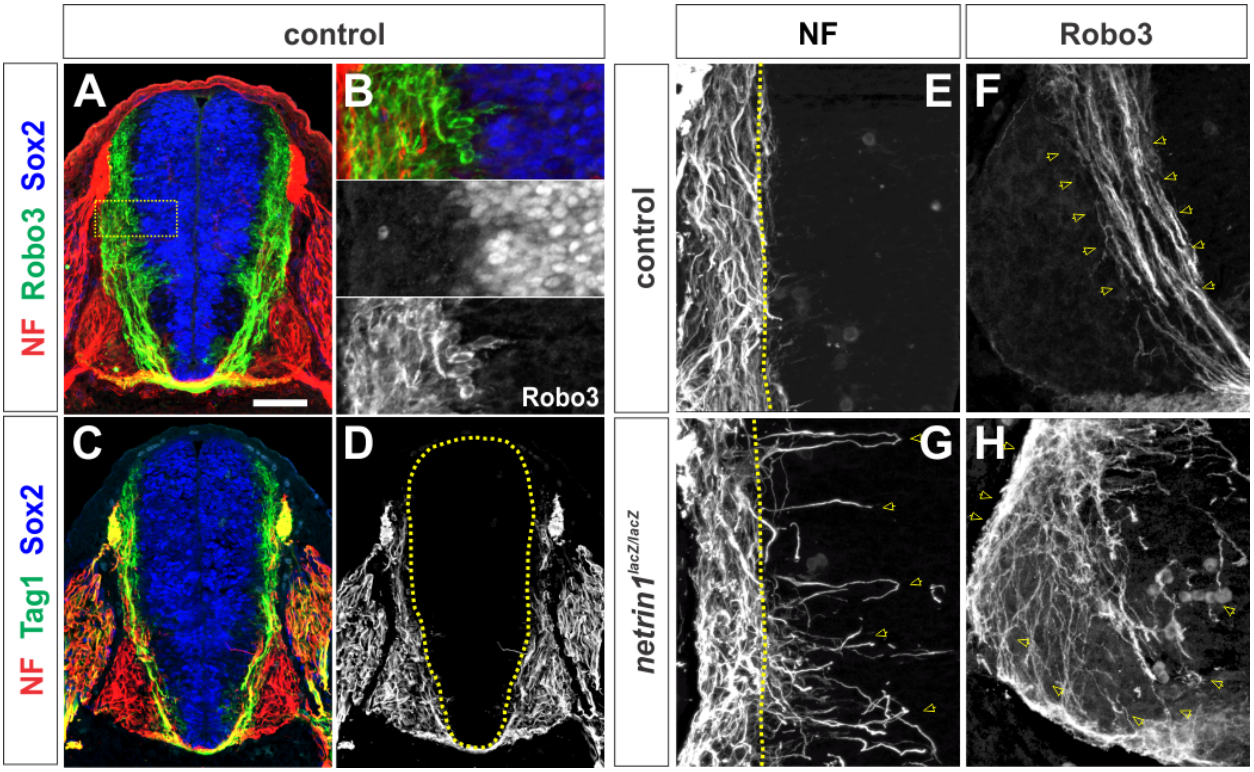
Figure S1 (related to **Figure 1** and **2):** Netrin1 is required for spinal axon orientation and fasciculation



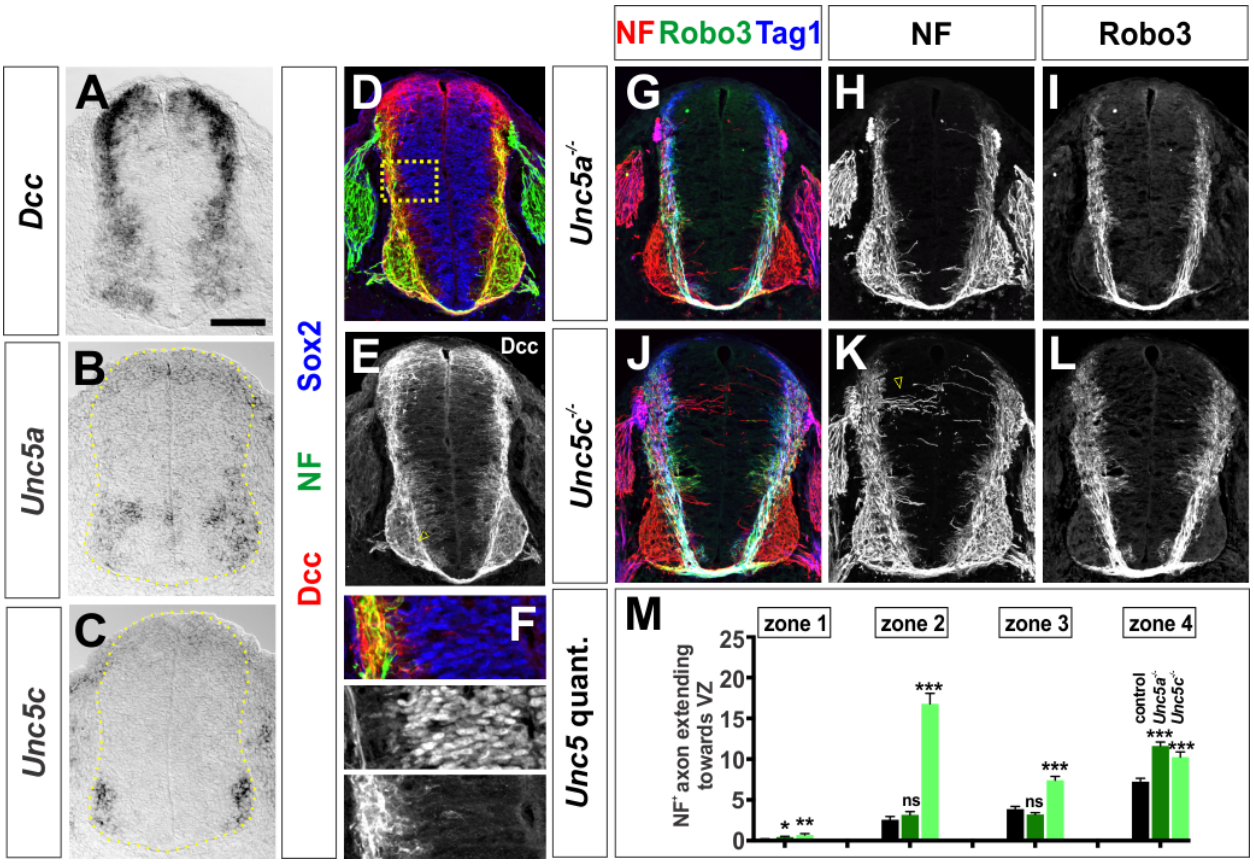
(A-D) All spinal axons, including NF^+ axons and the $Robo3^+$ and $Tag1^+$ commissural axons avoid the $Sox2^+$ VZ in E11.5 spinal cords (D). High magnification images (inset in A is shown in B) show that there is a precise, inverse relationship between all spinal axons and $Sox2^+$ neural progenitors (B).

(E, F) NF^+ axons grow just adjacent to the VZ in control spinal cords (yellow dotted line, E). $Robo3^+$ commissural axons project in a tightly fasciculated bundle, between the motor column and the VZ (arrows, F) as they extend towards the ventral midline.

(G, H) In contrast, axons profusely extend into the VZ in *netrin1*$^{lacZ/lacZ}$ mutant, while $Robo3^+$ are completely defasciculated in the ventral spinal cord, extending over the motor column (arrows, H).

Scale bar: 100 μm

Figure S2 (related to **Figure 4**): Axons are only mildly perturbed in Unc5 mutants



(A-L Transverse sections of E11.5 control (A-F), *Unc5a*$^{-/-}$ (G-I) and *Unc5c*$^{-/-}$ (J-L) mouse spinal cords mouse spinal cord.

(A) *Dcc* is expressed at high levels in many post-mitotic neurons in the dorsal spinal cord and at lower levels in the ventral spinal cord, including the motor columns, at all levels of the spinal cord.

(B) *Unc5a* is expressed transiently in the dorsal VZ and then subsequently in the motor columns.

(C) *Unc5c* is present both in the motor columns and in the DRGs at later stages.

(D-F) Dcc protein is present in at high levels in a broad swathe of commissural axons extending to the FP, and at low levels in the motor columns (E). It is not present in the Sox2^{+} VZ (F).

D-F, K-N), labeled with antibodies against NF (red), Robo3 (green) and Tag1 (blue).

(G-I) In the absence of *Unc5a*, there is a modest increase in the numbers of NF^{+} axons projecting into zone 4 (H), however Robo3^{+} commissural axons extend in an analogous manner to control littermates (I).

(J-L) Similarly a few NF^+ axons extend into zone 3 and 4 in *Unc5c* mutants (K), while the the $Robo3^+$ commissural axon trajectory is undistinguishable from controls (L). There is more robust *Unc5c*$^{-/-}$ axon growth into zone 2; however, these latter projections are NF^+ $Tag1^+$ $Robo3^-$ (arrows, K). This profile suggests that they not spinal axons, rather they are DRG axons precociously innervating the spinal cord as previously described (Masuda et al., 2008).
(M) The trajectories of NF^+ spinal axons in *Unc5* mutants are considerably less perturbed that those in *Dcc* mutants (Figure 4P). Control: n=36 sections from 5 embryos, *Unc5a*$^{-/-}$: n=67 sections from 7 embryos and *Unc5c*$^{-/-}$: n=57 sections from 4 embryos.

Movie S1 (related to **Figure 3**)

Transverse sections of E11.5 thoracic mouse spinal cord processed for netrin1 (red), nestin (green) and laminin (blue) staining. A series of Imaris renderings permits the visualization of all netrin1 labeling, following by the netrin1 specifically associated with nestin and then laminin.

Movie S2 and S3 (related to **Figure 4**)

Transverse sections of E11.5 thoracic control (movie 2) and *Dcc* mutant (movie 3) mouse spinal cord processed for netrin1 (red) and NF (green). Imaris rendering permits the visualization of netrin1 labeling specifically associated with NF^+ axons.