A

Family	Protein	BLAST score	Mitoprot Score
CEBPG	CEBPG	16.2	17
CEBP	CEBPA	27.7	12
	CEBPB	28.9	6
	CEBPD	27.7	2
	CEBPE	15.8	1
DDIT3	DDIT3	17.7	10
CREB	ATF1	18.9	11
	CREB-1	15.0	2
	CREM	25.4	4
OASISA	CREB3	30.4	0
	CREB3L3	38.1	0
	CREB3L4	17.3	0.4
OASISB	CREB3L1	22.7	0.7
ATF6	ATF6	25.0	0.7
	ATF6B	28.9	6
CREBZF	CREBZF	21.9	35
XBP1	XBP1	31.2	0.3
NFIL3	NFIL3	19.2	0.3
ATF2	ATF2	27.7	19
	ATF7	31.6	4.5
	CREB5	31.2	6
JUN	JUN	33.1	18
	JUNB	16.5	15
	JUND	31.6	4
FOS	FOS	20.8	3
	FOSB	15.4	11
	FOSL1	18.5	9
	FOSL2	18.5	7
ATF3	ATF-3	21.9	1
	JDP2	20.8	3
ATF4	ATF-4	42.0	12
	ATF-5	40.0	30
B-ATF	B-ATF	25.4	8.6
	B-ATF2	21.2	0
	B-ATF3	28.1	18
PAR	DBP	18.9	56
	HLF	21.9	10
	TEF	30.0	1
SMAF	MAFF	23.9	6
	MAFG	13.1	3
LMAF	MAFB	23.1	5
	MAF	23.9	3
	MAFA	NS	1
	NRL	NS	2
NFE2	NFE2-p45	25.0	20
	NFE2L1	25.0	10
	NFE2L2	26.9	0
	NFE2L3	26.9	59
BACH	BACH1	27.3	4
	BACH	26.6	3

B

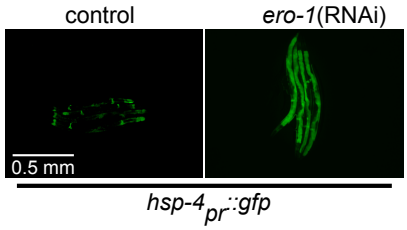

Figure S1, related to Figure 1
ATF5 has the highest homology to ATFS-1 and contains a putative mitochondrial targeting sequence.
(A) Table of bZip proteins with each protein's Mitoprot II and BLAST score. Mitoprot II score is the percentage likelihood of import into mitochondria.

(B) Photomicrographs of *hsp-4$_{pr}$::gfp* treatd with control or *ero-1*(RNAi). Scale bar, 0.5 mm.

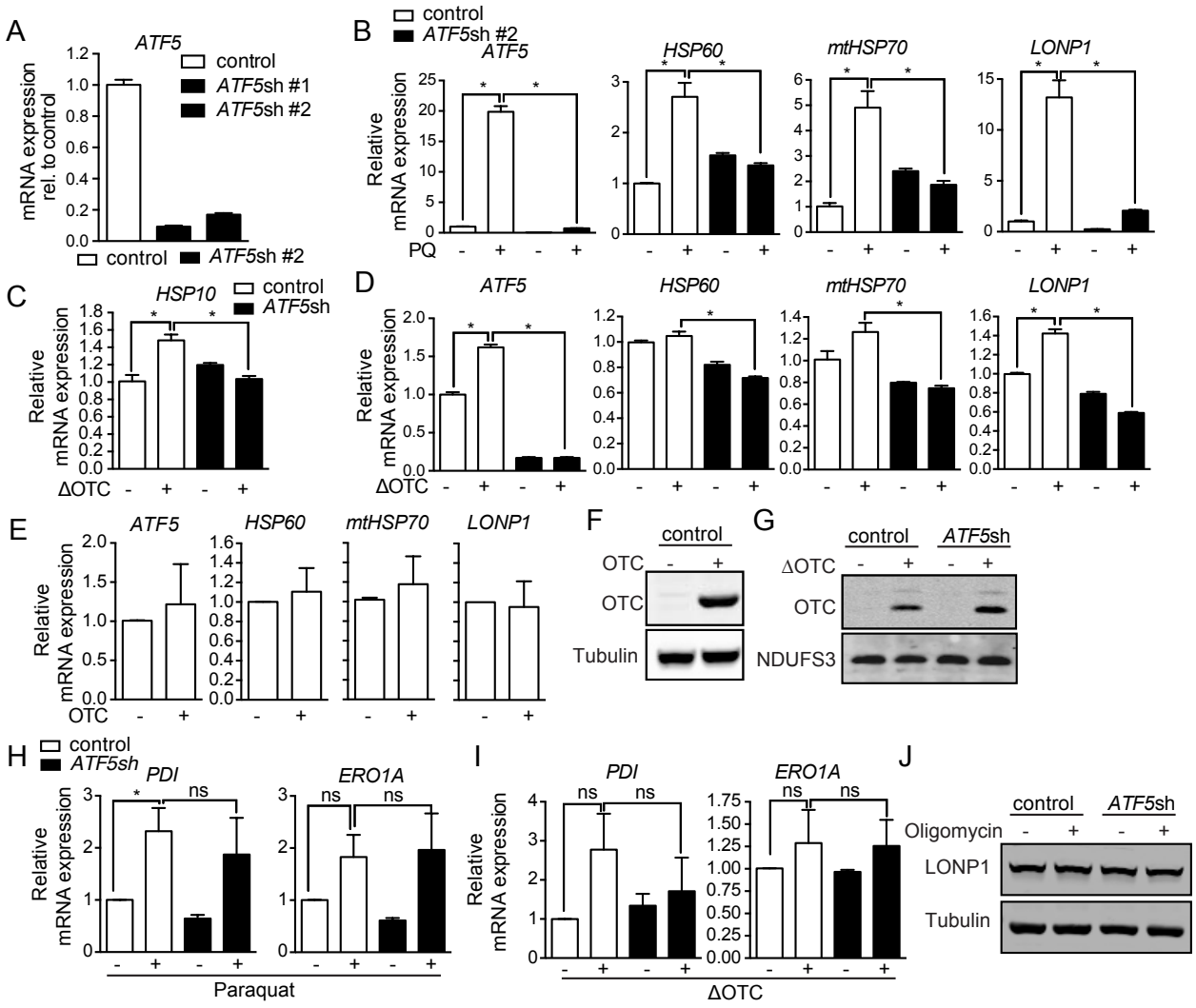

Figure S2, related to Figure 2

ATF5 depletion shows ATF5 is required for UPR^{mt} gene expression in response to stress.

(A) Expression levels of *ATF5* mRNA in HEK 293T cells treated with vector shRNA, *ATF5* shRNA #1, or *ATF5* shRNA #2 (see Experimental Procedures) (n=3, mean ± SEM, *p<0.05).

(B) Expression levels of *ATF5*, *HSP60*, *mtHSP70* and *LONP1* mRNA in control or *ATF5* shRNA #2 HEK 293T cells with or without paraquat (PQ) (n=3, mean ± SEM, p<0.05,).

(C) Expression levels of *HSP10* mRNA in control or *ATF5* shRNA #1 HEK 293T with or without ΔOTC expression (n=3, mean ± SEM *p<0.05).

(D) Expression levels of *ATF5*, *HSP60*, *mtHSP70* and *LONP1* mRNA in control or *ATF5* shRNA #2 HEK 293T cells with or without ΔOTC expression (n=3, mean ± SEM, p<0.05,).

(E) Expression levels of *ATF5, HSP60*, *mtHSP70*, and *LONP1* mRNA in HEK 293T cells with or without OTC expression (n=2 combined experiments, mean ± SEM, p<0.05).

(F-G) Immunoblots from control or *ATF5* shRNA HEK293T cell lysates with or without OTC (F), or ΔOTC (G) expression.

(H-I) Expression levels of *PDI*, and *ERO1A* mRNA in control or *ATF5* shRNA HEK293T cells treated with either (H) PQ, or (I) expressing ΔOTC (n=3 combined experiments, mean ± SEM, *p<0.05).

(J) Immunoblots from control or *ATF5* shRNA HEK293T cells with or without oligomycin treatment.

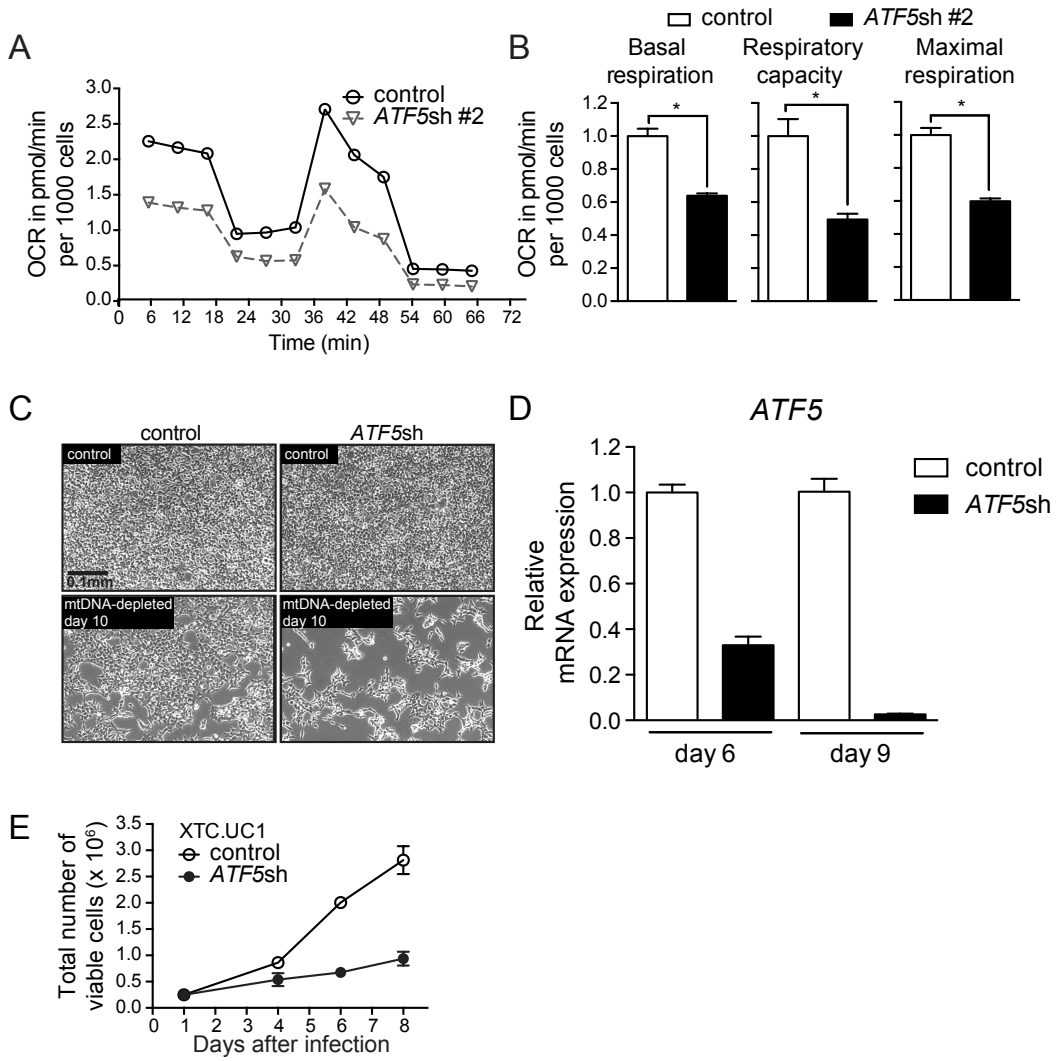

Figure S3, related to Figure 4
ATF5 promotes survival and recovery from mitochondrial stress.
(A-B) Oxygen consumption rates (OCR) of control or *ATF5* shRNA #2 HEK 293T cells (n=15 (control) and n=16 (*ATF5*sh), mean ± SEM, *p<0.05).

(C) Photomicrographs of control and *ATF5* shRNA HEK 293T cells following 6 days of mtDNA depletion by EtBr treatment. The cells were imaged 4 days after removal of EtBr. Scale bar, 0.1 mm.

(D) Expression levels of *ATF5* mRNA in control or *ATF5* shRNA HEK293T cells following 6 days of mtDNA depletion by EtBr treatment (n=3, mean ± SEM).

(E) Growth curve of control or *ATF5* shRNA XTC.UC1 cells (n=2, mean ± SEM).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Worm strains and plasmids

The reporter strains *hsp-60$_{pr}$::gfp(zcIs9)V*, *hsp-4$_{pr}$::gfp(zcIs4)V*, *atfs-1(tm4525)V* and *hsp-60$_{pr}$*::gfp* and RNAi feeding conditions have been described previously [S1-3]. Induction of *hsp-4$_{pr}$::gfp* by heat shock was performed as in [S4]. Briefly, *hsp-4$_{pr}$::gfp* was incubated at 30°C for 1 hour to induce heat shock, then imaged.

To generate the *hsp-16$_{pr}$::ATF5* expression plasmid, *ATF5* cDNA was amplified and ligated into the *hsp-16$_{pr}$::atfs-1* plasmid replacing *atfs-1* [S1]. Generation of *hsp-16$_{pr}$::ATF4* was achieved by amplifying *ATF4* cDNA and ligating into *hsp-16$_{pr}$::ATF5* plasmid, replacing *ATF5*. The transgenic *C. elegans* lines were generated by co-injecting either the *hsp-16$_{pr}$::ATF5* or *hsp-16$_{pr}$::ATF4* plasmid (25 ng/µl) with a *myo-3$_{pr}$::mCherry* (60 ng/µl) marker plasmid, and pBluescript (65 ng/µl) into *hsp-60$_{pr}$::gfp; atfs-1(tm4525)* generating multiple stable extra-chromosomal arrays. The *hsp-16$_{pr}$::ATF5* transgene was crossed into the described reporter worms. The *myo-3$_{pr}$::ΔOTC* plasmid was generated by amplifying ΔOTC [S5] and ligating it into the *myo-3$_{pr}$::mCherry::ubl-5* plasmid by replacing the *ubl-5* open reading frame. The ATF5::GFP mammalian expression plasmid was generated by amplifying *ATF5* cDNA and ligating into the EGFP-N1 plasmid (Clontech). All plasmids were confirmed by sequencing.

Cell culture and plasmids

HeLa and HEK 293T cells were cultured in DMEM supplemented with 10 % FBS, 10 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin/100 µg/ml streptomycin. *ATF5*-knockdown cells were generated through lentiviral-driven gene silencing using two different shRNAs (ATF5sh #1(Thermo Scientific): 5'-AAGTCTTCCATCTGTTCCAGC-3'; ATF5sh #2(Sigma): 5'-TGTCTTGGATACTCTGGACTT-3') expressed by the pLKO.1 vector, while pLKO.1 was used as a control (Sigma). To induce mitochondrial stress, cells were either transfected with ΔOTC plasmid [S5], or treated with 400 µM paraquat for 48 hours unless stated otherwise. Treatment of HEK 293T cells with antimycin (Sigma), piericidin (Santa Cruz), and/or oligomycin (Sigma) was for 24 hours before collecting cells for qPCR as described below. Cellular doubling times were calculated as described [S6].

RNA Isolation, qRT-PCR and Western Blots

Total RNA was isolated using RNA Stat-60 (amsbio) or RNEasy Plus Mini-Kit (Qiagen). cDNA synthesis and qPCR was performed as described [S7]using the following primers: *ATF5* forward 5'-CTGGCTCCCTATGAGGTCCTTG-3' and reverse 5'-GAGCTGTGAAATCAACTCGCTCAG-3'; *HSP60* forward 5'-GATGCTGTGGCCGTTACAATG-3' and reverse 5'-GTCAATTGACTTTGCAACAGTCACAC-3'; *mtHSP70* forward 5'-CAAGCGACAGGCTGTCACCAAC-3' and reverse 5'-CAACCCAGGCATCACCATTGG-3'; *LONP1* forward 5'-CATTGCCTTGAACCCTCTC-3' and reverse 5'-ATGTCGCTCAGGTAGATGG-3'; *HD-5* forward 5' – ACCTTGCTATCTCCTTTGCAGG-3' and reverse 5' – CGGTTCGGCAATAGCAGGTG-3'; *HSP10* forward 5'-TGGCAGGACAAGCGTTTAG-3' and reverse 5'-GGTTACAGTTTCAGCAGCAC-3'; *PDI* forward 5'-TGAGAACATCGTCATCGCC -3 and reverse 5' – CGTTCCCCGTTGTAATCAATG – 3'; *ERO1A* forward 5' -CAAGGGACAAGTGAAGAGAAC -3' and reverse 5'- CCCCATTTCTTTTCTAACCAG -3'. And, *HPRT* forward 5'-CTTTGCTGACCTGCTGGATT-3' and reverse 5'-TCCCCTGTTGACTGGTCATT-3' was used as a reference gene.

SDS-Page, western blots and mouse liver sucrose fractionation were performed as described [S1, 8] using the following antibodies: OTC (Santa Cruz), Tubulin (Santa Cruz) NDUFS3 (Abcam), GFP (Roche), ATF5 [S9], and KDEL (Stressgen) and imaged on an Odyssey infrared imager (LI-COR).

Microscopy

HeLa cells were transfected using Lipofectamine 2000 (Life Technologies) with 1 µg of GFP, Histone 2B::GFP, or ATF5::GFP expressing plasmids. The cells were treated with 10 µM MG-132 for 6 hours, stained with MitoTracker Red FM (Life) for 30 minutes prior to fixation with 4% paraformaldehyde (Fisher Chemicals) and incubated with primary GFP antibody (Roche) followed by incubation with secondary antibody AlexaFluor488 (Life). Samples were imaged on a Nikon Eclipse T*i*.

C. elegans images were acquired as previously described [S1].

Respiration Analysis

Oxygen consumption was measured using a Seahorse Extracellular Flux Analyzer XF^{e}96 as described [S10]. Analysis plates were coated with Corning Cell-Tak. 64,000 cells were seeded per well in 30 µl of XF-Media by centrifugation. After a 25 minute incubation at 37°C in an incubator w/o CO_2, 145 µl of XF-Media was added to each well followed by another incubation period of 20 minutes, and then the plates were subjected to analysis using 1 µM oligomycin, 500 nM FCCP and 1 µM rotenone/antimycin as indicated. Data were normalized to cell number.

mtDNA Content
DNA was extracted from $2x10^6$ cells using phenol/chlorophorm/isoamyl alcohol (25:24:1) [S11] and mtDNA quantitation by qPCR was performed as described [S12] using the following primers: mtDNA 16S rRNA forward 5'-GCCTTCCCCCGTAAATGATA-3' and reverse 5'-TTATGCGATTACCGGGCTCT-3'; nDNA β2-M forward 5'-TGCTGTCTCCATGTTTGATGTATCT-3' and reverse 5'-TCTCTGCTCCCCACCTCTAAGT-3'.

Bioinformatics
Promoter search was performed using the Signal Search Analysis Server program FindM using the UPR^{mt} Element previously described [S2]. BLAST analysis against the whole sequence of ATFS-1 was done using the BLASTP 2.3.1+ program [S13].

Statistics
Unless stated otherwise, experiments were performed at least three times, or in triplicates and expressed as mean ± SEM. Bar graphs of qPCR and Seahorse data show one representative experiment. Group differences were assayed using two-tailed Student's t test. Significance was considered when $p \leq 0.05$. Pearson correlation coefficient was performed as described in [S14].

Supplemental References

S1. Nargund, A.M., Pellegrino, M.W., Fiorese, C.J., Baker, B.M., and Haynes, C.M. (2012). Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science *337*, 587-590.

S2. Nargund, A.M., Fiorese, C.J., Pellegrino, M.W., Deng, P., and Haynes, C.M. (2015). Mitochondrial and nuclear accumulation of the transcription factor ATFS-1 promotes OXPHOS recovery during the UPR(mt). Mol Cell *58*, 123-133.

S3. Calfon, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clark, S.G., and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature *415*, 92-96.

S4. Yoneda, T., Benedetti, C., Urano, F., Clark, S.G., Harding, H.P., and Ron, D. (2004). Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. J Cell Sci *117*, 4055-4066.

S5. Zhao, Q., Wang, J., Levichkin, I.V., Stasinopoulos, S., Ryan, M.T., and Hoogenraad, N.J. (2002). A mitochondrial specific stress response in mammalian cells. The EMBO journal *21*, 4411-4419.

S6. Chanvorachote, P., Luanpitpong, S., Chunhacha, P., Promden, W., and Sriuranpong, V. (2012). Expression of CA125 and cisplatin susceptibility of pleural effusion-derived human lung cancer cells from a Thai patient. Oncology letters *4*, 252-256.

S7. Pellegrino, M.W., Nargund, A.M., Kirienko, N.V., Gillis, R., Fiorese, C.J., and Haynes, C.M. (2014). Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. Nature *516*, 414-417.

S8. Graham, J.M. (2001). Purification of a crude mitochondrial fraction by density-gradient centrifugation. Current protocols in cell biology / editorial board, Juan S. Bonifacino ... [et al.] *Chapter 3*, Unit 3 4.

S9. Zhou, D., Palam, L.R., Jiang, L., Narasimhan, J., Staschke, K.A., and Wek, R.C. (2008). Phosphorylation of eIF2 directs ATF5 translational control in response to diverse stress conditions. The Journal of biological chemistry *283*, 7064-7073.

S10. Yoshida, S., Tsutsumi, S., Muhlebach, G., Sourbier, C., Lee, M.J., Lee, S., Vartholomaiou, E., Tatokoro, M., Beebe, K., Miyajima, N., et al. (2013). Molecular chaperone TRAP1 regulates a metabolic switch between mitochondrial respiration and aerobic glycolysis. Proceedings of the National Academy of Sciences of the United States of America *110*, E1604-1612.

S11. Guo, W., Jiang, L., Bhasin, S., Khan, S.M., and Swerdlow, R.H. (2009). DNA extraction procedures meaningfully influence qPCR-based mtDNA copy number determination. Mitochondrion *9*, 261-265.

S12. Venegas, V., and Halberg, M.C. (2012). Measurement of mitochondrial DNA copy number. Methods in molecular biology *837*, 327-335.

S13. Altschul, S.F., Wootton, J.C., Gertz, E.M., Agarwala, R., Morgulis, A., Schaffer, A.A., and Yu, Y.K. (2005). Protein database searches using compositionally adjusted substitution matrices. The FEBS journal *272*, 5101-5109.

S14. Dunn, K.W., Kamocka, M.M., and McDonald, J.H. (2011). A practical guide to evaluating colocalization in biological microscopy. American journal of physiology. Cell physiology *300*, C723-742.