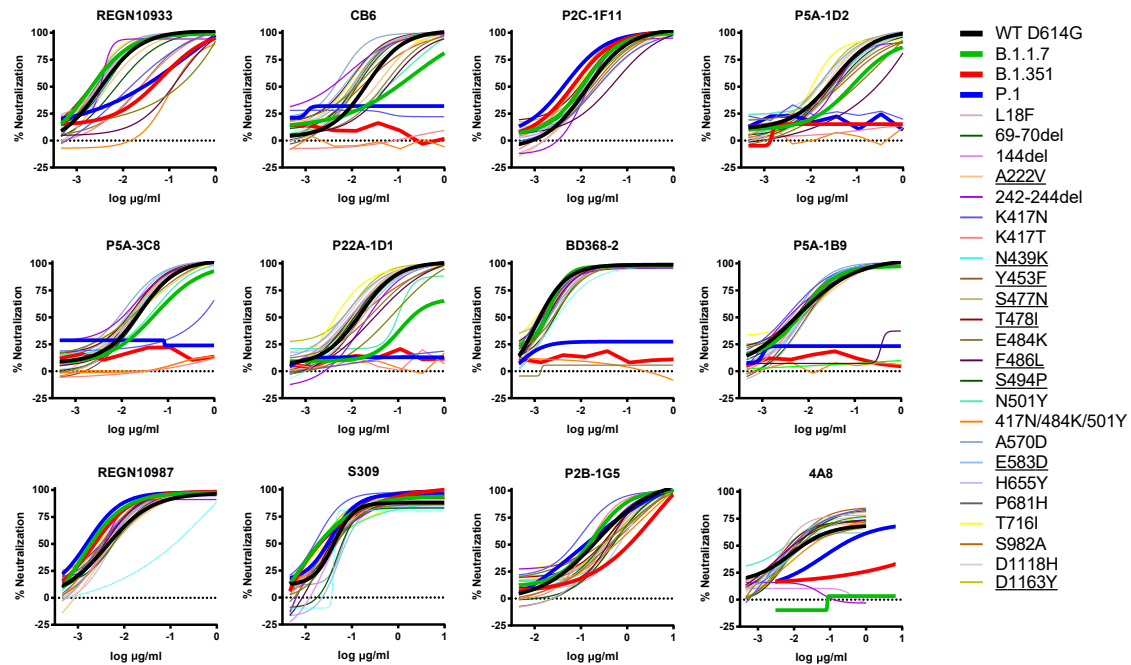


**Supplemental information**

**Analysis of SARS-CoV-2 variant mutations reveals  
neutralization escape mechanisms and the ability  
to use ACE2 receptors from additional species**

**Ruoke Wang, Qi Zhang, Jiwan Ge, Wenlin Ren, Rui Zhang, Jun Lan, Bin Ju, Bin Su, Fengting Yu, Peng Chen, Huiyu Liao, Yingmei Feng, Xuemei Li, Xuanling Shi, Zheng Zhang, Fujie Zhang, Qiang Ding, Tong Zhang, Xinquan Wang, and Linqi Zhang**

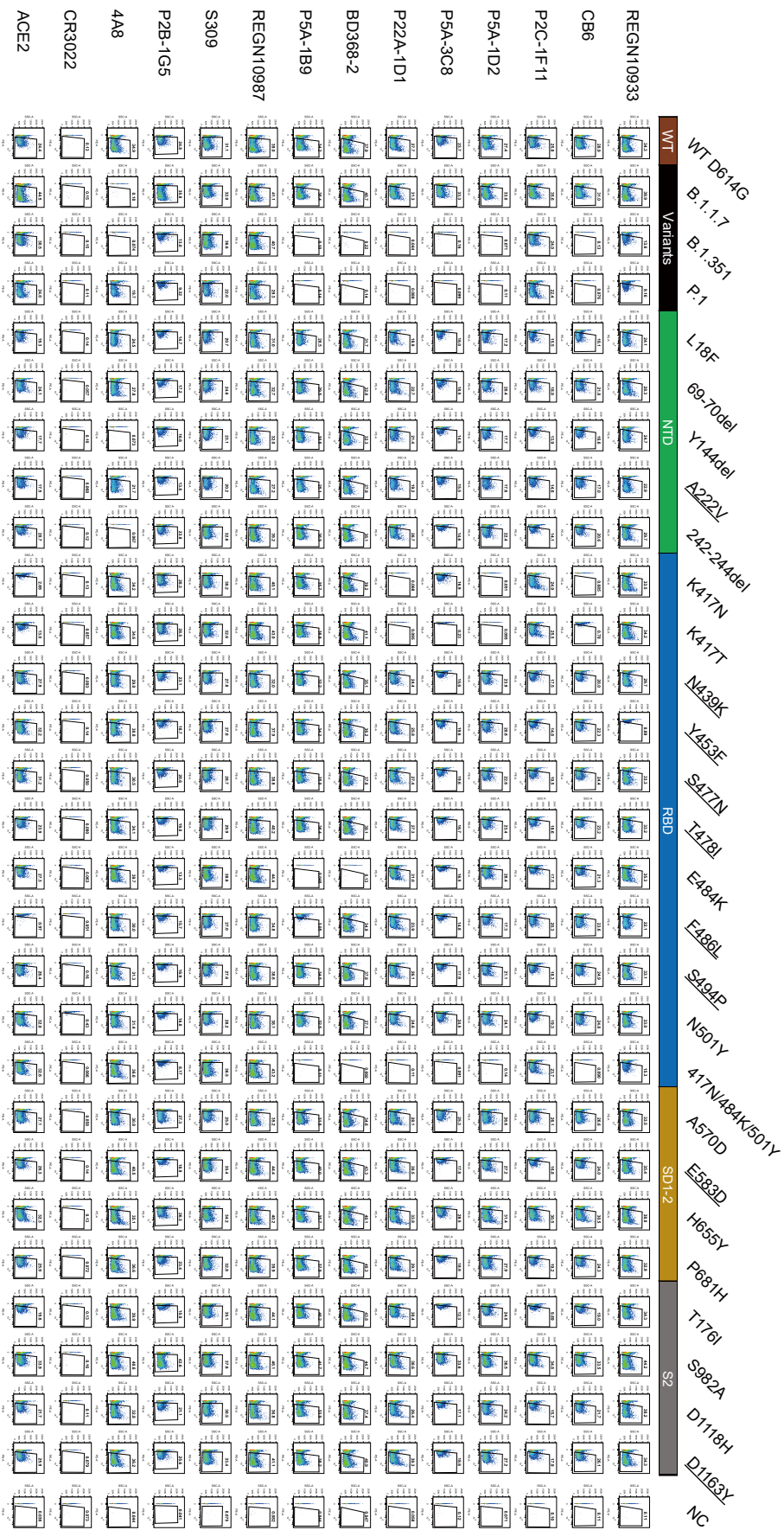
## Supplemental Information titles and legends



**Figure S1.**

### Neutralization of SARS-CoV-2 variants by each antibody, related to

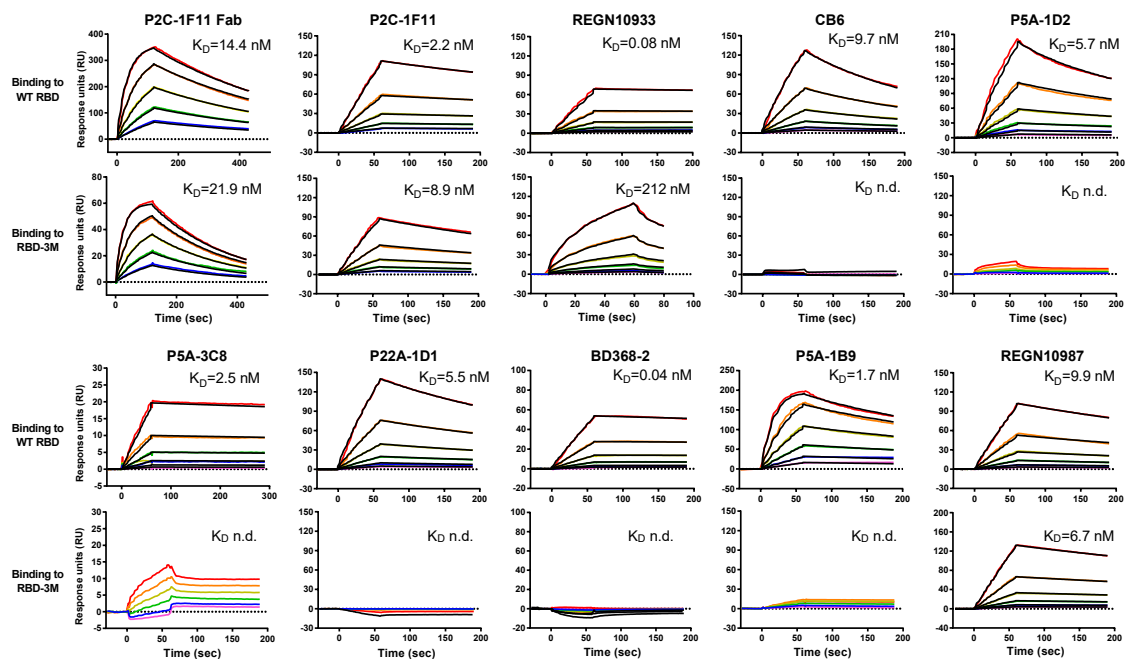
**Figure 1.** Pseudoviruses bearing the indicated mutations were tested against serial dilutions of each mAb. Neutralizing activity was defined as the percent reduction in luciferase activities compared to no antibody controls. Levels of resistance were calculated as the -fold change in IC50 between each mutant and WT D614G, as presented in Figure 1C. Results were calculated from three independent experiments.



## **Figure S2.**

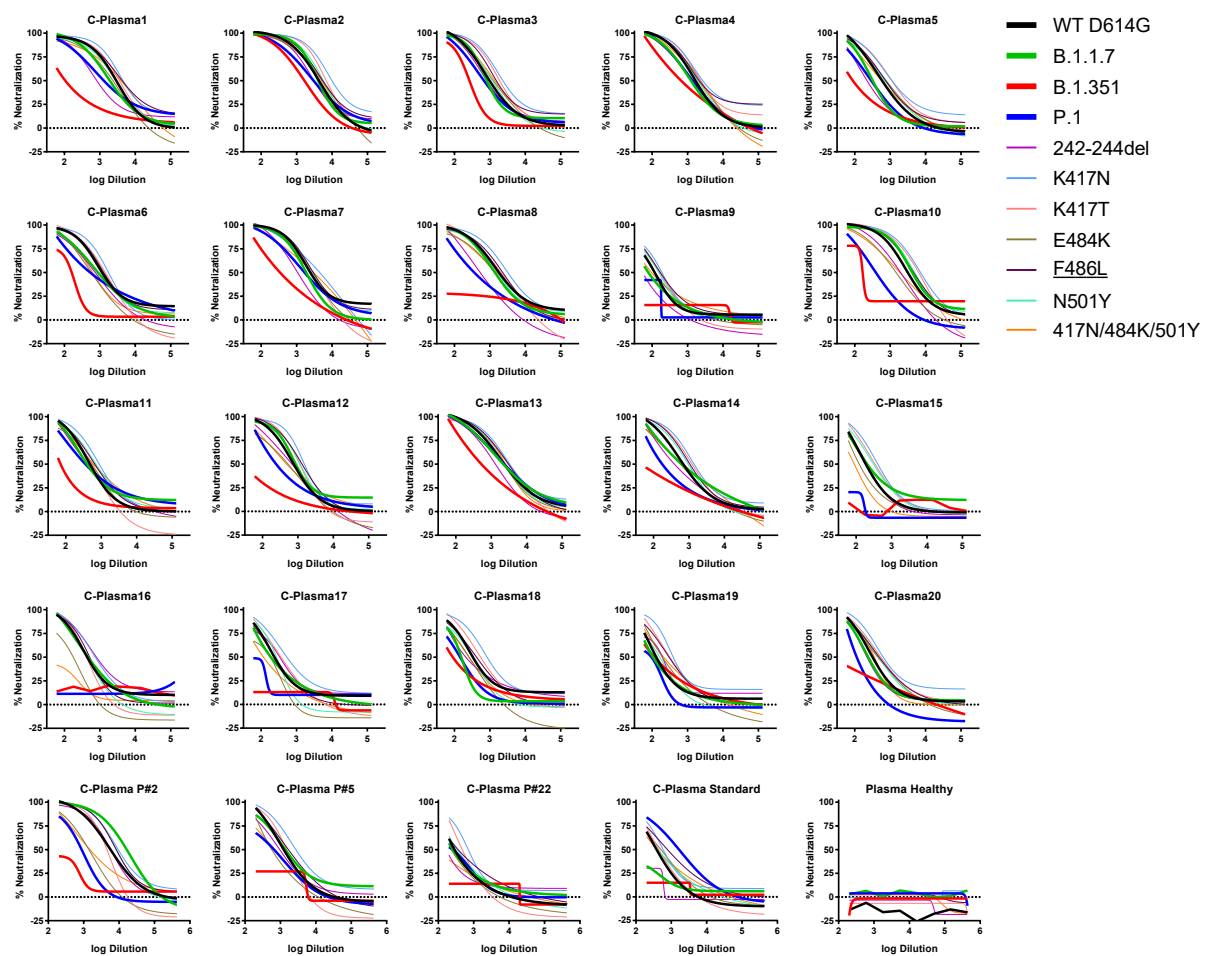
**Binding to cell surface expressed SARS-CoV-2 variants by each antibody, related to Figure 1.** Wildtype and mutant S proteins were expressed on the surface of HEK 293T, incubated with the mAbs or human soluble ACE2 under study, followed by staining with anti-human IgG Fc PE or anti-his PE, and analyzed by FACS. The gated cell percentages are shown. The fold changes in antibody binding, as shown in Figure 1D, was determined by comparing the total MFI in the selected gate between S variants and WT D614G. Data shown were calculated from three independent experiments. CR3022 is a negative control antibody. NC is HEK 293T cells with mock transfection.





**Figure S3.**

**Binding kinetics of mAb to WT RBD and RBD-3M measured by SPR, related to Figure 2.** The Fab and IgG form of mAbs were immobilized on a CM5 sensor and serial concentrations of either wildtype RBD or mutant RBD-3M were flowed through the system. Colored lines indicate the experimentally derived curves. Black lines represent best fitted curves based on the experimental data. The calculated  $K_D$ s for each mAb are shown against WT RBD and mutant RBD-3M. The names of mAb tested are indicated at the top of each graph.



**Figure S4.**

**Neutralization of SARS-CoV-2 variants by each convalescent plasma, related to Figure 3.** Pseudoviruses bearing the indicated mutations were tested against serial dilutions of convalescent plasma. Neutralization activity was defined as the percent reduction in luciferase activity relative to no serum control. The actual ID<sub>50</sub> and the -fold changes between each mutant and WT D614G pseudovirus were calculated to estimate the resistance levels shown in Figure 3. Results were calculated from three independent experiments.

**Table S1. Data collection and refinement statistics (molecular replacement), related to Figure 2.**

RBD-K417N-E484K-N501Y-P2C-1F11 complex	
<b>Data collection</b>	
Space group	C2
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	195.844, 85.973, 57.872
<i>a</i> , <i>b</i> , <i>g</i> (°)	90, 99.75, 90
Resolution (Å)	50.00-2.094 (2.15-2.094) <sup>a</sup>
Unique reflections	50710 (3033)
<i>R</i> <sub>sym</sub> or <i>R</i> <sub>merge</sub>	0.088(0.384)
<i>I</i> / <i>sI</i>	17.2(3.2)
Completeness (%)	90.92 (54.79)
CC <sub>1/2</sub> (%)	99.7 (76.2)
Redundancy	5.3 (2.3)
<b>Refinement</b>	
Resolution (Å)	20.98-2.094
No. reflections	50684
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub>	16.9/19.7
No. atoms	
Protein	4728
Ligand/ion	14
Water	505
<i>B</i> -factors	
Protein	41.18
Ligand/ion	81.75
Water	46.02
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	0.89
Ramachandran statistics (%)	
Favored	97.19
Allowed	2.64
Disallowed	0.17

<sup>a</sup> One crystal for the data, values in parentheses are for highest-resolution shell.

**Table S2. Molecular interaction between P2C-1F11 and mutant RBD-K417N-E484K-N501Y, related to Figure 2.**

		<b>P2C-1F11</b>	<b>Length (Å)</b>	<b>Interactions</b>
<b>P2C-1F11/RBD</b>	E/K417/N[N]	H/Y52/CE2[C]	3.84	Hydrogen bond
		H/Y52/OH[O]	3.87	
	E/K417/CG[C]	H/Y52/CE2[C]	3.85	
		H/Y52/CZ[C]	3.97	
		H/Y52/OH[O]	3.32	
	E/K417/CE[C]	H/Y52/OH[O]	3.69	
	E/K417/NZ[N]	H/Y52/OH[O]	3.19	
	E/K417/CD[C]	H/Y33/OH[O]	4.02	
	E/K417/CE/[C]	H/Y33/OH[O]	4.03	
<b>P2C-1F11/RBD-3M</b>	E/N417/N[N]	H/Y52/CE2[C]	3.82	
		H/Y52/OH[O]	3.53	
	E/Y453/CE1[C]	L/Y33/OH[O]	3.50	
	E/Y453/CZ[C]	L/Y33/OH[O]	3.43	
	E/Y453/OH[O]	L/Y33/CE1[C]	3.58	
		L/Y33/CZ[C]	3.47	
		L/Y33/OH[O]	2.52	
	E/Q493/CG[C]	L/Y33/OH[O]	3.62	
	E/Q493/NE2[N]	L/S32/CB[C]	3.96	
		L/S32/OG/[O]	3.77	
	E/T500/C[C]	L/S28/OG[O]	3.90	
	E/T500/O[O]	L/S28/CB[C]	3.30	
		L/S28/OG[O]	3.01	
	E/Y501/CA[C]	L/S28/OG[O]	3.87	
	E/Y501/CE1[C]	L/V29/O[O]	3.79	
	E/Y501/CZ[C]	L/S30/CA[C]	3.94	
	E/Y501/OH[O]	L/S30/CA[C]	3.82	
		L/S30/CB[C]	3.85	
L/S30/OG[O]		3.85		

**Table S3. Information of the study subjects, related to Figure 3.**

Patients	Gender	Age	Severity
C-Plasma1	male	60	severe
C-Plasma2	male	57	severe
C-Plasma3	male	57	severe
C-Plasma4	female	81	severe
C-Plasma5	female	62	mild
C-Plasma6	male	35	mild
C-Plasma7	male	55	mild
C-Plasma8	female	52	mild
C-Plasma9	female	36	mild
C-Plasma10	male	63	mild
C-Plasma11	female	63	severe
C-Plasma12	male	75	mild
C-Plasma13	male	78	severe
C-Plasma14	male	62	mild
C-Plasma15	male	30	mild
C-Plasma16	male	30	mild
C-Plasma17	female	29	mild
C-Plasma18	female	43	mild
C-Plasma19	male	58	mild
C-Plasma20	female	67	severe
C-Plasma P#2	female	65	severe
C-Plasma P#5	female	63	severe
C-Plasma P#22	male	62	mild

**Table S4. Primers used in the construction of mutated clones of SARS-CoV-2 S, related to Figure 1.**

No.	Primer Name	sequence
1	Mut-1stMS-F	TTGGTACCGAGCTCGGATCCATGTTCTGCTGACCACCA AGAGAACCATGTTTCGTGTTCTGCTGGTG
2	Mut-S-R	CCACTGTGCTGGATATCTGCAGAATTCTCAGGTGTAGTG CAGCTTCAC
3	nCov-SL18F-F	GTGAGCAGCCAGTGCCTGAATTTACCACCAGAAC
4	nCov-SL18F-R	GTTCTGGTGGTCAAATTCACGCACTGGCTGCTCAC
5	nCov-SHV69- 70del-F	CGTGACCTGGTCCACGCCATCAGCGGCACCAATGGCAC CAAGAG
6	nCov-SHV69- 70del-R	CTCTTGGTGCCATTGGTGCCGCTGATGGCGTGGAACCAG GTCACG
7	nCov-SY144del antisense	CTCTTGTTGTTCTTATGATAAACACCCAGGAAAGGGTCAT
8	nCov-SY144del	ATGACCCTTTCCTGGGTGTTTATCATAAGAACAACAAGAG
9	nCov-SA222V-F	CAGGGCTTCAGCGTCCTGGAGCCTCTG
10	nCov-SA222V-R	CAGAGGCTCCAGGACGCTGAAGCCCTG
11	nCov-S242-244del- F	ACCAGATTCCAGACCCTGCACAGATCATATCTTACAC
12	nCov-S242-244del- R	GTGTAAGATATGATCTGTGCAGGGTCTGGAATCTGGT
13	nCov-SK417N- Fg1251t	CAGGGCAGACCGGCAATATCGCCGAC
14	nCov-SK417N-R	GTCGGCGATATTGCCGGTCTGCCCTG
15	nCov-SK417T- Fa1250c	GGGCAGACCGGCACGATCGCCGACTAC
16	nCov-SK417T-R	GTAGTCGGCGATCGTGCCGGTCTGCCC
17	nCov-SN439K-F	CGTGATCGCGTGGAAGTCTAAGAATCTAGATTGAAA
18	nCov-SN439K-R	TTTCGAATCTAGATTCTTAGAGTTCCACGCGATCACG
19	nCov-SY453F- Fa1358t	GGCAATTACAATTACCTGTTTCAGACTGTTTCAGAAAGAGC
20	nCov-SY453F-R	GCTCTTTCTGAACAGTCTGAACAGGTAATTGTAATTGCC
21	nCov-SS477N- FG1430A	CTACCAGGCCGGCAACACACCCGTGTAATG
22	nCov-SS477N-R	CATTACACGGTGTGTTGCCGGCCTGGTAG
23	nCov-ST478I-F	TACCAGGCCGGCAGCATAACCGTGTAAATGG
24	nCov-ST478I-R	CCATTACACGGTATGCTGCCGGCCTGGTA
25	nCov-SE484K- Fg1450a	CACCGTGTAAATGGCGTGAAGGGCTTCAATTGCTAC
26	nCov-SE484K-R	GTAGCAATTGAAGCCCTTCACGCCATTACACGGTG

**Table S4 continued. Primers used in the construction of mutated clones of SARS-CoV-2 S, related to Figure 1.**

No.	Primer Name	sequence
27	nCov-SF486L-F	ATGGCGTGGAGGGCTTAAATTGCTACTTCCCTC
28	nCov-SF486L-R	GAGGGAAGTAGCAATTTAAGCCCTCCACGCCAT
29	nCov-SS494P-F	TACTTCCCTCTGCAGCCCTACGGCTTCCAGCC
30	nCov-SS494P-R	GGCTGGAAGCCGTAGGGCTGCAGAGGGAAGTA
31	nCov-SN501Y-Fa1501t	GCTTCCAGCCTACCTATGGCGTG GGCTAC
32	nCov-SN501Y-R	GTAGCCCACGCCATAGGTAGGCTGGAAGC
33	nCov-SA570D-F	CATCTGTGGTGTTCGTTCGATGTCTCTGCCG
34	nCov-SA570D-R	CGGCAGAGACATCGACGACACCACAGATG
35	nCov-SE583D-F	CCCTCAGACCCTGGATATCCTGGACATCACT
36	nCov-SE583D-R	AGTGATGTCCAGGATATCCAGGGTCTGAGGG
37	H655Y-F	GCCGGCTGCCTGATCGGCGCCGAGTACGTGAATAATAGC TACGAGTGCGACATCCCTA
38	H655Y-R	TAGGGATGTTCGACTCGTAGCTATTATTACGTA GACTCGGC GCCGATCAGGCAGCCGGC
39	nCov-SP681H-F	ACCCAGACCAATAGCCATAGAAGAGCCAGAAGC
40	nCov-SP681H-R	GCTTCTGGCTCTTCTATGGCTATTGGTCTGGGT
41	nCov-ST716I-F	TAGCATCGCCATCCCTATCAATTTACCATCAGCG
42	nCov-ST716I-R	CGCTGATGGTGAAATTGATAGGGATGGCGATGCTA
43	nCov-SS982A-F	GTACTCAACGATATCCTGGCCAGACTGGACAAGGTGGA
44	nCov-SS982A-R	TCCACCTTGTCCAGTCTGGCCAGGATATCGTTGAGTAC
45	nCov-ST1027I-Fc3080t	CCAATCTGGCCGCCATCAAGATGAGCGAGTG
46	nCov-ST1027I-R	CACTCGCTCATCTTGATGGCGGCCAGATTGG
47	nCov-SD1118H-F	GCTCACGAAGGTATTGTGGGTGGTGTGATGATCTGAG
48	nCov-SD1118H-R	CTCAGATCATCACCACCCACAATACCTTCGTGAGC
49	nCov-SD1163Y-F	CAAGAATCACACCAGCCCTTATGTGGACCTCGGTGATATTT
50	nCov-SD1163Y-R	AAATATCACCGAGGTCCACATAAGGGCTGGTGTGATTCTTG
51	nCov-SV1176F-F	TTCGGGAATCAATGCCAGCTTCGTGAATATCCAGAAGGAAA
52	nCov-SV1176F-R	TTTCCTTCTGGATATTCACGAAGCTGGCATTGATTCCCGAA