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Supplemental Information

Translational Regulation of Non-autonomous Mitochondrial Stress Response Promotes Longevity

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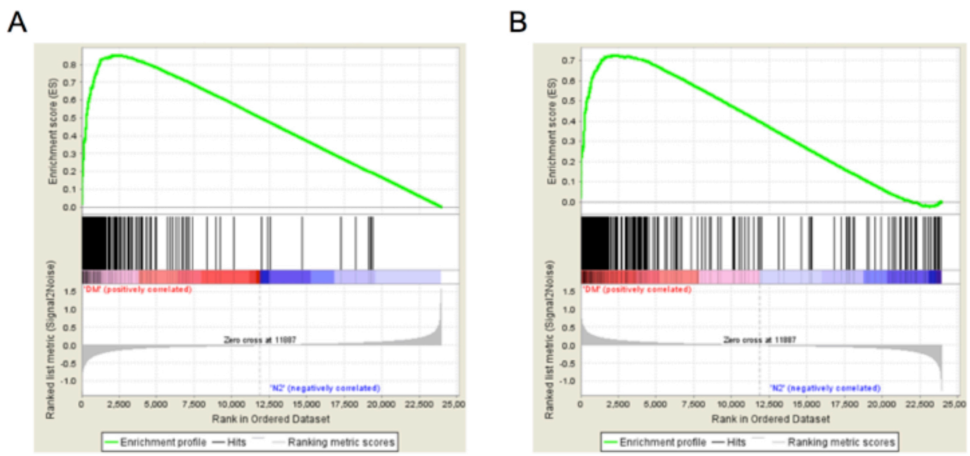

Figure S1. Gene Set Enrichment Analysis (GSEA) of genes transcriptionally regulated in the *daf-2 rsks-1* mutant (related to Figure 1).

(A-B) GSEA comparing the lists of genes transcriptionally down-regulated (A) or up-regulated (B) in the *daf-2 rsks-1* double mutant (DM) based on RNA-Seq versus the previous microarray experiment. The top enrichment profile shows the cumulative enrichment score as each ranked gene in the list is compared to the gene set. Where each gene in the list falls in the gene set is represented by black bars in the middle section. The bottom of the plot depicts the ranked list metric, which measures a gene's correlation with the treatment. Both lists were significantly enriched (FDR < 0.001) as compared to the microarray study.

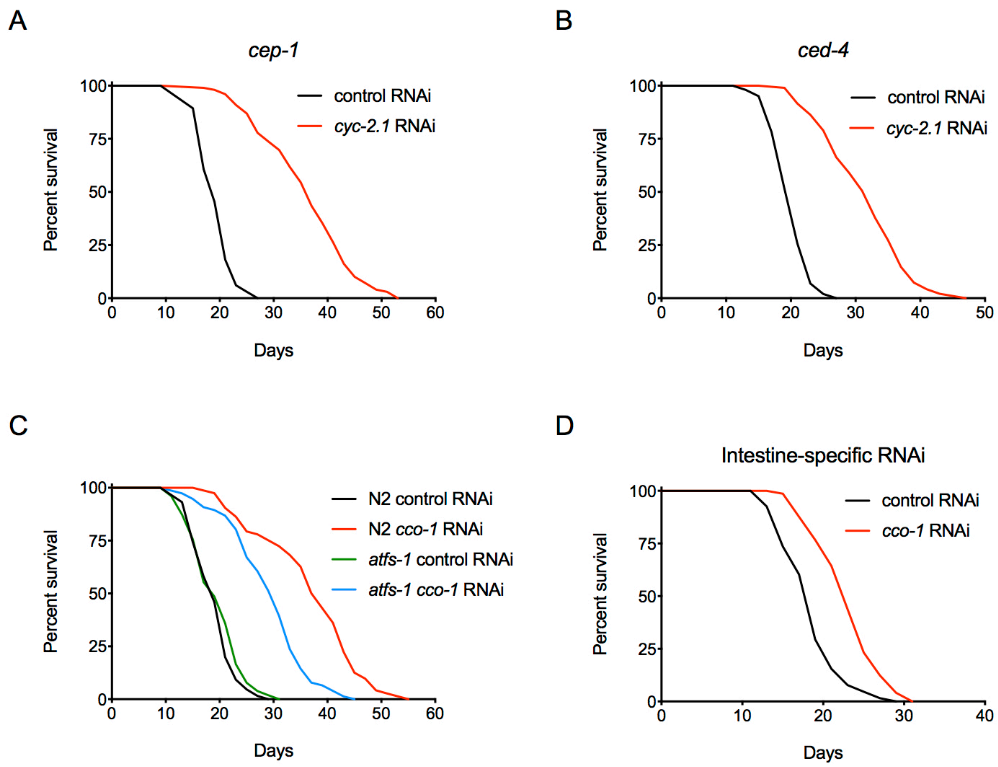

Figure. S2. *cyc-2.1* knockdown and other mitochondrial ETC perturbation function through different mechanisms to regulate lifespan (related to Figure 2, 3).

(A) Survival curves of the *cep-1* deletion mutant treated with either control or *cyc-2.1* RNAi ($p < 0.0001$, log-rank test). (B) Survival curves of the *ced-4* deletion mutant treated with either control or *cyc-2.1* RNAi ($p < 0.0001$, log-rank test). (C) Survival curves of N2 and the *atfs-1* deletion mutant treated with either control or *cco-1* RNAi. (D) Survival curves of animals treated with the intestine-specific control or *cco-1* RNAi ($p < 0.0001$, log-rank test).

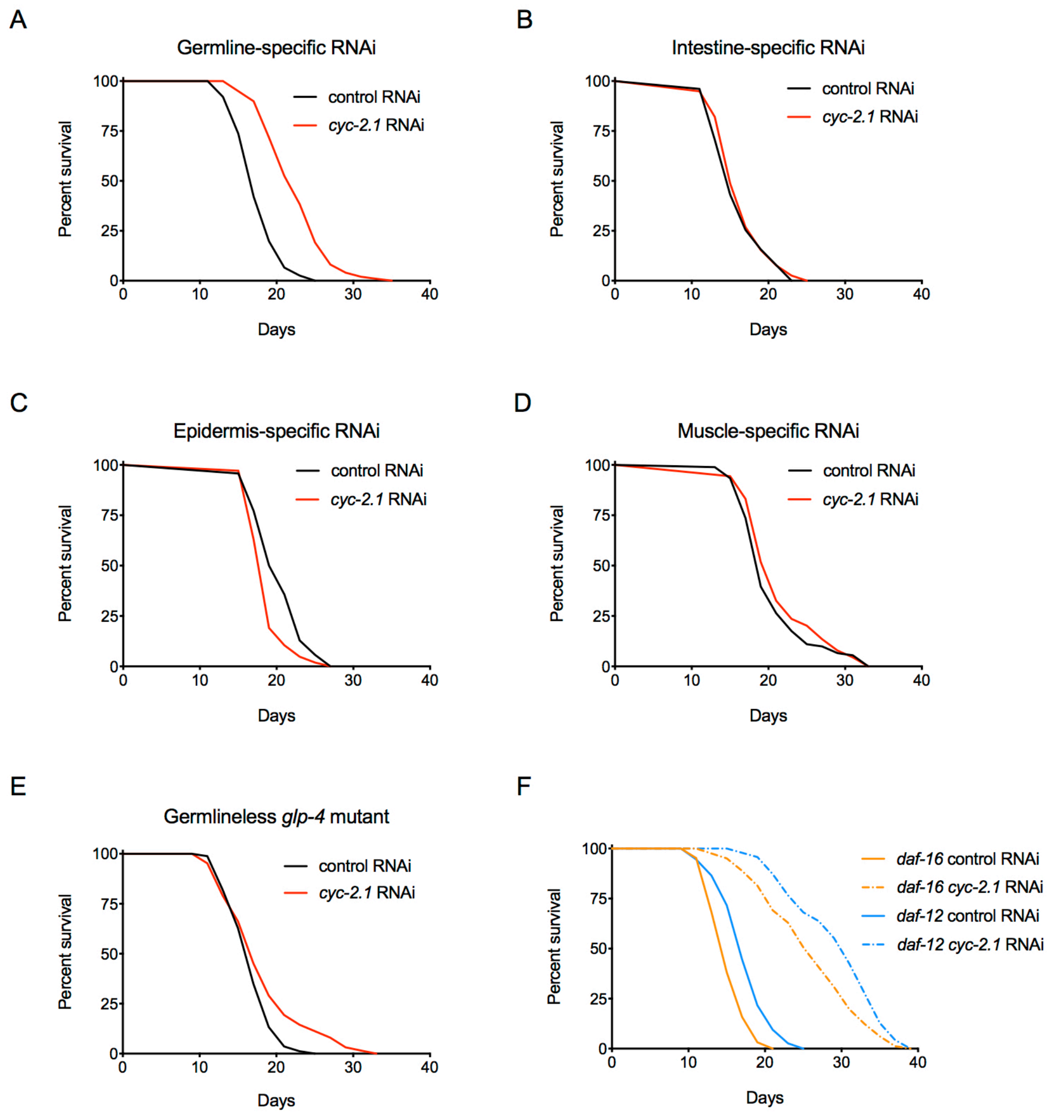

Figure S3. The germline plays an important role in *cyc-2.1* knockdown-induced lifespan extension (related Figure 3).

(A-D) Survival curves of animals treated with germline-specific (A), intestine-specific (B), epidermis-specific (C) and muscle-specific (D) control or *cyc-2.1* RNAi. (E) Survival curves of the germline-less *glp-4* mutant treated with control RNAi or *cyc-2.1* RNAi (p = 0. 0123, log-rank test). (F) Survival curves of the *daf-16* KO and *daf-12* KO mutants treated with the control or *cyc-2.1* RNAi (p < 0. 0001, log-rank tests).

Table S2. List of lifespan determinant genes that are translationally repressed in the *daf-2 rsks-1* mutant (related to Figure 1, 2)

RNAi	Lifespan (days)		Lifespan extension [c]	n [d]	Function	Essentiality [e]
	Mean [a]	Max [b]				
control	19.1	27	/	79	/	/
rpl-5	22.5	31	18% ****	82	mRNA translation	Yes
rpl-6	23.2	35	22% ****	76	mRNA translation	Yes
rpl-7	23.5	33	23% ****	85	mRNA translation	Yes
rpl-13	23.1	33	21% ****	89	mRNA translation	Yes
rpl-14	22.1	31	16% ****	86	mRNA translation	Yes
rpl-15	21.6	35	13% ****	73	mRNA translation	Yes
rpl-18	22.1	33	16% ****	74	mRNA translation	Yes
rpl-25.2	22.1	29	16% ****	87	mRNA translation	Yes
rpl-30	22.3	35	17% ****	74	mRNA translation	Yes
rpl-33	23.6	37	24% ****	82	mRNA translation	Yes
rpl-35	22.1	31	16% ****	78	mRNA translation	Yes
rpl-36	23.8	33	25% ****	73	mRNA translation	Yes
rps-3	22.8	33	20% ****	77	mRNA translation	Yes
rps-12	22.3	33	17% ****	63	mRNA translation	Yes
rps-20	22.2	35	17% ****	80	mRNA translation	Yes
rps-26	21.2	31	11% ***	66	mRNA translation	Yes
rps-30	22.7	33	17% ****	77	mRNA translation	Yes
cytb-5.2	22.1	33	16% ****	73	metabolism	No
cyc-2.1	33.8	49	77% ****	78	mitochondria	No
sym-1	20.9	31	9% **	57	development	No
ncam-1	21.4	31	12% ***	70	development	No
pqn-48	20.6	29	8% *	69	protein modification	No
pqn-65	20.8	29	9% **	70	unknown	No
T10B5.7	22.0	31	15% ****	75	unknown	No

[a] Mean lifespan.
[b] Maximum lifespan.
[c] Mean lifespan extension compared to the control RNAi treatment. ****, $p <$ 0.0001; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$ (log-rank tests).
[d] Numbers of animals scored.
[e] Essential genes are those, RNAi knocking-down of which during development led to larval arrest. Lifespan assays of these genes were performed with RNAi treatments only during adulthood.

Table S3 Statistical analyses of lifespan experiments (related to Figure 2-6)

Genotype	RNAi	Tissue [a]	Lifespan (days) Mean	Max	Percent of control [b]	n [c]	*p* [d]
Effects of *cyc-2.1* RNAi on lifespan of N2, *rsks-1*, *daf-2* and *daf-2 rsks-1* (Figure 2A)							
N2	control		18.9, 20.0	29, 29	/	72, 88	/
	cyc-2.1		32.2, 31.6	49, 47	170%, 158%	67, 94	<0.0001, <0.0001
rsks-1	control		22.4, 21.9	35, 29	/	68, 64	/
	cyc-2.1		31.2, 28.8	51, 51	139%, 131%	79, 62	<0.0001, <0.0001
daf-2	control		41.1, 42.2	61, 57	/	69, 106	/
	cyc-2.1		68.8, 68.5	105, 97	167%, 162%	82, 156	<0.0001, <0.0001
daf-2 rsks-1	control		68.1, 67.2	99, 101	/	114, 140	/
	cyc-2.1		65.4, 65.3	101, 105	96%, 97%	103, 141	0.2820, 0.7378
cyc-2.1 RNAi induced lifespan extension requires ATFS-1 and AAK-2 (Figure 2D, G)							
atfs-1	control		19.1, 19.7	31, 29	/	120, 83	/
	cyc-2.1		19.8, 20.3	33, 35	104%, 103%	84, 64	0.2355, 0.2985
aak-2	control		15.9, 16.0	21, 21	/	71, 55	/
	cyc-2.1		15.9, 16.0	23, 23	100%, 100%	53, 56	0.9647, 0.9295
Effects of tissue-specific *cyc-2.1* RNAi on lifespan (Figure 3A)							
mkcSi13; rde-1	control	germline	17.7, 17.7, 17.5	25, 23, 23	/	76, 84, 75	/
	cyc-2.1		22.7, 22.0, 22.5	35, 31, 33	128%, 124%, 129%	99, 94, 89	<0.0001, <0.0001, <0.0001
rde-1; kbIs7	control	intestine	17.9, 19.4, 16.2	25, 27, 23	/	62, 94, 51	/
	cyc-2.1		17.9, 17.7, 16.6	25, 29, 25	100%, 91%, 102%	74, 98, 78	0.9741, 0.0050, 0.4904
rde-1; kzIs9	control	epidermis	19.5, 20.6, 20.5	29, 29, 27	/	114, 101, 70	/
	cyc-2.1		19.0, 19.0, 18.9	27, 27, 27	97%, 92%, 92%	99, 112, 105	0.1543, 0.0010, 0.0002
rde-1; neIs9	control	muscle	19.4, 19.0, 20.7	27, 31, 33	/	66, 91, 91	/
	cyc-2.1		18.5, 18.8, 21.6	29, 29, 33	95%, 99%, 105%	70, 90, 89	0.3438, 0.5818, 0.2178
Intestinal AAK-2 is sufficient to restore *cyc-2.1* RNAi-induced lifespan extension in the *aak-2* mutant (Figure 3E)							
mkcSi51; aak-2	control		15.5, 16.0, 15.4	21, 23, 21	/	70, 82, 77	/
	cyc-2.1		23.3, 22.7, 21.8	41, 37, 33	150%, 142%, 142%	73, 64, 60	<0.0001, <0.0001, <0.0001
The *cup-4* mutation blocks germline-specific *cyc-2.1* RNAi-induced lifespan extension (Figure 3L)							
mkcSi13; cup-4; rde-1	control	germline	21.6, 23.5	31, 33	/	114, 110	/
	cyc-2.1		21.6, 24.8	33, 33	100%, 106%	115, 98	0.8629, 0.1545
The *drp-1* mutant shows reduced lifespan extension by *cyc-2.1* RNAi compared to N2 (Figure 4E, F)							
N2	control		19.5, 18.7, 19.1	27, 25, 27	/	102, 94, 104	/
	cyc-2.1		33.5, 29.3, 32.4	53, 51, 49	172%, 157%, 169%	93, 98, 63	<0.0001, <0.0001, <0.0001
drp-1	control		21.3, 21.4, 18.1	33, 35, 31	/	87, 88, 91	/
	cyc-2.1		28.6, 29.7, 27.8	45, 43, 39	134%, 139%, 154%	84, 67, 64	<0.0001, <0.0001, <0.0001
Roles of UPR^{mt} pathway transcriptional factors in the regulation of N2 and *daf-2 rsks-1* mutant lifespan (Figure 5D, E, F)							
N2	control		20.4, 19.8, 19.7	29, 27, 25	/	64, 86, 58	/
	dve-1		15.8, 14.2, 15.1	21, 19, 21	78%, 72%, 77%	83, 97, 93	<0.0001, <0.0001, <0.0001
	ubl-5		20.6, 19.4, 20.0	27, 29, 27	101%, 98%, 102%	77, 95, 92	0.8181, 0.6945, 0.1015
	atfs-1		21.3, 20.1, 19.8	31, 31, 27	104%, 102%, 100%	73, 93, 49	0.2259, 0.2192, 0.7948
daf-2 rsks-1	control		55.7, 49.9, 51.7	97, 85, 87	/	58, 68, 119	/
	dve-1		18.5, 18.7, 22.5	31, 27, 41	33%, 37%, 43%	74, 92, 208	<0.0001, <0.0001, <0.0001
	ubl-5		34.9, 36.2, 32.5	59, 55, 55	63%, 72%, 63%	85, 80, 192	<0.0001, <0.0001, <0.0001
	atfs-1		42.6, 39.5, 44.1	69, 59, 83	76%, 79%, 85%	73, 62, 139	<0.0001, <0.0001, 0.0013
Effects of *gld-1* RNAi on lifespan of N2, *rsks-1*, *daf-2* and *daf-2 rsks-1* (Figure 6F)							
N2	control		18.0, 18.2	27, 29	/	67, 68	/
	gld-1		16.6, 16.5	25, 23	92%, 91%	64, 62	0.0167, 0.0025
rsks-1	control		21.2, 21.2	33, 35	/	35, 35	/
	gld-1		17.4, 16.9	23, 25	82%, 79%	45, 46	<0.0001, <0.0001
daf-2	control		46.1, 43.3	63, 63	/	50, 40	/
	gld-1		39.3, 41.1	61, 63	85%, 95%	34, 36	0.0945, 0.9598
daf-2 rsks-1	control		65.2, 59.1	105, 91	/	53, 47	/
	gld-1		49.6, 52.1	69, 75	76%, 88%	43, 45	<0.0001, 0.0135

[a] Tissue in which RNAi is effective.
[b] Changes in mean lifespan compared to the control.
[c] Numbers of animals scored.
[d] *p* values for log-rank tests.

Table S4. Sequences of oligonucleotides (Related to STAR methods)

Oligonucleotides	Source	Identifier
rps-0-RT-F: CGTATCGATCATCAGGCTGTCAC	This paper	N/A
rps-0-RT-R: CCGATGTCGATCAACTTGAGTGG	This paper	N/A
rps-3-RT-F: GAATCCCTCAGATACAAGCTCGT	This paper	N/A
rps-3-RT-R: CCAGAGACGATAACCTCAACTCCT	This paper	N/A
rpl-5-RT-F: ACTTACCAACTACGCCGCTG	This paper	N/A
rpl-5-RT-R: TAGTCTTCTCCAGTAAGCTCTTCGT	This paper	N/A
rpl-25.2-RT-F: GGCTGTTAAGGTCAACACCCTG	This paper	N/A
rpl-25.2-RT-R: TGGCAACATCAAGAGCATCGT	This paper	N/A
pmp-2-RT-F: AGGATTGCGATGGCTCGATTG	This paper	N/A
pmp-2-RT-R: TACATGGCTCCTTCAACATCAAC	This paper	N/A
hsp-6-RT-F: GCTGGAGATAAGATCATCGCTG	This paper	N/A
hsp-6-RT-R : GTGGACTTGACCTCGAAGAC	This paper	N/A
dnj-10-RT-F: GCGGGCTCATTCATCGATCTGTAC	This paper	N/A
dnj-10-RT-R: CAGATTTTTTGTCGACACCCAAAG	This paper	N/A
drp-1-RT-F: TGGATTCCTTGGATTATTCGGC	This paper	N/A
drp-1-RT-R: AGTTGCGTCTCTGGCACTTCTG	This paper	N/A
timm-17-RT-F: GATTGTTGTCTTGTCGCCATCC	This paper	N/A
timm-17-RT-R: ATCACCTTTGGTCCTGAACGG	This paper	N/A
cyc-2.1-RT-F: TAACAAGAACAAGGGAGTCGTCTG	This paper	N/A
cyc-2.1-RT-R: CGTCAGCCTTCTTGAGTCCA	This paper	N/A
ubl-5 RNAi-F: GGGGTACCAGTAGAGCTCGAAATTGAATCCC	This paper	N/A
ubl-5 RNAi-R: AACTGCAGCATGCGCCTTTAAGTTTACTGAT	This paper	N/A
cyc-2.1-sgRNA1-F: AGATCTCATCAAGTACATCGGTTTTAGAGCTAGAAATAGCAAGT	This paper	N/A
cyc-2.1-sgRNA-2-F: GAATATTTAGAGGGACTTGGGTTTTAGAGCTAGAAATAGCAAGT	This paper	N/A
sgRNAs-R: CAAGACATCTCGCAATAGG	This paper	N/A
sgRNA-seq: GGTGTGAAATACCGCACAGA	This paper	N/A
cyc-2.1-5’HR-F: CCCAAGCTTTGCAATGATCCATAAGGCATCGG	This paper	N/A
*cyc-2.1*5’HR-R: CGGGATCCGAGGGACTTGGCGGATTCA	This paper	N/A
cyc-2.1-3’HR-F: CGGGATCCGAGGGACTTGGCGGATTCA	This paper	N/A
cyc-2.1-3’HR-R: GGACTAGTAGCGGGAAATTTGAATAGGCG	This paper	N/A
GFP-seq-5R: CCATCTAATTCAACAAGAATTGGGACAAC	This paper	N/A
GFP-seq-3F: GGTCCTTCTTGAGTTTGTAAC	This paper	N/A
cyc-2.1-3xflag-F: GGGGTACCGACTACAAAGACCATGACGGTGA	This paper	N/A
cyc-2.1-3xflag-R: AACTGCAGTTACTTGTCATCGTCATCCTTG	This paper	N/A
cyc-2.1 (SITE DIRECTED M)-F: CAAGTACATCGAaGTTGAATCCGC	This paper	N/A
cyc-2.1 (SITE DIRECTED M)-R: GCGGATTCAACtTCGATGTACTTG	This paper	N/A
cyc-2.1 KI-F: GTGTGGTTTGAATCCTGTTTGTTCAGC	This paper	N/A
3xflag KI-genotyping-R: GATCTTTATAATCACCGTCATGGTC	This paper	N/A
gld-1-sgRNA1-F: CCCGAGTGGAGCAAGTCCCTGTTTTAGAGCTAGAAATAGCAAGT	This paper	N/A
gld-1-sgRNA2-F: CGAGGGACTTGCTCCACTCGGTTTTAGAGCTAGAAATAGCAAGT	This paper	N/A
gld-1-5’HR-F: GCAGAAGTTGATCAGCGAGGAAGACTTGTAAAAAGTTCACATTTATAACTCACACTCC	This paper	N/A
gld-1-5’HR-R: GGAAACAGCTATGACCATGTTATCGATTAGTGAGATTTGAACATATCTAACTAGCG	This paper	N/A
gld-1-3’HR-F: CACGACGTTGTAAAACGACGGCCAGTCAAGCAGCACTCGAACAGGTCAAGAAGTT	This paper	N/A
gld-1-3’HR-R: CCTGAGGCTCCCGATGCTCCGAAAGAGGTGTTGTTGACTGAAGAAGCTGAGGGACTTGC	This paper	N/A
gld-1 (SITE DIRECTED M)-F: GTCGCCGAGTGGAGCAAGTCCCTC	This paper	N/A
gld-1 (SITE DIRECTED M)-R: ACATTGGTGTTTGTTTGATTGGCAAAAGAAGATG	This paper	N/A
gld-1 KI-F: CTCGAAGACGATCTGCACG	This paper	N/A
mKate2 KI-genotyping-R: GTTGTTGACGGTTCCCTCC	This paper	N/A