# nature portfolio

Corresponding author(s): Ruslan Medzhitov

Last updated by author(s): 05/25/2023

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

n/a	Cor	firmed
	$\square$	The exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	$\square$	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	$\boxtimes$	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
	$\boxtimes$	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)
	$\boxtimes$	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 statistics for biologists contains articles on many of the points above.

### Software and code

Policy information about availability of computer code						
Data collection	No software was used.					
Data analysis	Fiji-Image J, QuPath v3.0, Flowjo 10.8.1, Graphpad prism v9.5.0					

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 <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

The data availability statement was added to the manuscript.

### Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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

### Life sciences study design

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

Sample size	No formal sample size calculations were performed prior to experimentation. Sample size was determined based on prior literature performing similar measurements (Mirotti et al.; 2009).
Data exclusions	Exclusion of data was pre-established for the two bottle preference tests. Samples with cumulative licks of 0 or over 100/minute were excluded.
Replication	Each experiment reported in the manuscript was repeated at least two times with independent cohorts of mice with >3 technical replicates of individual mice per cohort.
Randomization	Allocation of individual animals in control or experimental groups was random.
Blinding	Fos-positive cells in the brain were manually quantified by a blinded investigator throughout the entire procedure. For all other assays, blinding was not required as they do not reflect subjective scorings.

### 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 n/a Involved in the study $\mathbf{X}$ ChIP-seq Antibodies $\boxtimes$ Eukaryotic cell lines Flow cytometry Palaeontology and archaeology $\boxtimes$ MRI-based neuroimaging $\mathbb{N}$ Animals and other organisms Clinical data $\square$ Dual use research of concern $\mathbf{X}$

### Antibodies

Antibodies used

APC-Cy7-CD117 (clone 2B8; Biolegend #105826) APC/eFluor780-MHCII (clone M5/114.15.2; eBioscience 47-5321-82) APC/eFluor780-CD19 (clone eBio1D3; eBioscience 47-0193-82) APC/eFluor780-CD4 (clone RM4-5; Invitrogen 47-0042-82)

PE-FceRI (clone M	AR-1; eBioscience 12-5898-82)
PE-SiglecF (clone E	50-2440; BD Pharmingen #552126)
PE-Gata3 (clone I)	WAJ; eBioscience #12-9966-42)
eFluor450-CD45 (d	clone 30-F11; eBioscience #48- 0451-82)
eFluor450-FceRI (d	clone MAR-1; eBioscience #48-5898-82)
APC-CD11b (clone	M1//0; eBioscience #1/-0112-82)
APC-Ly6c (clone H	K1.4; eBioscience #17-5932-82)
APC-TCRb (clone F	157-597; Biolegend #109212)
APC-SA (eBioscien	ce #17-4317-82)
APC-MHCII (clone	M5/114.15.2; eBioscience #17-5321-82)
Alexa700-CD3 (clo	ne 17A2; Biolegend 15 #100216)
Alexa700-CD45 (cl	one 30-F11; Biolegend #103128)
Alexa700-CD19 (cl	one 6D5; Biolegend #B189284)
PE/Cy7-CD3e (clor	ne 145-SC11, eBioscience #25-0031-82)
PE/Cy7-Ly6G (clon	e RB6-8C5; eBioscience #25-5931-82)
PE/Cy7-CD117 (clc	one 2B8; eBioscience #25-1171-82)
PE/Cy7-Tbet (clone	e eBio4B10; eBioscience #25-5825-82)
PE/Cy7-CD4 (clone	e GK1.5; Biolegend #100422)
PE/Cy7-CD11b (clo	one M1/70; eBioscience #25-0112-82)
PE/Cy7-CD45R (clo	one RA3-6B2; eBioscience #25-0452-82)
PE/Cy7-NK1.1 (clo	ne PK136; BD Pharmingen #552878)
FITC-CD11c (clone	N418; eBioscience #11-0114-85)
FITC-IgE (clone R3	5-72; BD Pharmingen #553415)
FITC-CD11b (clone	• M1/70; eBioscience #11-0112-85)
FITC-CD19 (clone e	eBio1D3; eBioscience #11-0193-82)
FITC-Gr1 (Ly6G/Ly	6c) (clone RB6-8C5; Biolegend#108406)
FITC-NK1.1 (clone	PK136; Biolegend #108706)
FITC-Ter119 (clone	e Ly76; Biolegend #116206)
FITC-CD49b (clone	DX5; Biolegend #108906)
FITC-Lin (clones 14	15- 2C11, RB6-8C5, RA3-6B2, Ter-119, M1/70; Biolegend #133301)
FITC-MHCII (clone	M5/114.15.2; eBioscience #11-5321-82)
biotin-IgE (clone R	35-72; BD Pharmingen #553414)
BV711-F4/80 (clor	ne T45-2342; BD Horizon #565612)
BV421-CD11b (clo	ne M1/70; BD Horizon #562605)
BV421-RORgt (clor	ne Q31-378; BD Horizon #562894)
BUV395-CD45(cloi	ne 30-F11; BD Horizon #564279)
BUV737-CD90.2 (c	lone 53-2.1; BD Bioscience #741701)
PECy5.5-Foxp3 (clo	one FJK-16s; eBioscience #35-5773-82)
Rabbit monoclona	l anti-c-Fos primary antibody (Cell Signaling #2250S)
Rabbit Polyclonal a	anti-fluoro-gold (Fluorochrome)
, Alexa Fluor 594-do	onkey anti-rabbit IgG secondary antibody (Invitrogen, #A-21207)
Purified Rat Anti-N	Aouse IgE (clone R35-72; BD Biosciences #553413)
Purified Mouse Ig	E,k Isotype Control (clone C38-2; BD Biosciences #557079)
Purified Mouse Ig	E,k Isotype Control (clone C48-2; BD Biosciences #557080)
Biotin Rat Anti-Mc	buse IgE (clone R35-118; BD Biosciences #553419)
Streptavidin-HRP (	BD Biosciences #553419)
Ovalbumin antibo	dy (anti-OVA IgE) (clone 2C6; BioRad MCA2259)
Ovalbumin, Biotin	Labeled (Nanocs OVA1-BN-1)
Purified Mouse Ig	G1,k Isotype Control (clone MOPC-31C; BD Biosciences #557273)
Biotin Rat Anti-Mc	use IgG1 (clone A85-1; BD Biosciences #553441)
All antibodies wer	e previously validated for flow cytometry by the manufacturer, unless otherwise noted.
CD16/CD32 (Fc blo	ock) was validated by ThermoFisher using THP-1 cells.
Ethidium monoazi	de bromide was validated by ThermoFisher using Jurkat cells.
Zombie Yellow Fix	able Viability Kit was validated by Biolegend using mouse splenocytes.
CD11c-FITC was va	alidated by eBioscience using mouse splenocytes.
IgE-FITC was valid:	ated in our lab by using mouse peritoneal cells.
CD11b-FITC was va	alidated by eBioscience using mouse bone marrow cells.
CD19-FITC was val	idated by eBioscience using mouse splenocytes.
Gr1 (I v6G/I v6c)-F	TC was validated by Biolegend using mouse hone marrow cells
NK1.1-FITC was va	lidated by Biolegend using mouse splenocytes
Ter119-FITC was v	alidated by Biolegend using mouse bone marrow cells
CD49b-FITC was v	alidated by Biolegend using mouse splenocytes
Lin-EITC was valids	and the Biolegend using mouse hone marrow cells
	Jidatad hy aRiocciance using mouse solenocytes
SiglecE-DE was vali	idated by BD Pharmingen using mouse bone marrow cells
EcoPI DE Was valid	nated by operations using the mouse polle fild(100 Cells.
Cata2 DE was valla	ated by ebioscience using the mouse most cell life, MC/9.
	dated by epidoscience using mouse chlorestee.
LYOU-APE Was Valle	Jaceu by epioscience using mouse spienocyles.
	andated by Biologond using mouse pone marrow cens.
ICKD-APC Was Vali	uareu by Бюгедела using mouse spienoCytes.
IVITICII-APC Was Va	nualeu by ebioscience using mouse spienocytes.
	Led by ebioscience using now cytometry.
SA-APC was valida	
IgE-biotin was valida	dated in our lab by using mouse peritoneal cells.
gE-biotin was valida gE-biotin was vali CD3e-PE/Cy7 was	dated in our lab by using mouse peritoneal cells. validated by eBioscience using mouse splenocytes.

Validation

CD117-PE/Cy7 was validated by eBioscience using mouse bone marrow cells. Tbet-PE/Cy7 was validated by eBioscience using normal human peripheral blood cells. CD4-PE/Cy7 was validated by Biolegend using mouse splenocytes. CD11b-PE/Cy7 was validated by eBioscience using mouse bone marrow cells. CD45R-PE/Cy7 was validated by eBioscience using mouse splenocytes. NK1.1-PE/Cy7 was validated by BD Pharmingen using mouse splenocytes. MHCII-APC/Cy7 was validated by eBioscience using mouse splenocytes. CD19-APC/Cy7 was validated by eBioscience using mouse splenocytes. CD4-APC/Cy7 was validated by eBioscience using mouse splenocytes. CD117-APC/Cy7 was validated by Biolegend using mouse bone marrow cells. F4/80-BV711 was validated by BD Horizon using mouse splenocytes. CD11b-BV421 was validated by BD Horizon using mouse bone marrow cells. RORgt-BV421 was validated by BD Horizon using mouse thymocytes. CD45-BUV395 was validated by BD Horizon using mouse splenocytes. CD3-Alexa700 was validated by Biolegend using mouse splenocytes. CD19-Alexa700 was validated by Biolegend using mouse splenocytes. CD45-Alexa700 was validated by Biolegend using mouse splenocytes. CD45-e450 was validated by eBioscience using mouse bone marrow cells. FceRI-e450 was validated by eBioscience using a mouse mast cell line, MC/9. CD90.2-BUV737 was validated in our lab using mouse splenocytes. Foxp3-PECy5.5 was validated by eBioscience using mouse splenocytes. c-fos primary and secondary antibodies were validated using mouse brains. Purified Rat Anti-Mouse IgE was validated by ELISA. Purified Mouse IgE, Isotype Control was validated by ELISA. Purified Mouse IgE, Isotype Control was validated by ELISA. Biotin Rat Anti-Mouse IgE was validated by ELISA. Streptavidin-HRP was validated by ELISA. Ovalbumin antibody (anti-OVA IgE) was validated by ELISA. Ovalbumin, Biotin Labeled was validated by ELISA. Purified Mouse IgG1, Isotype Control was validated by ELISA. Biotin Rat Anti-Mouse IgG1 was validated by ELISA.

### 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	Female mice at 6-10 weeks of age were used for all experiments. BALB/cJ (000651), C57BL/6J (000664), C57BL/6 FceRI KO (B6.129S2(Cg)-Fcer1atm1Knt/J, 010512), DdblGATA1 (C.129S1(B6)-Gata1tm6Sho/J, 005653), BALB/c Il4ra KO (BALB/c-Il4ratm1Sz/J, 003514), and C57BL/6 substance P KO (B6.Cg-Tac1tm1Bbm/J, 004103) mice were purchased from The Jackson Laboratories and maintained in our facilities. BALB/c IgE KO were generously provided by H. C. Oettgen (Harvard University), RMB (B6. Ms4a2tm1Mal) were generously provided by P. Launay (Université Paris Diderot), and C57BL/6 Trpm5-/- mice were provided by W. Garret (Harvard University). RMB, Fc RI KO, IgE KO, or Trpm5-/- mice were backcrossed more than eight times onto BALB/cJ or C57BL/6J for this study. We used littermate controls in all experiments.
Wild animals	The study did not involve wild animals.
Reporting on sex	Since testosterone was shown to have a suppressive effect on mast cell activation and secretion of anaphylactic mediators (Mackey et al., 2019), we did not use male mice for this study.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal care and experimentation were approved by the Institutional Animal Care and Use Committee of Yale University School of Medicine and consistent with the National Institutes of Health, USA, guidelines.

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

Single-cell suspensions were treated with anti-CD16/32 (Fc block) and stained with one of two live/dead markers: (1)

Instrument	BD LSRII analyser equipped with the following lasers: 355 nm (UV), 405 nm (violet), 488 nm (blue), and 633 nm (red).
Software	BD FACS Diva software was used to collect data. Data was analyzed using FlowJo software.
Cell population abundance	Cell sorting was not performed.
Gating strategy	Enteric Mast Cell gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA-CD3- CD19- CD11b- CD117+ FceRI+
	Peritoneal Mast Cell gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- CD3- CD19- CD11b- CD117 + FceRI+
	Dendritic Cell gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets. CD45+ EMA- CD11c+ MHCII+
	Macrophage gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets. CD45+ EMA- F4/80+ MHCII+
	Monocyte gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- F4/80- CD11b+ Ly6c+
	Neutrophil gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- CD11b+ Gr1+ MHCII-
	Eosinophil gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- SiglecF+ CD11b+ MHCII-
	ILC2 gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- Lin- CD4- TCRb- CD90.2+ Gata3+
	CD4+ T Cell gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- Lin- CD4+ TCRb+
	RORgt+T Cell gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- Lin- CD4+ TCRb+ RORgt+
	Tbet+ T Cell gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- Lin- CD4+ TCRb+ Tbet+
	Gata3+ T Cell gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- Lin- CD4+ TCRb+ Gata3+
	Foxp3+ T Cell gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- Lin- CD4+ TCRb+ Foxp3+
	Basophil gating: SSC-H vs. SSC-A and FSC-A vs. FSC-H to exclude doublets, CD45+ EMA- Lin-CD49b+ IgE+.

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

Sample preparation