

1 **Transmission, infectivity, and antibody neutralization of an emerging SARS-CoV-**
2 **2 variant in California carrying a L452R spike protein mutation**

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36 **Abstract**

37 We identified a novel SARS-CoV-2 variant by viral whole-genome sequencing of
38 2,172 nasal/nasopharyngeal swab samples from 44 counties in California. Named
39 B.1.427/B.1.429 to denote its 2 lineages, the variant emerged around May 2020 and
40 increased from 0% to >50% of sequenced cases from September 1, 2020 to January
41 29, 2021, exhibiting an 18.6-24% increase in transmissibility relative to wild-type
42 circulating strains. The variant carries 3 mutations in the spike protein, including an
43 L452R substitution. Our analyses revealed 2-fold increased B.1.427/B.1.429 viral
44 shedding in vivo and increased L452R pseudovirus infection of cell cultures and lung
45 organoids, albeit decreased relative to pseudoviruses carrying the N501Y mutation
46 found in the B.1.1.7, B.1.351, and P.1 variants. Antibody neutralization assays showed
47 4.0 to 6.7-fold and 2.0-fold decreases in neutralizing titers from convalescent patients
48 and vaccine recipients, respectively. The increased prevalence of a more transmissible
49 variant in California associated with decreased antibody neutralization warrants further
50 investigation.

51

52 **Key words:** SARS-CoV-2; coronavirus; pandemic; COVID-19; viral whole-genome
53 sequencing; genomic surveillance; molecular dating; genomic epidemiology; spike
54 protein; L452R mutation; variant; antibody neutralization; vaccine; N501Y mutation;
55 B.1.427/B.1.429; 20C/L452R; pseudovirus infectivity studies; antibody neutralization

56

57

58 **Introduction**

59 Genetic mutation provides a mechanism for viruses to adapt to a new host and/or
60 evade host immune responses. Although SARS-CoV-2 has a slow evolutionary rate
61 relative to other RNA viruses ($\sim 0.8 \times 10^{-3}$ substitutions per site per year) (Day et al.,
62 2020), an unabating COVID-19 pandemic with high viral transmission has enabled the
63 virus to acquire significant genetic diversity since its initial detection in Wuhan, China in
64 December 2019 (Zhu et al., 2020), thereby facilitating the emergence of new variants
65 (Fontanet et al., 2021). Among numerous SARS-CoV-2 variants now circulating
66 globally, those harboring a D614G mutation have predominated since June of 2020
67 (Korber et al., 2020), possibly due to enhanced viral fitness and transmissibility (Hou et
68 al., 2020; Plante et al., 2020; Zhou et al., 2021).

69 Emerging variants of SARS-CoV-2 that harbor genome mutations that may
70 impact transmission, virulence, and immunity have been designated “variants of
71 concern” (VOCs). Beginning in the fall of 2020, 3 VOCs have emerged globally, each
72 carrying multiple mutations across the genome, including several in the receptor-binding
73 domain (RBD) of the spike protein. The B.1.1.7 variant, originally detected in the United
74 Kingdom (UK) (Chand et al., 2020), has accumulated 17 lineage-defining mutations,
75 including the spike protein N501Y mutation that confers increased transmissibility over
76 other circulating viruses (Leung et al., 2021; Rambaut et al., 2020b; Volz et al., 2020).
77 Preliminary data suggest that B.1.1.7 may also cause more severe illness (Davies et al.,
78 2021). As of early 2021, the B.1.1.7 variant has become the predominant lineage
79 throughout the UK and Europe, with reported cases also rising in the United States (US)
80 (Washington et al., 2021). The other two VOCs, B.1.351 detected in South Africa
81 (Tegally et al., 2020) and P.1 first detected in Brazil (Sabino et al., 2021), carry E484K

82 and K417N/K417T in addition to N501Y mutations. Multiple studies have reported that
83 the E484K mutation in particular may confer resistance to antibody neutralization (Cole
84 et al., 2021; Wang et al., 2021; Wibmer et al., 2021; Wu et al., 2021; Xie et al., 2021),
85 potentially resulting in decreased efficacy of currently available vaccines (Liu et al.,
86 2021; Wise, 2021). This phenotype may have also contributed to widespread reinfection
87 by P.1 in an Amazon community that had presumptively achieved herd immunity (Buss
88 et al., 2021; Sabino et al., 2021).

89 In January 2021, we and others independently reported the emergence of a
90 novel variant in California carrying an L452R mutation in the RBD of the spike protein
91 (CDPH, 2021; Zhang et al., 2021). Here we used viral whole-genome sequencing of
92 nasal/nasopharyngeal (N/NP) swab samples from multiple counties to characterize the
93 emergence and spread of this L452R-carrying variant in California from September 1,
94 2020, to January 29, 2021. We also combined epidemiologic, clinical, and in vitro
95 laboratory data to investigate transmissibility and susceptibility to antibody neutralization
96 associated with infection by the variant.

97

98 **Results**

99 **Viral genomic surveillance**

100 We sequenced 2,172 viral genomes across 44 California counties from remnant
101 N/NP swab samples testing positive for SARS-CoV-2 (**Supplementary Tables 1 and**
102 **2**). The counties with proportionally higher representation in the dataset included Santa
103 Clara County (n=725, 33.4%), Alameda County (n=228, 10.5%), Los Angeles County
104 (n=168, 7.7%) and San Francisco County (n=155, 7.1%) (**Figure 1A**). A novel variant,

105 subsequently named 20C/L452R according to the NextStrain nomenclature system
106 (Bedford et al., 2021) or B.1.427/B.1.429 according to the Pango system (Rambaut et
107 al., 2020a) (henceforth referred to using the Pango designation to distinguish between
108 the B.1.427 and B.1.429 lineages), was identified in 21.1% (459 of 2,172) of the
109 genomes (**Supplementary Table 1**). The frequency of this variant in California
110 increased from 0% at the beginning of September 2020 to >50% of sequenced cases by
111 the end of January 2021. The rise in the proportion of sequenced cases due to the
112 variant was rapid, with an estimated increase in transmission rate of the
113 B.1.427/B.1.429 variant relative to circulating non-B.1.427/B.1.429 lineages of 18.6-
114 24.2% and an approximate doubling time of 18.6 days (**Figure 1B**). Similar epidemic
115 trajectories were observed from multiple counties (**Figure 1C-1E, Supplementary**
116 **Figure 1**), despite different sampling approaches used for sequencing. Specifically,
117 genomes from San Francisco County were derived from COVID-19 patients being
118 tested at University of California, San Francisco (UCSF) hospitals and clinics; genomes
119 from Alameda County were derived from community testing; genomes from Santa Clara
120 County were derived from congregate facility, community, and acute care testing; and
121 genomes from Los Angeles County were derived from coroner, community, and
122 inpatient testing.

123

124 **Phylogenetic and molecular dating analyses**

125 Bayesian phylogenetic analysis of 1,166 genomes subsampled from a 2,519-
126 genome dataset consisting of the 2,172 California genomes sequenced in this study
127 and 347 representative global genomes (Bedford and Neher, 2020) identified two

128 distinct lineages in clade 20C (Nextstrain designation) associated with the novel variant,
129 B.1.427 and B.1.429 (**Figure 2B**). Both lineages share a triad of coding mutations in the
130 spike protein (S13I, W152C, and L452R), one coding mutation in the orf1b protein
131 (D1183Y), and an additional 2 non-coding mutations (**Figure 2A**). Four additional
132 mutations, one of them a coding mutation orf1a:l4205V, were specific to B.1.429, while
133 3 additional non-coding mutations were specific to B.1.427. Using a previously reported
134 algorithm to assess divergence time dating (Drummond et al., 2012), we estimated that
135 the most recent common ancestor emerged on May 20, 2020 (95% highest posterior
136 density [HPD] interval: April 29 -June 9). The branches giving rise to the B.1.427 and
137 B.1.429 lineages were predicted to have diverged on July 27 (95% HPD: June 6-
138 September 8) and June 9 (95% HPD: May 23-June 23), respectively (**Figure 2C**).

139

140 **Increased transmissibility and infectivity**

141 Analysis of data from 2,126 (97.8%) of the 2,172 sequenced genomes in the
142 current study revealed that the median PCR cycle threshold (Ct) value associated with
143 B.1.427/B.1.429 variant infections was significantly lower ($p=3.47 \times 10^{-6}$) than that
144 associated with non-variant viruses (**Figure 3C**). We estimated that in swab samples
145 N/NP viral RNA is approximately 2-fold higher in B.1.427/B.1.429 than in non-variant
146 viruses (Drew et al., 2020).

147 Analysis of the SARS-CoV-2 spike protein complexed to its human ACE2
148 receptor (Lan et al., 2020) revealed that the L452 residue does not directly contact the
149 receptor. Instead, L452 together with F490 and L492 form a hydrophobic patch on the
150 surface of the spike RBD (**Figure 4A**). To understand the effects of L452R RBD

151 mutation on viral entry, pseudoviruses carrying D614G with L452R or W152C, or
152 D614G alone were generated and used for infection of 293T cells stably expressing the
153 ACE2 cell entry receptor and TMPRSS2 cofactor for SARS-CoV-2 (Hoffmann et al.,
154 2020) and human airway lung organoids (HAO) stably expressing ACE2. We observed
155 increased entry by pseudoviruses carrying the L452R mutation compared to D614G
156 alone, with a 6.7 to 22.5-fold increase in 293T cells and a 5.8 to 14.7-fold increase in
157 HAOs (**Figure 4B and 4C**). This increase in infection with L452R mutation is slightly
158 lower than the increase observed with the N501Y mutation (11.4 to 30.9-fold increase in
159 293T cells and 23.5 to 37.8-fold increase in HAO relative to D614G alone), which has
160 previously been reported to increase pseudovirus entry (Hu et al., 2021). Pseudoviruses
161 carrying the W152C mutation demonstrated small increases in infection of 293T cells
162 and HAO relative to the D614 control, although these increases were not as
163 pronounced as those observed for the L452R and N501Y pseudoviruses.

164

165 **Reduced susceptibility to neutralizing antibodies from convalescent patients and** 166 **vaccine recipients**

167 To examine the effect of the L452R mutation on antibody binding, we performed
168 neutralizing antibody assays. We cultured a B.1.429 lineage virus from a patient's NP
169 swab sample in Vero TMPRSS2 cells. We then performed plaque reduction
170 neutralization tests (PRNT) using 21 plasma samples from convalescent patients and
171 vaccine recipients to compare neutralization titers between the B.1.429 isolate and a
172 control isolate USA-WA1/2020 (**Figure 5A, Supplementary Table 3, and**
173 **Supplementary Figure 2**). Twelve samples were collected from individuals after

174 receiving both doses of either the Pfizer BNT16b2 or Moderna mRNA-1273 vaccine,
175 with samples collected 4-28 days after the second dose. Nine samples were
176 convalescent plasma collected from patients clinically diagnosed with COVID-19 from
177 August 20 to December 10, 2020, with samples collected 18 to 71 days after symptom
178 onset. Measurable neutralizing antibody responses in the assay range were not
179 observed for 1 convalescent patient and 1 vaccine recipient.

180 We found that in comparison to USA-WA1/2020, 7 of 8 (88%) convalescent
181 patients and 6 of 11 (55%) vaccine recipients, showed reduced PRNT₅₀ titers to a
182 B.1.429 lineage virus, with 6.7-fold ($p=0.016$) and 2-fold ($p=0.031$) median reductions,
183 respectively (**Figure 5A**). There were no differences in neutralization between WA1 or
184 D614G isolates by convalescent or post-vaccination plasma (**Figure 5A, right**).

185 Next, we independently evaluated neutralizing antibody responses against a
186 cultured B.1.427 lineage virus. The TCID₅₀, or median tissue culture infective dose at
187 which 50% of cultures exhibit cytopathic effect (CPE), was determined for 10 different
188 convalescent plasma samples collected from COVID-19 patients from June 19 to
189 August 19, 2020, with samples collected 21 to 85 days after symptom onset. Nine of 10
190 (90%) convalescent patients showed reduced TCID₅₀ titers to a B.1.427 lineage virus,
191 with 5.3 ($p=0.0039$) and 4.0-fold ($p=0.0039$) median reductions for USA-WA1/2020) and
192 D614G isolates, respectively.

193

194 **Discussion**

195 As of early 2021, multiple SARS-CoV-2 variants have emerged in different
196 regions of the world, each rapidly establishing itself as the predominant lineage within a

197 few months after its initial detection (Chand et al., 2020; Sabino et al., 2021; Tegally et
198 al., 2020). In the current study, we describe the spread of a novel B.1.427/B.1.429
199 variant in California carrying a characteristic triad of spike protein mutations (S13I,
200 W152C, and L452R) that is predicted to have emerged in May 2020 and increased in
201 frequency from 0% to >50% of sequenced cases from September 2020 to January
202 2021. Importantly, this variant was found to comprise 2 separate lineages, B.1.427 and
203 B.1.429, with each lineage rising in parallel in California as well as in multiple other
204 states (Gangavarapu et al., 2020). We also observed a moderate resistance to
205 neutralization by antibodies elicited by prior infection (4.0 to 6.7-fold) or vaccination (2-
206 fold). These findings indicate that the B.1.427/B.1.429 variant warrants close monitoring
207 and further investigation regarding its potential to cause future surges in COVID-19
208 cases, accumulate further mutations, and/or decrease vaccine efficacy.

209 The results here highlight the urgent need for implementation of a robust
210 genomic surveillance system in the US and globally to rapidly identify and monitor
211 SARS-CoV-2 variants. Although our findings suggest that the B.1.427/B.1.429 variant
212 emerged as early as May 2020, the first cases of B.1.427 and B.1.429 in the US were
213 not identified by sequencing until September 28, 2020, and July 13, 2020, respectively.
214 Sparse genomic sequencing of circulating viruses likely contributed to delayed
215 identification of the B.1.427/B.1.429 variant. Furthermore, unlike in countries such as
216 the UK (consortiumcontact@cogconsortium.uk, 2020) and South Africa (Msomi et al.,
217 2020), the US lacks an organized system for real-time analysis and reporting of variants
218 that is tied to actionable public health responses. The first public disclosure of the
219 existence of this variant, initiated by us in coordination with local and state public health

220 agencies and the US CDC, did not occur until January 17, 2021 (CDPH, 2021), by
221 which time the variant had already become the dominant lineage in several California
222 counties and spread to multiple other states (Gangavarapu et al., 2020). Earlier
223 identification and monitoring of the variant may have guided focused contact tracing
224 efforts by public health to slow its spread, as well as enabled more timely investigation
225 of its potential significance. Our identification of the B.1.427/B.1.429 variant was made
226 possible by California COVIDNet, a collaborative sequencing network working to track
227 transmission and evolution of SARS-CoV-2 in the state by viral whole-genome
228 sequencing (CDPH, 2021).

229 The B.1.427/B.1.429 variant carries 4 new coding mutations, including 3 in the
230 spike protein, that are not found in the 3 SARS-CoV-2 VOCs (B.1.1.7, B.1.351, and P.1)
231 or in other major circulating lineages. The sudden appearance of several new mutations
232 in a new variant is not unexpected. Indeed, the B.1.1.7 and B.1.351 variants each carry
233 over 8 missense mutations in the spike protein (Rambaut et al., 2020b; Tegally et al.,
234 2020). The evolutionary mechanism underlying the unusual genetic divergence of these
235 emerging variants, with the accumulation of many mutations over a short time period,
236 remains unexplained, but this divergence may potentially be due to accelerated viral
237 quasispecies evolution in chronically infected patients (Avanzato et al., 2020; Choi et
238 al., 2020; Kemp et al., 2021). Another possible explanation for the absence of genomes
239 directly ancestral to B.1.427/B.1.429 is the aforementioned limited genomic sampling of
240 SARS-CoV-2 in California and the US to date.

241 Prior studies have suggested that the L452R mutation may stabilize the
242 interaction between the spike protein and its human ACE2 receptor and thereby

243 increase infectivity (Chen et al., 2020; Teng et al., 2020). Our findings of enhanced
244 infection of 293T cells and lung organoids by pseudoviruses carrying L452R confirm
245 these early predictions. Notably, the L452 residue does not directly contact the ACE2
246 receptor, unlike the N501 residue that is mutated to Y501 in the highly transmissible
247 B.1.1.7, B.1.351 and P.1 variants (**Figure 4A**). However, given that L452 is positioned
248 in a hydrophobic patch of the spike RBD, it is plausible that the L452R mutation causes
249 structural changes in the region that promote the interaction between the spike protein
250 and its ACE2 receptor. Notably, our findings reveal that the infectivity of L452R
251 pseudoviruses was higher than D614G, but slightly reduced compared to that of N501Y
252 pseudoviruses in 293T cells and human airway lung organoids. Thus, whether the
253 L452R-carrying B.1.427/B.1.429 will continue to remain the predominant circulating
254 strain in California, or whether it will eventually be replaced by the N501Y-carrying
255 B.1.1.7 variant (Washington et al., 2021) remains unclear.

256 The L452R mutation in the B.1.427/B.1.429 variant has been observed
257 previously in rare, mostly singleton cases, first reported from Denmark on March 17,
258 2020, and also reported from multiple US states and the UK prior to September 1, 2020
259 (Gangavarapu et al., 2020). Given our findings of increased infectivity of L452R
260 pseudoviruses, it is unclear why surges in L452R-carrying lineages have not occurred
261 earlier. We speculate that although these lineages may have been more infective,
262 transmission may not have reached a critical threshold locally or may have been
263 influenced by other factors such as population density and/or public health
264 interventions. An alternative (but not mutually exclusive) possibility is that the additional
265 mutations in B.1.427/B.1.429, especially the W152C and S13I mutations in the spike

266 protein, may contribute to increased infectivity of the variant relative to lineages carrying
267 the L452R mutation alone. Indeed, in the current study we observed smaller but
268 statistically significant increases in infection of 293T cell and lung organoids by
269 pseudoviruses carrying W152C. Studies of pseudoviruses carrying the 3 spike
270 mutations or the full complement of mutations in the B.1.427/B.1.429 variant are needed
271 to address these hypotheses.

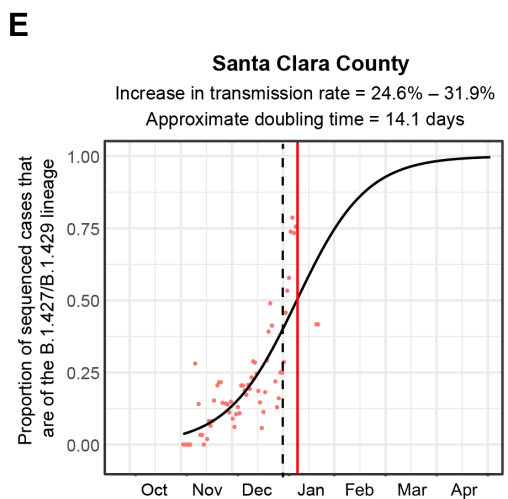
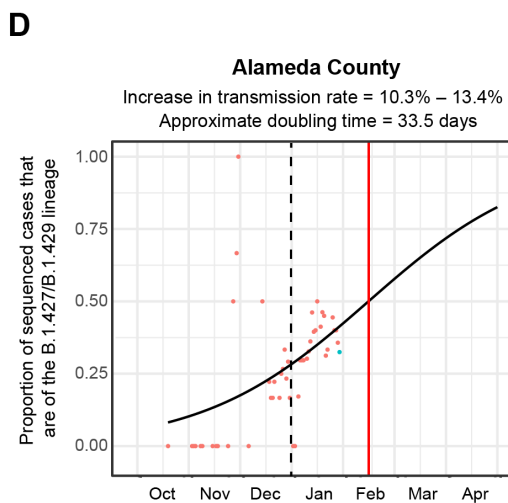
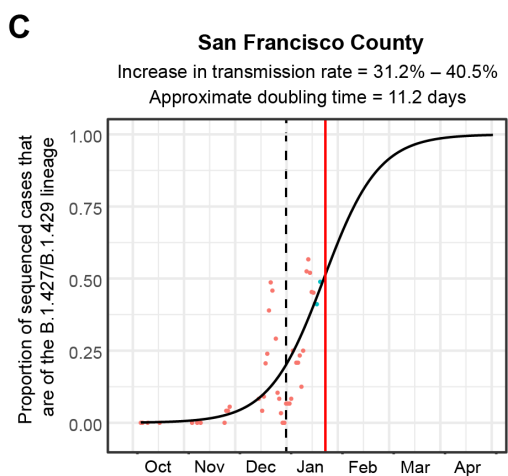
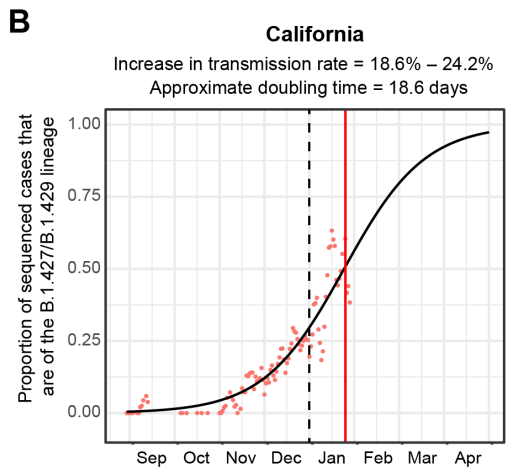
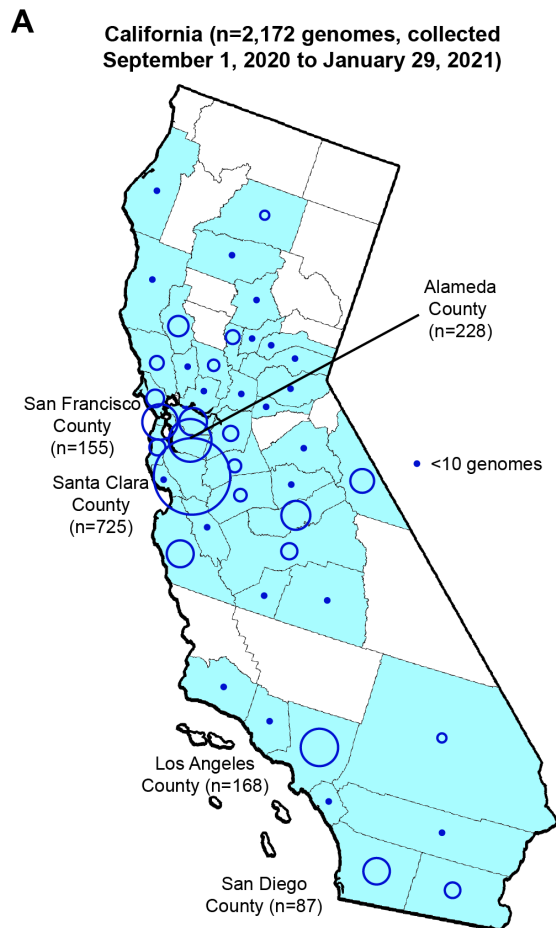
272 Our neutralization findings are consistent with a prior report showing decreased
273 binding of L452R-carrying pseudoviruses by antibodies from previously infected COVID-
274 19 patients and escape from neutralization in 3 of 4 convalescent plasma samples (Liu
275 et al., 2020). We speculate that mutation of the L452 residue in a hydrophobic pocket
276 may induce conformational changes in the RBD that impact neutralizing antibody
277 binding. Of note, a >4-fold decrease in neutralizing antibody titers in convalescent
278 plasma suggest that immune selection pressure from a previously exposed population
279 may be partly driving the emergence of L452R variants. These data also raise
280 questions regarding potential higher risk of re-infection and the therapeutic efficacy of
281 monoclonal antibodies and convalescent plasma to treat COVID-19 disease from the
282 B.1.427/B.1.429 variant.

283 Overall, the modest 2-fold decrease in neutralizing antibody titers in vaccine
284 recipients to the B.1.429 variant is an indication of the robust neutralizing antibody
285 responses elicited by mRNA vaccines in the face of variants under immune selection
286 pressure. Indeed, a reduction in neutralization associated with the L452R mutation has
287 been reported following vaccination, although the observed decrease in neutralizing
288 antibody titers was similar at 2.9-fold (Garcia-Beltran et al., 2021). The use of a B.1.429

289 isolate in the present study, carrying the full complement of mutations that characterize
290 the lineage, may account for some of the differences between the Garcia-Beltran et al.
291 study and ours, and the contribution of epistatic mutations to neutralization phenotypes
292 for SARS-CoV-2 variants merits further study. In addition, as neutralizing antibodies in
293 natural infection have been shown to wane over time (Lau et al., 2021; Seow et al.,
294 2020), longitudinal serologic studies are needed to determine whether these modest
295 decreases will affect the long-term durability of vaccine-elicited immune responses to
296 the B.1.427/B.1.429 variant. Of concern is also the possibility that B.1.427/B.1.429
297 lineages may accumulate additional mutations in the future that may further enhance
298 the escape phenotype.

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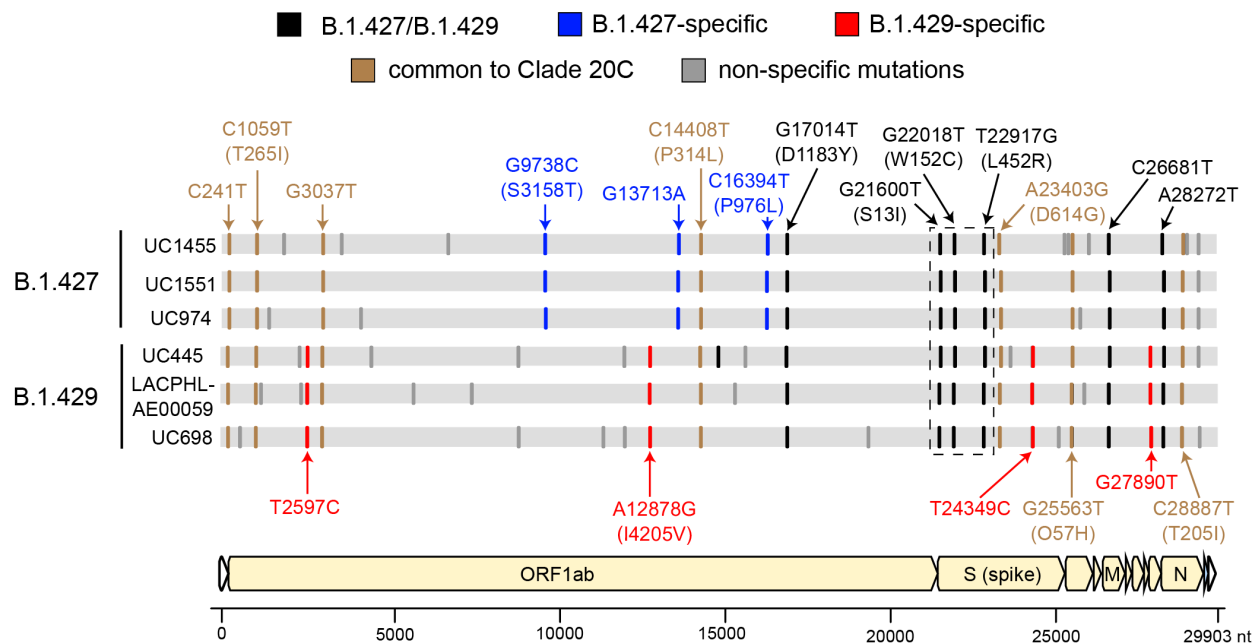
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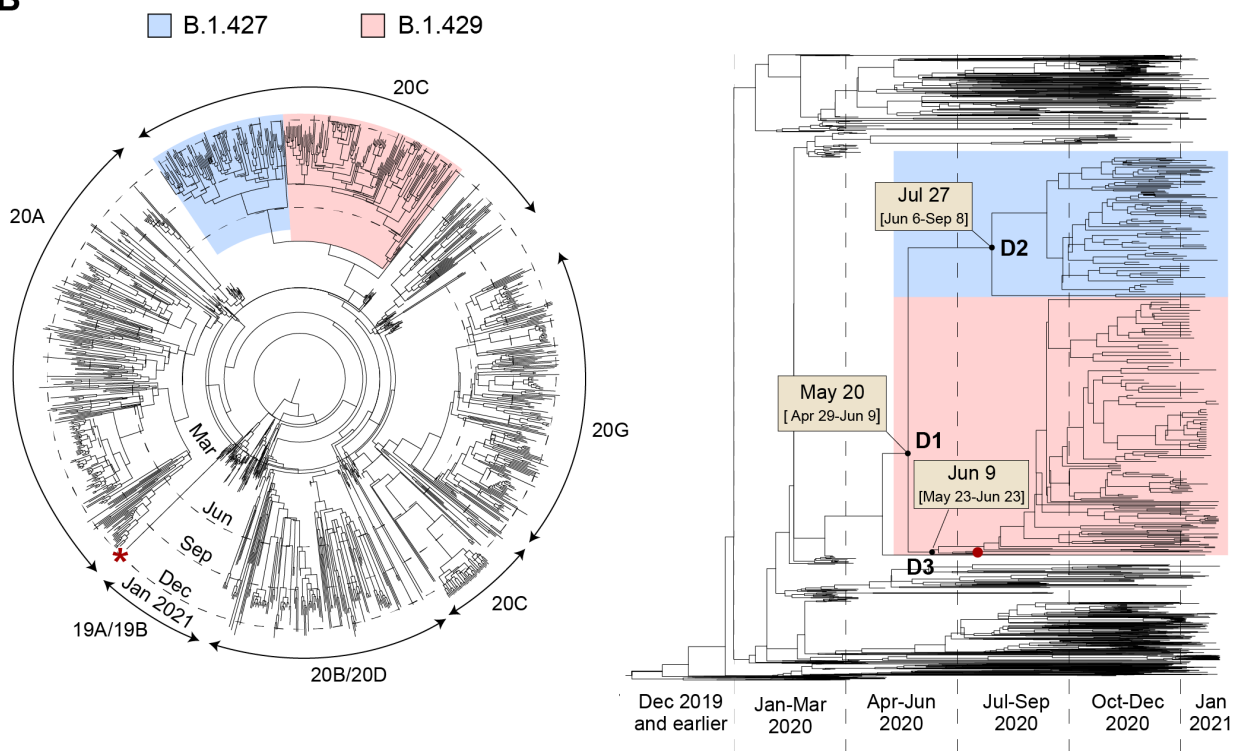
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304 **Figure 1. Increasing frequency of the B.1.427/B.1.429 variant in California from**
305 **September 1, 2020 to January 29, 2021. (A)** County-level representation of the 2,172
306 newly sequenced SARS-CoV-2 genomes in the current study. Counties from which at
307 least 1 genome were sequenced are colored in sky blue. The size of the circle is
308 proportionally to the number of genomes sequenced from each county, while points
309 designate counties for fewer than 10 genomes were sequenced. Logistic growth curves
310 fitting the 5-day rolling average of the estimated proportion of B.1.427/B.1.429 variant
311 cases in **(B)** California, **(C)** San Francisco County, **(D)** Alameda County, and **(E)** Santa
312 Clara County. The predicted time when the growth curve crosses 0.5 is indicated by a
313 vertical red line. A vertical black dotted line denotes the transition from 2020 to 2021.
314 The increase in transmission rate is defined as the change in the relative proportion of
315 B.1.427/B.1.429 variant cases relative to circulating non-B.1.427/B.1.429 variant
316 lineages as estimated from the logistic growth model (Volz et al., 2020; Washington et
317 al., 2021).
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A



B



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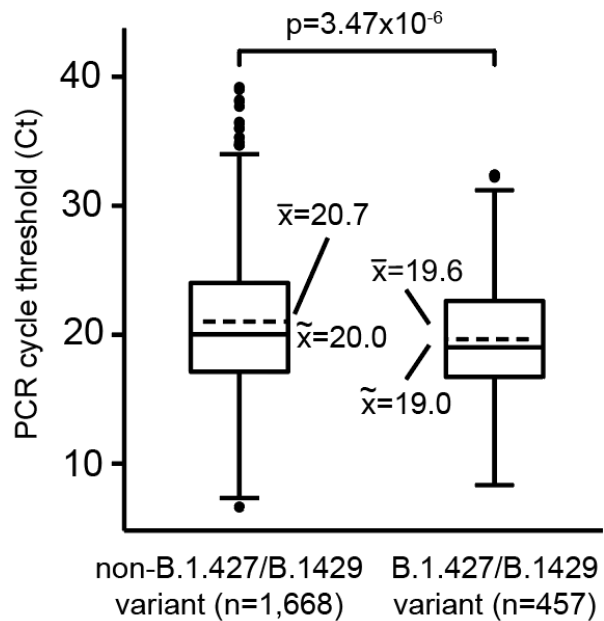
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324 **Figure 2. Genomic, phylogenetic, and molecular clock analyses of the**
325 **B.1.427/B.1.429 variant in California. (A)** A multiple sequence alignment of 6
326 representative B.1.427/B.1.429 genomes, 3 from the B.1.427 lineage and 3 from the
327 B.1.429 lineage, using the prototypical Wuhan Hu-1 genome as a reference. Defining
328 single nucleotide polymorphisms (SNPs) in the B.1.427 and B.1.429 lineages are
329 compared to each other and to other SARS-CoV-2 viruses in Nextstrain clade 20C. The
330 SNPs are color coded as follows: black SNPs are shared between the B.1.427 and
331 B.1.429 lineages, blue SNPs are specific to B.1.427, red SNPs are specific to B.1.429,
332 brown SNPs are shared among nearly all clade 20C viruses, and gray SNPs are
333 specific to individual viruses. **(B)** Bayesian phylogenetic tree of 1,166 subsampled
334 genomes constructed using molecular clock analysis from a complete dataset
335 consisting of the 2,172 genomes recovered in the current study and 347 representative
336 global genomes. The left panel shows a radial view of the tree, with marking of
337 segments corresponding to the major clades. The right panel shows the divergence
338 dates and associated 95% highest posterior density (HPD) distributions, or confidence
339 intervals, for the B.1.427/B.1.429 variant (D1), B.1.427 lineage (D2), and B.1.429
340 lineage (D3), as estimated from TMRCA (time to most recent common ancestor)
341 calculations. The B.1.427 lineage is colored in blue, and the B.1.429 lineage in red. The
342 red asterisk denotes a UK B.1.1.7 variant genome. The red dot denotes the first
343 reported genomic sequence of the B.1.429 variant from Los Angeles County from a
344 sample collected July 13, 2020.

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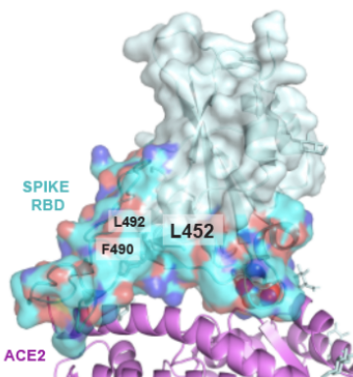
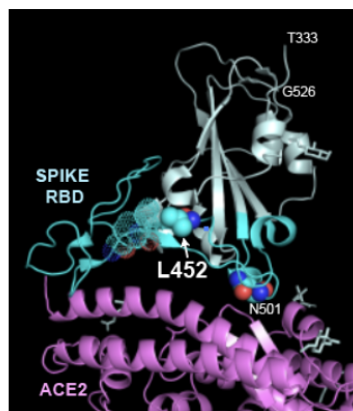


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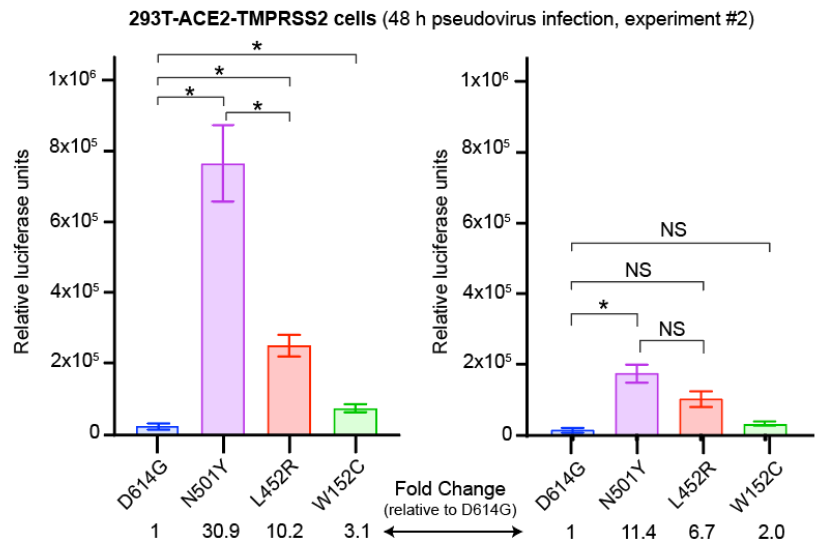
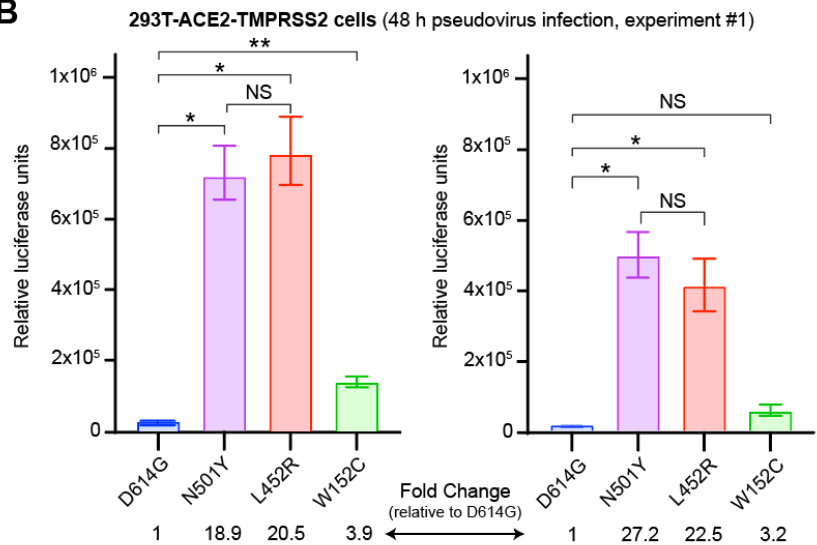
348 **Figure 3. Higher viral loads in infections from the B.1.427/B.1.429 variant as**
349 **compared to non-B.1.427/B.1.429 variant lineages.** Box and whisker plots of
350 available PCR cycle threshold (C_t) values for B.1.427/B.1.429 variant (left) and non-
351 variant (right) samples sequenced in the current study. Note that a C_t difference of 1
352 represents a 2-fold difference in the virus concentration (Drew et al., 2020). The dashed
353 horizontal line in the box denotes the median value, the solid horizontal lines the mean
354 value. The interquartile ranges are calculated with respect to the median value.

355

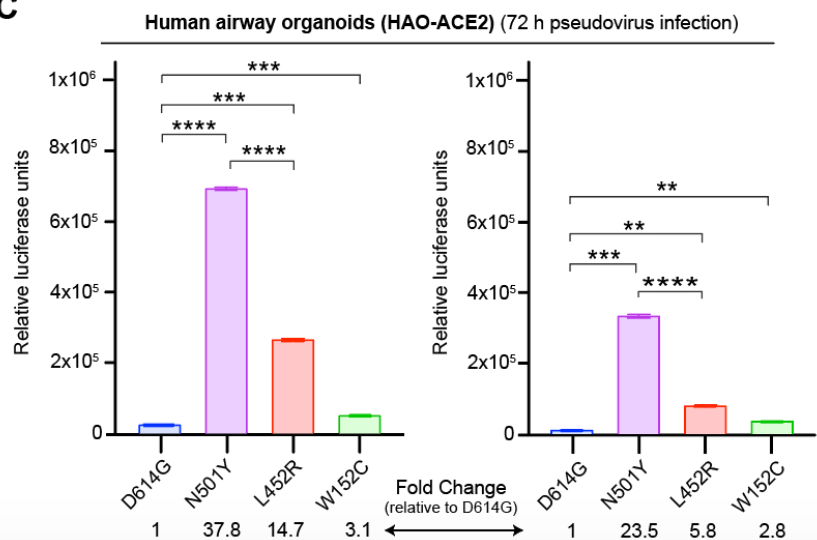
A



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C



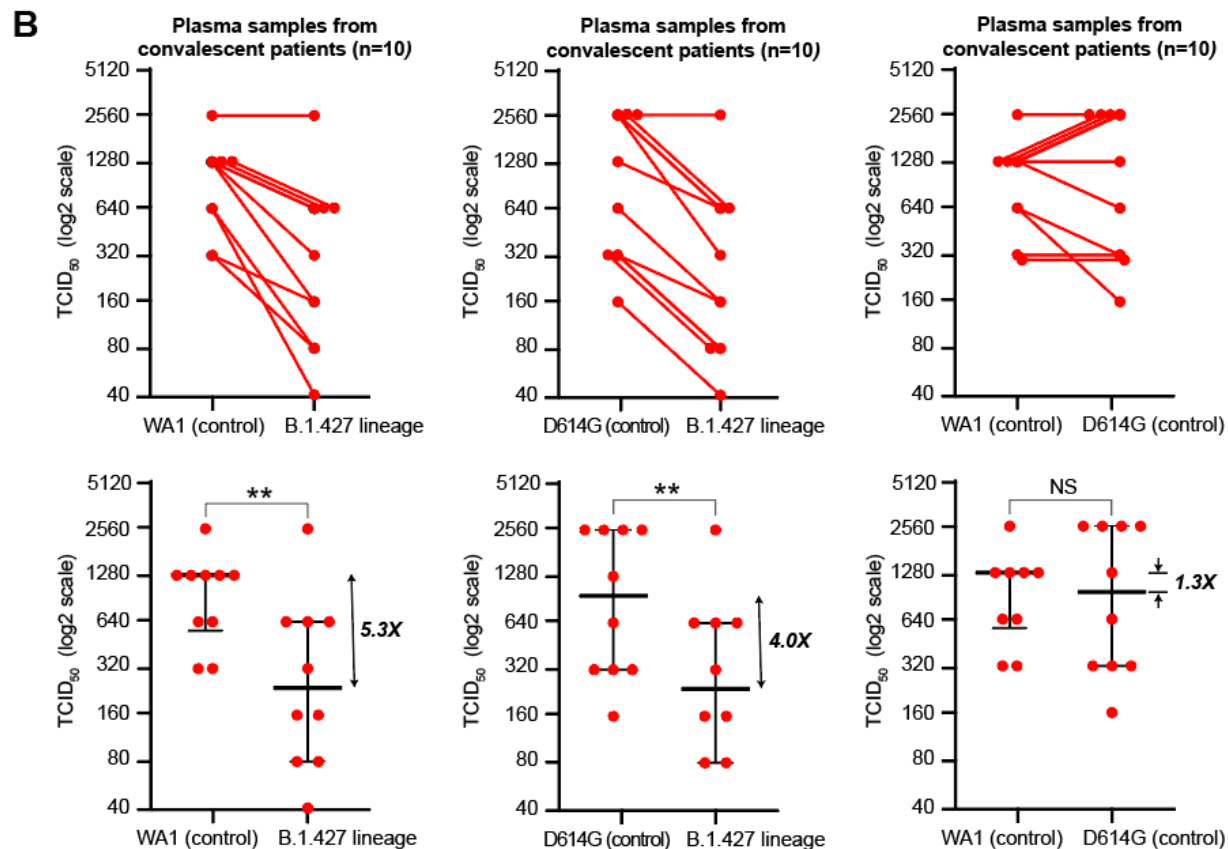
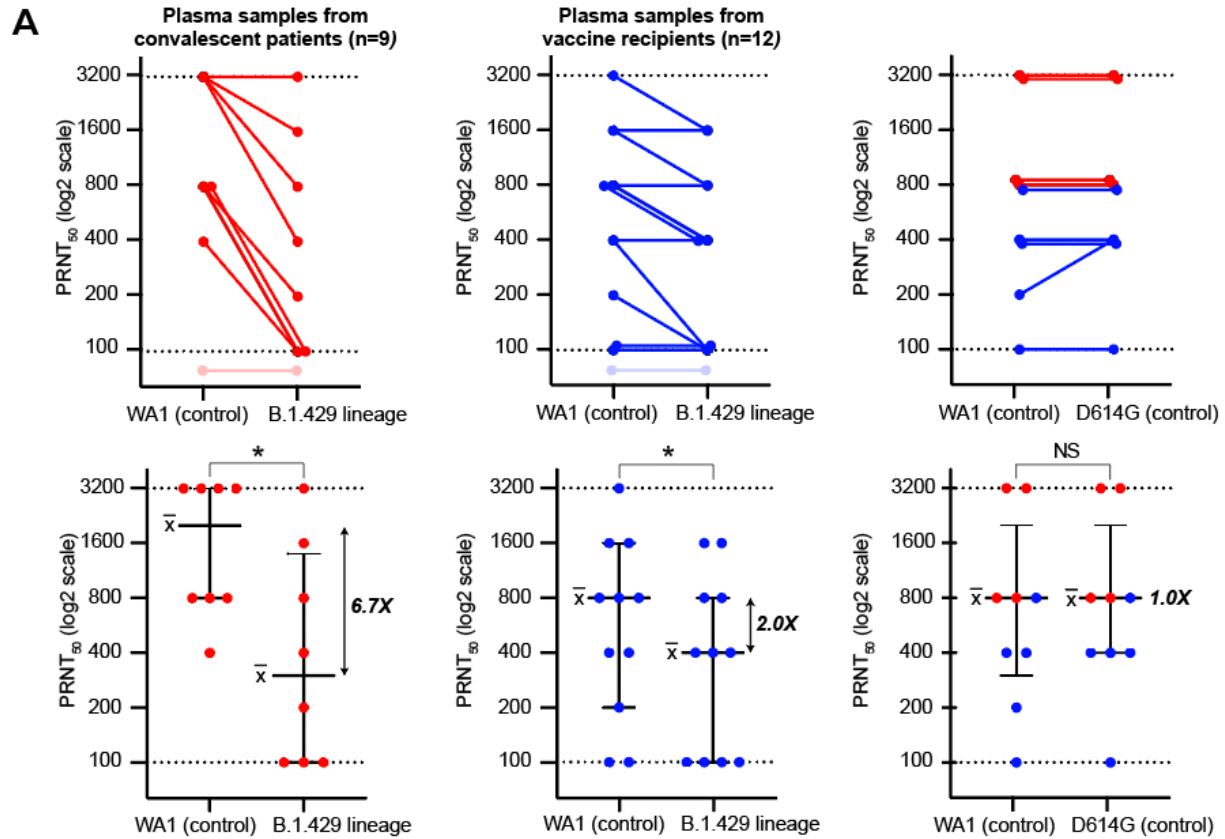
357 **Figure 4. Increased infectivity of L452R-carrying pseudoviruses (A) Upper panel:**
358 Ribbon diagram of the SARS-CoV-2 spike RBD in cyan bound to ACE2 receptor in
359 magenta (PDB ID 6M0J). The receptor-binding motif of RBD is colored in dark cyan with
360 L452 in solid spheres and F490 and L492 with dotted spheres. Sugars and Zn²⁺ are
361 shown in grey. The position of N501 in direct contact with the ACE2 receptor is also
362 shown for purposes of comparison. **Lower panel:** Surface representation of the spike
363 RBD showing the hydrophobic patch outlined by L452, F490, and L492. **(B)** Levels of
364 infection of SARS-CoV-2 spike pseudoviruses carrying D614G alone or D614G with
365 N501Y, L452R, or W152C mutations in 293T cells stably expressing ACE2 and
366 TMPRSS2. 293T cells were seeded in 96-well plates and infected with high (6 ng, **left**)
367 or low (3 ng, **right**) concentrations of the indicated pseudoviruses for 48 h. Two
368 biological replicates were assessed in two independent experiments, with 3 technical
369 replicates per experiment. **(C)** Levels of infection in human lung airway organoids (HAO)
370 stably expressing ACE2. HAO were seeded in 24-well plates and infected with high (4
371 ng, **left**) or low (2 ng, **right**) concentrations of the indicated pseudoviruses for 72 h.
372 Pseudovirus cell entry was measured with a luciferase assay. The error bars represent
373 the standard deviation of 3 technical replicates. Dunn's multiple comparisons test was
374 used to determine significance. Note that each of the N501Y, L452R, and W152G
375 pseudoviruses also carries D614G. Abbreviations: NS, not significant.

376

377

378

379



381 **Figure 5. B.1.427/B.1.429 variant resistance to antibody neutralization in vitro. (A)**
382 Antibody neutralization titers from 9 convalescent patients and 12 vaccine recipients
383 against cultured WA1 (control), D614G (control), and B.1.429 viral isolates were
384 assessed using a PRNT assay. Lines connect the individual plasma samples tested
385 pairwise for neutralization (**top row**). Only a subset of the plasma samples were tested
386 with the WA1 and D614G head-to-head comparisons (**top row, right**). The dotted lines
387 denote the upper and lower bounds for the PRNT assay (1:100 to 1:3200). Plasma
388 samples that did not exhibit detectable neutralizing activity at titers above the lower
389 threshold are shown as transparent. Individual PRNT₅₀ measurements are plotted along
390 with error bars denoting the median and standard deviation (**bottom row**). **(B)** Antibody
391 neutralization titers from 10 convalescent patients against cultured WA1 (control),
392 D614G (control) and B.1.427 viral isolates were assessed by 50% CPE endpoint
393 dilution. Lines connect the individual plasma samples tested pairwise for neutralization
394 (**top row**). Individual TCID₅₀ measurements are plotted along with error bars denoting
395 the median and standard deviation (**bottom row**). A Wilcoxon matched pairs signed
396 rank test was used to determine significance.
397 Abbreviations: NS, not significant; PRNT, plaque-reduction neutralization test; CPE,
398 cytopathic effect; TCID, tissue culture infective dose.

399

400

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410 opinions of the California Department of Public Health or the California Health and
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412

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420

421 **Author contributions**

422 CYC, MO, and RA conceived and designed the study. CYC, XD, MAG-K, VS,
423 CW, and GRK coordinated the sequencing efforts and laboratory studies. XD, MAG-K,

424 MMK, VS, CW, AS-G, DRG, KRR, CSSM, BS, P-YC, US-G, TYT, JMH, CRS, PVL, YX,
425 and MKM performed experiments. CYC, SF, and XD assembled and curated viral
426 genomes. CYC performed the phylogenetic and molecular clock analyses. CYC, XD,
427 MAG-K, VS, CW, KRR, ASG, NPR, JB, JT, JC, GRK, and CYC analyzed data. VS, CW,
428 AS-G, ASG, NPR, KRR, JAS, and SM collected and sequenced SARS-CoV-2 samples
429 from UCSF and throughout California. PH and NMG collected and sequenced samples
430 from Los Angeles County. CA and DF collected and sequenced samples from Monterey
431 County. FL, PAF, HS, and SKW collected and sequenced samples from Alameda
432 County. CYC, XD, MAGK, VS, and CW wrote the manuscript. CYC, MAGK, GRK, and
433 VS prepared the figures. CYC, XD, MAGK, VS, DAW, JKH, and CW edited the
434 manuscript. All authors read the manuscript and agree to its contents.

435

436 **Competing interests**

437 Dr. Charles Chiu receives support for SARS-CoV-2 research unrelated to this
438 study from Abbott Laboratories and Mammoth Biosciences. The other authors declare
439 no competing interests.

440

441 **Resource Availability**

442 **Lead Contact**

443 Further information and requests for resources and reagents should be directed
444 to and will be fulfilled by the Lead Contact, Charles Chiu (charles.chiu@ucsf.edu).

445 **Materials Availability**

446 This study did not generate any new reagents.

447 **Data Availability**

448 Assembled SARS-CoV-2 genomes in this study were uploaded to GISAID (Elbe
449 and Buckland-Merrett, 2017; Shu and McCauley, 2017) (accession numbers in
450 Supplementary Table 1) and can be visualized in NextStrain. Viral genomes were also
451 submitted to the National Center for Biotechnology Information (NCBI) GenBank
452 database (accession numbers pending). Raw sequence data were submitted to the
453 Sequence Read Archive (SRA) database (BioProject accession number PRJNA171119,
454 Chiu laboratory at UCSF; BioProject accession number PRJNA639591, Wyman
455 laboratory at UC Berkeley).

456

457 **Cell lines for virus culture**

458 Vero E6 cells and Vero cells expressing human TMPRSS2 were used for SARS-
459 CoV-2 viral culture. The culture was maintained in a humidified incubator at 37°C in 5%
460 CO₂ in the indicated media and passaged every 3-4 days.

461

462 **Methods**

463 **Sample collection and diagnostic assay of SARS-CoV-2**

464 Remnant nasal/nasopharyngeal (N/NP) swab samples in universal transport
465 media (UTM) or viral transport media (VTM) (Copan Diagnostics, Murrieta, CA, USA)
466 from RT-PCR positive COVID-19 patients were obtained from the University of
467 California, San Francisco (UCSF) Clinical Microbiology Laboratory, the Innovative
468 Genomics Institute (IGI) at University of California, Berkeley, California Department of
469 Public Health, Santa Clara County and Los Angeles County for SARS-CoV-2 genome
470 sequencing. A small fraction of swab samples (<1%) were obtained from the anterior
471 nares. Clinical samples from state and county public health laboratories were collected
472 and sequenced as part of routine public health surveillance activities. Clinical samples
473 from the IGI were sequenced under a waiver from the UC Berkeley Office for the
474 Protection of Human Subjects. Clinical samples from UCSF were collected for a
475 biorepository and sequenced according to protocols approved by the UCSF Institutional
476 Review Board (protocol number 10-01116, 11-05519).

477 Due to variation in results reported by different clinical testing platforms used at
478 UCSF, the Taqpath™ Multiplex Real-time RT-PCR test, which includes nucleoprotein
479 (N) gene, spike (S) gene, and orf1ab gene targets, was used to determine cycle
480 threshold (Ct) values for PCR-positive samples. The Taqpath™ assay was also used for
481 determining Ct values for PCR-positive samples from Alameda County that were
482 sequenced by the University of California, Berkeley IGI and from the California
483 Department of Public Health.

484 **Viral Genome Sequencing**

485 NP swab samples were prepared using 100 uL of the primary sample in UTM or
486 VTM mixed with 100uL DNA/RNA shield (Zymo Research, # R1100-250). The 1:1
487 sample mixture was then extracted using the Omega BioTek MagBind Viral DNA/RNA
488 Kit (Omega Biotek, # M6246-03) on KingFisher™ Flex Purification System with a 96
489 deep-well head (ThermoFisher, 5400630). Extracted RNA was reverse transcribed to
490 complementary DNA and tiling multiplexed amplicon PCR was performed using SARS-
491 CoV-2 primers Version 3 according to a published protocol (Quick et al., 2017).
492 Amplicons were ligated with adapters and incorporated with barcodes using NEBNext
493 Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, # E7645L). Libraries
494 were barcoded using NEBNext Multiplex Oligos for Illumina (96 unique dual-index
495 primer pairs) (New England Biolabs, # E6440L) and purified with AMPure XP
496 (Beckman-Coulter, #. Amplicon libraries were then sequenced on either Illumina MiSeq
497 or Novaseq 6000 as 2x150 paired-end reads (300 cycles).

498

499 **Genome Assembly and Variant Calling**

500 Genome assembly of viral reads and variant calling were performed using an in-
501 house developed bioinformatics pipeline as previously described (Deng et al., 2020). In
502 short, Illumina raw paired-end reads were first screened for SARS-CoV-2 sequences
503 using BLASTn (BLAST+ package 2.9.0) alignment against viral reference genome
504 NC_045512, and then processed using the BBTools suite, v38.87 (Bushnell, 2021).
505 Adapter sequences were trimmed and low-quality reads were removed using BBDuk,

506 and then mapped to the NC_045512 reference genome using BMap. Variants were
507 called with CallVariants and a depth cutoff of 5 was used to generate the final assembly.
508 A genome coverage breadth of $\geq 70\%$ was required for inclusion in the study.

509 Multiple sequence alignment of 6 B.1.427/B.1.429 genomes and the Wuhan Hu-1
510 prototypical genome (GISAID ID: EPI_ISL_402125, GenBank accession number
511 MN908947) was performed using the MAFFT aligner v7.388 (Kato and Standley,
512 2013) as implemented in Geneious v11.1.5 (Kearse et al., 2012).

513

514 **Phylogenetic Analysis**

515 High-quality SARS-CoV-2 genomes (n=2,519, 2,172 generated in the current
516 study and 347 used as representative global genomes) were downloaded from the
517 Global Initiative on Sharing of All Influenza Data (GISAID) database and processed
518 using the NextStrain bioinformatics pipeline Augur using IQTREE v1.6. Branch locations
519 were estimated using a maximum-likelihood discrete traits model. The resulting tree
520 was visualized in the NextStrain web application Auspice and in Geneious v11.1.5
521 (Kearse et al., 2012). Molecular clock analysis of SARS-CoV-2 for estimating the
522 TMRCA (time to most recent common ancestor) and divergence dates for the
523 B.1.426/B.1.427 variant was performed using the Markov chain Monte Carlo (MCMC)
524 method implemented by Bayesian Evolutionary Analysis on Sampling Trees (BEAST)
525 software v.2.63 (Drummond et al., 2012). Briefly, a HKY85 nucleotide substitution model
526 was used, using a strict clock model and exponential population growth. All models
527 were run using default priors except for the exponential growth rate (Laplace

528 distribution) for which the scale was set to 100. The chain length was set to 10 million
529 states with a 10% burn-in. Convergence was evaluated using Tracer v1.7.1 (Rambaut
530 et al., 2018). The resulting maximum clade credibility (MCC) tree was generated using
531 TreeAnnotator v.2.6.3 (Drummond et al., 2012) and visualized using FigTree v.1.4.4
532 (Rambaut, 2021).

533

534 **Cell culture**

535 Cells were maintained in a humidified incubator at 37°C in 5% CO₂ in the
536 indicated media and passaged every 3-4 days. Vero E6 cells were cultured in MEM
537 supplemented with 1x penicillin-streptomycin-glutamine (Gibco) and 10% fetal calf
538 serum (FCS). Vero cells overexpressing human TMPRSS2 were a kind gift from the
539 Whelan lab (Case et al., 2020) and were maintained in DMEM supplemented with 1x
540 sodium pyruvate, 1x penicillin-streptomycin-glutamine and 10% FCS.

541

542 **SARS-CoV-2 isolation and passages**

543 For the B.1.429 neutralization studies, a non-B.1.427/B.1.429 variant SARS-
544 CoV-2/human/USA/CA-UCSF-0001C/2020 clinical isolate carrying the D614G spike
545 mutation was isolated as previously described (Samuel et al., 2020) and passaged in
546 A549-ACE22 expressing cells. For isolation of the B.1.429 lineage virus, 100 µL of a NP
547 swab sample from a COVID-19 patient that was previously sequenced and identified as
548 B.1.429 was mixed 1:1 with serum free DMEM (supplemented with 1x sodium pyruvate
549 and 1x penicillin-streptomycin-glutamine), and two-fold serial dilutions were made of the
550 sample over six wells of a 96-well plate. 100 µL of freshly trypsinized Vero TMPRSS2

551 cells resuspended in DMEM (supplemented with 1x sodium pyruvate, 2x penicillin-
552 streptomycin-glutamine, 5 µg/mL amphotericin B and 10% FCS) was added to each well
553 and mixed. The culture was incubated at 37°C in 5% CO₂ for 4-6 days and cytopathic
554 effect (CPE) on cells was evaluated daily using a light microscope. The contents of
555 wells positive for CPE were collected and stored at -80°C as a passage 0 stock (P0). P1
556 stocks were made following infection of four near confluent wells of a 24-well plates with
557 Vero TMPRSS2 using the P0 stock. Supernatants were harvested 48 hours later after
558 centrifugation at 800g for 7 minutes. P2 stocks were similarly made after infection of a
559 near confluent T25 plate seeded with Vero E6 cells. All steps for isolation of the B.1.429
560 lineage virus were done in a Biosafety Level 3 lab using protocols approved by the
561 Institutional Biosafety Committee at UCSF.

562 For the B.1.427 neutralization studies, Vero-81 cells were cultured with MEM
563 supplemented with 1x penicillin-streptomycin (Gibco) and glutamine (Gibco) and 5%
564 FCS (Hyclone). For isolation of B.1.427 and non-B.1.427/B.1.429 variant D614G
565 viruses, 100 µL each from NP swab samples from COVID-19 patients identified as
566 being infected by the B.1.427 or non-B.1.427/B.1.429 variant D614G lineage was
567 diluted 1:5 in PBS supplemented with 0.75% bovine serum albumin (BSA-PBS) and
568 added to confluent Vero-81 cells in a T25 flask. After adsorption for 1 h, additional
569 media was then added, and the flask was incubated at 37°C with 5% CO₂ for 3-4 days
570 with daily monitoring for CPE. The contents were collected, clarified by centrifugation
571 and stored at -80C as passage 0 stock. P1 stock was made by inoculation of Vero-81
572 confluent T150 flasks with 1:10 diluted p0 stock and similarly monitored and harvested
573 to approximately 50% confluency. All steps for isolation of the B.1.427 lineage virus

574 were done in a Biosafety Level 3 lab at the Viral and Rickettsial Disease Laboratory
575 (VRDL) at the California Department of Public Health (CDPH).

576 For both the B.1.429 and B.1.427 neutralization studies, the SARS-CoV-2 USA-
577 WA1/2020 strain (BEI resources) was passaged in Vero E6 cells (ATCC CRL-1586) or
578 Vero-81 cells and used as a control. All stocks were resequenced and the consensus
579 assembled viral genomes were identical to the genomes derived from the primary NP
580 samples and carried all of the expected mutations.

581

582 **Plaque reduction neutralization tests using a B.1.429 lineage virus**

583 Conventional PRNT assays were done using P2 stocks of B.1.429 lineage
584 viruses and the USA-WA1/2020 isolate passaged on Vero E6 cells. Patient plasma was
585 heat inactivated at 56°C for 30 minutes, clarified by centrifugation at 10,000 relative
586 centrifugal force (rcf) for 5 minutes and aliquoted to minimize freeze thaw cycles. Serial
587 2-fold dilutions were made of plasma in PBS supplemented with 0.75% bovine serum
588 albumin (BSA). Plasma dilutions were mixed with ~100 plaque forming units (pfu) of
589 viral isolates in serum free MEM in a 1:1 ratio and incubated for 1 hr at 37°C. Final
590 plasma dilutions in plasma-virus mixtures ranged from 1:100 to 1:3200. 250 µL of
591 plasma-virus mixtures were inoculated on a confluent monolayer of Vero E6 cells in 6-
592 well plates, rocked and incubated for 1 h in a humidified incubator at 37°C in 5% CO₂.
593 After incubation, 3 mL of a mixture of MEM containing a final concentration of 2% FCS,
594 1x penicillin-streptomycin-glutamine and 1% melted agarose, maintained at ~56°C, was
595 added to the wells. After 72 h of culture as above, the wells were fixed with 4%
596 paraformaldehyde for 2 h, agarose plugs were removed, and wells were stained with

597 0.1% crystal violet solution. Plaques were counted and the PRNT₅₀ values were defined
598 as the serum dilution at which 50% or more of plaques were neutralized. Assays were
599 done in duplicate, and a positive control and negative control were included using
600 plasma with known neutralizing activity (diluted 1:50) and from a SARS-CoV-2
601 unexposed individual (1:20 dilution), respectively. All steps were done in a Biosafety
602 Level 3 lab using protocols approved by the Institutional Biosafety Committee at UCSF.

603

604 **CPE endpoint neutralization assays using a B.1.427 lineage virus**

605 CPE endpoint neutralization assays were done following the limiting dilution
606 model (Wang et al., 2005) and using P1 stocks of B.1.427, D614G, and USA-WA1/2020
607 lineages. Convalescent patient plasma was diluted 1:10 and heat inactivated at 56°C for
608 30 min. Serial 2-fold dilutions of plasma were made in BSA-PBS. Plasma dilutions were
609 mixed with 100 TCID₅₀ of each virus diluted in BSA-PBS at a 1:1 ratio (220 µL plasma
610 dilution and 220 µL virus input) and incubated for 1 hour at 37°C. Final plasma dilutions
611 in plasma-virus mixture ranged from 1:20 to 1:2560. 100 µL of the plasma-virus
612 mixtures were inoculated on confluent monolayer of Vero-81 cells in 96-well plates in
613 quadruplicate and incubated at 37°C with 5% CO₂ incubator. After incubation 150 µL of
614 MEM containing 5% FCS was added to the wells and plates were incubated at 37°C
615 with 5% CO₂ until consistent CPE was seen in virus control (no neutralizing plasma
616 added) wells. Positive and negative controls were included as well as cell control wells
617 and a viral back titration to verify TCID₅₀ viral input. Individual wells were scored for CPE
618 as having a binary outcome of ‘infection’ or ‘no infection’. The TCID₅₀ was calculated as

619 the dose that produced cytopathic effect in >50% of the inoculated wells. All steps were
620 done in a Biosafety Level 3 lab using approved protocols.

621

622 **SARS-CoV-2 receptor binding domain mutagenesis and pseudovirus infection** 623 **assay**

624 SARS-CoV-2 spike mutants (D614G, D614G+W152C, D614G+L452R, and
625 D614G+N501Y) were cloned using standard site-directed mutagenesis and PCR.

626 Pseudoviruses typed with these spike mutants were generated as previously described
627 with modifications (Crawford et al., 2020). Briefly, 293T cells were transfected with
628 plasmid DNA (per 6-well plate: 340 ng of spike mutants, 1 μ g CMV-Gag-Pol (pCMV-
629 d Δ R8.91), 125 ng pAdvantage (Promega), 1 μ g Luciferase reporter) for 48 h.

630 Supernatant containing pseudovirus particles was collected, filtered (0.45 μ m), and
631 stored in aliquots at -80°C. Pseudoviruses were quantified with a p24 assay

632 (Takara #632200), and normalized based on titer prior to infection for entry assays.

633 Human airway organoids (HAO) stably expressing ACE2 (HAO-ACE2) or 293T cells
634 stably expressing ACE2 and TMPRSS2 (293T-ACE2-TMPRSS2) were infected with an
635 equivalent amount of the indicated pseudoviruses in the presence of 5-10 μ g/ml of
636 polybrene for 72h. Pseudovirus entry was assayed using a luciferase assay (Promega
637 #E1501) and luminescence was measured in a plate reader (TECAN, Infinite 200 Pro M
638 Plex). Two independent experiments were run for the 293T pseudovirus assays (2
639 biological replicates), with 3 technical replicates run per experiment. The HAO
640 pseudovirus assays were run as a single experiment with 3 technical replicates.

641 **Statistical analyses**

642 The proportion of B.1.427/B.1.429 was estimated by dividing the number of
643 B.1.427/B.1.429 variant cases by the total number of samples sequenced at a given
644 location and collection date. A logistic growth curve fitting to the data points was
645 generated using a non-linear least squares approach, as implemented by the nls()
646 function in R(version 4.0.3). We estimated the increase in relative transmission rate of
647 the B.1.427/B.1.429 variant by multiplying the logistic growth rate, defined as the
648 change in the proportion of B.1.427/B.1.429 cases per day, by the serial interval (5 days
649 for SARS-CoV2 (Rai et al., 2021)), as previously described (Volz et al., 2020;
650 Washington et al., 2021). Similar to the analyses in Washington, et al., the doubling time
651 was approximated using the formula: $\log(2) / \text{logistic growth rate}$.

652 Welch's t-test, as implemented in R (version 4.0.3) using the rstatix_0.7.0
653 package, was used to compare the N gene Ct values between B.1.427/B.1.429 variant
654 and non-B.1.427/B.1.429 groups. For the in vitro pseudovirus infectivity studies, a one-
655 way ANOVA test was used to determine significance. For the PRNT studies, a Wilcoxon
656 matched pairs signed rank test was used to determine significance.

657

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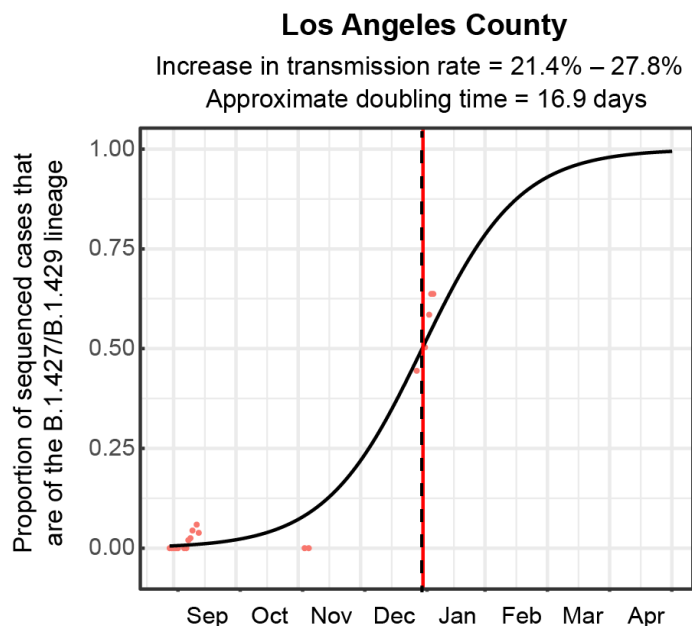
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662 **Supplementary Figures and Tables**

663

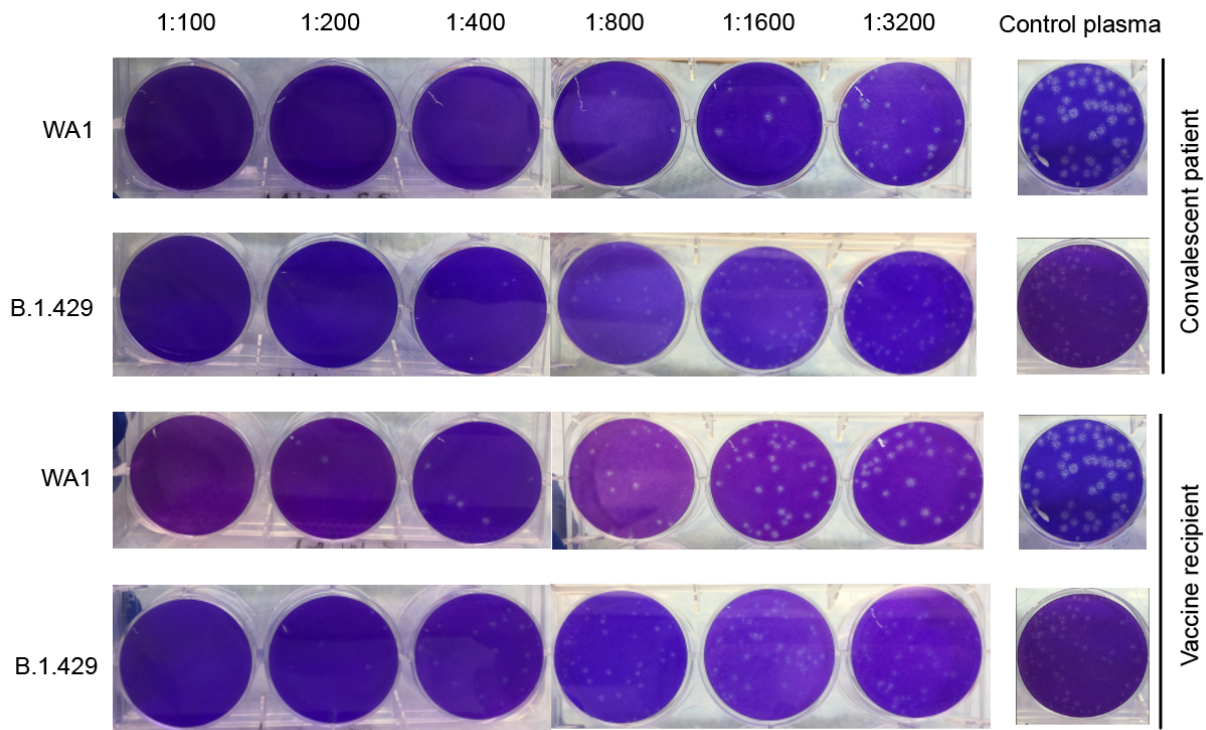


664

665

666 **Supplementary Figure 1. Increasing frequency of the B.1.427/B.1.429 variant in**
667 **Los Angeles County from September 1, 2020 to January 29, 2021.** Logistic growth
668 curves fitting the 5-day rolling average of the estimated proportion of B.1.427/B.1.429
669 variant cases in Los Angeles County. A vertical black dotted line is used to denote the
670 transition from 2020 to 2021.

671



672

673 **Supplementary Figure 2. Differential neutralization of WA1 and B.1.429 viruses as**

674 **measured by plaque-reduction neutralization tests.** Representative 6-well plates

675 arranged in one line showing viral plaques formed after co-culture with plasma samples

676 from a convalescent patient and vaccine recipient. The same negative control well

677 image is shown in line with the respective viral strain for both vaccine and convalescent

678 samples. The plaques from B.1.429 lineage virus are observed to be small and lighter

679 than those from control WA1 virus. The larger plaques for WA1 are likely due to

680 adaptation in Vero E6 cells; these adaptation mutations have been reported not to

681 impact neutralization responses (Klimstra et al., 2020).

682

683

684 **Supplementary Table 1. Metadata for the 2,172 genomes analyzed in this study.**

685 “SupplementaryTable1.xlsx”

686

687 **Supplementary Table 2. List of California counties and the B.1.427/B.1.429**
 688 **genomes sequenced from each county**

name of county	# of genomes	# of B.1.427/B.1.429 genomes	% of B.1.427/B.1.429 genomes
Santa_Clara_County	605	120	19.8%
Alameda_County	156	72	46.2%
San_Francisco_County	123	32	26.0%
Los_Angeles_County	115	53	46.1%
Madera_County	99	1	1.0%
San_Diego_County	79	8	10.1%
Monterey_County	66	25	37.9%
Contra_Costa_County	65	31	47.7%
Mono_County	55	13	23.6%
Lake_County	38	12	31.6%
Mendocino_County	34	0	0.0%
Marin_County	31	7	22.6%
Fresno_County	30	2	6.7%
Imperial_County	26	4	15.4%
San_Mateo_County	25	6	24.0%
San_Joaquin_County	25	3	12.0%
Sutter_County	23	1	4.3%
Sonoma_County	21	3	14.3%
Stanislaus_County	15	6	40.0%
Solano_County	14	2	14.3%
Merced_County	14	5	35.7%
Shasta_County	10	0	0.0%
Sacramento_County	9	0	0.0%
San_Bernardino_County	8	2	25.0%
Yuba_County	7	0	0.0%
Riverside_County	7	1	14.3%
El_Dorado_County	7	0	0.0%
Butte_County	7	0	0.0%

Placer_County	5	0	0.0%
Nevada_County	5	1	20.0%
Yolo_County	4	0	0.0%
Tulare_County	3	1	33.3%
Santa_Cruz_County	3	1	33.3%
Tehama_County	2	1	50.0%
Napa_County	2	1	50.0%
Kings_County	2	1	50.0%
Humboldt_County	2	0	0.0%
Ventura_County	1	1	100.0%
Tuolumne_County	1	0	0.0%
Santa_Barbara_County	1	0	0.0%
San_Benito_County	1	1	100.0%
Orange_County	1	0	0.0%
Mariposa_County	1	1	100.0%
Amador_County	1	0	0.0%

689

690 **Supplementary Table 3. Plasma samples from convalescent COVID-19 patients**
 691 **and SARS-CoV-2 vaccine recipients used for evaluating neutralizing activity**
 692 **against B.1.427 and B.1.429 lineage viruses**

#	Sample Type	Vaccine type	Days post symptom onset	Days post second dose	PRNT50 or 50% CPE endpoint titer		
					WA1	B.1.427 (#1-21) or B.1.429 (#22-31)	D614G
1	Convalescent Plasma	-	60	-	3200	800	NT
2	Convalescent Plasma	-	18	-	ND	ND	NT
3	Convalescent Plasma	-	70	-	3200	3200	NT
4	Convalescent Plasma	-	71	-	800	200	NT
5	Convalescent Plasma	-	15	-	400	100	NT
6	Convalescent Plasma	-	49	-	800	100	800
7	Convalescent Plasma	-	23	-	800	100	800
8	Convalescent Plasma	-	36	-	3200	1600	3200
9	Convalescent Plasma	-	31	-	3200	400	3200
10	Vaccine Recipient Plasma	BNT162b2 (Pfizer)	-	28	800	800	NT
11	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	4	100	100	NT
12	Vaccine Recipient Plasma	BNT162b2 (Pfizer)	-	14	800	400	NT
13	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	4	200	100	NT
14	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	14	ND	ND	NT
15	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	18	1600	800	NT
16	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	10	800	400	NT
17	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	10	3200	1600	NT
18	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	11	3200	1600	NT
19	Vaccine Recipient Plasma	BNT162b2 (Pfizer)	-	15	200	100	400
20	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	7	400	100	400
21	Vaccine Recipient Plasma	mRNA-1273 (Moderna)	-	5	400	400	400
22	Convalescent Plasma	-	-	55	2560	2560	2560
23	Convalescent Plasma	-	-	44	1280	640	2560
24	Convalescent Plasma	-	-	85	320	160	320
25	Convalescent Plasma	-	-	72	640	40	160
26	Convalescent Plasma	-	-	62	320	80	320
27	Convalescent Plasma	-	-	56	1280	640	2560
28	Convalescent Plasma	-	-	21	1280	640	1280
29	Convalescent Plasma	-	-	72	1280	160	640
30	Convalescent Plasma	-	-	30	1280	320	2560
31	Convalescent Plasma	-	-	42	640	80	320

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