Quirós et al., https://doi.org/10.1083/jcb.201702058

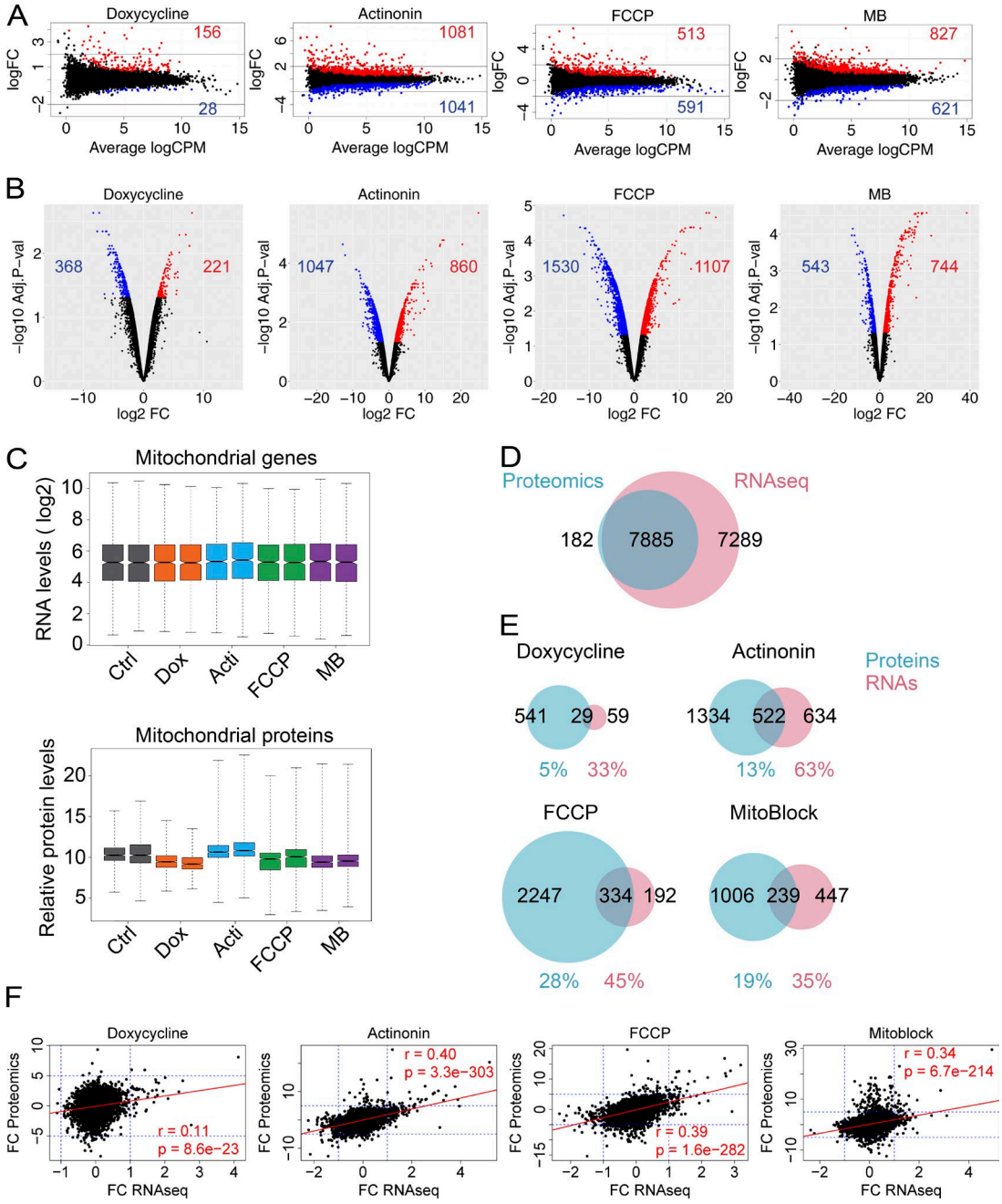


Figure S1. **RNA sequencing and proteomic analysis upon mitochondrial stress.** (A) Smear plots of RNA sequencing analysis in each condition, representing the relation of the logarithm base 2 of the fold change (logFC) relative to control and the mean of counts per million (logCPM) of each gene. (B) Volcano plots of quantitative proteomic analysis in each treatment, representing the relation of the logarithm base 10 of the p-value using a Bonferroni correction and the logarithm base 2 of fold change (log2FC). For both smear and volcano plots, genes significantly up-regulated are depicted in red and those significantly down-regulated are in blue using an FDR < 0.05. Also, the number of transcripts and proteins up- and down-regulated are shown. (C) Boxplots showing profiles of mitochondrial transcripts and proteins in RNA sequencing and quantitative proteomic analysis, respectively, showing changes in mitochondrial proteins upon treatment with the different drugs without changes in mRNA levels. (D) Venn diagram comparing the number of transcripts and proteins detected in both RNA sequencing and quantitative proteomic analysis. (E) Venn diagrams of differential expressed transcripts and proteins in RNA sequencing and proteomics in each condition using the 7,885 common transcripts and proteins detected in both analysis. Overlapping percentage of differential expressed proteins and transcripts for each condition is represented. (F) Spearman correlation analysis between the logarithm base 2 of the fold change in proteomics and RNA sequencing using the previous 7,885 transcripts/proteins. Spearman coefficient and p-value are depicted in each plot. Acti, actinonin; Dox, doxycycline; MB, MitoBloCK-6.

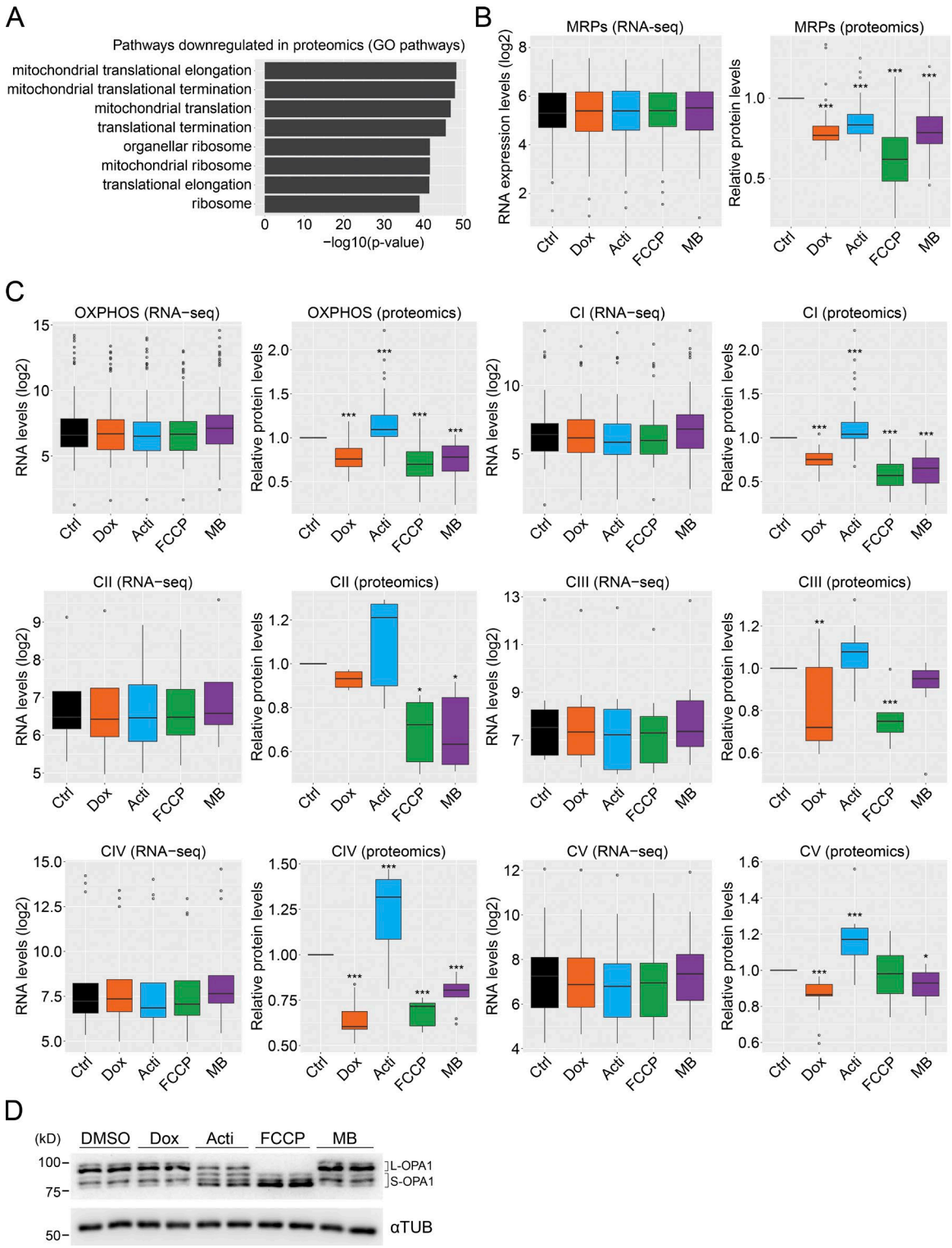
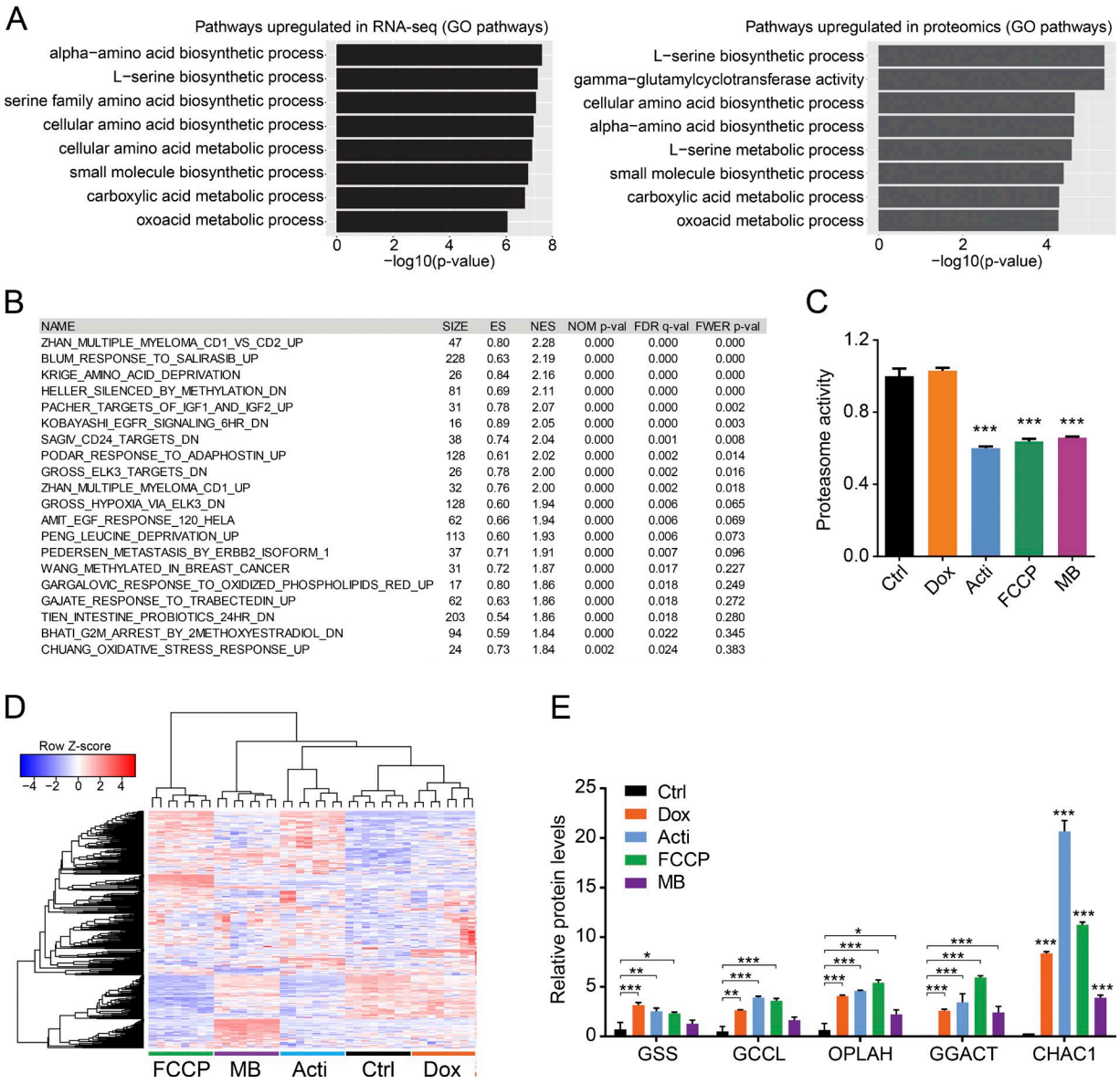


Figure S2. **Mitochondrial stress inhibits mitochondrial translation and decreases MRPs and OXPHOS complexes.** (A) Gene enrichment analysis using Gene Ontology (GO) categories of the 101 commonly down-regulated proteins. Enrichment is represented as negative of log10 of P value after Bonferroni correction. (B) Boxplots showing the expression of the transcripts and the relative amount of proteins of the mitochondrial ribosomal proteins (MRPs) in each condition. (C) Boxplots showing the transcript levels and relative amount of proteins of the OXPHOS components in each condition. All total complexes together (OXPHOS) or individual complexes are shown. Note that except for actinonin, in which are greatly increased, all treatments decreased the expression of all respiratory complexes. (D) Western blot analysis of OPA1 upon treatment for 6 h with each stressor. Long OPA1 isoforms (L-OPA1) and short OPA1 isoforms (S-OPA1) are represented. Acti, actinonin; Dox, doxycycline; MB, MitoBloCK-6.



NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
ZHAN_MULTIPLE_MYELOMA_CD1_VS_CD2_UP	47	0.80	2.28	0.000	0.000	0.000
BLUM_RESPONSE_TO_SALIRASIB_UP	228	0.63	2.19	0.000	0.000	0.000
KRIGE_AMINO_ACID_DEPRIVATION	26	0.84	2.16	0.000	0.000	0.000
HELLER_SILENCED_BY_METHYLATION_DN	81	0.69	2.11	0.000	0.000	0.000
PACHER_TARGETS_OF_IGF1_AND_IGF2_UP	31	0.78	2.07	0.000	0.000	0.002
KOBAYASHI_EGFR_SIGNALING_6HR_DN	16	0.89	2.05	0.000	0.000	0.003
SAGIV_CD24_TARGETS_DN	38	0.74	2.04	0.000	0.001	0.008
PODAR_RESPONSE_TO_ADAPHOSTIN_UP	128	0.61	2.02	0.000	0.002	0.014
GROSS_ELK3_TARGETS_DN	26	0.78	2.00	0.000	0.002	0.016
ZHAN_MULTIPLE_MYELOMA_CD1_UP	32	0.76	2.00	0.000	0.002	0.018
GROSS_HYPOXIA_VIA_ELK3_DN	128	0.60	1.94	0.000	0.006	0.065
AMIT_EGF_RESPONSE_120_HELA	62	0.66	1.94	0.000	0.006	0.069
PENG_LEUCINE_DEPRIVATION_UP	113	0.60	1.93	0.000	0.006	0.073
PEDERSEN_METASTASIS_BY_ERBB2_ISOFORM_1	37	0.71	1.91	0.000	0.007	0.096
WANG_METHYLATED_IN_BREAST_CANCER	31	0.72	1.87	0.000	0.017	0.227
GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_RED_UP	17	0.80	1.86	0.000	0.018	0.249
GAJATE_RESPONSE_TO_TRABECTEDIN_UP	62	0.63	1.86	0.000	0.018	0.272
TIEN_INTESTINE_PROBIOTICS_24HR_DN	203	0.54	1.86	0.000	0.018	0.280
BHATI_G2M_ARREST_BY_2METHOXYESTRADIOL_DN	94	0.59	1.84	0.000	0.022	0.345
CHUANG_OXIDATIVE_STRESS_RESPONSE_UP	24	0.73	1.84	0.002	0.024	0.383

Figure S3. **Mitochondrial stress increases biosynthesis of amino acids.** (A) Gene enrichment analysis of the 59 genes (left; black bars) and 17 proteins (right; gray bars) commonly up-regulated in mitochondrial stress using GO categories. Enrichment is represented as negative of log10 of P value after Bonferroni correction. (B) Table representing the results obtained from GSEA of all mitochondrial stress conditions from RNA sequencing. GSEA was performed against the gene sets from CGP category (chemical and genetic perturbations). (C) Proteasome activity measured in cells treated with selected drugs for 24 h. Bars depict the relative slope in fluorogenic suc-LLVY-AMC peptide cleavage compared with control cells (n = 3 independent experiments; mean ± SEM). (D) Heatmap and hierarchical clustering analysis of the more than 300 unique metabolites detected in our mitochondrial stress experiment. (E) Relative protein levels of glutathione cycle enzymes. Expression data were extracted from the proteomic analysis (mean values ± SEM). *, P < 0.05; **, P < 0.01; ***, P < 0.001. Acti, actinonin; Dox, doxycycline; ES, enrichment score; FDR q-val, false discovery rate adjusted p-value; FWER, family-wise error rate; MB, MitoBloCK-6; NES, normalized enrichment score; NOM p-val, nominal p-value.

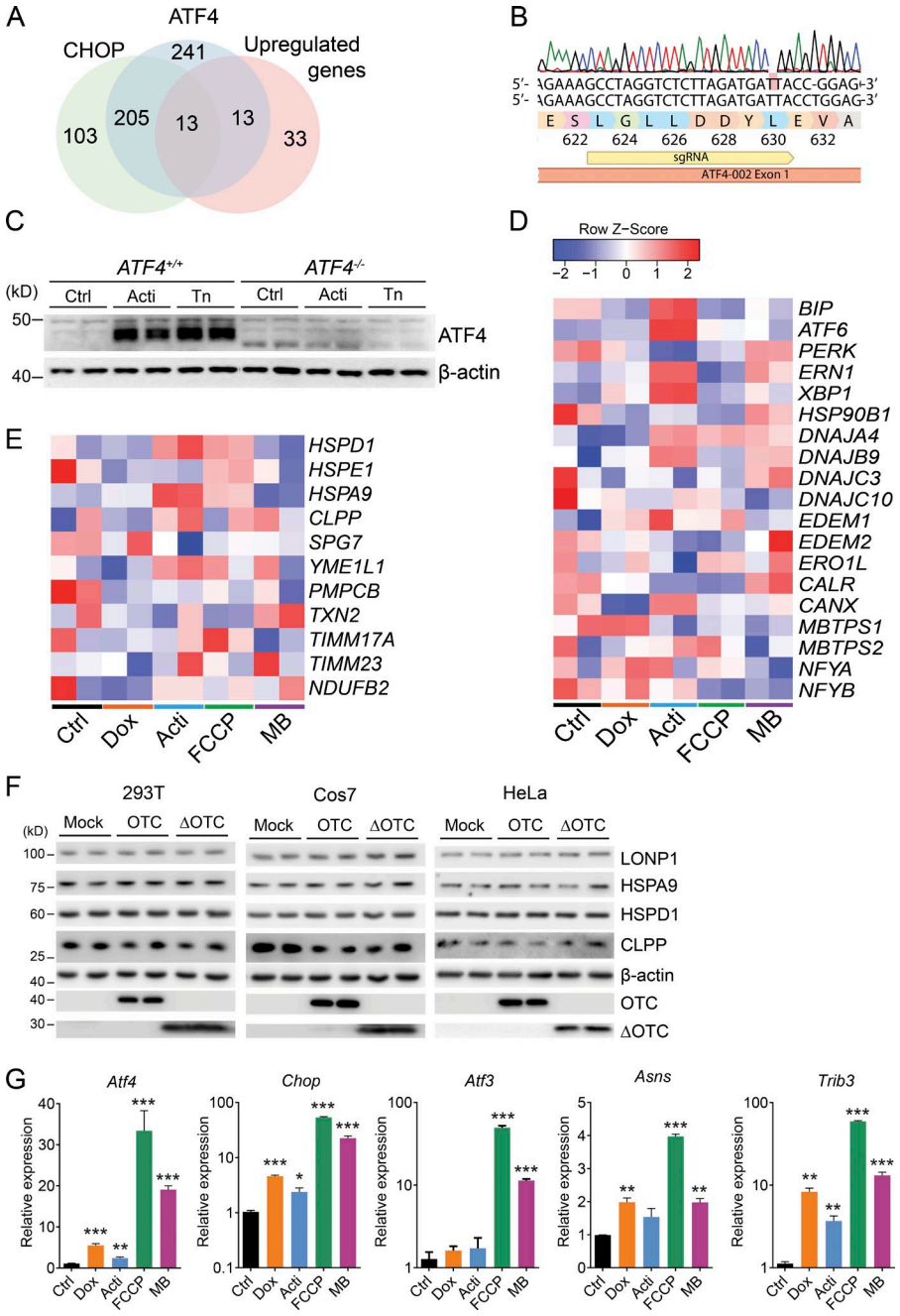


Figure S4. **ATF4 mediates the mitochondrial stress response independent of the UPR^{er} and UPR^{mt}.** (A) Triple Venn diagram of ATF4 and CHOP targets with common up-regulated genes, showing that 26 genes overlap with ATF4, of which 13 overlap with CHOP targets. (B) Sanger sequencing of individual deletion clone of ATF4, showing a biallelic deletion c.995delT in the exon 1. (C) Western blot analysis showing the absence of ATF4 upon addition of 50 µM actinonin (Acti) or 2.5 µg/ml tunicamycin (Tn) for 6 h. (D) Heatmap analysis of UPR^{er} transcript levels in RNA sequencing showing that this pathway was not activated under mitochondrial stress. (E) Heatmap analysis of prototypical UPR^{mt} genes in RNA sequencing showing that this pathway was not activated in a coordinated fashion by the different mitochondrial stress conditions. (F) Western blot analysis of mitochondrial stress protease LONP1 and UPR^{mt} proteins (HSPA9, HSPD1, and CLPP) upon transfection with empty plasmid pCAGGS (mock), wild-type ornithine transcarbamylase (OTC), and ΔOTC in HEK293T, HeLa, and Cos7 cells for 48 h. Note: ΔOTC was only detected in the insoluble fraction. (G) mRNA expression analysis of *Atf4* and its target genes, *Chop (Ddit3)*, *Atf3*, *Asns*, and *Trib3* in mouse embryonic fibroblasts (MEFs) 24 h after the addition of different mitochondrial stressors at the same concentration as was used in HeLa cells. Data are presented as mean ± SEM of two independent experiments and were analyzed using multi *t* test against control (Ctrl) cells. *, P < 0.05; **, P < 0.01; ***, P < 0.001. Dox, doxycycline; MB, MitoBloCK-6.

Table S1. **List of primers**

Gene	Organism	Forward (5′–3′)	Reverse (5′–3′)
ACTB	Human	GTCATCACCATTGGCAATGAG	CGTCATACTCCTGCTTGCTG
ASNS	Human	ATCACTGTCGGGATGTACCC	TGATAAAAGGCAGCCAATCC
ATF3	Human	GGAGCCTGGAGCAAAATGATG	AGGGCGTCAGGTTAGCAAAA
ATF4	Human	CAGCAAGGAGGATGCCTTCT	CCAACAGGGCATCCAAGTC
ATF5	Human	GAGCCCCTGGCAGGTGAT	CAGAGGGAGGAGAGCTGTGAA
BIP	Human	CACAGTGGTGCCTACCAAGA	TGTCTTTTGTCAGGGGTCTTT
CHAC1	Human	GTGGTGACGCTCCTTGAAGA	TTCAGGGCCTTGCTTACCTG
DDIT3	Human	AGCCAAAATCAGAGCTGGAA	TGGATCAGTCTGGAAAAGCA
CLPP	Human	AAGCACACCAAACAGAGCCT	AAGATGCCAAACTCCTGGG
GAPDH	Human	TTGGTATCGTGGAAGGACTC	ACAGTCTTCTGGGTGGCAGT
HSPA9	Human	TGGTGAGCGACTTGTTGGAAT	ATTGGAGGCACGGACAATTTT
HSPD1	Human	GGGTAACCGAAGCATTTCTGC	CTGCACTCTGTCCCTCACTC
HSPE1	Human	AGTAGTCGCTGTTGGATCGG	TGCCTCCATATTCTGGGAGA
LONP1	Human	CCCGCGCTTTATCAAGATT	AGAAAGACGCCGACATAAGG
PCK2	Human	CATCCGAAAGCTCCCCAAGT	GCTCTCTACTCGTGCCACAT
PHGDH	Human	GCAAAGAGGAGCTGATAGCG	TTCTCAGCTGCGTTGATGAC
PSPH	Human	GAGGACGCGGTGTCAGAAAT	GGTTGCTCTGCTATGAGTCTCT
FH	Human	GGAGGTGTGACAGAACGCAT	CATCTGCTGCCTTCATTATTGC
HRI	Human	GTTCGTCAGGATCCTTGTGAGG	ACGTGAAGTTTGTGCTTCCAA
PKR	Human	TCGTTGCTTATGAATGGTCTCAG	AGGTCAAATCTGGGTGCCAA
PERK	Human	GCACTCAGATGGAGAGAGTCA	AACCATCACGTACTCACAAGGA
GCN2	Human	GTGAAATTGAAATTCTGGCTGTGG	ATGCCTGTTCATCAGCATCC
tRNA-Leu (mtDNA)	Human	GATGGCAGAGCCCGGTAATCGC	TAAGCATTAGGAATGCCATTGCG
POLG (nDNA)	Human	AGCGACGGGCAGCGGCGGCGGCA	CCCTCCGAGGATAGCACTTGCGGC
Actb	Mouse	TGTTACCAACTGGGACGACA	GGGGTGTTGAAGGTCTCAAA
Asns	Mouse	GAGAAACTCTTCCCAGGCTTTG	CAAGCGTTTCTTGATAGCGTTGT
Atf3	Mouse	ATGATGCTTCAACACCCAGGC	TTAGCTCTGCAATGTTCCTTC
Atf4	Mouse	GCCGGTTTAAGTTGTGTGCT	CTGGATTCGAGGAATGTGCT
Chop	Mouse	CGGAACCTGAGGAGAGAGTG	CGTTTCCTGGGGATGAGATA
Gapdh	Mouse	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGAT
Trib3	Mouse	TGTCTTCAGCAACTGTGAGAGGA	CAGTCATCACGCAGGCATC

Table S2. **List of shRNAs**

Gene	Clone
HRI	TRCN0000194754
HRI	TRCN0000195265
PKR	TRCN0000197012
PKR	TRCN0000196400
PERK	TRCN0000197197
PERK	TRCN0000262380
GCN2	TRCN0000300851
GCN2	TRCN0000300850
LONP1	TRCN0000291803
LONP1	TRCN0000310154
FH	TRCN0000052464
FH	TRCN0000052465

Provided online are six Excel tables. Table S3 shows RNA sequencing results of mitochondrial stress. Table S4 shows TMT-based quantitative proteomic analysis of mitochondrial stress. Table S5 shows common differential expressed genes and proteins in mitochondrial stress. Table S6 shows gene enrichment analysis of each stressor in RNA sequencing analysis. Table S7 shows enrichment analysis of each stressor in TMT-quantitative proteomic analysis. Table S8 shows nontargeted metabolomics raw data of mitochondrial stress.