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Supplemental information

Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization

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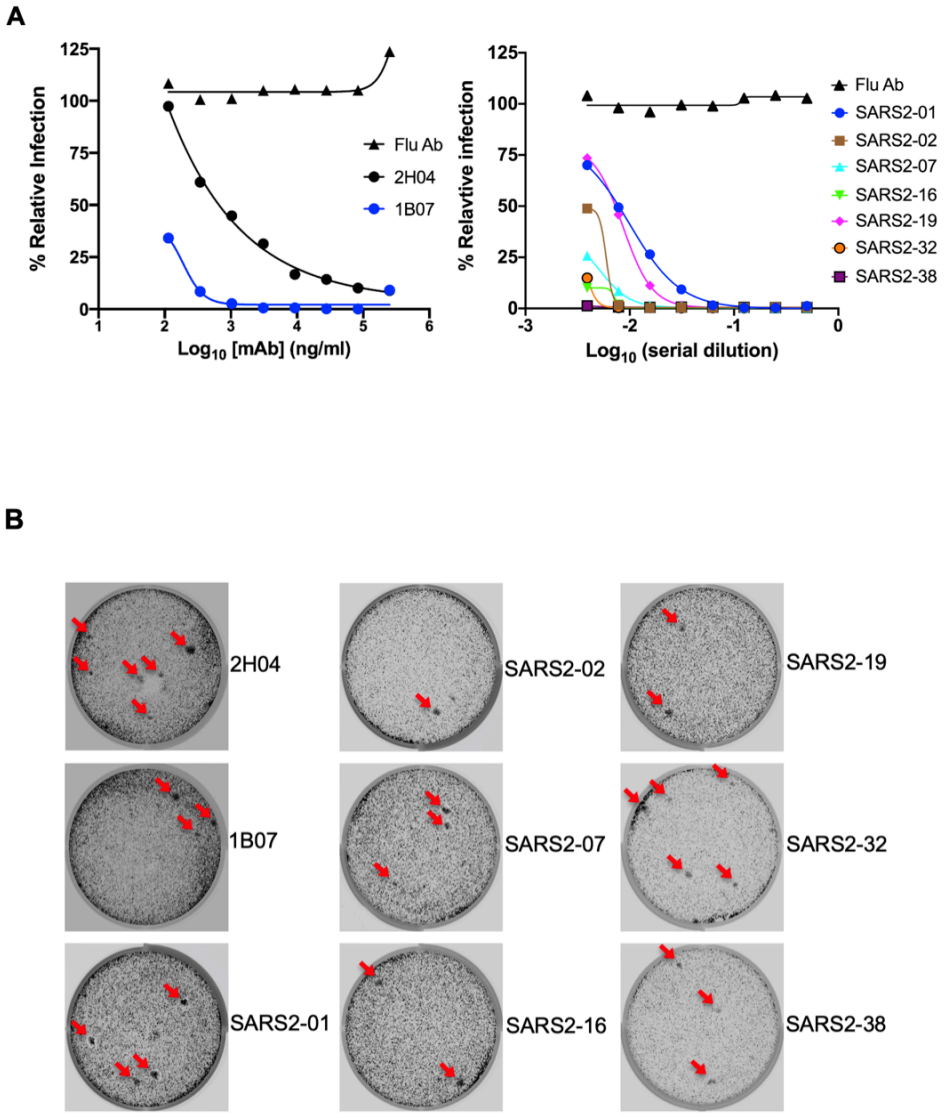

Figure S1. Isolation of VSV-SARS-CoV-2 escape mutants by plaque assay. Related to Fig 2. (**A**) RBD-specific antibodies were tested for neutralizing activity against VSV-SARS-CoV-2. MAbs in the left panel were purified from Expi293F cells transfected with antibody expression vector (pABVec6W) expressing heavy chain V-D-J and light Chain V-J cloned from single B cells. MAbs in the right panel were from hybridomas that bound to SARS-CoV-2-infected Vero CCL81 cells by flow cytometry. Data are representative of two independent experiments. (**B**) Plaque assays were performed to isolate the VSV-SARS-CoV-2-S escape mutant on Vero E6 TMPRSS2 cells in the present of the indicated mAb in the overlay. Representative images of two independent experiments are shown.

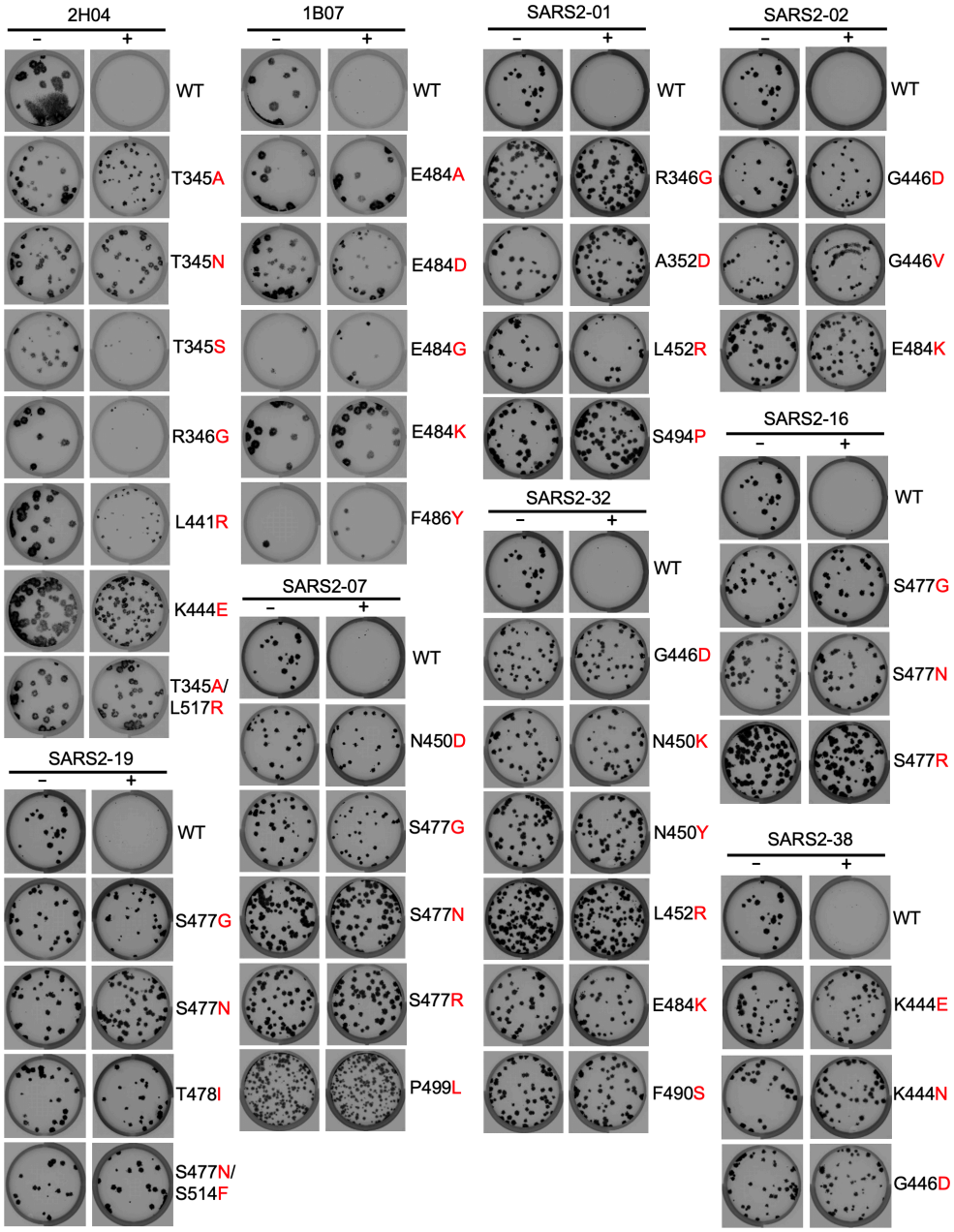

Figure S2. Validation of selected VSV-SARS-CoV-2 mutants. Related to Fig 2. Plaque assays were performed to validate the VSV-SARS-CoV-2 mutant on Vero cells in the presence and absence of the mAb in the overlay. MAb concentrations added in the overlay were the same as those used to select the escape mutants. Representative images of two independent experiments are shown.

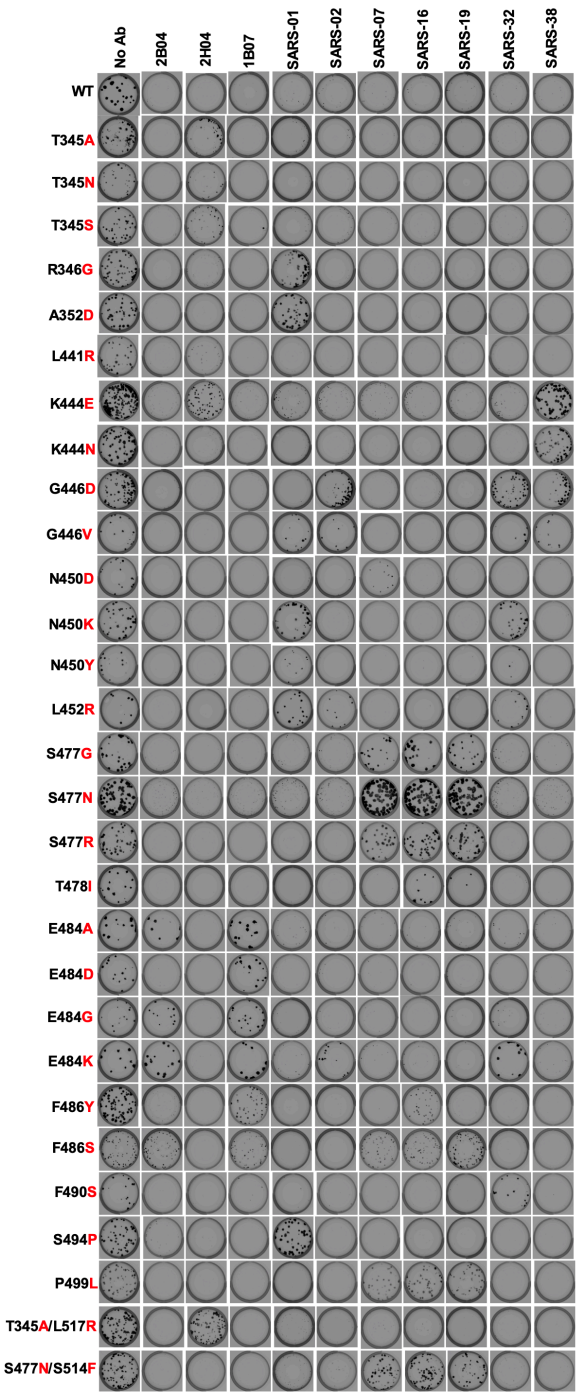

Figure S3. Plaque assay validation of cross-neutralization of VSV-SARS-CoV-2 mutants. Related to Fig 3A. Wild-type and identified VSV-SARS-CoV-2 mutants were tested for neutralizing activity using a plaque assay with the indicated mAb in the overlay. MAb concentrations added were the same as those used to select the escape mutants. Representative images of two independent experiments are shown.

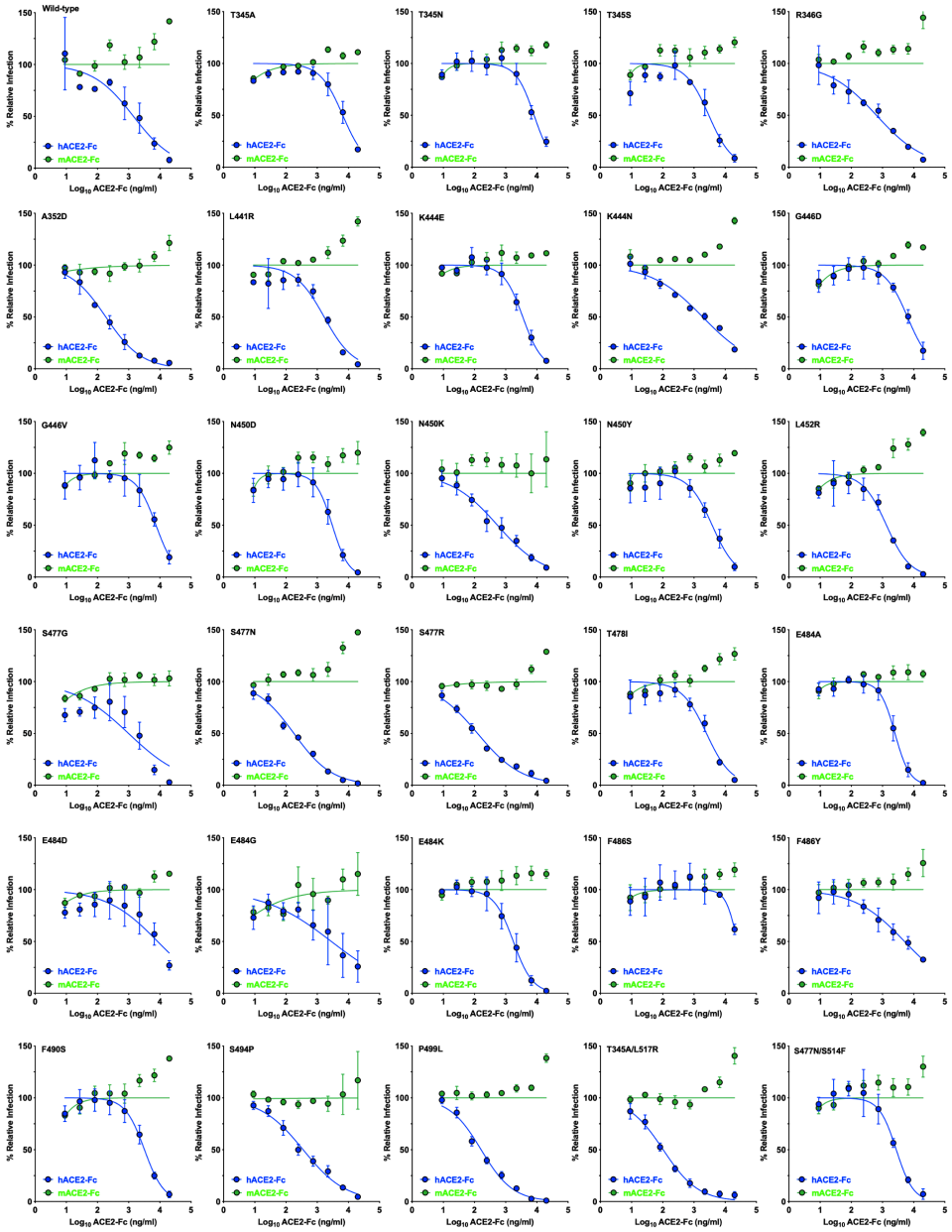

Figure S4. Neutralization of VSV-SARS-CoV-2 mutants by hACE2 decoy receptors. Related to Fig 3B and 3C. hACE2-Fc or mACE2-Fc were tested for neutralizing activity against wild-type and mutant VSV-SARS-CoV-2 (n=3). Error bars represent the SEM. Data are representative of three independent experiments.

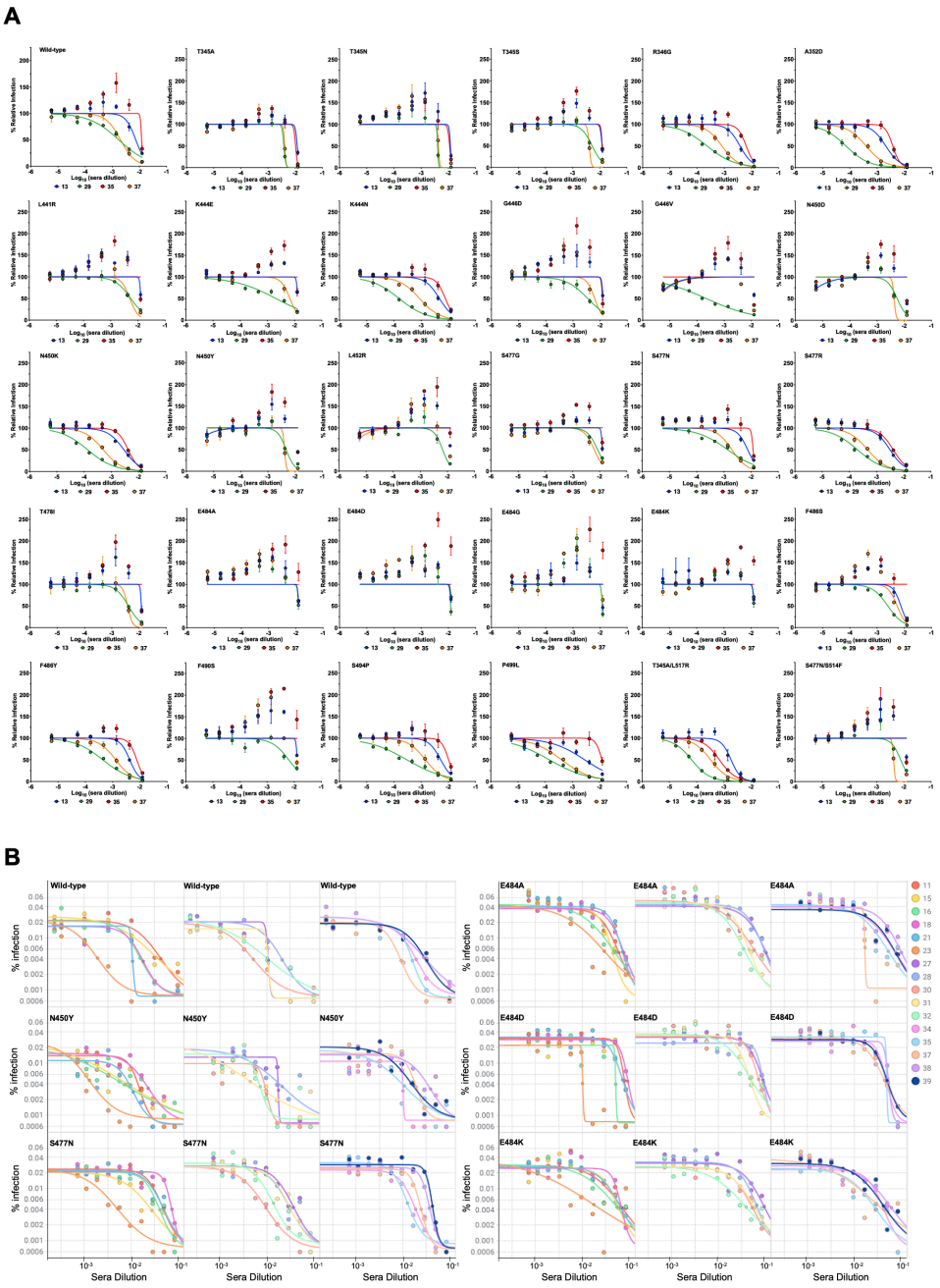

Figure S5. Neutralization of VSV-SARS-CoV-2 mutants by human sera. Related to Fig 4. (**A**) Four human sera were tested for neutralization of wild-type and mutant VSV-SARS-CoV-2 (n = 3). Error bars represent the SEM. Data are representative of three independent experiments. Related to **Fig 4A and 4B**. (**B**) Sixteen human sera were tested for neutralization of wild-type and 5 mutant VSV-SARS-CoV-2 (n = 1). Related to **Fig 4C**.

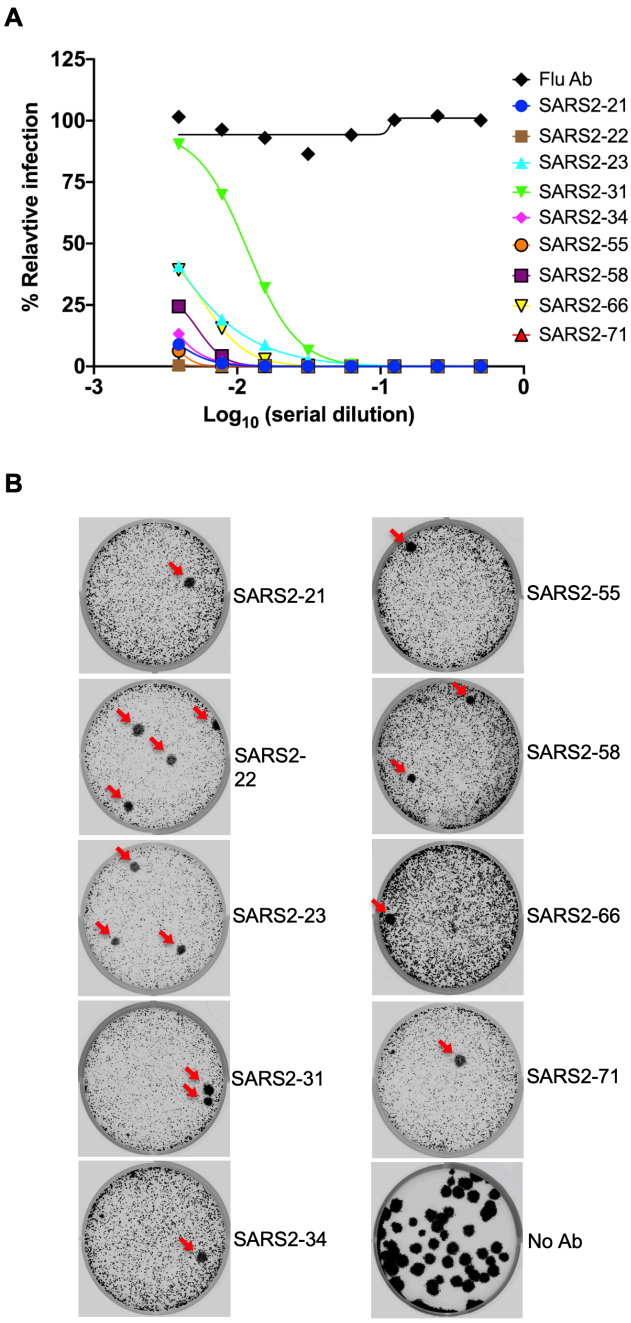

Figure S6. A second neutralization escape selection campaign with nine additional mAbs. Related to Fig 5. (**A**) Nine additional RBD-specific antibodies were tested for neutralization activity against VSV-SARS-CoV-2. Data are representative of two independent experiments. (**B**) Plaque assays were performed to isolate the VSV-SARS-CoV-2 escape mutant on Vero E6 TMPRSS2 cells in the presence of the indicated mAb in the overlay. Representative images of six independent experiments are shown.

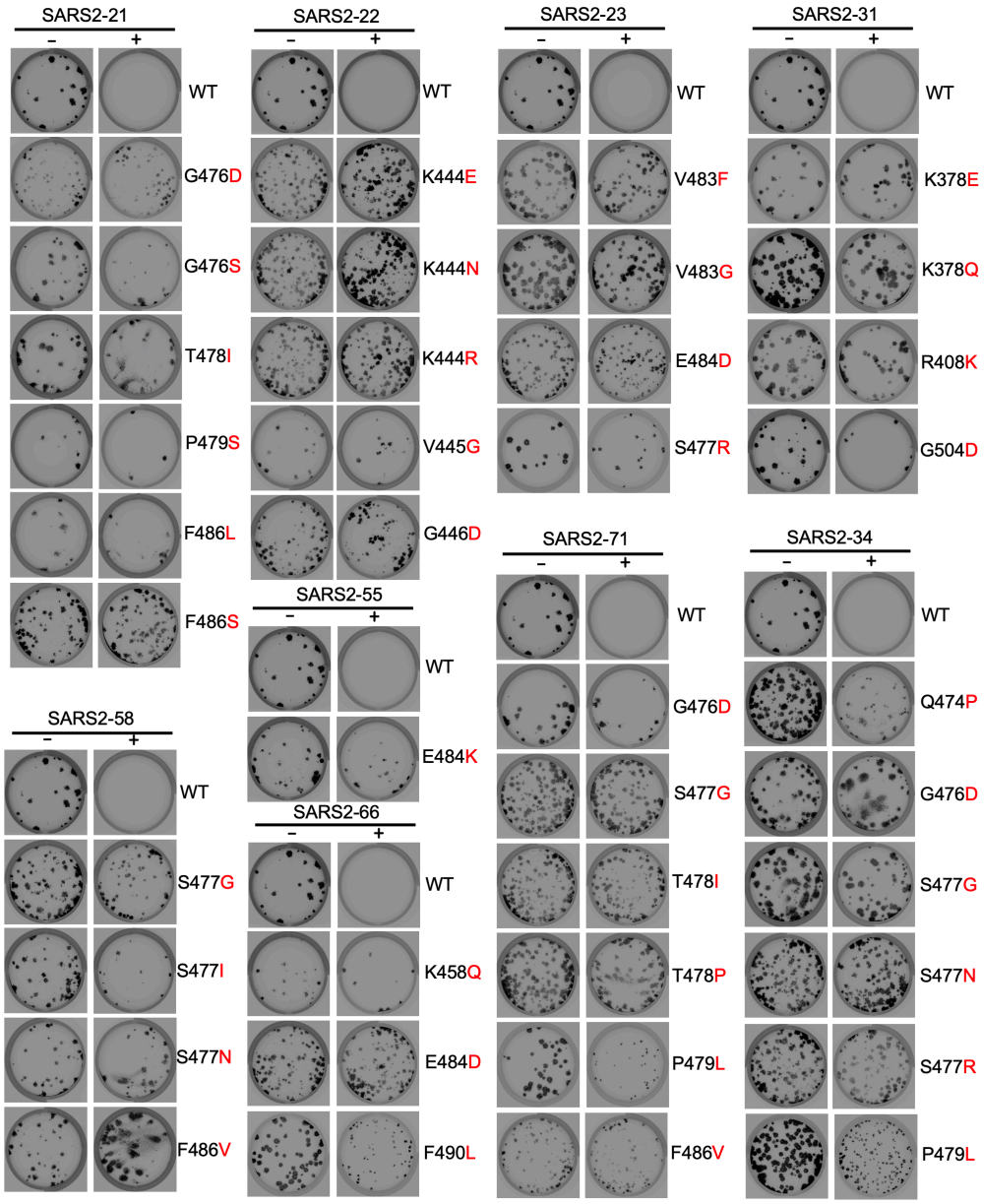

Figure S7. Validation of selected VSV-SARS-CoV-2 mutants. Related to Fig 5. Plaque assays were performed to validate the VSV-SARS-CoV-2 mutant on Vero cells in the presence and absence of mAb in the overlay. MAb concentration added in the overlay were the same as those used to select the escape mutants. Representative images of two independent experiments are shown.

Table S1. List of mutants

Campaign 1

mAb	Nucleotide	Amino Acid
2B04	A1451C	E484A
	G1450A	E484K
	T1457C	F486S
2H04	A1033G	T345A
	C1034A	T345N
	A1033T	T345S
	A1036G	R346G
	T1322G	L441R
	A1330G	K444E
	A1033G /T1550G	T345A /L517R
1B07	A1451C	E484A
	A1452C	E484D
	A1451G	E484G
	G1450A	E484K
	T1457A	F486Y
SARS2-01	A1036G	R346G
	C1055A	A352D
	T1355G	L452R
	T1480C	S494P
SARS2-02	G1337A	G446D
	G1337T	G446V
	G1450A	E484K
SARS2-07	A1348G	N450D
	A1429G	S477G
	G1430A	S477N
	C1431G	S477R
	C1496T	P499L
SARS2-16	A1429G	S477G
	G1430A	S477N
	C1431A	S477R
SARS2-19	A1429G	S477G
	G1430A	S477N
	C1433T	T478I
	G1430A /C1541T	S477N /S514F
SARS2-32	G1337A	G446D
	T1350G	N450K
	A1348T	N450Y
	T1355G	L452R
	G1450A	E484K
	T1469C	F490S
SARS2-38	A1330G	K444E
	G1332T	K444N
	G1337A	G446D

Campaign 2

mAb	Nucleotide	Amino Acid
SARS2-21	G1427A	G476D
	G1426A	G476S
	C1433T	T478I
	C1435T	P479S
	T1456C	F486L
	T1457C	F486S
SARS2-22	A1330G	K444E
	G1332T	K444N
	A1331G	K444R
	T1334G	V445G
	G1337A	G446D
SARS2-23	C1431G	S477R
	G1447T	V483F
	T1448G	V483G
	A1452C	E484D
SARS2-31	A1132G	K378E
	A1132C	K378Q
	G1223A	R408K
	G1511A	G504D
SARS2-34	A1421C	Q474P
	G1427A	G476D
	A1429G	S477G
	G1430A	S477N
	A1429C	S477R
	C1436T	P479L
SARS2-55	G1450A	E484K
SARS2-58	A1429G	S477G
	G1430T	S477I
	G1430A	S477N
	T1456G	F486V
SARS2-66	A1372C	K458Q
	A1452C	E484D
	T1468C	F490L
SARS2-71	G1427A	G476D
	A1427G	S477G
	C1433T	T478I
	A1432C	T478P
	C1436T	P479L
	T1456G	F486V

Campaign 3

Virus	mAb	Nucleotide	Amino Acid
VSV-SARS-CoV-S E484A	2H04	A1033G/A1451C	T345A/E484A
		A1036G/A1451C	T346G/E484A
		A1330G/A1451C	K444E/E484A
VSV-SARS-CoV-S E484K	2H04	G1037A/G1450A	R346K/E484K
		G1114A/G1450A	A372T/E484K
		A1330G/G1450A	K444E/E484K
VSV-SARS-CoV-S F486S	2H04	A1033T/T1457C	T345S/F486S

Sanger sequencing of isolated escape variants selected for by each mAb. The mutated nucleotides and residues in the RBD region of S are highlighted in red.