Cell Reports

Supplemental Information

The Apoptotic Engulfment Machinery Regulates Axonal Degeneration in *C. elegans* Neurons

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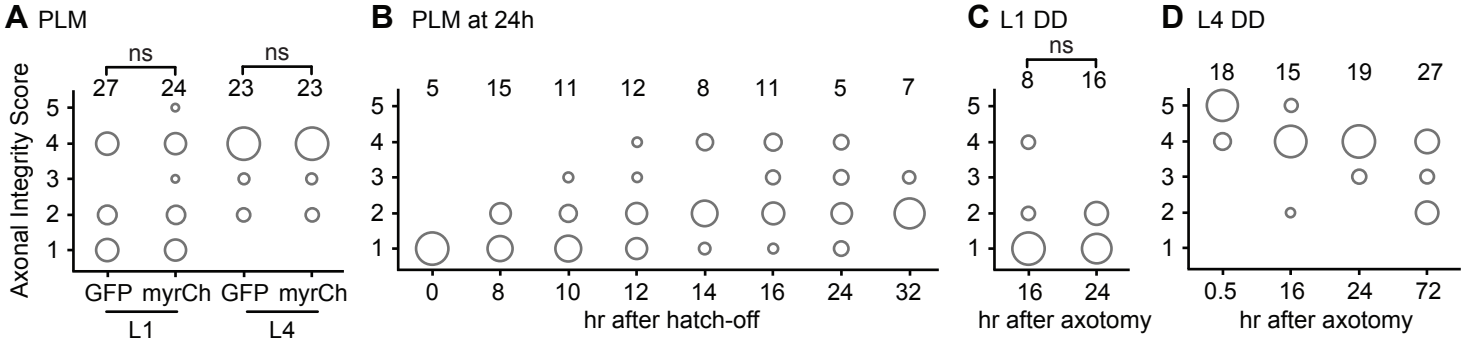
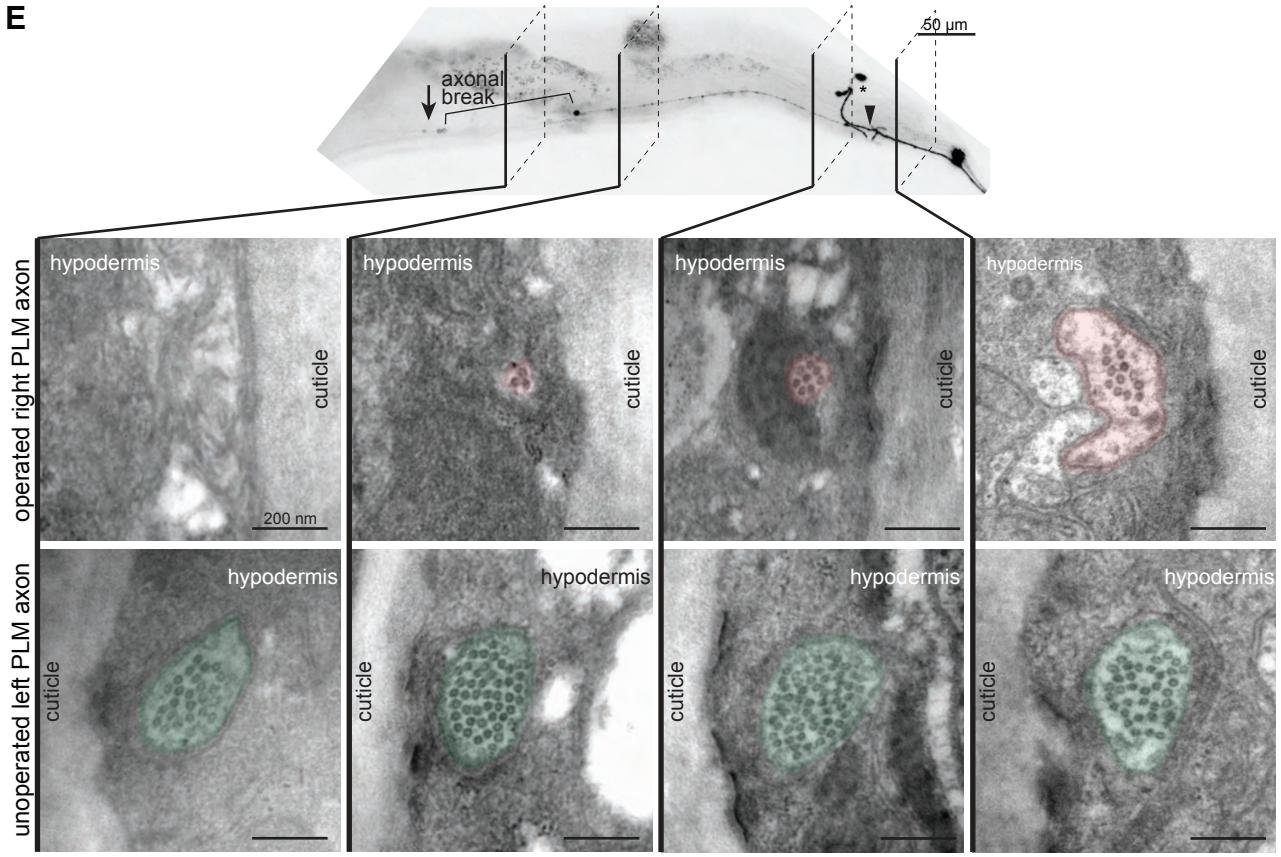
Figure S1


A PLM
Axonal Integrity Score
ns
ns
27
24
23
23
GFP
myrCh
GFP
myrCh
L1
L4
B PLM at 24h
5
15
11
12
8
11
5
7
0
8
10
12
14
16
24
32
hr after hatch-off
C L1 DD
ns
8
16
16
24
hr after axotomy
D L4 DD
18
15
19
27
0.5
16
24
72
hr after axotomy


E
axonal break
50 µm
operated right PLM axon
unoperated left PLM axon
hypodermis
cuticle
200 nm

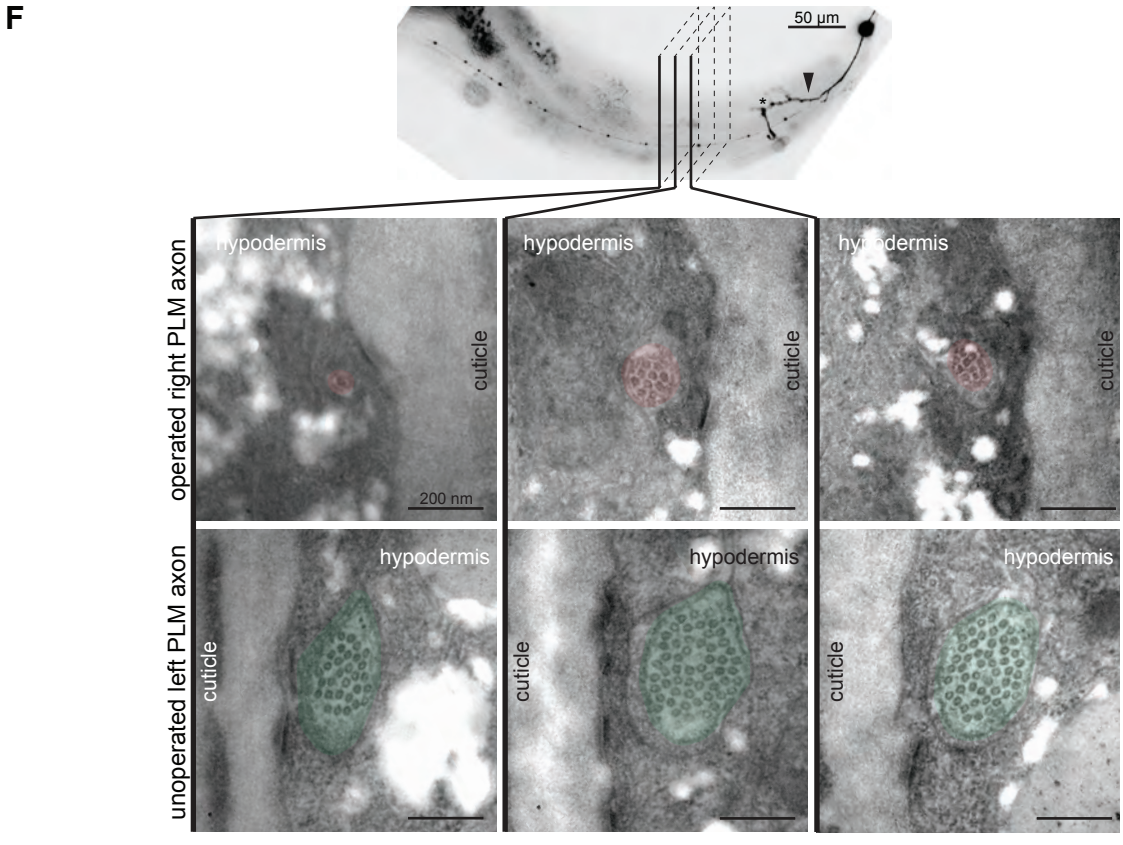

F
50 µm
operated right PLM axon
unoperated left PLM axon
hypodermis
cuticle
200 nm

Figure S1. Additional characterization of axonal degeneration in *C. elegans* neurons following laser-induced axotomy, related to Figure 1. (**A**) Axotomies of neurons expressing a membrane-bound fluorophore (*Pmec-4::MYR::mCherry*) together with cytoplasmic GFP show highly correlated levels of degeneration at both the L1 and L4 stages (scored at 16 and 72 hours, respectively). For each axon, degeneration was first scored using MYR::mCherry (myrCh), followed by GFP, thereby generating two scores for each axon. (**B**) Axotomies of the PLM neuron where performed at increasing time points after hatching, and degeneration was scored 24 hours post-axotomy. Axonal degeneration at multiple time points post-axotomy of the DD3 and DD5 motor neurons at the L1 (**C**) or L4 (**D**) stages. Each animal was imaged at only one time point. The area of each circle represents the proportion of data points that fall into that category; n value indicated on top of each bubble graph. Significance was tested for by a Kruskal-Wallis and Dunn's post-test or a Mann-Whitney test. (**E, F**) Representative images of EM serial transverse sections of two unilaterally axotomized L4 animals. For each animal, the fluorescence image shows the axotomized PLM neuron 72 hours post-axotomy, immediately before fixation for EM imaging. Arrowheads point to the location of the axotomy, asterisks indicate the regrowing axon, the arrow in (**E**) points to a distal separated fragment, and the bracket in (**E**) indicates a break in the axon. For each transverse section presented here, an electron micrograph of the operated neuron is presented in the top panel (operated right PLM axon, axon highlighted in red), and an electron micrograph of the healthy contralateral neuron is presented in the bottom panel (unoperated left PLM axon, axon highlighted in green). Note the reduced diameter and number of microtubules of the degenerating axon (thinning), compared to the healthy axon on the contralateral side (**E, F**). The axon is completely absent in sections corresponding to an axonal break in animal (**E**).

Figure S2

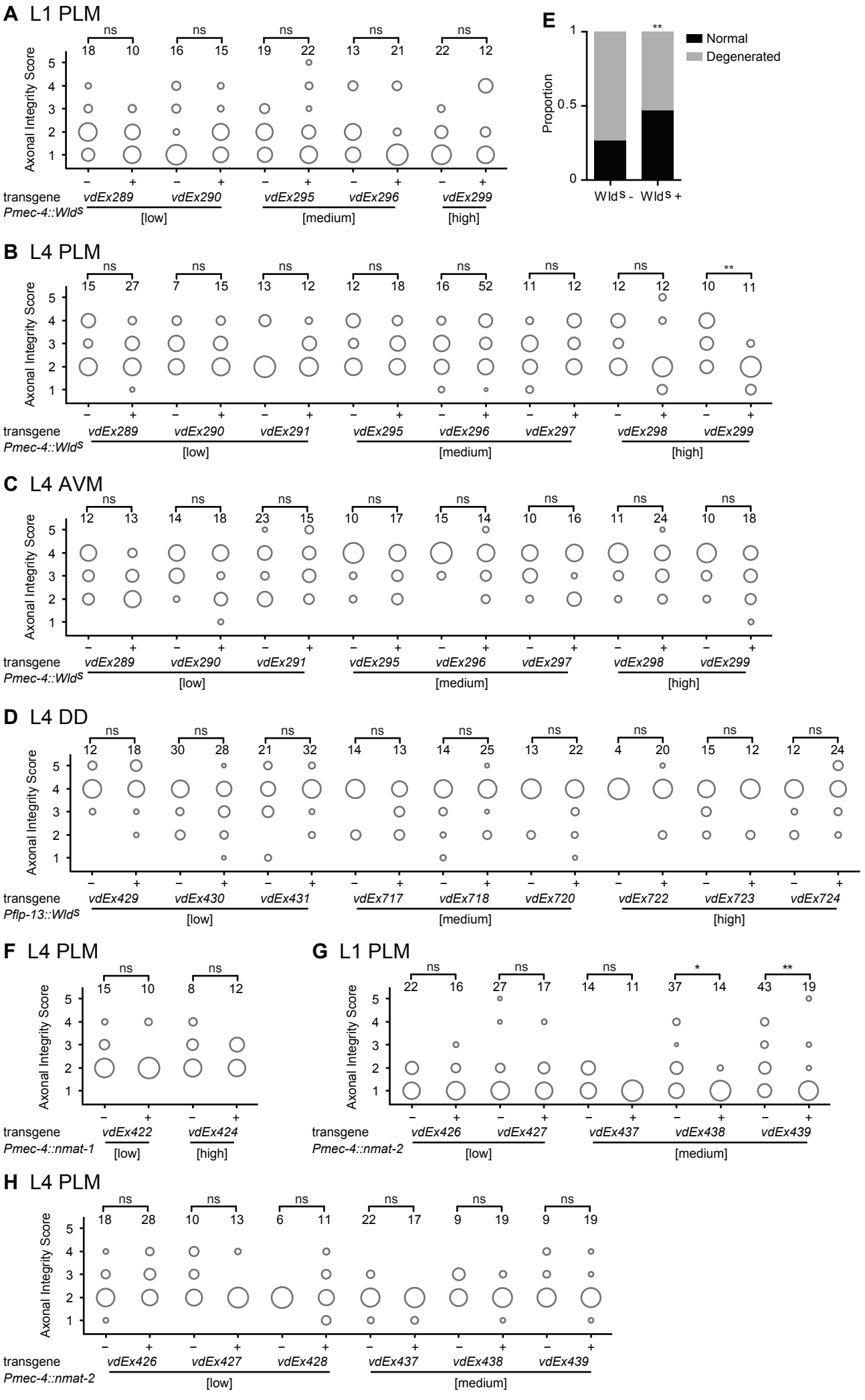

A L1 PLM
B L4 PLM
C L4 AVM
D L4 DD
E
F L4 PLM
G L1 PLM
H L4 PLM
Axonal Integrity Score
Proportion
Normal
Degenerated
WldS -
WldS +
transgene
Pmec-4::WldS
Pflp-13::WldS
Pmec-4::nmat-1
Pmec-4::nmat-2
[low]
[medium]
[high]
ns
vdEx289
vdEx290
vdEx291
vdEx295
vdEx296
vdEx297
vdEx298
vdEx299
vdEx429
vdEx430
vdEx431
vdEx717
vdEx718
vdEx720
vdEx722
vdEx723
vdEx724
vdEx422
vdEx424
vdEx426
vdEx427
vdEx428
vdEx437
vdEx438
vdEx439

Figure S2. Overexpression of *Wld^S* or the endogenous *Nmnat* genes has no protective effect against axonal degeneration after axotomy, related to Figure 2. Axonal degeneration of PLM axons quantified (**A**) 16 hours post-axotomy in L1 animals or (**B**) 72 hours post-axotomy in L4 animals. Animals expressed *Pmec-4::Wld^S* at low (5 ng/µL), medium (10 ng/µL), or high (20 ng/µL) concentrations. (**C**) Axonal degeneration of the AVM neuron 72 hours post-axotomy at the L4 stage in animals expressing the *Pmec-4::Wld^S* transgene at low (5 ng/µL), medium (10 ng/µL), or high (20 ng/µL) concentrations. (**D**) Quantification of axonal degeneration 48 hours after axotomy in DD3 and DD5 motor neurons in animals carrying the *Pflp-13::Wld^S* transgene at low (5 ng/µL), medium (10 ng/µL), or high (20 ng/µL) concentrations. (**E**) WLD^S significantly protects against *mec-4(d)* induced neurodegeneration of AVM; n ≥100. (**F**) Quantification of axonal degeneration in PLM neurons 72 hours post-axotomy in L4 in animals carrying the *Pmec-4::nmat-1* transgene at low (5 ng/µL), or high (20 ng/µL) concentrations. (**G**) Quantification of axonal degeneration in PLM neurons 24 hours after axotomy in L1 at low (5 ng/µL) and medium (10 ng/µL) concentrations, or (**H**) 72 hours post-axotomy in L4 at low (5 ng/µL) and medium (10 ng/µL) concentrations of the *Pmec-4::nmat-2* transgene. The area of each circle represents the proportion of data points that fall into that category; n value indicated on top of each bubble graph. * $p<0.05$, ** $p<0.005$ as determined by a Kruskal-Wallis and Dunn's or a Mann-Whitney test, or a Z test for proportions (**E**).

Figure S3

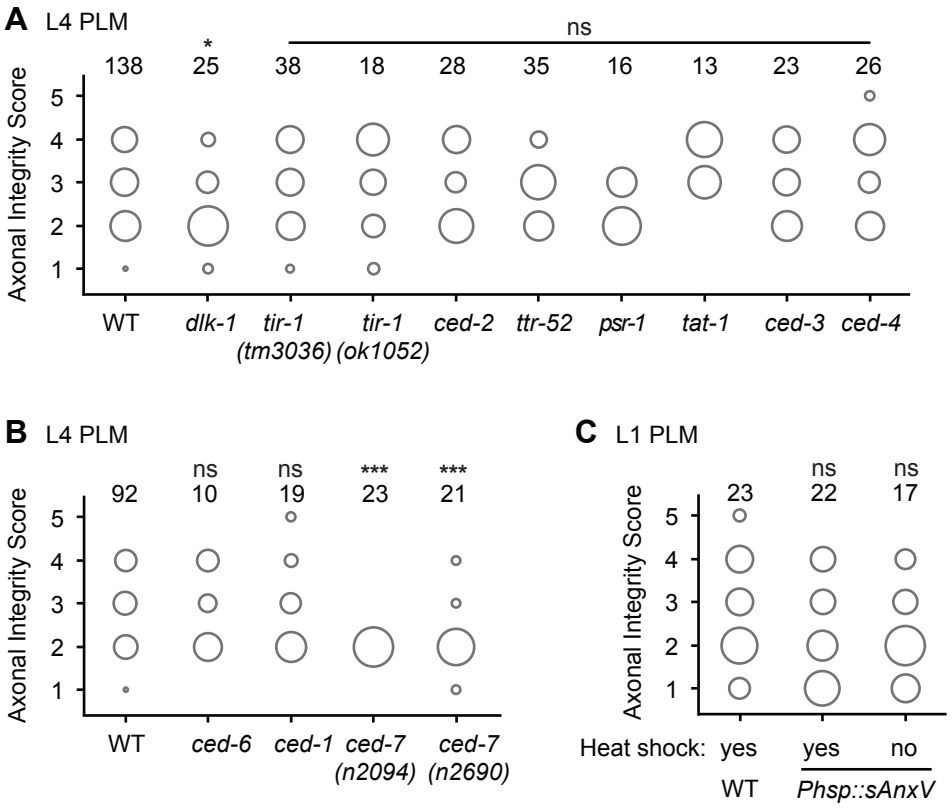

A
L4 PLM
ns
*
138
25
38
18
28
35
16
13
23
26
Axonal Integrity Score
5
4
3
2
1
WT
dlk-1
tir-1 (tm3036)
tir-1 (ok1052)
ced-2
ttr-52
psr-1
tat-1
ced-3
ced-4
B
L4 PLM
ns
ns
***
***
92
10
19
23
21
Axonal Integrity Score
WT
ced-6
ced-1
ced-7 (n2094)
ced-7 (n2690)
C
L1 PLM
ns
ns
23
22
17
Axonal Integrity Score
Heat shock:
yes
yes
no
WT
Phsp::sAnxV

Figure S3. Conserved genes associated with axonal degeneration in other species do not have a similar role in *C. elegans*, related to Figure 3. (**A**) Mutations in *dlk-1(ju476)*, *tir-1(tm3036)*, *tir-1(ok1052)*, *ced-2(e1752), ttr-52*(*sm211*), *psr-1(ok714), tat-1(tm3117), ced-3(n2452),* and *ced-4(n1162)* do not protect against axonal degeneration 72 hours post-axotomy at the L4 stage. (**B**) Quantification of axonal degeneration 72 hours post-axotomy at the L4 stage in animals carrying mutations in *ced-6(n1813)*, *ced-1(e1735)*, *ced-7(n2094),* or *ced-7(n2690)*. (**C**) Quantification of axonal degeneration following exposure to an mRFP-tagged version of Annexin V, driven by a heat shock promoter. The area of each circle represents the proportion of data points that fall into that category; n value indicated on top of each bubble graph. * $p<0.05$, *** $p<0.0005$ as measured by a Kruskal-Wallis and Dunn's test comparing groups to wild-type (WT) or a Tukey's multiple comparisons test to compare between mutants.

Figure S4

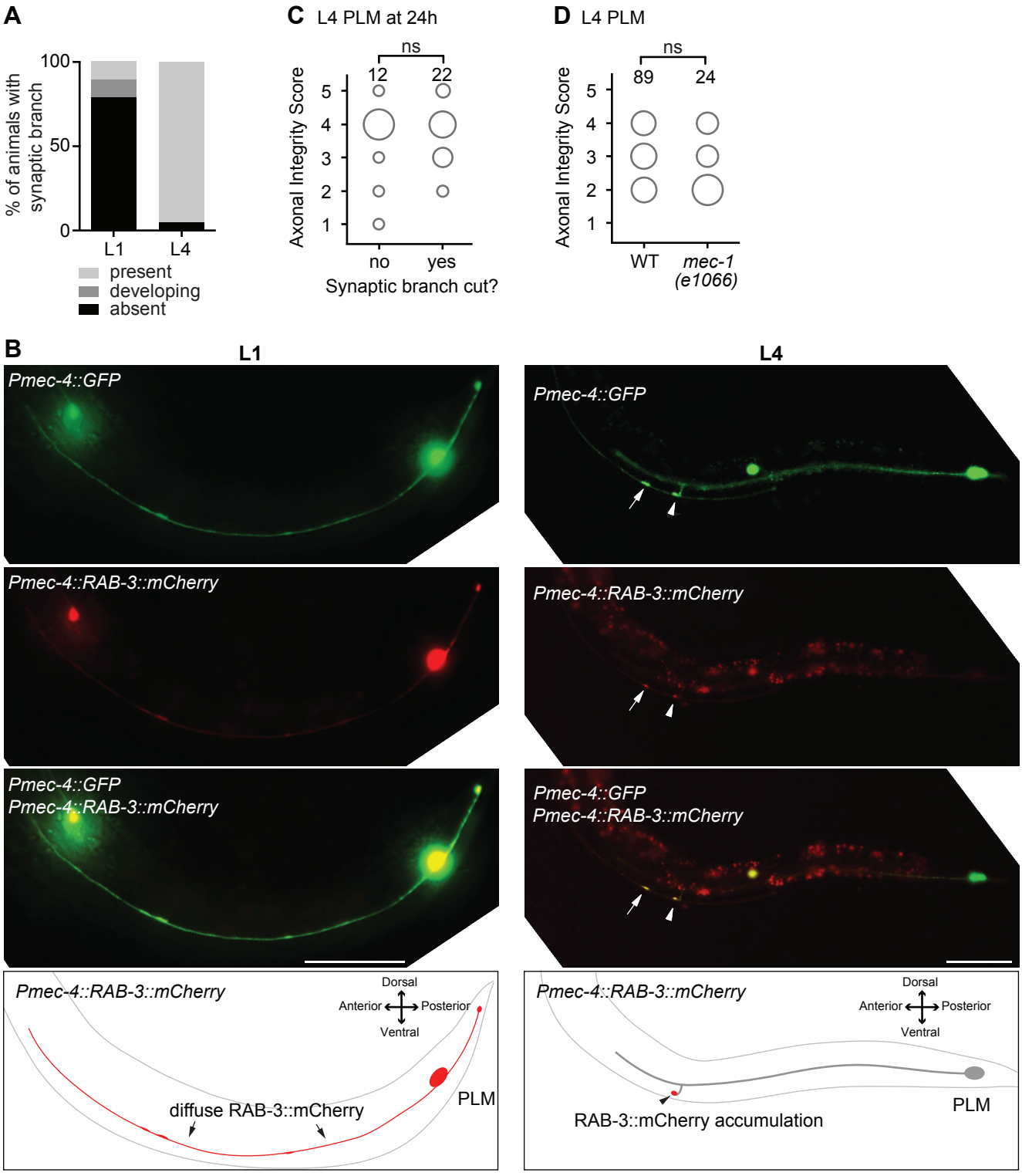

A
100
50
0
% of animals with synaptic branch
L1
L4
present
developing
absent
C L4 PLM at 24h
ns
12
22
Axonal Integrity Score
5
4
3
2
1
no
yes
Synaptic branch cut?
D L4 PLM
ns
89
24
Axonal Integrity Score
WT
mec-1 (e1066)
B
L1
L4
Pmec-4::GFP
Pmec-4::RAB-3::mCherry
Pmec-4::GFP
Pmec-4::RAB-3::mCherry
Dorsal
Anterior
Posterior
Ventral
diffuse RAB-3::mCherry
PLM
RAB-3::mCherry accumulation

Figure S4. During development, axonal degeneration of PLM occurs independently of the presence of the synaptic branch, related to Figure 5. (**A**) The synaptic branch develops after the extension of the PLM axon and is absent in 79% (n=19) of L1 animals but only 5% (n=20) of L4 animals. (**B**) The lack of PLM presynaptic loci at the L1 stage is supported by the absence of accumulation of the synaptic vesicle associated small guanosine triphosphatase RAB-3 tagged with mCherry on the PLM branch. The arrowhead indicates the presynaptic locus with RAB-3 localization on the left PLM neuron; the arrow points to the locus of the right PLM. (**C**) Severing the synaptic branch after axotomy does not change the average degeneration score after 24 hours in L4 animals. (**D**) Degeneration of PLM in L4 wild-type (WT) and *mec-1(e1066)* mutant animals, 72 hours after axotomy. The area of each circle represents the proportion of data points that fall into that category; n value indicated on top of each bubble graph. Significance was tested using the Mann-Whitney test.

Figure S5

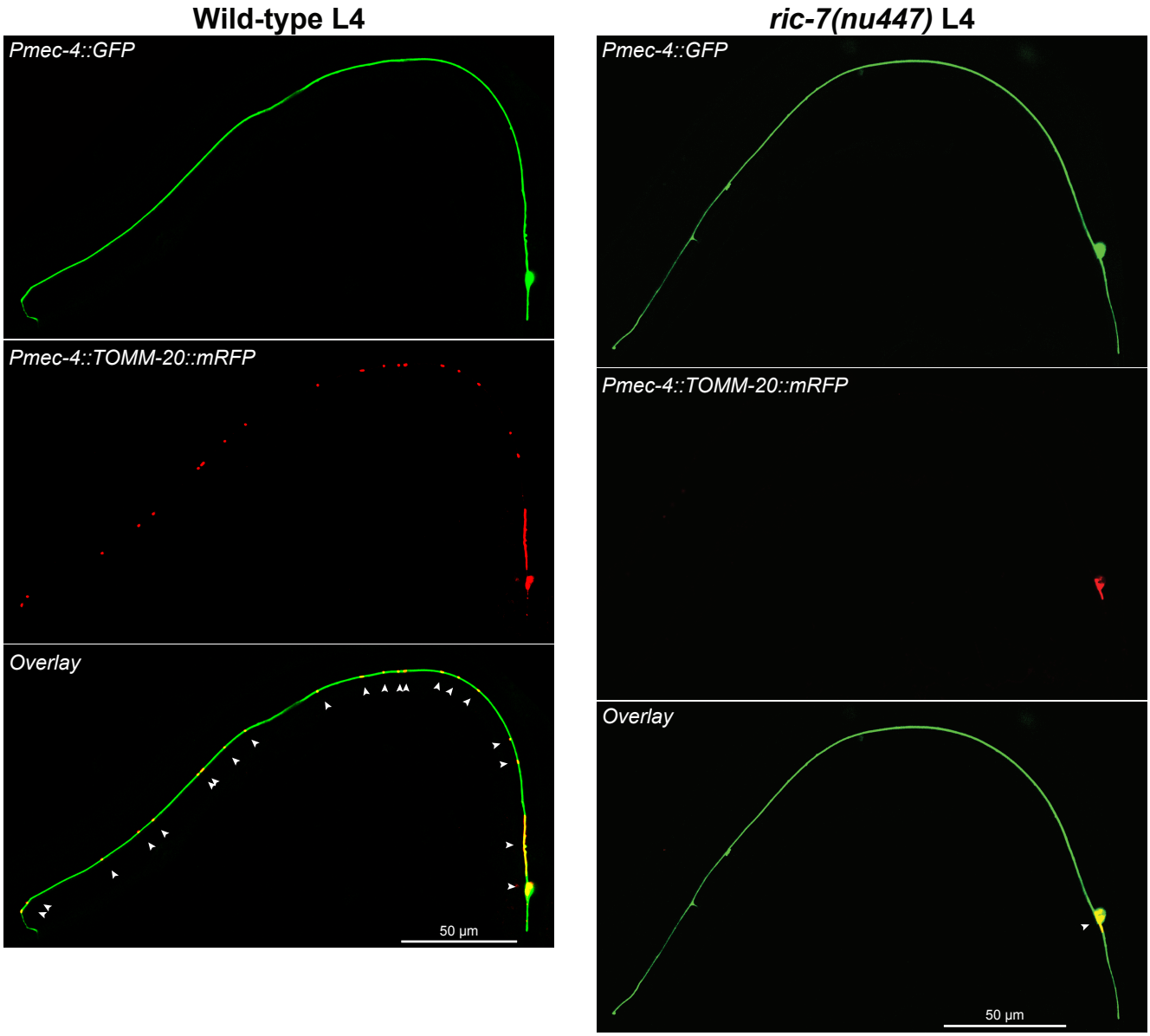

Wild-type L4
ric-7(nu447) L4
Pmec-4::GFP
Pmec-4::TOMM-20::mRFP
Overlay
Pmec-4::GFP
Pmec-4::TOMM-20::mRFP
Overlay
50 µm
50 µm

Figure S5. Mitochondria are largely absent from the PLM axon in *ric-7* mutant animals, related to Figure 5. TOMM-20::mRFP was used to fluorescently label mitochondria in wild-type (left panels) and *ric-7(nu447)* (right panels) animals at the L4-stage. Note the lack of mitochondria within the PLM axon of *ric-7* mutants. Arrowheads in the overlay images point to each mitochondrion.