JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain

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Supplemental Figure 1

Supplemental Figure 2

Supplemental Figure 3

Supplemental Figure 4

Supplemental Figure 5

Supplemental Figure 6
Supplemental Figure 1. Immunocytochemistry shows increased MCP-1 expression in cultured astrocytes following TNF-α stimulation (10 ng/ml, 1 h). Triple staining of GFAP, MCP-1, and DAPI in non-stimulated control astrocytes (A-D) and TNF-α-stimulated astrocytes (E-H). D and H are merged images of triple staining. Scale bar, 100 μm.
Supplemental Figure 2. Intrathecal injection of TNF-α induces heat hyperalgesia in a dose-dependent manner. Heat hyperalgesia was tested 3 h after injection. * P<0.05 vs saline control, n=4.
**Supplemental Figure 3.** Spinal injection of TNF-α induces mechanical allodynia (A), heat hyperalgesia (B), and MCP-1 upregulation in the spinal cord (C) in wildtype mice. These changes are significantly reduced in TNFR1 null mice (A-C). * P<0.05 vs wild type mice, n=3.
Supplemental Figure 4. Intrathecal application of the JNK inhibitor D-JNKI-1 (2 nmol), once a day for 3 days, starting before nerve injury (indicated by small arrows) attenuates SNL-induced mechanical allodynia. * P<0.05, vs saline control, n=4.
Supplemental Figure 5. Immunohistochemistry shows MCP-1 expression in the DRG (A) and spinal cord dorsal horn (B). In the DRG, MCP-1 is constitutively expressed in neurons (A). In the spinal cord, MCP-1 is partially colocalized with CGRP, a marker for primary afferents, in the superficial dorsal horn (B). Scale bars, 100 μm in A and 50 μm in B.
Supplemental Figure 6. Bath application of MCP-1 increases the spontaneous excitatory postsynaptic currents (sEPSCs) in both young mice (3-4 week old) and adult mice (8 week old). MCP-1 induces comparable increases of both frequency (A) and amplitude (B) of sEPSCs. * P<0.05, vs pre-treatment baseline.