**LETTER**

**Caenorhabditis elegans** pathways that surveil and defend mitochondria

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Mitochondrial function is challenged by toxic by-products of metabolism as well as by pathogen attack. Here, we show that, in the nematode *Caenorhabditis elegans*, mitochondrial dysfunction activates the nuclear genome to defend mitochondria against stress. We used genome-wide RNA interference (RNAi) screens to identify 18% of bacterial species in the wild-caught *C. elegans* population that induce stress responses when fed to animals. We also identified a novel mitochondrial stress-induced gene, *isp-1*, which is necessary for averting stress responses. These findings reveal a fundamental role for the nucleus in responding to mitochondrial dysfunction.

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is an attractive iron depot for bacteria. Bacterial toxins, siderophores or virulence factors from these many bacterial strains may target the mitochondrion.

To identify the molecular basis behind the detection of animal mitochondrial dysfunction and its coupling to a detoxification response, we performed a genome-wide RNAi screen for gene inactivations that render *C. elegans* unresponsive to mitochondrial dysfunction (Extended Data Figs 1 and 2). Gene inactivations that caused a failure to induce *hsp-6p-gfp* in antimycin were re-screened for failure to upregulate other genes normally induced by mitochondrial dysfunction: the detoxification gene *ugt-61::gfp* in the presence of antimycin, *hsp-6p-gfp* induction in the *isp-1(qm150)* mutant, or *hsp-6p-gfp* induction in response to RNAi targeting the ATP synthase *atp-2*. Mitochondrial surveillance and response was disrupted by 45 gene inactivations (Extended Data Table 1), including the known components *atsf-1, clpp-1* and *dve-1* (refs 4, 6).

One of the gene inactivations that potently disrupts response to mitochondrial dysfunction, *sptl-1*, encodes serine palmitoyltransferase, which has a role in the sphingolipid biosynthesis pathway (Extended Data Fig. 3). Inactivation of *sptl-1* by RNAi inhibited the induction of *hsp-6p-gfp* upon antimycin treatment or *spg-7(RNAi)* (Fig. 3a, b), whereas *sptl-1(RNAi)* alone without antimycin treatment did not induce the expression of *hsp-6p-gfp* (Extended Data Fig. 4b). *sptl-1(RNAi)* did not affect the activation of endoplasmic reticulum stress reporter *hsp-4p-gfp* by the drug tunicamycin (Fig. 3c), indicating a specific role in mitochondrial surveillance. A probable null mutation in *sptl-1* (Extended Data Fig. 5a) impaired the induction of *hsp-6, cyp-14A3* and *ugt-61* after mitochondrial disruption by *spg-7(RNAi)* (Fig. 3d). A double mutant in the two ceramide synthase genes of the sphingolipid biosynthetic pathway also attenuated the induction of *hsp-6* upon mitochondrial damage (Extended Data Fig. 5b). Treatment with myriocin, a fungal inhibitor of mammalian serine palmitoyltransferase, disrupted antimycin-induced *hsp-6p-gfp* induction (Fig. 3e), *sptl-1(RNAi)* (with normal movement, Supplementary Videos 1 and 2) attenuated food avoidance induced by antimycin or *spg-7(RNAi)*, suggesting that this behavioural response is also coupled to *sptl-1* (Fig. 3g and Extended Data Fig. 4d, e). *sptl-1* expression is upregulated 2.5-fold by mitochondrial damage (Extended Data Fig. 4a), suggesting that increased sphingolipid levels during mitochondrial disruption may act as a signal rather than as a membrane component required for another signal.

The morphology of the normally extensive mitochondrial network of *C. elegans* and other animals is responsive to mutation or inactivation of mitochondrial components11. Mitochondria in *sptl-1(RNAi)* animals hyperfused into larger, longer mitochondria (Fig. 3f), suggesting that *sptl-1* is critical for mitochondrial homeostasis. The *isp-1* mitochondrial mutant had a severe synthetic growth defect on a *sptl-1(RNAi)* background, whereas wild-type development was only slightly delayed on *sptl-1(RNAi)* (Fig. 3h). Sphingolipid signalling may act in the homeostatic response to the mitochondrial defect caused by the *isp-1(qm150)* mutation; in the absence of that response, *isp-1(qm150)* may cause a more severe mitochondrial defect and growth arrest. *sptl-1(RNAi)* animals were also more sensitive to the mitochondrial inhibitor antimycin (Extended Data Fig. 4f). In addition, *sptl-1(RNAi)* animals were unable to sense mitochondrial inhibition and activate the mitochondrial surveillance pathway when challenged by a set of the wild microbes that disrupt mitochondrial function (Extended Data Fig. 4g).

Ceramide supplementation rescued the *sptl-1* defect in mitochondrial surveillance (Fig. 4a and Extended Data Fig. 5d), whereas ceramide in the absence of mitochondrial damage did not induce *hsp-6p-gfp* (Extended Data Fig. 5e), suggesting that ceramide is not a sufficient signal to induce the suite of responses to mitochondrial dysfunction in the absence of true mitochondrial dysfunction. Ceramide supplementation also partially rescued the mitochondrial morphology defect (Fig. 4b) and the impaired food-avoidance behaviour caused by *sptl-1(RNAi)* (Extended Data Fig. 6a). Dihydroceramide, a ceramide precursor with no signalling function in mammals, could not rescue the *sptl-1(RNAi)* mitochondrial surveillance defect (Fig. 4a). Two *C. elegans* ceramide synthases, HYL-1 and HYL-2, synthesize different ceramide species (C24 to C26 and C20 to C22, respectively12). On testing C16, C20, C22 and C24 ceramides, only C24 rescued the deficiency of mitochondrial surveillance caused by *sptl-1(RNAi)* (Fig. 4c).

During apoptosis and mitophagy, ceramide accumulates on the outer membrane of mitochondria13–15. Using antibodies to stain ceramide and Complex IV (COX-IV; a mitochondrial protein in the electron transport chain), ceramide and mitochondrial protein colocalization was shown to markedly increase after *C. elegans* mitochondrial inhibition (Fig. 4d).
The increased colocalization preceded induction of hsp-6p::gfp (Extended Data Fig. 6b, c). Thus, ceramide may participate in an early step of mitochondrial surveillance by marking domains of dysfunction.

To map ceramide relative to other gene inactivations that render animals unable to respond to mitochondrial damage, we tested whether ceramide could rescue the mitochondrial surveillance defects of other hits from the RNAi screen. Five other gene inactivations were rescued by ceramide: ran-4, a nuclear transport component; Y47G6A.29, a phosphatidylinositol signalling component; F40F12.7, a zinc finger protein; Y54E10BR.5, a signal peptidase component; and ceh-20, a homeobox transcription factor (Extended Data Fig. 6e). These gene inactivations may disrupt mitochondrial surveillance upstream of ceramide production.

ATFS-1 is a transcription factor that activates hsp-6 and hsp-60 during mitochondrial targeting sequence as well as a carboxy-terminal nuclear localization signal; when mitochondria are damaged, nuclear accumulation of ATFS-1 is favoured. Deletion of the N-terminal 1–32 amino acids causes constitutive nuclear accumulation of ATFS-1 (Extended Data Fig. 6d) and activates hsp-60p::gfp. Activation of hsp-60p::gfp by ATFS-1(1-32).myc did not require splt-1 gene activity (Fig. 4f), suggesting that ceramide works upstream of ATFS-1 and has a role in the early detection of mitochondrial dysfunction.

hmgs-1, which encodes an HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) synthase of the mevalonate synthetic pathway, is also a strong hit from the genome-wide screen. hmg-1 gene inactivation inhibited antimycin-induced hsp-6p::gfp induction and food avoidance (Extended Data Fig. 7a–d). hmg-1(RNAi) also induced abnormal mitochondrial morphology (Extended Data Fig. 7e). Supplementing mevalonate to hmg-1(RNAi) animals rescued the deficiency of antimycin-induced hsp-6p::gfp (Extended Data Fig. 7f).

Statins are cholesterol-lowering drugs that inhibit the mevalonate pathway (Extended Data Fig. 8a). Treating C. elegans with statins also abrogates their ability to sense mitochondrial damage and activate protective programs (Extended Data Figs 7g and 8b). A common side effect of statin therapy is muscle toxicity. By inhibiting the mevalonate pathway, statins inhibit ubiquinone synthesis, a key component of the electron transport chain (Extended Data Fig. 8a). Our data suggest that a combined inhibition of ubiquinone synthesis and mitochondrial surveillance may contribute to the muscle toxicity of statins. In support of a role for mitochondrial surveillance in statin action, a gain-of-function mutation in C. elegans atfs-1 confers statin resistance. Treating human embryonic kidney HEK293T cells with statin impaired the mitochondrial network and morphology (Extended Data Figs 7h and 9). Statin treatment also decreased ATP production in mouse C2C12 myotubes (Extended Data Fig. 7i).

Eukaryotic mitochondrial surveillance pathway components are likely to be targets of microbial toxins and virulence factors. If the mitochondrial surveillance pathway in an animal is disabled by a bacterial toxin or virulence factor, other anti-mitochondrial toxins, siderophores or virulence factors would be rendered more effective. To detect such bacterial anti-surveillance activities, we screened our collection of C. elegans flora for bacterial species that, when co-cultured with C. elegans, could disrupt the induction of hsp-6p::gfp by antimycin. Six wild bacterial strains of the genus Pseudomonas from three species (vranovensis, breneri...
and asplenii) disrupt mitochondrial surveillance (Fig. 2d, e). The dozens of C. elegans genes we have identified in the mitochondrial surveillance pathway are candidate targets for toxins or virulence factors from these Pseudomonas strains.

Our studies have revealed roles of ceramide and mevalonate in mitochondrial surveillance. The products of these pathways either constitute signals that are transferred within or between cells, or structures within those cells that are necessary for other signals to emerge. We favour that these constitute signals because the expression of these biosynthetic pathways are induced by the mitochondrial insults (Extended Data Fig. 4a) and because these molecules are localized to the site of injury (Fig. 4d). Ceramides are upstream elements of the stress response of plasma membrane, endoplasmic reticulum and mitochondria in multiple species19,20, and can act as hormonal signals21. But it is also possible that these constitute membrane elements required for other signals. Variations in the animal pathways for mitochondrial surveillance identified here, many of which involve conserved proteins, may underlie variation in the symptoms caused by the same mitochondrial mutations or variation in response to mitochondrial toxins such as statins. Microbial secondary metabolites that render animals unresponsive to their mitochondrial dysfunctions (Fig. 2d, e) may have therapeutic potential in the treatment of a predicted upregulation of detoxification and antibacterial pathways, which may contribute to the devastating symptoms of some mitochondrial diseases. The anti-surveillance activities of such natural products may also treat other disorders of dysregulated detoxification and innate immunity, such as autoimmunity.

**METHODS SUMMARY**

RNAi clones in E. coli were seeded to 24-well worm plates and, 40 worms per well were assayed at 48 h. For drug screens, GFP expression was assayed after 24 h. Wild microbes were seeded onto 24-well plates in duplicate and, 40 synchronized L1 worm were added to the wells. Animals were scored after 48 h.

**Online Content** Any additional Methods, 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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Supplementary Information is available in the online version of the paper.

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Author Contributions Y.L. and G.R. designed experiments; Y.L., B.S.S. and P.C.B. carried out experiments. Y.L., B.S.S. and G.R. wrote the paper. G.R. supervised the project.

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 G.R. (ruvkun@molbio.mgh.harvard.edu).
METHODS

C. elegans strains. hsp-6p::gfp(zc113)V, dpy-5(e907)уст-61p::gfp(Ex15171), hsp- 4p::gfp(zc145)V, myo-3p::GFPPnt(zc140), is-1(usp150), spdt(1·k6933), lagr-1(ок976)IV, hyl-1(ок796)IV, spdt(1·k766)X, hyl-2(ок171)X and asm-3(ок1744)IV were obtained from the Caenorhabditis Genetics Center (CGC).

Primers for genotyping. hsp-6p::gfp (forward AACCAATTGCAAGAA; reverse GCAATTGGAGGTCATG) for gfp expression include Achromobacter, Curtobacterium, Enterobacter, Leucobacter, Mycolicola, Myroides, Raoultella and Rhodococcus.

Microscopy. Comparable GFP reporter images were obtained by a Zeiss AxioImager Z1 using the same exposure time. Mitochondrial morphology of C. elegans or HEK293T cells was visualized under an Olympus Fluoview 1000 confocal microscope.

Genome-wide RNAi screen. Primary screen was performed by seeding individual bacterial clones each bearing a distinct C. elegans dsRNA to initiate RNAi onto 24-well RNAi plates. Dried plates were kept at room temperature overnight to allow IPTG induction of double-stranded RNA (dsRNA) expression. Synchronized L1 worms (~40 worms per well) were raised on the RNAi plates at 20 °C. Fluorescence was assayed at 48 h.

Activation of GFP reporter by RNAi. RNAi clones were grown in LB containing 50 μg ml⁻¹ carbencillin at 37 °C overnight and seeded 100 μl per well to 24-well worm plates with 5 mM IPTG. Dried plates were kept at room temperature overnight to allow IPTG induction of dsRNA expression. Synchronized L1 worms (~40 worms per well) were raised on the RNAi plates at 20 °C. Fluorescence was assayed after 48 h.

Activation of GFP reporter by drugs. Synchronized L1 worms (~40 worms per well) were raised on a 24-well plate at 20 °C for 48 h. At this time, each well was treated with 0.5 μg antimycin (total volume 20 μl) in M9 buffer (3 g KH₂PO₄, 6 g Na₂HPO₄, 5 g NaCl, 1 ml 1 M MgSO₄, H₂O to 1 litre), or 1.5 μg tunicamycin in DMSO (total volume 20 μl). GFP expression was assayed after 24 h (antimycin), or 7 h (tunicamycin).

SYBR GREEN PCR Master Mix (Bio-Rad). Quantification of transcripts was normalized to rpl-32 and relative expression levels were calculated as described previously22.

Food-avoidance assays. Each gene inactivation that disrupted the response to mitochondrial dysfunction was grown in 1 ml LB containing 50 μg ml⁻¹ carbencillin at 37 °C overnight, pelletted, re-suspended with 50 μl of LB and dropped in the centre of a well of 6-well plates containing 5 mM IPTG. Dried plates were kept at room temperature overnight to allow IPTG induction of dsRNA expression. Only circular larvae of uniform size and density were used for food-avoidance assays. Synchronized L1 worms (~100 animals per well) were dropped into the centre of each bacterial lawn. Food-avoidance phenotypes were scored at 48 h. For antimycin treatment, synchronized L1 worms (~100 animals per well) were dropped into the centre of each bacterial lawn, and grown at 20 °C for 48 h. At this time, 50 μg antimycin (total volume 80 μl) was added directly to the bacteria lawn. Food avoidance was scored 8 h after drug treatment.

Synthetic growth defect on splt-1 (RNAi). Wild-type N2 or mitochondrial mutant allele isp-1 animals were synchronized at the L1 stage. Animals were then raised on control or splt-1(RNAi). isp-1 animals were grown on control RNAi for 3 days versus on splt-1(RNAi) for 5 days to match the developmental stage of wild-type N2 animals.

Ceramide biosynthetic mutant analyses. To dissect the pathway of splt-1 function in mitochondrial surveillance, we tested other mutations in the sphingolipid biosynthetic pathway (Extended Data Figs 4 and 5a). Inactivation of another serine palmitoyltransferase gene splt-3 had no effect on the induction of hsp-6 upon mitochondrial damage (Extended Data Figs 4, 5a, b). A double mutation of hyl-1 and lagr-1, which encode ceramide synthases, reduced the induction of hsp-6 upon mitochondrial damage (Extended Data Fig. 5b), whereas hyl-1 or lagr-1 single mutants were less defective (Extended Data Fig. 5c). Only the C24 ceramide, synthesized by Lagr-1, rescued the defect of mitochondrial surveillance caused by splt-1(RNAi). This is consistent with the defect in hsp-6 induction shown by hyl-1(lagr-1) double mutant (Extended Data Fig. 5b).

Immunostaining. Immunostaining of dissected animals was carried out according to ref. 23 with minor modifications. Specifically, dissected animals were blocked with 0.5% BSA in PBS for 1 h at room temperature, and stained with anti-ceramide antibody (MID1584, Alexis Biochemicals, diluted 1:600 (v/v)) in 0.2% BSA in PBS and anti-human OxPhos Complex IV antibody (Invitrogen diluted 1:300 (v/v)) at room temperature for 30 min and 4 °C overnight. These were then washed three times with PBS (10 min each time), and slides were then incubated with Cy3 anti-mouse secondary antibody (diluted 1:200 (v/v)) for 1 h at room temperature. After three further washes with PBS (10 min each time), slides were mounted and visualized under an Olympus Fluoview 1000 confocal microscope.

HEK293T cells (ATCC) were washed twice with ice cold PBS and fixed with 4% formaldehyde at 4 °C for 1 h. After fixation, cells were permeabilized by 0.1% Triton in PBS at room temperature for 8 min, and then blocked with 0.5% BSA in PBS for 1 h at room temperature, and stained with anti-human OxPhos Complex IV antibody (Invitrogen diluted 1:300 (v/v)) at room temperature for half hour and 4 °C overnight. Following three washes with PBS (10 min each time), slides were mounted and visualized under an Olympus Fluoview 1000 confocal microscope.

APL levels. C2C12 myoblasts (ATCC) were grown in DMEM supplemented with 10% (v/v) FBS and antibiotics (100 mg ml⁻¹ penicillin/streptomycin mix) at 37 °C. Differentiation into myotubes was induced at ~90% density by changing the medium to DMEM supplemented with 2% (v/v) horse serum. Differentiation occurred after 5 days. Myotubes were then treated with DMSO, 10 μM simvastatin or 10 μM mevastatin. After incubation for 48 h, CellTiter-Glo reagent (Promega) was added to cell-culture medium, and luminescence was measured after 10 min incubation.

Primers for quantitative RT–PCR. hsp-6: forward CAAACTCTCGTGTGCATG ATCATGGAAGG, reverse GTCGGTGCTGACAACTTTGAGG; cyp-16: forward GACCTTGGCTTGCCGAAAAAGCCTTGG, reverse CAGTGGGC GTCTTCTTTGAAGCTCCACG; ugt-61: forward GGTGACAGAATGCGAGGA ACGTAATCATG, reverse GGGAAAGAATGGTGCCAGCATTCATGTT; splt-3: forward GGAAGAGTATACGAACTTGCAGGAR, reverse TGTAGAAGTCGATGGAGGACGATG; spftl-3: forward CTTGGGTGTCCCTTTCGTG, reverse ATCCCAATGTGACCGAAATCGAGTTGAG; hyl-1: forward GCCCGTTAATAATGGACCAA, reverse TGCTGTTGCTTCTCAGTCC; hyl-2: forward GGGGAGTGTAGGGAGAAAT, reverse TTGCGCAACATGAGAAGAGAAGATG.

Primers for genotyping. splt-1: forward AGCCAAAGGACCAATCTTCC, reverse AACAGCAATTGTGAAGTCCG; spftl-3: forward CTGGTGGTTCCCTGTGTTGGTT, reverse ATCCCAATGTGACCGAAATCGAGTTGAG; hyl-1: forward GCCCGTTAATAATGGACCAA, reverse TGCTGTTGCTTCTCAGTCC; hyl-2: forward GGGGAGTGTAGGGAGAAAT, reverse TTGCGCAACATGAGAAGAGAAGATG.

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TTACACGTTCTCGGTTTAAG; sphk-1: forward ATGTTCATAGTAGTGTAAC, reverse CTAGGCAGTTGATGAGAAAACG.

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Extended Data Figure 1 | Mitochondrial dysfunction activates homeostatic, detoxification and pathogen responses. a. Drug-induced food-avoidance phenotypes on live or dead bacteria. Dead bacteria were obtained by heating bacteria at 90 °C for 30 min. Avoidance behaviour was also observed when antimycin was added to dead bacteria, showing that the drug acts directly on *C. elegans* and is not transformed by the bacteria. b. Quantification of the food-avoidance phenotypes of Extended Data Fig. 1a (*n* = 4). Error bars represent s.d. c. A dose response of hsp-6p::gfp induction with the addition of antimycin.
Extended Data Figure 2 | Diagram of the genome-wide RNAi screen workflow. For the detailed experimental procedure, see Methods.
Extended Data Figure 3 | Diagram of the sphingolipid metabolism pathway and the corresponding genes.

| Gene product         | Sequence name | C. elegans gene | Alleles |
|----------------------|---------------|-----------------|---------|
| 1. Serine Palmitoyl Transferase | C23H3.4       | spl1-1          | ok1693  |
|                      | T22G5.5       | spl1-3          | ok1927  |
| 2. Ceramide Synthase  | K02G10.6      | hyl-2           | gnv1    |
|                      | C09G4.1       | hyl-1           | ok976   |
|                      | Y6B3B.10      | lagr-1          | gk327   |
| 3. Sphingomyelinase   | W03G1.7a,b    | asm-3           | tm2384  |
|                      | T27F6.6       |                 | tm2178  |
| 4. Ceramidase         | F27E5.1       |                 | ok864   |
| 5. Ceramide Kinase    | T10B11.2      |                 | ok1252  |
| 6. Sphingosine Kinase | C34C6.5a,b    | sphk-1          | ok1097  |

Extended Data Figure 3 | Diagram of the sphingolipid metabolism pathway and the corresponding genes.
Extended Data Figure 4 | sptl-1 is required for mitochondrial surveillance.

a, A graph showing the fold change in *C. elegans* sptl-1 transcript level compared to control (n = 3). Error bars represent s.d.

b, hsp-6p:gfp worms raised on control or sptl-1(RNAi) (n = 40). c, Body wall muscle animals expressing a mitochondrially localized GFP reporter. The animals were subjected to sptl-1(RNAi) for 36 h and transferred onto the second RNAi (control, fzo-1, fis-1 or drp-1). The hyperfusion of mitochondria observed under sptl-1(RNAi) is dependent on the fusion machinery as disruption of mitochondrial fusion by inactivating fzo-1 or fis-1 partially restored the tubular structure. By contrast, inhibition of the gene drp-1 that governs fission led to mitochondrial hyperfusion. The images were taken 36 h after they were placed on the second RNAi. d, A graph showing the percentage of worms which avoid the spg-7(RNAi) bacteria lawn 48 h after they were initially placed on the plates (n = 4). Error bars represent s.d. e, A graph showing the percentage of worms that avoid the bacteria lawn 8 h after the addition of antimycin (n = 4). Error bars represent s.d. f, Wild-type N2 or sptl-1(ok1693) mutant animals raised in the presence or absence of 0.8 μM antimycin. Photos were taken 4 days after the synchronized L1 worms were placed on the plates. g, hsp-6p:gfp animals were raised on sptl-1(RNAi) for 36 h and transferred onto a subset of wild microbes. The images were taken after 2 days.
Extended Data Figure 5 | Ceramide biosynthesis is required for mitochondrial surveillance.  

a. Genotyping of the sphingolipid metabolism pathway mutant alleles.  
b. c. Fold difference in hsp-6 transcript levels in wild type or sphingolipid metabolism pathway mutants (b) and hyl-1 or lagr-1 mutants (c) (n = 3). Error bars represent s.d.  
d. hsp-6p::gfp worms raised on sptl-1(RNAi) in the presence of increasing amounts of ceramide.  
e. hsp-6p::gfp in the presence or absence of ceramide.
Extended Data Figure 6 | Ceramide biogenesis is required for mitochondrial surveillance. a, The percentage of worms that avoid the bacterial lawn 8 h after the addition of antimycin. Animals were pre-treated with control RNAi, control RNAi with ceramide, sptl-1 RNAi or sptl-1 RNAi with ceramide (n = 4). Error bars represent s.d. b, Time course experiment for the induction of hsp-6p::gfp with antimycin. c, Dissected young adults after 4 h antimycin treatment were stained with anti-COX-IV antibody (green) and anti-ceramide antibody (red). d, Nomarski (top) and fluorescent (bottom) images of intestinal cells in atfs-1;hsp-16::atfs-1 D1-32.myc::gfp transgenic animals. e, hsp-6p::gfp worms raised on indicated RNAi in the presence or absence of ceramide.
Extended Data Figure 7 | Inhibition of the mevalonate pathway disrupts mitochondrial surveillance.  

**a**, *hsp-6p:gfp* animals raised on control or *hmgs-1(RNAi)* in the presence of antimycin.  

**b**, Immunoblotting of GFP expressed by *hsp-6p:gfp* animals, with or without antimycin.  

**c**, Antimycin-induced food avoidance in control or *hmgs-1(RNAi)* animals.  

**d**, Quantification of food avoidance (*n* = 4). Error bars represent s.d.  

**e**, Body wall muscle of control or *hmgs-1(RNAi)* animals expressing a mitochondrially localized GFP reporter.  

**f**, *hsp-6p:gfp* animals raised on *hmgs-1(RNAi)*, or *hmgs-1(RNAi)* with addition of mevalonate exposed to antimycin.  

**g**, *hsp-6p:gfp* animals treated with antimycin after pre-treatment with simvastatin or mevastatin.  

**h**, Mitochondrial immunostaining in HEK293T cells.  

**i**, ATP levels in C2C12 myotubes after treating with simvastatin or mevastatin (*n* = 3). Error bars represent s.d.
Extended Data Figure 8 | Statin treatment impairs mitochondrial surveillance. a, Diagram of the mevalonate pathway for the biosynthesis of cholesterol, ubiquinone and haem A, and protein N-glycosylation and prenylation. b, hsp-6p::gfp animals treated with antimycin after pre-treatment with increasing concentration of simvastatin. c, hsp-6p::gfp animals with mock, 80 μg/ml simvastatin or 80 μg/ml mevastatin treatment. d, hsp-6p::gfp animals raised on control RNAi, hmsg-1(RNAi), or hmsg-1(RNAi) with the addition of geranylgeranyl pyrophosphate. The animals were then treated with antimycin to induce mitochondrial damage. Statin toxicity has been proposed to be caused by the inhibition of Rab prenylation. Geranylgeranyl pyrophosphate (GGPP), a precursor of protein prenylation rescued the statin side effect in cell culture. GGPP also partially rescued the deficiency of mitochondrial surveillance and activated hsp-6p::gfp in antimycin-treated hmsg-1(RNAi) animals.
Extended Data Figure 9 | Mitochondrial immunostaining in HEK293T cells. The cells were treated with DMSO, 10 μM simvastatin or 10 μM mevastatin for 2 days.
### Extended Data Table 1 | Full list of genes identified in the genome-wide RNAi screen for mitochondrial surveillance

The table was sorted by the severity in the defect of the reporter gene induction. The intensity of blue denotes the severity of the defect in mitochondrial inactivation-induced gene response for each gene inactivation, with darker blue showing the most severe failure to upregulate the response genes. Yellow indicates previously reported genes for mitochondria unfolded response. N/A indicates that the gene inactivations render the *isp-1; hsp-6p::gfp* animals sick or lethal, which prevented assessment of GFP levels. The synthetic lethality of these mitochondrial surveillance-defective gene inactivations with a mitochondrial defect in *isp-1(qm150)* endorses their role in the homeostatic response that may allow the *isp-1(qm150)* mutant to be viable.

| gene | hsp-6p::gfp | hsp-6p::gfp on atp-2 (RNAi) | ural-6p::gfp on atp-2 (RNAi) | isp-1; hsp6p::gfp | Function |
|------|-------------|-------------------------------|-----------------------------|-------------------|----------|
| empty vector | | | | | |
| splt-1 | | | | | serine palmitoyl transferase |
| ran-4 | | | | | nuclear transport |
| tag-214 | | | | | E3 ubiquitin ligase |
| skp-1 | | | | | transcriptional cofactor |
| npo-6 | | | | | nuclear pore complex |
| Y4766A.29 | | | | | uncharacterized protein |
| let-70 | | | | | E2 ubiquitin conjugating enzyme |
| thoc-2 | | | | | transcription factor/nuclear export |
| syx-5 | | | | | lethal, syntaxin, vesicular transport |
| snap-1 | | | | | lethal, vesicular transport |
| gsp-2 | | | | | lethal, Phosphatase |
| F40F12.7 | | | | | lethal, CREB binding protein |
| atfs-1 | | | | | transcription factor |
| W04A4.5 | | | | | uncharacterized protein |
| imb-3 | | | | | nuclear transport |
| smg-1 | | | | | SMG-associated and Lethal |
| hmps-1 | | | | | HMG-CoA synthase |
| YS4E10BR.5 | | | | | signal peptidase complex subunit |
| F18F11.5 | | | | | protein kinase |
| pas-3 | | | | | proteasome subunit |
| nxt-1 | | | | | nuclear export protein |
| ccpp-1 | | | | | mitochondrial protease |
| Y82E9BR.13 | | | | | uncharacterized protein |
| fat-6 | | | | | fatty acid desaturase |
| dve-1 | | | | | DNA-binding protein |
| vps-32.2 | | | | | vacuolar protein sorting |
| ast-1 | | | | | transcription factors |
| wnk-1 | | | | | protein kinase |
| unc-60 | | | | | actin depolymerizing factor |
| inx-17 | | | | | INNexin |
| pno-92 | | | | | glutamine/asparagine-rich |
| itr-1 | | | | | inositol triphosphate receptor |
| dss-1 | | | | | 26S proteasome subunit |
| M03F4.6 | | | | | epidermal growth factor-like |
| sos-1 | | | | | guanine nucleotide exchange |
| Y48G10A.4 | | | | | uncharacterized protein |
| dcp-66 | | | | | lethal, transcriptional repressor |
| hda-1 | | | | | histone deacetylase 1 |
| Y1707B.18a | | | | | Methytransferase |
| celh-20 | | | | | homeodomain co-factor |
| elo-3 | | | | | fatty acid elongase |
| pkc-3 | | | | | protein kinase |
| hpo-10 | | | | | Hypersensitive to Pore-forming toxin |
| ketni-1 | | | | | transcription regulated by hypoxia |
| cdc-42 | | | | | RHO GTPase |

Lethal *isp-1; hsp-6p::gfp* animals were lethal at the time of scoring GFP. Thus the failure to respond to *isp-1* mitochondrial dysfunction in these gene inactivations is deleterious.

**from the strongest inhibition to the weakest inhibition of GFP inducer**

**genes identified previously to be involved in mitochondrial stress response**

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