Inhibitor screening using microarray identifies the high capacity of neutralizing antibodies to Spike variants in SARS-CoV-2 infection and vaccination

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Supplementary tables

Table S1: The worldwide cumulative prevalence of SARS-CoV-2 spike protein mutations in GISAID as of December 28, 2021

Mutations	Mutation found		when found**	
	total	cumulative prevalence*	first	last
S1 (D614G)	6,239,957	99%	26-Jan-20	23-Dec-21
RBD (L452R)	3,749,640	59%	26-Jan-20	23-Dec-21
RBD (N501Y)	1,345,483	21%	7-Feb-20	23-Dec-21
S1 (A222V, D614G)	561,725	9%	2-Feb-20	23-Dec-21
S1 (L18F, D614G)	229,364	4%	28-Jan-20	20-Dec-21
RBD (S477N)	124,442	2%	1-Mar-20	17-Dec-21
S1 (T20N, D614G)	115,372	2%	28-Jan-20	23-Dec-21
RBD (K417N)	58,007	1%	16-Mar-20	14-Dec-21
RBD (N439K)	37,149	1%	15-Feb-20	23-Dec-21
RBD (K417N, E484K, N501Y)	33,998	1%	27-Mar-20	15-Dec-21
RBD (N440K)	20,878	< 0.5%	28-Jan-20	13-Dec-21
RBD (E484Q)	17,709	< 0.5%	27-Jan-20	14-Dec-21
RBD (S494P)	13,439	< 0.5%	3-Mar-20	7-Dec-21
RBD (G446S)	13,261	< 0.5%	5-Mar-20	14-Dec-21
RBD (G446V)	10,960	< 0.5%	19-Mar-20	19-Dec-21
RBD (A520S)	9,366	< 0.5%	18-Mar-20	18-Dec-21
RBD (S477I)	8,331	< 0.5%	10-Mar-20	20-Dec-21
RBD (A522V)	6,810	< 0.5%	26-Mar-20	12-Nov-21
RBD (P384L)	3,783	< 0.5%	25-Feb-20	16-Dec-21

RBD (V367F)	3,146	< 0.5%	13-Mar-20	18-Dec-21
RBD (A348S)	2,581	< 0.5%	21-Nov-21	21-Nov-21
RBD (L455F)	2,398	< 0.5%	15-Mar-20	20-Dec-21
RBD (Q414R)	2,026	< 0.5%	15-Mar-20	23-Dec-21
RBD (R408I)	1,984	< 0.5%	13-Mar-20	18-Dec-21
RBD (A475V)	1,967	< 0.5%	31-Mar-20	10-Dec-21
RBD (F490L)	1,701	< 0.5%	7-Apr-20	15-Dec-21
RBD (S477R)	1,567	< 0.5%	18-Mar-20	2-Dec-21
RBD (A352S)	1,518	< 0.5%	28-Mar-20	18-Dec-21
RBD (Y453F)	1,405	< 0.5%	1-Feb-20	14-Dec-21
RBD (Y508H)	1,195	< 0.5%	28-Jan-20	9-Dec-21
RBD (F338L)	1,126	< 0.5%	31-Mar-20	21-Nov-21
RBD (E471Q)	1,082	< 0.5%	6-Apr-20	6-Dec-21
RBD (T478I)	917	< 0.5%	3-Mar-20	18-Dec-21
RBD (N370S)	758	< 0.5%	30-Mar-20	8-Dec-21
RBD (P499R)	742	< 0.5%	18-Mar-20	14-Dec-21
RBD (V503F)	640	< 0.5%	28-Mar-20	20-Dec-21
RBD (A435S)	540	< 0.5%	2-Apr-20	15-Dec-21
RBD (S359N)	528	< 0.5%	25-Mar-20	18-Dec-21
RBD (F456L)	516	< 0.5%	8-Apr-20	3-Dec-21
RBD (V483I)	485	< 0.5%	29-Mar-20	29-Mar-20
RBD (V483A)	456	< 0.5%	15-Mar-20	14-Aug-21
RBD (N354D)	445	< 0.5%	29-Feb-20	17-Dec-21
RBD (E406Q)	372	< 0.5%	1-Mar-20	14-Dec-21
RBD (P521S)	338	< 0.5%	17-Mar-20	20-Dec-21
RBD (K378N)	310	< 0.5%	10-Apr-20	15-Dec-21
RBD (A520V)	304	< 0.5%	10-Mar-20	23-Dec-21
RBD (V445F)	266	< 0.5%	15-Apr-20	16-Dec-21
RBD (P337S)	242	< 0.5%	13-Mar-20	16-Dec-21
RBD (V341I)	229	< 0.5%	14-Jan-21	14-Dec-21
RBD (K458R)	181	< 0.5%	1-Apr-20	13-Dec-21
RBD (G485S)	176	< 0.5%	6-Apr-20	15-Dec-21
RBD (K378R)	88	< 0.5%	30-Mar-20	15-Dec-21
RBD (F377L)	87	< 0.5%	8-Oct-20	14-Dec-21
RBD (F342L)	74	< 0.5%	6-May-20	18-Dec-21
RBD (N481D)	67	< 0.5%	19-Feb-20	15-Dec-21
RBD (A372T)	61	< 0.5%	15-Dec-20	9-Dec-21
RBD (F486S)	46	< 0.5%	4-Apr-20	23-Dec-21
RBD (A372S)	44	< 0.5%	8-Jul-20	16-Dec-21
RBD (V395I)	26	< 0.5%	1-Apr-20	15-Dec-21
RBD (T385A)	22	< 0.5%	8-Jun-20	7-Dec-21
RBD (W436R)	7	< 0.5%	3-Mar-20	18-Dec-21
RBD (Q414E)	1	< 0.5%	18-Mar-20	19-Dec-21
RBD (T393P)	1	< 0.5%	29-Jul-20	15-Dec-21

S1 (N234Q)	0	not detected
S1 (HV69-70 deltion, N501Y, D614G)	0	not detected
RBD (Y505C)	0	not detected
RBD (F456E)	0	not detected
RBD (N487R)	0	not detected

Note: The prevalences were calculated by Outbreak.info Mutation Situation Reports (https://outbreak.info/situation-reports#voi) with 6,315,868 sequences from GISAID as of 28 December 2021.

* Apparent cumulative prevalence is the ratio of the sequences containing mutation to all sequences collected since the identification of mutation in that location.

** Dates are based on the sample collection date.

Table S2: Clinical information of the convalescent COVID-19 patients

Gender	n	Age, median	Weeks post discharge
Female	11	49 (18 ~ 64)	2 or 4
Male	14	36 (5 ~ 68)	2 or 4
Total	25	43 (5 ~ 68)	2 or 4

Table S3: Clinical information of people in the study 4 weeks after receiving the second dose of COVID-19 inactivated vaccine

Gender	n	Age, median	Weeks post the second vaccine dose
Female	6	36 (21 ~ 53)	4
Male	24	37 (23 ~ 57)	4
Total	30	37 (21 ~ 57)	4

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Table S4: Clinical information of patients in the study 24 weeks after receiving the second dose of COVID-19 inactivated vaccine

Gender	n	Age, median	Weeks post the second vaccine dose
Female	18	38.5 (27 ~ 55)	24
Male	7	36 (31 ~ 46)	24
Total	25	38 (27 ~ 55)	24

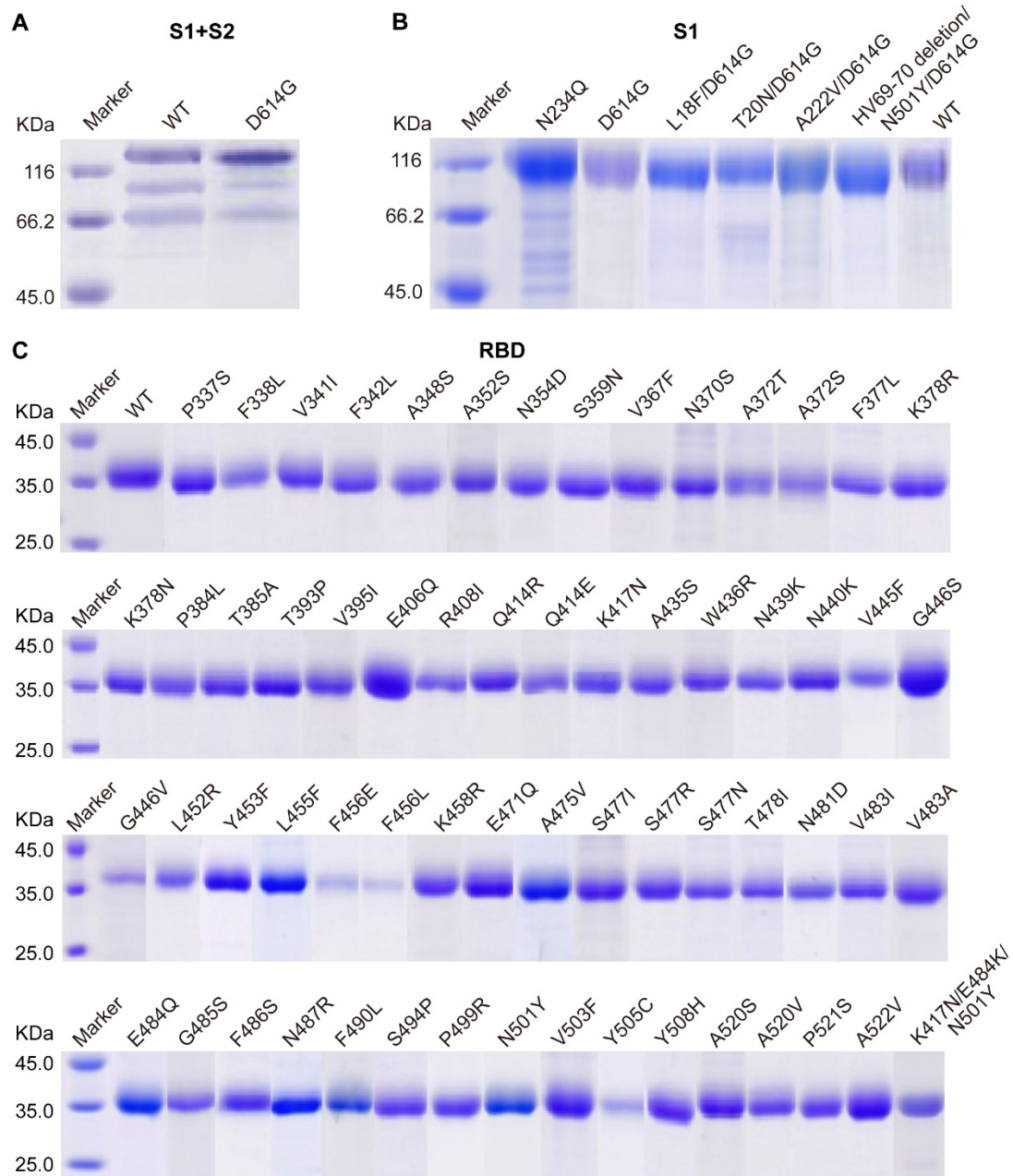67

Table S5： Clinical information of validation cohort 4 weeks after receiving the second dose of COVID-19 inactivated vaccine

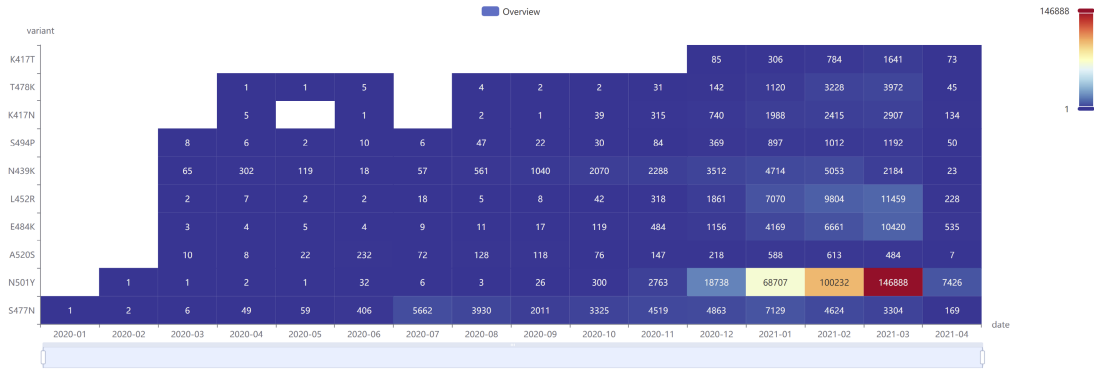| Gender	n	Age, median	Weeks post the second vaccine dose
Female	11	46 (21 ~ 70)	4
Male	93	39 (23 ~ 70)	4
Total	104	39 (21 ~ 70)	4

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Supplementary Figures

Figure S1. SDS-PAGE analysis of purified recombinant SARS-CoV-2 S variants.

(A-C) are SDS-PAGE analyses of S1+S2, S1 and RBD wild-type and variant proteins,

respectively. WT: wild-type.

variant	2020-01	2020-02	2020-03	2020-04	2020-05	2020-06	2020-07	2020-08	2020-09	2020-10	2020-11	2020-12	2021-01	2021-02	2021-03	2021-04
K417T												85	306	784	1641	73
T478K				1	1	5		4	2	2	31	142	1120	3228	3972	45
K417N			5		1		2	1	39	315	740	1988	2415	2907	134	
S494P			8	6	2	10	6	47	22	30	84	369	897	1012	1192	50
N439K			65	302	119	18	57	561	1040	2070	2288	3512	4714	5053	2184	23
L452R			2	7	2	2	18	5	8	42	318	1861	7070	9804	11459	228
E484K			3	4	5	4	9	11	17	119	484	1156	4169	6661	10420	535
A520S			10	8	22	232	72	128	118	76	147	218	588	613	484	7
N501Y		1	1	2	1	32	6	3	26	300	2763	18738	68707	100232	146888	7426
S477N	1	2	6	49	59	406	5662	3930	2011	3325	4519	4863	7129	4624	3304	169

Figure S2. Timeline of when the top 20 RBD mutations emerged. The graph was obtained from the COVID-19 virus mutation tracker database (https://www.cbrc.kaust.edu.sa/covmt/index.php?p=top-rbd-variants-heatmap) [1].

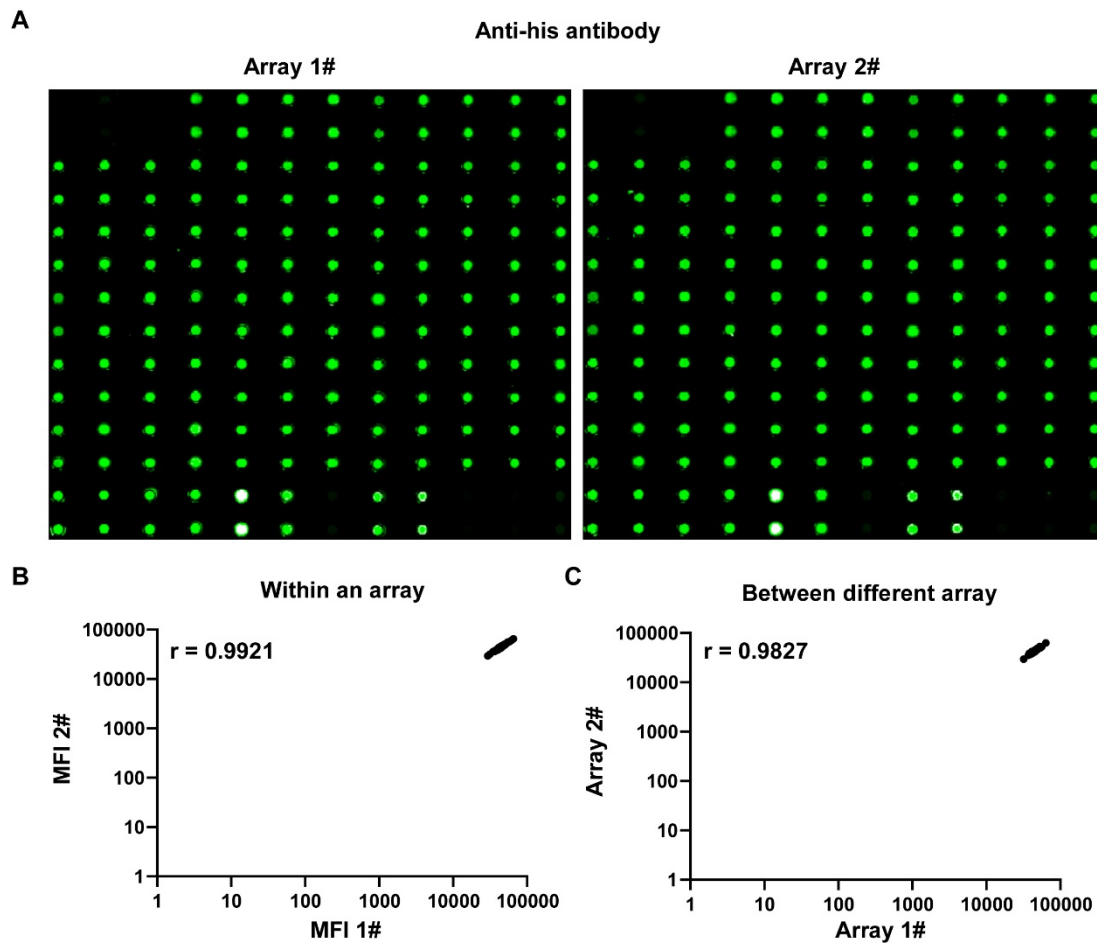|	1	2	3	4	5	6	7	8	9	10	11	12
1	buffer	N	buffer	S1+S2	S1+S2(D614G)	S1	S1(N234Q)	S1(D614G)	S1(A222V/D614G)	S1(T20N/D614G)	S1(L18F/D614G)	S1(HV69-70 deltion/N501Y/D614G)
2	buffer	N	buffer	S1+S2	S1+S2(D614G)	S1	S1(N234Q)	S1(D614G)	S1(A222V/D614G)	S1(T20N/D614G)	S1(L18F/D614G)	S1(HV69-70 deltion/N501Y/D614G)
3	RBD insect	RBD 293	RBD (V367F)	RBD(R408I)	RBD(V341I)	RBD(Y508H)	RBD(N439K)	RBD(V503F)	RBD(A522V)	RBD(S494P)	RBD(A372S)	RBD(N354D)
4	RBD insect	RBD 293	RBD (V367F)	RBD(R408I)	RBD(V341I)	RBD(Y508H)	RBD(N439K)	RBD(V503F)	RBD(A522V)	RBD(S494P)	RBD(A372S)	RBD(N354D)
5	RBD(A520S)	RBD(Q414E)	RBD(P384L)	RBD(A348S)	RBD(F338L)	RBD(F377L)	RBD(L452R)	RBD(P521S)	RBD(T478I)	RBD(V483I)	RBD(S359N)	RBD(K378R)
6	RBD(A520S)	RBD(Q414E)	RBD(P384L)	RBD(A348S)	RBD(F338L)	RBD(F377L)	RBD(L452R)	RBD(P521S)	RBD(T478I)	RBD(V483I)	RBD(S359N)	RBD(K378R)
7	RBD(A372T)	RBD(A520V)	RBD(A435S)	RBD(E406Q)	RBD(K378N)	RBD(N370S)	RBD(Q414R)	RBD(S477I)	RBD(S477N)	RBD(T385A)	RBD(T393P)	RBD(V395I)
8	RBD(A372T)	RBD(A520V)	RBD(A435S)	RBD(E406Q)	RBD(K378N)	RBD(N370S)	RBD(Q414R)	RBD(S477I)	RBD(S477N)	RBD(T385A)	RBD(T393P)	RBD(V395I)
9	RBD(V483A)	RBD(A475V)	RBD(G446V)	RBD(G485S)	RBD(N440K)	RBD(E471Q)	RBD(A352S)	RBD(K417N)	RBD(F342L)	RBD(P337S)	RBD(S477R)	RBD(L455F)
10	RBD(V483A)	RBD(A475V)	RBD(G446V)	RBD(G485S)	RBD(N440K)	RBD(E471Q)	RBD(A352S)	RBD(K417N)	RBD(F342L)	RBD(P337S)	RBD(S477R)	RBD(L455F)
11	RBD(K458R)	RBD(N481D)	RBD(F456L)	RBD(Y505C)	RBD(F456E)	RBD(F486S)	RBD(N487R)	RBD(G446S)	RBD(P499R)	RBD(V445F)	RBD(Y453F)	RBD(E484Q)
12	RBD(K458R)	RBD(N481D)	RBD(F456L)	RBD(Y505C)	RBD(F456E)	RBD(F486S)	RBD(N487R)	RBD(G446S)	RBD(P499R)	RBD(V445F)	RBD(Y453F)	RBD(E484Q)
13	RBD(N501Y)	RBD(F490L)	RBD(W436R)	RBD(K417N/E484K/N501Y)	MERS-CoV RBD	SARS-CoV RBD	RBD-hFc	RBD-mFc	RBD-mFc (V367F)	RBD-rabbitFc	RBD	buffer
14	RBD(N501Y)	RBD(F490L)	RBD(W436R)	RBD(K417N/E484K/N501Y)	MERS-CoV RBD	SARS-CoV RBD	RBD-hFc	RBD-mFc	RBD-mFc (V367F)	RBD-rabbitFc	RBD	buffer

Figure S3. Layout of SARS-CoV-2 spike variant protein microarray. The buffer and nucleocapsid (N) protein served as the negative controls. The RBD protein served as the positive control.

A

Anti-his antibody

Array 1#

Array 2#

B

Within an array

r = 0.9921

MFI 2#

MFI 1#

C

Between different array

r = 0.9827

Array 2#

Array 1#

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94 **Figure S4. Reproducibility of protein microarray preparation.** (A) Fluorescent

95 staining of immobilized proteins on the glass-based microarray using an anti-his

96 antibody; (B-C) are the intra- and inter-array reproducibility, respectively.
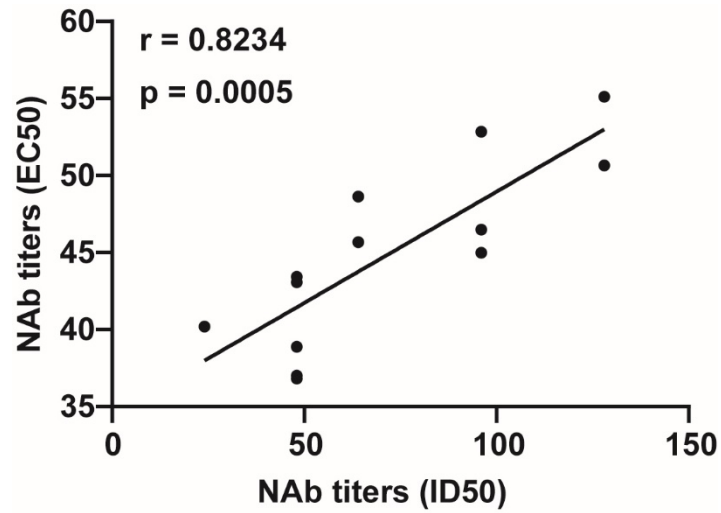
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Figure S5. Correlation of the mSAIS assay and live SARS-CoV-2 neutralization

assay. Pearson's correlation coefficient and linear regression analyses were

performed by the GraphPad Prism software 8.3. Statistical significance was

determined using the two-tailed t-test.

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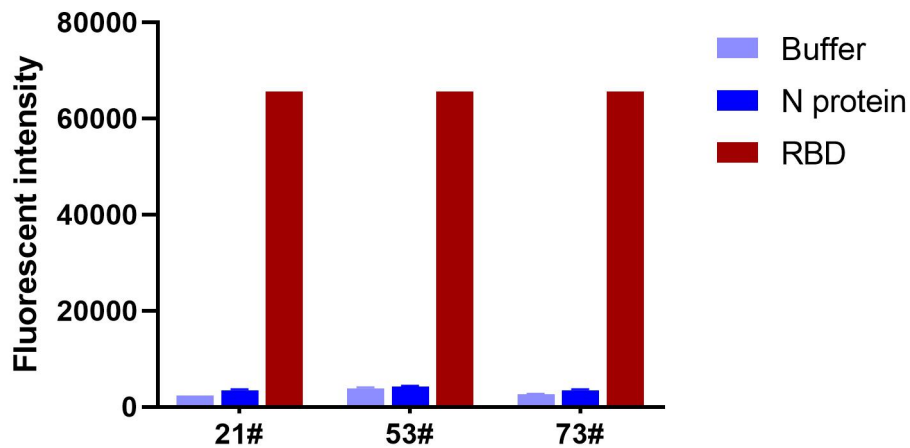
Figure S6. Detection of different anti-RBD antibodies binding to immobilized RBD protein using the SARS-CoV-2 proteome peptide microarray. The nucleocapsid (N) protein and buffer served as the negative controls.

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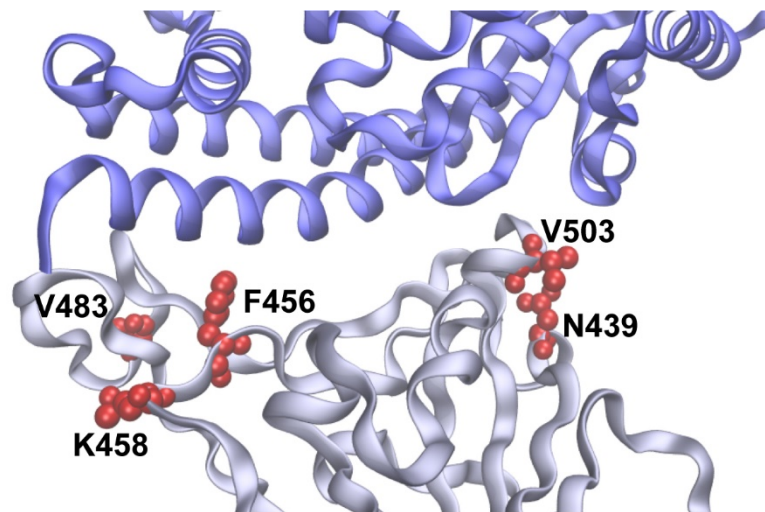
Figure S7. Structural analysis of spike mutations located at the RBD-ACE2 interaction interface that are resistant to antibody #21. The interaction structure of the SARS-CoV-2 RBD and human ACE2 were derived from the Protein Data Bank with PDB ID 6M0J. The S protein is colored in gray, the ACE2 protein is colored in blue, and RBD mutations are labeled in red.
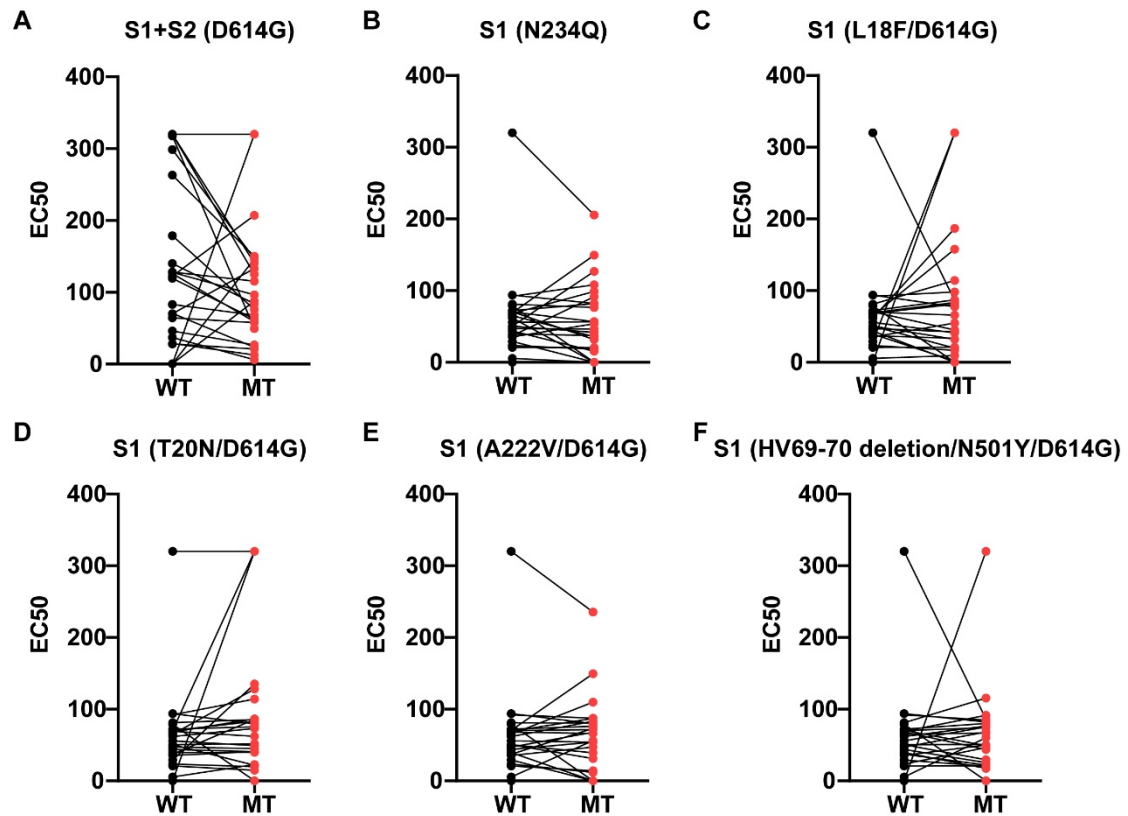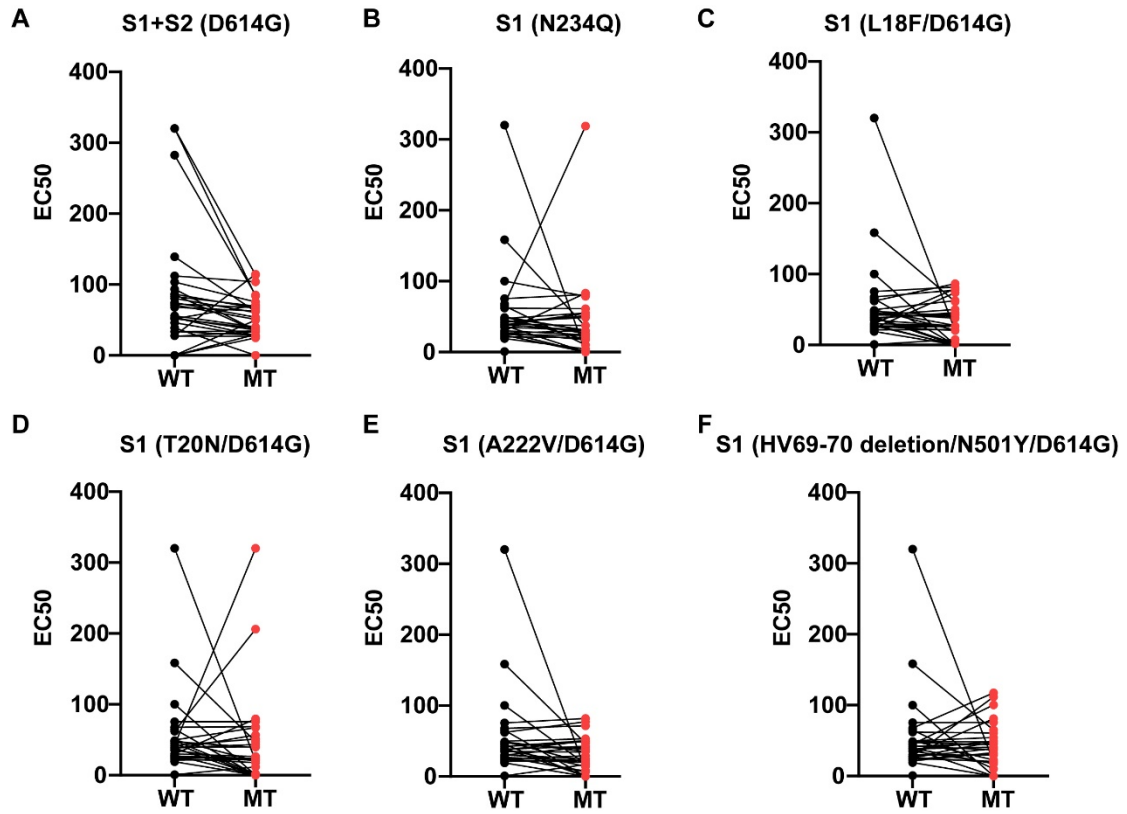
Figure S8. NAb titers to the wild type and variant S proteins in convalescent COVID-19 patients. (A-F) are the NAb titers of convalescent serum to different S variants with D614G, N234Q, L18F/D614G, T20N/D614G, A222V/D614G and hv69-70 deletion/N501Y/D614G, respectively. WT and MT represent the wild type and mutants, respectively.

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135 **Figure S9. NAb titers to the wild type and variant S proteins in vaccinees.** (A-F)

136 are the NAb titers of vaccinees serum to different S variants with D614G, N234Q,

137 L18F/D614G, T20N/D614G, A222V/D614G and hv69-70 deletion/N501Y/D614G,

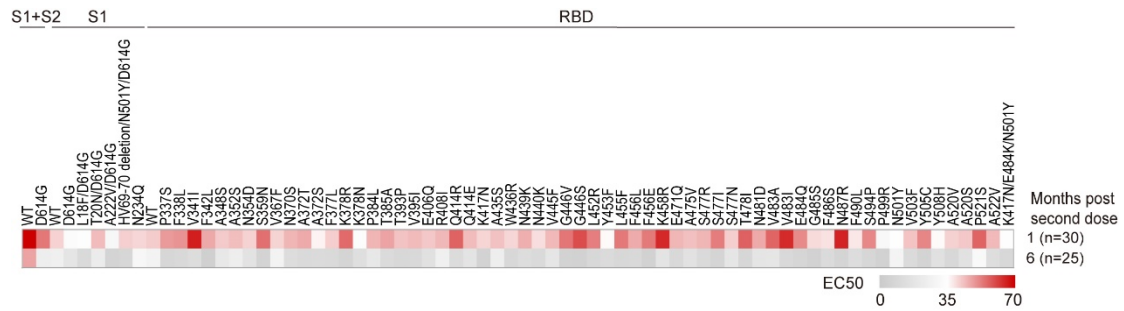138 respectively. WT and MT represent the wild type and mutants, respectively.

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Figure S10. Heat map of NAbs titers to S variants in vaccinees' sera after the second dose. The NAb titers of 30 and 25 vaccinees one (1) and six (6) months, respectively, after receiving the second dose of the inactivated COVID-19 vaccine were measured using the mSAIS assay. The heat map was generated using the mean EC50. The rainbow color from gray to red correspond to the EC50 from low to high, respectively. WT = wild-type.

References:

1.   Alam I, Radovanovic A, Incitti R, Kamau AA, Alarawi M, Azhar EI, et al. CovMT: an interactive SARS-CoV-2 mutation tracker, with a focus on critical variants. Lancet Infect Dis. 2021; 21: 602.