

1 **SARS-CoV-2 evolved during advanced HIV disease immunosuppression has**
2 **Beta-like escape of vaccine and Delta infection elicited immunity**

3
4 Sandile Cele^{1,2}, Farina Karim^{1,2}, Gila Lustig³, James Emmanuel San⁴, Tandile Hermanus^{5,6},
5 Houriiyah Tegally⁴, Jumari Snyman^{1,7}, Thandeka Moyo-Gwete, Eduan Wilkinson^{4,8}, Mallory
6 Bernstein¹, Khadija Khan^{1,2}, Shi-Hsia Hwa^{1,9}, Sasha W. Tilles¹⁰, Lavanya Singh⁴, Jennifer
7 Giandhari⁴, Ntombifuthi Mthabela¹, Matilda Mazibuko¹, Yashica Ganga¹, Bernadett I. Gosnell¹¹,
8 Salim Abdool Karim³, Willem Hanekom^{1,9}, Wesley C. Van Voorhis¹⁰, Thumbi Ndung'u^{1,7},
9 COMMIT-KZN Team[§], Richard J. Lessells^{2,3,4}, Penny L. Moore^{3,5,6}, Mahomed-Yunus S.
10 Moosa¹¹, Tulio de Oliveira^{2,3,4,8,12}, Alex Sigal^{1,2,13*}

11
12 ¹Africa Health Research Institute, Durban, South Africa. ²School of Laboratory Medicine and
13 Medical Sciences, University of KwaZulu-Natal, Durban, South Africa. ³Centre for the AIDS
14 Programme of Research in South Africa, Durban, South Africa. ⁴KwaZulu-Natal Research
15 Innovation and Sequencing Platform, Durban, South Africa. ⁵National Institute for
16 Communicable Diseases of the National Health Laboratory Service, Johannesburg, South
17 Africa. ⁶MRC Antibody Immunity Research Unit, School of Pathology, Faculty of Health
18 Sciences, University of the Witwatersrand, Johannesburg, South Africa. ⁷Centre for Epidemic
19 Response and Innovation, School of Data Science and Computational Thinking, Stellenbosch
20 University, Stellenbosch, South Africa. ⁸HIV Pathogenesis Programme, University of KwaZulu-
21 Natal, Durban, South Africa. ⁹Division of Infection and Immunity, University College London,
22 London, UK. ¹⁰Center for Emerging and Re-emerging Infectious Diseases, University of
23 Washington, Seattle, USA. ¹¹Department of Infectious Diseases, Nelson R. Mandela School of
24 Clinical Medicine, University of KwaZulu-Natal, Durban, South Africa. ¹²Department of Global
25 Health, University of Washington, Seattle, USA. ¹³Max Planck Institute for Infection Biology,
26 Berlin, Germany.

27
28 § A list of authors and affiliations appears at the end of the paper.

29
30 * Corresponding author. Email: alex.sigal@ahri.org

31
32
33
34

35 **Highlights**

36

37 • A prolonged ancestral SARS-CoV-2 infection pre-dating the emergence of Beta and Delta

38 resulted in evolution of a Beta-like serological phenotype

39

40 • Serological phenotype includes strong escape from Delta infection elicited immunity,

41 intermediate escape from ancestral virus immunity, and weak escape from Beta immunity

42

43 • Evolved virus showed substantial but incomplete escape from antibodies elicited by

44 BNT162b2 vaccination

45

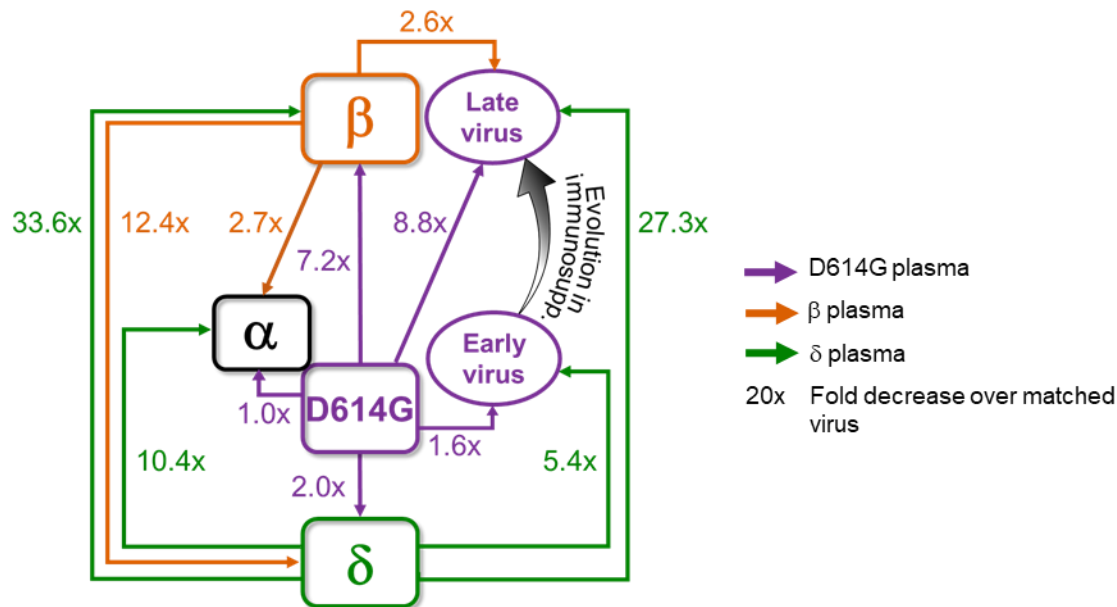
46

47

48 **Graphical abstract**

49

50



51

52

53

54 **Keywords**

55

56 SARS-CoV-2; HIV; Advanced HIV disease; Beta variant; Delta variant; Evolution; Immune

57 escape; Neutralization; Variants of concern

58

59 **Summary**

60

61 **Characterizing SARS-CoV-2 evolution in specific geographies may help predict the**
62 **properties of variants coming from these regions. We mapped neutralization of a SARS-**
63 **CoV-2 strain that evolved over 6 months from the ancestral virus in a person with advanced**
64 **HIV disease. Infection was before the emergence of the Beta variant first identified in South**
65 **Africa, and the Delta variant. We compared early and late evolved virus to the ancestral,**
66 **Beta, Alpha, and Delta viruses and tested against convalescent plasma from ancestral,**
67 **Beta, and Delta infections. Early virus was similar to ancestral, whereas late virus was**
68 **similar to Beta, exhibiting vaccine escape and, despite pre-dating Delta, strong escape of**
69 **Delta-elicited neutralization. This example is consistent with the notion that variants**
70 **arising in immune-compromised hosts, including those with advanced HIV disease, may**
71 **evolve immune escape of vaccines and enhanced escape of Delta immunity, with**
72 **implications for vaccine breakthrough and reinfections.**

73

74 Neutralization is highly predictive of vaccine efficacy (1, 2). Some SARS-CoV-2 variants show
75 decreased neutralization by vaccine elicited immunity. This may make vaccines less effective at
76 reducing the frequency of infection (3). The Alpha variant shows relatively little escape (4-7). The
77 Beta (4, 6-12), Gamma (13), Lambda (14) and Mu (15) variants show neutralization escape to
78 different degrees from neutralization elicited by ancestral SARS-CoV-2 infection or vaccines. The
79 Delta variant has evolved a strong transmission advantage over other SARS-CoV-2 strains (16).
80 It does not show a high degree of neutralization escape from plasma immunity elicited by
81 ancestral strains but does show escape from Beta antibody immunity (17).

82

83 Escape from neutralization and enhanced transmission involves substitutions and deletions in the
84 spike glycoprotein of the virus which binds the ACE2 receptor on the cell surface (18). Mutations
85 associated with neutralization escape are found in the receptor binding domain (RBD) (18-20),
86 and N-terminal domain of spike (21-24). RBD mutations for the Beta variant include the K417N,
87 E484K and N501Y (see <https://covdb.stanford.edu/page/mutation-viewer/>). E484K and N501Y
88 are shared with Gamma, which has the K417T instead of K417N. Alpha shares N501Y and in
89 some cases E484K. Lambda has L452Q and F490S. Delta has L452R and T478K. There are
90 also extensive differences in the NTD. For example, Beta NTD substitutions include L18F, D80A,
91 D215G, and a 241-243 deletion. In contrast, Delta has T19R, G142D, E156G, and a 157-158
92 deletion.

93

94 An important consideration in some geographical areas is high prevalence of co-infection of
95 SARS-CoV-2 and HIV (25). HIV can attenuate immunity to other infections. The mechanism may
96 differ between co-infecting pathogens but thought to involve a compromised antibody response
97 because of depletion and dysregulation of CD4 helper T cells (26, 27). ART allows people living
98 with HIV to avoid the worst consequences of HIV infection, which are the result of severe depletion
99 of CD4 T cells (28). However, lack of adherence to ART and development of drug resistance
100 mutations leads to ongoing HIV replication, which, if left to persist for years, results in advanced
101 HIV disease mediated immune suppression (28). We and others have recently shown that
102 advanced HIV disease may lead to delayed clearance of SARS-CoV-2 and evolution of the SARS-
103 CoV-2 virus (29, 30), similar to what is observed in immune suppression caused by other factors
104 (31-35).

105

106 Here we mapped neutralization of ancestral, Beta, Alpha and Delta variant viruses and a virus
107 evolved in advanced HIV disease immune suppression by antibodies elicited by each strain or
108 variant. These strains were isolated from infections in South Africa. The SARS-CoV-2 antibody
109 neutralization response tested was from the blood of convalescent individuals infected in one of
110 three SARS-CoV-2 infection waves in South Africa, where the first wave was composed of
111 infections by ancestral strains with the D614G substitution, the second dominated by the Beta
112 variant, and the third by the Delta variant (Fig 1A). We obtained viral isolates from the upper
113 respiratory tract and blood derived plasma from convalescent individuals for each infection wave,
114 and we sequenced viruses eliciting plasma immunity to validate the infecting virus where possible
115 (Table S1). A phylogenetic tree of the variants shows the genetic relationships between the Alpha,
116 Beta, Gamma, Delta and Lambda variants (Fig 1B), as well as the virus evolved from infection of
117 an ancestral SARS-CoV-2 strain in a person with advanced HIV disease (described below).

118

119 We used a live virus neutralization assay (LVNA) to quantify neutralization. LVNA reads out as
120 the reduction in the number of infection foci at different neutralizing plasma dilutions which is used
121 to obtain the plasma dilution needed for 50% inhibition. We report focus reduction neutralization
122 test (FRNT)₅₀, the reciprocal of this dilution (2). We observed that neutralization capacity of
123 ancestral virus elicited plasma declined 7.2-fold against the Beta virus (Fig 1C). In contrast, it
124 declined only two-fold against the Delta virus (Fig 1D). Neutralization did not decline against the
125 Alpha variant (Fig 1E). Using Beta-elicited plasma, we observed a 12.4-fold decline of
126 neutralization of Delta virus relative to Beta virus (Fig 1F). Alpha virus was well neutralized by

127 Beta elicited plasma, with a 2.7-fold decline compared to the matched Beta virus (Fig 1G). A
128 dramatic decline was observed when Delta plasma was used to neutralize Beta virus. This
129 resulted in a 33.6-fold drop compared to Delta virus neutralization (Fig 1H). Alpha also showed
130 relatively high neutralization escape from Delta elicited plasma, at 10.4-fold relative to Delta virus
131 (Fig 1I), although this was considerably lower than Beta escape. Because we have not previously
132 observed the degree of escape in terms of fold-change seen with Beta virus neutralization by
133 Delta elicited plasma, we repeated the experiments using a pseudovirus neutralization assay (10).
134 We observed increased sensitivity to neutralization with this system relative to LVNA. However,
135 the drop in neutralization of Beta virus with Delta plasma, at 26.1-fold, was very similar to the
136 LVNA results (Fig 1J).

137
138 We next characterized SARS-CoV-2 that had evolved in a person with advanced HIV diagnosed
139 in late September 2020 with SARS-CoV-2 infected with the ancestral lineage B.1.1.273 which we
140 previously described in a case report (30). The study participant was discharged following clinical
141 recovery 10 days post-diagnosis according to South Africa guidelines and remained
142 asymptomatic for most study visits. HIV viremia persisted up to day 190 post-diagnosis due to
143 irregular ART adherence and SARS-CoV-2 titer was high, ranging from a Ct of 16 to 27 (30).
144 Diagnosis of SARS-CoV-2 infection was 16 days post-symptom onset and study enrollment was
145 6 days post-diagnosis (30). Last positive qPCR result was early May 2021. Phylogenetic mapping
146 is consistent with a single infection event (Fig 1B). The CD4 count was <10 at enrollment (Fig 2A
147 top row). It increased at later timepoints, possibly due to the improved adherence to ART and a
148 switch to dolutegravir based therapy which reduced the HIV viral load to below the level of clinical
149 detection (30). SARS-CoV-2 was detected by qPCR until day 216 post-diagnosis (Fig 2A second
150 row). We attempted to isolate live virus up to and including the day 216 post-diagnosis swab
151 sample. While there was insufficient sample to isolate virus from the day 0 swab, we isolated and
152 expanded SARS-CoV-2 from subsequent swabs until and including day 190 post-SARS-CoV-2
153 diagnosis (Fig 2A third row). Successful isolation indicates that live virus was shed at that time.
154 RBD specific IgG antibodies in the blood were at borderline detection levels (slightly above the
155 mean negative control + 2 std) at the early timepoints but were detected at higher levels starting
156 day 190 (Fig 2A fourth row, Fig S1).

157
158 Outgrown virus was sequenced to detect majority and minority variants (Fig 2B). The mutations
159 found in the outgrown virus were representative of the virus in the swab from the matched time
160 point (Tables S2-S3) except for the R682W substitution at the furin cleavage site on the day 6

161 sample. This mutation evolves *in vitro* during expansion in VeroE6 cells and likely confers
162 moderate neutralization escape (36). E484K was first detected on day 6 (Fig 2B). This mutation
163 persisted at day 20 and 34 but was replaced with the F490S substitution starting on day 71 when
164 the K417T mutation was also detected. The N501Y mutation was detected in the virus isolated
165 on day 190 post-diagnosis. Mutations were clustered in the RBD, including K417T, F490S, and
166 N501Y in the day 190 viral isolate (Fig 2C, see Fig S2 for mutations per timepoint). Among the
167 RBD mutations in the day 190 isolate, K417T is found in the Gamma variant, and F490S is found
168 in the Lambda variant. Among NTD mutations, T95I is found in Mu, and R190K is at the same
169 location as the R190S in Gamma. N501Y is found in Beta, among others. Interestingly the
170 Omicron variant has emerged as this work was being revised and has mutations at many of the
171 same sites as the evolving virus described here, also shared with some of the other variants
172 (https://covdb.stanford.edu/page/mutation-viewer/#sec_b-1-351). This includes the D796Y
173 mutation only found in Omicron among the major variants (Fig 2B).

174
175 We tested three of the isolates for neutralization: viruses outgrown from day 6 and day 20 swabs
176 (designated D6, D20) representing viruses from early infection, and virus outgrown from the day
177 190 swab (D190), after substantial evolution. Neutralization of the D6, D20, and D190 isolates by
178 self-plasma was low at the early timepoints (Fig 2D). However, neutralization of D6 and D20 was
179 evident by plasma sampled from day 190 and was more pronounced in the plasma sampled from
180 day 216. The D6 isolate was the most sensitive to neutralization by day 216 plasma. Neutralization
181 declined for D20 and further for D190, suggesting sequential evolution of escape (Fig 2D). The
182 ancestral virus and Beta and Delta variants were also tested for neutralization using day 216
183 plasma. Neutralization was lower for all three non-self strains relative to self-derived virus. The
184 strongest neutralization was of ancestral virus. Delta was neutralized to a lesser degree, and Beta
185 was not detectably neutralized (Fig 2D).

186
187 We also tested the D6, D20, and D190 isolates against plasma from other convalescent
188 participants infected with ancestral virus. Neutralization of D190 by ancestral infection elicited
189 plasma was decreased dramatically relative to D6, with FRNT50 for D190 being 9.3-fold lower
190 despite the presence of the E484K mutation in D6 (Figure 2E). The difference was smaller
191 between D190 and D20 (5.1-fold, Figure 2F), consistent with evolution of some neutralization
192 escape in D20 relative to D6. We also tested neutralization of D190 virus using Pfizer BNT162b2
193 vaccinated participants (Table S4 lists participant characteristics). BNT162b2-elicited plasma
194 neutralization capacity was decreased 5-fold against D190 relative to ancestral virus with the

195 D614G mutation (Fig 2G). We compared neutralization of Beta, D6, D20, and D190 on a subset
196 of remaining BNT162b2 plasma samples from 5 participants 5-6 months post-vaccine, where
197 neutralization declined to relatively low levels. Despite this limitation, neutralization showed a
198 pattern consistent with the other results: D190 neutralization escape was very similar to Beta,
199 while D6 and D20 showed no escape from BNT162b2 elicited neutralization (Fig S3). A 5-fold
200 reduction is less than the fold-drop we previously obtained for the Beta variant with convalescent
201 plasma from previous infection (9) and confirmed below. Moreover, BNT162b2 is effective against
202 Beta, albeit with some reduction. Therefore, these results are consistent with substantial but
203 incomplete escape of D190 from BNT162b2.

204
205 We next assessed the serological distance between D190, D6, the ancestral strain, Beta, Delta,
206 and Alpha variants. We tested against convalescent plasma obtained from participants infected
207 with ancestral strains or Beta or Delta variants. Neutralization by ancestral plasma immunity
208 declined 8.8-fold relative to ancestral virus for the D190 isolate (Fig 2H), similar to the Beta variant.
209 In contrast, there was only a 1.6-fold decline for the D6 isolate (Fig 2I). D190 virus was neutralized
210 relatively well by Beta variant infection elicited plasma, with a 2.6-fold reduction (Fig 2J). This was
211 similar to D6 neutralization, which was reduce 2.3-fold relative to Beta (Fig 2K). A much more
212 dramatic decline was observed with Delta variant elicited immunity: a 27.1-fold drop in
213 neutralization capacity compared to neutralization of Delta virus (Fig 2L). Escape from Delta
214 elicited immunity was much more moderate for D6, with a 5.4-fold decline compared to Delta virus
215 (Fig 2M).

216
217 Mapping the results (Fig 2N) shows that the Beta and Delta variants are serologically far apart,
218 with ancestral virus forming a hub. The greater distance of Beta relative to Delta from ancestral
219 is consistent with Beta being an escape variant and so evolving antibody escape mutations in the
220 RBD as well as mutations and deletions in the NTD. The Alpha variant was serologically like the
221 ancestral strain and was well neutralized by Beta plasma. However, it did escape Delta elicited
222 neutralization. D6 was serologically similar to the ancestral strain even though it had the E484K
223 substitution, and the in vitro evolved R682W which is reported to confer a moderate decrease in
224 neutralization. In contrast, the D190 virus, which underwent extensive evolution, was serologically
225 like Beta despite sequence differences. It had escape from ancestral and a much more
226 pronounced escape from Delta elicited plasma, similar to the Beta virus. We did not map Gamma,
227 but neutralization escape of the Delta but not the Beta variant from Gamma elicited neutralization
228 (17) may suggest that Beta and Gamma are serologically similar.

229

230 We have shown that Delta, Beta, Alpha, and the D190 virus we characterize here, are all escape
231 variants/strains in the sense that each can escape from neutralizing immunity elicited by at least
232 one other variant. This is even though D190 virus and Beta and Alpha variants predate Delta and
233 so Delta escape could not have been selected for. Furthermore, Delta shows weak and Alpha no
234 escape from immunity elicited by ancestral strains, indicating they may have evolved to better
235 transmit and not to escape. This could happen because antibodies are elicited to preferred sites
236 on spike and these differ between variants. Antibodies against the RBD of ancestral strains are
237 concentrated around the E484 site (class 2) which differs between ancestral and Beta (19). In
238 contrast, Beta elicits a stronger response to the class 3 epitope spanning sites 443 to 452 in the
239 RBD (37), where Beta and ancestral do not differ and cross-neutralization by Beta immunity is
240 effective. Delta and Beta do differ at class 3 sites, possibly leading to Delta escape of Beta
241 immunity. Differences in the NTD would further decrease cross-neutralization.

242

243 D190 did show escape from BNT162b2 elicited neutralization, although a 5-fold reduction is less
244 than the fold-drop we obtain for the Beta variant with convalescent ancestral plasma. BNT162b2
245 is effective against Beta, albeit with some reduction (38). Therefore, these results are consistent
246 with substantial but incomplete escape of D190 from BNT162b2.

247

248 The driving force behind the evolution of D190 may have been the presence of very low levels of
249 antibody to SARS-CoV-2 which may select antibody escape mutations. While the participant
250 described here did develop a neutralization response and did clear the virus, the response was
251 strongest against the ancestral-like virus early in infection and weak for the Beta-like D190 isolate,
252 indicating a considerable lag between SARS-CoV-2 evolution and neutralization. Consistent with
253 this, neutralization was detectable for the ancestral strain but not Beta virus. Delta virus
254 neutralization was also low. All non-participant derived viruses were neutralized more poorly than
255 participant derived viruses. This may be a source of vulnerability for future SARS-CoV-2 infections
256 in the participant.

257

258 A limitation of this study is that we have characterized one case. Out of 93 PLWH in our cohort at
259 the time of analysis (25), 13 had persistent CD4<200 but only one (~1%) showed extensive
260 evolution of SARS-CoV-2 as described here. However, as there are about 8 million PLWH in
261 South Africa, (<https://www.unaids.org/en/regionscountries/countries/southafrica>), assuming a
262 frequency of 1% would translate to 80,000 people where SARS-CoV-2 evolution could occur. We

263 have previously reported on a 2-fold decrease in the frequency of detectable HIV antiretroviral
264 therapy and 2-fold increase in detectable HIV viremia in SARS-CoV-2 infected people in the
265 second versus first infection wave in South Africa (25). If immunosuppression by advanced HIV
266 drives SARS-CoV-2 evolution, ART coverage should be increased to prevent it.

267
268 Antigenic cartography has been extensively used in influenza (39). Genetic distance may be
269 partially but not completely a proxy for antigenic distance, as some substitutions can lead to large
270 changes in antigenicity while others lead to minor effects (39). It is unclear whether genetic
271 distance is a good measure for antigenic differences between SARS-CoV-2 variants. For
272 example, based on the phylogenetic tree (Fig 1B) it is not obvious that Beta elicited immunity
273 would neutralize Alpha virus better than Delta immunity. Perhaps this is because SARS-CoV-2
274 evolution seems be closer to HIV, where multiple strains radiate from a common ancestor (40),
275 than to the stepwise progression in influenza (39). How far the variants will diverge for each other
276 is unclear, but if further divergence occurs, SARS-CoV-2 may form serotypes such as occur with
277 polio (41) and dengue (42) viruses. With serotypes, antibody immunity is developed to the
278 infecting but not the other serotypes. This may imply that a vaccine which is based on one variant
279 or strain or previous infection with that variant may generate immunity which is vulnerable to
280 infection by another variant.

281
282 What surprised us in the participant with prolonged infection was that, over the 6-month period
283 where SARS-CoV-2 titer was high, infection was for the most part asymptomatic. One explanation
284 is that the virus evolved attenuated pathogenicity or had low pathogenicity to begin with. Another
285 may that high pathogen levels over time are tolerated because immunosuppression reduces the
286 inflammatory response. This is observed in tuberculosis (43). No attenuated pathogenicity was
287 observed with the Beta escape variant (25).

288
289 As this work is being revised, the Omicron variant has been detected in South Africa. Omicron
290 (B.1.1.529) is derived from strains which were common in the first infection wave in South Africa,
291 1.5 years ago, but were then supplanted first by Beta, then Delta. There may be several
292 possibilities for how this virus persisted so long without being detected. One explanation is that
293 Omicron may have evolved in a region with less developed genomic surveillance relative to South
294 Africa and may have arrived in South Africa recently. Alternatively and without excluding the
295 former possibility, it may have persisted in a single immune compromised host until it acquired a
296 critical mass of mutations that would allow it to effectively transmit in a population where the

297 prevalence of previous infection is high (44, 45). In support of this, previous studies investigating
298 SARS-CoV-2 evolution in people immune-compromised for reasons other than advanced HIV
299 disease have established that such evolution leads to immune escape mutations (31, 33).
300 Therefore, evolution of escape is not related directly to HIV but rather to advanced unsuppressed
301 disease which leads to severe damage to the immune response. If the B.1.1.273 strain described
302 in this work and Omicron share a common evolutionary mechanism, Omicron may have Beta-like
303 serology, substantial but incomplete escape from neutralizing immunity elicited by mRNA
304 vaccines, and strong escape of Delta elicited immunity which may lead to reinfections. Whether
305 other features could also be shared, such as potentially mild pathogenicity, is yet unknown.
306
307
308
309

310 **Methods**

311

312 Ethical statement

313 Nasopharyngeal and oropharyngeal swab samples and blood samples were obtained from
314 hospitalized adults with PCR-confirmed SARS-CoV-2 infection or vaccinated individuals who
315 were enrolled in a prospective cohort study approved by the Biomedical Research Ethics
316 Committee at the University of KwaZulu–Natal (reference BREC/00001275/2020).

317

318 Whole-genome sequencing, genome assembly and phylogenetic analysis

319 cDNA synthesis was performed on the extracted RNA using random primers followed by gene-
320 specific multiplex PCR using the ARTIC V.3 protocol ([https://www.protocols.io/view/covid-19-](https://www.protocols.io/view/covid-19-artic-v3-illumina-library-construction-an-bibtkann)
321 [artic-v3-illumina-library-construction-an-bibtkann](https://www.protocols.io/view/covid-19-artic-v3-illumina-library-construction-an-bibtkann)). In brief, extracted RNA was converted to cDNA
322 using the Superscript IV First Strand synthesis system (Life Technologies) and random hexamer
323 primers. SARS-CoV-2 whole-genome amplification was performed by multiplex PCR using
324 primers designed using Primal Scheme (<http://primal.zibraproject.org/>) to generate 400-bp
325 amplicons with an overlap of 70 bp that covers the 30 kb SARS-CoV-2 genome. PCR products
326 were cleaned up using AmpureXP purification beads (Beckman Coulter) and quantified using the
327 Qubit dsDNA High Sensitivity assay on the Qubit 4.0 instrument (Life Technologies). We then
328 used the Illumina Nextera Flex DNA Library Prep kit according to the manufacturer's protocol to
329 prepare indexed paired-end libraries of genomic DNA. Sequencing libraries were normalized to 4
330 nM, pooled and denatured with 0.2 N sodium acetate. Then, a 12-pM sample library was spiked
331 with 1% PhiX (a PhiX Control v.3 adaptor-ligated library was used as a control). We sequenced
332 libraries on a 500-cycle v.2 MiSeq Reagent Kit on the Illumina MiSeq instrument (Illumina). We
333 assembled paired-end fastq reads using Genome Detective 1.126
334 (<https://www.genomedetective.com>) and the Coronavirus Typing Tool. We polished the initial
335 assembly obtained from Genome Detective by aligning mapped reads to the reference sequences
336 and filtering out low-quality mutations using the bcftools 1.7-2 mpileup method. Mutations were
337 confirmed visually with BAM files using Geneious software (Biomatters). We analyzed sequences
338 from the six different time points (D6, D20, D34, D71, D106 and D190) against a global reference
339 dataset of 3883 genomes using a custom build of the SARS-CoV-2 NextStrain
340 (<https://github.com/nextstrain/ncov>). The workflow performs alignment of genomes, phylogenetic
341 tree inference, tree dating and ancestral state construction and annotation. The phylogenetic tree
342 was visualized using ggplot and ggtree. All sequences from the participant clustered in a

343 monophyletic clade ([https://nextstrain.org/groups/ngs-sa/COVID19-AHRI-](https://nextstrain.org/groups/ngs-sa/COVID19-AHRI-2021.05.27?label=clade:HIV%20patient)
344 [2021.05.27?label=clade:HIV%20patient](https://nextstrain.org/groups/ngs-sa/COVID19-AHRI-2021.05.27?label=clade:HIV%20patient)) that are well separated from the rest of the phylogeny.

345

346 Cells

347 Vero E6 cells (ATCC CRL-1586, obtained from Cellonex in South Africa) were propagated in
348 complete DMEM with 10% fetal bovine serum (Hylone) containing 1% each of HEPES, sodium
349 pyruvate, L-glutamine and nonessential amino acids (Sigma-Aldrich). Vero E6 cells were
350 passaged every 3–4 days. The H1299-E3 cell line for first-passage SARS-CoV-2 expansion,
351 derived as described in (9), was propagated in complete RPMI with 10% fetal bovine serum
352 containing 1% each of HEPES, sodium pyruvate, L-glutamine and nonessential amino acids.
353 H1299 cells were passaged every second day. Cell lines have not been authenticated. The cell
354 lines have been tested for mycoplasma contamination and are mycoplasma negative.

355

356 Virus expansion

357 All work with live virus was performed in Biosafety Level 3 containment using protocols for SARS-
358 CoV-2 approved by the AHRI Biosafety Committee. We used ACE2-expressing H1299-E3 cells
359 for the initial isolation (P1 stock) followed by passaging in Vero E6 cells (P2 and P3 stocks, where
360 P3 stock was used in experiments). ACE2-expressing H1299-E3 cells were seeded at 4.5×10^5
361 cells in a 6 well plate well and incubated for 18–20 h. After one DPBS wash, the sub-confluent
362 cell monolayer was inoculated with 500 μ L universal transport medium diluted 1:1 with growth
363 medium filtered through a 0.45- μ m filter. Cells were incubated for 1 h. Wells were then filled with
364 3 mL complete growth medium. After 8 days of infection, cells were trypsinized, centrifuged at
365 300 rcf for 3 min and resuspended in 4 mL growth medium. Then 1 mL was added to Vero E6
366 cells that had been seeded at 2×10^5 cells per mL 18–20 h earlier in a T25 flask (approximately
367 1:8 donor-to-target cell dilution ratio) for cell-to-cell infection. The coculture of ACE2-expressing
368 H1299-E3 and Vero E6 cells was incubated for 1 h and the flask was then filled with 7 mL of
369 complete growth medium and incubated for 6 days. The viral supernatant (P2 stock) was
370 aliquoted and stored at -80°C and further passaged in Vero E6 cells to obtain the P3 stock used
371 in experiments.

372

373 Live virus neutralization assay

374 Vero E6 cells were plated in a 96-well plate (Corning) at 30,000 cells per well 1 day pre-infection.
375 Plasma was separated from EDTA-anticoagulated blood by centrifugation at 500 rcf for 10 min
376 and stored at -80°C . Aliquots of plasma samples were heat-inactivated at 56°C for 30 min and

377 clarified by centrifugation at 10,000 rcf for 5 min. GenScript A02051 anti-spike neutralizing
378 monoclonal antibody was added as a positive control to one column of wells. Virus stocks were
379 used at approximately 50-100 focus-forming units per microwell and added to diluted plasma.
380 Antibody–virus mixtures were incubated for 1 h at 37 °C, 5% CO₂. Cells were infected with 100
381 µL of the virus–antibody mixtures for 1 h, then 100 µL of a 1X RPMI 1640 (Sigma-Aldrich, R6504),
382 1.5% carboxymethylcellulose (Sigma-Aldrich, C4888) overlay was added without removing the
383 inoculum. Cells were fixed 18 h post-infection using 4% PFA (Sigma-Aldrich) for 20 min. Foci
384 were stained with a rabbit anti-spike monoclonal antibody (BS-R2B12, GenScript A02058) at 0.5
385 µg/mL in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% BSA (Sigma-
386 Aldrich) and 0.05% Tween-20 (Sigma-Aldrich) in PBS. Plates were incubated with primary
387 antibody overnight at 4 °C, then washed with wash buffer containing 0.05% Tween-20 in PBS.
388 Secondary goat anti-rabbit horseradish peroxidase (Abcam ab205718) antibody was added at 1
389 µg/mL and incubated for 2 h at room temperature with shaking. TrueBlue peroxidase substrate
390 (SeraCare 5510-0030) was then added at 50 µL per well and incubated for 20 min at room
391 temperature. Plates were imaged in an ELISPOT instrument with built-in image analysis (C.T.L).

392

393 Pseudovirus neutralization assay

394 SARS-CoV-2 pseudotyped lentiviruses were prepared by co-transfecting the HEK 293T cell line
395 with either the SARS-CoV-2 Beta spike (L18F, D80A, D215G, K417N, E484K, N501Y, D614G,
396 A701V, 242-244 del) or the Delta spike (T19R, R158G L452R, T478K, D614G, P681R, D950N,
397 156-157 del) plasmids in conjunction with a firefly luciferase encoding lentivirus backbone
398 plasmid. For the neutralization assay, heat-inactivated plasma samples from vaccine recipients
399 were incubated with the SARS- CoV-2 pseudotyped virus for 1 hour at 37°C, 5% CO₂.
400 Subsequently, 1x10⁴ HEK 293T cells engineered to over-express ACE-2 were added and
401 incubated at 37°C, 5% CO₂ for 72 hours upon which the luminescence of the luciferase gene was
402 measured. CB6 was used as a positive control.

403

404 Receptor binding domain ELISA

405 Plasma samples were tested for anti-SARS-CoV-2 IgG. Flat bottom microplates (ThermoFisher
406 Scientific) were coated with 500 ng/mL of the receptor binding domain (RBD) protein (provided
407 by Dr Galit Alter from the Ragon Institute) and incubated overnight at 4°C. Plates were blocked
408 with a 200 µL/well tris-buffered saline containing 1% BSA (TBSA) and incubated at room
409 temperature (RT) for 1h. Samples were diluted in TBSA with 0.05% Tween-20 (TBSAT) to 1:100.
410 Subsequently, goat anti-human IgG (1:5000)- horseradish peroxidase conjugated secondary

411 antibodies (Jackson ImmunoResearch) were added a 100 μ L/well and incubated at RT for 1h.
412 Bound secondary antibodies were detected using 100 μ L/well 1-step Ultra TMB substrate
413 (ThermoFisher Scientific). Plates were incubated at RT for 3 min in the dark before addition of 1
414 N sulphuric acid stop solution at 100 μ L/well. Plates were washed with 1X high salt TBS containing
415 0.05% Tween-20, three times each after coating and blocking, and five times each after the
416 sample and secondary antibody. The concentration of anti-RBD expressed as ng/mL equivalent
417 of anti-SARS-CoV-2 monoclonal, CR3022 (Genscript). We used pre-pandemic plasma samples
418 as negative controls to define seroconversion cut-offs calculated as mean + 2 std of the negative
419 samples.

420

421 Statistics and fitting

422 All statistics and fitting were performed using MATLAB v.2019b. Neutralization data were fit to

423

$$424 \quad T_x = 1 / (1 + (D / ID_{50}))$$

425

426 Here T_x is the number of foci normalized to the number of foci in the absence of plasma on the
427 same plate at dilution D and ID_{50} is the plasma dilution giving 50% neutralization. $FRNT_{50} =$
428 $1 / ID_{50}$. Values of $FRNT_{50} < 1$ are set to 1 (undiluted), the lowest measurable value.

429

430 Acknowledgements

431 This study was supported by the Bill and Melinda Gates award INV-018944 (AS), National
432 Institutes of Health award R01 AI138546 (AS), South African Medical Research Council awards
433 (AS, TdO, PLM) and National Institutes of Health U01 AI151698 (WVV). PLM is supported by the
434 South African Research Chairs Initiative of the Department of Science and Innovation and the
435 NRF (Grant No 9834). The funders had no role in study design, data collection and analysis,
436 decision to publish, or preparation of the manuscript. We thank Hylton Rodel for advice on figure
437 preparation.

438

439 COMMIT-KZN Team

440 Moherndran Archary, Department of Paediatrics and Child Health, University of KwaZulu-Natal,
441 Durban, South Africa.

442

443 Philip Goulder, Africa Health Research Institute and Department of Paediatrics, Oxford, UK.

444

445 Nokwanda Gumede, Africa Health Research Institute, Durban, South Africa.

446

447 Ravindra K. Gupta, Africa Health Research Institute and Cambridge Institute of Therapeutic
448 Immunology & Infectious Disease, Cambridge, UK.
449
450 Guy Harling, Africa Health Research Institute and the Institute for Global Health, University
451 College London, London, UK.
452
453 Rohen Harrichandparsad, Department of Neurosurgery, University of KwaZulu-Natal, Durban,
454 South Africa.
455
456 Kobus Herbst, Africa Health Research Institute and the South African Population Research
457 Infrastructure Network, Durban, South Africa.
458
459 Prakash Jeena, Department of Paediatrics and Child Health, University of KwaZulu-Natal,
460 Durban, South Africa.
461
462 Zesuliwe Jule, Africa Health Research Institute, Durban, South Africa.
463
464 Thandeka Khoza, Africa Health Research Institute, Durban, South Africa.
465
466 Nigel Klein, Africa Health Research Institute and the Institute of Child Health, University College
467 London, London, UK.
468
469 Henrik Klooverpris, Africa Health Research Institute, Durban, South Africa.
470
471 Alasdair Leslie, Africa Health Research Institute, Durban, South Africa.
472
473 Rajhmun Madansein, Department of Cardiothoracic Surgery, University of KwaZulu-Natal,
474 Durban, South Africa.
475
476 Mohlopheni Marakalala, Africa Health Research Institute, Durban, South Africa.
477
478 Yoliswa Miya, Africa Health Research Institute, Durban, South Africa.
479
480 Mosa Moshabela, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.
481
482 Nokukhanya Msomi, Department of Virology, University of KwaZulu-Natal, Durban, South Africa.
483
484 Kogie Naidoo, Centre for the AIDS Programme of Research in South Africa, Durban, South Africa.
485
486 Zaza Ndhlovu, Africa Health Research Institute, Durban, South Africa.
487
488 Kennedy Nyamande, Department of Pulmonology and Critical Care, University of KwaZulu-Natal,
489 Durban, South Africa.
490
491 Vinod Patel, Department of Neurology, University of KwaZulu-Natal, Durban, South Africa.
492
493 Dirhona Ramjit, Africa Health Research Institute, Durban, South Africa.
494
495 Kajal Reedoy, Africa Health Research Institute, Durban, South Africa.
496
497 Theresa Smit, Africa Health Research Institute, Durban, South Africa.

498
499 Adrie Steyn, Africa Health Research Institute, Durban, South Africa.
500
501 Emily Wong, Africa Health Research Institute, Durban, South Africa.
502

503 **References**

- 504
- 505 1. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, Subbarao K, Kent
506 SJ, Triccas JA, Davenport MP. Neutralizing antibody levels are highly predictive of immune
507 protection from symptomatic SARS-CoV-2 infection. *Nature medicine*. 2021:1-7.
 - 508 2. Earle KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, Dull P,
509 Plotkin SA. Evidence for antibody as a protective correlate for COVID-19 vaccines. *Vaccine*.
510 2021;39(32):4423-8.
 - 511 3. Madhi SA, Baillie V, Cutland CL, Voysey M, Koen AL, Fairlie L, Padayachee SD, Dheda
512 K, Barnabas SL, Borat QE, Briner C, Kwatra G, Ahmed K, Aley P, Bhikha S, Bhiman JN,
513 Borat AE, du Plessis J, Esmail A, Groenewald M, Horne E, Hwa SH, Jose A, Lambe T,
514 Laubscher M, Malahleha M, Masenya M, Masilela M, McKenzie S, Molapo K, Moultrie A,
515 Oelofse S, Patel F, Pillay S, Rhead S, Rodel H, Rossouw L, Taoushanis C, Tegally H,
516 Thombrayil A, van Eck S, Wibmer CK, Durham NM, Kelly EJ, Villafana TL, Gilbert S, Pollard AJ,
517 de Oliveira T, Moore PL, Sigal A, Izu A, Group N-S, Wits VCG. Efficacy of the ChAdOx1 nCoV-
518 19 Covid-19 Vaccine against the B.1.351 Variant. *N Engl J Med*. 2021;384(20):1885-98.
 - 519 4. Garcia-Beltran WF, Lam EC, Denis KS, Nitido AD, Garcia ZH, Hauser BM, Feldman J,
520 Pavlovic MN, Gregory DJ, Poznansky MC. Multiple SARS-CoV-2 variants escape neutralization
521 by vaccine-induced humoral immunity. *Cell*. 2021;184(9):2372-83. e9.
 - 522 5. Supasa P, Zhou D, Dejnirattisai W, Liu C, Mentzer AJ, Ginn HM, Zhao Y, Duyvesteyn
523 HME, Nutalai R, Tuekprakhon A, Wang B, Paesen GC, Slon-Campos J, Lopez-Camacho C,
524 Hallis B, Coombes N, Bewley KR, Charlton S, Walter TS, Barnes E, Dunachie SJ, Skelly D,
525 Lumley SF, Baker N, Shaik I, Humphries HE, Godwin K, Gent N, Sienkiewicz A, Dold C, Levin
526 R, Dong T, Pollard AJ, Knight JC, Klenerman P, Crook D, Lambe T, Clutterbuck E, Bibi S,
527 Flaxman A, Bittaye M, Belij-Rammerstorfer S, Gilbert S, Hall DR, Williams MA, Paterson NG,
528 James W, Carroll MW, Fry EE, Mongkolsapaya J, Ren J, Stuart DI, Screaton GR. Reduced
529 neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. *Cell*.
530 2021;184(8):2201-11 e7.
 - 531 6. Planas D, Bruel T, Grzelak L, Guivel-Benhassine F, Staropoli I, Porrot F, Planchais C,
532 Buchrieser J, Rajah MM, Bishop E, Albert M, Donati F, Prot M, Behillil S, Enouf V, Maquart M,
533 Smati-Lafarge M, Varon E, Schortgen F, Yahyaoui L, Gonzalez M, De Seze J, Pere H, Veyer D,
534 Seve A, Simon-Loriere E, Fafi-Kremer S, Stefic K, Mouquet H, Hocqueloux L, van der Werf S,
535 Prazuck T, Schwartz O. Sensitivity of infectious SARS-CoV-2 B.1.1.7 and B.1.351 variants to
536 neutralizing antibodies. *Nat Med*. 2021;27(5):917-24.
 - 537 7. Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, Wang M, Yu J, Zhang B, Kwong PD,
538 Graham BS, Mascola JR, Chang JY, Yin MT, Sobieszczyk M, Kyratsous CA, Shapiro L, Sheng
539 Z, Huang Y, Ho DD. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature*.
540 2021;593(7857):130-5.
 - 541 8. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, Doolabh D,
542 Pillay S, San EJ, Msomi N, Mlisana K, von Gottberg A, Walaza S, Allam M, Ismail A, Mohale T,
543 Glass AJ, Engelbrecht S, Van Zyl G, Preiser W, Petruccione F, Sigal A, Hardie D, Marais G,
544 Hsiao NY, Korsman S, Davies MA, Tyers L, Mudau I, York D, Maslo C, Goedhals D, Abrahams
545 S, Laguda-Akingba O, Alisoltani-Dehkordi A, Godzik A, Wibmer CK, Sewell BT, Lourenco J,
546 Alcantara LCJ, Kosakovsky Pond SL, Weaver S, Martin D, Lessells RJ, Bhiman JN, Williamson

- 547 C, de Oliveira T. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature*.
548 2021;592(7854):438-43.
- 549 9. Cele S, Gazy I, Jackson L, Hwa SH, Tegally H, Lustig G, Giandhari J, Pillay S, Wilkinson
550 E, Naidoo Y, Karim F, Ganga Y, Khan K, Bernstein M, Balazs AB, Gosnell BI, Hanekom W,
551 Moosa MS, Network for Genomic Surveillance in South A, Team C-K, Lessells RJ, de Oliveira
552 T, Sigal A. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma.
553 *Nature*. 2021;593(7857):142-6.
- 554 10. Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B,
555 Lambson BE, de Oliveira T, Vermeulen M, van der Berg K, Rossouw T, Boswell M,
556 Ueckermann V, Meiring S, von Gottberg A, Cohen C, Morris L, Bhiman JN, Moore PL. SARS-
557 CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nat Med*.
558 2021;27(4):622-5.
- 559 11. Zhou D, Dejnirattisai W, Supasa P, Liu C, Mentzer AJ, Ginn HM, Zhao Y, Duyvesteyn
560 HME, Tuekprakhon A, Nutalai R, Wang B, Paesen GC, Lopez-Camacho C, Slon-Campos J,
561 Hallis B, Coombes N, Bewley K, Charlton S, Walter TS, Skelly D, Lumley SF, Dold C, Levin R,
562 Dong T, Pollard AJ, Knight JC, Crook D, Lambe T, Clutterbuck E, Bibi S, Flaxman A, Bittaye M,
563 Belij-Rammerstorfer S, Gilbert S, James W, Carroll MW, Klenerman P, Barnes E, Dunachie SJ,
564 Fry EE, Mongkolsapaya J, Ren J, Stuart DI, Screaton GR. Evidence of escape of SARS-CoV-2
565 variant B.1.351 from natural and vaccine-induced sera. *Cell*. 2021;184(9):2348-61 e6.
- 566 12. Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, Schaefer-Babajew
567 D, Cipolla M, Gaebler C, Lieberman JA, Oliveira TY, Yang Z, Abernathy ME, Huey-Tubman KE,
568 Hurley A, Turroja M, West KA, Gordon K, Millard KG, Ramos V, Da Silva J, Xu J, Colbert RA,
569 Patel R, Dizon J, Unson-O'Brien C, Shimeliovich I, Gazumyan A, Caskey M, Bjorkman PJ,
570 Casellas R, Hatzioannou T, Bieniasz PD, Nussenzweig MC. mRNA vaccine-elicited antibodies
571 to SARS-CoV-2 and circulating variants. *Nature*. 2021;592(7855):616-22.
- 572 13. Dejnirattisai W, Zhou D, Supasa P, Liu C, Mentzer AJ, Ginn HM, Zhao Y, Duyvesteyn
573 HME, Tuekprakhon A, Nutalai R, Wang B, Lopez-Camacho C, Slon-Campos J, Walter TS,
574 Skelly D, Costa Clemens SA, Naveca FG, Nascimento V, Nascimento F, Fernandes da Costa
575 C, Resende PC, Pauvolid-Correa A, Siqueira MM, Dold C, Levin R, Dong T, Pollard AJ, Knight
576 JC, Crook D, Lambe T, Clutterbuck E, Bibi S, Flaxman A, Bittaye M, Belij-Rammerstorfer S,
577 Gilbert SC, Carroll MW, Klenerman P, Barnes E, Dunachie SJ, Paterson NG, Williams MA, Hall
578 DR, Hulswit RJG, Bowden TA, Fry EE, Mongkolsapaya J, Ren J, Stuart DI, Screaton GR.
579 Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell*. 2021;184(11):2939-54 e9.
- 580 14. Acevedo ML, Alonso-Palomares L, Bustamante A, Gaggero A, Paredes F, Cortés CP,
581 Valiente-Echeverría F, Soto-Rifo R. Infectivity and immune escape of the new SARS-CoV-2
582 variant of interest Lambda. *medRxiv*. 2021:2021.06.28.21259673.
- 583 15. Uriu K, Kimura I, Shirakawa K, Takaori-Kondo A, Nakada TA, Kaneda A, Nakagawa S,
584 Sato K, Genotype to Phenotype Japan C. Neutralization of the SARS-CoV-2 Mu Variant by
585 Convalescent and Vaccine Serum. *N Engl J Med*. 2021.
- 586 16. Mlcochova P, Kemp S, Dhar MS, Papa G, Meng B, Ferreira I, Datir R, Collier DA,
587 Albecka A, Singh S, Pandey R, Brown J, Zhou J, Goonawardane N, Mishra S, Whittaker C,
588 Mellan T, Marwal R, Datta M, Sengupta S, Ponnusamy K, Radhakrishnan VS, Abdullahi A,
589 Charles O, Chattopadhyay P, Devi P, Caputo D, Peacock T, Wattal DC, Goel N, Satwik A,
590 Vaishya R, Agarwal M, Indian S-C-GC, Genotype to Phenotype Japan C, Collaboration C-NBC-
591 Mavousian A, Lee JH, Bassi J, Silacci-Fegni C, Saliba C, Pinto D, Irie T, Yoshida I, Hamilton
592 WL, Sato K, Bhatt S, Flaxman S, James LC, Corti D, Piccoli L, Barclay WS, Rakshit P, Agrawal
593 A, Gupta RK. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature*.
594 2021.
- 595 17. Liu C, Ginn HM, Dejnirattisai W, Supasa P, Wang B, Tuekprakhon A, Nutalai R, Zhou D,
596 Mentzer AJ, Zhao Y, Duyvesteyn HME, Lopez-Camacho C, Slon-Campos J, Walter TS, Skelly
597 D, Johnson SA, Ritter TG, Mason C, Costa Clemens SA, Gomes Naveca F, Nascimento V,

- 598 Nascimento F, Fernandes da Costa C, Resende PC, Pauvolid-Correa A, Siqueira MM, Dold C,
599 Temperton N, Dong T, Pollard AJ, Knight JC, Crook D, Lambe T, Clutterbuck E, Bibi S, Flaxman
600 A, Bittaye M, Belij-Rammerstorfer S, Gilbert SC, Malik T, Carroll MW, Klenerman P, Barnes E,
601 Dunachie SJ, Baillie V, Serafin N, Ditse Z, Da Silva K, Paterson NG, Williams MA, Hall DR,
602 Madhi S, Nunes MC, Goulder P, Fry EE, Mongkolsapaya J, Ren J, Stuart DI, Screaton GR.
603 Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. *Cell*.
604 2021;184(16):4220-36 e13.
- 605 18. Barnes CO, Jette CA, Abernathy ME, Dam KA, Esswein SR, Gristick HB, Malyutin AG,
606 Sharaf NG, Huey-Tubman KE, Lee YE, Robbiani DF, Nussenzweig MC, West AP, Jr., Bjorkman
607 PJ. SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature*.
608 2020;588(7839):682-7.
- 609 19. Greaney AJ, Loes AN, Crawford KH, Starr TN, Malone KD, Chu HY, Bloom JD.
610 Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect
611 recognition by polyclonal human plasma antibodies. *Cell host & microbe*. 2021;29(3):463-76.
612 e6.
- 613 20. Greaney AJ, Starr TN, Barnes CO, Weisblum Y, Schmidt F, Caskey M, Gaebler C, Cho
614 A, Agudelo M, Finkin S, Wang Z, Poston D, Muecksch F, Hatziioannou T, Bieniasz PD,
615 Robbiani DF, Nussenzweig MC, Bjorkman PJ, Bloom JD. Mapping mutations to the SARS-CoV-
616 2 RBD that escape binding by different classes of antibodies. *Nature Communications*.
617 2021;12(1):4196.
- 618 21. McCallum M, De Marco A, Lempp FA, Tortorici MA, Pinto D, Walls AC, Beltramello M,
619 Chen A, Liu Z, Zatta F, Zepeda S, di Iulio J, Bowen JE, Montiel-Ruiz M, Zhou J, Rosen LE,
620 Bianchi S, Guarino B, Fregni CS, Abdelnabi R, Foo SC, Rothlauf PW, Bloyet LM, Benigni F,
621 Cameroni E, Neyts J, Riva A, Snell G, Telenti A, Whelan SPJ, Virgin HW, Corti D, Pizzuto MS,
622 Veesler D. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2.
623 *Cell*. 2021;184(9):2332-47 e16.
- 624 22. Cerutti G, Guo Y, Zhou T, Gorman J, Lee M, Rapp M, Reddem ER, Yu J, Bahna F,
625 Bimela J. Potent SARS-CoV-2 neutralizing antibodies directed against spike N-terminal domain
626 target a single supersite. *Cell Host & Microbe*. 2021;29(5):819-33. e7.
- 627 23. Suryadevara N, Shrihari S, Gilchuk P, VanBlargan LA, Binshtein E, Zost SJ, Nargi RS,
628 Sutton RE, Winkler ES, Chen EC. Neutralizing and protective human monoclonal antibodies
629 recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell*. 2021;184(9):2316-
630 31. e15.
- 631 24. Chi X, Yan R, Zhang J, Zhang G, Zhang Y, Hao M, Zhang Z, Fan P, Dong Y, Yang Y. A
632 neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-
633 2. *Science*. 2020;369(6504):650-5.
- 634 25. Karim F, Gazy I, Cele S, Zungu Y, Krause R, Bernstein M, Khan K, Ganga Y, Rodel HE,
635 Mthabela N, Mazibuko M, Muema D, Ramjit D, Ndung'u T, Hanekom W, Gosnell B, Team C-K,
636 Lessells RJ, Wong EB, de Oliveira T, Moosa Y, Lustig G, Leslie A, Klooverpris H, Sigal A. HIV
637 status alters disease severity and immune cell responses in beta variant SARS-CoV-2 infection
638 wave. *Elife*. 2021;10.
- 639 26. Avelino-Silva VI, Miyaji KT, Mathias A, Costa DA, de Carvalho Dias JZ, Lima SB,
640 Simoes M, Freire MS, Caiaffa-Filho HH, Hong MA. CD4/CD8 ratio predicts yellow fever vaccine-
641 induced antibody titers in virologically suppressed HIV-infected patients. *JAIDS Journal of*
642 *Acquired Immune Deficiency Syndromes*. 2016;71(2):189-95.
- 643 27. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover
644 of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature*. 1995;373(6510):123-6.
- 645 28. Murphy EL, Collier AC, Kalish LA, Assmann SF, Para MF, Flanigan TP, Kumar PN,
646 Mintz L, Wallach FR, Nemo GJ. Highly active antiretroviral therapy decreases mortality and
647 morbidity in patients with advanced HIV disease. *Annals of internal medicine*. 2001;135(1):17-
648 26.

- 649 29. Hoffman SA, Costales C, Sahoo MK, Palanisamy S, Yamamoto F, Huang C, Verghese
650 M, Solis DC, Sibai M, Subramanian A. SARS-CoV-2 Neutralization Resistance Mutations in
651 Patient with HIV/AIDS, California, USA. *Emerging Infectious Diseases*. 2021;27(10).
- 652 30. Karim F, Moosa MY, Gosnell B, Sandile C, Giandhari J, Pillay S, Tegally H, Wilkinson E,
653 San EJ, Msomi N. Persistent SARS-CoV-2 infection and intra-host evolution in association with
654 advanced HIV infection. *medRxiv*. 2021.
- 655 31. Chen L, Zody MC, Di Germanio C, Martinelli R, Mediavilla JR, Cunningham MH,
656 Composto K, Chow KF, Kordalewska M, Corvelo A, Oschwald DM, Fennessey S, Zetkalic M,
657 Dar S, Kramer Y, Mathema B, Germer S, Stone M, Simmons G, Busch MP, Maniatis T, Perlin
658 DS, Kreiswirth BN. Emergence of Multiple SARS-CoV-2 Antibody Escape Variants in an
659 Immunocompromised Host Undergoing Convalescent Plasma Treatment. *mSphere*.
660 2021;6(4):e0048021.
- 661 32. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, Solomon IH, Kuo HH,
662 Boucau J, Bowman K, Adhikari UD, Winkler ML, Mueller AA, Hsu TY, Desjardins M, Baden LR,
663 Chan BT, Walker BD, Lichterfeld M, Brigl M, Kwon DS, Kanjilal S, Richardson ET, Jonsson AH,
664 Alter G, Barczak AK, Hanage WP, Yu XG, Gaiha GD, Seaman MS, Cernadas M, Li JZ.
665 Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. *N Engl J Med*.
666 2020;383(23):2291-3.
- 667 33. Clark SA, Clark LE, Pan J, Coscia A, McKay LGA, Shankar S, Johnson RI, Brusica V,
668 Choudhary MC, Regan J, Li JZ, Griffiths A, Abraham J. SARS-CoV-2 evolution in an
669 immunocompromised host reveals shared neutralization escape mechanisms. *Cell*.
670 2021;184(10):2605-17 e18.
- 671 34. Kemp SA, Collier DA, Datir RP, Ferreira I, Gayed S, Jahun A, Hosmillo M, Rees-Spear
672 C, Mlcochova P, Lumb IU, Roberts DJ, Chandra A, Temperton N, Collaboration C-NBC-,
673 Consortium C-GU, Sharrocks K, Blane E, Modis Y, Leigh KE, Briggs JAG, van Gils MJ, Smith
674 KGC, Bradley JR, Smith C, Doffinger R, Ceron-Gutierrez L, Barcenas-Morales G, Pollock DD,
675 Goldstein RA, Smielewska A, Skittrall JP, Gouliouris T, Goodfellow IG, Gkrania-Klotsas E,
676 Illingworth CJR, McCoy LE, Gupta RK. SARS-CoV-2 evolution during treatment of chronic
677 infection. *Nature*. 2021;592(7853):277-82.
- 678 35. Weigang S, Fuchs J, Zimmer G, Schnepf D, Kern L, Beer J, Luxenburger H, Ankerhold
679 J, Falcone V, Kemming J, Hofmann M, Thimme R, Neumann-Haefelin C, Ulferts S, Grosse R,
680 Hornuss D, Tanriver Y, Rieg S, Wagner D, Huzly D, Schwemmler M, Panning M, Kochs G.
681 Within-host evolution of SARS-CoV-2 in an immunosuppressed COVID-19 patient as a source
682 of immune escape variants. *Nat Commun*. 2021;12(1):6405.
- 683 36. Johnson BA, Xie X, Bailey AL, Kalveram B, Lokugamage KG, Muruato A, Zou J, Zhang
684 X, Juelich T, Smith JK, Zhang L, Bopp N, Schindewolf C, Vu M, Vanderheiden A, Winkler ES,
685 Swetnam D, Plante JA, Aguilar P, Plante KS, Popov V, Lee B, Weaver SC, Suthar MS, Routh
686 AL, Ren P, Ku Z, An Z, Debbink K, Diamond MS, Shi P-Y, Freiberg AN, Menachery VD. Loss of
687 furin cleavage site attenuates SARS-CoV-2 pathogenesis. *Nature*. 2021;591(7849):293-9.
- 688 37. Greaney AJ, Starr TN, Eguia RT, Loes AN, Khan K, Karim F, Cele S, Bowen JE, Logue
689 JK, Corti D, Veesler D, Chu HY, Sigal A, Bloom JD. A SARS-CoV-2 variant elicits an antibody
690 response with a shifted immunodominance hierarchy. *bioRxiv*. 2021:2021.10.12.464114.
- 691 38. On YB, Noor E, Gottlieb N, Sigal A, Milo R. The importance of time post-vaccination in
692 determining the decrease in vaccine efficacy against SARS-CoV-2 variants of concern.
693 *medRxiv*. 2021:2021.06.06.21258429.
- 694 39. Smith DJ, Lapedes AS, De Jong JC, Bestebroer TM, Rimmelzwaan GF, Osterhaus AD,
695 Fouchier RA. Mapping the antigenic and genetic evolution of influenza virus. *science*.
696 2004;305(5682):371-6.
- 697 40. Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, Hahn B, Wolinsky S,
698 Bhattacharya T. Timing the ancestor of the HIV-1 pandemic strains. *science*.
699 2000;288(5472):1789-96.

- 700 41. Minor PD, Ferguson M, Evans DM, Almond JW, Icenogle JP. Antigenic structure of
701 polioviruses of serotypes 1, 2 and 3. *Journal of General Virology*. 1986;67(7):1283-91.
- 702 42. Guzman MG, Alvarez M, Rodriguez-Roche R, Bernardo L, Montes T, Vazquez S, Morier
703 L, Alvarez A, Gould EA, Kourí G. Neutralizing antibodies after infection with dengue 1 virus.
704 *Emerging infectious diseases*. 2007;13(2):282.
- 705 43. Meintjes G, Stek C, Blumenthal L, Thienemann F, Schutz C, Buyze J, Ravinetto R, van
706 Loen H, Nair A, Jackson A. Prednisone for the prevention of paradoxical tuberculosis-
707 associated IRIS. *New England Journal of Medicine*. 2018;379(20):1915-25.
- 708 44. Khan K, Lustig G, Bernstein M, Archary D, Cele S, Karim F, Smith M, Ganga Y, Jule Z,
709 Reedoy K, Miya Y, Mthabela N, Team C-K, Lessells R, de Oliveira T, Gosnell BI, Karim SA,
710 Garrett N, Hanekom W, Bekker LG, Gray G, Blackburn JM, Moosa M-YS, Sigal A.
711 Immunogenicity of SARS-CoV-2 infection and Ad26.CoV2.S vaccination in people living with
712 HIV. *medRxiv*. 2021:2021.10.08.21264519.
- 713 45. Sykes W, Mhlanga L, Swanevelder R, Glatt TN, Grebe E, Coleman C, Pieterse N,
714 Cable R, Welte A, van den Berg K. Prevalence of anti-SARS-CoV-2 antibodies among blood
715 donors in Northern Cape, KwaZulu-Natal, Eastern Cape, and Free State provinces of South
716 Africa in January 2021. *Research Square*. 2021.
- 717
- 718
- 719

720 **Figure legends**

721

722 **Figure 1: Neutralization distance between variants.** (A) Infection waves and variant
723 frequencies in South Africa. (B) Maximum-likelihood phylogenetic tree with evolved virus
724 sequences (red) at 6 time-points in relation to 3883 global sequences with variants shown. (C-E)
725 Neutralization of the Beta (C), Delta (D) and Alpha (E) virus compared to D614G ancestral virus
726 by plasma from convalescent participants infected by ancestral strains (n=8). (F-G) Neutralization
727 of the Delta (F) and Alpha (G) compared to Beta virus by plasma from Beta infections (n=9). (H-
728 I) Neutralization of the Beta (H) and Alpha (I) compared to Delta virus by plasma from Delta
729 infections (n=10). Experiments presented in panels C-I performed by a live virus neutralization
730 assay (LVNA). (J) Neutralization of Beta compared to Delta virus same plasma as (I) using a
731 pseudo-virus neutralization assay (PNA). Red horizontal line denotes most concentrated plasma
732 tested. Numbers in black above each virus strain are geometric mean titers (GMT) of the
733 reciprocal plasma dilution (FRNT50 for LVNA, ID50 for PNA) for 50% neutralization. Numbers in
734 red denote fold-change in GMT between virus strain on the left and the virus strain on the right.
735 p-Values are * <0.05-0.01; ** <0.01-0.001; *** < 0.001-0.0001, **** < 0.0001 as determined by the
736 Wilcoxon rank sum test.

737

738 **Figure 2: Mapping neutralization of variants and evolved virus.** (A) Participant characteristics
739 over 233 days from SARS-CoV-2 diagnosis: CD4 T cell count (cells/ μ L), SARS-CoV-2 by qPCR,
740 virus outgrowth, and presence of anti-RBD IgG. Because IgG levels were close to the background
741 for some timepoints, they were marked as borderline. (B) Majority and minority SARS-CoV-2
742 genotypes in the swab (day 0) and outgrowth (day 6 to 190). X-axis lists substitutions and
743 deletions in spike sequence and positions where mutations are found in variants are highlighted.
744 AF: allele frequency. (C) Cryo-EM structure of the SARS-CoV-2 spike protein. The mutations in
745 day 190 isolated virus (D190) shown as red spheres. (D) Neutralization of day 6 isolated (D6),
746 day 20 isolated (D20), and D190 virus by self-plasma collected days 6 to 216 and the ancestral
747 D614G, Beta and Delta viruses with plasma collected day 216. (E-F) Neutralization of D6 (E) and
748 D20 (F) relative to D190 virus by ancestral infection elicited plasma (n=8). (G) Neutralization of
749 D190 compared to D614G by Pfizer BNT162b2 plasma (n=12). (H-I) Neutralization of D190 (H)
750 and D6 (I) compared to D614G by ancestral plasma (n=8). (J-K) Neutralization of D190 (J) and
751 D6 (K) compared to Beta by Beta plasma (n=9). (L-M) Neutralization of D190 (L) and D6 (M)
752 compared to Delta by Delta plasma (n=10). Red horizontal line denotes most concentrated
753 plasma tested. Numbers in black are GMT FRNT50. Numbers in red are fold-change in GMT

754 between virus strain on left and right. p-values are * <0.05-0.01; ** <0.01-0.001, *** <0.001-0.0001
755 as determined by the Wilcoxon rank sum test. (N) Summary map (not to scale) of serological
756 distances as measured by fold-decrease in neutralization. For clarity, Beta plasma neutralization
757 of D6 not shown.

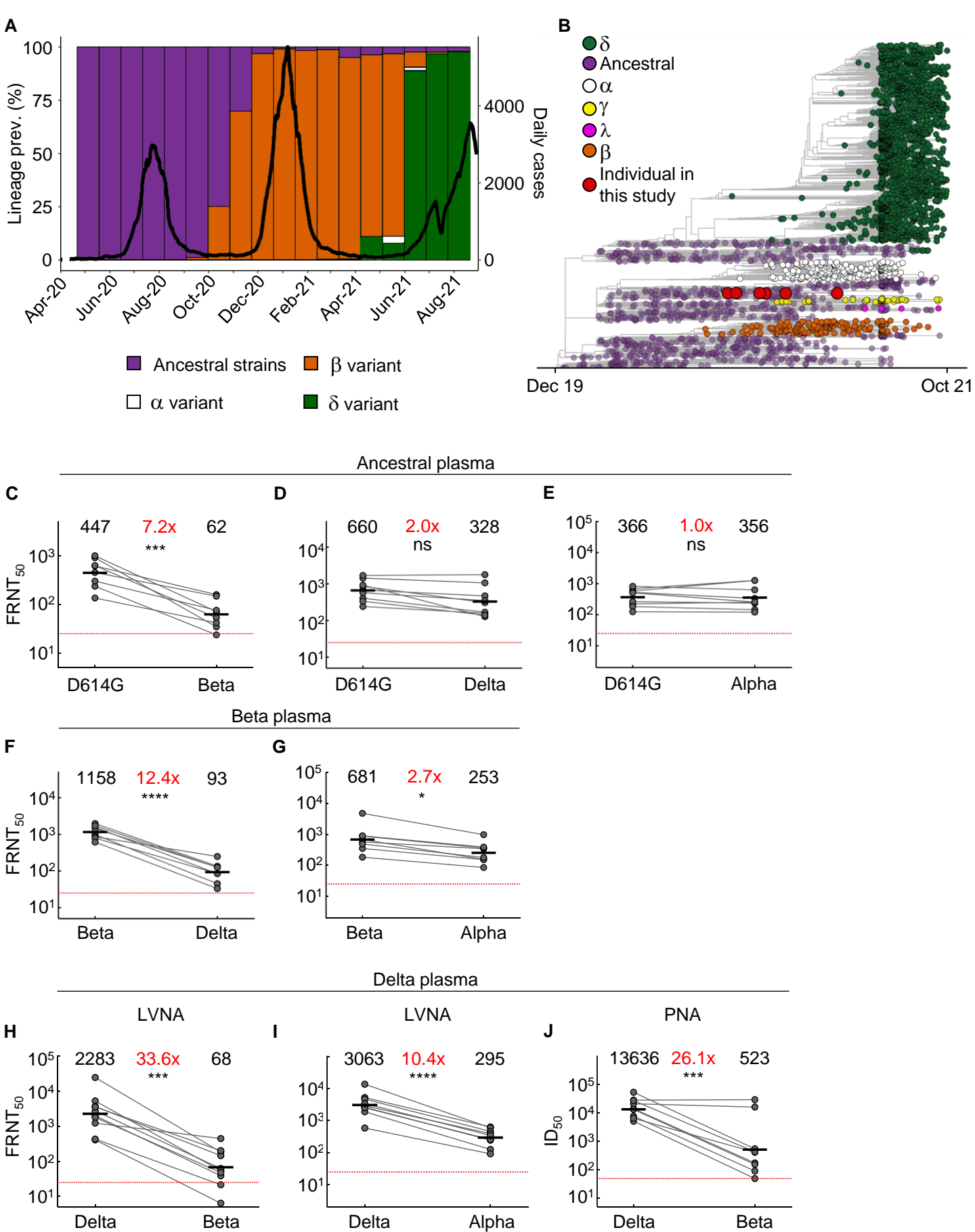


Figure 1

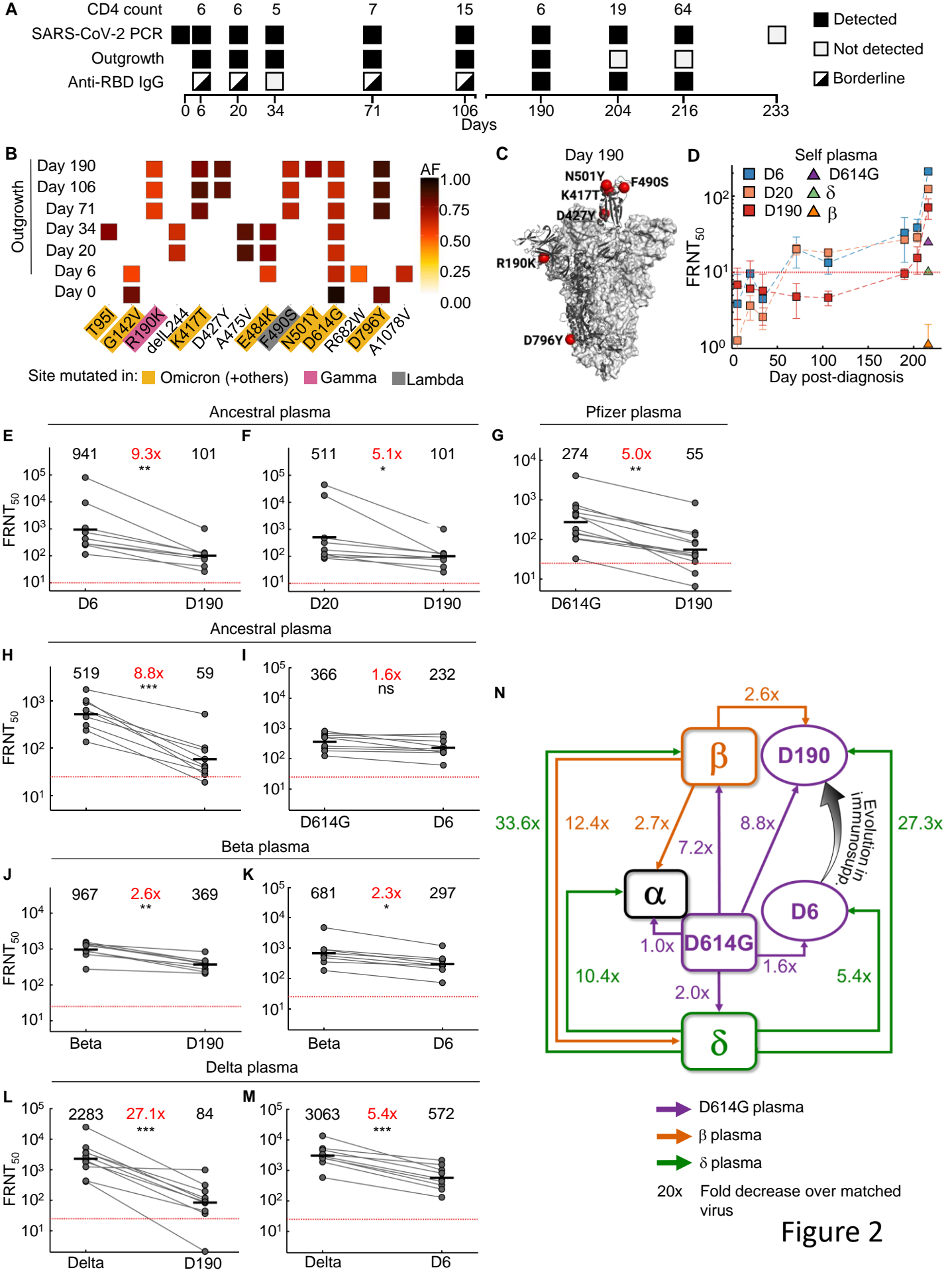


Figure 2

Supplementary materials

SARS-CoV-2 evolved during advanced HIV disease
immunosuppression has Beta-like escape of vaccine
and Delta infection elicited immunity

Cele et al.

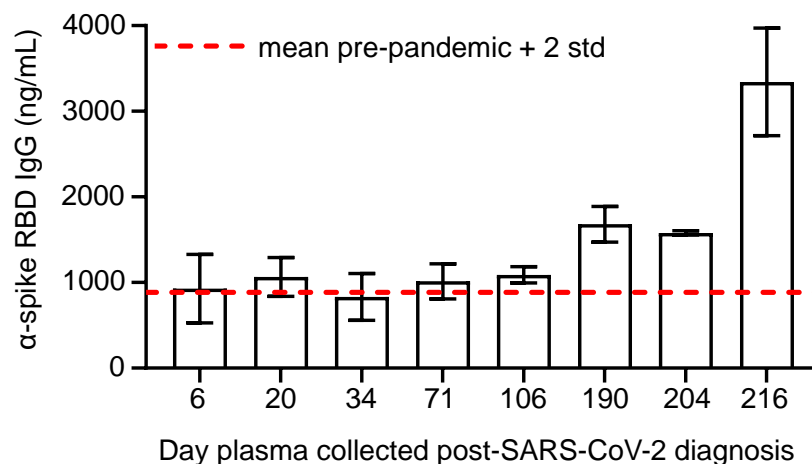


Fig S 1: Spike specific antibody levels with time post-SARS-CoV-2 diagnosis. Shown are mean ($n=4$ replicates per timepoint) and standard deviation of anti-spike RBD antibody concentrations measured in the plasma of the participant with advanced HIV disease by ELISA. Red dashed line denotes the mean + 2 standard deviations of signal from a set of 6 control samples, including plasma of pre-pandemic controls ($n=4$) and pre-pandemic commercial human serum ($n=2$).

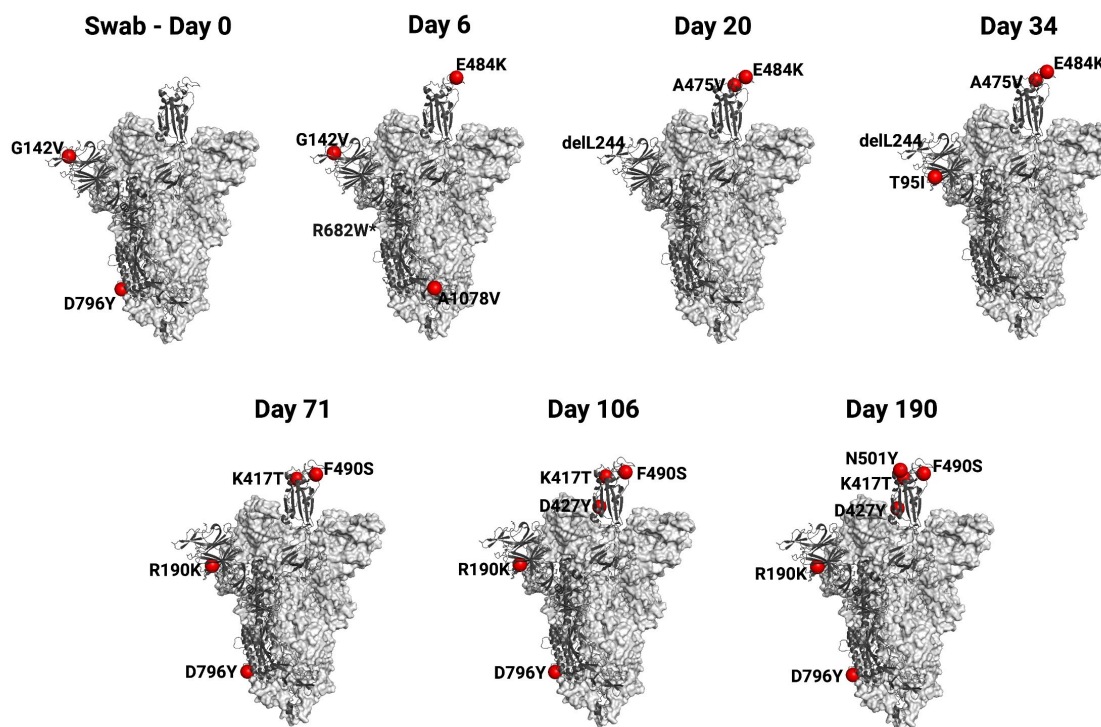


Fig S 2: Location of spike substitutions at different timepoints. Cryo-EM structure of the SARS-CoV-2 spike protein with one RBD protomer in the “up” conformation (PDB ID: 7A94). The mutations which accumulated in the SARS-CoV-2-infected immunocompromised individual over 190 days are shown in red spheres. One protomer is shown in cartoon representation (dark grey) and the other two protomers in surface representation (light grey). Analysis of the structure was performed in Pymol version 2.0.7. The R682 residue is not present in the structure.

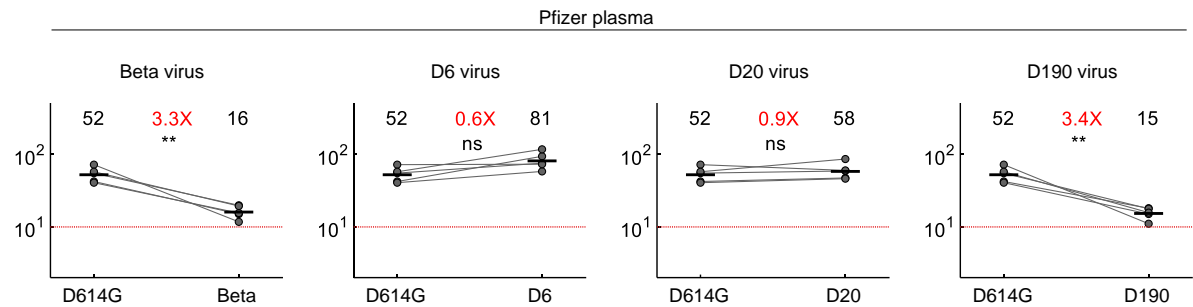


Fig S 3: Neutralization of Beta variant and evolved virus by a subset of BNT162b2 plasma donors. Neutralization of Beta, D6, D20, and D190 compared to D614G by Pfizer BNT162b2 plasma (n=5). Plasma donors were 136072, 136074, 136075, 136076, 136078. Red horizontal line denotes most concentrated plasma tested. Numbers in black are GMT FRNT50. Numbers in red are fold-change in GMT between virus strain on left and right. ** <0.01-0.001, as determined by the Wilcoxon rank sum test.

Table S1: Characteristics of SARS-CoV-2 convalescent study participants

Cohort ID	Variant/strain by infection date	Variant/strain by sequencing	Sequence ID	GISAID Accession	Age range (y)	Sex	Days between symptom onset and plasma collection
039-02-0027	Ancestral	B.1.1.273	K008646	EPI_ISL_2397308	30-39	F	6, 20, 26, 34, 71, 106, 190
039-02-0005	Ancestral	B.1.1	K003667	EPI_ISL_602623	50-59	M	29
039-02-0011	Ancestral	B.1.1.273	K003675	EPI_ISL_602631	40-49	F	32
039-02-0013	Ancestral	B.1.1.117	K003668	EPI_ISL_602624	70+	F	29
039-02-0014	Ancestral	B.1.1	K004289	EPI_ISL_660170	60-69	F	27
039-02-0017	Ancestral	B.1.140	K004295	EPI_ISL_660176	60-69	F	28
039-13-0018	Ancestral	B.1.1.84	K003673	EPI_ISL_602629	40-49	F	28
039-13-0033	Ancestral	B.1	K004291	EPI_ISL_660172	30-39	F	30
039-13-0062	Ancestral	C.9	K004302	EPI_ISL_660181	60-69	M	26
039-02-0030	Beta	Beta	K008635	N/A*	40-49	F	30
039-02-0031	Beta	Beta	K008636	N/A*	40-49	F	41
039-02-0032	Beta	Beta	K008628	N/A*	40-49	M	32
039-02-0033	Beta	Beta	K008637	EPI_ISL_1229368	50-59	M	42
039-02-0034	Beta	N/A [§]	N/A [§]	N/A	30-39	F	32
039-02-0046	Beta	Beta	K010372	N/A*	30-39	M	33
039-02-1011	Beta	Beta	K010356	N/A*	70+	F	48
039-09-0001	Beta	Beta	K008633	EPI_ISL_1229367	60-69	F	29
039-02-0045	Beta	Beta	K010370	N/A*	30-39	F	31
039-02-0104	Delta	Delta	K021407	EPI_ISL_3722338	40-49	F	26
039-02-0106	Delta	Delta	K021399	EPI_ISL_3722335	40-49	M	23 [#]
039-02-0108	Delta	Delta	K021401	N/A*	50-59	M	31
039-02-0109	Delta	Delta	K021225	N/A*	40-49	M	13 [#]
039-13-0153	Delta	Delta	K021400	N/A*	40-49	M	44
039-13-0140	Delta	Delta	K021226	N/A*	50-59	M	44
039-13-0141	Delta	Delta	K020186	EPI_ISL_3939068	40-49	M	31
039-13-0142	Delta	Delta	K020187	EPI_ISL_3939088	30-39	M	31
039-13-0149	Delta	Delta	K020214	EPI_ISL_3447779	50-59	F	30 [#]
039-13-0157	Delta	Delta	K021404	N/A*	30-39	M	32

* <90% coverage. Not submitted to GISAID but sufficient sequence for variant call. [§] Not sequenced. [#] Asymptomatic at diagnosis, date post-diagnostic test used instead of symptom onset date.

Table S2: Read numbers at nucleotides which led to amino acid substitutions or deletions in virus sequenced from the swab

Amino Acid change	Nucleotide change	Codon change	Day 0	Day 6	Day 20	Day 34	Day 71	Day 106	Day 190
<u>T95I</u>	21846C>T	21845 ACT>ATT	ACT – 143 ATT – 0	ACT – 6105 ATT - 9	ACT – 2667 ATT - 298	ACT – 935 ATT – 1626	ACT – 1379 ATT – 36	ACT –11843 ATT – 576	ACT – 231 ATT – 10
<u>G142V</u>	21987G>T	21986 GGT>GTT	GGT – 10 GTT – 40	GGT – 1386 GTT - 622	GGT – 880 GTT - 313	GGT – 954 GTT – 96	GGT – 480 GTT – 5	GGT – 3621 GTT – 12	GGT – 133 GTT – 22
<u>R190K</u>	22131G>A	22130 AGG>AAG	AGG – 46 AAG – 0	AGG – 5591 AAG – 8	AGG – 1702 AAG – 1	AGG – 1685 AAG - 2	AGG – 222 AAG – 1096	Gap in sequence	AGG – 13 AAG – 40
<u>*L244del</u>	22293ACA >del	22811 ACA>del	ACA – 12 del – 1	ACA – 1378 del - 0	ACA – 50 del – 158	ACA – 17 del – 176	ACA – 414 del – 4	ACA – 11 del – 0	ACA – 64 del – 0
<u>K417T</u>	22812A>G	22811 AAG>ACG	AAG – 117 ACG – 0	AAG – 3237 ACG - 1	AAG – 3209 ACG – 0	AAG – 2587 ACG – 1	AAG – 299 ACG – 1362	AAG – 32 ACG – 2	Gap in sequence
<u>D427Y</u>	22841G>T	22925 GAT>TAT	GAT – 107 TAT – 0	GAT – 3856 TAT – 3	GAT – 3719 TAT – 0	GAT – 3434 TAT – 0	GAT – 3434 TAT – 0	GAT – 51 TAT – 4	GAT – 18 TAT – 103
<u>L455F</u>	22927G>T	22925 TTG>TTT	TTG – 11 TTT – 0	TTG – 3714 TTT – 9	TTG – 3219 TTT – 0	TTG – 4331 TTT – 2	TTG – 2983 TTT – 4	TTG – 527 TTT – 317	TTG – 114 TTT – 0
<u>F456L</u>	22928T>C	22928 TTT>CTT	TTT – 12 CTT – 0	TTT – 3739 CTT – 6	TTT – 3248 CTT – 0	TTT – 4361 CTT – 0	TTT – 2971 CTT – 3	TTT – 537 CTT – 310	TTT – 138 CTT – 0
<u>A475V</u>	22986C>T	22985 GCC>GTC	GCC – 17 GTC – 0	GCC – 3409 GTC - 11	GCC – 3629 GTC – 1218	GCC – 2874 GTC – 2421	GCC – 3396 GTC – 101	GCC – 901 GTC – 23	GCC – 177 GTC – 14
<u>E484K</u>	23012G>A	23012 GAA>AAA	GAA – 17 AAA – 0	GAA – 56 AAA - 2063	GAA – 1632 AAA – 2881	GAA – 2395 AAA – 2139	GAA – 3147 AAA – 196	GAA – 688 AAA – 40	GAA – 180 AAA – 53

<u>F490S</u>	23031T>C	23030 TTT>TCT	TTT - 18 TCT - 0	TTT - 1605 TCT - 2	TTT - 4067 TCT - 0	TTT - 4007 TCT - 8	TTT - 341 TCT - 2912	TTT - 182 TCT - 446	TTT - 89 TCT - 125
<u>N501Y</u>	23063A>T	23063 AAT>TAT	AAT - 13 TAT - 0	AAT - 810 TAT - 2	AAT - 2034 TAT - 293	AAT - 2855 TAT - 29	AAT - 2510 TAT - 14	AAT - 282 TAT - 13	AAT - 19 TAT - 180
<u>D614G</u>	23403A>G	23402 GAT>GGT	GAT - 3 GGT - 158	GAT - 8 GGT 6668	GAT - 0 GGT - 5299	GAT - 3 GGT - 2863	GAT - 3 GGT - 1560	GAT - 1 GGT - 26	GAT - 2 GGT - 173
<u>R682W</u>	23606C>T	23606 CGG>TGG	CGG - 98 TGG - 1	CGG - 1831 TGG - 4	CGG - 27 TGG - 0	CGG - 59 TGG - 0	CGG - 124 TGG - 0	CGG - 12 TGG - 1	CGG - 40 TGG - 4
<u>D796Y</u>	23948G>T	23948 GAT>TAT	GAT - 16 TAT - 70	GAT - 4293 TAT - 16	GAT - 1235 TAT - 477	GAT - 568 TAT - 649	GAT - 97 TAT - 791	GAT - 7 TAT - 0	GAT - 2 TAT - 11
<u>A1078V</u>	24795C>T	24974 GCT>GTT	GCT - 219 GTT - 3	GCT - 772 GTT - 4833	GCT - 4397 GTT - 1	GCT - 2519 GTT - 12	GCT - 1462 GTT - 30	GCT - 13695 GTT - 74	GCT - 286 GTT - 17

*Only deletions where the adjacent codon was preserved were counted.

Table S3: Read numbers at nucleotides which led to amino acid substitutions or deletions in virus sequenced from outgrown stock

Amino Acid change	Nucleotide change	Codon change	Day 0 (Swab)	Day 6	Day 20	Day 34	Day 71	Day 106	Day 190
<u>T95I</u>	21846C>T	21845 ACT>ATT	ACT – 143 ATT – 0	ACT – 1342 ATT - 10	ACT – 1540 ATT - 33	ACT – 96 ATT – 1328	ACT – 1609 ATT – 27	ACT – 1593 ATT – 29	ACT – 1325 ATT – 16
<u>G142V</u>	21987G>T	21986 GGT>GTT	GGT – 10 GTT – 40	GGT – 316 GTT - 808	GGT – 1352 GTT - 4	GGT – 1238 GTT – 14	GGT – 1416 GTT – 2	GGT – 1400 GTT – 5	GGT – 977 GTT – 6
<u>R190K</u>	22131G>A	22130 AGG>AAG	AGG – 46 AAG – 0	AGG – 119 AAG – 0	AGG – 357 AAG – 3	AGG – 324 AAG - 2	AGG – 26 AAG – 378	AGG – 22 AAG – 468	AGG – 419 AAG – 33
<u>*L244del</u>	22293ACA >del	22811 ACA>del	ACA – 12 del – 1	ACA – 371 del - 4	ACA – 0 del – 635	ACA – 17 del – 556	ACA – 692 del – 3	ACA – 693 del – 7	ACA – 653 del – 1
<u>K417T</u>	22812A>G	22811 AAG>ACG	AAG – 117 ACG – 0	AAG – 141 ACG - 0	AAG – 122 ACG – 0	AAG – 96 ACG – 0	AAG – 6 ACG – 172	AAG – 0 ACG – 60	AAG – 0 ACG – 17
<u>D427Y</u>	22841G>T	22841 GAT>TAT	GAT – 107 TAT – 0	GAT – 1281 TAT – 7	GAT – 1273 TAT – 1	GAT – 1164 TAT - 1	GAT – 1079 TAT – 42	GAT – 13 TAT – 743	GAT – 6 TAT – 529
<u>L455F</u>	22927G>T	22925 TTG>TTT	TTG – 11 TTT – 0	TTG – 886 TTT – 0	TTG – 978 TTT – 2	TTG – 888 TTT – 3	TTG – 784 TTT – 2	TTG – 636 TTT – 0	TTG – 454 TTT – 1
<u>F456L</u>	22928T>C	22928 TTT>CTT	TTT – 12 CTT – 0	TTT – 1112 CTT – 31	TTT – 1109 CTT – 19	TTT – 1011 CTT – 20	TTT – 882 CTT – 19	TTT – 725 CTT – 2	TTT – 544 CTT – 24
<u>A475V</u>	22986C>T	22985 GCC>GTC	GCC – 17 GTC – 0	GCC – 1180 GTC - 30	GCC – 11 GTC – 1369	GCC – 43 GTC – 1224	GCC – 956 GTC – 22	GCC – 839 GTC – 24	GCC – 687 GTC – 19
<u>E484K</u>	23012G>A	23012 GAA>AAA	GAA – 17 AAA – 0	GAA – 363 AAA - 917	GAA – 27 AAA – 1276	GAA – 55 AAA – 1133	GAA – 1120 AAA – 22	GAA – 1001 AAA – 5	GAA – 814 AAA – 4

<u>F490S</u>	23031T>C	23030 TTT>TCT	TTT – 18 TCT – 0	TTT- 1196 TCT - 15	TTT – 1226 TCT – 10	TTT – 1031 TCT – 30	TTT – 62 TCT – 935	TTT – 31 TCT – 784	TTT – 21 TCT – 669
<u>N501Y</u>	23063A>T	23063 AAT>TAT	AAT – 13 TAT – 0	AAT – 1428 TAT – 2	AAT – 1498 TAT – 1	AAT – 1354 TAT – 0	AAT – 1193 TAT – 2	AAT – 1026 TAT – 0	AAT – 6 TAT – 726
<u>D614G</u>	23403A>G	23402 GAT>GGT	GAT – 3 GGT – 158	GAT – 4 GGT 811	GAT – 6 GGT – 904	GAT – 14 GGT – 886	GAT – 6 GGT – 702	GAT – 10 GGT – 827	GAT – 3 GGT – 834
<u>R682W</u>	23606C>T	23606 CGG>TGG	CGG – 98 TGG – 1	CGG – 189 TGG – 407	CGG – 618 TGG – 5	CGG – 607 TGG – 3	CGG – 523 TGG – 6	CGG – 471 TGG – 5	CGG – 424 TGG – 3
<u>D796Y</u>	23948G>T	23948 GAT>TAT	GAT – 16 TAT – 70	GAT – 1155 TAT – 0	GAT – 1409 TAT – 5	GAT – 1313 TAT – 10	GAT – 38 TAT – 1257	GAT – 6 TAT – 1438	GAT – 12 TAT – 1223
<u>A1078V</u>	24795C>T	24794 GCT>GTT	GCT – 219 GTT – 3	GCT – 772 GTT - 358	GCT – 1319 GTT – 18	GCT – 1270 GTT – 45	GCT – 1418 GTT – 85	GCT – 1418 GTT – 36	GCT – 1264 GTT – 29

*Only deletions where the adjacent codon was preserved were counted.

Table S4: Characteristics of Pfizer BNT162b2 vaccinated participants

Cohort ID	Age range (y)	Sex	Days post-second dose
136023	60-69	M	11
136024	60-69	M	10
136025	50-59	M	18
136026	40-49	F	9
136028	60-69	M	10
136029	20-29	F	8
136071	60-69	M	128
136072	30-39	F	134
136074	20-29	M	131
136075	50-59	M	152
136076	30-39	F	153
136078	30-39	M	158