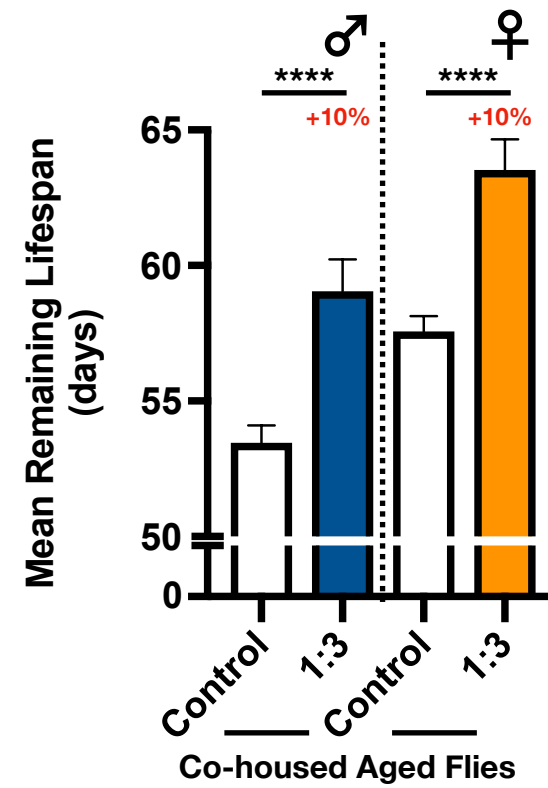
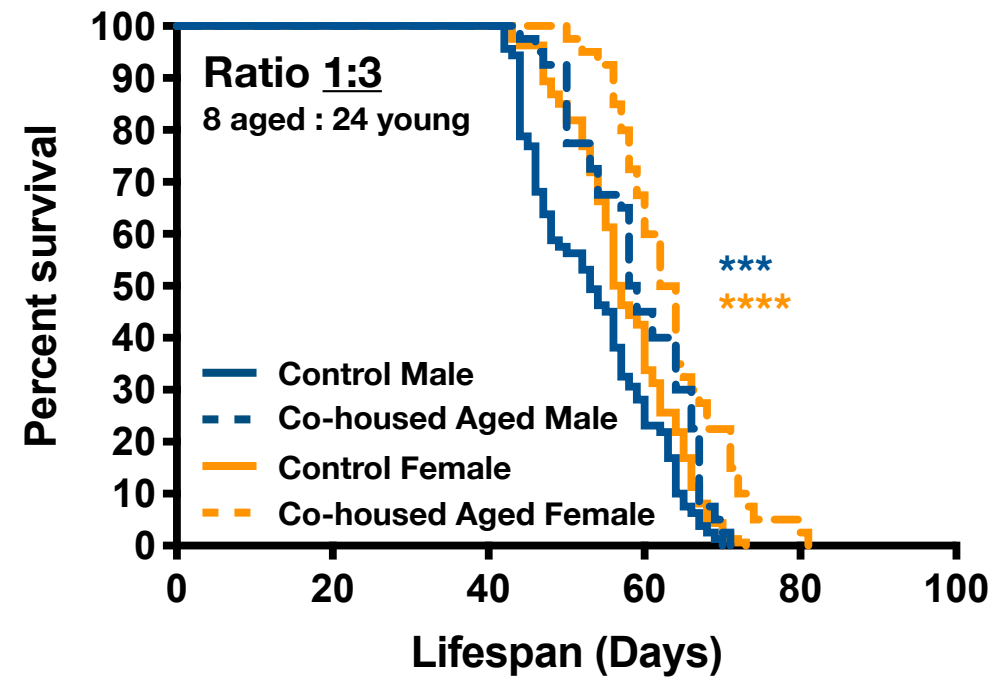


**d**

| Mean Lifespan (Days) ± SEM | Control      | n= | Co-housed Aged (40D) Flies | n= |
|----------------------------|--------------|----|----------------------------|----|
| <b>Male</b>                |              |    |                            |    |
| Canton-S                   | 59.76 ± 1.58 | 50 | 68.30 ± 1.36               | 50 |
| Oregon-R                   | 62.14 ± 1.34 | 50 | 62.78 ± 1.35               | 50 |
| w <sup>1118</sup>          | 68.40 ± 1.77 | 60 | 75.94 ± 1.57               | 50 |
| <b>Female</b>              |              |    |                            |    |
| Canton-S                   | 57.74 ± 1.39 | 50 | 72.08 ± 1.49               | 50 |
| Oregon-R                   | 52.60 ± 0.93 | 50 | 59.68 ± 0.90               | 50 |
| w <sup>1118</sup>          | 67.18 ± 1.07 | 60 | 73.10 ± 1.52               | 50 |

# Supplementary Figure 1

e



Ratio 1:3  
8 aged : 24 young

Control

Co-housed Aged (40D) Flies

|        | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) ± SEM | n=  | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) ± SEM | n= |
|--------|---------------------------|-------------------------------|-----|---------------------------|-------------------------------|----|
| Male   | 53                        | 53.46 ± 0.65                  | 160 | 58.5                      | 59.05 ± 1.18                  | 40 |
| Female | 56.5                      | 57.58 ± 0.56                  | 160 | 63                        | 63.53 ± 1.13                  | 40 |

# Supplementary Figure 1

**f**

|           | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) ± SEM | n= |
|-----------|---------------------------|-------------------------------|----|
| Male      |                           |                               |    |
| Control   | 58.5                      | 60.08 ± 1.76                  | 50 |
| Ratio 1:1 | 62.5                      | 61.10 ± 1.37                  | 50 |
| Control   | 60                        | 59.76 ± 1.58                  | 50 |
| Ratio 1:3 | 69                        | 68.30 ± 1.36                  | 50 |
| Control   | 59                        | 58.35 ± 2.44                  | 20 |
| Ratio 1:9 | 68.5                      | 71.80 ± 2.81                  | 20 |
| Control   | 62                        | 62.84 ± 1.82                  | 50 |
| Ratio 3:1 | 63                        | 63.26 ± 1.47                  | 50 |
| Female    |                           |                               |    |
| Control   | 61.5                      | 62.40 ± 1.57                  | 50 |
| Ratio 1:1 | 68                        | 64.88 ± 1.38                  | 50 |
| Control   | 57                        | 57.74 ± 1.39                  | 50 |
| Ratio 1:3 | 71                        | 72.08 ± 1.49                  | 50 |
| Control   | 60                        | 61.20 ± 2.72                  | 20 |
| Ratio 1:9 | 72                        | 71.50 ± 2.19                  | 20 |
| Control   | 63                        | 64.48 ± 1.26                  | 50 |
| Ratio 3:1 | 67                        | 67.20 ± 1.58                  | 50 |

g

|                                         | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) ± SEM | n= |
|-----------------------------------------|---------------------------|-------------------------------|----|
| Male                                    |                           |                               |    |
| Control                                 | 60                        | 59.76 ± 1.58                  | 50 |
| Aged (40D) Flies<br>+ Fresh Young Flies | 60.5                      | 61.46 ± 1.54                  | 50 |
| Female                                  |                           |                               |    |
| Control                                 | 57                        | 57.74 ± 1.39                  | 50 |
| Aged (40D) Flies<br>+ Fresh Young Flies | 67.5                      | 67.46 ± 1.22                  | 50 |

## Supplementary Figure 2

|                               | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) $\pm$ SEM | n= |
|-------------------------------|---------------------------|-----------------------------------|----|
| Male                          |                           |                                   |    |
| Control                       | 57                        | 55.24 $\pm$ 2.18                  | 49 |
| Co-housed Aged (30D)<br>Flies | 57                        | 57.22 $\pm$ 2.19                  | 49 |
| Female                        |                           |                                   |    |
| Control                       | 61                        | 59.56 $\pm$ 1.48                  | 50 |
| Co-housed Aged (30D)<br>Flies | 64                        | 64.86 $\pm$ 1.88                  | 50 |

|                               | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) $\pm$ SEM | n= |
|-------------------------------|---------------------------|-----------------------------------|----|
| Male                          |                           |                                   |    |
| Control                       | 61                        | 62.40 $\pm$ 1.36                  | 50 |
| Co-housed Aged (50D)<br>Flies | 64                        | 64.08 $\pm$ 1.19                  | 50 |
| Female                        |                           |                                   |    |
| Control                       | 61.5                      | 63.90 $\pm$ 1.42                  | 50 |
| Co-housed Aged (50D)<br>Flies | 70.5                      | 71.24 $\pm$ 1.71                  | 50 |

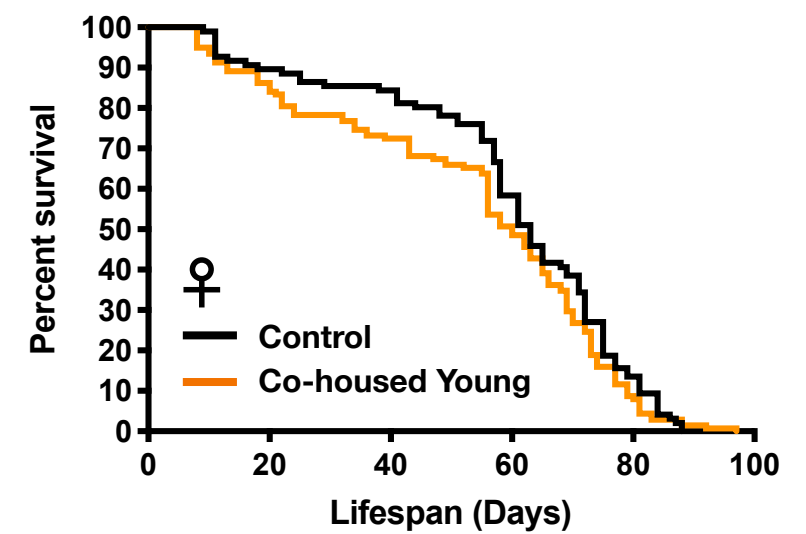
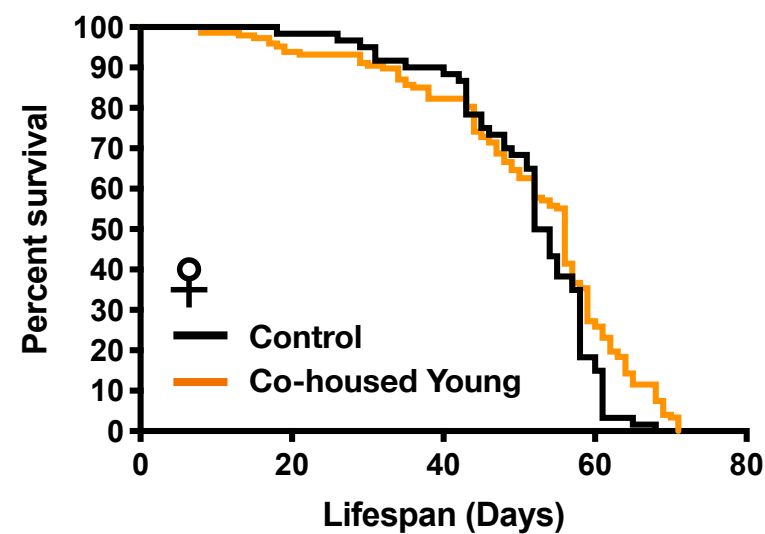
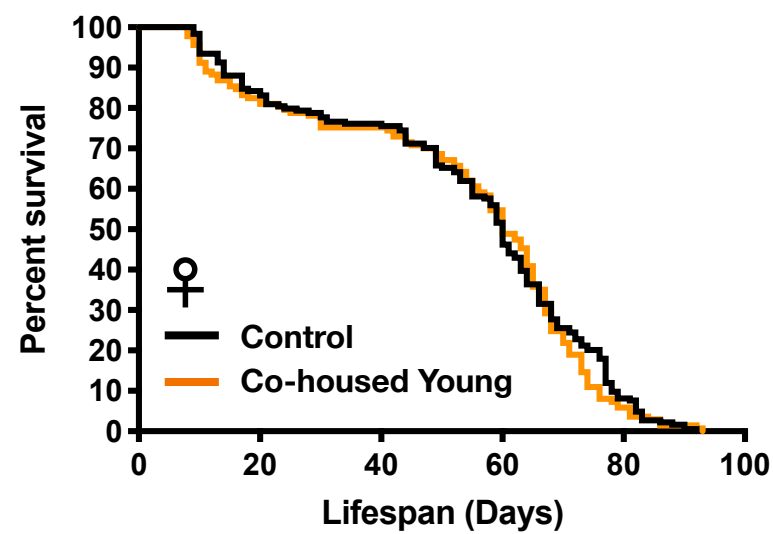
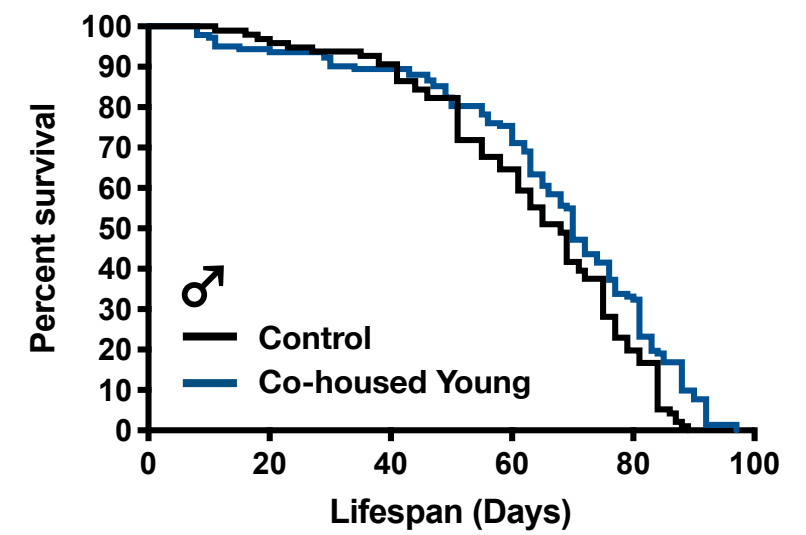
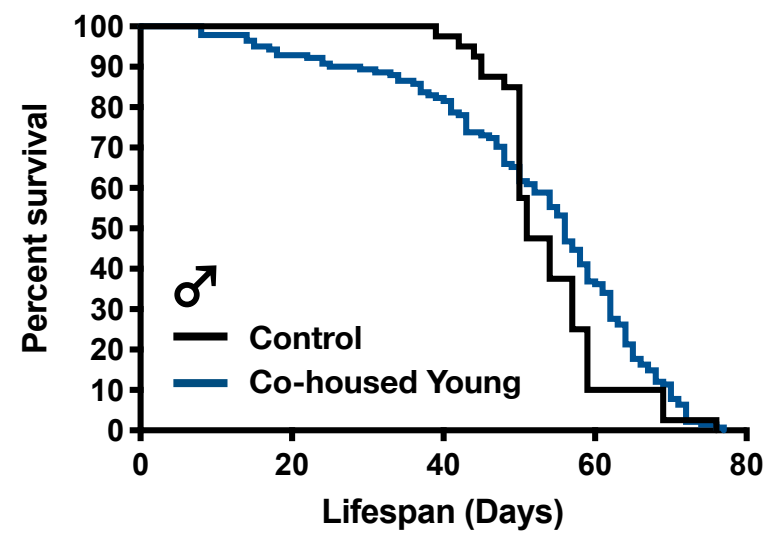
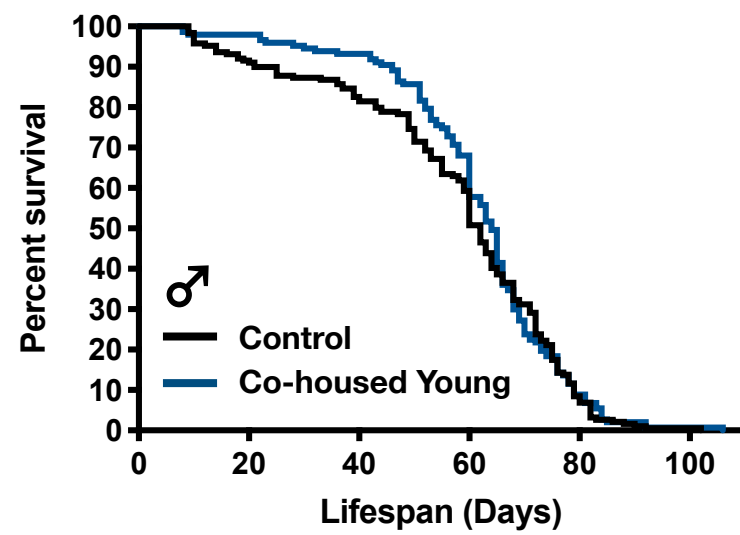
# Supplementary Figure 3

**a**

**Canton-S**

**Oregon-R**

**w<sup>1118</sup>**



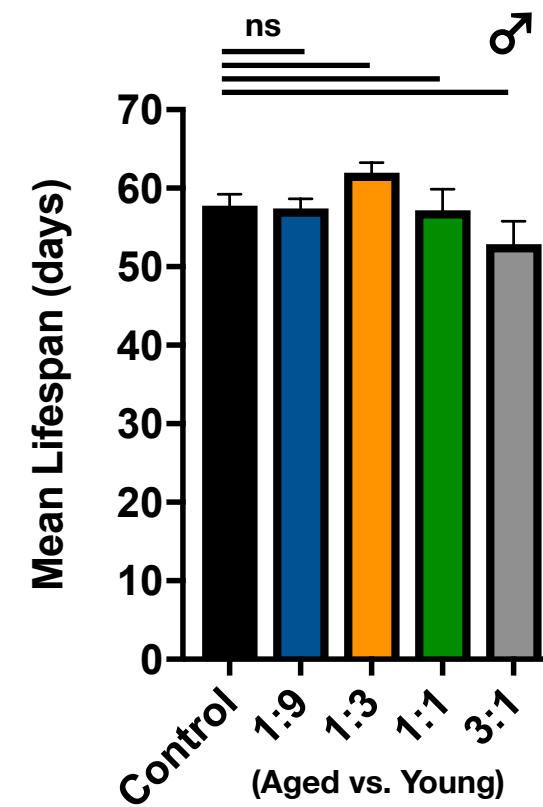
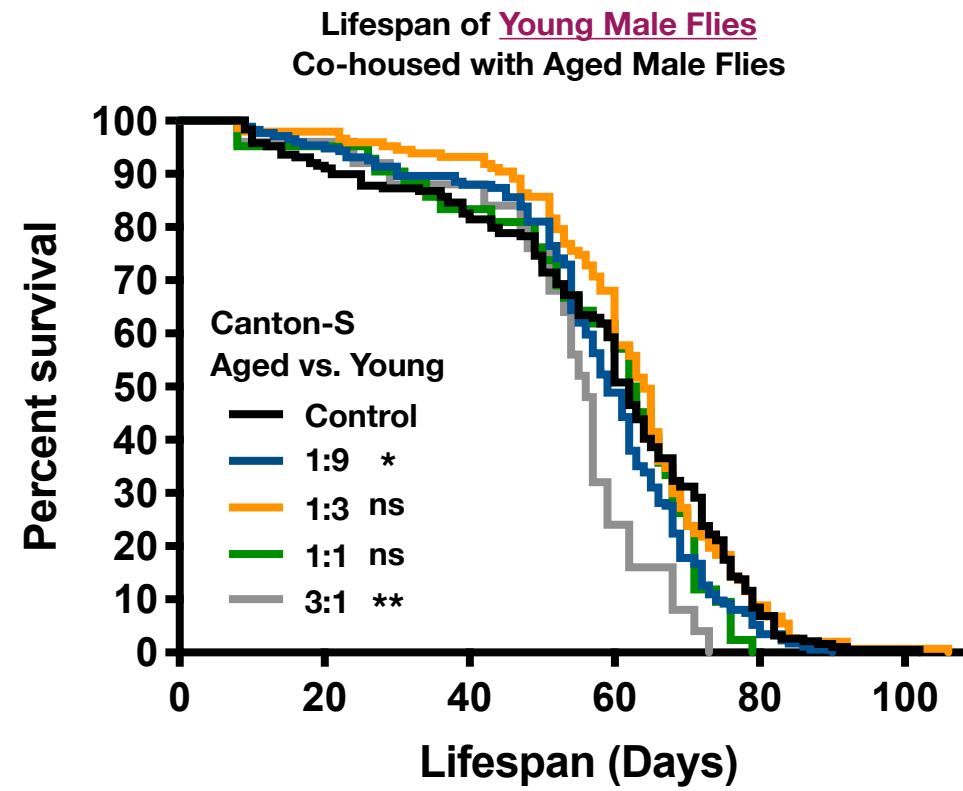
# Supplementary Figure 3

**b**

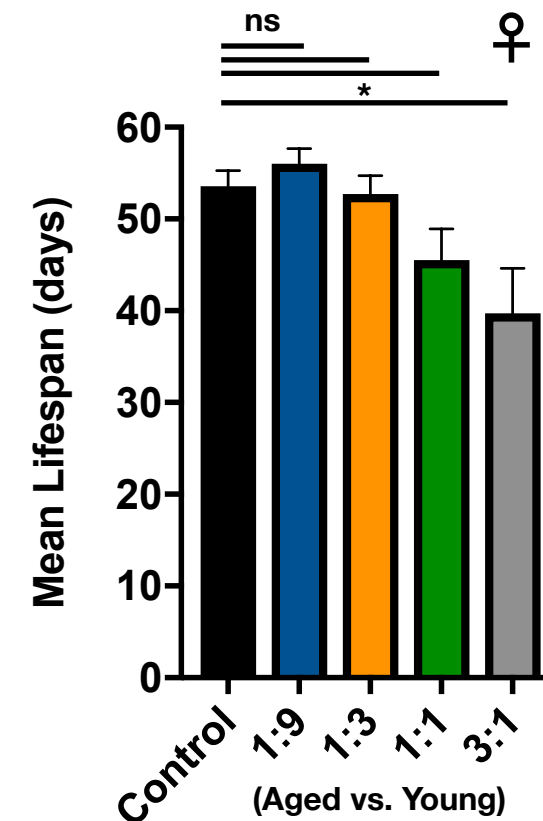
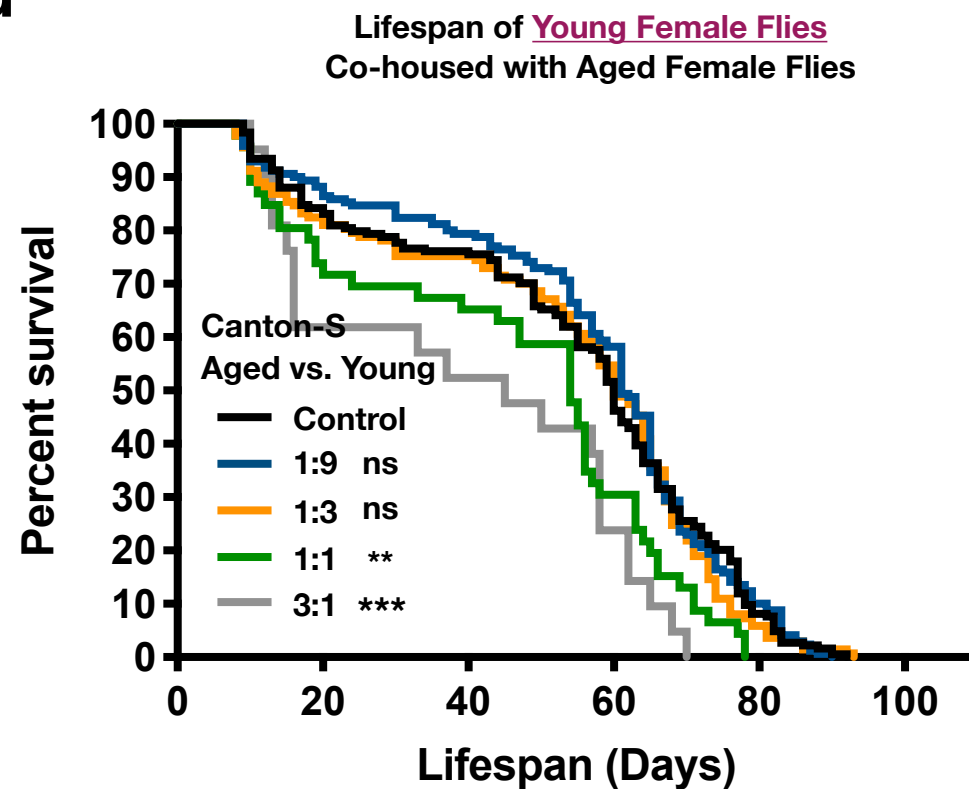
| Canton-S                 | Median<br>Lifespan<br>(Days) | Mean<br>Lifespan<br>(Days) ± SEM | n=  | Oregon-R                 | Median<br>Lifespan | Mean<br>Lifespan<br>(Days) ± SEM | n=  | w <sup>1118</sup>        | Median<br>Lifespan | Mean<br>Lifespan<br>(Days) ± SEM | n=  |
|--------------------------|------------------------------|----------------------------------|-----|--------------------------|--------------------|----------------------------------|-----|--------------------------|--------------------|----------------------------------|-----|
| Male                     |                              |                                  |     | Male                     |                    |                                  |     | Male                     |                    |                                  |     |
| Control                  | 62                           | 57.76 ± 1.46                     | 189 | Control                  | 51                 | 53.88 ± 1.20                     | 40  | Control                  | 68                 | 63.51 ± 1.85                     | 96  |
| Co-housed<br>Young Flies | 64                           | 61.97 ± 1.29                     | 147 | Co-housed<br>Young Flies | 56                 | 52.18 ± 1.34                     | 141 | Co-housed<br>Young Flies | 70                 | 66.71 ± 1.77                     | 142 |
| Female                   |                              |                                  |     | Female                   |                    |                                  |     | Female                   |                    |                                  |     |
| Control                  | 60                           | 53.55 ± 1.71                     | 184 | Control                  | 53                 | 51.22 ± 1.29                     | 60  | Control                  | 63                 | 59.09 ± 2.17                     | 96  |
| Co-housed<br>Young Flies | 60                           | 52.72 ± 2.01                     | 137 | Co-housed<br>Young Flies | 56                 | 51.56 ± 1.16                     | 147 | Co-housed<br>Young Flies | 60                 | 53.46 ± 2.02                     | 138 |

# Supplementary Figure 3

**c**



**d**



# Supplementary Figure 3

e

|         | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) $\pm$ SEM | n=  |
|---------|---------------------------|-----------------------------------|-----|
| Male    |                           |                                   |     |
| Control | 62                        | 57.76 $\pm$ 1.46                  | 189 |
| 1:9     | 59                        | 57.45 $\pm$ 1.23                  | 174 |
| 1:3     | 64                        | 61.97 $\pm$ 1.29                  | 147 |
| 1:1     | 62.5                      | 57.19 $\pm$ 2.72                  | 42  |
| 3:1     | 56                        | 52.88 $\pm$ 2.92                  | 25  |
| Female  |                           |                                   |     |
| Control | 60                        | 53.55 $\pm$ 1.71                  | 184 |
| 1:9     | 61                        | 56.00 $\pm$ 1.67                  | 170 |
| 1:3     | 60                        | 52.72 $\pm$ 2.01                  | 137 |
| 1:1     | 54                        | 45.52 $\pm$ 3.39                  | 46  |
| 3:1     | 45                        | 39.71 $\pm$ 4.93                  | 21  |

## Supplementary Figure 4

|            | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) $\pm$ SEM | n= |
|------------|---------------------------|-----------------------------------|----|
| Male       |                           |                                   |    |
| Control    | 60                        | 59.76 $\pm$ 1.58                  | 50 |
| Aged + 1d  | 69                        | 68.30 $\pm$ 1.36                  | 50 |
| Aged + 10d | 61                        | 60.08 $\pm$ 1.16                  | 50 |
| Aged + 20d | 62                        | 61.94 $\pm$ 1.29                  | 50 |
| Aged + 30d | 63                        | 62.26 $\pm$ 1.23                  | 50 |
| Female     |                           |                                   |    |
| Control    | 57                        | 57.74 $\pm$ 1.39                  | 50 |
| Aged + 1d  | 71                        | 72.08 $\pm$ 1.49                  | 50 |
| Aged + 10d | 61.5                      | 65.54 $\pm$ 1.42                  | 50 |
| Aged + 20d | 63                        | 63.50 $\pm$ 1.13                  | 50 |
| Aged + 30d | 67                        | 64.46 $\pm$ 1.62                  | 50 |

# Supplementary Figure 5

**a**

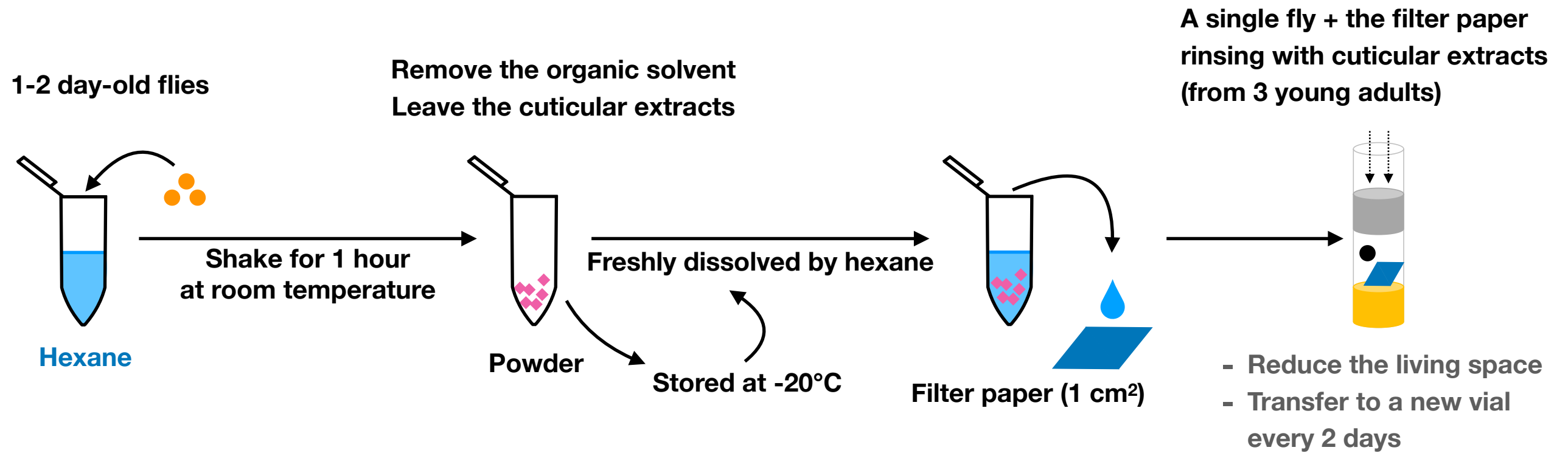
|                         | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) ± SEM | n=  |
|-------------------------|---------------------------|-------------------------------|-----|
| Male                    |                           |                               |     |
| <i>w<sup>1118</sup></i> | 65                        | 61.21 ± 2.11                  | 100 |
| <i>or83b (-/-)</i>      | 66                        | 61.44 ± 1.65                  | 100 |
| <i>ppk23 (-/-)</i>      | 60                        | 62.96 ± 0.95                  | 118 |
| Female                  |                           |                               |     |
| <i>w<sup>1118</sup></i> | 61                        | 56.97 ± 2.33                  | 100 |
| <i>or83b (-/-)</i>      | 82                        | 74.44 ± 2.20                  | 96  |
| <i>ppk23 (-/-)</i>      | 77                        | 77.16 ± 0.91                  | 119 |

**b**

| <i>or83b (-/-)</i>                                      | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) ± SEM | n= |
|---------------------------------------------------------|---------------------------|-------------------------------|----|
| Male                                                    |                           |                               |    |
| Control                                                 | 70                        | 69.72 ± 1.09                  | 25 |
| co-housed 1d-old <i>w<sup>1118</sup></i><br>flies (1:3) | 72                        | 73.68 ± 1.48                  | 25 |
| co-housed 1d-old <i>w<sup>1118</sup></i><br>flies (1:9) | 75                        | 75.35 ± 0.98                  | 26 |
| Female                                                  |                           |                               |    |
| Control                                                 | 80                        | 79.76 ± 1.73                  | 25 |
| co-housed 1d-old <i>w<sup>1118</sup></i><br>flies (1:3) | 83                        | 83.84 ± 1.54                  | 25 |
| co-housed 1d-old <i>w<sup>1118</sup></i><br>flies (1:9) | 88                        | 87.35 ± 1.25                  | 26 |
| <i>ppk23 (-/-)</i>                                      | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) ± SEM | n= |
| Male                                                    |                           |                               |    |
| Control                                                 | 74                        | 75.28 ± 1.92                  | 25 |
| co-housed 1d-old <i>w<sup>1118</sup></i><br>flies (1:3) | 77                        | 78.88 ± 1.84                  | 25 |
| co-housed 1d-old <i>w<sup>1118</sup></i><br>flies (1:9) | 81.5                      | 82.42 ± 1.12                  | 26 |
| Female                                                  |                           |                               |    |
| Control                                                 | 85                        | 82.60 ± 1.73                  | 25 |
| co-housed 1d-old <i>w<sup>1118</sup></i><br>flies (1:3) | 84                        | 85.32 ± 1.64                  | 25 |
| co-housed 1d-old <i>w<sup>1118</sup></i><br>flies (1:9) | 88.5                      | 89.62 ± 1.41                  | 26 |

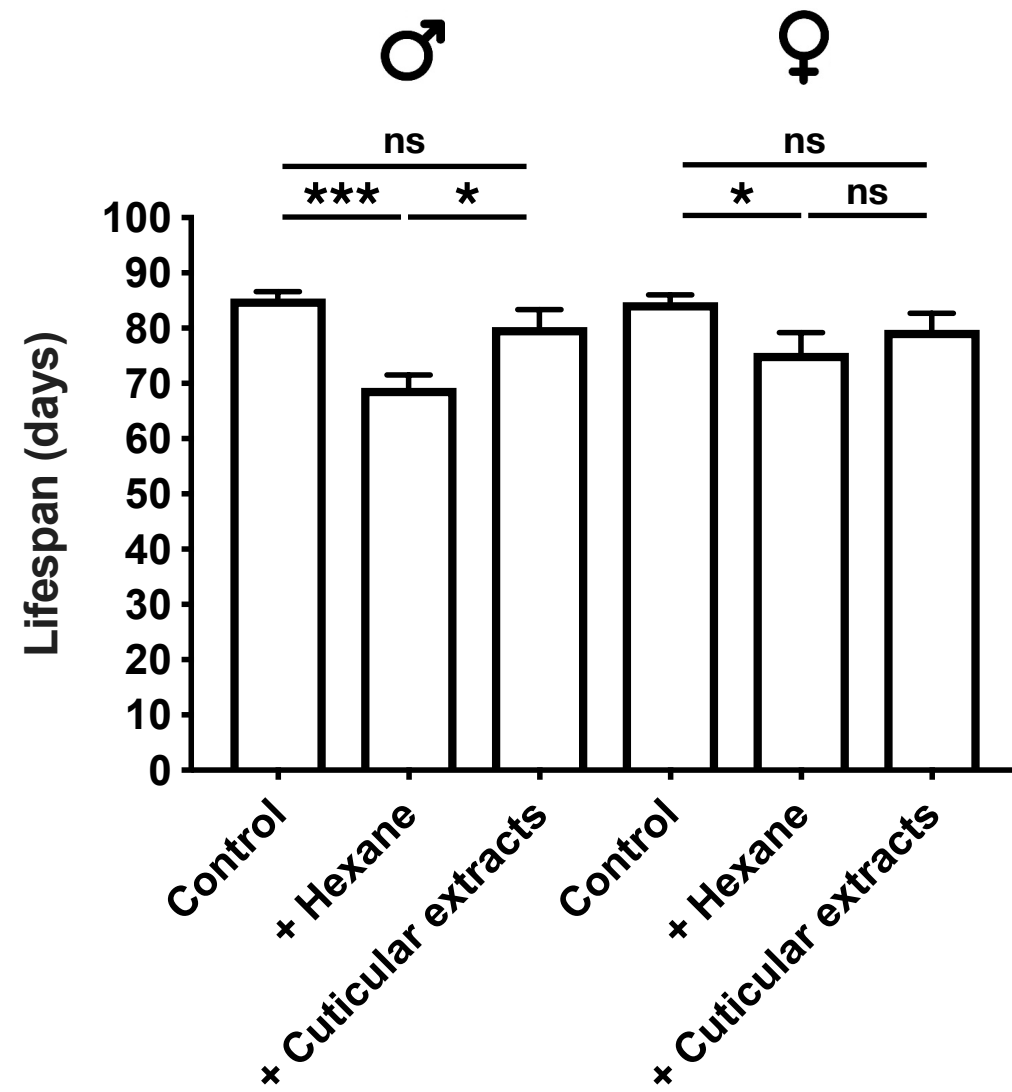
## Supplementary Figure 6

**a**



# Supplementary Figure 6

**b**



| Mean Lifespan<br>(Days) ± SEM | Male        | n= |
|-------------------------------|-------------|----|
| Control                       | 85.4 ± 1.20 | 10 |
| Hexane                        | 69.1 ± 2.39 | 10 |
| Cuticular extracts            | 80.2 ± 3.18 | 10 |
| Mean Lifespan<br>(Days) ± SEM | Female      | n= |
| Control                       | 84.7 ± 1.36 | 10 |
| Hexane                        | 75.5 ± 3.67 | 10 |
| Cuticular extracts            | 79.7 ± 2.99 | 10 |

## Supplementary Figure 7

| Aged Flies<br>Co-housed with     | Median lifespan<br>(Days) | Mean Lifespan<br>(Days) $\pm$ SEM | n= |
|----------------------------------|---------------------------|-----------------------------------|----|
| Male                             |                           |                                   |    |
| Young Control                    | 69                        | 68.30 $\pm$ 1.36                  | 50 |
| OK72>FASN <sup>CG3524</sup> RNAi | 58                        | 57.56 $\pm$ 1.53                  | 25 |
| OK72>Cyp4g1 RNAi                 | 56                        | 55.32 $\pm$ 0.96                  | 25 |
| Female                           |                           |                                   |    |
| Young Control                    | 60                        | 72.08 $\pm$ 1.49                  | 50 |
| OK72>FASN <sup>CG3524</sup> RNAi | 57                        | 61.28 $\pm$ 1.23                  | 25 |
| OK72>Cyp4g1 RNAi                 | 61.5                      | 59.28 $\pm$ 0.91                  | 25 |

# Raw Images of Western Blots

