mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants

Zijun Wang ${ }^{1, *}$, Fabian Schmidt2,*, Yiska Weisblum ${ }^{2, *}$, Frauke Muecksch ${ }^{2, *}$, Christopher O. Barnes $^{3, *}$, Shlomo Finkin ${ }^{1, *}$, Dennis Schaefer-Babajew ${ }^{1, *}$, Melissa Cipolla ${ }^{1 *}$, Christian Gaebler ${ }^{1 *}$, Jenna A. Lieberman ${ }^{4, *}$, Zhi Yang ${ }^{3}$, Morgan E. Abernathy ${ }^{3}$, Kathryn E. Huey-Tubman ${ }^{3}$, Arlene Hurley ${ }^{5}$, Martina Turroja ${ }^{1}$, Kamille A. West ${ }^{6}$, Kristie Gordon $^{1}$, Katrina G. Millard ${ }^{1}$, Victor Ramos ${ }^{1}$, Justin Da Silva ${ }^{2}$, Jianliang Xu $^{4}$, Robert A. Colbert ${ }^{7}$, Roshni Patel ${ }^{1}$, Juan Dizon ${ }^{1}$, Cecille Unson-O'Brien ${ }^{1}$, Irina Shimeliovich ${ }^{1}$, Anna Gazumyan ${ }^{1}$, Marina Caskey ${ }^{1}$, Pamela J. Bjorkman ${ }^{3, \#}$, Rafael Casellas ${ }^{4,8, \#}$, Theodora Hatziioannou ${ }^{2, \#}$, Paul D. Bieniasz ${ }^{2,9, \#}$, Michel C. Nussenzweig ${ }^{1,9, \#}$
${ }^{1}$ Laboratory of Molecular Immunology, The Rockefeller University, New York, NY 10065, USA.
${ }^{2}$ Laboratory of Retrovirology, The Rockefeller University, New York, NY 10065, USA.
${ }^{3}$ Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA.
${ }^{4}$ Lymphocyte Nuclear Biology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892.
${ }^{5}$ Hospital Program Direction, The Rockefeller University, New York, NY 10065, USA.
${ }^{6}$ Department of Transfusion Medicine, National Institutes of Health Clinical Center, Bethesda, MD 20892.
${ }^{7}$ Pediatric Translational Research Branch and Office of the Clinical Director, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892.
${ }^{8}$ The NIH Regulome Project, National Institutes of Health, Bethesda, MD 20892.
${ }^{9}$ Howard Hughes Medical Institute
*Equal contribution
\#Send correspondence to Paul Bieniasz: pbieniasz@rockefeller.edu; Pamela Bjorkman:
bjorkman@caltech.edu; Rafael Casellas: rafael.casellas@nih,gov; Theodora Hatziioannou:
thatziio@rockefeller.edu; Michel C. Nussenzweig: nussen@rockefeller.edu

To date severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has infected nearly $\mathbf{1 0 0}$ million individuals resulting in over two million deaths. Many vaccines are being deployed to prevent coronavirus disease-2019 (COVID-19) including two novel mRNA-based vaccines ${ }^{1,2}$. These vaccines elicit neutralizing antibodies and appear to be safe and effective, but the precise nature of the elicited antibodies is not known ${ }^{3-5}$. Here we report on the antibody and memory $B$ cell responses in a cohort of 20 volunteers who received either the Moderna (mRNA-1273) or Pfizer-BioNTech (BNT162b2) vaccines. Consistent with prior reports, 8 weeks after the second vaccine injection volunteers showed high levels of IgM, and IgG anti-SARS-CoV-2 spike protein (S), receptor binding domain (RBD) binding titers ${ }^{3,5}$. Moreover, the plasma neutralizing activity, and the relative numbers of RBD-specific memory $B$ cells were equivalent to individuals who recovered from natural infection ${ }^{6,7}$. However, activity against SARS-CoV-2 variants encoding E484K or N501Y or the K417N:E484K:N501Y combination was reduced by a small but significant margin. Consistent with these findings, vaccine-elicited monoclonal antibodies (mAbs) potently neutralize SARS-CoV-2, targeting a number of different RBD epitopes epitopes in common with mAbs isolated from infected donors. Structural analyses of mAbs complexed with $S$ trimer suggest that vaccine- and virus-encoded $S$ adopts similar conformations to induce equivalent anti-RBD antibodies. However, neutralization by 14 of the 17 most potent mAbs tested was reduced or abolished by either K417N, or E484K, or N501Y mutations. Notably, the same mutations were selected when recombinant vesicular stomatitis virus (rVSV)/SARS-CoV-2 S was cultured in the presence of the vaccine elicited mAbs. Taken together the results suggest that the monoclonal antibodies in clinical use
should be tested against newly arising variants, and that mRNA vaccines may need to be updated periodically to avoid potential loss of clinical efficacy.

Between 19 October 2020 and 15 January 2021, 20 volunteers who received two doses of the Moderna ( $\mathrm{n}=14$ ) or Pfizer-BioNTech mRNA ( $\mathrm{n}=6$ ) vaccines were recruited for blood donation and analyzed. Ages of the analyzed volunteers ranged from 29-69 years (median 43); 12 ( $60 \%$ ) were male and $8(40 \%)$ female. 16 participants identified as Caucasian, 2 as Hispanic, and 1 as African American or Asian, respectively. The time from the second vaccination to sample collection varied between 3-14 weeks with an average of 8 weeks. None of the volunteers had a history of prior SARS-CoV-2 infection and none experienced serious adverse events after vaccination (Extended Data Table 1).

## Vaccinee plasma binding and neutralizing activity against SARS-CoV-2

Plasma IgM and IgG anti-SARS-CoV-2 S and RBD were measured by enzyme-linked immunosorbent assay (ELISA) ${ }^{6,7}$. All individuals tested showed reactivity to S and RBD that was significantly higher compared to pre-COVID-19 historic controls (Extended Data Fig 1). As might be expected anti-S and -RBD IgG levels were higher than $\operatorname{IgM}$ or $\operatorname{IgA}$. Moreover, there was greater variability in the anti-RBD than the anti-S response but the two were positively correlated (Extended Data Fig 1).

Plasma neutralizing activity was determined using HIV-1 pseudotyped with SARS-CoV-2 S protein ${ }^{6-8}$. In agreement with previous reports, there was a broad range of plasma neutralizing activity 3-14 weeks after the second vaccine dose that was similar to that elicited by natural infection in a convalescent cohort after 1.3 months, and greater than the activity at 6.2 months after infection (Fig. 1a, Extended Data Table 1). There was no significant difference in neutralizing activity between the Moderna and Pfizer-BioNTech vaccines (Fig. 1b). As
expected, plasma neutralizing activity was directly correlated to anti-S and -RBD binding titers in ELISAs ${ }^{6,7}$ (Fig. 1c, d, and Extended Data Fig. 2a-d). Finally, RBD and $S$ binding, and neutralizing activities were directly correlated to the time between the first vaccine dose and blood donation with significantly reduced levels in all 3 measurements with time (Fig. 1e-g, and Extended Data Fig. 2e-h) ${ }^{9}$.

To determine whether plasma from vaccinated individuals can neutralize circulating SARS-CoV2 variants of concern and mutants that arise in vitro under antibody pressure ${ }^{10,11}$, we tested vaccinee plasma against a panel of 10 mutant pseudotype viruses including recently reported N501Y (B1.1.7 variant), K417N, E484K and the combination of these 3 RBD mutations (501Y.V2 variant) ${ }^{12-17}$. Vaccinee plasma was significantly less effective in neutralizing the HIV1 virus pseudotyped with certain SARS-CoV-2 mutant S proteins (Fig. 1h and i and Extended Data Fig. 2j). Among the volunteer plasmas tested there was an 1 to $3,1.3$ to 2.5 , and 1.1 to 3 fold decrease in neutralizing activity against E484K, N501Y and the K417N:E484K:N501Y combination respectively ( $p=0.0033, p=0.0002$, and $p<0.0001$, respectively, Fig. 1 h and i). We conclude that the plasma neutralizing activity elicited by mRNA vaccination is variably but significantly less effective against particular RBD mutants in the tested panel.

## Vaccine-elicited SARS-CoV-2 RBD-specific monoclonal antibodies

Although circulating antibodies derived from plasma cells wane over time, long-lived immune memory can persist in expanded clones of memory B cells ${ }^{6}$. We used flow cytometry to enumerate the circulating SARS-CoV-2 RBD-specific memory B cells elicited by mRNA immunization ${ }^{6,7}$ (Fig. 2a, Extended Data Fig. 3a and b). Notably, the percentage of RBD-binding
memory B cells in vaccinees was significantly greater than in naturally infected individuals assayed after 1.3 months, but similar to the same individuals assayed after 6.2 months (Fig. 2b). The percentage of RBD-binding memory B cells in vaccinees was not correlated to the time after vaccination (Extended Data Fig. 3c). Thus, mRNA vaccination elicits a robust SARS-CoV-2 RBD-specific B cell memory response that resembles natural infection.

To examine the nature of the antibodies produced by memory B cells in response to vaccination, we obtained 1,409 paired antibody heavy and light chains from RBD binding single B cells from 14 individuals ( $\mathrm{n}=10$ Moderna and $\mathrm{n}=4$ Pfizer-BioNTech vaccinees) (Extended Data Table 2). Expanded clones of cells comprised 4-50\% of the overall RBD binding memory B cell compartment (Fig. 2c and d, and Extended Data Fig. 3d). Similar to natural infection, IGVH 353, and 3-30 and some IGVL genes were significantly over-represented in the RBD-binding memory B cell compartment of vaccinated individuals (Fig. 2e, Extended Data Fig. 4a). In addition, antibodies that share the same combination of IGHV and IGLV genes in vaccinees comprised $39 \%$ of all the clonal sequences (Extended Data Fig. 4b) and 59\% when combined with naturally infected individuals ${ }^{6,7}$ (Fig. 2f), and some of these antibodies were nearly identical (Extended Data Table 2 and 3). The number of V gene nucleotide mutations in vaccinees is greater than in naturally infected individuals assayed after 1.3 months, but lower than that in the same individuals assayed after 6.2 months (Fig. 2g and Extended Data Fig. 5a). The length of the IgH CDR3 was similar in both natural infected individuals and vaccinees and hydrophobicity was below average ${ }^{18}$ (Fig. 2h and Extended Data Fig. 5a and b). Thus, the IgG memory response is similar in individuals receiving the Pfizer-BioNTech and Moderna vaccines and both are rich in recurrent and clonally expanded antibody sequences.

Eighty-four representative antibodies from 4 individuals were expressed and tested for reactivity to the RBD (Extended Data Table 4). The antibodies included: (1) 58 that were randomly selected from those that appeared only once, and (2) 26 representatives of expanded clones. Of the antibodies tested $99 \%$ ( 83 out of the 84 ) bound to RBD indicating that single cell sorting by flow cytometry efficiently identified B cells producing anti-RBD antibodies (Extended Data Fig. 6a and $b$ and Table 4). In anti-RBD ELISAs the mean half-maximal effective concentration $\left(\mathrm{EC}_{50}\right)$ was higher than that observed in infected individuals after 6 months but not significantly different from antibodies obtained 1.3 months after infection (Extended Data Fig. 6a, and Table 4 and ${ }^{6,7}$ ). To examine memory B cell antibodies for binding to circulating SARS-CoV-2 variants and antibody resistant mutants we performed ELISA assays using mutant RBDs ${ }^{10,13,19-22}$. Twenty-two (26\%) of the antibodies showed at least 5-fold decreased binding to at least one of the mutant RBDs (Extended data Fig. 6c-1, and Table 4).

SARS-CoV-2 S pseudotyped viruses were used to measure the neutralizing activity of all 84 antibodies ${ }^{6-8}$ (Fig. 3a, Extended Data Table 4). Consistent with the plasma neutralization results, the geometric mean neutralization half-maximal inhibitory concentration of the vaccinee antibodies ( $\mathrm{IC}_{50}=151 \mathrm{ng} / \mathrm{ml}$ ) was not significantly different to antibody collections obtained from naturally infected individuals 1.3 or 6.2 months after infection (Fig. 3a and ${ }^{6,7}$ ).

To examine the neutralizing breadth of the monoclonal antibodies and begin to map their target epitopes we tested the 17 most potent antibodies (Extended data Table 5), 8 of which carried IgHV3-53, against a panel of 12 SARS-CoV-2 variants: A475V is resistant to class 1 antibodies
(structurally defined as described ${ }^{20}$ ); E484K and Q493R are resistant to class 2
antibodies ${ }^{6,7,10,11,20,21,23,24}$; while R346S, N439K, and N440K are resistant to class 3
antibodies ${ }^{6,7,10,11,20,24}$. Additionally, K417N, Y453F, S477R, N501Y, D614G, and R683G represent circulating variants some of which have been associated with rapidly increasing case numbers ${ }^{12,13,24-27}$. Based on their neutralizing activity against the variants, all but 3 of the antibodies were provisionally assigned to a defined antibody class or a combination (Fig. 3b). As seen in natural infection, a majority of the antibodies tested (9/17) were at least ten-fold less effective against pseudotyped viruses carrying the E484K mutation ${ }^{6,10,20}$. In addition, 5 of the antibodies were less potent against K 417 N and 4 against N 501 Y by ten-fold or more (Fig. 3b).

To determine whether antibody-imposed selection pressure could also drive the emergence of resistance mutations in vitro, we cultured an rVSV/SARS-CoV-2 recombinant virus in the presence of each of 18 neutralizing monoclonal antibodies. All of the tested antibodies selected for RBD mutations. Moreover, in all cases the selected mutations corresponded to residues in the binding sites of their presumptive antibody class (Fig. 3b and c). For example, antibody 627, which was assigned to class 2 based on sensitivity to E484K mutation, selected for the emergence of the E484K mutation in vitro (Fig 3c). Notably, 6 of the antibodies selected for K417N E or T, 5 selected for E484K and 3 selected for N501Y, T or H, which coincide with mutations present in the circulating B1.1.17, 501Y.V2 and B1.1.28/501.V3 variants that have been associated with rapidly increasing case numbers in particular locales ${ }^{12,15,17,28}$.

## Cryo-EM mapping of antibody epitopes

To further characterize antibody epitopes and mechanisms of neutralization, we characterized seven complexes between mAb Fab fragments and the prefusion, stabilized ectodomain trimer of SARS-CoV-2 S glycoprotein ${ }^{29}$ using single-particle cryo-EM (Fig. 4 and Extended Data Table 6). Overall resolutions ranged from 5-8 $\AA$ and coordinates from $S$ trimer and representative Fab crystal structures were fit by rigid body docking into the cryo-EM density maps to provide a general assessment of antibody footprints/RBD epitopes. Fab-S complexes exhibited multiple RBD-binding orientations recognizing either 'up'/'down' (Fig. 4a-j) or solely 'up' (Fig. 4k-n) RBD conformations, consistent with structurally defined antibody classes from natural infection (Fig. 4o $)^{20}$. The majority of mAbs characterized (6 of 7) recognized epitopes that included RBD residues involved in ACE2 recognition, suggesting a neutralization mechanism that directly blocks ACE2-RBD interactions. Additionally, structurally defined antibody epitopes were consistent with RBD positions that were selected in rVSV/SARS-CoV-2 recombinant virus outgrowth experiments, including residues K417, N439/N440, E484, and N501 (Fig. 3c and Fig. 4f-j,m,n). Taken together, these data suggest that functionally similar antibodies are raised during vaccination and natural infection, and that the RBDs of spike trimers translated from the mRNA delivered by vaccination adopt a similar distribution of 'up'/'down' conformations as observed on SARS-CoV-2 virions.

## Discussion

The mRNA-based SARS-CoV-2 vaccines are safe and effective and being deployed globally to prevent infection and disease. The vaccines elicit antibody responses against the RBD, the major target of neutralizing antibodies ${ }^{30-35}$, in a manner that resembles natural infection. Notably, the neutralizing antibodies produced by mRNA vaccination target the same epitopes as natural
infection. The data is consistent with SARS-CoV-2 spike trimers translated from the injected RNA adopting the range of conformations available to spikes on the surfaces of virions. Moreover, different individuals immunized with either the Moderna (mRNA-1273) or PfizerBioNTech (BNT162b2) vaccines produce closely related and nearly identical antibodies.

Human neutralizing monoclonal antibodies to the SARS-CoV-2 RBD can be categorized as belonging to 4 different classes based on their target regions on the $\mathrm{RBD}^{20}$. Class 1 and 2 antibodies are among the most potent and also the most abundant antibodies ${ }^{6,7,30,31,34}$. These antibodies target epitopes that overlap or are closely associated with RBD residues K417, E484 and N 501 . They are frequently sensitive to mutation in these residues and select for K 417 N , E484K and N501Y mutations in both yeast and VSV expressing SARS-CoV-2 S proteins ${ }^{10,13,24}$. To avert selection and escape, antibody therapies should be composed of combinations of antibodies that target non-overlapping epitopes ${ }^{7,10,11,24,36-38}$.

A number of circulating SARS-CoV-2 variants that have been associated with rapidly increasing case numbers and have particular prevalence in the UK (B1.1.7/501Y.V1), South Africa (501Y.V2) and Brazil (B1.1.28/501.V3) ${ }^{12,15,17,28,39}$. Our experiments indicate that these variants, and potentially others that carry K417N/T, E484K and N501Y mutations, can reduce the neutralization potency of vaccinee plasma. The comparatively modest effects of the mutations on viral sensitivity to plasma reflects the polyclonal nature of the neutralizing antibodies in vaccinee plasma. Nevertheless, emergence of these particular variants is consistent with the dominance of the class 1 and 2 antibody response in infected or vaccinated individuals and raises the possibility that they emerged in response to immune selection in individuals with non-sterilizing
immunity. What the long-term effect of accumulation of mutations on the SARS-CoV-2 pandemic will be is not known, but the common cold coronavirus $\mathrm{HCoV}-229 \mathrm{E}$ evolves antigenic variants that are comparatively resistant to the older sera but remain sensitive to contemporaneous sera ${ }^{40}$. Thus, it is possible that these mutations and others that emerge in individuals with suboptimal or waning immunity will erode the effectiveness of natural and vaccine elicited immunity. The data suggests that SARS-CoV-2 vaccines may need to be updated and immunity monitored in order to compensate for viral evolution.


Fig. 1. Plasma neutralizing activity. a, SARS-CoV-2 pseudovirus neutralization assay. $\mathrm{NT}_{50}$ values for COVID-19 convalescent plasma measured at 1.3 months $^{7}$ and 6.2 months $^{6}$ as well as plasma from mRNA-vaccinees. $\mathrm{NT}_{50}$ values lower than 10 were plotted at 10 . Mean of 2 independent experiments. Red bars and indicated values represent geometric mean $\mathrm{NT}_{50}$ values. Statistical significance was determined using the two-tailed Mann-Whitney U-test. b, NT 50 values for Moderna (black) and Pfizer-BioNTech (red) vaccine recipients. Red bars and indicated values represent geometric mean $\mathrm{NT}_{50}$ values. Statistical significance was determined using the two-tailed Mann-Whitney U-test. c, Anti-RBD IgG AUC (Y axis) plotted against $\mathrm{NT}_{50}$ ( X axis) $\mathrm{r}=0.84, \mathrm{p}<0.0001$. d, Anti-S IgG AUC ( Y axis) plotted against $\mathrm{NT}_{50}$ ( X axis) $\mathrm{r}=0.83$, $\mathrm{p}<0.0001$. e, Anti-RBD IgG AUC (Y axis) plotted against time between first dose and blood draw ( X axis) $\mathrm{r}=-0.60 \mathrm{p}=0.0055$. f, Anti-S IgG AUC (Y axis) plotted against time between first
dose and blood draw (X axis) $\mathrm{r}=-0.62 \mathrm{p}=0.0038 . \mathbf{g}, \mathrm{NT}_{50}$ (Y axis) plotted against time between first dose and blood draw ( X axis) $\mathrm{r}=-0.69 \mathrm{p}=0.0008$. The r and p values for correlations in $\mathbf{c - g}$ were determined by two-tailed Spearman correlation. Moderna vaccinees are in black and PfizerBioNTech in red. h. Examples of neutralization assays, comparing the sensitivity of pseudotyped viruses with WT and RBD mutant SARS-CoV-2 S proteins to vaccinee plasma. i, NT50 values for vaccinee plasma $(\mathrm{n}=15)$ neutralization of pseudotyped viruses with WT and the indicated RBD-mutant SARS-CoV-2 S proteins. Statistical significance was determined using one tailed ttest. All experiments were performed a minimum of 2 times. Pfizer-BioNTech vaccinees in red.


Fig. 2. Memory B cell antibodies. a, Representative flow cytometry plots showing dual AlexaFluor-647-RBD and PE-RBD binding B cells for 4 vaccinees. $\mathbf{b}$, as in a, dot plot summarizes the percentage of RBD binding B cells in 19 vaccinees, in comparison to a cohort of infected individuals assays 1.3 and 6.2 months after infection ${ }^{6,7}$. Individuals who received the Moderna vaccine are shown in black and Pfizer-BioNTech vaccine recipients in red. Red horizontal bars indicate mean values. Statistical significance was determined using two-tailed Mann-Whitney U-tests. c, Pie charts show the distribution of antibody sequences from the 4 individuals in a. The number in the inner circle indicates the number of sequences analyzed. Pie slice size is proportional to the number of clonally related sequences. The black outline indicates the frequency of clonally expanded sequences. $\mathbf{d}$, as in $\mathbf{c}$, graph shows relative clonality among 14 vaccinees assayed, individuals who received the Moderna vaccine are shown in black and Pfizer-BioNTech vaccine recipients in red. Red horizontal bars indicate mean values. Statistical significance was determined using two-tailed Mann-Whitney U-tests. e, Graph shows relative abundance of human IGVH genes Sequence Read Archive accession SRP010970 (orange), and vaccinees (blue). A two-sided binomial test was used to compare the frequency distributions, significant differences are denoted with stars ( ${ }^{*} \mathrm{p}<0.05$, ${ }^{* *} \mathrm{p}<0.01,{ }^{* * *} \mathrm{p}<0.001,{ }^{* * * *}=\mathrm{p}<$ 0.0001). f, Clonal relationships between sequences from 14 vaccinated individuals (Moderna in black, Pfizer-BioNTech in red Extended Data Table 2) and naturally infected individuals (in green, from ${ }^{6,7}$ ). Interconnecting lines indicate the relationship between antibodies that share V and J gene segment sequences at both IGH and IGL. Purple, green and grey lines connect related clones, clones and singles, and singles to each other, respectively. $\mathbf{g}$, Number of somatic nucleotide mutations in the IGVH (top) and IGVL (bottom) in vaccinee antibodies (Extended Data Table 2) compared to natural infection obtained 1.3 or 6.2 months after infection ${ }^{6,7}$.

Statistical significance was determined using the two-tailed Mann-Whitney U-tests and red horizontal bars indicate mean values. $\mathbf{h}$, as in $\mathbf{g}$, but for CDR3 length.
a


C


Fig. 3: Anti-SARS-CoV-2 RBD monoclonal antibody neutralizing activity. a, SARS-CoV-2 pseudovirus neutralization assay. $\mathrm{IC}_{50}$ values for antibodies cloned from COVID-19 convalescent patients measured at 1.3 and 6.2 months ${ }^{6,7}$ as well as antibodies cloned from mRNA-vaccinees. Antibodies with $\mathrm{IC}_{50}$ values above $1000 \mathrm{ng} / \mathrm{ml}$ were plotted at $1000 \mathrm{ng} / \mathrm{ml}$. Mean of 2 independent experiments. Red bars and indicated values represent geometric mean $\mathrm{IC}_{50}$ values in $\mathrm{ng} / \mathrm{ml}$. Statistical significance was determined using the two-tailed Mann-Whitney U-test. b, IC ${ }_{50}$ values for 17 selected mAbs for neutralization of wild type and the indicated mutant SARS-CoV-2 pseudoviruses. Color gradient indicates IC so $_{0}$ values ranging from 0 (white)
to $1000 \mathrm{ng} / \mathrm{ml}$ (red). c, Antibody selection pressure can drive emergence of S variants in cell culture; the percentage of sequence reads encoding the indicated RBD mutations after a single passage of rVSV/SARS-CoV-2 in the presence of the indicated antibodies is tabulated. Color gradient indicates percentage of sequence reads bearing the indicated mutation ranging from 0 (white) to 100 (red). Positions for which no sequence read was detected are shown in grey. K417N, E484K/R683G and N501 are highlighted in b and c as they constitute important circulating variants.


Fig. 4. Cryo-EM reconstructions of Fab-S complexes. Cryo-EM densities for Fab-S complexes ( $\mathbf{a - e} ; \mathbf{k} \mathbf{k}$ ) and close-up views of antibody footprints on RBDs ( $\mathbf{f} \mathbf{-} \mathbf{j} ; \mathbf{m}-\mathbf{n}$ ) are shown for neutralizing mAbs. As expected due to Fab inter-domain flexibility, cryo-EM densities (a-e; k-l) were weak for the $\mathrm{Fab} \mathrm{C}_{\mathrm{H}}-\mathrm{C}_{\mathrm{L}}$ domains. Models of antibody footprints on RBDs ( $\mathbf{f} \mathbf{- j} ; \mathbf{m} \mathbf{- n}$ ) are
presented as Fab $\mathrm{V}_{\mathrm{H}}-\mathrm{V}_{\mathrm{L}}$ domains (cartoon) complexed with the RBD (surface). To generate models, coordinates of stabilized S trimer (PDB 6XKL) and representative Fab fragments (PDB 6XCA or 7 K 8 P ) with CDR3 loops removed were fit by rigid body docking into the cryo-EM density maps. a,f, C669; b,g, C643; c,h, C603; d,i, C601; e,j, C670; k,m, C666; and l,n, C663. RBD residues K417, N439, N440, E484, and N501 are highlighted as red surfaces. The N343 glycan is shown as a teal sphere. $\mathbf{o}$, Composite model illustrating targeted epitopes of RBDspecific neutralizing mAbs (shown as $\mathrm{V}_{\mathrm{H}}-\mathrm{V}_{\mathrm{L}}$ domains in colors from panels a-I) elicited from mRNA vaccines.

## Methods

## Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

## Study participants.

To isolate and characterize anti-SARS-CoV-2 RBD antibodies from vaccinees, a cohort of 20 individuals that participated in either the Moderna or Pfizer-BioNTech phase 3 vaccine clinical trials and did not report prior history of SARS-CoV-2 infection was recruited at the NIH Blood Center and the Rockefeller University Hospital for blood donation. Eligible participants included adults, at least 18 years of age with no known heart, lung, kidney disease or bleeding disorders, no history of HIV-1 or malaria infection. All participants were asymptomatic at the time of the study visit and had received a complete 2 dose regimen of either mRNA vaccine. Informed consent was obtained from all participants and the study was conducted in accordance with Good Clinical Practice. The study visits and blood draws were reviewed and approved under the National Institutes of Health's Federalwide Assurance (FWA00005897), in accordance with Federal regulations 45 CFR 46 and 21 CFR 5 by the NIH Intramural Research Program IRB committee (IRB\# 99CC0168, Collection and Distribution of Blood Components from Healthy Donors for In Vitro Research Use) and by the Institutional Review Board of the Rockefeller University (IRB\# DRO-1006, Peripheral Blood of Coronavirus Survivors to Identify VirusNeutralizing Antibodies). For detailed participant characteristics see Extended Data Table SI 1.

## Blood samples processing and storage.

Samples collected at NIH were drawn from participants at the study visit and processed within 24 hours. Briefly, whole blood samples were subjected to Ficoll gradient centrifugation after 1:1 dilution in PBS. Plasma and PBMC samples were obtained through phase separation of plasma layer and Buffy coat phase, respectively. PBMCs were further prepared through centrifugation, red blood cells lysis and washing steps, and stored in CellBanker cell freezing media (Amsbio). All samples were aliquoted and stored at $-80^{\circ} \mathrm{C}$ and shipped on dry ice. Prior to experiments, aliquots of plasma samples were heat-inactivated $\left(56^{\circ} \mathrm{C}\right.$ for 1 hour) and then stored at $4^{\circ} \mathrm{C}$. Peripheral Blood Mononuclear Cells (PBMCs) obtained from samples collected at Rockefeller University were purified as previously reported ${ }^{6,7}$ by gradient centrifugation and stored in liquid nitrogen in the presence of FCS and DMSO. Heparinized plasma samples were aliquoted and stored at $-20^{\circ} \mathrm{C}$ or less. Prior to experiments, aliquots of plasma samples were heatinactivated $\left(56^{\circ} \mathrm{C}\right.$ for 1 hour) and then stored at $4^{\circ} \mathrm{C}$.

## ELISAs

ELISAs ${ }^{41,42}$ to evaluate antibodies binding to SARS-CoV-2 S (BioHub), RBD and additional mutated RBDs were performed by coating of high-binding 96-half-well plates (Corning 3690) with $50 \mu \mathrm{l}$ per well of a $1 \mu \mathrm{~g} / \mathrm{ml}$ protein solution in PBS overnight at $4^{\circ} \mathrm{C}$. Plates were washed 6 times with washing buffer ( $1 \times$ PBS with $0.05 \%$ Tween-20 (Sigma-Aldrich)) and incubated with $170 \mu \mathrm{l}$ per well blocking buffer ( $1 \times$ PBS with $2 \%$ BSA and $0.05 \%$ Tween-20 (Sigma)) for 1 h at room temperature. Immediately after blocking, monoclonal antibodies or plasma samples were added in PBS and incubated for 1 h at room temperature. Plasma samples were assayed at a

1:66.6 (RU samples) or a 1:33.3 (NIH samples) starting dilution and 7 additional threefold serial dilutions. Monoclonal antibodies were tested at $10 \mu \mathrm{~g} / \mathrm{ml}$ starting concentration and 10 additional fourfold serial dilutions. Plates were washed 6 times with washing buffer and then incubated with anti-human IgG, IgM or IgA secondary antibody conjugated to horseradish peroxidase (HRP) (Jackson Immuno Research 109-036-088 109-035-129 and Sigma A0295) in blocking buffer at a 1:5,000 dilution (IgM and $\operatorname{IgG}$ ) or 1:3,000 dilution (IgA). Plates were developed by addition of the HRP substrate, TMB (ThermoFisher) for 10 min (plasma samples) or 4 minutes (monoclonal antibodies), then the developing reaction was stopped by adding $50 \mu \mathrm{l} 1 \mathrm{M} \mathrm{H}_{2} \mathrm{SO}_{4}$ and absorbance was measured at 450 nm with an ELISA microplate reader (FluoStar Omega, BMG Labtech) with Omega and Omega MARS software for analysis. For plasma samples, a positive control (plasma from participant $\operatorname{COV} 72^{6,7}$, diluted 66.6 -fold and with seven additional threefold serial dilutions in PBS) was added to every assay plate for validation. The average of its signal was used for normalization of all of the other values on the same plate with Excel software before calculating the area under the curve using Prism V8.4 (GraphPad). For monoclonal antibodies, the EC50 was determined using four-parameter nonlinear regression (GraphPad Prism V8.4).

## Expression of RBD proteins

Mammalian expression vectors encoding the RBDs of SARS-CoV-2 (GenBank MN985325.1; S protein residues 319-539) and eight additional mutant RBD proteins (E484K, Q493R, R346S, N493K, N440K, V367F, A475V, S477N and V483A) with an N-terminal human IL-2 or Mu phosphatase signal peptide were previously described ${ }^{21}$.

## Cells and viruses

293T/ACE2.cl22 and HT1080/ACE2.cl14 cells ${ }^{8}$ were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with $10 \%$ fetal bovine serum (FBS) at $37^{\circ} \mathrm{C}$ and $5 \% \mathrm{CO}_{2}$. Cells were periodically tested for contamination with mycoplasma or retroviruses.
rVSV/SARS-CoV-2/GFP chimeric virus stocks were generated by infecting 293T/ACE2.cl22 cells. Supernatant was harvested 1 day post infection (dpi), cleared of cellular debris, and stored at $-80^{\circ} \mathrm{C}$. A plaque purified variant designated rVSV/SARS-CoV-2/GFP ${ }_{2 \mathrm{EI} 1}$ that encodes D215G/R683G substitutions was used in these studies ${ }^{8}$.

## Selection and analysis of antibody escape mutations

For the selection of monoclonal antibody-resistant spike variants, a rVSV/SARS-CoV-2/GFP ${ }_{2 \mathrm{E} 1}$ (for details see ${ }^{8}$ ) population containing $10^{6}$ infectious units was incubated with of monoclonal antibodies ( $10 \mu \mathrm{~g} / \mathrm{ml}$ passage $10-40 \mu \mathrm{~g} / \mathrm{ml}$ for 1 hr at $37^{\circ} \mathrm{C}$. Then, the virus-antibody mixtures were incubated with $5 \times 10^{5} 293$ T/ACE2cl. 22 cells in 6 -well plates. At 1 day post infection media was replaced with fresh media containing the equivalent concentrations of antibodies.

Supernatant was harvested 2 days after infection and $150 \mu \mathrm{l}$ of the cleared supernatant was used to infect cells for passage 2 , while $150 \mu \mathrm{l}$ was subjected to RNA extraction and sequencing.

For identification of putative antibody resistance mutations, RNA was extracted using NucleoSpin 96 Virus Core Kit (Macherey-Nagel). The RNA was reversed transcribed using the SuperScript VILO cDNA Synthesis Kit (Thermo Fisher Scientific). KOD Xtreme Hot Start DNA Polymerase (Millipore Sigma) was used for amplification of cDNA using primers flanking the Sencoding sequence. The PCR products were purified and sequenced as previously described ${ }^{6,10}$. Breifly, tagmentation reactions were performed using 1ul diluted cDNA, $0.25 \mu \mathrm{l}$ Nextera TDE1

Tagment DNA enzyme (catalog no. 15027865), and $1.25 \mu 1$ TD Tagment DNA buffer (catalog no. 15027866; Illumina). Next, the DNA was ligated to unique i5/i7 barcoded primer combinations using the Illumina Nextera XT Index Kit v2 and KAPA HiFi HotStart ReadyMix (2X; KAPA Biosystems) and purified using AmPure Beads XP (Agencourt), after which the samples were pooled into one library and subjected to paired-end sequencing using Illumina MiSeq Nano 300 V2 cycle kits (Illumina) at a concentration of 12 pM .

For analysis of the sequencing data, the raw paired-end reads were pre-processed to remove trim adapter sequences and to remove low-quality reads (Phred quality score $<20$ ) using BBDuk. Reads were mapped to the rVSV/SARS-CoV-2/GFP sequence using Geneious Prime (Version 2020.1.2). Mutations were annotated using Geneious Prime, with a P-value cutoff of $10^{-6}$.

## SARS-CoV-2 pseudotyped reporter virus

A panel of plasmids expressing RBD-mutant SAR-CoV-2 spike proteins in the context of pSARS-CoV-2-S ${ }_{\Delta 19}$ have been described previously ${ }^{10}$. Additional substitutions were introduced using either PCR primer-mediated mutagenesis or with synthetic gene fragments (IDT) followed by Gibson assembly. The mutants E484K and KEN (K417N+E484K+N501Y) were constructed in the context of a pSARS-CoV-2-S $\mathrm{S}_{\Delta 19}$ variant with a mutation in the furin cleavage site (R683G). The NT50s and IC50 of these pseudotypes were compared to a wildtype SARS-CoV-2 (NC_045512) spike sequence carrying R683G in the subsequent analyses, as appropriate. Generation of SARS-CoV-2 pseudotyped HIV-1 particles was performed as previously described ${ }^{7}$. Briefly, 293T cells were transfected with pNL4-3 ${ }^{\text {E Env-nanoluc and pSARS-CoV-2- }}$ $S_{\Delta 19}$ and pseudotyped virus stocks were harvested 48 hours after transfection, filtered and stored at $-80^{\circ} \mathrm{C}$.

## SARS-CoV-2 pseudotype neutralization assays

Plasma or monoclonal antibodies from vaccine recipients were four-fold or five-fold serially diluted and then incubated with SARS-CoV-2 pseudotyped HIV-1 reporter virus for 1 h at $37^{\circ} \mathrm{C}$. The antibody and pseudeotype virus mixture was added to $293 \mathrm{~T}_{\text {Ace2 }}$ cells ${ }^{7}$ (for comparisons of plasma from COVID-19-convalescents and vaccine recipients) or HT1080ACE2.cl14 cells ${ }^{8}$ (for analysis of spike mutants with vaccine recipient plasma or monoclonal antibodies). After 48 h cells were washed with PBS and lysed with Luciferase Cell Culture Lysis $5 \times$ reagent (Promega) and Nanoluc Luciferase activity in lysates was measured using the Nano-Glo Luciferase Assay System (Promega) with the Glomax Navigator (Promega). The relative luminescence units were normalized to those derived from cells infected with SARS-CoV-2 pseudotyped virus in the absence of plasma or monoclonal antibodies. The half-maximal neutralization titers for plasma $\left(\mathrm{NT}_{50}\right)$ or half-maximal and $90 \%$ inhibitory concentrations for monoclonal antibodies $\left(\mathrm{IC}_{50}\right.$ and $\mathrm{IC}_{90}$, respectively) were determined using four-parameter nonlinear regression (least squares regression method without weighting; constraints: top=1, bottom=0) (GraphPad Prism).

## Biotinylation of viral protein for use in flow cytometry

Purified and Avi-tagged SARS-CoV-2 RBD was biotinylated using the Biotin-Protein LigaseBIRA kit according to manufacturer's instructions (Avidity) as described before ${ }^{7}$. Ovalbumin (Sigma, A5503-1G) was biotinylated using the EZ-Link Sulfo-NHS-LC-Biotinylation kit according to the manufacturer's instructions (Thermo Scientific). Biotinylated ovalbumin was conjugated to streptavidin-BV711 (BD biosciences, 563262) and RBD to streptavidin-PE (BD Biosciences, 554061) and streptavidin-AF647 (Biolegend, 405237) ${ }^{7}$.

## Flow cytometry and single cell sorting

Single-cell sorting by flow cytometry was performed as described previously ${ }^{7}$. Briefly, peripheral blood mononuclear cells were enriched for $B$ cells by negative selection using a pan-B-cell isolation kit according to the manufacturer's instructions (Miltenyi Biotec, 130-101-638). The enriched B cells were incubated in FACS buffer ( $1 \times$ PBS, $2 \%$ FCS, 1 mM EDTA ) with the following anti-human antibodies (all at 1:200 dilution): anti-CD20-PECy7 (BD Biosciences, 335793), anti-CD3-APC-eFluro 780 (Invitrogen, 47-0037-41), anti-CD8-APC-eFluor 780 (Invitrogen, 47-0086-42), anti-CD16-APC-eFluor 780 (Invitrogen, 47-0168-41), anti-CD14-APC-eFluor 780 (Invitrogen, 47-0149-42), as well as Zombie NIR (BioLegend, 423105) and fluorophore-labelled RBD and ovalbumin (Ova) for 30 min on ice. Single CD3-CD8-CD14-CD16-CD20+Ova-RBD-PE+RBD-AF647+ B cells were sorted into individual wells of 96 -well plates containing $4 \mu \mathrm{l}$ of lysis buffer $(0.5 \times$ PBS, 10 mM DTT, 3,000 units/ml RNasin Ribonuclease Inhibitors (Promega, N2615) per well using a FACS Aria III and FACSDiva software (Becton Dickinson) for acquisition and FlowJo for analysis. The sorted cells were frozen on dry ice, and then stored at $-80^{\circ} \mathrm{C}$ or immediately used for subsequent RNA reverse transcription.

## Antibody sequencing, cloning and expression

Antibodies were identified and sequenced as described previously ${ }^{7}$. In brief, RNA from single cells was reverse-transcribed (SuperScript III Reverse Transcriptase, Invitrogen, 18080-044) and the cDNA stored at $-20^{\circ} \mathrm{C}$ or used for subsequent amplification of the variable IGH, IGL and IGK genes by nested PCR and Sanger sequencing. Sequence analysis was performed using

MacVector. Amplicons from the first PCR reaction were used as templates for sequence- and ligation-independent cloning into antibody expression vectors. Recombinant monoclonal antibodies and Fabs were produced and purified as previously described ${ }^{7}$.

## Cryo-EM sample preparation

Expression and purification of SARS-CoV-2 6P stabilized S trimers ${ }^{29}$ was conducted as previously described ${ }^{43}$. Purified Fab and S 6P trimer were incubated at a 1.1:1 molar ratio per protomer on ice for 30 minutes prior to deposition on a freshly glow-discharged 300 mesh, 1.2/1.3 Quantifoil copper grid. Immediately before $3 \mu 1$ of complex was applied to the grid, fluorinated octyl-malotiside was added to the Fab-S complex to a final detergent concentration of $0.02 \% \mathrm{w} / \mathrm{v}$, resulting in a final complex concentration of $3 \mathrm{mg} / \mathrm{ml}$. Samples were vitrified in $100 \%$ liquid ethane using a Mark IV Vitrobot after blotting for 3 s with Whatman No. 1 filter paper at $22^{\circ} \mathrm{C}$ and $100 \%$ humidity.

## Cryo-EM data collection and processing

Data collection and processing followed a similar workflow to what has been previously described in detail ${ }^{20}$. Briefly, micrographs were collected on a Talos Arctica transmission electron microscope (Thermo Fisher) operating at 200 kV for all Fab-S complexes. Data were collected using SerialEM automated data collection software ${ }^{44}$ and movies were recorded with a K3 camera (Gatan). For all datasets, cryo-EM movies were patch motion corrected for beaminduced motion including dose-weighting within cryoSPARC v2.155 after binning super resolution movies. The non-dose-weighted images were used to estimate CTF parameters using cryoSPARC implementation of the Patch CTF job. Particles were picked using Blob picker and
extracted 4x binned and 2D classified. Class averages corresponding to distinct views with secondary structure features were chosen and ab initio models were generated. 3D classes that showed features of a Fab-S complex were re-extracted, unbinned ( $0.869 \AA /$ pixel $)$ and homogenously refined with C1 symmetry. Overall resolutions were reported based on gold standard FSC calculations.

## Cryo-EM Structure Modeling and Refinement

Coordinates for initial complexes were generated by docking individual chains from reference structures into cryo-EM density using UCSF Chimera ${ }^{46}$ (S trimer: PDB 6KXL, Fab: PDB 6XCA or 7K8P after trimming CDR3 loops and converting to a polyalanine model). Models were then refined into cryo-EM maps by rigid body and real space refinement in Phenix ${ }^{47}$ If the resolution allowed, partial CDR3 loops were built manually in $\operatorname{Coot}^{48}$ and then refined using real-space refinement in Phenix.

## Computational analyses of antibody sequences

Antibody sequences were trimmed based on quality and annotated using Igblastn v.1.14. with IMGT domain delineation system. Annotation was performed systematically using Change-O toolkit v.0.4.540 ${ }^{49}$. Heavy and light chains derived from the same cell were paired, and clonotypes were assigned based on their V and J genes using in-house R and Perl scripts (Extended data Fig. 2). All scripts and the data used to process antibody sequences are publicly available on GitHub (https://github.com/stratust/igpipeline).

The frequency distributions of human V genes in anti-SARS-CoV-2 antibodies from this study was compared to $131,284,220 \mathrm{IgH}$ and IgL sequences generated by ${ }^{50}$ and downloaded from cAb-Rep ${ }^{51}$, a database of human shared BCR clonotypes available at https://cabrep.c2b2.columbia.edu/. Based on the 97 distinct V genes that make up the 4186 analyzed sequences from Ig repertoire of the 14 participants present in this study, we selected the $\operatorname{IgH}$ and IgL sequences from the database that are partially coded by the same V genes and counted them according to the constant region. The frequencies shown in (Fig 2e and Extended Data Fig 3a) are relative to the source and isotype analyzed. We used the two-sided binomial test to check whether the number of sequences belonging to a specific $\operatorname{IgHV}$ or $\operatorname{IgLV}$ gene in the repertoire is different according to the frequency of the same $\operatorname{IgV}$ gene in the database. Adjusted p-values were calculated using the false discovery rate (FDR) correction. Significant differences are denoted with stars.

Nucleotide somatic hypermutation and CDR3 length were determined using in-house R and Perl scripts. For somatic hypermutations, IGHV and IGLV nucleotide sequences were aligned against their closest germlines using Igblastn and the number of differences were considered nucleotide mutations. The average mutations for V genes were calculated by dividing the sum of all nucleotide mutations across all participants by the number of sequences used for the analysis. To calculate the GRAVY scores of hydrophobicity ${ }^{52}$ we used Guy H.R. Hydrophobicity scale based on free energy of transfer $(\mathrm{kcal} / \mathrm{mole})^{53}$ implemented by the R package Peptides (the Comprehensive R Archive Network repository; https://journal.r-project.org/archive/2015/RJ-2015-001/RJ-2015-001.pdf). We used 1409 heavy chain CDR3 amino acid sequences from this study and $22,654,256$ IGH CDR3 sequences from the public database of memory B cell receptor
sequences ${ }^{54}$. The two-tailed Wilcoxon nonparametric test was used to test whether there is a difference in hydrophobicity distribution.

Data availability statement: Data are provided in SI Tables 1-8. The raw sequencing data and computer scripts associated with Fig. 2 have been deposited at Github (https://github.com/stratust/igpipeline). This study also uses data from "A Public Database of Memory and Naive B-Cell Receptor Sequences" ${ }^{54}$, PDB (6VYB and 6NB6) and from "High frequency of shared clonotypes in human B cell receptor repertoires" ${ }^{50}$. Cryo-EM maps associated with data reported in this manuscript will be deposited in the Electron Microscopy Data Bank (EMDB: https://www.ebi.ac.uk/pdbe/emdb/).

## Data presentation

Figures arranged in Adobe Illustrator 2020.

Competing interests: The Rockefeller University has filed a provisional patent application in connection with this work on which Z.W. and M.C.N. are inventors (US patent 63/021,387).

Code availability statement: Computer code to process the antibody sequences is available at GitHub (https://github.com/stratust/igpipeline).

Acknowledgements: We thank all study participants who devoted time to our research; Drs. Barry Coller and Sarah Schlesinger, the Rockefeller University Hospital Clinical Research Support Office and nursing staff; Charles M. Rice and all members of the M.C.N. laboratory for
helpful discussions and Maša Jankovic for laboratory support; and Dr. Jost Vielmetter and the Protein Expression Center in the Beckman Institute at Caltech for expression assistance. Electron microscopy was performed in the Caltech Beckman Institute Resource Center for Transmission Electron Microscopy and we thank Drs. Songye Chen and Andrey Malyutin for technical assistance. This work was supported by NIH grant P01-AI138398-S1 (M.C.N. and P.J.B.) and 2U19AI111825 (M.C.N.); the Caltech Merkin Institute for Translational Research and P50 AI150464-13 (P.J.B.), a George Mason University Fast Grant (P.J.B.); R37-AI64003 to P.D.B.; R01AI78788 to T.H.; We thank Dr. Jost Vielmetter and the Protein Expression Center in the Beckman Institute at Caltech for expression assistance C.O.B. is supported by the HHMI Hanna Gray and Burroughs Wellcome PDEP fellowships. C.G. was supported by the Robert S. Wennett Post-Doctoral Fellowship, in part by the National Center for Advancing Translational Sciences (National Institutes of Health Clinical and Translational Science Award program, grant UL1 TR001866), and by the Shapiro-Silverberg Fund for the Advancement of Translational Research. P.D.B. and M.C.N. are Howard Hughes Medical Institute Investigators.

## Author Contributions:

P.D.B., P.J.B., R.C., T.H., M.C.N, Z.W., F.S., Y.W. F.M. C.O.B, S.F., D.S.B., M.Cipolla. conceived, designed and analyzed the experiments. M.Caskey, C.G., J.A. L, K.W. D.S.B. designed clinical protocols Z.W., F.S., Y.W. F.M. C.O.B, S.F., D.S.B., M.Cipolla, J.D.S. A.G. Z.Y., M.E.A., K.E.H. carried out experiments. C.G., M.Caskey, K.W. D., R.A.C., A.H., K.G.M. recruited participants and executed clinical protocols. I.S., R.P, J.D., J.X. and C.U.O. processed clinical samples. T.Y.O. and V.R. performed bioinformatic analysis. R.C. P.D.B., P.J.B., T.H., and M.C.N. wrote the manuscript with input from all co-authors.

## Extended Data Figures



## Extended Data Fig. 1. Plasma antibodies against SARS-CoV-2. a-f, Results of ELISAs

measuring plasma reactivity to $S(\mathbf{a}, \mathbf{c}, \mathbf{e})$ and RBD protein (b,d,f) of 20 vaccinees (grey curves) and 8 controls (black curves). a, Anti-S IgG. b, Anti-RBD IgG. c, Anti-S IgM. d, Anti-RBD IgM. e, Anti-S IgA. f, Anti-RBD IgA. Left, optical density at 450 nm (OD 450 nm ) for the indicated reciprocal plasma dilutions. Right, normalized area under the curve (AUC) values for the 8 controls and 20 vaccinees. Horizontal bars indicate geometric mean. Statistical significance was determined using the two-tailed Mann-Whitney U-test. Average of two or more experiments. g-i, Correlations of plasma antibodies measurements. g, Normalized AUC for IgG anti-S plotted against normalized AUC for IgG anti-RBD. h, Normalized AUC for IgM anti-S plotted against normalized AUC for IgM anti-RBD. i, Normalized AUC for IgA anti-S plotted against normalized AUC for IgA anti-RBD. The $r$ and $p$ values in $\mathbf{g}-\mathbf{i}$ were determined with the
two-tailed Spearman's correlation test. Moderna vaccinees are in black and Pfizer-BioNTech in red.


Extended Data Fig. 2: Plasma neutralizing activity. a, Anti-S IgM AUC (Y axis) plotted against $\mathrm{NT}_{50}$ ( X axis) $\mathrm{r}=0.12, \mathrm{p}<0.6179$. b, Anti-S IgA AUC (Y axis) plotted against $\mathrm{NT}_{50}$ ( X axis) $\mathrm{r}=0.79, \mathrm{p}<0.0001$. $\mathbf{c}$, Anti-RBD IgM AUC (Y axis) plotted against NT50 (X axis) $\mathrm{r}=-0.05$ $\mathrm{p}=0.8502 . \mathbf{d}$, Anti-RBD IgA AUC (Y axis) plotted against NT50 (X axis) $\mathrm{r}=0.70 \mathrm{p}=0.0006 \mathbf{e}$, $\mathrm{NT}_{50}$ (Y axis) plotted against time between last dose and blood draw (X axis) $\mathrm{r}=-0.63 \mathrm{p}=0.0032$. $\mathbf{f}, \mathrm{NT}_{50}$ (Y axis) plotted against time between doses (X axis) $\mathrm{r}=0.03 \mathrm{p}=0.8906$. g, Anti-RBD $\operatorname{IgG}$ AUC (Y axis) plotted against time between last dose and blood draw ( X axis) $\mathrm{r}=-0.57 \mathrm{p}=0.0084$. h, Anti-S IgG AUC (Y axis) plotted against time between last dose and blood draw (X axis) r=$0.59 \mathrm{p}=0.0064$. i, Age (Y axis) plotted against $\mathrm{NT}_{50}$ (X axis) $\mathrm{r}=-0.06 \mathrm{p}=0.8150$. The r and p values were determined by two-tailed Spearman's. Moderna vaccinees in black and PfizerBioNTech in red. $\mathbf{j}$, NT50 values for vaccinee plasma $(\mathrm{n}=15)$ neutralization of pseudotyped
viruses with WT and the indicated RBD-mutant SARS-CoV-2 S proteins; p-values determined using one tailed t -test.


C


d


Extended Data Fig. 3: Flow cytometry. a, Gating strategy used for cell sorting. Gating was on singlets that were CD20+ and CD3-CD8-CD16-OVA-. Sorted cells were RBD-PE+ and RBDAF647+. b, Flow cytometry showing the percentage of RBD-double positive memory B cells from a pre-COVID-19 control (HD) and 15 vaccinees, who received the Moderna vaccine are shown in black and Pfizer-BioNTech vaccine recipients are in red. $\mathbf{c}$, the percentage of RBDbinding memory B cells in vaccinees (Y axis) plotted against time between first dose and blood draw ( X axis) $\mathrm{r}=0.4028 \mathrm{p}=0.0873$ (left panel), and between last dose and blood draw ( X axis) $\mathrm{r}=0.3319 \mathrm{p}=0.1651$ (right panel). Moderna vaccinees in black and Pfizer-BioNTech in red. The r and $p$ values for correlations were determined by two-tailed Spearman's. d, Pie charts show the distribution of antibody sequences from 10 individuals in $\mathbf{b}$. The number in the inner circle indicates the number of sequences analyzed. Pie slice size is proportional to the number of clonally related sequences. The black outline indicates the frequency of clonally expanded sequences. The $r$ and $p$ values for correlations in $\mathbf{c}$ were determined by the two-tailed Spearman correlation test.


Extended Data Fig. 4: Frequency distributions of human VL genes.

Graph shows relative abundance of human IGVK (left) and IgVL (right) genes of Sequence Read Archive accession SRP010970 (orange) ${ }^{55}$, and vaccinees (blue). Two-sided binomial tests with unequal variance were used to compare the frequency distributions., significant differences are denoted with stars. ( ${ }^{*} \mathrm{p}<0.05,{ }^{* *} \mathrm{p}<0.01,{ }^{* * *} \mathrm{p}<0.001,{ }^{* * * *}=\mathrm{p}<0.0001$ ). b. Sequences from 14 individuals (Extended Data Table 2) with clonal relationships depicted as in c.

Interconnecting lines indicate the relationship between antibodies that share V and J gene segment sequences at both IGH and IGL. Purple, green and grey lines connect related clones, clones and singles, and singles to each other, respectively.


Extended Data Fig. 5: Antibody somatic hypermutation, and CDR3 length. a, Number of somatic nucleotide mutations in both the IGVH and IGVL in 14 participants (left). Individuals who received the Moderna vaccine are shown in black and Pfizer-BioNTech vaccine recipients in red. For each individual, the number of the amino acid length of the CDR3s at the IGVH and IGVL is shown (right). The horizontal bars indicate the mean. The number of antibody sequences (IGVH and IGVL) evaluated for each participant are $\mathrm{n}=68$ (MOD1), $\mathrm{n}=45$ (MOD2), $\mathrm{n}=117$ (MOD3), $\mathrm{n}=123$ (MOD4), $\mathrm{n}=110$ (MOD6), $\mathrm{n}=109$ (MOD7), $\mathrm{n}=144$ (MOD8), $\mathrm{n}=102$ (MOD9), $\mathrm{n}=132$ (PFZ10), $\mathrm{n}=109$ (MOD11), $\mathrm{n}=91$ (PFZ12), $\mathrm{n}=78$ (C001), $\mathrm{n}=66$ (C003), and $\mathrm{n}=115$ (C004). $\mathbf{b}$, Distribution of the hydrophobicity GRAVY scores at the IGH CDR3 compared to a public database (see Methods for statistical analysis). The box limits are at the lower and upper quartiles, the center line indicates the median, the whiskers are $1.5 \times$ interquartile range and the dots represent outliers. Statistical significance was determined using two-tailed Wilcoxon matched-pairs signed rank test (n.s.=non-significant, ${ }^{* * * *}=\mathrm{p}<0.0001$ ).


b


C

g


d


i



I

|  |  | 3 | $2_{3}^{30^{65}}$ |  | ${\sqrt{\left(x^{3}\right.}}^{()^{x}}$ | $2+1 x^{10}$ |  | $s^{\hat{x^{10}}}$ | $y^{x^{\circ}}$ | $\psi_{0}^{80^{9}\left(x^{2}\right)}$ | $0^{x^{x^{3}}}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Class 1 | C682 | 2.4 | 2.2 | 2.6 | 3.6 | 3.5 | 35.0 | 3.3 | 2.8 | 3.7 | 4.1 |
|  | C613 | 2.0 | 1.9 | 1.8 | 3.3 | 2.5 | 126.1 | 2.4 | 2.3 | 2.2 | 4.9 |
|  | C663 | 1.9 | 1.8 | 1.7 | 2.7 | 2.5 | 12.6 | 2.3 | 2.0 | 2.4 | 2.2 |
|  | C614 | 1.4 | 1.6 | 1.6 | 2.0 | 1.9 | 2.5 | 1.8 | 1.9 | 1.6 | 1.8 |
|  | C660 | 2.0 | 2.0 | 2.1 | 2.3 | 2.7 | 2.5 | 2.1 | 2.2 | 2.4 | 2.2 |

Class $1 / 2 \mid$ C653 | 2.1 | 2.1 | 2.1 | 2.5 | 2.6 | 2.2 | 2.1 | 2.3 | 4.0 | 3.1 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |

Class 2

| C627 | 1.6 | 1.6 | 1.8 | 2.0 | 2.0 | 2.1 | 1.9 | 1.8 | 1000 | 1000 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| C602 | 2.0 | 1.8 | 1.9 | 2.1 | 2.2 | 2.2 | 2.1 | 3.1 | 1000 | 1.9 |
| C671 | 2.3 | 1.7 | 1.5 | 2.4 | 2.4 | 2.5 | 2.0 | 1.8 | 1000 | 2.5 |
| C643 | 2.1 | 2.2 | 2.3 | 2.0 | 2.2 | 1.7 | 1.8 | 2.5 | 1000 | 1.9 |


| Class 2/3 | C603 | 2.1 | 1.7 | 1.7 | 2.1 | 2.2 | 2.3 | 2.0 | 1.9 | 1000 | 1.9 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | C628 | 2.7 | 1.9 | 2.0 | 4.3 | 3.2 | 3.1 | 2.4 | 2.4 | 10.0 | 3.2 |
| Class 3 | C669 | 2.3 | 2.1 | 2.2 | 950.6 | 291.8 | 2.1 | 2.1 | 2.2 | 2.4 | 2.2 |
|  | C670 | 2.9 | 2.1 | 2.3 | 210.7 | 2.8 | 3.0 | 2.6 | 2.4 | 3.3 | 3.3 |
| Unclassified | C666 | 1.9 | 1.7 | 1.8 | 2.0 | 2.1 | 1.5 | 1.8 | 1.9 | 2.1 | 1.8 |
|  | C601 | 1.1 | 1.8 | 2.1 | 2.2 | 2.2 | 1.9 | 1.9 | 2.2 | 12.1 | 2.0 |
|  | C675 | 1.3 | 1.1 | 1.4 | 1.8 | 1.7 | 3.5 | 1.4 | 1.5 | 1.6 | 1.4 |

Extended Data Fig. 6: Monoclonal antibody ELISAs. a, Graph shows antibody binding to SARS-CoV-2 RBD. ELISA $\mathrm{EC}_{50}$ (half-maximal response) values for 84 antibodies isolated from Moderna vaccinees measured at 8 weeks after the boost and from convalescent individuals at 1.3 and 6.2 months ${ }^{6,7}$. Horizontal bars indicate geometric mean. Statistical significance was determined using the two-tailed Mann-Whitney U-test. Average of two or more experiments. b$\mathbf{k}$, Graphs show ELISA titrations for antibodies all 84 antibodies against the indicated RBD variants. $\mathrm{n}=84$ samples and isotype antibody as indicated in the figure. Low-binding antibodies are indicated in colors. Data are representative of two independent experiments. I, Table shows a heat map summary of $\mathrm{EC}_{50}$ values for binding to wild type RBD and the indicated mutants for 17 top neutralizing antibodies.

Supplementary Tables
Supplementary Table 1: Individual vaccinee characteristics


Supplementary Table 2: Antibody sequences from vaccinees is provided as a separate Excel
file.

Supplementary Table 3: CDR3 alignment of highly identical clonal sequences.


Supplementary Table 4: Sequences, half maximal effective concentrations (EC50s) and inhibitory concentrations (IC50s) of the cloned monoclonal antibodies is provided as a separate Excel file.

Supplementary Table 5: Neutralization activity of mAbs against mutant SARS-CoV-2 pseudoviruses.

|  | wt |  | R683G |  | R346S |  | K417N |  | N439K |  | N440K |  | Y453F |  | A475V |  | S477R |  | R683G/E484K |  | Q493R |  | N501Y |  | D614G |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\begin{gathered} \hline \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml}]} \\ \hline \end{gathered}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml]}} \end{gathered}$ | $\begin{gathered} \hline \text { IC50 } \\ \text { [ng/mi] } \end{gathered}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml}]} \end{gathered}$ | $\begin{array}{\|c} \hline \text { IC50 } \\ \text { [ng/mi] }] \\ \hline \end{array}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml]}} \\ \hline \end{gathered}$ | $\begin{gathered} \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml}]} \end{gathered}$ | $\begin{gathered} \text { IC90 } \\ \text { [ng/mi] } \\ \hline \end{gathered}$ | $\begin{array}{\|c\|} \hline \text { IC50 } \\ \text { [ng/ml] } \\ \hline \end{array}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml}]} \end{gathered}$ | $\begin{gathered} \hline \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml}]} \\ \hline \end{gathered}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml}]} \\ \hline \end{gathered}$ | $\begin{gathered} \hline \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml}]} \end{gathered}$ | $\begin{array}{c\|} \hline \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml]}} \end{array}$ | $\begin{gathered} \hline \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml}]} \\ \hline \end{gathered}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml}]} \\ \hline \end{gathered}$ | $\begin{gathered} \hline \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml]}} \\ \hline \end{gathered}$ | $\begin{gathered} \text { IC90 } \\ \text { [ng/mi] } \\ \hline \end{gathered}$ | $\begin{gathered} \hline \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml}]} \\ \hline \end{gathered}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml]}} \\ \hline \end{gathered}$ | $\begin{array}{\|c\|} \hline \text { IC50 } \\ \text { [ng/mI] } \\ \hline \end{array}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml]}} \\ \hline \end{gathered}$ | $\begin{gathered} \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml}]} \end{gathered}$ | $\begin{gathered} \text { IC90 } \\ \text { [ng/mi] } \\ \hline \end{gathered}$ | $\begin{array}{\|c\|c} \hline \text { IC50 } \\ {[\mathrm{ng} / \mathrm{ml]}} \end{array}$ | $\begin{gathered} \text { IC90 } \\ {[\mathrm{ng} / \mathrm{ml]}} \\ \hline \end{gathered}$ |
| C601 | 2.0 | 9.2 | 1.4 | 6.3 | 1.9 | 11.5 | 1.4 | 6.4 | 1.8 | 8.5 | 2.2 | 10.2 | 1.2 | 7.9 | 1.8 | 11.9 | 1.9 | 8.3 | 112.2 | 630.3 | 2.6 | 13.5 | 4.9 | 13.8 | 2.5 | 8.5 |
| C602 | 13.5 | 70.2 | 10.3 | 142.3 | 7.8 | 75.8 | 4.8 | 37.0 | 15.8 | 224.3 | 16.3 | 185.2 | 13.1 | 210.4 | 11.2 | 111.9 | 13.5 | 116.3 | >1000 | >1000 | 57.6 | >1000 | 82.4 | 684.6 | 9.9 | 70.6 |
| C603 | 14.3 | 29.9 | 6.7 | 89.5 | 8.7 | >1000 | 4.9 | 38.8 | 11.8 | 0.2 | 13.2 | 115.1 | 11.7 | 3.0 | 19.1 | 208.3 | 11.3 | 112.5 | >1000 | >1000 | 7.1 | 78.5 | 56.1 | 292.9 | 10.8 | 130.7 |
| C613 | 20.4 | 127.1 | 16.4 | 93.8 | 12.4 | 97.7 | 3.1 | >1000 | 45.3 | 224.7 | 22.3 | 119.4 | 46.7 | 311.3 | >1000 | >1000 | 32.9 | 261.2 | 30.0 | 105.7 | 236.3 | >1000 | >1000 | >1000 | 13.3 | 105.0 |
| C614 | 5.0 | 20.2 | 3.7 | 16.9 | 4.1 | 26.7 | >1000 | >1000 | 3.9 | 20.7 | 5.7 | 23.1 | 4.7 | 34.6 | 31.7 | 215.3 | 4.4 | 20.2 | 9.4 | 26.6 | 6.6 | 41.5 | 16.1 | 37.1 | 4.2 | 17.6 |
| 27 | 4.8 | 39.3 | 2.5 | 21.8 | 3.7 | 43.6 | 6.6 | 104.1 | 3.4 | 25.7 | 4.5 | 35.6 | 4.2 | 46.7 | 3.9 | 43.6 | 2.7 | 16.3 | >1000 | >1000 | >1000 | >1000 | 18.3 | 75.2 | 4.2 | 22.5 |
| 28 | 18.8 | 169.9 | 11.6 | 144.3 | 8.6 | 95.3 | 5.2 | 63.4 | 257.3 | >1000 | 29.8 | 216.2 | 36.5 | 570.6 | 16.1 | 120.1 | 17.9 | 147.2 | >1000 | >1000 | 146.8 | >1000 | >1000 | >1000 | 13.6 | 98.4 |
| 43 | 1.5 | 9.9 | 0.9 | 3.6 | 1.4 | 10.5 | 1.5 | 6.6 | 1.8 | 12.6 | 2.0 | 10.4 | 0.7 | 5.2 | 1.6 | 9.9 | 1.8 | 7.2 | >1000 | >1000 | 1.3 | 10.9 | 6.3 | 14.5 | 2.5 | 10.6 |
| 53 | 9.7 | 45.7 | 7.4 | 42.4 | 6.6 | 47.5 | >1000 | >1000 | 7.7 | 36.0 | 9.3 | 43.0 | 18.7 | 109.8 | 20.0 | 126.2 | 10.0 | 41.1 | >1000 | >1000 | 168.3 | 875.2 | 34.3 | 100.7 | 8.4 | 39.9 |
| 60 | 5.8 | 35.4 | 4.4 | 25.8 | 5.1 | 46.1 | 412.7 | >1000 | 8.7 | 40.6 | 7.8 | 1.4 | 5.2 | 38.2 | 7.5 | 39.0 | 5.0 | 28.8 | 0.7 | 27.1 | 8.2 | 47.7 | 16.7 | 44.4 | 5.2 | 24.5 |
| 63 | 11 | 103.7 | 8.5 | 90.6 | 7.6 | 91.7 | 79.6 | >1000 | 18.9 | 222.4 | 14.4 | 1.7 | 14.4 | 158.5 | 382.1 | >1000 | 16 | 154.1 | 31.7 | 143.3 | 60.2 | 922.1 | >1000 | >1000 | 11.5 | 117.8 |
| 66 | 3.7 | 19.2 | 2.3 | 1.7 | 3.6 | 20.6 | 2.1 | 10.5 | 3.0 | 14.7 | 4.0 | 19.5 | 2.8 | 17.0 | 6.7 | 72.9 | 3.4 | 13.2 | 239.8 | 901.3 | 4.2 | 32.8 | 10.0 | 29.7 | 3.7 | 13.6 |
| 69 | 15.5 | 183.0 | 17.5 | 164.1 | 9.4 | 169.1 | 12.0 | 96.1 | >1000 | >1000 | >1000 | >1000 | 20.4 | 154.0 | 13.3 | 172.5 | 13.5 | 146.3 | 24.1 | 258.5 | 13.1 | 177.3 | 141.0 | 469.8 | 16.7 | 84.6 |
| C670 | 13.8 | 168.9 | 12.1 | 125.2 | 10.1 | 148.8 | 7.6 | 73.7 | >1000 | >1000 | 15.9 | 87.7 | 16.6 | 219.6 | 12.4 | 129.6 | 10.8 | 156.0 | 50.4 | 246.3 | 15.4 | 296.2 | >1000 | >1000 | 13.2 | 85.1 |
| C671 | 5.4 | 37.8 | 3.1 | 22.7 | 5.9 | 47.1 | 4.5 | 29.2 | 4.8 | 33.1 | 6.9 | 35.3 | 5.6 | 38.8 | 5.9 | 42.3 | 4.7 | 34.5 | >1000 | >1000 | 282.8 | >1000 | 27.7 | 84.4 | 5.1 | 24.3 |
| C675 | 19.5 | 111.6 | 12.3 | 92.9 | 15.0 | 129.3 | 11.3 | 76.8 | 17.2 | 78.9 | 22.7 | 105.5 | 18.1 | 140.4 | 38.3 | 225.3 | 15.0 | 91.5 | 23.9 | 78.1 | 13.7 | 110.8 | 71.3 | 190.9 | 13.3 | 82.3 |
| C682 | 45.3 | 196.0 | 29.4 | 191.9 | 25.9 | 199.6 | >1000 | >1000 | 23.3 | 132.0 | 43.2 | 215.0 | 12.4 | 85.3 | >1000 | >1000 | 62.3 | 367.1 | 110.4 | 478.6 | 214.3 | >1000 | 383.2 | >1000 | 28.0 | 232.8 |

Supplementary Table 6: Cryo-EM data collection and processing statistics

|  | $\begin{gathered} \text { C601 Fab } \\ \text { SARS-CoV-2 S 6P } \end{gathered}$ | $\begin{gathered} \text { C603 Fab } \\ \text { SARS-CoV-2 S 6P } \end{gathered}$ | $\begin{gathered} \text { C643 Fab } \\ \text { SARS-CoV-2 S 6P } \end{gathered}$ | $\begin{gathered} \text { C663 Fab } \\ \text { SARS-CoV-2 S 6P } \end{gathered}$ | $\begin{gathered} \text { C666 Fab } \\ \text { SARS-CoV-2 S 6P } \end{gathered}$ | $\begin{gathered} \text { C669 Fab } \\ \text { SARS-CoV-2 S 6P } \end{gathered}$ | $\begin{gathered} \text { C670 Fab } \\ \text { SARS-CoV-2 S 6P } \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| EMD | xxxx | xxxx | xxxx | xxxx | xxxx | xxxx | xxxx |
| Data collection conditions |  |  |  |  |  |  |  |
| Microscope | Talos Arctica | Talos Artica | Talos Artica | Talos Artica | Talos Arctica | Talos Artica | Talos Artica |
| Camera | Gatan K3 Summit | Gatan K3 Summit | Gatan K3 Summit | Gatan K3 Summit | Gatan K3 Summit | Gatan K3 Summit | Gatan K3 Summit |
| Magnification | 45,000x | 45,000x | 45,000x | 45,000x | 45,000x | 45,000x | 45,000x |
| Voltage (kV) | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Recording mode | counting | counting | counting | counting | counting | counting | counting |
| Dose rate (e/pixel/s) | 13.5 | 13.5 | 13.8 | 13.3 | 13.5 | 13.8 | 13.3 |
| Electron dose (e $/ 1 A^{2}$ ) | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Defocus range ( $\mu \mathrm{m}$ ) | 0.7-2.0 | 0.7-2.0 | 0.7-2.0 | 0.7-2.0 | 0.7-2.0 | 0.7-2.0 | 0.7-2.0 |
| Pixel size (A) | 0.8689 | 0.8689 | 0.8689 | 0.8689 | 0.8689 | 0.8689 | 0.8689 |
| Micrographs collected | 846 | 1,053 | 1,849 | 1,200 | 2,059 | 1,961 | 1,863 |
| Micrographs used | 796 | 1,053 | 1,487 | 1,005 | 1,975 | 1,720 | 1,863 |
| Total extracted particles | 187,627 | 490,663 | 340,563 | 268,485 | 256,500 | 265,364 | 596,506 |
| Refined particles | 101,271 | 57,573 | 117,987 | 108,407 | 127,936 | 129,917 | 140,171 |
| Particles in final refinement | 37,665 | 57,573 | 54,392 | 48,088 | 42,582 | 54,129 | 140,171 |
| Symmetry imposed | C1 | C1 | C1 | C1 | C1 | C1 | C1 |
| FSC=0.143 Resolution (A) | 6.5 | 6.4 | 5.0 | 8.3 | 5.1 | 4.9 | 6.9 |

## References

1 Gaebler, C. \& Nussenzweig, M. C. All eyes on a hurdle race for a SARS-CoV-2 vaccine. Nature 586, 501-502, doi:10.1038/d41586-020-02926-w (2020).
2 Krammer, F. SARS-CoV-2 vaccines in development. Nature 586, 516-527, doi:10.1038/s41586-020-2798-3 (2020).
3 Jackson, L. A. et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. $N$ Engl J Med 383, 1920-1931, doi:10.1056/NEJMoa2022483 (2020).
4 Polack, F. P. et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. $N$ Engl J Med 383, 2603-2615, doi:10.1056/NEJMoa2034577 (2020).
5 Walsh, E. E. et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. $N$ Engl J Med 383, 2439-2450, doi:10.1056/NEJMoa2027906 (2020).
6 Gaebler, C. et al. Evolution of Antibody Immunity to SARS-CoV-2. bioRxiv, doi:10.1101/2020.11.03.367391 (2020).
7 Robbiani, D. F. et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature 584, 437-442, doi:10.1038/s41586-020-2456-9 (2020).
8 Schmidt, F. et al. Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses. J Exp Med 217, doi:10.1084/jem. 20201181 (2020).
9 Widge, A. T. et al. Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. $N$ Engl J Med 384, 80-82, doi:10.1056/NEJMc2032195 (2021).
10 Weisblum, Y. et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. Elife 9, doi:10.7554/eLife. 61312 (2020).
11 Baum, A. et al. Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. Science 369, 1014-1018, doi:10.1126/science.abd0831 (2020).
12 Davies, N. G. et al. Estimated transmissibility and severity of novel SARS-CoV-2 Variant of Concern 202012/01 in England. medRxiv, 2020.2012.2024.20248822, doi:10.1101/2020.12.24.20248822 (2020).
13 Greaney, A. J. et al. Comprehensive mapping of mutations to the SARS-CoV-2 receptorbinding domain that affect recognition by polyclonal human serum antibodies. 588, 682635, doi:10.1101/2020.12.31.425021 (2021).
14 Greaney, A. J. et al. Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. Cell Host Microbe 29, 4457 e49, doi:10.1016/j.chom.2020.11.007 (2021).
15 Japan, N. I. o. I. D. Brief report: New Variant Strain of SARS-CoV-2 Identified in Travelers from Brazil. doi:https://www.niid.go.jp/niid/images/epi/corona/covid19-33-en210112.pdf (2021).

16 Lauring, A. S. \& Hodcroft, E. B. Genetic Variants of SARS-CoV-2-What Do They Mean? JAMA, doi:10.1001/jama.2020.27124 (2021).
17 Tegally, H. et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv, 2020.2012.2021.20248640, doi:10.1101/2020.12.21.20248640 (2020).

18 Briney, B., Inderbitzin, A., Joyce, C. \& Burton, D. R. Commonality despite exceptional diversity in the baseline human antibody repertoire. Nature 566, 393-397, doi:10.1038/s41586-019-0879-y (2019).

19 Andreano, E. et al. SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma. bioRxiv 5, 237-236, doi:10.1101/2020.12.28.424451 (2020).
20 Barnes, C. O. et al. SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. Nature 588, 682-687, doi:10.1038/s41586-020-2852-1 (2020).
21 Barnes, C. O. et al. Structures of Human Antibodies Bound to SARS-CoV-2 Spike Reveal Common Epitopes and Recurrent Features of Antibodies. Cell 182, 828-842 e816, doi:10.1016/j.cell.2020.06.025 (2020).
22 Thomson, E. C. et al. The circulating SARS-CoV-2 spike variant N439K maintains fitness while evading antibody-mediated immunity. bioRxiv, 2020.2011.2004.355842, doi:10.1101/2020.11.04.355842 (2020).
23 Tortorici, M. A. et al. Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. Science 370, 950-957, doi:10.1126/science.abe3354 (2020).

24 Starr, T. N. et al. Prospective mapping of viral mutations that escape antibodies used to treat COVID-19. bioRxiv, 2020.2011.2030.405472, doi:10.1101/2020.11.30.405472 (2020).

25 Lauring, A. S. \& Hodcroft, E. B. Genetic Variants of SARS-CoV-2-What Do They Mean? JAMA, 1-3, doi:10.1001/jama. 2020.27124 (2021).
26 Singer, J., Gifford, R., Cotten, M. \& Robertson, D. CoV-GLUE: A Web Application for Tracking SARS-CoV-2 Genomic Variation. Preprints, doi:10.20944/preprints202006.0225.v1 (2020).
27 Elbe, S. \& Buckland-Merrett, G. Data, disease and diplomacy: GISAID's innovative contribution to global health. Glob Chall 1, 33-46, doi:10.1002/gch2.1018 (2017).
28 Voloch, C. M. et al. Genomic characterization of a novel SARS-CoV-2 lineage from Rio de Janeiro, Brazil. medRxiv (2020).
29 Hsieh, C. L. et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science 369, 1501-1505, doi:10.1126/science.abd0826 (2020).
30 Brouwer, P. J. M. et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science 369, 643-650, doi:10.1126/science.abc5902 (2020).

31 Cao, Y. et al. Potent Neutralizing Antibodies against SARS-CoV-2 Identified by HighThroughput Single-Cell Sequencing of Convalescent Patients' B Cells. Cell 182, 73-84 e16, doi:10.1016/j.cell.2020.05.025 (2020).
$32 \mathrm{Ju}, \mathrm{B}$. et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature 584, 115-119, doi:10.1038/s41586-020-2380-z (2020).
33 Liu, L. et al. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. Nature 584, 450-456, doi:10.1038/s41586-020-2571-7 (2020).
34 Rogers, T. F. et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science 369, 956-963, doi:10.1126/science.abc7520 (2020).
35 Zost, S. J. et al. Potently neutralizing and protective human antibodies against SARS-CoV-2. Nature 584, 443-449, doi:10.1038/s41586-020-2548-6 (2020).
36 Schafer, A. et al. Antibody potency, effector function, and combinations in protection and therapy for SARS-CoV-2 infection in vivo. J Exp Med 218, doi:10.1084/jem. 20201993 (2021).

37 Mendoza, P. et al. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. Nature 561, 479-484, doi:10.1038/s41586-018-0531-2 (2018).
38 Bar-On, Y. et al. Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals. Nat Med 24, 1701-1707, doi:10.1038/s41591-018-0186-4 (2018).
39 Li, Q. et al. The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. Cell 182, 1284-1294 e1289, doi:10.1016/j.cell.2020.07.012 (2020).
40 Eguia, R. et al. A human coronavirus evolves antigenically to escape antibody immunity. bioRxiv 5, 52-28, doi:10.1101/2020.12.17.423313 (2020).
41 Amanat, F. et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med 26, 1033-1036, doi:10.1038/s41591-020-0913-5 (2020).
42 Grifoni, A. et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell 181, 1489-1501 e1415, doi:10.1016/j.cell.2020.05.015 (2020).
43 Cohen, A. A. et al. Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice. Science, doi:10.1126/science.abf6840 (2021).
44 Mastronarde, D. N. Automated electron microscope tomography using robust prediction of specimen movements. J Struct Biol 152, 36-51, doi:10.1016/j.jsb.2005.07.007 (2005).
45 Punjani, A., Rubinstein, J. L., Fleet, D. J. \& Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. Nat Methods 14, 290-296, doi:10.1038/nmeth. 4169 (2017).
46 Goddard, T. D. et al. UCSF ChimeraX: Meeting modern challenges in visualization and analysis. Protein Sci 27, 14-25, doi:10.1002/pro. 3235 (2018).
47 Terwilliger, T. C., Adams, P. D., Afonine, P. V. \& Sobolev, O. V. A fully automatic method yielding initial models from high-resolution cryo-electron microscopy maps. Nat Methods 15, 905-908, doi:10.1038/s41592-018-0173-1 (2018).
48 Emsley, P., Lohkamp, B., Scott, W. G. \& Cowtan, K. Features and development of Coot. Acta Crystallogr D Biol Crystallogr 66, 486-501, doi:10.1107/S0907444910007493 (2010).

49 Gupta, N. T. et al. Change-O: a toolkit for analyzing large-scale B cell immunoglobulin repertoire sequencing data. Bioinformatics 31, 3356-3358, doi:10.1093/bioinformatics/btv359 (2015).
50 Soto, C. et al. High frequency of shared clonotypes in human B cell receptor repertoires. Nature 566, 398-402, doi:10.1038/s41586-019-0934-8 (2019).
51 Guo, Y., Chen, K., Kwong, P. D., Shapiro, L. \& Sheng, Z. cAb-Rep: A Database of Curated Antibody Repertoires for Exploring Antibody Diversity and Predicting Antibody Prevalence. Front Immunol 10, 2365, doi:10.3389/fimmu.2019.02365 (2019).
52 Kyte, J. \& Doolittle, R. F. A simple method for displaying the hydropathic character of a protein. J Mol Biol 157, 105-132, doi:10.1016/0022-2836(82)90515-0 (1982).
53 Guy, H. R. Amino acid side-chain partition energies and distribution of residues in soluble proteins. Biophys $J$ 47, 61-70, doi:10.1016/S0006-3495(85)83877-7 (1985).
54 DeWitt, W. S. et al. A Public Database of Memory and Naive B-Cell Receptor Sequences. PLoS One 11, e0160853, doi:10.1371/journal.pone. 0160853 (2016).
55 Rubelt, F. et al. Onset of immune senescence defined by unbiased pyrosequencing of human immunoglobulin mRNA repertoires. PLoS One 7, e49774, doi:10.1371/journal.pone. 0049774 (2012).
bioRxiv preprint doi: https://doi.org/10.1101/2021.01.15.426911; this version posted January 19, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

