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Reporting Summary

Nature Research 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 Research 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	Cryo-EM data was collected using Leginon 3.4.beta. Sequencing of memory B cell clones done using Illumina NextSeq 500. Cell sorting was performed on FACSDiva version 8.0.1.
Data analysis	Cryo-EM data was processed using cryoSPARC v2.14.2, MotionCor2, Topaz v0.2.4, 3DFSC v3.0, UCSF Chimera v1.13.1, ChimeraX v0.93, ISOLDE v1.0b5, Phenix v1.18, and COOT v0.8.9.2. Next-generation sequencing data of antibody repertoires were processed using Cell ranger v3.1.0, SONAR V1, BLAST v2.2.25, CLUSTALO1.2.3, and USEARCH v9.2.64. FlowJo 10.4 was used for analyzing FACS data. For 10X Genomics; cellranger 3.1.0 for BCL to FASTQ conversion, and gene counting was used. GraphPad Prism 8 was used for plotting 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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

We confirm we have deposited the sequencing dataset into Genbank and they will become available publicly within two business day. Once the accession numbers are assigned, we will add the data availability statement in the manuscript and here. Healthy donor antibody repertoires were from previous study with SRA ID PRJNA336331. The following data availability statement will be included in the final version of the manuscript: "The 19 neutralizing antibodies were deposited to Genbank with accession numbers: ACXXXXXXXX. Coordinates for the antibody 2-4 complex are deposited in the Protein Data Bank as PDB 6XEY. Cryo-EM maps and

data are deposited in EMDB with deposition codes EMDB-22156 for antibody 2-4, EMDB-22158 and EMDB-22159 for antibody 4-8, and EMDB-22275 for antibody 2-43. These data are used in Fig. 4 and Extended Data Figs. 7, 8, 9, 10, and 11."

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.

- [x] 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	40 patients detected positive for SARS-CoV-2 using diagnostic RT-PCR tests were used for screening of neutralization abilities of their plasma samples. Based on the neutralization profile of the plasma, patients with most potent plasma were downselected for sorting of the memory B-cells and antibody isolation and cloning. The sample size is appropriate within technical capability to downselect multiple patients with potent neutralizing plasma.
Data exclusions	None
Replication	All experiments were performed and verified in multiple replicates as indicated in their methods/figure legends of the manuscript.
Randomization	All samples were selected for their ability to produce neutralization antibodies and all PBMCs were randomly processed from the 5 patients with potent neutralization of the plasma using baits specific for their ability to measure neutralization (SARS-CoV-2 S trimer). The screens for the binding and neutralization assays were also performed without any bias for selection and efficacy determined solely by the potency of the individual clones/antibodies.
Blinding	Blinded scoring of the neutralization of SARS-CoV-2 virus associated cytopathic effects were performed and average of the scores was converted to percentage of the neutralization. The results were plotted as mean +/- SEM. All other experiments in the study were predesigned with the hypothesis and strategies were laid out so as to use instruments that were calibrated to report the data. This feature led to the non-relevance of blinding for any of those experiments. Experiments were validated using technical and/or biological replicates in all cases.

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	
[ ]	[x]	Antibodies
[ ]	[x]	Eukaryotic cell lines
[x]	[ ]	Palaeontology and archaeology
[x]	[ ]	Animals and other organisms
[ ]	[x]	Human research participants
[ ]	[x]	Clinical data
[x]	[ ]	Dual use research of concern

Methods

n/a	Involved in the study	
[x]	[ ]	ChIP-seq
[ ]	[x]	Flow cytometry
[x]	[ ]	MRI-based neuroimaging

Antibodies

Antibodies used	For S trimer-specific B cells sorting and single-cell BCR sequencing, anti-human CD3 PE-CF594 (BD Biosciences, Cat.562406, Clone SP34-2, Lot.9325656, 1:20 dilution), anti-human CD19 397 PE-Cy7 (Biolegend, Cat.302216, Clone HIB19, Lot.B276834, 1:20 dilution), anti-human CD20 APC-Cy7 (Biolegend, Cat.302314, Clone 2H7, Lot.B288789, 1:20 dilution), anti-human IgM V450 (BD Biosciences, Cat.561286, Clone G20-127, Lot.9003910, 1:20 dilution), anti-human CD27 PerCP-Cy5.5 (BD Biosciences, Cat.560612, Clone M-T271, Lot.9283016, 1:20 dilution), anti-His PE (Biolegend, Cat.362603, Clone J095G46, Lot.B269138, 1:20 dilution), Human Hashtag 3 (Biolegend, Cat.394665, Clone LNH-94,Lot.B282244, 1:20 dilution). For epitope mapping by ELISA, anti-human IgG (Jackson ImmunoResearch, Cat. 109-035-003, Polyclonal, Lot.146269, 1: 10,000 dilution), Streptavidin-APC (Biolegend, Cat.405243, Lot.B266052, 1: 2,000 dilution), Avidin-HRP (Invitrogen, Cat.18-4100-51, Lot.2197902, 1: 500 dilutions), anti-Strep-HRP (Strep-TagII - HRP, EMD Millipore, Cat.71591, Lot.3393843, 1: 2,000 dilution).
Validation	All validations are available from the commercial website under the validation sheet link for the catalogued item. 1. Anti-human CD3 PE-CF594 (BD Biosciences, Cat # 562406), https://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-non-human-primate-antibodies/cell-surface-antigens/pe-cf594-mouse-anti-human-cd3-sp34-2/

p/562406
2. Anti-human CD19 397 PE-Cy7 (Biolegend, Cat# 302216), https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd19-antibody-1911
3. Anti-human CD20 APC-Cy7 (Biolegend, Cat# 302314), https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd20-antibody-1901
4. Anti-human IgM V450 (BD Biosciences, Cat # 561286), https://www.bdbiosciences.com/eu/applications/research/b-cell-research/immunoglobulins/human/v450-mouse-anti-human-igm-g20-127/p/561286
5. Anti-human CD27 PerCP-Cy5.5 (BD Biosciences, Cat# 560612), https://www.bdbiosciences.com/eu/applications/research/b-cell-research/surface-markers/human/percp-cy55-mouse-anti-human-cd27-m-t271/p/560612
6. Human Hashtag 3 (Biolegend, Cat # 394665), https://www.biolegend.com/en-us/products/totalseq-c0253-anti-human-hashtag-3-antibody-17164
7. Anti-His PE (Biolegend, Cat# 362603), https://www.biolegend.com/en-us/products/pe-anti-his-tag-antibody-9861
8. Anti-human IgG (Jackson ImmunoResearch, Cat# 109-035-003), https://www.jacksonimmuno.com/catalog/products/109-035-003
9. Streptavidin-APC (Biolegend, Cat# 405243), https://www.biolegend.com/en-us/products/apc-streptavidin-high-concentration-10081
10. Avidin-HRP (Invitrogen, Cat# 18-4100-51), https://www.thermofisher.com/order/catalog/product/18-4100-51#/18-4100-5
11. Anti-Strep-HRP (Strep-TagII –HRP, EMD Millipore, Cat# 71591), https://www.emdmillipore.com/US/en/product/StrepTag-II-Antibody-HRP-Conjugate,EMD_BIO-71591

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Vero-E6 (ATCC), Expi293 (Thermofisher), 293T (ATCC)

Authentication

Obtained from authenticated vendors. Cells were recovered as healthy logarithmically growing cells within 4 to 7 days after thawing. Viability was measured and found to be >90%.

Mycoplasma contamination

Mycoplasma is negative (Detected mycoplasma contamination using Mycoplasma PCR ELISA ,Sigma,catalog number is 11663925910)

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used in the study.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Eligibility criteria include: (1) greater than age 18 (inclusive) (2) confirmed COVID-19 infection by a FDA- approved molecular based assay (including those under emergency use authorization) of respiratory or blood specimens; (3) If symptomatic with COVID-19, must have evidence of improvement of symptoms and a duration of at least 4 weeks from the onset of symptoms to day of enrollment; (4) If asymptomatic, must have a duration of at least 4 weeks from first positive molecular based COVID-19 assay to day of enrollment. Among the 40 participants enrolled in this study, the mean age was 50 (20-84) and 53% were male. Among those with race/ethnicity information, 21% were Black/African American, 38% Latinx, 3% Asian, and 38% non-Hispanic white.

Recruitment

This is a prospective study to enroll participants who have recovered from coronavirus disease (COVID-19) for the purpose of obtaining blood specimens to isolate monoclonal antibodies against SARS-CoV2 that can be developed into preventive or therapeutic agents. Potential participants were referred by health care providers from within the Columbia University Irving Medical Center/New York Presbyterian Hospital system and from outside institutions. Potential participants were contacted by study staff and informed consent signed prior to performance of study procedures. All participants with severe COVID-19 were recruited during or after prolonged hospitalization at a single medical center in New York City, while participants with mild COVID-19 were self-referred through online recruitment. All participants were recruited in March and April, 2020 during the early stages of the epidemic in New York. These factors may impact the generalizability of our findings.

Ethics oversight

This protocol, "Acquiring convalescent specimens to isolate and identify potent monoclonal antibodies against COVID-19" (AAAS9517) was approved by the Columbia University Institutional Review Board. Informed consent was obtained from all participants or surrogates. This statement is added to the manuscript.

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 ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.

Clinical trial registration

NCT04342195

Study protocol

The protocol "Acquiring convalescent specimens to isolate and identify potent monoclonal antibodies against COVID-19" is accessible by sending request to Dr. Michael Yin <mty4@cumc.columbia.edu>.

Data collection	The study protocol was approved on 3/13/2020 and the last participant enrolled for this analysis was on 4/7/2020. All data were collected at Columbia University Irving Medical Center, New York NY. Recruitment and data collection occurred between 3/25/2020 and 4/7/2020.
Outcomes	The primary outcome for the clinical study was the SARS-CoV-2 antibody response as measured by the S-trimer and nucleocapsid ELISA and pseudovirus assays.

Flow Cytometry

Plots

Confirm that:

- [x] The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- [x] 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).
- [x] All plots are contour plots with outliers or pseudocolor plots.
- [x] A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Peripheral blood mononuclear cells from five patients and one healthy donor were stained with LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit (Invitrogen) at ambient temperature for 20 mins, followed by washing with RPMI-1640 complete medium and incubation with 10 μg/mL of S trimer at 4°C for 45 mins. Afterwards, the cells were washed again and incubated with a cocktail of flow cytometry and hashtag antibodies, containing CD3 PE-CF594 (BD Biosciences), CD19 PE-Cy7 (Biolegend), CD20 APC-Cy7 (Biolegend), IgM V450 (BD Biosciences), CD27 PerCP Cy5.5 (BD Biosciences), anti-His PE (Biolegend), and human Hashtag 3 (Biolegend) at 4°C for 1hr. Stained cells were then washed, resuspended in RPMI-1640 complete medium and sorted for S trimer-specific memory B cells (CD3-CD19+CD27+S trimer+ live single lymphocytes).
Instrument	BD FACSAriaII (P69500149)
Software	FACSDiva version 8.0.1
Cell population abundance	S trimer bait positive cells were purified from the PBMCs of the 5 patients using the gating strategy used below. Purified trimer positive memory B cells were obtained from 5 patients and compared to healthy donor (negative control) as shown in extended data figure 1.
Gating strategy	As shown in Supplementary Figure 1b, sorting of the PBMC was performed in identical manner for all the samples including healthy donor. The summary of the gating is provided herewith: All PBMCs were initially gated using FSC-A and SSC-A gates for lymphocyte populations. The lymphocytes were gated using SSC-H and SSC-W initially followed by FSC-H and FSC-W to isolate the singlets in the population. The singlets was gated based on the fluorescence from the LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit for live cells. This step was followed by selecting for CD3- population by gating the SSC-A versus CD3-PE-CF594 stained population on the Texas Red channel. The negative population was gated for B-cells by first selecting for CD19 + cells followed by CD27+ cells on the respective fluorescent channels. The subsets of CD19+ cells were then selected for S-trimer bait positive by selecting for the cells bound to anti-Histag-PE on the trimer.

- [x] Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.