nature portfolio

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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	EPIC EHR software (2020 May released) (retrospective EMR review and clinical data aggregation) and REDCap 9.3.6 (clinical data aggregation).
Data analysis	Jmp Pro 15.0.0 (SAS Institute) (graphs/statistics), GraphPad Prism 8.4.3(graphs/statistics), FlowJo software version 10.6 software (Tree Star), R 3.4.3 or 4.0.1 (graphs/statistics), iVar version 1.3.1 (data analysis), and Nextclade v.1.5.0 (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 data generated during the current study is available: raw data is available at the source data files; genomes of SARS-CoV-2 isolates are uploaded to GenBank and accession numbers are provided in the supplement and the aligned consensus genomes are available on GitHub (https://github.com/grubaughlab/paper_2021_Nab-variants).

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 statistical methods were used to calculate the sample size. Sample size was determined based on the number of adults health care workers (≥ 18 years old) from the Yale-New Haven Hospital (YNHH) that received the mRNA vaccine (Moderna or Pfizer) between November 2020 and January 2021, and were recruited and consented with the current study. This study enrolled 40 volunteers under IRB and HIC approved protocol #2000028599. The sample size number was sufficient once it kept errors at an acceptably low levels. Informed consent was obtained by trained staff and sample collection commenced immediately upon study enrollment. HCWs were followed serially post-vaccination. Clinical specimens were collected were collected at baseline (previous to vaccination), 7- and 28- post first vaccination dose, and 7-, 28- and 70-days post second vaccination dose. Sixteen SARS-CoV-2 isolates belonging to 12 lineages, were selected from the Yale SARS-CoV-2 Genomic Surveillance Initiative's weekly surveillance program in Connecticut, US. The sixteen isolates represent variants of concern/ interest, lineages with mutations of concern/interest, and controls that were identified within the surveillance program.
Data exclusions	One participant that received an adenovirus- based vaccine was excluded from this study. For the current study we had only included participants that received mRNA vaccines.
Replication	Neutralization assays were done in duplicate with 6 fold dilution for each sample. ELISAs were done in duplicate with 4 fold dilutions for each samples. Replications were successful. The flow cytometry findings were not replicated due to samples availability limitations, however longitudinal analyses were performed from human individuals.
Randomization	Vaccinated donors were stratified in two major groups, previously infected with SARS-CoV2 (recovered) on uninfected (naive), confirmed by RT-qPCR and serology.
Blinding	The clinical data were collected using EPIC EHR May 2020 and REDCap 9.3.6 software. Blood acquisition was performed and recorded by a separate team. Vaccinated HCW's clinical information and time points of collection information was not available until after processing and analyzing raw data by flow cytometry and ELISA. ELISA, neutralizations, and flow cytometry analyses were blinded.

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	All antibodies used in this study are against human proteins. BB515 anti-hHLA-DR (G46-6) (1:400) (BD Biosciences # 564516), BV605 anti-hCD3 (UCHT1) (1:300) (BioLegend #300460), BV785 anti-hCD19 (SJ25C1) (1:300) (BD Biosciences # 363028), BV785 anti-hCD4 (SK3) (1:200) (BioLegend # 344642), APCFire750 or BV711 anti-hCD8 (SK1) (1:200) (BioLegend # 344746), AlexaFluor 700 anti-hCD45RA (HI100) (1:200) (BD Biosciences # 560673), PE anti-hPD1 (EH12.2H7) (1:200) (BioLegend # 621608), APC or PE-CF594 anti-hTIM3 (F38-2E2) (1:50) (BioLegend # 345012), BV711 anti-hCD38 (HIT2) (1:200) (BioLegend # 303528), BB700 anti-hCXCR5 (RF8B2) (1:50) (BD Biosciences # 566470), PE-CF594 anti-hCD25 (BC96) (1:200) (BD Biosciences #562403), AlexaFluor 700 anti-hTNFa (MAb11) (1:100) (BioLegend # 506338), PE or APC/Fire750 anti-hIFNy (4S.B3) (1:60) (BioLegend # 343536), FITC anti-hGranzymeB (GB11) (1:200) (BioLegend # 515403), BV785 anti-hCD19 (SJ25C1) (1:300) (BioLegend # 302240), BV421 anti-hCD138 (MI15) (1:300) (BioLegend # 356516), AlexaFluor700 anti-hCD20 (2H7) (1:200) (BioLegend # 302310), AlexaFluor 647 anti-hCD27 (M-T271) (1:350) (BioLegend # 356434), PE/Dazzle594 anti-hIgD (IA6-2) (1:400) (BioLegend # 348240), Percp/Cy5.5 anti-hCD137 (4B4-1) (1:150) (BioLegend #309814) and PE anti-CD69 (FN-50) (1:200) (BioLegend # 310906), APC anti-hCD40L (24-31) (1:100) (BioLegend #

313008), HRP anti-Human IgG Antibody (#A00166) (GenScript) (1:5,000), SARS-CoV-2 Human Anti-Spike (AM006415) (1:500) (Active Motif #91351), SARS-CoV-2 Human anti-Nucleocapsid (1A6) (1:500) (Active Motif # MA5-35941).

Validation

All antibodies used in this study are commercially available, and all have been validated by the manufacturers and used by other publications. All antibodies listed below used for flow cytometry were quality control tested by immunofluorescent staining with flow cytometric analysis. HRP anti-Human IgG Antibody (#A00166) (GenScript), SARS-CoV-2 Human Anti-Spike (AM006415) (Active Motif #91351), SARS-CoV-2 Human anti-Nucleocapsid (AM006415) (Active Motif #91351) were quality control tested by ELISA. Likewise, we titrated these antibodies according to our own our staining conditions. The following were validated in the following species: BB515 anti-hHLA-DR (G46-6) (BD Biosciences) (Human, Rhesus, Cynomolgus, Baboon), BV605 anti-hCD3 (UCHT1) (BioLegend) (Human, Chimpanzee), BV785 anti-hCD19 (SJ25C1) (BD Biosciences) (Human), BV785 anti-hCD4 (SK3) (BioLegend) (Human), APCFire750 or PE-Cy7 or BV711 anti-hCD8 (SK1) (BioLegend) (Human, Cross-Reactivity: African Green, Chimpanzee, Cynomolgus, Pigtailed Macaque, Rhesus, Sooty Mangabey), AlexaFluor 700 anti-hCD45RA (HI100) (BD Biosciences) (Human), PE anti-hPD1 (EH12.2H7) (BioLegend) (Human, African Green, Baboon, Chimpanzee, Common Marmoset, Cynomolgus, Rhesus, Squirrel Monkey), APC anti-hTIM3 (F38-2E2) (BioLegend) (Human), BV711 anti-hCD38 (HIT2) (BioLegend) (Human, Chimpanzee, Horse), BB700 anti-hCXCR5 (RF8B2) (BD Biosciences) (Human), PE-CF594 anti-hCD25 (BC96) (BD Biosciences) (Human, Rhesus, Cynomolgus, Baboon), AlexaFluor 700 anti-hTNFa (MAb11) (BioLegend) (Human, Cat, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus, Pigtailed Macaque, Sooty Mangabey, Swine), PE or APC/Fire750 anti-hIFNy (4S.B3) (BioLegend) (Human, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus), FITC anti-hGranzymeB (GB11) (BioLegend) (Human, Mouse, Cross-Reactivity: Rat), BV785 anti-hCD19 (SJ25C1) (BioLegend) (Human), BV421 anti-hCD138 (MI15) (BioLegend) (Human), AlexaFluor700 anti-hCD20 (2H7) (BioLegend) (Human, Baboon, Capuchin Monkey, Chimpanzee, Cynomolgus, Pigtailed Macaque, Rhesus, Squirrel Monkey), AlexaFluor 647 anti-hCD27 (M-T271) (BioLegend) (Human, Cross-Reacitivity: Baboon, Cynomolgus, Rhesus), PE/Dazzle594 anti-hIgD (IA6-2) (BioLegend) (Human), Percp/Cy5.5 anti-hCD137 (4B4-1) (BioLegend), Human, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus), PE anti-CD69 (FN-50) (BioLegend) (Human, African Green, Baboon, Chimpanzee, Cynomolgus, Pigtailed Macaque, Rhesus), APC anti-hCD40L (24-31) (BioLegend) (Human, African Green, Baboon, Cynomolgus, Rhesus), HRP anti-Human IgG Antibody (#A00166) (GenScript) (Human), SARS-CoV-2 Human Anti-Spike (AM006415) (Active Motif) (Human), SARS-CoV-2 Human anti-Nucleocapsid (1A6) (Active Motif) (Human).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	TMPRSS2-VeroE6 kidney epithelial cell line was obtained from the ATCC
Authentication	TMPRSS2-VeroE6 was obtained from ATCC, tested and authenticated by morphology, karyotyping, and PCR based approaches.
Mycoplasma contamination	The cell line tested negative for contamination with mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Cohort characteristics: age (average, 46.19), sex (Male 19.44% / Females 80.56.%). Full demographic data is included in Extended data table 1.
Recruitment	HCW volunteers from the Yale New Haven Hospital (YNHH) were recruited between November 2020 and January 2021 during SARS-CoV-2 vaccination program. Participants were enrolled during the vaccination program with no self selection. The study goal (charactherization of the immune response post vaccination) was explained to the participants. Participants interested in the study , consented with the current study and were recruited. Informed consent was obtained by trained staff and sample collection commenced immediately upon study enrollment. Plasma and PBMCs samples were collected at baseline (previous to vaccination), 7- and 28- post first vaccination dose, and 7-, 28- and 70-days post second vaccination dose.
Ethics oversight	Yale Human Research Protection Program Institutional Review Boards. Informed consents were obtained from all enrolled healthcare workers. • Our research protocol was reviewed and approved by the Yale School of Medicine IRB and HIC (#2000028599). Informed consent was obtained by trained staff and records maintained in our research database for the duration of our study. There were no minors included on this study.

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	Frozen isolated PBMCs were stained for live and dead markers, blocked with Human TruStan FcX , stained for surface markers and then fixed with PFA 4%. For intracellular cytokine staining following stimulation , cells were surface stained, washed and fixed in 4% PFA. After permeabilization with 1X Permeabilization Buffer cells were stained for intracellular cytokines analysis.
Instrument	Cells were acquired on an Attune NXT (ThermoFisher).
Software	Data were analysed using FlowJo software version 10.6 software (Tree Star).
Cell population abundance	Cell population abundance: Cells populations were reported in various formats including proportion of live, single PBMC (% of Live), or as a proportion of a parent gate (% of CD4 T cells, % of Monocytes, etc.). The full gating path for clarification is included in the extended figure 4.
Gating strategy	SSC-A and FSC-A parameters were used to select leukocytes from isolated PBMCs. Live and dead cells were defined based on aqua staining. Singlets were separated based on SSC/ FSC parameters. Leukocytes were gated based on to identify lymphocytes (CD3/CD4/CD8/CD19 markers). Activated T cells were defined using HLA-DR, CD38, CCR7,CD127, PD1, TIM-3, CXCR5, CD45RA, CD25.

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