# Ablation of the integrin CD11b mac-1 limits deleterious responses to traumatic spinal cord injury and improves functional recovery in mice

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## Introduction

Spinal cord injury (SCI) causes major neurological impairments including long-term sensorimotor deficits and posttraumatic neuropathic pain, with no effective treatment. In part, this reflects incomplete understanding of the complex secondary pathobiological mechanisms involved. SCI triggers microglial/macrophage activation with distinct pro-inflammatory or inflammation-resolving phenotypes, which potentiate tissue damage or facilitate functional repair, respectively. However, little work has addressed underlying molecular mechanisms that determine the balance of microglial/macrophage phenotype responses. Identifying such pathways could provide novel targets for more effective therapeutic interventions. The major integrin Mac-1 (CD11b/CD18, αMβ2 or CR3), a heterodimer consisting of αM (CD11b) and β2 (CD18) chains, is generally regarded as a pro-inflammatory receptor in neurotrauma. Multiple immune cells of the myeloid lineage express CD11b, including microglia, macrophages, and neutrophils. In the present study, we examined the effects of genetically manipulating CD11b on posttraumatic neuroinflammation and functional outcomes after SCI. We **hypothesize** that CD11b functions as a key mechanism in regulating the inflammatory phenotype of microglia/macrophages, thus affecting long-term neurological outcome after SCI.

## Methods

**SCI:** Adult C57BL/6J and CD11b KO female mice were subjected to a moderate SCI at T10. **Functional tests**: BMS, von Frey filament method. **Flow Cytometry:** Spinal cord tissue (1 cm lesion area) was harvested from injury area after transcardial perfusion, and cells were isolated with a percoll gradient following digestion in a mixture of papain, DNase, and collagenase. Neuroinflammation **analysis by Nanostring and RNAseq**: Total RNA was extracted from injured spinal cord tissue using Qiagen RNEasy Mini Kit. The neuroinflammation panel was selected for analysis by NanoString nCounterTM technologies (Nanostring, Seattle, WA) against mouse genes. Bulk RNAseq was performed on injury groups for further transcriptional analysis.

## CD11b is dysregulated after SCI

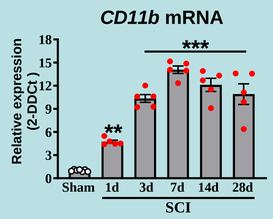


**Fig 1.** CD11b is dysregulated after SCI. Tme course experiment of C57BL/6 female mice showed significant increase of CD11b mRNA level from 1d post-injury and up to 28d after SCI. N=5 mice/group, **p<0.01, ***p<0.001 vs Sham. N=5mice/group

## CD11b deletion leads to reduced number of microglia/macrophage at lesion site

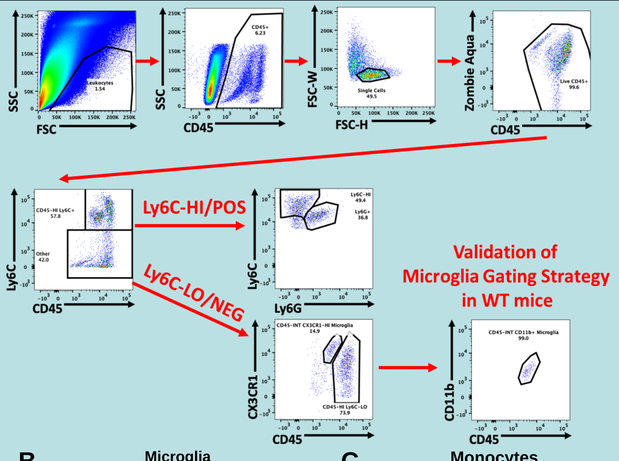


**Fig 2.** Deleting CD11b leads to decreased microglia proliferation and monocyte infiltration. (**A**) Gating strategy for flow cytometry. (**B-C**) Cell count normalized by tissue weight. *p<0.05, ***p<0.001 vs. Sham. ##p<0.01 vs. SCI/WT. N=6 mice/group.

## CD11b depletion leads to reduced ROS production after SCI

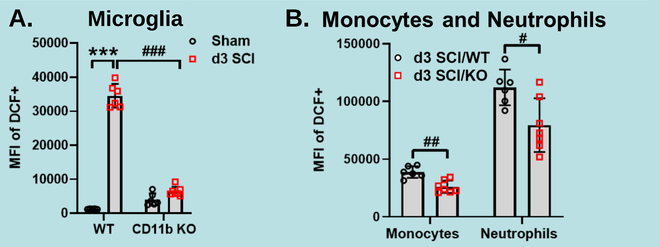


**Fig 3.** CD11b KO mice showed decreased ROS signaling by flow cytometry (DCF dye). (**A**) microglia. (**B**) monocytes and neutrophils. ***p<0.001 vs. Sham/WT, #p<0.05, ##p<0.01, ###p<0.001 vs. SCI/WT. N=6 mice/group.

## Deleting CD11b leads to Improved resolution of neuroinflammation

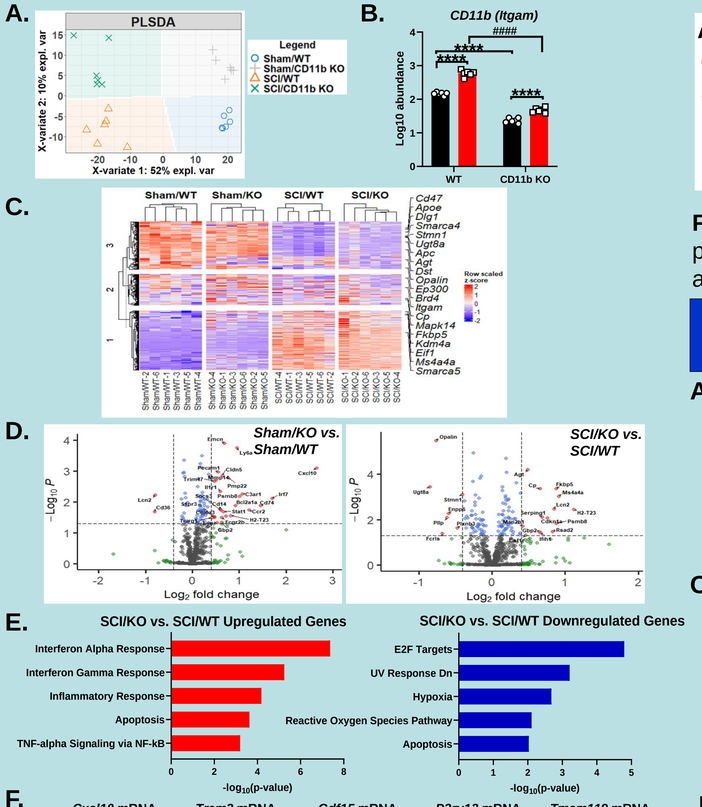


**Fig 4.** CD11b deletion leads to robust changes of SCI-induced neuroinflammation at 1d post-injury. (**A**) PLSDA of Normalized transcriptional data from Nanostring neuroinflammation panel. (**B**) Log10 abundance of CD11b gene. (**C**) Heatmap of all normalized transcriptomic data with annotation of top 20 DEGs. (**D**) Volcano plots of all DEGs in pairwise analysis of genotype effect. (**E**) Pathway analysis of DEGs in SCI/KO vs. SCI/WT comparison using the MsigDB2020 database. (**F**) qPCR was used to examine inflammatory responses in the injured spinal cord at 1d SCI. ****p<0.0001 vs. Sham groups. ####p<0.0001 vs. SCI/WT. N=6 mice/group.

## CD11b KO show upregulation of neuroregeneration pathways

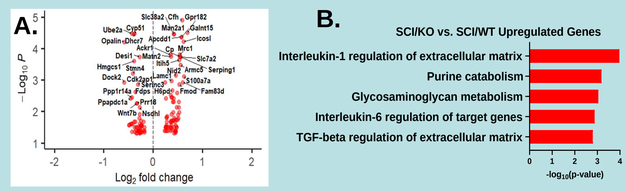


**Fig 5.** RNAseq of SCI/KO and SCI/WT lesion sites at 1d post-injury. (**A**) Volcano plot of all DEGs. (**B**) Pathway analysis of upregulated genes in pairwise.

## CD11b depletion promotes function recovery after injury

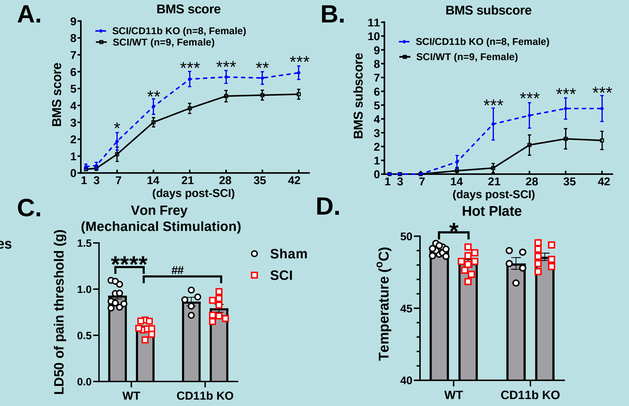


**Fig 6.** (**A-B**) CD11b KO mice show improved motor functional recovery after SCI. (**C-D**) CD11b KO mice showed reduced mechanical and thermal allodynia in Von Frey monofilament and Hot plate test at 6w after SCI. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. WT groups. ##p<0.01 vs. SCI/WT N=8-10 mice/group.

## CD11b KO mice show reduced tissue damage after injury

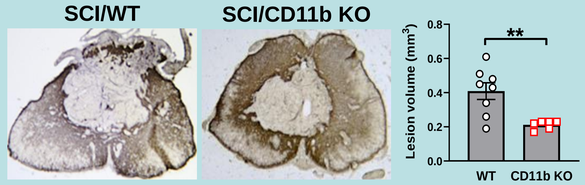


**Fig 7.** CD11b KO showed decreased lesion volume (by GFAP staining of glial scar) compared to SCI/WT mice. **p<0.01, vs. SCI/WT. N=6-10 mice/group.

## Summary

1. **SCI causes a rapid and persistent up-regulation of CD11b mRNA in the injured spinal cord after a moderate contusion.**
2. **CD11b KO mice exhibit significantly attenuated neuroinflammation, ROS production and cytokines.**
3. **Depletion of CD11b in KO mice reduces SCI-induced tissue damage and improves motor function and hyperesthesia.**
4. **Taken together our data suggest an important role for CD11b in regulating neuroinflammation and functional damage post-SCI.**