Neutralization of Mu and C.1.2 SARS-CoV-2 Variants by Vaccine-elicited Antibodies in Individuals With and Without Previous History of Infection

Takuya Tada[1†], Hao Zhou[1†], Belinda M. Dcosta[1†], Marie I. Samanovic[2], Amber Cornelius[2], Ramin S. Herati[2], Mark J. Mulligan[2], and Nathaniel R. Landau[1*]

Affiliation:

[1]Department of Microbiology, NYU Grossman School of Medicine, New York, NY, USA.

[2]NYU Langone Vaccine Center and Department of Medicine, NYU Grossman School of Medicine, New York, NY, USA.

[†]Contributed equally to this study

*Corresponding author:

Nathaniel R. Landau, Ph.D.

NYU Grossman School of Medicine

430 East 29th Street, Alexandria West Building, Rm 509, New York, NY 10016

Email: nathaniel.landau@med.nyu.edu

Phone: (212) 263-9197

Abstract

Recently identified SARS-CoV-2 variants Mu and C.1.2 have mutations in the receptor binding domain and N- and C-terminal domains that might confer resistance to natural and vaccine-elicited antibody. Analysis with pseudotyped lentiviruses showed that viruses with the Mu and C.1.2 spike proteins were partially resistant to neutralization by antibodies in convalescent sera and those elicited by mRNA and adenoviral vector-based vaccine-elicited antibodies. Virus with the C.1.2 variant spike, which is heavily mutated, was more neutralization-resistant than that of any of variants of concern. The resistance of the C.1.2 spike was caused by a combination of the RBD mutations N501Y, Y449H and E484K and the NTD mutations. Although Mu and C.1.2 were partially resistant to neutralizing antibody, neutralizing titers elicited by mRNA vaccination remained above what is found in convalescent sera and thus are likely to remain protective against severe disease. The neutralizing titers of sera from infection-experienced BNT162b2-vaccinated individuals, those with a history of previous SARS-CoV-2 infection, were as much as 15-fold higher than those of vaccinated individuals without previous infection and effectively neutralized all of the variants. The findings demonstrate that individuals can raise a broadly neutralizing humoral response by generating a polyclonal response to multiple spike protein epitopes that should protect against current and future variants.

Introduction

SARS-CoV-2 isolates have been classified by the World Health Organization (WHO) as variants of concern (VOC; Alpha (B.1.1.7), Beta (B.1.351), Gamma (B.1.1.248) and Delta (B.1.617.2) and variants of interest (VOI) that include Lambda (C.37)) and newly classified Mu (B.1.621)[1]. In addition, a yet unclassified C.1.2 variant was identified in South Africa[2] that appears to be increasing in prevalence and spreading to neighboring countries and a variant termed Delta+N501S was identified in Japan, currently at low frequency. Mu[3] and C.1.2[2,4] have mutations in the receptor binding domain (RBD) of the spike protein that could contribute to increased transmissibility and cause resistance to neutralization by convalescent sera and vaccine-elicited and therapeutic monoclonal antibodies.

In this study, we measured the infectivity of viruses with the Mu, C.1.2 and Delta+N501S spike proteins and determined their susceptibility to neutralization by convalescent and vaccine-elicited antibodies, both in unexperienced and experienced individuals. We also tested their neutralization by therapeutic monoclonal antibodies. Viruses with the variant spikes were partially resistant to neutralization. The C.1.2 variant, which is highly mutated, was the most resistant. Sera from experienced patients vaccinated with BNT162b2 had very high neutralizing titer against all of the variants, providing a strong rationale for the vaccination of previously infected individuals.

Results

Prevalence and infectivity of Mu, C.1.2 and Delta+N501S variants.

As of October 2021, the prevalence of the Mu was highest in the British Virgin Islands and Colombia where its accounts for 64% and 43% of sequenced cases **(Figure 1A)**. It is present at low frequency in the Central and South America. The virus has also been found in the United States and Europe although frequencies have not yet been accurately determined. C.1.2 is present with a prevalence of 5% in Swaziland, 1% in South Africa and small numbers of cases have been sequenced in as many as 10 other countries **(Figure 1A)**. In addition, a variant of Delta was recently identified in a handful of cases, termed here Delta+N501S, and has not yet been further characterized.

The variants have unique mutations in the RBD and NTD **(Figure 1B and S1A)**. The Mu spike has RBD mutations R346K, E484K and N501Y**;** C.1.2 has Y449H, E484K and N501Y; and Delta+N501S has L452R, T478K **(Figure 1B and S1A)**. To evaluate the function and sensitivity of the variant spikes to antibody neutralization, we generated lentiviruses pseudotyped with the Mu, C.1.2 and Delta+N501S spike proteins and, in addition, a pseudotype with the C.1.2 RBD mutations (Y449H, E484K, N501Y) and pseudotypes with the individual RBD mutations of each variant spike. The variant spike proteins were similarly expressed and proteolytically processed in transfected cells and were incorporated into lentiviral virions at a level similar to that of the parental D614G spike protein **(Figure S1B)**.

Analysis of the infectivity of viruses with the variant spike proteins on ACE2.293T and ACE2.A549 cells showed a slight decrease for the Beta spike compared to D614G (1.8-fold) on ACE2.293T cells while Delta, Delta+N501S and Mu were slightly increased **(Figure 1C)**. The pattern of infectivity was similar on ACE2.A549 cells, except that the infectivity differences were somewhat great, most likely due to the low level of ACE2 on these cells. Analysis of the individual point mutations **(Figure S1C)** showed the individual Beta and Delta RBD mutations (R346K, Y449H, E484K, N501Y, N501S) did not significantly increase infectivity. A spike protein with the NTD C.1.2 mutations (P9L-C136F-Δ144-190S-D215G-Δ242-243) also had wild-type infectivity while a spike with the C.1.2 RBD mutations had a significant increase in infectivity (1.9-fold). A spike containing the CTD mutations (H655Y, N679K, T716I, T859) of C.1.2 was decreased 15-fold. Similar infectivity ratios were obtained on ACE2.A549 cells.

Neutralization of variants by convalescent and vaccine-elicited antibodies.

To determine the susceptibility of the viruses with the variant spike proteins to antibody neutralization, we analyzed the neutralizing titers of serum antibodies elicited by the BNT162b2 and mRNA-1273 mRNA vaccines and the Ad26.COV2.S adenoviral vector-based vaccine on the variants. The vaccine sera analyzed were collected from individuals at similar time-points post-final injection, (a mean of 90 days for BNT162b2, 80 for mRNA-1273 and 82 for Ad26.COV2.S; **Table S1**) and all participants tested negative for antibodies against the SARS-CoV-2 N protein suggesting no history of SARS-CoV-2 infection **(Table S1)**. Convalescent sera neutralized D614G spike with a mean titer of 334. Neutralization of Beta, Delta, Delta+ and Mu variants showed a modest 4-9-fold decrease

in neutralizing titer while C.1.2 was more resistant to neutralization with a 9-fold decrease **(Figure 2A)**. BNT162b2 sera neutralized virus with the D614G spike with a mean titer of 862, a 2.6-fold increase compared to convalescent sera. The neutralizing titers against Beta, Delta and Delta+N501S were decreased 4.8-, 3.4- and 3.4-fold, respectively. Mu and C.1.2 were somewhat more resistant with a 6.8 and 7.9-fold decrease in titer respectively. mRNA-1273 vaccinated sera showed a similar pattern of neutralization with C.1.2 being the most resistant (11.2-fold decreased titer). Neutralizing antibody titers of sera from Ad26.COV2.S-immunized individuals neutralized D614G with an average titer of 245 and showed a similar pattern of variant neutralization. Titers against C.1.2 fell into a range below 50, the minimum detectable by the assay **(Figure 2B).** Presentation of the data grouped by variant shows decreased neutralizing titers against the variants by sera of the Ad26.COV2.S-vaccinated individuals **(Figure 2C)**. Analysis of the spike proteins with individual variant mutations showed that the neutralization resistance of Mu was caused by R346K and E484K while resistance of C.1.2 was caused by E484K, Y449H and the NTD (P9L-C136F-Δ144-190S-D215G-Δ242-243) **(Figure 2D)**.

Analysis of neutralization by the sera of donors who had a history of COVID-19 pre-BNT162b2 vaccination showed an overall higher neutralizing titer against all of the variants. The neutralizing titer of sera from unexperienced donors against D614G was 1087 on average **(Figure 2E)** with Beta, Delta, Delta+N501S, Mu and C.1.2 having a 1.4-14-fold decrease in titer. In contrast, experienced-vaccinated donor sera were significantly increased in titer against D614G (2.8-fold) and the titers remained high for all of the variants **(Figure 2E)**. After two doses of vaccination, infection-experienced donors

had a 10.6-fold increase in neutralizing titer against the Beta variant compared to unexperienced donors. Titers were increased 3.7-fold for Delta and Delta+N501S. Overall, the neutralization titers of sera from experienced donors were 8.5-8.9-fold greater against Mu and C.1.2 variants compared with unexperienced individuals. (**Table S2**)

Variant spike avidity for ACE2.

To measure the ACE2 binding avidity of the variant spikes, we established an ACE2 avidity assay in which the variant spike proteins were expressed in 293T cells and then incubated with a serially diluted soluble ACE2:nanoluciferase fusion protein (sACE2-Nluc) **(Figure 3A)**. Similar cell surface spike protein expression levels on the transfected 293T cells was confirmed by flow cytometry (not shown). The analysis showed increased ACE2 binding affinity of the Beta, Delta, Delta+N501S and Mu spikes (2.2-, 1.7-, 1.4-, 1.7-fold, respectively) as indicated by a decrease in the concentration required to achieve 50% occupancy of the spike protein. In contrast, C.1.2 bound ACE2 with decreased affinity, requiring 1.4-fold higher concentration of ACE2 for 50% binding compared to D614G and 2.4-fold decrease as compared to the high affinity Beta variant spike protein **(Figure 3B)**. Analysis of the point-mutated spike proteins showed that the increased affinity of Beta, Delta, Mu with ACE2 was attributed to N501Y and L452R **(Figure 3B)**, consistent with previous studies[6]. The decreased affinity C.1.2 for ACE2 was due to the combination of the Y449H in the RBD and the mutated NTD. These results were confirmed in a virion binding assay in which pseudotyped virions were incubated with sACE2 and then added to ACE2.293T cells and the amount of bound virions was then measured **(Figure S2)**. In this assay, virions with D614G, Beta, Delta, Delta+N501S, C.1.2 (RBD) and Mu spikes

bound similarly to ACE2 while C.1.2 binding was decreased. These findings suggest that the C.1.2 spike protein binds ACE2 with a relatively lower affinity than the other spike protein variants.

SARS-CoV-2 spike protein mediated cell:cell fusion.

To test the ability of the variant spike proteins to mediate the fusion reaction upon ACE2 binding, we established an assay for SARS-CoV-2 spike-mediated cell:cell fusion. The assay is based on the alpha-complementation of beta galactosidase strategy that we previously established for the analysis of HIV-1 envelope glycoprotein-mediated fusion[5]. Cells expressing the SARS-CoV-2 spike protein and α peptide were mixed with cells expressing ACE2 and ω fragment **(Figure S3)** and β-galactosidase activity was measured between 0-5 hours using a luminescent substrate. The results showed that fusion activity could be detected as early as 30 minutes post-mixing and reached a near maximum by 4 hours (>$1x10^6$ cps). Analysis of the variant spike proteins with this assay showed that Delta, Delta+N501S, Mu and C.1.2 increased fusion activity compared to D614G **(Figure. 3C)**. Although the fusion activity was same between C.1.2 (RBD) and D614G, C.1.2. (CTD) and C.1.2 (NTD) were higher than D614G, suggesting that the mutations in NTD (P9L-C136F-Δ144-190S-D215G-Δ242-243) and CTD (H655Y, N679K, T716I, T859) affect fusion activity **(Figure 3C)**.

Therapeutic antibodies neutralize Mu and C.1.2.

Regeneron monoclonal antibodies maintained their ability to neutralize Delta, Delta+N501S, Mu and C.1.2. REGN10933 lost titer (50-fold) against virus with the Beta

179 spike **(Figure S4A)**, as previously reported[6-8] but maintained neutralizing activity against
180 the others while REGN10987 maintained activity against all variants **(Figure S4B)** and
181 the combination of the two mAbs was highly active against all of the variants **(Figure**
182 **S4C)**.
183
184

Discussion

Virus with the newly described Mu and C.1.2 spike proteins were partially resistant to neutralization by antibodies in convalescent sera and to those elicited by mRNA and adenoviral vector-based vaccine-elicited antibodies. The C.1.2 variant spike, which is heavily mutated, was the most neutralization-resistant of the variant spike proteins tested here and was more resistant than those on which we have previously reported[6]. The resistance of the C.1.2 spike was caused by a combination of the RBD mutations N501Y, Y449H and E484K and the NTD mutations.

Mathematical modeling by Khoury *et al.* predicts that 50% protection from SARS-CoV-2 infection is provided by a titer that is 20% that of the convalescent titer[9]. In this study, mean convalescent titer was 334 (**Table S1**), indicating that 50% protection would correspond to an IC50 of 67. The titer required to protect against severe disease is predicted to be 3% that of the mean titer of convalescent sera, corresponding to a titer of 10 in this study, suggesting that vaccination should remain protective against severe disease resulting from infection with Mu or C.1.2.

Interestingly, the neutralizing titers of sera from infection-experienced BNT162b2-vaccinated individuals, those with a history of previous SARS-CoV-2 infection, were on average 6.4-fold higher than those of vaccinated individuals without previous infection and effectively neutralized all of the variants. The findings demonstrate that individuals can raise a broadly neutralizing humoral response[10,11], presumably by generating a polyclonal response to multiple spike protein epitopes, that will protect against current and most likely, future variants. In addition, Regeneron therapeutic monoclonal antibodies retained their ability to neutralize Mu and C.1.2 variants.

An unexplained finding in our analysis regards the effect of the T716I mutation in the C.1.2 spike. The mutation in the C.1.2 spike caused an 18-fold decrease in infectivity which was alleviated when the mutation was taken out and replaced with a threonine at position 716 (**Figure S1D**). The mutation is also present in the Alpha variant spike protein where it similarly caused a marked decrease in the infectivity[6]. To ensure that the mutation did not affect the findings of our study, the C.1.2 spike protein used here contained all of the mutations except T716I. The reason for decreased infectivity of the fully mutated C.1.2 spike is unclear. It may be a result of producing the spike protein in 293T cells perhaps caused by the proximity of T716 to a potential glycosylation site at position 717. The T716I mutation had no effect on expression of the protein in transfected cells, packaging into virions or ACE2 avidity. The mutation also had no effects on antibody neutralization profile (data not shown). Thus, the T716I mutation did not influence the results but this effect should be considered in studies with pseudotypes using spike proteins with this mutation.

It is interesting that the C.1.2 spike protein has a decreased affinity for ACE2 compared to that of the other variant spikes. Its RBD has the N501Y mutation that confers increased ACE2 affinity[12,13] but this is counteracted by the novel Y449H mutation that decreases ACE2 affinity. The decreased ACE2 affinity is unexpected as it is thought the virus is mutating to increase ACE2 affinity and thereby increase transmissibility. This would suggest that Y449H was selected as an antibody escape mutation, a finding consistent with the increased resistance to antibody neutralization indicated in our data. The

231 decrease in ACE2 affinity of the C.1.2 spike protein may cause a decrease in
232 transmissibility, and thereby limit spread of the virus, despite its relative resistance to
233 antibody neutralization. It suggests that as the virus is selected to escape the humoral
234 immune response, it becomes less fit, unable to evolve a highly transmissible,
235 neutralization resistant variant.
236
237 The Mu variant does not appear to present any additional concerns over Delta with which
238 it is nearly identical. The C.1.2 variant is currently at low prevalence and has a restricted
239 geographic distribution but given the large number of NTD mutations coupled with RBD
240 and CTD mutations and relative neutralization resistance, the spread of the variant should
241 be closely monitored. The high titers of antibody against all of the variants in experienced
242 patients is encouraging because it demonstrates the ability of individuals to mount a
243 broadly neutralizing antibody response that will may be impervious to current and future
244 variants. By extrapolation, the finding suggests that vaccine booster immunization might
245 result in a similarly broad antibody response.
246
247 **Acknowledgements**
248 The work was funded by grants from the NIH to N.R.L. (DA046100, AI122390 and
249 AI120898) and to M.J.M. (UM1AI148574).
250
251 **Author contributions**
252 T.T., H.Z. and N.R.L. designed the experiments. T.T., H.Z. and B.M.D. carried out the
253 experiments and analyzed data. T.T., H.Z., B.M.D. and N.R.L. wrote the manuscript.

M.I.S., A.C., R.H. and M.J.M supervised specimen selection and the collection of clinical information, did the ELISAs and provided reagents and key insights. All authors provided critical comments on manuscript.

Declaration of Interests.

The authors declare no competing interests except M.J.M. who received research grants from Lilly, Pfizer, and Sanofi, and serves on advisory boards for Pfizer, Merck, and Meissa Vaccines.

Figure legends

Figure 1. Mu (B.1.621), C.1.2 and Delta+501S variant prevalence and spike protein mutations

(A) The global prevalence of Mu and C.1.2 variants is shown for countries with the highest prevalence or cases (extracted from https://outbreak.info/).

(B) Mutations in Mu, C.1.2 and Delta+N501S variant spikes are shown on the three-dimensional spike protein structure. A single RBD in each is shown in gray (side view). The PDB file of spike protein (7BNM)[15] was downloaded from the Protein Data Bank. 3D view of protein was obtained using PyMOL.

(C) The infectivity of Beta, Delta, Delta+N501S, Mu, C.1.2 variant spikes pseudotyped lentiviruses on ACE2.293T and ACE2.A549 cells is shown. The viruses were normalized for RT activity and measured in triplicate with error bars that indicate the standard deviation. The experiment was done three times with similar results.

Figure 2. Neutralization of variant spike pseudotyped viruses by convalescent sera, antibodies elicited by RNA and adenoviral vector vaccines.

(A) Neutralization of pseudotyped viruses with D614G, Beta, Delta, Delta+N501S, Mu, C.1.2 variant spikes by convalescent serum samples from 8 donors was tested. The serum was collected at 32-57 days after infection. Each dot represents the IC50 for a single donor. Neutralization titers of variants were compared with that of D614G.

(B-C) Neutralizing titers of serum samples from BNT162b2 vaccinated individuals (n=9), mRNA-1273 vaccinated donors (n=8), Ad26.COV2.S vaccinated individuals (n=10) was

measured. Sera were collected at 90, 80, 82 days on average post-last immunization. IC50 of neutralization of virus from individual donors are shown. Significance was based on two-sided testing.

(D) Neutralization titers of viruses with single point mutations by antibodies elicited by BNT162b2. Neutralizing titers of serum samples from BNT162b2 vaccinated individuals (n=5). Aera were collected 7 days post-second immunization. Each dot represents the IC50 for a single donor.

(E) Neutralizing titers of serum samples from BNT162b2 vaccinated individuals with (n=5) or without previous SARS-CoV-2 experience (n=12) was measured. The neutralization IC50 of virus from individual donors is shown. The sera were collected 7 days post-second immunization. Significance between variants and D614G was determined by student-t test or Nonparametric ANOVA test. (*P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001). The experiment was done twice with similar results.

Figure 3. Binding and fusion of variant spikes to ACE2.

(A) The diagram shows the principle of the ACE2 avidity assay in which 293T cells transfected with variant spike protein expression vector are incubated with serially diluted sACE2-nluc protein. Following a 30-minute incubation, the unbound fusion protein is removed and the bound protein measured by luciferase assay. (B) ACE2 avidity of the indicated variant spike proteins is shown as curves with maximal binding defined as luciferase activity upon binding of the ACE2.nLuc fusion protein at 50 mg.ml set to 100% (left two panels). The histogram on the right shows 50% of maximal binding. (C) Cell:cell

fusion kinetics of the variant spike proteins is shown as measured in an α-complementation assay. The experiment was done twice with similar results.

Supplementary Figure 1.

Infectivity of variant spike viruses and spike expression levels.

(A) The domain structure of the SARS-CoV-2 spikes of Mu (B.1.621), C.1.2 and Delta+N501S is diagrammed. NTD, N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; SD1 subdomain 1; SD2, subdomain 2; HR1, heptad repeat 1; HR2, heptad repeat 2; TM, transmembrane region; IC, intracellular domain.

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

(C) Infectivity of virus pseudotyped by Beta, Delta, Delta+N501S, Mu, C.1.2 variant individual spikes pseudotyped lentivirus in ACE2.293T and ACE2.A549 cells.

(D) Infectivity of virus pseudotyped by C.1.2 (full), C.1.2. (-T716I) variant spikes and T716I variant individual spike pseudotyped lentivirus in ACE2.293T cells. The experiment was done twice with similar results.

Supplementary Figure 2.

Neutralization of spike protein variants by sACE2.

Serially diluted sACE2 was mixed with variant pseudotypes (D614G, Mu, C.1.2) (left) and single point mutated virus (N501Y, N501S, R346K, Y449H) (right) for 30 minutes. The mixture was added on ACE2.293T cells. After 2 days of infection, luciferase activity was measured. The experiment was done three times with similar results.

Supplementary Figure 3.

α-Complementation cell:cell fusion assay.

(A) The assay is based on the enzymatically inactive β-galactosidase α peptide (red) and C-terminal ω fragment (yellow). The fragments are expressed separately in transfected cells. Upon mixing of cells separately expressing a spike protein and ACE2, enzymatically active β-galactosidase tetramers are formed. Effector 293T cells that express alpha-N85 and SARS-CoV-2-Δ19 spike were incubated with target 293T cells that express ω and ACE2. β-galactosidase activity was measured after 4 and 20 hours of incubation. (B) 293T cells expressing alpha and spike were mixed with target cells. After 4 and 20 hours of incubation, β-galactosidase activity was measured. The data are displayed as the mean ± SD and significance as calculated in the student-t test.

Supplementary Figure 4.

Neutralization of spike protein variants by monoclonal antibodies REGN10933 and REGN10987.

(A-C) Neutralization of D614G, Beta, Delta, Delta+N501S, Mu, C.1.2 variant spikes by REGN10933 and REGN10987. Neutralization of viruses by REGN10933 (A), REGN10987 (B), and 1:1 mixture of REGN10933 and REGN10987 (C) was measured.

352 The table shows the calculated IC50 for each curve. The experiment was done three
353 times with similar results.

354 **Methods**

355 **Plasmids**

356 Plasmids used in the production of lentiviral pseudotyped virus have been previously
357 described[14]. Mutations were introduced into pcCOV2.Δ19.D614G by overlap extension
358 PCR and confirmed by DNA sequencing.

359

360 **Human sera and monoclonal antibodies**

361 Convalescent sera were collected 32-57 days post-symptom onset. BNT162b2-
362 vaccinated sera were collected 90 days (mean) post-second immunization and mRNA-
363 1273-vaccinated sera were collected 80 (mean) days post-second immunization.
364 Ad26.COV2.S-vaccinated sera were collected 82 days (mean) post-immunization.
365 COVID-19 experienced serum samples were collected 7 days post-second immunization
366 with BNT162b2. Participants reported experiencing COVID symptoms were confirmed
367 COVID-19-experienced by direct PCR or antibody testing. The clinical study was
368 conducted at the NYU Vaccine Center with participant's written consent under IRB-
369 approved protocols (18-02035 and 18-02037). sACE2 was generated as previously
370 described[14].

371

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

373 Lentivirus pseudotyped by variant SARS-CoV-2 spikes were produced as previously
374 reported[14]. Viruses were concentrated by ultracentrifugation and normalized for reverse
375 transcriptase (RT) activity. Sera and monoclonal antibodies were serially diluted and then
376 incubated with pseudotyped virus (approximately 2.5 X 10^7 cps) for 30 minutes at room

377 temperature and then added to target cells. Luciferase activity was measured 2 days post-
378 infection.
379
380 **Binding assay**
381 293T cells were transfected with mutated spike variant expression vectors using
382 lipofectamine 2000 and seeded in a 96-well plate at 1 x 10^4 / well. Serially diluted sACE2
383 protein fused with nano-Luciferase was added to the cells. Following incubation for 30
384 minutes at 37^{o}C, the unbound proteins were washed and luciferase activity was
385 measured using Nano-Glo substrate (Nanolight) in an Envision 2103 microplate
386 luminometer (PerkinElmer).
387
388 **Neutralization assay by soluble ACE2**
389 Briefly, pseudotyped virus was incubated with serially diluted recombinant soluble ACE2
390 protein for 1 hour at room temperature and subsequently added to 1 X 10^4 ACE2.293T
391 cells. After 2 days, the cell medium was removed and 50 μl Nano-Glo luciferase substrate
392 (Nanolight) was added. The luminescence signal was read in an Envision 2103 microplate
393 luminometer.
394
395 **α-complementation assay**
396 293T (4 X 10^6) were cotransfected with pcΔ19S and 5 μg pSCTZ-alpha N85 or variable
397 amounts of pLenti.ACE2-HA and 5 μg of pSCTZ-omega by lipofection with lipofectamine
398 2000 (Invitrogen). After 24 hours, the medium was changed and the following day, the
399 transfected cells were collected with PBS/5 mM EDTA. Spike protein+alpha-N85

transfected cells (2 X 10^5) were incubated with patient serum or ACE2 microbody for 30 min at room temperature and then mixed with an equal number of ACE2+omega transfected cells in a volume of 100 μl in a 96-well culture plate. After 4 hours, β-galactosidase activity was measured using the Galacto-Light Plus β-Galactosidase Reporter Gene Assay System (Thermo Fisher). The cells were lysed in 100 μl Tropix Lysis Buffer for 10 min at room temperature and then 20 μl of the lysate was mixed with 70 μl Galacto-Plus substrate diluted 1:100 in Tropix Galacto Reaction Buffer Diluent. After incubation for 30 min at room temperature, 100 μl of Tropix Accelerator II was added and the luminescence was read in an Envision 2103 microplate luminometer (PerkinElmer). For β-Galactosidase detection with FDG, the cells were lysed in 100 μl of buffer containing 10 mM Tris pH 8.0, 150 mM NaCl and 0.1% triton X-100. After 5 min, 10 μl of the lysate was mixed with 100 μl of Tropix Galacto Reaction Buffer Diluent and 10 μl of 2 mM FDG (Thermo Fisher) in 50% DMSO. The reactions were incubated for 30 min in the dark after which luminescence was visualized by illumination with 365 nm UV light or on an iBright gel documentation instrument (Thermo Fisher).

Immunoblot analysis

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

Statistical Analysis

All experiments were performed in technical duplicates or triplicates and the data were analyzed using GraphPad Prism 8. Statistical significance was determined by the two-tailed unpaired t-test or Nonparametric ANOVA test. Significance was based on two-sided testing and attributed to $p < 0.05$. Confidence intervals are shown as the mean ± SD or SEM (*P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001).

References

1. WHO. 2021:https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/. (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/).
2. Scheepers C, Everatt J, Amoako DG, et al. Emergence and phenotypic characterization of C.1.2, a globally detected lineage that rapidly accumulated mutations of concern. medRxiv 2021:2021.08.20.21262342. DOI: 10.1101/2021.08.20.21262342.
3. outbreak.info. Archived from the original on September 1, 2021. "Mu Lineage Report" 2021.
4. outbreak.info. “C.1.2 Lineage Report". Archived from the original, 2021 2021.
5. Holland AU, Munk C, Lucero GR, Nguyen LD, Landau NR. Alpha-complementation assay for HIV envelope glycoprotein-mediated fusion. Virology 2004;319(2):343-52. (Research Support, Non-U.S. Gov't

Research Support, U.S. Gov't, P.H.S.) (In eng). DOI: 10.1016/j.virol.2003.11.012.

6. Tada T, Dcosta BM, Samanovic MI, et al. Convalescent-Phase Sera and Vaccine-Elicited Antibodies Largely Maintain Neutralizing Titer against Global SARS-CoV-2 Variant Spikes. mBio 2021;12(3):e0069621. DOI: 10.1128/mBio.00696-21.
7. Wang P, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 2021. DOI: 10.1038/s41586-021-03398-2.
8. Chen RE, Winkler ES, Case JB, et al. In vivo monoclonal antibody efficacy against SARS-CoV-2 variant strains. Nature 2021;596(7870):103-108. DOI: 10.1038/s41586-021-03720-y.

9. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nature Medicine 2021;27(7):1205-1211. DOI: 10.1038/s41591-021-01377-8.

10. Wu J, Liang B, Chen C, et al. SARS-CoV-2 infection induces sustained humoral immune responses in convalescent patients following symptomatic COVID-19. Nature Communications 2021;12(1):1813. DOI: 10.1038/s41467-021-22034-1.

11. Robbiani DF, Gaebler C, Muecksch F, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature 2020;584(7821):437-442. DOI: 10.1038/s41586-020-2456-9.

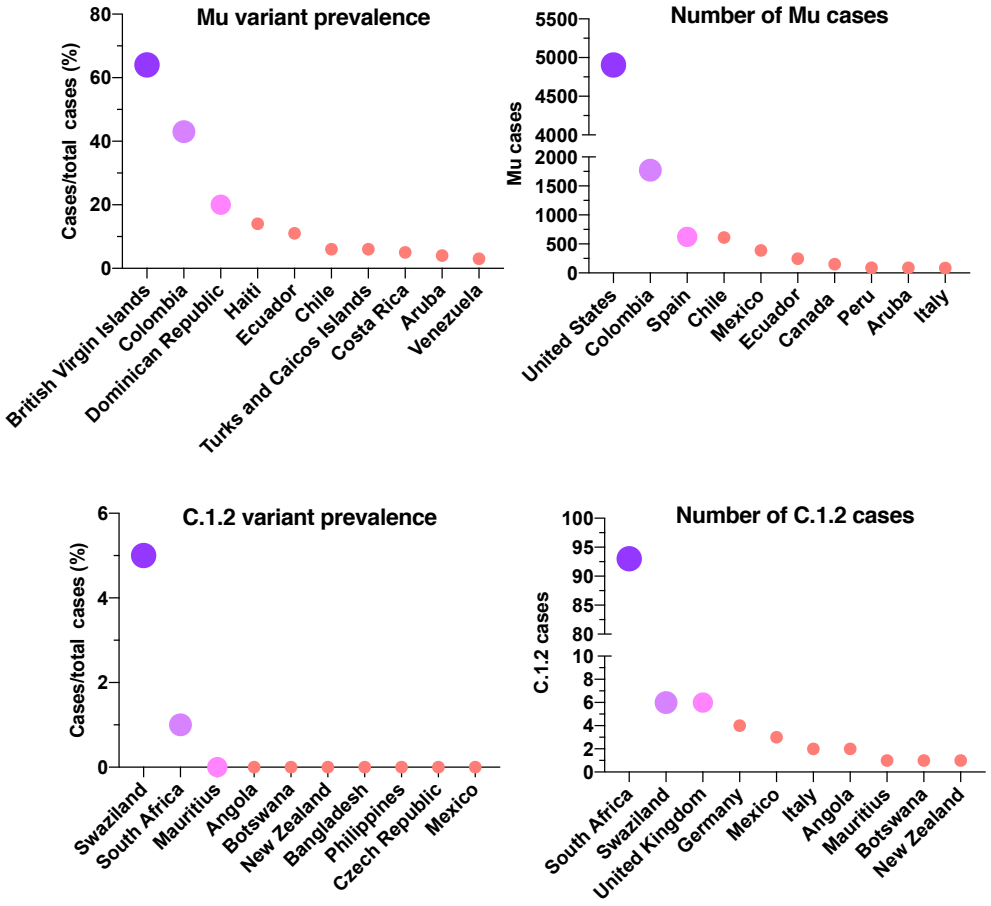
12. Gu H, Chen Q, Yang G, et al. Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. Science 2020;369(6511):1603-1607. DOI: 10.1126/science.abc4730.

13. Starr TN, Greaney AJ, Hilton SK, et al. Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. Cell 2020;182(5):1295-1310 e20. DOI: 10.1016/j.cell.2020.08.012.

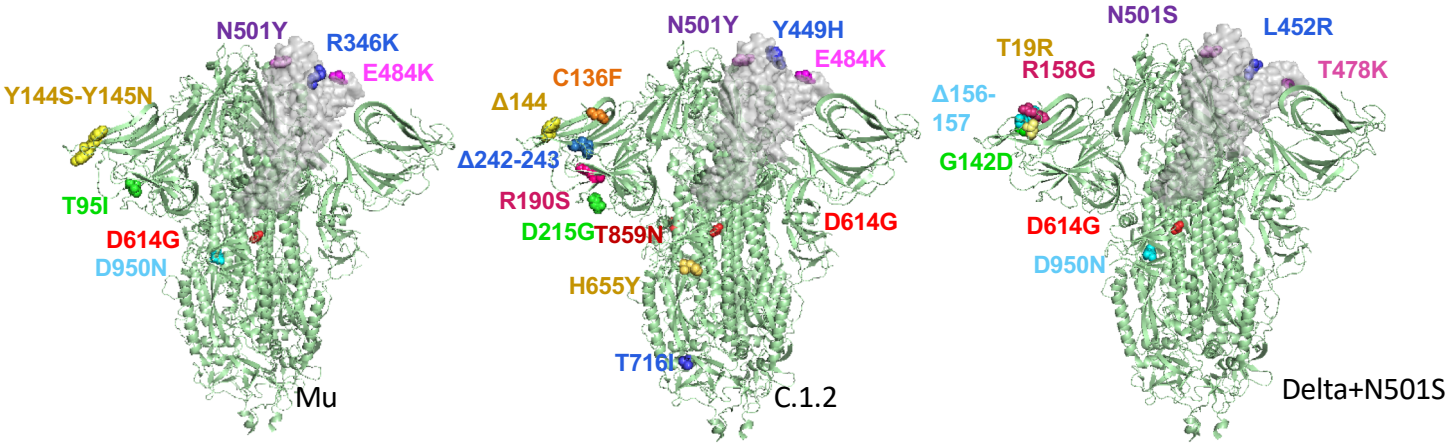
14. Tada T, Fan C, Chen JS, et al. An ACE2 Microbody Containing a Single Immunoglobulin Fc Domain Is a Potent Inhibitor of SARS-CoV-2. Cell Rep 2020;33(12):108528. DOI: 10.1016/j.celrep.2020.108528.

15. Benton DJ, Wrobel AG, Roustan C, et al. The effect of the D614G substitution on the structure of the spike glycoprotein of SARS-CoV-2. Proc Natl Acad Sci U S A 2021;118(9). DOI: 10.1073/pnas.2022586118.

A



B



C

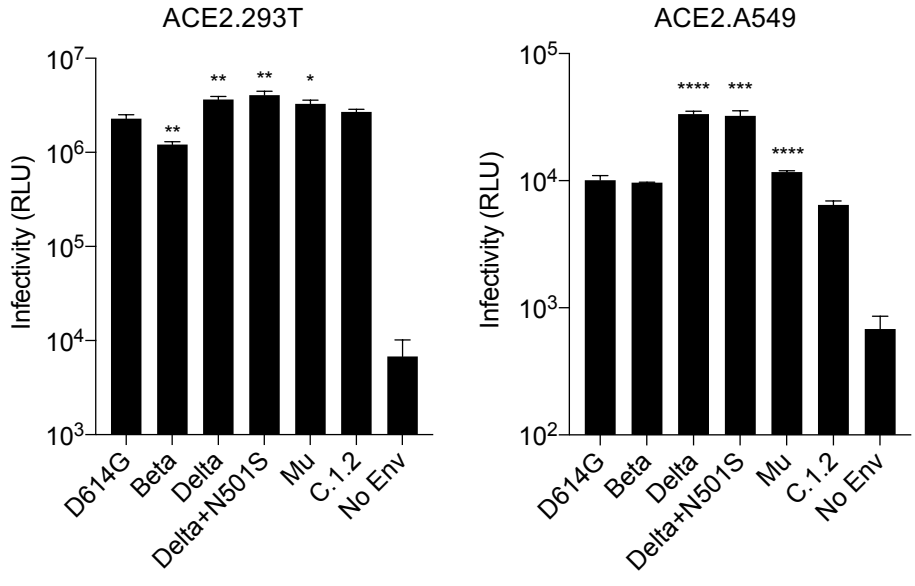


Figure. 1

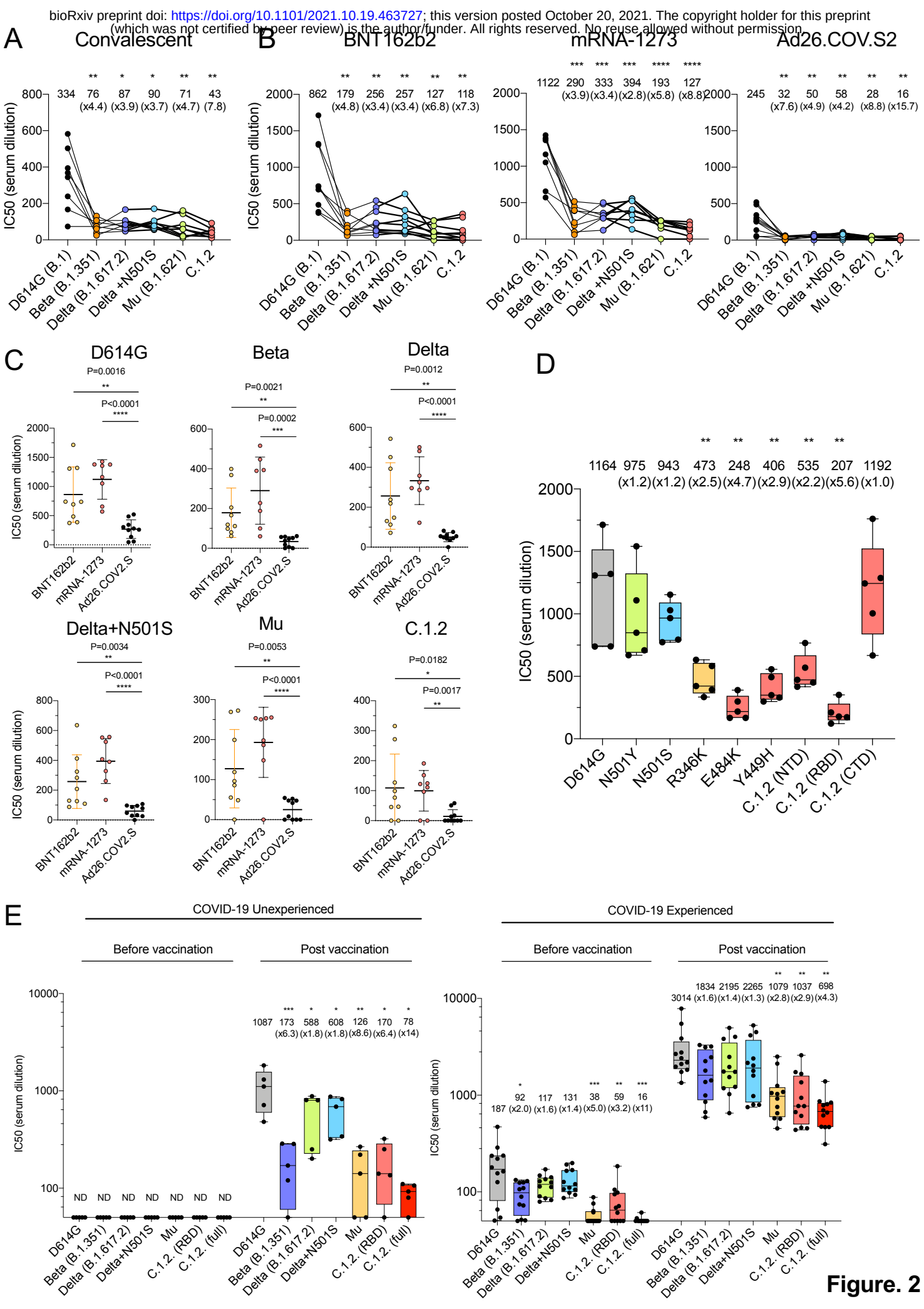


Figure. 2

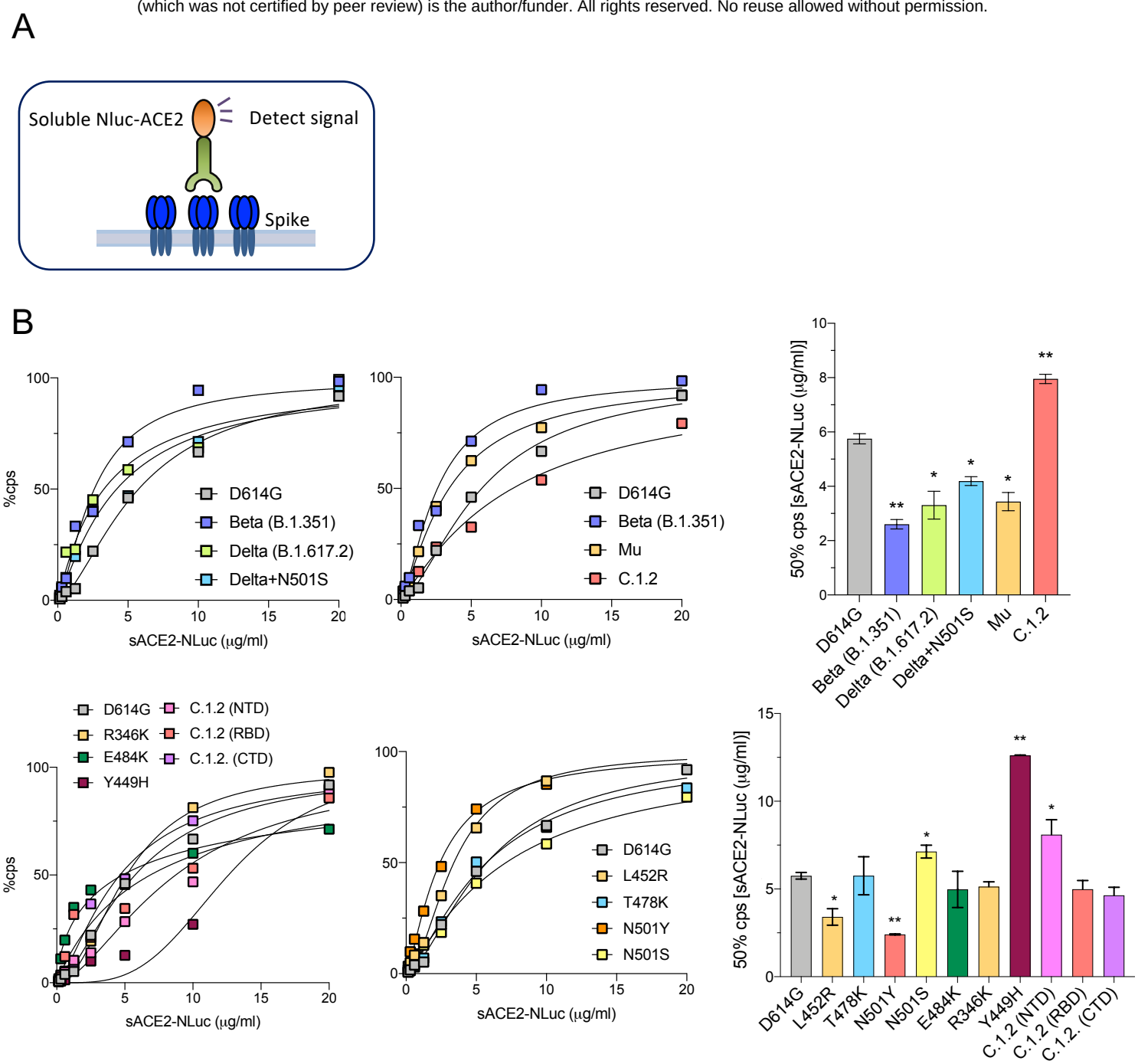
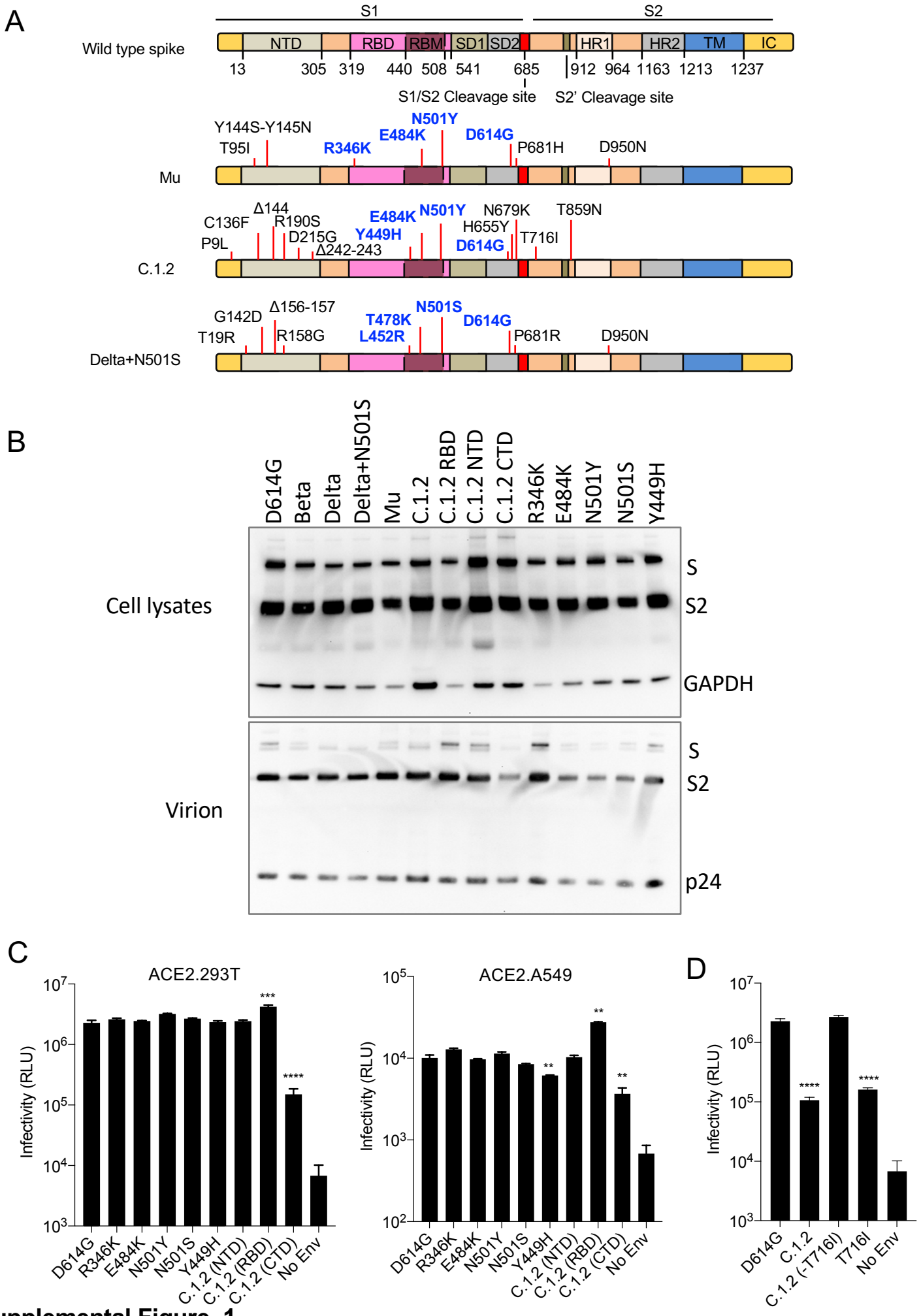
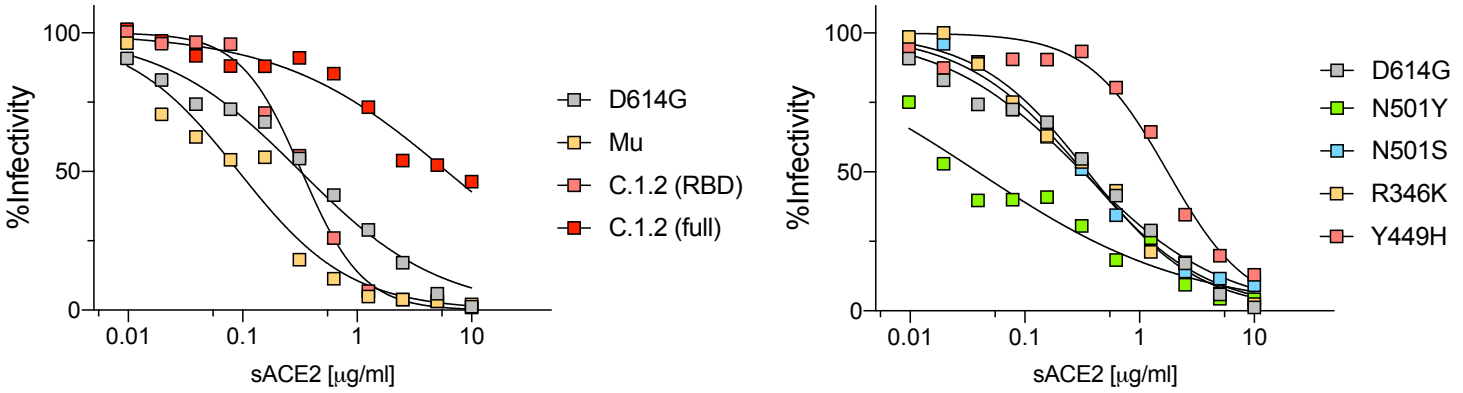


Figure. 3

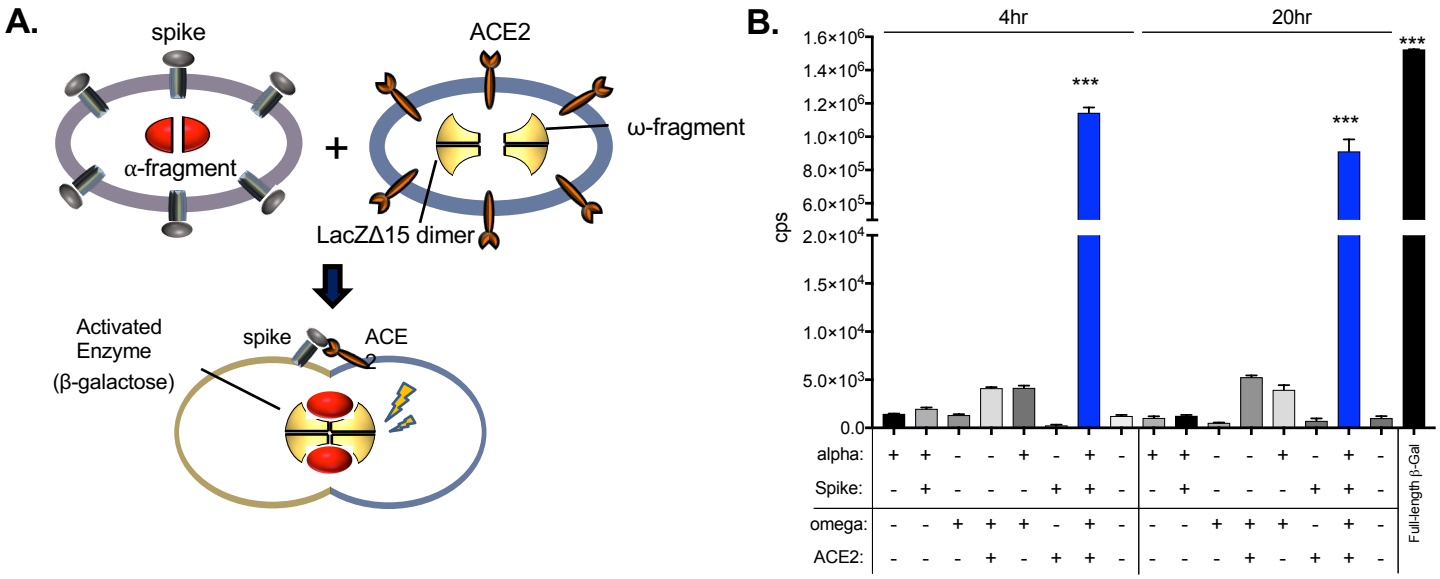


Supplemental Figure. 1

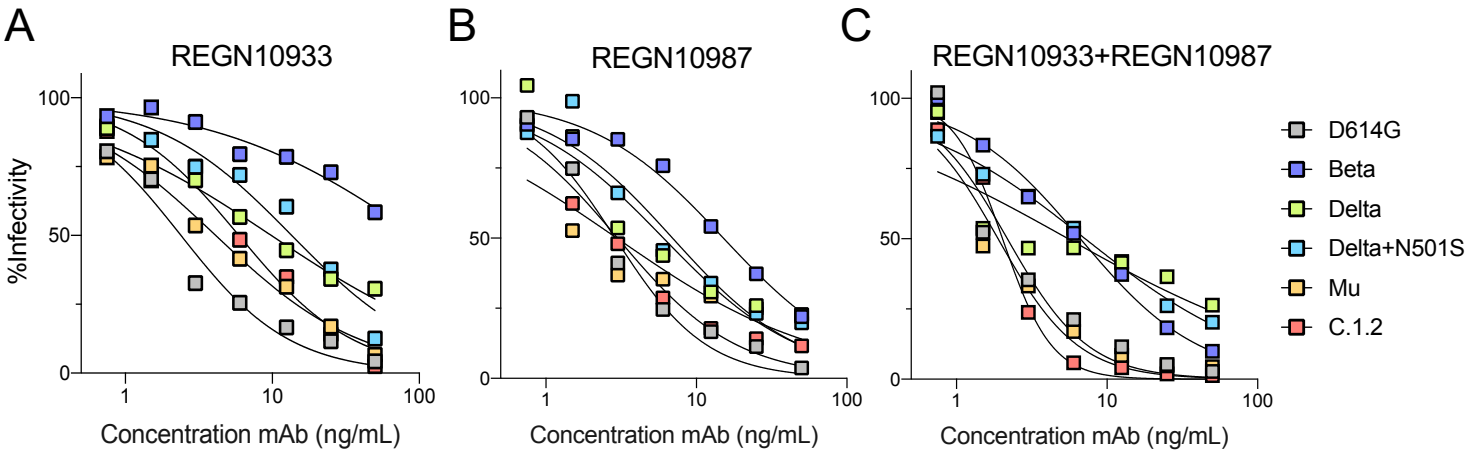


IC50 (sACE2; μg/ml)							
D614G	Mu	C.1.2 (RBD)	C.1.2 (full)	R346K	H449H	N501Y	N501S
0.32	0.09	0.33	6.08	0.35	1.76	0.04	0.33

Supplemental Figure. 2



Supplemental Figure. 3



	IC50 (ng/ml)					
	D614G	Beta	Delta	Delta+N501S	Mu	C.1.2
REGN10933	2.4	96.3	9.8	13.6	4.1	5.9
REGN10987	2.9	15.1	6.0	6.9	3.0	3.0
REGN-COV2	2.2	4.0	4.3	5.1	1.9	2.0

Supplemental Figure. 4

Table S1. IC50 of Convalescent, BNT162b2, mRNA-1273 and Ad26.COV.S elicited antibodies against viruses with variant spike proteins. Age, Sex and Comorbidities are shown.

	Convalescent					
	IC_{50}(serum dilution)					
donor	D614G (B.1)	Beta (B.1.351)	Delta (B.1.617.2)	Delta +N501S	Mu (B.1.621)	C.1.2
1	239	72	52	96	25	17
2	167	97	77	92	61	10
3	74	74	167	171	143	61
4	394	39	48	56	85	41
5	583	61	98	59	59	27
6	369	131	62	105	161	92
7	503	107	106	66	12	54
8	345	25	83	77	19	40
Mean (SD)	334 (169)	76 (35)	87 (38)	90 (37)	71 (56)	43 (26)

BNT162b2										
					IC_{50}(serum dilution)					
donor	Days post 2nd dose	Age	Sex	Comorbidities	D614G	Beta	Delta	Delta +N501S	Mu	C.1.2
1	84	39	F	None	740	98	219	283	86	99
2	52	23	F	None	1713	119	451	636	96	77
3	101	26	F	Asthma	1320	399	543	412	273	315
4	109	33	F	None	738	205	393	325	199	127
5	60	35	F	Hypothyroidism, Psoriasis	1308	80	113	88	49	46
6	81	42	F	Asthma	374	62	72	109	0	0
7	108	26	F	None	694	369	148	126	269	271
8	107	24	M	None	389	166	131	130	55	0
9	110	35	M	None	485	112	238	209	119	46
Mean (SD)	90 (22)	31 (7)			862 (474)	179 (124)	256 (167)	257 (180)	127 (98)	109 (112)

mRNA-1273										
					IC_{50}(serum dilution)					
donor	Days post 2nd dose	Age	Sex	Comorbidities	D614G	Beta	Delta	Delta +N501S	Mu	C.1.2
1	89	26	M	None	1378	188	296	557	198	111
2	92	53	M	None	1379	390	358	390	254	124
3	61	67	M	Prediabetes	1053	100	122	407	251	97
4	93	33	F	None	570	60	296	135	0	0
5	44	32	M	None	1162	447	325	553	255	191
6	100	29	F	None	1355	516	481	524	148	0
7	52	33	F	None	1426	228	500	328	187	122
8	105	55	F	Asthma	656	394	286	262	253	151
Mean (SD)	80 (24)	41			1122 (339)	290 (169)	333 (119)	394 (150)	193 (88)	100 (68)

Ad26.COV2.S										
					IC_{50}(serum dilution)					
donor	Days post 2nd dose	Age	Sex	Comorbidities	D614G	Beta	Delta	Delta +N501S	Mu	C.1.2
1	57	42	F	None	47	58	43	32	54	14
2	58	28	F	None	138	38	49	97	48	58
3	66	36	F	None	521	ND	ND	ND	48	ND
4	92	33	F	None	344	18	36	29	ND	ND
5	87	39	F	Prediabetes	253	ND	45	107	ND	ND
6	72	32	M	None	280	61	61	26	ND	ND
7	92	39	F	None	260	52	55	95	42	17
8	71	75	F	None	304	57	82	87	8	ND
9	105	30	M	None	58	6	76	48	52	51
10	115	33	F	None	487	48	55	79	ND	ND
Mean (SD)	82 (20)	39 (13)			245 (180)	32 (25)	50 (23)	58 (37)	28 (25)	16 (22)

Table 2. IC50 of sera from unexperienced and experienced individuals before and after vaccination with BNT162b2 against viruses with variant spike proteins. Age, Sex and Comorbidities are shown.

Covid Unexperienced BNT162b2 Note: Covid experienced samples had no detectable levels of antibodies against variants before vaccination											
					IC_{50}(serum dilution) (post vaccination)						
donor	Days post 2nd dose	Age	Sex	Comorbidities	D614G	Beta	Delta	Delta +N501S	Mu	C.1.2 (RBD)	C.1.2 (full)
1	8	62	Male	none	1824	285	882	871	141	136	80
2	8	34	Male	none	1307	171	802	825	267	322	0
3	8	40	Female	none	1109	287	804	687	221	141	93
4	8	39	Female	none	713	120	251	317	0	251	110
5	9	39	Male	none	481	0	201	341	0	0	107
Mean (SD)	8.2 (0.4)	43 (11)			1087 (524)	173 (121)	588 (332)	608 (264)	126 (123)	170 (123)	78 (45)

					IC_{50}(serum dilution)											
donor	Days post 2nd dose	Age	Sex	Comorbidities	D614G		Beta		Delta		Delta +N501S		Mu		C.1.2		C.1.2 (full)	
					Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	10	34	M	none	66	5477	107	2462	129	1957	165	2157	31	755	69	1660	29	838
2	11	46	M	none	163	7844	51	3183	171	4964	192	4157	17	2118	71	2585	3	660
3	7	60	M	none	287	3852	129	3133	125	3823	120	5268	76	2501	184	1731	53	807
4	9	42	F	none	470	2179	40	853	90	652	86	752	62	570	0	939	0	863
5	12	43	M	none	237	1829	51	1421	79	1539	111	979	0	1247	61	771	0	311
6	8	37	F	none	236	2760	89	3299	112	4007	105	4488	63	1029	94	668	61	696
7	10	54	F	Hypertension, History of obesity	156	2101	125	2246	148	1178	168	794	23	1051	105	461	12	468
8	7	34	F	none	220	1739	72	1368	141	1218	199	1592	11	1080	98	454	0	468
9	7	32	F	Anemia, Asthma, GERD	54	1970	74	667	86	1025	107	797	88	453	0	437	22	471
10	11	24	F	none	179	2412	109	1793	114	2485	93	2027	20	577	15	771	13	785
11	10	49	F	Hypertension, Rhinosinusitis	126	1343	133	591	135	1742	127	2395	35	651	9	610	5	618
12	6	44	F	Hereditary Hemorrhagic Telangiectasias, Eosinophilic Esophagitis	51	2664	125	993	82	1754	98	1783	28	921	0	1357	0	1388
Mean (SD)	9 (2)	42 (11)			187 (118)	3014 (1885)	92 (34)	1834 (1008)	118 (29)	2195 (1359)	131 (39)	2266 (1554)	38 (28)	1079 (629)	59 (57)	1037 (665)	17 (21)	698 (279)