Supplementary Information (Figures S1-S14 and Table S1)

Tiam1 Coordinates Synaptic Structural and Functional Plasticity Underpinning the Pathophysiology of Neuropathic Pain

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Figures S1-S14

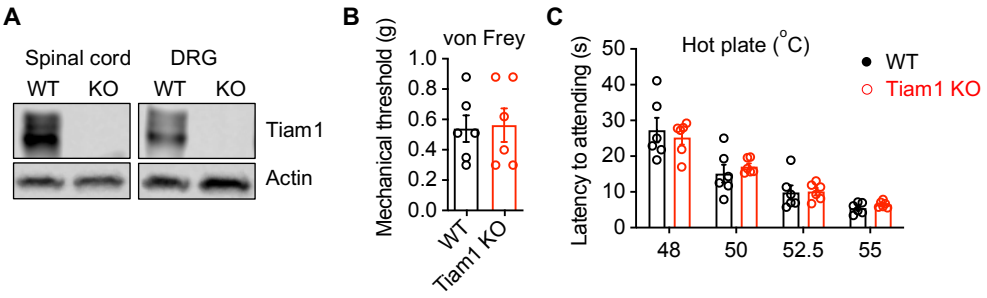

Figure S1. Tiam1 global KO mice. Related to Figure 1.

(**A**) Western blots of Tiam1 from the spinal cord and dorsal root ganglion (DRG) samples of WT mice and global *Tiam1* KO mice. GAPDH was used as the internal loading control.

(**B, C**) Global *Tiam1* KO mice do not display altered basal sensitivity to mechanical (B) or thermal (C) stimuli as compared to the WT mice. Two-tailed Student's *t*-test (n = 6 mice/group. WT vs KO).

Data are presented as means ± s.e.m.

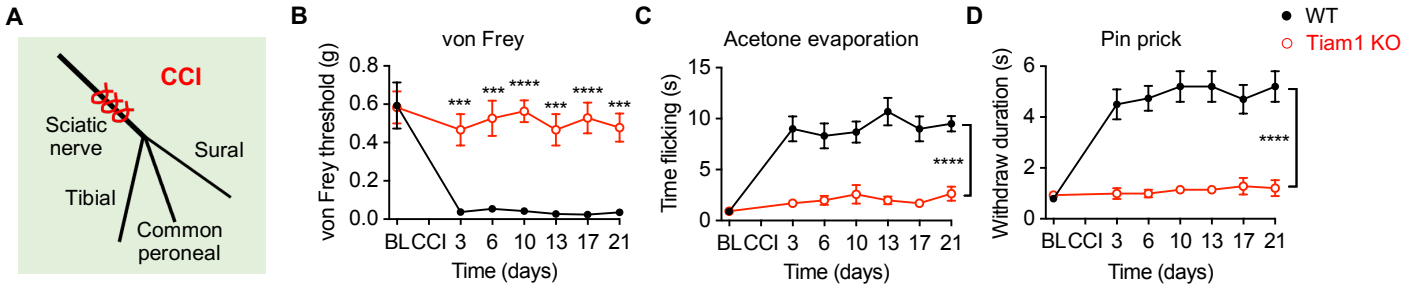

Figure S2. Tiam1 deletion prevents chronic contrition injury (CCI)-induced neuropathic pain hypersensitivity. Related to Figure 1.

(**A**) Depiction of the CCI neuropathic pain model.

(**B-D**) Time course of changes in von Frey withdrawal thresholds (B), acetone evaporation time flicking (C), and pinprick withdraw duration (D) in Tiam1 global KO mice and WT littermates before and after CCI surgery. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 5-7 mice/group. von Frey: $P < 0.0001$, $F_{6,70} = 5.649$; acetone: $P < 0.0001$, $F_{6,70} = 11.55$; pinprick: $P < 0.0001$, $F_{6,70} = 10.46$).

Data are presented as means ± s.e.m. ***$P < 0.001$, ****$P < 0.0001$.

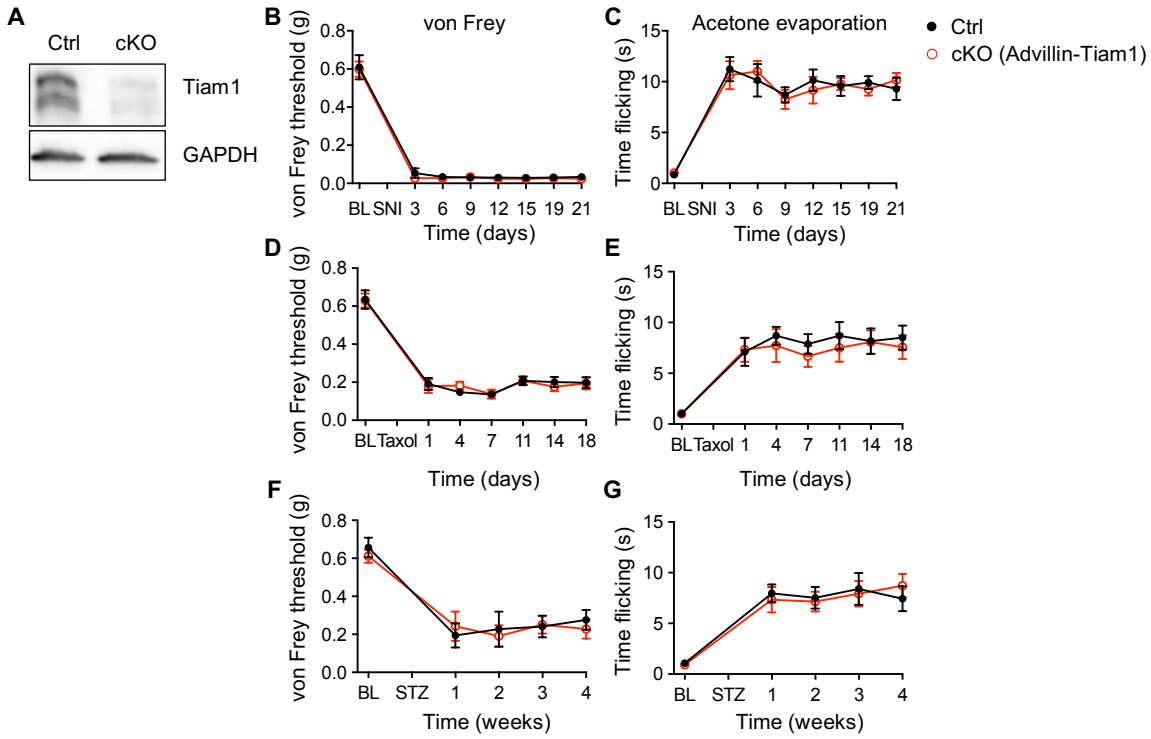

Figure S3. Tiam1 deletion from DRG neurons does not alter neuropathic pain development. Related to Figure 2.

(**A**) Representative western blots of Tiam1 from DRG samples of Tiam1 DRG conditional KO mice (cKO, *Advillin-Cre::Tiam1*$^{fl/fl}$) and littermate controls (Ctrl, *Tiam1*$^{fl/fl}$). GAPDH was used as the internal loading control.

(**B, C**) Time courses for changes in von Frey withdrawal thresholds (B) and acetone evaporation time flicking (C) in *Advillin-Tiam1* cKO mice and littermates (Ctrl) before (baseline, BL) and after SNI surgery. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 6-7 mice/group. von Frey: $P = 0.9991$, $F_{7,88} = 0.08009$; acetone: $P = 0.9694$, $F_{7,88} = 0.2546$).

(**D, E**) Time courses for changes in von Frey withdrawal thresholds (D) and acetone evaporation time flicking (E) in *Advillin-Tiam1* cKO mice and littermate controls (Ctrl) before and after paclitaxel (Taxol) treatment. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 6-7 mice/group. von Frey: $P = 0.9721$, $F_{6,77} = 0.2177$; acetone: $P = 0.9898$, $F_{6,77} = 0.1436$).

(**F, G**) Time courses for changes in von Frey withdrawal thresholds (F) and acetone evaporation time flicking (G) in *Advillin-Tiam1* cKO mice and littermate controls (Ctrl) before and after streptozotocin (STZ) treatment. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 6-7 mice/group. von Frey: $P = 0.9110$, $F_{4,55} = 0.2457$; acetone: $P = 0.9001$, $F_{4,55} = 0.2636$). Data are presented as means ± s.e.m.

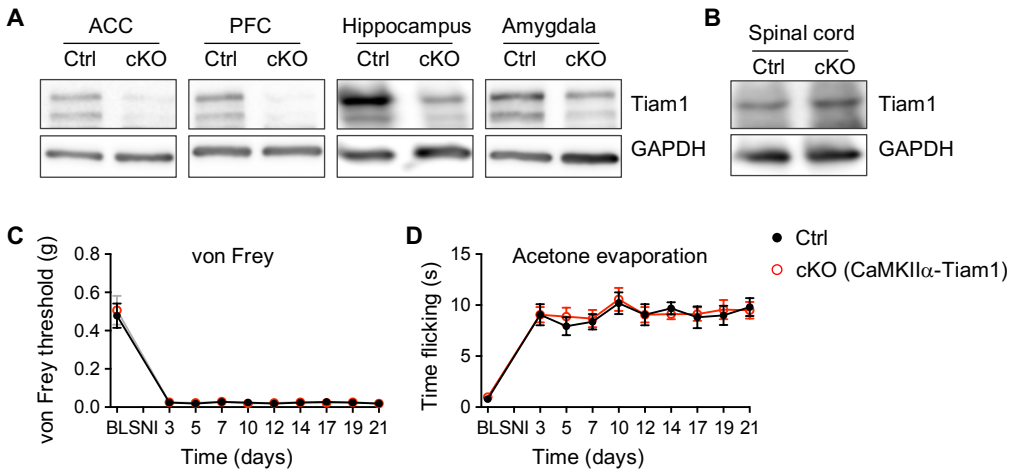

Figure S4. Tiam1 deletion from postnatal forebrain excitatory neurons does not alter spared nerve injury (SNI)-induced neuropathic pain hypersensitivity. Related to Figure 2.

(**A**) Western blots showing Tiam1 loss in the cortical and subcortical structures that are involved in chronic pain processing, including the anterior cingulate cortex (ACC), prefrontal cortex (PFC), hippocampus, and amygdala of Tiam1 postnatal forebrain excitatory neuron cKO mice (cKO, *CaMKIIα-Cre::Tiam1$^{fl/fl}$*) compared to littermate controls (Ctrl, *Tiam1$^{fl/fl}$*). GAPDH was used as an internal control.

(**B**) Western blots of Tiam1 from the spinal cord samples of *CaMKIIα-Tiam1* cKO mice (cKO) and littermate controls (Ctrl). GAPDH was used as an internal control.

(**C, D**) Time courses for changes in von Frey withdrawal thresholds (B) and acetone evaporation time flicking (C) in *CaMKIIα-Tiam1* cKO mice and littermate controls (Ctrl) before (baseline, BL) and after SNI. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 5-8 mice/group. von Frey: $P = 0.9998$, $F_{9,100} = 0.08144$; acetone: $P = 0.9988$, $F_{9,140} = 0.1317$).

Data are presented as means ± s.e.m.

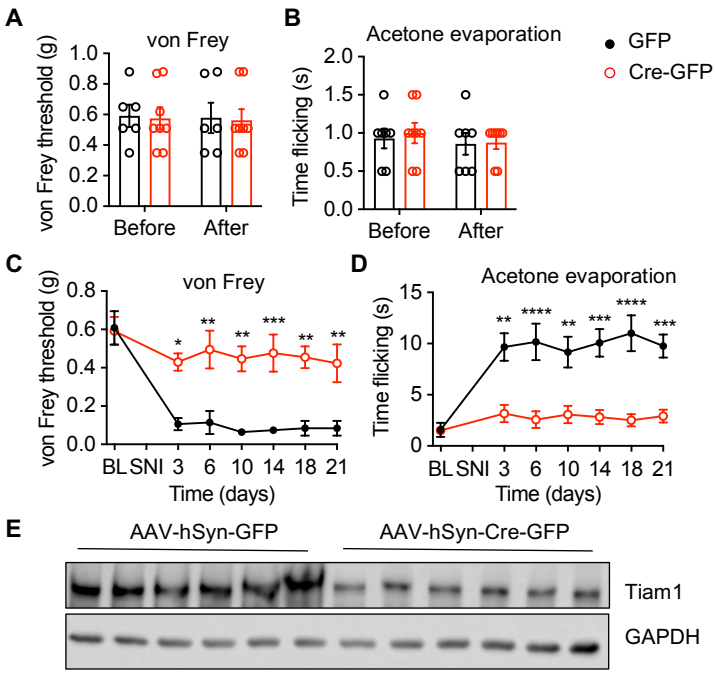

Figure S5. Tiam1 deletion from spinal dorsal horn neurons prevents the development of neuropathic pain. Related to Figure 2.

(**A, B**) Tiam1 deletion from spinal dorsal horn neurons does not affect basal nociceptive sensitivity. (A) and time flicking with acetone evaporation (B) of *Tiam1*$^{fl/fl}$ mice before and 2 weeks after intra-spinal dorsal horn microinjection of rAAV8-hSyn-GFP (GFP) or rAAV8-hSyn-Cre-GFP (Cre-GFP). Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 6-8 mice/group. von Frey: $P = 0.9959$, $F_{1,24} = 2.720e\text{-}005$; acetone: $P = 0.8296$, $F_{1,26} = 0.04727$).

(**C, D**) Time course for changes in von Frey withdrawal thresholds (C) and time flicking with acetone evaporation (D) of *Tiam1*$^{fl/fl}$ mice infected with rAAV8-hSyn-GFP (GFP) or rAAV8-hSyn-Cre-GFP (Cre) before and after SNI. Two-way ANOVA analysis followed by Dunnett's post-hoc test (n = 6 mice/group. von Frey: $P < 0.0001$, $F_{6,63} = 6.613$; acetone: $P = 0.0001$, $F_{6,70} = 5.481$).

(**E**) Western blots of Tiam1 from the spinal cord dorsal horn samples of *Tiam1*$^{fl/fl}$ mice microinjected with rAAV8-hSyn-GFP (6 mice) or rAAV8-hSyn-Cre-GFP (6 mice) after behavioral testing in C and D show that the variation for Tiam1 deletion efficacy across animals was minimal. GAPDH was used as the internal loading control.

Data are presented as means ± s.e.m.

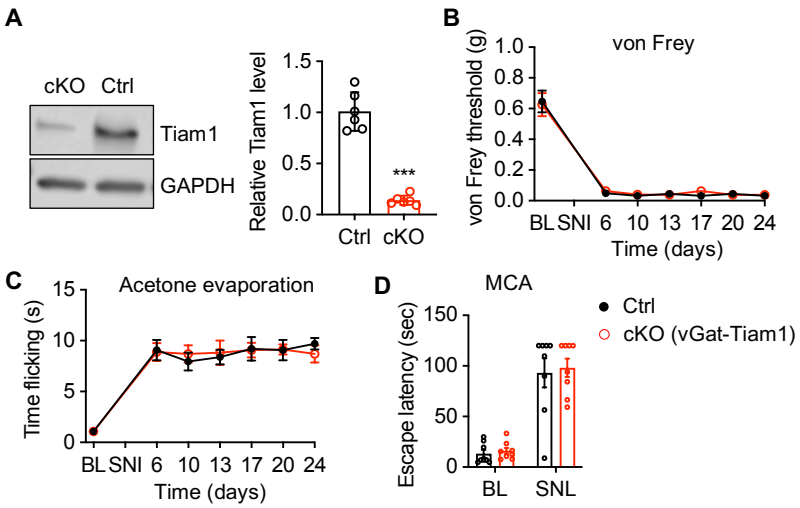

Figure S6. Tiam1 deletion from inhibitory neurons does not alter the development of neuropathic pain. Related to Figure 3.

(A) Western blots and quantification showing Tiam1 deletion from vGat-expressing inhibitory neurons in *vGat-Tiam1* cKO mice (cKO, *vGat-Cre::Tiam1*$^{fl/fl}$) compared to littermate controls (Ctrl, *vGat-Cre*). Cre-dependent AAV vector expressing eGFP (rAAV8-pCAG-DIO-eGFP) was injected into the spinal dorsal horn of control mice (*vGat-Cre*) and *vGat-Tiam1* cKO mice via intra-spinal dorsal horn microinjection. eGFP-expressing spinal dorsal horn neurons were isolated with flow cytometry for western blot analysis. Two-tailed Student's *t*-test (n = 6). GAPDH was used as the internal loading control.

(**B-D**) Tiam1 deletion from vGat-expressing neurons does not alter spared nerve injury (SNI)-induced neuropathic pain hypersensitivity, (B) von Frey withdrawal thresholds, (C) time flicking with acetone evaporation, and (D) escape latency in the MCA assay of *vGat-Tiam1* cKO mice and littermate controls before and after SNI. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 8 mice/group. von Frey: $P = 0.9868$, $F_{6,98} = 0.1590$; acetone: $P = 0.9706$, $F_{6,98} = 0.2170$; MCA: $P = 0.8993$, $F_{1,28} = 0.01631$).

Data are presented as means ± s.e.m.

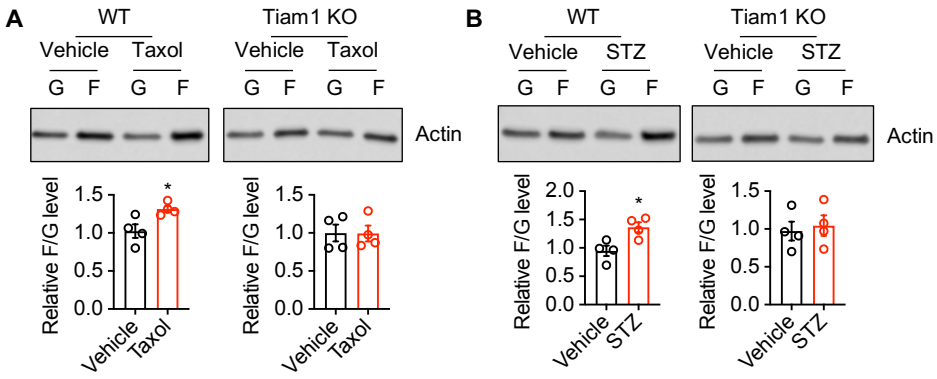

Figure S7. Tiam1 mediates paclitaxel (Taxol)- and streptozotocin (STZ)-stimulated actin polymerization in the spinal dorsal horn. Related to Figure 4.

(**A, B**) Western blots and quantification revealed that paclitaxel (Taxol) (A) and streptozotocin (STZ) (B) increase the F- to G-actin ratio in the spinal dorsal horn of WT mice but not Tiam1 global KO mice. Mann-Whitney *U*-test (n = 4).

Data are presented as means ± s.e.m. **P* < 0.05.

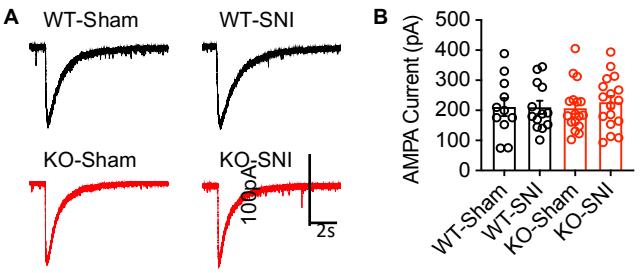

Figure S8. AMPAR currents are unaltered in neuropathic pain mice. Related to Figure 5.

(**A, B**) Representative traces and mean changes in AMPAR currents elicited by puff application of 200 μM AMPA to spinal dorsal horn neurons of WT and global *Tiam1* KO mice 3 weeks after sham or SNI surgery. One-way ANOVA followed by Tukey's *post hoc* test (n = 11-17 neurons from 3 mice in each group. $P = 0.9135$, $F_{3,54} = 0.1741$).

Data are presented as mean ± s.e.m.

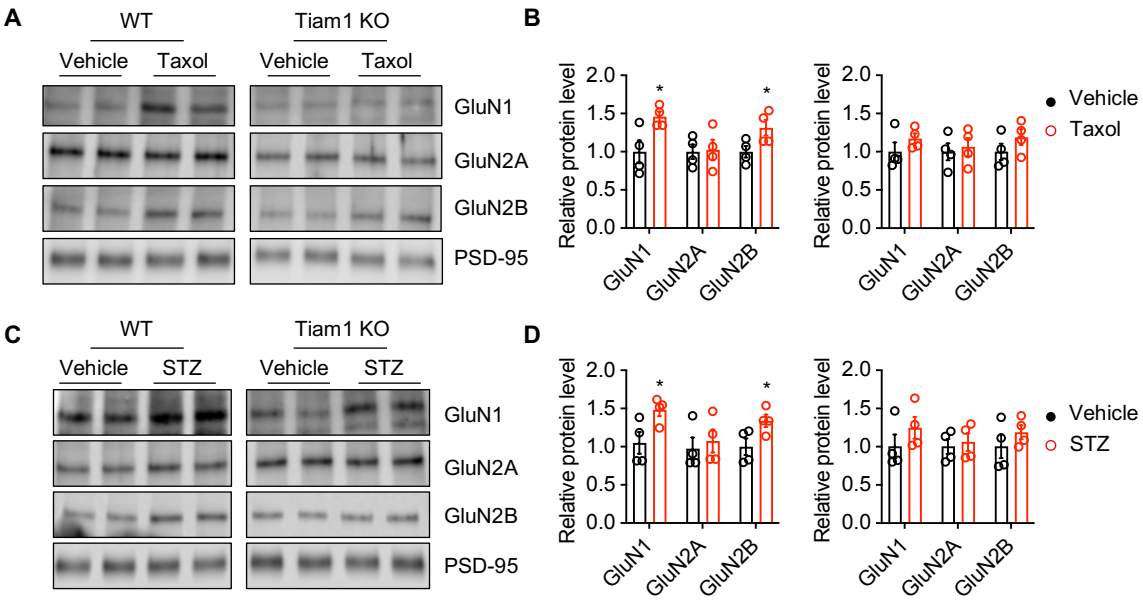

Figure S9. Tiam1 mediates paclitaxel- and streptozotocin-induced increases in synaptic NMDAR subunit protein levels in the spinal dorsal horn. Related to Figure 5.

(**A-D**) In WT mice, NMDAR subunits (GluN1 and GluN2B) are significantly increased in the spinal dorsal horn after paclitaxel (Taxol) (A, B) or streptozotocin (STZ) (C, D) treatment. In contrast, in global *Tiam1* KO mice, synaptic NMDAR subunits in the spinal dorsal horn do not significantly increase after drug treatment. PSD-95 was used as the internal loading control. Mann-Whitney *U*-test (n = 4. vehicle vs drug).

Data are presented as means ± s.e.m. **P* < 0.05.

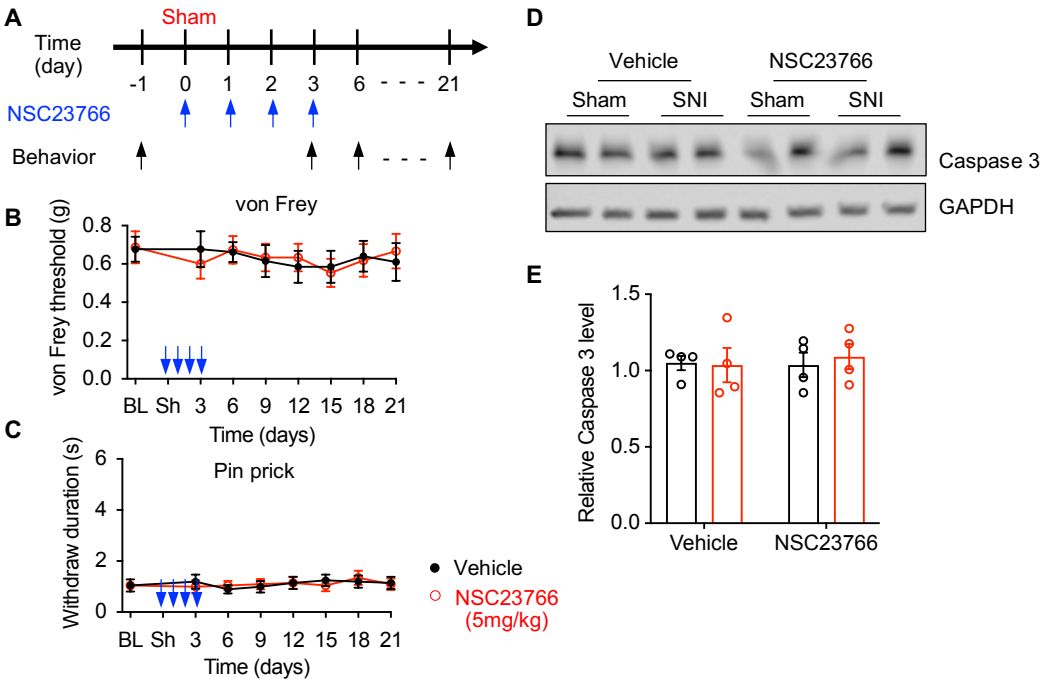

Figure S10. NSC23766 treatment does not affect basal pain sensitivity or spinal motor neuron death. Related to Figure 7.

(**A**) Experimental paradigm. Blue arrows represent NSC23766 treatment, and black arrows represent behavioral analyses.

(**B, C**) Time course for changes in von Frey thresholds (B) and withdrawal duration to pinprick (C) of sham mice treated with 4 consecutive daily injections of vehicle or NSC23766 starting at the time of sham surgery. BL, baseline. Sh, sham surgery. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 10 mice/group. von Frey: $P = 0.9942$, $F_{7,144} = 0.1464$; pinprick: $P = 0.9880$, $F_{7,144} = 0.1856$).

(**D, E**) Western blots and quantification of apoptosis marker caspase-3 activity in the spinal ventral horn of NSC23766- or vehicle-treated SNI and sham mice. GAPDH was used as the internal loading control. Two-way ANOVA analysis followed by Dunnett's *post-hoc* test (n = 4. $P = 0.6937$, $F_{1,12} = 0.1628$).

Data are presented as means ± s.e.m.

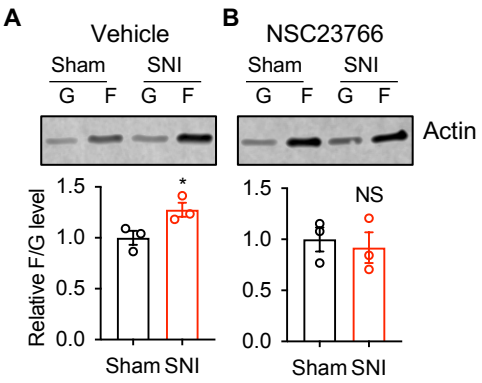

Figure S11. NSC23766 treatment attenuates nerve injury-induced actin polymerization in the spinal cord dorsal horn. Related to Figure 7.

(**A, B**) Western blots and quantification show that 4-day treatment with NSC23766 starting 2 weeks after SNI surgery normalizes the increase in the F-actin to G-actin ratio. Mann-Whitney *U*-test (n = 3).

Data are presented as means ± s.e.m. **P* < 0.05, NS, no significant difference.

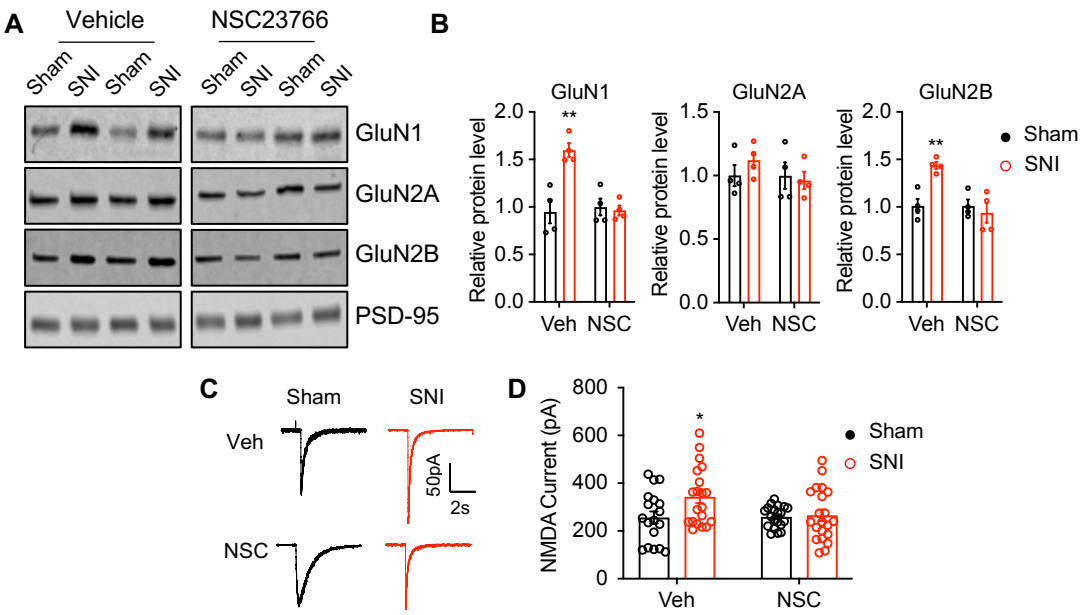

Figure S12. NSC23766 normalizes nerve injury-induced increases in the levels and activity of synaptic NMDARs. Related to Figure 7.

(**A, B**) Western blots and quantification show that four-day treatment with NSC23766 starting 2 weeks after SNI normalized the increase in the amount of synaptic NMDAR subunits in the spinal dorsal horn. PSD-95 was used as the internal loading control. Mann-Whitney *U*-test (n = 4. sham vs SNI). Veh, vehicle. NSC, NSC23766.

(**C, D**) Representative current traces and mean changes show that four-day treatment with NSC23766 starting 2 weeks after SNI surgery normalizes the increases in NMDAR currents elicited by puff application of 100 μM NMDA to spinal dorsal horn neurons. Two-way ANOVA followed by Tukey's *post-hoc* test (n = 18-21 neurons from 3 mice in each group. $P = 0.0419$, $F_{1,75} = 4.283$).

Data are presented as means ± s.e.m. *$P < 0.05$, **$P < 0.01$.

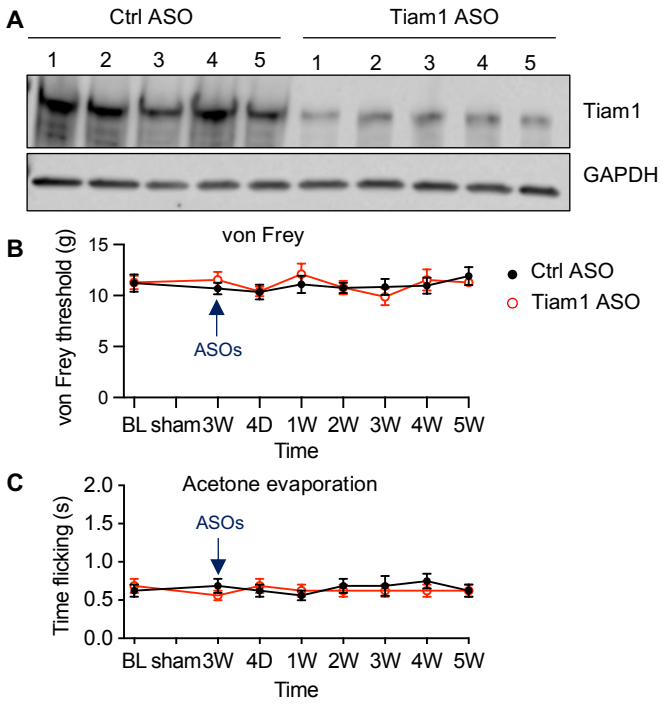

Figure S13. Tiam1 ASO reduces spinal Tiam1 protein levels. Related to Figure 8.

(**A**) Western blots showing Tiam1 levels from spinal dorsal horn samples of neuropathic pain rats (3 weeks after SNI) treated with control ASO (Ctrl ASO, 5 rats) or Tiam1 ASO-1 (Tiam1 ASO, 5 rats) 2 weeks after a single lumbar puncture injection of 100 μg ASO. Note that the variation for Tiam1 knockdown efficacy across animals was minimal. GAPDH was used as the internal loading control.

(**B, C**) Tiam1 ASO or control ASO does not affect basal pain sensitivity. (B) von Frey thresholds and (C) time flicking with acetone evaporation (C) of sham rats show that ASOs injection does not alter basal pain sensitivity. Two-way ANOVA followed by Tukey's *post-hoc* test (n = 8 rats/group. von Frey: $P = 0.9015$, $F_{7,112} = 0.3985$; acetone: $P = 0.8765$, $F_{7,112} = 0.4378$).

Data are presented as means ± s.e.m.

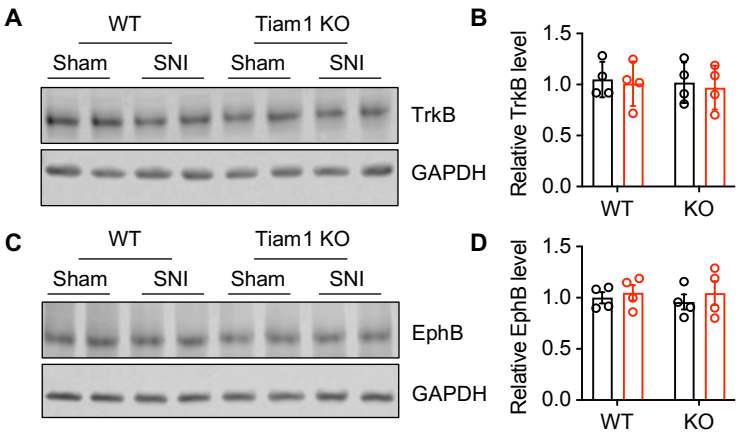

Figure S14. Tiam1 deletion does not affect the expression level of TrkB or EphB receptors in the spinal cord. Related to Figure 8.

(**A, B**) Western blots and quantification showed no significant difference in spinal TrkB receptors between WT mice and global *Tiam1* KO mice 3 weeks after sham or SNI surgery. GAPDH was used as the internal loading control. Mann-Whitney *U*-test (n = 4. sham vs SNI).

(**C, D**) Western blots and quantification showed no significant difference in spinal EphB receptors between WT mice and global *Tiam1* KO mice 3 weeks after sham or SNI surgery. GAPDH was used as an internal loading control. Mann-Whitney *U*-test (n = 4. sham vs SNI).

Data are presented as means ± s.e.m.

Table S1. Sequences of the antisense oligonucleotides (ASOs) used in this study. Related to Figure 8.

ASO name	Sequence	Start site
ASO-1	5’ mA*mUmGmAmUA*T*G*A*A*C*A*G*T*G*mCmAmG*mU*mG 3’	6732
ASO-2	5’ mC*mUmCmAmGA*T*C*T*G*A*G*A*G*T*mGmAmG*mA*mA 3’	747
ASO-3	5’ mG*mAmUmAmGG*A*C*T*C*A*A*G*C*T*mUmGmU*mU*mG 3’	5175
ASO-4	5’ mA*mUmAmUmGA*A*C*A*G*T*G*C*A*G*mUmGmG*mU*mA 3’	6748
ASO-5	5’ mA*mGmAmUmGT*C*T*G*C*A*G*A*T*T*mUmAmG*mA*mG 3’	707
Control	5’ mG*mUmUmUmUC*A*A*A*T*A*C*A*C*C* mUmUmC*mA*mU 3’	

*: Phosphorothioate bond; m: 2’O-methoxy-ethyl (MOE) base. U: chimeric antisense.