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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	All the raw sequence files were processed with CellRanger (v.6.0.2)
Data analysis	Python (v.3.1.12), R (v.4.2.2), Scanpy (v.1.9.1), Scrublet (v.0.2.3), BBKNN (v.1.5.1), GSEApy (v.1.0.4), CellChat (v.1.6.1), Monocle3 (v.1.3.1), sceasy (v.0.0.7), pySCENIC (v.0.12.0), arboreto (v.0.1.6), ctxcore (v.0.2.0), umap-learn (v.0.5.3), statannot (v.0.2.3), scipy (v.1.7.1). The codes used in this study is available through Zenodo repository (https://zenodo.org/records/11203839).

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 single-cell RNA sequencing dataset used in this study is publicly available through the Gene Expression Omnibus with the accession number GSE239574. Processed data is available through figshare (https://doi.org/10.6084/m9.figshare.24547210.v2). 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	N/A. No human participants, data, or biological materials are included in this study.
Reporting on race, ethnicity, or other socially relevant groupings	N/A. No human participants, data, or biological materials are included in this study.
Population characteristics	N/A. No human participants, data, or biological materials are included in this study.
Recruitment	N/A. No human participants, data, or biological materials are included in this study.
Ethics oversight	N/A. No human participants, data, or biological materials are included in 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

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 the sample size for the single-cell RNA sequencing. 19 samples from individual mice were used for the single-cell transcriptome data generation.
Data exclusions	Low-quality cells meeting any one of the following criteria were excluded in the downstream analysis: (1) UMI gene count <1000, (2) number of genes detected <500, (3) number of genes detected >7000, (4) fraction of mitochondrial genes <10%, and (5) the predicted doublets identified by Scrublet.
Replication	For most of the experiments conducted in this study, 4-8 biological replicates were used for statistical comparison.
Randomization	For all experiments, mice were allocated randomly to the control and experiment groups.
Blinding	Researchers had to track the mice that were given shots during the mice experiment, therefore blinding was not suitable. Sequencing data generation and data pre-processing steps were performed blindly.

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	antibody specific for mouse IFN-gamma (890895, R&D Systems)

Antibodies used	anti-IFN-β antibody (Clone HDb-4A7, B659-1MG Lenico Technologies) APC/Cyanine7 anti-mouse CD3 Antibody (Clone 17A2, 100222, BioLegend) PerCP/Cyanine5.5 anti-mouse CD8a Antibody (Clone 53-6.7, 100734, BioLegend) TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody (Clone S17011E, 156604, BioLegend) BD Pharmingen™ APC Rat Anti-Mouse IFN-γ (Clone XMG1.2, 554413, BD)
Validation	Antibodies were validated by the supplier with citations: IFN-gamma antibody (https://www.rndsystems.com/products/mouse-ifn-gamma-elispot-kit_el485), anti-IFN-beta antibody (https://www.leinco.com/p/anti-mouse-ifn-purified-functional-grade-gold-4/), anti-CD3 antibody (https://www.biolegend.com/fr-ch/products/apc-cyanine7-anti-mouse-cd3-antibody-6068), CD8a antibody (https://www.biolegend.com/de-at/products/percp-cyanine5-5-anti-mouse-cd8a-antibody-4255), and the Fc blocker (https://www.biolegend.com/de-at/products/trustain-fcx-plus-anti-mouse-cd16-32-antibody-17085)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Vero E6 cell line was isolated from the kidney of an African green monkey
Authentication	Authenticated by ATCC
Mycoplasma contamination	Not detected
Commonly misidentified lines (See ICLAC register)	None

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 BALB/c mice aged 4–6 weeks were purchased from Samtako Bio Korea. Mice were housed and bred in the specific pathogen–free mouse facility, where temperature, humidity, and light cycle were controlled. Animal experiments were conducted in an animal biosafety level 2 facility.
Wild animals	No wild animals were used in this study
Reporting on sex	Female BALB/c mice were used in this study
Field-collected samples	No filed-collected samples were used in this study
Ethics oversight	The animal protocol used in this study was reviewed and approved by the Institutional Animal Care and Use Committee of the Korea Centers for Disease Control and Prevention (KCDC-IACUC-22-004).

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

Plants

Seed stocks	N/A. No plant-related materials are included in this study.
Novel plant genotypes	N/A. No plant-related materials are included in this study.
Authentication	N/A. No plant-related materials are included in this study.

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	Spleens were collected 35 d after prime (14 d after boost) and cells were isolated by mechanical disruption using a gentleMAX machine. After filtering with strainers, red blood cells were removed using an ACK lysis buffer. Cells were suspended in RPMI containing 10% FBS and 1% P/S and seeded at 1 × 106 cells per 100 µL in 96-well U-shaped plate. Cells were restimulated with 100 ng/well SARS-CoV-2 Spike Glycoprotein-crude (RP30020, GeneScript) in complete RPMI 1640 medium. GolgiPlug (51-2301KZ, BD) was added to each well and incubated in a 37°C incubator for 18 to 24 hours. After collecting the stimulated cells in a tube, wash the cells with PBS. For Live/Dead staining, cells were stained with Aqua Fluorescent Reactive Dye (1:300 dilution; L34965, Invitrogen) and incubated for 30 min at 4°C. After washing steps, cells were stained with following surface marker antibodies: anti-CD3 (1:200 dilution; APC/Cyanine7; 100222, BioLegend), anti-CD8 (1:200 dilution; PerCP/Cyanine5.5; 100734, BioLegend), for 30 minutes at 4ºC, and FC Receptor block antibody anti-CD16/32 (1:500 dilution; 156604, BioLegend) was added to Flow cytometry staining buffer (00-4222-26, eBioscience). For intracellular cytokine staining, cells were washed with the Cytofix/Cytoperm solution (51-2090KZ, BD) added staining buffer according to Cytofix/CytopermTM Plus (555028, BD) protocol, and the cells were incubated for 40 minutes at 4ºC and washed twice with 1X Perm/Wash buffer (51-2091KZ, BD). We added anti-IFN-γ (1:100 dilution, APC, 554413, BD) antibodies to the cells and incubated for 1 hour at 4°C. Finally, cells were washed with 1X Perm/Wash buffer and resuspended with the Flow cytometry staining buffer.
Instrument	Data were acquired on a CytoFLEX LX flow cytometer (Beckman Coulter)
Software	FlowJo analysis software v10
Cell population abundance	Cell population abundances were anlyzed using FLowJo software. This study do not involve cell-sorting experiments.
Gating strategy	Lymphocytes were first gated using FSC-A and SSC-A, doublets were sequentially excluded by combining SSC-A and SSC-H. Cells were labeled with viability dye to exclude dead cells, and with anti-CD3, -CD8 antibodies followed by fixation and permeabilization and then stained with anti-IFN-γ. IFN-γ producing T-cells were defined as CD3+CD8+IFN-γ+ or CD3+CD8-IFN-γ+ cells.

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