

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

Neutralizing antibody data were collected using a Luminoskan luminometer.
Flow cytometry data were acquired using a BD FACSymphony A5 flow cytometer (BD Biosciences).

Data analysis

Neutralizing antibody data analysis (inhibition curve fitting and ID50 determinations) was done using Monogram proprietary analysis software. Flow cytometry data were analyzed using FlowJo version 9.9.4 (FlowJo LLC).
All analyses were done reproducibly on the basis of publicly available R scripts hosted on the GitHub collaborative programming platform at https://github.com/CoVpN/correlates_reporting2/tree/R40. The repository hosts multiple modular workflows for correlates of risk/protection analyses and automated reporting of analytic results. A complementary repository that handles the upstream data processing aspects of the analysis workflow is available at https://github.com/CoVpN/correlates_processing. The R scripts are also available on Zenodo (<https://doi.org/10.5281/zenodo.18217537>) (reference number 54).
CoVpN/correlates_reporting2 analysis modules used in the present work, along with the figures/tables they were used to generate are listed on https://github.com/CoVpN/correlates_reporting2/blob/R40/manuscripts/COVAILTcellCoP_DataandCode.md.
Additional code in Supplementary Software 1 was used to generate the following figures/tables: clustering: 002A_extract_flow_exprs_data_CD4+T.R (obtain fluorescence intensities), 002Ba_Leiden_k=30.R (run Leiden and some downsampling for visualization), and 002D_MIMOSA_CD4+T.R (run MIMOSA positivity calls): used in clustering figures (Supplementary Figs. 29-31); 002_High-Dimensional-ICS-Analysis_CD4+T.Rmd (clustering analysis, Supplementary Fig. 30); 002C_UMAP-emb.R: generate UMAP plot of Leiden clusters (Supplementary Fig. 29).

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 trial dataset will be available to appropriate academic parties on request from the corresponding author, in accordance with the data sharing policies of the Division of Microbiology and Infectious Diseases of NIAID with input from the investigator group subject to submission of a suitable study protocol and analysis plan. Requests should be directed to Dr. Paul C. Roberts (paul.roberts@nih.gov), and will be responded to within a month.

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

Cell lines: The pseudovirus neutralization assay used the HEK 293 cell line. The HEK 293 cell line was derived from a human fetus, with Lin et al. (<https://doi.org/10.1038/ncomms5767>) having reported evidence that HEK 293 cells are of female provenance (i.e. the complete absence of any Y-chromosome-derived sequence in high-coverage genomic sequencing data). It is unknown whether alternative neutralization assays would provide different results if based on a HEK cell line derived from a male donor.

In the COVAIL trial, information on sex was self-reported according to a binary choice (male/female). Responses were recorded by the research team.

There were no target recruitment goals for ensuring that a given percentage of the total cohort would be a certain sex. Randomization was not stratified to any given sex. None of the statistical analyses adjusted for sex as a covariate. However, sex (male/female) was among the baseline demographic input variables used to build the risk score (see section 9.1 in the Statistical Analysis Plan, included in the supplementary material).

Of the total 387 (68.0%) naïve participants in the PP-IAS, 183 were female (mean age 51.6 years) and 204 were male (mean age 52.2 years). The remaining 182 (32.0%) were non-naïve (73 female, mean age 48.1 years and 109 male, mean age 46.7 years), almost all of whom (172/182, 94.5%) had a positive anti-Nucleocapsid test result at baseline. The remaining 10 non-naïve participants (5.5%) tested anti-Nucleocapsid negative at baseline and were classified as non-naïve due to self-reporting a previous SARS-CoV-2 infection.

- Supplementary Data 1 presents geometric mean response magnitudes and 95% confidence intervals for D1 CD4+ IFN-g and-or IL-2 Spike BA.4/5, D1 CD8+ IFN-g and-or IL-2 Spike BA.4/5, D15 CD4+ IFN-g and-or IL-2 Spike BA.4/5, and D15 CD8+ IFN-g and-or IL-2 Spike BA.4/5 (as well as D1 CD4+ IFN-g and-or IL-2 N Index and D1 CD8+ IFN-g and-or IL-2 N Index, in the non-naïve cohort only), separately by each vaccine arm, separately among the naïve and non-naïve cohorts, stratified by sex (male, female). This was a post hoc analysis.

- Supplementary Data 2 presents analogous information as shown in Supplementary Data 1, except for Spike BA.4/5 functionality score (means and 95% confidence intervals). This was a post hoc analysis.

- Supplementary Figures 20 and 21 present covariate-adjusted hazard ratios (HRs) and 95% confidence intervals of COVID-19 per 3-fold increase in CD4+ or CD8+ T-cell response magnitude (or, in Supplementary Figure 21, per 0.01 unit increase in functionality score) at D1 or at D15 in the one-dose mRNA group, separately for the naïve and non-naïve cohorts, separately by sex (male, female). The only exception is that analyses in females in the non-naïve cohort were not done due to <10 endpoints (only 5). This was a post hoc analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Relevant terms for race and ethnicity were provided by the researchers, and participants could select multiple choices of boxes to tick. Participants were classified into the different categories by self report.

Race and ethnicity were each included among the baseline demographic variables that were included for building the risk score (see section 9.1 in the SAP, included in the Supplementary Material).

Population characteristics

Of the total 387 (68.0%) naïve participants in the PP-IAS, 183 were female (mean age 51.6 years) and 204 were male (mean age 52.2 years). The remaining 182 (32.0%) were non-naïve (73 female, mean age 48.1 years and 109 male, mean age 46.7 years), almost all of whom (172/182, 94.5%) had a positive anti-Nucleocapsid test result at baseline. The remaining 10 non-naïve participants (5.5%) tested anti-Nucleocapsid negative at baseline and were classified as non-naïve due to self-reporting a previous SARS-CoV-2 infection.

Recruitment

As described in Branche et al. Nat Med 2023, participants were recruited from 22 geographically diverse sites in the US with a goal of ensuring at least 25% of the total cohort would be non-white race or Hispanic ethnicity. Sites independently created a recruitment plan which may have included but were not limited to social media strategies, print media ads, radio and television ads, institutional news letters and the use of database registries. These specific methods were not tracked centrally by the sponsor but overseen by the central IRB. There may be inherent biases related to site specific recruitment strategies which might impact which participants were enrolled that could not be accounted for within the scope of the study.

Ethics oversight

The trial (NCT05289037) was reviewed and initially approved by the Advarra central institutional review board on March 22, 2022 and overseen by an independent Data and Safety Monitoring Board. All participants provided written informed consent before enrollment. A stipend was provided for participation in the study, which was determined by each enrolling site.

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](https://www.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

The SAP in Zhang et al. 2025 Nature Communications contains information on the prespecified numbers of evaluable COVID-19 endpoints for immune correlates analyses to be conducted in that paper. Section 3.2 defined three time periods of follow-up and specifies that for each analysis for each time-period, if there a total of fewer than 25 evaluable COVID-19 endpoints available to include in the analysis, then the analysis will be canceled. However, some of the Super Learner analyses required a larger number of endpoints (at least 50) to be conducted, as shown in Table 2 of the SAP of Zhang et al.

The same requirements for evaluable endpoints applied to this T cell correlates manuscript.

Table 1 shows the numbers of participants with T cell markers measured: 122 cases and 209 non-cases among mRNA naïve; 22 cases and 117 non-cases among mRNA non-naïve; 13 and 43 for Sanofi naïve; and 4 and 39 for Sanofi non-naïve.

Data exclusions

For flow cytometry, data are excluded if the number of CD4+ T cells acquired on the cytometer for a particular stimulation are <10,000, or CD8+ T cells <5,000. These criteria are pre-established in the assay SOP. Selected data may be excluded if the data are determined to be unreliable by the laboratory. The reliability is based on the review of the data as analyzed in the FlowJo analysis software. It is very rare for data to be determined to be unreliability and this would typically be due to unusual staining profiles.

For the statistical analyses, the SAP provides the following information on data exclusions:

Section 3:

For D15 immune marker correlates analyses, follow-up for COVID-19 is right-censored at the date of receipt of the second dose (in all analyses), to avoid the complexity of a systematic perturbation in antibody response partway through follow-up for one study arm but not for others, which could complicate the interpretability of results. Descriptives show that zero COVID-19 endpoints are evaluable for correlates analyses for Arm 3 participants. Therefore Arm 3 participants contribute essentially no information about correlates. Accordingly, Arm 3 is excluded from the correlates analyses.

Section 5:

* D1 % D614 N peptides-stimulated CD8+ T cells expressing IFN γ and/or IL-2 will be included in the immunogenicity analysis and plots but excluded from correlates analyses because it did not make the positivity screening (positivity lower than 10% among one-dose mRNA non-naïves).

* N peptides-stimulated markers will not be studied as correlates for naïve participants.

We adopt a screening criterion for primary, secondary and exploratory markers: if a marker's response rate in mRNA NN is >10%, it will be studied in all cohorts, otherwise it will not.

Section 8:

T-cell markers were not measured in Arms 16 and 17, so they were dropped from SL analysis.

Replication

For flow cytometry, study samples were assayed only one time, therefore assay results for study samples were not replicated. The intracellular cytokine staining assay itself has been previously validated for multiple parameters, including reproducibility. Additionally, each batch of samples assayed contained an internal control with known CD4+ and CD8+ IFN γ and/or IL-2 responses to CMV pp65 stimulation. Internal control responses were trended over all batches assayed to verify assay performance.

For the neutralizing antibody assay, test samples were assayed in singlicate titrations per plate. Each plate included a SARS-CoV-2 positive control, a SARS-CoV-2 negative control, a daily positive control, and a specificity control.

Randomization

In the COVAIL trial, eligible participants were stratified by age (18-64 and $\geq$ 65 years) and history of confirmed SARS-CoV-2 infection, and randomly assigned across arms within each stage in an equal ratio. Randomization was stratified by previous infection status determined by self-report only, due to the delay in N-antibody testing.

Blinding

For flow cytometry, samples were assayed by group or "stage" (the different groups/stages reflected which vaccine platform the participants received) and it was also known in advance which timepoint each sample belonged to. However, within each stage, samples were blinded as to which group they belonged to.

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

Detector Antibody Fluorochrome Manufacturer Catalog Number Clone Dilution

U395 TNF BUV395 BD Biosciences 563996 Mab11 1:500

U450 Viability Live/Dead Fixable Blue Invitrogen L34962 N/A 1:500

U500 CD45RA BUV496 BD Biosciences 624283/Custom HI100 1:1667

U570 CD19 BUV563 BD Biosciences 612916 SJ25C1 1:200

U660 CD14 BUV661 BD Biosciences 624285/Custom MOP9 1:500

U730 CD154 BUV737 BD Biosciences 624286/Custom TRAP1 1:125

U780 CD8 BUV805 BD Biosciences 612889 SK1 1:1250

V450 IFN- V450 BD Biosciences 560371 B27 1:50

V510 CD4 BV480 BD Biosciences 566104 SK3 1:500

V570 CD16 BV570 BioLegend 302036 3G8 1:200

V610 CCR7 BV605 BioLegend 353224 G043H7 1:10

V655 CD25 BV650 BD Biosciences 563719 M-A251 1:20

V710 PD1 BV711 BioLegend 329928 EH12.2H7 1:10

V750 CD56 BV750 BioLegend 362556 5.1H11 1:100

V780 CCR6 BV785 BioLegend 353422 G034E3 1:50

B515 Perforin FITC BioLegend 353310 B-D48 1:50

B610 IL-5* BB630 BD Biosciences 624294/Custom TRFK5 1:385

B610 IL-13* BB630 BD Biosciences 624294/Custom JES10-5A2 1:200

B660 Ki67 BB660 BD Biosciences 624295/Custom B56 1:100

B710 IL-4 BB700 BD Biosciences 624381/Custom MP4-25D2 1:1000

G575 CXCR5 PE BioLegend 356904 J252D4 1:100

G610 IL-21 PE-CF594 BD Biosciences 624352/Custom 3A3-N2.1 1:500

G660 CXCR3 PE-Cy5 BD Biosciences 551128 IC6/CXCR3 1:5

G710 FOXP3 PE-Cy5.5 Invitrogen 35-4776-42 PCH101 1:50

G780 IL-17a PE-Cy7 BioLegend 512315 BL168 1:833

R660 IL-2 APC BioLegend 500310 MQ1-17H12 1:200

R710 Granzyme B Axl700 BD Biosciences 560213 GB11 1:200

R780 CD3 APC-Fire750 BioLegend 300470 UCHT1 1:500

V655 CD4** BV650 BD Biosciences 563875 SK3 1:50

V710 CD4** BV711 BD Biosciences 344648 SK3 1:200

B610 CD4** BB630 BD Biosciences 624294/Custom SK3 1:1000

B660 CD4** BB660 BD Biosciences 624295-Custom SK3 1:5000

B710 CD4** BB700 BD Biosciences 566392 SK3 1:50

G610 CD4** PE-CF594 BD Biosciences 566914 SK3 1:250

*IL-5 and IL-13 are detected on the same channel.

**Used for compensation control only

Validation

All antibodies were titrated upon receipt using human PBMC to verify performance and select the optimal titer. Titrations took place in the context of the intended use of the antibody in the assay. Antibodies detecting cytokines or other activation markers were titrated on PBMC that had been stimulated with SEB. Where a given lot of antibody was insufficient to assay all samples, the new lot of antibody was titrated upon receipt and bridged with the current lot to ensure equivalent performance.

Eukaryotic cell lines

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

Cell line source(s)

The pseudovirus neutralization assay used the HEK 293 cell line, sourced from the Master Cell Bank (LC0027490) established by Monogram Biosciences in 2001.

Cell lines: The pseudovirus neutralization assay used the HEK 293 cell line. The HEK 293 cell line was derived from a human fetus, with Lin et al. (<https://doi.org/10.1038/ncomms5767>) having reported evidence that HEK 293 cells are of female provenance (i.e. the complete absence of any Y-chromosome-derived sequence in high-coverage genomic sequencing data).

Authentication

No formal authentication was performed. The HEK293 cell line has been in continuous use at Monogram Biosciences since 1996.

Mycoplasma contamination

Mycoplasma testing is routinely performed per Monogram Standard Operating Procedure, and the HEK293 cell line is confirmed to test negative for mycoplasma contamination.

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

Not applicable

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

ClinicalTrials.gov ID NCT05289037

Study protocol

The full study protocol for the COVAIL trial is provided in the supplemental methods of Branche et al. 2023 Nature Medicine.

Data collection

Participants were recruited at 22 US sites comprised of research clinics associated with academic medical institutions. These sites are listed in the Supplementary Material. All clinical data was collected during scheduled visits. Recruitment for Stages 1-3 occurred from March 2022-June 2022. Data collection was completed for these stages in August 2023. Recruitment for Stage 4 occurred October 2022 and data collection was completed in November 2023.

Outcomes

Branche et al. 2023 Nat Med and Branche et al. 2023 CID describe the primary and secondary outcomes of the COVAIL trial. In the present analysis, the COVID-19 endpoint was a self-reported positive SARS-CoV-2 test (RT-PCR or antigen test) or study-conducted positive SARS-CoV-2 test (nasal swab and subsequent nucleic acid amplification test at an unscheduled illness visit) with onset date the earliest positive test date. Almost all (98%) COVID-19 endpoints met the CDC clinical criteria and supportive laboratory criteria for a COVID-19 surveillance case (Zhang et al. 2025, Nat Commun).

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

SARS-CoV-2 Spike- and Nucleocapsid-specific CD4+ and CD8+ T-cell responses were evaluated using a validated (FDA Drug Master File 026862) 27-color ICS assay essentially as described,³⁵ with the slight modification that CXCR5, PD-1, and IL-21 (for circulating T follicular helper cell detection) were used in replacement of CRTh2, CD32, and CD64. This switch was made due to increased interest in follicular helper T cells (Tfh) cells and their role in protective immunity. All antibodies were titrated upon receipt using human PBMC to verify performance and select the optimal titer. Titrations took place in the context of the intended use of the antibody in the assay. Antibodies detecting cytokines or other activation markers were titrated on PBMC that had been stimulated with Staphylococcus aureus enterotoxin B. Where a given lot of antibody was insufficient to assay all samples, the new lot of antibody was titrated upon receipt and bridged with the current lot to ensure equivalent performance. For the ICS assay, after thaw and overnight rest at 37 degrees C maintained with 5% CO2, peripheral blood mononuclear cells

(PBMCs) were plated at 1M-1.2M cells per well for each condition and stimulated ex vivo for 6 h with pooled peptides covering the SARS-CoV-2 D614G Spike subunit 1 (S1; comprising the N-terminal domain and the receptor-binding domain) and Spike subunit 2 (S2), SARS-CoV-2 Spike BA.4/5 S1 and S2, and the SARS-CoV-2 Nucleocapsid Index protein (final concentration of 1 µg/mL per 15-mer peptide, overlapping by 11 amino acids; Bio-Synthesis Inc., Lewisville, TX), in the presence of the secretory inhibitor brefeldin A (10 µg/mL; Sigma-Aldrich, St. Louis, MO) and the co-stimulatory antibodies CD28 and CD49d (each at 1 µg/mL; Becton Dickinson Biosciences, San Jose, CA). Fluorescently labeled CXCR5 phycoerythrin antibody (BioLegend, San Diego, CA) was also included in the stimulation cocktail. Stimulation with staphylococcal enterotoxin B (0.25 µg/mL; Sigma-Aldrich) served as a positive control. Peptide diluent [dimethyl sulfoxide (DMSO) at a final concentration of 0.5%] served as the negative control. The peptide pools had varying concentrations of DMSO, depending on the number of peptides in the pool and/or the amount of DMSO required for reconstituting. The final concentration of DMSO in the negative control (0.5%) was chosen such that it would always be higher than the final concentration of DMSO in the peptide pools. This has been our standard practice and is how the assay was validated (for the peptide pools used in this assay, the final concentrations of DMSO were: COV2 CON S1: lot 20K072A, 0.12% DMSO; COV2 CON S2: lot 20K073A, 0.12% DMSO; Spike BA.4/5 S1: lot 22Q070A, 0.12% DMSO; Spike BA.4/5 S2: lot 22Q075A, 0.18% DMSO; Nucleocapsid Index: lot 22P078A, 0.06% DMSO). [The COV2 CON S1 peptide pool is the Index strain but the pool also contains additional peptides covering the D614G amino acid mutation.]

Cells were incubated for 6 hours at 37 degrees C maintained with 5% CO₂. After the incubation, 20 µL 20 mM EDTA 1X PBS was added to each well and the plates were transferred to 4°C for overnight storage. The next day, cells were washed and stained with viability dye (Thermo Fisher, Waltham, MA) in 1X PBS for 20 min in the dark. The cells were washed again and incubated with the surface staining antibodies [Supplementary Table 4, panel adapted from Cohen et al.³⁶] in FACS Wash (2% FBS in 1X PBS) for 20 min in the dark. Subsequently, cells were fixed (BD FACS Lyse; BD Biosciences) for 10 min, centrifuged and permeabilized (BD FACS Perm II; BD Biosciences) for 10 min followed by two washes. Cells were then incubated with intracellular antibodies in FACS Wash in the dark for 30 min, followed by two washes and a final resuspension in FACS Wash prior to overnight storage at 4°C. Cells were acquired on a custom BD FACSymphony A5 (BD Biosciences). Data were acquired using BD FACSymphony A5 instruments equipped with 28 fluorescence detectors and 5 lasers (355 nm UV laser, 405 nm violet laser, 488 nm blue laser, 561 nm green laser, and 640 nm red laser). All instruments were previously characterized and standardized based on a slightly modified approach to the procedure described by Perfetto et al.³⁷ Instruments are also standardized to each other, allowing for interchangeable use. Prior to each acquisition, instruments were quality controlled to their optimal settings using hard dyed beads.

Instrument

Data were acquired using a BD FACSymphony A5 flow cytometer (BD Biosciences)

Software

Data were analyzed using FlowJo version 9.9.4 (FlowJo LLC)

Cell population abundance

Not applicable. Samples were not sorted.

Gating strategy

As visualized in Supplementary Fig. 36, cells were initially gated by Time followed by additional gates to remove aggregates/non-specific staining and dead cells. Further gates identified lymphocytes, singlets, CD3+ T cells, and CD4+ and CD8+ T-cell subsets. Additional upstream gates also identified monocytes, B cells, natural killer (NK), and natural killer T (NKT) cells. For each of CD4+ and CD8+, the expression of functional and phenotypic markers was determined using marginal gates. Where applicable, placement of gates was determined using Fluorescence Minus One (FMO) testing, which defined the lowest possible bound of the gate. For cytokine gate placement, the lower bounds of the gate were placed higher than the FMO to improve specificity without negatively impacting sensitivity. Select cytokine and phenotyping gates were also applied to the monocytes, B cells, NK, and NKT cells.

Spike-specific and Nucleocapsid-specific CD8 T cell responses were defined as live, CD14-, FSClow SSClow, singlet, CD19-, CD16-, CD56-, CD3+, and CD8+ T cells that expressed the designated cytokines/markers. Spike-specific and Nucleocapsid-specific CD4 T cell responses were defined as live, CD14-, FSClow SSClow, singlet, CD19-, CD16-, CD56-, CD3+, and CD4+ T cells that expressed the designated cytokines/markers. Cytokine/marker positive Spike- and/or Nucleocapsid-specific CD4+ memory T cell populations were further characterized as naïve (CD45RA+CCR7+), effector memory (CD45RA-CCR7-), central memory (CD45RA-CCR7+), and terminally differentiated effector memory T cells (TEMRA; CD45RA+CCR7-).

Representative flow plots (from the same participant) after stimulation with Spike BA.4/5 S1 and after negative control stimulation are shown in Supplementary Figs. 37 and 38, respectively.

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