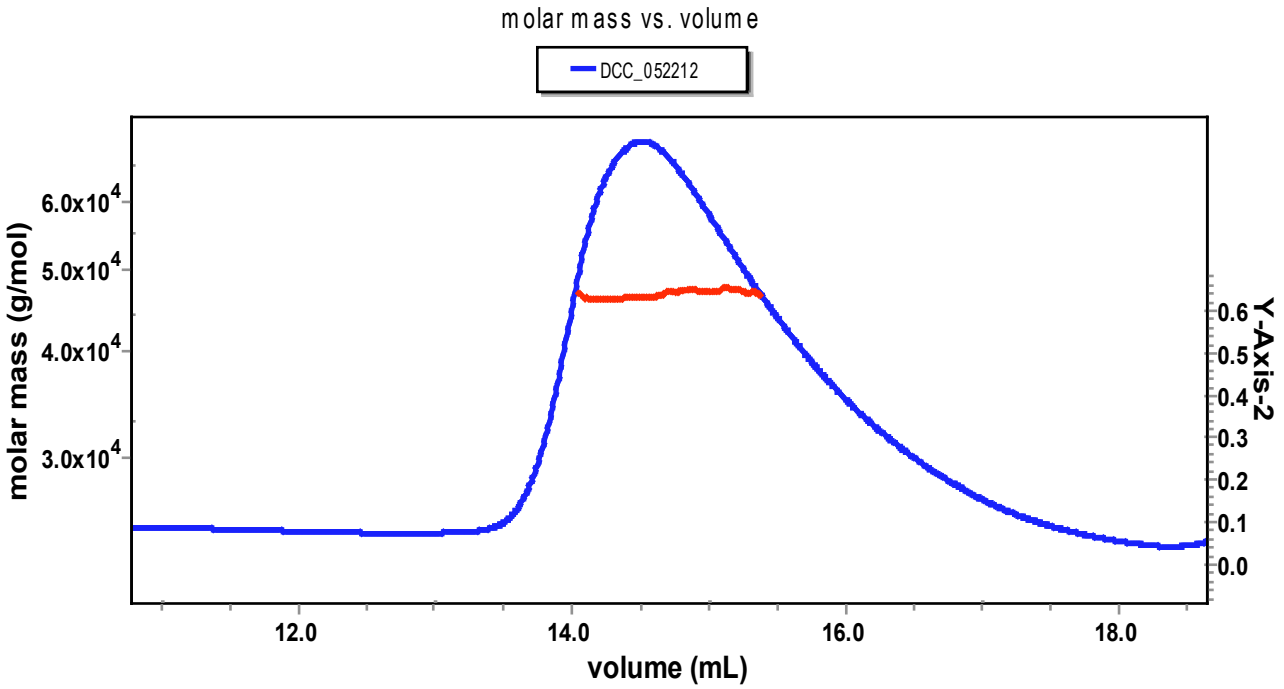

Fig. S1. Combined Size Exclusion Chromatography/ Multi Angle Light Scattering analysis to measure the molecular weight of DCC N-terminal 4 Ig domains. The *M*r was determined to be 47.52×10^3, with 1% uncertainty (10 mg/ml, 200 µl DCC sample). The theoretic *M*r of this DCC recombinant protein is 44.1×10^3, and there are four glycosylation sites. So the DCC N-terminal 4 Ig fragment exists as a monomer in solution.

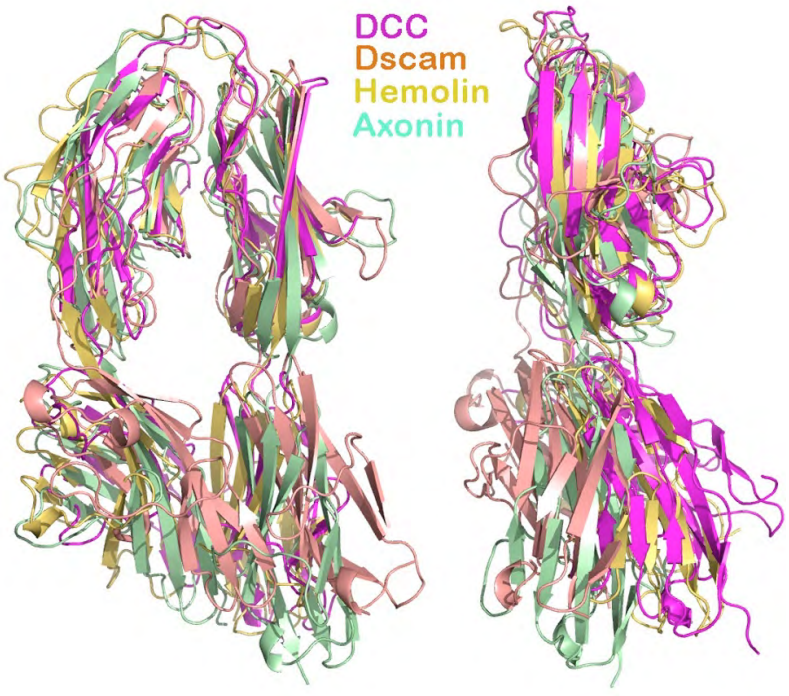

Fig. S2. Overlay of four horseshoe structures. The two views are related roughly by 90° around a vertical axis.

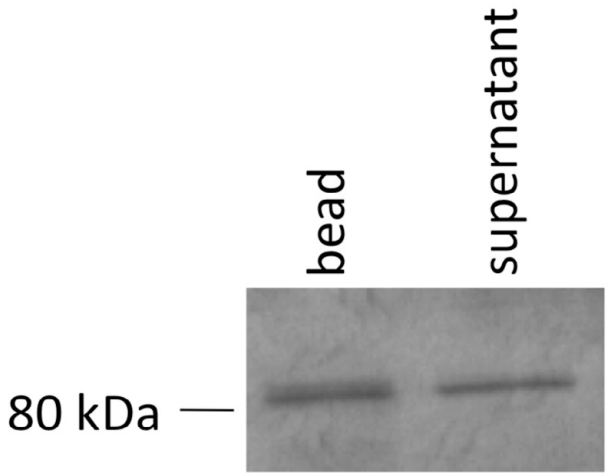

Fig. S3. Western blot to show netrin released from the beads.

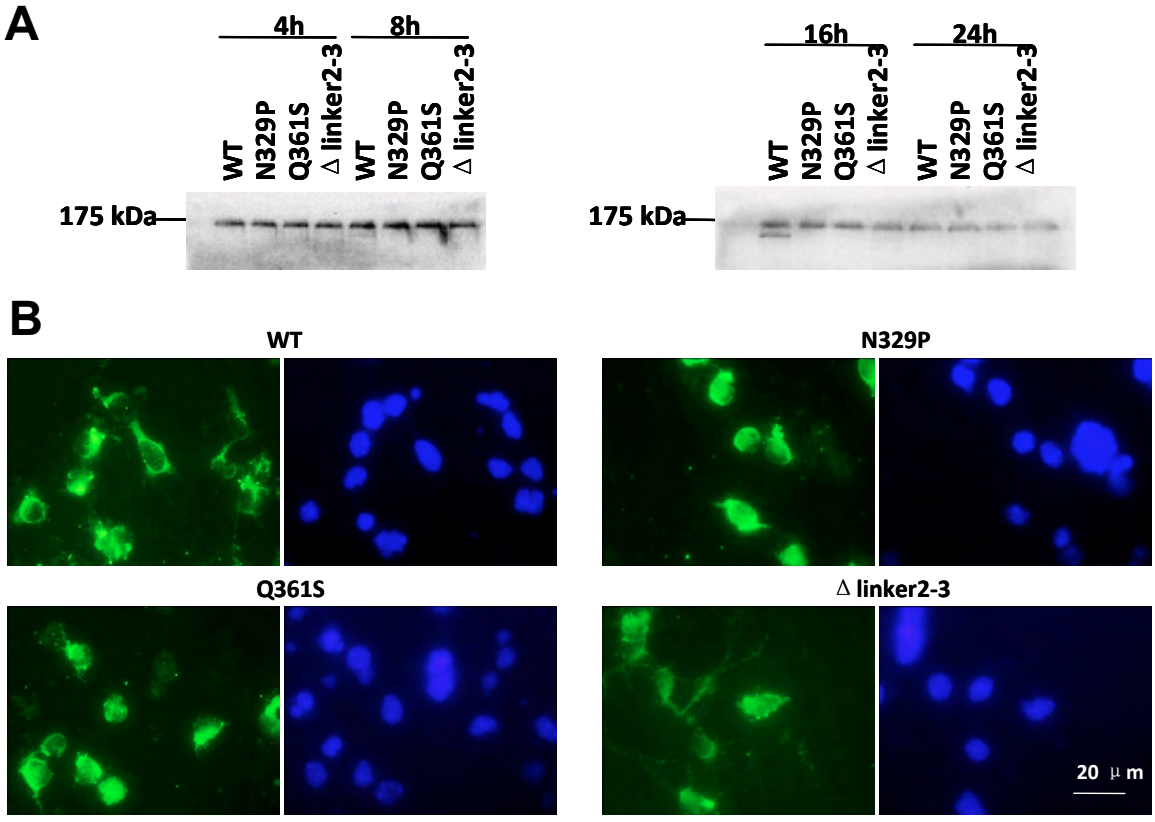

Fig. S4. Stable expression of wild type (WT) DCC, DCC-N329P, DCC-Q361S and DCC-Δlinker2-3. **A)** Immunoprecipitation of biotinylated cell surface DCC showed that at 4, 8, 16 and 24 hours, similar levels of wild type (WT) DCC, DCC-N329P, DCC-Q361S and DCC-Δlinker2-3. **B)** COS-7 cells were transfected with WT DCC, DCC-N329P, DCC-Q361S and DCC-Δlinker2-3 constructs, respectively. DCC immunostaining with DCC antibody showed that at 24 hours after transfection, all constructs were expressed (green fluorescent) at roughly similar levels at cell surface. DAPI (blue fluorescent) staining showed the cell population. Scale bar: 20 μm.

Table S1. Genome-wide search for potential horseshoe-containing proteins

Species	*H. sapiens*	*D. melanogaster*	*C. elegans*
Genes	23,000	18,424	13,601
IgSF	778	153	70
4 Ig-like	105	42	25
Horseshoe	23	6	3-5
Neural Receptor	22	4	3