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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

Data analysis

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 sequencing data generated in this study have been deposited in the Gene Expression Omnibus database under accession code GSE248984 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE248984>]. The remaining data generated in this study are provided in the Manuscript, Supplementary Information and Source Data file.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	Sample sizes were determined based on the outcome and variance calculations from experiments performed before in the laboratory. Sample sizes are chosen as such that biologically relevant differences can be observed and detected using statistical tests with sufficient power.
Data exclusions	no data was excluded
Replication	Experiments are the result of multiple reproducible experiments. Data for each experiment is given in the different figures in the manuscript.
Randomization	Samples were randomized during the experiment, with unique identifiers given to each sample to de-identify data for visualization
Blinding	Data was analysed by an investigator that was blinded to the groups. After analysis, groups were unblinded for data representation

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	anti-SARS-CoV-2-N monoclonal antibody (1C7C7), anti-mouse IgG HRP-conjugated antibody (ThermoFisher Scientific), Flow Cytometry: Staining was performed with Viability stain and anti-CD45, CD3 CD11b, Ly6C, CD11c, CD19, Ly6G, MHCII, B220 and SiglecF antibodies. All antibodies are purchased from BD. T cell depletion: CD4 (clone YTS 177, BioXCell) and CD8 (clone 53-5.8, BioXCell) depleting antibodies are used.
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Validation

Antibody binding is tested by western blot, flow cytometry, ELISA or immune fluorescence

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Vero-TMPRSS2 cells (BPS Bioscience, Cat# 78081)

Authentication

visual inspection under microscope

Mycoplasma contamination

Confirmed negative

Commonly misidentified lines
(See [ICLAC](#) register)

NA

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

mice: 3-months old heterozygous K18-hACE2 C57BL/6J (strain 2B6.Cg-Tg(K18-ACE2)2PrImn/J) and 129S1 (strain 129S1/SvImJ) female mice were procured from The Jackson Laboratory

Wild animals

NA

Reporting on sex

Sex is considered as a variable in our experiments in the laboratory. In this manuscript we mainly report on work in female mice, however follow up publications will also address the differences we observe between male and female mice.

Field-collected samples

NA

Ethics oversight

All animal experiments were approved by the Institutional Biosafety committee and by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai (ISMMS) (PROTO202100007). This data is included in the manuscript.

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

Plants

Seed stocks

NA

Novel plant genotypes

NA

Authentication

NA

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 T-cell analysis, whole lungs collected at 30 DPI were dissociated into a single cell suspension using gentleMACS™ Octo Dissociator (Miltenyi Biotec) and Mouse Lung Dissociation Kit, mouse (Miltenyi Biotec) as per manufacturer's instructions. The single cell suspension was then treated with eBioscience™ 1X RBC Lysis Buffer (ThermoFisher Scientific) to remove erythrocytes. The treated cells were then washed twice with PBS and resuspended in eBioscience™ Flow Cytometry Staining Buffer (ThermoFisher Scientific) containing 1:50 Mouse BD Fc Block™ (BD biosciences) antibody and incubated at room temperature for 15 mins, followed by staining for viability-e780 (Fixable Viability Dye, eBioscience) and with 1L of each

antibody: anti-CD3-FITC (Clone 145-2C11, BD Bioscience), CD8a-PerCP (Clone 53-6.7, BD Bioscience), CD44-PE-CF549 (Clone IM7, BD Bioscience), CD69-PE-Cy7 (H1.2F3, BioLegend), CD103-APC antibodies (Clone M290, BD biosciences) and SARS-CoV-2 spike tetramer (VNFNFNGL-PE, NIH core tetramer) for 15 mins at room temperature in the dark. The cells were washed twice with eBioscience™ Flow Cytometry Staining Buffer (ThermoFisher Scientific) and fixed by adding 10% formaldehyde (5% final concentration in staining buffer) and incubating for 48hrs at 4°C. Fixed cells were washed twice with staining buffer and filtered through a 100µm cell strainer (Falcon) to remove clumped cells. Filtered single cell suspensions were then analyzed using the Gallios Flow Cytometer (Beckman Coulter) and FlowJo software.

To confirm successful of T-cell depletion, 200 µL of blood was collected by terminal bleeding 4 days after the B.1.351 challenge into tubes containing 50µL of EDTA 0.5M (Invitrogen). RBC lysis, washes and Fc Block were performed as described above. Staining was performed with viability stain-eFluor520 (Fixable Viability Dye, eBioscience) and 1L of each antibody: anti-CD3-BV480 (Clone 145-2C11, BD Bioscience), CD4-BV750 (Clone RM4-5, BD Bioscience) and CD8a-BB700 (Clone 53-6.7, BD Bioscience) antibodies and incubated for 30 mins at 4°C. Cells were then fixed as described above and prepared for analysis in the cytometer. Analysis was performed in a using spectral flow Cytometer (Northern Lights, Cytek) and subsequent analysis and representation was performed with FlowJo software. We could confirm complete CD8+ T cell depletion until 4 DPI, the end of the experiment. At this time point, a population of CD4+ T cells started to reappear, be it with lower mean fluorescence intensity staining than the original CD4+ T cell population.

Characterization of the CD11c+ alveolar compartment was performed using bronchoalveolar lavage fluid obtained from mice 7 and 30 DPI. A total of 1.2 mL of cold PBS-EDTA was used for the lavage, performed in two separate 600 µl lavages. RBC lysis, washes and Fc Block were performed as described above. Staining was performed with 0.5L of viability dye-eFluor520 ((Fixable Viability Dye, eBioscience), 1L of anti-CD45-PE-CF594 (Clone 30F-11, BD Biosciences), 1L of CD3-AF532 (Clone 17A2, eBioscience) 0.5L of CD11b-BV570 (Clone M1/70, BioLegend), 1.25L Ly6C-BV510 (Clone HK1.4, BioLegend), 1L of CD11c-eFluor450 (Clone N418, eBioscience), 1L of Ly6G-BV450 (Clone 1A8-Ly6g, eBioscience), 0.5L of MHCII-PerCP (Clone M5/114.15.2, BioLegend), 2L of B220-BV605 (Clone RA3-6B2, BioLegend) and 1L of SiglecF-BV786 (Clone E50-2440, BD bioscience) antibodies and incubated for 30 mins at 4°C. Cells were then fixed as described above and prepared for analysis in the cytometer.

Instrument

For T cell analysis, Filtered single cell suspensions were then analyzed using the Gallios Flow Cytometer (Beckman Coulter). For CD11c+ cell analysis, a spectral flow cytometry (Northern Lights, Cytek) and subsequent analysis and representation was performed with FlowJo (v10.0.0) software, Downsample (v3.3.1), FlowSOM (v4.1.0) and UMAP_R (v4.0.4) plugins.

Software

Analysis and representation was performed with FlowJo (v10.0.0) software, Downsample (v3.3.1), FlowSOM (v4.1.0) and UMAP_R (v4.0.4) plugins.

Cell population abundance

NA

Gating strategy

For all samples, cells were gated on single cells using FSC/SSC gates and dead cells were excluded using viability dyes. Next cells are gated for the specific lineage markers (CD3+, CD11c+, ...) and activation markers to define the specific populations as described in the text and depicted in the gating schemes.

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