

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	16S rRNA sequencing analysis was sequenced by using Novaseq 6000 (Illumina) as described in the Methods section. No commercial, open source or custom code was used to collect data.
Data analysis	Graphpad Prism software, v 9.5 Fluidigm CyTOF software, v 6.7.1014. R, v 4.0.5. FlowJo software, v 10.6.1. SPSS Statistics, v 25.0. ImageJ ® software, v 1.53q. CytExpert software, v 2.0.

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 [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](#)

The 16S rRNA sequencing data used in this study has been deposited in the NCBI Sequence Read Archive under the accession codes PRJNA1162303, PRJNA1162283, and PRJNA1162236. The mass spectrometry datasets supporting the findings of this study have been uploaded to the Zenodo database under the accession codes 13773165 and 13773748. All other data generated or analyzed in this study are included in this published article and its supplementary information files. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	The sex of the study participants was determined through self-report during the initial demographic data collection. We did not perform any sex-specific analyses as this is out of the scope of this project.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, and socially relevant grouping were not utilized in this study.
Population characteristics	Ten healthy participants consisted of 5 male and 5 female donors.
Recruitment	All donors who reported no current or past history of allergic reactions were chose for volunteers. We are not aware of any self-selection bias or other biases that would be expected to impact the results.
Ethics oversight	Zhejiang Gongshang University Ethics Review Committee (No. 2024030401) approved this study.

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](https://www.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 statistical methods were used to determine sample size. According to the 3R principle, we chose the minimal replicate number sufficient to ascertain statistics.
Data exclusions	In some animal experiments, one or two samples with extreme deviations from the same group were excluded from data analysis.
Replication	All the experiments were repeated twice or more, and reproducibility was confirmed.
Randomization	Animals used in this study were randomly assigned to their respective groups before the experiments were performed.
Blinding	Allergy clinical symptom scores after allergen challenge and H&E staining, immunohistochemistry (IHC), and immunofluorescence (IF) of tissues were assessed by blinded observers. In other experiments, the investigators were not blinded since randomization minimized their influence on experiments.

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

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

FITC anti-mouse CD4 antibody (BD, Cat #. 553650)
 APC anti-mouse IFN- γ antibody (BD, Cat #. 554413)
 PE anti-mouse IFN- γ antibody (BD, Cat #. 554412)
 PE anti-mouse IL-4 antibody (BD, Cat #. 554435)
 APC anti-mouse IL-4 antibody (BD, Cat #. 554436)
 FITC anti-mouse CD11c antibody (BD, Cat #. 553801)
 BV605 anti-mouse CD11c antibody (BD, Cat #. 563057)
 PE anti-mouse CD11b antibody (BD, Cat #. 553311)
 PerCP-Cyanine5.5 anti-mouse CD80 antibody (BD, Cat #. 560526)
 APC anti-mouse CD86 antibody (BD, Cat #. 558703)
 FITC anti-mouse CD103 antibody (BD, Cat #. 557494)
 FITC anti-mouse MHC-II antibody (BD, Cat #. 553623)
 Brilliant Violet 421™ anti-human CD284 (TLR4) Antibody (BioLegend, Cat #. 312811)
 APC anti-human NF- κ B p65 antibody (BioLegend, Cat #. 653006)
 FITC anti-human CD4 antibody (BioLegend, Cat #. 344604)
 PE/Cyanine7 anti-human IL-4 antibody (BioLegend, Cat #. 500824)
 PerCP/Cyanine5.5 anti-human IFN- γ antibody (BioLegend, Cat #. 506528)
 PE anti-RAGE antibody[EPR21171] (Abcam, Cat #. ab237363)
 FITC anti-mouse CD4 antibody (Biolegend, Cat #. 100406)
 APC anti-mouse IL-4 antibody (Biolegend, Cat #. 504106)
 Brilliant Violet 421™ anti-mouse IFN- γ antibody (Biolegend, Cat #. 505829)
 PE labeled I-Ab OVA323-339 Tetramer (MBL, Cat #. TS-M710-1)
 FITC anti-mouse CD11c antibody (Biolegend, Cat #. 117306)
 PE anti-mouse/human CD11b antibody (Biolegend, Cat #.101208)
 APC anti-mouse CD80 antibody (Biolegend, Cat #.104714)
 Alexa Fluor® 700 anti-mouse CD86 antibody (Biolegend, Cat #.159214)
 Brilliant Violet 421™ anti-mouse CD103 antibody (Biolegend, Cat #.121422)
 Alexa Fluor® 700 anti-mouse I-A/I-E antibody (Biolegend, Cat #.107622)
 APC anti-mouse CD252 (OX40L) antibody (Biolegend, Cat #.108812)

Goat anti-Mouse IgG2a Secondary Antibody, HRP (Thermo Fisher Scientific, Cat #. M32207)
 Goat anti-Mouse IgG1 Secondary Antibody, HRP (Thermo Fisher Scientific, Cat #. PA1-74421)
 Goat anti-Mouse IgE Secondary Antibody, HRP (Thermo Fisher Scientific, Cat #. SA5-10263)
 Anti -Claudin 2 Mouse mAb (Servicebio, Cat #. GB14068-50)
 Anti-ZO1 tight junction protein Mouse mAb (Servicebio, Cat #. GB12195-100)
 Anti -Occludin Rabbit pAb (Servicebio, Cat #. GB111401-100)
 Anti -TLR2 Rabbit pAb (Servicebio, Cat #. GB113378-100)
 Anti -TLR4 Mouse mAb (Servicebio, Cat #. GB12186-100)
 Anti -RAGE Rabbit pAb (Servicebio, Cat #. GB11278-100)
 Anti- Mast Cell Chymase Rabbit pAb (Servicebio, Cat #. GB111085-50)
 Anti-Mast cell tryptase Mouse mAb (Servicebio, Cat #. GB12109-100)
 Cy3 conjugated goat anti-mouse IgG (Servicebio, Cat #. GB21303)
 Antibodies for CyTOF were provided by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

Validation

1) FITC anti-mouse CD4 antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fic-rat-anti-mouse-cd4.553650>

2) APC anti-mouse IFN- γ antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-rat-anti-mouse-ifn.554413>

3) PE anti-mouse IFN- γ antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-rat-anti-mouse-ifn.554412>

- 4) PE anti-mouse IL-4 antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-rat-anti-mouse-il-4.554435>
 - 5) APC anti-mouse IL-4 antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-rat-anti-mouse-il-4.554436>
 - 6) FITC anti-mouse CD11c antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-hamster-anti-mouse-cd11c.553801>
 - 7) BV605 anti-mouse CD11c antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-hamster-anti-mouse-cd11c.563057>
 - 8) PE anti-mouse CD11b antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-rat-anti-cd11b.553311>
 - 9) PerCP-Cyanine5.5 anti-mouse CD80 antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/percp-cy-5-5-hamster-anti-mouse-cd80.560526>
 - 10) APC anti-mouse CD86 antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-rat-anti-mouse-cd86.558703>
 - 11) FITC anti-mouse CD103 antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-rat-anti-mouse-cd103.557494>
 - 12) FITC anti-mouse MHC-II antibody (Verified reactivity, Mouse; For Flow Cytometry analysis)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-rat-anti-mouse-i-a-i-e.553623>
 - 13) Brilliant Violet 421™ anti-human CD284 (TLR4) Antibody (Verified reactivity, Human; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-human-cd284-trl4-antibody-7257>
 - 14) APC anti-human NF-κB p65 Antibody (Verified reactivity, Human; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/apc-anti-nf-kappab-p65-antibody-9921>
 - 15) FITC anti-human CD4 antibody (Verified reactivity, Human; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/fitc-anti-human-cd4-antibody-6206>
 - 16) PE/Cyanine7 anti-human IL-4 Antibody (Verified reactivity, Human; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-human-il-4-antibody-6514>
 - 17) PerCP/Cyanine5.5 anti-human IFN-γ Antibody (Verified reactivity, Human; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/percp-cyanine5-5-anti-human-ifn-gamma-antibody-12334>
 - 18) PE Anti-RAGE antibody[EPR21171] (Verified reactivity, Human; For Flow Cytometry analysis)
<https://www.abcam.cn/products/primary-antibodies/pe-rage-antibody-epr21171-ab237363.html>
 - 19) FITC anti-mouse CD4 antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd4-antibody-248?GroupID=BLG4211>
 - 20) APC anti-mouse IL-4 antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/apc-anti-mouse-il-4-antibody-891>
 - 21) Brilliant Violet 421™ anti-mouse IFN-γ antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-mouse-ifn-gamma-antibody-7154>
 - 22) PE labeled I-Ab OVA323-339 Tetramer (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.mblbio.com/bio/g/dtl/T/?pcd=TS-M710-1>
 - 23) FITC anti-mouse CD11c antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd11c-antibody-1815>
 - 24) PE anti-mouse/human CD11b antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/pe-anti-mouse-human-cd11b-antibody-349>
 - 25) APC anti-mouse CD80 antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd80-antibody-2340>
 - 26) Alexa Fluor® 700 anti-mouse CD86 antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/alexa-fluor-700-anti-mouse-cd86-antibody-24671>
 - 27) Brilliant Violet 421™ anti-mouse CD103 antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-mouse-cd103-antibody-7329>
 - 28) Alexa Fluor® 700 anti-mouse I-A/I-E antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/alexa-fluor-700-anti-mouse-i-a-i-e-antibody-3413>
 - 29) APC anti-mouse CD252 (OX40L) antibody (Verified reactivity, mouse; For Flow Cytometry analysis)
<https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd252-ox40l-antibody-8174>
- Each lot of conjugated antibody is quality control tested by CyTOF analysis of stained cells using the appropriate positive and negative cell staining and/or activation controls.

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	C57BL/6J (female, 4- to 6-week-old) or BALB/c mice (female, 4- to 6-week-old) were purchased from the Laboratory Animal Research Center of Zhejiang Academy of Medical Sciences (Zhejiang, China). Female RAGE ^{-/-} and TLR4 ^{-/-} mice on a C57BL/6J mice genetic background were generated at the Shanghai Model Organisms Center, Inc. (Shanghai, China). Mice were housed in 12-hour light–dark cycle, at 22 ± 1 °C, and about 55% ± 10% humidity.
Wild animals	No wild animals were used in the study.
Reporting on sex	Data presented here is based on female mice, but the findings presented in this study were not restricted to female mice.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All animal experiments were carried out in strict accordance with the National Guidelines for the Care and Use Of Laboratory Animals of China and were approved by the Zhejiang Gongshang University Ethics Review Committee (No. 2022032001).

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

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>

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	For analysis of Th1 and Th2 cell proportions in MLN lymphocytes, single-cell suspensions of MLN were stimulated with eBioscience™ Cell Stimulation Cocktail containing PMA, ionomycin, brefeldin A, and monensin for 6 h at 37 °C.
Instrument	Flow-cytometric analysis was performed using CytoFLEX Flow cytometer (Beckman Coulter, Inc., Brea, CA, USA).
Software	Flow cytometry data were analyzed using FlowJo software (version 10.6.1, BD Biosciences) and CytExpert software (version 2.0).
Cell population abundance	Purity for all cell populations were confirmed to be greater than 95%.
Gating strategy	Each cell lineage was identified in normal flow cytometry as follows: T helper type 1 (Th1) cells (CD4+ IFN-gamma+), T helper type 2 (Th2) cells (CD4+ IL4+), Dendritic Cells (DCs) (CD11b+ CD11c+). Each cell lineage was identified in CyTOF as follows: Conventional Dendritic Cells (cDCs) (CD45+ CD11b+ CD11c+ MHCII+), B Cells (CD45+ CD19+), CD4+ T cells (CD45+ CD3+ CD4+), CD8+ T cells (CD45+ CD3+ CD8+), Th1 cells (CD45+ CD3+ CD4+ IFN-gamma+), Th2 cells (CD45+ CD3+ CD4+ IL-4+), Treg (CD45+ CD3+ CD4+ CD25+ Foxp3+).
☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.	