Supplemental information

The NAD$^+$/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling

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Supplementary data

Extended experimental procedures

C. elegans strains and RNAi experiments

C. elegans strains were cultured at 20°C on nematode growth media agar plates seeded with *E. coli* strain OP50 unless stated otherwise. Strains used were wild-type Bristol N2, RB1042 *pme-1(ok988)* I, CF1038 *daf-16(mu86)* I, CF1139 *daf-16(mu86)* I; muEx50[*daf16*::GFP(pKL78) + *rol-6*(pRF4)], VC199 *sir-2.1(ok434)* IV, KN259 (huIs33[*sod-3*::GFP + pRF4(*rol-6*(su1006))]), MV390(*gels101*(*rol-6*(su1006)), MV389(g*els3*(P_{int}*sir-2.1*(+),(*rol-6*(su1006)), NL2099 *rrf-3(pk1426)* II, SJ4100 (zcIs13[*hsp-6*::GFP]), SJ4103 (zcIs14[*myo-3*::GFP(mit)]) and SJ4005 (zcIs4[*hsp-4*::GFP]). Strains were provided by the *Caenorhabditis* Genetics Center (University of Minnesota). MV390 and MV389 strains were outcrossed six times and were wild type for the dye-filling (dyf) mutation (Viswanathan and Guarente, 2011). *pme-1(ok988)* were outcrossed two times with N2 wild type worms.

Bacterial feeding RNAi experiments were carried out as described (Kamath et al., 2001). Clones used were *daf-16* (R13H8.1), *pme-1* (Y71F9AL.18), *qns-1* (C24F3.4), *sir-2.1* (R11A8.4) and *ubl-5* (F46F11.4). Clones were purchased from GeneService and sequenced.

Worm lifespan analysis and stress resistance assays

Lifespan tests were performed as described (Mouchiroud et al., 2011). Briefly, 60-100 animals were used per conditions and scored every other day. All lifespan experiments were performed at 20°C unless stated otherwise. Animals that crawled off the plate or had an «exploded vulva» phenotype were censored.

Treatments with PARP inhibitors— AZD2281 (also known as KU59436, olaparib), and ABT-888 (also known as veliparib)—or NAD^+ precursors—nicotinamide riboside (NR) and nicotinamide (NAM)— were added at the indicated concentration just before pouring the plates. Animals were exposed to compounds during the full life from eggs until death. To ensure a permanent exposure to the compound, plates were changed twice a week. All the compounds used in this study were dissolved in a water stock solution. Paraquat assay was performed as described (Mouchiroud et al., 2011).

Fluorescence analysis

Quantification of GFP expression and endogenous gut fluorescence were carried out according to the protocol previously described (Yamamoto et al., 2011). Briefly, GFP was monitored in day 1 or day 3 adults. Fluorimetric assays were performed using a Victor X4 multilabel plate reader (Perkin-Elmer Life Science). Eighty worms were picked at random (20 worms per well of a black-walled 96-well plate) and each well was read four times and averaged. Each experiment was repeated at least three times. Endogenous gut fluorescence was monitored at day 1, day 4 and day 8 by following the same protocol as described before.

Confocal microscopy and images processing

Worms were immobilized with 6 mM solution of tetramisole hydrochloride (Sigma) in M9 and mounted on 6% agarose pads on glass slides. Images of worms were acquired using Zeiss LSM 700 Upright confocal microscope (Carl Zeiss AG) under non-saturating exposure conditions. All the snapshots were taken from the same part of *C. elegans*: muscles from the upper part of the worm, excluding the regions of oesophagus and vulva. For each condition multiple worms were observed and imaged. Image processing was performed with the Fiji software (http://imagej.nih.gov/ij; version 1.47b). One of the critical issues during the imaging process was the position of the worm, as it influences the level of background. For the uniformity of the represented images, contrast and brightness were adjusted in order to eliminate the undesirable background signal. Neither of these manipulations was affecting the mitochondrial shape. Tracing of the mitochondrial network contour was done by the use of Gaussian blur filter followed by the application of Laplacian operator.

MitoSox staining

MitoSox staining was performed as previously described with slight modification (Yang and Hekimi, 2010). Briefly, a population of 100 worms was recovered in 1 ml of M9 buffer,

washed three times to remove residual bacteria, and resuspended in 200 µl of 1:200 MitoSox stock solution (initial stock solution was dissolved at 1mM in DMSO). After 20 minutes of treatment, worms were washed five times in 1ml of M9 buffer to eliminate the MitoSox reagent, and then transferred in a black-walled 96-well plate for reading. Fluorescence produced by the Mitosox reaction was measured as described before (Yamamoto et al., 2011).

Worm respiration assays

Oxygen consumption was measured using the Seahorse XF96 equipment (Seahorse Bioscience Inc.) as described (Yamamoto et al., 2011). Typically, 100 animals per conditions were recovered from NGM plates with M9 medium, washed three times in 2 mL M9 to eliminate residual bacteria, and resuspended in 500 µL M9 medium. Worms were transferred in 96-well standard Seahorse plates (#100777-004) (10 worms per well) and oxygen consumption was measured 6 times. Respiration rates were normalized to the number of worms in each individual well.

Quantitative real-time PCR for mRNA and DNA quantification

Total RNA was prepared using TRIzol (Invitrogen) according to the manufacturer's instructions. RNA was treated with DNase, and 2 µg of RNA was used for reverse transcription (RT). 50X diluted cDNA was used for RT-quantitative PCR (RT-qPCR) reactions. The RT-qPCR reactions were preformed using the Light-Cycler system (Roche Applied Science) and a qPCR Supermix (Qiagen) with the indicated primers. *act-1* and Y45F10D.4 were used as normalization controls. The average of at least three technical repeats was used for each biological data point. Primer sequences are shown in Table S3.

Absolute quantification of the mtDNA copy number in worms was performed by real-time PCR as previously described (Bratic et al., 2010). Relative values for *nd-1* and *act-3* were compared within each sample to generate a ratio representing the relative level of mtDNA per nuclear genome. The results obtained were confirmed with a second mitochondrial gene

MTCE.26. The average of at least three technical repeats was used for each biological data point. Primer sequences are shown in Table S3.

Mitochondrial DNA in mouse cells was quantified as described with slight modifications (Lagouge et al., 2006). Briefly, total DNA was extracted with the Nucleospin tissue kit (Macherey Nagel). Fourty nanograms of total DNA was assessed by real-time PCR using a Light Cycler 480 (Roche). The reaction was performed in a final volume of 8 µl with 1x SYBR green master mix (Roche) and 1.25µM of the reverse and forward primers. HK2 primers were used as endogenous control for nuclear DNA and 16s as marker for mitochondrial DNA. Primer sequences are shown in Table S3.

Quantification of ATP levels

Total ATP content was measured by the CellTiter-Glo luminescent cell viability assays (Promega). Typically, the luminescence was recorded with a Victor X4 plate reader (PerkinElmer) and values are normalized by the total protein concentration determined using a Bradford assay.

NAD measurement by HPLC

For NAD^+ quantification, approximately 1000 worms were collected in M9 buffer, washed five times to remove residual bacteria and flash-frozen in liquid nitrogen and stored at -80ºC until analysis. Extraction was started by adding 250 µL of 1.6M perchloric acid to frozen samples, followed by extensive homogenization in a Qiagen tissuelyser, and centrifugation at 20.000g for 15 min. The supernatant was recovered and neutralized with a 3M potassium carbonate solution. After centrifugation, 100 µl of supernatant was filtered and used for NAD measurement using an HPLC system (Agilent) with a Supelco LC-18-T column (Sigma), as described (Yoshino et al., 2011).

Generation of Sirt1 floxed ($Sirt1^{L2/L2}$) mice

For the generation of *Sirt1* floxed (*Sirt1*$^{L2/L2}$) mice, genomic DNA covering the *Sirt1* locus was amplified from the 129Sv strain by using high-fidelity PCR. The resulting DNA fragments were assembled into the targeting vector of the Institut Clinique de la Souris (Strasbourg, France). The construct in which exons 5, 6 and 7 were flanked by LoxP sites was then electroporated into 129Sv embryonic stem (ES) cells. G418-resistant colonies were selected and analyzed for homologous recombination by PCR and positive clones were verified by Southern blot hybridization. The karyotype was verified and several correctly targeted ES cell clones were injected into blastocysts from C57BL/6J mice. These blastocysts were transferred into pseudopregnant females, resulting in chimeric offspring that were mated to female C57BL/6J mice that express the Flp recombinase under the control of the ubiquitous cytomegalovirus promoter (Rodriguez et al., 2000). Offspring that transmitted the mutated allele, in which the selection marker was excised, and that lost the Flp transgene (*Sirt1*$^{L2/WT}$ mice) were then selected, and backcrossed to C57BL/6J mice for ten generations.

Cell experiments

Primary hepatocytes were isolated using collagenase perfusion as described previously with minor modifications (Dentin et al., 2004; Oosterveer et al., 2012). Hepatocytes from *Sirt1*$^{L2/L2}$ (generation of mice - see above) or C57BL/6J mice were plated in DMEM 1 g/L glucose with 10% FBS. Four hours after plating on separate dishes, hepatocytes isolated from a single congenic 10-week-old C57BL/6J male *Sirt1*$^{L2/L2}$ mouse were transduced either with an Ad-GFP or an Ad-Cre virus at a MOI=5 to generate matched *Sirt1*$^{+/+}$ (wild type) and *Sirt1*$^{-/-}$ (knock-out) hepatocytes, respectively. After overnight infection, culture medium was removed and *Sirt1*$^{+/+}$ and *Sirt1*$^{-/-}$ hepatocytes cultures were treated or not with NR or AZD2281. To obtain gain-of-Sirt1-function hepatocytes, Ad-mSirt1 (Adgene 8438) or Ad-GFP virus was used at the same MOI to infect primary hepatocytes obtained from a 10-week-old male C57BL/6J mouse to generate hepatocytes overexpressing *Sirt1* and GFP (control hepatocytes), respectively.

The mouse hepatocytes cell line AML-12 (alpha mouse liver 12) was obtained from ATCC and grown at 37°C in a humidified atmosphere of 5% CO_2/95% air in Dulbecco's Modified Eagle Medium **/** Nutrient Mixture F-12 (DMEM／F-12) supplemented with 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 5 ng/ml selenium, 40 ng/ml dexamethasone, and 10% fetal bovine serum without antibiotics.

Cells were collected after 48 hours treatment in ice-cold RIPA buffer, containing 25 mM Tris, pH7.4, 150 mM NaCl, 1% (v/v) NP-40, 1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS). NR was dissolved in water and AZD in DMSO.

Superoxide dismutase activity

AML12 cells were lysed in a buffer (150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 8.0) with protease inhibitors. Total protein concentration was determined using the Bradford assay. The protein lysates were normalized to 1ug/uL. SOD activity was determined at indicated times after NR and AZD treatment by the SOD Assay Kit (Cayman Chemical) according to the manufacturer's instructions. Absorbance was determined with a Victor X4 multilabel plate reader (Perkin-Elmer Life Science) at 450 nm. Results are expressed in U/ml according to the standard curve.

Hsp60 and Mfn2 luciferase reporter assays

Human Hsp60 (-603 to +735 for Hsp60) and MfN2 promoter fragments (-805 to +424 for Mfn2) were amplified and ligated into the pGL3 basic luciferase reporter vector (Promega, Madison, Wisconsin, USA). Primers used are HSP60-forward (5'-GACA ACGCGTAACAAAAGAGGGGCGTCAG-3'), HSP60-reverse (5'-GACACTCGAGCCCT GAGAAACCAAGTCAGC-3'), MFN2-forward (5'-GACAGGTACCCCTCCCGAGT GCACTATCAT -3') and MFN2-reverse (5'-GACA GCTAGC GCTTGACTGCATCCCAGAC -3'). The primers were tailed with MluI site (forward) and XhoI site (reverse) for Hsp60, KpnI site (forward) and NheI site (reverse) for Mfn2. Transfections of the mouse hepatocyte cell line AML-12 with these Hsp60 and Mfn2 luciferase reporters were performed in 96-well

plates using jetPEI (PolyPlus). Each well contained 30 ng of luciferase reporter and 10 ng of Renilla expression plasmid (Promega). After 6h of incubation with the DNA–jetPEI complexes, the transfection medium was exchanged for medium with or without NR and AZD. Luciferase and Renilla activity were measured with the Dual Glo luciferase assay system (Promega) in the Victor X4 (PerkinElmer).

Western blot

Poly(ADP-Ribose) detection was performed as previously described with slight modifications (Bai et al., 2011). Briefly, PAR was detected by using a monoclonal anti-PAR antibody (Alexis, Lausanne Switzerland) by Western blotting of total protein lysates, using either 50 μg of total worm protein lysates, 50 μg of liver protein lysate or 200 μg of muscle total protein extracts. PGC-1α acetylation was determined using PGC-1α immunoprecipitates from 2 mg of protein extracts, as described (Canto et al., 2009). Other immunoblots were performed with antibodies against, HSP60 (Santa Cruz Biotechnology), HSP90 (BD Transduction Laboratories), CLPP (Sigma Aldrich), SOD2 (Santa Cruz Biotechnology), β-actin, (Santa Cruz Biotechnology), ATP5A (H28O16.1 in worms; Abcam) and MTCO1/COX1 (MTCE.26 in worms; Abcam), and HRP-labeled anti-goat and anti-mouse secondary antibodies (Jackson Laboratory),

Statistics

Survival analyses were performed using the Kaplan Meier method and the significance of differences between survival curves calculated using the log rank test. Differences between two groups were assessed using two-tailed t-tests. To compare the interaction between age and genotype, two-way ANOVA tests were performed. Analysis of variance, assessed by Bonferroni's multiple comparison test, was used when comparing more than two groups. We used R (R software version 2.9.0) for the calculation of mean lifespan and SEM, and

GraphPad Prism 5 (GraphPad Software, Inc.) for all other statistical analyses. All p-values <0.05 were considered significant.

Table S1. Summary of lifespan experiments

strains and culture conditions	mean lifespan ± SE (days)	variation compared to control (%)	*P*-values against control	N(trials)
N2	14,6 ± 0,52			75/5(1)
N2 +paraquat 4mM	5,5 ± 0,11	- 62,3	$<10^{-3}$	78/2(1)
rrf-3(pk1426) empty vector	20,5 ± 0,82			56/24(1)
rrf-3(pk1426) qns-1	14,4 ± 0,57	- 29,7	$<10^{-3}$	66/14(1)
N2	19,7 ± 0,61			96/24(2)
pme-1(ok988)	25,5 ± 0,52	+ 29,4	$<10^{-3}$	91/29(2)
N2	15,7 ± 0,57			52/8(1)
pme-1(ok988) *	19,2 ± 0,53	+ 22,3	0.0001	51/9(1)
rrf-3(pk1426) empty vector	10,3 ± 0,40			56/4(1)
rrf-3(pk1426) pme-1 **	12,0 ± 0,48	+ 16,5	0.0016	51/9(1)
N2 +water	20,1 ± 0,59			138/42(3)
N2 +AZD 100nM	24,7 ± 0,55	+ 22,9	$<10^{-3}$	141/39(3)
N2 +water	17,0 ± 0,58			126/14(2)
N2 +ABT 100nM	19,8 ± 0,64	+ 16,5	0.0007	120/20(2)
N2 +water	19,8 ± 0,87			53/7(1)
pme-1(ok988) +water	27,2 ± 0,55	+ 37,4	$<10^{-3}$	49/11(1)
N2 +AZD 100 nM	22,8 ± 0,9	+ 15,2	0,02	48/12(1)
pme-1(ok988) +AZD 100nM	26,1 ± 0,78	+ 31,2	ns [a]	49/11(1)
N2 +water	18,5 ± 0,54			121/19(2)
N2 +NAM 200µM	22,3 ± 0,68	+ 20,5	$<10^{-3}$	95/45(2)
N2 +water	19,5 ± 0,51			188/52(4)
N2 +NR 500µM	23,5 ± 0,56	+ 20,1	$<10^{-3}$	172/68(4)
N2 +water	16,1 ± 0,64			75/5(1)
N2 +AZD 100nM	19,7 ± 0,79	+ 22,3	0,0004	71/9(1)
N2 + NR 500µM	18,7 ± 0,80	+ 16,1	0,01	69/11(1)
N2 +AZD +NR	18,6 ± 0,74	+ 15,5	0,02	63/17(1)
N2 +water	20,1 ± 0,64			74/6(1)
N2 +AZD 10nM	21,5 ± 0,65	+ 6,9	ns	76/4(1)
N2 + NR 100µM	21,1 ± 0,68	+ 4,9	ns	75/5(1)
N2 +AZD +NR	22,3 ± 0,66	+ 11,0	0,01	70/10(1)
N2 +water	20,5 ± 0,737			83/37(2)
sir-2.1(ok434) +water	22,9 ± 0,624	+ 11,7	ns	88/32(2)
N2 +AZD 100nM	24,7 ± 0,669	+ 20,5	$<10^{-3}$	89/31(2)
sir-2.1(ok434) +AZD 100nM	21,9 ± 0,528	+ 6,7	ns [b]	84/36(2)
N2 +water	20,7 ± 0,80			79/41(2)
sir-2.1(ok434) +water	23,4 ± 0,67	+ 13,0	ns	80/40(2)
N2 +NR 500µM	24,9 ± 0,68	+ 20,3	0,0008	86/34(2)
sir-2.1(ok434) +NR 500µM	23,2 ± 0,61	+ 12,0	ns [b]	76/44(2)
N2 +water empty vector	20,0 ± 0,62			40/40(1)
N2 +AZD empty vector	22,0 ± 0,50	+ 10,0	0,0076	36/44(1)
N2 +water *ubl-5* RNAi	16,9 ± 0,64	- 15,5	0,0011	47/33(1)
N2 +AZD *ubl-5* RNAi	16,7 ± 0,62	- 16,5	$<10^{-3}$ [c]	47/33(1)

N2 +water empty vector	17,5 ± 0,67			51/29(1)
N2 +NR empty vector	20,6 ± 0,63	+ 17,7	0,0006	30/50(1)
N2 +water *ubl-5* RNAi	17,6 ± 0,57	+ 0,5	ns	45/35(1)
N2 +NR *ubl-5* RNAi	16,9 ± 0,61	- 3,4	$<10^{-3}$ [d]	50/30(1)
N2 +water	21,7 ± 1,24			30/30(1)
daf-16(mu86) +water	17,5 ± 0,69	- 19,3	0,0009	36/24(1)
N2 +AZD 100nM	26,5 ± 0,77	+ 22,1	0,004	41/19(1)
daf-16(mu86) +AZD 100nM	18,2 ± 0,76	- 16,1	ns [e]	37/23(1)
N2 +water	20,7 ± 0,80			79/41(2)
daf-16(mu86) +water	17,1 ± 0,47	- 17,4	$<10^{-3}$	76/44(2)
N2 +NR 500µM	24,9 ± 0,68	+ 20,3	0,0008	86/34(2)
daf-16(mu86) +NR 500µM	19,1 ± 0,51	- 7,8	0,01 [e]	88/32(2)
MV390 +water	17,6 ± 0,61			98/22(2)
MV389 +water	23,0 ± 0,57	+ 30,7	$<10^{-3}$	106/14(2)
MV390 +NR 500µM	20,1 ± 0,61	+ 14,2	0,0081	89/51(2)
MV389 +NR 500µM	23,6 ± 0,54	+ 34,1	$<10^{-3}$	92/48(2)
MV390 empty vector	18,9 ± 0,63			51/29(1)
MV389 empty vector	24,7 ± 0,55	+ 30,7	$<10^{-3}$	30/50(1)
MV390 *sir-2.1* RNAi	18,5 ± 0,65	- 2,1	ns	45/35(1)
MV389 *sir-2.1* RNAi	20,8 ± 0,69	+ 10,0	0,0004 [f]	50/30(1)
MV390 *daf-16* RNAi	14,8 ± 0,44	- 21,7	$<10^{-3}$	42/38(1)
MV389 *daf-16* RNAi	16,0 ± 0,41	- 15,3	$<10^{-3}$ [f]	47/33(1)
MV390 empty vector	17,6 ± 0,56			95/45(2)
MV389 empty vector	23,7 ± 0,56	+ 34,7	$<10^{-3}$	94/46(2)
MV390 *ubl-5* RNAi	17,8 ± 0,47	+ 1,1	ns	94/46(2)
MV389 *ubl-5* RNAi	19,9 ± 0,52	+ 13,0	$<10^{-3}$ [f]	97/43(2)

Table S2. Summary of lifespan experiments

strains and culture conditions	mean lifespan ± SE (days)	variation compared to control (%)	*P*-values against control	N(trials)
N2 +water	18,3 ± 0,54			122/18(2)
N2 +AZD 100nM	22,5 ± 0,62	+ 22,9	$<10^{-3}$	112/28(2)
N2 +AZD 500nM	20,6 ± 0,60	+ 12,5	0,008	112/28(2)
N2 +AZD 1000nM	21,2 ± 0,96	+ 15,8	0,01	53/27(1)
N2 +water	17,0 ± 0,58			126/14(2)
N2 +ABT 100nM	19,3 ± 0,62	+ 13,5	0.02	124/16(2)
N2 +ABT 500nM	19,8 ± 0,64	+ 16,5	0.0007	120/20(2)
N2 +ABT 1000nM	18,1 ± 0,66	+ 6,5	ns	123/17(2)
N2 +water	17,7 ± 0,49			121/19(2)
N2 +NR 200μM	20,7 ± 0,55	+ 16,9	$<10^{-3}$	104/36(2)
N2 +NR 500μM	20,6 ± 0,54	+ 16,4	$<10^{-3}$	106/34(2)
N2 +NR 1000μM	19,8 ± 0,49	+ 11,8	0,004	123/17(2)
N2 +water	18,5 ± 0,54			121/19(2)
N2 +NAM 200μM	22,3 ± 0,68	+ 20,5	<10-3	95/45(2)
N2 +NAM 500μM	22,5 ± 0,55	+ 21,6	<10-3	120/20(2)
N2 +NAM 1000μM	20,7 ± 0,51	+ 11,9	0,01	120/120(2)

Table S3. Q-RT-PCR primer list

Gene name	Sequence
sir-2.1-fw	TTCTTGTGTTGACTGGCGCT
sir-2.1-rv	GTCCTGGGAAGATTTCTCTCG
cts-1-rv	GGTACAGGTTGCGATAGATGATAGC
cts-1-fw	CTCGACAACTTCCCAGATAACC
cox-4-rv	AGGTTGGCGGCAGTTCTGGG
cox-4-fw	GCCCCAATTCGCGCCAAGGA
hxk-1-rv	CTAGAGATGACGTCACACACTTCTC
hxk-1-fw	GTGCGACGAGTACTTTCTCAACTG
pyc-1-rv	GTGATCATACATCCTGGTCTACTGC
pyc-1-fw	TCCAACTACTCCTCTTGCTACTGAC
fzo-1-rv	AGTCGGCATTCCCCTGATTCCG
fzo-1-fw	TCTGCAGGTTGAAGGTTCAGAAGGC
opa-1-rv	CTTGGCCATCCATTCTGCCCA
opa-1-fw	TCGCGGCTAGAACGTGGTATGA
drp-1-rv	ACCGGTTGTAGCTGCGCTCT
drp-1-fw	AGGCTGAAGCCCACCAATGAGC
prdx-2-rv	GGGTCGATGATGAAGAGTCCACGG
prdx-2-fw	TTGTGCTCGCCGCTTCCACC
gst-4-rv	CCAGCGAGTCCAAATTTTCTTGCCA
gst-4-fw	TGCTCTTGCTGAGCCAATCCGT
sod-1-rv	ACTGGGGAGCAGCGAGAGCA
sod-1-fw	CCGACACGCTCGTCACGCTT
sod-2-rv	TCCTTTGGAGACCGCCTCGTGA
sod-2-fw	ACAGGAGTCGCTGCTGTTCGC
hsf-1-rv	GGTGGATGAGGTGGAAGTCG
hsf-1-fw	GAATGCGACTAGGCAAATGGC
hsp-4-rv	TGCTGGCACGGTGACAACGG
hsp-4-fw	GCACTGGCCGTTCAAGATCGTCG
sod-3-fw	CTAAGGATGGTGAACCTTCA

sod-3-rv	CGCGCTTAATAGTGTCCATCAG
hsp-6-fw	AACCACCGTCAACAACGCCG
hsp-6-rv	AGCGATGATCTTATCTCCAGCGTCC
act-1-fw	GCTGGACGTGATCTTACTGATTACC
act-1-rv	GTAGCAGAGCTTCTCCTTGATGTC
Y45F10D.4-fw	GTCGCTTCAAATCAGTTCAGC
Y45F10D.4-rv	GTTCTTGTCAAGTGATCCGACA
HK2-Forward	GCCAGCCTCTCCTGATTTTAGTGT
HK2-Reverse	GGGAACACAAAAGACCTCTTCTGG
16s-Forward	CCGCAAGGGAAAGATGAAAGAC
16s-Reverse	TCGTTTGGTTTCGGGGTTTC
act-3-rv	GGTGGTTCTCCGGAAAGAA
act-3-fw	TGCGACATTGATATCCGTAAGG
nd-1-rv	AAGCTTGTGCTAATCCCATAAATGT
nd-1-fw	AGCGTCATTTATTGGGAAGAAGAC
MTCE.26-rv	CAGGGTGCCCCATTGTTCTT
MTCE.26-fw	GGTTGTGGGACTAGGTGAACA

Figure legends

Figure S1. PARP activity and NAD^+ in aged mammals and worms. Related to Figure 1.

(A) Total protein PARylation was evaluated in liver and muscle of young (6 months) and aged (24 months) C57BL/6J mice, and was accompanied by (B) decreased NAD^+ levels, and (C) PGC-1α hyperacetylation.

Bar graphs are expressed as mean±SEM, *** $p \leq 0.001$.

Figure S2. Lifespan analyses with different concentrations of PARP inhibitors or NAD^+ precursors. Related to Figures 1 and 2.

(A-B) Worm lifespan was measured after treatment with PARP inhibitors AZD2281 (A) or ABT-888 (B) at the indicated concentrations.

(C-D) Worm lifespan was measured after treatment with NAD^+ precursors NR (C) or NAM (D) at the concentrations indicated.

See Table S1 and S2 for statistics.

Figure S3. The NAD precursor NAM increases NAD^+ levels, respiration and mitochondrial content. Related to Figure 2.

(A) 100 nM ABT-888 increased NAD^+ levels in *C. elegans* at day 3 of adulthood.

(B) 200 µM NAM increased NAD^+ levels in *C. elegans* at day 3 of adulthood.

(C) Oxygen consumption was increased in day 3 adult worms after NAM.

Bar graphs are expressed as mean±SEM, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Figure S4. Early and late phase stress response after *pme-1* RNAi. Related to Figures 3 and 4.

(A) At day 1 of adulthood, *pme-1* RNAi activates UPR^{mt} response (*hsp-6*::GFP) but not ROS defense (*sod-3*::GFP).

(B) At day 3 of adulthood, *pme-1* RNAi activates both UPR^{mt} response (*hsp-6*::GFP) and ROS defense (*sod-3*::GFP).

Bar graphs are expressed as mean±SEM, * $p \leq 0.05$; ** $p \leq 0.01$.

Figure S5. The NAD precursor NAM induces paraquat resistance and *daf-16* nuclear translocation. Related to Figure 4.

(A) Supplementation of the NAD^+ precursor NAM (200 μM) increases lifespan of wild type N2 worms treated with 4 mM paraquat.

(B) Representative images of *daf-16::*GFP reporter worms treated with either vehicle or NAM, showing nuclear accumulation of *daf-16* following treatment.

(C) Quantification of *daf-16* nuclear translocation following treatment with NAM.

Bar graphs are expressed as mean±SEM, *** $p \leq 0.001$.

Figure S6. Summary of the role of NAD^+ in lifespan control.

Scheme summarizing how we hypothesize that NAD^+ precursors and PARP inhibitors increase lifespan through activation of *sir-2.1*, *ubl-5*, and *daf-16*.

In the normal unstressed situation (left panel) *sir-2.1* controls normal lifespan through 1) maintaining normal mitochondrial function, and 2) maintaining proper *daf-16* signaling. If this axis is disturbed, for instance in *sir-2.1* or *daf-16* mutants, lifespan is shortened.

In the *sir-2.1* gain-of-function situation (right panel), achieved by either *sir-2.1* overexpression or increasing the load of its substrate NAD^+ (as happens in caloric restriction) (Canto et al., 2009; Canto et al., 2012; Chen et al., 2008), lifespan is extended. In this situation, *sir-2.1* induces mitochondrial biogenesis leading to mitonuclear protein imbalance, i.e. the production of proteins encoded by nDNA is not matched to that of mtDNA. The mitonuclear protein imbalance induces the protective UPR^{mt}, which in turn is essential for longevity. At the same time, the *daf-16*/*sod-3* pathway is induced to protect against ROS, but it is not fully understood how this pathway is activated.

Table legends

Table S1. Summary of lifespan experiments

Summary of mean lifespan and statistical analysis (*P*-values) for lifespan experiments including all RNAi feeding conditions and different treatments displayed in Figures 1D-I, 2B, 3E-F, 4H, 5A, 5C-D, 5H. *P*-values from a log rank test comparing RNAi treatment population to the vector control or to specific groups ([a]versus *pme-1(ok988)* +water; [b]*sir-2.1(ok434)* +water; [c]N2 +AZD empty vector; [d]N2 +NR empty vector; [e]*daf-16(mu86)* +water; [f]MV389 empty vector). *P*-values less than 0,05 are considered statistically significant, demonstrating that the two lifespan populations are different. The total number of individuals scored is shown followed by the total number of censored worms and the total number of independent experiments. *the *pme-1(ok988)* strain used in this experiment was backcrossed two times and compared with the corresponding wild type. **this experiment was performed at 25°C.

Table S2. Summary of lifespan experiments using different treatments with AZD, ABT, NR and NAM

Summary of mean lifespans and statistical analysis (*P*-values) for lifespan experiments including different treatments with AZD, ABT, NR and NAM displayed in Figures 1I-J, 2A-B, S2A-D. *P*-values less than 0,05 are considered statistically significant, demonstrating that the two lifespan populations are different. The total number of individuals scored is shown followed by the number of individuals censored due to bursting vulva or crawling off the agar. The total number of individuals scored is shown followed by the total number of independent experiments.

Table S3. Q-RT-PCR primer list

Summary of Q-RT-PCR primer sequences used for experiments displayed in Figures 2H, 2J, 3B, 4B, 4D, 4D-E, 5B-C, 5G, 6A-B.

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