

Supporting Information

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SI Materials and Methods

von Frey Testing. Mice were tested using the up-down staircase method of Dixon (1, 2). Mice were placed on a perforated metal floor (with 5-mm diameter holes placed 7-mm apart) within small Plexiglas cubicles, and a set of eight calibrated von Frey fibers (Stoelting Touch Test Sensory Evaluator Kit; ranging from ~0.04 g to ~2.0 g of force) were applied to the plantar surface of the hind paw until the fibers bowed, and then held for 3 s. The threshold force required to elicit withdrawal of the paw (median 50% withdrawal) was determined twice on each hind paw (and averaged) on each testing day, with sequential measurements separated by at least 5 min. In both sham and destabilization of the medial meniscus (DMM) mice, baseline thresholds were assessed before surgery, and thresholds were assessed at the following times postsurgery: weeks 4, 8, 12, and 16.

LABORAS (Assessment of Spontaneous Behavior). Locomotive behaviors (distance, climbing, and rearing) were assessed by using the Laboratory Animal Behavior Observation Registration Analysis System (LABORAS; Metris). LABORAS is an automated system that analyzes vibrations evoked by movement of a single mouse in a cage. Pattern-recognition software then classifies and quantifies behaviors, including distance traveled, climbing, and rearing (3). Operated mice were monitored in the system for 16 h overnight, with the first hour used as an acclimation period and therefore not analyzed.

Histopathology of the Knee. At 8 wk postsurgery, histopathology of the operated knees was evaluated according to Osteoarthritis Research Society International recommendations (4) (Alison Bendele, Bolder BioPATH, Inc., Boulder CO). Joints were fixed in 10% formalin for 48 h, decalcified for 3 wk in 14% EDTA, pH 7.2, and incubated for 2 d in 10% formic acid. Knee joints were trimmed of extraneous tissue, embedded in the frontal plane, and sectioned. One section was taken from each joint at the approximate midpoint of the frontal plane. All sections were 8 μ m and were stained with Toluidine blue. Medial and lateral femoral and tibial cartilage degeneration were scored for severity of cartilage degeneration on a scale of 0–5, using the following system: 0 = no damage, 1 = superficial damage, tangential layer of collagen absent over 50% or greater of the zone surface or up to 10% loss of proteoglycan or chondrocytes in focal or diffuse distribution in zone; 2 = matrix loss extends into upper one-fourth of 50% or greater area of the zone or up to 25% loss of proteoglycan or chondrocytes in focal or diffuse distribution in zone; 3 = matrix loss extends through cartilage thickness over 50% or greater of the zone or there are focal areas of full thickness loss that are up to 25% of the width of the zone or up to 50% loss of proteoglycan or chondrocytes in focal or diffuse distribution in zone; 4 = matrix

loss extends through three-fourths of cartilage thickness over 50% or greater of the zone or there are focal areas of full thickness loss that are 26–50% of the width of the zone or up to 75% loss of proteoglycan or chondrocytes in focal or diffuse distribution in zone; 5 = matrix loss extends through entire cartilage thickness over 50% or greater of the zone or up to 100% loss of proteoglycan or chondrocytes in focal or diffuse distribution in zone. Scores were assigned with attention to zonal (inside, middle and outside) distribution of lesions and ultimately the scores (0–5) for each third were summed for each area of the joint (medial tibial plateau, medial femoral condyles, lateral tibial plateau, lateral femoral condyles) and then for the whole joint.

Scoring of the osteophytes (largest on tibial or femoral surface under evaluation) and categorization into small, medium and large was done with an ocular micrometer.

Osteophytes were scored from 0–3 using the following system: 0 = no osteophytes; 1 = small osteophytes (up to 150 μ m); 2 = medium osteophytes (151–300 μ m); 3 = large osteophytes (>301 μ m).

Total joint score is the sum of cartilage degeneration scores in the medial tibial, medial femoral, lateral medial, and lateral tibial compartments and the osteophyte scores in the medial and lateral compartments (maximum score of 66).

Calcium Imaging. The response of cultured dorsal root ganglia (DRG) neurons to the chemokine monocyte chemoattractant protein (MCP-1) (200 ng/mL; R&D Systems) was recorded through intracellular Ca^{2+} -imaging, following standard protocols (5). Cells were loaded with the fluorescent calcium-binding dye Fura-2AM (2 μ M; Life Technologies) for 30 min at room temperature in a balanced salt solution (BSS) [NaCl (140 mM), Hepes (10 mM), $CaCl_2$ (2 mM), $MgCl_2$ (1 mM), Glucose (10 mM), KCl (5 mM)]. The cells were rinsed with BSS and allowed to de-esterify for 30 min before being mounted onto a chamber that was placed onto the inverted microscope and continuously perfused with BSS. Intracellular free calcium concentration was measured by digital video microfluorometry with an intensified CCD camera coupled to a microscope and Metafluor software. Cells were illuminated with a 150W xenon arc lamp, and excitation wavelengths (340/380 nm) were selected by a filter changer. MCP-1 was applied for 3 min by adding 1 mL of solution at its final concentration directly to the bath chamber after stopping the flow. Cells were washed for 3 min before applying positive control solution (Potassium, 50 mM) (6). Three independent experiments each using DRG cells pooled from three to four mice were performed. The number of cells responding to chemokines was counted. Total number of neurons counted for each condition: age-matched naïve (4 wk, $n = 631$ neurons; 8 wk, $n = 416$), sham (4 wk, $n = 258$; 8 wk, $n = 339$), and DMM mice (4 wk, $n = 306$; 8 wk, $n = 498$).

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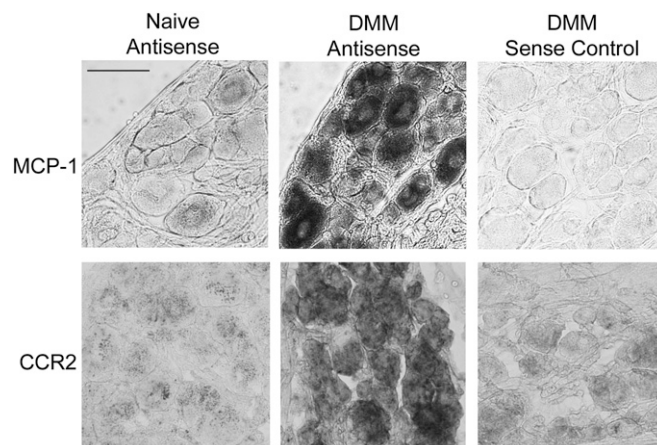


Fig. S1. Representative images of in situ hybridization using antisense probes for MCP-1 and CCR2 in DRG sections (L3–L5) taken from DMM wild-type mice at 8 wk postsurgery and age-matched naïve mice. 40× magnification. (Scale bar, 50 μ m.)

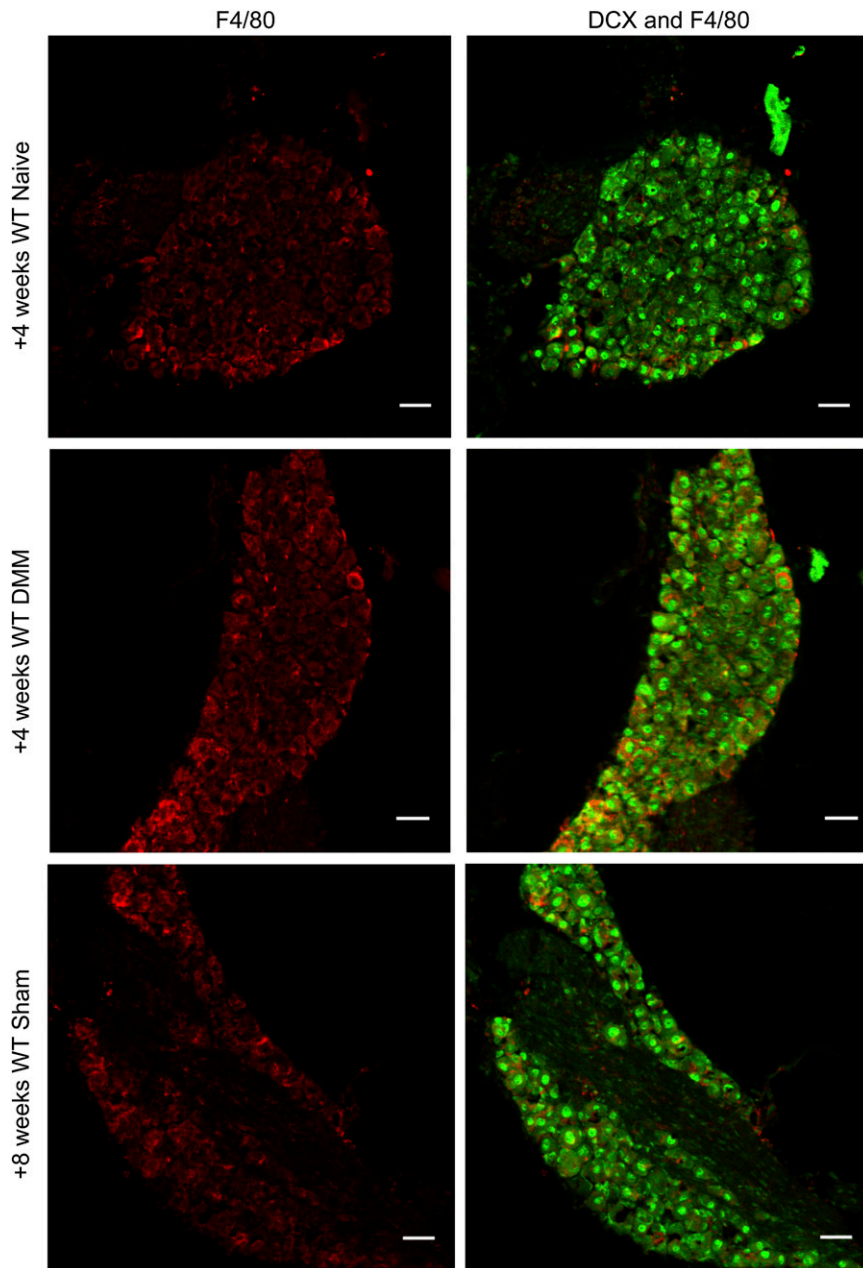


Fig. S2. Representative immunofluorescence photographs at 20 \times magnification showing F4/80 (red) and combined F4/80 and doublecortin (DCX, green) staining. (Scale bars, 50 μ m.)

