

Supplementary Information

Role of Sirtuins in Lifespan Regulation is Linked to Methylation of Nicotinamide

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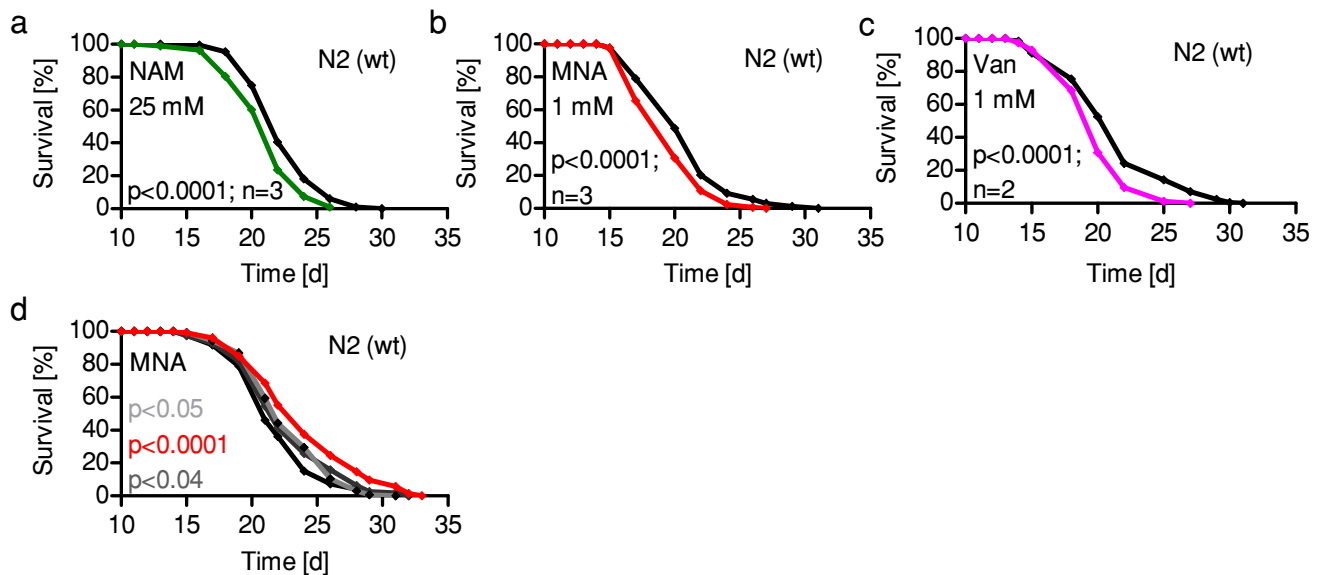
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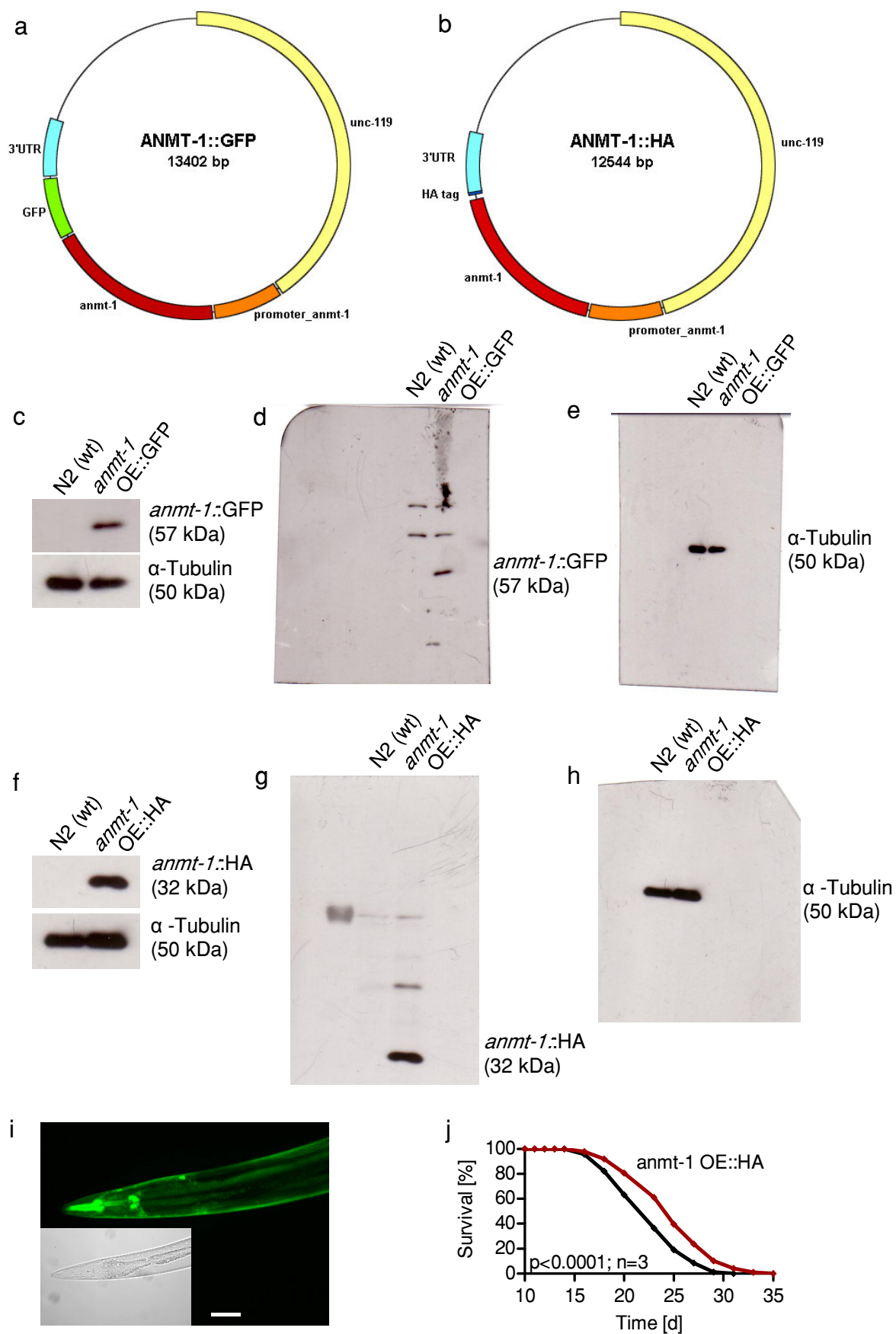
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Supplementary Figure 1



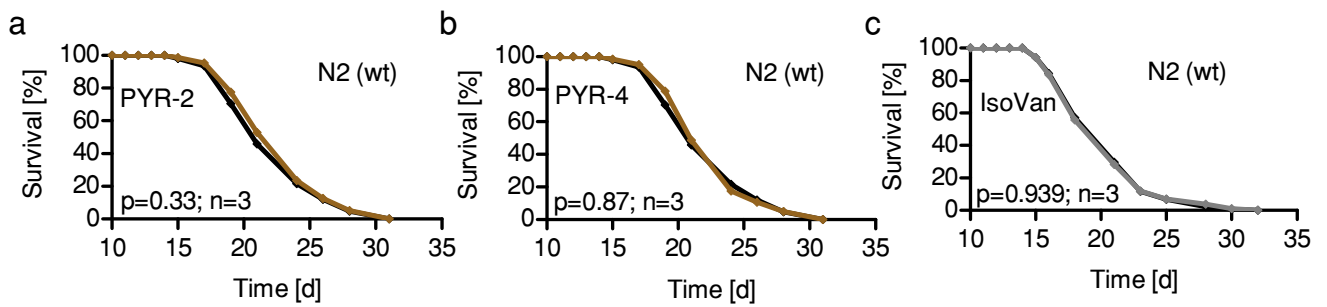
Suppl. Fig. 1: Effects of different high-dose supplements on *C. elegans* lifespan

a Lifespan analyses of wildtype (wt) nematodes exposed to 25 mM nicotinamide (green) compared with untreated worms (black). **b** Lifespan analyses of wt nematodes exposed to 1 mM 1-methylnicotinamide (MNA, red). **c** Lifespan analyses of wt nematodes exposed to 1 mM vanillin (pink). **d** Lifespan analyses of wt nematodes exposed to 0.1 μ M MNA (light grey, $n=3$), 1 μ M MNA (red, $n=6$), and 10 μ M MNA (dark grey, $n=3$).

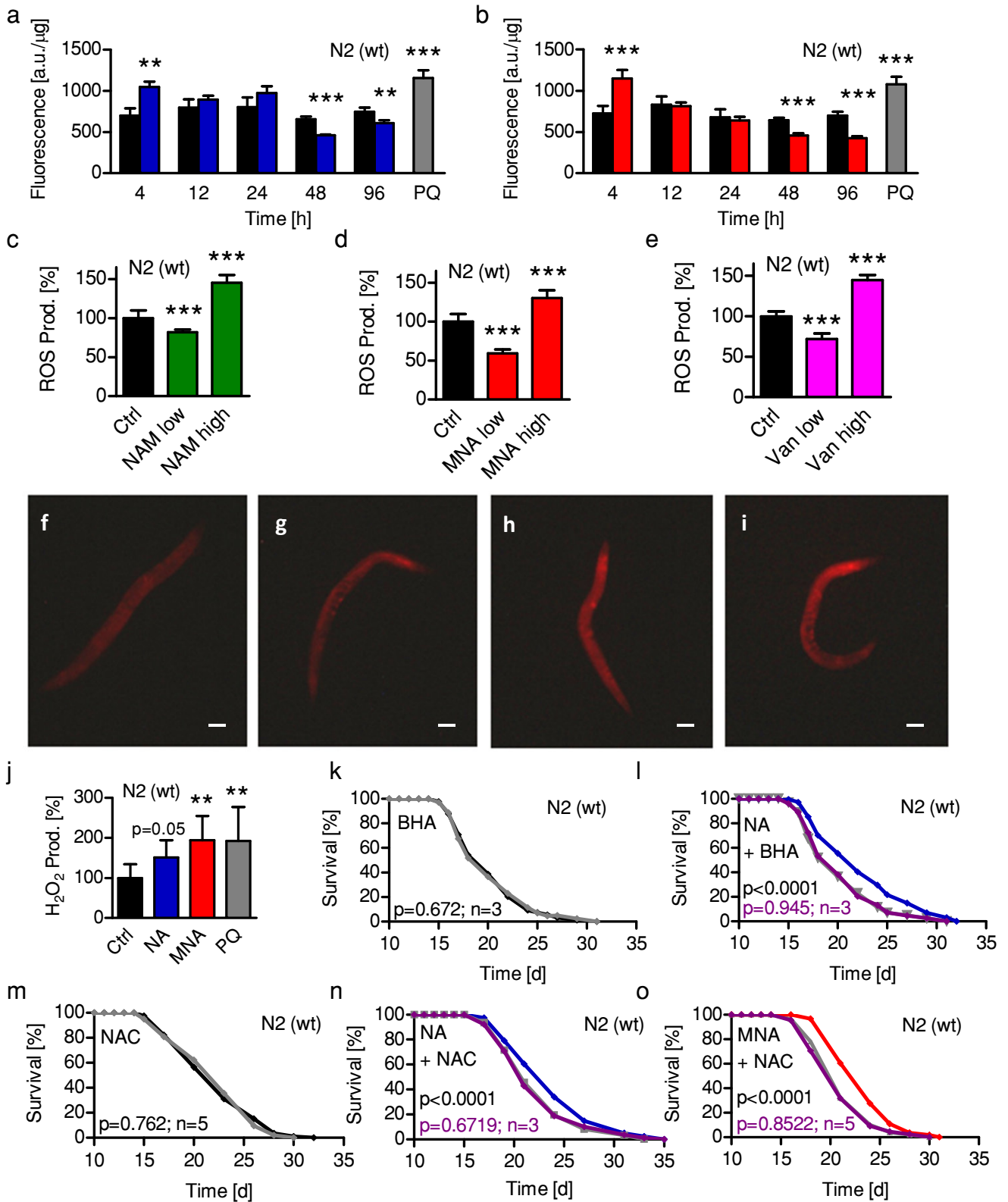


Suppl. Fig. 2: Overexpressing 1-nicotinamide-methyltransferase/ANMT-1

a Plasmid with GFP marker. **b** Plasmid with hemagglutinin (HA) marker. Plasmid maps were generated with Plasm V 2.0.4.29. **c – e** Immunoblotting against GFP in wild-type (wt) nematodes and overexpressors of *anmt-1* with a C-terminally fused GFP tag (*anmt-1* OE::GFP). **f – h** Immunoblotting against hemagglutinin in wt nematodes and overexpressors of *anmt-1* with a C-terminally fused HA tag (*anmt-1* OE::HA). Immunoblotting against α -tubulin was used to normalize protein amount. **i** Expression pattern of GFP as a fused surrogate marker of ANMT-1 protein expression in the head of *anmt-1* OE::GFP nematodes. Scale bar, 50 μ m. **j** Lifespan analyses of *anmt-1* OE::HA (dark red) compared with wt nematodes (black).

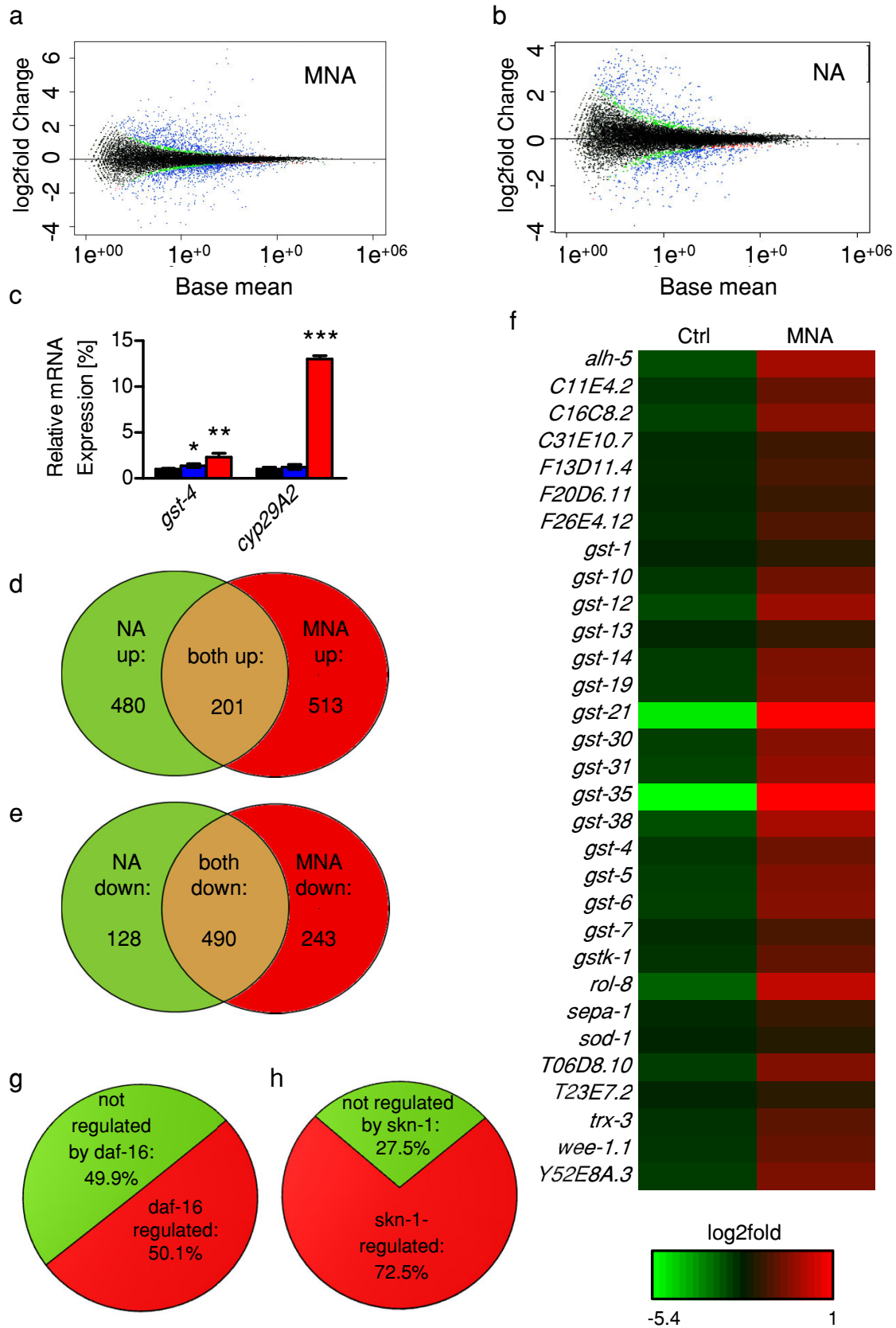


Suppl. Fig. 3: Effects of aldehyde oxidase/GAD-3 products and an inhibitor on *C. elegans* lifespan
a Lifespan analyses of wild-type (wt) nematodes exposed to 1 μ M of the 1-methylnicotinamide (MNA) metabolite 1-methyl-2-pyridone-5-carboxamide (PYR-2; brown). **b** Lifespan analyses of wt nematodes exposed to 1 μ M of the MNA metabolite 1-methyl-4-pyridone-5-carboxamide (PYR-4; brown). **c** Lifespan analyses of wt nematodes exposed to 1 μ M of the AOX1/GAD-3 inhibitor iso-vanillin (IsoVan; grey).



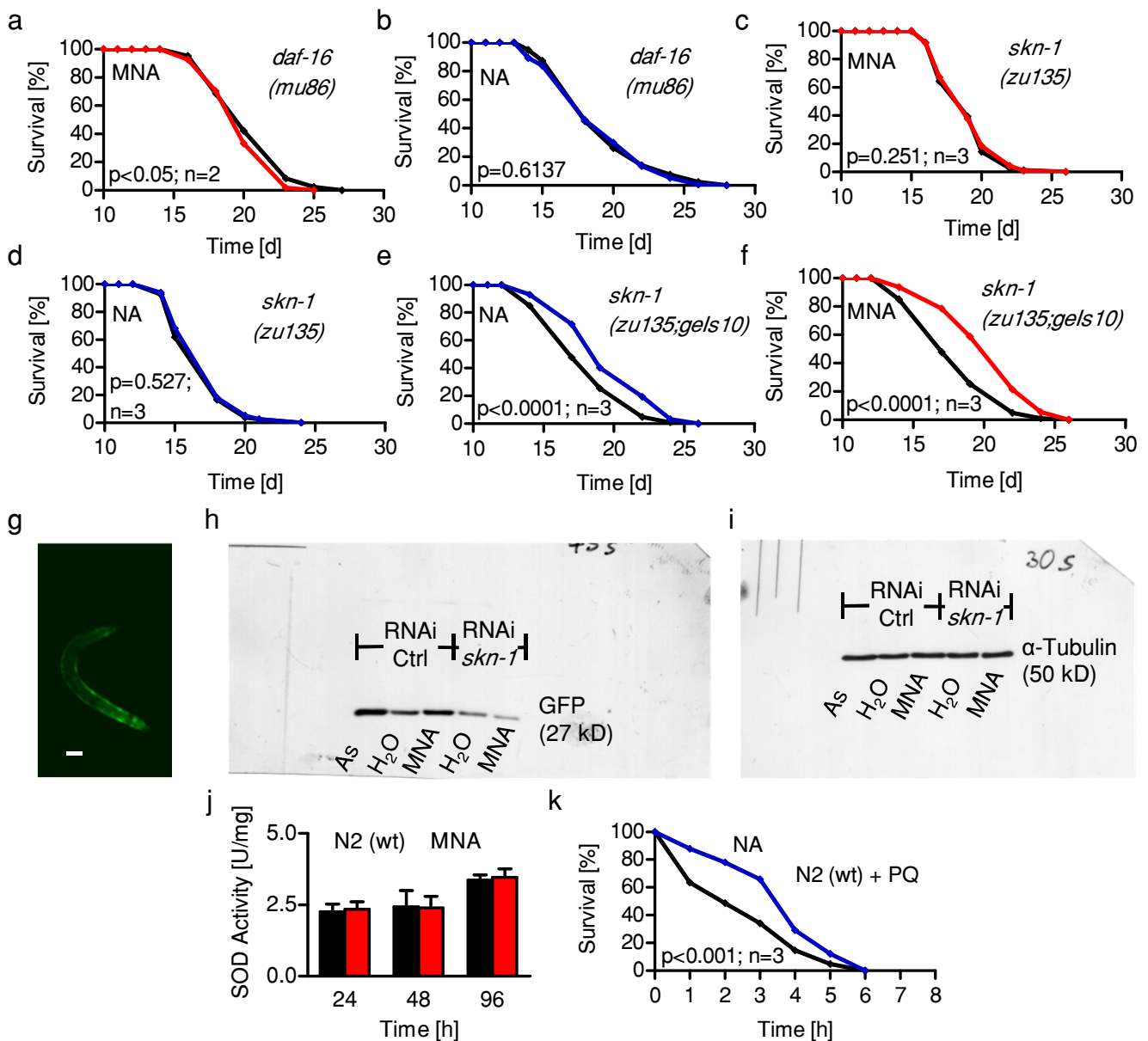
Suppl. Fig. 4: ROS production and effects of nicotinic acid (NA) and its metabolites in presence and absence of N-acetyl-cysteine (NAC) and butylated hydroxyanisole (BHA)

a ROS levels following 1 mM NA (blue bars) exposure compared with untreated wild-type (wt) nematodes (black bars) at different time points (absolute fluorescence). **b** ROS levels following 1 μ M 1-methylnicotinamide (MNA; red bars) exposure. **c** ROS levels in wt nematodes following 96 hrs exposure to 100 μ M nicotinamide (NAM low) and 25 mM NAM (NAM high; green bars). **d** ROS levels in wt nematodes following 96 hrs exposure to 1 μ M MNA (MNA low) and 1 mM MNA (MNA high; red bars). **e** ROS levels in wt nematodes following 7 days exposure to 1 μ M vanillin (Van low) and 1 mM vanillin (Van high; pink bars). **f** Fluorescent microphotographs of MitoTracker Red CM-H2X-treated wt nematodes **f** after 4 hr on control plates, **g** after 4 hr exposed to NA, **h** after 4 hr exposed to MNA and **i** after 1 hr PQ treatment. Scale bar, 100 μ m. **j** H_2O_2 production following 4 hrs exposure to 1 mM NA and 1 μ M MNA. Data represent mean values with standard deviation of at least 2 independent experiments. **k** Lifespan analyses of wt nematodes exposed to 10 μ M BHA (grey) compared with untreated worms (black). **l** Lifespan analyses of wt nematodes exposed to NA in presence (purple) or absence of BHA (blue) compared with BHA-treated worms. **m** Lifespan analyses of wt nematodes exposed to 1 mM NAC (grey). **n** Lifespan analyses of wt nematodes exposed to NA in presence (purple) or absence of NAC (blue) compared to NAC-treated worms. **o** Lifespan analyses of wt nematodes exposed to MNA in presence (purple) or absence of NAC (red) compared to NAC-treated worms.



Suppl. Fig. 5: Transcriptome analyses identify DAF-16/FOXO and SKN-1/NRF-2 signaling as molecular pathways for MNA-mediated lifespan extension

a Differentially expressed genes as quantified by deep sequencing analysis after 48 hr treatment of wild-type nematodes with 1 μ M 1-methylnicotinamide (MNA) in comparison with untreated nematodes. Black dots indicate no differential regulation, red and green dots indicate regulation according to one statistical method only. Blue dots indicate regulation according to both statistical methods (used for further analysis). **b** Differentially expressed genes after 48 hr treatment of wt nematodes with 1 mM nicotinic acid (NA). **c** Relative mRNA levels of *gst-4* and *cyp29A2* followed 48 hr treatment of wt nematodes with NA and MNA. Data represent mean values with standard deviation of 3 independent experiments. **d** and **e** Similar regulated genes by NA and MNA identified by Venn analyses, **d** upregulated and **e** downregulated genes. **f** Regulation of genes associated with oxidative stress response after 48 hr MNA treatment in comparison with untreated control (cutoff for two sets, $p = 0.05$). **g** and **h** Promoter analyses identified 50.1 and 72.5 per cent of all differentially expressed genes after MNA treatment as under transcriptional control of **g** DAF-16 and **h** SKN-1, respectively.



Suppl. Fig. 6: Additional findings on downstream signaling and parameters of stress resistance

a Lifespan analyses of *daf-16(mu86)* nematodes exposed to 1 μ M 1-methylnicotinamide (MNA; red) compared with untreated worms (black). **b** Lifespan analyses of *daf-16(mu86)* nematodes exposed to 1 mM nicotinic acid (NA; blue). **c** Lifespan analyses of *skn-1(zu135)* nematodes exposed to 1 μ M MNA. **d** Lifespan analyses of *skn-1(zu135)* nematodes exposed to 1 mM NA (blue). **e** Lifespan analyses of intestine-reconstituted *skn-1(zu135;gels10)* nematodes exposed to 1 mM NA. **f** Lifespan analyses of intestine-reconstituted *skn-1(zu135;gels10)* nematodes exposed to 1 μ M MNA. **g** Fluorescent microphotograph of untreated *gst-4::GFP* nematodes. Scale bar, 100 μ m. **h** and **i** Western Blot against GFP (**h**) as a surrogate marker of GST-4 protein expression in *gst-4::GFP* nematodes and α -tubulin (**i**) in the presence or absence of RNAi against *skn-1* after 48 hr MNA treatment compared with untreated nematodes. 48 hr arsenite (As) treatment acts as positive control. **j** Activity of superoxide dismutase in wild-type (wt) nematodes exposed to 1 μ M MNA (red bars) in comparison with untreated nematodes (black bars) at different time points. Data represent mean values with standard deviation of 3 independent experiments. **k** Survival of wt nematodes in liquid medium containing 50 mM Paraquat after 7 days NA (blue) exposure in comparison with untreated nematodes (black). Data were expressed as mean values of 2 independent experiments and 50 examined nematodes/condition each.

Supplemental Table 1: Results and statistical analyses of *C. elegans* lifespan assays

Strain, RNAi, Substance, Solvent	Maximum Life Span [d] [+/- SEM] ¹	Mean Life Span [d] [+/- SEM]	P-Value (vs. control, see footnotes)	Number of Experiments [n]	Number of Nematodes [n]
N2 H ₂ O	25.0 +/-1.4	22.57 +/-0.3		6	565
N2 NA/H ₂ O	26.3 +/-1.7	24.04 +/-0.3	<0.0001 ^a	6	600
N2 H ₂ O	24.3 +/-0.5	22.59 +/-0.1		6	655
N2 NAM/H ₂ O	25.7 +/-0.5	24.02 +/-0.2	<0.0001 ^a	6	513
N2 NAM high/H ₂ O	24.0 +/-0.9	20.90 +/-0.2	<0.0001 ^a	3	337
N2 H ₂ O	24.3 +/-1.2	22.05 +/-0.2		6	535
N2 MNA/H ₂ O	27.0 +/-1.4	23.75 +/-0.2	<0.0001 ^a	6	516
N2 MNA high/H ₂ O	22.0 +/-0.6	20.09 +/-0.2	<0.0001 ^a	3	331
N2 H ₂ O	24.0 +/-0.5	22.63 +/-0.2		3	370
N2 PYR-2/H ₂ O	24.0 +/-0.9	22.93 +/-0.1	n.s. ^a	3	341
N2 PYR-4/H ₂ O	24.0 +/-1.2	22.61 +/-0.3	n.s. ^a	3	353
N2 (<i>gad-3</i> RNAi) H ₂ O	26.3 +/-0.9	23.02 +/-0.3		3	257
N2 (<i>gad-3</i> RNAi) MNA/H ₂ O	23.0 +/-0.5	22.17 +/-0.2	0.0042 ^b	3	224
N2 DMSO/H ₂ O	24.7 +/-0.7	22.54 +/-0.2	n.s. ^a	3	311
N2 IsoVan/DMSO/H ₂ O	25.1 +/-0.8	22.49 +/-0.2	n.s. ^c	3	268
N2 IsoVan/DMSO/MNA/H ₂ O	25.0 +/-0.5	22.08 +/-0.2	n.s. ^c	3	250
N2 DMSO	25.0 +/-0.2	21.97 +/-0.2	n.s. ^a	3	396
N2 vanillin/DMSO	26.3 +/-0.6	23.16 +/-0.3	<0.0001 ^d	2	232
N2 vanillin high/DMSO	24.5 +/-0.3	21.20 +/-0.2	<0.0001 ^d	2	168
N2 DMSO	25.0 +/-1.0	22.51 +/-0.2	n.s. ^a	3	323
N2 DMSO/ H ₂ O	25.3 +/-1.5	22.42 +/-0.3	n.s. ^a	3	301
N2 BHA/DMSO/ H ₂ O	25.5 +/-0.7	22.51 +/-0.3	n.s. ^a	3	330
N2 NA/H ₂ O/BHA/DMSO	25.0 +/-0.0	22.68 +/-0.2	n.s. ^e	3	318
N2 MNA/H ₂ O/BHA/DMSO	24.5 +/-0.5	21.67 +/-0.2	0.0074 ^e	3	314
N2 NAC/H ₂ O	24.5 +/-1.5	22.23 +/-0.2	n.s. ^a	5	455
N2 NA/NAC/H ₂ O	23.0 +/-0.6	22.01 +/-0.3	n.s. ^f	3	279
N2 MNA/NAC/H ₂ O	24.5 +/-1.5	22.08 +/-0.2	n.s. ^f	5	513
<i>sir-2.1(ok434)</i> (MIR14) H ₂ O	25.0 +/-2.0	22.34 +/-0.4		3	274
<i>sir-2.1(ok434)</i> (MIR14) NA/H ₂ O	25.0 +/-0.0	22.01 +/-0.3	n.s. ^g	3	249
<i>sir-2.1(ok434)</i> (MIR14) NAM/H ₂ O	28.0 +/-1.0	24.44 +/-0.3	<0.0001 ^g	3	240
<i>sir-2.1(ok434)</i> (MIR14) MNA/H ₂ O	27.5 +/-1.5	24.06 +/-0.3	<0.0001 ^g	3	226
N2 <i>rol-6</i> H ₂ O	24.3 +/-1.0	21.79 +/-0.4		2	174
N2 <i>rol-6</i> NA/H ₂ O	26.0 +/-0.5	24.30 +/-0.6	<0.0001 ^h	2	181
<i>sir-2.1</i> OE (GA468) H ₂ O	28.5 +/-1.5	24.25 +/-0.5	<0.0001 ^h	2	184
<i>sir-2.1</i> OE (GA468) NA/H ₂ O	30.0 +/-1.0	27.46 +/-0.5	<0.0001 ⁱ	2	166
<i>sir-2.1</i> OE Ctrl (LG390) H ₂ O	26.5 +/-0.5	24.09 +/-0.4		2	191
<i>sir-2.1</i> OE Ctrl (LG390) NA/H ₂ O	29.0 +/-1.0	26.35 +/-0.4	<0.0001 ^j	2	204
<i>sir-2.1</i> OE (LG389) H ₂ O	27.5 +/-1.5	25.83 +/-0.3	0.0019 ^j	2	170

Continuation of Supplemental Table 1

Strain, RNAi, Substance, Solvent	Maximum Life Span [d] [+/- SEM] ¹	Mean Life Span [d] [+/- SEM]	P-Value (vs. control, see footnotes)	Number of Experiments [n]	Number of Nematodes [n]
<i>sir-2.1</i> OE (LG389) NA/H ₂ O	32.0 +/-2.0	27.63 +/-0.4	0.0002 ^k	2	173
<i>sir-2.1</i> OE (GA468)	26.0 +/-1.0	23.08 +/-0.3		2	168
<i>sir-2.1</i> OE (GA468) (<i>gad-3</i> RNAi)	26.0 +/-1.0	23.36 +/-0.3	n.s. ^l	2	153
<i>anmt-1</i> (gk457) (MIR16) H ₂ O	25.0 +/-1.4	22.76 +/-0.1		6	667
<i>anmt-1</i> (gk457) (MIR16) NA/H ₂ O	24.7 +/-0.9	22.77 +/-0.1	n.s. ^m	6	695
<i>anmt-1</i> (gk457) (MIR16) NAM/H ₂ O	24.0 +/-0.5	23.07 +/-0.1	n.s. ^m	4	465
<i>anmt-1</i> (gk457) (MIR16) MNA/H ₂ O	26.0 +/-1.0	23.94 +/-0.1	<0.0001 ^m	6	615
N2 <i>rol-6</i>	25.0 +/-0.0	21.91 +/-0.2		4	419
<i>sir-2.1</i> OE/ <i>anmt-1</i> (gk457) (MIR22)	23.0 +/-1.0	20.81 +/-0.3	0.0017 ⁿ	3	252
N2	24.0 +/-1.0	21.85 +/-0.1		4	401
<i>anmt-1</i> OE::GFP (MIR8)	25.3 +/-0.5	23.32 +/-0.2	<0.0001 ^o	6	557
<i>anmt-1</i> OE::HA tag (MIR12)	27.0 +/-0.0	25.01 +/-0.2	<0.0001 ^o	3	306
<i>daf-16</i> (<i>mu86</i>) H ₂ O	22.5 +/-0.5	20.31 +/-0.2		4	321
<i>daf-16</i> (<i>mu86</i>) NA/H ₂ O	22.0 +/-0.0	19.30 +/-0.3	n.s. ^p	2	151
<i>daf-16</i> (<i>mu86</i>) MNA/H ₂ O	22.5 +/-0.5	20.71 +/-0.2	0.048 ^p	2	136
<i>skn-1</i> (<i>zu135</i>) H ₂ O	19.5 +/-1.5	18.60 +/-0.2		3	338
<i>skn-1</i> (<i>zu135</i>) NA/H ₂ O	19.5 +/-1.0	18.51 +/-0.1	n.s. ^q	3	316
<i>skn-1</i> (<i>zu135</i>) MNA/H ₂ O	20.0 +/-0.0	19.05 +/-0.1	n.s. ^q	3	213
<i>skn-1</i> (<i>zu135;gels10</i>) H ₂ O	22.0 +/-0.5	17.75 +/-0.2		3	450
<i>skn-1</i> (<i>zu135;gels10</i>) NA/H ₂ O	22.5 +/-1.5	19.79 +/-0.2	<0.0001 ^r	3	389
<i>skn-1</i> (<i>zu135;gels10</i>) MNA/H ₂ O	22.0 +/-0.0	19.43 +/-0.2	<0.0001 ^r	3	366

¹ 75% quantile

Controls: ^a N₂ H₂O, ^b N2 (*gad-3* RNAi) H₂O, ^c N2 DMSO/H₂O, ^d N2 DMSO, ^e N2 BHA/DMSO/H₂O, ^f N2 NAC/H₂O, ^g *sir-2.1(ok434)* H₂O, ^h N2 *rol-6* H₂O, ⁱ *sir-2.1* OE (GA468) H₂O, ^j *sir-2.1* OE Ctrl (LG390) H₂O, ^k *sir-2.1* OE (LG389) H₂O, ^l *sir-2.1* OE (GA468), ^m *ANMT-1(gk457)* H₂O, ⁿ N2 *rol-6*, ^o N₂, ^p *daf-16(mu86)* H₂O, ^q *skn-1(zu135)* H₂O, ^r *skn-1(zu135;gels10)* H₂O

Supplementary Table 2: Selection of functional categories affiliated with MNA-upregulated genes

Category	Hits	P-value
catalase reaction	12	<0.0001
cellular sensing and response to external stimulus	159	<0.0001
chemoperception and response	139	<0.0001
defense related proteins	127	<0.0001
detoxification by modification	30	<0.0001
detoxification involving cytochrome P450	13	<0.0001
disease, virulence and defense	150	<0.0001
glutathione conjugation reaction	19	<0.0001
inflammatory response	110	<0.0001
lipid, fatty acid and isoprenoid metabolism	180	<0.0001
metabolism of thioredoxin, glutaredoxin, glutathione	16	<0.0001
oxidation of fatty acids	29	<0.0001
oxidative stress response	31	<0.0001
oxygen and radical detoxification	33	<0.0001
secondary metabolism	95	<0.0001
stress response	150	<0.0001
DNA repair	7	<0.0005
lipid/fatty acid transport	29	<0.0006
chemical agent resistance	14	<0.008
DNA damage response	2	<0.03