

Figure S1. The *phm-2(lf)* extended lifespan phenotype was observed at multiple temperatures and in multiple genetic backgrounds, Related to Figures 1 and 2

Animals were cultured on NGM dishes with live *E. coli* OP50 bacteria. (A-B) Survival curves of wild-type (blue) and *phm-2(am117)* (red) animals cultured at 15°C and 25°C. See Table S1 for summary statistics of lifespan assays, number of animals and number of independent experiments.

(C-D) Survival and self-fertile reproduction curves at 20°C for the FOXO transcription factor gene *daf-16(mu86), daf-16(mgDf47);daf-2(e1370)* and *daf-16(mu86) phm-2(am117);daf-2(e1370)* mutant animals. The *phm-2* extended lifespan and reproductive span phenotypes were not suppressed by *daf-16(lf)* (For panel D, N=8-9 animals).

(E) The percent of animals that form dauer larvae at three temperatures was determined for the insulin/IGF-1 receptor gene *daf-2(e1370)* and *phm-2(am117); daf-2(e1370)* animals. *phm-2(lf)* did not significantly affect the temperature sensitive Daf-c phenotype of *daf-2* mutants (Three biological replicates with N>100 animals each; n.s., not significant, P > 0.05 by Student's *t*-test). (F-G) Survival curves at 20°C for *phm-2(am117)*, the AMP kinase gene *aak-2(ok524), phm-2(am117); aak-2(ok524),* the target of rapamycin complex 2 gene *rict-1(mg360),* and *phm-2(am117); rict-1(mg360).* See Table S1 for summary statistics of lifespan assays, number of animals and number of independent experiments.

(H) *daf-2(e1370)* mutant animals were cultured on *pha-4* RNAi bacteria or control RNAi bacteria. See Table S4 for summary statistics of lifespan assays, number of animals and number of independent experiments.

(I) Quantification of whole animal fluorescence for wild type, *phm-2(am117)* and *eat-2(ad1116)* adult animals cultured with green fluorescent microspheres for 10 minutes. Values in arbitrary units (AU) are the average (+/-S.D.) of five biological replicates (N=35-61 animals per replicate). Tukey post hoc HSD; *, P < 0.05; **, P < 0.01.

(J-L) Wild-type, *phm-2(am117)* and *osm-3(p802)* animals were incubated with dye DiO that allows visualization of amphid neurons that project to the outside of the body. Representative fluorescence microscope images show the head region. WT and *phm-2(am117)* animals displayed strong DiO staining in amphid neuron axons and their cell bodies in the pharyngeal region. *osm-3(p802)* animals, which have defective amphid neuron morphology, did not display staining (the Dyf phenotype). Scale bar = $10 \mu m$.



Figure S2. Diet affected the scrawny body morphology phenotype of *phm-2(lf)* mutants, Related to Figures 1 and 6.

Eggs from wild-type (left column) or *phm-2(am117*) (right column) hermaphrodites were cultured with the bacterial food sources labeled in each row. Bright field images of representative live animals were captured using a dissecting microscope with identical magnification on day four after the L4 stage. Live *E. coli* OP50, *E. coli* HB101, *Comamonas* DA1877, and *Bacillus subtilis* proliferated on the dish, whereas dead *E. coli* OP50 was treated with UV light. Scale bar = 100 μ m.



Figure S3. The wild-type F32B4.4a cDNA partially rescued the *phm-2(am117)* extended lifespan and scrawny body morphology phenotypes, Related to Figure 3

(A) Transgenic animals contained the chromosomal mutation *phm-2(am117)* and an extrachromosomal array composed of plasmid pSK2 with the upstream *phm-2* promoter driving

the F32B4.4a cDNA and the plasmid pRF4 that causes a dominant Rol phenotype used to identify transgenic animals. Five independently-derived transgenic strains were analyzed, named *amEx321*, *amEx322*, *amEx324*, *amEx328*, and *amEx331*. Lifespan assays were initiated with L4 stage transgenic animals that displayed the Rol phenotype, and animals were cultured on NGM dishes with live *E. coli* OP50. All five transgenic strains displayed shorter mean (14-23%) and maximum (12-22%) lifespans than *phm-2(am117)* mutant animals. See Table S1 for summary statistics of lifespan assays and number of animals analyzed.

(B) Bars represent the average volume of individual worms (+/-S.D) four days after the L4 stage determined by analyzing dissecting microscope images with the wormsizer algorithm. To control for the effects of the dominant Rol marker used to make transgenic strains, we used the integrated insertion sequence allele *acls101* [F35E12.5p::GFP + *rol-6(su1006)*] to generate *acls101;phm-2(am117)* animals that displayed the Rol and scrawny body morphology phenotypes. Transgenic *phm-2(am117);amEx328* animals displayed a significantly higher (42%) volume compared *acls101;phm-2(am117)* animals when cultured on NGM dishes with live *E. coli* OP50 (N=30-43 animals analyzed: Tukey post hoc HSD; **, *P* < 0.01).

am117 (R44stop)

| Ce Dm Hs Mm | MPLESG-KLINDLRVSELKTELEKRGLSTOGVKWLTVRLNKALRDEGLDPADHVFEHAVSPMKKSTRRSNEMARAAAA MPEAG-KKIAELRVCDLKSELEKRELETVGFKAVLIERLEKSLRAEGLDPATHLIVPGAKAKKP | 78 63 97 97 |
|----------------------|--|---------------------------|
| Ce Dm Hs Mm | AAAEKVEKGAGDEGNDENVLVEEKEEEKEEDSHDLQIIEDHELEVPSDEKDDTIVEDEEFEEAEQVEPEPEA | 151 120 195 195 |
| Ce Dm Hs Mm | VEPVVEEKPEEKLEEKPEEKLEEKPEEKPVEPEVVIVEEPVAEVIEVEKAVAEPVELKEKPEKEPEVVIVEEPVEQLENEPEVVPMEVEE DDECVILDDDDEDEDHVIDDEPQSGDGDAEGKAQDDGAENNGDDKAIIHGEERNTGDTG | 241 194 275 292 |
| Ce Dm Hs Mm | AKKSDE_DGEDEFEEDDSSSDIEIIEPTL@ESEPLAEEKVEKKEKKPEIPHNLE@NEPISMETEEKVEEEVIILNSSINNVSCDDEIVLDYEEDLLEDP DE@KLLHDEASDDKSIKSVKPANKSEKSNSKDADKKNDDGARSKKRDEKSGDKKDSSE@KSSGKSSNQKDDKEKS ESTAHAQSSKADSLLAVVKREPAEQPGDGERTDCEPVGLEPAVEQSSAASELAEASSEELAEAPTEAPSPEARDSKEDGKKFDFDAC SSKSESPSTRC@WSEADAPLAVVKREPADAPGGGTGMDREPVGLEEPVEQSSTAAQLPEATSUELVRAPTAALSPEP(DSKEDVKKFAFDAC | 341 269 362 384 |
| Ce Dm Hs Mm | LDEPIEUKEPKAAEPTPKULUKVEASTPOATPSKAASSSAGSGKSMLFGDDVKKTSIWIRGMTPATKASSVKULASUYGKVIUSKIFNSKAADANGDVKN TAAPGGATSSTASSAAGSKSGSGSSAGNGTGANSINIK-OATLSRNL | 441 353 447 469 |
| Ce Dm Hs Mm | CFALIQFADVTAMELAMTSLHCKNYQGRVLRVEKVSESHLTSSAEKLAREKHVAEAASTMSTSPAPTPTPEPVVTTTTTTSAAPKRKEPIHAPESSSSEG CYGYVTMSSSADASRCIENLHRTELHGRIISVERTKNEIGGSLNSKEGKGKAPGDAGNKKKEDDSGKKSGSGKGSSSNSGDKKGGDGNGDSK CYGFVTMSTAEEATKCINNLHKTELHGKMISVEKAKNEPVGKKTSDKRDSDGKKEKSSNSDRSTNLKRDDKCDRDDAKKGDDGSGEK CYGFVTMSTAEEATKCISHLHKTELHGKMISVEKAKSEPTGKRVPDRDGDSKKEKASTSDRSANLKREEKGERKDDAKKTDDGSTEK RRM_SAFB | 541 446 535 557 |
| Ce Dm Hs Mm | QRAKRRAIEAPVRSRSRSRGGGGEPRSSRKPITFDREEESNRDSRRTIAAAPPARTSRMARSPLRAPLRAARGSESSRSSTRGGGGGGESLTISSRVD SVGCDLKRDCKESNRARSRNDDRGKSLASQDRPRIDRERSAKGSQDHBSGRNPRDVDRELQQRQRERERQREM | 639 529 610 632 |
| Ce Dm Hs Mm | TSGCRGGAIK-RSVERSVPNNISTSELRRKHHQIHVTVQQDAPRASYQTEQYARERSSSTTTSSRRRQVSPDRSEQRRHRDEPPPRRAPQEQ ERERORLRERERELREERRRREARERORIEEERLQEERRKLAVERERLEREKAELLRMERERQKLEREKIELERLELKRQQMKIMESRDD PRKSRDSESHSRVRERSEREQRMQAQWEREERERLEIARERLAFORORLERERMERERLERERMHVEHERRREQERIHREREELRRQQELRYE SRKSRDSESR-RERERSEREQRLQAQWEREERERLEIARERLAFHRHRLERERMERERLERERMHVEQERRREQERIHREREELRRQQELRYE | 730 620 703 656 |
| Ce Dm Hs Mm | PSRRRGASPPEPPRQEGARRSEEPERRRLFDEREQLAHIMAAKDMYAEQQKIRAEKALIAFQMQQLEKKKLEAELQIAQKA PGKRGVSKRADDRYGDVTDRKRTAIFDAPPPPRFDTNLVSSRSTYDKKHDDYGSSSSTKRVLDDYSSSSKRLDYSSK-RNDYSSS-SGSKRG QERRPAVRRPY-DLD-RRDDAYWPEAKRAALDERYHSDFNQDRFHDFDHRDRGRYPDH-SVDRR- QERRPAVRRPY-EVDGRRDDAYWPEAKRAALDRYHSDFSQDRFHDFDHRDRGRYPNH-SVDRR- ad597 (5 nucleotide deletion) | 812 710 765 719 |
| Ce Dm Hs Mm | LLMQQAALGGGGALVVDGGALVAGGGAVGYHHUEHRSSYGGGGSSSNGGSSNRRQTRRSPSPPQQHHSSSRRQRRDSGGAGRYRQSTSSSNNR AVDDYTAVPTKRSNDYSASSSSKDDYKRDEVRHLPLSGTVGGGSSSS-YLHKNNTAGGGASSYVHKNNTSGTGGRYNDSDRS -EGSRSMMGEREGQHYPERHGGPERHGRDSRDGWGGYGSDKRMSEGRGLPPPPRGRRDWGDHGRR-EDDRS EGSRSMMGDREGQHYPERHGGPERHGRDSRDGW-GYGSNKRLSEGRGLPPPPRRDWGEHGRRLEDDRA | 907 792 835 785 |
| Ce Dm Hs Mm | NSNSGGRNLVVTATTTNNTNATNAGRSYGIQSVPDASSYSTNNYQQRSSAASAYDLQITATMPQAGAANSGAAYHQPYGNVYQHNTAAYPVWGGLDAQGH NTSSYRRDDSHSLGNKRYDNSSGGGGAYGSSSNSVNIWAPSSGGSSSHLGSGGGGGGQQIKSYGNNGMSS WQGTADGGMMDRDHKRWQGGERSMSGHSGP-GHMMNRGGMSGRGS | 1007 861 879 829 |
| Ce Dm | -MAMDTNWSQNAATPSTSTSSGGGGQQWQQQSYGSNQHQHHQNNNSSQPSSSNRRGNDYGNYRGNY 1072 | |

 Dm
 NMHSTNSWUKSAPVDDNGWRUVPARPDRSYNERSTGYUGUSGGGAAGMYGASKPAUDKYSGYDRI
 92/

 Hs
 ------FAPGGASRGHPIPHGGMD-----GGFGGQSRGSRPSDARFT---RRY
 918

 Mm
 ------FAPGGASRGHVIPRGGMD-----AGFGGOSRGSRPSDARFT---RRY
 868

Figure S4. Alignment of PHM-2 protein with insect and mammalian SAFB proteins, related to Figure 3

The predicted *C. elegans* (Ce) PHM-2 (F32B4.4a) protein is aligned with homologous Scaffold attachment factor B (SAF-B) proteins from the insect *Drosophila melanogaster* (Dm) and the mammals *Homo sapiens* (Hs) and *Mus musculus* (Mm). The *am117* nonsense mutation (red line) and the *ad597* deletion mutation (red line) are marked. Two highly conserved domains are highlighted: the SAP domain (purple) is a putative DNA binding domain and the RRM_SAF domain (green) is a putative RNA recognition motif (Aravind and Koonin, 2000; Townson et al., 2004).



Figure S5. Transcriptional regulation of innate immune genes and HLH-30 nuclear localization, Related to Figure 4

Fluorescence photomicrographs show transgenic animals cultured with live *E. coli* OP50 (A-C) or *P. aeruginosa* strain PA14 (D-F) for 24 hours. Images were captured at the same time with the same camera exposure. Each of the three transgenic strains contained a promoter driving GFP. *irg-1* (left, *irg-1p::GFP*) and *F35E15.5* (middle, *F35E15.5p::GFP*) are established innate immune response genes, and both strains displayed increased GFP fluorescence when cultured on *P. aeruginosa* compared to live *E. coli*. Animals expressing *phm-2* (right, *phm-2p::GFP*) did not display increased GFP fluorescence when cultured on *P. aeruginosa* compared to live *E. coli*. Red fluorescence in the left panels is a marker that is expressed in the pharynx and used to identify transgenic animals. Scale bar=100µm.

(G) Schematic of method (upper), and representative bright field photographs (lower). Green fluorescent animals were not observed in wild-type but were observed in *phm-2(am117)* and *eat-2(ad465)* mutants.

(H-I) Quantification of number of fluorescent animals; adult animals were cultured with green fluorescent *E. coli* for 24 hours. N(n) indicates total number of animals and number of independent trials. (H) Wild-type or *phm-2(am117)* hermaphrodites were cultured on *pha-4* RNAi bacteria or control RNAi bacteria until adulthood, then transferred to RNAi plates seeded with a 1:3 mixture of GFP expressing *E. coli* OP50 and RNAi bacteria. WT with *pha-4* RNAi was compared to WT with control, and *phm-2* with *pha-4* RNAi was compared to *phm-2(am117)*, *rsks-1(ok1255)*, *and phm-2;rsks-1* adult animals were cultured with green fluorescent *E. coli* for 24 hours. ANOVA; ns, *P* > 0.05; *rsks-1(ok1255)* animals were compared to WT, and *phm-2;rsks-1* animals were compared to *phm-2(am117)*.

(J) Representative fluorescence micrographs of animals that were *phm-2(+)* or *phm-2(am117)*, that express HLH-30::RFP, and were cultured on live *E. coli* OP50 or live *Comamonas*. N≥100 animals. Scale bar = 100 μ m. Only *phm-2(lf)* animals cultured on live *E. coli* displayed nuclear localized fluorescence (White arrows).

(K-O)Bars represent mRNA levels (+/-S.D) for *clec-7*, *clec-60*, *clec-82*, *F53A9.8* and *lys-5* pathogen response genes determined by qPCR in wild-type, *phm-2(am117)*, *eat-2(ad465)*, *hlh-30(tm1978)*, and *phm-2;hlh-30* animals cultured on live *E. coli* OP50 (M-O as indicated and K-L) or live *Comamonas* bacteria (M-O as indicated). Values in arbitrary units (AU) are the average of three to five biological replicates. ANOVA; *, **, P < 0.05, P < 0.005; ns, not significant, P > 0.05.



Figure S6. *phm-2(lf)* bacterial avoidance behavior does not appear to be mediated by a secreted factor, Related to Figure 4

(A-K) "Avoidance" is the average percent of animals (+/- SD) outside the bacterial lawn at the larval stages L1, L2, L3, L4, day 1 adult (YA) and day 2 adult (LA). The *phm-2* allele was *am117*. (A,B) The antibiotics tetracycline (A) or kanamycin (B) were added to the NGM medium. Values are the average of three biological replicates, N=120-152 animals. WT and *phm-2* were not significantly different, P > 0.05, by Student's *t*-test.

(C) Concentrated *Comamonas* bacteria were dissolved in supernatant from live *E. coli* OP50. Values are the average of three to five biological replicates, N=139-295 animals. WT and *phm-2* were not significantly different, P > 0.05, by Student's *t*-test.

(D) Concentrated live *E. coli* OP50 were dissolved in supernatant from *Comamonas* bacteria. Values are the average of three to five biological replicates, N=139-295 animals. WT and *phm-2* were significantly different, P < 0.001, by Student's *t*-test.

(E) Concentrated live *E. coli* OP50 were dissolved in S medium buffer. Values are the average of three biological replicates, N=148-150 animals. WT and *phm-2* were significantly different, *P* < 0.001, by Student's *t*-test.

(F) A block of NGM agar with *Comamonas* bacteria was attached to the inside of the lid, and animals were cultured with live *E. coli* OP50. Values are the average of two biological replicates, N=89-100 animals. WT and *phm-2* were significantly different, P < 0.001, by Student's *t*-test.

(G) A block of NGM agar with live *E. coli* OP50 was attached to the inside of the lid, and animals were cultured with *Comamonas* bacteria. Values are the average of three biological replicates, N=89-100 animals. WT and *phm-2* were not significantly different, P > 0.05, by Student's *t*-test. (H-I) Wild-type (H) and *phm-2(am117)* (I) animals were cultured with a mixture of live *E. coli* OP50 and *Comamonas* DA1877 in the following ratios: 100:0, 90:10, 50:50, 10:90 and 0:100. Values are the average of three or four biological replicates, N≥100 animals. Tukey post hoc HSD showed *phm-2(am117)* animals cultured with 50:50 was significantly different than 100:0 and 0:100 (P < 0.01).

(J-K) WT and *phm-2(am117*) animals were grown on either *Comamonas* or live *E. coli* OP50 bacteria from embryo to the young adult stage and then tested on live *E. coli* OP50 or *Comamonas*. Only *phm-2(am117*) animals on live *E. coli* OP50 displayed bacterial avoidance behavior (N=119-200 animals, 5-6 biological replicates, significance Student's *t*-test; n.s., not significant, P > 0.05).

(L) Schematic of learning paradigm.



phm-2

0

WT

Figure S7. *phm-2(lf)* and wild type displayed different food preferences but no evidence of learning in a food choice learning assay, Related to Figure 4

(A-E) "Inside Lawn" is the average percent of animals (+/- SD) inside the specified bacterial lawn 24 hours after transfer to the test dish. Schematics (top) illustrate the method and bacteria used in each panel. Wild-type and *phm-2(am117*) eggs were transferred to the training dish and allowed to develop to adults. Adult animals were transferred the test dish containing two small bacterial lawns, and the number of animals inside each lawn was scored after 24 hours. (A-D) Values are the average of four biological replicates (N=149-180 animals: Tukey post hoc HSD; *, P < 0.05; **, P < 0.01) (E) The training dish contained two small bacterial lawns. Values are the average of three biological replicates, (N=77-114 animals: Tukey post hoc HSD; ns, not significant, P>0.05; **, P < 0.01).





Table S1. *phm-2(lf)* extended mean and maximum life span, Related to Figures 1, 2 and 4, and Figures S1 and S3.

| Genotype ¹ | Mean ^{2,3} | % | Maximum ^{2,3} | % | N (n) ⁴ |
|--|----------------------------------|------------|---------------------------------|--------------|--------------------|
| | lifespan | Change | lifespan | Change in | |
| | ± SD (days) | in mean | ± SD (days) | maximum | |
| WT 20°C | 15.8 ± 3.7 | | 21.9±1.2 | | 316 (6) |
| <i>phm-2(am117)</i> 20°C | 21.1±3.1** | +34 | 27.3±1.2** | +24 | 279 (6) |
| | | | | | |
| WT 15°C | 20.5±4.3 | | 28.2±1.4 | | 99 (2) |
| <i>phm-2(am117)</i> 15°C | 30.5±5.4** | +49 | 38.2±1.0** | +36 | 140 (2) |
| NET 250C | 0.0+2.0 | | 17 (1 1 | | 105 (2) |
| W1 25°C | 9.8±3.0 | . 71 | 15.6±1.1 | 126 | 185 (3) |
| <i>phm-2(am11/)</i> 25°C | 16./±26** | +/1 | 21.2±0.6** | +36 | 140 (3) |
| | 16.1 ± 3.4 | | 22.2 ± 1.3 | | 171 (3) |
| phm-2(ad538) | 21.0±4.0** | +30 | 28.5±1.2** | +28 | 148 (3) |
| phm-2(ad597) | 21.4±3.3** | +33 | 28.2±0.8** | +27 | 192 (3) |
| <i>phm-2(am117)</i> | 21.8±3.1 | | 27.7±1.4 | | 154 (3) |
| daf-2(e1370) | 36.3±9.9 | | 53.3±3.4 | | 239 (3) |
| phm-2;daf-2 | 58±24.6** | +60 | 100.1±5.6** | +88 | 156 (3) |
| $phm_{-}2(am117)$ | 21 1+3 1 | | 27 1+1 1 | | 103 (1) |
| $daf_{16}(m_{18})$ | 117+23 | | 163+11 | | 219(4) |
| $daf_{16} nhm_{-2}$ | 11.7 ± 2.3 18 5 \pm 2 7** | +58 | 10.3 ± 1.1 23 3 $\pm0.0**$ | +13 | 183(4) |
| ug-10 pnm-2 | 10.3±2.7 | + 50 | 23.3±0.7 | T T 3 | 105 (+) |
| daf-16(mu86) | 11.6±2.4 | | 16.3±1.1 | | 104 (2) |
| daf-16;daf-2 | 13.0±2.2 | | 16.7±0.8 | | 128 (2) |
| daf-16 phm-2;daf-2 | 21.9±3.3** | +68 | 26.8±0.7** | +60 | 168 (2) |
| phm-2(am117) | 21.4±2.5 | | 27.2±1.3 | | 180 (4) |
| isp-1(qm150) | 21.3±6.8 | | 33.3±1.4 | | 106 (4) |
| phm-2;isp-1 | 28.8±9.9** | +35 | 43.8±1.2** | +32 | 91 (4) |
| $phm_{-}2(am117)$ | 21 3+3 1 | | 27.7+1.2 | | 1/1/(3) |
| $aak_{-2}(ak_{11})$ | 143+23 | | 19 6+0 7 | | 174(3) |
| $nhm_2(0k)(24)$ | 20.4+2.7** | +42 | 27 1+0 9** | +38 | 170(3) 133(3) |
| | 20.1-2.7 | 172 | 27.1±0.9 | 130 | 155 (5) |
| phm-2(am117) FudR ⁵ | 26±4.4 | | 32.5 ± 1.0 | | 83 (2) |
| <i>eat-2(ad1116)</i> FudR ³ | 23.2±3.8 | | 29.5±1 | | 112(2) |
| phm-2;eat-2 FudR ³ | 22.9±4.5 ^{ns} | -1 | 30 ± 0.9^{ns} | +2 | 90 (2) |
| <i>phm-2(am117)</i> | 21.3±3.6 | | 27.5±0.9 | | 254 (3) |
| rict-1(mg360) | 10.2±2.2 | | 14.2±0.6 | | 138 (3) |
| phm-2;rict-1 | 19.7±2.8** | +93 | 24.2±0.9** | +70 | 220 (3) |
| nhm 2(av. 117) | 21.2+2.6 | | 27.5+0.0 | | 254 (2) |
| pnm-2(am11/) | 21.3 ± 3.0 | | $2/.5\pm0.9$ | | 234(3) |
| $r_{SKS-1}(OK1255)$ | $1/.9\pm4.4$ | 12 | 23.3 ± 1.2 | 1 | 232(3) |
| pmm-2;rsks-1 | 10.3±3.0 | <i>τ</i> ∠ | 23.4±1.3 | 1 1 | 1/9(3) |

| phm-2(am117) | 20.2±3.1 | | 26.4±1.0 | | 117 (2) |
|--|-------------------------|-----|-------------------------|-----|---------|
| hlh-30(tm1978) | 14.8±2.2 | | 18.9 ± 0.8 | | 232 (2) |
| <i>phm-2;hlh-30</i> ⁶ | 17.3±2.7** | -14 | 21.7±1.5** | -18 | 154 (2) |
| phm-2(am117) | 17.2±0.45 | | 22.85±0.26 | | 86 (2) |
| raga-1(ok386) | 17.3 ± 0.48 | | 27.4±0.57 | | 100 (2) |
| phm-2;raga-1 | 18.1±0.38 ^{ns} | +5 | 26.2±0.62 ^{ns} | -4 | 113 (2) |
| | | | | | |
| phm-2(am117) | 21.2±4.1 | | 28.0±1.0 | | 47 |
| <i>phm-2(am117);amEx321</i> ⁷ | 16.2±3.0** | -23 | 21.7±1.1** | -22 | 25 |
| <i>phm-2(am117);amEx322</i> ⁷ | 16.2±3.6** | -23 | 23.0±1.0** | -18 | 26 |
| <i>phm-2(am117);amEx324</i> ⁷ | 18.3±4.4** | -14 | 24.7±0.6** | -12 | 22 |
| <i>phm-2(am117);amEx328</i> ⁷ | 16.6±4.5** | -21 | 23.7±1.4** | -15 | 64 |
| <i>phm-2(am117);amEx331</i> ⁷ | 17.1±3.5* | -19 | 22.2±0.9** | -20 | 35 |

¹Genotype: Wild-type hermaphrodites or the indicated mutant strains were analyzed.

²Lifespan: L4 stage animals were cultured on standard NGM dishes with a small lawn of live *E. coli* OP50 bacteria at 20°C, except those labeled 25°C and 15°C.

³Mean, Maximum, % Change: Maximum adult lifespan is the mean lifespan of the 10% of the population that had the longest lifespans. Comparisons are to the matched WT control (rows 1-9), the non-*phm-2* single mutant strain (rows 10-15, 19-33, and 37-39) or to *daf-16; daf-2* (rows 16-18). The statistical test was one-way ANOVA with Tukey post hoc HSD; *, P < 0.05; **, P < 0.01; ns, not significant, P>0.05.

⁴N(n): Total number of hermaphrodites analyzed, and the number of independent experiments. ⁵Hermaphrodites were exposed FudR, a drug that prevents embryos from hatching, to avoid matricidal hatching and allow the observation of aging.

⁶In rows 34-36, comparison is to *phm-2(am117)*.

⁷Transgenic animals contained the chromosomal mutation *phm-2(am117)* and an extrachromosomal array composed of plasmid pSK2 with the upstream *phm-2* promoter driving the F32B4.4a cDNA and the plasmid pRF4 that causes a dominant Rol phenotype used to identify transgenic animals. Five independently-derived transgenic lines were analyzed, named *amEx321*, *amEx322*, *amEx324*, *amEx328*, and *amEx331*.

| Genotype ¹ | Bacteria ² | Live/ | % Avoidance ³ | % Avoidance ³ | N (n) ⁴ |
|-----------------------|-----------------------|-------------------|--------------------------|--------------------------|--------------------|
| | | Dead ² | \pm SD | \pm SD | |
| | | | Young adult (YA) | Late adult (LA) | |
| WT | E. coli OP50 | Live | 11.6±5.4 | 11.4±4.2 | 410 (9) |
| phm-2(am117) | | Live | 87.2±5.0** | 88.1±5.2** | 453 (9) |
| | | | | | |
| WT | <i>E. coli</i> OP50 | Live | 9.2±3.7 | 10.7±2.4 | 136 (3) |
| phm-2(ad538) | | Live | 78.7±3.9** | 82.2±0.9** | 185 (3) |
| phm-2(ad597) | | Live | 83.0±4.7** | 85.3±3.1** | 160 (3) |
| | | | | | |
| WT | <i>E. coli</i> HB101 | Live | 8.6±4.1 | 9.4±4.0 | 273 (5) |
| phm-2(am117) | | Live | 62.9±7.2** | 70.4±4.1** | 296 (5) |
| WT | Comment | T : | 107.0 | 10.0+1.7 | 252 (5) |
| | Comamonas | Live | $19./\pm0.9$ | 18.0 ± 1.7 | 252(5) |
| pnm-2(am11/) | DA18// | Live | 25.0±4.9 ^{ns} | 1/.9±3.6 ^{ns} | 269 (5) |
| | D. J. J. | | | 10 - 11 | |
| WT | B. subtilis | Live | 15.1±3.9 | 13.7±4.4 | 284 (5) |
| phm-2(am117) | | Live | 13.4 ± 3.4^{ns} | 17.4 ± 7.2^{ns} | 257 (5) |
| | | | | | |
| WT | E. coli OP50 | Dead | 13.0±2.9 | 16.6±1.4 | 162 (3) |
| <i>phm-2(am117)</i> | | Dead | 12.7 ± 0.7^{ns} | 16.9±0.6 ^{ns} | 155 (3) |
| | | | | | |
| WT | E. coli OP50 | Live | 9.1±2.9 | 11.5±2.3 | 307 (7) |
| eat-2(ad465) | | Live | 44.5±2.3** | 54.1±3.5** | 334 (7) |
| | | | | | |
| WT | Comamonas | Live | 19.5±3.9 | 19.9±4.0 | 123 (3) |
| <i>eat-2(ad465)</i> | DA1877 | Live | 20.9±5.0 ^{ns} | 21.3±2.8 ^{ns} | 118 (3) |
| | | | | | , í |
| WT | E. coli OP50 | Dead | 11.4±2.2 | 14.1±2.4 | 203 (5) |
| <i>eat-2(ad465)</i> | | Dead | 13.3±3.0 ^{ns} | 11.6 ± 0.7^{ns} | 249 (5) |
| (| | | | | - (-) |

Table S2. Avoidance behavior is influenced by bacteria, Related to Figures 1, 6, and 7.

¹Genotype: Wild-type hermaphrodites or the indicated mutants were analyzed.

²Bacteria: Bacteria of the indicated species and strain were spotted in the center of the NGM dish to form a small lawn. To generate dead *E. coli*, we spotted bacteria on the dish and then treated with UV light.

³Avoidance: Animals were scored as inside or outside the bacterial lawn (Avoidance= N_{out}/N_{total}) as one day old adults (YA) and two day old adults (LA); Comparisons are to the matched WT, and the statistical test was one-way ANOVA with Tukey post hoc HSD; *, P < 0.05; **, P < 0.01; ns, not significant, P>0.05.

⁴N(n): Total number of hermaphrodites analyzed, and the number of independent experiments.

| Day | Genotype ¹ | Pharyngeal pumps per | $N(n)^{3}$ |
|-----|-----------------------|------------------------------|------------|
| | | minute \pm SD ² | |
| | WT | 279±25 | 55(2) |
| 2 | phm-2(am117) | 263±23** | 50(2) |
| | <i>eat-2(ad1116)</i> | 74±17** | 49(2) |
| | WT | 253±22 | 46(2) |
| 4 | phm-2(am117) | 216±26** | 47(2) |
| | <i>eat-2(ad1116)</i> | 51±14** | 57(2) |
| | WT | 201±34 | 45(2) |
| 6 | phm-2(am117) | 205±35 ^{ns} | 44(2) |
| | <i>eat-2(ad1116)</i> | 45±15** | 44(2) |
| | WT | 138±47 | 45(2) |
| 8 | phm-2(am117) | 196±33** | 43(2) |
| | eat-2(ad1116) | 44±16** | 43(2) |
| | WT | 127±43 | 47(2) |
| 10 | phm-2(am117) | 189±35** | 44(2) |
| | eat-2(ad1116) | 29 ±18** | 50(2) |
| | WT | 65±43 | 43(2) |
| 12 | phm-2(am117) | 168±40** | 46(2) |
| | <i>eat-2(ad1116)</i> | 25±17** | 46(2) |
| | WT | 36±47 | 37(2) |
| 14 | phm-2(am117) | 105±29** | 36(2) |
| | <i>eat-2(ad1116)</i> | 15±13* | 35(2) |
| | WT | 12±16 | 33(2) |
| 16 | phm-2(am117) | 96±44** | 30(2) |
| | <i>eat-2(ad1116)</i> | 16 ± 14^{ns} | 34(2) |
| | WT | 3±5 | 15(2) |
| 18 | phm-2(am117) | 58±46** | 24(2) |
| | eat-2(ad1116) | $7\pm8^{ m ns}$ | 16(2) |

Table S3. Pharyngeal pumping rate of *phm-2* and *eat-2* mutants, Related to Figures 1 and 2.

¹Genotype: Wild-type hermaphrodites or the indicated mutants were analyzed.

²Pharyngeal pumps per minute: Pharyngeal pumping rate was measured by counting using a dissecting microscope for 10 seconds. The results were multiplied by 6 to calculate the number of pharyngeal pumps per minute. Comparisons are to the matched WT control. Statistical test was Student's *t*-Test; *, P < 0.05; **, P < 0.01; ns, not significant, P>0.05.

 ${}^{3}N(n)$: Total number of hermaphrodites analyzed, and the number of independent experiments.

| Genotype ¹ | RNAi ² | Mean ³ | %Change | Maximum ³ | % | N (n) ⁴ |
|-----------------------|-------------------|-------------------------|---------|----------------------|-----------|--------------------|
| | | lifespan | in mean | lifespan | Change in | |
| | | ±SD (days) | | \pm SD (days) | maximum | |
| WT | Control | 16.5±3.5 | | 22.6±0.9 | | 234 (5) |
| WT | pha-4 | 12.2±2.9** | -26 | 18.0±1.3** | -20 | 203 (5) |
| phm-2(am117) | Control | 20.7±4.8 | | 30.2±1.2 | | 128 (5) |
| phm-2(am117) | pha-4 | 11.5±2.7** | -44 | 16.2±1.2** | -46 | 267 (5) |
| WT | pha-4 | 12.2±2.9 | | 18.0±1.3 | | 203 (5) |
| phm-2(am117) | pha-4 | 11.5±2.7 ^{ns} | -6 | 16.2±1.2** | -10 | 267 (5) |
| daf-2(e1370) | Control | 39.3±9.6 | | 53.3±2.7 | | 117 (3) |
| daf-2(e1370) | pha-4 | 39.9±11.2 ^{ns} | +2 | 57.0±2.0** | +7 | 115 (3) |

Table S4. pha-4 RNAi affected Mean and Maximum life span, Related to Figure 2 and Figure S1.

¹Genotype: Wild-type hermaphrodites or the indicated mutant strains were analyzed.

²RNAi: Animals were cultured with bacteria containing the control RNAi plasmid (L4440) or the *pha-4* RNAi plasmid starting at the L4 stage.

³Mean, Maximum, % Change: Maximum adult lifespan is the mean lifespan of the 10% of the population that had the longest lifespans. Comparisons are between rows 1 and 2, 3 and 4, 5 and 6, or 7 and 8. Statistical test was one-way ANOVA with Tukey post hoc HSD; *, P < 0.05; **, P < 0.01; ns, not significant, P>0.05.

 ${}^{4}N(n)$: Total number of hermaphrodites analyzed, and the number of independent experiments.

| Genotype ¹ | % Avoidance ² ± SD | % Avoidance ² ± SD | $N(n)^{3}$ |
|-----------------------|-------------------------------|-------------------------------|------------|
| | Young adult (YA) | Late adult (LA) | |
| tph-1(mg280) | 15.1±3.9 | 15.8±3.3 | 292 (5) |
| phm-2(am117) | 85.3±4.2 | 84.3±3.9 | 250 (5) |
| phm-2;tph-1 | 25.0±5.7** | 71.7±1.9** | 304 (5) |
| daf-2(e1370) | 5.8±2.2 | 7.7±2.3 | 431 (9) |
| phm-2(am117) | 85.0±3.9 | 83.5±3.9 | 465 (9) |
| phm-2;daf-2 | 59.8±10.3** | 58.2±9.0** | 485 (9) |
| daf-16(mu86) | 16.0±1.7 | 10.3±2.2 | 258 (3) |
| phm-2(am117) | 84.8±5.2 | 83.2±5.0 | 135 (3) |
| daf-16 phm-2 | 86.9 ± 8.4^{ns} | 87.9±3.0 ^{ns} | 210 (3) |
| daf-16;daf-2 | 17.9±6.9 | 10.8 ± 3.8 | 199 (3) |
| daf-16 phm-2;daf-2 | 88.9±1.6** | 91.0±3.2** | 190 (3) |
| isp-1(qm150) | 31.0±5.2 | 42.7±1.8 | 266 (4) |
| phm-2(am117) | 85.4±4.3 | 84.3±3.9 | 250 (4) |
| phm-2;isp-1 | 85.2±5.9 ^{ns} | 86.5±6.8 ^{ns} | 212 (4) |
| aak-2(ok524) | 24.2±4.8 | 30.7±6.5 | 210 (4) |
| phm-2(am117) | 84.6±4.0 | 82.5±4.3 | 215 (4) |
| phm-2;aak-2 | 66.1±7.3** | 73.7±6.0 ^{ns} | 213 (4) |
| rict-1(mg360) | 7.7±3.0 | 14.6±3.5 | 177 (3) |
| phm-2(am117) | 86.2±2.9 | 84.2±2.9 | 166 (3) |
| phm-2;rict-1 | 71.4±1.4** | 81.0±2.2 ^{ns} | 174 (3) |
| rsks-1(ok1255) | 5.1±2.8 | 8.8±5.8 | 176 (3) |
| phm-2(am117) | 86.2±2.9 | 84.2±2.9 | 166 (3) |
| phm-2;rsks-1 | 81.7±6.0 ^{ns} | 82.8±5.7 ^{ns} | 181 (3) |
| hlh-30(tm1978) | 6.4±3.2 | 10.0±1.7 | 159 (3) |
| phm-2(am117) | 84.8±5.2 | 83.2±4.9 | 135 (3) |
| phm-2;hlh-30 | 86.4±5.3 ^{ns} | 84.4±2.6 ^{ns} | 165 (3) |
| pmk-1(ok2471) | 11.4±2.6 | 14.4±4.7 | 240 (4) |
| phm-2(am117) | 85.5±4.9 | 83.6±4.1 | 204 (4) |
| phm-2;pmk-1 | 76.8±4.7* | 80.7±3.3 ^{ns} | 229 (4) |
| mlk-1(ok2471) | 9.8±2.8 | 10.3±4.6 | 183 (4) |
| phm-2(am117) | 75.8±1.8 | 80.6±1.1 | 191 (4) |
| phm-2;mlk-1 | 82.9±1.9** | 89.5±2.6** | 211 (4) |
| npr-1(ur89) | 17.5±1.9 | 18.9±4.4 | 183 (4) |
| phm-2(am117) | 85.5±4.9 | 83.7±4.1 | 191 (4) |
| phm-2;npr-1 | 71.3±2.4** | 80.3 ± 3.7^{ns} | 211 (4) |
| | | | |

Table S5. Bacterial avoidance behavior of mutant strains, Related to Figure 4 and Figure S8.

¹Genotype: The indicated mutant strains were cultured with a small lawn of live *E. coli* OP50 bacteria on NGM dishes at 20°C.

²Avoidance: Animals were scored as inside or outside the bacterial lawn (Avoidance= N_{out}/N_{total}) as one day old adults (YA) and two day old adults (LA); Comparisons are to the *phm-2* single mutant, and the statistical test was one-way ANOVA with Tukey post hoc HSD; *, *P* < 0.05; **, *P* < 0.01; ns, not significant, P>0.05.

³N(n): Total number of hermaphrodites analyzed, and the number of independent experiments.

| Genotype ¹ | Bacteria ² | Mean ³ | % | Maximum ³ | % | N (n) ⁴ |
|----------------------------------|-----------------------|------------------------|---------|---------------------------|-----------|--------------------|
| | | lifespan | Change | lifespan | Change in | |
| | | \pm SD (days) | in mean | \pm SD (days) | maximum | |
| WT | Live E. coli | 15.8±3.7 | | 21.9±1.2 | | 316 (6) |
| phm-2(am117) | OP50 | 21.1±3.1** | +34 | 27.3±1.2** | +24 | 279 (6) |
| | | | | | | |
| WT | Live E. coli | 15.2±2.7 | | 19.8±0.9 | | 158 (3) |
| phm-2(am117) | HB101 | 20.6±3.5** | +36 | 26.7±0.8** | +35 | 93 (3) |
| | | | | | | |
| WT | Live | 14.7±2.4 | | 18.8±0.6 | | 83 (2) |
| phm-2(am117) | Comamonas | 15.2 ± 2.4^{ns} | +4 | 19.3±0.5* | +3 | 141 (2) |
| | | | | | | |
| WT | Live B. subtilis | 20.4±4.7 | | 27.7±1.4 | | 68 (3) |
| phm-2(am117) | | 20.5±4.4 ^{ns} | +1 | 27.6±1.2 ^{ns} | -1 | 59 (3) |
| | | | | | | |
| WT | Dead E. coli | 17.9±3.6 | | 24.5±1.1 | | 78 (2) |
| phm-2(am117) | OP50 | 16.2±3.1** | -10 | 22.0±0.8** | -10 | 69 (2) |
| | | | | | | |
| WT | Begin on Live | 15.2 ± 0.4 | | 24.2±0.4 | | 97 (2) |
| phm-2(am117) | <i>E. coli</i> OP50, | 21.1±0.6** | +39 | 29.8±0.3** | +23 | 103 (2) |
| | transfer to Live | | | | | |
| | E. coli OP50 | | | | | |
| WT | Begin on Live | 14.5 ± 0.4 | | 22.0±0.5 | | 87 (2) |
| phm-2(am117) | Comamonas, | 19.96±0.4** | +32 | 26.4±0.5** | +20 | 100 (2) |
| | transfer to Live | | | | | |
| | E. coli OP50 | | | | | |
| WT | Live E. coli | 17.0±3.7 | | 23.0±1.0 | | 116 (2) |
| eat-2(ad465) | OP50 | 22.2±4.7** | +30 | 31.0±2.4** | +36 | 115 (2) |
| | | | | | | |
| WT | Live | 14.4 ± 2.8 | | 19.4±1.0 | | 72 (2) |
| eat-2(ad465) | Comamonas | 14.8 ± 2.8 ns | +3 | $20.1\pm0.9^{\text{ ns}}$ | -1 | 76 (2) |
| | | | | | | |
| WT | Dead E. coli | 20.1±4.8 | | 27.4±1.1 | | 231 (5) |
| eat-2(ad465) | OP50 | 22.3±7.1** | +11 | 34.0±1.8** | +24 | 275 (5) |
| | | | | | | |
| WT ⁵ | Live E. coli | 12.6±4.4 | | 19.6±1.3 | | 146 (6) |
| <i>phm-2(am117)</i> ⁵ | OP50, | 8.6±3.8** | -31 | 16.9±2.3** | -14 | 172 (6) |
| | Large Lawn | | | | | |
| <i>phm-2(am117)</i> ⁵ | Live E. coli | 18.8 ± 5.5 | | 28.6±1.0 | | 163 (3) |
| <i>tph-1(mg280)</i> ⁵ | OP50, | 13.3±4.3 | | 21±1.4 | | 275 (3) |
| <i>phm-2;tph-1</i> ⁵ | Small Lawn | 11.9±6.9* | -37 | 24.4±1.5** | -15 | 142 (3) |
| | | | | | | |

Table S6. The bacterial strain and lawn size affected mean and maximum life span, Related to Figures 4, 5, 6 and 7.

¹Genotype: Wild-type hermaphrodites or the indicated mutants were analyzed.

²Bacteria: Bacterial species of the indicated strains were spotted in the center of the NGM dish to form a small lawn, except rows 21-22 where bacteria were spread to form a large lawn. To generate dead *E. coli*, we spotted bacteria on the dish and then treated with UV light. For rows 11-14, animals were cultured on one bacterial strain from egg to adulthood, and then transferred to a second strain for the remainder of the lifespan.

³Mean, Maximum, % Change: Maximum adult lifespan is the mean lifespan of the 10% of the population that had the longest lifespans. Comparisons are to the matched WT control; row 25 was compared to row 23. Statistical test was one-way ANOVA with Tukey post hoc HSD; *, P < 0.05; **, P < 0.01; ns, not significant, P>0.05.

⁴N(n): Total number of hermaphrodites analyzed, and the number of independent experiments. ⁵For rows 21-25, animals that died due to matricidal hatching were not censored from the data.