

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplemental Appendix

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Cohort Description and Methods

Cohort

Plasma samples were obtained from hospitalized COVID-19 patients (n=89) with moderate disease admitted to Groote Schuur Hospital cohort, Cape Town from 30 December 2020 – 15 January 2021 during the second wave in South Africa. All patients were aged ≥ 18 years and were HIV negative. This study received ethics approval from the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town (R021/2020). All patients had polymerase chain reaction (PCR) confirmed SARS-CoV-2 infection a median of 7 days (IQR 3-11) before blood collection. Clinical folders were consulted for 87/89 participants and none showed evidence of prior symptomatic COVID-19 disease.

We also used data from a previously described cohort infected with the original D614G variant during the first wave, for comparison¹. The Pretoria COVID study cohort consisted of adults (age > 18 years) who had PCR-confirmed SARS-CoV-2 infections, with moderate to severe COVID-19, and were admitted to Steve Biko Academic Hospital (Pretoria, South Africa) from April to September 2020. Blood samples were collected at admission and a second follow-up visit, or at discharge from hospital (whichever was sooner). Admission occurred at a median of 3 days (IQR:2-5) after PCR testing, and follow-up samples were taken a median of 10 days (IQR:9-13) after testing. Ethics approval was received from the University of Pretoria, Human Research Ethics Committee (Medical) (247/2020).

SARS-CoV-2 spike genome sequencing

Sequencing of the spike was performed using swabs obtained from 28 randomly collected Groote Schuur Hospital patients. Swabs were stored as follows: from mid-December 2020 onwards, all SARS-CoV-2 positive swabs collected at the Groote Schuur Hospital from the wards or testing center were stored. For patients at Groote Schuur who had swabs taken elsewhere, a maximum of 500 swabs per week were stored. If the total number of swabs exceeded 500 per week, a regional random proportional selection algorithm was applied due to storage restrictions. A CT cutoff of less than 30 was used as these would likely be successfully sequenced. Swabs from the other participants were not stored. RNA sequencing was performed as previously published². Briefly, RNA extracted participants was used to synthesize cDNA using the Superscript IV First Strand synthesis system (Life Technologies, Carlsbad, CA) and random hexamer primers. SARS-CoV-2 whole genome amplification was performed by multiplex PCR using primers designed on Primal Scheme (<http://primal.zibraproject.org/>) to generate 400 bp amplicons with a 70 bp overlap covering

the SARS-CoV-2 genome. PCR products were cleaned up using AMPure XP magnetic beads (Beckman Coulter, CA) and quantified using the Qubit dsDNA High Sensitivity assay on the Qubit 3.0 instrument (Life Technologies Carlsbad, CA). The Illumina[®] DNA Prep kit was used to prepare indexed paired end libraries of genomic DNA. Sequencing libraries were normalized to 4 nM, pooled and denatured with 0.2 N sodium hydroxide. Libraries were sequenced on the Illumina MiSeq instrument (Illumina, San Diego, CA, USA). Paired-end fastq reads were assembled using Genome Detective 1.132

(<https://www.genomedetective.com>) and the Coronavirus Typing Tool³. The initial assembly obtained from Genome Detective was polished by aligning mapped reads to the references and filtering out low-quality mutations using bcftools 1.7-2 mpileup method. Mutations were confirmed visually with bam files using Geneious software (Biomatters Ltd, New Zealand).

Clade and Lineage classification and phylogenetic analysis

To assign the sequenced samples to their lineage and clade, we used the dynamic lineage classification method proposed by Rambault et al. via the Phylogenetic Assignment of named Global Outbreak LINEages (PANGOLIN) software suite (<https://github.com/hCoV-2019/pangolin>)⁴, and Nextclade⁵, respectively. We confirmed clade classification by analyzing our sequenced genomes against a global reference dataset using a custom pipeline based on a local version of NextStrain (<https://github.com/nextstrain/ncov>)⁵. The pipeline contains several python scripts that manage the analysis workflow. It performs alignment of genomes in MAFFT⁶, phylogenetic tree inference in IQ-Tree V1.6.9⁷, tree dating and ancestral state construction and annotation (<https://github.com/nextstrain/ncov>). The phylogenetic trees were visualized using ggplot and ggtree^{8,9}.

Expression and purification of SARS-CoV-2 antigens

SARS-CoV-2 spike and receptor binding domain + subdomain 1 proteins were expressed in Human Embryonic Kidney (HEK) 293F suspension cells by transfecting the cells with SARS CoV-2 plasmid DNA. After incubating for six days at 37 °C, 70% humidity and 10% CO₂, proteins were first purified using a nickel resin followed by size-exclusion chromatography. Relevant fractions were collected and frozen at -80 °C until use.

Enzyme-linked Immunosorbent Assay (ELISA)

Two µg/ml of full spike or receptor binding domain + subdomain 1 proteins were used to coat 96-well, high-binding plates and incubated overnight at 4 °C. The plates were blocked in blocking buffer consisting of 5% skimmed milk powder, 0.05% Tween 20, 1X PBS. Plasma

samples were diluted to 1:100 starting dilution in blocking buffer and added to the plates. Secondary antibody was diluted to 1:3000 in blocking buffer and added to the plates followed by 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Thermofisher Scientific). Upon stopping the reaction with 1M H₂SO₄, absorbance was measured at a 450nm wavelength. In all instances, mAbs CR3022 or palivizumab were used as positive and negative controls, respectively.

SARS-CoV-2 pseudovirus based neutralization assay

SARS-CoV-2 pseudotyped lentiviruses were prepared by co-transfecting the HEK 293T cell line with either the SARS-CoV-2 614G spike (D614G), SARS-CoV2 501Y.V2-receptor binding domain spike (K417N, E484K and N501Y, D614G) or SARS-CoV-2 501Y.V2 spike (L18F, D80A, D215G, K417N, E484K, N501Y, D614G, A701V, 242-244 del) and the SARS-CoV-2 501Y.V3 spike (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F) plasmids in conjunction with a firefly luciferase encoding lentivirus backbone plasmid and a murine leukemia virus backbone plasmid. The parental plasmids were kindly provided by Drs Elise Landais and Devin Sok (IAVI). For the neutralization assay, heat-inactivated plasma samples from COVID-19 convalescent donors were incubated with the SARS-CoV-2 pseudotyped virus for 1 hour at 37°C, 5% CO₂. Subsequently, 1x10⁴ HEK293T cells engineered to over-express ACE-2, kindly provided by Dr Michael Farzan (Scripps Research), were added and incubated at 37°C, 5% CO₂ for 72 hours upon which the luminescence of the luciferase gene was measured. CB6, CA1, CR3022 and palivizumab were used as controls.

Figures and Tables

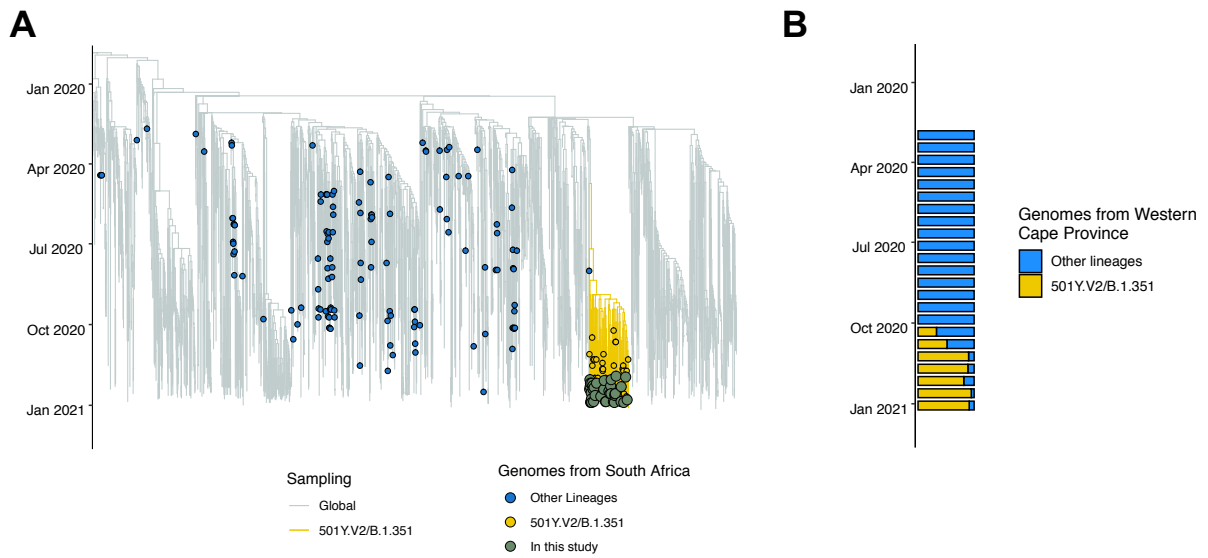


Figure S1. SARS-CoV-2 genome sequencing confirms dominance of infection by the 501Y.V2 variant in Western Cape, South Africa. Genomic sequencing was conducted in the Western Cape Province of South Africa (the same province where our GSH cohort is based) on an approximately weekly basis from the second week of March 2020 to the second week of January 2021. Swabs from 28 individuals from the GSH cohort were sequenced to confirm the infecting variant. **(A)** A time-resolved maximum clade credibility phylogeny of 2621 SARS-CoV-2 sequences, 209 of which are from South Africa and denoted with tip points. The 501Y.V2 (B.1.351) cluster is highlighted in yellow, and the genomes from this study are shown in green, all falling in that cluster. One genome, although assigned to the 501Y.V2/B.1.351 lineage by mutations, was excluded from the phylogenetic tree because of low coverage. **(B)** The bar plot shows the progressive proportion of the 501Y.V2/B.1.351 lineage in the Western Cape.

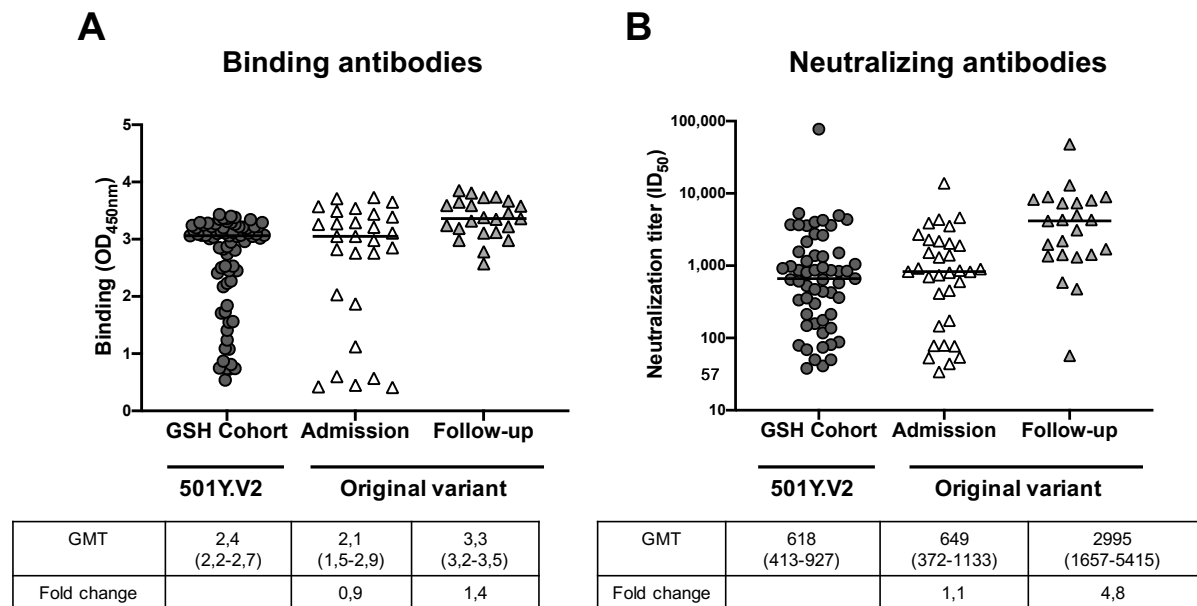


Figure S2. 501Y.V2 elicits robust binding and neutralizing antibody responses. Plasma samples from hospitalized individuals infected with either the 501Y.V2 variant (n=89) or the original variant (n=62) were tested for **(A)** Binding (OD_{450nm}) to sequence-matched SARS-CoV-2 spike antigen and **(B)** neutralizing activity (ID_{50}) against sequence-matched SARS-CoV-2 pseudovirus. Binding was assessed at a single dilution of 1:100, and neutralization titers from a starting dilution of 1:20. For both binding and neutralization, only individuals with binding or neutralizing responses are shown. For the 501Y.V2 cohort, binding data are shown for 75 patients, and neutralization data for 57. For the original variant cohort, 28 samples were measured at admission and 23 at follow-up for binding responses; 33 at admission and 23 at follow-up for neutralization assays. Samples were scored as positive when binding was greater than an $OD_{450nm} > 0.4$ and the threshold of detection for the neutralization assay is $ID_{50} > 20$. All experiments were performed in duplicate and the average value is shown. The median for each dataset is indicated. Geometric mean titers (GMT) (95% confidence interval) were calculated for each group and the fold change relative to the titers of the Groote Schuur Cohort are shown.

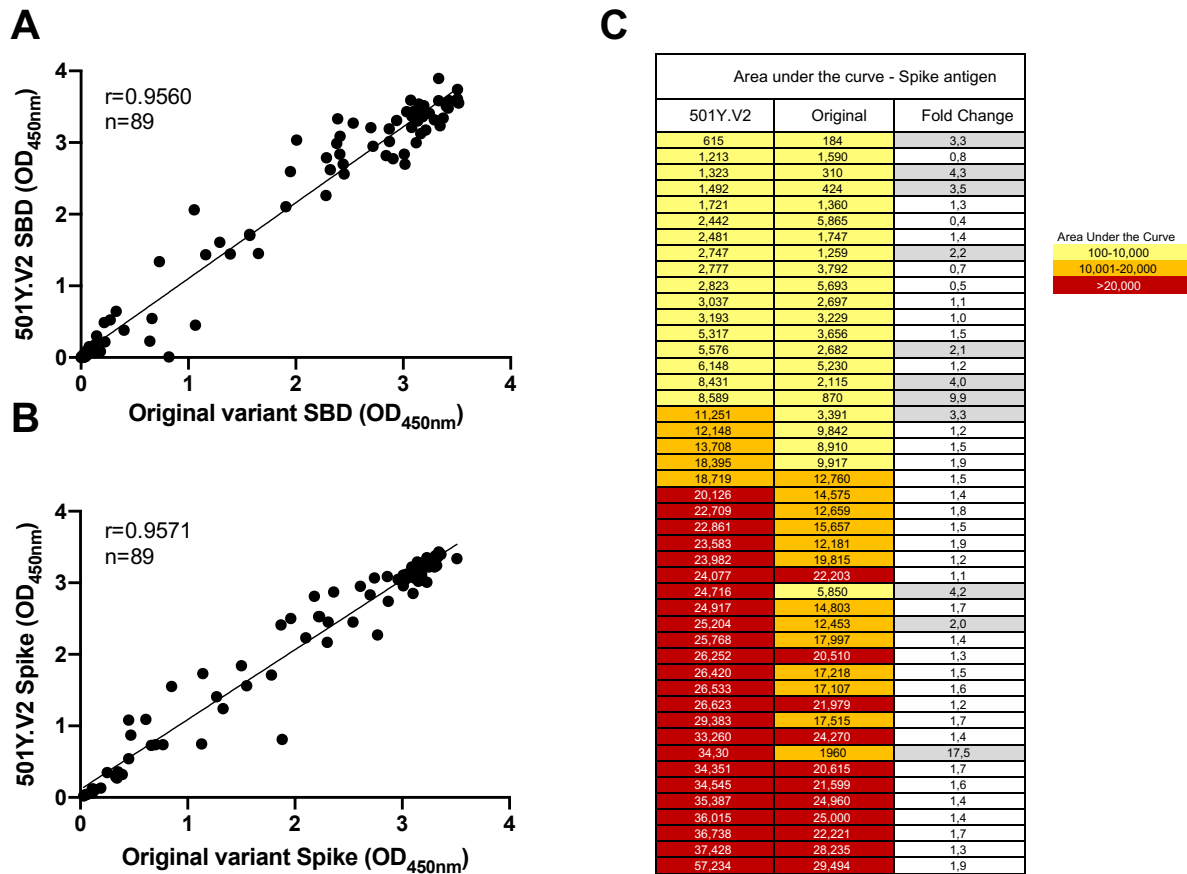


Figure S3. Plasma binding antibodies in 501Y.V2 infected individuals are cross-reactive. Plasma samples from 89 GSH patients were tested for binding to (A) receptor binding domain + S1 domain (SBD) (original variant and 501Y.V2) and (B) full spike (original variant and 501Y.V2). Binding in (A) and (B) was assessed at a single plasma dilution of 1:100. (C) Plasma samples from a subset of $n=46$ individuals were titrated out in full against the 501Y.V2 spike (column 1) and the spike from the original variant (column 2). Data are plotted as area under the curve, ranked by titers against 501Y.V2, and colored according to magnitude. The fold differences between binding to each of the two lineages is shown in column 3, with grey shading denoting fold changes ≥ 2 . All experiments were performed in duplicate and the average value is shown.

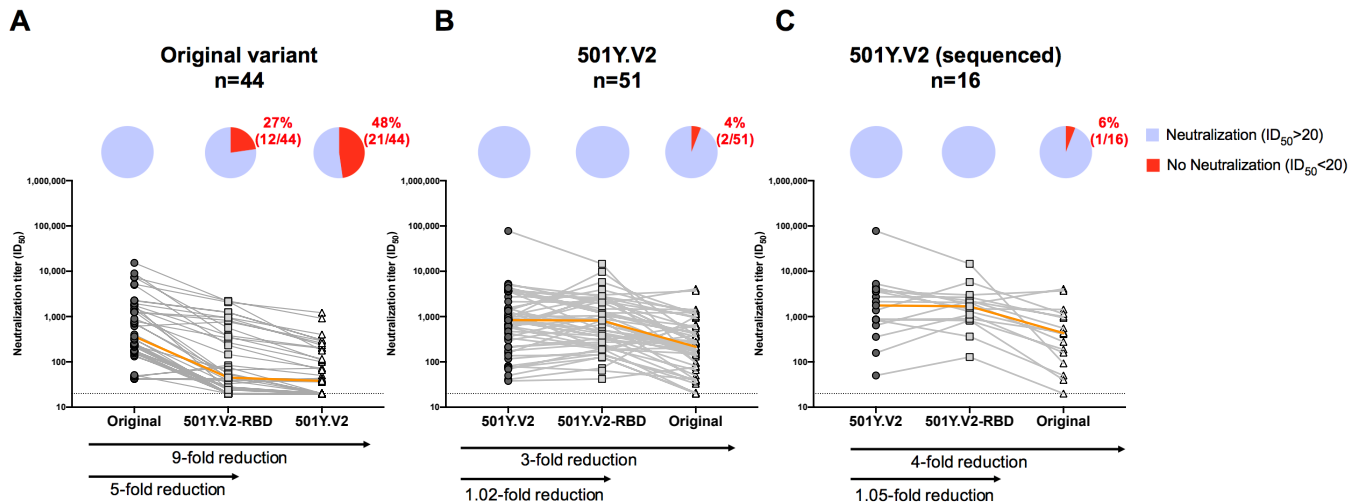


Figure S4. Antibody cross-reactivity against the full 501Y.V2, 501Y.V2-RBD and the original variant in plasma from patients infected with 501Y.V2. (A) Plasma samples from patients infected with the original variant and (B-C) 501Y.V2-infected GSH cohort samples were compared for their neutralization cross-reactivity against 501Y.V2, 501Y.V2-RBD and D614G (n=51). In (C), the analysis was limited to those samples where sequencing confirmed infection by 501Y.V2 (n=16). The construct that contains only the three receptor binding domain changes in 501Y.V2 (K417N, E484K, N501Y) showed similar titers to the full 501Y.V2 (GMT:669 (462-969) compared to 501Y.V2: GMT:686 (448-1052), for this subset of n=51). The orange line indicates the slope between the median neutralization potency of the samples tested. Two plasma samples showed the opposite trend, with higher neutralization of the original variant (>5 fold-higher), compared to 501Y.V2. We do not have sequencing data for these samples but it is likely that these individuals were infected with the original variant and not 501Y.V2. In the pie charts, purple indicates the proportion of samples with neutralization activity and red the proportion of samples with no detectable neutralization activity. The threshold of detection for the neutralization assay is $ID_{50} > 20$. All experiments were performed in duplicate and the average value is shown. Data for the original virus plasma was taken from *Wibmer et al., 2021, Nature Medicine*.

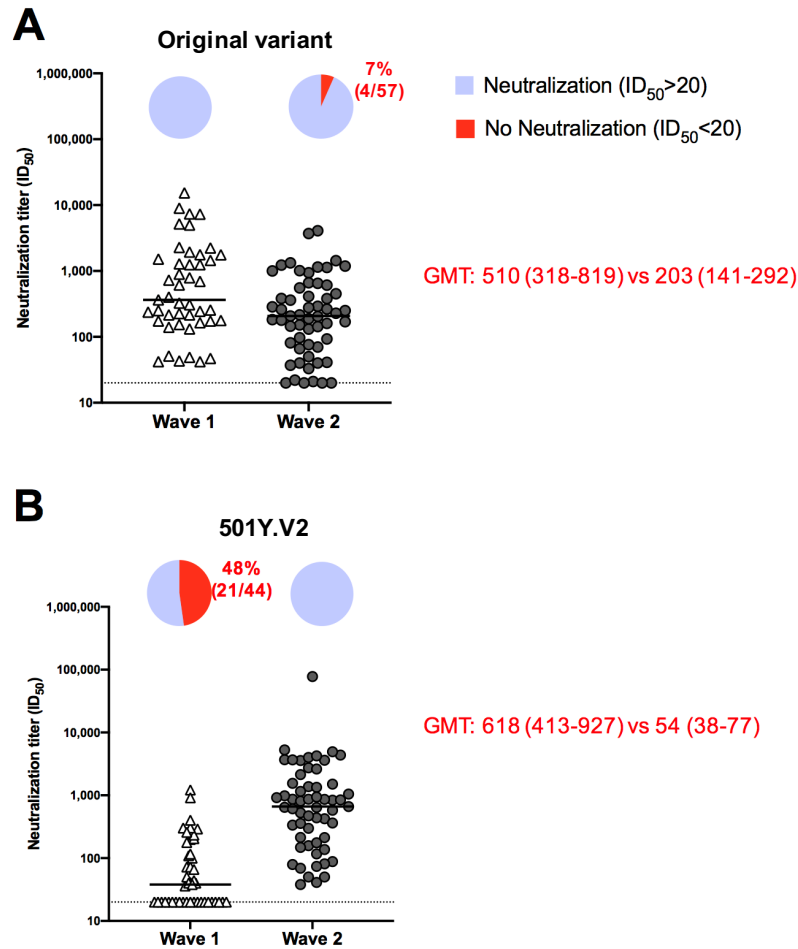


Figure S5. Plasma elicited by 501Y.V2 infection (Wave 2) is more cross-reactive than plasma from the original virus infection (Wave 1). Plasma from patients infected with 501Y.V2 or the original variant were tested against (A) the original virus or (B) the 501Y.V2 variant. We compared titers from wave 1, where infection was by the original variant (May to September 2020) and the wave 2 Groote Schuur Hospital plasma (501Y.V2 infection, December 2020 to January 2021) against both the original variant (Figure S3A) and 501Y.V2 (Figure S3B). The original variant was neutralized by both wave 1 and wave 2 sera (GMT: 510 and 203, respectively). However, the 501Y.V2 variant was neutralized 11-fold better by the wave 2 sera (GMT: 618) than wave 1 sera (GMT: 54) which was obtained from both hospitalized and non-hospitalized individuals (Figure S3A, B). 95% confidence intervals for each geometric mean are shown in brackets. In the pie charts, purple indicates the proportion of samples with neutralization activity and red the proportion of samples with no detectable neutralization activity. The threshold of detection for the neutralization assay is $ID_{50} > 20$. All experiments were performed in duplicate and the average value is shown. Data for the original virus plasma was taken from *Wibmer et al., 2021, Nature Medicine*.

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Author Contributions

T.M-G. performed and designed experiments, analyzed data and wrote the manuscript. M.M performed neutralization experiments and analyzed data. Z.M and F.A performed ELISA binding experiments. D.M, B.O and B.E.L made molecular constructs. P.K performed neutralization assays. H.T, T.dO, A.I, D.D, L.T and C.W sequenced the viral genomes. L.R.C, M.M, S.S and G.M aided in sample collection, preparation and cohort organization. C.K.W and J.N.B designed molecular constructs and provided intellectual input to the study design. J.N.B, C.W and T.d.O identified the 501Y.V2 lineage. T.R, M.B and V.U provided plasma samples for the study. W.A.B and N.N provided samples for the study and clinical data on the participants. L.M aided in the establishment of the pseudovirus assay and intellectual input to the study design. P.L.M conceptualized the study, designed experiments and wrote the manuscript.

Conflicts of Interest

None of the authors declare any conflicts of interest

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