Males induce premature demise of the opposite sex by multifaceted strategies

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Interactions between the sexes negatively impact health in many species. In Caenorhabditis, males shorten the lifespan of the opposite sex—hermaphrodites or females. Here we use transcriptomic profiling and targeted screens to systematically uncover conserved genes involved in male-induced demise in C. elegans. Some genes (for example, delm-2, acbp-3), when knocked down, are specifically protective against male-induced demise. Others (for example, sri-40), when knocked down, extend lifespan with and without males, suggesting general mechanisms of protection. In contrast, many classical long-lived mutants are impacted more negatively than wild type by the presence of males, highlighting the importance of sexual environment for longevity. Interestingly, genes induced by males are triggered by specific male components (seminal fluid, sperm and pheromone), and manipulating these genes in combination in hermaphrodites induces stronger protection. One of these genes, the conserved ion channel delm-2, acts in the nervous system and intestine to regulate lipid metabolism. Our analysis reveals striking differences in longevity in single sex versus mixed sex environments and uncovers elaborate strategies elicited by sexual interactions that could extend to other species.

Sexual interactions influence organismal health independently of reproduction in nematodes, flies and mammals1–4. For example, males induce weight gain and shorten lifespan in female mice, independent of fertilization5–7. However, the impact of sexual interactions on health is largely uncharacterized, in large part because most experiments are conducted in single-sex environments. Because of its short lifespan, C. elegans is an ideal model organism to systematically examine how sexual interactions affect longevity. Sexual interactions with males shorten the lifespan of the opposite sex (females or hermaphrodites) in Caenorhabditis1–4, and this phenomenon has been shown to involve components from both sexes. Indeed, males promote the premature death of hermaphrodites using male sperm and seminal fluid during mating1 as well as male pheromones and secreted compounds1,10 (especially when large numbers of males are present11). In hermaphrodites, several molecular and cellular pathways mediate aspects of male-induced demise, including transcription factors (for example, FOXO/DAF-16 and TFEB/HLH-30)12,13, chromatin regulators (for example, KDM6A/UTX-1), insulin ligands (for example, INS-11 and INS-7)12,13 and even self-sperm itself14,15. However, a systematic investigation of the pathways driving the negative impact of males on lifespan, and how they overlap with known longevity pathways, is still missing.

Results

Male-induced gene expression changes. To systematically assess the impact of sexual interactions with males on the opposite sex in C. elegans, we performed RNA sequencing (RNA-seq) of young (first day of adulthood or day 3 of life) and middle-aged (day 7 of life) hermaphrodites in the presence or absence of males (Fig. 1a; Methods). The males were present for a brief exposure (1 day) or a long exposure (5 consecutive days) (Fig. 1a). To eliminate possible effects of sexual interactions during development16,17 and avoid confounds from embryo transcripts, we initiated exposure to males at the onset of adulthood in sterile (gfp-1[c2144]) hermaphrodites (Fig. 1a). Male-induced demise occurs robustly and reproducibly under these conditions (Fig. 1b)18.

Principal component analysis (PCA) separated the transcriptomes of the samples based on age (Fig. 1c and Extended Data Fig. 1a) and exposure to males, especially after longer exposure (Fig. 1d–e). Prolonged exposure to males resulted in a marked increase in the degree and number of gene expression changes compared with the brief exposure to males (Fig. 1f). The genes that were induced in hermaphrodites following a brief (1 day) or long (5 days) interaction with males partially overlap (for example, ins-11, sri-40, acbp-3) (Fig. 1g). Importantly, many of the gene expression changes that we observed in sterile, gfp-1 hermaphrodites following sexual interactions with males were also observed in wild-type, fertile hermaphrodites (Extended Data Fig. 1c–e)19. Genes upregulated in response to males were enriched for lipid metabolism and transport, collagens and stress response (detoxification) gene categories (Fig. 1h and Extended Data Fig. 1e). Thus, males induce striking transcriptional changes in hermaphrodites, with a greater number and magnitude of changes in response to long interactions. As both brief and prolonged exposure to males shortens hermaphrodite lifespan12 (Fig. 1b), genes that are induced in both conditions might be more functionally relevant to elicit demise and we focused on these for the remainder of the study.

Identification of functional male-induced demise genes. To understand the functional role of these genes in the male-induced demise of hermaphrodites, we performed a targeted RNA-mediated
Fig. 1 | Sexual interactions induce premature death and specific transcriptional changes in *C. elegans* hermaphrodites. **a**, Scheme describing the ages of the glp-1(e2144) hermaphrodites (Her) used in RNA-seq experiments and length of sexual interactions with males. Pink dots, age (day of life) of hermaphrodites at sample collection times; blue line, period of time (1 day versus 5 days) with males. Three to eight biological replicates, each consisting of 75 hermaphrodites per condition. **b**, The presence of males during adulthood (either all of adulthood (dashed dark red line) or for 1 day (dotted pink line), the presence of males during adulthood (either all of adulthood (dashed dark red line) or for 1 day (dotted pink line)) shortens the lifespan of sterile glp-1(e2144) *C. elegans* hermaphrodites (*P* < 0.0001 for any condition compared with hermaphrodites only). Lifespan data plotted as Kaplan–Meier survival curves, *P* value calculated using Mantel–Cox log ranking. A total of 98–114 hermaphrodites were tested. **c–e**, PCA of normalized read counts from the hermaphrodite transcriptomes after removal of male-enriched genes (Extended Data Fig. 1a,b; Methods). In e, data from all hermaphrodite age groups were used, and in **d** and **e**, the read counts from day 3 or day 7 only were normalized and analyzed. **f**, Male-induced hermaphrodite gene expression changes following 1 or 5 days of interaction between the sexes. After filtering the data for male-enriched genes, the log2 (fold change) for detected genes is displayed and differentially expressed genes (*P* ≤ 0.05) in the presence of males are in red. Complete analysis results are in Supplementary Data 1. **g**, Heatmap of differentially expressed genes. **h**, Gene category enrichment: common differentially expressed genes.

Our screen identified genes that, when knocked down, exhibited a significant and protective interaction with the presence of males by Cox proportional hazard model—a statistical method to test for association between lifespan and different parameters (Methods).
Fig. 2 | Male-induced demise is mediated by specific genes and more general health genes. a, Results of our RNAi-based screen for functionally important hermaphrodite genes in male-induced demise (MID). Color of squares on the left indicate significant and protective (green), significant and detrimental (purple) or NS (yellow and gray) interaction between male-induced demise and a gene knockdown using the Cox proportional hazard model (see gene classification criteria in Methods). Yellow and gray squares are distinguished based on the ability of the gene knockdown to extend lifespan in the presence of males (yellow, significant lifespan extension; gray, no significant lifespan extension). Circle colors indicate hazard ratios of specific gene knockdowns versus control, EV RNAi in hermaphrodite only conditions (left circles) or in the presence of males (right circles). Circle sizes indicate P values (calculated using Cox proportional hazard model). b–d, Knockdown of genes encoding the ion channel DEML-2 (b), collagen COL-43 (c), acyl-CoA binding protein ACPB-3 (d) or acyl-CoA oxidase ACOX1.3 (Extended Data Fig. 2b) partially protected hermaphrodites from male-induced demise (dashed lines) but did not detectably extend lifespan in the absence of males (solid lines) (significant and protective interaction by Cox proportional hazard model). Delm-2 knockdown can extend lifespan in the absence of males in some but not all experiments (Extended Data Fig. 2a); ctrl, control. e,f, Knockdown of the genes encoding the cytochrome P450 protein CYP-25A3 (e), thioesterase T05E7.1 (f) or other genes (Extended Data Fig. 2) extended hermaphrodite lifespan in the presence of males (no significant interaction by Cox proportional hazard model). g, Knockdown of serpentine receptor gene sri-40 extended hermaphrodite lifespan in the absence of males (solid lines) of males (no significant interaction by Cox proportional hazard model). Lifespan data plotted as Kaplan–Meier survival curves and for P values calculated by Mantel–Cox log ranking. For the screen, 31–59 wild-type hermaphrodites were tested; the complete results of the lifespan assays can be found in Supplementary Table 1.
Fig. 3 | Classical longevity mutants are susceptible to male-induced demise. 

a, Comparison of classical longevity mutants, knockdown and overexpression in the presence and absence of males. Color of squares and color and size of circles as in Fig. 2a. b, Mutation of the mitochondria electron transport chain gene *nuo-6* extended lifespan in a hermaphrodite only setting (solid lines). In the presence of males, *nuo-6* hermaphrodites lived longer than wild type (WT), but this extension was blunted compared with the extension of lifespan in the absence of males (no significant interaction by Cox proportional hazard model). c, The presence of males decreases the expression of a mitochondrial UPR reporter (*hsp-6p::GFP*) under conditions that activate the mitochondrial UPR (*nuo-6* mutation). Representative images (top) and quantification (bottom, two-tailed Mann–Whitney test) are shown. The fluorescence of 29–33 hermaphrodites were measured per condition (Extended Data Fig. 2j; Source Data). d, e, Overexpression (OE) of the histone demethylase gene *jmjd-1.2* (*d*) or mutation in the insulin/IGF1 receptor gene *daf-2* (*e*) extended hermaphrodite lifespan in a hermaphrodite only environment (solid lines) but not in the presence of males (dashed lines) (significant and detrimental interaction by Cox proportional hazard model). The lifespans of 72–114 hermaphrodites were measured per condition in b, d and e. A complete list of lifespan data is presented in Supplementary Table 1. f, FOXO/DAF-16 localization after heat shock. g, Summary of the lifespan results from our screen and additional replicates. Box plot minima and maxima are the first and third quartile, respectively, and the center line shows the median. Whiskers extend to the largest and smallest values. Hazard ratios for each lifespan assay were determined using the Cox proportional hazard model to model the interaction between male-induced demise and a gene knockdown or mutation. Inverted log₁₀ scale of the y axis. Some replicates of the *utx-1* RNAi, *daf-2(e1370)* and *glp-1(e2144)* results were published previously¹. All other lifespan results are from this manuscript (Supplementary Table 1).
identified in our screen. The detrimental effect of males on classical longevity mutants may be due to males repressing downstream components of longevity pathways, including FOXO/DAF-16 transcription factor nuclear localization (Fig. 3f) and mitochondrial unfolded protein response (UPR) upregulation (Fig. 3c and Extended Data Fig. 2). Thus, the context in which an individual lives (for example, with or without mates) can drastically impact the effect of a longevity intervention (Fig. 3g).

Genes induced by male sperm, seminal fluid and pheromones. We asked whether the genes we identified are induced differently by the various male components that contribute to premature demise (male sperm, seminal fluid and pheromones\(^{2,11}\)), as this could lead to the development of combined interventions against male-induced demise. We measured gene expression in young (day 4–5 of life) hermaphrodites following 1 day of mating with both wild-type males and sperm-less (fer-6\(\text{hc6}\)) males (Fig. 4a,b and Extended Data Fig. 2) (thereby distinguishing male sperm from seminal fluid) or on male-conditioned plates (MCP) for 5 days (isolating male pheromones) (Fig. 4c). Interestingly, each male component elicited a distinct transcriptional response from hermaphrodites (Extended Data Fig. 4e), consistent with the different physiological changes (for example, fat loss and body shrinking) observed in response to these male components\(^{2,11}\).

The subset of genes induced by male sperm (that is, expressed in response to mating with wild-type but not sperm-deficient males) were enriched for metabolism genes, and included genes encoding...
**Fig. 5 | Strategies to strongly protect hermaphrodites from male-induced demise.** a. A model for the transcriptomics and male-induced demise lifespan data showing that male sperm, seminal fluid and pheromones each induce a different set of functionally important male-induced demise genes in hermaphrodites. b. Reduction of acbp-3 or delm-2 individually by RNAi partially protected hermaphrodites from male-induced demise (acbp-3 or delm-2 P < 0.0001 compared with control RNAi, Mantel–Cox log ranking). Reduction of acbp-3 and delm-2 simultaneously by double RNAi (dashed pink line) protected hermaphrodites from male-induced demise to a greater extent than knockdown of either acbp-3 or delm-2 alone (P < 0.0001 compared with either single gene knockdown, Mantel–Cox log ranking). c. Knockdown of acbp-3 and delm-2 simultaneously extended hermaphrodite lifespan. In the presence of males (dashed lines), loss of acbp-3 and delm-2 increased lifespan and resulted in a lifespan almost comparable with that of control hermaphrodites in a single-sex setting (black solid line versus dashed pink line: P = 0.03, Mantel–Cox log ranking). In the absence of males, loss of acbp-3 and delm-2 increased lifespan (P < 0.0001, Mantel–Cox log ranking) compared with control RNAi (solid black line). A significant, protective interaction between simultaneous delm-2 and acbp-3 knockdown and male-induced demise was detected by Cox proportional hazard model (P = 1.43 × 10^-8). d. Average MACS2 score of transcription factor ChIP–seq binding peaks within ± 5 kb of the transcription start site of male-induced functional important genes. For complete transcription factor enrichment analysis, see Supplementary Data 9. e. Knockdown of the gene encoding the transcription factor ceh-60 significantly extended lifespan in both the presence and absence of males (P < 0.0001, Mantel–Cox log ranking). Significant, protective interaction between ceh-60 knockdown and male-induced demise by Cox proportional hazard model (P = 4.39 × 10^-8). In b, c and e, lifespan data displayed as Kaplan–Meier survival curves. In all, 105-124 hermaphrodites were used. Complete list of lifespan assays and statistical tests are presented in Supplemental Table 1.

RNAi to delm-2 or acd-1 probably target all three paralogs (delm-1, delm-2 and acd-1) (Extended Data Fig. 3a). To dissect the role of each paralog in male-induce demise, we measured the lifespans of single, double and triple mutants for delm-1, delm-2 and acd-1. While the single mutants for delm-1, delm-2 or acd-1, and the delm-2delm-1 double mutant did not exhibit specific protection against male-induced demise (no significant interaction detected by Cox proportional hazard model, grey square, Extended Data Fig. 3d–g), the acd-1 delm-2delm-1 triple mutant did show a specific protection against male-induced demise (significant and protective interaction in the presence of males by Cox proportional hazard model, green square (Extended Data Fig. 3h). The acd-1 delm-2delm-1 triple mutant still exhibited some lifespan extension in the absence of males (though proportionally less than in the presence of males), whereas the delm-2 RNAi knockdown did not show consistent lifespan extension in the absence of males—perhaps due to differences between knockout and knockdown (Extended Data Fig. 2a).
**Fig. 6 | DELM-2 and ACBP-3 act in the nervous and digestive systems to regulate lifespan.** a. Heatmaps showing the predicted tissue expression patterns of the male sperm, seminal fluid and pheromone regulated hermaphrodite genes. The weighted average score of each tissue was calculated as the mean prediction score for the male-induced genes divided by mean prediction score for all genes detected in the microarrays. b–e. UMAP plots of larval stage 2 (L2) hermaphrodite cells (b and e). Cells with detectable expression of delm-2, acd-1 (b) and acbp-3 (e) are shown. Previously published data was reanalyzed to generate these plots. Reduction of delm-2 (green lines) in the nervous system (c) or the intestine (d) by RNAi is sufficient to extend lifespan of hermaphrodites in the presence of males (dashed lines, \( P = 0.00041 \)) and conferred specific protection against male-induced demise as shown by modeling the interaction between male-induced demise and tissue-specific rNAi using Cox proportional hazard modeling (\( P = 0.137 \times 10^{-15} \)). f, Knockdown of acbp-3 (blue lines) by RNAi specifically in the nervous system had no detectable impact on hermaphrodite lifespan in the presence (dashed lines) or absence (solid lines) of males. g, In contrast, RNAi knockdown of acbp-3 specifically in the intestine extended lifespan of hermaphrodites when in the presence of males (dashed lines, \( P = 0.0001 \)) but not in the absence of males (\( P = 3.02 \times 10^{-16} \)). Lifespan data are plotted as Kaplan–Meier survival curves and \( P \) values calculated by Mantel–Cox log ranking. A total of 98–111 hermaphrodites were used per condition. See Supplementary Table 1 for a complete list of all lifespan data.
Effective strategies to counter male-induced demise. Different genetic pathways in the hermaphrodite mediate the demise induced by male sperm, seminal fluid and pheromones. We assessed the role of delm-2 (induced by male seminal fluid) in combination withacb-3 (induced by male sperm). While reduction of each gene individually resulted in partial protection from male-induced demise, knockdown of delm-2 (and paralogs) together with acbp-3 resulted in additive protection against male-induced demise (Fig. 3b). Interestingly, knockdown of delm-2 (and paralogs) and acbp-3 in combination fully protected hermaphrodites from the lifespan-shortening impact of sexual interactions with males (Fig. 4c), and these hermaphrodites lived approximately the same lifespan as hermaphrodites in the absence of males (Fig. 4c). Thus, a combined loss of function in the hermaphrodite pathways targeted by male sperm and male seminal fluid strongly improves protection from male-induced demise.

Analysis of the regulatory regions of the genes induced by parallel male strategies revealed key transcription factor binding sites, with some of them being shared among different male-induced pathways (for example, PQM-1 (ref. 25), PPARD/NHR-28 and PBX3.4/CEH-60) (Fig. 5a and Extended Data Fig. 5). We tested the functional role of one of these key upstream regulators (the conserved homeobox transcription factor PBX3.4/CEH-60) and found that ceh-60 RNAi strongly and specifically protected hermaphrodites from male-induced demise (Fig. 5e). Collectively, these results suggest that targeting the different genes induced by males in combination (using several genes or a common transcription factor) is more effective to counter male-induced demise than targeting a single gene or pathway.

Tissue-specific regulation of male-induced demise. We asked whether the genes induced by males act in specific cells and tissues to mediate male-induced demise. Using a prediction tool based on tissue-specific expression26,27, we found that the genes differentially expressed by exposure to male sperm, seminal fluid and pheromones were all enriched in the nervous system and the digestive system (for example, intestine) (Fig. 6a). Genes involved in the response to sperm and seminal fluid were also enriched in the reproductive and epithelial system (Fig. 6a). We focused on two of the top genes mediating male-induced demise: the seminal fluid-responsive gene delm-2 and the sperm-responsive gene acbp-3. To analyze their expression in different cells, we used publicly available single-cell RNA-seq data (I2 hermaphrodites)28 (Fig. 6b,c). This analysis revealed that delm-2 and paralog acbp-1 were each expressed in intestinal and nervous system cells, and that delm-2 was expressed in pharyngeal epithelial cells (Fig. 6b). acbp-3 was expressed in intestinal and epithelial cells (Fig. 6e). Together, this analysis suggests a multi-tissue response, with contribution from both the nervous and digestive systems.

We next determined if DELM-2 (and paralogs) and ACBP-3 could functionally mediate male-induced demise by acting in these tissues. Using tissue-specific RNAi, we showed that reduction of delm-2 and paralogs in either the nervous system or the intestine was sufficient to protect against male-induced demise (Fig. 6c,d). In contrast, reduction of acbp-3 in the intestine, but not in the nervous system, protected against male-induced demise (Fig. 6f,g). Hence, DELM-2 and paralogs function in at least two tissues—the nervous system and the intestine—to mediate male-induced demise.

Regulation of fat metabolism by the ion channel DELM-2. How might DELM-2 regulate male-induced demise? Males induce changes in lipid metabolism in the opposite sex in several species29,30, and generate pheromones that potentiate the male-induced demise. Using a prediction tool based on tissue-specific expression, we found that the genes induced by males act in specific cells and tissues to mediate male-induced demise. We therefore determined whether DELM-2 could regulate lipid metabolism in the absence and presence of males. To measure neutral lipids, we performed Oil Red O (ORO) staining on fixed hermaphrodites. We found that delm-2 RNAi or mutation of delm-2 and its paralogs significantly prevented the depletion of neutral lipids induced by males, especially at middle age (Fig. 7a–c; Source Data). Reduction of delm-2 (and paralogs) either in the intestine or the nervous system increased neutral lipids both in the presence and absence of males, and, interestingly, reduction of delm-2 (and paralogs) in the nervous system prevented the depletion of neutral lipids produced by males.
of neutral lipids that is normally induced by males (Fig. 7d,e). Knockdown of delm-2 (and paralogs) also upregulated expression of enzymes (for example, FAT-5 and FAT-7) involved in lipid metabolism, notably the production of mono-unsaturated fatty acids (MUFA) (Fig. 7f,g). These results are consistent with the possibility that loss of the ion channel DELM-2 and paralogs could protect hermaphrodites from male-induced demise by regulating MUFA synthesis and preventing fat loss induced by males. These observations also suggest a multi-tissue site of action for DELM-2 and paralogs in regulating fat metabolism.
Discussion
Here we have identified genes that are not only regulated by but are also functionally important for the response of hermaphrodites to sexual interactions (male-induced demise). Our analysis uncovers specific regulators of male-induced demise (for example, ACBP-3 and DELM-2 and paralogs). It also identifies regulators whose reduction extends lifespan both in the absence and presence of males (for example, SRI-40) and that have been missed by previous genetic screens. In contrast, several classical longevity mutants are, in fact, more susceptible to the presence of males than wild type, with males inhibiting components of classical longevity pathways. Thus, several classical long-lived mutants may have an Achilles’ heel: their susceptibility to the opposite sex. Our study reveals that targeting genes in a mixed- versus a single-sex environment can have different outcomes on lifespan, which has important implications for health. Sexual interactions may therefore represent a particularly potent biological force distinct from other types of stresses.

Why have hermaphrodites not evolved to turn the genes induced by males off? One possibility could be that these genes provide some evolutionary benefit to the hermaphrodites that outweighs their cost. Alternatively, negative selection may be weak on these genes because C. elegans males are not normally abundant and because hermaphrodites succumb to male-induced demise after reproducing. A third possibility is that the induction of these genes by males provides a benefit to the next generation that might offset the costs of males to maternal health.

By investigating these genes through different ‘lenses’ we have found that they fall into different categories both in terms of the male signal to which they respond (male sperm, seminal fluid or pheromones) and the specificity of their regulation of lifespan (specific to male-induced demise versus broad longevity regulation). Strikingly, many classical longevity mutants are more susceptible than wild-type animals are to male-induced demise, possibly through repression of their downstream pro-longevity effectors (for example, DAF-16/FOXO2). Future studies on the effects of sexual interactions on classic longevity pathways will be important to further elucidate the mechanism of action. Many of the genes identified in this study are conserved in mammals and could play a role in lifespan and health in humans. The placement of male-induced demise genes in different pathways allowed us to predict which combination of strategies could lead to greater protection from male-induced demise. Indeed, knockdown of delm-2 (and paralogs) and acbp-3 in combination (or knockdown of a gene encoding a potential common regulator, the transcription factor CEH-60) strongly extends the lifespan of hermaphrodites in the presence of males to the lifespan of hermaphrodites in the absence of males. This is important because it could help identify additional combinations of strategies that more robustly extend lifespan.

Finally, we find that DELM-2, a conserved ion channel, is specifically involved in male-induced demise and regulates lipid metabolism. The ability of DELM-2 and paralogs to impact the enzymes involved in MUFA production is consistent with the observation that MUFA s themselves partially protect hermaphrodites from male-induced demise59,60. DELM-2 and paralogs act in the nervous system and intestine to regulate lifespan and lipid metabolism in response to sexual signals received in the reproductive system (male seminal fluid). This observation suggests that communication between tissues is important for male-induced demise. The specific molecules involved in the tissue-to-tissue communication are unknown, but intriguingly, mammalian orthologs of delm-2 (SCNN1G and ASIC1,2,3) are involved in sensing specific lipids53,55. Overall, our study reveals that longevity is highly dependent on the sexual environment (single sex versus mixed sex) and that sexual interactions trigger an elaborate network of functional regulation, which could extend to other species and be used to counter aging and age-related diseases.

**Methods**

**Worm strains and maintenance.**

| C. elegans strain | Genotype | Source |
|-------------------|----------|--------|
| N2 | Wild type | M.-W. Tan & CGC* |
| CFI903 | glp-1(e2144) III | CGC |
| CB4037 | glp-1(e2144) III | CGC |
| CB1467 | him-5(q1467V) F | CGC |
| BA6 | fer-6(hc6) I | CGC |
| CB4108 | fog-2(q71) V | CGC |
| RB1523 | delm-2(ok1822) I | CGC and CDMC<sub>1</sub> |
| RB1771 | delm-1(ok1226) IV | CGC and CDMC |
| BLC100 | delm-1(ok1226) IV; delm-2(ok1822) I | L. Bianchi |
| ZB90 | acd-1(tz90) I | L. Bianchi |
| CB1611 | mec-4(e1611) X | M. Goodman |
| ABR212 | acd-1(sta6) delm-2(ok1822) I | This study |
| ABR225 | acd-1(sta6) delm-2(ok1822) I ; delm-1(ok1226) IV | This study |
| MQ1333 | nuo-6(q2000) I | CGC |
| MQ887 | isp-1(ym150) IV | CGC |
| CB4876 | clk-1(e2519) III | CGC |
| CB1370 | daf-2(e1370) III | CGC |
| AGD1505 | utih-404[q(myo-2p:td tomato, sur-5p:;jmjd-1.2u:3'UTR unc-54)] | A. Dillin |
| SJ4100 | zcs13 [hsp-6p:GFP + lin-15(+)] | CGC |
| ABR213 | nuo-6(q2000) I ; zcs13 [hsp-6p:GFP + lin-15(+)] | This study |
| ABR214 | isp-1(ym150) IV ; zcs13 [hsp-6p:GFP + lin-15(+)] | This study |
| TJ356 | zls356 [+;daf-16p;daf-16a/b::GFP + rol-6(su1006)] | CGC |
| TU3401 | sid-1(pk3321) V; uba69 [pcPF700(myo-2p:mCherry)+ unc-119::sid-1] | CGC |
| VP303 | rde-1(ne219) V; kbi-17 [nxa-2p::rde-1 + rol-6(su1006)] | CGC |
| IG1839 | Rde-1(ne300) V; fts17 [lin-2p::rde-1.3'UTR II, fts17 [nlp-29p::GFP + col-12p::Daf-4(Red) IV] | CGC |
| BX113 | lin15B, A(c675S) X; waEx15 [fat-7:GFP + lin15(+)] | CGC |
| BX150 | lin15B, A(c675S) X; waEx18 [fat-5::GFP + lin15(+)] | CGC |

<sup>*CGC, Caenorhabditis Genetics Center °CDMC, C. elegans Deletion Mutant Consortium</sup>

All C. elegans wild-type and mutant strains used in this study are listed above. All strains were maintained on Nematode Growth Media (NGM) plates with 50 μg/ml streptomycin (Gibco) and a lawn of OP50-1 bacteria (a gift from M.-W. Tan) from stationary phase cultures. Nematodes were grown at 20°C, except temperature-sensitive mutants (glp-1(e2144) and (e2141)), which were maintained at 15°C (permissive temperature). When temperature-sensitive mutants were used for assays, they were grown at the restrictive temperature (25°C). The genotype of strains was verified by genotyping PCR and Sanger sequencing and the strains were backcrossed three times into our laboratory’s N2 strain (in addition to the backcrossing that was performed when the mutants were initially isolated).

**RNA-sequencing.** To better understand how males induce premature hermaphrodite demise, we characterized the transcriptions of sterile glp-1(e2144) hermaphrodites that have a shortened lifespan after interacting with wild-type males for 1 day or 5 days to those that never interacted with males.

Individuals were age-synchronized using a brief, 3- to 4-h egg lay (Lifespan assays) on 10-cm NGM plates seeded with OP50-1 bacteria and grown at 25°C during development and adulthood. The day of the egg lay is considered day 0 of life. For the longer (5 day) exposure to males, 75 glp-1(e2144) hermaphrodites were placed onto a 10-cm NGM plates seeded with OP50-1 bacteria and with 75 young (day 3–5 of life) wild-type males starting on day 2 of life (young adults) until day 7 of life. At day 5 of life, the hermaphrodites were moved to fresh plates and the males were replaced with new, young wild-type males. For samples in
which hermaphrodites interacted with males for a single day, 75 glp-1(e2144) hermaphrodites were transferred onto two 10-cm NGM plates to bacteria and 75 young (day 3–5 of life) wild-type males were added starting on either day 2 of life (young adults) or on day 6 of life. Hermaphrodites that interacted with males starting on day 5 of life were maintained on 10-cm NGM plates with OP50-1 at a density of 150 hermaphrodites starting from day 2 of life. At the same time, hermaphrodites from the same cohort of age-synchronized glp-1(e2144) individuals were also aged on fresh plates with males (150 hermaphrodites per 10-cm plate to maintain a similar density). After 1 or 5 days, 75 hermaphrodites from each condition (individuals that either interacted with males or never interacted with males) were collected by removing either 75 hermaphrodites (for the ‘no males’ condition) or the 75 males from the plates. For each sample, these remaining 75 hermaphrodites were immediately washed three times with ice-cold M9 buffer (22 mM KH2PO4, 42 mM Na2PO4, 86 mM NaCl and 1 mM MgSO4) and the worm pellets were flash frozen in liquid nitrogen. In parallel with the RNA-seq sample collection, glp-1(e2144) hermaphrodites and wild-type males form the same batches of age-synchronization as their corresponding RNA-seq samples were used to measure the effects of RNAi knockdown (see Supplementary Table 1 for all data). While blinding was not possible for comparing hermaphrodite in the absence or presence of males, blinding was done for comparing genotypes and/or RNAi knockdown.

For conditions in which the effect of sexual interactions was assessed, we used one of three methods, as indicated. For the long-term exposure method (described previously1,2), young males (day 1 to 2 of adulthood) were added to the hermaphrodites at the onset of adulthood. For lifespan experiments in which the hermaphrodites were exposed to males for their entire adulthood, males were added in a 1:1 ratio with hermaphrodites and the number of males remained fixed, even as hermaphrodites began to die or censored. Male worms were replaced every other day at the time the hermaphrodites were transferred to new plates. Approximately every other day for the entirety of the lifespan assay, the lifespan experiments in which hermaphrodites were exposed to males for only 1 day, young males were added in a 2:1 male to hermaphrodite ratio. Following 24h of exposure, hermaphrodites were moved to new plates and did not encounter a male again throughout their lifespan. For the male-conditioned media lifespan assays, hermaphrodites were transferred onto MCP from late L4 stage and stayed on MCP for the remainder of their life. MCP were prepared throughout the course of the lifespan assays: 30 day of life males (fog-2(q72), essentially wild type) were transferred onto each plate (35-mm NGM plates); 2 days later, the males were removed and hermaphrodites for lifespan assays were immediately transferred onto these MCP. When males were used, matured individuals, and so on were randomly assigned to the ‘no males’ or ‘+ males’ conditions by picking them onto fresh plates in an alternating manner to avoid selection bias. Similarly, the males used for mating with individuals of different genotypes or RNAi treatments were from the same sets of males in each assay and were allocated randomly in an alternating manner. For each single biological replicate, approximately 35 individuals were placed on each of three to six plates (each plate represents a technical replicate). The number of individuals per plate and number of technical replicates were chosen based on field standards1,2.

For sterile mutants, slight modifications were made to the methods. The sterile glp-1(e2144) mutant and wild-type control parents were used for an egg lay at the permissive temperature (15 °C). Following the egg lay, the individuals used for the assay were kept at 25 °C for the remainder of the assay.

The number of animals (n) used for each assay and the number of independent biological replicates (N) can be found in Supplementary Table 1. Lifespan data were plotted as Kaplan–Meier survival curves and pairwise statistical analyses were performed using the logrank (Mantel–Cox) test in Prism v.8 and the Cox proportional hazard model in R (v.3.5.1) to model the effect of a gene knockdown or mutation in either the presence or absence of males. Figure panels summarizing lifespan assay results (for example, box plots) were made in R using ggplot2 (v.3.3.0). The R code used for Cox proportional hazard modeling and for graphing data is available at https://github.com/brunetlab/Booth-et-al.-2022.

RNA knockdown. To knock down expression of specific genes, we fed worms HT115 (Escherichia coli) bacteria expressing double-strand RNA targeting a specific gene. For whole-worm RNAi knockdown, wild-type (N2) hermaphrodites were used. For tissue-specific RNAi knockdown, TUC401 (neuron-specific), VP303 (intestine-specific) or IG1839 (intestine-specific) hermaphrodites were used. Worms were cultured on NGM containing ampicillin (100 μg ml−1, Sigma) and isopropyl-β-D-thiogalactoside (IPTG) (0.4 mM, Invitrogen). During development, worms were fed HT115 bacteria (grown to stationary phase, RNAi expression induced for 2–4 h with 0.4 mM IPTG, and the bacteria concentrated to 200 μg ml−1 carrying control empty vector (EV)). Upon adulthood (day 3 of life), worms were placed onto plates with HT115 bacteria (grown to stationary phase, RNAi expression induced for 2–4 h with 0.4 mM IPTG, and the bacteria concentrated to 20x) carrying the appropriate RNAi clone. RNAi clones in HT115 E. coli were isolated from the Ahringer RNAi library1 (a gift from A. Fire) or, if unavailable, from the Vidal RNAi library2 (Dharmacon). The inclusion of the plasmids encoding the RNAi clones used in this study is documented in Supplementary data 1 and 3. For RNAi knockdown assays, the identity of the RNAi clone was blinded until the lifespan assay was completed.

Supplementary Data 1 and 3 are available as supplemental datasets and their analyses are included in this study.
We note that the RNAi construct that targets delm-2 shares high sequence similarity to two delm paralogs: delm-1 and acd-1 (Extended Data Fig. 3). Interestingly, acd-1 expression was also induced by males (Fig. 1g and Extended Data Fig. 4d) and loss of acd-1 by RNAi knockdown partially protected hermaphrodites from male-induced demise (Fig. 1m and Supplementary Table 1). However, single mutations in these genes are not sufficient to protect hermaphrodites from male-induced demise (Extended Data Fig. 3), suggesting that these changes in gene expression are not all that are required for male-induced demise. Cox proportional hazard model—a statistical method to examine association of the presence of males (that is, specific and protective to male-induced demise) (green squares).

(2) Genes that, when knocked down or mutated, do not exhibit a significant interaction with the presence of males (independent of males) (yellow squares).

(3) Genes that, when knocked down or mutated, do not exhibit a significant interaction with the presence of males and do not have an impact on lifespan extension (purple squares).

(4) Genes that, when knocked down or mutated, exhibit a significant and detrimental interaction with the presence of males (red squares).

Thus, this classification based on Cox proportional hazard model yields four categories of genes: (with a P value cut-off of 0.01 for the screen and 0.05 for all other lifespan assays):

(1) Genes that, when knocked down or mutated, exhibit a significant and protective interaction with the presence of males (that is, specific and protective to male-induced demise) (green squares).

To perform double RNAi, we combined equal amounts of bacteria expressing delm-2 targeting dsRNA and ach-3 targeting dsRNA. This was compared with control RNAi bacteria (with EV) and to single RNAi knockdown. The single RNAi knockdown for these experiments were diluted 50% using control (EV) RNAi expressing bacteria. We note that RNAi knockdown does not result in complete loss of function (that is, it is not a null). Therefore, a caveat to the interpretation of double RNAi results is that effects on lifespan may be due to intensifying the loss of function in a single pathway rather than targeting two parallel pathways.

RNAi-based screen. To determine whether the male-induced gene expression changes in hermaphrodites functionally contribute to their premature demise, we performed a targeted RNAi-based screen. The specific genes that we included in our screen were chosen because they were upregulated in several datasets: glp-1 sterile hermaphrodites for long and short interactions (Fig. 1g) and wild-type or feminized individuals mated with males for 2 h (Extended Data Fig. 1d). We also included several genes that were highly male-enriched (lys-3, C29F7.2, T02B5.3 and T16G1.6) and that were not significantly enriched (hkb-1 and K09C4.5) as controls to test whether genes that we filtered from our RNA-seq analysis were functionally important for male-induced demise. None of these genes significantly extended hermaphrodite lifespan when knocked down (Supplementary Table 1).

As a positive control, we used RNAi knockdown of the male-induced gene utx-1 (ref. 1). RNAI treatments were blinded until the last animals died. For the screen, we performed lifespan assays using a single plate of approximately 35 N2 (wild type) hermaphrodites for each ‘no males’ condition and two plates of approximately 18 N2 (wild type) hermaphrodites and 18 him-5(e1467) males for each + males’ condition (Lifespan assays).

Classification of RNAI and mutant lifespan results using the Cox proportional hazard model. To test whether there is a significant interaction between a specific gene perturbation and the presence of males for impact on lifespan, we used the Cox proportional hazard model—a statistical method to examine association between survival (lifespan) and other parameters (in this case, gene perturbation) (Extended Data Fig. 3). To test this possibility, we generated an acd-1 and delm-2 double mutant and an acd-1, delm-2, and delm-1 triple mutant (see below; CRISPR–Cas9 knockout of acd-1).

To perform the Cox proportional hazard model, we fitted a Cox regression model to the data, allowing us to estimate the hazard ratio between survival (lifespan) and other parameters (in this case, gene perturbation). The hazard ratio indicates the relative risk of death between two groups. A hazard ratio of 1 indicates no difference in survival, while a hazard ratio greater than 1 indicates a higher risk of death in the group with the higher value of the parameter being studied. In this case, we were interested in the hazard ratio between the presence of males and the absence of males.

To test whether there is a significant interaction between a specific gene and the presence of males, we used a Cox proportional hazard model. The hazard ratio between survival (lifespan) and other parameters (in this case, gene perturbation) was estimated using the Cox regression model. The hazard ratio indicates the relative risk of death between two groups. A hazard ratio of 1 indicates no difference in survival, while a hazard ratio greater than 1 indicates a higher risk of death in the group with the higher value of the parameter being studied. In this case, we were interested in the hazard ratio between the presence of males and the absence of males.

CRISPR–Cas9 knockout of acd-1. The paralogs acd-1 and delm-2 are present on the same chromosome in the C. elegans genome. To generate a double delm-2 delm-2 mutant strain, we used CRISPR–Cas9 gene editing as described previously to knockout acd-1 in a delm-2(ok1822) background (strain RB1523). A CRISPR RNA (crRNA) for acd-1 was designed using the predesigned Alt-R CRISPR–Cas9 guide RNA tool from IDT. To generate the crRNA-trans-activating crRNA (tracrRNA) duplexes, we used 200 µM Alt-R CRISPR crRNA for acd-1, 200 µM tracrRNA and 20 µg crRNA for dpy-10, which were annealed for 5 min at 95°C followed by 5 min at room temperature. Next, 27 µM crRNA:tracrRNA duplex was mixed with 27 µM Cas9-NLS protein (IDT) and incubated for 5 min at room temperature. Finally, the injection mix was assembled with components having the final concentrations of: 17.5 µM Cas9 protein, 17.5 µg mRNA:tracrRNA duplexes and 0.5 µM single-stranded DNA repair template for dpy-10 (ref. 2). The addition of the dpy-10 crRNA and repair template allows for a rapidly identifiable marker of CRISPR editing that is crossed out of the strain during backcrossing. Young delm-2(delk1822) mutant hermaphrodites were injected and left to recover on individual plates. After 3–5 days, plates were screened for rollers or dumpy worms and then subsequently screened on a greater scale using Sanger sequencing.

The resulting strain was backcrossed three times (ABR212, delm-2(ok1822) acl-1(st56)). A triple mutant (ABR225) was made by crossing ABR212 with RB177 (delm-1(ok1226)).

Guide and repair template sequences.

dpy-10 crRNA: GCCUCCAUAAGGCACCAGAG

dpy-10 repair template ssDNA:

CACCACCATGCTTAACGGGCAAGATGAGAATGGACGAAACCGT

ACCCGATGCCGGTGCCTAATGGTAGCGGACAATGGCTTACAG

GCACAACAGCTAT

crd RNA: CATAATGGCTCGGGTCTCC

Alignment of delm-2 RNAI targeting sequence. The delm-1 and acl-1 unsplitted transcript sequences were downloaded from WormBase (WS275) and aligned to the Ahringer library RNAi constructs that target delm-2 using Clustal Omega. The pairwise alignments were visualized using jAlign.

Fluorescent reporters. To quantify the mitochondrial UPR induction and FAT-5 expression, transgenic reporters (Worm strains and maintenance) were used. For the mitochondrial UPR, hsp-6::GFP reporters were crossed to the mitochondrial mutants nus-6(qm200) and isp-1(quin150) to generate strains ABR213 and ABR214, respectively. Hermaphrodites were synchronized using a timed egg lay and were cultured on RNAI plates seeded with HT115 E. coli containing either EV or delm-2 targeting dsRNA (individuals were selected randomly for each condition in an alternating manner). Starting on adult day 1, synchronized hermaphrodites were randomly selected in an alternating fashion to be either cultured only with other hermaphrodites (around 35 per 6-cm plate) or
microarrays were used for expression analysis. Significantly differentially expressed primarily cytoplasmic, primarily nuclear or nuclear and cytoplasmic DAF-16::GFP localization of DAF-16, all images were blinded and individual worms scored as within a given experiment. When determining nuclear versus cytoplasmic on a Nikon Eclipse confocal with identical exposure settings for all conditions within a given experiment. When determining nuclear versus cytoplasmic localization of DAF-16, FOXO/DAF-16 localization.

To measure nuclear localization of FOXO/DAF-16, we used a fluorescent DAF-16 reporter \( ^7 \) (Worm strains and maintenance). Hermaphrodites were anesthetized using a 1:1,3-hexadecanol solution and were mounted on a glass slide on agar pads and imaged. To avoid the stress of anesthesia and mounting to influence expression changes that were very similar to the transcriptional response of males \( ^{11} \), we reanalyzed published single-cell RNA-seq data from larval stage 2 (L2) hermaphrodites \( ^{31} \). Briefly, the data from the two single-cell experiments were merged and clustered using Seurat v.3.2.3 and Harmony (v.1.0.10 reference). We performed uniform manifold approximation and projection (UMAP) clustering on the first 35 principal components after performing PCA on the 2,000 most variable genes. Expression of \( daf-2 \) and \( acpb-3 \) was projected onto the UMAP with a maximum cut-off of three. The original, published cell type and tissue identifiers (for example intestine, body wall muscle) were used.

Oro staining. ORO staining was used to detect neutral lipids in fixed worms. Wild-type hermaphrodites were age-synchronized using a timed egg lay on RNAi plates seeded with EV HT115 E. coli. At adult day 1 (day 3 of life), hermaphrodites were placed randomly on either EV RNAi or MCP and cultured either with EV HT115 RNAi and cultured either with males (around 15 hermaphrodites and around 30 adult day 1 males). After 2 days (adult day 3), half of the hermaphrodites were exposed to a 2-h heat shock at 30 °C by wrapping the plates in Paraffilm and submerging in a waterbath. The worms were immediately anesthetized in M9 \( (22 \text{mM KH}_2\text{PO}_4, 42 \text{mM Na}_2\text{HPO}_4, 86 \text{mM NaCl and 1mM MgSO}_4) \) containing 50 mM sodium azide and mounted on 2% agar pads and imaged. To avoid the stress of anesthesia and mounting to influence FOXO/DAF-16 localization, FOXO/DAF-16 was expressed using a timereporter strain (daf-16::GFP) was used to determine the enrichment of chromatin immunoprecipitation–sequencing (ChIP–seq) peaks at the male sperm, seminal fluid and pheromone regulated genes. These data were identified using a timereporter strain (daf-16::GFP) and were used as input. The tissue expression patterns of the male sperm, seminal fluid and pheromone regulated genes were calculated using the webtool https://puma.princeton.edu. The male-induced, differentially expressed genes were identified by microarrays and were used as input. The tissue expression patterns were clustered and displayed as a heatmap using the R package pheatmap (v.1.0.12). The weighted average expression in a gene set was calculated as the mean male-induced expression prediction score divided by the mean expression prediction score for all genes detected by microarray.

Single-cell RNA-seq reanalysis. To identify cell- and tissue-specific expression patterns for \( daf-2 \) and \( acpb-3 \), we reanalyzed published single-cell RNA-seq data from larval stage 2 (L2) hermaphrodites \( ^{11} \). Briefly, the data from the two single-cell experiments were merged and clustered using Seurat v.3.2.3 (refs. \( ^{62-64} \)) and Harmony (v.1.0.10 reference). We performed uniform manifold approximation and projection (UMAP) clustering on the first 35 principal components after performing PCA on the 2,000 most variable genes. Expression of \( daf-2 \) and \( acpb-3 \) was projected onto the UMAP with a maximum cut-off of three. The original, published cell type and tissue identifiers (for example intestine, body wall muscle) were used.
the experiments were performed blinded (for example, the RNAi treatment or genotypes without a phenotype that could be discerned by eye). However, in some cases blinding was not possible (for example, comparing the effects of the presence versus absence of males on lifespan). Data were excluded if they failed to meet pre-established quality control criteria. For example, two RNA-seq libraries were excluded before sequencing because they did not meet quality control criteria for insert size distribution and purity. For lifespan assays, worms were scored as censored if they crawled off the media or died due to bagging (internal hatching) or vulval rupture, following field standards. Data from these censored worms were included up until the point of censorship (see Supplementary Table 1 for all data). In many cases, experiments were repeated by independent investigators or results were verified by an orthogonal method. See, for example, Supplementary Table 1 for a complete list of lifespan assays replicated by independent researchers. In addition, our transcriptomic experiments (RNA-seq and microarrays) were performed by different researchers at different institutes. These experiments show highly similar results.

Lifespan data were plotted as Kaplan–Meier survival curves and pairwise statistical differences were performed using the logrank (Mantel–Cox) test and the Cox proportional hazard model to model the effect of a gene knockdown or mutation in either the presence or absence of males. The Cox proportional hazard model was also used to test for an interaction between the effects of the presence of males and a specific mutation and RNAi treatment on Hermaphrodite lifespan. For the ORO and fluorescence microscopy experiments, conditions were compared by a two-tailed, Mann–Whitney test and two-way ANOVA.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All RNA-seq reads are available on NCBI Sequence Read Archive (PRJNA642294). Figure 1 is associated with raw data that can be found under this accession code. The results from the microarrays (Fig. 4) are associated with raw data that is included up until the point of censorship (see Supplementary Table 1 for all data). In addition, our transcriptomic experiments (RNA-seq and microarrays) were performed by different researchers at different institutes. These experiments show highly similar results. Lifespan data were plotted as Kaplan–Meier survival curves and pairwise statistical differences were performed using the logrank (Mantel–Cox) test and the Cox proportional hazard model to model the effect of a gene knockdown or mutation in either the presence or absence of males. The Cox proportional hazard model was also used to test for an interaction between the effects of the presence of males and a specific mutation and RNAi treatment on Hermaphrodite lifespan. For the ORO and fluorescence microscopy experiments, conditions were compared by a two-tailed, Mann–Whitney test and two-way ANOVA. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Code availability**

All code is available on GitHub (https://github.com/brunelab/Booth-et-al-2022).
Author contributions
This study was designed by L.N.B., C.S., C.T.M. and A.B. L.N.B. performed the RNA-seq experiments and data were analyzed by L.N.B. and K.H. Microarrays were performed by C.S. and analyzed by C.S., L.N.B. and R.W.Y. All code written by L.N.B., K.H. and M.T.B. was checked independently by R.W.Y. and L.N.B. Lifespan assays were performed and analyzed by L.N.B., C.S. and C.T. (see Supplementary Table 1 for the assays that were performed by each researcher). C.N.H. performed assay validation and mating efficiency assays with L.N.B. J.W.M. generated a mutant line for the delm-2 ortholog acl-1 using CRISPR. T.J.M. and L.N.B. performed DAF-16 localization assay. M.T.B helped with single-cell analysis. The figures were prepared by L.N.B. with feedback from A.B., C.T.M. and C.S. The original manuscript draft was written by L.N.B. with advice and editing from A.B., C.T.M. and C.S.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | RNA-seq of *C. elegans* hermaphrodites and males. (a) Principal Component Analysis of the normalized read counts of *glp-1(e2144)* hermaphrodites and wild-type males' transcriptomes prior to removal of male-enriched genes (see Methods). (b) Enriched gene categories in genes expressed higher in wild-type males versus *glp-1* hermaphrodites (left) and the genes expressed higher in *glp-1* hermaphrodites that experienced a sexual interaction for one day (starting day 6 of life) versus no sexual interaction (right). These two sets of differentially expressed genes share several enriched gene categories, including those linked with sperm such as the major sperm proteins, tau tubulin kinases96, and phosphatases/kinases64 suggesting that male sperm-derived transcripts are detected by whole-worm RNA-seq of mated hermaphrodites. (c) Pearson's correlation of the Deseq2 calculated log2(fold change) differential expression of all shared, detected genes in wild-type, sterile *glp-1(e2144)*, and feminized *fem-1(hc17)* hermaphrodites following a sexual interaction with males versus no sexual interaction. Data are from this manuscript and Booth et al. eLife 2019. (d) Heatmap of genes that are differentially expressed in at least three of the five conditions. Data are displayed as log2(fold change). (e) Enriched gene categories in the genes that are upregulated (red) or downregulated (blue) following sexual interactions with males in at least three of the five datasets from *glp-1* sterile (this manuscript), *fem-1* feminized, and wild-type hermaphrodites97. In panels b and e, the number of differentially expressed genes is indicated by the size of the circle and significance of enrichment by the color of the circle (Fisher's exact test, Bonferroni corrected). Gene annotations are nested with the broadest categories listed in all capital letters and the middle categories listed with the first letter capitalized, and the most specific categories in gray. Complete differential expression and gene set analysis results are in Supplementary Data 1 to 4.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | RNAi Screen Results. (a) Hazard ratios comparing the effect of RNAi knockdown or mutation to control in the absence (pink) and presence (hollow blue) of males measured by Cox Proportional Hazard Model for each independent lifespan assay. Box plot minima and maxima are the first and third quartile, respectively and center line shows the median. Whiskers extend to largest and smallest values. Some replicates of utx-1 RNAi, daf-2(e1370) and glp-1(e2144) results were published previously. (b–h) The Kaplan-Meier survival curves of the screen hits (b–g) and isp-1(qm150) (h). Several control RNAi data are identical because these genes were tested together in a blinded group with one control RNAi. (b–g) 32-59 wild-type hermaphrodites tested and (h) 55-113 hermaphrodites were tested. p-values (Mantel-Cox log ranking) shown. See Supplementary Table 1. (i) Percent of hermaphrodites that mated. There was no detectable mating efficiency difference compared to wild type. >5 plates of approximately 20 hermaphrodites per condition and replicate were tested, N=2. (j) Whole worm quantification of a mitochondrial UPR reporter (hsp-6p::GFP) reporter in a wild type, nuo-6(qm200), or isp-1(qm150) background. Hermaphrodites were in the absence (filled points) or presence (hollow points) of males starting at adult day 1 and fluorescence was measured at adult day 3. In the presence of males, activation of mitochondrial UPR is blunted for nuo-6 mutants but not WT or isp-1 mutants (unpaired, two-tailed Mann-Whitney test for pairwise comparisons, two-way ANOVA across allele background). 29-45 hermaphrodites tested per condition. (k) Percent of hermaphrodites with primarily nuclear localization of FOXO/DAF-16. Hermaphrodites were in the presence or absence of males starting at adult day 1. Heat shock (right) and images on adult day 3. Each point for a given condition represents an independent experiment (N=3) consisting of >30 hermaphrodites per condition. Mean and standard deviation shown and p-values calculated by a two-tailed, unpaired t-test.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Ion channel paralogs and impact on lifespan. (a) DNA sequence alignment of the *delm-2* RNAi targeting sequence from the Ahringer RNAi library with the aligning portions of the *delm-1* and *acd-1* unspliced transcripts. (b) A schematic showing the CRISPR–Cas9 mutation we created in *acd-1* (top) and the predicted impact on the ACD-1 protein (bottom). (c) RNAi targeting *acd-1* extends lifespan of hermaphrodites in the presence of males. (d-g) Loss of function mutations in *acd-1*, *delm-1*, or *delm-2* alone (panels d-f) were not sufficient to extend hermaphrodite lifespan in the presence or absence of males, nor did *delm-1*, *delm-2* double mutation (panel g). (h) Combined mutation of *delm-2*, *acd-1*, and *delm-1* extended lifespan in the absence of males and, to a greater extent, in the presence of males as determined by Cox Proportional Hazard Modeling. These data suggest that ACD-1, DELM-2, and DELM-1 act redundantly. 89-138 hermaphrodites were tested in. Lifespans are plotted as Kaplan Meier survival curves, *p*-values calculated by Mantel-Cox log ranking, and 89-138 hermaphrodites were used per condition. See Supplementary Table 1 for extended statistics.
Extended Data Fig. 4  |  See next page for caption.
Extended Data Fig. 4 | Comparison of RNA-seq and microarray results. (a) Pearson’s correlation scores comparing the wild-type male-induced fold-change in hermaphrodite gene expression detected in the *glp-1* hermaphrodite RNA-seq and microarray. (b) Venn diagrams of the wild-type male-induced differentially expressed hermaphrodite genes detected by microarray and RNA-seq. The number of overlapping differentially expressed genes is higher than expected by chance (upregulated: \(p=6.15\times10^{-21}\), downregulated: \(p=1.69\times10^{-93}\), hypergeometric test). (c) Enriched gene categories in the hermaphrodite genes that are upregulated (red) and downregulated (blue) by wild-type males in the microarray and the RNA-seq results. The number of differentially expressed genes in each gene set is indicated by the size of the circle and the significance of the enrichment by the color of the circle. Gene annotations are nested with the broadest categories listed in all capital letters and the middle categories listed with the first letter capitalized, and the most specific categories in gray. (d) A heatmap of the microarray results from *glp-1* hermaphrodites mated with WT males for one day. The genes that we identified as functionally important for male-induced demise (Fig. 1i and Extended Data Fig. 2) are shown, including *ins-11*. The majority of genes that were differentially expressed in our RNA-seq and identified as functionally important in our screen were not investigated further. *sre-28* was not detected in the microarray. (e) Venn diagrams of the significantly up- and down-regulated hermaphrodite genes detected by microarray following one day of interacting (mating) with WT males or *fer-6(hc6)* sperm-less males (blue and yellow, respectively) or five days on male-conditioned plates (that is male-secreted compounds and pheromones, purple). Data from the RNA-seq and microarrays can be found in Supplementary Data 1, 2 and 5 to 8.
Extended Data Fig. 5 | Male-conditioned plate screen results. (a) A scheme describing the experimental set up for the male-conditioned plate lifespan assays. For complete details, see Methods. (b-f) The lifespans of hermaphrodites fed control empty vector RNAi (b) or RNAi against the male-conditioned plate induced genes \textit{cdr-1} (c), \textit{cyp-35D1} (d), C33G8.3 (e), or \textit{hmit-1.1} (f). Hermaphrodite worms were either exposed to media conditioned by 30 \textit{fog-2(q71)} males for 2 days (removed prior to placing the hermaphrodites on the plate) (dotted lines) or were placed on normal plates (solid lines). Results are plotted as Kaplan-Meier survival curves and \(p\)-values calculated using Mantel-Cox log ranking. Percent change in median lifespan compared to normal plates is shown in each panel. 118-132 hermaphrodites were tested in each condition. See Supplementary Table 1 for a complete list of all lifespan assay results.
Extended Data Fig. 6 | Transcription factor binding enrichment. (a) The enrichments of selected transcription factor binding peaks within 2kb +/- the transcription start site of the male sperm, seminal fluid, and pheromone downregulated and upregulated genes are shown. Fold enrichment at the gene set of interest compared to genome-wide is indicated by the size of each circle and Q-value (Benjamini-Hochberg false discovery corrected two-tailed Fisher’s exact test p-value) or p-value (two-tailed Fisher’s exact test) by the color of each circle. A complete list of the transcription factor binding enrichment is presented in Supplementary Data 9. (b) Enrichment of ‘known’ transcription factor binding motifs within 300bp +/- the transcription start site of the differentially expressed genes. The Bonferroni-corrected, one-tailed Fisher’s exact test p-values for the motif enrichment for each gene set is shown.
Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a | Confirmed
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☐ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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☐ | The statistical test(s) used AND whether they are one- or two-sided
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☐ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐ | For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  > Give P values as exact values whenever suitable.
☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐ | Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

*Our web collection on statistics for biologists contains articles on many of the points above.*

Software and code

Policy information about availability of computer code

**Data collection**

No software was used

**Data analysis**

Data analysis was performed using R (v3.2.4 or 3.5.1). Key packages used were: STAR (v2.5.4a), DESeq2 (v1.10.1), Biobase (v2.30.0 and v2.42.0), ggplot2 (v3.3.0), pheatmap (v1.0.12), vennerable (v3.1.0.9000), Seurat (v3.2.3), and Harmony (v1.0). Prism v8.4 was used. All custom code used for the study are publicly available on GitHub (https://github.com/brunelab/Booth-et-al-2022). For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy.

All RNA-seq reads are available on NCBI Sequence Read Archive (PRJNA642294). Figure 1 is associated with raw data which can be found under this accession code. The results from the microarrays (Figure 3) are associated with raw data and these data are available at http://puma.princeton.edu [see Methods]. In
addition, we have included the complete results of the RNA-seq and microarray analyses and the raw data for each worm measured with microscopy [fluorescence intensity and Oil Red O staining] as Source Data. The complete list of all lifespan assays (including statistics and number of animals) is presented in Supplementary Table 1.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| Reporting on sex and gender | n/a |
|----------------------------|-----|
| Population characteristics | n/a |
| Recruitment                | n/a |
| Ethics oversight           | n/a |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

The chosen sample size for all lifespan assays was determined using field standards (Lucanic et al. Nature Communications, 2017). The sample sizes for each experimental condition are listed in Supplementary Table 1.

Data exclusions

Data was excluded if it failed to meet pre-established quality control criteria. Specifically, two RNA-seq libraries failed our quality controls (BioAnalyzer quality control for insert size distribution and purity) prior to sequencing and were excluded.

For lifespan assays, worms that bagged, ruptured or crawled off of the plate were censored, consistent with field standards. The number of worms censored for each assay is listed in Supplementary Table 1.

Replication

All lifespan replicates are shown and includes results that were replicated independently by different researchers (see Supplementary Table 1 which includes all stats and the identity of the researcher for each experiment). In addition, our transcriptomic experiments (RNA-seq and microarray) were performed by two different researchers at different institutes. These experiments show highly similar results (see Extended Data Fig. 4). All microscopy experiments were performed at least twice and the data from the independent replicates are in Source Data.

Randomization

Worms were split from the same egg lay between control and treatment groups. Both male and hermaphrodite worms were randomly assigned to groups in an alternating and blinded fashion to avoid selection bias. Randomization was performed for the worms used for the RNA-seq, microarrays, microscopy, and lifespan assays.

Blinding

For all RNAi and mutant lifespan assays the identity of the RNAi treatment or mutation was blinded until the last animal died (Fig. 2a-j, 3d-f, 1, 4c, d, f, g and Extended Data Fig. 2a-h, 3c-h, 5). Blinding on the basis of the presence or absence of males in lifespan assays is not possible.

The RNAi treatments for the worms used in the mating efficiency assay were also blinded (Extended Data Fig. 2i). For Figure 2I and Extended Data Figure 2k, the hermaphrodites were blinded based on treatment when nuclear vs. cytoplasmic localization was determined. For the fluorescence (Figure 2k, 5f, g and Extended Data Figure 2j) and Oil Red O quantification (Figure 5a-e), treatments were not blinded but quantification of the entire worm was performed using Fiji and the experimenter used identical methods across all conditions.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
Materials & experimental systems

- Involved in the study
  - Antibodies
  - Eukaryotic cell lines
  - Palaeontology and archaeology
  - Animals and other organisms
  - Clinical data
  - Dual use research of concern

Methods

- Involved in the study
  - ChIP-seq
  - Flow cytometry
  - MRI-based neuroimaging

Animals and other research organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

| Laboratory animals | C. elegans hermaphrodite and male worms were used. A complete strain list is available in the Methods section. |
|--------------------|----------------------------------------------------------------------------------------------------------------|
| Wild animals       | n/a                                                                                                               |
| Reporting on sex   | This study was performed with hermaphrodites and male C. elegans and the sex of the animals in each experiment is clearly labeled. |
| Field-collected samples | n/a                                                                                         |
| Ethics oversight   | No ethical approval is required for studies with nematodes.                                                   |

Note that full information on the approval of the study protocol must also be provided in the manuscript.