SUPPLEMENTARY METHODS:

Histopathology:

Animals were perfused through the left cardiac ventricle with histological fixatives at 1, 2, 5, and 10 weeks post-MIA injections. For this, animals were first deeply anesthetized with an intra-peritoneal injection of Equithesin (a mixture of 12.75 mg chloral hydrate, 3 mg sodium pentobarbital, and 6.4 mg magnesium sulfate in a volume of 0.3 mL per 100 g of body weight). Following a vascular rinse (0.1% $NaNO_2$ and 0.05% $NaHCO_3$ in PBS), rats were perfused with 4 % paraformaldehyde in 0.1 M phosphate buffer (PFA), pH 7.4, with 15% (v/v) saturated picric acid, for 30 minutes. Spinal cords and L4-L6 DRGs were extracted and post-fixed for 2 hours at 4˚C in 4% PFA, then placed in 30 % sucrose in 0.1 M phosphate buffer. Spinal cords were sectioned in 50 µm-thick transverse sections in a sledge freezing microtome (Leica) and placed in PBS with 0.2% Triton X-100 (PBS+T) in wells of a 24-well tissue culture plate. DRGs were sectioned in 50 µm-thick transverse sections in a sledge freezing microtome (Leica) and collected directly on gelatin-subbed slides. Ankle joints were extracted and post-fixed in the same fixative for 4 hours at 4^{o}C, and subsequently placed in 10 % sucrose and 0.1 M cacodylate buffer solution overnight, at 4^{o}C. Ankle joints were then decalcified in 10 % ethylenediaminetetraacetic acid (EDTA) in phosphate buffered saline for 4 weeks at 4^{o}C on a shaker; the solution was changed twice a week. Subsequently, ankle joints were cryo-protected in 30% sucrose solution in cacodylate buffer at 4^{o}C overnight. To standardize sectioning of one ankle to the next, ankles were placed flat with the medial side facing down on the cryostat (Leica) specimen holder and covered with O.C.T. compound (Tissue-Tek, Torrance, CA, USA). After freezing, the ankles were trimmed by cutting 100 µm sections until a point was reached in which the talus appeared bean-shaped. Then, serial sections, 25 µm in thickness, were collected on gelatin-subbed slides, 3 sections per slide. Slides were numbered in order of collection. For each ankle-joint staining described below, 4 sections per animal were utilized, respecting the same slide numbering for each animal. This was done in order to normalize the region of interest of the tibial articular joint across all animals.

Immunohistochemistry:

Ankle joints:

Decalcified ankle joints were pre-incubated on-slide with excess PBS+T for 24 hours at 4°C. Slides were then incubated with 30 minutes of 50% ethanol and 30 minutes of 0.3% H_2O_2, with three 10 min washes with PBS between each incubation. Slides were left for 1 h at room temperature in 10% normal goat or donkey serum (Invitrogen) in PBS to block unspecific labeling. To detect immunoreactivity of the peptidergic and sympathetic fiber populations, the sections were then incubated for 48 hours at room temperature using a rabbit anti-calcitonin gene-related peptide (CGRP) (Sigma #C8198) antibody or goat anti-vesicular monoamine transporter-2 (VMAT-2) antibody (abm #Y213391) at dilutions of 1:2500 and 1:250, respectively. Following 3 rinses in PBS+T, sections were incubated at 4°C for 24 hours in goat anti-rabbit IgG biotinylated antibody (Vector #BA-1000; 1:400) and donkey anti-goat IgG biotinylated antibody (Jackson #705-066-147; 1:250). The A+B solution was prepared (1:400) in PBS+T (Vector Elite PK-6100) and added to the slides for incubation at room temperature for 1 hour. Diaminobenzidine (DAB) was added to the sections on-slide for 15 minutes, and then H_2O_2 was added for 8 to 10 minutes. Slides were subsequently rinsed with PBS and PBS+T, dehydrated with ascending concentrations of ethanol, cleared with xylene, and cover-slipped with Entellan mounting medium (Electron Microscopy Sciences). Bright field micrographs of the tibial articular surface of the tibio-talar joint were obtained using a Zeiss Axioplan 2 imaging microscope with a 40x oil-immersion objective, a high-resolution color camera and the Zeiss Zen software version 2.3.

Quantification involved six animals per time point, four sections per animal, and five images per section (of subchondral bone of tiba in the tibio-talar junction and synovium connecting the tibia and talus bones) for each time point (1, 2, 5, and 10 weeks post MIA injection).

DRGs and spinal cords:

On-slide DRG sections and free-floating spinal cord sections were washed with three 10-minute incubations in PBS+T, and then blocked in 10% normal donkey serum (Invitrogen) for 1 hour at room temperature on a shaker. DRG sections were incubated in 5% NDS in PBS+T and rabbit monoclonal anti-activating transcription factor 3 (ATF3; Abcam #ab207434; 1:500) primary antibody overnight at 4 ˚C. Spinal cord sections were incubated in 5% NDS in PBS+T and rabbit anti-ionized calcium binding adaptor molecule 1 (Iba1; Wako # 019-19741; 1:1000) and mouse anti-glial fibrillary acidic protein (GFAP; Cell Signaling # 3670S; 1:1000) primary antibodies

overnight at 4 ˚C. The following day, sections and slides were washed with three 10-minutes incubations in PBS+T and then incubated with fluorochrome-conjugated secondary antibodies in PBS+T for 2 hours at room temperature (donkey anti-rabbit Alexa 568 (Invitrogen Thermo Fisher Cat # A10042, 1:800) and donkey anti-mouse Alexa 488 (Invitrogen Thermo Fisher Cat # A21202, 1:800) for spinal cord sections, or donkey anti-rabbit Alexa 488 (Invitrogen Thermo Fisher Cat # A21206, 1:800) for DRG slides). Finally, sections were washed with three10-minute incubations of PBS+T. DRG slides were incubated with red Nissl (Neurotrace 530/615 N-21482; 1:100) in PBS-T for 30 minutes on a shaker at room temperature, washed for 10 minutes in PBS-T, followed by two 10-minute washes in PBS, and cover-slipped with mounting medium (Aqua Polymount). Spinal cord sections were mounted on gelatin subbed-slides and cover-slipped with mounting medium (Aqua Polymount). Immunofluorescence micrographs were obtained using a Zeiss Axioplan 2 imaging microscope (with 10x or 20x objectives), a high-resolution camera monochrome camera and the Zeiss Zen software version 2.3.

Quantification of DRG neurons and ATF3 expression:

Low magnification images (10X) used for quantification were taken to cover the whole DRG, and higher magnification images (20X) were obtained in order to show localization of the ATF3 signal in the nuclei of DGR neurons. At the lower magnification, one image per DRG section and 3 sections per animal were taken. The total number of cells stained with red Nissl and the number of ATF3-positive cells were counted and averaged for each animal (n = 4).

Quantification of glial cell density and dimensional ratio:

Each image was taken so that the upper limit of the photographic field was at the junction of laminae I and II and comprised part of laminae II-III. For each time point (1, 2, 5, and 10 weeks post MIA injection), quantification involved 5-6 animals, 6 sections per animal, and 1 image for each ipsilateral and contralateral dorsal horn. Extended depth of focus images were analyzed and Iba1+ and GFAP+ cell counts were performed and dimensional ratios (Iba1+ only) were obtained using an ImageJ macro as previously described [1].

Antibody specificity

We assessed the quality of all staining by omitting either primary or secondary antibodies in some sections. In absence of these antibodies, we observed no labelling. Specificity of the primary antibodies used in the experiments has been well validated in the literature. Anti-ATF3 was generated in rabbit and recognizes ATF3 in rat tissue; its specificity was tested via western blot of ATF3 knock outs in HeLa and HAP1 cell lysates (data supplied by abcam). The anti-Iba1 antibody was generated in rabbit and recognizes rat tissue. Its specificity has been validated using Iba1 expression in COS-7 cells ligated with an Iba1 cDNA fragment, where untreated COS-7 and anti-sense COS-7 showed no Iba1 signal on a western blot [2]. Anti-GFAP was generated in mouse and its specificity has been tested in SNB19 cells that show strong anti-GFAP labelling, while the HeLa cells used for negative control did not (data supplied by Cell Signalling). Anti-CGRP was generated in rabbit and its specificity was previously assessed via dot-blot against rat CGRP conjugated to BSA (data supplied by Sigma), and staining has been shown to be abolished when preincubated with rat CGRP [3]. Anti-VMAT2 was generated in goat against the SYPIGEDEESESD peptide sequence and the antibody was purified by affinity chromatography and shown to recognize VMAT2 in several species (Human, Mouse, Rat, Bovine) (data supplied by abm); we tested its specificity in lab by performing a co-localization study with another sympathetic fiber marker, a dopamineβ-hydroxylase monoclonal antibody (Medimabs Canada) and co-localization was 100%.

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