Supplementary Materials

Materials and Methods

Animals

Mice with DRG-specific expression of GCaMP6 were obtained by crossing Cre-dependent GCaMP6 mice (Rosa26-floxed-STOP-GCaMP6, Jackson Laboratory) with the sensory-neuron-specific Cre line *Advillin*^{Cre} [1] (courtesy of Fan Wang). To specifically label *Trpv1*$^{+}$ DRG neurons, we crossed *Trpv1*^{Cre} mice (Jackson Laboratory) with a Cre-dependent reporter mice (Ai14, Rosa-CAG-LSL-tdTomato-WPRE::ΔNeo, Jackson Laboratory). *Cx3cr1*^{GFP} mice (Jackson Laboratory) were used to sort GFP^{+} macrophages (MΦ) in the skin. The animals weighed 20–35 g at the time of experimentation. Adult male ICR mice (8–10 weeks) were used for behavioral and biochemical studies. All the animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of Zhejiang University. All animals were housed under a 12-h light/dark cycle with food and water available *ad libitum*. Sample sizes were estimated based on our previous studies of similar types of behavioral and biochemical analyses.

Mouse Model of Inflammatory Pain and Intraplantar Injections

Inflammatory pain was induced by intraplantar injection of carrageenan (CRG, 1.5%, 20 μL) into a hind paw. For local intraplantar injection, drugs (20 μL) were injected using a Hamilton microsyringe with a 30-gauge needle. To deliver siRNA *in vivo*, we used polyethyleneimine, a cationic polymer, as a delivery vehicle to prevent degradation and enhance cell membrane penetration of siRNAs as previously described.

Reagents

We purchased chemerin, chemerin antibody, and ELISA kit from R&D Systems, and pHrodo® Red Zymosan Bioparticles® Conjugate from Thermo Scientific (Catalog: P35364). We also purchased capsaicin, carrageenan, and ATP from Sigma.

Enzyme-linked Immunosorbent Assay (ELISA)

Mouse ELISA kits for chemerin were from R&D system (Minneapolis, MN). ELISA was performed using spinal cord and hindpaw skin tissues. Tissues were homogenized in a lysis buffer containing protease and phosphatase inhibitors. ELISA was conducted according to the manufacturer's instructions. The standard curve was included in each experiment.

Flow Cytometry

To confirm the expression of ChemR23 on DRG neurons and MΦ, we sorted *Trpv1*$^{+}$ sensory neurons from DRGs and *Cx3cr1*$^{+}$ MΦ from skin *via* fluorescence-activated cell sorting (FACS). The DRG tissue was collected from $Trpv1^{Cre+/-}$:$Ai14^{fl/-}$ mice, digested with Collagenase A (0.2 mg/mL)/Dispase-II (3 mg/mL) for 60 min at 37ºC, and then mechanically triturated by pipette. The cell suspension was centrifuged at 500 g for 5 min, and washed with Neurobasal medium, and filtered through a 100-µm mesh. The hind-paw skin tissue was collected from $Cx3cr1^{GFP}$ mice, dissociated into small strips 1–2 mm thick, and digested with Collagenase A (1 mg/mL)/Dispase-II (5 mg/mL) for 1.5 h at 37°C with continuous shaking at 150 rpm. Then, the skin cells were mechanically triturated by pipette, washed with Dulbecco's modified Eagle's medium (DMEM), and filtered through a 40-µm mesh. The FACS sorting was conducted in a Beckman moflo Astrios EQ sorter. Then, RFP^{+} or GFP^{+} cells were each collected and used for RNA extraction.

Reverse-transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from mouse spinal cord, DRGs, skin, peritoneal MΦ, and cells collected *via* FACS with the RNeasy Plus Mini Kit (Qiagen), and quantified using A260/A280 and

A260/A230 absorption. The first-strand cDNA was synthesized using the Superscript III First-Strand cDNA Synthesis Kit (Invitrogen) in accordance with the manufacturer's specifications. PCR primers were designed as follows:

Table S1 DNA primer sequences designed for RT-PCR

Target gene	primers	Genbank No.
GAPDH	F: CGTCCCGTAGACAAAATGGT R: TTGATGGCAACAATCTCCAC	NM_001289726.1
ChemR23	F: CTCCCCGTCCCTTGTCTTC R: CTGTGGAAACTACTTGCGAGT	NM_001359060.1
chemerin	F: TGCTTGCTGATCTCCCTAGC R: AACTGCACAGGTGGGTGTTT	NM_001347167.1

The mRNA levels of *chemerin* and *ChemR23* were analyzed by quantitative real-time RT-PCR using the CFX96 Real-Time RT-PCR system (Bio-Rad). The quantitative PCR reaction system contained the same amount of reversed cDNA product, 10 μL of 2 × SYBR-green mix (TaKaRa) and 200 nmol/L of forward and reverse primers in a final volume of 20 μL. *GAPDH* was used as an internal control. Relative quantities of target mRNA was analyzed by the standard $2^{-\Delta\Delta Ct}$ method. RT-PCR was performed with Taq DNA Polymerase (Takara). The cDNA samples were amplified for 35 cycles and then separated on 2% agarose gel.

Peritoneal Macrophage Culture

Peritoneal MΦ were collected by peritoneal lavage with 10 mL warm PBS containing 1 mmol/L EDTA. Cells were incubated in DMEM supplemented with 10% FBS, 50 U/mL penicillin, and 50

μg/mL streptomycin at 37°C for 2 h in Petri dishes and washed with PBS to eliminate non-adherent cells. The adherent cells were used as peritoneal MΦ. Peritoneal MΦ were used after 3 days in culture.

Phagocytosis Assay

The phagocytosis assay was modified from a previously described protocol [2]. pHrodo® Red Zymosan Bioparticles (diameter, 3 μm, Thermo Scientific, Catalog: P35364) were rinsed and reconstituted in culture medium. Particles were added onto adherent peritoneal MΦ at a concentration of 0.5 mg/mL to synchronize binding and internalization. After 30 min incubation at 37°C, non-adherent beads were removed with cold PBS and cells were fixed with 2% PFA for 10 min. Three optical fields were photographed with epifluorescence microscopy (Nikon) for quantification. Quantification of zymosan particles ingested by MΦ was conducted on at least 200 MΦ/condition, and triplicates were included for statistical analysis.

Whole-cell Patch Clamp Recordings in Dissociated Mouse DRG Neurons

DRGs were removed from mice and digested with collagenase (0.2 mg/mL)/dispase-II (3 mg/mL) for 60 min. Cells were placed on glass coverslips coated with poly-D-lysine and grown in a Neurobasal defined medium (10% fetal bovine serum and 2% B27 supplement) at 37°C with 5% CO_2 for 24 h before experiments. Whole-cell voltage-clamp recordings were performed to record capsaicin-induced currents with a MultiClamp 700B amplifier. The pipette solution contained the following (in mmol/L): 120 K-gluconate, 20 KCl, 2 $MgCl_2$, 0.5 EGTA, 2 NaATP, and 0.5 MgGTP, adjusted to pH 7.4 with KOH and osmolarity 295–300 mOsm. Extracellular solution was prepared by adding 0 mmol/L $CaCl_2$ and 2 mmol/L EGTA for Ca^{2+} chelation. It contained the following (in mmol/L): 140 NaCl, 5 KCl, 2 EGTA, 1 $MgCl_2$, 10 HEPES, 10 glucose, adjusted to pH 7.4 with NaOH. The recording chamber was continuously superfused (2–3 mL/min).

100 nmol/L capsaicin was applied for 5 s to induce an inward current. The resistance of a typical patch pipette was 5–6 MΩ. Signals were filtered at 2 kHz and digitized at 10 kHz. The recording data were analyzed using pClamp10.7 software. Voltage-clamp was performed at a holding potential of –60 mV.

Calcium Imaging

DRG neurons from Advillin-Cre;GCaMP6 (Advillin-GCaMP6) mice were cultured as described above, placed into a perfusion chamber attached to an upright microscope (Olympus BX51WIF), and imaged using a dipping objective (20×). During each imaging experiment, the cells were continuously perfused with normal external buffer (in mmol/L: 140 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 10 glucose, titrated to pH 7.4 with NaOH) using a gravity perfusion system. The fluorescence dynamics of GCaMP6 (excitation, 480 ± 10 nm) were captured with a digital camera (Hamamatsu C11440). Images were acquired at 0.5 Hz and analyzed using ImageJ software. Capsaicin, chemerin, or ATP was applied onto cells using a solenoid valve-controlled pressurized perfusion system (ALA-VM8, ALA Scientific Instruments).

Behavioral Analysis in Mice

The behavioral measurements were conducted in a blinded manner. Animals were habituated to the testing environment daily for at least 2 days before baseline testing. The room temperature and humidity remained stable for all experiments. *Hargreaves test for thermal pain*: Thermal sensitivity was tested using a Hargreaves radiant heat apparatus (IITC Life Science), the basal paw withdrawal latency in naive mice was adjusted to 9–12 s, with a cutoff of 20 s to prevent tissue damage.

Statistical Analyses

All the data are expressed as the mean ± SEM, as indicated in the figure legends. Statistical analyses were completed with Prism GraphPad 6.0. Biochemical and behavioral data were analyzed using two-tailed Student's *t*-test (two groups) and one-way or two-way ANOVA followed by the *post-hoc* Bonferroni test. The criterion for statistical significance was $P < 0.05$.

References

1. Zhou X, Wang L, Hasegawa H, Amin P, Han BX, Kaneko S, *et al*. Deletion of PIK3C3/Vps34 in sensory neurons causes rapid neurodegeneration by disrupting the endosomal but not the autophagic pathway. Proc Natl Acad Sci USA 2010, 107: 9424–9429.
2. Hong S, Tian H, Lu Y, Laborde JM, Muhale FA, Wang Q, *et al*. Neuroprotectin/protectin D1: Endogenous biosynthesis and actions on diabetic macrophages in promoting wound healing and innervation impaired by diabetes. Am J Physiol Cell Physiol 2014, 307: C1058–C1067.

Supplementary Figure and Figure Legend

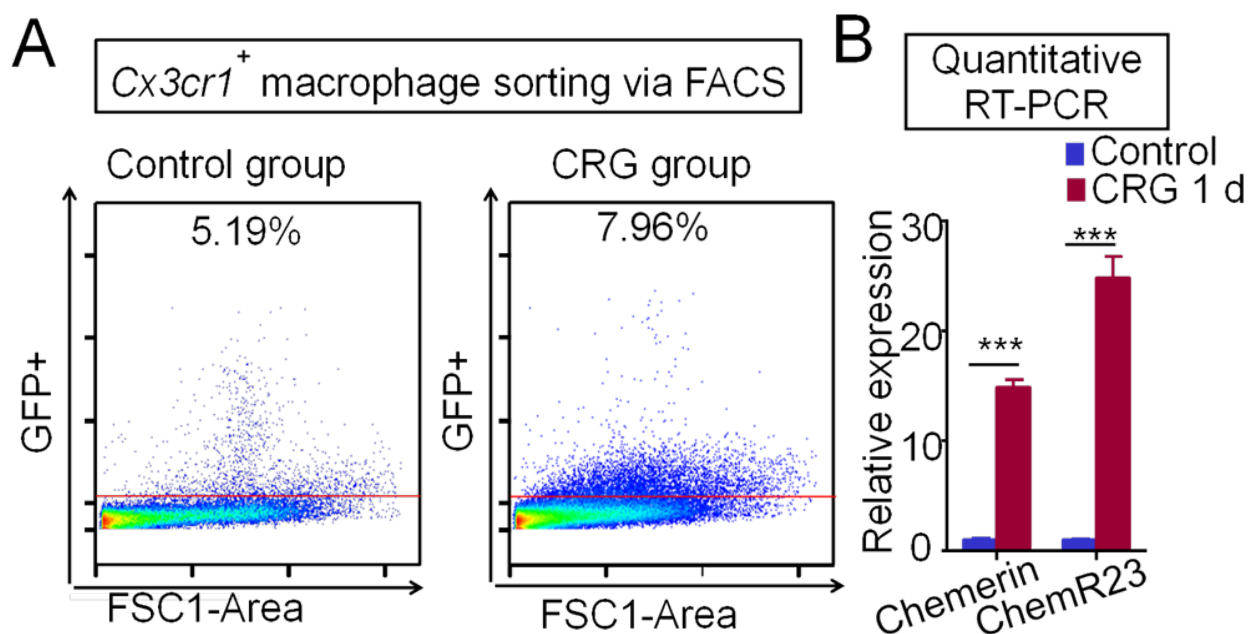

Fig. S1 *Chemerin* and *ChemR23* are strikingly upregulated in *Cx3cr1*-positive macrophages sorted *via* FACS after CRG treatment. **A** Flow cytometry showing macrophage sorting from hind paw skin of *Cx3cr1*^{GFP} mice. The GFP-positive cells with fluorescence signal exceeding the threshold (red line) were collected and used for RNA extraction (*n* = 2–3 mice/group). **B** *Chemerin* and *ChemR23* mRNA are boosted in sorted *Cx3cr1*-positive macrophages from hindpaw skin after CRG treatment (****P* <0.001 *vs* Control, unpaired Student's *t*-test. *n* = 2–3 mice/group). Data are the mean ± SEM.