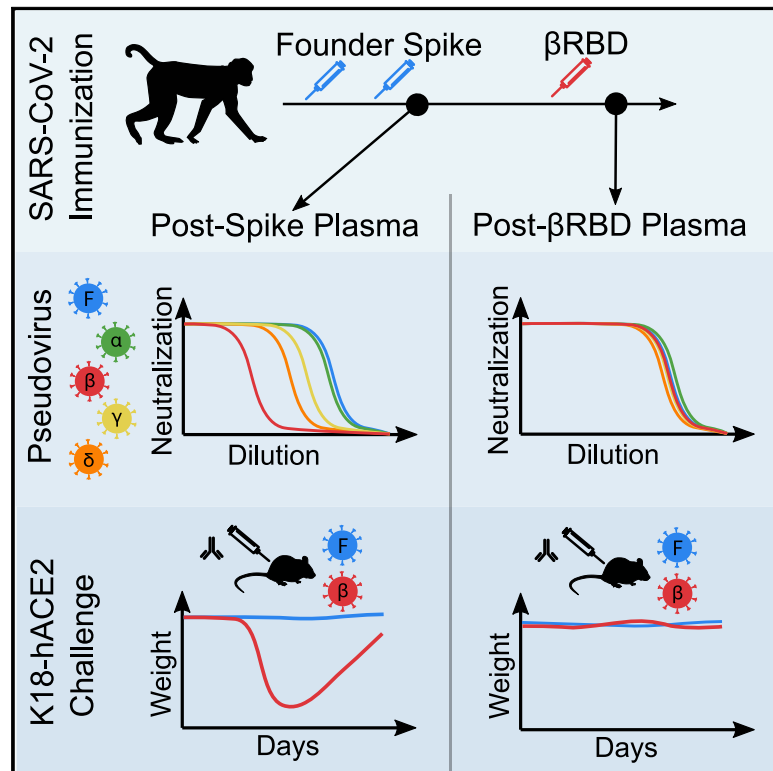


Beta RBD boost broadens antibody-mediated protection against SARS-CoV-2 variants in animal models

Graphical abstract



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In brief

The emergence and spread of antibody-resistant SARS-CoV-2 variants of concern (VOCs) threatens to diminish vaccine efficacy. Sheward et al. show, in rhesus macaques and K18-hACE2 mice, that reduced vaccine protection against VOCs can be restored by broadening antibody responses with a third, heterotypic RBD booster immunization.

Highlights

- Heterotypic RBD boost elicits cross-neutralizing antibody responses in macaques
- No evidence that original antigenic sin hinders booster immunizations with beta RBD
- Pre-boost plasma only partially protects K18-hACE2 mice from beta variant challenge
- Post-boost plasma affords full protection from beta variant challenge



Article

Beta RBD boost broadens antibody-mediated protection against SARS-CoV-2 variants in animal models

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SUMMARY

Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) variants of concern (VOCs) with resistance to neutralizing antibodies are threatening to undermine vaccine efficacy. Vaccination and infection have led to widespread humoral immunity against the pandemic founder (Wu-Hu-1). Against this background, it is critical to assess the outcomes of subsequent immunization with variant antigens. It is not yet clear whether heterotypic 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 memory B cell repertoire would bridge the gap between Wu-Hu-1 and VOCs. We show, in macaques immunized with Wu-Hu-1 spike, that a single dose of adjuvanted beta variant receptor binding domain (RBD) protein broadens neutralizing antibody responses to heterologous VOCs. Passive transfer of plasma sampled after Wu-Hu-1 spike immunization only partially protects K18-hACE2 mice from lethal challenge with a beta variant isolate, whereas plasma sampled following heterotypic RBD boost protects completely against disease.

INTRODUCTION

At least 27 candidate severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) vaccines have already entered phase 3 clinical trials. A number of these demonstrated high efficacy,^{1–5} 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-1.⁶

Driven by multiple evolutionary forces,⁷ SARS-CoV-2 is evading immune responses and threatening to undermine current prevention and mitigation strategies. Globally, novel variants of concern (VOCs) are increasingly dominating the pandemic (Figure 1). Of particular concern is the surge of variants harboring spike mutations that confer resistance to prior immunity, such as 501Y.V2 (B.1.351, “beta”).^{8–11} This underpins the substantially reduced vaccine efficacies observed in trials in South Africa, where this variant was circulating at high frequency.^{12,13} Recently, significant numbers of vaccine breakthrough infections have been observed during infection waves dominated by the delta (B.1.617.2) variant, which also displays reduced sensitivity to neutralization.^{14–16} Updated vaccines are likely required to protect against current and future mutated variants. Importantly, by the time these are rolled out, a significant propor-

tion of the global population are likely to be seropositive from either infection or immunization with Wu-Hu-1-based vaccines. A relevant question now is whether a single additional dose will be sufficient to induce robust neutralizing antibody responses to VOCs in seropositive individuals and whether these boosts are sufficient to confer protection. Importantly, the first exposure to a pathogen can shape future responses to mutated variants. This immunological imprinting or original antigenic sin¹⁷ is well described for influenza A virus, where protection is highest against the first strain encountered and diminished against those encountered later in life.^{18,19} It is crucial for the design of updated vaccines and regimens to determine whether existing immunity dampens antibody responses to new VOCs or whether a heterotypic boost can efficiently recruit cross-protective memory responses.

RESULTS

To address this, we immunized three rhesus macaques with two doses of soluble prefusion-stabilized Wu-Hu-1 spike protein (2 µg), adjuvanted with saponin-based Matrix-M (Novavax AB, Uppsala, Sweden), with a 1-month interval between doses, mimicking an immunization schedule for approved SARS-CoV-2 vaccines. After a single dose, neutralizing antibodies were



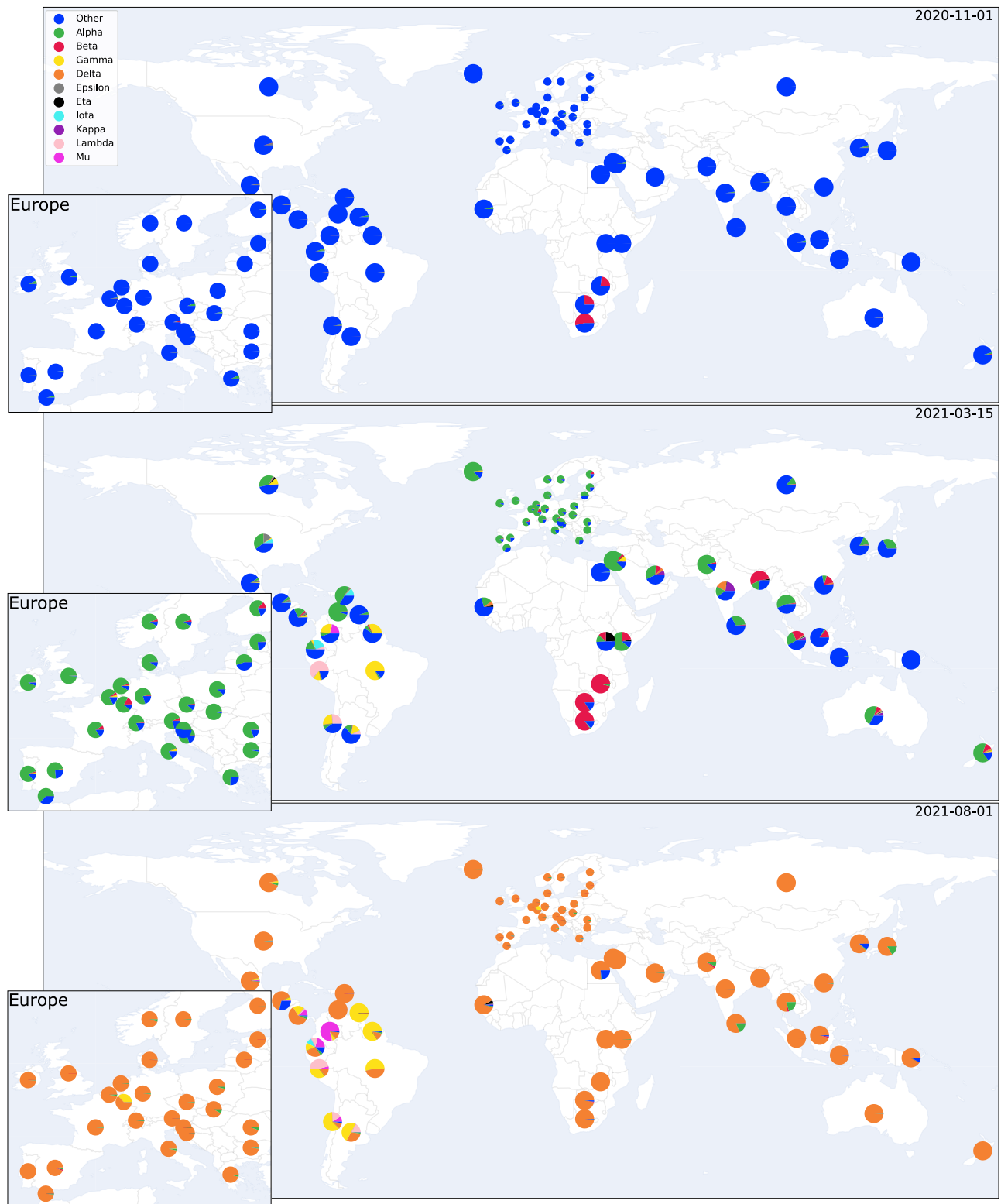


Figure 1. SARS-CoV-2 variants can rapidly come to dominate the global genomic landscape

The global distribution and estimated country-level proportions of deposited SARS-CoV-2 genomes for eight variants, shown for 1 November 2020 (top), 4.5 months later for 15 March 2021 (middle), and as of 1 August 2021 (bottom). Proportions over time are estimated from GISAID²⁰ genome metadata, using a locally weighted multinomial regression model (see STAR Methods).

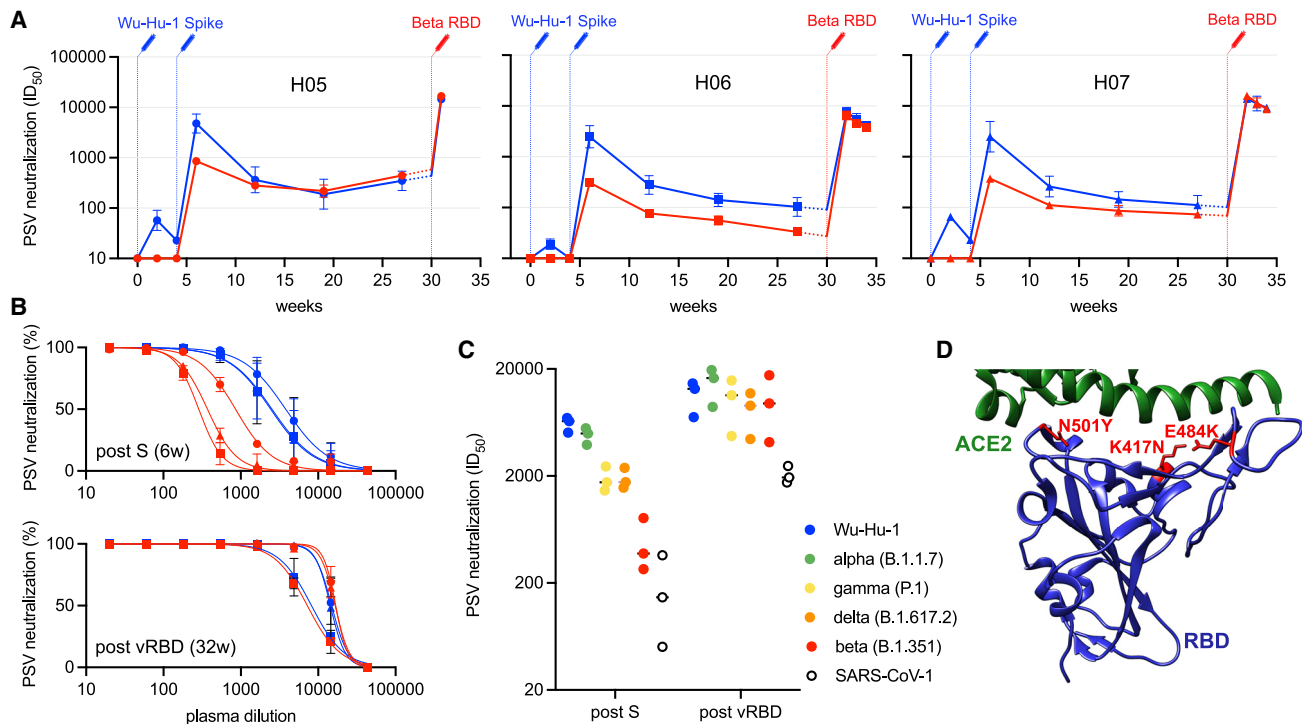


Figure 2. Heterotypic RBD boost drives a potent cross-neutralizing antibody response

(A) Neutralizing antibody responses over time to Wu-Hu-1 (blue) and beta (red) pseudotyped viruses (PSVs) are shown for three immunized macaques: H05; H06; and H07. Syringes indicate the timing of immunizations (blue: Wu-Hu-1 spike at 0 and 4 weeks; red: beta RBD at 30 weeks). Titers from 27 to 30 weeks (shown with dashed lines) have been extrapolated for clarity. Error bars depict the geometric SD from at least 3 technical repeats.

(B) Although PSV neutralization (reduction in infectivity, as a %) of beta was significantly reduced at 6 weeks, corresponding to peak responses 2 weeks following the second spike dose (post-S), neutralization was restored following subsequent heterotypic RBD boost (post-vRBD) such that beta (red) and Wu-Hu-1 (blue) were potently neutralized at similar titers in all three animals. Error bars depict the SD from at least 3 technical repeats.

(C) Heterotypic RBD boost also restored the reduced neutralizing antibody titers against VOCs alpha (green), gamma (yellow), and delta (orange) as well as improved the neutralization of the more distantly related SARS-CoV-1 (open circles).

(D) Depiction of the RBD immunogen (PDB: 6MOJ),²² used as a heterotypic boost in this study, that incorporates the three RBD mutations (located in red) defining lineage 20H/501Y.V2 (beta). The cellular receptor, ACE2, is shown in green.

detectable against Wu-Hu-1, but not against the beta variant (Figure 2). Neutralizing antibody responses against Wu-Hu-1 were substantially boosted by the second immunization (“post-S”), with a peak geometric mean titer (GMT) of 3,980, and then waned over the following months (Figure 2), as also reported in immunized humans.²¹ Notably, the beta VOC was on average 9-fold (range: 5.6- to 12.2-fold) less potently neutralized (GMT = 451 at peak), consistent with the responses observed in humans following vaccination.^{9–11}

6 months after their first immunization, macaques were boosted with either 2 μg (H05), 10 μg (H06), or 50 μg (H07) of soluble 501Y.V2 RBD 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 two other macaques (H06 and H07) were followed for 4 weeks. In all three animals, beta RBD efficiently boosted responses (Figure S1) that potently cross-neutralized both Wu-Hu-1 and beta, with similar titers (Figures 2A–2C; Wu-Hu-1 GMT = 11,795; beta GMT = 12,595). This suggests that the heterotypic boost may have preferentially recruited cross-neutral-

izing memory B cells. Indeed, heterotypic boost improved neutralization of other VOCs, including delta, alpha, and gamma, as well as the more distantly related SARS-CoV-1 (Figure 2C). In contrast, for macaques previously immunized with three doses of Wu-Hu-1 spike,²³ the reduced neutralization of beta (and other VOCs) compared to Wu-Hu-1 remained after the third homotypic spike immunization (Figure S2).

To determine whether recovery of neutralizing antibody titers to beta afforded a biologically relevant improvement in protective immunity, mice transgenic for human ACE2 (K18-hACE2)²⁴ were passively immunized intraperitoneally (i.p.) with plasma samples taken either 2 weeks following the second spike immunization (n = 8) (post-S) or 1 to 2 weeks following the RBD booster immunization (n = 8; “post-vRBD”). Passive immunization conferred titers approximately 10-fold lower than donor plasma (Figure S3), and macaque polyclonal antibodies were not rapidly cleared following xenotransfusion, with an unchallenged mouse still maintaining titers >1,400 after 5 days (data not shown). Mice were then challenged intranasally with 2.4 × 10⁶ RNA copies of either beta or “wild-type” (WT) (encoding a spike matching Wu-Hu-1) virus (corresponding to 100 plaque-forming units [PFUs]

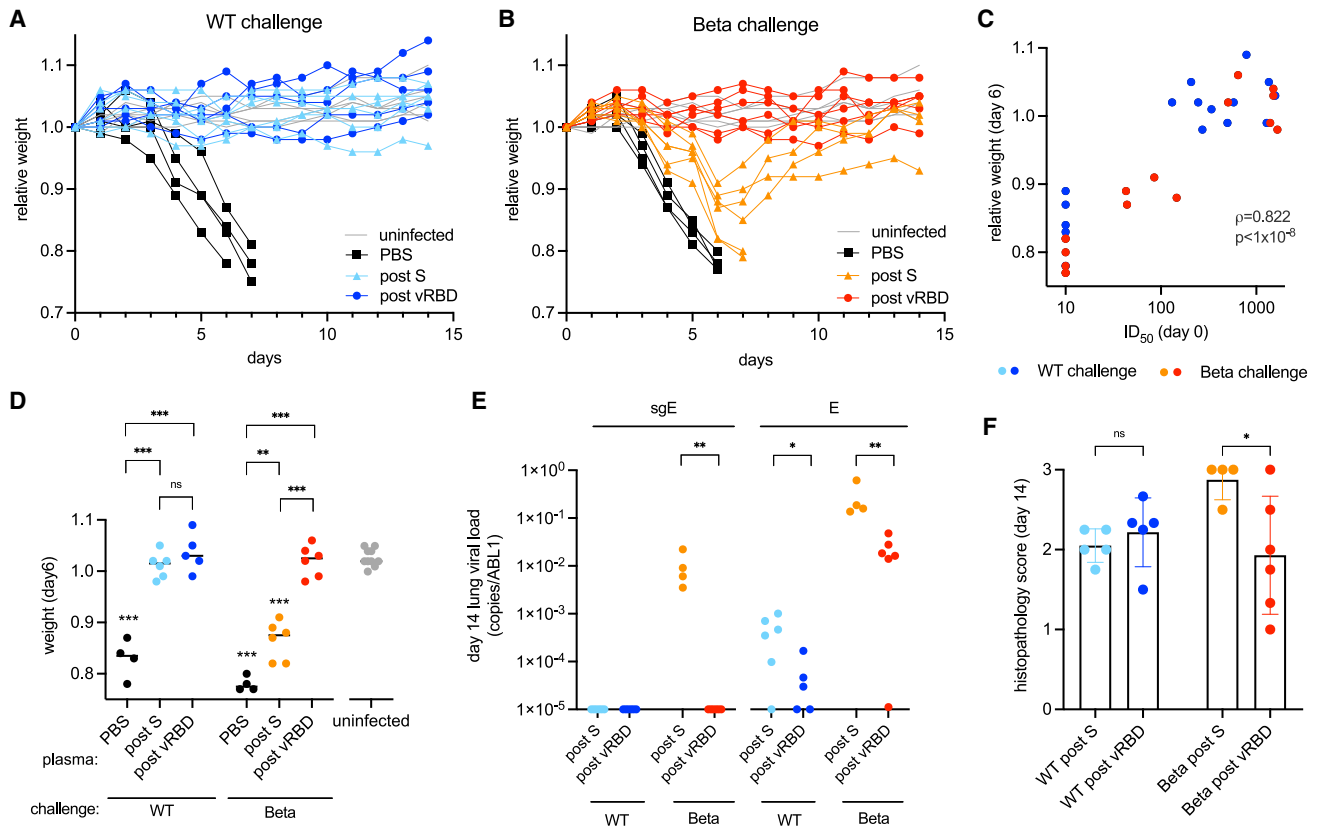


Figure 3. Heterotypic RBD boost restores protection against 501Y.V2 in passively immunized k18-hACE2 mice

(A and B) Weight loss following challenge with either (A) “wild-type” (“WT”) or (B) 501Y.V2 virus for K18-hACE2 mice passively immunized with macaque plasma sampled post-S or post-vRBD. Control mice mock immunized with PBS and subsequently challenged (“PBS”) are shown in black, and uninfected littermates housed in the same cages (“uninfected”) are shown in gray.

(C) Pseudovirus-neutralizing antibody titers against the challenge spike (infective dose 50 [ID₅₀]) in passively immunized mice on the day of challenge are associated with infection and disease severity summarized as weight loss 6 days following challenge. Titers below the limit of detection of the assay (20) are plotted as 10.

(D) Weight loss at day 6 for each group. Unchallenged littermates housed in the same cages (gray) are shown; PBS, mock immunized mice (black). Post-S, passive immunization with plasma following the second spike immunization (6-week plasma); post-vRBD, passive immunization with plasma from macaques boosted with variant (beta) RBD (31- or 32-week plasma). Groups displaying significant weight loss compared to uninfected mice are annotated above the points for that group.

(E) Viral loads in lung tissue on day 14 quantified as the ratio of the copies of either viral genomic envelope (E) or subgenomic transcripts (sgE) to the number of copies of housekeeping gene ABL1. Undetectable copies are plotted on the baseline (1×10^{-5}).

(F) Pulmonary pathology scores in H&E-stained lung sections (89 slides in total, with a mean of 3.3 slides per mouse; range: 2–5). All statistical comparisons are summarized as **p < 0.01 and ***p < 0.001; ns, not significant. Error bars depict SDs.

of beta or 86 PFUs of WT) and monitored for weight loss, a reliable proxy for disease severity (Figure 3).²⁵

Across all groups, protection was strongly correlated with the neutralizing antibody titers to the challenge virus on the day of challenge (Spearman’s $\rho = 0.822$; $p < 1 \times 10^{-8}$; Figure 3C). All control mice that did not receive plasma (PBS only) succumbed to disease when challenged with either variant, showing precipitous weight loss starting around 3 days post-challenge (Figures 3A, 3B, and 3D). Passive transfer of post S plasma conferred complete protection against weight loss following infection with WT virus (Figures 3A and 3D), but not from beta (Figures 3B and 3D), clearly demonstrating that evasion of the antibody response by this VOC was sufficient to cause disease. Notably, passive transfer of post-vRBD plasma pro-

tected against both WT and beta (Figures 3A–3D). Analysis of lung viral loads at day 14 post-infection demonstrated heightened viral clearance in mice transfused with post-vRBD plasma, in particular, in mice infected with beta (Figure 3E). In addition, hematoxylin and eosin staining was performed on lungs 14 days post-infection to assess the pathological effects of virus infection. WT and beta virus both induced signs of alveoli septa thickening and infiltration of inflammatory cells, although mice infected with beta also demonstrated a loss in general tissue architecture (Figures S5 and 3F). Even among only the surviving mice, those transfused with post-vRBD plasma displayed significantly reduced pathological scores following beta infection than did mice that received post-S plasma (Figure 3F).

DISCUSSION

For many licensed SARS-CoV-2 vaccines, reduced efficacy has been observed against the beta variant.^{12,26,27} Moreover, the decay of vaccine-elicited antibody titers^{21,28} suggests that, over time, protection will wane further. Consistent with reduced vaccine efficacy against beta, we show that breakthrough infection and disease occur in K18-hACE2 mice passively immunized with plasma from rhesus macaques immunized with two doses of adjuvanted WT spike protein.

Cross-species passive immunization and challenge experiments have been used to show antibody-mediated protection conferred by influenza vaccination (human to mouse)²⁹ and, more recently, to characterize protection from WT SARS-CoV-2 (macaque to hamster).³⁰ Our data further demonstrate the utility of this approach for investigating protection against SARS-CoV-2 variants.

The ability of vaccines to broaden existing responses to new variants is still largely unclear. Despite weak immunogenicity of soluble, monomeric RBD as a priming antigen,²³ heterotypic RBD administered as a boost elicited a potent recall response in non-human primates. This was robust to the boosting dose, and effective as low as 2 μ g, possibly aided by a dose-sparing effect of Matrix-M.³¹ Although reduced neutralization of beta was evident following 2 doses of Wu-Hu-1 spike, both Wu-Hu-1 and beta were potently neutralized following heterotypic (beta) RBD boost. In animal challenge models, neutralizing antibodies following passive immunization represented a robust correlate of protection such that the restoration of neutralizing antibody titers to beta also translated into protective immunity.

The potent, cross-neutralizing antibody response that arises following a heterotypic boost indicates that original antigenic sin does not represent a significant barrier to the acquisition of protective immunity against current SARS-CoV-2 VOCs. This is largely consistent with recently reported results from beta spike encoding mRNA (mRNA1273.351, Moderna) booster vaccinations.^{32,33} The observation that immunization with RBD (and not whole spike) was capable of inducing robust neutralizing antibody responses is particularly promising as RBD is a small, stable protein that can be rapidly synthesized and efficiently expressed. Here, in the immunized animal sampled only 5 days post-vRBD boost, neutralizing titers (against both Wu-Hu-1 and beta) were already elevated, suggesting these titers are the product of a rapidly activated population of antibody-secreting cells. Further, this time course indicates that successive rounds of affinity maturation likely were not required for neutralization of beta but rather that vRBD-specific antibody responses could be boosted from the pool of existing cross-neutralizing memory B cells primed by Wu-Hu-1. This is consistent with the neutralization of multiple VOCs (including alpha, gamma, and delta) with comparable potency. Cross-neutralization of delta is particularly important, given the high transmissibility and global prevalence of this variant.

Taken together, these data indicate that potent, cross-neutralizing, and cross-protective antibody responses can be recruited with heterotypic SARS-CoV-2 immunogens following a primary exposure and identify soluble RBD booster immunizations as

an attractive strategy to broaden vaccine protection from new SARS-CoV-2 variants.

Limitations of the study

Although we show that a heterotypic RBD immunization can efficiently boost protective cross-neutralizing antibody responses, these results are based on only a small number of non-human primates, and we did not characterize the development and breadth of individual antibody lineages. Furthermore, we did not have a well-controlled, homotypic boost arm for comparison. The animals in the previous study that received three doses of WT spike were immunized with a substantially higher dose and received the third dose significantly sooner, preventing a direct comparison. Thus, although a heterotypic RBD boost elicits potent cross-neutralizing responses, further work is required to quantify the benefits of this approach over a third homotypic spike (or RBD) boost.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xcrm.2021.100450>.

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AUTHOR CONTRIBUTIONS

Conceptualization, D.J.S., M.M., E.U., J.M.C., G.B.K.H., and B.M.; formal analysis, D.J.S. and B.M.; funding acquisition, G.M.M., J.M.C., G.B.K.H., and B.M.; investigation, D.J.S., M.M., E.U., C.K., A.P., N.L.S., and X.C.D.; methodology, D.J.S., M.M., E.U., A.P., N.L.S., J.M.C., G.B.K.H., and B.M.; resources, C.K., L.H., and L.P.V.; software, B.M.; supervision, L.H., G.M.M., J.M.C., G.B.K.H., and B.M.; visualization, D.J.S., A.P., and B.M.; writing – original draft, D.J.S. and B.M.; writing – review & editing, all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Wild-type SARS-CoV-2 isolate	Jonas Klingström	N/A
501Y.V2 isolate	Alex Sigal	Cele et al. ³⁴
Biological samples		
Plasma from NHPs	This study	N/A
Chemicals, peptides, and recombinant proteins		
Recombinant SARS-CoV-2 WT Spike	This study	N/A
Recombinant SARS-CoV-2 501Y.V2 RBD	This study	N/A
Polyethylenimine	Sigma-Aldrich	Cat# 764604
Matrix-M	Novavax AB	N/A
Lipofectamine 3000	Invitrogen	Cat# L3000075
Bright-Glo Luciferase Assay System	Promega	Cat# E2620
TRlzol Reagent	Thermo Fisher Scientific	Cat# 15596026
GlycoBlue Coprecipitant	Thermo Fisher Scientific	Cat# AM9515
SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase	Thermo Fisher Scientific	Cat# 12574018
mMESSAGE mMACHINE T7 Transcription Kit	Thermo Fisher Scientific	Cat# AM1344
GIBCO FreeStyle MAX Reagent	Thermo Fisher Scientific	Cat# 16447100
Gibson Assembly Mastermix	New England Biolabs	Cat# E2611S
Deposited data		
SARS-CoV-2 lineage metadata	GISAID	https://www.gisaid.org
Experimental models: cell lines		
Human: GIBCO FreeStyle 293-F cells	Thermo Fisher Scientific	Cat# R79009
Human: HEK293T-ACE2	Hanke et al. ³⁵	N/A
Human: Calu-3	Jonas Klingström	N/A
African Green Monkey: Vero E6 cell	ATCC	Cat# CRL-1586; RRID: CVCL_0574
Experimental models: organisms/strains		
NHP: Macaca mulatta	N/A	N/A
Mouse: K18-hACE2	Jackson Laboratory, McCray et al. ²⁴	Cat# 034860; RRID: MSR_JAX:034860
Oligonucleotides		
gBlocks Gene Fragments	Integrated DNA Technologies	N/A
Recombinant DNA		
SARS-CoV-2 Spike ectodomain plasmid (expression)	Hsieh et al. ³⁶	Addgene: 154754; RRID: Addgene_154754
SARS-CoV-2 B.1.351/501Y.V2 RBD plasmid	This study	N/A
SARS-CoV-2 WT Spike plasmid	James Voss	Rogers et al. ³⁷
SARS-CoV-2 B.1.1.7 Spike plasmid	David Nemazee	Addgene: 170451; RRID: Addgene_170451
SARS-CoV-2 B.1.351 Spike plasmid	Penny Moore	Wibmer et al. ³⁸
SARS-CoV-2 P.1 Spike plasmid	David Nemazee	Addgene: 170450; RRID: Addgene_170450
SARS-CoV-2 B.1.617.2 Spike plasmid	G2P-UK National Virology consortium	Spencer et al. ³⁹
SARS-CoV-1 Spike plasmid	James Voss	Rogers et al. ³⁷
Lentiviral backbone: pCMV delta R8.2	Bob Weinberg	Addgene: 8455; RRID: Addgene_8455

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Luciferase transfer plasmid	James Voss	Rogers et al. ³⁷
Software and algorithms		
GraphPad Prism v9.0.0	GraphPad Software Inc.	https://www.graphpad.com/scientific-software/prism/ ; RRID: SCR_002798
Julia v1.6	The Julia Programming Language	https://julialang.org/
Non-linear Multinomial Regression for VOC frequency estimation	This study	https://github.com/MurrellGroup/VOCfreq ; https://doi.org/10.5281/zenodo.5562800
Other		
HiLoad® 16/600 Superdex® 200 pg	Cytivia	Cat# 28-9893-35
His-Pur Ni-NTA resin	Thermo Fisher Scientific	Cat# 88222

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources or reagents should be directed to and will be fulfilled by the Lead Contact, Ben Murrell (benjamin.murrell@ki.se).

Materials availability

All unique/stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

Data and code availability

Original data reported in this paper will be shared by the Lead Contact upon request.

All original code has been deposited at <https://github.com/MurrellGroup/VOCfreq> and is publicly available as of the date of publication. DOIs are listed in the [Key resources table](#).

Any additional information required to reanalyze the data reported in this work paper is available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics statement

The animal work was conducted with the approval of Stockholms Jordbruksverket (10513-2020, 18427-2019 and 10895-2020). All animal procedures were performed according to approved guidelines.

Animal models

Rhesus macaques

Three female 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.

K18-hACE2 mice

Mice transgenic for human ACE2 under control of the cytokeratin 18 (K18) promoter²⁴ were obtained from the Jackson Laboratory. Mice were maintained as a hemizygous line, with hACE2 transgene presence confirmed using Sanger sequencing as per the Jackson Laboratory protocol. All mice were 12-21 weeks old at the start of the study, and experiments were conducted in BSL3 facilities at the Comparative Medicine department (KM-F) at Karolinska Institutet. Mice (24 male, 20 female) were housed in individually ventilated cages, had access to food and water *ad libitum*, and cage enrichment included shredded cardboard and paper rolls. Cage and water changes were performed on a weekly basis and general monitoring of all mice was performed daily by technical staff.

Cell lines

HEK293T and HEK293T-hACE2 (Human, female) cells were cultured in a humidified 37°C 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.

Calu-3 cells (Human, male), a lung-derived adenocarcinoma cell line, were obtained from Jonas Klingstrom (Karolinska Institutet). Calu-3 cells were maintained in Dulbecco's Modified Eagle Medium supplemented with Ham's F-12 (Thermo Fisher Scientific), 2% Fetal Bovine Serum and 1% Penicillin/Streptomycin in a humidified 37°C incubator (5% CO₂) and were passaged using 0.5X Trypsin-EDTA.

Vero E6 cells (ATCC-CRL-1586, African Green Monkey) were maintained in DMEM (GIBCO) supplemented with 2% fetal calf serum and 1% penicillin-streptomycin in a humidified incubator with 5% CO₂ at 37°C.

METHOD DETAILS

Immunizations

501Y.V2 RBD (encoding amino acid mutations K417N, E484K, and N501Y, and a C-terminal His-tag) was synthesized (Integrated DNA Technologies), and cloned into a mammalian expression vector (pcDNA3.1), using a Gibson Assembly Mastermix (New England Biolabs). Spike ectodomain (prefusion stabilized with 6 prolines³⁶) and RBD were produced by the transient transfection of Freestyle 293-F cells using FreeStyle MAX reagent (Thermo Fisher) or polyethylenimine (PEI), respectively. 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.

Macaques were immunized intramuscularly (i.m.) with half of each dose administered in each quadriceps. 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°C for 60 min.

Pseudotyped virus neutralization assays

All plasma and serum samples were heat inactivated at 56°C for 60 min. Pseudotyped lentiviruses displaying spikes (with C-terminal truncations) from the SARS-CoV-2 pandemic founder variant (Wu-Hu-1)³⁷ or from variants of concern³⁸⁻⁴⁰ and packaging a firefly 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 h after transfection, and pseudotyped viruses were harvested at 48- and 72-h post-transfection, clarified by centrifugation, and stored at -80°C 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°C in a 96-well plate, and then ~15,000 HEK293T-hACE2 cells were added to each well. Plates were incubated at 37°C for 48 h, and luminescence was then measured using Bright-Glo (Promega) per the manufacturer's protocol, on a GM-2000 luminometer (Promega).

Virus production and quantification

Viral isolates expanded from clinical samples of 'wild-type' and 501Y.V2³⁴ were kind gifts from Jonas Klingström (Karolinska Institutet) and Alex Sigal (The African Health Research Institute) respectively. Isolates were propagated in Calu-3 cells for 72 h and harvested from the supernatant to generate replication-competent SARS-CoV-2 stocks for animal challenge. All virus challenge stocks harbored no cell-culture adaptation mutations detectable by Sanger sequencing of the full-length spike gene (see [Figure S4](#)). Viral titers were quantified by a plaque-forming assay in Vero E6 cells, as previously described⁴¹.

Viral RNA was quantified from clarified viral supernatant in TRIzol (ThermoFisher Scientific) and extracted using the manufacturer's protocol but with the following modifications: Total RNA was precipitated with isopropanol in the presence of Glycoblue (Thermo Fisher Scientific) coprecipitant for 45 min at -20°C, and RNA pellets were resuspended in warm RNase-free water.

RT-qPCR reactions were performed using the Superscript III one step RT-qPCR system with Platinum Taq Polymerase (Invitrogen) with 400 nM of each primer and 200 nM of probe. Primers and probes for the CoV-E gene target were as previously described⁴². Thermal cycling consisted of RT at 55°C for 10 min, denaturation at 95°C for 3 min, and 45 cycles of 95°C for 15 s and 58°C for 30 s. Reactions were carried out using a CFX96 Connect Real-Time PCR Detection System (Bio-Rad) following manufacturer instructions. To generate standard curves, a synthetic DNA template gBlock (Integrated DNA Technologies) was transcribed using the mMessage mMachine T7 Transcription Kit (Invitrogen) and serially diluted. For each viral stock, RT-qPCR was run on a 10-fold dilution series ranging from 1:10 to 1:10,000 to ensure linearity of the assay and avoid saturation at high copy numbers.

K18-hACE2 mice challenge

One day prior to challenge, K18-hACE2 mice were passively immunized with 200 µL of macaque plasma, administered intraperitoneally (i.p.) under isoflurane sedation. Each plasma sample was administered to four mice (two to be challenged with wild-type virus, and two to be challenged with 501Y.V2). The following day, mice were bled from the tail vein (to obtain serum for the characterization of neutralizing antibody titers), moved to a BSL3 facility and challenged intranasally with a standardized dose (2.4x10⁶ RNA copies) of either 'wild-type' or 501Y.V2 virus stock in a total challenge volume of 40 µL in PBS. All challenges were performed under

light isoflurane sedation. Weight and general body condition was monitored daily until weight loss was evident, after which mice were monitored twice daily. Throughout the experiment, weight loss, changes in general health, breathing, body movement and posture, piloerection and eye health were monitored. Mice were euthanized when they experienced weight loss of at least 20% of their starting body weight, or when movement was greatly impaired and/or they experienced difficulty breathing that was considered to reach a severity level of 0.5 on Karolinska Institutet's veterinary plan for monitoring animal health.

QUANTIFICATION AND STATISTICAL ANALYSIS

Non-linear Multinomial Regression for VOC frequency estimation

For plotting maps in [Figure 1](#), SARS-CoV-2 lineage metadata was obtained from GISAID (gisaid.org - 2021-10-09 metadata release), comprising 4,197,215 genomes. Pango lineages without WHO labels were collapsed up the Pango lineage tree until a minimum of 50,000 observations for each lineage was obtained, and daily counts of genomes were aggregated at the country level. We used a multinomial regression to infer the frequencies at three selected dates, weighting the observations around the target dates using a radial basis function. Note: the lineage frequencies vary over time, but the relative advantage of one variant over another is assumed by the model to be locally constant around the target date. Inference was performed with the GLMNet.jl Julia package, using an $\alpha = 0.5$, and maps were plotted with the Cartopy python package (<https://github.com/SciTools/cartopy>). Jupyter notebooks implementing this are available at <https://github.com/MurrellGroup/VOCfreq>.

ID₅₀ titers

Neutralizing antibody ID₅₀ titers were calculated in Prism 9 (GraphPad Software) by fitting a four-parameter logistic curve bounded between 0 and 100, and interpolating the reciprocal plasma/serum dilution where RLUs were reduced by 50% relative to control wells (N = 8) in the absence of plasma.

Histology

Pulmonary pathology in blinded H&E-stained lung sections (N = 89 slides in total, with a mean of 3.3 slides per mouse (range: 2-5) was scored according to a four-point scale, as described previously⁴³. Briefly, normal architecture with thin alveolar septa and distinct bronchioles is scored as 0. Sections with thickened alveolar septa and reduced alveolar space are scored as 1. Thickening of the alveolar septa with reduced alveolar space and invasion of inflammatory cells is scored as 2. Sections displaying widespread loss of tissue architecture are scored as 3. The score for each animal represents the mean of the pathology scores for all sections from that animal. Representative images from each group are shown in [Figure S5](#).

Statistical Analysis

All statistical analyses were performed in Prism 9 (Graphpad Software) unless otherwise stated. Differences in day 6 weights between groups were assessed using Mann-Whitney tests. Reduction in pathology scores was assessed with a one-tailed Mann-Whitney test. Depicted symbols summarize the following: ns = $p > 0.05$ (not significant), * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. The association between neutralizing antibody titers and weight loss was assessed with a Spearman's rank correlation.

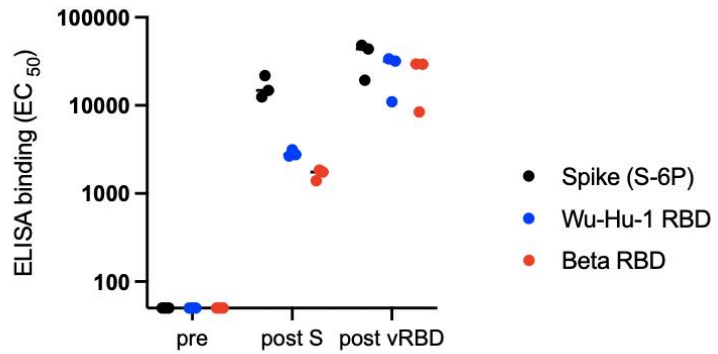
Cell Reports Medicine, Volume 2

Supplemental information

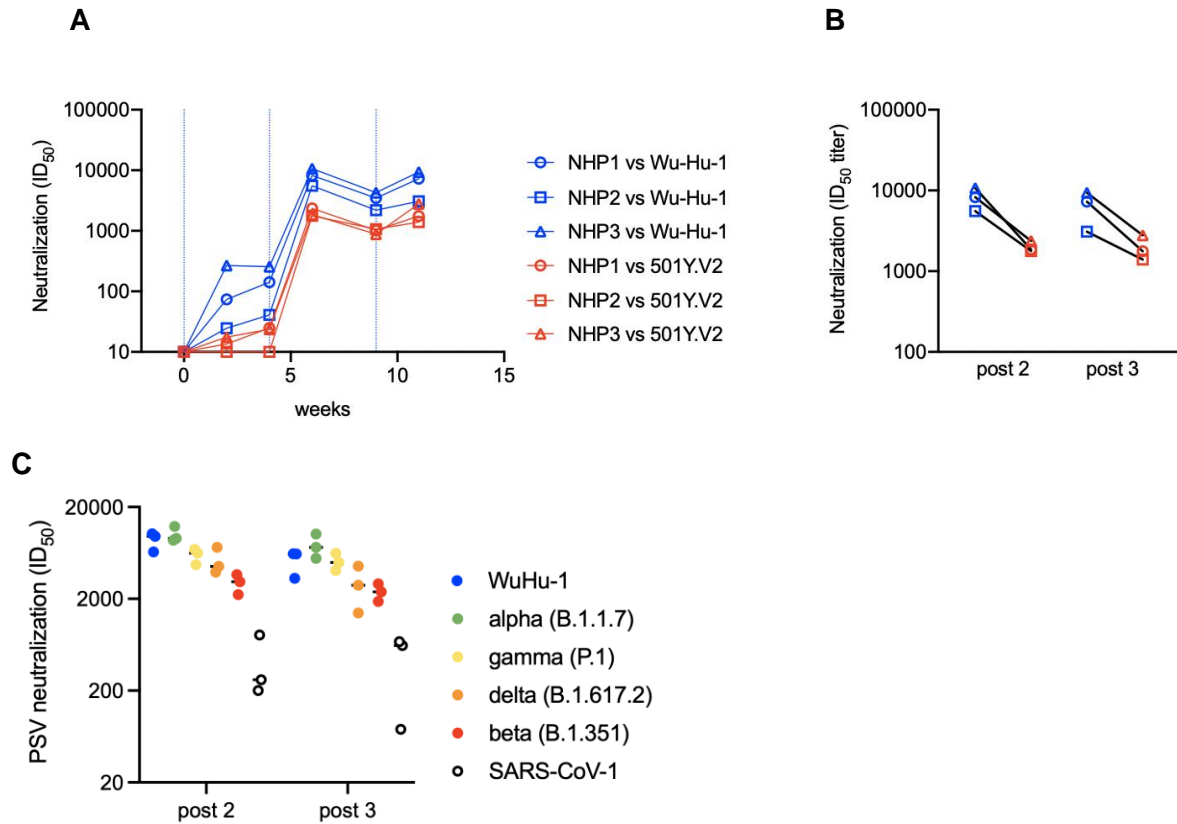
**Beta RBD boost broadens
antibody-mediated protection against
SARS-CoV-2 variants in animal models**

Daniel J. Sheward, Marco Mandolesi, Egon Urgard, Changil Kim, Leo Hanke, Laura Perez Vidakovics, Alec Pankow, Natalie L. Smith, Xaquín Castro Dopico, Gerald M. McInerney, Jonathan M. Coquet, Gunilla B. Karlsson Hedestam, and Ben Murrell

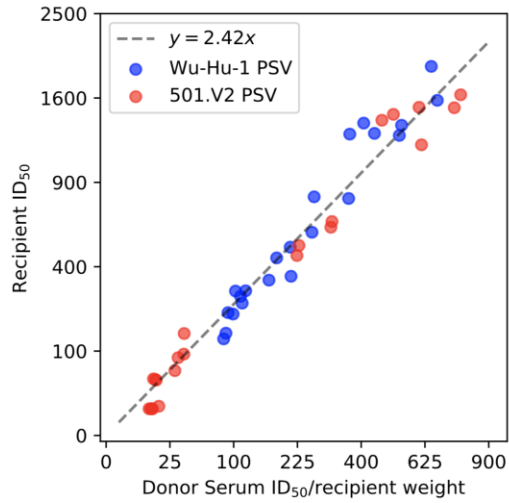
Supplementary Figures



Supplementary figure 1. ELISA spike and RBD binding titers. Binding antibody responses by ELISA are shown for samples prior to immunization (pre) and 2 weeks following the second spike immunization (post S) or variant RBD boost (post vRBD). Binding was assessed against Spike ectodomain with 6 Proline mutations stabilizing the pre-fusion conformation (S-6P, black), as well as against RBD matching Wu-Hu-1 (blue) and the Beta VOC (red). Related to Figure 2.



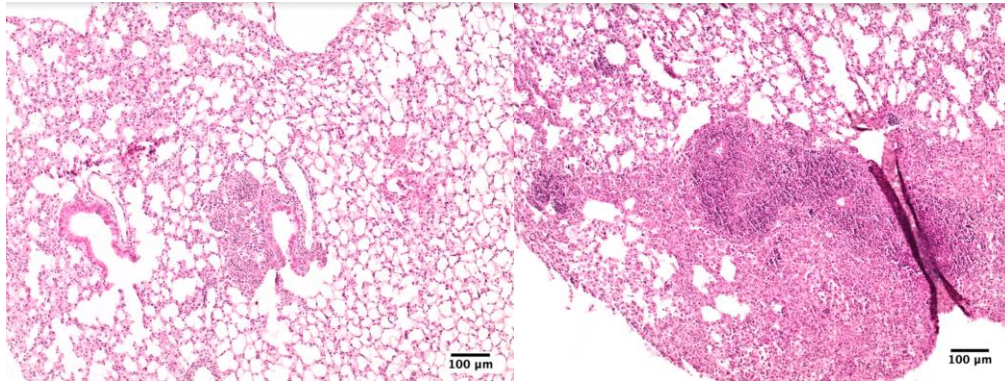
Supplementary figure 2. (a) Longitudinal neutralizing antibody responses against Wu-Hu-1 (blue) and 501Y.V2 (red) for plasma samples from ²¹, where three rhesus macaques (NHP1-NHP3) were immunized with three doses of Wu-Hu-1 spike (100 µg) in Matrix-M™ adjuvant. Vertical blue lines indicate the timing of immunizations (at 0, 4, and 9 weeks). (b-c) Comparison of the neutralizing antibody titers at 6 weeks (post 2) and 11 weeks (post 3) illustrating that reduced titers to beta (red) and other VoCs (c) compared to Wu-Hu-1 (blue) persisted after a third homotypic spike boost. Related to Figure 2.



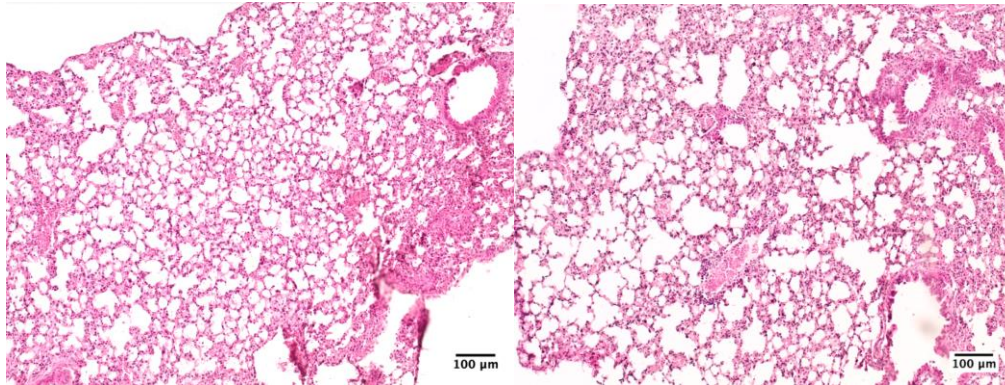
Supplementary figure 3: Relationship between donor and recipient titers following passive immunization. Mice were passively immunized with 200 ul of plasma, resulting in titers approximately 10-fold lower than in the donor. Correcting for weight, Spearman's $r = 0.98$ (after a variance-stabilizing square-root transform). Related to Figure 3.

WT challenge

Beta challenge



post S



post vRBD

Supplementary figure 5. Lung histopathology in surviving mice. Representative images of Hematoxylin and Eosin stained lung sections from surviving mice at day 14, illustrating typical histopathology in each group. Scale bar represents 100 μm. Related to Figure 3f.