

1 **Post-entry, spike-dependent replication advantage of B.1.1.7 and B.1.617.2 over B.1 SARS-
2 CoV-2 in an ACE2-deficient human lung cell line**

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58 **KEYWORDS**

59 SARS-CoV-2; B.1.1.7; B.1.617.2; NCI-H1299 cells; ACE2

60
61 **ABSTRACT**

62 Epidemiological data demonstrate that SARS-CoV-2 variants of concern (VOC) B.1.1.7 and
63 B.1.617.2 are more transmissible and infections are associated with a higher mortality than non-
64 VOC virus infections. Phenotypic properties underlying their enhanced spread in the human
65 population remain unknown. B.1.1.7 virus isolates displayed inferior or equivalent spread in most
66 cell lines and primary cells compared to an ancestral B.1 SARS-CoV-2, and were outcompeted
67 by the latter. Lower infectivity and delayed entry kinetics of B.1.1.7 viruses were accompanied by
68 inefficient proteolytic processing of spike. B.1.1.7 viruses failed to escape from neutralizing
69 antibodies, but slightly dampened induction of innate immunity. The bronchial cell line NCI-H1299
70 supported 24- and 595-fold increased growth of B.1.1.7 and B.1.617.2 viruses, respectively, in the
71 absence of detectable ACE2 expression and in a spike-determined fashion. Superior spread in
72 NCI-H1299 cells suggests that VOCs employ a distinct set of cellular cofactors that may be
73 unavailable in standard cell lines.

74
75 **INTRODUCTION**

76 Since its emergence, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has
77 genetically diversified, giving rise to variants with altered phenotypic properties (Rambaut et al.
78 2020). In May 2021 the WHO announced a scheme for labeling SARS-CoV-2 lineages with

79 evidence for increased transmissibility, severity, and escape from immunity
80 (<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants>). The B.1.1.7 lineage was
81 labeled Alpha as it was the first variant of concern (VOC) (O'Toole et al., 2021). First detected in
82 the United Kingdom in September 2020 (<https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563>), it shows a 50-100% higher reproduction number than previously circulating virus
85 (Volz et al., 2021). Furthermore, the estimated hazard of death associated with B.1.1.7 is 61%
86 higher than with pre-existing variants (Davies et al., 2021). B.1.617.2 was identified in India in
87 October 2020 and was labelled VOC Delta in May 2021. In July 2021, the ECDC reported an
88 increase of weekly COVID-19 cases observed in 20 European countries of 64.3%. Delta is the
89 currently predominant circulating strain.

90 Genetic hallmarks of B.1.1.7 comprise a set of mutations resulting in nonsynonymous
91 changes within the spike gene including deletion of amino acids 69, 70 and 144 in the amino-
92 terminal domain, an N⁵⁰¹Y exchange in the receptor-binding domain, an A⁵⁷⁰D exchange in
93 subdomain 1, P⁶⁸¹H and T⁷¹⁶I exchanges in the proximity of the furin cleavage site and the S1 /
94 S2 domain junction, as well as S⁹⁸²A and D¹¹¹⁸H in the S2 domain. In addition to SNPs in ORF1ab
95 and nucleoprotein, ORF8 in variant B.1.1.7. contains a premature stop codon. The functional role
96 of ORF8 in SARS-CoV-2 is unclear. A variant with deleted ORF8 that circulated in Singapore
97 during spring 2020 showed limited evidence for changes in *in-vitro* transcription profile (Gamage
98 et al., 2020) and clinical outcome (Young et al., 2020).

99 To date, a functional correlate for the enhanced transmission and pathogenicity of B.1.1.7
100 and B.1.617.2 is missing. Studies of viral loads demonstrated that B.1.1.7- and B.1.617.2-infected
101 individuals shed viral RNA of increased levels (Jones et al., 2021; Wang et al., 2021) and for
102 prolonged time (Kissler et al., 2021b; Ong et al., 2021). Individual mutations in B.1.1.7 and
103 B.1.617.2 spike have been investigated with regards to protein structure (Yang et al., 2021), *in*
104 *vitro* ACE2-binding (Ramanathan et al., 2021), spike processing (Liu et al., 2021; Lubinski et al.,

105 2021a) and stability (Motozono et al., 2021), as well as fitness (Motozono et al., 2021). In contrast
106 to B.617.2 (Hoffmann et al., 2021a; Mlcochova et al., 2021; Planas et al., 2021), B.1.1.7 displays
107 only modest, if any, alteration of sensitivity to neutralizing antibodies (Hoffmann et al., 2021b;
108 Shen et al., 2021; Widera et al., 2021), suggesting a limited contribution of antibody-dependent
109 immune escape to the observed phenotype of B.1.1.7. *In vitro* and *in vivo* replication of B.1.1.7
110 was found to differ depending on the model used. Some epithelial cell cultures and hamster
111 models showed equal, slightly superior, or inferior replication for B.1.1.7 (Brown et al., 2021;
112 Nuñez et al., 2021; Touret et al., 2021; Ulrich et al., 2021), while B.1.1.7 generally exhibited
113 marginally superior replication in primates and ferrets (Rosenke et al., 2021; Ulrich et al., 2021).
114 However, animal models may be limited in their capability to reflect adaptive processes that occur
115 in a virus establishing endemicity in humans. Here, we studied the replication of B.1.1.7 viruses
116 in different cell and organ models as well as dwarf hamsters, and identified a human cell line that
117 reflects the replicative phenotype of B.1.1.7 as well as of B.1.617.2.

118

119 **RESULTS**

120 **B.1 and B.1.1.7 SARS-CoV-2 display similar replication kinetics in immortalized cell lines**
121 We first studied virus replication kinetics in a panel of immortalized cell lines. Compared to an
122 early lineage (Non-VOC) B.1 strain carrying the D⁶¹⁴G mutation (BavPat1/2020, (Wölfel et al.,
123 2020)), two different clade B.1.1.7 isolates displayed smaller plaque size three days post-infection
124 (Fig. 1A) and a delayed manifestation of cytopathogenic effects (CPE) in Vero E6 cells (Fig. 1B
125 and Supplementary Movies), accompanied by a delay in infectious particle production during the
126 initial 40 hours post-infection of Vero E6 cells (Fig. 1C).

127 Unlike Vero E6 cells, Caco-2 and Calu-3 cells express the transmembrane protease serine
128 subtype 2 (TMPRSS2) and are capable of producing type I interferons (IFNs). They supported the
129 growth of B.1. equally or more efficiently than B.1.1.7 (Fig. 1D-E). Interestingly, B.1.1.7 production

130 was particularly delayed in the very early phase of replication (Fig. 1F). Cultivation of infected cells
131 at 32°C in order to resemble the temperature in the upper respiratory tract did not alter relative
132 replication efficiencies (Fig. 1G). Under competitive passaging in Calu-3 cells, B.1 outcompeted
133 B.1.1.7, even when the starting inoculum contained a nine-fold excess of B.1.1.7 (Fig. 1H). In sum,
134 immortalized cell models failed to establish a clear growth advantage of B.1.1.7 that correlates
135 with its enhanced transmissibility and pathogenicity *in vivo*.

136

137 **Absence of detectable fitness advantages of B.1.1.7 in primary human respiratory cells,
138 organoids and hamsters**

139 To detect potential variant-specific differences that may not become evident in immortalized cell
140 lines, we studied more complex, physiologically relevant, primary human models of mucosal
141 infection. Infection experiments in differentiated air-liquid interface cultures of human nasal (Fig.
142 2A), human bronchial (Fig. 2B) airway epithelial cultures (hBAECs), as well as epithelial intestinal
143 organoids (Fig. 2C) failed to reveal a growth advantage for B.1.1.7 isolates compared to B.1.
144 Growth of B.1.1.7 was even slightly inferior to B.1 in adult stem cell-derived human lung organoids
145 (Fig. 2D). Virus production in the lung of infected dwarf hamsters was not different between any
146 of the viruses used. In addition, virus production in the lungs of contact animals co-housed with
147 infected animals did not show variant-specific quantitative differences in preliminary analyses (Fig.
148 2E).

149

150 **B.1.1.7 spike protein shows decreased proteolytic processing**

151 To identify potential consequences of B.1.1.7 spike mutations on expression and proteolytic
152 processing of the glycoprotein, we analyzed lysates of HEK293T cells transfected with plasmids
153 expressing SARS-CoV-2 spike-HA. Overall expression levels of spike constructs encoding
154 individual or all B.1.1.7-specific mutations did not differ significantly from B.1 constructs in
155 quantitative immunoblots (Fig. 3A, Fig. S1A). However, quantification of the proportion of S2-HA

156 spike relative to the total spike-HA signal revealed a 1.8-fold reduction of proteolytic processing of
157 the B.1.1.7 glycoprotein compared to that of B.1 (Fig. 3A). No single B.1.1.7-defining mutation,
158 including P⁶⁸¹H, fully recapitulated this property, even though deletion of H⁶⁹/V⁷⁰ showed a trend
159 towards more efficient processing, in agreement with (Meng et al., 2021), and T⁷¹⁶H showed a
160 trend towards decreased proteolytic processing. The data suggest that a combination of amino
161 acid exchanges is required for rendering proteolytic processing of B.1.1.7 spike less efficient.
162 Reduced processing of B.1.1.7 spike was accompanied by a 2.3-fold decrease of spike levels
163 associated with lentiviral particles, when compared to particles containing B.1 spike (Fig. 3B).
164 Interestingly, the individual T⁷¹⁶H mutation was sufficient for reduction of spike quantities
165 associating to lentiviral particles. In accordance with previous reports (Kemp et al., 2021; Meng et
166 al., 2021), deletion of H⁶⁹/V⁷⁰ increased spike abundance in pseudotyped particles.

167 We challenged these findings by analysis of SARS-CoV-2-infected cells. B.1.1.7 spike in
168 infected Vero E6 cells showed a 2.4-fold reduced processing efficiency (Fig. 3C, left panel and
169 immunoblot, Fig. S1B). In virus particles, the ratio of spike per nucleocapsid signal appeared
170 intact, suggesting that inefficient proteolytic cleavage does not translate into a decreased
171 association of mature S2 into virions (Fig. 3C, middle panel and immunoblot). Overall, expression
172 levels of spike did not differ significantly from B.1 in quantitative immunoblots (Fig. S1C).

173

174 **Enhanced cell-cell fusion and reduced virus particle entry by B.1.1.7 SARS-CoV-2 spike**

175 We next analyzed fusogenicity of individual spike proteins in a cell-cell fusion assay based on co-
176 cultures of CHO cells transiently expressing HIV-1 Tat and individual SARS-CoV-2 spike proteins,
177 and ACE2/TMPRSS2-transfected, LTR-luciferase-expressing target TZM-bl cells. Compared to
178 B.1 spike, no significant changes in membrane fusion activity were detected with any single
179 mutation present in B.1.1.7 spike (Fig. 4A). However, full B.1.1.7 spike was more prone to induce
180 cell-cell fusion. Furthermore, entry of lentiviral pseudotypes mediated by the same set of spike
181 proteins was quantified after transduction of Calu-3 cells using p24 capsid normalized inocula in

182 a luciferase-based assay (Fig. 4B). Whereas most individual mutations did not significantly alter
183 the ability of SARS-CoV-2 spike to mediate entry into Calu-3 cells, T⁷¹⁶I and the full B.1.1.7 spike
184 mediated reduced entry (Fig. 4B, Supplemental Fig. S2A). The inferior ability of B.1.1.7 spike-
185 containing lentiviral pseudotypes to transduce Calu-3 cells was corroborated in titration
186 experiments on ACE2/TMPRSS2-positive A549 cells (Fig. 4C) and is potentially related to the
187 lower levels of incorporated spike when compared to B.1 spike-pseudotyped particles.

188 We next investigated entry kinetics in Calu-3 and A549-ACE2 cells with authentic SARS-
189 CoV-2. In single-cycle entry experiments, virus inocula were absorbed at 4°C for one hour, cells
190 were washed to remove excessive virus particles, and the cells eventually incubated at 37°C to
191 initiate synchronized entry. *De novo*-synthesized, cell-associated subgenomic transcripts served
192 for detection of early signs of virus replication after entry (Fig. 4D and 4E). Reminiscent of our
193 results obtained with the lentiviral pseudotypes, B.1.1.7 initiated slightly lower levels of virus
194 replication in Calu-3, A549-ACE2 and A549-ACE2/TMPRSS2 cells when compared to B.1 at the
195 earliest time point at which subgenomic RNA production was detectable. ACE2 seems to be
196 required for entry into A549, as levels of subgenomic viral RNA remained at background levels in
197 parental A549. Entry of both viruses in Calu-3 cells depended on TMPRSS2 and furin and less on
198 a cathepsin L-dependent pathway. In addition, clathrin inhibition resulted in decreased endocytic
199 entry of B.1 and B.1.1.7 in Calu-3 cells (Fig. S2B), as was previously described for SARS-CoV
200 (Wang et al., 2008). Inocula were back-titrated to ensure that equal amounts of virus were used
201 for infection. In conclusion, B.1.1.7 spike-pseudotyped lentiviral particles and authentic B.1.1.7 are
202 less efficient in entering susceptible target cells than their B.1 counterparts. This inefficiency may
203 be related to the detected lower efficiency of spike processing.

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207 **B.1.1.7 fails to escape from neutralizing antibodies and may dampen induction of innate**
208 **immunity**

209 In accordance with reports by others (Bates et al., 2021; Supasa et al., 2021; Widera et al., 2021),
210 B.1 infection- and vaccination-induced antibodies efficiently neutralized both B.1 and B.1.1.7 in a
211 plaque reduction neutralization test, whereas such antibodies failed to effectively neutralize
212 B.1.351 (Fig. 5A). Interestingly, binding of B.1.1.7 RBD, which differs from that of B.1 solely at one
213 position (N⁵⁰¹Y), to ACE2 in a surrogate neutralization test was slightly less sensitive to inhibition
214 by antibodies from non-VOC convalescents and vaccinees when compared to antibodies raised
215 following B.1.1.7 infection (Fig. 5B), indicating that antibodies targeting other regions than the RBD
216 may contribute to the neutralization of infection. However, B.1.351 RBD was clearly more resistant
217 to ACE2 binding inhibition than the B.1 and B.1.1.7 RBDs. These results suggest a large absence
218 of escape from humoral immunity by B.1.1.7, as opposed to B.1.351.

219 We next hypothesized that B.1.1.7 has evolved superior ability to prevent or evade cell-
220 intrinsic immunity. In the basal medium of infected differentiated bronchial airway epithelial cell
221 cultures, no significant variant-specific differences were identified for various cytokines and other
222 secreted proteins related to innate immunity, including IFN- α , IFN- γ , and IP-10 (Fig. 5C). In
223 infected Calu-3 cells, expression of *IFNB*, *MXA*, *CCL5*, *IL6* and *TNFA* was induced to similar levels
224 by both variants (Fig. 5D). Interestingly, B.1.1.7 infection appeared to induce slightly lower levels
225 of *IFNL* expression than B.1 (Fig. 5D), suggesting that B.1.1.7 may be able to suppress innate
226 immune responses more efficiently.

227 In conclusion, absence of a detectable escape from neutralizing antibodies suggest that
228 the enhanced B.1.1.7 transmissibility and pathogenicity *in vivo* involves other immune evasion
229 mechanisms, potentially the ability to dampen induction of an antiviral state in infected cells.

230
231 **B.1.1.7 and B.1.617.2 display a spike-dependent, ACE2-independent post-entry replication**
232 **advantage in NCI-H1299 cells**

233 We reasoned that common cell culture models used up to this point may have failed to reflect the
234 predominant spread of B.1.1.7 observed in the human population. Upon further exploration of
235 susceptible cell lines, we identified that the human bronchial cell line NCI-H1299 (Phelps et al.,
236 1996) supports B.1.1.7 growth with up to 24-fold higher efficiency than B.1 growth (Fig. 6A) in
237 multi-cycle growth kinetics. The smaller plaque size of B.1.1.7 virus originating from Vero E6 cells
238 (see Fig. 1A, lower panel) was corroborated with NCI-H1299 cell-derived virus.

239 Interestingly, there was no advantage in entry of lentiviral particles based on the B.1.1.7
240 spike protein in NCI-H1299 cells (Fig. 6B). Also, there was no advantage for authentic SARS-
241 CoV-2 B.1.1.7. virus in entering NCI-H1299 cells in a synchronized entry assay (Fig. 6C),
242 indicating that the more efficient replication of B.1.1.7 may not necessarily be determined by
243 improved entry. Even more surprisingly, replication of B.1.1.7 in NCI-H1299 cells occurred in the
244 absence of detectable ACE2 protein (Fig. 6D), and ACE2 and TMPRSS2 mRNAs were only
245 weakly expressed as compared to other SARS-CoV-2-susceptible cell cultures (Fig. 6E). In order
246 to exclude the possibility that SARS-CoV-2 infection in NCI-H1299 cells was maintained by minute
247 traces of ACE2 expressed below the detection limit of our system, we blocked ACE2 by antibodies.
248 Individual incubation of Vero E6 and Calu-3 cells with three ACE2-neutralizing antibodies
249 abolished and diminished SARS-CoV-2 infection, respectively (Fig. 6F, Fig. S3A-B). In contrast,
250 anti-ACE2 antibody treatment did not and only very modestly modulate the susceptibility of NCI-
251 H1299 to B.1 and B.1.1.7 SARS-CoV-2 infection (Fig. 5G and Fig. S3C), reinforcing the notion
252 that this cell line supports infection via a largely ACE2-independent mechanism.

253 To analyze the specificity of the observed phenotypic changes for the spike protein, we
254 generated, by reverse genetics, a Wuhan-1 virus with a D⁶¹⁴G mutation (representing a B.1 virus,
255 rB.1), as well as a Wuhan-1 virus carrying the full spike protein of B.1.1.7 (rB.1/Spike B.1.1.7).
256 Moreover, we engineered a virus consisting of the B.1.1.7 backbone but the Wuhan-1 D⁶¹⁴G spike,
257 representing a prototypic B.1 spike (rB.1.1.7/Spike B.1, Fig. 5H-J). As expected, all recombinant
258 viruses grew to similar titers in Vero E6 cells (Fig. 6H). In Calu-3 cells, growth of rB.1.1.7/Spike

259 B.1 was increased 9-fold over rB.1 at 72 hours post-infection, suggesting that the B.1.1.7
260 backbone may confer replication advantages that do not depend on the spike protein (Fig. 6I). In
261 NCI-H1299 cells, the virus expressing the B.1.1.7 spike in the backbone of B.1 grew to
262 considerably higher titers (up to 65-fold increased titer) than the reciprocal virus and the original
263 rB.1 virus (up to 15-fold increased titer, Fig. 6J). This suggests the replicative advantage of B.1.1.7
264 in NCI-H1299 cells, while probably exerted on entry, is mediated by the spike protein.

265 We next reasoned that NCI-H1299 cell-specific increased viral growth may be a property
266 that is shared by the current, predominating VOC Delta. In Calu-3 cells and in hBAEC cultures, a
267 B.1.617.2 isolate grew to similar and increased titers, respectively, compared to B.1 and B.1.1.7
268 (Fig. 6K and Fig. 6L). Remarkably, in multi-cycle infection experiments in NCI-H1299 cells,
269 B.1.617.2 grew to 264 (min. 37 -max. 595)-fold higher titers than B.1 and 24 (min. 3 -max. 59)-
270 fold higher titers than B.1.1.7, respectively (Figure 6M). Together, these data suggest that VOCs
271 B.1.1.7 and B.1.617.2 may utilize partially common mechanisms of replication enhancement that
272 are spike-dependent but do not improve entry efficiency.

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277 **DISCUSSION**

278 To date, our understanding of molecular mechanisms that underlie the rapid spread and/or
279 increased pathogenicity of SARS-CoV-2 VOCs is still in its infancy. As SARS-CoV-2 productively
280 infects epithelial cells (Hou et al., 2020), and B.1.1.7-infected patients shed 10-fold more viral RNA
281 (Jones et al., 2021), we hypothesized that B.1.1.7 has a replication advantage in human epithelial
282 cell cultures. Although all immortalized, primary, and organoid cultures tested were highly
283 permissive for SARS-CoV-2 infection, we failed to detect a B.1.1.7-specific growth advantage with
284 one notable exception. Our findings corroborate reports by others showing a similar growth rate

285 of B.1.1.7 and B.1. viruses in common culture cells and primary human airway epithelial (HAE)
286 cells (Brown et al., 2021; Touret et al., 2021; Ulrich et al., 2021). Despite monitoring infected
287 cultures for up to ten days, we failed to observe temporally increased virus production in human
288 organoid models as seen by others (Lamers et al., 2021). *In vivo*, B.1.1.7 has been proposed to
289 display a fitness advantage in experimentally infected ferrets, in hACE2-K18Tg-transgenic mice
290 and hACE2-KI transgenic mice (Ulrich et al., 2021), but not in Syrian hamsters (Nuñez et al., 2021;
291 Ulrich et al., 2021). In line with these results, our data confirm the absence of significant growth
292 differences between B.1.1.7 and B.1 variants in experimentally infected dwarf hamsters
293 (Abdelnabi et al., 2021). However, a recent study did observe a transmission advantage of B.1.1.7
294 upon low dose infection of Syrian hamsters (Mok et al., 2021). Finally, in intranasally infected
295 African green monkeys, B.1.1.7 infection generated higher levels of viral RNA in the respiratory
296 tract than B.1 infection (Rosenke et al., 2021). Together, the superiority of B.1.1.7 spread can be
297 recapitulated in some animal models, but is not detectable in most cell culture systems.

298 B.1 SARS-CoV-2 productively infects susceptible cells via binding of the spike protein to
299 ACE2 and TMPRSS2-mediated priming of spike (Hoffmann et al., Cell 2020). Compared to B.1
300 viruses, B.1.1.7 viruses and B.1.1.7 spike-decorated lentiviral particles were equally or even less
301 efficient in entering ACE2-expressing cell lines. Intriguingly, B.1.1.7 spike appeared to be more
302 fusogenic in cell-cell fusion assays, in accordance with published work (Meng et al., 2021; Rajah
303 et al., 2021). Proteolytic processing and viral packaging of coronaviral spike can be rate-limiting
304 during infectious virus production. Our findings of reduced proteolytic cleavage in spike plasmid-
305 transfected and SARS-CoV-2-infected cells are consistent with reduced furin-mediated
306 processing of B.1.1.7 spike as shown by a biochemical peptide cleavage assay (Lubinski et al.,
307 2021b), and challenge data reporting intact processing of cell-associated spike when expressed
308 in spike plasmid-transfected cells (Mlcochova et al., 2021). Of note, the latter study used plasmids
309 expressing spikes lacking 19 C-terminal amino acids, an experimental modification that has been
310 widely accepted for artificial enhancement of cell surface expression and lentiviral incorporation

311 of spike (Yu et al., 2021), and that we refrained to adopt in order to maintain the expression context
312 as physiological as possible. The observation of reduced processing translated into lower levels
313 of lentivirus-associated spike, which may be the cause of the reduced infectivity of particles. Even
314 though impaired maturation did not detectably alter virion-associated spike levels under our
315 experimental conditions, we cannot exclude that it may still modulate the kinetics of virus particle
316 secretion and/or the quality of secreted SARS-CoV-2 particles.

317 Whereas increased B.1.1.7 replication has been observed in patients, the initial phase of
318 virus infection is difficult to capture in clinical observations due to late sampling, making cell culture
319 studies potentially more insightful. On the contrary, cell cultures may fail to reflect differences in
320 virus production in later stages of tissue infection due to the limiting effect of cytopathogenic effects
321 *in vitro*. Our observation of reduced growth of B.1.1.7 in cell culture does not necessarily contradict
322 clinical observations. We observed a slower ramp-up of virus production, delayed onset of
323 cytopathic effect, and slightly reduced levels of *IFNL* expression for B.1.1.7, which, overall, is
324 consistent with a stealthy invasion of tissue with initially limited production of PAMPs and more
325 efficient evasion of cell-intrinsic innate immunity, as suggested by others. The previously reported
326 transcriptional changes affecting the N gene reading frame, leading to expression of ORF9b
327 potentially acting as an antagonist of IFN induction, as well as the reported increase of production
328 of the IFN signaling antagonist ORF6, may contribute to this phenotype (Parker et al., 2021;
329 Thorne et al., 2021). Accordingly, infected air-liquid interface cultures of alveolar type 2 cells
330 produced more infectious B.1.1.7 virus when compared to B.1 only in a late phase of infection
331 (Lamers et al., 2021). Also, replication of B.1 and B.1.1.7 variants in the upper respiratory tract of
332 experimentally infected African green monkeys differed only from day 5 post-infection (Rosenke
333 et al., 2021). Together, this suggests a delayed but extended phase of infectious B.1.1.7 virion
334 shedding, potentially resulting in a prolonged phase of heightened transmission probability, which
335 is also supported by preliminary viral load studies (Jones et al., 2021; Kissler et al., 2021a).

336 While cell-intrinsic immunity may be involved, its onset and extent is still dependent on
337 fundamental factors such as receptor usage and cell entry, often reflected by changes in the spike
338 protein. Paradoxically, ACE2 expression levels in the respiratory tract are low and common cell
339 culture models may not fully recapitulate *in vivo* properties of primary target tissues of SARS-CoV-
340 2 infection (Hikmet et al., 2020). Our exploration of other cell cultures may thus have uncovered a
341 cell line that has remained understudied with regard to SARS-CoV-2 infection phenotypes. While
342 caveats and limitations of neoplastic cell lines apply, NCI-H1299 cells yielded higher levels of
343 replication and infectious virus production not only for B.1.1.7, but also for B.1.617.2. This cell line
344 of epithelial morphology is devoid of detectable ACE2 protein and remains susceptible to SARS-
345 CoV-2 infection even in the presence of ACE2-neutralizing antibodies, suggesting the existence
346 of an alternative, but not variant-specific mode of entry. This finding is reminiscent of a recent
347 study (Puray-Chavez et al., 2021) that identified SARS-CoV-2 infection of the H522 lung cell line
348 in an ACE2-independent fashion (in this study the alternative way of infection could only be utilized
349 by viruses carrying an E⁴⁸⁴D substitution within the spike protein RBD).

350 Surprisingly, while experiments with reciprocal chimeras established that the B.1.1.7
351 spread advantage in NCI-H1299 cells is mediated to a large extent by its spike, the entry process
352 *per se* was similarly or even less efficient for B.1.1.7 than for B.1. Notably, a virus consisting of
353 the B.1.1.7 backbone with B.1 spike displayed a slightly better replication efficiency than the
354 original B.1 virus in type-I IFN-competent Calu-3 cells, confirming that other genetic determinants
355 of B.1.1.7 beyond spike contribute to enhanced replication. These findings suggest a post-entry,
356 yet largely spike-dependent replication advantage of B.1.1.7 that manifests itself in NCI-H1299
357 cells. Remarkably, we conducted preliminary studies of replication for B.1.617.2 and found a highly
358 similar phenotype, i.e., a similar level of replication in common ACE2-positive cell lines but a
359 specific increase of replication in NCI-H1299 cells that does not seem to be determined by
360 improved entry. In addition, B.1.617.2 displayed a spreading advantage in human BAECs that
361 manifested itself not before four days post-infection. The extent of growth advantage for B.1.617.2

362 in NCI-H1299 cells was higher than for B.1.1.7, which would be expected given the observed
363 differences in epidemic growth rates for these VOCs. More work will be necessary to understand
364 the nature of the alternative entry mechanism, the identity of the entry-independent and variant-
365 specific difference in replication as a function of spike, as well as the possibility that two VOCs
366 may have undergone convergent evolution towards utilization of this same unknown mechanism.
367 Also, whether the ACE2-independent entry is linked to the replication advantage, e.g. by
368 potentially more efficient virion release due to ACE2 scarcity or absence, remains unclear at this
369 time. Mere absence of ACE2 expression is not sufficient for B.1.1.7-specific growth advantage as
370 illustrated by the refractoriness of ACE2-negative A549 cells to SARS-CoV-2 infection. Future
371 studies are required for the identification of a hypothetical alternative receptor of SARS-CoV-2 and
372 for the elucidation of the specific cellular environment of NCI-H1299 cells that confers a replication
373 advantage to B.1.1.7 and B.1.617.2 SARS-CoV-2.

374

375 **MATERIALS AND METHODS**

376

377 *Samples from COVID-19 patients and vaccinees*

378 Sera were available through a study on convalescent plasma donors, who recovered from mild to
379 moderate COVID-19 before the emergence of any SARS-CoV-2 VOCs (Schlickeiser et al., 2020).
380 Additional sera were available through a study on SARS-CoV-2 infection and COVID-19
381 vaccination (Hillus et al., 2021), a prospective observational cohort study Pa-COVID-19 (Thibeault
382 et al., 2021) including its study arm RECAST (Understanding the increased resilience of children
383 compared to adults in SARS-CoV-2 infection) and from RT-PCR confirmed B.1.1.7-infected
384 patients. The use of clinical samples (sera) was approved by the Institutional Review Board at
385 Charité - Universitätsmedizin Berlin (EA1/068/20, EA2/092/20 and EA2/066/20) and is in
386 accordance with the Berlin State Hospital Law, allowing for pseudonymized scientific analysis of
387 routine patient data.

388

389 *Cells and culture conditions*

390 A549 parental (ATCC CCL-185), A549-ACE2, A549-TMPRSS2, A549-ACE2 + TMPRSS2
391 (Widera et al. 2021), Caco-2 (ATCC HTB-37), Calu-3 (HTB-55), CHO (HIV Reagent Program
392 ARP-2238), NCI-H1299 (ATCC CRL-5803), HEK-293T (ATCC CRL-3216), TZM-bl (HIV Reagent
393 Program ARP-8129) and Vero E6 (ATCC CRL-1586) cells were maintained at 37°C and 5% CO₂
394 in a humidified atmosphere and cultured in Dulbecco's Modified Eagle's Medium (DMEM,
395 ThermoFisher Scientific) supplemented with 10% fetal bovine serum (FBS, ThermoFisher
396 Scientific), 1% non-essential amino acids 100x concentrate (NEAA, ThermoFisher Scientific) and
397 1% sodium pyruvate 100 mM (NaP, ThermoFisher Scientific) and split twice a week. For seeding
398 and cultivation, cells were washed with phosphate buffered saline (PBS, ThermoFisher Scientific)
399 and detached with 0.05% trypsin-EDTA solution (ThermoFisher Scientific).

400

401 *Virus strains*

402 Infection experiments were done with BetaCoV/Munich/ChVir984/2020 (B.1, EPI_ISL_406862),
403 hCoV-19/Germany/BY-ChVir21652/2020 (B.1.1.7/v1, EPI_ISL_802995), BetaCoV/Baden-
404 Wuertemberg/ChVir21528/2021 (B.1.1.7/v2, EPI_ISL_754174) and hCoV-19/Germany/BW-
405 ChVir22131/2021 (B.1.351, EPI_ISL_862149). A virus of the B.1.617.2 ("Delta") clade (hCoV-
406 19/Germany/SH-ChVir25702_4/2021) was isolated from a patient in Schleswig-Holstein,
407 Germany, and its sequence deposited in Gisaid (EPI_ISL_2500366). Due to the observation of
408 rapid cell culture-induced mutations at the spike polybasic furin cleavage motif (spike amino acid
409 no. 681-685), all virus stocks were sequenced by next generation sequencing to confirm the
410 absence of minority variants. Unless otherwise stated, only virus stocks with no or variant
411 frequencies below 20% of all sequence reads were included in downstream infection experiments.
412 Virus isolation and all SARS-CoV-2-related infection experiments were performed under biosafety
413 level-3 (BSL-3) conditions with enhanced respiratory personal protection equipment.

414

415 *Plasmids*

416 Codon-optimized, C-terminally tagged spike cDNAs in pCG were generated using pCG-SARS-
417 CoV-2 spike Wuhan as a template (Hoffmann et al., 2020) in which the N-terminus was repaired
418 and D⁶¹⁴G was introduced via site-directed mutagenesis. The individual B.1.1.7-characteristic
419 mutations were introduced individually and in combination by site-directed mutagenesis. All
420 constructs were confirmed by Sanger sequencing.

421

422 *Virus isolation*

423 Virus was isolated from naso- or oropharyngeal swabs using Vero E6 cells. Cells were seeded at
424 a density of 175,000 cells per well in 24-well plates one day prior to isolation. For virus isolation,
425 the medium was removed and cells were rinsed once with 1x PBS (ThermoFisher Scientific) and
426 inoculated with 200 µl of swab sample. After one hour incubation, 800 µl of isolation medium
427 (DMEM, supplemented with 2% FBS, 1% penicillin-streptomycin and 1% amphotericin B,
428 ThermoFisher Scientific) was added to each well. Cells were monitored for cytopathic effects
429 (CPE) every day. Four days post-inoculation, viral RNA was isolated and quantified from the
430 supernatant as described below. Isolation success was determined when CPE was detectable
431 and viral RNA concentrations were above a threshold of 100,000 genome equivalents per µl. Virus
432 stocks were produced from all positive cultures.

433

434 *Virus stock production*

435 Vero E6 cells were seeded in T175 tissue culture flasks allowing the cells to reach 90% confluence
436 on the following day. Cells were washed once with PBS and inoculated with 100 µl of low passage
437 (passage 1-2) virus stock solution (approximately 1,000,000 PFU per ml) in 20 ml virus infection
438 medium (DMEM supplemented with 2% FBS, 1% NEAA, 1% sodium phosphate). Three days post-
439 inoculation, supernatant was harvested and virus particles were purified from cytokines and

440 concentrated using Vivaspin 20 (Sartorius, filtration units with a size exclusion of 100 kDa)
441 according to the manufacturer's instructions. Virus concentrate was resuspended in 2-3 ml PBS,
442 diluted 1:2 in virus preservation medium (0.5% gelatine in OptiPRO serum free medium) and
443 stored at -80°C. Infectious titers were determined in three independent plaque titration
444 experiments and viral RNA concentration was quantified by real-time RT-PCR (E gene assay). All
445 stocks were sequenced by next generation sequencing methods and the absence of additional
446 mutations was confirmed to occur in less than 20% of the virus-specific reads.

447

448 *Virus infection and virus growth kinetics in cell cultures*

449 Vero E6, Caco-2, NCI-H1299 and A549 cells were seeded at a density of 350,000 cells per ml
450 and Calu-3 cells at a density of 500,000 cells per ml one day prior to infection. For infection, virus
451 stocks were diluted in OptiPRO SFM (ThermoFisher Scientific) serum-free medium according to
452 the desired MOI. For virus adsorption 200 µl (24-well) or 1 ml (6-well) of virus master mix was
453 added to the cells and incubated at 37°C in a 5% CO₂ atmosphere with 95% humidity. After one
454 hour, virus dilutions were removed, cells were washed three times with PBS and wells were refilled
455 with DMEM infection medium. To determine infectious titers, supernatants were harvested at the
456 indicated time points, and diluted 1:2 in virus preservation medium and stored at -80°C until
457 conducting plaque titration assay.

458

459 *Infection bronchial epithelial cells*

460 Human bronchial airway epithelial (hBAE, SmallAirTM) cell cultures applied in Figure 2B were
461 obtained from Epithelix Sàrl, Geneva Switzerland. All other experiments were conducted with
462 hBAECs isolated from explanted lungs which were obtained from the Hannover Lung Transplant
463 Program after patients informed consent, ethical vote 2923-2015. For isolation of hBAECs, human
464 bronchial tissue was cut into small pieces in Hank's buffer (Thermo Fisher Scientific) containing

465 0.18% protease XIV and incubated for two hours at 37°C. After thorough pipetting with a 25/50 ml
466 serological pipette, cell solution was filtered through a 100 µm cell strainer (Corning) to remove
467 clumps and 10 ml RPMI supplemented with 10% FCS (Thermo Fisher Scientific) was added. After
468 centrifugation for 10 min at 500g and 4°C, supernatant was removed and cells were resuspended
469 in SAGM™ (Lonza) + Primocin (InvivoGen) + Penicillin-Streptomycin (P/S) (Sigma-Aldrich). For
470 air-liquid interface cultures, 200.000 hBAECs were seeded onto PureCol- (Advanced BioMatrix)
471 coated 12 well inserts (Greiner Bio-One) in SAGM™ + Primocin + P/S. 48 hours post seeding,
472 culture medium in apical and basal chamber was changed to PneumaCult-ALI medium
473 (STEMCELL Technologies). Air-Lift was performed 48 hours later by gently removing medium
474 from the apical chamber. Homogenous distributed cilia were visible latest three weeks after air-lift
475 and inserts were used for infections.

476 For infection, the apical surface was washed up to five times with 200 µl PBS to remove
477 mucus. Virus stocks were diluted in OptiPRO and hBHAE were infected with an absolute infectious
478 dose of 50,000 PFU (SmallAir™) or 100,000 PFU (in-house hBAECs). Cells were incubated for
479 1.5 hours at 37°C in a 5% CO₂ atmosphere with 95% humidity. After adsorption, virus dilutions
480 were removed and the cells were washed three times with 200 µl PBS. Samples were taken at
481 the indicated time points from the apical surface by applying 200 µl PBS to the cells. PBS was
482 incubated on the cells for 10 minutes at 37°C to ensure that virus particles diffuse into the solution
483 before collecting the supernatant samples. Basolateral medium (SmallAir™ Medium for SmallAir™
484 cultures or PneumaCult-ALI for in-house hBAECs) was exchanged every 48 hours.

485

486 *Infection of nasal airway epithelial cells*

487 Primary human nasal airway epithelial cells (hNAECs) were collected from healthy individuals by
488 nasal brushings. Informed consent was obtained from all volunteers and the study was approved
489 by the Charité Ethics Committee (EA2/161/20, EA2/066/20). Cultivation of hNAECs was

490 performed as previously described (Gentzsch et al., 2017). Briefly, cells were expanded using the
491 conditionally reprogrammed cell (CRC) culture method, then p.2 or p.3 cells were seeded on
492 porous Transwell or Snapwell 1.1 cm² supports (Corning) in UNC-ALI medium and differentiated
493 at air-liquid interface for at least three weeks prior to infection. Approximately 200,000 hNAECs
494 were infected with SARS-CoV-2 B.1 or B.1.1.7/v2 at an MOI of 0.1 in 150 µl D-PBS containing 0.3
495 % BSA for one hour at 37°C in a 5% CO₂ atmosphere with 95% humidity. Afterwards, cells were
496 washed apically with D-PBS and fresh medium was added basolaterally. Samples were taken at
497 the indicated time points from the apical surface by applying 100 µl D-PBS to the cells. PBS was
498 incubated on the cells for 30 minutes at 37°C to ensure that virus particles diffuse into the solution
499 before collecting the supernatant samples. A 250 µl sample was taken from the basolateral side
500 and medium was replenished. All samples were titrated on Vero E6 cells by plaque assay to
501 determine infectious titers.

502

503 *Infection of lung organoids*

504 Human lung organoids were established as previously published (Youk et al., 2020). Informed
505 consent was obtained from all volunteers and the study was approved by the Charité Ethics
506 Committee (project 451, EA2/079/13). For infection, Matrigel was liquefied and removed on ice
507 and organoids were broken up by repeated resuspension using a disposable syringe with needle
508 (27G). Virus stocks were diluted at the desired MOI in organoid infection medium (Advanced
509 DMEM/F12 with 10 mM HEPES and 1x GlutaMax, ThermoFisher Scientific) and dilution was
510 inoculated for one hour at 37°C and in 5% a humidified CO₂ atmosphere. After infection, organoids
511 were washed twice with PBS and resuspended in Cultrex 3-D Culture Matrix (R&D Systems) for
512 30 min before organoid medium (as described above) was added. Samples were taken from
513 supernatants at the indicated time points and analyzed by plaque titration assay as described
514 previously.

516 *Infection of intestinal organoids*

517 Human normal colon organoids were established from non-cancerous parts of colorectal cancer
518 resection tissue and cultured as previously published (Sato et al., 2011) under the ethics approval
519 no. EA4/164/19 (to Markus Morkel). For infection studies, organoids were harvested, Matrigel
520 (Corning, #356231) was removed by resuspension and centrifugation. Subsequently, organoids
521 were infected in solution with an MOI of 0.05 (SARS-CoV-2 WT and B.1.1.7 strains) at 37°C for
522 one hour. Infected organoids were seeded in Matrigel and were supplemented with medium.
523 Samples were taken from supernatants at 24, 48 and 72 hours post-infection and analyzed by
524 real-time RT-PCR as described (Corman et al., 2020).

525

526 *Synchronized infection experiments*

527 Synchronized infection experiments were performed to determine entry efficiency of the virus
528 variants. Infection of cells was performed on ice and cells were immediately transferred to 4°C for
529 one hour after virus dilutions were added to ensure synchronized virus uptake and start of
530 replication. After virus adsorption, cells were washed five times with PBS to remove excess of
531 virus particles. Cells were lysed either immediately, or incubated with infection medium until four
532 or six hours post-infection. At the indicated time points, medium was removed and cells were lysed
533 with MagNA Pure 96 external lysis buffer (Roche, Penzberg, Germany). Isolation of RNA from cell
534 lysates and quantitative RT-PCR on subgenomic nucleocapsid RNA was performed as described
535 elsewhere (Corman et al., 2020; Kreye et al., 2020). Entry inhibitors were dissolved in DMSO in
536 the indicated concentrations, added one hour prior to virus infection and were supplied for the
537 entire duration of the experiment.

538

539 *Plaque assay*

540 Plaque assay was performed to determine the titer of stocks and the infectious dose of
541 supernatants harvested from infected cells. 175.000 Vero E6 cells were seeded in a 24-well plate

542 one day prior to infection. After washing the cells once with PBS, cells were inoculated in
543 duplicates with 200 μ l of 1:10 serially diluted cell culture supernatants from infected cells. After
544 adsorption for one hour at 37°C, the virus dilutions were removed and 500 μ l of a highly viscous
545 overlay (1:1 mix of 2.4% avicel and 2x concentrated DMEM supplemented with 5% FBS, 2%
546 NEAA and 2% NaP) was added to each well. The overlay was discarded at three days post-
547 infection. Cells were fixed for 30 min in 6% formaldehyde, washed once with PBS and stained for
548 15 min with crystal violet solution. Plaques were counted from one to two dilutions for which distinct
549 plaques (in a range between 1-100 plaques) were detectable. To calculate the titer, the number
550 of all plaques counted was divided by the respective inoculation volume and multiplied with the
551 inversed dilution factor.

552

553 *Competition assay*

554 Calu-3 cells were infected in 24-well plates with a mixture of two SARS-CoV-2 variants, using
555 three different ratios (1:1 and 9:1 and 1:9) and an initial, total infectious dose of 10.000 PFU
556 (corresponding to an MOI of 0.04). Serial infections were performed by sampling the supernatant
557 of the previous passage at 24 hours post-infection and infecting naive Calu-3 cells with a 1:50
558 dilution of this sample. This process was repeated until completion of five passages. As a control
559 for genome stability over five passages, single infections were performed. Viral RNA was isolated
560 from the initial inoculum and from the supernatant of all five passages. To confirm that the virus is
561 detectable over five passages, concentration of viral RNA was analyzed by quantitative RT-PCR
562 (E gene assay) from each passage. To determine the variant frequency in each passage, RNA
563 samples were sequenced using next generation sequencing techniques (Illumina technology). For
564 virus sequence analysis, the raw sequences were trimmed, matched and presorted for SARS-
565 CoV-2-specific sequence reads. The processed sequence reads were mapped to the
566 BetaCoV/Munich/ChVir984/2020 genome (here referred to as SARS-CoV-2 2019-nCoV strain) in
567 Geneious (version 9.1.8). The two virus variants in each sample were distinguished from each

568 other by their lineage-specific mutations. For evaluation, the relative variant frequencies were
569 calculated for each variable position. This was conducted for a total of 19 lineage-specific
570 mutations which were distributed over the entire genome.

571

572 *Next generation sequencing*

573 Viral RNA was extracted using MagNA Pure 96 System (Roche, Penzberg, Germany) according
574 to the manufacturers' recommendations. The RNA-seq library was prepared from viral RNA
575 extracts using the KAPA RNA HyperPrep Kit (Roche, Penzberg, Germany) and KAPA DI adaptors
576 according to the manufacturers' instructions. The RNA library was subjected to next generation
577 sequencing on a NextSeq System (Illumina) using a NextSeq 500/550 v2.5 Kit (Illumina).
578 Sequences were analyzed using the geneious software, version 9.1.8, and sequence reads were
579 assembled by mapping reads to the respective reference sequences.

580

581 *Live cell imaging*

582 For live cell imaging, Vero E6 cells were infected with SARS-CoV-2 984, B1.1.7 Passau or B1.1.7
583 Baden-Württemberg at an MOI of 0.01 or 0.001 for one hour with subsequent replacement of the
584 inoculum with full culture medium. Cells were imaged with the Zeiss LSM800 Airyscan Confocal
585 Microscope over 72 hours with 30 min intervals in a 5% CO₂ supplemented, humidified
586 environment. Images were analyzed for the onset of visible cytopathic effects and merged using
587 Zeiss ZEN Blue 3.0 and ImageJ 1.53c.

588

589 *In vivo infections*

590 Animals: Animal procedures were performed according to the European Guidelines for Animal
591 Studies after approval by the relevant state authority (Landesamt für Gesundheit und Soziales,
592 Berlin, permit number 0086/20). Per group, nine male and female Roborovski dwarf hamsters

593 (*Phodopus roborovskii*) obtained via the German pet trade were used. Animals were housed in
594 groups of three-six hamsters in GR-900 IVC cages (Tecniplast, Buguggiate, Italy) and provided
595 with bountiful enrichment and nesting materials (Carfil, Oud-Turnhout, Belgium). Hamsters of the
596 same sex were randomly distributed into experimental groups and individually marked with a
597 subcutaneously implanted IPPT-300 transponder (BMDS, Seaford (DE), USA) that allows remote
598 identification and measurement of body temperature.

599 Infection and transmission experiments: To determine virus production *in vivo*, nine
600 hamsters were inoculated with 100,000 PFU of either WT or B.1.1.7. as previously described
601 (Trimpert et al., 2020). Briefly, anaesthetized hamsters received 100,000 PFU SARS-CoV-2 in 20
602 µL MEM by intranasal instillation. At 24 hours post-inoculation, contact to uninfected hamsters
603 was enabled by placing three infected animals into a cage containing three uninfected animals of
604 the same sex. Hamsters were monitored twice daily for clinical signs of infection. Body weight and
605 temperature was recorded daily. Hamsters were sacrificed to determine virological parameters of
606 infection on days 2, 3, 5 and 7 post-infection or contact, or once an individual reached a defined
607 humane endpoint.

608 Virus titrations, RNA extractions and RT-qPCR: To determine virus titers from 50 mg of
609 lung tissue, tissue homogenates were prepared using a bead mill (Analytic Jena) and 10-fold serial
610 dilutions were prepared in MEM, which were then added to Vero E6 cells in 12-well plates. The
611 dilutions were removed after two hours and cells were overlaid with 1.25% microcrystalline
612 cellulose (Avicel) in MEM supplemented with 10% FBS and penicillin/streptomycin. Two days
613 later, cells were formalin-fixed, stained with crystal violet, and plaques were counted. RNA was
614 extracted from 25 mg of lung homogenates and oropharyngeal swabs using the innuPREP Virus
615 RNA kit (Analytic Jena). Viral RNA copies were quantified in 10% of the obtained eluate volume
616 with a one-step RT-qPCR reaction using a standard curve and the Luna Universal Probe One-
617 Step RT-qPCR kit (New England Biolabs) and previously published TaqMan primers and probe
618 (Corman et al., 2020) on a StepOnePlus RealTime PCR System (Thermo Fisher Scientific).

619

620 *Reverse genetics*

621 We employed the previously described in-yeast transformation-associated recombination (TAR)
622 cloning method (Thi Nhu Thao et al., 2020) for the generation of infectious SARS-CoV-2 B.1.1.7
623 cDNA clones. Overlapping DNA fragments were obtained by first strand cDNA synthesis of viral
624 RNA extracts from infected Vero E6 cells using SuperScript III reverse transcriptase (Invitrogen)
625 followed by a nested Phusion PCR (Invitrogen). Primers for TAR fragment generation were used
626 as previously described (Thi Nhu Thao et al., 2020) with B.1.1.7-specific deviations for two
627 fragments, as specified in Table S1. For generation of the D⁶¹⁴G mutant we performed site-
628 directed mutagenesis PCR (NEB) on synthetic viral subgenomic fragments cloned into pUC57
629 vectors (Thi Nhu Thao et al., 2020). Assembly of purified DNA fragments was performed by TAR
630 cloning as previously described (Thi Nhu Thao et al., 2020). Clones were screened for correctly
631 assembled DNA fragments by multiplex PCR using the QIAGEN Multiplex PCR kit (QIAGEN)
632 according to the manufacturers' instructions. Clones tested positive for all junctions were
633 expanded, plasmid DNA was extracted, linearized and subjected to T7-based *in vitro* RNA
634 transcription (Thermo Fisher Scientific). Capped viral RNA was electroporated into baby hamster
635 kidney cells and supernatant was subsequently transferred to Vero E6 cells one day after
636 electroporation for stock production. Successful virus rescue was confirmed by SARS-CoV-2-
637 specific RT-PCR. Virus stocks were harvested three days post-infection, purified and deep
638 sequenced as described above.

639

640 *Isolation of viral RNA and quantitative real-time RT-PCR assay*

641 For isolation of viral RNA, 50 µl of supernatant was diluted in 300 µl of MagNA Pure 96 external
642 lysis buffer (Roche, Penzberg, Germany). All samples were heat inactivated for ten minutes at
643 70°C prior to export from the BSL-3. Isolation and purification of viral RNA was performed using
644 the MagNA Pure 96 System (Roche, Penzberg, Germany) according to the manufacturers'

645 recommendations. Viral RNA was quantified using real-time RT-PCR (E gene assay) as previously
646 described (Corman et al., 2020).

647

648 *Isolation of total RNA, cDNA synthesis and quantitative PCR*

649 For extraction of total RNA, the MagNa Pure 96 System (Roche, Penzberg, Germany) was used
650 according to the manufacturers' instructions. Briefly, cells were washed once with PBS before 350
651 µl of external lysis buffer (Roche, Penzberg, Germany) was added to the cells. Lysed cells were
652 resuspended 2-3 times and transferred to the reaction tube. Samples were heat-inactivated for
653 ten min at 70°C and exported from the BSL-3 laboratory. For quantitative RT-PCR, a 12.5 µl
654 reaction with 2.5 µl RNA was done with the SuperScript III one-step reverse transcriptase-PCR
655 system (Invitrogen) with the Platinum Taq DNA polymerase according to the manufacturers'
656 protocol and the primers indicated in Table S2. Probes contained a 5' FAM-520 reporter dye and
657 a ZEN/Iowa Black FQ 3' quencher (Integrated DNA technologies). The RT-PCR was performed
658 using a thermocycling protocol with reverse transcription for 15 min at 55°C and a subsequent
659 denaturation step for two min at 95°C to restore Taq DNA polymerase activity, followed by PCR
660 amplification by 45 cycles of 95°C for 15 sec and 58°C for 30 sec. Fluorescence signals were
661 detected after the elongation step of each cycle. The mean fold change in gene expression was
662 calculated by the delta-delta ct method and by using expression of TATA-binding protein (TBP)
663 as a reference.

664 To determine early virus replication, a quantitative RT-PCR targeting the subgenomic RNA
665 encoding the nucleocapsid (sgN) was performed. Viral RNA was extracted from cell lysates which
666 were previously lysed by external lysis buffer (Roche, Penzberg, Germany) as described above.
667 RT-PCR was done with the following primers and probe: nCoV sgN Fwd: 5'-CGA TCT CTT GTA
668 GAT CTG TTC TC-3', nCoV sgN Rev: 5'-CAG TAT TAT TGG GTA AAC CTT GG-3' and nCoV
669 sgN prb: 5'-56-FAM/ CAG TAA CCA GAA TGG AGA ACG CAG /3BHQ-1-3 ((Kreye et al., 2020)).

670 For quantification, values were normalized to the housekeeping gene TBP levels by delta ct
671 method.

672

673 *Tat-mediated cell-cell fusion assay*

674 CHO and TZM-bl cells were retrieved from the NIH AIDS Reagent Program and propagated as
675 recommended. CHO cells were transiently transfected with expression plasmids for HIV-1 Tat and
676 individual pCG-spike-HA or empty vector control for 48 hours, using Lipofectamine LTX Reagent
677 with PLUS™ Reagent (Invitrogen). TZM-bl cells, stably expressing LTR-driven luciferase, were
678 transfected with a plasmid encoding human ACE2 and human myc-TMPRSS2. CHO and TZM-bl
679 cells were cocultured for eight hours. Subsequently, cells were washed once with PBS, lysed
680 using cell culture lysis buffer (Promega), and Tat-dependent increase of luciferase enzyme activity
681 in cell lysates was determined with the Luciferase Assay system (Promega). Luminometric activity
682 was analyzed with a Mithras luminometer.

683

684 *Lentivirus production and transduction experiments*

685 SARS-CoV-2 spike-HA-pseudotyped lentiviral particles were produced in triple-transfected
686 HEK293T cells. Cells were transfected with individual pCG-SARS-CoV-2 spike-HA plasmids, the
687 HIV-1-based packaging plasmid deltaR8.91 (Zufferey et al., 1997) and the luciferase transfer
688 plasmid pCSII-luciferase (Agarwal et al., 2006) via calcium phosphate precipitation. Virus-
689 containing supernatant was harvested 40 and 64 hours post transfection and sterile-filtered.
690 Particles were concentrated via ultracentrifugation through a 20% sucrose cushion. Indicated cell
691 lines were transduced for 72 hours with identical p24 capsid equivalents as quantified by
692 immunoblotting of particle lysates. Transduction efficiency was quantified luminometrically three
693 days post-transduction.

694

695 *Immunoblotting*

696 To determine incorporation and processing of Spike in lentiviral particles, transduced cells and
697 lentiviral particles were lysed with M-PER Mammalian Protein Extraction Reagent (Pierce) and
698 Triton X-100, respectively. The lysate was mixed with Laemmli buffer and boiled for ten minutes
699 at 95°C. Proteins were separated on a 10% SDS-PAGE and immobilized on a nitrocellulose
700 membrane (GE Healthcare) using the Trans-Blot Turbo system (BioRad). Blocked membranes
701 were incubated with the following antibodies: mouse anti-HIV-1 p24 capsid (ExBio, 1:1000), rabbit
702 anti-S2 spike (Novusbio, NB100-56578, 1:1000), mouse anti-HA (Sigma, H3663, 1:1400), rabbit
703 anti-tubulin (Cell Signaling Technology, 2144S, 1:1000). Secondary antibodies conjugated to
704 Alexa680/800 fluorescent dyes were used for detection and quantification by Odyssey Infrared
705 Imaging System (LI-COR Biosciences). Spike processing efficiency was calculated as the
706 percentage of S2 from total spike signal. Relative levels of spike-HA abundance in lentiviral
707 pseudotypes were quantified by calculating the signal intensity of S2-HA per HIV-1 p24 capsid.

708 To determine the processing and incorporation of spike from infected cells, cells and
709 purified virus particles were lysed with RIPA (ThermoFisher Scientific) buffer supplemented with
710 complete protease inhibitor cocktail (Roche) for 30 min at 4°C. Subsequently, lysates were
711 centrifuged for 15 min at 4°C and 15,000 rpm to remove cell debris. The supernatants were mixed
712 with 4x Laemmli buffer, which was supplemented with 10% beta-mercaptoethanol, and lysates
713 were boiled for ten minutes at 95°C to ensure protein denaturation and virus inactivation. Protein
714 concentration was determined by BCA protein assay (ThermoFisher Scientific) and 20 µg total
715 protein was loaded. Proteins were separated by SDS-PAGE on a 6% gel and transferred to a
716 nitrocellulose membrane (0.45 µm pore size, GE Healthcare) by Trans-Blot Turbo system
717 (BioRad). Membranes were blocked with 5% dried milk in 0.1% PBS-Tween (0.9% NaCl, 10 mM
718 Tris-HCl [pH 7.5], 0.1% Tween 20) for 30 min at room temperature. Blocked membranes were
719 incubated with the following antibodies: rabbit anti-S2 spike (Novusbio, NB100-56578, 1:1000),
720 rabbit anti-SARS-CoV-2 nucleocapsid (GeneTex, GTx135361, 1:1000). Secondary antibodies
721 conjugated with horseradish peroxidase (HRP) were used for chemiluminescence-based

722 detection by Fusion Fx7 (Peqlab Biotechnologie GmbH). Detection was performed using
723 SuperSignal™ West Femto substrate (ThermoFisher Scientific). Quantification was done by the
724 use of ImageJ 1.48v software. Spike processing efficiency was calculated as the percentage of
725 S2 from total spike signal. Relative levels of spike abundance in concentrated virion preparations
726 were quantified by calculating the signal intensity of S2 per nucleocapsid.

727

728 *MagPix Luminex*

729 To assay cytokine levels in airway epithelial cell supernatant, 25 μ l of supernatant were sampled
730 prior to infection and at 24 h and 48 h post-infection with SARS-CoV-2 WT, B.1.1.7/v1, and
731 B.1.1.7/v2. Cytokine quantification was performed using a Human Cytokine/Chemokine/Growth
732 Factor Panel A 48-Plex Premixed Magnetic Bead Multiplex Assay (Merck Millipore), using the
733 Luminex MAGPIX System in 96-well plate format, according to the manufacturer's instructions.
734 Plate washing steps were performed using HydroFlex Microplate Washer (Tecan). Calibration,
735 verification, and quality control checks were met for all of the analytes. However, Analyte 15 (FGF-
736 2) was omitted because its standard curve had an R^2 value of 0.82 and Analyte 53 (IL-17F) was
737 omitted because it had a high limit of detection and several extreme outliers from the rest of the
738 dataset. All other analytes were reported.

739

740 *PRNT assays*

741 Plaque reduction neutralization tests (PRNT) were performed as previously described (Kreye et
742 al., 2020; Wölfel et al., 2020). Briefly, heat-inactivated sera were serially diluted starting at 1:40 in
743 OptiPro, mixed 1:1 with 200 μ L virus solution containing 200 plaque forming units of SARS-CoV-
744 2 (strains B.1, B.1.1.7/v1, and B.1.351) and 200 μ L of the mix were incubated in duplicates on
745 Vero E6 cells (160,000 cells per well) seeded in 24-well plates on the previous day. After one hour
746 incubation at 37°C, the supernatant was discarded and cells were washed with PBS and overlaid
747 with 1.2% Avicel solution in supplemented DMEM. After three days at 37°C, the supernatants

748 were removed and cells were inactivated and fixed with a 6% formaldehyde/PBS solution and
749 stained with crystal violet. Serum dilutions with a mean plaque reduction of 50% and 90% are
750 referred to as PRNT₅₀ or PRNT₉₀. For numerical calculations, titers <40 were set to 20, and titers
751 >1:640 were set to 1:1,280.

752

753 *Surrogate neutralization assay*

754 Neutralizing capacity of patients' sera against B.1, B.1.1.7, and B.1.351 was assessed by a
755 surrogate virus neutralization test (cPass Assay, Medac, Wedel, Germany) as described
756 previously (Momsen Reincke et al., 2021; von Rhein et al., 2021). Briefly, sera of infected and
757 vaccinated patients were diluted 1:10 with sample dilution buffer, mixed 1:1 with B.1-HRP-RBD,
758 B.1.1.7-HRP-RBD, and B.1.351-HRP-RBD (provided by Medac, Wedel, Germany) solution and
759 incubated at 37°C for 30 minutes. Afterwards, the mixture was added to the hACE2-coated plate
760 and incubated at 37°C for 15 minutes. After washing, 3'3,5,5-tetramethylbenzidine solution was
761 added, and the plate was incubated in the dark at room temperature for 15 minutes. Stop solution
762 was then added and the optical density at 450 nm was measured using a Tecan Infinite 200 PRO
763 plate reader. For calculation of the relative inhibition of ACE2/RBD binding, the following formula
764 was applied: Inhibition score (%) = (1 – OD value sample/OD value negative control) × 100%.

765 Values below zero were set to zero.

766

767 **Data Presentation and Statistical Analysis**

768 If not stated otherwise, bars and symbols show the arithmetic mean of the indicated amount of
769 independent replicates. Error bars indicate S.D. from at least three or S.E.M. from the indicated
770 amount of individual experiments. Statistical analysis was performed with GraphPad Prism (V
771 8.3.0 or 9.1.2) using two-tailed unpaired Student's t-tests or for comparing neutralizing activities
772 the Friedman test and Dunn's multiple comparison unless indicated differently. *P* values <0.05

773 were considered significant (*), <0.01 (**), <0.001 (***), <0.0001 (****); n.s. = not significant
774 (≥ 0.05).

775

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796

797 **AUTHOR CONTRIBUTIONS**

798 Conceptualization: DN, CG, CD

799 Methodology: DN, KF, SSch, FWe, JT, CG, CD, AB, MAM
800 Investigation: DN, KF, SSch, FWe, JT, AR, SSt, JJ, JE, JK, FP, LMJ, RO, MCJ, BT, JP, JH, FWa,
801 MLS, NH, EMB, TV, MB, AB, JS, CM, MAM
802 Resources: KH, MW, TTNT, SC, LGH, UM, MM, MAMü, CG, CD, AB, MAM, LH, VMC
803 Writing – Original Draft: DN, CG, CD
804 Writing – Review & Editing: DN, AB, MAM, KO, MaMü, VMC, CG, CD
805 Visualization: DN, SSchr, KF, JT, AR, SSt, JE, JK, FP, LMJ
806 Supervision: DN, SC, MAM, ACH, VT, KO, TW, UM, MAMü, VMC, CG, CD
807 Funding Acquisition: AB, MAM, ACH, TW, VMC, CG, CD
808

809 **DECLARATION OF INTERESTS**

810 Technische Universität Berlin, Freie Universität Berlin and Charité - Universitätsmedizin have filed
811 a patent application for siRNAs inhibiting SARS-CoV-2 replication with DN as co-author. MAMü
812 and VMC are named together with Charité - Universitätsmedizin Berlin and Euroimmun
813 Medizinische Labordiagnostika AG on a patent application (EP3715847) filed recently regarding
814 the diagnostic of SARS-CoV-2 by antibody testing. The other authors declare no competing
815 interests.

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840 **LEGENDS**

841

842 **Figure 1. B.1 and B.1.1.7 SARS-CoV-2 display similar replication kinetics in immortalized
843 cell lines**

844 (A) Plaque morphology on Vero E6 cells which were infected with 1:100 diluted (B.1 and
845 B.1.1.7/v1) or undiluted (B.1.1.7/v2) supernatants of infected Vero E6 cells.

846 (B) Vero E6 cells were infected at the indicated MOI and onset of CPE was monitored by live cell
847 imaging until 70 hours post-infection.

848 (C-E) Virus growth was quantified in Vero E6 (C) Caco-2 (D) and Calu-3 (E) cells infected at
849 indicated MOIs. Supernatant collected at the respective time points was titrated by plaque assay.

850 Growth kinetic experiments in Vero E6 and Caco-2 cells were each performed in triplicates. One
851 representative experiment out of two is shown for Calu-3 cells.
852 (F) Virus growth in Calu-3 cells infected at an MOI of 0.1 was quantified at early time points after
853 infection.
854 (G) Virus growth kinetics in Calu-3 cells at 37°C (left) and 32°C (right).
855 (H) Competition assay. Calu-3 cells were infected with a mixture of B.1 and B.1.1.7/v1 at indicated
856 ratios. After serial passaging, viral RNA from the supernatant was isolated, sequenced and the
857 relative proportion of B.1- and B.1.1.7-corresponding sequences (discriminated by a mutation in
858 nsp12) was plotted. Data show arithmetic means of one experiment performed in triplicates.
859 Dashed horizontal lines indicate the lower detection limit of the plaque assay. Inoc.: Inoculum,
860 MOI: multiplicity of infection, PFU: plaque forming units, p0-p5: passage 0 - passage 5.

861
862 **Figure 2. Comparison of fitness in primary human respiratory cells, organoids and dwarf**
863 **hamsters**

864 (A) Virus growth kinetics were performed in infected human nasal airway epithelial cultures
865 (hNAECs) (MOI 0.1). Samples were collected from the apical and basal side at indicated time
866 points and titrated by plaque assay. n=3 biological replicates.

867 (B) Virus growth kinetics was conducted in infected bronchial AEC (MOI 0.5). Samples were
868 collected from the apical side and titrated by plaque assay. Data are derived from one experiment
869 conducted in triplicates.

870 (C) Intestinal organoids were infected (MOI 0.05) and viral load in supernatant (left) and organoid
871 lysates (right) was quantified at indicated time points by E-gene specific quantitative RT-PCR.
872 Data are derived from four independent experiments.

873 (D) Virus replication was monitored in infected lung organoids (MOI 1). Samples harvested at
874 indicated time points were titrated by plaque assay. Data are derived from three independent
875 experiments.

876 (E) Dwarf hamsters were intranasally infected (100,000 PFU) and infectious virus particles from
877 lung homogenates were quantified using plaque assay (left). Donor hamsters were co-housed
878 with naive animals and transmission efficiency was determined from lung homogenates at the
879 indicated time points (right). n=1-3 animals per experimental condition.

880 Dotted horizontal lines indicate the lower detection limit of the plaque assays. GE: genome
881 equivalents, n.d.: not detected

882

883 **Figure 3. B.1.1.7 spike displays decreased proteolytic processing**

884 (A) Spike processing in lysates of HEK293T cells expressing empty vector or SARS-CoV-2 spike-
885 HA encoding individual or all B.1.1.7-corresponding mutations was quantified by immunoblotting
886 (upper panel). Shown is one representative immunoblot (bottom panel) out of four.

887 (B) Protein in lysed lentiviral particles pseudotyped with SARS-CoV-2 spike-HA was quantified by
888 immunoblotting (upper panel). Shown is one representative immunoblot (bottom panel) out of four.

889 (C) Vero E6 cells were infected with SARS-CoV-2 (MOI 5). Cells and virus-containing
890 supernatants were harvested at 48 hours post-infection and processed for detection of spike and
891 nucleocapsid by immunoblotting. Processing of spike in cell lysates (left panel) and spike
892 incorporation in concentrated virion preparations (middle panel) was quantified. One
893 representative blot out of two is shown (right panel).

894 Black and white arrowheads indicate the bands of the uncleaved spike-HA precursor and of the
895 cleaved S2-HA subunit, respectively. Statistical significance was calculated by a two-tailed, paired
896 Student's T-test. kDa: kilodalton, UI: uninfected

897

898 **Figure 4. Membrane fusion and entry based on lentiviral pseudotypes and SARS-CoV-2
899 particles.**

900 (A) For Tat-mediated cell-cell fusion assay, CHO cells were co-transfected with plasmids
901 expressing indicated spike-HA and HIV-1 Tat. LTR-luciferase-expressing target TZM-bl cells were

902 transfected with plasmids encoding human ACE2 and TMPRSS2. Transfected cells were co-
903 cultured for eight hours and luciferase expression resulting from intercellular Tat transfer was
904 quantified luminometrically. All values were normalized to B.1 spike (indicated by a dotted line).
905 Shown are results from three-six biological replicates, each performed in triplicates.
906 (B) Calu-3 cells were transduced with lentiviral pseudoparticles expressing luciferase and
907 decorated with indicated spike-HA. Transduction efficiency was quantified luminometrically.
908 Dotted line indicates background levels of luciferase non-transduced cultures. Shown are results
909 from six biological replicates, each performed in triplicates, indicated by symbols.
910 (C) Indicated A549 cells were transduced with increasing quantities (0.5 μ l, 5 μ l and 50 μ l) of
911 lentiviral, luciferase-expressing particles pseudotyped with B.1- or B.1.1.7-spike. Transduction
912 efficiency was determined luminometrically. Dotted line indicates luciferase background level of
913 luciferase detected in non-transduced cells. Symbols represent individual values of three
914 biological replicates, each performed in triplicates.
915 (D, E) Calu-3 (D) and indicated A549 (E) cells were infected at 4°C with B.1 or B.1.1.7 isolates
916 (MOI 1) to allow synchronized infection. Total cellular RNA was isolated at the indicated time
917 points and nucleocapsid-encoding subgenomic RNA was quantified by RT-PCR.
918 Del: deletion, RLU: relative light units, sgRNA N: subgenomic nucleocapsid RNA, conc.:
919 concentration
920

921 **Figure 5. B.1.1.7 fails to escape from neutralizing antibodies and may dampen induction of**
922 **innate immunity**

923 (A) Neutralizing titers against the indicated virus strains were determined in plaque reduction
924 neutralization tests (PRNT). Red line indicates median titers per group.
925 (B) Inhibition of ACE2/RBD interaction was measured using surrogate virus neutralization assays.
926 Sera were tested using RBD proteins of B.1, B.1.1.7 and B.1.351 as indicated.

927 Red lines indicate median values. The same set of samples was measured in (A) and (B),
928 vaccinees n=19, Non-VOC convalescent donors n=50, B1.1.7 patients n=13.
929 (C) Concentration (pg/ml) of cytokines and chemokines in the basal medium of infected bronchial
930 airway epithelial cells (MOI 0.5). Concentration of cytokines and chemokines was determined by
931 MagPix Luminex technology.
932 (D) Calu-3 cells were infected at indicated MOIs or left uninfected (UI) and cell lysates were
933 generated 16 hours post-infection. Total RNA was extracted and expression of the indicated genes
934 was determined by quantitative real-time PCR. Shown is the mean fold change +/- S.D.. The
935 experiment was performed in triplicates.

936

937 **Figure 6. B.1.1.7 and B.1.617.2 display a spike-dependent, ACE2-independent post-entry**
938 **replication advantage in NCI-H1299 cells.**

939 (A) Virus growth of B.1 and B.1.1.7/v1 was assessed on NCI-H1299 cells. Cells were infected
940 (MOI 0.01) and supernatants of indicated time points were titrated on Vero E6 cells. Plaque
941 morphology of NCI-H1299-derived B.1 and B.1.1.7/v1 on Vero E6 cells is shown.

942 (B) NCI-H1299 cells were transduced with increasing amounts of lentiviral particles pseudotyped
943 with indicated spike proteins. Pseudotype entry was analyzed luminometrically in cell lysates. Data
944 from two biological replicates, each performed in triplicates, is shown. White symbols represent
945 arithmetic means of the biological replicates.

946 (C) H1299 cells were infected in triplicates at 4°C with B.1 or B.1.1.7 isolates (MOI 1) to allow
947 synchronized entry. Relative quantities of cell-associated nucleocapsid-specific subgenomic RNA
948 were determined by quantitative RT-PCR. Two independent experiments were performed, each
949 conducted in 3-4 replicates. Symbols represent the arithmetic means of each experiment.

950 (D) ACE2 expression levels were analyzed by immunoblotting. Beta-actin was used as a loading
951 control.

952 (E) Expression of *TMPRSS2* and *ACE2* was quantified by quantitative RT-PCR in indicated cells.

953 (F, G) Calu-3 (F) and NCI-H1299 (G) cells were pretreated with 20 µg/ml anti-ACE2 antibody for
954 one hour prior to infection with B.1 and B.1.1.7 isolates (MOI of 0.01). At 48 hours post-infection,
955 viral replication was quantified from the supernatant by the use of E-gene assay. Results from two
956 independently performed experiments, each conducted in triplicates, are shown.
957 (H-J) Vero E6 (H), Calu-3 (J) and NCI-H1299 (J) cells were infected (MOI 0.01) and supernatant
958 was titrated on Vero E6 cells. The growth experiment in Vero E6 cells was performed once in
959 duplicates. Growth experiments in Calu-3 and NCI-H1299 cells were performed once in triplicates.
960 (K-M) Virus growth of B.1, B.1.1.7 and B.1.617.2 isolates (MOI 0.01) was quantified in Calu-3 (K),
961 human bronchial airway epithelial cells (hBAECs) (L) and NCI-H1299 (M) cells.
962 Dashed horizontal lines indicate the lower limit of detection of the plaque assay.

963

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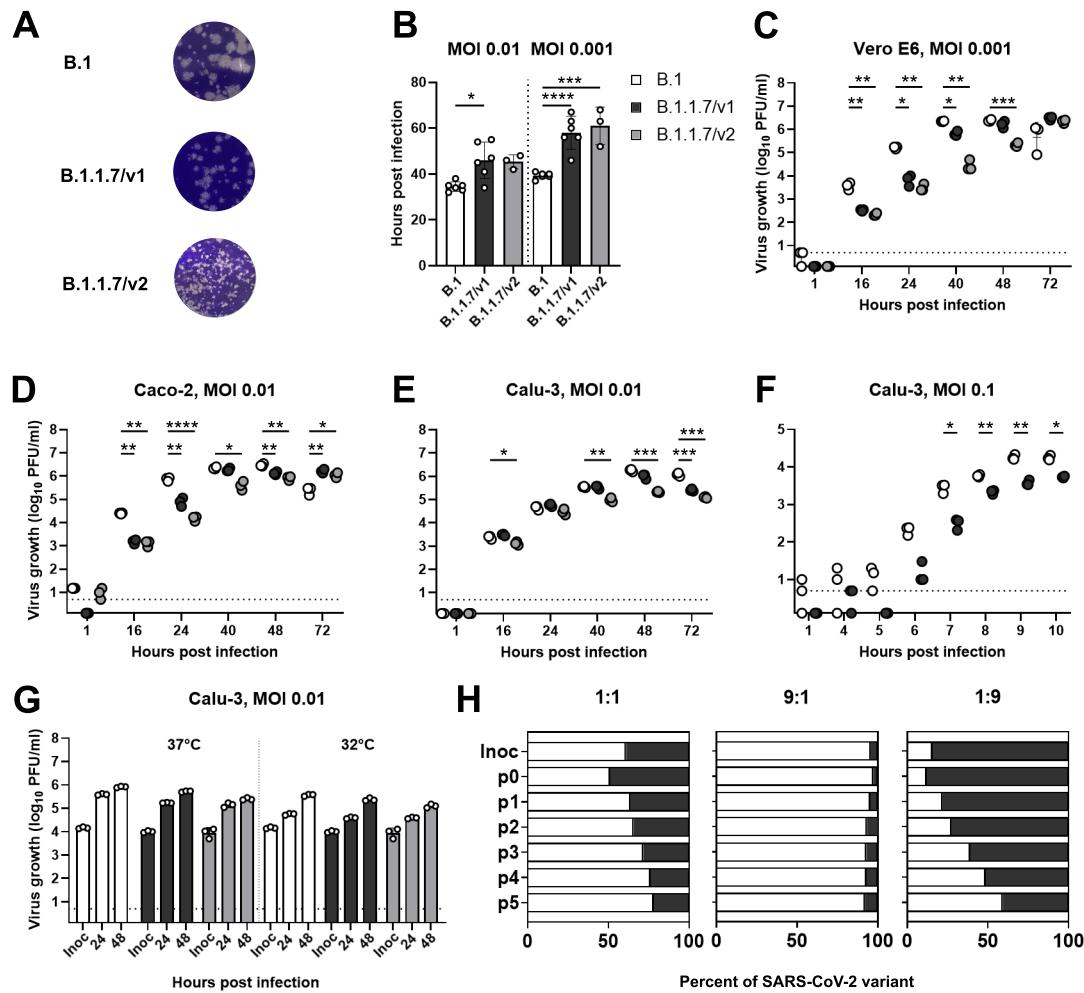
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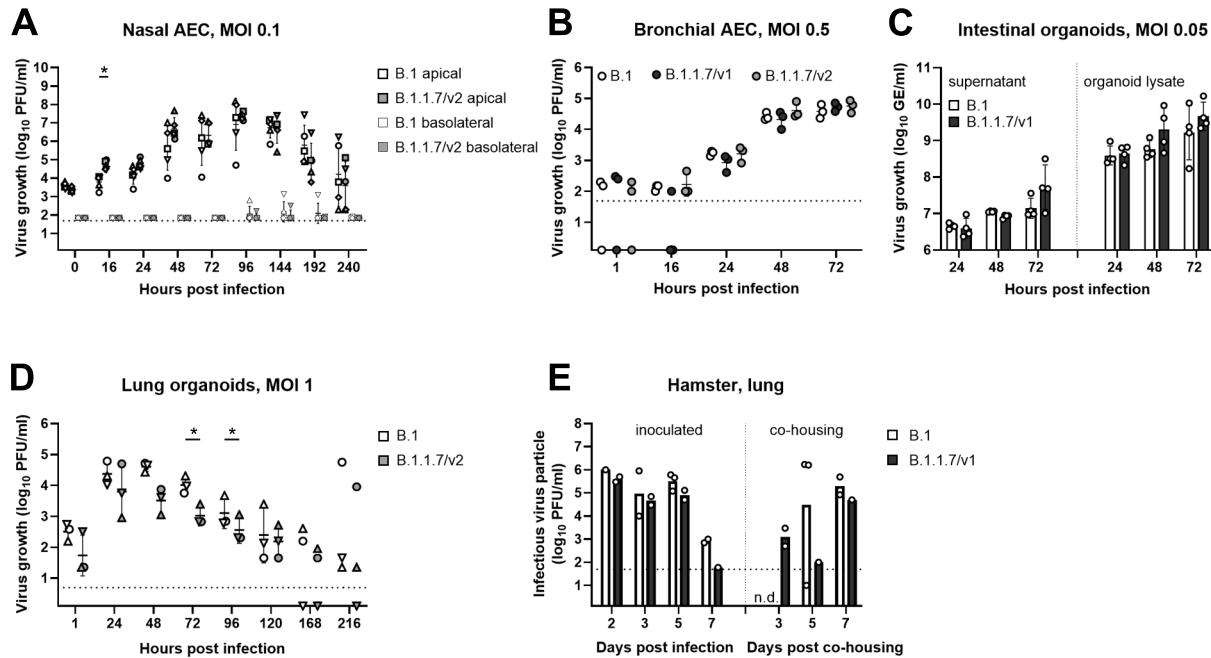
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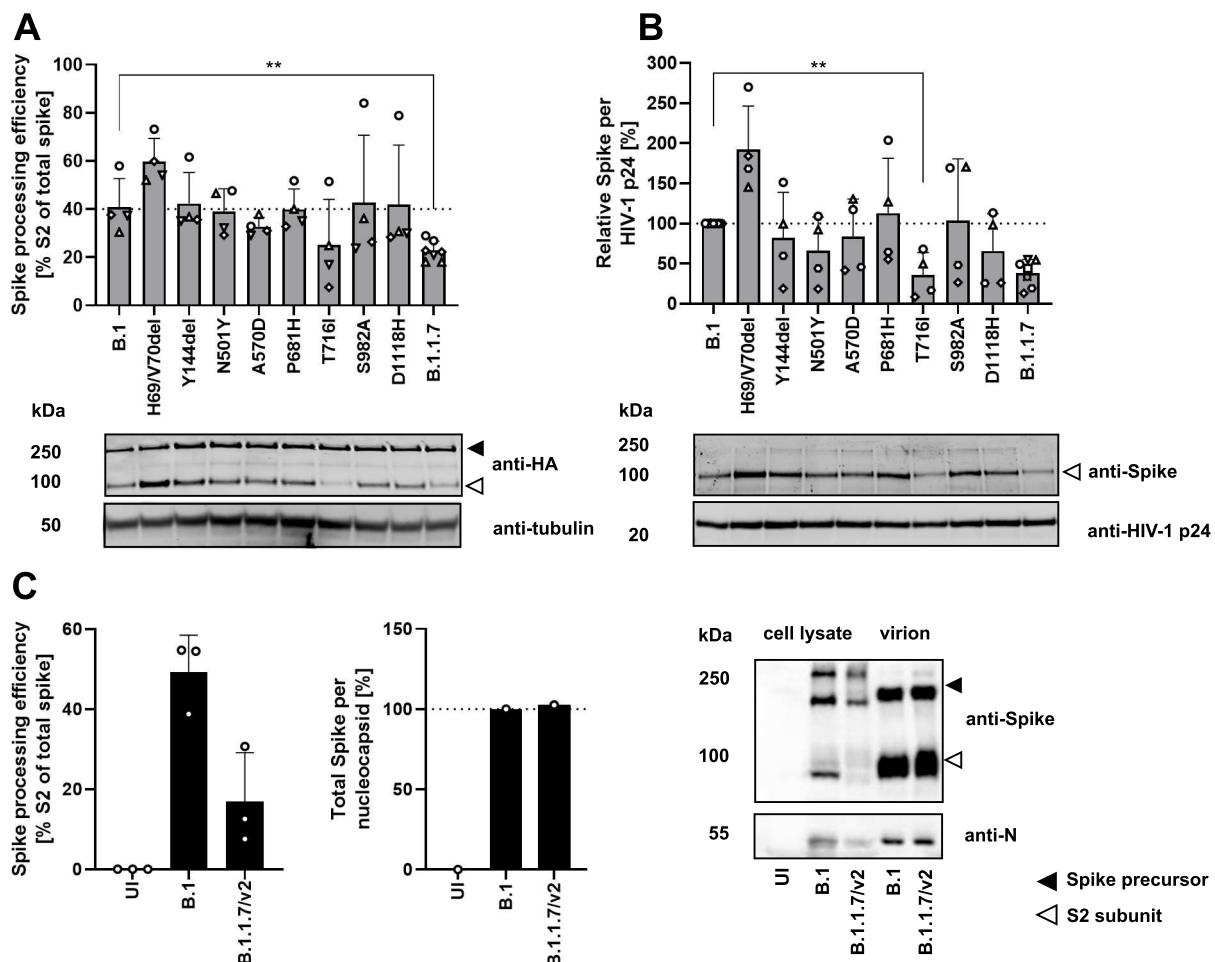
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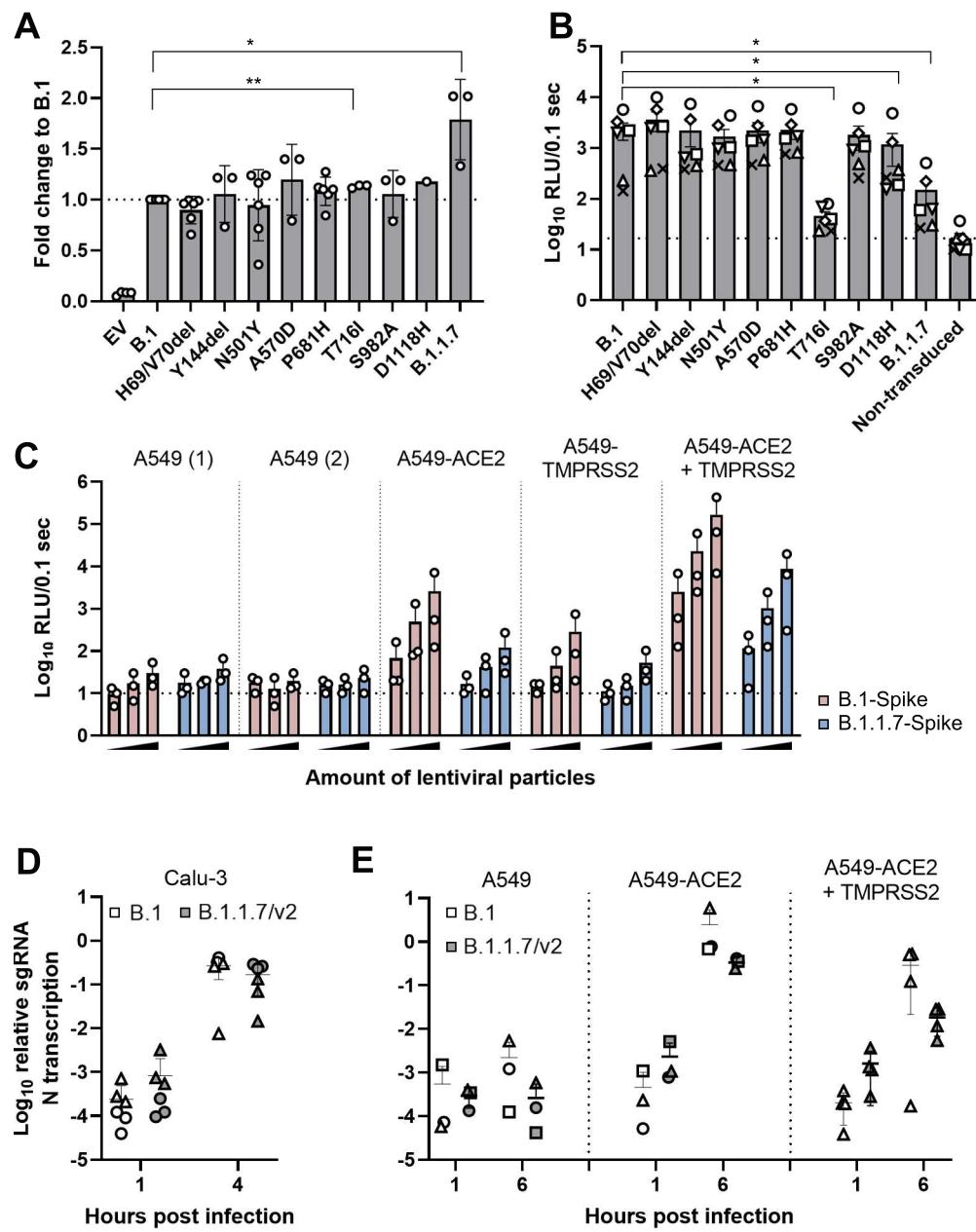
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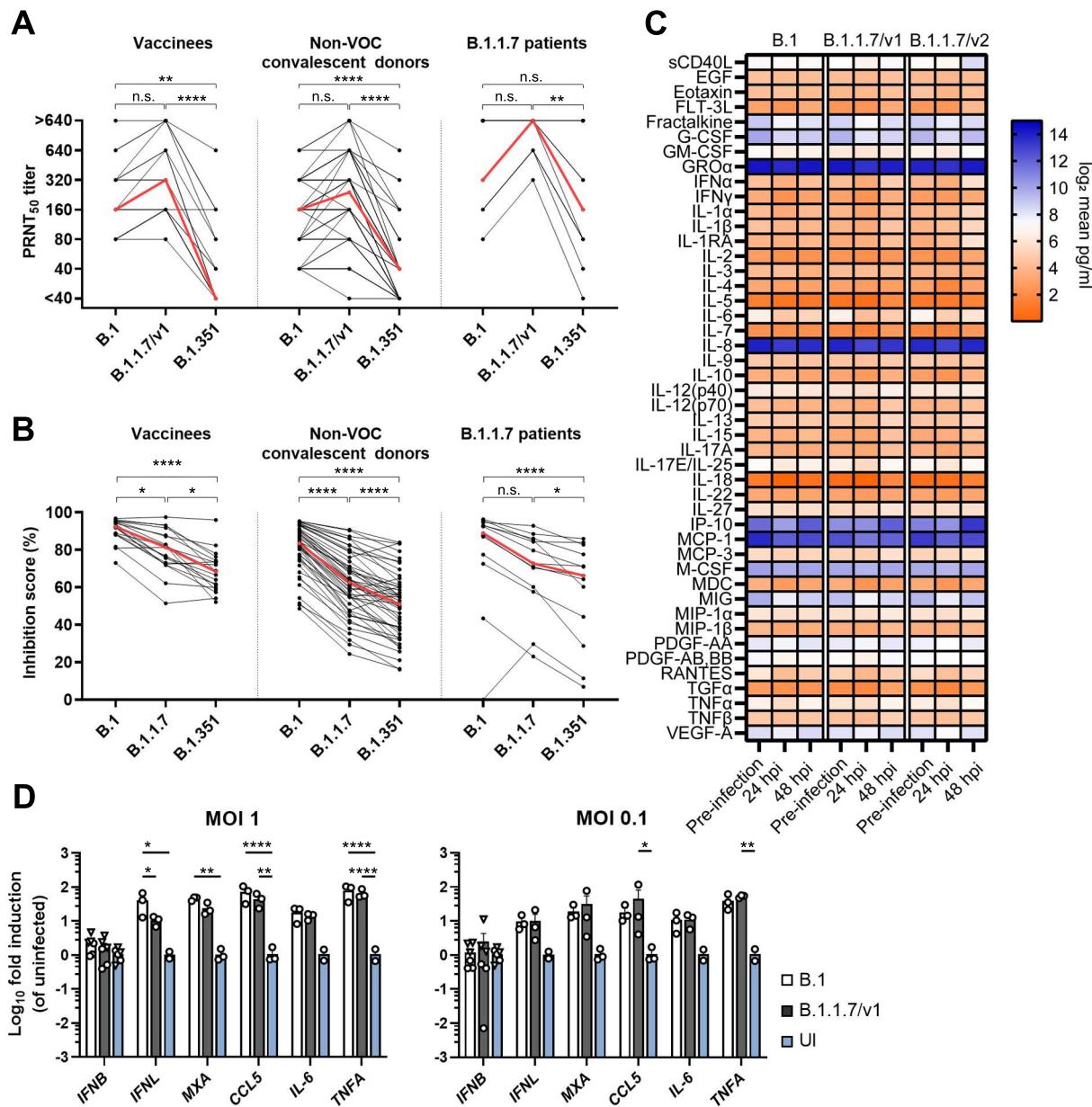
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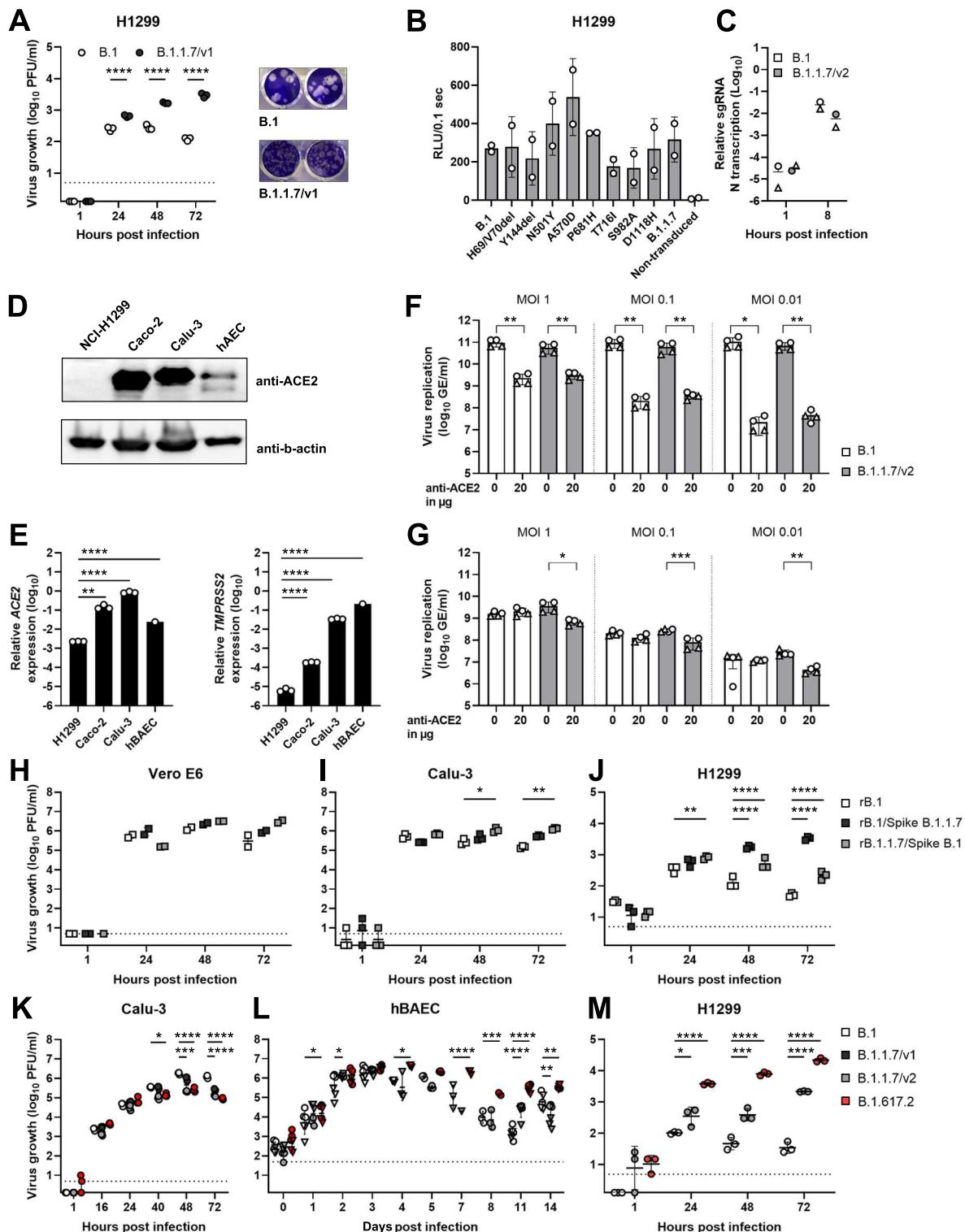
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Post-entry, spike-dependent replication advantage of B.1.1.7 and B.1.617.2 over B.1

SARS-CoV-2 in an ACE2-deficient human lung cell line

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SUPPLEMENTAL INFORMATION

Supplemental Figures (Figures S1-S3)

Supplemental Tables (Tables S1-S2)

Supplemental Movies

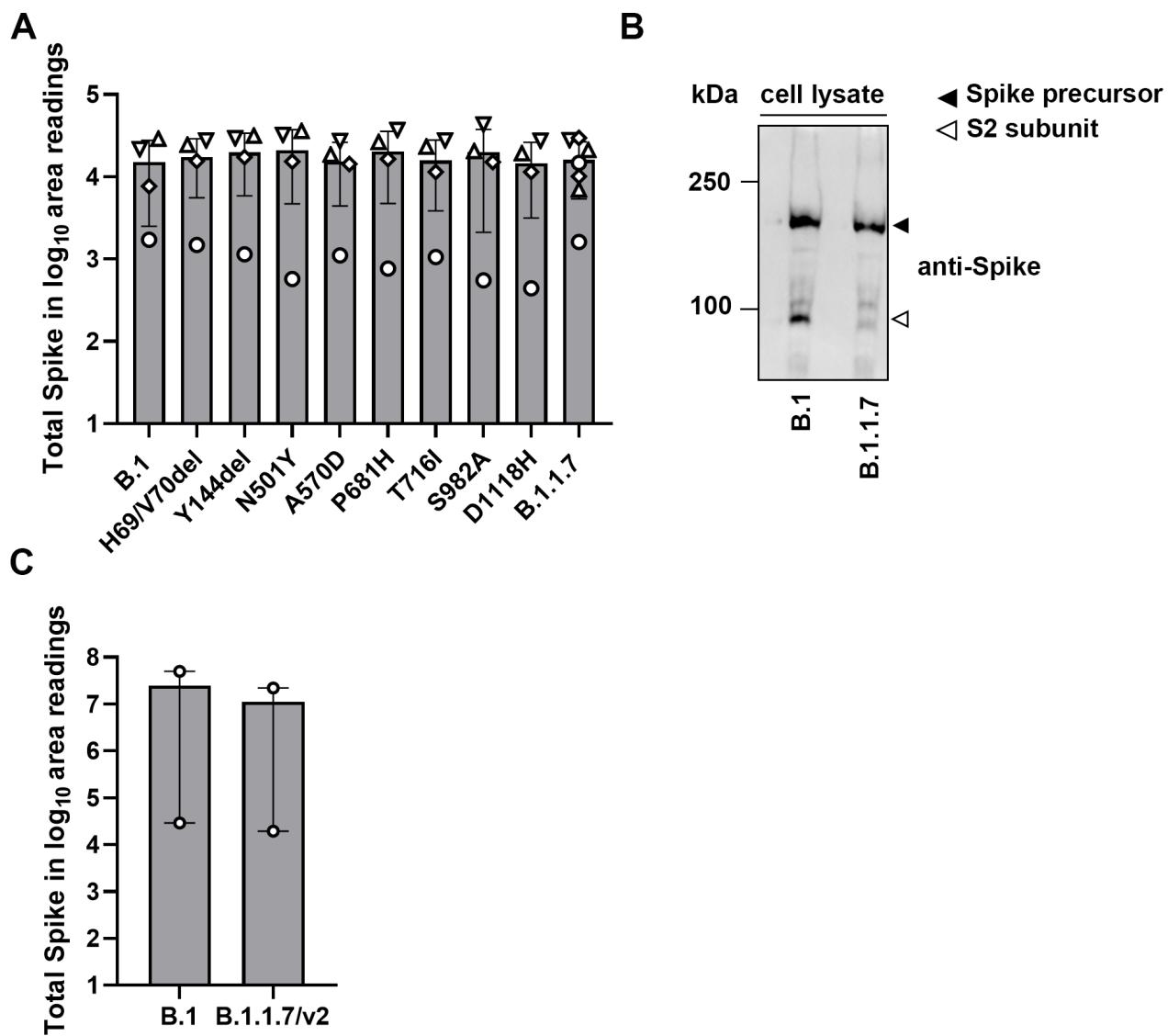


Figure S1. B.1 and B.1.1.7 spike is expressed at similar levels.

(A) Expression of total spike in HEK-293T cells. Symbols represent independently performed experiments.

(B) Vero E6 cells were infected with SARS-CoV-2 (MOI 5). Cells and virus-containing supernatants were harvested at 48 hours post-infection and processed for detection of spike by immunoblotting.

(C) Expression of total Spike in Vero E6 cells was quantified by the use of ImageJ 1.48v.

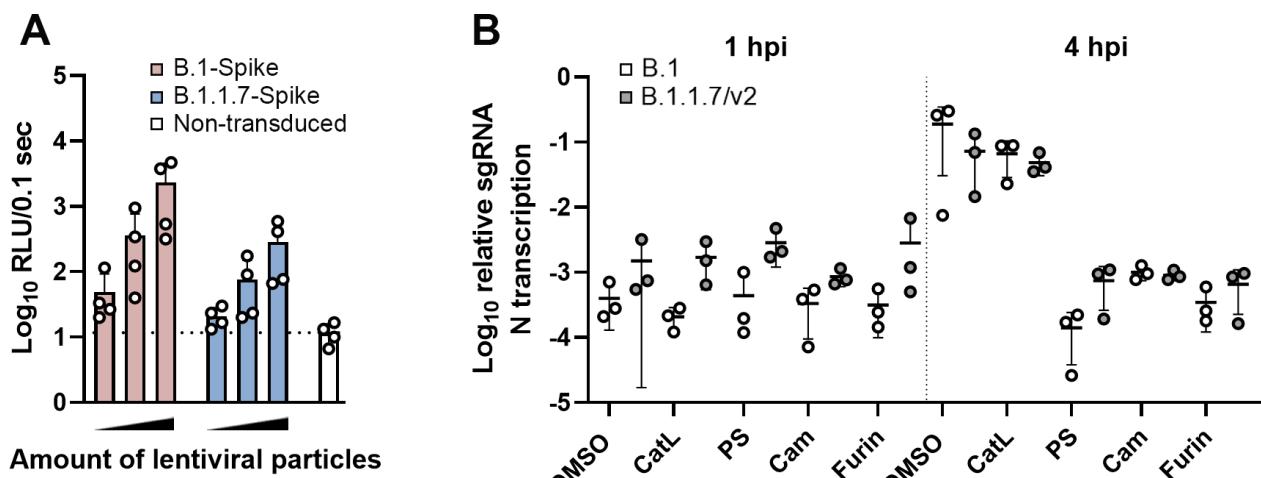


Figure S2. B.1.1.7 spike is not superior in mediating entry compared to B.1 spike

(A) Calu-3 cells were transduced for 72 hours with increasing amounts of lentiviral particles (0.1 μ l, 1 μ l and 10 μ l) pseudotyped with either WT- or B.1.1.7-spike proteins. Pseudotype entry was analyzed luminometrically in cell lysates.

(B) Calu-3 cells were pretreated with 25 μ M MDL28170 (Cathepsin L inhibitor), 25 μ M pitstop II (clathrin inhibitor), 100 μ M Camostat (TMPRSS2 inhibitor) or 15 μ M CMK (furin inhibitor), infected and entry efficiency was determined by sgN quantitative RT-PCR.

DMSO: Dimethylsulfoxide, CatL: Cathepsin L, PS: PitStop, Cam: Camostat mesylate

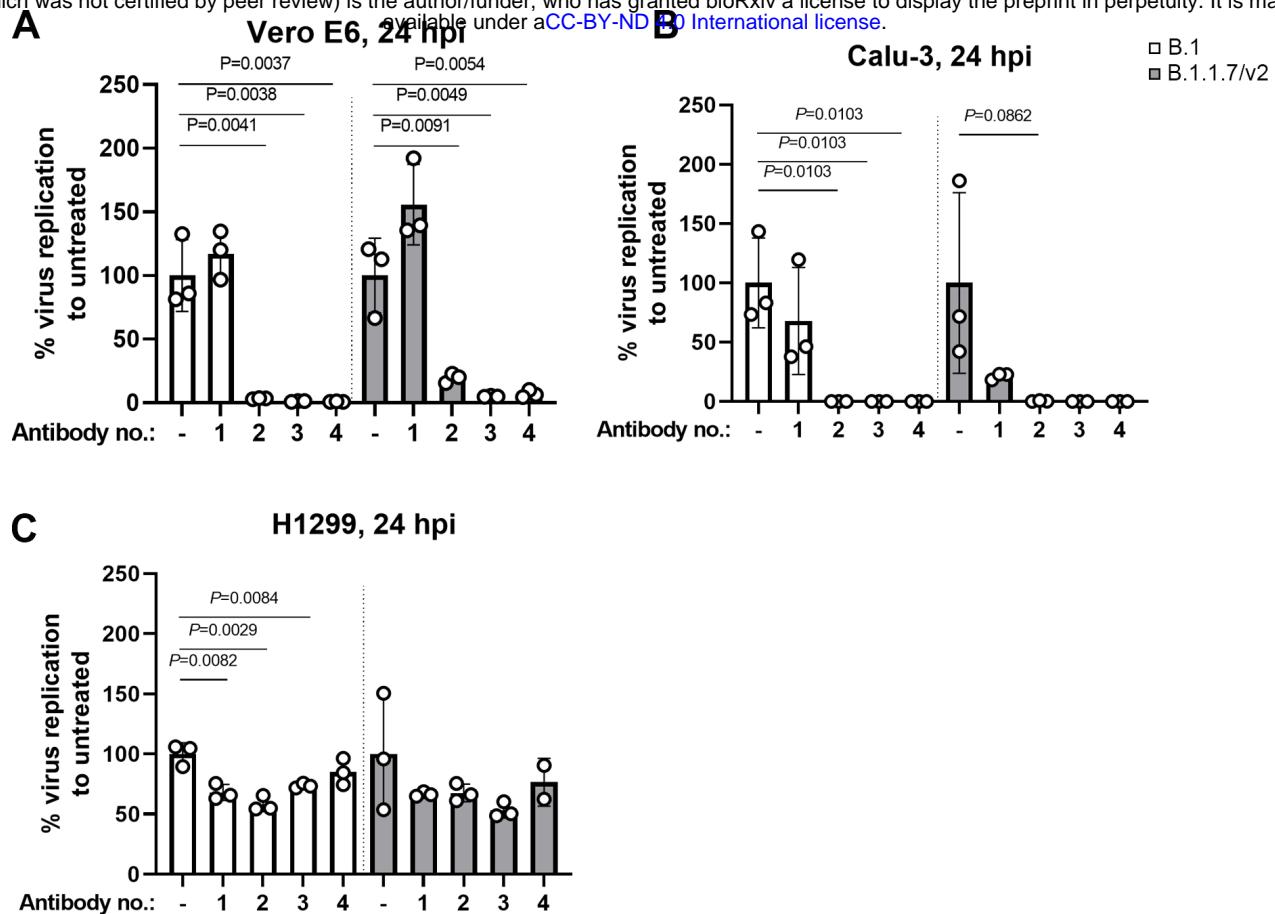


Figure S3. ACE2-dependent growth of SARS-CoV-2 in Vero E6 and Calu-3 cells, but not in NCI-H1299 cells

(A-C) Vero E6 (A), Calu-3 (B) and NCI-H1299 (C) cells were pretreated with four different anti-ACE2 antibodies (each applied at final concentration of 20 µg/ml) for one hour prior to infection with B.1- and B.1.1.7 isolates (MOI of 0.01). At 24 hours post-infection, viral replication was quantified from the supernatant by the use of E-gene assay. Replication was normalized to the respective untreated cells. Results from one experiment, conducted in triplicates, are shown.

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Supplemental Tables

Table S1. Primers used for reconstruction of recombinant SARS-CoV-2

Side directed mutagenesis:	
F10 D614G F	TCTTTATCAGGGTGTAACTGCAC
F10 D614G R	ACAGCAACCTGGTTAGAAGTATTG
RT PCR:	
5R	TGTTTAGCAAGATTGTGTCCGCT
10R	AACAGTATTCTTGCTATAGTAGTCGG
15R	AAAGGTGTGAACATAACCATCCACTG
19R	TCTAAGCATAAGTAAAAGCATTGTCTG
23R	CTGATAGCAGCATTACCATCCTG
28R	GAGCCCTGTGATGAATCAACAGT
33R	CCTTAGAAACTACAGATAATCTGGGA
38R	ACTAGCGCATATACCTGCACC
42R	TGAGTACAGCTGGTAATAGTCTGAAG
47R	CGGCCAATGTTGTAATCAGTTCC
Nested PCR and TAR fragment generation:	
Primers used as published with exception for F3 and F9	
B.1.1.7 F3 F	TGGCTTCACATATGTATTGTTCTTT
B.1.1.7 F9 R	GCATCAGTAGTGTCATCAATGTC

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Table S2. Primers used for quantitative RT-PCR

Name	Sequence 5' to 3'
hulFNB1-F	AGGATTCTGCATTACCTGAAGG
hulFNB1-R	GGCTAGGAGATCTTCAGTTCG
hulFNB1-P	TCCACTCTGACTATGGTCCAGGCA
hulFNL1-F	GACGCCCTGGAAGAGTCACTC
hulFNL1-R	CCTACCTGGAGAAGCCTAG
IL29_P	AGTTGCAGCTCTCCTGTCTCCCCG
hMXA_F	TTCAGACCTGATGGCCTATC
hMXA_R	TGGATGATCAAAGGGATGTGG
hMXA_P	CAGGAGGCCAGCAAGCGCCATC
hIL6_F	GGATTCAATGAGGAGACTTGC
hIL6_R	CACAGCTCTGGCTTGTCC
hIL6_P	AATCATCACTGGCTTTGGAGTTGAGG
hTNF-alpha_F	TGGCCCAGGCAGTCAGA
hTNF-alpha_R	TGTAGCCCATGTTGTAGCAAACC
hTNF-alpha_P	CATCTTCTCGAACCCCGAGTGACAAGC
hTBP_F	GCTGCGGTAATCATGAGGATAAG
hTBP_P	AGCCACGAACCACGGCACTGATT
hTBP_R	TGCACACCATTCCCAGAA
hACE2_F	TGCCTATCCTCCTATATCAGTCAA
hACE2_R	GAGTACAGATTGTCCAAAATCTAC
hACE2_P	ATGCCTCCCTGCTCATTGCTTGGT
hTMPRSS2	Hs01122322_m1 (ThermoFisher Scientific)

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Supplemental Movies 1 and 2: Delayed cytopathic onset of B.1.1.7 SARS-CoV-2 infection

Vero E6 cells were infected with B.1, B.1.1.7/v1 and B.1.1.7/v2 (MOI 0.01, supplemental movie 1; MOI 0.001, supplemental movie 2). Onset of CPE was monitored by live cell imaging until 70 hours post infection.