Supplementary Information

Microglia-independent peripheral neuropathic pain in male and female mice

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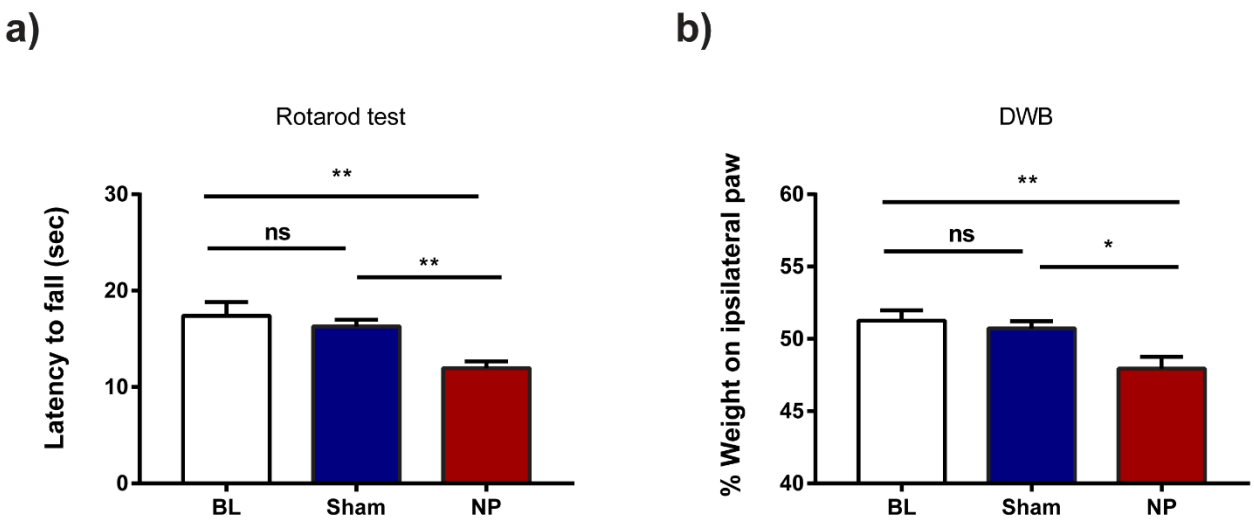
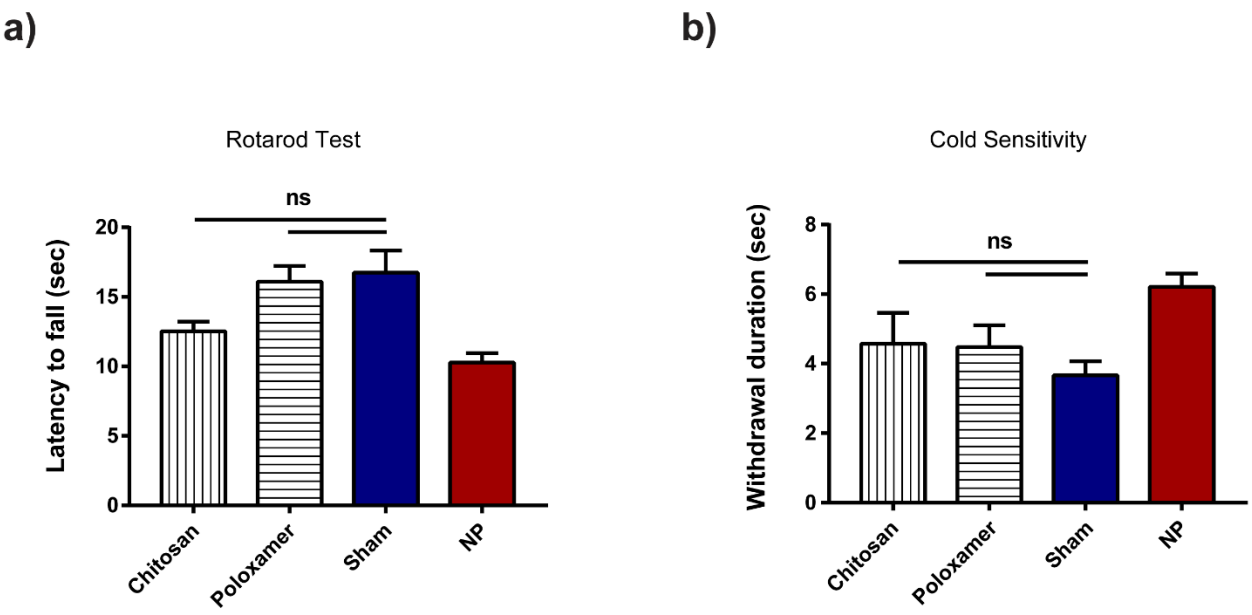
This Supplementary Information file includes:

Supplementary Figures 1 to 10
Supplementary Table 1



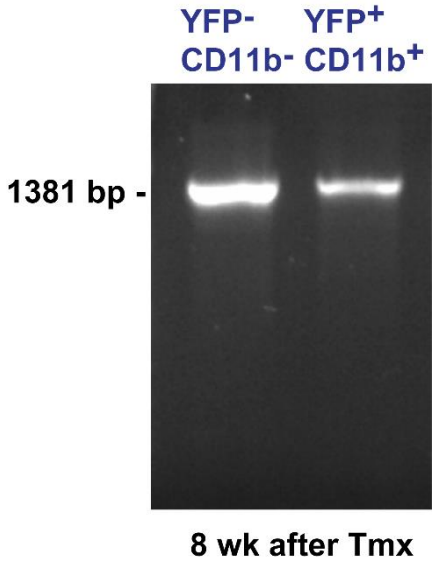
Supplementary Figure 1. Rotarod test and dynamic weight bearing test after the NP exposure at day 5.

(a) Accelerating rotarod test. Average latency time on the rod was measured at baseline (BL) and 5 days after the NP exposure in sham-operated (n=23) and NP-exposed mice (n=26). **(b)** Dynamic weight bearing test of sham-operated (n=10) and NP-exposed (n=13) mice at baseline (BL) and 5 days after NP exposure. Comparisons were made by Kruskal-Wallis test with Dunn's multiple comparisons test **(a)**, or one-way ANOVA **(b)**. *p<0.05, **p<0.01; ns>0.05. All data are mean ± SEM.



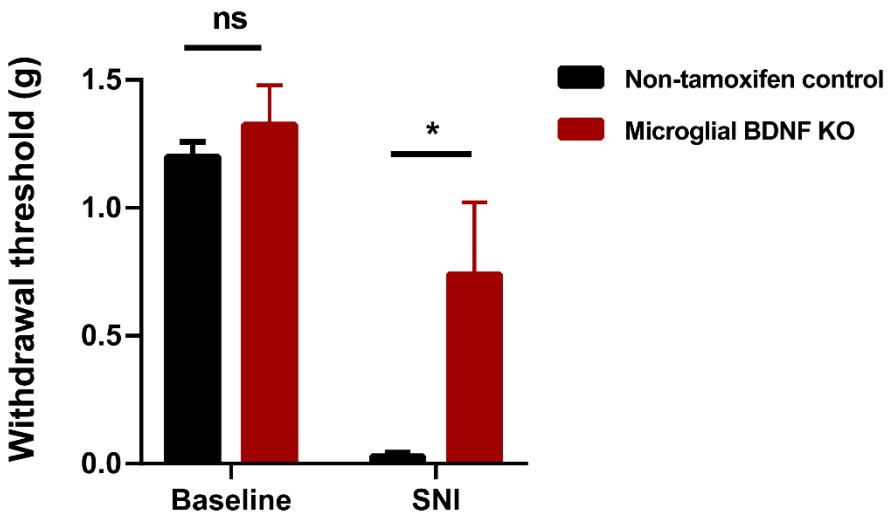
Supplementary Figure 2. Rotarod and acetone test after the exposure of space-filling mass at day 5.

(a) Accelerating rotarod test. Average latency time (in seconds) on the rod was measured 5 days after surgery for sham (n=4) and applications of chitosan, poloxamer or NP (n=7 per group) on the sciatic nerve. **(b)** Cold sensitivity, measured by withdrawal duration (in seconds) using acetone test, in the ipsilateral paws of sham controls (n=4) and animals with the application of chitosan, poloxamer or NP (n=7 per group) at day 5. Comparisons were made by Kruskal-Wallis test, Dunn's multiple comparisons test **(a, b)**. **p<0.01, ****p<0.0001; ns>0.05. All data are mean ± SEM.



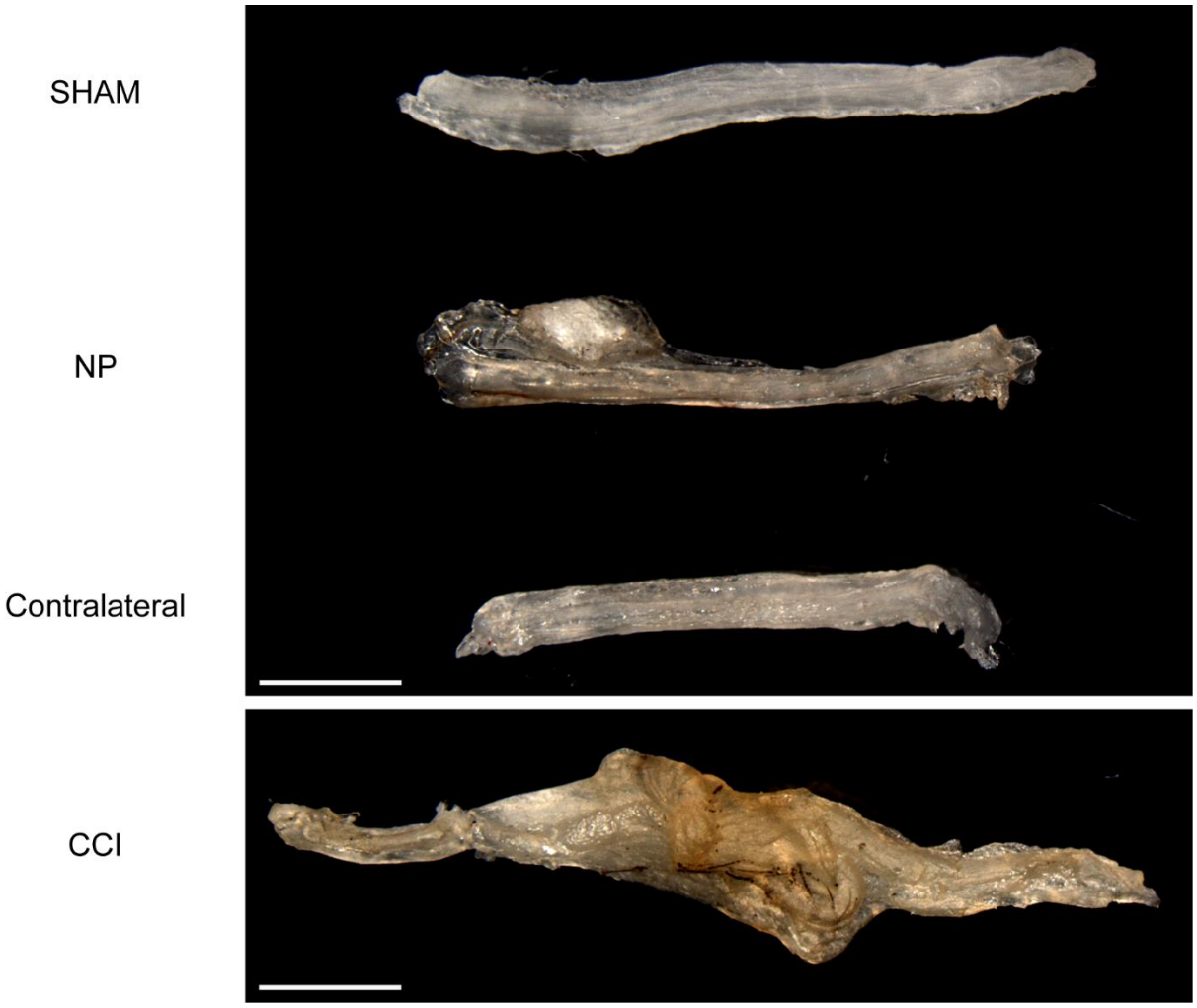
Supplementary Figure 3. PCR analysis of BDNF recombination from the blood of *CX$_3$CR1$^{CreER/+}$:BDNF$^{fl/fl}$* male mice.

PCR analysis of the YFP$^-$/CD11b$^-$ or YFP$^+$/CD11b$^+$ cells sorted from the blood of *CX$_3$CR1$^{CreER/+}$:BDNF$^{fl/fl}$* mice, in which YFP is constitutively expressed in CreER-expressing cells (see Methods), eight weeks after tamoxifen treatment.



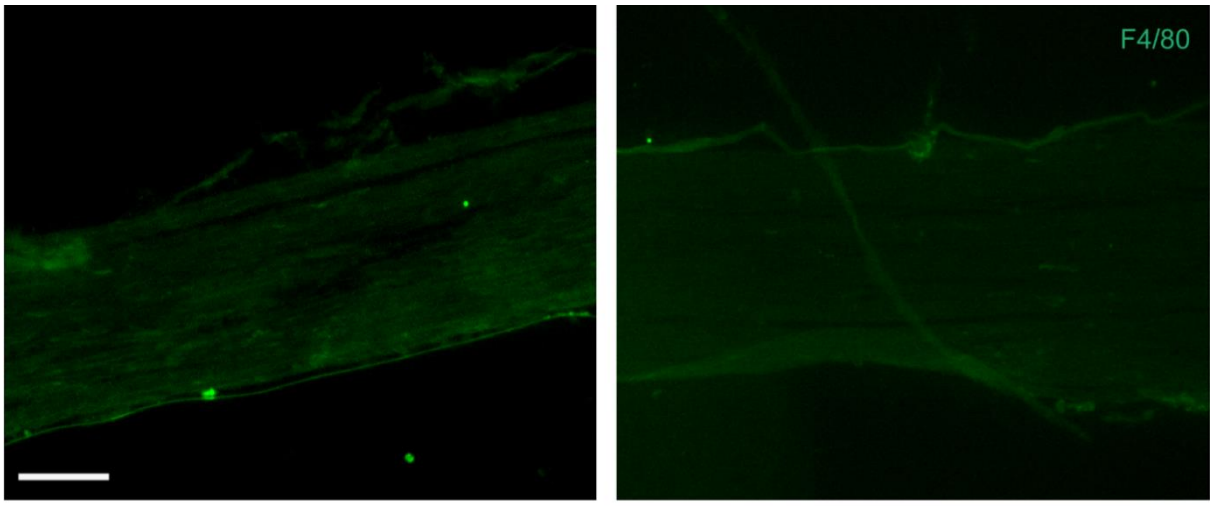
Supplementary Figure 4. Microglial BDNF knockout (KO) male mice fail to develop mechanical sensitivity following spared nerve injury (SNI).

Withdrawal threshold from von Frey filaments in *CX$_3$CR1$^{CreER/+}$:BDNF$^{fl/fl}$* male mice that had received tamoxifen (microglial BDNF KO), at baseline and eight weeks post SNI surgery (*n*=4 mice/condition). Corn oil served as vehicle. The control mice were the identical genotype but received corn oil without tamoxifen. Comparisons were made by unpaired T-test. *p<0.05. All data are mean ± SEM.



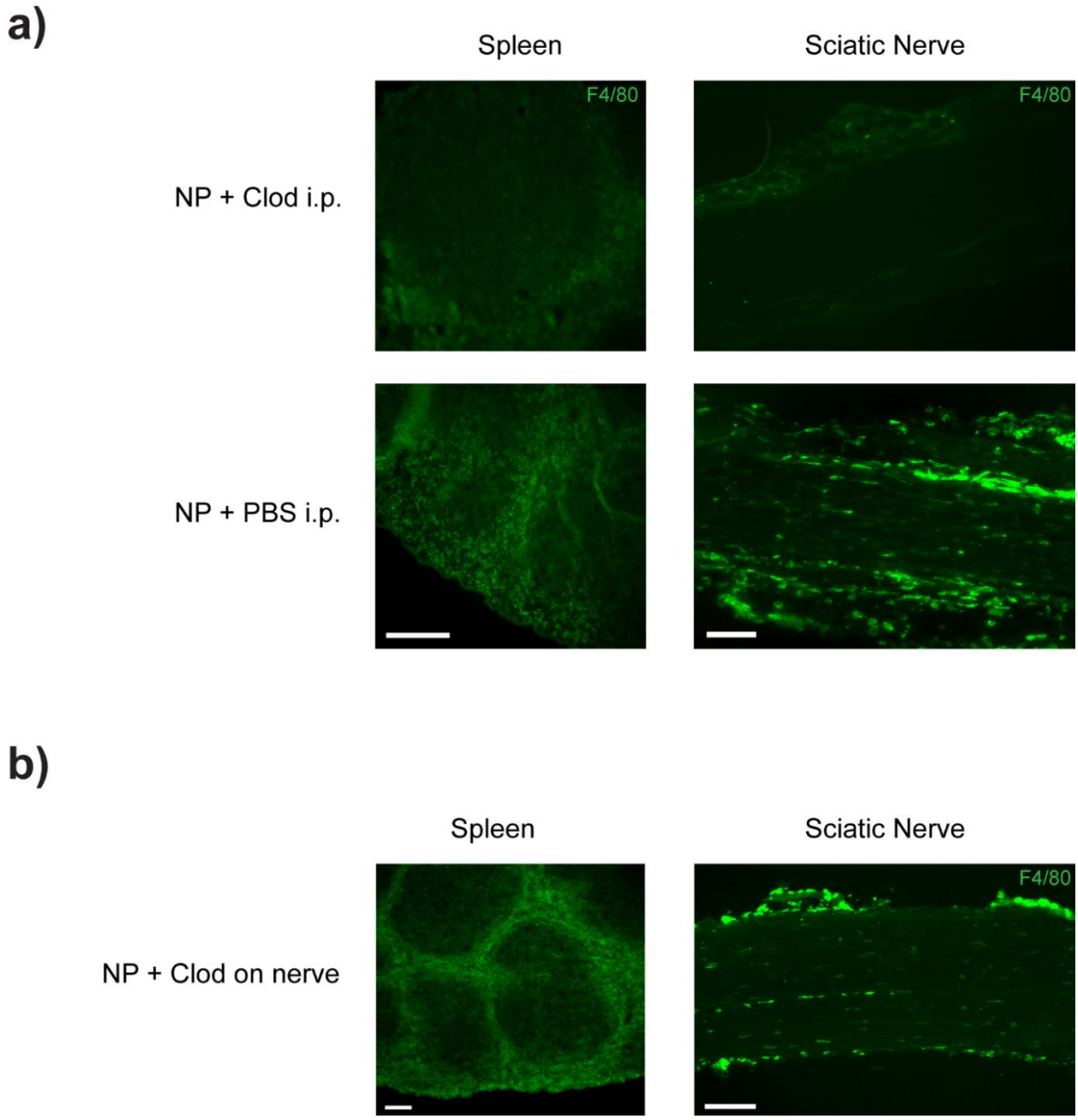
Supplementary Figure 5. Stereoscopic view of sciatic nerves following sham, NP and CCI surgery.

Stereoscopic view of sciatic nerve following sham (upper), NP (ipsilateral and contralateral, center) and CCI (lower) surgery. Scale bar, 360μm.



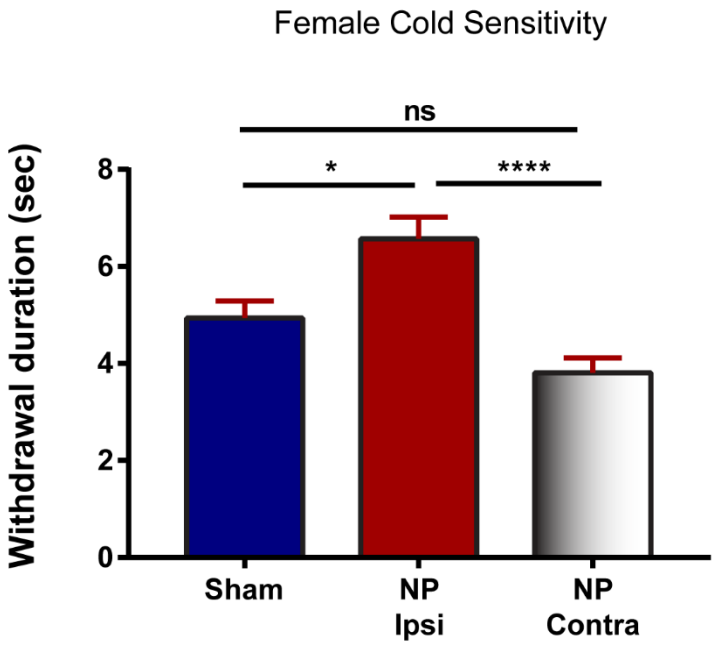
Supplementary Figure 6. F4/80 staining of sciatic nerves from animals receiving chitosan or poloxamer at day 7.

Scale bar, 120μm.



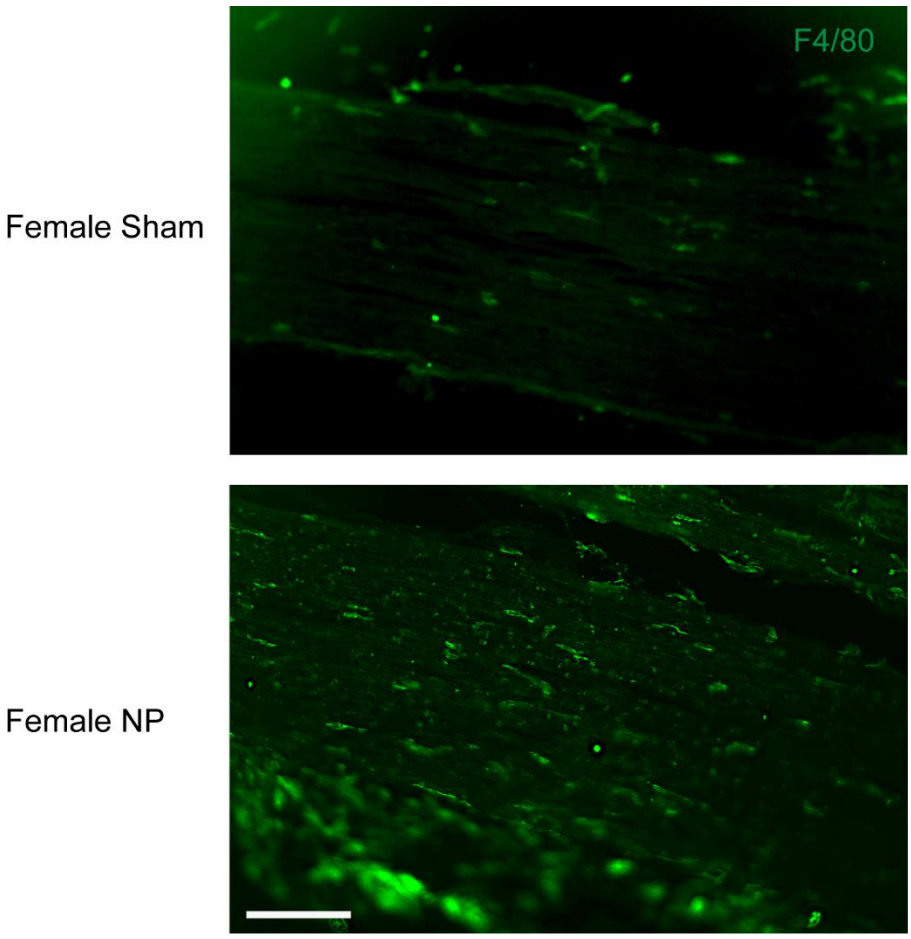
Supplementary Figure 7. F4/80 staining of spleen and sciatic nerves from NP-exposed animals following the administration of clodronate liposome.

(a) F4/80 staining of spleen and sciatic nerves from NP-exposed animals receiving PBS or clodronate liposome (Clod) by intraperitoneal injection (i.p.). Scale bar, 15μm for spleen; 70μm for sciatic nerve. **(b)** F4/80 staining in spleen and sciatic nerve from NP-exposed animals with clodronate liposome applied on the nerve. Scale bar, 130μm for spleen and sciatic nerve.



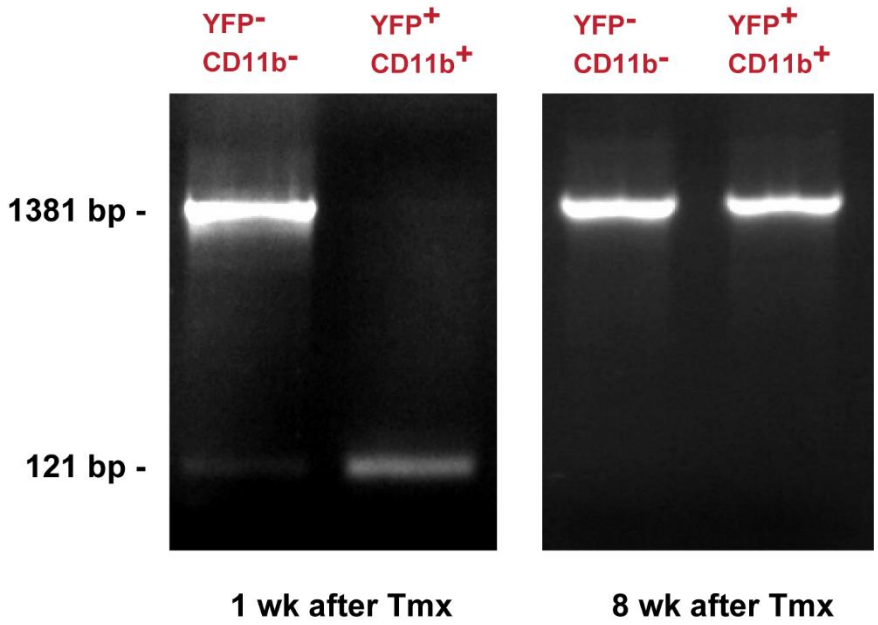
Supplementary Figure 8. Acetone test in female mice.

Cold sensitivity, measured by withdrawal duration (in seconds) using acetone test, in the ipsilateral and contralateral paws of NP-exposed female mice (n=24) and sham controls (n=20) 5 days after surgery. *p<0.05, ****p<0.0001; ns>0.05 by Kruskal-Wallis test with Dunn's multiple comparisons test. Data are mean ± SEM.



Supplementary Figure 9. F4/80 staining in sciatic nerves from sham control or NP-exposed female mice at day 7.

Scale bar, 100μm.



Supplementary Figure 10. PCR analysis of BDNF recombination from the blood of *CX$_3$CR1$^{CreER/+}$:BDNF$^{fl/fl}$* female mice.

PCR analysis of the YFP$^-$/CD11b$^-$ or YFP$^+$/CD11b$^+$ cells sorted from the blood of *CX$_3$CR1$^{CreER/+}$:BDNF$^{fl/fl}$* female mice one or eight weeks after tamoxifen treatment.

	$NeuN^+$ Cells	$ATF3^+$ Cells	
Sham	3898	31	
NP	6285	346	ns
SNI	3367	1619	****
CCI	3122	1250	****

Supplementary Table 1. Total number of $NeuN^+$ and $ATF3^+$ cells quantified in DRG neurons.

The number of $NeuN^+$ and $ATF3^+$ neurons was counted in the L4/5 DRGs from sham controls and animals with NP, SNI and CCI (4-7sections, n= 4-7 animals per group). ns>0.05, **** $p< 0.0001$ compared to corresponding sham controls by Kruskal-Wallis test with Dunn's multiple comparisons test.