

1 **SARS-CoV-2 Lambda variant exhibits higher infectivity and immune**  
2 **resistance**

3

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46 **Conflict of interest:** The authors declare that no competing interests exist.

47 **Short title:** Evolution and epidemics of the Lambda variant (45/50 characters)

48 **Keywords:** SARS-CoV-2; COVID-19; spike protein; C.37; Lambda

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51 **Highlights** (85 characters including spaces)

52 ● Lambda S is highly infectious and T76I and L452Q are responsible for this  
53 property

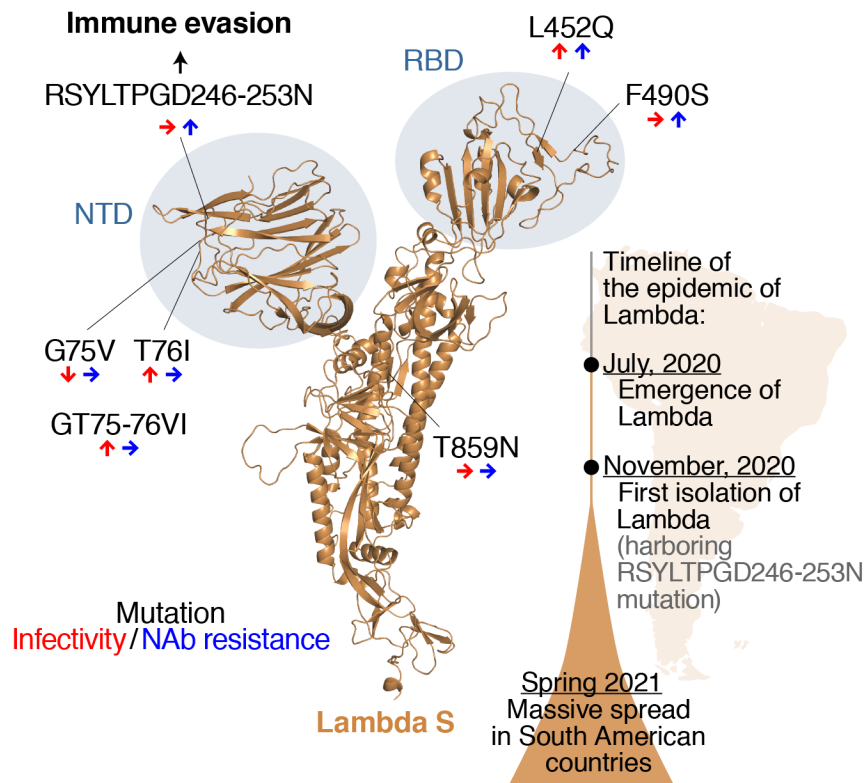
54 ● Lambda S is more susceptible to an infection-enhancing antibody

55 ● RSYLTPGD246-253N, L452Q and F490S confer resistance to antiviral immunity

56

57

58 **Graphical Abstract**



61 **Summary**

62 SARS-CoV-2 Lambda, a new variant of interest, is now spreading in some South  
63 American countries; however, its virological features and evolutionary trait remain  
64 unknown. Here we reveal that the spike protein of the Lambda variant is more  
65 infectious and it is attributed to the T76I and L452Q mutations. The RSYLTPGD246-  
66 253N mutation, a unique 7-amino-acid deletion mutation in the N-terminal domain of  
67 the Lambda spike protein, is responsible for evasion from neutralizing antibodies.  
68 Since the Lambda variant has dominantly spread according to the increasing  
69 frequency of the isolates harboring the RSYLTPGD246-253N mutation, our data  
70 suggest that the insertion of the RSYLTPGD246-253N mutation is closely  
71 associated with the massive infection spread of the Lambda variant in South America.

## 72 **Introduction**

73 During the pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-  
74 2), the causative agent of coronavirus disease 2019 (COVID-19), has been  
75 diversified. As of July 2021, there are four variants of concerns (VOCs), Alpha  
76 [B.1.1.7 lineage; the lineage classification is based on Phylogenetic Assignment of  
77 Named Global Outbreak (PANGO): [https://cov-  
79 lineages.org/resources/pangolin.html](https://cov-<br/>78 lineages.org/resources/pangolin.html)], Beta (B.1.351 lineage), Gamma (P.1 lineage)  
80 and Delta (B.1.617.2 lineage), and four variants of interests (VOIs), Eta (B.1.525  
81 lineage), Iota (B.1.526 lineage), Kappa (B.1.617.1 lineage) and Lambda (C.37  
82 lineage) ([WHO, 2021a](#)). These variants are considered to be the potential threats to  
83 the human society.

84 VOCs and VOIs harbor multiple mutations in their spike (S) protein and are  
85 relatively resistant to the neutralizing antibodies (NAbs) that are elicited in  
86 convalescent and vaccinated individuals ([Chen et al., 2021](#); [Collier et al., 2021](#);  
87 [Garcia-Beltran et al., 2021](#); [Hoffmann et al., 2021](#); [Liu et al., 2021a](#); [Liu et al., 2021b](#);  
88 [Planas et al., 2021](#); [Wall et al., 2021a](#); [Wang et al., 2021a](#); [Wang et al., 2021b](#)).  
89 Because the receptor binding domain (RBD) of the SARS-CoV-2 S protein is  
90 immunodominant, mutations in this domain can lead to the immune evasion ([Piccoli  
91 et al., 2020](#)). Additionally, the mutations in the N-terminal domain (NTD) of the  
92 SARS-CoV-2 S protein are associated with the escape neutralization ([McCallum et  
93 al., 2021](#)). Moreover, the antibodies that enhance viral infectivity [enhancing  
94 antibodies (EABs)] were detected in severe COVID-19 patients, and these EABs  
95 target NTD ([Li et al., 2021](#); [Liu et al., 2021c](#)). Because natural mutations in the S  
96 NTD crucially influence the sensitivity to antibodies ([Gobeil et al., 2021](#)), the  
97 accumulation of mutations in this domain is closely associated with the infection  
98 spread of VOCs and VOIs.

99 The Lambda variant (also known as the C.37 lineage) is the newest VOI  
100 (designated on June 14, 2021) ([WHO, 2021a](#)) and is currently spreading in South  
101 American countries such as Peru, Chile, Argentina, and Ecuador ([WHO, 2021a](#)).  
102 Based on the information data from the Global Initiative on Sharing All Influenza Data  
103 (GISAID) database (<https://www.gisaid.org>; as of June 29, 2021), the Lambda  
104 variant has been isolated in 26 countries. Notably, the vaccination rate in Chile is  
105 relatively high; the percentage of the people who received at least one dose of  
106 COVID-19 vaccine was ~60% on June 1, 2021 ([https://ourworldindata.org/covid-  
108 vaccinations](https://ourworldindata.org/covid-<br/>107 vaccinations)). A recent paper also suggested that the vaccines have effectively  
109 prevented COVID-19 in Chile ([Jara et al., 2021](#)). Nevertheless, a big COVID-19  
110 surge has occurred in Chile in Spring 2021 ([WHO, 2021b](#)), suggesting that the  
111 Lambda variant is proficient in escaping from the antiviral immunity elicited by

110 vaccination. In this study, we reveal the evolutionary trait of the Lambda variant by  
111 molecular phylogenetic analysis. We further demonstrate that the RSYLTPGD246-  
112 253N mutation, a unique mutation in the NTD of the Lambda S protein, is responsible  
113 for the virological phenotype of the Lambda variant that can associate with the  
114 massive infection spread mainly in South American countries.

## 115 **Results**

### 116 **Epidemic dynamics of the Lambda variant in South American countries**

117 As of June 29, 2021, 1,908 genome sequences of the Lambda variant belonging to  
118 the PANGO C.37 lineage have been isolated from 26 countries and deposited in  
119 GISAID. Although it is considered that the Lambda variant was first detected in Peru  
120 in December 2020 ([WHO, 2021a](#)), our in-depth analysis revealed that the Lambda  
121 variant was first detected in Argentina on November 8, 2020 (GISAID ID:  
122 EPI\_ISL\_2158693) (**Figure 1A**; see **STAR★METHODS** for the detail). The fact that  
123 the percentage of the Lambda sequence is increasing in South American countries  
124 including Peru, Chile, and Argentina (**Figure 1A and Table S1**) suggests that the  
125 Lambda variant is spreading predominantly in these countries (**Table S2**). We then  
126 generated a maximum likelihood-based phylogenetic tree of the Lambda variant  
127 (C.37). Although there are some isolates that have been misclassified as the  
128 Lambda, which could be a sister group of the Lambda variant, our phylogenetic tree  
129 indicated the monophyly of genuine Lambda variant isolates (**Figure S1**).

130

### 131 **Association of the lambda variant spread with the increasing frequency of the** 132 **isolates harboring the RSYLTPGD246-253N mutation**

133 The S protein of the consensus sequence of the Lambda variant bears six  
134 substitution mutations (G75V, T76I, L452Q, F490S, D614G and T859N) and a 7-  
135 amino-acid deletion in the NTD (RSYLTPGD246-253N) (**Table S3**). The analysis  
136 using the 1,908 sequences of the Lambda variant (C.37 lineage) showed that the six  
137 substitution mutations are relatively highly (> 90%) conserved (**Figure 1B and Table**  
138 **S3**). Although a large deletion in the NTD, the RSYLTPGD246-253N mutation, is  
139 also highly conserved, 287 out of the 1,908 sequences (15.0%) of the Lambda (C.37  
140 lineage) genomes do not harbor this mutation (**Figure 1C and Table S3**). To ask  
141 whether the epidemic dynamics of the Lambda variant is associated with the  
142 emergence of the RSYLTPGD246-253N mutation, we examined all amino acid  
143 replacements in the S protein of the SARS-CoV-2 genomes deposited in the GISAID  
144 database (2,084,604 sequences; as of June 29, 2021). The RSYLTPGD246-253N  
145 mutation was first found in Argentina on November 8, 2020 (GISAID ID:  
146 EPI\_ISL\_2158693), which is the first isolate of the Lambda variant (**Figure 1A**),  
147 suggesting that this deletion event uniquely occurred in the ancestral lineage of the  
148 Lambda variant. We then analyzed the molecular evolutionary dynamics of the  
149 Lambda variants by performing the Bayesian tip-dating analysis. We showed that  
150 the Lambda variant bearing the RSYLTPGD246-253N mutation emerged around  
151 July 12, 2020 (95% CI, January 5, 2020 – October 22, 2020) (**Figure 1C and Figure**  
152 **S1B**). To infer the population dynamics of the lineage, we performed the Bayesian

153 skyline plot analysis. This analysis showed that the effective population size of the  
154 Lambda variant has increased at the beginning of 2021 (**Figure 1D**). Intriguingly,  
155 when we plot the proportion of the Lambda variant that bears RSYLTPGD246-253N  
156 mutation, it was increased after the emergence of the Lambda variant and closely  
157 associated with the increase of effective population size (**Figure 1D**). These results  
158 suggest that the emergence of the RSYLTPGD246-253N mutation is associated with  
159 the outbreak of the Lambda variant in South America.

160

### 161 **Higher infectivity and resistance to NABs of the Lambda variant**

162 To address the virological phenotype of the Lambda variant, we prepared the viruses  
163 pseudotyped with the S proteins of the Lambda variant as well as four VOCs, Alpha  
164 (B.1.1.7), Beta (B.1.351), Gamma (P.1) and Delta (B.1.617.2) (**Table S4**). We also  
165 prepared the pseudoviruses with the S proteins of the D614G-bearing parental  
166 isolate (B.1) and the Epsilon (B.1.427/429) variant (**Table S4**), which is used to be a  
167 VOC/VOI by July 6, 2021 ([WHO, 2021a](#)), as controls of this experiment. As shown  
168 in **Figure 2A**, the infectivities of the Alpha and Beta variants were significantly lower  
169 than that of the parental D614G S, and the infectivities of Gamma variant and the  
170 parental D614G S were comparable. On the other hand, the infectivities of the Delta,  
171 Epsilon and Lambda variants were significantly higher than that of the parental  
172 D614G (**Figure 1A**). This pattern was independent of the input dose of  
173 pseudoviruses used (**Figure S2A**).

174 To assess the effect of a characteristic mutation of the Lambda variant, the  
175 RSYLTPGD246-253N mutation (**Figure 1**), on viral infectivity, we prepared the  
176 pseudovirus with the Lambda S derivative recovering this deletion mutation  
177 ("Lambda+N246-253RSYLTPGD"). The infectivity of this mutant was comparable to  
178 that of the Lambda S (**Figures 2A and S2A**), suggesting that the 7-amino-acid  
179 deletion in the NTD does not affect viral infection.

180 Because the NAb resistance is a remarkable phenotype of most VOCs  
181 [reviewed in ([Harvey et al., 2021](#))], we next analyzed the sensitivity of the Lambda S  
182 to the NABs induced by BNT162b2 vaccination. As shown in **Figure 2B**, the Lambda  
183 S is 1.5-fold in average (2.63-fold at a maximum) more resistant to the BNT162b2-  
184 induced antisera than the parental D614G S ( $P = 0.0077$  by Wilcoxon matched-pairs  
185 signed rank test). On the other hand, the neutralization level of the Lambda+N246-  
186 253RSYLTPGD was similar to the D614G pseudovirus ( $P = 0.21$  by Wilcoxon  
187 matched-pairs signed rank test), and this recovered variant was 1.37-fold more  
188 sensitive to the vaccine-induced neutralization than the Lambda ( $P = 0.0016$  by  
189 Wilcoxon matched-pairs signed rank test) (**Figure 2B**). These results suggest that  
190 the Lambda S is highly infectious and resistant to the vaccine-induced humoral

191 immunity, and the robust resistance of the Lambda S to the vaccine-induced  
192 neutralization is determined by a large deletion in the NTD.

193

### 194 **Effect of the consensus mutations in the Lambda S on viral infectivity and NAb** 195 **sensitivity**

196 We next plotted six substitution mutations (G75V, T76I, L452Q, F490S, D614G and  
197 T859N) and a deletion mutation (RSYLTPGD246-253N) of the Lambda variant on  
198 the structure of the SARS-CoV S protein. Structural analysis showed that the three  
199 mutations, G75V, T76I and RSYLTPGD246-253N are in the NTD (**Figure 2C**), and  
200 the RSYLTPGD246-253N mutation is located in a loop structure, which is designated  
201 as loop 5 structure (residues 246-260) in a previous study ([Chi et al., 2020](#)) (**Figure**  
202 **2D**). The L452Q and F490S mutations are in the RBD (**Figures 2C and 2D**), but  
203 neither residues are located on the ACE2 interface (**Figure S2D**). The T859N  
204 mutation is in the heptad repeat 1 of the S2 subunit (**Figure 2C**).

205 To investigate the effects of these seven consensus mutations in the  
206 Lambda S on viral infectivity and NAb sensitivity, we prepared the viruses  
207 pseudotyped with the D614G S-based derivatives that possess respective mutations  
208 of the Lambda variant. **Figure 2E** showed that the G75V mutation significantly  
209 reduces viral infectivity, while the T76I and GT75-76VI mutations significantly  
210 increases it. Additionally, 91.5% (1,746/1,908) of the Lambda variant sequence  
211 possesses these two mutations, and the phylogenetic tree of the Lambda variant  
212 indicated that the variant harboring either G75V or T76I sporadically emerged during  
213 the epidemic of the Lambda variant (**Figure S1A**). These findings suggest that T76I  
214 is a compensatory mutation to recover the decreased infectivity by the G75V  
215 mutation.

216 Similar to the experiment using the pseudoviruses with the Lambda S and  
217 Lambda+N246-253RSYLTPGD (**Figure 2A**), the insertion of the RSYLTPGD246-  
218 253N mutation did not affect viral infectivity (**Figure 2E**). The infectivity of the T859N  
219 mutation was also similar to that of the parental D614G pseudovirus (**Figure 2E**).  
220 When we focus on the effect of the mutations in the RBD, the L452Q and  
221 L452Q/F490S mutations significantly increased viral infectivity, while the F490S sole  
222 mutation did not (**Figure 2E**). Taken together, these results suggest that the T76I  
223 and L452Q mutations are responsible for the higher infectivity of the Lambda S  
224 (**Figure 2A**). The effect of each mutation was similar in different amounts of  
225 pseudoviruses used and in the target cells without TMPRSS2 expression (**Figure**  
226 **S2E**).

227 We next assessed the sensitivity of these pseudoviruses with the mutated  
228 S proteins to BNT162b2-induced antisera. As shown in **Figure 2F**, the G75V, T76I,



229 GT75-76VI and T852N mutations did not affect the vaccine-induced neutralization.  
230 On the other hand, the RSYLTPGD246-253N mutant exhibited a significant  
231 resistance to the vaccine-induced neutralization ( $P = 0.027$  by Wilcoxon matched-  
232 pairs signed rank test; **Figure 2F**), which is relevant to the experiment with the S  
233 proteins of the Lambda variant and the Lambda+N246-253RSYLTPGD derivative  
234 (**Figure 2B**). Additionally, we found that the L452Q and F490S mutations confer  
235 resistance to the vaccine-induced antisera (**Figure 2F**). The results that the F490S  
236 mutation does not affect viral infectivity (**Figure 2E**) but confers the resistance to the  
237 vaccine-induced antisera (**Figure 2F**) suggest that this mutation has acquired to be  
238 resistant to antiviral humoral immunity. On the other hand, the L452Q mutation not  
239 only increases viral infectivity (**Figure 2E**) but also augments the resistance to the  
240 vaccine-induced antisera (**Figure 2F**), suggesting that that this mutation can be  
241 critical for the viral dissemination in the human population.

242 To further assess the association of the mutations in the Lambda S,  
243 particularly those in the NTD, we used two monoclonal antibodies that targets the  
244 NTD: an NTD-targeting NAb, clone 4A8 ([Chi et al., 2020](#)), and an EAb, clone COV2-  
245 2490, that recognizes the NTD and enhances viral infectivity ([Liu et al., 2021c](#)). As  
246 shown in **Figure 2G**, the 4A8 antibody inhibited the pseudovirus infections of the  
247 parental D614G, Lambda+N246-253RSYLTPGD derivative, G75V, T76I and GT75-  
248 75VI in dose-dependent manners. Intriguingly, the pseudoviruses with the S proteins  
249 of the Lambda and the RSYLTPGD246-253N mutant were resistant to the antiviral  
250 effect mediated by the 4A8 antibody (**Figure 2G**). These results suggest that the  
251 RSYLTPGD246-253N mutation critically affects the sensitivity to certain NAb  
252 targeting the NTD. On the other hand, the COV2-2490 antibody enhanced the  
253 infectivities of the parental D614G, the Lambda, and the Lambda+N246-  
254 253RSYLTPGD derivative (**Figure 2H**). Particularly, the infectivities of the Lambda  
255 and the Lambda+N246-253RSYLTPGD derivative were more significantly enhanced  
256 than the parental D614G (**Figure 2H**). These data suggest that the Lambda S is  
257 more susceptible to the EAb-mediated virus infection enhancement.

## 258 **Discussion**

259 In this study, we demonstrated that three mutations, the RSYLTPGD246-253N,  
260 L452Q and F490S mutations, respectively confer resistance to the vaccine-induced  
261 antiviral immunity. Additionally, the T76I and L452Q mutations contribute to  
262 enhanced viral infectivity. Our data suggest that there are at least two virological  
263 features on the Lambda variant: increasing viral infectivity (by the T76I and L452Q  
264 mutations) and exhibiting resistance to antiviral immunity (by the RSYLTPGD246-  
265 253N, L452Q and F490S mutations).

266 Virological experiments demonstrated that a large 7-amino-acid deletion,  
267 the RSYLTPGD246-253N mutation, does not affect viral infectivity but is responsible  
268 for the resistance to the vaccine-induced neutralization as well as an NTD-targeting  
269 NAb. Additionally, molecular phylogenetic analyses showed that the transition of the  
270 proportion of the Lambda variant harboring a large 7-amino-acid deletion, the  
271 RSYLTPGD246-253N mutation, is associated with the increase of the effective  
272 population size of this variant. Therefore, the emergence of the RSYLTPGD246-  
273 253N mutation could be one of a driving forces behind the spread of this variant in  
274 the human population. In fact, here we showed that the Lambda S is more resistant  
275 to the vaccine-induced antisera than the Lambda+N246-253RSYLTPGD S  
276 derivative. Our results suggest that the resistance of the Lambda variant against  
277 antiviral humoral immunity was conferred by the RSYLTPGD246-253N mutation.  
278 Importantly, the RSYLTPGD246-253N mutation overlaps with a component of the  
279 NTD “supersite” (Chi et al., 2020). Altogether, these observations suggest that the  
280 NTD “supersite” is immunodominant and closely associate with the efficacy of the  
281 vaccine-induced neutralization, and further support the possibility that the  
282 emergence of the RSYLTPGD246-253N mutation triggered the massive spread of  
283 the Lambda variant.

284 We showed that the infectivity of the viruses pseudotyped with the Lambda  
285 S is significantly higher than that with the parental D614G S. Additionally, consistent  
286 with our previous reports (Mlcochova et al., 2021; Motozono et al., 2021b), the  
287 infectivity of the viruses pseudotyped with the S proteins of the Delta and Epsilon  
288 variants was significantly higher than that of the parental D614G S. A common  
289 feature of the Lambda, Delta and Epsilon variants is the substitution in the L452 of  
290 SARS-CoV-2 S protein: the Lambda variant harbors the L452Q mutation, while the  
291 Delta and Epsilon variants possess the L452R mutation (Mlcochova et al., 2021;  
292 Motozono et al., 2021b). Here we demonstrated that the L452Q mutation increases  
293 viral infectivity. Together with our previous observation that the L452R mutation  
294 enhances viral infectivity (Motozono et al., 2021b), it is strongly suggested that the  
295 relatively higher infectivity of the Lambda, Delta and Epsilon variants is attributed to

296 the L452Q/R mutation. The fact that the Lambda and Delta variants are currently a  
297 VOI and a VOC, respectively, suggests that their increasing spread in the human  
298 population is partly attributed to their higher infectivity compared to the parental  
299 SARS-CoV-2. In contrast, nevertheless of its higher infectivity, the Epsilon variant,  
300 an ex VOC, has been excluded from the VOC/VOI classification on July 6, 2021  
301 because this variant was stamped out ([WHO, 2021a](#)). The transient and  
302 unsuccessful (compared to the other VOCs) spread of the Epsilon variant in the  
303 human population implies that increasing viral infectivity is insufficient to maintain  
304 efficient spread in the human population. In addition to increasing viral infectivity, the  
305 Delta variant exhibits higher resistance to the vaccine-induced neutralization  
306 ([Mlcochova et al., 2021](#); [Wall et al., 2021b](#)). Similarly, here we showed that the  
307 Lambda variant equips not only increased infectivity but also resistance against  
308 antiviral immunity. These observations suggest that acquiring at least two virological  
309 features, increased viral infectivity and evasion from antiviral immunity, is pivotal to  
310 the efficient spread and transmission in the human population.

311 Gobeil et al. have suggested that the mutations in the S NTD drive viral  
312 transmission and escape from antiviral immunity ([Gobeil et al., 2021](#)). In fact, three  
313 out of the current four VOCs harbor the deletions in the S NTD [reviewed in ([Harvey  
314 et al., 2021](#))]. The Alpha variant bears the 2-amino-acid deletion, the HV69-70  
315 deletion, in the NTD. Meng et al. showed that the HV69-70 deletion does not affect  
316 the NAb sensitivity but increase viral infectivity ([Meng et al., 2021](#)), suggesting that  
317 the virological significance of the deletion of a portion of the NTD between the Alpha  
318 (the HV69-70 deletion) and the Lambda (the RSYLTPGD246-253N mutation) is  
319 different. A cluster of the Beta variant also possesses the 3-amino-acid deletion,  
320 the LAL242-244 deletion. This deletion does not critically affect the sensitivity to the  
321 vaccine-induced neutralization but exhibits resistance to some NTD-targeting NAb  
322 such as 4A8 ([Liu et al., 2021b](#); [Wang et al., 2021b](#)). Interestingly, the Delta variant  
323 (B.1.617.2 lineage), an VOC, harbors the 2-amino-acid deletion, the EFR156-8G  
324 mutation, while its relative VOI, the Kappa variant (B.1.617.1 lineage), does not  
325 ([WHO, 2021a](#)). Although the virological significance of the EFR156-8G mutation  
326 remains unclear, this mutation in the S NTD may associate with the spread of the  
327 Delta variant worldwide. Together with our findings that the RSYLTPGD246-253N  
328 confers resistance to the vaccine-induced antisera and an NTD-targeting NAb, 4A8,  
329 the accumulative mutations in the S NTD may closely associate with the virological  
330 feature of the variants that can explain their spreading efficacy in the human  
331 population. Particularly, 4A8 targets the NTD “supersite” ([Harvey et al., 2021](#); [Lok,  
332 2021](#)) that includes RSYLTPGD246-253 ([Chi et al., 2020](#)). Because the  
333 RSYLTPGD246-253N mutation is responsible for the resistance to the vaccine-

334 induced antiviral effect of the Lambda variant, our data support the possibility that  
335 the NTD “supersite” is immunodominant and acquiring immune escape mutations in  
336 this region associates with the efficacy of viral dissemination in the human population,  
337 which is proposed in previous reports ([Harvey et al., 2021](#); [McCallum et al., 2021](#)).  
338 Moreover, we revealed that at least an EAb, COV2-2490, more preferentially  
339 enhances the Lambda S-mediated infection. Although the enhancing effect is  
340 independent of the RSYLTPGD246-253N mutation, such enhancement may  
341 associate with feasible spread of the Lambda variant.

342 By molecular phylogenetic analyses and virological experiments, here we  
343 elucidated how the Lambda variant was originated and acquired virological  
344 properties. Because the Lambda variant is a VOI, it might be considered that this  
345 variant is not an ongoing threat compared to the pandemic VOCs. However, because  
346 the Lambda variant is relatively resistant to the vaccine-induced antisera, it might be  
347 possible that this variant is feasible to cause breakthrough infection ([Hacisuleyman  
348 et al., 2021](#); [Jacobson et al., 2021](#); [Nixon and Ndhlovu, 2021](#); [Rana et al., 2021](#)).  
349 Moreover, elucidating the evolutionary trait of threatening SARS-CoV-2 variants can  
350 explain the possibility to lead to wider epidemic, and revealing the virological features  
351 of the respective mutations acquired in VOCs and VOIs should be important to  
352 prepare the risk of newly emerging SARS-CoV-2 variants in the future.

353 **STAR★METHODS**

354 ● KEY RESOURCES TABLE

355 ● RESOURCE AVAILABILITY

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370

371 **Supplemental Information**

372 Supplemental Information includes 2 figures and 5 tables and can be found with this  
373 article online.

374 **Consortia**

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377 Suganami, Miyoko Takahashi, Miyabishara Yokoyama

378

379 **Acknowledgments**

380 We would like to thank all members belonging to The Genotype to Phenotype Japan  
381 (G2P-Japan) Consortium. The super-computing resource was provided by Human  
382 Genome Center at The University of Tokyo and the NIG supercomputer at ROIS  
383 National Institute of Genetics.

384 This study was supported in part by AMED Research Program on Emerging  
385 and Re-emerging Infectious Diseases 20fk0108163 (to A.S.), 20fk0108146 (to  
386 K.Sato), 20fk0108270 (to K.Sato) and 20fk0108413 (to S.N. and K.Sato); AMED  
387 Research Program on HIV/AIDS 21fk0410033 (to A.S.) and 21fk0410039 (to  
388 K.Sato); AMED Japan Program for Infectious Diseases Research and Infrastructure  
389 20wm0325009 and 21wm0325009 (to A.S.); JST SICORP (e-ASIA) JPMJSC20U1  
390 (to K.Sato); JST SICORP JPMJSC21U5 (to K.Sato), JST CREST JPMJCR20H6 (to  
391 S.N.) and JPMJCR20H4 (to K.Sato); JSPS KAKENHI Grant-in-Aid for Scientific  
392 Research C 19K06382 (to A.S.), Scientific Research B 18H02662 (to K.Sato) and  
393 21H02737 (to K.Sato); JSPS Fund for the Promotion of Joint International Research  
394 (Fostering Joint International Research) 18KK0447 (to K.Sato); JSPS Core-to-Core  
395 Program JPJSCCA20190008 (A. Advanced Research Networks) (to K.Sato); JSPS  
396 Research Fellow DC1 19J20488 (to I.K.); ONO Medical Research Foundation (to  
397 K.Sato); Ichiro Kanehara Foundation (to K.Sato); Lotte Foundation (to K.Sato);  
398 Mochida Memorial Foundation for Medical and Pharmaceutical Research (to  
399 K.Sato); Daiichi Sankyo Foundation of Life Science (to K.Sato); Sumitomo  
400 Foundation (to K.Sato); Uehara Foundation (to K.Sato); Takeda Science Foundation  
401 (to K.Sato); The Tokyo Biochemical Research Foundation (to K.Sato); a Grant for  
402 Joint Research Projects of the Research Institute for Microbial Diseases, Osaka  
403 University (to A.S.); and Joint Usage/Research Center program of Institute for  
404 Frontier Life and Medical Sciences, Kyoto University (to K.Sato).

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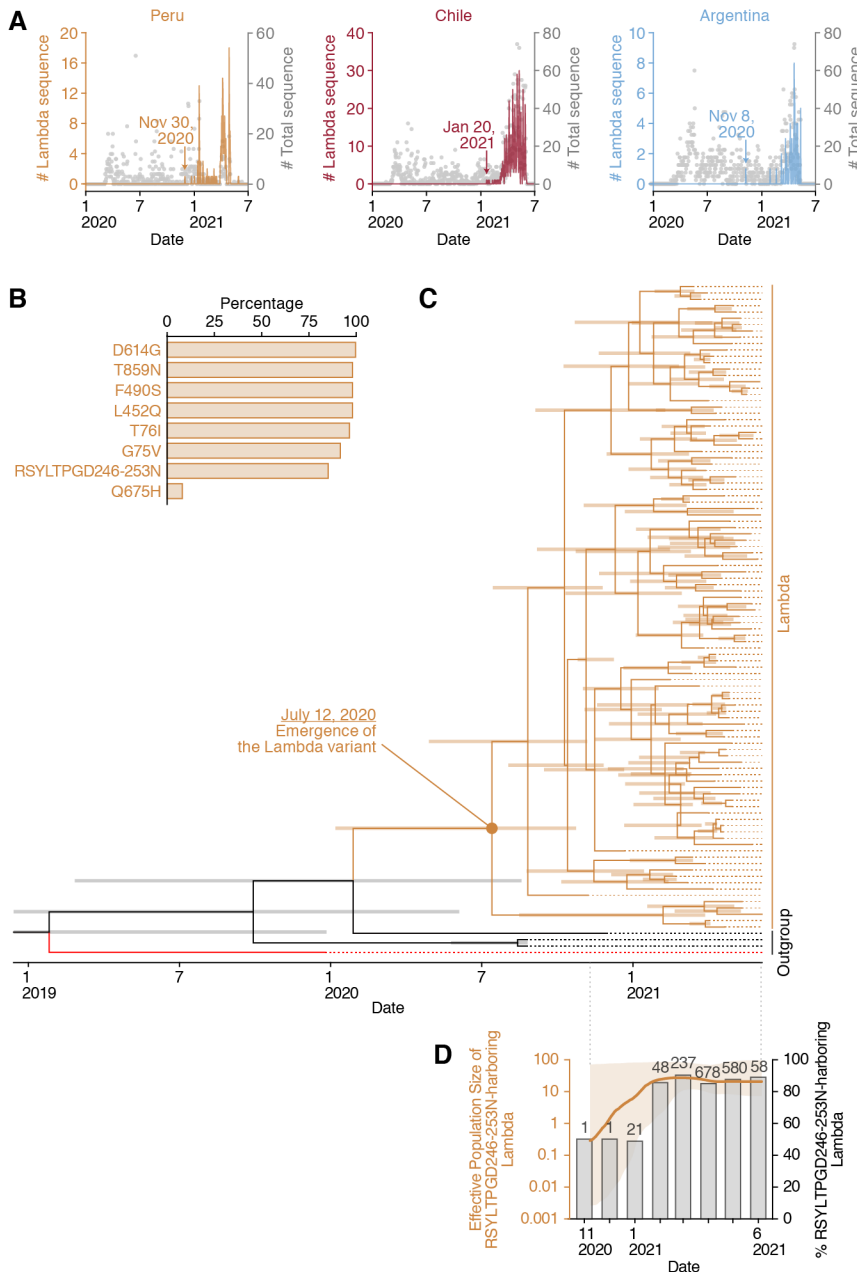
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534

## 535 Figures



536

537

### 538 **Figure 1. Epidemic and evolutionary dynamics of the Lambda variant.**

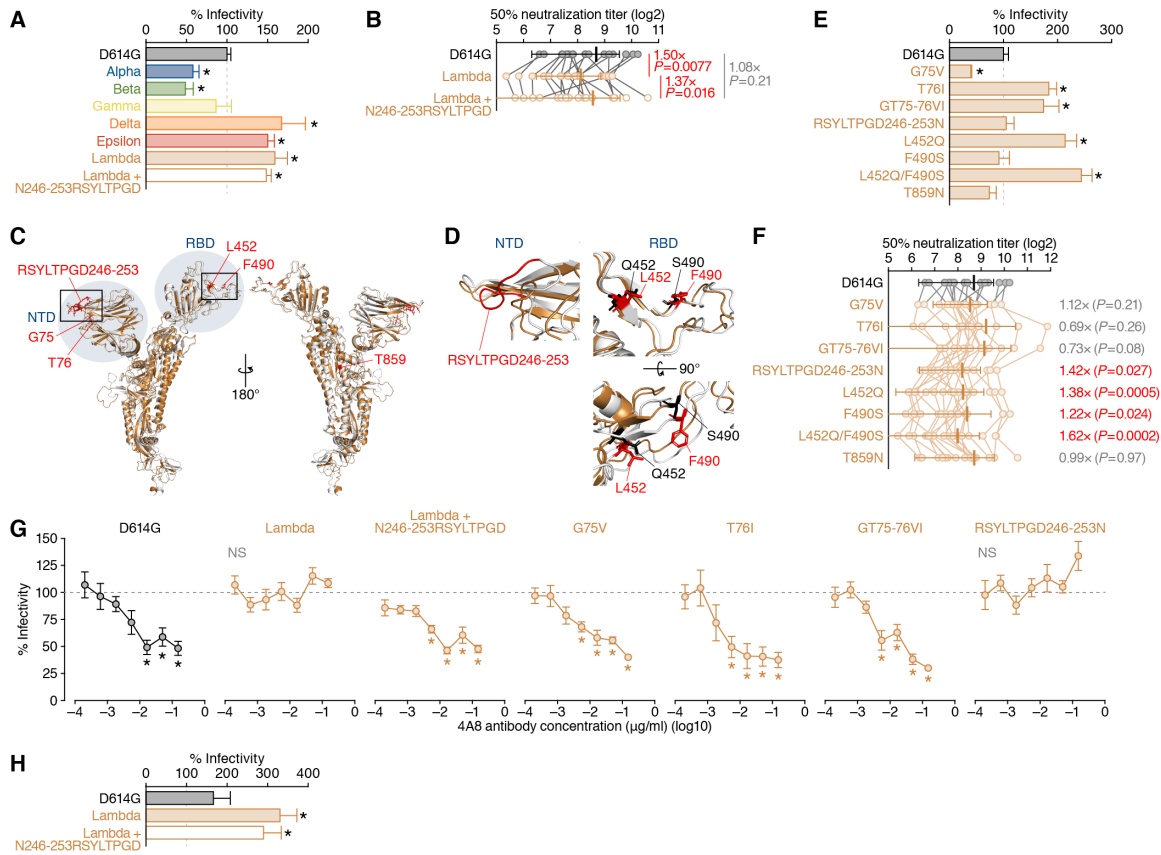
539 (A) Epidemic dynamics of the Lambda variant in three South American countries.  
 540 The numbers of the Lambda variant (C.37 lineage) deposited per day from Peru  
 541 (brown), Chile (dark red), and Argentina (pale blue) are indicated in lines. Grey dots  
 542 indicate the numbers of SARS-CoV-2 genome sequences deposited in the GISAID  
 543 database per day from respective countries. The raw data are summarized in **Tables**  
 544 **S1 and S2.**

545 (B) Proportion of amino acid replacements in the Lambda variant (C.37 lineage). The  
546 top 8 replacements conserved in the S protein of the Lambda variant (C.37 lineage)  
547 are summarized. The raw data are summarized in **Table S3**.

548 (C) An evolutionary timetree of the Lambda variant (C.37 lineage). The estimated  
549 date of the emergence of the Lambda variant is indicated in the figure. The three  
550 sister sequences of the genuine C.37 lineage [GISAID ID: EPI\_ISL\_1532199  
551 (B.1.1.1 lineage), EPI\_ISL\_1093172 (B.1.1.1 lineage) and EPI\_ISL\_1534656 (C.37  
552 lineage)] are used as an outgroup and indicated in black. Wuhan-Hu-1 (GISAID ID:  
553 EPI\_ISL\_1532199), the oldest SARS-CoV-2 (isolated on December 26, 2019), is  
554 indicated in red. Bars on the internal nodes correspond to the 95% highest posterior  
555 density (HPD). The tree noted with the GISAID ID and sampling date at each terminal  
556 node is shown in **Figure S1B**.

557 (D) Transition of the effective population size of the Lambda variant and the  
558 proportion of the Lambda variant harboring the RSYLTPGD246-253N mutation. The  
559 effective population size of the Lambda variant harboring the RSYLTPGD246-253N  
560 mutation (left y-axis) was analyzed by the Bayesian skyline plot. The initial date is  
561 when the first Lambda variant was sampled (November 8, 2020). The 95% highest  
562 posterior density (HPD) is shaded in brown. In the same panel, the proportion of the  
563 Lambda variants harboring RSYLTPGD246-253N mutation for each month (right y-  
564 axis) is also plotted. The number at each time point indicates the number of the  
565 Lambda variants harboring the RSYLTPGD246-253N mutation the mutation. The  
566 number in parentheses indicates the number of the Lambda variants deposited in  
567 the GISAID database.

568 See also **Figure S1** and **Tables S1-S3**.



569

570

## 571 **Figure 2. Virological and immunological features of the Lambda variant.**

572 (A) Pseudovirus assay. The HIV-1-based reporter viruses pseudotyped with the  
 573 SARS-CoV-2 S proteins of the parental D614G (B.1), Alpha (B.1.1.7), Beta (B.1.351),  
 574 Gamma (P.1), Delta (B.1.617.2), Epsilon (B.1.427), Lambda (C.37) variants as well  
 575 as the Lambda+N246-253RSYLTPGD derivative were prepared as described in  
 576 **STAR★METHODS**. The mutations in each variant are listed in **Table S4**. The  
 577 pseudoviruses were inoculated into HOS-ACE2/TMPRSS2 cells at 1,000 ng HIV-1  
 578 p24 antigen, and the percentages of infectivity compared to the virus pseudotyped  
 579 with parental S D614G are shown.

580 (B) Neutralization assay. Neutralization assay was performed using the  
 581 pseudoviruses with the S proteins of parental D614G, Lambda and Lambda+N246-  
 582 253RSYLTPGD and 18 BNT162b2-vaccinated sera as described in  
 583 **STAR★METHODS**. The raw data are shown in **Figure S2B**. The number in the  
 584 panel indicates the fold change of neutralization resistance of the Lambda S to the  
 585 D614G S or the Lambda+N246-253RSYLTPGD derivative.

586 (C and D) Structural insights of the mutations in the Lambda S. (C) Overlaid  
 587 overviews of the crystal structure of SARS-CoV-2 S (PDB: 6ZGE, white) (Wrobel et  
 588 al., 2020) and a homology model of the Lambda S (brown). The mutated residues in

589 the Lambda S and the regions of NTD and RBD are indicated in red and blue. The  
590 squared regions are enlarged in **(D)**. Mutated residues in the NTD (left) and RBD  
591 (right) of the Lambda S. The residues in the parental S and the Lambda S are  
592 indicated in red and black.

593 **(E)** Pseudovirus assay. The HIV-1-based reporter viruses pseudotyped with the  
594 SARS-CoV-2 S proteins bearing respective mutations of the Lambda variant as well  
595 as the D614G S were prepared. The pseudoviruses were inoculated into HOS-  
596 ACE2/TMPRSS2 cells at 1,000 ng HIV-1 p24 antigen, and the percentages of  
597 infectivity compared to the virus pseudotyped with parental S D614G are shown.

598 **(F)** Neutralization assay. Neutralization assay was performed using the  
599 pseudoviruses used in **Figure 2B** and 18 BNT162b2-vaccinated sera as described  
600 in **STAR★METHODS**. The raw data are shown in **Figure S2B**. The number in the  
601 panel indicates the fold change of neutralization resistance to the D614G S.

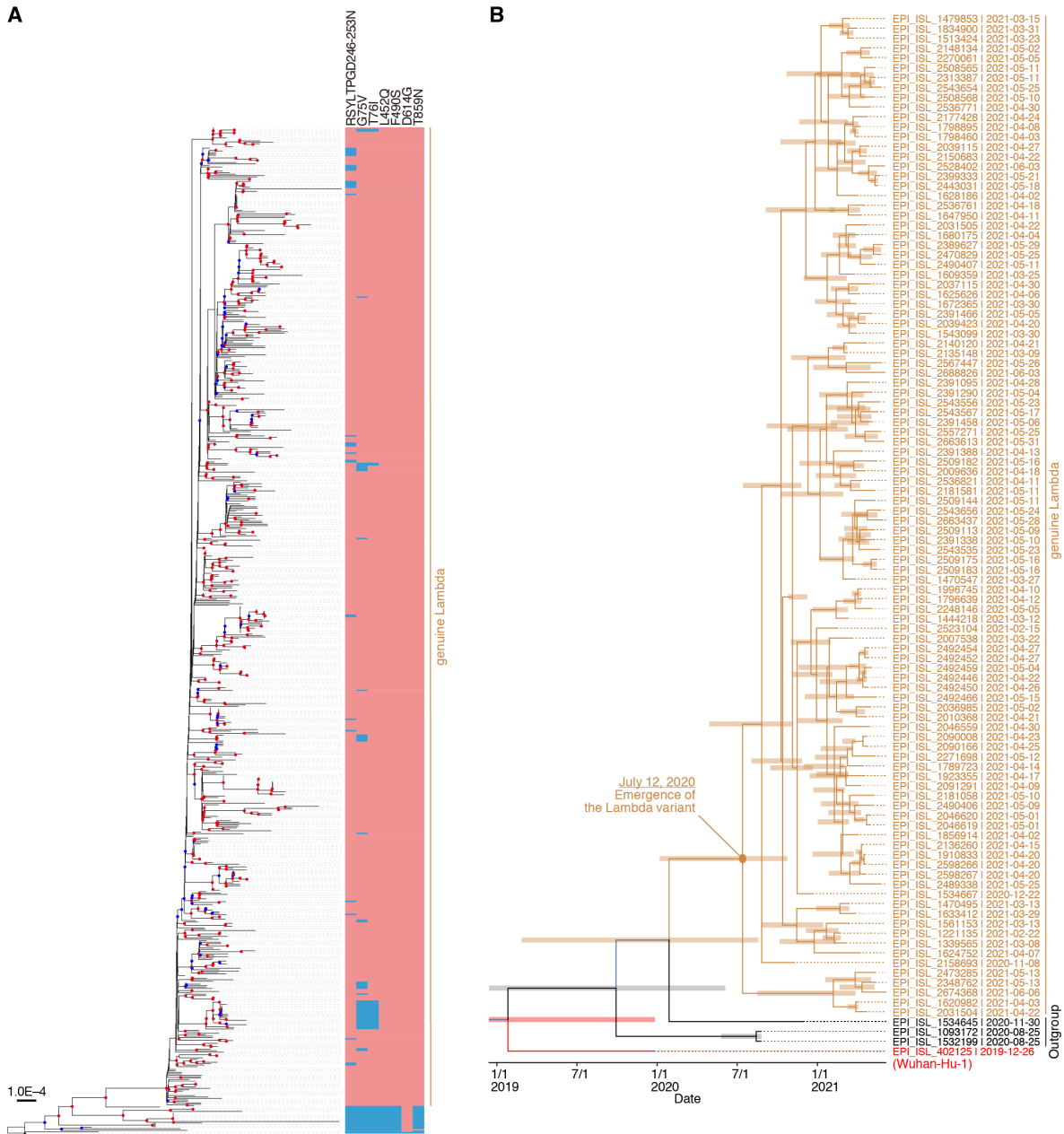
602 **(G and H)** Effect of monoclonal antibodies. **(G)** Antiviral effect of an NTD-targeting  
603 NAb clone 4A8 ([Chi et al., 2020](#)). **(H)** Enhancing effect of an EAb clone COV2-2490  
604 ([Liu et al., 2021c](#)). The percentages of infectivity compared to the virus without  
605 antibodies are shown.

606 In **A, E and H**, assays were performed in triplicate, and the average is shown with  
607 SD. Statistically significant differences (\*,  $P < 0.05$ ) versus the D614G S were  
608 determined by Student's *t* test.

609 In **B and F**, assays were performed in triplicate, and the average is shown with SD.  
610 Statistically significant differences were determined by Wilcoxon matched-pairs  
611 signed rank test. The *P* values are indicated in the figure.

612 In **G**, assays were performed in quadruplicate, and the average is shown with SD.  
613 Statistically significant differences (\*,  $P < 0.05$ ) versus the value without antibody  
614 were determined by Student's *t* test. NS, no statistical significance.

615 See also **Figure S2 and Table S4**.



616  
617

618 **Figure S1. A maximum likelihood-based phylogenetic tree and an evolutionary**  
619 **timetree of the Lambda variant (C.37 lineage) (Related to Figure 1).**

620 (A) The 696 SARS-CoV-2 genome sequences were used for the analysis. Wuhan-  
621 Hu-1 (GISAID ID: EPI\_ISL\_1532199), located in the bottom of the tree, was  
622 indicated by a black star. EPI\_ISL\_1532199 and EPI\_ISL\_1093172 belonging to the  
623 B.1.1.1 lineage were indicated by grey stars. Red or blue circle on the branch was  
624 shown in each internal node if the bootstrap value was  $\geq 80$  or  $\geq 50$  ( $n = 1,000$ ). A  
625 color box in pink or pale blue indicates the mutation in the S protein exist or not,  
626 respectively.

627 **(B)** An evolutionary timetree of the Lambda variant (C.37 lineage). The estimated  
628 date of the emergence of the Lambda variant is indicated in the figure. The GISAID  
629 ID and sampling date is noted at each terminal node. The three sister sequences of  
630 the genuine C.37 lineage [GISAID ID: EPI\_ISL\_1532199 (B.1.1.1 lineage),  
631 EPI\_ISL\_1093172 (B.1.1.1 lineage) and EPI\_ISL\_1534656 (C.37 lineage)] are used  
632 as an outgroup and indicated in black. Wuhan-Hu-1 (GISAID ID: EPI\_ISL\_1532199),  
633 the oldest SARS-CoV-2 (isolated on December 26, 2019), is indicated in red. Bars  
634 on the internal nodes correspond to the 95% highest posterior density (HPD).





644 antigen), and percentages of infectivity compared to the virus pseudotyped with  
645 parental S D614G (1,000 ng HIV-1 p24 antigen) are shown. Assays were performed  
646 in triplicate. Note that the data of 1,000 ng HIV-1 p24 antigen are identical to those  
647 shown in **Figure 1B**.

648 In **A**, assays were performed in triplicate, and the average is shown with SD.  
649 Statistically significant differences (\*,  $P < 0.05$ ) versus the D614G S were determined  
650 by Student's *t* test.

651 **(B)** Neutralization assay. Eighteen vaccinated sera were used for the neutralization  
652 assay. The 50% neutralization titers of respective serum against respective virus are  
653 shown. The values are summarized in **Figures 2B and 2F**.

654 **(C)** Amino acid and nucleotide sequences of the residues 245-254 of the SARS-  
655 CoV-2 S. The amino acid sequences (residues 245-254, bold) and nucleotide  
656 sequences of the parental S (top) and the Lambda S (bottom) are shown. The  
657 nucleotides shaded in red in the parental S are deleted in the Lambda S, resulting in  
658 the RSYLTPGD246-253N mutation.

659 **(D)** Positions of the residues L452 and F490. The residues L452Q and F490S are  
660 labeled in the cocrystal structure of SARS-CoV-2 and human ACE2 (PDB: 6M17)  
661 ([Yan et al., 2020](#)) in red.

662 **(E)** Pseudovirus assay. The HIV-1-based reporter viruses pseudotyped with the  
663 SARS-CoV-2 S proteins bearing respective mutations of the Lambda variant as well  
664 as the D614G S were prepared. The pseudoviruses were inoculated into HOS-  
665 ACE2/TMPRSS2 cells (top) or HOS-ACE2 cells (bottom) at 4 different doses (1,000,  
666 500, 250 or 125 ng HIV-1 p24 antigen), and percentages of infectivity compared to  
667 the virus pseudotyped with parental S D614G (1,000 ng HIV-1 p24 antigen) in HOS-  
668 ACE2 cells are shown. Assays were performed in triplicate. Note that the data of  
669 1,000 ng HIV-1 p24 antigen in HOS-ACE2/TMPRSS2 cells are identical to those  
670 shown in **Figure 2E**.

671 In **E**, statistically significant differences (\*,  $P < 0.05$ ) versus the D614G S were  
672 determined by Student's *t* test.

673 **Table S1.** Frequency of the Lambda variant (C.37 lineage) and all SARS-CoV-2  
674 genomes sampled in Chile, Argentina, and Peru per day, related to Figure 1

675

676 **Table S2.** Number of the Lambda variant sequences deposited from 26 countries,  
677 related to Figure 1

678

679 **Table S3.** Mutations in the S proteins of the Lambda variants obtained from the  
680 GISAID database (as of June 29, 2021), related to Figure 1

681

682 **Table S4.** Mutations in the S proteins of SARS-CoV-2 variants used in this study,  
683 related to Figure 2

684

685 **Table S5.** Primers for the construction of S derivatives, related to Figure 2

686 **STAR★METHODS**

687

688 **KEY RESOURCES TABLE**

689 **RESOURCE AVAILABILITY**

690 **Lead Contact**

691 Further information and requests for resources and reagents should be directed to  
692 and will be fulfilled by the Lead Contact, Kei Sato ([KeiSato@g.ecc.u-tokyo.ac.jp](mailto:KeiSato@g.ecc.u-tokyo.ac.jp)).

693

694 **Materials Availability**

695 All unique reagents generated in this study are listed in the Key Resources Table  
696 and available from the Lead Contact with a completed Materials Transfer Agreement.

697

698 **Data and Code Availability**

699 Additional Supplemental Items are available from Mendeley Data at <http://...>

700

701 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

702 **Ethics Statement**

703 For the use of human specimen, all protocols involving human subjects recruited at  
704 Kyoto University were reviewed and approved by the Institutional Review Boards of  
705 Kyoto University (approval number G0697). All human subjects provided written  
706 informed consent.

707

708 **Collection of BNT162b2-Vaccinated Sera**

709 Peripheral blood were collected four weeks after the second vaccination of  
710 BNT162b2 (Pfizer-BioNTech), and the sera of 18 vaccinees (average age: 40, range:  
711 28-59, 22% male) were isolated from peripheral blood. Sera were inactivated at 56°C  
712 for 30 min and stored at -80°C until use.

713

714 **Cell Culture**

715 HEK293T cells (a human embryonic kidney cell line; ATCC CRL-3216), and HOS  
716 cells (a human osteosarcoma cell line; ATCC CRL-1543) were maintained in  
717 Dulbecco's modified Eagle's medium (high glucose) (Wako, Cat# 044-29765)  
718 containing 10% fetal calf serum and 1% PS.

719 HOS-ACE2/TMPRSS2 cells, the HOS cells stably expressing human ACE2 and  
720 TMPRSS2, were prepared as described previously ([Ferreira et al., 2021](#); [Ozono et al., 2021](#)).

721

722 HOS-ACE2 cells, the HOS cells stably expressing human ACE2, were prepared as  
723 described previously (Saito et al., 2021).

724

## 725 **METHOD DETAILS**

### 726 **Viral Genome Sequence Analysis**

727 All SARS-CoV-2 genome sequences and annotation information used in this study  
728 were downloaded from GISAID (<https://www.gisaid.org>) as of June 29, 2021  
729 (2,084,604 sequences). We obtained 1,908 genomes of SARS-CoV-2 Lambda  
730 variant (C.37 lineage based on the PANGO annotation) in the GISAID metadata. We  
731 confirmed all of them are isolated from humans. To estimate when a Lambda variant  
732 harboring the RSYLTPGD246-253N deletion mutation in the S protein occurred, we  
733 screened 1,908 Lambda variants by removing genomes 1) containing more than 5  
734 undetermined nucleotides at coding regions and 2) having an unknown sampling  
735 date. We then collected 644 and 49 viral genomes with and without RSYLTPGD246-  
736 253N deletion mutation in the Lambda S protein. We used Wuhan-Hu-1 strain  
737 isolated in China on December 31, 2019 (GenBank ID: NC\_045512.2 and GISAID  
738 ID: EPI\_ISL\_402125) as the outgroup for tree inference. We aligned entire genome  
739 sequences by using the FFT-NS-1 program in MAFFT suite v7.407 (Kato and  
740 Standley, 2013) and deleted gapped regions in the 5' and 3' regions. We constructed  
741 a phylogenetic tree using IQ-TREE 2 v2.1.3 software (Minh et al., 2020) with 1,000  
742 bootstraps (Figure S1A). GTR+G substitution model is utilized based on BIC  
743 criterion. We found that several sequences without the RSYLTPGD246-253N  
744 mutation also clustered with the genomes carrying the RSYLTPGD246-253N  
745 mutation (Figure S1A), which could be due to reversible mutation(s) and/or  
746 recombination (Jackson et al., 2021). Thus, these sequences were excluded from  
747 the further analysis.

748 To estimate the emerging time of the Lambda variant (C.37 lineage), we  
749 collected all Lambda sequences carrying the RSYLTPGD246-253N mutation that  
750 were sampled in 2020 (2 sequences) and randomly sampled 100 sequences in 2021.  
751 We also added the following 4 SARS-CoV-2 genomes as the outgroup: strain  
752 Wuhan-Hu-1 (GISAID ID: EPI\_ISL\_1532199, isolated on December 26, 2019),  
753 EPI\_ISL\_1093172 (isolated on August 25, 2020), and EPI\_ISL\_1534645 (isolated  
754 on November 30, 2020). Note that the two viral genomes isolated in Peru on August  
755 25, 2020 (EPI\_ISL\_1532199 and EPI\_ISL\_1093172) were categorized in the B.1.1.1  
756 lineage, although they were previously categorized as the C.37 lineage. We carefully  
757 examined these two sequences and found that they could be used as a sister group  
758 of the C.37 lineage. As for EPI\_ISL\_1534645, it does not contain any typical  
759 mutations in the S protein, but it the closed to the genuine Lambda variant (Figure

760 S1A). Therefore, we included these four sequences in the analysis. We conducted  
761 the Bayesian tip-dating analysis using BEAST v1.10.4 (Suchard et al., 2018). We  
762 used GTR+Gamma model for nucleotide substitution model. For the assumption of  
763 rate variations, we applied uncorrelated relaxed clock, assuming that the distribution  
764 of rates followed a Gamma distribution. We carefully checked the effective sample  
765 size of each parameter and confirmed that all are > 200. The emerging time is  
766 estimated as 2020.7585 (95% CI, 2020.245–2020.8525). A timetree was  
767 summarized using TreeAnnotator software in the BEAST package and visualized by  
768 using FigTree v1.4.4 (Figure 1C and Figure S1B). Reconstruction of the population  
769 history, namely the changing on effective population size across time (Figure 1D),  
770 was conducted by Bayesian skyline plot using the same software and parameter  
771 settings using the sampled Lambda sequences as noted in the tip-dating analysis.

772

### 773 Protein Structure Homology Model

774 All protein structural analyses were performed using Discovery Studio 2021  
775 (Dassault Systèmes BIOVIA). In Figures 2C and 2D, the crystal structure of SARS-  
776 CoV-2 S (PDB: 6ZGE) (Wrobel et al., 2020) was used as the template, and 40  
777 homology models of the SARS-CoV-2 S of the Lambda variant were generated using  
778 Build Homology Model protocol MODELLER v9.24 (Fiser et al., 2000). Evaluation of  
779 the homology models were performed using PDF total scores and DOPE scores and  
780 the best model for the Lambda S was selected. In Figure S2D, the cocrystal  
781 structure of SARS-CoV-2 and human ACE2 (PDB: 6M17) (Yan et al., 2020) was  
782 used.

783

### 784 Plasmid Construction

785 Plasmids expressing the SARS-CoV-2 S proteins of the parental D614G (B.1)  
786 (Ozono et al., 2021) and the Epsilon (B.1.427) variant (Motozono et al., 2021b) were  
787 prepared in our previous studies. Plasmids expressing the S proteins of the Alpha  
788 (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Lambda (C.37) variants  
789 and the point mutants were generated by site-directed overlap extension PCR using  
790 pC-SARS2-S D614G (Ozono et al., 2021) as the template and the following primers  
791 listed in Table S4. The resulting PCR fragment was digested with Acc65I or KpnI  
792 and NotI and inserted into the corresponding site of the pCAGGS vector (Niwa et al.,  
793 1991). Nucleotide sequences were determined by DNA sequencing services  
794 (Fasmac or Eurofins), and the sequence data were analyzed by Sequencher v5.1  
795 software (Gene Codes Corporation).

796

### 797 Pseudovirus Assay

798 Pseudovirus assay was performed as previously described ([Motozono et al., 2021a](#);  
799 [Ozono et al., 2021](#)). Briefly, the pseudoviruses, lentivirus (HIV-1)-based, luciferase-  
800 expressing reporter viruses pseudotyped with the SARS-CoV-2 S protein and its  
801 derivatives, HEK293T cells ( $1 \times 10^6$  cells) were cotransfected with 1  $\mu$ g of psPAX2-  
802 IN/HiBiT ([Ozono et al., 2020](#)), 1  $\mu$ g of pWPI-Luc2 ([Ozono et al., 2020](#)), and 500 ng  
803 of plasmids expressing parental S or its derivatives using Lipofectamine 3000  
804 (Thermo Fisher Scientific, Cat# L3000015) or PEI Max (Polysciences, Cat# 24765-  
805 1) according to the manufacturer's protocol. At two days posttransfection, the culture  
806 supernatants were harvested, centrifuged. The amount of pseudoviruses prepared  
807 was quantified using the HiBiT assay as previously described ([Ozono et al., 2021](#);  
808 [Ozono et al., 2020](#)). The pseudoviruses prepared were stored at  $-80^{\circ}\text{C}$  until use.  
809 For the experiment, HOS-ACE2 cells and HOS-ACE2/TMPRSS2 cells (10,000  
810 cells/50  $\mu$ l) were seeded in 96-well plates and infected with 100  $\mu$ l of the  
811 pseudoviruses prepared at 4 different doses. At two days postinfection, the infected  
812 cells were lysed with a One-Glo luciferase assay system (Promega, Cat# E6130),  
813 and the luminescent signal was measured using a CentroXS3 plate reader  
814 (Berthold Technologies) or GloMax explorer multimode microplate reader 3500  
815 (Promega).

816

### 817 **Antibody Treatment**

818 Antibody treatment for neutralization and infectivity enhancement were performed  
819 as previously described ([Saito et al., 2021](#)). Briefly, this assay was performed on  
820 HOS-ACE2/TMPRSS2 cells using the SARS-CoV-2 S pseudoviruses expressing  
821 luciferase (see "Pseudovirus Assay" above). The SARS-CoV-2 S pseudoviruses  
822 (counting  $\sim 20,000$  relative light unit) were incubated with serially diluted heat-  
823 inactivated human sera, a NTD-targeting NAb clone 4A8 ([Chi et al., 2020](#)) or an EAb  
824 clone COV2-2490 ([Liu et al., 2021c](#)) at  $37^{\circ}\text{C}$  for 1 h. The pseudoviruses without  
825 sera/antibodies were included as controls. Then, the 80  $\mu$ l mixture of pseudovirus  
826 and sera/antibodies was added into HOS-ACE2/TMPRSS2 cells (10,000 cells/50  $\mu$ l)  
827 in a 96-well white plate and the luminescence was measured as described above  
828 (see "Pseudovirus Assay" above). 50% neutralization titer was calculated using  
829 Prism 9 (GraphPad Software).

830

### 831 **QUANTIFICATION AND STATISTICAL ANALYSIS**

832 Data analyses were performed using Prism 9 (GraphPad Software). Data are  
833 presented as average with SD.