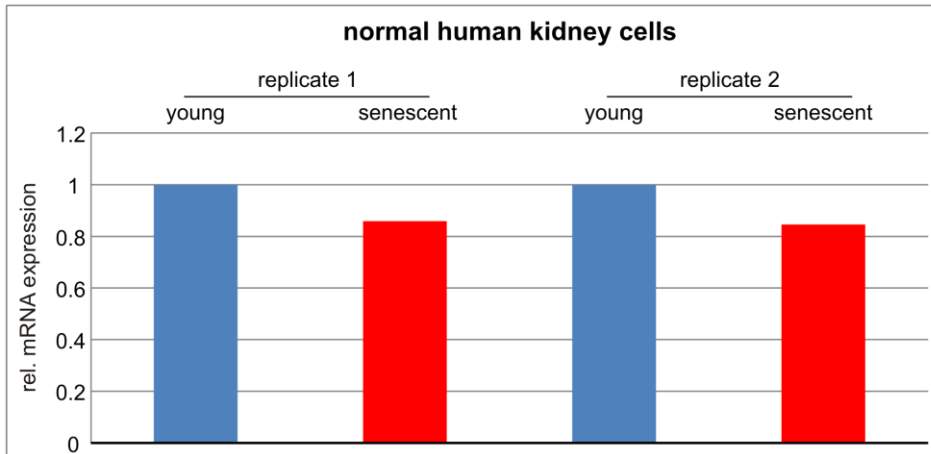
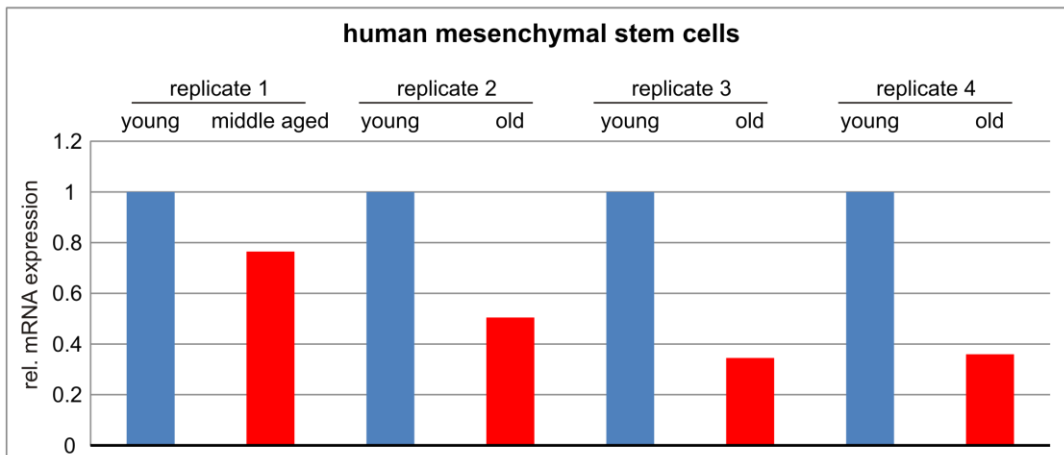
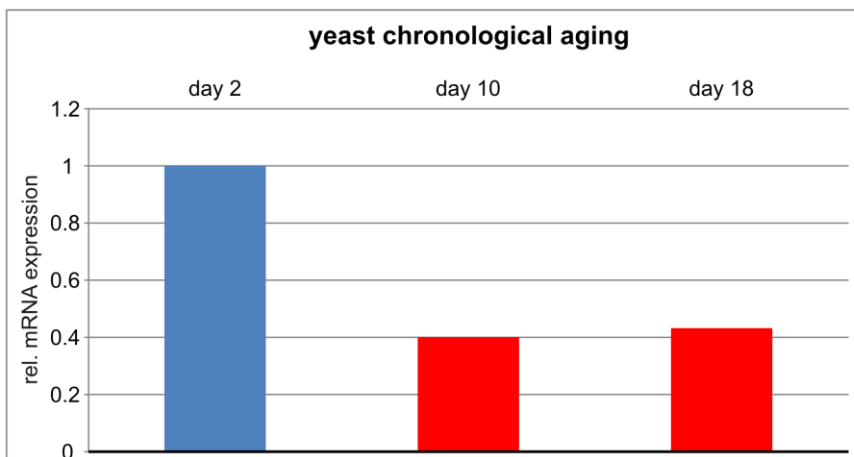
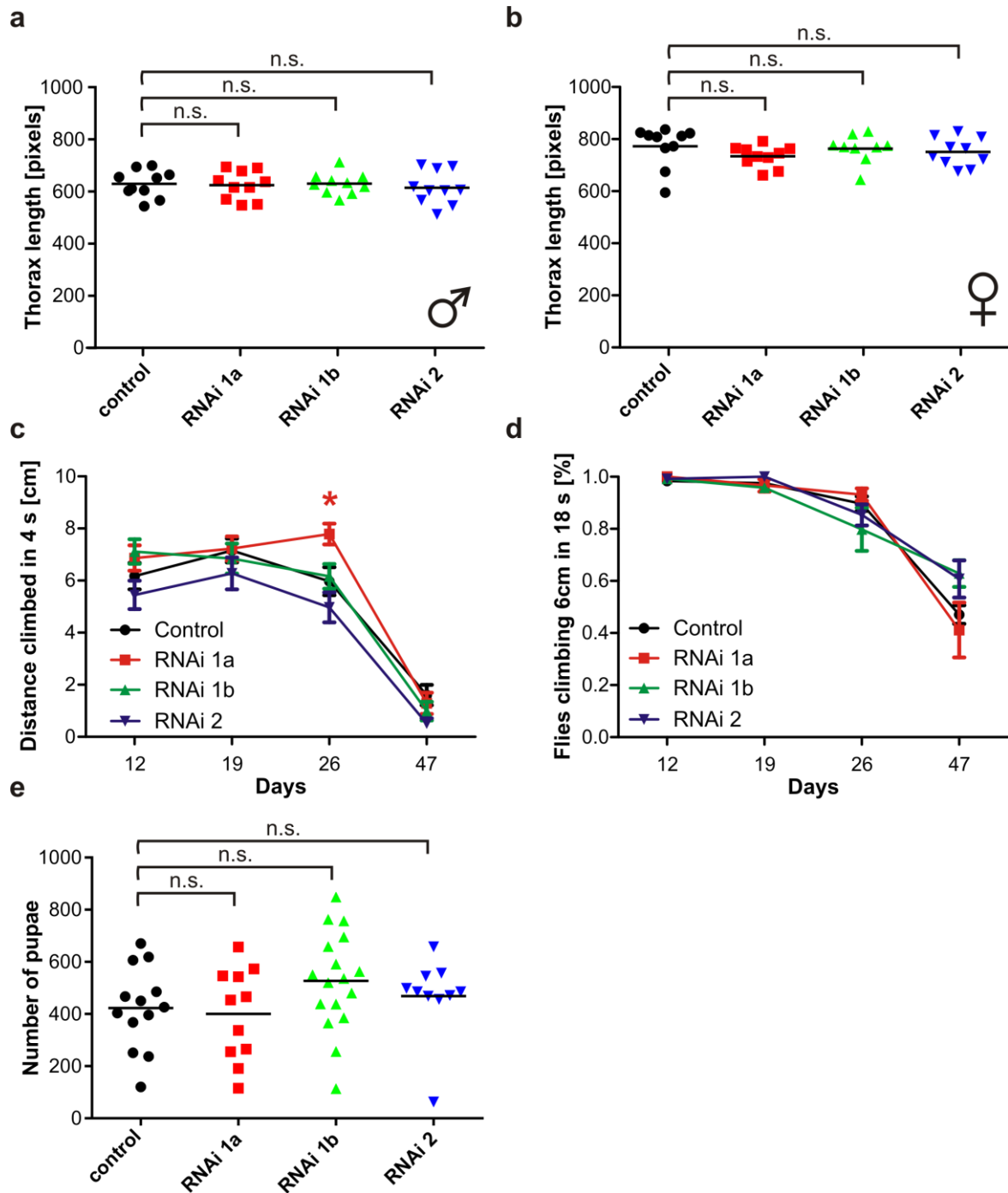
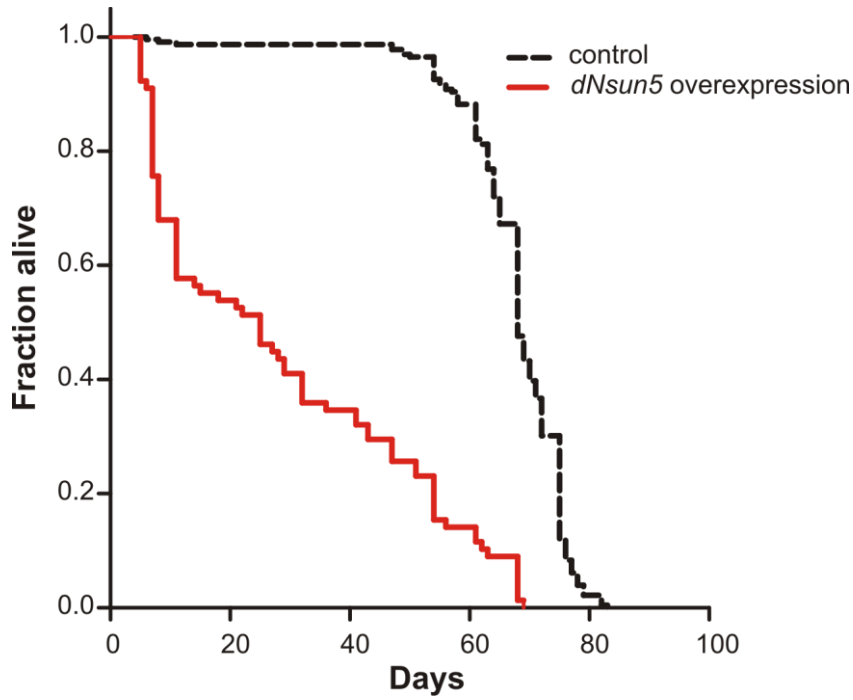


**a****b****c**

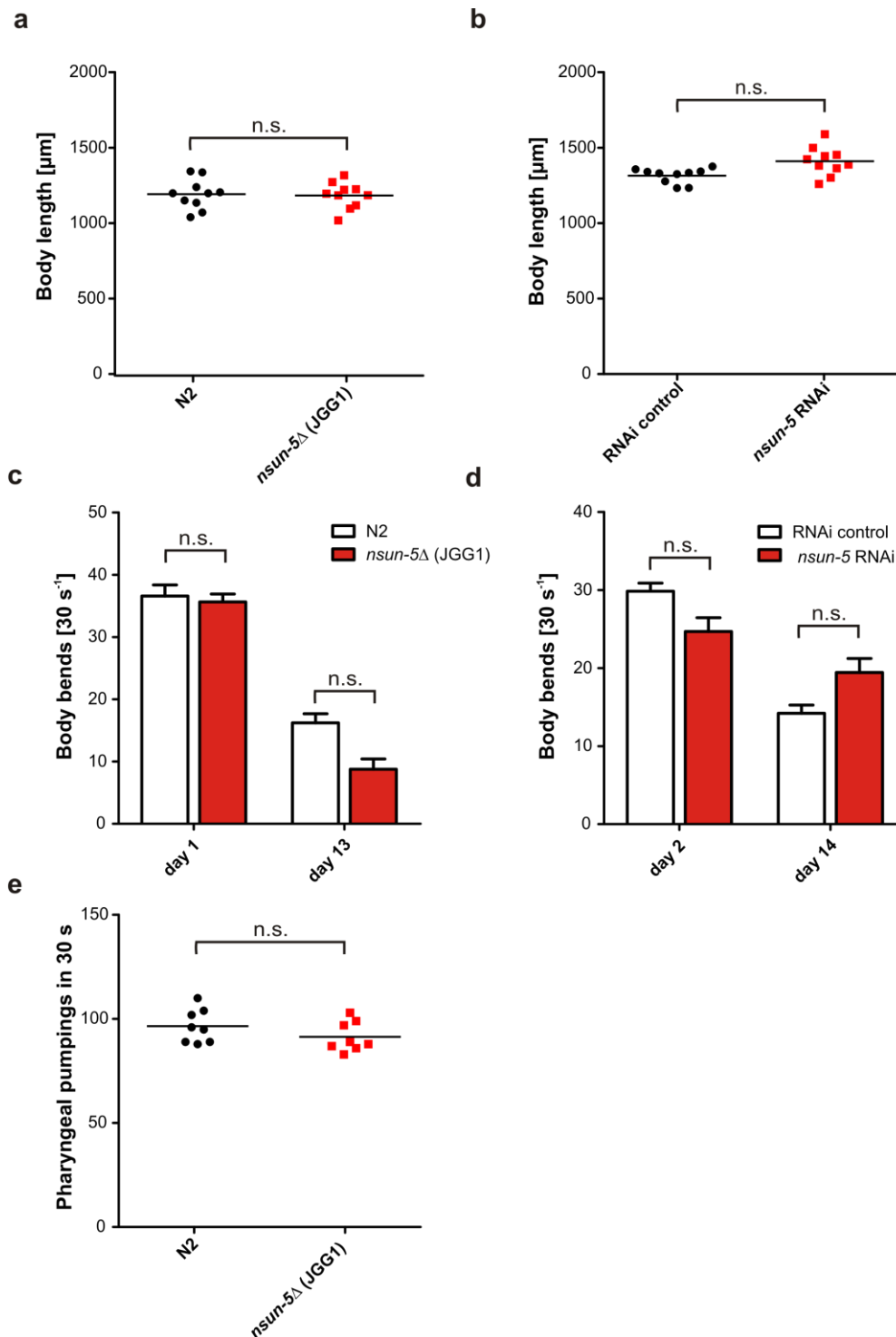
**Supplementary Figure 1 | NSUN5/RCM1 mRNA is less abundant in aged cells.** Gisao.db database derived mRNA expression values of young and replicative-senescent normal human kidney cells from two different donors (**a**), of mesenchymal stem cells from young, middle-aged and old donors (**b**) and of chronologically aged yeast (**c**). For human cells (**a,b**) the mean expression of two probesets is shown. For yeast (**c**), only one probeset for *RCM1* was available.



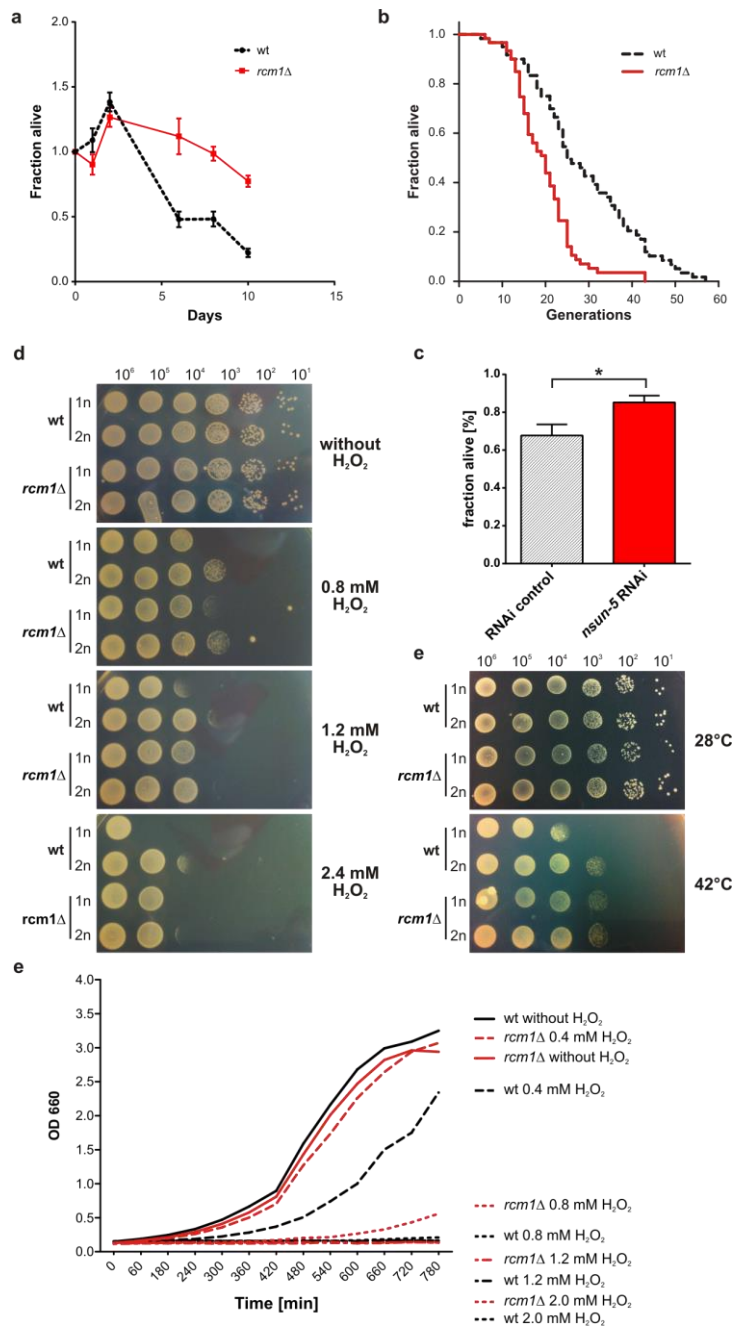
**Supplementary Figure 2 | Phenotypic characterization of *dNsun5* knockdown flies. a-b,** Mean thorax length in pixels of male (**a**) and female (**b**) control and three different *dNsun5* RNAi fly lines. Error bars represent S.E.M. N = 10,  $\alpha = 0.05$ , One-way ANOVA with Dunnett's post test not significant for all conditions. **d-e,** Locomotion of control and three different *dNsun5* RNAi fly lines at different ages measured by climbed distance in 4 s per fly, N = 40 (**c**) or number of flies climbing 6 cm in 18 s, N = 4 (**d**). Two-way ANOVA with Bonferroni post tests at  $\alpha = 0.05$  was not significant unless marked otherwise, \* $p < 0.05$ . **e,** Number of pupae produced over 4 weeks by control and three different *dNsun5* RNAi fly lines. Error bars represent S.E.M. N = 20,  $\alpha = 0.05$ , one-way ANOVA with Dunnett's post test not significant.



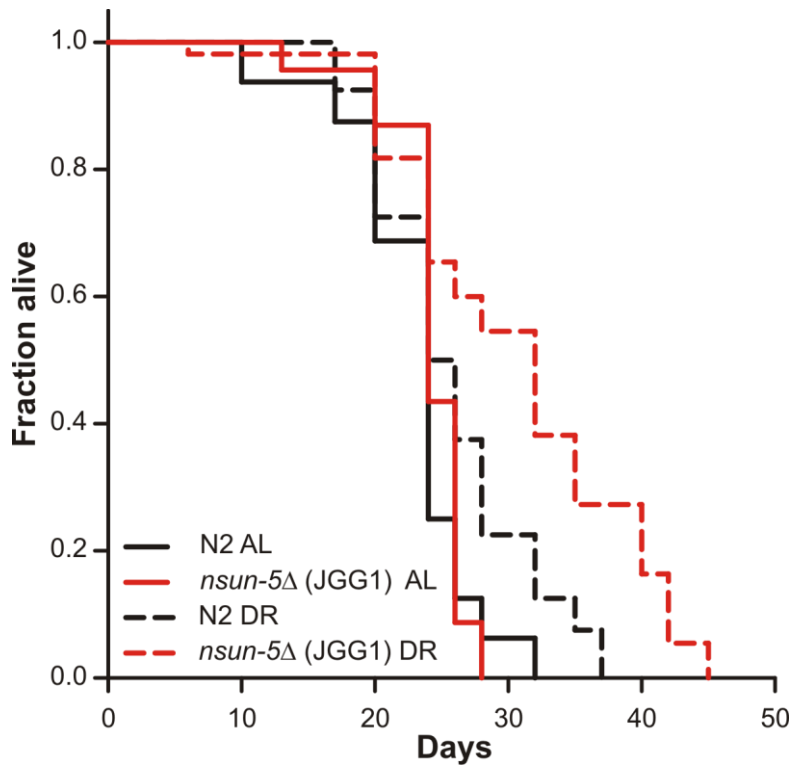
**Supplementary Figure 3 | Overexpression of *dNsun5* shortens *Drosophila* lifespan.** Kaplan-Meier survival curves of control and *dNsun5* overexpressing male *D. melanogaster* (average N per group = 78, log-rank  $p < 0.001$ ).



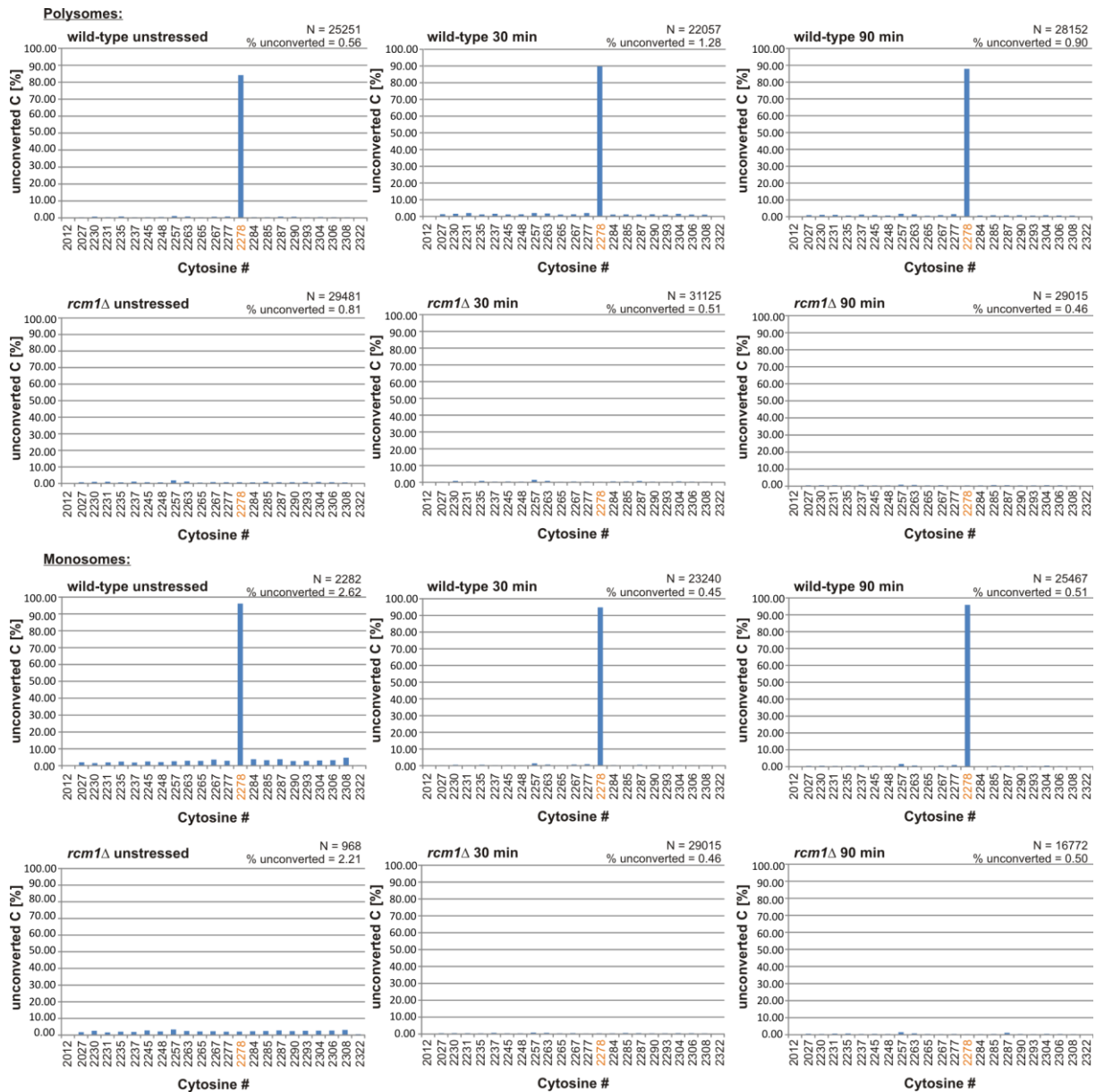
**Supplementary Figure 4 | Phenotypic characterization of *nsun-5* knockout worms. a-b,** Mean body length in  $\mu\text{m}$  of N2 vs. *nsun-5* knockouts (JGG1) (a) or NL2099 subjected to control or *nsun-5* RNAi (b) hermaphrodites. Error bars represent S.E.M.  $N = 10$ ,  $\alpha = 0.05$ , two-sided Student's t-Test not significant. **c-d,** Locomotion activity at different timepoints of wild-type vs. *nsun-5* knockouts (JGG1) (c) or NL2099 subjected to control or *nsun-5* RNAi (d) hermaphrodites. Error bars represent S.E.M.  $N = 20$ ,  $\alpha = 0.05$ , two-sided Student's t-Test at different timepoints not significant. **e,** Pharyngeal pumping of wild-type vs. *nsun-5* knockout (JGG1) hermaphrodites. Error bars represent S.E.M.  $N = 20$ ,  $\alpha = 0.05$ , two-sided Student's t-Test not significant.



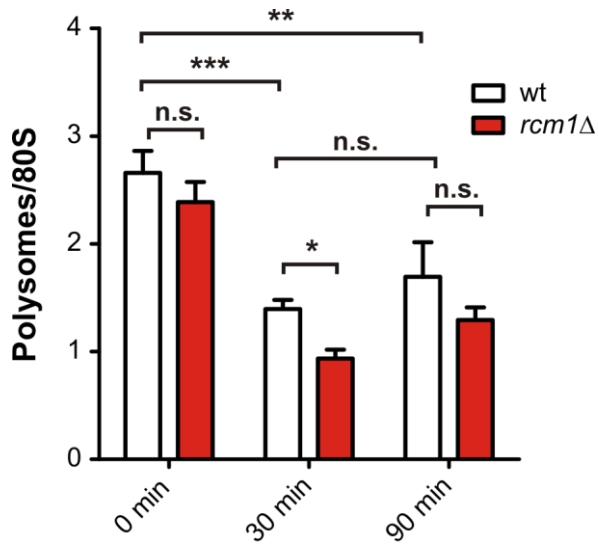
**Supplementary Figure 5 | NSUN5 modulates stress resistance and chronological lifespan.** **a**, Deletion of *rcm1* in haploid *S. cerevisiae* increases chronological lifespan in water. Error bars represent S.E.M. of 2-3 biological replicates. **b**, Replicative lifespan assay of haploid wild-type *S. cerevisiae* vs. a haploid *rcm1* knockout strain (N = 60, log-rank,  $p < 0.001$ ). **c**, RNAi against *nsun-5* increases the paraquat resistance of N2 wild-type worms. Survival after 3 days of treatment with 20 mM paraquat are shown. N = 4 biological replicates with 30 worms each,  $\alpha = 0.05$ , two-tailed t-test \* $p < 0.05$ . Error bars represent S.E.M. **d, e**, Yeast serial dilution growth assays demonstrate increased resistance of haploid (1n) *rcm1Δ* cells compared to the haploid wild-type to H<sub>2</sub>O<sub>2</sub> (**d**) and to heatshock (**e**). Diploid (2n) heterozygously deleted *rcm1* cells compared to the diploid wild-type did not show increased survival, suggesting that a single copy of *RCM1* is sufficient. **f**, Liquid growth assay with increasing amounts of H<sub>2</sub>O<sub>2</sub> in the medium shows increased survival of haploid *rcm1Δ* cells (red) compared to the wild-type (black).



**Supplementary Figure 6 | Lifespans of *nsun-5* mutants are only extended upon complete removal of food.** Dietary restriction (DR) induced by complete removal of food during adulthood extends the lifespan of an *nsun-5* deletion mutant JGG1 (average N per group = 73, log-rank  $p = 0.03$ ).



**Supplementary Figure 7 | Bisulfite treatment demonstrates Rcm1-dependent methylation of C2278 in 25S rRNA.** RNA isolated from ribosome fractions was bisulfite-sequenced. The percentage of unconverted (= C) to all (= C + T) cytosines at the indicated positions is represented by bars. C2278 is highlighted in orange. The number of full sequence reads is indicated (N), as well as the average percentage of unconverted cytosines at all positions except C2278.



**Supplementary Figure 8 | Quantification of polysomes to monosomes ratio of wt and *rcm1* knockout cells.** The area under the curves of all recorded ribosome profiles was measured and the ratio of polysomes to monosomes was calculated. Error bars represent S.E.M. (N = 3-5,  $\alpha = 0.05$ , Two-way ANOVA with Turkey's multiple comparisons test for testing effect of stress in wt, multiple comparison two-sided t-test using the Holm-Sidak method without assuming constant S.D. for comparing wild-type and *rcm1Δ* at different timepoints, \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ ).





**Supplementary Figure 9 | Verification of translational profiling microarrays by RT-qPCR.** Relative expression levels of a subset of genes from the microarray (red) were validated by RT-qPCR (blue) on translatoe and transcriptome level and show similar trends. Normalization was performed against *TDH-3*, *ACT-1* and *TUB-1*. The average target gene expression relative to all three housekeeping genes is depicted. Error bar represent S.D., N = 3 biological replicates,  $\alpha = 0.05$ , one-way ANOVA with Dunnett's post test, \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.001$ .

Strain	Mean lifespan +/- s.e.m. [days]	N	P	mean lifespan extension [%]
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*dNsun5* RNAi (normal diet):

initial screen:

RNAi control	55.9 +/- 0.8	147		
<i>dNsun5</i> RNAi 1a	58.9 +/- 0.6	20	0.0077	5.37
<i>dNsun5</i> RNAi 1b	59.1 +/- 2.0	20	0.0097	5.72
<i>dNsun5</i> RNAi 2	53.6 +/- 1.2	20	0.0071	-4.11

replicate 1:

RNAi control	50.8 +/- 1.2	168		
<i>dNsun5</i> RNAi 1a	60.4 +/- 1.2	80	0.0002	18.90
<i>dNsun5</i> RNAi 1b	59.0 +/- 1.6	80	< 0.0001	16.14
<i>dNsun5</i> RNAi 2	61.4 +/- 1.3	82	< 0.0001	20.87

replicate 2:

RNAi control	56.0 +/- 0.9	240		
<i>dNsun5</i> RNAi 1a	59.8 +/- 1.3	74	0.0015	6.79
<i>dNsun5</i> RNAi 1b	62.1 +/- 1.6	85	0.0010	10.89
<i>dNsun5</i> RNAi 2	61.6 +/- 1.9	84	0.0002	10.00

*dNsun5* overexpression:

replicate 1 (25°C):

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control	67.7 +/- 0.7	229		
<i>dNsun5</i> construct #1	28.7 +/- 2.5	78	< 0,001	-57.61
<i>dNsun5</i> construct #2		embryonic lethal		

**replicate 2 (20°C):**

control	67.3 +/- 0.5	291		
<i>dNsun5</i> construct #1	60.8 +/- 1.2	102	< 0,001	-9.66
<i>dNsun5</i> construct #2		embryonic lethal		

***dNsun5* RNAi (sugar and yeast rich diet):**

**replicate 1:**

RNAi control	56.8 +/- 1.2	85		
<i>dNsun5</i> RNAi 1a	50.2 +/- 1.3	93	0.00322	-11.62
<i>dNsun5</i> RNAi 1b	60.2 +/- 1.0	89	0.140	5.99
<i>dNsun5</i> RNAi 2	61.8 +/- 0.7	84	0.128	8.80

**replicate 2:**

RNAi control	61.9 +/- 1.0	73		
<i>dNsun5</i> RNAi 1a	50.4 +/- 1.6	98	0.000153	-18.58
<i>dNsun5</i> RNAi 1b	61.9 +/- 1.2	90	0.318	0.00
<i>dNsun5</i> RNAi 2	63.9 +/- 0.9	91	0.0728	3.23

**Supplementary Table 1 | Overview of *D. melanogaster* lifespans**

Strain	Mean +/- s.e.m. days	Number of animals that died/total	P	mean lifespan extension [%]
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**NL2099 (reduced diet):**

**replicate1:**

RNAi control	15.2 +/- 0.4	108/120		
<i>nsun-5</i> RNAi	21.0 +/- 0.7	88/120	<0.001	38.16

**replicate 2:**

RNAi control	15.4 +/- 0.3	113/120		
<i>nsun-5</i> RNAi	17.3 +/- 0.3	118/120	0.004	12.34

**replicate 3:**

RNAi control	15.2 +/- 0.3	116/120		
<i>nsun-5</i> RNAi	17.5 +/- 0.3	103/120	<0.001	15.13

**replicate 4:**

RNAi control	14.6 +/- 0.3	116/120		
<i>nsun-5</i> RNAi	18.2 +/- 0.2	114/120	<0.001	24.65

**NL2099 (ad libidum fed):**

**replicate1:**

RNAi control	23.5 +/- 0.4	103/120		
<i>nsun-5</i> RNAi	23.8 +/- 0.3	104/120	0.552	1.28

**replicate 2:**

RNAi control	31.6 +/- 0.7	34/120		
<i>nsun-5</i> RNAi	30.7 +/- 0.6	53/120	0.513	-2.85

**N2 vs. JGG1 (normal diet):**

**replicate1:**

<b>N2</b>	18.8 +/- 0.3	74/120		
<b>JGG1</b>	18.1 +/- 0.3	96/120	0.144	-3.72

**replicate2:**

<b>N2</b>	17.7 +/- 0.3	109/120		
<b>JGG1</b>	17.8 +/- 0.3	111/120	0.777	0.56

**N2 vs. JGG1 (complete removal of food):**

**replicate1:**

<b>N2</b>	25.9 +/- 0.9	40/73		
<b>JGG1</b>	30.7 +/- 2.0	19/74	0.033	18.53

**replicate 2:**

<b>N2</b>	11.2 +/- 3.0	6/47		
<b>JGG1</b>	15.6 +/- 2.3	9/48	0.145	39.29

**DA1116 (*eat-2* mutation):**

**replicate 1:**

<b>RNAi control</b>	23.7 +/- 0.8	55/120		
<b><i>nsun-5</i> RNAi</b>	27.4 +/- 0.9	51/120	<0.001	15.61

**replicate 2:**

<b>RNAi control</b>	25.4 +/- 0.6	90/120		
<b><i>nsun-5</i> RNAi</b>	28.0 +/- 0.6	93/120	<0.001	10.24

**Supplementary Table 2 | Overview of *C. elegans* lifespans**

**qPCR primers:**

DDR2_qPCR_sense	TCTGCCATCTCTGTCTTCGG
DDR2_qPCR_as	TCAAAAAGGCCAAAGCACCA
HSP30_qPCR_sense	GCTAATTGCAGTCAGCCGTG
HSP30_qPCR_as	TCTGCTTCAGGTTCTGGGTTCT
SRX1_qPCR_sense	CTCTAGAACAGGCTGAGGCG
SRX1_qPCR_as	CAGGTACATGCGGATTTGGC
HXK1_qPCR_sense	AATGTCTGAAGGCCACGATGT
HXK1_qPCR_as	CCGTTGACACCAGTACCGAA
SOD2_qPCR_sense	CTCCCGCAAACGCAAGAAAA
SOD2_qPCR_as	CTCGTCCAGACTGCCAAACT
TMA10_qPCR_sense	TAACGGCAACTTTGGGGAGT
TMA10_qPCR_as	TGGGAGTTAGAGCCCCTTCT
ZPS1_qPCR_sense	ACCGCTGAAATCACCGCTTA
ZPS1_qPCR_as	TAGCTGCACACAAGCCATCA
ZRT1_qPCR_sense	ATGGACCCTGCTTATGGTGC
ZRT1_qPCR_as	TCGTGGGTATGGTCATGGGA
ACT1_qPCR_sense	TGAGAATCGATTTGGCCGGT
ACT1_qPCR_as	GATTGAGCAGCGTTTGCAT
TDH3_qPCR_sense	TGCCATGGGGTTCTTCCAACG
TDH3_qPCR_as	TGGGGCGGTGGAAGATGGAG
TUB1_qPCR_sense	ACTGGTTCCGGTCTAGGGTC
TUB1_qPCR_as	TGCAAAGCTTGGTCTTGGGA

**Bisulfite Sequencing primers:**

SC-25S-rRNA_bisulfite_sense	TGGTTAGAAAGTGATGTT
SC-25S-rRNA_bisulfite_as	TCTCATTAAATCCATTCA
CE-28S-rRNA_bisulfite_sense	TGAATGTTAAATTGTAGTAATT
CE-28S-rRNA_bisulfite_as	TCTCGTTAAATCCATTCA

**Barcode primers:**

SC-25S-rRNA_torrent_code1f	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGTGCCTTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code1r	CCTCTCTATGGGCAGTCGGTGATACGAGTGCCTTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code2f	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCTCGACATTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code2r	CCTCTCTATGGGCAGTCGGTGATACGCTCGACATCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code3f	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACGCACTCTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code3r	CCTCTCTATGGGCAGTCGGTGATAGACGCACTCTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code4f	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCACTGTAGTTTGAATGTTAAAGTGAA

SC-25S-rRNA_torrent_code4r	CCTCTCTATGGGCAGTCGGTGATAGCACTGTAGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code5f	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCAGACACGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code5r	CCTCTCTATGGGCAGTCGGTGATATCAGACACGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code6f	CCATCTCATCCCTGCGTGTCTCCGACTCAGATATCGCGAGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code6r	CCTCTCTATGGGCAGTCGGTGATATATCGCGAGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code7f	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGTCTCTATTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code7r	CCTCTCTATGGGCAGTCGGTGATCGTGTCTCTATCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code8f	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTCGCGTGTCTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code8r	CCTCTCTATGGGCAGTCGGTGATCTCGCGTGTCTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code9f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCTATGCGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code9r	CCTCTCTATGGGCAGTCGGTGATTCTCTATGCGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code10f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGATACGTCTTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code10r	CCTCTCTATGGGCAGTCGGTGATTGATACGTCTTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code11f	CCATCTCATCCCTGCGTGTCTCCGACTCAGATAGTAGTGTGTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code11r	CCTCTCTATGGGCAGTCGGTGATATAGTAGTGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code12f	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGAGAGATATTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code12r	CCTCTCTATGGGCAGTCGGTGATCGAGAGATATCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code13f	CCATCTCATCCCTGCGTGTCTCCGACTCAGATACGACGTATTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code14f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACGTACTATTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code15f	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTCTAGTACTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code16f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTACGTAGCTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code17f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTACTACTTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code18f	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGACTACAGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code19f	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTAGACTAGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code20f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACGAGTATGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code21f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACTCTCGTGTGTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code22f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGAGACGAGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code23f	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTCGCTCGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code24f	CCATCTCATCCCTGCGTGTCTCCGACTCAGACATACGCGTTTGAATGTTAAAGTGAA
SC-25S-rRNA_torrent_code13r	CCTCTCTATGGGCAGTCGGTGATATACGACGTATCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code14r	CCTCTCTATGGGCAGTCGGTGATTCACGTACTATCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code15r	CCTCTCTATGGGCAGTCGGTGATCGTCTAGTACTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code16r	CCTCTCTATGGGCAGTCGGTGATTCTACGTAGCTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code17r	CCTCTCTATGGGCAGTCGGTGATTGTACTACTTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code18r	CCTCTCTATGGGCAGTCGGTGATACGACTACAGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code19r	CCTCTCTATGGGCAGTCGGTGATCGTAGACTAGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code20r	CCTCTCTATGGGCAGTCGGTGATTACGAGTATGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code21r	CCTCTCTATGGGCAGTCGGTGATTACTCTCGTGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code22r	CCTCTCTATGGGCAGTCGGTGATTAGAGACGAGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code23r	CCTCTCTATGGGCAGTCGGTGATTCTGTCGCTCGTCTCATTAAATCCATTCA
SC-25S-rRNA_torrent_code24r	CCTCTCTATGGGCAGTCGGTGATACGAGTGC GTTCTCATTAAATCCATTCA
CE-28S-rRNA_torrent_code1f	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGTGC GTTGAATGTTAAATTGTAGTAATT
CE-28S-rRNA_torrent_code2f	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCTCGACATGAATGTTAAATTGTAGTAATT
CE-28S-rRNA_torrent_code3f	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACGCACTCTGAATGTTAAATTGTAGTAATT
CE-28S-rRNA_torrent_code4f	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCACTGTAGTGAATGTTAAATTGTAGTAATT

CE-28S-rRNA_torrent_code5f	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCAGACACGTGAATGTTAAATTGTAGTAATT
CE-28S-rRNA_torrent_code6f	CCATCTCATCCCTGCGTGTCTCCGACTCAGATATCGCGAGTGAATGTTAAATTGTAGTAATT
CE-28S-rRNA_torrent_code7f	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGTCTCTATGAATGTTAAATTGTAGTAATT
CE-28S-rRNA_torrent_code1r	CCTCTCTATGGGCAGTCGGTGATACGAGTGC GTTCTCGTTAATCCATTCA
CE-28S-rRNA_torrent_code2r	CCTCTCTATGGGCAGTCGGTGATACGCTCGACATCTCGTTAATCCATTCA
CE-28S-rRNA_torrent_code3r	CCTCTCTATGGGCAGTCGGTGATAGACGCACTCTCTCGTTAATCCATTCA
CE-28S-rRNA_torrent_code4r	CCTCTCTATGGGCAGTCGGTGATAGCACTGTAGTCTCGTTAATCCATTCA
CE-28S-rRNA_torrent_code5r	CCTCTCTATGGGCAGTCGGTGATATCAGACACGTCTCGTTAATCCATTCA
CE-28S-rRNA_torrent_code6r	CCTCTCTATGGGCAGTCGGTGATATATCGCGAGTCTCGTTAATCCATTCA
CE-28S-rRNA_torrent_code7r	CCTCTCTATGGGCAGTCGGTGATCGTGTCTCTATCTCGTTAATCCATTCA

**Supplementary Table 3 | Primers used in this study**