

## Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

### Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a Confirmed

- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of 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  
*Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- 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 statistics 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.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
*Give  $P$  values as exact values whenever suitable.*
- 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  $d$ , Pearson's  $r$ ), indicating how they were calculated
- Clearly defined error bars  
*State explicitly what error bars represent (e.g. SD, SE, CI)*

*Our web collection on [statistics for biologists](#) may be useful.*

### Software and code

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

#### Data collection

Leica TCS SP 5 II CLSM data were collected using Las AF software version 2.7.3.9723. Spinning Disc Confocal Microscopy (SDCM) was performed by using a Zeiss Spinning Disc Axio Observer.Z1 and images were collected via the Zen Blue image acquisition software Version 2.3. Light sheet fluorescence microscopy (LSFM) data were generated using an Ultramicroscope II (LaVision BioTech GmbH) and collected with Inspector software Version 5.1.304. MRI scans were performed using a ClinScan 70/30 7 Tesla MRI System (Bruker). Flow cytometry data and cell sorting data were acquired by using a CytoFlex S (Beckman Coulter) and a MoFlo XDP (Beckman Coulter) system.

Individual settings for data acquisitions via the systems listed above are described in detail in the experimental procedures.

#### Data analysis

CLSM and SDCM data were processed and analysed using Huygens professional software Version 17.10 (Scientific Volume Imaging), Imaris software Version 9.1 (Bitplane) and Image J software Version 1.8.0\_112. LSFM data were processed using Imaris software Version 9.1 (Bitplane) and Image J software version 1.8.0\_112. MRI data were processed via Horos LGPL Version 3.0. Flow cytometry data and cell sorting data were analyzed via the Summit Software System, MacsQuantify (Miltenyi Biotec, Version 2.5), CytExpert (Beckman Coulter, Version 2.2.0.97), FlowJo (FlowJo, Version 7.6.5), and Kaluza software (Beckman Coulter, Version 1.5a). Statistical data analysis was performed with GraphPad Prism 5.

Differential expression analysis was performed with the DESeq2 package v.1.20.0 and volcano plots were generated with ggplot2 package in R software.  
For single cell RNA sequencing analysis Cell Ranger 2.1.0, FastQC v0.11.7, the Seurat (Version 2.3) package for R and Monocle 2 package were used.

All data analysis strategies and softwares are described precisely in the experimental procedures and supplementary table 3.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available on reasonable request from the corresponding author [G.K, S.C., A.G.]. The data are not publicly available due to comprised information that could compromise research participant privacy.

## Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

## Life sciences study design

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

|                 |  |
|-----------------|--|
| Sample size     | Sample size was determined by statistical power analysis including high significance levels ( $p < 0.05$ ). For calculation of statistical significance GraphPad Prism 5 was used. Data are presented as mean $\pm$ SEM and were analyzed using Student's t-test, Mann-Whitney U-Test, or Kruskal-Wallis H-Test with Dunn's multiple comparisons test as post hoc procedure. P values less than 0.05 were considered significant. Differential expression analysis for Bulk RNA sequencing has been performed with DESeq2. Wald test was used to calculate two-sided p-values; adjustment for multiple comparisons was performed with the Benjamini-Hochberg method. For PANTHER Overrepresentation Test Fisher's exact Test with False Discovery Rate correction. Cluster markers of SingleCell Sequencing data sets were identified using the Wilcoxon Rank Sum test. Adjusted p-values based on Bonferroni correction using all genes in the dataset. |
| Data exclusions | No data were excluded from analysis.   |
| Replication     | Minimum three independent measurements per experiment were performed and successfully confirmed results.   |
| Randomization   | Experimental groups were randomly allocated. Treated groups were housed together with control groups.  |
| Blinding        | Data analysis was performed in a blinded fashion. The results were confirmed by two investigators, who analyzed the blindet data independently.  |

## Reporting for specific materials, systems and methods

## Materials &amp; experimental systems

| n/a                                 | Involvement                         | Involved in the study       |
|-------------------------------------|-------------------------------------|-----------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Unique biological materials |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Antibodies                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Palaeontology               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Animals and other organisms |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Human research participants |

## Methods

| n/a                                 | Involvement                         | Involved in the study  |
|-------------------------------------|-------------------------------------|------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | ChIP-seq               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | MRI-based neuroimaging |

## Unique biological materials

Policy information about [availability of materials](#)

## Obtaining unique materials

All unique biological materials (genetically modified mouse strains) are available from standard commercial sources:

- C57BL/6J  
Charles River  
# 632
- C57BL/6Rj  
Janvier Labs
- Tg(Cx3cr1-cre)MW126Gsat/Mmucd MMRRC  
# 036395-UCD
- B6;129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J The Jackson Laboratory  
# 007905
- STOCK Tg(ACTB-DsRed\*MST)1Nagy/J, DsRed.T3 The Jackson Laboratory  
# 005441
- B6.129P2(C)-Cx3cr1tm2.1(cre/ERT2)Jung/J The Jackson Laboratory  
# 020940
- FVB-Tg(Csf1r-cre/Esr1\*)1Jwp/J The Jackson Laboratory  
# 019098
- C57BL/6-Gt(ROSA)26Sortm1(HBEGF)Awai/J  
The Jackson Laboratory  
# 007900
- B6;129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J The Jackson Laboratory  
# 007905
- C57BL/6-Tg(Csf1r-HBEGF/mCherry)1Mnz/J The Jackson Laboratory  
# 024046
- B6.129P-Cx3cr1tm1Litt/J The Jackson Laboratory  
# 005582

All used mouse strains are additionally listed in supplementary table 1.

## Antibodies

## Antibodies used

Antibodies used in this study are:

- ApoE  
unconjugated, Thermo Fisher, Cat: 701241, Lot: 1984882, Clone: 16H22L18, Dilution: 1:200
- CD1c-PerCP/Cy5.5  
BioLegend, Cat: 331513, Lot: B267879, Clone: L161, Dilution: 1:500
- CD11b-PE/Cy7  
BioLegend, Cat: 101216, Lot: B185646, Clone: M1/70, Dilution: 1:500
- CD11b-Alexa Fluor 488

BioLegend, Cat: 393107, Lot: B261594, Clone: LM2, Dilution: 1:500

- CD 14 PE-Cy7

BioLegend, Cat: 367111, Lot: B252403, Clone: 63D3, Dilution: 1:500

- CD15-Brilliant Violet 421

BioLegend, Cat: 232039, Lot: B263781, Clone: W6D3, Dilution: 1:500

- CD20-Brilliant Violet 421

BioLegend, Cat: 302329, Lot: B257594, Clone: 2H7, Dilution: 1:500

- CD31-PE

BioLegend, Cat: 102507, Lot: B129965, Clone: MEC13.3, Dilution: 1:500

- CD31-Alexa Fluor 647

BioLegend, Cat: 102516, Lot: B234197, Clone: MEC13.3, Dilution: 2.5µg/mouse

- CD45-Brilliant Violet 421,

BioLegend, Cat: 103133, Lot: B263588, Clone: 30-F11, Dilution: 1:500

- CD45-Alexa Fluor 700,

BioLegend, Cat: 368513, Lot: B248833, Clone: 2D1, Dilution: 1:500

- CD45.2-Alexa Fluor 700

BioLegend, Cat: 109822, Lot: B202497, Clone: 104, Dilution: 1:500

- CD68-Alexa Fluor 594

BioLegend, Cat: 137020, Lot: B239125, Clone: FA-11, Dilution: 1:400

- CD68-Alexa Fluor 647

BioLegend, Cat: 137004, Lot: B153907, Clone: FA-11, Dilution: 1:400

- CD68 unconjugated

BioLegend, Cat: 333801, Lot: B200949, Clone: Y1/82A, Dilution: 1:200

- CD68 unconjugated

Abcam, Cat: ab955, Lot: GR3230929-1, Clone: KP1, Dilution: 1:200

- CD68 -Alexa Fluor 594

R&D systems, Cat: IC20401T, Lot: 1471045, Clone: 298807, Dilution: 1:200

- CSF1R-APC

BioLegend, Cat: 135510, Lot: B183456, Clone: AFS98, Dilution: 1:500

- CSF1R-Alexa Fluor 647

BioLegend, Cat: 135530, Lot: , Clone: AFS98, Dilution: 1:200

- Claudin 2 unconjugated

Abcam, Cat: ab53032, Lot: GR314368-11, Clone: polyclonal, Dilution: 1:200

- Claudin 5 unconjugated

Abcam, Cat: ab15106, Lot: GR3182385, Clone: polyclonal, Dilution: 1:200

- Claudin 13 unconjugated

Invitrogen, Cat: PA1-24420, Lot: TA2507851, Clone: polyclonal, Dilution: 1:200

- Connexin 43 unconjugated

Sigma Aldrich, Cat: C6219, Lot: 027144804V, Clone: polyclonal, Dilution: 1:200

- Donkey anti-Rabbit IgG Alexa Fluor 647

Life Technologies, Cat: A-31573, Lot: 1563697, Clone: polyclonal, Dilution: 1:200

- Donkey anti-Rabbit IgG Alexa Fluor 488

Life Technologies, Cat: A-21206, Lot: 1644644, Clone: polyclonal, Dilution: 1:200

- E-Cadherin-PE,

BioLegend, Cat: 147303, Lot: B260705, Clone: DECMA-1, Dilution: 1:500

- F4/80-Alexa Fluor 647,

BioLegend, Cat: 123122, Lot: B212680, Clone: BM8, Dilution: 1:400

- F4/80-FITC,

BioLegend, Cat: 123108, Lot: B177257, Clone: BM8, Dilution: 1:400

- HLA-DR PE,

BioLegend, Cat: 361605, Lot: B261328, Clone: Tü36, Dilution: 1:500

- Ki67-Af647,

BioLegend, Cat: 652407, Lot: B238782, Clone: 16A8, Dilution: 1:200

- Ly6C-Alexa Fluor 488,

BioLegend, Cat: 128022, Lot: B248739, Clone: HK1.4, Dilution: 1:400

- Ly6G-Brilliant Violet,

BioLegend, Cat: 127627, Lot: B193096, Clone: 1A8, Dilution: 1:400

- Ly6G-FITC,

BioLegend, Cat: 127606, Lot: B175677, Clone: 1A8, Dilution: 1:400

- Ly6G-Alexa Fluor 488,

BioLegend, Cat: 127626, Lot: B240194, Clone: 1A8, Dilution: 1:400

- Ly6G-Alexa Fluor 647,

BioLegend, Cat: 127610, Lot: B204928, Clone: 1A8, Dilution: 1:200

- MHC II-PE,

BioLegend, Cat: 107608, Lot: B130064, Clone: M5/114.15.2, Dilution: 1:200

- Relm alpha

Abcam, Cat: ab39626, Lot: GR1287151, Clone: polyclonal, 1:200

- Trem 2 unconjugated

Abcam, Cat: ab86491, Lot: GR3207091-11, Clone: RM0139-5J46, Dilution: 1:200

- Trem2-APC,

R&D systems, Cat: FAB17291A, Lot: AADSO17111, Clone: 237920, Dilution: 1:500

- ZO-1 unconjugated, EMD Millipore, Cat: AB2272, Lot: 2905383, Clone: polyclonal, Dilution: 1:100

All used antibodies are listed in more detail in supplementary table 2, including information regarding antigen, conjugation, concentration, isotype, host reactivity, clone, source, Cat#, Lot#, dilution, and application.

## Validation

Exclusively commercially available antibodies were used. Antibody specificity, concentration and quality validation were performed by the manufacturers. Validation statements of the manufacturers can be found on their webpages:

- Abcam: <https://www.abcam.com/primary-antibodies/improving-reproducibility-with-better-antibodies>

- BioLegend: <https://www.biolegend.com/reproducibility>

- Invitrogen: <https://www.thermofisher.com/de/de/home/life-science/antibodies/invitrogen-antibody-validation.html>

- LifeTechnologies: <https://www.thermofisher.com/de/de/home/life-science/antibodies/invitrogen-antibody-validation/independent-antibody-validation.html>

- R&D Systems: <https://www.rndsystems.com/tags/antibody-validation>

- Sigma-Aldrich: <https://www.sigmaaldrich.com/technical-documents/articles/biology/antibody-standard-validation.html>

- ApoE, unconjugated, Thermo Fisher, Cat: 701241

[https://assets.thermofisher.com/TFS-Assets/LSG/certificate/Certificates-of-Analysis/701241\\_1984882.PDF](https://assets.thermofisher.com/TFS-Assets/LSG/certificate/Certificates-of-Analysis/701241_1984882.PDF)

- CD1c-PerCP/Cy5.5, BioLegend, Cat: 331513

<https://www.biolegend.com/en-us/global-elements/pdf-popup/percp-cyanine5-5-anti-human-cd1c-antibody-5182?filename=PerCPCyanine55%20anti-human%20CD1c%20Antibody.pdf&pdfgen=true>

- CD11b-PE/Cy7, BioLegend, Cat: 101216

<https://www.biolegend.com/en-us/global-elements/pdf-popup/pe-cy7-anti-mouse-human-cd11b-antibody-1921?filename=PECy7%20anti-mousehuman%20CD11b%20Antibody.pdf&pdfgen=true>

- CD11b-Alexa Fluor 488, BioLegend, Cat: 393107

<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-488-anti-human-cd11b-antibody-16010?filename=Alexa%20Fluor%20488%20anti-human%20CD11b%20Antibody.pdf&pdfgen=true>

- CD 14 PE-Cy7, BioLegend, Cat: 367111

<https://www.biolegend.com/en-us/global-elements/pdf-popup/pe-cy7-anti-human-cd14-antibody-12794?filename=PECy7%20anti-human%20CD14%20Antibody.pdf&pdfgen=true>

- CD15-Brilliant Violet 421, BioLegend, Cat: 232039 falsch! ? 323039

<https://www.biolegend.com/en-us/global-elements/pdf-popup/brilliant-violet-421-anti-human-cd15-ssea-1-antibody-12371?filename=Brilliant%20Violet%20421%20anti-human%20CD15%20SSEA-1%20Antibody.pdf&pdfgen=true>

- CD20-Brilliant Violet 421, BioLegend, Cat: 302329

<https://www.biolegend.com/en-us/global-elements/pdf-popup/brilliant-violet-421-anti-human-cd20-antibody-7192?filename=Brilliant%20Violet%20421%20anti-human%20CD20%20Antibody.pdf&pdfgen=true>

- CD31-PE, Biolegend, Cat:102507  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/pe-anti-mouse-cd31-antibody-379?filename=PE%20anti-mouse%20CD31%20Antibody.pdf&pdfgen=true>
- CD31-Alexa Fluor 647, Biolegend, Cat: 102516  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-647-anti-mouse-cd31-antibody-3094?filename=Alexa%20Fluor%20647%20anti-mouse%20CD31%20Antibody.pdf&pdfgen=true>
- CD45-BrilliantViolet 421, Biolegend, Cat:103133  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/brilliant-violet-421-anti-mouse-cd45-antibody-7253?filename=Brilliant%20Violet%20421%20anti-mouse%20CD45%20Antibody.pdf&pdfgen=true>
- CD45-Alexa Fluor 700, Biolegend, Cat: 368513  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-700-anti-human-cd45-antibody-12399?filename=Alexa%20Fluor%20700%20anti-human%20CD45%20Antibody.pdf&pdfgen=true>
- CD45.2-Alexa Fluor 700, BioLegend, Cat: 109822  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-700-anti-mouse-cd45-2-antibody-3393?filename=Alexa%20Fluor%20700%20anti-mouse%20CD45%20Antibody.pdf&pdfgen=true>
- CD68-Alexa Fluor 594, BioLegend, Cat: 137020  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-594-anti-mouse-cd68-antibody-9671?filename=Alexa%20Fluor%20594%20anti-mouse%20CD68%20Antibody.pdf&pdfgen=true>
- CD68-Alexa Fluor 647, BioLegend, Cat: 137004  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-647-anti-mouse-cd68-antibody-6422?filename=Alexa%20Fluor%20647%20anti-mouse%20CD68%20Antibody.pdf&pdfgen=true>
- CD68 unconjugated, BioLegend, Cat: 333801  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/purified-anti-human-cd68-antibody-4835?filename=Purified%20anti-human%20CD68%20Antibody.pdf&pdfgen=true>
- CD68 unconjugated, Abcam, Cat: ab955  
<https://www.abcam.com/cd68-antibody-kp1-ab955.html?productWallTab=Questions>
- CD68 -Alexa Fluor 594, R&D systems, Cat: IC20401T <https://resources.rndsystems.com/pdfs/datasheets/ic20401t.pdf>
- CD115-APC, BioLegend, Cat: 135510
- Claudin 2 unconjugated, Abcam, Cat: ab53032 <https://www.abcam.com/claudin-2-antibody-ab53032.html>
- Claudin 5 unconjugated, Abcam, Cat: ab15106 <https://www.abcam.com/claudin-5-antibody-ab15106.html>
- Claudin 13 unconjugated, Invitrogen, Cat: PA1-24420  
[https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fcertificate%2FCertificates-of-Analysis%2FMA191114\\_TA2507851.PDF&title=TG9zLU5yLiZuYnNwO1RBMjUwNzg1MQ==](https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fcertificate%2FCertificates-of-Analysis%2FMA191114_TA2507851.PDF&title=TG9zLU5yLiZuYnNwO1RBMjUwNzg1MQ==)
- Connexin 43 unconjugated, Sigma Aldrich, Cat: C6219 <https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/3/c6219dat.pdf>
- CSF1R-APC, Biolegend, Cat: 135510  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/apc-anti-mouse-cd115-csf-1r-antibody-6336?filename=APC%20anti-mouse%20CD115%20CSF-1R%20Antibody.pdf&pdfgen=true>
- CSF1R-Alexa Fluor 647, Biolegend, Cat: 135530  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-647-anti-mouse-cd115-csf-1r-antibody-12485?filename=Alexa%20Fluor%20647%20anti-mouse%20CD115%20CSF-1R%20Antibody.pdf&pdfgen=true>
- Donkey anti-Rabbit IgG Alexa Fluor 647, Life Technologies, Cat: A-31573  
[https://assets.thermofisher.com/TFS-Assets/LSG/certificate/Certificates%20of%20Analysis/1563697\\_A31573.pdf](https://assets.thermofisher.com/TFS-Assets/LSG/certificate/Certificates%20of%20Analysis/1563697_A31573.pdf)
- Donkey anti-Rabbit IgG Alexa Fluor 488, Life Technologies, Cat: A-21206  
[https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fcertificate%2FCertificates-of-Analysis%2F1644644\\_A21202.pdf&title=TG9zLU5yLiZuYnNwOzE2NDQ2NDQ=](https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fcertificate%2FCertificates-of-Analysis%2F1644644_A21202.pdf&title=TG9zLU5yLiZuYnNwOzE2NDQ2NDQ=)
- E-Cadherin-PE, Biolegend, Cat: 147303  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/pe-anti-mouse-human-cd324-e-cadherin-antibody-9276?filename=PE%20anti-mousehuman%20CD324%20E-Cadherin%20Antibody.pdf&pdfgen=true>
- F4/80-Alexa Fluor 647, BioLegend, Cat: 123122  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-647-anti-mouse-f4-80-antibody-4074?filename=Alexa%20Fluor%20647%20anti-mouse%20F480%20Antibody.pdf&pdfgen=true>
- F4/80-FITC, BioLegend, Cat: 123108  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/fitc-anti-mouse-f4-80-antibody-4067?filename=FITC%20anti->

mouse%20F480%20Antibody.pdf&pdfgen=true  
 - HLA-DR PE, BioLegend, Cat: 361605  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/pe-anti-human-hla-dr-antibody-9390?filename=PE%20anti-human%20HLA-DR%20Antibody.pdf&pdfgen=true>

- Ki67-AF647, BioLegend, Cat: 652407 <https://www.biolegend.com/Default.aspx?id=18921>

- Ly6C-Alexa Fluor 488, BioLegend, Cat: 128022  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-488-anti-mouse-ly-6c-antibody-6756?filename=Alexa%20Fluor%20488%20anti-mouse%20Ly-6C%20Antibody.pdf&pdfgen=true>

- Ly6G-Brilliant Violet, BioLegend, Cat: 127627  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/brilliant-violet-421-anti-mouse-ly-6g-antibody-7161?filename=Brilliant%20Violet%20421%20anti-mouse%20Ly-6G%20Antibody.pdf&pdfgen=true>

- Ly6G-FITC, BioLegend, Cat: 127606  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/fitc-anti-mouse-ly-6g-antibody-4775?filename=FITC%20anti-mouse%20Ly-6G%20Antibody.pdf&pdfgen=true>

- Ly6G-Alexa Fluor 488, BioLegend, Cat: 127626  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-488-anti-mouse-ly-6g-antibody-7085?filename=Alexa%20Fluor%20488%20anti-mouse%20Ly-6G%20Antibody.pdf&pdfgen=true>

- Ly6G-Alexa Fluor 647, BioLegend, Cat: 127610  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/alexa-fluor-647-anti-mouse-ly-6g-antibody-4780?filename=Alexa%20Fluor%20647%20anti-mouse%20Ly-6G%20Antibody.pdf&pdfgen=true>

- MHC II-PE, BioLegend, Cat: 107608  
<https://www.biolegend.com/en-us/global-elements/pdf-popup/pe-anti-mouse-i-a-i-e-antibody-367?filename=PE%20anti-mouse%20I-AI-E%20Antibody.pdf&pdfgen=true>

- Trem 2 unconjugated, Abcam, Cat: ab86491 <https://www.abcam.com/trem2-antibody-rm0139-5j46-ab86491.html>

- Trem2-APC, R&D systems, Cat: FAB17291A <https://resources.rndsystems.com/pdfs/datasheets/fab17291a.pdf>

- ZO-1 unconjugated, EMD Millipore, Cat: AB2272  
[http://www.merckmillipore.com/DE/de/product/Anti-ZO-1-Antibody,MM\\_NF-AB2272?ReferrerURL=https%3A%2F%2Fwww.google.com%2F#documentation](http://www.merckmillipore.com/DE/de/product/Anti-ZO-1-Antibody,MM_NF-AB2272?ReferrerURL=https%3A%2F%2Fwww.google.com%2F#documentation)

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

### Laboratory animals

In this study the following mouse lines were used:

- C57BL/6  
Strain: C57BL/6J, Source: Charles River, Identifier: 632
- C57BL/6  
Strain: C57BL/6JRj, Source: Janvier Labs
- Cx3cr1cre:R26-tdTomato  
Strain: Tg(Cx3cr1-cre)MW126Gsat/Mmucd, Source: MMRRC, Identifier: 036395-UCD
- DsRed  
Strain: STOCK Tg(ACTB-DsRed\*MST)1Nagy/J, DsRed.T3, Source: The Jackson Laboratory, Identifier: 005441
- Cx3cr1creER  
Strain: B6.129P2(C)-Cx3cr1tm2.1(cre/ERT2)Jung/J, Source: The Jackson Laboratory, Identifier: 020940
- CSF1RcreER  
Strain: FVB-Tg(Csf1r-cre/Esr1\*)1Jwp/J, Source: The Jackson Laboratory, Identifier: 019098
- iDTR  
Strain: C57BL/6-Gt(ROSA)26Sortm1(HBEGF)Awai/J, Source: The Jackson Laboratory, Identifier: 007900
- tdTomato  
Strain: B6;129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J, Source: The Jackson Laboratory, Identifier: 007905
- CD115DTR  
Strain: C57BL/6-Tg(Csf1r-HBEGF/mCherry)1Mnz/J, Source: The Jackson Laboratory, Identifier: 024046
- Cx3cr1gfp  
Strain: B6.129P-Cx3cr1tm1Litt/J, Source: The Jackson Laboratory, Identifier: 005582

All mice were housed under "specific pathogen-free" (SPF) conditions at the animal facilities of the University of Erlangen, Germany. Male and female mice at an age of 8-18 weeks were used.

Wild animals

This study does not include wild animals.

Field-collected samples

This study does not include field-collected samples.

## Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Synovial biopsies were obtained from knee joints of patients diagnosed with osteoarthritis (OA) and rheumatoid arthritis (RA), respectively. RA patients fulfilled the 2010 EULAR/ACR criteria of RA. All patients were  $\geq 18$  years of age.

Recruitment

OA patients were recruited at the Department of Trauma Surgery, Universitätsklinikum Erlangen and RA patients were recruited at the Department of Internal Medicine 3 - Rheumatology and Immunology, Universitätsklinikum Erlangen. All patients signed an informed consent.

## Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Sample preparation is described in detail in the supplemental experimental procedures.

Instrument

Flow cytometry was performed with a CytoFLEX S, Beckman Coulter. Sorting of cells was performed with a MoFlo XDP, Beckman Coulter.

Software

Flow cytometry data and cell sorting data were analyzed via the Summit Software System, CytExpert, FlowJo, and Kaluza software.

Cell population abundance

Purity of sorted cells was confirmed by flow cytometry analysis.

Gating strategy

FACS strategies are provided in detail in the extended data and supplementary experimental procedures.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.