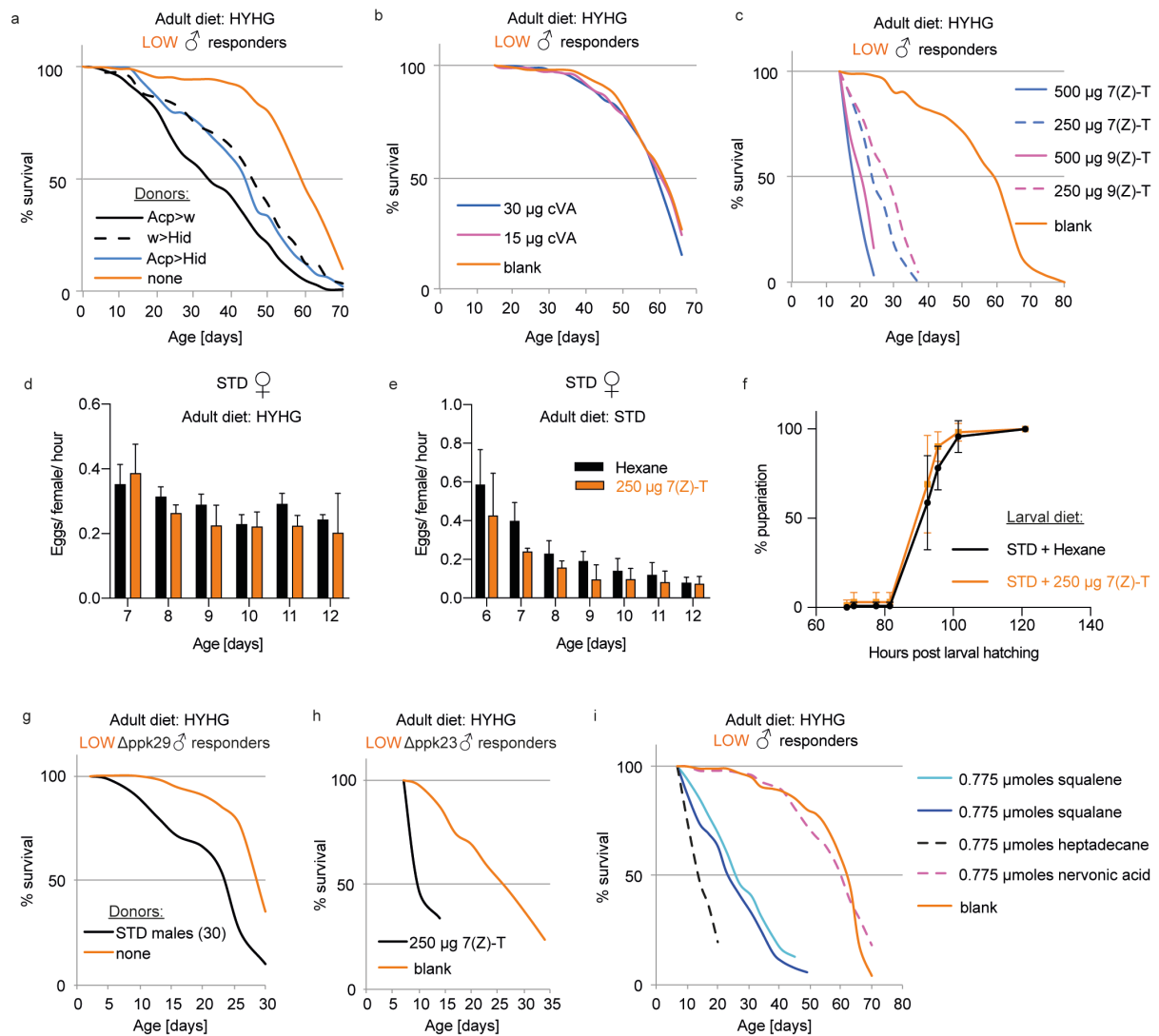


Supplementary Figure 1: Lifespan and body mass effects of larval dietary manipulations.

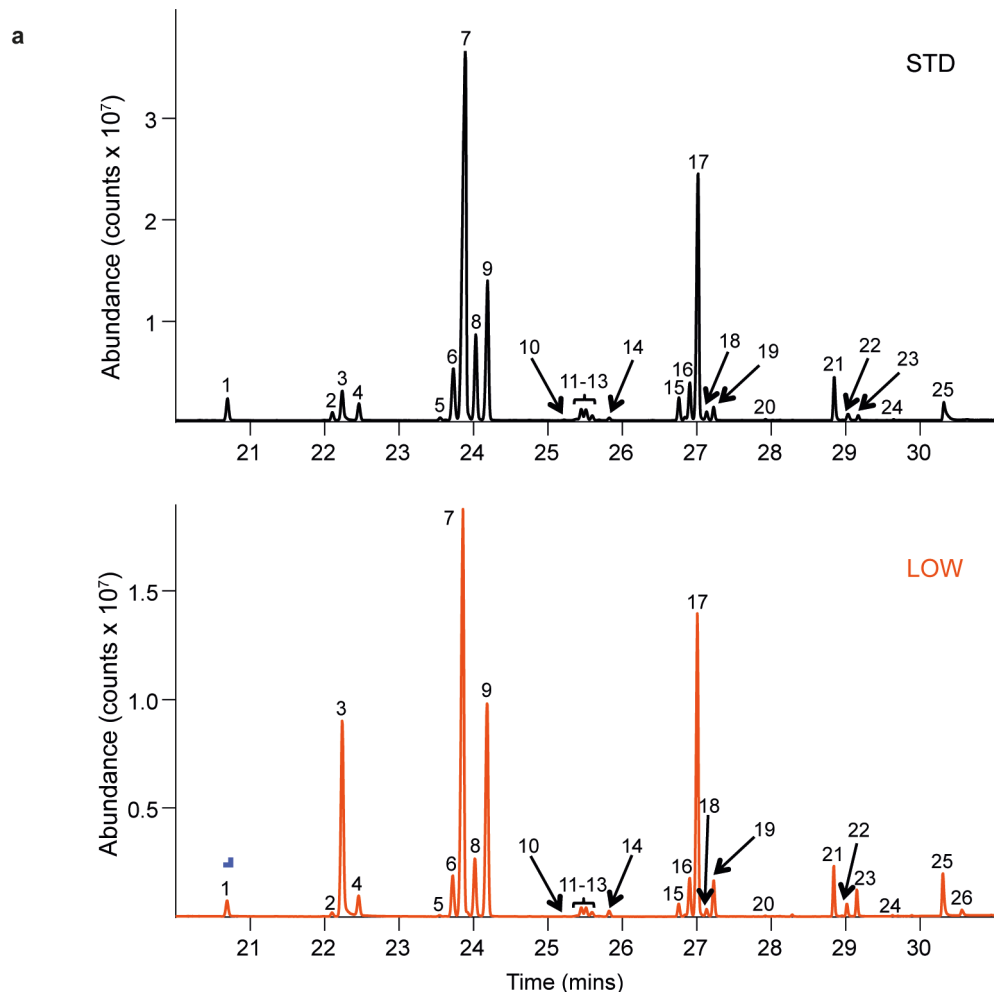
a, Wet body masses of individually-weighed STD ($n=23$) and LOW ($n=23$) *iso31* adult males aged for 1 wk on HYHG diet. **b**, Percentage dry body mass (hatched) and water content (unhatched) of STD and LOW *iso31* adult males aged for 1 wk on HYHG. In both cases: 20 males per sample, $n=5$ samples. **c,d**, Survival of STD (**c**) or LOW (**d**) adult males on four different adult diets. These survival curves are also shown in Fig. 1c, but are replotted here to allow comparisons between adult diets. **e**, Wet body masses of individually-weighed STD ($n=7$) and LOW ($n=26$) adult females aged for 1 wk on HYHG. **f**, Survival of STD or LOW adult females on HYHG or LYHG diets. For panels a, b and e: error bars show 1 s.d.



Supplementary Figure 2: The response to autotoxins does not require *ppk23* or *ppk29*.

a, Survival of LOW male responders is shortened to a similar extent by cohousing with STD donors of a control genotype (*w>hid* and *Acp>w*) or those expressing the pro-apoptotic gene *hid* in main cells of the accessory glands (*Acp>hid*) **b**, Survival of LOW males is not significantly altered by exposure to 15 or 30 µg of synthetic *cis*-vacacenyl acetate (cVA) compared to solvent alone (blank). **c**, Survival of LOW males is decreased by 250 or 500 µg of synthetic 7(Z)-tricosene or 9(Z)-tricosene, compared to hexane solvent alone (blank). **d-e**, Rate of egg laying by STD females at 1 wk of age on HYHG (d) or STD (e) adult diets is not affected significantly ($p>0.05$; two-way ANOVA followed by Sidak's multiple comparisons test) by exposure to 250 µg of synthetic 7(Z)-tricosene compared to hexane solvent alone ($n=3$ independent experiments and each experiment used 6 vials containing 15 females each). **f**, Larval time to pupariation and survival are not affected by supplementing STD larval diet with 250 µg of synthetic 7(Z)-tricosene (STD + 250 µg 7(Z)-T), compared to solvent alone (STD + hexane). $n=10$ vials containing 10 larvae each and one representative experiment of three independent experiments is shown. **g**, Survival of $\Delta ppk29$ males is decreased by autotoxins in vials preconditioned with 30 STD males. **h**, Survival of $\Delta ppk23$ males is decreased in vials supplemented with 250 µg synthetic 7(Z)-tricosene (7(Z)-T) compared to hexane solvent alone (blank). **i**, Survival is decreased by 0.775 µmol (molar equivalent of 250 µg 7(Z)-T) of squalene

(C30:6), squalane (C30:0) or heptadecane (C17:0) compared to nervonic acid (C24:1) or hexane solvent alone (blank). All survival curves used 15 LOW male responders per HYHG vial. Error bars show 1 s.d in panels d,e and f.



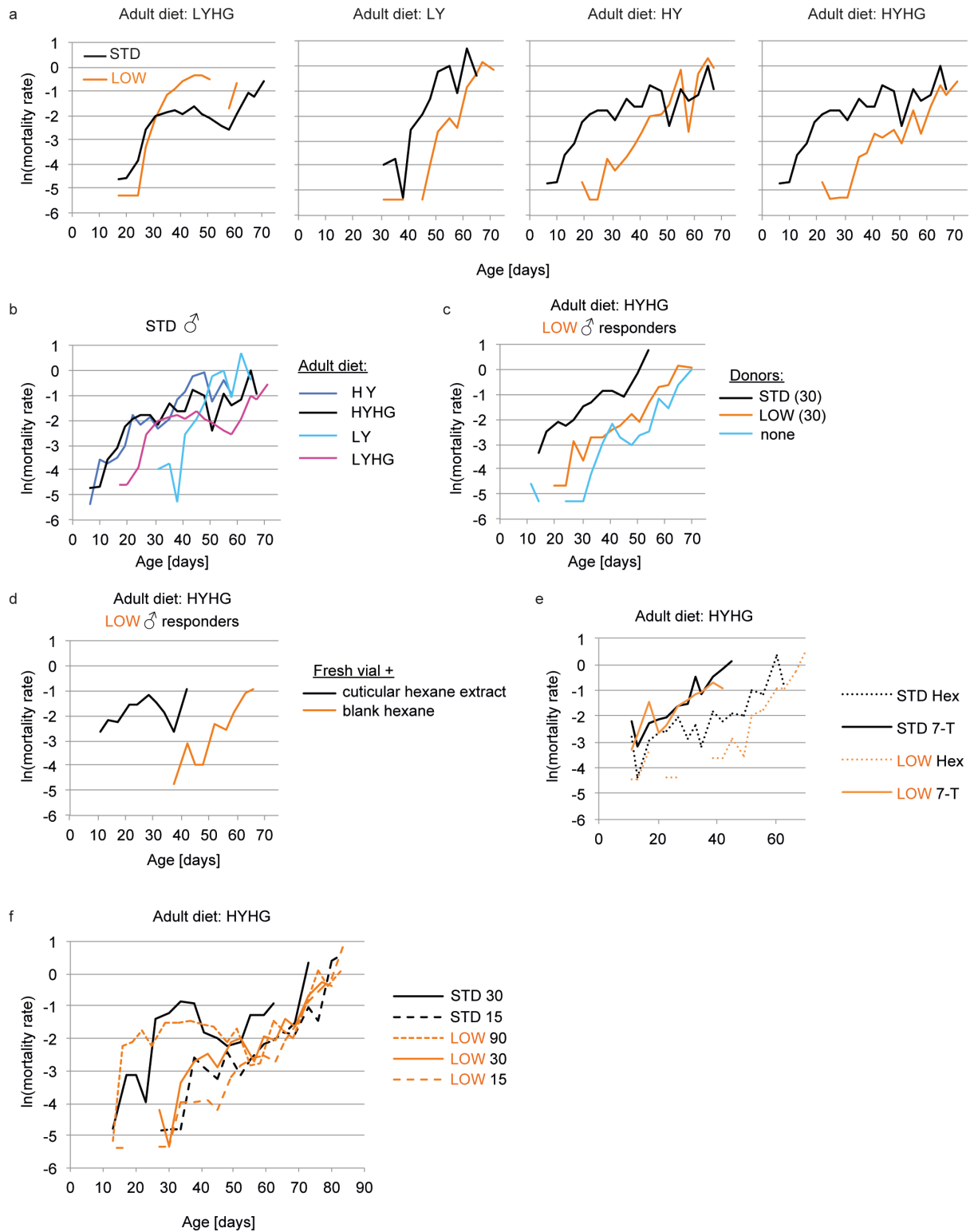
b

| Peak no. | Hydrocarbon | STD | LOW |
|----------|-------------------------|--------------|-------------|
| 1 | Heneicosane | 0.34 ± 0.04 | 0.11 ± 0.03 |
| 3 | 11-cis-Vaccenyl Acetate | 1.52 ± 1.28 | 1.97 ± 0.97 |
| 4 | Docosane | 0.21 ± 0.02 | 0.10 ± 0.02 |
| 6 | 9(Z)-Tricosene | 1.45 ± 0.13 | 0.43 ± 0.12 |
| 7 | 7(Z)-Tricosene | 14.58 ± 1.20 | 4.59 ± 1.02 |
| 9 | Tricosane | 1.75 ± 0.12 | 0.99 ± 0.19 |
| 14 | Tetracosane | 0.03 ± 0.00 | 0.02 ± 0.00 |
| 17 | 7(Z)-Pentacosene | 5.32 ± 0.19 | 2.11 ± 0.54 |
| 19 | Pentacosane | 0.11 ± 0.01 | 0.10 ± 0.01 |
| 23 | Heptacosane | 0.03 ± 0.00 | 0.04 ± 0.01 |
| 26 | Nonacosane | n.d. | 0.01 ± 0.00 |

Supplementary Figure 3. GC-MS analysis of STD and LOW cuticular extracts.

a, Representative chromatograms from GC-MS analyses of cuticular hexane extracts from STD and LOW adult males on HYHG at 3 wks of age. Note the difference in y-axis scales between the two chromatograms. Key to peaks: 1, Heneicosane; 2, B-Docosene; 3, 11-cis-Vaccenyl acetate; 4, Docosane; 5, Unknown (281); 6, 9(Z)-Tricosene; 7, 7(Z)-Tricosene; 8, C-Tricosene; 9, Tricosane; 10, Unknown (295); 11, A-Tetracosene; 12, B-Tetracosene; 13, C-Tetracosene; 14, Tetracosane; 15, Unknown (309); 16, A-Pentacosene; 17, 7(Z)-

Pentacosene; 18, C-Pentacosene; 19, Pentacosane; 20, Unknown (323); 21, Unknown (337); 22, B-Heptacosene; 23, Heptacosane; 24, Unknown (351); 25, Unknown (365); 26, Nonacosane. Definitive identifications were assigned to peaks only if this was verified by direct comparison to a pure synthetic standard. A letter at the start of the name of an unsaturated hydrocarbon denotes that the position of the double bond is not known. 'Unknowns' are unidentified hydrocarbon-like molecules, with their diagnostic ion in brackets. Note that all peaks except cVA (peak 3) are absent or severely reduced in adult males expressing *Cyp4g1* RNAi under the control of *PromE^{TS}-GAL4³⁷*. **b**, Quantifications of identified cuticular hydrocarbons are expressed as abundance normalised to an internal standard (a.u.) \pm 1 s.d. n=6 for STD and LOW flies. In all cases, extracts were prepared from 5 male flies per sample.



Supplementary Figure 4. Age-specific mortality analysis for the effects of diet, autotoxins and housing density. Ln (mortality rate) plots for survival data shown in Fig. 1c (panel a), Supplementary Fig. 1c (panel b), Fig. 3g (panel c), Fig. 5b (panel d), Fig. 7e (panel e) and Fig. 9b, c (panel f).