Supplemental material

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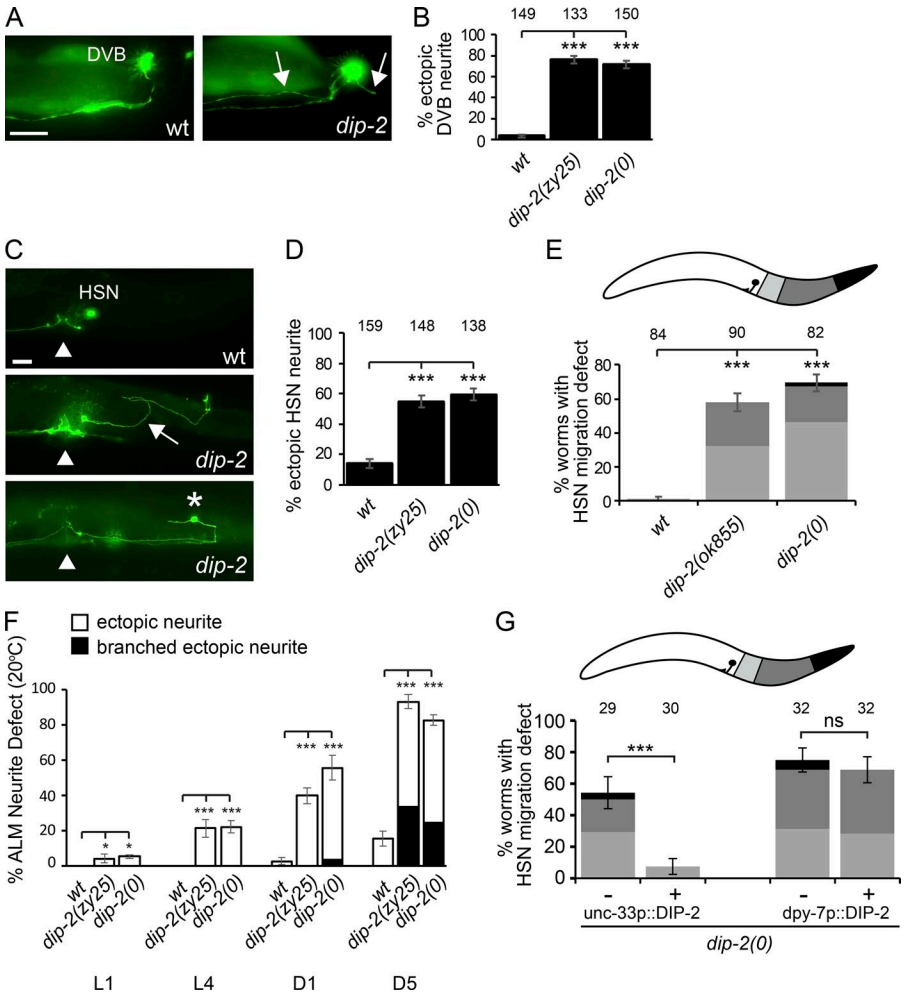

Figure S1. ***dip-2* mutants display neuronal morphology and migration defects. (A–D)** Images and quantification of DVB (A and B) and HSN (C and D) neuronal morphology defects in *dip-2* mutants. *dip-2* mutants display ectopic neurites (arrows) from cell bodies. **(C and E)** Images and quantification of HSN neuronal migration defects in *dip-2* mutants. In C, an asterisk marks a typical HSN neuron in a *dip-2* mutant that fails to migrate to its normal position adjacent to the vulva (arrowhead). **(F)** ALM neurons in *dip-2* mutants cultured at 20°C display a cumulative increase in ectopic neurite formation with increasing age. Neuronal morphology defects in *dip-2* mutants are temperature sensitive (compare with 25°C quantification in Fig. 2 B). **(G)** Neuron-specific expression of DIP-2 rescues neuronal migration defects in *dip-2* mutants. Bars, 20 µm. Error bars in B and D indicate SEM of proportion with number of neurons (n = 133–159) above. Error bars in E and G indicate SEM of proportion for the entire stacked column with the number of animals (n = 29–90) above. Error bars in F indicate SD for data collected from three independent counts of 40–60 neurons. Significance compared with WT using one-way ANOVA with Tukey post hoc test (B, D, E, and F) or two-tailed t test (G). *, P < 0.05; ***, P < 0.001.

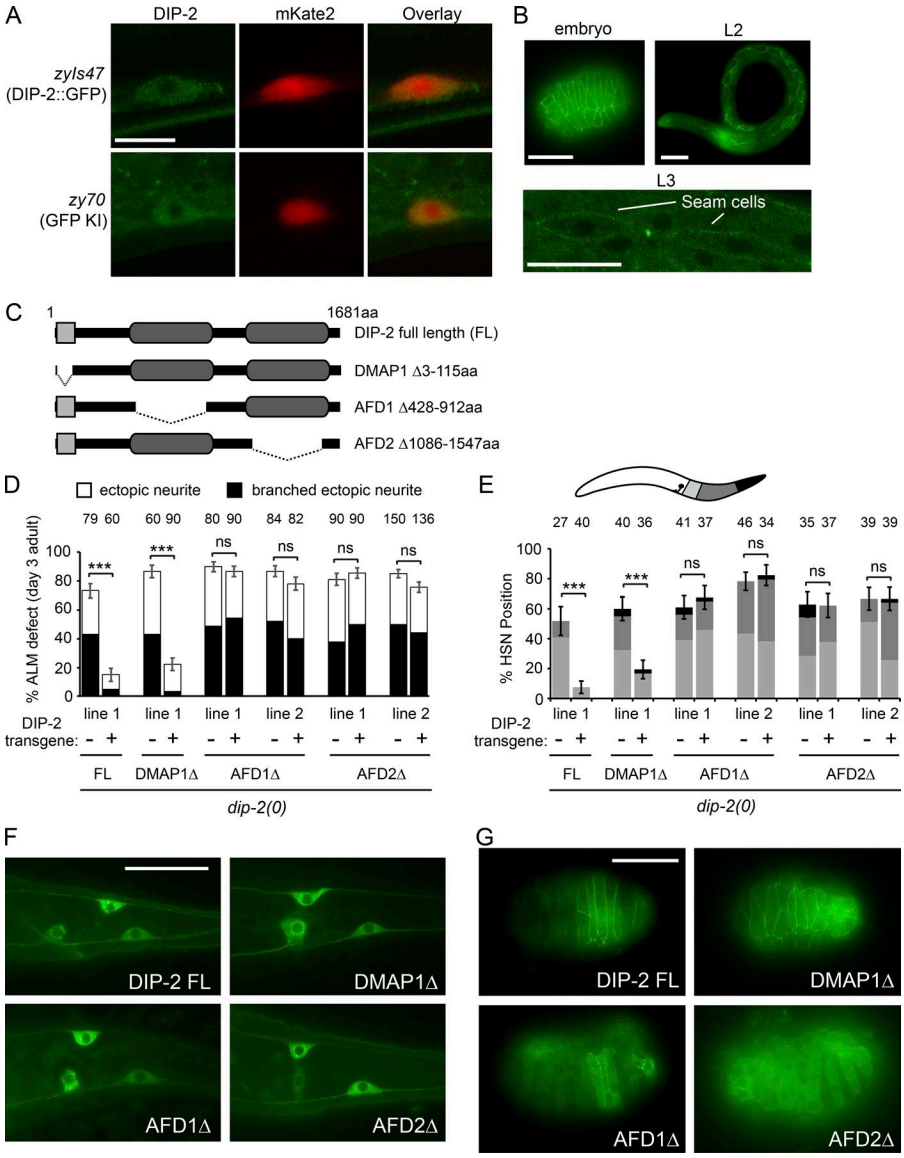

Figure S2. **Requirements for the DMAP1 binding and AFD domains for DIP-2 neuronal development and protein localization. (A and B)** Endogenous GFP::DIP-2 (*zy70*) shows cytoplasmic localization in mechanosensory neurons (A) and membrane localization in epidermal cells (B) indistinguishable from those expressed in transgenic (*zyEx78* and *zyIs47*) animals. Shown are confocal images of PLM neurons marked by Pmec-4–mKate2 (*juSi329*) in young adults (A) and L3 stage epidermal seam cells (B, bottom) and wide-field images of embryonic epidermal cells (B, top left) and L2 stage seam cells (B, top right). **(C)** Schematics of FL, DMAP1-binding, and AFD domain-deleted DIP-2 proteins. **(D and E)** ALM neuronal morphology and HSN migration defects in *dip-2* mutants were rescued with transgenes expressing FL and DMAP1-binding domain-deleted but not AFD-deleted DIP-2 proteins. Error bars indicate SEM of proportion with number of neurons (n = 60–150; D) or animals (n = 27–46; E) indicated above. Significance compared between transgene-negative and transgene-positive siblings using two-tailed t tests. ***, $P < 0.001$. **(F and G)** Representative images of *dip-2* promoter–driven FL DIP-2::GFP and domain-deleted DIP-2::GFP proteins expressed in L4-stage tail neurons and embryonic epidermal cells. FL and DMAP1 and AFD domain–deleted DIP-2::GFP proteins show similar localization patterns in late-stage neuronal cells. However, in contrast with FL and DMAP1-deleted DIP-2::GFP, AFD1- and AFD2-deleted DIP-2::GFP proteins displayed a partial or complete loss, respectively, of membrane localization in epidermal cells. Bars: 10 µm (A); 20 µm (B, F, and G).

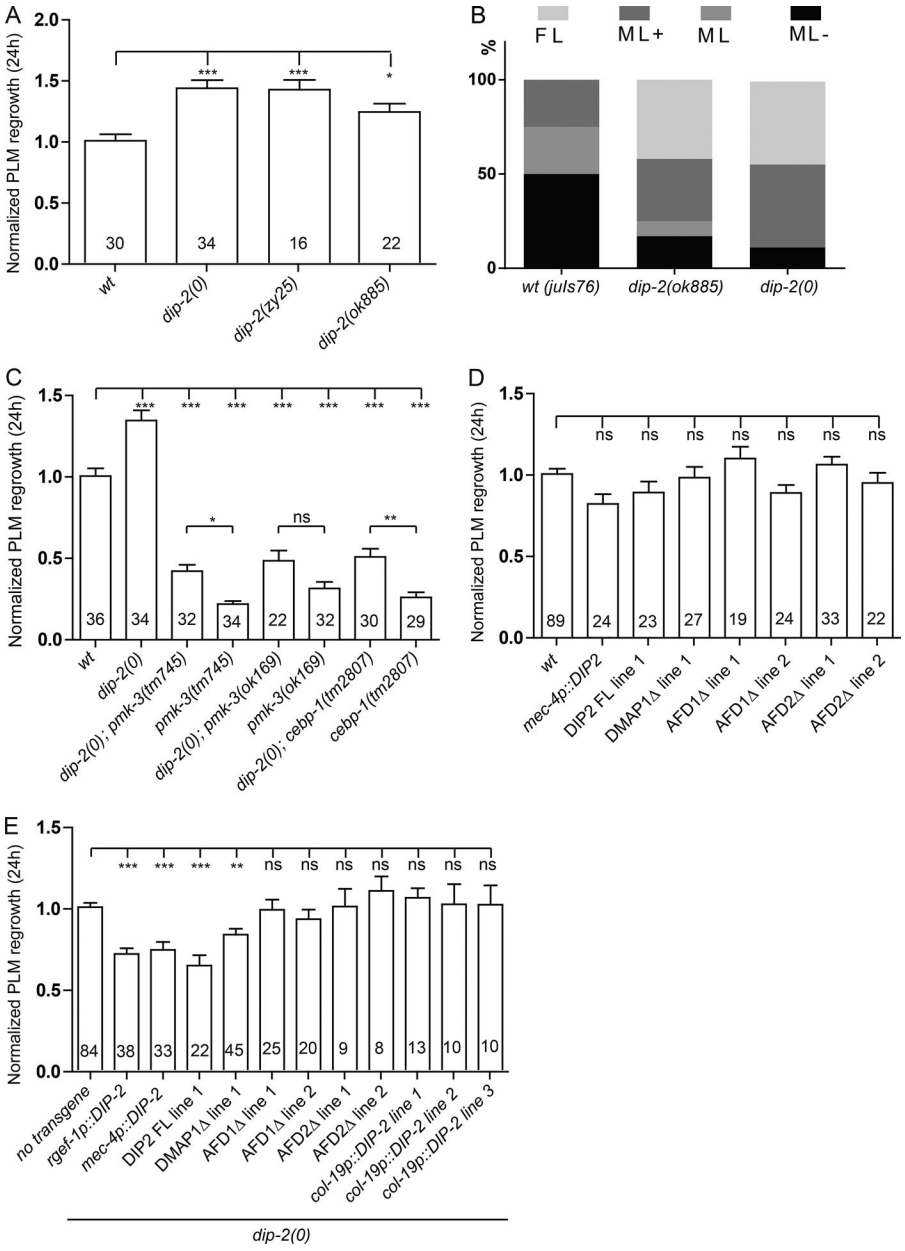

Figure S3. **Supporting evidence for *dip-2* in PLM axon regeneration. (A)** Multiple alleles of *dip-2* result in enhanced PLM regrowth; shown are normalized regrowth data >24 h after axotomy. **(B)** Loss of *dip-2* results in enhanced regrowth of D-type motor neurons. D-type neuron commissures were axotomized at mid-lateral (ML) position along dorsal–ventral axis, and regrowth was scored by the final position of regrowing tip of severed axon. FL, full regrowth to dorsal nerve cord; ML+, partial regrowth to above midlateral position along dorsal–ventral axis; ML, no regrowth; ML–, below midpoint, no regrowth. *n* = 8 for WT, 12 for *dip-2(ok885)*, and 9 for *dip-2(gk913988)*. **(C)** PLM regrowth analysis in double mutants of *dip-2* with *pmk-3*, *cebp-1*, consistent with *dip-2* acting in parallel to the DLK-1–PMK-3–CEBP-1 pathway. **(D)** DIP-2 transgenes do not affect PLM regrowth in a WT background. The graph shows normalized PLM regrowth 24 h after axotomy. *dip-2* transgenic lines are FL, DMAP1-binding, and AFD domain-deleted DIP-2 proteins (as indicated in Fig. S2). **(E)** PLM regrowth depends on AFD not DMAP1 binding domains. Same transgenic lines as in D in *dip-2(0)* background. Statistics for A and C–E, one-way ANOVA. *, P < 0.05; **, P < 0.01; ***, P < 0.001. Error bars are SEM.