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Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice

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Protection against SARS-CoV-2 and SARS-related emergent zoonotic coronaviruses is urgently needed. We made homotypic nanoparticles displaying the receptor-binding domain (RBD) of SARS-CoV-2 or co-displaying SARS-CoV-2 RBD along with RBDs from animal betacoronaviruses that represent threats to humans (mosaic nanoparticles; 4–8 distinct RBDs). Mice immunized with RBD-nanoparticles, but not soluble antigen, elicited cross-reactive binding and neutralization responses. Mosaic-RBD-nanoparticles elicited antibodies with superior cross-reactive recognition of heterologous RBDs compared to sera from immunizations with homotypic SARS-CoV-2–RBD-nanoparticles or COVID-19 convalescent human plasmas. Moreover, sera from mosaic-RBD-immunized mice neutralized heterologous pseudotyped coronaviruses equivalently or better after priming than sera from homotypic SARS-CoV-2–RBD-nanoparticle immunizations, demonstrating no immunogenicity loss against particular RBDs resulting from co-display. A single immunization with mosaic-RBD-nanoparticles provides a potential strategy to simultaneously protect against SARS-CoV-2 and emerging zoonotic coronaviruses.

SARS-CoV-2, a newly-emergent betacoronavirus, resulted in a global pandemic in 2020, infecting millions and causing the respiratory disease COVID-19 (1, 2). Two other zoonotic betacoronaviruses, SARS-CoV and MERS-CoV, also resulted in outbreaks within the last 20 years (3). All three viruses presumably originated in bats (4), with SARS-CoV and MERS-CoV adapting to intermediary animal hosts before jumping to humans. SARS-like viruses circulate in bats and serological surveillance of people living near caves where bats carry diverse coronaviruses demonstrates direct transmission of SARS-like viruses with pandemic potential (5), suggesting a pan-coronavirus vaccine is needed to protect against future outbreaks and pandemics. In particular, the bat WIV1 and SHC014 strains are thought to represent an ongoing threat to humans (6, 7).

Most current SARS-CoV-2 vaccine candidates include the spike trimer (S), the viral protein that mediates target cell entry after one or more of its receptor-binding domains (RBDs) adopt an “up” position to bind a host receptor (Fig. 1A). The RBDs of human coronaviruses SARS-CoV-2, SARS-CoV, HCoV-NL63, and related animal coronaviruses (WIV1 and SCH014) use angiotensin-converting enzyme 2 (ACE2) as their host receptor (1, 8, 9), while other coronaviruses use receptors such as dipeptidyl peptidase 4 (10) or sialic acids

(11, 12). Consistent with its function in viral entry, S is the primary target of neutralizing antibodies (13–22), with many targeting the RBD (14–18, 21–26).

Multivalent display of antigen enhances B cell responses and can provide longer-lasting immunity than monovalent antigens (27, 28), thus protein-based vaccine candidates often involve a nanoparticle that enables antigen multimerization. Many nanoparticles and coupling strategies have been explored for vaccine design (29), with “plug and display” strategies being especially useful (30, 31). In one such approach, multiple copies of an engineered protein domain called SpyCatcher fused to subunits of a virus-like particle form spontaneous isopeptide bonds to purified antigens tagged with a 13-residue SpyTag (29–32). The SpyCatcher-SpyTag system was used to prepare multimerized SARS-CoV-2 RBD or S trimer that elicited high titers of neutralizing antibodies (33, 34). Although promising for protection against SARS-CoV-2, coronavirus reservoirs in bats suggest future cross-species transmission (6, 7, 35), necessitating a vaccine that protects against emerging coronaviruses as well as SARS-CoV-2. Here we prepared SpyCatcher003-mi3 nanoparticles (31, 36) simultaneously displaying SpyTagged RBDs from human and animal coronaviruses to evaluate whether mosaic particles can elicit cross-reactive antibody

responses, as previously demonstrated for influenza head domain mosaic particles (37). We show that mice immunized with homotypic or mosaic nanoparticles produced broad binding and neutralizing responses, in contrast to plasma antibodies elicited in humans by SARS-CoV-2 infection. Moreover, mosaic nanoparticles showed enhanced heterologous binding and neutralization properties against human and bat SARS-like betacoronaviruses (sarbecoviruses) compared with homotypic SARS-CoV-2 nanoparticles.

We used a study of sarbecovirus RBD receptor usage and cell tropism (38) to guide our choice of RBDs for co-display on mosaic particles. From 29 RBDs that were classified into distinct clades (clades 1, 2, 1/2, and 3) (38), we identified diverse RBDs from SARS-CoV, WIV1, and SHC014 (clade 1), SARS-CoV-2 (clade 1/2), Rs4081, Yunnan 2011 (Yun11), and Rf1 (clade 2), and BM48-31 (clade 3), of which SARS-CoV-2 and SARS-CoV are human coronaviruses and the rest are bat viruses originating in China or Bulgaria (BM48-31). We also included RBDs from the GX pangolin clade 1/2 coronavirus (referred to here as pang17) (39), RaTG13, the bat clade 1/2 virus most closely related to SARS-CoV-2 (40), RmYN02, a clade 2 bat virus from China (41), and BtKY72, a Kenyan bat clade 3 virus (42). Mapping of the sequence conservation across selected RBDs showed varying degrees of sequence identity (68-95%), with highest sequence variability in residues corresponding to the SARS-CoV-2 ACE2 receptor-binding motif (Fig. 1, A to D, and fig. S1). We chose 8 of the 12 RBDs for making three types of mosaic nanoparticles: mosaic-4a (coupled to SARS-2, RaTG13, SHC014, and Rs4081 RBDs), mosaic-4b (coupled to pang17, RmYN02, Rf1, and WIV1 RBDs), and mosaic-8 (coupled to all eight RBDs), and compared them with homotypic mi3 particles constructed from SARS-CoV-2 RBD alone (homotypic SARS-2). RBDs from SARS, Yun11, BM-4831, and BtKY72, which were not coupled to mosaic particles, were used to evaluate sera for cross-reactive responses.

SpyTag003-RBDs were coupled to SpyCatcher003-mi3 (60 potential conjugation sites) (36, 43) to make homotypic and mosaic nanoparticles (Fig. 2A). Particles were purified by size exclusion chromatography (SEC) and analyzed by SDS-PAGE, revealing monodisperse SEC profiles and nearly 100% conjugation (Fig. 2, B and C). Representative RBDs were conjugated to SpyCatcher003-mi3 with similar or identical efficiencies (fig. S2), suggesting that mosaic particles contained approximately equimolar mixtures of different RBDs.

We immunized mice with either soluble SARS-CoV-2 spike trimer (SARS-2 S), nanoparticles displaying only SARS-2 RBD (homotypic SARS-2), nanoparticles co-displaying RBDs (mosaic-4a, mosaic-4b, mosaic-8), or unconjugated nanoparticles (mi3). IgG responses were evaluated after prime or boost immunizations (Fig. 3A) by ELISA

against SARS-2 S (Fig. 3B) or a panel of RBDs (Fig. 3, C to F, and fig. S3). Sera from unconjugated nanoparticle-immunized animals (black in Fig. 3 and fig. S3) showed no responses above background. Anti-SARS-2 S trimer and anti-SARS-2 RBD serum responses were similar (Fig. 3, B and C), demonstrating that antibodies elicited against RBDs can access their epitopes on SARS-2 S trimer. We also conducted *in vitro* neutralization assays using a pseudotyped virus assay that quantitatively correlates with authentic virus neutralization (44) for strains known to infect 293T_{ACE2} target cells (SARS-CoV-2, SARS, WIV1 and SHC104). Neutralization and ELISA titers were significantly correlated (fig. S4), thus suggesting ELISAs are predictive of neutralization results when pseudotyped neutralization assays were not possible due to unknown viral entry receptor usage.

Mice immunized with soluble SARS-2 S trimer (brown bars) showed no binding or neutralization except for autologous responses against SARS-2 after boosting (Fig. 3, C to F). By contrast, sera from RBD-nanoparticle-immunized animals (red, green, yellow, and blue bars) exhibited binding to all RBDs (Fig. 3, C to F, and fig. S3A) and neutralization against all four strains after boosting (Fig. 3, C to E), consistent with increased immunogenicities of multimerized antigen on nanoparticles versus soluble antigen (27, 28). Homotypic SARS-2 nanoparticles, but not soluble SARS-2 trimer, induced heterologous responses to zoonotic RBDs and neutralization of heterologous coronaviruses (Fig. 3, D to F). To address whether co-display of SARS-2 RBD along with other RBDs on mosaic-4a and mosaic-8 versus homotypic display of SARS-2 RBD (homotypic SARS-2) diminished anti-SARS-2 responses, we compared SARS-2-specific ELISA and neutralization titers for mosaic versus homotypic immunizations (Fig. 3C): there were no significant differences in IgG anti-SARS-2 titers for animals immunized with homotypic (red in Fig. 3C) versus mosaic nanoparticles (green and blue in Fig. 3C). Thus there was no advantage of immunization with a homotypic RBD-nanoparticle versus a mosaic-nanoparticle that included SARS-2 RBD in terms of the magnitude of immune responses against SARS-2.

We next compared serum responses against matched RBDs (RBDs present on an injected nanoparticle; gray horizontal shading) versus mismatched RBDs (RBDs not present on injected nanoparticle; red horizontal shading) (Fig. 3 and fig. S3). Although SARS-2 RBD was not presented on mosaic-4b, antibody titers elicited by mosaic-4b immunization (yellow) were not significantly different than titers elicited by matched nanoparticle immunizations (homotypic SARS-2 (red), mosaic-4a (green), and mosaic-8 (blue)), and sera from boosted mosaic-4b-immunized mice neutralized SARS-2 pseudovirus (Fig. 3C). In other matched versus mismatched comparisons, sera showed binding and neutralization of SHC014 and WIV1 regardless of whether these RBDs

were included on the injected nanoparticle (Fig. 3D), underscoring sharing of common epitopes among RBDs (Fig. 1A).

Demonstrating advantages of mosaic versus homotypic SARS-2 nanoparticles, sera from mosaic-8-immunized mice bound SHC014 and WIV1 RBDs significantly better after the prime than sera from homotypic SARS-2-immunized mice and retained better binding to SHC014 RBD after boosting (Fig. 3D). Thus the potential increased avidity of the homotypic SARS-2 nanoparticle displaying only one type of RBD over the mosaic-8 nanoparticles did not confer increased breadth. Moreover, mosaic-8-immunized and boosted sera were 7-44-fold more potent than sera from homotypic SARS-2-immunized animals in neutralizing SHC014 and WIV1 (Fig. 3D). Neutralization of the SHC014 and WIV1 pseudoviruses by mosaic-8 sera suggests that combining RBDs on a mosaic nanoparticle does not diminish the immune response against a particular RBD, also suggested by ELISA binding of sera to Rs4081 and RaTG13 (fig. S3, A and B).

To further address whether RBD-nanoparticles elicited antibodies that recognized totally mismatched strains and SARS-CoV-2 RBD mutants, we evaluated sera for binding to SARS, Yun11, BM-4831, and BtKY72 RBDs (Fig. 3, E and F), SARS-2 RBD mutants (fig. S3C), MERS-CoV RBD (fig. S3D), and for neutralization in SARS pseudovirus assays (Fig. 3E). We found no reductions in SARS-2 RBD binding as a result of mutations (Y453F, the “Danish mink variant” (45) or a Q493K/Q498Y/P499T triple mutant (46)) (fig. S3C), no binding of any elicited sera to MERS-CoV RBD (fig. S3D), and higher and more cross-reactive antibody responses for mosaic immunizations compared with homotypic SARS-2 immunizations: e.g., mosaic-8-primed and boosted animals showed significantly higher titers against SARS RBD than sera from homotypic SARS-2-immunized mice (Fig. 3E). After the prime, sera from the homotypic SARS-2-immunized animals did not neutralize SARS, whereas the mosaic-4b and mosaic-8 sera were neutralizing (Fig. 3E), perhaps facilitated by these nanoparticles including WIV1 RBD, which is related by 95% amino acid identity to SARS RBD (Fig. 1D). After boosting, SARS-2 and mosaic-4a sera were also neutralizing, although titers were ~4-fold lower than for mosaic-8-immunized animals (Fig. 3E). ELISA titers against other mismatched RBDs (Yun11, BM-4831, BtKY72) were significantly higher for sera collected after mosaic-8 priming compared to sera from homotypic SARS-2 priming, and heightened binding was retained after boosting (Fig. 3F). Thus mosaic nanoparticles, particularly mosaic-8, induce higher antibody titers against mismatched RBDs than homotypic SARS-2 nanoparticles, again favoring the co-display approach for inducing broader anti-coronavirus responses, especially after a single prime.

We investigated the potential for cross-reactive recog-

nition using flow cytometry to ask whether B cell receptors on IgG⁺ splenic B-cells from RBD-nanoparticle-boosted animals could simultaneously recognize RBDs from SARS-2 and Rs4081 (related by 70% sequence identity) (Fig. 1D and fig. S5). Whereas control animals were negative, all other groups showed B-cells that recognized SARS-2 and Rs4081 RBDs simultaneously, suggesting the existence of antibodies that cross-react with both RBDs (fig. S5E).

To compare antibodies elicited by RBD-nanoparticle immunization to antibodies elicited by SARS-CoV-2 infection, we repeated ELISAs against the RBD panel using IgGs from COVID-19 plasma donors (47) (Fig. 4). Most of the convalescent plasmas showed detectable binding to SARS-2 RBD (Fig. 4A). However, binding to other sarbecovirus RBDs (RaTG13, SHC014, WIV1, Rs4081 and BM-4831) was significantly weaker than binding to SARS 2 RBD, with many human plasma IgGs showing no binding above background (Fig. 4, B to G). In addition, although convalescent plasma IgGs neutralized SARS-CoV-2 pseudoviruses, they showed weak or no neutralization of SARS, SHC014, or WIV1 pseudoviruses (Fig. 4H). These results are consistent with little to no cross-reactive recognition of RBDs from zoonotic coronavirus strains resulting from SARS-CoV-2 infection in humans.

In conclusion, we confirmed that multimerization of RBDs on nanoparticles enhances immunogenicity compared with soluble antigen (33, 48) and further showed that homotypic SARS-2 nanoparticle immunization produced IgG responses that bound zoonotic RBDs and neutralized heterologous coronaviruses after boosting. By contrast, soluble SARS-2 S immunization and natural infection with SARS-CoV-2 resulted in weak or no heterologous responses in plasmas. Co-display of SARS-2 RBD along with diverse RBDs on mosaic nanoparticles showed no disadvantages for eliciting neutralizing antibodies against SARS-CoV-2 compared with homotypic SARS-2 nanoparticles, suggesting mosaic nanoparticles as a candidate vaccine to protect against COVID-19. Furthermore, compared with homotypic SARS-2 RBD particles, the mosaic co-display strategy demonstrated advantages for eliciting neutralizing antibodies against zoonotic sarbecoviruses, thus potentially also providing protection against emerging coronaviruses with human spillover potential. Neutralization of matched and mismatched strains was observed after mosaic priming, suggesting a single injection of a mosaic-RBD nanoparticle might be sufficient in a vaccine. Since COVID-19 convalescent plasmas showed little to no recognition of coronavirus RBDs other than SARS-CoV-2, COVID-19-induced immunity in humans may not protect against another emergent coronavirus. However, the mosaic nanoparticles described here could be used as described or easily adapted to present RBDs from newly-discovered zoonotic coronaviruses.

REFERENCES AND NOTES

1. P. Zhou, X.-L. Yang, X.-G. Wang, B. Hu, L. Zhang, W. Zhang, H.-R. Si, Y. Zhu, B. Li, C.-L. Huang, H.-D. Chen, J. Chen, Y. Luo, H. Guo, R.-D. Jiang, M.-Q. Liu, Y. Chen, X.-R. Shen, X. Wang, X.-S. Zheng, K. Zhao, Q.-J. Chen, F. Deng, L.-L. Liu, B. Yan, F.-X. Zhan, Y.-Y. Wang, G.-F. Xiao, Z.-L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270–273 (2020). [doi:10.1038/s41586-020-2012-7](https://doi.org/10.1038/s41586-020-2012-7) [Medline](#)
2. F. Wu, S. Zhao, B. Yu, Y.-M. Chen, W. Wang, Z.-G. Song, Y. Hu, Z.-W. Tao, J.-H. Tian, Y.-Y. Pei, M.-L. Yuan, Y.-L. Zhang, F.-H. Dai, Y. Liu, Q.-M. Wang, J.-J. Zheng, L. Xu, E. C. Holmes, Y.-Z. Zhang, A new coronavirus associated with human respiratory disease in China. *Nature* **579**, 265–269 (2020). [doi:10.1038/s41586-020-2008-3](https://doi.org/10.1038/s41586-020-2008-3) [Medline](#)
3. E. de Wit, N. van Doremalen, D. Falzarano, V. J. Munster, SARS and MERS: Recent insights into emerging coronaviruses. *Nat. Rev. Microbiol.* **14**, 523–534 (2016). [doi:10.1038/nrmicro.2016.81](https://doi.org/10.1038/nrmicro.2016.81) [Medline](#)
4. W. Li, Z. Shi, M. Yu, W. Ren, C. Smith, J. H. Epstein, H. Wang, G. Crameri, Z. Hu, H. Zhang, J. Zhang, J. McEachern, H. Field, P. Daszak, B. T. Eaton, S. Zhang, L. F. Wang, Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**, 676–679 (2005). [doi:10.1126/science.1118391](https://doi.org/10.1126/science.1118391) [Medline](#)
5. N. Wang, S.-Y. Li, X.-L. Yang, H.-M. Huang, Y.-J. Zhang, H. Guo, C.-M. Luo, M. Miller, G. Zhu, A. A. Chmura, E. Hagan, J.-H. Zhou, Y.-Z. Zhang, L.-F. Wang, P. Daszak, Z.-L. Shi, Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. *Viol. Sin.* **33**, 104–107 (2018). [doi:10.1007/s12250-018-0012-7](https://doi.org/10.1007/s12250-018-0012-7) [Medline](#)
6. V. D. Menachery, B. L. Yount Jr., K. Debbink, S. Agnihothram, L. E. Gralinski, J. A. Plante, R. L. Graham, T. Scobey, X.-Y. Ge, E. F. Donaldson, S. H. Randell, A. Lanzavecchia, W. A. Marasco, Z.-L. Shi, R. S. Baric, A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat. Med.* **21**, 1508–1513 (2015). [doi:10.1038/nm.3985](https://doi.org/10.1038/nm.3985) [Medline](#)
7. V. D. Menachery, B. L. Yount Jr., A. C. Sims, K. Debbink, S. S. Agnihothram, L. E. Gralinski, R. L. Graham, T. Scobey, J. A. Plante, S. R. Royal, J. Swanstrom, T. P. Sheahan, R. J. Pickles, D. Corti, S. H. Randell, A. Lanzavecchia, W. A. Marasco, R. S. Baric, SARS-like WIV1-CoV poised for human emergence. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 3048–3053 (2016). [doi:10.1073/pnas.1517719113](https://doi.org/10.1073/pnas.1517719113) [Medline](#)
8. W. Li, M. J. Moore, N. Vasilieva, J. Sui, S. K. Wong, M. A. Berne, M. Somasundaran, J. L. Sullivan, K. Luzuriaga, T. C. Greenough, H. Choe, M. Farzan, Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450–454 (2003). [doi:10.1038/nature02145](https://doi.org/10.1038/nature02145) [Medline](#)
9. M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T. S. Schiergens, G. Herrler, N.-H. Wu, A. Nitsche, M. A. Müller, C. Drosten, S. Pöhlmann, SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **181**, 271–280.e8 (2020). [doi:10.1016/j.cell.2020.02.052](https://doi.org/10.1016/j.cell.2020.02.052) [Medline](#)
10. V. S. Raj, H. Mou, S. L. Smits, D. H. W. Dekkers, M. A. Müller, R. Dijkman, D. Muth, J. A. A. Demmers, A. Zaki, R. A. M. Fouchier, V. Thiel, C. Drosten, P. J. M. Rottier, A. D. M. E. Osterhaus, B. J. Bosch, B. L. Haagmans, Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* **495**, 251–254 (2013). [doi:10.1038/nature12005](https://doi.org/10.1038/nature12005) [Medline](#)
11. R. Vlasak, W. Luytjes, W. Spaan, P. Palese, Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4526–4529 (1988). [doi:10.1073/pnas.85.12.4526](https://doi.org/10.1073/pnas.85.12.4526) [Medline](#)
12. X. Huang, W. Dong, A. Milewska, A. Golda, Y. Qi, Q. K. Zhu, W. A. Marasco, R. S. Baric, A. C. Sims, K. Pyrc, W. Li, J. Sui, Human Coronavirus HKU1 Spike Protein Uses O-Acetylated Sialic Acid as an Attachment Receptor Determinant and Employs Hemagglutinin-Esterase Protein as a Receptor-Destroying Enzyme. *J. Virol.* **89**, 7202–7213 (2015). [doi:10.1128/JVI.00854-15](https://doi.org/10.1128/JVI.00854-15) [Medline](#)
13. T. S. Fung, D. X. Liu, Human Coronavirus: Host-Pathogen Interaction. *Annu. Rev. Microbiol.* **73**, 529–557 (2019). [doi:10.1146/annurev-micro-020518-115759](https://doi.org/10.1146/annurev-micro-020518-115759) [Medline](#)
14. 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). [doi:10.1126/science.abc5902](https://doi.org/10.1126/science.abc5902) [Medline](#)
15. Y. Cao, B. Su, X. Guo, W. Sun, Y. Deng, L. Bao, Q. Zhu, X. Zhang, Y. Zheng, C. Geng, X. Chai, R. He, X. Li, Q. Lv, H. Zhu, W. Deng, Y. Xu, Y. Wang, L. Qiao, Y. Tan, L. Song, G. Wang, X. Du, N. Gao, J. Liu, J. Xiao, X. D. Su, Z. Du, Y. Feng, C. Qin, C. Qin, R. Jin, X. S. Xie, Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell* **182**, 73–84.e16 (2020). [doi:10.1016/j.cell.2020.05.025](https://doi.org/10.1016/j.cell.2020.05.025) [Medline](#)
16. C. Kreer, M. Zehner, T. Weber, M. S. Ercanoglu, L. Giesemann, C. Rohde, S. Halwe, M. Korenkov, P. Schommers, K. Vanshylla, V. Di Cristanziano, H. Janicki, R. Brinker, A. Ashurov, V. Kräling, A. Kupke, H. Cohen-Dvashi, M. Koch, J. M. Eckert, S. Lederer, N. Pfeifer, T. Wolf, M. J. G. T. Vehreschild, C. Wendtner, R. Diskin, H. Gruell, S. Becker, F. Klein, Longitudinal Isolation of Potent Near-Germine SARS-CoV-2-Neutralizing Antibodies from COVID-19 Patients. *Cell* **182**, 843–854.e12 (2020). [doi:10.1016/j.cell.2020.06.044](https://doi.org/10.1016/j.cell.2020.06.044)
17. 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). [doi:10.1038/s41586-020-2571-7](https://doi.org/10.1038/s41586-020-2571-7) [Medline](#)
18. 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. Shmeliovich, P. Mendoza, H. Hartweg, 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). [doi:10.1038/s41586-020-2456-9](https://doi.org/10.1038/s41586-020-2456-9) [Medline](#)
19. R. Shi, C. Shan, X. Duan, Z. Chen, P. Liu, J. Song, T. Song, X. Bi, C. Han, L. Wu, G. Gao, X. Hu, Y. Zhang, Z. Tong, W. Huang, W. J. Liu, G. Wu, B. Zhang, L. Wang, J. Qi, H. Feng, F.-S. Wang, Q. Wang, G. F. Gao, Z. Yuan, J. Yan, A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature* **584**, 120–124 (2020). [doi:10.1038/s41586-020-2381-y](https://doi.org/10.1038/s41586-020-2381-y) [Medline](#)
20. S. J. Zost, P. Gilchuk, R. E. Chen, J. B. Case, J. X. Reidy, A. Trivette, R. S. Nargi, R. E. Sutton, N. Suryadevara, E. C. Chen, E. Binshtein, S. Shrihari, M. Ostrowski, H. Y. Chu, J. E. Didier, K. W. MacRenaris, T. Jones, S. Day, L. Myers, F. Eun-Hyung Lee, D. C. Nguyen, I. Sanz, D. R. Martinez, P. W. Rothlauf, L. M. Bloyet, S. P. J. Whelan, R. S. Baric, L. B. Thackray, M. S. Diamond, R. H. Carnahan, J. E. Crowe Jr., Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat. Med.* **26**, 1422–1427 (2020). [Medline](#)
21. T. F. Rogers, F. Zhao, D. Huang, N. Beutler, A. Burns, W. T. He, O. Limbo, C. Smith, G. Song, J. Woehl, L. Yang, R. K. Abbott, S. Callaghan, E. Garcia, J. Hurtado, M. Parren, L. Peng, J. Ricketts, M. J. Ricciardi, S. A. Rawlings, D. M. Smith, D. Nemazee, J. R. Teijaro, J. E. Voss, R. Andrabi, B. Briney, E. Landais, D. Sok, J. G. Jardine, D. R. Burton, Rapid isolation of potent SARS-CoV-2 neutralizing antibodies and protection in a small animal model. *Science* **369**, 956–963 (2020). [doi:10.1126/science.abc7520](https://doi.org/10.1126/science.abc7520) [Medline](#)
22. E. Seydoux, L. J. Homad, A. J. MacCamy, K. R. Parks, N. K. Hurlburt, M. F. Jennewein, N. R. Akins, A. B. Stuart, Y.-H. Wan, J. Feng, R. E. Whaley, S. Singh, M. Boeckh, K. W. Cohen, M. J. McElrath, J. A. Englund, H. Y. Chu, M. Pancera, A. T. McGuire, L. Stamatatos, Analysis of a SARS-CoV-2-Infected Individual Reveals Development of Potent Neutralizing Antibodies with Limited Somatic Mutation. *Immunity* **53**, 98–105.e5 (2020). [doi:10.1016/j.immuni.2020.06.001](https://doi.org/10.1016/j.immuni.2020.06.001) [Medline](#)

23. S. J. Zost, P. Gilchuk, J. B. Case, E. Binshtein, R. E. Chen, J. P. Nkolola, A. Schäfer, J. X. Reidy, A. Trivette, R. S. Nargi, R. E. Sutton, N. Suryadevara, D. R. Martinez, L. E. Williamson, E. C. Chen, T. Jones, S. Day, L. Myers, A. O. Hassan, N. M. Kafai, E. S. Winkler, J. M. Fox, S. Shrihari, B. K. Mueller, J. Meiler, A. Chandrashekar, N. B. Mercado, J. J. Steinhardt, K. Ren, Y.-M. Loo, N. L. Kallewaard, B. T. McCune, S. P. Keeler, M. J. Holtzman, D. H. Barouch, L. E. Gralinski, R. S. Baric, L. B. Thackray, M. S. Diamond, R. H. Carnahan, J. E. Crowe Jr., Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443–449 (2020). [doi:10.1038/s41586-020-2548-6](https://doi.org/10.1038/s41586-020-2548-6) [Medline](#)
24. C. O. Barnes, C. A. Jette, M. E. Abernathy, K. 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). [Medline](#)
25. 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. Veessler, D. Corti, Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290–295 (2020). [doi:10.1038/s41586-020-2349-y](https://doi.org/10.1038/s41586-020-2349-y) [Medline](#)
26. L. Piccoli, Y. J. Park, M. A. Tortorici, N. Czudnochowski, A. C. Walls, M. Beltramello, C. Silacci-Fregni, D. Pinto, L. E. Rosen, J. E. Bowen, O. J. Acton, S. Jaconi, B. Guarino, A. Minola, F. Zatta, N. Sprugasci, J. Bassi, A. Peter, A. De Marco, J. C. Nix, F. Mele, S. Jovic, B. F. Rodriguez, S. V. Gupta, F. Jin, G. Piumatti, G. Lo Presti, A. F. Pellanda, M. Biggiogero, M. Tarkowski, M. S. Pizzuto, E. Cameroni, C. Havenar-Daughton, M. Smithy, D. Hong, V. Lepori, E. Albanese, A. Ceschi, E. Bernasconi, L. Elzi, P. Ferrari, C. Garzoni, A. Riva, G. Snell, F. Sallusto, K. Fink, H. W. Virgin, A. Lanzavecchia, D. Corti, D. Veessler, Mapping neutralizing and immunodominant sites on the SARS-CoV-2 spike receptor-binding domain by structure-guided high-resolution serology. *Cell* **183**, 1024–1042.e21 (2020). [Medline](#)
27. J. López-Sagaseta, E. Malito, R. Rappuoli, M. J. Bottomley, Self-assembling protein nanoparticles in the design of vaccines. *Comput. Struct. Biotechnol. J.* **14**, 58–68 (2015). [doi:10.1016/j.csbj.2015.11.001](https://doi.org/10.1016/j.csbj.2015.11.001) [Medline](#)
28. M. K. Slifka, I. J. Amanna, Role of Multivalency and Antigenic Threshold in Generating Protective Antibody Responses. *Front. Immunol.* **10**, 956 (2019). [doi:10.3389/fimmu.2019.00956](https://doi.org/10.3389/fimmu.2019.00956) [Medline](#)
29. K. D. Brune, M. Howarth, New Routes and Opportunities for Modular Construction of Particulate Vaccines: Stick, Click, and Glue. *Front. Immunol.* **9**, 1432 (2018). [doi:10.3389/fimmu.2018.01432](https://doi.org/10.3389/fimmu.2018.01432) [Medline](#)
30. K. D. Brune, D. B. Leneghan, I. J. Brian, A. S. Ishizuka, M. F. Bachmann, S. J. Draper, S. Biswas, M. Howarth, Plug-and-Display: Decoration of Virus-Like Particles via isopeptide bonds for modular immunization. *Sci. Rep.* **6**, 19234 (2016). [doi:10.1038/srep19234](https://doi.org/10.1038/srep19234) [Medline](#)
31. T. U. J. Bruun, A. C. Andersson, S. J. Draper, M. Howarth, Engineering a Rugged Nanoscaffold To Enhance Plug-and-Display Vaccination. *ACS Nano* **12**, 8855–8866 (2018). [doi:10.1021/acs.nano.8b02805](https://doi.org/10.1021/acs.nano.8b02805) [Medline](#)
32. B. Zakeri, J. O. Fierer, E. Celik, E. C. Chittock, U. Schwarz-Linek, V. T. Moy, M. Howarth, Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E690–E697 (2012). [doi:10.1073/pnas.1115485109](https://doi.org/10.1073/pnas.1115485109) [Medline](#)
33. T. K. Tan *et al.*, A COVID-19 vaccine candidate 1 using SpyCatcher multimerization of the SARS-CoV-2 spike protein receptor-binding domain induces potent neutralising antibody responses. *bioRxiv* **275701** [preprint]. 31 August 2020.
34. B. Zhang, C. W. Chao, Y. Tsybovsky, O. M. Abiona, G. B. Hutchinson, J. I. Moliva, A. S. Olia, A. Pegu, E. Phung, G. B. E. Stewart-Jones, R. Verardi, L. Wang, S. Wang, A. Werner, E. S. Yang, C. Yap, T. Zhou, J. R. Mascola, N. J. Sullivan, B. S. Graham, K. S. Corbett, P. D. Kwong, A platform incorporating trimeric antigens into self-assembling nanoparticles reveals SARS-CoV-2-spike nanoparticles to elicit substantially higher neutralizing responses than spike alone. *Sci. Rep.* **10**, 18149 (2020). [doi:10.1038/s41598-020-74949-2](https://doi.org/10.1038/s41598-020-74949-2) [Medline](#)
35. B. Hu, L. P. Zeng, X. L. Yang, X. Y. Ge, W. Zhang, B. Li, J. Z. Xie, X. R. Shen, Y. Z. Zhang, N. Wang, D. S. Luo, X. S. Zheng, M. N. Wang, P. Daszak, L. F. Wang, J. Cui, Z. L. Shi, Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLOS Pathog.* **13**, e1006698 (2017). [doi:10.1371/journal.ppat.1006698](https://doi.org/10.1371/journal.ppat.1006698) [Medline](#)
36. R. Rahikainen, P. Rijal, T. K. Tan, H. J. Wu, A. C. Andersson, J. R. Barrett, T. A. Bowden, S. J. Draper, A. R. Townsend, M. Howarth, Overcoming Symmetry Mismatch in Vaccine Nanoassembly through Spontaneous Amidation. *Angew. Chem. Int. Ed.* **60**, 321–330 (2021). [Medline](#)
37. M. Kanekiyo, M. G. Joyce, R. A. Gillespie, J. R. Gallagher, S. F. Andrews, H. M. Yassine, A. K. Wheatley, B. E. Fisher, D. R. Ambrozak, A. Creanga, K. Leung, E. S. Yang, S. Boyoglu-Barnum, I. S. Georgiev, Y. Tsybovsky, M. S. Prabhakaran, H. Andersen, W.-P. Kong, U. Baxa, K. L. Zephir, J. E. Ledgerwood, R. A. Koup, P. D. Kwong, A. K. Harris, A. B. McDermott, J. R. Mascola, B. S. Graham, Mosaic nanoparticle display of diverse influenza virus hemagglutinins elicits broad B cell responses. *Nat. Immunol.* **20**, 362–372 (2019). [doi:10.1038/s41590-018-0305-y](https://doi.org/10.1038/s41590-018-0305-y) [Medline](#)
38. M. Letko, A. Marzi, V. Munster, Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat. Microbiol.* **5**, 562–569 (2020). [doi:10.1038/s41564-020-0688-y](https://doi.org/10.1038/s41564-020-0688-y) [Medline](#)
39. T. T.-Y. Lam, N. Jia, Y.-W. Zhang, M. H.-H. Shum, J.-F. Jiang, H.-C. Zhu, Y.-G. Tong, Y.-X. Shi, X.-B. Ni, Y.-S. Liao, W.-J. Li, B.-G. Jiang, W. Wei, T.-T. Yuan, K. Zheng, X.-M. Cui, J. Li, G.-Q. Pei, X. Qiang, W. Y.-M. Cheung, L.-F. Li, F.-F. Sun, S. Qin, J.-C. Huang, G. M. Leung, E. C. Holmes, Y.-L. Hu, Y. Guan, W.-C. Cao, Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. *Nature* **583**, 282–285 (2020). [doi:10.1038/s41586-020-2169-0](https://doi.org/10.1038/s41586-020-2169-0) [Medline](#)
40. K. G. Andersen, A. Rambaut, W. I. Lipkin, E. C. Holmes, R. F. Garry, The proximal origin of SARS-CoV-2. *Nat. Med.* **26**, 450–452 (2020). [doi:10.1038/s41591-020-0820-9](https://doi.org/10.1038/s41591-020-0820-9) [Medline](#)
41. H. Zhou, X. Chen, T. Hu, J. Li, H. Song, Y. Liu, P. Wang, D. Liu, J. Yang, E. C. Holmes, A. C. Hughes, Y. Bi, W. Shi, A Novel Bat Coronavirus Closely Related to SARS-CoV-2 Contains Natural Insertions at the S1/S2 Cleavage Site of the Spike Protein. *Curr. Biol.* **30**, 2196–2203.e3 (2020). [doi:10.1016/j.cub.2020.05.023](https://doi.org/10.1016/j.cub.2020.05.023) [Medline](#)
42. Y. Tao, S. Tong, Complete Genome Sequence of a Severe Acute Respiratory Syndrome-Related Coronavirus from Kenyan Bats. *Microbiol. Resour. Announce.* **8**, e00548-19 (2019). [doi:10.1128/MRA.00548-19](https://doi.org/10.1128/MRA.00548-19) [Medline](#)
43. A. H. Keeble, P. Turkki, S. Stokes, I. N. A. Khairil Anuar, R. Rahikainen, V. P. Hytönen, M. Howarth, Approaching infinite affinity through engineering of peptide-protein interaction. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 26523–26533 (2019). [doi:10.1073/pnas.1909653116](https://doi.org/10.1073/pnas.1909653116) [Medline](#)
44. F. Schmidt, Y. Weisblum, F. Muecksch, H.-H. Hoffmann, E. Michailidis, J. C. C. Lorenzi, P. Mendoza, M. Rutkowska, E. Bednarski, C. Gaebler, M. Agudelo, A. Cho, Z. Wang, A. Gazumyan, M. Cipolla, M. Caskey, D. F. Robbiani, M. C. Nussenzweig, C. M. Rice, T. Hatzioannou, P. D. Bieniasz, Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses. *J. Exp. Med.* **217**, e20201181 (2020). [doi:10.1084/jem.20201181](https://doi.org/10.1084/jem.20201181) [Medline](#)
45. L. van Dorp *et al.*, Recurrent mutations in SARS-CoV-2 genomes isolated from mink point to rapid host-adaptation. *bioRxiv* **384743** [preprint]. 16 November 2020.
46. S. R. Leist, K. H. Dinno 3rd, A. Schäfer, L. V. Tse, K. Okuda, Y. J. Hou, A. West, C. E. Edwards, W. Sanders, E. J. Fritch, K. L. Gully, T. Scobey, A. J. Brown, T. P. Sheahan, N. J. Moorman, R. C. Boucher, L. E. Gralinski, S. A. Montgomery, R. S. Baric, A Mouse-Adapted SARS-CoV-2 Induces Acute Lung Injury and Mortality in Standard Laboratory Mice. *Cell* **183**, 1070–1085.e12 (2020). [doi:10.1016/j.cell.2020.09.050](https://doi.org/10.1016/j.cell.2020.09.050) [Medline](#)
47. C. O. Barnes, A. P. West Jr., K. E. Huey-Tubman, M. A. G. Hoffmann, N. G. Sharaf, P. R. Hoffman, N. Koranda, H. B. Gristick, C. Gaebler, F. Muecksch, J. C. C. Lorenzi, S. Fink, T. Häggelöf, A. Hurley, K. G. Millard, Y. Weisblum, F. Schmidt, T. Hatzioannou, P. D. Bieniasz, M. Caskey, D. F. Robbiani, M. C. Nussenzweig, P. J. Bjorkman, Structures of Human Antibodies Bound to SARS-CoV-2 Spike Reveal

- Common Epitopes and Recurrent Features of Antibodies. *Cell* **182**, 828–842.e16 (2020). [doi:10.1016/j.cell.2020.06.025](https://doi.org/10.1016/j.cell.2020.06.025) [Medline](#)
48. 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. Dinno 3rd, 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. Veessler, N. P. King. Elicitation of Potent Neutralizing Antibody Responses by Designed Protein Nanoparticle Vaccines for SARS-CoV-2. *Cell* **183**, 1367–1382.e17 (2020). [doi:10.1016/j.cell.2020.10.043](https://doi.org/10.1016/j.cell.2020.10.043) [Medline](#)
 49. M. Landau, I. Mayrose, Y. Rosenberg, F. Glaser, E. Martz, T. Pupko, N. Ben-Tal, ConSurf 2005: The projection of evolutionary conservation scores of residues on protein structures. *Nucleic Acids Res.* **33**, W299–W302 (2005). [doi:10.1093/nar/gki370](https://doi.org/10.1093/nar/gki370) [Medline](#)
 50. H. B. Gristick, L. von Boehmer, A. P. West Jr., M. Schamber, A. Gazumyan, J. Golijanin, M. S. Seaman, G. Fätkenheuer, F. Klein, M. C. Nussenzweig, P. J. Bjorkman, Natively glycosylated HIV-1 Env structure reveals new mode for antibody recognition of the CD4-binding site. *Nat. Struct. Mol. Biol.* **23**, 906–915 (2016). [doi:10.1038/nsmb.3291](https://doi.org/10.1038/nsmb.3291) [Medline](#)
 51. F. Sievers, A. Wilm, D. Dineen, T. J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert, J. Söding, J. D. Thompson, D. G. Higgins, Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **7**, 539 (2011). [doi:10.1038/msb.2011.75](https://doi.org/10.1038/msb.2011.75) [Medline](#)
 52. S. Guindon, J.-F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, O. Gascuel, New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol.* **59**, 307–321 (2010). [doi:10.1093/sysbio/syq010](https://doi.org/10.1093/sysbio/syq010) [Medline](#)
 53. 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). [doi:10.1126/science.abd0826](https://doi.org/10.1126/science.abd0826) [Medline](#)
 54. A. A. Cohen *et al.*, Construction, characterization, and immunization of nanoparticles that display a diverse array of influenza HA trimers. *bioRxiv* [911388](https://doi.org/10.1101/2020.01.19.311388) [preprint], 19 January 2020.
 55. D. Angeletti, J. S. Gibbs, M. Angel, I. Kosik, H. D. Hickman, G. M. Frank, S. R. Das, A. K. Wheatley, M. Prabhakaran, D. J. Leggat, A. B. McDermott, J. W. Yewdell, Defining B cell immunodominance to viruses. *Nat. Immunol.* **18**, 456–463 (2017). [doi:10.1038/ni.3680](https://doi.org/10.1038/ni.3680) [Medline](#)
 56. K. H. D. Crawford, R. Eguia, A. S. Dingens, A. N. Loes, K. D. Malone, C. R. Wolf, H. Y. Chu, M. A. Tortorici, D. Veessler, M. Murphy, D. Pettie, N. P. King, A. B. Balazs, J. D. Bloom, Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays. *Viruses* **12**, 513 (2020). [doi:10.3390/v12050513](https://doi.org/10.3390/v12050513) [Medline](#)
 57. A. P. West Jr., L. Scharf, J. Horwitz, F. Klein, M. C. Nussenzweig, P. J. Bjorkman, Computational analysis of anti-HIV-1 antibody neutralization panel data to identify potential functional epitope residues. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 10598–10603 (2013). [doi:10.1073/pnas.1309215110](https://doi.org/10.1073/pnas.1309215110) [Medline](#)
 58. T. N. Starr, A. J. Greaney, S. K. Hilton, D. Ellis, K. H. D. Crawford, A. S. Dingens, M. J. Navarro, J. E. Bowen, M. A. Tortorici, A. C. Walls, N. P. King, D. Veessler, J. D. Bloom, Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295–1310.e20 (2020). [doi:10.1016/j.cell.2020.08.012](https://doi.org/10.1016/j.cell.2020.08.012) [Medline](#)

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S5

References (50–58)

MDAR Reproducibility Checklist

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Fig. 1. Properties of RBDs chosen for this study. (A) Left: Structure of SARS-CoV-2 S trimer (PDB 6VXX) with one RBD (dashed circle) in an "up" position. Middle and right: Sequence conservation of 12 RBDs calculated by the ConSurf Database (49) plotted on a surface representation of the RBD structure (PDB 7BZ5). Epitopes for representatives from defined classes of RBD-binding antibodies (class 1-class 4) (24) indicated by dashed lines. (B) Summary of properties of the viral strains from which the 12 sarbecovirus RBDs were derived. (C) Phylogenetic tree of human and selected other coronaviruses based on RBD protein sequences. Red shading indicates strains known to use ACE2 as a receptor. (D) Heat map showing percent amino acid sequence identities between 12 sarbecovirus RBDs.

Fig. 2. Construction of RBD nanoparticles. (A) Left: SpyTagged RBDs were attached to SpyCatcher003-mi3 to make a homotypic particle and three mosaic particles. 10 of 60 potential coupling sites on mi3 are shown for clarity. (B) SEC profile showing separation of RBD nanoparticles and free RBD proteins. (C) Coomassie-stained SDS-PAGE of RBD-coupled nanoparticles, free RBD proteins, and uncoupled SpyCatcher003-mi3 particles (SC3-mi3).

Fig. 3 (preceding page). RBD nanoparticles induce cross-reactive IgG responses in immunized mice. Red and gray rectangles below ELISA and neutralization data represent mismatched strains (red; RBD from that strain was not present on the immunized particle) or matched strains (gray; RBD was present on the immunized particle). **(A)** Left: Immunization schedule. Adjuvant = AddaVax (Invivogen). Right: Key for immunizations; number of mice in each cohort is indicated. **(B to F)** Mice were immunized with soluble SARS-CoV-2 S trimer (SARS-2 S; brown bars), or the following nanoparticles: homotypic SARS-2 (red), mosaic-4a (green), mosaic-4b (yellow), mosaic-8 (blue), or unconjugated SpyCatcher003-mi3 (mi3; black). ELISA data from serum IgG responses to SARS-2 spike trimer (B) or RBDs [(C) to (F)] shown as area under the curve (AUC). For (C) to (E), neutralization potencies are presented as half-maximal inhibitory dilutions (ID_{50} values) of sera against the pseudoviruses from the indicated coronavirus strains. Dashed horizontal lines correspond to the lowest dilution representing the limit of detection. Each dot represents serum from one animal, with means and standard deviations for vaccinated cohorts represented by rectangles (mean) and horizontal lines (SD). Significant differences between groups linked by horizontal lines are indicated by asterisks and p-values. NS = not significant. [(B) to (F)] Neutralization and/or binding data for serum IgGs for recognition of (B) SARS-2 spike trimer, (C) SARS-2 RBD and SARS-2 pseudovirus, (D) SHC014 and WIV1 RBDs and corresponding pseudoviruses, (E) SARS RBD and SARS pseudovirus, (F) Yun 11, BM-4831, and BtKY72 RBDs.

Fig. 4 (next page). IgGs from convalescent COVID-19 plasma (18, 24) show little to no cross-reactive responses. **(A to F)** Plasma IgG responses were evaluated by ELISA (data shown as binding curves with plasma names (18) listed) against RBDs from (A) SARS-2, (B) RaTG13, (C) SHC014, (D) WIV1, (E) Rs4081, and (F) BM-4831. Data points are plotted as the mean and standard deviation of duplicate measurements. IOMA, an anti-HIV-1 IgG (50), was used as a control. **(G)** ELISA results from (A) to (F) presented as area under the curve (AUC), where each dot represents one plasma sample, with means and standard deviations represented by rectangles (mean) and horizontal lines (SD). Significant differences between groups linked by horizontal lines are indicated by asterisks and p-values. **(H)** IC_{50} values for pseudotyped neutralization assays using IgGs from COV7, COV21, and COV72 plasmas (18) (evaluated at top concentrations of 1500 $\mu\text{g/mL}$) against the indicated strains. Mean = arithmetic mean IC_{50} ; SD = standard deviation.