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Reporting Summary

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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	We used the 10X Genomics cellranger (version 2.1.1) [https://support.10xgenomics.com/single-cell-gene-expression/software/overview/welcome] bioinformatics processing pipeline. We began by first running the cellranger mkfastq program to generate FASTQ files that were then fed into the cellranger vdj program to generate CSV and JSON files containing processed data. All processed heavy and light chain sequences were then reprocessed using our PyIR (version 1.0) [https://github.com/crowelab/PyIR] processing pipeline. PyIR is a Python wrapper that parses out VDJ assignment from IgBLAST [PMID: 23671333].
Data analysis	We used a customized Python script, 10x-filter.py, for downstream selection of heavy-light pairs for expression and testing. The script begins by filtering out all ambiguous heavy and light chain pairings. It then bins all unique heavy chain somatic variants with the same V3J clonotype (i.e., V germline gene, J germline gene and CDR3 amino acid sequence). The heavy chain somatic variants are then rank-ordered within each V3J clonotype bin from most to least mutated. The user has the option to output only the most mutated sequence or least mutated sequence from each V3J clonotype bin for downstream expression and characterization. Code used for sequence processing for selecting sequences for synthesis and for analyses that appear in the paper is available at https://github.com/crowelab/cov2-panel-scripts. For ELISAs binding data and neutralization assays, analyses of data were performed using Prism 8.0 (GraphPad Inc). For generation of structural schematics for Figure 3, PyMOL (Schrödinger) was used to visualize previously deposited cryo EM and crystal structures. For IgG quantification, data were analyzed using ForeCyt software version 6.2 (IntelliCyt Corp). For RTCA neutralization assays, sensograms were visualized and analyzed using RTCA HT software version 1.0.1 (ACEA Biosciences Inc). For VSV-SARS-CoV-2 neutralization assays, images were analyzed using the Multi Target Analysis Module of the InCell Analyzer 1000 Workstation Software (GE Healthcare). GFP-positive cells were identified using the top hat segmentation method and counted within the

InCell Workstation software.

For negative-stain electron microscopy analysis, image processing was performed using the cryoSPARC software package.

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/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

The main data supporting the results in this study are available within the paper and Supplementary Information. The ImMunoGeneTics database is available from http://www.imgt.org/. The analysis pipeline PyIR (https://github.com/crowelab/PyIR) and the specific scripts used for sequence analysis (https://github.com/crowelab/cov2-panel-scripts) are available. Structures deposited by other groups for the full-length spike trimer (6VYB) and the RBD–hACE2 complex (6M0J) that were used for visualization in this paper are publicly available (www.rcsb.org). Sequences for mAbs described in this study have been deposited at GenBank and are available under the following accession codes: MT665032 - MT665070, MT665419 - MT665457, MT665071 - MT665418, and MT665458 - MT665805. Datasets are available from the corresponding authors upon reasonable request.

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 sample-size calculations were performed. For mAb isolation, a large number of antibodies cloned and synthesized yielded mAbs targeting multiple epitopes on the spike glycoprotein, suggesting that the pandel described here represents a diverse repertoire from these donors. Details about research subjects groups are provided in Supplementary information.
Data exclusions	No data were excluded from the analysis
Replication	Key experimental findings that include identification of SARS-CoV2-reactive and neutralizing human monoclonal antibodies were confirmed in two or more independent experiments. Initial neutralization screening results by the RTCA assay were confirmed using a VSV-SARS-CoV-2 virus neutralization assay, and the neutralization activity of several of these mAbs was confirmed using a SARS-CoV-2 luciferase reporter virus in a BSL-3 neutralization assay. All mAbs that neutralized in the cell impedance assay showed neutralizing activity in subsequent neutralization assays. For antigen-specific staining of PBMCs from the human subjects studied, analytical flow cytometry was performed prior to initiating processing of the larger sample. The antigen specific frequencies we observed for a given donor sample were similar across multiple days and independent staining reactions. Following sorting, a small fraction of sorted cells was analyzed by analytical flow cytometry to verify the purity of the sorted population prior to initiating single cell sequencing or B cell stimulation or expansion.
Randomization	Antibody sequences were randomly allocated to different 96 well plates for DNA synthesis and for the initial antibody expression and screening assays, with the end result that antibodies with highly similar sequences and phenotypes were present across plates and experimental replicates.
Blinding	The initial antibody expression and screening for antigen reactivity and neutralization activity was done in a blinded fashion, as a given antibody sequence was not known to the investigator at time of analysis. Importantly, multiple mAbs discovered by independent workflows were closely related and some had identical amino acid sequences and exhibited similar phenotypes in both antigen binding and neutralization assays. For the mAb validation experiments, investigators were not blinded to the study groups. Quantitative data analysis and validation controls were used, minimizing the risk of introducing bias through the absence of blinding.

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
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

B cell phenotyping flow cytometry antibodies included APC mouse anti-human CD19 (BioLegend clone HIB19 Cat# 982406, Lot B270238), FITC anti-human IgM (BioLegend clone MHM-88, Cat# 314506, Lot B218736), and FITC anti-human IgD (BioLegend clone IA6-2, Cat# 348206, lot B258195).
Polyclonal goat anti-human IgG-HRP antibody (Southern Biotech Cat 2040-05, Lot B3919-XD29) was used for antigen binding ELISA assays.
For FRNT assay, a previously described anti-SARS S-protein human antibody CR3022 (PMID: 32245784) was used as a primary antibody and the detection was performed using a goat anti-human IgG (γ-chain specific)-peroxidase antibody (Sigma-Aldrich, Cat# A6029).
For antigen binding and hACE2 blocking screening assays on Berkeley Lights' Beacon instrument, goat anti-human IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 568 (ThermoFisher Scientific Cat# A-21090), and rat anti-FLAG Alexa Fluor 647 antibody (BioLegend clone L5, Cat #637315, Lot B265929) were used.

Validation

All antibodies used in this study except anti-S human antibody CR3022 (PMID: 32245784) are commercially available. Antibodies used in a specific species or application have been appropriately validated by manufacturers and this information is provided on their website and information datasheets as follows:
APC mouse anti-human CD19 (https://www.biolegend.com/en-us/products/apc-anti-human-cd19-antibody-14024);
FITC anti-human IgM (https://www.biolegend.com/en-us/products/fitc-anti-human-igm-antibody-2880);
FITC anti-human IgD (https://www.biolegend.com/en-gb/products/fitc-anti-human-igd-antibody-6683);
Goat anti-human IgG-HRP (https://www.southernbiotech.com/?catno=2040-05&type=Polyclonal#&panel1-1&panel2-1);
Goat anti-human IgG (γ-chain specific)-peroxidase antibody (https://www.sigmaaldrich.com/catalog/product/sigma/a6029?lang=en&region=US).
Goat anti-human IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 568 (https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_secondary&productId=A-21090&version=105)
Rat anti-FLAG Alexa Fluor 647 (https://www.biolegend.com/fr-ch/global-elements/pdf-popup/alexa-fluor-647-anti-dykddddk-tag-14979?filename=Alexa%20Fluorreg%20647%20anti-DYKDDDDK%20Tag%20Antibody.pdf&pdfgen=true)
Activity of newly discovered SARS-CoV-2-specific monoclonal antibodies are validated via multiple assays described in this paper.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Vero E6 (CRL-1586, (American Type Culture Collection [ATCC})
Vero CCL81 (CCL-81, ATCC)
HEK293T (CRL-3216 ATCC)
Vero-furin cells were obtained from T. Pierson (NIH) and have been described previously (reference 2, Online Methods).
Expi293F (ThermoFisher Scientific, A1452)
ExpiCHO (ThermoFisher Scientific, A29127)

Authentication

None of the cell lines used were authenticated

Mycoplasma contamination

All cell lines were tested and confirmed negative for Mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)

None

Human research participants

Policy information about studies involving human research participants

Population characteristics

We studied five subjects in the United States, four with SARS-CoV2 exposure history and one healthy subject. Research subject demographics and SARS-CoV-2 exposure history are found in Supplemental Table 3.
SARS-CoV-2-infected subjects:
Subject 1: Male, 35 years old
Subject 2: Female, 52 years old
Subject 3: Male, 56 years old

	Subject 4: Female, 56 years old Healthy control subject: Subject 5, Male, 58 years old
Recruitment	Samples were obtained after written informed consent was obtained by the Vanderbilt Clinical Trials Center. Participants were selected for inclusion in the study based on PCR-confirmed diagnosis with SARS-CoV-2 infection and having convalesced. There was no potential self-selection bias in recruiting patients.
Ethics oversight	The studies were approved by the Institutional Review Board of Vanderbilt University Medical Center

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	The frequency of SARS-CoV-2 spike antigen-specific B cells was enumerated from B cells pre-enriched from fresh or frozen PBMCs. B cells were labeled using a soluble recombinant S2Pecto protein produced in our laboratory and commercially available receptor-binding domain (RBD)-mouse Fc-fusion recombinant protein (Sino Biological). Functionality of antigens was validated using a conventional ACE2 binding assay. Briefly, B cells were purified magnetically (STEMCELL Technologies) and stained with anti-CD19, -IgD, -IgM, phenotyping antibodies (BD Biosciences) and biotinylated S protein. 4',6-diamidino-2- phenylindole (DAPI) was used as a viability dye to discriminate dead cells. Antigen-labeled class-switched memory B cell-S complexes (CD19+IgM-IgD-SARS-CoV2 S+DAPI-) were detected with phycoerythrin (PE)-labeled streptavidin conjugate and quantified using a SH800 cell sorter (Sony). After identification of the two subjects with the highest B cell response against S2Pecto protein, target-specific memory B cells were isolated by FACS using an SH800 cell sorter (from pooled PBMCs of these subjects, after labeling of B cells with biotinylated S protein. These details are also found in the Methods section.
Instrument	A SH800 cell sorter (Sony) was used for FACS and analytical flow cytometry studies
Software	SH800 software and FlowJo version 10 (Tree Star Inc.).
Cell population abundance	Frequency of antigen-specific B cells ranged from 0.2 to approx. 1 % of class-switched memory B cells. Antigen specificity of sorted cells was validated in functional assays after production of recombinant antibodies, which included antigen binding and virus neutralization.
Gating strategy	PBMCs were pre-enriched for B cells using magnetic negative selection with commercial (STEMCELL Technologies) kit. Extended Data Fig. 2 indicates gating strategy for sorting of antigen-labeled class switched B cells, that included staining with phenotyping anti-CD19, anti-IgM, and anti-IgD antibodies. Pre-enriched memory B cells were first gated by forward and side scatter. Dead cells were excluded using a viability dye (DAPI). Class-switched memory B cells were gated from this viable population as CD19 +,IgM-,IgD-. The gate for the antigen-specific subset was placed based on staining of B cells isolated from a non- immune healthy donor (no exposure history to SARS-CoV-2). Specificity of antigen labeling was validated in functional assays after production of recombinant antibodies, which included antigen binding and virus neutralization.

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