Fig. S1



D PVD sax-7(nj48);mnr-1(wy758) A/PLM::SAX-7; A/PLM::MNR-1 D::SAX-7; D::MNR-1























Fig. S3















Fig. S5



■ diffused SAX-7 ■ partial SAX-7 stripes ■ completed SAX-7 stripes





# Fig. S1, related to Fig. 1. Gain-of-function SAX-7 manipulations generate predictable patterns of 4° dendrites

A-C) Confocal images of *sax-7(nj48)* mutant expressing PVD::mCherry and A/PLM::SAX-7::GFP. White arrowhead shows one PVD 3° branch, and yellow arrowhead indicates the PLM neurite. Yellow arrow marks the synaptic branch of PLM, and the white arrow shows a PVD branch that follows the synaptic branch of PLM. D-F) Confocal images of *sax-7(nj48)*; *mnr-1(wy758)* double mutant expressing SAX-7 and MNR-1 (SAX-7's co-ligand) together in the D type neurons (DD and VD) and the A/PLM neurons. Yellow arrows indicate the commissures of D type neurons, and the white arrows show the PVD dendrites that follow the D type neurons' commissures. Yellow arrowheads indicate the ALM neurites, and the white arrowheads show the PVD 3° branch. G) Quantification of ratio between 4° and 3° branch of worms that SAX-7 ectopically expressed in A/PLM. H) Quantification of the ratio that 2° branches cross the sub-lateral line when *sax-7* ectopically expressed in D type neuron. Error bars, SEM. \*\*\*p < 0.001 by Student's t test. NS: not significant. n>20 for each genotype. I-K) Confocal images of *dma-1(wy686*) mutant expressing hypodermal SAX-7::YFP and PVD::mCherry. Arrow indicates the 3° branch and arrowhead shows one of the SAX-7 stripes. n=20. Scale bar is 10 µm.

## Fig. S2, related to Fig. 1. *lin-22* worms produce five PVDs and have denser 4° branches but still follow the SAX-7 stripes

A-B) Confocal images of young adult wild type and *lin-22 (wy50001)* worm. The arrows indicate the cell bodies of PVDs. C-F) A *lin-22 (wy50001)* worm expressing both PVD::mCherry and Pdpy-7::SAX-7::YFP. Note that all the PVD 4° branches (red) co-localized with SAX-7 stripes

## Fig. S3, related to Fig. 2. *unc-52(e998)* affects PVD dendrites and SAX-7 pattern, while *unc-52(gk3)* does not affect PVD dendrites development

A-C) Florescent images of young adult wild type, *unc-52(e998)* and *unc-52(gk3)* expressing PVD::GFP. Arrow indicates one 3° branch and arrowhead shows one 4° branch. Scale bar is 10  $\mu$ m. D) Quantification of the percentage of the 2° branches which contain "L" or "T" shaped 4° branches in *unc-52(e998)*, *unc-52(e998)* and the *unc-52* genomic DNA rescued worm, and *unc-52(gk3)* mutant. E) Quantification of percentage of the 2° branches which contain less than three 4° branches in *unc-52(e998)*, *unc-52(e998)* and the *unc-52* genomic DNA rescued worm, and *unc-52(gk3)* mutant. E) Quantification of percentage of the 2° branches which contain less than three 4° branches in *unc-52(e998)*, *unc-52(e998)* and the *unc-52* genomic DNA rescued worm, and *unc-52(gk3)* mutant. Error bars, SEM. \*\*\*p < 0.001 by ANOVA. NS: not significant. n>20 for each genotype. F-H) Confocal images of *unc-52(e998)*. In *unc-52(e998)* mutants the disorganized SAX-7 stripes were always followed by the disorganized PVD 4° branches. Arrows indicate the PVD tertiary branch and the SAX-7 sub-lateral tripe. Arrowheads mark one of the PVD quaternary branch and the co-localized SAX-7 stripe. Scale bar is 10  $\mu$ m.

Fig. S4, related to Fig. 3. UNC-52 antibody staining is affected in *unc-52* mutants but not *sax-7(nj48)* 

A-D) GM1 antibody staining confocal images of young adult animals of wild type, *sax-7(nj48)*, *unc-52(e1421)* and *unc-52(e998)*. Compared with wild type, *sax-7(nj48)* did not change the regular pattern of UNC-52 (illustrated by GM1 staining). In *unc-52(e1421)* and *unc-52(e998)* the staining of UNC-52 was greatly reduced and the fluorescence becomes diffusely localized. n>10 for each genotype. Scale bar is 10  $\mu$ m.

#### Fig. S5, related to Fig. 6. Overexpression MUA-6 blocked PVD 4° branches growth

A-D) Confocal images of overexpressed MUA-6::mCherry and PVD::GFP. Arrow indicates one PVD 4° branch and arrowhead shows one FO stripe. E) Quantification of 4° branches in *mua-6(rh85)* and *mua-6* overexpression worms. Error bars, SEM. \*\*\*p < 0.001 by ANOVA. n>20. *mua-6(rh85)* mutant was very sick and also disrupted 2° and 3° branches, therefore, only identifiable 3° branches were quantified. F) Quantification of the SAX-7 stripes formation time. Diffused SAX-7 means no stripes were observed; partial SAX-7 stripes means SAX-7 stripes formed in some regions; completed SAX-7 stripes means SAX-7 stripes formed in all the regions. n=20 for each stage. Scale bar is 10 µm.

## Table S1, related to experimental procedures "*C. elegans* Strains, Genetics and RNAi" section. Mutant Alleles and Transgenes Used in This Study

### A. Mutant alleles

Allele	Reference
unc-52(e998)	(Gregory P. Mullen et al., 1999)
unc-52(e1421)	(Gregory P. Mullen et al., 1999)
sax-7(nj48)	(Xintong Dong et al.,2013)
lin-22(wy50001)	This study
dma-1(wy686)	(Liu and Shen, 2012)
mua-6(rh85)	(Vera Hapiak et al.,2003)

### **B.** Integrated transgenes

Allele	Chromosome	Constructs	Co-injection marker
wyIs50001	Х	pXD26(15ng/ul),pOL036(5ng/ul)	Podr-1::RFP
wyIs50005	?	pOL036(20ng/ul),POL057(5ng/ul)	Podr-1::RFP

### C. Extrachromosomal arrays

Allele	Constructs	Co-injection marker
wyEx5147	pXD28(15ng/ul),pOL036(15ng/ul)	Podr-1::RFP
wyEx5312	pXD30(15ng/ul),pOL036(15ng/ul)	Podr-1::RFP
wyEx6519	pXD26(15ng/ul),pOL036(5ng/ul)	Podr-1::RFP
wyEx6522	pXD26(0.1ng/ul),pOL036(5ng/ul)	Podr-1::RFP
wyEx50017	unc-52 genomic DNA(25ng/ul)	Pegl-17::myri-mCherry
wyEx50028	pXM41(30ng/ul),pXM3(20ng/ul)	
wyEx50029	pXM41(6ng/ul),pOL057(15ng/ul)	
wyEx50030	pXM41(6ng/ul),pOL020(50ng/ul)	
wyEx50034	pXM41(30ng/ul),pOL057(15ng/ul)	

Table S2, related to experimental procedures "Molecular Biology and Transgenes"section. Plasmid Used in This Study

Plasmid	Genotype
pOL020	ser-2Prom3::myr-GFP
pXD26	Pdpy-7::SAX-7S::YFP
pOL036	ser-2Prom3::myr-Cherry
pOL057	Pdpy-7::SAX-7S::GFP
pXD28	Pdpy-7::SAX-7S Del Ex::YFP
pXD30	Pdpy-7::SAX-7S Del Cyto::YFP
pXM3	ser-2Prom3::GFP
pXM41	Pmua-6::MUA-6::Cherry