

Figure S1.

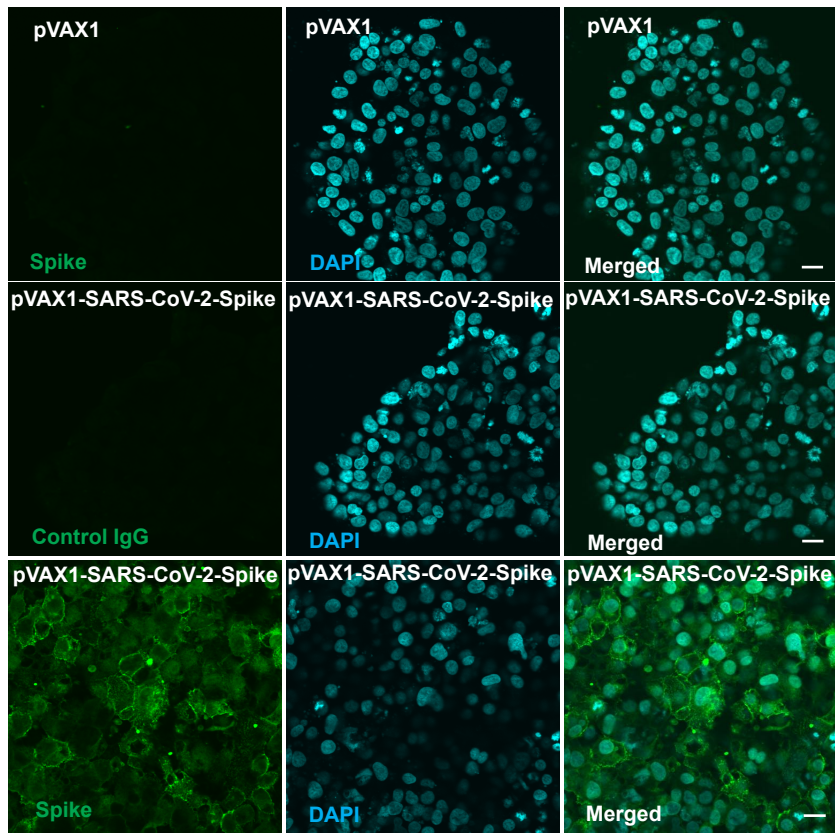


Figure S1. The membrane localization of the spike glycoprotein.

The localization of pVAX1-SARS-CoV-2 Spike in HEK293 cells assessed by immunostaining. The membrane was permeabilized by Triton X-100. The transfected spike protein was stained with a polyclonal spike antibody and a secondary antibody labeled with Alexa Fluor 488 (green). The nucleus was stained with DAPI (blue). Scale bar=20 μ m. See also Figure 1.

Figure S2.

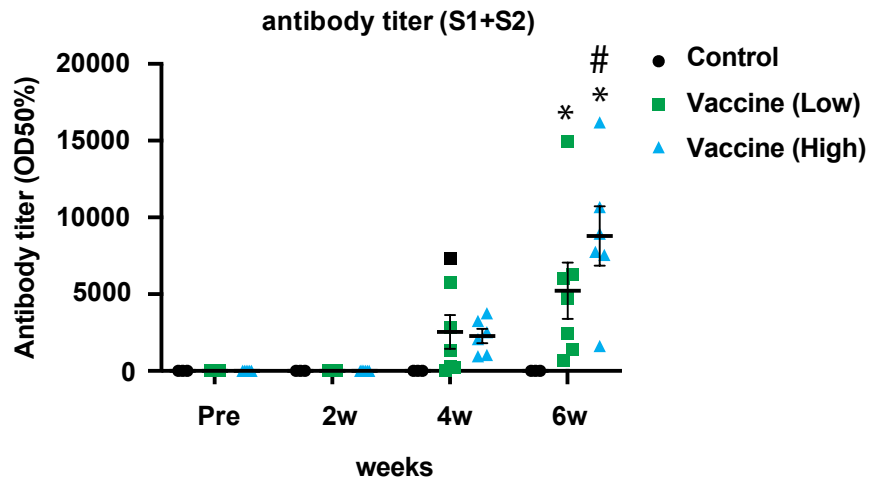


Figure S2. Dose-dependent DNA vaccine-induced anti-spike antibody production in rats.

Different doses of DNA vaccine (low dose: 66.6 $\mu\text{g}/\text{rat}$, high dose: 666.6 $\mu\text{g}/\text{rat}$) was injected into SD rats (female) 3 times at 2 weeks interval (at 0, 2, 4wks). Blood samples were collected at pre, 2w, 4w, and 6w to measure anti-Spike antibody titer. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. Vaccine (Low).

Figure S3.

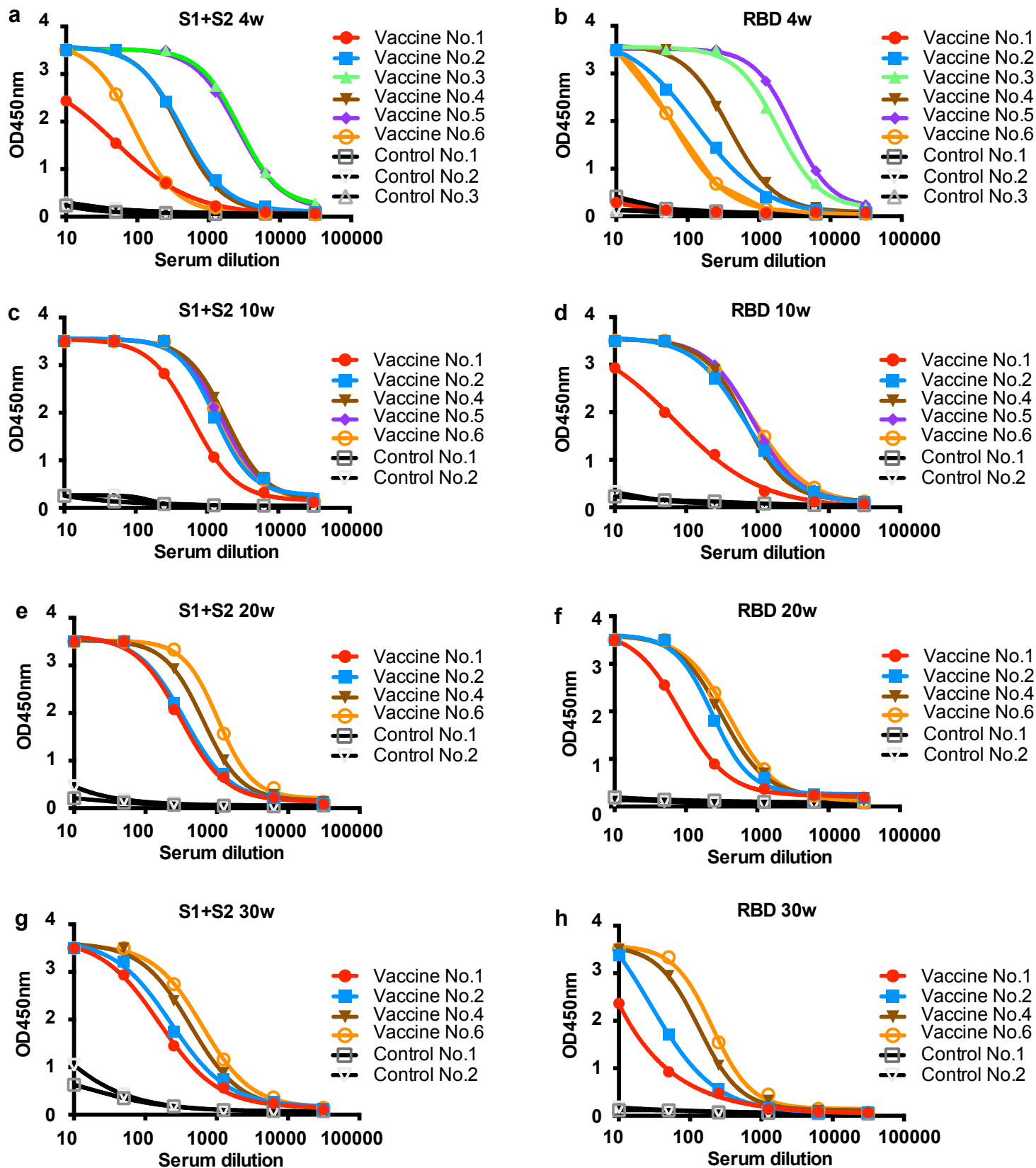


Figure S3. Spike glycoprotein-specific antibody titer from 4 weeks through 30 weeks.

Antibody titers for recombinant S1+S2 and RBD protein measured by ELISA. Serum dilution from 10x to x31250. (a) 4 weeks: S1+S2. (b) 4 weeks: RBD. (c) 10 weeks: S1+S2. (d) 10 weeks: RBD. (e) 20 weeks: S1+S2. (f) 20 weeks: RBD. (g) 30 weeks: S1+S2. (h) 30 weeks: RBD. See also Fig.2.

Figure S4.

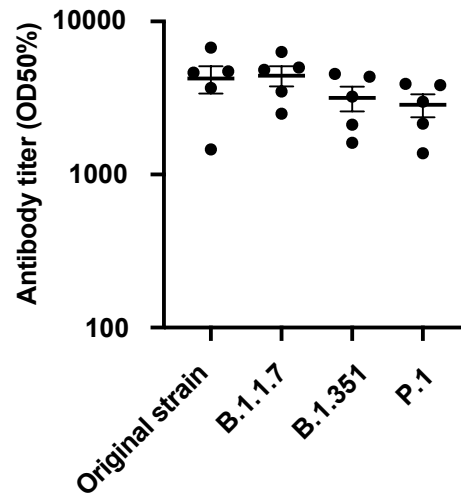


Figure S4. Antibody titer for the spike glycoprotein from original strain and variants (B.1.1.7, B. 1. 351, and P.1)

Antibody titer for recombinant spike glycoprotein from original strain or variants assessed by ELISA at 10 weeks after the 1st vaccination. Serum dilution from 10x to x31250. The antibody titer is shown as the serum dilution exhibiting half maximum binding at optical density at 450 nm (OD50%). Data are shown as mean \pm SEM. See also Fig.2.

Figure S5.

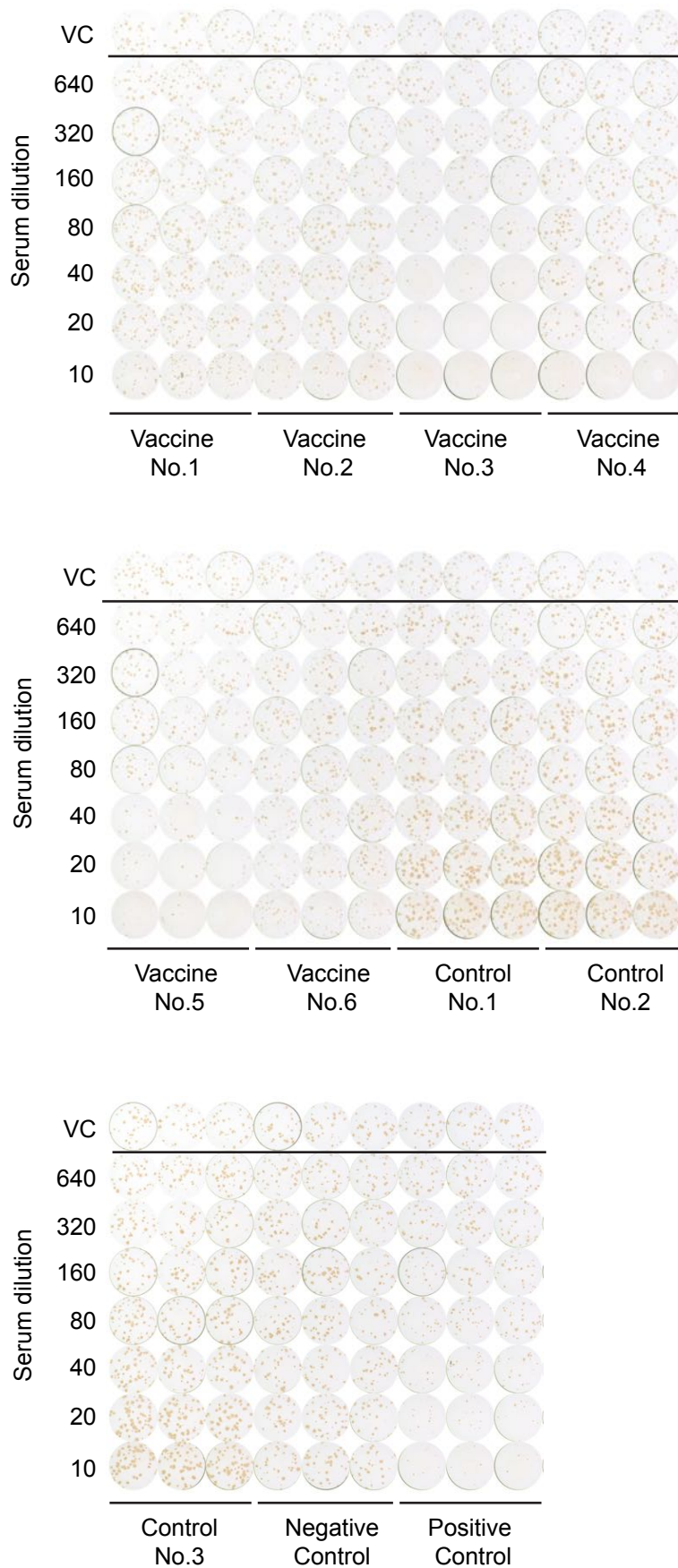


Figure S5. Neutralization activity of vaccinated serum against live SARS-CoV-2 by FRNT. Neutralization activity of serial dilution of vaccinated or control serum at 8 w was performed with live SARS-CoV-2 infection. VC: viral control. Acute-phase sera of SARS-CoV-2 infected patients was used as negative control, convalescent-phase sera serum of SARS-CoV-2 infected patients was used as positive control. Vaccine No.3 and Control No.3: 7 weeks sample. See also Fig.4.

Figure S6.

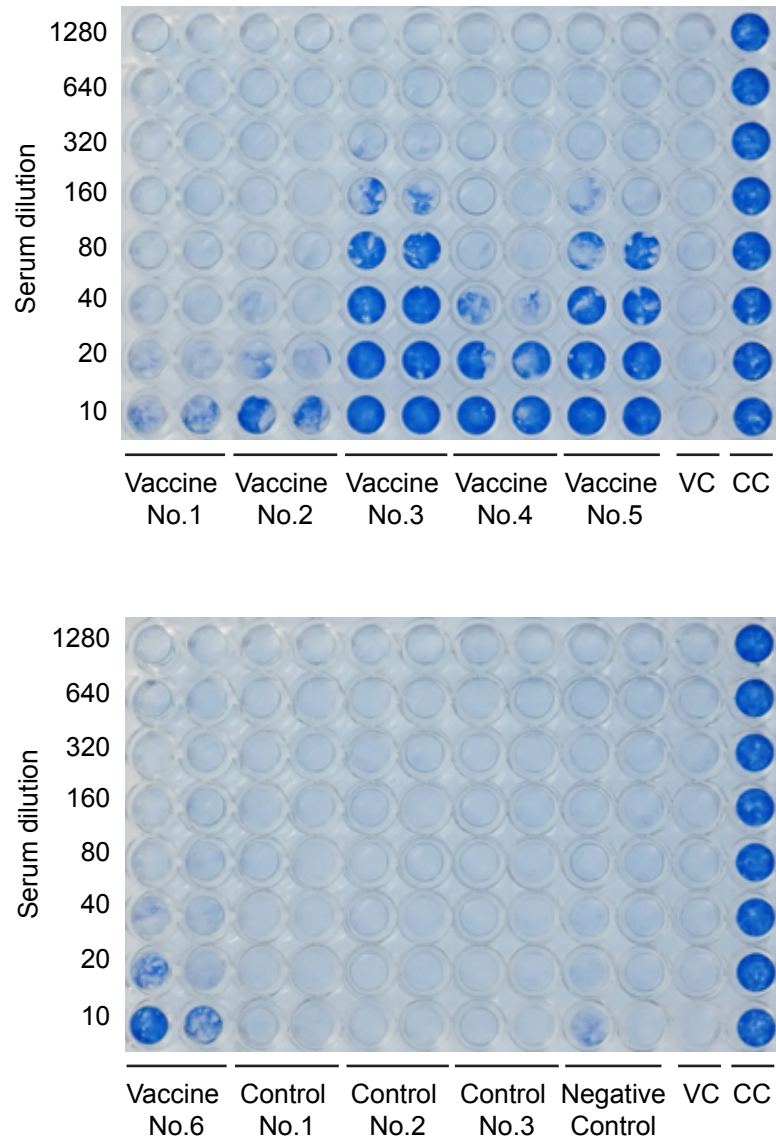


Figure S6. Neutralization activity of vaccinated serum against live SARS-CoV-2 by TCID. Neutralization activity of serial dilution of vaccinated or control serum at 8w was performed with live SARS-CoV-2 infection. VC: viral control. CC: cell control. Acute-phase sera of SARS-CoV-2 infected patients was used as negative control. Vaccine No.3 and Control No.3: 7 weeks sample. See also Fig.4.

Figure S7.

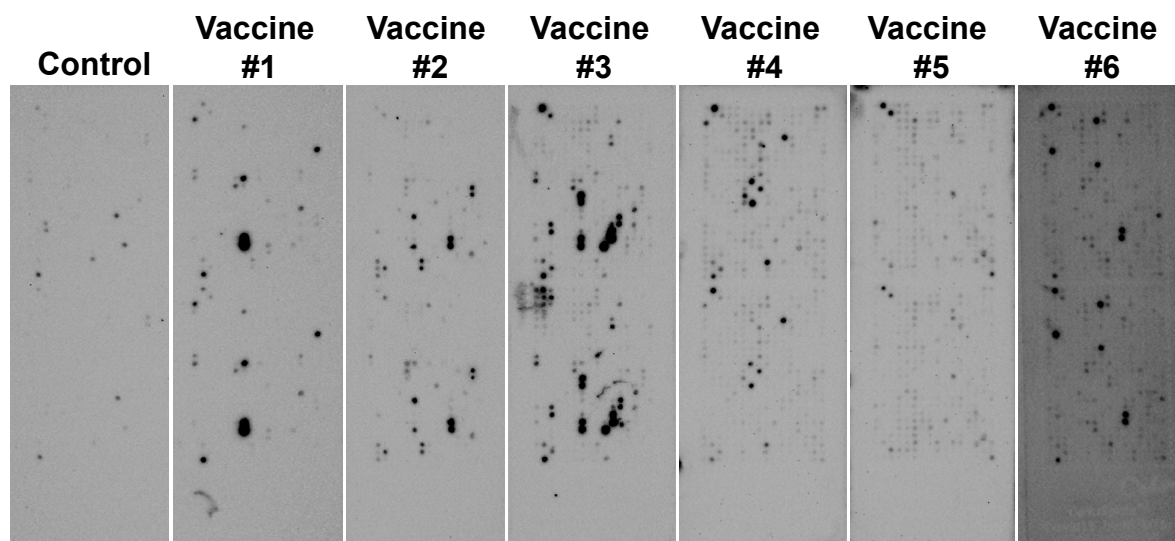


Figure S7. Epitope profiles of vaccine-induced antibodies (peptide array coated with the SARS-CoV-2 Spike glycoprotein). Spike peptide-coated membranes treated with immunized or nonimmunized rat serum at 6 weeks were developed by chemiluminescence. Images were acquired by a Bio-Rad instrument. See also Fig.5, Table 2, and Table S1.

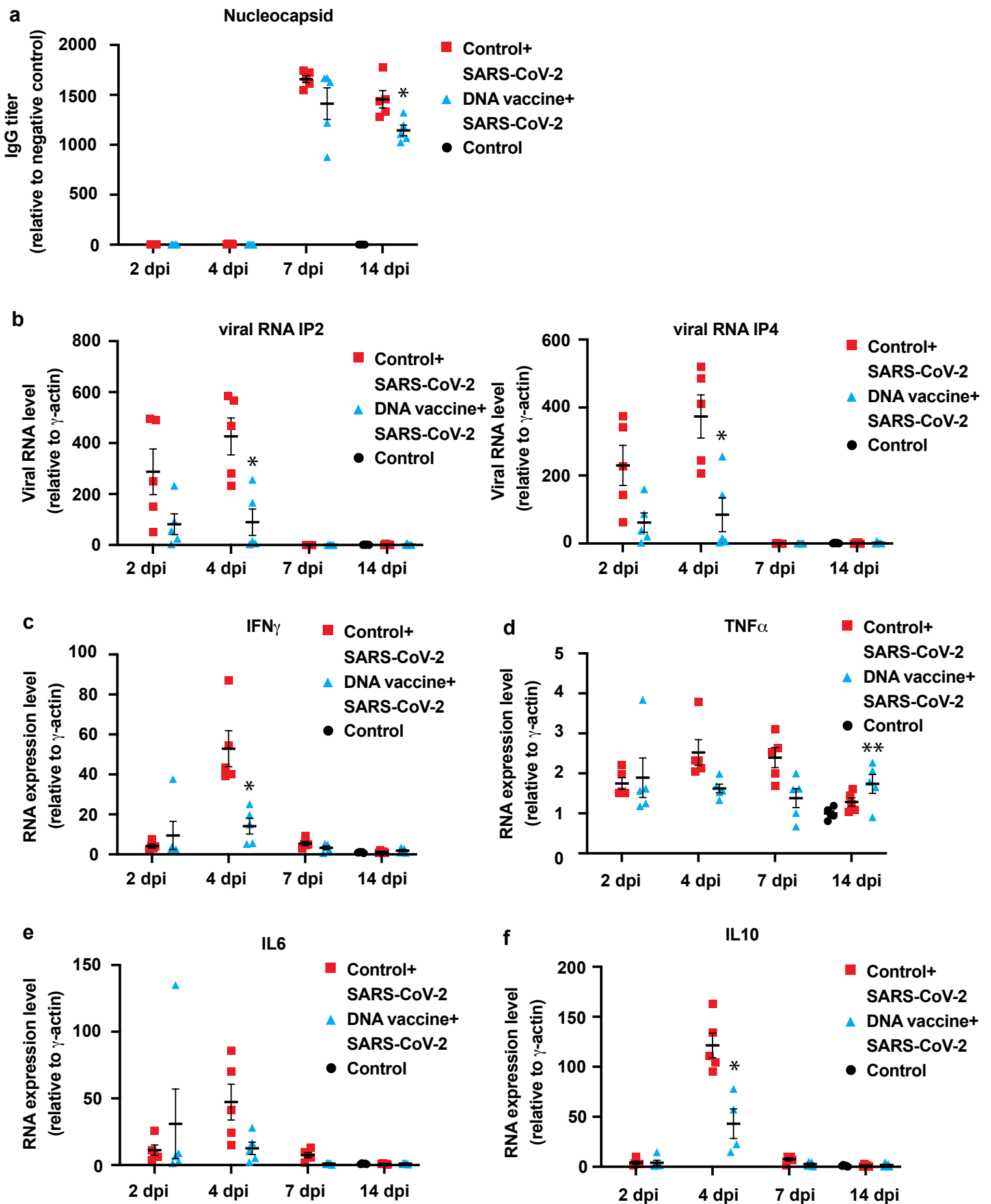
Figure S8

Figure S8. Antibody titer in serum, viral RNA level in lung, and expression of inflammation-related cytokines in lung of hamster after infection. (a) IgG antibody titer specific for nucleocapsid. (b) viral RNA level using IP2, IP4 primers in the lung. The value was normalized with γ -actin. (c) IFN γ , (d) TNF α , (e) IL6, (f) IL10 expression in the lung at 2, 4, 7, and 14 dpi with SARS-CoV-2 challenge. In IL10 (f), One sample from DNA vaccine + SARS-CoV-2 group at 4 dpi was below detection limit. The values were normalized with γ -actin.* $p < 0.05$ vs. Control+SARS-CoV-2. ** $p < 0.05$ vs. Control. Data are shown as mean \pm SEM. ANOVA followed by Bonferroni comparison. See also Fig.7.

Figure S9.

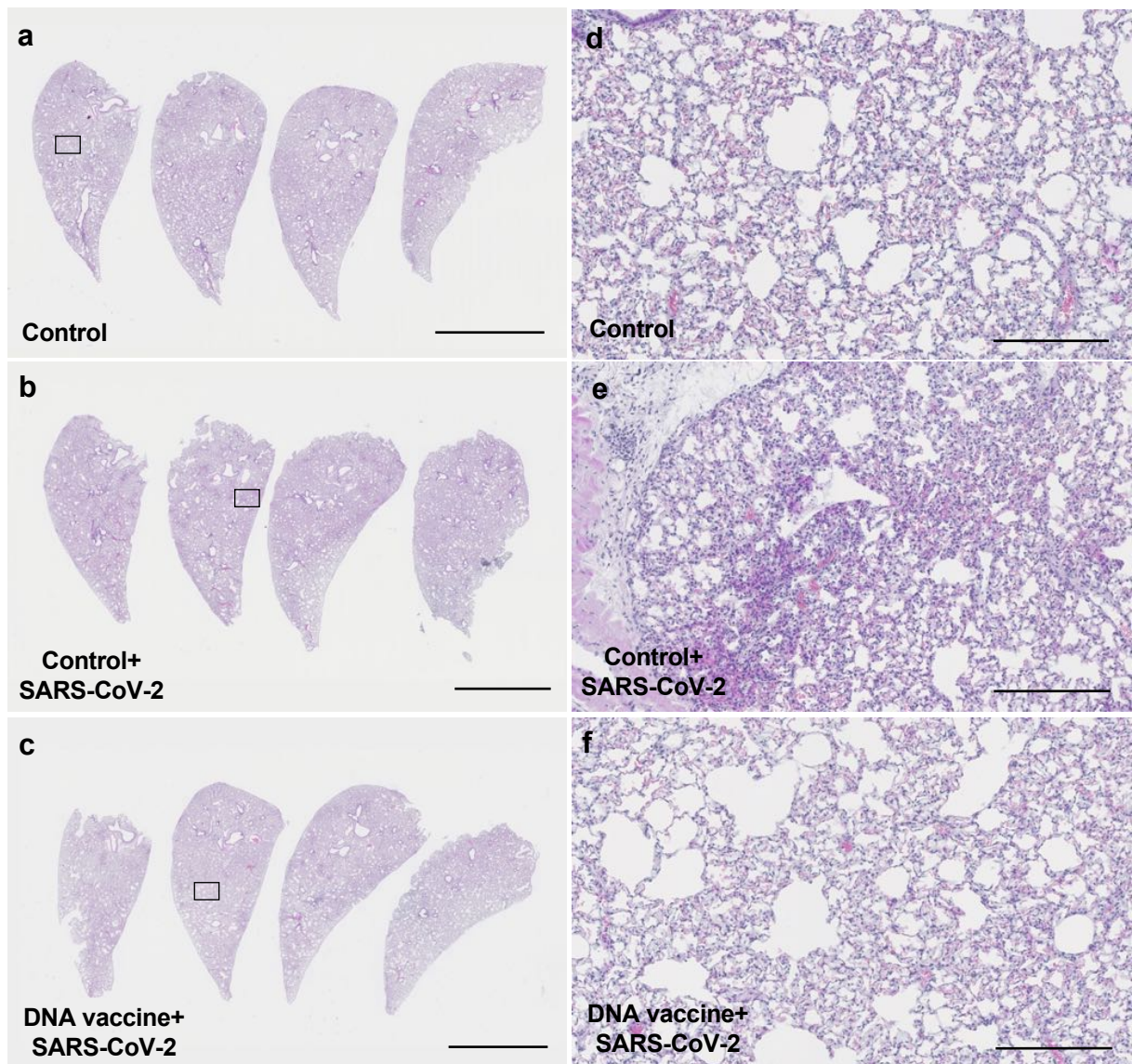


Figure S9. Histological analysis in the lung of hamster at 14 days after infection. Paraffin embedded lung was sectioned and stained with Hematoxylin-Phloxin (H&P) to visualize histomorphometric changes (inflammation). Shown are representative image from each groups, (a and d) Control, (b and e) Control+SARS-CoV-2 infection, (c and f) DNA vaccine + SARS-CoV-2 infection. (c-f) Enlarged images of the area with black rectangle from a-c respectively. (a)-(c) Scale bar=5mm. (d)-(f) Scale bar=250µm. See also Fig.7.

Table S1. Top 30 strongest epitope recognized by vaccine-induced antibody from each animal (No.1-No.6) : red color indicates RBD. See also Fig.5, Fig.S7, and Table 2.

No.1	Position	Amino Acid Sequence
1	1176 - 1190	V-V-N-I-Q-K-E-I-D-R-L-N-E-V-A
2	1171 - 1185	G-I-N-A-S-V-V-N-I-Q-K-E-I-D-R
3	1131 - 1145	G-I-V-N-N-T-V-Y-D-P-L-Q-P-E-L
4	31 - 50	S-F-T-R-G-V-Y-Y-P-D-K-V-F-R-S
5	1056 - 1070	A-P-H-G-V-V-F-L-H-V-T-Y-V-P-A
6	1166 - 1180	L-G-D-I-S-G-I-N-A-S-V-V-N-I-Q
7	311 - 325	G-I-Y-Q-T-S-N-F-R-V-Q-P-T-E-S
8	1256 - 1270	F-D-E-D-D-S-E-P-V-L-K-G-V-K-L
9	1181 - 1195	K-E-I-D-R-L-N-E-V-A-K-N-L-N-E
10	1051 - 1065	S-F-P-Q-S-A-P-H-G-V-V-F-L-H-V
11	1096 - 1110	V-S-N-G-T-H-W-F-V-T-Q-R-N-F-Y
12	816 - 830	S-F-I-E-D-L-L-F-N-K-V-T-L-A-D
13	286 - 300	T-D-A-V-D-C-A-L-D-P-L-S-E-T-K
14	1136 - 1150	T-V-Y-D-P-L-Q-P-E-L-D-S-F-K-E
15	1251 - 1265	G-S-C-C-K-F-D-E-D-D-S-E-P-V-L
16	456 - 470	F-R-K-S-N-L-K-P-F-E-R-D-I-S-T
17	821 - 835	L-L-F-N-K-V-T-L-A-D-A-G-F-I-K
18	81 - 95	N-P-V-L-P-F-N-D-G-V-Y-F-A-S-T
19	1066 - 1080	T-Y-V-P-A-Q-E-K-N-F-T-T-A-P-A
20	36 - 55	V-Y-Y-P-D-K-V-F-R-S-S-V-L-H-S
21	151 - 165	S-W-M-E-S-E-F-R-V-Y-S-S-A-N-N
22	1061 - 1075	V-F-L-H-V-T-Y-V-P-A-Q-E-K-N-F
23	786 - 800	K-Q-I-Y-K-T-P-P-I-K-D-F-G-G-F
24	106 - 120	F-G-T-T-L-D-S-K-T-Q-S-L-L-I-V
25	891 - 905	G-A-A-L-Q-I-P-F-A-M-Q-M-A-Y-R
26	26 - 40	P-A-Y-T-N-S-F-T-R-G-V-Y-Y-P-D
27	291 - 305	C-A-L-D-P-L-S-E-T-K-C-T-L-K-S
28	76 - 95	T-K-R-F-D-N-P-V-L-P-F-N-D-G-V
29	331 - 345	N-I-T-N-L-C-P-F-G-E-V-F-N-A-T
30	1126 - 1040	C-D-V-V-I-G-I-V-N-N-T-V-Y-D-P

No.2	Position	Amino Acid Sequence
1	576 - 590	V-R-D-P-Q-T-L-E-I-L-D-I-T-P-C
2	571 - 585	D-T-T-D-A-V-R-D-P-Q-T-L-E-I-L
3	176 - 190	L-M-D-L-E-G-K-Q-G-N-F-K-N-L-R
4	1156 - 1170	F-K-N-H-T-S-P-D-V-D-L-G-D-I-S
5	181 - 195	G-K-Q-G-N-F-K-N-L-R-E-F-V-F-K
6	1176 - 1190	V-V-N-I-Q-K-E-I-D-R-L-N-E-V-A
7	1066 - 1080	T-Y-V-P-A-Q-E-K-N-F-T-T-A-P-A
8	221 - 235	S-A-L-E-P-L-V-D-L-P-I-G-I-N-I
9	1071 - 1085	Q-E-K-N-F-T-T-A-P-A-I-C-H-D-G
10	1251 - 1265	G-S-C-C-K-F-D-E-D-D-S-E-P-V-L
11	1256 - 1270	F-D-E-D-D-S-E-P-V-L-K-G-V-K-L
12	566 - 580	N-K-K-F-L-P-F-Q-Q-F-G-R-D-I-A
13	851 - 865	C-A-Q-K-F-N-G-L-T-V-L-P-P-L-L
14	456 - 470	F-R-K-S-N-L-K-P-F-E-R-D-I-S-T
15	786 - 800	K-Q-I-Y-K-T-P-P-I-K-D-F-G-G-F
16	1131 - 1145	G-I-V-N-N-T-V-Y-D-P-L-Q-P-E-L
17	311 - 325	G-I-Y-Q-T-S-N-F-R-V-Q-P-T-E-S
18	451 - 465	Y-L-Y-R-L-F-R-K-S-N-L-K-P-F-E
19	691 - 705	S-I-I-A-Y-T-M-S-L-G-A-E-N-S-V
20	1261 - 1275	S-E-P-V-L-K-G-V-K-L-H-Y-T
21	416 - 430	G-K-I-A-D-Y-N-Y-K-L-P-D-D-F-T
22	581 - 595	T-L-E-I-L-D-I-T-P-C-S-F-G-G-V
23	296 - 310	L-S-E-T-K-C-T-L-K-S-F-T-V-E-K
24	461 - 475	L-K-P-F-E-R-D-I-S-T-E-I-Y-Q-A
25	561 - 575	P-F-Q-Q-F-G-R-D-I-A-D-T-T-D-A
26	301 - 315	C-T-L-K-S-F-T-V-E-K-G-I-Y-Q-T
27	696 - 710	T-M-S-L-G-A-E-N-S-V-A-Y-S-N-N
28	1216 - 1230	I-W-L-G-F-I-A-G-L-I-A-I-V-M-V
29	556 - 570	N-K-K-F-L-P-F-Q-Q-F-G-R-D-I-A
30	1211 - 1225	K-W-P-W-Y-I-W-L-G-F-I-A-G-L-I

No.3	Position	Amino Acid Sequence
1	816 - 830	S-F-I-E-D-L-L-F-N-K-V-T-L-A-D
2	691 - 705	S-I-I-A-Y-T-M-S-L-G-A-E-N-S-V
3	686 - 700	S-V-A-S-Q-S-I-I-A-Y-T-M-S-L-G
4	1176 - 1190	V-V-N-I-Q-K-E-I-D-R-L-N-E-V-A
5	1141- 1155	L-Q-P-E-L-D-S-F-K-E-E-L-D-K-Y
6	1171 - 1185	G-I-N-A-S-V-V-N-I-Q-K-E-I-D-R
7	1146 - 1160	D-S-F-K-E-E-L-D-K-Y-F-K-N-H-T
8	556 - 570	N-K-K-F-L-P-F-Q-Q-F-G-R-D-I-A
9	561 - 575	P-F-Q-Q-F-G-R-D-I-A-D-T-T-D-A
10	571 - 585	D-T-T-D-A-V-R-D-P-Q-T-L-E-I-L
11	681 - 695	P-R-R-A-R-S-V-A-S-Q-S-I-I-A-Y
12	626 - 640	A-D-Q-L-T-P-T-W-R-V-Y-S-T-G-S
13	311 - 325	G-I-Y-Q-T-S-N-F-R-V-Q-P-T-E-S
14	886 - 900	W-T-F-G-A-G-A-A-L-Q-I-P-F-A-M
15	811 - 825	K-P-S-K-R-S-F-I-E-D-L-L-F-N-K
16	1256 - 1270	F-D-E-D-D-S-E-P-V-L-K-G-V-K-L
17	696 - 710	T-M-S-L-G-A-E-N-S-V-A-Y-S-N-N
18	1166 - 1180	L-G-D-I-S-G-I-N-A-S-V-V-N-I-Q
19	321 - 335	Q-P-T-E-S-I-V-R-F-P-N-I-T-N-L
20	821 - 835	L-L-F-N-K-V-T-L-A-D-A-G-F-I-K
21	1151 - 1165	E-L-D-K-Y-F-K-N-H-T-S-P-D-V-D
22	621 - 635	P-V-A-I-H-A-D-Q-L-T-P-T-W-R-V
23	491 - 505	P-L-Q-S-Y-G-F-Q-P-T-N-G-V-G-Y
24	1251 - 1265	G-S-C-C-K-F-D-E-D-D-S-E-P-V-L
25	1131 - 1145	G-I-V-N-N-T-V-Y-D-P-L-Q-P-E-L
26	836 - 850	Q-Y-G-D-C-L-G-D-I-A-A-R-D-L-I
27	176 - 190	L-M-D-L-E-G-K-Q-G-N-F-K-N-L-R
28	971 - 985	G-A-I-S-S-V-L-N-D-I-L-S-R-L-D
29	566 - 580	G-R-D-I-A-D-T-T-D-A-V-R-D-P-Q
30	806 - 820	L-P-D-P-S-K-P-S-K-R-S-F-I-E-D

No.4	Position	Amino Acid Sequence
1	1146 - 1160	D-S-F-K-E-E-L-D-K-Y-F-K-N-H-T
2	621 - 635	P-V-A-I-H-A-D-Q-L-T-P-T-W-R-V
3	1131 - 1145	G-I-V-N-N-T-V-Y-D-P-L-Q-P-E-L
4	1016 - 1030	A-E-I-R-A-S-A-N-L-A-A-T-K-M-S
5	946 - 960	G-K-L-Q-D-V-V-N-Q-N-A-Q-A-L-N
6	1261 - 1275	S-E-P-V-L-K-G-V-K-L-H-Y-T
7	986 - 1000	K-V-E-A-E-V-Q-I-D-R-L-I-T-G-R
8	1006 - 1020	T-Y-V-T-Q-Q-L-I-R-A-A-E-I-R-A
9	996 - 1010	L-I-T-G-R-L-Q-S-L-Q-T-Y-V-T-Q
10	1266 - 1280	E-P-V-L-K-G-V-K-L-H-Y-T
11	851 - 865	C-A-Q-K-F-N-G-L-T-V-L-P-P-L-L
12	121 - 135	N-N-A-T-N-V-V-I-K-V-C-E-F-Q-F
13	1101 - 1115	H-W-F-V-T-Q-R-N-F-Y-E-P-Q-I-I
14	846 - 860	A-R-D-L-I-C-A-Q-K-F-N-G-L-T-V
15	1096 - 1110	V-S-N-G-T-H-W-F-V-T-Q-R-N-F-Y
16	971 - 985	G-A-I-S-S-V-L-N-D-I-L-S-R-L-D
17	456 - 470	F-R-K-S-N-L-K-P-F-E-R-D-I-S-T
18	1256 - 1270	F-D-E-D-D-S-E-P-V-L-K-G-V-K-L
19	1 - 15	M-F-V-F-L-V-L-L-P-L-V-S-S-Q-C
20	1211 - 1225	K-W-P-W-Y-I-W-L-G-F-I-A-G-L-I
21	951 - 965	V-V-N-Q-N-A-Q-A-L-N-T-L-V-K-Q
22	181 - 195	G-K-Q-G-N-F-K-N-L-R-E-F-V-F-K
23	811 - 825	K-P-S-K-R-S-F-I-E-D-L-L-F-N-K
24	116 - 130	S-L-L-I-V-N-N-A-T-N-V-V-I-K-V
25	506 - 520	Q-P-Y-R-V-V-V-L-S-F-E-L-L-H-A
26	126 - 140	V-V-I-K-V-C-E-F-Q-F-C-N-D-P-F
27	231 - 245	I-G-I-N-I-T-R-F-Q-T-L-L-A-L-H
28	1251 - 1265	G-S-C-C-K-F-D-E-D-D-S-E-P-V-L
29	991 - 1005	V-Q-I-D-R-L-I-T-G-R-L-Q-S-L-Q
30	431 - 445	G-C-V-I-A-W-N-S-N-N-L-D-S-K-V

No.5	Position	Amino Acid Sequence
1	116- 130	S-L-L-I-V-N-N-A-T-N-V-V-I-K-V
2	221 - 235	S-A-L-E-P-L-V-D-L-P-I-G-I-N-I
3	661 - 675	E-C-D-I-P-I-G-A-G-I-C-A-S-Y-Q
4	1211 - 1225	K-W-P-W-Y-I-W-L-G-F-I-A-G-L-I
5	621 - 635	P-V-A-I-H-A-D-Q-L-T-P-T-W-R-V
6	346 - 360	R-F-A-S-V-Y-A-W-N-R-K-R-I-S-N
7	616 - 630	N-C-T-E-V-P-V-A-I-H-A-D-Q-L-T
8	506 - 520	Q-P-Y-R-V-V-V-L-S-F-E-L-L-H-A
9	1221 - 1235	I-A-G-L-I-A-I-V-M-V-T-I-M-L-C
10	106 - 120	F-G-T-T-L-D-S-K-T-Q-S-L-L-I-V
11	121 - 135	N-N-A-T-N-V-V-I-K-V-C-E-F-Q-F
12	811 - 825	K-P-S-K-R-S-F-I-E-D-L-L-F-N-K
13	326 - 340	I-V-R-F-P-N-I-T-N-L-C-P-F-G-E
14	1216 - 1230	I-W-L-G-F-I-A-G-L-I-A-I-V-M-V
15	1121 -1135	F-V-S-G-N-C-D-V-V-I-G-I-V-N-N
16	851 - 865	C-A-Q-K-F-N-G-L-T-V-L-P-P-L-L
17	971 - 985	G-A-I-S-S-V-L-N-D-I-L-S-R-L-D
18	451 - 465	Y-L-Y-R-L-F-R-K-S-N-L-K-P-F-E
19	1126 - 1240	C-D-V-V-I-G-I-V-N-N-T-V-Y-D-P
20	76 - 95	T-K-R-F-D-N-P-V-L-P-F-N-D-G-V
21	1 - 15	M-F-V-F-L-V-L-L-P-L-V-S-S-Q-C
22	486 - 500	F-N-C-Y-F-P-L-Q-S-Y-G-F-Q-P-T
23	706 - 720	A-Y-S-N-N-S-I-A-I-P-T-N-F-T-I
24	946 - 960	G-K-L-Q-D-V-V-N-Q-N-A-Q-A-L-N
25	1056 - 1070	A-P-H-G-V-V-F-L-H-V-T-Y-V-P-A
26	876 - 890	A-L-L-A-G-T-I-T-S-G-W-T-F-G-A
27	586 - 600	D-I-T-P-C-S-F-G-G-V-S-V-I-T-P
28	176 - 190	L-M-D-L-E-G-K-Q-G-N-F-K-N-L-R
29	111 - 125	D-S-K-T-Q-S-L-L-I-V-N-N-A-T-N
30	846 - 860	A-R-D-L-I-C-A-Q-K-F-N-G-L-T-V

No.6	Position	Amino Acid Sequence
1	971 - 985	G-A-I-S-S-V-L-N-D-I-L-S-R-L-D
2	691 - 705	S-I-I-A-Y-T-M-S-L-G-A-E-N-S-V
3	686 - 700	S-V-A-S-Q-S-I-I-A-Y-T-M-S-L-G
4	1001 - 1015	L-Q-S-L-Q-T-Y-V-T-Q-Q-L-I-R-A
5	76 - 95	T-K-R-F-D-N-P-V-L-P-F-N-D-G-V
6	946 - 960	G-K-L-Q-D-V-V-N-Q-N-A-Q-A-L-N
7	851 - 865	C-A-Q-K-F-N-G-L-T-V-L-P-P-L-L
8	116 - 130	S-L-L-I-V-N-N-A-T-N-V-V-I-K-V
9	1256 - 1270	F-D-E-D-D-S-E-P-V-L-K-G-V-K-L
10	1176 - 1190	V-V-N-I-Q-K-E-I-D-R-L-N-E-V-A
11	846 - 860	A-R-D-L-I-C-A-Q-K-F-N-G-L-T-V
12	621 - 635	P-V-A-I-H-A-D-Q-L-T-P-T-W-R-V
13	491 - 505	P-L-Q-S-Y-G-F-Q-P-T-N-G-V-G-Y
14	1251 - 1265	G-S-C-C-K-F-D-E-D-D-S-E-P-V-L
15	681 - 695	P-R-R-A-R-S-V-A-S-Q-S-I-I-A-Y
16	1211 - 1255	I-A-G-L-I-A-I-V-M-V-T-I-M-L-C
17	106 - 120	F-G-T-T-L-D-S-K-T-Q-S-L-L-I-V
18	1171 - 1185	G-I-N-A-S-V-V-N-I-Q-K-E-I-D-R
19	191 - 205	E-F-V-F-K-N-I-D-G-Y-F-K-I-Y-S
20	1131 - 1145	G-I-V-N-N-T-V-Y-D-P-L-Q-P-E-L
21	321 - 335	Q-P-T-E-S-I-V-R-F-P-N-I-T-N-L
22	1216 - 1230	I-W-L-G-F-I-A-G-L-I-A-I-V-M-V
23	326 - 340	I-V-R-F-P-N-I-T-N-L-C-P-F-G-E
24	431 - 445	G-C-V-I-A-W-N-S-N-N-L-D-S-K-V
25	416 - 430	G-K-I-A-D-Y-N-Y-K-L-P-D-D-F-T
26	91 - 105	Y-F-A-S-T-E-K-S-N-I-I-R-G-W-I
27	456 - 470	F-R-K-S-N-L-K-P-F-E-R-D-I-S-T
28	906 - 920	F-N-G-I-G-V-T-Q-N-V-L-Y-E-N-Q
29	221 - 235	S-A-L-E-P-L-V-D-L-P-I-G-I-N-I
30	136 - 150	C-N-D-P-F-L-G-V-Y-Y-H-K-N-N-K

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