UNC-129 regulates the balance between UNC-40 dependent and independent UNC-5 signaling pathways
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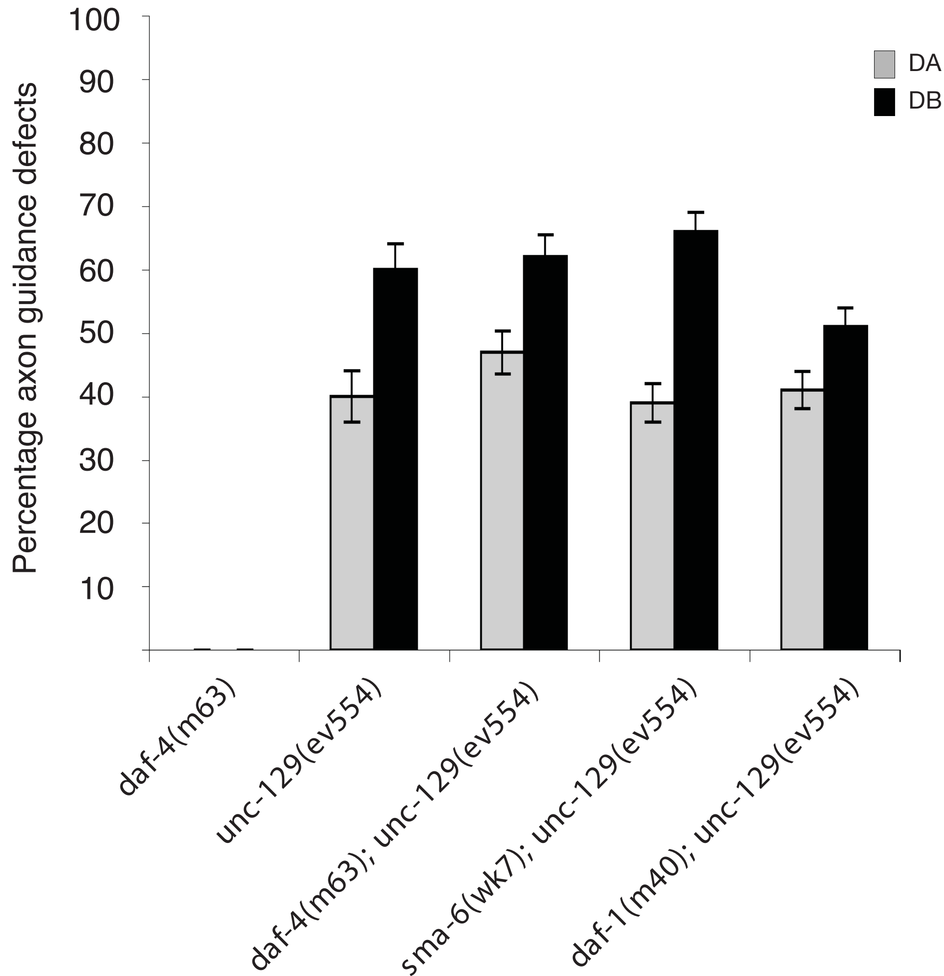
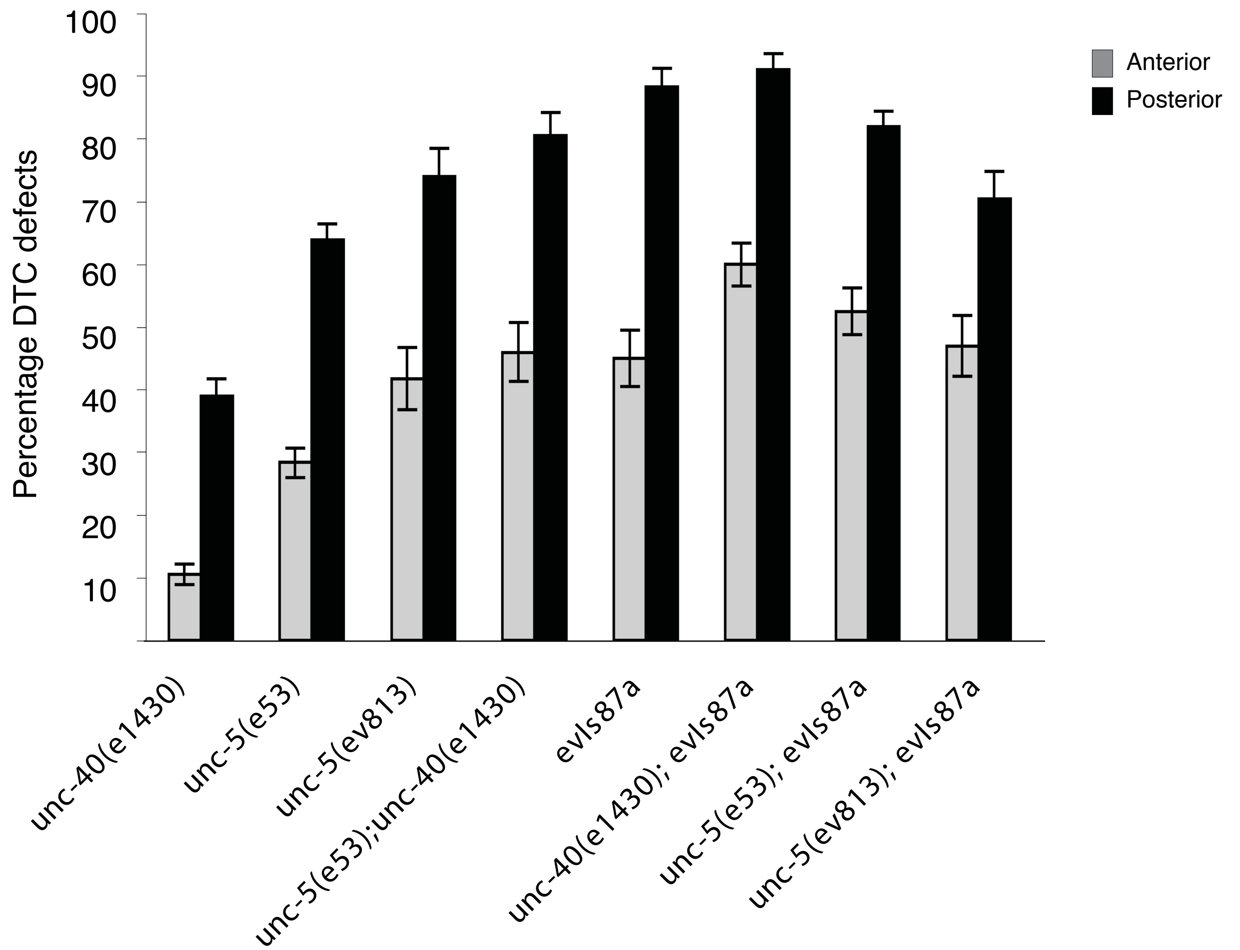
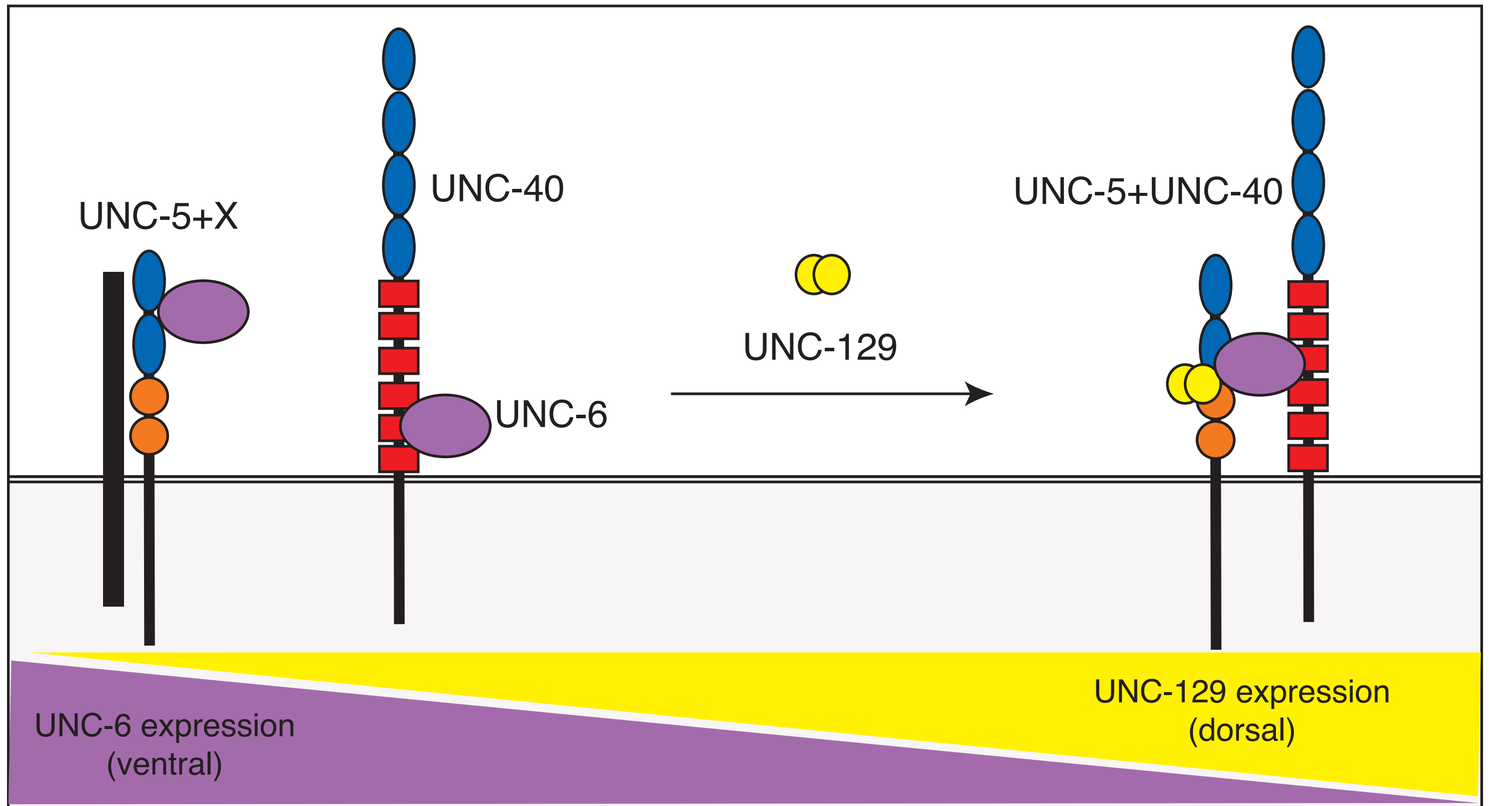


Supplementary Fig. S1. UNC-129 functions independent of the canonicalTGF-β signaling pathway. *evIs82b [unc-129::GFP]* was used to score DA and DB motor-axons in the indicated genetic backgrounds. The percentage of misguided DA (grey) and DB (black) axons of the indicated genetic backgrounds are plotted as the mean plus and minus the standard error. n>100 animals for each genotype scored.



Supplementary Fig. S2. Quantification of phase two DTC defects in *evIs87a [myo3::unc-129]* animals. The percentage of misguided anterior (grey) and posterior (black) DTCs of the indicated genetic backgrounds are plotted as the mean plus and minus the standard error. The difference between *evIs87a* and *unc-40(e1430); evIs87a* for anterior DTCs is statistically significant with a p-value of 0.0004 by the Chi-square statistic.



Supplementary Figure S3. Model for UNC-129 changing UNC-6 set point sensitivity.
As a growth cone is repelled by UNC-6 and moves down (left to right) the UNC-6 gradient (violet) it simultaneously moves up the UNC-129 gradient (yellow). UNC-129 resets sensitivity to UNC-6/netrin, as levels of UNC-6/netrin decrease and levels of UNC-129 increase, UNC-129 increases sensitivity to UNC-6 by switching receptor complexes from less sensitive UNC-40 independent UNC-5 signaling to more sensitive 'UNC-5+UNC-40' signaling.

Culotti Figure 6

Supplementary Methods

Gaussia Luciferase binding assay:

In order to express a Gaussia-luciferase tagged UNC-129 (Gluc-UNC-129), we used the human activin pro-domain to ensure proper processing of the ligand. Downstream of the RXXR cleavage site in activin, we fused Gaussia luciferase in-frame followed by the mature UNC-129 ligand. Luciferase tagged ligands were produced in 293T cells. Cells were transfected with luciferase encoding constructs, media was changed into 0.2% serum 12 hours after transfection and collected 24 hours later. Media was concentrated 10-fold using Amicon protein concentrators (5K cutoff) (Millipore). 293T cells were transiently transfected with UNC-5HA, TGF-βRIHA or a control plasmid. Triplicate samples were set up for each condition. 48 hours after transfection, cells were incubated for 4 hours at 4° C with prepared ligands. Following incubation, cells were washed twice in PBS containing 0.2% BSA and twice in PBS alone. Cells were lysed in TNTE buffer containing 0.5% Triton-X and immunoprecipitations were performed using 12CA5 anti-HA. Immunoprecipitations were washed twice in lysis buffer, 5 times in TNTE containing 0.1% Triton-X and twice in PBS to remove detergent. Immunoprecipitations were then quantitated for luciferase activity and receptor expression was verified by western blot.