

**SUPPLEMENTARY TABLES**

**Supplementary Table SI. Summary and statistical analysis of survival assays and Seahorse experiments.**

Gene/ RNAi clone	RNAi mild power	Lifespan change (%) <sup>a</sup>	P-value vs control <sup>b</sup>	Sample size/ n trials (censored)	Seahorse Parameters (nmol O <sub>2</sub> /min/ mg protein) <sup>^</sup>						
					Basal OCR	ATP- Linke d OCR	Proton Leak	Maximal OCR	Spare Capacity	Non- mitochondrial OCR	Sample size/ n trials
pL4440					36.1	16	10.0	102.9	61.4	11.7	~20000/6
T20H4.5	undiluted	+30.96	< 0.0001	250/3 (26)	36.6	21.9	9.8	98.8	53.7	9.8	~6000/4
<i>tag-316</i>	undiluted	+25.1	< 0.0001	125/2 (21)	39.8	21.7	9.3	103.5	56.5	11.6	~6000/4
F53F4.10	undiluted	+14.7	< 0.0001	320/4 (67)	30.8	19.0	12.5	129.6	62.3	10.5	~6000/4
<i>nuo-5</i>	undiluted	+20.2	< 0.0001	200/3 (49)	25.2	10.5	7.4	82.8	40.6	7.4	~6000/4
<i>lpd-5</i>	undiluted	+5.6	< 0.0001	220/3 (48)	32.2	12.7	12.4	107.6	57.7	10.7	~6000/4
<i>spg-7</i>	undiluted	+19.7	< 0.0001	230/3 (30)	31.2	11.8	12.3	96.9	30.9	19.5	~6000/4
<i>nuo-1</i>	diluted*	+31.8	< 0.0001	200/3 (21)	43.2	27.0	8.0	99.7	54.5	15.0	~6000/4
<i>tag-61</i>	diluted*	+21.4	< 0.0001	250/3 (19)	33.5	20.8	8.1	73.1	33.5	8.4	~6000/4
<i>atp-3</i>	diluted*	+79.24 (Ventura et al., 2009)			66.8	60.7	12.2	117.2	45.2	11.0	~6000/4
<i>cco-1</i>	diluted*	+53.5 (Ventura et al., 2009)			40	19.3	9.1	98.6	54.0	15.6	~6000/4
<i>isp-1</i>	diluted*	+86.8 (Ventura et al., 2009)			33.1	18.5	10.6	80.5	41.6	6.5	~6000/4
<i>nuo-2</i>	diluted*	+34.3 (Maglioni et al, 2014)			33.6	14.9	7.8	83.8	47.4	16.4	~6000/4
<i>spg-7</i>	diluted*	+22.4	< 0.0001	230/3 (25)	ND	ND	ND	ND	ND	ND	ND
<i>hmg-5</i>	undiluted	+9.29	0.0005	135/2 (34)	ND	ND	ND	ND	ND	ND	ND
<i>pdr-1</i>	diluted*	+15.3	< 0.0001	135/2 (33)	ND	ND	ND	ND	ND	ND	ND
<i>tag-61</i>	undiluted	+12.1	< 0.0001	120/2 (17)	ND	ND	ND	ND	ND	ND	ND
<i>eat-3</i>	undiluted	+16.99	< 0.0001	120/2 (21)	ND	ND	ND	ND	ND	ND	ND

<sup>a</sup> % increase normalized mean lifespan compared to control; <sup>b</sup> Kaplan-Meier survival analysis, Log-rank test against control.

\* Diluted RNAi is always 1:10 with empty vector pL4440 expressing bacteria, except for *pdr-1* where is 1:50.

ND not determined.

<sup>^</sup>For Seahorse measurement and analysis see Experimental Procedure.

**Supplementary Table SII. List of clones not selected for further analysis.**

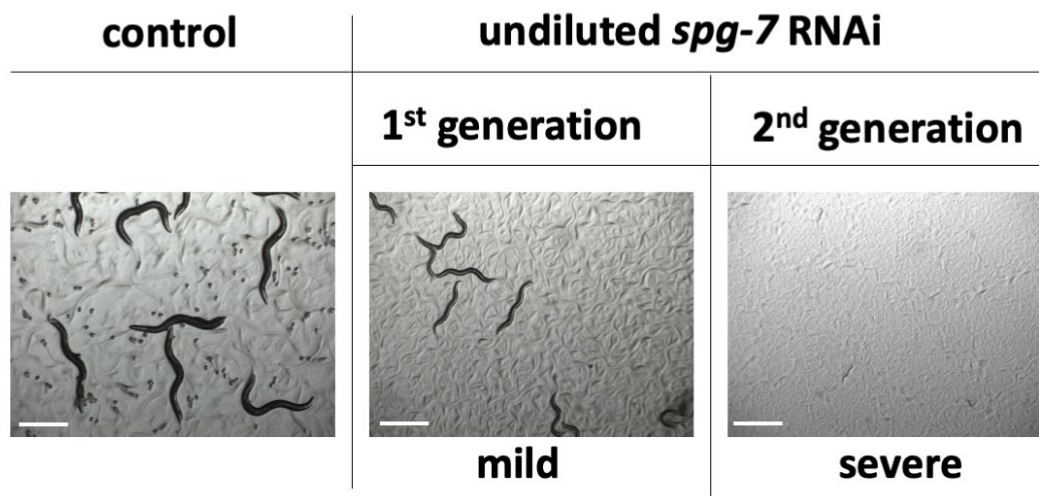
Gene/ RNAi clone	RNAi power	Lifespan change (%) <sup>a</sup>	P value vs control <sup>b</sup>	Sample size/ n trials
<i>sco-1</i> #	undiluted *	+4.38	0.06	240/3
<i>sod-1</i> #	undiluted *	- 9.91	< 0.0001	240/3
<i>cox-10</i> #	undiluted *	- 7.11	0.01	160/2
<i>fum-1</i> #	undiluted *	- 31.15	< 0.0001	160/2
<i>aco-2</i>	undiluted *	- 9.92	0.013	140/2
<i>ymel-1</i> #	undiluted *	0	0.8697	150/2
<i>drp-1</i> #	undiluted *	-1.69	0.37	140/2
<i>ogdh-1</i> #	undiluted *	-2.86	2.89	240/3
<i>hmgs-1</i> #	diluted*	-35.49	0	130/2
<i>fis-2</i> #	undiluted *	-15.21	0.0002	140/2
<i>sdha-1</i> #	undiluted *	-22.99	< 0.0001	120/2
<i>aldo-2</i>	undiluted *	NC	NC	120/2
<i>dlst-1</i> #	undiluted *	NC	NC	240/3
<i>dlat-1</i> #	undiluted *	NC	NC	180/3

<sup>a</sup> % increase normalized mean lifespan compared to control; <sup>b</sup> Kaplan-Meier survival analysis, Log-rank test against control.

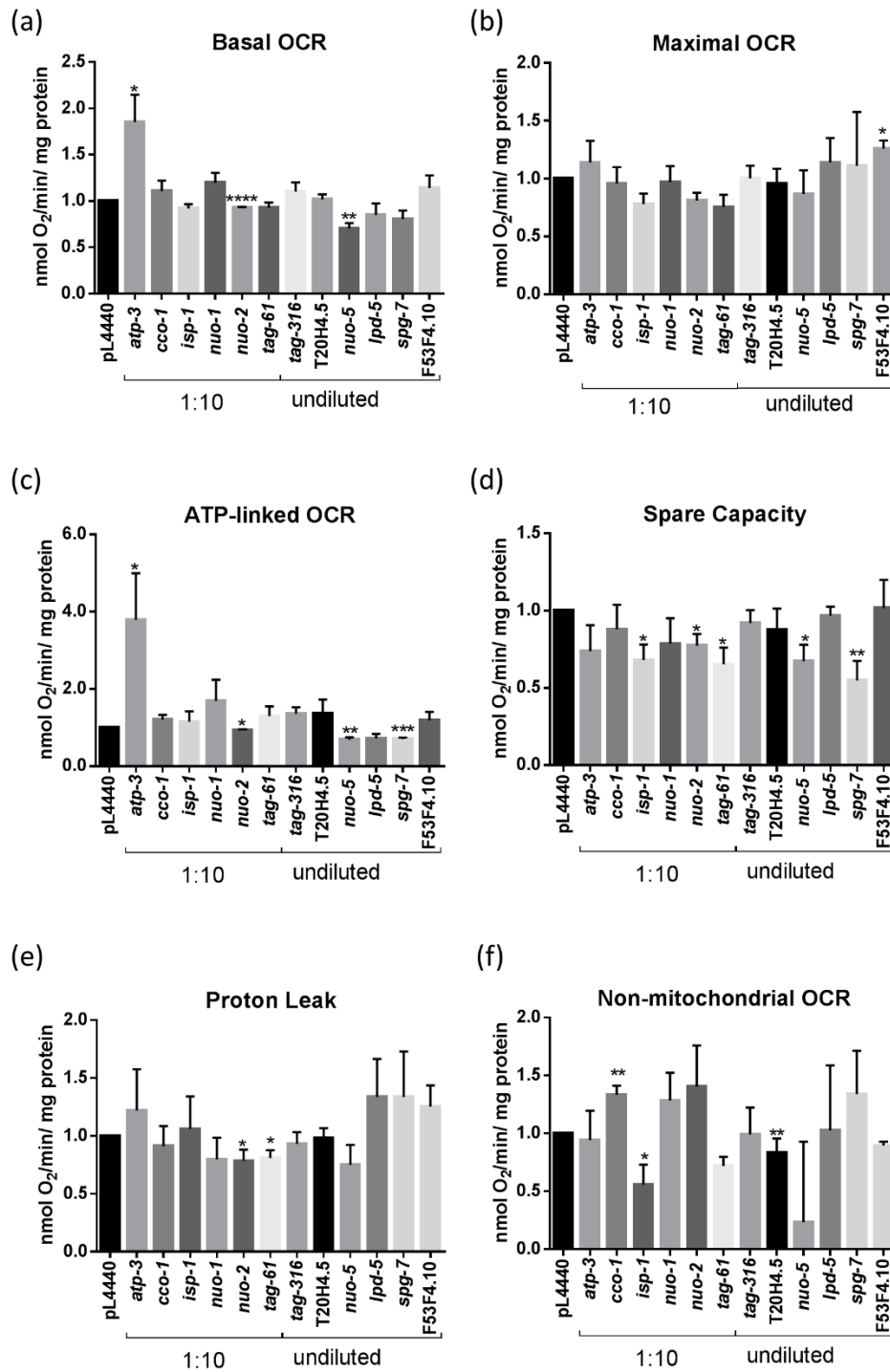
\* Diluted RNAi is 1:10 with empty vector pL4440 expressing bacteria.

# Clones that didn't produce clearly distinct mild and strong phenotypes.

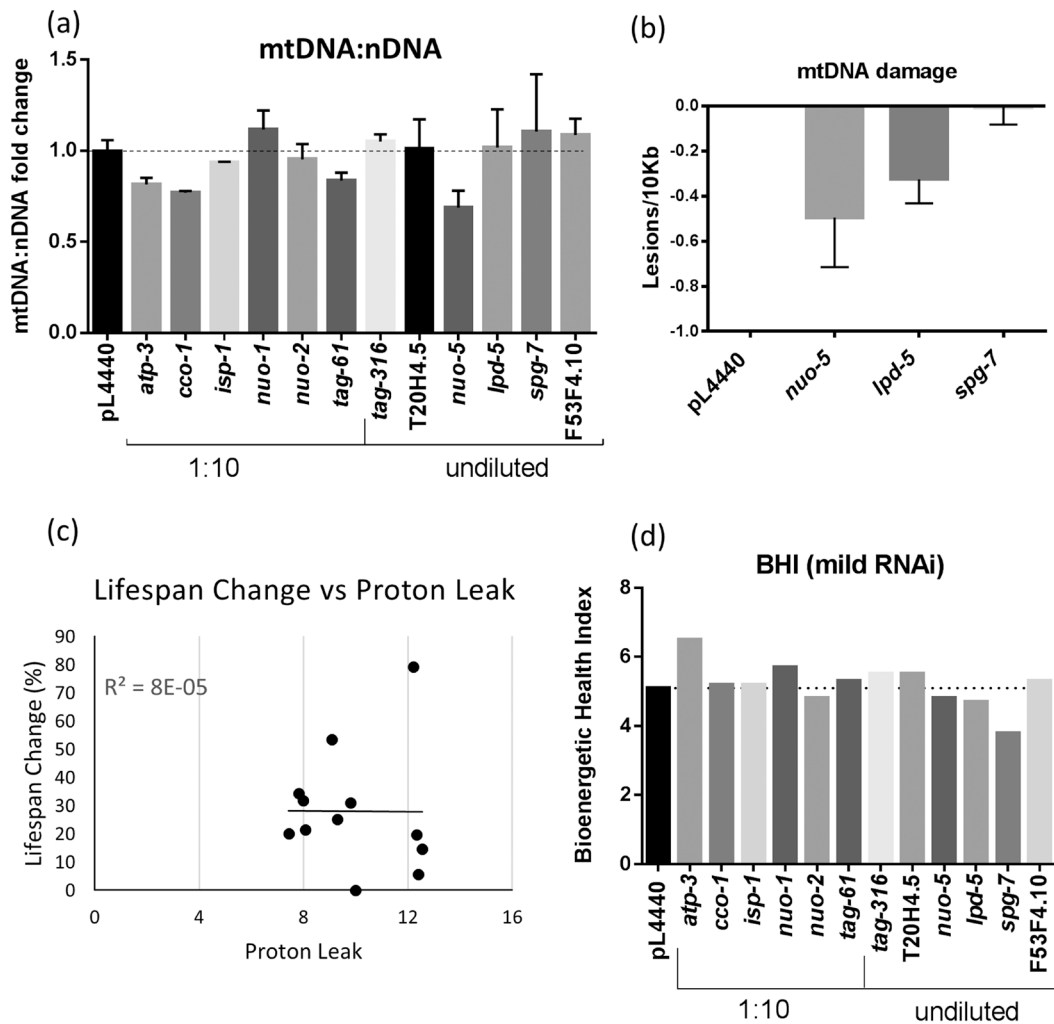
NC not consistent results on survival assay.



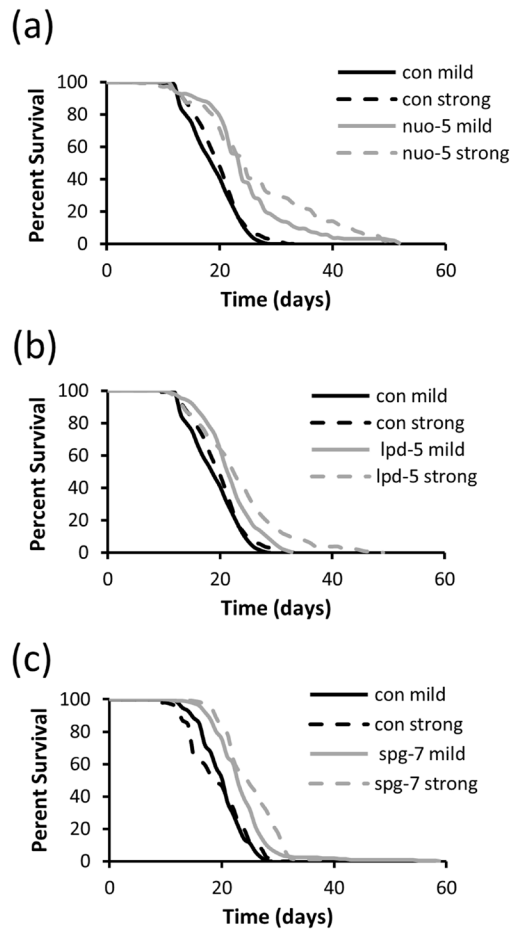
**Figure S1. Different degrees of mitochondrial stress lead to different phenotypic readouts in *C. elegans*.** Representative pictures of mild and strong phenotypes obtained in our screen of knockdown of mitochondrial proteins. HT115 bacteria expressing dsRNA targeting *spg-7* were fed to N2 (wild-type) animals in undiluted form, from the time of hatching for one (middle picture, mild) or two (right picture, severe) generations. Animals were photographed 4 days after hatching at the same magnification. Scale bar 500  $\mu$ m. Control worms were fed pL4440 (empty vector) expressing bacteria. When *spg-7* and *tag-61* are diluted 1:10 in the parental generation, similar mild phenotypes are obtained.



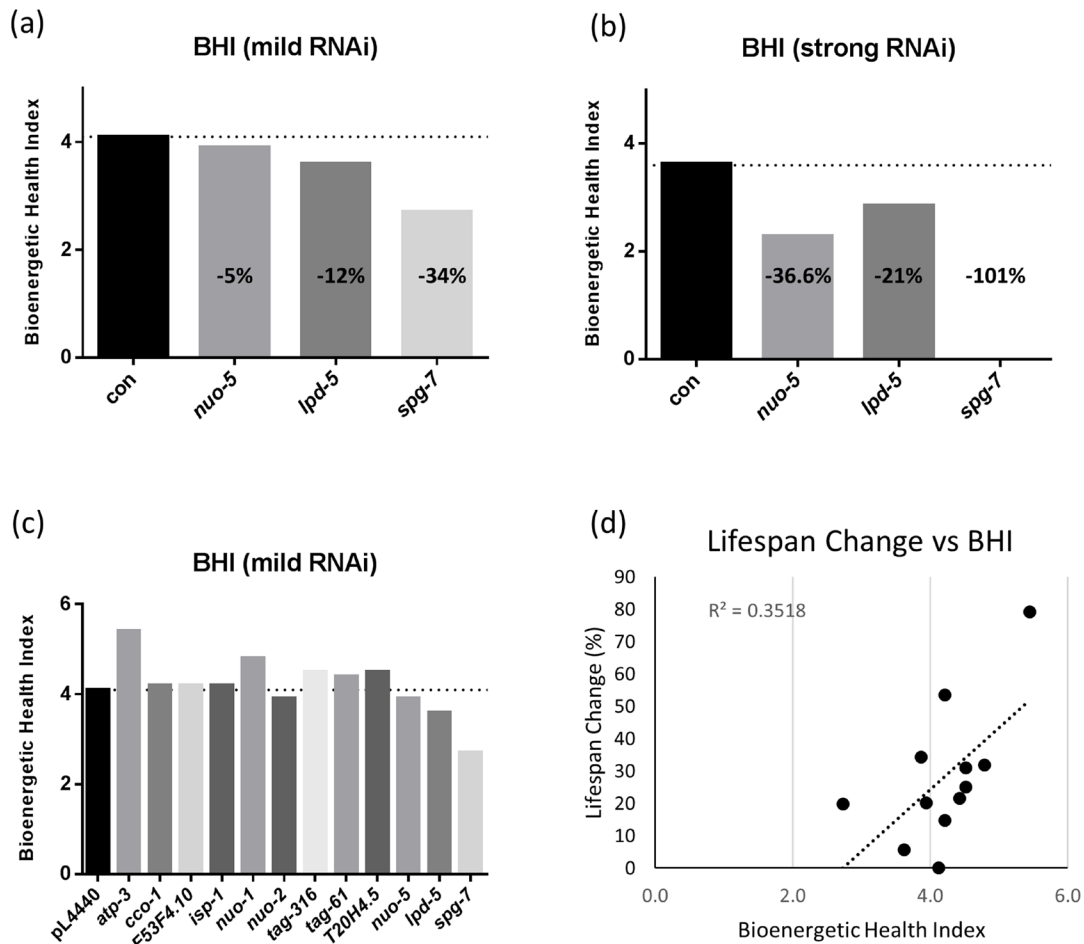
**Figure S2. Seahorse measurements of different mitochondrial parameters.** (a) Basal OCR, (b) Maximal OCR, (c) ATP-linked OCR, (d) Spare Capacity, (e) Proton Leak, and (f) Non-mitochondrial OCR. Refer to Supplementary Table S1 and supporting information for complete information.



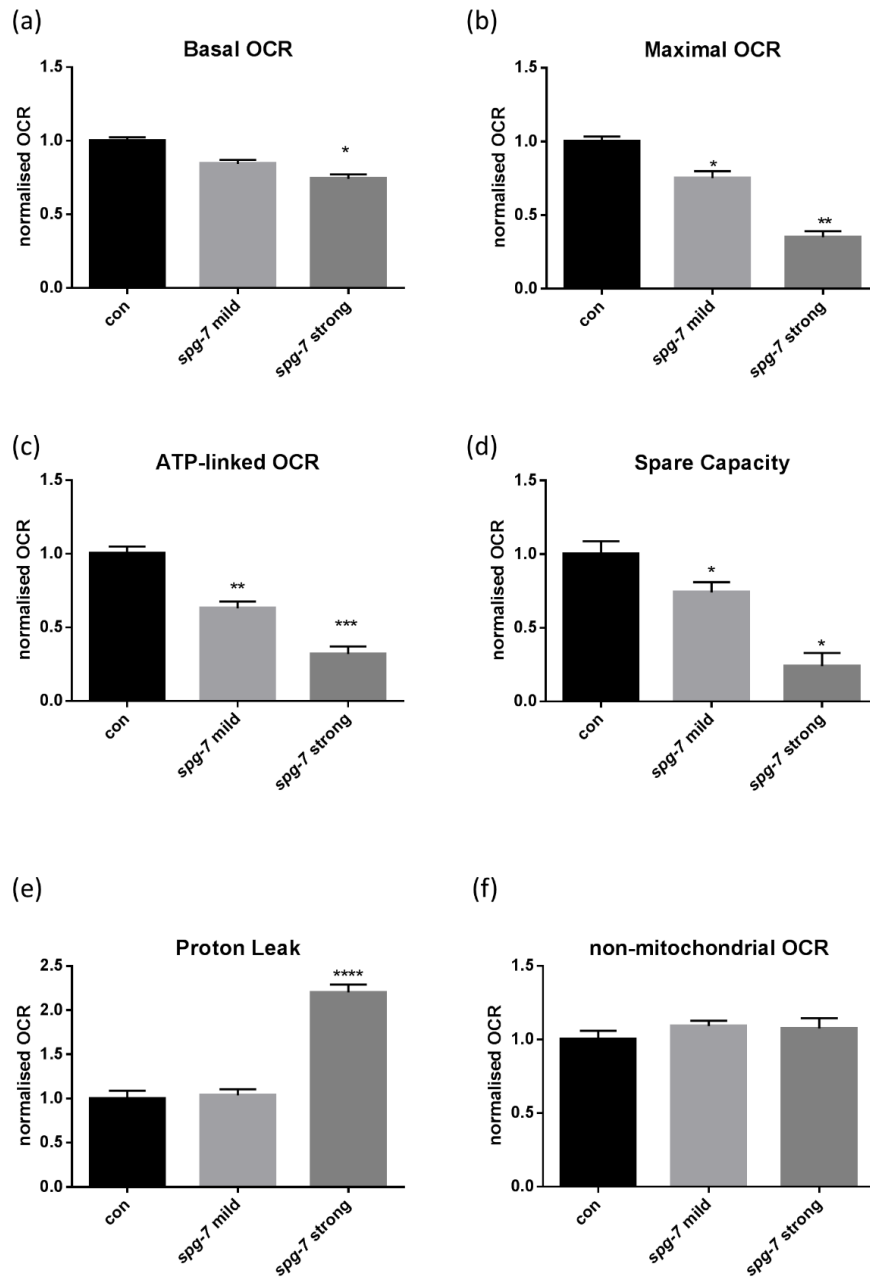
**Figure S3. Additional mitochondrial analyses.** Quantification of (a) mtDNA:nDNA ratio and (b) mtDNA damage normalized to control (empty vector pL4440) animals. The differences are not statistically significant. (c) % increase in mean lifespan plotted against proton leak in animals with mild suppression of different mitochondrial proteins. Each dot indicates an average value obtained from independent experiments with variable population sizes for each condition (for lifespan experiments an average of 60 animals per condition per replicate was used; for Seahorse experiment an average of 1500 animals per condition per replicate were employed; see Table S1 for details). (d) Bioenergetic Health Index (BHI) calculated for all clones under study (mild RNAi).



**Figure S4. Lifespan is extended upon mild and strong suppression of three mitochondrial proteins.** Kaplan-Meier survival curves of wild-type animals fed bacteria transformed with empty vector pL4440 (con) or with pL4440 vector expressing dsRNA targeting the indicated mitochondrial proteins. (a) *nuo-5*, (b) *lpd-5*, (c) *spg-7*. Mild suppression of the mitochondrial proteins is achieved in the parental (P0) generation of animals using undiluted RNAi, and strong suppression is observed in the second generation (F1). For all three clones the F1 animals arrest as L3 larvae and are therefore infertile. An average of 60 animals per condition was used in each replica and survival curves of pooled populations of animals coming from three independent replicas are shown.



**Figure S5. Alternative BHI calculation weighting proton leak more heavily.** (a-b) Bioenergetic Health Index (BHI) using the alternative equation:  $BHI = \log[(\text{spare capacity})^2 \times (\text{ATP-linked OCR})^3 / ((\text{non-mitochondrial OCR})^1 \times (\text{proton leak})^2)]$ , calculated for mild (a) and strong (b) RNAi-mediated suppression of *nuo-5*, *lpd-5* and *spg-7*. (c) % decrease of BHI compared to control is shown on the corresponding bar. BHI using the alternative equation calculated for all clones under study (mild RNAi). (d) Correlation plot between % increase in mean lifespan and BHIs (calculated with the alternative equation) in animals with mild suppression of different mitochondrial proteins.



**Figure S6. Mitochondrial respiratory parameters upon mild and strong suppression of *spg-7*.** Comparison of respiratory parameters upon *spg-7* RNAi (mild and strong), normalized to the respective controls (stage-matched animals treated with empty vector pL4440). (a) Basal OCR, (b) maximal OCR, (c) ATP-linked OCR, (d) spare respiratory capacity, (e) proton leak and (f) non-mitochondrial OCR.