Appendix Information

Mitochondrial chaperone HSP-60 regulates anti-bacterial immunity via p38 MAP kinase signaling

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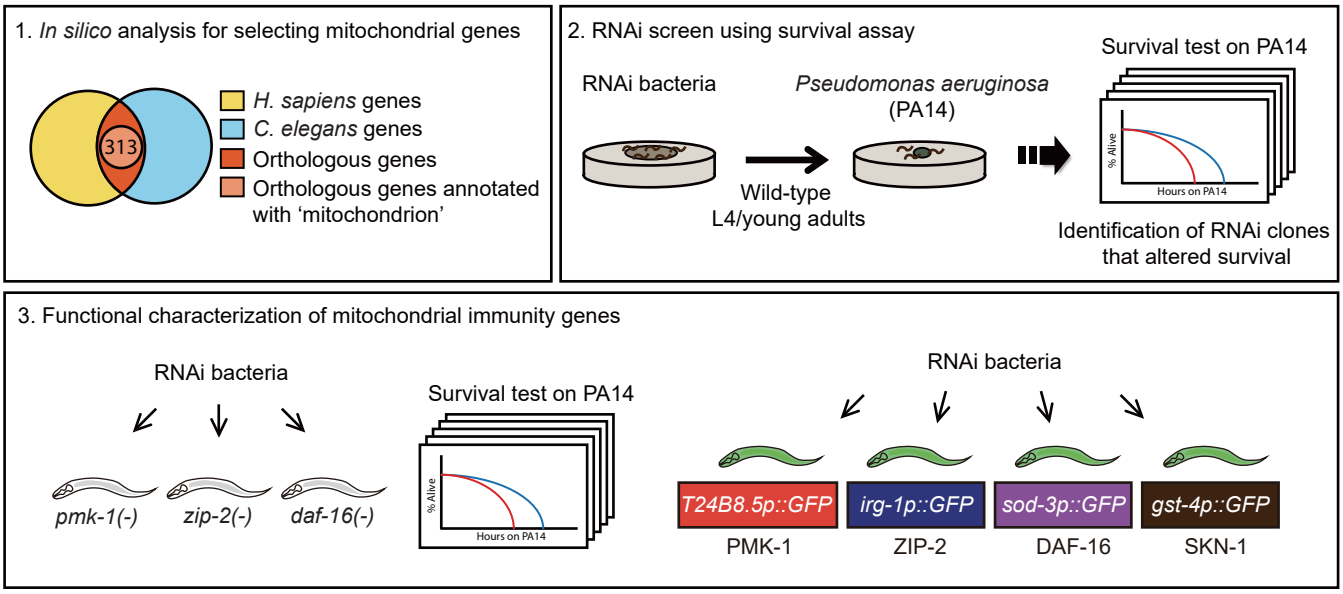
Appendix Figure S1

A



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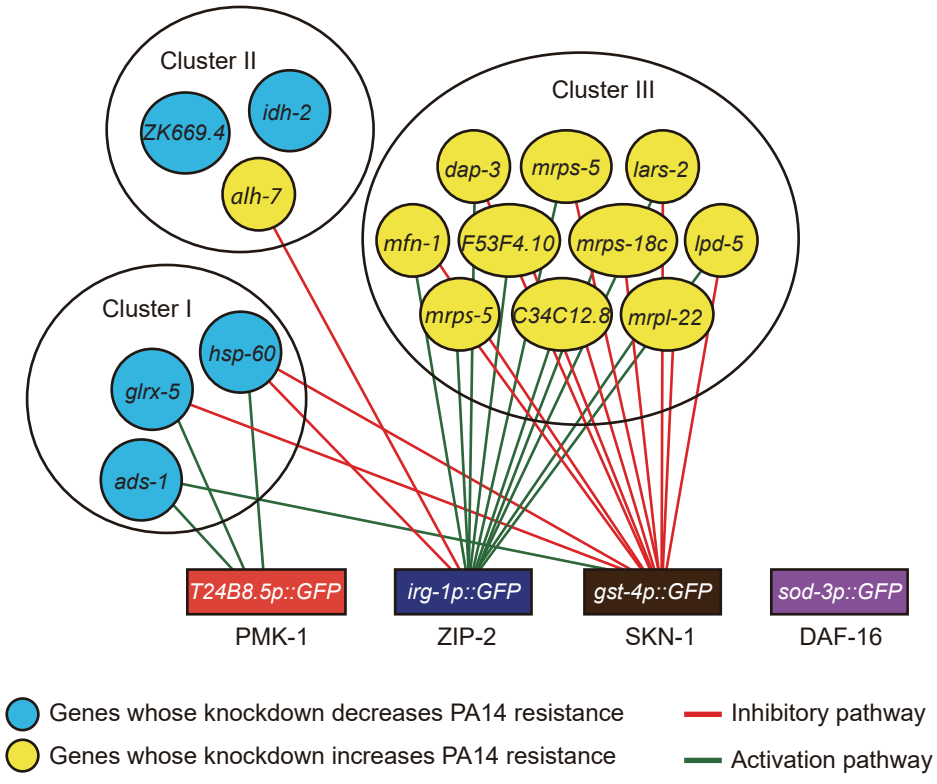


Appendix Figure S1. RNAi screen for mitochondrial components that affect *C. elegans* immunity against PA14.

A Schematic of overall primary and secondary RNAi screens. 1. Among 3,146 *C. elegans*-human orthologous genes, we filtered 313 mitochondrial genes whose human orthologs were annotated as Gene Ontology term "mitochondrion" (Ashburner et al, 2000; Gene Ontology, 2015) and included in human MitoCarta (Pagliarini et al, 2008) (See Materials and Methods for detail). 2. Standard PA14 slow killing assays were performed upon knocking down each of the 220 mitochondrial genes whose RNAi clones were available and did not cause severe developmental phenotypes. The survival time of *C. elegans* on PA14 was blindly measured and subsequently analyzed. 3. A secondary RNAi screen was carried out by performing a survival test on PA14 using immune-compromised mutants (*pmk-1(km25)*, *zip-2(tm4067)*, and *daf-16(mu86)*), and by measuring the levels of GFP reporters for known immune effectors (*T24B8.5p::GFP* for PMK-1, *irg-1p::GFP* for ZIP-2, *gst-4p::GFP* for SKN-1, and *sod-3p::GFP* for DAF-16).

B A schematic diagram that shows the effects of genes identified from our RNAi screen shown in panel **A**. Light blue circles and yellow circles indicate genes whose inhibition decreased and increased the survival of wild-type animals on PA14, respectively. Red and green lines indicate genes whose knockdown enhanced and suppressed the expression of the corresponding GFP reporters, respectively. The cluster I genes include a mitochondrial chaperone (*hsp-60*), glutaredoxin-related protein 5 (*glrx-5*), and alkyldihydroxyacetonephosphate synthase 1 (*ads-1*). Inhibition of the cluster I genes increased the susceptibility of wild-type animals to PA14 infection, and reduced the level

of *T24B8.5p::GFP* (Fig 1B-C and Dataset EV2). The PA14 resistance-decreasing effects of RNAi clones in this cluster were markedly reduced in *pmk-1* mutant animals (Fig 1B). These results suggest that genes in this cluster are required for resistance against PA14 via modulating PMK-1. The cluster II genes include mitochondrial isocitrate dehydrogenase 2 (*idh-2*), aldehyde dehydrogenase 7 (*alh-7*), and a dihydrolipoamide-branched chain transacylase (*ZK669.4*). RNAi clones targeting cluster II genes tend not to affect the expression of GFP reporters for known immune effector proteins that we tested (Fig 1C and Dataset EV2). Thus, genes in the cluster II may modulate unknown or other untested immune effectors to affect the PA14 susceptibility of *C. elegans*. The cluster III mostly consists of genes implicated in the mitochondrial mRNA translation and respiration. These include a tRNA synthetase (*lars-2*), mitochondrial ribosomal proteins (*mrps-35*, *mrpl-22*, *dap-3*, *mrps-18c*, and *mrps-5*), mitochondrial ETC components [an NADH-ubiquinone oxidoreductase flavoprotein 2 (F53F4.10/NDUFV2) and an NADH-coenzyme Q reductase (*lpd-5*/NDUFS4)], a mitochondrial iron transporter (*mfn-1*), and a component of presequence translocase-associated motor (PAM) complex (C34C12.8). Iron transporter and PAM complex are known to play roles in mitochondrial respiration in mammals (Chen et al, 2015; Kulawiak et al, 2013). RNAi clones targeting genes in this cluster increased the survival of animals on PA14 (Fig 1B and Dataset EV2). In addition, these RNAi clones generally increased the expression of the SKN-1 target *gst-4p::GFP*, while suppressing the induction of the ZIP-2 target *irg-1p::GFP* upon PA14 infection (Fig 1C and Dataset EV2). In contrast, the expression of PMK-1/p38 MAPK target reporter *T24B8.5p::GFP* was largely unaffected (Fig 1C and Dataset EV2). The effects of the RNAi clones on PA14 resistance decreased or

disappeared in *zip-2* or *daf-16* mutant animals (Fig 1B). Together, mitochondrial components that influence immunity against PA14 appear to form functional modules to regulate the activities of immune proteins.

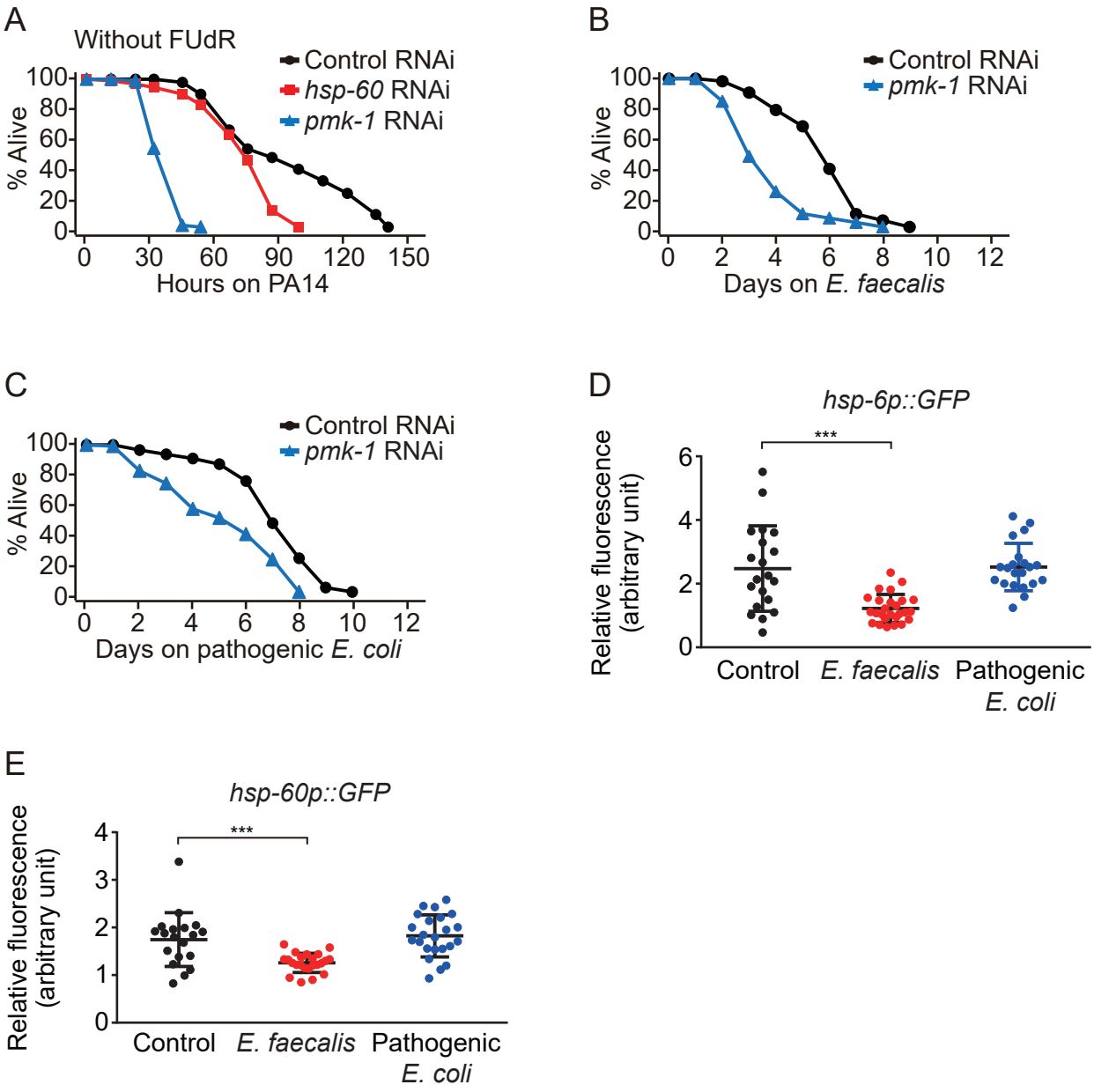
Interestingly, five RNAi clones targeting mitochondrial ribosomal proteins increased PA14 resistance. Recent studies have shown that depletion of mitochondrial ribosomal proteins extends *C. elegans* lifespan through activating mitochondrial stress responses (Houthkooper et al, 2013). Although enhanced PA14 resistance is not always linked to longevity, many *C. elegans* longevity mutants are resistant to bacterial pathogens (Kim, 2013). Thus, it seems likely that RNAi targeting mitochondrial ribosomal protein genes promotes longevity, which may contribute to increased survival on PA14. Furthermore, depletion of mitochondrial ribosomal proteins activates UPR^{MT}, which leads to the induction of anti-microbial genes (Nargund et al, 2012; Pellegrino et al, 2014) and mitochondrial chaperone genes, including *hsp-60*. The induction of *hsp-60* may then contribute to PA14 resistance, as we showed in this study.

Among the 16 RNAi clones, we noticed that RNAi clones targeting six genes (*dap-3*, *mrps-5*, *mrpl-22*, *mrps-35*, *mfn-1*, and *hsp-60*) were reported to induce UPR^{MT} (Bennett et al, 2014). As five of these six RNAi clones were included in the cluster III and increased PA14 resistance, these five RNAi clones may activate UPR^{MT} to enhance immunity in *C. elegans* (Pellegrino et al, 2014).

Knocking down each of four genes (*mrps-5*, *mrps-35*, *F53F4.10*, and *mfn-1*) in the cluster III suppresses the induction of *nlp-29*, an epidermal anti-microbial peptide, upon infection with a fungal pathogen (Couillault et al, 2004; Zugasti et al, 2016). RNAi

targeting each of five genes (*dap-3*, *mrpl-22*, *F53F4.10*, *mfn-1*, and *lpd-5*) in the cluster III also reduces the induction of an osmotic-response reporter *gpdh-1::GFP* caused by mutations in a mucin-like gene *osm-8* (Rohlfing et al, 2011). Transcriptional changes by osmotic stress and fungal infection are similar (Rohlfing et al, 2010), and resistance to osmotic stress correlates with immunity against fungal infection (Pujol et al, 2008). Therefore, inhibition of genes in the cluster III may promote the resistance against the pathogenic bacteria, PA14, while reducing protective responses to fungal pathogens and osmotic stresses.

Appendix Figure S2



Appendix Figure S2. **The effects of *hsp-60* RNAi or *pmk-1* RNAi on PA14 resistance without FUdR treatment and resistance against pathogenic *E. faecalis* or pathogenic *E. coli*.**

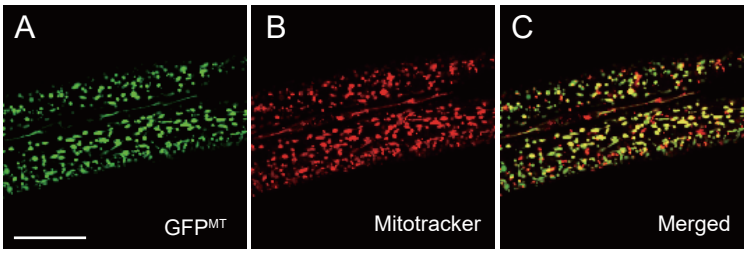
A *hsp-60* RNAi reduced resistance to PA14 without fluoro-2'-deoxyuridine (FUdR) treatment.

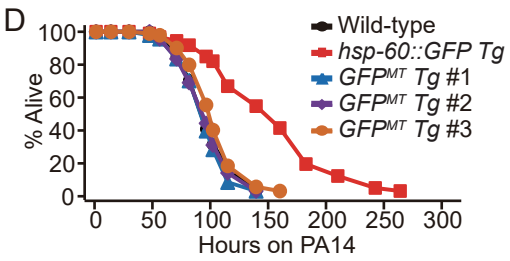
B-C Knockdown of *pmk-1* reduced the survival of worms infected with *E. faecalis* (**B**) or pathogenic *E. coli* (**C**). *pmk-1* RNAi in this figure was used as a positive control for data in Fig 2H-I.

D-E Relative intensities for GFP expression were quantified using animals expressing *hsp-6p::GFP* (**D**) and *hsp-60p::GFP* (**E**) on control bacteria, *E. faecalis*, or pathogenic *E. coli*. Infection with *E. faecalis* or pathogenic *E. coli* did not increase the levels of *hsp-6p::GFP* or *hsp-60p::GFP*.

Data information: See Appendix Table S2 for additional repeats and statistical analysis for the survival and the lifespan data shown in this Figure. Error bars represent SEM. *p* values were calculated by using two-tailed Student's t-test (***$p<0.001$).

Appendix Figure S3





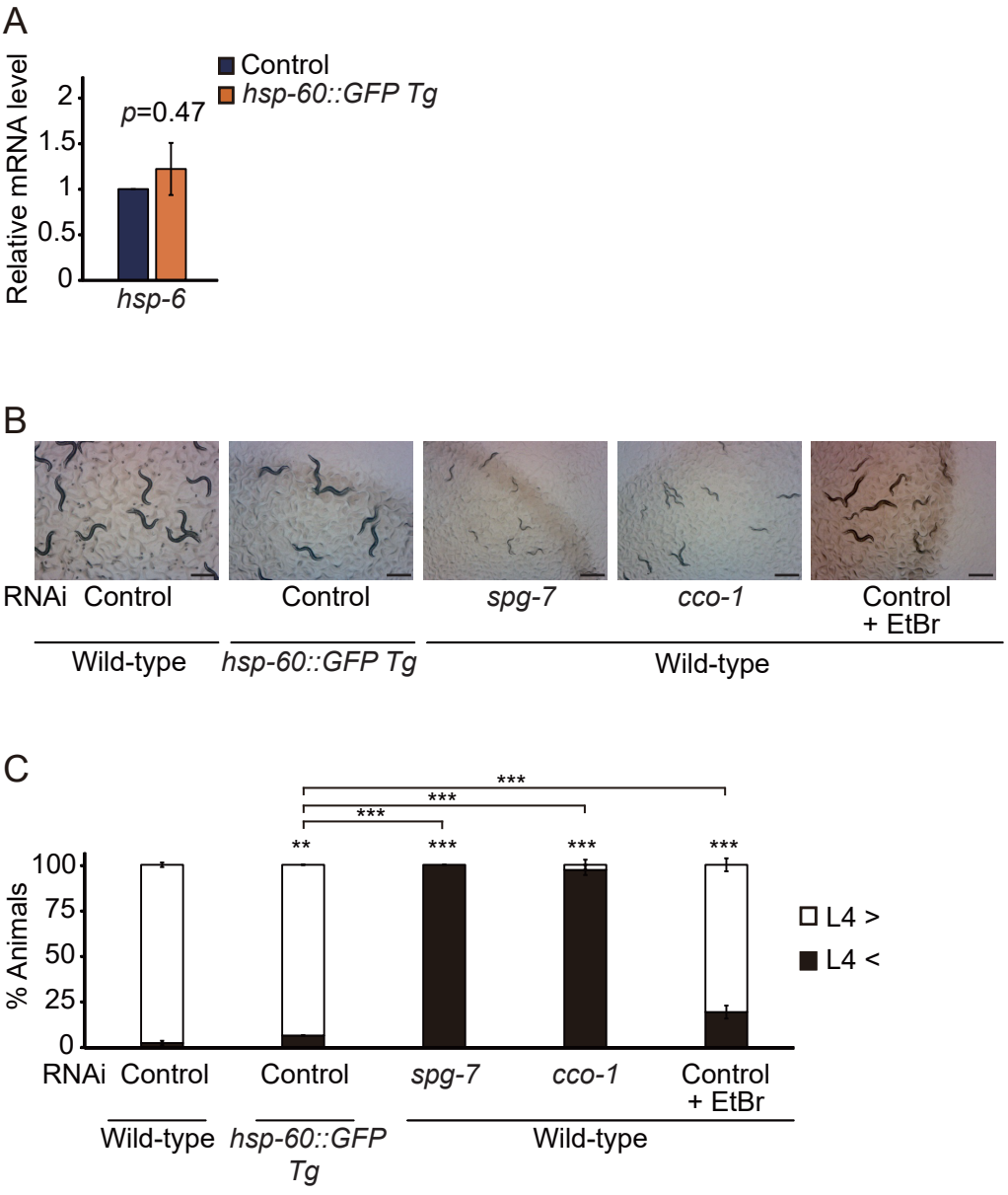
Appendix Figure S3. Expression of GFP in mitochondria did not affect resistance against PA14.

A-C Representative images of L2 larval animals that expressed GFP in the mitochondria (GFP^{MT}) with N-terminal 60 amino acids (predicted mitochondrial targeting sequence) of HSP-60. GFP^{MT} (**A**) and mitochondria stained with the Mitotracker CMXRos (**B**) were co-localized (**C**). Scale bar indicates 25 μm.

D Transgenic expression of only GFP in mitochondria (*GFP^{MT} Tg*) did not affect the resistance of animals on PA14.

Data information: See Appendix Table S9 for additional repeats and statistical analysis for the survival and the lifespan data shown in this Figure.

Appendix Figure S4



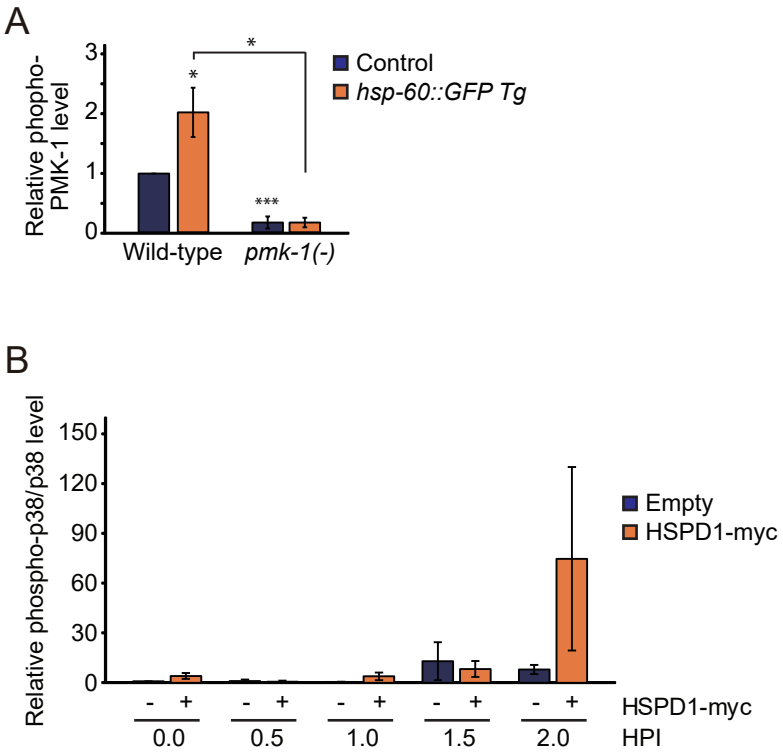
Appendix Figure S4. *hsp-60::GFP* expression does not appear to induce mitochondrial stress.

A *hsp-60::GFP Tg* did not affect the expression of *hsp-6*, another mitochondrial chaperone that was known to be induced by UPR^{MT} (Yoneda et al, 2004).

B Representative pictures of wild-type and *hsp-60::GFP Tg* animals upon treating with control, *spg-7* or *cco-1* RNAi, or with EtBr (30 μg/ml). Scale bar indicates 500 μm.

C Quantification of data in panel **B** (n≥347 from at least two independent experiments). Error bars represent SEM (Chi-squared test, $**p<0.01$, $***p<0.001$). *hsp-60* expression is known to be increased in response to stresses that trigger UPR^{MT} (Pellegrino et al, 2013). Therefore, the activation of UPR^{MT} upon PA14 infection might sequester a certain amount of HSP-60 away from the SEK-1/PMK-1 signaling pathway, and this might not allow the activation of a fully functional defense response. If so, one can expect that the induction of UPR^{MT} is detrimental to animals upon PA14 infection. However, it does not seem to be the case as *spg-7* RNAi, which induces UPR^{MT}, actually enhances resistance to PA14 (Pellegrino et al, 2014).

Appendix Figure S5



Appendix Figure S5. Increased expression of *hsp-60*/*HSPD1* up-regulates the level of phospho-p38 MAPK in *C. elegans* and cultured human cells.

A Quantification of data in Fig 7D (n=4).

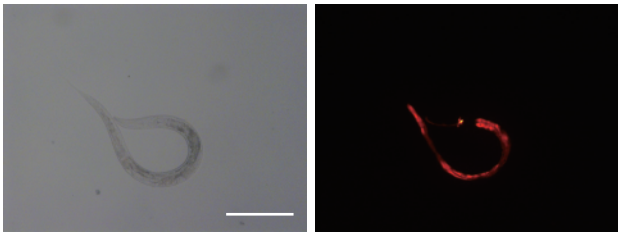
B Quantification of data in Fig 7E (n≥3). HPI: hours post PA14 infection. See Fig 7E for a representative blot and the Source Data 1 that include all six Western blot data used for quantification.

Data information: Error bars represent SEM. *p* values were calculated by two tailed Student's t test (**$p<0.01$, ***$p<0.001$).

Appendix Figure S6

A

Intestinal HSP-60::tagRFP



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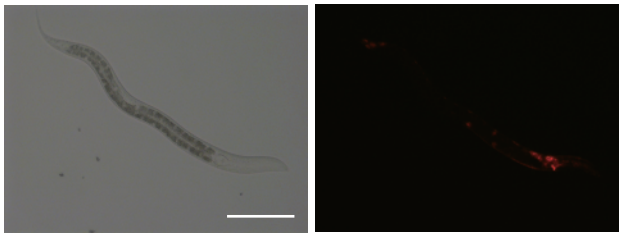
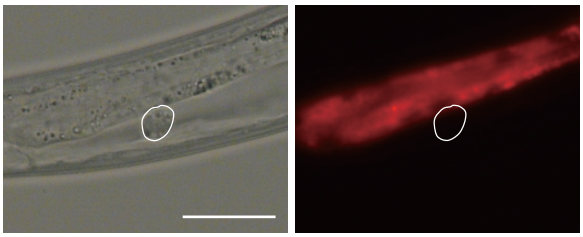
Neuronal HSP-60::tagRFP



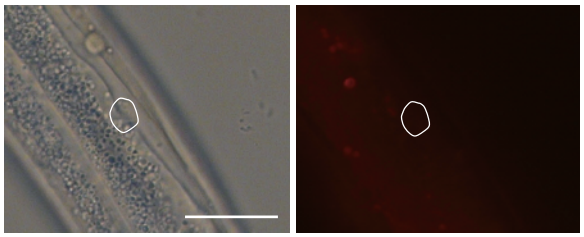
C

Intestinal HSP-60::tagRFP



D

Neuronal HSP-60::tagRFP



Appendix Figure S6. HSP-60 expressed in the intestine or neurons is not localized in coelomocytes.

A-**B** Representative bright field (left) and fluorescence (right) images of the transgenic animals that expressed HSP-60::tagRFP in the intestine by using a *vha-6* promoter (**A**) or in neurons by using an *unc-119* promoter (**B**). Scale bars indicate 200 μm.

C-**D** Representative bright field (left) and fluorescence (right) images showing that HSP-60::GFP expressed in the intestine (**C**) or neurons (**D**) was not detected in coelomocytes marked with white circles. Scale bars indicate 25 μm.

Appendix Tables

Appendix Table S1. The effects of RNAi clones targeting mitochondrial components on the survival of wild-type animals, and *pmk-1*, *zip-2* and *daf-16* mutants.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
Wild-type /Control RNAi	PA14	93.7±2.4	101		109/120		Fig 1B
Wild-type /*hsp-60* RNAi	PA14	59.2±0.9	76	-37%	115/120	<0.001	Fig 1B
Wild-type /*glrx-5* RNAi	PA14	69.4±1.3	76	-26%	117/120	<0.001	Fig 1B
Wild-type /*alh-7* RNAi	PA14	109.4±2.6	129	17%	108/120	<0.001	Fig 1B
Wild-type /Control RNAi	PA14	111.3±2.24	134		113/120		Fig 1B
Wild-type /*ZK669.4* RNAi	PA14	90.2±1.8	100	-19%	111/120	<0.001	Fig 1B
Wild-type /*idh-2* RNAi	PA14	97.3±2.1	114	-12%	106/120	<0.001	Fig 1B
Wild-type /Control RNAi	PA14	92.8±2.1	104		108/120		Fig 1B
Wild-type /*ads-1* RNAi	PA14	68.7±1.5	78	-26%	111/120	<0.001	Fig 1B
Wild-type /*lars-2* RNAi	PA14	118.7±3.6	137	28%	80/90	<0.001	Fig 1B
Wild-type /*mrpl-22* RNAi	PA14	129.7±3.5	162	40%	103/120	<0.001	Fig 1B
Wild-type /*mrps-35* RNAi	PA14	132.9±3.2	149	43%	104/120	<0.001	Fig 1B
Wild-type /Control RNAi	PA14	108.0±2.4	121		115/120		Fig 1B
Wild-type /*F53F4.10* RNAi	PA14	130.3±3.9	164	21%	87/90	<0.001	Fig 1B
Wild-type /Control RNAi	PA14	108.5±2.5	127		103/120		Fig 1B
Wild-type /*lpd-5* RNAi	PA14	119.6±3.3	135	10%	109/120	<0.01	Fig 1B

Wild-type /*mfn-1* RNAi	PA14	153.0±4.0	183	41%	110/120	<0.001	Fig 1B
Wild-type /Control RNAi	PA14	104.7±2.8	126		112/120		Fig 1B
Wild-type /*C34C12.8* RNAi	PA14	132.8±4.6	186	27%	111/120	<0.001	Fig 1B
Wild-type /*mrps-5* RNAi	PA14	135.1±4.1	168	29%	114/120	<0.001	Fig 1B
Wild-type /*mrps-18c* RNAi	PA14	138.2±4.5	174	32%	115/120	<0.001	Fig 1B
Wild-type /*dap-3* RNAi	PA14	140.9±4.3	174	35%	114/120	<0.001	Fig 1B
pmk-1(km25) /Control RNAi	PA14	51.0±1.1	65		111/120		Fig 1B and 4G
pmk-1(km25) /*hsp-60* RNAi	PA14	50.9±0.9	65	0%	119/120	0.4613	Fig 1B and 4G
pmk-1(km25) /*dap-3* RNAi	PA14	69.0±1.8	80	35%	117/120	<0.001	Fig 1B and 4G
pmk-1(km25) /Control RNAi	PA14	47.3±1.6	56		120/120		Fig 1B
pmk-1(km25) /*hsp-60* RNAi	PA14	61.0±1.4	73	29%	120/120	<0.001	Fig 1B
pmk-1(km25) /*dap-3* RNAi	PA14	70.2±1.9	79	49%	120/120	<0.001	Fig 1B
pmk-1(km25) /Control RNAi	PA14	46.7±1.2	52		94/120		Fig 1B
pmk-1(km25) /*ads-1* RNAi	PA14	45.3±1.6	52	-3%	44/60	0.5341	Fig 1B
pmk-1(km25) /*glrx-5* RNAi	PA14	44.1±1.1	52	-6%	82/110	0.1535	Fig 1B
pmk-1(km25) /*ZK669.4* RNAi	PA14	41.1±1.1	52	-12%	93/120	<0.01	Fig 1B
pmk-1(km25) /*idh-2* RNAi	PA14	47.9±1.1	58	3%	108/120	0.3541	Fig 1B
pmk-1(km25) /*lpd-5* RNAi	PA14	63.2±1.9	70	35%	111/120	<0.001	Fig 1B
pmk-1(km25) /*alh-7* RNAi	PA14	47.4±1.2	52	2%	103/120	0.5916	Fig 1B
pmk-1(km25) /*F53F4.10* RNAi	PA14	75.0±1.7	81	61%	96/120	<0.001	Fig 1B
pmk-1(km25) /*C34C12.8* RNAi	PA14	63.8±1.7	75	37%	95/120	<0.001	Fig 1B

pmk-1(km25) /Control RNAi	PA14	52.5±1.4	70		114/120		Fig 1B
pmk-1(km25) /*ads-1* RNAi	PA14	46.2±1.7	54	-12%	92/100	<0.001	Fig 1B
pmk-1(km25) /*glrx-5* RNAi	PA14	59.8±1.5	74	14%	105/111	<0.01	Fig 1B
pmk-1(km25) /*ZK669.4* RNAi	PA14	41.1±1.4	48	-22%	104/114	<0.001	Fig 1B
pmk-1(km25) /*idh-2* RNAi	PA14	54.8±1.2	69	4%	118/120	0.3174	Fig 1B
pmk-1(km25) /*lpd-5* RNAi	PA14	71.2±1.8	80	36%	114/120	<0.001	Fig 1B
pmk-1(km25) /Control RNAi	PA14	49.6±1.2	58		117/120		Fig 1B
pmk-1(km25) /*alh-7* RNAi	PA14	46.8±1.0	58	-6%	116/120	<0.05	Fig 1B
pmk-1(km25) /*F53F4.10* RNAi	PA14	63.6±1.7	71	28%	116/120	<0.001	Fig 1B
pmk-1(km25) /*C34C12.8* RNAi	PA14	82.0±2.3	98	65%	85/90	<0.001	Fig 1B
pmk-1(km25) /Control RNAi	PA14	50.5±1.3	58		110/120		Fig 1B
pmk-1(km25) /*lars-2* RNAi	PA14	61.9±1.2	71	22%	108/120	<0.001	Fig 1B
pmk-1(km25) /*mrps-5* RNAi	PA14	63.8±2.1	71	26%	112/120	<0.001	Fig 1B
pmk-1(km25) /*mrps-18c* RNAi	PA14	66.7±2.2	71	32%	112/120	<0.001	Fig 1B
pmk-1(km25) /*mrpl-22* RNAi	PA14	66.7±2.2	82	32%	114/120	<0.001	Fig 1B
pmk-1(km25) /*mfn-1* RNAi	PA14	64.1±1.7	82	27%	113/120	<0.001	Fig 1B
pmk-1(km25) /*mrps-35* RNAi	PA14	61.6±1.7	71	22%	108/120	<0.001	Fig 1B
pmk-1(km25) /Control RNAi	PA14	45.6±1.2	57		101/120		Fig 1B
pmk-1(km25) /*lars-2* RNAi	PA14	63.2±1.8	72	39%	111/120	<0.001	Fig 1B
pmk-1(km25) /*mrps-5* RNAi	PA14	75.4±2.3	91	65%	112/120	<0.001	Fig 1B

pmk-1(km25) /*mrps-18c* RNAi	PA14	70.2±1.7	79	54%	116/120	<0.001	Fig 1B
pmk-1(km25) /*mrpl-22* RNAi	PA14	68.1±2.0	79	49%	114/120	<0.001	Fig 1B
pmk-1(km25) /*mfn-1* RNAi	PA14	59.6±1.6	66	31%	105/120	<0.001	Fig 1B
pmk-1(km25) /*mrps-35* RNAi	PA14	70.8±2.0	79	55%	116/120	<0.001	Fig 1B
zip-2(tm4067) /Control RNAi	PA14	91.1±2.4	100		77/90		Fig 1B and 4H
zip-2(tm4067) /*hsp-60* RNAi	PA14	64.3±0.8	72	-30%	114/120	<0.001	Fig 1B and 4H
zip-2(tm4067) /Control RNAi	PA14	88.2±1.9	93		115/120		Fig 1B
zip-2(tm4067) /*hsp-60* RNAi	PA14	75.5±1.0	82	-15%	113/120	<0.001	Fig 1B
zip-2(tm4067) /Control RNAi	PA14	86.8±2.1	98		116/120		Fig 1B
zip-2(tm4067) /*ads-1* RNAi	PA14	64.6±1.4	72	-26%	120/122	<0.001	Fig 1B
zip-2(tm4067) /*glrx-5* RNAi	PA14	81.3±2.0	92	-6%	115/120	0.0791	Fig 1B
zip-2(tm4067) /*ZK669.4* RNAi	PA14	83.7±1.9	92	-4%	107/120	0.2519	Fig 1B
zip-2(tm4067) /*idh-2* RNAi	PA14	84.9±1.9	92	-2%	114/120	0.4756	Fig 1B
zip-2(tm4067) /*lpd-5* RNAi	PA14	87.1±2.1	98	0%	111/120	0.8901	Fig 1B
zip-2(tm4067) /*alh-7* RNAi	PA14	77.3±1.3	92	-11%	107/120	<0.001	Fig 1B
zip-2(tm4067) /*F53F4.10* RNAi	PA14	74.8±2.1	79	-14%	106/120	<0.001	Fig 1B
zip-2(tm4067) /Control RNAi	PA14	87.2±1.9	104		108/120		Fig 1B
zip-2(tm4067) /*ads-1* RNAi	PA14	70.2±1.6	80	-20%	116/120	<0.001	Fig 1B
zip-2(tm4067) /*glrx-5* RNAi	PA14	94.3±2.4	103	8%	116/120	0.1149	Fig 1B
zip-2(tm4067) /*ZK669.4* RNAi	PA14	85.3±1.9	92	-2%	123/125	<0.05	Fig 1B

zip-2(tm4067) /*idh-2* RNAi	PA14	97.0±2.5	116	11%	115/120	0.0946	Fig 1B
zip-2(tm4067) /*lpd-5* RNAi	PA14	89.2±2.3	103	2%	125/127	0.7772	Fig 1B
zip-2(tm4067) /*alh-7* RNAi	PA14	89.8±2.1	103	3%	116/120	0.9841	Fig 1B
zip-2(tm4067) /*F53F4.10* RNAi	PA14	85.4±3.0	102	-2%	104/110	0.3744	Fig 1B
zip-2(tm4067) /Control RNAi	PA14	108.0±2.8	119		79/90		Fig 1B
zip-2(tm4067) /*C34C12.8* RNAi	PA14	134.9±4.7	165	25%	79/90	<0.001	Fig 1B
zip-2(tm4067) /*dap-3* RNAi	PA14	136.9±5.0	176	27%	84/90	<0.001	Fig 1B
zip-2(tm4067) /Control RNAi	PA14	110.4±3.1	130		87/90		Fig 1B
zip-2(tm4067) /*C34C12.8* RNAi	PA14	125.2±6.0	166	13%	85/90	<0.001	Fig 1B
zip-2(tm4067) /*dap-3* RNAi	PA14	150.3±6.7	202	36%	86/90	<0.001	Fig 1B
zip-2(tm4067) /Control RNAi	PA14	139.1±2.6	154		116/120		Fig 1B
zip-2(tm4067) /*lars-1* RNAi	PA14	118.8±3.9	143	-15%	190/120	<0.05	Fig 1B
zip-2(tm4067) /*mrps-5* RNAi	PA14	168.4±5.3	213	21%	112/120	<0.001	Fig 1B
zip-2(tm4067) /*mprs-18c* RNAi	PA14	147.8±4.9	191	6%	115/120	<0.01	Fig 1B
zip-2(tm4067) /*mrpl-22* RNAi	PA14	129.7±4.8	154	-7%	117/120	0.8495	Fig 1B
zip-2(tm4067) /*mfn-1* RNAi	PA14	123.7±4.0	165	-11%	117/120	0.2006	Fig 1B
zip-2(tm4067) /*mrps-35* RNAi	PA14	154.6±5.1	202	11%	116/120	<0.001	Fig 1B
zip-2(tm4067) /Control RNAi	PA14	135.1±3.2	153		104/120		Fig 1B
zip-2(tm4067) /*lars-1* RNAi	PA14	120.5±3.9	123	-11%	91/120	<0.05	Fig 1B
zip-2(tm4067) /*mrps-5* RNAi	PA14	147.8±5.1	189	9%	104/120	<0.01	Fig 1B

zip-2(tm4067) /*mprs-18c* RNAi	PA14	141.6±5.2	178	5%	104/120	0.1886	Fig 1B
zip-2(tm4067) /*mrpl-22* RNAi	PA14	129.8±5.0	141	-4%	103/120	0.3946	Fig 1B
zip-2(tm4067) /*mfn-1* RNAi	PA14	125.7±4.5	141	-7%	100/120	0.2709	Fig 1B
zip-2(tm4067) /*mrps-35* RNAi	PA14	132.0±4.4	153	-2%	108/120	0.8304	Fig 1B
daf-16(mu86) /Control RNAi	PA14	104.8±1.7	113		117/120		Fig 1B and 4I
daf-16(mu86) /*hsp-60* RNAi	PA14	75.8±1.2	93	-28%	112/120	<0.001	Fig 1B and 4I
daf-16(mu86) /*glrx-5* RNAi	PA14	90.4±2.0	102	-14%	117/120	<0.001	Fig 1B
daf-16(mu86) /*ZK669.4* RNAi	PA14	80.7±2.1	92.5	-23%	98/107	<0.001	Fig 1B
daf-16(mu86) /*idh-2* RNAi	PA14	84.7±1.9	102	-19%	116/120	<0.001	Fig 1B
daf-16(mu86) /Control RNAi	PA14	96.1±2.4	111		112/120		Fig 1B
daf-16(mu86) /*hsp-60* RNAi	PA14	77.1±1.7	88	-20%	87/120	<0.001	Fig 1B
daf-16(mu86) /*glrx-5* RNAi	PA14	74.8±2.1	88	-22%	107/120	<0.001	Fig 1B
daf-16(mu86) /*ZK669.4* RNAi	PA14	84.2±2.1	100	-16%	117/120	<0.001	Fig 1B
daf-16(mu86) /*idh-2* RNAi	PA14	87.4±2.4	111	-9%	116/120	<0.05	Fig 1B
daf-16(mu86) /Control RNAi	PA14	102.0±1.8	114		99/120		Fig 1B
daf-16(mu86) /*ads-1* RNAi	PA14	94.3±2.3	114	-8%	111/120	<0.001	Fig 1B
daf-16(mu86) /*lpd-5* RNAi	PA14	96.8±2.0	114	-5%	112/120	0.0722	Fig 1B
daf-16(mu86) /*alh-7* RNAi	PA14	98.5±2.3	114	-4%	92/120	0.0906	Fig 1B
daf-16(mu86) /*F53F4.10* RNAi	PA14	113.7±2.2	125	11%	113/120	0.4087	Fig 1B
daf-16(mu86) /*C32C12.8* RNAi	PA14	12.8±3.2	149	25%	103/120	<0.001	Fig 1B

daf-16(mu86) /Control RNAi	PA14	99.5±2.5	121		104/120		Fig 1B
daf-16(mu86) /*ads-1* RNAi	PA14	93.1±2.4	111	-6%	110/120	0.0553	Fig 1B
daf-16(mu86) /*lpd-5* RNAi	PA14	87.7±1.7	111	-12%	120/120	<0.001	Fig 1B
daf-16(mu86) /*alh-7* RNAi	PA14	99.5±2.3	121	0%	120/120	0.8827	Fig 1B
daf-16(mu86) /*F53F4.10* RNAi	PA14	84.6±1.8	95	-15%	117/120	<0.001	Fig 1B
daf-16(mu86) /*C32C12.8* RNAi	PA14	91.8±2.8	111	-8%	120/120	0.1850	Fig 1B
daf-16(mu86) /Control RNAi	PA14	103.0±2.5	120		110/120		Fig 1B
daf-16(mu86) /*lars-2* RNAi	PA14	102.9±2.9	120	0%	108/120	0.6444	Fig 1B
daf-16(mu86) /*mrps-5* RNAi	PA14	131.1±3.3	153	27%	103/120	<0.001	Fig 1B
daf-16(mu86) /*mrps-18c* RNAi	PA14	119.7±3.7	153	16%	96/120	<0.001	Fig 1B
daf-16(mu86) /*mrpl-22* RNAi	PA14	110.5±3.5	141	7%	96/120	<0.05	Fig 1B
daf-16(mu86) /*mfn-1* RNAi	PA14	122.9±3.5	141	19%	99/120	<0.001	Fig 1B
daf-16(mu86) /*mrps-35* RNAi	PA14	120.1±3.8	153	17%	107/120	<0.001	Fig 1B
daf-16(mu86) /Control RNAi	PA14	90.5±2.8	105		94/120		Fig 1B
daf-16(mu86) /*lars-2* RNAi	PA14	90.9±2.5	105	1%	100/120	0.5847	Fig 1B
daf-16(mu86) /*mrps-5* RNAi	PA14	112.2±3.6	139	24%	111/120	<0.001	Fig 1B
daf-16(mu86) /*mrps-18c* RNAi	PA14	104.4±3.8	129	15%	106/120	<0.01	Fig 1B
daf-16(mu86) /*mrpl-22* RNAi	PA14	96.4±3.4	117	7%	96/120	0.1677	Fig 1B
daf-16(mu86) /*mfn-1* RNAi	PA14	95.8±3.8	117	6%	104/120	0.3531	Fig 1B
daf-16(mu86) /*mrps-35* RNAi	PA14	104.5±3.4	129	16%	98/120	<0.01	Fig 1B

daf-16(mu86) /Control RNAi	PA14	114.3±2.0	132		85/90		Fig 1B
daf-16(mu86) /*dap-3* RNAi	PA14	132.1±3.6	147	16%	78/90	<0.001	Fig 1B
daf-16(mu86) /Control RNAi	PA14	113.0±2.4	130		90/90		Fig 1B
daf-16(mu86) /*dap-3* RNAi	PA14	128.8±3.8	155	14%	86/90	<0.001	Fig 1B

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival and *p* values of the animals treated with each RNAi clone were calculated against the animals treated with control RNAi. All the *p* values were calculated using the log-rank (Mantel-Cox method) test.

Appendix Table S2. The effects of *hsp-60* RNAi on the survival of *C. elegans* on various bacteria.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
Wild-type /control RNAi	PA14	109.2±2.5	130		113/120		Fig 2A
Wild-type /*hsp-60* RNAi	PA14	61.9±1.1	73	-43%	118/120	<0.001	Fig 2A
Wild-type /control RNAi	PA14	129.0±3.2	153		116/120		
Wild-type /*hsp-60* RNAi	PA14	75.2±1.1	92	-42%	118/125	<0.001	
Wild-type /control RNAi	PA14	150.2±4.2	169		80/90		
Wild-type /*hsp-60* RNAi	PA14	110.8±2.9	132	-26%	76/90	<0.001	
Wild-type /control RNAi	PA14	129.5±2.8	143		83/90		
Wild-type /*hsp-60* RNAi	PA14	93.3±2.2	106	-28%	89/90	<0.001	
[#]Wild-type /control RNAi	PA14	98.9±4.0	142		53/90		AF S2A
[#]Wild-type /*hsp-60* RNAi	PA14	77.9±2.1	104	-21%	82/90	<0.001	AF S2A
[#]Wild-type /*pmk-1* RNAi	PA14	40.5±0.8	-	-59%	85/90	<0.001	AF S2A
[#]Wild-type /control RNAi	PA14	94.6±4.5	126		66/90		
[#]Wild-type /*hsp-60* RNAi	PA14	83.7±2.3	102	-12%	72/90	<0.05	
[#]Wild-type /*pmk-1* RNAi	PA14	45.3±0.7	-	-52%	78/90	<0.001	
Wild-type /control RNAi	*E. coli* (HT115)	20.5±0.4	23		107/120		Fig 2G
Wild-type /*hsp-60* RNAi	*E. coli* (HT115)	19.2±0.3	22	-6%	114/120	<0.01	Fig 2G
Wild-type /control RNAi	*E. coli* (HT115)	15.7±0.3	20		108/150		

Wild type /*hsp-60* RNAi	*E. coli* (HT115)	14.9±0.3	17	-5%	112/150	<0.05	
[#]Wild-type /control RNAi	*E. faecalis*	5.9±0.2	7		43/120		Fig 2H and AF S2B
[#]Wild-type /*hsp-60* RNAi	*E. faecalis*	6.2±0.2	8	6%	80/120	0.1907	Fig 2H
[#]Wild-type /*pmk-1* RNAi	*E. faecalis*	3.7±0.2	5	-36%	61/120	<0.001	AF S2B
[#]Wild-type /control RNAi	*E. faecalis*	6.8±0.3	8		58/120		
[#]Wild-type /*hsp-60* RNAi	*E. faecalis*	5.9±0.2	7	-13%	104/120	<0.01	
[#]Wild-type /*pmk-1* RNAi	*E. faecalis*	4.5±0.2	6	-34%	74/120	<0.001	
Wild-type /control RNAi	Pathogenic *E. coli*	7.2±0.2	9		103/120		Fig 2I and AF S2C
Wild-type /*hsp-60* RNAi	Pathogenic *E. coli*	6.8±0.1	9	-6%	98/120	<0.001	Fig 2I
Wild-type /*pmk-1* RNAi	Pathogenic *E. coli*	5.2±0.2	7	-27%	88/120	<0.001	AF S2C
Wild-type /control RNAi	Pathogenic *E. coli*	8.5±0.2	10		120/120		
Wild-type /*hsp-60* RNAi	Pathogenic *E. coli*	7.8±0.1	10	-8%	94/120	<0.001	
Wild-type /*pmk-1* RNAi	Pathogenic *E. coli*	6.4±0.2	9	-24%	114/120	<0.001	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival and *p* values of the animals treated with *hsp-60* or *pmk-1* RNAi were calculated against the animals treated with control RNAi. All the *p* values were calculated using the log-rank (Mantel-Cox method) test. [#] indicates that survival analysis was performed without FUdR. AF indicates Appendix Figure.

Appendix Table S3. The effects of tissue-specific *hsp-60* RNAi on the survival of *C. elegans* exposed to PA14.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
rde-1(ne219) /control RNAi	PA14	98.0±2.2	116		80/90		Fig 3E
rde-1(ne219) /*hsp-60* RNAi	PA14	97.6±2.3	116	0%	88/90	0.9838	Fig 3E
rde-1(ne219) /control RNAi	PA14	92.6±2.0	102		75/90		
rde-1(ne219) /*hsp-60* RNAi	PA14	97.7±2.5	115	5%	61/90	0.1110	
rde-1(ne219); nhx-2p::rde-1 /control RNAi	PA14	129.9±2.9	142		75/90		Fig 3A
rde-1(ne219); nhx-2p::rde-1 /*hsp-60* RNAi	PA14	104.0±1.5	116	-20%	90/90	<0.001	Fig 3A
rde-1(ne219); nhx-2p::rde-1 /control RNAi	PA14	119.4±3.6	137		77/90		
rde-1(ne219); nhx-2p::rde-1 /*hsp-60* RNAi	PA14	101.0±2.0	115	-15%	78/90	<0.001	
rde-1(ne219); hlh-1p::rde-1 /control RNAi	PA14	105.9±3.6	128		57/60		Fig 3C
rde-1(ne219); hlh-1p::rde-1 /*hsp-60* RNAi	PA14	103.1±6.0	128	-3%	27/30	0.7746	Fig 3C
rde-1(ne219); hlh-1p::rde-1 /control RNAi	PA14	89.1±2.5	102		61/90		
rde-1(ne219); hlh-1p::rde-1 /*hsp-60* RNAi	PA14	81.5±2.9	90	-9%	52/90	0.0682	
rde-1(ne219); lin-26p::rde-1 /control RNAi	PA14	116.6±2.9	142		87/90		Fig 3D

rde-1(ne219); lin-26p::rde-1 /hsp-60 RNAi	PA14	111.0±3.2	128	-5%	85/90	0.3467	Fig 3D
rde-1(ne219); lin-26p::rde-1 /control RNAi	PA14	103.1±3.0	126		76/90		
rde-1(ne219); lin-26p::rde-1 /hsp-60 RNAi	PA14	97.9±3.3	115	-5%	80/90	0.4159	
sid-1(pk3321) /control RNAi	PA14	136.6±3.5	157		84/90		Fig 3F
sid-1(pk3321) /hsp-60 RNAi	PA14	134.9±3.6	157	-1%	86/90	0.9094	Fig 3F
sid-1(pk3321) /control RNAi	PA14	161.0±4.2	192		85/90		
sid-1(pk3321) /hsp-60 RNAi	PA14	151.2±4.2	181	-6%	79/90	0.1009	
sid-1(pk3321); unc-119p::sid-1 /control RNAi	PA14	123.0±4.0	143		75/90		Fig 3B
sid-1(pk3321); unc-119p::sid-1 /hsp-60 RNAi	PA14	83.9±1.8	95	-32%	86/90	<0.001	Fig 3B
sid-1(pk3321); unc-119p::sid-1 /control RNAi	PA14	139.8±4.7	169		70/90		
sid-1(pk3321); unc-119p::sid-1 /hsp-60 RNAi	PA14	84.3±1.8	95	-40%	73/90	<0.001	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival and *p* values of the animals treated with *hsp-60* RNAi were calculated against the animals treated with control RNAi. All the *p* values were calculated using the log-rank (Mantel-Cox method) test.

Appendix Table S4. The effects of genetic inhibition of UPR^{MT} components on the survival of *C. elegans* on PA14.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
Wild-type /control RNAi	PA14	91.1±1.6	96		111/120		Fig EV1A
Wild-type /*dve-1* RNAi	PA14	48.2±0.8	60	-47%	110/120	<0.001	Fig EV1A
Wild-type /control RNAi	PA14	77.6±2.2	91		108/120		
Wild-type /*dve-1* RNAi	PA14	42.0±0.8	54	-45%	113/120	<0.001	
Wild-type /control RNAi	PA14	115.2±2.0	126		106/120		Fig EV1B
Wild-type /*ubl-5* RNAi	PA14	96.6±2.6	117	-16%	116/120	<0.001	Fig EV1B
Wild-type /control RNAi	PA14	110.9±2.3	122		120/120		
Wild-type /*ubl-5* RNAi	PA14	88.7±3.5	107	-20%	113/120	<0.001	
Wild-type	PA14	96.5±2.9	126		102/120		Fig EV1C and EV1D
atfs-1(gk3094)	PA14	75.7±2.0	86	-22%	105/120	<0.001	Fig EV1C
haf-1(ok705)	PA14	93.0±2.8	110	-4%	108/120	0.3885	Fig EV1D
Wild-type	PA14	85.0±3.1	104		105/120		
atfs-1(gk3094)	PA14	67.8±2.3	82	-20%	79/101	<0.001	
haf-1(ok705)	PA14	78.0±2.5	101	-8%	100/120	<0.05	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival

and *p* values of the animals treated with RNAi targeting each of UPR^{MT} components were calculated against the animals treated with control RNAi. Percent (%) changes in mean survival and *p* values of mutant animals were calculated against wild-type (N2) animals. All the *p* values were calculated using the log-rank (Mantel-Cox method) test.

Appendix Table S5. The effects of *hsp-60* RNAi on the survival of *pmk-1*, *zip-2*, and *daf-16* mutant animals on PA14.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
pmk-1(km25) /control RNAi	PA14	51.0±1.1	65		111/120		Fig 1B and 4G
pmk-1(km25) /*hsp-60* RNAi	PA14	50.9±0.9	65	0%	119/120	0.4613	Fig 1B and 4G
pmk-1(km25) /control RNAi	PA14	57.3±1.3	73		113/120		
pmk-1(km25) /*hsp-60* RNAi	PA14	49.1±1.1	65	-14%	117/120	<0.001	
pmk-1(km25) /control RNAi	PA14	47.3±1.6	56		120/120		Fig 1B
pmk-1(km25) /*hsp-60* RNAi	PA14	61.0±1.4	73	29%	120/120	<0.001	Fig 1B
zip-2(tm4067) /control RNAi	PA14	91.1±2.4	100		77/90		Fig 1B and 4H
zip-2(tm4067) /*hsp-60* RNAi	PA14	64.3±0.8	72	-30%	114/120	<0.001	Fig 1B and 4H
zip-2(tm4067) /control RNAi	PA14	88.2±1.9	93		115/120		Fig 1B
zip-2(tm4067) /*hsp-60* RNAi	PA14	75.5±1.0	82	-15%	113/120	<0.001	Fig 1B
zip-2(tm4067) /control RNAi	PA14	103.2±2.1	119		117/120		
zip-2(tm4067) /*hsp-60* RNAi	PA14	49.8±1.9	65	-52%	113/120	<0.001	
daf-16(mu86) /control RNAi	PA14	104.8±1.7	113		117/120		Fig 1B and 4I
daf-16(mu86) /*hsp-60* RNAi	PA14	75.8±1.2	93	-28%	112/120	<0.001	Fig 1B and 4I
daf-16(mu86) /control RNAi	PA14	96.1±2.4	111		112/120		Fig 1B
daf-16(mu86) /*hsp-60* RNAi	PA14	77.1±1.7	88	-20%	87/120	<0.001	Fig 1B

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival and *p* values of the animals treated with *hsp-60* RNAi were calculated against the animals treated with control RNAi. All the *p* values were calculated using the log-rank (Mantel-Cox) test.

Appendix Table S6. The effects of *hsp-60* RNAi on the survival of *tir-1* gain-of-function (gf) mutant animals on PA14.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
Wild-type /control RNAi	PA14	120.1±2.2	130		116/120		Fig 5C
Wild-type /*hsp-60* RNAi	PA14	73.5±1.2	83	-39%	115/120	<0.001	Fig 5C
tir-1(yz68gf) /control RNAi	PA14	138.5±3.3	157	15%	100/120	<0.001	Fig 5C
tir-1(yz68gf) /*hsp-60* RNAi	PA14	81.9±1.0	101	-41% (12%$^{hsp-60i}$)	91/120	<0.001 (<0.001 $^{hsp-60i}$)	Fig 5C
Wild-type /control RNAi	PA14	101.6±2.4	115		82/90		
Wild-type /*hsp-60* RNAi	PA14	91.5±1.5	105	-10%	86/90	<0.001	
tir-1(yz68gf) /control RNAi	PA14	126.0±2.6	152	24%	76/90	<0.001	
tir-1(yz68gf) /*hsp-60* RNAi	PA14	83.8±1.4	92	-34% (-8%$^{hsp-60i}$)	84/90	<0.001 (<0.001 $^{hsp-60i}$)	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival and *p* values of the animals treated with *hsp-60* RNAi were calculated against the animals treated with control RNAi. Percent (%) changes in mean survival and *p* values of *tir-1(gf)* animals treated with *hsp-60* RNAi were obtained against *tir-1(gf)* animals treated with control RNAi. *p* values in parentheses were calculated against wild-type animals treated with *hsp-60* RNAi ($^{hsp-60i}$), and $\%^{hsp-60i}$ indicates percent (%) changes in mean survival compared to wild-type animals treated with *hsp-60* RNAi. All the *p* values were calculated using the log-rank (Mantel-Cox) test.

Appendix Table S7. The effects of *hsp-60::GFP* on the survival of *C. elegans* on PA14.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
Wild-type	PA14	119.1±2.4	130		84/90		Fig 6G
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	183.9±5.5	224	54%	77/90	<0.001	Fig 6G
hsp-60p::hsp-60::GFP; odr-1p:: RFP #2	PA14	174.2±5.8	211	46%	73/90	<0.001	Fig 6G
Wild-type	PA14	121.5±3.0	145		77/90		
hsp-60p::hsp-60::GFP; odr-1p:: RFP #1	PA14	196.9±8.2	254	62%	78/90	<0.001	
hsp-60p::hsp-60::GFP; odr-1p:: RFP #2	PA14	161.2±6.4	194	33%	78/89	<0.001	
odr-1p::RFP	PA14	122.1±2.0	134		111/120		Fig EV3E
hsp-60p::hsp-60::GFP; odr-1p:: RFP #3	PA14	184.9±8.4	227	51%	41/50	<0.001	Fig EV3E
hsp-60p::hsp-60::GFP; odr-1p:: RFP #4	PA14	137.5±3.7	155	13%	116/120	<0.001	Fig EV3E
hsp-60p::hsp-60::GFP; odr-1p:: RFP #5	PA14	147.1±4.6	167	21%	79/90	<0.001	Fig EV3E
odr-1p::RFP	PA14	92.6±2.7	112		114/120		
hsp-60p::hsp-60::GFP; odr-1p:: RFP #3	PA14	156.8±5.4	195	69%	74/90	<0.001	
hsp-60p::hsp-60::GFP; odr-1p:: RFP #4	PA14	109.7±5.4	122	18%	49/50	0.0034	

hsp-60p::hsp-60::GFP; odr-1p:: RFP #5	PA14	120.7±9.1	145	30%	23/25	<0.001	
Wild-type /control RNAi	PA14	135.3±4.1	165		85/90		Fig 6H
Wild-type /*hsp-60* RNAi	PA14	74.4±1.7	92	-45%	88/90	<0.001	Fig 6H
hsp-60p::hsp-60::GFP; odr-1p::RFP #1 /control RNAi	PA14	208.5±7.9	262	54%	71/90	<0.001	Fig 6H
hsp-60p::hsp-60::GFP #1 /*hsp-60* RNAi	PA14	78.5±2.4	92	-62% (5%$^{hsp\text{-}60i}$)	86/90	<0.001 (0.0221$^{hsp\text{-}60i}$)	Fig 6H
Wild-type /control RNAi	PA14	101.7±2.9	120		78/90		
Wild-type /*hsp-60* RNAi	PA14	78.4±1.3	95	-23%	85/90	<0.001	
hsp-60p::hsp-60::GFP; odr-1p::RFP #1 /control RNAi	PA14	152.8±4.6	191	50%	80/89	<0.001	
hsp-60p::hsp-60::GFP; odr-1p::RFP #1 /*hsp-60* RNAi	PA14	82.1±2.5	95	-46% (5%$^{hsp\text{-}60i}$)	55/60	<0.001 (0.0464$^{hsp\text{-}60i}$)	
Day 6 wild-type	PA14	66.4±4.0	81		79/89		Fig 6J
Day 6 *hsp-60p::hsp-60::GFP; odr-1p::RFP* #1	PA14	115.4±7.0	152	74%	76/90	<0.001	Fig 6J
Day 6 wild-type	PA14	76.6±4.7	104		77/90		
Day 6 *hsp-60p::hsp-60::GFP; odr-1p::RFP* #1	PA14	96.5±6.7	143	26%	74/90	0.0108	
fasn-1p::GFP	PA14	91.7±2.5	106		85/90		Fig EV3F
hsp-60p::hsp-60; fasn-1p::GFP #1	PA14	102.4±2.9	129	12%	82/90	<0.01	Fig EV3F

hsp-60p::hsp-60; fasn-1p::GFP #2	PA14	113.0±3.0	129	23%	74/90	<0.001	Fig EV3F
fasn-1p::GFP	PA14	107.6±2.5	122		81/90		
hsp-60p::hsp-60; fasn-1p::GFP #1	PA14	117.7±3.4	136	9%	78/90	<0.01	
hsp-60p::hsp-60; fasn-1p::GFP #2	PA14	122.7±3.3	147	14%	78/90	<0.001	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival and *p* values of transgenic animals were calculated against control animals. Percent (%) changes in mean survival and *p* values of *hsp-60p::hsp-60::GFP* animals treated with *hsp-60* RNAi were obtained against *hsp-60p::hsp-60::GFP* animals treated with control RNAi. All the *p* values were calculated using the log-rank (Mantel-Cox method) test.

Appendix Table S8. The effects of transgenic expression of *hsp-60::GFP* on the lifespan of wild-type *C. elegans*.

Strain /treatment	Bacteria	Mean lifespan ±s.e.m. (days)	Days at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
Wild-type	*E. coli* (OP50)	18.7±0.4	22		101/120		Fig 6I
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	*E. coli* (OP50)	19.3±0.6	24	3%	82/120	0.1292	Fig 6I
hsp-60p::hsp-60::GFP; odr-1p::RFP #2	*E. coli* (OP50)	16.9±0.4	19	-10%	80/120	0.0024	Fig 6I
Wild-type	*E. coli* (OP50)	19.9±0.4	23		108/120		
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	*E. coli* (OP50)	19.2±0.6	23	-4%	73/120	0.8126	
hsp-60p::hsp-60::GFP; odr-1p::RFP #2	*E. coli* (OP50)	18.2±0.5	21	-9%	74/90	0.0400	
odr-1p::RFP	*E. coli* (OP50)	18.7±0.4	23		105/120		Fig EV3G
hsp-60p::hsp-60::GFP; odr-1p::RFP #3	*E. coli* (OP50)	16.9±0.5	21	-10%	78/120	<0.001	Fig EV3G
odr-1p::RFP	*E. coli* (OP50)	17.8±0.5	21		71/90		
hsp-60p::hsp-60::GFP; odr-1p::RFP #3	*E. coli* (OP50)	16.3±0.6	21	-8%	71/90	0.2187	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean lifespan and *p* values of transgenic animals that express *hsp-60p::hsp-60::GFP* were calculated against control animals. All the *p* values were calculated using the log-rank (Mantel-Cox method) test.

Appendix Table S9. The effects of transgenic expression of mitochondrial GFP (*GFP^{MT}*) on the survival of *C. elegans* on PA14.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
odr-1p::RFP	PA14	100.0±2.4	116		81/90		AF S3D
hsp-60p::GFP^{MT}; odr-1p::RFP #1	PA14	96.7±2.2	116	-3%	80/90	0.3508	AF S3D
hsp-60p::GFP^{MT}; odr-1p::RFP #2	PA14	98.8±2.4	116	-1%	81/90	0.7841	AF S3D
hsp-60p::GFP^{MT}; odr-1p::RFP #3	PA14	105.0±2.5	116	5%	79/90	0.1346	AF S3D
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	155.1±6.2	213	55%	60/90	<0.001	AF S3D
odr-1p::RFP	PA14	95.0±2.6	115		85/90		
hsp-60p::GFP^{MT}; odr-1p::RFP #1	PA14	101.4±2.4	115	7%	84/90	0.1693	
hsp-60p::GFP^{MT}; odr-1p::RFP #2	PA14	90.3±2.3	115	-5%	87/90	0.1171	
hsp-60p::GFP^{MT}; odr-1p::RFP #3	PA14	97.0±2.9	115	2%	85/90	0.5348	
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	118.0±4.9	134	24%	82/90	<0.001	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival

and *p* values of transgenic animals were calculated against control animals. All the *p* values were calculated using the log-rank (Mantel-Cox method) test. AF indicates Appendix Figure.

Appendix Table S10. The effects of transgenic expression of *hsp-60::GFP* on the survival of *pmk-1*, *sek-1*, *nsy-1*, and *tir-1* mutant animals on PA14.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
Wild-type	PA14	121.7±2.5	139		89/90		Fig 7F
pmk-1(km25)	PA14	54.1±0.7	68	-56%	87/90	<0.001	Fig 7F
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	190.2±5.1	222	56%	61/88	<0.001	Fig 7F
pmk-1(km25); hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	67.0±1.9	80	-65% (24%$^{pmk-1}$)	89/90	<0.001 (<0.001^{pmk-1})	Fig 7F
Wild-type	PA14	133.7±3.8	153		81/90		
pmk-1(km25)	PA14	59.9±1.2	68	-55%	90/90	<0.001	
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	187.1±7.4	237	40%	58/81	<0.001	
pmk-1(km25); hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	77.9±2.2	91	-58% (30%$^{pmk-1}$)	85/90	<0.001 (<0.001^{pmk-1})	
Wild-type	PA14	126.9±2.5	140		84/90		Fig 7G
sek-1(km4)	PA14	41.1±0.9	54	-68%	89/90	<0.001	Fig 7G
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	174.6±5.9	212	38%	77/90	<0.001	Fig 7G
sek-1(km4); hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	46.9±1.6	68	-73% (14%$^{sek-1}$)	90/90	<0.001	Fig 7G

Wild-type	PA14	144.9±2.5	165		80/90		
sek-1(km4)	PA14	17.6±2.0	54	-88%	90/90	<0.001	
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	187.3±8.1	214	29%	44/90	<0.001	
sek-1(km4); hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	33.0±2.5	54	-82% (88%$^{sek-1}$)	90/90	<0.001 (<0.001^{sek-1})	
Wild-type	PA14	128.9±3.5	143		83/90		Fig 7H
nsy-1(ok293)	PA14	68.4±2.1	84	-47%	85/90	<0.001	Fig 7H
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	162.3±8.3	229	26%	61/90	<0.001	Fig 7H
nsy-1(ok293); hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	110.4±4.5	133	-32% (61% $^{nsy-1}$)	68/90	<0.001 (<0.001^{nsy-1})	Fig 7H
Wild-type	PA14	139.5±3.0	156		83/90		
nsy-1(ok293)	PA14	75.1±1.9	108	-46%	85/90	<0.001	
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	172.1±6.5	252	23%	73/90	<0.001	
nsy-1(ok293); hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	108.7±3.7	134	-37% (45%$^{nsy-1}$)	81/90	<0.001 (<0.001^{nsy-1})	
Wild-type	PA14	120.0±2.1	129		88/90		Fig 7I
tir-1(tm3036)	PA14	61.2±1.6	77	-49%	88/90	<0.001	Fig 7I
hsp-60p::hsp-60::GFP; odr-1p::RFP	PA14	204.9±7.6	284	71%	67/85	<0.001	Fig 7I
tir-1(tm3036); hsp-60p::hsp-60::GFP; odr-1p::RFP	PA14	126.9±5.0	176	-38% (107%$^{tir-1}$)	50/90	<0.001 (<0.001^{tir-1})	Fig 7I

Wild-type	PA14	108.1±2.0	119		85/90		
tir-1(tm3036)	PA14	48.8±0.7	57	-55%	90/90	<0.001	
hsp-60p::hsp-60::GFP; odr-1p::RFP	PA14	178.1±4.8	201	65%	84/90	<0.001	
tir-1(tm3036); hsp-60p::hsp-60::GFP; odr-1p::RFP	PA14	97.4±4.0	106	-45% (83%$^{tir-1}$)	81/90	<0.001 (<0.001^{tir-1})	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival and *p* values of transgenic animals were calculated against control animals. Percent (%) changes in mean survival and *p* values of *pmk-1*, *sek-1*, *nsy-1*, or *tir-1* mutants that expressed *hsp-60p::hsp-60::GFP* were obtained against wild-type that expressed *hsp-60p::hsp-60::GFP*. *p* values in parentheses were calculated against wild-type animals treated with *hsp-60* RNAi ($^{hsp-60i}$), or *pmk-1* ($^{pmk-1}$), *sek-1* ($^{sek-1}$), *nsy-1* ($^{nsy-1}$) or *tir-1* ($^{tir-1}$) mutant animals. %$^{pmk-1}$, %$^{sek-1}$, %$^{nsy-1}$ and %$^{tir-1}$ indicate percent (%) changes in mean survival compared to *pmk-1*, *sek-1*, *nsy-1* and *tir-1* mutants, respectively. All the *p* values were calculated using the log-rank (Mantel-Cox method) test.

Appendix Table S11. The effects of transgenic expression of cytosol-specific *hsp-60::GFP* (*cythsp-60::GFP*) on survival upon PA14 infection.

Strain /treatment	Bacteria	Mean survival ±s.e.m. (hours)	Hours at 75% mortality	% change	Number of animals that died/total	*p* value vs. control	Figure in text
odr-1p::RFP	PA14	95.9±2.4	113		78/90		Fig 9F
hsp-60p::hsp-60::GFP; odr-1p::RFP #1	PA14	134.6±6.3	182	41%	74/90	<0.001	Fig 9F
hsp-60p::cythsp-60::GFP; odr-1p::RFP #1	PA14	144.8±5.8	169	51%	76/90	<0.001	Fig 9F
hsp-60p::cythsp-60::GFP; odr-1p::RFP #2	PA14	124.4±4.7	161	30%	70/90	<0.001	Fig 9F
hsp-60p::cythsp-60::GFP; odr-1p::RFP #3	PA14	131.3±5.0	145	37%	68/90	<0.001	Fig 9F
odr-1p::RFP	PA14	87.7±2.4	97		83/90		
hsp-60p::cythsp-60::GFP; odr-1p::RFP #1	PA14	197.0±6.5	239	125%	77/90	<0.001	
hsp-60p::cythsp-60::GFP; odr-1p::RFP #2	PA14	188.7±6.3	239	115%	78/90	<0.001	
hsp-60p::cythsp-60::GFP; odr-1p::RFP #3	PA14	156.1±4.8	185	78%	84/90	<0.001	

The experiments for the survival data within the solid line were done in parallel. Statistical analysis was done for the data sets within the solid line. Percent (%) changes in mean survival and *p* values of transgenic animals were calculated against control animals. All the *p* values were calculated using the log-rank (Mantel-Cox method) test.

Appendix References

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* **25:** 25-29

Bennett CF, Vander Wende H, Simko M, Klum S, Barfield S, Choi H, Pineda VV, Kaeberlein M (2014) Activation of the mitochondrial unfolded protein response does not predict longevity in *Caenorhabditis elegans*. *Nat Commun* **5**: 3483

Chen YC, Wu YT, Wei YH (2015) Depletion of mitoferrins leads to mitochondrial dysfunction and impairment of adipogenic differentiation in 3T3-L1 preadipocytes. *Free Radic Res* **49:** 1285-1295

Couillault C, Pujol N, Reboul J, Sabatier L, Guichou JF, Kohara Y, Ewbank JJ (2004) TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. *Nat Immunol* **5:** 488-494

Gene Ontology Consortium (2015) Gene Ontology Consortium: going forward. *Nucleic Acids Res* **43:** D1049-1056

Houtkooper RH, Mouchiroud L, Ryu D, Moullan N, Katsyuba E, Knott G, Williams RW, Auwerx J (2013) Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature* **497**: 451-7

Kim DH (2013) Bacteria and the aging and longevity of *Caenorhabditis elegans*. *Annu Rev*

Genet **47:** 233-246

Kulawiak B, Hopker J, Gebert M, Guiard B, Wiedemann N, Gebert N (2013) The mitochondrial protein import machinery has multiple connections to the respiratory chain. *Biochim Biophys Acta* **1827:** 612-626

Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM (2012) Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science* **337:** 587-590

Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, Hill DE, Vidal M, Evans JG, Thorburn DR, Carr SA, Mootha VK (2008) A mitochondrial protein compendium elucidates complex I disease biology. *Cell* **134:** 112-123

Pellegrino MW, Nargund AM, Haynes CM (2013) Signaling the mitochondrial unfolded protein response. *Biochim Biophys Acta* **1833:** 410-416

Pellegrino MW, Nargund AM, Kirienko NV, Gillis R, Fiorese CJ, Haynes CM (2014) Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. *Nature* **516:** 414-417

Pujol N, Zugasti O, Wong D, Couillault C, Kurz CL, Schulenburg H, Ewbank JJ (2008) Anti-fungal innate immunity in *C. elegans* in enhanced by evolutionary diversification of antimicrobial peptides. *PLoS Pathog* **4**:e1000105

Rohlfing AK, Miteva Y, Hannenhalli S, Lamitina T (2010) Genetic and physiological activation of osmosensitive gene expression mimics transcriptional signatures of pathogen

infection in *C. elegans*. *PLoS One* **5**(2): e9010

Rohlfing AK, Miteva Y, Moronetti L, He L, Lamitina T (2011) The *Caenorhabditis elegans* mucin-like protein OSM-8 negatively regulates osmosensitive physiology via the transmembrane protein PTR-23. *PLoS Genet* **7**: e1001267

Yoneda T, Benedetti C, Urano F, Clark SG, Harding HP, Ron D (2004) Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. *J Cell Sci* **117:** 4055-4066

Zugasti O, Thakur N, Belougne J, Squiban B, Kurz L, Soule J, Omi S, Tichit L, Pujol N, Ewbank JJ (2016) A quantitative genome-wide RNAi screen in *C. elegans* for antifungal innate immunity genes. *BMC Biol* **14**:35