

## Accelerated Article Preview

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# SARS-CoV-2 RBD antibodies that maximize breadth and resistance to escape

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Tyler N. Starr<sup>1,15</sup>, Nadine Czudnochowski<sup>2,15</sup>, Zhuoming Liu<sup>3,15</sup>, Fabrizia Zatta<sup>4</sup>, Young-Jun Park<sup>5</sup>, Amin Addetia<sup>1</sup>, Dora Pinto<sup>4</sup>, Martina Beltramello<sup>4</sup>, Patrick Hernandez<sup>2</sup>, Allison J. Greaney<sup>1,6</sup>, Roberta Marzi<sup>4</sup>, William G. Glass<sup>7</sup>, Ivy Zhang<sup>7,8</sup>, Adam S. Dingens<sup>1</sup>, John E. Bowen<sup>5</sup>, M. Alejandra Tortorici<sup>5</sup>, Alexandra C. Walls<sup>5</sup>, Jason A. Wojcechowskyj<sup>2</sup>, Anna De Marco<sup>4</sup>, Laura E. Rosen<sup>2</sup>, Jiayi Zhou<sup>2</sup>, Martin Montiel-Ruiz<sup>2</sup>, Hannah Kaiser<sup>2</sup>, Josh Dillen<sup>2</sup>, Heather Tucker<sup>2</sup>, Jessica Bassi<sup>4</sup>, Chiara Silacci-Fregni<sup>4</sup>, Michael P. Housley<sup>2</sup>, Julia di Iulio<sup>2</sup>, Gloria Lombardo<sup>4</sup>, Maria Agostini<sup>2</sup>, Nicole Sprugasci<sup>4</sup>, Katja Culap<sup>4</sup>, Stefano Jaconi<sup>4</sup>, Marcel Meury<sup>2</sup>, Exequiel Dellota<sup>2</sup>, Rana Abdelnabi<sup>9</sup>, Shi-Yan Caroline Foo<sup>9</sup>, Elisabetta Cameroni<sup>4</sup>, Spencer Stumpf<sup>3</sup>, Tristan I. Croll<sup>10</sup>, Jay C. Nix<sup>11</sup>, Colin Havenar-Daughton<sup>2</sup>, Luca Piccoli<sup>4</sup>, Fabio Benigni<sup>4</sup>, Johan Neyts<sup>9</sup>, Amalio Telenti<sup>2</sup>, Florian A. Lempp<sup>2</sup>, Matteo S. Pizzuto<sup>4</sup>, John D. Chodera<sup>7</sup>, Christy M. Hebner<sup>2</sup>, Herbert W. Virgin<sup>2,12,13</sup>, Sean P. J. Whelan<sup>3</sup>, David Veesler<sup>5</sup>, Davide Corti<sup>4,14</sup>, Jesse D. Bloom<sup>1,6,14</sup> & Gyorgy Snell<sup>2,15</sup>

An ideal anti-SARS-CoV-2 antibody would resist viral escape<sup>1–3</sup>, have activity against diverse SARS-related coronaviruses (sarbecoviruses)<sup>4–7</sup>, and be highly protective through viral neutralization<sup>8–11</sup> and effector functions<sup>12,13</sup>. Understanding how these properties relate to each other and vary across epitopes would aid development of antibody therapeutics and guide vaccine design. Here, we comprehensively characterize escape, breadth, and potency across a panel of SARS-CoV-2 antibodies targeting the receptor-binding domain (RBD). Despite a tradeoff between *in vitro* neutralization potency and breadth of sarbecovirus binding, we identify neutralizing antibodies with exceptional sarbecovirus breadth and a corresponding resistance to SARS-CoV-2 escape. One of these antibodies, S2H97, binds with high affinity across all sarbecovirus clades to a previously undescribed cryptic epitope and prophylactically protects hamsters from viral challenge. Antibodies targeting the ACE2 receptor binding motif (RBM) typically have poor breadth and are readily escaped by mutations despite high neutralization potency. Nevertheless, we characterize one potent RBM antibody (S2E12<sup>8</sup>) with breadth across sarbecoviruses related to SARS-CoV-2 and a high barrier to viral escape. These data highlight principles underlying variation in escape, breadth, and potency among antibodies targeting the RBD, and identify epitopes and features to prioritize for therapeutic development against the current and potential future pandemics.

The most potently neutralizing antibodies to SARS-CoV-2—including those in clinical use<sup>14</sup> and dominant in polyclonal sera<sup>15,16</sup>—target the spike receptor-binding domain (RBD). Mutations in the RBD that reduce binding by antibodies have emerged among SARS-CoV-2 variants<sup>17–21</sup>, highlighting the need for antibodies and vaccines that are robust to viral escape. We have previously described an antibody, S309<sup>9</sup>, that exhibits its potent effector functions and neutralizes all current SARS-CoV-2

variants<sup>22,23</sup> and the divergent sarbecovirus SARS-CoV-1. S309 forms the basis for an antibody therapy (VIR-7831, recently renamed sotrovimab) that has received Emergency Use Authorization from the FDA for treatment of COVID-19<sup>24</sup>. Longer term, antibodies with broad activity across SARS-related coronaviruses (sarbecoviruses) would be useful to combat potential future spillovers<sup>6</sup>. These efforts would be aided by a systematic understanding of the relationships among antibody epitope,

<sup>1</sup>Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, 98109, USA. <sup>2</sup>Vir Biotechnology, San Francisco, CA, 94158, USA. <sup>3</sup>Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, 63110, USA. <sup>4</sup>Humabs BioMed SA, a subsidiary of Vir Biotechnology, 6500, Bellinzona, Switzerland. <sup>5</sup>Department of Biochemistry, University of Washington, Seattle, WA, 98195, USA. <sup>6</sup>Department of Genome Sciences, University of Washington, Seattle, WA, 98195, USA. <sup>7</sup>Computational and Systems Biology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA. <sup>8</sup>Tri-Institutional PhD Program in Computational Biology and Medicine, Weill Cornell Graduate School of Medical Sciences, New York, NY, 10065, USA. <sup>9</sup>Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, KU Leuven, Leuven, Belgium. <sup>10</sup>Cambridge Institute for Medical Research, Department of Haematology, University of Cambridge, Cambridge, CB2 0XY, UK. <sup>11</sup>Molecular Biology Consortium, Advanced Light Source, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA. <sup>12</sup>Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis, MO, 63110, USA. <sup>13</sup>Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX, 75390, USA. <sup>14</sup>Howard Hughes Medical Institute, Seattle, WA, 98109, USA. <sup>15</sup>These authors contributed equally: Tyler N. Starr, Nadine Czudnochowski, Zhuoming Liu. ✉e-mail: dcorti@vir.bio; jlbloom@fredhutch.org; gsnell@vir.bio

resistance to viral escape, and breadth of sarbecovirus cross-reactivity. Here we address this question by comprehensively characterizing a diverse panel of antibodies, including S309, using deep mutational scanning, pan-sarbecovirus binding assays, *in vitro* selection of viral escape, and biochemical and structural analyses.

### Potency, escapability, and breadth in a panel of RBD antibodies

We identified a panel of anti-SARS-CoV-2 antibodies with distinct properties (Fig. 1a, Extended Data Table 1), including six antibodies newly described in this study. These antibodies bind different epitopes within the receptor-binding motif (RBM) and the non-RBM “core” of the RBD. The antibody panel spans a range of neutralization potencies and binding affinities (Fig. 1a, Extended Data Fig. 1a-c).

We used deep mutational scanning to map how all amino-acid mutations in the SARS-CoV-2 RBD affect binding by each antibody<sup>3</sup> (Fig. 1b,c and Extended Data Fig. 2). Some antibodies have narrowly focused functional epitopes (the set of residues where mutations abolish binding<sup>25</sup>), with binding-escape mutations at just a few key residues (e.g., S309, S2D106), while other antibodies have wider functional epitopes (e.g., S2H13; tabulations at right in Fig. 1b,c). We previously measured how all RBD mutations affect folded RBD expression and ACE2 binding affinity<sup>26</sup> (letter colors in Fig. 1b,c). We used the combined measures of how mutations affect antibody binding and RBD function to compute the “escapability” of each antibody, which reflects the extent to which mutations that escape antibody binding are functionally tolerated (Fig. 1b,c and Extended Data Fig. 3a,b). We also investigated the sensitivity of each antibody to mutations among SARS-CoV-2 sequences reported in GISAID (heatmap below logoplots in Fig. 1b,c; Extended Data Fig. 3c), and found that some antibodies are more affected by natural SARS-CoV-2 mutations than others, including mutations found in SARS-CoV-2 variants of concern (Extended Data Fig. 1d)<sup>27-29</sup>.

We next extended our deep mutational scanning platform to measure binding of each antibody to a pan-sarbecovirus panel of 45 RBDs (Fig. 1d and Extended Data Fig. 4a-f). The four antibodies that bind the core RBD exhibit cross-reactive binding to RBDs from SARS-CoV-1 and related ACE2-utilizing bat sarbecoviruses, and from sarbecoviruses isolated in Europe and Africa. Antibodies S304 and S2H97 also bind RBDs of the most divergent clade from Asia that have an average 64% amino acid identity with SARS-CoV-2. S2H97 exhibits notably tight binding to all RBDs tested (Fig. 1d and Extended Data Fig. 4f), making it the broadest pan-sarbecovirus RBD antibody described to date. Antibodies that bind epitopes within the RBM exhibit more limited cross-reactivity, typically binding only SARS-CoV-2 and the closely related GD-Pangolin-CoV RBD. S2E12 stands out among the RBM antibodies we evaluated as it also binds the RaTG13 and GX-Pangolin-CoV RBDs, showing that even within the evolutionarily plastic RBM<sup>19,26</sup> there are epitopes that enable greater breadth than others.

### The pan-sarbecovirus S2H97 antibody

To understand the structural basis for cross-reactive sarbecovirus binding, we determined the structures of S2H97 Fab (X-ray crystallography, 2.65 Å resolution), S2X35 Fab (X-ray crystallography, 1.83 Å resolution), and S2E12 Fab (X-ray crystallography, 2.95 Å resolution) bound to SARS-CoV-2 RBD (Fig. 2a and Extended Data Table 2). This panel of cross-reactive antibodies emphasizes the core RBD as a general target of broad antibody binding due to its conservation among sarbecoviruses, reflected in the diverse core RBD surfaces targeted by the broadest of these antibodies (Fig. 2a and Extended Data Fig. 5a-f).

The exceptionally cross-reactive S2H97 antibody targets a previously undescribed cryptic antigenic site, which we designated site V (Fig. 2a,b). S2H97 binding is facilitated by packing of the heavy chain CDR3 into an RBD crevice at the center of the epitope, together with

polar contacts with all three heavy chain CDRs and the light chain CDR2 (Extended Data Fig. 5f). Molecular dynamics simulation of the S2H97 Fab:RBD complex highlights the durability of many of these interactions (Fig. 2b). The surface bound by S2H97 is constrained by the deleterious effects of mutations on folded RBD expression (Fig. 2b)<sup>26</sup>, and this constraint is likely enhanced by quaternary packing with the NTD in the closed spike trimer (Extended Data Fig. 6a). Consistent with the conservation of the S2H97 epitope, S2H97 neutralizes diverse sarbecoviruses (Fig. 2c and Extended Data Fig. 4g) and SARS-CoV-2 variants (Fig. 2d).

To understand the evolution of S2H97 breadth, we measured breadth of binding by its germline form, S2H97<sub>GL</sub>, in which we reverted the 13 somatic mutations (Extended Data Fig. 4h,i). S2H97<sub>GL</sub> bound all tested sarbecovirus RBDs and exhibited particularly high affinity for SARS-CoV-2-related RBDs. Somatic mutations enhanced affinity across all sarbecoviruses by two orders of magnitude. This general increase in affinity together with the absence of non-conservative amino acid replacements among paratope residues suggests that framework mutations may contribute to a general improvement in S2H97 binding affinity.

To characterize the mechanism of S2H97 neutralization, we determined a cryoEM structure of S2H97 bound to SARS-CoV-2S (Extended Data Fig. 5i-l and Extended Data Table 3). S2H97 binding requires extensive opening of the RBD to unmask its cognate epitope (Extended Data Fig. 6b), even more than is required to access the cryptic antigenic site II<sup>15</sup>. Like other antibodies that only bind the open RBD<sup>30,31</sup>, S2H97 induces rapid and premature refolding of spike into the post-fusion state (Fig. 2e), promotes S1 shedding of cell-surface-expressed spike (Extended Data Fig. 6c), and induces a low level of syncytia formation among spike-expressing cells (Extended Data Fig. 6d). S2H97 does not interfere with ACE2 binding (Extended Data Fig. 6e). Like other non-ACE2-competitive antibodies<sup>31,32</sup>, S2H97 neutralization is attenuated in cells that over-express ACE2 (Extended Data Fig. 6f). Consistent with its ability to neutralize spike-mediated viral entry, S2H97 inhibits spike-mediated cell-cell fusion (Extended Data Fig. 6g). Taken together, these experiments suggest that the S2H97 mechanism of neutralization involves receptor-independent conversion of S to the post-fusion state<sup>30</sup>, thereby inhibiting ACE2-mediated cell entry.

Next, we determined the prophylactic efficacy of S2H97 *in vivo* using a Syrian hamster model of infection. We administered hamsters with S2H97 at 25 mg/kg two days prior to intranasal challenge with SARS-CoV-2 and assessed viral RNA load and infectious viral titers in the lungs four days post-infection. S2H97 prophylaxis reduced RNA copies by >10,000-fold relative to control in the four animals that had detectable circulating antibody levels at the time of challenge and reduced infectious viral titers to the lower detection limit in these animals (Fig. 2f). The two animals without a reduction in viral load had circulating S2H97 levels below the limit of quantification (50 ng/ml) at the time of viral challenge (Extended Data Fig. 6h), which may reflect a failure in the intraperitoneal administration procedure. Therefore, S2H97 demonstrates that antibodies to the newly identified antigenic site V can be protective *in vivo*.

Last, we performed serum blockade of binding experiments<sup>15</sup>, demonstrating that antibodies competing with S2H97 binding are rare in infection- and vaccine-elicited sera (Fig. 2g). This sub-dominance of antigenic site V may be explained by the inaccessibility of the epitope as illustrated in the cryoEM structure. However, the protective nature and exceptional breadth of S2H97 suggests that updated immunogen designs, such as those based on the RBD<sup>33-35</sup>, could unmask antigenic site V to better elicit S2H97-like antibodies.

### Variation in breadth and escapability among RBM epitopes

Our survey reveals variation in the escapability and breadth of antibodies that target the RBM (Fig. 1c,d), which is immunodominant (Fig. 2g)

but variable over sarbecovirus and SARS-CoV-2 evolution. We performed *in vitro* selection experiments to identify spike-expressing VSV mutants that emerge in the presence of each of seven monoclonal antibodies (Fig. 3a and Extended Data Fig. 7a,b) to further understand escape from these antibodies.

Many RBM antibodies such as S2X58 and S2D106 select mutations present in SARS-CoV-2 variants of concern (e.g., L452R and E484K)<sup>27–29</sup>. In contrast, S2E12 selects viral mutants at sites that do not exhibit substantial variation among circulating SARS-CoV-2, and S2E12 correspondingly neutralizes a diverse panel of SARS-CoV-2 variants (Fig. 3b)<sup>17</sup>. S2E12 is also unique in its breadth among RBM antibodies (Fig. 1d), neutralizing VSV pseudotyped with each of the four SARS-CoV-2 clade sarbecovirus spikes (Fig. 3c and Extended Data Fig. 4j). As with S2H97, somatic mutations in S2E12 enhanced affinity across sarbecoviruses, though the increase in affinity was more modest than for S2H97 (Extended Data Fig. 4k,l).

Conservation of the S2E12 epitope among SARS-CoV-2 variants could reflect the relative rarity of S2E12-like antibodies in polyclonal sera leading to little antigenic pressure at these sites (Fig. 2g), together with functional constraint in the S2E12 epitope (escapability being the lowest for S2E12 and S2H97 among the 12 antibodies evaluated). Indeed, the strong antibody-escape mutations that emerged in S2E12 viral escape selections decrease ACE2 binding affinity (Fig. 3a)<sup>26</sup> and reduce replicative fitness in a bulk competition experiment between spike-expressing VSV variants passaged in the absence of antibody (Fig. 3d).

To understand the structural basis for the unique breadth and robustness of S2E12 to escape, we compared its structure to that of S2D106 Fab (cryoEM, 4.0 Å resolution local refinement) bound to SARS-CoV-2 RBD (Fig. 3e,f, Extended Data Fig. 5g,h,m-p and Extended Data Tables 2, 3). We also integrated evolutionary, functional, and structural details for the sites in each antibody's structural footprint (Fig. 3g,h). S2E12 and S2D106 bind the receptor-binding ridge, with 8 residues shared between their footprints. S2E12 binding is oriented toward extensive packing of the ACE2-contact residue F486<sub>RBD</sub> within a cavity lined by aromatic residues at the antibody light/heavy-chain interface (Fig. 3e and Extended Data Fig. 5g), as was seen with the homologous antibody COV2-2196<sup>36</sup>. Sites within the S2E12 footprint that exhibit less functional constraint (e.g., E484, S477) are located at the periphery of the interface, explaining the robustness of S2E12 toward SARS-CoV-2 variants (Fig. 3b,g). This structural interface also explains the breadth of S2E12 toward RaTG13 and GX-Pangolin-CoV (Fig. 1d), as the F486L mutation present in these sarbecoviruses retains the central hydrophobic packing.

In contrast to S2E12, S2D106 binding is centered on residue E484<sub>RBD</sub> which may form a salt bridge with R96<sub>LC</sub>, in addition to nonpolar contacts between F490<sub>RBD</sub> and residues in the heavy chain CDR2 (Fig. 3f and Extended Data Fig. 5h). Although the long heavy chain CDR3 packs intimately across the surface of the RBD, there are no crucial CDRH3:RBD contacts that are sensitive to mutation. S2D106 escape is therefore highly focused on E484 and F490, which are functionally tolerant and exhibit variation among SARS-CoV-2 sequences (Fig. 3h). This comparison between S2E12 and S2D106 highlights how small differences in the RBD:antibody interface impact the breadth and robustness of each antibody to viral escape.

## The landscape of RBD epitopes

Last, we examined how escapability, breadth, and neutralization potency relate to one another and to RBD epitope. We used our binding-escape maps (Fig. 1b,c), together with comparable maps published for other RBD antibodies<sup>3,20,21,36,37</sup>, to project antibodies into a two-dimensional space based on similarities in sites of binding-escape mutations (Fig. 4a).

We annotated our projection of epitope space by antibody properties such as *in vitro* neutralization potency, breadth, and escapability

(Fig. 4b–d and Extended Data Fig. 7c,d). The most potently neutralizing antibodies (e.g., S2E12, S2D106) bind epitopes in the RBM, while antibodies targeting the core RBD are less potently neutralizing (Fig. 4b). It is important to note that RBD antibodies can protect *in vivo* through other mechanisms beyond neutralization<sup>12,13,22</sup>. Antibodies with broad sarbecovirus binding target the core RBD (Fig. 4c). Our panel therefore extends prior observations<sup>4,5,32,38</sup> to highlight a general tradeoff between sarbecovirus breadth and potency of SARS-CoV-2 neutralization (Fig. 4e). Nonetheless, some cross-reactive antibodies exhibit intermediate *in vitro* neutralization potency (e.g., S309, S2X259<sup>37</sup>), and the highly potent RBM-directed antibody S2E12 exhibits modest breadth, highlighting the existence of antibodies that balance neutralization potency and breadth.

The size of an antibody's functional epitope (Fig. 1b,c) is not strongly influenced by the epitope's structural location (Extended Data Fig. 7c)—instead, narrower functional epitopes are associated with higher Fab:RBD binding affinity (Fig. 4f). However, an antibody's escapability, which integrates how escape mutations affect RBD folding and ACE2 affinity, is influenced by variation in these functional constraints across the RBD structure. For example, antibodies that cluster with S2E12 exhibit lower escapability (Extended Data Fig. 7c) and frequency of natural SARS-CoV-2 escape mutants (Fig. 4d). As highlighted by S2E12 and S2H97 above, antibodies with a modest degree of sarbecovirus breadth show a greatly reduced frequency of escape mutations among circulating SARS-CoV-2 variants (Fig. 4g).

## Principles for optimizing antibody and vaccine development

Ongoing SARS-CoV-2 evolution<sup>19,27–29</sup>, long-term antigenic evolution of other human coronaviruses<sup>39,40</sup>, and the spillover potential of diverse sarbecovirus lineages<sup>6,7</sup> indicate the importance of developing antibodies and vaccines that are robust to viral evolution. In this work, we identify antibody and epitope features which can guide this process. Although *in vitro* neutralization potency is often prioritized for lead selection, our results suggest this will bias antibodies toward RBM epitopes, many of which are poorly conserved in the short-term evolution of SARS-CoV-2<sup>19</sup> and the long-term evolution of sarbecoviruses<sup>7</sup>. Our results suggest that additional prioritization of high affinity binding and at least a moderate degree of sarbecovirus breadth will yield antibodies with improved resistance to viral escape<sup>4,5</sup>.

A long-term goal is to develop antibodies and vaccines that cross-react with distant sarbecovirus lineages capable of zoonotic spillover. We have identified a cryptic epitope capable of eliciting pan-sarbecovirus immunity, represented by S2H97. Though S2H97-like antibodies are rare in polyclonal sera, the protective capacity and exceptional breadth of S2H97 indicates that pan-sarbecovirus vaccines could seek to improve responses to this epitope by unmasking this and other cryptic broadly neutralizing epitopes<sup>5,37,41</sup>. Broader cross-reactivity among betacoronavirus lineages including MERS and OC43 has been reported for antibodies that bind the spike S2 domain<sup>32,38,42</sup>. Though S2H97 breadth does not extend beyond sarbecoviruses, its discovery expands our view of what can be achieved via a potent RBD-directed antibody response.

The global emergence of variants of concern (VOC) has been an important feature of the pandemic<sup>27–29</sup>. Mutations in VOC occur in immunodominant RBM epitopes (e.g., residues E484, K417 and L452) and impact binding by polyclonal serum and some therapeutic antibodies<sup>17–21</sup>. We cannot predict which mutations will next rise to prominence as SARS-CoV-2 continues to evolve, but it seems likely that they will include additional RBM mutations that impact recognition by infection- and vaccine-elicited antibodies<sup>1,2,15,16,19</sup>. Therefore, antibody discovery efforts focused on breadth<sup>4,5</sup>, aided by high-resolution differentiation among antibody epitopes as generated herein, can inform the development of antibody and vaccine countermeasures with greater robustness

to immune escape in the current SARS-CoV-2 pandemic and utility for potential future sarbecovirus spillovers.

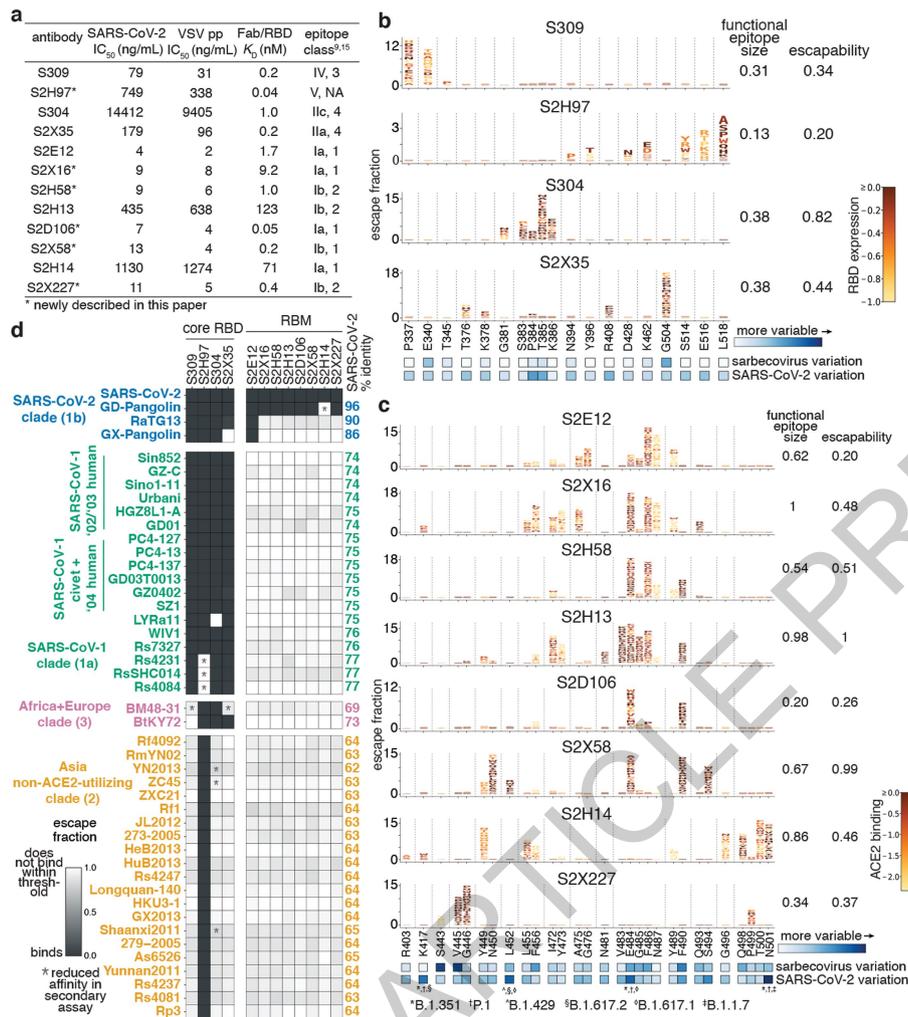
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Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-021-03807-6>.

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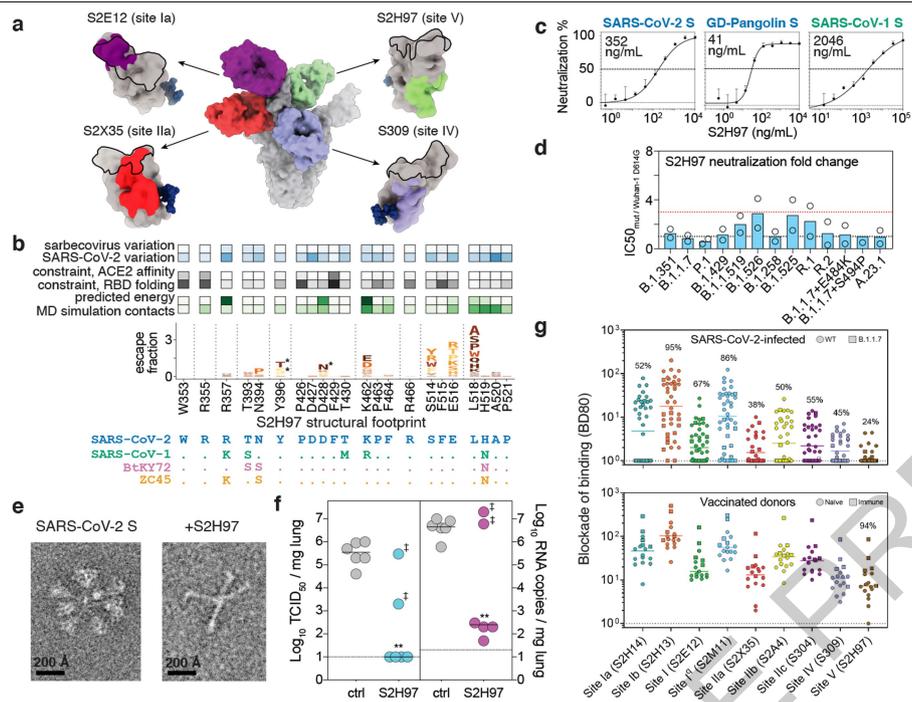
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**Fig. 1 | Potency, escapability, and breadth of a panel of RBD antibodies.**

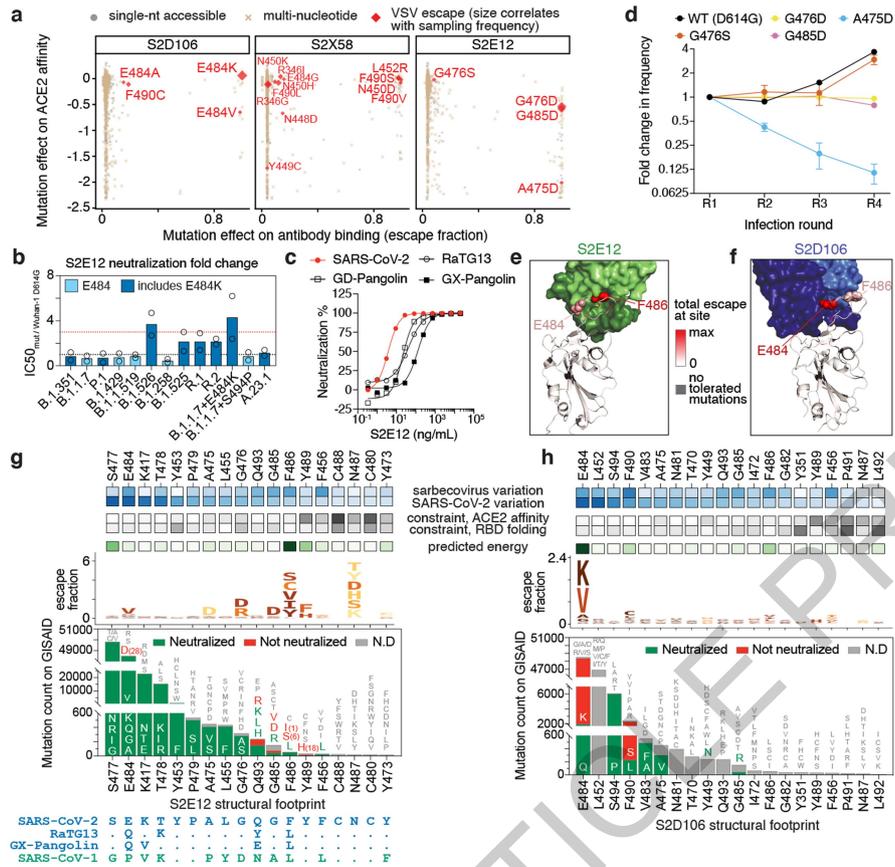
**a**, SARS-CoV-2 neutralization potency (authentic virus [ $n=3$ ] and spike-pseudotyped VSV particles [ $n=3$  to 8] on Vero E6 cells), Fab:RBD binding affinities measured by SPR [ $n=2$  to 4], and epitope classifications. Additional details in Extended Data Table 1. **b, c**, Deep mutational scanning maps of mutations that escape binding by antibodies targeting the core RBD (**b**) or the RBM (**c**). Letter height indicates that mutation's strength of escape from antibody binding. Letters colored by effect on folded RBD expression (**b**) or

ACE2 binding affinity (**c**)<sup>26</sup>. Relative "functional epitope size" and "escapability" are tabulated at right, scaling from 0 (no escape mutations) to 1 (largest epitope/most escapable antibody). Heatmaps, bottom, illustrate variability among sarbecoviruses or SARS-CoV-2 sequences. **d**, Antibody binding to a pan-sarbecovirus RBD panel. Heatmap illustrates binding from FACS-based selections (scale bar, bottom left). Asterisks, reduced-affinity binding in secondary binding assays (Extended Data Fig. 4a-f).



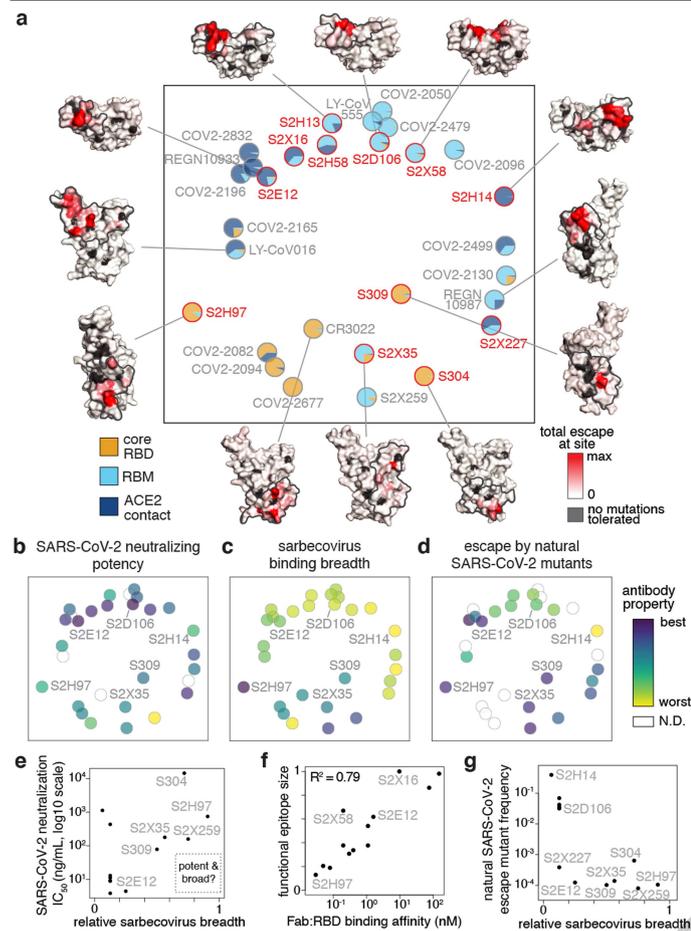
**Fig. 2 | The pan-sarbecovirus S2H97 antibody.** **a**, Composite model of the SARS-CoV-2 trimer with cross-reactive antibodies. Epitopes recognized by each Fab are shown as colored surface and ACE2 footprint as a black outline. **b**, Integrative features of the S2H97 structural footprint (5 Å cutoff). Heatmaps illustrate evolutionary variability (blue), functional constraint from prior deep mutational scans (gray), and energetic contribution to binding from the static crystal structure (predicted energy) or molecular dynamics simulation (green). Logoplot as in Fig. 1b. Asterisk, introduction of N-linked glycosylation motifs. **c**, S2H97 breadth of neutralization (spike-pseudotyped VSV on Vero E6 cells). Curves representative of at least two independent experiments. Points represent means, error bars standard deviation from three technical replicates, and IC50 geometric mean of experiments. **d**, S2H97 neutralization of SARS-CoV-2 variants (Extended Data Fig. 1d; spike-pseudotyped VSV on Vero

E6 cells). Points represent individual measurements and bar mean fold-change in neutralization potency. **e**, Negative stain EM imaging of native-like soluble prefusion S trimer (left) alone or incubated with S2H97 (right). Micrographs representative of 51 (SARS-CoV-2 S) and 173 (+S2H97) micrographs. **f**, S2H97 prophylactic efficacy in Syrian hamsters. Infectious virus titers (left) and RNA levels (right) measured in hamsters four days after SARS-CoV-2 challenge in animals prophylactically dosed with 25 mg/kg S2H97 or isotype control. Two animals with undetectable S2H97 levels (<50 ng/mL) at the time of viral challenge are marked by ‡. \*\* p=0.0048 (virus titer) and p=0.0048 (RNA) vs control, two-sided Mann-Whitney test (animals with no detectable serum antibody excluded). **g**, Blockade of binding<sup>15</sup> by sera from SARS-CoV-2-infected (top) or vaccinated (bottom) individuals. Percentage of samples with blockade above the lower detection limit are indicated.



**Fig. 3 | Breadth and escapability among RBM antibodies.** **a**, Escape mutations in spike-expressing VSV passaged in the presence of monoclonal antibody. Plot shows mutation effects on antibody (x-axis) and ACE2 (y-axis) binding. **b**, Neutralization of SARS-CoV-2 variants by S2E12 (spike-pseudotyped VSV on Vero E6 cells), as in Fig. 2d. **c**, S2E12 breadth of neutralization (spike-pseudotyped VSV on 293T-ACE2 cells). Points represent mean of biological duplicates. **d**, Replicative fitness of S2E12 escape mutations identified in (a) on Vero E6 cells. Points represent mean and error bars standard error from triplicate experiments. **e, f**, Structures of S2E12 Fab (e) and S2D106 Fab (f) bound to SARS-CoV-2 RBD. RBD sites colored by escape (scale bar,

center). The E484 side chain is included for visualization purposes only but was not included in the final S2D106-bound structure due to weak density. **g, h**, Integrative features of the structural footprints (5 Å cutoff) of S2E12 (g) and S2D106 (h). Sites are ordered by the frequency of observed mutants among SARS-CoV-2 sequences on GISAID. Heatmaps as in Fig. 2b. Logoplots as in Fig. 1c, but only showing amino acid mutations accessible via single-nucleotide mutation from Wuhan-Hu-1 for comparison with (a). Barplots illustrate frequency of SARS-CoV-2 mutants and their validated effects on antibody neutralization (spike-pseudotyped VSV on Vero E6 cells). Red, >10-fold increase in IC50 due to mutation.



**Fig. 4 | Antibody epitope, potency, breadth, and escapability.**

**a**, Multidimensional scaling projection of similarities in antibody binding-escape maps from this (red) and prior (gray) studies. Pie charts illustrate the RBD sub-domains where mutations confer escape (bottom left). Structural projections of escape arrayed around the perimeter (scale bar, bottom right), with gray outlines tracing structural footprints. **b-d**, Projected epitope space from (a) annotated by antibody properties. For each property, antibodies are colored such that purple reflects the most desirable antibody (scale bar, right): most potent neutralization (log10 scale), highest breadth, and lowest natural frequency of escape mutants (log10 scale). **e**, Relationship between SARS-CoV-2 neutralization potency and sarbecovirus breadth for antibodies described in this work and in a parallel study (S2X259)<sup>37</sup>. **f**, Relationship between functional epitope size and SARS-CoV-2 RBD binding affinity. **g**, Relationship between natural SARS-CoV-2 escape mutant frequency (Extended Data Fig. 3c) and sarbecovirus breadth.

## Methods

### Mammalian cell lines

Cell lines were received from ATCC (Vero E6, Vero, BHK-21, CHO-K1, HEK293T/17), Takara (Lenti-X 293T) and Thermo Fisher Scientific (ExpicoHO-S, Expi293F and Freestyle 293-F). MA104 cells were a gift from Harry Greenberg. 293T-ACE2 cells are described in references<sup>31</sup> and<sup>43</sup>. Vero and MA104 cell lines tested negative for mycoplasma contamination. Other cell lines were not tested. No authentication was performed beyond manufacturer standards.

### Isolation of peripheral blood mononuclear cells (PBMCs), plasma and sera

Samples from three SARS-CoV-2 recovered individuals, designated as donors S2H (age 36, male), S2D (age 70, male) and S2X (age 52, male) were obtained under study protocols approved by the local Institutional Review Board (Canton Ticino Ethics Committee, Switzerland). All donors provided written informed consent for the use of blood and blood components (such as PBMCs, sera or plasma). Blood drawn from donor S2X was obtained at day 48 (S2X16, S2X35 and S2X58 antibodies) and 75 (S2X227) after symptoms onset. Blood from donor S2H was obtained at day 17 (S2H13 and S2H14), day 45 (S2H58) and day 81 (S2H97) after symptoms onset. Blood from donor S2D was obtained at day 98 (S2D106) after symptoms onset.

PBMCs were isolated from blood draw performed using tubes pre-filled with heparin, followed by Ficoll density gradient centrifugation. PBMCs were either used fresh for SARS-CoV-2 Spike protein-specific memory B cell sorting or stored in liquid nitrogen for later use. Sera were obtained from blood collected using tubes containing clot activator, followed by centrifugation and storage at -80 °C.

Sera for blockade of binding serological assays were obtained from 3 cohorts of SARS-CoV-2 convalescent (average age 52, range 25–78, 55% male) or vaccinated (average age 49, range 28–69, 65% male) individuals under study protocols approved by the local Institutional Review Boards (Canton Ticino Ethics Committee, Switzerland, the Ethical Committee of Luigi Sacco Hospital, Milan, Italy, and WCG North America, Princeton, NJ, USA). All donors provided written informed consent for the use of blood and blood components (such as PBMCs, sera or plasma) and were recruited at hospitals or as outpatients.

### B-cell isolation and recombinant mAb production

Discovery and initial characterization of six antibodies in our panel was previously reported (S309 and S304<sup>4,15</sup>, S2X35, S2H13 and S2H14<sup>15</sup>, and S2E12<sup>8</sup>), and six new antibodies are first described here (S2H97, S2X16, S2H58, S2D106, S2X58, S2X227). Starting from freshly isolated PBMCs or upon cells thawing, B cells were enriched by staining with CD19 PE-Cy7 (BD Bioscience 557835, 1:50) and incubation with anti-PE MicroBeads (Miltenyi Biotec 130-048-801, 1:100), followed by positive selection using LS columns. Enriched B cells were stained with anti-IgM (BioLegend 314508, 1:20), anti-IgD (BD Bioscience 555779, 1:40), anti-CD14 (BD Bioscience 562691, 1:50) and anti-IgA (Southern Biotech 2050-09, 1:400), all PE labeled, and perfusion SARS-CoV-2S with a biotinylated Avi-tag (in house produced) conjugated to Streptavidin Alexa-Fluor 647 (Life Technologies S21374, 1:40). SARS-CoV-2S-specific IgG<sup>+</sup> memory B cells were sorted by flow cytometry via gating for PE negative and Alexa-Fluor 647 positive cells. Cells were cultured for the screening of positive supernatants. Antibody VH and VL sequences were obtained by RT-PCR and mAbs were expressed as recombinant human Fab fragment or as IgG1 (G1m3 allotype) carrying the half-life extending M428L/N434S (LS) mutation in the Fc region. ExpicoHO-S cells (Thermo Fisher Scientific) were transiently transfected with heavy and light chain expression vectors as previously described<sup>4</sup>. Affinity purification was performed on ÄKTA Xpress FPLC (Cytiva) operated by UNICORN software version 5.11 (Build 407) using HiTrap Protein A columns (Cytiva) for full length human mAbs and CaptureSelect

CHI-XL MiniChrom columns (Thermo Fisher Scientific) for Fab fragments, using PBS as mobile phase. Buffer exchange to the appropriate formulation buffer was performed with a HiTrap Fast desalting column (Cytiva). The final products were sterilized by filtration through 0.22 µm filters and stored at 4 °C.

Using the Database IMG (http://www.imgt.org), the VH and VL germline gene family and the number of somatic mutations were determined by analyzing the homology of the VH and VL sequences to known human V, D and J genes. Germline-reverted sequences of the VH and VL were constructed using IMG/V-QUEST. The S2E12 and S2H97 germline-reverted antibodies (G1m17 allotype) were produced by ATUM. S2E12 and S2H97 germline-reverted Fabs were generated by digestion of the corresponding IgGs.

Epitope classes shown in Figs. 1a and 2g are defined as in Piccoli et al.<sup>15</sup> Briefly, the classification of these epitope classes results from Octet binning experiments using structurally characterized antibodies, structural insights to define the recognition of open-only RBD and ability of antibodies to interfere with RBD binding to ACE2. In particular, site Ia is accessible only in the open state of RBD and largely overlaps with ACE2 footprint; site Ib is accessible in both open and closed RBD states and overlaps in part with ACE2 footprint; site IIa is in the core RBD (accessible only in the open RBD state) and antibodies binding to this site interfere with binding to ACE2, site IIc is also in the core RBD but targeted by antibodies that do not interfere with binding to ACE2; site IV is fully accessible on both open and closed RBDs and is defined by the footprint of S309 antibody.

### Neutralization of authentic SARS-CoV-2 by entry-inhibition assay

Neutralization was determined using SARS-CoV-2-Nluc, an infectious clone of SARS-CoV-2 (based on strain 2019-nCoV/USA\_WA1/2020) which encodes nanoluciferase in place of the viral ORF7 and demonstrated comparable growth kinetics to wildtype virus<sup>44</sup>. Vero E6 cells (ATCC, CRL-1586) were seeded into black-walled, clear-bottom 96-well plates at  $2 \times 10^4$  cells/well and cultured overnight at 37 °C. The next day, 9-point 4-fold serial dilutions of mAbs were prepared in infection media (DMEM + 10% FBS). SARS-CoV-2-Nluc was diluted in infection media at a final MOI of 0.01 PFU/cell, added to the mAb dilutions and incubated for 30 minutes at 37 °C. Media was removed from the Vero E6 cells, mAb-virus complexes were added and incubated at 37 °C for 24 hours. Media was removed from the cells, Nano-Glo luciferase substrate (Promega) was added according to the manufacturer's recommendations, incubated for 10 minutes at room temperature and the luciferase signal was quantified on a VICTOR Nivo plate reader (Perkin Elmer).

### SARS-CoV-2 spike pseudotyped VSV generation and neutralization assay

Replication defective VSV pseudoviruses<sup>45</sup> expressing SARS-CoV-2 spike protein were generated as previously described<sup>46</sup> with some modifications. Plasmids encoding SARS-CoV-2 spike single-mutant variants were generated by site-directed mutagenesis of the wild-type plasmid, pcDNA3.1(+)-spike-D19<sup>47</sup>, and plasmids encoding multiply mutated SARS-CoV-2 variants of concern were generated using a multi-step overlap extension PCR protocol<sup>23,48</sup>, in which sequential, overlapping fragments were designed to introduce all mutations, which were PCR assembled and cloned into the pcDNA3.1 vector using the Takara In-fusion HD cloning kit following manufacturer's instructions.

Lenti-X 293T (Takara, 632180) cells were seeded in 10-cm dishes at a density of  $1 \times 10^5$  cells/cm<sup>2</sup> and the following day transfected with 5 µg of spike expression plasmid with TransIT-Lenti (Mirus, 6600) according to the manufacturer's instructions. For the neutralization assays with variants of concern (Figs. 2d, 3b), Lenti-X 293T cells were seeded in 10-cm dishes at a density of  $5 \times 10^6$  cells/cm<sup>2</sup>, and transfected the following day with 10 µg of spike expression plasmid. One day post-transfection, cells were infected with VSV (G\*ΔG-luciferase) (Kerafast, EH1020-PM)

## Article

for 1 h, rinsed three times with PBS, then incubated for an additional 24 h in complete media at 37 °C. The cell supernatant was clarified by centrifugation, filtered (0.45 µm), aliquoted, and frozen at -80 °C.

For VSV pseudovirus neutralization assays, Vero E6 cells (ATCC, CRL-1586) were grown in DMEM supplemented with 10% FBS and seeded into clear bottom white 96 well plates (Costar, 3903) at a density of  $2 \times 10^4$  cells per well. The next day, mAbs were serially diluted in pre-warmed complete media, mixed at a 1:1 ratio with pseudovirus and incubated for 1 h at 37 °C in round bottom polypropylene plates. Media from cells was aspirated and 50 µL of virus-mAb complexes were added to cells and then incubated for 1 h at 37 °C. An additional 100 µL of prewarmed complete media was then added on top of complexes and cells incubated for an additional 16-24 h. Conditions were tested in duplicate wells on each plate and at least six wells per plate contained uninfected, untreated cells (mock) and infected, untreated cells ('no mAb control').

Virus-mAb-containing media was then aspirated from cells and 100 µL of a 1:4 dilution of Bio-glo (Promega, G7940) in PBS was added to cells. For neutralization assays with variants of concern, 50 µL of a 1:2 dilution of SteadyLite Plus (Perkin Elmer) in PBS with  $\text{Ca}^{2+}$ / $\text{Mg}^{2+}$  was added to cells in place of Bio-glo. Plates were incubated for 10 min at room temperature and then were analyzed on the Envision plate reader (PerkinElmer), or for variants of concern assays, a Synergy HI Hybrid Multi-Mode reader (Biotek).

Relative light units (RLUs) for infected wells were subtracted by the average of RLU values for the mock wells (background subtraction) and then normalized to the average of background subtracted "no mAb control" RLU values within each plate. Percent neutralization was calculated by subtracting from 1 the normalized mAb infection condition. Data were analyzed and visualized with Prism (Version 8.4.3).  $\text{IC}_{50}$  values were calculated from the interpolated value from the log(inhibitor) versus response - variable slope (four parameters) nonlinear regression with an upper constraint of <100. Neutralization experiments with wildtype SARS-CoV-2 S and single-mutant variants were conducted on three independent days, i.e., biological replicates, where each biological replicate contains a technical duplicate.  $\text{IC}_{50}$  values across biological replicates are presented as geometric mean. The loss or gain of neutralization potency across spike variants was calculated by dividing the variant  $\text{IC}_{50}$  by the parental  $\text{IC}_{50}$  within each biological replicate. Neutralization experiments with SARS-CoV-2 S variants of concern were conducted in biological duplicates, with  $\text{IC}_{50}$  values normalized by the corresponding wildtype measurement, and presented as arithmetic mean of the duplicate experiments.

### SARS-CoV-2 spike pseudotyped VSV neutralization on 293T-ACE2 cells

To investigate the effect of ACE2 expression on S2H97 neutralization, Vero E6 cells were seeded at 20,000 cells per well in black clear-bottom 96-well plates. 293T-ACE2 cells<sup>31</sup> were seeded at 35,000 cells per well in black clear-bottom 96-well plates that had been pre-coated with poly-D-Lysine (Gibco). The next day, SARS-CoV-2 spike-pseudotyped VSV neutralizations with S2E12, S309 and S2H97 were performed as described above. Neutralizations were performed in triplicate wells.

### Sarbecovirus spike pseudotyped VSV neutralization by S2H97

Mammalian expression constructs (pcDNA3.1(+)) or pTwist-CMV encoding the spike proteins from various sarbecoviruses with a C-terminal deletion of 19 amino acids (D19) were synthesized for SARS-CoV-2 (Genbank: QOU99296.1), SARS-CoV-1 Urbani (Genbank: AAP13441.1), hCoV-19/pangolin/Guangdong/1/2019 (GD-Pangolin-CoV, Genbank: QLR06867.1), Pangolin coronavirus Guanxi-2017 (GX-Pangolin-CoV, Genbank: QIA48623.1), and bat sarbecovirus WIV1 (WIV1, Genbank: AGZ48828.1). Lenti-X 293T cells (Takara, 632180) were seeded in 15 cm dishes such that the cells would reach 80% confluency after culturing overnight. The following day, cells were transfected using TransIT-Lenti (Mirus, 6600) according to the manufacturer's instructions. One day

post-transfection, cells were infected with VSV ( $\text{G}^*\Delta\text{G}$ -luciferase) (Kerafast, EH1020-PM). The supernatant containing sarbecovirus pseudotyped VSV was collected 2 days post-transfection, centrifuged at  $1000 \times g$  for 5 minutes, aliquoted and frozen at -80 °C.

For neutralization assays, cells supporting robust pseudovirus infection were seeded into clear bottom white-walled 96-well plates at 20,000 cells/well in 100 µL culture media. Vero E6 cells were used for VSV-SARS-CoV-2, VSV-SARS-CoV-1, and VSV-GD-Pangolin-CoV. BHK-21 cells (ATCC, CCL-10) stably expressing ACE2 were used for VSV-GX-Pangolin-CoV and VSV-WIV1. After culturing cells overnight, 1:3 serial dilutions of antibody were prepared in DMEM in triplicate. Pseudovirus was diluted in DMEM and added to each antibody dilution such that the final dilution of pseudovirus was 1:20. Pseudovirus:antibody mixtures were incubated for 1 hour at 37 °C. Media was removed from the cells and 50 µL of pseudovirus:antibody mixtures were added. One hour post-infection, 50 µL of culture media was added to wells containing pseudovirus:antibody mixtures and incubated overnight at 37 °C. Media was then removed and 100 µL of 1:1 diluted DPBS:Bio-Glo (Promega, G7940) luciferase substrate was added to each well. The plate was shaken at 300 RPM at room temperature for 10 minutes after which RLUs were read on an EnSight (Perkin Elmer) microplate reader. Percent neutralization was determined by first subtracting the mean background (cells with luciferase substrate alone) RLU values of 6 wells per plate for all data points. Percent neutralization for each antibody concentration was calculated relative to no antibody control wells for each plate. Percent neutralization data were analyzed and graphed using Prism (GraphPad, v9.0.1). Absolute  $\text{IC}_{50}$  values were calculated by fitting a curve using a non-linear regression model (variable slope, 4 parameters) and values were interpolated from the curve at  $y=50$ . The geometric mean from at least two independent experiments was calculated using Excel (Microsoft, Version 16.45).

### Sarbecovirus spike pseudotyped VSV neutralization by S2E12

Spikes from SARS-CoV-2 (CAD0240757.1), RaTG13 (QHR63300.2), GD-Pangolin (QLR06867.1), GX-Pangolin (QIA48623.1), SARS-CoV-1 Tor2 (YP009825051), WIV1 (AGZ48831.1) and WIV16 (ALK02457.1) were used to pseudotype VSV. To produce pseudotyped viruses, HEK293T/17 (ATCC, CRL-11268) seeded in 10 cm dishes in DMEM supplemented with 10% FBS, 1% PenStrep were transfected with plasmids using lipofectamine 2000 (Life Technologies) following manufacturer's instructions. One day post-transfection, cells were infected with VSV ( $\text{G}^*\Delta\text{G}$ -luciferase) for 2 h and washed four times with DMEM, before adding medium supplemented with anti-VSV-G antibody (II-mouse hybridoma supernatant at 1:50 dilution, from CRL-2700, ATCC). Pseudotyped particles were harvested 18 h post-inoculation, clarified by centrifugation at  $2000 \times g$  for 5 min, concentrated  $10 \times$  with a 30 kDa cutoff membrane filter, and stored at -80 °C. For S2E12 neutralization experiments, 293T cells stably expressing ACE2 (BEI #NR-52511)<sup>43</sup> in DMEM supplemented with 10% FBS and 1% PenStrep were seeded at 40,000 cells/well in clear-bottom white-walled 96-well plates and cultured overnight at 37 °C. Twelve 3-fold serial dilutions of S2E12 antibody were prepared in DMEM, and antibody dilutions were mixed 1:1 with pseudotyped VSV in the presence of 1:50 diluted anti-VSV-G antibody. After 45 min incubation at 37 °C, 40 µL of antibody-virus mixture was added to cells, and 40 µL DMEM was added 2 h post-infection. After 17-20 h, 50 µL One-Glo-EX substrate (Promega) was added to the cells. Cells were incubated in the dark for 5-10 min prior to luminescence reading on a Varioskan LUX plate reader (Thermo Fisher Scientific). Relative luciferase unit values were converted to percentage of neutralization and plotted with a nonlinear regression curve fit in GraphPad Prism. Measurements were performed in duplicate with two independent productions of pseudotyped virus.

### Recombinant protein production

SARS-CoV-2 RBD WT proteins for SPR binding assays (with N-terminal signal peptide and C-terminal thrombin cleavage site-TwinStrep-8xHis-tag)

were expressed in Expi293F (Thermo Fisher Scientific) cells at 37 °C and 8% CO<sub>2</sub>. Transfections were performed using the ExpiFectamine 293 Transfection Kit (Thermo Fisher Scientific). Cell culture supernatants were collected three days after transfection and supplemented with 10x PBS to a final concentration of 2.5x PBS (342.5 mM NaCl, 6.75 mM KCl and 29.75 mM phosphates). SARS-CoV-2 RBDs were purified using 1 or 5 mL HisTALON Superflow cartridges (Takara Bio) and subsequently buffer exchanged into 1x HBS-N buffer (Cytiva) or PBS using a Zeba Spin Desalting (Thermo Fisher Scientific) or HiPrep 26/10 (Cytiva) desalting column.

SARS-CoV-2 RBD WT for crystallization (with N-terminal signal peptide and 'ETGT', and C-terminal 8xHis-tag) was expressed similarly as described above in the presence of 10 μM kifunensine. Cell culture supernatant was collected four days after transfection and supplemented with 10x PBS to a final concentration of 2.5x PBS. Protein was purified using a 5 ml HisTALON Superflow cartridge followed by size exclusion chromatography on a Superdex 200 Increase 10/300 GL column (Cytiva) equilibrated in 20 mM Tris-HCl pH 7.5, 150 mM NaCl. For crystallization of the RBD-S2X259-S2H97 and RBD-S2E12-S304-S309 Fab complexes, RBD was deglycosylated by overnight incubation with EndoH glycosidase at 4 °C.

RBDs from other sarbecoviruses for SPR (with N-terminal signal peptide and C-terminal thrombin cleavage site-TwinStrep-8xHis-tag) were expressed in Expi293F cells at 37 °C and 8% CO<sub>2</sub>. Cells were transfected using PEI MAX (Polysciences) at a DNA:PEI ratio of 1:3.75. Transfected cells were supplemented three days after transfection with 3 g/L glucose (Bioconcept) and 5 g/L soy hydrolysate (Sigma-Aldrich Chemie GmbH). Cell culture supernatant (423 mL) was collected seven days after transfection and supplemented with 47 mL 10x binding buffer (1M Tris-HCl, 1.5M NaCl, 20 mM EDTA, pH 8.0) and 25 mL BioLock (IBA GmbH) and incubated on ice for 30 min. Proteins were purified using a 5 mL Strep-Tactin XT Superflow high-capacity cartridge (IBA GmbH) followed by buffer exchange to PBS using HiPrep 26/10 desalting columns (Cytiva).

Prefusion-stabilized SARS-CoV-2 spike proteins for SPR (residues 14-1211, either D614 or D614G), containing the 2P and Furin cleavage site mutations<sup>49</sup> with a mu-phosphatase signal peptide and a C-terminal Avi-8xHis-C-tag or C-terminal 8xHis-Avi-C-tag were expressed in Freestyle 293-F cells (Thermo Fisher Scientific, R79007) at 37 °C and 8% CO<sub>2</sub>. Transfections were performed using 293fectin as a transfection reagent. Cell culture supernatant was collected after three days and purified over a 5 mL C-tag affinity matrix. Elution fractions were concentrated and injected on a Superose 6 Increase 10/300 GL column (Cytiva) with 50 mM Tris-HCl pH 8.0 and 200 mM NaCl as running buffer.

SARS-CoV-2 HexaPro spike protein for cryoEM analysis was produced in Freestyle 293-F cells grown in suspension using Freestyle 293 expression medium (Life Technologies) at 37 °C in a humidified 8% CO<sub>2</sub> incubator rotating at 130 RPM. The cultures were transfected using PEI (9 μg/mL) with cells grown to a density of 2.5 million cells per mL and cultivated for three days. The supernatants were harvested and cells resuspended for another three days, yielding two harvests. Spike proteins were purified from clarified supernatants using a 5 mL Cobalt affinity column (Cytiva, HiTrap TALON crude), concentrated and flash frozen in a buffer containing 20 mM Tris pH 8.0 and 150 mM NaCl prior to analysis.

SARS-CoV-2 S native-like ectodomain trimer for refolding assays was engineered with a mu-phosphatase signal peptide beginning at 14Q, a mutated S1/S2 cleavage site (SGAR), and a TEV cleavage, fold-on trimerization motif, and 8x His tag appended to the C-terminus (K1211). Native-like spike was expressed and purified as described for SARS-CoV-2 HexaPro spike above.

Recombinant hACE2 for SPR (residues 19-615 from Uniprot Q9BYF1 with a C-terminal AviTag-10xHis-GGG-tag, and N-terminal signal peptide) was produced by ATUM. Protein was purified via Ni Sepharose resin followed by isolation of the monomeric hACE2 by size exclusion

chromatography using a Superdex 200 Increase 10/300 GL column (Cytiva) pre-equilibrated with PBS.

### SPR binding assays

SPR binding measurements were performed using a Biacore T200 instrument with CM5 sensor chip covalently immobilized with Strep-Tactin XT to capture recombinant RBD proteins (data in Fig. 1a and Extended Data Fig. 4f,i,l). Running buffer was Cytiva HBS-EP+ (pH 7.4). All measurements were performed at 25 °C. Fab (or hACE2) analyte concentrations were 11, 33, 100, and 300 nM, run as single-cycle kinetics. Double reference-subtracted data were fit to a 1:1 binding model using Biacore T200 Evaluation (version 3.1) or Biacore Insight Evaluation (version 2.0.15) software. K<sub>D</sub> above 1 μM were determined from fits where R<sub>max</sub> was set as a constant based on results for higher affinity analytes binding to the same RBD at the same surface density. Data are representative of duplicate or triplicate measurements (except measurements with germline Fabs were singleton measurements).

To corroborate the SARS-CoV-2 RBD binding measurements, experiments were also performed in two additional formats, both with monovalent analytes (data in Extended Data Table 1): (1) Fab binding to SARS-CoV-2 spike ectodomain was measured using CM5 sensor chips immobilized with anti-AviTag pAb (Genscript, A00674-40) for capturing S, other experiment parameters same as above, and (2) RBD binding to IgG was measured using CM5 sensor chips immobilized with anti-human Fc pAb (Southern Biotech, 2014-01) for capturing IgG, with RBD analyte concentrations of 3.1, 12.5, and 50 nM, other experiment parameters same as above. Fit results yield an "apparent K<sub>D</sub>" for the spike-binding experiments because the kinetics also reflect spike conformational dynamics. Spike ectodomain was D614G with C-terminal 8xHis-Avi-C-tag for all measurements except S2X58 binding was performed with D614 spike with C-terminal Avi-8xHis-C-tag. For the comparison of mature and germline-reverted antibody binding to RaTG13, the data reported are from experiment format (2) with IgG as ligand. These data were fit to a Heterogeneous Ligand model, due to an artifactual kinetic phase with very slow dissociation that often arises when RBD is an analyte; the lower affinity of the two K<sub>D</sub>s reported by the fit is given as the K<sub>D</sub> (the two K<sub>D</sub>s are separated by at least one order of magnitude).

### Deep mutational scanning mutant escape profiling

We used a previously described deep mutational scanning approach<sup>3</sup> to comprehensively identify RBD mutations that escape binding by each antibody. This approach leverages duplicate RBD mutant libraries<sup>26</sup>, which contain virtually all of the 3,819 possible amino acid mutations in the background of the Wuhan-Hu-1 RBD sequence. Library variants were previously linked to short identifier barcode sequences and sorted to purge the library of variants that strongly decrease ACE2 binding affinity or expression of folded RBD<sup>3</sup>.

We first used an isogenic yeast strain expressing the unmutated SARS-CoV-2 RBD and flow cytometry to identify the EC90 of each antibody's binding to yeast-displayed SARS-CoV-2 RBD. We then performed library selections as previously described<sup>3,20</sup>, labeling libraries with the EC90 concentration of antibody to standardize escape mutation sensitivity across selections. Briefly, libraries of yeast were induced for surface expression, washed, and labeled with the primary antibody for one hour at room temperature. Cells were washed, and secondarily labeled with 1:200 PE-conjugated goat anti-human-IgG antibody (Jackson ImmunoResearch 109-115-098) to label for bound antibody, and 1:100 FITC-conjugated chicken anti-Myc-tag (Immunology Consultants Lab, CYMC-45F) to label for RBD surface expression. We prepared controls for setting FACS selection gates by labeling yeast expressing the unmutated SARS-CoV-2 RBD with the same antibody concentration as library selections (1x), 100x reduced antibody concentration to illustrate the effect of mutations with 100x-reduced affinity, and 0 ng/mL antibody to illustrate complete loss of antibody

# Article

binding. Representative selection gates are shown in Extended Data Fig. 2b. Gates were set and sorting performed with FACSDiva software (version 6.1.3). We sorted approximately  $7.5 \times 10^6$  RBD+ cells per library on a BD FACSAria II, collecting yeast cells from the antibody-escape sort bin (fractions of library falling into antibody escape bin given in Extended Data Fig. 2c). Sorted cells were recovered overnight, plasmids were extracted from the pre-sort and antibody-escape populations, and variant-identifier barcode sequences were PCR amplified and sequenced on an Illumina HiSeq 2500<sup>3,26</sup>.

As previously described<sup>3,20</sup>, sequencing counts pre- and post-selection were used to estimate the “escape fraction” for each library variant, which reflects the fraction of yeast expressing a variant that fall into the antibody-escape FACS bin. Briefly, we used the `dms_variants` package ([https://jbloomlab.github.io/dms\\_variants/](https://jbloomlab.github.io/dms_variants/), version 0.8.2) to process Illumina sequences into variant counts pre- and post-selection using the barcode/RBD variant lookup table from Starr et al.<sup>26</sup>. We then computed per-variant escape fractions as:  $E_v = F \times (n_v^{post} / N_{post}) / (n_v^{pre} / N_{pre})$ , where  $F$  is the total fraction of the library that escapes antibody binding (Extended Data Fig. 2c),  $n_v^{post}$  and  $n_v^{pre}$  are the sequencing counts of variant  $v$  in the RBD library after and before FACS selection (with a pseudo-count of 0.5 added to all counts), and  $N_{post}$  and  $N_{pre}$  are the total counts of all variants after and before FACS selection. We then applied computational filters to remove variants with low pre-sort sequencing counts or highly deleterious mutations that might cause artefactual antibody escape due to global unfolding or loss of expression of RBD on the cell surface. Specifically, we filtered out variants whose pre-selection sequencing counts were lower than the 99<sup>th</sup> percentile counts of variants containing premature stop codons, which were largely purged by the prior sorts for RBD expressing and ACE2-binding RBD variants. We also removed variants with ACE2 binding scores  $< -2.35$  or RBD expression scores  $< -1$ , and variants containing individual mutations with effects below these thresholds, using the variant- and mutation-level deep mutational scanning measurements of Starr et al.<sup>26</sup>. We also filtered out rare mutations with low coverage in the libraries, retaining mutations that were sampled on at least one single-mutant barcoded variant or at least two multiply-mutated variants in each replicate. Last, to decompose single-mutation escape fractions for each antibody, we implemented global epistasis models<sup>50</sup> using the `dms_variants` package to estimate the effect of each individual amino acid mutation, exactly as described in ref.<sup>20</sup>.

Antibody escape selections were conducted in full duplicate using independently generated and assayed SARS-CoV-2 mutant libraries (see correlations in Extended Data Fig. 2e,f). The reported escape fractions throughout the paper are the average across the two replicates, and these final per-mutation escape fractions are provided on GitHub: [https://github.com/jbloomlab/SARS-CoV-2-RBD\\_MAP\\_Vir\\_mAbs/blob/main/results/supp\\_data/vir\\_antibodies\\_raw\\_data.csv](https://github.com/jbloomlab/SARS-CoV-2-RBD_MAP_Vir_mAbs/blob/main/results/supp_data/vir_antibodies_raw_data.csv). Interactive visualizations of antibody escape maps ([https://jbloomlab.github.io/SARS-CoV-2-RBD\\_MAP\\_Vir\\_mAbs](https://jbloomlab.github.io/SARS-CoV-2-RBD_MAP_Vir_mAbs)) were created using `dms-view`<sup>51</sup>.

## Sarbecovirus library binding assays

A curated set of all unique sarbecovirus RBD amino acid sequences was gathered, including the sarbecovirus RBD sequence set reported by Letko et al.<sup>7</sup>, along with additional unique RBD sequences among SARS-CoV-1 epidemic strains reported by Song et al.<sup>52</sup>, BtKY72<sup>53</sup> and new sarbecovirus sequences RmYN02<sup>54</sup>, GD-Pangolin-CoV (consensus RBD reported in Fig. 3a of Lam et al.<sup>55</sup>), and GX-Pangolin-CoV<sup>55</sup> (P2V, ambiguous nucleotide within codon 515 (SARS-CoV-2 spike numbering) resolved to retain F515, which is conserved in all other sarbecoviruses). A list of all RBDs and sequence accession numbers is available on GitHub: [https://github.com/jbloomlab/SARSr-CoV\\_RBD\\_MAP/blob/main/data/RBD\\_accessions.csv](https://github.com/jbloomlab/SARSr-CoV_RBD_MAP/blob/main/data/RBD_accessions.csv)

To define clades of sarbecovirus RBDs, an alignment of amino acid RBD sequences was generated using `mafft`<sup>56</sup> with gap opening penalty 4.5 (alignment available on GitHub: <https://github.com/jbloomlab/>

[SARSr-CoV\\_RBD\\_MAP/blob/main/data/RBD\\_aa\\_aligned.fasta](https://github.com/jbloomlab/SARSr-CoV_RBD_MAP/blob/main/data/RBD_aa_aligned.fasta)). The corresponding nucleotide sequence alignment was generated from the amino acid alignment using `PAL2NAL`<sup>57</sup>. The gene sequence phylogeny was inferred using `RAXML` version 8.2.12<sup>58</sup>, with the `GTRGAMMA` substitution model and a partition model with separate parameters for first, second, and third codon positions. The Hibecovirus RBD sequence Hp-Zhejiang2013 (Genbank: KF636752) was used as an outgroup for rooting of the sarbecovirus phylogeny.

All unique sarbecovirus RBD protein-coding sequences were ordered from IDT, Twist, and Genscript, and cloned into our yeast display vector<sup>26</sup>. Sequences were pooled and appended with downstream 16-nt barcode sequences according to the protocol described in Starr et al.<sup>26</sup>. Long read circular consensus sequences spanning the 16-nt barcode and RBD genotype were gathered on a PacBio Sequel v2.0 and processed exactly as described in Starr et al.<sup>26</sup>. This yielded a barcode:variant lookup table for the sarbecovirus RBD library analogous to that used for SARS-CoV-2 mutant libraries. This table is available on GitHub: [https://github.com/jbloomlab/SARSr-CoV\\_RBD\\_MAP/blob/main/data/barcode\\_variant\\_table.csv](https://github.com/jbloomlab/SARSr-CoV_RBD_MAP/blob/main/data/barcode_variant_table.csv).

The pooled sarbecovirus RBD library was labeled, sorted, and quantified as described for the SARS-CoV-2 mutant libraries above, except we only sorted ~1 million RBD+ cells due to the reduced library size. Sequencing and quantification of per-variant antibody escape was conducted as described above. Data for the HKU3-8 RBD is not shown, as this RBD did not express in our yeast-display platform. For several antibodies, we performed a secondary experiment, selecting the sarbecovirus RBD library with a more stringent “full escape” gate to select out only variants exhibiting complete loss of binding (Extended Data Fig. 2b,c).

For follow-up quantitative binding assays, select sarbecovirus RBDs were cloned into the yeast-display platform as isogenic stocks. Binding assays were conducted across a titration series of antibody in 96-well plates, and binding at each antibody concentration (geometric mean fluorescence intensity in the PE channel among RBD+ (FITC+) cells) was determined via flow cytometry and fit to a four-parameter Hill curve to identify the EC50 (midpoint).

## Analysis of mutations in natural SARS-CoV-2 sequences

All spike sequences on GISAID<sup>59</sup> as of May 2, 2021, were downloaded and aligned via `mafft`<sup>56</sup>. Sequences from non-human origins, sequences with gaps or ambiguous characters in the RBD, and sequences with more than 8 amino acid differences from the Wuhan-Hu-1 reference sequence (Genbank MN908947, residues N331-T531) were removed. We determined mutation frequencies compared to Wuhan-Hu-1 reference from this final alignment of 1,190,241 sequences. We acknowledge all contributors to the GISAID EpiCoV database for their sharing of sequence data. All contributors to GISAID EpiCoV listed at: [https://github.com/jbloomlab/SARS-CoV-2-RBD\\_MAP\\_Vir\\_mAbs/blob/main/data/gisaid\\_hcov-19\\_acknowledgement\\_table\\_2021\\_03\\_04.pdf](https://github.com/jbloomlab/SARS-CoV-2-RBD_MAP_Vir_mAbs/blob/main/data/gisaid_hcov-19_acknowledgement_table_2021_03_04.pdf).

## Quantitative summary metrics of antibody properties

The relative epitope size of an antibody was calculated as the sum of per-mutant escape fractions that are at least five times the global median escape fraction (to minimize the impact of variation in background noise on the summation). For this summation, escape fractions were normalized to the maximum per-mutation escape fraction, to account for slight variation in the largest per-mutation escape fraction measured between selections.

The relative escapability of an antibody was calculated the same as relative epitope size, but each mutation was multiplied by two weighting factors scaled from 0 to 1 that reflect the impact of that mutation on ACE2-binding affinity and RBD expression as measured in our prior deep mutational scan<sup>26</sup>. The relationship between weighting factors and mutation effect on each property is shown in Extended Data Fig. 3a. Mutations with  $< -1$  effect on either property are effectively zeroed out

in the escapability summation. Mutations with effects between -1 and 0 have intermediate weights, and mutations with 0 or positive effects are assigned weight factors of 1.

Antibody susceptibility to escape by natural SARS-CoV-2 mutations was calculated as the summed GISAID frequencies of all escape mutations, where escape mutations (all labels in Extended Data Fig. 3c) are defined as those with escape fraction greater than five times the median escape fraction as above. These summed natural escape frequencies are tabulated in the plot headers in Extended Data Fig. 3c.

The summary breadth of an antibody was calculated from the sarbecovirus RBD library escape selection using the standard gating (Extended Data Fig. 4b), only. Although we have various follow-up binding data illustrating reduced affinity binding for some “escaped” sarbecovirus RBDs, these follow-up experiments were not conducted systematically for all antibody/RBD combinations, and therefore would bias breadth estimates. Breadth of binding was calculated as the frequency of all sarbecovirus RBDs that are bound with affinity within the FACS selection gating threshold, weighted by clade representation. Breadth was normalized to give equal representation to each of the four sarbecovirus clades to account for different depth of sampling. Within the SARS-CoV-1 clade, all human 02/03 strains and civet + human 03/04 strains were similarly down-weighted to each represent 1/8 of the possible breadth within the SARS-CoV-1 clade (together with the six bat sarbecoviruses in this clade). As an example, breadth for S304 is calculated as  $[4/4 + ([6/6] + [6/6] + 5)/8 + 2/2 + 0/21]/4 = 0.72$ , based on the data shown in Extended Data Fig. 4b.

#### Multidimensional scaling projection of antibody epitopes

Multidimensional scaling projection in Fig. 4 was performed using the Python scikit-learn package. We first computed the similarity and dissimilarity in the sites of escape between each pair of antibodies, exactly as described in Greaney et al.<sup>3</sup>, and performed metric multidimensional scaling with two components on the matrix of dissimilarities between all antibody pairs. Antibodies in this layout were colored with pie charts proportional to the total squared site-wise escape that falls into the labeled structural regions (RBM = residues 437 to 508, ACE2 contact defined as 4Å cutoff based on 6MOJ crystal structure<sup>60</sup>, and core RBD otherwise). In this layout, we included all of our previously published antibodies for which we have performed escape mapping via this same approach. These antibodies and their citations include: S2X259<sup>37</sup>; LY-CoV555<sup>21</sup>; COV2-2196 and COV2-2130<sup>36</sup>; REGN10933, REGN10987, and LY-CoV016<sup>20</sup>; and all other COV2 antibodies and CR3022<sup>3</sup>.

For Fig. 4b–d and Extended Data Fig. 7c, we colored the antibodies within this layout according to various antibody properties. When appropriate, we also colored these previously assayed antibodies, as described below. Extended Data Fig. 7d and the scatterplots in Fig. 4e–g show the relationships between properties for antibodies specifically in this study (and S2X259) for the most direct comparability.

Antibody neutralization potencies illustrated in Fig. 4b incorporate the authentic SARS-CoV-2 neutralization IC50s as reported in this study (Fig. 1a), together with the live SARS-CoV-2 neutralization IC50s for the COV2 antibodies reported by Zost et al.<sup>10</sup>. We acknowledge that it is imperfect to compare neutralization potencies reported from different labs on different antibody batches, though in this case, both sets are indeed neutralization potencies with authentic virus. We therefore do not directly compare these two sets of measurements in a quantitative manner, but we do note that their joint inclusion in Fig. 4b supports the dichotomy between neutralization potency of core RBD versus RBM antibodies which is supported by either neutralization panel alone.

Sarbecovirus breadth illustrated in Fig. 4c incorporates the pan-sarbecovirus breadth measurements reported in the current study together with more limited breadth measurements for antibodies reported in our prior publications. These previously published experiments determined binding within a more restricted sarbecovirus RBD set present in our libraries (SARS-CoV-2, RaTG13, GD-Pangolin,

SARS-CoV-1 [Urbani], LYRa11, and WIVI). We calculated breadth from this incomplete sarbecovirus sequence set for comparison, but note that these antibodies are limited to a relative breadth of 0.5 because no RBDs from the Africa/Europe or non-ACE2-utilizing Asia clades were included. However, as with neutralization, inclusion of these antibodies nonetheless emphasizes the core RBD/RBM dichotomy in sarbecovirus breadth established by our primary panel.

For illustrations of epitope size and escapability in Fig. 4d and Extended Data Fig. 7c, we calculated these quantities for our previously profiled antibodies as described above. We excluded the antibodies profiled in Greaney et al.<sup>3</sup>, as these assays were performed on a prior version of our SARS-CoV-2 mutant library that exhibited different quantitative features of absolute escape, complicating its quantitative comparison to extent of escape for antibodies profiled in this and our other studies, which all use the same library.

Structural mappings around the perimeter of Fig. 4a were created by mapping total site-wise escape to the b-factor column of PDB structures. Footprints were defined as residues within a 5Å cutoff of antibody heavy atoms. Structures used were those described in this paper, or previously published structures: ACE2-bound RBD (6MOJ)<sup>60</sup>, CR3022-bound RBD (6W41)<sup>61</sup>, REGN10987- and REGN10933-bound RBD (6XDG)<sup>62</sup>, CB6- (LY-CoV016) bound RBD (7C01)<sup>63</sup>, and S304, S309, and S2H14-bound RBD (7JX3)<sup>15</sup>.

#### RBD ELISA

96 half area well-plates (Corning, 3690) were coated over-night at 4 °C with 25 µL of sarbecovirus RBD proteins at 5 µg/mL in PBS pH 7.2. Plates were blocked with PBS 1% BSA (Sigma-Aldrich, A3059) and subsequently incubated with mAb serial dilutions for 1 h at room temperature. After 4 washing steps with PBS 0.05% Tween 20 (PBS-T) (Sigma-Aldrich, 93773), goat anti-human IgG secondary antibody (Southern Biotech, 2040-04) was added and incubated for 1 h at room temperature. Plates were then washed 4 times with PBS-T and 4-NitroPhenyl phosphate (pNPP, Sigma-Aldrich, 71768) substrate added. After 30 min incubation, absorbance at 405 nm was measured by a plate reader (Biotek) and data plotted using GraphPad Prism.

#### Binding to cell surface expressed sarbecovirus S proteins by flow cytometry

ExpiCHO-S cells were seeded at  $6 \times 10^6$  cells/mL in a volume of 5 mL in a 50 mL bioreactor. Spike coding plasmids were diluted in cold Opti-PRO SFM, mixed with ExpiFectamine CHO Reagent (Life Technologies) and added to the cells. Transfected cells were then incubated at 37 °C with 8% CO<sub>2</sub> with an orbital shaking speed of 120 RPM (orbital diameter of 25 mm) for 42 hours. Transiently transfected ExpiCHO-S cells were harvested and washed two times in wash buffer (PBS 1% BSA, 2 mM EDTA). Cells were counted and distributed into round bottom 96-well plates (Corning) and incubated with 10 µg/mL S2H97, S2X35 or S309 mAb. Alexa Fluor647-labelled Goat Anti-Human IgG secondary Ab (Jackson ImmunoResearch 109-607-003) was prepared at 1.5 µg/mL added onto cells after two washing steps. Cells were then washed twice and resuspended in wash buffer for data acquisition on a ZE5 cytometer (Biorad).

#### Crystallization, data collection, structure determination, and analysis

To form RBD-Fab complexes for crystallization, SARS-CoV-2 RBD was mixed with a 1.3-fold molar excess of each Fab and incubated on ice for 20-60 min. Complexes were purified on a Superdex 200 Increase 10/300 GL column (Cytiva) preequilibrated with 20 mM Tris-HCl pH 7.5 and 150 mM NaCl. Crystals of the RBD-Fab complexes were obtained at 20 °C by sitting drop vapor diffusion.

For the SARS-CoV-2 RBD-S2X35-S309 complex, a total of 200 nL complex at 5.4 mg/mL was mixed with 100 nL mother liquor solution containing 1.85 M Ammonium Sulfate, 0.1 M Tris pH 8.17, 0.8% (w/v)

polyvinyl alcohol, 1% (v/v) 1-propanol, and 0.01 M HEPES pH 7. Crystals were flash frozen in liquid nitrogen using the mother liquor solution supplemented with 20% glycerol for cryoprotection. Data were collected at Beamline 9-2 of the Stanford Synchrotron Radiation Lightsource facility in Stanford, CA and processed with the XDS software package (version Jan 31, 2020)<sup>64</sup> to 1.83 Å in space group C222. The RBD-S2X35-S309 Fab complex structure was solved by molecular replacement using Phaser<sup>65</sup> from a starting model consisting of RBD-S309 Fab (PDB ID: 7JX3) and a homology model for the S2X35 Fab built using the Molecular Operating Environment (MOE) software package from the Chemical Computing Group (<https://www.chemcomp.com>).

For the SARS-CoV-2-RBD-S2H97-S2X259 Fab complex, 200 nL complex at 5.7 mg/mL were mixed with 200 nL mother liquor solution containing 0.12 M Monosaccharides mix, 20% (v/v) Ethylene glycol, 10% (w/v) PEG 8000, 0.1 M Tris (base)/bicine pH 8.5, 0.02 M Sodium chloride, 0.01 M MES pH 6 and 3% (v/v) Jeffamine ED-2003. Crystals were flash frozen in liquid nitrogen. Data were collected at Beamline 9-2 of the Stanford Synchrotron Radiation Lightsource facility in Stanford, CA. Data were processed with the XDS software package (version Jan 31, 2020)<sup>64</sup> for a final dataset of 2.65 Å in space group P2<sub>1</sub>. The RBD-S2H97-S2X259 Fab complex structure was solved by molecular replacement using phaser from a starting model consisting of SARS-CoV-2 RBD (PDB ID: 7JX3) and homology models for the S2H97 and S2X259 Fabs built using the MOE software package.

For the SARS-CoV-2-RBD-S2E12-S304-S309 Fab complex, 200 nL complex at 4.5 mg/mL were mixed with 100 nL of 0.09 M Phosphate/Citrate pH 5.5, 27% (v/v) PEG Smear Low, 4% (v/v) Polypropylene glycol 400 and 0.02 M Imidazole pH 7 or 100 nL of 0.09 M Phosphate/Citrate pH 5.5, 27% (v/v) PEG Smear Low, 0.01 M Potassium/sodium phosphate pH 7, 1% (v/v) PPGBA 230 and 1.5% (v/v) PPGBA 400. Crystals were flash frozen in liquid nitrogen. Data were collected at the Molecular Biology Consortium beamline 4.2.2 at the Advanced Light Source synchrotron facility in Berkeley, CA. Datasets from two crystals from the two conditions were individually processed and then merged with the XDS software package (version Jan 31, 2020)<sup>64</sup> for a final dataset of 2.93 Å in space group I4<sub>1</sub>22. The RBD-S2E12-S304-S309 Fab complex structure was solved by molecular replacement using phaser from starting models consisting of RBD-S304-S309 Fab (PDB ID: 7JX3) and S2E12 (PDB ID: 7K3Q).

For all structures, several subsequent rounds of model building and refinement were performed using Coot (version 0.9.5)<sup>66</sup>, ISOLDE (ChimeraX version 1.1/ISOLDE version 1.1)<sup>67</sup>, Refmac5 (version 5.8.0267)<sup>68</sup>, and MOE (version 2019.0102) (<https://www.chemcomp.com>), to arrive at the final models. For all complexes, epitopes on the RBD protein were determined by identifying all RBD residues within a 5.0 Å distance from any Fab atoms. The analysis was performed using the MOE software package and the results were manually confirmed.

### Cryo-electron microscopy

SARS-CoV-2 HexaProS<sup>69</sup> at 1.2 mg/mL was incubated with 1.2 fold molar excess of recombinantly purified S2D106 or S2H97 at 4 °C before application onto a freshly glow discharged 2.0/2.0 UltrAuFoil grid (200 mesh). Plunge freezing used a vitrobot MarkIV (Thermo Fisher Scientific) using a blot force of 0 and 6.5 second blot time at 100% humidity and 23 °C.

For the S/S2D106 data set, Data were acquired using an FEI Titan Krios transmission electron microscope operated at 300 kV and equipped with a Gatan K2 Summit direct detector and Gatan Quantum GIF energy filter, operated in zero-loss mode with a slit width of 20 eV. Automated data collection was carried out using Legimon<sup>70</sup> at a nominal magnification of 130,000x with a pixel size of 0.525 Å. The dose rate was adjusted to 8 counts/pixel/s, and each movie was acquired in super-resolution mode fractionated in 50 frames of 200 ms. 2,166 micrographs were collected with a defocus range between -0.5 and -2.5 µm. Movie frame alignment, estimation of the microscope contrast-transfer function

parameters, particle picking, and extraction were carried out using Warp<sup>71</sup>. Particle images were extracted with a box size of 800 binned to 400 pixels<sup>2</sup> yielding a pixel size of 1.05 Å.

For the S/S2H97 data set, data were acquired on an FEI Glacios transmission electron microscope operated at 200 kV equipped with a Gatan K2 Summit direct detector. Automated data collection was carried out using Legimon<sup>70</sup> at a nominal magnification of 36,000x with a pixel size of 1.16 Å. The dose rate was adjusted to 8 counts/pixel/s, and each movie was acquired in counting mode fractionated in 50 frames of 200 ms. 3,138 micrographs were collected in a single session with a defocus range comprised between -0.5 and -3.0 µm. Preprocessing was performed using Warp<sup>71</sup> and particle images were extracted with a box size of 400 pixels<sup>2</sup>.

For the S/S2D106 and S/S2H97 datasets, two rounds of reference-free 2D classification were performed using CryoSPARC to select well-defined particle images<sup>72</sup>. These selected particles were subjected to two rounds of 3D classification with 50 iterations each (angular sampling 7.5° for 25 iterations and 1.8° with local search for 25 iterations), using our previously reported closed SARS-CoV-2 S structure as initial model<sup>49</sup> (PDB 6VXX) in Relion<sup>73</sup>. 3D refinements were carried out using non-uniform refinement<sup>74</sup> along with per-particle defocus refinement in CryoSPARC. Selected particle images were subjected to the Bayesian polishing procedure<sup>75</sup> implemented in Relion3.0 before performing another round of non-uniform refinement in CryoSPARC followed by per-particle defocus refinement and again non-uniform refinement.

To further improve the density of the S2D106 Fab, the particles were then subjected to focus 3D classification without refining angles and shifts using a soft mask on RBD and Fab variable domains with a tau value of 60 in Relion. Particles belonging to classes with the best resolved local density were selected and subject to local refinement using CryoSPARC. Local resolution estimation, filtering, and sharpening were carried out using CryoSPARC. Reported resolutions are based on the gold-standard Fourier shell correlation (FSC) of 0.143 criterion and Fourier shell correlation curves were corrected for the effects of soft masking by high-resolution noise substitution<sup>76</sup>. UCSF Chimera<sup>77</sup> and Coot<sup>78</sup> were used to fit atomic models into the cryoEM maps. Spike-RBD/S2D106 Fab model was refined and relaxed using Rosetta using sharpened and unsharpened maps<sup>79</sup>.

### S2H97-induced spike refolding

10 µM native-like SARS-CoV-2 S was incubated with 13 µM S2H97 Fab for 1 hour at room temperature. Samples were diluted to 0.01 mg/mL immediately prior to adsorption to glow-discharged carbon-coated copper grids for -30 sec prior to a 2% uranyl formate staining. Micrographs were recorded using the Legimon software<sup>70</sup> on a 120 kV FEI Tecnai G2 Spirit with a Gatan Ultrascan 4000 4k x 4k CCD camera at 67,000 nominal magnification. The defocus ranged from -1.0 to -2.0 µm and the pixel size was 1.6 Å.

### Cell-surface antibody-mediated S1 shedding

CHO-K1 cells stably expressing the prototypic SARS-CoV-2 spike protein were harvested, washed in wash buffer (PBS + 1% BSA, 2 mM EDTA) and resuspended in PBS. 90,000 cells per well were dispensed into round bottom 96-well plates (Corning), and treated with 10 µg/mL TPCK-Trypsin (Worthington Biochem) for 30 min at 37 °C. Cells were washed and incubated with 15 µg/mL antibody across 5, 30, 60, 120, and 180 min timepoints at 37 °C. Cells were washed with ice-cold wash buffer, and stained with 1.5 µg/mL Alexa Fluor647-conjugated goat anti-human IgG secondary antibody (Jackson ImmunoResearch) for 30 min on ice in the dark. Cells were washed twice with cold wash buffer and analyzed using a ZE5 cytometer (Biorad) with acquisition chamber at 4 °C. Binding at each time point was measured as mean fluorescence intensity (MFI), normalized to the MFI at the 5 min labeling time point. Data was analyzed and plotted using GraphPad Prism v. 9.0.1.

### Cell-cell fusion of CHO-S cells

Cell-cell fusion between S-expressing CHO-K1 cells was performed as described by Lempp et al.<sup>31</sup>. CHO-K1 cells stably expressing the prototypic SARS-CoV-2 spike protein were seeded in 96-well plates (Thermo Fisher Scientific) at 12,500 cells/well. The following day, antibody and nuclei marker Hoechst (final dilution 1:1000) were added to cells and incubated for 24 h. Cell-cell fusion was visualized using the Cytation 5 Imager (BioTek), and an object detection protocol was used to detect nuclei and measure their size. The nuclei of fused cells (syncytia) are aggregated at the center of the syncytia and recognized as a uniquely large object that is gated according to its size. To quantify cell-cell fusion, we report the area of objects in fused cells divided by the total area of all objects, multiplied by 100 to represent as a percentage.

### Antibody blockade of RBD binding to ACE2

ACE2 blockade ELISA was performed as described by Piccoli et al.<sup>15</sup>. Unlabeled antibodies were serially diluted, mixed with RBD mouse Fc-tagged antigen (Sino Biological, final concentration 20 ng/mL) and incubated for 30 min at 37 °C. The mix was added for 30 min to ELISA 96-well plates (Corning) pre-coated overnight at 4 °C with 2 µg/mL human ACE2 in PBS. Plates were washed and RBD binding was revealed using secondary goat anti-mouse IgG (Southern Biotech 1030-04). After washing, pNPP substrate was added and plates were read at 405 nm. The percentage of inhibition was calculated as:  $(1 - (\text{OD sample} - \text{OD neg ctrl}) / (\text{OD pos ctrl} - \text{OD neg ctrl})) \times 100$ .

### Inhibition of spike-mediated cell-to-cell fusion

Cell-to-cell fusion inhibition assays were performed as described by McCallum et al.<sup>80</sup>. Vero E6 cells were seeded in 96 well plates at 15,000 cells per well in 70 mL DMEM with high glucose and 2.4% FBS (Hyclone). After 16 h at 37 °C with 8% CO<sub>2</sub>, the cells were transfected as follows: for 10 wells, 0.57 mg plasmid SARS-CoV-2-S-D19\_pcDNA3.1 was mixed with 1.68 mL X-tremeGENE HP in 30 mL OPTIMEM. After 15 min incubation, the mixture was diluted 1:10 in DMEM medium and 30 mL was added per well. 4-fold antibody serial dilutions were prepared and added to the cells, with a starting concentration of 20 µg/mL. The following day, 30 µL 5X concentrated DRAQ5 in DMEM was added per well and incubated for 2 h at 37 °C. Nine images of each well were acquired with a Cytation 5 equipment for analysis.

### S2H97 prophylactic protection in Syrian hamsters

We used a validated SARS-CoV-2 Syrian Golden hamster model of infection<sup>81,82</sup> to test S2H97 prophylactic efficacy. Experiments were performed in the high-containment A3 and BSL3+ facilities of the KU Leuven Rega Institute (3CAPS) under licenses AMV 30112018 SBB 219 2018 0892 and AMV 23102017 SBB 219 20170589 according to institutional guidelines.

Syrian hamsters (*Mesocricetus auratus*) were purchased from Janvier Laboratories. Hamsters were housed per two in ventilated isolator cages (IsoCage N Biocontainment System, Tecniplast) with ad libitum access to food, water, and cage enrichment (wood block). Housing conditions and experimental procedures were approved by the ethical committee of animal experimentation of KU Leuven (license P065-2020). Sample sizes of 6 hamsters was determined in order to have a significant difference of at least 1 log viral RNA level (effect size  $d=2.004$ ) between control and treatment groups, by using a 2-tail t-test with 80% power and an alpha of 0.05, calculated with G\*Power 3.1 software. 6-10-week-old female hamsters were randomized for administration of 25 mg/kg S2H97 antibody or 20 mg/kg human isotype control via intraperitoneal injection. Approximately 5 h before infection, animals were anesthetized with isoflurane to allow collection of a blood sample from the jugular vein to be used for antibody quantification. Forty-eight hours post antibody injection, hamsters were infected intranasally with  $1.89 \times 10^6$  TCID<sub>50</sub> SARS-CoV-2 virus in 50 µL inoculum. The challenge virus was

a SARS-CoV-2 Wuhan isolate from February, 2020 (EPI\_ISL\_407976), passaged on Vero E6 cells. Passage 6 stock titer was determined by end-point dilution on Vero E6 cells by the Reed and Muench method<sup>83</sup>, expressed as 50% tissue culture infectious dose (TCID<sub>50</sub>).

Hamsters were monitored for appearance, behavior, and weight. At day 4 post-infection, hamsters were euthanized by intraperitoneal injection of 500 µL Dolethal (200 mg/mL sodium pentobarbital, Vétocquinol SA). Lungs were collected, homogenized via bead disruption (Precellys) in 350 µL RLT buffer (RNeasy Mini kit, Qiagen) and centrifuged (10,000 rpm, 5 min, 4 °C) to pellet cell debris. RNA was extracted using a NucleoSpin kit (Macherey-Nagel) according to manufacturer instructions. RT-qPCR was performed on a LightCycler96 platform (Roche) using the iTaq Universal Probes One-Step RT-qPCR kit (BioRad) with N2 primers and probes targeting the nucleocapsid<sup>81</sup>. Standards of SARS-CoV-2 cDNA (IDT) were used to express viral genome copies per mg tissue. To quantify infectious SARS-CoV-2 particles, endpoint titrations were performed on confluent Vero E6 cells in 96-well plates. Viral titers were calculated as above, and were expressed as TCID<sub>50</sub> per mg tissue. The circulating antibody levels were measured by Mesoscale bridging ELISA, using an anti-human LS mutation mAb as a capture and anti-human CH2 mAb as detection. Technicians performing RNA, virus, and antibody quantification were blinded to the treatment groups of processed samples. RNA and viral levels were compared between treatment and control via 2-tailed Mann-Whitney test, excluding the two treatment animals with undetectable S2H97 levels at time of viral challenge.

### Blockade of binding serology competition assays

Sera blockade of antibody binding was performed as described in Piccoli et al.<sup>15</sup>. Briefly, human IgG1 antibodies were biotinylated using the EZ-link NHS-PEO solid phase biotinylation kit (Pierce). Each labeled antibody was tested for binding to RBD by ELISA, and a concentration for each antibody competition experiment was selected to achieve 80% maximal binding (EC<sub>80</sub>). ELISA 96-well plates (Corning) were pre-coated overnight at 4 °C with 1 µg/mL of mouse Fc-tagged RBD antigen (Sino Biological) in PBS. Unlabeled sera/plasma were serially diluted and added to ELISA plates for 30 min, followed by addition of biotinylated anti-RBD antibody at its EC<sub>80</sub> concentration. After 30 min incubation, plates were washed and antibody binding was detected using alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch). Plates were washed, pNPP substrate (Sigma-Aldrich) was added, and plates were read at 405 nm. The percentage of inhibition of antibody binding was calculated as:  $(1 - (\text{OD}_{\text{sample}} - \text{OD}_{\text{neg ctrl}}) / (\text{OD}_{\text{pos ctrl}} - \text{OD}_{\text{neg ctrl}})) \times 100$ .

### Selection of VSV-SARS-CoV-2 monoclonal antibody resistance mutants (MARMS)

VSV-SARS-CoV-2 S chimera was used to select for SARS-CoV-2 S monoclonal antibody resistant mutants (MARMS) as previously described<sup>184</sup>. Briefly, MARMS were recovered by plaque isolation on Vero E6 cells (ATCC, CRL-1586) with the indicated mAb in the overlay. The concentration of mAb in the overlay was determined by neutralization assays at a multiplicity of infection (MOI) of 100. Escape clones were plaque-purified on Vero cells (ATCC, CCL-81) in the presence of mAb, and plaques in agarose plugs were amplified on MA104 cells (Gift from Harry Greenberg) with the mAb present in the medium. Viral stocks were amplified on MA104 cells at an MOI of 0.01 in Medium 199 containing 2% FBS and 20 mM HEPES pH 7.7 (Millipore Sigma) at 34 °C. Viral supernatants were harvested upon extensive cytopathic effect and clarified of cell debris by centrifugation at 1,000 xg for 5 min. Aliquots were maintained at -80 °C. Viral RNA was extracted from VSV-SARS-CoV-2 mutant viruses using RNeasy Mini kit (Qiagen), and S was amplified using OneStep RT-PCR Kit (Qiagen). The mutations were identified by Sanger sequencing (GENEWIZ). Their resistance was verified by subsequent virus infection in the presence or absence of antibody. Briefly,

# Article

Vero cells were seeded into 12 well plates for overnight. The virus was serially diluted using DMEM and cells were infected at 37 °C for 1 h. Cells were cultured with an agarose overlay in the presence or absence of mAb at 34 °C for 2 days. Plates were scanned on a biomolecular imager and expression of eGFP is shown at 48 h post-infection. The S2X58-selected mutation S494L is not shown in Fig. 3a, as its effect on RBD expression was below the deep mutational scanning computational filter.

## Viral replication fitness assays

Vero E6 cells (ATCC, CRL-1586) were seeded at  $1 \times 10^6$  cells per well in 6-well plates. Cells were infected with multiplicity of infection (MOI) of 0.02, with WT and four mutant VSV-SARS-CoV-2 S chimeras mixed at equal (0.20) frequencies. Following 1 h incubation, cell monolayers were washed three times with HBBS and cultures were incubated for 72 h in humidified incubators at 34 °C. To passage the progeny viruses, virus mixture was continuously passaged four times in Vero E6 cells at MOI of 0.02. Cellular RNA samples from each passages were extracted using RNeasy Mini kit (QIAGEN) and subjected to next-generation sequencing as described previously to confirm the introduction and frequency of substitutions<sup>84</sup>.

## Molecular dynamics simulations

Full details of molecular dynamics workflow and analysis are available on GitHub: <https://github.com/choderalab/rbd-ab-contact-analysis>. The RBD:S309 complex was constructed from PDB ID 7JX3 (Chains A, B, and R). 7JX3 was first refined using ISOLDE<sup>67</sup>. Refinement included adjusting several rotamers, flipping several peptide bonds, fixing several weakly resolved waters, and building in a missing four-residue-long loop. Though the N343 glycan N-Acetylglucosamine (NAG) was present in 7JX3, ISOLDE was used to construct a complex glycan at N343. The full glycosylation pattern was determined from Shajahan et al.<sup>85</sup> and Watanabe et al.<sup>86</sup>. The glycan structure used for N343 (FA2G2) corresponds to the most stable conformer obtained from multi microsecond molecular dynamics (MD) simulations of cumulative sampling<sup>87</sup>. The base NAG residue in FA2G2 was aligned to the corresponding NAG stub in the RBD:S309 model and any resulting clashes were refined in ISOLDE. The same process was repeated for the RBD:S2H97 crystal structure.

The refined glycosylated RBD:S309 and RBD:S2H97 complexes were prepared for simulation using tleap from AmberTools20<sup>88</sup>. All relevant disulfide bridges and covalent connections in glycan structures were specified. The glycosylated proteins were parameterized with the Amber ff14SB<sup>89</sup> and GLYCAM\_06j-1<sup>90</sup> force fields. The systems were solvated using the TIP3P rigid water model<sup>91</sup> in a truncated octahedral box with 2.2 nm solvent padding on all sides. The solvent box's shape and size were chosen to prevent the protein complex from interacting with its periodic image. The solvated systems were then neutralized with 0.15 M NaCl using the Li/Merz ion parameters of monovalent ions for the TIP3P water model (12-6 normal usage set)<sup>92</sup>. Virtual bonds were added across chains that should be imaged together to aid the post-processing of trajectories.

Each system was energy-minimized with an energy tolerance of 10 kJ mol<sup>-1</sup> and equilibrated five times independently using the OpenMMTools 0.20.0 (<https://github.com/choderalab/openmmtools>) BAOAB Langevin integrator<sup>93</sup> for 20 ns in the NPT (p=1 atm, T=310 K) ensemble with a timestep of 4.0 femtoseconds, a collision rate of 1.0 picoseconds<sup>-1</sup>, and a relative constraint tolerance of  $1 \times 10^{-5}$ . Hydrogen atom masses were set to 4.0 amu by transferring mass from connected heavy atoms, bonds to hydrogen were constrained, and center of mass motion was not removed. Pressure was controlled by a molecular-scaling Monte Carlo barostat with an update interval of 25 steps. Non-bonded interactions were treated with the Particle Mesh Ewald method<sup>94</sup> using a real-space cutoff of 1.0 nm and the OpenMM default relative error tolerance of 0.0005, with grid spacing selected automatically. The simulations were subsequently packaged to seed for production simulation on Folding@home<sup>95,96</sup>. Default parameters were used unless noted otherwise.

The equilibrated structures (five per complex) were used to initiate parallel distributed MD simulations on Folding@home<sup>95,96</sup>. Simulations were run with OpenMM 7.4.2 (compiled into Folding@home core22 0.0.13). Production simulations used the same Langevin integrator as the NPT equilibration described above. 5000 and 4985 independent MD simulations were generated on Folding@home for RBD:S309 and RBD:S2H97, respectively. Conformational snapshots (frames) were stored at an interval of 1 ns/frame for subsequent analysis. The final datasets contained 1.1 ms and 623.7  $\mu$ s of aggregate simulation time for RBD:S309 and RBD:S2H97, respectively. This trajectory dataset (without solvent) are available at the MolSSI COVID-19 Molecular Structure and Therapeutics Hub: <https://covid.molssi.org/simulations/#foldinghome-simulations-of-the-sars-cov-2-spike-rbd-bound-to-mono-clonal-antibody-s309> and <https://covid.molssi.org/simulations/#foldinghome-simulations-of-the-sars-cov-2-spike-rbd-bound-to-mono-clonal-antibody-s2h97>.

The first 100 ns of each trajectory was discarded (to allow relaxation away from the crystal structure), yielding total simulation times of 644.3 and 262.9  $\mu$ s used for analysis of RBD:S309 and RBD:S2H97 systems, respectively. All trajectories had solute structures aligned to their first frame and centered using MDTraj<sup>97</sup>. Residues were considered to be at the interface if they were within 10 Å of any antibody Fab / RBD residue (with the exception of RBD N343 glycans, where all glycan residues were considered). The minimum distance of heavy atoms between every pair of interface residues was computed for every frame (1 ns) using MDAnalysis<sup>98,99</sup>. A close contact was counted if the minimum distance between a residue pair was below 3.5 Å (if one of the residues was hydrophobic, a 4.5 Å cutoff was used). The contribution of each RBD residue to close contacts was calculated as a percentage by summation of the number of close contacts for a particular RBD residue and normalizing by the total number of close contact interactions over all frames of each simulation.

## Materials Availability

The SARS-CoV-2 RBD mutant libraries (#1000000172) and unmutated parental plasmid (#166782) are available on Addgene. Other materials generated in this study will be made available on request and may require a material transfer agreement

## Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

## Data Availability

Interactive escape maps and structural visualizations can be found at: [https://jbloomlab.github.io/SARS-CoV-2-RBD\\_MAP\\_Vir\\_mAbs/](https://jbloomlab.github.io/SARS-CoV-2-RBD_MAP_Vir_mAbs/). Raw Illumina sequencing data from deep mutational scanning experiments are available on NCBI SRA, BioSample SAMN18315604 (SARS-CoV-2 mutant selection data) and BioSample SAMN18316011 (sarbecovirus RBD selection data). PacBio sequencing data used to link N16 barcodes to sarbecovirus RBD variant are available on NCBI SRA, BioSample SAMN18316101. Complete table of deep mutational scanning antibody escape fractions is provided on GitHub: [https://github.com/jbloomlab/SARS-CoV-2-RBD\\_MAP\\_Vir\\_mAbs/blob/main/results/supp\\_data/all\\_antibodies\\_raw\\_data.csv](https://github.com/jbloomlab/SARS-CoV-2-RBD_MAP_Vir_mAbs/blob/main/results/supp_data/all_antibodies_raw_data.csv). This table includes both antibodies first described in this study (Fig. 1b,c), and all other antibody selections that were re-processed to generate Fig. 4a. The X-ray structure data and model has been deposited with accession code PDB 7R6W for RBD-S2X35-S309, PDB 7M7W for RBD-S2H97-S2X259 and PDB 7R6X for RBD-S2E12-S304-S309. CryoEM structure data and model are available with accession codes EMD-24300 for S/S2D106, EMD-24299 and PDB 7R7N for the S/S2D106 local refinement, and EMD-24301 for S/S2H97. The raw and processed molecular dynamics trajectory data are available at the MolSSI COVID-19 Molecular Structure and Therapeutics

Hub: <https://covid.molssi.org//simulations/#foldinghome-simulations-of-the-sars-cov-2-spike-rbd-bound-to-monoclonal-antibody-s309> and <https://covid.molssi.org//simulations/#foldinghome-simulation-s-of-the-sars-cov-2-spike-rbd-bound-to-monoclonal-antibody-s2h97>. Source data for Fig. 2f and Extended Data Fig. 6h are provided with the paper. All other datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Source data are provided with this paper.

## Code Availability

Repository containing all code, analysis, and summary notebooks for the analysis of the SARS-CoV-2 deep mutational scanning escape selections available on GitHub: [https://github.com/jbloomlab/SARS-CoV-2-RBD\\_MAP\\_Vir\\_mAbs](https://github.com/jbloomlab/SARS-CoV-2-RBD_MAP_Vir_mAbs). Repository containing code and analysis of the sarbecovirus RBD library binding experiments available on GitHub: [https://github.com/jbloomlab/SARSr-CoV\\_RBD\\_MAP](https://github.com/jbloomlab/SARSr-CoV_RBD_MAP). Repository containing code and analysis of molecular dynamics simulations is available on GitHub: <https://github.com/choderlab/rbd-ab-contact-analysis>.

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**Author contributions** Conceived research and designed study: TNS, NC, HWV, DC, JDB, GS. Antibody discovery: FZ, DP, MB, RM, ADM, EC, MSP, DC. Expression and purification of proteins: NC, PH, GL, NS, JEB, ACW, KC, SJ, MM. Antibody functional experiments: YJP, ZL, FZ, DP, MB, RM, JEB, MAT, ACW, JAW, ADM, LER, JZ, MMR, HK, JD, HT, JB, CSF, MPH, MA, ED, SS, CHD, LP, FB, FAL, SPJW. Deep mutational scanning experiments and analysis: TNS, AA, AJG, ASD. Hamster model: RA, SCF, FB, JN. Bioinformatics analysis: JDI, AT. Structure determination: NC, YJP, PH, JEB, TIC, JCN, DV, GS. Molecular dynamics simulation and analysis: WGG, IZ, JDC. Supervision: MSP, JDC, CMH, SPJW, DV, DC, JDB, GS. Wrote the initial draft: TNS, NC, DC, JDB, GS. Edited the final version: all authors.

**Competing interests** NC, FZ, DP, MB, PH, RM, JAW, ADM, LER, JZ, MMR, HK, JD, HT, JB, CSF, MPH, JDI, GL, MA, NS, KC, SJ, MM, ED, EC, CHD, LP, FB, AT, FAL, MSP, CMH, HWV, DC and GS are or were employees of Vir Biotechnology and may hold shares in Vir Biotechnology. DC is currently listed as an inventor on multiple patent applications, which disclose the subject matter described in this manuscript. After the submission of the initial version of this study, JDB began consulting for Moderna on viral evolution and epidemiology. JDB has the potential to receive a share of IP revenue as an inventor on a Fred Hutchinson Cancer Research Center-licensed technology/patent (application WO2020006494) related to deep mutational

scanning of viral proteins. HWV is a founder of PierianDx and Casma Therapeutics. Neither company provided funding for this work nor is performing related work. JCN, TIC, and DV are consultants for Vir Biotechnology Inc. The Veesler laboratory has received a sponsored research agreement from Vir Biotechnology Inc. JDC is a current member of the Scientific Advisory Boards of OpenEye Scientific Software, Interline Therapeutics, and Redesign Science. The Chodera laboratory receives or has received funding from the National Institute of Health, the National Science Foundation, the Parker Institute for Cancer Immunotherapy, Relay Therapeutics, Entasis Therapeutics, Silicon Therapeutics, EMD Serono (Merck KGaA), AstraZeneca, Vir Biotechnology, XtalPi, Interline Therapeutics, and the Molecular Sciences Software Institute, the Starr Cancer Consortium, the Open Force Field Consortium, Cycle for Survival, a Louis V. Gerstner Young Investigator Award, and the Sloan Kettering Institute. A complete funding history for the Chodera lab can be found at <http://choderalab.org/funding>. The other authors declare no competing interests.

## Additional information

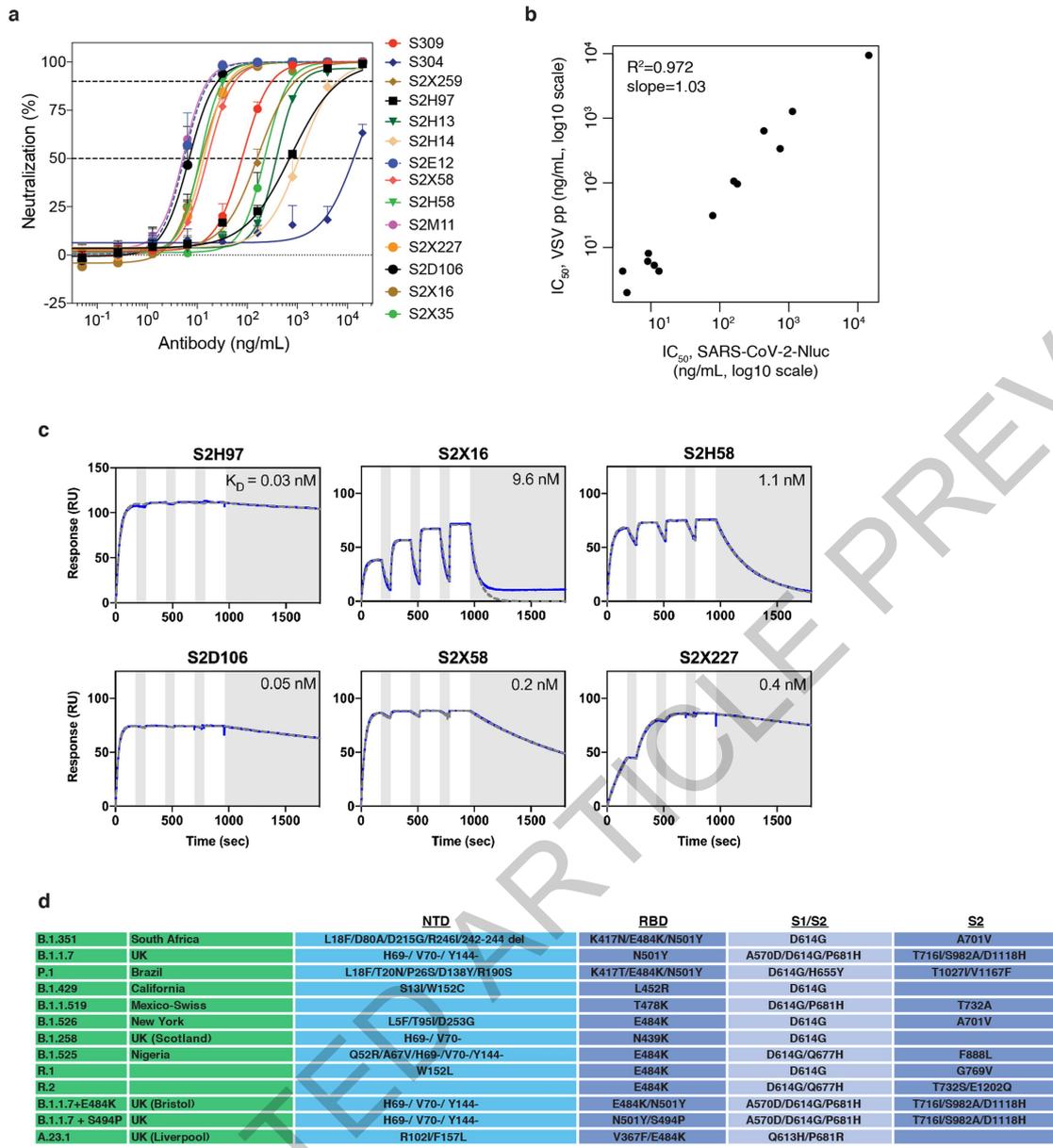
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**Correspondence and requests for materials** should be addressed to D.C., J.D.B. or G.S.

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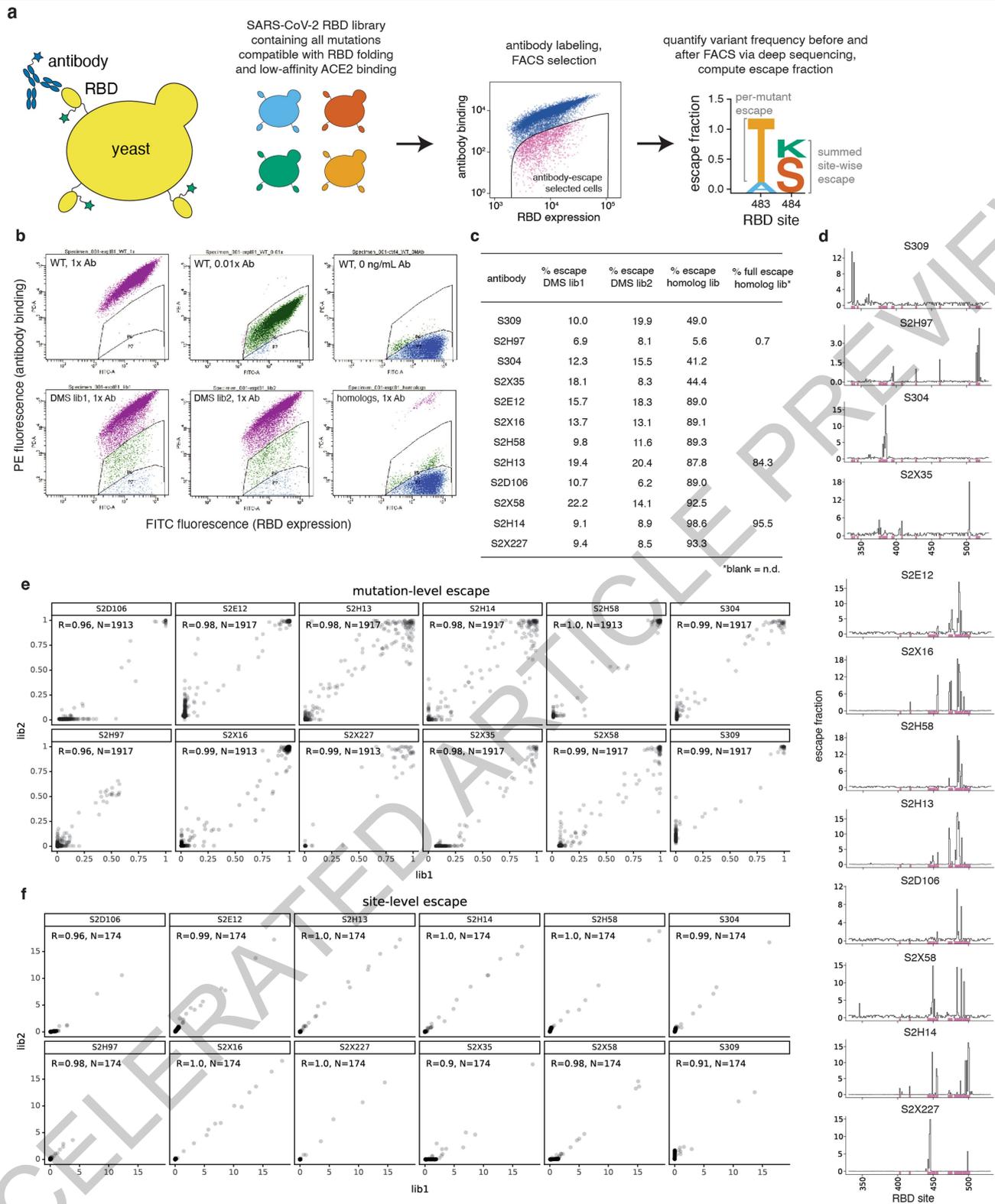
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**Extended Data Fig. 1 | Antibody neutralization and binding data.**

**a**, Neutralization of authentic SARS-CoV-2 (SARS-CoV-2-Nluc) by 14 antibodies. Shown are representative live virus neutralization plots, measured with entry into Vero E6 cells. Symbols are means  $\pm$  SD of technical triplicates. Dashed lines indicate  $IC_{50}$  and  $IC_{90}$  values. All antibodies were measured at each concentration point in the series, with hidden points due to overplotting reflecting overlap at the upper and lower neutralization limits. **b**, Correlation in antibody neutralization  $IC_{50}$  as determined in spike-pseudotyped VSV particles

( $n = 3$  to 8) versus authentic SARS-CoV-2 ( $n = 3$ ). **c**, Representative SPR sensorgrams of Fab fragments of the six newly described antibodies binding to the SARS-CoV-2 RBD. White and gray stripes indicate association and dissociation phases, respectively. Binding affinities for previously described antibodies shown in Fig. 1a are consistent with measurements from Piccoli et al. (S304, S309, S2X35, S2H13, S2H14)<sup>15</sup> and Tortorici et al. (S2E12)<sup>8</sup>. **d**, Identifiers and spike genotypes of SARS-CoV-2 variants tested in neutralization assays in Figs. 2d and 3b.

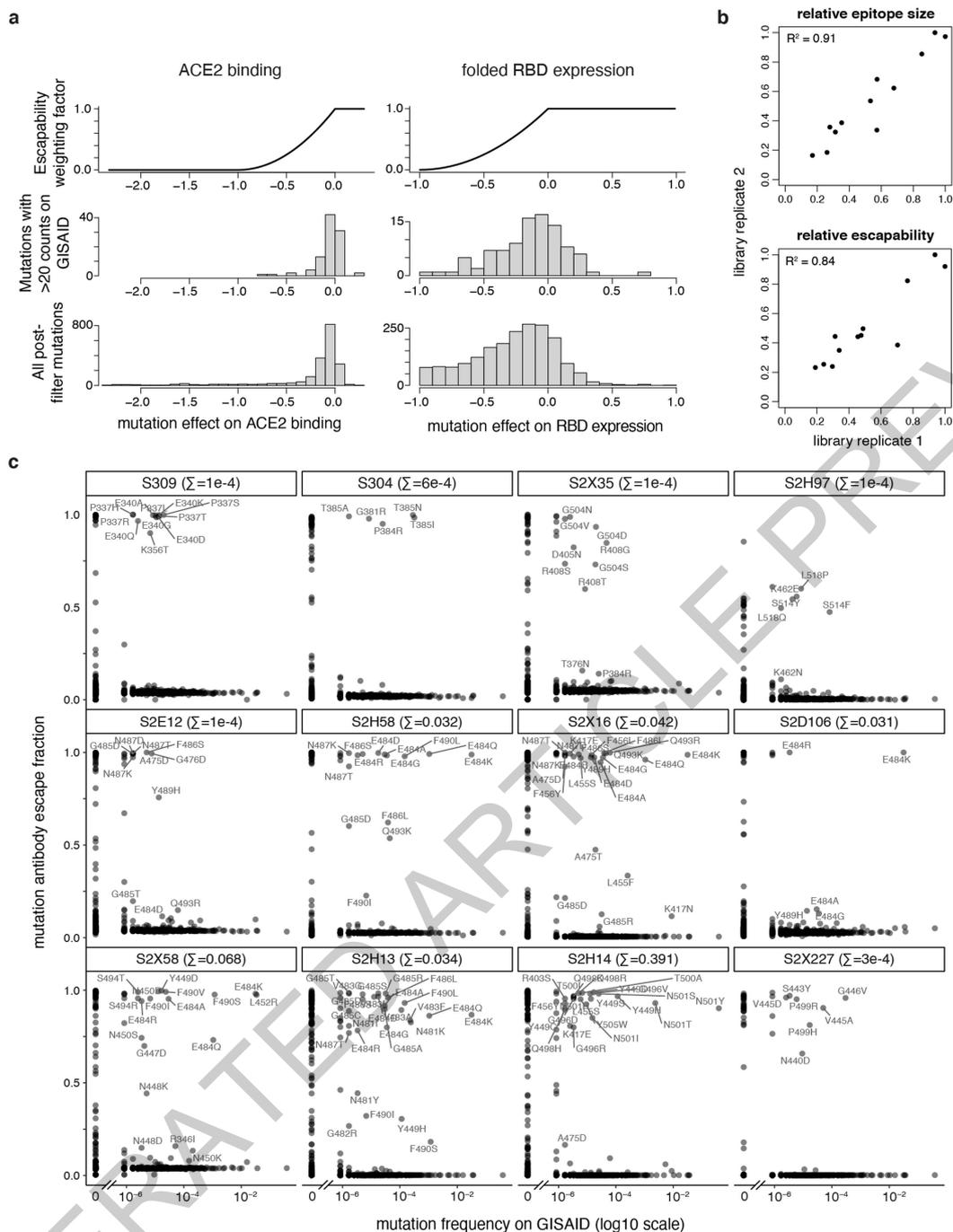


Extended Data Fig. 2 | See next page for caption.

**Extended Data Fig. 2 | Deep mutational scanning to map mutations that escape antibody binding.** **a**, Scheme of the deep mutational scanning assay. Conformationally intact RBD is expressed on the surface of yeast, where RBD expression and antibody binding is detectable via fluorescent labeling. We previously constructed mutant libraries containing virtually all of the 3,819 possible amino acid mutations in the SARS-CoV-2 RBD<sup>26</sup> and sorted the library to eliminate mutations that destabilize the RBD or strongly reduce ACE2-binding affinity<sup>3</sup>. We incubate the library with a sub-saturating antibody concentration and use fluorescence-activated cell sorting (FACS) to isolate yeast cells expressing RBD mutants with reduced antibody binding. Deep sequencing quantifies mutant frequencies before and after FACS selection, enabling calculation of the "escape fraction" of each amino acid mutation, which reflects the fraction of cells carrying that mutation that fall into the antibody-escape bin. Mutation escape fractions are represented in logoplots, where the height of a letter reflects the extent of escape from antibody

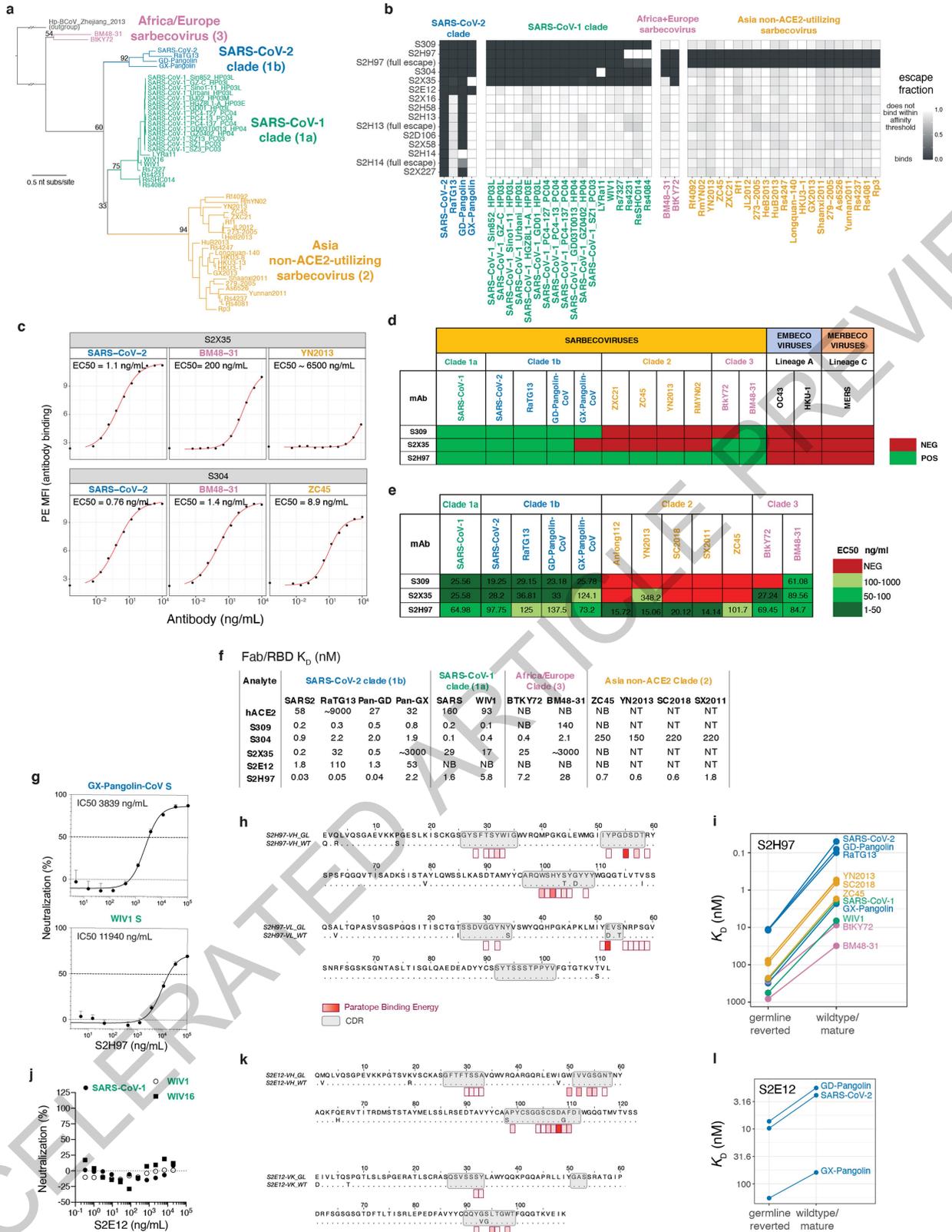
binding. **b**, Representative selection gates, after gating for single cells expressing RBD as in Greaney et al.<sup>3</sup>. Yeast expressing the SARS-CoV-2 RBD (top panels) are labeled at 1x, 0.01x and no antibody to guide selection gates. Mutant RBDs that reduce binding (green, gate drawn to capture 0.01x WT control) are sorted and sequenced for calculation of mutant escape fractions. This same gate was used to quantify escape within libraries of yeast expressing all sarbecovirus RBD homologs. For several antibodies, we also selected the sarbecovirus RBD library with a more stringent "full escape" gate (blue, gate drawn to capture 0 ng/mL WT control). **c**, Fraction of library cells falling into escape bins for each antibody selection. **d**, Line plots showing total escape at all RBD sites for each antibody. Sites of strong escape illustrated in logoplots in Fig. 1b,c shown with pink indicators. **e,f**, Correlation in per-mutation (**e**) and per-site (**f**, sum of per-mutation) escape fractions for duplicate libraries that were independently generated and assayed. *N*, number of mutations (**e**) or sites (**f**) in the correlation.

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**Extended Data Fig. 3 | Antibody escapability from deep mutational scanning measurements and in natural SARS-CoV-2 mutants.** **a**, To calculate antibody escapability (Fig. 1b,c), mutation escape fractions were weighted by their deleterious consequences for ACE2 binding or RBD expression. Top plots show the weighting factor (y-axis) for mutation effects on ACE2 binding (left) and RBD expression (right). This weight factor was multiplied by the mutation escape fraction in the summation to calculate antibody escapability as described in the Methods. Histograms show the distribution of mutation effects on ACE2 binding (left) and RBD expression (right) for all mutations that pass our computational filtering steps (bottom), and mutations that are found with at least 20 sequence counts on GISAID (middle). **b**, Correlation in antibody

relative epitope size (top) and escapability (bottom) calculated from independent deep mutational scanning replicates, compared to the averaged replicates shown in Fig. 1b,c.  $R^2$ , squared Pearson correlation coefficient. **c**, Scatterplots illustrate the degree to which a mutation escapes antibody binding (escape fraction, y-axis) and its frequency among 1,190,241 high-quality human-derived SARS-CoV-2 sequences present on GISAID as of May 2, 2021. Large escape mutations (>5x global median escape fraction) for each antibody with non-zero mutant frequencies are labeled. Plot labels report the sum of mutant frequencies for all labeled mutations, corresponding to the natural SARS-CoV-2 mutant escape frequency for antibodies shown in Fig. 4d,g.



Extended Data Fig. 4 | See next page for caption.

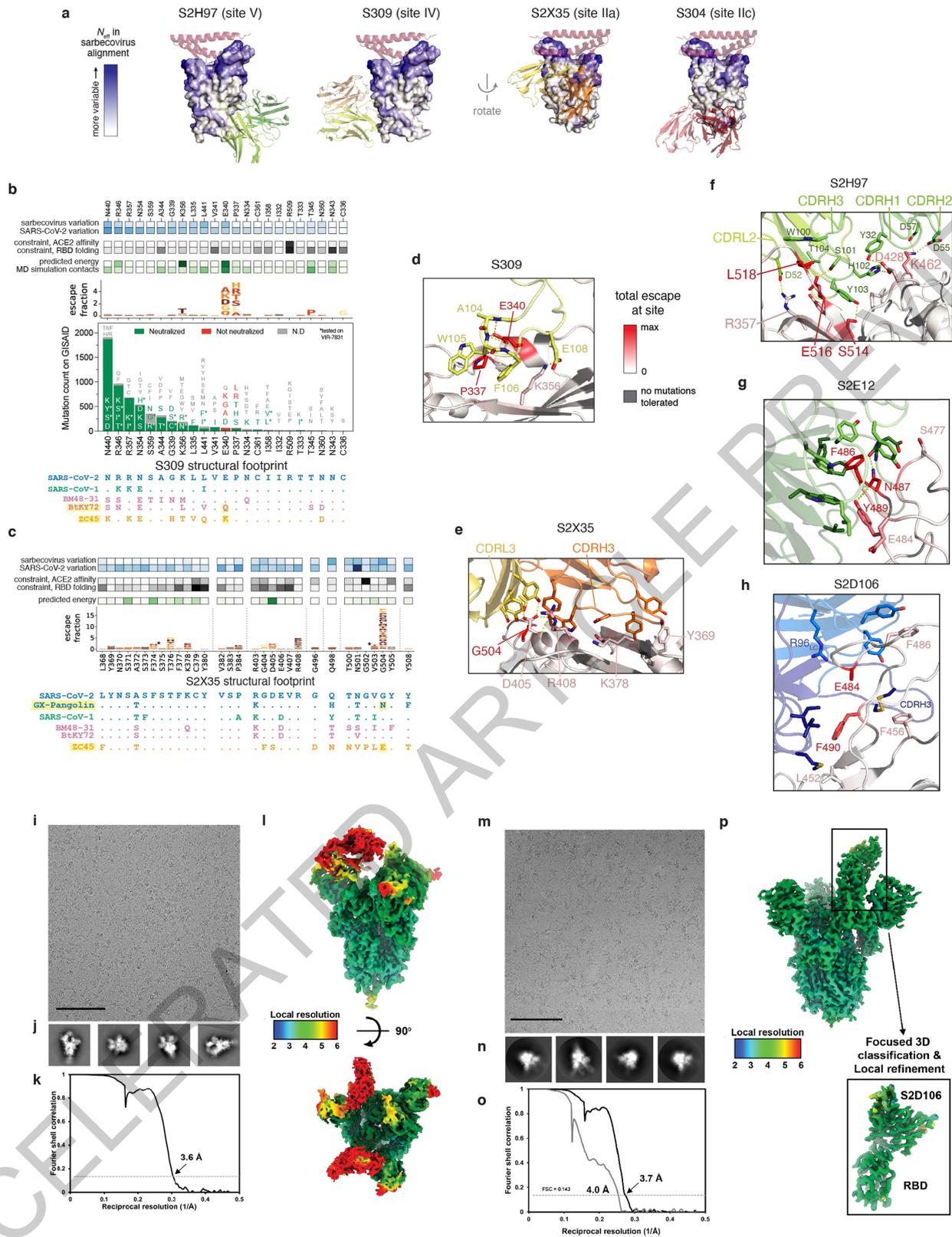
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### Extended Data Fig. 4 | Breadth of antibody binding across sarbecoviruses.

**a**, Phylogenetic relationship of sarbecovirus RBDs inferred from aligned nucleotide sequences, with the four sarbecovirus clades labeled in separate colors used throughout the text. Node support values are bootstrap support values. **b**, Breadth of sarbecovirus binding by each antibody to a panel of yeast-displayed sarbecovirus RBDs. Data as in Fig. 1d, with the addition of secondary “full escape” selection data for S2H97, S2H13, and S2H14 (0 ng/mL WT control, Extended Data Fig. 2b,c), enabling differentiation of RBDs with intermediate binding (e.g., S2H97/RsSHC014) versus complete loss of binding. Escape fractions are calculated as the mean of replicate barcoded genotypes internal to the library. Median number of barcodes per RBD is 249, with a range of 104 to 566. The median SEM across escape fraction measurements is 0.019, with a range of 0.00005 to 0.038 across all RBD/antibody pairs. **c**, Flow cytometry detection of antibody binding to isogenic yeast-displayed RBD variants. **d**, Flow cytometry detection of antibody binding to mammalian-surface displayed spikes. **e**, ELISA binding of antibody to purified RBD proteins. **f**, SPR measurement of binding of cross-reactive antibodies (Fab) and human ACE2 to

select sarbecovirus RBDs. NB, no binding; NT, not tested. Values from single replicates. **g**, S2H97 neutralization of VSV pseudotyped with select sarbecovirus spikes, with entry measured in ACE2-transduced BHK-21 cells. Curves are representative of two independent experiments. Points represent means, error bars standard deviation from three technical replicates, and IC50 geometric mean of experiments. IC50 values are not comparable to other experiments on Vero E6 cells (e.g. Fig. 2c) due to ACE2 overexpression and its impact on S2H97 neutralization. **h**, Alignment of germline-reverted and mature S2H97 heavy- (top) and light-chain (bottom) amino acid sequences. CDR sequences shown in grey box. Heatmap overlay indicates the predicted energetic contribution of antibody paratope residues from the crystal structure. **i**, Binding of germline-reverted and mature S2H97 to select sarbecovirus RBDs as measured by SPR. **j**, Neutralization of select sarbecoviruses by S2E12 (spike-pseudotyped VSV on 293T-ACE2 cells). Details as in Fig. 3c. **k**, Alignment of germline-reverted and mature S2E12. Details as in (h). **l**, Binding of germline-reverted and mature S2E12 to select sarbecovirus RBDs as measured by SPR.

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Extended Data Fig. 5 | See next page for caption.

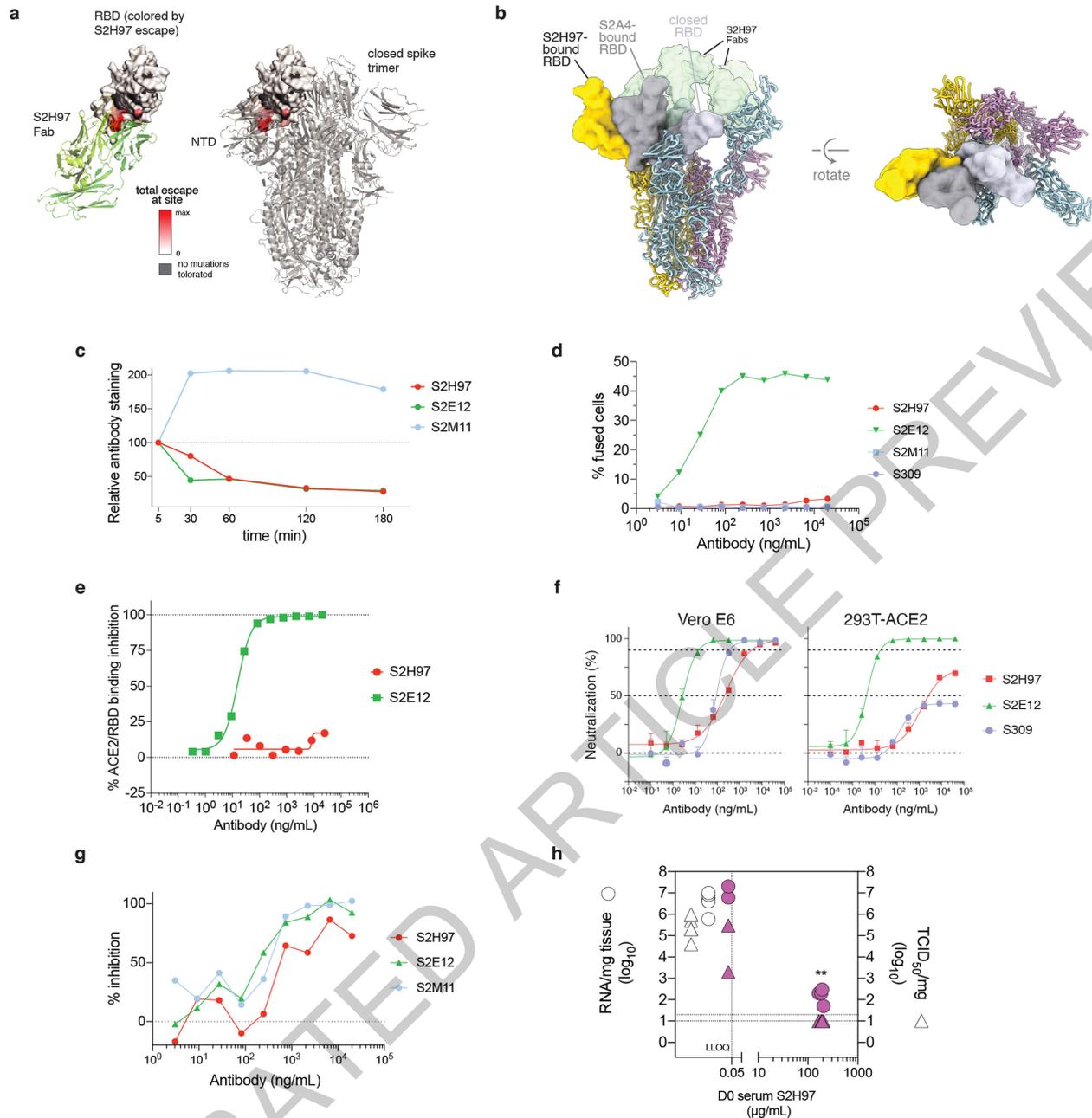
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### Extended Data Fig. 5 | Structures and epitopes of Fab:RBD complexes.

**a**, Surfaces targeted by broadly binding RBD antibodies. RBD surface is colored by site variability across sarbecoviruses. ACE2 key motifs shown in transparent red cartoon. Antibody variable domains shown as cartoon, with darker shade indicating the heavy chain. **b,c**, Integrative features of the S309 (**b**) and S2X35 (**c**) structural epitopes. Details as in Fig. 3g,h and Fig. 2b. **d-h**, Zoomed in view of the RBD bound to S309 (**d**), S2X35 (**e**), S2H97 (**f**), S2E12 (**g**), and S2D106 (**h**), with important contact and escape residues labeled. RBD residues colored by total site escape [scale bar, right of (**d**)]. **i,j**, Representative electron micrograph and 2D class averages of SARS-CoV-2 S in complex with the S2H97 Fab embedded in vitreous ice. Scale bar: 400 Å. Micrographs representative of 3138 micrographs. **k**, Gold-standard Fourier shell correlation curve for the S2H97-

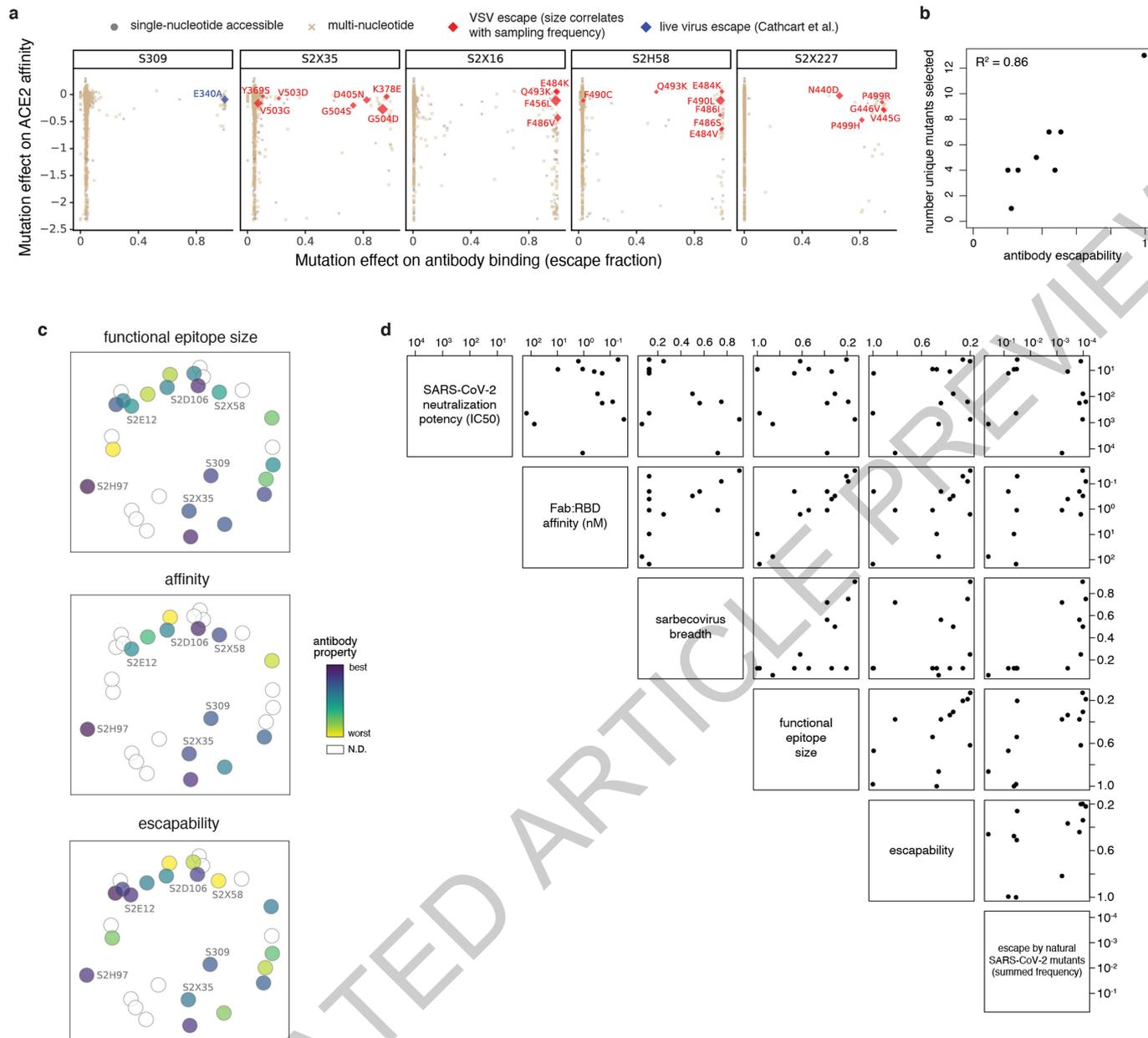
bound SARS-CoV-2 S trimer reconstruction. The 0.143 cutoff is indicated by a horizontal dashed line. **l**, Local resolution map calculated using cryoSPARC for the whole reconstruction with two orthogonal orientations. **m,n**, Representative electron micrograph and 2D class averages of SARS-CoV-2 S in complex with the S2D106 Fab embedded in vitreous ice. Scale bar: 400 Å. Micrographs representative of 2166 micrographs **o**, Gold-standard Fourier shell correlation curves for the S2D106-bound SARS-CoV-2 S trimer (black line) and locally refined RBD/S2D106 variable domains (gray line). The 0.143 cutoff is indicated by a horizontal dashed line. **p**, Local resolution map calculated using cryoSPARC for the whole reconstruction and the locally refined RBD/S2D106 variable domain region.

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**Extended Data Fig. 6 | Mechanism of action of S2H97 neutralization and protection.** **a**, Quaternary context of the S2H97 epitope. Left, S2H97-bound RBD, with RBD sites colored by S2H97 escape (scale bar, bottom). Right, RBD in the same angle as left, in the closed spike trimer. **b**, CryoEM structure of S2H97 Fabs (green surfaces) bound to SARS-CoV-2 S indicating the extensive opening of the RBD (yellow surface) necessary to access the S2H97 epitope. Closed RBD (light purple surface, PDB 7K43) and site II Fab S2A4 bound open RBD (gray surface, PDB 7JVC) are shown for comparison. Spike protomers are shown in yellow, blue, and pink. **c**, Antibody-mediated  $S_1$  shedding from cell-surface expressed SARS-CoV-2 S as determined by flow cytometry. **d**, Cell-cell fusion of CHO cells expressing SARS-CoV-2 S (CHO-S) incubated with variable concentrations of antibody. **e**, Antibody competition with RBD-ACE2 binding determined by ELISA. Points represent mean of technical duplicates. **f**, S2H97 neutralization of SARS-CoV-2 S pseudotyped VSV on ACE2-overexpressing cells

(293T-ACE2) compared to Vero E6 cells. Points reflect mean and error bars reflect standard deviation from triplicate measurements. Curves are representative of two biological replicates. **g**, Antibody inhibition of cell-to-cell fusion of Vero E6 cells transfected with SARS-CoV-2 S. **h**, Influence of circulating S2H97 level on prophylactic efficacy in Syrian hamsters. Infectious virus titers (right y-axis, triangles) and RNA levels (left y-axis, circles) reflect the data represented in Fig. 2f, measured in hamsters four days after SARS-CoV-2 challenge in animals prophylactically dosed with 25 mg/kg S2H97 (magenta symbols) or isotype control (white symbols). The levels of circulating S2H97 (D0, before infection,  $\mu\text{g/mL}$ ) are shown on the x-axis (LLOQ, lower level of quantification). \*\*  $p=0.0048$  (virus titer) and  $p=0.0048$  (RNA) vs control isotype, two-sided Mann-Whitney test (the 2 animals shown with no detectable serum antibody were excluded from the comparison).



**Extended Data Fig. 7 | Escapability and the relationships among antibody properties.** **a**, Additional spike-VSV viral escape selections, as in Fig. 3a, and an illustration of the authentic SARS-CoV-2 escape data for S309 reported in Cathcart et al.<sup>22</sup>. **b**, Correlation between the number of unique mutations selected across viral escape selection experiments and antibody escapability as tabulated in Fig. 1b,c, plus S2X259<sup>37</sup>. **c**, Projected epitope space from Fig. 4a annotated by antibody properties as in Fig. 4b–d. For each property, antibodies are colored such that purple reflects the most desirable antibody (scale bar, right; N.D., not determined): narrowest functional epitope, tightest binding

affinity ( $K_D$ , log10 scale), lowest escapability. **d**, Pairwise scatterplots between all antibody properties discussed in the main text. Select scatterplots from this panel are shown in Figs. 4e–g. Details of each property described in Methods. All axes are oriented such that moving up on the y-axis and right on the x-axis corresponds to moving in the "preferred" direction for an antibody property (lower neutralization IC50, lower  $K_D$ , higher breadth, narrower epitope size, lower escapability, lower total frequency of SARS-CoV-2 escape mutants among sequences on GISAID).

**Extended Data Table 1 | Characteristics of the antibodies described in this study**

| Mab ID | Site | Cross-reactive | ACE2 blocker | VH     | HCDR3 length (amino acids) | VH identity to GL | VK/VL  | IC50 (ng/ml)       |        |                    | Spike Apparent Affinity <sup>§</sup> by SPR (nM) | RBD Affinity by SPR (nM)<br>(RBD ligand/Fab analyse: IgG ligand/RBD analyse) |                    | S1 shedding      | Effector function | RBD open vs closed | Days from symptoms onset | Somatoic Hyper Mut. H:LC |     |
|--------|------|----------------|--------------|--------|----------------------------|-------------------|--------|--------------------|--------|--------------------|--|--|--------------------|------------------|-------------------|--------------------|--------------------------|--------------------------|-----|
|        |      |                |              |        |                            |                   |        | VSV-pp             | IFituc | Fab                |  | Fab  | IgG                |                  |                   |                    |                          |                          |     |
| S2E12  | Ia   | No             | Yes          | VH1-58 | 16                         | 97.2%             | VK3-20 | 2.0 (0.9-3.4)      | 12.4   | 4.5 (3-6)          | 14.0   | 2.5 <sup>1</sup>   | 1.7 (1.61-1.76)    | 0.7              | Yes               | No                 | open                     | 51                       | 6/4 |
| S2X58  | Ib   | No             | Yes          | VH1-46 | 12                         | 99.0%             | VK1-33 | 4.3 (2.7-8.8)      | 69.0   | 13.1 (7-20)        | 49.1   | 0.6  | 0.2 (0.20-0.24)    | 0.2              | Yes               | No                 | open                     | 48                       | 3/3 |
| S2H58  | Ib   | No             | Yes          | VH1-2  | 15                         | 97.9%             | VK2-28 | 6.1 (1.7-15.7)     | 123.0  | 9.0 (6-11)         | 28.7   | 4.8  | 1.0 (0.99-1.08)    | 1.5              | No                | Yes                | NA                       | 45                       | 6/3 |
| S2D106 | Ia   | No             | Yes          | VH1-69 | 19                         | 97.2%             | VK1-39 | 4.3 (3.5-6.5)      | 25.6   | 6.8 (5-9)          | 23.6   | 0.17   | 0.05 (0.046-0.049) | 0.04             | Yes               | No                 | open                     | 98                       | 5/3 |
| S2X227 | Ib   | No             | Yes          | VH1-46 | 14                         | 97.9%             | VK4-1  | 5.3 (4.4-6.2)      | 26.9   | 11.2 (9-13)        | 36.0   | 0.11   | 0.4 (0.41-0.46)    | 0.1              | No                | Yes                | NA                       | 75                       | 3/2 |
| S2X16  | Ia   | No             | Yes          | VH1-69 | 18                         | 97.6%             | VK2-28 | 8.1 (2.6-14.9)     | 62.1   | 9.2 (4-16)         | 39.7   | 11.1   | 9.2 (8.83-9.59)    | 6.1 <sup>1</sup> | Yes               | No                 | NA                       | 48                       | 3/2 |
| S304   | Iic  | Yes            | No           | VH3-13 | 14                         | 98.6%             | VK1-39 | 9405 (8827-9795)   | n/a    | 14412 (7573-29889) | n/a  | 8.7*   | 1.0 (0.93-1.17)    | 0.4              | Yes               | No                 | open                     | 10y                      | 5/6 |
| S309   | IV   | Yes            | No           | VH1-18 | 20                         | 97.2%             | VK3-20 | 31.1 (23.0-58.7)   | 160.9  | 78.9 (52-121)      | 300.3  | -0.2*  | 0.2 (0.17-0.31)    | 0.2 <sup>1</sup> | No                | Yes                | open/closed              | 10y                      | 8/6 |
| S2X35  | Ila  | Yes            | Yes          | VH1-18 | 21                         | 98.6%             | VL1-40 | 96.2 (67.8-144.9)  | 530.5  | 179.4 (121-219)    | 592.5  | -0.1*  | 0.2 (0.18-0.22)    | 0.06             | Yes               | No                 | open                     | 48                       | 4/1 |
| S2H97  | V    | Yes            | No           | VH5-51 | 13                         | 98.3%             | VL2-14 | 337.6 (277-406)    | 3656.1 | 748.7 (540-1120)   | 3290.7   | ~2   | 0.04 (0.031-0.060) | 0.04             | Yes               | No                 | open                     | 81                       | 7/6 |
| S2H13  | Ib   | No             | Yes          | VH3-7  | 13                         | 97.9%             | VL7-46 | 637.6 (494-856)    | 6745.9 | 435.3 (331-656)    | 1390.1   | 119*   | 123 (108-149)      | 33 <sup>1</sup>  | No                | Yes                | open/closed              | 17                       | 4/1 |
| S2H14  | Ia   | No             | Yes          | VH3-15 | 17                         | 100.0%            | VL6-57 | 1274.0 (1027-1436) | 8735.3 | 1130.3 (522-2583)  | 5121.8   | 90.1*  | 71 (56-77)         | 65 <sup>1</sup>  | Yes               | No                 | open                     | 17                       | 0/0 |

VH and VL percent identity refers to V gene segment identity compared to germline (as per the International Immunogenetics Information System (<http://www.imgt.org/>)). HCDR3 length was determined using IMGT. SARS-CoV-2 neutralization potency (authentic virus [ $n=3$ ] and spike-pseudotyped VSV particles [ $n=3$  to 8] on Vero E6 cells), Fab:RBD binding affinities measured by SPR [ $n=2$  to 4]. Some binding affinities for previously described antibodies were measured in Piccoli et al. (S304, S309, S2X35, S2H13, S2H14)\*, Tortorici et al. (S2E12)<sup>1</sup> and Cathcart et al. (S309)<sup>1</sup>. Values in brackets are minimum and maximum determined values. <sup>§</sup>Spike binding data are "apparent affinity" or  $K_{d,app}$  because RBD conformational dynamics affect the kinetics. S2H97 Fab binding to spike doesn't fit well to 1:1 binding, presumably because of changing epitope accessibility. <sup>1</sup>Biphasic kinetics; Fit result is for fast phase

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Extended Data Table 2 | Crystallographic data collection and refinement statistics

|   | RBD/S2X35/S309<br>PDB 7R6W | RBD/S2H97/S2X259<br>PDB 7M7W | RBD/S2E12/S309/S304<br>PDB 7R6X |
|---|----------------------------|------------------------------|---------------------------------|
| <b>Data collection</b>                              |                            |                              |                                 |
| Space group   | C222                       | P2 <sub>1</sub>              | I4 <sub>1</sub> 22              |
| Cell dimensions                                     |                            |                              |                                 |
| <i>a</i> , <i>b</i> , <i>c</i> (Å)                  | 106.27, 239.37, 129.81     | 86.19, 66.40, 237.66         | 245.87, 245.87, 237.31          |
| $\alpha$ , $\beta$ , $\gamma$ (°)                   | 90.00, 90.00, 90.00        | 90.00, 94.34, 90.00          | 90.00, 90.00, 90.00             |
| Resolution (Å)                                      | 39.52-1.83 (1.86-1.83)     | 63.94-2.65 (2.70-2.65)       | 49.00-2.93 (2.99-2.93)          |
| <i>R</i> <sub>merge</sub>                           | 0.085 (2.96)               | 0.149/2.494                  | 0.295/7.868                     |
| <i>I</i> / $\sigma$ <i>I</i>                        | 16.2 (0.7)                 | 10.9 (0.8)                   | 13.3 (0.5)                      |
| Completeness (%)                                    | 99.6 (99.5)                | 98.6 (98.3)                  | 100.00 (100.00)                 |
| Redundancy  | 6.7 (7.0)                  | 6.9 (6.8)                    | 28.9 (27.2)                     |
| <b>Refinement</b>                                   |                            |                              |                                 |
| Resolution (Å)                                      | 1.83                       | 2.65                         | 2.95                            |
| No. reflections                                     | 135,667                    | 73,189                       | 71,532                          |
| <i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub> | 0.211/0.232                | 0.221/0.271                  | 0.232/0.262                     |
| No. atoms   |                            |                              |                                 |
| Protein   | 8160                       | 16,162                       | 9,101                           |
| Ligand/ion  | 172                        | 28                           | 16                              |
| Water   | 584                        | 95                           | 3                               |
| <i>B</i> -factors                                   |                            |                              |                                 |
| Protein   | 39.56                      | 75.86                        | 116.53                          |
| Ligand/ion  | 75.00                      | 84.00                        | 122.00                          |
| Water   | 42.94                      | 50.09                        | 65.90                           |
| R.m.s. deviations                                   |                            |                              |                                 |
| Bond lengths (Å)                                    | 0.004                      | 0.002                        | 0.003                           |
| Bond angles (°)                                     | 1.259                      | 0.817                        | 0.936                           |

\*Values in parentheses are for highest-resolution shell

**Extended Data Table 3 | CryoEM data collection, refinement, and validation statistics**

|  | SARS-CoV-2<br>S/S2D106<br>(EMD-24300) | SARS-CoV-2<br>S/S2D106<br>(local<br>refinement)<br>(EMD-24299)<br>(PDB 7R7N) | SARS-CoV-2<br>S/S2H97<br>(EMD-24301) |
|--|---------------------------------------|--|--------------------------------------|
| <b>Data collection and processing</b>            |                                       |  |                                      |
| Magnification                                    | 130,000                               | 130,000  | 36,000                               |
| Voltage (kV)                                     | 300                                   | 300  | 200                                  |
| Electron exposure (e-/Å <sup>2</sup> )           | 70                                    | 70   | 60                                   |
| Defocus range (μm)                               | -0.5 – -2.5                           | -0.5 – -2.5  | -0.5 – -3.0                          |
| Pixel size (Å)                                   | 0.525                                 | 0.525  | 1.16                                 |
| Symmetry imposed                                 | C1                                    | C1   | C1                                   |
| Initial particle images (no.)                    | 175,479                               | 87,587   | 98,950                               |
| Map resolution (Å)                               | 3.7                                   | 4.0  | 3.6                                  |
| FSC threshold                                    | 0.143                                 | 0.143  | 0.143                                |
| Map sharpening <i>B</i> factor (Å <sup>2</sup> ) | -61                                   | -17  | -70                                  |
| <b>Validation</b>                                |                                       |  |                                      |
| MolProbity score                                 |                                       | 0.89   |                                      |
| Clashscore                                       |                                       | 0.58   |                                      |
| Poor rotamers (%)                                |                                       | 0.45   |                                      |
| Ramachandran plot                                |                                       |  |                                      |
| Favored (%)                                      |                                       | 96.78  |                                      |
| Allowed (%)                                      |                                       | 3.22   |                                      |
| Disallowed (%)                                   |                                       | 0  |                                      |

## 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

- |                                     |                                     |  |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | A description of all covariates tested   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | 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

As described in Methods:

- \* SPR binding data collected and analyzed using Biacore T200 Evaluation Software (v. 3.1) and Biacore Insight Evaluation Software (v. 2.0.15)
- \* FACSDiva software (version 6.1.3) for FACS data collection and cell sorting
- \* Legion software (version 3.4) for electron microscopy data collection
- \* OpenMM (version 7.4.2) and Folding@home (core 22 0.0.13) for molecular dynamics simulation
- \* protein chromatography performed with UNICORN software (version 5.11)
- \* G\*Power 3.1 for statistical power calculations

Data analysis

- \* Deep mutational scanning data was analyzed using custom code that is available on GitHub, specifically: Repository containing all code, analysis, and summary notebooks for the analysis of the SARS-CoV-2 deep mutational scanning escape selections available on GitHub: [https://github.com/jbloomlab/SARS-CoV-2-RBD\\_MAP\\_Vir\\_mAbs](https://github.com/jbloomlab/SARS-CoV-2-RBD_MAP_Vir_mAbs). Repository containing code and analysis of the sarbecovirus RBD library binding experiments available on GitHub: [https://github.com/jbloomlab/SARSr-CoV\\_RBD\\_MAP](https://github.com/jbloomlab/SARSr-CoV_RBD_MAP)
- \* Neutralization assays, ELISA, cell biology, and serology assays analyzed using PRISM (versions 8.4.3 and 9.0.1) and Excel (version 16.45) as described in Methods.
- \* CryoEM data analysis performed with Warp (v. 1.09), Relion (v. 3.1) and CryoSPARC (version 3.2)
- \* Crystallography data analysis performed with XDS (VERSION Jan 31, 2020 BUILT=20200417), Coot (v. 0.9.5), ChimeraX (v. 1.1)/ISOLDE (v. 1.1), Refmac5 (v. 5.8.0267), and MOE (v. 2019.0102)
- \* Phylogenetic analysis performed with RAxML (version 8.2.12) and sequence alignment with MAFFT (version 7)
- \* MD simulation analyzed with custom code, available on GitHub: <https://github.com/choderalab/rbd-ab-contact-analysis>

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

- \* Raw Illumina sequencing data from deep mutational scanning experiments are available on NCBI SRA, BioSample SAMN18315604 (SARS-CoV-2 mutant selection data) and BioSample SAMN18316011 (sarbecovirus RBD selection data).
- \* PacBio sequencing data used to link N16 barcodes to sarbecovirus RBD variant are available on NCBI SRA, BioSample SAMN18316101.
- \* Complete table of deep mutational scanning antibody escape fractions is provided on GitHub: [https://github.com/jbloomlab/SARS-CoV-2-RBD\\_MAP\\_Vir\\_mAbs/blob/main/results/supp\\_data/all\\_antibodies\\_raw\\_data.csv](https://github.com/jbloomlab/SARS-CoV-2-RBD_MAP_Vir_mAbs/blob/main/results/supp_data/all_antibodies_raw_data.csv). This table includes both antibodies first described in this study (Fig. 1b,c), and all other antibody selections that were re-processed to generate Fig. 4a.
- \* The X-ray structure data and model has been deposited with accession code PDB 7R6W for RBD-S2X35-S309, PDB 7M7W for RBD-S2H97-S2X259 and PDB 7R6X for RBD-S2E12-S304-S309.
- \* CryoEM structure data and model are available with accession codes EMD-24300 for S/S2D106, EMD-24299 and PDB 7R7N for the S/S2D106 local refinement, and EMD-24301 for S/S2H97
- \* The raw and processed molecular dynamics trajectory data are available at the MolSSI COVID-19 Molecular Structure and Therapeutics Hub: <https://covid.molssi.org//simulations/#foldinghome-simulations-of-the-sars-cov-2-spike-rbd-bound-to-monoclonal-antibody-s309> and <https://covid.molssi.org//simulations/#foldinghome-simulations-of-the-sars-cov-2-spike-rbd-bound-to-monoclonal-antibody-s2h97>
- \* source data associated with hamster experiments in fig. 2f and extended data fig. 6g are included as source data provided with the paper

## 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](https://www.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     | For hamster prophylaxis experiments, sample size = 6 was determined in order to have a significant difference of at least 1 log viral RNA (Effect Size $d = 2.004$ ) between control and treated groups, by using a 2-tail T test with 80% power and an alpha error of 0.05, calculated with G*Power 3.1 software  |
| Data exclusions | For S2H97 prophylaxis experiments in hamster, two hamsters in the S2H97 arm were excluded from the statistical comparison. These hamsters had no detectable circulating antibody (LOD: 50ng/mL) at time of viral challenge (ELISA), which is a known technical failure in i.p. administration of hamster. The data justifying this exclusion in the statistical test is clearly shown in Extended Data Fig. 6h. No data were excluded from other experiments.                          |
| Replication     | Experimental assays were performed in biological duplicate or triplicate (or more) according to or exceeding standards in the field. Specifically, we perform deep mutational scans using two completely independently synthesized mutant libraries. We conducted all neutralization and antibody functional assays in biological duplicate, triplicate, or more, as indicated in relevant figure legends. In all cases, representative figure displays were appropriately replicated. |
| Randomization   | Hamsters were randomized to S2H97 and control treatment. For other experiments, randomization was not a relevant feature as we were applying a uniform set of techniques across a panel of candidate monoclonal antibodies   |
| Blinding        | The hamster protection samples for RNA quantification and viral load titration were run by technicians who were blinded to treatment group of the analyzed samples   |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials &amp; experimental systems

| n/a                                 | Involvement   |
|-------------------------------------|---|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Human research participants |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |

## Methods

| n/a                                 | Involvement  |
|-------------------------------------|--|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Antibodies

## Antibodies used

\* For antibody isolation, B cells were stained with CD19 PE-Cy7 (BD Biosciences 557835), anti-IgM (BioLegend 314508), anti-IgD (BD Bioscience 555779), anti-CD14 (BD Bioscience 562691), and anti-IgA (Southern Biotech 2050-09), and spike labeled with streptavidin Alexa-Fluor 647 (Life Technologies S21374)

\* For FACS during deep mutational scanning, we used PE-conjugated goat anti-human-IgG (Jackson ImmunoResearch 109-115-098) and FITC-conjugated chicken anti-Myc (Immunology Consultants Lab, CYMC-45F).

\* For VSV pseudotyped neutralization assays, media was supplemented with anti-VSV-G antibody (I1-mouse hybridoma supertant, ATCC CRL-2700)

\* For SPR, immobilization was performed with anti-AviTag pAb (Genscript Cat # A00674-40) and goat anti-human IgG Fc pAb, (Southern Biotech Cat # 2014-01)

\* For RBD ELISA, antibody binding was detected via goat anti-human IgG (Southern Biotech, 2040-04)

\* For antibody blockade of ACE2 binding, RBD was detected via goat anti-mouse IgG (Southern Biotech 1030-04)

\* For detection of circulating mAb levels in hamster experiments, an anti-human LS mutation antibody was custom-made by BioRad AbD Serotec, and anti-human CH2 antibody from BioRad cat# MCA5748G clone R10Z8E9

\* Monoclonal antibodies (S309, S2H97, S304, S2X35, S2E12, S2X16, S2H58, S2H13, S2D106, S2X58, S2H14, S2X227) were produced in-house using recombinant protein purification as described in the Methods. S2H97\_GL and S2E12\_GL were produced by ATUM.

## Validation

We discovered and describe six new primary antibodies in this study. Target validation of the new antibodies was done with multiple binding assays (Extended Data Table 1), in addition to cryoEM and x-ray crystallography structures determined for two of the new antibodies. The other primary antibodies used in this study were reported previously in Pinto et al. Nature (doi: 10.1038/s41586-020-2349-y.), Tortorici et al. Science (PMID doi: 10.1126/science.abe3354), and Piccoli et al. Cell (doi: 10.1016/j.cell.2020.09.037).

Reactivity of primary antibodies listed above is based on the information on manufacturer's homepages.

## Eukaryotic cell lines

Policy information about [cell lines](#)

## Cell line source(s)

Cell lines were received from ATCC (Vero E6, Vero, BHK-21, CHO-K1), Takara (Lenti-X 293T) and Thermo Fisher Scientific (ExpiCHO-S, Expi293F and Freestyle 293-F). MA104 cells were a gift from Harry Greenberg. 293T-ACE2 cells are described in Ref 31 and Ref 43.

## Authentication

No authentication was performed beyond manufacturer standards

## Mycoplasma contamination

Vero and MA104 cell lines tested negative for mycoplasma contamination. Other cell lines were not tested.

Commonly misidentified lines  
(See [ICLAC](#) register)

No commonly misidentified cell lines were used in the study.

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

## Laboratory animals

Experiments used Syrian hamsters (*Mesocricetus auratus*) from Janvier Laboratories. Female hamsters were used, at 6-10 week age

## Wild animals

The study did not involve wild animals

## Field-collected samples

The study did not involve field studies

## Ethics oversight

Housing conditions and experimental procedures were approved by the ethical committee of animal experimentation of KU Leuven (license P065-2020). Experiments were performed in the high-containment A3 and BSL3+ facilities of the KU Leuven Rega Institute (3CAPS) under licenses AMV 30112018 SBB 219 2018 0892 and AMV 23102017 SBB 219 20170589 according to institutional guidelines.

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

## Human research participants

Policy information about [studies involving human research participants](#)

|                            |   |
|----------------------------|---|
| Population characteristics | <p>Participants were identified by prior SARS-CoV-2 infection for PBMC isolation. Blood drawn from donor S2X was obtained at day 48 (S2X16, S2X35 and S2X58 mAbs) and 75 (S2X227) after symptoms onset. Blood from donor S2H was obtained at day 17 (S2H13 and S2H14), day 45 (S2H58) and day 81 (S2H97) after symptoms onset. Blood from donor S2D was obtained at day 98 (S2D106) after symptoms onset. Ages and sex for patients for mAb isolation: S2D: Age 70, male; S2H: Age 36, Male; S2X: Age 52, Male.</p> <p>For vaccinated and convalescent cohorts used in serology assays, average age (and range) for was 52 (25, 78) and 49 (28, 69) for convalescent and vaccinated cohorts, respectively. Gender breakdown was 54.8% and 64.7% male for convalescent and vaccinated cohorts, respectively.</p> |
| Recruitment                | <p>Patients were recruited on the basis of prior SARS-CoV-2 infection or vaccination in the hospital or outpatient setting. Patients were healthy volunteers who donated blood after being informed about the study. The only exclusion criteria used were HIV or other debilitating disease, but other information about diagnosis and treatment was not collected.</p>  |
| Ethics oversight           | <p>Study protocols for antibody isolation were approved by the local Institutional Review Board (Canton Ticino Ethics Committee, Switzerland), and all donors provided written informed consent for the use of blood and blood components. Study protocols for serological assays were approved by the local Institutional Review Boards relevant for each of three cohorts of samples (Canton Ticino Ethics Committee, Switzerland, the Ethical Committee of Luigi Sacco Hospital, Milan, Italy, and WCG North America, Princeton, NJ, USA). All donors provided written informed consent for the use of blood and blood components (such as PBMCs, sera or plasma) and were recruited at hospitals or as outpatients.</p>   |

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        | Yeast were induced for cell-surface expression, incubated with the primary antibody from the panel, and secondary labeled with anti-Myc and anti-Fc fluorescent conjugates as described in the Methods.  |
| Instrument                | FACSAria II  |
| Software                  | FACSDiva Version 6.1.3   |
| Cell population abundance | We work with homogenous yeast libraries in our deep mutational scanning experiments, therefore cell population abundance is not a detail of our approach.  |
| Gating strategy           | Yeast expressing unmutated SARS-CoV-2 RBD are labeled at 1x and 0.01x the concentration used for library labeling. Gates are drawn on the basis of these controls to standardize selection stringency across experiments. Gating schemes are shown in Extended Data Fig. 2 |

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