

Supplementary Materials for

**UNC-49 is a redox-sensitive GABA<sub>A</sub> receptor that regulates the mitochondrial unfolded protein response cell nonautonomously**

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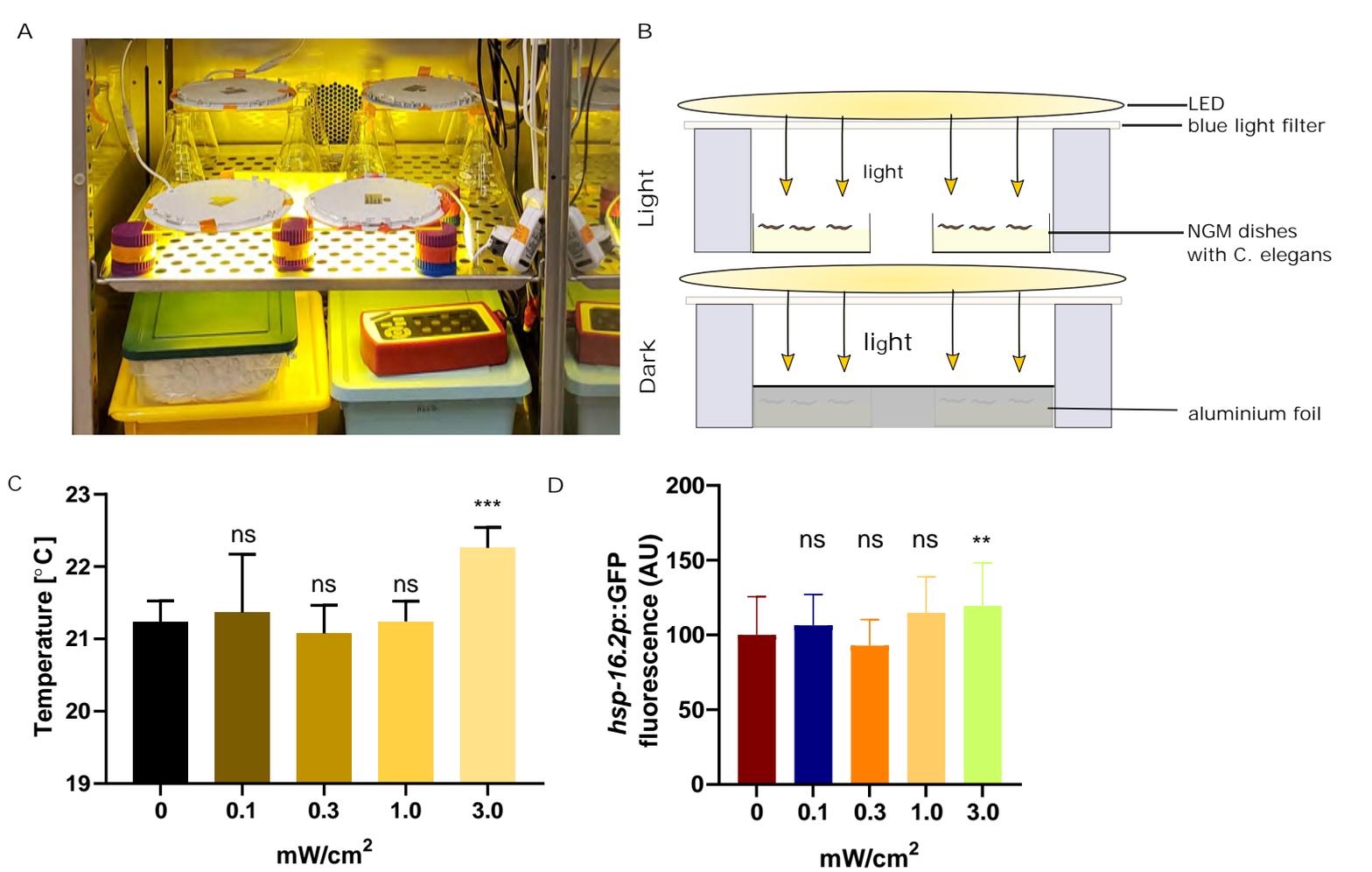
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**The PDF file includes:**

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

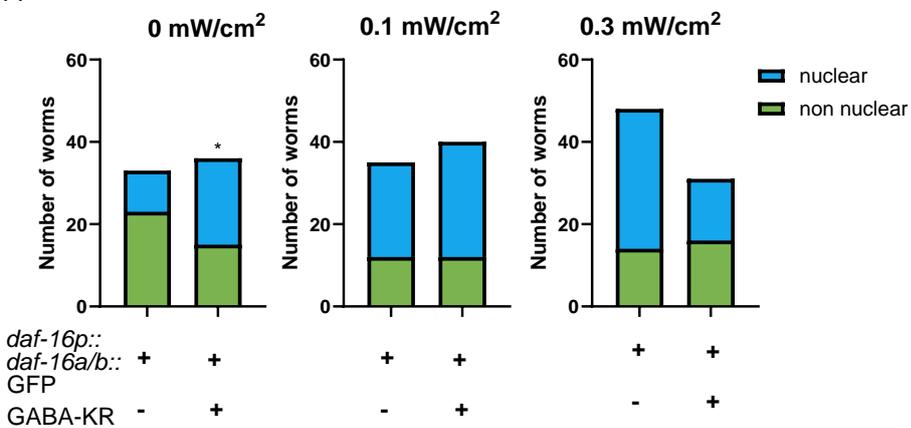
**Other Supplementary Material for this manuscript includes the following:**

Tables S3 to S5

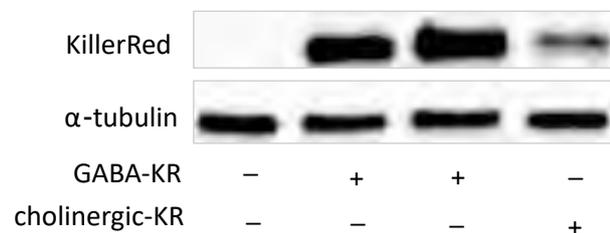


**Figure S1| Optogenetic platform used to induce ROS production by KR and its effect on worm plate temperature (goes with Fig. 1)**  
**A)** Incubator set-up for KillerRed light activation using LED lights. **B)** Schematic representation of set-up for KillerRed light activation using LED lights. **C)**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

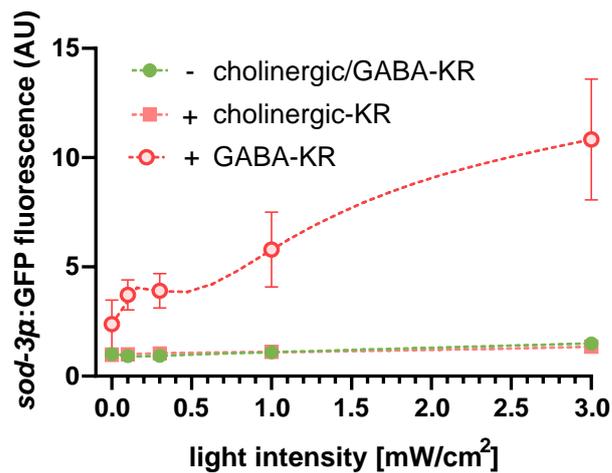
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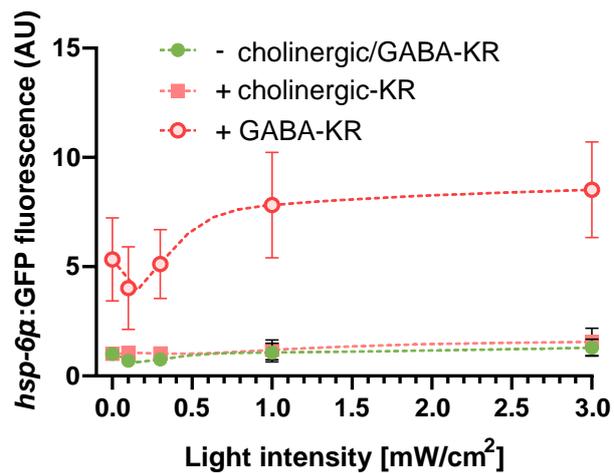
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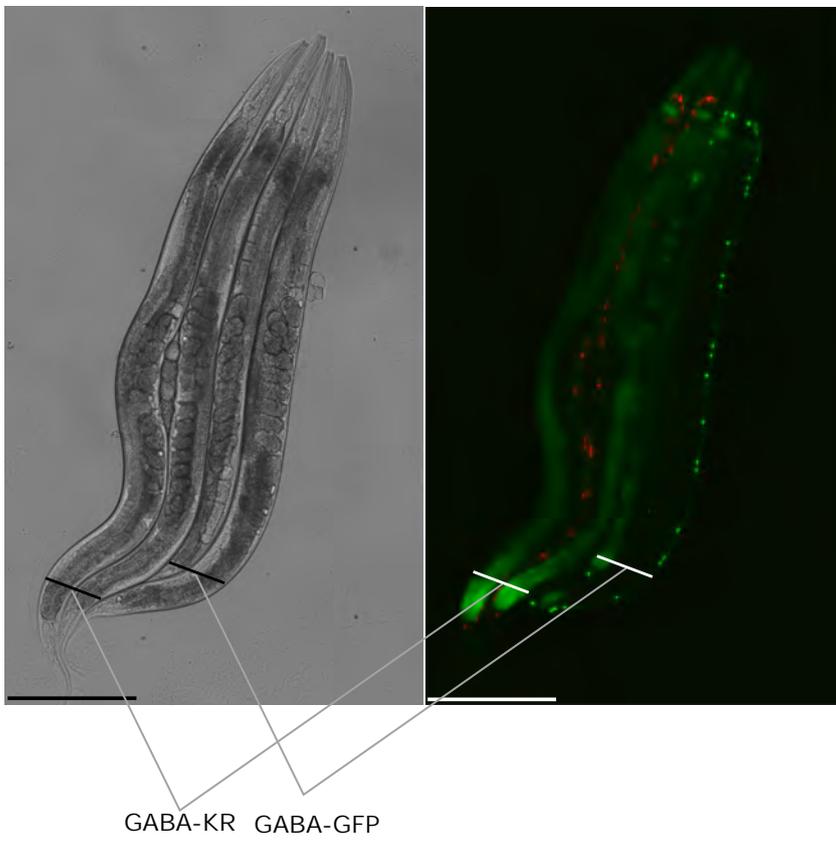
D



**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/cm<sup>2</sup>, Fisher Exact test (two-sided), n=3.

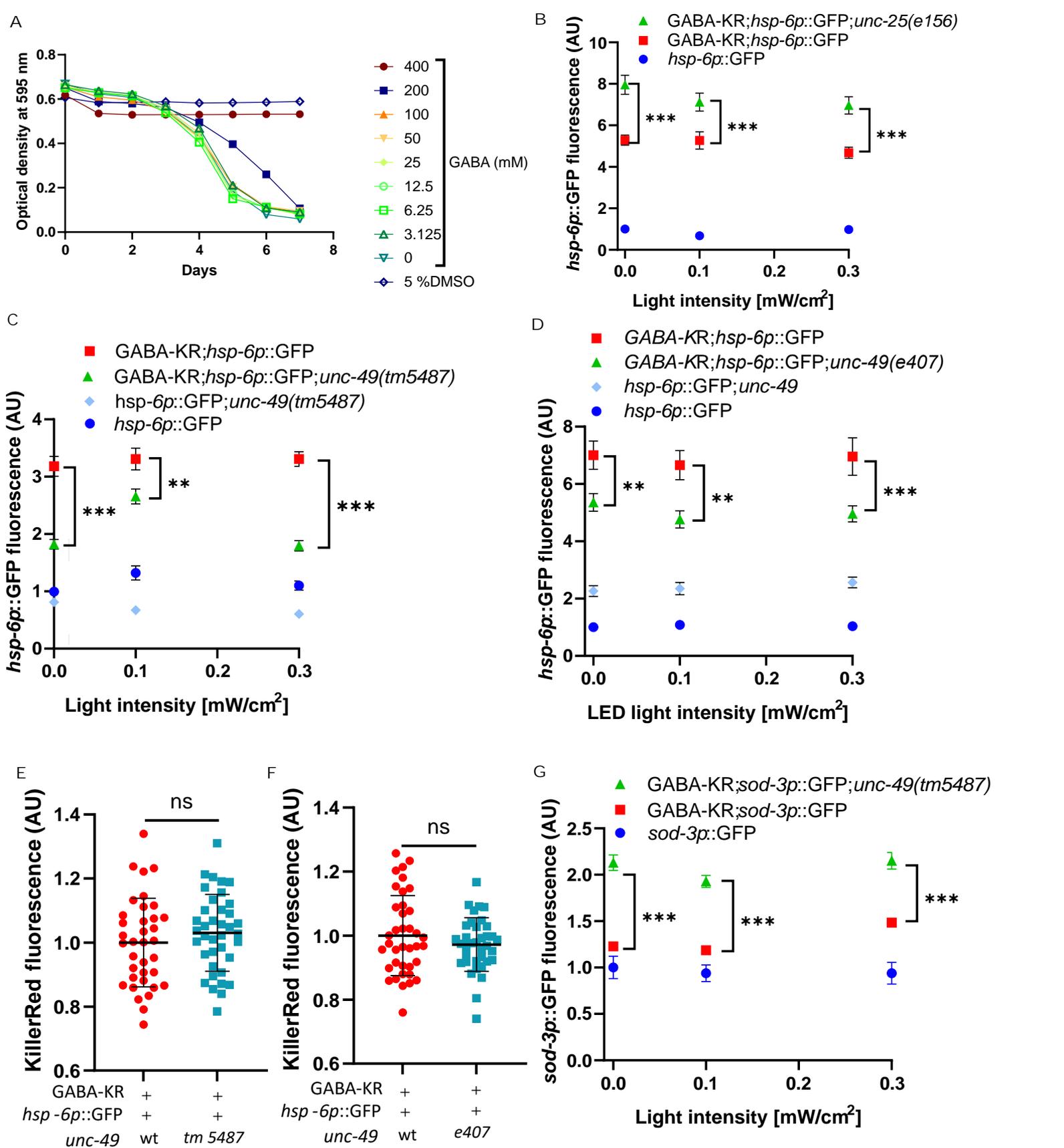
**B)** Western blot analysis of KillerRed expression in WT, GABA-KR, GABA-KR;*hsp-6p::GFP* and cholinergic-KR strains, representative of two biological replicates. **C)** GFP intensity measurements of *sod-3p::GFP* in the presence of GABA-KR (red, ring) and presence of cholinergic-KR (green, circle) between 0-3.0 mW/cm<sup>2</sup>. **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/cm<sup>2</sup>.



GABA-KR GABA-GFP

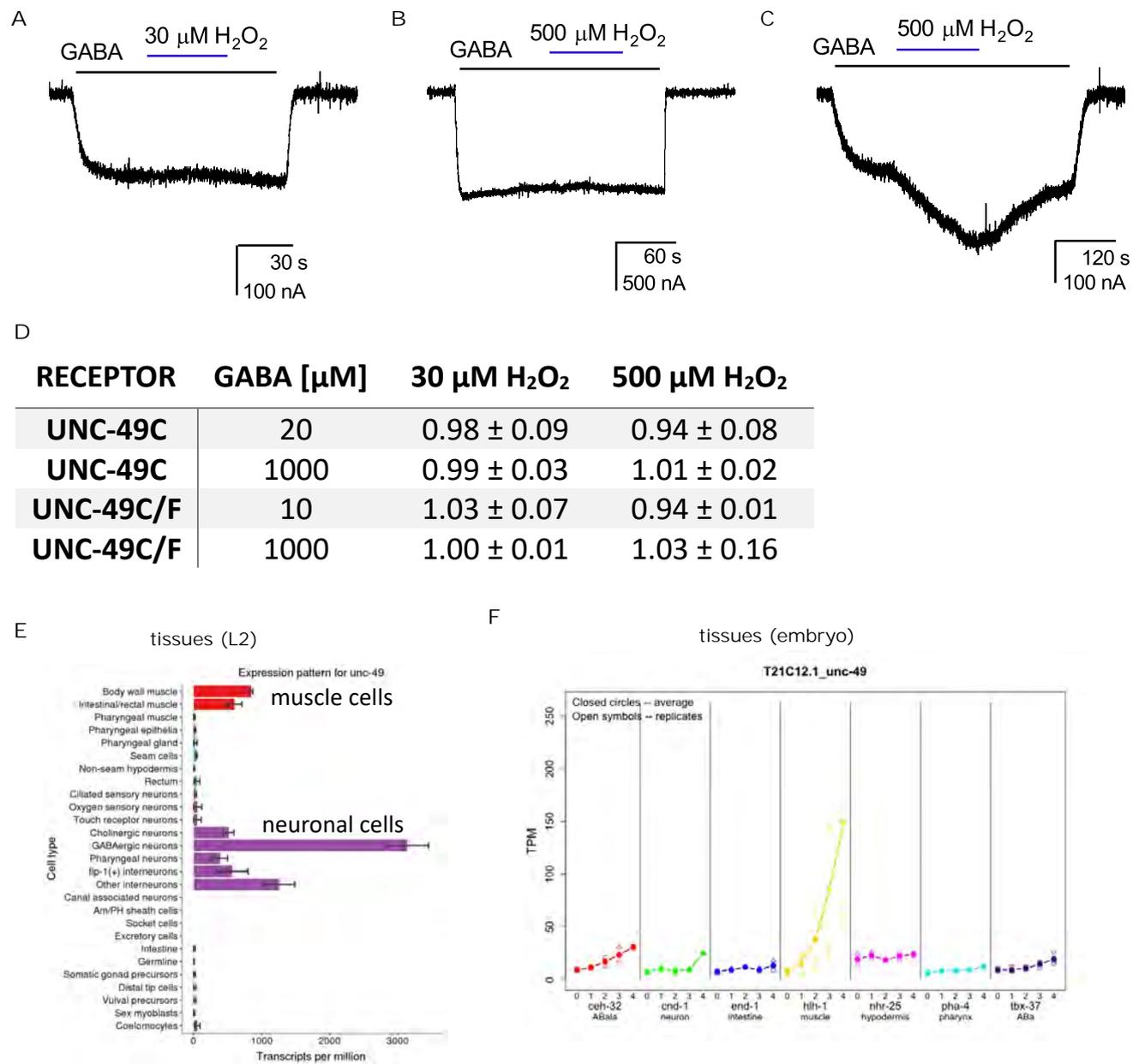
**Figure S3 | Effect of GABAergic KR vs. GABAergic GFP on *hsp-6p::GFP* expression (goes with Fig. 3)**

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  $\mu$ 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.



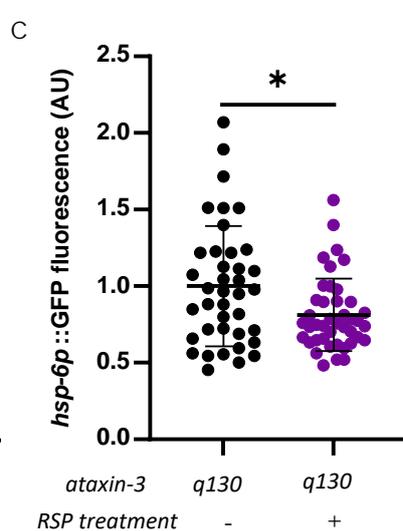
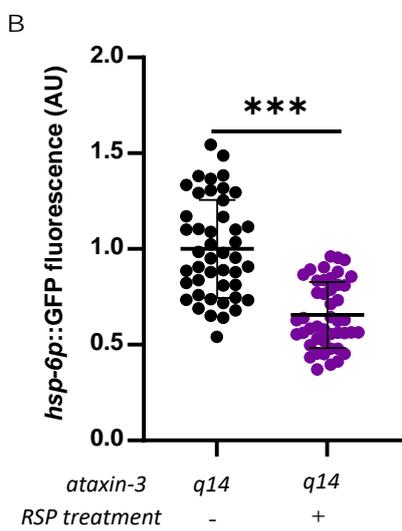
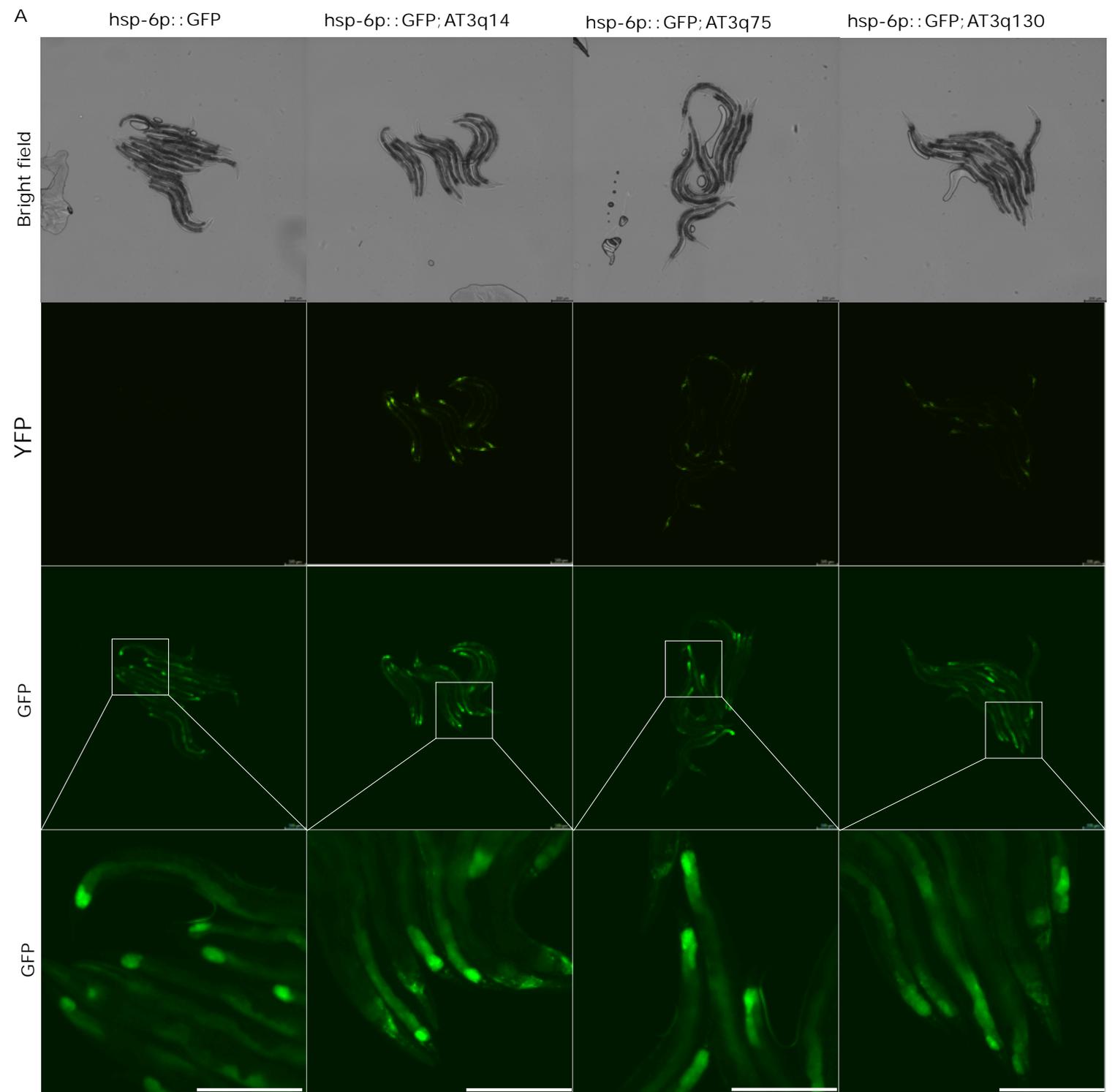
**Figure S4 | GABA drug toxicity in *C. elegans*, and effect of *unc-49* mutation on KR expression levels (goes with Fig. 4)**

**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  $\text{mW}/\text{cm}^2$ . 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  $\text{mW}/\text{cm}^2$ . 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  $\text{mW}/\text{cm}^2$ . 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  $\text{mW}/\text{cm}^2$ . 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 |  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$ . In **A** and **B**, the receptors were activated by 20  $\mu\text{M}$  GABA (EC2-4). In **C**, the receptors were activated by 0.4  $\mu\text{M}$  GABA (EC15). GABA applications are indicated by a black line.  $\text{H}_2\text{O}_2$  applications are indicated by a blue line. **D)** No modulation of GABA evoked currents in UNC-49C and UNC-49C/F receptors by  $\text{H}_2\text{O}_2$ . The table gives the modulating effect of 30  $\mu\text{M}$  and 500  $\mu\text{M}$   $\text{H}_2\text{O}_2$  on GABA currents in UNC-49C and UNC-49C/F receptors. The effects of  $\text{H}_2\text{O}_2$  were determined on both low (10 or 20  $\mu\text{M}$ ) and high (1000  $\mu\text{M}$ ) 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  $\mu$ 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/cm<sup>2</sup>).**

	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/cm <sup>2</sup>	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/cm <sup>2</sup>	1	65	20	0.6508	8	>0.9999	15	0.9993

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

<sup>a</sup> Maximum lifespan is the longest lived worms lifespan in days (mean ± standard deviation)

<sup>b</sup> 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</i> [ <i>Punc-47::krd-1</i> ] X	Marc Hammarlund, Yale	(15)
XE1158	GABA-KR,GFP	<i>juls76</i> [ <i>unc-25p::GFP + lin-15(+)</i> ] II; <i>wpls15</i> [ <i>unc-47p::KillerRed</i> ] X	Marc Hammarlund, Yale	(15)
XE1142	cholinergic-KR	<i>wpls14</i> [ <i>unc-17p::KillerRed + unc-122p::GFP</i> ] X	Marc Hammarlund, Yale	(15)
CF1553	<i>sod-3::GFP</i>	<i>muls84</i> [(pAD76) <i>sod-3p::GFP + rol-6(su1006)</i> ]	CGC	(91)
CL2070	<i>hsp-16.2::GFP</i>	<i>dvl570</i> [ <i>hsp-16.2p::GFP + rol-6(su1006)</i> ]	CGC	(92)
TJ356	<i>daf-16p::daf-16a/b::GFP</i>	<i>zls356</i> [ <i>daf-16p::daf-16a/b::GFP + rol-6(su1006)</i> ]	CGC	(45)
SJ4100	<i>hsp-6::GFP</i>	<i>zcls13</i> [ <i>hsp-6::GFP</i> ] V	CGC	(93)
CL2166	<i>gst-4::GFP</i>	<i>dvl519</i> [(pAF15) <i>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</i> [ <i>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</i> [PF25B3.3AT3v1-1q14::yfp]	Patricia Maciel/Andreia Teixeira-Castro, Braga, Portugal	(67)
MAC037/ AM519	AT3q75	<i>rmls237</i> [PF25B3.3AT3v1-1q75::yfp]	Patricia Maciel/Andreia Teixeira-Castro, Braga, Portugal	(67)
MAC001/ AM685	AT3q130	<i>rmls263</i> [PF25B3.3AT3v1-1q130::yfp] II	Patricia Maciel/Andreia Teixeira-Castro, Braga, Portugal	(67)
CL2122	A-beta control	<i>dvl515</i> [(pPD30.38) <i>unc-54</i> (vector) + (pCL26) <i>mtl-2::GFP</i> ]	CGC	(96)

<b>GMC101</b>	A-beta	<i>dvIs100 [unc-54p::A-beta-1-42::unc-54 3'-UTR + mtl-2p::GFP]</i>	CGC	(64)
<b>NM5548</b>	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)
<b>NM6007</b>	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>muls84</i> [(pAD76) <i>sod-3p::GFP</i> + <i>rol-6(su1006)</i> ]; <i>wpls[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[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[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[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>muls84</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[Punc-47::krd-1]</i> X; <i>unc-49(tm5487)</i> III	Held lab (F.Pohl)
JMH015	<i>hsp-6p::GFP</i> ;GABA-KR; <i>wt unc-49(tm5487)</i>	Cross: SJ4100;XE1150; <i>tm5487</i>	<i>zcls13</i> [ <i>hsp-6::GFP</i> ] V; <i>wpls[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[Punc-47::krd-1]</i> X; <i>unc-25(e156)</i> III	Held lab (F.Pohl)
JMH020	<i>hsp-6p::GFP</i> ;GABA-KR; <i>wt unc-25(e156)</i>	Cross: SJ4100;XE1150;CB156	<i>zcls13</i> [ <i>hsp-6::GFP</i> ] V; <i>wpls[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)

<b>JMH024</b>	<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)
<b>JMH028</b>	<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)
<b>JMH029</b>	<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)
<b>JMH030</b>	<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)
<b>JMH031</b>	GABA-KR; AT3q14	Cross: XE1150;MAC003/AM509	<i>wpls[Punc-47::krd-1]X; rmls227 [PF25B3.3AT3v1-1q14::yfp]</i>	Held lab (F.Pohl)
<b>JMH032</b>	GABA-KR; AT3q75	Cross: XE1150;MAC037/AM519	<i>wpls[Punc-47::krd-1]X; rmls237 [PF25B3.3AT3v1-1q75::yfp]</i>	Held lab (F.Pohl)
<b>JMH033</b>	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)
<b>JMH039</b>	Muscle A $\beta_{1-42}$ 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)
<b>JMH040</b>	Muscle A $\beta_{1-42}$ ;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)
<b>JMH049</b>	<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)
<b>JMH051</b>	<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)
<b>JMH052</b>	<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)
<b>JMH053</b>	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)
<b>JMH054</b>	<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)

<b>JMH056</b>	<i>hsp-6p::GFP;AT3q130;unc-49(e407)</i>	Cross: SJ4100; MAC001/AM685;CB407	<i>zcls13[hsp-6::GFP] V; rms263 [PF25B3.3AT3v1-1q130::yfp] II; unc-49(e407)III</i>	Held lab (F.Pohl)
<b>JMH057</b>	<i>hsp-6p::GFP; AT3q130;wt unc-49(e407)</i>	Cross: SJ4100; MAC001/AM685;CB407	<i>zcls13[hsp-6::GFP] V; rms263 [PF25B3.3AT3v1-1q130::yfp] II</i>	Held lab (F.Pohl)
<b>JMH058</b>	<i>AT3q130; unc-49(e407)</i>	Cross: SJ4100; MAC001/AM685;CB407	<i>rms263 [PF25B3.3AT3v1-1q130::yfp] II; unc-49(e407)III</i>	Held lab (F.Pohl)
<b>JMH059</b>	<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)
<b>JMH064</b>	<i>hsp-6p::GFP;AT3q130;unc-49(tm5487)</i>	Cross: SJ4100; MAC001/AM685; <i>tm5487</i>	<i>zcls13[hsp-6::GFP] V; rms263 [PF25B3.3AT3v1-1q130::yfp] II; unc-49(tm5487)III</i>	Held lab (F.Pohl)
<b>JMH065</b>	<i>hsp-6p::GFP; AT3q130;wt unc-49(tm5487)</i>	Cross: SJ4100; MAC001/AM685; <i>tm5487</i>	<i>zcls13[hsp-6::GFP] V; rms263 [PF25B3.3AT3v1-1q130::yfp] II</i>	Held lab (F.Pohl)
<b>JMH066</b>	<i>AT3q130; unc-49(tm5487)</i>	Cross: SJ4100; MAC001/AM685; <i>tm5487</i>	<i>rms263 [PF25B3.3AT3v1-1q130::yfp] II; unc-49(tm5487)III</i>	Held lab (F.Pohl)
<b>JMH067</b>	<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)
<b>NM6388</b>	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
<b>NM6402</b>	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
<b>NM6418<sup>#</sup></b>	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

<b>NM6419</b>	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
<b>NM6421<sup>#</sup></b>	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
<b>NM6414<sup>#</sup></b>	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
<b>NM6416<sup>#</sup></b>	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
<b>NM6417</b>	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
<b>NM6452</b>	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

<sup>#</sup> 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**

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