nature portfolio

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Last updated by author(s): 10/26/2021

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	BD FACSDiva (v.8.0) for collection of flow cytometry and FACS data. BLI analysis software (Fortebio/Sartorius, version 7.1) was used for collection of biolayer-interferometry data.
Data analysis	For single cell analysis, we used the cell ranger pipeline (v.6.0.1) from 10X genomics, and R package Seurat (v.4.0.2). FlowJo was used for data analysis of flow cytometry and FACS data. R version 4.0.3 and GraphPad Prism 9.1.0 was used for statistical analyses. BLI analysis software (Fortebio/Sartorius, version 7.1) was used for collection of biolayer-interferometry data.

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 are available on NCBI sequence read archive (SRA) under BioProject accession number PRJNA775994. Additionally, processed data will be made available upon request. Sequencing data was aligned to GRCh38 and IMGT (05/2021).

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	Our single-cell transcriptomic analysis includes four serial timepoint measurements of n=9 individuals (36 samples), yielding a total of 131,138 single cells transcriptomes. The study size is in line with prior single-cell RNA-sequencing studies in COVID-19 patients (Wilk AJ, et al. Nat Med. 2020;26(7):1070-1076; Yang AC, et al. Nature. 2021;595(7868):565-571; Combes AJ, et al., Nature. 2021;591(7848):124-130). In addition, vaccine study samples tend to be more uniform than samples from individuals with infectious diseases, due to the defined dose of the immunogen and its application at a specific timepoint. Quality control during analysis assured that all identified B cell populations were present in all individuals at all four timepoints, as shown in Extended Data Fig. 1a.
Data exclusions	No data were excluded from the study.
Replication	All regimens of biological and technical replication are annotated in the methods section. Briefly, ELISA and neutralization experiments were performed at 1-2 times in at least two technical replicates. Flow cytometry experiments were performed with n=9 biological replicates (study participants), and the experiment was performed twice. Bio-layer interferometry experiments were performed 1-2 times, each in triplicate dilutions, and cross-checked with ELISA-results. Single-cell transcriptome and repertoire sequencing was performed in n=9 individual samples at 4 timepoints. For droplet-based single-cell sequencing, n=9 biological replicates for each timepoint were performed, and all nine samples were used in the downstream analysis. All attempts at replication were successful.
Randomization	As this study does not explore group differences, no specific randomization strategy has been applied for the selection of study participants. However, we aimed to capture a cross-section of the population with regard to gender and age (Extended Data table 1: female=4, male=5; age: 23-52). For antigen specific B cell analysis, the antigen group was defined by CLR counts of the two barcoded, florescent tetramers for each antigen. For analysis of mAbs used in the manuscript, the groups were defined by the reactivity of the mAbs to specific antigens by ELISA.
Blinding	Participants were de-identified. As this study does not explore group differences, investigators were not blinded to the de-identified sample names. The study does not rely on subjective measures but analyzes quantitative sequencing data. Differences across timepoints were assessed using a computational pipeline that was applied equally to all samples. Investigators were not blinded during the additional experiments, including FACS, ELISAs, and plasma neutralization. The additional experiments involving samples from participants all have quantitative readouts, and methods were applied equally to all samples.

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. IgA-FITC Antibody, anti-human, Miltenyi, 130-113-475
2. APC/Cy7 anti-human IgM Antibody, Biolegend, 314520
3. PE/Dazzle 594 anti-human CD3 Antibody, BioLegend, 317346
4. BUV395 Mouse Anti-Human CD19, BD Biosciences, B563549
5. CD20 Monoclonal Antibody (HI47), Pacific Orange, Thermo Fisher Scientific, MHCD2030
6. BV605 Mouse Anti-Human CD27, BD Biosciences, 562656
7. PE-Cy7 Mouse Anti-Human CD38, BD Biosciences, 335790
8. BUV737 Mouse Anti-Human IgD, BD Biosciences, 612798

9. TotalSeq-C0050 anti-human CD19 Antibody, Biolegend, 302265
10. TotalSeq-C0571 anti-mouse IgD Antibody, Biolegend, 405747
11. TotalSeq-C0154 anti-human CD27 Antibody, Biolegend, 302853
12. TotalSeq-C0389, anti-human CD38 Antibody, Biolegend, 303543
13. TotalSeq-C0951 PE Streptavidin, Biolegend, 405261
14. TotalSeq-C0952 PE Streptavidin, Biolegend, 405263
15. TotalSeq-C0953 PE Streptavidin, Biolegend, 405265
16. TotalSeq-C0956 APC Streptavidin, Biolegend, 405283
17. TotalSeq-C0957 APC Streptavidin, Biolegend, 405285
18. TotalSeq-C0958 APC Streptavidin
19. TotalSeq-C0251 anti-human Hashtag 1 Antibody, Biolegend, 394661
20. TotalSeq-C0252 anti-human Hashtag 2 Antibody, Biolegend, 394663
21. TotalSeq-C0253 anti-human Hashtag 3 Antibody, Biolegend, 394665
22. TotalSeq-C0254 anti-human Hashtag 4 Antibody, Biolegend, 394667
23. TotalSeq-C0255 anti-human Hashtag 5 Antibody, Biolegend, 394669
24. TotalSeq-C0256 anti-human Hashtag 6 Antibody, Biolegend, 394671
25. TotalSeq-C0257 anti-human Hashtag 7 Antibody, Biolegend, 394673
26. TotalSeq-C0258 anti-human Hashtag 8 Antibody, Biolegend, 394675
27. TotalSeq-C0259 anti-human Hashtag 9 Antibody, Biolegend, 394677
28. Antibodies derived from participants were made in-house, see methods and Extended Data Table 3
29. HRP-conjugated goat anti-human IgG, Bethyl, A80-104P
30. HRP-conjugated goat anti-human IgA, Bethyl, A80-102P

Validation

1. https://www.miltenyibiotec.com/US-en/products/iga-antibody-anti-human-is11-8e10.html#fitc:100-tests-in-200-ul
2. https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-igm-antibody-7403?GroupID=BLG4120
3. https://www.biolegend.com/en-us/search-results/pe-dazzle-594-anti-human-cd3-antibody-11986
4. https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-mouse-anti-human-cd19.563549
5. https://www.thermofisher.com/antibody/product/CD20-Antibody-clone-HI47-Monoclonal/MHCD2030
6. https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-mouse-anti-human-cd27.562656
7. https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/clinical-discovery-research/single-color-antibodies-ruo-gmp/pe-cy-7-mouse-anti-human-cd38.335790
8. https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv737-mouse-anti-human-igd.612798
9. https://www.biolegend.com/en-us/punchout/search-results/totalseq-c0050-anti-human-cd19-antibody-16832
10. https://www.biolegend.com/en-us/punchout/search-results/totalseq-c0571-anti-mouse-igd-antibody-19765
11. https://www.biolegend.com/en-us/punchout/search-results/totalseq-c0154-anti-human-cd27-antibody-16840
12. https://www.biolegend.com/en-us/products/totalseq-c0389-anti-human-cd38-antibody-17311
13. https://www.biolegend.com/en-us/products/totalseq-c0951-pe-streptavidin-18176
14. https://www.biolegend.com/en-us/products/totalseq-c0952-pe-streptavidin-18177
15. https://www.biolegend.com/en-us/products/totalseq-c0953-pe-streptavidin-18178
16. https://www.biolegend.com/en-us/products/totalseq-c0956-apc-streptavidin-19607
17. https://www.biolegend.com/en-us/products/totalseq-c0957-apc-streptavidin-19606
18. https://www.biolegend.com/en-us/products/totalseq-c0958-apc-streptavidin-19883
19. https://www.biolegend.com/en-us/search-results/totalseq-c0251-anti-human-hashtag-1-antibody-17162
20. https://www.biolegend.com/en-us/products/totalseq-c0252-anti-human-hashtag-2-antibody-17163?GroupID=GROUP28
21. https://www.biolegend.com/en-us/search-results/totalseq-c0253-anti-human-hashtag-3-antibody-17164?GroupID=GROUP28
22. https://www.biolegend.com/en-us/search-results/totalseq-c0254-anti-human-hashtag-4-antibody-17165?GroupID=GROUP28
23. https://www.biolegend.com/en-us/search-results/totalseq-c0255-anti-human-hashtag-5-antibody-17166
24. https://www.biolegend.com/en-us/search-results/totalseq-c0256-anti-human-hashtag-6-antibody-18373
25. https://www.biolegend.com/en-us/products/totalseq-c0257-anti-human-hashtag-7-antibody-18374?GroupID=GROUP28
26. https://www.biolegend.com/en-us/clone-search/totalseq-c0258-anti-human-hashtag-8-antibody-18375
27. https://www.biolegend.com/en-gb/search-results/totalseq-c0259-anti-human-hashtag-9-antibody-18376
28. Antibodies derived from participants were validated by ELISA for their specific antigens and for polyreactive antigens, by Octet for their specific antigens, and in neutralization experiments with SARS-CoV-2 pseudovirus, as described in this manuscript (see Fig. 4a,e,f; Extended Data Fig. 8; Extended Data Fig. 9, Extended Data Fig. 10)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Lenti-X 293T Cell Line (human, Takara, 632180), Expi293F (human, Thermo Fisher Scientific, A14527), HeLa-ACE2 (human, gift from Dennis Burton, see ref. 44). HeLa-hACE2 were generated in the lab of Dennis Burton. Briefly, HeLa-hACE2 were generated through transduction using a lentivirus carrying the human ACE2 gene. The pBOB-hACE2 construct was co-transfected into HEK293T cells along with lentiviral packaging plasmids pMDL, pREV, and pVSV-G (Addgene #12251, #12253, #8454) by Lipofectamine 2000 (Thermo Fisher Scientific, 11668019) according to the manufacturer's instructions. Supernatants were collected 48 h after transfection, then were transduced to pre-seeded HeLa or A549 cells. 12 h after transduction, stable cell lines were collected, scaled up and stored for neutralization assay (ref. 44).

Authentication

Cells from commercial sources were confirmed by short tandem repeat by the commercial sources. HeLa-ACE2 were not authenticated. However, we tested infectivity of both HeLa and HeLa-ACE2, and determined that only HeLa-ACE2 were able to be infected by SARS-CoV-2 pseudovirus.

Mycoplasma contamination	Expi293F were tested and were found negative for mycoplasma contamination. Other cell lines were not tested for mycoplasma contamination but no indication of contamination was observed.
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	Human participants were 5 males (ages 23 - 38) and 4 females (ages 26 - 52). All participants were naive to prior SARS-CoV-2 infection. Blood samples were collected before BNT162b2 first vaccination, as well as 7-9 days, 21-23 days, and 28 days after the first vaccination.
Recruitment	Participants were recruited as a part of elective vaccination campaigns for VA and Stanford with the BNT162b2 mRNA vaccine (Pfizer/BioNTech) at the VA Hospital Palo Alto and the Stanford Hospital. As the BNT162b2 vaccination was not widely available to the general population at the time, participants were personnel of the VA and Stanford hospitals. As mentioned above, it was still possible to select a relatively diverse group considering gender and age. As our study does not compare several groups and as all investigated parameters are quantitatively measurable parameters, the danger of introducing selection biases is low. Major factors known to alter the vaccine response are comorbidities and therapeutics that lead to immunosuppression. All participants were questioned on these matters to avoid immunosuppression. Comorbidities and immunosuppressive therapeutics were denied by all participants. We have no reason to assume that our study group diverges from the general population in significant ways.
Ethics oversight	Stanford University Institutional Review Board (IRB-3780)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	n/a
Study protocol	n/a
Data collection	n/a
Outcomes	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	PBMCs were thawed at 37C°, treated for 15min with DNAse and washed in complete RPMI. PBMCs were enriched for B cells using the EasySep Human Pan-B Cell Enrichment Kit (Stem Cell Technologies) according to the manufacturer instructions. B cell samples without antigen enrichment were stained with CD19, IgD, CD27, CD38 TotalSeq-C antibodies (all Biolegend). For antigen-sorted B cell samples, cells were stained with the following fluorescently labeled antibodies according to standard protocols: CD19, CD20, CD38 (all BD Biosciences), CD3, CD27, IgM, IgD, (all BioLegend), IgA (Miltenyi Biotec), Sytox blue (Thermo Fisher Scientific), and S-antigen tetramers (Extended Data Fig. 6a,b,d). Additionally, samples were labeled with TotalSeq-C hashtag 1-9 antibodies (Biolegend) for demultiplexing individual samples in downstream analysis. Single cells were sorted with a FACSAria II cell sorter (BD Biosciences) into cooled 1.5 ml tubes (BioRad).
Instrument	FACSAria II cell sorter (BD Biosciences)
Software	FACS Diva
Cell population abundance	IgA+/IgG+ switched memory B cells and plasmablasts (~20%) were sorted from enriched B cell blood samples. Purity was >95% when analyzed downstream in Seurat (v.4.0.2).

Gating strategy	Details of the gating strategy are annotated in Extended Data Fig. 5 a and b. Briefly, we applied forward and side scatter parameters (FSC-A, FSC-W, SSC) to select for live lymphocytes. We gated on SytoxBlue-/CD3-/CD19+/IgD-/CD27+/IgM- for the sorted cells. We gated on SytoxBlue-/CD3-/CD19+/CD20+/CD27+/IgM- for analysis of SARS-CoV-2 antigen staining of switched memory B cells. We gated on SytoxBlue-/CD3-/CD19+/CD20-/CD27+/CD38+ for analysis of SARS-CoV-2 antigen staining of plasmablasts.

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