Supplemental information

Table S1. Mutant alleles and transgenes used in this study

A. Mutant alleles

Allele	**Reference**
sax-7(nj48)	(Sasakura et al., 2005)
dma-1(wy686)	(Liu and Shen, 2012)
mnr-1(wy758)	This study

B. Integrated transgenes

Allele	**Chromosome**	**Constructs**	**Co-injection marker**
wyIs378	X	pOL020, pOL090	
wyIs369	IV	pOL071	P*myo-2::mCherry*

C. Extrachromosomal arrays

Allele	**Constructs**	**Co-injection marker**
wyEx3869	pRP13	P*myo-2::mCherry*
wyEx4003	Engineered fosdmid WRM063aC06	P*odr-1::gfp*
wyEx5668	pXD65	P*odr-1::gfp*
wyEx5776	Engineered fosmid WRM0618aD06	P*odr-1::gfp*
wyEx5134	pXD13+pXD26+pOL036	P*odr-1::rfp*
wyEx5781	pXD38+pXD86+pOL036	P*odr-1::gfp*
wyEx5937	pXD86	P*myo-2::mCherry*
wyEx5950	pXD20+pXD97	P*odr-1::gfp*
wyEx5946	pXD105+pXD106	P*odr-1::gfp*
wyEx5948	pXD86+pXD97+pXD105+pXD106	P*odr-1::gfp*
wyEx4897	pOL036+pOL057	P*odr-1::rfp*
wyEx5938	pXD103	P*odr-1::gfp*
wyEx6039	pXD118	P*odr-1::rfp*
wyEx4286	pOL035	P*odr-1::rfp*
wyEx5790	pXD46+pOL036	P*odr-1::gfp*
wyEx6034	pXD91+pOL036	P*odr-1::rfp*

Table S2. Plasmids used in this study

A. Plasmids

Plasmid	**Description**	**Note**
pOL020	*ser2prom3::myr-gfp*	
pOL035	*ser2prom3::dma-1::gfp*	
pOL036	*ser2prom3::myr-mCherry*	
pOL057	P*dpy-7::sax-7s::gfp*	
pOL071	P*nhr-81::sax-7s*	
pOL090	P*rab-3::myr-mCherry*	

pRP13	P*dpy-7::sax-7s*	A gift of Oliver Hobert. (Pocock et al., 2008)
pXD13	P*rab-3::cfp*	
pXD20	P*mec-17::BFP*	
pXD26	P*dpy-7::sax-7s:yfp*	
pXD38	P*mec-17::sax-7s::yfp*	
pXD46	P*dpy-7::sax-7sΔIg*	All 4 Ig domains of SAX-7 deleted by yeast recombineering using primers 5'-ttcttgtcacaagtttgaacccacgcagagtgaacagccacaggaacatcattcacgtactgctctgttgg -3' and 5'- gatgttcctgtggctgttca -3' (Kevin R. et al., 1997)
pXD65	P*dpy-7::mnr-1*	cDNA of mnr-1 cloned with primers 5'-ATGATGATATCAATACTATTGGTTT -3' and 5'-TTAAAGTAGGTAGATCATTAGGAG -3'
pXD86	P*mec-17::mnr-1*	
pXD91	P*dpy-7::sax-7sΔFnIII*	All 5 FnIII domains of SAX-7 deleted by yeast recombineering using primers 5'-aacatccttaaattgaattttga -3' and 5'-ctaaattggatcaagttgaaaaggaaatcaaaattcaatttaaggatgttggtgaaacgacgaaaacgt -3' (Kevin R. et al., 1997)
pXD97	P*mec-17::sax-7s*	
pXD103	P*dpy-7::mnr-1ΔTM*	
pXD105	P*unc-47::mnr-1*	
pXD106	P*unc-47::sax-7s*	
pXD118	Pdpy-7::mCherry::mnr-1	

B. Engineered fosmids

Construct	**Original fosmid**
P*sax-7::sax-7::gfp::sl2::mCherry*	WRM063aC06
P*mnr-1::mnr-1::gfp::sl2::mCherry*	WRM0618aD06

Table S3. S2 cell aggregation assay

A. Plasmids used in S2 cell transfection

Plasmid	**Description**
pAWG	P*actin::gfp*
pAWR	P*actin::rfp*
pXD49	P*actin::sax-7s::gfp*
pXD54	P*actin::dma-1::rfp*
pXD85	P*actin::mnr-1::gfp*
pXD53	P*actin::sax-7s::HA*
pXD95	P*actin::dma-1 ecto-domain::myc*
pXD84	P*actin::mnr-1::Flag*

pXD130	P*actin::sax-7sΔIg::gfp*
pXD116	P*actin::sax-7sΔFnIII::gfp*

B. S2 cell aggregation assay

Green cells	**Red cells**	**Result**
GFP (pAWG)	DMA-1::RFP (pXD54)	No aggregation
SAX-7::GFP(pXD49)	RFP (pAWR)	No aggregation
MNR-1::GFP(pXD85)	RFP (pAWR)	No aggregation
SAX-7::FP(pXD49)	DMA-1::RFP (pXD54)	No aggregation
MNR-1::GFP(pXD85)	DMA-1::RFP (pXD54)	No aggregation
SAX-7::GFP(pXD49) + MNR-1::GFP(pXD85)	DMA-1::RFP (pXD54)	Aggregation
SAX-7ΔIg::GFP(pXD130) + MNR-1::GFP(pXD85)	DMA-1::RFP (pXD54)	Aggregation
SAX-7ΔFnIII::GFP(pXD116) + MNR-1::GFP(pXD85)	DMA-1::RFP (pXD54)	No aggregation

Supplemental figure legends

Figure S1. Phylogenetic analysis of MNR-1 and schematic structures of SAX-7, MNR-1 and DMA-1 proteins. Related to Figure 1.

(A) Phylogenetic tree of *C. elegans* MNR-1 and its orthologues in other species. (B) Schematic of SAX-7, MNR-1 and DMA-1 proteins. Ig: Immunoglobulin domain. FnIII: Fibronectin III domain. F: FERM binding motif. A: Ankyrin binding motif. P: PDZ binding motif. LRR: Leucine rich repeat. LRR-NT: LRR N-terminal domain. LRR-CT: LRR C-terminal domain.

Figure S2. SAX-7 was expressed and localized early in development independent of MNR-1 or PVD dendrites. Related to Figure 2 and Figure 3.

(A-B) SAX-7 was expressed and subcellularly localized in hypodermal cells in (A) L2 and (B) L3 stages. (C-E) The expression and localization of SAX-7 did not depend on MNR-1 or PVD dendrites. (C) Fluorescent image showing disrupted PVD morphology in *mnr-1(wy758)* mutant. (D) SAX-7 localization in the hypodermal cells revealed by P*dpy-7::sax-7s::gfp* was not altered in *mnr-1(wy758)* mutants despite the largely abnormal PVD structure. (E) Overlay of (C) and (D). Scale bar: 10μm

Figure S3. MNR-1 was partially secreted. Related to Figure 5.

(A) Expressing MNR-1 without its transmembrane domain partially rescued the "T" formation phenotype of *mnr-1(wy758)*. (B) Full length MNR-1 tagged with mCherry at its N-terminus could be detected in the ceolomocytes. Arrowheads: Ceolomocytes.

Figure S4. PVD dendrites in *dma-1* mutants did not follow PLM and ALM neurons expressing SAX-7 and MNR-1. Related to Figure 6.

(A-C) PLM overexpression of SAX-7 and MNR-1 failed to alter dendritic morphology in *dma-1(wy686)* mutants. (A) PLM and ALM neurons labeled by P*mec-17::sax-7::yfp.* (B) Dendritic structure of *dma-1(wy686)* mutant worms was unaltered by overexpression of SAX-7 and MNR-1. No additional T's were formed when 2^{o} PVD dendrites made contact with the PLM and ALM neurons. (C) Overlay between (A) and (B). Scale bar: 10 μm.

Figure S5. DMA-1 was the neuronal receptor for SAX-7 and MNR-1. Related to Figure 6.
(A) PVD dendritic phenotype of *dma-1(wy686); mnr-1(wy758)* mutants were identical to that of *dma-1(wy686)* single mutants. (B) PVD dendritic phenotype of *dma-1(wy686); sax-7(nj48); mnr-1(wy758)* mutants were identical to that of *dma-1(wy686)* single mutants. (C) Over-expressing DMA-1 in PVD caused overbranching. (D-E) PVD morphology of *sax-7(nj48)* mutant (D) or *mnr-1(wy758)* mutant (E) was not altered by DMA-1 overexpression. Scale bar: 10 μm.

Figure S6. Structure-function analysis of SAX-7. Related to Figure 7.
(A) Summary of structure-function analysis of SAX-7. (B) SAX-7ΔIg could rescue the PVD dendrite phenotype of *sax-7(nj48)* mutant. Scale bar: 10μM (C) SAX-7ΔFnIII failed to rescue. (D) S2 cells transfected with *sax-7ΔIg::gfp* and *mnr-1::gfp* strongly aggregated with cells expressing DMA-1-RFP. Scale bar: 100 μm (E) Cells transfected with *sax-7ΔFnIII::gfp* and *mnr-1::gfp* failed to aggregate with cells expressing DMA-1-RFP. (F) Schematic representation of proposed model: SAX-7 and MNR-1 formed pre-patterned lines in hypodermal cell (left). When PVD 2^{o} branches expressing DMA-1 reached the SAX-7 and MNR-1 enriched line (middle), 3 ^{o} branches formed and grew along the pre-patterned line. Thus the precise location of branch formation and growth direction was specified through interaction between pre-patterned cell adhesion molecules and their neuronal receptor.

Movies S1-S4. Time-lapse movies of PVD dendrite development. Related to Figure 4.
S1. Development of WT PVD.
S2. Development of PVD dendrites in *sax-7(nj48)* mutant.
S3. Development of PVD dendrites in *mnr-1(wy758)* mutant.
S4. Development of PVD dendrites in *dma-1(wy686)* mutant

Supplemental experimental procedures

Fluorescence microscopy and confocal imaging
Images of fluorescent proteins were captured in live animals using a Plan-Apochromat 40x/1.3 objective on a Zeiss LSM710 confocal microscope (Carl Zeiss). Worms were immobilized on 2% agarose pads using a mixture of 225 mM 2,3-butanedione monoxime and 2.5 mM levamisole (Sigma-Aldrich). Z-stacks were collected and the maximum intensity projection was used for additional analysis.

S2 cell aggregation and co-IP assays

S2 cells were transfected with P*actin::sax-7::gfp*, P*actin::mnr-1::gfp*, P*actin::sax-7::gfp* +P*actin::mnr-1::gfp*, or P*actin::dma-1::rfp*. Cells transfected with P*actin::gfp* and P*actin-rfp* were used as controls. 3 days after transfection, cells were washed with 5 ml phosphate buffered saline (PBS) and resuspended in S2 medium at 10^6 cell/ml. 500 μL of green cells were mixed with 500 μL red cells and rotated at 30 rpm for 3 hours at room temperature. 3 μL of each mixture was immediately spotted on glass slides for imaging and quantification.

Similarly, for co-IP assays S2 cells were transfected with P*actin::sax-7::HA*, P*actin::mnr-1::FLAG*, both P*actin::sax-7::HA* and P*actin::mnr-1::FLAG*, or P*actin::dma-1::ecto-myc*. 3 days after transfection, cells transfected with SAX-7 and/or MNR-1 were collected and lysed in lysis buffer (50mM Tris-HCl pH 7.4, 10mM $MgCl_2$, 150mM NaCl, 1mM EGTA, 10% Glycerol, 0.5% Nonidet P-40 and protease inhibitor (Roche)). Cultured medium from P*actin::dma-1::ecto-myc* transfected cells was collected. The cell lysates or cultured medium containing the desired proteins were mixed with anti-HA antibody coated agarose beads (Sigma) and incubated at 4^oC for 3 hours with rotation and washed thoroughly with lysis buffer. Proteins were eluted at 65^oC using 2% SDS elution buffer and detected using western blot analysis with mouse antibody to HA (1:1000, Roche), rabbit antibody to Myc (1:2000, Santa Cruz Biotechnology), mouse antibody to FLAG (1:5000, Sigma) and HRP-conjugated goat antibodies to mouse or rabbit (1:20,000, Jackson Immuno Research).

Supplemental references

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