1 **The 18S rRNA Methyltransferase DIMT-1 Regulates Lifespan in the Germline Later in Life**

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Abstract: Ribosome heterogeneity has emerged as an important regulatory control feature for determining which proteins are synthesized, however, the influence of age on ribosome heterogeneity is not fully understood. Whether mRNA transcripts are selectively translated in young versus old cells 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 the 18S rRNA N6'-dimethyl adenosine ($m^{6,2}A$) methyltransferase, *dimt-1,* as a regulator of *C. elegans* lifespan and stress resistance. Lifespan extension induced by *dimt-1* deficiency required a functional germline and was dependent on the known regulator of protein translation, the Rag GTPase, *raga-1,* which links amino acid sensing to the mechanistic target of rapamycin complex (mTORC)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 knock-down 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 a new layer of proteome dysfunction, beyond protein synthesis and degradation, as an important regulator of aging. Our findings highlight a new role for ribosome heterogeneity, and specific rRNA modifications, in maintaining appropriate translation later in life to promote healthy aging.

dimt-1 rRNA methyltransferase knock-down 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[1–3], which has been shown to mask the effect of some genes on lifespan[4,5]. 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 knock-down of several rRNA methyltransferases could significantly shorten or extend *C. elegans* lifespan (**Fig. 1a**). As previously reported[6], knock-down of *T01C3.7/fib-1* extended *C. elegans* lifespan (~9% p<0.0005, **Fig. 1a**). FIB-1 is a homologue of the 2'-O-methyltransferase fibrillarin, which is conserved in animals and plants. We also found that knock-down 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*[7]. DIMT-1 is a conserved methyltransferase that methylates conserved adjacent adenosines on the 18S rRNA in yeast and humans[8–10].

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 knock-down 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[11], knock-down 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[12], knock-down 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

80   both UV stress (43.7% p<0.0001) and heat stress resistance (231.3% p<0.0001) (**Figs. 1b, c**). As
81   *dimt-1* knock-down caused the most significant lifespan extension (**Fig. 1a**), we focused
82   subsequent analyses on determining how DIMT-1 can regulate longevity. To determine whether
83   *dimt-1* was regulating longevity by altering protein homeostasis, we measured the levels of 3 stress
84   induced chaperone proteins important for stabilizing misfolded proteins, HSP-4[13,14], HSP-6[15,16],
85   and HSP-16.2[17,18]. These chaperones are indicators of misfolded protein loading in response to
86   endoplasmic reticulum (ER) stress, mitochondrial stress, and cytosolic stress, respectively. While
87   HSP-6 and HSP-16.2 showed no change upon *dimt-1* knock-down (Extended Data **Fig. 1b, c**),
88   HSP-4 had a significant reduction in expression levels when *dimt-1* was knocked down, indicating
89   that when levels of DIMT-1 are decreased, the level of misfolded protein loading in the
90   endoplasmic reticulum (ER) is also lowered, likely due to better protein turnover compared to
91   control (Extended Data **Fig. 1d**). To determine whether there is a better ER specific protein
92   turnover, we performed a proteotoxicity survival assay using the ER stress inducer tunicamycin
93   (TM), which block N-linked glycosylation. We found that *dimt-1* knock-down caused a significant
94   increase in tunicamycin survival relative to WT control worms (Extended Data **Fig. 1e**).

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

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99   **rRNA modifications are dynamically regulated throughout the lifespan**

100 Although we found that rRNA methyltransferases regulate lifespan and stress resistance, it was
101 still unclear whether rRNA modifications themselves change during the life of the organism.
102 Changes in specific modifications with age may indicate a regulatory role for rRNA methylation
103 events. We first wanted to see if the rRNA methyltransferase genes are dynamically expressed
104 during the life of *C. elegans.* Examination of previously published transcriptional profiles of *C.*
105 *elegans* during aging[19], revealed that a number of putative rRNA methyltransferases are
106 dynamically expressed with age (Extended Data **Fig. 2a**). There were genes which are strongly
107 expressed in early life and declined towards the end such as *ZK1128.2/mett-10, W01B11.3/nol-58,*
108 and *W07E6.1/nsun-1*. Alternatively, genes such as *E02H1.1/dimt-1* and *C18A3.1/damt-1* showed
109 the opposite trend, peaking near the end of the lifespan (Extended Data **Fig. 2a**). We independently
110 confirmed that *E02H1.1/dimt-1* expression increases as *C. elegans* age (Extended Data **Fig. 2b**)
111 suggesting that knock-down of *dimt-1* is reverting the worm to a more youthful state.

112 To test if the rRNA modifications themselves showed similar patterns during aging, we
113 performed ultra-high performance liquid chromatography coupled with mass spectrometry
114 (UHPLC-MS) with wildtype (WT) N2 worms harvested in an age gradient at days 4, 7, 10, 13, 16,
115 and 19. We measured rRNA modification levels in the 26S and 18S rRNA subunits in 4 biological
116 replicates. We observed a similarly dynamic pattern of change in rRNA modifications across
117 lifespan (**Fig. 2a**). 2'-O-ribose methylation increased on all four nucleosides (Am, Cm, Gm, Um)
118 as the worm gets older in both the 26S and 18S rRNAs (**Fig. 2a** and Extended Data **Fig. 2c**). Other
119 18S rRNA modifications such as m^6A and surprisingly, $m^{6,2}A$, showed the inverse trend with a
120 higher level in early life which declines as the worm ages (**Fig. 2a** and Extended Data **Fig. 2c**).
121 Since knock-down of the 18S rRNA $m^{6,2}A$ methyltransferase, *dimt-1,* increased *C. elegans* lifespan
122 we had hypothesized that $m^{6,2}A$ would have increased as *C. elegans* age. This finding could
123 suggest a dynamic and tissue specific change in 18S rRNA $m^{6,2}A$ which might be masked by

124 examination of changes in rRNA methylation across all tissues. These results suggest that both
125 rRNA methyltransferases and modifications are dynamically regulated throughout the lifespan of
126 *C. elegans.*

127

128 We had found that the lifespan extension phenotypes observed from the RNAi screen is
129 sometimes associated with increased stress resistance in survival assays (**Fig. 1**). We wanted to
130 determine if there were associated changes in rRNA methyltransferases and modifications when
131 *C. elegans* are exposed to stress. When we examined a previously published transcriptomic
132 dataset[11], we found that most rRNA methyltransferases increased expression in response to 37°C
133 heat stress (Extended Data **Fig. 2d**). We found that rRNA modifications in wildtype N2 worms
134 were less consistently dynamic in response to 37°C heat shock or UV stress exposure (**Fig. 2b,c**).
135 Some rRNA modifications did change in response to the stresses, for example, 2'-O-ribose
136 adenosine methylation (Am) in 26S rRNA was significantly increased and 2'-O-ribose cytosine
137 methylation (Cm) in 18S rRNA subunit was significantly decreased in response to heat stress
138 (Extended Data **Fig. 2e**). We also observed a significant increase in 2'-O-ribose guanosine
139 methylation (Gm) and Am in 26S rRNA in response to UV exposure (Extended Data **Fig. 2f**). As
140 with the changes in rRNA methylation during aging (**Fig. 2a**), lack of changes in specific rRNA
141 methylation in response to stress could reflect tissue specific changes, which change in opposite
142 directions in different tissues or an inability to capture the correct window to observe changes due
143 to dynamic rRNA modification changes after stress exposure. Nevertheless, taken together, these
144 results indicate that rRNA methyltransferases and rRNA modifications are dynamically regulated
145 during the lifespan of *C. elegans*, and can undergo changes in response to environmental stresses.
146 This suggests that rRNA modifications could be the regulatory changes that rRNA
147 methyltransferases induce to regulate longevity and stress resistance.

148

149 *dimt-1 catalytic activity is required for lifespan and stress resistance regulation*

150 Due to the paradoxical decrease in $m^{6,2}A$ in whole worms as *C. elegans* age when depletion
151 of *dimt-1,* the $m^{6,2}A$ methyltransferase[7], causes an extension in organismal lifespan we wished to
152 determine whether DIMT-1's regulation of lifespan and stress resistance was dependent on its
153 catalytic activity. We generated a catalytic inactive DIMT-1 by mutating the conserved glutamic
154 acid E79 to alanine that we had previously demonstrated was necessary for DIMT-1's catalytic
155 activity *in vitro*[7]. We found that *dimt-1* (E79A) mutant worms were viable but had no detectable
156 18S rRNA $m^{6,2}A$ (**Fig. 3a**). *dimt-1* (E79A) mutant worms displayed a significant increase in
157 lifespan (39.49% p<0.0001, **Fig. 3b**) and a significant increase in response to the ER stress inducer
158 tunicamycin (Extended Data **Fig. 1e**). Therefore, DIMT-1 methyltransferase activity is required
159 for lifespan and stress resistance regulation.

160

161 *dimt-1* **regulates lifespan through the DAF-16/FOXO and TOR pathways and requires the**
162 **germline**

163 Previous studies have elucidated various regulators of lifespan which are involved in pathways
164 such as insulin-signaling, heat shock response, and target of rapamycin (TOR)[20,21]. In order to
165 identify putative mechanisms by which DIMT-1 could regulate lifespan, we performed genetic

166 epistasis experiments by measuring lifespans of wildtype worms and mutants of specific longevity
167 pathways, grown on either empty vector control or *dimt-1* RNAi plates. We found that *dimt-1*
168 knock-down increased lifespan to a similar extent in mutants of the acetylcholine receptor, *eat-2,*
169 which has reduced pharyngeal pumping and therefore decreased food intake, and has been
170 proposed as a genetic mimic of dietary restriction[22,23] (23.65% in WT, 30.55% in *eat-2*; p=0.7021
171 by 2-way ANOVA, **Fig. 3c**). Similarly, *dimt-1* knock-down increased lifespan to a similar extent
172 in wildtype worms and in mutants of the insulin receptor, *daf-2*[24] (40.16% in WT, 33.98% in daf-
173 2; p= 0.0806 by 2-way ANOVA, **Fig. 3d**), mutants of the ubiquinone biosynthesis gene, *clk-1*[23]
174 (23.65% in WT, 35.2% in clk-1; p=0.0855 by 2-way ANOVA, Extended Data **Fig. 3a**), and in
175 mutants of the heat-shock response transcription factor, *hsf-1*[25,26] (10.72% in WT, 22.00% in *hsf-*
176 *1*; p=0.4245 by 2-way ANOVA, **Fig. 3e**).

177   Next, we tested if *daf-16,* which is a FOXO transcription factor that mediates longevity
178 regulation downstream of several signaling pathways[26–32], has an effect on *dimt-1* knock-down-
179 induced lifespan extension. We found that *dimt-1* knock-down extended wildtype worm lifespan
180 but failed to increase the lifespan of *daf-16* mutant *C. elegans* (p=0.0907), indicating that *daf-16*
181 is functioning in the same genetic signaling pathway as *dimt-1* (23.65% in WT, 5.13% in *daf-16*;
182 p<0.0001 by 2-way ANOVA, **Fig. 3f**). We also tested *raga-1*, which is a Rag GTPase that links
183 amino acid sensing to mechanistic target of rapamycin complex (mTORC)1, where *raga-1*
184 mutation causes an increase in lifespan[33]. Again *dimt-1* knock-down increased WT worm lifespan
185 but failed to increase the long lifespan of *raga-1* mutant worms (22.67% in WT, 0.53% in raga-1,
186 p=0.0327 by 2-way ANOVA, **Fig. 3g**), suggesting that DIMT-1 functions in the same signaling
187 pathway as RAGA-1 and TOR. To determine if an intact germline is necessary for *dimt-1* induced
188 lifespan extension, we performed *dimt-1* knock-down in *glp-1(e2141ts)* mutant worms, which
189 develop 5-15 meiotic germ cells instead of ~1,500 when shifted to the restrictive temperature[34]
190 and *pgl-1(bn101ts)* mutant worms, which have defective germ granules and are sterile[35]. Knock-
191 down of *dimt-1* extended the lifespan of WT worms but failed to increase the lifespan of either
192 *glp-1(e2141ts)* or *pgl-1(bn101ts)* mutant worms (28.32% or 40.16% in WT, -5.23% in *glp-1,*
193 3.34% in *pgl-1*; p<0.0001 by 2-way ANOVA, **Fig. 3h, i**), suggesting that a functional germline is
194 required for the *dimt-1* knock-down induced lifespan extension. Taken together, these results show
195 that *dimt-1* is likely affecting or regulating lifespan through the DAF-16 and TOR pathways and
196 either functions in or requires the germline for longevity regulation.

197

dimt-1 functions in the germline to regulate lifespan

199 To resolve the paradox of depletion of *dimt-1* extending lifespan while 18S rRNA m^{6,2}A increases
200 in whole worms with age (**Fig. 2a** and Extended Data **Fig. 2c**), we next wished to determine
201 whether DIMT-1 was functioning in specific tissues to regulate lifespan. To examine DIMT-1's
202 tissue specific function we created an auxin-inducible degron (AID) tagged DIMT-1 worm strain
203 and crossed it with strains that express TIR1 in a tissue-specific manner to allow for tissue specific
204 auxin-dependent depletion of DIMT-1[36,37]. We found that DIMT-1 depletion ubiquitously led to a
205 significant decrease in 18S rRNA m^{6,2}A relative to control strains as assessed by UHPLC-MS/MS,
206 suggesting that our AID-tagged DIMT-1 strains were effective (**Fig. 4a**). We found that when
207 DIMT-1 was depleted using two independent ubiquitous *eft-3* promoters or the germline specific
208 *mex-5* promoter to drive TIR1 expression we observed a significant lifespan extension relative to
209 control AID-tagged DIMT-1 strains that did not express TIR1 (29.71% p<0.0001 and 47.97%

210 p<0.0001 respectively, **Fig. 4b** and Extended Data **Fig. 3b**). In contrast, we observed no lifespan
211 extension when DIMT-1 was depleted in muscle, intestine, or neurons (**Fig. 4b**). This finding
212 suggests that DIMT-1 not only requires the presence of a functional germline to regulate lifespan
213 (**Fig. 3h, i**), but indeed functions in the germline to regulate lifespan.

214 To validate our findings from the AID/TIR1 experiments, we performed an orthogonal
215 approach by using tissue-specific RNAi strains to knock-down *dimt-1* and measure its effect on
216 lifespan. When we specifically knocked down *dimt-1* in muscle, intestine, or neurons, we failed to
217 observe a significant increase in lifespan; however, germline-specific knock-down of *dimt-1*
218 caused a significant increase in lifespan that was comparable to knock-down of *dimt-1* in WT
219 worms (16.93% in WT, 15.93% in germline-specific; p =0.8536 by 2-way ANOVA, **Fig. 4c, d**).
220 Further bolstering the importance of the germline for the longevity effects of *dimt-1*, we found that
221 treating worms with 5-fluorodeoxyuridine (FUdR), which inhibits proliferation of germline stem
222 cells, the production of intact eggs in adults, and extends longevity[4,38], abolished the effect of *dimt-*
223 *1* knock-down on lifespan (**Fig. 4e**).

224

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

226 We had previously found that knock-down of *dimt-1* in the parental generation caused a significant
227 misregulation of both transcription and ribosome binding to transcripts involved in the
228 determination of adult lifespan in the eggs of progeny[7]. To specifically examine which transcripts
229 displayed altered binding by ribosomes after a decrease in DIMT-1 at later stages of life in the
230 germline, we performed germline-specific Translating Ribosome Affinity Purification (TRAP)[39,40]
231 in four independent biological replicates from worms that were grown on empty vector (EV)
232 control or *dimt-1* RNAi. We first validated that *dimt-1* knock-down extended longevity in this
233 strain (Extended Data **Fig. 3c**). We next analyzed the transcription changes in response to *dimt-1*
234 knock-down in post reproduction worms. Transcriptional changes would not be predicted to be
235 direct consequences of manipulating the rRNA methylation, however, a natural consequence of
236 changes in translation will lead to changes in transcription[41,42]. We found that 5,765 genes were
237 differentially expressed, with 3,700 genes that were significantly upregulated and 2065 genes that
238 were significantly downregulated upon *dimt-1* depletion (Extended Data **Fig. 4a** and
239 Supplementary Table 3). Pathway analysis revealed increased expression of genes involved in
240 longevity regulation, xenobiotic detoxifications, TGF-β, WNT, and MAPK signaling pathways,
241 as well as degradation pathways including proteasome, peroxisome, and autophagy genes
242 (Extended Data **Fig. 4b, 4c**, and Supplementary Table 3). Downregulated genes were enriched for
243 protein processing, mTOR and FOXO signaling pathway, and ribosome biogenesis genes
244 (Extended Data **Fig. 4d, 4e**, and Supplementary Table 3).

245 These altered transcriptional pathways could help to explain the extended longevity in
246 response to *dimt-1* depletion, and the decreased expression of FOXO/DAF-16 and mTOR
247 signaling pathways expression could help explain the *daf-16* and *raga-1* dependency of the
248 lifespan extension (**Fig. 3f, g**). To determine if *dimt-1* depletion also altered the ribosome binding
249 levels of specific transcripts, we sequenced ribosome-bound RNAs from the same four biological
250 replicates isolated from the germline by TRAP and normalized that to the levels of the transcripts
251 to measure translation efficiency. We found that 2082 genes were differently bound to ribosomes,
252 with 1,666 genes that were significantly more bound and 416 genes that had significantly lower

ribosome binding (**Fig. 5a** and Supplementary Table 4). 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**, Extended Data **Fig. 5**, and Supplementary Table 4). 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.

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[43,44]. 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. 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.

Interestingly one of the transcripts which displayed decreased ribosome occupancy 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[45–48]. We therefore tested whether the DAF-9/DAF-12 signaling pathway could be responsible for DIMT-1 regulated lifespan, 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%, 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,

297 it was still unclear if the egg-laying phase is important for this phenotype, as we had previously
298 found that DIMT-1 depletion causes a reduction in fertility[7] and there is a well-known anti-
299 correlation between reproduction and longevity[34]. We therefore examined the lifespan of our
300 germline-specific TIR1 DIMT-1-AID tagged strain and introduced auxin at young adult, post egg-
301 laying and mid-life phases. Surprisingly, we observed significant lifespan extension in all three
302 stages compared to the untreated control (21.61%, 25.01%, and 18.93% respectively $p<0.0001$,
303 **Fig. 6d**). This suggests that DIMT-1 affects lifespan post-developmentally, after egg-laying is
304 complete, and in mid-life.

305

Discussion

307 Here, we show that DIMT-1, an evolutionarily conserved 18S rRNA $m^{6,2}A$
308 methyltransferase[7,9,10,49], regulates lifespan in *C. elegans*. Reduction of DIMT-1 causes a lifespan
309 extension that requires the DAF-16/FOXO transcription factor and TOR signaling. We found that
310 DIMT-1 functions in the germline to regulate *C. elegans* lifespan and also causes differential
311 binding of the ribosome to specific subsets of mRNA transcripts. One of the altered transcripts we
312 identified was *daf-9* and we found that reduction of DIMT-1 caused a lifespan extension that
313 required both the cytochrome P450 enzyme DAF-9 and its downstream nuclear hormone receptor
314 transcription factor DAF-12. These results suggest an overall model whereby DIMT-1 regulates
315 organismal lifespan by selective translation in the germline of specific mRNA transcripts which
316 subsequently leads to altered germline to soma signaling and lifespan. Furthermore, while most
317 longevity regulators identified to date function early in life to "lock-in" aging rates, we found that
318 DIMT-1 can regulate lifespan after middle age. Together, this study provides evidence of selective
319 translation, via ribosome heterogeneity, playing a significant role in the regulation of aging.

320   A major feature of aging is the loss of proteostasis, where maintenance of the appropriate
321 amounts of proteins in the cell, is significantly disrupted later in life[50,51]. Previous studies have
322 examined the levels of misfolded proteins which become increasingly prevalent in older
323 organisms, causing breakdown of normal cellular function[52,53]. However, little is known about how
324 transcripts are selected for translation during the aging process. Ribosomes were initially believed
325 to be non-discriminatory, translating any mRNA transcripts they were presented with[54], but recent
326 work has suggested that specialized ribosomes can translate unique sets of transcripts under
327 specific stress conditions[11,55–58]. We found that perturbations in many of the enzymes that regulate
328 rRNA modifications have substantial effects on overall health and duration of lifespan in *C.*
329 *elegans* (**Fig. 1**).

330   We[7], and others[59], have recently shown that DIMT-1's function to N6-dimethylate two
331 adjacent adenines on the 18S rRNA can direct the ribosome to specific subsets of transcripts for
332 translation. Our previous work suggests that while DIMT-1 binding to rRNAs during rRNA
333 processing is important for appropriate processing of rRNAs, the decreased expression of *dimt-1*
334 that we see in response to *dimt-1* knock-down is not sufficient to significantly alter rRNA
335 processing[7]. We also demonstrated that rRNAs are the predominant, if not the only, substrate of
336 DIMT-1[7]. Here we additionally demonstrate that a complete ablation of DIMT-1's catalytic
337 activity is not lethal and even leads to increased lifespan and stress resistance (**Fig. 3a, b** and
338 Extended Data **Fig. 1d**). These findings suggests that DIMT-1 is regulating lifespan through
339 altering ribosome specialization, rather than through changing available amounts of processed
340 rRNAs. In this work we found that reducing 18S rRNA $m^{6,2}A$ caused changes in ribosome binding

341 in a set of selective mRNAs that are mostly involve in longevity, metabolisms, cellular
342 detoxifications, protein homeostasis, degradation and recycling pathways specifically in the
343 germline after reproduction.

344 Regulation of aging has been shown to involve both germline and somatic tissues[34].
345 However, most findings related to the germline regulation of lifespan have been shown to extend
346 lifespan due to defects in germline development or reducing the energy diverted towards producing
347 the next generation of progeny[34,60]. We showed that DIMT-1 functions through the germline,
348 however, its role in lifespan extension takes place after development and reproduction (**Fig. 6**).
349 This indicates that DIMT-1 is amenable to therapies or manipulation once the developmental or
350 reproductive stage of an organism has passed.

351 It is interesting to note that global $m^{6,2}A$ levels decrease in whole worms as *C. elegans* age
352 (**Fig. 2a**). This finding runs counter to the observation that *dimt-1* levels increase as *C. elegans* age
353 (Extended Data **Fig. 2a, b**), and that depletion of *dimt-1* causes an increase in lifespan. Our
354 working hypothesis to explain this apparent paradox is that this could reflect that $m^{6,2}A$ increases
355 in some tissues or in some specific cells while it decreases in other tissues as organisms age. One
356 other potential explanation for this apparent paradox could be that $m^{6,2}A$ could be important in
357 responding to immediate environmental stresses in *C. elegans,* and rather than the absolute levels
358 of $m^{6,2}A$ at particular ages being important for regulating lifespan, this modifications capacity to
359 rapidly change could deteriorate with age. This notion is supported by the fact that $m^{6,2}A$ increases
360 in response to both UV stress and heat stress (**Fig. 2b, c**). It will be interesting in future experiments
361 to examine whether $m^{6,2}A$ levels increase specifically in the germline with age or if the capacity
362 of this rRNA modification to respond to stresses later in life is diminished.

363 Several recent studies have demonstrated that there are proteomic, epigenetic, and
364 epitranscriptomic changes associated with aging, and that enzymes which regulate these processes
365 can also regulate aging[4,12,61–65]. Most regulators of aging determine organismal lifespan at early
366 developmental stages[66–68], however, several exceptions, including dietary restriction[69] and this
367 study, can increase lifespan later in life. Pathways which may be manipulated later in life to extend
368 lifespan offer exciting possibilities to potentially address aging-associated diseases with
369 interventions. Thus, altering rRNA modifications late in life may represent a new approach for
370 addressing aging-related disorders and increasing health span. Nevertheless, our results provide
371 evidence of selective translation, via ribosome heterogeneity, playing a significant role in the
372 regulation of aging.

373

Methods:

Strains used

376 N2 Bristol strain was used as the WT background. Worms were grown on *dam⁻dcm⁻* on standard
377 nematode growth medium (NGM) plates in all experiments except for RNAi and auxin-inducible
378 degron experiments. TIR1 expressing strains CA1200, DV3801, DV3803, DV3805, JDW221, and
379 JDW225, the tissue specific RNAi strains DCL569, IGL1839, NR350, XE1474, the Flag tagged
380 RPL-4 strain for germline specific ribosome purifications EV484, *eat-2(ad1116), daf-16(mu86),*
381 *daf-2(e1370), hsf-1(sy441), clk-1(e2519), raga-1(ok386), glp-1(e2141ts), pgl-1(bn101ts), daf-*
382 *9(rh50),* and *daf-12(rh61rh412)* were obtained from the Caenorhabditis Genetics Center which is

383 supported by the NIH office of research infrastructure programs P40OD010440. The AID-tagged
384 DIMT-1 and the *dimt-1* E79A strains were generated by SunyBiotech. TIR1 strains were crossed
385 with the AID-tagged DIMT-1 strain to generate double homozygous strains. Each strain was
386 assayed by PCR to confirm the genotypes.

387

388 *Single-worm genotyping*

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

397

398 *Targeted RNAi screen*

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

405

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

407 NGM plates containing streptomycin (300 µg/ml) and 1-Napthaleneacetic acid (NAA, auxin,
408 150µM), were seeded with OP50-1 bacteria. Worms were grown on the auxin plates at specific
409 worm stages for specific lengths of time according to the experiment shown in the results.

410

411 *Ultra-High Performance Liquid Chromatography coupled with Mass Spectrometry (UHPLC-*
412 *MS/MS)*

413 Total RNA was extracted by addition of 1 ml TRIzol (Invitrogen) to 100 µl of packed worm pellet.
414 Six freeze-thaw cycles were performed in liquid nitrogen. RNA extraction was performed
415 according to the manufacturer's protocol (Invitrogen, TRIzol). To isolate 26S, 18S and 5.8/5S
416 rRNAs, total RNA was run on agarose electrophoresis gels to separate rRNAs. rRNA bands were
417 excised from the gel and purified with Zymoclean Gel RNA Recovery Kit (Zymo). For digestion
418 to nucleosides, 250 ng of RNA samples were digested at 37°C for 2 hours with Nucleoside
419 Digestion Mix (New England Biolabs, M069S). Digested RNA samples were diluted to 100 µl
420 with double-distilled water and filtered through 0.22 µm Millex Syringe Filters. 5 µl of filtered
421 solution was injected for LC-MS/MS analysis and analyzed using the Agilent 1290 UHPLC system
422 with C18 reversed-phase column (2.1 mm by 50 mm; 1.8 m) as in[7]. Mobile Phase A is composed

423 of water with 0.1% (v/v) formic acid, and mobile phase B is composed of acetonitrile with 0.1%
424 (v/v) formic acid. MS detection was performed using an Agilent 6470 triple quadrupole mass
425 spectrometer in positive electrospray ionization mode, and data was quantified in dynamic multiple
426 reaction monitoring mode by monitoring mass transition $268 \rightarrow 136$ for adenosine, $282 \rightarrow 136$ for
427 Am, $282 \rightarrow 150$ for m^6A, $296 \rightarrow 164$ for m^6_2A, $284 \rightarrow 152$ for guanine, $298 \rightarrow 152$ for Gm, 298
428 $\rightarrow 166$ for m^7G, $244.1 \rightarrow 112$ for cytosine, $258 \rightarrow 112$ for Cm, $245 \rightarrow 113$ for uracil, $259 \rightarrow 113$
429 for Um, $245 \rightarrow 125$ for PseudoU.

430

431 *Longevity assays*

432 Worm lifespan assays were performed at 20°C and 25°C. For longevity assays involving RNAi,
433 worm populations were synchronized by placing L1 worms on NGM RNAi plates. Resulting eggs
434 from the P^{-1} generation were transferred to new RNAi plates and hatching day for the P^0 generation
435 was counted as day 1 for all lifespan measurements. For longevity assay involving the auxin-
436 inducible degron system, worm populations were synchronized by placing eggs for the P^0
437 generation, on NGM plates seeded with OP50-1 bacteria either with or without 150µM auxin.
438 Worms were changed every other day to new plates to avoid confounding progeny and were scored
439 as dead or alive. Dead worms were scored if they did not respond to repeated prods with a platinum
440 pick. Worms were censored if they died from vulval bursting or crawled off the plate. Each life-
441 span assay used 90 worms on 3 plates (30 worms/plate). Data was plotted with Kaplan-Meier
442 survival curves and statistical significance was tested using log-rank (Mantel-Cox) tests. Life-span
443 assays were repeated at least once and showed similar trends in relative life-span effects.

444

445 *UV stress assays*

446 For survival assays involving RNAi, the P^0 generation was prepared as described in the longevity
447 assays. Hatched eggs were allowed to grow to L4 stage on 3 plates per condition with 30 worms
448 per plate (90 worms per assay). Worms were exposed to 0.8 Joules (J), then grown at 20°C,
449 assessed every 24 hours for survival, and scored as dead or alive as described in the longevity
450 assays. For UHPLC-MS/MS analysis, L4 worms were synchronized on NGM plates seeded with
451 OP50-1 bacteria and exposed to 0.8 J. Then, the worms were allowed to recover for either 1 or 2
452 hours at 20°C, before being processed as described for the UHPLC-MS/MS analysis.

453

454 *Heat stress*

455 For survival assays involving RNAi, the P^0 generation was prepared as described in the longevity
456 assays. Hatched eggs were allowed to grow to L4 stage on 3 plates per condition with 30 worms
457 per plate (90 worms per assay). Worms were grown at 37°C for 6-7 hours, then grown at 20°C,
458 assessed every 24 hours for survival, and scored as dead or alive as described in the longevity
459 assays. For UHPLC-MS/MS analysis, L4 worms were synchronized on NGM plates seeded with
460 OP50-1 bacteria and were grown at 37°C for 6-7 hours. Then the worms were allowed to recover
461 for either 1 or 2 hours at 20°C, before being processed as described for the UHPLC-MS/MS
462 analysis.

463

464 *Genetic epistasis*

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

468

469 *Transcriptomic analysis*

470 Transcriptomic analysis for the gene expression profiles of putative rRNA methyltransferases was
471 performed using a publicly available dataset[19].

472

473 *Translating Ribosome Affinity Purification (TRAP)*

474 Synchronized EV484 (*efIs155[Cbr-unc-119(+) + Pmex-5::rpl-4::FLAG::tbb-2 3'UTR] II*) worms
475 (germline-specific FLAG-tagged ribosomal protein *rpl-4*) were grown on NGM RNAi plates
476 seeded with overnight grown HT1115 bacteria containing empty vector (control RNAi) till young
477 adult stage. Young adult animals were than collected in M9 buffer and divided into two groups.
478 Half of the worms were put into the fresh empty vector control RNAi plate and other half into the
479 *dimt-1* RNAi plate for its knock-down (HT1115 bacteria containing a vector expressing double
480 stranded RNA for *dimt-1* gene). In the background, we have also checked the lifespan extension
481 phenotype of *dimt-1* using EV484 as a control experiment. Worms were grown at 20°C till the end
482 of their reproductive phase (7-day old worms). Each day the P^0 generation (parental) worms were
483 filtered through a 20-micron mesh to remove confounding progenies during the egg-laying phase.
484 On day 7, worms were washed thrice with M9 containing 1 mM cycloheximide (Sigma) and once
485 with Lysis Buffer I (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4,
486 150 mM KCl, 5 mM MgCl2, 0.5 mM DTT, 1 mM cycloheximide, Mini cOmplete Protease
487 Inhibitor). Then, flash-frozen worm pearls were made using liquid nitrogen and homogenized
488 using ice cold glass Dounce tissue homogenizer (around 30-40 strokes). Lysis Buffer II (Lysis
489 Buffer I containing 0.5% v/v NP-40 (Sigma), 0.4 U/μL RNasin (Promega), 10 mM ribonucleoside
490 vanadyl complex (RVC by NEB), 33 mM 1,2-diheptanoylsn-glycero-3-phosphocholine (DHPC
491 by Merck) and 1% w/v sodium deoxycholate (Sigma)) was added to the sample and incubated on
492 ice for 30 minutes. Samples were centrifuged for 12000 x rcf for 15 minutes at 4°C and the
493 supernatant was collected (around 2 ml). 200 μl clear supernatant was collected for total RNA
494 extraction (for mRNA sequencing). Remaining samples were added to washed Protein G coated
495 DynaBeads for pre-clearing, incubated for 1 hour at 4°C rotating. Then, samples were incubated
496 with 5 μL anti-FLAG antibody(1mg/ml)-coupled beads (F1804, sigma) for 2 hours at 4°C rotating
497 and beads were washed 4 times with Wash Buffer (10 mM HEPES pH 7.4, 350 mM KCl, 5 mM
498 MgCl2, 1% v/v NP-40). For RNA elution, the beads were incubated in 350 μL of RLT buffer
499 (Qiagen RNeasy Kit) including 1% v/v β-mercaptoethanol for 10 min at room temperature. The
500 eluate was separated from the beads and RNA was purified using RNeasy Plus Mini Kit
501 (QIAGEN) according to manufacturer's protocol.

502

503

504

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 (2×150bp) (reads trimmed to 2×100bp) to get 20M 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[70]. All subsequent steps were performed in R using WormBase gene IDs. After filtering of genes with low numbers of reads, the raw total mRNA-seq counts were normalized using edgeR[71]. 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[71] 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[72], heatmaps were generated using pheatmap package, and Revigo plots were generated using R code obtained from the Revigo webserver[73]. Sequence motif analysis for 5' UTR regions of identified mRNA transcripts was performed using the MEME suite (https://meme-suite.org/meme/tools/meme)[74].

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UHPLC-ms/ms, E.L.G. helped complete Fig. 1, generated Figs. 3a-d, 3f, 3g, 4b-d, 5, S1a, S1c,and
692 S2a-b. All authors discussed the results and commented on the manuscript.

703 **Fig. 1 | Ribosomal RNA methyltransferases regulate lifespan, heat and UV stress response.**
704 **a-c,** Directed RNAi screen of putative rRNA methyltransferases reveals changes in **a**) lifespan, **b**)
705 UV stress survival, and **c**) heat stress survival relative to empty vector control worms. * $p < 0.05$,
706 ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Some RNAi clones were not replicated (without error
707 bars) due to no effect being observed.

709 **Fig. 2 | Ribosomal RNA modifications in 26S and 18S are dynamically regulated throughout**
710 **life and change in response to UV and heat stress. a,** 26S and 18S rRNA extracted from *C.*
711 *elegans* at different ages from 4 days to 19 days reveal changes in rRNA methylation as assessed
712 by UHPLC-ms/ms. This heat map represents the relative changes of methylation in 4 biological
713 replicates. Changes in individual modifications can be seen in fig. S2C. **b-c**, 26S and 18S rRNA
714 extracted from *C. elegans* after UV stress (b) or 37°C heat stress (c) relative to worms not exposed
715 to UV (ctl) or grown at 20°C reveal some changes in rRNA methylation as assessed by UHPLC-
716 ms/ms. These heat maps represent the relative changes of methylation in 3 biological replicates.
717 Changes in individual modifications can be seen in Extended Data Fig. 2e-f.

719 **Fig. 3 | Longevity induced by *dimt-1* deficiency requires the FoxO and TOR signaling**
720 **pathways and requires an intact germline. a,** Mutation of glutamic acid 79 to an alanine (E79A)
721 in *dimt-1* caused a complete elimination of 18S rRNA $m^{6,2}A$ as assessed by UHPLC-ms/ms.
722 Statistics represent an unpaired t-test with Welch's correction. **b,** Mutation of E79A in *dimt-1*
723 caused lifespan extension relative to WT worms. **c,** *dimt-1* knock-down extends the lifespan of
724 both WT and *eat-2(ad1116)* mutant worm lifespan to a similar extent (p=0.7021 by 2-way
725 ANOVA). **d,** *dimt-1* knock-down extends the lifespan of both WT and *daf-2(e1370)* mutant worm
726 lifespan to a similar extent (p=0.0806 by 2-way ANOVA). **e**, *dimt-1* knock-down extends the
727 lifespan of both WT and *hsf-1(sy441)* mutant worm lifespan to a similar extent (p=0.4245 by 2-
728 way ANOVA). **f,** *dimt-1* knock-down extends the lifespan of WT but not *daf-16(mu86)* mutant
729 worm lifespan (p<0.0001 by 2-way ANOVA). **g,** *dimt-1* knock-down extends the lifespan of WT
730 but does not further extend the long lifespan of *raga-1(ok386)* mutant worms (p=0.0327 by 2-way
731 ANOVA). **h,** *dimt-1* knock-down 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 2-way ANOVA). **i,** *dimt-1* knock-down 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 2-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.

Fig. 4 | DIMT-1 functions in the germline to regulate lifespan and affects 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. **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 knock-down of *dimt-1* in the germline increases lifespan to a similar extent as in ubiquitous knock-down while knock-down of *dimt-1* in the muscle, intestine, or neurons has no significant effect on longevity. **e,** *dimt-1* knock-down does not extend the lifespan of worms treated with 5-fluorodeoxyuridine (FUdR), a drug that inhibits 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.

Fig. 5 | DIMT-1 affects translation of specific mRNA transcripts. a, Heat maps of the 2082 differentially ribosome bound transcripts in the ribosome after *dimt-1* knock-down 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* knock-down 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 Tables 3 and 4. **c,** Sequence motifs enriched in the 5' UTR of more bound mRNA transcripts after *dimt-1* knock-down . **d,** *dimt-1* knock-down extends the lifespan of WT but not *daf-9* mutant worms (p=0.004 by 2-way ANOVA). **e,** *dimt-1* knock-down extends the lifespan of WT but not *daf-12* mutant worms (p=0.0005 by 2-way ANOVA).

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. **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 5. ns; not-significant, *; $p < 0.05$, ***; $p < 0.001$, ****; $p < 0.0001$ as calculated by log-rank (mantel-cox) statistical test.

Extended Data Fig. 1. DIMT-1 knock-down affects endoplasmic reticulum unfolding protein response (UPR^{ER}). a, No difference in HSP-16.2 expression in response to *dimt-1* knock-down as assessed by GFP fluorescence 5 hours after 30 minutes of heat shock at 37°C in *Phsp-16.2::gfp* (zSi3000) (UPR^{cytosol}) worms. **b**, No difference in HSP-6 expression in response to *dimt-1* knock-down as assessed by GFP fluorescence after 5 hours of ethidium bromide (25 μg/mL) treatment in *Phsp-6::gfp* (zcIs13) (UPR^{mitochondria}) worms. **c**, Decrease in HSP-4 expression in response to *dimt-1* knock-down as assessed by GFP fluorescence after 5 hours of tunicamycin (5 μg/mL) treatment in *Phsp-4::gfp(zcIs4)* (UPR^{ER}) worms. All quantification was done using ImageJ software. Statistics represent unpaired t-test with Welch's correction. ns not significant, ** $p < 0.01$, **d**, Knock-down of *dimt-1* or mutation of the catalytic domain (E79A) caused increased survival on the ER stress inducer tunicamycin.

Extended Data Fig. 2. Ribosomal RNA methyltransferases and modifications are dynamically regulated throughout life and change in response to heat stress. a, Gene expression profile of putative rRNA methyltransferases in *C. elegans* from day 4 to day 19 age gradient. This data was generated by an analysis of gene expression data from[19]. **b**, *dimt-1* transcript levels increased with age relative to actin control as assessed by quantitative RT PCR. **c**, UHPLC-ms/ms analysis of rRNA modification levels in 26S and 18S rRNA subunits in an age gradient. Experiments were performed with 4 biological replicates. **d**, Gene expression profile of putative rRNA methyltransferases in *C. elegans* exposed to heat stress compared to control. This data was generated by an analysis of gene expression data from[7]. **e**, UHPLC-ms/ms analysis of rRNA modification levels in 26S and 18S rRNA subunits in worms exposed to heat stress compared to control. Experiments were performed with 4 biological replicates. **f**, UHPLC-ms/ms analysis of rRNA modification levels in 26S and 18S rRNA subunits in worms exposed to UV stress compared to control. Experiments were performed with 4 biological replicates. ns not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Extended Data Fig. 3. Lifespan extension by DIMT-1 does not require *clk-1* and occurs in the germline. a, *dimt-1* knock-down increases in WT and *clk-1(e2519)* mutant worm lifespan to a similar extent (p=0.0855 by 2-way ANOVA). **b**, AID-induced DIMT-1 depletion extends lifespan in two different ubiquitous driver strains and when depleted specifically in the germline. **c**, *dimt-1* knock-down increases lifespan of EV484 mutant worms. Statistics and replicate experiments are presented in Supplementary Tables 1 and 2. ns; not-significant, *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$, ****; $p < 0.0001$ as calculated by log-rank (mantel-cox) statistical test.
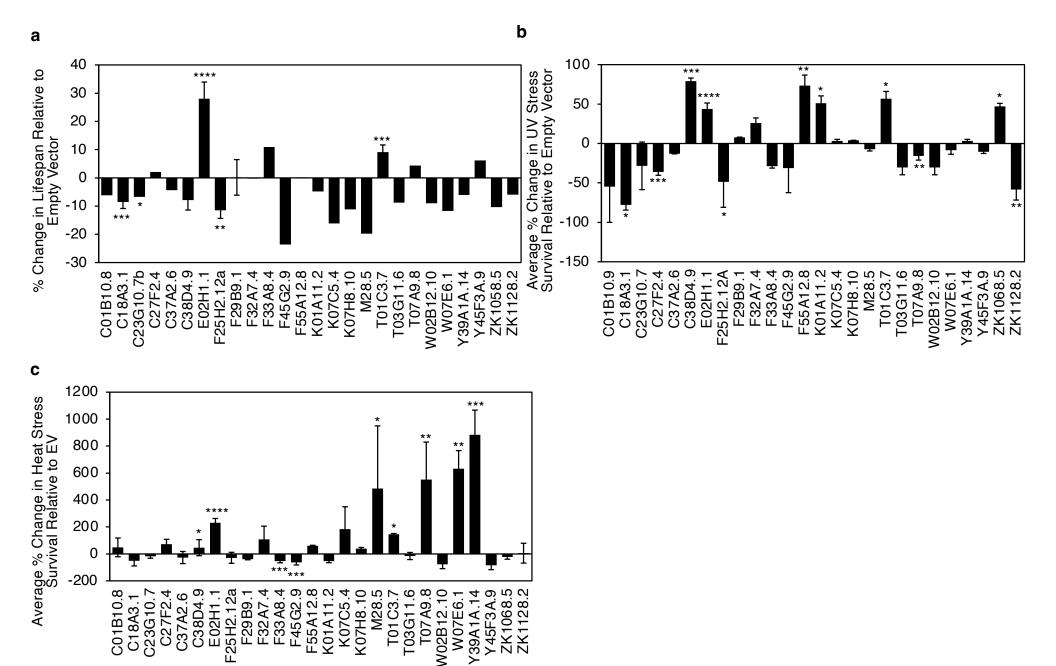
Extended Data Fig. 4. *dimt-1* **depletion causes a misregulation of expression of genes involved in longevity regulation, MTOR and FoxO/DAF-16 signaling and degradation pathways. a,** A heat map of 5,765 differentially transcribed genes in response to *dimt-1* knock-down in day 7 worms. *dimt-1* was knocked down from the L4 stage until day 7. Each column represents an independent biological replicate. **b-c,** Pathway analysis of genes which show increased expression in response to *dimt-1* knock-down reveal genes involved in longevity regulation, TGF-β, WNT and MAPK signaling pathways, as well as degradation pathways including proteasome, peroxisome, and autophagy genes. **d-e,** Pathway analysis of genes which showed decreased expression in response to *dimt-1* knock-down reveal genes involved in protein processing, MTOR and FoxO signaling pathway, and ribosome biogenesis genes. Gene details and gene ontology pathways is presented in Supplementary Table 3.

Extended Data Fig. 5. *dimt-1* **depletion causes altered binding of the ribosome to transcripts involved in longevity regulation, oxidative phosphorylation, degradation pathways, and glutathione metabolism in the germline of day 7 worms. a,** Pathway analysis of 2082 differentially bound transcripts in the germline of day 7 worms treated with *dimt-1* RNAi from the L4 stage until day 7 reveals transcripts involved in longevity regulation, oxidative phosphorylation, degradation pathways and glutathione metabolism. **b-c,** Pathway analysis of transcripts that show increased binding in response to *dimt-1* depletion include genes involved in longevity regulation, fatty acid metabolism, glutathione metabolism, metabolism, and various degradation pathways. **d-e,** Pathway analysis of transcripts that show decreased binding in response to *dimt-1* depletion in the germline include genes involved in the ribosome, proteasome, oxidative phosphorylation and longevity regulation. Gene details and gene ontology pathways is presented in Supplementary Table 4.

Supplementary Table 1. Dimt-1 depletion extends lifespan in a raga-1 and germline dependent manner The figure panels in which specific experiments are shown or used are indicated in the right column. The mean lifespan and SD values were calculated by Prism from triplicate samples of 30 worms each (90 worms total). # worms: number of observed dead worms at the end of the experiment/number of alive worms at the beginning of the experiment. The difference between both numbers corresponds to the number of censored worms (worms that underwent "matricide", exhibited ruptured vulva, or crawled off the plates). P values are calculated by log rank (Mantel-Cox) statistical test.

Supplementary Table 2. DIMT-1 functions in the germline to regulate lifespan The figure panels in which specific experiments are shown or used are indicated in the right column. The mean lifespan and SD values were calculated by Prism from triplicate samples of 30 worms each (90 worms total). # worms: number of observed dead worms at the end of the experiment/number of alive worms at the beginning of the experiment. The difference between both numbers corresponds to the number of censored worms (worms that underwent "matricide", exhibited ruptured vulva, or crawled off the plates). P values are calculated by log rank (Mantel-Cox) statistical test.

Supplementary Table 5. DIMT-1 functions after mid-life to regulate lifespan The figure panels in which specific experiments are shown or used are indicated in the right column. The mean lifespan and SD values were calculated by Prism from triplicate samples of 30 worms each (90 worms total). # worms: number of observed dead worms at the end of the experiment/number of alive worms at the beginning of the experiment. The difference between both numbers corresponds to the number of censored worms (worms that underwent "matricide", exhibited ruptured vulva, or crawled off the plates). P values are calculated by log rank (Mantel-Cox) statistical test.

Rothi MH, Sarkar GC, Al Hadad J et al Figure 1

a

26S rRNA

Am, Gm, Cm, Um, m³C,m⁵C, m¹A, m⁶A, PseudoU

18S rRNA

Am, Gm, Cm, Um, m³C,m⁵C, m¹A, m⁶A, m⁶,²A, m⁷G, PseudoU

Replicate: 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
Age (days): 4 7 10 13 16 19

b

26S rRNA

Am, Gm, Cm, Um, m³C,m⁵C, m¹A, m⁶A, PseudoU

18S rRNA

Am, Gm, Cm, Um, m³C,m⁵C, m¹A, m⁶A, m⁶,²A, m⁷G, PseudoU

Replicate: 1 2 3 1 2 3
Ctl UV

c

26S rRNA

Am, Gm, Cm, Um, m³C,m⁵C, m¹A, m⁶A, PseudoU

18S rRNA

Am, Gm, Cm, Um, m³C,m⁵C, m¹A, m⁶A, m⁶,²A, m⁷G, PseudoU

Replicate: 1 2 3 1 2 3
20°C 37°C

Normalized Signal

0   0.5   1

Rothi MH, Sarkar GC , Al Hadad J et al Figure 2

a

m^{6,2}A/A

0.0025
0.0020
0.0015
0.0010
0.0005
0.0000

*

18S rRNA
dimt-1::AID;P*eft-3*::TIR1

Control   Auxin

b

Percent Alive

100
80
60
40
20
0

No TIR1
Ubiquitous TIR1 P*eft-3* +29.71% ****
Muscle TIR1 P*unc-54*
Intestine TIR1 P*ges-1*
Neuronal TIR1 P*rgef-1*
Germline TIR1 P*mex-5* +47.97% ****

Age (Days)
0   10   20   30   40

c

Percent Alive

100
80
60
40
20
0

WT EV
WT *dimt-1*
Muscle P*hlh-1::rde-1* EV
Muscle P*hlh-1::rde-1 dimt-1*
Intestine P*mtl-2::rde-1* EV
Intestine P*mtl-2::rde-1 dimt-1*

29.01% ****
-2.17% ns
4% ns

Age (Days)
0   10   20   30

d

Percent Alive

100
80
60
40
20
0

WT EV
WT *dimt-1*
Germline P*sun-1::rde-1* EV
Germline P*sun-1::rde-1 dimt-1*
Neuronal P*dat-1::rde-1* EV
Neuronal P*dat-1::rde-1 dimt-1*

16.39% *
15.93% ***
2.71% ns

Age (Days)
0   10   20   30

e

Percent Alive

100
80
60
40
20
0

FUdR
EV
dimt-1 -3.33% ns

Age (Days)
0   10   20

Rothi MH, Sarkar GC, Al Hadad J et al Figure 4

a Translation efficiency

EV   *dimt-1*

2082 genes

b Differentially bound transcripts after *dimt-1* KD

Lysosome
Metabolism of xenobiotics by cytochrome P450
Glutathione metabolism
Pyruvate metabolism
Proteasome
Drug metabolism
Propanoate metabolism
Oxidative phosphorylation
Sphingolipid metabolism
Ribosome
Fatty acid metabolism
Lysine degradation
Phagosome
Fatty acid degradation
Glycolysis/Gluconeogenesis
Longevity regulating pathway
Autophagy
Peroxisome
Carbon metabolism
Metabolic pathways

Fold Enrichment

Genes
● 25
● 50
● 75
● 100
● 125

-log10(FDR)
5
10
15
20

c

d

22.66% ****
-3.84% ns

— WT EV
-·- WT *dimt-1*
— *daf-9* EV
-·- *daf-9 dimt-1*

Percent Alive
Age (Days)

e

17.02% ****
-1.13% ns

— WT EV
-·- WT *dimt-1*
— *daf-12 EV*
-·- *daf-12 dimt-1*

Percent Alive
Age (days)

Rothi MH, Sarkar GC, Al Hadad J et al Figure 5

a

Parental generation | Assayed generation

# of Days: 2 * 3 4 4

Control:
P-1:
Birth:
Birth->y.a.:
y.a.:
Post egg lay:
Mid-life:

150 µM Auxin: →
NGM plates: →
*: Birth of assayed generation

b

Germline TIR1
P*mex-5*
Control
P-1
Birth
Birth->y.a.
y.a.

52.19% ****
41.33% ****
0.48% ns
38.17% ****

Percent Alive
Age (Days)

c

Ubiquitous TIR1
P*eft-3*
Control
P-1
Birth
Birth->y.a.
y.a.

42.45% ****
30.47% ****
10.15% *
35.45% ****

Percent Alive
Age (Days)

d

Germline TIR1
P*mex-5*
Control
young adult
Post egg lay
Mid-life

21.61% ****
25.01% ****
18.93% ****

Percent Alive
Age (Days)

Rothi MH, Sarkar GC, Al Hadad J et al Figure 6