# nature portfolio

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# **Reporting Summary**

Nature Portfolio 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 Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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
	$\square$ The exact sample size (n) 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  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 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>
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\times$	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 <u>statistics for biologists</u> contains articles on many of the points above.

#### Software and code

Policy information about availability of computer code

Data collection Images of micrographs were captured using a confocal laser scanning microscope (C2 ver4.10, Nikon).

 $Immunostained \ cell \ were \ collected \ using \ FACSAria \ by \ BD \ FACSDiva \ 4.1, \ or \ SONY \ cell \ sorter \ SH \ 800 \ series \ by \ software \ ver \ 2.1 \ .$ 

Data analysis Adobe Photoshop CC 2018, ImageJ, Statcel-the Useful Addin Forms on Excel-4th ed, FlowJo software (V10), IBM SPSS (V23).

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 <u>availability of data</u>

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

All data supporting the findings of this study are found within the manuscript and its Supplementary information, and are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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Field-specific reporting				
Please select the o	ne below that is the best fit for your r	research. If you are not sure, read the appropriate sections before making your selection. iences Ecological, evolutionary & environmental sciences		
For a reference copy of t	the document with all sections, see <u>nature.com</u> ,	<u>/documents/nr-reporting-summary-flat.pdf</u>		
Life scier	nces study design			
All studies must dis	sclose on these points even when the	disclosure is negative.		
Sample size	minimum of three biologically independ	determine sample size, but our sample sizes are similar to those reported in previous publications. A lent samples were tested, and experiments performed in at least 2 independent instances (mostly 3) sed on pilot experiments and previous experience with similar experiments in the laboratory. Data and to achieve power.		
Data exclusions	No data was excluded.			
Replication	Behavioral experiments on mice were conducted with a sufficient number of animals (6 to 33) to take individual differences into account. Experiments were conducted at least five times. All experiments were performed in multiple independent experiments as indicated in the figure legends. All attempts for littermates were successfully performed by multiple investigators using independent litter of mice.			
Randomization	Transgenic mice were predetermined by mouse genotype and therefore could not be randomized. Littermates were assigned into the control or knockout groups after genotyping. In all the behavioral tests, however, examiners were always blind to the genotypes of mice, the kinds of treatments, and the sides of hind paws that received injections. After the evaluation was done, the behavioral data were analyzed by a different researcher. C57BL/6J mice were randomly assigned to treatment and control groups. All mice were age-matched.			
Blinding	Experimentors was blinded to the identify of mice being analyzed in behavioral tests. For experiments other than behavioral tests, experimentors were not blinded. Blinding was not possible as predominately one person was responsible for performing each experiment and carrying out data analysis. Blinding was not relevant because the quantification of signal intensity in Immunoblot or images, and analysis of gene expression level using thermal cycler were performed under the same conditions as in the control group, respectively.			
Reportin	g for specific ma	terials, systems and methods		
'		terials, experimental systems and methods used in many studies. Here, indicate whether each material, t sure if a list item applies to your research, read the appropriate section before selecting a response.		
Materials & ex	Materials & experimental systems Methods			
n/a Involved in the study		a Involved in the study		

Antibodies

Eukaryotic cell lines Palaeontology and archaeology

Animals and other organisms Human research participants

Clinical data

Dual use research of concern

ChIP-seq

| Flow cytometry

MRI-based neuroimaging

# **Antibodies**

Antibodies used

Mouse anti-NF200 (N0142, Sigma-Aldrich, clone N52) 1:1000, AB 477257 Mouse anti-PGP9.5 (ab8189, Abcam, clone 13C4 / I3C4) 1:500, AB 306343 Mouse anti-NeuN (MAB377, Millipore, clone A60) 1:150, AB\_2298772 Mouse anti-GFAP (MAB360, Millipore, clone GA5) 1:500, AB\_11212597 Rabbit anti-c-Fos (226003, Synaptic Systems) 1:10000, AB\_2231974 Rabbit anti-TRPV1 (KM018, Trans Genic) 1:100, AB\_1627247 Rabbit anti-TrkA (ab76291, Abcam) 1:150-1:1000, AB 1524514 Rabbit anti-Iba1 (019-19741, Wako) 1:500, AB\_839504 Rabbit anti-F4/80 (28463-1-AP, Proteintech) 1:2000, AB\_2881149 Rabbit anti-NGF (sc-548, Santa Cruz Biotechnology) 1:200-1:1000, AB\_632011 Rabbit anti-GFP (A6455, Thermo Fisher Scientific) 1:5000, AB\_221570 Rabbit anti-SNX25 (13294-1-AP, Proteintech) 1:500, AB 2192549 Rabbit anti-Nrf2 (sc-722, Santa Cruz Biotechnology) 1:500, AB\_2108502

Rabbit anti-HO-1 (ADI-SPA-896, Enzo Life Sciences,) 1:500, AB\_10614948

Rabbit anti-TGFbRI (sc-398, Santa Cruz Biotechnology) 1:200, AB 632493 Rabbit anti-GAPDH (ABS16, Merk Millipore) 1:2000, AB\_10806772 Rat anti-MHCII (NBP1-43312, Novus Biologicals) 1:100, AB 10006677 Rat anti-F4/80 (NB600-404, Novus Biologicals) 1:500, AB\_10003219 Rat anti-CD117 (MAB1356, R&D Systems) 1:100, AB 2131131 Rat anti-CCR2 (NBP1-48337, Novus Biologicals) 1:200, AB 10011101 Rat anti-GFP (04404-84, Nacalai Tesque) 1:5000, AB\_10013361 Goat anti-CD206 (AF2535, R&D Systems) 1:500-1:1000, AB 2063012 Alexa Fluor 488-anti-CD11b (101219, BioLegend, clone, M1/70) 1:100, AB 493545 Alexa Fluor 647-anti-F4/80 (123121, Biolegend, clone, BM8) 1:100, AB\_893480 APC anti-CD64 (139306, BioLegend, clone, X54-5/7.1) 1:25, AB 11219391 APC/Cyanine7 Ly6C (128025, BioLegend, clone HK1.4) 1:100, AB\_10643867 Biotin anti-CD4 (100403, BioLegend, clone GK1.5) 1:100-1:200, AB\_312688 Biotin anti-CD8a (100703, BioLegend, clone 53-6.7) 1:100-1:200, AB 312742 Biotin anti-Ly6G/Ly6C (108403, BioLegend, clone Gr1) 1:100-1:200, AB\_313368 Biotin anti-NK1.1 (108703, BioLegend, clone PK136) 1:100-1:200, AB 313390 Biotin-anti-CD19 (115504, BioLegend, clone 6D5) 1:100, AB\_313639 Biotin anti-CD19 (13-0193-81, eBioscience, clone 1D3) 1:100-1:200, AB\_657657 Biotin anti-MHCII (107603, BioLegend, clone M5/114.15.2) 1:100, AB\_313318 Biotin anti-F4/80 (123105, BioLegend, clone BM8) 1:100-1:500, AB 893499 Biotin anti-CD3 (100243, BioLegend, clone 17A2) 1:100, AB\_2563946 Biotin anti-TER119 (116203, BioLegend, clone TER-119) 1:100, AB\_313704 Biotin anti-Ly6G (127603, BioLegend, clone 1A8) 1:100, AB\_1186105 FITC-anti-MHCII (107605, BioLegend, clone M5/114.15.2) 1:100, AB\_313320 PE/Cyanine7 anti-CD45 (103113, BioLegend, clone 30-F11) 1:100, AB 312979 PE anti-CD11b (101207, BioLegend, clone M1/70) 1:100, AB\_312790 PE anti-CD45 (103106, BioLegend, clone 30-F11) 1:100, AB\_312971 BV421 anti-mouse CD45 (103131, BioLegend, clone 30-F11) 1:00, AB 10899570 Brilliant violet 421 anti-CD24 (101825, BioLegend, clone M1/69) 1:100, AB\_10901159 PerCP-Cyanine5.5 streptavidin (405214, BioLegend) 1:200, AB 2716577 APC-Streptavidin (405207, BioLegend) 1:200 TruStain FcX™ PLUS (CD16/32 antibody, 156604, BioLegend, clone S17011E) 1:100, AB\_2783138

#### Validation

All antibodies used are commercially available as described in the manuscript. We selected antibody clones that have been extensively used in the literature. We also titrated all antibodies prior to experiments.

Mouse anti-NF200 (N0142, Sigma-Aldrich, clone N52)

https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/355/551/n0142dat.pdf

Mouse anti-PGP9.5 (ab8189, Abcam, clone 13C4 / I3C4)

https://www.abcam.co.jp/pgp95-antibody-13c4--i3c4-ab8189.html

Mouse anti-NeuN (MAB377, Millipore, clone A60)

https://www.merckmillipore.com/JP/ja/product/Anti-NeuN-Antibody-clone-A60,MM\_NF-MAB377

Mouse anti-GFAP (MAB360, Millipore, clone GA5)

 $https://www.merckmillipore.com/JP/ja/product/Anti-Glial-Fibrillary-Acidic-Protein-Antibody-clone-GA5, MM\_NF-MAB360-MIR-$ 

Rabbit anti-c-Fos (226003, Synaptic Systems)

https://sysy.com/product/226003

Rabbit anti-TRPV1 (KM018, Trans Genic)

 $https://www.sceti.co.jp/images/upload/export/1013\_KM018\_p.pdf$ 

Rabbit anti-TrkA (ab76291, Abcam)

https://www.abcam.co.jp/pan-trk-antibody-ep1058y-ab76291.html

Rabbit anti-Iba1 (019-19741, Wako)

https://labchem-wako.fujifilm.com/us/product/detail/W01W0101-1974.html

Rabbit anti-F4/80 (28463-1-AP, Proteintech)

https://www.ptglab.co.jp/products/F4-80-Antibody-28463-1-AP.htm

Rabbit anti-NGF (sc-548, Santa Cruz Biotechnology)

https://datasheets.scbt.com/sc-548.pdf

Rabbit anti-GFP (A6455, Thermo Fisher Scientific)

https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-6455

Rabbit anti-SNX25 (13294-1-AP, Proteintech)

https://www.ptglab.co.jp/products/SNX25-Antibody-13294-1-AP.htm

Rabbit anti-Nrf2 (sc-722, Santa Cruz Biotechnology)

https://datasheets.scbt.com/sc-722.pdf

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Rabbit anti-HO-1 (ADI-SPA-896, Enzo Life Sciences,)
https://www.enzolifesciences.com/ADI-SPA-896/ho-1-polyclonal-antibody/
Rabbit anti-TGFbRI (sc-398, Santa Cruz Biotechnology)
https://datasheets.scbt.com/sc-398.pdf
Rabbit anti-GAPDH (ABS16, Merk Millipore)
https://www.merckmillipore.com/JP/ja/product/Anti-GAPDH-Antibody,MM NF-ABS16
Rat anti-MHCII (NBP1-43312, Novus Biologicals)
https://www.novusbio.com/products/mhc-class-ii-i-a-i-e-antibody-m5-114152 nbp1-43312
Rat anti-F4/80 (NB600-404, Novus Biologicals)
https://www.novusbio.com/products/f4-80-antibody-ci-a3-1 nb600-404
Rat anti-CD117 (MAB1356, R&D Systems)
https://www.rndsystems.com/products/mouse-cd117-c-kit-antibody-180627_mab1356
Rat anti-CCR2 (NBP1-48337, Novus Biologicals)
https://www.novusbio.com/products/ccr2-antibody nbp1-48337
Rat anti-GFP (04404-84, Nacalai Tesque)
https://www.nacalai.co.jp/ss/ec2/EC-srchdetl.cfm?jump=EC-srchdetl&syohin=0440484&syubetsu=3
Goat anti-CD206 (AF2535, R&D Systems)
https://www.rndsystems.com/products/mouse-mmr-cd206-antibody_af2535
Alexa Fluor 488-anti-CD11b (101219, BioLegend, clone, M1/70)
https://www.biolegend.com/ja-jp/clone-search/alexa-fluor-488-anti-mouse-human-cd11b-antibody-2700
Alexa Fluor 647-anti-F4/80 (123121, Biolegend, clone, BM8)
https://www.biolegend.com/ja-jp/sean-tuckers-tests/alexa-fluor-647-anti-mouse-f4-80-antibody-4074
APC anti-CD64 (139306, BioLegend, clone, X54-5/7.1)
https://www.biolegend.com/ja-jp/clone-search/apc-anti-mouse-cd64-fcgammari-antibody-7874
APC/Cyanine7 Ly6C (128025, BioLegend, clone HK1.4)
https://www.biolegend.com/ja-jp/explore-new-products/apc-cyanine7-anti-mouse-ly-6c-antibody-6758?GroupID=BLG5853
Biotin anti-CD4 (100403, BioLegend, clone GK1.5)
https://www.biolegend.com/ja-jp/products/biotin-anti-mouse-cd4-antibody-247
Biotin anti-CD8a (100703, BioLegend, clone 53-6.7)
https://www.biolegend.com/ja-jp/productstab/biotin-anti-mouse-cd8a-antibody-152
Biotin anti-Ly6G/Ly6C (108403, BioLegend, clone Gr1)
https://www.biolegend.com/ja-jp/neuroscience-1/biotin-anti-mouse-ly-6g-ly-6c-gr-1-antibody-457?GroupID=BLG4876
Biotin anti-NK1.1 (108703, BioLegend, clone PK136)
https://www.biolegend.com/ja-jp/productstab/biotin-anti-mouse-nk-1-1-antibody-428
Biotin-anti-CD19 (115504, BioLegend, clone 6D5)
https://www.biolegend.com/ja-jp/products/biotin-anti-mouse-cd19-antibody-1527
Biotin anti-CD19 (13-0193-81, eBioscience, clone 1D3)
https://www.thermofisher.com/antibody/product/CD19-Antibody-clone-eBio1D3-1D3-Monoclonal/16-0193-81
Biotin anti-MHCII (107603, BioLegend, clone M5/114.15.2)
https://www.biolegend.com/fr-lu/products/biotin-anti-mouse-i-a-i-e-antibody-365?
pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Biotin%20anti-mouse%20I-A/I-E%
20Antibody.pdf
Biotin anti-F4/80 (123105, BioLegend, clone BM8)
https://www.biolegend.com/ja-jp/products/biotin-anti-mouse-f4-80-antibody-4066?GroupID=BLG5319
Biotin anti-CD3 (100243, BioLegend, clone 17A2)
https://production.biolegend.com/ja-jp/products/biotin-anti-mouse-cd3-antibody-10023
Biotin anti-TER119 (116203, BioLegend, clone TER-119)
https://www.biolegend.com/ja-jp/productstab/biotin-anti-mouse-ter-119-erythroid-cells-antibody-1864? Group ID=Imported GROUP 1-10-erythroid-cells-antibody-1864. Group ID=Imported GROUP
Biotin anti-Ly6G (127603, BioLegend, clone 1A8)
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https://www.biolegend.com/ja-jp/products/biotin-anti-mouse-ly-6g-antibody-4772

FITC-anti-MHCII (107605, BioLegend, clone M5/114.15.2)

https://www.biolegend.com/ja-jp/clone-search/fitc-anti-mouse-i-a-i-e-antibody-366?GroupID=BLG11931

PE/Cyanine7 anti-CD45 (103113, BioLegend, clone 30-F11)

https://www.biolegend.com/ja-jp/products/pe-cyanine7-anti-mouse-cd45-antibody-1903

PE anti-CD11b (101207, BioLegend, clone M1/70)

https://www.biolegend.com/ja-jp/products/pe-anti-mouse-human-cd11b-antibody-349

PE anti-CD45 (103106, BioLegend, clone 30-F11)

https://www.biolegend.com/ja-jp/explore-new-products/pe-anti-mouse-cd45-antibody-100

BV421 anti-mouse CD45 (103131, BioLegend, clone 30-F11)

https://www.biolegend.com/ja-jp/explore-new-products/brilliant-violet-421-anti-mouse-cd45-antibody-7253

Brilliant violet 421 anti-CD24 (101825, BioLegend, clone M1/69)

https://www.biolegend.com/ja-jp/explore-new-products/brilliant-violet-421-anti-mouse-cd24-antibody-7323

PerCP-Cyanine5.5 streptavidin (405214, BioLegend)

https://www.biolegend.com/ja-jp/products/percp-cyanine5-5-streptavidin-4212

AAPC-Streptavidin (405207, BioLegend)

https://www.biolegend.com/ja-jp/explore-new-products/apc-streptavidin-1470

TruStain FcX™ PLUS (CD16/32 antibody, 156604, BioLegend, clone S17011E)

https://www.biolegend.com/en-us/punchout/punchout-products/trustain-fcx-plus-anti-mouse-cd16-32-antibody-17085? GroupID=GROUP20

### Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) 293T (RRID:CVCL\_0063), RAW264.7 (RRID:CVCL\_0493)

Authentication 293T cells (ECACC 12022001), RAW264.7 (ECACC 91062702)

Mycoplasma contamination Cell-lines were not tested for mycoplasma contamination.

Commonly misidentified lines (See <u>ICLAC</u> register)

N/A

# Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

C57BL/6J, RRID:IMSR JAX:000664

Mlc1Tgmice (B6; CBB6(129)-Tg(Mlc1-tTA)2Rhn), RBRC05450

 $Snx25\ constitutive\ KO\ (Snx25+/-)\ mice\ (C57BL/6N-Atm1Brd\ Snx25tm1a(KOMP)Wtsi/NjuMmucd,\ strain\ number,\ T001400)\ ,\ RRID:MMRRC\_068035-UCD$ 

CAG-Flpo mice (B6.Cg-Tg(CAG-FLPo)/10sb), RBRC09982

Advillin-Cre mice (B6.Cg-Tg(Avil-Cre/ERT2)AJwo/J) (Jackson Laboratory, Stock No: 032027), RRID:IMSR\_JAX:032027

Cx3cr1CreERT2 mice (B6.129P2(C)-Cx3cr1tm2.1(Cre/ERT2)Jung/J) (Jackson Laboratory, Stock No: 020940), RRID:IMSR\_JAX:020940

Ai39 mice, RCL-eNpHR3.0-EYFP, Jackson Laboratory, Stock No: 014539), RRID:IMSR\_JAX:014539

Ai32 mice, RCL-ChR2(H134R)/EYFP, Jackson Laboratory, Stock No: 012569), RRID:IMSR\_JAX:012569

GFP mice (C57BL/6-Tg (CAG-EGFP)), RBRC00267

All the protocols for the animal experiments were approved by the Animal Care Committee of Nara Medical University in accordance with the policies established in the NIH Guide for the Care and Use of Laboratory Animals. This study was also carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org/).

Wild animals

No wild animals were used in this study.

Field-collected samples

No field collected samples were used in this study.

Ethics oversight

All the protocols for the animal experiments were approved by the Animal Care Committee of Nara Medical University in accordance with the policies established in the NIH Guide for the Care and Use of Laboratory Animals. This study was also carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org/).

# 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 Skin from mice were collected and dissociated using Multi Tissue Dissociation Kit 1 (Miltenyi Biotec, Germany).

Instrument FACSAria (BD), Cell Sorter SH800 (Sony)

Software Data was collected using BD FACSDiva Software and analyzed using FlowJo software (Tree Star).

Cell population abundance Purity of sorted macrophages was more than 94%.

Gating strategy

For flow cytometry gating strategy for skin macrophages under inflammatory condition, cells isolated from hind paw skin were stained with CD11b, CD45 and F4/80. Living leukocytes were identified as 7-AAD negative CD45+ cells within medium forward scatter (FSC) and low side scatter (SSC) population. Macrophage compartment was identified as CD11b+F4/80+ cells

dendritic cells (CD64- Ly6C- MHCII+) in the lineage- CD11b+ live cells were sorted.

within living leukocytes.

Dermal macrophages are defined by Fc-g receptor 1 (CD64) expression in lineage (CD3, CD19, Ly6G, NK1.1, TER119, and Langerhans cell marker CD24) negative CD45+ CD11b+ Ly6C- population and subdivided by the expression of MHCII. CD45 positive cells were isolated using magnetic beads from enzymatically digesting saline-perfused mouse skins, and then myeloid populations, including dermal macrophages (CD64+ Ly6C- MHCII+), dermal monocytes (CD64- Ly6C+ MHCII lo), and dermal

For flow cytometry gating strategy for dermal myeloid cells including macrophages in mixed BM chimeric mice, dermal myeloid cells including macrophages were selectively collected from the skin of the mixed BM chimeric mice by FACS using FVS700, CD11b, CD45.1, CD45.2 and lineage (CD3, CD19, NK1.1, TER119, Ly6G) marker expression.

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