

Figure 1. The D614G mutation is associated with enhanced infectivity. Cryo-EM structure of S1 (grey) and S2 (orange) heterodimer (PBD 6VXX). The residues 581-676, a C-terminal region of the S1 domain involved in S2 interaction, is shown in green. Aspartic acid 614 is shown in light green. The area indicated with a black square is presented magnified at the right. Residues within 5.5 Å of D614 are shown in a ball-and-stick representation. **b**, A representation of the SARS-CoV-2 S protein (upper panel) and D/G variation at the residue 614 presented in logo plots at different time points between January 1st and May 30th, 2020 (lower panel). Total number of sequences analyzed: 17 in January, 33 in February, 293 in March, 1511 in April, and 2544 in May. NTD: N-terminal domain, RBD: Receptor-binding domain, FP: Fusion peptide, HR1 and HR2: Heptad-repeat region 1 and 2, respectively, TM: Transmembrane region, CT: Cytoplasmic tail. **c,d**, Mock- and hACE2-293T cells on 96-well plates were infected with MLV PV (5×10^8 vector genome per well) expressing GFP and pseudotyped with the indicated viral glycoprotein and analyzed 24 h later. Representative histograms (c) or mean \pm SEM (d) of five experiments conducted using two independent PV preparations are shown. Each dot in (d) indicates an average value of a duplicated experiment. Significant differences were analyzed by two-way ANOVA with Sidak multiple comparisons test. PV titers are presented in Extended Data Fig. 1. FKO: Furin-cleavage knockout mutant.

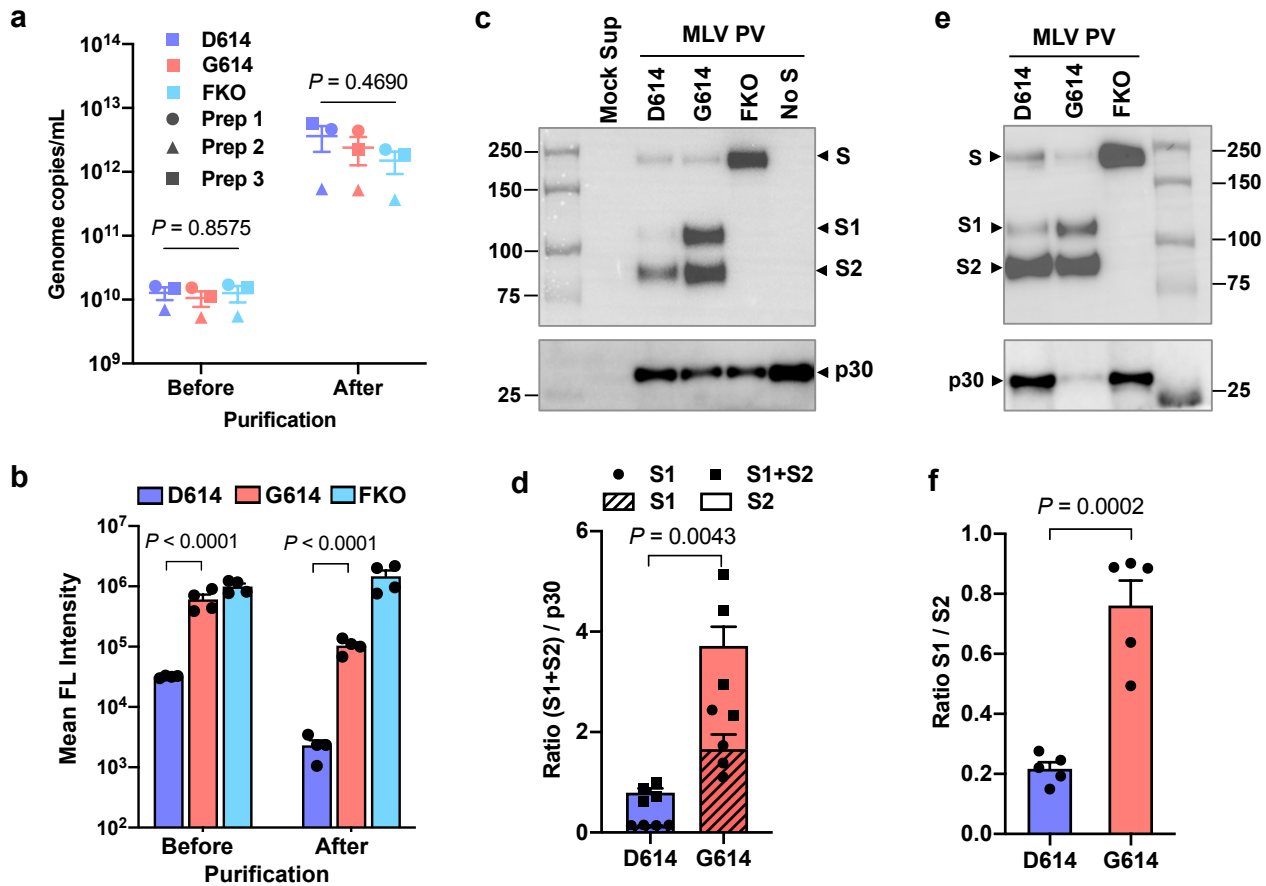


Figure 2. Superior infectivity of G614 results from decreased S1 shedding and higher level of S protein in the virion. a-f, Indicated MLV PVs produced with the S protein containing the Flag tag at both the N- and C-termini were partially purified and concentrated by pelleting through a 20% sucrose layer. PV titers were assessed by RT-qPCR (a). The same symbols in different PV groups indicate they are from the same batch. The same PVs were assessed for their infectivity in Mock- and hACE2-293T cells (b). Each symbol in (a,b) indicates an average value of a duplicated experiment. Mean \pm SEM of three independently prepared PVs (a) and four experiments using those three PV batches (b) are shown. The same amount (1×10^{10} vg per lane) (c,d) or to more accurately compare the S1 and S2 ratio, different amount (e) of the purified PVs were analyzed by WB using the anti-Flag M2 antibody or anti-p30 MLV gag antibody. A similar experiment performed with an independently prepared batch of PVs is shown in Extended Data Fig 2a. The same PVs visualized by silver stain is shown in Extended Data Fig. 2b. Total virion S protein (d) and the S1/S2 ratio (f) of PV^{D614} and PV^{G614} were calculated from four (d) or five (f) WBs performed with three independently prepared PV batches and presented as mean \pm SEM. Significant differences were analyzed by one-way ANOVA (a), two-way ANOVA with Sidak multiple comparison tests of log-transformed data (b), or unpaired Student's t-test (d,f).

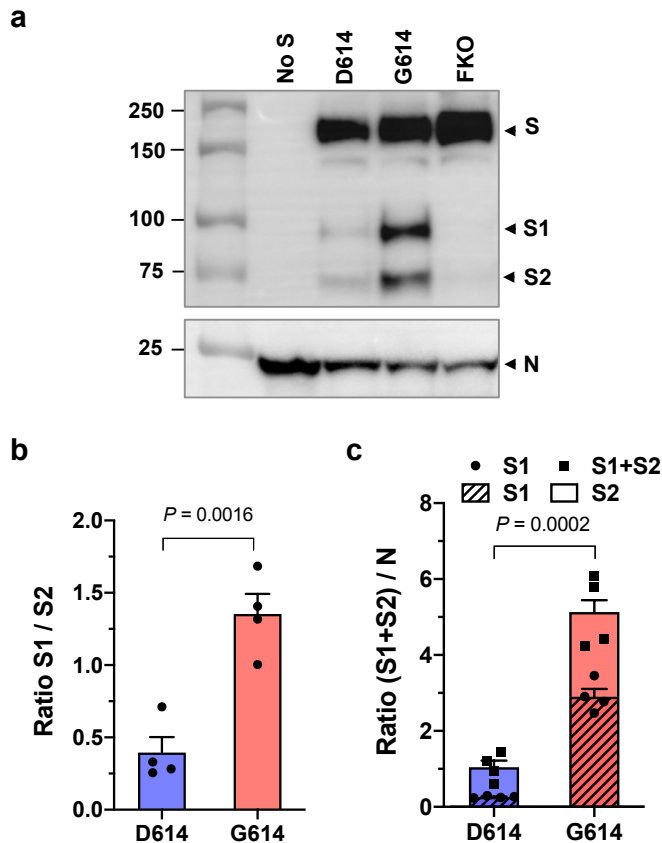


Fig. 3. SARS-CoV-2 VLP^{G614} also exhibits decreased S1 shedding and increased total virion S protein. a-c, VLPs were produced from HEK293T cell transfection of the M, N, E, and S proteins of SARS-CoV-2. VLPs were harvested from the culture supernatant and partially purified as MLV PVs. The S protein bands were visualized using the anti-Flag tag M2 antibody and the N protein band using pooled convalescent plasma (a). A representative of WBs performed with three independently prepared VLPs is shown. The S1/S2 ratio (b) and the difference in total virion S protein (c) incorporated into the VLP^{D614} and VLP^{G614} were calculated from four WBs performed with three independent VLP preparations and presented as mean \pm SEM. Significant differences were analyzed by unpaired Student's t-test.

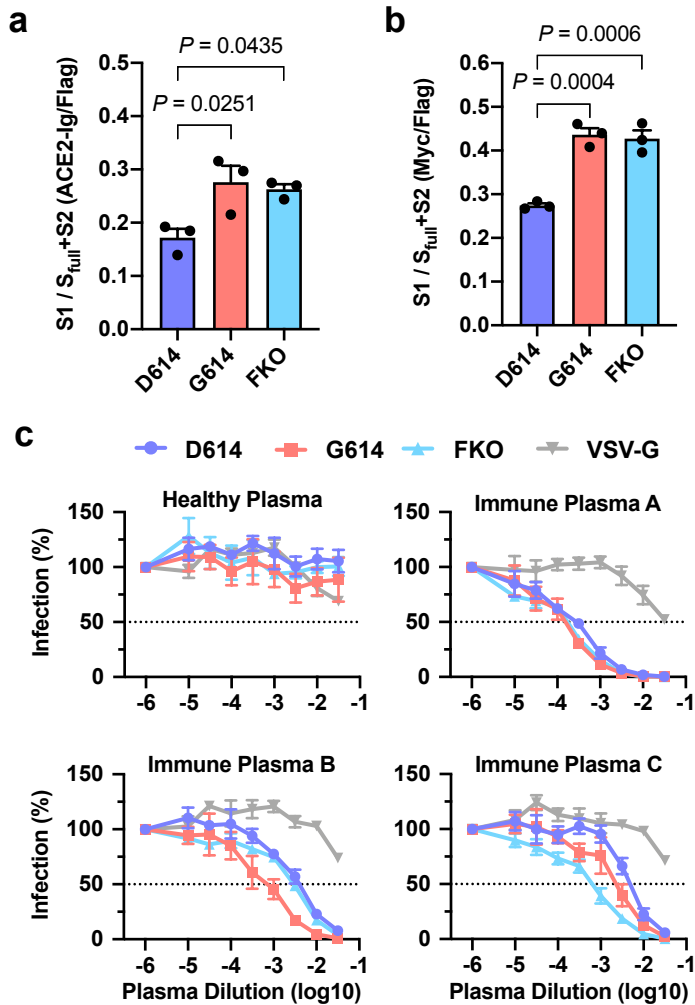
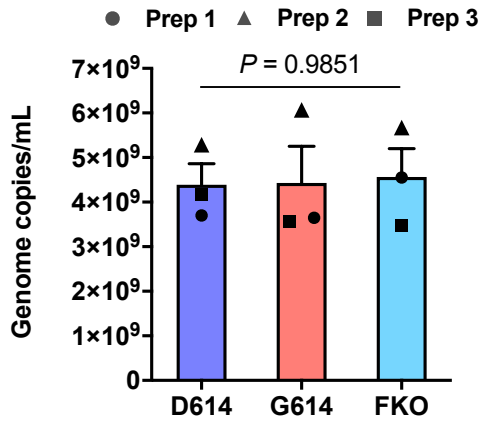
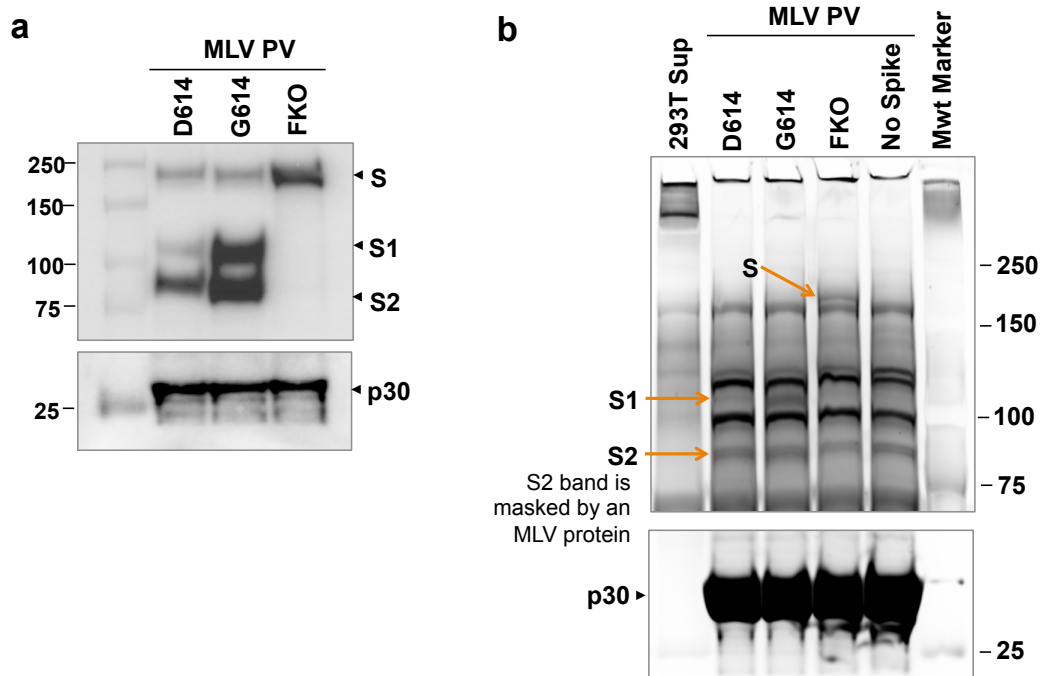


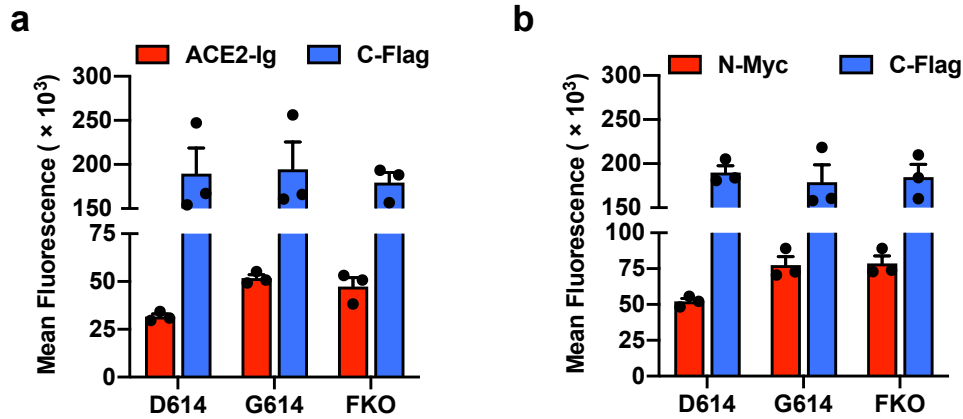
Fig 4. PV^{G614} is not more resistant to neutralization than does PV^{D614}. **a**, The S protein containing C-terminal Flag tag is transfected into HEK293T cells and assessed for hACE2-NN-Ig binding. Total S protein was measured by detecting the Flag tag in the permeabilized cells. The ratio of hACE2-NN-Ig binding to Flag-tag staining is shown. **b**, Experiments similar to those in (a) except the S protein contains N-Myc and C-Flag tags, and S1 level was assessed using an anti-Myc antibody. Each symbol in (a,b) indicates an average value of a duplicated experiment. The data in (a,b) before normalization are presented in Extended Data Fig. 3a,b. Mean \pm SEM of three independent experiments are presented. Significant differences were analyzed by one-way ANOVA and Sidak multiple comparisons test. **c**, MLV PVs expressing firefly luciferase and pseudotyped with the indicated S protein or VSV G protein were preincubated without (presented at $x = -6$) or with serially diluted plasmas derived from convalescent COVID-19 patients or a SARS-CoV naïve individual. hACE2-293T cells were infected with these preincubated mixes and infection was assessed 24 h later by measuring luciferase activity. Mean \pm SEM of three-five independent experiments are presented.



Extended Data Fig. 1. Titers of various MLV PVs. The titers of the MLV PVs used in the experiments shown in Fig. 1c,d were quantified by RT-qPCR. The same symbols in different PV groups indicate they were from the same batch. Each symbol indicates an average value of a duplicated experiment. Similar PV titers validate that enhanced infectivity of PV^{G614} was not resulted from a large difference in virus titers and/or normalization thereof. Mean \pm SEM of two independent PV preparations are shown. Significant differences were analyzed by one-way ANOVA.



Extended Data Fig. 2. Superior infectivity of G614 results from decreased S1 shedding and higher level of S protein in the virion. a, A similar experiment as that shown in Fig. 2c but performed with an independently prepared PV batch. **b**, The same PVs used in the experiment shown in Fig. 2c were analyzed by SDS-PAGE and silver stain to avoid a potential bias caused by using the M2 antibody that recognizes N- and C-terminal Flag tags with different affinity. Although the S2 band is masked by an MLV-derived protein, the S1 band is clearly separated and much weaker in PV^{D614} compared to that in PV^{G614}.



Extended Data Fig. 3. S^{G614} binding to hACE2 is not increased. The same data presented in Fig. 4a and b before normalized to total S protein level. **a**, The S protein containing C-terminal Flag tag is transfected into HEK293T cells and assessed for hACE2-NN-Ig binding. Total S protein was measured by detecting the Flag tag in the permeabilized cells. **b**, Experiments similar to those in (a) except the S protein contains N-Myc and C-Flag tags, and S1 level was assessed using an anti-Myc antibody. Each symbol in (a,b) indicates an average value of a duplicated experiment. Mean \pm SEM of three independent experiments are presented.