

Title: Regulation of age-related structural integrity in neurons by protein with tau-like repeats (PTL-1) is cell autonomous

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Legends

Supplementary Figure S1

Pan-neuronal but not TRN-specific re-expression of PTL-1 rescues the short-lived phenotype of the *ptl-1(ok621)* null mutant. The survival curves shown were obtained independently from data shown in **Figure 1bii** and **cii**. Lifespan assays for a) pan-neuronal transgenic worms and b) TRN-transgenic worms. Results of statistical analysis are indicated by p-values underneath each graph. Survival curves for control wild-type and *ptl-1(ok621)* animals in both graphs were obtained in the same experiment. For all strains, n = 120 at day 0. Details of p-values are shown in **Supplementary Table S2**.

Supplementary Figure S2

Neuronal aging phenotypes rescued by pan-neuronal or TRN-specific Tg lines at 20 °C also rescue at 25 °C in TRNs and GABAergic neurons. The TRNs were visualised using the *Pmec-*

4::gfp (*zdis5*) reporter and the GABAergic neurons using the *Punc-47::gfp* (*oxIs12*) reporter. The presence of the *ptl-1(ok621)* mutation in the genetic background of each transgenic line is indicated by the addition of “*ptl-1(ok621)*” in the strain name. a) Neuron imaging assay conducted for pan-neuronal transgenic worms (“Pan-neuronal Tg”) showing (i) TRN cell body branching, (ii) TRN axon blebbing and (iii) GABAergic commissure branching. b) Neuron imaging assay conducted for TRN-specific transgenic worms (“TRN Tg”) showing (i) TRN cell body branching, (ii) TRN axon blebbing and (iii) GABAergic commissure branching. Data for wild-type and *ptl-1(ok621)* animals in TRN graphs were obtained in the same experiment, similarly data for wild-type and *ptl-1(ok621)* animals in GABAergic neuron graphs were obtained from the same experiment. n for each time-point is indicated under the graphs. The chi-squared statistical test was used to determine statistical significance. P-value is indicated by ns = not significant, * <0.05 . Details of p-values are shown in **Supplementary Table S2**. Experiments were conducted twice independently, and the representative data shown are from one experiment.

Supplementary Figure S3

Knockdown of *ptl-1* in a TRN *SID-1* transgenic animal expressing PTL-1::GFP results in loss of fluorescence in TRNs only. The strain used for imaging expresses a PTL-1::GFP fusion protein and TRN-specific *SID-1* in a *sid-1* mutant background. Micrographs are shown for empty vector (EV) RNAi controls (a,c,e) and for *ptl-1* RNAi treatment (b,d,f) with PTL-1::GFP shown on the left and phase images shown on the right. For all micrographs showing fluorescence, boxed regions (solid lines) are shown as a magnified inset that is bordered by dashed lines. a) The ALM neuron in the EV control is clearly visible at a 200 ms exposure time, indicated by the

white arrow. b) The approximate location of the ALM neuron in an animal exposed to *ptl-1* RNAi treatment shows no fluorescence visible at 200 ms. (c,d) At a longer exposure time (5000 ms), the ALM neuron is clearly seen in the EV control, indicated by the white arrow (c) and as a faint signal in the *ptl-1* RNAi-treated animal (d). (e,f) The PLM neuron cell body is clearly visible in the EV control, indicated by the white arrow (e) but not with *ptl-1* RNAi treatment (f) at a 200 ms exposure time. Fourth larval stage/first day adult animals were imaged after being fed RNAi bacteria for two generations at 20 °C.

Supplementary Table S1

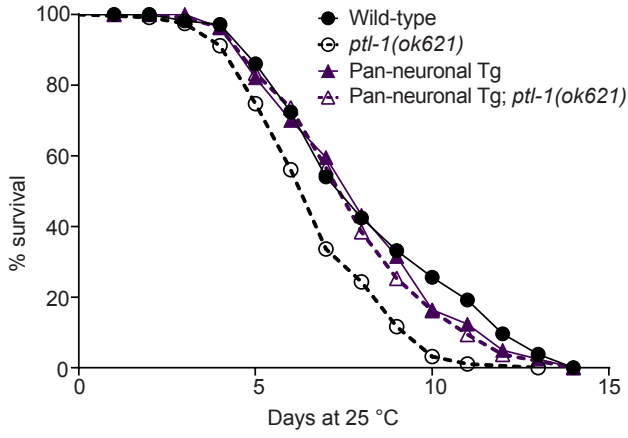
Tables indicate the exact p-value (to two decimal places) of statistical testing conducted in this investigation shown in Figures 1-4. Neuronal imaging experiments both for transgenic lines and knockdown by RNAi treatment were analysed using Pearson's chi-squared test (Microsoft Excel). Survival curves were analysed with GraphPad Prism 6 (GraphPad Software Inc.) using log-rank and Wilcoxon tests. For more detail on statistical methods, please see the **Materials and Methods** section.

Supplementary Table S2

Tables indicate the exact p-value (to two decimal places) of statistical testing conducted for lifespan and neuronal imaging experiments shown in Supplementary Figures S1 and S2. Neuronal imaging experiments for transgenic lines were analysed using Pearson's chi-squared test (Microsoft Excel). Survival curves were analysed with GraphPad Prism 6 (GraphPad Software Inc.) using log-rank and Wilcoxon tests. For more detail on statistical methods, please see the **Materials and Methods** section.

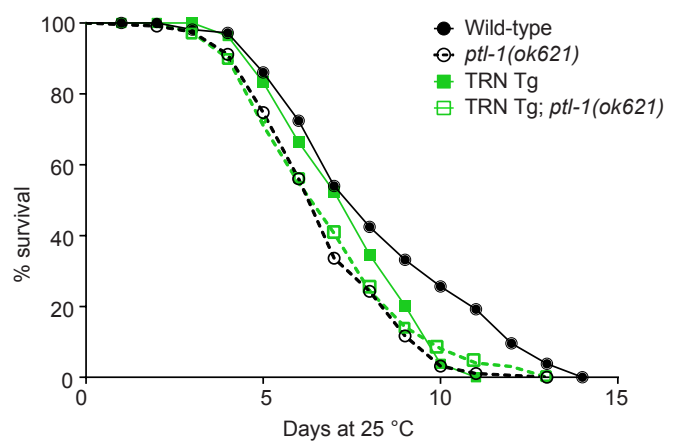
Lifespan experiments for pan-neuronal and TRN Tg lines - independent experiment #2

a Lifespan assay: Pan-neuronal Tg



	Log rank test	Gehan-Breslow-Wilcoxon test
Wild-type vs. <i>ptl-1(ok621)</i>	<0.05	<0.05
Wild-type vs. "Pan-neuronal Tg"	Not significant	Not significant
Wild-type vs. "Pan-neuronal Tg; <i>ptl-1(ok621)</i> "	Not significant	Not significant

b Lifespan assay: TRN Tg



	Log rank test	Gehan-Breslow-Wilcoxon test
Wild-type vs. <i>ptl-1(ok621)</i>	<0.05	<0.05
Wild-type vs. "TRN Tg"	<0.05	Not significant
Wild-type vs. "TRN Tg; <i>ptl-1(ok621)</i> "	<0.05	<0.05

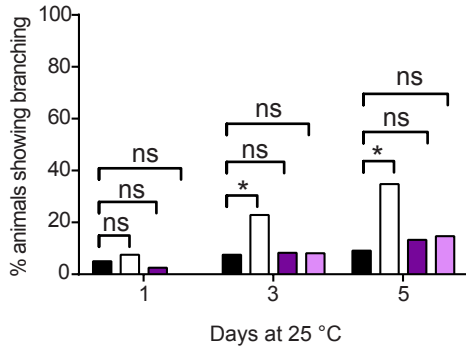
Neuron imaging experiments at 25 °C

Pan-neuronal Tg

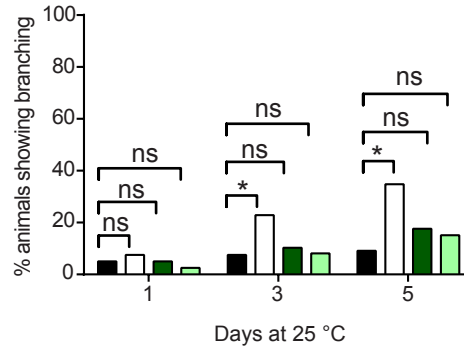
TRN-specific Tg



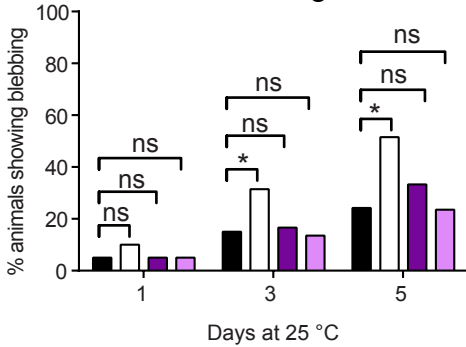
a i TRN cell body branching



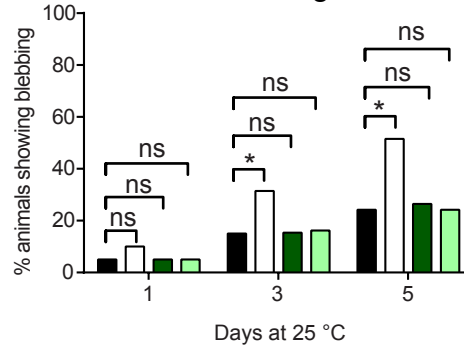
b i TRN cell body branching



ii TRN axon blebbing



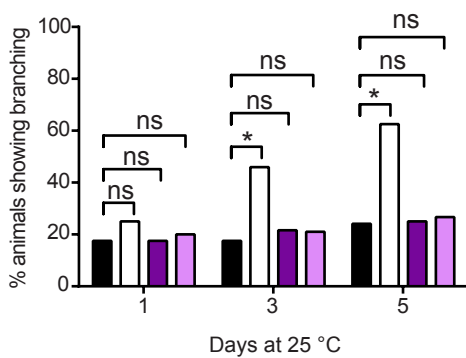
ii TRN axon blebbing



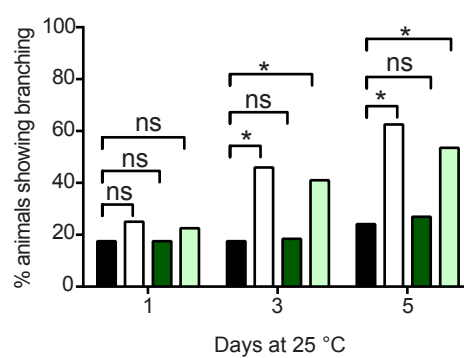
n	D1	D3	D5
Wild-type	40	40	33
<i>ptl-1(ok621)</i>	40	35	23
Pan-neuronal Tg	40	36	30
Pan-neuronal Tg; <i>ptl-1(ok621)</i>	40	37	34

n	D1	D3	D5
Wild-type	40	40	33
<i>ptl-1(ok621)</i>	40	35	23
TRN Tg	40	39	34
TRN Tg; <i>ptl-1(ok621)</i>	40	37	33

iii GABAergic neuron branching



iii GABAergic neuron branching

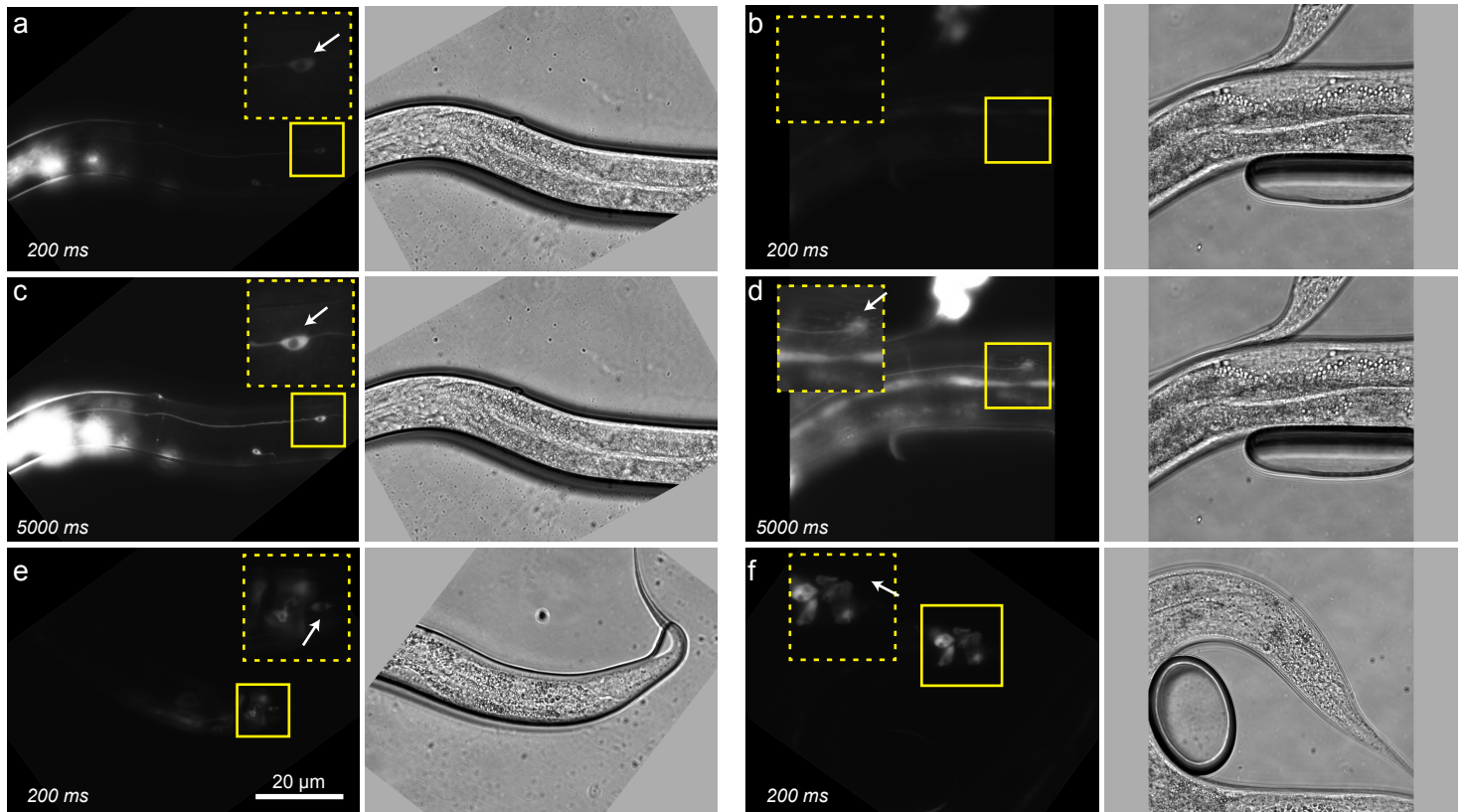


n	D1	D3	D5
Wild-type	40	40	29
<i>ptl-1(ok621)</i>	40	37	16
Pan-neuronal Tg	40	37	28
Pan-neuronal Tg; <i>ptl-1(ok621)</i>	40	38	30

n	D1	D3	D5
Wild-type	40	40	33
<i>ptl-1(ok621)</i>	40	35	23
TRN Tg	40	38	26
TRN Tg; <i>ptl-1(ok621)</i>	40	39	28

TRN-specific RNAi knockdown of *ptl-1* in a PTL-1::GFP transgenic line

EV RNAi

ptl-1 RNAi

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Supplementary Table S1

Neuron imaging assay using Pan-neuronal Tg and TRN Tg lines

Table indicates p-values for chi-squared statistical test between wild-type (set as expected value) and other strains tested

Anterior TRNs - cell body branching

Day	<i>ptl-1(ok621)</i>	Pan-neuronal Tg	Pan-neuronal Tg; <i>ptl-1(ok621)</i>	TRN Tg	TRN Tg; <i>ptl-1(ok621)</i>
1	0.55	0.55	0.23	0.55	0.55
5	0.01	0.34	0.16	0.16	0.48
7	2.69E-05	0.72	0.26	0.51	0.28
9	2.63E-04	0.67	0.44	0.50	0.21

Anterior TRNs - axon blebbing

Day	<i>ptl-1(ok621)</i>	Pan-neuronal Tg	Pan-neuronal Tg; <i>ptl-1(ok621)</i>	TRN Tg	TRN Tg; <i>ptl-1(ok621)</i>
1	not calculated - wild-type value is 0%				
5	not calculated - wild-type value is 0%				
7	1.37E-07	0.74	0.21	0.44	0.01
9	1.92E-09	0.05	0.05	0.11	0.09

GABAergic neurons - branching

Day	<i>ptl-1(ok621)</i>	Pan-neuronal Tg	Pan-neuronal Tg; <i>ptl-1(ok621)</i>	TRN Tg	TRN Tg; <i>ptl-1(ok621)</i>
1	0.34	0.63	1.00	0.63	1.00
5	5.90E-07	0.10	0.26	0.11	8.46E-07
7	1.72E-04	0.44	0.51	0.85	2.88E-04
9	7.41E-07	0.57	0.57	0.36	3.85E-06

Lifespan assay

p-values for survival curves using log-rank or Gehan-Breslow-Wilcoxon (Wilcoxon) comparisons between wild-type and other strains tested

Test	<i>ptl-1(ok621)</i>	Pan-neuronal Tg	Pan-neuronal Tg; <i>ptl-1(ok621)</i>	TRN Tg	TRN Tg; <i>ptl-1(ok621)</i>
Log-rank	< 0.0001	0.28	0.28	0.01	1.00E-04
Wilcoxon	3.00E-04	0.53	0.47	0.15	2.10E-03

Neuron imaging assay using *ptl-1* RNAi

p-values for chi-squared statistical test between empty vector (EV) treatment (set as expected value) and *ptl-1* treatment for each genotype

Anterior TRNs - cell body branching

Day	<i>zDIs5</i>	<i>sid-1(qt2)</i> ; TRN SID-1
1	0.54	0.65
3	1.00	0.41
5	0.69	0.01
7	0.70	0.01

Anterior TRNs - axon blebbing

Day	<i>zDIs5</i>	<i>sid-1(qt2)</i> ; TRN SID-1
1	n.c.	1.00
3	1.00	0.22
5	0.46	0.00
7	1.00	0.01

n.c. = not calculated - wild-type value is 0%

Anterior TRNs - axon branching

Day	<i>zDIs5</i>	<i>sid-1(qt2)</i> ; TRN SID-1
1	0.31	0.54
3	n.c.	0.00
5	n.c.	0.00
7	n.c.	0.11

n.c. = not calculated - wild-type value is 0%

GABAergic neurons - branching

Day	<i>zDIs5</i>	<i>sid-1(qt2)</i> ; TRN SID-1
1	1.00	1.00
3	0.62	1.00
5	1.00	0.39
7	0.45	0.82

n.c. = not calculated - wild-type value is 0%

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Supplementary Table S2

Lifespan assay

p-values for survival curves using log-rank or Gehan-Breslow-Wilcoxon (Wilcoxon) comparisons between wild-type and other strains tested

Test	<i>ptl-1(ok621)</i>	Pan-neuronal Tg	Pan-neuronal Tg; <i>ptl-1(ok621)</i>	TRN Tg	TRN Tg; <i>ptl-1(ok621)</i>
Log-rank	< 0.0001	0.51	0.31	0.00	4.00E-04
Wilcoxon	4.00E-04	0.76	0.63	0.12	1.00E-03

Neuron imaging assay using Pan-neuronal Tg and TRN Tg lines

Table indicates p-values for chi-squared statistical test between wild-type (set as expected value) and other strains tested

Anterior TRNs - cell body branching

Day	<i>ptl-1(ok621)</i>	Pan-neuronal Tg	Pan-neuronal Tg; <i>ptl-1(ok621)</i>	TRN Tg	TRN Tg; <i>ptl-1(ok621)</i>
1	0.04	1.00	1.00	1.00	1.00
3	0.00	0.52	0.24	0.87	0.63
5	9.94E-03	0.43	0.48	0.48	0.47

Anterior TRNs - axon blebbing

Day	<i>ptl-1(ok621)</i>	Pan-neuronal Tg	Pan-neuronal Tg; <i>ptl-1(ok621)</i>	TRN Tg	TRN Tg; <i>ptl-1(ok621)</i>
1	0.15	1.00	1.00	1.00	1.00
3	0.01	0.49	0.59	0.86	0.61
5	0.00	0.22	0.84	0.72	1.00

GABAergic neurons - branching

Day	<i>ptl-1(ok621)</i>	Pan-neuronal Tg	Pan-neuronal Tg; <i>ptl-1(ok621)</i>	TRN Tg	TRN Tg; <i>ptl-1(ok621)</i>
1	0.21	1.00	0.68	1.00	0.41
3	1.06E-05	0.43	0.52	0.73	1.33E-04
5	3.25E-04	0.83	0.71	0.52	3.42E-04