Supporting Information

Zhao and Ma 10.1073/pnas.0906880106

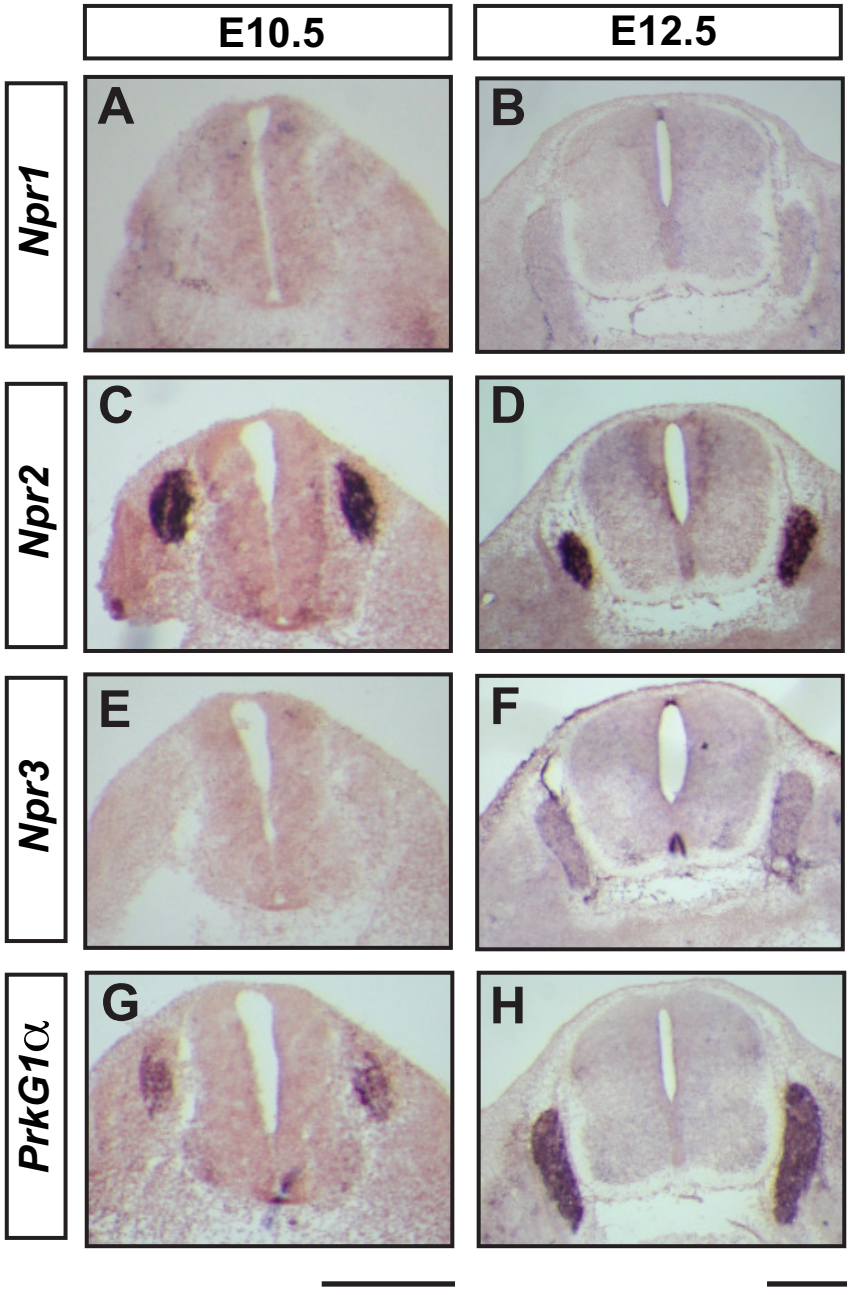


Fig. S1. Expression of Npr receptors in the developing spinal cord and DRG. RNA in situ hybridization by using digoxigenin-labeled anti-sense probes detects the transcripts of Npr receptors *Npr1* (*A* and *B*), *Npr2* (*C* and *D*), and *Npr3* (*E* and *F*) and *PrkG1* (*G* and *H*) in the spinal cord and the DRG from E10.5 (*A*, *C*, *E*, and *G*) and E12.5 (*B*, *D*, *F*, and *H*) mouse embryos. (Scale bar: 500 μm.)

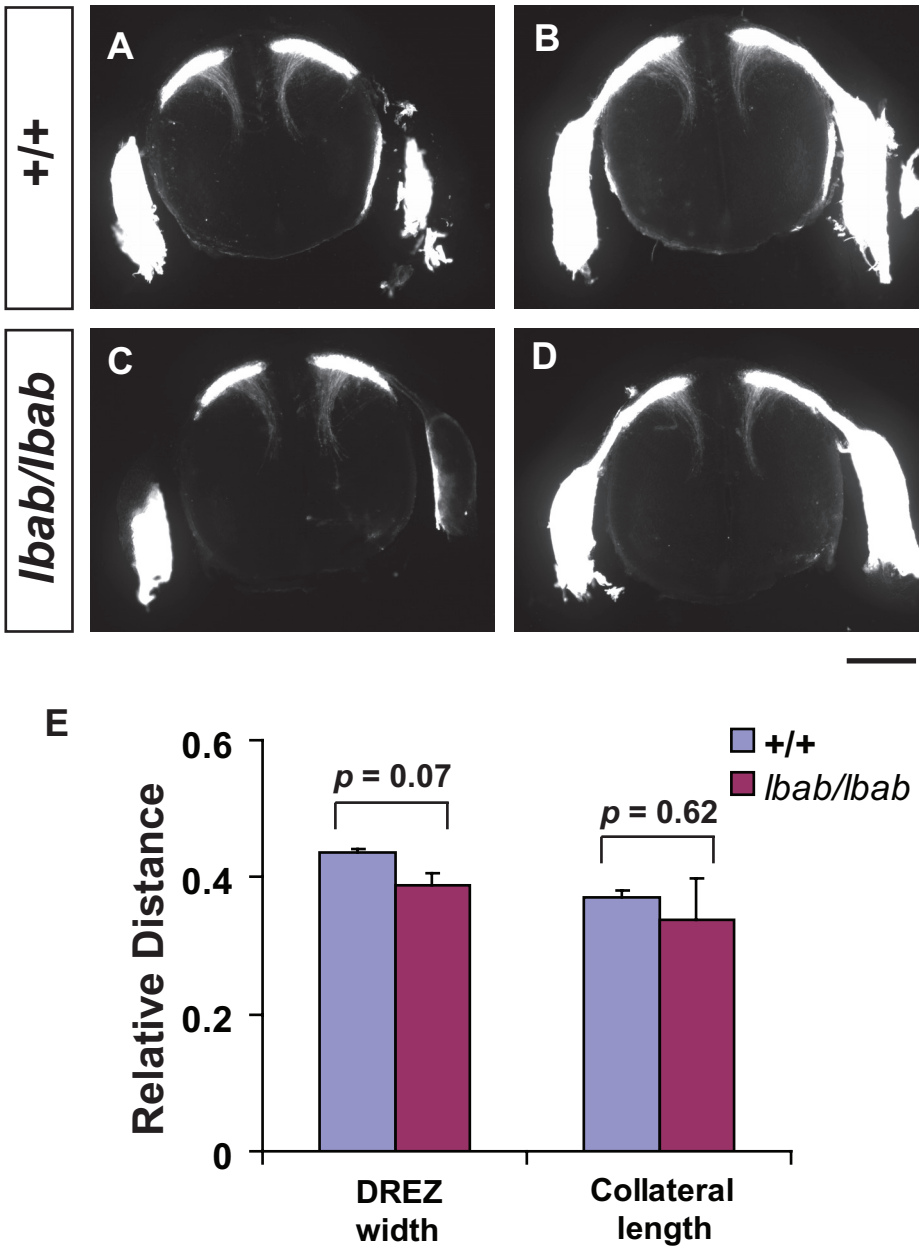


Fig. S2. Collaterals are formed normally in the *lbab* mutant spinal cord. Collaterals from Ia sensory neurons are visualized by DiI labeling in the cross sections of E13.5 spinal cords isolated from the wild-type (+/+, *A* and *B*) or the mutant (*lbab/lbab*, *C* and *D*) animals. Note the normal trajectory of the Ia fibers in the mutants as compared to that in the wild type. The relative width of the DREZ and the relative length of the collaterals (*E*, mean ± SEM, $n = 3$) showed no significant difference ($P = 0.07$ or 0.62, *t* test) between the 2 genotypes after being normalized to the height of the spinal cord.

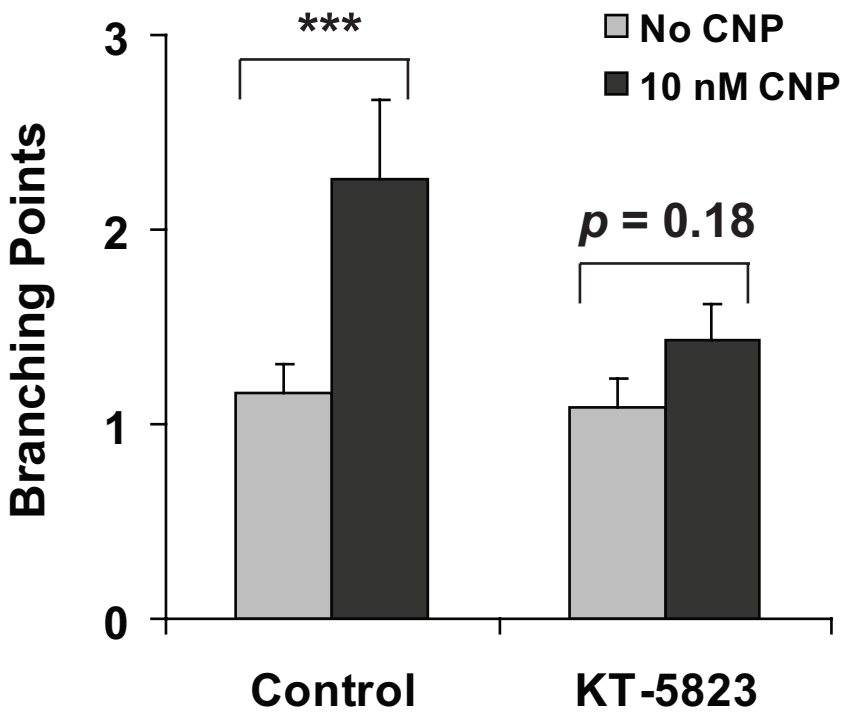


Fig. S3. KT-5823 blocks axon branching induced by CNP in culture. Dissociated DRG neurons were cultured in collagen gels in the presence of NGF for 24 h and then treated with buffer or CNP (10 nM) in the presence or absence of KT-5823 (10 μM) for another day. The increase in branch formation by CNP (***, $P < 0.001$, *t* test) is nearly reversed by KT-5823 ($P = 0.18$). For each condition, 50 neurons were analyzed, and the averaged number of branching points was plotted as mean $\pm$ SEM.

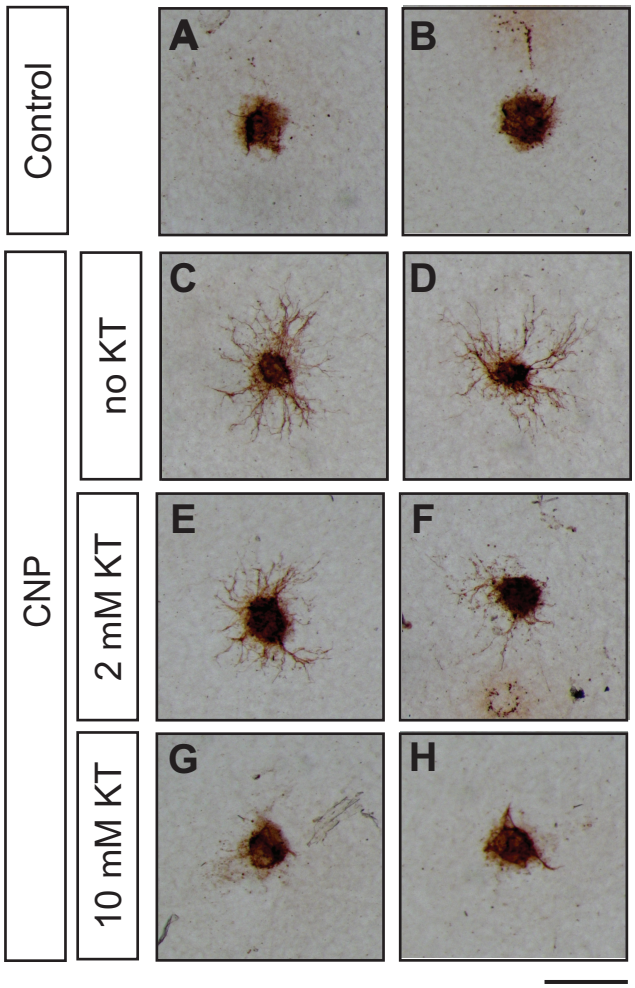


Fig. S4. KT-5823 blocks axon outgrowth from DRG explants induced by CNP. DRG explants from E14 rat embryos were cultured in collagen gels in the absence of NGF, but treated with buffer (*A* and *B*) or CNP (10 nM, *C–H*). In some cultures, KT-5823 was included (*E–H*). Axon outgrowth induced by CNP (*C* and *D*) was reduced by 2 μM KT-5823 (*E* and *F*), but totally blocked by 10 μM KT-5823 (*G* and *H*). (Scale bar: 200 μm.)

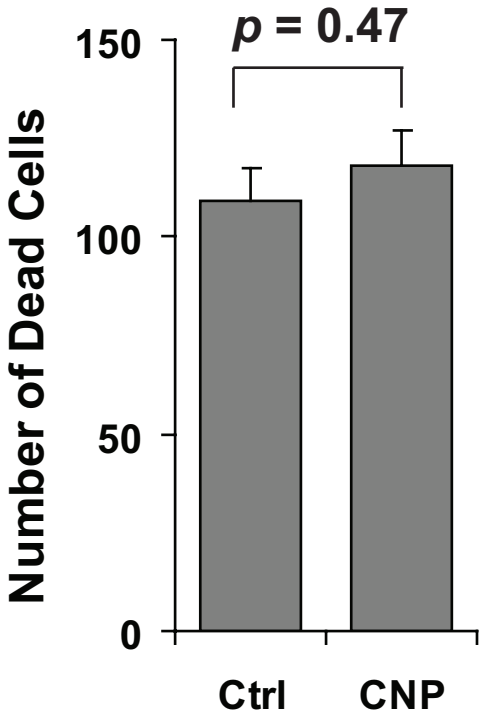


Fig. S5. CNP does not promote neuronal survival in the absence of NGF. DRG explants from E14 embryos were cultured in collagen gels in the absence of NGF but treated with buffer (Ctrl) or CNP (100 nM). After 2 days in culture, they were fixed and analyzed for cell death by TUNEL staining. The number of TUNEL positive cells was counted from a quarter of the explant (6 explants per condition) and plotted as mean ± SEM. No significant change in cell death was found ($P = 0.47$, $n = 6$, Mann–Whitney test).

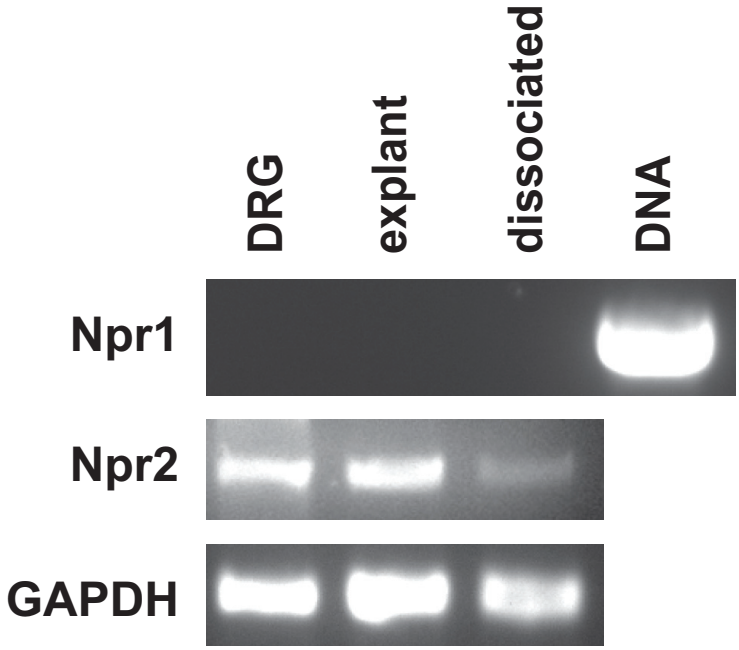
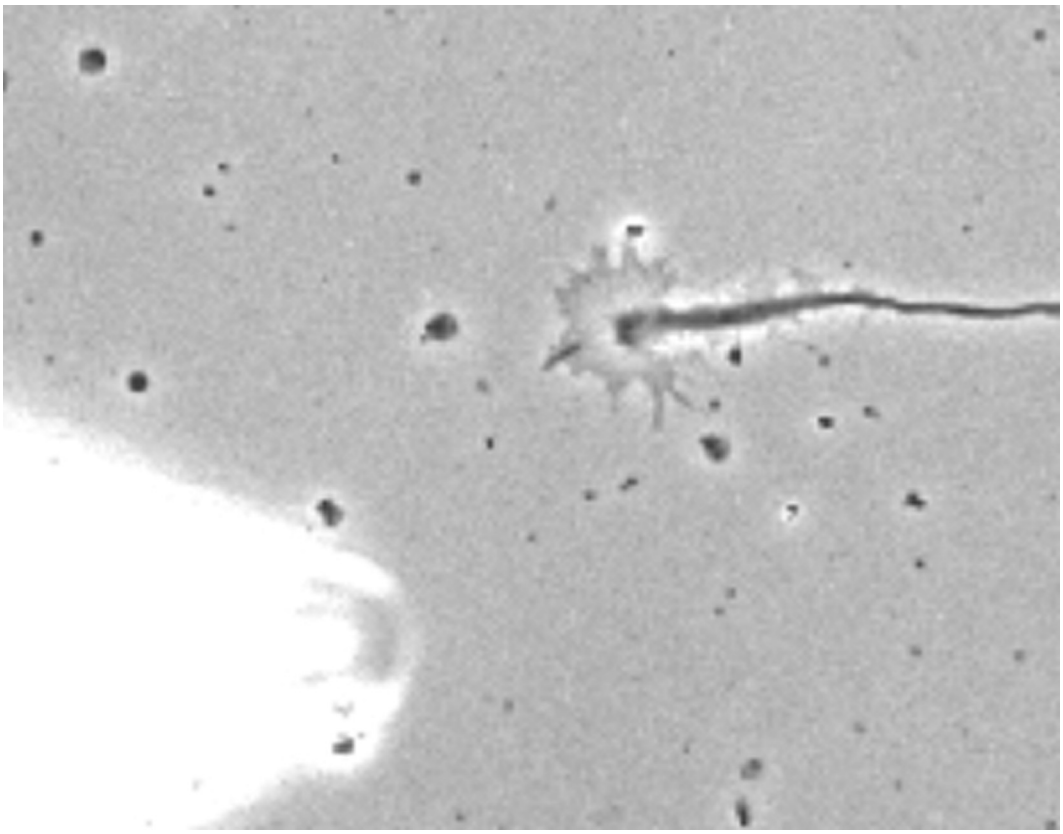


Fig. S6. Expression of Npr receptors in cultured DRG neurons. RT-PCR analysis is shown of Npr1 and Npr2 expression in the freshly isolated DRG, in cultured explants, and in dissociated DRG neurons. GAPDH was used as an internal control. Npr2 is present in all samples, but Npr1 is not. An Npr1-containing plasmid DNA was used as a positive control.



Movie S1. The time-lapse movie is constructed from phase contrast images captured every 15 sec for a DRG growth cone in a period of 60 min, and the play was sped up by 225× the normal speed. A CNP gradient was created from a pipette filled with 10 μM CNP that was ejected by pulse application (2 Hz) of a positive pressure (3 psi). Note the growth cone was attracted to the pipette tip and followed it after repositioning.

Movie S1 (MP4)