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Supplemental information

18S rRNA methyltransferases DIMT1 and BUD23 drive intergenerational hormesis

Noa Liberman, M. Hafiz Rothi, Maxim V. Gerashchenko, Christiane Zorbas, Konstantinos Boulias, Fiona G. MacWhinnie, Albert Kejun Ying, Anya Flood Taylor, Joseph Al Haddad, Hiroki Shibuya, Lara Roach, Anna Dong, Scarlett Dellacona, Denis L.J. Lafontaine, Vadim N. Gladyshev, and Eric Lieberman Greer

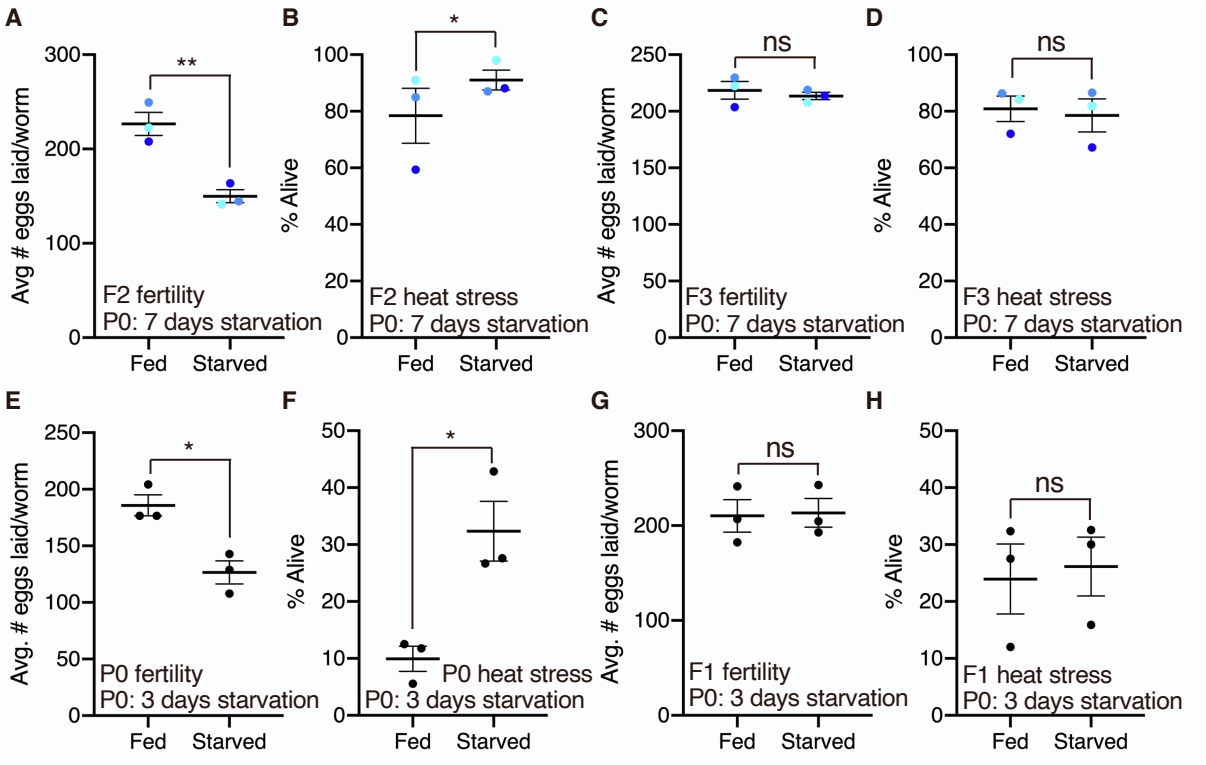


Fig. S1 Starvation induces transgenerational reduced fertility and increased heat stress resistance that revert in the F3 generation, related to Figure 1.

A, Naïve F2 progeny whose grandparents were starved for seven days have reduced fertility relative to progeny whose grandparents were fed. Each column represents the mean ± SEM of 3 independent experiments performed in three plates with 10 worms per plate. Dots are color coded to display matched independent experiments. * $p<0.05$ as assessed by unpaired t test. **B,** Naïve F2 progeny whose grandparents were starved for seven days display an increase in survival in response to 37ºC heat stress for 6 hours relative to progeny whose grandparents were fed. Each column represents the mean ± SEM of 3 independent experiments performed in three plates with 30 worms per plate. Dots are color coded to display matched independent experiments. * $p<0.05$ as assessed by two-way ANOVA. **C,** Naïve F3 progeny whose great grandparents were starved for seven days have similar fertility relative to progeny whose great grandparents were fed. Each column represents the mean ± SEM of 3 independent experiments performed in three plates with 10 worms per plate. Dots are color coded to display matched independent experiments. ns, not significant as assessed by unpaired t test. **D,** Naïve F3 progeny whose great grandparents were starved for seven days do not display an increase in survival in response to 37ºC heat stress for 6 hours relative to progeny whose great grandparents were fed. Each column represents the mean ± SEM of 3 independent experiments performed in three plates with 30 worms per plate. Dots are color coded to display matched independent experiments. ns, not significant as assessed by two-way ANOVA. **E,** Starvation for three days causes a reduction in reproduction. Each column represents the mean ± SD of three plates with 10 worms per plate. * $p<0.05$ as assessed by paired t test. **F,** Starvation for three days causes an increase in survival in response to 37ºC heat stress for 6.5 hours. Each column represents the mean ± SD of three plates with 30 worms per plate. * $p<0.05$ as assessed by paired t test. **G,** Naïve F1 progeny whose parents were starved for three days have similar reproduction relative to progeny whose

parents were fed. Each column represents the mean ± SD of three plates with 10 worms per plate. ns, not significant as assessed by unpaired t test. **H,** Naïve F1 progeny whose parents were starved for three days display similar survival in response to 37ºC heat stress for 6 hours. Each column represents the mean ± SD of three plates with 30 worms per plate. ** $p<0.01$ as assessed by paired t test. ns, not significant as assessed by unpaired t test.

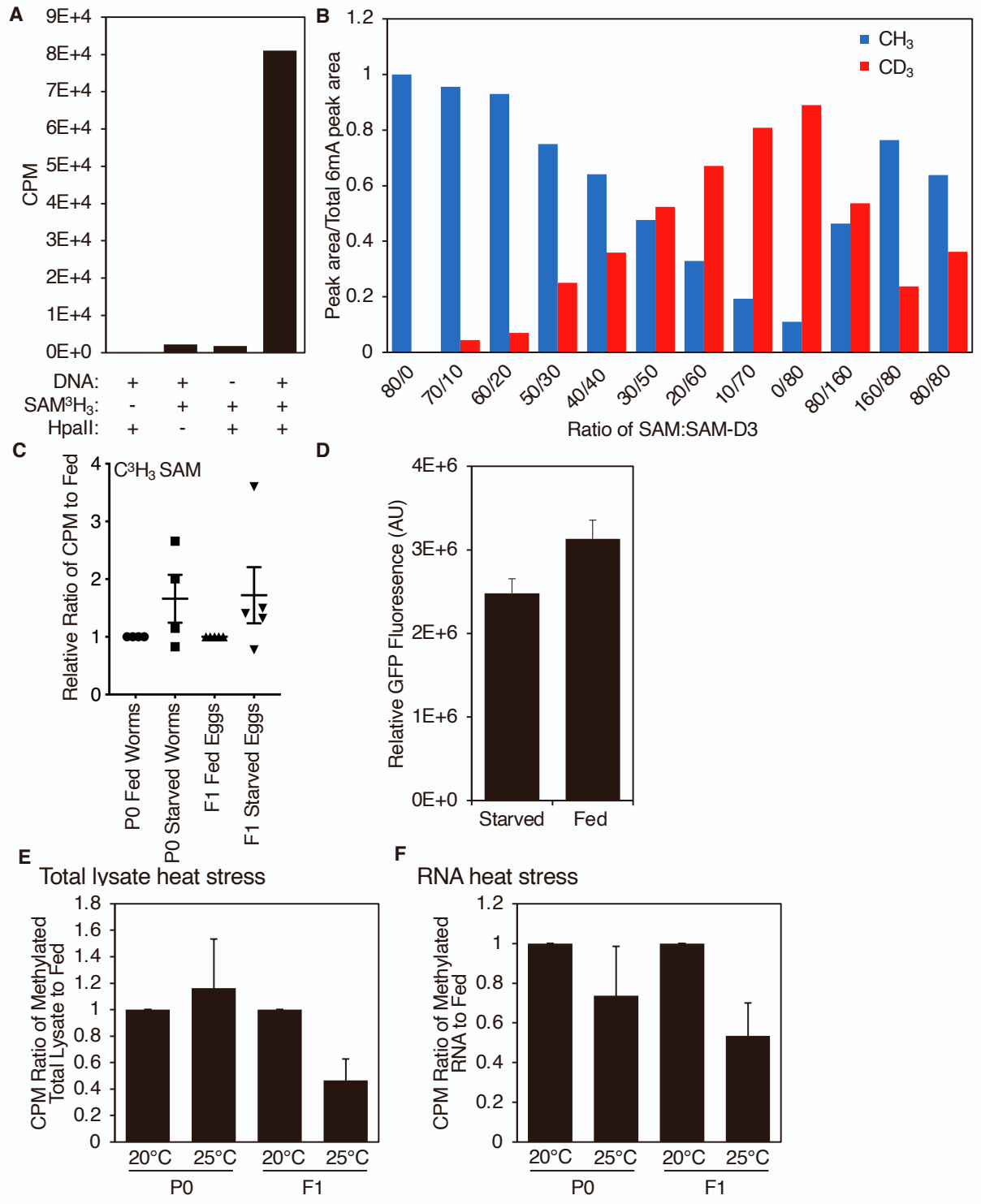


Fig. S2 Deuterated and tritiated SAM can be utilized by enzymes with ~equal activity, starved descendants eat similar amounts of OP50 to fed descendants, and heat stress causes no consistent heritable change in methylation, related to Figure 2.

A, The C5-cytosine methyltransferase HpaII can utilize tritiated SAM to methylate DNA as detected by scintillation counting. **B,** The DNA adenine methylase *dam* can utilize deuterated SAM to methylate DNA as assessed by UHPLC-ms/ms. As increasing concentrations of

deuterated SAM were incubated with *dam* and DNA there was an increased relative incorporation of deuterated N6-methyladenosine relative to hydrogen methyl groups. SAM was used more efficiently than deuterated SAM. The numbers in the x axis represent the relative amount of [SAM] to [SAM-D_3]. **C,** Increased radioactive signal is detected in the RNA of both the P0 worms as well as their naïve F1 progeny when the P0 generation is starved relative to fed P0 worms and F1 progeny when fed SAM-C^3H_3. Each column represents the mean ± SEM of 4 or 5 independent experiments. **D,** Starved worms do not consume more food than fed worms after recovering on food for 2 days as assessed by GFP fluorescence in the intestine of worms fed OP50 expressing GFP. **E,** After a 25ºC heat stress there was no increase in radioactive methyl groups incorporated into P0 parental worms or their F1 naïve descendants as assessed by scintillation counting of total lysate using tritiated SAM as the methyl donor. **F,** After a 25ºC heat stress there was no increase in radioactive methyl groups incorporated into RNA of P0 parental worms or their F1 naïve descendants as assessed by scintillation counting using tritiated SAM as the methyl donor.

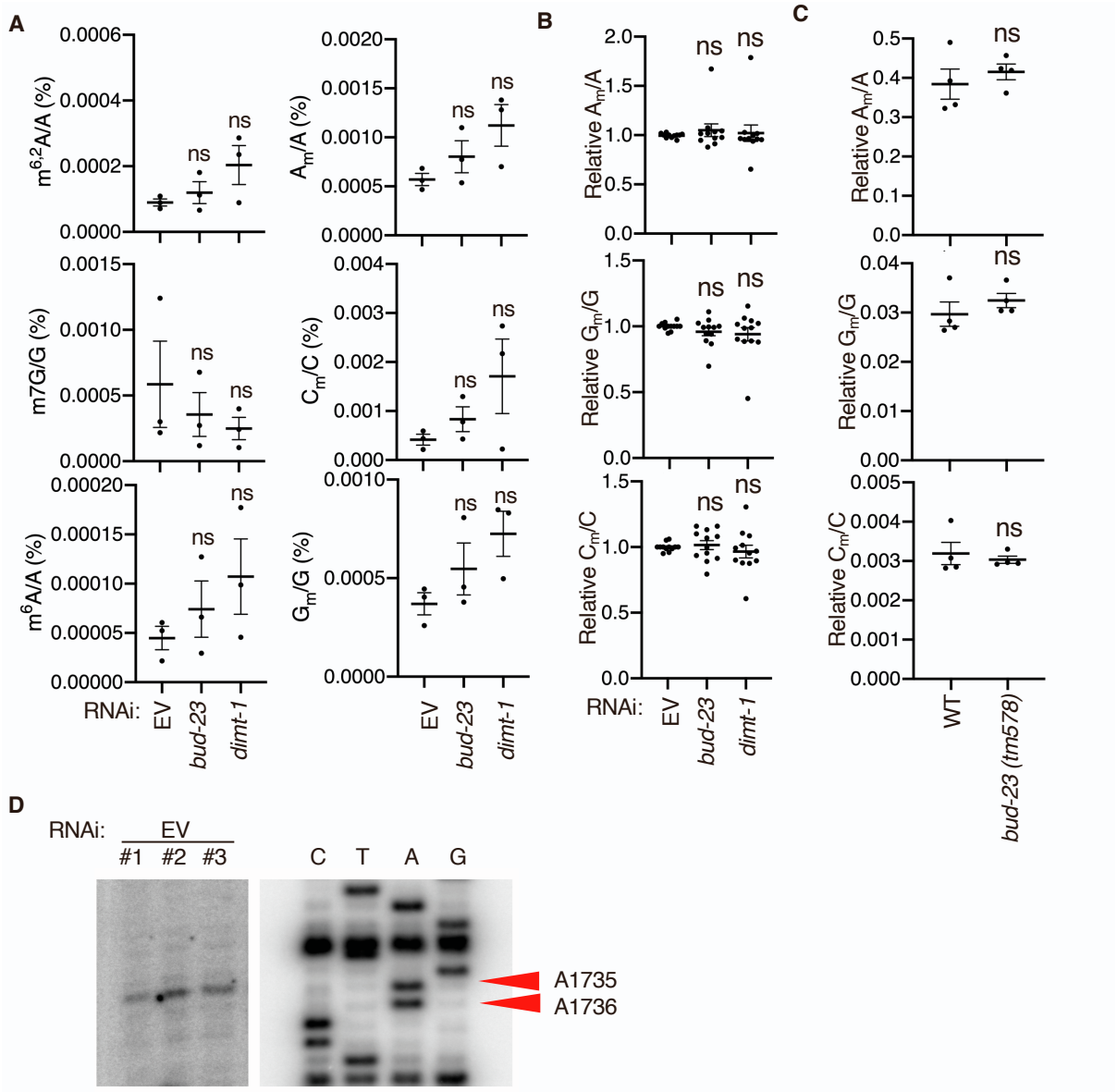


Fig. S3 There is no change in 2'O methylation modifications on the 18S rRNA after deletion of *bud-23* or knock-down of *bud-23* or *dimt-1*, related to Figure 3.
A, Knock-down of *dimt-1* or *bud-23* caused no change in mRNA methylation at $m^{6,2}A$, m^7G, m^6A, A_m, C_m, or G_m relative to empty vector (EV) control knock-down as assessed by UHPLC-ms/ms. **B,** Knock-down of *dimt-1* or *bud-23* caused no change in A_m/A, G_m/G, or C_m/C levels on 18S rRNA relative to empty vector (EV) control knock-down as assessed by UHPLC-ms/ms. Each bar represents the mean ± SEM of 12 independent replicates. ns, not significant as assessed by one-way ANOVA with Dunnett's multiple comparison test. **C,** *bud-23(tm5768)* mutant strain displays no change in A_m/A, G_m/G, or C_m/C levels on 18S rRNA relative to WT control worms as assessed by UHPLC-ms/ms. Each bar represents the mean ± SEM of 4 independent experiments. ns, not significant as assessed by paired t test. **D,** Primer extension assays reveals that A1735 is modified on *C. elegans* 18S rRNA. Each lane represents and independent replicate sample on 5 µg of total RNA extracted from worms.

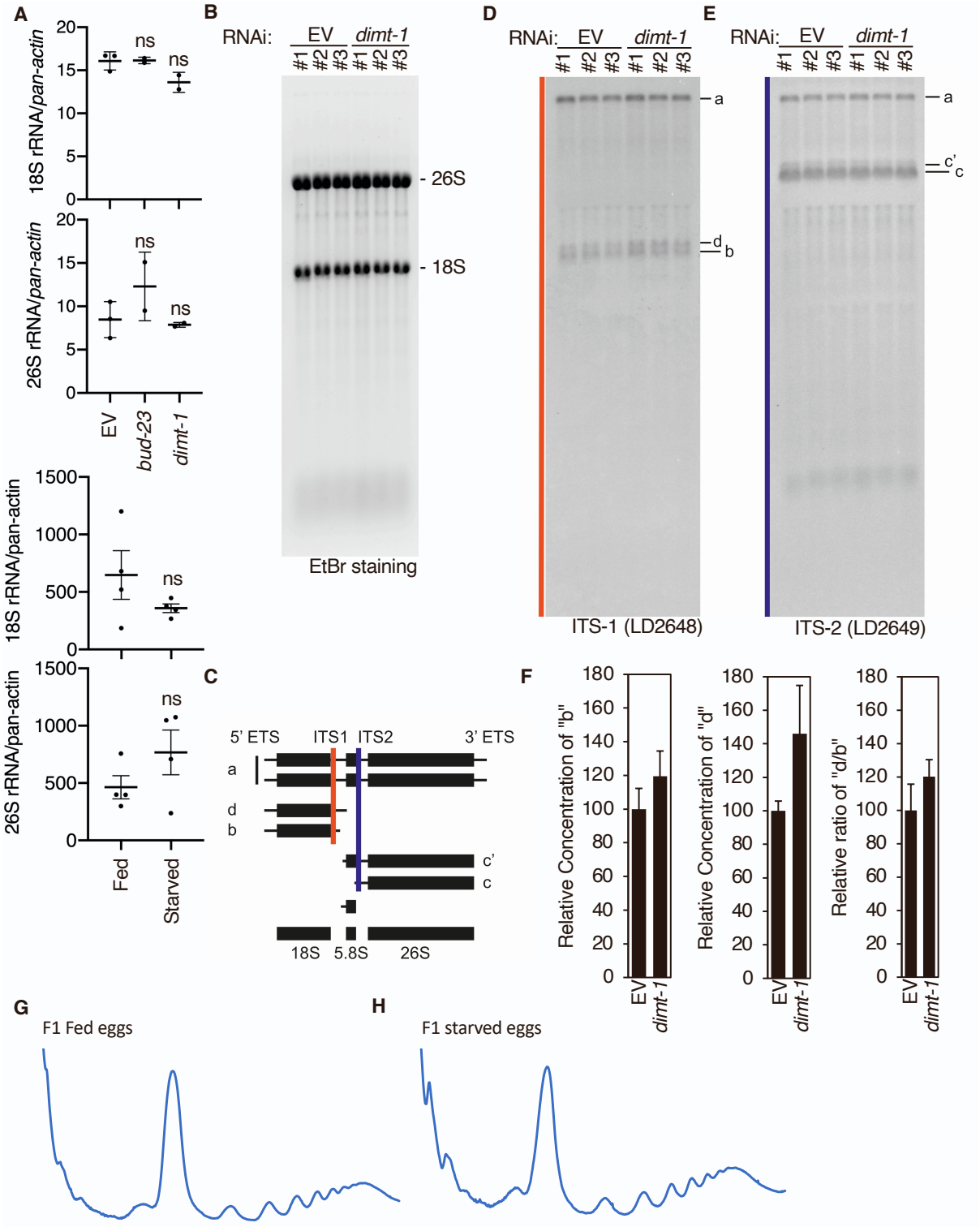
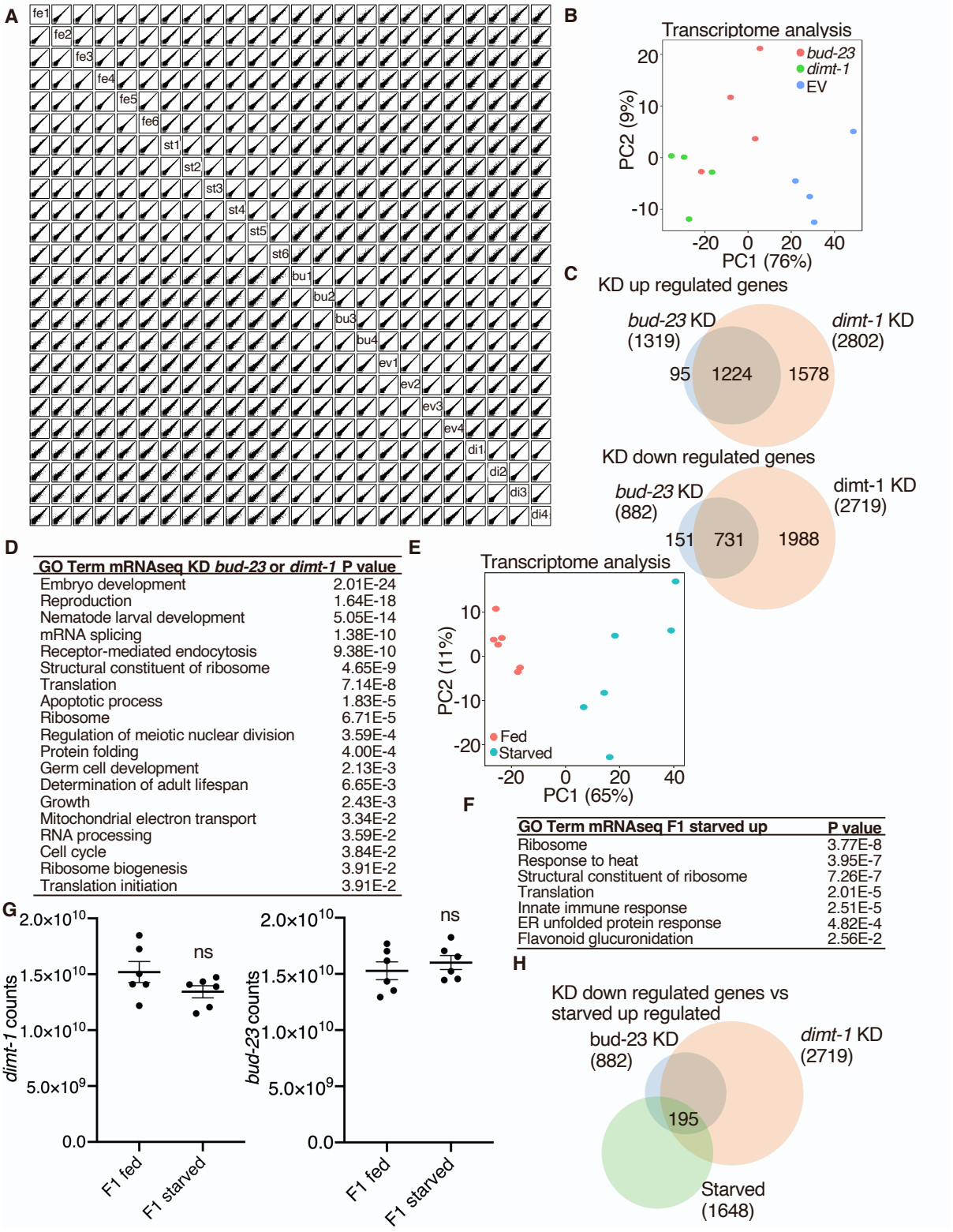


Fig. S4 *dimt-1* knock-down has no significant effect on pre-rRNA processing and F1 eggs from fed and starved parents have similar polysome profiles, related to Figure 4.
A, Knock-down of *bud-23* or *dimt-1* (top) or parental starvation (bottom) had no effect on amounts of mature 18S or 26S rRNA, as assessed by real time RT PCR. Each column represents the mean ± SD of 2-4 independent replicates of 10-60,000 eggs. ns, not significant as assessed by

one way ANOVA (left) or t test (right). **B,** Ethidium bromide staining of RNA extracted from empty vector (EV) control or *dimt-1* knock-down treated worms shows no dramatic difference in steady state rRNA. **C-F,** *dimt-1* knock-down caused a subtle change in the pathway of synthesis of 18S rRNA (seen as increased amounts of the "d" and "b" precursors, and "d/b ratio", (**D**) without changes in the pathway of synthesis of 26S rRNA (**E**) as assessed by northern blotting with probes directed against different portions of the pre-ribosomal RNAs (**C**). **G-H**, Polysome profiles of descendants from (**G**) fed parents were indistinguishable from polysome profiles of descendants from (**H**) starved parents. This graph is a representative experiment where UV absorbance at OD_{254} (optical density at 254 nm) is monitored continuously.


A
fe1
fe2
fe3
fe4
fe5
fe6
st1
st2
st3
st4
st5
st6
bu1
bu2
bu3
bu4
ev1
ev2
ev3
ev4
di1
di2
di3
di4
B
Transcriptome analysis
bud-23
dimt-1
EV
PC2 (9%)
PC1 (76%)
C
KD up regulated genes
bud-23 KD (1319)
dimt-1 KD (2802)
95
1224
1578
KD down regulated genes
bud-23 KD (882)
dimt-1 KD (2719)
151
731
1988
D
GO Term mRNAseq KD bud-23 or dimt-1 P value
Embryo development 2.01E-24
Reproduction 1.64E-18
Nematode larval development 5.05E-14
mRNA splicing 1.38E-10
Receptor-mediated endocytosis 9.38E-10
Structural constituent of ribosome 4.65E-9
Translation 7.14E-8
Apoptotic process 1.83E-5
Ribosome 6.71E-5
Regulation of meiotic nuclear division 3.59E-4
Protein folding 4.00E-4
Germ cell development 2.13E-3
Determination of adult lifespan 6.65E-3
Growth 2.43E-3
Mitochondrial electron transport 3.34E-2
RNA processing 3.59E-2
Cell cycle 3.84E-2
Ribosome biogenesis 3.91E-2
Translation initiation 3.91E-2
E
Transcriptome analysis
Fed
Starved
PC2 (11%)
PC1 (65%)
F
GO Term mRNAseq F1 starved up P value
Ribosome 3.77E-8
Response to heat 3.95E-7
Structural constituent of ribosome 7.26E-7
Translation 2.01E-5
Innate immune response 2.51E-5
ER unfolded protein response 4.82E-4
Flavonoid glucuronidation 2.56E-2
G
dimt-1 counts
bud-23 counts
ns
ns
F1 fed
F1 starved
H
KD down regulated genes vs starved up regulated
bud-23 KD (882)
dimt-1 KD (2719)
195
Starved (1648)

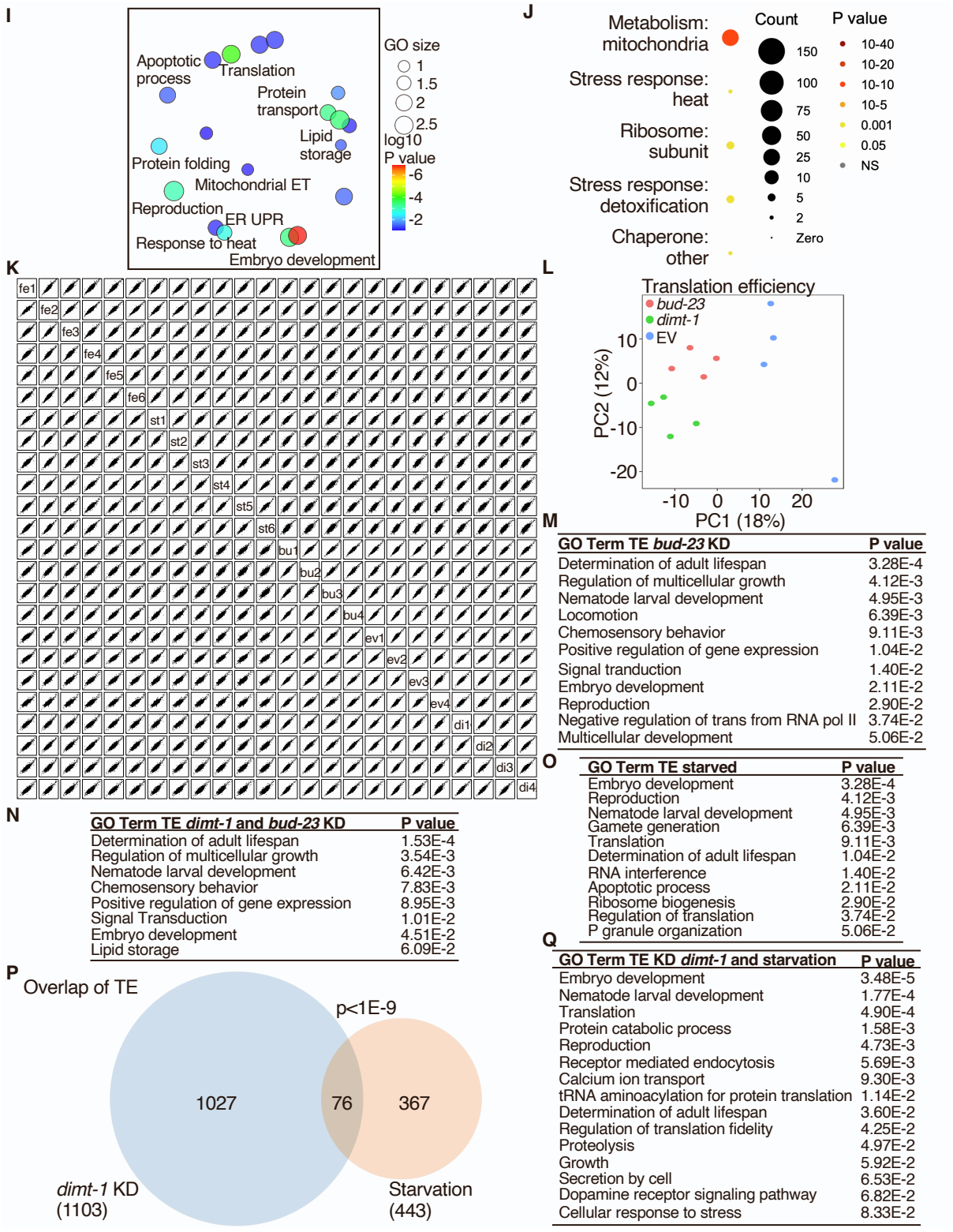


Fig. S5 Knock down of *bud-23* or *dimt-1* or parental starvation induce dysregulation of gene expression and similar changes in translation efficiency, related to Figure 4.

A, Scatter plots of RNA sequencing show pair wise comparisons of F1 fed (fe) and starved (st) worms and WT worms after knockdown of *bud-23* (bu), *dimt-1* (di), or an empty vector (EV)

control. **B,** Principal component analysis of RNA sequencing of four independent biological replicates reveals that *bud-23* and *dimt-1* knockdown cause a misregulation of similar sets of genes relative to an empty vector (EV) control. **C,** Venn diagrams display a high degree of overlap between genes which are dysregulated upon *bud-23* knockdown to those which are dysregulated upon *dimt-1* knockdown. **D,** GO analysis of genes which are coordinately differentially transcribed after *bud-23* and *dimt-1* knockdown reveals the importance of 18S methylation in regulation of development, reproduction, longevity, and translation. **E**, Principal component analysis of RNA sequencing of six independent biological replicates reveals that parental starvation cause a misregulation of a large number of genes. **F,** GO analysis of genes which are differentially upregulated after parental starvation reveals an effect on translation, the response to heat and the endoplasmic reticulum unfolded protein response. **G**, Parental starvation does not affect *dimt-1* (left) or *bud-23* (right) expression levels. ns, not significant as assessed by t test. **H,** Venn diagrams display overlap between genes which are dysregulated upon *bud-23* or *dimt-1* knockdown and those genes which become upregulated in response to parental starvation. **I,** Revigo plots reveal relative enrichment of coordinately dysregulated gene transcription in response to parental starvation and *bud-23* and *dimt-1* knockdown. Proximity of bubbles reflects the similarity of terms, color intensity represents p value of enrichment, and size of the bubbles reflects how many genes are in the gene set depicted. **J,** WormCat gene ontology analysis [50] reveal relative enrichment of coordinately dysregulated gene transcription in response to parental starvation and *bud-23* and *dimt-1* knockdown. **K,** Scatter plots of translation efficiency show pair wise comparisons of F1 fed and starved worms and WT worms after knockdown of *bud-23, dimt-1,* or an empty vector (EV) control. **L,** Principal component analysis of translation efficiency after knockdown of *bud-23* and *dimt-1* reveals that *bud-23* and *dimt-1* knockdown cause a similar change in binding of the ribosome to transcripts relative to an empty vector (EV) control. **M,** GO analysis of transcripts that are differentially bound after *bud-23* knockdown reveals an effect on pathways involved in regulation of development, reproduction, and longevity. **N,** GO analysis of transcripts that are coordinately differentially bound after *bud-23* and *dimt-1* knockdown reveals an effect on pathways involved in regulation of development, growth, regulation of gene expression, and longevity. **O,** GO analysis of transcripts that are differentially bound after parental starvation reveals an effect on pathways involved in regulation of development, reproduction, longevity, and translation. **P**, Venn diagram display overlap between dysregulated translation efficiency upon *dimt-1* knockdown and parental starvation. $p<1E-9$ by hypergeometric probability. **Q,** GO analysis of transcripts that are coordinately differentially bound after *dimt-1* knockdown and starvation reveals an effect on pathways involved in regulation of development, reproduction, cellular response to stress, and longevity.

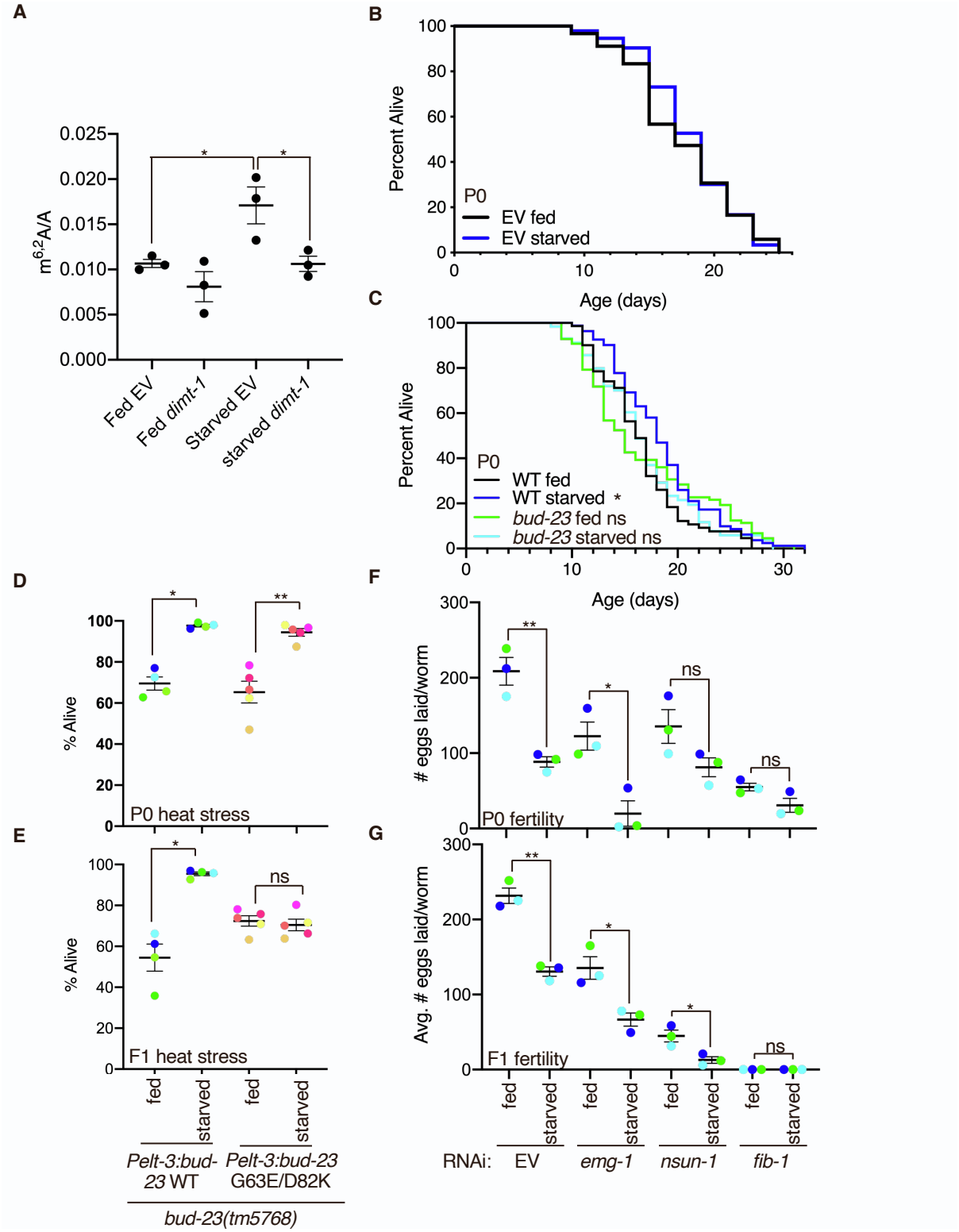


Fig. S6 *dimt-1* is necessary for the heritable increase in $m^{6,2}A$ on the 18S rRNA in response to parental starvation and the catalytic activity of *bud-23* is necessary for descendant heat stress resistance while other rRNA methylases are dispensable, related to Figure 5.

A, Naïve F1 progeny whose parents were starved display elevated $m^{6,2}A/A$ levels on the 18S rRNA relative to F1 progeny whose parents were fed as detected by UHPLC-ms/ms and this increase was dependent on *dimt-1*. P0 parents were fed Methionine-CD_3 and RNA was extracted from F1 eggs. Each dot represents an independent replicate. * $p<0.05$ as assessed by one way ANOVA with Tukey's multiple comparison test. **B,** Starvation causes no significant change in lifespan when both fed and starved worms are placed on HT115 empty vector expressing bacteria. Each condition represents three plates of ~30 worms per plate. This is a representative experiment which has been performed 2 times. **C,** Starvation causes a subtle increase in longevity of WT worms but not *bud-23(tm5768)* mutant worms. Each condition represents three plates of ~30 worms per plate. This is a representative experiment which has been performed 3 times. Statistics of independent experiments are presented in Supplementary Table 1. Ns, not significant, * $p<0.05$ as assessed by Log-rank (Mantel-Cox) test. Statistics of independent experiments are presented in Supplementary Table 1. **D-E,** Rescue of *bud-23(tm5768)* with a WT but not a catalytically dead version of *bud-23* rescues the capacity to transmit the starvation induced increase in 37ºC heat stress resistance after 8-9 hours to naïve well fed children. Each column represents the mean ± SEM of 4-5 independent experiments performed in three plates with 30 worms per plate. **F-G**, Knock down of other rRNA methyltransferases that have been proposed to be important for rRNA processing has no effect on the transmission of decreased fertility to naïve well fed children in response to parental starvation. Each column represents the mean ± SEM of 3 independent experiments performed in three plates with 30 worms per plate. Dots are color coded to display matched independent experiments. ns, not significant, * $p<0.05$, ** $p<0.01$, as assessed by one way ANOVA with Tukey's or Holm-Sidak's multiple comparisons test.

Generation	Strain	Mean +/- SD	Median	p values	# worms	Figure
P0	WT fed	15.692	15		78/98	1c
P0	WT starved	17.548	17	0.0009	73/94	1c
F1	WT fed	15.951	17		82/96	1f
F1	WT starved	17.416	17	0.0051	77/96	1f
P0	WT fed	17.120	17		83/96	
P0	WT starved	20.358	19	<0.0001	81/96	
F1	WT fed	16.816	17		87/96	
F1	WT starved	18.671	19	0.0463	81/96	
P0	WT fed	17.841	17		88/96	
P0	WT starved	19.543	19	0.0432	81/95	
F1	WT fed	17.706	19		86/96	
F1	WT starved	20.101	19	0.0030	89/96	
P0	WT fed Empty vector	17.488	17		86/96	S6a
P0	WT starved Empty vector	18.130	19	0.6368	92/96	S6a
P0	WT fed Empty vector	19.132	19		91/96	
P0	WT starved Empty vector	20.241	19	0.0350	87/96	
P0	WT fed	16.448	16		67/77	S6b
P0	WT starved	18.457	18	0.0120	81/84	S6b
P0	*bud-23(tm5768)* fed	16.652	15	0.3866	92/98	S6b
P0	*bud-23(tm5768)* starved	16.315	16	0.7249	54/62	S6b
P0	WT fed	18.190	19		84/96	
P0	WT starved	19.597	19	0.0198	67/96	
P0	*bud-23(tm5768)* fed	15.370	15	0.0742	54/71	
P0	*bud-23(tm5768)* starved	16.176	15	0.5224	68/96	
P0	WT fed	16.183	16		83/97	
P0	WT starved	17.405	18	0.0725	42/96	
P0	*bud-23(tm5768)* fed	16.871	15	0.1077	85/96	
P0	*bud-23(tm5768)* starved	16.377	16	0.9069	53/96	
F1	WT fed	17.649	17		77/87	
F1	WT starved	19.557	19	0.0159	79/89	
F1	*bud-23(tm5768)* fed	16.729	15	0.9979	59/93	
F1	*bud-23(tm5768)* starved	16.92	17	0.9201	50/90	
F1	WT fed	15.568	15		88/98	
F1	WT starved	17.169	17	0.0081	83/99	
F1	*bud-23(tm5768)* fed	16.313	15	0.1350	67/97	
F1	*bud-23(tm5768)* starved	14.463	15	0.0425	67/94	

Supplementary Table 1. Parental starvation causes an increase in lifespan in the P0 and F1 generation in WT worms but not in *bud-23(tm5768)* mutant worms, related to Figure 1. 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.