Materials and Methods

Fly stocks and general husbandry

The standard laboratory stocks *yw*, w^{1118}, and *Canton-S* were originally obtained from the Bloomington Stock Center. The recently caught wild-type strain, NJ-99, was generously provided by A. Gibbs. *Npf-GAL4*, UAS-shi^{ts} and the oenocyte driver, *OK72-GAL4,* were obtained from the Bloomington Stock Center. *PromE(800)*-GAL4 flies were generously provided by J. Levine. *UAS-TRPA1* flies were provided by P. Garrity. $Poxn^{\Delta M22\text{-}B5}$-*ΔXB* and $Poxn^{\Delta M22\text{-}B5}$-*Full1* were provided by J. Alcedo. *UAS-tra, UAS-tra-RNAi* and *Fruitless*/*Sb* were provided by B. J. Dickson. The *ppk23* mutant stock and *ppk23-GAL4* were generously provided by K. Scott. Males with feminized CHC profiles were created from the progeny of the cross of *OK72-GAL4* or *PromE(800)-GAL4* to *UAS-tra*. Unless otherwise noted, feminized males are *OK72-GAL4; UAS-tra*. Females with masculinized pheromone were generated by crossing *OK72-GAL4* to UAS-*tra-RNAi.*

For all experiments, larvae were cultured in cornmeal-sugar-yeast media. Same-age adults were collected within a 24hr window and transferred to 10% Sugar/Yeast (SY) food. Unless otherwise noted, all flies were maintained at 25 °C and 60% relative humidity in a 12:12 h light:dark cycle. Flies were transferred to fresh vials every two or three days.

Cuticular hydrocarbon measures

Cuticular hydrocarbon extraction and detection were as follows. For each sample, 5 flies were placed in 100 µl of hexane, which contained 10 µg/ml of the synthetic hydrocarbon hexacosane (Sigma-Aldrich) as a spike-in standard. Extractions were allowed to incubate at room temperature for 30 min. The cuticular extract was removed, placed in a clean glass vial and then evaporated under a chemical hood. Extracts were stored at -80°C and re-dissolved in 70 µl of hexane prior to GC-MS analysis.

The GC-MS analysis was performed with a QP2010 system (Shimadzu) equipped with a DB-5 column (5%-Phenyl-methylpolysiloxane column; 30 m length, 0.25 mm ID, 0.25 µm film thickness; Agilent). Ionization was achieved by electron ionization (EI) at 70 eV. One microliter of the sample was injected using a splitless injector. The helium flow was set at 1.9 mL/min. The column temperature program began at 50°C, increased to 210°C at a rate of 35°C /min, then increased to 280°C at a rate of 3°C/min. A mass spectrometer was set to unit mass resolution and 3 scans/sec, from *m*/*z* 37 to 700. Chromatograms and mass spectra were analyzed using GCMSsolution software (Shimadzu).

Standard Exposure Protocol

As illustrated in Fig 1A, the standard exposure protocol involved housing five experimental flies with 25 donor flies that were expressing either male or female cuticular hydrocarbons. Experimental and donor flies were reared separately under controlled larval conditions. Adults from each group were transferred to 10% Sugar/Yeast (SY) food for two days where they were allowed to mate freely. At two days post-eclosion, sexes were separated using light CO_2 anesthesia (flies were anesthetized for 3-5min under 100% CO_2 flowing at 5 l/min through a Flow Buddy and standard fly pushing pad from FlyStuff.com) and subsequently aged to 10 days on 10% Sugar/Yeast medium (SY). At 10 days post-eclosion, exposure cohorts were

established by randomly placing 5 experimental flies with 25 donor flies. At least 10 replicate exposure vials were established for each treatment. The exposure period was 48hr, after which starvation resistance or triglyceride (TAG) amounts were measured. For experiments involving *UAS-TRPA1* and *UAS-shi^{ts}*, flies were normally maintained at 23°C and shifted to 29°C only during the time they were being exposed to donor flies. The *w^{1118}* laboratory strain is naturally lean, and flies from this strain were aged a further 4 days on 30% sugar/ 5% yeast to increase TAG amounts and provide a sensitized background from which to measure pheromone effects. The phenotypes of experimental flies in each donor group were then compared. It is important to note that comparisons always involved experimental flies of identical genotype; only their social environment (as defined by the donor flies' pheromone profiles) were different. Experimental flies could be distinguished from donor flies by eye color or body color. In cases where eye or body color was not distinguishing (e.g., when measuring pheromone effects in mutant or transgenic flies), the wings were notched with a razor blade to mark donor animals.

Exposure with purified 7(Z) ,11(Z)-Heptacosadiene (7,11-HD)

Age-matched, experimental yw flies were prepared as described in the previous section. Purified 7,11-HD was purchased from Cayman Chemicals (Ann Arbor, MI). Approximately 2 mg of purified 7,11-HD in ethanol was applied to ¼ piece of Whatman filter paper (25 mm circles). The ethanol was allowed to evaporate in the fume hood. The dry filter paper was then placed at the bottom of a vial on top of the SY10 food. Control vials contained ¼ piece of Whatman filter paper that had been soaked with an equivalent volume of purified ethanol and evaporated in the fume hood. Ten *yw* flies were placed in each vial, with a total of 5 vials used for each treatment. After 24 hrs, the flies were flipped into freshly prepared vials with ¼ filter paper containing 2 mg 7,11-HD or ethanol alone. After another 24 hr period (for a total of 48hrs exposure), the flies were placed into 1% agar only vials and starvation resistance was followed by counting the number of dead flies approximately every 2-5 hrs.

Survival assays

Following the creation of treatment cohorts (see *Exposure Protocol* above) lifespans were measured using established protocols. Normally, 20 replicate vials (100 experimental flies) were established for each treatment. Flies were transferred to fresh media every 2-3 days, at which time dead flies were removed and recorded. Flies were kept in constant temperature (25°C) and humidity (60%) conditions with a 12:12hr light:dark cycle. Unless otherwise noted experimental flies were co-housed with donor flies throughout their lifespan. Donor flies were replenished at least once, at roughly 70% survivorship of the feminized donor cohorts. For shibere and TRPA1 experiments, experimental flies were housed at 29°C throughout their lifespan.

Starvation assays

After the designated exposure period (see *Exposure Protocol* above), donor flies were removed and experimental flies were placed in fresh vials containing 1% agar. The number of dead flies was recorded approximately every 2-5 hrs.

TAG assays

After the designated exposure period (see *Exposure Protocol* above), donor flies were removed and 5 experimental males were homogenized in 200 µl PBS/0.05% Triton-X. The homogenate (10 µL) was added into 200 µl of Infinity Triglyceride Reagent (Thermo Electron Corp.) and incubated at 37°C for 10 minutes with constant agitation. TAG concentrations were determined by the absorbance at 520nm and estimated by a known triglyceride standard. Average TAG values were based on 5-10 independent biological replicates (of five flies each) from multiple vials.

Quantitative PCR

Flies were handled according to the procedures outlined above for the survival assays. Total RNA was extracted from 10-day old flies using Trizol (Invitrogen). For each treatment 10 independent extractions were prepared using 5 experimental flies for each extraction. Extracted RNA was treated with 1 unit DNAse I (Invitrogen), and cDNA was synthesized using the Superscript III first strand synthesis kit, Invitrogen. Real time PCR was performed using RT^2 SYBR green/Rox PCR master mix from SA Biosciences and an ABI StepOnePlus. The following primers were used: npfF-RA-L1 (TGAACCAGAACTATGTGCCAAA), *npf*-RA-R1 (TTGTCCATCTCGTGATTCCTC); RP49F (ACTCAATGGATACTGCCAG), RP49R (CAAGGTGTCCCACTAATGCAT).

RNA-seq

After the 48 hour exposure period (see Exposure Protocol above), RNA was extracted from three independent sets of five flies per treatment group (*w1118* and *ppk23* mutant flies each exposed to male and female pheromone). Thus, for each of four treatment groups we had three biological replicates. RNA was first isolated using Trizol (Invitrogen) and then further purified using the RNeasy kit (Qiagen). Aliquots of 1.1ug of RNA (as determined by nanodrop) from each sample were delivered to the University of Michigan Sequencing Core, which performed cDNA library creation using Poly-A selection followed by 50bp paired-end high-throughput sequencing using an Illumina HiSeq 2000 sequencer. Sequencing was completed in three separate lanes, each multiplexed with samples prepared from each of the four treatment groups. Sequencing data were processed using the Bowtie-TopHat-Cufflinks pipeline (26). Sequences were aligned to NCBI build5.3 of the *Drosophila melanogaster* genome, and the FPKM (Fragments Per Kilobase of transcript per Million mapped reads) per annotated gene was used as the expression measure. Cluster/GO analysis was carried out using cummeRbund software to identify genes differentially expressed upon pheromone exposure, and functional classification analysis was completed using DAVID and a significant category enrichment Bonferonni $P< 0.001$.

Aggression assays

Aggression was monitored in an arena chamber with 12 evenly spaced cells as described in (27). The insides of the cells were coated with Fluon (Northern Products) to prevent the flies from walking on the walls of the cell. The chamber was placed on a bed of food medium identical to that used during pheromone exposure (10%SY). We measured aggressive

interactions among 24 pairs of experimental and donor flies (1:1 experimental vs. donor). Note that we observed significant effects of pheromone exposure in different housing conditions including 1:1 (experiment:donor) as well as 15:15 and 5:25. Because flies are generally less aggressive under grouped conditions, our 1:1 pairing is a conservative test because it is in these conditions that we expect the maximum amount of aggression, if it is present at all. Each pair of 10 day old males was introduced in each arena through a loading hole in the cover plate that was plugged with a small rubber plug. When all the males were loaded, the cover plate was gently moved up so that the loading holes aligned just above each arena. The chamber was then filmed for 30 min after a 2-min adjustment phase, and movies were analyzed with the CADABRA package for Matlab. The chambers were also visually monitored for multiple parameters of aggression as previously described (27), but we focused our analysis on lunges. Overall, aggression was very low under our pheromone exposure protocol, and there were no differences between males exposed to male vs. female pheromone.

Respirometry

CO_2 production was measured using a Sable Systems Respirometry System, including a LiCor LI-7000 carbon dioxide analyzer, a Mass Flow Controllers (MFC2), and UI-2 analog signal unit. Immediately prior to analysis, flies were transferred, without anesthesia, into glass, cylindrical respirometry chambers. Flies were allowed to acclimate to the new environment for 10min before CO_2 collection began. Six chambers were analyzed simultaneously using stop-flow analysis and the Sable Systems multiplexer (MUX). For each group, three measures of CO_2 production over a period of 20min were collected, and these three measures were averaged to determine a final, single estimate of the rate of CO_2 production per group. A sample size of N=5 groups was applied to each genotype and treatment. CO_2 production values were obtained using the EXPDATA software from Sable Systems, following adjustment using a proportional baseline.

Activity assays

Fly activity count measurements were collected using the *Drosophila* Activity Monitoring System (TriKinetics; Waltham, MA). Except for social exposure experiments where two male flies were co-housed, an individual fly was contained in a 5 mm diameter by 65 mm length polycarbonate plastic tube with a 10% SY food source at one end of the tube. For pheromone exposure activity experiments, either 7-11-heptacosadiene (Cayman Chemical; Ann Arbor, MI) or 100% ethanol was applied to the interior walls of the activity tube and allowed to dry completely. Total activity counts were calculated by summing all activity counts recorded for an individual fly during a specified time interval and averaging across the group. Experiments were performed for at least 2 days at 25°C (except for TRPA1 experiments, which were performed at 29°C) under 12-hour light:12-hour dark cycling conditions unless otherwise noted.

Statistics

Unless otherwise indicated, pairwise comparisons between different treatment survivorship curves (both lifespan and starvation resistance) were carried out using the statistical package R with DLife, a survival analysis package developed in the Pletcher Laboratory. P-values were

obtained using log-rank test. For comparisons involving TAG amounts or gene expression, pairwise t-tests were carried using independent homogenates or RNA extractions as the unit of observation. Two-tailed P-values are reported for initial observations (e.g., Fig 1) but one-tailed P-values are reported when testing genetic manipulations whose predicted effects were noted *a priori*. Mortality rates were calculated using standard methods (2), and values in Figs. 4C and S12 were smoothed using a kernel smooth over a three-census window. Significant differences in mortality rates were identified using Aalen regression, which allows for variability in the shapes of the individual hazards and provides the ability to examine transient differences in mortality (28).

Supplementary Figure Legends

Fig. S1 CHC profiles comparing male, female, and feminized males. (A) Representative GC-MS profile of cuticular hydrocarbons extracted from control donor males, which carry the *UAS-tra* transgene only. **(B)** Representative GC-MS profile of cuticular hydrocarbons extracted from females of the same control genotype. **(C)** Representative GC-MS profile of hydrocarbons extracted from males with hydrocarbon profiles that have been feminized by driving *UAS-tra* in the oenocytes with the *PromE(800)-GAL4* driver (see methods). **(D)** Representative GC-MS profile of hydrocarbons extracted from males with hydrocarbon profiles that have been feminized by driving UAS-*tra* in the oenocytes using the *OK72-GAL4* driver (see methods). Each profile was obtained by extracting cuticular hydrocarbons from five animals and is a representative example from five replicate extractions. Note the prevalence of the dominant female pheromones (7,11-HD, 7,11ND) in the transformed males. cVA, cis-vaccenyl acetate; T, tricosene; P, pentacosene; HD, heptacosadiene; ND, nonacosadiene.

Fig. S2 Perception of male pheromones affects female longevity. *yw* females exposed to female donor pheromone were modestly, but significantly, longer lived than genetically identical females exposed to male donor pheromone. Exposure procedure mimicked that shown in Figure 1 for males. P-value determined via log-rank test.

Fig. S3 The effects of pheromone exposure are robust across strains. (**A-D**) Experimental males exposed to male donor pheromone (solid black line) were significantly more starvation resistant than experimental males exposed to female donor pheromone generated using two different GAL4 drivers (*OK72-GAL4,* solid red line*;* and *Prom E(800)-GAL4,* solid blue line, see Methods). The experimental strains included: (**A**) *yw* males (*OK72-GAL4* vs. control: P = 0.009; *PromE(800)-GAL4* vs. control: P = 0.0003); (**B**) *Canton-S* males (OK72-GAL4: P ≤ 0.0001; *PromE(800)-GAL4*: P ≤ 0.0001); (**C**) w^{1118} (*OK72-GAL4*: P ≤ 0.0001; *PromE(800)-GAL4*: P ≤0 .0001); (**D**) NJ-99 (*OK72-GAL4*: p = 0.006; *Prom E(800)-GAL4*: P = 0.03),which is a recently caught wild-type strain. Sample size were N=50 for all cohorts except those exposed to feminized donor flies from the *PromE(800)-GAL4;UAS-tra* pheromones manipulations, which ranged from N = 23-50. All of the *P* values were determined by paired log-rank test of feminized vs. control cohorts. **(E)** TAG amounts were also significantly affected by donor pheromone in all four fly strains. Feminized and control donor males carried the *OK72-GAL4* construct. **E**ach point represents a biological replicate composed of 5 flies (N = 50 flies total per treatment). Boxes in box plots represent SEM, the horizontal black line indicates the median, and the colored line indicates the mean. *P* values for TAG assays were determined by a two-tailed t-test.

Fig. S4 The physiological effects of pheromone exposure persist with different cohort compositions. (**A-C**) Male *yw* experimental flies placed at variable compositions with donor males (represented as experimental:donor) experienced significant effects on starvation resistance (left panels) and TAG amounts (right panels). The 1:1 experiment rules out interactions between multiple donor flies as causal for these phenotypes. Sample sizes are as follows: for all starvation cohorts, N = 50 flies per treatment. For TAG assays, N = 50 flies, with

each point representing a biological replicate composed of 5 flies. Boxes in box plots represent SEM, the horizontal black line indicates the median, and the colored line indicates the mean. *P* values for starvation experiments were determined by log-rank test, while *P* values for TAG assays were determined by a one-tailed t-test.

Fig. S5 The effects of female pheromone perception on male starvation resistance is reversible. Differences in starvation resistance are reversed when the experimental flies are separated from donor flies for 48 hrs prior to testing (N=95 and 105 for male and female donor pheromone, respectively. Compare these results to Fig 1C). P-value is by log-rank test.

Fig. S6 Large behavioral differences between treatments are not responsible for the effects of pheromone exposure. (A) Experimental males exposed to feminized males do not experience differences in aggressive interactions compared with those exposed to control males. Aggression was measured by direct observation and CADABRA video analysis software. **(B)** Total activity is modestly increased in males exposed to feminized males compared to those exposed to control males, but **(C)** this does not lead to changes in overall metabolic rate (as measured by CO_2 production). **(D)** Exposure of experimental flies to synthetic pheromone 7-11 HD had no effect on total activity. **(E)** Feeding is not disrupted in experimental flies exposed to female pheromone. Feeding score was based on the intensity of blue color in the abdomen after 3 hours of feeding (0=no blue, 3= intense blue). **(F,G)** Activation of *ppk23*-expressing neurons via heat-activated TRPA1, which is sufficient to phenocopy the effects of pheromone exposure, does not cause changes in total activity (panel F) or respiration rate (panel G). **(H)** Activation of *ppk23*-expressing neurons has no significant effect on feeding. **(I)** Consistent behavioral disruption during exposure did not eliminate the exposure effects. The vortex was activated at maximal speed for 3 seconds every 30 second interval throughout the two-day exposure period. P-values for panels A-H were determined by t-test. P-values for panel I were determined by log-rank test.

Fig. S7 Odorant perception is not required for pheromone-induced changes in starvation resistance. *Or83b* mutant flies are broadly anosmic, yet exhibit normal changes in starvation resistance in response to differential pheromone exposure (N = 25 and 30 experimental flies exposed to control and feminized donor flies, respectively). P-value determined by log-rank test.

Fig. S8 A screen of candidate pheromone receptors identified *ppk23* as required for the effects of pheromone exposure on starvation resistance. Flies homozygous mutant for loss of function alleles in putative pheromone receptors were tested for significant effects of pheromone exposure on starvation resistance. All of the lines tested, with the exception of *ppk23* mutants, exhibited significant effects of exposure to male donor pheromone (solid lines) relative to female donor pheromone (dashed lines). Exposure used the standard 5:25 (experimental:donor) ratio and lasted for two days prior to the analysis (see Supplementary Methods). Sample sizes of experimental flies in each cohort ranged from N = 30-50 flies per treatment. P-values were determined by log-rank test.

Fig. S9 Surgical removal of the forelegs abrogates the effects of pheromone exposure on starvation resistance. Summary of four replicate amputation experiments illustrating the mean starvation resistance (with standard error) of control and amputee flies exposed to male or female donor pheromones. Injury control represents males that were poked in the thorax using a sterile tungsten needle. The total sample sizes are as follows: N = 200 and 196 for unharmed animals exposed to male or female donor pheromones, respectively. N = 150 for injured animals in both treatments. N = 135 and 129 for amputee animals exposed to male or female donor pheromones, respectively. P-values were determined by log-rank and t-test.

Fig. S10 A candidate "brain screen" identified *npf*-expressing neurons as required for the effects of pheromone exposure on starvation resistance. Flies containing both the *UAS-shibire* temperature-sensitive construct and a single enhancer-trap-GAL4, which targeted neuronal inhibition to specific functional regions of the brain, were tested for significant effects of differential pheromone exposure on starvation resistance. All lines tested except *npf-GAL4; UAS-shi^{ts}* exhibited significant effects of exposure to male donor pheromone (solid lines) relative to female donor pheromone (dashed lines). Exposure used the standard 5:25 (experimental:donor) ratio and lasted for two days prior to the analysis (see Methods). Sample sizes of experimental flies in each cohort ranged from N = 37-50 per treatment. P-values were determined by log-rank test.

Driver	Target Regions
Corazonin (*Crz*)	Protocerebrum
1471	Suboesophageal ganglion (SOG)
Lilli	Ellipsoid body, mushroom body (MB), antennal lobe, chemosensory input, large GABA-expressing & segmental nerves
C119	Ellipsoid body
C5	Fan shaped body, large field neurons, wing, protocerebrum, mushroom body
C205	Fan shaped body & SOG
Cib[p]	Mushroom body and ellipsoid body
P247	Mushroom body

Fig. S11 Exposure to female donor pheromones results in a significant increase in *npf* mRNA levels. For each treatment, expression data represent 10 independent RNA extractions from five experimental males each (see Supplementary Methods). Fold-change is relative to experimental males exposed to male donor pheromone. P-value was by t-test.

Fig. S12 When access to females is limited (1:1 sex ratio), male costs of reproduction may be predominantly due to pheromone perception. Age-specific mortality rates for *yw* males exposed to feminized males (dashed black line) and females (solid blue line) is significantly higher than mortality rates of flies exposed to control donor males (solid black line; P=0.0004 and P=0.01, respectively by Aalen regression). Mortality rates of flies exposed to feminized donor males is not different from those of flies exposed to females (P = 0.22 by Aalen

regression). Cohorts consisted of five experimental males together with five control donor males (solid black line), five feminized donor males (dashed black line) or five females (solid blue line). 20 replicate cohorts were measured for each treatment (i.e., N=100 experimental flies for each treatment).

Fig. S13 High levels of mating partially reverse changes in male TAG amounts caused by exposure to female pheromone. When females are in excess (3:1 sex ratio), differences in male TAG amounts caused by exposure to sex-specific pheromones is partially reversed (P=0.01). These data mirror those observed for age-specific mortality (see Fig 4C). Also similar to age-specific mortality (see Fig S12), when access to females is limited (1:1 sex ratio), there is no significant effect on the impact of pheromone perception (P=0.32). N = 50 flies, with each point representing a biological replicate composed of 5 flies. Boxes in box plots represent SEM, the horizontal black line indicates the median, and the colored line indicates the mean. *P* values were determined by t-test.

Fig. S14 A model for organism-non-autonomous effects on aging. We propose a model whereby insulin/TOR signaling, which are known to modulate aging within organisms through cell autonomous and non-autonomous mechanisms and to have direct effects on attractiveness, may also exert organism non-autonomous effects on lifespan and physiology in other individuals through neural circuits involved in sensory perception and sexual reward.

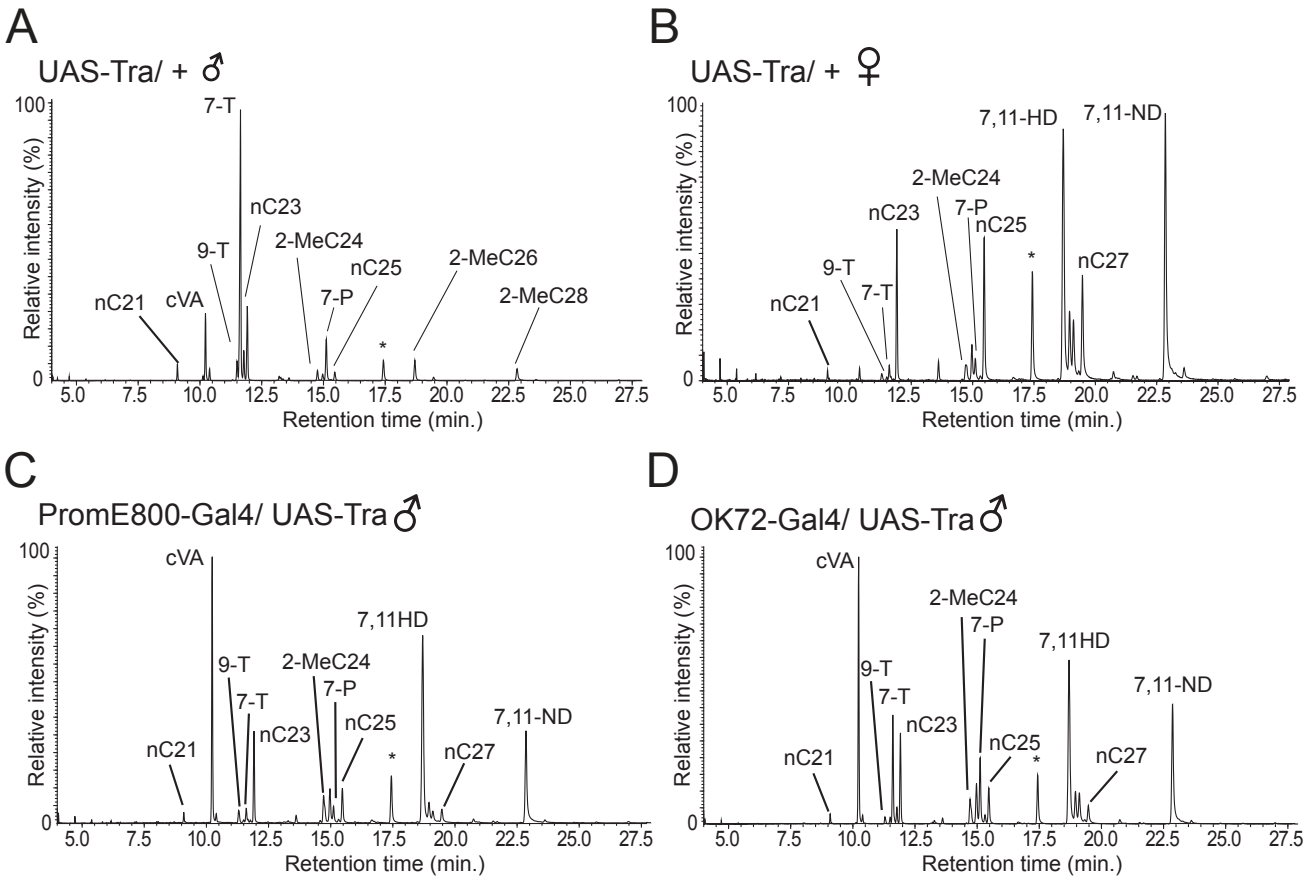
A
UAS-Tra/ + ♂
B
UAS-Tra/ + ♀
C
PromE800-Gal4/ UAS-Tra ♂
D
OK72-Gal4/ UAS-Tra ♂
Relative intensity (%)
Retention time (min.)
nC21
cVA
9-T
7-T
nC23
2-MeC24
7-P
nC25
2-MeC26
2-MeC28
7,11HD
7,11-HD
7,11-ND
nC27
*

Figure S1

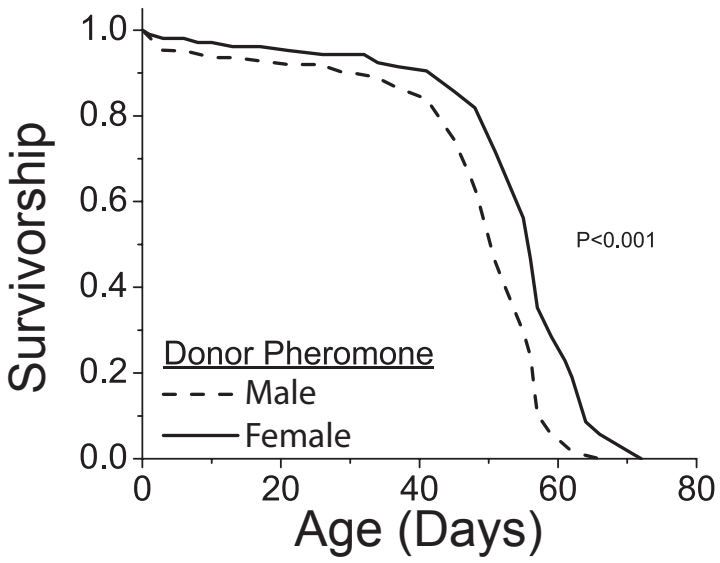
1.0
0.8
0.6
0.4
0.2
0.0
Survivorship
0
20
40
60
80
Age (Days)
P<0.001
Donor Pheromone
Male
Female

Figure S2

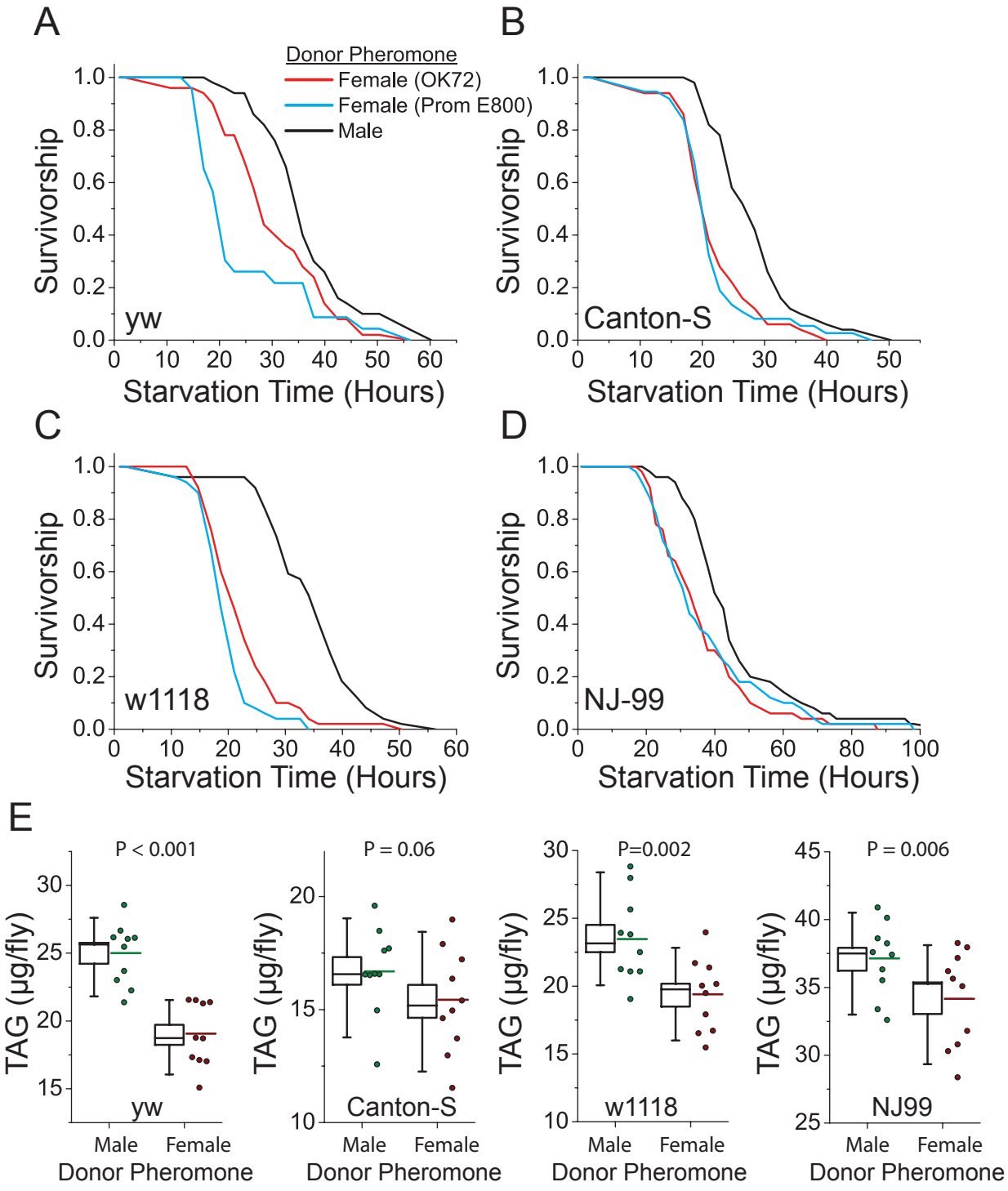
A
Donor Pheromone
Female (OK72)
Female (Prom E800)
Male
Survivorship
Starvation Time (Hours)
yw
B
Canton-S
C
w1118
D
NJ-99
E
P < 0.001
P = 0.06
P=0.002
P = 0.006
TAG (µg/fly)
yw
Canton-S
w1118
NJ99
Male
Female
Donor Pheromone

Figure S3

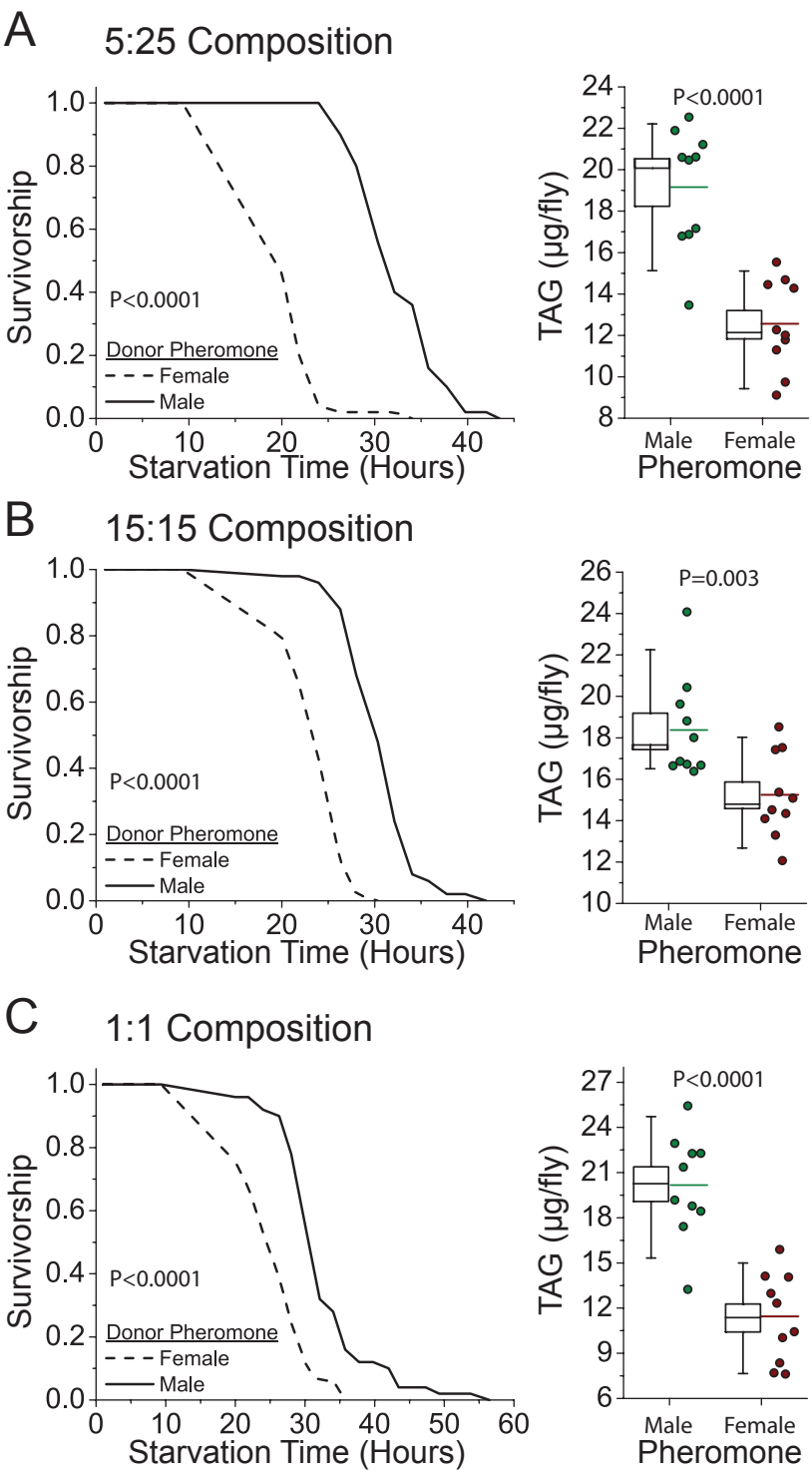
A
5:25 Composition
Survivorship
Starvation Time (Hours)
P<0.0001
Donor Pheromone
Female
Male
TAG (µg/fly)
P<0.0001
Male
Female
Pheromone
B
15:15 Composition
Survivorship
Starvation Time (Hours)
P<0.0001
Donor Pheromone
Female
Male
TAG (µg/fly)
P=0.003
Male
Female
Pheromone
C
1:1 Composition
Survivorship
Starvation Time (Hours)
P<0.0001
Donor Pheromone
Female
Male
TAG (µg/fly)
P<0.0001
Male
Female
Pheromone

Figure S4

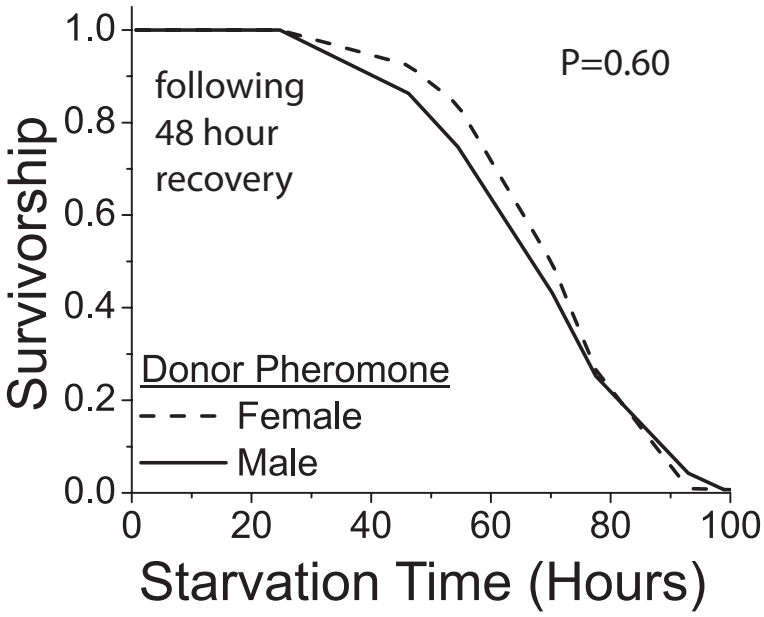
1.0
0.8
0.6
0.4
0.2
0.0
Survivorship
following
48 hour
recovery
P=0.60
Donor Pheromone
Female
Male
0
20
40
60
80
100
Starvation Time (Hours)

Figure S5

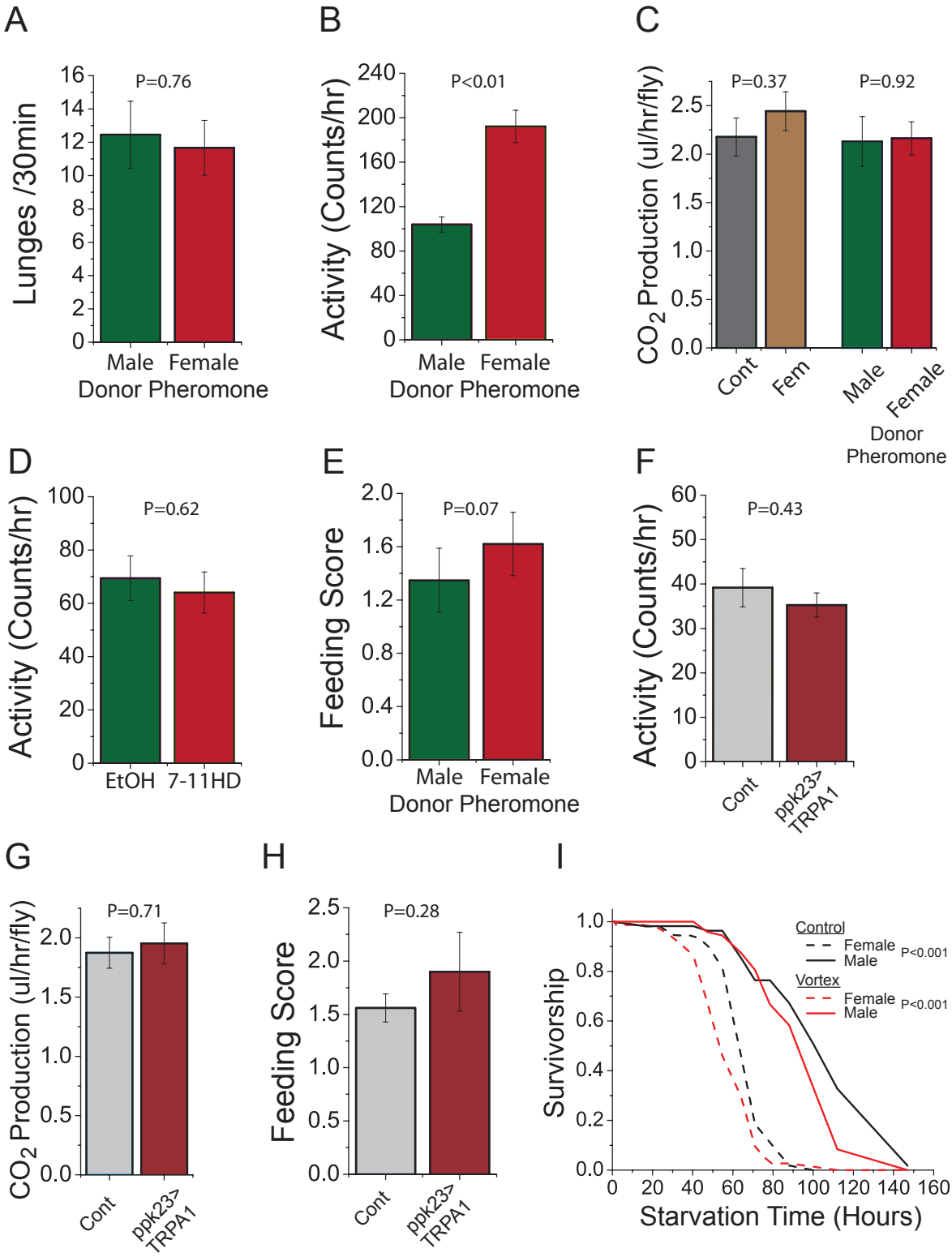
A
Lunges /30min
P=0.76
Male Female
Donor Pheromone
B
Activity (Counts/hr)
P<0.01
Male Female
Donor Pheromone
C
CO2 Production (ul/hr/fly)
P=0.37
P=0.92
Cont
Fem
Male
Female
Donor Pheromone
D
Activity (Counts/hr)
P=0.62
EtOH 7-11HD
E
Feeding Score
P=0.07
Male Female
Donor Pheromone
F
Activity (Counts/hr)
P=0.43
Cont
ppk23> TRPA1
G
CO2 Production (ul/hr/fly)
P=0.71
Cont
ppk23> TRPA1
H
Feeding Score
P=0.28
Cont
ppk23> TRPA1
I
Survivorship
Control
Female
Male
P<0.001
Vortex
Female
Male
P<0.001
Starvation Time (Hours)

Figure S6

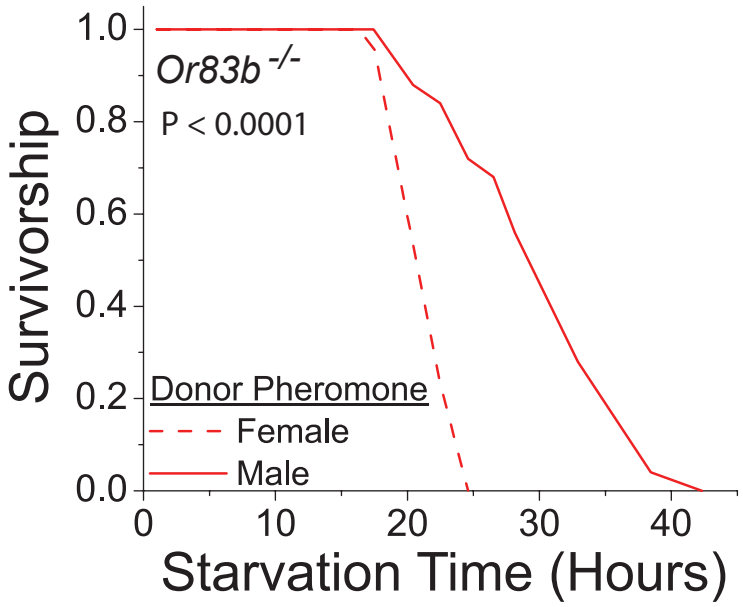
Survivorship
1.0
0.8
0.6
0.4
0.2
0.0
Or83b -/-
P < 0.0001
Donor Pheromone
Female
Male
0
10
20
30
40
Starvation Time (Hours)

Figure S7

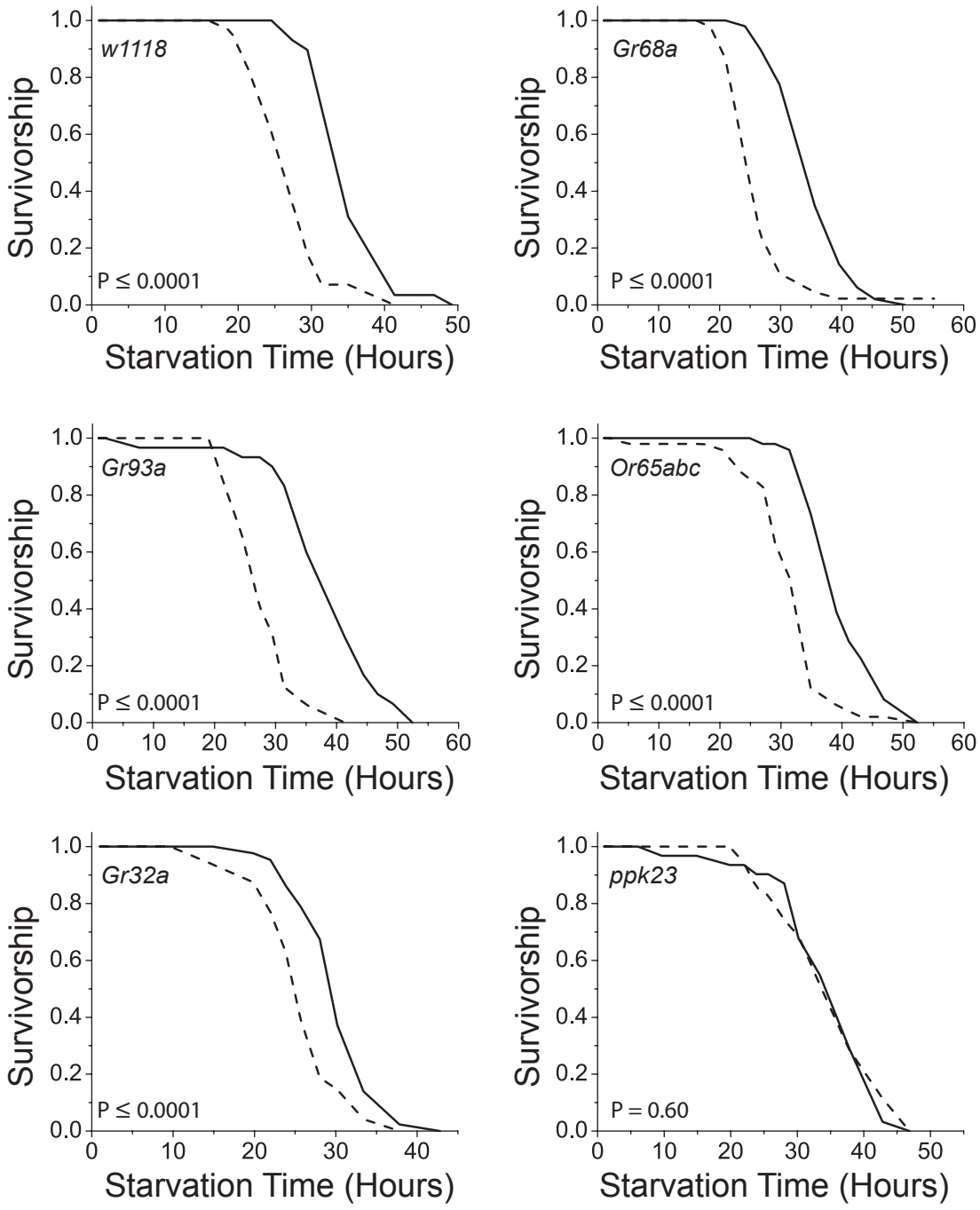
w1118
P ≤ 0.0001
Gr68a
P ≤ 0.0001
Gr93a
P ≤ 0.0001
Or65abc
P ≤ 0.0001
Gr32a
P ≤ 0.0001
ppk23
P = 0.60
Survivorship
Starvation Time (Hours)

Figure S8

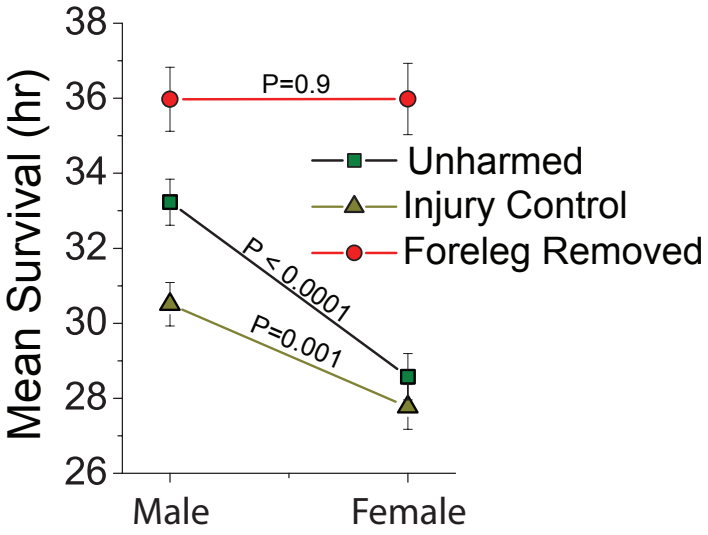
Mean Survival (hr)
38
36
34
32
30
28
26
Male
Female
P=0.9
P < 0.0001
P=0.001
Unharmed
Injury Control
Foreleg Removed

Figure S9

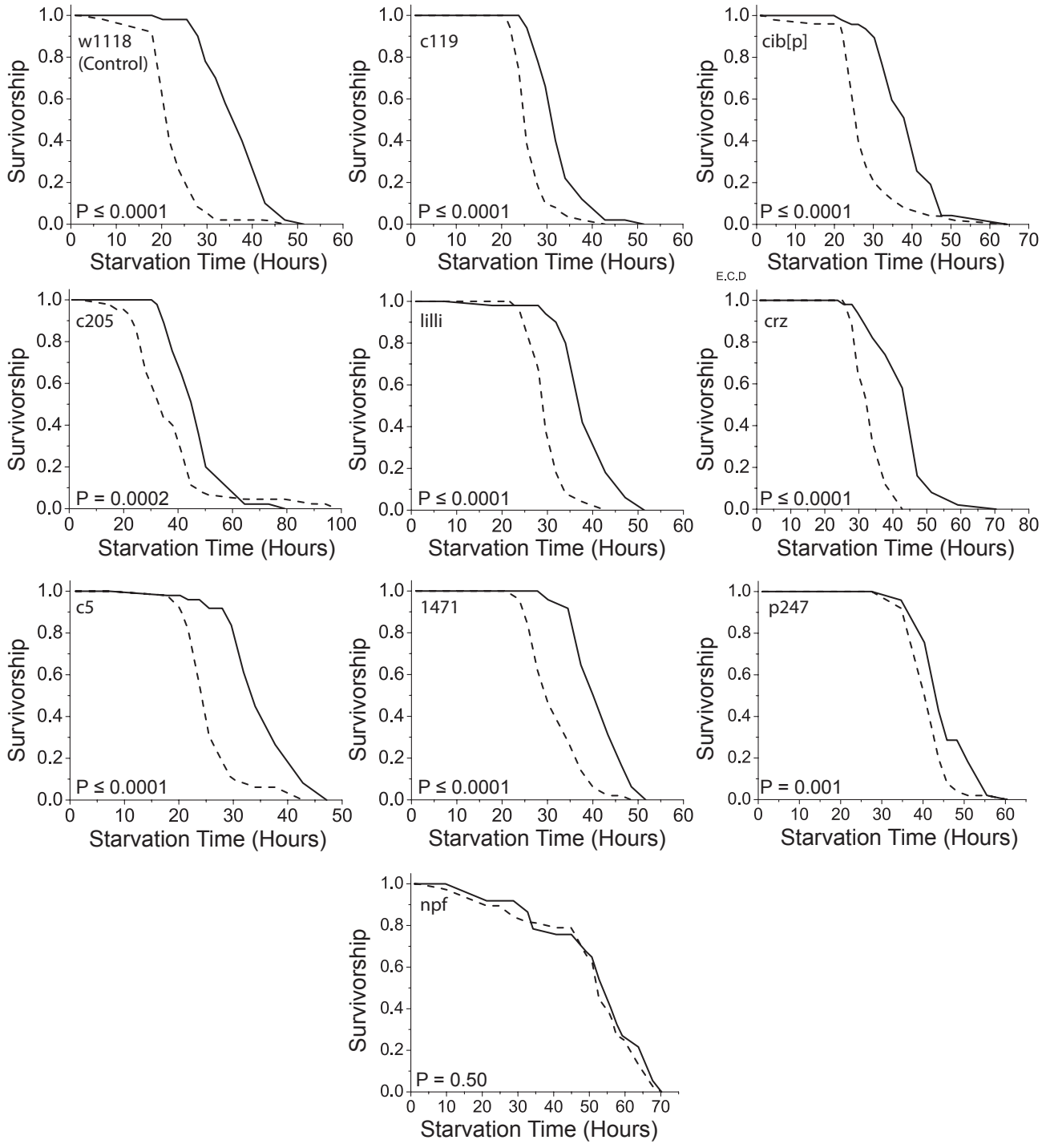
w1118
(Control)
P ≤ 0.0001
c119
P ≤ 0.0001
cib[p]
P ≤ 0.0001
c205
P = 0.0002
lilli
P ≤ 0.0001
crz
P ≤ 0.0001
c5
P ≤ 0.0001
1471
P ≤ 0.0001
p247
P = 0.001
npf
P = 0.50
Survivorship
Starvation Time (Hours)

Figure S10

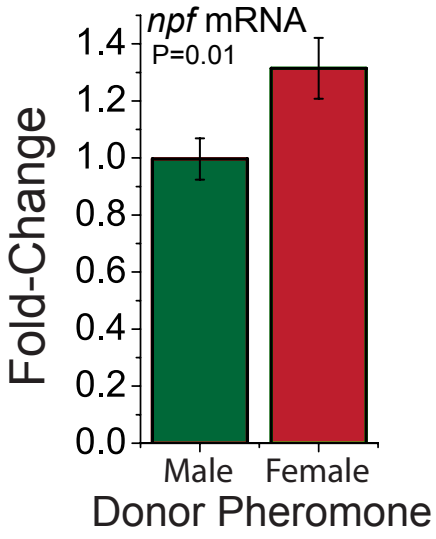
npf mRNA
P=0.01
Fold-Change
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Male
Female
Donor Pheromone

Figure S11

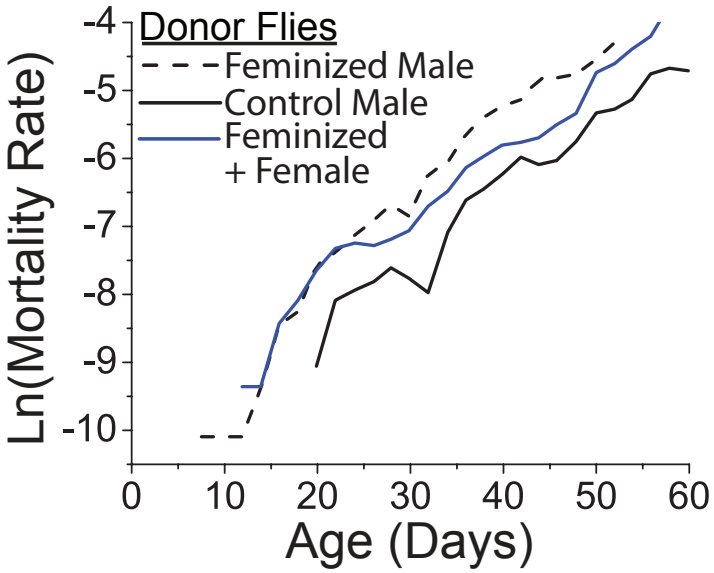
Donor Flies
Feminized Male
Control Male
Feminized
+ Female
Ln(Mortality Rate)
-4
-5
-6
-7
-8
-9
-10
0
10
20
30
40
50
60
Age (Days)

Figure S12

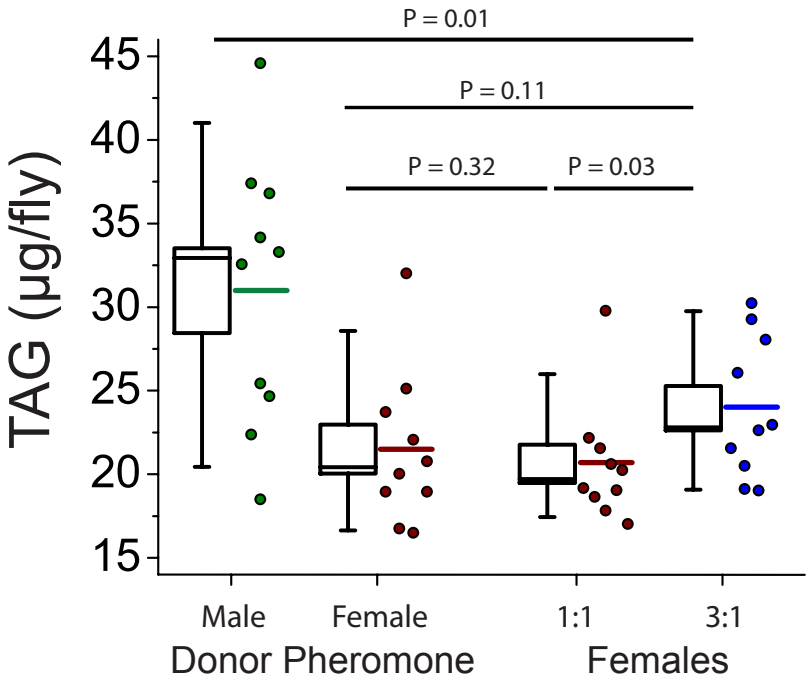
P = 0.01
P = 0.11
P = 0.32
P = 0.03
45
40
35
30
25
20
15
TAG (µg/fly)
Male
Female
1:1
3:1
Donor Pheromone
Females

Figure S13

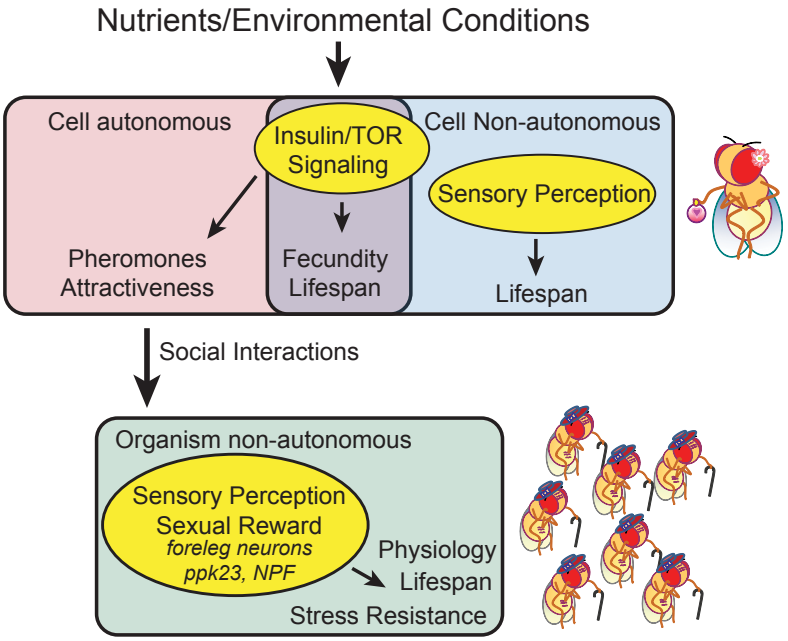
Nutrients/Environmental Conditions
Cell autonomous
Insulin/TOR Signaling
Cell Non-autonomous
Sensory Perception
Pheromones Attractiveness
Fecundity Lifespan
Lifespan
Social Interactions
Organism non-autonomous
Sensory Perception
Sexual Reward
foreleg neurons
ppk23, NPF
Physiology
Lifespan
Stress Resistance

Figure S14