

1 **Sequence analysis of SARS-CoV-2 in nasopharyngeal samples from patients with COVID-19**
2 **illustrates population variation and diverse phenotypes, placing the in vitro growth properties**
3 **of B.1.1.7 and B.1.351 lineage viruses in context.**

4

5 **Running title:** Genetic diversity of SARS-CoV-2 and growth kinetics

6 **Keywords:** SARS-CoV-2, COVID-19, clinical samples, minor variants, growth kinetics

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28 **Abstract**

29 New variants of SARS-CoV-2 are continuing to emerge and dominate the regional and global
30 sequence landscapes. Several variants have been labelled as Variants of Concern (VOCs) because
31 of perceptions or evidence that these may have a transmission advantage, increased risk of
32 morbidity and/or mortality or immune evasion in the context of prior infection or vaccination.
33 Placing the VOCs in context and also the underlying variability of SARS-CoV-2 is essential in
34 understanding virus evolution and selection pressures. Sequences of SARS-CoV-2 in
35 nasopharyngeal swabs from hospitalised patients in the UK were determined and virus isolated.
36 The data indicated the virus existed as a population with a consensus level and non-synonymous
37 changes at a minor variant. For example, viruses containing the nsp12 P323L variation from the
38 Wuhan reference sequence, contained minor variants at the position including P and F and other
39 amino acids. These populations were generally preserved when isolates were amplified in cell
40 culture. In order to place VOCs B.1.1.7 (the UK 'Kent' variant) and B.1.351 (the 'South African'
41 variant) in context their growth was compared to a spread of other clinical isolates. The data
42 indicated that the growth in cell culture of the B.1.1.7 VOC was no different from other variants,
43 suggesting that its apparent transmission advantage was not down to replicating more quickly.
44 Growth of B.1.351 was towards the higher end of the variants. Overall, the study suggested that
45 studying the biology of SARS-CoV-2 is complicated by population dynamics and that these need
46 to be considered with new variants.

47 **Importance**

48 SARS-CoV-2 is the causative agent of COVID-19. The virus has spread across the planet causing a
49 global pandemic. In common with other coronaviruses, SARS-CoV-2 genetic material (genomes)
50 can become quite diverse as a consequence of replicating inside cells. This has given rise to
51 multiple variants from the original virus that infected humans. These variants may have different
52 properties and in the context of a widespread vaccination program may render vaccines less
53 ineffective. Our research confirms the degree of genetic diversity of SARS-CoV-2 in patients. By
54 isolating viruses from these patients, we show that there is a 100-fold range in growth of even
55 normal variants. Interestingly, by comparing this to the pattern seen with two Variants of
56 Concern (UK and South African variants), we show that at least in cells the ability of the B.1.1.7
57 variant to grow is not substantially different to many of the previous variants.

58 Introduction

59 SARS-CoV-2 emerged late 2019 in Wuhan, China and causes COVID-19 (1). This can be a
60 fatal infection with severe immunopathology in the respiratory system (2). The virus has since
61 spread worldwide and resulted in more than 2.5 million deaths (3) placing large burdens on
62 healthcare infrastructures and global economies. Several vaccines have been granted emergency
63 licensure and these appear to be driving down cases in countries with large scale vaccine roll
64 outs. However, multiple variants have been identified worldwide and these have the potential
65 for vaccine evasion and immune escape, leading to the label of Variants of Concern (VOCs).

66 SARS-CoV-2 has a single stranded positive sense RNA genome about 30kb in length. The
67 first two thirds of the genome is translated to give the viral non-structural proteins (NSP1-16),
68 which includes the viral RNA dependent RNA polymerase (NSP12). Several viral RNA synthesis
69 processes occur during infection including replication of the genome and transcription of a
70 nested set of subgenomic mRNAs (sgmRNAs). This latter process requires discontinuous
71 transcription during negative strand synthesis (4). As a natural consequence, coronaviruses have
72 high levels of recombination. This can result in both deletions and insertions and template
73 switching as well as the formation of defective RNAs. An example of this is the probable insertion
74 of the furin cleavage site in the spike glycoprotein (5). Although SARS-CoV-2 and other
75 coronaviruses have some type of proof-reading capability (6), this is generally thought to help
76 maintain their large genomes, without entering error catastrophe. Otherwise the accumulation
77 of deleterious mutations would result in a rapid loss of fitness and extinction of a viral population
78 (7). Additionally, potential genome modifications can result from nucleotide changes through the
79 action of cellular proteins involved in RNA processing (8). SARS-CoV-2 accumulates mutations at

80 roughly the same frequency as Ebola virus (9). These drivers of genetic diversity and the numbers
81 of people infected has led to multiple lineages and variants of SARS-CoV-2 being identified
82 worldwide.

83 The sgRNAs encode for the main structural proteins, including the envelope protein (E)
84 protein, the membrane (M) protein, the nucleocapsid (N) protein and the spike (S) glycoprotein.
85 The S protein is a component of the enveloped virion and interacts with the angiotensin
86 converting enzyme-2 receptor (ACE-2) found on human cells. The S protein is also the major
87 source of neutralising epitopes and therefore under selection pressure in coronaviruses (and
88 SARS-CoV-2). Other viral proteins are involved in modulating the innate immune response.

89 Many variations in the coronavirus genome occur in the S gene (10-12) and this also has
90 been identified for SARS-CoV-2. For example, the D614G substitution in the SARS-CoV-2 S
91 protein, which emerged by March 2020, demonstrated improved transmissibility compared to
92 Wuhan variants, and proceeded to dominate worldwide subsequently. This mutation is most
93 often accompanied with another amino acid substitution in NSP12, P323L (13). In September
94 2020, a variant of concern, VOC 202012/01 (B.1.1.7 lineage) was detected in Kent in the UK which
95 possessed 23 mutations distinct from the Wuhan reference sequence, including the N501Y
96 substitution in the receptor binding domain of the S protein. This may increase the affinity of
97 spike protein to ACE-2 receptor (14). Initial data suggested this variant could be related to an
98 increased risk of hospitalisation and death (15). The variant has now spread to several countries
99 and modelling studies have suggested increased transmissibility (16). Preliminary experiments in
100 hamsters have identified increased viral shedding compared to the D614G variant (17). However,

101 *in vitro* studies suggest that the B.1.1.7 VOC does not have any replicative advantage in primary
102 airway epithelial cells (18).

103 As variants are likely to continue to emerge on a background of incomplete vaccination
104 globally, understanding the significance of such variants both *in vitro* and *in vivo* is important to
105 provide biological mechanistic data rather than rely on *in silico* modelling to determine their
106 potential threat to vaccines or transmission advantage. To investigate the genetic and phenotypic
107 diversity of SARS-CoV-2 in patients and in the context of the emergence of the B.1.1.7 and B.1.351
108 lineage viruses and concerns around potential higher viral loads, the growth of these viruses was
109 bench marked against the Victoria isolate and clinical isolates from other samples taken during
110 the outbreak.

111 **Results**

112 Although consensus genomes for SARS-CoV-2 are reported on global databases from the
113 sequencing of clinical specimens, in reality the virus will exist as a population within an individual
114 and may also include defective RNAs. Likewise, in some pipelines, viral genomes or variants
115 containing out of place stop codons within ORFs will not be returned as consensus even though
116 they may be dominant. In this case, at a minor variant level, which could represent 49% of other
117 genomes within the same individual, the wildtype protein may be expressed, and counterbalance
118 any aberrantly functioning proteins. To investigate the sequence diversity of SARS-CoV-2 within
119 a patient and to compare the growth of these viral populations to recent VOCs, nasopharyngeal
120 swabs were taken from patients with COVID-19, sequenced and the genotypes and variants of
121 isolated viruses and their growth properties compared in cell culture (Figure 1).

122

123 **Sequence variation of SARS-CoV-2 in clinical swabs compared to Wuhan reference strain**

124 Nine swabs representing different time points in the outbreak in the UK contained
125 recoverable virus that could be isolated and grown. The virus population in these swabs was
126 sequenced and both consensus genomes and minor variants determined. Consensus sequence
127 variation was compared to the reference genome (NC_045512; Wuhan-Hu-1) to see how far the
128 isolates had diverged and with minor variants listed for the secondary and tertiary positions
129 (Supplementary Table 1). Most viruses demonstrated a few amino acid variations compared to
130 the reference sequence. For example, SCV2-006, a lineage B virus sequenced from the swab of
131 a patient from the Diamond Princess cruise ship (February 2020) had only one substitution
132 present, R203K in the N protein (Figure 2). In comparison, sequence analysis of SCV2-009, a virus

133 isolated from a swab sampled from a patient in the UK in March 2020 (Figure 2) now possessed
134 the D614G and P323L substitutions in the spike glycoprotein and NSP12, respectively. These are
135 in contrast to the B.1.1.7 variant, which emerged later in 2020 and is characterised by the
136 presence of 23 amino acid differences from the reference genome. Analysis of the virus
137 population present in the nasopharyngeal isolate of SCV2-009 illustrated the diversity associated
138 with the virus. For example, taking the P323L substitution in NSP12, out of an amino acid
139 coverage of 202, 170 amino acids mapped to L, 12 to P and 9 to F. For the D614G substitution in
140 the spike glycoprotein, out of an amino acid coverage of 3452, 3360 amino acids mapped to G,
141 24 to S and 21 to V. This general pattern is reflected in other clinical isolates. For example, in
142 isolate SCV2-010, in NSP12, out of an amino acid coverage of 285, 273 mapped to L, 50 to I and
143 3 to P. In isolate SCV2-008, in NSP12, out of an amino acid coverage of 153, 130 mapped to L, 9
144 to P and 7 to F. This suggests, for NSP12, that at the minor variant level the reference sequence
145 amino acid is still present, but other amino acids such as F may be common (Supplementary Table
146 1), and subject to selection pressure. In some clinical swabs, for example in N at position 204, the
147 second most common feature is a stop codon. SCV2-011 and SCV2-018 were variants isolated
148 from clinical swabs taken from the same patient but three days apart, these did not vary at the
149 consensus between each other, but did at the minor variant level. SCV2-007 and SCV2-017 were
150 also variants isolated from clinical swabs taken from the same patient but three days apart and
151 did not vary at the consensus between each other in swabs, but did at the minor variant level.

152

153 **Comparison of sequence variation in stocks and after 72 hours in hACE2-A549 cells**

154 In order to assess the biology of the viruses isolated from the clinical swabs and compare
155 their growth to B.1.1.7 and B.1.351, sufficient stocks had to be grown. To isolate SARS-CoV-2
156 from the clinical swabs, the nasopharyngeal sample was filtered and placed on VeroE6 cells with
157 antibiotics and antifungals until CPE was observed. The supernatant was collected from these
158 cells to generate sufficient stocks for infectivity assays and comparisons.

159 Growing virus for stocks may have introduced or selected for specific variants. One of
160 these, that has been characterised for SARS-CoV-2, is a deletion of the furin cleavage site in the
161 spike glycoprotein when grown in Vero E6 cell (19). Therefore, viral stocks were sequenced to
162 ensure they did not possess the deletion and to determine if variation occurred compared to
163 when the virus was sequenced directly from clinical swabs. Comparator viruses of known
164 provenance were obtained from collaborators. The comparator viruses were the B.1.1.7 ('Kent'
165 UK VOC) virus (termed SCV2-019 in this study) obtained at P4, the SARS-CoV-2/Victoria/01/2020
166 (an isolate from Australia) obtained at P3 (termed SCV2-021), and the B.1.351 virus ('South
167 African' VOC) (termed SCV2-022 in this study). These were grown in Vero/hSLAMS as a precaution
168 to prevent selection for the furin deletion. These were also sequenced to ensure they had the
169 variant defining mutations present. For these three comparator viruses, the sequencing showed
170 at the consensus level the furin cleavage site was intact and the other defining variations
171 separating these variants from the Wuhan reference sequence were present (Figure 3).

172 Analysis of the genome diversity between viruses sequenced in swabs from patients and
173 the virus stock used to infect cells indicated that most consensus variations from the Wuhan
174 reference sequence were still present (Supplementary Table 1). The minor variants at selected
175 positions were also still present. For example, in the stock preparation for SCV2-009, at position

176 323 in NSP12, this was read with an amino acid depth of 517. The L was present at a depth of
177 499, P with a depth of 6 and F with a depth of 5, indicating that the consensus level amino acid
178 was still present with P and F at a minor level. For some stock viruses, variation from the
179 reference sequence was lost during preparation of the stock virus. The growth of these viruses
180 from the stocks was compared to a B.1.1.7. and a B.1.351 lineage virus and SARS-CoV-
181 2/Victoria/01/2020, obtained from near the start of the COVID-19 pandemic.

182

183 **Growth comparison of different SARS-CoV-2 variants to Variants of Concern (VOCs).**

184 In order to identify whether the B.1.1.7 (SCV2-019) and B.1.351 (SCV2-022) displayed a
185 growth advantage over less recent strains of the virus, three different cell lines were infected
186 with the viruses at an MOI of 0.01 over the course of 72 hours and the resultant supernatants, at
187 24, 48 and 72 hours titrated by plaque assay on Vero E6 cells. The three different cell lines were
188 Vero E6 (commonly used to grow viral stocks and initial isolates from clinical samples),
189 Vero/hSLAM (reported to prevent deletion of the furin cleavage site in the spike glycoprotein)
190 and hACE2-A549 cells. This latter cell line is based on A549 cells, which are respiratory epithelium
191 in origin, commonly used to study respiratory viruses in cell culture but overexpress the ACE2
192 protein. A549 cells mount an interferon response to virus infection.

193 In Vero E6 cells, eleven SARS-CoV-2 variants followed a similar pattern of growth, with
194 the exception of SCV2-021 (SARS-CoV-2/Victoria/01/2020) which grew at significantly reduced
195 levels compared to other variants by 72 hours post infection (mean 5.7×10^4 PFU/ml, $p=0.006$)
196 (Figure 4A). A similar pattern of growth was observed in all twelve variants in Vero/hSLAM cells,
197 but there was no significant difference ($p>0.05$) in the titres of any of the viruses produced by 72

198 hours post infection (Figure 4B). In contrast, in hACE2-A549 cells, there was more heterogeneity
199 observed between variants, with the range of viral titres being much lower (7.9×10^1 - 3.01×10^4
200 PFU/ml) than that observed in Vero cells at 72 hours post infection. The B.1.1.7 variant (SCV2-
201 019) had the lowest titre at 24 hours post-infection in growth assays in hACE2-A549 cells, before
202 growing to reach a final titre 2.71×10^3 PFU/ml at 72 hours post-infection (Figure 4C). We
203 observed that between variants at the growth extremes at 72 hrs post-infection in hACE2-A549
204 cells there was an approximately <2 log difference between titres of SCV2-007 and SCV2-018,
205 despite the same amount of virus being used as the inoculum (MOI=0.01). For the VOC B.1.1.7
206 the growth at 72 hrs post-infection in hACE2-A549 cells was in the middle of the other variants
207 tested (2.71×10^3 PFU/ml) while the VOC B.1.351 had the second highest final titre of 2.62×10^4
208 PFU/ml. (Figure 4C). In addition, we note that the B.1.351 lineage variant had the highest viral
209 titres in both VeroE6 and Vero/hSLAMs at 24 and 48 hrs post-infection.

210 Comparing viruses grown from the same patient but sampled three days apart (SCV2-007
211 and SCV2-017, and SCV2-011 and SCV2-018 at day one and day three respectively, showed
212 differences in their growth in hACE2-A549 cells. (Supplementary Table 2). There was a 2-log
213 difference in the growth of SCV2-007 and SCV2-017, while there was little difference in the
214 growth of SCV2-011 and SCV2-018.

215

216 **The phenotype of the variants differed widely between cell lines, displaying mixed plaque** 217 **morphology and growth characteristics**

218 The phenotype of the plaques formed by each virus stock was observed in the three
219 different cell lines used at 72 hours post-infection. The appearance of the plaques from the

220 variants differed (Figure 5). SCV2-006, SCV2-011, SCV2-016, SCV2-018 and SCV2-022 had a larger
221 plaque phenotype after growth in Vero E6 cells, compared with SCV2-019 (B.1.1.7) and SCV2-021
222 (SARS-CoV-2/Victoria/01/2020). Equally, some variants displayed a mixed phenotype of both
223 large and small plaques in Vero E6 cells, as seen for SCV2-011, suggesting mixed viral species
224 were present (Figure 5). After growth in Vero/hSLAMS, SCV2-011 and SCV2-018 showed a mixed
225 phenotype after plaque assay. SCV2-019 (B.1.1.7) and SCV2-021 had the smallest plaque
226 phenotypes. After growth in hACE2-A549 cells, SCV2-006 and SCV2-022 (B.1.351) had the largest
227 plaque phenotypes, while SCV2-021 had the smallest plaque phenotype. SCV2-006 and SCV2-016
228 had mixed morphology of both large and small plaques. This illustrates the potential diversity
229 with a viral population.

230

231 **Genetic diversity of variants after passage in the three different cell types**

232 We hypothesised that differences in the phenotypic appearance of viruses and their
233 reproduction might reflect the presence of minor variants and stop codons in their underlying
234 sequences and this was investigated at 72 hrs post-infection in hACE2-A549 cells. All variants
235 have a consensus level genome but also minor variants. In SCV2-016, at 72 hrs post-infection
236 there was a stop codon at consensus level in ORF3A that was also present in the viral stock
237 (Supplementary Table 1). In SCV2-019 (the B.1.1.7. lineage virus) there was a stop codon in ORF8
238 at the consensus level at 72 hr post-infection, which was also present in the stock (Supplementary
239 Table 1). We note that both of these were low read depth, and other amino acids were present
240 at the minor variant level. Stop codons were also present in the variants at a minor variant level
241 (Supplementary Table 1).

242 Discussion

243 Sequence analysis of SARS-CoV-2 in clinical swabs from patients revealed a heterogenous
244 and diverse population from the Wuhan reference sequence. At the minor variant level, a
245 number of variants had genomes which contained premature stop codons. Examples of SARS-
246 CoV-2 genomes encoding non-functioning proteins have been previously identified in the human
247 population. For example, a cohort of patients in Singapore were identified with a deletion in ORF8
248 that was associated with a milder infection (20), although the variant disappeared either through
249 control measures or lack of fitness. This potential disconnect is not restricted to SARS-CoV-2. The
250 balance between consensus and minor variants and the presence of stop codons in virus
251 populations within individual patients has been shown to influence the activity of the Ebola virus
252 RNA dependent RNA polymerase and correlate with outcome in patients with Ebola virus disease
253 (21). Within an individual person with SARS-CoV-2, these mixtures of functioning and presumably
254 non-functioning viral proteins will potentially influence viral load.

255 Recent VOCs include SARS-CoV-2 variants from Nigeria (B.1.525). One of the differences
256 in this variant from the Wuhan consensus sequence is P323F in NSP12. This variation was also
257 identified in a cluster of patients in Northern Nevada in the USA (22). The analysis of variants in
258 this study, isolated earlier in 2020, indicated that an F at position 323 existed at a minor variant
259 level. Therefore, we hypothesize that if an F at this position was advantageous (e.g. altered RdRp
260 activity) then the variant would have been selected during passage. However, this was not the
261 case, and therefore we speculate that the emergence of an F at position 323 in NSP12 may be
262 through founder effect.

263 The growth of different variants, and a variant from near the start of the COVID-19
264 pandemic, were compared in three different cell lines to the growth of two VOCs and the
265 Australia Victoria variant. These VOCs were from the B.1.1.7 and B.1.351 lineages and represent
266 viruses that have an apparent transmission advantage in the general population and/or may be
267 less refractive to currently approved vaccines. There was an approximately 2 log difference in
268 growth at 72 hrs in the hACE2-A549 cells by the different variants. The growth of VOCs in this cell
269 line were within these limits. Extrapolating this observation to the perceived transmission
270 advantage of B.1.1.7 in the human population, would suggest this is not down to the VOC growing
271 to higher titres in cells *in vivo* compared with other variants, though we acknowledge that out *in*
272 *vitro* experiments may not correlate exactly with growth rates *in vivo*. With B.1.1.7, the same
273 heterogenous patterns of disease in humans as other variants, although we note that B.1.1.7 has
274 been associated with a decrease in Ct values from nasopharyngeal swabs and also an increase in
275 mortality in some populations. In contrast, different variants of the coronavirus infectious
276 bronchitis (IBV) can individually cause different spectrums of organ specific disease, and
277 therefore current variations in the genome of SARS-CoV-2 may not automatically equate to
278 radically different disease as observed with IBV. Due to the promiscuous nature of coronavirus
279 RNA synthesis, variants have and will occur all of the time. This emphasises the need for genotype
280 to phenotype studies to place newly emerged variants that have perceived differences in context.

281 Comparison of viruses isolated from the same patients at different time points revealed
282 intriguing differences. While the viruses SCV2-007 and SCV2-017 differed by 2-logs in hACE2-
283 A549 cells at 72 hours, there was little difference observed between the twin viruses SCV2-011
284 and SCV2-018. Notably, the SCV2-017 virus had picked up an additional mutation at 72 hours

285 post-infection – a change from A in the reference genome at position 1120 in NSP3 to a V. It is
286 possible this may be responsible for the difference in growth of this virus to its founder, SCV2-
287 007, and could reflect viral adaptation to the immune response in this individual over the course
288 of infection (Supplementary table 2).

289 The analysis of virus in the nasopharyngeal swabs clearly paints a picture of a diverse
290 population of SARS-CoV-2. When studying isolates, even when grown in cell culture, that
291 population still continues. Thus, whilst lineage defining variations are present at a consensus
292 level, minor variants are present underneath that may have an impact on biology. This would
293 suggest that the study of specific genotypes requires either plaque purification or reverse
294 genetics. However, the study suggests that the viral population (consensus and minor variants)
295 should be taken into account when studying the transmission of SARS-CoV-2.

296 **Methods**

297

298 **Cells.** African green monkey kidney C1008 (Vero E6) cells (Public Health England, PHE) were
299 cultured in Dulbecco's minimal essential medium (DMEM) (Sigma) with 10% foetal bovine serum
300 (FBS) (Sigma) and 0.05mg/ml gentamicin at 37°C/5% CO₂. Vero/hSLAM cells (PHE) were grown in
301 DMEM with 10% FBS and 0.05mg/ml gentamicin (Merck) with the addition of 0.4mg/ml Geneticin
302 (G418; Thermofisher) at 37°C/5% CO₂. Human ACE2-A549 (hACE2-A549), a lung epithelial cell line
303 which overexpresses the ACE-2 receptor, were the kind gift of Oliver Schwartz (23) and were
304 cultured in DMEM with 10% FBS and 0.05mg/ml gentamicin with the addition of 10µg/ml
305 Blastidicin (Invitrogen). Only passage 3-10 cultures were used for experiments.

306

307 **Virus isolation.** The SARS-CoV-2/human/Liverpool/REMRQ0001/2020 isolate (Genbank ID
308 MW041156.1), was used at passage 3. The fourth passage of virus (here named SCV2-
309 006_stockP4) was cultured in Vero E6 cells with DMEM containing 4% FBS and 0.05mg/ml
310 gentamicin at 37°C/5% CO₂ and harvested 48 hours post inoculation. Virus stocks were aliquoted
311 and stored at -80°C.

312 Viruses named SCV2-007 to SCV2-018 were grown from nasopharyngeal swabs of
313 patients using the following method. One hundred microlitres of viral transport media from the
314 swab was mixed with 100µl DMEM with 4% FBS, 0.05mg/ml gentamicin, 25µg/ml plasmocin
315 (Invivogen) and 2.5µg/ml amphotericin B (Merck). These were then filtered using ultrapure MC
316 0.22µm filters (Merck) and the filtrate placed onto cells in a 24 well plate of Vero E6 cells for 1
317 hour. After one hour, the media was topped up with DMEM (2% FBS, 0.05 mg/ml gentamicin,

318 25µg/ml plasmocin, 2.5 µg/ml amphotericin B). Cells were observed daily for cytopathic effect
319 (CPE) and the cell supernatant harvested once CPE was evident. This provided the first passage
320 virus. Stocks of these were then grown in Vero E6 as described above and frozen down in aliquots
321 at -80°C and named SCV2-007 to SCV2-018_stockP2.

322 The B.1.1.7 and B.1.351 isolates were used at passage 4. The fifth passage (here named
323 SCV2-019_stockP5 and SCV2-022_stockP5) were cultured in Vero/hSLAM cells with DMEM
324 containing 4% FBS, 0.05mg/ml gentamicin and 0.4mg/ml geneticin and harvested 72 hours post
325 inoculation. Virus stocks were aliquoted and stored at -80°C. SARS-CoV-2 Victoria/01/2020 was
326 passaged three times in Vero/hSLAM cells. The fourth passage stock (here named SCV2-
327 021_stockP4) was cultured in Vero/hSLAM cells DMEM containing 4% FBS, 0.05mg/ml
328 gentamicin and 0.4mg/ml geneticin and harvested 72 hours post inoculation. Virus stocks were
329 aliquoted and stored at -80°C (Supplementary table 2).

330

331 **Virus titration.** Viral titres of stocks were calculated using plaque assays. Briefly, confluent 24-
332 well plates of Vero E6 cells were inoculated with serial ten-fold dilutions of the stocks in duplicate
333 for one hour at 37°C/5% CO₂. Plates were overlaid with DMEM containing 2% FBS, 0.05mg/ml
334 gentamicin and 2% low melting point agarose (Lonza) and incubated at 37°C/5% CO₂ for 72 hours.
335 Plates were fixed using 10% formalin, the overlay removed, and plates stained using crystal violet
336 solution (Sigma). Virus titre was measured in plaque forming units per ml (PFU/ml).

337

338 **Virus growth kinetics.** Vero E6, Vero/hSLAM and hACE2-A549 cells were grown in 96 well plates
339 for viral growth kinetic experiments. For infection, media was removed from plates and virus

340 inoculum added at an MOI of 0.01 in DMEM containing 2% FBS, 0.05mg/ml gentamicin and the
341 respective selective antibiotics for each cell line (6 wells per timepoint). Plates were incubated at
342 37°C/5% CO₂ for one hour. The inoculum was removed, and cells were washed once with PBS
343 (Sigma). The respective media with 2% FBS (100µl) was added to each well. The cell supernatant
344 was removed from wells and combined (0hrs post infection) and plates incubated further.
345 Supernatants were likewise removed at 24, 48 and 72 hours post infection. Approximately 250µl
346 of the supernatants were aliquoted directly into tubes containing 750µl Trizol LS (Fisher) to
347 inactivate the virus. All supernatants and inactivated supernatants were stored at -80°C until viral
348 titration and RNA extraction could be performed. All infections were performed at least three
349 times in independent experiments.

350

351 **RNA extraction and amplification of viral nucleic acids.** RNA from clinical samples was extracted
352 and DNase treated as described previously. Samples from patients were sequenced using the
353 RLSA approach (24). RNA from viral stocks and from 72-hour post infection cultures were
354 sequenced by Oxford Nanopore long read length sequencing on flow cells run on MinION or
355 GridION.

356

357 **Nanopore sequencing.** Sequencing libraries for amplicons generated by RSLA (24) or ARTIC were
358 prepared following the 'PCR tiling of SARS-CoV-2 virus with Native Barcoding' protocol provided
359 by Oxford Nanopore Technologies using LSK109 and EXP-NBD104/114.

360

361 **Variant calling.** The artic-ncov2019 pipeline v1.2.1 (<https://artic.network/ncov-2019/ncov2019->
362 [bioinformatics-sop.html](https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html)) was used to filter the passed Fastq files produced by Nanopore
363 sequencing with lengths between 800 and 1600 for RSLA, and 400 and 700 for ARTIC. This
364 pipeline was then used to map the filtered reads on the reference SARS-CoV-2 genome
365 (NC_045512.2) by minimap2 and assigned each read alignment to a derived amplicon and
366 excluded primer sequences based on the RSLA and ARTIC V3 primer schemes in the bam files.
367 These bam files were further analysed using DiversiTools
368 (<http://josephhughes.github.io/btctools/>) with the “-orfs” function to generate the ratio of
369 amino acid change in the reads and coverage at each site of protein in comparison to the
370 reference SARS-CoV-2 genome (NC_045512.2). The amino acids with highest ratio and coverage
371 > 10 were used to assemble the consensus protein sequences.

372

373 **Statistics.** Viral titre data was log transformed and one-way ANOVAs performed with post-hoc
374 Bonferroni tests performed to determine if any significant difference at T=72 hours post infection
375 occurred between the SCV2-019 (B.1.1.7) and other viruses in different cell lines.

376

377 **Ethics and clinical information.** The patients from which the virus samples were obtained gave
378 informed consent and were recruited under the International Severe Acute Respiratory and
379 emerging Infection Consortium (ISARIC) WHO Clinical Characterisation Protocol CCP. Ethical
380 approval for data collection and analysis by ISARIC4C was given by the South Central-Oxford C
381 Research Ethics Committee in England (reference 13/SC/0149), and by the Scotland A Research
382 Ethics Committee (reference 20/SS/0028). Samples were use with consent from patients or

383 consultees. The ISARIC WHO CCP-UK study was registered at
384 <https://www.isrctn.com/ISRCTN66726260> and designated an Urgent Public Health Research
385 Study by NIHR. Protocol, patient information sheets, consents, case report forms and process of
386 data and sample access request are available at <https://ISARIC4C.net>.

387

388 **Biosafety.** All work was performed in accordance with risk assessments and standard operating
389 procedures approved by the University of Liverpool Biohazards Sub- Committee and by the UK
390 Health and Safety Executive. Work with SARS-CoV-2 was performed at containment level 3 by
391 personnel equipped with respirator airstream units with filtered air supply.

392

393 **Funding.** This work was funded by U.S. Food and Drug Administration Medical Countermeasures
394 Initiative contract (75F40120C00085) awarded to JAH. The article reflects the views of the
395 authors and does not represent the views or policies of the FDA. This work was also supported
396 by the MRC (MR/W005611/1) G2P-UK: A national virology consortium to address phenotypic
397 consequences of SARS-CoV-2 genomic variation (co-I JAH). JAH is also funded by the Centre of
398 Excellence in Infectious Diseases Research (CEIDR) and the Alder Hey Charity. The ISARIC4C
399 sample collection and sequencing in this study was supported by a grants from the Medical
400 Research Council (grant MC_PC_19059), the National Institute for Health Research (NIHR; award
401 CO-CIN-01) and the Medical Research Council (MRC; grant MC_PC_19059). JAH, GLH, MWC and
402 LT are supported by the NIHR Health Protection Research Unit (HPRU) in Emerging and Zoonotic
403 Infections at University of Liverpool in partnership with Public Health England (PHE), in
404 collaboration with Liverpool School of Tropical Medicine and the University of Oxford (award

405 200907). LT is supported by a Wellcome Trust fellowship [205228/Z/16/Z]. For the purpose of
406 Open Access, the authors have applied a CC BY public copyright licence to any Author Accepted
407 Manuscript version arising from this submission. The views expressed are those of the authors
408 and not necessarily those of the funders.

409 **Author Contributions**

410 TP, XD, RP-R, NR, CH, HG, BJ, JD, GLH, GS, ERA and EIP performed the experiments,
411 sequencing, bioinformatics and isolated virus. Data was analysed by TP, XD, RP-R and
412 JAH. MWC, LT, JPS and JAH supervised the project. MGS, JKB and PJMO established
413 the ISARIC4C consortium that was used to obtain some of the UK clinical isolates used
414 in the study. TP, XD, MWC and JAH wrote the manuscript, all authors provided editing
415 and final approval.

416

417 **Acknowledgements**

418 Clinical isolates used in this study were gathered under the auspices of the ISARIC Coronavirus
419 Clinical Characterisation Consortium (ISARIC4C) and processed at the University of Liverpool. We
420 would like to acknowledge all members of the consortia. Consortium Lead Investigator: J.
421 Kenneth Baillie; Chief Investigator: Malcolm G. Semple; Co-Lead Investigator: Peter J.M.
422 Openshaw; ISARIC Clinical Coordinator: Gail Carson; Co-Investigators: Beatrice Alex, Benjamin
423 Bach, Wendy S. Barclay, Debby Bogaert, Meera Chand, Graham S. Cooke, Annemarie B. Docherty,
424 Jake Dunning, Ana da Silva Filipe, Tom Fletcher, Christopher A. Green, Ewen M. Harrison, Julian
425 A. Hiscox, Antonia Ying Wai Ho, Peter W. Horby, Samreen Ijaz, Saye Khoo, Paul Klenerman,
426 Andrew Law, Wei Shen Lim, Alexander J. Mentzer, Laura Merson, Alison M. Meynert, Mahdad
427 Noursadeghi, Shona C. Moore, Massimo Palmarini, William A. Paxton, Georgios Pollakis, Nicholas
428 Price, Andrew Rambaut, David L. Robertson, Clark D. Russell, Vanessa Sancho-Shimizu, Janet T.
429 Scott, Thushan de Silva, Louise Sigfrid, Tom Solomon, Shiranee Srisakandan, David Stuart,

430 Charlotte Summers, Richard S. Tedder, Emma C. Thomson, A.A. Roger Thompson, Ryan S.
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434 and Catherine A. Shaw; Data and Information System Managers: Jo Dalton, James Lee, Daniel
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436 Connor, Sophie Halpin, Clare Jackson, and Carrol Gamble; Data Integration and Presentation:
437 Gary Leeming, Andrew Law, Murray Wham, Sara Clohisey, Ross Hendry, and James Scott-Brown;
438 Material Management: William Greenhalf, Victoria Shaw, and Sarah McDonald; Patient
439 Engagement: Seán Keating; Outbreak Laboratory Staff and Volunteers: Katie A. Ahmed, Jane A.
440 Armstrong, Milton Ashworth, Innocent G. Asimwe, Siddharth Bakshi, Samantha L. Barlow, Laura
441 Booth, Benjamin Brennan, Katie Bullock, Benjamin W.A. Catterall, Jordan J. Clark, Emily A. Clarke,
442 Sarah Cole, Louise Cooper, Helen Cox, Christopher Davis, Oslem Dincarslan, Chris Dunn, Philip
443 Dyer, Angela Elliott, Anthony Evans, Lorna Finch, Lewis W.S. Fisher, Terry Foster, Isabel Garcia-
444 Dorival, William Greenhalf, Philip Gunning, Catherine Hartley, Antonia Ho, Rebecca L. Jensen,
445 Christopher B. Jones, Trevor R. Jones, Shadia Khandaker, Katharine King, Robyn T. Kiy, Chrysa
446 Koukorava, Annette Lake, Suzannah Lant, Diane Latawiec, L. Lavelle-Langham, Daniella Lefteri,
447 Lauren Lett, Lucia A. Livoti, Maria Mancini, Sarah McDonald, Laurence McEvoy, John McLauchlan,
448 Soeren Metelmann, Nahida S. Miah, Joanna Middleton, Joyce Mitchell, Shona C. Moore, Ellen G.
449 Murphy, Rebekah Penrice-Randal, Jack Pilgrim, Tessa Prince, Will Reynolds, P. Matthew Ridley,
450 Debby Sales, Victoria E. Shaw, Rebecca K. Shears, Benjamin Small, Krishanthi S. Subramaniam,
451 Agnieska Szemiel, Aislynn Taggart, Jolanta Tanianis-Hughes, Jordan Thomas, Erwan Trochu, Libby

452 van Tonder, Eve Wilcock, and J. Eunice Zhang; Local Principal Investigators: Kayode Adeniji, Daniel
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454 Shahedal Bari, Gavin Barlow, Stella Barnass, Nicholas Barrett, Christopher Bassford, David Baxter,
455 Michael Beadsworth, Jolanta Bernatoniene, John Berridge, Nicola Best, Pieter Bothma, David
456 Brealey, Robin Brittain-Long, Naomi Bulteel, Tom Burden, Andrew Burtenshaw, Vikki Caruth,
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459 Dawson, Samir Dervisevic, Phil Donnison, Sam Douthwaite, Ingrid DuRand, Ahilanadan
460 Dushianthan, Tristan Dyer, Cariad Evans, Chi Eziefula, Christopher Fegan, Adam Finn, Duncan
461 Fullerton, Sanjeev Garg, Sanjeev Garg, Atul Garg, Effrossyni Gkrania-Klotsas, Jo Godden, Arthur
462 Goldsmith, Clive Graham, Elaine Hardy, Stuart Hartshorn, Daniel Harvey, Peter Havalda, Daniel B.
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464 Agilan Kaliappan, Vidya Kasipandian, Stephen Kegg, Michael Kelsey, Jason Kendall, Caroline
465 Kerrison, Ian Kerslake, Oliver Koch, Gouri Koduri, George Koshy, Shondipon Laha, Steven Laird,
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470 Katherine Mortimore, Samuel Moses, Mbiye Mpenge, Rohinton Mulla, Michael Murphy, Megan
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472 Paraiso, Brij Patel, Natalie Pattison, Justin Pepperell, Mark Peters, Mandeep Phull, Stefania
473 Pintus, Jagtur Singh Pooni, Frank Post, David Price, Rachel Prout, Nikolas Rae, Henrik Reschreiter,

474 Tim Reynolds, Neil Richardson, Mark Roberts, Devender Roberts, Alistair Rose, Guy Rousseau,
475 Brendan Ryan, Taranprit Saluja, Aarti Shah, Prad Shanmuga, Anil Sharma, Anna Shawcross,
476 Jeremy Sizer, Manu Shankar-Hari, Richard Smith, Catherine Snelson, Nick Spittle, Nikki Staines,
477 Tom Stambach, Richard Stewart, Pradeep Subudhi, Tamas Szakmany, Kate Tatham, Jo Thomas,
478 Chris Thompson, Robert Thompson, Ascanio Tridente, Darell Tupper-Carey, Mary Twagira,
479 Andrew Ustianowski, Nick Vallotton, Lisa Vincent-Smith, Shico Visuvanathan, Alan Vuylsteke,
480 Sam Waddy, Rachel Wake, Andrew Walden, Ingeborg Welters, Tony Whitehouse, Paul Whittaker,
481 Ashley Whittington, Meme Wijesinghe, Martin Williams, Lawrence Wilson, Sarah Wilson,
482 Stephen Winchester, Martin Wiselka, Adam Wolverson, Daniel G. Wooton, Andrew Workman,
483 Bryan Yates, and Peter Young.

484 **References**

485

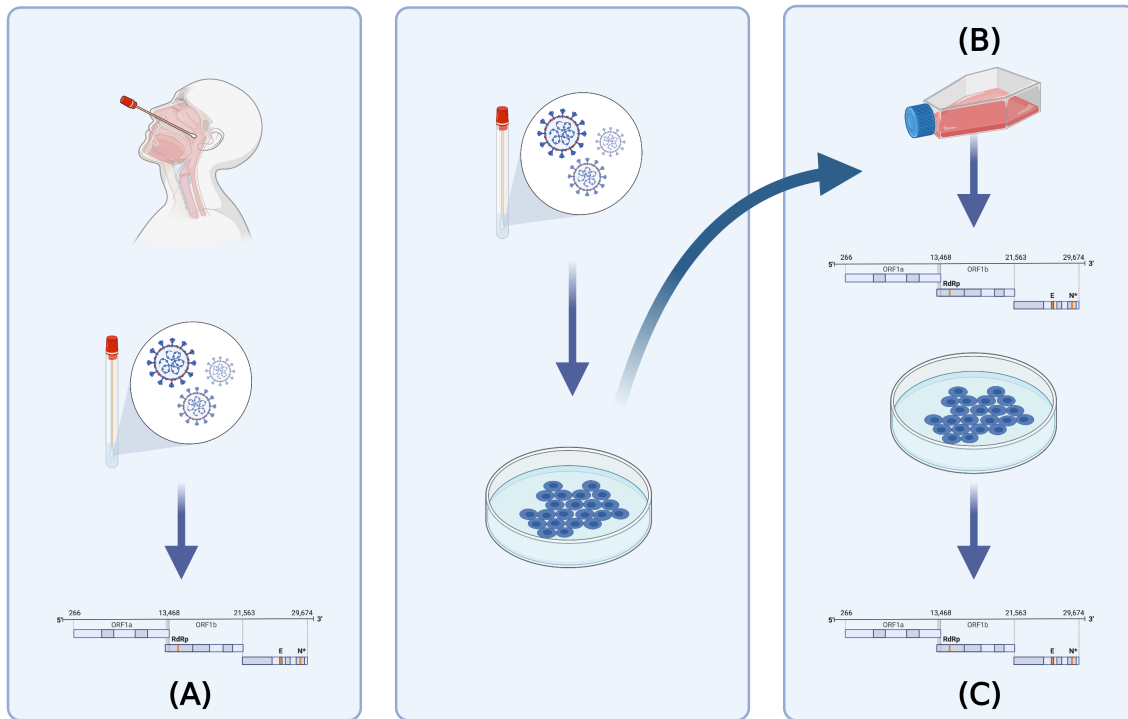
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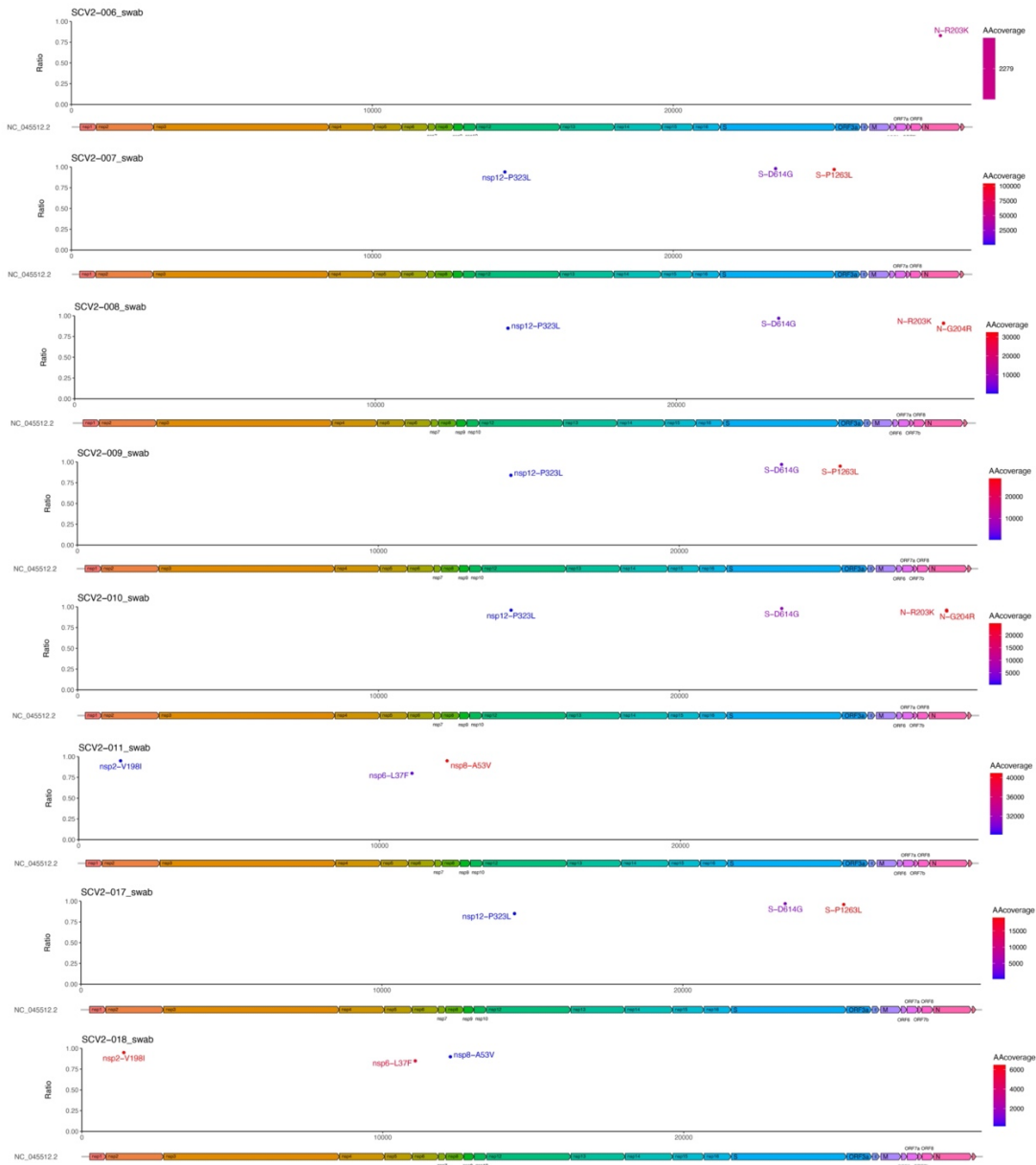
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590

591 **Figure 1.** Testing strategy. (A) Nasopharyngeal swabs from patients with COVID-19 recruited to
592 the ISARIC-4C study were sequenced using an amplicon based approach on the Oxford Nanopore
593 MinION (P0). Virus was isolated from the same nasopharyngeal swabs(P1). (B) Viral isolates from
594 the ISARIC-4C study, B.1.1.7, B.1.351 and Victoria isolates were grown up into stocks which were
595 then sequenced. (C) Viral stocks were titrated and used to infect hACE2-A549 cells, and 72-
596 hour post infection supernatants were sequenced.



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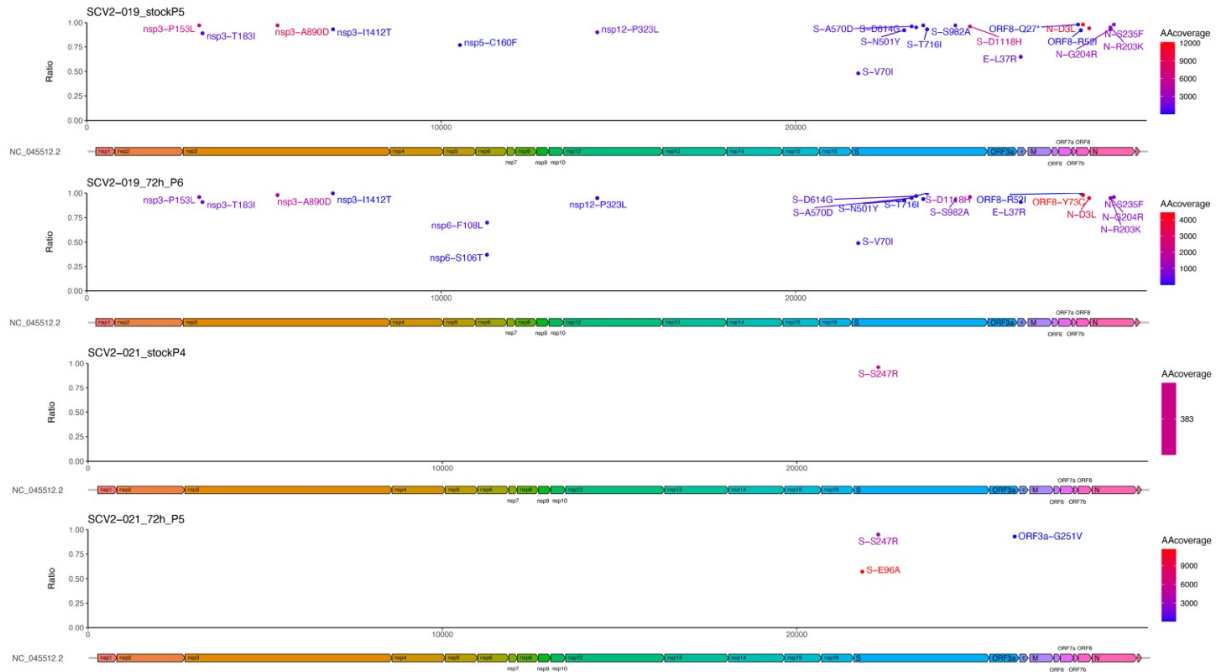
598 **Figure 2.** (A) Comparison of the consensus sequence SARS-CoV-2 in ISARIC4C swabs collected

599 from patients in hospital with COVID-19. Variations from the Wuhan reference sequence are

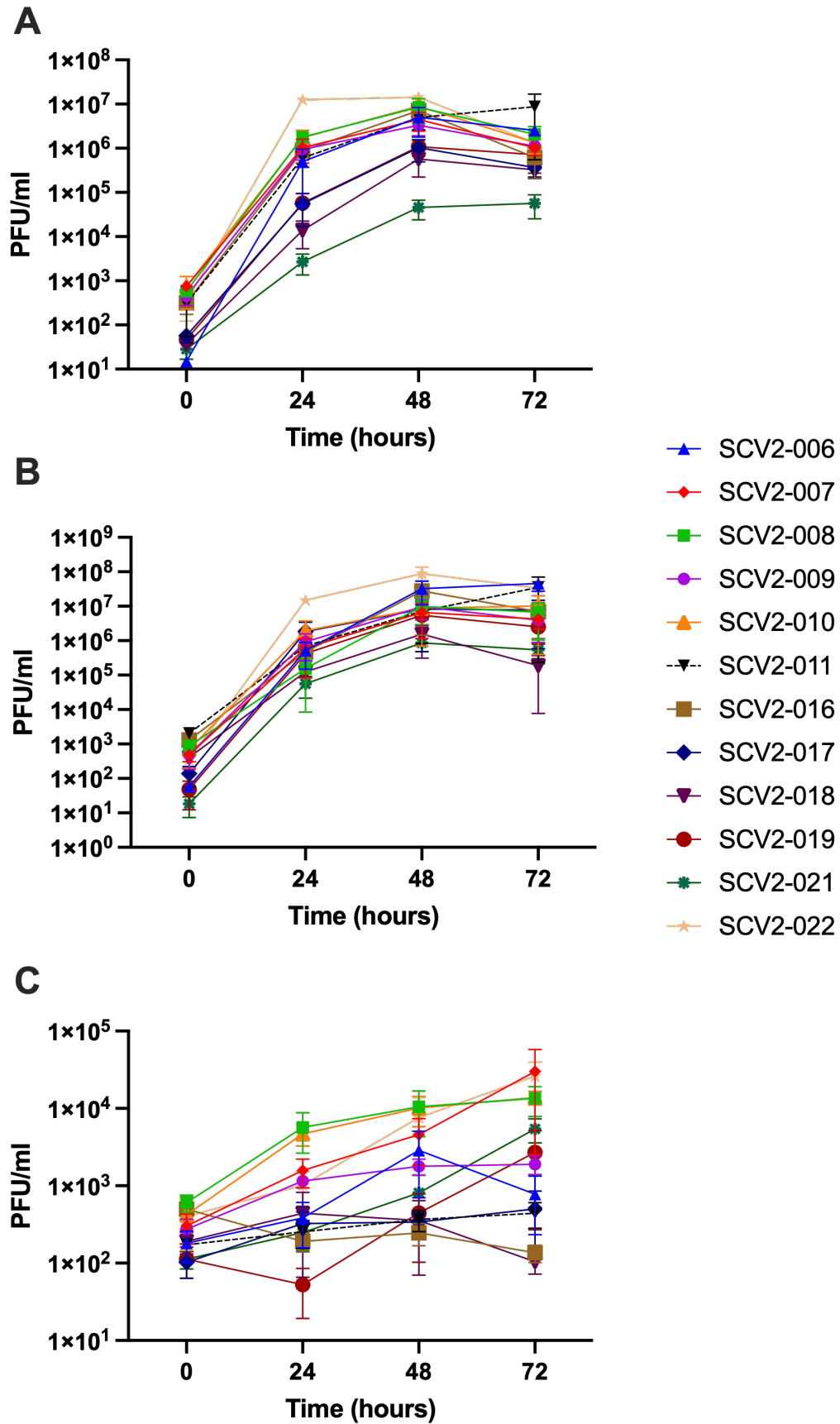
600 plotted with the location on the viral genome against the percentage (ratio) of reads where the

601 variation is observed. Variants are coloured to demonstrate the number of reads achieved (>10).

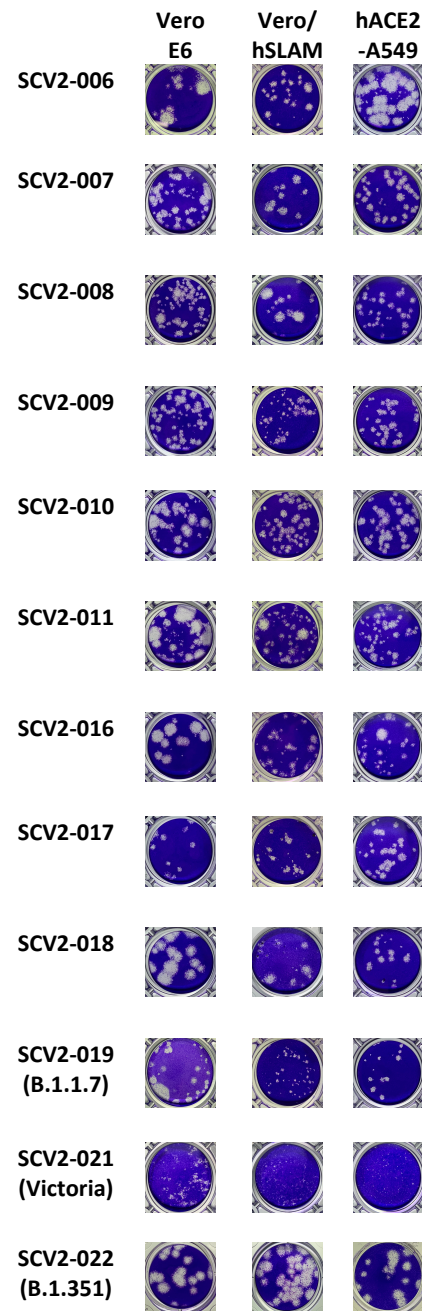
602



603
 604 **Figure 3.** Comparison of the UK 'Kent' VOC (SCV2-019) and the Australian Victoria isolate (SCV2-
 605 021) with the Wuhan reference sequence. Variations from the Wuhan reference sequence are
 606 plotted with the location on the viral genome against the percentage (ratio) of reads where the
 607 variation is observed. Variants are coloured to demonstrate the number of reads achieved (> 10).



609 **Figure 4.** Growth over time of 11 different viral isolates in three different cell lines compared to
610 the Variants of Concern SCV2-019 (UK 'Kent' VOC) and SCV2-022 ('South African' VOC).
611 Comparison viruses included the Australian Victoria variant (SCV2-021). (A) Growth of viruses in
612 plaque-forming units (PFU) per ml over times in Vero E6 African green monkey kidney cells. (B)
613 Growth of viruses in Vero cells expressing the human signalling lymphocytic activation module
614 (SLAM) gene (Vero/hSLAM). (C) Growth of viruses in human ACE-2 expressing A549 cells (hACE2-
615 A549). All experiments were repeated in triplicate using supernatant from 6 wells (n=3).



617 **Figure 5.** Phenotypic appearance of plaque assays from variants grown in three different cell
618 lines; (i) Vero E6, (ii) Vero/hSLAM and (iii) hACE2-A549 cells. Plaque assays were performed on
619 VeroE6 cells. Variants of Concern are SCV2-019 (UK 'Kent' VOC) and SCV2-022 ('South African'
620 VOC). Comparison viruses include the Australian Victoria variant (SCV2-021).