

Supplemental Figure 1. PLM axon termination does not require electric synapse formation with BDU. (A) Schematic showing ALM, PLM and BDU neurons. Box highlights region visualized by epifluorescent microscopy. Bottom left image (BDU present) shows BDU axon terminating (pink arrow) and PLM axon terminating (white arrow) where electrical synapses form (Zhang et al., 2013). Bottom right image shows normal PLM axon termination when BDU neuron is ablated by expression of CED-3. (B) Quantitation of PLM axon termination when BDU is present, or genetically ablated by transgenic CED-3. Shown are averages from three counts (minimum 10 worms/count) for animals with and without BDU. Error bars represent standard error of mean. Significance assessed using Student's *t*-test with Bonferonni correction. ns = not significant. Scale bar 20µm.

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### **Supplemental Figure 2**

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**Supplemental Figure 2. PLM axonal growth cones are dynamic or static.** Time lapse imaging was used to assess if PLM growth cones are dynamic or static. Immediately after hatching, 40% of growth cones are dynamic (black) and 60% of growth cones are static (grey) in wildtype. The frequency of dynamic and static growth cones is not significantly different in *rpm-1* mutants. n = 30 growth cones for wt, and n = 21 growth cones for *rpm-1*.

# **Supplemental Figure 3**

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Supplemental Figure 3. Transgenic RPM-1::GFP rescues axon termination defects in rpm-1 mutants. To study RPM-1 localization (Figure 5), we generated rpm-1 mutants that carried transgenic extrachromosomal arrays coexpressing RPM-1::GFP and tdTOMATO. These arrays expressing transgenic RPM-1::GFP rescued termination defects rpm-1 mutants axon in compared to extrachromosomal arrays expressing only tdTOMATO. Shown are averages from minimum of 4 counts (at least 20 worms/count) from two transgenic lines for each genotype. Significance assessed using Student's *t*-test with Bonferonni correction. \*\*\* p < 0.001.



**Supplemental Movie 1. Dynamic growth cone in wild-type animal, 0-1.5 hrs PH.** Time-lapse imaging shows a dynamic PLM growth cone with active extension and retraction of membrane protrusions (0 to 102 minutes PH). Still frames for this movie are shown in Figure 4A.



**Supplemental Movie 2.** Static growth cone in wild-type animal, 0-1.5 hrs PH. Time lapse-imaging shows a static PLM growth cone (0 to 97.5 minutes PH). Note the static growth cone does not have changes in membrane protrusions. Still frames for this movie are shown in Figure 4B.



Supplemental Movie 3. Dynamic growth cone transitions to static in wildtype animal, 0-6 hrs PH. Time lapse-imaging shows a dynamic to static growth cone transition in a wild-type animal (0 to 369 minutes PH). Once the growth cone becomes static it does not become dynamic again during the imaging session.



**Supplemental Movie 4. Dynamic growth cone in** *rpm-1* **mutant, 0-1.5 hrs PH.** Time-lapse imaging shows dynamic PLM growth cone in an *rpm-1* mutant (0 to 104 minutes PH). Still frames for this movie are shown in Figure 5A.



**Supplemental Movie 5. Static growth cone in** *rpm-1* **mutant, 0-2 hrs PH.** Timelapse imaging shows static PLM growth cone in an *rpm-1* mutant (0 to 122 minutes PH). Still frames for this movie are shown in Figure 5B.



Supplemental Movie 6. Static growth cone in *rpm-1* mutant decreases in size but fails to fully collapse, 4-6 hrs PH. Time lapse-imaging shows a static growth in an *rpm-1* mutant that decreases in size but fails to fully collapse (240-378 minutes PH). Still frames for this movie are shown in Figure 5C.

## Table S1. Transgenic extrachromosomal arrays and injection conditions

Reference	Transgene	Figure	Injection Conditions	Genotype
Strain				
XMN828	bggEx130	1B	- P <sub>ttx-3</sub> ::RFP (pBG-41), 50ng/µl	muls32
			- P <sub>mec-7</sub> ::mRFP-UtrCH (pBG-GY755, partial utrophin cDNA), 20ng/µl	
			- pBluescript (pBG-49), 30ng/µl	
	2 lines	2C, 3C	- P <sub>rpm-1</sub> RPM-1 (pCZ160, genomic clone), 25ng/µl	rpm-1; muls32
			- P <sub>myo-3</sub> RFP (pBG-24), 2ng/µl	
			- pBluescript (pBG-49), 75ng/µl	
XMN768	bggEx127	6, S3	- P <sub>mec-3</sub> RPM-1::GFP (pBG-46, genomic clone), 20ng/µl	rpm-1
			- P <sub>mec-7</sub> tdTOMATO (pBG-GY575), 15ng/µl	
			- P <sub>ttx-3</sub> GFP (pBG-40), 50ng/µl	
			- pBluescript (pBG-49), 15ng/µl	
	4 lines	7D	- P <sub>rgef-1</sub> RHO-1 DN (pBG-GY673, <i>rho-1</i> T91N cDNA),10ng/µl	zdls5
			- P <sub>ttx-3</sub> RFP (pBG-41), 50ng/µl	
			- pBluescript (pBG-49), 40ng/µl	
	4 lines	7D	- P <sub>rgef-1</sub> RHO-1 DN (pBG-GY673, <i>rho-1</i> T91N cDNA), 10ng/μl	rpm-1; zdls5
			- P <sub>ttx-3</sub> RFP (pBG-41), 50ng/µl)	
			- pBluescript (pBG-49), 40ng/µl)	
	4 lines	8B	- Pmec-3MIG-2 (pBG-GY693, mig-2 cDNA), 5ng/µl	rpm-1; mig-2; zdIs5
			- P <sub>ttx-3</sub> RFP (pBG-41), 50ng/µl	
			- pBluescript (pBG-49), 45ng/µl	
	7 lines	9B	- Pmec-17MEC-17 PCR product (native promoter, open reading frame	mec-17; zdls5
			and 3' UTR), 0.02ng/µl	
			- P <sub>ttx-3</sub> RFP (pBG-41), 50ng/µl	
	6 lines	9B	- P <sub>ptL1</sub> PTL-1 PCR product (native promoter, open reading frame and	ptl-1: zdls5
			3' UTR). 5ng/ul	
			- P <sub>ttx-3</sub> RFP (pBG-41), 50ng/µl	
	4 lines	9C	- Pmec-7tdTOMATO (pBG-GY575), 15ng/ul	N2
			- P <sub>ttx-3</sub> GFP (pBG-40), 50ng/µl	
	4 lines	9C	- Pmec-7tdTOMATO (pBG-GY575), 15ng/µl	N2
			- Pmec-3RPM-1::GFP (pBG-46), 50ng/ul	
			- P <sub>ttx-3</sub> GFP (pBG-40), 50ng/µl	
	4 lines	S3	- Pmec-7tdTOMATO (pBG-GY575), 15ng/µl	rpm-1
		-	- P <sub>ttx-3</sub> GFP (pBG-40), 50ng/µl	'
			- pBluescript (pBG-49), 35ng/ul	
			r	