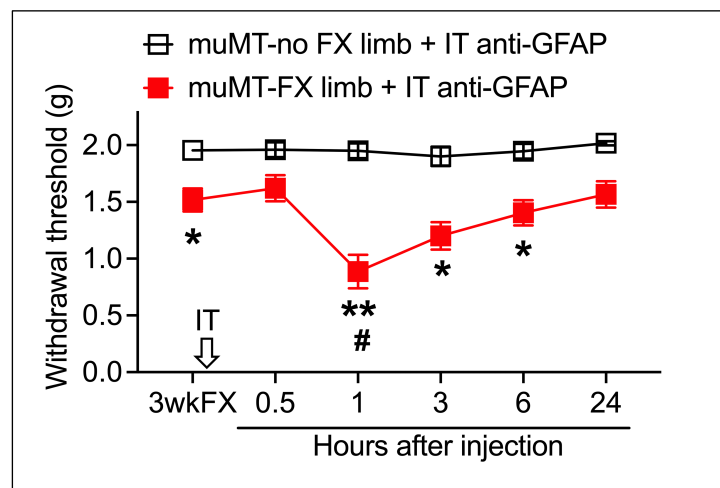


Supplemental Figure 1. Intrathecal injection of anti-GFAP antibody in muMT fracture mice caused nociceptive sensitization in the fracture hindlimb without contralateral effect.

The effects of intrathecally injecting a commercially available mouse polyclonal IgG antibody targeting a broad range of human GFAP epitopes were evaluated in muMT fracture mice.

Intrathecal injection of anti-GFAP IgG antibody (1ug/5ul, i.t.) reduced von Frey withdrawal thresholds in the fracture limb at 1 hour post injection, compared to the ipsilateral 3-week post fracture baseline. Intrathecal injection of anti-GFAP had no effect on nociceptive thresholds in the contralateral nonfracture hindlimb. A 2-way repeated measures analysis of variance was used to test treatment effects over time, using a Sidak correction test for post hoc contrasts. Data are expressed as mean values $\pm$ SEM, $n = 7$ mice per cohort. * $P < 0.05$, and ** $P < 0.01$ vs threshold in the contralateral nonfracture hindlimb at the corresponding timepoint, # $P < 0.05$ vs threshold at 3 weeks post fracture in the same group. muMT: mice lacking B cells, FX limb: fracture limb, no FX limb: the contralateral nonfracture limb, IT: intrathecal injection, GFAP: glial fibrillary acidic protein, 3wkFX: 3 weeks after fracture

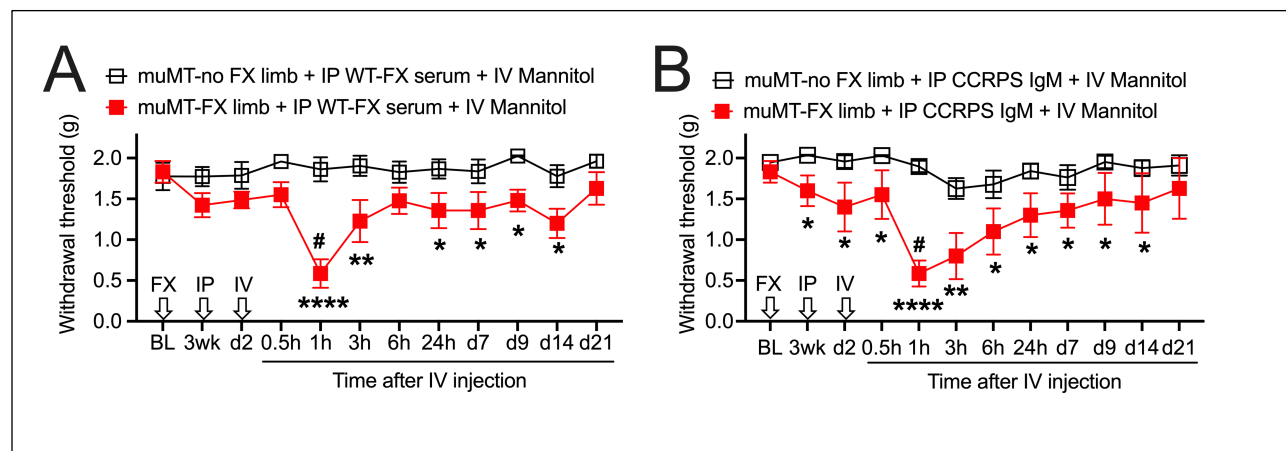


Supplemental Figure 2. Systemic injection of chronic CRPS patient IgM was pronociceptive in fracture mice after osmotic disruption of the blood spinal cord barrier.

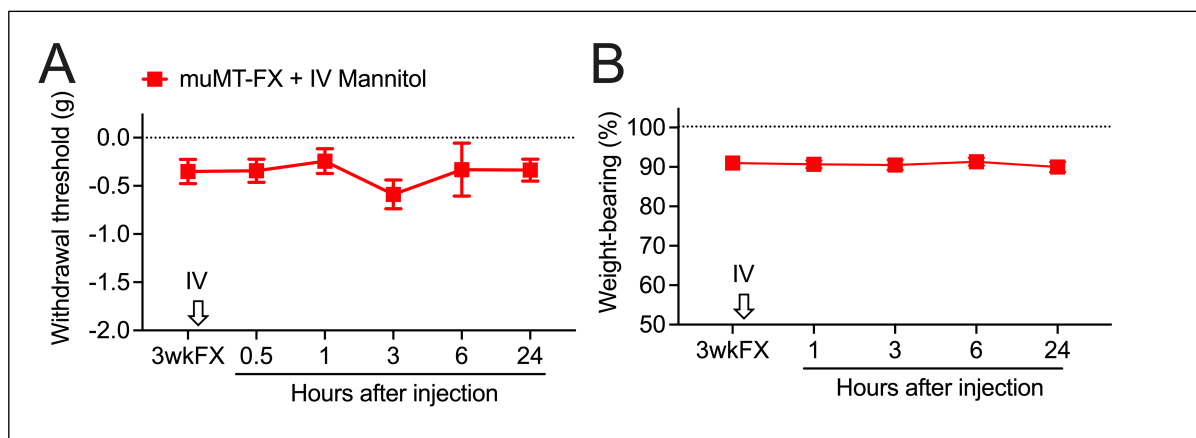
This experiment used intravenous mannitol injection to disrupt the blood spinal cord barrier in fracture mice. Three-week post fracture muMT mice were injected with 3-week wildtype

fracture mouse serum (0.5 ml, i.p.) and then 2 days later the mice were injected with mannitol (2mg/g body weight, i.v.). Mannitol injected mice developed increased fracture limb von Frey allodynia within an hour of injection, relative to 3-week post fracture thresholds (A).

Intraperitoneal injection of wildtype fracture mouse serum followed by mannitol injection 2 days later had no effect on nociceptive thresholds in the contralateral nonfracture hindlimb. When intravenous mannitol was injected into muMT fracture mice that had previously been injected with pooled chronic CRPS patient IgM (0.5 mg, i.p.), the fracture mice developed increased allodynia in the fracture limb within an hour, relative to 3-week post fracture thresholds (B). Intraperitoneal injection of chronic CRPS patient IgM followed by mannitol injection 2 days later had no effect on nociceptive thresholds in the contralateral nonfracture hindlimb. A 2-way repeated measures analysis of variance was used to test the effects treatment (fracture vs nonfracture) on the dependent variable (von Frey threshold) over time, using a Sidak correction test for post hoc contrasts. Data are expressed as mean values $\pm$ SEM, $n = 6$ mice per cohort * $P < 0.05$, ** $P < 0.01$, and **** $P < 0.0001$ vs threshold in the contralateral nonfracture hindlimb at the corresponding timepoint, # $P < 0.05$ vs threshold at 3 weeks post fracture in the same group. WT: wildtype mice, muMT: mice lacking B cells, FX: fracture, FX limb: fracture limb, no FX limb: the contralateral nonfracture limb, IP: intraperitoneal injection, IV: intravenous injection, BL: baseline prior to fracture, 3wk: 3 weeks after fracture, CCRPS: chronic complex regional pain syndrome patient

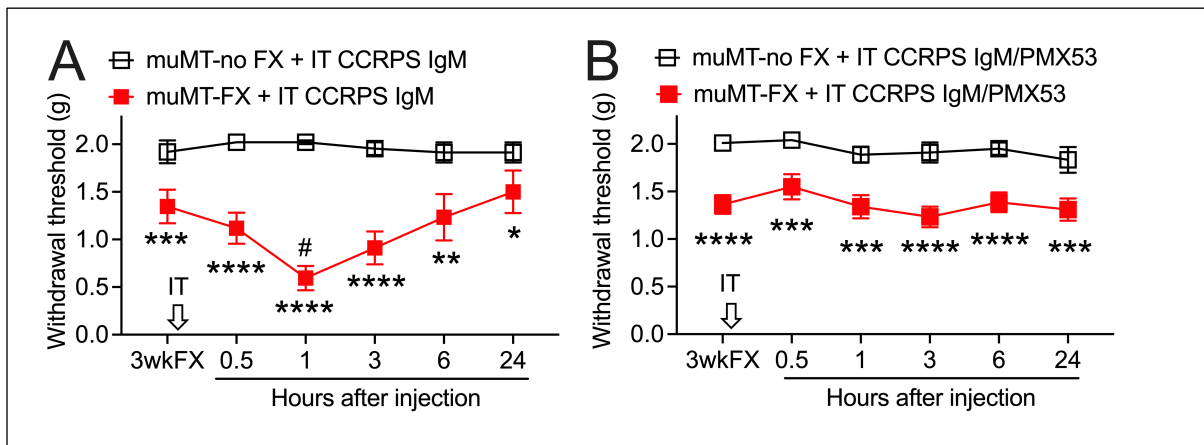


Supplemental Figure 3. Mannitol osmotic disruption of the blood spinal cord barrier in muMT fracture mice had no effect on nociceptive thresholds. This experiment used intravenous mannitol injection to disrupt the blood spinal cord barrier in muMT fracture mice lacking mature B cells and antibodies. At 3 weeks after tibia fracture and casting, the cast was removed and the next day the mice underwent hindpaw von Frey allodynia (A) and unweighting (B) testing, and then the mice were intravenously injected with mannitol (2mg/g body weight, i.v.) and nociceptive testing was repeated at 0.5, 1, 3, 6, and 24 hours post injection. Mannitol injection had no effect on fracture limb von Frey allodynia or on hindlimb unweighting. Data are expressed as mean values $\pm$ SEM, $n = 6$ mice per cohort. muMT: mice lacking B cells, FX: fracture, IV: intravenous injection, 3wk: 3 weeks after fracture



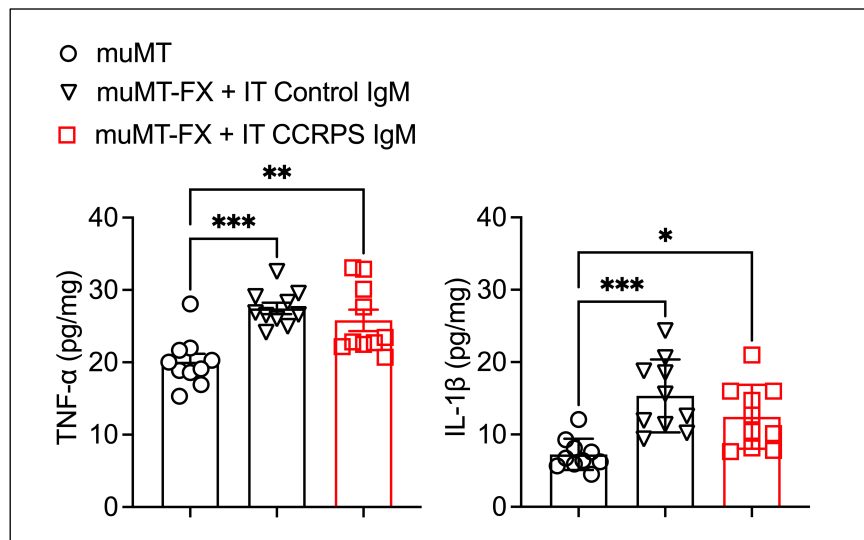
Supplemental Figure 4. C5a complement spinal signaling mediated the pronociceptive effects of intrathecal chronic CRPS patient IgM in fracture mice. Intrathecal injection of pooled chronic CRPS patient IgM (1ug/5ul, i.t.) in 3-week post fracture muMT mice increased hindpaw von Frey allodynia in the fracture limb at 1 hour post-injection, but had no pronociceptive effect in the contralateral nonfractured hindlimb (A). When the C5a receptor antagonist PMX53 (200ng, i.t.) was intrathecally co-injected with chronic CRPS patient IgM, it completely blocked IgM antibody mediated pronociceptive effects in the fracture limb and had no effect on von Frey thresholds in the contralateral nonfracture hindlimb (B). A 2-way repeated measures analysis of variance was used to test the effects of each treatment group on the dependent variables over time, using a Sidak correction test for post hoc contrasts. Data are expressed as mean values $\pm$ SEM, $n = 7$ mice per cohort. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$,

and **** $P < 0.0001$ vs threshold in the contralateral nonfracture hindlimb at the corresponding timepoint, # $P < 0.05$ vs threshold at 3 weeks post fracture in the same group. muMT: mice lacking B cells, FX: fracture limb, no FX: the contralateral nonfracture limb, IT: intrathecal injection, 3wk: 3 weeks after fracture, CCRPS: chronic complex regional pain syndrome patient, PMX53: C5a complement receptor antagonist



Supplemental Figure 5. Fracture increased spinal cytokine levels in the lumbar spinal cord. TNF and IL-1 protein levels were quantified in right side lumbar spinal cord samples collected from muMT nonfracture control mice and from 3-week post right tibia fracture muMT mice. The fracture mouse spinal cord samples were collected 1 hour after intrathecal injection of either control subject IgM or chronic CRPS patient IgM. Right tibia fracture increased TNF and IL-1 protein levels in the right lumbar spinal cord at 3 weeks post fracture (muMT fracture mice injected with control IgM vs muMT nonfracture control mice), consistent with our previous observations of neuropeptide dependent upregulated mRNA and protein expression of TNF and IL-1 pronociceptive cytokines in the lumbar spinal cord of tibia fracture mice and rats. {Shi, 2015 #2321; Wei, 2016 #2570} TNF or IL-1 protein spinal cord levels were unchanged at 1 hour post injection of chronic CRPS patient IgM, vs control subject IgM, consistent with our experimental hypothesis (Fig. 7) that intrathecal injection of chronic CRPS patient pronociceptive IgM antibodies preferentially target apoptotic neoantigens in the spinal cord, thus forming immune complexes capable of activating the C1 complex that initiates the classical complement pathway, thus generating the C5a complement components capable of binding the

C5aR receptors on dermal macrophages and spinal microglia to induce the production and secretion of pronociceptive IL-1, IL-6, and TNF cytokines. {Zhang, 2007 #2175;Ristoiu, 2013 #2177;Yan, 2012 #2626} Intrathecal injection of chronic CRPS IgM into fracture muMT mice would not be expected to alter lumbar spinal cord cytokine protein levels within an hour post injection, but could activate C5a complement within minutes of injection and stimulate microglia release of pronociceptive TNF and IL-1 protein in less than an hour. {Ji, 2016 #2994;Okusawa, 1988 #2995;Schaeffer, 2007 #2996} A one-way analysis of variance was performed, followed by a Sidak correction test for post hoc contrasts. Data are expressed as mean values $\pm$ SEM, n = 10 mice per cohort. ** P < 0.01, *** P < 0.001, and **** P < 0.0001 vs the control group. muMT: mice lacking B cells, FX: fracture, IT: intrathecal injection, CCRPS: chronic complex regional pain syndrome patient, control: normal subject



Supplemental Figure 6. Spinal cytokine signaling mediated pronociceptive effects of intrathecal chronic CRPS patient IgM in fracture muMT mice.

Intrathecal injection of pooled chronic CRPS patient IgM (1ug/5ul, i.t.) in 3-week post fracture muMT mice increased hindpaw von Frey allodynia in the fracture limb at 1 hour post-injection, but had no effect on the contralateral nonfracture hindlimb von Frey thresholds (A). A separate cohort of 3-week post fracture muMT mice underwent von Frey testing and then the mice were treated with the global cytokine inhibitor pentoxifylline (200mg/kg by gavage once daily for 2 days). Intrathecal injection of pooled chronic CRPS patient IgM into pentoxifylline pretreated

fracture mice had no pronociceptive effects in the fracture or nonfracture limbs (B). A 2-way repeated measures analysis of variance was used to test the effects of each treatment group on the dependent variables over time, using a Sidak correction test for post hoc contrasts. Data are expressed as mean values $\pm$ SEM, $n = 6$ mice per cohort * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ vs threshold in the contralateral nonfracture hindlimb at the corresponding timepoint, # $P < 0.05$ vs threshold at 3 weeks post fracture in the same group. muMT: mice lacking B cells, FX: fracture limb, no FX: the contralateral nonfracture limb, IT: intrathecal injection, PO: orally administered, 3wk: 3 weeks after fracture, CCRPS: chronic complex regional pain syndrome patient, Pent: pentoxifylline, a global cytokine inhibitor

