Supplementary Information

"*Crmp4* deletion promotes recovery from spinal cord injury
by neuroprotection and limited scar formation"

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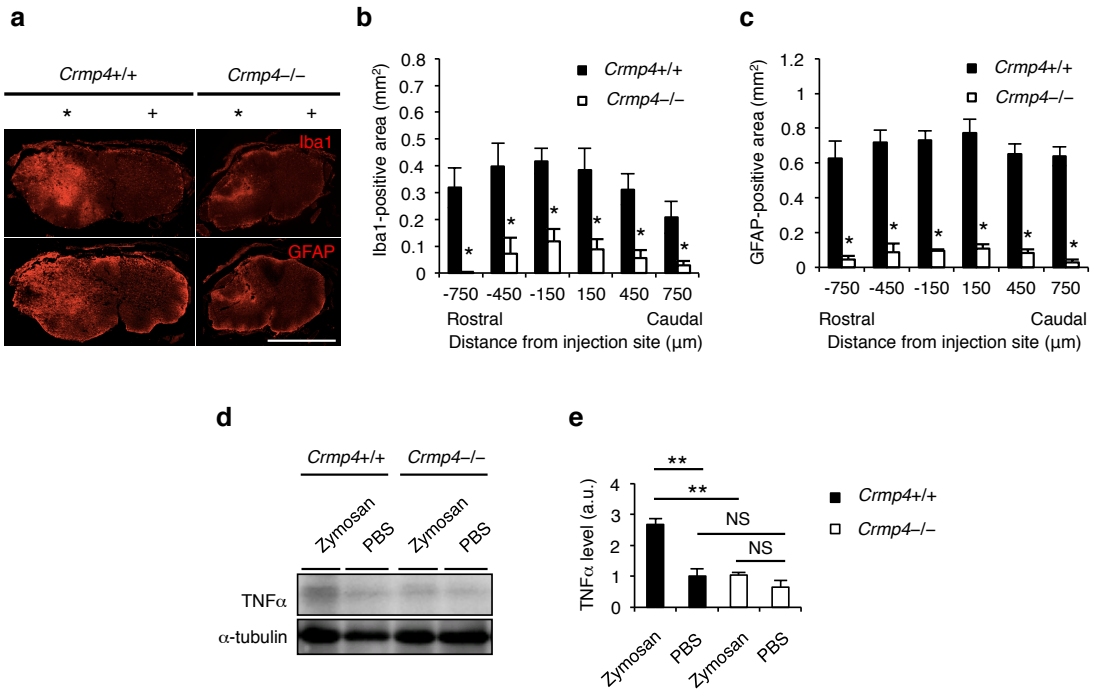
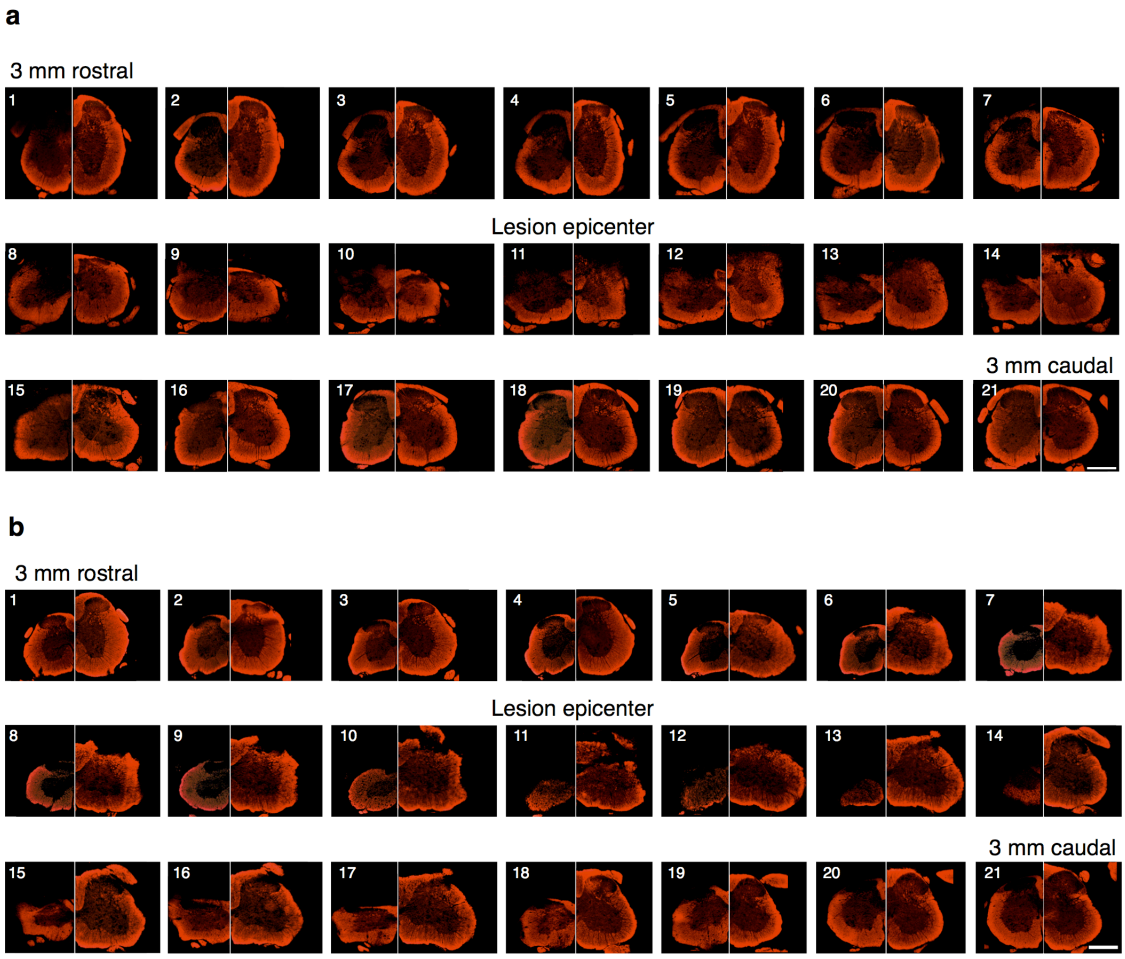
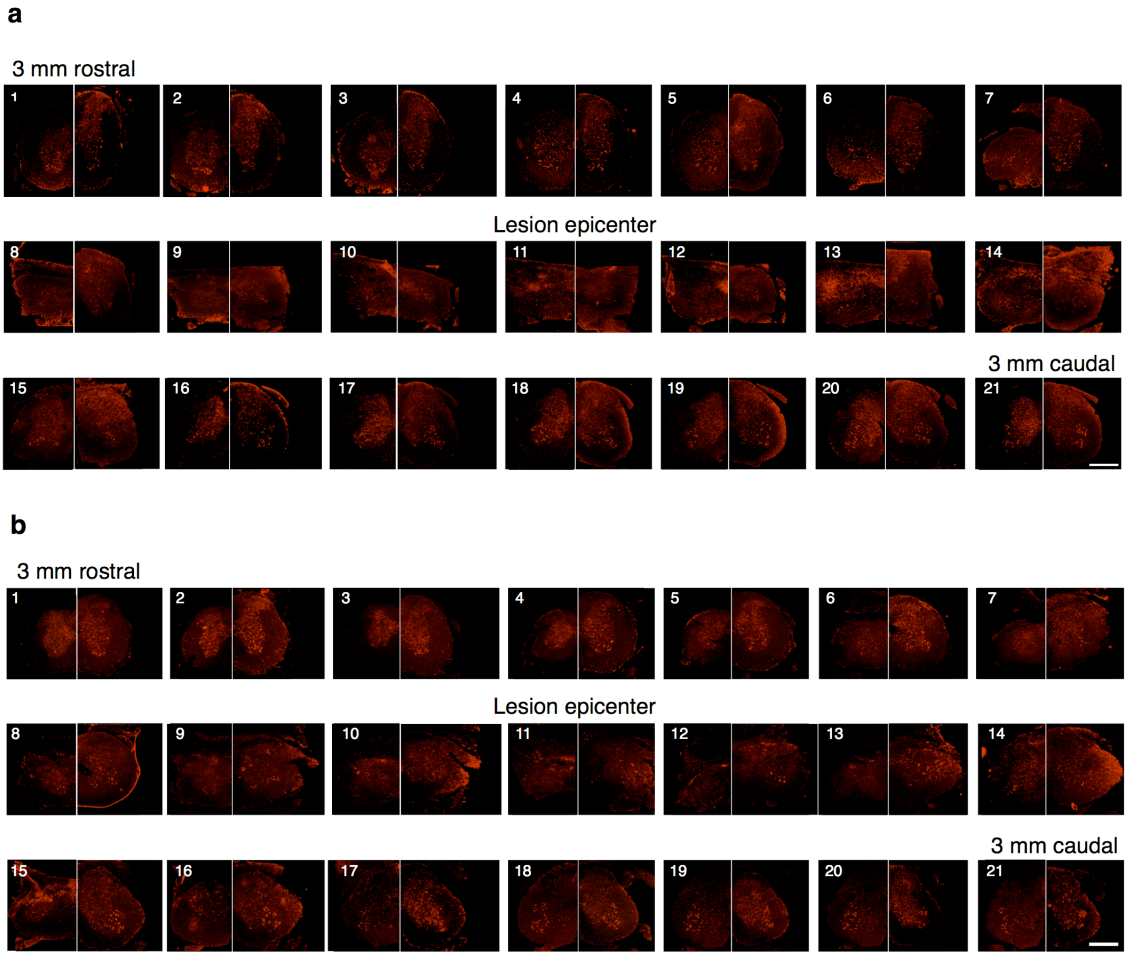
Supplementary Fig. S1 Nagai et al.



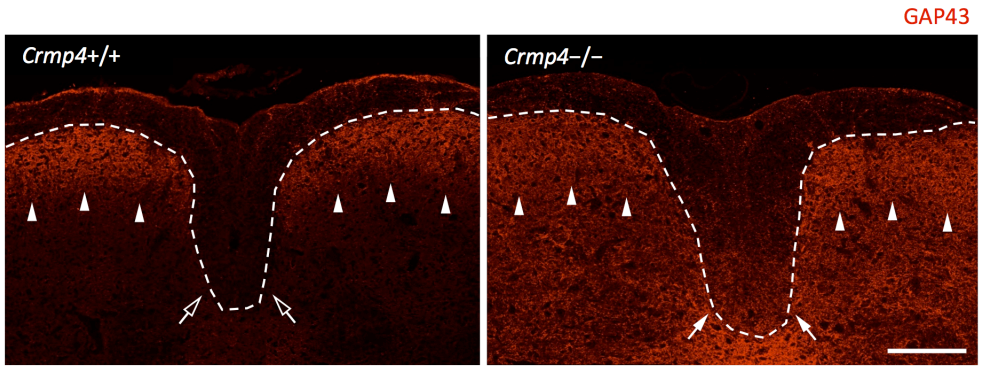
Supplementary Fig. S2 Nagai et al.



Supplementary Fig. S3 Nagai et al.



Supplementary Fig. S4 Nagai et al.



Supplementary Figure 1. CRMP4 deletion reduces non-traumatic inflammation. (**a**) Representative transverse images of thoracic spinal cords from mice with CRMP4 deletion stained with anti-Iba1 (marker for microglia/macrophages) and anti-GFAP (marker for astrocytes*)* antibodies at 3 days post induction of non-traumatic inflammation by Zymosan A. *, Zymosan A injection site; +, PBS injection site. Intraspinal micropipette insertion is confirmed to be non-traumatic by failure of PBS microinjection to activate microglia and astroglia. Scale bars = 1000 μm. (**b, c**) Areas of quantified microglial and astroglial activation in transverse sections taken every 300 μm around the injection site. n = 3. *, $P < 0.05$ compared with *Crmp4*+/+ controls. Statistical analysis by unpaired Student's *t* test. (**d, e**) Western blotting analysis with anti-Tumor necrosis factor alpha antibody after Zymosan A- or buffer-injection of spinal cords (n = 3 animals). **, $P < 0.01$. Statistical analysis by one-way ANOVA followed by Tukey's multiple comparison test. Data are mean ± S.E.M. PBS, phosphate-buffered saline; TNFα, Tumor necrosis factor alpha; NS, not significant.

Supplementary Figure 2. Serial cross sections from *Crmp4*+/+ and *Crmp4*–/– mice after SCI with myelin staining. Myelin staining of serial cross sections taken every 300 μm reveals demyelination through the lesion epicenter (#11). Sections #1–10 are rostral and #12–21 are caudal to the injury site. The left half of each spinal cord section illustrated is from a *Crmp4*+/+ mouse and the right half is from a *Crmp4*–/– mouse. The areas of myelin-positive white matter are not different among samples at 1 week post SCI, indicating that the dorsal transections to the animals were of uniform extent (**a**). However, at 4 weeks post injury, *Crmp4*–/– spinal cord exhibits significantly increased sparing of tissue, including white matter, when compared with control (**b**). The images of #11 and #14 are shown in Fig. 5d. Scale bar: 500 μm.

Supplementary Figure 3. Serial cross sections from *Crmp4*+/+ and *Crmp4*–/– mice after SCI with Nissl staining. Nissl staining of serial cross sections taken every 300 μm reveals demyelination through the lesion epicenter (#11). Sections #1–10 are rostral and #12–21 are caudal to the injury site. The left halves of the spinal cord sections are from a *Crmp4*+/+ mouse and the right halves are from a *Crmp4*–/– mouse at 1 week post injury (**a**) or at 4 weeks post injury (**b**). The images of #11 and #16 are shown in Fig. 5c. Scale bar: 500 μm.

Supplementary Figure S4. GAP43-immunolabeling in cross sections of injured spinal cords from *Crmp4*+/+ and *Crmp4*–/– mice. Axonal growth in cross sections at 1.5 mm caudal to lesion site at 4 weeks after injury was detected by immunostaining with anti-GAP43 antibody. Although there was absent of GAP43-positive fibers at the location of descending corticospinal tracts in a control spinal cord (open arrows), GAP43 signals were apparent there in a *Crmp4*–/– spinal cord (arrows). Ascending fibers from dorsal root ganglion were GAP43-positive in both genotypes (arrowheads). Scale bar: 100 μm.

Supplementary Movie 1. Locomotor recovery after spinal cord injury in mice lacking CRMP4. Example of a *Crmp4*+/+ mouse demonstrating inability to move with hindlimbs and a *Crmp4*–/– mouse bearing its body weight at 4 weeks after spinal cord injury.