

Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma

Sandile Cele^{1,2}, Inbal Gazy^{2,3,4}, Laurelle Jackson¹, Shi-Hsia Hwa^{1,5}, Houriiyah Tegally³, Gila Lustig⁶, Jennifer Giandhari³, Sureshnee Pillay³, Eduan Wilkinson³, Yeshnee Naidoo³, Farina Karim^{1,2}, Yashica Ganga¹, Khadija Khan¹, Mallory Bernstein¹, Alejandro B. Balazs⁷, Bernadett I. Gosnell⁸, Willem Hanekom^{1,5}, Mahomed-Yunus S. Moosa⁸, NGS-SA[§], COMMIT-KZN Team^{§§}, Richard J. Lessells^{2,3,6}, Tulio de Oliveira^{2,3,6,9*}, Alex Sigal^{1,2,10*}

¹Africa Health Research Institute, Durban 4001, South Africa. ²School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban 4001, South Africa. ³KwaZulu-Natal Research Innovation and Sequencing Platform, Durban 4001, South Africa. ⁴Department of Biochemistry and Molecular Biology, The Institute for Medical Research Israel-Canada, Hadassah Medical School, The Hebrew University of Jerusalem, 91120, Jerusalem, Israel. ⁵Division of Infection and Immunity, University College London, London WC1E 6BT, UK. ⁶Centre for the AIDS Programme of Research in South Africa, Durban 4001, South Africa. ⁷Ragon Institute of MGH, Harvard, and MIT, Cambridge, USA. ⁸Department of Infectious Diseases, Nelson R. Mandela School of Clinical Medicine, University of KwaZulu-Natal, Durban 4001, South Africa. ⁹Department of Global Health, University of Washington, Seattle, USA. ¹⁰Max Planck Institute for Infection Biology, Berlin 10117, Germany.

* Corresponding authors. Email: deoliveira@ukzn.ac.za, alex.sigal@ahri.org

1 Abstract

2 SARS-CoV-2 variants of concern (VOC) have arisen independently at multiple locations
3 and may reduce efficacy of current vaccines targeted at the spike glycoprotein. We re-
4 cently described the emergence of VOC in South Africa (501Y.V2 or PANGO lineage
5 B.1.351) with mutations in the spike receptor-binding domain (RBD) and N-terminal do-
6 main (NTD). Here, using a live virus neutralization assay (LVNA), we compared neutral-
7 ization of a first wave virus (B.1.1.117) versus the 501Y.V2 variant using plasma collected
8 from adults hospitalized with COVID-19 from two South African infection waves, with the
9 second wave dominated by 501Y.V2 infections. Sequencing demonstrated that infections in
10 first wave plasma donors were with viruses harbouring none of the 501Y.V2-defining RBD
11 or NTD mutations, except for one with E484K. 501Y.V2 virus was effectively neutralized
12 by plasma from second wave infections and first wave virus was effectively neutralized
13 by first wave plasma. In cross-neutralization, 501Y.V2 virus was poorly neutralized by
14 first wave plasma, with an 8.4-fold drop in neutralization relative to first wave virus and
15 a 15.1-fold drop relative to 501Y.V2 neutralization by second wave plasma. In contrast,
16 second wave plasma neutralization of first wave virus was more effective, showing 4.1-fold
17 decline relative to 501Y.V2 virus neutralization and 2.3-fold decline relative to first wave
18 plasma neutralization. While we only tested one plasma elicited by E484K alone, this po-
19 tently neutralized both variants. The observed effective neutralization of first wave virus
20 by 501Y.V2 infection elicited plasma provides preliminary evidence that vaccines based
21 on VOC sequences could retain activity against other circulating SARS-CoV-2 lineages.

22 Through genomic surveillance of the severe acute respiratory syndrome-related coronavirus 2 (SARS-
23 CoV-2), a number of new variants have been identified with multiple mutations in the spike glycoprotein.
24 We recently described the emergence of the 501Y.V2 (B.1.351) variant in South Africa, characterized
25 by the K417N, E484K, and N501Y mutations in the spike receptor binding domain (RBD) as well as
26 four substitutions and a deletion in the N-terminal domain (NTD) [1]. This variant was first detected
27 in October 2020, and has rapidly become the dominant variant in South Africa with a frequency in
28 January 2021 of 97% according to the GISAID (<https://www.gisaid.org/hcov19-mutation-dashboard/>).

29 The RBD is the main target of neutralizing antibodies (NAbs) elicited by SARS-CoV-2 infection,
30 with the remaining activity directed at the NTD [2, 3]. All three amino acid residues in the RBD that
31 carry mutations in 501Y.V2 interact directly with the human angiotensin-converting enzyme 2 (hACE2)
32 receptor and form part of the epitopes for hACE2-blocking NAbs [4]. The E484 residue specifically is a
33 hotspot for binding of highly potent NAbs [4]. In a number of separate *in vitro* studies using monoclonal
34 antibodies (mAbs), mutations at E484 have emerged as immune escape mutations, often conferring broad
35 cross-resistance to panels of mAbs [5, 6, 7, 8]. E484K also emerged during passage with convalescent
36 plasma, leading to substantial drops in neutralization [9, 10]. Using a deep mutation scanning approach
37 to determine the effect of individual mutations on neutralization by polyclonal sera, mutations at E484
38 were associated with the largest drops in neutralization [11].

39 South Africa experienced two SARS-CoV-2 infection waves to date (<https://coronavirus.jhu.edu/map.html>).
40 The first wave peaked in July 2020 and consisted of viral variants which usually showed the D614G mu-
41 tation but none of the defining mutations of 501Y.V2. These variants have been almost completely
42 supplanted by 501Y.V2 variants in the second South African infection wave, peaking January 2021.

43 Coinciding with our initial report, there have been multiple studies showing that 501Y.V2 decreases
44 neutralization capacity of polyclonal antibodies elicited by non-501Y.V2 SARS-CoV-2 infection or vac-
45 cination. The decrease ranges from relatively moderate [12, 13, 14, 15] to severe [16, 17, 18, 19, 20, 21].
46 Importantly, three clinical trials performed in South Africa during the second, 501Y.V2 infection wave
47 reported dramatic decreases in vaccine efficacy. The Novavax NVX-CoV2373 subunit vaccine demon-
48 strated a decrease in efficacy from 89.3% to 49.4% ([https://ir.novavax.com/news-releases/news-release-
49 details/novavax-covid-19-vaccine-demonstrates-893-efficacy-uk-phase-3](https://ir.novavax.com/news-releases/news-release-details/novavax-covid-19-vaccine-demonstrates-893-efficacy-uk-phase-3)). This trial also detected SARS-
50 CoV-2 seroprevalence, and in the placebo arm there was no difference in infection frequency between

51 participants who were seropositive for SARS-CoV-2 relative to those who were negative, indicating that
52 previous infection with first wave, non-501Y.V2 virus does not protect against re-infection with 501Y.V2.
53 The Johnson and Johnson adenovirus vectored single dose vaccine showed a reduced efficacy from 72% in
54 the US to 57% in South Africa. (<https://www.jnj.com/johnson-johnson-announces-single-shot-janssen-covid-19-vaccine-candidate-met-primary-endpoints-in-interim-analysis-of-its-phase-3-ensemble-trial>). Most
55 strikingly, the AstraZeneca ChAdOx1 AZD1222 chimpanzee adenoviral vectored vaccine showed only
56 10% efficacy against 501Y.V2 variants, compared to 75% efficacy against earlier variants circulating in
57 South Africa [22]. The roll-out of this vaccine in South Africa is currently paused.

59 Here, using a live virus neutralization assay (LVNA), we measure the degree to which 501Y.V2
60 virus compromises neutralization elicited by natural infection with non-501Y.V2 variants. In addition,
61 we measure the degree to which the earlier variants could escape the neutralizing response elicited to
62 501Y.V2 virus (Figure 1A). The possible relevance to vaccination is that an effective vaccine should be
63 broadly protective against multiple variants, and this may depend on choice of variant sequence used in
64 the design.

65 We used plasma samples from our ongoing longitudinal cohort that tracks COVID-19 cases en-
66 rolled at hospitals in Durban, South Africa [23]. We sampled participants weekly for the first month
67 post-enrollment. At each timepoint a blood draw and combined nasopharyngeal/oropharyngeal swab
68 was performed to obtain both the plasma and infecting virus. Swabs positive for SARS-CoV-2 were
69 sequenced.

70 We chose plasma from 14 participants from the first South African infection wave where the infecting
71 virus was successfully sequenced (Materials and Methods). Plasma samples were from blood drawn
72 approximately 1 month post-symptom onset (Table S1), close to the antibody response peak [24]. Of
73 the 14 participants, 13 did not show RBD or NTD mutations in the infecting virus. A single participant
74 sampled in October 2020 showed the E484K escape mutation in the absence of the other 501Y.V2
75 mutations (Tables S2-S4). We had fewer participants from the second infection wave at the time of
76 writing as most have not yet reached the 1 month post-symptom onset time-point for sampling. The
77 second wave participants in this study were infected late December or early January 2021 (Figure 1B,
78 Table S1). We were able to sequence three second wave participants where sequence allowed variant
79 calling, two of which had good spike coverage (Figure 1B, Table S4). In all cases, the infecting variant
80 was 501Y.V2. It is extremely likely that 501Y.V2 was also the infecting variant for the rest of the
81 participants from infection wave 2, given the complete dominance of this variant in January 2021. For
82 each second wave participant, our clinical team conducted a telephonic interview and examined clinical
83 records to determine if the participant was also infected in the first South African infection wave. None
84 of the participants showed evidence of being previously infected.

85 We outgrew first wave virus (Materials and Methods) from one participant during the first infection
86 wave, and second wave, 501Y.V2 virus from a sample obtained in November 2020 through our genomic
87 surveillance program (Figure 1B). We used a microneutralization live virus focus forming assay [25]
88 which relies on a methylcellulose overlay to limit cell-free viral spread, resulting in a local infection
89 focus. The focus is detected by an anti-SARS-CoV-2 spike antibody (Materials and Methods). We
90 normalized the number of foci to the number of foci in the absence of plasma on the same plate to
91 obtain the transmission index (Tx, [26]). This controls for experimental variability in the input virus
92 dose between experiments. We mixed the virus with serially diluted plasma, then added the mixture
93 to Vero E6 cells and counted infection foci after 28 hours using automated image analysis (Figure S1A,
94 Figure 2A, Materials and Methods).

95 There was a clear reduction in neutralization capacity of 501Y.V2 by first wave plasma relative to
96 neutralization of the homologous, first wave variant (Figure S1). 501Y.V2 also showed larger foci, likely
97 reflecting a larger number of cells infected by one infected cell, or more rapid infection cycles (Figure
98 2A, Figure S1A). In order to compare foci of similar size we reduced the incubation time of 501Y.V2
99 infection to 18 hours. In order to detect some effect of first wave plasma on the 501Y.V2 variant, we
100 tested more concentrated plasma (Figure 2A,B). To rule out infection saturation effects, we obtained
101 a positive control monoclonal antibody with similar neutralization of first wave and 501Y.V2 variants.
102 We then repeated the experiments (Figures S2-S4 show representative neutralization experiments for
103 each participant plasma).

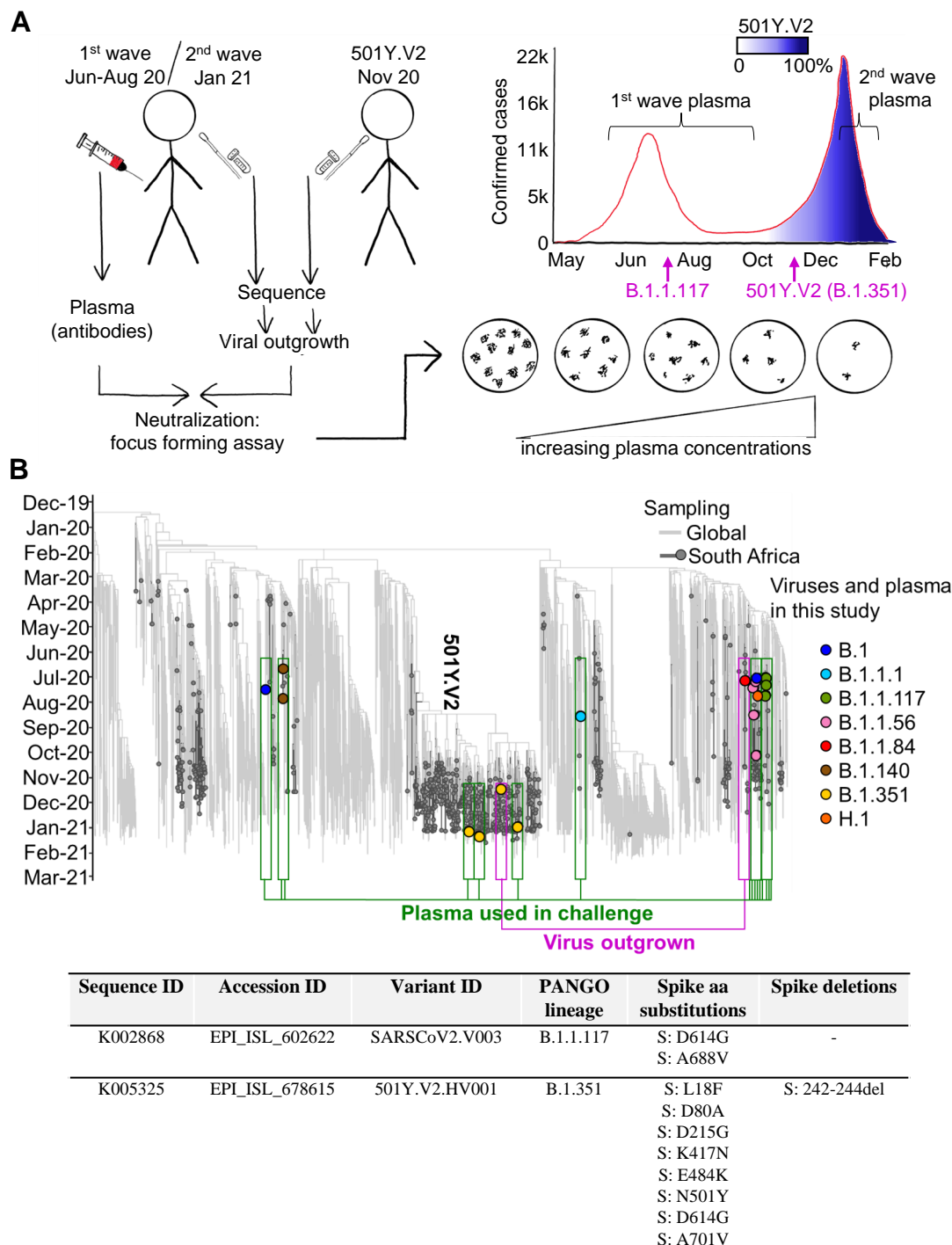


Figure 1: Study design and sequences of SARS-CoV-2 variants. (A) We obtained convalescent plasma and sequenced the matching infecting virus in the first and second SARS-CoV-2 infection waves in South Africa. A first wave variant lacking the RBD and NTD mutations characterizing 501Y.V2 was outgrown from one participant infected in the first South African infection wave, and 501Y.V2 was outgrown from a participant at the beginning of the second wave. Neutralization performed was by i) first wave plasma of first wave virus; ii) second wave plasma of 501Y.V2 virus; iii) first wave plasma of 501Y.V2 virus; iv) second wave plasma of first wave virus. A focus forming microneutralization assay was used to quantify neutralization. (B) Phylogenetic relationships and mutations in virus sequences. Variants which elicited the antibody immunity in the plasma samples are highlighted in green boxes. Variants which were outgrown are highlighted in magenta boxes. Y-axis denotes time of sampling. Table shows mutations present in spike for the SARS-CoV-2 variants used in the LVNA. See Tables S2-S4 for a complete list of mutations in the viral genomes of both the variants used in LVNA, and the sequenced variants eliciting the plasma immunity.

104 We observed the same trend in neutralization capacity as with the first set of experiments: there was
105 a decline in the number of foci when first wave plasma was added to the homologous, first wave virus.
106 This was strongly attenuated with 501Y.V2 (Figure 2B). When second wave, 501Y.V2 elicited plasma
107 was used, it effectively neutralized the homologous, 501Y.V2 variant (Figure 2C). In contrast to the first
108 wave plasma, the neutralization of the heterologous, first wave virus was clearly observed. Some of the
109 foci were smaller at the higher antibody concentrations (Figure 2C, Figures S2-S4), possibly indicative
110 of some reduction in cell-to-cell spread by neutralizing antibodies in the Vero E6 cell line.

111 The data from the focus forming assay at each dilution approximated a normal distribution (Figure
112 S5) and we therefore used parametric statistics to describe it. We fitted the data for each participant to
113 a sigmoidal function [27] with the dilution required to inhibit 50% of infection (ID_{50}) as the only free
114 parameter (Materials and Methods). For clarity, we plotted the data for each neutralization experiment
115 as percent neutralization ($(1 - Tx) \times 100\%$, Materials and Methods, [16]), with neutralization represented
116 by the 50% plaque reduction neutralization titer ($PRNT_{50}$, [15]), the reciprocal of the ID_{50} .

117 The A2051 monoclonal antibody was used as a positive control in each experiment (Figures S2-
118 S4) and showed a similar neutralization response between variants (Figure 2D), indicating that focus
119 number/size was not saturating. We also used a plasma pool from 3 study participants who did not have
120 any indications of SARS-CoV-2 infection, and this plasma pool did not appreciably neutralize either
121 variant (Figure 2D).

122 We then proceeded to quantify neutralization of the homologous virus and cross-neutralization.
123 First wave virus infection was neutralized by first wave plasma, with some variability in neutralization
124 capacity between first wave infected participants. It was also cross-neutralized by second wave, 501Y.V2
125 elicited plasma (Figure 2D). There was overlap between neutralization capacity of first wave and second
126 wave plasma. In contrast, when the 510Y.V2 variant was used as the infecting virus, there was a clear
127 separation between the neutralization capacity of the homologous second wave (Figure 2D) versus the
128 heterologous first wave plasma. While the homologous plasma effectively neutralized 501Y.V2, the
129 cross-neutralization mediated by first wave plasma was weaker, consistent with what is apparent when
130 viewing the raw number of foci (Figure 2B-C, Figures S2-S4). Plasma elicited by the variant with the
131 E484K mutation alone showed much stronger neutralization of both the first wave and 501Y.V2 virus
132 relative any of the other plasma samples (Figure 2D).

133 The $PRNT_{50}$ values showed a strong reduction in first wave plasma neutralization capacity of
134 501Y.V2 virus relative to the homologous first wave virus (Figure 2E). Excluding the plasma elicited by
135 the virus with E484K mutation alone, which showed a very high $PRNT_{50}$ for both variants, first wave
136 plasma $PRNT_{50}$ declined between 3.2 to 41.9-fold with the 501Y.V2 variant. In contrast, the decline in
137 $PRNT_{50}$ in cross-neutralization of first wave virus by second wave plasma was more attenuated. Here,
138 the decline ranged between 1.6 to 7.2-fold relative to the homologous 501Y.V2 virus (Figure 2E).

139 Given the data approximated a normal distribution, we derived the mean neutralization across first
140 wave (excluding the plasma elicited with the E484K only virus) and second wave participants (Figure 3).
141 In both cases, neutralization showed a separation across all dilutions tested between the homologous and
142 heterologous virus, where heterologous neutralization was lower. However, the separation was distinctly
143 less for first wave virus neutralization by first wave versus second wave plasma (Figure 3). To quantify
144 homologous versus heterologous neutralization capacity, we repeated the sigmoidal fit to the participant
145 means and obtained the combined $PRNT_{50}$. For first wave plasma neutralization of the homologous,
146 first wave virus, $PRNT_{50}$ was 344.0 with fit 95% confidence intervals of 275.4-458.0 (Figure 3 summary
147 table, top left blue rectangle). For second wave plasma neutralization of the homologous, 501Y.V2
148 virus (Figure 3 summary table, bottom right blue rectangle), $PRNT_{50}$ was 619.7 (517.8-771.5). Hence,
149 501Y.V2 elicits a robust antibody response in the participants tested. For cross-neutralization, first
150 wave plasma neutralization of the heterologous, 501Y.V2 virus (Figure 3, bottom left yellow rectangle)
151 was strongly attenuated across participants, with $PRNT_{50} = 41.1$. In contrast, second wave plasma
152 neutralization of the heterologous, first wave virus (Figure 3, top right yellow rectangle) was more
153 effective at $PRNT_{50} = 149.7$ (132.1-172.8). 95% confidence intervals did not overlap.

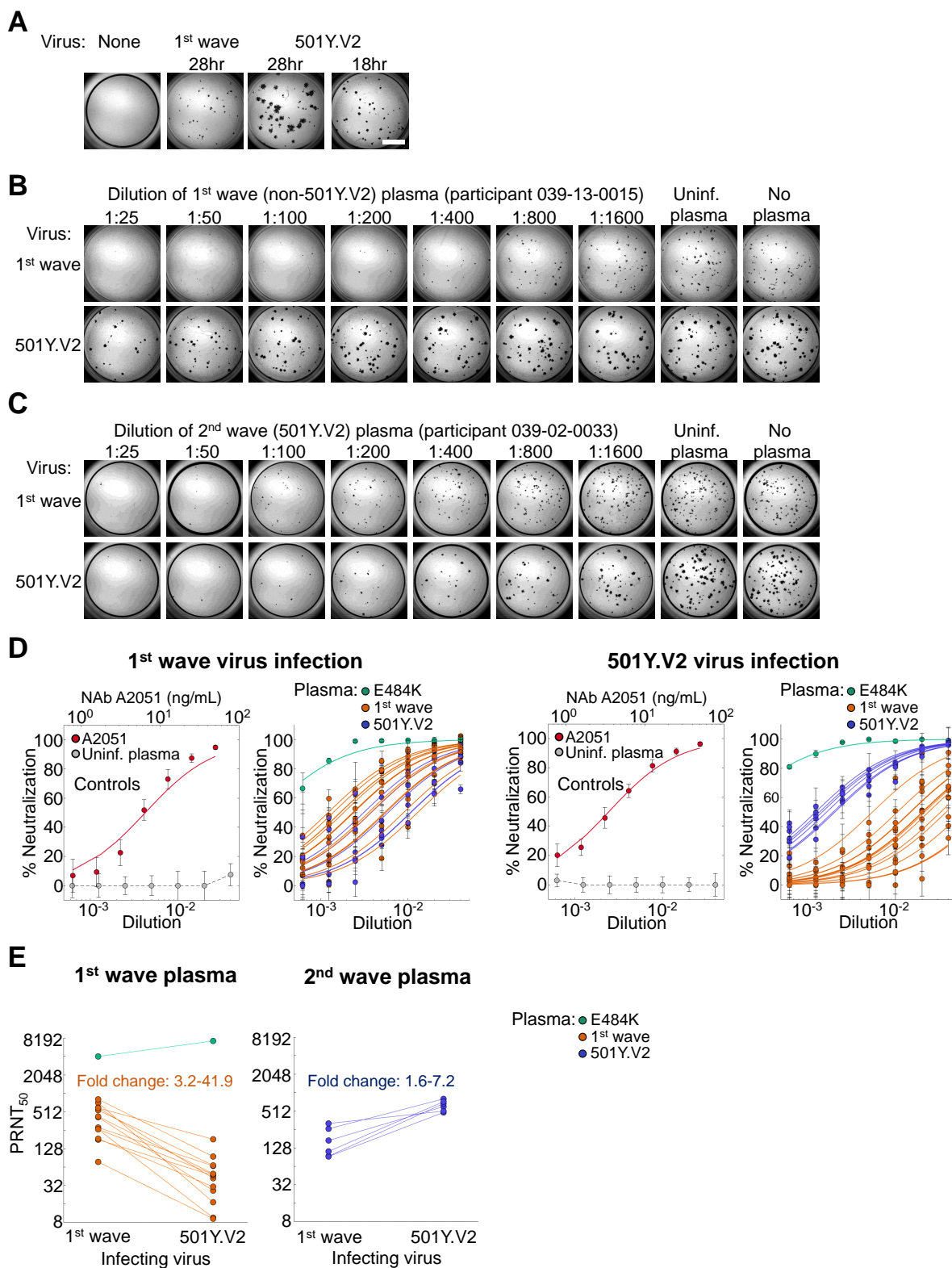


Figure 2: Neutralization of first infection wave and 501Y.V2 variants by convalescent plasma from South African first and second wave infections. (A) Focus formation in the absence of plasma when infection is by a first wave versus the 501Y.V2 viral variant. Incubation time for 501Y.V2 focus formation was reduced to 18 hours to obtain similar focus size. Scale bar is 2mm. (B) A representative focus forming assay using plasma from first wave infected participant 039-13-0015. First row is neutralization of infection by first wave virus, second row is neutralization of 501Y.V2. Columns are plasma dilutions, ranging from 1:25 to 1:1600. Last two columns are plasma from a pool of uninfected participants and the no plasma control. (C) Representative neutralization using plasma from second wave infected participant 039-02-0033. (D) Left two plots represent controls and plasma neutralization when infection is with first wave virus and right two plots when infection is with 501Y.V2. Points are means and standard errors of percent neutralization from 3 or 4 independent experiments for convalescent plasma from first wave (n=14) or second wave (n=6) participants, or 10 independent experiments for the controls. Solid lines of the corresponding colour are fitted values per participant using a fit to a sigmoidal equation. First plot is neutralization of first wave virus by neutralizing antibody A2051. $PRNT_{50} = 6.5$ ng/mL (3.9-9.1 ng/mL). Neutralization by plasma from uninfected participants is represented by the grey points. Second plot is neutralization of first wave virus by plasma from convalescent participants. Groups are first infection wave are (red), second wave (blue), and virus with E484K only (green). Third plot is the control neutralization of the 501Y.V2 variant by A2051. $PRNT_{50} = 3.5$ ng/mL (2.9-4.1 ng/mL). Fourth plot is neutralization of 501Y.V2 virus by plasma from convalescent participants. Groups are first infection wave are (red), second wave (blue), and virus with E484K only (green). (E) Decline in $PRNT_{50}$ in cross-neutralization of heterologous virus. Left plot is first wave plasma neutralization of first wave versus 501Y.V2 virus, and right plot is second wave plasma neutralization of 501Y.V2 versus first wave virus. For first wave plasma fold-change in $PRNT_{50}$ was calculated as $PRNT_{50}$ of first wave divided by $PRNT_{50}$ of 501Y.V2. For second wave plasma, fold change was calculated as $PRNT_{50}$ of 501Y.V2 divided by $PRNT_{50}$ of first wave virus. Fold change ranged from 3.2 to 41.9 for first wave plasma, and 1.6 to 7.2 for second wave plasma. For first wave plasma, $PRNT_{50}$ of plasma elicited by E484K virus was excluded.

154 Fold-change decrease of first wave plasma neutralization of 501Y.V2 compared to homologous virus
155 was 8.4. Fold-change decrease of second wave plasma neutralization of first wave compared to homol-
156 ogous virus was 4.1. However, absolute 501Y.V2 plasma neutralization capacity of first wave virus
157 dropped only 2.3-fold compared to first wave plasma. In contrast, it decreased 15.1-fold when 501Y.V2
158 was cross-neutralized by first wave plasma (Figure 3).

159 The significance of these results is that 501Y.V2 is poorly neutralized by plasma elicited by non-
160 501Y.V2 variants. However, 501Y.V2 infection elicited plasma not only effectively neutralized 501Y.V2,
161 but also cross-neutralized the earlier variant within the observed range of the first wave plasma (Figure
162 2). This cross-neutralization is within the lower part of the neutralization capacity range elicited by the
163 Pfizer BNT162b2 mRNA vaccine [12, 15, 13]. Due to potentially higher immunogenicity of 501Y.V2
164 virus according to the $PRNT_{50}$, the plasma it elicits does not greatly under-perform the plasma elicited
165 by earlier, non-501Y.V2 variants when neutralizing these earlier variants.

166 The larger focus size in 501Y.V2 relative to first wave virus is unlikely to influence these results. We
167 performed 501Y.V2 infections with larger foci using the same infection incubation time as first wave
168 virus, and also 501Y.V2 infections where focus size was similar using a shorter 501Y.V2 incubation
169 time. The results showed similar trends. Furthermore, neutralization by the monoclonal antibody
170 control indicated that the LVNA system could effectively read out neutralization for both variants
171 (Figure 2D, Figure S2). 501Y.V2 variants vary in some of their mutations. The variant we used has an
172 L18F mutation in the NTD which currently occurs in about a quarter of 501Y.V2 variants (GISAID).
173 Other current and future 501Y.V2 variants can be examined to track changes in neutralization and
174 cross-neutralization. An important question in the interpretation of the results is whether the second
175 wave infected participants were also infected in the first infection wave. Our clinical team conducted
176 telephonic interviews and investigated the clinical charts and found no evidence of previous SARS-CoV-2
177 infection. While previous infection may still be missed despite these measures, we it is unlikely to have
178 occurred in all the second wave participants, yet the neutralization response between the participants
179 was very similar (Figure 2). Lastly, while we and others in the field measured plasma neutralization,
180 how well this correlates to protection at the mucosal surface where initial infection takes place is yet
181 unclear.

182 The plasma elicited by virus with the E484K mutation alone showed the strongest neutralization
 183 both of the first wave and the 501Y.V2 virus relative to any of the other plasma samples tested (Figure
 184 2). Because we only found one participant in this category, this result is difficult to interpret: it may be
 185 due to high immunogenicity or because of participant specific factors. Our clinical data does not show
 186 prolonged SARS-CoV-2 shedding in this participant or other unusual features (Table S1). This result
 187 highlights the importance of sequencing the infecting virus, and requires further investigation.

188 The recent Novavax, Johnson and Johnson, and AstraZeneca South African vaccine trial results
 189 indicate that the 501Y.V2 variant may lead to a decrease in vaccine efficacy. The loss of neutralization
 190 capacity in 501Y.V2 infection we quantified among the vaccinated participants in the AstraZeneca trial
 191 [22] shows that loss of neutralization may be associated with loss of vaccine efficacy. Loss of vaccine
 192 efficacy may also be mediated by escape from T cell immunity, although we believe this is less likely
 193 due to the diversity of HLA alleles in the population, which may curtail the ability of an escape variant
 194 which evolved in one individual to escape T cell immunity in another. If loss of vaccine efficacy proves
 195 to require vaccine redesign, the results presented here may be the first indication that a vaccine designed
 196 to target 501Y.V2 may also effectively target other SARS-CoV-2 variants.

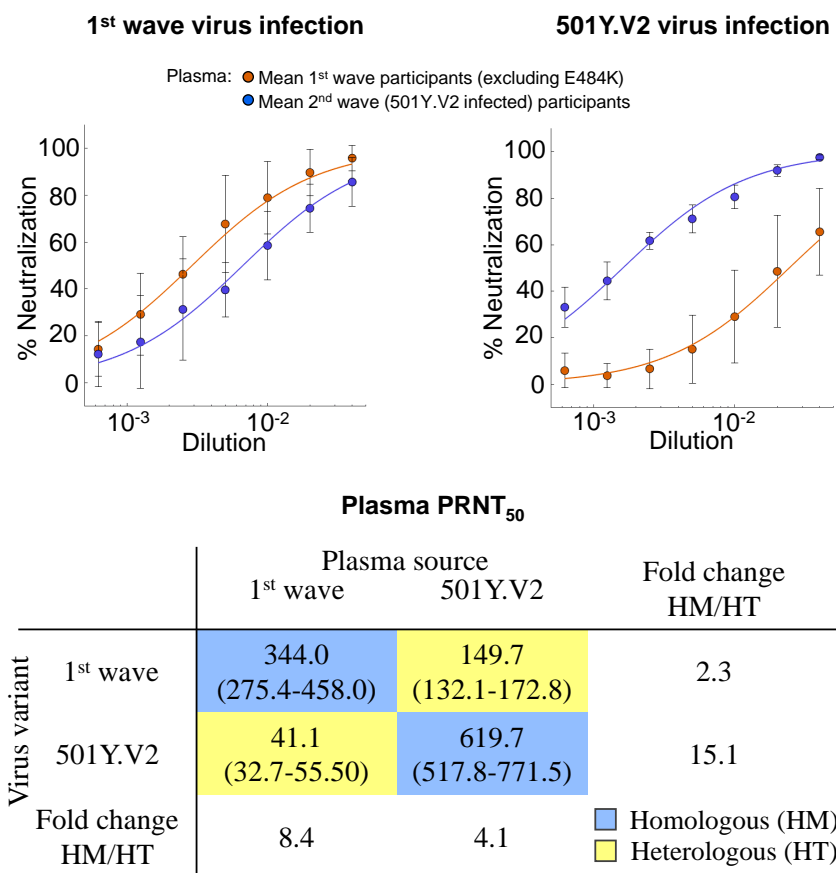


Figure 3: Cross-neutralization of first infection wave and 501Y.V2 virus across all participants from two infection waves. Left plot shows neutralization of infection by first wave virus, and right plot shows neutralization of 501Y.V2. Sigmoidal fits were performed to the means of first (red points) and second wave (blue points) plasma neutralization results across all participants excluding the participant with plasma immunity elicited by the viral variant containing the E484K mutation alone. Shown are means and standard deviations of n=13 first wave infected plasma donors and n=6 second wave infected plasma donors. Summary table shows plasma $PRNT_{50}$ as a function of plasma source (columns) and infecting viral variant (rows). Blue rectangles highlight homologous neutralization where virus and infection wave are matched, and yellow rectangles highlight cross-neutralization where virus and plasma are from different infection waves.

197 **Material and methods**

198 **Ethical statement**

199 Nasopharyngeal/oropharyngeal swab samples and plasma samples were obtained from six hospital-
200 ized adults with PCR-confirmed SARS-CoV-2 infection enrolled in a prospective cohort study ap-
201 proved by the Biomedical Research Ethics Committee (BREC) at the University of KwaZulu-Natal
202 (reference BREC/00001275/2020). The 501Y.V2 variants were obtained from residual nasopharyn-
203 geal/oropharyngeal samples used for routine SARS-CoV-2 diagnostic testing by the National Health
204 Laboratory Service, through our SARS-CoV-2 genomic surveillance program (BREC approval reference
205 BREC/00001510/2020).

206 **Whole genome sequencing, genome assembly and phylogenetic analysis**

207 cDNA synthesis was performed on the extracted RNA using random primers followed by gene specific
208 multiplex PCR using the ARTIC V3 protocol. Briefly, extracted RNA was converted to cDNA us-
209 ing the Superscript IV First Strand synthesis system (Life Technologies, Carlsbad, CA) and random
210 hexamer primers. SARS-CoV-2 whole genome amplification was performed by multiplex PCR using
211 primers designed on Primal Scheme (<http://primal.zibraproject.org/>) to generate 400bp amplicons with
212 an overlap of 70bp that covers the 30Kb SARS-CoV-2 genome. PCR products were cleaned up us-
213 ing AmpureXP purification beads (Beckman Coulter, High Wycombe, UK) and quantified using the
214 Qubit dsDNA High Sensitivity assay on the Qubit 4.0 instrument (Life Technologies Carlsbad, CA).
215 We then used the Illumina® Nextera Flex DNA Library Prep kit according to the manufacturer's
216 protocol to prepare indexed paired end libraries of genomic DNA. Sequencing libraries were normalized
217 to 4nM, pooled and denatured with 0.2N sodium acetate. 12pM sample library was spiked with 1%
218 PhiX (PhiX Control v3 adapter-ligated library used as a control). We sequenced libraries on a 500-
219 cycle v2 MiSeq Reagent Kit on the Illumina MiSeq instrument (Illumina, San Diego, CA). We assembled
220 paired-end fastq reads using Genome Detective 1.126 (<https://www.genomedetective.com>) and the Coro-
221 navirus Typing Tool. We polished the initial assembly obtained from Genome Detective by aligning
222 mapped reads to the references and filtering out low-quality mutations using the bcftools 1.7-2 mpileup
223 method. Mutations were confirmed visually with bam files using Geneious software (Biomatters Ltd,
224 Auckland, New Zealand). All of the sequences were deposited in GISAID (<https://www.gisaid.org/>).
225 We retrieved all South African SARS-CoV-2 genotypes from the GISAID database as of 11 January
226 2021 (N=2704). We initially analyzed South African genotypes against the global reference dataset
227 (N=2592) using a custom pipeline based on a local version of NextStrain. The pipeline contains several
228 python scripts that manage the analysis workflow. It performs alignment of genotypes in MAFFT,
229 phylogenetic tree inference in IQ-Tree20, tree dating and ancestral state construction and annotation
230 (<https://github.com/nextstrain/ncov>).

231 **Cells**

232 Vero E6 cells (ATCC CRL-1586, obtained from Cellonex) were propagated in complete DMEM with 10%
233 fetal bovine serum (Hylone) containing 1% each of HEPES, sodium pyruvate, L-glutamine, and non-
234 essential amino acids (Sigma-Aldrich). Cells were passaged every 3-4 days. H1299 cells were propagated
235 in complete RPMI with 10% fetal bovine serum containing 1% each of HEPES, sodium pyruvate, L-
236 glutamine, and non-essential amino acids and and passaged every second day.

237 **H1299-E3 cell line for first passage SARS-CoV-2 outgrowth**

238 The H1299-H2AZ clone with nuclear labelled YFP was constructed to overexpress ACE2 as follows:
239 VSVG-pseudotyped lentivirus containing the human ACE2 was generated by co-transfecting 293T cells
240 with the pHAGE2-EF1aInt-ACE2-WT plasmid along with the lentiviral helper plasmids HDM-VSVG,
241 HDM-Hgpm2, HDM-tat1b and pRC-CMV-Rev1b using TransIT-LT1 (Mirus) transfection reagent. Su-
242 pernatant containing the lentivirus was harvested two days after infection, filtered through a 0.45µm

243 filter (Corning) and used to spinfect H1299-H2AZ at 1000 rcf for 2 hours at room temperature in the pres-
244 ence of 5 $\mu\text{g}/\text{mL}$ polybrene (Sigma-Aldrich). ACE-2 transduced H1299-H2AZ cells were then subcloned
245 at the single cell density in 96-well plates (Eppendorf) in conditioned media derived from confluent cells.
246 After 3 weeks, wells were trypsinized (Sigma-Aldrich) and plated in two replicate plates, where the first
247 plate was used to determine infectivity and the second was stock. The first plate was screened for the
248 fraction of mCherry positive cells per cell clone upon infection with SARS-CoV-2 mCherry expressing
249 spike pseudotyped lentiviral vector 1610-pHAGE2/EF1a Int-mCherry3-W produced by transfecting as
250 above. Screening was performed using a Metamorph-controlled (Molecular Devices, Sunnyvale, CA)
251 Nikon TiE motorized microscope (Nikon Corporation, Tokyo, Japan) with a 20x, 0.75 NA phase ob-
252 jective, 561 laser line, and 607 nm emission filter (Semrock, Rochester, NY). Images were captured
253 using an 888 EMCCD camera (Andor). Temperature (37°C), humidity and CO₂ (5%) were controlled
254 using an environmental chamber (OKO Labs, Naples, Italy). The clone with the highest fraction of
255 mCherry expression was expanded from the stock plate and denoted H1299-E3. This clone was used in
256 the outgrowth.

257 Viral Outgrowth

258 All live virus work was performed in Biosafety level 3 containment using Africa Health Research Institute
259 biosafety committee approved protocols for SARS-CoV-2. For first wave virus, a T25 flask (Corning)
260 was seeded with Vero E6 cells at 2×10^5 cells/ml and incubated for 18-20 hours. After 1 DPBS wash,
261 the sub-confluent cell monolayer was inoculated with 500 μL universal transport medium (UTM) diluted
262 1:1 with growth medium and filtered through a 0.45 μM filter. Cells were incubated for 1 hour. Flask
263 was then filled with 7mL of complete growth medium and checked daily for cytopathic effect (CPE).
264 Four days post infection, supernatants of the infected culture were collected, centrifuged at 300 rcf for
265 3 minutes to remove cell debris, and filtered using a 0.45 μM filter. Viral supernatant was aliquoted and
266 stored at -80°C. For 501Y.V2 variants, we used H1299-ACE2-E3 cells for initial isolation followed by
267 passage into Vero E6 cells. H1299-ACE2-E3 cells were seeded at 1.5×10^5 cells/ml and incubated for
268 18-20 hours. After 1 DPBS wash, the sub-confluent cell monolayer was inoculated with 500 μL universal
269 transport medium (UTM) diluted 1:1 with growth medium and filtered through a 0.45 μM filter. Cells
270 were incubated for 1 hour. Wells were then filled with 3mL of complete growth medium. 8 days post-
271 infection, cells were trypsinized, centrifuged at 300 rcf for 3 minutes and resuspended in 4mL growth
272 medium. 1mL was added to Vero E6 cells that had been seeded at $t \ 2 \times 10^5$ cells/ml 18-20 hours
273 earlier in a T25 flask (approximately 1:8 donor-to-target cell dilution ratio) for cell-to-cell infection.
274 Coculture of H1299-ACE2-E3 and Vero E6 cells was incubated for 1 hour and flask was then filled with
275 7mL of complete growth medium and incubated for 6 days. Viral supernatant was aliquoted and stored
276 at -80°C or further passaged in Vero E6 cells as above. Two isolates were outgrown, 501Y.V2.HV001
277 and 501Y.V2.HVdF002. The second isolate showed fixation of furin cleavage site mutations during
278 outgrowth in Vero E6 cells and was not used except for data presented in Figure S1.

279 Microneutralization using focus forming assay

280 For plasma from first wave donors, we first quantified spike RBD IgG by enzyme-linked immunosor-
281 bent assay (ELISA) using monoclonal antibody CR3022 as a quantitative standard, (n = 13 excluding
282 participant 039-13-0103 for which ELISA data was not available). The mean concentration was 23.7
283 $\mu\text{g}/\text{mL}$ *pm* 19.1 $\mu\text{g}/\text{mL}$, (range 5.7 - 62.6 $\mu\text{g}/\text{mL}$). In comparison, uninfected donor controls had a mean
284 of 1.85 $\mu\text{g}/\text{mL}$ *pm* 0.645 $\mu\text{g}/\text{mL}$. To quantify neutralization, Vero E6 cells were plated in an 96-well
285 plate (Eppendorf or Corning) at 30,000 cells per well 1 day pre-infection. Importantly, before infection
286 approximately 5ml of sterile water was added between wells to prevent more rapid drying of wells at the
287 edge which we have observed to cause edge effects (lower number of foci). Plasma was separated from
288 EDTA-anticoagulated blood by centrifugation at 500 rcf for 10 minutes and stored at -80°C. Aliquots of
289 plasma samples were heat-inactivated at 56°C for 30 minutes, and clarified by centrifugation at 10,000
290 rcf for 5 minutes, where the clear middle layer was used for experiments. Inactivated plasma was stored
291 in single use aliquots to prevent freeze-thaw cycles. For experiments, plasma was serially diluted two-fold

292 from 1:100 to 1:1600, where this is the concentration during the virus-plasma incubation step before
293 addition to cells and during the adsorption step. As a positive control, the GenScript A02051 anti-spike
294 mAb was added at concentrations listed in the figures. Virus stocks were used at approximately 50
295 focus-forming units (FFU) per microwell and added to diluted plasma; antibody-virus mixtures were incu-
296 bated for 1 hour at 37°C, 5% CO₂. Cells were infected with 100µL of the virus-antibody mixtures for
297 one hour, to allow adsorption of virus. Subsequently, 100µL of a 1x RPMI 1640 (Sigma-Aldrich R6504),
298 1.5% carboxymethylcellulose (Sigma-Aldrich C4888) overlay was added to the wells without removing
299 the inoculum. Cells were fixed at 28 hours post-infection using 4% paraformaldehyde (Sigma-Aldrich)
300 for 20 minutes. For staining of foci, a rabbit anti-spike monoclonal antibody (mAb BS-R2B12, Gen-
301 Script A02058) was used at 0.5µg/mL as the primary detection antibody. Antibody was resuspended
302 in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% BSA (Sigma-Aldrich), and
303 0.05% tween (Sigma-Aldrich) in PBS. Plates were incubated with primary antibody overnight at 4°C,
304 then washed with wash buffer containing 0.05% tween in PBS. Secondary goat anti-rabbit horseradish
305 peroxidase (Abcam ab205718) was added at 1 µg/mL and incubated for 2 hours at room temperature
306 with shaking. The TrueBlue peroxidase substrate (SeraCare 5510-0030) was then added at 50µL per
307 well and incubated for 20 minutes at room temperature. Plates were then dried for 2 hours and imaged
308 using a Metamorph-controlled Nikon TiE motorized microscope with a 2x objective. Automated image
309 analysis was performed using a Matlab2019b (Mathworks) custom script, where focus detection was
310 automated and did not involve user curation. Image segmentation steps were stretching the image from
311 minimum to maximum intensity, local Laplacian filtering, image complementation, thresholding and
312 binarization. Two plasma donors initially measured from the South African second infection wave did
313 not have detectable neutralization of either 501Y.V2 or the first wave variant and were not used in the
314 study.

315 **Statistics and fitting**

316 All statistics and fitting were performed using Matlab2019b. Neutralization data was fit to

$$Tx = 1/1 + (D/ID_{50}).$$

317 Here Tx is the number of foci normalized to the number of foci in the absence of plasma on the
318 same plate at dilution D. To visualize the data, we used percent neutralization, calculated as (1-Tx)
319 × 100%. Negative values (Tx < 1, enhancement) was represented as 0% neutralization. Fit to a normal
320 distribution used Matlab2019b function normplot, which compared the distribution of the Tx data to
321 the normal distribution (see <https://www.mathworks.com/help/stats/normplot.html>).

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325 **§ Network for Genomic Surveillance in South Africa (NGS-SA)**

326 Shareef Abrahams¹, Luiz Carlos Junior Alcantara², Arghavan Alisoltani-Dehkordi^{3,4}, Mushal Allam⁵,
327 Jinal N Bhiman^{5,6}, Mary-Ann Davies^{7,8}, Deelan Doolabh⁹, Susan Engelbrecht¹⁰, Vagner Fonseca¹¹,
328 Marta Giovanetti², Allison J Glass^{6,12}, Adam Godzik⁴, Dominique Goedhals¹³, Diana Hardie¹⁴, Mar-
329 vin Hsiao¹⁴, Arash Iranzadeh⁴, Arshad Ismail⁵, Stephen Korsman¹⁴, Sergei L Kosakovsky Pond¹⁵,
330 Oluwakemi Laguda-Akingba^{1,16}, Jose Lourenco¹⁷, Gert Marais¹⁴, Darren Martin^{9,18}, Caroline Maslo¹⁹,
331 Koleka Mlisana^{20,21}, Thabo Mohale⁵, Nokukhanya Msomi²², Innocent Mudau⁹, Francesco Petruccione^{23,24},
332 Wolfgang Preiser¹⁰, Emmanuel James San¹¹, Bryan Trevor Sewell²⁵, Lynn Tyers⁹, Gert Van Zyl¹⁰,
333 Anne von Gottberg^{5,6}, Sibongile Walaza^{5,26}, Steven Weaver¹⁵, Constantinos Kurt Wibmer⁵, Carolyn
334 Williamson^{9,14,21}, Denis York²⁷.

335 ¹National Health Laboratory Service, Port Elizabeth, South Africa. ²Laboratorio de Flavivirus, Fun-
336 dacao Oswaldo Cruz, Rio de Janeiro, Brazil. ³Division of Medical Virology, Department of Pathology,
337 University of Cape Town, Cape Town, South Africa. ⁴Division of Biomedical Sciences, University of
338 California Riverside School of Medicine, Riverside, California, USA. ⁵National Institute for Commu-
339 nicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa. ⁶School of
340 Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
341 ⁷Centre for Infectious Disease Epidemiology and Research, University of Cape Town, Cape Town, South
342 Africa. ⁸Western Cape Government: Health, Cape Town, South Africa. ⁹Division of Medical Virology,
343 Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South
344 Africa. ¹⁰Division of Medical Virology at NHLS Tygerberg Hospital and Faculty of Medicine and Health
345 Sciences, Stellenbosch University, Cape Town, South Africa. ¹¹KwaZulu-Natal Research Innovation and
346 Sequencing Platform (KRISP), Department of Laboratory Medicine and Medical Sciences, University
347 of KwaZulu-Natal, Durban, South Africa. ¹²Department of Molecular Pathology, Lancet Laboratories,
348 Johannesburg, South Africa. ¹³Division of Virology at NHLS Universitas Academic Laboratories, Uni-
349 versity of The Free State, Bloemfontein, South Africa. ¹⁴Division of Medical Virology at NHLS Groote
350 Schuur Hospital, University of Cape Town, Cape Town, South Africa. ¹⁵Institute for Genomics and
351 Evolutionary Medicine, Temple University, Philadelphia, Pennsylvania, USA. ¹⁶Department of Labo-
352 ratory Medicine and Pathology, Faculty of Health Sciences, Walter Sisulu University, Mthatha, South
353 Africa. ¹⁷Department of Zoology, University of Oxford, Oxford, United Kingdom. ¹⁸Computational Bi-
354 ology Division, Department of Integrative Biomedical Sciences, University of Cape Town, Cape Town,
355 South Africa. ¹⁹Department of Quality Leadership, Netcare Hospitals, Johannesburg, South Africa.
356 ²⁰National Health Laboratory Service, Johannesburg, South Africa. ²¹Centre for the AIDS Programme
357 of Research in South Africa (CAPRISA), Durban, South Africa. ²²Discipline of Virology, University of
358 KwaZulu-Natal, School of Laboratory Medicine and Medical Sciences and National Health Laboratory
359 Service, Durban, South Africa. ²³Centre for Quantum Technology, University of KwaZulu-Natal, Dur-
360 ban, South Africa ²⁴National Institute for Theoretical Physics (NITheP), KwaZulu-Natal, South Africa.
361 ²⁵Structural Biology Research Unit, Department of Integrative Biomedical Sciences, University of Cape
362 Town, Rondebosch, South Africa. ²⁶School of Public Health, Faculty of Health Sciences, University
363 of the Witwatersrand, Johannesburg, South Africa. ²⁷Molecular Diagnostics Services, Durban, South
364 Africa.

365 § § COMMIT-KZN Team

366 Moherndran Archary¹, Kaylesh J. Dullabh², Philip Goulder^{3,4}, Guy Harling^{3,5}, Rohen Harrichandparsad⁶,
367 Kobus Herbst^{3,7}, Prakash Jeena¹, Thandeka Khoza³, Nigel Klein^{3,8}, Henrik Kløverpris^{3,9,10}, Alasdair
368 Leslie^{3,9}, Rajhmun Madansein², Mohlopheni Marakalala^{3,9}, Matilda Mazibuko³, Mosa Moshabela¹¹,
369 Ntombifuthi Mthabela³, Kogie Naidoo¹², Zaza Ndhlovu^{3,13}, Thumbi Ndung'u^{3,9,14,15}, Kennedy Nyamande¹⁶,
370 Nesri Padayatchi¹², Vinod Patel¹⁷, Theresa Smit³, Adrie Steyn^{3,18}, Emily Wong^{3,18}.

371 ¹Department of Paediatrics and Child Health, University of KwaZulu-Natal, Durban, South Africa.
372 ²Department of Cardiothoracic Surgery, University of KwaZulu-Natal, Durban, South Africa. ³Africa
373 Health Research Institute, Durban, South Africa. ⁴Department of Paediatrics, Oxford, UK. ⁵Institute
374 for Global Health, University College London, UK. ⁶Department of Neurosurgery, University of KwaZulu-
375 Natal, Durban, South Africa. ⁷South African Population Research Infrastructure Network, Durban,
376 South Africa. ⁸Institute of Child Health, University College London, UK. ⁹Division of Infection and
377 Immunity, University College London, London, UK. ¹⁰Department of Immunology and Microbiology,
378 University of Copenhagen, Copenhagen, Denmark. ¹¹College of Health Sciences, University of KwaZulu-
379 Natal, Durban, South Africa. ¹²Centre for the AIDS Programme of Research in South Africa, Durban,
380 South Africa. ¹³Ragon Institute of MGH, MIT and Harvard, Boston, USA. ¹⁴HIV Pathogenesis Pro-
381 gramme, The Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban, South
382 Africa. ¹⁵Max Planck Institute for Infection Biology, Berlin, Germany. ¹⁶Department of Pulmonology
383 and Critical Care, University of KwaZulu-Natal, Durban, South Africa. ¹⁷Department of Neurology,

384 University of KwaZulu-Natal, Durban, South Africa. ¹⁸Division of Infectious Diseases, University of
385 Alabama at Birmingham.

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389 Msomi, Koleka Mlisana, Anne von Gottberg, Sibongile Walaza, Mushal Allam, Arshad Ismail,
390 Thabo Mohale, Allison J Glass, Susan Engelbrecht, Gert Van Zyl, Wolfgang Preiser, Francesco
391 Petruccione, Alex Sigal, Diana Hardie, Gert Marais, Marvin Hsiao, Stephen Korsman, Mary-
392 Ann Davies, Lynn Tyers, Innocent Mudau, Denis York, Caroline Maslo, Dominique Goedhals,
393 Shareef Abrahams, Oluwakemi Laguda-Akingba, Arghavan Alisoltani-Dehkordi, Adam Godzik,
394 Constantinos Kurt Wibmer, Bryan Trevor Sewell, José Lourenço, Luiz Carlos Junior Alcantara,
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404 M. S. Pizzuto, E. Camerini, C. Havenar-Daughton, M. Smithy, D. Hong, V. Lepori, E. Albanese,
405 A. Ceschi, E. Bernasconi, L. Elzi, P. Ferrari, C. Garzoni, A. Riva, G. Snell, F. Sallusto, K. Fink,
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411 Alexandra C. Walls, Martina Beltramello, Alex Chen, Zhuoming Liu, Fabrizia Zatta, Samantha
412 Zepeda, Julia di Iulio, John E. Bowen, Martin Montiel-Ruiz, Jiayi Zhou, Laura E. Rosen, Siro
413 Bianchi, Barbara Guarino, Chiara Silacci Fregni, Rana Abdelnabi, Shi-Yan Caroline Foo, Paul W.
414 Rothlauf, Louis-Marie Bloyet, Fabio Benigni, Elisabetta Camerini, Johan Neyts, Agostino Riva,
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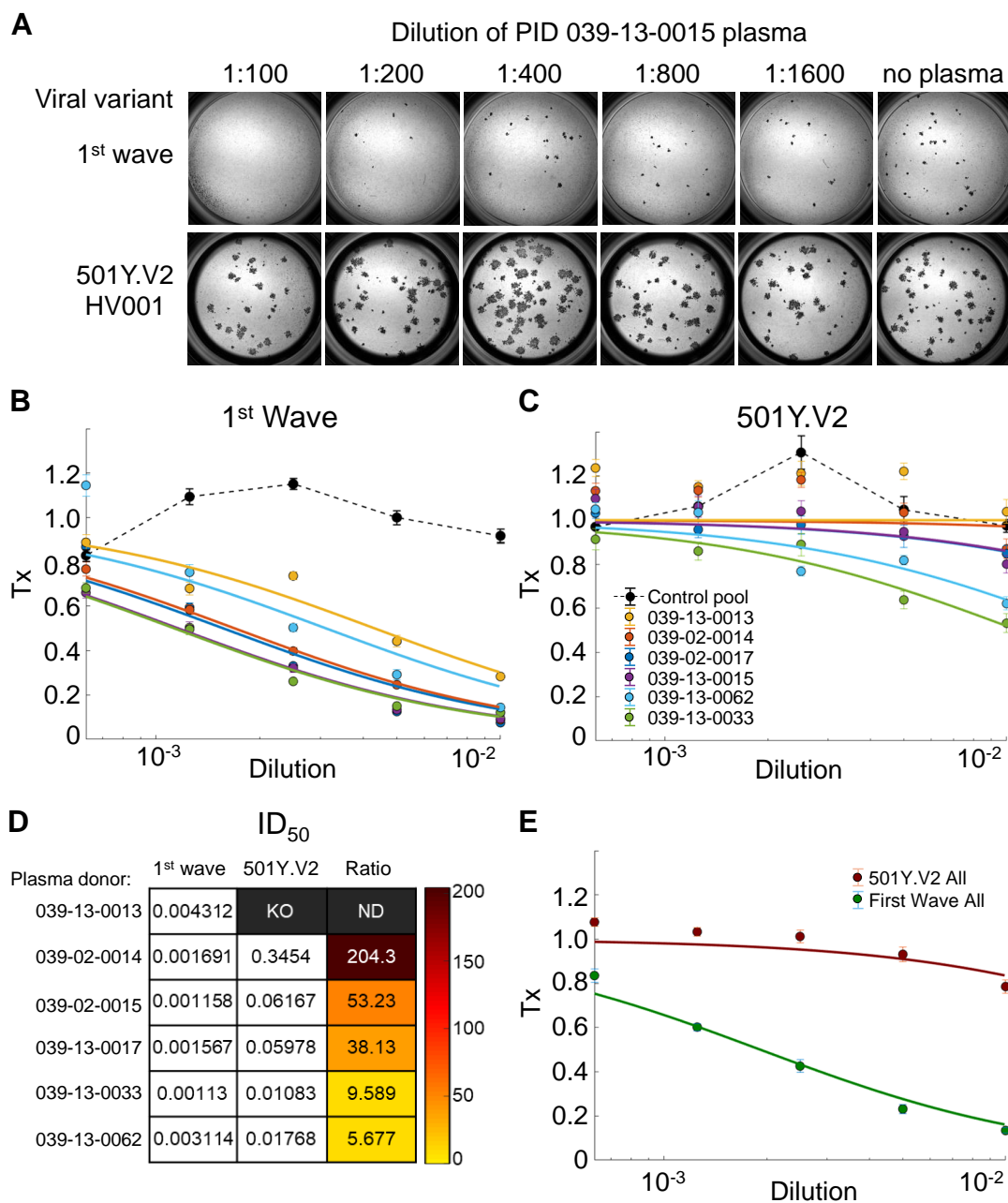


Figure S 1: Neutralization of first wave and 501Y.V2 variants by convalescent plasma from first wave infections using equal infection incubation times. (A) A representative focus forming assay using plasma from participant 039-13-0015. Plasma neutralization of (B) first wave virus and (C) 501Y.V2 variants (501Y.V2.HV001 and 501Y.V2.HVdF002). Colored circles represent means and standard errors from 8 independent neutralization experiments using plasma from 6 convalescent participants who were infected by first wave variants in the first peak of the pandemic in South Africa. Correspondingly colored lines are fits of the sigmoidal equation with ID_{50} as the fitted parameter. Data from both 501Y.V2 variants was combined as separate experiments to obtain a more accurate fit of the data using a sigmoidal function since the declines in 501Y.V2 infection were small in the range of plasma concentrations used. The matched infections with first wave virus which were done in parallel with each 501Y.V2 variant were also combined. One experiment was removed in the process of quality control due to plate edge effects, which were subsequently corrected by adding sterile water between wells. Black points represent a pool of plasma from three uninfected controls. The transmission index (Tx) is the number of foci in the presence of the plasma dilution normalized by the number of foci in the absence of plasma. (D) Plasma ID_{50} values and ratios for first wave and 501Y.V2 variants. Knockout (KO) was scored as $ID_{50} > 1$. ND, not defined. (E) Mean and standard error across all plasma donors.

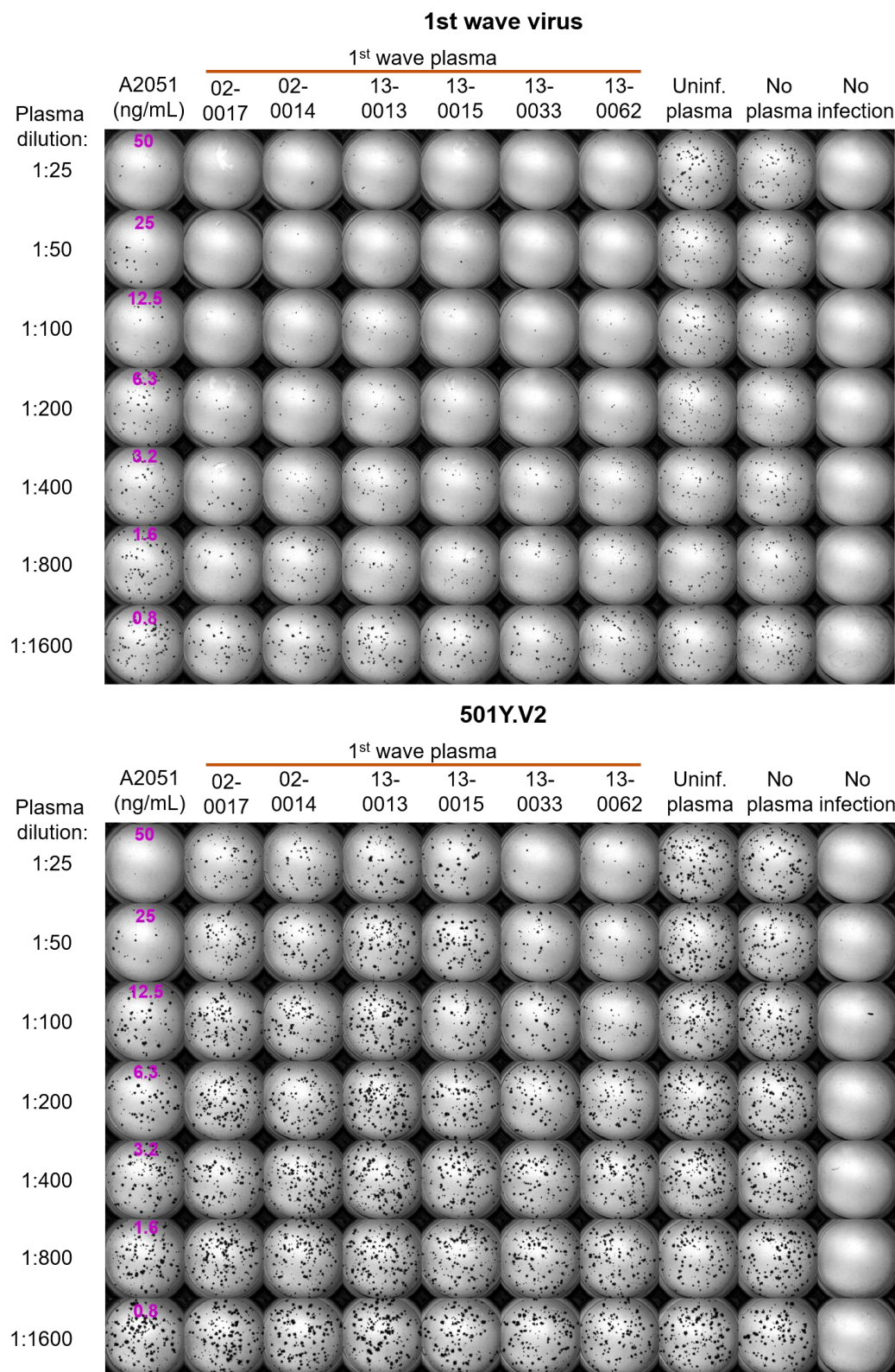


Figure S 2: Neutralization of first wave and 501Y.V2 by convalescent plasma: Representative experiments of first set of participant plasma tested. Top montage shows neutralization of first wave virus, bottom montage shows neutralization of 501Y.V2. Rows are plasma dilutions, ranging from 1:25 to 1:1600. Last three columns are plasma from a pool of uninfected participants, the no plasma control, and no virus, respectively. First column is the A2051 NAb, with antibody concentrations in ng/mL (magenta). First wave plasma donors are marked with a red line, second wave plasma donors are marked with a blue line.

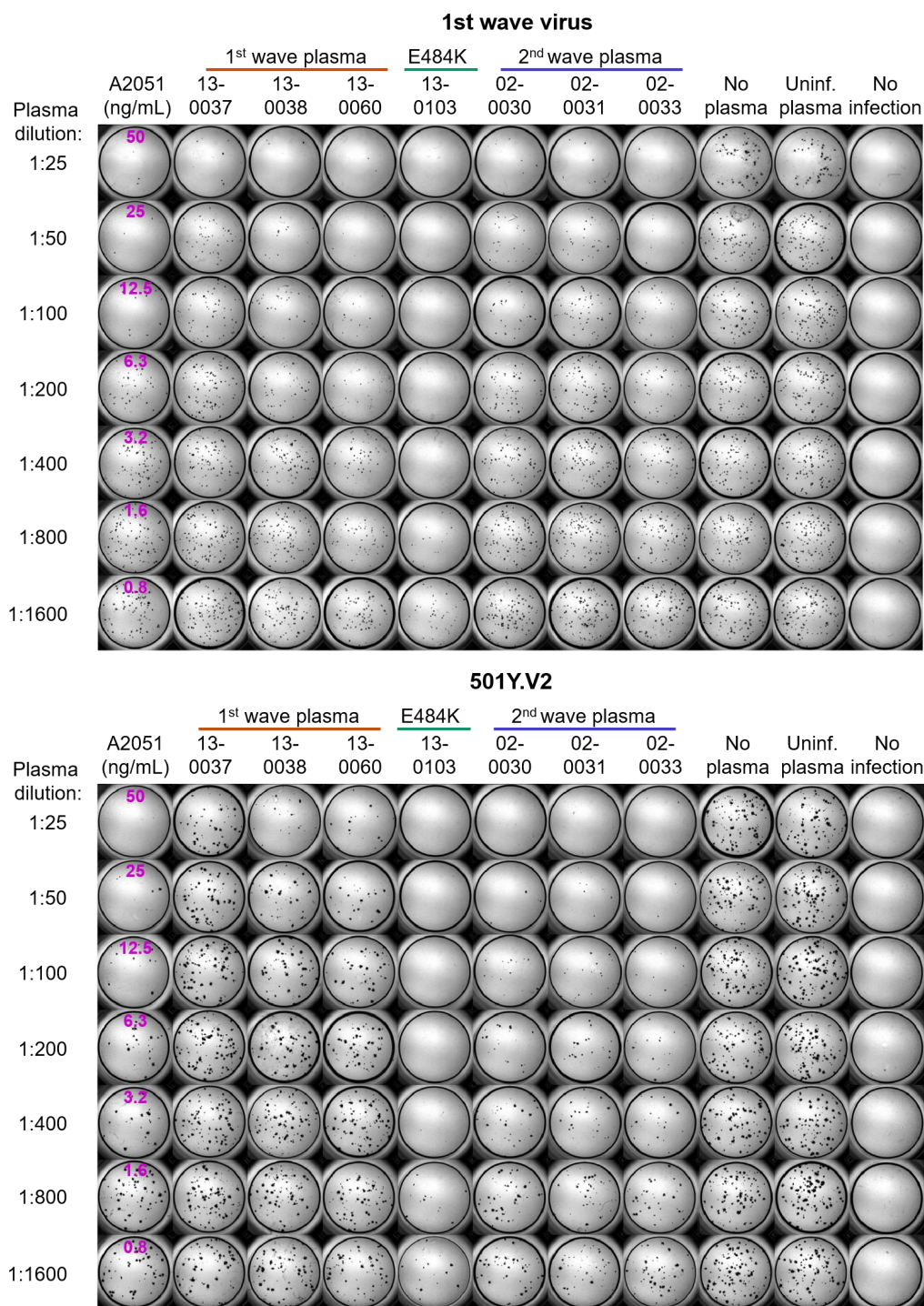


Figure S 3: Neutralization of first wave and 501Y.V2 by convalescent plasma: Representative experiments of second set of participant plasma tested. Top montage shows neutralization of first wave virus, bottom montage shows neutralization of 501Y.V2. Rows are plasma dilutions, ranging from 1:25 to 1:1600. Last three columns are plasma from a pool of uninfected participants, the no plasma control, and no virus, respectively. First column is the A2051 NAb, with antibody concentrations in ng/mL (magenta). First wave plasma donors are marked with a red line, second wave plasma donors are marked with a blue line, the plasma donor showing the E484K mutation only is marked with a green line.

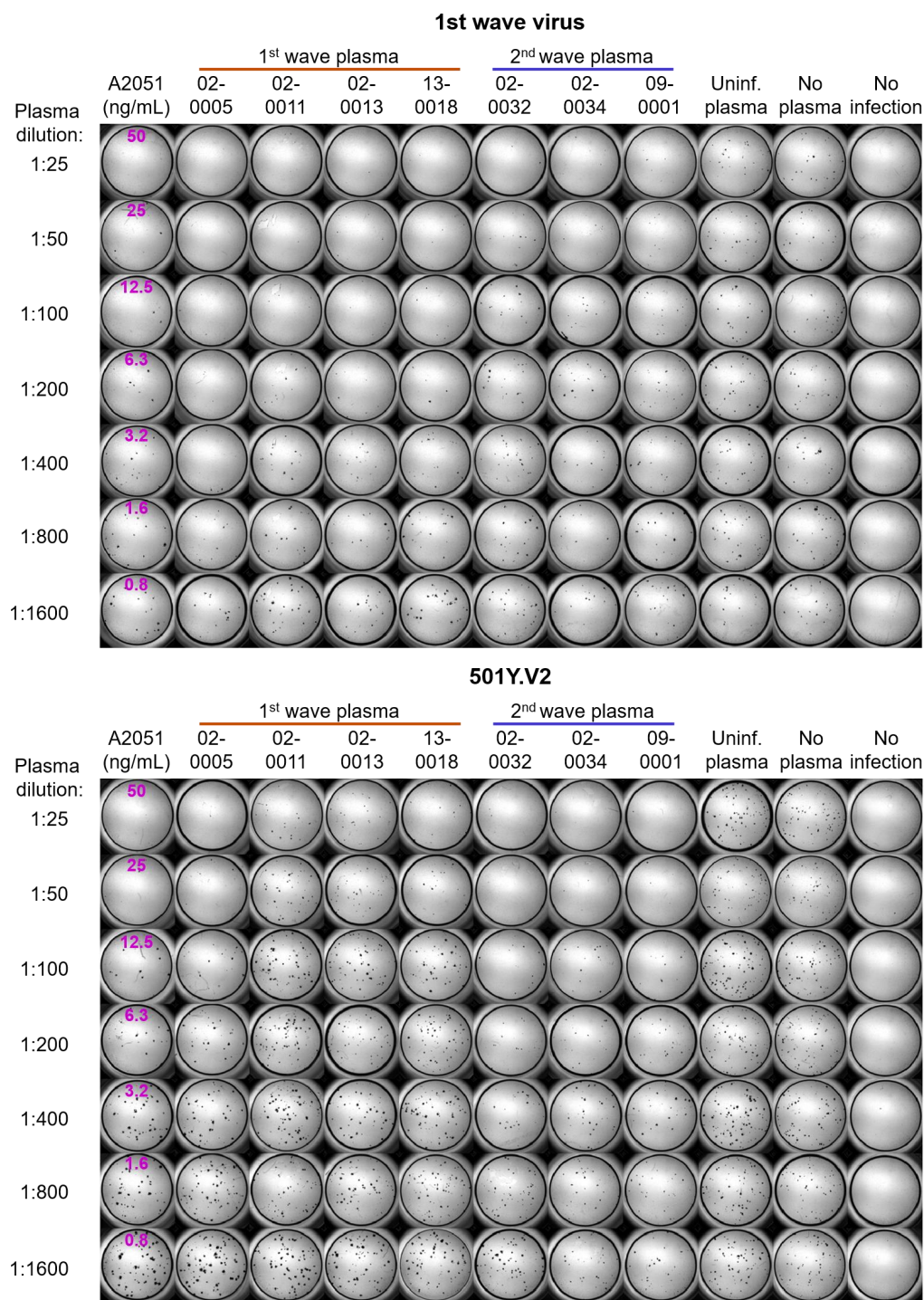


Figure S 4: Neutralization of first wave and 501Y.V2 by convalescent plasma: Representative experiments of third set of participant plasma tested. Top montage shows neutralization of first wave virus, bottom montage shows neutralization of 501Y.V2. Rows are plasma dilutions, ranging from 1:25 to 1:1600. Last three columns are plasma from a pool of uninfected participants, the no plasma control, and no virus, respectively. First column is the A2051 NAb, with antibody concentrations in ng/mL (magenta). First wave plasma donors are marked with a red line, second wave plasma donors are marked with a blue line.

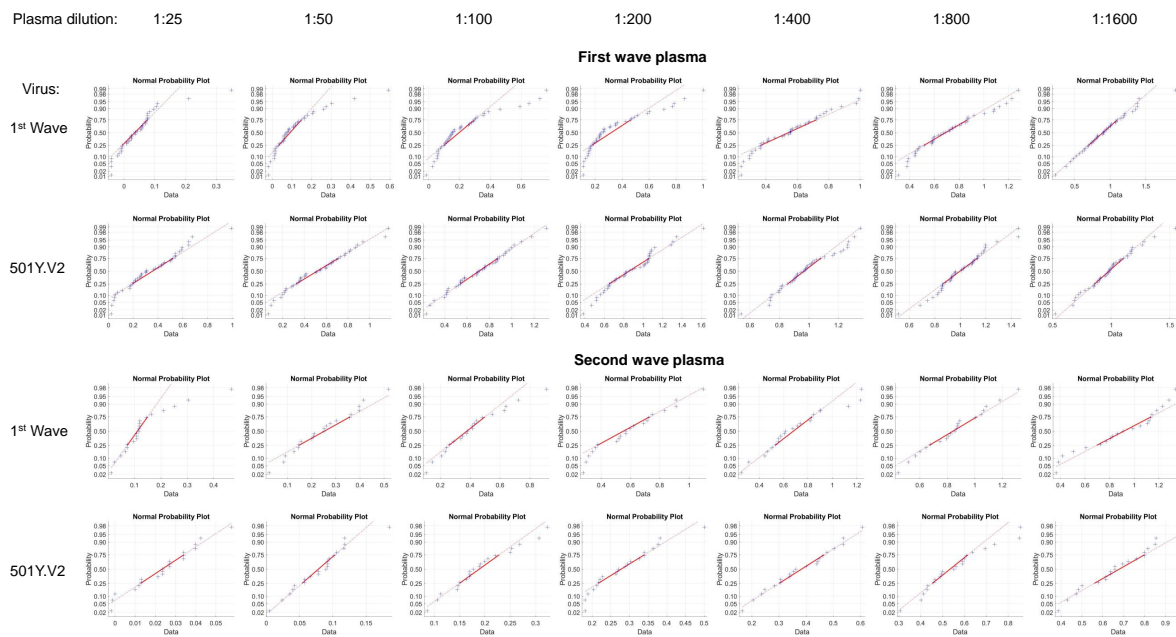


Figure S 5: Fit of combined data for each plasma dilution to a normal distribution. The Matlab2019b function normplot was used to assess the fit of the data (blue crosses) to a normal distribution (solid red line). For each plot, one data point is the Tx result for one experiment for one participant at the specified dilution. Number of total experiments per viral variant was $n=42$ for first wave plasma, and $n=21$ for second wave plasma. Lack of pronounced curvature of the data in the range of the solid line indicates that the data is a reasonably good fit to a normal distribution. see <https://www.mathworks.com/help/stats/normplot.html> for additional information.

Table S 1: Plasma donor characteristics

Cohort ID	Sex	Age (yrs)	HIV status	HIV viral load (copies/mL)	Supplemental oxygen	Date of symptom onset	Days between symptom onset and plasma collection	Days between symptom onset and last positive qPCR
039-02-0005	M	50-59	Negative	-	No	Mid-June	29	8
039-13-0015	F	40-49	Negative	-	No	Mid-June	26	12
039-13-0033	F	30-39	Negative	-	No	Late June	30	23
039-02-0013	F	70+	Negative	-	Yes	Late June	29	15
039-13-0013	F	50-59	Positive	<40	No	Late June	30	10
039-13-0018	F	40-49	Negative	-	No	Late June	28	14
039-02-0014	F	60-69	Negative	-	No	Early July	27	20
039-02-0011	F	40-49	Positive	<40	No	Early July	32	32
039-13-0060	M	40-49	Positive	<40	No	Mid-July	38	31
039-02-0017	F	60-69	Negative	-	Yes	Mid-July	28	7
039-13-0062	M	60-69	Negative	-	No	Early August	26	12
039-13-0103	M	60-69	Negative	-	Yes	Late September	37	17
039-13-0037	M	30-39	Positive	<40	No	Asymptomatic	29*	8†
039-13-0038	M	30-39	Negative	-	No	Asymptomatic	29*	8†
039-02-0031	F	40-49	Negative	-	Yes	Late December	34	13
039-02-0032	M	40-49	Positive	<40	Yes	Late December	43	12
039-02-0033	M	50-59	Negative	-	No	Late December	35	42
039-02-0030	F	40-49	Positive	<40	Yes	Late December	30	9
039-02-0034	F	30-39	Negative	-	Yes	Late December	32	5‡
039-09-0001	F	60-69	Negative	-	Yes	Early January	29	29

*Asymptomatic cases; plasma collected 29 days after positive diagnostic swab for these two participants

†Last positive qPCR collected 8 days after diagnostic swab collection for two participants

‡Only single qPCR test positive at diagnosis

Table S 2: Mutation profile for the genomes of the outgrown viruses and for the infecting viruses of convalescent plasma donors

Sequence ID	Accession ID*	Cohort ID	Date of collection	PANGO lineage†	Spike aa substitutions	Spike deletions	Other aa substitutions	Other deletions
<i>Outgrown viruses</i>								
K002868‡	EPI_ISL_602622	039-13-0013	02-Jul-2020	B.1.1.117	S: D614G S: A688V	-	N: L319F N: R203K N: G204R ORF14: G50N ORF1a: D1481N ORF1b: P314L	-
K005325	EPI_ISL_678615	-	16-Nov-2020	B.1.351	S: L18F S: D80A S: D215G S: K417N S: E484K S: N501Y S: D614G S: A701V	S: 242-244del	E: P71L N: T205I ORF14: L52F ORF1a: T265I ORF1a: K1655N ORF1a: K3353R ORF1b: P314L ORF3a: Q57H ORF3a: W131L ORF3a: S171L ORF7a: V93F	ORF1ab: 3675-3677del
<i>Plasma donors (first wave)</i>								
K003667	EPI_ISL_602623	039-02-0005	22-Jun-2020	B.1.140	S: D614G	-	ORF1a: D75E ORF1a: F741L ORF1a: S2224F ORF1b: P314L	-
K004285	EPI_ISL_660167	039-13-0015	03-Jul-2020	B.1	S: D614G	-	E: L73P ORF1a: D3728N ORF1b: P314L	-
K003673	EPI_ISL_602629	039-13-0018	06-Jul-2020	B.1.1.84	S: A653V S: D614G	-	N: R203K N: G204R ORF1b: P314L ORF14: G50R/E	-
K003675	EPI_ISL_602631	039-02-0011	07-Jul-2020	B.1.1.56	S: D614G	-	N: R203K N: G204R ORF1b: P314L ORF1b: P970L ORF14: G50R/E	-

Table S 3: Mutation profile for the genomes of the outgrown viruses and for the infecting viruses of convalescent plasma donors
continued

K003668	EPI_ISL_602624	039-02-0013	10-Jul-2020	B.1.1.117	S: A688V S: D614G	-	-	N: L139F N: R203K N: G204R ORF1b: P314L ORF14: G50R/E
K004289	EPI_ISL_660170	039-02-0014	14-Jul-2020	B.1.1.56	S: D614G	-	-	E: L73P N: R203K N: G204R ORF14: G50N ORF1b: P314L ORF1b: T152I
K004293	EPI_ISL_660174	039-13-0038	16-Jul-2020	H.1	S: D614G S: Q677H	-	-	N: R203K N: G204R ORF1a: P1497L ORF1a: D3728N ORF1b: P314L ORF14: G50N
K004291	EPI_ISL_660172	039-13-0033	17-Jul-2020	B.1	S: D614G	-	-	N: T148A ORF10: A28V ORF1a: K2511R ORF1a: V3858I ORF1b: P314L
K004292	EPI_ISL_660173	039-13-0037	24-Jul-2020	B.1.1.117	S: D614G S: A688V S: M731I	-	-	N: L139F N: R203K N: G204R ORF3a: Q245K ORF1b: P314L
K004295	EPI_ISL_660176	039-02-0017	28-Jul-2020	B.1.140	S: D614G	-	-	ORF1a: F1178S ORF1b: P314L
K004300	EPI_ISL_660180	039-13-0060	17-Aug-2020	B.1.1.56	S: D614G	-	-	E: L73P N: R203K N: G204R ORF1a: R2267G ORF1b: P314L ORF1b: A2589T ORF14: G50N
K004302	EPI_ISL_660181	039-13-0062	18-Aug-2020	B.1.1.1	S: D614G	-	-	N: R203K N: G204R

Table S 4: Mutation profile for the genomes of the outgrown viruses and for the infecting viruses of convalescent plasma donors continued

							ORF14:G50N ORF1a:T1246I ORF1a:G3278S ORF1b:P314L
K004306	EPI_ISL_660185	039-13-0103	05-Oct-2020	B.1.1.56	S: D614G S: E484K	-	N: R203K N: G204R ORF1a: H110Y ORF1a: R2267G ORF1a: V3718F ORF1b: P314L ORF9b: R13H ORF14: G50N
Plasma donors (second wave)							
K008637	039-02-0033	05-Jan-2021	05-Jan-2021	B.1.351	S: D80A S: K417N S: E484K S: N501Y S: D614G S: A701V	S: 242-244del	E: P71L N: T205I ORF1a: T265I ORF1a: K1655N ORF1a: S2285F ORF1a: K3353R ORF1b: P314L ORF3a: Q57H ORF3a: S171L ORF1a: 3675-3677del
K008633	039-09-0001	11-Jan-2021	11-Jan-2021	B.1.351	S: D80A S: D215G S: K417N S: E484K S: N501Y S: D614G S: A701V	S: 242-244del	E: P71L N: T205I ORF1a: T265I ORF1a: V721I ORF1a: S1612L ORF1a: K1655N ORF1a: K3353R ORF1b: P314L ORF3a: Q57H ORF3a: S171L ORF1a: 3675-3677del

Amino acid mutation nomenclature includes open reading frame, wild-type amino acid, ORF position and amino-acid mutation (e.g. S:D80A, Spike D to A substitution at position 80), del refers to deletion between stated positions. Amino acid mutations are annotated based on mature protein region of coding sequence (CDS) of SARS-CoV-2 reference sequence NC_045512.2.

* Accession ID refers to GISAID EpiCoV™ database (www.gisaid.org)

† Lineage classification performed by Pangolin software application version v2.1.7 (<https://cov-fineages.org/pangolin.html>)

‡ Also plasma donor (first wave virus)