

UNC-6/Netrin mediates dendritic self-avoidance

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Supplemental Table 1. Self-avoidance requires specific axon guidance molecules in the UNC-6/Netrin signaling pathway. Mutants of known axon guidance molecules were tested for PVD 3⁰ branch self-avoidance defects with the PVD::GFP reporter. Transcripts enriched ($\geq 1.5X$, $FDR \leq 1\%$) in PVD (Smith et al., 2010) are denoted with bold lettering. Only mutants of the UNC-6/Netrin signaling pathway showed self-avoidance defects that were significantly different from wt (+ indicates $p < 0.01$, Student's t-test). $N \geq 20$

Function	Gene	Self-avoidance defect
Ligand	<i>unc-6/Netrin</i>	+
	<i>unc-129/TGF beta family</i>	-
	<i>vab-1/Ephrin</i>	-
	<i>slt-1/Slit</i>	-
Receptor	<i>sax-3/Robo</i>	-
	<i>vab-2/Eph</i>	-
	<i>ptp-3/Lar</i>	-
	<i>unc-5/Unc-5H</i>	+
	<i>unc-40/DCC</i>	+
	<i>madd-2/NetrinR</i>	-
ECM component	<i>nid-1/Nidogen</i>	-

Supplemental Figure Legends

Supplemental Figure 1. Mutants of *unc-40*, *unc-5* and *unc-6* show a range of dendritic morphogenesis phenotypes in addition to the self-avoidance defect. (a)

PVD 1^o processes project along the lateral nerve cord in the wild type but deviate from

a strict A/P trajectory in >75% of *unc-40*, *unc-5* or *unc-6* mutants. **(b)** Wild-type (wt)

PVD neurons show an asymmetric pattern of lateral branching that results in more

dorsal than ventral menorahs in 100% of cases examined (n > 15) (wild-type

distribution). In UNC-6/Netrin pathway mutants, this asymmetry is disrupted resulting in

PVD neurons that have more ventral menorahs than dorsal menorahs or an equal

number of menorahs on each side ~50% of the time (defective distribution), an outcome

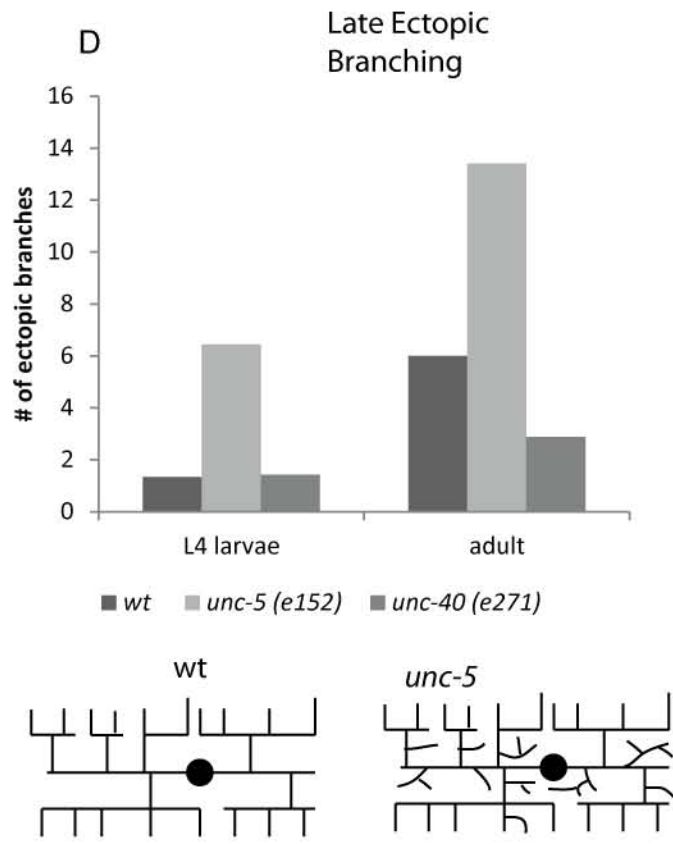
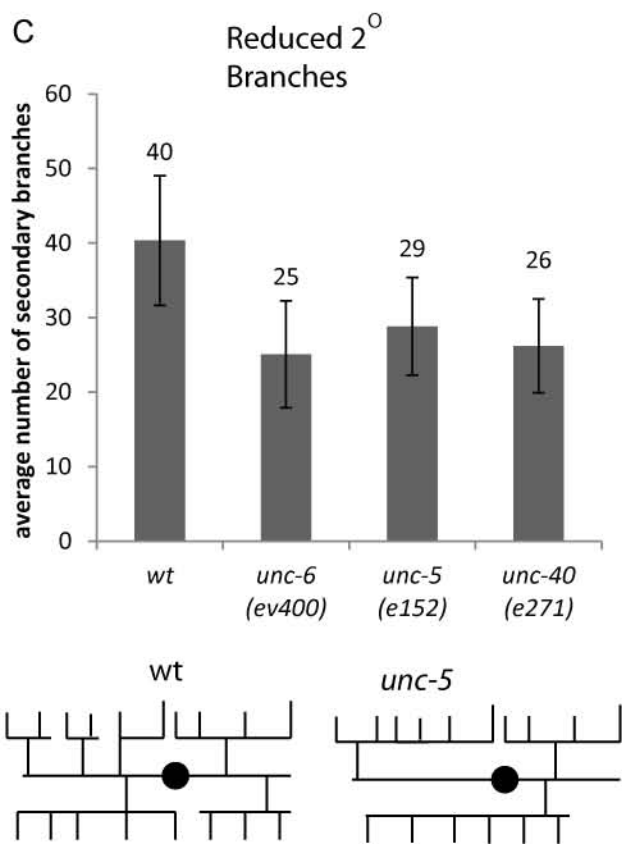
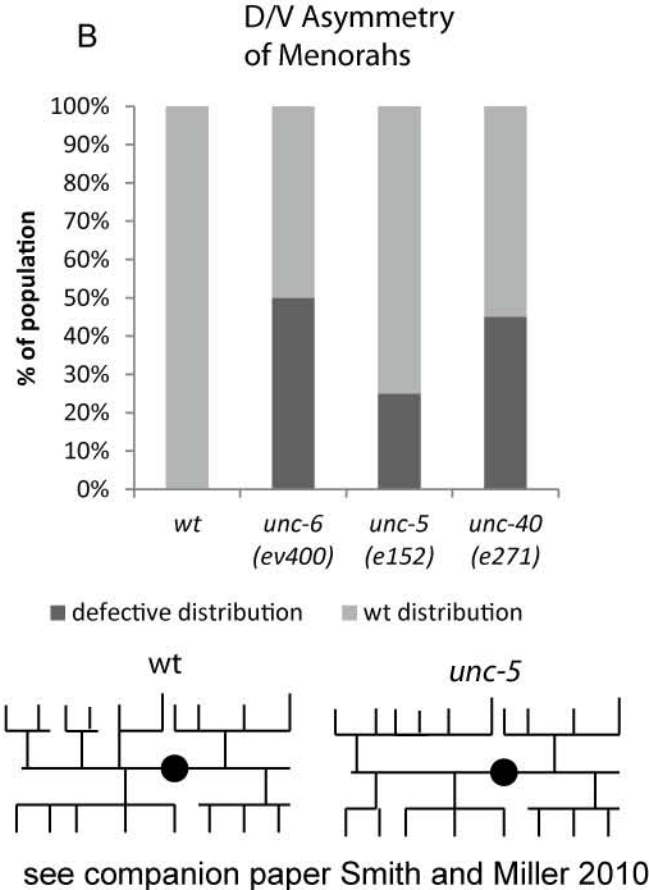
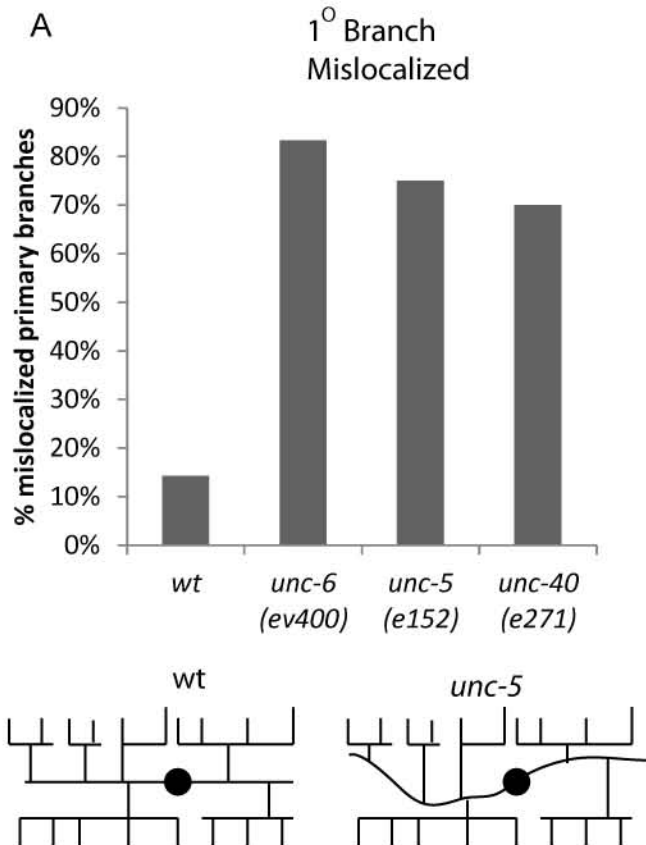
that is consistent with a randomized probability of dorsal vs ventral initiation of 2⁰

branches (CJ Smith and DM Miller, manuscript in preparation). **(c)** The average number

of 2^o dendrites/PVD neuron in *unc-6*, *unc-5* and *unc-40* mutants is reduced in

comparison to wild-type PVD neurons. **(d)**. Ectopic branching in adults is more frequent

in *unc-5(e271)* than in either wild type (wt) or *unc-40(e271)*.



Supplemental figure 2. *unc-40* and *unc-5* mutants show defects in contact-

dependent self-avoidance. Quantification from movies of self-avoidance events in

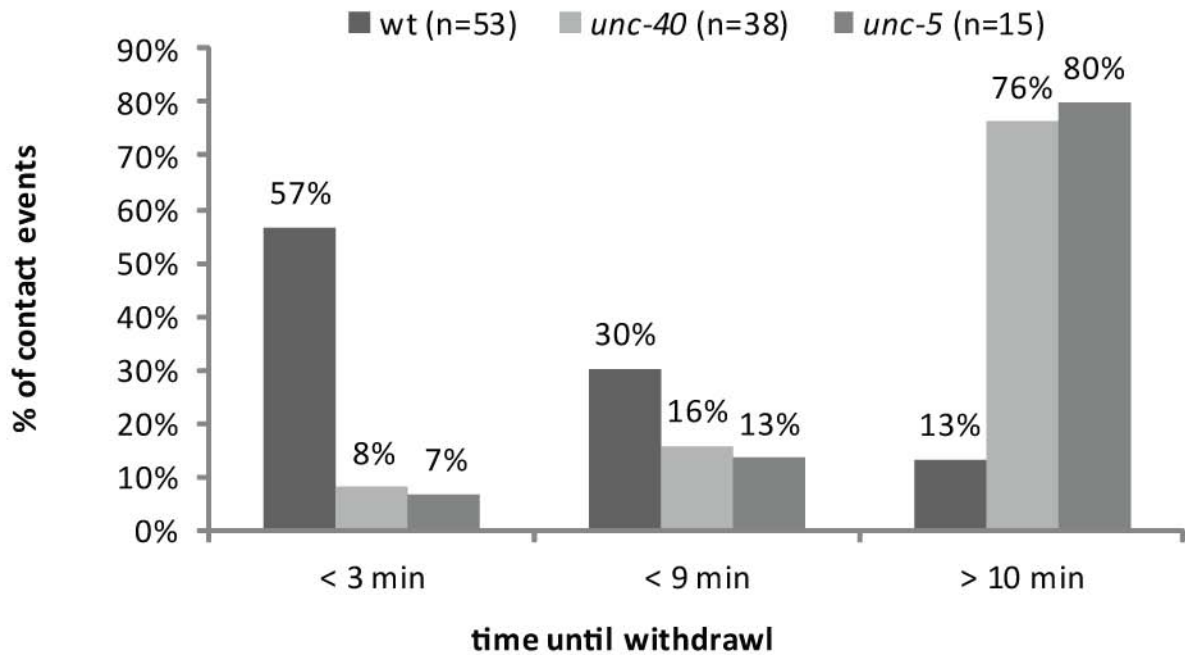
wild type (wt), *unc-5 (e152)* and *unc-40 (e271)* show that 3^o branches in *unc-5 (e152)*

and *unc-40 (e271)* do not retract as quickly as in wild-type animals; a majority (>75%) of

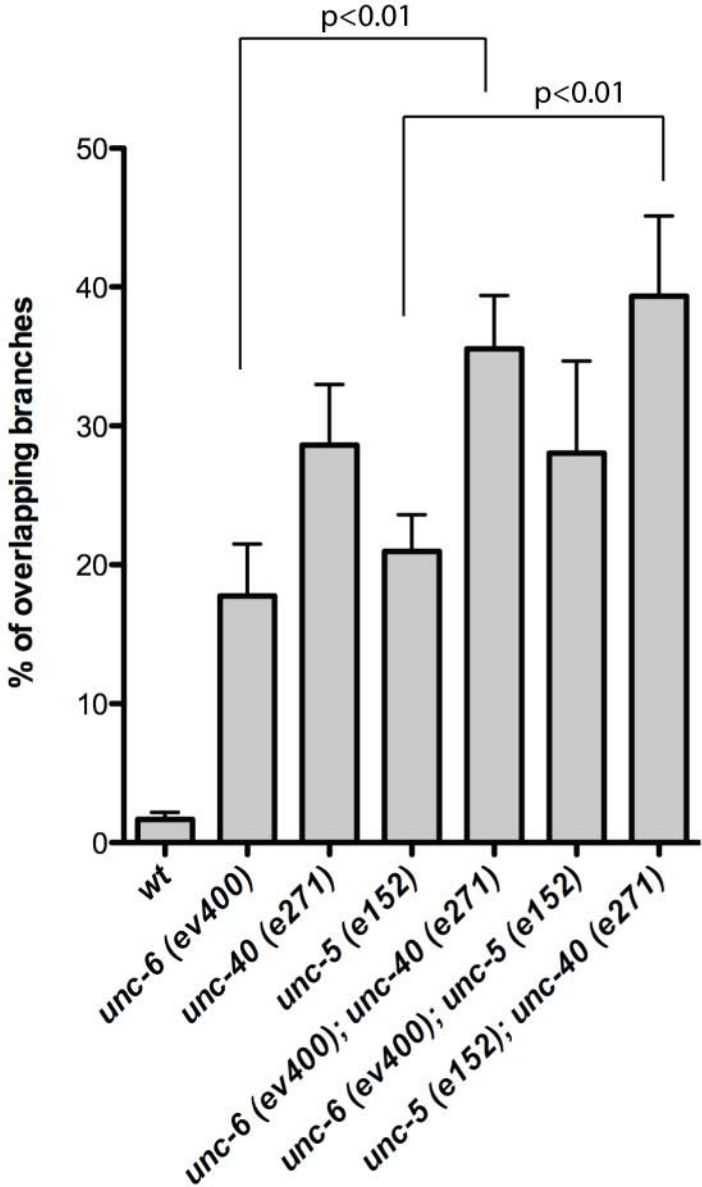
3^o branches have failed to retract up to 10 minutes after initial contact in *unc-40* and

unc-5 mutants whereas only 13% of 3^o dendrites are still overlapping at this time point

in wild type.

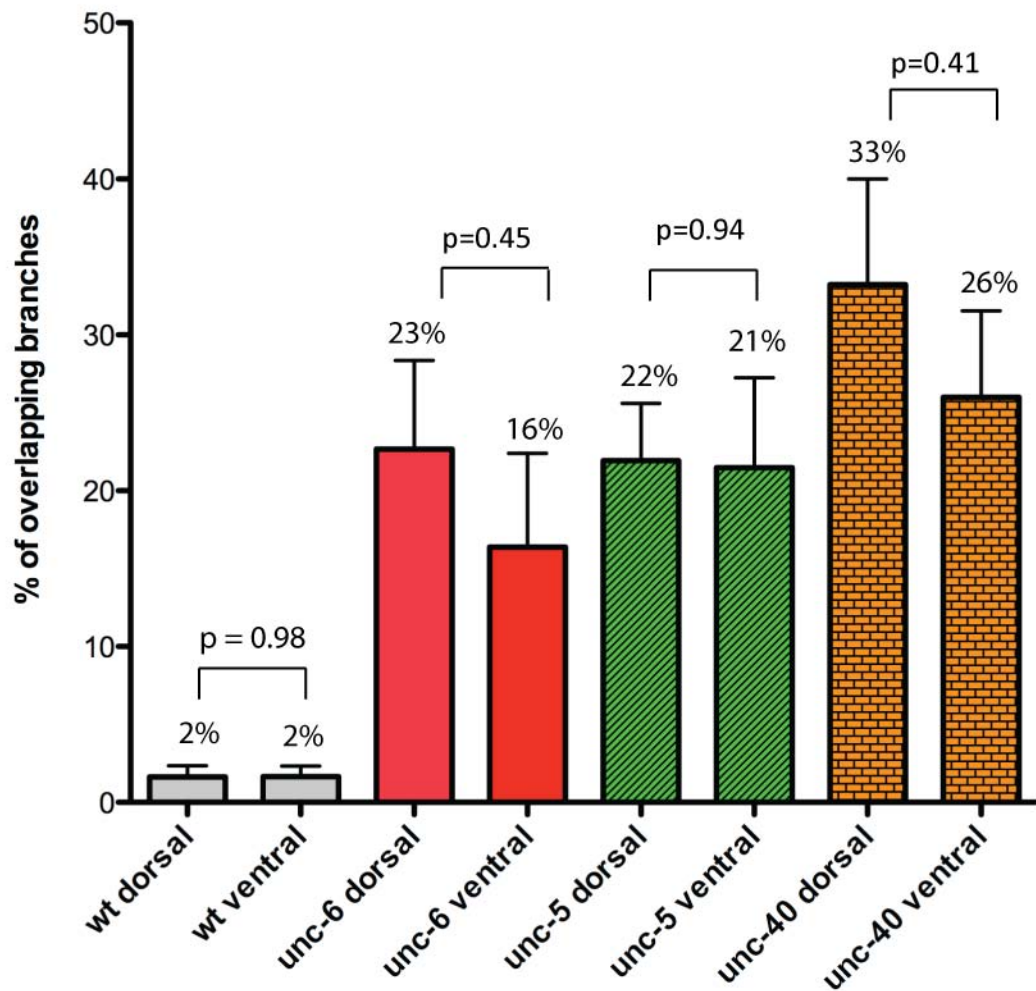


Supplemental Figure 3. Genetic interactions of *unc-40*, *unc-5* and *unc-6*. Single mutants of *unc-5* (*e152*), *unc-40* (*e271*) and *unc-6* (*ev400*) show comparable self-avoidance defects that are not statistically different from each other. The self-avoidance defect of the double mutant *unc-5* (*e152*); *unc-6* (*ev400*) is not significantly different from either *unc-5* (*e152*) or *unc-6* (*ev400*) single mutant which suggests that *unc-5* and *unc-6* function in a common pathway. *unc-40* (*e271*); *unc-5* (*e152*) double mutants do not show enhancement of the PVD self-avoidance defect vs *unc-40* (*e271*) but do show a more severe self avoidance defect than *unc-5* (*e152*) alone ($p < 0.01$, $n = 20$, Students t-test). *unc-40* (*e271*); *unc-6* (*ev400*) double mutants show enhancement of self-avoidance defects compared to *unc-6* (*ev400*) but not to *unc-40* (*e271*) ($p = 3E-3$ vs *unc-6* (*ev400*)). These results suggest that *unc-40* fulfills an additional *unc-5/unc-6*-independent role in self-avoidance.



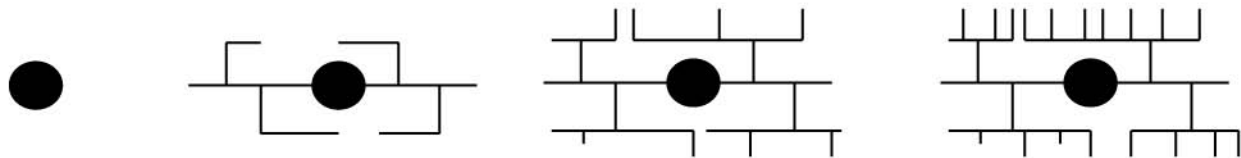
Supplemental Figure 4. UNC-6/Netrin signaling mutants do not show differences in dorsal vs. ventral 3⁰ dendrite self-avoidance phenotypes. The fraction of overlapping 3⁰ branches in dorsal vs ventral regions was scored for *unc-6(ev400)*, *unc-5(e152)* and *unc-40(e271)*. N = 20 animals

dorsal vs ventral



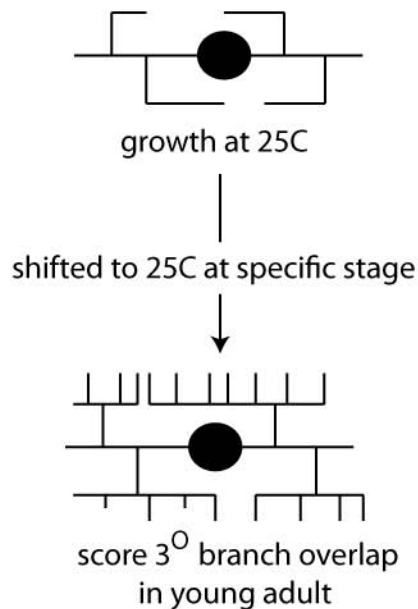
Supplemental Figure 5. UNC-6/Netrin is required for self-avoidance during the L3 larval stage. **(a)** Schematic of PVD development showing the elaboration of dendritic branches during larval development. **(b)** Experimental design for temperature shifts with the temperature sensitive mutant *unc-6(rh46)* to determine the temporal requirement for UNC-6 in PVD 3⁰ dendritic branch self-avoidance. **(c)** Histogram showing fraction of overlapping 3⁰ branches resulting from maintenance at either the permissive (15C) (15C control) or restrictive (25C) (25C control) temperatures and from upshift experiments (15C>25C) in which animals grown at permissive temperature are shifted to growth at the restrictive temperature. Note that the extent of overlapping 3⁰ branches after shifting to restrictive temperature at the L2/L3 larval transition is not significantly different from the self-avoidance defect resulting from continuous exposure to 25 C whereas shifts to restrictive temperature at later developmental periods (*i.e.*, L3/L4 transition, L4/adult transition) result in a significantly lower fraction of overlapping 3⁰ dendritic branches that is not significantly different from the PVD self-avoidance defect from 15C control animals. These results indicate that UNC-6/Netrin function is required before the L3 larval stage for 3⁰ branch self-avoidance but is not necessary in older animals.

a. mid L2 larvae L2/L3 transition L3/L4 larvae transition L4 larvae/adult transition

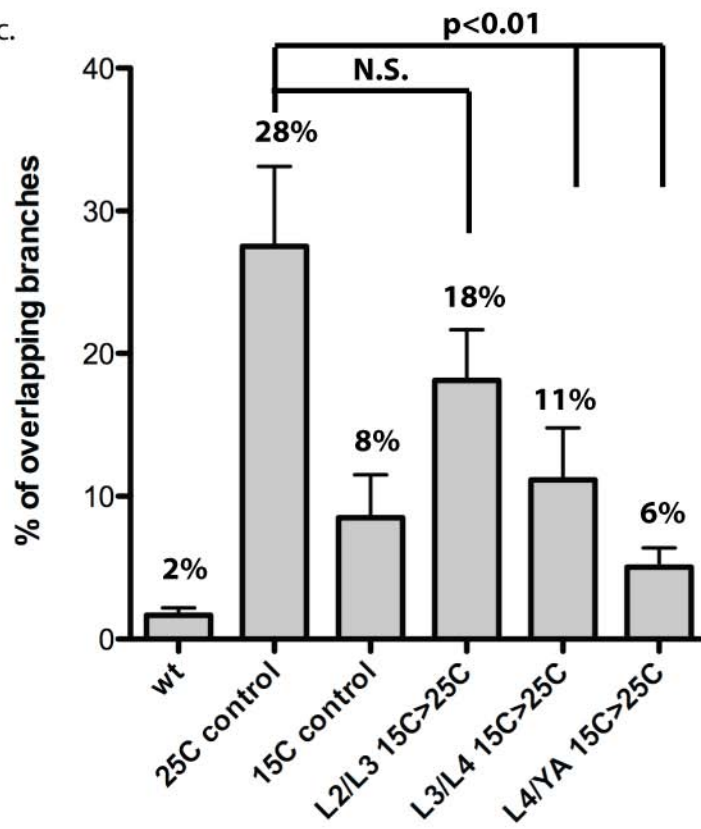


b.

Experimental Design



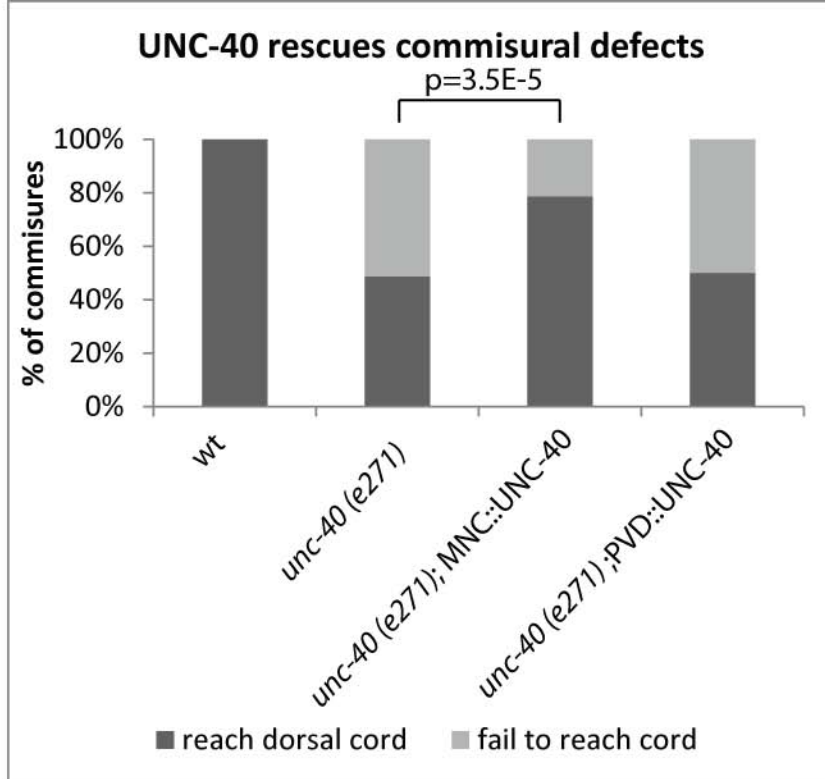
c.



Supplemental Figure 6. Expression of UNC-40/DCC in ventral cord motor

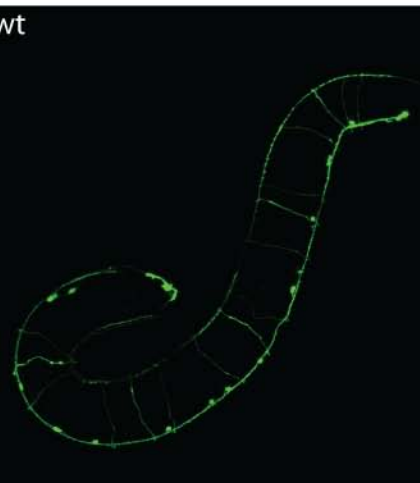
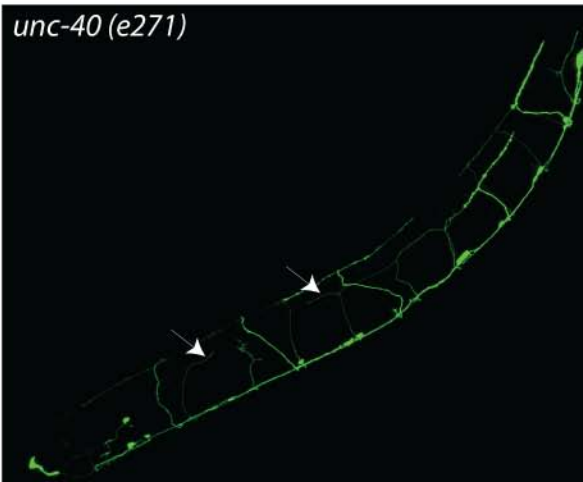
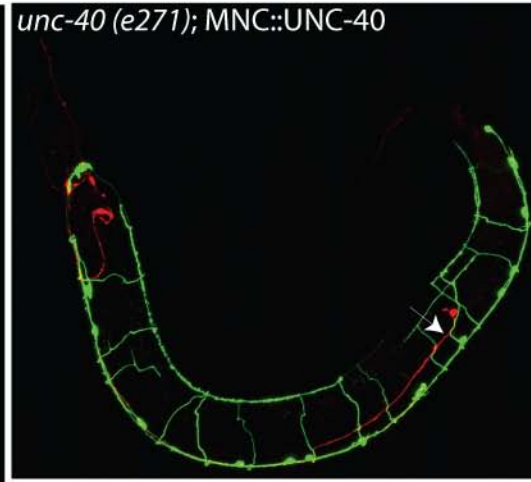
neurons rescues motor axon guidance defects. (a) Histogram showing that 100% of *unc-25::GFP*-labeled GABAergic motor neurons extend circumferential commissures (MNCs) to the dorsal cord whereas only ~45% of MNCs reach the dorsal nerve cord in *unc-40 (e271)* (n = 20). MNC guidance defects are largely rescued by expression of UNC-40 in ventral cord motor neurons with the *unc-25* promoter (MNC::*UNC-40*). **(b)** Representative confocal images of wild type (wt), *unc-40 (e271)* and *unc-40 (e271); MNC::*UNC-40** adults. Arrows point to MNCs that fail to reach the dorsal nerve cord in *unc-40(e271)*. Axon guidance defects are not rescued in the PDE neuron that is labeled by a co-injected marker (*dat-1::mcherry*) in which expression of UNC-40 is not restored (arrow in MNC::*UNC-40*)

A



B

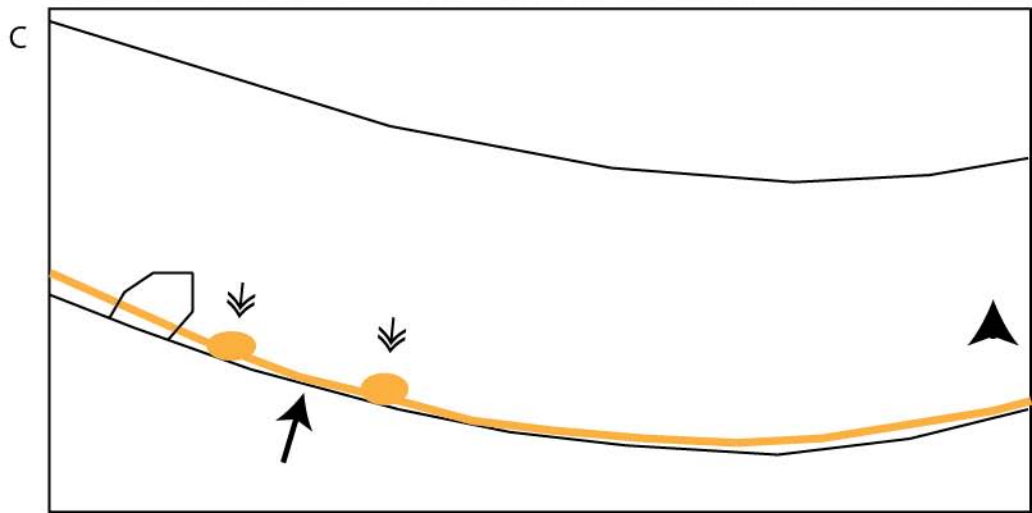
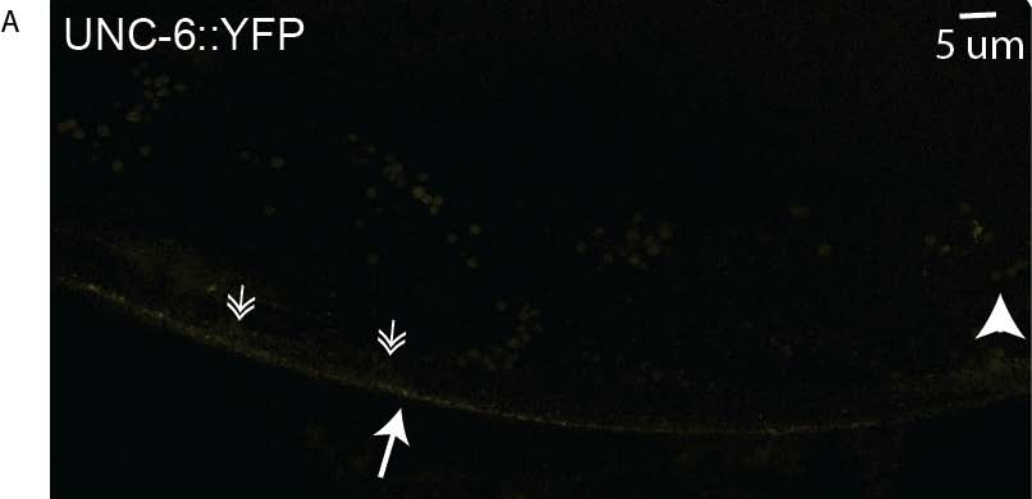
wt

*unc-40 (e271)**unc-40 (e271); MNC::UNC-40*

Supplemental Figure 7. Expression of UNC-6::YFP in ventral motor neurons

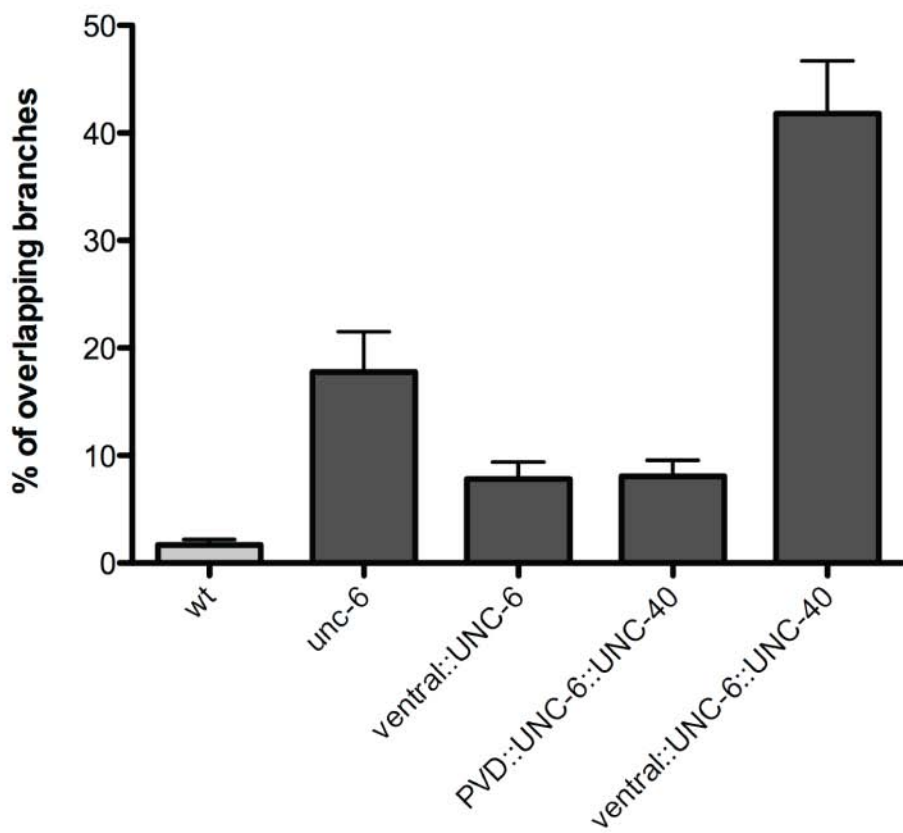
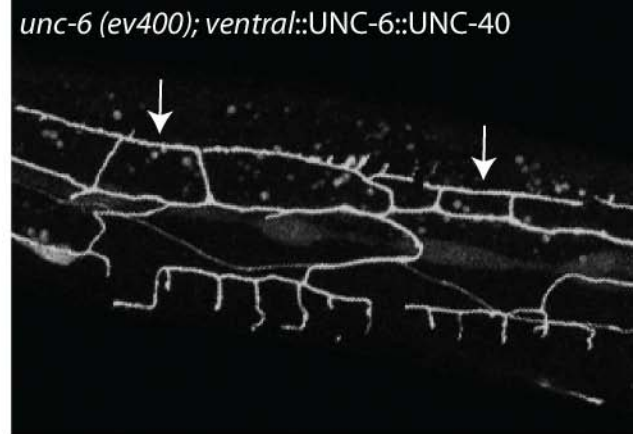
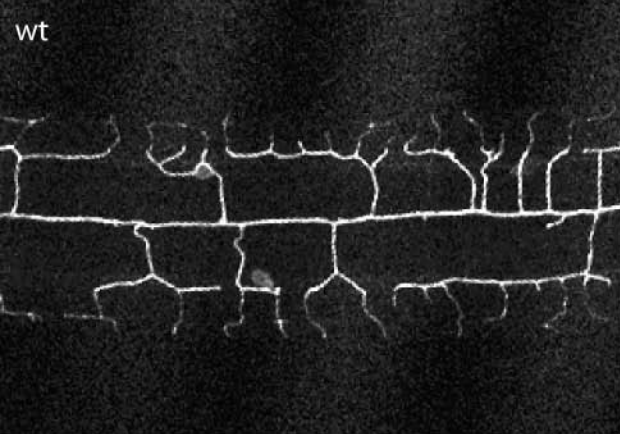
labels the ventral nerve cord but is not detected at the wild-type PVD neuron. (a,b)

In a wild-type animal, YFP-labeled UNC-6 (UNC-6::YFP) is detected in the cell body of ventral cord motor neurons (double-headed arrowheads) where it is expressed (*unc-6* promoter) and in the adjoining ventral nerve cord (arrow) but is not detectable in posterior lateral region in which the wild-type PVD neuron (arrowhead) and its dendritic arbor reside. **(c)** Schematic representation of UNC-6::YFP localization.

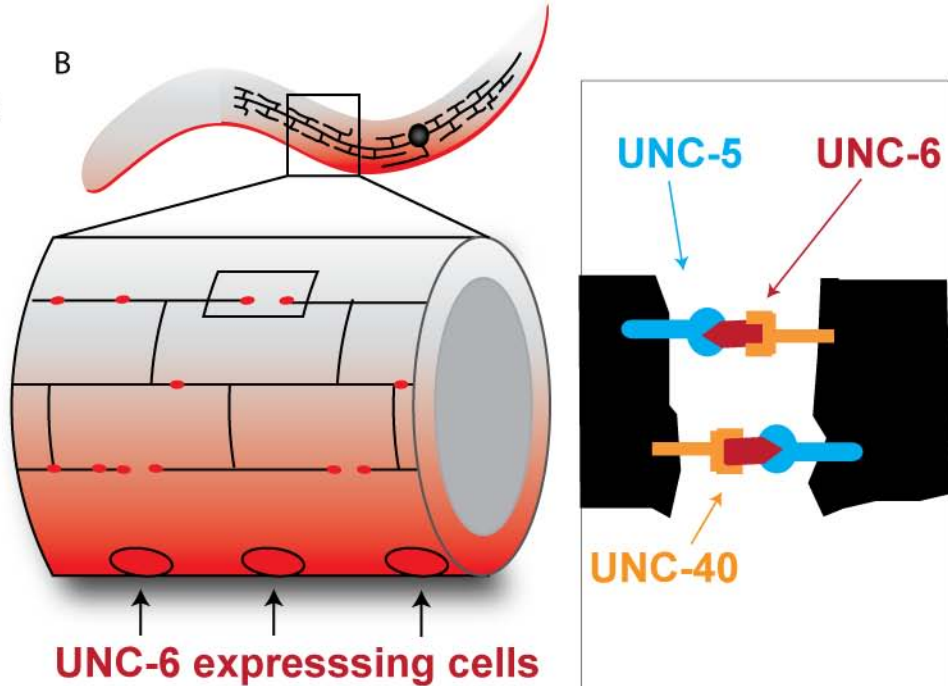
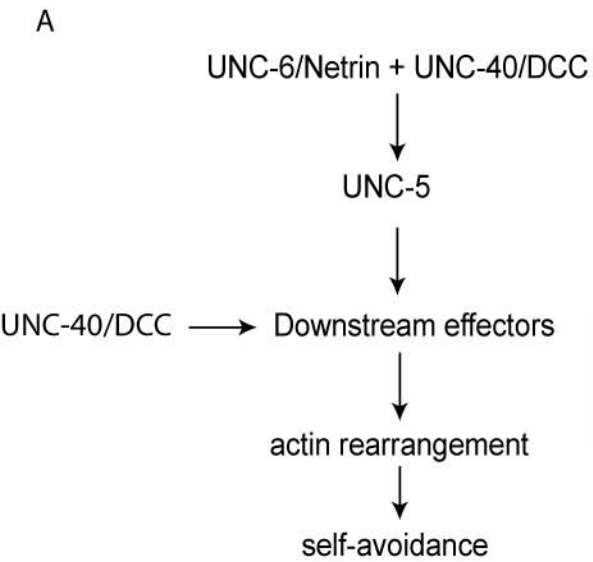


Supplemental figure 8. Expression of the UNC-6::UNC-40 chimeric protein in ventral neurons does not rescue the Unc-6 PVD self-avoidance defect.

Expression of ventral::UNC-6::UNC-40 in *unc-6 (ev400)* does not restore self-avoidance (*unc-6* vs ventral::UNC-6::UNC-40) whereas expression of a secreted form of UNC-6 in ventral neurons (ventral::UNC-6) or membrane-tethered UNC-6 in PVD (PVD:UNC-6::UNC-40) does rescue the Unc-6 self-avoidance defect. We note that expression of UNC-6::UNC-40 in ventral neurons enhances the PVD self avoidance defect of *unc-6(ev400)*; the mechanism of this effect is unclear. For histogram, genetic backgrounds are wild type (wt) (light grey box) or *unc-6(ev400)* (dark grey boxes).



Supplemental figure 9. Model: UNC-40/DCC captures UNC-6/Netrin at the tips of growing dendrites to mediate UNC-5-dependent mutual repulsion. (a) UNC-6/Netrin functions with UNC-40 and UNC-5 through downstream effectors to reorganize the actin cytoskeleton for self-avoidance. UNC-40 also signals through an UNC-6/Netrin-independent pathway **(b)** Schematic showing distribution of UNC-6/Netrin expressed from ventral cells and focal UNC-6/Netrin localization to PVD dendritic branches. **(c)** Inset depicts the tips of adjacent sister dendrites where UNC-40/DCC captures UNC-6/Netrin for contact with UNC-5 and mutual repulsion.



Supplemental movie 1. Wildtype self-avoidance. Time-lapse confocal movie of PVD::GFP in wild-type background. 3^o dendrites contact but quickly retract (arrows). Note the intervening distance between 3^o dendrites at the end of the movie is comparable to distance visualized in mature PVD neurons. Arrows indicate location of contact-dependent self-avoidance.

Supplemental movie 2. Self-avoidance defect in *unc-40* (e271). Time-lapse confocal movie of PVD::GFP in *unc-40* (e271). 3^o dendrites grow toward each other but upon contact fail to retract. Arrow indicates location of failed self-avoidance.

Supplemental movie 3. Self-avoidance defect in *unc-5* (e152). Imaging of *unc-5* (e152) shows PVD dendrites fail to retract after contact. Arrow indicates location of failed self-avoidance.