Driving potent neutralization of a SARS-CoV-2 Variant of Concern with a heterotypic boost.

Daniel J. Sheward[*,✉], Marco Mandolesi[*], Changil Kim , Leo Hanke , Laura Perez Vidakovics , Gerald McInerney , Gunilla B. Karlsson Hedestam[†,✉], and Ben Murrell[†,✉]

Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden
[*]These authors contributed equally
[†]These authors contributed equally

The emergence of SARS-CoV-2 Variants of Concern (VOCs) with mutations in key neutralizing antibody epitopes threatens to undermine vaccines developed against the pandemic founder variant (Wu-Hu-1). Widespread vaccine rollout and continued transmission are creating a population that has antibody responses of varying potency to Wu-Hu-1. Against this background, it is critical to assess the outcomes of subsequent booster vaccination with variant antigens. It is not yet known whether such heterotypic vaccine boosts would be compromised by original antigenic sin, where pre-existing responses to a prior variant dampen responses to a new one, or whether the primed memory B cell repertoire would bridge the gap between Wu-Hu-1 and VOCs. Here, we show that a single adjuvanted dose of receptor binding domain (RBD) protein from VOC 501Y.V2 (B.1.351) drives an extremely potent neutralizing antibody response capable of cross-neutralizing both Wu-Hu-1 and 501Y.V2 in rhesus macaques previously immunized with Wu-Hu-1 spike protein.

Correspondence: ***daniel.sheward@ki.se, gunilla.karlsson.hedestam@ki.se, benjamin.murrell@ki.se***

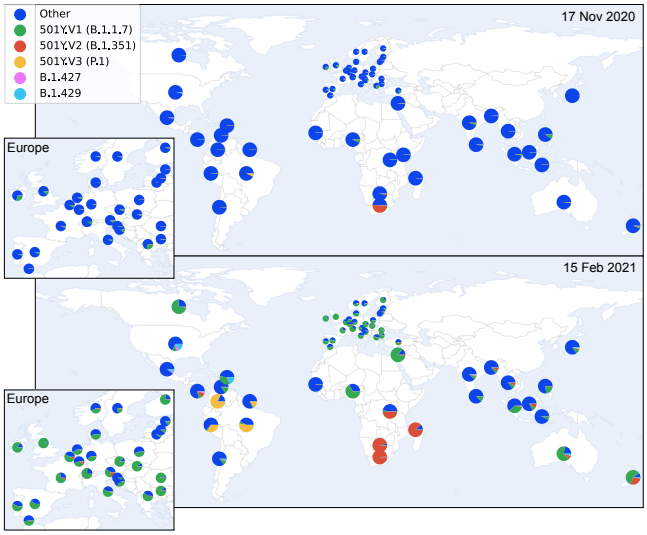

Fig. 1. Concern over Variants. The global distribution and estimated country-level proportions of deposited SARS-CoV-2 genomes for 5 CDC-recognized VOCs (with all non-VOC lineages in blue), shown for 17th Nov 2020 (top), and 90 days later for 15th Feb 2021 (bottom), demonstrating how rapidly these VOCs are coming to dominate the SARS-CoV-2 genomic landscape. Proportions over time are estimated from GISAID genome metadata, using a temporally non-linear multinomial regression model (see Methods).

At least 20 candidate SARS-CoV-2 vaccines have already entered phase 3 clinical trials[1]. A number of these demonstrated high efficacy[2–6], significantly reducing morbidity and mortality, and are being rolled-out globally. This first generation of vaccines all encode or deliver a spike glycoprotein derived from the pandemic founder strain, Wu-Hu-1[7].

Driven by multiple evolutionary forces[8], SARS-CoV-2 is rapidly evading our response. Globally, a number of VOCs are rising in frequency (see Fig 1), each harbouring spike mutations that confer resistance to prior immunity. Of particular concern is the surge of variant 501Y.V2[9], with multiple mutations in dominant neutralizing antibody epitopes making it several fold more resistant to antibodies elicited by current vaccines[10–12]. This underpins the substantially reduced vaccine efficacies in South Africa, where this variant is circulating at high frequency[13,14]. Updated vaccines are likely required to protect against current and future mutated variants. Importantly, by the time these are rolled out, a significant proportion of the global population are likely to be seropositive as a result of either infection, or immunization with Wu-Hu-1 based vaccines.

The first exposure to a pathogen can shape future responses to mutated variants. This immunological imprinting or original antigenic sin[15] is well-described for influenza A virus where protection is highest against the first strain encountered, and diminished against those encountered later in life[16,17]. It is crucial for the design of updated vaccines and regimens to determine if existing immunity dampens antibody responses to new VOCs, or if a heterotypic boost can efficiently recruit cross-protective memory responses.

To address this, we immunized three rhesus macaques with two doses of soluble prefusion-stabilized Wu-Hu-1 spike protein (2 µg), adjuvanted with 50 µg of saponin-based Matrix-M™ (Novavax AB, Uppsala, Sweden), with a one-month interval between doses, mimicking an immunization schedule for approved SARS-CoV-2 vaccines. After a single dose, neutralizing antibodies were detectable against Wu-Hu-1 but not 501Y.V2 (Fig. 2). Neutralizing antibody responses against Wu-Hu-1 were substantially boosted by the second immunization (GMT = 3980), and then waned over the following months (Fig. 2), as also reported in immunized humans[19]. Notably, the circulating VOC 501Y.V2 was on average 9-fold (range: 5.6 - 12.2 fold) less potently neutralized (GMT = 451 at peak), with this difference less pronounced in one of the animals (H05), consistent with the responses observed in humans following vaccination[10–12].

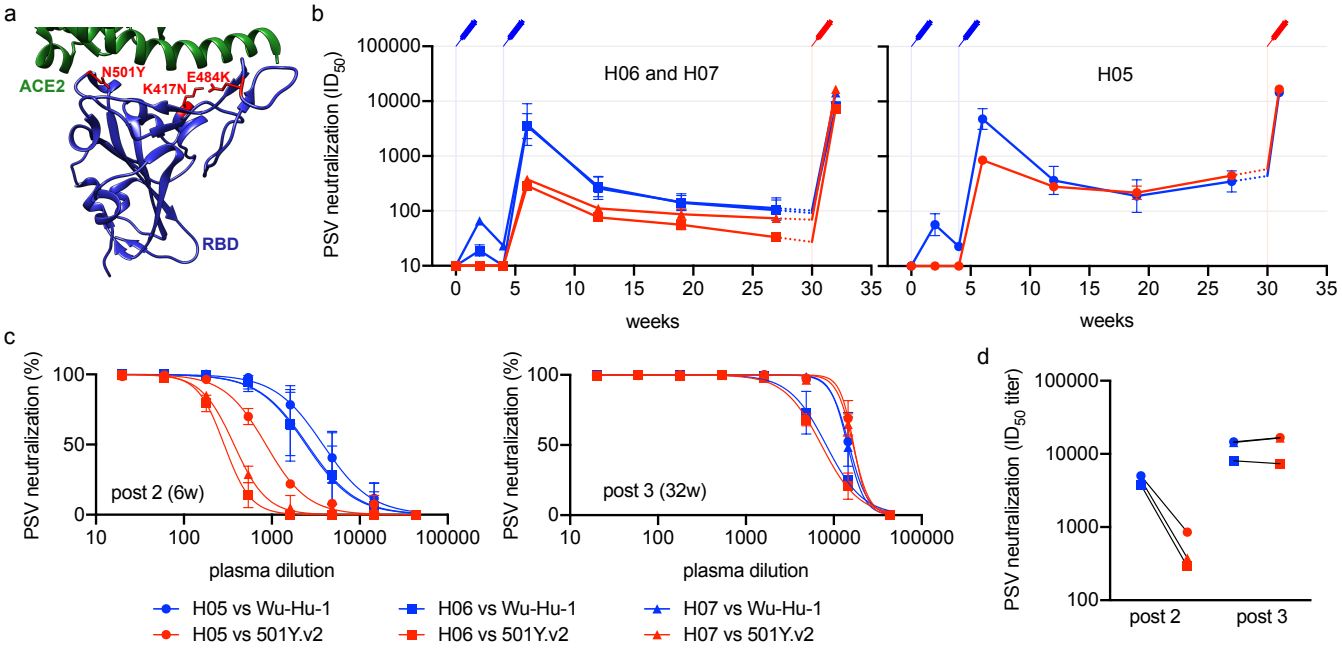

Fig. 2. Heterotypic RBD boost drives a potent cross-neutralizing antibody response. (**a**) Depiction of the RBD immunogen (PDB:6MOJ[18]) used as a heterotypic boost in this study, that incorporates the three RBD mutations (located in red) defining lineage 20H/501Y.V2. The cellular receptor, ACE2, is shown in green. (**b-d**) Neutralizing antibody responses over time to Wu-Hu-1 (blue) and 501Y.V2 (red) pseudotyped viruses (PSV) are shown for three immunized macaques: (**b**) H06 and H07 (left) and H05 (right), plotted separately as they exhibit different trajectories prior to the heterotypic boost. Syringes indicate the timing of immunizations (blue: Wu-Hu-1 spike at 0 and 4-weeks, red: 501Y.V2 RBD at 30-weeks). Titers from 27-30 weeks (shown with dashed lines) have been extrapolated for clarity. Error bars depict the geometric SD. (**c**) While neutralization of 501Y.V2 was significantly reduced at 6 weeks, corresponding to peak responses 2 weeks following the second spike dose (left), neutralization was restored following subsequent heterotypic RBD boost (right), such that 501Y.V2 (red) and Wu-Hu-1 (blue) were potently neutralized at similar titers (**d**) in all three animals.

Six months after their first immunization, macaques were boosted with soluble 501Y.V2 RBD, with either a 2 μg (H05), 10 μg (H06), or 50 μg (H07) dose in 50 μg Matrix-M™ adjuvant. One macaque (H05) was terminated 5 days after immunization, due to an unrelated illness that had begun prior to the third immunization, and was sampled for detailed follow-up studies of antibody specificities. The other two (H06 and H07) were followed for 2 weeks. In all three animals, 501Y.V2 RBD efficiently boosted responses that potently cross-neutralized both Wu-Hu-1 and 501Y.V2, with similar titers (Fig. 2a-c; Wu-Hu-1 GMT = 11795, 501Y.V2 GMT = 12595). In contrast, for macaques previously immunized with three doses of Wu-Hu-1 spike[20], the reduced neutralization of 501Y.V2 compared to Wu-Hu-1 remained after the third homotypic spike immunization (Supp. Fig. 1). Despite weak immunogenicity as a priming antigen[20], soluble monomeric heterotypic RBD elicited a potent recall response. This was robust to the boosting dose, and effective as low as 2 μg, possibly aided by a dose-sparing effect of Matrix-M[21]. This is particularly promising as RBD is a small, stable protein that can be rapidly synthesized and efficiently expressed.

Taken together, these data indicate that potent, cross-neutralizing antibody responses can be recruited with heterotypic SARS-CoV-2 immunogens following a primary exposure, and that soluble RBD booster immunizations represent an attractive strategy to broaden vaccine protection from new SARS-CoV-2 variants.

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AUTHOR CONTRIBUTIONS

Conceptualization: DJS, MM, GKH, BM; Formal Analysis: DJS, BM; Funding acquisition: GMM, GKH, BM; Investigation: DJS, MM, CK; Methodology: DJS, MM, GKH, BM; Resources: CK, LH, LPV; Software: BM; Supervision: LH, GMM, GKH, BM; Visualization: DJS, BM; Writing – original draft: DJS, BM; Writing – review editing: all authors.

METHODS

Non-linear Multinomial Regression for VOC frequency estimation: SARS-CoV-2 lineage metadata was obtained from GISAID (gisaid.org - 2021-03-24 metadata release) comprising 850203 genomes. For each of the lineages in Figure 1, we aggregated daily counts of genomes at the country level, requiring at least 30 samples in the 30 days before 15th Feb, 2020, which was chosen because sequence data diminished rapidly beyond this point. Using a Generalized Linear Model, we model the daily variant counts with a multinomial distribution (and a log-link function), with underlying frequencies parameterized by a linear combination of 400 randomly drawn Fourier basis features (aka. a "Random Kitchen Sink"[22]) to allow frequencies to vary non-linearly as a function of time. We estimate the model parameters with an L2 norm on the random feature coefficients, using the GLMNet.jl Julia package, plotting the map with Cartopy (https://github.com/SciTools/cartopy). Code available at https://github.com/MurrellGroup/VOCfreq.

Ethics statement: The animal work was conducted with the approval of the regional Ethical Committee on Animal Experiments (Stockholms Norra Djurförsöksetiska Nämnd). All animal procedures were performed according to approved guidelines.

Protein production: 501Y.V2 RBD (encoding amino acid mutations K417N, E484K, and N501Y, and a C-terminal His-tag) was synthesized (IDT eBlocks), and cloned into a mammalian expression vector (pcDNA3.1), using a Gibson Assembly Mastermix (New England Biolabs). Spike ectodomain (prefusion stabilized with 6 prolines[23]) and RBD were produced by the transient transfection of Freestyle 293-F cells using FreeStyle MAX reagent (Thermo Fisher) or polyethylenimine (PEI), re-

spectively. The HIS-tagged Spike ectodomain and RBD were purified from filtered supernatant using nickel IMAC resin (HisPur Ni-NTA, Thermo Fisher Scientific) followed by size-exclusion chromatography on a Superdex 200 (Cytiva) in PBS. On the day of immunization, indicated doses were mixed with 50 µg Matrix-M™ adjuvant (Novavax AB, Uppsala, Sweden) in a final inoculation volume of 800 µl.

Animal Model: Rhesus macaques (Macaca mulatta) of Chinese origin, 5-6 years old, were housed at the Astrid Fagraeus Laboratory at Karolinska Institutet. Housing and care procedures complied with the provisions and general guidelines of the Swedish Board of Agriculture. The facility has been assigned an Animal Welfare Assurance number by the Office of Laboratory Animal Welfare (OLAW) at the National Institutes of Health (NIH). The macaques were housed in groups in enriched 14 m3 cages. They were habituated to the housing conditions for more than six weeks before the start of the experiment and subjected to positive reinforcement training in order to reduce the stress associated with experimental procedures. The macaques were weighed at each sampling. All animals were confirmed negative for simian immunodeficiency virus, simian T cell lymphotropic virus, simian retrovirus type D and simian herpes B virus. Macaques were immunized intramuscularly (i.m.) with half of each dose administered in each quadricep. All immunizations and blood samplings were performed under sedation with 10-15 mg/kg ketamine (Ketaminol, Intervet, Sweden) administered i.m.. Blood plasma was isolated by centrifugation, and heat inactivated at 56℃ for 60 minutes.

Pseudotyped neutralization assays: HEK293T and HEK293T-hACE2 (human:female) cells were cultured in a humidified 37℃ incubator (5% CO2) in Dulbecco's Modified Eagle Medium (Gibco) supplemented with 10% Fetal Bovine Serum and 1% Penicillin/Streptomycin, and were passaged when nearing confluency using 1X Trypsin-EDTA. Pseudotyped lentiviruses displaying either the SARS-CoV-2 pandemic founder variant (Wu-Hu-1) or 501Y.V2 variant spike [24] and packaging a luciferase reporter gene were generated by the co-transfection of HEK293T cells using Lipofectamine 3000 (Invitrogen) per the manufacturer's protocols. Media was changed 12-16 hours after transfection, and pseudotyped viruses were harvested at 48- and 72-hours post-transfection, clarified by centrifugation, and stored at -80℃ until use. Pseudotyped viruses sufficient to generate 50,000 relative light units (RLUs) were incubated with serial dilutions of plasma for 60 min at 37℃ in a 96-well plate, and then 15,000 HEK293T-hACE2 cells were added to each well. Plates were incubated at 37℃ for 48 hours, and luminescence was then measured using Bright-Glo (Promega) per the manufacturer's protocol, on a GM-2000 luminometer (Promega). ID50 titers were interpolated as the reciprocal plasma dilution where RLUs were reduced by 50% relative to control wells in the absence of serum, fitting a four-parameter logistic curve in Prism 9 (GraphPad Software).

Bibliography

1. Draft landscape of COVID-19 candidate vaccines. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines. Accessed: 2021-2-16.
2. Lindsey R Baden, Hana M El Sahly, Brandon Essink, Karen Kotloff, Sharon Frey, Rick Novak, David Diemert, Stephen A Spector, Nadine Rouphael, C Buddy Creech, John McGettigan, Shishir Khetan, Nathan Segall, Joel Solis, Adam Brosz, Carlos Fierro, Howard Schwartz, Kathleen Neuzil, Larry Corey, Peter Gilbert, Holly Janes, Dean Follmann, Mary Marovich, John Mascola, Laura Polakowski, Julie Ledgerwood, Barney S Graham, Hamilton Bennett, Rolando Pajon, Conor Knightly, Brett Leav, Weiping Deng, Honghong Zhou, Shu Han, Melanie Ivarsson, Jacqueline Miller, Tal Zaks, and COVE Study Group. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.*, 384(5):403–416, February 2021.
3. Fernando P Polack, Stephen J Thomas, Nicholas Kitchin, Judith Absalon, Alejandra Gurtman, Stephen Lockhart, John L Perez, Gonzalo Pérez Marc, Edson D Moreira, Cristiano Zerbini, Ruth Bailey, Kena A Swanson, Satrajit Roychoudhury, Kenneth Koury, Ping Li, Warren V Kalina, David Cooper, Robert W Frenck, Jr, Laura L Hammitt, Özlem Türeci, Haylene Nell, Axel Schaefer, Serhat Ünal, Dina B Tresnan, Susan Mather, Philip R Dormitzer, Uğur Şahin, Kathrin U Jansen, William C Gruber, and C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. *N. Engl. J. Med.*, December 2020.
4. Merryn Voysey, Sue Ann Costa Clemens, Shabir A Madhi, Lily Y Weckx, Pedro M Folegatti, Parvinder K Aley, Brian Angus, Vicky L Baillie, Shaun L Barnabas, Qasim E Bhorat, Sagida Bibi, Carmen Briner, Paola Cicconi, Andrea M Collins, Rachel Colin-Jones, Clare L Cutland, Thomas C Darton, Keertan Dheda, Christopher J A Duncan, Katherine R W Emary, Katie J Ewer, Lee Fairlie, Saul N Faust, Shuo Feng, Daniela M Ferreira, Adam Finn, Anna L Goodman, Catherine M Green, Christopher A Green, Paul T Heath, Catherine Hill, Helen Hill, Ian Hirsch, Susanne H C Hodgson, Alane Izu, Susan Jackson, Daniel Jenkin, Carina C D Joe, Simon Kerridge, Anthonet Koen, Gaurav Kwatra, Rajeka Lazarus, Alison M Lawrie, Alice Lelliott, Vincenzo Libri, Patrick J Lillie, Raburn Mallory, Ana V A Mendes, Eveline P Milan, Angela M Minassian, Alastair McGregor, Hazel Morrison, Yama F Mujadidi, Anusha Nana, Peter J O'Reilly, Sherman D Padayachee, Ana Pittella, Emma Plested, Katrina M Pollock, Maheshi N Ramasamy, Sarah Rhead, Alexandre V Schwarzbold, Nisha Singh, Andrew Smith, Rinn Song, Matthew D Snape, Eduardo Sprinz, Rebecca K Sutherland, Richard Tarrant, Emma C Thomson, M Estée Török, Mark Toshner, David P J Turner, Johan Vekemans, Tonya L Villafana, Marion E E Watson, Christopher J Williams, Alexander D Douglas, Adrian V S Hill, Teresa Lambe, Sarah C Gilbert, Andrew J Pollard, and Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in brazil, south africa, and the UK. *Lancet*, December 2020.
5. Cheryl Keech, Gary Albert, Iksung Cho, Andreana Robertson, Patricia Reed, Susan Neal, Joyce S Plested, Mingzhu Zhu, Shane Cloney-Clark, Haixia Zhou, Gale Smith, Nita Patel, Matthew B Frieman, Robert E Haupt, James Logue, Marisa McGrath, Stuart Weston, Pedro A Piedra, Chinar Desai, Kathleen Callahan, Maggie Lewis, Patricia Price-Abbott, Neil Formica, Vivek Shinde, Louis Fries, Jason D Lickliter, Paul Griffin, Bethanie Wilkinson, and Gregory M Glenn. Phase 1–2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. *N. Engl. J. Med.*, 383(24):2320–2332, December 2020.
6. Denis Y Logunov, Inna V Dolzhikova, Dmitry V Shcheblyakov, Amir I Tukhvatulin, Olga V Zubkova, Alina S Dzharullaeva, Anna V Kovyrshina, Nadezhda L Lubenets, Daria M Grousova, Alina S Erokhova, Andrei G Botikov, Fatima M Izhaeva, Olga Popova, Tatiana A Ozharovskaya, Ilias B Esmagambetov, Irina A Favorskaya, Denis I Zrelkin, Daria V Voronina, Dmitry N Shcherbinin, Alexander S Semikhin, Yana V Simakova, Elizaveta A Tokarskaya, Daria A Egorova, Maksim M Shmarov, Natalia A Nikitenko, Vladimir A Gushchin, Elena A Smolyarchuk, Sergey K Zyryanov, Sergei V Borisevich, Boris S Naroditsky, Alexander L Gintsburg, and Gam-COVID-Vac Vaccine Trial Group. Safety and efficacy of an rad26 and rad5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in russia. *Lancet*, 397(10275):671–681, February 2021.
7. Fan Wu, Su Zhao, Bin Yu, Yan-Mei Chen, Wen Wang, Zhi-Gang Song, Yi Hu, Zhao-Wu Tao, Jun-Hua Tian, Yuan-Yuan Pei, Ming-Li Yuan, Yu-Ling Zhang, Fa-Hui Dai, Yi Liu, Qi-Min Wang, Jiao-Jiao Zheng, Lin Xu, Edward C Holmes, and Yong-Zhen Zhang. A new coronavirus associated with human respiratory disease in china. *Nature*, 579(7798):265–269, March 2020.
8. Darren P Martin, Steven Weaver, Houryiah Tegally, Emmanuel James San, Stephen D Shank, Eduan Wilkinson, Jennifer Giandhari, Sureshnee Naidoo, Yeshnee Pillay, Lavanya Singh, Richard J Lessells, NGS-SA, COVID-19 Genomics UK (COG-UK), Ravindra K Gupta, Joel O Wertheim, Anton Nekturenko, Ben Murrell, Gordon W Harkins, Philippe Lemey, Oscar A MacLean, David L Robertson, Tulio de Oliveira, and Sergei L Kosakovsky Pond. The emergence and ongoing convergent evolution of the N501Y lineages coincides with a major global shift in the SARS-CoV-2 selective landscape. *medRxiv*, March 2021.
9. Houriiyah Tegally, Eduan Wilkinson, Marta Giovanetti, Arash Iranzadeh, Vagner Fonseca, Jennifer Giandhari, Deelan Doolabh, Sureshnee Pillay, Emmanuel James San, Nokukhanya Msomi, Koleka Mlisana, Anne von Gottberg, Sibongile Walaza, Mushal Allam, Arshad Ismail, Thabo Mohale, Allison J Glass, Susan Engelbrecht, Gert Van Zyl, Wolfgang Preiser, Francesco Petruccione, Alex Sigal, Diana Hardie, Gert Marais, Marvin Hsiao, Stephen Korsman, Mary-Ann Davies, Lynn Tyers, Innocent Mudau, Denis York, Caroline Maslo, Dominique Goedhals, Shareef Abrahams, Oluwakemi Laguda-Akingba, Arghavan Alisoltani-Dehkordi, Adam Godzik, Constantinos Kurt Wibmer, Bryan Trevor Sewell, José Lourenço, Luiz Carlos Junior Alcantara, Sergei L Kosakovsky Pond, Steven Weaver, Darren Martin, Richard J Lessells, Jinal N Bhiman, Carolyn Williamson, and Tulio de Oliveira. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in south africa. December 2020.
10. Pengfei Wang, Manoj S Nair, Lihong Liu, Sho Iketani, Yang Luo, Yicheng Guo, Maple Wang, Jian Yu, Baoshan Zhang, Peter D Kwong, Barney S Graham, John R Mascola, Jennifer Y Chang, Michael T Yin, Magdalena Sobieszczyk, Christos A Kyratsous, Lawrence Shapiro, Zizhang Sheng, Yaoxing Huang, and David D Ho. Antibody resistance of SARS-CoV-2 variants b.1.351 and b.1.1.7. *Nature*, March 2021.
11. Daming Zhou, Wanwisa Dejnirattisai, Piyada Supasa, Chang Liu, Alexander J Mentzer, Helen M Ginn, Yuguang Zhao, Helen M E Duyvesteyn, Aekkachai Tuekprakhon, Rungtiwa Nutalai, Beibei Wang, Guido C Paesen, Cesar Lopez-Camacho, Jose Slon-Campos, Bassam Hallis, Naomi Coombes, Kevin Bewley, Sue Charlton, Thomas S Walter, Donal Skelly, Sheila F Lumley, Christina Dold, Robert Levin, Tao Dong, Andrew J Pollard, Julian C Knight, Derrick Crook, Teresa Lambe, Elizabeth Clutterbuck, Sagida Bibi, Amy Flaxman, Mustapha Bittaye, Sandra Belij-Rammerstorfer, Sarah Gilbert, William James, Miles W Carroll, Paul Klenerman, Eleanor Barnes, Susanna J Dunachie, Elizabeth E Fry, Juthathip Mongkolsapaya, Jingshan Ren, David I Stuart, and Gavin R Screaton. Evidence of escape of SARS-CoV-2 variant b.1.351 from natural and vaccine induced sera. *Cell*, February 2021.
12. Kai Wu, Anne P Werner, Juan I Moliva, Matthew Koch, Angela Choi, Guillaume B E Stewart-Jones, Hamilton Bennett, Seyhan Boyoglu-Barnum, Wei Shi, Barney S Graham, Andrea Carfi, Kizzmekia S Corbett, Robert A Seder, and Darin K Edwards. mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. *bioRxiv*, January 2021.
13. Shabir A Madhi, Vicky Baillie, Clare L Cutland, Merryn Voysey, Anthonet L Koen, Lee Fairlie, Sherman D Padayachee, Keertan Dheda, Shaun L Barnabas, Qasim E Bhorat, Carmen Briner, Gaurav Kwatra, Khatija Ahmed, Parvinder Aley, Sutika Bhikha, Jinal N Bhiman, As'ad E Bhorat, Jeanine du Plessis, Aliasgar Esmail, Marisa Groenewald, Elizea Horne, Shi-Hsia Hwa, Aylin Jose, Teresa Lambe, Matt Laubscher, Mookho Malahleha, Masebole Masenya, Mduduzi Masilela, Shakeel McKenzie, Kgaogelo Molapo, Andrew Moultrie, Suzette Oelofse, Faeezah Patel, Sureshnee Pillay, Sarah Rhead, Hylton Rodel, Lindie Rossouw, Carol Taoushanis, Houriiyah Tegally, Asha Thombrayil, Samuel van Eck, Constantinos K Wibmer, Nicholas M Durham, Elizabeth J Kelly, Tonya L Villafana, Sarah Gilbert, Andrew J Pollard, Tulio de Oliveira, Penny L Moore, Alex Sigal, Alane Izu, and NGS-SA Group Wits–VIDA COVID Group. Efficacy of the ChAdOx1 nCoV-19 covid-19 vaccine against the b.1.351 variant. *N. Engl. J. Med.*, March 2021.
14. Vivek Shinde, Sutika Bhikha, Zaheer Hoosain, Mohernndran Archary, Qasim Bhorat, Lee Fairlie, Umesh Lalloo, Mduduzi S L Masilela, Dhayendre Moodley, Sherika Hanley, Leon Fouche, Cheryl Louw, Michele Tameris, Nishanta Singh, Ameena Goga, Keertan Dheda, Coert Grobbelaar, Gertruida Kruger, Nazira Carrim-Ganey, Vicky Baillie, Tulio de Oliveira, Anthonet Lombard Koen, Johan J Lombaard, Rosie Mngqibisa, As'ad Ebrahim Bhorat, Gabriella Benadé, Natasha Lalloo, Annah Pitsi, Pieter-Louis Vollgraaff, Angelique Luabeya, Aliasgar Esmail, Friedrich G Petrick, Aylin Oommen Jose, Sharne Foulkes, Khatija Ahmed, Asha Thombrayil, Lou Fries, Shane Cloney-Clark, Mingzhu Zhu, Chijioke Bennett, Gary Albert, Emmanuel Faust, Joyce S Plested, Andreana Robertson, Susan Neal, Iksung Cho, Greg M Glenn, Filip Dubovsky, Shabir A Madhi, and for the 2019nCoV-501 Study Group. Preliminary efficacy of the NVX-CoV2373 covid-19 vaccine against the b.1.351 variant. March 2021.
15. Thomas Francis. On the doctrine of original antigenic sin. *Proc. Am. Philos. Soc.*, 104(6): 572–578, 1960.
16. Justin Lessler, Steven Riley, Jonathan M Read, Shuying Wang, Huachen Zhu, Gavin J D Smith, Yi Guan, Chao Qiang Jiang, and Derek A T Cummings. Evidence for antigenic

seniority in influenza a (H3N2) antibody responses in southern china. *PLoS Pathog.*, 8(7): e1002802, July 2012.

17. Katelyn M Gostic, Monique Ambrose, Michael Worobey, and James O Lloyd-Smith. Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. *Science*, 354(6313):722–726, November 2016.
18. Jun Lan, Jiwan Ge, Jinfang Yu, Sisi Shan, Huan Zhou, Shilong Fan, Qi Zhang, Xuanling Shi, Qisheng Wang, Linqi Zhang, and Xinquan Wang. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, 581(7807):215–220, May 2020.
19. Alicia T Widge, Nadine G Rouphael, Lisa A Jackson, Evan J Anderson, Paul C Roberts, Mamodikoe Makhene, James D Chappell, Mark R Denison, Laura J Stevens, Andrea J Pruijssers, Adrian B McDermott, Britta Flach, Bob C Lin, Nicole A Doria-Rose, Sijy O'Dell, Stephen D Schmidt, Kathleen M Neuzil, Hamilton Bennett, Brett Leav, Mat Makowski, Jim Albert, Kaitlyn Cross, Venkata-Viswanadh Edara, Katharine Floyd, Mehul S Suthar, Wendy Buchanan, Catherine J Luke, Julie E Ledgerwood, John R Mascola, Barney S Graham, John H Beigel, and mRNA-1273 Study Group. Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. *N. Engl. J. Med.*, December 2020.
20. Marco Mandolesi, Daniel J Sheward, Leo Hanke, Junjie Ma, Pradeepa Pushparaj, Laura Perez Vidakovics, Changil Kim, Karin Loré, Xaquin Castro Dopico, Jonathan M Coquet, and Others. SARS-CoV-2 protein subunit vaccination elicits potent neutralizing antibody responses. *BioRxiv*, 2020.
21. Jing-Hui Tian, Nita Patel, Robert Haupt, Haixia Zhou, Stuart Weston, Holly Hammond, James Logue, Alyse D Portnoff, James Norton, Mimi Guebre-Xabier, Bin Zhou, Kelsey Jacobson, Sonia Maciejewski, Rafia Khatoon, Malgorzata Wisniewska, Will Moffitt, Stefanie Kluepfel-Stahl, Betty Ekechukwu, James Papin, Sarathi Boddapati, C Jason Wong, Pedro A Piedra, Matthew B Frieman, Michael J Massare, Louis Fries, Karin Lövgren Bengtsson, Linda Stertman, Larry Ellingsworth, Gregory Glenn, and Gale Smith. SARS-CoV-2 spike glycoprotein vaccine candidate NVX-CoV2373 immunogenicity in baboons and protection in mice. *Nat. Commun.*, 12(1):372, January 2021.
22. Ali Rahimi and Benjamin Recht. Weighted sums of random kitchen sinks: replacing minimization with randomization in learning. In *Nips*, pages 1313–1320, 2008.
23. Ching-Lin Hsieh, Jory A Goldsmith, Jeffrey M Schaub, Andrea M DiVenere, Hung-Che Kuo, Kamyab Javanmardi, Kevin C Le, Daniel Wrapp, Alison G Lee, Yutong Liu, Chia-Wei Chou, Patrick O Byrne, Christy K Hjorth, Nicole V Johnson, John Ludes-Meyers, Annalee W Nguyen, Juyeon Park, Nianshuang Wang, Dzifa Amengor, Jason J Lavinder, Gregory C Ippolito, Jennifer A Maynard, Ilya J Finkelstein, and Jason S McLellan. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. *Science*, 369(6510):1501–1505, September 2020.
24. Constantinos Kurt Wibmer, Frances Ayres, Tandile Hermanus, Mashudu Madzivhandila, Prudence Kgagudi, Brent Oosthuysen, Bronwen E Lambson, Tulio de Oliveira, Marion Vermeulen, Karin van der Berg, Theresa Rossouw, Michael Boswell, Veronica Ueckermann, Susan Meiring, Anne von Gottberg, Cheryl Cohen, Lynn Morris, Jinal N Bhiman, and Penny L Moore. SARS-CoV-2 501Y.V2 escapes neutralization by south african COVID-19 donor plasma. *Nat. Med.*, March 2021.

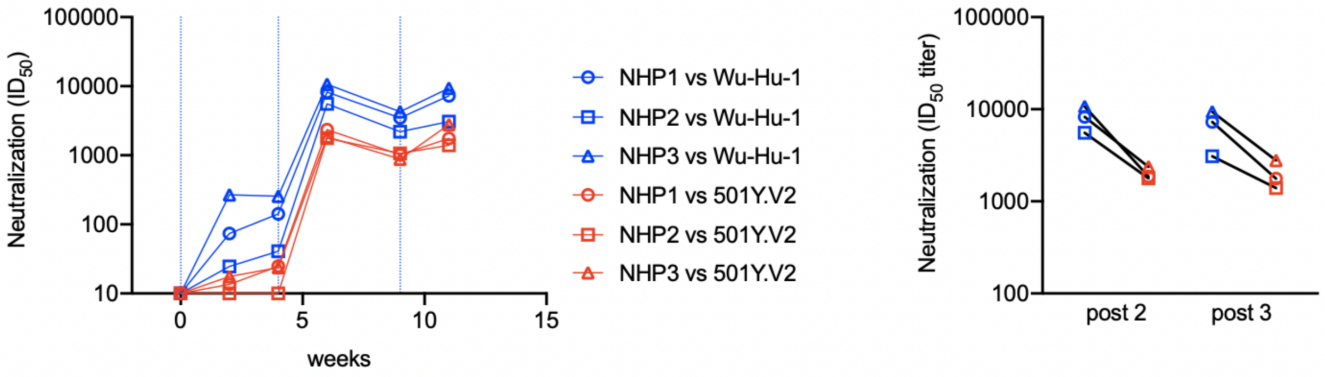

Fig. SI1. (left) Longitudinal neutralizing antibody responses against Wu-Hu-1 (blue) and 501Y.V2 (red) for plasma samples from Mandolesi et al.[20], where three rhesus macaques (NHP1-NHP3) were immunized with three doses of Wu-Hu-1 spike (100 µg) in Matrix-M™ adjuvant. Vertical blue lines indicate the timing of immunizations (at 0, 4, and 9 weeks). (right) Comparison of the titers at 6 weeks (post 2) and 11 weeks (post 3) illustrating that reduced titers to 501Y.V2 (red) compared to Wu-Hu-1 (blue) were maintained after a third homotypic spike boost.