Heller et al., http://www.jcb.org/cgi/content/full/jcb.201307057/DC1

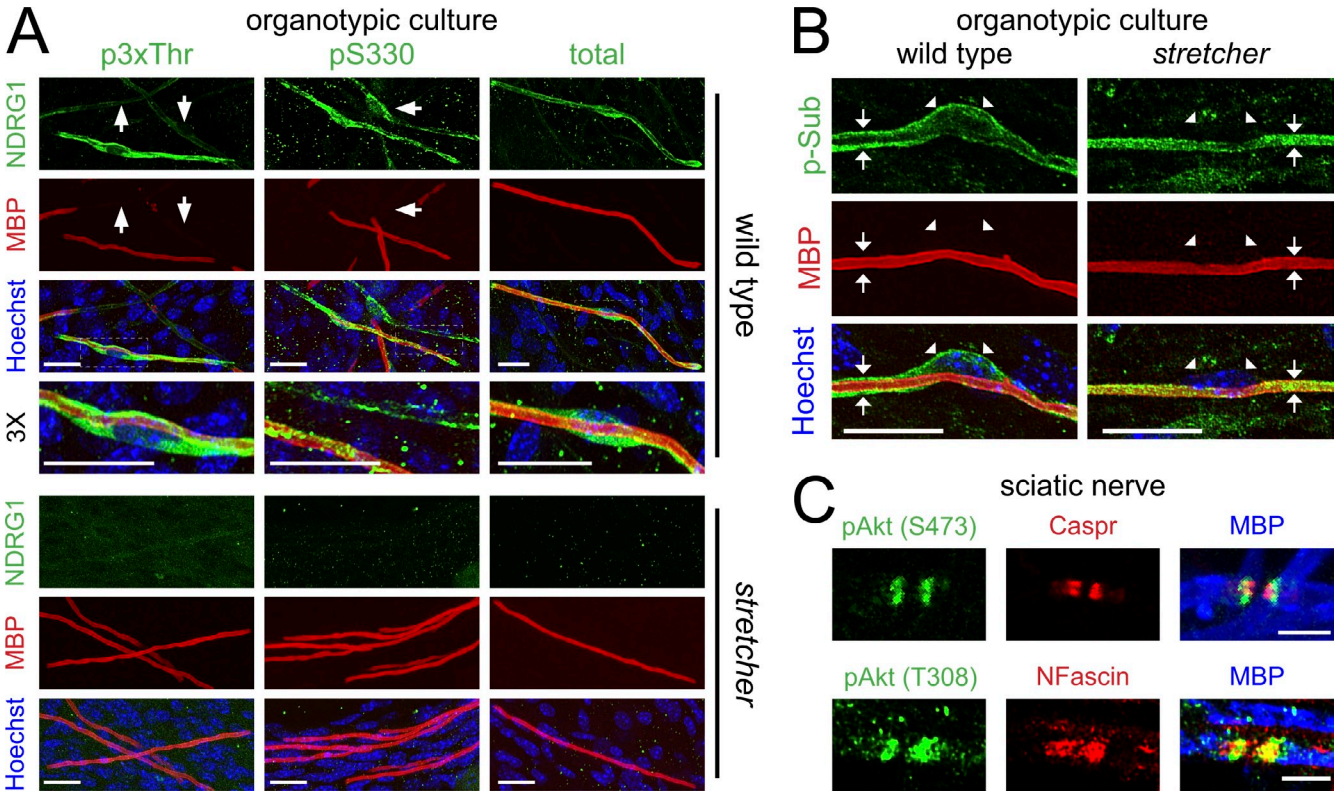


Figure S1. **Subcellular localization of phospho-substrates.** (A) Organotypic cultures obtained from wild-type (top) or *stretcher* (bottom) littermate embryos were stained for NDRG1 (green), MBP (red), and Hoechst (blue). Total and p-NDRG1 are present in all myelinated and some premyelinating Schwann cells (arrowheads). NDRG1 staining is completely abolished in the *stretcher* cultures, underscoring specificity of the staining. Bar, 20 µm. (B) Organotypic cultures obtained from wild-type (left) or *stretcher* (right) littermate embryos were stained with p-Sub (green), MBP (red), and Hoechst (blue). Abaxonal staining (arrows) is significantly reduced in the *stretcher* mutant and completely absent around the soma (arrowheads) of myelinating Schwann cells. NDRG1 is therefore a major but not exclusive phospho-substrate detected by the p-Sub antibody in myelinating Schwann cells. Bar, 10 µm. (C) P16 sciatic nerve stained for p-Akt (S473, green), Caspr (red), and MBP (blue) shows localized p-Akt activity in the glial paranode. The p-Akt surrounds the Caspr stain and appears in a region slightly distal to the node of Ranvier. P9 sciatic nerve (bottom) stained for p-Akt T308 exhibits a similar paranodal localization using a pan-Neurofascin antibody (red) as a marker for the glial paranode and axonal node. Bar, 5 µm.

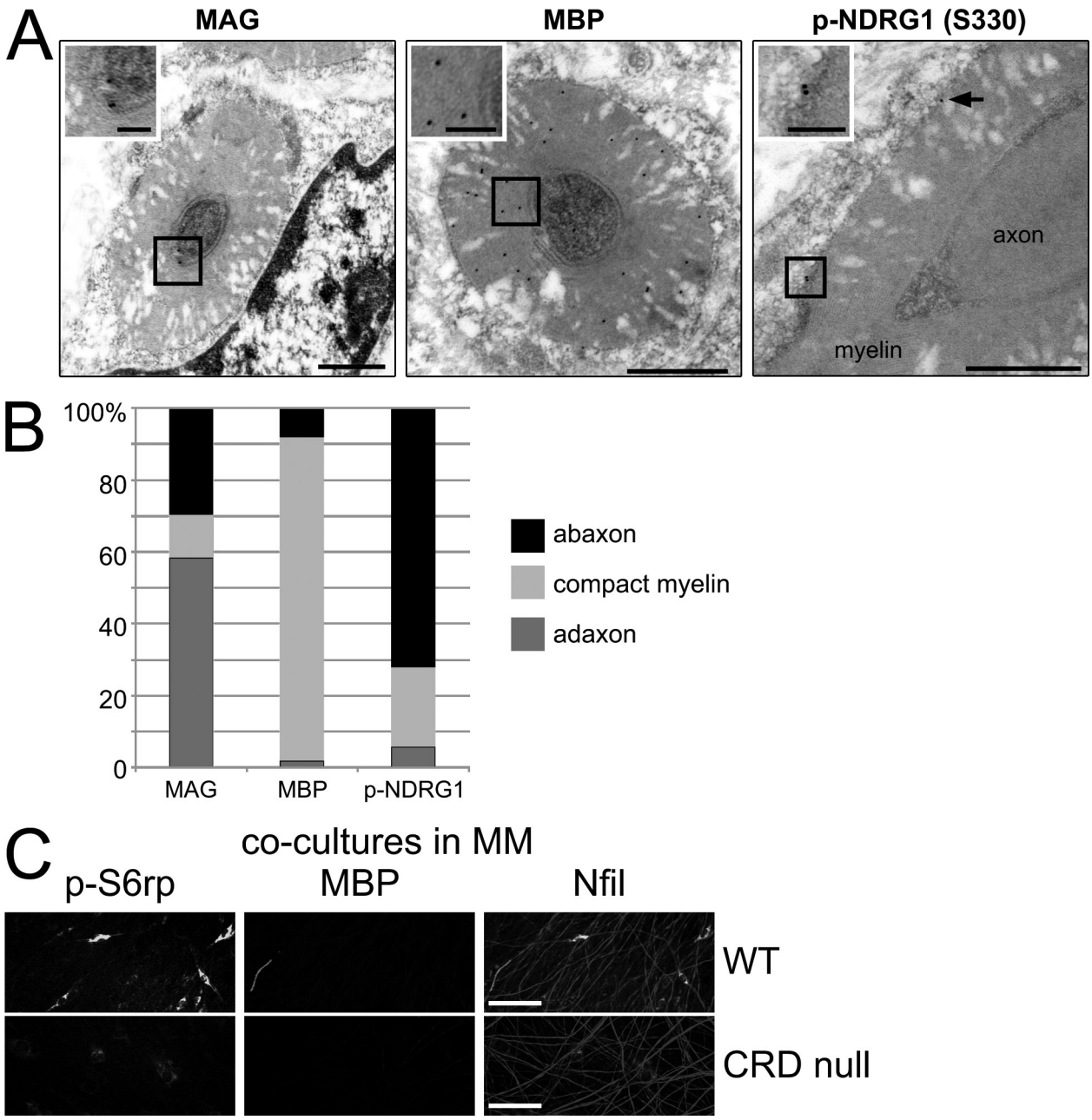


Figure S2. **p-NDRG1 is localized to the adaxon; p-S6rp staining is Neuregulin1 dependent.** (A) Localization of phospho-NDRG1 in myelinated co-cultures by immunoelectron microscopy is shown; primary antibodies were detected with gold-conjugated anti-IgG. Phosphorylated NDRG1 (S330) is enriched in the abaxon (inset and arrow). Myelin-associated glycoprotein (MAG) and MBP are enriched in the adaxon and compact myelin, respectively. Bar (main panels) 500 nm; (insets) 125 nm. (B) Percentages of gold particles in the abaxon, compact myelin, and adaxon for MAG ($n$ = 206 particles), MBP ($n$ = 87 gold particles), and p-NDRG1 S330 ($n$ = 217 particles) are shown from a single experiment. (C) Cultures of wild-type (WT) and Neuregulin1 type III–null (CRD null) mouse sensory neurons seeded with wild-type rat Schwann cells were maintained under myelinating conditions and stained for p-S6rp (green), MBP (red), and Neurofilament (Nfil, blue). Phosphorylated S6rp is only detectable in wild-type cultures. The myelin segment on the far left of the WT field does not stain for p-S6rp. Bar, 50 µm.

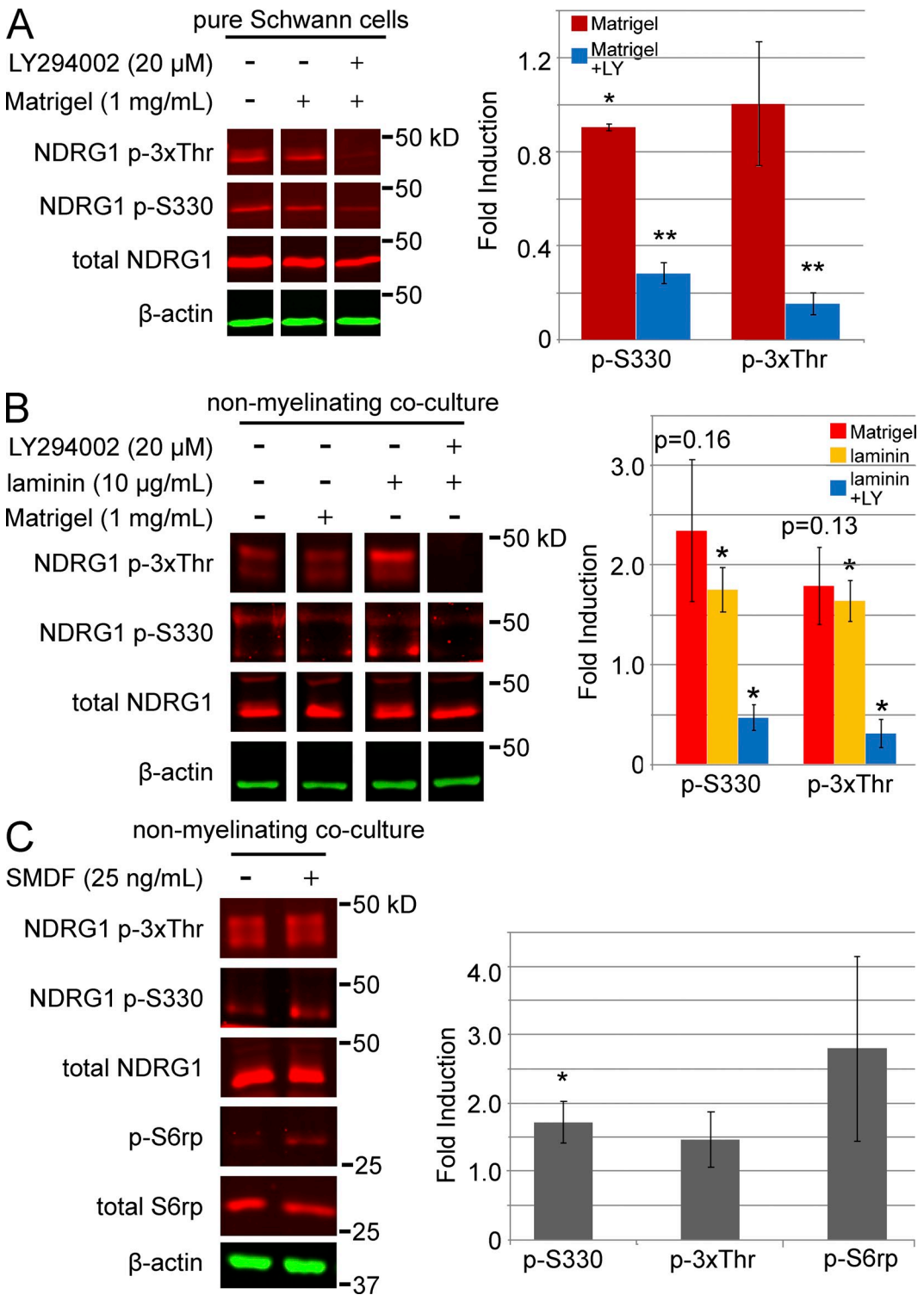


Figure S3. **Axon contact is necessary for laminin-induced NDRG1 phosphorylation.** (A) Western blotting of pure Schwann cell cultures serum starved and treated with 1 mg/ml Matrigel (for 30 min) does not induce NDRG1 phosphorylation. A small decrease in p-S330 is observed in induced conditions. 20 μM LY-294002 lowers the levels of NDRG1 phosphorylation well below baseline. p-NDRG1 is normalized to total NDRG1 for quantifications. Mean ± SEM; *, $P < 0.05$; **, $P < 0.01$ by two-tailed Student's *t* test. (B) Western blotting of nonmyelinating co-cultures treated with 10 μg/ml laminin (for 30 min) or 1 mg/ml Matrigel (for 30 min) in the presence or absence of 20 μM LY294002. NDRG1 phosphorylation is induced by laminin; this induction is blocked by the LY inhibitor. p-NDRG1 is normalized to total NDRG1 for quantifications. Mean ± SEM; *, $P < 0.05$; **, $P < 0.01$ by two-tailed Student's *t* test ($n = 4$). (C) Western blotting of nonmyelinating co-cultures treated with soluble Neuregulin1 type III, or SMDF (25 ng/ml, 30 min). Both NDRG1 and S6rp phosphorylation are induced by Neuregulin1. Phosphorylated protein is normalized to total protein for quantifications. Mean ± SEM; *, $P < 0.05$ by two-tailed Student's *t* test ($n = 4$).

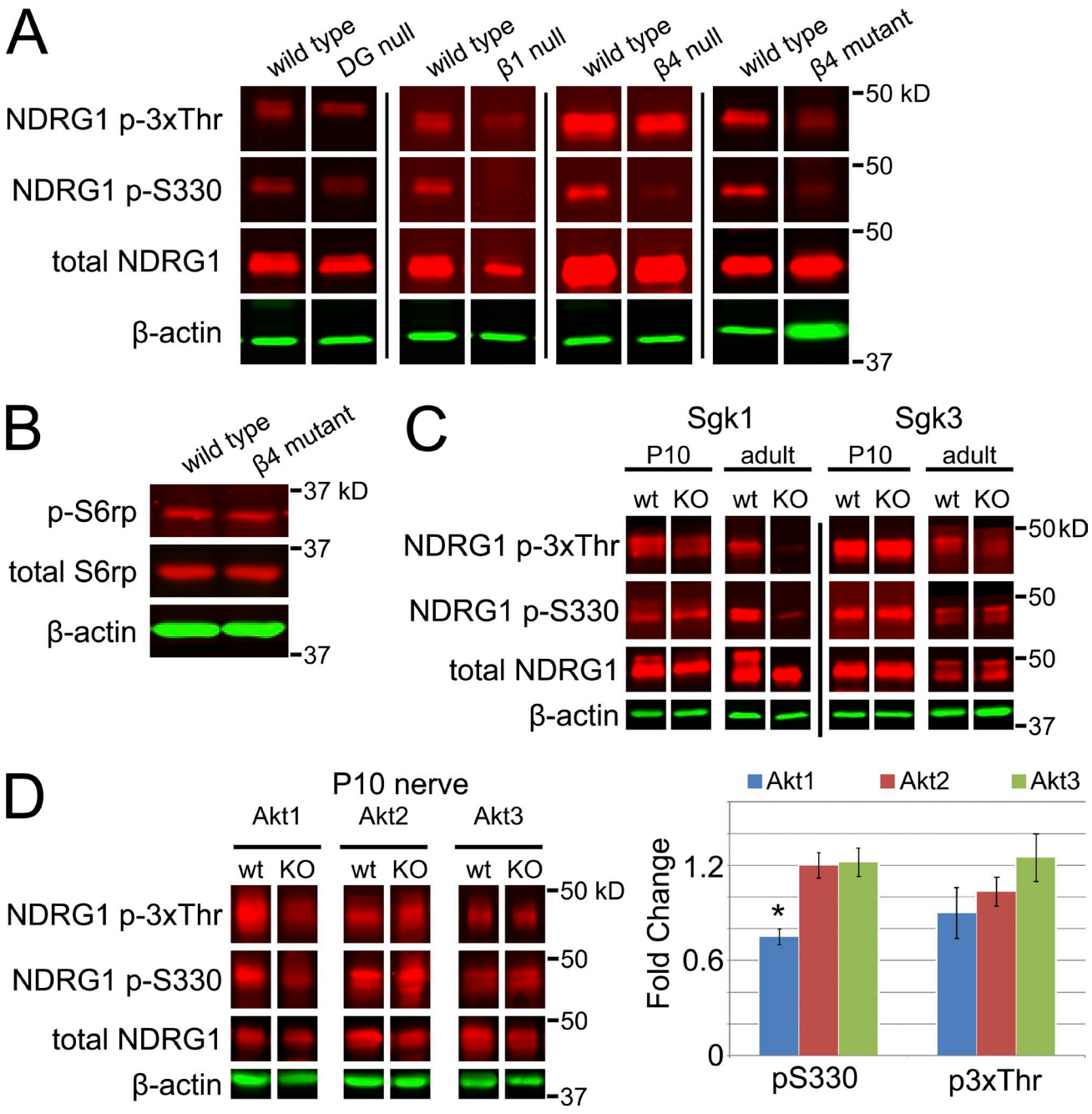


Figure S4. **Akt1, Sgk1, and β4 integrin are required for proper NDRG1 phosphorylation at P10.** (A) Western blotting data for Fig. 7 A. (B) Western blotting data for Fig. 7 B. (C) Western blotting data for Fig. 8 . (D) Western blots and quantifications of P10 sciatic nerve from isoform-specific knockouts of Akt1, Akt2, and Akt3. Akt1 loss affects p-Ser330 only; loss of other isoforms does not cause a defect in NDRG1 phosphorylation. p-NDRG1 is normalized to total NDRG1 for quantification. Mean ± SEM, $n \geq 3$; *, $P < 0.05$ by two-tailed Student's *t* test.

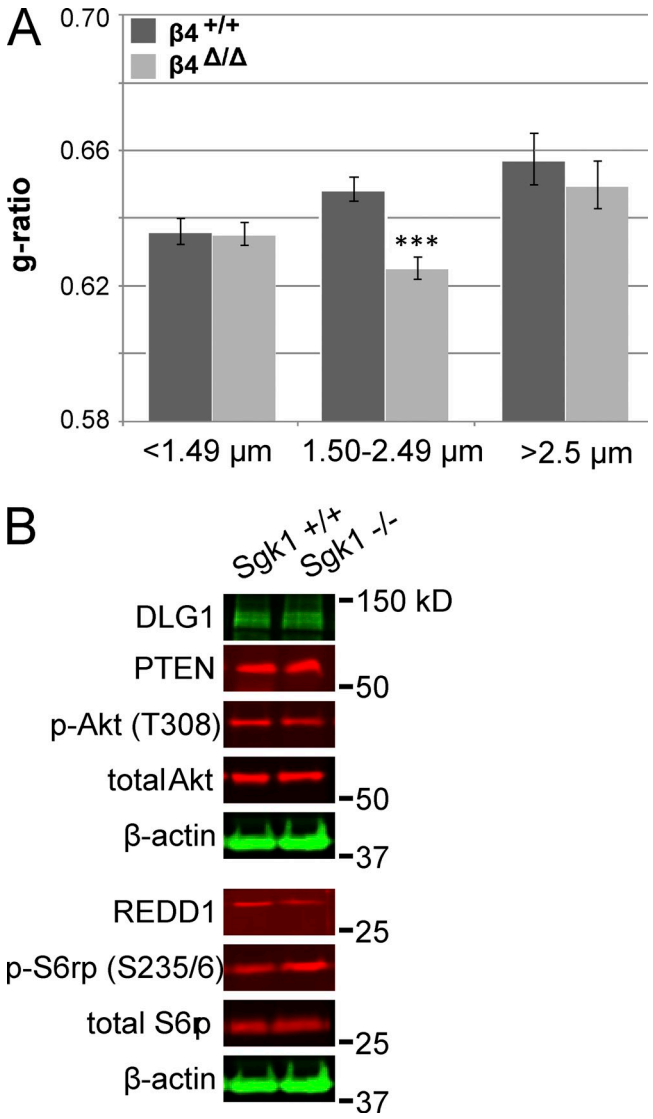


Figure S5. **Analysis of the β4-signaling mutant and Sgk1-null mice.** (A) Quantification of the g-ratios in P10 sciatic nerves from wild-type and β4 mutant mice shown in Fig. 9 D. The loss of β4 integrin signaling results in a modest hypermyelination, particularly for smaller-caliber axons. An entire cross section of proximal nerve was quantified from two wild-type (875 axons total) and two β4 mutant mice (892 axons total). Mean g-ratios are shown ± SEM; ***, $P < 0.001$ by ANOVA. (B) Lysates from P10 *Sgk1*$^{+/+}$ and *Sgk1*$^{-/-}$ nerves were analyzed for levels of PTEN, DLG1, total and p-Akt (T308), REDD1, and total and p-S6rp (S235/6). There is no change in PTEN, DLG, or Akt levels; however, REDD1 decreases and p-S6rp increases but the difference is not statistically significant; $P > 0.05$ ($n = 4$) by two-tailed Student's *t* test.