Colombelli et al., http://www.jcb.org/cgi/content/full/jcb.201403111/DC1

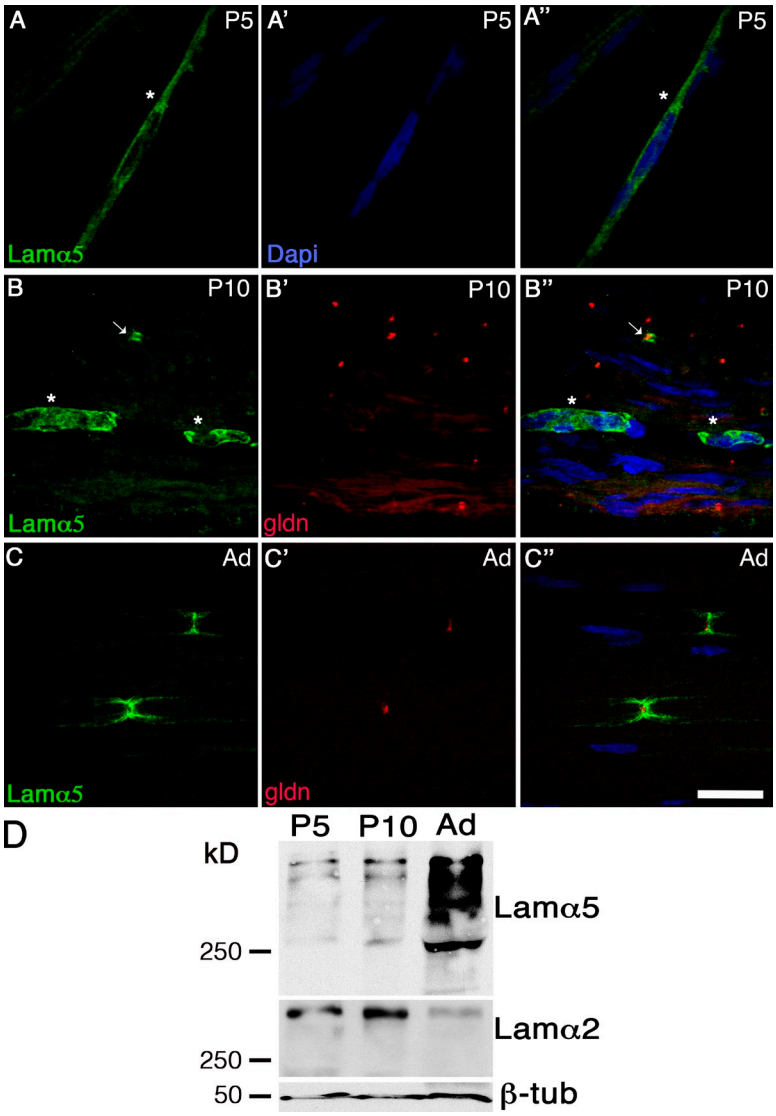

Figure S1. **Laminin 511 accumulates to nodes after they are formed.** Teased fibers from sciatic nerves of P5, P10, and adult (Ad) rats stained for laminin α5 (green; A–C), gliomedin (red; B′–C′), and DAPI (blue) show that at P5 SCs contain perinuclear laminin α5 (asterisk), which accumulates to nodes after P10 (arrow). Bar, 17.5 µm. (D) Western blot of sciatic nerve lysates for laminin α5 confirms late expression of laminin 511. Multiple α5-laminin bands at the expected size of 450, 380, 320, and 210 kD are detected (Miner et al., 1997). For comparison, the same blot was incubated with anti–laminin α2 chain antibody.

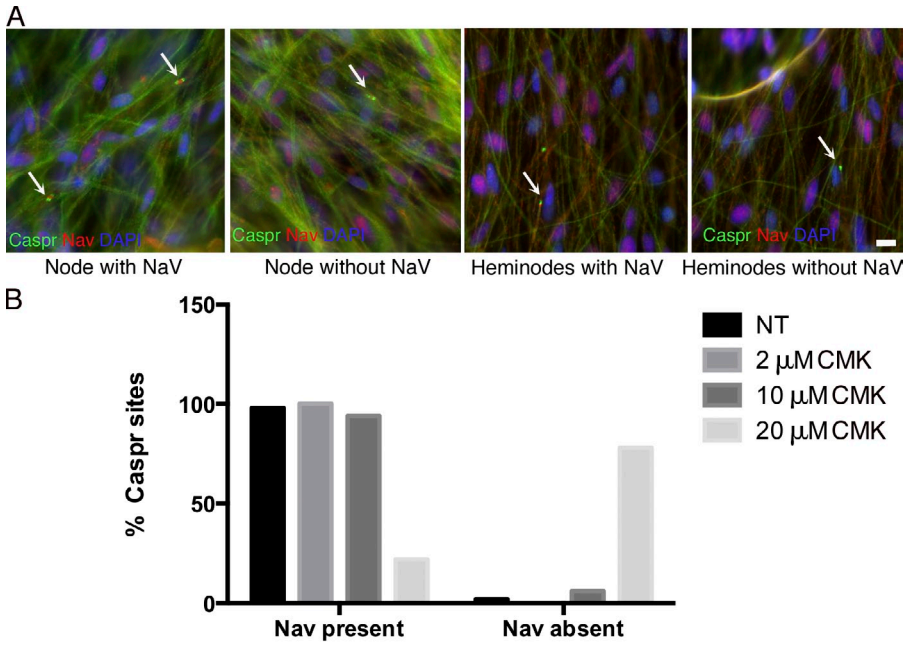

Figure S2. **Furin inhibition reduces clustering of Nav channels in vitro.** (A) Myelinating DRG-SC co-coltures were treated with CMK and stained for Caspr (green), Nav (red), and DAPI (blue). Nodes and heminodes are indicated with arrows. (B) The percentage of paranodes (marked with Caspr) flanked by Nav channels was quantified. 20 μM CMK reduced myelination (not depicted) and the percentage of paranodes flanked by Nav channels ($P < 0.0001$ by χ^2 test). Data from a representative experiment out of two repeats. For the experiment shown, $n = 333$ nodes. Bar, 10 μm.

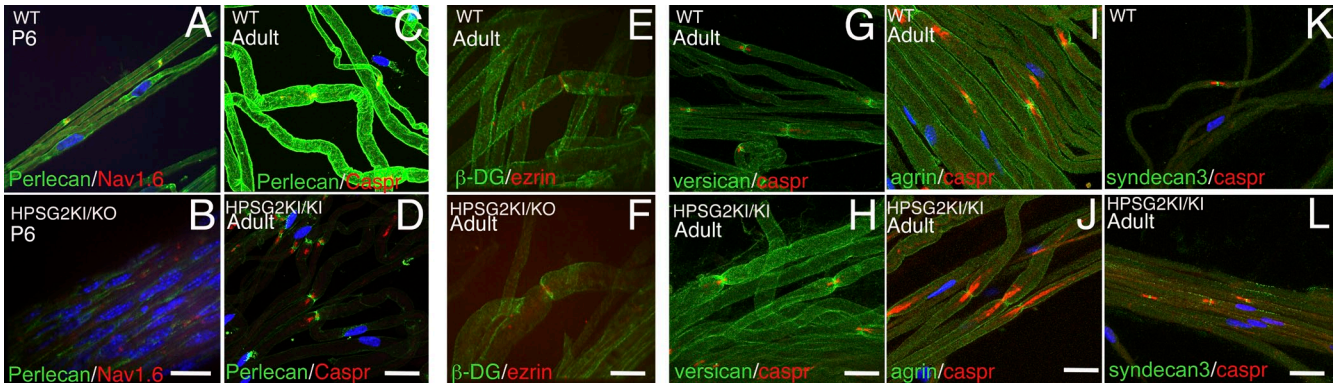

Figure S3. **The expression of other proteoglycans is mantained in perlecan mutants.** Teased fibers from P6 (A and B) or adult (C–L) sciatic nerves from wild-type (A, C, E, G, I, and K), *Hspg2*$^{KI/KO}$ (B and D), or *Hspg2*$^{KI/KI}$ (F, H, J, and L) mice. Staining for perlecan or the indicated proteoglycans at nodes (green), counterstained with nodal and paranodal markers (red), shows that perlecan is absent at nodes in *Hspg2*$^{KI/KO}$ mutants (B) and decreased in *Hspg2*$^{KI/KI}$ mutants (D). The green signal in C and D was increased to show residual perlecan in D. Other proteoglycans at nodes are maintained in *Hspg2* mutants. Fibers are counterstained with DAPI (blue). Bars, 25 μm.

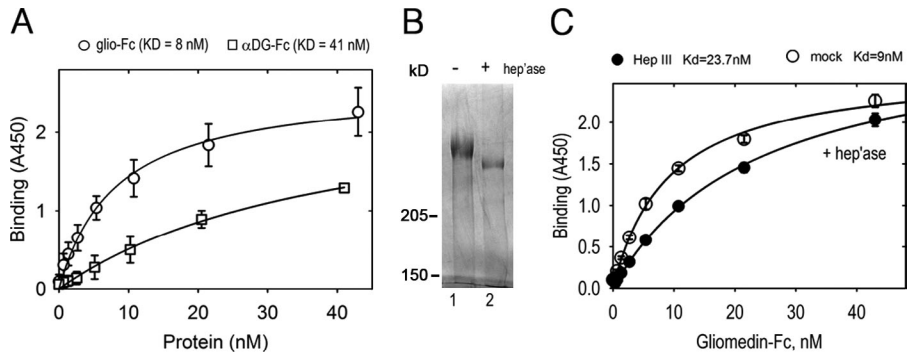

Figure S4. **Gliomedin binds with high affinity to perlecan HS chain and core protein.** (A) Binding of gliomedin-Fc or DG-Fc5 to perlecan by solid-phase assay shows apparent dissociation constants of 8 and 41 nM, respectively. Mean and SD of three data points for different protein concentrations are shown for $n = 3$ experiments. (B) Heparitinase treatment of perlecan shows a shift in the mobility consistent with removal of HS chains. (C) Solid-phase assay shows that binding is preserved after heparinase treatment of perlecan, even if the affinity is decreased. Mean and SD of three data points for all different protein concentrations are shown.

Reference

Miner, J.H., B.L. Patton, S.I. Lentz, D.J. Gilbert, W.D. Snider, N.A. Jenkins, N.G. Copeland, and J.R. Sanes. 1997. The laminin α chains: expression, developmental transitions, and chromosomal locations of α1-5, identification of heterotrimeric laminins 8–11, and cloning of a novel α3 isoform. *J. Cell Biol.* 137:685–701. http://dx.doi.org/10.1083/jcb.137.3.685