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	REDCap (Research Electronic Data Capture) v8.5.27
Data analysis	Trimmomatic (v0.38.0), STAR (v2.6.0b), UMI-tools (v0.5.3), subread (v1.6.2), R (v3.6.1, 4.1.0, 4.1.1), variancePartition (v1.16.1), limma (v3.42.2, 3.50.1), clusterProfiler (v3.17.0), ggplot2(v3.3.5), ggpubr (v0.4.0), RUVSeq (v1.18), GSVA (v1.30.0, 1.42.0), edgeR (v3.26.8), impute (v1.60.0), CellRanger (v6.0.1), demuxlet (v2), Seurat (v4.0.3, 4.1.0), dsb (v0.3.0), Circlize (v0.4.14), ComplexHeatmap (v2.10.0), eNetXplorer (v1.1.3), SpectroFlo (v2.2.0), scRepertoire (v1.4.0), DEseq2 (v1.34.0), MASS (v7.3-53), FlowJo (v10)

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 and processed data from the whole blood bulk RNA-seq and single-cell CITE-seq are available from the NCBI Gene Expression Omnibus, accession numbers GEO: GSE194378 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE194378) and GEO:GSE206265 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE206265), respectively (will be released to public at time of publication). Additional datasets, including clinical, proteomics, flow cytometry, CITE-seq, and

influenza antibody measurements, are available at: https://doi.org/10.5281/zenodo.5935845 (will be released to public at time of publication). The influenza infection dataset we utilized was downloaded directly from GEO: GSE68310 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68310).

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	n = 73 (COVR-F=17; HC-F=21; COVR-M = 16; HC-M = 19). No sample size calculations were done prior to enrollment, in part because there were no reliable effect size estimates related to the impact of prior COVID-19 infection on vaccine responses. The number of subjects in the study was the number that were able to be recruited during the recruitment period.
Data exclusions	None
Replication	Technical replicates were used to assess technical variability of mRNA expression in bulk RNA-seq and single cell CITE-seq, and genes whose technical variance was higher than a predefined threshold were removed from downstream analysis. In addition, the linear models constructed to assess group differences leveraged technical replicates to control for batch and other technical effects.
Randomization	Because this study looked at the cellular and molecular responses to influenza vaccination, there was no indication to randomize individuals to receive influenza vaccination or placebo. The covariates age, self-reported influenza vaccination history (of the past 10 years) and pre-vaccination (baseline) influenza antibody titers were accounted for in our results as they have been included as co-variates in our statistical model for assessing immune response differences among the groups. We model post-vaccination titer as the outcome variable with influenza vaccination history and pre-vaccination titer included as covariates. This approach has also been used for influenza vaccine evaluation by the Food and Drug Administration (e.g., see https://www.fda.gov/media/135687/download page 27).
Blinding	Blinding was not performed as individuals were recruited based on a known history of SARS-CoV-2 infection. Furthermore, as part of the study, an individual's SARS-CoV-2 infection history was confirmed by antibody test in order to allocate them to the correct subject group. Finally, because this study looked at the cellular and molecular responses to influenza vaccination, we did not administer any placebo vaccine, only an FDA-approved influenza vaccine.

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	TotalSeq™-C Human Universal Cocktail, V1.0; Fluorochrome-conjugated antibodies as indicated in Methods Table 1, 2 and 3.
Validation	See Supplementary Tables 11-14 for antibody data, including validation information from the commercial companies who made the products.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Lenti-X- 293T cells were obtained from Takara Bio (Cat. No. 632180). 293T-ACE2 cells were obtained from ATCC.

Authentication	Cell line was checked for expression of ACE2 and validated by FACS analysis. None of the cell lines were authenticated by karyotyping or other genomic techniques.
Mycoplasma contamination	Negative for Mycoplasma
Commonly misidentified lines (See ICLAC register)	No misidentified cell lines were used in the study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	COVID-19-recovered males (n=16; ages 21-67; median time since COVID-19 diagnosis: 186 days). COVID-19-recovered females (n=17; ages 23-70; median time since COVID-19 diagnosis: 172 days). Healthy control males (n=19; ages 24-69). Healthy control females (n=21; ages 22-70).
Recruitment	Subjects were recruited from the community using email listserv messages, newspaper advertisements, and follow-up contact from a COVID-19 convalescent plasma study. All subjects were self-selected based on interest in participating in COVID-19-related research, ability to participate in study visits, and exposure to recruitment materials. As influenza vaccine titers and responses are not routinely measured outside of research settings, it is unlikely that individuals who opted to participate in the study introduced systemic bias as a result of prior knowledge about their individual cellular or molecular responses to the influenza vaccine.
Ethics oversight	Institutional Review Board of the National Institutes of Health. Informed consent was obtained from all study participants prior to the onset of study procedures.

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	NCT04025580
Study protocol	19-I-0126 (Systems analyses of the immune response to the seasonal influenza vaccine). Trial protocol described on clinicaltrials.gov.
Data collection	Subjects were recruited between August and December 2020. Study visits occurred at the National Institutes of Health (NIH) Clinical Center (CC) in Bethesda, Maryland, USA. Blood samples were collected by phlebotomy staff at the NIH CC. Samples were collected between September 2020 and April 2021.
Outcomes	Primary outcome to be measured was serum microneutralization titers to the four influenza strains in the FDA-approved seasonal influenza vaccines used in the study (Flucelvax and high dose Fluzone).

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	Whole blood was collected from study participants, PBMCs were isolated using a modified Ficoll procedure, and cells were frozen and stored at -80C. Thawed PBMC were washed in RPMI culture medium containing 50U/ml benzonase nuclease and then washed by PBS. Cells were incubated with LIVE/DEAD Fixable Blue Dye, which was used to exclude dead cells from analysis. Cells were incubated with fluorochrome-conjugated B, H1, H3 probes and fluorochrome-conjugated antibodies against IgM, IgA, CD21, CD85J, FCRL5, CD20, IgG, CD38, CD14, CD56, CD3, CD27, CD71, CD19, IgD etc as indicated in the Method for 30 min at 4 C in the dark. After incubation with antibodies for 30 minutes, cells were washed two times with FACS buffer (0.1%BSA/PBS (pH7.4)) and fixed in 1% paraformaldehyde.
Instrument	Cytek Auora (Cytek Biosciences), BD Symphony (BD Biosciences)
Software	SpectroFlo version 2.2.0 (Cytek Biosciences) and BD FACSDiva Software were used for collecting the data and FlowJo version 10 (BD Biosciences) was used for flow cytometry data analysis.

Cell population abundance	Post-sort fractions not evaluated.
Gating strategy	Gating strategies provided in Supplementary Information Figures 1, 3 and 6.

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