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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	As described in Methods: * protein purification monitored by UNICORN software version 5.11 * SPR binding assays collected and analyzed using Biacore Evaluation and Biacore Insight software * FACSDiva (version 6.1.3) and Everest 3.0 software for FACS data collection and cell sorting * Identification of escape mutants in virological assay was performed by Sanger sequencing (GENEWIZ)
Data analysis	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. This code uses packages and versions as described in the Methods for various steps of computation. Binding, Neutralization assays analyzed using PRISM (Version 9.1.0) as described in Methods. 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)

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

• All datasets generated and information presented in the study are available from the corresponding author on reasonable request.
• CryoEM structure data and model are available with accession code PDB 7RA8 (EMD-24347) and PDB 7RAL (EMD-24365)
• The X-ray structure data and model has been deposited with accession code PDB 7M7W for RBD-S2H97-S2X259.
• Interactive escape maps and structural visualizations can be found at: 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).
• 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.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For in vivo studies the sample size was chosen based on prior experience with this animal model.
Data exclusions	Lack of detection of the human monoclonal antibody in the hamster sera at 48 post-administration (prophylactic setting) or at 4 days post-infection (therapeutic setting) was considered a criteria to identify animals that were not properly administered and to exclude them from the analysis
Replication	Experimental assays were performed in biological duplicate or triplicate (or more) according to or exceeding standards in the field. 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 S2X259 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 derived 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 & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	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 S1 shedding and binding FACS analysis, we used AF647-conjugated goat anti-human-IgG (Jackson ImmunoResearch 109-606-098) * 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 and S2X259) were produced in-house using recombinant protein purification as described in the Methods. S2H97_GL and S2E12_GL were produced by ATUM. All the commercial antibodies used in the study have been indicted with supplier name, catalog number. The monoclonal antibodies used as controls or for combination experiments were previously described by our group.
Validation	We discovered and describe S2X259 monoclonal antibody in this study. Target validation of this antibody was performed with multiple binding assays, in addition to cryoEM and x-ray crystallography structures were determined. 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 used in this study were obtained from ATCC (HEK293T, Vero and Vero-E6), ThermoFisher Scientific (Expi CHO cells, FreeStyle™ 293-F cells and Expi293F™ cells) or were generated via lentiviral trasduction (Expi CHO-S, HEK293T-ACE2).
Authentication	None of the cell lines used were authenticated
Mycoplasma contamination	Cell lines were tested for mycoplasma contamination
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	6-10 week-old female Syrian hamsters (Mesocricetus auratus) were Syrian hamsters (Mesocricetus auratus) were purchased from Janvier Laboratories and used in the study
Wild animals	The study did not involve wild animals
Field-collected samples	No field collected samples were used in the study
Ethics oversight	This work was conducted 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. Housing conditions and experimental procedures were approved by the ethical committee of animal experimentation of KU Leuven (license P065-2020).

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	Participants were identified by prior SARS-CoV-2 infection for PBMC isolation. Blood drawn from donor S2X (male, 52 years-old) was obtained at day 75 after symptoms onset. For recovered individual samples were collected between 14 and 75 days after symptoms onset, for vaccinees samples were collected between 13 and 20 days after the second dose of mRNA-based SARS-CoV-2 vaccine.
Recruitment	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.
Ethics oversight	Study protocols for antibody isolation were approved by the local Institutional Review Board (Canton Ticino Ethics

Ethics oversight	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.

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 and ZE5 BioRad
Software	FACSDiva and Everest 3.0
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 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 expreriments. Gating schemes are shown in Extended Data Fig. 1c and 7a

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