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# Supplemental information

# Structural mechanism of SARS-CoV-2 neutralization

#### by two murine antibodies targeting the RBD

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**Figure S1: Cryo-EM validation for 2B04 and 2H04 maps. Related to figure 1.** (**A**) Example micrographs and CTF estimates for 2B04-spike datasets imaged on holey lacey carbon grids or lacey carbon grids with ultra-thin carbon film. (**B**) Example micrographs and CTF estimates for 2H04-spike datasets imaged with holey lacey carbon grids or lacey carbon grids or lacey carbon grids or lacey carbon film. (**C**) Particle orientation distribution for 2B04-spike up/down/down reconstruction. (**D**) Particle orientation for 2H04-spike down/down reconstruction. (**E**) GSFSC curve for 2B04-spike up/down/down refinement. (**F**) GSFSC curve for 2H04-spike down/down/down refinement. (**G**) Local resolution map for 2B04-spike up/down/down map. (**H**) Local resolution map for 2H04-spike down/down/down map.



**Figure S2:** Cryo-EM processing strategy. Related to figures 1 and 2. (A) Flowchart depicting cryo-EM data processing steps for the 2B04-spike up/down/down and 2B04/RBD locally refined maps. (B) Flowchart depicting cryo-EM data processing steps for the 2H04-spike down/down/down and 2H04/RBD locally refined maps.



**Figure S3**: **Model fits into maps and validation for locally refined maps. Related to figures 1 and 2.** (A) Density map for 2B04 U/D/D spike reconstruction shown as an outline, with the model shown internally as a cartoon. S1/S2 is depicted in green, with the RBD portion shown in yellow. 2B04 is shown in cyan. (B) Density map for 2B04 U/U/D spike reconstruction shown as an outline, with the model shown internally as a cartoon and colored as in A. (C) Density map for 2H04 D/D/D spike reconstruction shown as an outline, with the model shown internally as a cartoon. S1/S2 is depicted in green, with the RBD portion shown in yellow. 2H04 is shown in magenta. (D) Density map for 2H04 U/D/D spike reconstruction shown as an outline, with the model shown internally as a cartoon and colored as in C. (E) GSFSC curve for the locally refined 2B04/RBD map. (F) GSFSC curve for the locally refined 2H04/RBD map. (G) Example density and model fits for an RBD beta-strand (left) and at the 2B04/RBD interface (right). RBD is colored in yellow, 2B04 heavy chain is shown in magenta, while the light chain is shown in violet.



**Figure S4: 2H04 contacts the core fucose of the N343 glycan on SARS-CoV-2 RBD. Related to figure 2.** 2H04 is depicted as a surface, with the heavy chain colored magenta and the light chain colored violet. The N343 glycan is depicted as atoms in blue, with the remainder of the N-terminal strand of the RBD depicted in silver as a cartoon ribbon. Residues at the interface between the core fucose and 2H04 are labelled in black text.



**Figure S5: Binding affinity of 2B04 and 2H04 to mammalian cell-derived SARS-CoV-2 RBD. Related to figure 6. (A)** Binding of 2B04 to mammalian cell-derived SARS-CoV-2 RBD. (**B**) Binding of 2H04 to mammalian cell-derived SARS-CoV-2 RBD. (**C**) Binding of 2H04 to bacterially derived SARS-CoV RBD. (**D**) Binding of 2B04 to bacterially der



**Figure S6: Neutralization profiles of 2B04 and 2H04 IgG versus Fab. Related to figure 6.** Serial dilutions of 2B04 or 2H04 mAbs or Fabs were pre-mixed with GFP expressing VSV-SARS-CoV-2 for 1 hour, followed by addition of the mixture to Vero E6-TMPRSS2 cells. GFP<sup>+</sup> infected cells were quantified by flow cytometry 8 hours post of infection. Data are pooled from three independent experiments performed in duplicate. Error bars indicate SEM.



Figure S7: Comparison of 2B04 and 2H04 to germline Ig sequences. Related to figure 3. (A) Sequence alignment of 2B04  $V_{\rm H}$  to IgH2-9-1 (2B04  $V_{\rm H}$  parent germline sequence) and IgHV3-53\*02 (germline sequence of human antibodies targeting an overlapping RBM epitope). Residues are colored from black to white according to degree of conservation. 2B04 contact residues on the SARS-CoV-2 RBD are shown as cyan triangles. (B) Sequence alignment of 2B04  $V_{\rm L}$  to IgVL1\*01 (2B04  $V_{\rm L}$  parent germline sequence), colored as in A. (C) Sequence alignment of 2H04  $V_{\rm H}$  to IgHV1-55\*01 (2H04  $V_{\rm H}$  parent germline sequence). Residues are colored from black to white according to degree of conservation. 2H04  $V_{\rm H}$  to IgHV1-55\*01 (2H04  $V_{\rm H}$  parent germline sequence). Residues are colored from black to white according to degree of conservation. 2H04  $V_{\rm H}$  to IgHV1-55\*01 (2H04  $V_{\rm H}$  parent germline sequence) are shown as pink triangles. (D) Sequence alignment of 2H04  $V_{\rm L}$  to IgKV5-48\*01, colored as in C.

### Table S1: Cryo-EM data collection, processing, refinement, and validation statistics. Related to Figures 1 and 2.

	SARS-CoV-2 spike U/D/D with Fab 2B04 (full)	SARS-CoV-2 spike U/D/D with Fab 2B04 (local)	SARS-CoV-2 spike U/U/D with Fab 2B04 (full)	SARS-CoV-2 spike D/D/D with Fab 2H04 (full)	SARS-CoV-2 spike D/D/D with Fab 2H04 (local)	SARS-CoV-2 spike U/D/D with Fab 2H04 (full)
	EMD-22748	EMD-22749	EMD-22752	EMD-22750	EMD-22751	EMD-22753
Data collection						
Magnification	105,000x	105,000x	105,000x	105,000x	105,000x	105,000x
Voltage (kV)	300	300	300	300	300	300
Electron exposure ( $e^{-}/Å^{2}$ )	67	67	67	67	67	67
Defocus range (µm)	0.5-2.5	0.5-2.5	0.5-2.5	0.5-2.5	0.5-2.5	0.5-2.5
Pixel size (Å/pixel)	1.1	1.1	1.1	1.1	1.1	1.1
Data processing						
Symmetry imposed	C1	C1	C1	C3	C1	C1
Initial partile images (no.)	1,842,978	1,842,978	1,842,978	877,481	877,481	877,481
Final particle images (no.)	162,281	263,342 (C3 exp)	165,840	155,896	304,667 (C3 exp)	109,868
Map resolution (Å)	3.2	3.3	3.35	3.0	3.14	3.55
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143
Map sharpening factor	-78.4	-61	-75	-94.3	-84.7	-64.1
Model refinement						
Initial PDB model used	6VYB, 6W41, 1GIG	6W41, 1GIG		6VXX, 6DG2, 1K4C	6VXX, 6DG2, 1K4C	
Model resolution (Å)	3.4	3.7		3.3	3.6	
FSC threshold	0.5	0.5		0.5	0.5	
Model composition						
Non-hydrogen atoms	27,022	3,295		29,766	3202	
Protein residues	3,391	423		3627	402	
Ligand residues	105	1		111	4	
B-factors (Å <sup>2</sup> )						
protein residues	115	93		100	82	
ligands (glycans)	118	99		140	76	
RMS deviations						
Bond lengths (Å)	0.006	0.005		0.004	0.006	
Bond angles ( <sup>o</sup> )	0.724	0.710		0.737	0.927	
Validation						
Molprobity score	1.82	1.83		1.73	1.82	
Clashscore	10.02	11.92		8.76	11.03	
Poor rotamers (%)	0.18	0.00		0.57	0.00	
Ramachandran						
Favored (%)	95.70	96.40		96.18	96.19	
Allowed (%)	4.30	3.60		3.82	3.81	
Outliers (%)	0.00	0.00		0.00	0.00	

# Table S2. GISAID citations. Related to Figure 4.



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#### All Submitters of data may be contacted directly via www.gisaid.org

Accession ID	Originating Laboratory	Submitting Laboratory	Authors
EPI_ISL_430518	Victorian Infectious Diseases Reference Laboratory (VIDRL)	Microbiological Diagnostic Unit Public Health Laboratory and Victorian Infectious Diseases Reference Laboratory, The Pater Doherty Institute for Infection and Immunity	Caly 1 Seemann T., Sait, M., Schultz M., Druce J., Sheny, N.
EPI_ISL_444976	Hospital Universitari Vall d'Hebron - Vall d'Hebron Institut de Recerca	Hospital Universitari Vall d'Hebron	Cristina Andres, Maria Piñana, Damir Garcia-Ceffic, Mercedes Guerrero Murillo, Ariadno Rando, Juliana Esperalba, María Gerna Codina, Tomás Pumarola, Josep Quer, Andrés Antón
EPI_ISL_447508	Servicio de Microbiologia. Hospital Clínico Universitario de Valencia.	Sequencing and Sconformatics Service and Molecular Epidemiology Research Group: FISADIO-Public Health	Nariana Reym-Prieto, Vicente Soriano Chruma, Ivan Ansant, Licia Martinez-Priego, Guaeppe Yauria, David Navarm, Elison Albert, Maria Alma Bracho, Lidia Ruiz Roldan, Neris Garcia-Gonzalez, Imma Galán Vendrell, Sandra Cartio, Loreto Fernin Abaid, Paula Ruiz-Rauso, Fernando Gonzalez-Caridelan
EPI_IGL_448386	Quadram institute Bioscience	COVID-19 Generics UK (COG-UK) Consertium	Dave J. Baker, Germin L. Kay, Alp Aydin, Thanh Le-Viet, Steven Ruibler, Ana P. Tedim, Anastasia Koiyva, Maria Diaz, Leonards de Giveira Martins, Nabil-Fareed Alikhan, Lizzie Meadows, Rachael Stamley, Napoli Elumoga, Muhammed Yasir, Nichelas N. Thomson, Alexander J. Trotter, Rachel Giroy, Samuel Biomrifield, Clare Souart, Andrew Boll, Reemish Prakash, Samir Derviseric, Alson E. Mather, John Wahr, Nark Webzer, Andrew J. Beer, Territori, Alexander J. Trotter, Rachel Giroy, Samuel Biomrifield, Clare Souart, Andrew Boll, Reemish Prakash, Samir Derviseric, Alson E. Mather, John Wahr, Nark Webzer, Andrew J. Beer, Julio O'Chally
EPI ISL 454530	NW influenza	MV Influenza	Podar V
EPC,ISL_455588	Trang Hospital	National Institute of Health: Department of medical Sciences, Ministry of Public Health, Thailand	Plaiuk.Okada; Sirjapporn, Phuygun; Thanutiapa, Thanadachakul; Sittiporn, Parnmen, Warawan, Wongboot; Sunthaneeya, Waichansen; Malinee, Chittaganptich
EPI_ISL_460933	Dutch COVID-19 response feam	Erasmus Medical Center	Bas Oude Munnink, David Meuwerhuise, Keina Sikkema, Claudia Schapendork, Kina Chestakovo, Anne van der Linden, Theo Bestebroer, Stefan van Nieuwkoop, Mark Fronk, Pascal Lewmont, Corieri Swaan, Manon Hererkate, Madelef Nollers, Mant Stein, Sandra Kenge Kanga Mobou, jereen van Kangen, jolnato Vioermans, Aum Timen, Corieri Geurswarktessel, Annemiek van der Ejk, Richard Nolerkamp, Narion Koopmans, on behalf of the Dutch national COPDL3 response team.
EPL/SL_461895	Queens Nedical Centre, Clinical Microbiology Department / DeepSeq Nottingham	COVE-19 Genomics UK (CDG-UK) Consortium	Gernma Clark, Wenity Smith, Nanjinder Rhakh, Hannah Howson-Wells, Jonathan Ball, Patrick McClare, Joseph Chappell, Theocharis Toolenials, Nadine Holmes, Natthew Carlisle, Christopher Moore, Fei Sang, Johnny Debebe, Victoria Wright, Matthew Laose
EM_ISL_463539	Washington State Department of Health	Sevittle Fix Study	Chu et al
EPI_H4_464376.EPI_H4_465520	Respiratory Virus Linit, Microbiology Services Colindate, Public Health England	Respiratory Virus Unit, Nicrobiology Services Colimbale, Public Health England	PHE Covid Sequencing Team
EPI_/SL_466306	Max von Pettenkoler Institute, Virology, National Reference Center for Retroviruses, LMU München	Laboratory for Functional Genome Analysis, Dept. Genomics, Gene Center of the LWU Mursich	Max Muunchhoff, Stefan Krebs, Alexander Graf, Oliver Kappler, Hehmut Blum
Eff_ISL_467270	Hospital Clinice Universitario de Santiago de Compostela	SeeCOVID-SPAIN consertium/IBV(CSIC)	José Javier Costa Alcalde, Antonio Aguilera Guirao, MP Luisa Pérez del Holino Bernal, Ampany Coira Nieto, Gerna Barbeito Castineiras, Recie Trastoy Pena and SegCOVID-SPARI censoritum
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EPL_6L_471833	Hichigan Department of Health and Human Services, Bureau of Laboratories	Hichigan Department of Health and Human Services, Bureau of Laboratories	Blankership HN, Riner D, Saetnien MK
EPL/6L_472405	Queens Medical Centre, Clinical Microbiology Department / DeepSeq Nottingham	COVID-19-Genomics UK (CDG-UK) Consertium	Germa Clark, Wendy Smith, Manjoser Khakh, Vicki M Fleming, Nichelle H Lister, Hannah Howson-Welle, Jonathan Ball, Patrick NcClure, Joseph Chappell, Theocharis Tuolenists, Nadine Holmes, Nathew Carlisie, Christopher Moore, Fei Sang, Johnny Debete, Victoria Wright, Matthew Loose
EP1.ISL 472846, EP1.ISL 473042, EP1.ISL 473071	Wales Specialist Virology Centre Sequencing Juli: Pathogen Genumics Unit	COVID-19 Genomics UK (CDG-UK) Consortium	Catharine Noore, Johnsthan Evans, Laura Gifford, Makele Peny, Simon Cottrell, Angela Marchbark, Alec Brohley, Alaxander Aslama, Amy Gaskin, Brier Gatsa-Wilkos, Javon Cosmbes, Joel Southgate, Laurén Gibert, Lee Graham, Nicole Pacchiante, Sara Kurziene-Summerhayes, Sarah Teylor, Sophie Jones, Sara Rey, Matthew Bull, Joanne Walkins, Sally Corden, Tom Comor
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EPI_ISL_490363	University of Wisconsin-Madison AIDS Vaccine Research Laboratories	University of Wisconsin-Madison AIDS Vaccine Research Lationatories	Gage Moreno, Katarina Braun, et al. AIDS Vaccine Research Laboratories
EPI/051_430948	Piorida Bureau of Public Health Laboratories	Florida Barnina of Public Health Laboratories	Sarah Schmedos, Janon Blantinh
EPI_ISL_481886. EPI_ISL_488423	PHE South West Regional Laboratory. National Infection Service	Wellcome Sanger institute for the COVID-19 Genumics UK Consortium	Stephanie Hutchings, Hennah Pymont, Dr Peter Muir, Barry Vapond, Rich Hopes; and Alex Aderton, Roberto Amato, Sania Gencalves, Ewan Harrison, David K. jackson, Ian johnstan, Daminic Kwlatkowski, Cordelia Langford, John Silvitoe on behalf of the Welcome Sanger matikute COVID-19 Sunveillance Team (http://www.sanger.ac.ut/covid.team)
EPI 151, 494170, EPI 151, 494194, EPI 152, 494240, EPI 151, 494256, EPI 151, 494279, EPI 151, 494311, EPI 151, 494335, EPI 151, 494348, EPI 151, 494352	Originating lab: Wales Specialist Vockogy Centre Sequencing lab: Pathogen Genomics Unit	COVID-19 Generation UK (COG-UK) Consortium	Catherine Noore, Johnsthan Evans, Laura Gifferd, Malorie Peny, Sinon Ozitiveli, Angela Harthbark, Alec Birchley, Alexander Adams, Amy Gaskin, Bree Gabua-Wikos, Jason Coombes, Joel Southgate, Lauren Gébert, Lee Graham, Nicole Facchlarin, Sara Namoiene-Summerhayes, Sarah Taylor, Sophe Jones, Sara Rey, Matthew Bull, Joanne Walkin, Solly Corden, Tom Cornor
EPT_ISL_494618	Scripps Medical Laboratory	Andersen lab at Scripps Research	SEARCH Allance San Diego with Michael Duigley, Ellen Stefanski, Ian Michardy
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EPI_ISL_501014	Regional Virus Laboratory, Belfast Health and Social Carle Trust	Wellcome Sanger institute for the COVID-19 Genomics UK Consortium	Conall McCaughey, James McKenna, Tanya Curran, Susan Feerey, Alison Watt. Clara Cox, Mairead Connor, Zotan Molnar, David Simpson, Derok Fairley, and Alex Alderton, Roberts Amato, Sonia Goncalves, Ewan Hamson, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langferd, John Silitoe on benall of the Welicome Sanger Institute COVID-19 Surveillance Team (http://www.sanger.ac.uk/covid-team)
184_161_509133	OHSU Lab Services Molecular Microbiology Lab	Dregon SARS-CoV-2 Genome Sequencing Center	Brendan L. O'Cannell, Rath V. Nichols, Safy B. Grindstaff, Alec J. Hirsch, Guang Fan, Daniel N. Stribilow, William B. Messer, Andrew C. Adey, Benjamin N. Bimber, Brian J. O'Roak
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EPI_ISL_512239	San Diego County Public Health Laboratory	Andersen lab at Scripps Research	SEARCH Allence San Diego with Tracy Basler, jovan Shephand, Brett Austin
EPI_ISL_512381. EPI_ISL_512382. EPI_ISL_514452, EPI_ISL_514453	Queens Medical Centre, Clinical Microbiology Department / DeepSeq Nottingham	COVID-19-Genamics UK (COG-UK) Consortium	Germa Clark, Wendy Smith, Manjinder Khalih, Vicki M Fleming, Nichelle M Lister, Haonah Howson-Wells, jonathan Ball, Patrick NcClure, Joseph Chappell, Theocharis Tsolerialis, Nadine Holmes, Natthew Carlisle, Christigher Moore, Fei Sang, Johnny Debele, Victoria Wright, Matthew Loose
EPI_ISL_515409	Medical Disagnostics Services (MDS)	KRISP, KZN Research Innovation and Sequencing Platform	Gandhert J, Pilley S, Lesselli R, ChimukangaraB, Mdlaiose K, York D, Khan S, Tegally H, Wilkinson E, de Oliveira T
EPI /SL 516595	Viollier AG	Department of Biosystems Science and Engineering, ETH Zürich	Christian Betsel, Sarah Nadeau, Ivan Topolsky, Pedro Ferreira, Philipp Jalionski, Susana Posado-Cilspeties, Tobias Schär, Iva Nissen, Natascha Santacroce, Elodie Burcklen, Christiane Beckmann, Maurice Redondo,
EPI (51_518926	Microbiological Diagnostic Unit - Public Health	NDU-PHL	Olivier Kubell, Christoph Noppen, Sophie Seidel, Noemie Santamaria de Ssuza, Nike Berneneinkel, Tanja Stadler Seemann T., Schultz M., Sak, M., Sherry, N.
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EPI_ISL_566030	Michigan Department of Health and Human Services, Bureau of Laboratories	Michigan Department of Health and Human Services, Bureau of Laboratories

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