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 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

Confocal images were collected with Zeiss LSM900 and ThermoFisher EVOS S1000
Data for histology were collected with 3D histech image analysis platform.
Transmission electron microscopy images were collected with KBSI Bio-HVEM system (JEM-1400Plus and JEM-1000BEF , JEOL) and Oneview camera with DM Software.
Flow cytometry data were acquired with BD FACSDiscover S8 (NFEC-2025-02-303724)
RNA seq data were collected with Illumina NovaSeq.
Data for ELISA were collected with VersaMAX Microplate Readers (Molecular Devices)
Data from the q-PCR experiments were colllected using Qiagen Rotor-Gene Q 2plex.
RNA extraction was performed on FFPE samples utilizing the RNeasy FFPE Kit (#73504, Qiagen), followed by DV 200 evaluation using an Agilent TapeStation.
FFPE tissue samples were then prepared according to the Visium Spatial Gene Expression for FFPE protocol (#CG000409, 10xGenomics Pleasanton, CA, USA).
Libraries were constructed utilizing the Visium Spatial Gene Expression for FFPE Kit, Mouse Transcriptome v.1 (PN-1000521, 10xGenomics) along with the Dual Index Kit TS Set A (PN-1000251, 10xGenomics).
Subsequent sequencing was conducted on an Illumina NovaSeq6000 S1 Rgt Kit v1.5 200cycles, employing paired-end 200bp FlowCells.
Visium Matrix and H&E image were collected using Seurat v.4.0 (R 4.1).
Cell dissociation was performed using Liberase TL (Roche, #05401020001), and quality control was assessed with the LUNA-FX7™ Automated Cell Counter (Logos Biosystems) using Acridine Orange/Propidium Iodide Stain.
Bulk RNA sequencing of BMDMs, mouse lung tissues, and Mycobacterium tuberculosis H37Rv was performed on the Illumina NovaSeq platform (Macrogen). Spatial transcriptomic libraries were generated using the 10x Genomics Visium Spatial Gene Expression for FFPE Kit

(Mouse Transcriptome v.1) and sequenced on the Illumina NovaSeq X+ platform; raw reads and tissue images were processed with the SpaceRanger pipeline (v4.0.1). Fecal and cecal 16S rRNA gene sequencing data were generated on the Illumina MiSeq platform after DNA extraction from stool and cecal contents. Untargeted metabolomic data of cecal contents were acquired on a Vanquish UHPLC system coupled to a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific) using Xcalibur software (v4.1.31.9). Targeted bile acid profiling of serum, cecum, and lung tissue was performed using the Biocrates® Bile Acids Kit on an Agilent 1200 HPLC system coupled to an AB Sciex API 4000 triple quadrupole mass spectrometer, with data acquired by MetIDQ® software (Biocrates Life Sciences AG).

Data analysis

Flowjo (version) 10.8.1 was used to analyze flow cytometric data.
GraphPad Prism (version 8) and Microsoft excel 365 were used analyze the data.
Microscopy data was processed using Zeiss Zen 3.7 and analyzed using Image J (version 1.52)
Bulk RNA-seq raw reads were processed using a Snakemake workflow (v9.14.8 for host; v9.19.0 with snakemake-wrappers v9.4.0 for M. tuberculosis) with fastp for quality control and adapter trimming. Host reads were quantified with Salmon against the GRCm39 reference, while M. tuberculosis reads were aligned with STAR (v2.7.11b) to the MycoBrowser reference. Transcript abundances were imported into R (v4.5.2) using tximport (v1.38.2), and differential expression analysis was conducted with DESeq2 (v1.38.3). Functional enrichment (GO/KEGG) was performed with clusterProfiler (v4.18.4), and results were visualized with ggplot2 (v4.0.2), pheatmap (v1.0.13), and ComplexHeatmap (v2.26.1). Spatial transcriptomic data were analyzed in R using Seurat (v5.4.0) with Harmony for batch correction, and cell type deconvolution was performed using Cell2location (v0.1.5) with the GSE151974 single-cell RNA-seq dataset as the reference. Downstream microbiota analyses were conducted in QIIME2 (v2024.10) using the DADA2 plugin for denoising, the SILVA database (v138.1) for taxonomic classification, ANCOM-BC for differential abundance analysis, and PICRUSt2 (v2.5.2) for functional pathway prediction; data were visualized in R using the qiime2R (v0.99.6) and ggplot2 packages. Untargeted metabolomic spectra were converted to mzML format using ProteoWizard MSConvert (v3.0.23132) and processed in MetaboAnalyst 6.0 with metabolite annotation against the HMDB database; sPLS-DA was performed in R using mixOmics (v6.28.0), car (v3.1-3), and vegan (v2.6-8). Targeted bile acid data were quantified with MetIDQ® software. Statistical analyses were performed using GraphPad Prism (v10.2.1) and R (v4.5.2). All custom analysis scripts are publicly available at https://github.com/hgod2356/Ino_Mtb.
All figures were arranged using Adobe Illustrator (v26.4.1).

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

Reviewer access information
All sequencing data (16S rRNA (cecal & fecal microbiota), BMDM bulk RNA-seq, Bacterial RNA-seq, Lung bulk RNA-seq) generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1446238:
[https://dataview.ncbi.nlm.nih.gov/object/PRJNA1446238?reviewer=oq1vf3lr3cif43chv2dj2rc550]
Spatial transcriptomics data : GSE330354 (GEO)
Enter reviewer token klsxecawvzmhvmv at [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE330354]
Cecal untargeted metabolomics data : MTBLS14469 (MetaboLights): [https://www.ebi.ac.uk/metabolights/reviewer790fec90-f94c-4423-a9ff-2ba2ee26f1e4]

All datasets are provided in detail below.
The 16S rRNA gene sequencing data for cecal and fecal microbiota generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number: PRJNA1446238 (SAMN56816553–SAMN56816591) [https://dataview.ncbi.nlm.nih.gov/object/PRJNA1446238?reviewer=oq1vf3lr3cif43chv2dj2rc550].

BMDM bulk RNA-seq data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number: PRJNA1446238 [https://dataview.ncbi.nlm.nih.gov/object/PRJNA1446238?reviewer=oq1vf3lr3cif43chv2dj2rc550] (SAMN57114323–SAMN57114328)

Bacterial RNA-seq data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number: PRJNA1446238 [https://dataview.ncbi.nlm.nih.gov/object/PRJNA1446238?reviewer=oq1vf3lr3cif43chv2dj2rc550] (SAMN58893990–SAMN58893993)

Lung bulk RNA-seq data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number: PRJNA1446238 [https://dataview.ncbi.nlm.nih.gov/object/PRJNA1446238?reviewer=oq1vf3lr3cif43chv2dj2rc550] (SAMN59142067–SAMN59142072)

The Visium spatial transcriptomics data generated in this study are available in the Gene Expression Omnibus (GEO) under accession number: GSE330354 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE330354]
: Enter reviewer tokens klsxecawvzmhvmv into the box

The cecal untargeted metabolomics data generated in this study have been deposited in the MetaboLights repository under accession number: MTBLS14469 [https://www.ebi.ac.uk/metabolights/reviewer790fec90-f94c-4423-a9ff-2ba2ee26f1e4]

The public scRNA-seq datasets reused in this study are available in GEO under accession number: GSE151974 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE151974]

The publicly available metagenomic dataset analyzed in this study is available in the NCBI Sequence Read Archive (SRA) under BioProject accession number: PRJNA390657
[https://www.ncbi.nlm.nih.gov/bioproject/PRJNA390657/]

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	Samples were obtained from female participants.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	Fecal samples were obtained from a healthy female volunteer in her 30s for the sole purpose of bacterial strain isolation. No additional clinical, demographic, or phenotypic data from the donor were collected or used in this study.
Recruitment	A healthy adult donor was recruited from Kyung Hee University. No incentives were provided.
Ethics oversight	The protocol for human fecal sample collection was strictly reviewed and approved by the Institutional Review Board (IRB) of Kyung Hee University (Approval No. KHSIRB-19-241-1(RA)). Prior to enrollment, all participants received a detailed explanation of the study's objectives and procedures. Subsequently, written informed consent was obtained from all individuals in accordance with the ethical guidelines approved by the Kyung Hee University

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 statistical method was used to predetermine sample size.
Data exclusions	No samples was excluded in our analysis.
Replication	All experimental findings are reproducible, confirmed by repeating the experiments independently with proper controls. There are at least 3 independent replicates performed for every experiments. Figures shown in this study are represetative of the replicates or pooled from all experiments performed, as indicated in each figure legend.
Randomization	All samples used in this study were randomly allocated into different experimental groups. For experiments related to mice, age (6-8 weeks)- and sex-matched littermate mice were randomized and used for experiments.
Blinding	Histological assessment of the mouse lung tissue was performed blindly by a pathologist. The investigators were not blinded to allocation during experiments, but unbiased quantifications were applied to obtain the results. Blinding was carried out during the software analysis step in all experiments, including flow cytometry, immunofluorescence, qPCR, ELISA.

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
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Human/Mouse Myeloperoxidase/MPO Antibody, R&D SYSTEMS, Cat#AF3667
Histone H3 [Citrulline Arg17, Citrulline Arg2, Citrulline Arg8] Antibody, Novus Biologicals, Cat#NB100-57135
Anti-S100A8 + S100A9 antibody [RM1038], Abcam, Cat#ab288715
IL-6 Antibody (10E5), santa cruz, Cat#sc-57315
F4/80 (C-7), santa cruz, Cat#sc-377009
InVivoPlus anti-mouse Ly6G, BioX Cell, Cat#BP0075
Alexa Fluor® 488 Conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (BT4JAC-111-545-003), Invitrogen, Cat#A17041
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594, Invitrogen, Cat#A-21207
Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488, Invitrogen, Cat#A-11055
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594, Cat#A-11007
BD Pharmingen™ PE Rat Anti-CD11b, BD Biosciences, Cat#557397
Zombie-violet Live-Dead stain, Biolegend, Cat#423114
Alexa Fluor™ 700 rat anti-mouse CD45, Invitrogen, Cat#56-0451-82
APC anti-mouse CD64 (FcγRI), Biolegend, Cat#161006
PE anti-mouse MERTK (Mer), Biolegend, Cat#151506
PE-Cyanine7 Rat Anti-Ly6G, Invitrogen, Cat#25-9668-82
APC-Cy™7 Rat Anti-CD11b, BD Bioscience, Cat#557657

Validation

Human/Mouse Myeloperoxidase/MPO Antibody, R&D SYSTEMS, Cat#AF3667
https://www.novusbio.com/products/myeloperoxidase-mpo-antibody_af3667
Histone H3 [Citrulline Arg17, Citrulline Arg2, Citrulline Arg8] Antibody, Novus Biologicals, Cat#NB100-57135
https://www.novusbio.com/products/histone-h3-antibody_nb100-57135
Anti-S100A8 + S100A9 antibody [RM1038], Abcam, Cat#ab288715
https://www.abcam.com/en-us/products/primary-antibodies/s100a8-s100a9-antibody-rm1038-ab288715
IL-6 Antibody (10E5), santa cruz, Cat#sc-57315
https://www.scbt.com/p/il-6-antibody-10e5?srsltid=AfmBOoq7VoIFUMAD89wjZQXZ8r3BqDJ5hhRWl4W8eU1vqUALYvKDy5G9
F4/80 (C-7), santa cruz, Cat#sc-377009
https://www.scbt.com/p/f4-80-antibody-c-7?srsltid=AfmBOop_Zvtnq6_kGwVmXsBvkk9WJgc_OSWe_QsUK4FnD3LP14-NOaUc
InVivoPlus anti-mouse Ly6G, BioX Cell, Cat#BP0075
https://bioxcell.com/invivomab-anti-mouse-ly6g-be0075-1?https://bioxcell.com/invivomab-anti-mouse-ly6g-be0075-1?queryID=bb1d2f62acd4a8ff60dab671a79a000f&objectID=30551&indexName=bioxcell_live_default_products
Alexa Fluor® 488 Conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (BT4JAC-111-545-003):
https://www.thermofisher.com/order/catalog/product/A17041?SID=srch-hj-a17041
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594, Invitrogen, Cat#A-21207:
https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21207
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594
https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11032
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594, Cat#A-11007
https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11007
BD Pharmingen™ PE Rat Anti-CD11b, BD Bioscience, Cat#557397:
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-rat-anti-cd11b.557397
Zombie-violet Live-Dead stain, Biolegend, Cat#423114:
https://www.biolegend.com/ja-jp/products/zombie-violet-fixable-viability-kit-9341?GroupID=BLG2181
Alexa Fluor™ 700 rat anti-mouse CD45, Invitrogen, Cat#56-0451-82:
https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/56-0451-82
APC anti-mouse CD64 (FcγRI), Biolegend, Cat#161006:
https://www.biolegend.com/de-de/products/apc-anti-mouse-cd64-fc-gammari-21552
PE anti-mouse MERTK (Mer), Biolegend, Cat#151506:
https://www.biolegend.com/ja-jp/products/pe-anti-mouse-mertk-mer-antibody-13406?GroupID=BLG15085

PE-Cyanine7 Rat Anti-Ly6G, Invitrogen, Cat#25-9668-8:
https://www.thermofisher.com/antibody/product/Ly-6G-Antibody-clone-1A8-Ly6g-Monoclonal/25-9668-82
APC-Cy™7 Rat Anti-CD11b, BD Bioscience, Cat#557657:
https://www.bdbiosciences.com/ko-kr/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-cy-7-rat-anti-cd11b.557657?tab=product_details:
InVivoMAb anti-mouse Ly6G,,BioXCell, Cat#BE0075-1:
https://bioxcell.com/invivomab-anti-mouse-ly6g-be0075-1?https://bioxcell.com/invivomab-anti-mouse-ly6g-be0075-1?queryID=bb1d2f62acd4a8ff60dab671a79a000f&objectID=30551&indexName=bioxcell_live_default_products

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	In this study, sex-matched mice aged 8–10weeks were used. C57BL/6 mice were obtained from Samtako Bio Korea. C3HeB/FeJ mice were obtained from the Jackson Laboratory (JAX Stock#000658). Mice were housed at 21-22°C, with 30-70% humidity on a 12-hour light/dark cycle with ad libitum access to food and water.
Wild animals	This study did not involve wild animals.
Reporting on sex	All experiments were performed on mixed gender animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee, Chungnam National University College of Medicine (202109A-CNU-180; Daejeon, South Korea). These procedures were conducted in compliance with the guidelines of the Korean Food and Drug Administration.

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

Plants

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	For neutrophil staining, murine lung tissues were perfused with 10 ml of cold PBS via right ventricular injection prior to harvest. Each lung lobe was dissected into small pieces and digested using a mouse lung dissociation kit (Miltenyi Biotec, Cat# 130-095-927) with GentleMACS™ Dissociator treatment according to the manufacturer's protocol. Tissue homogenates were resuspended in working solution and passed through a 70 µm cell strainer to remove debris. Red blood cells were lysed by incubation with 1× RBC lysis buffer (Invitrogen, Cat# 00-4333-57) for 3 min at room temperature, followed by two washes with PBS containing 2% FBS (v/v). Cell pellets were then stained with the following antibodies for 30 min at 4°C in the dark: Zombie Violet Live/Dead stain (1:1000; BioLegend, Cat# 423114), Alexa Fluor 700 rat anti-mouse CD45 (1:250; Invitrogen, Cat# 56-0451-82), APC anti-mouse CD64 (FcγRI) (1:250; BioLegend, Cat# 161006), PE anti-mouse MerTK (1:250; BioLegend, Cat# 151506), PE-Cyanine7 rat anti-mouse Ly6G (1:250; Invitrogen, Cat# 25-9668-82), and APC-Cy7 rat anti-mouse CD11b (1:250; BD Biosciences, Cat# 557657). Following two washes with PBS containing 2% FBS (v/v), stained cells were resuspended in 2% paraformaldehyde

	and immediately acquired on a BD FACSDiscover™ S8 cell sorter (BD Biosciences).
Instrument	We used BD FACSDiscover S8
Software	Flow cytometry data were collected and analyzed using BD FACSChorus™ Software and FlowJo software (Tree Star, Ashland,
Cell population abundance	No cell sorting was performed in this study.
Gating strategy	For lung cell analysis, Subsequently, doublets were excluded using single-cell gating based on ccentricity/Radical movement. Mouse neutrophils (not macrophages) were identified as Live CD45+MerTK-CD64-CD11b+Ly6G+ cells. The information above is described in Figure legends and Methods.

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