nature communications

Article

https://doi.org/10.1038/s41467-025-62323-7

The 18S rRNA methyltransferase DIMT-1 regulates lifespan in the germline later in life

Received: 23 October 2024

Accepted: 17 July 2025

Published online: 28 July 2025

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Specialized ribosomes help determine which proteins are synthesized, however, the influence of age on ribosome heterogeneity and whether dysregulation of this process drives organismal aging is unknown. Here we examined the role of ribosomal RNA (rRNA) methylation in maintaining appropriate translation as organisms age. In a directed RNAi screen, we identified 18S rRNA N6′-dimethyl adenosine ($m^{6,2}A$) methyltransferase, *dimt-1*, as a regulator of *C. elegans* lifespan and stress resistance. We demonstrate that DIMT-1 functions in the germline after mid-life to regulate lifespan. Depletion of *dimt-1* leads to selective translation of transcripts important for stress resistance and lifespan regulation in the *C. elegans* germline including the cytochrome P450 *daf-9*, which synthesizes a steroid that signals from the germline to the soma. *dimt-1* induced lifespan extension is dependent on the *daf-9* signaling pathway. Our findings highlight ribosome heterogeneity, and specific rRNA modifications, in maintaining appropriate translation later in life to promote healthy aging.

Disruption of the proteome is a hallmark of aging. Protein homeostasis or proteostasis is maintained by the processes of regulated protein degradation and production. There is increased dysregulation of protein homeostasis with age, and its misregulation can lead to protein aggregation diseases, including Alzheimer's and Huntington's diseases[1,2]. Depletion of the ribosome or attenuation of protein synthesis have been shown to increase lifespan in yeast, *C. elegans*, and *Drosophila*[3–6]. Research of the past two decades has focused on understanding how aggregation, degradation, shuttling, and translation have regulated proteostasis and how they go awry during aging or diseases[7–11]. Having the capacity to express the appropriate protein in response to environmental cues is an essential and evolutionarily conserved process. Therefore, preserving the proteome is critical for maintaining organismal health and healthy aging. However, how aging-responsive mRNAs are selectively translated is unknown.

More than 100 distinct post-transcriptional chemical modifications, termed the "epitranscriptome", have been identified in cellular RNAs of all kingdoms of life[12,13]. The identification of these residues, as well as the enzymes that add, remove, and recognize the modified bases, has revealed added complexity that controls virtually every aspect of RNA processing[14–18]. Ribosomal modifications help specify which transcripts are translated under different environmental conditions[19–21], providing an additional layer of control to gene regulation. Ribosomal RNAs (rRNA; 28S, 18S, 5.8S, and 5S in eukaryotes) are encoded by many copies of ribosomal DNA throughout the genome, which display tissue-specific expression patterns[22], raising the possibility that different combinations of rRNAs and ribosomal proteins could form ribosomes that are specialized for translation of subsets of mRNAs[23]. Some of these modifications (2′-*O*-methylation and pseudouridinylation) occur in substoichiometric frequency[24,25], suggesting that specialized ribosomes might regulate differential

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translation of distinct transcripts. The ribosome consists of four rRNAs and ~80 core ribosomal proteins[23]. Historically, ribosomes were believed to translate whatever transcript they were presented with, but recent studies suggest that ribosome composition varies and plays a significant role in the regulation of translation. Whether rRNA methylation becomes dysregulated during aging and could play a role in preserving proteome integrity as organisms age is still unclear. We have recently demonstrated that the N6-dimethyladenosine ($m^{6,2}A$) methyltransferase, DIMT-1, which methylates adenosines 1735 and 1736 on 18S rRNA, regulates selective ribosomal binding and translation of specific mRNAs to regulate intergenerational transmission of the response to ancestral starvation[26]. However, whether rRNA methylation more broadly can regulate age and stress-responsive translation and how this dysregulation drives the aging process is still unknown.

Here, we performed a directed RNAi screen of all putative rRNA methyltransferases to identify whether rRNA methyltransferases could regulate lifespan and stress resistance in the nematode *C. elegans*. We also examined how rRNA methylation changes with age. We found that the 18S rRNA $m^{6,2}A$ methyltransferase, *dimt-1*, regulates *C. elegans* lifespan and stress resistance. Lifespan extension induced by *dimt-1* deficiency required the known regulator of mRNA translation, the Rag GTPase, *raga-1*. Using an auxin-inducible degron-tagged version of *dimt-1*, we demonstrate that DIMT-1 functions in the germline after mid-life to regulate lifespan. We further found that knockdown of *dimt-1* leads to selective translation of transcripts important for stress resistance and lifespan regulation in the *C. elegans* germline in mid-life, including the cytochrome P450 *daf-9*, which synthesizes a steroid that signals from the germline to the soma to regulate lifespan. We found that *dimt-1*-induced lifespan extension was dependent on the *daf-9* signaling pathway. This finding reveals an additional layer of proteome dysfunction, beyond protein synthesis and degradation, as an important regulator of aging. Our findings highlight ribosome heterogeneity, and specific rRNA modifications, in maintaining appropriate translation later in life to promote healthy aging.

Results

dimt-1 rRNA methyltransferase knockdown extends lifespan and increases resistance to stress

Previous genome-wide RNAi screens for genes that regulate lifespan in *C. elegans* have been performed in worms where progeny production was inhibited[27–29], which has been shown to mask the effect of some genes on lifespan[30,31]. An understudied aspect of proteostasis during aging is ribosome heterogeneity, and one of the key factors for heterogeneity are rRNA modifications such as methylation, which are catalyzed by rRNA methyltransferases. Hence, we performed a targeted RNAi screen for putative rRNA methyltransferases present in fertile *C. elegans* to test whether manipulation of rRNA methylation could regulate lifespan or stress resistance. We knocked down 26 putative rRNA methyltransferases and discovered that knockdown of several rRNA methyltransferases could significantly shorten or extend *C. elegans* lifespan (Fig. 1a). As previously reported[32], knockdown of *T01C3.7/fib-1* extended *C. elegans* lifespan (~9% $p < 0.0005$, Fig. 1a). FIB-1 is a homolog of the 2′-*O*-methyltransferase fibrillarin, which is conserved in animals and plants. We also found that knockdown of *E02H1.1/dimt-1* caused the most significant extension of lifespan (22–33% $p < 0.0001$) (Fig. 1a). We had previously reported that DIMT-1 is the 18S rRNA N6-dimethyladenosine ($m^{6,2}A$) methyltransferase for adenosines 1735 and 1736 in *C. elegans*[26]. DIMT-1 is a conserved methyltransferase that methylates conserved adjacent adenosines on the 18S rRNA in yeast and humans[33–35].

Although lifespan extension can be beneficial, it may also come with detrimental effects to the organism. To determine if the effects on longevity by rRNA methyltransferase knockdown were associated with improved health, we performed UV stress and heat stress survival assays using the same targeted RNAi screen. As we had found previously[21], knockdown of *C38D4.9/metl-5* increased both UV stress and heat stress resistance (79%, $p = 0.001$ and 46.5% $p < 0.05$, Fig. 1b, c). Additionally, as previously reported[36], knockdown of *W07E6.1/nsun-1* increased heat stress resistance without having a significant effect on overall lifespan (633%, $p = 0.0036$ and −11.7%, $p = 0.6713$, Fig. 1). *E02H1.1/dimt-1* also displayed a significant increase in both UV stress (43.7% $p < 0.0001$) and heat stress resistance (231.3% $p < 0.0001$) (Fig. 1b, c). As *dimt-1* knockdown caused the most significant lifespan extension (Fig. 1a), we focused subsequent analyses on determining how DIMT-1 can regulate longevity. To determine whether *dimt-1* was regulating longevity by altering protein homeostasis, we measured the levels of three stress-induced chaperone proteins important for stabilizing misfolded proteins, HSP-4[37,38], HSP-6[39,40], and HSP-16.2[41,42] using fluorescent reporter strains. These chaperones are indicators of misfolded protein loading in response to endoplasmic reticulum (ER) stress, mitochondrial stress, and cytosolic stress, respectively. While HSP-6 and HSP-16.2 showed no change upon *dimt-1* knock-down (Supplementary Fig. 1a, b), HSP-4 had a significant reduction in expression levels when *dimt-1* was knocked down, indicating that when

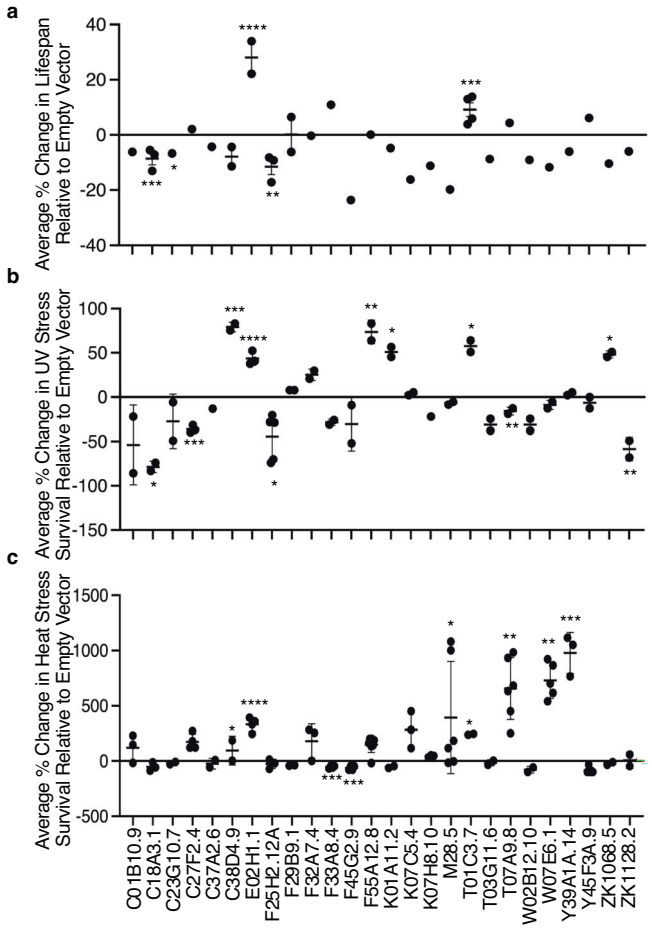

Fig. 1 | Ribosomal RNA methyltransferases regulate lifespan, heat, and UV stress response. a–c Directed RNAi screen of putative rRNA methyltransferases reveals changes in **a** lifespan, **b** UV stress survival, and **c** heat stress survival relative to empty vector control worms. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. Each dot represents an independent experiment with 30 worms per plate in three plates. One-way ANOVA with Dunnett's multiple comparisons was used to calculate statistics within a single experiment for stress assays, and log-rank (Mantel–Cox) test were performed for longevity assays. Fisher's combined method was used to calculate *p* values across multiple independent experiments. All columns represent the mean ± SEM (**a**) or SD (**b**, **c**). Some RNAi clones were not replicated (without error bars) due to no effect being observed.

https://doi.org/10.1038/s41467-025-62323-7

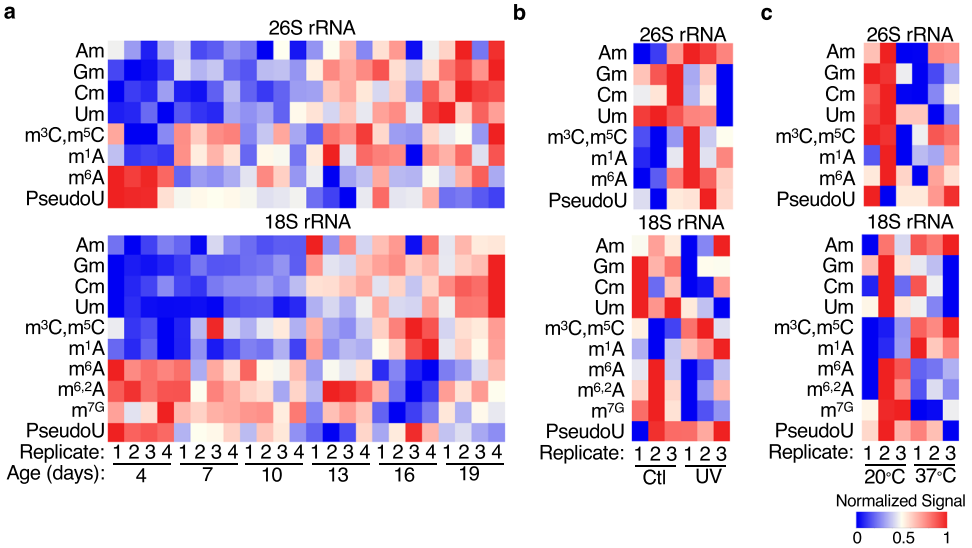

Fig. 2 | Ribosomal RNA modifications in 26S and 18S are dynamically regulated throughout life and change in response to UV and heat stress. a 26S and 18S rRNA extracted from *C. elegans* at different ages, from 4 days to 19 days, reveal changes in rRNA methylation as assessed by UHPLC-ms/ms. This heat map represents the relative changes of methylation in 4 biological replicates. Changes in individual modifications can be seen in Fig. S2C. **b**, **c** 26S and 18S rRNA extracted from *C. elegans* after UV stress (**b**) or 37 °C heat stress (**c**) relative to worms not exposed to UV (ctl) or grown at 20 °C reveal some changes in rRNA methylation as assessed by UHPLC-ms/ms. These heatmaps represent the relative changes of methylation in three biological replicates. Changes in individual modifications can be seen in Supplementary Fig. 2e, f.

levels of DIMT-1 are decreased, the level of misfolded protein loading in the endoplasmic reticulum (ER) is also lowered, likely due to changes in protein turnover compared to control (Supplementary Fig. 1c). To determine whether there is better ER specific protein turnover, we performed a proteotoxicity survival assay using the ER stress inducer tunicamycin (TM), which blocks N-linked glycosylation. We found that *dimt-1* knockdown caused a significant increase in tunicamycin survival relative to WT control worms (Supplementary Fig. 1d).

Taken together, these findings suggest that rRNA methyltransferases play significant roles in regulating lifespan and responses to UV and heat stress and more specifically that *dimt-1* deficiency increases longevity and stress resistance.

rRNA modifications are dynamically regulated throughout the lifespan

Although we found that rRNA methyltransferases regulate lifespan and stress resistance, it was still unclear whether rRNA modifications themselves change during the life of the organism. Changes in specific modifications with age may indicate a regulatory role for rRNA methylation events. We first wanted to see if the rRNA methyltransferase genes are dynamically expressed during the life of *C. elegans*. Examination of previously published transcriptional profiles of *C. elegans* during aging[43], revealed that a number of putative rRNA methyltransferases are dynamically expressed with age (Supplementary Fig. 2a). There were genes which are strongly expressed in early life and declined towards the end, such as *ZK1128.2/mett-10, W01B11.3/nol-58*, and *W07E6.1/nsun-1*. Alternatively, genes such as *E02H1.1/dimt-1* and *C18A3.1/damt-1* showed the opposite trend, peaking near the end of the lifespan (Supplementary Fig. 2a). We independently confirmed that *E02H1.1/dimt-1* expression increases as *C. elegans* age (Supplementary Fig. 2b), suggesting that knockdown of *dimt-1* is reverting the worm to a more youthful state.

To test if the rRNA modifications themselves showed similar patterns during aging, we performed ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) with wild-type (WT) N2 worms harvested in an age gradient at days 4, 7, 10, 13, 16, and 19. We measured rRNA modification levels in the 26S and 18S rRNA subunits in four biological replicates. We observed a similarly dynamic pattern of change in rRNA modifications across lifespan (Fig. 2a). 2′-*O*-ribose methylation increased on all four nucleosides (Am, Cm, Gm, Um) as the worm gets older in both the 26S and 18S rRNAs (Fig. 2a and Supplementary Fig. 2c). Other 18S rRNA modifications such as m^6A and surprisingly, $m^{6,2}A$, showed the inverse trend with a higher level in early life which declines as the worm ages (Fig. 2a and Supplementary Fig. 2c). Since knockdown of the 18S rRNA $m^{6,2}A$ methyltransferase, *dimt-1*, increased *C. elegans* lifespan, we had hypothesized that $m^{6,2}A$ would have increased as *C. elegans* age. This finding could suggest a dynamic and either a tissue or cell-specific change in 18S rRNA $m^{6,2}A$, which might be masked by examination of changes in rRNA methylation across all tissues. It could also reflect that the age gradient was generated under conditions using the drug 5-fluorodeoxyuridine (FUdR), which inhibits proliferation of germline stem cells, the production of intact eggs in adults, and extends longevity[30,44] which could affect the $m^{6,2}A$ levels. These results suggest that both rRNA methyltransferases and modifications are dynamically regulated throughout the lifespan of *C. elegans*.

We had found that the lifespan extension phenotypes observed from the RNAi screen is sometimes associated with increased stress resistance in survival assays (Fig. 1). We wanted to determine if there were associated changes in rRNA methyltransferases and modifications when *C. elegans* are exposed to stress. When we examined a previously published transcriptomic dataset[21], we found that most rRNA methyltransferases decreased expression in response to 37 °C heat stress (Supplementary Fig. 2d). We found that rRNA modifications in wild-type N2 worms were less consistently dynamic in response to 37 °C heat shock or UV stress exposure (Fig. 2b, c). Some rRNA modifications did change in response to the stresses, for example, 2′-*O*-ribose adenosine methylation (Am) in 26S rRNA was significantly increased and 2′-*O*-ribose cytosine methylation (Cm) in 18S rRNA subunit was significantly decreased in response to heat stress (Supplementary Fig. 2e). We also observed a significant increase in 2′-*O*-ribose guanosine methylation (Gm) and Am in 26S rRNA in response to UV exposure (Supplementary Fig. 2f). As with the changes in rRNA methylation during aging (Fig. 2a), lack of changes in specific rRNA methylation in response to stress could reflect tissue-specific changes, which change in opposite directions in different tissues or an inability to capture the correct window to observe changes due to dynamic

https://doi.org/10.1038/s41467-025-62323-7

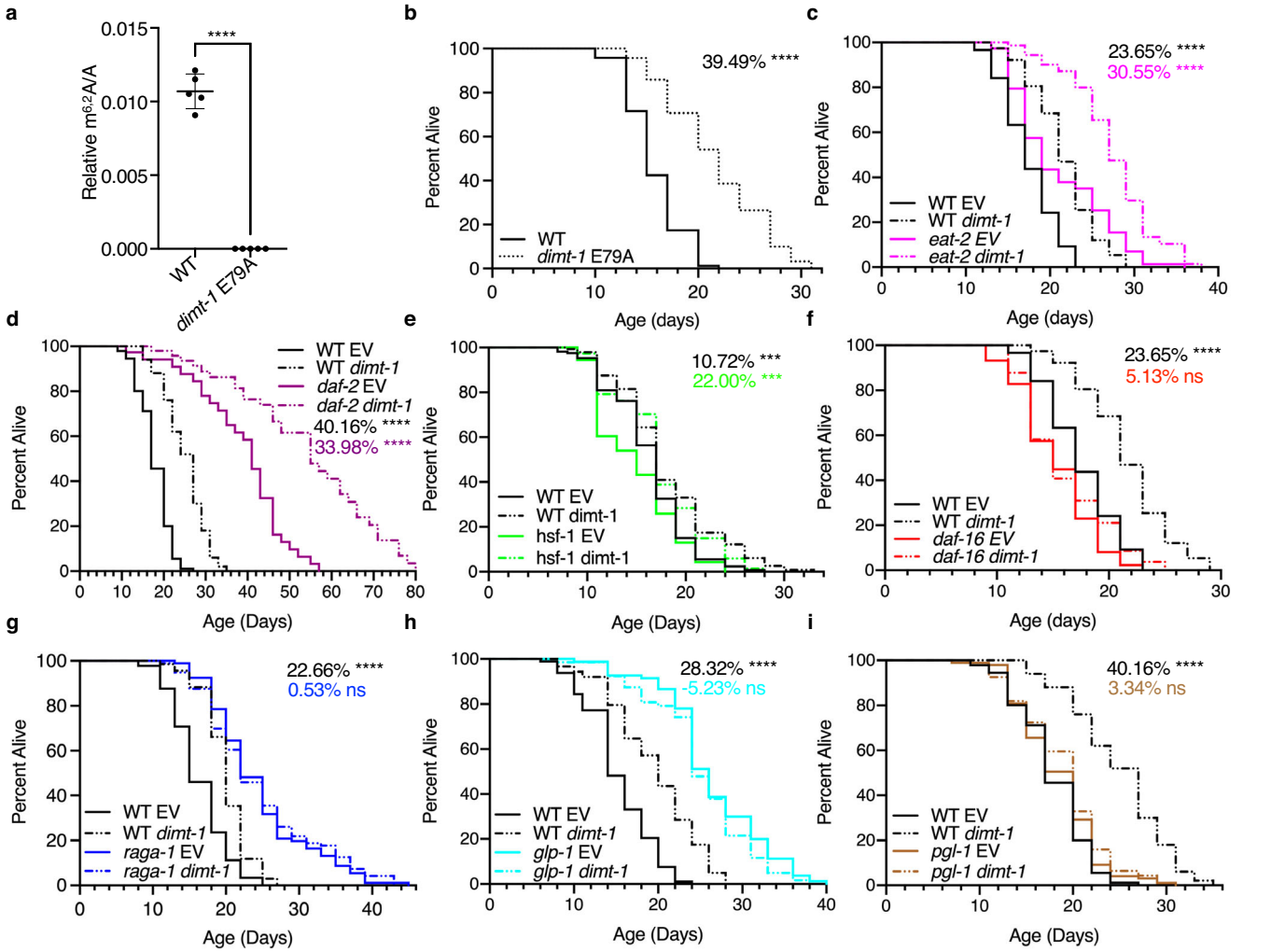

Fig. 3 | Longevity induced by *dimt-1* deficiency requires the FoxO and TOR signaling pathways and requires an intact germline. a Mutation of glutamic acid 79 to an alanine (E79A) in *dimt-1* caused a complete elimination of 18S rRNA $m^{6,2}A$ as assessed by UHPLC-ms/ms. Statistics represent an unpaired two-tailed *t*-test with Welch's correction. **b** Mutation of E79A in *dimt-1* caused lifespan extension relative to WT worms. **c** *dimt-1* knockdown extends the lifespan of both WT and *eat-2(ad1116)* mutant worm lifespan to a similar extent ($p = 0.7021$ by two-way ANOVA). **d** *dimt-1* knockdown extends the lifespan of both WT and *daf-2*(*e1370*) mutant worm lifespan to a similar extent ($p = 0.0806$ by two-way ANOVA). **e** *dimt-1* knockdown extends the lifespan of both WT and *hsf-1*(*sy441*) mutant worm lifespan to a similar extent ($p = 0.4245$ by two-way ANOVA). **f** *dimt-1* knockdown extends the lifespan of WT but not *daf-16*(*mu86*) mutant worm lifespan ($p < 0.0001$ by two-way ANOVA). **g** *dimt-1* knockdown extends the lifespan of WT but does not further extend the long lifespan of *raga-1*(*ok386*) mutant worms ($p = 0.0327$ by two-way ANOVA). **h** *dimt-1* knockdown extends the lifespan of WT but does not further extend the long lifespan of germline deficient *glp-1*(*e2141ts*) mutant worms that were shifted to the restrictive temperature at the L1 stage ($p < 0.0001$ by two-way ANOVA). **i** *dimt-1* knockdown extends the lifespan of WT but not sterile *pgl-1*(*bn101ts*) mutant worms whose mothers were shifted to the restrictive temperature (25.5 °C) at the L4 stage ($p < 0.0001$ by two-way ANOVA). Statistics and replicate experiments are presented in Supplementary Table 1. ns not-significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ as calculated by log-rank (Mantel–Cox) statistical test.

rRNA modification changes after stress exposure. Nevertheless, taken together, these results indicate that rRNA methyltransferases and rRNA modifications are dynamically regulated during the lifespan of *C. elegans*, and can undergo changes in response to environmental stresses. This suggests that rRNA modifications could be the regulatory changes that rRNA methyltransferases induce to regulate longevity and stress resistance.

DIMT-1 catalytic activity is required for the regulation of lifespan and stress resistance

Due to the paradoxical decrease in $m^{6,2}A$ in whole worms as *C. elegans* age, considering that we observe an extension in organismal lifespan when we deplete *dimt*-1, we wished to determine whether DIMT-1's regulation of lifespan and stress resistance was dependent on its catalytic activity. We generated a catalytically inactive DIMT-1 by mutating the conserved glutamic acid E79 to alanine, that we had previously demonstrated was necessary for DIMT-1's catalytic activity in vitro[26]. We found that *dimt-1* (E79A) mutant worms were viable but had no detectable 18S rRNA $m^{6,2}A$ (Fig. 3a). *dimt-1* (E79A) mutant worms displayed a significant increase in lifespan (39.49% $p < 0.0001$, Fig. 3b) and a significant increase in response to the ER stress inducer tunicamycin (Supplementary Fig. 1e). Therefore, DIMT-1 methyltransferase activity is required for lifespan and stress resistance regulation.

DIMT-1 regulates lifespan through the DAF-16/FOXO and TOR pathways and requires the germline

Previous studies have elucidated various regulators of lifespan which are involved in pathways such as insulin-signaling, heat shock response, and target of rapamycin (TOR)[45,46]. In order to identify putative mechanisms by which DIMT-1 could regulate lifespan, we performed genetic epistasis experiments by measuring the lifespans of wild-type worms and mutants of specific longevity pathways, grown on

either empty vector control or *dimt-1* RNAi plates. We found that *dimt-1* knockdown increased lifespan to a similar extent in mutants of the acetylcholine receptor, *eat-2*, which has reduced pharyngeal pumping and therefore decreased food intake, and has been proposed as a genetic mimic of dietary restriction[47,48] (23.65% in WT, 30.55% in *eat-2*; $p = 0.7021$ by two-way ANOVA, Fig. 3c). Similarly, *dimt-1* knockdown increased lifespan to a similar extent in wild-type worms and in mutants of the insulin receptor, *daf-2*[49] (40.16% in WT, 33.98% in daf-2; $p = 0.0806$ by two-way ANOVA, Fig. 3d), mutants of the ubiquinone biosynthesis gene, *clk-1*[48] (23.65% in WT, 35.2% in clk-1; $p = 0.0855$ by two-way ANOVA, Supplementary Fig. 3a), and in mutants of the heat-shock response transcription factor, *hsf-1*[8,50] (10.72% in WT, 22.00% in *hsf-1*; $p = 0.4245$ by two-way ANOVA, Fig. 3e).

Next, we tested if *daf-16*, which is a FOXO transcription factor that mediates longevity regulation downstream of several signaling pathways[8,51–56], has an effect on *dimt-1* knockdown-induced lifespan extension. We found that *dimt-1* knockdown extended wild-type worm lifespan but failed to increase the lifespan of *daf-16* mutant *C. elegans* ($p = 0.0907$), indicating that *daf-16* is functioning in the same genetic signaling pathway as *dimt-1* (23.65% in WT, 5.13% in *daf-16*; $p < 0.0001$ by two-way ANOVA, Fig. 3f). We also tested *raga-1*, which is a Rag GTPase that links amino acid sensing to mechanistic target of rapamycin complex (mTORC)1, where *raga-1* mutation causes an increase in lifespan[57]. Again, *dimt-1* knockdown increased WT worm lifespan but failed to increase the long lifespan of *raga-1* mutant worms (22.67% in WT, 0.53% in raga-1, $p = 0.0327$ by two-way ANOVA, Fig. 3g), suggesting that DIMT-1 functions in the same signaling pathway as RAGA-1 and TOR. To determine if an intact germline is necessary for *dimt-1* induced lifespan extension, we performed *dimt-1* knockdown in *glp-1(e2141ts)* mutant worms, which develop 5–15 meiotic germ cells instead of ~1500 when shifted to the restrictive temperature[58] and *pgl-1(bn101ts)* mutant worms, which have defective germ granules and are sterile[59]. Knockdown of *dimt-1* extended the lifespan of WT worms but failed to increase the lifespan of either *glp-1(e2141ts)* or *pgl-1(bn101ts)* mutant worms (28.32 or 40.16% in WT, −5.23% in *glp-1*, 3.34% in *pgl-1*; $p < 0.0001$ by two-way ANOVA, Fig. 3h, i), suggesting that a functional germline is required for the *dimt-1* knockdown induced lifespan extension. Taken together, these results show that *dimt-1* is likely affecting or regulating lifespan through the DAF-16 and TOR pathways and either functions in or requires the germline for longevity regulation.

DIMT-1 functions in the germline to regulate lifespan

To resolve the paradox of depletion of *dimt-1* extending lifespan while 18S rRNA $m^{6,2}A$ decreases in whole worms with age (Fig. 2a and Supplementary Fig. 2c), we next wished to determine whether DIMT-1, which is a ubiquitously expressed protein, was functioning in specific tissues to regulate lifespan. To examine DIMT-1's tissue-specific function, we created an auxin-inducible degron (AID) tagged DIMT-1 worm strain and crossed it with strains that express TIR1 in a tissue-specific manner to allow for tissue-specific auxin-dependent depletion of DIMT-1[60,61]. We found that DIMT-1 depletion ubiquitously led to a significant decrease in 18S rRNA $m^{6,2}A$ relative to control strains as assessed by UHPLC-MS/MS, suggesting that our AID-tagged DIMT-1 strains were effective (Fig. 4a). We found that when DIMT-1 was depleted using two independent ubiquitous *eft-3* promoters or the germline-specific *mex-5* promoter to drive TIR1 expression we observed a significant lifespan extension relative to control AID-tagged DIMT-1 strains that did not express TIR1 (29.71% $p < 0.0001$ and 47.97% $p < 0.0001$ respectively, Fig. 4b and Supplementary Fig. 3b). In contrast, we observed no lifespan extension when DIMT-1 was depleted in muscle, intestine, or neurons (Fig. 4b). This finding suggests that DIMT-1 not only requires the presence of a functional germline to regulate lifespan (Fig. 3h, i), but indeed functions in the germline to regulate lifespan.

To validate our findings from the AID/TIR1 experiments, we performed an orthogonal approach by using tissue-specific RNAi strains to knock down *dimt-1* and measure its effect on lifespan. When we specifically knocked down *dimt-1* in muscle, intestine, or neurons, we failed to observe a significant increase in lifespan; however, germline-specific knockdown of *dimt-1* caused a significant increase in lifespan that was comparable to knockdown of *dimt-1* in WT worms (16.93% in WT, 15.93% in germline-specific; $p = 0.8536$ by two-way ANOVA, Fig. 4c, d). Further bolstering the importance of the germline for the longevity effects of *dimt-1*, we found that treating worms with 5-fluorodeoxyuridine (FUdR), which inhibits proliferation of germline stem cells, the production of intact eggs in adults, and extends longevity[30,44], abolished the effect of *dimt-1* knockdown on lifespan (Fig. 4e). This finding could also explain why, in our aging gradient, which out of technical necessity was generated using FUdR, we observed a decrease in 18S rRNA $m^{6,2}A$ levels (Fig. 2a and Supplementary Fig. 2c).

DIMT-1 affects ribosome binding to specific mRNA transcripts in the germline

We had previously found that knockdown of *dimt-1* in the parental generation caused a significant misregulation of both transcription and ribosome binding to transcripts involved in the determination of adult lifespan in the eggs of progeny[26]. Presumably, all effects on transcription come as a secondary consequence of alterations in translation. To specifically examine which transcripts displayed altered binding by ribosomes after a decrease in DIMT-1 at later stages of life in the germline, we performed germline-specific Translating Ribosome Affinity Purification (TRAP)[62,63] in four independent biological replicates from worms that were grown on empty vector (EV) control or *dimt-1* RNAi. We used a flag-tagged RPL-4 driven by the germline-specific promoter *mex-5*, which has previously been shown not to have a negative effect on ribosome function[63]. We first validated that *dimt-1* knockdown extended longevity in this strain (Supplementary Fig. 3c). We next analyzed the transcription changes in response to *dimt-1* knockdown in post reproduction worms. Transcriptional changes would not be predicted to be direct consequences of manipulating the rRNA methylation; however, a natural consequence of changes in translation will lead to changes in transcription[64,65]. We found that 5765 genes were differentially expressed, with 3700 genes that were significantly upregulated and 2065 genes that were significantly downregulated upon *dimt-1* depletion compared to the empty vector control (Supplementary Fig. 4a and Supplementary Data 1). Pathway analysis revealed increased expression of genes involved in longevity regulation, xenobiotic detoxification, TGF-β, WNT, and MAPK signaling pathways, as well as degradation pathways including proteasome, peroxisome, and autophagy genes (Supplementary Fig. 4b, c and Supplementary Data 1). Downregulated genes were enriched for protein processing, mTOR and FOXO/DAF-16 signaling pathway, and ribosome biogenesis genes (Supplementary Fig. 4d, e and Supplementary Data 1). These altered transcriptional pathways could help to explain the extended longevity in response to *dimt-1* depletion, and the decreased expression of FOXO/DAF-16 and mTOR signaling pathways expression could help explain the *daf-16* and *raga-1* dependency of the lifespan extension (Fig. 3f, g). As RAGA-1 is a RAG GTPase that links amino acid sensing to the mechanistic target of rapamycin complex mTORC1, these findings suggest that *raga-1* dependency is mediated by both transcriptional and translational changes induced by *dimt-1* depletion.

To determine if *dimt-1* depletion also altered the ribosome binding levels of specific transcripts, we sequenced ribosome-bound RNAs from the same four biological replicates isolated from the germline by TRAP and normalized them to the levels of the transcripts to measure translation efficiency. We found that 2082 genes were differently bound to ribosomes, with 1666 genes that were significantly more

https://doi.org/10.1038/s41467-025-62323-7

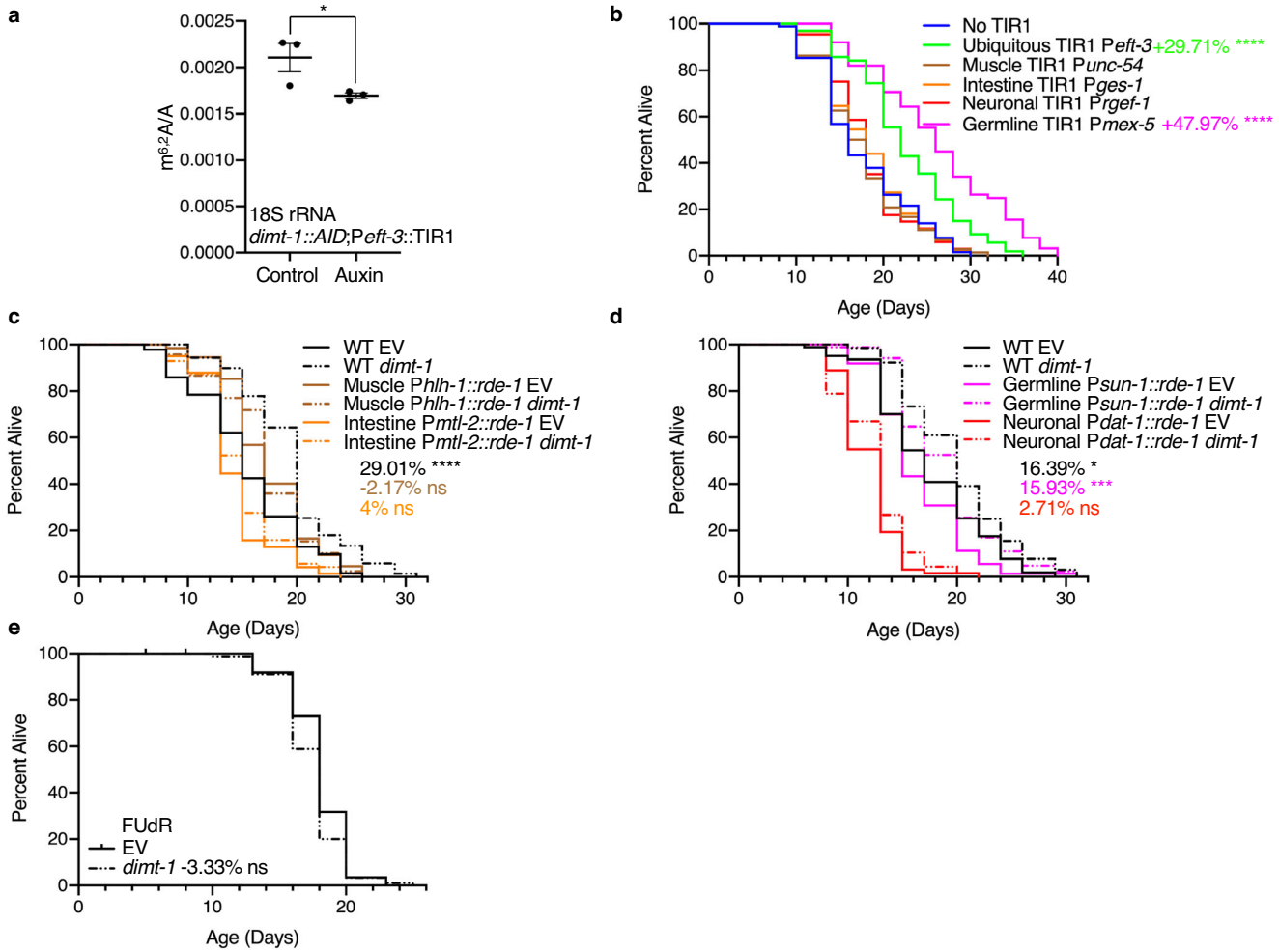

Fig. 4 | DIMT-1 functions in the germline to regulate lifespan and affects the translation of specific mRNA transcripts. a Auxin-inducible degron (AID) ubiquitous degradation of DIMT-1 protein leads to significant decrease in $m^{6,2}A$ levels in the 18S rRNA subunit as assessed by UHPLC-ms/ms. Statistics represent an unpaired two-tailed *t*-test $p = 0.0286$. **b** Ubiquitous and germline-specific AID-induced DIMT-1 protein degradation causes a lifespan extension, while DIMT-1 depletion in the muscle, intestine, or neurons has no effect on lifespan extension. **c**, **d** Tissue-specific knockdown of *dimt-1* in the germline increases lifespan to a similar extent as in ubiquitous knockdown, while knockdown of *dimt-1* in the muscle, intestine, or neurons has no significant effect on longevity. **e** *dimt-1* knockdown does not extend the lifespan of worms treated with 5-fluorodeoxyuridine (FUdR), a drug that inhibits the proliferation of germline stem cells and the production of intact eggs. Statistics and replicate longevity experiments are presented in Supplementary Table 2. ns not-significant, $*p < 0.05$, $***p < 0.001$, $****p < 0.0001$ as calculated by log-rank (Mantel–Cox) statistical test.

bound and 416 genes that had significantly lower ribosome binding in *dimt-1*-depleted samples compared to empty vector controls (Fig. 5a and Supplementary Data 2). Pathway analysis revealed that *dimt-1* depletion significantly altered the ribosomal binding to a selective subset of mRNAs involved in longevity regulation, degradation pathways, cellular detoxifications, glutathione metabolism, and oxidative phosphorylation (Fig. 5b, Supplementary Fig. 5, and Supplementary Data 2). The differences in ribosome-bound transcripts could help to explain the altered longevity and stress resistance observed upon *dimt-1* depletion. Some of the pathways which were dysregulated on a transcriptional level were further dysregulated on a translational level as well, while some other categories of genes appeared to only be misregulated transcriptionally or translationally, as would be expected.

To determine if the *dimt-1* depletion-induced altered ribosome binding was specific to germline-regulated genes, we additionally performed TRAP specifically in the muscle cells with or without *dimt-1* depletion. We found that 2556 genes were differently bound to ribosomes, with 2175 genes that were significantly more bound and 381 genes that had significantly lower ribosome binding in *dimt-1*-depleted muscle samples compared to empty vector controls (Supplementary Fig. 5f and Supplementary Data 3). Pathway analysis revealed that *dimt-1* depletion significantly altered the ribosomal binding to a selective subset of mRNAs in the muscle involved in transport and phosphorylation (Supplementary Fig. 5g). Unsurprisingly the set of differently bound transcripts in the muscle was not enriched for longevity regulating pathways and was different from the set of differently bound transcripts in the germline (Supplementary Fig. 5h). Together, these results suggest that altered ribosome binding specifically in the germline is what is driving the altered longevity.

Differentially binding to mRNA transcripts by ribosomes could be due to specific features present in the 5′UTR, such as specific sequences that may be translationally regulated[66,67]. To test if 18S $m^{6,2}A$ absence caused an enrichment of sequence motifs, we examined the 5′ UTR regions of mRNA transcripts which were ribosome-bound when *dimt-1* was depleted in comparison to empty vector control in the germline. We identified 49 genes which have the sequence motif GRVRAMGAHGRMGRHGRWGVR and 11 genes which have the sequence motif GRCTCCGCCCACTTT (e-values 6.8E-4 and 5.4E4, respectively, Fig. 5c). This indicates that the presence of $m^{6,2}A$ on the 18S rRNA could regulate which transcripts are bound to the ribosome based partly on sequence features.

https://doi.org/10.1038/s41467-025-62323-7

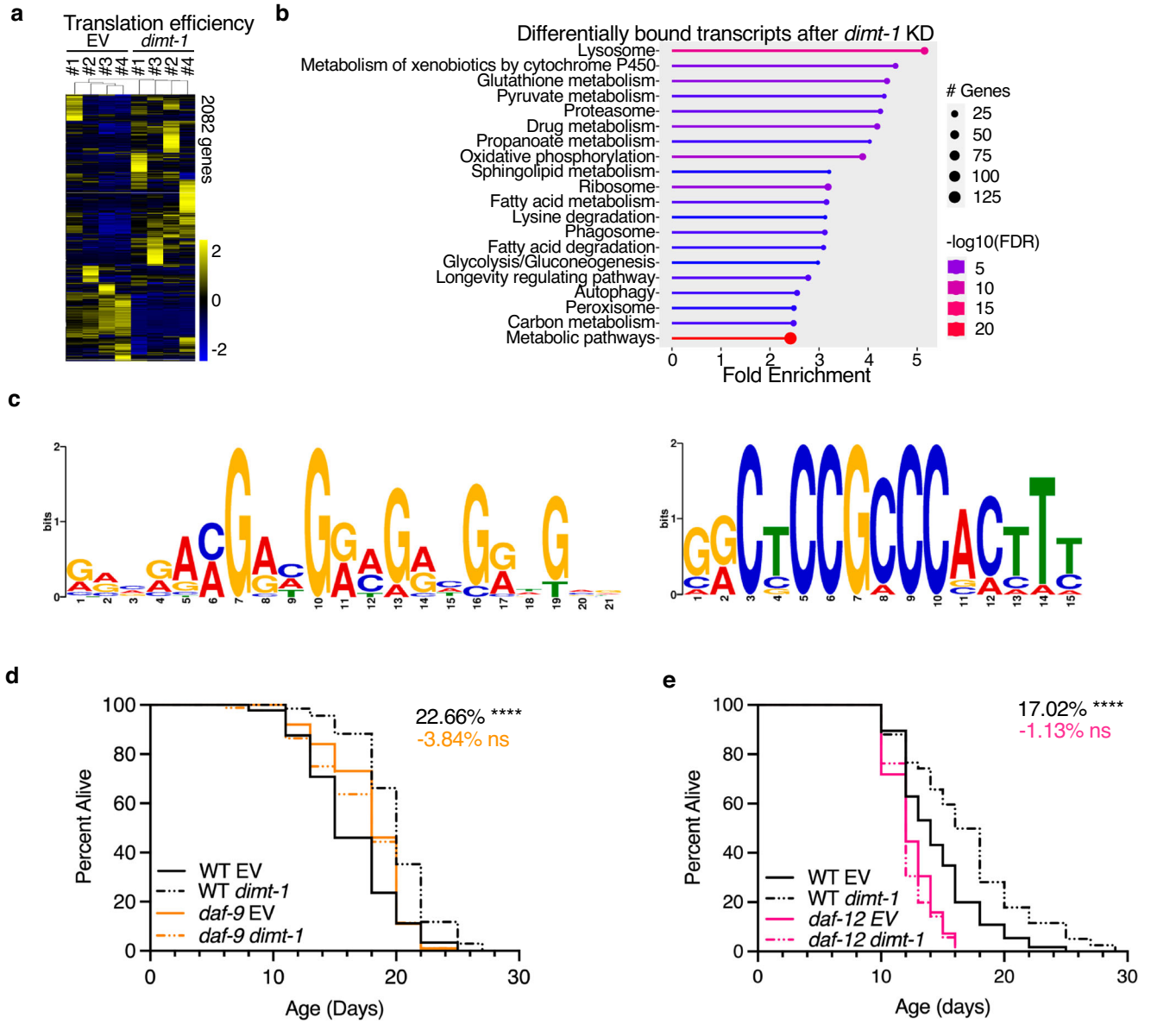

Fig. 5 | DIMT-1 affects the translation of specific mRNA transcripts. a Heatmaps of the 2082 differentially ribosome-bound transcripts in the ribosome after *dimt-1* knockdown from day 7 worms. Ribosome binding was normalized to total RNA expression to give translation efficiency. *Dimt-1* was knocked down from the L4 stage until day 7. Each column represents an independent biological replicate from ribosome sequencing after TRAP. **b** Pathway analysis of differentially bound transcripts after *dimt-1* knockdown revealed altered ribosome binding to transcripts involved in longevity regulation, degradation, fatty acid metabolism, the ribosome, and oxidative phosphorylation. RNAseq and translation efficiency significantly regulated genes and gene ontology categories are presented in Supplementary Data 1, 2. **c** Sequence motifs enriched in the 5′ UTR of more bound mRNA transcripts after *dimt-1* knockdown. **d** *dimt-1* knockdown extends the lifespan of WT but not *daf-9* mutant worms ($p = 0.004$ by two-way ANOVA). **e** *dimt-1* knockdown extends the lifespan of WT but not *daf-12* mutant worms ($p = 0.0005$ by two-way ANOVA).

Interestingly, one of the transcripts, which showed no change in transcription but did display decreased ribosome occupancy in the germline when *dimt-1* was depleted, was the cytochrome P450 enzyme, *daf-9*. DAF-9 has previously been demonstrated to produce a signaling lipid, dafachronic acid, which signals from the germline to the soma to activate the nuclear hormone receptor DAF-12, which inhibits organismal longevity[68–71]. We therefore tested whether the DAF-9/DAF-12 signaling pathway could be responsible for DIMT-1-regulated lifespan as translational targets, as we found that DIMT-1 functions in the worm germline to regulate organismal lifespan. We found that *dimt-1*-dependent lifespan extension was abolished in both *daf-9* and *daf-12* mutant worms (Fig. 5d, e). This indicates that *daf-9* and *daf-12* are required for *dimt-1*-induced lifespan extension. Taken together, these results suggest that *dimt-1* regulates organismal lifespan by selective translation in the germline of specific mRNA transcripts, which subsequently leads to altered germline-to-soma signaling and lifespan.

DIMT-1 regulates lifespan in later life

We were next interested in determining when DIMT-1 functioned to regulate organismal longevity. We performed a series of lifespan assays with AID-tagged *dimt-1* strains crossed with germline and ubiquitous TIR1 strains, where DIMT-1 was knocked out with auxin treatment at specific stages in the life of the worm (Fig. 6a). We found that when auxin was administered starting at the previous generation and for the entirety of the tested generation, starting at birth, or starting at the young adult stage, a consistent increase in lifespan was observed (52.19, 41.33, and 38.17% $p < 0.0001$ in germline strain and 42.45, 30.47,

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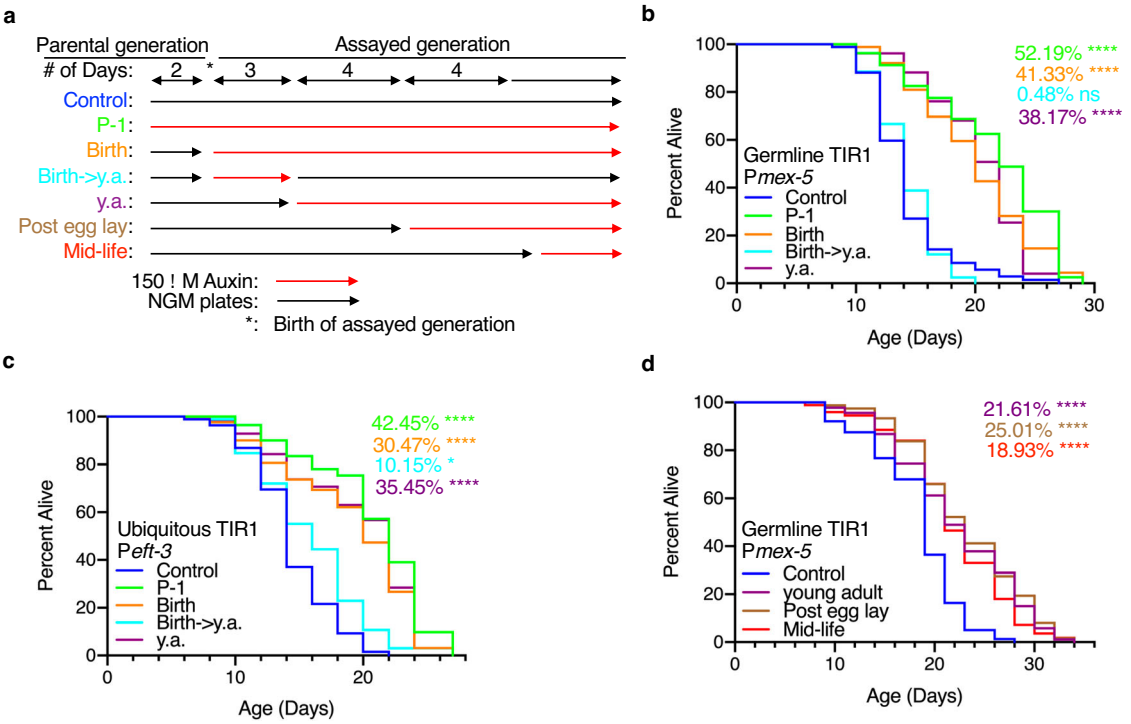

Fig. 6 | DIMT-1 regulates lifespan after mid-life. a Experimental design for the AID-tagged DIMT-1 temporal knock out experiments (y.a. young adults). Red lines indicate when strains were placed on 150 uM auxin and when DIMT-1 is knocked down. **b** AID-induced depletion of DIMT-1 in the germline extends lifespan when depleted in the previous generation and for the entirety of the assayed generation, starting at birth, or from young adulthood for the remainder of the lifespan but does not extend lifespan when depleted only from birth until young adulthood. **c** AID-induced depletion of DIMT-1 ubiquitously extends lifespan when depleted in the previous generation and for the entirety of the assayed generation, starting at birth, or from young adulthood for the remainder of the lifespan and causes a less dramatic extension in lifespan when depleted only from birth until young adulthood. **d** AID-induced depletion of DIMT-1 in the germline extends lifespan to a similar extent when depleted from young adulthood, after reproduction, or starting in mid-life. Statistics and replicate longevity experiments are presented in Supplementary Table 3. ns not-significant, $*p < 0.05$, $***p < 0.001$, $****p < 0.0001$ as calculated by log-rank (Mantel–Cox) statistical test.

and 35.45% $p < 0.0001$ in ubiquitous strain, Fig. 6b, c). When auxin was only introduced from birth to the young adult stage and then worms were removed from auxin, we failed to observe a significant lifespan extension in the germline-specific DIMT-1 depletion strain (0.48% $p = 0.9570$, Fig. 5b) and we only observed a modest lifespan extension in the ubiquitous DIMT-1 depletion strain (10.15% $p = 0.0101$, Fig. 6c). Together, these results suggest that DIMT-1 functions in the germline after development to regulate lifespan. Although we determined that *dimt-1* regulates longevity following development, it was still unclear if the egg-laying phase is important for this phenotype, as we had previously found that DIMT-1 depletion causes a reduction in fertility[26] and there is a well-known anti-correlation between reproduction and longevity[58]. We therefore examined the lifespan of our germline-specific TIR1 DIMT-1-AID-tagged strain and introduced auxin at young adult, post egg-laying and mid-life phases. Surprisingly, we observed significant lifespan extension in all three stages compared to the untreated control (21.61, 25.01, and 18.93%, respectively $p < 0.0001$, Fig. 6d). This suggests that DIMT-1 affects lifespan post-developmentally, after egg-laying is complete, and in mid-life.

Discussion

Here, we show that DIMT-1, an evolutionarily conserved 18S rRNA $m^{6,2}A$ methyltransferase[26,34,35,72], regulates lifespan in *C. elegans*. Reduction of DIMT-1 causes a lifespan extension that requires the DAF-16/FOXO transcription factor and TOR signaling. We found that DIMT-1 functions in the germline to regulate *C. elegans* lifespan and also causes differential binding of the ribosome to specific subsets of mRNA transcripts. One of the altered transcripts we identified was *daf-9*, and we found that reduction of DIMT-1 caused a lifespan extension that required both the cytochrome P450 enzyme DAF-9 and its downstream nuclear hormone receptor transcription factor DAF-12. These results suggest an overall model whereby DIMT-1 regulates organismal lifespan by selective translation in the germline of specific mRNA transcripts, which subsequently leads to altered germline-to-soma signaling and lifespan. Furthermore, while most longevity regulators identified to date function early in life to "lock-in" aging rates, we found that DIMT-1 can regulate lifespan after middle age. Together, this study provides evidence of selective translation, via ribosome heterogeneity, playing a significant role in the regulation of aging.

A major feature of aging is the loss of proteostasis, where maintenance of the appropriate amounts of proteins in the cell, is significantly disrupted later in life[73,74]. Previous studies have examined the levels of misfolded proteins, which become increasingly prevalent in older organisms, causing breakdown of normal cellular function[75,76]. However, little is known about how transcripts are selected for translation during the aging process. Ribosomes were initially believed to be non-discriminatory, translating any mRNA transcripts they were presented with[77], but recent work has suggested that specialized ribosomes can translate unique sets of transcripts under specific stress conditions[21,23,78–80]. We found that perturbations in many of the enzymes that regulate rRNA modifications have substantial effects on overall health and duration of lifespan in *C. elegans* (Fig. 1).

We[26], and others[81], have recently shown that DIMT-1's function to N6-dimethylate two adjacent adenines on the 18S rRNA can direct the ribosome to specific subsets of transcripts for translation. Our previous work suggests that while DIMT-1 binding to rRNAs during rRNA processing is important for the appropriate processing of rRNAs, the decreased expression of *dimt-1* that we see in response to *dimt-1* knockdown is not sufficient to significantly alter rRNA processing[26]. We also demonstrated that rRNAs are the predominant, if not the only,

substrate of DIMT-1[26]. Here, we additionally demonstrate that a complete ablation of DIMT-1's catalytic activity is not lethal and even leads to increased lifespan and stress resistance (Fig. 3a, b and Supplementary Fig. 1d). These findings suggest that DIMT-1 is regulating lifespan through altering ribosome specialization, rather than through changing available amounts of processed rRNAs. Additionally, increased longevity from DIMT-1 depletion could also be due to reduced ER stress from better protein homeostasis, where we observed lowered ER-UPR marker induction even after the worms were treated with tunicamycin (Supplementary Fig. 1c). In this work we found that reducing 18S rRNA $m^{6,2}A$ caused changes in ribosome binding in a set of selective mRNAs that are mostly involve in longevity, metabolisms, cellular detoxifications, protein homeostasis, degradation and recycling pathways specifically in the germline after reproduction.

Regulation of aging has been shown to involve both germline and somatic tissues[58]. However, many findings related to the germline regulation of lifespan have been shown to extend lifespan due to defects in germline development or reducing the energy diverted toward producing the next generation of progeny[58,82]. We showed that DIMT-1 functions through the germline; however, its role in lifespan extension takes place after development and reproduction (Fig. 6). This indicates that DIMT-1 is amenable to therapies or manipulation once the developmental or reproductive stage of an organism has passed. In addition, given that DIMT-1 is expressed ubiquitously in the worm and that the catalytically dead mutant also shows extended lifespan, it is likely that the enzymatic activity of DIMT-1 in the germline has a specific effect on longevity.

It is interesting to note that global $m^{6,2}A$ levels decrease in whole worms as *C. elegans* age (Fig. 2a). This finding runs counter to the observation that *dimt-1* levels increase as *C. elegans* age (Supplementary Fig. 2a, b), and that depletion of *dimt-1* causes an increase in lifespan. Our working hypothesis to explain this apparent paradox is that this could reflect that $m^{6,2}A$ increases in some specific cells while it decreases in other tissues as organisms age. We found that $m^{6,2}A$ levels decrease in the isolated *C. elegans* germline with age (Supplementary Fig. 6) in addition to the whole worm, suggesting that this predicted change would have to occur in a subset of germline cells. Indeed, DAF-9, one of the effector downstream targets of DIMT-1, has been reported to be expressed in the somatic germline, suggesting that DIMT-1's critical site of action for regulating lifespan could be in the somatic gonad cells. An example of a molecular change having different effects in different cell types would be insulin signaling in gustatory neurons, which were reported to have differing effects on longevity dependent on the specific cell type[83]. An alternative reason for this paradox could lie in the fact that, out of necessity, we had to use the drug 5-fluorodeoxyuridine (FUdR), which inhibits proliferation of germline stem cells, the production of intact eggs in adults, and extends longevity[30,44], to generate our aging gradient and this drug could also indirectly affect the $m^{6,2}A$ levels. In support of this hypothesis we found that the use of FUdR eliminated the beneficial effects of *dimt-1* depletion on *C. elegans* lifespan (Fig. 4e). One other potential explanation for this apparent paradox could be that $m^{6,2}A$ could be important in responding to immediate environmental stresses in *C. elegans*, and rather than the absolute levels of $m^{6,2}A$ at particular ages being important for regulating lifespan, this modifications capacity to rapidly change could deteriorate with age. This notion is supported by the fact that $m^{6,2}A$ increases in response to both UV stress and heat stress (Fig. 2b, c). It will be interesting in future experiments to examine whether $m^{6,2}A$ levels increase specifically in certain cells of the germline with age or if the capacity of this rRNA modification to respond to stresses later in life is diminished.

Several recent studies have demonstrated that there are proteomic, epigenetic, and epitranscriptomic changes associated with aging, and that enzymes which regulate these processes can also regulate aging[3,4,10,11,30,36,84]. Most regulators of aging determine organismal lifespan at early developmental stages[85–87]; however, several exceptions, including dietary restriction[88] and this study, can increase lifespan later in life. Pathways which may be manipulated later in life to extend lifespan offer exciting possibilities to potentially address aging-associated diseases with interventions. Thus, altering rRNA modifications late in life may represent a new approach for addressing aging-related disorders and increasing health span. Nevertheless, our results provide evidence of selective translation, via ribosome heterogeneity, playing a significant role in the regulation of aging.

Methods

Strains used

The N2 Bristol strain was used as the WT background. Worms were grown on *dam*$^{-}$*dcm*$^{-}$ on standard nematode growth medium (NGM) plates in all experiments except for RNAi and auxin-inducible degron experiments. TIR1 expressing strains CA1200, DV3801, DV3803, DV3805, JDW221, and JDW225, the tissue-specific RNAi strains DCL569, IGL1839, NR350, XE1474, the Flag-tagged RPL-4 strain for germline-specific ribosome purifications EV484, *eat-2(ad1116), daf-16(mu86), daf-2(e1370), hsf-1(sy441), clk-1(e2519), raga-1(ok386), glp-1(e2141ts), pgl-1(bn101ts), daf-9(rh50)*, and *daf-12(rh61rh412)* were obtained from the *Caenorhabditis* Genetics Center which is supported by the NIH office of research infrastructure programs P40OD010440. The AID-tagged DIMT-1 and the *dimt-1* E79A strains were generated by SunyBiotech. TIR1 strains were crossed with the AID-tagged DIMT-1 strain to generate double homozygous strains. Each strain was assayed by PCR to confirm the genotypes.

Single-worm genotyping

Single worms were placed in 5 μl of worm lysis buffer [50 mM KCl, 10 mM Tris (pH 8.3), 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween 20, and proteinase K (60 mg/ml)] and incubated at −80 °C for 1 h, 60 °C for 1 h, and 95 °C for 15 min. PCRs were performed using the following primers: E02H1.1 SY373 (forward), 5′-CGTCGAGGATGAGCGAGAAA-3′, E02H1.1 SY373 (reverse), 5′-TGGCCATTCCATTTTCATTACA-3′; DV3801, DV3803, DV3805, JDW221, and JDW225 primers as described in (*40*); CA1200 primers as described in (*39*). PCRs were performed according to the manufacturer's protocol (New England Biolabs, M0273) and the PCRs were resolved on agarose gels.

Targeted RNAi screen

Bacteria expressing double-stranded RNA of putative ribosomal RNA methyltransferases were obtained from the Blackwell lab, with bacteria carrying the empty vector backbone as a negative control. Bacteria were grown at 37 °C and seeded on NGM plates containing ampicillin (100 μg/ml) and isopropylthiogalactoside (IPTG; 0.4 mM). For experiments involving FuDR, bacteria were seeded on NGM plates containing ampicillin (100 μg/ml), FuDR (100 mg/ml) and isopropylthiogalactoside (IPTG; 0.4 mM).

Tissue-specific and temporal knock-out with auxin-inducible degron-tagged DIMT-1

NGM plates containing streptomycin (300 μg/ml) and 1-napthaleneacetic acid (NAA, auxin, 150 μM), were seeded with OP50-1 bacteria. Worms were grown on the auxin plates at specific worm stages for specific lengths of time according to the experiment shown in the results.

Ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS/MS)

Total RNA was extracted by the addition of 1 ml TRIzol (Invitrogen) to 100 μl of packed worm pellet. Six freeze-thaw cycles were performed in liquid nitrogen. RNA extraction was performed according to the manufacturer's protocol (Invitrogen, TRIzol). To isolate 26S, 18S, and 5.8/5S rRNAs, total RNA was run on agarose electrophoresis gels to

separate rRNAs. rRNA bands were excised from the gel and purified with Zymoclean Gel RNA Recovery Kit (Zymo). For the digestion of nucleosides, 250 ng of RNA samples were digested at 37 °C for 2 h with Nucleoside Digestion Mix (New England Biolabs, M069S). Digested RNA samples were diluted to 100 µl with double-distilled water and filtered through 0.22 µm Millex Syringe Filters. About 5 µl of filtered solution was injected for LC-MS/MS analysis and analyzed using the Agilent 1290 UHPLC system with C18 reversed-phase column (2.1 mm by 50 mm; 1.8 m) as in ref. 26. Mobile Phase A is composed of water with 0.1% (v/v) formic acid, and mobile phase B is composed of acetonitrile with 0.1% (v/v) formic acid. MS detection was performed using an Agilent 6470 triple quadrupole mass spectrometer in positive electrospray ionization mode, and data was quantified in dynamic multiple reaction monitoring mode by monitoring mass transition 268 → 136 for adenosine, 282 → 136 for Am, 282 → 150 for m^6A, 296 → 164 for $m^6{}_2A$, 284 → 152 for guanine, 298 → 152 for Gm, 298 → 166 for m^7G, 244.1 → 112 for cytosine, 258 → 112 for Cm, 245 → 113 for uracil, 259 → 113 for Um, 245 → 125 for PseudoU. Data analysis was performed using the Agilent MassHunter software.

Longevity assays

Worm lifespan assays were performed at 20 and 25 °C. For longevity assays involving RNAi, worm populations were synchronized by placing L1 worms on NGM RNAi plates. Resulting eggs from the P^{-1} generation were transferred to new RNAi plates, and the hatching day for the P^0 generation was counted as day 1 for all lifespan measurements. For the longevity assay involving the auxin-inducible degron system, worm populations were synchronized by placing eggs for the P^0 generation, on NGM plates seeded with OP50-1 bacteria either with or without 150 µM auxin. Worms were changed every other day to new plates to avoid confounding progeny and were scored as dead or alive. The lifespan assay is started on the day the worm hatches. Dead worms were scored if they did not respond to repeated prods with a platinum pick. Worms were censored if they died from vulval bursting or crawled off the plate. Each independent lifespan assay experiment used 90 worms on three plates (30 worms/plate). Data were plotted with Kaplan–Meier survival curves, and statistical significance was tested using log-rank (Mantel–Cox) tests. Life-span assays were repeated at least once and showed similar trends in relative life-span effects. Two-way ANOVA tests were performed to determine whether the change in lifespan was statistically different in two different genotypes in Prism using the average lifespan, SEM, and sample size for each condition. Fisher's combined probability test was used to determine whether significant changes in lifespan across multiple independent experiments were significant in aggregate.

UV stress assays

For survival assays involving RNAi, the P^0 generation was prepared as described in the longevity assays. Hatched eggs were allowed to grow to the L4 stage on three plates per condition with 30 worms per plate (90 worms per assay). L4 worms were exposed to 0.8 Joules (J), then grown at 20 °C, assessed every 24 h for survival, and scored as dead or alive as described in the longevity assays. For UHPLC-MS/MS analysis, L4 worms were synchronized on NGM plates seeded with OP50-1 bacteria and exposed to 0.8 J. Then, the worms were allowed to recover for either 1 or 2 h at 20 °C, before being processed as described for the UHPLC-MS/MS analysis.

Heat stress

For survival assays involving RNAi, the P^0 generation was prepared as described in the longevity assays. Hatched eggs were allowed to grow to the L4 stage on three plates per condition with 30 worms per plate (90 worms per assay). L4 worms were then grown at 37 °C for 6–7 h, then grown at 20 °C, assessed every 24 h for survival, and scored as dead or alive as described in the longevity assays. For UHPLC-MS/MS analysis, L4 worms were synchronized on NGM plates seeded with OP50-1 bacteria and were grown at 37 °C for 6–7 h. Then the worms were allowed to recover for either 1 or 2 h at 20 °C, before being processed as described for the UHPLC-MS/MS analysis.

Analysis of protein homeostasis using stress-induced chaperone proteins

Different reporter strain for UPR were bleached, and the eggs were put either on control or *dimt-1* RNAi and grown until the L4 stage. At the L4 stage, worms were treated either with Tunicamycin (5 µg/mL, *Phsp-4::gfp*(*zcIs4)* (UPR^{ER})) for 5 h or heat shock treatment for 30 min at 37 °C in *Phsp-16.2::gfp* (zSi3000) ($UPR^{cytosol}$) or 5 h of ethidium bromide (25 µg/mL) treatment in *Phsp-6::gfp* (zcIs13) ($UPR^{mitochondria}$). To check the GFP expression, worms were mounted on 2% agarose slides using 10 mM sodium azide. The GFP fluorescence of worms was captured with a Leica DMi8 Microscope at 10X magnification (excitation 488 nm and absorbance at 520 nm). Quantification of GFP expression was done using NIH ImageJ software. Statistical analyses were done using GraphPad 9.0.

Genetic epistasis

Specific worm strains as described in the results section, were grown on NGM RNAi plates seeded with either bacteria expressing double-stranded RNA for *dimt-1* gene or carrying the empty vector backbone as the negative control.

Transcriptomic analysis

Transcriptomic analysis for the gene expression profiles of putative rRNA methyltransferases was performed using a publicly available dataset[43].

Translating ribosome affinity purification (TRAP)

Synchronized EV484 (*efIs155[Cbr-unc-119*(+) + *Pmex-5::rpl-4::FLAG::tbb-2 3′UTR] II*) worms (germline-specific FLAG-tagged ribosomal protein *rpl-4*) were grown on NGM RNAi plates seeded with overnight-grown HT1115 bacteria containing empty vector (control RNAi) till the young adult stage. Young adult animals were then collected in M9 buffer and divided into two groups. Half of the worms were put into the fresh empty vector control RNAi plate, and the other half into the *dimt-1* RNAi plate for its knock-down (HT1115 bacteria containing a vector expressing double-stranded RNA for *dimt-1* gene). In the background, we have also checked the lifespan extension phenotype of *dimt-1* using EV484 as a control experiment. Worms were grown at 20 °C till the end of their reproductive phase (7-day-old worms). Each day, the P^0 generation (parental) worms were filtered through a 20-micron mesh to remove confounding progenies during the egg-laying phase. On day 7, worms were washed thrice with M9 containing 1 mM cycloheximide (Sigma) and once with Lysis Buffer I (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4, 150 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 1 mM cycloheximide, Mini cOmplete Protease Inhibitor). Then, flash-frozen worm pearls were made using liquid nitrogen and homogenized using an ice-cold glass Dounce tissue homogenizer (around 30–40 strokes). Lysis Buffer II (Lysis Buffer I containing 0.5% v/v NP-40 (Sigma), 0.4 U/µL RNasin (Promega), 10 mM ribonucleoside vanadyl complex (RVC by NEB), 33 mM 1,2-diheptanoylsn-glycero-3-phosphocholine (DHPC by Merck) and 1% w/v sodium deoxycholate (Sigma)) was added to the sample and incubated on ice for 30 min. Samples were centrifuged for 12,000× rcf for 15 min at 4 °C, and the supernatant was collected (around 2 ml). 200 µl clear supernatant was collected for total RNA extraction (for mRNA sequencing). Remaining samples were added to washed Protein G-coated Dyna-Beads for pre-clearing, incubated for 1 h at 4 °C, rotating. Then, samples were incubated with 5 µL anti-FLAG antibody(1 mg/ml)-coupled beads (F1804, sigma) for 2 h at 4 °C, rotating, and the beads were washed four times with Wash Buffer (10 mM HEPES pH 7.4, 350 mM

KCl, 5 mM $MgCl_2$, 1% v/v NP-40). For RNA elution, the beads were incubated in 350 µL of RLT buffer (Qiagen RNeasy Kit) including 1% v/v β-mercaptoethanol for 10 min at room temperature. The eluate was separated from the beads, and RNA was purified using the RNeasy Plus Mini Kit (QIAGEN) according to the manufacturer's protocol. A similar procedure was performed with WBM1339 (wbmIs118(Pmyo-3::3xFLAG::rpl-22::SL2::wrmScarlet::unc-54 3'UTR) but synchronized L1 worms were placed on control or *dimt-1* RNAi plates until the young adult stage and were then collected for IP with anti-FLAG antibody for identifying muscle-specific ribosome-bound transcripts.

Transcriptome and ribosome profiling, sequencing, and analysis

RNA concentration was measured with Qubit using the RNA HS Assay kit. Libraries were prepared with TruSeq Stranded mRNA LT Sample Prep Kit according to TruSeq Stranded mRNA Sample Preparation Guide, Part # 15031047 Rev. E. Libraries were quantified using the Bioanalyzer (Agilent, Santa Clara, CA) and sequenced with Illumina NovaSeq6000 S4 (2 × 150 bp) (reads trimmed to 2 × 100 bp) to get 20 M read depth coverage per sample. The BCL (binary base calls) files were converted into FASTQ using the Illumina package bcl2fastq. Fastq files were mapped to the WBCel235 *C. elegans* genome, and gene counts were obtained with STAR v2.7. 2b[89]. All subsequent steps were performed in R using WormBase gene IDs. After filtering genes with low numbers of reads, the raw total mRNA-seq counts were normalized using edgeR[90]. Translation efficiency was calculated by dividing the raw ribosomal mRNA-seq counts by the raw total mRNA-seq counts. Differential gene expression analysis was performed using edgeR[90] and was corrected for multiple comparisons using the Benjamini–Hochberg method. Statistical significance was defined as having a False Discovery Rate (FDR, or adjusted p value) <0.05. GSEA was performed using the clusterProfiler package[91], heatmaps were generated using the pheatmap package, and Revigo plots were generated using R code obtained from the Revigo webserver[92]. Sequence motif analysis for 5′ UTR regions of identified mRNA transcripts was performed using the MEME suite (https://meme-suite.org/meme/tools/meme)[93]. Additional pathway analysis was performed using ShinyGo[94].

Statistics and reproducibility

No statistical method was used to predetermine sample size, as these sample sizes are all based on previous experience of what is required for lifespan and stress resistance assays. No data were excluded from the analyses. The Investigators were not blinded to allocation during experiments and outcome assessment.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Raw sequencing data can be accessed through the GEO repository, accession number GSE237802 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi]. Permanent reference for the code used in this study can be found at Zenodo[95].

Code availability

Bioinformatics pipelines and supplementary code are available at https://github.com/wtm09002/Greer_Dimt1.

References

1. Balch, W. E., Morimoto, R. I., Dillin, A. & Kelly, J. W. Adapting proteostasis for disease intervention. *Science* **319**, 916–919 (2008).
2. DiLoreto, R. & Murphy, C. T. The cell biology of aging. *Mol. Biol. Cell* **26**, 4524–4531 (2015).
3. Hansen, M. et al. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. *Aging Cell* **6**, 95–110 (2007).
4. Steffen, K. K. et al. Yeast life span extension by depletion of 60S ribosomal subunits is mediated by Gcn4. *Cell* **133**, 292–302 (2008).
5. Chiocchetti, A. et al. Ribosomal proteins Rpl10 and Rps6 are potent regulators of yeast replicative life span. *Exp. Gerontol.* **42**, 275–286 (2007).
6. Zid, B. M. et al. 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*. *Cell* **139**, 149–160 (2009).
7. Kaeberlein, M. & Kennedy, B. K. Protein translation, 2007. *Aging Cell* **6**, 731–734 (2007).
8. Hsu, A.-L., Murphy, C. T. & Kenyon, C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* **300**, 1142–1145 (2003).
9. Gidalevitz, T., Ben-Zvi, A., Ho, K. H., Brignull, H. R. & Morimoto, R. I. Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* **311**, 1471–1474 (2006).
10. Cohen, E., Bieschke, J., Perciavalle, R. M., Kelly, J. W. & Dillin, A. Opposing activities protect against age-onset proteotoxicity. *Science* **313**, 1604–1610 (2006).
11. Anisimova, A. S., Alexandrov, A. I., Makarova, N. E., Gladyshev, V. N. & Dmitriev, S. E. Protein synthesis and quality control in aging. *Aging* **10**, 4269–4288 (2018).
12. Machnicka, M. A. et al. MODOMICS: a database of RNA modification pathways—2013 update. *Nucleic Acids Res.* **41**, D262–D267 (2013).
13. Grosjean, H. RNA modification: the Golden Period 1995–2015. *RNA* **21**, 625–626 (2015).
14. Peer, E., Rechavi, G. & Dominissini, D. Epitranscriptomics: regulation of mRNA metabolism through modifications. *Curr. Opin. Chem. Biol.* **41**, 93–98 (2017).
15. Hsu, P. J., Shi, H. & He, C. Epitranscriptomic influences on development and disease. *Genome Biol.* **18**, 197 (2017).
16. Roundtree, I. A., Evans, M. E., Pan, T. & He, C. Dynamic RNA modifications in gene expression regulation. *Cell* **169**, 1187–1200 (2017).
17. Shen, L., Song, C.-X., He, C. & Zhang, Y. Mechanism and function of oxidative reversal of DNA and RNA methylation. *Annu. Rev. Biochem.* **83**, 585–614 (2014).
18. Boulias, K. & Greer, E. L. Put the pedal to the METTL1: adding internal m7G increases mRNA translation efficiency and augments miRNA processing. *Mol. Cell* **74**, 1105–1107 (2019).
19. Ramagopal, S. & Ennis, H. L. Regulation of synthesis of cell-specific ribosomal proteins during differentiation of *Dictyostelium discoideum*. *Proc. Natl Acad. Sci. USA* **78**, 3083–3087 (1981).
20. Simsek, D. et al. The mammalian ribo-interactome reveals ribosome functional diversity and heterogeneity. *Cell* **169**, 1051–1065.e18 (2017).
21. Liberman, N. et al. N6-adenosine methylation of ribosomal RNA affects lipid oxidation and stress resistance. *Sci. Adv.* **6**, eaaz4370 (2020).
22. Parks, M. M. et al. Variant ribosomal RNA alleles are conserved and exhibit tissue-specific expression. *Sci. Adv.* **4**, eaao0665 (2018).
23. Genuth, N. R. & Barna, M. The discovery of ribosome heterogeneity and its implications for gene regulation and organismal life. *Mol. Cell* **71**, 364–374 (2018).
24. Taoka, M. et al. Landscape of the complete RNA chemical modifications in the human 80S ribosome. *Nucleic Acids Res.* **46**, 9289–9298 (2018).
25. Sloan, K. E. et al. Tuning the ribosome: the influence of rRNA modification on eukaryotic ribosome biogenesis and function. *RNA Biol.* **14**, 1138–1152 (2017).
26. Liberman, N. et al. 18S rRNA methyltransferases DIMT1 and BUD23 drive intergenerational hormesis. *Mol. Cell* **83**, 3268–3282.e7 (2023).
27. Hamilton, B. et al. A systematic RNAi screen for longevity genes in *C. elegans*. *Genes Dev.* **19**, 1544–1555 (2005).
28. Lee, S. S. et al. A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. *Nat. Genet.* **33**, 40–48 (2003).

29. Hansen, M., Hsu, A.-L., Dillin, A. & Kenyon, C. New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a *Caenorhabditis elegans* genomic RNAi screen. *PLoS Genet.* **1**, e17 (2005).
30. Greer, E. L. et al. Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in *C. elegans*. *Nature* **466**, 383–387 (2010).
31. Anderson, E. N. et al. *C. elegans* lifespan extension by osmotic stress requires FUdR, base excision repair, FOXO, and sirtuins. *Mech. Ageing Dev.* **154**, 30–42 (2016).
32. Tiku, V. et al. Small nucleoli are a cellular hallmark of longevity. *Nat. Commun.* **8**, 16083 (2017).
33. Suvorov, A. N., van Gemen, B. & van Knippenberg, P. H. Increased kasugamycin sensitivity in *Escherichia coli* caused by the presence of an inducible erythromycin resistance (erm) gene of Streptococcus pyogenes. *Mol. Gen. Genet.* **215**, 152–155 (1988).
34. Lafontaine, D., Delcour, J., Glasser, A.-L., Desgres, J. & Vandenhaute, J. The *DIM1* gene responsible for the conserved m62Am62A dimethylation in the 3′-terminal loop of 18 S rRNA is essential in yeast. *J. Mol. Biol.* **241**, 492–497 (1994).
35. Shen, H., Stoute, J. & Liu, K. F. Structural and catalytic roles of the human 18S rRNA methyltransferases DIMT1 in ribosome assembly and translation. *J. Biol. Chem.* **295**, 12058–12070 (2020).
36. Heissenberger, C. et al. The ribosomal RNA m5C methyltransferase NSUN-1 modulates healthspan and oogenesis in *Caenorhabditis elegans*. *eLife* **9**, e56205 (2020).
37. Kapulkin, V., Hiester, B. G. & Link, C. D. Compensatory regulation among ER chaperones in *C. elegans*. *FEBS Lett.* **579**, 3063–3068 (2005).
38. Ient, B. et al. HSP-4 endoplasmic reticulum (ER) stress pathway is not activated in a *C. elegans* model of ethanol intoxication and withdrawal. *Invertebr. Neurosci.* **12**, 93–102 (2012).
39. Yoneda, T. et al. Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. *J. Cell Sci.* **117**, 4055–4066 (2004).
40. Govindan, J. A., Jayamani, E., Zhang, X., Mylonakis, E. & Ruvkun, G. Dialogue between *E. coli* free radical pathways and the mitochondria of *C. elegans*. *Proc. Natl Acad. Sci USA* **112**, 12456–12461 (2015).
41. Fonte, V. et al. Suppression of in vivo β-amyloid peptide toxicity by overexpression of the HSP-16.2 small chaperone protein. *J. Biol. Chem.* **283**, 784–791 (2008).
42. Burnaevskiy, N. et al. Chaperone biomarkers of lifespan and penetrance track the dosages of many other proteins. *Nat. Commun.* **10**, 5725 (2019).
43. Lund, J. et al. Transcriptional profile of aging in *C. elegans*. *Curr. Biol.* **12**, 1566–1573 (2002).
44. Mitchell, D. H., Stiles, J. W., Santelli, J. & Sanadi, D. R. Synchronous growth and aging of *Caenorhabditis elegans* in the presence of fluorodeoxyuridine1. *J. Gerontol.* **34**, 28–36 (1979).
45. Greer, E. L. & Brunet, A. Signaling networks in aging. *J. Cell Sci.* **121**, 407–412 (2008).
46. Kenyon, C. J. The genetics of ageing. *Nature* **464**, 504–512 (2010).
47. Avery, L. The genetics of feeding in *Caenorhabditis elegans*. *Genetics* **133**, 897–917 (1993).
48. Lakowski, B. & Hekimi, S. The genetics of caloric restriction in *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **95**, 13091–13096 (1998).
49. Kenyon, C., Chang, J., Gensch, E., Rudner, A. & Tabtiang, R. A. *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464 (1993).
50. Hajdu-Cronin, Y. M., Chen, W. J. & Sternberg, P. W. The L-type cyclin CYL-1 and the heat-shock-factor HSF-1 are required for heat-shock-induced protein expression in *Caenorhabditis elegans*. *Genetics* **168**, 1937–1949 (2004).
51. Ogg, S. et al. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* **389**, 994–999 (1997).
52. Lin, K., Dorman, J. B., Rodan, A. & Kenyon, C. daf-16: an HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* **278**, 1319–1322 (1997).
53. Henderson, S. T., Bonafè, M. & Johnson, T. E. daf-16 protects the nematode *Caenorhabditis elegans* during food deprivation. *J. Gerontology: Ser. A* **61**, 444–460 (2006).
54. Berman, J. R. & Kenyon, C. Germ-cell loss extends *C. elegans* life span through regulation of DAF-16 by *kri-1* and lipophilic-hormone signaling. *Cell* **124**, 1055–1068 (2006).
55. Greer, E. L. et al. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. *Curr. Biol.* **17**, 1646–1656 (2007).
56. Takahashi, Y. et al. Asymmetric arginine dimethylation determines life span in *C. elegans* by regulating forkhead transcription factor DAF-16. *Cell Metab.* **13**, 505–516 (2011).
57. Schreiber, M. A., Pierce-Shimomura, J. T., Chan, S., Parry, D. & McIntire, S. L. Manipulation of behavioral decline in *Caenorhabditis elegans* with the Rag GTPase raga-1. *PLoS Genet.* **6**, e1000972 (2010).
58. Arantes-Oliveira, N., Apfeld, J., Dillin, A. & Kenyon, C. Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. *Science* **295**, 502–505 (2002).
59. Kawasaki, I. et al. PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in *C. elegans*. *Cell* **94**, 635–645 (1998).
60. Zhang, L., Ward, J. D., Cheng, Z. & Dernburg, A. F. The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in *C. elegans*. *Development* **142**, 4374–4384 (2015).
61. Ashley, G. E. et al. An expanded auxin-inducible degron toolkit for *Caenorhabditis elegans*. *Genetics* **217**, iyab006 (2021).
62. Heiman, M. et al. A translational profiling approach for the molecular characterization of CNS cell types. *Cell* **135**, 738–748 (2008).
63. Nousch, M. RPL-4 and RPL-9-mediated ribosome purifications facilitate the efficient analysis of gene expression in *Caenorhabditis elegans* germ cells. *G3* **10**, 4063–4069 (2020).
64. Proshkin, S., Rahmouni, A. R., Mironov, A. & Nudler, E. Cooperation between translating ribosomes and RNA polymerase in transcription elongation. *Science* **328**, 504–508 (2010).
65. Burmann, B. M. et al. A NusE:NusG complex links transcription and translation. *Science* **328**, 501–504 (2010).
66. Bugaut, A. & Balasubramanian, S. 5′-UTR RNA G-quadruplexes: translation regulation and targeting. *Nucleic Acids Res.* **40**, 4727–4741 (2012).
67. Grayeski, P. J. et al. Global 5′-UTR RNA structure regulates translation of a SERPINA1 mRNA. *Nucleic Acids Res.* **50**, 9689–9704 (2022).
68. Motola, D. L. et al. Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans*. *Cell* **124**, 1209–1223 (2006).
69. Gerisch, B. et al. A bile acid-like steroid modulates Caenorhabditis elegans lifespan through nuclear receptor signaling. *Proc. Natl Acad. Sci. USA* **104**, 5014–5019 (2007).
70. Jia, K., Albert, P. S. & Riddle, D. L. DAF-9, a cytochrome P450 regulating *C. elegans* larval development and adult longevity. *Development* **129**, 221–231 (2002).
71. Luciani, G. M. et al. Dafadine inhibits DAF-9 to promote dauer formation and longevity of *Caenorhabditis elegans*. *Nat. Chem. Biol.* **7**, 891–893 (2011).
72. Sergiev, P. V., Aleksashin, N. A., Chugunova, A. A., Polikanov, Y. S. & Dontsova, O. A. Structural and evolutionary insights into ribosomal RNA methylation. *Nat. Chem. Biol.* **14**, 226–235 (2018).

73. Kaushik, S. & Cuervo, A. M. Proteostasis and aging. *Nat. Med.* **21**, 1406–1415 (2015).
74. Santra, M., Dill, K. A. & de Graff, A. M. R. Proteostasis collapse is a driver of cell aging and death. *Proc. Natl Acad. Sci. USA* **116**, 22173–22178 (2019).
75. Brehme, M. et al. A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep.* **9**, 1135–1150 (2014).
76. Walther, D. M. et al. Widespread proteome remodeling and aggregation in aging *C. elegans*. *Cell* **161**, 919–932 (2015).
77. Brenner, S., Jacob, F. & Meselson, M. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* **190**, 576–581 (1961).
78. McColl, G. et al. Insulin-like signaling determines survival during stress via posttranscriptional mechanisms in *C. elegans*. *Cell Metab.* **12**, 260–272 (2010).
79. Shenton, D. et al. Global translational responses to oxidative stress impact upon multiple levels of protein synthesis. *J. Biol. Chem.* **281**, 29011–29021 (2006).
80. Gerashchenko, M. V., Lobanov, A. V. & Gladyshev, V. N. Genome-wide ribosome profiling reveals complex translational regulation in response to oxidative stress. *Proc. Natl Acad. Sci. USA* **109**, 17394–17399 (2012).
81. Liu, K. et al. Regulation of translation by methylation multiplicity of 18S rRNA. *Cell Rep.* **34**, 108825 (2021).
82. Khodakarami, A., Saez, I., Mels, J. & Vilchez, D. Mediation of organismal aging and somatic proteostasis by the germline. *Front. Mol. Biosci.* **2**, 3 (2015).
83. Alcedo, J. & Kenyon, C. Regulation of *C. elegans* longevity by specific gustatory and olfactory neurons. *Neuron* **41**, 45–55 (2004).
84. Stein, K. C., Morales-Polanco, F., van der Lienden, J., Rainbolt, T. K. & Frydman, J. Ageing exacerbates ribosome pausing to disrupt cotranslational proteostasis. *Nature* **601**, 637–642 (2022).
85. Dillin, A. et al. Rates of behavior and aging specified by mitochondrial function during development. *Science* **298**, 2398–2401 (2002).
86. Curran, S. P. & Ruvkun, G. Lifespan regulation by evolutionarily conserved genes essential for viability. *PLoS Genet.* **3**, e56 (2007).
87. Chen, D., Pan, K. Z., Palter, J. E. & Kapahi, P. Longevity determined by developmental arrest genes in *Caenorhabditis elegans*. *Aging Cell* **6**, 525–533 (2007).
88. Mair, W., Goymer, P., Pletcher, S. D. & Partridge, L. Demography of dietary restriction and death in *Drosophila*. *Science* **301**, 1731–1733 (2003).
89. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
90. McCarthy, D. J., Chen, Y. & Smyth, G. K. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res.* **40**, 4288–4297 (2012).
91. Yu, G., Wang, L.-G., Han, Y. & He, Q.-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* **16**, 284–287 (2012).
92. Supek, F., Bošnjak, M., Škunca, N. & Šmuc, T. REVIGO Summarizes and visualizes long lists of gene ontology terms. *PLoS ONE* **6**, e21800 (2011).
93. Bailey, T. L. & Elkan, C. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **2**, 28–36 (1994).
94. Ge, S. X., Jung, D. & Yao, R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics* **36**, 2628–2629 (2020).
95. Rothi M. H. et al. The 18S rRNA methyltransferase DIMT-1 regulates lifespan in the germline later in life. *Zenodo* https://doi.org/10.5281/zenodo.15733649 (2025).

Acknowledgements

We thank J. Ward for advice about TIR1 strains. We thank the T.K. Blackwell lab for RNAi clones. We thank members of the Greer laboratory for discussions and feedback on the manuscript. This work was supported by NIH grants (DP2AG055947, R56AG076496, and R01AG084739) to E.L.G. and NIH grant T32 EB016652 to W.M.

Author contributions

E.L.G. conceived and planned the study. M.H.R. and E.L.G. wrote the paper. M.H.R. helped complete Fig. 1, generated Figs. 2, 3g, 4a, 4e, 5c, S2c-f, S5h, and performed replicate lifespan assays in Table S1. G.C.S. produced, isolated, and performed IPs of aged worms for ribosome sequencing, generated Figs. 4e, 5b, S1, S2b, S3c, S4, S5, and S6 and performed lifespan assays, J.A.H. produced Fig. 1, W.M. performed TRAP assay and ribosome sequencing analysis, generated Fig. 4F and S3A, and was advised by V.N.G., K.Y. helped conceive the project and optimize the UHPLC-ms/ms protocol and performed replicate lifespan assays, N.P. generated aging gradients for Fig. 2a and performed lifespan assays, R.S. performed replicate lifespan assays, J.N. helped produce Fig. 3c, S.D. helped M.H.R. isolate rRNAs and perform UHPLC-ms/ms, E.L.G. helped complete Fig. 1, generated Figs. 3a–f, 3h, i, 4b–d, 5d, e, 6, S2a, S3a, b. All authors discussed the results and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-025-62323-7.

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Peer review information *Nature Communications* thanks Martin Denzel; Markus Schosserer, who co-reviewed with Anja Wagner-Schrittwieser, and the other anonymous reviewer(s) for their contribution to the peer review of this work. A peer review file is available.