Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection

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Metazoans identify and eliminate bacterial pathogens in microbe-rich environments such as the intestinal lumen; however, the mechanisms are unclear. Host cells could potentially use intracellular surveillance or stress response programs to detect pathogens that target monitored cellular activities and then initiate innate immune responses1–3. Mitochondrial function is evaluated by monitoring mitochondrial protein import efficiency of the transcription factor ATFS-1, which mediates the mitochondrial unfolded protein response (UPRmt). During mitochondrial stress, mitochondrial import is impaired4, allowing ATFS-1 to traffic to the nucleus where it mediates a transcriptional response to re-establish mitochondrial homeostasis5. Here we examined the role of ATFS-1 in Caenorhabditis elegans during pathogen exposure, because during mitochondrial stress ATFS-1 induced not only mitochondrial protective genes but also innate immune genes that included a secreted lysozyme and anti-microbial peptides. Exposure to the pathogen Pseudomonas aeruginosa caused mitochondrial dysfunction and activation of the UPRmt. C. elegans lacking atfs-1 were susceptible to P. aeruginosa, whereas hyper-activation of ATFS-1 and the UPRmt improved clearance of P. aeruginosa from the intestine and prolonged C. elegans survival in a manner mainly independent of known innate immune pathways6–8. We propose that ATFS-1 import efficiency and the UPRmt are a means to detect pathogens that target mitochondria and initiate a protective innate immune response.

Animals harbour bacteria that are essential for normal physiology9; however, they must distinguish between commensal and pathogenic microbes to maintain homeostasis. Pathogenic bacteria can be recognized directly or by damage inflicted by the pathogen10 leading to activation of innate immune responses that limit pathogen growth. Recently it has been demonstrated that perturbations to protein synthesis, proteolysis or mitochondrial activity are sufficient to activate innate immune responses, suggesting the elegant hypothesis that host cells use intracellular stress responses to initiate innate immunity programs when pathogens perturb monitored cellular processes1–3.

Cells respond to mitochondrial dysfunction by activating the UPRmt, which is regulated by the transcription factor ATFS-1. In healthy cells, ATFS-1 is efficiently imported into mitochondria and degraded. However, during mitochondrial stress, mitochondrial import efficiency is reduced4–6, allowing a small percentage of ATFS-1 to accumulate in the cytosol. Because ATFS-1 has a nuclear localization sequence (NLS), it then traffics to the nucleus where it activates a protective transcriptional response (Fig. 1a). Our expression profiling studies indicated that ATFS-1 induces genes that promote mitochondrial protein folding, reactive oxygen species (ROS) detoxification and mitochondrial protein import, suggesting the UPRmt stabilizes the mitochondrial protein folding environment to promote organellar homeostasis.

Intriguingly, a number of transcripts induced during mitochondrial stress caused by inhibition of the mitochondrial protease SPG-7 encode innate immunity proteins11 (Extended Data Table 1), some of which were also found to be induced following exposure to the pathogen P. aeruginosa12 (Fig. 1b and Extended Data Table 2). The antimicrobial peptide abf-2 and the secreted lysozyme lys-2, both of which are required for resistance to pathogen infection13,14, were induced during mitochondrial stress (Fig. 1c, d), as were two C-type lectins, which are involved in pathogen recognition15 (Fig. 1e, f). Mitochondrial-specific stress also caused induction of antimicrobial peptides14 in mammalian cells (Fig. 1g–j), suggesting the response is conserved. In C. elegans, induction of innate immune genes by spg-7 RNA interference (spg-7(RNAi)) required ATFS-1 (Fig. 1c–f). Thus, in addition to inducing mitochondrial-protective genes, ATFS-1 also transcriptionally upregulated innate immune genes during mitochondrial stress. Therefore we hypothesized that ATFS-1 and the UPRmt are involved in regulating innate immunity during exposure to pathogens that perturb mitochondrial function.

P. aeruginosa produces virulence factors that target many cellular functions including the mitochondrial toxins cyanide and pyocyanin16,17. P. aeruginosa also produces exotoxin A, which impairs protein synthesis and leads to the induction of the innate immune gene irg-1 via the transcription factor ZIP-2 (refs 2, 3, 17). Mitochondrial stress also caused irg-1pe::gfp (pr, promoter) induction, which was blocked in atfs-1(tm4919) and partially so in zip-2(tm4248) worms (Fig. 1k), suggesting that multiple transcription factors and stressors influence innate immune gene expression. zip-2 mRNA was also induced during mitochondrial stress, which also required atfs-1 (Fig. 1l). F35E12.5, which is induced by the MAP kinase PMK-1 and the transcription factor ATF-7 during P. aeruginosa exposure18–20, was not induced during mitochondrial stress (Extended Data Fig. 1a). Thus, ATFS-1 regulates a subset of innate immune genes during mitochondrial stress in addition to its cytoprotective role in promoting mitochondrial homeostasis.

We next examined if P. aeruginosa exposure caused mitochondrial stress capable of activating the UPRmt. Slow-killing conditions were used in which the pathogen accumulates in the intestine leading to infection19. Interestingly, P. aeruginosa exposure caused intestinal cell mitochondria to elongate similar to spg-7(RNAi) treatment (Fig. 2a), consistent with the pathogen causing mitochondrial stress, and mitochondrial fusion providing protection20. Exposure to P. aeruginosa also caused striking developmental delays in combination with mild mitochondrial stresses such as ethidium bromide21, paraquat or the clk-1(qm30) allele22 (Fig. 2b), consistent with the pathogen causing modest mitochondrial stress. Importantly, P. aeruginosa exposure caused an atfs-1-dependent increase in mitochondrial chaperone reporter (hsp-6 and hsp-60pe::gfp) activation in the intestine (Fig. 2c and Extended Data Fig. 1b), which correlated with increased nuclear accumulation of ATFS-1:GFP and required the NLS in ATFS-1 (Fig. 2d and Extended Data Fig. 1c, d). Exposure to P. aeruginosa liquid-killing conditions, which requires pathogen-expressed iron chelating siderophores23, also induced mitochondrial chaperone genes, suggesting multiple P. aeruginosa virulence factors can activate the UPRmt (Extended Data Fig. 2a, b). Interestingly, both synthetic growth arrest and UPRmt activation required the P. aeruginosa global virulence activator gene gacA23 (Fig. 2b, c). Furthermore, exposure to P. aeruginosa strains lacking individual siderophore, pyocyanin or cytochrome c genes resulted in less UPRmt activation than wild-type P. aeruginosa.
(Extended Data Fig. 2d, e), suggesting that multiple pathogen toxins target mitochondrial function resulting in UPR\textsuperscript{mt} activation. However, UPR\textsuperscript{mt} activation may also be due to indirect damage associated with activation of a separate immune response\textsuperscript{44}.

We examined the role of ATFS-1 in the induction of innate immune genes during \textit{P. aeruginosa} exposure rather than specifically during mitochondrial stress. Similarly, abf-2, lys-2, clec-4 and clec-65 were induced upon \textit{P. aeruginosa} exposure independent of exogenous mitochondrial stress, which also required \textit{atfs-1} (Fig. 2e–h). Similar to the mitochondrial chaperones, both \textit{lys-2} and \textit{igf-1} expression were impaired in both \textit{atfs-1} knockout (Fig. 2i and Extended Data Fig. 3). Interestingly, increased \textit{igf-1} expression was impaired in both \textit{atfs-1} knockout and \textit{zip-2} (Extended Data Fig. 2i). Furthermore, \textit{zip-2} transcript induction on \textit{P. aeruginosa} was also partially impaired in \textit{atfs-1} mutant worms, suggesting \textit{atfs-1} can function upstream of \textit{zip-2} (Extended Data Fig. 4a).

Consistent with a role for ATFS-1 in inducing innate immune and mitochondrial protective genes\textsuperscript{5}, the survival of worms raised on \textit{atfs-1(RNAi)} was significantly reduced when exposed to \textit{P. aeruginosa}, but not \textit{E. coli} (Fig. 3a, b). \textit{atfs-1(RNAi)} treated worms were also susceptible to \textit{P. aeruginosa} liquid-killing (Extended Data Fig. 2c), supporting a role for ATFS-1 in activating a protective transcriptional response to pathogen exposure. RNAi was used to reduce \textit{atfs-1} activity for the survival studies rather than \textit{atfs-1(RNAi)} because of germline defects that complicate the analysis (Extended Data Fig. 4b, c).

We examined if UPR\textsuperscript{mt} activation is sufficient to protect against \textit{P. aeruginosa}. The UPR\textsuperscript{mt} was induced by allowing worms to develop on \textit{spg-7(RNAi)} for two days before pathogen exposure. UPR\textsuperscript{mt} pre-activation dramatically reduced the intestinal accumulation of \textit{P. aeruginosa} expressing GFP (\textit{P. aeruginosa}–GFP\textsuperscript{5}) (Fig. 3c, d). Importantly, \textit{P. aeruginosa}–GFP accumulated in the intestine of \textit{atfs-1(RNAi)} worms following \textit{spg-7(RNAi)} treatment indicating that UPR\textsuperscript{mt} activation promotes pathogen clearance. In addition to adapting transcription, worms are also able to avoid \textit{P. aeruginosa}, which was unaffected by \textit{atfs-1} knockout or pre-treatment with \textit{spg-7(RNAi)} (Extended Data Fig. 5a–e). Consistent with increased pathogen clearance via anti-microbial gene induction, UPR\textsuperscript{mt} pre-activation prolonged the survival of animals challenged with \textit{P. aeruginosa}, which required \textit{atfs-1} (Fig. 3e) and was independent of germline defects or feeding behaviour (Extended Data Fig. 5f, g).

Because mitochondrial stress can activate multiple stress response pathways in addition to the UPR\textsuperscript{mt} (refs 25, 26), we examined an \textit{atfs-1} gain-of-function mutant, which constitutively activates the UPR\textsuperscript{mt} independent of mitochondrial dysfunction. \textit{atfs-1(\textit{et}18)} worms express ATFS-1 with an amino acid substitution in the mitochondria targeting sequence that reduces mitochondrial import efficiency causing constitutive UPR\textsuperscript{mt} activation\textsuperscript{27} and innate immune induction (Extended Data Fig. 6a–e). We observed that \textit{atfs-1(\textit{et}18)} worms accumulated less \textit{P. aeruginosa}–GFP in the intestine (Fig. 3f, g) and survived longer than wild-type worms (Fig. 3h) indicating that UPR\textsuperscript{mt} activation is sufficient to provide resistance to \textit{P. aeruginosa}. Importantly, \textit{atfs-1(RNAi)} and \textit{lys-2(RNAi)} reduced \textit{atfs-1(\textit{et}18)} worm survival (Fig. 3h and Extended Data Fig. 6f), suggesting that ATFS-1-mediated innate immune gene induction provides resistance to \textit{P. aeruginosa}.

Inhibition of additional cellular activities including translation (\textit{etf-2}, also known as \textit{ef}-2), mRNA splicing (\textit{T08A11.2}), calcium transport (\textit{sla-1}) and the pentose phosphate pathway (\textit{T25B9.9}) also induce innate immune gene expression\textsuperscript{23} but do not induce the UPR\textsuperscript{mt} (Extended Data Fig. 7a, b). Thus, we examined if other stress-activated innate immune responses are also protective against \textit{P. aeruginosa}. Knockdown of \textit{etf-2}, \textit{T25B9.9}, \textit{sla-1} or \textit{T08A11.2} did not increase survival on \textit{P. aeruginosa} (Extended Data Fig. 7c), however, \textit{sla-1(RNAi)} and \textit{T08A11.2(RNAi)} decreased lifespan on \textit{E. coli}, indicating a reduction in general fitness (Extended Data Fig. 7d).
**Figure 3 | UPRmt activation provides resistance to P. aeruginosa.**

![Graph and images showing survival of worms and fluorescence units.](image)

**Figure 4 | UPRmt activation prolongs survival independently of innate immune pathways.**

![Graph and images showing survival of worms and fluorescence units.](image)

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The UPRmt pathway mediated by NSY-1/SEK-1/PMK-1 (refs 6, 7, 10), the MLK-1/MLK-8/PMK-1 pathway, or the MLK-1-space-1 pathway, all contribute to the survival of wild-type and atfs-1(RNAi) worms on PQ (ref. 17). Interestingly, pre-activation of the UPRmt enhanced the survival of the pmk-1 and sek-1 mutants (Fig. 4a, b), as well as the kgb-1 and mlk-1 mutants (Fig. 4c, d). Of note, increased survival by spg-7(RNAi) was further enhanced in kgb-1 and mlk-1 worms, consistent with kgb-1 being a negative regulator of the UPRmt (ref. 29) (Extended Data Fig. 7e). In contrast, knockdown of the mitochondrial ATP synthase subunit atp-2, which activates mitochondrial protective and innate immune gene expression (Extended Data Fig. 7a, b), prolonged survival during P. aeruginosa exposure (Extended Data Fig. 7c). Our data suggest the UPRmt provides protection from P. aeruginosa by coupling mitochondrial-protective and antimicrobial gene expression.

Lastly, we determined if ATFS-1 and the UPRmt interacted with established C. elegans innate immune pathways, which include a MAP kinase pathway mediated by NSY-1/SEK-1/PMK-1 (refs 6, 7, 10), the MLK-1/MLK-8/PMK-1 pathway, and the JUN pathway (ref. 17). Interestingly, pre-activation of the UPRmt enhanced the survival of the pmk-1 and sek-1 mutants (Fig. 4a, b), as well as the kgb-1 and mlk-1 mutants (Fig. 4c, d). Of note, increased survival by spg-7(RNAi) was further enhanced in kgb-1 and mlk-1 worms, consistent with kgb-1 being a negative regulator of the UPRmt (ref. 29) (Extended Data Fig. 7e). In contrast, knockdown of the mitochondrial ATP synthase subunit atp-2, which activates mitochondrial protective and innate immune gene expression (Extended Data Fig. 7a, b), prolonged survival during P. aeruginosa exposure (Extended Data Fig. 7c). Our data suggest the UPRmt provides protection from P. aeruginosa by coupling mitochondrial-protective and antimicrobial gene expression.
contrast, zip-2(tm4248) modestly reduced the enhanced resistance conferred by sgg-7(RNAi) (Fig. 4e), consistent with atfs-1 functioning in the same pathway as zip-2 during mitochondrial stress. Together, our data suggest that the UPRmt can function independently of the MAP and c-Jun kinase regulated innate immune pathways.

Our studies indicate that the UPRmt is activated by and protects against *P. aeruginosa*, and thus support a mechanistic means by which host cells can detect pathogens that target mitochondrial function (Fig. 4f), which is consistent with only a subset of bacterial species inducing the UPRmt (ref. 30). Because ATFS-1 responds directly to mitochondrial dys-function and induces a transcriptional response that is both mitochondrial protective and antimicrobial, the UPRmt is a uniquely positioned pathway to mitigate mitochondrial damage stemming from genetic defects or pathogen exposure (Fig. 4f).

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.M.H (haynesc@mskcc.org).
METHODS

Worm and bacterial strains. The atfs-1 (tm4919) mutant strain was a gift from the National BioResource Project and backcrossed twice to wild-type N2 worms. Worm strains were provided by the Caenorhabditis Genetics Center unless otherwise noted. Hermaphrodite worms were raised on the OP50 strain of E. coli unless they were treated with RNAi, in which case the HT115 E. coli strain expressing the described RNAi plasmid was used31,32. Where indicated, worms were exposed to the pathogenic strain of P. aeruginosa, PA14.

Cell culture. Expression of dominant-negative AFG3L2 was induced in stable HEK293 cells by the addition of 1 μg/ml tetracycline33 and the cells were collected 48 h later. The AOTC expression plasmid was transfected into Hela cells via Lipofectamine and the cells were collected after 72 h.

C. elegans slow-killing assay. Slow-killing experiments were performed as previously described34,35 with minor modifications. E. coli or P. aeruginosa overnight cultures were used to seed slow-killing nematode growth medium (NGM) agar plates (with 0.35% peptone). Plates were allowed to dry overnight at room temperature, incubated at 37°C for 24 h and allowed to equilibrate at room temperature. Synchronized L1 worms were allowed to develop on E. coli until the L4 stage and then transferred to P. aeruginosa slow-killing plates and incubated at 25°C. RNAi was performed as described previously36. For atfs-1(RNAi) (Fig. 3a, b), eri-1(mg366) (enhanced RNAi) worms37 were raised on control or atfs-1(RNAi) bacteria at 16°C until the L4 stage. All animals were transferred to fresh P. aeruginosa slow-killing plates in a randomized fashion. Animals were counted at the described times and were scored as dead if they failed to respond when touched. Fifty worms were used per experiment and those that had crawled off the plate or exploded at the vulva were excluded. All data related to the survival analysis is presented in Extended Data Table 3. Each experiment was performed in triplicate and the log rank (Mantel–Cox) statistical test was used to evaluate P values.

Intestinal mitochondrial morphology was visualized using ges-1::gfp::m worms38,39. The worms were synchronized by bleaching and allowed to hatch on plates containing P. aeruginosa and raised for 48 h at 25°C. Visualization of hsp-6::gfp, hsp-16::gfp and atfs-1::gfp was performed essentially as described36,37. P. aeruginosa was grown at 16°C for 24 h and seeded onto slow-killing plates. Plates were incubated overnight at room temperature. Synchronized L1 animals were transferred to P. aeruginosa plates and incubated at 20°C for 24 h before imaging.

To examine growth rates, eggs were allowed to hatch on plates containing P. aeruginosa and raised for 3 days at 25°C. 30 μg/ml ethidium bromide or 0.2 mM paraquat was added to E. coli or P. aeruginosa slow-killing plates. For clk-1(qm30) growth rates, worms were raised for 4 days at 25°C.

Statistics. All experiments were performed multiple times yielding similar results and comprised of biological replicates. The sample size and statistical tests were chosen based on previous studies with similar methodologies and the data met the assumptions for each statistical test performed. No statistical methods were used in deciding sample sizes, nor were any blinded experiments performed. For all figures, the mean ± standard deviation (s.d.) is represented unless otherwise noted.

C. elegans liquid-killing assay, gfp-4(b24) worms were raised at 25°C to sterilize them while being fed atfs-1(RNAi). At the L4-stage adult, the described worms were exposed to P. aeruginosa under conditions used for the liquid-killing assay37. RNA isolation and quantitative real-time PCR (qRT–PCR). Total RNA was obtained using the RNA STAT reagent (Tel-Test) and used for cDNA synthesis via the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). qRT–PCR was performed using Thermo-Scientific SyBr Green Maxima Mix. For Fig. 1c–f, worms were allowed to develop on control or atfs-1(RNAi) plates and incubated at 37°C for 24 h. To exclude pathogen avoidance as a means of decreased intestinal colonization, where indicated P. aeruginosa–GFP was also spread across the entire surface of the slow-killing plate (Extended Data Fig. 5d, e). Worms at the L4 stage were transferred to P. aeruginosa–GFP plates and allowed to feed for 24–48 h before examination. The extent of bacterial accumulation was scored as either ‘none/mild’, ‘moderate’ or ‘strong’ as indicated (Extended Data Fig. 5c). Plasmid construction. The hsp-16::atfs-1::gfp and hsp-16::atfs-1::mRl plasmids were described previously30. To construct the lys-2::mCherry plasmid, a 803 base pair fragment of the lys-2 promoter sequence upstream of the start codon was amplified using PCR and cloned into the HindIII and PstI sites of pPD95.73. lys-2::mCherry was microinjected into wild-type worms at a concentration of 20 ng μl−1 along with myo-2::mCherry at a concentration of 60 ng μl−1.

P. aeruginosa avoidance assay. Synchronized L1 wild-type and atfs-1(tm4919) worms were allowed to develop on control or spg-7(RNAi) plates to the L4 stage and then transferred to E. coli or P. aeruginosa slow-killing plates for 17 h when the worms were scored. The extent of avoidance was expressed as the per cent of animals off of the bacterial lawn over the total number of animals on the plate (Extended Data Fig. 5a, b).

Microscopy. C. elegans were imaged using a Zeiss AxioCam MRm mounted on a Zeiss Imager.Z2 microscope. Exposure times were the same in each experiment. 31. Yoneda, T. et al. Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. J. Cell Sci. 117, 4055–4066 (2004).
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Extended Data Figure 1 | Nuclear accumulation of ATFS-1 is required for UPR™ activation during *P. aeruginosa* exposure. **a**, Representative photomicrographs of *F35E12.5*:*pr::gfp* transgenic worms raised on control or *spg-7* (RNAi). No detectable increase in expression was observed following *spg-7* (RNAi) treatment. In contrast, strong expression of *F35E12.5*:*pr::gfp* was observed following exposure to *P. aeruginosa* compared to *E. coli* controls. Scale bar, 0.5 mm. **b**, Wild-type or *atfs-1(tm4525);hsp-60*:*pr::gfp* worms on *E. coli* or *P. aeruginosa*. Lower panels are magnified views of the intestine showing enhanced expression of *hsp-60*:*pr::gfp* (asterisks). Scale bars, 0.05 mm. **c**, Diagrams of wild-type ATFS-1 (ATFS-1FL) and ATFS-1 with a mutated nuclear localization signal (ATFS-1ΔNLS). **d**, Photomicrographs of *atfs-1(tm4525);hsp-60*:*pr::gfp* worms expressing ATFS-1FL or ATFS-1ΔNLS via the hsp-16 promoter exposed to *E. coli* or *P. aeruginosa*. Scale bar, 0.1 mm.
Extended Data Figure 2 | Multiple *P. aeruginosa* virulence genes contribute to UPR\textsuperscript{mt} activation. a, b, Expression of *hsp-60* and *hsp-6* mRNA for *glp-4(bn2)* worms exposed to *E. coli* or *P. aeruginosa* liquid-killing using qRT–PCR (*n = 3, ± s.d.*). Fold inductions are normalized to wild-type *E. coli* test group, *P < 0.05* (Student’s *t*-test). c, Quantification of survival for *glp-4(bn2)* worms raised on control or *atfs-1* (RNAi) and exposed to *P. aeruginosa* liquid-killing, *P < 0.0001* (Student’s *t*-test). d, List of *P. aeruginosa* toxin mutants. e, Quantification of the proportion of worms showing increased *hsp-6\textsubscript{pr}::gfp* expression in the intestine under slow-killing conditions. Exposure to *P. aeruginosa* caused *hsp-6\textsubscript{pr}::gfp* induction (*n = 3, ± s.e.m.*), *P < 0.05* (Student’s *t*-test). However, exposure to *P. aeruginosa* with mutations in the *pvdA*, *pvdD*, *pvdF*, *phzM*, *hcnB*, or *hcnC* toxin genes resulted in relatively less UPR\textsuperscript{mt} activation (*n = 3, ± s.e.m.*), **P < 0.05** (Student *t*-test).
Extended Data Figure 3 | Intestinal accumulation of lys-2 during mitochondrial stress and *P. aeruginosa* exposure requires ATFS-1.

**a**, Representative photomicrographs of wild-type and *atfs-1(tm4919)* worms carrying the *lys-2*:*pr:gfp* transgene raised on control or *spg-7*(RNAi). Scale bar, 0.1 mm.

**b**, Representative photomicrographs of wild-type and *atfs-1(tm4919)* worms carrying the *lys-2*:*pr:gfp* transgene exposed to *E. coli* or *P. aeruginosa*. Scale bar, 0.1 mm.
Extended Data Figure 4 | ATFS-1 partially regulates zip-2 expression during P. aeruginosa exposure. a, Expression levels of zip-2 mRNA in wild-type or atfs-1(tm4525) worms raised on E. coli or P. aeruginosa using qRT–PCR (n = 3, ± s.d.), * P < 0.05 (Student’s t test). b, Schematic diagram of the atfs-1 genomic open reading frame showing positions of exons 1–8 (boxes) and locations of the tm4525 (ref. 5) and tm4919 deletions in red. The tm4919 allele is a 334 base pair deletion beginning 107 base pairs upstream of the atfs-1 start codon and ends within the second intron of the atfs-1 genomic open reading frame. c, Representative photomicrographs of a germline in wild-type and atfs-1(tm4919) worms. Scale bar, 0.02 mm.
Extended Data Figure 5 | ATFS-1 is not required for pathogen avoidance during *P. aeruginosa* exposure. a, Quantification of avoidance behaviour for wild-type and *atfs-1(tm4919)* worms raised on *E. coli* or *P. aeruginosa*, expressed as a percentage of the number of animals off the bacterial lawn relative to the total number of worms (n = 4, ± s.d.). *P* < 0.0001. **P** = 0.1914 (Student’s t-test). b, Quantification of avoidance behaviour for wild-type worms raised on control or *spg-7* (RNAi) and exposed to *E. coli* or *P. aeruginosa*, expressed as a percentage of the number of animals off the bacterial lawn relative to the total number of worms (n = 3, ± s.d.). *P* < 0.0001, **P** = 0.8706 (Student’s t-test). c, Representative photomicrographs illustrating the scored level of infection for *P. aeruginosa* colonization assay using *P. aeruginosa*–GFP. Three categories of *P. aeruginosa*–GFP infection were used: none/mild, moderate and strong. Scale bar, 0.1 mm. d, Representative photomicrographs of wild-type and *atfs-1(tm4919)* worms raised on *spg-7* (RNAi) and exposed to a lawn of *P. aeruginosa*–GFP that completely covered the surface of the slow-killing plate for 24 h. Images are overlays of DIC and GFP. Scale bar, 0.1 mm. e, Quantification of *P. aeruginosa* intestinal colonization as shown in Extended Data Fig. 5d. White, grey and black bars denote no/mild infection, moderate infection and strong infection, respectively. Forty worms were analysed per treatment. f, Survival analysis of *glp-4(bn2)* and *atfs-1(tm4919); glp-4(bn2)* worms raised on control or *spg-7* (RNAi) and exposed to *P. aeruginosa*. Statistics for each survival analysis are presented in Extended Data Table 3. g, Quantification of pharyngeal pumping rate per minute for wild-type worms raised on control or *spg-7* (RNAi) (n = 10, ± s.d.). n.s., no significant difference (P = 0.10; Student’s t-test).
Extended Data Figure 6 | *atfs-1(et18)* gain of function mutant worms induce innate immune gene expression in the absence of mitochondrial stress. **a-d.** Expression levels of *abf-2*, *lys-2*, *clec-4* and *clec-65* mRNA in wild-type or *atfs-1(et18)* worms using qRT–PCR (*n* = 3 ± s.d.), *P* < 0.05 (Student’s *t* test). **e.** Representative photomicrographs of wild-type and *atfs-1(et18)* worms carrying the *irg-1pr::gfp* transgene raised on control or *zip-2*(RNAi). Scale bar, 0.10 mm. **f.** Survival analysis of wild-type and *atfs-1(et18)* worms raised on control or *lys-2*(RNAi) and exposed to *P. aeruginosa*. Statistics for each survival analysis are presented in Extended Data Table 3.
Extended Data Figure 7 | Mitochondrial protective and innate immune gene induction contributes to ATFS-1-mediated resistance to P. aeruginosa infection. a, Representative photomicrographs of wild-type hsp-60pr::gfp worms raised on control, atp-2(RNAi), eft-2(RNAi), sca-1(RNAi), T25B9.9(RNAi) or T08A11.2(RNAi). Scale bar is 0.1 mm. b, Representative photomicrographs of wild-type irg-1pr::gfp worms raised on control, atp-2(RNAi), eft-2(RNAi), sca-1(RNAi), T25B9.9(RNAi) or T08A11.2(RNAi). Scale bar is 0.1 mm. c, Survival analysis of wild-type worms raised on control, atp-2(RNAi), eft-2(RNAi), sca-1(RNAi), T25B9.9(RNAi) or T08A11.2(RNAi) and exposed to P. aeruginosa. Statistics for each survival analysis are presented in Extended Data Table 3. d, Survival analysis of wild-type worms raised on control, atp-2(RNAi), eft-2(RNAi), sca-1(RNAi), T25B9.9(RNAi) or T08A11.2(RNAi) and exposed to E. coli. Statistics for each survival analysis are presented in Extended Data Table 3. e, Representative photomicrographs of wild-type or kgb-1(km21);hsp-60pr::gfp worms raised on E. coli plates with or without 30 μg ml⁻¹ ethidium bromide, suggesting that the KGB-1 Jun kinase pathway negatively regulates the UPRmt during mitochondrial stress. Scale bar, 0.5 mm.
Extended Data Table 1 | ATFS-1-dependent innate immune genes upregulated when raised on *spg-7(RNAi)*

| Sequence Name | Gene symbol | KOG title, protein domain or function | Fold Induction Wild-type *spg-7(RNAi)/control* | Fold Induction *atfs-1(tm4525) spg-7(RNAi)/control* |
|---------------|-------------|--------------------------------------|-----------------------------------------------|---------------------------------------------------|
| **Antimicrobial peptides** |             |                                      |                                               |                                                   |
| g6714550      | *abf-2*     | antimicrobial peptide                | 10.456                                        | 2.33072                                           |
| R09B5.9       | *cnc-4*     | Caenorhabditis bacteriocin           | 4.58689                                       | 1.59881                                           |
| **Lysozyme**  |             |                                      |                                               |                                                   |
| Y22F5A.5      | *lys-2*     | N-acetylmuraminidase/lysozyme        | 4.81394                                       | 2.87725                                           |
| **C-type lectins** |         |                                      |                                               |                                                   |
| F35C5.9       | *clec-66*   | Lectin C-type domain/CUB domain      | 3.60596                                       | 2.09418                                           |
| F35C5.5       | *clec-62*   | Lectin C-type domain/CUB domain      | 3.81898                                       | 2.47423                                           |
| F35C5.8       | *clec-65*   | Lectin C-type domain                | 4.366                                         | 2.25587                                           |
| E03H4.10      | *clec-17*   | C-type lectin                       | 21.9148                                       | 7.58123                                           |
| C03H5.1       | *clec-10*   | C-type lectin                       | 3.47056                                       | 1.82945                                           |
| F31D4.4       | *clec-264*  | C-type lectin                       | 1.87899                                       | 1.24332                                           |
| T09F5.9       | *clec-47*   | C-type lectin                       | 5.57165                                       | -1.12101                                          |
| Y38E10A.5     | *clec-4*    | C-type lectin                       | 8.25168                                       | 3.66669                                           |
| M02F4.7       | *clec-265*  | C-type lectin                       | 8.40775                                       | 4.75704                                           |
| F08H9.7       | *clec-56*   | C-type lectin                       | 2.30962                                       | 1.32766                                           |
| **Galectin**  |             |                                      |                                               |                                                   |
| F38A5.3       | *lec-11*    | Galectin, galactose-binding lectin   | 2.29608                                       | 1.41767                                           |
| **Signaling** |             |                                      |                                               |                                                   |
| F08B1.1       | *vhp-1*     | Dual specificity phosphatase         | 2.39868                                       | 1.59839                                           |
## Extended Data Table 2 | ATFS-1-dependent UPR\textsuperscript{mt} genes in common with genes induced following *P. aeruginosa* exposure\textsuperscript{10}

| Sequence name | Gene symbol | KOG title, protein domain or function | Fold Induction Wild-type | Fold Induction PA14/OP50 | Fold Induction (Trometel et al. 2006 or this study) |
|---------------|-------------|--------------------------------------|--------------------------|--------------------------|-------------------------------------------------|
| R08F11.3      | cyp-33C8    | Cytochrome P450 CYP2 subfamily       | 52.7302                  | 4.1                      | 7.9                                             |
| T089.2        | cyp-13A5    | Cytochrome P450 family              | 35.697                   | 2.8                      | 7.9                                             |
| E03H4.10      | ciec-17     | C-type lecin                         | 21.914                   | 7.0                      | 7.9                                             |
| C54D10.1      | cdk-2       | glutathione S-transferase-like protein | 15.2001                 | 2.6                      | 2.6                                             |
| K01D12.11     | cia-1       | cadmium responsive                   | 11.9017                  | 4.0                      | 4.0                                             |
| g67F15.50     | atf-2       | antimicrobial peptide                | 10.450                   | 1.80*                    | 1.80*                                           |
| F15B6.6       |             |                                      | 8.3786                   | 2.7                      | 2.7                                             |
| K01D12.2      |             |                                      | 8.84434                  | 3.1                      | 3.1                                             |
| M2Z2F4.7      | ciec-265    | C-type Lecin                         | 8.40775                  | 3.9                      | 3.9                                             |
| Y3B5E1.0A.5   | ciec-4      | C-type Lecin                         | 8.29368                  | 11.1                     | 11.1                                            |
| C4G9G7.5      |             |                                      | 7.9102                   | 21.9                     | 21.9                                            |
| C18A11.1      |             |                                      | 7.43593                  | 3.1                      | 3.1                                             |
| F23H10.2      |             |                                      | 7.33184                  | 4.4                      | 4.4                                             |
| C4G9G7.10     |             |                                      | 7.14237                  | 9.7                      | 9.7                                             |
| C10C5.2       |             |                                      | 6.85214                  | 5.9                      | 5.9                                             |
| Y5B7A7.5      |             |                                      | 5.84850                  | 12.2                     | 12.2                                            |
| R11G11.12     | ntr-210     | Nuclear Hormone Receptor family      | 5.76055                  | 2.3                      | 2.3                                             |
| T12G3.1       |             | contains ZZ-type Zn-finger           | 5.74038                  | 3.0                      | 3.0                                             |
| ZK97G7.7      |             |                                      | 5.46349                  | 3.9                      | 3.9                                             |
| R19B5.9       | cne-4       | Caenorhabditis bacteriotoxin         | 4.58669                  | 4.2                      | 4.2                                             |
| C4G9G7.7      |             |                                      | 4.41335                  | 5.6                      | 5.6                                             |
| F35C5.8       | ciec-66     | Lectin C-type domain                 | 4.366                    | 2.9                      | 2.9                                             |
| Y5B7A7.3      |             |                                      | 4.34718                  | 5.7                      | 5.7                                             |
| C34H4.2       |             |                                      | 4.34064                  | 2.3                      | 2.3                                             |
| T15G1.5       |             | Predicted small molecule kinase      | 4.25195                  | 8.9                      | 8.9                                             |
| F01G10.3      | eoh-9       | Hydroxysterol-CoA dehydrogenase/lenoyl- | 4.17940                  | 5.0                      | 5.0                                             |
| T12G3.1       |             | CoA hydratase                        | 4.09383                  | 2.4                      | 2.4                                             |
| C56F4.1       |             | Uncharacterized conserved protein     | 4.07802                  | 2.1                      | 2.1                                             |
| B0218.2       | fsah-2      | Amidase                              | 3.89777                  | 2.2                      | 2.2                                             |
| P19B2.5       |             | Helicase-like transcription factor   | 3.81204                  | 2.3                      | 2.3                                             |
| C56F4.1       |             |                                      | 3.76713                  | 2.1                      | 2.1                                             |
| F35C5.9       | ciec-66     | Lectin C-type domain/CUB domain      | 3.60596                  | 6.2                      | 6.2                                             |
| M01G12.9      |             |                                      | 3.57904                  | 3.2                      | 3.2                                             |
| K11G4.3       |             |                                      | 3.58653                  | 2.2                      | 2.2                                             |
| C26H4.1       | ciec-10     | C-type lecin                         | 3.47056                  | 2.3                      | 2.3                                             |
| C34C6.7       |             |                                      | 3.17263                  | 2.8                      | 2.8                                             |
| Y22D7AR.9     | fbxa-74     | F-box A protein                      | 3.10471                  | 8.7                      | 8.7                                             |
| Y119G3.20     | fbxa-62     | F-box A protein                      | 3.06775                  | 4.1                      | 4.1                                             |
| F55C12.7      | tag-234     |                                      | 3.02488                  | 3.5                      | 3.5                                             |
| Y17G7B.8      |             |                                      | 3.02481                  | 2.2                      | 2.2                                             |
| C25F9.3       |             |                                      | 3.02333                  | 2.6                      | 2.6                                             |
| C34D1.5       | zip-5       | bZIP transcription factor            | 2.98077                  | 2.1                      | 2.1                                             |
| Y5B7A7.4      |             |                                      | 2.95558                  | 2.2                      | 2.2                                             |
| T01D3.6       |             | von Willebrand factor                | 2.91981                  | 2.6                      | 2.6                                             |
| ZK97G7.6      |             | Secreted surface protein             | 2.55033                  | 2.4                      | 2.4                                             |
| F45F1.6       |             |                                      | 2.48370                  | 17.8                     | 17.8                                            |
| K08D8.6       |             |                                      | 2.41167                  | 3.6                      | 3.6                                             |
| C09F12.1      | cip-1       | Claudin homolog                      | 2.36493                  | 2.3                      | 2.3                                             |
| T27F4.2       |             | bZIP transcription factor            | 2.35058                  | 2.8                      | 2.8                                             |
| F35A3.3       | lec-11      | Galectin, galectose-binding lectin   | 2.29068                  | 2.4                      | 2.4                                             |
| Y47H10A.5     |             |                                      | 2.24131                  | 3.9                      | 3.9                                             |
| Y43C5A.3      |             |                                      | 2.06783                  | 2.5                      | 2.5                                             |
| F23H12.3      |             |                                      | 1.77130                  | 2.1                      | 2.1                                             |
| E02C12.8      |             |                                      | 1.7586                   | 3.7                      | 3.7                                             |
| Y95B8A.6      |             |                                      | 1.67149                  | 2.5                      | 2.5                                             |
| F11D11.3      |             |                                      | 1.55814                  | 3.4                      | 3.4                                             |
| C50F4.9       |             |                                      | 1.54635                  | 3.4                      | 3.4                                             |
| Y22F5A.5      | lyp-2       | Lysosome                            | 4.81394                  | 10.6*                    | 10.6*                                           |

*This study*
## Extended Data Table 3 | Statistics for survival analysis

| Strain comparison | $p$ values     | Number of worms | Figure |
|-------------------|----------------|-----------------|--------|
| en(mg566) control vs en-1(mg566) stds-1(RNAi) | 0.0003 | en(mg566) control: 34/50, en-1(mg566) stds-1(RNAi): 39/50 | 3a     |
| en(mg566) control vs en-1(mg566) stds-1(RNAi) | 0.6986 | en(mg566) control: 50/50, en-1(mg566) stds-1(RNAi): 47/50 | 3b     |
| wild-type control vs wild-type spp-(RNAi) | <0.0001 | wild-type control: 37/50, wild-type spp-(RNAi): 45/50 | 3c     |
| wild-type control vs atts-1(mu891) spp-(RNAi) | <0.0001 | wild-type spp-(RNAi): 37/50, atts-1(mu891) spp-(RNAi): 50/50 | 3c     |
| wild-type control vs atts-1(mu891) spp-(RNAi) | 0.1322 | wild-type spp-(RNAi): 37/50, atts-1(mu891) spp-(RNAi): 50/50 | 3e     |
| wild-type control vs atts-1(e18) control | <0.0001 | wild-type control: 41/50, atts-1(e18) control: 30/50 | 3h     |
| wild-type control vs wild-type atts-1(RNAi) | 0.039  | wild-type control: 41/50, wild-type atts-1(RNAi): 45/50 | 3h     |
| atts-1(e18) control vs atts-1(e18) control | 0.0001 | atts-1(e18) control: 30/50, atts-1(e18) control: 37/50 | 3h     |
| wild-type control vs pmk-1(fem25) control | <0.0001 | wild-type control: 33/50, pmk-1(fem25) control: 42/50 | 3a     |
| wild-type control vs wild-type spp-(RNAi) | <0.0001 | wild-type control: 33/50, wild-type spp-(RNAi): 47/50 | 4a     |
| pmk-1(fem25) control vs pmk-1(fem25) spp-(RNAi) | <0.0001 | pmk-1(fem25) control: 42/50, pmk-1(fem25) spp-(RNAi): 23/50 | 4a     |
| wild-type control vs sek-(fkm4) control | <0.0001 | wild-type control: 35/50, sek-(fkm4) control: 35/50 | 4b     |
| wild-type control vs wild-type spp-(RNAi) | <0.0001 | wild-type control: 35/50, wild-type spp-(RNAi): 37/50 | 4b     |
| sek-(fkm4) control vs sek-(fkm4) spp-(RNAi) | <0.0001 | sek-(fkm4) control: 35/50, sek-(fkm4) spp-(RNAi): 38/50 | 4b     |
| wild-type control vs kgb-1(fem21) control | <0.0001 | wild-type control: 29/50, kgb-1(fem21) control: 38/50 | 4c     |
| wild-type control vs wild-type spp-(RNAi) | <0.0001 | wild-type control: 29/50, wild-type spp-(RNAi): 37/50 | 4b     |
| kgb-1(fem21) control vs kgb-1(fem21) spp-(RNAi) | <0.0001 | kgb-1(fem21) control: 38/50, kgb-1(fem21) spp-(RNAi): 26/50 | 4c     |
| wild-type control vs mkh-1(ok2471) control | 0.0001 | wild-type control: 38/50, mkh-1(ok2471) control: 29/50 | 4d     |
| wild-type control vs wild-type spp-(RNAi) | <0.0001 | wild-type control: 38/50, wild-type spp-(RNAi): 33/50 | 4d     |
| mkh-1(ok2471) control vs mkh-1(ok2471) spp-(RNAi) | <0.0001 | mkh-1(ok2471) control: 28/50 vs mkh-1(ok2471) spp-(RNAi): 48/50 | 4d     |
| wild-type control vs wild-type spp-(RNAi) | <0.0001 | wild-type control: 50/50, wild-type spp-(RNAi): 28/50 | 4e     |
| wild-type control vs atts-1(mu891) spp-(RNAi) | <0.0001 | wild-type control: 28/50, atts-1(mu891) spp-(RNAi): 28/50 | 4e     |
| wild-type control vs zip-2(mu246) spp-(RNAi) | 0.0004 | wild-type control: 28/50, zip-2(mu246) spp-(RNAi): 39/50 | 4e     |
| gpl-4(brn2) control vs gpl-4(brn2) spp-(RNAi) | <0.0001 | gpl-4(brn2) control: 32/50, gpl-4(brn2) spp-(RNAi): 19/50 | ED 5f  |
| gpl-4(brn2) spp-(RNAi) vs atts-1(mu891) spp-(RNAi) | <0.0001 | gpl-4(brn2) spp-(RNAi): 19/50, atts-1(mu891) spp-(RNAi): 32/50 | ED 5f  |
| wild-type control vs atts-1(e18) control | 0.0001 | wild-type control: 40/50, atts-1(e18) control: 35/50 | 4f     |
| wild-type control vs wild-type y2(RNAi) | 0.0419 | wild-type control: 40/50, wild-type y2(RNAi): 38/50 | 4f     |
| wild-type control vs atts-1(e18) y2(RNAi) | 0.0004 | y2(RNAi): 38/50, wild-type y2(RNAi): 38/50 | 4f     |
| wild-type control vs atts-1(e18) y2(RNAi) | 0.7317 | wild-type control: 40/50, atts-1(e18) y2(RNAi): 44/50 | 4f     |
| wild-type control vs wild-type atp-2 | <0.0001 | wild-type control: 36/50, wild-type atp-2: 31/50 | ED 7c  |
| wild-type control vs wild-type atf-2 | <0.0001 | wild-type control: 36/50, wild-type atf-2: 30/50 | ED 7c  |
| wild-type control vs wild-type acu-1 | <0.0001 | wild-type control: 36/50, wild-type acu-1: 34/50 | ED 7c  |
| wild-type control vs wild-type TGBA11.2 | <0.0001 | wild-type control vs wild-type TGBA11.2: 50/50 | ED 7c  |
| wild-type control vs wild-type atp-2 | <0.0001 | wild-type control: 37/50, wild-type atp-2: 31/50 | ED 7d  |
| wild-type control vs wild-type spp-(RNAi) | <0.0001 | wild-type control: 37/50, wild-type spp-(RNAi): 39/50 | ED 7d  |
| wild-type control vs wild-type atf-2 | <0.0001 | wild-type control: 37/50, wild-type atf-2: 28/50 | ED 7d  |
| wild-type control vs wild-type acu-1 | <0.0001 | wild-type control: 37/50, wild-type acu-1: 49/50 | ED 7d  |
| wild-type control vs wild-type TGBA11.2 | <0.0001 | wild-type control: 37/50, wild-type TGBA11.2: 38/50 | ED 7d  |
| wild-type control vs wild-type TGBA11.2 | <0.0001 | wild-type control: 37/50, wild-type TGBA11.2: 43/50 | ED 7d  |

**ID=** Extended Data

Statistical analysis was performed using the log rank (Mantel-Cox) statistical test. Number of worms represents the number of dead worms scored relative to the number of worms alive at the start of the experiment. The difference in numbers indicates those worms that were excluded (see Methods).