Supplemental Methods

Behavioral Studies

Mechanical allodynia testing was performed using von Frey fibers, and the up-down method to calculate a 50% paw withdrawal threshold as previously described (1,2). Briefly, mice were tested using the up-down staircase method of Dixon (3), where mice hind paws were poked with a von Frey fiber of a specific force, and if the mouse withdrew its paw, the tester would go down in force of fiber, and vice versa if the mouse did not withdraw its hind paw. The threshold force required to elicit withdrawal of the paw (median 50% withdrawal) was determined on each hind paw.

Knee hyperalgesia was determined using a Pressure Application Measurement device (Ugo Basile), which applies a specific range of forces directly to the knee joint to determine a quantitative withdrawal threshold (4-6). Briefly, mice were restrained by hand and the hind paw was lightly pinned to make the correct flexion at a similar angle for each mouse. The PAM transducer was pressed against the medial side of the knee and pressure applied against the knee. PAM software guided the user to apply a constantly increasing force (30 g/s) up to a maximum of 450 g. If the mouse tried to withdraw its knee, the force at which this occurred was recorded. Two measurements were taken and recorded per knee by an experimenter blinded to the mouse strain and the withdrawal force data were averaged. Since right and left knees showed similar trends for both mechanical allodynia and knee hyperalgesia, here we only showed the right leg data.

Grip strength testing was done on a square grid device using a grip force meter and normalized to mouse body weight. Briefly, mice were tested in 5 rounds

of 3 trials each (totaling 15 measurements), with at least 3 min in home cages between rounds. Mice were placed on the square grid and once all paws were firmly grasping the grid, mice were gently pulled along the axis of the force sensor until they were unable to retain their grip. The 15 grip strength measurements (in grams) were averaged. Mice were weighed immediately prior to the test and grip strength analyses were corrected for the body weight of each mouse (7, 8).

Histology

Following pain behavior testing, 6-month (n=10) and 20-month old (n=12) male and 6-month (n=6) and 20-month (n=6) female mice were sacrificed to collect right-side knees for histologic analysis, and peripheral blood and DRGs were collected for flow cytometry as described below. The knees were fixed in 10% natural buffered formalin, decalcified in EDTA for 3 weeks, and embedded in paraffin. The knees were serially sectioned at six-micron thickness in the coronal plane. Sections from the center of the joint were stained with 0.1% Safranin O/Fast Green for the evaluation of cartilage damage based on Osteoarthritis Research Society International recommendations (9,10). These analyses were done semi-quantitatively, and tissue sections were stained altogether when possible. Mice of different staining groups did not result in unwanted variability. For cartilage degeneration: Medial and lateral femoral condyles and tibial plateau were scored for severity of cartilage degeneration. For each cartilage surface, scores were assigned individually to each of three zones (inner, middle, outer) on a scale of 0–5, with 5 representing the most damage. The maximum score for the sum of femoral and tibial cartilage degeneration on either the medial or lateral side is 30. The

maximum possible total cartilage degeneration score for the whole joint (sum of medial and lateral sides) is 60.

We also measured chondrophyte/osteophyte width via an ocular micrometer if present. Chondrophytes (cartilage outgrowth) mature into osteophytes (fibrocartilage-capped bony outgrowth). In the process of developing into a mature osteophyte, a chondrophyte is at earlier stages of the development. Please see Suppl. Fig. 6 for representative images of the method used to quantify chondrophyte/osteophyte width regardless of maturity.

The synovial pathology was evaluated as follows: Changes in synovial hyperplasia, cellularity and fibrosis were evaluated at the synovial insertion of lateral femur, medial femur, lateral tibia, and medial tibia separately as described (11, 12). All four joint spaces were visible, except for capsule insertion in some instances, in which the score was considered 0 for that quadrant. Synovitis scoring was performed by 2 independent observers blinded to the groups. Synovial hyperplasia was defined as thickness of the lining layer with a score range of 0-3. Cellularity defined as the cell density of the synovial sublining with a score range of 0-3. Fibrosis defined as the extracellular matrix density in the synovium with a score range of 0-1. Each synovial pathology was reported as a sum score of all 4 quadrants was averaged for the two readers (AMO and JL) and reported per knee. Inter-rater reliability (weighted kappa) was calculated to be 0.579 for hyperplasia, 0.350 for cellularity, and 0.400 for fibrosis. Synovial pathologies were plotted by anatomical location in Suppl. Fig. 7.

Flow cytometry

To yield at least 1 million cells for flow cytometry, 6 DRGs were needed. For males, DRGs from 2 mice were pooled such that L3-L5 DRGs of either the right or left side from two mice were collected (3 DRGs per mouse = total 6 DRGs), *i.e.,* when the mouse number n=10, flow cytometry sample number n=5. Trends were similar on right and left sides, thus, flow cytometry plots show right side only data for males. Since we did not see differences between right and left side DRGs in males, we aimed to reduce the number of mice needed per sample for females (*i.e.,* one mouse instead of two mice per sample). Thus, for females, we pooled right and left side L3-L5 DRGs together for one mouse per sample (n=6). Lumbar levels 3-5 were chosen based on previous reports showing knee and hindpaw innervation at these levels via retrograde labeling. After dissection of the L3-L5 knee-innervating DRGs, tissue was digested with collagenase type IV (1.6mg/mL) and DNase I (200ug/mL) for 1 hour shaking at 37°C. Following digestion, cells were counted, and 1 million cells were stained with an immune cell panel of anti-mouse antibodies: PE-CD45, AF700-CD3, BV711-CD11b, PE/Cy7-MHCII, PerCP/Cy5.5-Ly6G, BV605-Ly6C, APC-F4/80, PE/Dazzle- CD206, BV650-CD11c, BV785-CX3CR1, BV421-CD163 (BioLegend), and Aqua-Live/Dead stain (ThermoFisher). After staining, sample data were acquired through an LSR Fortessa flow cytometer. Flow cytometry analysis was completed with FlowJo software (version 10). A representative gating strategy is shown in Suppl. Figs. 2,3 (DRG) and Suppl. Fig. 4 (Peripheral blood).

RNAscope

For human DRG RNAScope, slides were fixed in 4% paraformaldehyde for 30 min, followed by dehydration through 5 min of 50%, 75% and two 100% ethanol washes. Hydrogen peroxide (3%) was applied for 10 min, and target retrieval performed with a reduced time of 3 min. Protease III was applied for 30 min. After washing, sections were incubated with probes for *CCL2* and *CCL3* at 1:50 dilution at 40°C for 2 hours. The signal was amplified, and Opal dyes (Akoya Biosciences) were added to detect respective probes at 1:100 dilution. Slides were counterstained with Vectashield containing DAPI. Tissue slides were imaged on a Fluoview FV10i confocal microscope. Laser intensity for all samples was ≤9.9%. Single planes of focus were selected and processed using Fiji software, with only brightness and contrast tools used to adjust images.

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