

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 [Editorial Policies](#) and the [Editorial Policy Checklist](#).

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 (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. 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

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

For imaging data collection: Zen Blue (v 2012) and Zen Black (v8.1) (Carl Zeiss Microscopy)
For FACS data collection: FACSDiva (BD, v8.0.1).
For Western Blot data collection: Image Lab TM Touch Software (Bio-Rad, v6)

Data analysis

Kymographs were analyzed using a Matlab program which is available at GitHub (<https://github.com/KMGlaser/Bambach-et-al.git>). Mast cell clusters were analyzed using ClusterQuant2D. ClusterQuant2D code is provided at: <https://zenodo.org/record/7684553>.
Single-cell RNA sequencing analysis was done with the RaceID3 (v0.2.3) algorithm (Herman et al, 2018 Nat Methods 15, 379) and is available on CRAN (<https://cran.r-project.org/>) and RStudio (v4.0.2 GUI 1.72, source code as described previously, DOI: <https://doi.org/10.1038/nature12175>). Paired-end reads were aligned to the transcriptome using bwa (version 0.6.2-r126; <https://bioweb.pasteur.fr/packages/pack@bwa@0.6.2>).

For Imaging data analysis: Imaris (Bitplane, v9.1.2 and v9.5.1), Fiji (v2.1.0/1.53) and Huygens (SVI; v20.04)
For FACS data analysis: FlowJo (BD, v10.6.1)
For qPCR data analysis: Excel (Microsoft, v16)

For statistical data analysis and generation of graphs: Graphpad Prism (v9.3.1)

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](#)

Single-cell RNA-sequencing data have been deposited in Gene Expression Omnibus (GEO) with the accession code GSE205412 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE205412>). Genome annotation ENCODE VM9 (<https://hgdownload.soe.ucsc.edu/gbdb/mm10/>) was used for mapping single-cell RNA-sequencing data. All other data supporting the findings of this study are included in the article, its supplementary materials or source data.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

| | |
|-----------------------------|--|
| Reporting on sex and gender | <input type="text" value="This study doesn't include human research participants."/> |
| Population characteristics | <input type="text" value="N/A"/> |
| Recruitment | <input type="text" value="N/A"/> |
| Ethics oversight | <input type="text" value="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

Life sciences study design

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

| | |
|-----------------|---|
| Sample size | <input type="text" value="No statistical methods were used to predetermine sample size. They were determined based on our previous publications, prior experience or pilot experiments."/> |
| Data exclusions | <input type="text" value="For most experiments: No data exclusion methods were used. For image analysis of mast cell proximity to arterioles: Cells at ACTA2-positive structures without characteristic arteriolar morphology (capillaries) were manually excluded from the analysis. Sc-RNAseq analysis: A very small cell subset showed additional macrophage features (cluster 10) (ED Fig. 5c), which we excluded from further analysis."/> |
| Replication | <input type="text" value="Reproducibility of the experimental findings was verified using biological replicates and independent experiments. The numbers are indicated in the respective figures (individual data points). Most experimental findings were replicated by independent experiments through several lab members."/> |
| Randomization | <input type="text" value="Experimental groups were defined by inhibitor treatment or by the genotype. Mast cells and mast cell clusters were randomly chosen for tracking, cell size analysis or cluster size determination, respectively."/> |
| Blinding | <input type="text" value="Experimentalists were blinded regarding the genotype of mice or BMCC cultures. Mouse numbers were used as identifiers."/> |

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

Methods

| n/a | Involved in the study |
|-------------------------------------|---|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Antibodies |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern |

| n/a | 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 |

Antibodies

Antibodies used

IgE coating of mast cells
 Mouse Anti-Dinitrophenyl (DNP) IgE, clone: SPE-7; provided by Marc Schmidt-Supprian, TU Munich, Germany; used in all assays 1:300 (1 μ g/ml)

BMMC & PMC validation by FACS

Rat Anti-CD117 Brilliant Violet 421-conjugated; BioLegend; Cat# 105827; 1:200
 Armenian Hamster Anti-Fc ϵ R1 α Alexa Fluor 647-conjugated; BioLegend; Cat# 134309; 1:200

Fibronectin staining

Rabbit Anti-fibronectin; Sigma-Aldrich; Cat# F3648; 1:200
 Donkey Anti-rabbit Alexa Fluor 488-conjugated; Invitrogen; Cat# A-21206; 1:400

Mast cell maturation and integrin expression

Rat Anti-CD16/CD32 Antibody; BD Biosciences; Cat# 553142; 1:250
 Armenian Hamster Anti-Fc ϵ R1 α Alexa Fluor 647-conjugated; BioLegend; Cat# 134309; 1:200
 Armenian Hamster Anti-CD29 (Integrin beta 1) PE-conjugated; BioLegend; Cat# 102208; 1:400
 Rat Anti-CD117 Brilliant Violet 421-conjugated; BioLegend; Cat# 105827; 1:800
 Rat Anti-CD49f (Integrin alpha 6) Alexa Fluor[®] 488-conjugated; BioLegend; Cat# 313608; 1:400
 Armenian Hamster IgG Isotype Control PE-conjugated; BioLegend; Cat# 400907; 1:400
 Rat Anti-CD11b (Integrin alpha M) PE-conjugated; BD Biosciences; Cat# 557397; 1:400
 Rat Anti-CD18 (Integrin beta 2) PE-conjugated; clone C71/16; BD Biosciences; Cat# 553293; 1:400
 Rat Anti-CD49e (Integrin alpha 5); PE-conjugated, BD Biosciences; Cat# 557447; 1:400
 Rat Anti-CD51 (Integrin alpha V) PE-conjugated BD; Biosciences; Cat# 551187; 1:400
 Armenian Hamster Anti-CD61 (Integrin beta 3) PE-conjugated; BD Biosciences; Cat# 553347; 1:400
 Rat Anti-Integrin β 7 Chain PE-conjugated; BD Biosciences; Cat# 557498; 1:400
 Rat Anti-CD41 (Integrin alpha IIb) FITC-conjugated; BD Bioscience; Cat# 553848; 1:400
 Rat Anti-CD49d (Integrin alpha 4) PE-conjugated; Thermo Fisher Scientific; Cat# 12-0492-82; 1:400
 Rat IgG2a kappa Isotype Control PE-conjugated; Thermo Fisher Scientific; Cat# 12-4321-80; 1:400
 Rat IgG2b kappa Isotype Control PE-conjugated; Thermo Fisher Scientific; Cat# 12-4031-82; 1:400
 Rat IgG2a Isotype Control Alexa Fluor 488-conjugated; Thermo Fisher Scientific; Cat# R2a20; 1:400
 Rat IgG1 kappa Isotype Control FITC-conjugated; Thermo Fisher Scientific; Cat# 11-4301-81; 1:400
 Rat Anti-CD18 (Integrin beta 2) FITC-conjugated; clone C71/16; BD Biosciences; Cat. 553292; 1:200
 Rat Anti-CD29 Alexa Fluor 488 conjugated (Integrin beta 1, HMBeta1-1); BioLegend; Cat# 102211; 1:200
 Rat IgG2b kappa isotype control FITC conjugated; ThermoFisher Scientific; Cat# 11-4031-82; 1:400
 Rat Anti-CD117 APC-conjugated; ThermoFisher Scientific; Cat# 17-1172-83; 1:200
 Armenian Hamster Anti-Fc ϵ R1 α PE/CY7-conjugated; BioLegend; Cat# 134318; 1:200
 Armenian Hamster IgG isotype control Alexa Fluor 488-conjugated; BD Bioscience; Cat# 53-4888-80; 1:200

Talin-1 depletion analysis

Mouse Anti-talin, clone:8d4; Sigma-Aldrich; Cat# T3287; 1:300 for FACS and 1:1000 for Western Blot
 Donkey Anti-mouse Cy3TM-conjugated; Jackson ImmunoResearch Labs; Cat# 715-165-150; 1:800
 Rabbit Anti-actin; Sigma-Aldrich; Cat# A2066; 1:2000
 Rabbit Anti-mouse HRP-conjugated Antibody; Agilent Dako; Cat# P0161; 1:5000
 Swine Anti-rabbit HRP-conjugated Antibody; Agilent Dako; Cat# P0217; 1:5000

Ear skin whole mount immunofluorescence analysis

Mouse Anti-smooth muscle actin (ACTA2) Alexa Fluor 405-conjugated; Abcam; Cat# ab210128; 1:500
 Mouse Anti-smooth muscle actin (ACTA2) Cy3TM-conjugated; Sigma-Aldrich; Cat# C6198; 1:500
 Rat anti-CD31; BD Bioscience; Cat# 550274; 1:150
 Rabbit Anti-collagen IV; Abcam; Cat# ab19808; 1:500
 Rat Anti-ACKR1 Alexa Fluor 488-conjugated, clone 6B7, provided by Aude Thiriou, Department of Immunology, Harvard Medical School, Boston; 1:300
 Rabbit Anti-Desmin; Abcam; Cat# ab32362; 1:300
 Rat Anti-endomucin; Invitrogen; Cat# 14-5851-81; 1:500
 Rabbit Anti-fibronectin; Sigma-Aldrich; Cat# F3648; 1:200
 Rabbit Anti-Lamc1; Sigma-Aldrich; Cat# HPA001909; 1:300
 Goat Anti-Mcpt6; R&D Systems; Cat# AF3736; 1:600
 Anti-MITF polyclonal; Atlas Antibodies; Cat# HPA003259; 1:100
 Rabbit Anti-NG2; Sigma-Aldrich; Cat# AB5320; 1:200

Rat Anti-PDGFR α ; Invitrogen; Cat# 14-4321-82; 1:200
 Rabbit Anti-Vimentin; Abcam; Cat# ab92547; 1:300
 Goat Anti-rabbit Alexa Fluor 405-conjugated; Thermo Fisher Scientific; Cat# A-31556; 1:300
 Donkey Anti-goat Alexa Fluor 488-conjugated; Thermo Fisher Scientific; Cat# A-11055; 1:500
 Donkey Anti-rabbit Alexa Fluor 568-conjugated; Thermo Fisher Scientific; Cat# A10042; 1:500
 Donkey Anti-rabbit Alexa Fluor 488-conjugated; Invitrogen; Cat# A-21206; 1:500
 Donkey Anti-rat Alexa Fluor 647-conjugated; Abcam; Cat# ab150155; 1:500
 Donkey Anti-rat Alexa Fluor 405-conjugated; Abcam; Cat# ab175670; 1:500
 Rabbit Anti-Red Fluorescent Protein (RFP); Rockland; Cat# 600-401-379; 1:1000
 Goat Anti-GFP Dylight™ 488-conjugated; Rockland; Cat# 600-141-215; 1:1000

Immunofluorescence analysis of adhesion structures in mast cells

Mouse Anti-talin, clone:8d4; Sigma-Aldrich; Cat# T3287; 1:200
 Mouse Anti-vinculin; Sigma-Aldrich; Cat# V9264; 1:200
 Rat Anti-CD29 (Integrin beta 1, 9EG7); BD Biosciences; Cat# 553715; 1:200
 Mouse Anti-paxillin; BD Biosciences; Cat# 610051; 1:200
 Rat Anti-CD49e (Integrin alpha 5); BD Biosciences; Cat# 557446; 1:200
 Goat Anti-rat Alexa Fluor 568-conjugated; Invitrogen; Cat# A-11077; 1:500
 Goat Anti-mouse Alexa Fluor 647-conjugated; Invitrogen; Cat# A-21237; 1:500
 Donkey Anti-mouse Cy3TM-conjugated; Jackson ImmunoResearch Labs; Cat# 715-165-150; 1:1000
 Rabbit Anti-fibronectin; Sigma-Aldrich; Cat# F3648; 1:200
 Goat Anti-rabbit Alexa Fluor 405-conjugated; Thermo Fisher Scientific; Cat# A-31556; 1:500
 anti-rat CyTM3; Jackson ImmunoResearch; Cat: 712-166-153; 1:1000

RNA-FISH

Rat Anti-nidogen (NG2); Thermo Fisher; Cat# MA1-06501; 1:300
 Donkey Anti-rat Alexa Fluor 405-conjugated; Abcam; Cat# ab175670; 1:500

FACS sort for single-cell RNA sequencing

Mouse Anti-CD45.2 Brilliant Violet 711™-conjugated; BioLegend; Cat# 109847; 1:100
 Armenian Hamster Anti-CD3e PE-conjugated; Thermo Fisher Scientific; Cat# 2-0031-83; 1:100
 Rat Anti-CD4; PE-conjugated BioLegend; Cat# 100407; 1:100
 Rat Anti-CD8a PE-conjugated; Thermo Fisher Scientific; Cat# 12-0081-82; 1:100
 Armenian Hamster Anti-CD11c (Integrin alpha X)PE-conjugated; BioLegend; Cat# 117307; 1:100
 Rat Anti-CD19 PE-conjugated; Thermo Fisher Scientific; Cat# 12-0193-83; 1:100
 Mouse Anti-NK-1.1 PE-conjugated; BioLegend; Cat# 108707; 1:100
 Rat Anti-F4/80 PE-conjugated; Thermo Fisher Scientific; Cat# MF48004; 1:100

Validation

All commercial antibodies, validation informations can be found on the antibody registry (<https://antibodyregistry.org>) or company websites:

Armenian Hamster Anti-CD11c (Integrin alpha X)PE-conjugated; PRID: AB_313776
 Armenian Hamster Anti-CD29 (Integrin beta 1) PE-conjugated; PRID: AB_312885
 Armenian Hamster Anti-CD3e PE-conjugated; PRID:AB_465496
 Armenian Hamster Anti-CD61 (Integrin beta 3) PE-conjugated; PRID: AB_394800
 Armenian Hamster Anti-Fc ϵ R1 α Alexa Fluor 647-conjugated; PRID: AB_1626097
 Armenian Hamster Anti-Fc ϵ R1 α PE/CY7-conjugated; PRID AB_10640122
 Armenian Hamster IgG Isotype Control PE-conjugated; PRID: AB_326593
 Donkey Anti-goat Alexa Fluor 488-conjugated; PRID: AB_2534102
 Rabbit Anti-Red Fluorescent Protein (RFP); PRID:AB_2209751
 Donkey Anti-mouse CyTM3-conjugated; PRID: AB_2340813
 Donkey Anti-rabbit Alexa Fluor 488-conjugated; PRID: AB_2535792
 Donkey Anti-rabbit Alexa Fluor 568-conjugated; PRID: AB_2534017
 Donkey Anti-rat Alexa Fluor 405-conjugated; <https://www.abcam.com/donkey-rat-igg-hl-alex-fluor-405-preadsorbed-ab175670.html>
 Donkey Anti-rat Alexa Fluor 647-conjugated; PRID: AB_2813835
 Goat Anti-GFP Dylight™ 488-conjugated; RRID: AB_1961516
 Goat Anti-Mcpt6; PRID: AB_884330
 Goat Anti-mouse Alexa Fluor 647-conjugated; PRID: AB_2535806
 Goat Anti-rabbit Alexa Fluor 405-conjugated; PRID: AB_221605
 Goat Anti-rat Alexa Fluor 568-conjugated; PRID: AB_2534121
 Mouse Anti-CD45.2 Brilliant Violet 711™-conjugated; PRID: AB_2616859
 Mouse Anti-Dinitrophenyl (DNP) IgE: Eshhar, Z., Ofarim, M. & Waks, T. Generation of hybridomas secreting murine reaginic antibodies of anti-DNP specificity. J Immunol 124, 775-780 (1980); PRID: AB_259249
 Mouse Anti-NK-1.1 PE-conjugated; PRID: AB_313394
 Mouse Anti-paxillin; PRID: AB_397463
 Mouse Anti-smooth muscle actin Alexa Fluor 405-conjugated; <https://www.abcam.com/alex-fluor-405-alpha-smooth-muscle-actin-acetyl-e3--actg2-acetyl-e3-antibody-e184-ab210128.html>
 Mouse Anti-smooth muscle actin CyTM3-conjugated; PRID: AB_476856
 Mouse Anti-vinculin; PRID: AB_10603627
 Rabbit Anti-actin ; PRID: AB_476693
 Rabbit Anti-collagen IV; RRID: AB_445160
 Rabbit Anti-Desmin; PRID: AB_731901
 Mouse Anti-talin; PRID: AB_477572

Rabbit Anti-fibronectin; PRID:AB_476976
 Rabbit Anti-Lamc1; PRID: AB_1079230
 Rabbit Anti-mouse HRP-conjugated Antibody; PRID: AB_2687969
 Rabbit Anti-NG2; AB_11213678
 Rabbit Anti-Vimentin; PRID: AB_10562134
 Rat Anti-CD117 Brilliant Violet 421-conjugated; PRID: AB_10898120
 Rat Anti-CD117 APC-conjugated; PRID AB_469434
 Rat Anti-CD11b (Integrin alpha M) PE-conjugated; PRID: AB_396680
 Rat Anti-CD16/CD32 Antibody; PRID:AB_394657
 Rat Anti-CD18 (Integrin beta 2) PE-conjugated; clone C71/16; PRID: AB_394762
 Rat Anti-CD18 (Integrin beta 2) FITC-conjugated; clone C71/16; PRID AB_394761
 Rat Anti-CD19 PE-conjugated; PRID: AB_657660
 Rat Anti-CD29 (Integrin beta 1, 9EG7); RRID:AB_395001
 Rat Anti-CD29 Alexa Fluor 488 conjugated (Integrin beta 1, HMBeta1-1); PRID: AB_492830
 Rat Anti-CD29 PE conjugated (Integrin beta 1, HMBeta1-1); PRID:AB_312884
 Rat anti-CD31; PRID:AB_393571
 Rat Anti-PDGFRa; RRID:AB_467491
 Rat Anti-CD4 PE-conjugated; PRID: AB_312692
 Rat Anti-CD41 (Integrin alpha IIb) FITC-conjugated; PRID: AB_395085
 Rat Anti-CD49d (Integrin alpha 4) PE-conjugated; PRID: AB_465697
 Rat Anti-CD49e (Integrin alpha 5); PRID: AB_396709
 Rat Anti-CD49e (Integrin alpha 5) PE-conjugated; PRID: AB_396710
 Rat Anti-CD49f (Integrin alpha 6) Alexa Fluor® 488-conjugated; PRID: AB_493635
 Rat Anti-CD51 (Integrin alpha V) PE -conjugated; PRID: AB_394088

Rat Anti-CD8a PE-conjugated; PRID: AB_465530
 Rat Anti-DARC Alexa Fluor 488-conjugated; Aude Thiriout, Carolina Perdomo, Guiying Cheng, Igor Novitzky-Basso, Sara McArdle, Jamie K. Kishimoto, Olga Barreiro, Irina Mazo, Robinson Triboulet, Klaus Ley, Antal Rot & Ulrich H. von Andrian. Differential DARC/ACKR1 expression distinguishes venular from non-venular endothelial cells in murine tissues. BMC Biol. 2017 May 19;15(1):45.
 doi: 10.1186/s12915-017-0381-7.
 Rat Anti-endomucin; PRID: AB_891529
 Rat Anti-F4/80 PE-conjugated; PRID: AB_10372666
 Rat Anti-Integrin β7 Chain PE-conjugated; PRID: AB_396735
 Rat Anti-nidogen; PRID: AB_558792
 Rat IgG1 kappa Isotype Control FITC-conjugated; PRID: AB_470008
 Rat IgG1 kappa Isotype Control PE-conjugated; PRID: AB_470046
 Rat IgG2a Isotype Control Alexa Fluor 488-conjugated; PRID: AB_2556535
 Rat IgG2a kappa Isotype Control PE-conjugated; PRID: AB_1834380
 Rat IgG2b kappa Isotype Control PE-conjugated; PRID: AB_470042
 Swine Anti-rabbit HRP-conjugated Antibody; PRID: AB_2728719
 Anti-MITF polyclonal; PRID: AB_1079381

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

| | |
|--|--|
| Cell line source(s) | CHO (Chinese hamster ovary) cells for SCF production; provided by Georg Häcker (University of Freiburg, Germany) Immortalized MEFs (mouse embryonic fibroblasts); provided by Susanna Minguet (University of Freiburg, Germany) WEHI-3 cells; provided by Rudolf Grosschedl (MPI of Immunobiology and Epigenetics, Freiburg, Germany); CVCL_3622 |
| Authentication | The used previously reported immortalized cell lines were not further authenticated. |
| Mycoplasma contamination | The used cell lines were negative for Mycoplasma contamination, proven by PCR analysis. |
| Commonly misidentified lines (See ICLAC register) | No misidentified cell lines were used in this study. |

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 | Mice between 7 to 12 weeks on C57BL/6J were used for this study. The following mouse strains were used in this study, detailed references are also given in the methods section of this manuscript: Itgb1tm1Ref, MGI: 1926498; Itgb2tm2Bay, MGI: 1861705; Itgb3tm1.1Wlbc, MGI: 5688572; Commd10Tg(Vav1-iCre)A2Kio, MGI: 2449949 Gt(ROSA)26Sortm1(EYFP)Cos, MGI: 2449041 (also referred to as Rosa26-LSL:YFP); Gt(ROSA)26Sor tm14(CAG-tdTomato)Hze, MGI: 3809524 (also referred to as Rosa26-LSL:Tom); Tg(Myh11-cre,-EGFP)2Mik, MGI: 2653286; Tyrc-2J (B6.Albino); MGI: 1855985 Tg(UBC-Brainbow1.0L)35Mal, MGI: 5645781 |
|--------------------|---|

Kitltm1.1Sjm, MGI: 5300819
 Tg(Kitl-ERT2Cre,-TdT), provided by Claus Nerlov (University of Oxford, United Kingdom), Buono et al 2016
 Tg(CAG-EGFP)#Rows, MGI: 4831036
 Tg(Cma1-cre)ARoer (brief: Mcpt5-Cre), MGI: 3785000
 Tln1tm4.1Crit, MGI:3770513
 Rag2tm1Fwa, MGI: 1858556
 Mcpt5-Cre Tln1-fl/fl mice and crosses with fluorescent reporter lines (Rosa26LSL:YFP, Tg(Ubow)) were on a Tyrc-2J/c-2J (C57BL/6J-Albino) background, as we initially planned intravital microscopy studies of ear skin in these mice.

| | |
|-------------------------|---|
| Wild animals | This study did not involve wild animals. |
| Reporting on sex | Both male and female mice were used for experiments. In comparative experiments, control and knockout mice were sex- and age-matched littermates. |
| Field-collected samples | No field-collected samples were used in this study. |
| Ethics oversight | Mouse breeding and husbandry were performed at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, in accordance with the guidelines provided by the Federation of European Laboratory Animal Science Association and as approved by German authorities (Regional Council of Freiburg). Mice were only used for organ removal after euthanasia by carbon dioxide exposure and thus not subject to experimental procedures and ethical approval according to §4 (3) Tierschutzgesetz. |

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

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 | Details on sample preparation for flow cytometry are provided in the Methods section (Flow Cytometry) |
| Instrument | BD LSR Fortessa & BD LSRII |
| Software | Data collection: BD FACSDiva software Data analysis: FlowJo software v10 (Tree Star, Ashland, OR, USA) |
| Cell population abundance | Sorted mast cells from skin were analyzed by single cell RNA sequencing and confirmed the expression of the mast cell marker Cpa3 of all sorted cells. |
| Gating strategy | Gating strategies have been included in Extended Data Figure 1 and 2. |

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