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>.

Statistics

For	all sta	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Con	firmed
	\square	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	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.
\boxtimes		A description of all covariates tested
	\boxtimes	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.
\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
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), 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	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 <u>availability of data</u>

All manuscripts must include a <u>data availability statement</u>. 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 RNAsequencing 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	This study doesn't include human research participants.
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 <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

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 size. They were determined based on our previous publications, prior experience or pilot experiments.
Data exclusions	For most experiments: No data exlcusion 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	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	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	Experimentalists were blinded regarding the genotype of mice or BMMC 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

MRI-based neuroimaging

☐ ChIP-seq
✓ Flow cytometry

n/a	Involved in the study		
	Antibodies		
	Eukaryotic cell lines		
\square	Palaeontology and archaeology		

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- Clinical data
 - Dual use research of concern

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 (1ug/ml) BMMC & PMC validation by FACS Rat Anti-CD117 Brilliant Violet 421-conjugated; BioLegend; Cat# 105827; 1:200 Armenian Hamster Anti-FcεRIα 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-FccRI 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 β7 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-FccRIa PE/CY7-conjugated; BioLegend; Cat# 134318; 1:200 Armenien 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 Thiriot, 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

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, Donkey Anti-rat Alexa Fluo	r 647-conjugated; Abcam; Cat# ab150155; 1:500
Donkey Anti-rat Alexa Fluo	r 405-conjugated; Abcam; Cat# ab175670; 1:500
Rabbit Anti-Red Fluorescer	1t Protein (RFP); Rockland; Cat# 600-401-379; 1:1000
Goat Anti-GFP Dylight™ 48	8-conjugated; Rockland; Cat# 600-141-215; 1:1000
Immunofluorescence analy	rsis of adhesion structures in mast cells
, Mouse Anti-talin, clone:8d	4; Sigma-Aldrich; Cat# T3287; 1:200
Mouse Anti-vinculin; Sigma	a-Aldrich; Cat# V9264; 1:200
Rat Anti-CD29 (Integrin bet	ta 1, 9EG7); BD Biosciences; Cat# 553715; 1:200
Mouse Anti-paxillin; BD Bic	sciences; Cat# 610051; 1:200
Rat Anti-CD49e (Integrin al	pha 5); BD Biosciences; Cat# 55/446; 1:200
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RNA-FISH	
Rat Anti-nidogen (NG2); Th	iermo Fisher; Cat# MA1-06501; 1:300
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Mouse Anti-CD45.2 Brillian	it Violet 711™-conjugated; BioLegend; Cat# 109847; 1:100
Armenian Hamster Anti-CD)3e PE-conjugated; Thermo Fisher Scientific; Cat# 2-0031-83; 1:100
Rat Anti-CD4; PE-conjugate	ed BioLegend; Cat# 100407; 1:100
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Validation

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 Thiriot, 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 <u>cell lines and Sex and Gender in Research</u>

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 <u>ICLAC</u> register)	No misidentified cell lines were used in this study.

Animals and other research organisms

Policy information about studies involving animals; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in</u> <u>Research</u>

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;
Itgb3tm1.1Wlbcr, 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

	Kittm1.1Sym, 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).

 \bigotimes All plots are contour plots with outliers or pseudocolor plots.

 \bigotimes 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.

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