

Extended Data

“Increased Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7 to Antibody Neutralization”

Methods

Patients and vaccinees. Plasma samples were obtained from patients convalescing from documented SARS-CoV-2 infection approximately one month after recovery or later. These cases were enrolled into an observational cohort study of convalescent patients followed at the Columbia University Irving Medical Center starting in the Spring of 2020. The study protocol was approved by the Institutional Review Board (IRB), and all participants provided written informed consent. From their documented clinical profiles, plasma samples from ten with severe Covid-19 were selected, along with plasma from 10 with non-severe infection, for this study. Sera were obtained from 12 participants in a Phase 1 clinical trial of Moderna SARS-CoV-2 mRNA-1273 Vaccine conducted at the NIH, under an IRB-approved protocol. Sera were also obtained from 10 health care workers who received the Pfizer BNT162b2 Covid-19 Vaccine as a part of the vaccine rollout among hospital employees. These cases were then enrolled into an IRB-approved protocol to assess immunological responses to SARS-CoV-2.

Monoclonal antibodies. Monoclonal antibodies tested in this study were constructed and produced at Columbia University as previously described¹⁸, except REGN10933, REGN10987, REGN10985, COV2-2196, and COV2-2130 were provided by Regeneron Pharmaceuticals, Inc., Bii-196 and Bii-198 were provided by Bii Biosciences, and CB6 was provided by B.Z. and P.D.K.

Construction and production of variant pseudoviruses. The original pCMV3-SARS-CoV-2-spike plasmid was kindly provided by Dr. Peihui Wang of Shandong University in China. Plasmids encoding for D614G variant, all the single-mutation variants found in B.1.1.7 or B.1.351, 8-mutation-combination variant (UKΔ8) and 9-mutation-combination variant (SAΔ9) were generated by Quikchange II XL site-directed mutagenesis kit (Agilent). Recombinant Indiana VSV (rVSV) expressing different SARS-CoV-2 spike variants were generated as previously described^{18,19,29}. HEK293T cells were grown to 80% confluency before transfection with the spike gene using Lipofectamine 3000 (Invitrogen). Cells were cultured overnight at 37°C with 5% CO₂, and VSV-G pseudo-typed ΔG-luciferase (G*ΔG-luciferase, Kerafast) was used to infect the cells in DMEM at an MOI of 3 for 2 hours before washing the cells with 1X DPBS three times. The next day, the transfection supernatant was harvested and clarified by centrifugation at 300 g for 10 min. Each viral stock was then incubated with 20% I1 hybridoma (anti-VSV-G, ATCC: CRL-2700) supernatant for 1 hour at 37°C to neutralize contaminating VSV-G pseudo-typed ΔG-luciferase virus before measuring titers and making aliquots to be stored at -80°C.

Pseudovirus neutralization assays. Neutralization assays were performed by incubating pseudoviruses with serial dilutions of mAbs or heat-inactivated plasma or sera, and scored by the reduction in luciferase gene expression^{18,19}. In brief, Vero E6 cells were seeded in a 96-well plate at a concentration of 2×10^4 cells per well. Pseudoviruses were incubated the next day with serial dilutions of the test samples in triplicate for 30 minutes at 37°C. The mixture was added to cultured cells and incubated for an additional 24 hours. The luminescence was measured by Luciferase Assay System (Promega). IC₅₀

was defined as the dilution at which the relative light units were reduced by 50% compared to the virus control wells (virus + cells) after subtraction of the background in the control groups with cells only. The IC₅₀ values were calculated using non-linear regression in GraphPad Prism.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. Materials used in this study will be made available but may require execution of a materials transfer agreement. Source data are provided herein.

Additional references:

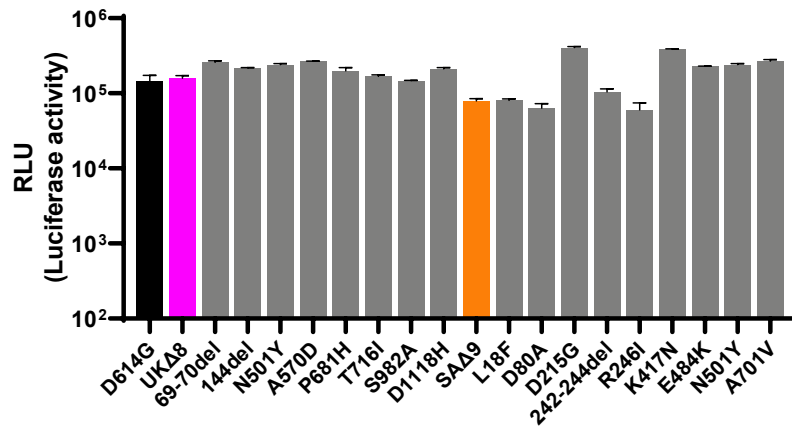
29. Han, Y., *et al.* Identification of SARS-CoV-2 inhibitors using lung and colonic organoids. *Nature* **589**, 270-275 (2021).
30. Henderson, R., *et al.* Controlling the SARS-CoV-2 spike glycoprotein conformation. *Nat Struct Mol Biol* **27**, 925-933 (2020).

Acknowledgements. We thank Stephen Goff and Brandon DeKosky for helpful discussions. This study was supported by funding from Andrew & Peggy Cherng, Samuel Yin, Barbara Picower and the JPB Foundation, Bria Biosciences, Roger & David Wu, and the Bill and Melinda Gates Foundation.

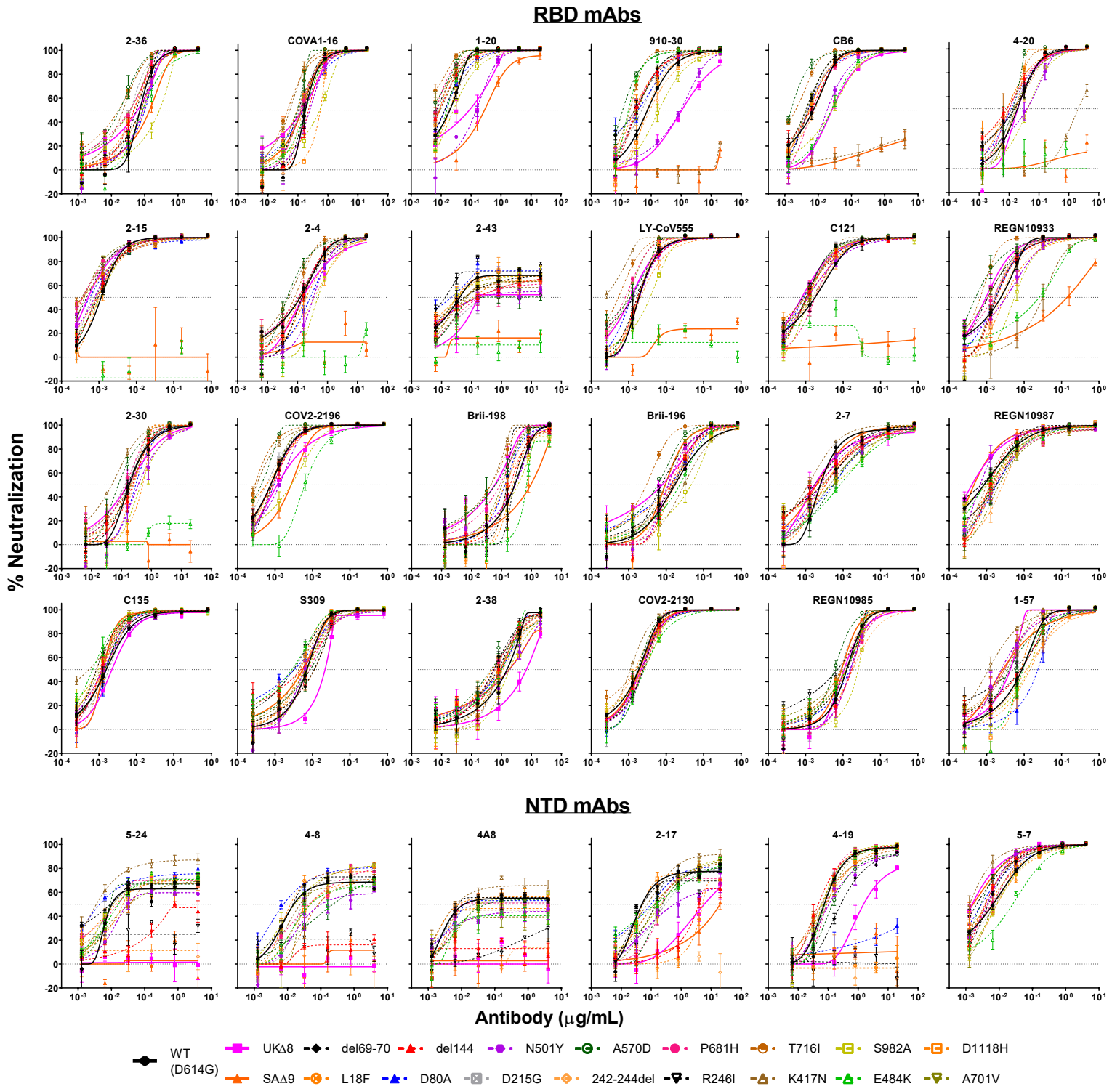
Author contributions. The study was conceptualized by D.D.H. The experiments were principally carried out by P.W., L.L., and S.I. with able assistance from M.W., J.Y., M.N. Structural interpretations were made by Y.G., Z.S., L.S., and P.D.K. B.Z., P.D.K., and C.A.K. provided mAbs. J.Y.C. and M.T.Y. provided plasma from convalescent patients.

B.S.G. and J.R.M. provided sera from participants in the Moderna vaccine trial; J.Y.C. and M.S. provided sera from health care workers immunized with the Pfizer vaccine. Y.H. and Y.L. helped to supervise the study. The manuscript was written by D.D.H. with editing by P.W., P.D.K., L.S., Y.L., and reviewed, commented, and approved by all the authors.

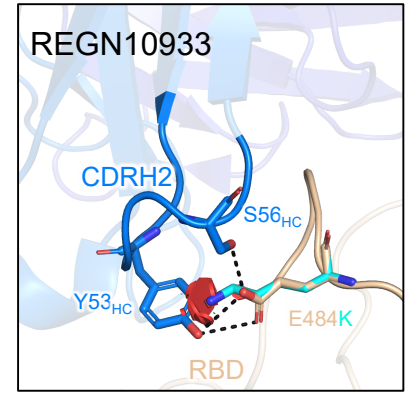
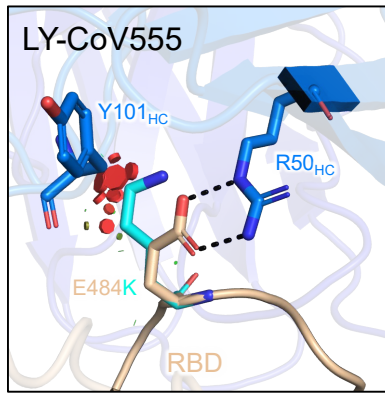
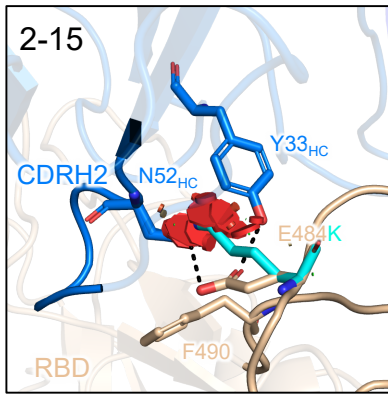
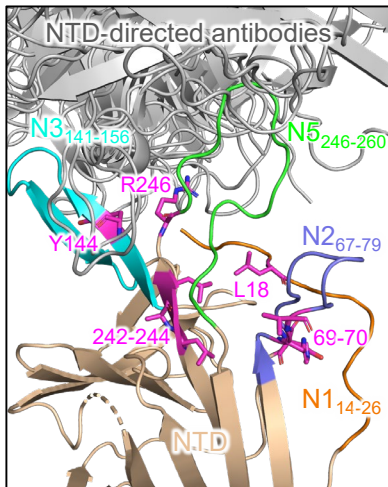
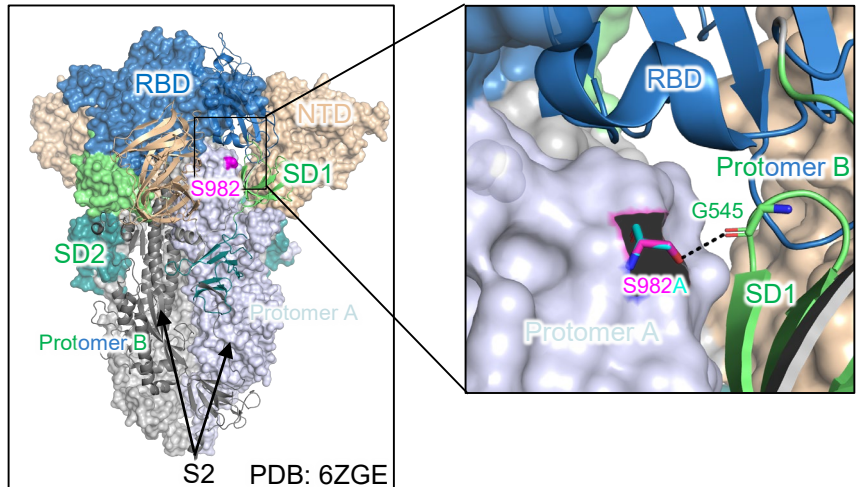
Competing interests: P.W., L.L., J.Y., M.N., Y.H., and D.D.H. are inventors on a provisional patent application on mAbs to SARS-CoV-2. D.D.H. is a member of the scientific advisory board of Bii Biosciences, which also has provided a grant to Columbia University to support this and other studies on SARS-CoV-2.



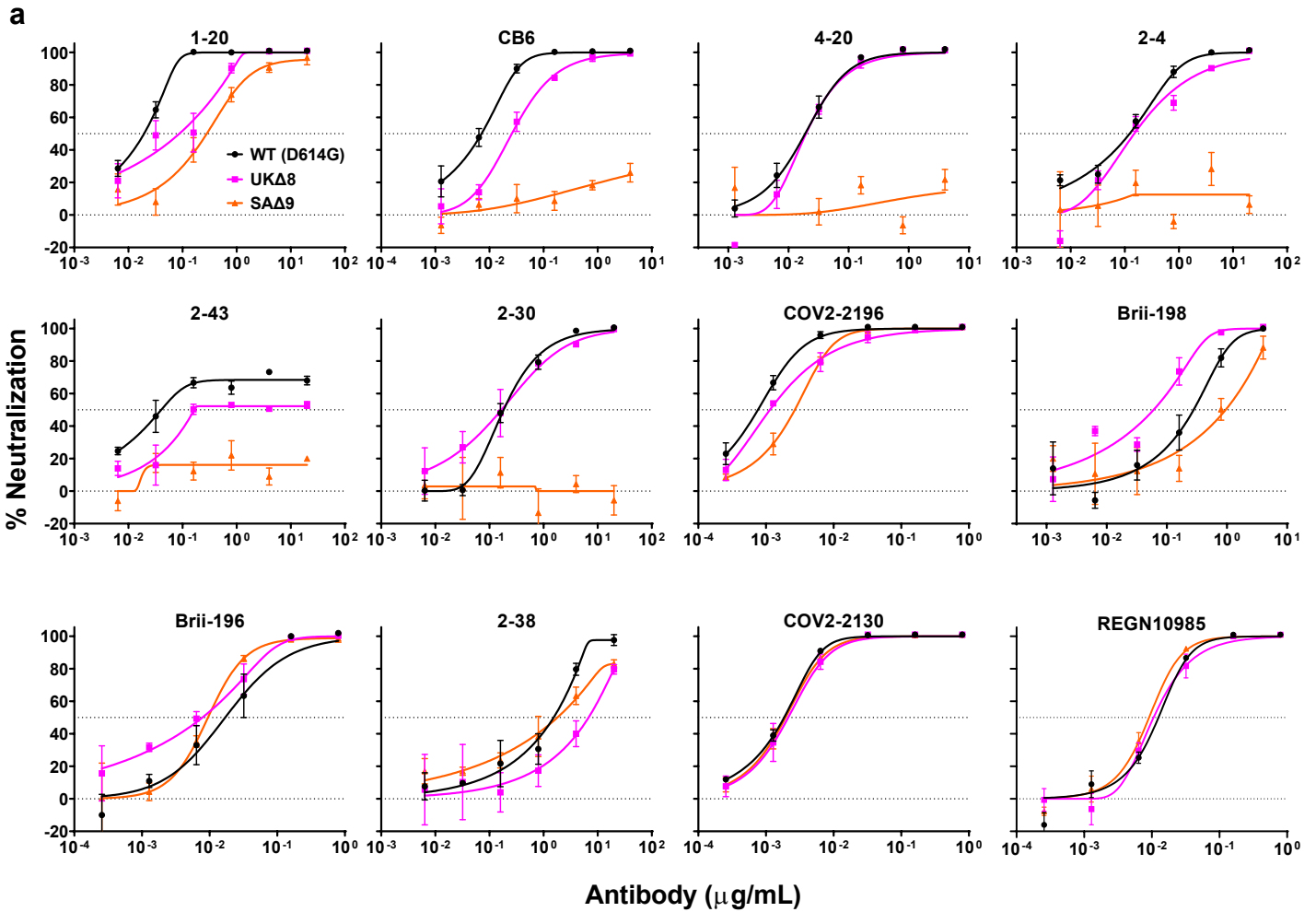
Extended Data Fig. 1 | Titers of WT (D614G) and the 18 mutant SARS-CoV-2 pseudoviruses. VSV-based pseudoviruses were generated^{18,19} and viral particles were quantified and normalized by VSV nucleocapsid protein by western blot. Equal amount of each pseudovirus was then used to infect Vero E6 cells and relative luciferase unit (RLU) was measured 16 hrs later.



Extended Data Fig. 2 | Neutralization profiles of mAbs against WT, UK Δ 8, and SA Δ 9, as well as single-mutation pseudoviruses. Data represent mean \pm SEM of technical triplicates.

a**b****c**

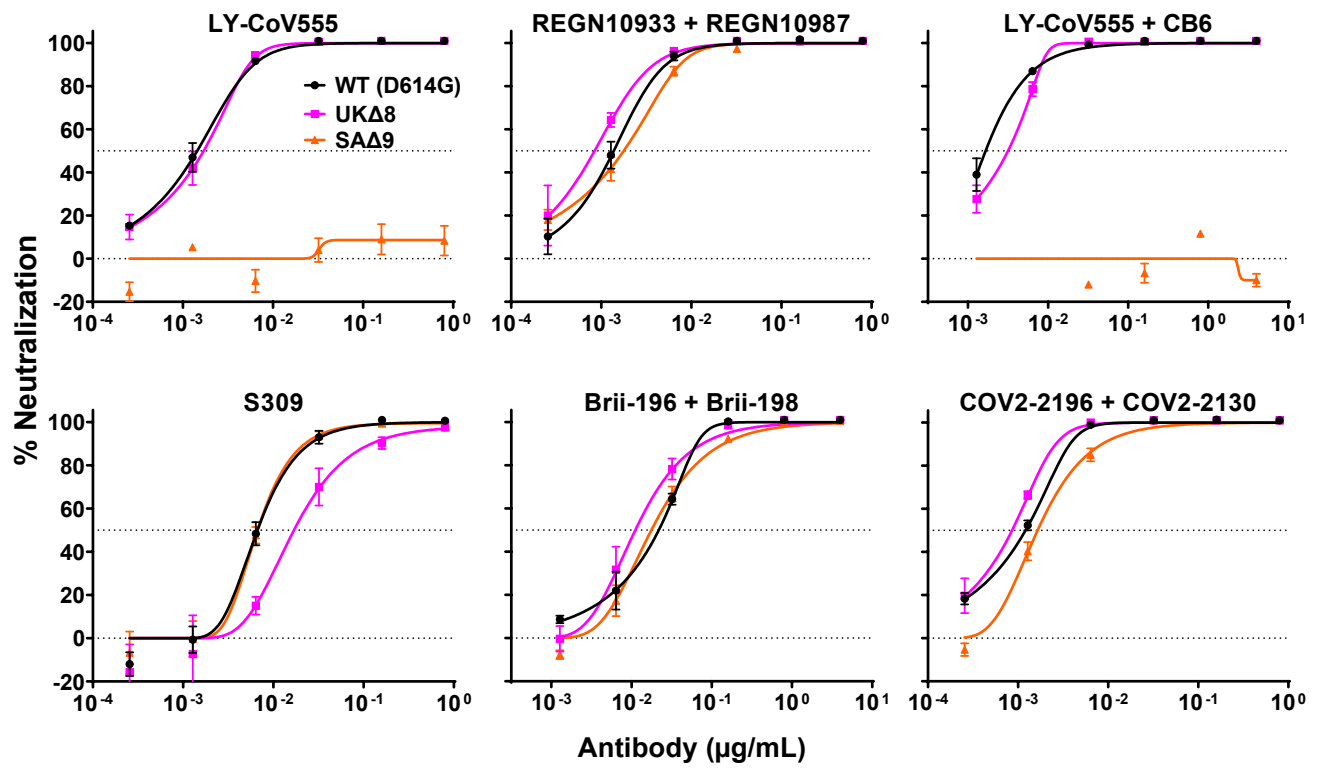
Extended Data Fig. 3 | Structural explanations on how critical mutations affect mAb activity. **a**, E484 forms hydrogen bonds with mAbs that target RBM. Mutation E484K causes steric clashes at antibody binding sites, and thus abolishes binding by these RBM-directed mAbs. Steric clashes are shown by red plates. **b**, Mutations at or near the NTD antigenic supersite – comprised of loops N1, N3, and N5 – that is recognized by many potent NTD-directed mAbs. **c**, S982 forms a hydrogen bond with G545 (SD1 domain) from an adjacent protomer. Because SD1 plays a role in modulating RBD conformation³⁰, the abolishment of the hydrogen bond by mutation S982A may affect RBD conformation dynamics.



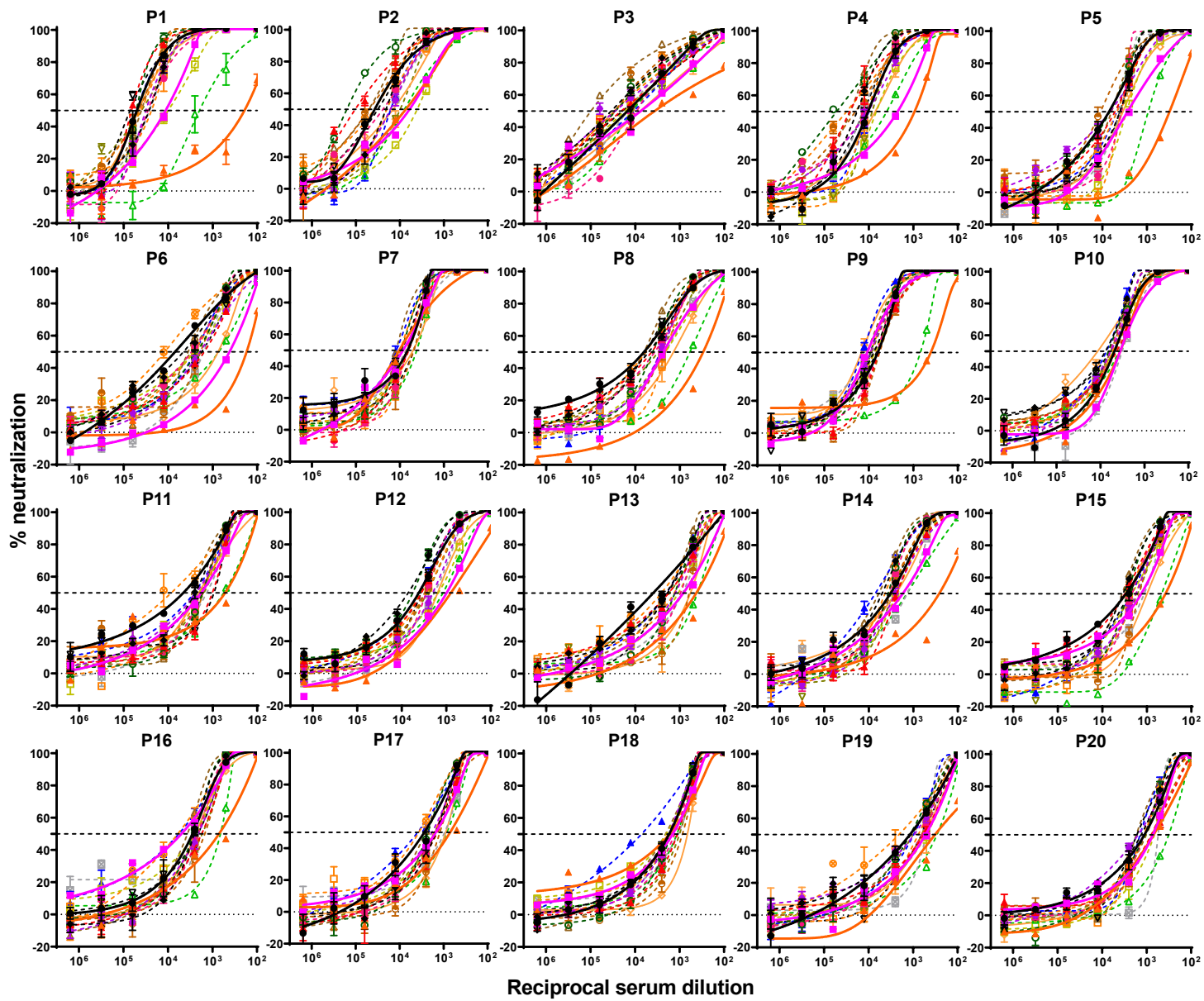
Fold change of IC50 from WT		RBD-directed mAbs											
		1-20	CB6	4-20	2-4	2-43	2-30	COV2-2196	Brie-198	Brie-196	2-38	COV2-2130	REGN10985
UK	UKΔ8	-4.5	-3.6	-1.0	-1.2	-4.1	1.1	-1.5	4.6	2.2	-4.4	-1.1	1.2
	69-70del	1.5	1.5	1.2	-1.2	-1.4	1.1	1.0	1.1	1.1	2.8	-1.3	-1.0
	144del	2.2	1.2	2.2	-1.2	-3.7	-1.3	-1.1	1.4	1.2	1.6	-1.0	-1.4
	N501Y	-7.7	-2.6	-2.1	-2.8	-4.8	-2.0	-1.7	-1.0	1.5	-1.2	-1.3	-1.3
	A570D	4.6	4.6	1.4	2.7	-5.3	2.3	1.8	4.4	2.9	3.4	1.1	2.1
	P681H	2.9	1.1	1.8	1.2	-4.5	1.3	-1.2	3.1	1.5	2.7	-1.4	-1.3
	T716I	10.0	3.4	1.9	1.5	-1.5	-1.1	2.6	6.3	5.7	1.7	1.2	1.4
	S982A	-1.3	-3.6	-2.2	-3.9	1.2	-2.4	-3.6	-1.3	-2.7	-1.2	-1.4	-2.0
D1118H	1.0	1.4	1.1	-1.6	1.2	-2.8	1.3	1.2	1.2	1.8	-1.2	1.0	
SA	SAΔ9	-14.4	<-1000	<-1000	<-1000	<-1000	<-1000	-3.5	-3.4	1.8	-1.2	-1.0	1.4
	L18F	2.2	1.3	2.2	-1.0	-2.8	-1.1	1.0	2.1	1.1	3.1	-1.2	1.1
	D80A	1.4	1.4	1.6	-2.4	1.9	-1.1	-1.2	2.1	1.4	1.6	-1.0	-1.2
	D215G	2.4	1.6	1.3	-1.4	-5.6	1.4	1.3	1.7	1.0	1.5	1.1	1.4
	242-244del	5.1	-1.1	1.5	-2.1	1.2	1.1	1.0	1.7	-1.1	1.4	1.3	-1.4
	R246I	2.8	1.7	1.3	-1.1	3.0	1.2	-1.1	1.9	2.3	2.0	-1.2	1.8
	K417N	1.1	<-1000	-108.3	2.5	-1.7	3.8	2.4	6.3	-1.8	2.5	1.7	1.1
	E484K	1.3	-3.5	<-1000	<-1000	<-1000	<-1000	-8.1	-2.5	-1.4	1.5	-1.4	1.0
	N501Y	-7.7	-2.6	-2.1	-2.8	-4.8	-2.0	-1.7	-1.0	1.5	-1.2	-1.3	-1.3
A701V	2.1	1.6	1.1	1.1	-1.6	-1.2	-1.1	2.7	1.8	1.7	-1.0	1.2	

Red: resistance >3 fold; Green: sensitization >3 fold

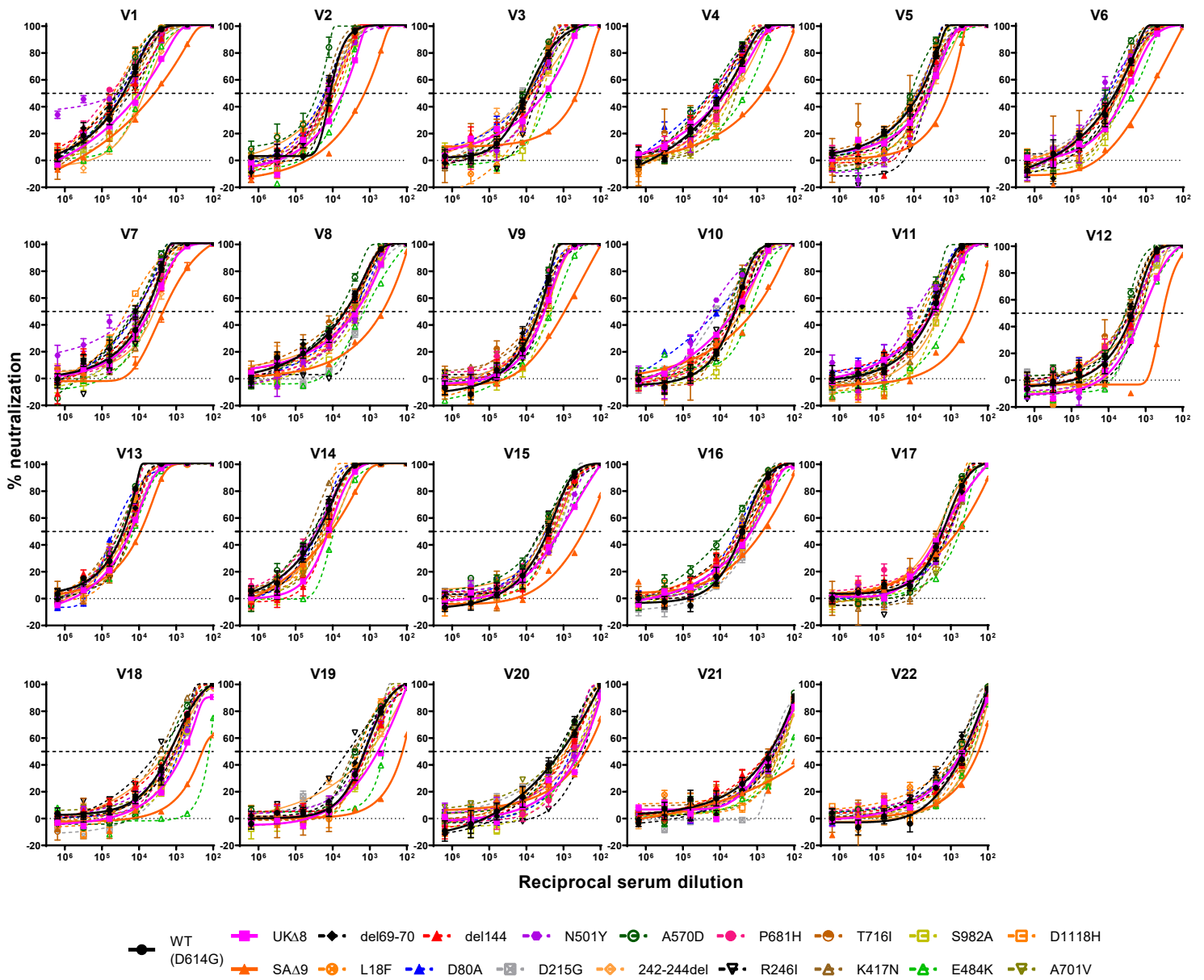
Extended Data Fig. 4 | Neutralization susceptibility of UK and SA variants to additional SARS-CoV-2 RBD-directed mAbs. **a**, Neutralization of WT, UKΔ8, and SAΔ9 pseudoviruses by additional RBD-directed mAbs. Data represent mean ± SEM of technical triplicates. **b**, Fold-change in IC50 of neutralizing mAbs against UKΔ8 and SAΔ9, as well as single-mutation pseudoviruses, relative to WT.



Extended Data Fig. 5 | Neutralization profiles of authorized or investigational therapeutic mAbs against WT, UKΔ8, and SAA9. Data represent mean ± SEM of technical triplicates.



Extended Data Fig. 6 | Neutralization profiles of convalescent patient plasma against WT, UKΔ8, SAΔ9, and single-mutation pseudoviruses. 20 plasma from SARS-CoV-2 convalescent patients were tested. Data represent mean ± SEM of technical triplicates.



Extended Data Fig. 7 | Neutralization profiles of vaccinee sera against WT, UKΔ8, SAΔ9, and single-mutation pseudoviruses. 12 sera from Moderna vaccinees (V1-V12) and 10 sera from Pfizer vaccinees (V13-V22) were tested. Data represent mean ± SEM of technical triplicates.