Supplemental Figure Legends

Supplemental Figure 1. G-CaMP sensors detect axotomy-induced calcium transients in PLM. (A) Comparison of fluorescence intensity change ($\Delta F/F_0$) in PLM after axotomy using GFP label (green trace, *zdIs5*), and in P*mec-4*-G-CaMP 2.0 (*juEx2116*) with (red) or without axotomy (blue). Traces are representative; fluorescence was measured in a 12 μm long ROI as in Figure 1. (B) Dot plot of maximal $\Delta F/F_0$ from the groups in (A). (C) PLM regrowth is not significantly different between P*mec-7*-GFP(*muIs32*), P*mec-4*-GFP(*zdIs5*), or P*mec-4*-G-CaMP 2.0 (*juEx2166*) transgenic backgrounds. (D) Incubation in liquid increases total regrowth. P*mec-4*-GFP(*zdIs5*) worms were grown on NGM agar plates, in M9 buffer, or in M9 buffer with DMSO. (E) Overnight incubation in 1 mM BAPTA-AM prior to axotomy did not affect $\Delta F/F_0$. (F) BAPTA-AM treatment reduces regrowth at 10 h. n (in bars) = total number of axons, at least two independent experimental groups. Statistics: ANOVA (panel D) or Student t test, *, $P < 0.05$; ***, $P < 0.001$.

Supplemental Figure 2 PLM morphology in mutants with elevated Ca^{2+} or cAMP. (A) The PLM branch is normally located close to the anterior end of the PLM process (~78% of axon length) in *muIs32* transgenic animals. In approximately 50% of *zdIs5* animals the branch forms more posteriorly (top panel), 50-60 μm from the cell body. This 'posterior branching' is not observed in *pde-4* or *egl-19(gf)* mutants in the *zdIs5* background (quantitation in c). Scale, 10 μm. (B) PLM process length measured in mid-L4 stage is slightly increased in *egl-19(gf)* but not in *pde-4(lf)* or *egl-19 pde-4* double mutants. Mean

± SD, $P < 0.01$ by ANOVA. (C) *zdIs5* transgenic animals display a posterior synaptic branch phenotype that is suppressed in *pde-4* and *egl-19(gf)* mutants.

Supplemental Figure 3 Contributions of calcium sources to regenerative growth (A) The partial loss of function mutation *egl-19(ad1006)* does not affect the amplitude of the G-CaMP transient. (B) PLM regrowth is not significantly affected by null mutations in *unc-2*, *unc-36*, *crt-1,* or *unc-68*. Partial loss of function mutations in *egl-19(ad1006)* or *itr-1(sa73)* do not affect regrowth. (C) Nemadipine-A treatment does not affect baseline G-CaMP intensity. Statistics: t test; ns, not significant.

Supplemental Figure 4 Morphology of PLM axon regrowth in *eff-1* mutants. (A) typical morphology proximal axon in *eff-1(ok1031) muIs32* animals; (B) the regrowing axon is highly branched around the distal fragment and does not reconnect. (C) Total levels of regrowth are reduced in *eff-1* mutants. Statistics, t test; *, $P < 0.05$.

Supplemental Video Legends

All videos, except #2, are spinning disk confocal movies of P*mec-4*-G-CaMP transgenic PLM neurons severed ~50 μm from the cell body, with intensity color coded. See Methods for imaging conditions.

Video 1. Representative P*mec-4*-G-CaMP (*juEx2116*) fluorescence in PLM before and after femtosecond laser axotomy.

Video 2. GFP fluorescence (transgene *zdIs5*) is unaffected by PLM axotomy.

Video 3. Although treatment with BAPTA-AM reduces the baseline fluorescence intensity of G-CaMP in PLM axon, it does not affect the relative increase following axotomy.

Supplemental Table 1. DNA constructs and transgenes

Gene/construct	DNA clone	Transgenic arrays
PDE-4	P*mec-4*-PDE-4 pCZGY562	*juEx1795-1798, 1800*, *2419*
G-CaMP 2.0	P*mec-4*-G-CaMP2.0 pCZGY905	*juEx2115, juEx2116*
ITR-1 Control Sponge	P*mec-4*-control sponge pCZGY911	*juEx2412-2414*
ITR-1 Sponge	P*mec-4*-sponge pCZGY910	*juEx2424, juEx2425*
Super Sponge	P*mec-4*-super sponge pCZGY912	*juEx2410, juEx2411, juEx2418*

Experimental DNAs were injected at 50 ng/μl with the exception of P*mec-4*-G-CaMP 2.0 (100 ng/ μl). All transgenes use P*ttx-3*-RFP or P*ttx-3*-GFP as coinjection marker to total DNA concentration of 100-150 ng/μl.