Figures and figure supplements

The *Caenorhabditis elegans* microtubule minus-end binding homolog PTRN-1 stabilizes synapses and neurites

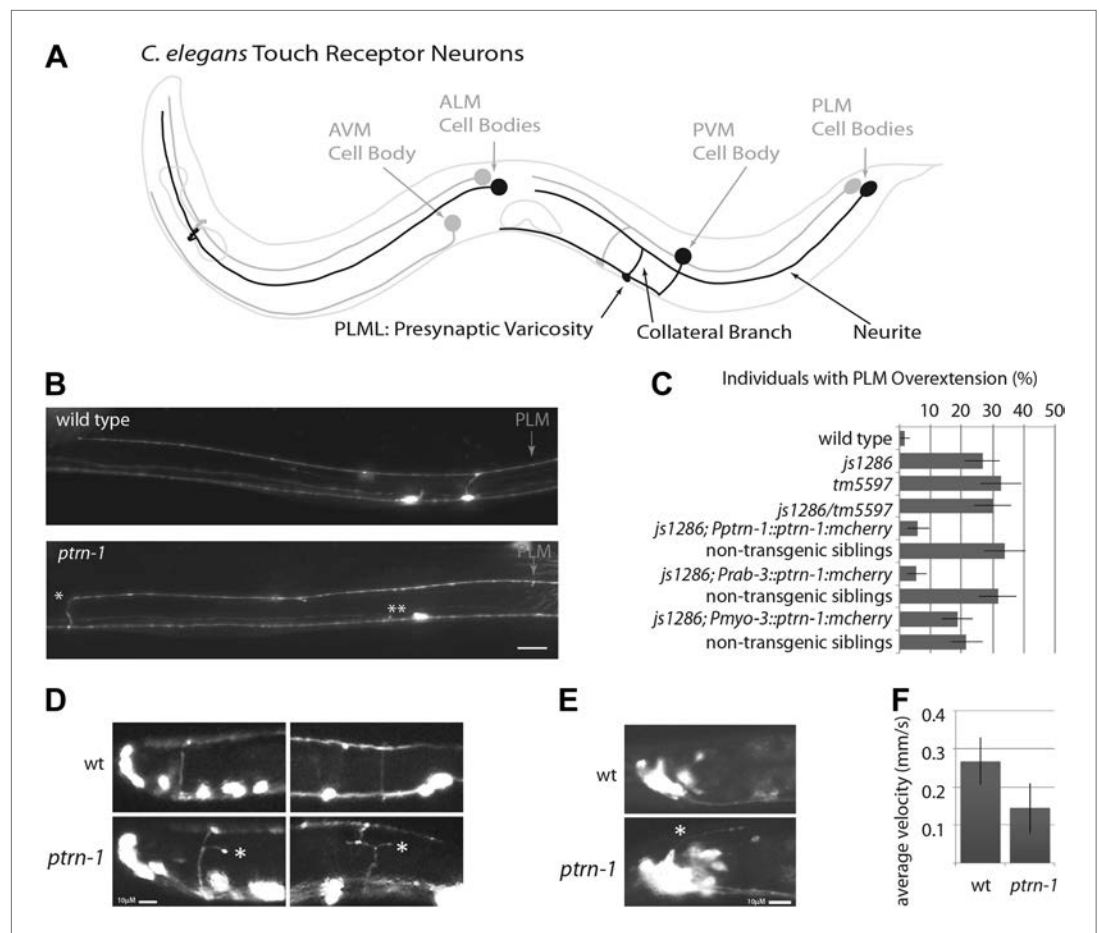**Jana Dorfman Marcette, et al.**

Figure 1. *ptrn-1* mutant neurite overextension and collateral branch retraction phenotypes. (**A**) Schematic diagram of an L4 *C. elegans* hermaphrodite showing the morphology of the touch receptor neurons. Neurons on the left side are in black, and those on the right side are in gray. (**B**) Morphology of mechanosensory neurons in wild-type and *ptrn-1* mutants, as seen by expression of mRFP under the control of the *mec-7* promoter. Defects in *ptrn-1* animals include PLM neurite overextension and ectopic formation of presynaptic varicosities (*), retraction of PLM collateral presynaptic varicosities and branches (**). Scale bar = 10 μm. Additional examples and variance in Ptrn-1 phenotypes are presented in *Figure 1—figure supplement 1*. (**C**) Quantification of the PLM neurite overextension phenotype at 22°C. Both *ptrn-1* alleles (*js1286* and *tm5597*) show similar defects, as do trans-heterozygotes. Also shown is the rescue of the PLM neurite overextension phenotype by expression of PTRN-1a-mCherry under a native promoter and in neurons under a *rab-3* promoter, but not under a *myo-3* promoter. Non-transgenic siblings were identified as individuals from mothers segregating the transgene, but that lacked detectable mCherry fluorescence. n = 50–60; error bars = SEP. (**D**) Aberrant formation and extension of neurites (*) was found in D-type motor neurons. Quantification is in *Figure 1—figure supplement 2*. (**E**) Aberrant formation and extension of neurites (*) was found in command interneurons. Quantification is in *Figure 1—figure supplement 2*. (**F**) Quantification of locomotory defects using L4 animals. Error bars represent standard deviation. N = 6.
DOI: 10.7554/eLife.01637.003
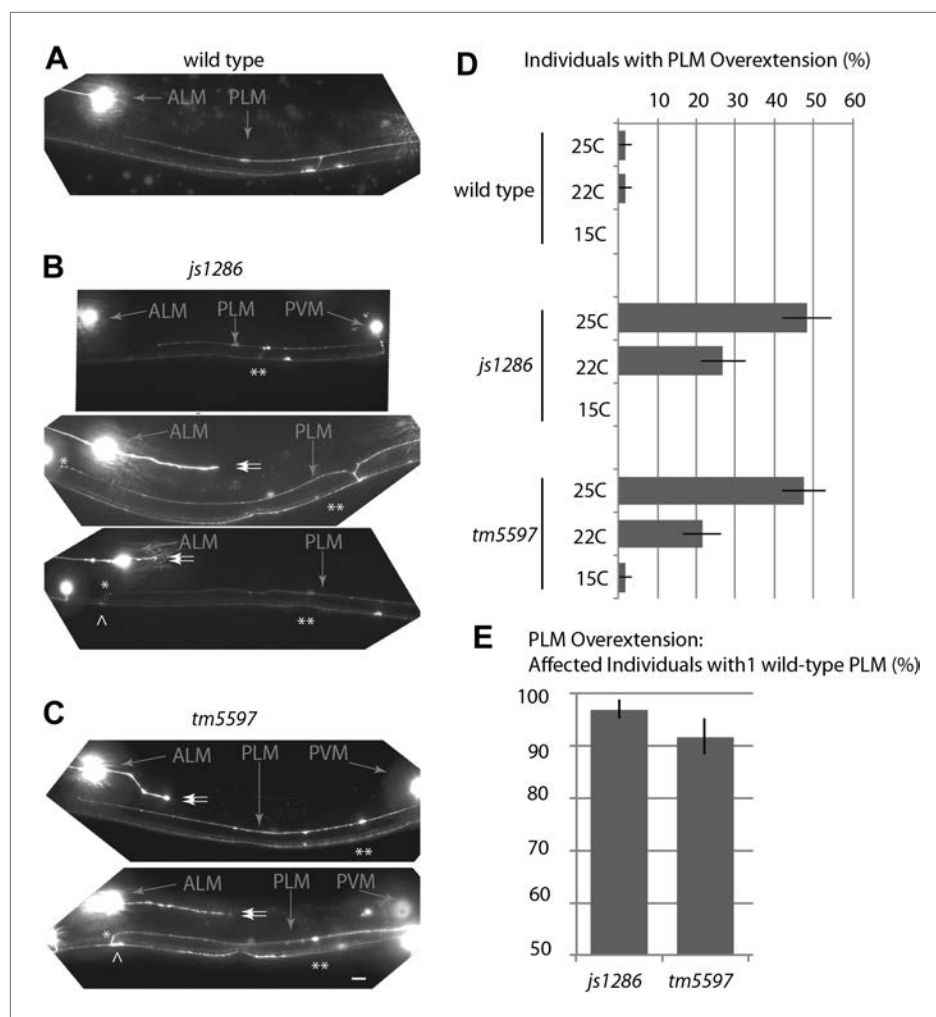
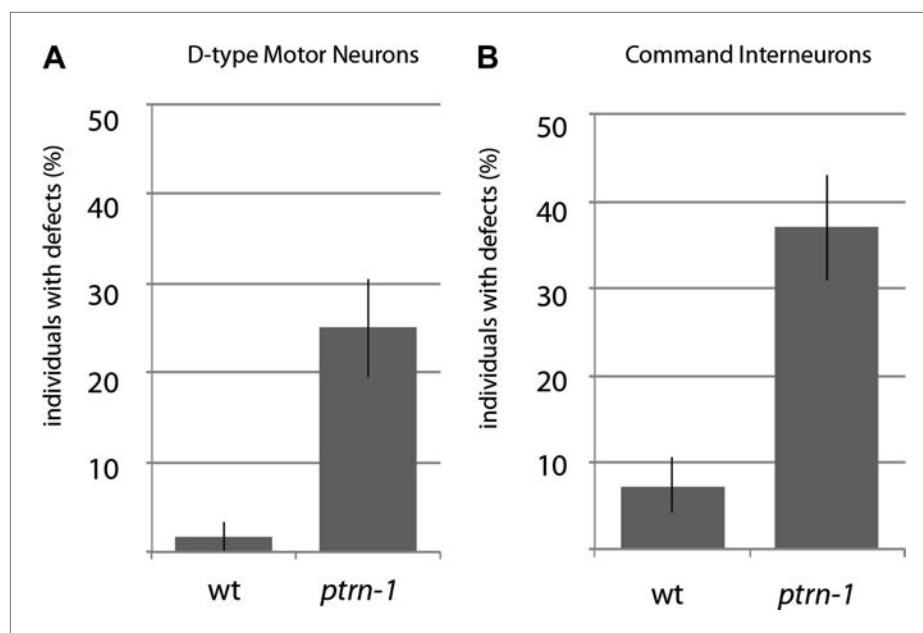**Figure 1—figure supplement 1**. Variance in the *ptrn-1* mutant TRN phenotypes.

Figure 1—figure supplement 2. Quantification of other morphology phenotypes in *ptrn-1* mutants.
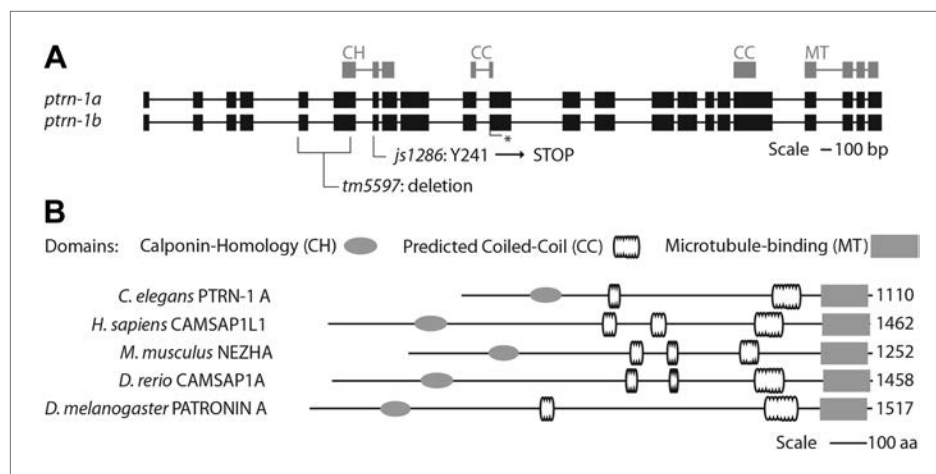DOI: 10.7554/eLife.01637.005

Figure 2. Gene and protein structure of *C. elegans ptrn-1*. (**A**) Exon (thick regions) and intron (thin regions) organization of the *ptrn-1* locus. The position of structural domains is demarcated above and the position of the genetic lesion for each of the *ptrn-1* alleles is shown below. *ptrn-1b* differs from *ptrn-1a* by the inclusion of 6 bp (2 amino acids) at the exon 10–11 boundary (*). Nucleotide sequences for *ptrn-1a* and *ptrn-1b* are listed in *Figure 2—figure supplements 1 and 2*, respectively. Omitted from the figure is a distinct *ptrn-1* isoform lacking the C-terminal half of the protein, which is not represented in RNA-seq data for which the sole support is a single compound EST yk1268c03. (**B**) The CAMSAP protein family consists of a calponin-homology domain followed by one or two 30–40 amino acid coiled-coil regions, a 70–85 amino acid coiled-coil region and a CKK microtubule-binding domain. CAMSAP sequences from *C. elegans* (this paper), *D. melanogaster* (AAO41362.1), *D. rerio* (NP_001159727.1), and *H. sapiens* (AAI25231.1) were used for homology analysis. Domains were annotated by running sequences through the NCBI Conserved Domain Database. Coiled-coil regions were defined as those predicted by both the Paircoil2 and COILS programs.
DOI: 10.7554/eLife.01637.006

ptrn-1a
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Figure 2—figure supplement 1. Nucleotide sequence of *ptrn-1a*.

ptrn-1b

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Figure 2—figure supplement 2. Nucleotide sequence of *ptrn-1b*.
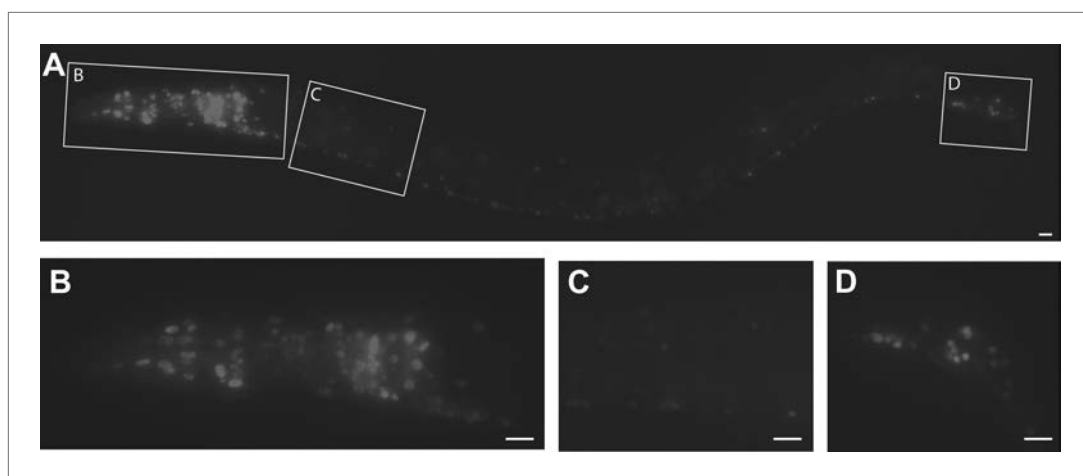DOI: 10.7554/eLife.01637.008

Figure 3. *C .elegans ptrn-1* is broadly expressed in neuronal tissue. (**A**) *ptrn-1* expression pattern from a transcriptional fusion between mCherry and a *ptrn-1* promoter segment sufficient to rescue Ptrn-1 TRN cellular phenotypes (see *Figure 1C* for rescue). 0.8 kb was chosen as the promoter fragment because of the presence of a 1.2 kb Mariner transposable element at this position 5' of the *ptrn-1* ATG. Scale bars = 10 µm. (**B–D**) Views at higher magnification of the head neuronal ganglion (**B**), ventral nerve cord (**C**), and pre-anal and lumbar neuronal ganglia (**D**). mCherry is primarily nuclear localized.

DOI: 10.7554/eLife.01637.009
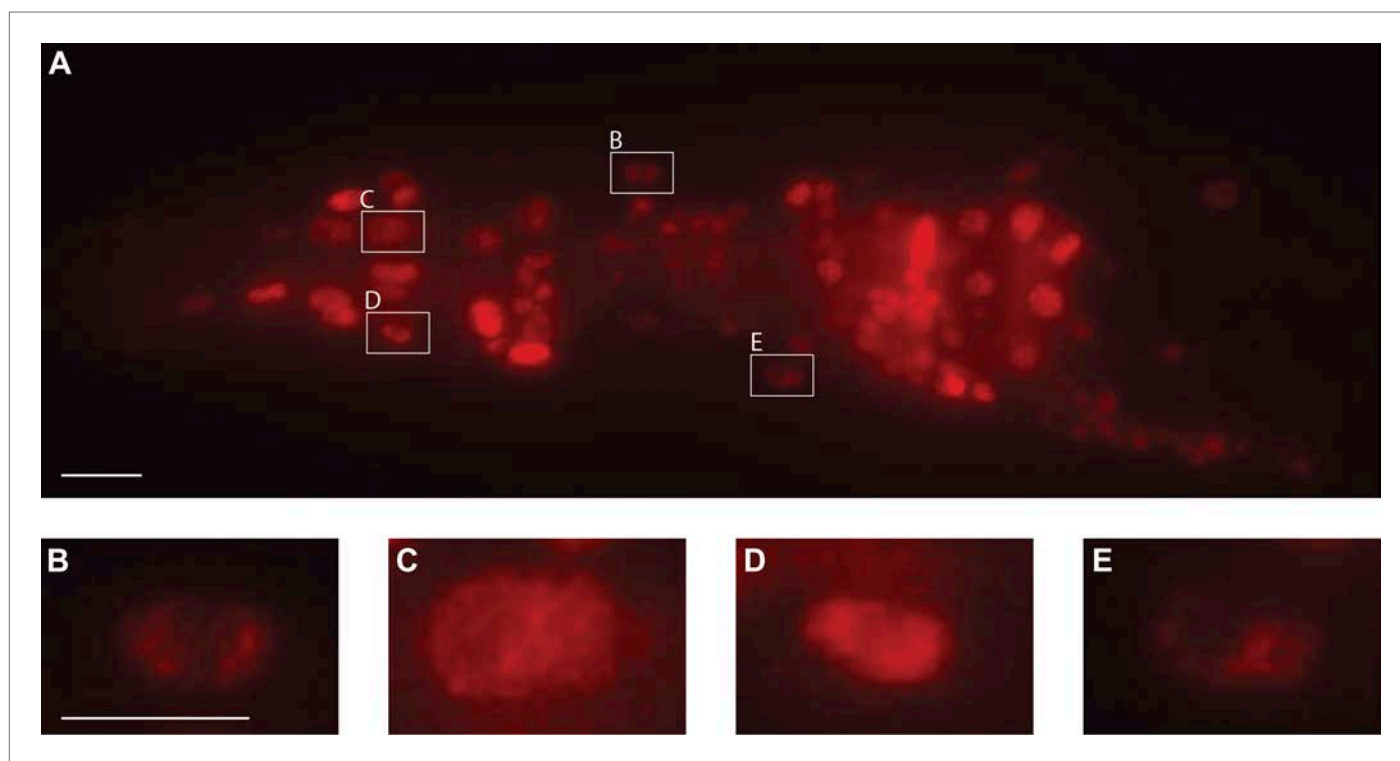
Figure 3—figure supplement 1. Close-up view of the *ptrn-1* expression pattern.
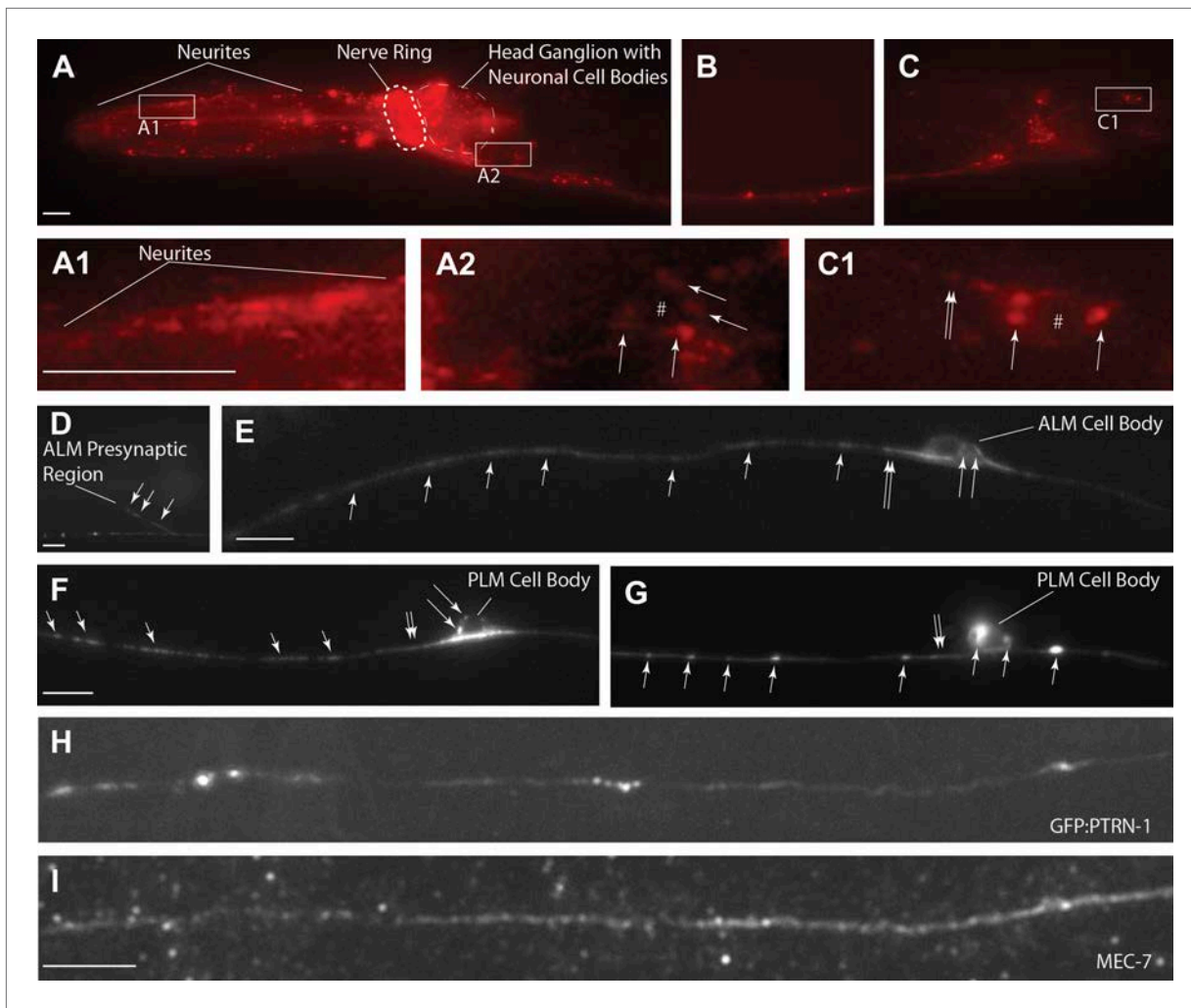DOI: 10.7554/eLife.01637.010

Figure 4. PTRN-1-localization pattern. Expression of PTRN-1-mcherry under a *ptrn-1* 0.8 kb promoter segment sufficient to rescue Ptrn-1 TRN cellular phenotypes (**A–C**) (see *Figure 1C* for rescue data). Enlarged views of neurites (**A1**), a head ganglion cell body (**A2**), and a tail neuron with isolated morphology (**C1**) are shown. Puncta (arrows), nuclear exclusion (*), and neurite (double arrow) are noted. Single-cell resolution showing a N-terminal GFP-PTRN-1 fusion protein under the control of the TRN *mec-7* promoter in ALM and PLM neurons (**D–G**). Puncta (arrows) and neurites (double arrows) are noted. Scale bars = 10 μm. Whole-mount immunofluorescent staining with anti-GFP (**H**) and anti-MEC-7 (**I**) antibodies. Co-localization of GFP-PTRN-1 with MEC-7 in mechanosensory neurons is shown.
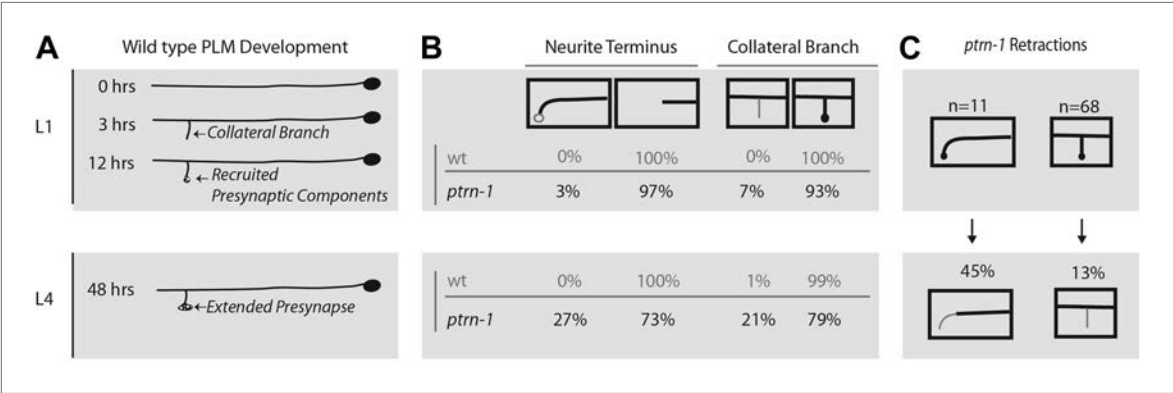DOI: 10.7554/eLife.01637.011

Figure 5. *ptrn-1* mutants fail to stabilize mature PLM morphology and synaptic contacts. (**A**) Schematic diagram illustrating wild-type PLM neuron developmental changes during the L1 larva from 0 to 12 hr after hatching, as well as the structure of PLM neurons in L4 larva—note that the figure is not drawn to scale. PLM neurons maintain their relative position within the animal from L1 to L4, but L4 are approximately four times longer than L1 animals. (**B**) Changes observed in PLM neuronal morphology in wild-type and *ptrn-1* (*js1286*) mutants from a synchronized population of animals analyzed at L1 (10 hr) and L4 (36 hr) stages. Animals were grown at 22°C. PLM neurons show both overextension and ventral targeting of the anterior neurite in *ptrn-1* mutants, but rarely in wild type. The ventral targeting posterior neurites display variable sized varicosities that are labeled with presynaptic components. *ptrn-1* animals also often exhibit loss of collateral branch and associated presynaptic structures. N = 90–98 neurites. (**C**) Retraction of collateral branch synaptic varicosities and overextended ectopic ventral neurite targeted varicosities in animals imaged as both L1 and L4 larva. N = 11 and 68 neurites. (**B** and **C**) Black lines and circles represent neurites and presynaptic varicosities respectively. Gray lines and open circles represent variability in *ptrn-1* animals.
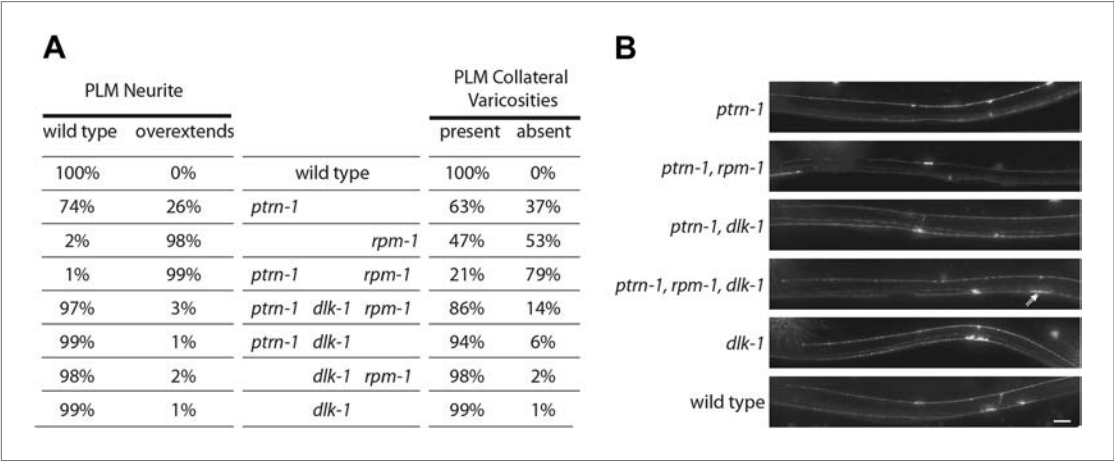DOI: 10.7554/eLife.01637.012

Figure 6. *dlk-1* MAP kinase mutants suppress Ptrn-1 overextension phenotypes. (**A**) Single, double, and triple mutant combinations of *ptrn-1*(*tm5597*), *dlk-1*(*km12*) and *rpm-1*(*ok364*) were grown at 22°C, scored as L4 larvae for PLM neurite morphology defects using *jsIs973*(P*mec-7*:mRFP), and for the presence of collateral branch presynaptic varicosities using *jsIs821*(P*mec-7*::GFP-RAB-3). Since PVM forms small GFP puncta in the ventral nerve cord (see *Figure 1A,B*), PLM collateral varicosities which retained a small GFP-RAB-3 puncta in the ventral nerve cord cannot be unambiguously distinguished from PLM collaterals completely lacking presynaptic components. n = 200 PLM cells from 100 animals. (**B**) Midbody of L4 animals expressing mRFP in TRNs under P*mec-7* (*jsIs973*) showing the wild-type and mutant PLM morphology associated with *ptrn-1* and *dlk-1*. Elongated varicosity in *ptrn-1*, *rpm-1*, *dlk-1* triple mutant identified with double arrows. Scale bar = 10 µm.
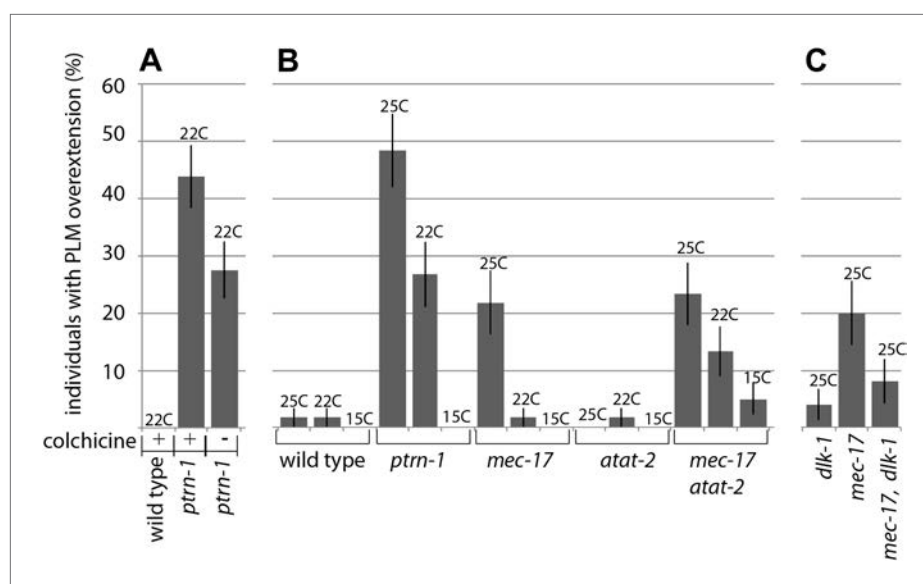DOI: 10.7554/eLife.01637.013

Figure 7. Manipulating the microtubule cytoskeleton leads to terminal neurite extension and ventral nerve cord targeting. (**A**) Wild-type and *ptrn-1*(*js1286*) mutants were grown for two generations on plates containing 1 mM colchicine and scored for PLM neurite overextension (n = 80). Error bars represent SEP. (**B**) Wild-type, *ptrn-1* mutants and mutants with lesions in microtubule acetylases (paralogs *mec-17* and *atat-2*) were scored for PLM neurite overgrowth defects at various temperatures (n = 55-60). Error bars represent SEP. (**C**) *dlk-1* suppresses the PLM neurite overextension phenotypes of *mec-17* (n = 50). Error bars represent SEP.
DOI: 10.7554/eLife.01637.014