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 ar	nalyses, 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 stateme	ent on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statis Only comm	tical test(s) used AND whether they are one- or two-sided non tests should be described solely by name; describe more complex techniques in the Methods section.
\boxtimes	A descrip	tion of all covariates tested
	A descrip	tion of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full deso	cription of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) ation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null h	ypothesis 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 less as exact values whenever suitable.
\boxtimes	For Bayes	ian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierar	chical 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.
So	ftware an	d code
Poli	cy information	about <u>availability of computer code</u>
Da	ata collection	N/A
Da	ata analysis	FlowJo versions 9 and 10 were used to analyze flow cytometry data. Prism version 9 and 10 were used to data graphing and statistical anlsysis
		g 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 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 generated or analyzed from which conclusions are drawn in this manuscript are available within the paper and Supplementary Information.

Research involving	human partici	pants, their	data. or	biological	material

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Reporting on sex and a	ender N/A. no human samples or material	
Reporting on race, eth other socially relevant groupings	nicity, or N/A. no human samples or material	
Population characteris	tics N/A. no human samples or material	
Recruitment	N/A. no human samples or material	
Ethics oversight	N/A. no human samples or material	
Note that full information o	n the approval of the study protocol must also be provided in the manuscript.	
Etalal assaut		
rieia-specii	ic reporting	
Please select the one be	ow that is the best fit for your research. If you are not sure, read the appropriate sections before making your selec	ition.
X Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences	
For a reference copy of the doo	ument with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>	
lifo coionoc	s study dosign	
Life science	es study design	
All studies must disclose	on these points even when the disclosure is negative.	
	ole sizes were not predetermined based on specific statistical methods. The number of animals per group in the study generated of g sufficient statistics for the effect sizes of interest.	data
Data exclusions No o	ata are excluded	
Replication For I	oiological replicates, 3-5 mice were randomly assigned to each group and experiments were performed at least twice	
Randomization Anin	nals were randomly assigned to groups in the study	
Blinding Inve	stigators were not blinded to the study. Blinding is not typically necessary in this type of study	
	or specific materials, systems and methods n authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each r	 material,
system or method listed is	elevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a res	ponse.
Materials & experir	nental systems Methods	
n/a Involved in the stu	n/a Involved in the study	
Antibodies	ChIP-seq	
Eukaryotic cell lii	les Flow cytometry	
Palaeontology ar	d archaeology MRI-based neuroimaging	
Animals and other	r organisms	
Clinical data		
Dual use research	n of concern	
Plants		
<u> Antibodies</u>		
Antihodies used	Antihodies: hamster anti-mouse KLRG1 (2F1: RD Biosciences), rat anti-mouse CD62L (MFL-14: Biolegend), rat anti-mouse CD4	(GK1 5

 $BioLegend), rat anti-mouse CD8\alpha \ (53-6.7; BioLegend), rat anti-mouse CD49d \ (R1-2, BioLegend), rat anti-mouse CD11a \ (M17/4; BD-18), rat anti-mouse CD11a \ (M17/4; BD-18$ Biosciences, San Jose, CA), rat anti-mouse CD103 (M290; BD Biosciences), and rat anti-mouse CD69 (H1.2F3; eBioscience), rat antimouse CD19 (1D3; BD Biosciences), rat anti-mouse B220 (RA3-6B2; BioLegend), rat anti-mouse IgM (B7-6), rat anti-mouse Fas (Jo2; BioLegend), and rat anti-mouse GL7 (GL7; Biolegend), rat anti-mouse CD38 (90; Biolegend), hamster anti-mouse CD69 (H1.2F3; Biolegend), rat anti-mouse CD73 (TY/11.8; Biolegend), and hamster anti-mouse CXCR3 (CXCR3-173; Biolegend), H2-Db NP366-374 tetramer, H2-Kb M1128-135 tetramer; rat anti-mouse Eomes (Dan11mag; Invitrogen), hamster anti-mouse/rat CD49a (Ha31/8; BD Biosciences), hamster anti-mouse CXCR3 (CXCR3-173; Biolegend), and anti-mouse CX3CR1 (SA011F11; Biolegend)

Validation

Validation was performed by vendors. Antibodies were titrated to determine optimal staining concentrations.

Animals and other research organisms

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

Laboratory animals	Female 8–12-week-old C57BI/6 mice purchased from Charles River Laboratories
Wild animals	N/A
Reporting on sex	Adult female mice were used
Field-collected samples	N/A
Ethics oversight	The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Iowa and comply with the NIH Guide for Care and Use of Laboratory Animals

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

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Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	- blood was collected in non-heparinized capillary tubes for serum collection. Blood samples were left at room temperature for 30 min, centrifuged at 16,000 × g for 20 min, and then collected and stored at −20°C until analysis. - Bronchial alveolar lavage (BAL) fluid was collected using a protocol modified from. Briefly, the tracheae were cannulated with a 22-gage catheter tube (attached to a 5cc syringe) and then washed once with 1 mL of sterile PBS. Samples were stored at −20°C until analysis - lungs and mediastinal lymph nodes were harvested, digested, homogenized in gentleMACS™ C tubes utilizing the gentleMACS™ Octo Dissociator and subsequently strained through 70 µm filters into single cell suspensions
Instrument	Data were acquired on a BD LSRII (BD Biosciences) or Cytek Aurora (Cytek Biosciences)
Software	Flow cytometry data was collected using FACSDiva or Spectroflo software and analyzed in FlowJo 9 and 10
Cell population abundance	No cell sorting was performed in this study
Gating strategy	Live (Viability dye), single cell (FSC-H vs FSC-A) lymphocytes (FSC-A vs SSC-A) were gated first. Boundaries between positive

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