

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☒	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	3D structure illumination images were acquired with DeltaVision-OMX SR (GE) Super-Resolution Microscope and reconstructed using Deltavision softWoRx 6.1.1 software (GE). Confocal images were captured with Zeiss LSM 880 with Airyscan and Zeiss Zen 2.3 (black edition) software (Zeiss Canada) Low-magnification images were captured with Zeiss Axioimager M2 imaging microscope with the Zeiss ZenPro software v.2.3 (Zeiss Canada) The cell counting, RNA scope analysis, and image intensity analysis were done using ImageJ (NIH, USA) Engulfment analysis was done with Imaris 9.7 (Oxford Instruments) Synapse quantifications and complement deposition analysis were done with Imaris 9.7 (Oxford Instruments) and Matlab 9.8 (R2020a) (The MathWorks Inc.) RNA-Seq data from GEO accession GSE111216 was analyzed using published applications in a standard fashion: Trimmomatic, tophat, bowtie, and cuffdiff. Volcano plot drawn using the R statistical package. All steps are available on demand.
Data analysis	All statistical analyses were carried out with Prism 8 software (Graphpad software) using one-way/two-way ANOVA or repeated measures ANOVA, as appropriate, followed by Bonferroni's multiple comparison tests. Unpaired two-tailed t-test was used for experimental designs with less than three groups and one variable.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

We used RNA-Seq data in figure 3a from a previously published source data: Parisien, M. et al. Genetic pathway analysis reveals a major role for extracellular matrix organization in inflammatory and neuropathic pain. Pain 160, 932-944, doi:10.1097/j.pain.0000000000001471 (2019): Omnibus accession GSE111216 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi>

We used RNA-Seq data in SuppFig. 3a from previously published source data: Tansley, S. et al. Single-cell RNA sequencing reveals time- and sex-specific responses of mouse spinal cord microglia to peripheral nerve injury and links ApoE to chronic pain. Nat Commun 13, 843, doi:10.1038/s41467-022-28473-8 (2022): Omnibus under the accession GSE162807. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE162807>

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender Study in rodents, N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample sizes. For behavior experiments our sample sizes are similar to those reported in the previous publication in the field (Mogil, J.S. et al. Pain 126, 24–34 (2006)). For the engulfment and synapse analyses our sample sizes were determined based on the pilot experiments to allow assessing sex differences in the mechanism studied. In the absence of sex difference, male and female mice were combined.
Data exclusions	In this work we included all the data generated from the experiments.
Replication	Similar increases in engulfment and synapse pruning-related proteins were found in two independent studies performed by two different experimenters. The PLX3397 and ANX M1.21 behavior, engulfment, and synapse quantification experiments were repeated twice independently in male mice and similar results were found. Results for the C3 KO studies was replicated in the C4 KO mice separately.
Randomization	The age, weight, and sex matched animals used in the experiments were randomly assigned to different experimental groups.
Blinding	For the pharmacology and KO experiments, the investigators were blind to treatment and genotype for all data acquisition and analysis. For the engulfment analysis in figure 1, the experimenter could not be blind to the conditions due to the evident morphological differences. However, for this experiment, blinding to groups was not considered necessary since the quantification procedure was fully automated.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies:

rabbit anti-Iba1 (Wako, 1:1000) cat. #019-19741
 guinea pig anti-Iba1 (Synaptic Systems, 1:500) cat. #234 004
 rat anti-CD68 (Bio-Rad, 1:500) cat. #MCA1957
 rabbit anti-CC3 (St John's Laboratory, 1:100) cat. #STJ97448
 mouse anti-VGAT (Synaptic Systems, 1:1000) cat. #131 011
 rabbit anti-VGAT (Synaptic Systems, 1:2000) cat. #131 002
 rat anti-CD11b (Bio-Rad, 1:100) cat: #MCA711
 guinea pig anti-VGLUT2 (Millipore, 1:2000) cat: #AB2251-I
 mouse anti-gephyrin (Synaptic Systems, 1:400) cat: #147 021
 rabbit anti-Homer1 (Synaptic Systems, 1:200) cat: #160 003
 goat anti-mouse C3 (MP Biomedicals, 1:100) cat: #55444
 rabbit-anti NeuN (Millipore, 1:1000) cat: #MABN140
 rabbit anti-C1q (Abcam, 1:400) cat: #182451
 goat anti-CX3CL1 (R&D systems, 1:500) cat: #AF537
 rabbit anti-CX3CR1 (Thermofisher, 1:200) cat: #702321
 Secondary antibodies: Alexa-fluorophore-conjugated secondary antibodies (Invitrogen, 1:800)
 Isolectin B4 (IB4) conjugated to Alexa 647 (Invitrogen, 1:200) cat: #I32450
 rabbit anti-ANX-M1.21 antibody (provided by Annexon Biosciences, 1:500) cat:NA
 mouse monoclonal anti-C3R (Hycult Biotech, 1:500) cat: # HM1123
 mouse monoclonal anti-C5R (Hycult Biotech, 1:500) cat: # HM1077

Validation

Antibodies used in this study have been used frequently in publications in the field (examples provided below) and/or were validated for use in IHC using KO validation.

Rabbit anti-Iba1 (Wako, 1:1000) is the most widely used antibody to label microglia and is raised against a synthetic peptide corresponding to C-terminus of Iba1: Bennett, Mariko L., et al. "New tools for studying microglia in the mouse and human CNS." *Proceedings of the National Academy of Sciences* 113.12 (2016): E1738-E1746.

Guinea pig anti-Iba1 (Synaptic Systems, 1:500) was raised against a synthetic peptide corresponding to AA 134 to 147 from rat Iba1: Masuda, Takahiro, et al. "Novel Hexb-based tools for studying microglia in the CNS." *Nature immunology* 21.7 (2020): 802-815.

Rat anti-CD68 (Bio-Rad, 1:500) monoclonal is clone FA-11 that recognizes mouse macrophage which is a homologue of human CD68: Schafer, Dorothy P., et al. "Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner." *Neuron* 74.4 (2012): 691-705.

Rabbit anti-CC3 (St John's Laboratory, 1:100) detects endogenous Active Caspase-3 protein: Györfy, Balázs A., et al. "Local apoptotic-like mechanisms underlie complement-mediated synaptic pruning." *Proceedings of the National Academy of Sciences* 115.24 (2018): 6303-6308.

Mouse anti-VGAT (Synaptic Systems, 1:1000) is a monoclonal raised against a synthetic peptide corresponding to AA 75 to 87 from rat VGAT: Lorenzo, Louis-Etienne, et al. "Enhancing neuronal chloride extrusion rescues $\alpha 2/\alpha 3$ GABA A-mediated analgesia in neuropathic pain." *Nature communications* 11.1 (2020): 1-23.

Rabbit anti-VGAT (Synaptic Systems, 1:2000) was raised against a synthetic peptide corresponding to AA 75 to 87 from rat VGAT: Babaev, Olga, et al. "IgSF9b regulates anxiety behaviors through effects on centromedial amygdala inhibitory synapses." *Nature communications* 9.1 (2018): 1-16.

Rat anti-CD11b (Bio-Rad, 1:100) monoclonal is from clone 5C6 and recognizes CD11b, also known as the integrin alpha M chain: Schafer, Dorothy P., et al. "Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner." *Neuron* 74.4 (2012): 691-705.

Guinea pig anti-VGLUT2 (Millipore, 1:2000) was raised against 18 amino acids from the C-terminal, cytoplasmic domain of rat Vesicular Glutamate Transporter 2: Gutierrez-Mecinas, Maria, et al. "Substance P-expressing excitatory interneurons in the mouse superficial dorsal horn provide a propriospinal input to the lateral spinal nucleus." *Brain Structure and Function* 223.5 (2018): 2377-2392.

Mouse anti-gephyrin (Synaptic Systems, 1:400) antibody was raised against AA 1 to 768 from rat Gephyrin: Gutierrez-Mecinas, Maria, et al. "Immunostaining for Homer reveals the majority of excitatory synapses in laminae I–III of the mouse spinal dorsal horn." *Neuroscience* 329 (2016): 171-181.

Rabbit anti-Homer1 (Synaptic Systems, 1:200) was raised against to AA 1 to 196 from human Homer1: Gutierrez-Mecinas, Maria, et al. "Immunostaining for Homer reveals the majority of excitatory synapses in laminae I–III of the mouse spinal dorsal horn." *Neuroscience* 329 (2016): 171–181.

Goat anti-mouse C3 (MP Biomedicals, 1:100) antibody was verified using our C3 KO mice.

Rabbit anti-NeuN (Millipore, 1:1000), from clone 27-4, is widely used to label neuronal soma: Schiweck, Juliane, et al. "Drebrin controls scar formation and astrocyte reactivity upon traumatic brain injury by regulating membrane trafficking." *Nature communications* 12.1 (2021): 1–16.

Rabbit anti-C1q (Abcam, 1:400) was verified in the C1q KO mice: Hong, Soyon, et al. "Complement and microglia mediate early synapse loss in Alzheimer mouse models." *Science* 352.6286 (2016): 712–716.

Goat anti-CX3CL1 (R&D systems, 1:500) was raised against recombinant rat CX3CL1/Fractalkine: Castro-Sánchez, Sara, et al. "Cx3cr1-deficiency exacerbates alpha-synuclein-A53T induced neuroinflammation and neurodegeneration in a mouse model of Parkinson's disease." *Glia* 66.8 (2018): 1752–1762.

Rabbit anti-ANX-M1.21 is an anti-idiotypic antibody that is designed to bind to the variable region of ANX-M1.21. This antibody was generated by Annexon biosciences

Rabbit anti-CX3CR1 (ThermoFisher, 1:200) was verified using our CX3CR1 KO mice.

Mouse anti-C3R is an antibody 14D4 reacts with mouse C3aR and has been validated by multiple groups: Kiss, Máté G., et al. "Cell-autonomous regulation of complement C3 by factor H limits macrophage efferocytosis and exacerbates atherosclerosis." *Immunity* 56.8 (2023): 1809–1824.

Mouse anti-C5R is a monoclonal antibody 10/92 recognizes the mouse receptor C5a (C5aR, CD88) and has been validated by multiple groups: Brennan, Faith H., et al. "The complement receptor C5aR controls acute inflammation and astrogliosis following spinal cord injury." *Journal of Neuroscience* 35.16 (2015): 6517–6531.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Species: Mouse Strain: C57BL/6 Genotype: Rosa26LSL-tdTomato reporter mouse line (Ai14) (JAX, stock #007908), Gad2-IRES-Cre mice (JAX, stock #019022), Tac1-IRES2-Cre-D mice (JAX, stock #021877), C3 KO (JAX, stock #003641), C57BL/6-Tmem119em1(cre/ERT2)Gfng/J (JAX, stock # 031820), C4 KO (provided by Dr. Michael Carroll), Cx3cr1GFP KO (JAX, stock #005582), Mrgprdtm1.1(cre/ERT2)Wql/J (JAX, stock #031286) and wild type controls Age: 6–8 weeks Sex: male and female
Wild animals	This study does not involve wild animals.
Reporting on sex	Animals of both sexes were used with the objective of detecting sex differences
Field-collected samples	N/A
Ethics oversight	Our animal protocol was approved by McGill university and followed the guidelines of the International Association for Study of Pain for pain-related studies

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A