Supporting Information

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SI Methods

Yeast Two-Hybrid Screen. The yeast transformation procedure was conducted using standard techniques. The intracellular domain (438 aa) coding region of CAM-1b was fused with pBTM116 (LexA DNA binding domain, Leu selection) as the bait to screen a *Caenorhabditis elegans* mixed-stage cDNA library. The screen was conducted as previously described (17).

Conditional Knockout of *Sax-3* in RME Cells. The RME-specific conditional knockout allele of *sax-3* was created by following the protocol reported previously (26). Two targeting sgRNA were inserted into the pDD162-P*eft-3*:Cas9+P*U6*::Empty sgRNA (#47549; Addgene) and the P*eft-3* promoter was replaced by the P*unc-25* promoter. The Cas-9 plasmids (40 ng/μL) were coinjected with P*unc-122*-RFP (coinjection marker, 50 ng/μL) into *wt*. After obtaining the transgenic lines, we checked the presence of the *sax-3* mutation by PCR using primers flanking the two targeting sites. A relatively short extension time (20 s) was used in the PCR-amplification protocol to detect the corresponding deletions.

Quantitative RT-PCR Analysis. The total mRNA was extracted using TRIzol method from mixture stage of worms. The quantitative PCR analysis was conducted by following the manufacturer's instructions (SYBR, Transgene). Primers designed for various regions of *cam-1* or *sax-3* gene were used. *act-1* was used as the internal control. All experimental samples were analyzed in triplicates. The $2^{-\triangle\triangle Ct}$ method was used to calculate the fold-changes for various mRNA samples.

Characterization of CAN Neuron Migration and Withered Tail. The *hpIs162* (*Pceh-10::wCherry; rol-6*) (a gift from Mei Zhen's laboratory, Lunenfeld-Tanenbaum Research Institute, Toronto, Canada) strain could label both CAN and RID neurons. The cell body of RID neuron is situated in the dorsal ganglion near the nerve region and remains unchanged in *sax-3* or *cam-1* mutant animals. The CAN migration ratio was calculated by the distance between CAN cell boy and RID cell body over the distance between RID cell body and anus. The CAN migration ratio in wild-type was normalized to 100% in wild-type. The girth of the region posterior to the vulva is reduced compared with that anterior to vulva is defined as withered tail phenotype.

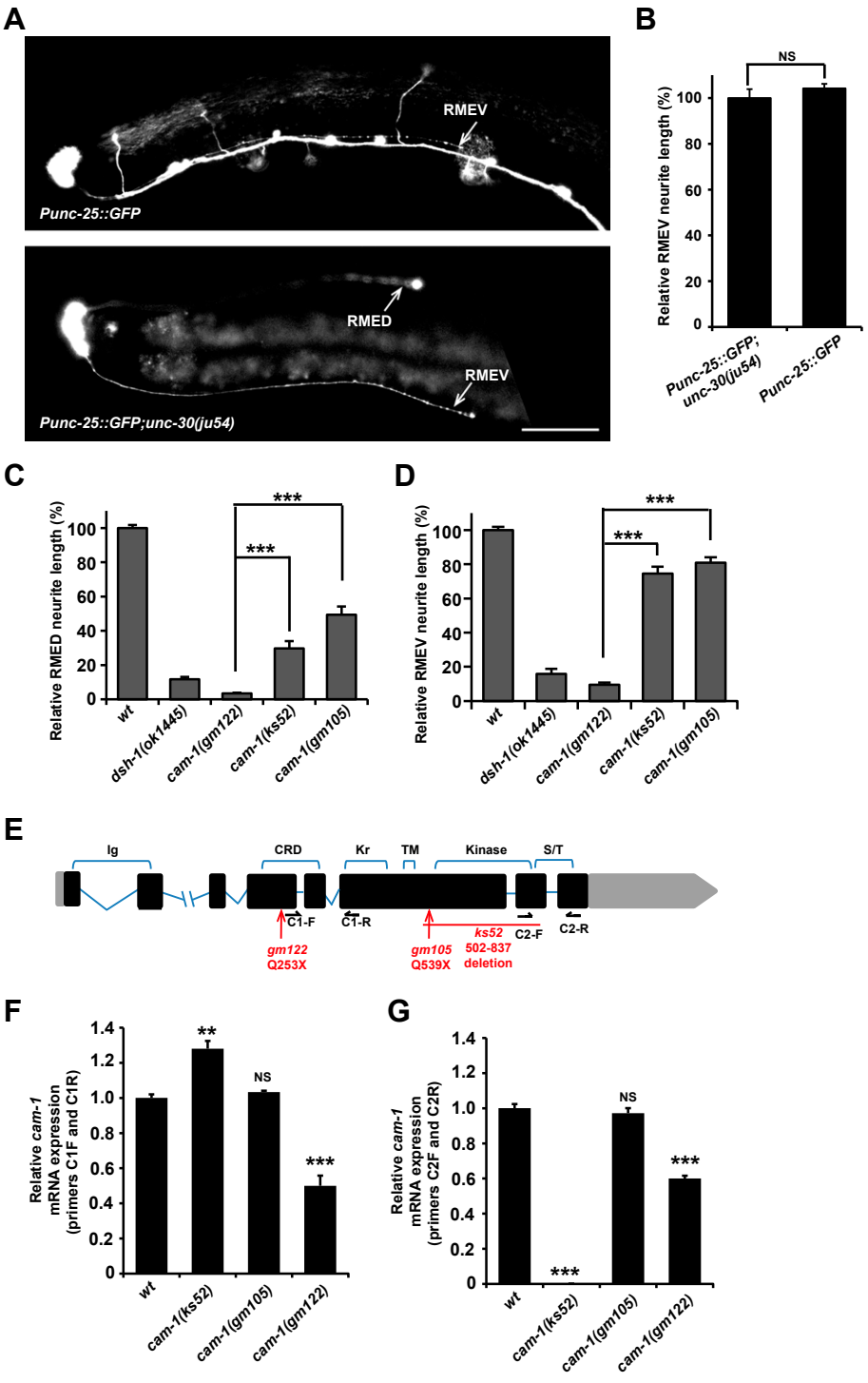

Fig. S1. The intracellular domain is important for RMED/V neurite outgrowth. (*A*) The RMEV (labeled with *Punc-25::GFP*) in wild-type or *unc-30(ju54)* animals. The posterior neurites of RMED or RMEV are indicated by white arrows. (Scale bar, 10 μm.) (*B*) Quantification of the neurite length of RMEV neurons. All data are expressed as mean ± SEM; $n \geq 20$ for each genotype. NS, not significant; one-way ANOVA with Bonferroni's test. (*C* and *D*) Quantification of the neurite length of RMED (*C*) and RMEV (*D*) neurons. All data are expressed as mean ± SEM; $n \geq 50$ for each genotype. ***$P < 0.001$; one-way ANOVA with Bonferroni's test. (*E*) The molecular lesions of *cam-1(gm122)*, *cam-1(gm105)* and *cam-1(ks52)* are labeled in red. The various protein domains are labeled (CRD, cysteine rich; kinase, kinase domain; Kr, Kringle region; S/T, Ser/Thr rich region; TM, transmembrane). C1-F, C1-R, C2-F, and C2-R indicate the primers for Quantitative RT-PCR. (*F* and *G*) The mRNA expression level of the *cam-1* gene in wild-type and different *cam-1* mutant animals. Data are expressed as mean ± SEM; ***$P < 0.001$; **$P = 0.007$; NS, not significant. One-way ANOVA with Dunnett's test.

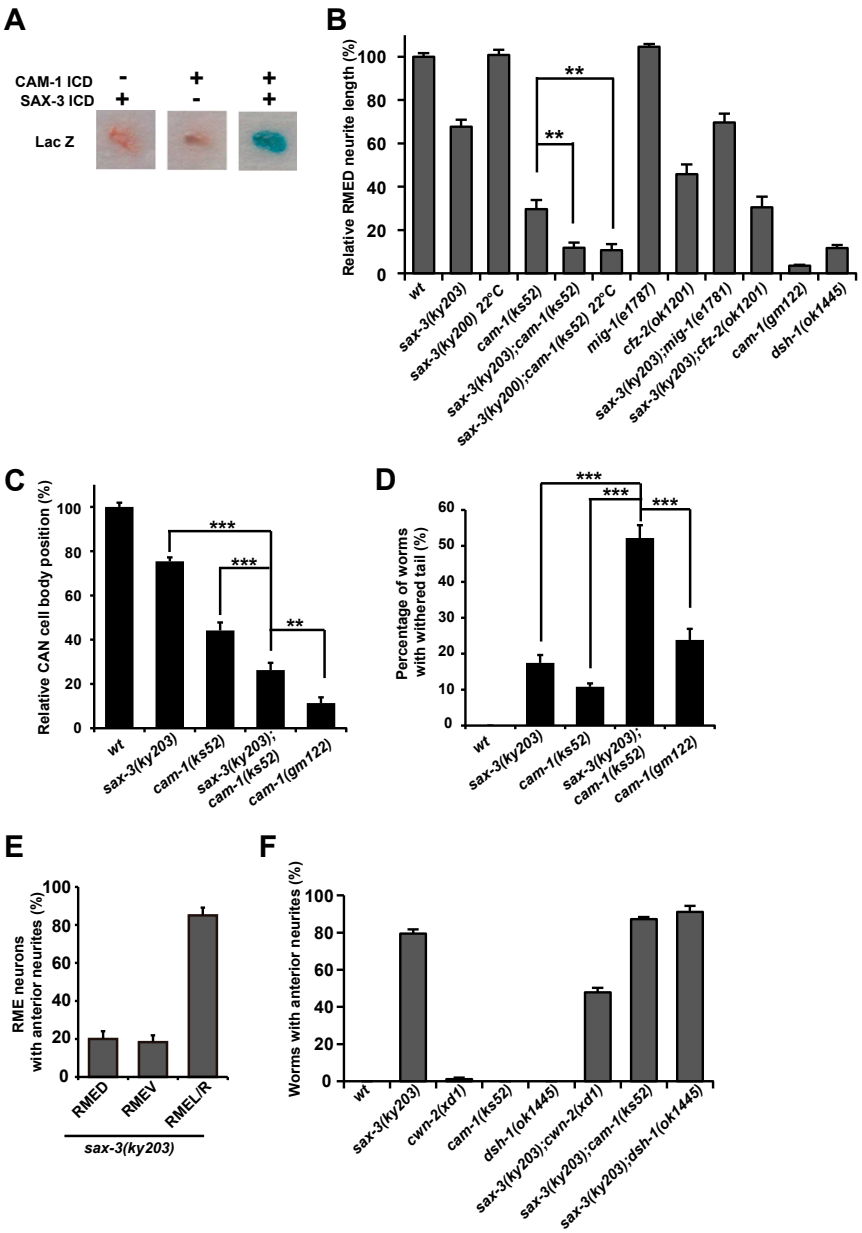

Fig. S2. SAX-3 functions with CAM-1 to regulate RMED/V neurite outgrowth and other developmental processes. (*A*) SAX-3 ICD binds to CAM-1 ICD in a yeast two-hybrid assay. (*B*) Quantification of the RMED neurite length in various genotypes. All data are expressed as mean ± SEM $n \geq 50$ for each genotype; $^{**}P < 0.01$; one-way ANOVA with Bonferroni's test. (*C*) Quantification of the CAN neuron migration in various genotypes. Data are expressed as mean ± SEM; $n \geq 30$ for each genotype; $^{***}P < 0.001$, $^{**}P = 0.006$; one-way ANOVA with Bonferroni's test. (*D*) Quantification of the withered tail phenotype in various genotypes. Data are expressed as mean ± SEM; $n \geq 60$ for each genotype; $^{***}P < 0.001$; one-way ANOVA with Bonferroni's test. (*E*) Quantification of the anterior neurite outgrowth in RMED, RMEV and RMEL/R neurons. $n \geq 100$ for each genotype). (*F*) Quantification of the anterior neurite outgrowth in various genotypes. $n \geq 100$ for each genotype.

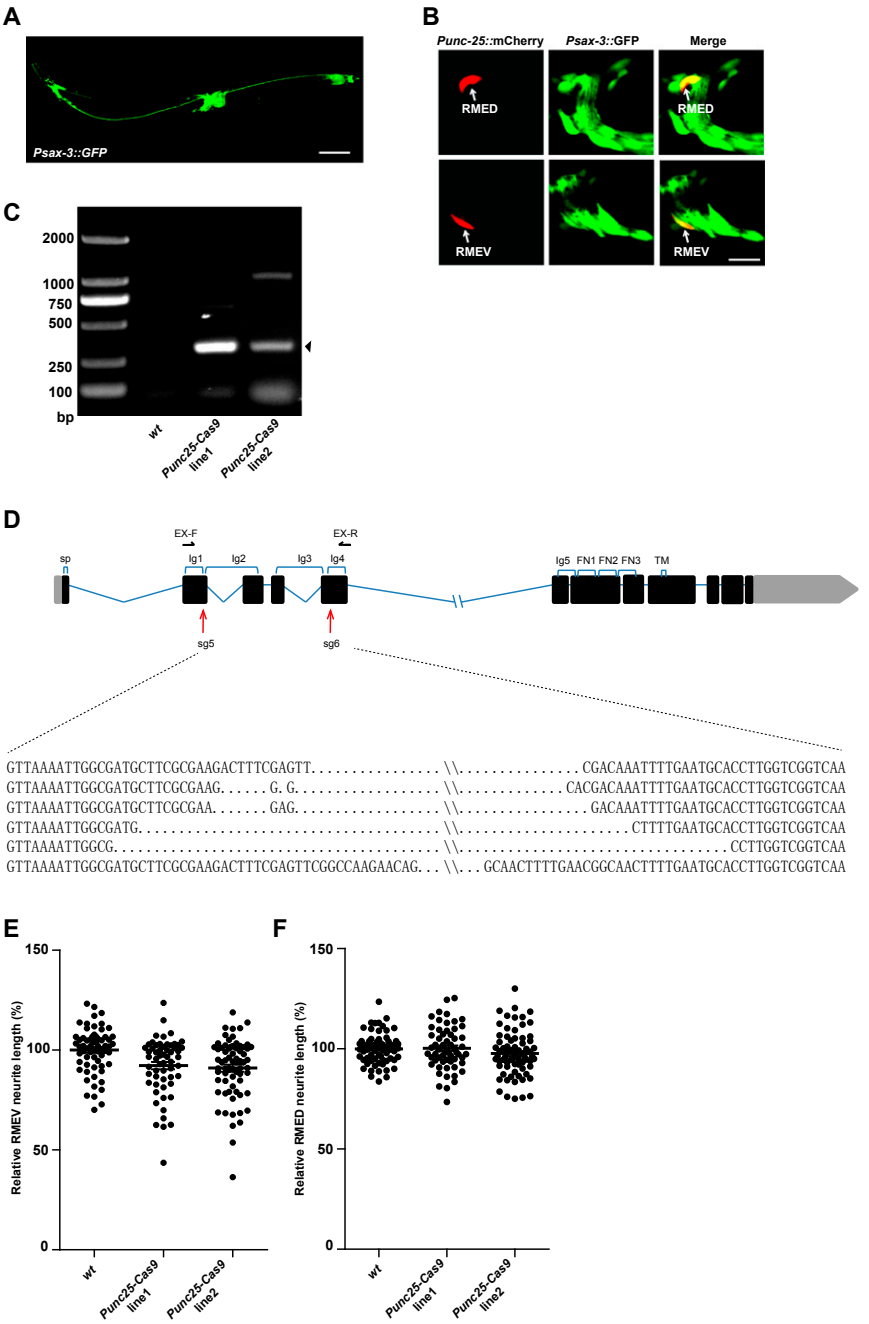

Fig. S3. SAX-3 functions in RME cells. (*A*) The expression of the *sax-3* reporter *Psax3::GFP* is shown (green). (Scale bar, 100 μm.) (*B*) *Psax-3::GFP* (green) colocalizes with P*unc-25::mCherry* (red). White arrows indicate RMED and RMEV neuron cell bodies. (Scale bar, 10 μm.) (*C*) PCR reactions to verify the creation of the RME-specific conditional *sax-3* knockout allele. The arrowhead indicates the PCR product from the *sax-3* deletion animals. (*D*) Sequencing data of the RME-specific *sax-3* knockout animals. EX-F and EX-R are primers used to amplify *sax-3* deletions. sg5 and sg6 indicate the location of sgRNAs. (*E* and *F*) Quantification of the RMEV (*C*) and RMED (*D*) neurite length in the RME-specific *sax-3* knockout lines.

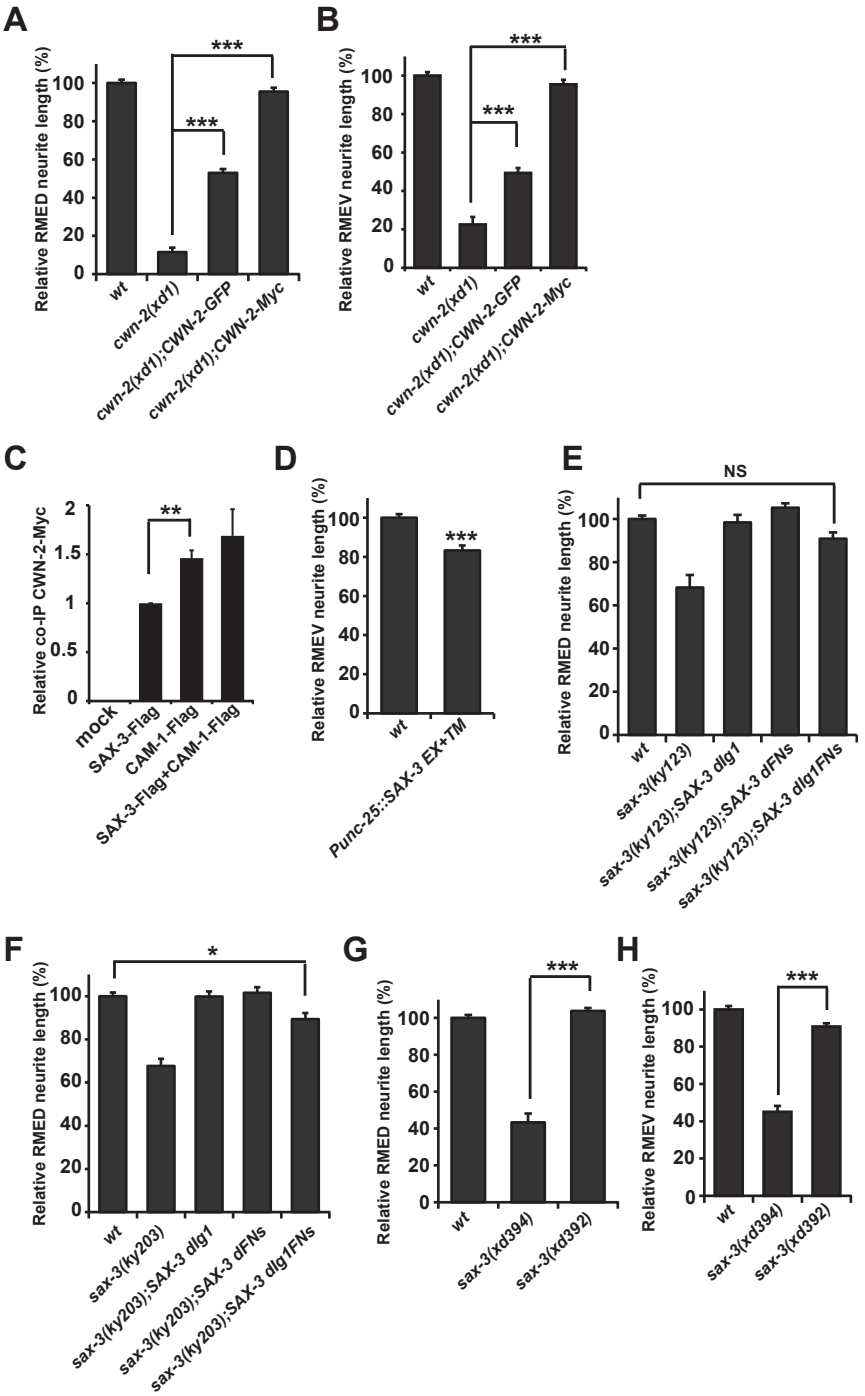

Fig. S4. The extracellular domain of SAX-3 functions in RMED/V neurite outgrowth. (*A* and *B*) CWN-2μLGFP and CWN-2μLMyc rescue the neurite outgrowth phenotype in RMED (*A*) and RMEV (*B*) cells in *cwn-2(xd1)* mutants. All data are expressed as mean ± SEM; $n \geq 50$ for each genotype; ***$P < 0.001$; one-way ANOVA with Bonferroni's test. (*C*) Quantification of CWN-2–Myc precipitated by SAX-3–Flag or CAM-1–Flag or both. Data are expressed as mean ± SEM; $n = 5$; **$P = 0.006$; one-way ANOVA with Fisher's LSD test. (*D*) Overexpression of the extracellular domain of SAX-3 reduces the neurite length of RMEV. Data are expressed as mean ± SEM; $n \geq 50$ for each genotype; ***$P < 0.001$; two-tailed unpaired Student's *t* test. (*E* and *F*) Quantification of the RMED neurite length in various rescuing lines. $n \geq 50$ for each genotype; NS, not significant; *$P = 0.03$; one-way ANOVA with Dunnett's test. dFNs, deletion of the fibronectin domain; dIg1, deletion of Ig1 domain; dIg1FNs, deletion of both the Ig1 domain and fibronectin domain. (*G* and *H*) Quantification of the RMED (*G*) and RMEV (*H*) neurite length. $n \geq 50$ for each genotype; ***$P < 0.001$; One-way ANOVA with Fisher's LSD test.

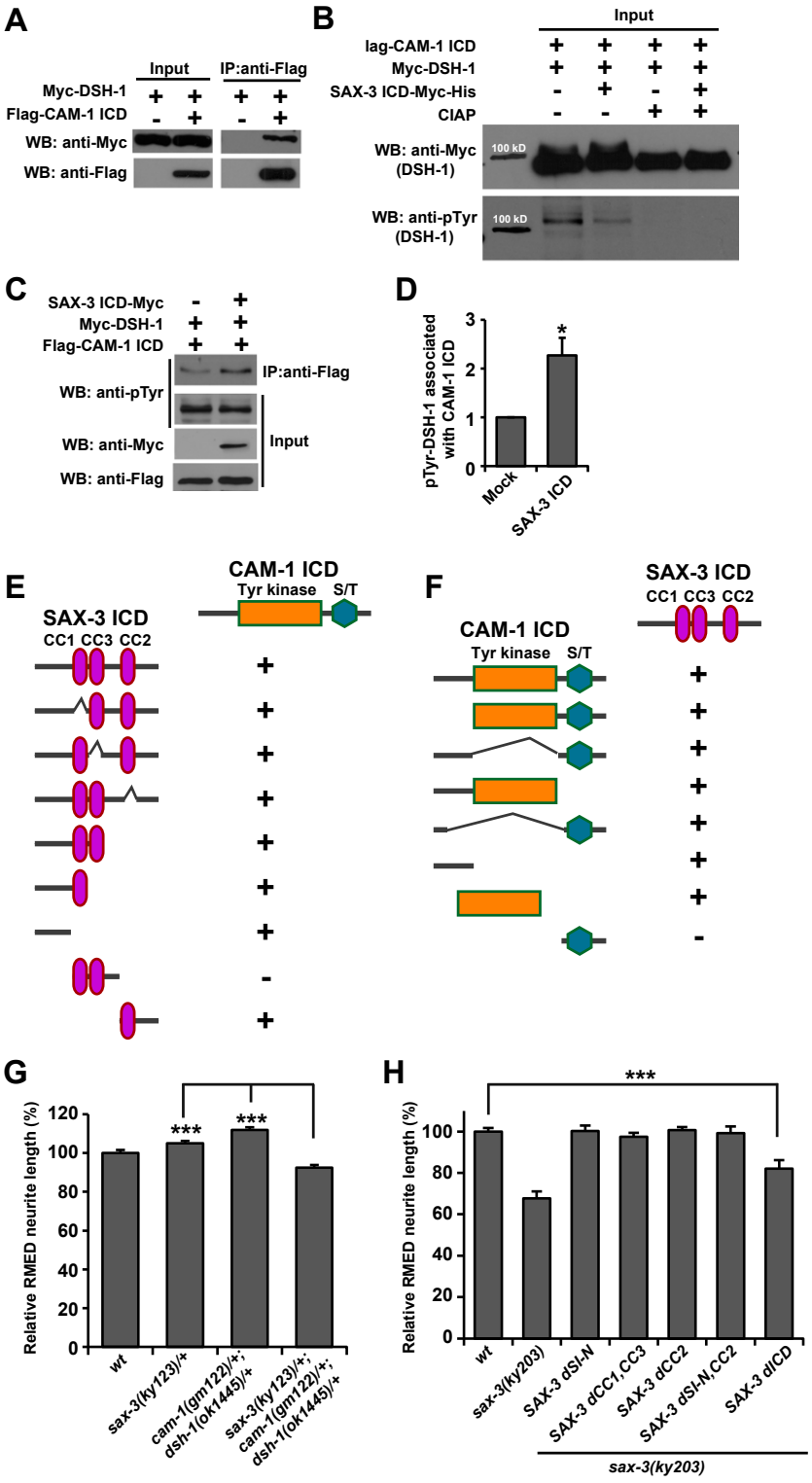

Fig. S5. The association between CAM-1, SAX-3 and DSH-1. (*A*) The association between Flag-tagged CAM-1 intracellular domain (CAM-1 ICD) and Myc–DSH-1. (*B*) An antibody against phosphorylated tyrosine (anti-pTyr) detects the phosphorylated MycμLDSH-1. (*C*) In the presence of Myc-tagged SAX-3 ICD, the amount of phosphorylated DSH-1 protein associated with CAM-1 ICD is increased. (*D*) Quantification of the level of phosphorylated DSH-1 associated with CAM-1 ICD. All data are expressed as mean ± SEM $n = 6$; $^*P < 0.05$ and $P = 0.02$; two-tailed paired Student's t test. (*E*) Protein–protein interactions between CAM-1 ICD and various truncated SAX-3 ICDs. (*F*) Protein–protein interactions between SAX-3 ICD and various truncated CAM-1 ICDs. (*G*) Quantification of the RMED neurite length. $n \geq 50$ for each genotype; $^{***}P < 0.001$; one-way ANOVA with Bonferroni's test. (*H*) Quantification of the RMED neurite length in various rescuing lines. $n \geq 50$ for each genotype; $^{***}P < 0.001$; One-way ANOVA with Dunnett's test. dCC1,CC3, deletion of both CC1 and CC3; dCC2, deletion of CC2; dICD, deletion of the entire intracellular domain; dSI-N, deletion of the membrane proximity region; dSI-N,CC2, deletion of both the membrane proximity region and CC2 region.

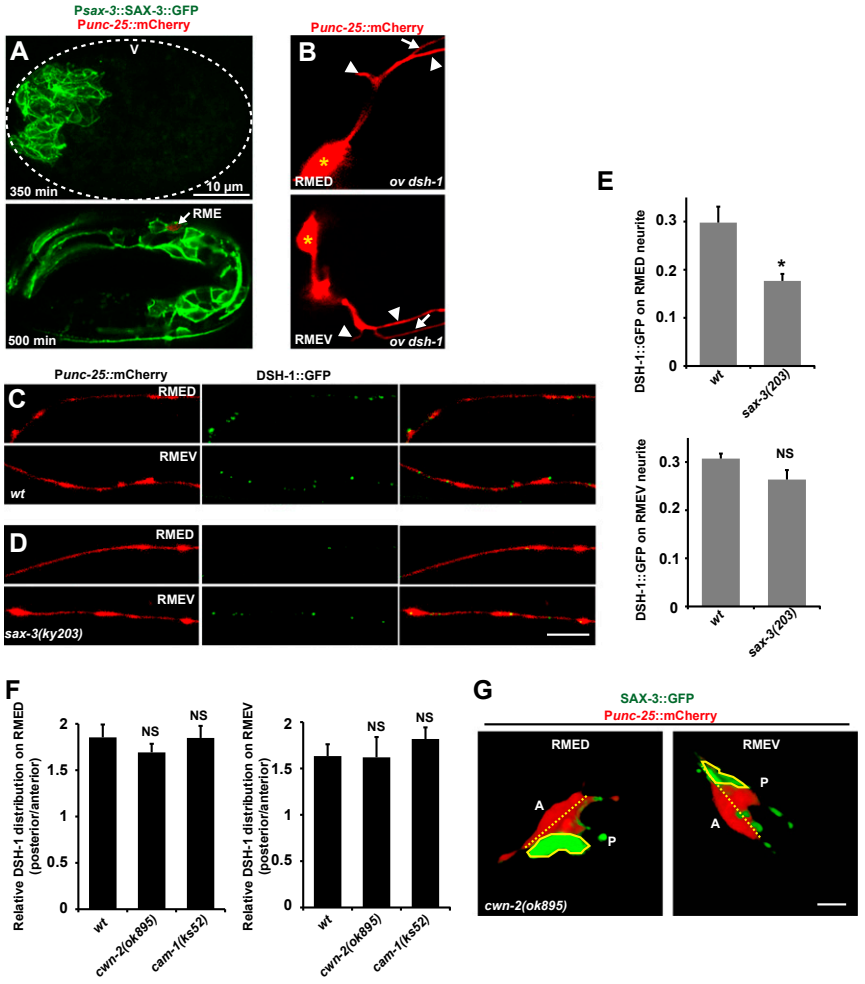

Fig. S6. *cwn-2* and *cam-1* are not required for the asymmetric distribution of DSH-1. (*A*) P*sax-3*::SAX-3::GFP (green) distribution in embryos at two different time-points. V, ventral. (Scale bar, 10 μm.) (*B*) Overexpression of *dsh-1* induces ectopic neurites (indicated by white arrowheads). White arrows indicate the posterior neurites of RMED/V. Yellow asterisks indicate the RMED/V cell bodies. (Scale bar, 10 μm.) (*C* and *D*) The DSH-1::GFP signal on RMED/V neurites in wild-type (*wt*) and *sax-3(ky203)* animals. (Scale bar, 10 μm.) (*E* and *F*) Quantification of DSH-1::GFP on RMED/V neurites. All data are expressed as mean ± SEM $n = 6$; $^*P < 0.05$ and NS, not significant; two-tailed paired Student's *t* test. (*F*) Quantification of the enrichment of DSH-1::GFP in wild-type, *cwn-2(ok895)*, and *cam-1 (ks52)* animals. Data are expressed as mean ± SEM. For RMED, *wt* $n = 13$, *cwn-2(ok895)* $n = 12$, and *cam-1(ks52)* $n = 5$; for RMEV, *wt* $n = 7$, *cwn-2(ok895)* $n = 8$, and *cam-1(ks52)* $n = 5$. NS, not significant; One-way ANOVA with Dunnett's test. (*G*) Three-dimensioinal reconstructions of SAX-3::GFP (green) distribution on RMED and RMEV neuron cell bodies (red) in *cwn-2(ok895)* mutants. The dashed yellow line divides the cell body into anterior and posterior domains. The yellow lines indicate the nerve ring region. (Scale bar, 2 μm.)