Comparison of 10 emerging SARS-CoV-2 Variants: infectivity, animal tropism, and antibody neutralization

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Abstract

Ten emerging SARS-CoV-2 variants—B.1.1.298, B.1.1.7, B.1.351, P.1, P.2, B.1.429, B.1.525, B.1.526-1, B.1.526-2, B.1.1.318—and seven corresponding single amino acid mutations in the receptor-binding domain were examined using SARS-CoV-2 pseudovirus. The results indicate that the current SARS-CoV-2 variants do not increase infectivity among humans. The K417N/T, N501Y, or E484K-carrying variants exhibited increased abilities to infect to mouse ACE2-overexpressing cells. The activities of Furin, TMPRSS2, and cathepsin L were increased against most of the variants. RBD amino acid mutations comprising K417T/N, L452R, Y453F, S477N, E484K, and N501Y caused significant immune escape from 11 of 13 monoclonal antibodies. However, the resistance to neutralization by convalescent serum or vaccines was mainly caused by the E484K mutation, while the neutralization of E484K-carrying variants was decreased by 1.1–6.2-fold. The convalescent serum from B.1.1.7- and B.1.351-infected patients neutralized the variants themselves better than other SARS-CoV-2 variants.

Introduction

Following the discovery of SARS-CoV-2, the emergence of multiple variants has been reported[1,2]. Mutations of the virus may cause changes in its infectivity and pathogenicity, resulting in the emergence of highly infectious or lethal mutant strains, they may also change the antigenicity of the virus, leading to failures of existing antibody treatments or the vaccine[1,3,4]. Additionally, mutations may cause cross-species transmission and the virus may undergo further evolution in the new host, triggering a new wave of virus spread[4]. Therefore, the mutations of SARS-CoV-2 have received close attention from scientists worldwide. Beginning in March 2020, the D614G mutant strain became the dominant strain globally, and the current prevalence has exceeded 95%[3]. In November 2020, the mink strain B.1.1.298 (cluster 5) was reported to spread between humans and minks[4,5]. Since December, increasing numbers of SARS-CoV-2 variants have been reported worldwide, among which B.1.1.7, B.1.351, and P.1 have been listed as viruses of concern (VOCs) by the WHO[6]. As of March 2021, B.1.1.7(VOC 202012/01 or 501Y.V1), which first appeared in the United Kingdom, has spread to 125 countries; this variant exhibits increased transmissibility, risk of hospitalization, severity and mortality[7-9]. B.1.351(501Y.V2) first appeared in South Africa and leads to immune escape of the spike protein because of mutation E484K in the RBD; this variant may influence the efficacies of vaccines and therapeutic monoclonal antibodies (mAbs) and sera[10,11]. P.1 (B.1.1.28.1/501Y.V3) and P.2, which first appeared in Brazil, led to the disappointment regarding Brazil's herd immunity dream and almost caused the collapse of Brazil's medical system[12,13]. Variants B.1.429 (or B.1.427) in California and B.1.526 in New York comprised the largest proportion of the new COVID-19 cases in those areas, eliciting widespread concern [1,14-17]. Moreover, the Nigerian variant B.1.525, which contains subsets of mutations previously observed in variants B.1.1.7 and B.1.351, has spread rapidly in Nigerian and the United Kingdom [18,19] . Additionally, a new variant B.1.1.318 recently appeared in the United Kingdom; this variant requires close attention because of its E484K mutation[18,20]. In this study, we investigated the 10 currently prevalent variants (B.1.1.298, B.1.1.7,

B.1.351, P.1, P.2, B.1.429, B. 1.525, B.1.526-1, B.1.526-2, and B.1.1.318) using the VSV-based pseudovirus system (Fig1). We compared infectivity, host tropism, and neutralization characteristics with the D614G reference strain, with the aim of providing clues for the prevention and control of COVID-19, particularly with respect to designing mAbs and vaccines.

Results

Infectivities of 10 SARS-CoV-2 variants

The infectivities of the 10 variants and seven RBD-located single mutations was first tested in four SARS-CoV-2-susceptible cell lines, including two human cell lines (Huh-7 and 293T-ACE2) and two non-human primate cell lines (LLC-MK2 and Vero). Notably, none of the examined variants had more than fourfold increased infectivity, compared with the D614G reference strain (Fig 2A-D). However, the L452R single mutation led to slightly increased infectivity, whereas the B.1.1.298 variant exhibited significantly decreased infectivity (Fig 2A-D).

The activity of Furin/TMPRSS2/Cathepsin L the ten SARS-CoV-2 variants

Because Furin, TMPRSS2, and Cathepsin L play important roles in SARS-CoV-2 infection[21-23], mutation-related structural changes in the virus may influence the functions of these enzymes. We found that the ability of the D614G reference strain to infect 293T-ACE2 cells was significantly increased when Furin, TMPRSS2, or Cathepsin L was overexpressed (Fig 2E). We subsequently investigated the infectivities of the variants in respective Furin-, TMPRSS2-, or Cathepsin L stable overexpressing cells. The results showed that the increased infectivity to the 293T-ACE2 cells in the presence of Furin, TMPRSS2 or Cathepsin L was further increased among all tested variants, as compared to the D614G reference strain (Fig 2F-H).

Animal tropism of 10 SARS-CoV-2 variants

To investigate changes in the animal tropism of the 10 SARS-CoV-2 variants, 14 ACE2s from different species were overexpressed in 293T cells. The infectivities of most variants were increased in the mouse ACE2 overexpressed cell lines. Notably, the infectivities of the B.1.1.7, B.1.351, P.1, B.1.525, and B.1.1.318 were significantly increased (by more than fourfold), compared with the D614G reference strain (Fig3). Single mutation analyses showed that K417T, K417N, E484K, and N501Y led to increased infectivity in mouse ACE2-overexpressed cell lines, thus explaining the dramatically increased infectivity of variants carrying these mutations (Fig3). However, the infectivities of other variants were not alerted in ACE2-overexpressed cell lines from other species.

The neutralization activity of mAbs to the ten variants.

To compare the effectivity of neutralizing mAbs against the 10 SARS-CoV-2 variants, the neutralizing activity of 13 mAbs targeting different areas of the receptor-binding domain were tested. Variants with

the highest escape frequencies were B.1.351 and P.1, which escaped from 10 of 13 mAbs (1F9, 2H10, 10D12, 10F9, 9G11, X593, CB6, A247, H00S022, and A261-262). These results were consistent with single-mutation findings involving K417T/N (1F9, 2H10, 10D12, CB6 and A247) and N501Y (1F9, 10D12, 10F9, CB6, A247 and H00S022) and E484K (9G11, X593and A261-262). The variant with the second highest escape frequency was B.1.1.7, against which seven of 13 mAbs (1F9, 2H10, 10D12, 10F9, CB6, A247 and H00S022) displayed significantly reduced neutralizing activity. Single-mutation analysis indicated that immune escape of the B.1.1.7 variant was mainly caused by the N501Y mutation. Similarly, the variants B.1.525, P.2, B.1.526-2 (E484K), B.1.1.318, as well as the E484K single-mutation strain, displayed similar patterns of neutralization sensitivity involving significantly reduced neutralization activity among three mAbs (9G11, X593and A261-262). Furthermore, the variant B.1.429 and L452R single-mutation strain showed reduced susceptibility to mAbs 9G11 and X593; the variant B.1.526-1(S477N) and S477N single-mutation strain showed reduced susceptibility to mAb 7B8; and variant B.1.1.298 and Y453F single-mutation strain showed reduced sensitivity to mAb 1F9. Additionally, the K417N/T mutation caused reduced neutralization activity involving five mAbs (1F9, 2H10, 10D12, CB6, and A247), whereas it increased neutralization sensitivity of one mAb (A261-262) for more than ten times (Fig4). These results indicate that the RBD mutations in SARS-CoV-2 variants are the major mutations that affect their neutralization sensitivities.

Neutralization activities of immunized sera against 10 SARS-COV-2 variants

To determine whether the antigenicity change in the 10 variants could change their neutralizing sensitivity with respect to vaccine immunization, animals were immunized with different types of SARS-CoV-2 antigens: trimer spike protein (in mice), pseudotyped virus (in guinea pigs), recombinant DNA containing full-length spike gene (in guinea pigs) or purified RBD protein (in horses). The neutralization reactions elicited by immunized sera generated with these different antigens was compared among the 10 SARS-CoV-2 variants. Of the 10 variants, only B.1.351, P1, P2, B.1.525, B.1.526-2, and B.1.1.318 displayed obviously reduced sensitivities to immunized sera. Notably, these variants all harbor the E484K mutation (Fig. 5A-E). The reduced sensitivities were observed regardless of SARS-CoV-2 immunogen and source of animal serum (Fig. 5A-E). Furthermore, the neutralization activity against the K417T/N single-mutation strain was increased among all serum samples (Fig 5E). The neutralization activities of the various immunogens against D614G reference strain were compared. The results showed that immunization with the RBD protein induced the highest antibody titers, whereas immunization with the trimer full-length protein or pseudovirus induced more robust titers than did immunization with the full-length spike DNA (Fig. 5F). Regarding the E484K-carrying variants, neutralization sensitivity of the RBD immunized sera reduced 2.6 to 6.2 folds, which is much obvious than other immunogens. On the other hand, the trimer protein immunized sera are much stable, the neutralization activity reduced only 1.1 to 1.8 folds. (Fig 5A-E). Additionally, although the variants B.1.351 and P.1 carry nearly identical RBD mutations, the variant B.1.351 exhibited a much greater reduction in neutralization sensitivity. Moreover, although P.2 only has one additional mutation (V1176F) in the S2 domain, its neutralization sensitivity differs from the sensitivity of the E484K single-mutation strain (Fig. 5A-E).

Neutralization activities of convalescence sera from patients infected with B.1.1.7 and B.1.351 variants

To evaluate the impacts of infection with the B.1.1.7 and B.1.351 variants on neutralization activities, convalescent sera from patients with the two variants and the D614G reference strain were analyzed. The mean neutralizing antibody levels (i.e., ID50 values) were comparable between D614G and B.1.1.7, whereas B.1.351 induced much lower neutralizing antibody production (Fig6A). Convalescent sera from D614G infected patients showed a neutralization pattern similar to the pattern exhibited by SARS-CoV-2 immunized animal sera (Fig6B). However, the B.1.351 variant-infected sera showed better neutralization activity against P.1, the variant itself and E484K-carrying variants, relative to the D614G reference strain (Fig. 6C). B.1.1.7 variant-infected sera also showed the highest neutralization activity against the variant itself; it was comparatively resistant to B.1.351 and other E484K-carrying sera (Fig. 6D).

Discussion

Recently, emerging SARS-CoV-2 variants have attracted the attention of scientists worldwide. After the onset of the SARS-CoV-2 outbreak, the D614G strain rapidly replaced the original virus strain and became the dominant variant[3]. Further studies showed that the host cell infectivity of D614G was increased, compared with the original virus, because of mutation-related structure changes[2,24]. Subsequently, many SARS-CoV-2 variants were reported to be spread rapidly in various countries. The

World Health Organization and the United States CDC have reported several variants of interest (VOIs), which are associated with established or suspected phenotypic implications and have been caused community transmission or been detected in multiple countries. As of March 30, 2021, there were nine VOIs, of which three were variants of concern (VOCs; e.g., B.1.1.7, B.1.351 and P.1.) that showed increased transmissibility and potentially reduced neutralization by convalescent and post-vaccination sera[6]. Furthermore, a previous study demonstrated increased binding of N501Y to mouse ACE2, which implies differences in host tropism[25]. Here, we evaluated the host tropism characteristics of 10 SARS-CoV-2 variants, which variants included most VOIs and additional potentially important variants. Pseudoviruses of multiple variants and single mutants at RBD sites presumably related to these variants were examined for their ability to infect four SARS-CoV-2 susceptible cells. No significant enhancements of infectivity were observed among the tested variants, compared with the D614G reference strain, while the L452R mutation and B.1.429 variant exhibited slightly increased infectivity; these findings were consistent with the reports by MaCallum et al[16]. In addition, we also found slight enhancements of infectivity involving the B.1.525, B.1.526-2, and B.1.1.318 variants, suggesting that these variants should receive close attention.

Furin, TMPRSS2 and Cathepsin L are important proteolytic enzyme, which are key regulators of SARS-CoV-2 infection[22,23]. Overexpression of these enzymes facilitates cellular infection. Surprisingly, almost all SARS-CoV-2 variants further enhanced the enzymes promoted SARS-CoV-2 infection, especially when Furin was overexpressed in 293T-ACE2 cells. When TMPRSS2 overexpressed infection was compared to mock 293T-ACE2 cells, the enhancement of infectivity by TMPRSS2 for the P.1 variant was almost

fourfold than for the D614G variant. The underlying mechanisms of these increased infectivities require further analyses.

Cross-species infections caused by viral mutations contribute to the extensive spreading of many animal-derived viruses in human populations. In this study, we evaluated the abilities of current SARS-CoV-2 variants to bind ACE2 proteins of 14 different animal species. We found that K417N/T, E484K, and N501Y mutations significantly increased the ability of SARS-CoV-2 to infect 293T cells overexpressing mouse ACE2; variants carrying these mutations (i.e., B.1.1.7, B.1.351, P.1, P.2, B.1.429, B.1.525, B.1.526-2, and B.1.1.318) showed similar changes in infectivity. These results suggest the need for careful monitoring of new variants in mice, which may lead to further virus mutations and prolong the spread of disease.

Regarding neutralization, we found that mutations in the RBD enabled escape from various mAbs; these escape findings were consistent with the activities of SARS-CoV-2 variants carrying the corresponding mutations. Variants with more mutations in the RBD region (e.g., B.1.1.7, B.1.351, and P.1) more frequently escaped from mAbs. The results indicate that the neutralization activities of mAbs against epidemic variants should be examined during the development of new therapeutic mAbs; additionally, specific mAbs are presumably more effective against specific variants, implying that cocktail therapy might be appropriate in clinical practice.

Analysis of serum neutralization resistance revealed that most single mutations, including those in the RBD region, could not directly and substantially change the serum neutralization effect on SARS-CoV-2. However, variants carrying the E484K mutation had distinct reductions in neutralization susceptibility. Moreover, some studies have shown that selective pressure from therapeutic mAbs or convalescent serum could induce E484K or E484Q mutations[26,27]. These results indicate the importance of E484 in the viral epitope. Our previous study revealed that the K417N mutation in the RBD region of B.1.351 led to the enhanced convalescence sera neutralization activity[10]. Here we found that the K417T mutation in the P.1 variant has a similar effect. Although variants B.1.351 and P.1 have similar RBD mutation sites, the neutralization resistance of P.1 was less robust than the resistance of B.1.351. Mutations in other regions of the virus (e.g., NTD or S2) may also cause antigenicity changes[28]. Our preliminary study found that mice immunized with the NTD fragment produced almost no neutralizing antibody, while mice immunized with S2 fragment produced neutralizing antibody. Because the S2 region is a key region that mediates virus fusion, mutation of this region may change viral structure and influence neutralization activity. These results emphasize the need to consider regions other than the RBD in vaccine design efforts. Notably, the RBD is a robust immunogen for neutralizing antibody production, and the corresponding antibody titer is higher than the titers elicited by other immunogens (e.g., pseudovirus, full-length DNA, and full-length protein trimer). However, modification of E484K in the RBD led to the most marked change in immune escape. Therefore, the RBD alone may not be an ideal immunogen for vaccine development.

Because of the B.1.1351 variant cam escape from a large number of mAbs and exhibits resistance to most vaccine-induced protection[10,11], the B.1.351 variant has been regarded as an important candidate

for new generations of vaccines. However, neutralization analyses of convalescent sera from D614G-, B.1.1.7- and B.1.351-infected patients indicate that B.1.351 variant induced much lower antibody production, compared with other variants. However, there were only three patients in our tests, more samples are needed to further analyze the immunogenicity of B.1.351.

In summary, we systematically analyzed the infectivity and host tropism of 10 SARS-CoV-2 variants. Although the infectivities of all tested SARS-CoV-2 variants were not significantly increased compared with the reference strain, these variants exhibited considerable enhancements of infectivity in the presence of furin, TMPRSS2, and cathepsin L. Our results demonstrate that the K417N/T, E484K, and N501Y mutations change host tropism, implying possible transmission of SARS-CoV-2 variants in mice. The neutralization activity of mAbs, immunized sera and convalescent sera from different variants infected patients were analyzed against the 10 variants, which would provide insights for the development of therapeutic antibodies and vaccine design.

Declarations

The human study was approved by Medical Ethics Committee of National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

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Authors' contributions

Y.W, L.Z and W.H conceived, designed and supervised the experiments; L.Z, J.N and Y.W wrote the manuscript; Z.C, Q.L, Y.Y, J.W, R.D, H.W, Y.Z and S.L performed the experiments. Z.C, Y.H and W.X provided convalescence sera and patients information. All of the authors approved the final manuscript.

Declaration of interests:

All authors declare no competing interest.

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Materials And Methods

Cells

Two human cell lines Huh-7 and 293T was from Japanese Collection of Research Bioresources (Cat: 0403) and American Type Culture Collection (ATCC, Cat: CRL-3216). Two monkey cell lines LLC-MK2 and Vero were from ATCC (Cat: CCL-7 and CCL-81). 293T-hACE2, 293T-hACE2-Furin, 293T-hACE2-TMPRSS2 and 293T-hACE2-Cathepsin L overexpressing cells were human ACE2, Furin, TMPRSS2, and Cathepsin L-stably expressing 293T cells. Receptor-transiently overexpressing cells were prepared by transfecting 293T cells with plasmids containing ACE2 from different species. Cells were cultured using Dulbecco's modified Eagle medium (DMEM, high glucose; Hyclone) supplied with 100 U/mL of Penicillin-Streptomycin solution (Gibco), 20mM N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES, Gibco) and 10% fetal bovine serum (FBS, Pansera ES, PAN-Biotech), in a 5% CO2 environment at 37 °C; cells were passaged at intervals of 2–3 days using 0.25% Trypsin-EDTA (Gibco). Lipofectamine 2000 (Invitrogen) was used as a transfection reagent. All cells were used 24-48 hours after transfection.

Monoclonal Antibodies

mAbs A261-262, A247 and 76A were from Professor Linqi Zhang of Tsinghua University. mAb H00S022 was from Sino Biological Company. mAbs 1F9, 7B8, 4E5, 2H10, 10D12, 10F9 and 9G11 were from Beijing Biocytogen Co. mAb X593 was from Prof. sunney Xie of Peking University. mAb CB6 was provided by Prof. Jinghua Yan from the Institute of Microbiology, Chinese Academy of Sciences.

Immunized Sera

The study protocol was approved by the Animal Care and Use Committee at the National Institutes for Food and Drug Control (NIFDC). The Animals were handled in accordance with the protocol and guidelines for laboratory animal care and use. Mice were immunized with purified Trimer protein with aluminum adjuvant (20μg per mouse, once per week for 3 weeks). Serum samples were collected at 4 weeks after the third immunization. Serum samples from 10 mice of each group were pooled to produce combined samples. Each two mice were combined to make one sample. Guinea pigs were immunized with SARS-CoV-2-Spike plasmid at 200μg per guinea pig or peudotyped virus at 6×10^5 TCID50 per guinea pig(once every 2 weeks for 6 weeks). Serum samples from five guinea pigs in each group were collected 28 days after the third immunization. Horses were immunized with SARS-CoV-2 RBD protein plus Freund's incomplete adjuvant (once every 10 days for 30 days) at doses of 3mg, 6mg and 12mg. Serum samples were collected at 1 week after the third immunization.

Convalescence sera

Twenty convalescence serum samples were collected from patients with COVID-19, 2-3 months after SARS-CoV-2 infection. Of these 10 samples, 5 were from D614G reference strain-infected patients, four were from B.1.1.7-infected patients, and three were from B.1.351-infected patients. All patients provided written informed consent to participate in the study.

SARS-CoV-2 pseudotyped virus

In accordance with our published method[2], the spike protein expression plasmid was constructed using GenBank sequence MN908947. Replication-defective SARS-CoV-2 viral particles were generated by transfection with pcDNA3.1- SARS-CoV-2 and concurrent infection with G*ΔG-VSV (Kerafast). The cell supernatant containing pseudotyped virus was harvest 24 and 48 hours later, then aliquoted and stored at −80 ℃. Site-directed mutagenesis based on circular PCR was used to construct mutants of SARS-CoV-2 pseudotyped virus. DpnI (NEB) was used to digest template DNA. Primers used for mutation are listed in supplementary Table 1. Pseudotyped SARS-CoV-2 variants were quantified via RT-PCR using VSV P protein as an internal control. Viruses of multiple variants were diluted to the same number of copies before use in experiments.

Infection and Neutralization assays

As described in our previous paper[2], for infection assays, similar copy numbers of different pseudotyped SARS-CoV-2 variants were mixed with the indicated cells. For neutralization assays, mAbs or sera were serially diluted and preincubated with pseudotyped virus at 37°C for 1 hour, then mixed with Huh-7 cells. The cells were then incubated for 20-28 hours at 37°C, in an environment containing 5% CO2. Chemiluminescence signals were collected by PerkinElmer Ensight using luciferase substrate (PerkinElmer). Duplicated wells were analyzed for each group. Each experiment was repeated for 2-5 times.

Statistical analysis

EC50 was calculated using the Reed-Muench method. Graphical representations were generated using GraphPad Prism 8. One-way ANOVA and Holm-Sidak's multiple comparisons test were used to identify differences relative to the D614G reference strain. Values are shown as means ±SEMs. For all figures, * P<0.05, ** P<0.01, *** P<0.005, and **** P<0.001.

Data availability

The raw data have been deposited in Figshare (https://figshare.com/articles/figure/Comparison_of_10_emerging_SARS-CoV-2_Variants_infectivity_animal_tropism_and_antibody_neutralization/14526894).

Figures

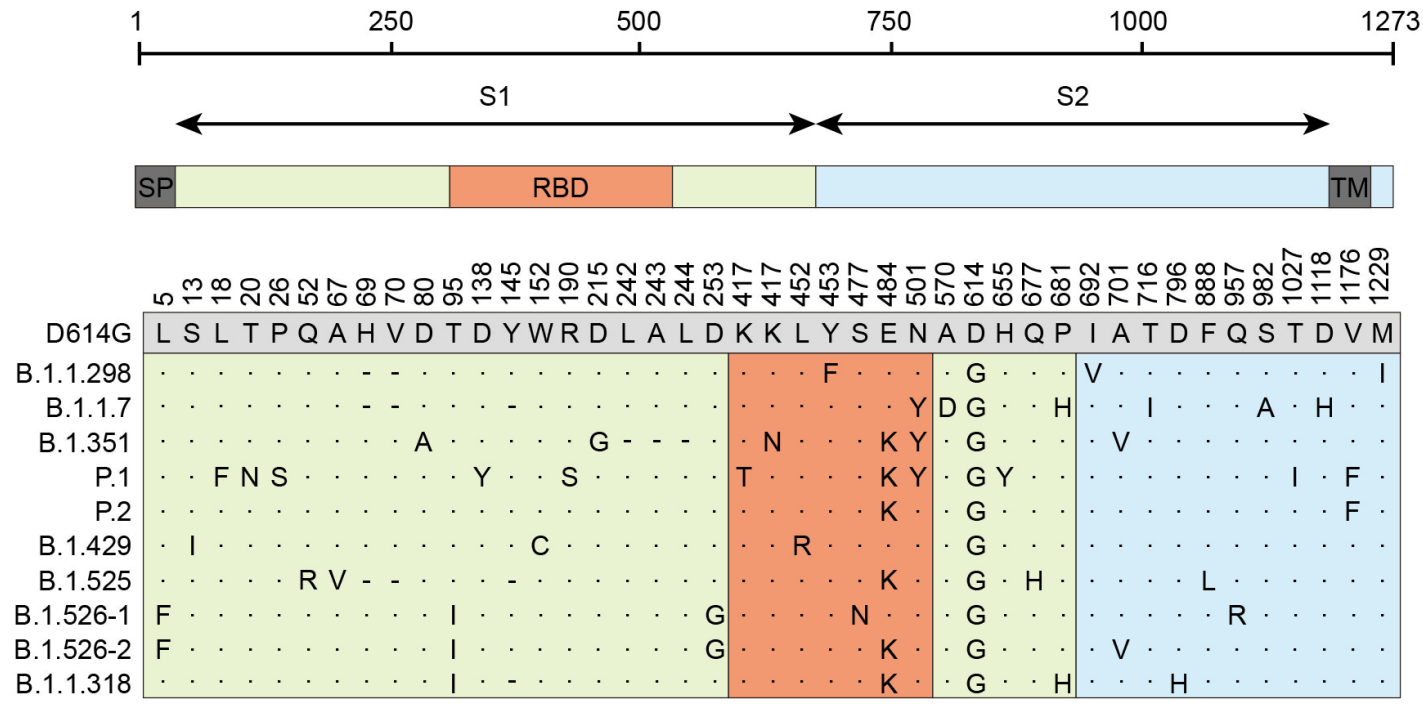

Figure 1

Schematic of SARS-CoV-2 variants. The most representative amino acid mutations of each variant were selected to construct pseudotyped virus for this study. Each mutation in each SARS-CoV-2 variant is indicated relative to the reference D614G sequence. A dot indicates an identical amino acid at the indicated position, while a dash indicates a deletion at that point. SP, sigmal peptide; TM, transmembrane domain; RBD, Receptor-binding domain.

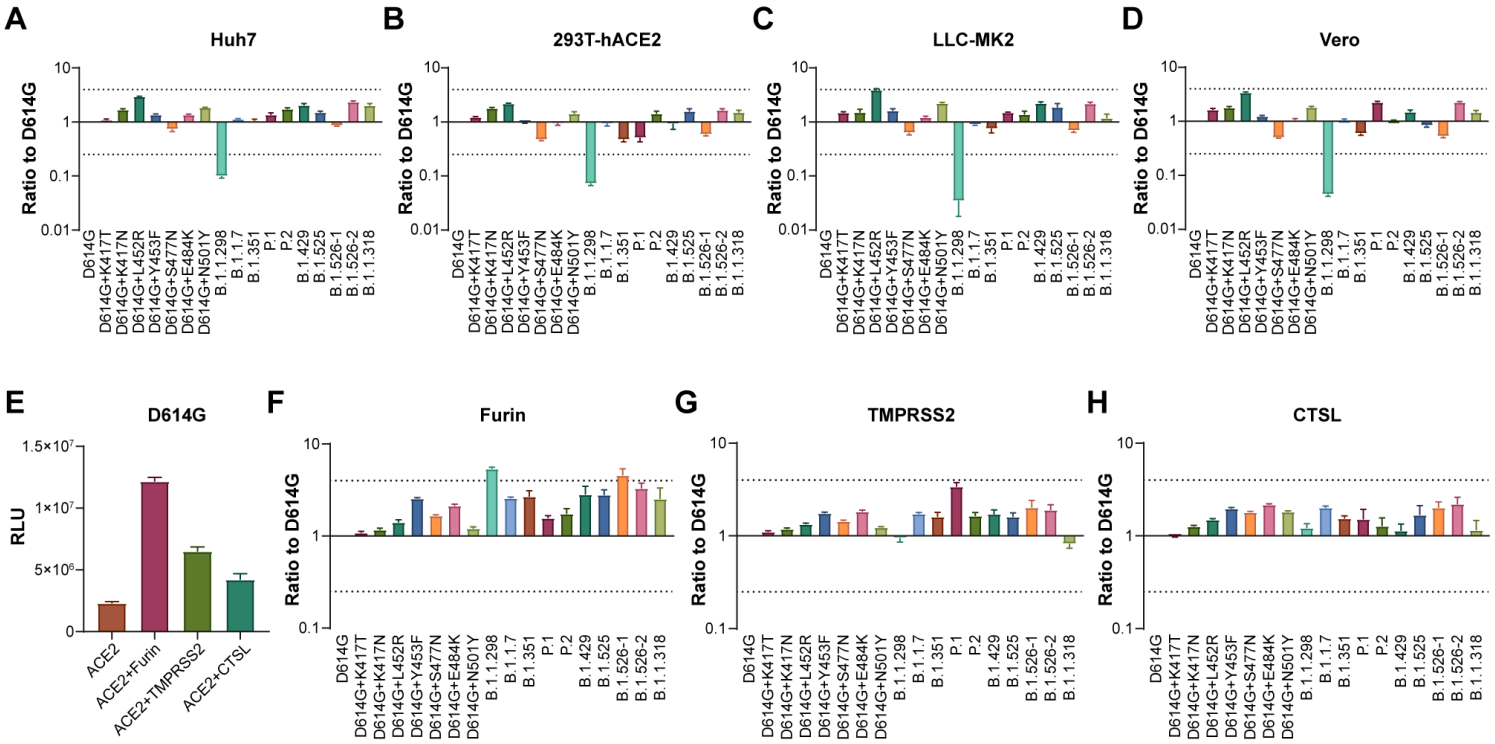

Figure 2

The infectivity of SARS-CoV-2 Variants in primate cell lines and Furin/TMPRSS2/CTSL expressed 293T-ACE2 cells A-D, SARS-CoV-2-susceptible cells as indicated were used to compare the infectivities among 10 SARS-CoV-2 variants. Pseudostyped virus was quantified via RT-PCR and all strains were diluted to the same copy number. Cells were harvested 24 hours after pseudostyped virus infection and analyzed for luminescence activities (RLUs). Relative infectivities compared with the D614G reference strain are displayed as the RLU ratio to D614G. E-H, Furin, TMPRSS2 and Cathepsin L (CTSL)-stably overexpressing 293T-hACE2 cells were used compared to mock 293T-hACE2 cells. All cells were infected with the same copy number of pseudotyped SARS-CoV-2 variants. Chemiluminescence signals were detected 24 hours later. Data shown indicate relative changes in infectivity due to the enzyme overexpression. Relative RLUs were compared with or without the indicated enzyme first, and then compared with the D614G reference strain. E, RLU of cells infected with D614G virus. F-H, Ratios by which enzyme overexpression induced enhanced infection, compared with D614G. The results were obtained from four independent experiments and the values shown indicate means ± SEMs. Dashed lines indicate the threshold of fourfold difference.

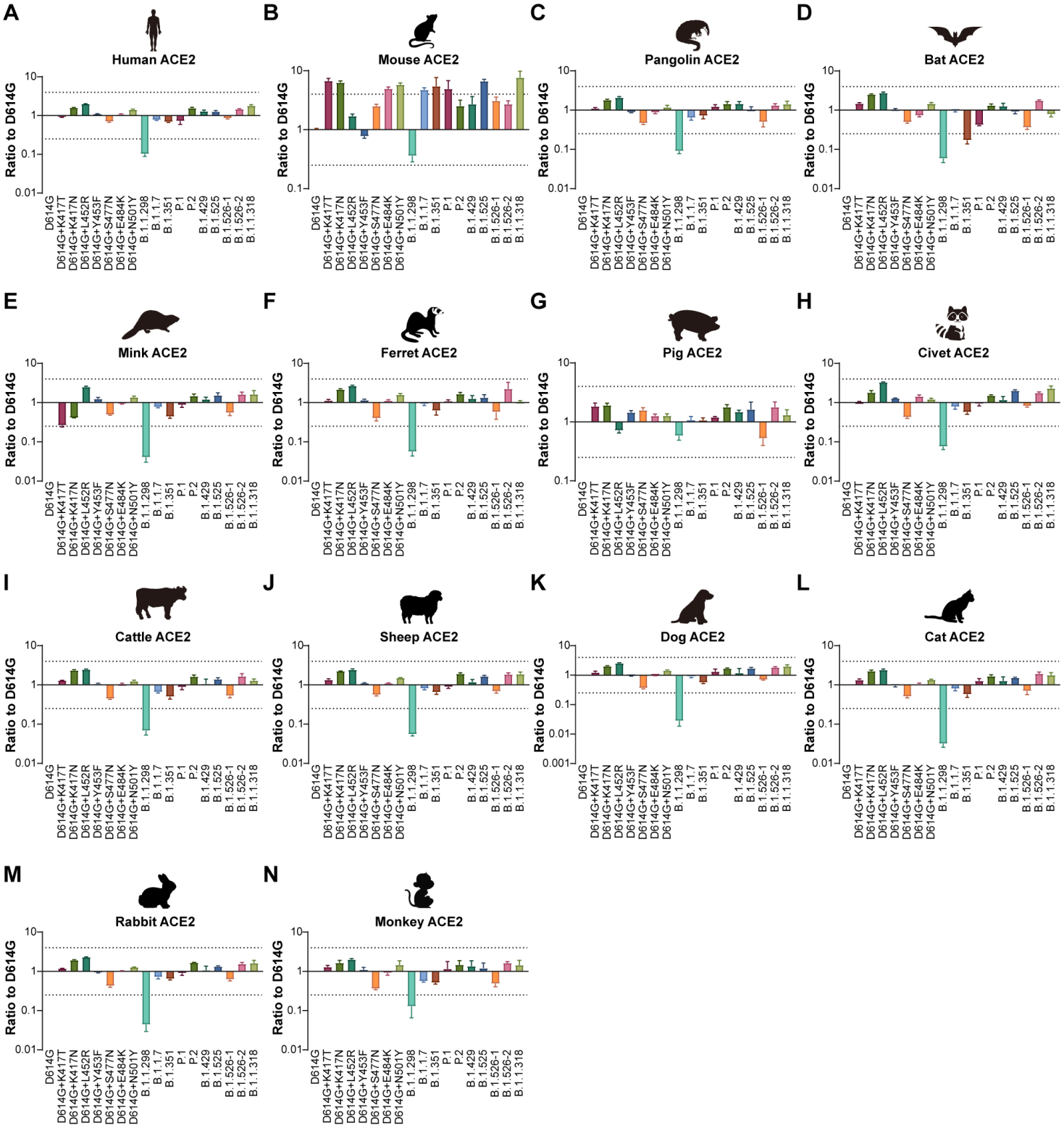

Figure 3

Infectivities of SARS-CoV-2 Variants in cell lines expressing ACE2 proteins from 14 different species. Equal amounts of overexpression plasmids carrying ACE2 sequences from different species were transfected into 293T cells. Transfection efficiency was confirmed by flow cytometry analysis of ACE2 surface expression. Cells were then infected with the same copy number of SARS-CoV-2 variants after quantification. Chemiluminescence signals were collected 24hours later. Ratios between variants and the

D614G reference strain were calculated. The results were obtained from four independent experiments and the values shown indicate means ± SEMs. Dashed lines indicate the threshold of fourfold difference.

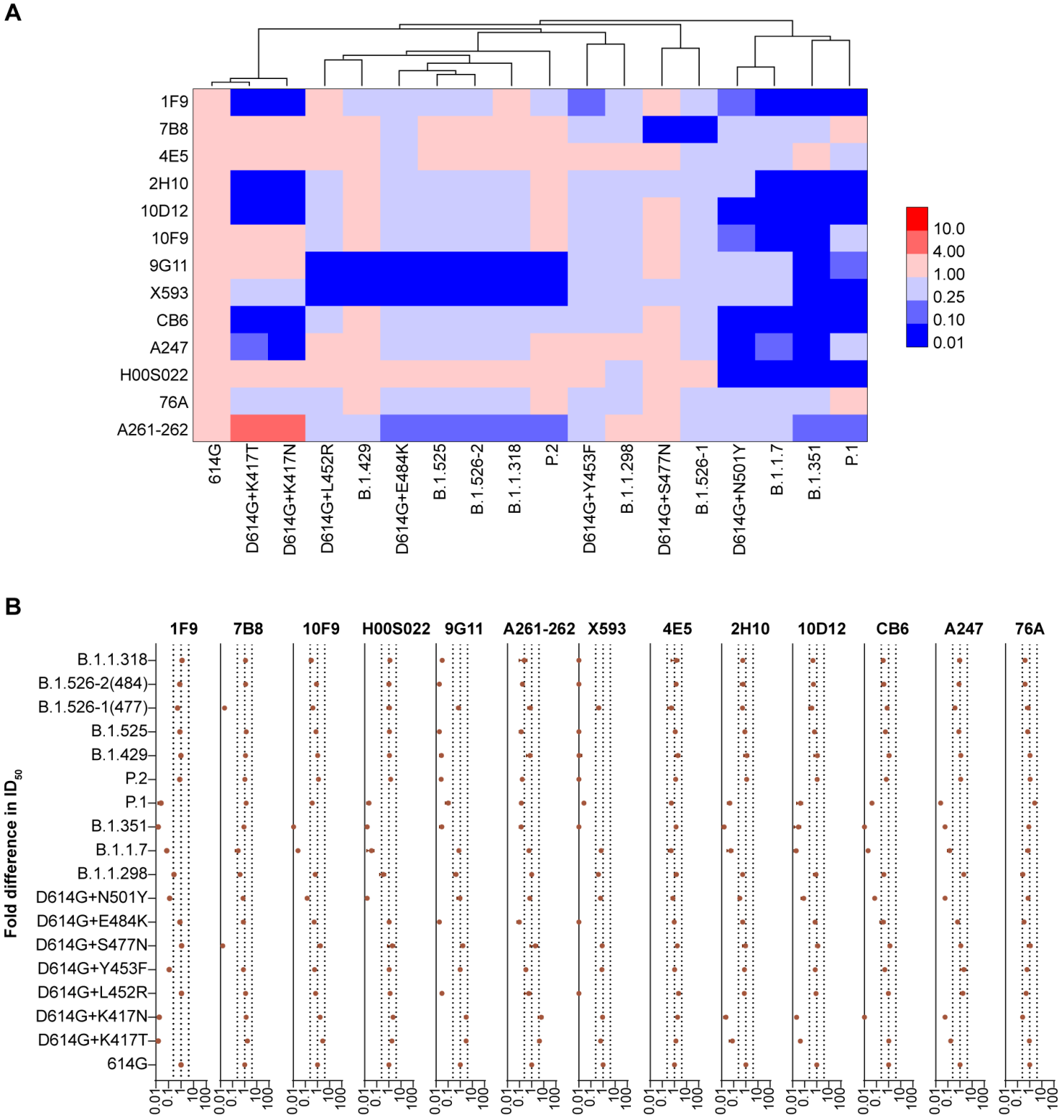

Figure 4

Neutralization analyses of mAbs. Thirteen mAbs were diluted serially and pre-incubated with pseudotyped SARS-CoV-2 variants at 37°C for 1 hour, then added to Huh-7 cell cultures. ID50 ratios between the variants and D614G reference strain are presented. A. Heat map of mean ID50 ratio,

compared with D614G. Red indicates increased neutralization activity and blue indicates decreased neutralization activity, as shown in the scale bar. B, ID50 ratios compared with D614G are presented as means±SEMs. Dashed lines indicate the threshold of fourfold difference. All experiments were repeated at least three times.

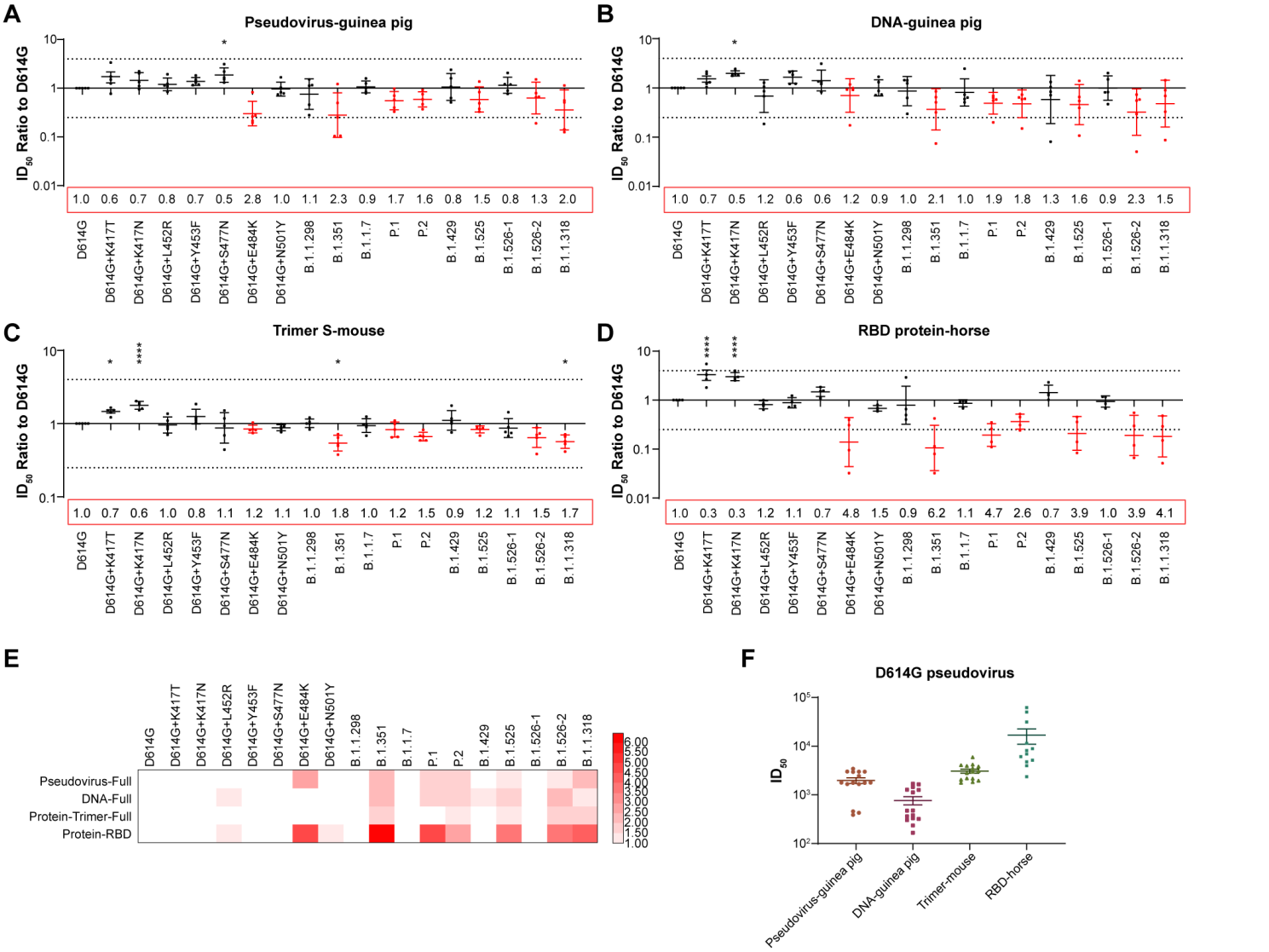

Figure 5

Neutralization analyses of animal immunized sera. Serially diluted serum preparations were incubated with pseudotyped SARS-CoV-2 variants as indicated. The remaining procedure is identical to the procedure in Fig. 5. ID50 ratios between the variants and D614G reference strain are presented. A-D, Dot plots of each serum sample against multiple variants. Data are presented as means±SEMs. Dashed lines indicate the threshold of fourfold difference. E484K-carrying variants are marked in red. Reduced neutralization, folds compared with the D614G reference strain, is labeled at the bottom of each plot. E. Heat map of mean ID50 ratio, compared with D614G. Shade of red indicates degree of reduced neutralization (folds), as shown in the scale bar. F. Neutralization ID50 values of different immunized serum samples against pseudotyped D614G virus. All experiments were repeated three times.

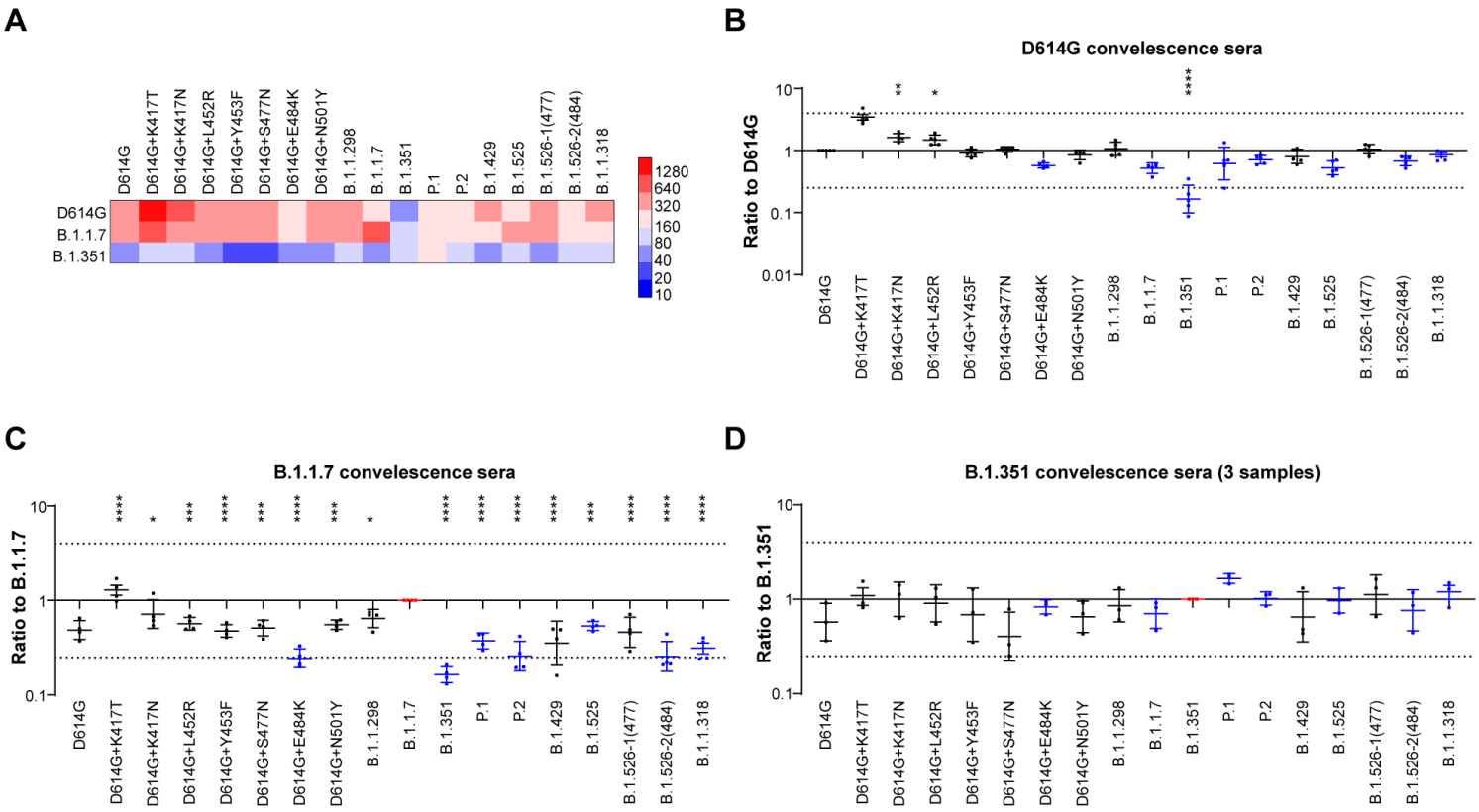

Figure 6

Neutralization analyses of convalescence sera from D614G-, B.1.1.7- and B.1.351- infected patients The procedures were identical to the methods used in animal serum neutralization analyses. A. Neutralization ID50 values of three different SARS-CoV-2 infected convalescence serum samples against pseudotyped SARS-CoV-2 variants. B, the ID50 ratios, compared with the D614G reference strain, are displayed using dot plots. C, the ID50 ratios, compared with the B.1.1.7 variant, are displayed using dot plots. D, the ID50 ratios, compared with the B.1.351 variant, are displayed using dot plots. Data are presented as means±SEMs. Dashed lines indicate the threshold of fourfold difference. The experiments were repeated twice due to the sample limitation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx