

Supplementary Materials for

UNC-49 is a redox-sensitive GABA_A receptor that regulates the mitochondrial unfolded protein response cell nonautonomously

Franziska Pohl *et al.*

Corresponding author: Jason M. Held, jheld@wustl.edu

Sci. Adv. **9**, eadh2584 (2023)
DOI: 10.1126/sciadv.adh2584

The PDF file includes:

Figs. S1 to S6
Tables S1 and S2
Legends for tables S3 to S5
References

Other Supplementary Material for this manuscript includes the following:

Tables S3 to S5

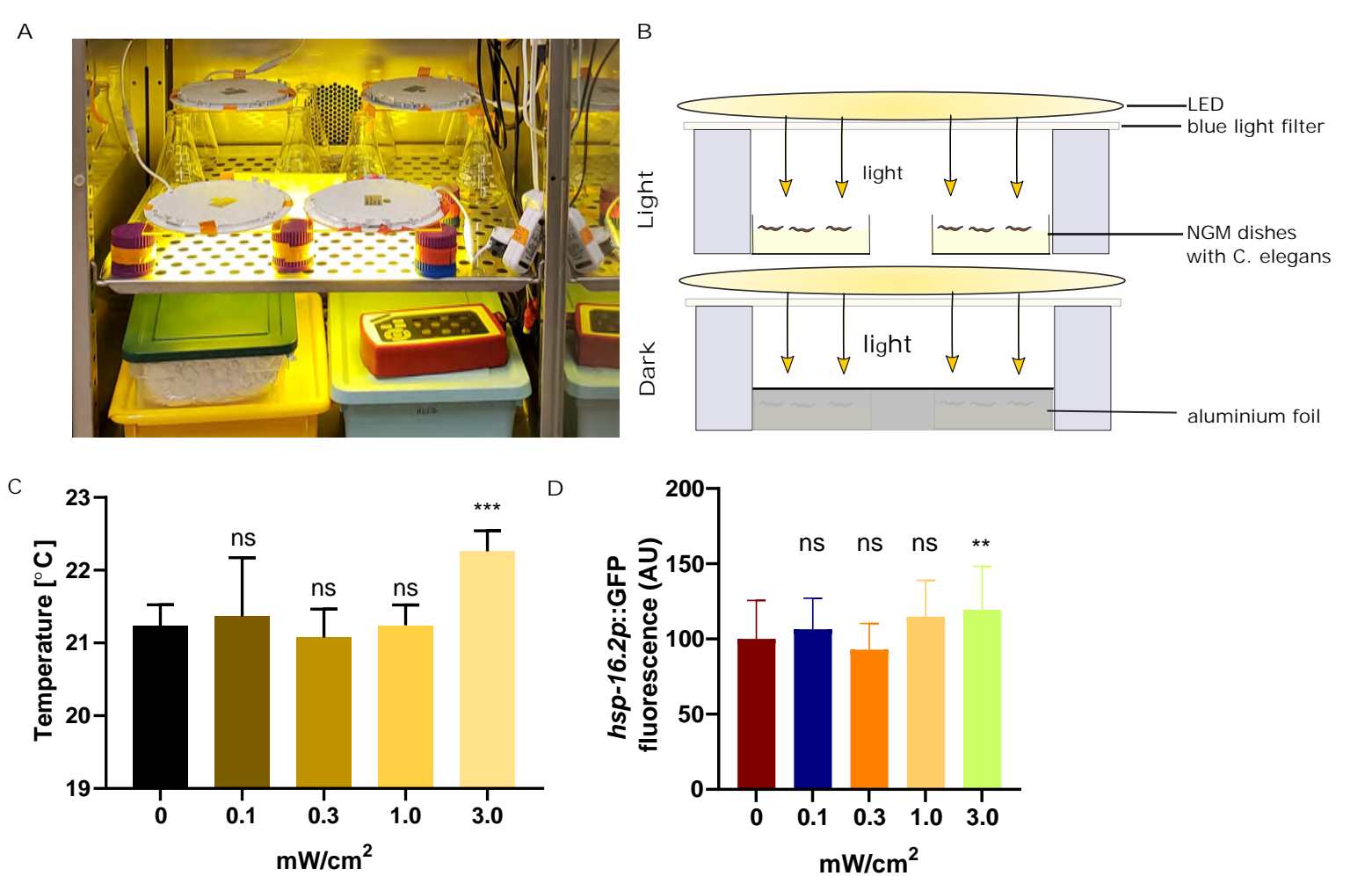
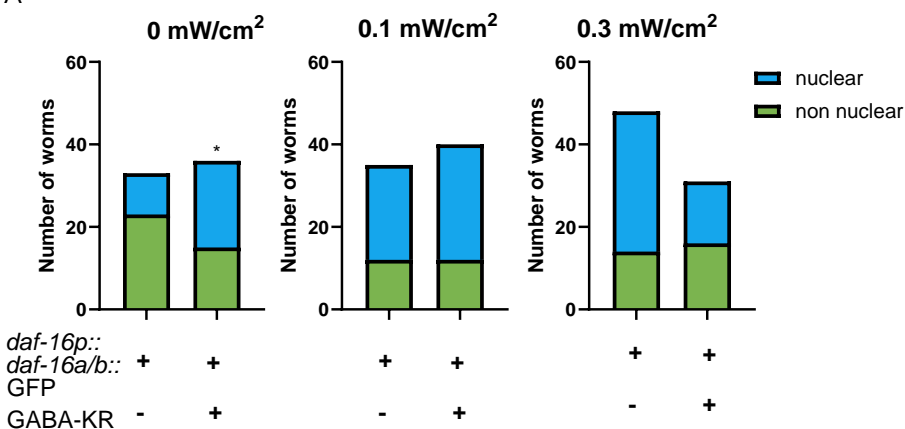
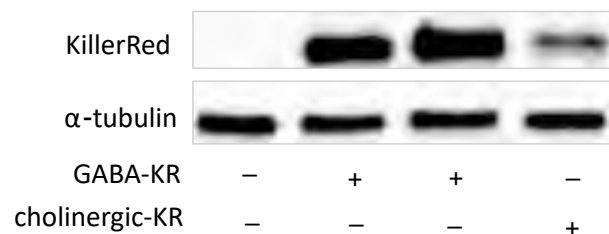


Figure S1| Optogenetic platform used to induce ROS production by KR and its effect on worm plate temperature (goes with Fig. 1)
A) Incubator set-up for KillerRed light activation using LED lights. **B)** Schematic representation of set-up for KillerRed light activation using LED lights. **C)** Average temperature of NGM dishes measured with infrared thermometer under light conditions ranging from 0-3.0 mW/cm^2 . Light conditions are compared to 0 (dark). **D)** GFP fluorescence expression of *hsp-16.2p::GFP* (heatshock) reporter strain between 0-0.3 mW/cm^2 . Light conditions are compared to 0.

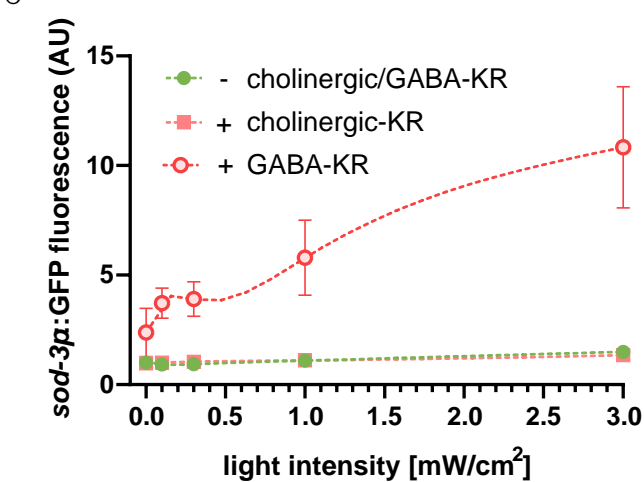
A



B



C



D

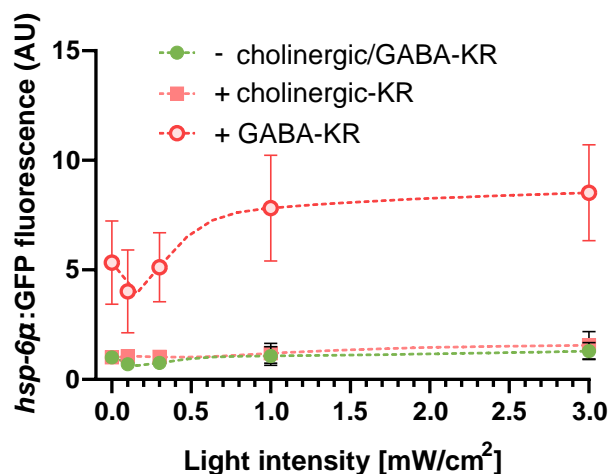


Figure S2 | Effect of cholinergic-KR on stress response pathways (goes with Fig. 2)

A) Quantification of *daf-16p::daf-16a/b::GFP* nuclear localization in the presence and absence of GABA-KR at 0, 0.1 and 0.3 mW/cm², Fisher Exact test (two-sided), n=3.

B) Western blot analysis of KillerRed expression in WT, GABA-KR, GABA-KR;*hsp-6p::GFP* and cholinergic-KR strains, representative of two biological replicates. **C)** GFP intensity measurements of *sod-3p::GFP* in the presence of GABA-KR (red, ring) and presence of cholinergic-KR (green, circle) between 0-3.0 mW/cm². **D)** GFP intensity measurements of *hsp-6p::GFP* in the presence of GABA-KR (red, ring) and cholinergic-KR (green, circle) between 0-3.0 mW/cm².



GABA-KR GABA-GFP

Figure S3 | Effect of GABAergic KR vs. GABAergic GFP on *hsp-6p::GFP* expression (goes with Fig. 3)

Bright field (left) and fluorescent image (right) of both GABA-KR;*hsp-6p::GFP* (two worms on left) and GABA-GFP; *hsp-6p::GFP* (two worms on right) strains, cultured in the dark for 72h, scale bar 200 μ m. Images show that although KillerRed expressed in GABAergic neurons increase mitoUPR expression, the expression of GFP in GABA neurons does not induce mitoUPR expression.

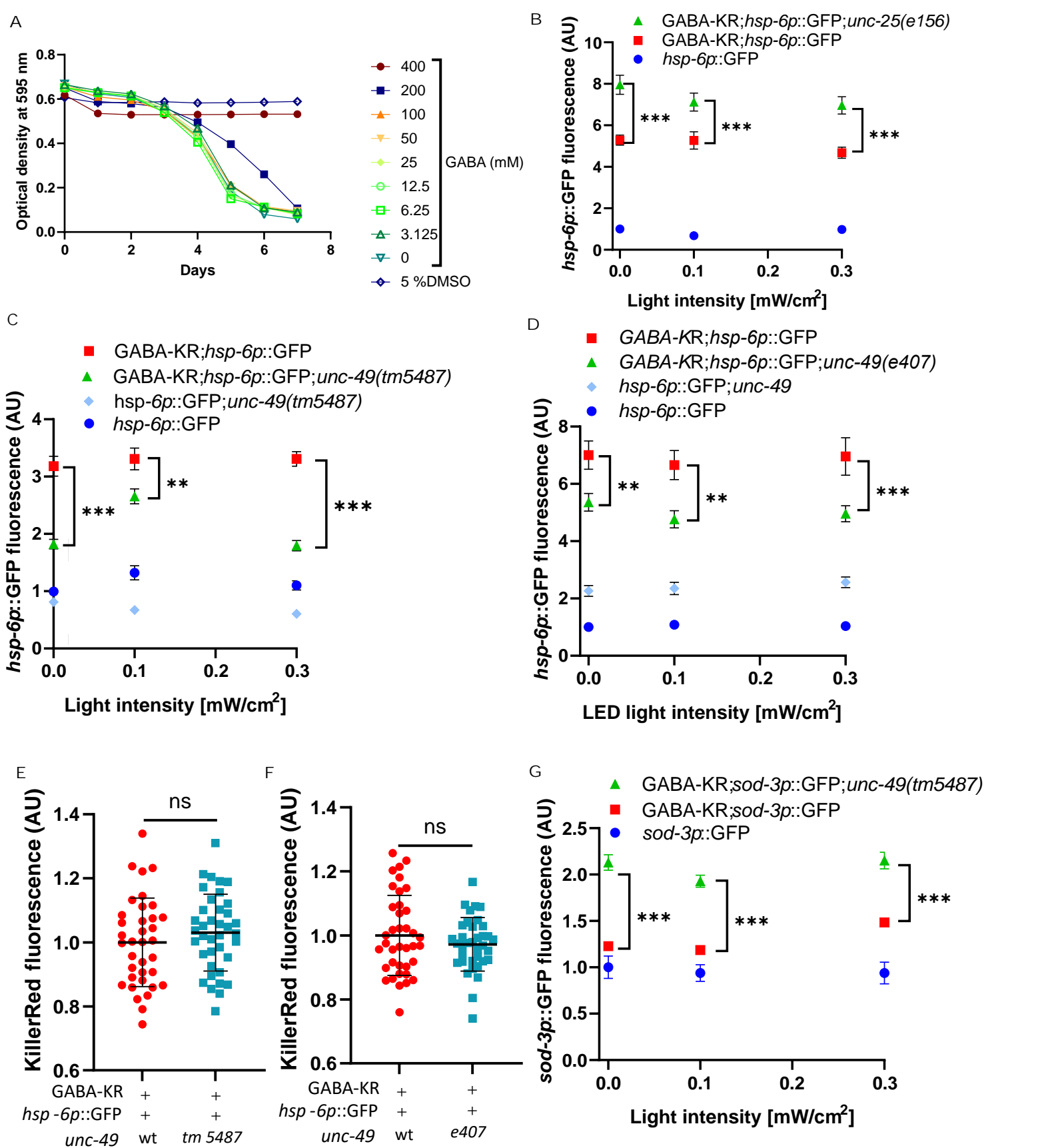


Figure S4 | GABA drug toxicity in *C. elegans*, and effect of *unc-49* mutation on KR expression levels (goes with Fig. 4)

A) Toxicity assessment of GABA using the food clearance assay in wild type worms. One independent experiment with five replicates (wells) per treatment concentration and DMSO at 5% was used as positive (toxic compound) control. The experiment starts on day 0 with eggs, the optical density of the OP50 bacteria is measured daily to assess GABA toxicity by food uptake. **B**) GFP intensity measurements of *hsp-6p::GFP* (blue, circle), *GABA-KR;hsp-6p::GFP* (red, square) and *GABA-KR;hsp-6p::GFP;unc-25(e156)* (green, triangle) between 0-0.3 mW/cm². Analyzed using ordinary two-way ANOVA and Tukey's multiple comparison test, with a single pooled variance, showing mean \pm SEM of three biological replicates with $N \geq 10$ for each replicate/condition. **C**) GFP intensity measurements of *hsp-6p::GFP* (blue, circle), *hsp-6p::GFP;unc-49(tm5487)* (light blue, diamond), *GABA-KR;hsp-6p::GFP* (red, square) and *GABA-KR;hsp-6p::GFP;unc-49(tm5487)* (green, triangle) between 0-0.3 mW/cm². Analyzed using ordinary two-way ANOVA and Tukey's multiple comparison test, with a single pooled variance, showing mean \pm SEM of three biological replicates with $N \geq 4$ for each replicate/condition. **D**) GFP intensity measurements of *hsp-6p::GFP* (blue, circle), *hsp-6p::GFP;unc-49(e407)* (light blue, diamond), *GABA-KR;hsp-6p::GFP* (red, square) and *GABA-KR;hsp-6p::GFP;unc-49(e407)* (green, triangle) between 0-0.3 mW/cm². Analyzed using ordinary two-way ANOVA and Tukey's multiple comparison test, with a single pooled variance, showing mean \pm SEM of two or more biological replicates with $N \geq 10$ for each replicate/condition. **E**) KillerRed fluorescence expression in *GABA-KR;hsp-6p::GFP* strain with *unc-49(+)* or mutant *unc-49(tm5487)*. Unpaired t test, mean \pm SD of 3 or more biological replicates with $N \geq 6$ for each condition. **F**) KillerRed fluorescence expression in *GABA-KR;hsp-6p::GFP* strain with *unc-49(+)* or mutant *unc-49(e407)*. Unpaired t test, mean \pm SD of 3 or more biological replicates with $N \geq 6$ for each condition. **G**) GFP intensity measurements of *sod-3p::GFP* (blue, circle), *GABA-KR;sod-3p::GFP* (red, square) and *GABA-KR;sod-3p::GFP;unc-49(tm5487)* (green, triangle) between 0-0.3 mW/cm². Analyzed using ordinary two-way ANOVA and Tukey's multiple comparison test, with a single pooled variance, showing mean \pm SEM of three or more biological replicates with $N \geq 10$ for each replicate/condition.

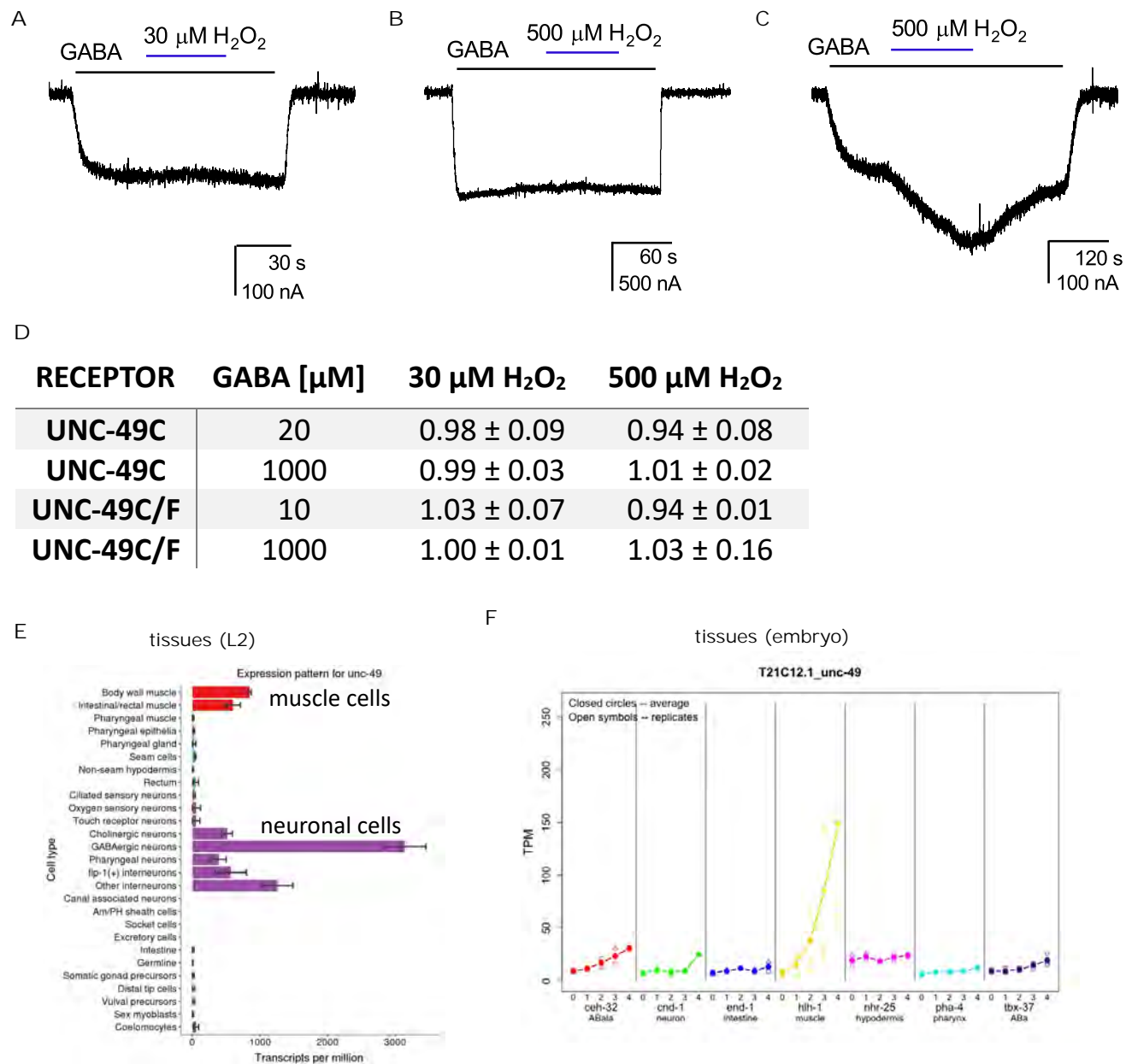


Figure S5 | H_2O_2 does not potentiate UNC-49C and UNC-49C/F in comparison to the human p1 GABAA receptors; unc-49 expression levels in *C. elegans* (goes with Fig. 5)

A-C) Comparison of H_2O_2 modulation of GABA-elicited currents in UNC-49C and human p1 GABAA receptors. The panels show sample current traces for UNC-49C (**A** and **B**) and p1 (**C**) and receptors activated by GABA in the absence and presence of H_2O_2 . In **A** and **B**, the receptors were activated by 20 μM GABA (EC2-4). In **C**, the receptors were activated by 0.4 μM GABA (EC15). GABA applications are indicated by a black line. H_2O_2 applications are indicated by a blue line. **D**) No modulation of GABA evoked currents in UNC-49C and UNC-49C/F receptors by H_2O_2 . The table gives the modulating effect of 30 μM and 500 μM H_2O_2 on GABA currents in UNC-49C and UNC-49C/F receptors. The effects of H_2O_2 were determined on both low (10 or 20 μM) and high (1000 μM) GABA-activated receptors. The modulating effect is given as fraction of control (mean \pm S.D.) from 3-4 cells per condition. **E**) Tissue specific expression pattern of unc-49 in *C. elegans* L2 from GExplore 1.4 (40). **F**) Tissue specific expression pattern of unc-49 in *C. elegans* embryo from GExplore 1.4 (40).

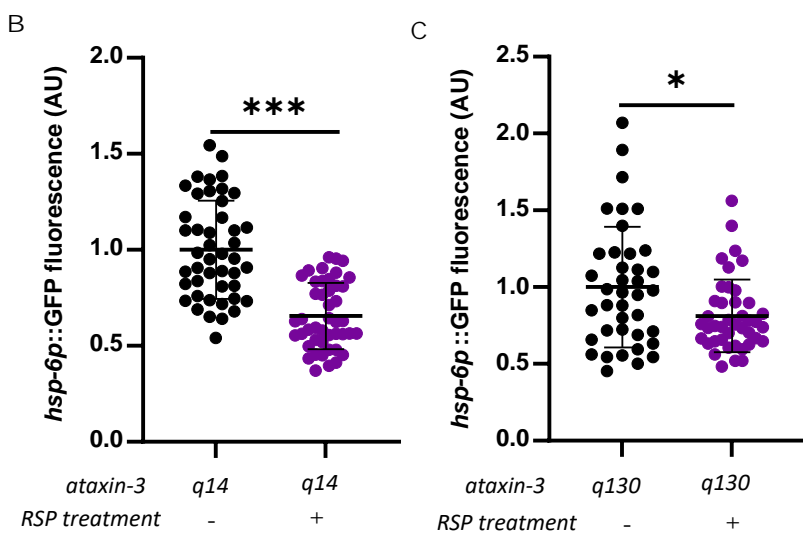
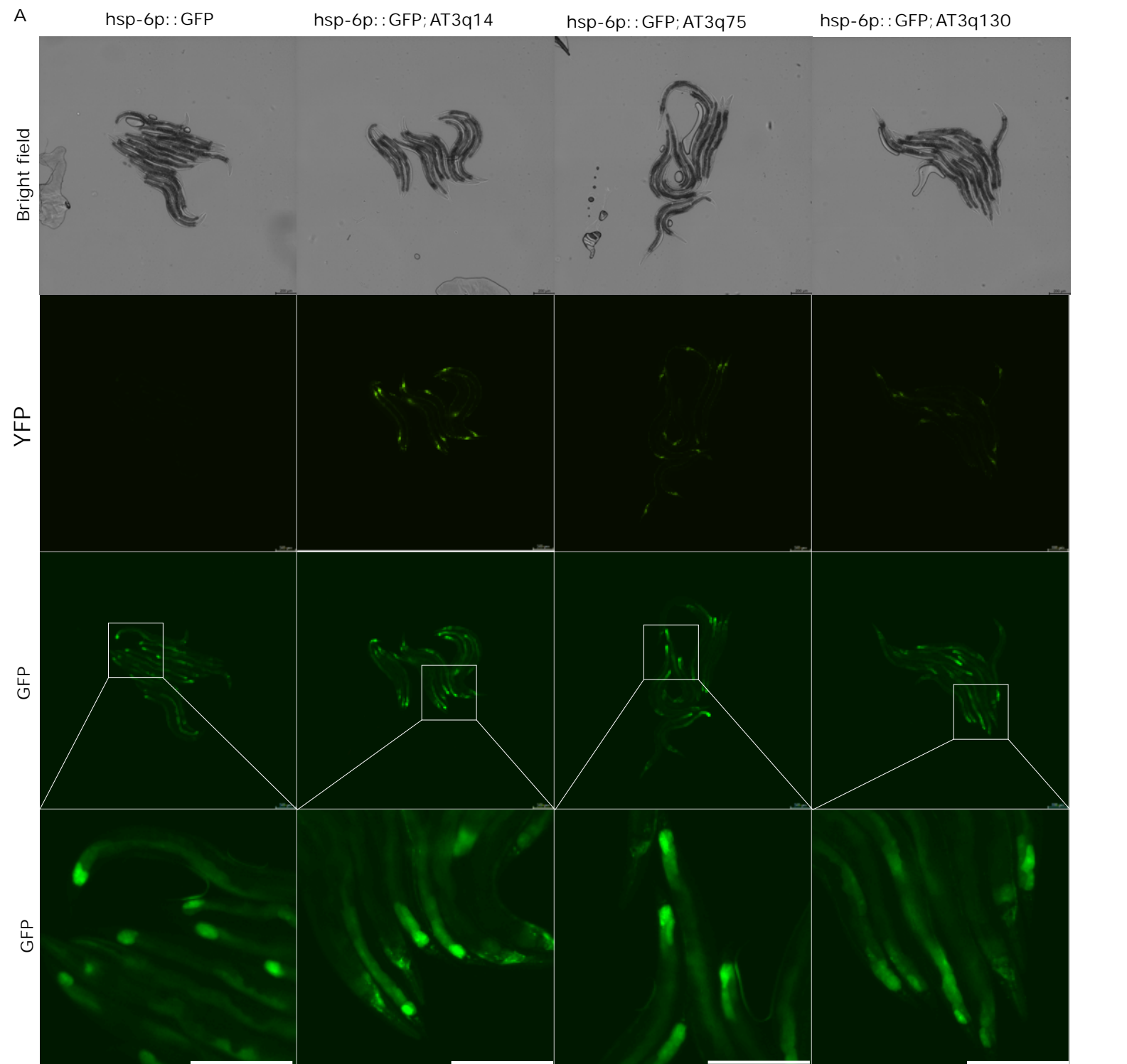


Figure S6| *hsp-6p::GFP* expression in SCA3 model (AT3q14, AT3q75 and AT3q130) in the absence and presence of RSP extract (goes with Fig. 6)

A) Bright field (top row) and fluorescence (bottom three, rows YFP and GFP) images of *hsp-6p::GFP* control and *hsp-6p;AT3q14*, *hsp-6p;AT3q75* and *hsp-6p;AT3q130* worms, cultured in the dark for 72h. The bottom row shows enlarged images of the third row, scale bar 200 μ m. **B)** *hsp-6p::GFP* expression in *hsp-6p;AT3q14* worms in the presence (+) and absence (-) of Rape seed pomace (RSP) extract (1 mg/mL); mean \pm SD, four independent biological replicates, with $N \geq 7$ for all replicates, unpaired t test. **C)** *hsp-6p::GFP* expression in *hsp-6p;AT3q130* worms in the presence (+) and absence (-) of Rape seed pomace (RSP) extract (1 mg/mL); mean \pm SD, three independent biological replicates, with $N \geq 11$ for all replicates, unpaired t test.

Table S1| Lifespan analysis of wild-type and GABA-KR worms cultured under different light conditions (0-0.3 mW/cm²).

	n bio rep	N total number of worms	Max lifespan (mean ± Std)	p-value (compared to WT)	minimum lifespan (mean ± Std)	p-value (compared to WT)	Median lifespan (mean ± Std)	p-value (compared to WT)
WT dark	4	290	23.00 ± 2.58	NA	8.00 ± 2.31	NA	14.75 ± 2.99	NA
GABA- KR dark	7	528	23.57 ± 2.64	0.975	7.29 ±1.11	0.8667	15.29 ± 1.89	0.9674
GABA- KR 0.1 mw/cm2	4	304	24.25 ± 2.87	0.8567	5.75 ± 2.22	0.2346	13.75 ± 2.22	0.8773
GABA- KR 0.3 mw/cm2	1	65	20	0.6508	8	>0.9999	15	0.9993

Kaplan-Meier simple survival analysis with Log-rank (Mantel-Cox) test.

^a Maximum lifespan is the longest lived worms lifespan in days (mean ± standard deviation)

^b Minimum lifespan is the shortest lived worms lifespan in days (mean ± standard deviation)

Table S2 | *C. elegans* Strains used

Name	Name in Paper	genotype	source	reference
N2	WT	wt (<i>C. elegans</i> wild isolate)	CGC	(20)
XE1150	GABA-KR	<i>wpls15[Punc-47::krd-1]</i> X	Marc Hammarlund, Yale	(15)
XE1158	GABA-KR,GFP	<i>juls76 [unc-25p::GFP + lin-15(+)] II; wpls15 [unc-47p::KillerRed]</i> X	Marc Hammarlund, Yale	(15)
XE1142	cholinergic-KR	<i>wpls14 [unc-17p::KillerRed + unc-122p::GFP]</i> X	Marc Hammarlund, Yale	(15)
CF1553	<i>sod-3::GFP</i>	<i>mul84 [(pAD76) sod-3p::GFP + rol-6(su1006)]</i>	CGC	(91)
CL2070	<i>hsp-16.2::GFP</i>	<i>dvl870 [hsp-16.2p::GFP + rol-6(su1006)]</i>	CGC	(92)
TJ356	<i>daf-16p::daf-16a/b::GFP</i>	<i>zls356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)]</i>	CGC	(45)
SJ4100	<i>hsp-6::GFP</i>	<i>zcls13[hsp-6::GFP]</i> V	CGC	(93)
CL2166	<i>gst-4::GFP</i>	<i>dvl819 [(pAF15)gst-4p::GFP::NLS]</i> III	CGC	(94)
SJ4197	<i>dve-1p::dve-1::GFP</i>	<i>zcls39</i> contains [<i>dve-1p::dve-1::GFP</i>]	CGC	(55)
EG1285	GABA-GFP	<i>oxls12 [unc-47p::GFP + lin-15(+)]</i>	CGC	(60)
<i>tm5487</i>	<i>unc-49(tm5487)</i>	<i>tm5487</i>	NBRP, Japan	(95)
CB156	<i>unc-25</i>	<i>e156</i>	CGC	(20)
CB407	<i>unc-49(e407)</i>	<i>e407</i>	CGC	(20)
MAC003/ AM509	AT3q14	<i>rmls227 [PF25B3.3AT3v1-1q14::yfp]</i>	Patricia Maciel/Andreia Teixeira-Castro, Braga, Portugal	(67)
MAC037/ AM519	AT3q75	<i>rmls237 [PF25B3.3AT3v1-1q75::yfp]</i>	Patricia Maciel/Andreia Teixeira-Castro, Braga, Portugal	(67)
MAC001/ AM685	AT3q130	<i>rmls263 [PF25B3.3AT3v1-1q130::yfp]</i> II	Patricia Maciel/Andreia Teixeira-Castro, Braga, Portugal	(67)
CL2122	A-beta control	<i>dvl815 [(pPD30.38) unc-54(vector) + (pCL26) mtl-2::GFP]</i>	CGC	(96)

GMC101	A-beta	<i>dvIs100 [unc-54p::A-beta-1-42::unc-54 3'-UTR + mtl-2p::GFP]</i>	CGC	(64)
NM5548	NA (only used for crosses, see below)	<i>jsSi1726 [loxP myo-2p FRT nlsCyOFP myo-2 3' mex-5p FLP D5 glh-2 3' FRT3] II</i>	M. Nonet, CGC	(87)
NM6007	NA (only used for crosses, see below)	<i>jsSi1901 [loxP FRT myo-2p nls-cyOFP let-858 3' mex-5p FLP D5 glh-2 3' FRT3] II</i>	M. Nonet, CGC	(87)

C. elegans Strains created in [this study](#)

Name	Name in Paper	Obtained from cross between/or injection	genotype	Source
JMH001	<i>sod-3p::GFP</i> ;GABA-KR	Cross: CF1553;XE1150	<i>mul84</i> [(pAD76) <i>sod-3p::GFP</i> + <i>rol-6(su1006)</i>]; <i>wpls</i> [<i>Punc-47::krd-1</i>]X	Held lab (F.Pohl)
JMH003	<i>gst-4p::GFP</i> ;GABA-KR	Cross: CL2166;XE1150	<i>dvls19</i> [(pAF15) <i>gst-4p::GFP::NLS</i>] III; <i>wpls</i> [<i>Punc-47::krd-1</i>]X	Held lab (F.Pohl)
JMH004	<i>hsp-6p::GFP</i> ;GABA-KR	Cross: SJ4100;XE1150	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>wpls</i> [<i>Punc-47::krd-1</i>]X	Held lab (F.Pohl)
JMH007	<i>hsp-16.2p::GFP</i> ;GABA-KR	Cross: CL2070;XE1150	<i>dvls70</i> [<i>hsp-16.2p::GFP</i> + <i>rol-6(su1006)</i>]; <i>wpls</i> [<i>Punc-47::krd-1</i>]X	Held lab (F.Pohl)
JMH008	<i>hsp-6p::GFP</i> ;cholinergic-KR	Cross: SJ4100;XE1142	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>wpls14</i> [<i>unc-17p::KillerRed</i> + <i>unc-122p::GFP</i>] X	Held lab (F.Pohl)
JMH009	<i>sod-3p::GFP</i> ;cholinergic-KR	Cross: CF1553;XE1142	<i>mul84</i> [(pAD76) <i>sod-3p::GFP</i> + <i>rol-6(su1006)</i>]; <i>wpls14</i> [<i>unc-17p::KillerRed</i> + <i>unc-122p::GFP</i>] X	Held lab (F.Pohl)
JMH011	<i>hsp-6p::GFP</i> ;GABA-GFP	Cross: SJ4100;EG1285	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>oxls12</i> [<i>unc-47p::GFP</i> + <i>lin-15(+)</i>]	Held lab (F.Pohl)
JMH014	<i>hsp-6p::GFP</i> ;GABA-KR; <i>unc-49(tm5487)</i>	Cross: SJ4100;XE1150; <i>tm5487</i>	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>wpls</i> [<i>Punc-47::krd-1</i>]X; <i>unc-49(tm5487)</i> III	Held lab (F.Pohl)
JMH015	<i>hsp-6p::GFP</i> ;GABA-KR;wt <i>unc-49(tm5487)</i>	Cross: SJ4100;XE1150; <i>tm5487</i>	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>wpls</i> [<i>Punc-47::krd-1</i>]X	Held lab (F.Pohl)
JMH019	<i>hsp-6p::GFP</i> ;GABA-KR; <i>unc-25(e156)</i>	Cross: SJ4100;XE1150;CB156	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>wpls</i> [<i>Punc-47::krd-1</i>]X; <i>unc-25(e156)</i> III	Held lab (F.Pohl)
JMH020	<i>hsp-6p::GFP</i> ;GABA-KR;wt <i>unc-25(e156)</i>	Cross: SJ4100;XE1150;CB156	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>wpls</i> [<i>Punc-47::krd-1</i>]X	Held lab (F.Pohl)
JMH021	<i>hsp-6p::GFP</i> ; <i>unc-25(e156)</i>	Cross: SJ4100;XE1150;CB156	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>unc-25(e156)</i> III	Held lab (F.Pohl)
JMH023	<i>hsp-6p::GFP</i> ; <i>unc-49(tm5487)</i>	Cross: SJ4100;XE1150; <i>tm5487</i>	<i>zcls13</i> [<i>hsp-6::GFP</i>] V; <i>unc-49(tm5487)</i> III	Held lab (F.Pohl)

JMH024	<i>dve-1p::dve-1::GFP;GABA-KR</i>	Cross: SJ4197;XE1150	<i>zcls39 [dve-1p::dve-1::GFP]; wpls[Punc-47::krd-1]X</i>	Held lab (F.Pohl)
JMH028	<i>hsp-6p::GFP;AT3q14</i>	Cross: SJ4100;MAC003/AM509	<i>zcls13[hsp-6::GFP] V; rmls227 [PF25B3.3AT3v1-1q14::yfp]</i>	Held lab (F.Pohl)
JMH029	<i>hsp-6p::GFP;AT3q75</i>	Cross: SJ4100;MAC037/AM519	<i>zcls13[hsp-6::GFP] V; rmls237 [PF25B3.3AT3v1-1q75::yfp]</i>	Held lab (F.Pohl)
JMH030	<i>hsp-6p::GFP;AT3q130</i>	Cross: SJ4100;MAC001/AM68	<i>zcls13[hsp-6::GFP] V; rmls263 [PF25B3.3AT3v1-1q130::yfp] II</i>	Held lab (F.Pohl)
JMH031	GABA-KR; AT3q14	Cross: XE1150;MAC003/AM509	<i>wpls[Punc-47::krd-1]X; rmls227 [PF25B3.3AT3v1-1q14::yfp]</i>	Held lab (F.Pohl)
JMH032	GABA-KR; AT3q75	Cross: XE1150;MAC037/AM519	<i>wpls[Punc-47::krd-1]X; rmls237 [PF25B3.3AT3v1-1q75::yfp]</i>	Held lab (F.Pohl)
JMH033	GABA-KR; AT3q130	Cross: XE1150;MAC001/AM68	<i>wpls[Punc-47::krd-1]X; rmls263 [PF25B3.3AT3v1-1q130::yfp] II</i>	Held lab (F.Pohl)
JMH039	Muscle A β ₁₋₄₂ control GABA-KR;	Cross: XE1150;CL2122	<i>wpls[Punc-47::krd-1]X; dvls15 [(pPD30.38) unc-54(vector) + (pCL26) mtl-2::GFP]</i>	Held lab (F.Pohl)
JMH040	Muscle A β ₁₋₄₂ ;GABA-KR	Cross: XE1150;GMC101	<i>wpls[Punc-47::krd-1]X; dvls100 [unc-54p::A-beta-1-42::unc-54 3'-UTR + mtl-2p::GFP]</i>	Held lab (F.Pohl)
JMH049	<i>daf-16p::daf-16a/b::GFP;GABA-KR</i>	Cross: TJ356;XE1150	<i>zls356 [daf-16p::daf-16a/b::GFP + rol-6(su1006); wpls[Punc-47::krd-1]X</i>	Held lab (F.Pohl)
JMH051	<i>hsp-6p::GFP;GABA-KR;unc-49(e407)</i>	Cross: SJ4100;XE1150;CB407	<i>zcls13[hsp-6::GFP] V; wpls[Punc-47::krd-1]X; unc-49(e407)III</i>	Held lab (F.Pohl)
JMH052	<i>hsp-6p::GFP;GABA-KR;wt unc-49(e407)</i>	Cross: SJ4100;XE1150;CB407	<i>zcls13[hsp-6::GFP] V; wpls[Punc-47::krd-1]X</i>	Held lab (F.Pohl)
JMH053	GABA-KR; <i>unc-49(e407)</i>	Cross: SJ4100;XE1150;CB407	<i>wpls[Punc-47::krd-1]X; unc-49(e407)III</i>	Held lab (F.Pohl)
JMH054	<i>hsp-6p::GFP; unc-49(e407)</i>	Cross: SJ4100;XE1150;CB407	<i>zcls13[hsp-6::GFP] V; unc-49(e407)III</i>	Held lab (F.Pohl)

JMH056	<i>hsp-6p::GFP;AT3q130;unc-49(e407)</i>	Cross: SJ4100; MAC001/AM685;CB407	<i>zcls13[hsp-6::GFP] V; rmsl263 [PF25B3.3AT3v1-1q130::yfp] II; unc-49(e407)III</i>	Held lab (F.Pohl)
JMH057	<i>hsp-6p::GFP; AT3q130;wt unc-49(e407)</i>	Cross: SJ4100; MAC001/AM685;CB407	<i>zcls13[hsp-6::GFP] V; rmsl263 [PF25B3.3AT3v1-1q130::yfp] II</i>	Held lab (F.Pohl)
JMH058	<i>AT3q130; unc-49(e407)</i>	Cross: SJ4100; MAC001/AM685;CB407	<i>rmsl263 [PF25B3.3AT3v1-1q130::yfp] II; unc-49(e407)III</i>	Held lab (F.Pohl)
JMH059	<i>hsp-6p::GFP; unc-49(e407)</i>	Cross: SJ4100; MAC001/AM685;CB407	<i>zcls13[hsp-6::GFP] V; unc-49(e407)III</i>	Held lab (F.Pohl)
JMH064	<i>hsp-6p::GFP;AT3q130;unc-49(tm5487)</i>	Cross: SJ4100; MAC001/AM685; <i>tm5487</i>	<i>zcls13[hsp-6::GFP] V; rmsl263 [PF25B3.3AT3v1-1q130::yfp] II; unc-49(tm5487)III</i>	Held lab (F.Pohl)
JMH065	<i>hsp-6p::GFP; AT3q130;wt unc-49(tm5487)</i>	Cross: SJ4100; MAC001/AM685; <i>tm5487</i>	<i>zcls13[hsp-6::GFP] V; rmsl263 [PF25B3.3AT3v1-1q130::yfp] II</i>	Held lab (F.Pohl)
JMH066	<i>AT3q130; unc-49(tm5487)</i>	Cross: SJ4100; MAC001/AM685; <i>tm5487</i>	<i>rmsl263 [PF25B3.3AT3v1-1q130::yfp] II; unc-49(tm5487)III</i>	Held lab (F.Pohl)
JMH067	<i>hsp-6p::GFP; unc-49(tm5487)</i>	Cross: SJ4100; MAC001/AM685; <i>tm5487</i>	<i>zcls13[hsp-6::GFP] V; unc-49(tm5487)III</i>	Held lab (F.Pohl)
NM6388	TG1 (control)	Cross: NM5548;JMH051	<i>jsSi1726 [loxP myo-2p FRT nlsCyOFP mex-5p FLP FRT3] II; unc-49(e407) III; zcls13 [hsp-6p::GFP + lin-15] V; wpls15 [unc-47p:killerRed] X</i>	M. Nonet
NM6402	TG2 (control)	Cross: NM6007;JMH051	<i>jsSi1901 [loxP FRT myo-2p nls-cyOFP mex-5p FLP FRT3] II; unc-49(e407) III; zcls13 [hsp-6p::GFP + lin-15] V; wpls15 [unc-47p:killerRed] X</i>	M. Nonet
NM6418[#]	NA	rRMCE of NMp4865 into NM6388	<i>jsSi2353 [loxP myo-2p FRT Scarlet 2X myo-2' 3' {rps-Op HygR unc-54 3'} ori {Amp} loxP myo-3p unc-49B tbb-2 3' FRT3] II; unc-49(e407) III; zcls13 [hsp-6p::GFP + lin-15] V; wpls15 [unc-47p:killerRed] X</i>	M. Nonet

NM6419	TG1	rRMCE of NMp4865 into NM6388	jsSi2354 [loxP myo-2p FRT Scarlet 2X myo-2' 3' {rps-Op HygR unc-54 3'} ori {Amp} loxP myo-3p unc-49B tbb-2 3' FRT3] II; unc-49(e407) III; zcIS13 [hsp-6p::GFP + lin-15] V; wpls15 [unc-47p:killerRed] X	M. Nonet
NM6421[#]	NA	rRMCE of NMp4865 into 5548	jsSi2356 [loxP myo-2p FRT Scarlet 2X myo-2' 3' {rps-Op HygR unc-54 3'} ori {Amp} loxP myo-3p unc-49B tbb-2 3' FRT3] II	M. Nonet
NM6414[#]	NA	rRMCE of NMp4865 into 5548	jsSi2357 [loxP myo-2p FRT Scarlet 2X myo-2' 3' {rps-Op HygR unc-54 3'} ori {Amp} loxP myo-3p unc-49B tbb-2 3' FRT3] II	M. Nonet
NM6416[#]	NA	rRMCE of NMp4866 into NM6007	jsSi2360 [loxP {rps-Op HygR unc-54 3'} ori {Amp} loxP myo-3p unc-49B sl2 Scarlet tbb-2 3' FRT3] II	M. Nonet
NM6417	NA, Only used to create NM6417	rRMCE of NMp4866 into NM6007	jsSi2361 [loxP {rps-Op HygR unc-54 3'} ori {Amp} loxP myo-3p unc-49B sl2 Scarlet tbb-2 3' FRT3] II	M. Nonet
NM6452	TG2	Cross of NM6417 with JMH051	jsSi2361 [loxP {rps-Op HygR unc-54 3'} ori {Amp} loxP myo-3p unc-49B sl2 Scarlet tbb-2 3' FRT3] II; unc-49(e407) III; zcIS13 [hsp-6p::GFP + lin-15] V; wpls15 [unc	M. Nonet

[#] duplicate independent alleles isolated but not used

Table S3 | Plasmids and DNA used (with ref #)

Table S4 | Oligonucleotides used

Table S5 | Transgenes and Alleles used/made with full designations, references and methodology

REFERENCES AND NOTES

1. M. Schieber, N. S. Chandel, ROS function in redox signaling and oxidative stress. *Curr. Biol.* **24**, R453–R462 (2014).
2. A. Miranda-Vizuete, E. A. Veal, *Caenorhabditis elegans* as a model for understanding ROS function in physiology and disease. *Redox Biol.* **11**, 708–714 (2017).
3. Y. Yang, S. M. Han, M. A. Miller, MSP hormonal control of the oocyte MAP kinase cascade and reactive oxygen species signaling. *Dev. Biol.* **342**, 96–107 (2010).
4. W. A. Edens, L. Sharling, G. Cheng, R. Shapira, J. M. Kinkade, T. Lee, H. A. Edens, X. Tang, C. Sullards, D. B. Flaherty, G. M. Benian, J. David Lambeth, Tyrosine cross-linking of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit gp91phox. *J. Cell Biol.* **154**, 879–892 (2001).
5. G. Li, J. Gong, H. Lei, J. Liu, X. Z. S. Xu, Promotion of behavior and neuronal function by reactive oxygen species in *C. elegans*. *Nat. Commun.* **7**, 13234 (2016).
6. D. Bazopoulou, D. Knoefler, Y. Zheng, K. Ulrich, B. J. Oleson, L. Xie, M. Kim, A. Kaufmann, Y.-T. Lee, Y. Dou, Y. Chen, S. Quan, U. Jakob, Developmental ROS individualizes organismal stress resistance and lifespan. *Nature* **576**, 301–305 (2019).
7. P. Back, W. H. de Vos, G. G. Depuydt, F. Matthijssens, J. R. Vanfleteren, B. P. Braeckman, Exploring real-time in vivo redox biology of developing and aging *Caenorhabditis elegans*. *Free Radic. Biol. Med.* **52**, 850–859 (2012).
8. J. Durieux, S. Wolff, A. Dillin, The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* **144**, 79–91 (2011).
9. D. J. Dues, C. E. Schaar, B. K. Johnson, M. J. Bowman, M. E. Winn, M. M. Senchuk, J. M. van Raamsdonk, Uncoupling of oxidative stress resistance and lifespan in long-lived *isp-1* mitochondrial mutants in *Caenorhabditis elegans*. *Free Radic. Biol. Med.* **108**, 362–373 (2017).

10. C. E. Schaar, D. J. Dues, K. K. Spielbauer, E. Machiela, J. F. Cooper, M. Senchuk, S. Hekimi, J. M. van Raamsdonk, Mitochondrial and cytoplasmic ROS have opposing effects on lifespan. *PLOS Genet.* **11**, 1–24 (2015).
11. J. M. van Raamsdonk, S. Hekimi, Reactive oxygen species and aging in *Caenorhabditis elegans*: Causal or casual relationship? *Antioxid. Redox Signal.* **13**, 1911–1953 (2010).
12. M. Waghray, Z. Cui, J. C. Horowitz, I. M. Subramanian, F. J. Martinez, G. B. Toews, V. J. Thannickal, Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts. *FASEB J.* **19**, 1–16 (2005).
13. A. P. Wojtovich, T. H. Foster, Optogenetic control of ROS production. *Redox Biol.* **2**, 368–376 (2014).
14. K. Takemoto, T. Matsuda, N. Sakai, D. Fu, M. Noda, S. Uchiyama, I. Kotera, Y. Arai, M. Horiuchi, K. Fukui, T. Ayabe, F. Inagaki, H. Suzuki, T. Nagai, SuperNova, a monomeric photosensitizing fluorescent protein for chromophore-assisted light inactivation. *Sci. Rep.* **3**, 2629 (2013).
15. D. C. Williams, R. El Bejjani, P. M. Ramirez, S. Coakley, S. A. Kim, H. Lee, Q. Wen, A. Samuel, H. Lu, M. A. Hilliard, M. Hammarlund, Rapid and permanent neuronal inactivation in vivo via subcellular generation of reactive oxygen with the use of KillerRed. *Cell Rep.* **5**, 553–563 (2013).
16. J. Kobayashi, H. Shidara, Y. Morisawa, M. Kawakami, Y. Tanahashi, K. Hotta, K. Oka, A method for selective ablation of neurons in *C. elegans* using the phototoxic fluorescent protein, KillerRed. *Neurosci. Lett.* **548**, 261–264 (2013).
17. A. P. Wojtovich, A. Y. Wei, T. A. Sherman, T. H. Foster, K. Nehrke, Chromophore-assisted light inactivation of mitochondrial electron transport chain complex II in *Caenorhabditis elegans*. *Sci. Rep.* **6**, 1–13 (2016).
18. L.-W. Shao, R. Niu, Y. Liu, Neuropeptide signals cell non-autonomous mitochondrial unfolded protein response. *Cell Res.* **26**, 1182–1196 (2016).

19. E. Yemini, T. Jucikas, L. J. Grundy, A. E. X. Brown, W. R. Schafer, A database of *Caenorhabditis elegans* behavioral phenotypes. *Nat. Method.* **10**, 877–879 (2013).
20. S. Brenner, The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94 (1974).
21. V. Adam-Vizi, Production of reactive oxygen species in brain mitochondria: Contribution by electron transport chain and non-electron transport chain sources. *Antioxid. Redox Signal.* **7**, 1140–1149 (2005).
22. M. E. Pamenter, Mitochondria: A multimodal hub of hypoxia tolerance. *Can. J. Zool.* **92**, 569–589 (2014).
23. M. V. Accardi, B. A. Daniels, P. M. G. E. Brown, J.-M. Fritschy, S. K. Tyagarajan, D. Bowie, Mitochondrial reactive oxygen species regulate the strength of inhibitory GABA-mediated synaptic transmission. *Nat. Commun.* **5**, 3168 (2014).
24. J. N. Cobley, M. L. Fiorello, D. M. Bailey, 13 reasons why the brain is susceptible to oxidative stress. *Redox Biol.* **15**, 490–503 (2018).
25. S. M. Kilbride, J. E. Telford, G. P. Davey, Age-related changes in H₂O₂ production and bioenergetics in rat brain synaptosomes. *Biochim. Biophys. Acta* **1777**, 783–788 (2008).
26. M. E. Rice, H₂O₂: A dynamic neuromodulator. *Neuroscientist* **17**, 389–406 (2011).
27. C. A. Massaad, E. Klann, Reactive oxygen species in the regulation of synaptic plasticity and memory. *Antioxid. Redox Signal.* **14**, 2013–2054 (2011).
28. J. W. Błaszczyk, Parkinson's disease and neurodegeneration: GABA-collapse hypothesis. *Front. Neurosci.* **10**, 269 (2016).
29. S. M. Garcia, M. O. Casanueva, M. C. Silva, M. D. Amara, R. I. Morimoto, Neuronal signaling modulates protein homeostasis in *Caenorhabditis elegans* post-synaptic muscle cells. *Genes Dev.* **21**, 3006–3016 (2007).

30. R. C. Taylor, K. M. Berendzen, A. Dillin, Systemic stress signalling: Understanding the cell non-autonomous control of proteostasis. *Nat. Rev. Mol. Cell Biol.* **15**, 211–217 (2014).
31. F. Yuan, J. Zhou, L. Xu, W. Jia, L. Chun, X. Z. S. Xu, J. Liu, GABA receptors differentially regulate life span and health span in *C. elegans* through distinct downstream mechanisms *Phys. Ther.* **317**, C953–C963 (2019).
32. Z. Zheng, X. Zhang, J. Liu, P. He, S. Zhang, Y. Zhang, J. Gao, S. Yang, N. Kang, M. I. Afridi, S. Gao, C. Chen, H. Tu, GABAergic synapses suppress intestinal innate immunity via insulin signaling in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2021063118 (2021).
33. A. N. Beltrán González, M. I. López Pazos, D. J. Calvo, Reactive oxygen species in the regulation of the GABA mediated inhibitory neurotransmission. *Neuroscience* **439**, 137–145 (2020).
34. A. N. Beltrán González, J. Gasulla, D. J. Calvo, An intracellular redox sensor for reactive oxygen species at the M3-M4 linker of GABA_A receptors. *Br. J. Pharmacol.* **171**, 2291–2299 (2014).
35. A. Amato, C. N. Connolly, S. J. Moss, T. G. Smart, Modulation of neuronal and recombinant GABA_A receptors by redox reagents. *J. Physiol.* **517**, 35–50 (1999).
36. Y. Fardghassemi, A. Tauffenberger, S. Gosselin, J. A. Parker, Rescue of ATXN3 neuronal toxicity in *Caenorhabditis elegans* by chemical modification of endoplasmic reticulum stress. *Dis. Model. Mech.* **10**, 1465–1480 (2017).
37. F. Pohl, A. Teixeira-Castro, M. D. Costa, V. Lindsay, J. Fiúza-Fernandes, M. Goua, G. Bermano, W. Russell, P. Maciel, P. K. Thoo Lin, P. Kong, T. Lin, V. Lindsay, GST-4-dependent suppression of neurodegeneration in *C. elegans* models of Parkinson's and Machado-Joseph disease by rapeseed pomace extract supplementation. *Front. Neurosci.* **13**, 1–13 (2019).
38. B. A. Bamber, R. E. Twyman, E. M. Jorgensen, Pharmacological characterization of the homomeric and heteromeric UNC-49 GABA receptors in *C. elegans*. *Br. J. Pharmacol.* **138**, 883–893 (2003).

39. B. A. Bamber, A. A. Beg, R. E. Twyman, E. M. Jorgensen, The *Caenorhabditis elegans* unc-49 locus encodes multiple subunits of a heteromultimeric GABA receptor. *J. Neurosci.* **19**, 5348–5359 (1999).
40. H. Hutter, J. Suh, GExplore 1.4: An expanded web interface for queries on *Caenorhabditis elegans* protein and gene function. *Worm* **5**, e1234659 (2016).
41. M. B. Goodman, P. Sengupta, How *Caenorhabditis elegans* senses mechanical stress, temperature, and other physical stimuli. *Genetics* **212**, 25–51 (2019).
42. S. L. Rea, D. Wu, J. R. Cypser, J. W. Vaupel, T. E. Johnson, A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*. *Nat. Genet.* **37**, 894–898 (2005).
43. R. P. Brandes, F. Rezende, K. Schröder, Redox regulation beyond ROS why ROS should not be measured as often. *Circ. Res.* **123**, 326–328 (2018).
44. Y. M. Go, J. J. Gipp, R. T. Mulcahy, D. P. Jones, H₂O₂-dependent activation of GCLC-ARE4 reporter occurs by mitogen-activated protein kinase pathways without oxidation of cellular glutathione or thioredoxin-1. *J. Biol. Chem.* **279**, 5837–5845 (2004).
45. S. T. Henderson, T. E. Johnson, daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr. Biol.* **11**, 1975–1980 (2001).
46. X. X. Lin, I. Sen, G. E. Janssens, X. Zhou, B. R. Fonslow, D. Edgar, N. Stroustrup, P. Swoboda, J. R. Yates, G. Ruvkun, C. G. Riedel, DAF-16/FOXO and HLH-30/TFEB function as combinatorial transcription factors to promote stress resistance and longevity. *Nat. Commun.* **9**, 1–15 (2018).
47. J. M. van Raamsdonk, S. Hekimi, Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. *PLOS Genet.* **5**, e1000361 (2009).
48. B. Leiers, A. Kampkötter, C. G. Grevelding, C. D. Link, T. E. Johnson, K. Henkle-Dührsen, A stress-responsive glutathione S-transferase confers resistance to oxidative stress in *Caenorhabditis elegans*. *Free Radic. Biol. Med.* **34**, 1405–1415 (2003).

49. K. M. Berendzen, J. Durieux, L.-W. Shao, Y. Tian, H.-E. Kim, S. Wolff, Y. Liu, A. Dillin, Neuroendocrine coordination of mitochondrial stress signaling and proteostasis. *Cell* **166**, 1553–1563.e10 (2016).
50. F. Muñoz-Carvajal, M. Sanhueza, The mitochondrial unfolded protein response: A hinge between healthy and pathological aging. *Front. Aging Neurosci.* **12**, 300 (2020).
51. D. Ganini, F. Leinisch, A. Kumar, J. J. Jiang, E. J. Tokar, C. C. Malone, R. M. Petrovich, R. P. Mason, Fluorescent proteins such as eGFP lead to catalytic oxidative stress in cells. *Redox Biol.* **12**, 462–468 (2017).
52. D. J. Kemble, G. Sun, Direct and specific inactivation of protein tyrosine kinases in the Src and FGFR families by reversible cysteine oxidation. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5070–5075 (2009).
53. Y. Liu, B. S. Samuel, P. C. Breen, G. Ruvkun, *Caenorhabditis elegans* pathways that surveil and defend mitochondria. *Nature* **508**, 406–410 (2014).
54. S. Bora, G. S. H. Vardhan, N. Deka, L. Khataniar, D. Gogoi, A. Baruah, Paraquat exposure over generation affects lifespan and reproduction through mitochondrial disruption in *C. elegans*. *Toxicology* **447**, 152632 (2021).
55. C. M. Haynes, K. Petrova, C. Benedetti, Y. Yang, D. Ron, ClpP mediates activation of a mitochondrial unfolded protein response in *C. elegans*. *Dev. Cell* **13**, 467–480 (2007).
56. G. K. Schwalfenberg, N-acetylcysteine: A review of clinical usefulness (an old drug with new tricks). *J. Nutr. Metab.* **2021**, 1–13 (2021).
57. F. Pohl, M. Goua, G. Bermano, W. R. Russell, L. Scobbie, P. Maciel, P. Kong Thoo Lin, Revalorisation of rapeseed pomace extracts: An in vitro study into its anti-oxidant and DNA protective properties. *Food Chem.* **239**, 323–332 (2018).

58. K. Yates, F. Pohl, M. Busch, A. Mozer, L. Watters, A. Shiryayev, P. Kong Thoo Lin, Determination of sinapine in rapeseed pomace extract: Its antioxidant and acetylcholinesterase inhibition properties. *Food Chem.* **276**, 768–775 (2019).
59. F. Pohl, M. Goua, K. Yates, G. Bermano, W. R. Russell, P. Maciel, P. K. Thoo Lin, Impact of rapeseed pomace extract on markers of oxidative stress and DNA damage in human SH-SY5Y cells. *J. Food Biochem.* **45**, e13592 (2021).
60. S. L. McIntire, R. J. Reimer, K. Schuske, R. H. Edwards, E. M. Jorgensen, Identification and characterization of the vesicular GABA transporter. *Nature* **389**, 870–876 (1997).
61. S. Pletnev, N. G. Gurskaya, N. Pletneva, K. A. Lukyanov, D. M. Chudakov, V. I. Martynov, V. O. Popov, M. Kovalchuk, A. Wlodawer, Z. Dauter, V. Pletnev, Structural basis for phototoxicity of the genetically encoded photosensitizer KillerRed. *J. Biol. Chem.* **284**, 32028–32039 (2009).
62. WormBase WS283 March, WormBase (2022), (available at <https://wormbase.org>).
63. H. S. Yi, J. Y. Chang, M. Shong, The mitochondrial unfolded protein response and mitohormesis: A perspective on metabolic diseases. *J. Mol. Endocrinol.* **61**, R91 (2018).
64. G. McColl, B. R. Roberts, T. L. Pukala, V. B. Kenche, C. M. Roberts, C. D. Link, T. M. Ryan, C. L. Masters, K. J. Barnham, A. I. Bush, R. A. Cherny, Utility of an improved model of amyloid-beta (A β 1_42) toxicity in *Caenorhabditis elegans* for drug screening for Alzheimer's disease. *Mol. Neurodegener.* **7**, 57 (2012).
65. X. Wang, K. Yi, Y. Zhao, Fucoidan inhibits amyloid- β -induced toxicity in transgenic *Caenorhabditis elegans* by reducing the accumulation of amyloid- β and decreasing the production of reactive oxygen species. *Food Funct.* **9**, 552–560 (2018).
66. V. Sorrentino, M. Romani, L. Mouchiroud, J. S. Beck, H. Zhang, D. D'Amico, N. Moullan, F. Potenza, A. W. Schmid, S. Rietsch, S. E. Counts, J. Auwerx, Enhancing mitochondrial proteostasis reduces amyloid- β proteotoxicity. *Nature* **552**, 187–193 (2017).

67. A. Teixeira-Castro, M. Ailion, A. Jalles, H. R. Brignull, J. L. Vilaça, N. Dias, P. Rodrigues, J. F. Oliveira, A. Neves-Carvalho, R. I. Morimoto, P. Maciel, Neuron-specific proteotoxicity of mutant ataxin-3 in *C. elegans*: Rescue by the DAF-16 and HSF-1 pathways. *Hum. Mol. Genet.* **20**, 2996–3009 (2011).
68. A. M. de Assis, J. A. M. Saute, A. Longoni, C. B. Haas, V. R. Torrez, A. W. Brochier, G. N. Souza, G. V. Furtado, T. C. Gheno, A. Russo, T. L. Monte, R. M. Castilhos, A. Schumacher-Schuh, R. D’Avila, K. C. Donis, C. R. de Mello Rieder, D. O. Souza, S. Camey, V. B. Leotti, L. B. Jardim, L. V. Portela, Peripheral oxidative stress biomarkers in spinocerebellar ataxia type 3/Machado–Joseph disease. *Front. Neurol.* **8**, 485 (2017).
69. C. C. Winterbourn, Reconciling the chemistry and biology of reactive oxygen species. *Nat. Chem. Biol.* **4**, 278–286 (2008).
70. J. M. Held, Redox systems biology: Harnessing the sentinels of the cysteine redoxome. *Antioxid. Redox Signal.* **32**, 659–676 (2020).
71. S. Xu, A. D. Chisholm, Highly efficient optogenetic cell ablation in *C. elegans* using membrane-targeted miniSOG. *Sci. Rep.* **6**, 1–13 (2016).
72. A. J. Trewin, L. L. Bahr, A. Almast, B. J. Berry, A. Y. Wei, T. H. Foster, A. P. Wojtovich, Mitochondrial reactive oxygen species generated at the complex-II matrix or intermembrane space microdomain have distinct effects on redox signaling and stress sensitivity in *Caenorhabditis elegans*. *Antioxid. Redox Signal.* **31**, 594–607 (2019).
73. I. Busack, F. Jordan, P. Sapir, H. Bringmann, The OptoGenBox—A device for long-term optogenetics in *C. elegans*. *J. Neurogenet.* **34**, 466–474 (2020).
74. S. Peña, T. Sherman, P. S. Brookes, K. Nehrke, The mitochondrial unfolded protein response protects against anoxia in *Caenorhabditis elegans*. *PLOS ONE* **11**, e0159989 (2016).
75. J. F. Cooper, E. Machiela, D. J. Dues, K. K. Spielbauer, M. M. Senchuk, J. M. van Raamsdonk, Activation of the mitochondrial unfolded protein response promotes longevity and dopamine neuron survival in Parkinson’s disease models. *Sci. Rep.* **7**, 1–16 (2017).

76. Á. Nemezc, M. S. Prevost, A. Menny, P. J. Corringer, Emerging molecular mechanisms of signal transduction in pentameric ligand-gated ion channels. *Neuron* **90**, 452–470 (2016).
77. Q. Jia, D. Sieburth, Mitochondrial hydrogen peroxide positively regulates neuropeptide secretion during diet-induced activation of the oxidative stress response. *Nat. Commun.* **12**, 1–22 (2021).
78. Q. Zhang, X. Wu, P. Chen, L. Liu, N. Xin, Y. Tian, A. Dillin, The mitochondrial unfolded protein response is mediated cell-non-autonomously by retromer-dependent Wnt signaling. *Cell* **174**, 870–883.e17 (2018).
79. Y.-T. Hsu, Y.-G. Chang, Y. Chern, Insights into GABAergic system alteration in Huntington's disease. *Open Biol.* **8**, 180165 (2018).
80. D. S. Fay, Classical genetic methods, in *WormBook*, The C. elegans Research Community, Ed. (2013), pp. 1–58.
81. T. Stiernagle, Maintenance of C. elegans, in *WormBook*, The C. elegans Research Community, Ed. (2006).
82. C. Voisine, H. Varma, N. Walker, E. A. Bates, B. R. Stockwell, A. C. Hart, Identification of potential therapeutic drugs for huntington's disease using Caenorhabditis elegans. *PLOS ONE* **2**, e504 (2007).
83. A. Teixeira-Castro, A. Jalles, S. Esteves, S. Kang, L. da Silva Santos, A. Silva-Fernandes, M. F. Neto, R. M. Briemann, C. Bessa, S. Duarte-Silva, A. Miranda, S. Oliveira, A. Neves-Carvalho, J. Bessa, T. Summavielle, R. B. Silverman, P. Oliveira, R. I. Morimoto, P. Maciel, Serotonergic signalling suppresses ataxin 3 aggregation and neurotoxicity in animal models of Machado-Joseph disease. *Brain*, **138**, 3221–3237 (2015).
84. P. Li, A. Khatri, J. Bracamontes, D. S. Weiss, J. H. Steinbach, G. Akk, Site-specific fluorescence reveals distinct structural changes induced in the human $\rho 1$ GABA receptor by inhibitory neurosteroids. *Mol. Pharmacol.* **77**, 539–546 (2010).

85. M. M. Eaton, Y. bin Lim, D. F. Covey, G. Akk, Modulation of the human $\rho 1$ GABAA receptor by inhibitory steroids. *Psychopharmacology* **231**, 3467–3478 (2014).
86. G. Akk, D. J. Shin, A. L. Germann, J. H. Steinbach, GABA type a receptor activation in the allosteric coagonist model framework: Relationship between EC50 and basal activity. *Mol. Pharmacol.* **93**, 90–100 (2018).
87. M. L. Nonet, Rapid generation of *Caenorhabditis elegans* single-copy transgenes combining recombination-mediated cassette exchange and drug selection. *Genetics* **224**, 1–15 (2023).
88. M. L. Nonet, Efficient transgenesis in *Caenorhabditis elegans* using Flp recombinase-mediated cassette exchange. *Genetics* **215**, 903–921 (2020).
89. A. Mottis, V. Jovaisaite, J. Auwerx, The mitochondrial unfolded protein response in mammalian physiology. *Mamm. Genome* **25**, 424–433 (2014).
90. E. M. Jorgensen, GABA, in *WormBook*, The C. elegans Research Community, Ed. (2005).
91. N. Libina, J. R. Berman, C. Kenyon, Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. *Cell* **115**, 489–502 (2003).
92. C. D. Link, J. R. Cypser, C. J. Johnson, T. E. Johnson, Direct observation of stress response in *Caenorhabditis elegans* using a reporter transgene. *Cell Stress Chaperones* **4**, 235–242 (1999).
93. T. Yoneda, C. Benedetti, F. Urano, S. G. Clark, H. P. Harding, D. Ron, Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. *J. Cell Sci.* **117**, 4055–4066 (2004).
94. C. D. Link, C. J. Johnson, Reporter transgenes for study of oxidant stress in *Caenorhabditis elegans*. *Methods Enzymol.* **353**, 497–505 (2002).
95. K. Gengyo-Ando, S. Mitani, Characterization of mutations induced by ethyl methanesulfonate, UV, and trimethylpsoralen in the nematode *Caenorhabditis elegans*. *Biochem. Biophys. Res. Commun.* **269**, 64–69 (2000).

96. D. S. Fay, A. Fluet, C. J. Johnson, C. D. Link, In vivo aggregation of β -amyloid peptide variants. *J. Neurochem.* **71**, 1616–1625 (1998).