

The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity

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Outline

- Previous studies have observed that the SARS-CoV-2 spike protein is mutating and is highly glycosylated.
- 106 spike protein mutations were selected to be analyzed for infectivity and antigenicity.
- D614G mutations, alone or in combination with other amino acid changes, are significantly more infectious.
- Deletion of both N331 and N343 glycosylation drastically reduced viral infectivity.
- Mutations such as N234Q, L452R, and V483A were markedly resistant to neutralizing antibodies.

The SARS-CoV-2 spike protein has been mutating and is highly glycosylated

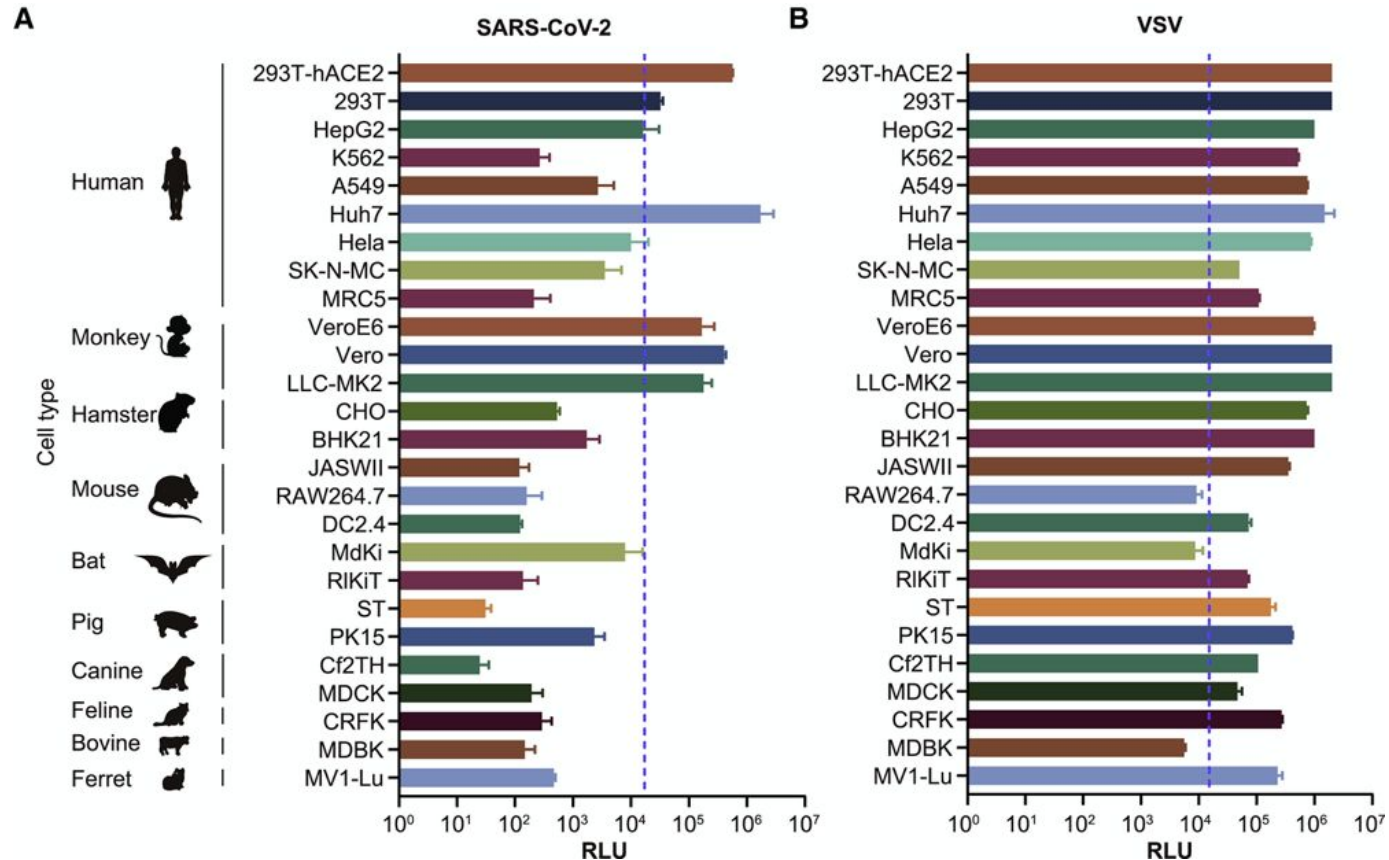
- Because SARS-CoV-2 enters target cells via the ACE2 receptor, the spike protein determines viral infectivity and transmissibility.
- Changes in the surface protein amino acids can impact viral function and interaction with the host's neutralizing antibodies.
- A previous study suggested that the D614G mutation could increase the fatality rate by altering S protein conformation and increasing infectivity.
- To gain a better understanding of natural variants, 106 S protein variants were analyzed for infectivity and reactivity to neutralizing antibodies.

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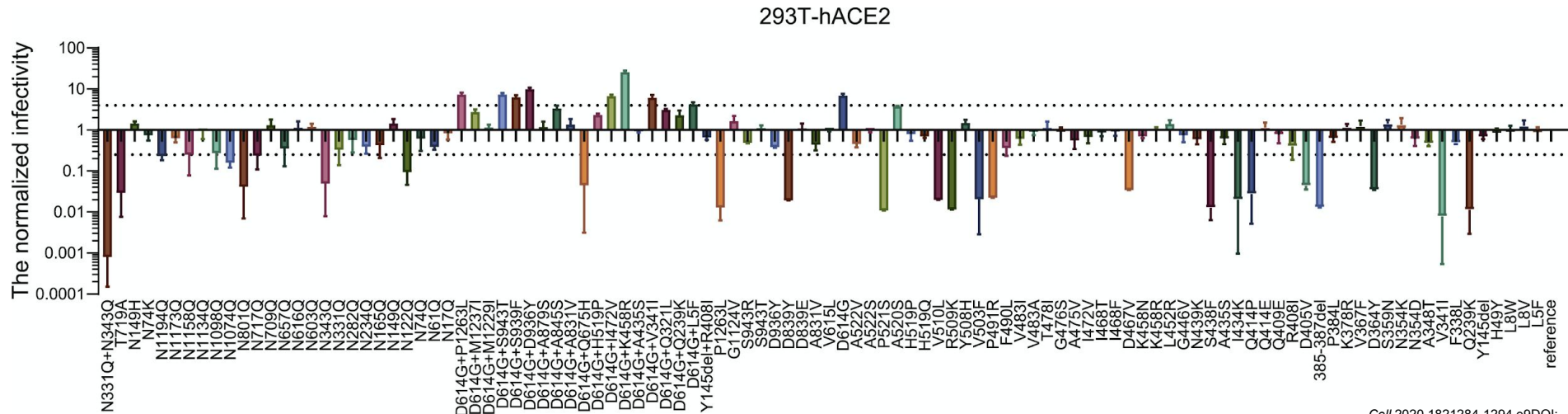
Fig. 2

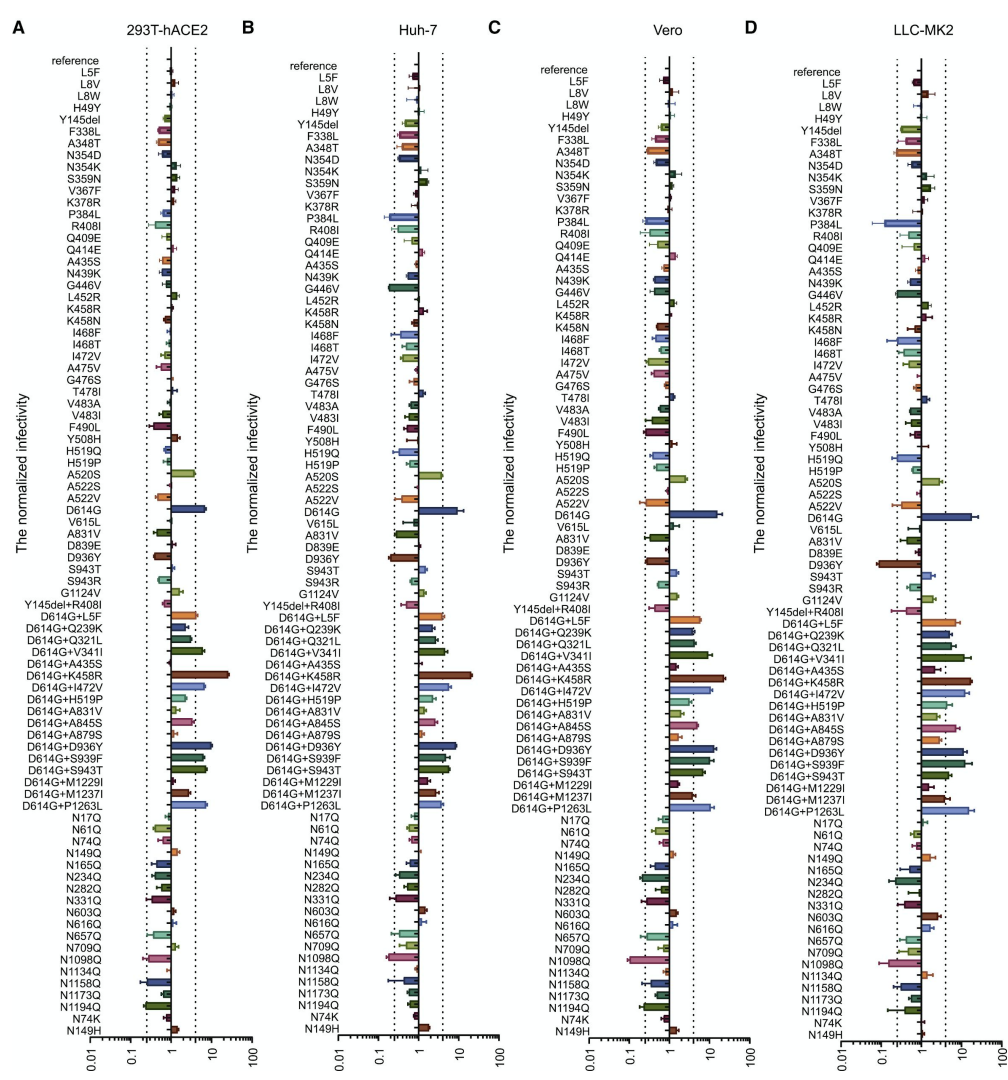
- A total of 26 different cell lines were infected with pseudotyped viruses with either SARS-CoV-2 S (A) or VSV G (B).
- The infected cell lysates were diluted by 10 \times and analyzed for luminescence activities (RLU).
- All results were obtained from three independent experiments (mean \pm SEM).
- When the RLU for the tested cell reached 1% of that for Huh-7, it was deemed as a permissive cell line.
- The dash line indicates the 1% RLU value of Huh-7 cell.



106 pseudotyped viruses show differing infectivity rates in the 293T-hACE2 cell line

- 24 strains were deemed low-infectivity, with RLU reading decreased by 4- to 100-fold, compared to the reference strain.
- Variant V341I and the investigative N331Q+N343Q glycosylation mutant showed no-infectivity, with over 100-fold decreases compared to the reference strain.





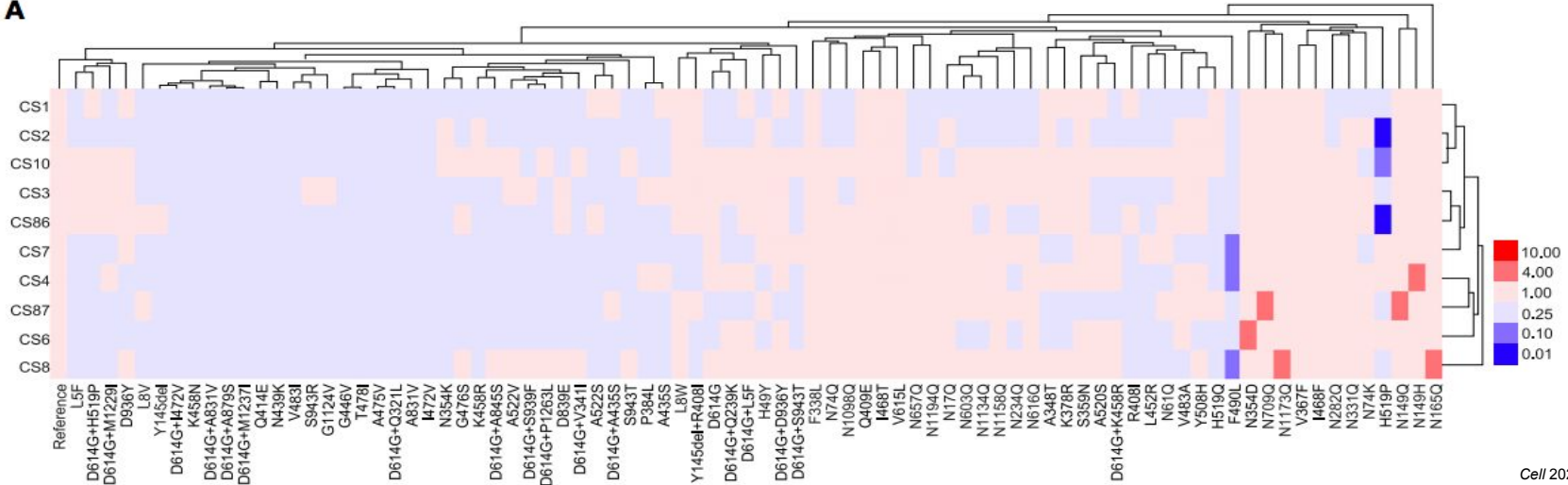
D614G mutations show significant increases in infectivity

- In all four cell lines, D614G variants showed increased infectivity compared to the reference strain.
- No difference was noted between single or combined variants, further indicating that D614G is responsible for the observed increase.

Differential Sensitivity of the Natural Variants and Experimental Mutants to a Panel of Convalescent Serum Samples

- Increase in neutralization activity in N165Q, N149H, N149Q, N1173Q, N709Q, N354D
- Major decrease in neutralization activity in H519P
- decrease in neutralization activity in F490L

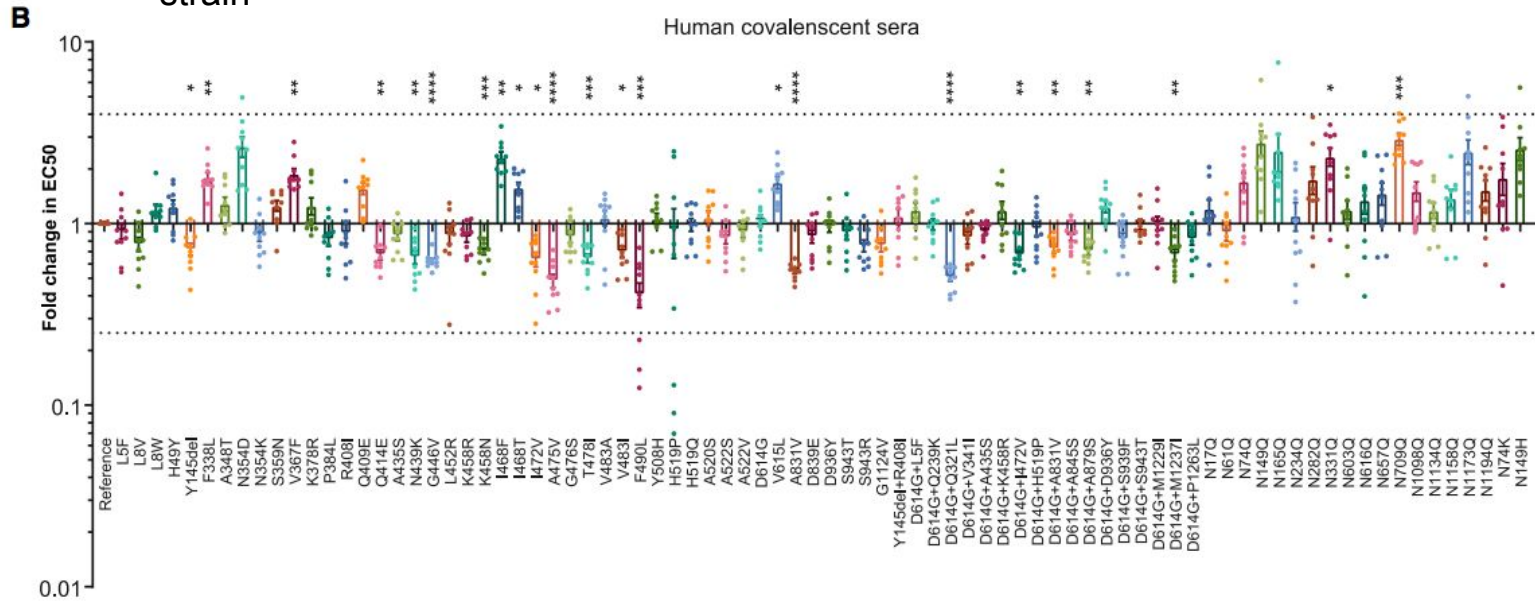
A



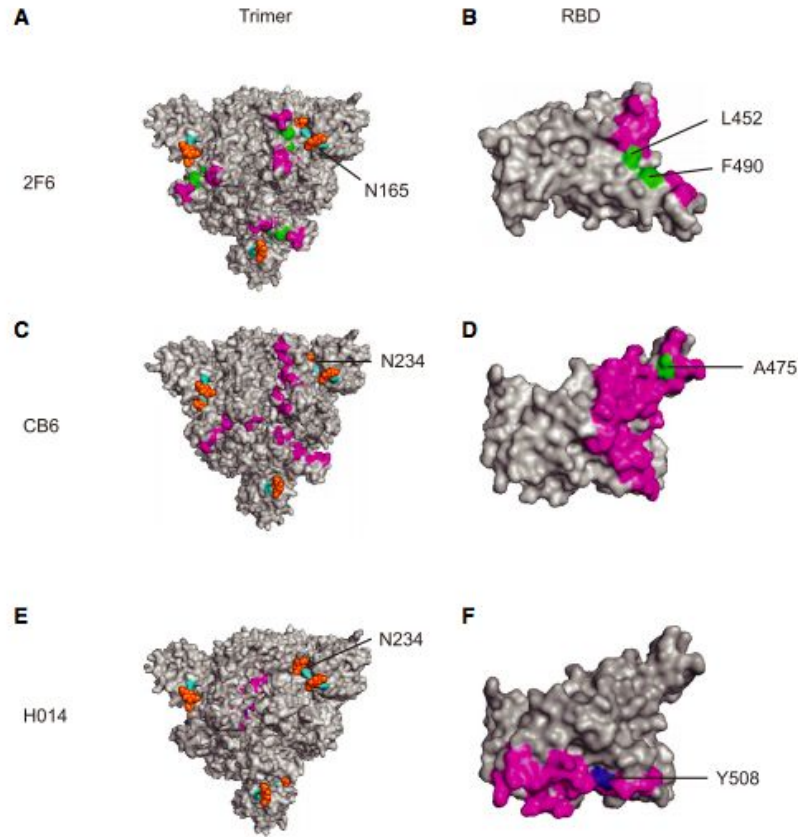
Summary of the data from ten serum samples, with the values presented as mean \pm SEM. The horizontal dashed lines indicate the threshold of 4-fold difference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$

- F338L, V367F, I468F, I468T, and V615L were even more sensitive to the convalescent sera compared with reference strain

- more variants were found to be resistant to the convalescent sera including single and double amino Acid Change



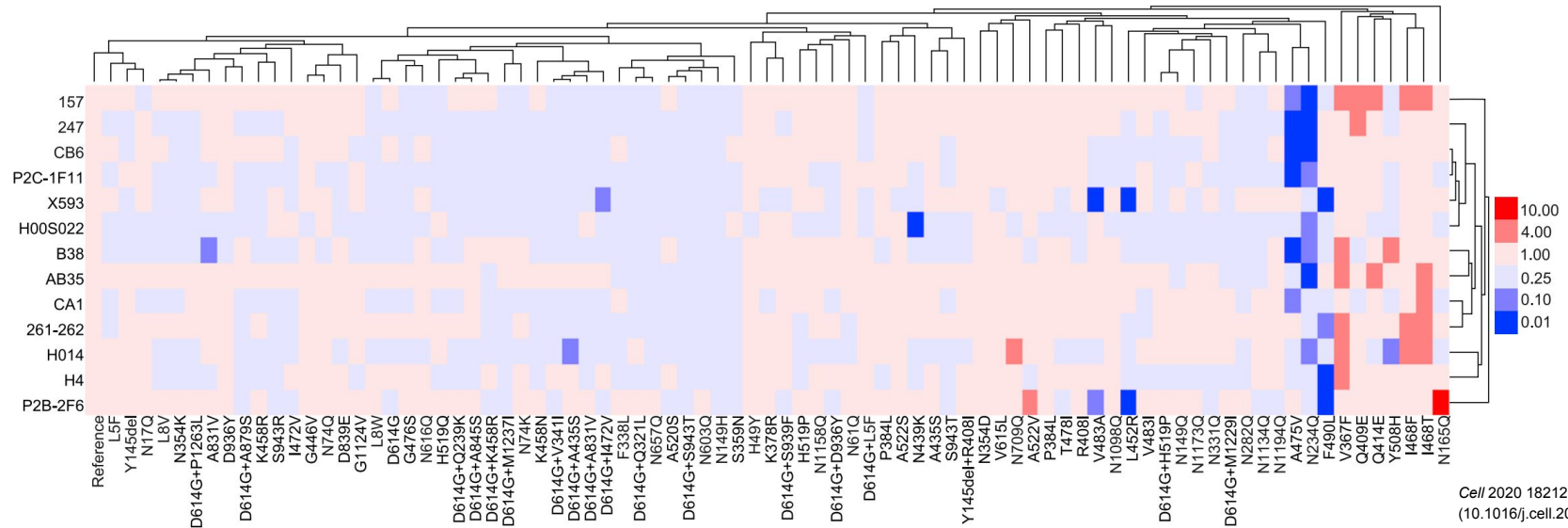
mAb Epitopes and Antigenicity Related Sites

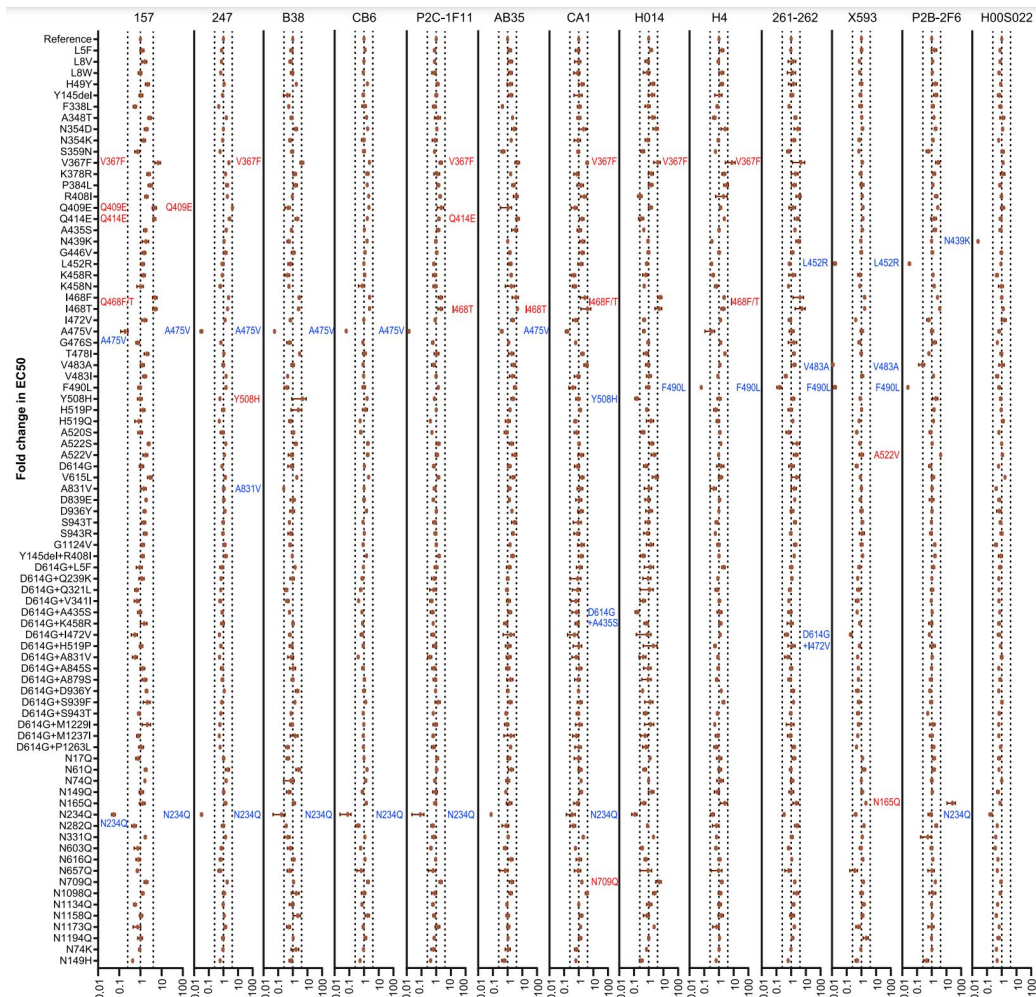


- A475V, L452R became resistant to some neutralizing antibodies
- N165Q became more sensitive
- N234Q was markedly resistant to neutralizing antibodies.
- F490L became resistant to some neutralizing antibodies

N165Q and N234Q were identified to be important binding sites for glycosylation

- A475V, N234Q, and F490L modifications all displayed less antibody effectiveness
- N165Q, I468T, I468F, V367F modifications all displayed increased antibody effectiveness





N165Q, V367F, A475V, and N234Q produced consistent results

- Sequences that had significant data did not vary substantially based on antibody.
- Sequences without significant data remained insignificant

N165Q H519P and F490L produced consistent results

- Both H519 and F490L were significantly less affected by antibodies
- Substantial variation among sequences that were affected more by antibodies

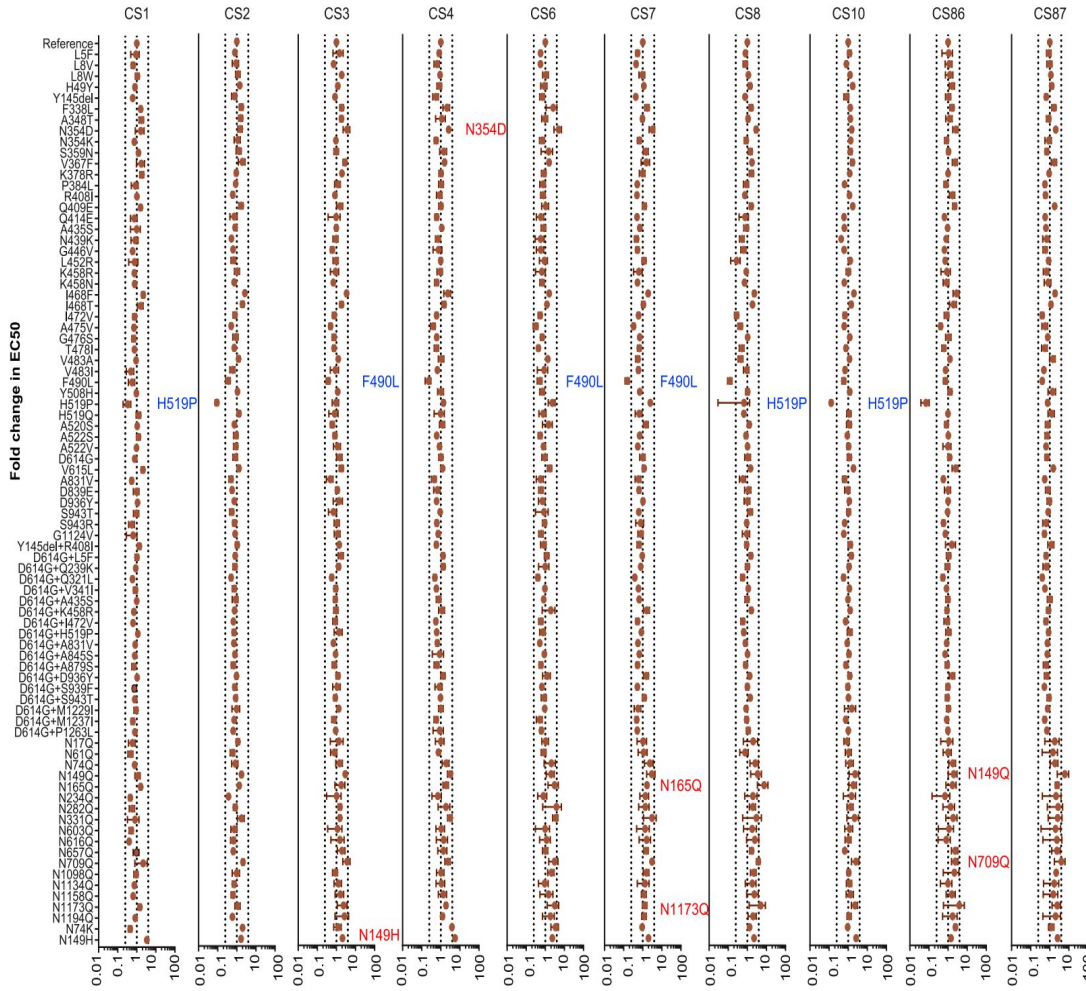


Table 1. Characteristics of Variants and Mutants

	Group A	Group B	Group C
Number of variants or mutants	29	51	26
Increased infectivity	D614G, D614G+L5F, D614G+D936Y, D614G+S939F, D614G+S943T	D614G+V341I, D614G+K458R, D614G+I472V ^a	none
Decreased infectivity	Q239K, D839Y, P1263L, D614G+Q675H	V341I, D364Y, 385-387del, D405V, Q414P, I434K, S438F, D467V, P491R, V503F, R509K, V510L, P521S	N122Q, N343Q, N717Q, T719A, N801Q, N1074Q, N331Q+N343Q
Increased sensitivity to neutralizing mAbs	none	V367F, Q409E, Q414E, I468F, I468T, Y508H, A522V	N165Q, N709Q
Decreased sensitivity to neutralizing mAbs	A831V	N439K, L452R, A475V, V483A, F490L, Y508H, D614G+A435S, D614G+I472V ^a	N234Q
Increased sensitivity to convalescent sera	V615L	F338L, V367F, I468F, I468T	N149H, N149Q, N165Q, N331Q, N354D, N709Q, N1173Q
Decreased sensitivity to convalescent sera	Y145del, A831V, D614G+A831V, D614G+A879S, D614G+M1237I	Q414E, N439K, G446V, K458N, I472V, A475V, T478I, V483I, F490L, H519P, D614G+Q321L, D614G+I472V ^a	none

^aD614G+I472V is the only variant with increased infectivity and decreased sensitivity to neutralizing mAb and convalescent sera. It is of note only one sequence is recorded in GISAID.

Variation infectivity and antigenicity can be used in vaccine and therapeutic antibody development

- Through analyzing 106 S protein variants, it was found that D614G is a particularly significant change, and has been rapidly accumulating.
- Most of the natural variants that affected sensitivity to neutralizing mAbs were located in the RBD region.
- The 13 mAbs used in the study appear to have different inhibitory effects on the variants.
 - It would be interesting to study the effects of a therapeutic regimen comprised of two or more mAbs.
- Glycosylation sites affect infectivity and antigenicity, indicating that glycan could impact SARS-CoV-2 replication and vaccine-induced immune response.

Summary

- Of 106 S protein variants, D614G mutations, alone or in combination with other amino acid changes, showed significantly higher infectivity.
- Deletion of both N331 and N343 glycosylation drastically reduced viral infectivity.
- Mutations such as N234Q, L452R, and V483A were markedly resistant to neutralizing antibodies.
- Glycosylation sites affect infectivity and sensitivity to neutralizing antibodies, indicating that glycan could impact SARS-CoV-2 replication and vaccine-induced immune response.

References

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Acknowledgements

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