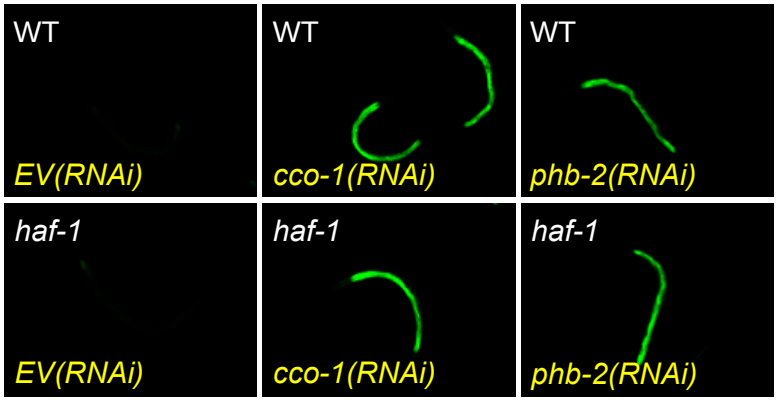
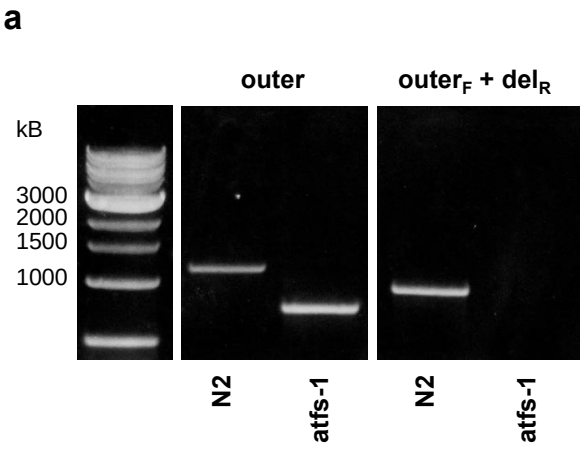
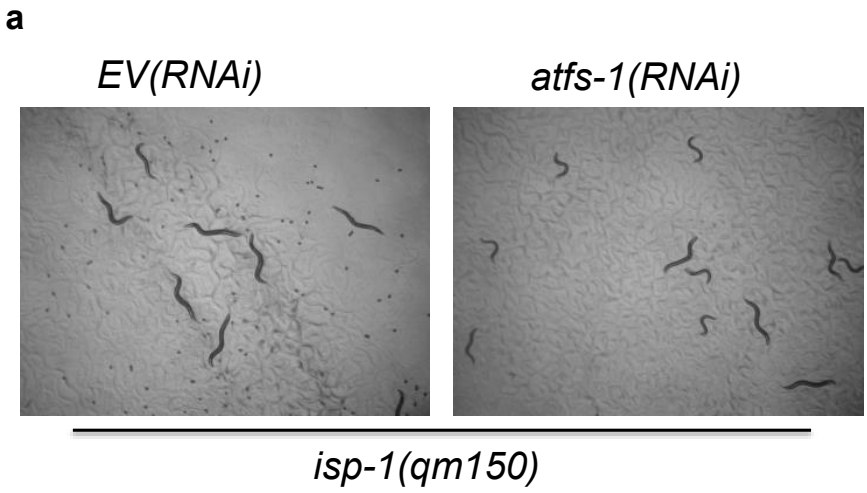
a



Supplementary Figure 1. HAF-1 is not required for UPR^{mt} induction caused by *cco-1* and *phb-2* RNAi. *hsp-6$_p$::gfp* induction was measured 3 days from hatch at 20°C in wild-type and *haf-1(ok705)* animals.



Supplementary Figure 2. Genotyping of *atfs-1(tm4525)* mutant. Outer primers, which bind outside the deletion, give a WT band of ~1200 bp and a *tm4525* band of ~800. The del$_R$ primer is inside the *tm4525* deletion. The outer$_F$ and del$_R$ give a WT band of ~1000 bp and no *tm4525* band.



Supplementary Figure 3. The developmental rate of *isp-1(qm150)* is delayed by *atfs-1(RNAi)*. (A) *isp-1(qm150)* were grown from egg on *EV(RNAi)* and *atfs-1(RNAi)* for 6 days.

Supplementary Table 1. Validated RNAi Inducers of the UPR^{mt}.

Gene Name	NCBI KOG Annotation	PPOD (Human | Yeast Orthologue)	Mito. Targeted	Extended Lifespan	Reported UPR^{mt} inducer
E04A4.5	Mitochondrial import inner membrane translocase, subunit TIM17	TIM17A/TIM17 B | TIM17	A, B, D		
F15D3.7	Mitochondrial import inner membrane translocase, subunit TIM23	TIMM23B | TIM23	A, B, D		G, H
T09B4.9	Mitochondrial import inner membrane translocase, subunit TIM44	TIM44 | TIM44	A, B, C, D		F
W02F12.5	Dihydrolipoamide succinyltransferase (2-oxoglutarate dehydrogenase, E2 subunit)	DLTS | KGD2	B, C, D		H
dnj-21	Molecular chaperone (DnaJ superfamily)	TIM14;DNAJC15; DNAJC19 | TIM14/PAM18	A, B, D		F
lpd-9			C, D		
phb-2		PHB2 | PHB2	A, B, D		F
C04C3.3	Pyruvate dehydrogenase E1, beta subunit	PDHB | PDB1	A, B, C, D		
wah-1	Programmed cell death 8 (apoptosis-inducing factor)	AIFM1 |	A, B, C, D		
F45G2.8	Uncharacterized conserved protein	PAM16 | PAM16	A, B, D		
Y54G9A.7			C, D		
ech-6	Enoyl-CoA hydratase	ECHS1 |	A, B, C, D		
Y110A7A.19	Uncharacterized conserved protein	PTCD3 |	B, C, D		
sco-1	Putative cytochrome C oxidase assembly protein	SCO1 | SCO1; SCO2	B, D	E	
cchl-1	Holocytochrome c synthase/heme-lyase	CCHL | CYC3	A, B, D	WBRNAi 00063981	I
ril-1			D	WBRNAi 00063960	I
tomm-22	Translocase of outer mitochondrial membrane complex, subunit TOM22	TOM22 |	B, D		
Y22D7AL.10	Mitochondrial chaperonin	HSPE1 | HSP10	B, C, D		
Y17G9B.5	Intermediate in Toll signal transduction pathway (ECSIT)	ECSIT |	B, C, D		
his-5	Histone H4	Histone H4 | HHF2	C		
letm-1	Ca2+-binding transmembrane protein LETM1/MRS7	LETM1 | MDM38	B, C		
C08F8.2	Mitochondrial RNA helicase SUV3, DEAD-box superfamily	SUPV3L1 | SUV3	A, B, C		

F02A9.4	3-Methylcrotonyl-CoA carboxylase	MCCC2 |	A, B, C		
hsp-60	Mitochondrial chaperonin, Cpn60/Hsp60p	HSPD1 | HSP60	A, B, C		F, H
Y38E10A.24					
tkt-1	Transketolase	TKTL1; TKTL2 | TKL1; TKL2		WBRNAi 00078549	H
ant-1.1	Mitochondrial ADP/ATP carrier proteins	ANT1; ANT2; ANT3 | AAC1; AAC2; AAC3	A, B	WBRNAi 00078570	
drp-1	Vacuolar sorting protein VPS1, dynamin, and related proteins	DRP1 | DNM1	A, B		
Y52B11C.1					
F15D3.6	Predicted member of the intramitochondrial sorting protein family	SLMO2 | UPS2; UPS3	B		
Y24D9A.8	Transaldolase	TALDO1 | TAL1			
W02B12.9	Mitochondrial carrier protein MRS3/4	MFRN | MRS3; MRS4	A, B		
AC8.6					
F21C3.10					

RNAi knockdown of the following 34 genes significantly induced the *hsp-6*$_p$*::gfp* reporter. The eukaryotic orthologous groups (KOGs) annotation and PPOD derived human and yeast orthologues for each gene are shown. Annotation for mitochondrial targeting is based off of Go Term "Mitochondrion" annotation (A), orthology to a known mitochondrial genes by PPOD (B), MitoProt II probability estimation >0.8 (C), or presence in a published *C. elegans* mitochondrial proteome data set by Li, J., et al., Proteomics, 2009 [1] (D). Reported effects on longevity are shown as WBRNAi experiments or Chen, D., et al., Aging Cell, 2007 [2] (E). Reported effects on the UPR^{mt} are based on the work of Yoneda, T., et al., Journal of Cell Science, 2004 [3] (F), and Nargund, A., et al., Science, 2012 [4] (G). Melo, J., et al., Cell, 2012 [5] (H). Shore, D., et al., PLoS Genet, 2012 [6] (I).

Supplementary Table 2. Non-Validated RNAi Inducers of the UPR^{mt}.

Gene Name	NCBI KOG Annotation	Putative Mito. Function	Mito. Targeted	Extended Lifespan	Reported UPR^{mt} inducer
F44G4.2	Unnamed protein	Complex I	✔		
B0491.5		Complex I	✔	C	
nuo-1	NADH:ubiquinone oxidoreductase, NDUFV1/51kDa subunit	Complex I	✔	WBRNAi 00066177	G
nduf-2.2	NADH:ubiquinone oxidoreductase, NDUFS2/49 kDa subunit	Complex I	✔	WBRNAi 00078565	
ZK809.3	NADH:ubiquinone oxidoreductase, NDUFB6/B17 subunit	Complex I	✔	WBRNAi 00078577	
F31D4.9		Complex I			
gas-1	NADH:ubiquinone oxidoreductase, NDUFS2/49 kDa subunit	Complex I	✔		
nduf-7	NADH-ubiquinone oxidoreductase, NUFS7/PSST/20 kDa subunit	Complex I	✔		
C16A3.5	NADH:ubiquinone oxidoreductase, NDUFB9/B22 subunit	Complex I	✔		
nduf-5	NADH:ubiquinone oxidoreductase, NDUFS5/15kDa	Complex I	✔		
D2030.4	NADH:ubiquinone oxidoreductase, NDUFB7/B18 subunit	Complex I	✔	WBRNAi 00065275	D, E, G
F59C6.5	NADH-ubiquinone oxidoreductase, subunit NDUFB10/PDSW	Complex I	✔	WBRNAi 00066189	D, G
T20H4.5	NADH:ubiquinone oxidoreductase, NDUFS8/23 kDa subunit	Complex I	✔	WBRNAi 00065279	E, G
nduf-6	NADH:ubiquinone oxidoreductase, NDUFS6/13 kDa subunit	Complex I	✔		D
Y51H1A.3	NADH:ubiquinone oxidoreductase, NDUFB8/ASHI subunit	Complex I			
F42G8.10	Uncharacterized conserved protein	Complex I			
nuo-4	NADH:ubiquinone oxidoreductase, NDUFA10/42kDa subunit	Complex I		WBRNAi 00065278	E, G
ucr-1	Mitochondrial processing peptidase, beta subunit, and related enzymes (insulinase superfamily)	Complex III	✔	WBRNAi 00078564	

T02H6.11	Ubiquinol cytochrome c reductase, subunit QCR7	Complex III	✔	B	
F45H10.2	Ubiquinol cytochrome c reductase, subunit QCR8	Complex III	✔		
R07E4.3	Ubiquinol cytochrome c reductase, subunit QCR8	Complex III	✔		
F29B9.11		Complex IV	✔		
cco-2	Cytochrome c oxidase, subunit Va/COX6	Complex IV	✔	WBRNAi 00063991	G
F29C4.2	Unnamed protein	Complex IV	✔	WBRNAi 00078571	
cco-1	Cytochrome c oxidase, subunit Vb/COX4	Complex IV	✔	WBRNAi 00063967	D, E, F
F26E4.6	Cytochrome c oxidase, subunit VIIc/COX8	Complex IV	✔	WBRNAi 00065276	D, E, G
atp-2	F0F1-type ATP synthase, beta subunit	Complex V	✔	WBRNAi 00066144	F, G
atp-4	Mitochondrial F1F0-ATP synthase, subunit Cf6 (coupling factor 6)	Complex V	✔	WBRNAi 00063979	G
R53.4	Mitochondrial F1F0-ATP synthase, subunit f	Complex V	✔	C	
F32D1.2	Mitochondrial F1F0-ATP synthase, subunit epsilon/ATP15	Complex V	✔		
F58F12.1	Mitochondrial F1F0-ATP synthase, subunit delta/ATP16	Complex V	✔		
H28O16.1	F0F1-type ATP synthase, alpha subunit	Complex V		WBRNAi 00066165	D, F, G
atp-3	Mitochondrial F1F0-ATP synthase, subunit OSCP/ATP5	Complex V		WBRNAi 00063970	G
R04F11.2	Mitochondrial F1F0-ATP synthase, subunit e	Complex V	✔		
asg-2	Mitochondrial F1F0-ATP synthase, subunit g/ATP20	Complex V	✔	WBRNAi 00065274	E, G
asg-1	Mitochondrial F1F0-ATP synthase, subunit g/ATP20	Complex V	✔		D
T26E3.7	F0F1-type ATP synthase, alpha subunit	Complex V			
Y82E9BR.3	Mitochondrial F1F0-ATP synthase, subunit c/ATP9/proteolipid	Complex V			
cyc-2.1	Cytochrome c	Cytochrome C	✔	WBRNAi 00078573	
F33D4.5	50S ribosomal protein L1	Mitochondrial Translation	✔	WBRNAi 00078562	A
C30C11.1	Mitochondrial ribosomal protein L32	Mitochondrial Translation	✔		
F09G8.3	Mitochondrial/chloroplast ribosomal protein S9	Mitochondrial Translation	✔	WBRNAi 00078568	
F59A3.3	Mitochondrial/chloroplast ribosomal protein L24	Mitochondrial Translation	✔	C	
K01C8.6	Mitochondrial ribosomal protein L10	Mitochondrial Translation	✔	C	

tag-264	Mitochondrial 28S ribosomal protein S30	Mitochondrial Translation	✔	C	D, A
tufm-1	Mitochondrial translation elongation factor Tu	Mitochondrial Translation	✔	WBRNAi 00078595	
T04A8.11		Mitochondrial Translation	✔		
dap-3	Mitochondrial ribosome small subunit component, mediator of apoptosis DAP3	Mitochondrial Translation	✔		
F45E12.5	Mitochondrial ribosomal protein L14	Mitochondrial Translation			
mrpl-37		Mitochondrial Translation	✔	A	A
mrps-5	Ribosomal protein S5	Mitochondrial Translation	✔	A	A
F56B3.8	Mitochondrial/chloroplast ribosomal protein L2	Mitochondrial Translation	✔	A	A
sars-2	Seryl-tRNA synthetase	Mitochondrial Translation	✔		
T13H5.5	Mitochondrial ribosomal protein S18b	Mitochondrial Translation	✔		
T23B12.2	Mitochondrial/chloroplast ribosomal protein L4	Mitochondrial Translation	✔		
T23B12.3	Mitochondrial/chloroplast ribosomal protein S2	Mitochondrial Translation	✔		
W04B5.4	Mitochondrial ribosomal protein L30	Mitochondrial Translation	✔		
Y39A1A.6	Mitochondrial/chloroplast ribosomal protein L22	Mitochondrial Translation	✔		
Y39B6A.39	Mitochondrial ribosomal protein S28	Mitochondrial Translation	✔		
Y48C3A.10	Mitochondrial/chloroplast ribosomal protein L20	Mitochondrial Translation	✔		
Y34D9A.1	Phosphatidylethanolamine binding protein	Mitochondrial Translation	✔		

This table represents 61 positive inducers of *hsp-6$_p$::gfp* identified in our screen that target components of the electron transport chain or mitochondrial translation machinery. Since similar hits have been reported to induce the UPR^{mt}, we focused our validation elsewhere, but this list is expected to represent genuine inducers of the UPR^{mt} and in some cases, have been validated by other groups. Putative Mitochondrial Function is based off KOG annotation, PPOD orthology analysis, or blastP homology analysis. Mitochondrial targeting is based of inclusion in the C. elegans mitochondrial proteome data set by Li, J., et al., Proteomics, 2009 [1]. Reported effects on longevity or UPR^{mt}

induction are shown as WBRNAi experiments or Houtkooper, R., et al., Nature, 2013 [7] (A), Lee, S.S., et al., Nature Gen, 2003 [8] (B), Chen, D., et al., Aging Cell, 2007 [2] (C), Yoneda, T., et al., Journal of Cell Science, 2004 [3] (D), Hamilton, B., et al., Genes Dev, 2005 [9] (E), Melo, J., et al., Cell, 2012 [5] (F), and Shore, D., et al., PLoS Genet, 2012 [6] (G).

Supplementary Table 3. Effects of 34 Validated RNAi inducers of the UPR^{mt} on other stress response reporters.

RNAi/cond.	*hsp-6$_p$::gfp*	*hsp-60$_p$::gfp*	*hsp-4$_p$::gfp*	*hsp-16.2$_p$::gfp*
EV	1 ± 0.02	1 ± 0.02	1 ± 0.04	1 ± 0.03
phb-2	12.29 ± 0.52	1.88 ± 0.07	1.23 ± 0.05	0.82 ± 0.07
EV + Heat Shock	#N/A	#N/A	#N/A	7.13 ± 0.46
EV + Tunicamycin	#N/A	#N/A	7.15 ± 0.63	#N/A
Y17G9B.5	1.4 ± 0.11	1.07 ± 0.05	0.92 ± 0.05	0.84 ± 0.04
his-5	2.27 ± 0.14	0.96 ± 0.03	1.18 ± 0.07	0.09 ± 0.01
tkt-1	2.42 ± 0.28	0.76 ± 0.02	0.98 ± 0.05	0.57 ± 0.02
ant-1.1	2.46 ± 0.33	0.86 ± 0.03	0.72 ± 0.04	0.83 ± 0.03
F21C3.10	2.56 ± 0.2	1.02 ± 0.05	1.09 ± 0.06	0.94 ± 0.06
drp-1	2.96 ± 0.39	0.92 ± 0.04	0.78 ± 0.03	1.02 ± 0.06
C04C3.3	3.19 ± 0.35	1.07 ± 0.05	1.19 ± 0.06	0.87 ± 0.04
Y24D9A.8	3.49 ± 0.25	0.75 ± 0.04	0.91 ± 0.08	0.55 ± 0.02
W02B12.9	3.58 ± 0.34	1.01 ± 0.03	1.16 ± 0.12	0.81 ± 0.05
sco-1	3.87 ± 0.59	1.16 ± 0.04	0.84 ± 0.09	0.92 ± 0.05
C08F8.2	5.08 ± 0.72	1.06 ± 0.05	0.9 ± 0.03	0.87 ± 0.05
Y52B11C.1	5.35 ± 0.39	1.35 ± 0.05	0.95 ± 0.06	1.1 ± 0.05
F15D3.6	6.03 ± 0.35	1.36 ± 0.07	0.82 ± 0.03	0.86 ± 0.05
cchl-1	8.2 ± 1.04	1.42 ± 0.04	0.84 ± 0.04	1.01 ± 0.04
ech-6	8.97 ± 1.37	1.45 ± 0.06	0.93 ± 0.04	0.93 ± 0.04
hsp-60	11.14 ± 0.78	1.01 ± 0.03	1.09 ± 0.04	0.59 ± 0.03
F02A9.4	11.17 ± 0.96	1.33 ± 0.06	0.87 ± 0.07	0.79 ± 0.03
AC8.6	11.62 ± 1	1.28 ± 0.06	0.76 ± 0.04	1 ± 0.04
tomm-22	11.93 ± 0.81	1.14 ± 0.05	1.19 ± 0.11	0.65 ± 0.04
T09B4.9	12.04 ± 0.73	1.65 ± 0.14	1.02 ± 0.04	0.7 ± 0.03
Y38E10A.24	12.16 ± 0.67	1.07 ± 0.05	1.44 ± 0.08	0.72 ± 0.02
Y22D7AL.10	12.85 ± 0.71	0.93 ± 0.03	0.76 ± 0.06	0.84 ± 0.04
wah-1	13.17 ± 1.99	1.36 ± 0.04	1.14 ± 0.04	0.93 ± 0.06
E04A4.5	13.33 ± 1.12	1.4 ± 0.11	0.97 ± 0.09	0.64 ± 0.02
Y54G9A.7	14.04 ± 1.31	1.28 ± 0.05	1.14 ± 0.08	0.92 ± 0.06
Y110A7A.19	14.41 ± 1.3	1.45 ± 0.08	0.84 ± 0.05	0.77 ± 0.03
ril-1	14.71 ± 2.81	1.05 ± 0.06	0.59 ± 0.04	0.35 ± 0.02
letm-1	16.06 ± 2.44	1.22 ± 0.05	0.83 ± 0.03	0.73 ± 0.03
phb-2	16.11 ± 2.36	1.99 ± 0.11	1.2 ± 0.06	0.55 ± 0.03
lpd-9	16.37 ± 1.45	1.46 ± 0.07	1.15 ± 0.08	0.97 ± 0.05
W02F12.5	16.47 ± 1.73	1.15 ± 0.06	1.1 ± 0.07	0.73 ± 0.03
F45G2.8	16.95 ± 1.4	1.37 ± 0.05	1.07 ± 0.09	0.81 ± 0.04
F15D3.7	17.52 ± 1.13	1.67 ± 0.08	0.93 ± 0.06	0.61 ± 0.04
dnj-21	22.12 ± 2.48	1.93 ± 0.1	1.19 ± 0.08	0.87 ± 0.04

RNAi knockdown of the shown genes significantly induced expression of the *hsp-6$_p$::gfp* reporter. The effect on mean GFP fluorescence from each RNAi clone is shown relative to empty vector (EV) treated animals (± SEM, N=3). Worms were grown at 20°C and

imaged 3 days from hatching. Gene knockdowns that significantly increased (student's t-test with Bonferroni correction) each reporter are highlighted in gray.

Supplementary Table 4. Primers used for qRT-PCR. qRT-PCR was used to measure expression of mitoUPR targets.

Primer	Sequence
hsp-6_F	TCGTGAACGTTTCAGCCAGA
hsp-6_R	CTCAGCGGCATTCTTTTCGG
hsp-60_F	GGGGAAGCCCAAAGATCACA
hsp-60_R	TCCAGCCTCCTCATTAGCCT
timm-23_F	CTCCGATCGATCTCAGTGCC
timm-23_R	ATAGGGTGTCATTTGCCGGG

SUPPLEMENTARY REFERENCES

1) Li, J., Cai, T., Wu, P., Cui, Z., Chen, X., Hou, J., Xie, Z., Xue, P., Shi, L., Liu, P., *et al.* (2009). Proteomic analysis of mitochondria from Caenorhabditis elegans. Proteomics *9*, 4539-4553.

2) Chen, D., Pan, K.Z., Palter, J.E., and Kapahi, P. (2007). Longevity determined by developmental arrest genes in Caenorhabditis elegans. Aging Cell *6*, 525-533.

3) Yoneda, T. et al. Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. J Cell Sci 117, 4055-4066, doi:10.1242/jcs.01275 (2004).

4) Nargund, A. M., Pellegrino, M. W., Fiorese, C. J., Baker, B. M. & Haynes, C. M. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science 337, 587-590, doi:10.1126/science.1223560 (2012).

5) Melo, J.A., and Ruvkun, G. Inactivation of Conserved C. elegans Genes Engages Pathogen- and Xenobiotic-Associated Defenses. Cell 149, 452–466 (2012).

6) Shore, D.E., Carr, C.E., and Ruvkun, G. Induction of Cytoprotective Pathways Is Central to the Extension of Lifespan Conferred by Multiple Longevity Pathways. PLoS Genet 8, e1002792 (2012).

7) Houtkooper, R. H. et al. Mitonuclear protein imbalance as a conserved longevity mechanism. Nature 497, 451-457, doi:10.1038/nature12188 (2013).

8) Lee, S. S. et al. A systematic RNAi screen identifies a critical role for mitochondria in C. elegans longevity. Nat Genet 33, 40-48, doi:10.1038/ng1056 (2003).

9) Hamilton, B. et al. A systematic RNAi screen for longevity genes in C. elegans. Genes Dev 19, 1544-1555 (2005).