Translational Regulation of Non-autonomous Mitochondrial Stress Response Promotes Longevity

Graphical Abstract

Highlights

- Longevity of daf-2 rsks-1 is mediated by translational repression of cyc-2.1
- Germline inhibition of cyc-2.1 activates intestinal UPR^{mt} and AMPK to extend lifespan
- Increased GLD-1 represses germline cyc-2.1 translation in the daf-2 rsks-1 mutant
- Translational regulation of cyc-2.1 and UPR^{mt} contribute to longevity of daf-2 rsks-1

Authors
Jianfeng Lan, Jarod A. Rollins, Xiao Zang, ..., Pankaj Kapahi, Aric N. Rogers, Di Chen

Correspondence
pkapahi@buckinstitute.org (P.K.), arogers@mdibl.org (A.N.R.), chendi@nju.edu.cn (D.C.)

In Brief
To understand how reduced translation delays aging, Lan et al. perform translational profiling in C. elegans and propose that, in the significantly long-lived daf-2 rsks-1 mutant, serial translational regulation leads to reduced cytochrome c in the germline, which non-autonomously activates UPR^{mt} and AMPK in the metabolic tissue to ensure longevity.

Lan et al., 2019, Cell Reports 28, 1050–1062
July 23, 2019 © 2019 The Author(s).
https://doi.org/10.1016/j.celrep.2019.06.078
Translational Regulation of Non-autonomous Mitochondrial Stress Response Promotes Longevity

Jianfeng Lan,1,4 Jarod A. Rollins,2,4 Xiao Zang,1,4 Di Wu,1,4 Lina Zou,1 Zi Wang,1 Chang Ye,1 Zixing Wu,1 Pankaj Kapahi,3,* Aric N. Rogers,2,* and Di Chen1,5,*
*Correspondence: pkapahi@buckinstitute.org (P.K.), arogers@mdibl.org (A.N.R.), chendi@nju.edu.cn (D.C.)

SUMMARY

Reduced mRNA translation delays aging, but the underlying mechanisms remain underexplored. Mutations in both DAF-2 (IGF-1 receptor) and RSKS-1 (ribosomal S6 kinase/S6K) cause synergistic lifespan extension in C. elegans. To understand the roles of translational regulation in this process, we performed polysomal profiling and identified translationally regulated ribosomal and cytochrome c (CYC-2.1) genes as key mediators of longevity. cyc-2.1 knockdown significantly extends lifespan by activating the intestinal mitochondrial unfolded protein response (UPRmt), mitochondrial fission, and AMP-activated kinase (AMPK). The germline serves as the key tissue for cyc-2.1 to regulate lifespan, and germline-specific cyc-2.1 knockdown non-autonomously activates intestinal UPRmt and AMPK. Furthermore, the RNA-binding protein GLD-1-mediated translational repression of cyc-2.1 in the germline is important for the non-autonomous activation of UPRmt and synergistic longevity of the daf-2 rsks-1 mutant. Altogether, these results illustrate a translationally regulated non-autonomous mitochondrial stress response mechanism in the modulation of lifespan by insulin-like signaling and S6K.

INTRODUCTION

Aging can be genetically modulated by perturbation of insulin/insulin-like signaling (IIS), target of rapamycin (TOR) pathway, and mitochondrial functions (Fontana et al., 2010; Kenyon, 2010; López-Otín et al., 2013). These genetic manipulations often lead to significant changes in gene expression at both transcriptional and translational levels. Inhibition of DAF-2, the C. elegans ortholog of the insulin growth factor 1 (IGF-1) receptor, doubles adult lifespan by activating the DAF-16 (FOXO) transcriptional factor to regulate downstream genes involved in stress resistance, detoxification, and metabolism (Kenyon et al., 1993; Kimura et al., 1997; Lin et al., 1997; McElwee et al., 2004; Murphy et al., 2003; Ogg et al., 1997). Quantitative proteomics and polysomal profiling studies revealed that the daf-2 mutant also shows altered mRNA translation in many genes, and translational regulation plays important roles in significantly prolonged longevity and extended survival during heat stress (Depuydt et al., 2013; Dong et al., 2007; McColl et al., 2010; Stout et al., 2013).

Inhibition of the TOR pathway significantly extends lifespan in many species (Kapahi et al., 2010). One important function of TOR is to regulate gene expression at the mRNA translation level through the ribosomal S6 kinase (S6K) and the translational initiation factor 4E-binding protein (4E-BP), both of which have been shown to play important roles in aging (Hansen et al., 2007; Kapahi et al., 2004; Pan et al., 2007; Zid et al., 2009). Deletion mutants of rsk-1, which encodes the C. elegans ribosomal S6K ortholog, lead to significant changes in development, lipid metabolism, reproduction, and longevity (Hansen et al., 2007; Korta et al., 2012; Pan et al., 2007; Shi et al., 2013). Previous studies have identified multiple mediators of the prolonged longevity of the rsk-1 mutant (Chen et al., 2009; McQuary et al., 2016; Selman et al., 2009; Seo et al., 2013; Sheaffer et al., 2008). However, genes translationally regulated by RSKS-1 that influence lifespan and the underlying molecular mechanisms remain to be characterized.

Serving as the key organelle in energy homeostasis, mitochondria play important but complex roles in aging. Mitochondrial dysregulation has been regarded as one of the major hallmarks of aging. However, mild perturbation of the mitochondrial electron transport chain (ETC) leads to significant lifespan extension in many species (Dillin et al., 2002; Houtkooper et al., 2013; Lee et al., 2003; López-Otín et al., 2013). Inhibition of mitochondrial ETC genes triggers the mitochondrial unfolded protein response (UPRmt) via transcriptional regulators such as DVE-1, UBL-5, and ATFS-1 (Haynes et al., 2007; Nargund et al., 2012). Perturbation of mitochondrial ETC functions in neurons releases a pro-longevity cue named mitokine to induce UPRmt in the intestine, a distal metabolic tissue in worms, and ensures lifespan extension (Durieux et al., 2011). Further studies have identified the neurotransmitter serotonin, neuropeptide FLP-2, and retromer-dependent Wnt signaling as the endocrine mediators of the neuron to intestine non-autonomous
mitochondrial stress response (Berendzen et al., 2016; Shao et al., 2016; Zhang et al., 2018). The trans-tissue mitochondrial stress response requires epigenetic modifications that ensure selective gene expression and prolonged longevity, and the epigenetic regulatory mechanisms are conserved in mammals (Merkwirth et al., 2016; Tian et al., 2016).

To study how insulin-like signaling interacts with the TOR pathway to modulate aging, we previously constructed a daf-2 rsks-1 double mutant and observed a synergistic longevity phenotype. Further analysis demonstrated an AMP-activated kinase (AMPK)-mediated positive feedback regulation of the DAF-16 transcriptional factor mechanism in the daf-2 rsks-1 mutant (Chen et al., 2013). However, genes that are translationally regulated in the daf-2 rsks-1 mutant and their roles in aging have not been determined. Because both daf-2 and rsks-1 have profound impacts on mRNA translation, we hypothesized that translational regulation plays important roles in the significantly prolonged longevity of daf-2 rsks-1 mutant animals. By genome-wide translational state analysis and genetic screens, we identified ribosomal protein genes and cyc-2, which encodes one of the worm cytochrome c orthologs, as negative regulators of longevity. The inhibition of cyc-2 results in a robust lifespan extension that requires UPR

**RESULTS**

**Genome-wide Translational State Analysis of the Significantly Long-Lived daf-2 rsks-1 Mutant**

To characterize the roles of mRNA translation in the significantly prolonged longevity of daf-2 rsks-1, we performed genome-wide translational state analysis via polysomal profiling coupled with RNA sequencing (RNA-seq) using wild-type N2 and daf-2 rsks-1 mutant animals (Figure 1A). Day 4 adult animals were collected for extraction of total mRNAs and translated mRNAs (≥2 ribosomes/transcript) for quantification via RNA-seq (Figure 1A). Gene set enrichment analysis (GSEA) was performed to compare RNA-seq results with our previous microarray studies (Chen et al., 2013). Transcriptionally up- and downregulated gene lists both showed significant concordance with the microarray results (false discovery rate [FDR] < 0.001), with normalized enrichment scores of 2.84 and 3.19, respectively (Figure S1). Changes in translation were determined by comparing the ratio of polysome-associated mRNAs to total mRNAs between N2 and daf-2 rsks-1 mutant animals. This is called the differential polysome association ratio (DPAR). Altogether, we identified 167 transcripts with differential translation but no changes at total mRNA levels. Among them, 52 genes are upregulated and 115 genes are downregulated in the daf-2 rsks-1 mutant (Figure 1B; Table S1). Gene Ontology (GO) enrichment analysis of biological processes revealed meiotic cell cycle, cell cycle, and organelle fission are the top three terms in the upregulated genes, whereas translation, ribosome biogenesis, and developmental process are the top three terms in the downregulated genes (Figure 1C).

To validate whether mRNAs with differential ribosome loading are regulated at the mRNA translational level, we compared expression of rps-0, rps-3, rpl-5, and rpl-25.2 at both mRNA and protein levels between N2 and daf-2 rsks-1 mutant animals. These genes were chosen based on the availability of antibodies (Liu et al., 2018) to detect their protein products. Consistent with the RNA-seq data (Table S1), mRNA levels of rps-0, rps-3, rpl-5, and rpl-25.2 show no significant changes between N2 and daf-2 rsks-1 mutant compared with N2 (Figures 1E and 1F). Previous quantitative proteomics studies also showed that the abundance of these ribosomal proteins is decreased in the daf-2 mutant compared with N2 (Depuydt et al., 2013; Stout et al., 2013). Altogether, these results indicate that polysomal profiling is a valid approach to quantitatively assess mRNA translation, which allowed us to identify differentially translated mRNAs in the daf-2 rsks-1 mutant.

**Genes Downregulated in the daf-2 rsks-1 Mutant Are Enriched with Lifespan Determinants**

Numerous studies have demonstrated that genes differentially expressed in long-lived mutants are key regulators of lifespan. We thus hypothesized that genes translationally downregulated in the long-lived daf-2 rsks-1 mutant are likely to be negative regulators of longevity, inhibition of which in the wild-type background could extend lifespan. Thus, we performed an RNAi-based genetic screen to individually knockdown those 115 translationally downregulated genes in N2 to test their effects on lifespan. To facilitate the survival assays, the primary screen was performed at 25°C using the spe-9; rrf-3 double mutant, which shows enhanced RNAi sensitivity, temperature-sensitive sterility, and normal lifespan. Intriguingly, 39 of the 115 RNAi treatments against these 39 genes only ground could extend lifespan. Thus, we performed an RNAi-based genetic screen to individually knockdown those 115 translationally downregulated genes in N2 to test their effects on lifespan. To facilitate the survival assays, the primary screen was performed at 25°C using the spe-9; rrf-3 double mutant, which shows enhanced RNAi sensitivity, temperature-sensitive sterility, and normal lifespan. Intriguingly, 39 of the 115 RNAi treatments against these 39 genes only during adulthood to test their lifespan phenotypes. After the re-test in the wild-type background at 20°C, we identified 24 genes, inhibition of which leads to significant lifespan extension (Table S2). Among them, 17 genes are essential ones that encode various ribosomal subunits. These results highlight the importance of developmentally essential genes and translationally regulated ribosomal biogenesis in lifespan determination.

**Inhibition of CYC-2.1 Results in Robust Lifespan Extension that Requires UPR\[\text{\textsuperscript{mt}}\] and AMPK**

Among all genes tested, cyc-2.1, which encodes one of the two highly conserved cytochrome c orthologs, showed the strongest...
lifespan extension upon RNAi knockdown (Table S2). cyc-2.1 was originally identified as a lifespan determinant from an RNAi screen for enhanced oxidative stress resistance (Kim and Sun, 2007). Knockdown of cyc-2.1 robustly extends lifespan in the wild-type, rsks-1 mutant, and daf-2 mutant backgrounds, but it does not further extend lifespan of the daf-2 rsks-1 double mutant (Figure 2A; Table S3). These results suggest that reduced CYC-2.1 might serve as the key mechanism in mediating the synergistic effect of daf-2 rsks-1 on longevity.

Cytochrome c functions in the mitochondrial ETC by transferring electrons from complex III to complex IV. Previous studies demonstrated that inhibition of certain mitochondrial ETC components extends lifespan, and the underlying mechanisms involve CEP-1/p53 (Baruah et al., 2014), the intrinsic apoptosis pathway (Yee et al., 2014), and UPRmt (Dillin et al., 2002; Durieux et al., 2011; Lee et al., 2003). Mutations in the C. elegans p53 ortholog CEP-1 or a key component of the apoptosis pathway CED-4 significantly suppress the lifespan extension...
by mitochondrial ETC mutants (Baruah et al., 2014; Yee et al., 2014). However, cyc-2.1 RNAi treatment significantly extends lifespan in cep-1 or ced-4 knockout mutant (Figures S2A and S2B), suggesting a different mechanism.

To examine the effect of cyc-2.1 knockdown on the mitochondrial stress response, we applied cyc-2.1 RNAi to transgenic animals carrying a gfp reporter driven by the hsp-6 promoter, which has been widely used to monitor UPRmt activation. Inhibition of cyc-2.1 significantly activates hsp-6p::gfp reporter expression (Figure 2B). Because the hsp-6p::gfp reporter is mainly expressed in the intestine, we performed micro-dissection to isolate the intestinal tissue of wild-type animals treated

**Figure 2. Knockdown of cyc-2.1 Significantly Extends Lifespan by Activating UPRmt and AMPK**

(A) Survival curves of the wild-type N2, rsks-1, daf-2, and daf-2 rsks-1 mutant animals treated with control or cyc-2.1 RNAi.

(B) Representative photographs of hsp-6p::gfp expression in animals treated with control or cyc-2.1 RNAi. Scale bar, 200 μm.

(C) qRT-PCR of hsp-6 (median with range) using RNAs extracted from dissected intestinal tissues of N2 treated with control or cyc-2.1 RNAi based on three biological replicates (**p < 0.0001, two-tailed t test). (D) Survival curves of the atfs-1 deletion mutant treated with control or cyc-2.1 RNAi (p = 0.2355, log-rank test).

(E and F) Immunoblots (E) and quantification (F) of phospho-AAK-2 (AMPKa) and actin in N2 animals treated with control or cyc-2.1 RNAi. Ratio of band intensity of phospho-AAK-2 to actin was normalized to the control RNAi-treated animals. Data are represented as mean ± SEM based on eight biological replicates (**p = 0.0041, two-tailed t test).

(G) Survival curves of the aak-2 deletion mutant treated with control or cyc-2.1 RNAi (p = 0.9647, log-rank test).

(H and I) Immunoblots (H) and quantification (I) of phospho-AAK-2 and actin in the atfs-1 deletion mutant treated with control or cyc-2.1 RNAi. Data are represented as mean ± SEM based on four biological replicates (ns, p = 0.5239, two-tailed t test).

(J) Representative photographs of hsp-6p::gfp expression in aak-2 deletion mutant animals treated with control or cyc-2.1 RNAi. Scale bar, 200 μm.

(K) qRT-PCR of hsp-6 (median with range) using RNAs extracted from dissected intestinal tissues of the aak-2 mutant treated with control or cyc-2.1 RNAi based on three biological replicates (**p < 0.0001, two-tailed t test).

See also Table S3.
Figure 3. Inhibition of cyc-2.1 in the Germline Extends Lifespan by Cell-Non-autonomous Activation of UPRmt and AMPK in the Intestine

(A) Tissue-specific cyc-2.1 RNAi-induced changes in the mean lifespan relative to the control RNAi treatments. Data are represented as mean ± SEM based on three biological replicates.

(B) qRT-PCR of UPRmt markers hsp-6, dnj-10, timm-17, and drp-1 (median with range) using RNAs extracted from dissected gonadal and intestinal tissues of N2 treated with global control or cyc-2.1 RNAi based on three biological replicates (ns, ***p < 0.001, p > 0.05, two-tailed t-tests).

(C and D) Immunoblots (C) and quantification (D) of phospho-AAK-2 and actin using proteins extracted from dissected gonadal and intestinal tissues of N2 treated with global control or cyc-2.1 RNAi. Data are represented as mean ± SEM based on three biological replicates (ns, **p < 0.01, p = 0.2436, two-tailed t-tests).

(E) Survival curves of the aak-2 mutant carrying an aak-2 transgene driven by the intestine-specific vha-6 promoter treated with the control or cyc-2.1 RNAi (p < 0.0001, log-rank test).

(F) qRT-PCR of UPRmt markers (median with range) using RNAs extracted from dissected intestinal tissues of animals treated with germline- or intestine-specific control versus cyc-2.1 RNAi based on three biological replicates (***p < 0.001, **p < 0.01, *p < 0.05, two-tailed t-tests).

(G and H) Immunoblots (G) and quantification (H) of phospho-AAK-2 and tubulin using proteins extracted from dissected intestinal tissues of animals treated with germline- or intestine-specific control versus cyc-2.1 RNAi. Data are represented as mean ± SEM based on three biological replicates (ns, **p < 0.001, p = 0.6141, two-tailed t-tests).

(legend continued on next page)
with the control or cyc-2.1 RNAi for qRT-PCR of the endogenous hsp-6 mRNA. Consistent with the hsp-6p::gfp reporter results, knockdown of cyc-2.1 significantly increases hsp-6 mRNA levels compared with the control RNAi treatment (Figure 2C). Previous studies have identified ATFS-1 as one of the key transcription factors that mediate the UPR\textsuperscript{mt} activation (Nargund et al., 2012). The cyc-2.1 RNAi-induced longevity phenotype is suppressed by a deletion mutant of atfs-1 (Figure 2D; Table S3). Therefore, activated mitochondrial stress response plays an essential role in cytochrome c knockdown-induced lifespan extension.

It has been reported that paraquat, a ROS (reactive oxygen species) generator, and hypomorphic mutations in mitochondrial ETC genes, such as isp-1, extend C. elegans lifespan by activating AMPK (Hwang et al., 2014). The synergistic lifespan extension by daf-2 rsks-1 also requires AMPK (Chen et al., 2013). We performed immunoblot to measure the levels of phosphorylated AAK-2 (AMPK\textsubscript{a}), which serve as an indicator of AMPK activation, in control or cyc-2.1 RNAI-treated animals. Knockdown of cyc-2.1 significantly increases phospho-AAK-2 levels compared with the control (Figures 2E and 2F). Consistently, cyc-2.1 RNAI fails to extend lifespan of the aak-2 deletion mutant (Figure 2G; Table S3). Therefore, inhibition of cyc-2.1 activates UPR\textsuperscript{mt} and AMPK to extend lifespan.

**AMPK Functions Downstream of UPR\textsuperscript{mt} upon cyc-2.1 Knockdown**

To characterize the relationship between UPR\textsuperscript{mt} and AMPK in animals treated with cyc-2.1 RNAI, we first measured phospho-AK-2 levels in the atfs-1 deletion mutant treated with either control or cyc-2.1 RNAI. Immunoblots and quantification results indicate that unlike in the N2 background (Figures 2E and 2F), cyc-2.1 RNAI does not increase phospho-AK-2 levels in the atfs-1 mutant (Figures 2H and 2I). We then crossed the hsp-6 promoter:gfp reporter into the aak-2 deletion mutant. In the absence of AAK-2, cyc-2.1 RNAI still significantly activates the hsp-6:gfp reporter (Figure 2J), as well as the endogenous hsp-6 transcription in the intestine (Figure 2K). Altogether, these results demonstrate that AMPK functions downstream of UPR\textsuperscript{mt} activation to promote lifespan extension produced by the cyc-2.1 RNAI treatment.

**cyc-2.1 Functions in the Germline to Regulate Lifespan**

To determine the key tissue in which cyc-2.1 functions to regulate lifespan, we performed tissue-specific RNAI experiments to knockdown cyc-2.1 in the germline, intestine, epidermis, and muscles. Spatially restricted RNAI knockdown was achieved by tissue-specific promoters driving transgene rescue of mutations in rde-1, which is essential for the RNAI machinery to be functional (Espelt et al., 2005; Qadota et al., 2007; Zou et al., 2019). Knockdown of cyc-2.1 in the germline significantly extends lifespan (Figure 3A; Figure S3A; Table S3), whereas knockdown of cyc-2.1 in the intestine, epidermis, or muscles does not extend lifespan (Figure 3A; Figures S3B–S3D; Table S3). The importance of germline in cyc-2.1 RNAI–induced lifespan extension is supported by evidence that knockdown of cyc-2.1 in the germline-less glp-4(ts) mutant (Beanan and Strome, 1992) only results in mild lifespan extension by 9% (Figure S3E). Altogether, these results demonstrate that germline is the key tissue in which CYC-2.1 functions to regulate lifespan.

**Germline Knockdown of cyc-2.1 Non-autonomously Activates UPR\textsuperscript{mt} and AMPK in the Intestine to Extend Lifespan**

To better understand whether cyc-2.1 RNAI activates UPR\textsuperscript{mt} in a tissue-specific manner, we performed qRT-PCR experiments to measure mRNA levels of several ATFS-1 direct target genes, including hsp-6, dnl-10, timm-17, and drp-1, transcription levels of which are elevated upon mitochondrial ETC perturbation (Nargund et al., 2012, 2015). Gonadal and intestinal tissues were dissected from wild-type animals treated with either control or cyc-2.1 RNAI for qRT-PCR assays. Because the gonad is 95% germline and 5% somatic gonad, and the germline and somatic gonad cannot be further dissected, we used the gonadal tissue as a proxy for the germline in subsequent experiments. Surprisingly, global cyc-2.1 RNAI treatment significantly activates UPR\textsuperscript{mt} markers in the intestine, but not in the germline (Figure 3B), although the latter is the tissue in which cyc-2.1 functions to regulate lifespan. Consistently, global cyc-2.1 RNAI treatment significantly activates AMPK in the intestine, but not in the gonad (Figures 3C and 3D). The aak-2 deletion mutant carrying a single-copy aak-2 transgene driven by the vha-6 intestine-specific promoter shows a 45% lifespan extension upon cyc-2.1 RNAI treatment (Figure 3E; Table S3). The intestinal AAK-2 is therefore sufficient to extend lifespan substantially upon cyc-2.1 RNAI knockdown.

**Germline cyc-2.1 Knockdown Induces Intestinal UPR\textsuperscript{mt} and AMPK Activation and Lifespan Extension**

We next tested whether tissue-specific knockdown of cyc-2.1 could activate UPR\textsuperscript{mt} and AMPK. Although both germline and intestinal-specific cyc-2.1 RNAI treatments significantly activate UPR\textsuperscript{mt} markers in the intestine (Figure 3F), only germline, not intestinal, cyc-2.1 RNAI treatment leads to increased phospho-AK-2 levels in the intestine (Figures 3G and 3H). Because there is no direct physical contact between gonad and intestine, these results suggest the existence of endocrine-like signaling for cell non-autonomous regulation.
C. elegans has six macrophage-like coelomocytes that take up soluble macromolecules in the body cavity via endocytosis. Coelomocytes have direct physical contact with the gonad and intestine. CUP-4, an ion channel, is required for endocytosis in coelomocytes (Patton et al., 2005). When treated with the germ-line-specific cyc-2.1 RNAi, the cup-4 mutant cannot activate UPRmt (Figure 3I) and AMPK (Figures 3J and 3K) in the intestine or extend lifespan (Figure 3L; Table S3). Altogether, these results indicate the existence of gonad to intestine signaling that regulates UPRmt, AMPK, and lifespan in response to cytochrome c reduction in the germline.

DRP-1-Mediated Mitochondrial Fission Plays an Important Role in cyc-2.1 Knockdown-Induced AMPK Activation and Lifespan Extension

RNAi knockdown of cyc-2.1 leads to the transcriptional upregulation of several ATFS-1 targets, including drp-1, which encodes a dynamin-related protein that is required for mitochondrial fission (Breckenridge et al., 2008). Using a transgenic line that expresses the TOMM-20 mitochondria outer membrane protein fused with the mKate2 fluorescent protein in the intestine (Ahier et al., 2018), we found that knockdown of cyc-2.1 significantly increases intestinal mitochondrial fragmentation (Figures 4A and 4B). Blocking the mitochondrial fission by a drp-1 knockout mutant significantly suppresses cyc-2.1 knockdown-induced AMPK activation (Figures 4C and 4D). cyc-2.1 RNAi treatment extends lifespan in wild-type animals by 66%, whereas the mean lifespan extension is significantly decreased to 42% in the drp-1 mutant (Figures 4E and 3F; Table S3). Therefore, DRP-1-mediated changes in mitochondrial dynamics serve as the key mechanism for cytochrome c reduction-induced lifespan extension.

Tissue-Specific Activation of UPRmt Contributes to the Significantly Prolonged Longevity of the daf-2 rsks-1 Mutant

cyc-2.1 was identified as one of the genes that show significantly decreased ribosomal loading in the daf-2 rsks-1 mutant compared with the wild-type N2 from the translational profiling analysis (Table S1). To examine whether CYC-2.1 is repressed at the protein level in the daf-2 rsks-1 mutant, we used a CRISPR/Cas9-based genome editing approach to knock in the mKate2 red fluorescent protein coding sequence to the 3’ of glp-1. Compared with the wild-type control, GLD-1::mKate2 protein levels are significantly increased in the germline of daf-2 rsks-1 mutant animals (Figures 6A and 6B). To examine whether GLD-1 is involved in the translational regulation of cyc-2.1, we applied glp-1 RNAi to the daf-2 rsks-1 mutant and found that CYC-2.1 protein levels are significantly elevated in the germline, but not in the intestine, when compared with the control RNAi-treated animals (Figures 6C and 6D). Consistently, RNAi knockdown of glp-1 significantly decreases UPRmt in the intestine of daf-2 rsks-1 mutant animals (Figure 6E). Although glp-1 RNAi treatment has little effect on lifespan in the wild-type and daf-2 mutant backgrounds, knockdown of glp-1 significantly decreases lifespan of the rsks-1 mutant and daf-2 rsks-1 double mutant (Figure 6F; Table S3). Altogether, these results demonstrate that GLD-1 functions as a translational repressor of cyc-2.1 in the germline to non-autonomously activate intestinal UPRmt and extend lifespan in the daf-2 rsks-1 mutant.

In summary, we have performed genome-wide translational state analysis via polysomal profiling coupled with RNA-seq and identified genes that are regulated at the translational level in the significantly long-lived daf-2 rsks-1 mutant. Mechanistically, we demonstrated that the GLD-1 RNA-binding protein is upregulated at the protein level in the germline of daf-2 rsks-1 mutant. Elevated GLD-1 leads to translational repression of CYC-2.1 in the germline, which non-autonomously activates UPRmt and AMPK in the intestine and significantly extends lifespan (Figure 6G). These results highlight the importance of translational regulation of a highly conserved mitochondrial gene in the significantly prolonged longevity exerted by inhibiting both insulin-like signaling and S6K.

GLD-1-Mediated Translational Repression of CYC-2.1 Is Important for the UPRmt Activation and Significantly Prolonged Longevity of the daf-2 rsks-1 Mutant

Specific translational regulations in many cases are mediated by RNA-binding proteins and their association with 5’ UTRs or 3’ UTRs. GLD-1, a K homology (KH) RNA-binding protein, is regulated by GLP-1/Notch signaling to negatively regulate target genes’ translation in the germline (Kimble and Crittenden, 2005). Our previous studies showed that a glp-1 gain-of-function mutation, which decreases GLD-1 expression, suppresses the significantly extended lifespan of daf-2 rsks-1 (Chen et al., 2013). The genome-wide translational state analysis indicates that glp-1 mRNAs have elevated ribosomal loading in the daf-2 rsks-1 mutant (Table S1). These results suggest that the germ-line-specific translational repressor GLD-1 might be involved in regulating CYC-2.1 protein levels in the germline of the daf-2 rsks-1 mutant.

To test whether GLD-1 is upregulated in the daf-2 rsks-1 mutant, we first performed CRISPR/Cas9-based genome editing experiments to knock in the mKate2 red fluorescent protein coding sequence to the 3’ of glp-1. Compared with the wild-type control, GLD-1::mKate2 protein levels are significantly increased in the germline of daf-2 rsks-1 mutant animals (Figures 6A and 6B). To examine whether GLD-1 is involved in the translational regulation of cyc-2.1, we applied glp-1 RNAi to the daf-2 rsks-1 mutant and found that CYC-2.1 protein levels are significantly elevated in the germline, but not in the intestine, when compared with the control RNAi-treated animals (Figures 6C and 6D). Consistently, RNAi knockdown of glp-1 significantly decreases UPRmt in the intestine of daf-2 rsks-1 mutant animals (Figure 6E). Although glp-1 RNAi treatment has little effect on lifespan in the wild-type and daf-2 mutant backgrounds, knockdown of glp-1 significantly decreases lifespan of the rsks-1 mutant and daf-2 rsks-1 double mutant (Figure 6F; Table S3). Altogether, these results demonstrate that GLD-1 functions as a translational repressor of cyc-2.1 in the germline to non-autonomously activate intestinal UPRmt and extend lifespan in the daf-2 rsks-1 mutant.

In summary, we have performed genome-wide translational state analysis via polysomal profiling coupled with RNA-seq and identified genes that are regulated at the translational level in the significantly long-lived daf-2 rsks-1 mutant. Mechanistically, we demonstrated that the GLD-1 RNA-binding protein is upregulated at the protein level in the germline of daf-2 rsks-1 mutant. Elevated GLD-1 leads to translational repression of CYC-2.1 in the germline, which non-autonomously activates UPRmt and AMPK in the intestine and significantly extends lifespan (Figure 6G). These results highlight the importance of translational regulation of a highly conserved mitochondrial gene in the significantly prolonged longevity exerted by inhibiting both insulin-like signaling and S6K.

Previous studies showed that transcriptional factors DVE-1 and ATFS-1, as well as UBL-5, a small ubiquitin-like factor that serves as the co-factor for DVE-1, are the key mediators of UPRmt (Haynes et al., 2007; Nargund et al., 2012). Inhibition of dve-1 by RNAI shortens N2 lifespan, whereas ubl-5 or atfs-1 RNAi treatment does not affect N2 lifespan (Figure 5D; Table S3). The prolonged longevity of daf-2 rsks-1 mutant animals can be significantly decreased by dve-1, ubl-5, or atfs-1 RNAi (Figure 5E; Table S3). Knockdown of these key transcriptional regulators of UPRmt significantly decreases the lifespan extension produced by the daf-2 rsks-1 double mutant (Figure 5F). Therefore, the daf-2 rsks-1 mutant shows germline reduction of CYC-2.1 and intestinal activation of UPRmt, which is required for significantly prolonged longevity.
DISCUSSION

Highly conserved IIS and the TOR pathway play an important role in aging across species (Fontana et al., 2010; Kenyon, 2010). To examine how these pathways interact with each other to modulate aging, we previously constructed a daf-2 rsks-1 double mutant that carries loss-of-function mutations in the DAF-2/IGF-1 receptor and TOR effector RSKS-1/S6K. The double mutant shows a synergistic effect, rather than an additive effect, on longevity, suggesting active interactions between these two important aging-related pathways. Functional genomics studies via transcriptome profiling helped to identify AMPK-mediated positive feedback regulation of the DAF-16/FOXO transcription factor mechanism in the daf-2 rsks-1 mutant (Chen et al., 2013). However, RSKS-1, which serves as a key regulator of mRNA translation, has not been well characterized for its roles in the significantly extended longevity of daf-2 rsks-1 mutant animals.

It has been well documented that inhibition of translation delays aging (Hansen et al., 2007; Kapahi et al., 2004, 2010; Pan et al., 2007; Rogers et al., 2011). One hypothesis is that reduced global translation helps organisms to maintain better protein homeostasis, dysregulation of which leads to aging and age-related pathologies. A linked hypothesis suggests that the anti-aging effect is achieved by the translational regulation of key modulators of aging. We reasoned that identification of genes that are translationally regulated in the daf-2 rsks-1 mutant and characterization of the translational regulation should help in gaining better mechanistic insights into the significantly extended longevity of the daf-2 rsks-1 double mutant.

Figure 4. cyc-2.1 Knockdown-Induced Mitochondria Fragmentation Plays an Important Role in AMPK Activation and Lifespan Extension

(A and B) Representative photographs (A) and quantification (B) of intestinal mitochondrial morphology in animals treated with control or cyc-2.1 RNAi (p < 0.0001, \( \chi^2 \) test). Scale bar, 10 \( \mu \m\). (C and D) Immunoblots (C) and quantification (D) of phospho-AAK-2 and actin in N2 and drp-1 mutant animals treated with control or cyc-2.1 RNAi. Data are represented as mean \( \pm \) SEM based on three biological replicates (ns, *p < 0.05, p = 0.3103, two-tailed t tests). (E) Survival curves of N2 and the drp-1 mutant treated with control or cyc-2.1 RNAi. (F) cyc-2.1 RNAi-induced changes in mean lifespan relative to the control RNAi treatments in N2 and drp-1 mutant backgrounds. Data are represented as mean \( \pm \) SEM based on three biological replicates (*p < 0.05, two-tailed t test).

See also Table S3.

Through genome-wide translational state analysis and genetic screens, we identified 24 negative regulators of longevity from the 115 genes that are translationally downregulated in the daf-2 rsks-1 mutant. One observation was that the lifespan determinants are enriched with developmentally essential genes (71%). Inhibition of these genes during development causes larval arrest, whereas RNAi knockdown only during adulthood leads to significant lifespan extension (Table S2). These results support the antagonistic pleiotropy theory of aging, which proposes that aging is adaptive, because natural selection favors genes that confer benefits during development but cause deleterious effects later in life (Williams, 1957). Thus, inhibition of developmentally essential genes during adulthood might extend lifespan (Chen et al., 2007; Curran and Ruvkun, 2007). All developmentally essential, lifespan-determinant genes encode various ribosomal subunits (Table S2), which is consistent with previous studies showing that TOR-mediated ribosomal biogenesis plays an important role in aging (Steffen et al., 2008). In addition, 13 of the 17 identified ribosomal genes showed decreased protein levels in the daf-2 mutant or in nutrients restricted animals from previous quantitative proteomics studies (Depuydt et al., 2013; Stout et al., 2013), which highlights the important role of reduced mRNA translation in the delay of aging.

Among the lifespan regulators that we identified, knockdown of cyc-2.1, which encodes a highly conserved cytochrome c ortholog that functions in the mitochondrial ETC, results in the most significant lifespan extension (Table S2; Figure 2A). Inhibition of genes encoding components of mitochondrial respiratory complexes extends lifespan (Dillin et al., 2002; Houtkooper et al., 2013; Lee et al., 2003), and the underlying mechanisms involve UPR\(^\text{mt}\) (Durieux et al., 2011), CEP-1/p53 (Baruah et al., 2014), and the intrinsic apoptosis pathway (Yee et al., 2014). CEP-1 and CED-4 from the intrinsic apoptosis pathway are not required...
for cyc-2.1 RNAi-induced lifespan extension (Figures S2A and S2B). Inhibition of cco-1, which encodes the cytochrome c oxidase-1 subunit Vb/COX4, significantly extends lifespan (Dillin et al., 2002). ATFS-1 is partially required for cco-1 RNAi-induced lifespan extension (Figure S2C), whereas prolonged longevity by cyc-2.1 RNAi can be suppressed by an atfs-1 deletion mutant (Figure 2D; Table S3). Unlike cyc-2.1, intestine-specific cco-1 RNAi also activates UPRmt, it fails to activate AMPK and extend lifespan (Figures 3A and 3F–3H; Figure S3B; Table S3). These results indicate that activation of UPRmt is required, but not sufficient, for the lifespan extension produced by cyc-2.1 knockdown. Because there is no direct contact between the germline and the intestine in C. elegans, we propose that reduced cytochrome c in the germ-line might produce an endocrine-like signaling, named gMitokine (germline-produced mitokine), to activate UPR mt in the distal tissue for prolonged survival.

The germline to intestine regulation can be blocked by the cup-4 mutant, which disrupts endocytosis in coelomocytes (Figures 3I–3L). Altogether, our findings demonstrate a cell non-autonomous signaling that is distinctive from the neuron to intestine mitokine pathways. Further characterization of this germline to intestine signal transduction process, especially identifying the gMitokine and its downstream effectors, will help in better understanding UPRmt-mediated anti-aging mechanisms.

The germline tissue plays an important role in C. elegans aging. Germline-less worms showed significant lifespan extension that depends on the DAF-16 FOXO transcription factor and DAF-12 nuclear hormone receptor. The prolonged longevity of germline-less animals is not due to sterility; rather, it is caused by diminished pro-aging signaling from the germline (Berman and Kenyon, 2006; Hsin and Kenyon, 1999). However, the
molecular identity of aging signals produced by germ cells has not been fully determined. We speculate that cyc-2.1 knockdown and germline deficiency function through different mechanisms to regulate lifespan, because unlike the long-lived germline-less animals, the cyc-2.1 RNAi-treated animals show significantly prolonged longevity independent of DAF-16 or DAF-12 (Figure S3F).

It has been shown that the germline is the key tissue for RSKS-1 to regulate lifespan. Knockdown of rsk-1 in the germline of daf-2 mutant produces a synergistic effect on longevity (Chen et al., 2013). The histone H3 lysine 3 trimethylation (H3K3me3) deficiency-induced lifespan extension is also mediated by downregulation of RSKS-1 in the germline, which leads to increased accumulation of mono-unsaturated fatty acids (MUFAs) in the distal intestine tissue (Han et al., 2017). It will be interesting to test whether inhibition of cyc-2.1 in the germline affects lipid metabolism in the intestine, and if so, whether UPRmt is involved in this process, in future studies.

In conclusion, genome-wide translational state analysis allowed us to identify a series of translational regulation of lifespan-determinant genes in the significantly long-lived daf-2 rsk-1 mutant. Functional studies revealed that RNA-binding protein GLD-1-mediated translational repression of cytochrome c in the germline leads to non-autonomous activation of UPRmt and AMPK in the intestine via germline-produced mitokine (gMitokine) signaling, which leads to significant lifespan extension in the daf-2 rsk-1 mutant. See also Table S3.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **LEAD CONTACT AND MATERIALS AVAILABILITY**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - C. elegans strains and maintenance
- **METHOD DETAILS**
  - Polysomal profiling
  - RNA-Seq and bioinformatics analysis
Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq—a Python framework to resolve patterns of genetic mosaicism. Nat. Cell Biol.

Ahier, A., Dai, C.-Y., Tweedie, A., Bezawork-Geleta, A., Kirmes, I., and Zuryn, L.L., Yee, C., Hekimi, S., Derry, W.B., and Lee, S.S. (2014). CEP-1, the p53 homolog, mediates opposing longevity outcomes in mitochondrial electron transport chain mutants. PLoS Genet. 10, e1004097.

Beanan, M.J., and Strome, S. (1992). Characterization of a germ-line proliferation mutation in C. elegans. Development 116, 755–768.

REFERENCES

Benjamin, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57, 289–300.

Berendzen, K.M., Durieux, J., Shao, L.-W., Tian, Y., Kim, H.-E., Wolff, S., Liu, Y., and Dillin, A. (2016). Neuroendocrine Coordination of Mitochondrial Stress Signaling and Proteostasis. Cell 166, 1553–1563.e10.

Berman, J.R., and Kenyon, C. (2006). Germ-cell loss extends C. elegans life span through regulation of DAF-16 by kri-1 and lipophilic-hormone-signaling. Cell 124, 1055–1068.

Breckenridge, D.G., Kang, B.-H., Koke, D., Mitani, S., Staehelin, L.A., and Xue, D. (2008). Caenorhabditis elegans drp-1 and f2-2 regulate distinct cell-death execution pathways downstream of ced-3 and independent of ced-9. Mol. Cell 31, 586–597.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics 77, 71–94.

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.

Chen, D., Thomas, E.L., and Kapahi, P. (2009). HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in Caenorhabditis elegans. PLoS Genet. 5, e1000486.

Chen, D., Li, P.W.-L., Goldstein, B.A., Cai, W., Thomas, E.L., Chen, F., Hubbard, A.E., Melov, S., and Kapahi, P. (2013). Germline signaling mediates the synergistically prolonged longevity produced by double mutations in daf-2 and rks-1 in C. elegans. Cell Rep. 5, 1600–1610.

Cox, D.R., and Reid, N. (1987). Parameter Orthogonality and Approximate Conditional Inference. J. R. Stat. Soc. Ser. B 49, 1–39.

Curran, S.P., and Ruvkun, G. (2007). Lifespan regulation by evolutionarily conserved genes essential for viability. PLoS Genet. 3, e56.

Depuydt, G., Xie, F., Petyuk, V.A., Shanmugam, N., Smolders, A., Dhondt, I., Brewer, H.M., Camp, D.G., 2nd, Smith, R.D., and Braeckman, B.P. (2013). Reduced insulin/insulin-like growth factor-1 signaling and dietary restriction inhibit translation but preserve muscle mass in Caenorhabditis elegans. Mol. Cell Proteomics 12, 3624–3639.

Dickinson, D.J., Ward, J.D., Reiner, D.J., and Goldstein, B. (2013). Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination. Nat. Methods 10, 1028–1034.

Dickinson, D.J., Pani, A.M., Heppert, J.K., Higgins, C.D., and Goldstein, B. (2015). Streamlined Genome Engineering with a Self-Excising Drug Selection Cassette. Genetics 200, 1035–1049.

Dillin, A., Hsu, A.-L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A.G., Kamath, R.S.,ahringer, J., and Kenyon, C. (2002). Rates of behavior and aging specified by mitochondrial function during development. Science 298, 2398–2401.

Dong, M.-Q., Venable, J.D., Au, N., Xu, T., Park, S.K., Cociorva, D., Johnson, J.R., Dillin, A., and Yates, J.R., 3rd. (2007). Quantitative mass spectrometry identifies insulin signaling targets in C. elegans. Science 317, 660–663.

Durieux, J., Wolff, S., and Dillin, A. (2011). The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell 144, 79–91.

Espelt, M.V., Estevez, A.Y., Yin, X., and Strange, K. (2005). Oscillatory Ca2+ signaling in the isolated Caenorhabditis elegans intestine: role of the inositol-1,4,5-trisphosphate receptor and phospholipases C beta and gamma. J. Gen. Physiol. 126, 379–392.

Fontana, L., Partridge, L., and Longo, V.D. (2010). Extending healthy life span—from yeast to humans. Science 328, 321–326.

Han, S., Schroeder, E.A., Silva-Garcia, C.G., Hebestreit, K., Mair, W.B., and Brunet, A. (2017). Mono-unsaturated fatty acids link H3K4me3 modifiers to C. elegans lifespan. Nature 544, 185–190.

Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.-J., and Kenyon, C. (2007). Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. Aging Cell 6, 95–110.

AUTHOR CONTRIBUTIONS

J.L., X.Z., and D.W. performed experiments. J.A.R. performed experiments and bioinformatics analysis. L.Z., Z. Wang, C.Y., and Z. Wu provided technical assistance. D.C., A.N.R., and P.K. conceived the project, designed experiments, and wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: December 20, 2018
Revised: June 4, 2019
Accepted: June 21, 2019
Published: July 23, 2019

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.06.078.

DATA AND CODE AVAILABILITY

- RNAi by feeding
- Lifespan assay
- RNAi screen for lifespan regulators
- Microscopy
- Western blot and antibodies
- RT-qPCR
- Worm tissue micro-dissection
- CRISPR/Cas9 alleles generation

QUANTIFICATION AND STATISTICAL ANALYSIS

ACKNOWLEDGMENTS

We thank Drs. Qian Bian, Shiqing Cai, Mengqiu Dong, Arjumand Ghazi, Cole Haynes, Ying Liu, Shohei Mitani, Billy Qi, Ye Tian, Xiaochen Wang, Zhiping Wang, and Steven Zuryn for worm strains, plasmids, antibodies, unpublished results and discussion. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010448), and some strains were provided by the Japanese National BioResource Project. This work was supported by grants from the National Natural Science Foundation of China (31471379 and 31671527) (to D.C.); by grants from the American Federation for Aging Research, the Larry L. Hillblom Foundation, the Impact Circle Award (Buck), and the NIH (AG053066 and AG045839) (to P.K.); and by grants from the NIH (AG056743), the Morris Scientific Discovery Award, and an Institutional Development Award from the National Institute of General Medical Sciences of the NIH (P20GM103423 and P20GM104318) (to A.N.R.).
Haynes, C.M., Petrova, K., Benedetti, C., Yang, Y., and Ron, D. (2007). ClpP mediates activation of a mitochondrial unfolded protein response in C. elegans. Dev. Cell 13, 467–480.

Houtkooper, R.H., Mouchiroud, L., Ryu, D., Moulian, N., Katsuya, E., Knott, G., Williams, R.W., and Auwerx, J. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. Nature 497, 451–457.

Hsin, H., and Kenyon, C. (1999). Signals from the reproductive system regulate the lifespan of C. elegans. Nature 399, 362–366.

Hwang, A.B., Ryu, E.-A., Artan, M., Chang, H.-W., Kabir, M.H., Nam, H.-J., Lee, D., Yang, J.-S., Kim, S., Mair, W.B., et al. (2014). Feedback regulation via AMPK and HIP-1 mediates ROS-dependent longevity in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 111, E4458–E4467.

Kamath, R.S., Martinez-Campos, M., Zipperlen, P., Fraser, A.G., and Ahringer, J. (2001). Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. Genome Biol 2, RESEARCH0002. PubMed.

Kapahi, P., Zid, B.M., Harper, T., Koslover, D., Sapin, V., and Benzer, S. (2004). Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Curr. Biol. 14, 885–890.

Kapahi, P., Chen, D., Rogers, A.N., Katewa, S.D., Li, P.W.-L., Thomas, E.L., and Kockel, L. (2010). With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. Cell Metab. 11, 453–465.

Kenyon, C.J. (2010). The genetics of aging. Nature 464, 504–512.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature 366, 461–464.

Kim, Y., and Sun, H. (2007). Functional genomic approach to identify novel genes involved in the regulation of oxidative stress resistance and animal lifespan. Aging Cell 6, 489–503.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcripts in the presence of insertions, deletions and gene fusions. Genome Biol. 14, R36.

Kimble, J., and Crittenden, S.L. (2005). Germline proliferation and its control. WormBook, 1–14.

Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). A forkhead family member that regulates longevity and diapause in C. elegans. Science 277, 942–946.

Korta, D.Z., Tuck, S., and Hubbard, E.J.A. (2012). S6K links cell fate, cell cycle and nutrient response in C. elegans germline stem/progenitor cells. Development 139, 859–870.

Lee, S.S., Lee, R.Y.N., Fraser, A.G., Kamath, R.S., Ahringer, J., and Ruvkun, G. (2005). ClpP degradation of the RNAi silencing machinery mediates activation of a mitochondrial unfolded protein response in C. elegans. Curr. Biol. 15, 1045–1050.

Lee, S.P., Paradies, S., Gottlieb, S., Patterson, G.J., Lee, L., Tissenbaum, H.A., and Ruvkun, G. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature 389, 994–999.

Pan, K.Z., Palter, J.E., Rogers, A.N., Olsen, A., Chen, D., Lithgow, G.J., and Kapahi, P. (2007). Inhibition of mRNA translation extends lifespan in Caenorhabditis elegans. Aging Cell 6, 111–119.

Patton, A., Knuth, S., Schaheen, B., Dang, H., Greenwald, I., and Fares, H. (2005). Endocytosis function of a ligand-gated ion channel homolog in Caenorhabditis elegans. Curr. Biol. 15, 1045–1050.

Qadota, H., Inoue, M., Hikita, T., Köppen, M., Hardin, J.D., Amano, M., Moerlein, D.G., and Kaibuchi, K. (2007). Establishment of a tissue-specific RNAi system in C. elegans. Gene 400, 166–173.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.

Roberts, E., Li, X., powers, M.C., and Bush, D.J. (2013). For the FBS-1/PHM system in C. elegans. J. Lipid Res. 54, 2504–2514.

Selman, C., Tullet, J.M.A., Wieser, D., Irvine, E., Lingard, S.J., Choudhury, A.I., Clare, M., Al-Qassab, H., Carmignac, D., Ramadan, F., et al. (2009). Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science 326, 140–144.

Seo, K., Choi, E., Lee, D., Jeong, D.-E., Jang, S.K., and Lee, S.-J. (2013). Heat shock factor 1 mediates the longevity conferred by inhibition of TOR and insulin/GF-1 signaling pathways in C. elegans. Aging Cell 12, 1073–1081.

Shao, L.-W., Niu, R., and Liu, Y. (2016). Neuropeptide signals cell non-autonomous mitochondrial unfolded protein response. Cell Res. 26, 1182–1196.

Sheaffer, K.L., Updike, D.L., and Mange, S.O. (2008). The Target of Rapamycin pathway antagonizes pha-4/FoxA to control development and aging. Curr. Biol. 18, 1355–1364.

Shi, X., Li, J., Zou, X., Greggain, J., Redkar, S.V., Fargeman, N.J., Liang, B., and Watts, J.L. (2013). Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase. J. Lipid Res. 54, 2504–2514.
Tian, Y., Garcia, G., Bian, Q., Steffen, K.K., Joe, L., Wolff, S., Meyer, B.J., and Dillin, A. (2016). Mitochondrial Stress Induces Chromatin Reorganization to Promote Longevity and UPR(mt). Cell 165, 1197–1208.

Williams, G.C. (1957). Pleiotropy, Natural Selection, and the Evolution of Senescence. Evolution 11, 398–411.

Yee, C., Yang, W., and Hekimi, S. (2014). The intrinsic apoptosis pathway mediates the pro-longevity response to mitochondrial ROS in C. elegans. Cell 157, 897–909.

Zhang, Q., Wu, X., Chen, P., Liu, L., Xin, N., Tian, Y., and Dillin, A. (2018). The Mitochondrial Unfolded Protein Response Is Mediated Cell-Non-autonomously by Retromer-Dependent Wnt Signaling. Cell 174, 870–883.e17.

Zid, B.M., Rogers, A.N., Katewa, S.D., Vargas, M.A., Koliopinski, M.C., Lu, T.A., Benzer, S., and Kapahi, P. (2009). 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in Drosophila. Cell 139, 149–160.

Zou, L., Wu, D., Zang, X., Wang, Z., Wu, Z., and Chen, D. (2019). Construction of a germline-specific RNAi tool in C. elegans. Sci. Rep. 9, 2354.
# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies** | | |
| β-Actin Antibody | Cell Signaling Technology | RRID: AB_330288; Cat# 4967 |
| Phospho-AMPKα (Thr172) (40H9) Rabbit mAb | Cell Signaling Technology | RRID: AB_331250; Cat# 2535S |
| Monoclonal Anti-α-Tubulin antibody | Sigma-Aldrich | RRID: AB_477582; Cat# T6074 |
| Monoclonal Anti-FLAG antibody | Sigma-Aldrich | RRID: AB_262044; Cat# F1804 |
| Anti-RPS-0 antibody | Liu et al., 2018 | N/A |
| Anti-RPS-3 antibody | Liu et al., 2018 | N/A |
| Anti-RPL-5 antibody | Liu et al., 2018 | N/A |
| Anti-RPL-25.2 antibody | Liu et al., 2018 | N/A |
| Goat Anti-Rabbit IgG (H+L) HRP | Bioworld | RRID: AB_2773728; Cat# BS13278 |
| Goat Anti-Mouse IgG (H+L) HRP | Bioworld | RRID: AB_2773727; Cat# BS12478 |
| **Bacterial and Virus Strains** | | |
| E. coli: Strain OP50 | Caenorhabditis Genetics Center | N/A |
| E. coli: Strain HT115 | Caenorhabditis Genetics Center | N/A |
| C. elegans RNAi Library (Ahringer) | Source BioScience | 3318_Cel_RNAi_complete |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Taq DNA Polymerase | ABM | Cat# G008 |
| T4 ligase | ABM | Cat# EL0011 |
| Ligation-Free Cloning Kit | ABM | Cat# E002 |
| Ampicillin | BBI | Cat# AB008 |
| Peptone | BD | Cat# 211677 |
| Agar | BD | Cat# 214010 |
| Tryptone | BD | Cat# 211705 |
| Yeast Extract | BD | Cat# 212750 |
| Agar | BD | Cat# 214010 |
| Biodlight Western Chemiluminescent HRP substrate | Bioworld | Cat# BLH0025050 |
| Protease K | Fermentas | Cat# EO0491 |
| FastAP Thermosensitive Alkaline Phosphatase | Fermentas | Cat# EF0654 |
| ExpressPlus PAGE Gel | GenScript | Cat# M42015C |
| Tris-MOPS-SDS Running Buffer | GenScript | Cat# M00138 |
| PVDF | Immobilon | Cat# IPVH00010 |
| cOmplete, EDTA-free Protease Inhibitor Cocktail | Roche | Cat# 4906845001 |
| PhosSTOP | Roche | Cat# D0056 |
| dNTP | Sangon | Cat# EX328 |
| Sodium chloride | Sangon | Cat# A100241 |
| Sodium dodecyl sulfate (SDS) | Sangon | Cat# S0227 |
| EDTA | Sangon | Cat# EB0185 |
| Tris (Base) | Sangon | Cat# TT1492 |
| Potassium chloride | Sangon | Cat# A501212-0500 |
| Magnesium sulfate anhydrous (MgSO₄) | Sangon | Cat# A601988-0250 |
| Calcium chloride (CaCl₂) | Sangon | Cat# 10035-04-8 |
| Isopropyl-b-D-thiogalactoside (IPTG) | Sangon | Cat# IB0168 |
| Potassium phosphate dibasic (K₂HPO₄) | Sangon | Cat# PT1212 |
| Potassium phosphate monobasic (KH₂PO₄) | Sangon | Cat# A600445 |

(Continued on next page)
Continued

| REAGENT or RESOURCE | SOURCE          | IDENTIFIER   |
|---------------------|----------------|--------------|
| Tetracycline        | Sangon         | Cat# G0167   |
| Protein ladder      | Sangon         | Cat# AB0072  |
| Glycine             | Sangon         | Cat# T0761   |
| APS, Ammonium persulfate | Sangon   | Cat# M0004   |
| TEMED               | Sangon         | Cat# SN319   |
| β-Mercaptoethanol   | Sangon         | Cat# NB0669  |
| 30% Acryl/Bis Solution | Sangon       | Cat# SN319   |
| NON-Fat Powdered Milk | Sangon            | Cat# NB0669  |
| Hygromycin B        | Sangon         | Cat# A600230-0001 |
| Triton X-100        | Sangon         | Cat# A110694 |
| NP-40               | Sigma-Aldrich  | Cat# 74388   |
| Tween-20            | Sigma-Aldrich  | Cat# P9416   |
| Cholesterol         | Sigma-Aldrich  | Cat# C3045   |
| FUDR                | Sigma-Aldrich  | Cat# F6627   |
| PrimeSTAR Max DNA Polymerase (HD) | TAKARA | Cat# R045A   |
| Trizol              | TAKARA         | Cat# 9108    |
| Hind III            | TAKARA         | Cat# 1060A   |
| BamH I              | TAKARA         | Cat# 1010A   |
| Spe I               | TAKARA         | Cat# 1086A   |
| Kpnl                | TAKARA         | Cat# 1068A   |
| PstI                | TAKARA         | Cat# 1073A   |
| Dpn I               | TAKARA         | Cat# 1235A   |
| SYBR Premix Ex TaqTM II | TAKARA             | Cat# RR820A  |
| PageRuler Prestained protein ladder | Thermo Scientific | Cat# 26616   |
| NuPAGE LDS Sample Buffer(4X) | Thermo Fisher      | Cat# NP0007  |
| Trans 2k plus DNA marker | Transgene              | Cat# BM111   |
| Agarose             | Vivantis       | Cat# PC0701  |

Critical Commercial Assays

| PCR clean-up Kit        | Axygen         | Cat# AP-PCR  |
| Plasmid mini prep       | Axygen         | Cat# AP-MN-P |
| Pure link Quick Plasmid mini prep kit | Invitrogen | Cat# K210011 |
| Pierce BCA Protein Assay Kit | Pierce        | Cat# 23227   |
| PrimeScript RT reagent Kit with gDNA Eraser | TAKARA  | Cat# RR047A  |
| KOD-Plus-Mutagenesis kit | TOYOBO        | Cat# SMK-101 |
| Direct-zol RNA mini prep | Zymo Research | Cat# R2052   |

Deposited Data

| RNA-Seqencing data of translated mRNA and total mRNA in N2 | This Study | GEO: GSE119485 |
| RNA-Seqencing data of translated mRNA and total mRNA in daf-2 raks-1 | This Study | GEO: GSE119485 |

Experimental Models: Organisms/Strains

| C. elegans: strain N2: wild isolate | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain AA86 daf-12(h61rh411) X | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain CB1370 daf-2(e1370) III | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain CF1038 daf-16(mu86) I | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain MT2547 ced-4(n1162) III | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain NR222 rde-1(ne219) V; kzl9[lil-26::NLS::GFP + lin-26::rde-1(+); rol-6] | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain SJ4100 zcls13[Phsp-6::gfp] V | Caenorhabditis Genetics Center | N/A |

(Continued on next page)
LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Di Chen (chendi@nju.edu.cn; cedauer@gmail.com). The germline-specific RNAi strain DCL569 has been deposited to the Caenorhabditis Genetics Center (https://cgc.umn.edu/strain/DCL569).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

C. elegans strains and maintenance

The following C. elegans strains used in this study were obtained from the Caenorhabditis Genome Center: Bristol (N2) strain as the wild-type strain, AA86 daf-12(rh61rh411) X, CB1370 daf-2(e1370) III, CF1038 daf-16(mu86) I, MT2547 ced-4(n1162) III, NR222

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| C. elegans: strain SJZ204 foxSi37[ges-1p::tomm-20::mKate2::HA::ttb-2 3'UTR] I | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain T91 cep-1(gk138) I | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain T916 spe-9[tc88] I; rrf-3(b286) II | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain WM118 rde-1(ne300) V; nels9[myo-3p::HA::rde-1(+) + rol-6] X | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain VP303 rde-1(ne219) V; kbs7[txh-2p::rde-1(+) + rol-6] | Caenorhabditis Genetics Center | N/A |
| C. elegans: strain CU6372 drp-1(tm1108) IV | Japanese National BioResource Project | N/A |
| C. elegans: strain DCL4 rsks-1(ok1255) III | This paper | N/A |
| C. elegans: strain DCL92 zcls13[Phsp-6::gfp] V; aak-2(ok524) X | This paper | N/A |
| C. elegans: strain DCL178 cyc-2.1[mkc6(cyc-2.1::3Xflag)] IV | This paper | N/A |
| C. elegans: strain DCL198 daf-2(e1370) rsks-1(ok1255) III; cyc-2.1[mkc6(cyc-2.1::3Xflag)] IV | This paper | N/A |
| C. elegans: strain DCL312 gfp-4(bn2) I | This paper | N/A |
| C. elegans: strain DCL374 att-1(gk3094) V | This paper | N/A |
| C. elegans: strain DCL419 gld-1[mkc28(gld-1::mKate2)] I | This paper | N/A |
| C. elegans: strain DCL430 gld-1[mkc28(gld-1::mKate2)] I; daf-2(e1370) rsks-1(ok1255) III | This paper | N/A |
| C. elegans: strain DCL569 mkcSi13[sun-1p::rde-1::sun-1 3'UTR + unc-119(+)] II; rde-1(mkc36) V | This paper | N/A |
| C. elegans: strain DCL606 mkcSi13[sun-1p::rde-1::sun-1 3'UTR + unc-119(+)] II; rde-1(mkc36) V | This paper | N/A |
| C. elegans: strain DCL701 mkcSi51[vha-6p::aak-2::x2::gfp::unc-54 3'UTR + Cbr-unc-119(+)] II; aak-2(ok542) X | This paper | N/A |
| C. elegans: strain XA8205 aak-2(ok524) X | This paper | N/A |
| C. elegans: strain XA8222 daf-2(e1370) rsks-1(ok1255) III | This paper | N/A |

Oligonucleotides

For primers used for RT-qPCR, RNAi constructs and gene editing via CRISPR/Cas9, see Table S4

Software and Algorithms

TopHat2 | Kim et al., 2013 | http://ccb.jhu.edu/software/tophat |
HTSeq | Anders et al., 2015 | http://htseq.readthedocs.io/en/ release_0.10.0/ |
edgeR | Robinson et al., 2010 | https://www.bioconductor.org/ |
Cox-Reid profile-adjusted likelihood method | Cox and Reid, 1987 | N/A |
Benjamin-Hochberg method | Benjamin and Hochberg, 1995 | N/A |
GraphPad Prism version 6 | GraphPad Software | https://www.graphpad.com/ |
Snapgene Viewer | Snapgene | http://www.snapgene.com/ |

Cell Reports 28, 1050–1062.e1–e6, July 23, 2019 e3
rde-1(ne219) V; kZts9[lin-26::NLS::GFP + lin-26::rde-1(+)] + rol-6] I; SJ4100 zcls13[Phsp-6::gfp] V; SJZ204 foxSi37[ges-1::tomm-20::mKate2::HA::tbb-2 3’sUTR] I; TJ1 cep-1(gk138) I; Tj1060 spe-9[hr88] I; nrf-3[26] II; VP303 rde-1(ne219) V; kbls7[nhx-2::rde-1(+)] + rol-6] III; and WM118 rde-1(ne300) V; nels9[myo-3::HA::rde-1(+)] + rol-6] I. The following biostrain used in this study was obtained from the National BioResource Project: drp-1(tm1108) IV. The following strains used in this study were generated in D.C. and P.K. labs: DCL4 rsks-1(ok1255) III; DCL124 zcls13[Phsp-6::gfp] V; aak-2(ok524) X; DCL178 cyc-2.1[mkc6(cyc-2.1::3 + flag)] IV; DCL198 daf-2(e1370) rsks-1(ok1255) III; cyc-2.1[mkc6(cyc-2.1::3 + flag)] IV; DCL312 glp-4(b2n) II; DCL374 atfs-1(gk3094) V; DCL419 gid-1[mc28(gid-1::mKate2)] I; DCL430 gid-1[mc28(gid-1::mKate2)] II; daf-2(e1370) rsks-1(ok1255) III; DCL589 mkcSi13 [sun-1::rde-1::sun-1 3’sUTR + unc-119(+)] II; daf-2(e1370) V; DCL606 mkcSi13[sun-1::rde-1::sun-1 3’sUTR + unc-119(+)] II; cup-4(rok837) III; daf-2(e1370) V; DCL701 mkcSi13[pha-6p::aak-2::si2::gfp::unc-54 3’sUTR + Cbr-unc-119(+)] II; aak-2(rok524) X; XA8205 aak-2(rok524) X, and XA8222 daf-2(e1370) rsks-1(ok1255) III.

Strains in this study were derived from the Bristol N2 background. Nematodes were cultured at 20°C on Nematode Growth Media (NGM) agar plates seeded with E. coli OP50 unless otherwise stated (Brenner, 1974).

**METHOD DETAILS**

**Polysomal profiling**

Four biological replicates of the wild-type N2 and daf-2 rsks-1 mutant animals at Day 4 of adulthood were collected for polysomal profiling. Around 10,000 worms per sample were homogenized on ice in 350 μl of lysis buffer (300 mM NaCl, 50 mM Tris-HCl [pH 8.0], 10 mM MgCl₂, 1 mM EGTA, 200 μg/ml heparin, 400 U/ml RNasin, 1.0 mM phenylmethylsulfonyl fluoride, 0.2 mg/ml cycloheximide, 1% Triton X-100, 0.1% Sodium Deoxycholate) by 60 strokes with a Teflon homogenizer. Each sample was then supplemented with 700 μl lysis buffer and incubated on ice for 30 minutes before centrifuging at 20,000 g for 15 minutes at 4°C. 0.9 mL of the supernatant was applied to the top of a 10%–50% sucrose gradient in the high salt resolving buffer (140 mM NaCl, 25 mM Tris-HCl [pH 8.0], 10 mM MgCl₂) and centrifuged in a Beckman SW41Ti rotor at 38,000 rpm for 90 min at 4°C. Gradients were fractionated using a Teledyne density gradient fractionator with continuous monitoring of absorbance at 252 nm. Translated mRNAs (≥2 ribosomes per mRNA) and total RNAs (from the original lysates) were extracted using the Trizol reagent.

**RNA-Seq and bioinformatics analysis**

Four biological replicates of translated and total mRNAs from N2 and daf-2 rsks-1 mutant animals were sent to the University of Minnesota Genomics Center for library construction and pair-ended sequencing with the length of 100 nucleotides, 11.5 million reads per sample on a HiSeq2000 machine (Illumina). Reads were aligned to the C. elegans genome (WS220) using the spliced-junction mapper TopHat2 (Kim et al., 2013). Aligned reads were counted per gene using the python script HTseq (Anders et al., 2015). Differential expression and dataset normalization were performed using the Biocconduct package edgeR (Robinson et al., 2010). Normalization in edgeR was adjusted for RNA composition to ensure that highly expressed genes, which consume a large portion of the RNA pool, did not result in the under-sampling of other genes. Dispersion of the gene counts were estimated tag-wise using the Cox-Reid profile-adjusted likelihood method (Cox and Reid, 1987). Only genes with an average counts per million (CPM) of eight or greater across all conditions were considered for differential expression. Dispersion of the gene counts were adjusted for multiple testing sing the Benjamin-Hochberg method (Benjamin and Hochberg, 1995). Changes in post-transcriptional processing were identified by comparing the ratio of polysomal-associated mRNA to total mRNA between the wild-type N2 and daf-2 rsks-1 mutant animals. This is referred to as Differential Polysome Association Ratio (DPAR), with a positive value indicating an increase in the percentage of a particular species of mRNA associated with polysomes in the daf-2 rsks-1 mutant.

**RNAi by feeding**

RNAi experiments were performed by feeding worms E. coli strain HT115 (DE3) transformed with either the empty vector L4440 as the control or gene-targeting constructs from the C. elegans Ahringer RNAi Collection (Kamath et al., 2001). Overnight bacterial culture in LB supplemented with Ampicillin (100 μg/ml) at 37°C was seeded onto NGM plates containing IPTG (1 mM) and Ampicillin (100 μg/ml) and incubated overnight at room temperature to induce double-stranded RNAs production. Embryos or L1 larvae were placed on RNAi plates and incubated at 20°C until adulthood to score phenotypes.

**Lifespan assay**

All lifespan assays were performed at 20°C unless otherwise stated. To prevent progeny production during, 20 μg/ml (+)-5-fluorodeoxyuridine (FUDR) was added onto NGM plates during the reproductive period (Day 1 to 7 of adulthood). The first day of adulthood is Day 1 on survival curves. Animals were scored as alive, dead or lost every other day. Animals that did not respond to gentle touch were scored as dead. Animals that died from causes other than aging, such as sticking to the plate walls, internal hatching or bursting in the vulval region, were scored as lost. Kaplan–Meier survival curves were plotted for each lifespan assay, and statistical analyses (log-rank tests) were performed using the Prism 6 software.
RNAi screen for lifespan regulators
The primary screen was performed using the strain TJ1060 spe-9(hc88) I; rrf-3(b26) II, which showed temperature sensitive sterility, enhanced RNAi sensitivity and normal lifespan. Synchronized TJ1060 L1 larvae were transferred onto plates with RNAi against 112 translationally downregulated genes at 25°C until animals reached Day 1 adulthood for survival assays. For the 39 RNAi treatments that caused larval arrest, synchronized TJ1060 L1 larvae were transferred to the control RNAi plates, and day 1 adult animals were then transferred to those 39 RNAi plates for survival assays. Animals were scored as alive, dead or lost every 4-5 days. RNAi treatments that caused significant lifespan extension (p < 0.05, log-rank tests) were selected for two rounds of re-tests in the wild-type N2 background at 20°C. RNAi treatments that caused significant lifespan extension in both re-test groups (p < 0.05, log-rank tests) were regarded as positive hits.

Microscopy
Animals were anaesthetized in 1% sodium azide and immediately imaged. The hsp-6p::GFP transgenic worms were imaged with a Leica MC165 FC dissecting microscope and a Leica DFC450 C digital camera. GLD-1::mKate2 animals were anaesthetized on 2% agarose pads and imaged with a Zeiss LSM880 confocal microscope. Mitochondria morphology was measured using the transgenic strain foxSi37[ges-1p::tomm-20::mKate2::HA::tbb-2 3′UTR]. Day 1 adult animals treated with either the control or cyc-2.1 RNAi during development were imaged with a Zeiss LSM880 confocal microscope. Filamented and fragmented were defined if majority of the mitochondrial filaments’ lengths were longer than 4 μm or shorter than 2 μm, respectively. The rest were defined as intermediate. In all the imaging studies, images within the same figure panel were taken with the same exposure time and adjusted with identical parameters using the Adobe Photosop or ImageJ.

Western blot and antibodies
For western blots using whole animals, either roughly equal numbers of synchronized Day 1 adult animals were manually transferred into the lysis buffer (150 mM NaCl, 1 mM EDTA, 0.25% SDS, 1.0% NP-40, 50 mM Tris-HCl [pH7.4], Roche complete protease inhibitors and phosphoSTOP phosphatase inhibitors) supplemented with the 4 × SDS loading buffer and immediately frozen at −80°C, or animals were collected for extracting the total proteins via sonication and quantifying the protein concentrations via Bradford assays. For western blots using dissected tissues, approximately equal amount of biomass in each sample was collected into the same lysis buffer supplemented with the 4 × SDS loading buffer. Samples were boiled for 10 minutes before resolving on precast SDS-PAGE gels (GenScript). Bands of interests were quantified using the ImageJ software and normalized to the intensities of the internal control, which is either α-tubulin or β-actin. Antibodies used in western blots include monoclonal anti-FLAG (Sigma, 1804), monoclonal anti-Tubulin Alpha (Sigma, T6074), anti-Actin (CST, 4967), anti-Phospho-AMPKα (CST, 2535S) and anti-RPS-0, RPS-3, RPL-5 and RPL-25.2 antibodies (Liu et al., 2019).

RT-qPCR
Synchronized Day 1 adult animals were collected, frozen in the Trizol reagent (Takara) and stored at −80°C until total RNA extraction using the Direct-zol RNA mini prep kit (ZYMO Research). The cDNA was synthesized by the reverse transcription system (Takara). The SYBR Green dye (Takara) was used for qPCR reactions carried out in triplicates on a Roche LightCycler 480 real-time PCR machine. Relative gene expression levels were calculated using the 2⁻ΔΔCT method (Livak and Schmittgen, 2001). RT-qPCR experiments were performed at least three times with consistent results using independent RNA preparations. mRNA levels of pmp-2 were used for normalization.

Worm tissue micro-dissection
Worms at Day 1 adulthood were transferred into the S buffer (100 mM NaCl and 50 mM potassium phosphate [pH 6.0]) on a glass slide. Heads of animals were cut off near the pharynx using syringe needles to collect the intestine and gonad. For RT-qPCR assays, 20-30 gonad or intestine tissues were collected in the Trizol reagent for total RNA extraction. For western blots, at least 100 gonad or intestine tissues were collected in the protein extraction buffer.

CRISPR/Cas9 alleles generation
CRISPR engineering to knock-in 3 × FLAG at the C-terminal of CYC-2.1 was performed by microinjection using the homologous recombination approach (Dickinson et al., 2013). The injection mix contained two plasmids that drive expression of two different Cas9-sgRNAs (50 ng / μl), a selection marker pCFJ90 (Pmyo-2::mCherry::unc-54-3′-UTR) (5 ng / μl, Addgene #19327) and a homologous recombination plasmid (50 ng / μl). To generate the sgRNA plasmids, primers were designed with the CRISPR DESIGN tool (https://zlab.bio/guide-design-resources) and inserted into the pDD162 vector (Addgene #47549) using the site-directed mutagenesis kit (TOYOBO SMK-101). To generate the homologous recombination plasmid, the 3 × FLAG coding sequence was first cloned to replace the GFP coding sequence on vector PD95.77, and two homologous arms (~1,000 bp each) corresponding to the 5′- and 3′-sides of the insertion site, respectively, were then cloned into the same vector. Successful knock-in events were screened by PCR genotyping from independent F1 transgenic animals’ progeny that did not carry the co-injection markers, and confirmed by Sanger sequencing.
CRISPR engineering with a self-excising drug selection cassette (SEC) was performed to knock-in the mKate2 fluorescent protein to the C-terminal of GLD-1 (Dickinson et al., 2015). The injection mix contained two plasmids that drive expression of two different Cas9-sgRNAs (50 ng/µl), a selection marker pCFJ90 (Pmyo-2::mCherry::unc-54-3′-UTR) (5 ng/µl, Addgene #19327) and a repair template FP-SEC vector (50 ng/µl). To generate the sgRNA plasmids, primers were designed with the CRISPR DESIGN tool (https://zlab.bio/guide-design-resources) and inserted into the pDD162 vector (Addgene #47549) using the site-directed mutagenesis kit (TOYOBO SMK-101). The ccdB sequences from the FP-SEC vector pDD287 (Addgene #70685) were replaced with two homologous arms (500-700 bp) to generate the repair template FP-SEC plasmid. Injected animals and their progeny were treated with Hygromycin B (350 µg/ml) to select successful knock-in events. Hygromycin B resistant roller animals were tested by PCR genotyping. The SEC was removed by heat shock and homozygous knock-in alleles were confirmed by PCR and DNA sequencing.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed via the Prism 6 (GraphPad) software. Detailed description of tests performed to determine statistical significance is included in figure legends. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001; ns, p > 0.05.

DATA AND CODE AVAILABILITY

RNA-Seq datasets are available at the NCBI under the accession number GEO: GSE119485.
Supplemental Information

Translational Regulation of Non-autonomous Mitochondrial Stress Response Promotes Longevity

Jianfeng Lan, Jarod A. Rollins, Xiao Zang, Di Wu, Lina Zou, Zi Wang, Chang Ye, Zxing Wu, Pankaj Kapahi, Aric N. Rogers, and Di Chen
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) | Function | Essentiality |
|-------|-----------------|----------|--------------|
|       | Mean | Max | extension | n |                        |           |
| 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 |
| cyt-b-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) | Percent of control b | n c | p d |
|----------|------|----------|-----------------|----------------------|-----|-----|
|          |      |          | Mean            | Max                  |     |     |
| 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 | / |
| rsks-1   | cyc-2.1 | 32.2, 31.6 | 49, 47 | 170%, 158% | 67, 94 | <0.0001, <0.0001 |
| daf-2    | cyc-2.1 | 31.2, 28.8 | 51, 51 | 139%, 131% | 79, 62 | <0.0001, <0.0001 |
| daf-2 rsks-1 | cyc-2.1 | 41.1, 42.2 | 61, 57 | / | 69, 106 | / |
| cy-c-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 | / |
| aak-2    | control | 16.4, 19.3 | 33, 35 | 104%, 103% | 84, 64 | 0.2355, 0.2985 |
| Effects of tissue-specific cyc-2.1 RNAi on lifespan (Figure 3A) |
| mksI13; re-1 | control | 17.7, 17.7, 17.5 | 25, 23, 23 | / | 76, 84, 75 | / |
| rde-1; kbs7 | control | 22.7, 22.0, 22.5 | 35, 31, 33 | 128%, 124%, 129% | 99, 94, 89 | <0.0001, <0.0001, <0.0001 |
| rde-1; kls9 | control | 17.9, 19.4, 16.2 | 25, 27, 23 | / | 62, 94, 51 | / |
| rde-1; nels9 | control | 19.5, 20.6, 20.5 | 29, 29, 27 | 100%, 91%, 102% | 74, 98, 78 | 0.9741, 0.0005, 0.4904 |
| Intestinal AAK-2 is sufficient to restore cyc-2.1 RNAi-induced lifespan extension in the aak-2 mutant (Figure 3E) |
| mksI13; aak-2 | control | 15.5, 16.0, 15.4 | 21, 23, 21 | / | 70, 82, 77 | / |
| Effect of tissue-specific cyc-2.1 RNAi on lifespan (Figure 3A) |
| mksI13; cup-4; re-1 | control | 21.6, 23.5 | 31, 33 | / | 114, 110 | / |
| mksI13; re-1; cup-4 | control | 21.6, 24.8 | 33, 33 | 100%, 106% | 115, 98 | 0.8629, 0.1545 |
| The cup-4 mutation blocks germline-specific cyc-2.1 RNAI-induced lifespan extension (Figure 3L) |
| mksI13; cup-4; control | cyc-2.1 | germline | 21.6, 23.5 | 31, 33 | / | 114, 110 | / |
| mksI13; cup-4; rsks-1 | control | cyc-2.1 | germline | 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 | / |
| daf-2 rsks-1 | control | 33.5, 29.3, 32.4 | 53, 51, 49 | 172%, 157%, 169% | 93, 98, 63 | <0.0001, <0.0001, <0.0001 |
| Roles of UPRme 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 | / |
| daf-2 rsks-1 | control | 20.6, 19.4, 20.0 | 27, 29, 27 | 101%, 98%, 102% | 77, 95, 92 | 0.8181, 0.6945, 0.1015 |
| 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 | / |
| rsks-1   | gld-1 | 16.6, 16.5 | 25, 23 | 92%, 91% | 64, 62 | 0.0167, 0.0025 |
| daf-2    | gld-1 | 17.4, 16.9 | 23, 25 | 82%, 79% | 45, 46 | <0.0001, <0.0001 |
| daf-2 rsks-1 | gld-1 | 46.1, 43.3 | 63, 63 | / | 50, 40 | / |
| Intestinal AAK-2 is sufficient to restore cyc-2.1 RNAI-induced lifespan extension in the aak-2 mutant (Figure 3E) |
| aak-2    | control | 15.9, 16.0 | 23, 23 | 100%, 100% | 53, 56 | 0.9647, 0.9295 |

Intestinal AAK-2 is sufficient to restore cyc-2.1 RNAI-induced lifespan extension in the aak-2 mutant (Figure 3E)
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.
| Oligonucleotides               | Source      | Identifier |
|-------------------------------|-------------|------------|
| rps-0-RT-F: CGTATCGATCATTAGGCTGTCAC | This paper | N/A        |
| rps-0-RT-R: CCGATGTCGATCAACTGAGTG | This paper | N/A        |
| rps-3-RT-F: GAATCCCTAGATACACGCTCTGT | This paper | N/A        |
| rps-3-RT-R: CCAGAGCGATACAACCCTAATCTCT | This paper | N/A        |
| rpl-5-RT-F: ACTTACAACTACGGCGCTG | This paper | N/A        |
| rpl-5-RT-R: TAGTCTTCCAGTAGCTTTCG | This paper | N/A        |
| rpl-2-5-RT-F: GGGGTATGCTGCTCGACGCTCT | This paper | N/A        |
| rpl-2-5-RT-R: TGGCAACATCAAGAGCAGCTCAG | This paper | N/A        |
| pmp-2-RT-F: AGGATTGCGATGCTGCGTACG | This paper | N/A        |
| pmp-2-RT-R: TACATGGCTTCTTTACATACAC | This paper | N/A        |
| hsp-6-RT-F: GCTGGAGAATAAGATCTCGT | This paper | N/A        |
| hsp-6-RT-R: GTGGACTTGACCTCGAGAGC | This paper | N/A        |
| dnrj-10-RT-F: GCCGGCTCATCTCATGCTGACG | This paper | N/A        |
| dnr-10-RT-R: CAGATTCTTTGTCGACACCAAG | This paper | N/A        |
| dnrj-1-RT-F: TGGATTCCTGGATATTCTCGG | This paper | N/A        |
| dnr-1-RT-R: AGTGGGCTTCTGCGAACCTCTG | This paper | N/A        |
| limm-17-RT-F: GATTTGTGTCTTGGCGCATCC | This paper | N/A        |
| limm-17-RT-R: ATCACTTCTTGGCTGACAGG | This paper | N/A        |
| cyc-2,1-RT-F: TAACAGAAGAAGGGAAGGTGCTGCTG | This paper | N/A        |
| cyc-2,1-RT-R: CGTCAACCTTCTTGAGTC | This paper | N/A        |
| ubI-5 RNA-F: GGGGTACAGTAGGCTGACAAATGGATCCC | This paper | N/A        |
| ubl-5 RNA-R: AATCTGCACTGCGCCTTTAAGTTTACTGAT | This paper | N/A        |
| cyc-2,1-sgRNA1-F: AGATTCTCATCAAGATGACATCGGTTTAGAGCTGAATAGACGAAT | This paper | N/A        |
| cyc-2,1-sgRNA2-F: GATAATTGAGGGAACCTTGAGCTGCAAAATAGCAGG | This paper | N/A        |
| sgRNAs-F: CAAGACATCTCCGCAATAGG | This paper | N/A        |
| sgRNA-seq: GGTGTAATAACCGGCAG | This paper | N/A        |
| cyc-2,1-5HR-F: CCCAACCTTTGCGATATCCGAAAGCTCAGG | This paper | N/A        |
| cyc-2,1-5HR-R: CGGGATCGAAGGAGCTGCGGATCA | This paper | N/A        |
| cyc-2-1,3HR-F: CGGATCGCGAGGAGTGGGCGATTCA | This paper | N/A        |
| cyc-2-1,3HR-R: GGAATCTGAGAAGGGAATAATGGATACGACG | This paper | N/A        |
| GFP-seq-5R: CCACCTATCTCAAAAGAAATGTCGACAA | This paper | N/A        |
| GFP-seq-3F: GTTCCTTTGTAGTGGTAC | This paper | N/A        |
| cyc-2,1-3xflag-F: GGGGTACCCGACTGACTACAAGGATGACCGGTTG | This paper | N/A        |
| cyc-2,1-3xflag-R: AATCGCTATCTGCTACTGCTCATCCTTG | This paper | N/A        |
| cyc-2,1 (SITE DIRECTED M)-F: CAAGATCATCGAAGGTAATC | This paper | N/A        |
| cyc-2,1 (SITE DIRECTED M)-R: GGAGATCTTTGACGTTGACTTG | This paper | N/A        |
| cyc-2,1 KI-F: GTGTGGTTGAAATCTGTTTGTCTG | This paper | N/A        |
| 3xflag KI-genotyping-R: GATCCTTTATTTATCACTTACCGTTGTC | This paper | N/A        |
| gld-1-sgRNA1-F: CCCAGGATGAGGAGATCTCCTCCTTTGTTAGCTGAGTAAATAGCAGG | This paper | N/A        |
| gld-1-sgRNA2-F: CGCAGAAGGATAGGAGAGATCTCCTCCTTTGTTAGCTGAGTAAATAGCAGG | This paper | N/A        |
| gld-1-5HR-F: GCCAGAAATGGTGTTACGCGAGGAAACTTGAAATTGTCAGATTTAATCTCAGCTC | This paper | N/A        |
| gld-1-5HR-R: GGGATCTGAGAAGGGAATAATGGATACGACG | This paper | N/A        |
| gld-1-3HR-F: CACGGAGTCTTTAAACGAGCGCCGAGTCAAGCACGGCATTACG | This paper | N/A        |
| gld-1-3HR-R: CCTGAGGCTCCCGCAGTGCTCCGAAAAAGGTTGTGTTGACTGAAGACTGAGGACGCTTC | This paper | N/A        |
| gld-1 (SITE DIRECTED M)-F: GTCCGGAGAGGAGGAGCCTC | This paper | N/A        |
| gld-1 (SITE DIRECTED M)-R: ACATTTGGTTTTGTAGTGGGAAAAGGAGGATG | This paper | N/A        |
| gld-1 KI-F: CTGAGGAGATGTCCTAGC | This paper | N/A        |
| mKate2 KI-genotyping-R: GTGGTGGACGCTTTCCCTCC | This paper | N/A        |