Science Advances
AAAS

Supplementary Materials for

Design of the SARS-CoV-2 RBD vaccine antigen improves neutralizing antibody response

Thayne H. Dickey *et al.*

Corresponding author: Niraj H. Tolia, niraj.tolia@nih.gov

Sci. Adv. **8**, eabq8276 (2022)
DOI: 10.1126/sciadv.abq8276

This PDF file includes:

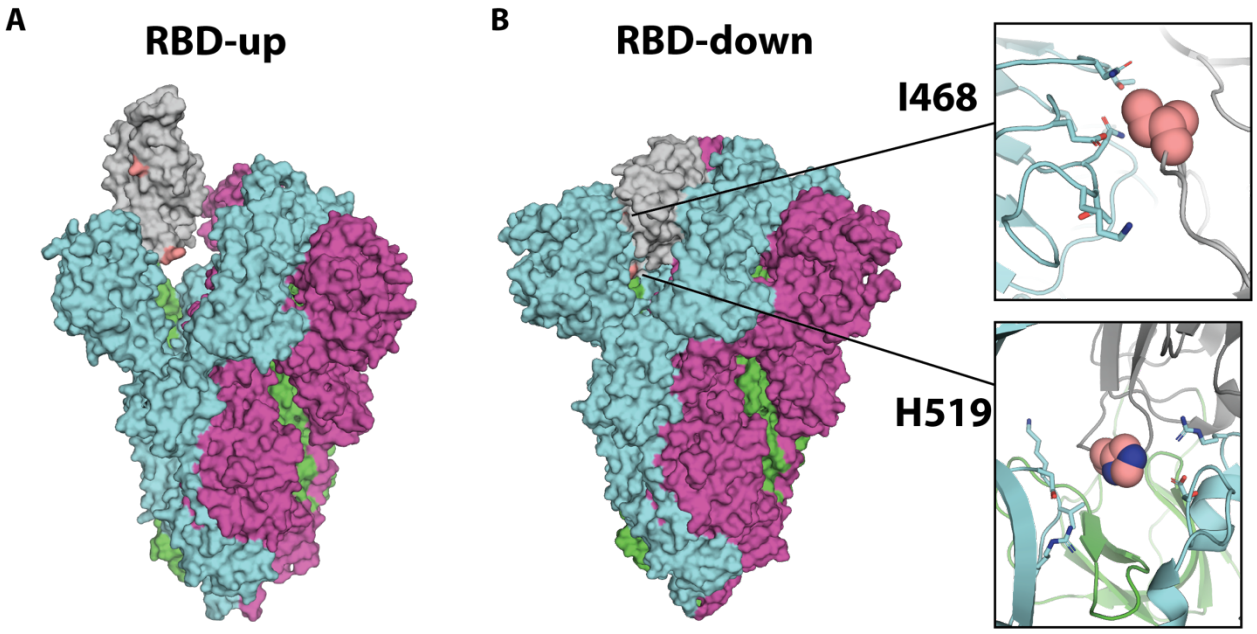

Fig. S1.

I468 and H519 are buried in the RBD-down conformation. A) I468 and H519 (pink) are solvent exposed when the RBD (grey) is in the up conformation (PDB 6VSB and 6MOJ) (*33, 72*). Protomers are shown in green, cyan, and magenta with the RBD of the green protomer in the up conformation. **B**) I468 and H519 (pink) become buried in the closed conformation where they make contacts with the neighboring protomer (cyan) (PDB 6XLU). Mutation of these residues to more hydrophilic residues could promote the RBD-up conformation.

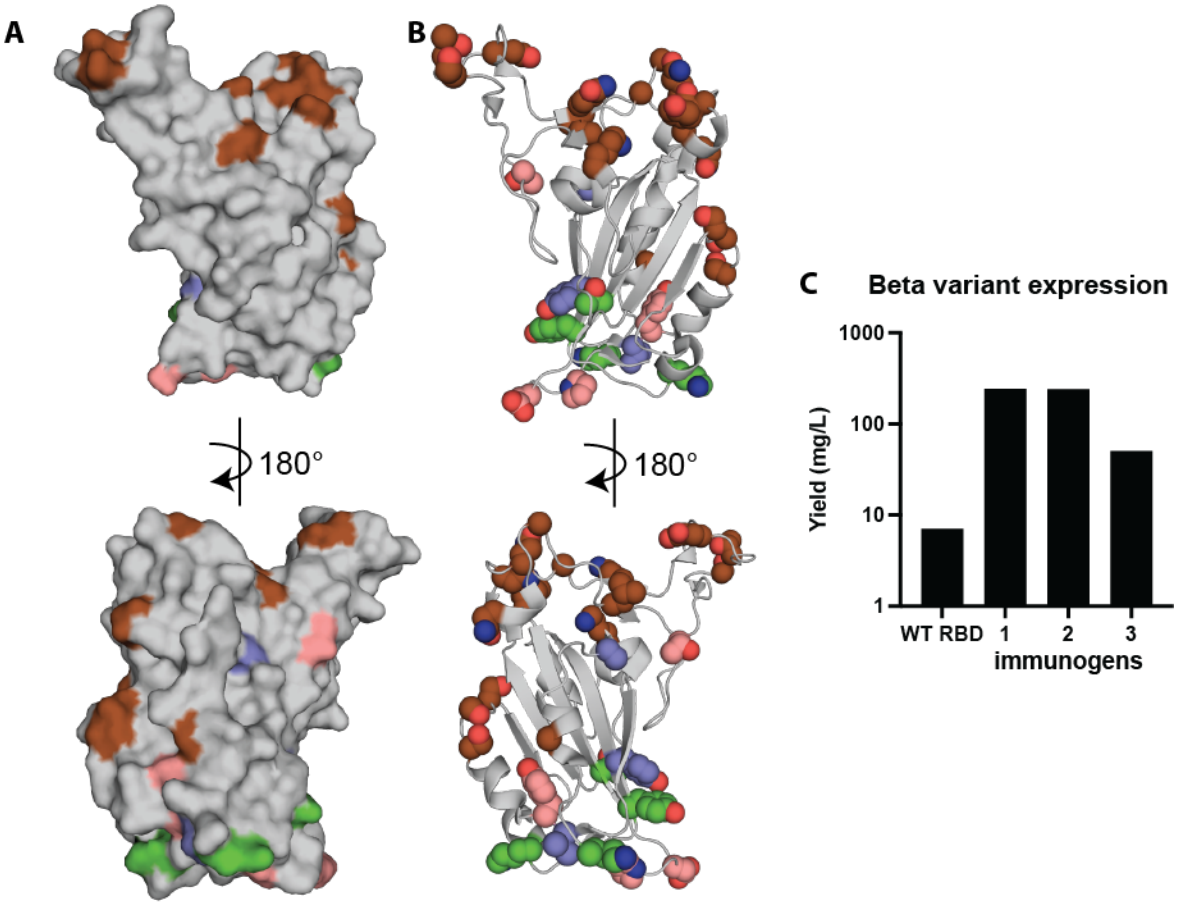

Fig. S2.

The amino acid changes in the immunogens are compatible with variants of concern. A, B Immunogen 3 structure with mutations in alpha, beta, delta, and omicron variants depicted in brown. Remaining molecule is colored as described in **Figure 3D**, with designed amino acid changes shared between immunogens (pink), changes specific to lead 1 (blue) and lead 3 (green) as surface (**A)** and cartoon (**B**) representation. **C**, Purification yields for immunogens containing the Beta variant amino acid changes.

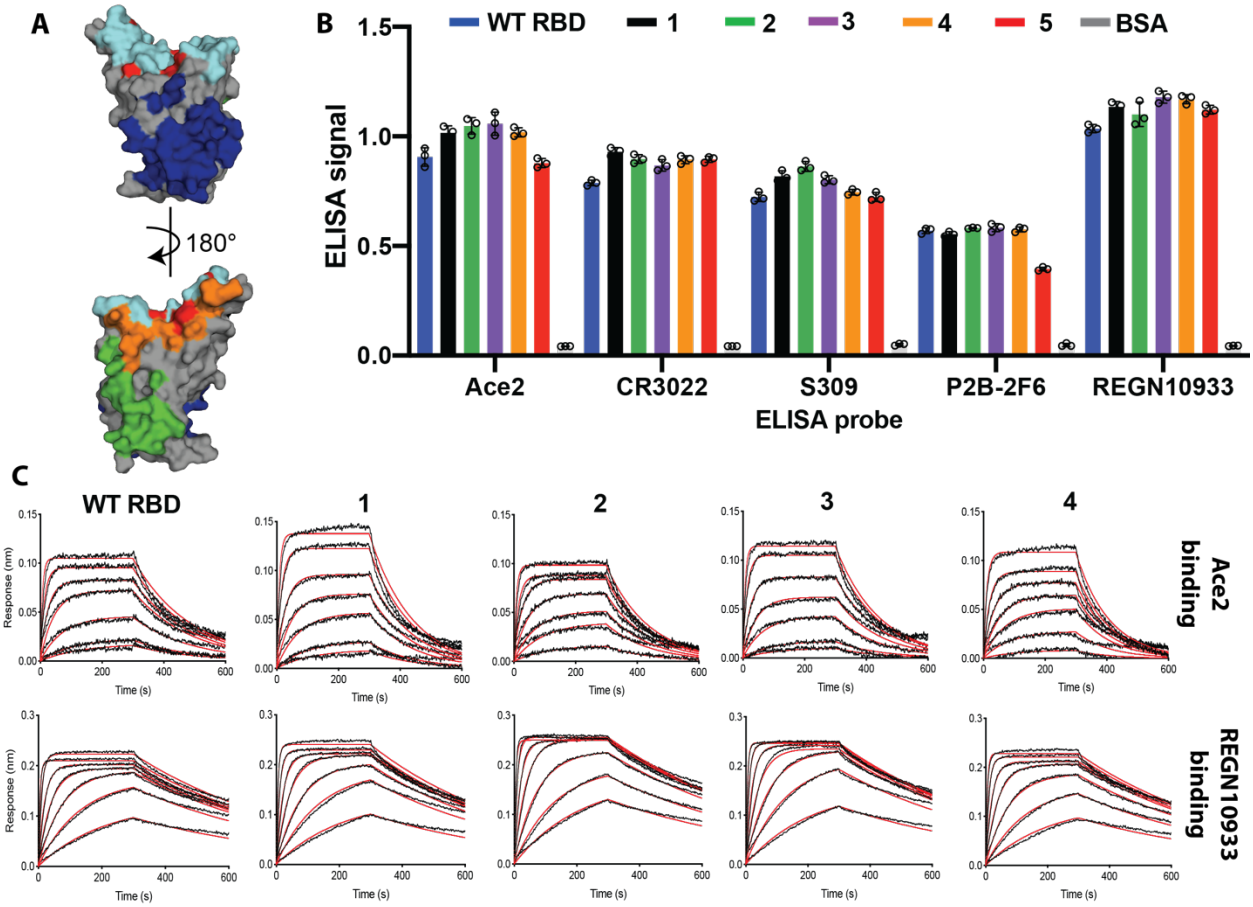

Fig. S3.

Neutralizing epitopes are unperturbed on RBD immunogens. A) Five distinct three-dimensional neutralizing epitopes covering the majority of the protein surface were probed for each immunogen (Ace2:cyan, REGN10933:red, P2B-2F6:orange, S309:green, CR3022:blue). **B)** ELISA probes bind to all five epitopes on the immunogens **C)** Representative BLI traces used to quantitatively measure the binding of the immunogens to two probes, demonstrating the high integrity of these epitopes. Immunogen concentrations begin at 150 nM and decrease in 2-fold increments.

name	sequence
Native RBD	etgTNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPT KLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVVLSFELLHAPATVCGgtggggskhhhhhh
Immunogen 1 (decoy 28)	etgMNLCPFGEVFNATRFPSVYAWNRKRISNCYYDYSVLYNSASFSTFKCYGVSPT KLNDLCFTQVFADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDTSTEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVVLSFELLDAPPTVCGgtggggskhhhhhh
Immunogen 2 (decoy 24)	etgTNLCPFGEVFNATRFASVYAWNRKRISNCQFDYSKLYNSASFSTFKCYGVSPT KLNDLCFTQVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDTSTEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVVLTFELLDAPATVCGgtggggskhhhhhh
Immunogen 3 (decoy 25)	etgKNLCPFGEVFNATRFASVYAWNRKRISNCVYDYSVLYNSASFSTFKCYGVSPT KLKDLCFTYVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDTSTEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVVLTFELLDAPPTVCGgtggggskhhhhhh
Immunogen 4 (decoy 26)	etgTNLCPFGEVFNATRFASVYAWNRKRISNCKYDYSALYNSASFSTFKCYGVSPT KLNDLCFTEVYADYFVVRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDTSTEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVVLTFELKDAPATVCGgtggggskhhhhhh
Immunogen 5 (decoy 21)	etgTNLCPFGEVFNATRFPAVYAWNRKRISNCVFDFSKLYNSASFSTFKCYGVSPT KLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLQPFERDTSEEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVVLNFELLDAPPTVCGgtggggskhhhhhh
Native/WT RBD Beta	etgTNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPT KLNDLCFTNVYADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVKGFNCYFPLQSYGF QPTYGVGYQPYRVVVLSFELLHAPATVCGgtkhhhhhh
Immunogen 1 Beta	etgMNLCPFGEVFNATRFPSVYAWNRKRISNCYYDYSVLYNSASFSTFKCYGVSPT KLNDLCFTQVFADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDTSTEIYQAGSTPCNGVKGFNCYFPLQSYGF QPTYGVGYQPYRVVVLSFELLDAPPTVCGgtkhhhhhh
Immunogen 2 Beta	etgTNLCPFGEVFNATRFASVYAWNRKRISNCQFDYSKLYNSASFSTFKCYGVSPT KLNDLCFTQVYADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDTSTEIYQAGSTPCNGVKGFNCYFPLQSYGF QPTYGVGYQPYRVVVLTFELLDAPATVCGgtkhhhhhh
Immunogen 3 Beta	etgKNLCPFGEVFNATRFASVYAWNRKRISNCVYDYSVLYNSASFSTFKCYGVSPT KLKDLCFTYVYADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDTSTEIYQAGSTPCNGVKGFNCYFPLQSYGF QPTYGVGYQPYRVVVLTFELLDAPPTVCGgtkhhhhhh

Table S1.

Sequences of lead immunogens after signal peptide cleavage. The RBD domain is shown in uppercase and cloning scars, tags, and linkers are shown in lowercase.

	Lead1/C144 PDB: 8DCE	Lead 3/P2B-2F6 PDB: 8DCC
Data collection		
Space group	I 2 2 2	P 21 21 21
Cell dimensions		
a, *b*, *c* (Å)	81.63 97.31 141.81	71.06, 91.97, 111.15
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	19.78-2.00 (2.05-2.00)	70.9-2.57 (2.66-2.57)
R_{meas} (%)	12.5 (84.5)	20.3 (226.2)
I /□	12.83 (2.78)	8.2 (1.02)
$CC_{1/2}$ (%)	99.7 (79.3)	99.4 (33.2)
Completeness (%)	99.68 (99.21)	99.9 (99.9)
Redundancy	5.73 (4.09)	6.6 (6.8)
Beamline	APS 23ID-D	APS 23ID-D
No. of complex/ASU	1	1
Refinement		
Resolution (Å)	19.78-2.00	56.23 - 2.6
No. reflections	38,255	23,032
R_{work} / R_{free}	17.60/20.32	22.1/27.2
No. atoms		
Protein	3,390	4,816
Ligand/ion	0	14
Water	232	31
B-factors		
Protein	40.39	62.9
Ligand/ion	-	93.3
Water	39.06	49.4
R.m.s. deviations		
Bond lengths (Å)	0.002	0.007
Bond angles (°)	0.53	1.11
Ramachandran plot		
Favored (%)	95.81	96.03
Allowed (%)	4.19	3.97
Disallowed (%)	0.00	0.00

Table S2.

X-ray data collection and refinement statistics

	K_D (x 10^{-9} ± SEM M)	k_a (x 10^5 ± SEM 1/Ms)	k_{dis} (x 10^{-3} ± SEM 1/s)	N
Ace2 with WT-RBD	8.7 ± 0.9	7.7 ± 0.4	6.5 ± 0.4	3
Ace2 with lead 1	9.3 ± 0.7	8.0 ± 0.2	7.4 ± 0.4	3
Ace2 with lead 2	15 ± 3	7 ± 1	9.1 ± 0.4	3
Ace2 with lead 3	11 ± 4	8 ± 1	7.5 ± 0.8	3
Ace2 with lead 4	14 ± 3	6.6 ± 0.6	7.1 ± 0.9	3
REG10933 with WT-RBD	1.8 ± 0.2	10.9 ± 0.8	1.9 ± 0.1	3
REG10933 with lead 1	1.6 ± 0.1	12.2 ± 0.2	1.9 ± 0.1	3
REG10933 with lead 2	1.4 ± 0.1	13.0 ± 0.3	1.8 ± 0.1	3
REG10933 with lead 3	1.6 ± 0.1	11.7 ± 0.3	1.9 ± 0.1	3
REG10933 with lead 4	1.5 ± 0.1	12.6 ± 0.2	1.8 ± 0.1	3

Table S3.

Binding affinities of Ace2 and REG10933 to RBD variants as determined by BLI. Binding data were fit using a 1:1 binding model. The averages and standard deviations of three biological replicates using independently purified immunogens are shown.

REFERENCES AND NOTES

1. E. Dong, H. Du, L. Gardner, An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect. Dis.* **20**, 533–534 (2020).

2. E. E. Walsh, R. W. Frenck Jr., A. R. Falsey, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, K. Neuzil, M. J. Mulligan, R. Bailey, K. A. Swanson, P. Li, K. Koury, W. Kalina, D. Cooper, C. Fontes-Garfias, P.Y. Shi, Ö. Türeci, K. R. Tompkins, K. E. Lyke, V. Raabe, P. R. Dormitzer, K. U. Jansen, U. Şahin, W. C. Gruber, Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *N. Engl. J. Med.* **383**, 2439–2450 (2020).

3. C. Keech, G. Albert, I. Cho, A. Robertson, P. Reed, S. Neal, J. S. Plested, M. Zhu, S. Cloney-Clark, H. Zhou, G. Smith, N. Patel, M. B. Frieman, R. E. Haupt, J. Logue, M. McGrath, S. Weston, P. A. Piedra, C. Desai, K. Callahan, M. Lewis, P. Price-Abbott, N. Formica, V. Shinde, L. Fries, J. D. Lickliter, P. Griffin, B. Wilkinson, G. M. Glenn, Phase 1-2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. *N. Engl. J. Med.* **383**, 2320–2332 (2020).

4. L. A. Jackson, E. J. Anderson, N. G. Rouphael, P. C. Roberts, M. Makhene, R. N. Coler, M. McCullough, J. D. Chappell, M. R. Denison, L. J. Stevens, A. J. Pruijssers, A. McDermott, B. Flach, N. A. Doria-Rose, K. S. Corbett, K. M. Morabito, S. O'Dell, S. . Schmidt, P. A. Swanson II, M. Padilla, J. R. Mascola, K. M. Neuzil, H. Bennett, W. Sun, E. Peters, M. Makowski, J. Albert, K. Cross, W. Buchanan, R. Pikaart-Tautges, J. E. Ledgerwood, B. S. Graham, J. H. Beigel; mRNA-1273 Study Group, An mRNA vaccine against SARS-CoV-2—Preliminary report. *N. Engl. J. Med.* **383**, 1920–1931 (2020).

5. J. Sadoff, M. L. Gars, G. Shukarev, D. Heerwegh, C. Truyers, A. M. de Groot, J. Stoop, S. Tete, W. Van Damme, I. Leroux-Roels, P.-J. Berghmans, M. Kimmel, P. Van Damme, J. de Hoon, W. Smith, K. E. Stephenson, S. C. De Rosa, K. W. Cohen, M. J. McElrath, E. Cormier, G. Scheper, D. H. Barouch, J. Hendriks, F. Struyf, M. Douoguih, J. Van Hoof, H. Schuitemaker, Interim results of a phase 1–2a trial of Ad26.COV2.S Covid-19 vaccine. *N. Engl. J. Med.* **384**, 1824–1835 (2021).

6. K. S. Corbett, D. K. Edwards, S. R. Leist, O. M. Abiona, S. Boyoglu-Barnum, R. A. Gillespie, S. Himansu, A. Schäfer, C. T. Ziwawo, A. T. DiPiazza, K. H. Dinnon, S. M. Elbashir, C. A. Shaw, A. Woods, E. J. Fritch, D. R. Martinez, K. W. Bock, M. Minai, B. M. Nagata, G. B. Hutchinson, K. Wu, C. Henry, K. Bahl, D. Garcia-Dominguez, L.Z. Ma, I. Renzi, W.P. Kong, S. D. Schmidt, L. Wang, Y. Zhang, E. Phung, L. A. Chang, R. J. Loomis, N. E. Altaras, E. Narayanan, M. Metkar, V. Presnyak, C. Liu, M. K. Louder, W. Shi, K. Leung, E. S. Yang, A. West, K. L. Gully, L. J. Stevens, N. Wang, D. Wrapp, N. A. Doria-Rose, G. Stewart-Jones, H. Bennett, G. S. Alvarado, M. C. Nason, T. J. Ruckwardt, J. S. McLellan, M. R. Denison, J. D. Chappell, I. N. Moore, K. M. Morabito, J. R. Mascola, R. S. Baric, A. Carfi, B. S. Graham, SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature* **586**, 567–571 (2020).

7. J. Pallesen, N. Wang, K. S. Corbett, D. Wrapp, R. N. Kirchdoerfer, H. L. Turner, C. A. Cottrell, M. M. Becker, L. Wang, W. Shi, W.P. Kong, E. L. Andres, A. N. Kettenbach, M. R. Denison, J. D. Chappell, B. S. Graham, A. B. Ward, J. S. McLellan, Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E7348–E7357 (2017).

8. R. Bos, L. Rutten, J. E. M. van der Lubbe, M. J. G. Bakkers, G. Hardenberg, F. Wegmann, D. Zuijdgeest, A. H. de Wilde, A. Koornneef, A. Verwilligen, D. van Manen, T. Kwaks, R. Vogels, T. J. Dalebout, S. K. Myeni, M. Kikkert, E. J. Snijder, Z. Li, D. H. Barouch, J. Vellinga, J. P. M. Langedijk, R. C. Zahn, J. Custers, H. Schuitemaker, Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses. *NPJ Vaccines* **5**, 91 (2020).

9. S. Scheiblhofer, J. Laimer, Y. Machado, R. Weiss, J. Thalhamer, Influence of protein fold stability on immunogenicity and its implications for vaccine design. *Expert Rev. Vaccines* **16**, 479–489 (2017).

10. F. B. Ntumngia, C. V. Pires, S. J. Barnes, M. T. George, R. Thomson-Luque, F. S. Kano, J. R. S. Alves, D. Urusova, D. B. Pereira, N. H. Tolia, C. L. King, L. H. Carvalho, J. H. Adams, An

engineered vaccine of the *Plasmodium vivax* Duffy binding protein enhances induction of broadly neutralizing antibodies. *Sci. Rep.* **7**, 13779 (2017).

11. I. Campeotto, A. Goldenzweig, J. Davey, L. Barfod, J. M. Marshall, S. E. Silk, K. E. Wright, S. J. Draper, M. K. Higgins, S. J. Fleishman, One-step design of a stable variant of the malaria invasion protein RH5 for use as a vaccine immunogen. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 998–1002 (2017).

12. F. Sesterhenn, C. Yang, J. Bonet, J. T. Cramer, X. Wen, Y. Wang, C.-I. Chiang, L. A. Abriata, I. Kucharska, G. Castoro, S. S. Vollers, M. Galloux, E. Dheilly, S. Rosset, P. Corthésy, S. Georgeon, M. Villard, C.-A. Richard, D. Descamps, T. Delgado, E. Oricchio, M.-A. Rameix-Welti, V. Más, S. Ervin, J.-F. Eléouët, S. Riffault, J. T. Bates, J.-P. Julien, Y. Li, T. Jardetzky, T. Krey, B. E. Correia, De novo protein design enables the precise induction of RSV-neutralizing antibodies. *Science* **368**, eaay5051 (2020).

13. B. E. Correia, J. T. Bates, R. J. Loomis, G. Baneyx, C. Carrico, J. G. Jardine, P. Rupert, C. Correnti, O. Kalyuzhniy, V. Vittal, M. J. Connell, E. Stevens, A. Schroeter, M. Chen, S. MacPherson, A. M. Serra, Y. Adachi, M. A. Holmes, Y. Li, R. E. Klevit, B. S. Graham, R. T. Wyatt, D. Baker, R. K. Strong, J. E. Crowe, P. R. Johnson, W. R. Schief, Proof of principle for epitope-focused vaccine design. *Nature* **507**, 201–206 (2014).

14. J. Jardine, J.-P. Julien, S. Menis, T. Ota, O. Kalyuzhniy, A. McGuire, D. Sok, P.-S. Huang, S. MacPherson, M. Jones, T. Nieusma, J. Mathison, D. Baker, A. B. Ward, D. R. Burton, L. Stamatatos, D. Nemazee, I. A. Wilson, W. R. Schief, Rational HIV immunogen design to target specific germline B cell receptors. *Science* **340**, 711–716 (2013).

15. J. B. Bale, S. Gonen, Y. Liu, W. Sheffler, D. Ellis, C. Thomas, D. Cascio, T. O. Yeates, T. Gonen, N. P. King, D. Baker, Accurate design of megadalton-scale two-component icosahedral protein complexes. *Science* **353**, 389–394 (2016).

16. L. Liu, P. Wang, M. S. Nair, J. Yu, M. Rapp, Q. Wang, Y. Luo, J. F.-W. Chan, V. Sahi, A. Figueroa, X. V. Guo, G. Cerutti, J. Bimela, J. Gorman, T. Zhou, Z. Chen, K.-Y. Yuen, P. D. Kwong, J. G. Sodroski, M. T. Yin, Z. Sheng, Y. Huang, L. Shapiro, D. D. Ho, Potent

neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* **584**, 450–456 (2020).

17. J. Hansen, A. Baum, K. E. Pascal, V. Russo, S. Giordano, E. Wloga, B. O. Fulton, Y. Yan, K. Koon, K. Patel, K. M. Chung, A. Hermann, E. Ullman, J. Cruz, A. Rafique, T. Huang, J. Fairhurst, C. Libertiny, M. Malbec, W.Y. Lee, R. Welsh, G. Farr, S. Pennington, D. Deshpande, J. Cheng, A. Watty, P. Bouffard, R. Babb, N. Levenkova, C. Chen, B. Zhang, A. Romero Hernandez, K. Saotome, Y. Zhou, M. Franklin, S. Sivapalasingam, D. C. Lye, S. Weston, J. Logue, R. Haupt, M. Frieman, G. Chen, W. Olson, A. J. Murphy, N. Stahl, G. D. Yancopoulos, C. A. Kyratsous, Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010–1014 (2020).

18. E. Andreano, E. Nicastri, I. Paciello, P. Pileri, N. Manganaro, G. Piccini, A. Manenti, E. Pantano, A. Kabanova, M. Troisi, F. Vacca, D. Cardamone, C. de Santi, J. L. Torres, G. Ozorowski, L. Benincasa, H. Jang, C. di Genova, L. Depau, J. Brunetti, C. Agrati, M. R. Capobianchi, C. Castilletti, A. Emiliozzi, M. Fabbiani, F. Montagnani, L. Bracci, G. Sautto, T. M. Ross, E. Montomoli, N. Temperton, A. B. Ward, C. Sala, G. Ippolito, R. Rappuoli, Extremely potent human monoclonal antibodies from COVID-19 convalescent patients. *Cell* **184**, 1821–1835.e16 (2021).

19. P. J. M. Brouwer, T. G. Caniels, K. van der Straten, J. L. Snitselaar, Y. Aldon, S. Bangaru, J. L. Torres, N. M. A. Okba, M. Claireaux, G. Kerster, A. E. H. Bentlage, M. M. van Haaren, D. Guerra, J. A. Burger, E. E. Schermer, K. D. Verheul, N. van der Velde, A. van der Kooi, J. van Schooten, M. J. van Breemen, T. P. L. Bijl, K. Sliepen, A. Aartse, R. Derking, I. Bontjer, N. A. Kootstra, W. J. Wiersinga, G. Vidarsson, B. L. Haagmans, A. B. Ward, G. J. de Bree, R. W. Sanders, M. J. van Gils, Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. *Science* **369**, 643–650 (2020).

20. W. Dejnirattisai, D. Zhou, H. M. Ginn, H. M. E. Duyvesteyn, P. Supasa, J. B. Case, Y. Zhao, T. S. Walter, A. J. Mentzer, C. Liu, B. Wang, G. C. Paesen, J. Slon-Campos, C. López-Camacho, N. M. Kafai, A. L. Bailey, R. E. Chen, B. Ying, C. Thompson, J. Bolton, A. Fyfe, S. Gupta, T. K. Tan, J. Gilbert-Jaramillo, W. James, M. Knight, M. W. Carroll, D. Skelly, C. Dold, Y. Peng,

R. Levin, T. Dong, A. J. Pollard, J. C. Knight, P. Klenerman, N. Temperton, D. R. Hall, M. A. Williams, N. G. Paterson, F. K. R. Bertram, C. A. Siebert, D. K. Clare, A. Howe, J. Radecke, Y. Song, A. R. Townsend, K.Y. A. Huang, E. E. Fry, J. Mongkolsapaya, M. S. Diamond, J. Ren, D. I. Stuart, G. R. Screaton, The antigenic anatomy of SARS-CoV-2 receptor binding domain. *Cell* **184**, 2183–2200.e22 (2021).

21. E. Andreano, E. Nicastri, I. Paciello, P. Pileri, N. Manganaro, G. Piccini, A. Manenti, E. Pantano, A. Kabanova, M. Troisi, F. Vacca, D. Cardamone, C. De Santi, J. L. Torres, G. Ozorowski, L. Benincasa, H. Jang, C. D. Genova, L. Depau, J. Brunetti, C. Agrati, M. R. Capobianchi, C. Castilletti, A. Emiliozzi, M. Fabbiani, F. Montagnani, L. Bracci, G. Sautto, T. M. Ross, E. Montomoli, N. Temperton, A. B. Ward, C. Sala, G. Ippolito, R. Rappuoli, Extremely potent human monoclonal antibodies from Covid-19 convalescent patients. *Cell* **184**, 1821–1835.e16 (2020).

22. Z. Lv, Y.-Q. Deng, Q. Ye, L. Cao, C.-Y. Sun, C. Fan, W. Huang, S. Sun, Y. Sun, L. Zhu, Q. Chen, N. Wang, J. Nie, Z. Cui, D. Zhu, N. Shaw, X.-F. Li, Q. Li, L. Xie, Y. Wang, Z. Rao, C.-F. Qin, X. Wang, Structural basis for neutralization of SARS-CoV-2 and SARS-CoV by a potent therapeutic antibody. *Science* **369**, eabc5881 (2020).

23. H. Liu, N. C. Wu, M. Yuan, S. Bangaru, J. L. Torres, T. G. Caniels, J. van Schooten, X. Zhu, C.-C. D. Lee, P. J. M. Brouwer, M. J. van Gils, R. W. Sanders, A. B. Ward, I. A. Wilson, Cross-neutralization of a SARS-CoV-2 antibody to a functionally conserved site is mediated by avidity. *Immunity* **53**, 1272–1280.e5 (2020).

24. C. G. Rappazzo, L. V. Tse, C. I. Kaku, D. Wrapp, M. Sakharkar, D. Huang, L. M. Deveau, T. J. Yockachonis, A. S. Herbert, M. B. Battles, C. M. O'Brien, M. E. Brown, J. C. Geoghegan, J. Belk, L. Peng, L. Yang, Y. Hou, T. D. Scobey, D. R. Burton, D. Nemazee, J. M. Dye, J. E. Voss, B. M. Gunn, J. S. McLellan, R. S. Baric, L. E. Gralinski, L. M. Walker, Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. *Science* **371**, 823–829 (2021).

25. D. Wrapp, N. Wang, K. S. Corbett, J. A. Goldsmith, C.-L. Hsieh, O. Abiona, B. S. Graham, J. S. McLellan, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260–1263 (2020).

26. A. C. Walls, Y.-J. Park, M. A. Tortorici, A. Wall, A. T. McGuire, D. Veesler, Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* **181**, 281–292.e6 (2020).

27. M. E. Toledo-Romani, M. García-Carmenate, C. Valenzuela-Silva, W. Baldoquín-Rodríguez, M. Martínez-Pérez, M. Rodríguez-González, B. Paredes-Moreno, I. Mendoza-Hernández, R. González-Mujica, O. Samón-Tabio, P. Velazco-Villares, Juan Pablo Bacallao-Castillo, E. Licea-Martín, M. Rodríguez-Ortega, N. Herrera-Marrero, E. Caballero-González, L. Egües-Torres, R. Duartes-González, S. García-Blanco, S. Pérez-Cabrera, S. Huete-Ferreira, K. Idalmis-Cisnero, O. Fonte-Galindo, D. Meliá-Pérez, I. Rojas-Remedios, View ORCID Profile Delaram Doroud, M. M. Gouya, A. Biglari, P. Van der Stuyft, S. Fernández-Castillo, Y. Climent-Ruiz, Y. Valdes-Balbín, D. García-Rivera, V. Verez-Bencomo; the SOBERANA Phase team, Safety and efficacy of the two doses conjugated protein-based SOBERANA-02 COVID-19 vaccine and of a heterologous three-dose combination with SOBERANA-PLUS: Double-blind, randomised, placebo-controlled phase 3 clinical trial. Medrxiv 2021.10.31.21265703 (2021).

28. S. Thuluva, V. Paradkar, K. Turaga, S. R. Gunneri, V. Yerroju, R. Mogulla, P. V. Suneetha, M. Kyasani, S. K. Manoharan, S. Adabala, A. S. Javvadi, G. Medigeshi, J. Singh, H. Shaman, A. Binayke, A. Zaheer, A. Awasthi, C. Singh, A Venkateshwar Rao, I. Basu, K. A. A. Kumar, A. K. Pandey, Immunogenic superiority and safety of Biological E's CORBEVAX™ vaccine compared to COVISHIELD™ (ChAdOx1 nCoV-19) vaccine studied in a phase III, single blind, multicenter, randomized clinical trial. Medrxiv 2022.03.20.22271891 (2022).

29. A. Goldenzweig, M. Goldsmith, S. E. Hill, O. Gertman, P. Laurino, Y. Ashani, O. Dym, T. Unger, S. Albeck, J. Prilusky, R. L. Lieberman, A. Aharoni, I. Silman, J. L. Sussman, D. S. Tawfik, S. J. Fleishman, Automated structure- and sequence-based design of proteins for high bacterial expression and stability. *Mol. Cell* **63**, 337–346 (2016).

30. C. L. Hsieh, J. A. Goldsmith, J. M. Schaub, A. M. DiVenere, H.-C. Kuo, K. Javanmardi, K. C. le, D. Wrapp, A. G. Lee, Y. Liu, C.W. Chou, P. O. Byrne, C. K. Hjorth, N. V. Johnson, J. Ludes-

Meyers, A. W. Nguyen, J. Park, N. Wang, D. Amengor, J. J. Lavinder, G. C. Ippolito, J. A. Maynard, I. J. Finkelstein, J. S. McLellan, Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. *Science* **369**, 1501–1505 (2020).

31. B. Ju, Q. Zhang, J. Ge, R. Wang, J. Sun, X. Ge, J. Yu, S. Shan, B. Zhou, S. Song, X. Tang, J. Yu, J. Lan, J. Yuan, H. Wang, J. Zhao, S. Zhang, Y. Wang, X. Shi, L. Liu, J. Zhao, X. Wang, Z. Zhang, L. Zhang, Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* **584**, 115–119 (2020).

32. D. F. Robbiani, C. Gaebler, F. Muecksch, J. C. C. Lorenzi, Z. Wang, A. Cho, M. Agudelo, C. O. Barnes, A. Gazumyan, S. Finkin, T. Hägglöf, T. Y. Oliveira, C. Viant, A. Hurley, H.-H. Hoffmann, K. G. Millard, R. G. Kost, M. Cipolla, K. Gordon, F. Bianchini, S. T. Chen, V. Ramos, R. Patel, J. Dizon, I. Shimeliovich, P. Mendoza, H. Hartweger, L. Nogueira, M. Pack, J. Horowitz, F. Schmidt, Y. Weisblum, E. Michailidis, A. W. Ashbrook, E. Waltari, J. E. Pak, K. E. Huey-Tubman, N. Koranda, P. R. Hoffman, A. P. West Jr., C. M. Rice, T. Hatziioannou, P. J. Bjorkman, P. D. Bieniasz, M. Caskey, M. C. Nussenzweig, Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* **584**, 437–442 (2020).

33. J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang, Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* **581**, 215–220 (2020).

34. M. Yuan, N. C. Wu, X. Zhu, C.-C. D. Lee, R. T. Y. So, H. Lv, C. K. P. Mok, I. A. Wilson, A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. *Science* **368**, 630–633 (2020).

35. D. Pinto, Y.-J. Park, M. Beltramello, A. C. Walls, M. A. Tortorici, S. Bianchi, S. Jaconi, K. Culap, F. Zatta, A. de Marco, A. Peter, B. Guarino, R. Spreafico, E. Cameroni, J. B. Case, R. E. Chen, C. Havenar-Daughton, G. Snell, A. Telenti, H. W. Virgin, A. Lanzavecchia, M. S. Diamond, K. Fink, D. Veesler, D. Corti, Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290–295 (2020).

36. C. O. Barnes, C. A. Jette, M. E. Abernathy, K.-M. A. Dam, S. R. Esswein, H. B. Gristick, A. G. Malyutin, N. G. Sharaf, K. E. Huey-Tubman, Y. E. Lee, D. F. Robbiani, M. C. Nussenzweig, A. P. West Jr., P. J. Bjorkman, SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature* **588**, 682–687 (2020).

37. F. Amanat, S. Strohmeier, R. Rathnasinghe, M. Schotsaert, L. Coughlan, A. García-Sastre, F. Krammer, Introduction of two prolines and removal of the polybasic cleavage site lead to higher efficacy of a recombinant spike-based SARS-CoV-2 vaccine in the mouse model. *MBio* **12**, e02648-20 (2021).

38. Z. Wang, F. Schmidt, Y. Weisblum, F. Muecksch, C. O. Barnes, S. Finkin, D. Schaefer-Babajew, M. Cipolla, C. Gaebler, J. A. Lieberman, T. Y. Oliveira, Z. Yang, M. E. Abernathy, K. E. Huey-Tubman, A. Hurley, M. Turroja, K. A. West, K. Gordon, K. G. Millard, V. Ramos, J. D. Silva, J. Xu, R. A. Colbert, R. Patel, J. Dizon, C. Unson-O'Brien, I. Shimeliovich, A. Gazumyan, M. Caskey, P. J. Bjorkman, R. Casellas, T. Hatziioannou, P. D. Bieniasz, M. C. Nussenzweig, mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *Nature* **592**, 616–622 (2021).

39. M. Hoffmann, N. Krüger, S. Schulz, A. Cossmann, C. Rocha, A. Kempf, I. Nehlmeier, L. Graichen, A.-S. Moldenhauer, M. S. Winkler, M. Lier, A. Dopfer-Jablonka, H.-M. Jäck, G. M. N. Behrens, S. Pöhlmann, The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. *Cell* **185**, 447–456.e11 (2022).

40. L. Liu, S. Iketani, Y. Guo, J. F.-W. Chan, M. Wang, L. Liu, Y. Luo, H. Chu, Y. Huang, M. S. Nair, J. Yu, K. K.-H. Chik, T. T.-T. Yuen, C. Yoon, K. K.-W. To, H. Chen, M. T. Yin, M. E. Sobieszczyk, Y. Huang, H. H. Wang, Z. Sheng, K.-Y. Yuen, D. D. Ho, Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature* **602**, 676–681 (2022).

41. A. Muik, B. G. Lui, A.K. Wallisch, M. Bacher, J. Mühl, J. Reinholz, O. Ozhelvaci, N. Beckmann, R. de la Caridad Güimil Garcia, A. Poran, S. Shpyro, A. Finlayson, H. Cai, Q. Yang, K. A. Swanson, Ö. Türeci, U. Şahin, Neutralization of SARS-CoV-2 Omicron by BNT162b2 mRNA vaccine–elicited human sera. *Science* **375**, 678–680 (2022).

42. P. Wang, M. S. Nair, L. Liu, S. Iketani, Y. Luo, Y. Guo, M. Wang, J. Yu, B. Zhang, P. D. Kwong, B. S. Graham, J. R. Mascola, J. Y. Chang, M. T. Yin, M. Sobieszczyk, C. A. Kyratsous, L. Shapiro, Z. Sheng, Y. Huang, D. D. Ho, Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* **593**, 130–135 (2021).

43. A. Pegu, S. E. O'Connell, S. D. Schmidt, S. O'Dell, C. A. Talana, L. Lai, J. Albert, E. Anderson, H. Bennett, K. S. Corbett, B. Flach, L. Jackson, B. Leav, J. E. Ledgerwood, C. J. Luke, M. Makowski, M. C. Nason, P. C. Roberts, M. Roederer, P. A. Rebolledo, C. A. Rostad, N. G. Rouphael, W. Shi, L. Wang, A. T. Widge, E. S. Yang; The mRNA-1273 Study Group, J. H. Beigel, B. S. Graham, J. R. Mascola, M. S. Suthar, A. B. McDermott, N. A. Doria-Rose, J. Arega, J. H. Beigel, W. Buchanan, M. Elsafy, B. Hoang, R. Lampley, A. Kolhekar, H. Koo, C. Luke, M. Makhene, S. Nayak, R. Pikaart-Tautges, P. C. Roberts, J. Russell, E. Sindall, J. Albert, P. Kunwar, M. Makowski, E. J. Anderson, A. Bechnak, M. Bower, A. F. Camacho-Gonzalez, M. Collins, A. Drobeniuc, V. V. Edara, S. Edupuganti, K. Floyd, T. Gibson, C. M. G. Ackerley, B. Johnson, S. Kamidani, C. Kao, C. Kelley, L. Lai, H. Macenczak, M. P. McCullough, E. Peters, V. K. Phadke, P. A. Rebolledo, C. A. Rostad, N. Rouphael, E. Scherer, A. Sherman, K. Stephens, M. S. Suthar, M. Teherani, J. Traenkner, J. Winston, I. Yildirim, L. Barr, J. Benoit, B. Carste, J. Choe, M. Dunstan, R. Erolin, J. ffitch, C. Fields, L. A. Jackson, E. Kiniry, S. Lasicka, S. Lee, M. Nguyen, S. Pimienta, J. Suyehira, M. Witte, H. Bennett, N. E. Altaras, A. Carfi, M. Hurley, B. Leav, R. Pajon, W. Sun, T. Zaks, R. N. Coler, S. E. Larsen, K. M. Neuzil, L. C. Lindesmith, D. R. Martinez, J. Munt, M. Mallory, C. Edwards, R. S. Baric, N. M. Berkowitz, E. A. Boritz, K. Carlton, K. S. Corbett, P. Costner, A. Creanga, N. A. Doria-Rose, D. C. Douek, B. Flach, M. Gaudinski, I. Gordon, B. S. Graham, L.S. Holman, J. E. Ledgerwood, K. Leung, B. C. Lin, M. K. Louder, J. R. Mascola, A. B. McDermott, K. M. Morabito, L. Novik, S. O'Connell, S. O'Dell, M. Padilla, A. Pegu, S. D. Schmidt, W. Shi, P. A. Swanson II, C. A. Talana, L. Wang, A. T. Widge, E. S. Yang, Y. Zhang, J. D. Chappell, M. R. Denison, T. Hughes, X. Lu, A. J. Pruijssers, L. J. Stevens, C. M. Posavad, M. Gale Jr, V. Menachery, P.Y. Shi, Durability of mRNA-1273 vaccine–induced antibodies against SARS-CoV-2 variants. *Science* **373**, 1372–1377 (2021).

44. T. K. Tan, P. Rijal, R. Rahikainen, A. H. Keeble, L. Schimanski, S. Hussain, R. Harvey, J. W. P. Hayes, J. C. Edwards, R. K. McLean, V. Martini, M. Pedrera, N. Thakur, C. Conceicao, I.

Dietrich, H. Shelton, A. Ludi, G. Wilsden, C. Browning, A. K. Zagrajek, D. Bialy, S. Bhat, P. Stevenson-Leggett, P. Hollinghurst, M. Tully, K. Moffat, C. Chiu, R. Waters, A. Gray, M. Azhar, V. Mioulet, J. Newman, A. S. Asfor, A. Burman, S. Crossley, J. A. Hammond, E. Tchilian, B. Charleston, D. Bailey, T. J. Tuthill, S. P. Graham, H. M. E. Duyvesteyn, T. Malinauskas, J. Huo, J. A. Tree, K. R. Buttigieg, R. J. Owens, M. W. Carroll, R. S. Daniels, J. W. McCauley, D. I. Stuart, K.Y. A. Huang, M. Howarth, A. R. Townsend, A COVID-19 vaccine candidate using SpyCatcher multimerization of the SARS-CoV-2 spike protein receptor-binding domain induces potent neutralising antibody responses. *Nat. Commun.* **12**, 542 (2021).

45. L. Dai, T. Zheng, K. Xu, Y. Han, L. Xu, E. Huang, Y. An, Y. Cheng, S. Li, M. Liu, M. Yang, Y. Li, H. Cheng, Y. Yuan, W. Zhang, C. Ke, G. Wong, J. Qi, C. Qin, J. Yan, G. F. Gao, A universal design of betacoronavirus vaccines against COVID-19, MERS, and SARS. *Cell* **182**, 722–733.e11 (2020).

46. A. C. Walls, B. Fiala, A. Schäfer, S. Wrenn, M. N. Pham, M. Murphy, L. V. Tse, L. Shehata, M. A. O'Connor, C. Chen, M. J. Navarro, M. C. Miranda, D. Pettie, R. Ravichandran, J. C. Kraft, C. Ogohara, A. Palser, S. Chalk, E.-C. Lee, K. Guerriero, E. Kepl, C. M. Chow, C. Sydeman, E. A. Hodge, B. Brown, J. T. Fuller, K. H. Dinnon III, L. E. Gralinski, S. R. Leist, K. L. Gully, T. B. Lewis, M. Guttman, H. Y. Chu, K. K. Lee, D. H. Fuller, R. S. Baric, P. Kellam, L. Carter, M. Pepper, T. P. Sheahan, D. Veesler, N. P. King, Elicitation of potent neutralizing antibody responses by designed protein nanoparticle vaccines for SARS-CoV-2. *Cell* **183**, 1367–1382.e17 (2020).

47. Y. F. Kang, C. Sun, Z. Zhuang, R.-Y. Yuan, Q. Zheng, J.-P. Li, P.-P. Zhou, X.-C. Chen, Z. Liu, X. Zhang, X.-H. Yu, X.-W. Kong, Q.-Y. Zhu, Q. Zhong, M. Xu, N.-S. Zhong, Y.-X. Zeng, G.-K. Feng, C. Ke, J.-C. Zhao, M.-S. Zeng, Rapid development of SARS-CoV-2 spike protein receptor-binding domain self-assembled nanoparticle vaccine candidates. *ACS Nano* **15**, 2738–2752 (2021).

48. X. Ma, F. Zou, F. Yu, R. Li, Y. Yuan, Y. Zhang, X. Zhang, J. Deng, T. Chen, Z. Song, Y. Qiao, Y. Zhan, J. Liu, J. Zhang, X. Zhang, Z. Peng, Y. Li, Y. Lin, L. Liang, G. Wang, Y. Chen, Q. Chen, T. Pan, X. He, H. Zhang, Nanoparticle vaccines based on the receptor binding domain

(RBD) and heptad repeat (HR) of SARS-CoV-2 elicit robust protective immune responses. *Immunity* **53**, 1315–1330.e9 (2020).

49. D. Wrapp, D. De Vlieger, K. S. Corbett, G. M. Torres, N. Wang, W. Van Breedam, K. Roose, L. van Schie; VIB-CMB COVID-19 Response Team, M. Hoffmann, S. Pöhlmann, B. S. Graham, N. Callewaert, B. Schepens, X. Saelens, J. S. McLellan, Structural basis for potent neutralization of betacoronaviruses by single-domain camelid antibodies. *Cell* **181**, 1004–1015.e15 (2020).

50. A. C. Walls, X. Xiong, Y.-J. Park, M. Alejandra Tortorici, J. Snijder, J. Quispe, E. Cameroni, R. Gopal, M. Dai, A. Lanzavecchia, M. Zambon, F. A. Rey, D. Corti, D. Veesler, Unexpected receptor functional mimicry elucidates activation of coronavirus fusion. *Cell* **176**, 1026–1039.e15 (2019).

51. J. E. Pak, C. Sharon, M. Satkunarajah, T. C. Auperin, C. M. Cameron, D. J. Kelvin, J. Seetharaman, A. Cochrane, F. A. Plummer, J. D. Berry, J. M. Rini, Structural insights into immune recognition of the severe acute respiratory syndrome coronavirus S protein receptor binding domain. *J. Mol. Biol.* **388**, 815–823 (2009).

52. W. C. Hwang, Y. Lin, E. Santelli, J. Sui, L. Jaroszewski, B. Stec, M. Farzan, W. A. Marasco, R. C. Liddington, Structural basis of neutralization by a human anti-severe acute respiratory syndrome spike protein antibody, 80R. *J. Biol. Chem.* **281**, 34610–34616 (2006).

53. P. Prabakaran, J. Gan, Y. Feng, Z. Zhu, V. Choudhry, X. Xiao, X. Ji, D. S. Dimitrov, Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. *J. Biol. Chem.* **281**, 15829–15836 (2006).

54. J. Sui, W. Li, A. Murakami, A. Tamin, L. J. Matthews, S. K. Wong, M. J. Moore, A. S. C. Tallarico, M. Olurinde, H. Choe, L. J. Anderson, W. J. Bellini, M. Farzan, W. A. Marasco, Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2536–2541 (2004).

55. J. T. Meulen, E. N. van den Brink, L. L. M. Poon, W. E. Marissen, C. S. W Leung, F. Cox, C. Y. Cheung, A. Q. Bakker, J. A. Bogaards, E. van Deventer, W. Preiser, H. W. Doerr, V. T. Chow, J. de Kruif, J. S. M Peiris, J. Goudsmit, Human monoclonal antibody combination against SARS coronavirus: Synergy and coverage of escape mutants. *PLOS Med.* **3**, e237 (2006).

56. LLC Schrodinger, The PyMOL Molecular Graphics System. Version 2.4 (2010).

57. S. F. Altschul, T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D. J. Lipman, Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402 (1997).

58. T. A. Whitehead, A. Chevalier, Y. Song, C. Dreyfus, S. J. Fleishman, C. de Mattos, C. A. Myers, H. Kamisetty, P. Blair, I. A. Wilson, D. Baker, Optimization of affinity, specificity and function of designed influenza inhibitors using deep sequencing. *Nat. Biotechnol.* **30**, 543–548 (2012).

59. W. Li, A. Godzik, Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**, 1658–1659 (2006).

60. A. R. Aricescu, W. Lu, E. Y. Jones, A time- and cost-efficient system for high-level protein production in mammalian cells. *Acta Crystallogr. D Biol. Crystallogr.* **62**, 1243–1250 (2006).

61. E. Gasteiger, A. Gattiker, C. Hoogland, I. Ivanyi, R. D. Appel, A. Bairoch, ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* **31**, 3784–3788 (2003).

62. K. K. Chan, D. Dorosky, P. Sharma, S. A. Abbasi, J. M. Dye, D. M. Kranz, A. S. Herbert, E. Procko, Engineering human ACE2 to optimize binding to the spike protein of SARS coronavirus 2. *Science* **369**, 1261–1265 (2020).

63. C. Vonrhein, C. Flensburg, P. Keller, A. Sharff, O. Smart, W. Paciorek, T. Womack, G. Bricogne, Data processing and analysis with the *autoPROC* toolbox. *Acta Crystallogr. D Biol. Crystallogr.* **67**, 293–302 (2011).

64. W. Kabsch, XDS. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 125–132 (2010).

65. M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. W. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin, K. S. Wilson, Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* **67**, 235–242 (2011).

66. P. R. Evans, G. N. Murshudov, How good are my data and what is the resolution? *Acta Crystallogr. D Biol. Crystallogr.* **69**, 1204–1214 (2013).

67. P. D. Adams, P. V. Afonine, G. Bunkóczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L.-W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger, P. H. Zwart, PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 213–221 (2010).

68. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 486–501 (2010).

69. V. B. Chen, W. B. Arendall III, J. J. Headd, D. A. Keedy, R. M. Immormino, G. J. Kapral, L. W. Murray, J. S. Richardson, D. C. Richardson, MolProbity: All-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 12–21 (2010).

70. A. Morin, B. Eisenbraun, J. Key, P. C. Sanschagrin, M. A. Timony, M. Ottaviano, P. Sliz, Collaboration gets the most out of software. *eLife* **2**, e01456 (2013).

71. X. Liu, C. Luongo, Y. Matsuoka, H.-S. Park, C. Santos, L. Yang, I. N. Moore, S. Afroz, R. F. Johnson, B. A. P. Lafont, C. Martens, S. M. Best, V. J. Munster, J. Hollý, J. W. Yewdell, C. le Nouën, S. Munir, U. J. Buchholz, A single intranasal dose of a live-attenuated parainfluenza virus-vectored SARS-CoV-2 vaccine is protective in hamsters. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2109744118 (2021).

72. T. Zhou, Y. Tsybovsky, J. Gorman, M. Rapp, G. Cerutti, G.-Y. Chuang, P. S. Katsamba, J. M. Sampson, A. Schön, J. Bimela, J. C. Boyington, A. Nazzari, A. S. Olia, W. Shi, M. Sastry, T.

Stephens, J. Stuckey, I.-T. Teng, P. Wang, S. Wang, B. Zhang, R. A. Friesner, D. D. Ho, J. R. Mascola, L. Shapiro, P. D. Kwong, Cryo-EM structures of SARS-CoV-2 spike without and with ACE2 reveal a pH-dependent switch to mediate endosomal positioning of receptor-binding domains. *Cell Host Microbe* **28**, 867–879.e5 (2020).