

1 **SARS-CoV-2 Lambda Variant Remains Susceptible to Neutralization by mRNA**
2 **Vaccine-elicited Antibodies and Convalescent Serum**

3

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23

24 **Abstract**

25 The SARS-CoV-2 lambda variant (lineage C.37) was designated by the World Health
26 Organization as a variant of interest and is currently increasing in prevalence in South
27 American and other countries. The lambda spike protein contains novel mutations within
28 the receptor binding domain (L452Q and F490S) that may contribute to its increased
29 transmissibility and could result in susceptibility to re-infection or a reduction in protection
30 provided by current vaccines. In this study, the infectivity and susceptibility of viruses with
31 the lambda variant spike protein to neutralization by convalescent sera and vaccine-
32 elicited antibodies was tested. Virus with the lambda spike had higher infectivity and was
33 neutralized by convalescent sera and vaccine-elicited antibodies with a relatively minor
34 2.3-3.3-fold decrease in titer on average. The virus was neutralized by the Regeneron
35 therapeutic monoclonal antibody cocktail with no loss of titer. The results suggest that
36 vaccines in current use will remain protective against the lambda variant and that
37 monoclonal antibody therapy will remain effective.

38

39

40 **Introduction**

41 The continued emergence of severe acute respiratory syndrome coronavirus 2 (SARS-
42 CoV-2) variants with increased transmissibility poses concerns with regards to re-
43 infection and diminished vaccine protection. The spread of variants also raises concerns
44 regarding potential decrease in the efficacy of anti-spike protein monoclonal antibody
45 therapy that has been shown to reduce disease symptoms and the rate of hospitalization¹.
46 Variants B.1.351 (Beta), B.1.617.2 (Delta), B.1.427/B.1.429 (Epsilon), B.1.526 (Iota), and
47 B.1.1.248 (Gamma) encode spike proteins with L452R, E484K, E484Q mutations in the
48 spike protein receptor binding domain (RBD) that provide a degree of resistance to
49 neutralization by serum antibodies of vaccinated and convalescent individuals²⁻⁷.

50

51 The lambda variant is prevalent in Peru and is increasing in prevalence in neighboring
52 Argentina, Ecuador, Chile and Brazil⁸. In June, the World Health Organization designated
53 the variant (C.37 lineage) a variant of interest⁹. The variant spike protein is characterized
54 by a novel deletion and mutations (Δ 246-252, G75V, T76I, L452Q, F490S, T859N),
55 L452Q and F490S of which are novel mutations in the RBD.

56

57 The increasing prevalence of the lambda variant raises concerns as to whether the
58 current vaccines will contain its spread. In this study, we tested the sensitivity of viruses
59 with the lambda variant spike protein to neutralization by convalescent sera, vaccine-
60 elicited antibodies and Regeneron therapeutic monoclonal antibodies REGN10933 and
61 REGN10987.

62 **Results**

63 **Lambda spike protein-pseudotyped lentiviruses.** The lambda spike protein has
64 mutations L452Q and F490S in the RBD, and G75V, T76I mutations and 246-252
65 deletions in the N-terminal domain (NTD) (**Figure S1A**). To analyze antibody
66 neutralization of the variant spike protein, we generated expression vectors for the variant
67 and its constituent mutations and used these to produce pseudotyped lentiviral virions
68 encoding GFP and nano-luciferase reporters. The use of such pseudotypes to determine
69 antibody neutralizing titers has been shown to yield results consistent with those obtained
70 with the live virus plaque reduction neutralization test¹⁰. Immunoblot analysis of
71 transfected pseudotype virus producer cells and virus-containing supernatants showed
72 that the variant spike proteins were well expressed, proteolytically processed and
73 incorporated into lentiviral virions at a level similar to that of the parental D614G spike
74 protein (**Figure S1B**). Analysis of the infectivity of the pseudotyped viruses on ACE2.293T
75 cells, normalized for particle number, showed that the lambda spike protein increased
76 infectivity by 2-fold. The increase was due to the L452Q mutation; the other mutations
77 (G75V-T76I, F490S, T859N and Δ 246-252) had no significant effect on infectivity (**Figure**
78 **S1C**).

79

80 **Neutralization of the lambda variants by convalescent sera and vaccine-elicited**
81 **antibody.** Analysis of serum specimens from convalescent patients who had been
82 infected prior to the emergence of the variants showed that viruses with the lambda
83 variant spike protein were 3.3-fold resistant to neutralization by convalescent sera as

84 compared to neutralization of virus with the parental D614G spike, similar to the 4.9-fold
85 resistance of the B.1.351 variant to neutralization (**Figure 1A**).

86

87 Analysis of serum samples from individuals vaccinated with Pfizer BNT162b2 showed
88 that virus with the lambda spike was about 3-fold resistant to neutralization (**Figure 1B**).

89 Serum samples from individuals vaccinated with the Moderna mRNA-1273 vaccine were
90 on average 2.3-fold resistant to neutralization (**Figure 1C**). The resistance was attributed
91 to the L452Q and F490S mutations in the lambda spike protein (**Figure 1A, B, C**).

92

93 **L452Q increases spike protein affinity for ACE2.** N501Y and L452R mutations in the
94 RBD of earlier variants increase spike protein affinity for ACE2, an effect that most likely
95 is a primary contributor to the increased transmissibility of the alpha, beta and delta
96 variants¹¹. To determine whether the lambda variant has an increased affinity for ACE2,
97 we used a sACE2 neutralization assay in which pseudotyped virions were incubated with
98 different concentrations of sACE2 and the infectivity of the treated virions was measured
99 on ACE2.293T cells. The results showed that the lambda spike caused a 3-fold increase
100 sACE2 binding. The increase was caused by the L452Q mutation and was similar to the
101 increase provided by the N501Y mutation^{12,13} (**Figure 1D**). The F490S mutation did not
102 have a detectable effect on sACE2 binding. The findings suggest that L452Q, like L452R
103 in the delta variant, increases virus affinity for ACE2, likely contributing to increased
104 transmissibility.

105

106 **Neutralization by REGN10933 and REGN10987 monoclonal antibodies.** Analysis of
107 REGN10933 and REGN10987, the monoclonal antibodies that constitute the Regeneron
108 REGN-COV2 therapy, showed that virus with the lambda variant spike protein was about
109 3.6-fold resistant to neutralization by REGN10987. The resistance was attributed to the
110 L452Q mutation (**Figure 2A and B**). Virus with the lambda variant spike protein was
111 neutralized by REGN10933 with no decrease in titer. The REGN10933 and REGN10987
112 cocktail neutralized the virus with no decrease in titer relative to virus with the D614G
113 spike protein (**Figure 2A and B**).

114

115

116 **Discussion**

117 Virus with the lambda spike protein, like several VOC variant spike proteins showed a
118 partial resistance to neutralization by vaccine-elicited antibodies and convalescent sera;
119 however the average 3-fold decrease in neutralizing titer against the variant is not likely
120 to cause a significant loss of protection against infection as the average neutralization
121 IC50 titer by the sera of BNT162b2 and mRNA-1273 vaccinated individuals was about
122 1:600, a titer that is above that in the sera of individuals who recovered from infection with
123 the parental D614G virus. A small fraction of vaccinated individuals had serum antibody
124 titers less than average but whether this will lead to reduced protection from variant
125 infection will need to be determined in epidemiological studies.

126

127 The resistance of the lambda variant to antibody neutralization was caused by the L452Q
128 and F490S mutations. The L452R mutation of the California B.1.427/B.1.429 is
129 associated with a 2-fold increase in virus shedding by infected individuals and a 4-6.7-
130 fold and 2-fold decrease in neutralizing titer by the antibodies of convalescent and
131 vaccinated donors, respectively¹⁴. The degree of neutralization resistance provided by
132 L452Q was similar to that of L452R. Amino acid residues 490 and 484 lie close together
133 on the top of the RBD and are therefore in a position to affect the binding of neutralizing
134 antibody. The E484K mutation in the B.1.351, B.1.526, P.1 and P.3 spike proteins causes
135 partial resistance to neutralization²⁻⁷. Similarly, the F490S mutation also caused a 2-3-
136 fold resistance to neutralization, demonstrating the importance of the amino acid as an
137 antibody recognition epitope. While the lambda variant was slightly resistant to
138 REGN10987, it was neutralized well by the cocktail with REGN10933.

139

140 This study suggests that the L452Q and F490S mutations of the lambda variant spike
141 protein caused a partial resistance to vaccine elicited serum and Regeneron monoclonal
142 antibodies. While our findings suggest that current vaccines will provide protection against
143 variants identified to date, the results do not preclude the possibility that novel variants
144 will emerge that are more resistant to current vaccines. The findings highlight the
145 importance of wide-spread adoption of vaccination which will protect individuals from
146 disease, decrease virus spread and slow the emergence of novel variants.

147

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153 **Author contributions**

154 T.T. and N.R.L. designed the experiments. H.Z., T.T. and B.M.D. carried out the
155 experiments and analyzed data. T.T., H.Z. and N.R.L. wrote the manuscript. M.I.S. and
156 M.J.M. provided key reagents and useful insights. All authors provided critical comments
157 on manuscript.

158

159 **Declaration of Interests.**

160 The authors declare no competing interests.

161 **Figure legends**

162

163 **Figure 1. Neutralization of variant spike protein pseudotyped viruses by**
164 **convalescent sera, vaccine-elicited antibodies, monoclonal antibodies and soluble**
165 **ACE2.**

166 (A) Neutralization of lambda variant spike protein viruses pseudotyped virus by
167 convalescent serum (n=8). Dots represent the IC50 of single donors.

168 (B) Neutralizing titers of serum samples from BNT162b2 vaccinated individuals (n=15).
169 Each dot represents the IC50 for a single donor.

170 (C) Neutralizing titers of serum samples from mRNA-1273 vaccinated donors (n=6). The
171 neutralization IC50 from individual donors is shown. Significance is based on the two-
172 sided test. (**P≤0.05, ***P≤0.001, ****P≤0.0001).

173 (D) Neutralization of beta (B.1.351) and lambda variant spike protein variants by
174 REGN10933 and REGN10987 monoclonal antibodies. Neutralization of D614G and
175 lambda variant pseudotyped viruses by REGN10933 (left), REGN10987 (middle), and 1:1
176 ratio of REGN10933 and REGN10987 (right). The IC50s of REGN10933, REGN10987
177 and the cocktail is shown in the table.

178 (E) Neutralization of individual mutated spikes by REGN10933 (left), REGN10987
179 (middle), and cocktail (right). The table shows the IC50 of REGN10933, REGN10987 and
180 the cocktail.

181 (F) Neutralization of lambda variant spike protein variants by soluble sACE2. Viruses
182 pseudotyped with variant spike proteins were incubated with a serially diluted
183 recombinant sACE2 and then applied to ACE2.293T cells. Each plot represents the

184 percent infectivity of D614G and other mutated spike pseudotyped virus. The diagram
185 shows the IC₅₀ for each curve.

186

187 **Supplementary Figure 1.**

188 (A) The domain structure of the SARS-CoV-2 spike is diagrammed with the variant amino
189 acid residues indicated. NTD, N-terminal domain; RBD, receptor-binding domain; RBM,
190 receptor-binding motif; SD1 subdomain 1; SD2, subdomain 2; FP, fusion peptide; HR1,
191 heptad repeat 1; HR2, heptad repeat 2; TM, transmembrane region; IC, intracellular
192 domain. Key mutations are shown in 3D structure (top view).

193 (B) Immunoblot analysis of the variant spike proteins in transfected 293T cells.
194 Pseudotyped viruses were produced by transfection of 293T cells. Two days post-
195 transfection, virions were analyzed on an immunoblot probed with anti-spike antibody and
196 anti-HIV-1 p24. The cell lysates were probed with anti-spike antibody and anti-GAPDH
197 antibodies as a loading control.

198 (C) Infectivity of virus pseudotyped by lambda variant and D614G spike proteins. Viruses
199 were normalized for RT activity and applied to target cells. Infectivity of viruses
200 pseudotyped with the lambda variant protein or the individual lambda mutations were
201 tested on ACE2.293T. Luciferase activity was measured two days post-infection.
202 Significance was based on two-sided testing. (**P≤0.05, ***P≤0.001).

203

204 **Methods**

205 **Plasmids**

206 Mutations in the spike were introduced into pcCOV2.Δ19.D614GS by overlap extension
207 PCR and confirmed by DNA sequencing. Plasmids used in the production of lentiviral
208 pseudotyped virus have been previously described².

209

210 **Human Sera and monoclonal antibodies**

211 Convalescent sera and BNT162b2 or Moderna-vaccinated sera were collected on day 28,
212 7 days post-second immunization, at the NYU Vaccine Center with written consent under
213 IRB approved protocols (IRB 18-02035 and IRB 18-02037). Donor age and gender were
214 not reported. Regeneron monoclonal antibodies (REGN10933 and REGN10987) were
215 generated as previously described¹¹.

216

217 **SARS-CoV-2 spike lentiviral pseudotypes**

218 Lentivirus pseudotyped by variant SARS-CoV-2 spikes were produced as previously
219 reported². Viruses were concentrated by ultracentrifugation and normalized for reverse
220 transcriptase (RT) activity. To determine neutralizing activity, sera or monoclonal
221 antibodies were serially diluted and then incubated with pseudotyped virus (approximately
222 2.5×10^7 cps) for 30 minutes at room temperature and then added to target cells.
223 Luciferase activity was measured 2 days post infection².

224

225 **Soluble ACE2 Neutralization assay**

226 Serially diluted recombinant soluble ACE2 protein prepared from transfected CHO cells
227 was incubated with pseudotyped virus for 30 minutes at room temperature and added to
228 ACE2.293T cells. Luciferase activity was measured using Nano-Glo luciferase substrate
229 (Nanolight) in an Envision 2103 microplate luminometer (PerkinElmer).

230

231 **Immunoblot analysis**

232 Proteins were analyzed on immunoblots probed with mouse anti-spike monoclonal
233 antibody (1A9) (GeneTex), anti-p24 monoclonal antibody (AG3.0) and anti-GAPDH
234 monoclonal antibody (Life Technologies) followed by goat anti-mouse HRP-conjugated
235 secondary antibody (Sigma) as previously described².

236

237 **Statistical Analysis**

238 All experiments were in technical duplicates or triplicates and the data were analyzed
239 using GraphPad Prism 8. Statistical significance was determined by the two-tailed,
240 unpaired t-test. Significance was based on two-sided testing and attributed to $p < 0.05$.
241 Confidence intervals are shown as the mean \pm SD or SEM. (* $P \leq 0.05$, ** $P \leq 0.01$,
242 *** $P \leq 0.001$, **** $P \leq 0.0001$). The PDB file of SARS-CoV-2 spike protein (7BNM)¹⁵ was
243 downloaded from the Protein Data Bank. 3D view of protein was obtained using PyMOL.

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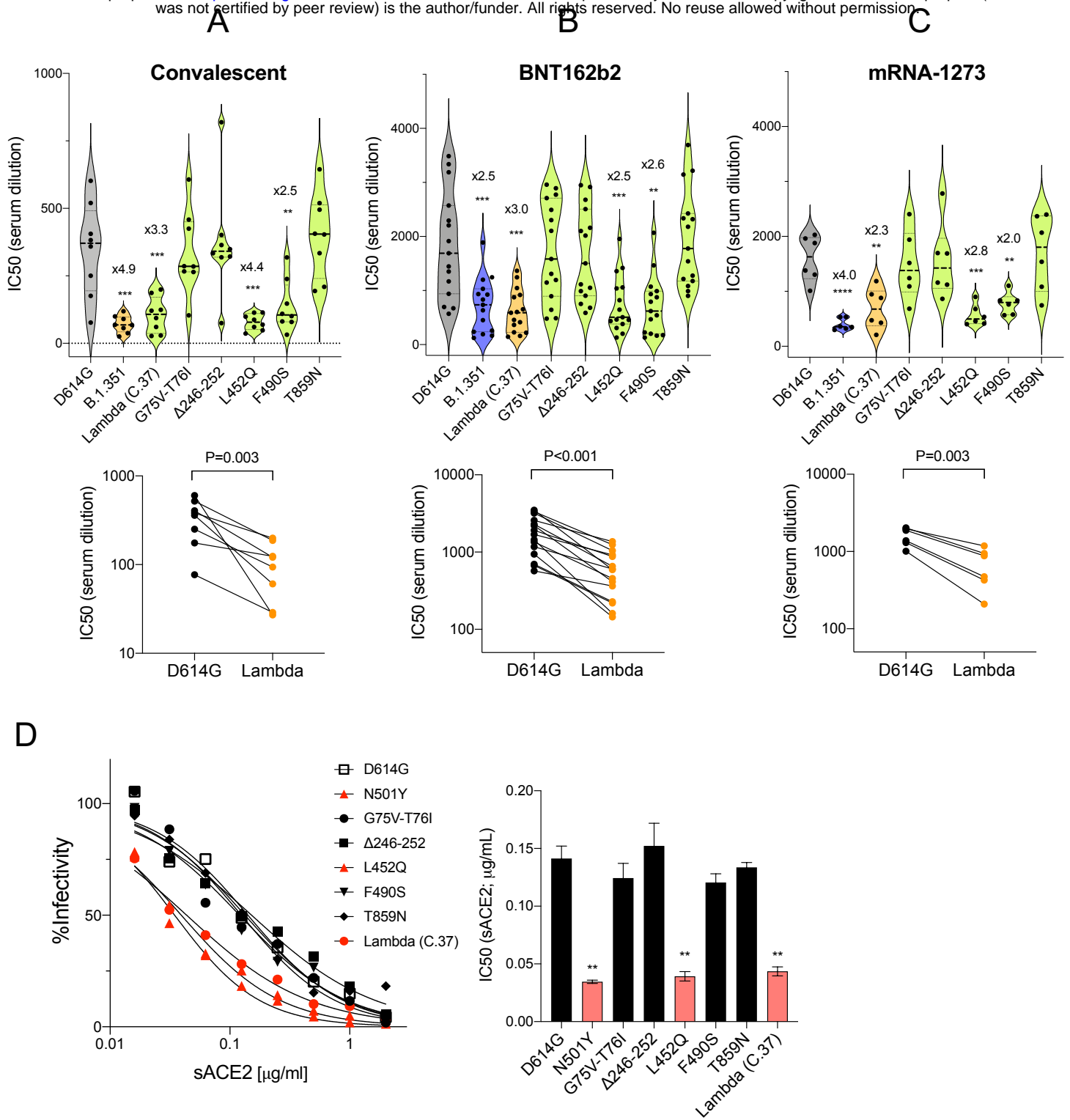
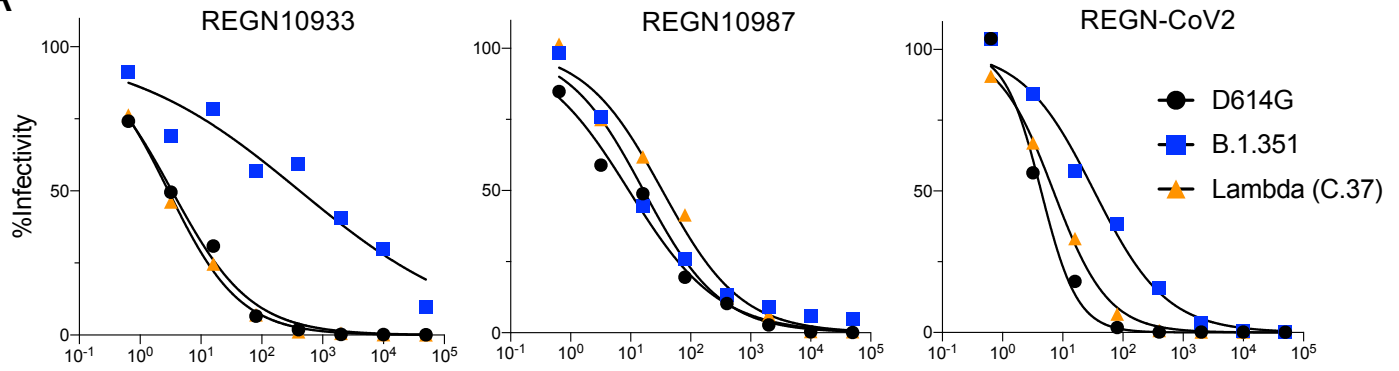


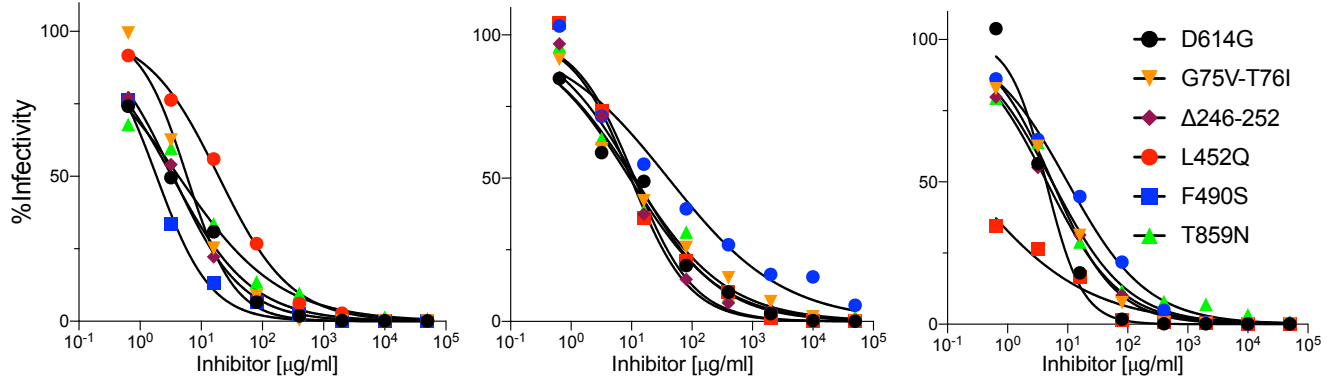
Figure 1

A



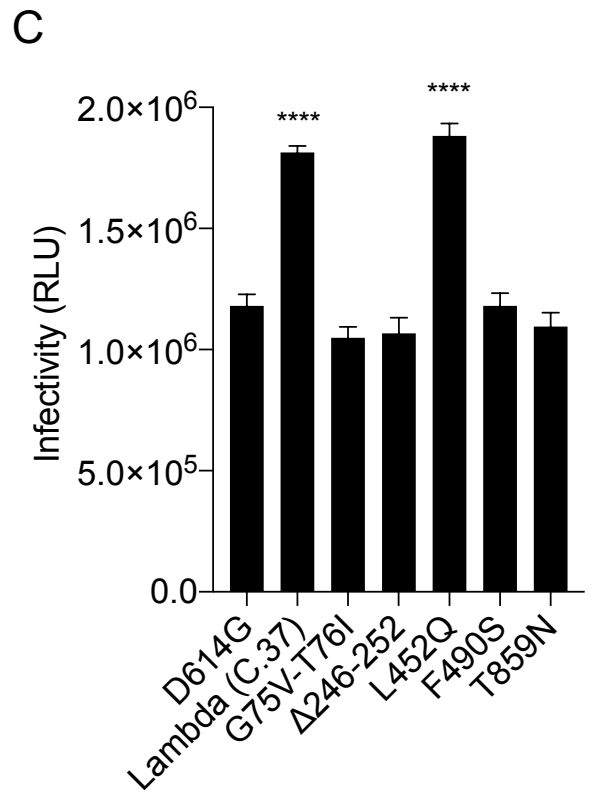
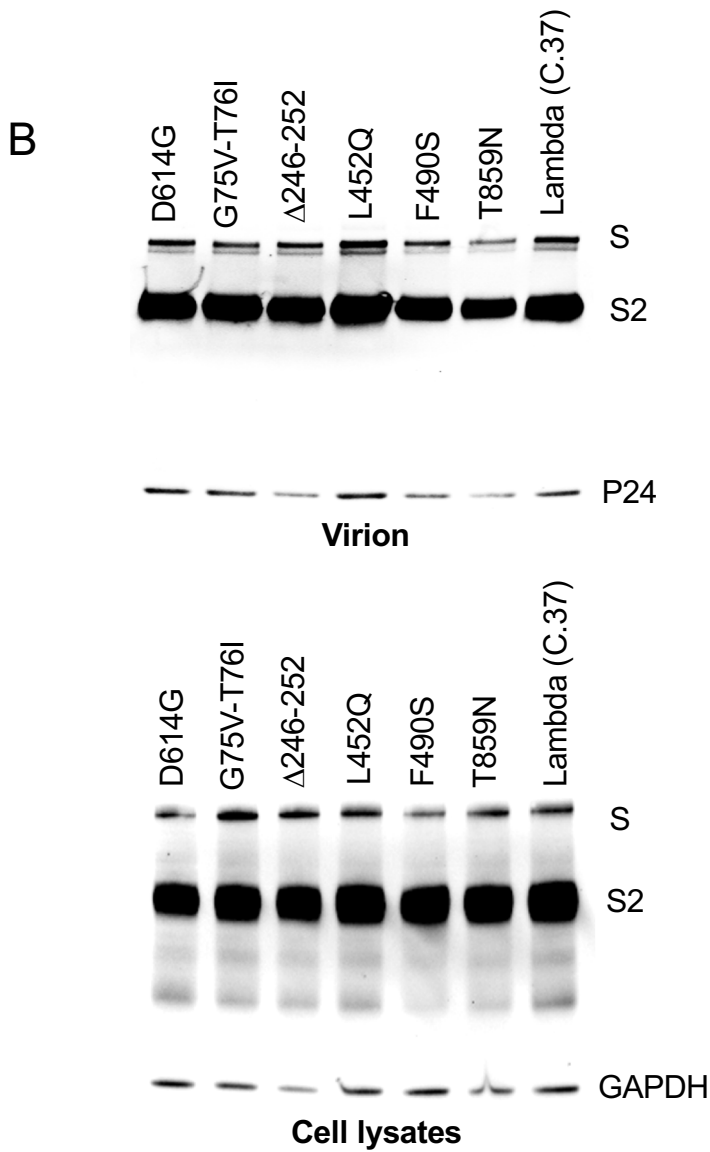
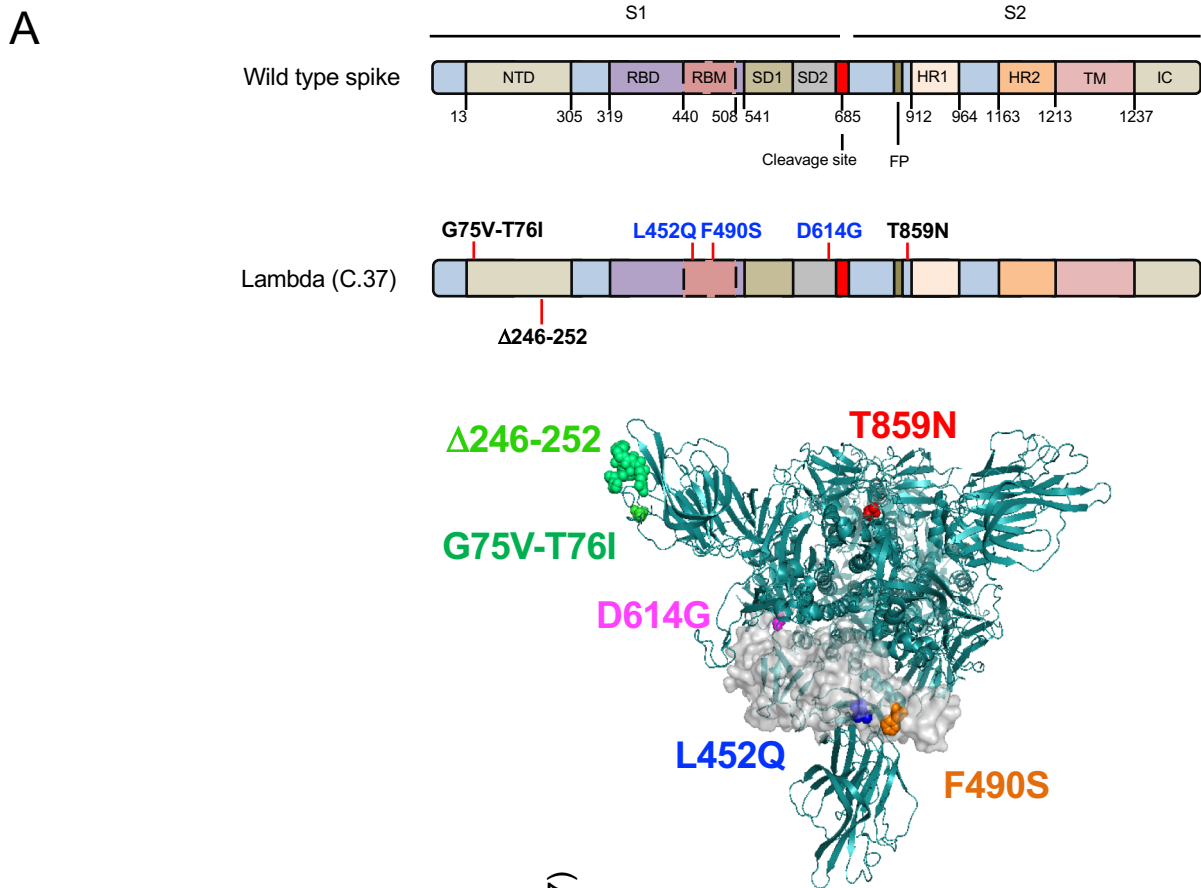
	IC50(ng/ml)		
	D614G	B.1.351	Lambda
REGN10933	3.3	420.4	2.9
REGN10987	9.3	16.4	33.7
REGN-CoV2	4.4	33.1	7.0

B



	IC50(ng/ml)				
	G75V-T76I	Δ246-252	L452Q	F490S	T859N
REGN10933	5.8	3.5	19.3	1.9	4.3
REGN10987	10.7	9.7	40.5	11.2	11.5
REGN-CoV2	5.6	4.5	9.9	0.2	5.5

Figure 2



Supplemental Figure 1