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Reporting Summary

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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	cDNA libraries were constructed starting with 10 ng of total RNA from lung tissues of each sample that was extracted using a MagMax mirVana Total RNA isolation kit (Thermo Scientific). cDNA was generated using the Seqplex kit (Sigma-Aldrich) with amplification of 20 cycles. Library construction was performed using 100 ng of cDNA undergoing end repair, A tailing, ligation of universal TruSeq adapters and amplification of 8 cycles to incorporate unique dual index sequences. Libraries were sequenced on the NovaSeq 6000 (Illumina, San Diego, CA) targeting 40 million read pairs and extending 150 cycles with paired end reads. Flow cytometry data were acquired on a BD-X20 cytometer (BD Biosciences) . Respiratory mechanics data was acquired using a flexiVent computer-controlled piston ventilator (SCIREQ Inc.).
Data analysis	RNA-seq reads were aligned to the mouse Ensembl data (GRCh38.76 primary assembly) and Genbank data (SARS-CoV-2 NCBI NC_045512 Wuhan-Hu-1 genome) with STAR program (version 2.5.1a). Gene counts were derived from the number of uniquely aligned unambiguous reads by Subread:featureCount (version 1.4.6-p5). The ribosomal fraction, known junction saturation, and read distribution over known gene models were quantified with RSeQC (version 2.6.2). All gene counts were preprocessed with the R package EdgeR v4.0.1 to adjust samples for differences in library size using the trimmed mean of M values (TMM) normalization procedure. Ribosomal genes and genes not expressed at a level greater than or equal to 1 count per million reads in the smallest group size were excluded from further analysis. The R package limma with voomWithQualityWeights v4.0.1 function was utilized to calculate the weighted likelihoods for all samples, based on the observed mean-variance relationship of every gene and sample. Differentially expressed genes were defined as those with at least 2-fold difference between two individual groups at the Benjamini-Hochberg false-discovery rate (FDR) adjusted p-value, i.e. q-value < 0.05. This study used commercially available Graphpad prism software v8.2.1 for data representation and statistical analysis. FlowJo v 10.5.3 was used to analyze all flow cytometry data. Flexiware v8.1, service pack 3 was used to analyze Flexivent data.

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All data supporting the findings of this study are found within the paper and its Extended Data Figures, and are available from the corresponding author upon request. RNA sequencing data sets generated in this study are available at GEO: GSE154104.

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 sample-size calculations were performed to power each study. Sample sizes for mouse studies were determined based on previous results for similar in vivo experiments which showed that the use of 5-12 mice per group represents a minimally sufficient sample to produce a study power of >80% (PMID: 32553273). No experiments not utilizing mice or downstream analysis of samples collected from mice was included in this study.
Data exclusions	One mouse was excluded from donwstream analysis due to an injury during handling. This mouse was excluded from all downstream analysis (cytokine analysis, blood chemistry analysis, flexivent analysis, RNAseq analysis). As the injury occured at 6 dpi, weight loss data and treadmill data from this mouse that occurred prior to the injury was still included in final analysis in there two parameters.
Replication	All experiments were performed in at least 2 independent biological repeats. All attempts at replication were successful.
Randomization	Mice were randomly assigned to each treatment group (mock, 2 dpi, 4, dpi and 7 dpi). No experiments not utilizing mice or downstream analysis of the mice was included in this study.
Blinding	Investigators were not blinded for the following analyses (clinical measurements, viral burden analysis and immunological analysis) due to the biosafety concerns associated with handling these samples. For histological analyses slides were blinded prior to scoring. For RNAseq data, investigators were unblinded as to sample identities only after initial analysis. Investigators were unblinded after initial analysis in order to dissect biological pathways in the context of the other data included in this paper that could be mechanistically informative in the RNAseq data.

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
☒	☐	Human research participants
☒	☐	Clinical data
☒	☐	Dual use research of concern

Methods

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

Antibodies

Antibodies used

AF700 anti-CD45 (Biolegend, Cat #103127, clone 30 F-11), APC-Cy7 anti-CD11c (Biolegend, Cat #117323, clone N418), PE anti-Siglec F (BD, Cat #552126, clone E50-2440), PE-Cy7 anti-Ly6G (Biolegend, Cat # 127607, clone 1A8), BV605 anti-Ly6C (Biolegend, Cat # 128035, clone HK1.4) BV 711 anti-CD11b (Biolegend, Cat #101241, clone M1/70), APC anti-CD103 (eBioscience, Cat# 17-1031-82, clone 2E7), Pacific Blue anti-CD3 (Biolegend, Cat # 100213, clone 17A2), APC anti-CD4 (Biolegend, Cat # 100515, clone RM4-5), PE-Cy7 anti-CD8 (Biolegend, Cat # 100721, clone53-6.7), FITX anti-NK1.1 (Biolegend, Cat #108705 clone PK136), BV605 anti-TCR γ/δ (Biolegend, Cat # 118129, clone GL3), PE-Cy5 anti-Foxp3 (eBioscience, Cat# 15-5773-82, clone FJK-16s), Fc Block (Biolegend, Cat#101301, clone 93), BUV395 CD45 (BD, Cat #564279 clone Clone 30-F11), APC anti-mouse CD3 Antibody (Biolegend, Cat# 100235, Clone 17A2), PE anti-mouse CD19 Antibody (Biolegend, Cat#152407, Clone 1D3), Brilliant Violet 650™ anti-mouse Ly-6G Antibody (Biolegend, Cat #127641, Clone 1A8), Pacific Blue™ anti-mouse Ly-6C Antibody (Biolegend, Cat #128013, Clone HK1.4), PE/Dazzle™ 594 anti-mouse/human CD11b Antibody (Biolegend, Cat# 101255, Clone , Brilliant Violet 605™, anti-mouse/human CD11b Antibody (Biolegend, Cat #101237, Clone M1/70), PerCP/Cyanine5.5 anti-mouse CD8a Antibody (Biolegend., Cat # 100733, Clone 53-6.7), Fixable Viability Dye eFluor™ 506 (eBioscience™, Cat #65-0866-14)

Validation

The antibody panels has been used previously for the same application (PMID 29511063, PMID: 32668198).

For primary antibody validation product citations on the the manufacturers website are listed below.

1. AF700 anti-CD45 (Biolegend, Cat #103127, clone 30 F-11): PMID: 22611244, PMID: 22844125, PMID: 25404286
2. APC-Cy7 anti-CD11c (Biolegend, Cat #117323, clone N418): PMID: 23065153
3. PE anti-Siglec F (BD, Cat #552126, clone E50-2440): PMID: 11579105
4. PE-Cy7 anti-Ly6G (Biolegend, Cat # 127607, clone 1A8): PMID: 22474024
5. BV605 anti-Ly6C (Biolegend, Cat # 128035, clone HK1.4) : PMID: 30318149
6. BV 711 anti-CD11b (Biolegend, Cat #101241: PMID: 25964477
7. APC anti-CD103 (eBioscience, Cat# 17-1031-82, clone 2E7: PMID: 28533230
8. Pacific Blue anti-CD3 (Biolegend, Cat # 100213, clone 17A2): PMID: 19234143
9. PE-Cy7 anti-CD8 (Biolegend, Cat # 100721, clone53-6.7): PMID: 22952867
10. APC anti-CD4 (Biolegend, Cat # 100515, clone RM4-5): PMID: 17277158
11. FITC anti-NK1.1 FITC (Biolegend, Cat #108705 clone PK136),PMID: 25155355
12. BV605 anti-TCR γ/δ (Biolegend, Cat # 118129, clone GL3), PMID: 26587585
13. PE-Cy5 anti-Foxp3 (eBioscience, Cat# 15-5773-82, clone FJK-16s)PMID: 30410056
14. Fc Block (Biolegend, Cat#101301, clone 93) : PMID: 15699180
15. BUV395 CD45 (BD, Cat #564279 clone Clone 30-F11) : PMID: 11062533
16. APC anti-mouse CD3 Antibody (Biolegend, Cat# 100235, Clone 17A2): PMID: 26169940
17. PE anti-mouse CD19 Antibody (Biolegend, Cat#152407, Clone 1D3): PMID: 30127434
18. Brilliant Violet 650™ anti-mouse Ly-6G Antibody (Biolegend, Cat #127641, Clone 1A8): PMID: 27731313
19. Pacific Blue™ anti-mouse Ly-6C Antibody (Biolegend, Cat #128013, Clone HK1.4): PMID: 22043017
20. PE/Dazzle™ 594 anti-mouse/human CD11b Antibody (Biolegend, Cat# 101255, Clone M1/70): PMID: 26604307
21. anti-mouse/human CD11b Antibody (Biolegend, Cat #101237, Clone M1/70): PMID: 24043758
22. PerCP/Cyanine5.5 anti-mouse CD8a Antibody (Biolegend., Cat # 100733, Clone 53-6.7): PMID: 23460738
23. Fixable Viability Dye eFluor™ 506 (eBioscience™, Cat #65-0866-14): PMID: 32668198

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

In this study we used the following cell lines: Vero E6 (ATCC, Cat# CRL-1586) , Vero CCL81 (ATCC Cat#CCL81) and Vero-Furin cells (Gift of T. Pierson, NIH; originally described in PMID: 27420797)

Authentication

Morphology for each cell line was assessed by microscope. Permissiveness of each cell line was assessed through observation of CPE by microscope and through quantification of virus produced by plaque assay, focus forming assay, and qPCR for viral RNA.

Mycoplasma contamination

All cells are tested on a monthly basis.

Commonly misidentified lines
(See ICLAC register)

None

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

In this study, the following mouse strain was used B6.Cg-Tg(K18-ACE2)2Prlmn/J (https://www.jax.org/strain/034860). For viral challenge male and female eight-week old mice were challenged intranasally with SARS-CoV-2.

Mice were housed in groups of up to 5 mice/cage at 18 degrees C-24 degrees C ambient temperatures with 40-60% humidity. Mice were fed a 20% protein diet (PicoLab 5053, Purina) and maintained on a 12 hour light/ dark cycle 6 am to 6 pm. Food and water were available ad libitum.

Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Animal studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols were approved by the Institutional Animal Care and Use Committee at the Washington University School of Medicine (assurance number A3381–01). Virus inoculations were performed under anesthesia that was induced and maintained with ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.

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).

☒ 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	Single cell suspensions of BAL and lung digests were preincubated with Fc Block antibody (BD PharMingen) in PBS + 2% heat-inactivated FBS for 10 min at room temperature before staining. Cells were incubated with antibodies against the following markers: AF700 anti-CD45 (clone 30 F-11), APC-Cy7 anti-CD11c (clone N418), PE anti-Siglec F (clone E50-2440; BD), PE-Cy7 anti-Ly6G (clone 1A8), BV605 anti-Ly6C (clone HK1.4; Biolegend), BV 711 anti-CD11b (clone M1/70), APC anti-CD103 (clone 2E7; eBioscience), PB anti-CD3 (clone 17A2), PE-Cy7, APC anti-CD4 (clone RM4-5), PE-Cy7 anti-CD8 (clone53-6.7), anti-NK1.1 (clone PK136), and BV605 anti-TCR γ/δ (clone GL3). All antibodies were used at a dilution of 1:200. Cells were stained for 20 min at 4°C, washed, fixed and permeabilized for intracellular staining with Foxp3/Transcription Factor Staining Buffer Set (eBioscience) according to manufacturer's instructions. Cells were incubated overnight at 4°C with PE-Cy5 anti-Foxp3 (clone FJK-16s), washed, re-fixed with 4% PFA (EMS) for 20 min and resuspended in permeabilization buffer. For analysis of peripheral blood leukocytes, peripheral blood was obtained from the submandibular vein. Erythrocytes were lysed twice with ammonium chloride-potassium (ACK) lysing buffer (Gibco), and remaining cells were resuspended in PBS supplemented with 2% FBS and 1 mM EDTA and maintained on ice. After blockade of Fcγ receptors with anti-CD16/32 (eBioscience; clone 93) and confirmation of viability (eBioscience; FVD eFluor 506), staining for cell surface antigens CD45 BUV395, CD3 APC, CD19 PE, Ly6G BV650, Ly6C Pacific Blue, CD11b PE/Dazzle 594, NK1.1 FITC, CD4 BV605, and CD8 PerCPCy5.5 was performed at dilutions of 1:200. Cells were incubated for 20 min at 4°C, fixed with 4% PFA for 20 min, and washed prior to resuspension in PBS supplemented with 2% FBS and 1 mM EDTA. Absolute cell counts were determined using TruCount beads (BD Biosciences). Flow cytometry data were acquired on a BD-X20 cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star).
Instrument	Flow cytometry data were acquired on a BD-X20; BD Biosciences
Software	FlowJo v10.5.3
Cell population abundance	N/A. No cell sorting was performed in this study.
Gating strategy	All gating strategies are shown in Extended Data Figures. For lung tissues and BAL to myeloid cells: FSC SSC to remove debris SSC-H by SSC-A to define single cells Autofluorescent by CD45 to identify hematopeitic cells FSC by Live/Dead to identify live cells SiglecF by CD11c to define alveolar macrophages For not alveolar macrophages, CD11b+SigF+ were identified as eosiniphils For not eosinophils, SSC by CD11c to define CD11c+ and CD11c- cells For CD11c- cells, Ly6G by Ly6C to define neutrophils and monocytes respectively For CD11c+ cells, SSC by MHCII+ to define dendritic cells SSC by CD103+ to define, CD103+ DCs For CD103-, CD11b+ to define CD11b+DCs For lung tissues and BAL to lymphoid cells: FSC SSC to remove debris SSC-H by SSC-A to define single cells Autofluorescent by CD45 to identify hematopeitic cells FSC by Live/Dead to identify live cells SSC by NK1.1 to define NK+ cells

SSC by CD19+ to define B cells
FSC by gd TCR to define gamma-delta T cells
For gdTCR- cells, CD4 by CD8 to define CD4+ and CD8+ T cells
For CD4+ T cells, Fox3P to define Tregs
For CD8+ T cells, CD44 to define activated CD44+ T cells

For Peripheral blood:
FSC SSC to remove debris
FSC-H by FSC-A to define single cells
FSC by Live/Dead to identify live cells
FSC by CD45 to identify hematopoeitic cells
FSC by CD19+ to define B cells
For CD19-, FSC by CD3+ to define T cells
For CD3+, CD4 by CD8 to define CD4+ and CD8+ T cells
For CD3-, CD11b by Ly6G to define neutrophils
For Ly6G-, CD11b by Ly6C to define monocytes

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