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Reporting Summary

Nature Research 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 Research 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	No software used
Data analysis	R v4.0.0, Prism v8 (Graph Pad), FlowJo v10.6.2

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

The authors declare that the data supporting the findings of this study are available within the paper and it's supplementary information files.

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 size for animal experiments was determined based on criteria set by institutional ACUC.
Data exclusions	No data were excluded.
Replication	Animal studies were completed once. All immunoassay testing was completed in duplicate or triplicate with 1 replicate, unless otherwise stated.
Randomization	Allocation of animals was not random.
Blinding	Blinding was not completed as assays were completed by the same team that immunized animals.

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	CR3022 (made in house, citation below) | For ICS, a surface stain cocktail containing the following antibodies: I-A/I-E PE (BD, cat. 557000, clone M5/114.15.2, 1/2500), CD8a BUV805 (BD, cat. 612898, clone 53-6.7, 1/80), CD44 BUV395 (BD, cat. 740215, clone IM7, 1/800), CD62L BV605 (Biolegend, cat. 104418, clone MEL-14, 1/5000), and CD4 BV480 (BD, cat. 565634, clone RM4-5, 1/500)
Validation	Jan ter Meulen, J. et al. Human Monoclonal Antibody Combination against SARS Coronavirus: Synergy and Coverage of Escape Mutants. PLOS Medicine 3, e237, doi:10.1371/journal.pmed.0030237 (2006).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi293 (ThermoFisher), HEK293T/17 (ATCC #CRL-11268), Vero E6 (ATCC), Huh7.5 cells (provided by Deborah R. Taylor, US Food and Drug Administration), ACE-2-expressing 293T (ATCC) cells (provided by Michael Farzan, Scripps Research Institute). Huh7.5 cells are a derivative of Huh7 cells (ATCC).
Authentication	Cell lines were not authenticated.
Mycoplasma contamination	All cells tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines are in this study.

Animals and other organisms

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

Laboratory animals	6-8-week-old female BALB/c (Charles River), BALB/cJ, C57BL/6J, or B6C3F1/J mice (Jackson Laboratory) | 16-20-week-old male and female 288/330+/+mice
Wild animals	There were no wild animals used in this study
Field-collected samples	There were no field-collected samples.
Ethics oversight	Animal experiments were carried out in compliance with all pertinent US National Institutes of Health regulations and approval from the Animal Care and Use Committee of the Vaccine Research Center, Moderna Inc., or University of North Carolina at Chapel Hill.

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	Mononuclear single cell suspensions from whole mouse spleens were generated using a gentleMACS tissue dissociator (Miltenyi Biotec) followed by 70 µm filtration and density gradient centrifugation using Fico/Lite-LM medium (Atlanta Biologicals). Cells from each mouse were resuspended in R10 media (RPMI 1640 supplemented with Pen-Strep antibiotic, 10% HI-FBS, Glutamax, and HEPES) and incubated for 6 hr at 37°C with protein transport inhibitor cocktail (eBioscience) under three conditions: no peptide stimulation, and stimulation with two spike peptide pools (JPT product PM-WCPV-S-1). Peptide pools were used at a final concentration of 2 µg/mL each peptide. Cells from each group were pooled for stimulation with cell stimulation cocktail (eBioscience) as a positive control. Following stimulation, cells were washed with PBS prior to staining with LIVE/DEAD Fixable Blue Dead Cell Stain (Invitrogen) for 20 min at RT. Cells were then washed in FC buffer (PBS supplemented with 2% HI-FBS and 0.05% NaN3) and resuspended in BD Fc Block (clone 2.4G2) for 5 min at RT prior to staining with a surface stain cocktail containing the following antibodies purchased from BD and Biolegend: I-A/I-E (M5/114.15.2) PE, CD8a (53-6.7) BUV805, CD44 (IM7) BUV395, CD62L (MEL-14) BV605, and CD4 (RM4-5) BV480 in brilliant stain buffer (BD). After 15 min, cells were washed with FC buffer then fixed and permeabilized using the BD Cytofix/Cytoperm fixation/permeabilization solution kit according to manufacturer instructions. Cells were washed in perm/wash solution and stained with Fc Block (5 min at RT), followed by intracellular staining (30 min at 4°C) using a cocktail of the following antibodies purchased from BD, Biolegend, or eBioscience: CD3e (17A2) BUV737, IFN-γ (XMG1.2) BV650, TNF-α (MP6-XT22) BV711, IL-2 (JES6-5H4) BV421, IL-4 (11B11) Alexa Fluor 488, and IL-5 (TRFK5) APC in 1x perm/wash diluted with brilliant stain buffer. Finally, cells were washed in perm/wash solution and resuspended in 0.5% PFA-FC stain buffer prior to running on a Symphony A5 flow cytometer (BD). Analysis was performed using FlowJo software, version 10.6.2 according to the gating strategy outlined in Extended Data Figure 9. Background cytokine expression in the no peptide condition was subtracted from that measured in the S1 and S2 peptide pools for each individual mouse.
Instrument	Symphony A5 flow cytometer (BD)
Software	FlowJo software, version 10.6.2
Cell population abundance	Concatenated files shown were generated using the same number of randomly selected events from each animal across the different stimulation conditions.
Gating strategy	Extended Data Fig. 10 shows a hierarchical gating strategy was used to unambiguously identify single, viable CD4+ and CD8+ T cells. Gating summary of SARS-CoV-2 S-specific CD4 (b-c) and CD8 (d-e) T cells. Antigen-specific T cell responses following peptide pool re-stimulation were defined as CD44hi/cytokine+.

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