nature portfolio

Corresponding author(s): Ali H. Ellebedy

Last updated by author(s): Jan 31, 2022

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	Flow cytometry data were acquired using SpectroFlo software v.2.2.
Data analysis	Flow cytometry data were analyzed using FlowJo v.10 and Prism v.9. ELISA and ELISpot were analyzed using Prism v.9. Sequencing data were analyzed using pRESTO v.0.6.2, Cell Ranger v.6.0.1, GRCh38 human reference, IgBLAST v.1.17.1, IMGT/GENE-DB release 202113-2, Change-O v.1.0.2, TIgGER v.1.0.0, Alakazam v.1.1.0, fastcluster v.1.2.3, R v.4.1.0, ggplot2 v.3.3.5, Prism v.9, circlize v.0.4.13, SHazaM v.1.0.2, IgPhyML v.1.1.3, ggtree v.3.0.4, SCANPY v.1.7.2, and Python v.3.8.8. GRNT data were analyzed using IN Cell Analyzer 1000 Workstation Software v3.7. BLI data were analyzed using BIAevaluation v4.1.

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

Raw sequencing data and transcriptomics count matrix are deposited at Sequence Read Archive and Gene Expression Omnibus respectively under BioProject PRJNA777934. Processed transcriptomics and BCR data are deposited at https://doi.org/10.5281/zenodo.5895181 on Zenodo. Previously reported bulk-sequenced

BCR data used in this study were deposited under PRJNA731610 and PRJNA741267 on SRA, and at https://doi.org/10.5281/zenodo.5042252 and https://doi.org/10.5281/zenodo.5040099 on Zenodo.

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	Total 43 healthy participants were enrolled based on recruitment, of whom 42 provided peripheral blood, 15 provided axillary lymph node samples and 11 provided bone marrow samples. Thirteen out of 43 healthy participants have a history of prior SARS-CoV-2 infection. Non-vaccinated 48 convalescent patients were enrolled based on recruitment. Sample size was not determined by statistical methods, but gave sufficient statistics of the effect sizes of interest.
Data exclusions	No data were excluded
Replication	Human samples were collected from 43 participants. ELISA and GFP-reduction neutralization test were performed once with two technical replicates. Affinity analysis via biolayer interferometry was performed at least two technical replicates according to the fitting-curve. ELISpot and flow cytometry experiments were performed once for each sample at each time point due to insufficient specimens. All atttempts at replication were successful.
Randomization	Different experimental groups were not used.
Blinding	This is not relevant, as this is an observational study.

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

1. Donkey anti-human IgG (H+L) (Jackson ImmunoResearch, 709-005-149)
2. HRP-conjugated goat anti-human IgG (H+L) (Jackson ImmunoResearch, 109-035-088)
3. HRP-conjugated goat anti-Human IgG Fcγ fragment (Jackson ImmunoResearch, 109-035-190)
4. HRP-conjugated goat anti-human serum IgA α chain (Jackson ImmunoResearch, 109-035-011)
5. HRP-conjugated goat anti-human IgM (Caltag, H15007)
6. BCL6-PE (K112-91, BD Pharmingen, 561522)
7. BLIMP1-A700 (646702, R&D, IC36081N)
8. CD38-BB700 (HIT2, BD Horizon, 566445)
9. CD45-A532 (HI30, Thermo, 58-0459-42)
10. IgA-FITC (M24A, Millipore, CBL114F)
11. IgG-BV480 (goat polyclonal, Jackson ImmunoResearch, 109-685-098)
12. PD-1-BB515 (EH12.1, BD Horizon, 564494)
13. CD3-FITC (HIT3a, BioLegend, 300306)
14. CD3-APC-Fire810 (SK7, BioLegend, 344858)
15. CD4-Spark685 (SK3, BioLegend, 344658)
16. CD4-Alexa-Fluor-700 (SK3, BioLegend, 344622)
17. CD8-BV570 (RPA-T8, BioLegend, 301038)

18. CD14-PerCP (HCD14, BioLegend, 325632)
19. CD19-BV750 (HIB19, BioLegend, 302262)
20. CD19-PE (HIB19, BioLegend, 302254)
21. CD19-APC (HIB19, BioLegend, 302212)
22. CD20-Pacific Blue (2H7, BioLegend, 302320)
23. CD27-BV510 (O323, BioLegend, 302836)
24. CD38-PE-Cy7 (HIT2, BioLegend, 303516)
25. CD71-PE (CY1G4, BioLegend, 334106)
26. CD71-PE-Cy7 (CY1G4, BioLegend, 334112)
27. CD71-APC (CY1G4, BioLegend, 334108)
28. CXCR5-PE-Dazzle 594 (J252D4, BioLegend, 356928)
29. FOXP3-BV421 (206D, BioLegend, 320124)
30. HLA-DR-BV650 (L243, BioLegend, 307650)
31. IgD-PE-Cy5 (IA6-2, BioLegend, 348250)
32. IgD-PerCP-Cy5.5 (IA6-2, BioLegend, 348208)
33. IgM-BV605 (MHM-88, BioLegend, 314524)
34. Ki-67-BV711 (Ki-67, BioLegend, 350516)
35. T-bet-BV785 (4B10, BioLegend, 644835)

Validation

All commercial antibodies were validated by their manufacturers as detailed in their product information and titrated in the lab for the indicated assay by serial dilution. We validated PB-, GC B cell-, LNPC, and BMPC-derived mAbs generated in our lab in preliminary ELISAs to SARS-CoV-2 spike, bovine serum albumin, and anti-Ig. The threshold of positivity for mAbs was set as two times the optical density of background binding to BSA at 10 ug/ml of each mAb.
1. https://www.jacksonimmuno.com/catalog/products/709-005-149
2. https://www.jacksonimmuno.com/catalog/products/109-035-088
3. https://www.jacksonimmuno.com/catalog/products/109-035-190
4. https://www.jacksonimmuno.com/catalog/products/109-035-011
5. https://www.thermofisher.com/antibody/product/Goat-anti-Human-IgM-Secondary-Antibody-Polyclonal/SA5-10293?imageId=710449
6. https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-mouse-anti-bcl-6.561522
7. https://www.rndsystems.com/products/human-blimp1-prdm1-alexa-fluor-700-conjugated-antibody-646702_ic36081n
8. https://www.bdbiosciences.com/en-pt/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bb700-mouse-anti-human-cd38.566446
9. https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-HI30-Monoclonal/58-0459-42
10. https://www.emdmillipore.com/US/en/product/Mouse-Anti-Human-IgA-Antibody-clone-M24A-heavy-chain-FITC-conjugated,MM_NF-CBL114F?ReferrerURL=https%3A%2F%2Fwww.google.co.kr%2F
11. https://www.jacksonimmuno.com/catalog/products/109-685-098
12. https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bb515-mouse-anti-human-cd279-pd-1.564494
13. https://www.biolegend.com/nl-be/products/fitc-anti-human-cd3-antibody-751?Clone=HIT3a
14. https://www.biolegend.com/en-us/search-results/apc-fire-810-anti-human-cd3-antibody-19515?GroupID=BLG7576&Clone=SK7
15. https://www.biolegend.com/en-us/products/spark-nir-685-anti-human-cd4-antibody-18516?Clone=SK3
16. https://www.biolegend.com/en-us/search-results/alexa-fluor-700-anti-human-cd4-antibody-9354?Clone=SK3
17. https://www.biolegend.com/fr-ch/products/brilliant-violet-570-anti-human-cd8a-antibody-7371?Clone=RPA-T8
18. https://www.biolegend.com/en-us/products/percp-anti-human-cd14-antibody-9564?Clone=HCD14
19. https://www.biolegend.com/en-us/search-results/brilliant-violet-750-anti-human-cd19-antibody-15900?GroupID=BLG10095&Clone=HIB19
20. https://www.biolegend.com/en-us/products/pe-anti-human-cd19-antibody-719?Clone=HIB19
21. https://www.biolegend.com/en-us/products/apc-anti-human-cd19-antibody-715?Clone=HIB19
22. https://www.biolegend.com/en-us/products/pacific-blue-anti-human-cd20-antibody-3330?Clone=2H7
23. https://www.biolegend.com/en-us/search-results/brilliant-violet-510-anti-human-cd27-antibody-8005?Clone=O323
24. https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd38-antibody-5418?Clone=HIT2
25. https://www.biolegend.com/en-us/products/pe-anti-human-cd71-antibody-4908?Clone=CY1G4
26. https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd71-antibody-9328?Clone=CY1G4
27. https://www.biolegend.com/en-us/products/apc-anti-human-cd71-antibody-7517?Clone=CY1G4
28. https://www.biolegend.com/en-us/products/pe-dazzle-594-anti-human-cd185-cxcr5-antibody-9860?Clone=J252D4
29. https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-human-foxp3-antibody-12045?Clone=206D
30. https://www.biolegend.com/en-us/search-results/brilliant-violet-650-anti-human-hla-dr-antibody-8875?Clone=L243
31. https://www.biolegend.com/en-us/products/pe-cyanine5-anti-human-igd-antibody-19969?Clone=IA6-2
32. https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-human-igd-antibody-6811?Clone=IA6-2
33. https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-human-igm-antibody-8746?Clone=MHM-88
34. https://www.biolegend.com/en-us/search-results/brilliant-violet-711-anti-human-ki-67-antibody-7946?Clone=Ki-67
35. https://www.biolegend.com/fr-ch/products/brilliant-violet-785-anti-t-bet-antibody-15077?Clone=4B10
We validated mAbs generated in our lab in preliminary ELISAs to SARS-CoV-2 spike, bovine serum albumin, and anti-Ig.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Expi293F, Vero

Authentication

The cell line was not authenticated.

Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination. Growth rates were consistent with manufacturer's published data.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Study participants demographics are detailed in Extended Data Table 1.
Recruitment	Study participants were recruited from the St. Louis metropolitan area by the Washington University Clinical Trials Unit. Recruitment was open to all eligible adults who were supposed to receive the two-dose series of Pfizer-BioNTech SARS-CoV-2 mRNA vaccine (BNT162b2). All participants privided written informed consent before participation in the study. Participants were asked to provide details of SARS-CoV-2 infection history, and side effects and comorbidities after vaccination. Other than the criteria listed herein, no other parameters were used to select participants. Potential self-selection and recruiting biases are unlikely to affect the parameters we measured.
Ethics oversight	The study was approved by the Washington University IRB.

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	Peripheral blood and bone marrow mononuclear cells were isolated from EDTA anticoagulated blood and bone marrow aspirates, respectively using density gradient centrifugation, and remaining RBCs were lysed with ammonium chloride lysis buffer. Bone marrow plasma cells were magnetically enriched from bone marrow mononuclear cells and immediately used for ELISpot or cryopreserved in 10% dimethylsufoxide in FBS for flow cytometric analysis. PBMCs were immediately used or cryopreserved in 10% DMSO in FBS. Fine needle aspirates of axillary LNs were flushed from needles with 3 mL of RPMI supplemented with 10% FBS and 100 U/mL penicillin/streptomycin, followed by three 1 mL rinses. Red blood cells were lysed with ammonium chloride buffer, washed twice with PBS supplemented with 2% FBS, 2mM EDTA and immediately used or cryopreserved in 10% DMSO in FBS.
Instrument	Cytek Aurora
Software	Flow cytometry data was acquired using Cytek SpectroFlo and analyzed using FlowJo (Treestar) v10.
Cell population abundance	Bulk sorts directly into lysis buffer were not amenable to post-sort purity analysis.
Gating strategy	Details of gating strategies are shown in Extended Data Figures. Briefly, forward and side scatter parameters (FSC-A/H/W, SSC-A,H,W), and Zombie dyes (BioLegend) were used to select for live singlet lymphocytes. For analyzing SARS-CoV-2 S binding cells, GC B cells were gated on CD3-/CD19+/IgD-/CD38int/BCL6+ live singlet lymphocytes; LNPCs were gated on CD3-/CD19+/IgD-/CD20low/CD38+/BLIMP+/CD71+ live singlet lymphocytes; and MBCs from PBMCs were gated on CD3-/CD19+/IgD-/CD20+/CD38- live singlet lymphocytes. Blood and tonsillectomy samples collected before the COVID-19 pandemic were stained as a negative control for S-binding cells. For sorting, plasmablasts from PBMCs were gated on CD3-/CD19+/IgD-/CD20-/CD38+/CD71+ live singlet lymphocytes; GC B cells were gated on CD4-/CD19+/IgD-/CD20high/CD38int live singlet lymphocytes; and LNPCs were gated on CD4-/CD19+/IgD-/CD20low/CD38+ live singlet lyphocytes.

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