

## Supporting Online Material

### Materials and Methods

#### Strains

Nematodes were cultivated according to standard protocols and maintained at 20°C unless stated otherwise (1). The following alleles were used: LGI, *unc-40(e1430)*, *zDis5[mec-4::gfp, lin-15(+)]*; LGII, *clr-1(e1745)*, *clr-1(cy14)*; LGIV, *ced-10(n1993)*, *evIs82[unc-129::gfp, pMH86]*, *zDis4[mec-4::gfp, lin-15(+)]*; LGV, *unc-34(gm104)*; LGX, *unc-6(ev400)*, *sax-3(ky123)*, *slt-1(eh15)*, *slt-1(ok255)*, *unc-115(ky275)*, *egl-15(n484)*, *egl-15(n1477)*, *egl-17(e1313)*, *egl-17(n1377)*. Transgenes maintained as extrachromosomal arrays included: *kyEx456[mec-7::myr::unc-40, str-1::gfp]*, *kyEx637[mec-7::myr::unc-40(ΔP2), odr-1::dsred]*, and *kyEx639[mec-7::myr::unc-40(ΔP1), odr-1::dsred]*. Strains that were not derived in the Tessier-Lavigne and Bargmann laboratories were kindly provided by Scott Clark (*zDis4* and *zDis5*), Gian Garriga (*unc-34(gm104)*), or Theresa Stiernagle of the *Caenorhabditis* Genetics Center. Double and triple mutants were constructed in the absence of marker mutations using standard genetic methods and confirmed by either complementation tests or genotyping. Detailed information is available upon request.

#### Microscopic Examination of Axon Trajectories

Axonal processes of the touch cell neurons and DA/DB motoneurons were visualized with the integrated *mec-4::gfp* transgene *zDis5* and *unc-129::gfp* transgene *evIs82*, respectively. Animals were placed on 5% Noble Agar pads in M9 buffer containing 20 mM sodium azide and examined with a Plan-NEOFLUAR 40x objective, using

fluorescence optics in a Zeiss Axioplan 2 imaging system. Images were captured using a SPOT camera (RT Slider Diagnostic Instruments, Inc.). The statistics was performed by comparing two proportions from different mutants.

### ***clr-1* Allele Sequencing**

To identify the molecular lesion in *clr-1(cy14)*, PCR was performed on genomic DNA from mutants, and the products purified and sequenced. The mutation was identified by aligning the sequence with the reported genomic sequence from the *C. elegans* Genome Sequencing Consortium. The consequence of the splice site mutation was determined by PCR amplification of reverse-transcribed RNA from mutants and wild-type animals. PCR amplification for 40 cycles of wild-type and *clr-1(cy14)* reverse-transcribed RNA was performed with two pairs of primers surrounding intron 5. RT-PCR products were sequenced or separated on a 3% agarose gel.

### **Molecular Biology**

Standard molecular biology techniques were used. *mec-7::clr-1* was generated by cloning the *clr-1* cDNA into NheI and KpnI sites of pPD96.41, which contains *mec-7* promoter. pPD96.41 was a gift of Andrew Fire (Carnegie Institute of Washington). The *clr-1* cDNA was provided by Michael Stern (Yale University).

### **Transgenic Animals**

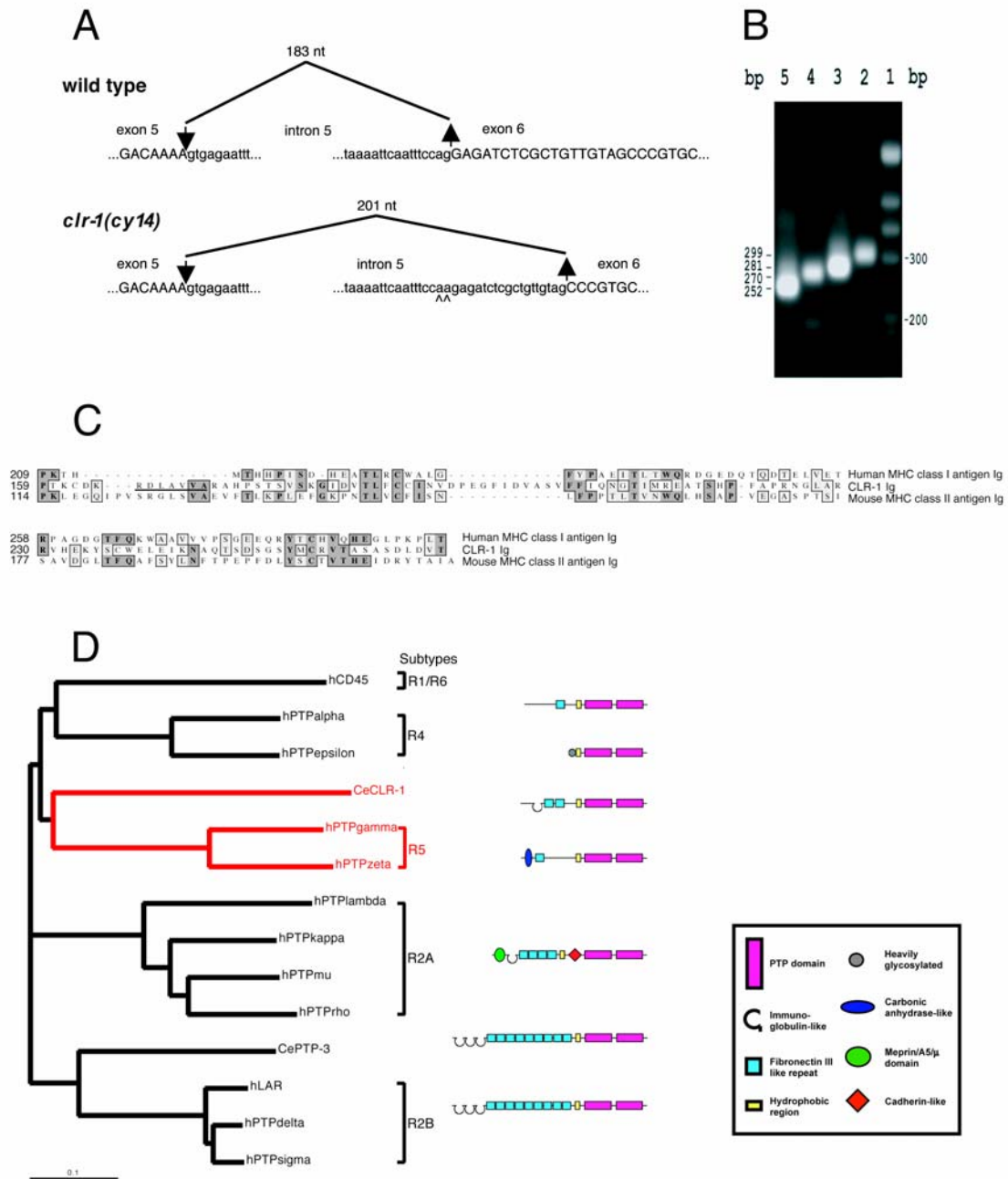
Germline transformation of *C. elegans* was performed using standard techniques (2). The *mec-7::clr-1* promoter fusion was injected at 100 ng/μl along with the coinjection marker

*odr-1::dsred* at 50 ng/μl. Transgenic lines were maintained by following *odr-1::dsred* fluorescence. For the *clr-1* cell autonomy experiment, *mec-7::clr-1* was injected with *odr-1::dsred* into *slt-1(eh15)* background. The resulting transgenic lines were crossed to *clr-1* mutants to generate *clr-1; slt-1; cyEx(mec-7::clr-1)* or crossed into wild type to generate *cyEx(mec-7::clr-1)*. Two independently isolated transgenic lines were analyzed. The data shown are from one representative line.

### **Binding Assays**

The full-length *clr-1* cDNA was cloned into XbaI/KpnI sites of pCDNA3 and the partial *clr-1* cDNA, which encodes the cytoplasmic domain 767-1409 amino acid residues, was cloned into NcoI/KpnI sites of pSPUTK *in vitro* translation vector (Stratagene, La Jolla, California). Both cDNAs are under the control of the SP6 promoter to generate a 35[S]-methionine-labeled probe by *in vitro* transcription/translation (TNT SP6 Quick Coupled Transcription/Translation Kit, Promega, Madison, WI). Expression vectors for UNC-40-GST (1106-1415), SAX-3-GST (978-1224) or GST proteins were constructed in pGEX-4T-1 (Amersham, Piscataway, New Jersey). GST proteins were expressed in *E. coli* BL21-CodonPlus RIL (Stratagene, La Jolla, California), purified using glutathione agarose beads (Sigma), and quantified by SDS-PAGE and Coomassie staining. For binding assays, 2 μg of GST protein was mixed with 10 μl of 35[S]-methionine-labeled CLR-1 probes in 10mM Tris 7.5, 150 mM NaCl, 0.1% Triton and 0.05% BSA. Samples were incubated for two hours at 4°C and then washed three times in the same buffer. BSA was omitted from the last wash. Bound proteins were separated by SDS-PAGE, soaked in Amplify (Amersham), dried down under vacuum and exposed to film.

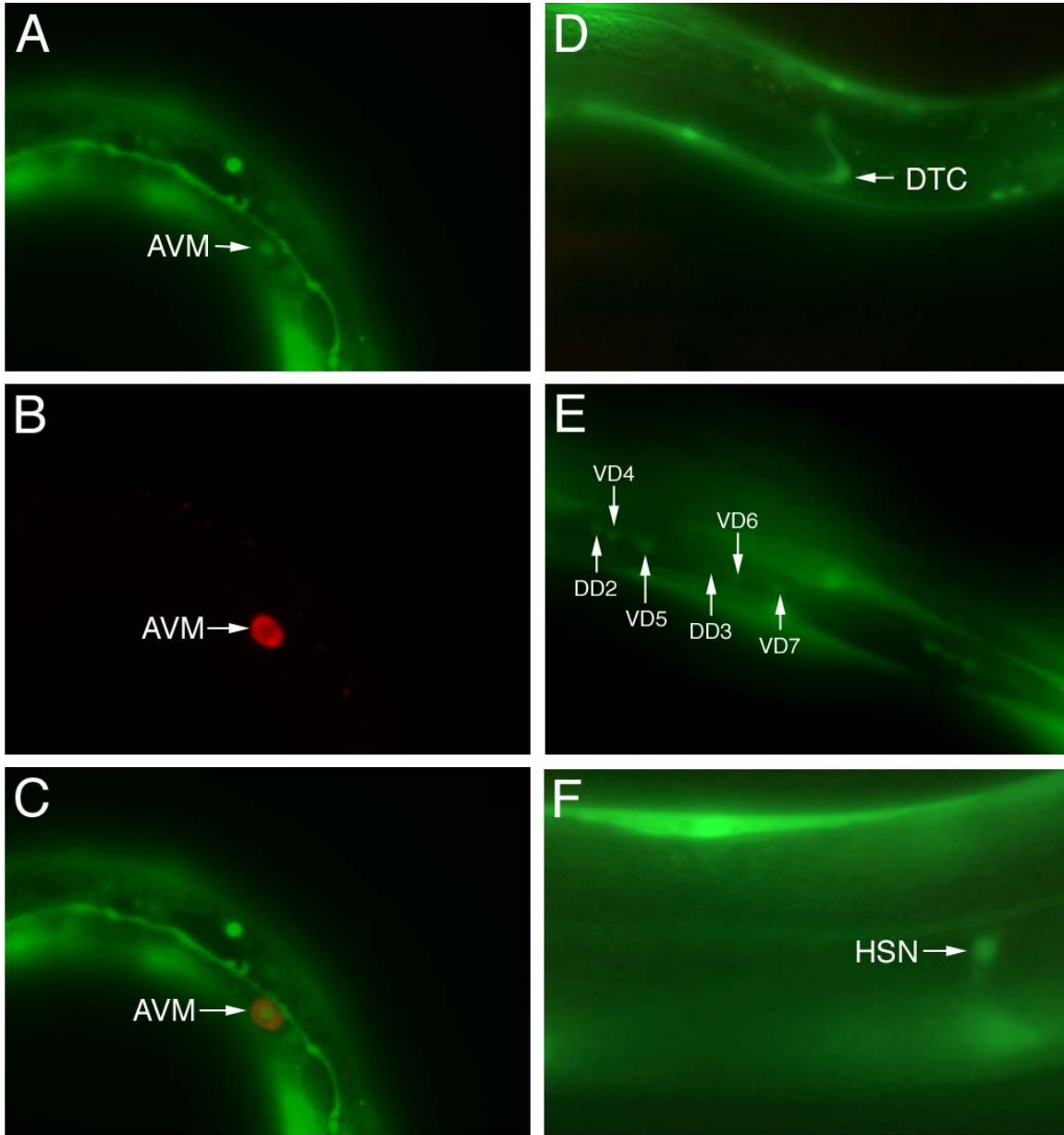
## Supporting Figures



**Fig. S1. Identification of the molecular lesion of *clr-1(cy14)*.**

(A) Sequence of the *clr-1(cy14)* mutation. The upper panel shows the genomic sequence around the exon 5–intron 5 and intron 5–exon 6 boundaries. Exons are shown in

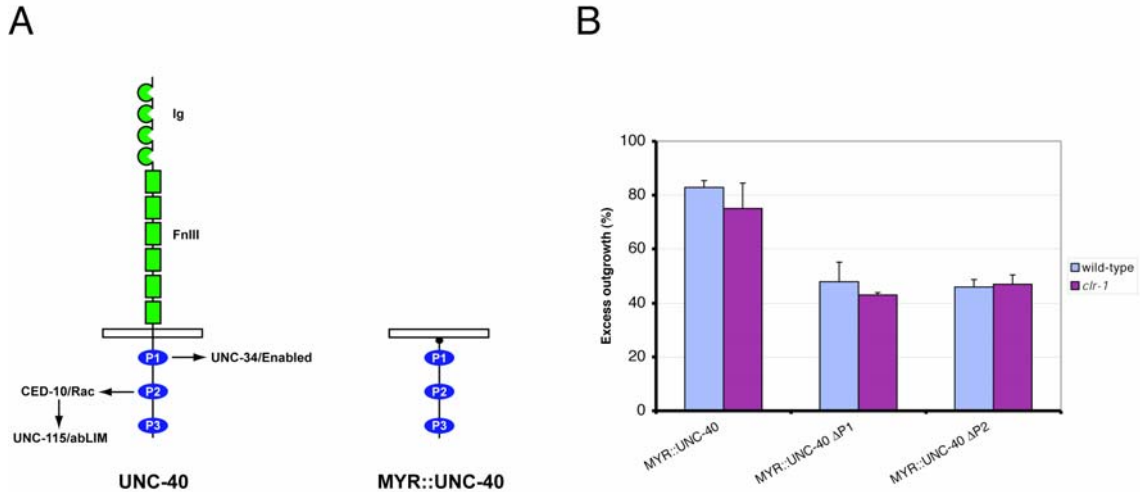
uppercase lettering, introns in lowercase. Upper panel, wild type; lower panel, *clr-1(cy14)*. The large downward-pointing arrow indicates the 5' location of the wild-type splice. The upward-pointing arrow indicates the 3' splice junction. The mutation is indicated by carets. The 3' splice acceptor has shifted due to the mutation. **(B)** RT-PCR analysis of wild-type and *clr-1(cy14)* RNA. Lanes: 1, 1 kb ladder; 2 and 4, amplification of wild-type cDNA; 3 and 5, amplification of *clr-1(cy14)* cDNA. Correctly spliced mRNA yields a product of 299 or 270 bp (correct joining of intron 5 and exon 6), depending on which primer set was used. In *clr-1(cy14)* animals, a 281 or a 252 bp product results from usage of a downstream cryptic splice acceptor at nucleotide 1,534 of cosmid F56D1.4a and removal of 18 bp from exon 6. **(C)** Sequence alignment of Ig domains from CLR-1, Human MHC class I antigen, and Mouse MHC class II antigen. Dark gray boxes indicate amino acid identities, and light gray boxes indicate conservative changes. Seven amino acids deleted in *clr-1(cy14)* are underlined. **(D)** Phylogram of the evolutionary relationships of *C. elegans* CLR-1 and five representative subtypes of vertebrate RPTPs. Shown is an unrooted tree derived from the alignment of five subtypes of vertebrate RPTPs with CeCLR-1 and CePTP-3, indicating the similar origin of CeCLR-1 and R5 subtype. The tree was drawn by the neighbor-joining method (3) and the proteins were selected to reflect the clustering in the neighbor-joining tree. The horizontal distance represents the degree of sequence divergence, and the scale bar at the bottom corner corresponds to 10% substitution events.



**Fig. S2. *clr-1* expression analysis.**

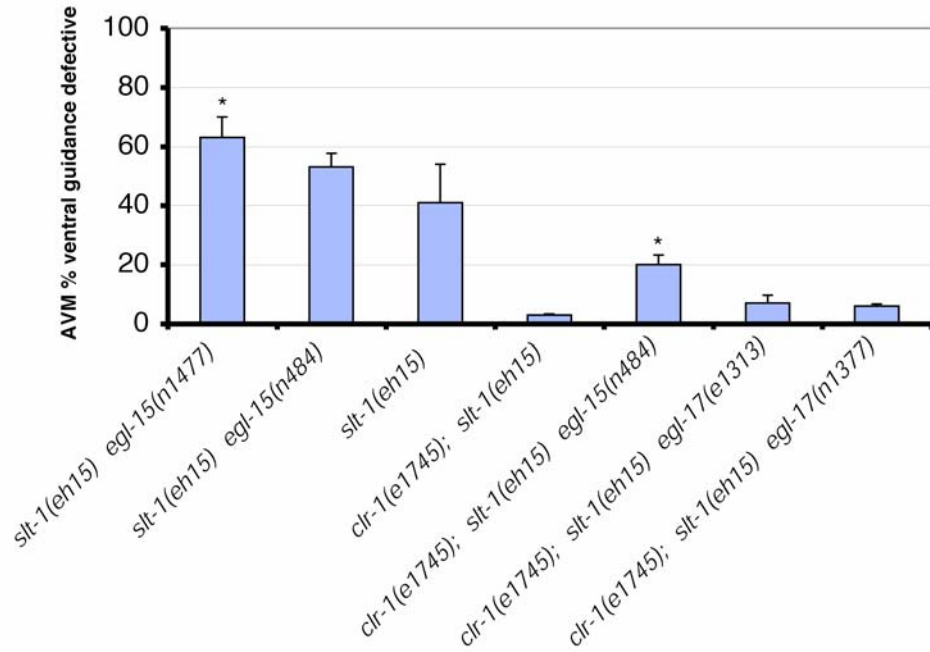
Expression pattern of *clr-1* promoter was obtained from an extrachromosomal array containing GFP with a nuclear localization sequence (NLS) (NH#950) (a gift from M. Stern). 4.5kb of *clr-1* promoter drives expression in a restricted subset of motile neurons and mesodermal cells. (A-C) *clr-1* is expressed in the AVM neuron from mid-L1, a stage

when AVM sends out its axon. *clr-1::gfp* expression in the AVM neuron is enriched in the nucleus because of the NLS (**A**). *mec-4::dsred* was used to label the AVM neuron mainly in its cytosol (**B**). The superimposed image identifies the GFP expressing cell as the AVM neuron (**C**). (**D-F**) 4.5kb of *clr-1* upstream sequence exhibits promoter activity in many other cells, including distal tip cells (DTC) (**D**), DD/VD neurons (**E**), and HSN neuron (**F**).



**Fig. S3. CLR-1 regulates the guidance rather than the outgrowth function of UNC-40.**

(A) Schematic of UNC-40 and its signaling pathways. The conserved cytoplasmic P1 and P2 motifs mediate distinct downstream pathways for UNC-40 function in the axon outgrowth: UNC-34 in one pathway, and CED-10 and UNC-115 in the other (4). MYR::UNC-40 consists of the UNC-40 cytoplasmic domain fused to a membrane-targeting myristoylation signal (black circle). The P1 and P2 domains of MYR::UNC-40 are deleted in  $\Delta P1$  and  $\Delta P2$ , respectively. (B) Mutations in *clr-1* do not enhance the excessive outgrowth phenotypes associated with MYR::UNC-40 expression. The percentage of excess AVM outgrowth, detected with a *mec-4::gfp* transgene, was determined for animals carrying the MYR::UNC-40, MYR::UNC-40  $\Delta P1$ , or MYR::UNC-40  $\Delta P2$  transgene alone or in combination with the *clr-1(e1745)* mutation.



**Fig. S4. *egl-15*/FGFR promotes AVM ventral guidance.** *egl-15(n484)* is a moderate hypomorphic allele and *egl-15(n1477ts)* is a temperature sensitive allele affecting the FGF receptor EGL-15; *egl-17(e1313)* and *egl-17(n1377)* are candidate null alleles of one of its two FGF ligands. *egl-15* mutations could significantly enhance the defect of either *slt-1* or *clr-1*; *slt-1* strains (asterisks).

### **Supporting References and Notes**

1. S. Brenner, *Genetics* **77**, 71 (1974).
2. C. Mello, A. Fire, *Methods Cell Biol.* **48**, 451 (1995).
3. N. Saitou, M. Nei, *Mol. Biol. Evol.* **4**, 406 (1987).
4. Z. Gitai, T. W. Yu, E. A. Lundquist, M. Tessier-Lavigne, C. I. Bargmann, *Neuron* **37**, 53 (2003).