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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	No software was used in this study to collect data
Data analysis	Statistical analyses were determined using GraphPad Prism version 10. Population gating and analysis of fluorescence intensity were performed with iQue Forecyt 9.0 software or FlowJo (v10.9.0).

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

All data supporting the findings of this study are available within the paper, its Supplemental or Extended Data files, or Source files. Any additional information related to the study is available from the corresponding author upon request. All reagents are available through a Material Transfer Agreement.

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	Eligible participants included healthy male and female adults >18 years of age.
Reporting on race, ethnicity, or other socially relevant groupings	The study was blinded to the investigators for race, ethnicity, or other socially relevant groupings
Population characteristics	The study was blinded to the investigators for population characteristics but included adults 18 years and older
Recruitment	The trials are being conducted across 23 sites in the United States of America, in accordance with the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Good Clinical Practice guidelines. Samples were obtained from open-label phase 2/3 studies (NCT04405076 and NCT04927065) that evaluate the immunogenicity, safety, and reactogenicity of mRNA booster vaccines in adults (18 years and older, male and female sex, healthy volunteers) who had already received 2-dose primary series (100μg) of mRNA-1273. Participants were enrolled in a sequential, non-randomized manner and received boosters of 50 μg of mRNA-1273, mRNA-1273.351, or mRNA-1273.211 (cohort 1), 50 μg of mRNA-1273 followed by 50 μg of mRNA-1273.214 or mRNA-1273.222 (cohort 2), or 50 μg of mRNA-1273 followed by 50 μg of mRNA-1273.222 and 50 μg of mRNA-1273.231 (cohort 3) or 50 μg of mRNA-1273 followed by 50 μg of mRNA-1273.222 and then 50 μg of mRNA-1273.815 (cohort 4).
Ethics oversight	The central Institutional Review Board/Ethics Committee (Advarra, Inc., 6100 Merriweather Drive, Columbia, MD 21044) approved the protocol and consent forms. All participants provided written informed consent.

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 sample sizes were chosen a priori but instead estimated based on prior knowledge of anticipated experimental differences among groups. All experiments with statistical analysis were repeated at least two independent times, each with multiple technical replicates. Experimental size of animal and human cohorts was determined based on prior experience performing studies in mice and on availability of samples.
Data exclusions	No data was excluded.
Replication	All experiments had multiple biological and/or technical replicates and are indicated the Figure legends.
Randomization	For animal studies, mice were randomly assigned from large batches obtained from the vendor to different experimental groups in an age-matched distribution. For human studies, participants were randomized in accordance with the specific clinical trial.
Blinding	No blinding was performed in mouse studies as we needed to know which animals received control or specific vaccines. Human samples were blinded to the investiigators conducting the experiments.

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

anti-mouse IgG-HRP (Sigma Aldrich, Cat. #A5278, 1:750); anti-mouse IgG Alexa Fluor™ 647 (ThermoFisher, Cat. #A-21235, 1:3000); anti-human IgG Alexa Fluor™ 647 (ThermoFisher, Cat. #A-21445, 1:3000); MAbs CV3-25 (spike and S2), SARS2-38 (RBD), or SARS2-57 (NTD) (published by the Diamond laboratory or cloned from public sequences; used at full dose response of dilutions; anti-VSV nucleoprotein antibody (Sigma Aldrich, Cat. #MABF2348, clone 10G4, 1:5,000) or anti-VSV matrix protein antibody (Sigma Aldrich, Cat. #MABF2347, clone 23H12, 1:5,000).

For competition mAbs: class 1: rCov2-3731; class 3: S309, rCoV2-3872, rCoV2-3967, and rCoV2-3889; class 4: rCoV2-4030)30 and the S2 stem helix region (CC40.8 and CV3-25). These mAbs were produced in the Diamond and Crowe laboratories and used at concentrations indicated in the Methods.

For MBC studies, we used the following antibodies: IgD FITC (1:160, goat polyclonal, Southern Biotech), IgM PerCP-Cy5.5 (1:20, clone G20-127, BD Biosciences), IgA Dylight 405 (1:80, goat polyclonal, Jackson Immunoresearch Inc), CD20 BV570 (1:40, clone 2H7, Biolegend), CD27 BV650 (1:20, clone O323, Biolegend), CD14 BV785 (1:80, clone M5E2, Biolegend), CD16 BUV496 (1:80, clone 3G8, BD Biosciences), CD4 BUV737 (1:320, clone SK3, BD Biosciences), CD19 APC (1:80, clone J3-119, Beckman), IgG Alexa 700 (1:20, clone G18-145, BD Biosciences), CD3 APC-Cy7 (1:40, clone SP34-2, BD Biosciences), CD38 PE (1:160, clone OKT10, Caprico Biotechnologies), CD21 PE-Cy5 (1:40, clone B-ly4, BD Biosciences) and CXCR5 PE-Cy7 (1:40, clone MU5UBEE, Thermo Fisher Scientific).

Validation

All primary Abs were validated using purified viral proteins by ELISA or transfected/infected cells by flow cytometry by the Diamond laboratory. All secondary antibodies were validated against indicated proteins by the manufacturer per their associated Data Sheets and are included on their websites.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

African green monkey Vero-TMPRSS2 cells were generated in the Diamond laboratory. Vero CCL-81 cells were obtained from the ATCC (CCL-81). Expi293F cells were purchased commercially (ThermoFisher, Cat # A14527). FreeStyle 293F cells were purchased comercially (ThermoFisher, Cat. #R79007). MA104 cells were obtained from the ATCC (CRL-2378.1).

Authentication

These cells grew as expected and propagated virus as expected. Also, we confirmed expression of specific transgenes (TMPRSS2) using antibodies and flow cytometry. Other cells were not specifically authenticated.

Mycoplasma contamination

All cell lines are routinely tested each month and were negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

This study did not involve any commonly misidentified cell lines.

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

Six-week-old female C57BL/6J mice (Cat # 000664, Jackson Laboratory) were used for all immunization experiments. Mice were housed in groups of 3 to 5. Photoperiod = 12 hr on:12 hr off dark/light cycle. Ambient animal room temperature is 70° F, controlled within ±2° and room humidity is 50%, controlled within ±5%.

Wild animals

No wild animals were used in this study.

Reporting on sex

Female mice were used to allow co-housing in groups without animal fighting, which could adversely impact immune responses.

Field-collected samples

No field collected samples were used in this study.

Ethics oversight

All experiments were conducted with approval of the Institutional Animal Care and Use Committee at the Washington University School of Medicine (Assurance number A3381-01)

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	Recombinant biotinylated spike proteins (spike, RBD, NTD, S2) from Wuhan-1, B.1.351, BA.1, BA.5, and XBB.1.5 strains of SARS-CoV-2 were incubated with different fluorescence intensity peaks of the SPHERO Streptavidin fluorescent Yellow Particles (Spherotech, Cat. #SVF3-2552) at 20 ng per µg beads for 30 min at room temperature on an end-over-end mixer. Free biotin (Avidity, Cat. #BIO200) was added to the beads at 5 µM and incubated for 15 min at room temperature. Beads of different intensity and loaded with different spike proteins were then pooled and washed with PBS supplemented with 2% FBS, 2 mM EDTA, and 0.1% sodium azide (FACS buffer), mixed with serially diluted pre-cleared serum samples or mAbs, and incubated for 30 min at room temperature. Beads were washed twice with FACS buffer and stained with a mixture of anti-mouse IgG Alexa Fluor™ 647 (ThermoFisher, Cat. #A-21235, 1:3000) and anti-human IgG Alexa Fluor™ 647 (ThermoFisher, Cat. #A-21445, 1:3000) for 10 min at room temperature in dark. After two washes with FACS buffer, beads were resuspended in FACS buffer and acquired on flow cytometer iQue®3 For MBC studies, cryopreserved PBMC were thawed, washed briefly with RPMI and 4% heat inactivated newborn calf serum, and incubated with aqua live/dead viability dye (Thermo Fisher Scientific) for 20 min at room temperature. Cells were stained with the following antibodies (monoclonal unless indicated) for 20 min at room temperature: IgD FITC (1:160, goat polyclonal, Southern Biotech), IgM PerCP-Cy5.5 (1:20, clone G20-127, BD Biosciences), IgA Dylight 405 (1:80, goat polyclonal, Jackson Immunoresearch Inc), CD20 BV570 (1:40, clone 2H7, Biolegend), CD27 BV650 (1:20, clone O323, Biolegend), CD14 BV785 (1:80, clone M5E2, Biolegend), CD16 BUV496 (1:80, clone 3G8, BD Biosciences), CD4 BUV737 (1:320, clone SK3, BD Biosciences), CD19 APC (1:80, clone J3-119, Beckman), IgG Alexa 700 (1:20, clone G18-145, BD Biosciences), CD3 APC-Cy7 (1:40, clone SP34-2, BD Biosciences), CD38 PE (1:160, clone OKT10, Caprico Biotechnologies), CD21 PE-Cy5 (1:40, clone B-ly4, BD Biosciences) and CXCR5 PE-Cy7 (1:40, clone MU5UBEE, Thermo Fisher Scientific). Stained cells were then incubated with streptavidin-BV605 (BD Biosciences) labeled BA.1 S-2P (1:6.25), streptavidin-BUV661 (BD Biosciences) labeled S-2P WA-1 (1:25), and either streptavidin-BUV395 labeled S-2P BA.5 (1:25) or streptavidin-BUV395 labeled S-2P BQ.1.1 (1:25) for 30 min at 4°C (protected from light). Cells were washed and fixed in 0.5% formaldehyde (Tousimis Research Corp) prior to data acquisition. All antibodies were titrated on human PBMC to determine the optimal concentration. Samples were acquired on an BD FACSymphony cytometer and analyzed for B cell phenotype using FlowJo version 10.9.0
Instrument	iQue®3 (Sartorius), BD FACSymphony flow cytometer
Software	Population gating and analysis of fluorescence intensity were performed with iQue Forecyt software and FlowJo version 10.9.0.
Cell population abundance	Sorting was not done
Gating strategy	Population gating and analysis of fluorescence intensity were performed with iQue Forecyt software and FlowJo version 10.9.0.

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