Immunodominant SARS Coronavirus Epitopes in Humans Elicited both Enhancing and Neutralizing Effects on Infection in Non-Human Primates

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Supplements

Table S1. Neutralization and enhancement of SARS-CoV infection of Vero E6 cells in the presence of human antisera of convalescent SARS patients*

[#]Enhancement of SARS-CoV infection						[#]Blockage of SARS-CoV infection	
SARS patient code	**Neutralization**	**SARS patient code**	**Neutralization**	**SARS patient Code**	**Neutralization**	**SARS patient code**	**Neutralization**
W1	–117.00% ± 13.73	W49	–53.00% ± 11.14	S204	–58.00% ± 9.98	W33	29.00% ± 6.28
W9	–92.00% ± 10.15	W61	–49.00% ± 16.08	S101	–91.00% ± 8.50	W29	48.00% ± 6.00
W35	–100.00% ± 11.56	W53	–53.00% ± 8.91	S245	–46.00% ± 7.69	W73	50.00% ± 7.77
W38	–65.00% ± 8.41	S102	–43.00% ± 1.95	S98	–62.00% ± 12.58	S100	25.00% ± 11.92
W41	–48.00% ± 9.82	S196	–46.00% ± 10.29			3E7	36.00% ± 2.27
W45	–42.00% ± 10.92	S241	–50.00% ± 9.35			1G4	56.00% ± 0.77

*Total 116 antisera (1:500 dilution) were tested in the neutral red staining(NRS) assay.

[#]For neutralization, six antisera with > 20% inhibition were defined as significantly blocking SARS-CoV infection; for enhancement, 17 antisera with > 40% augmentation were defined as significantly enhancing SARS-CoV infection.

Table S2 Design and synthesized new peptides

Novel peptides from the Spike glycoprotein
S11–38: TSGSDLDRCTTFDDVQAPNYTQHTSSMR
S119–132: NSTNVVIRACNFEL
S138–158: FAVSKPMGTQTHTMIFDNAFN
S159–184: CTFEYISDAFSLDVSEKSGNFKHLRE
S162–180: EYISDAFSLDVSEKSGNFK
S188–211: KNKDGFLYVYKGYQPIDVVRDLPSG
S342–368: RKKISNCVADYSVLYNSTFFSTFKCYG (Disulfide)
S391–404: GDDVRQIAPGGTGV
S416–434: FMGCVLAWNTRNIDATSTG
S436–459: YNYKYRYLRHGKLRPFERDISNVP
S471–490: ALNCYWPLNDYGFYTTTGIG
S491–502: YQPYRVVVLSFE
S522–535: NQCVNFNFNGLTGT
S597–625: LYQDVNCTDVSTAIHADQLTPAWRIYSTG

S679–695: MSLGADSSIAYSNNTIA

S686–713: SIAYSNNTIAIPTNFSISITTEVMPVSM

S803–828: LLFNKVTLADAGFMKQYGECLGDINA

S946–960: KQLSSNFGAISSVLN

S965–986: RLDKVEAEVQIDRLITGRLQSL

S993–1012: QLIRAAEIRASANLAATKM

S1084–1110: WFITQRNFFSPQIITTD

S1138–1154: FKNHTSPDVDLGDISG

S1155–1178: NASVVNIQKEIDRLNEVAKNLNE

Table S3. Generated antipeptide mAbs * NF=not functional.

No.	Clone no.		Isotype		Function*	Binding affinity to the peptide
1	$S_{471–503}$	2F7	IgG1	κ	NF	1.10E–09
2		3E10	IgG1	κ	NF	2.95E–09
3		4E5	IgG1	κ	Neutralizing	4.67E–09
4		4A10	IgG1	κ	Neutralizing	2.96E–09
5		6G5	IgG2b	κ	Neutralizing	5.82E–09
6		6A10	IgG1	κ	NF	7.57E–09
7		9A6	IgG1	κ	NF	4.94E–09
8	$S_{604–625}$	2B4	IgG1	κ	NF	4.75E–06
9		3B10	IgG1	κ	NF	2.17E–07
10		4H11	IgG1	κ	NF	1.68E–08
11		9A6	IgG1	κ	NF	2.62E–09
12		3A2	IgG2a	κ	NF	8.16E–09

13		11B1	IgG2a	κ	Neutralizing	2.32E–09
14		11B3	IgG2a	κ	NF	6.94E–08
15		14F1	IgG2b	κ	NF	1.58E–06
16		6D5	IgG1	κ	NF	8.77E–09
17	$S_{597–625}$	37-9-5	IgG1	κ	NF	3.94E–10
18		43-3-14	IgG1	κ	Enhancing	1.21E–11
19		65-5-10	IgG1	κ	NF	2.21E–10
20		219-10-28	IgG1	κ	Enhancing	1.20E–08
21	$S_{1164–1191}$	126-10	IgG1	κ	Neutralizing	4.59E–9
22		581-39	IgG1	κ	NF	1.56E–9
23		608-25	IgG1	κ	NF	1.68E–9

Table S4 Alanine walking peptides

L597A: AYQDVNCTDVSTAIHADQLTPAWRIYSTG
Y598A: LAQDVNCTDVSTAIHADQLTPAWRIYSTG
Q599A: LYADVNCTDVSTAIHADQLTPAWRIYSTG
D600A: LYQAVNCTDVSTAIHADQLTPAWRIYSTG
V601A: LYQDANCTDVSTAIHADQLTPAWRIYSTG
N602A: LYQDVACTDVSTAIHADQLTPAWRIYSTG
C603A: LYQDVNATDVSTAIHADQLTPAWRIYSTG
T604A: LYQDVNCADVSTAIHADQLTPAWRIYSTG
D605A: LYQDVNCTAVSTAIHADQLTPAWRIYSTG
V606A: LYQDVNCTDASTAIHADQLTPAWRIYSTG
mAb43-3-14 specifically binding short peptides
Leu-6-Asn(S597–602): LYQDVN
Leu-7-Cys(S597–603): LYQDVNC
Leu-8-Thr(S597–604): LYQDVNCT

Table S5 Monkeys for peptide vaccine immunization against SARS-CoV

Vac1 (Control group)			**Vac2 (MAP-$S_{597–625}$ formulated with FCA/IFA)**			**Vac3 (MAP-$S_{471–503}$, MAP-$S_{604–625}$, MAP-$S_{1164–1191}$ formulated with FCA/IFA)**			**Vac4 (MAP-$S_{471–503}$, MAP-$S_{597–625}$, MAP-$S_{1164–1191}$ formulated with FCA/IFA)**		
Animal code	**Gender**	**Body Weight (kg)**	**Animal code**	**Gender**	**Body weight (kg)**	**Animal Code**	**Gender**	**Body weight (kg)**	**Animal code**	**Gender**	**Body weight (kg)**
041870 (A1)	F	5.2	040888 (B1)	F	5.6	041057 (C1)	M	6.1	041642 (D1)	F	4.6
031860 (A2)	F	4.8	040390 (B2)	F	5.2	030874 (C2)	F	5.5	030177 (D2)	M	5.4
040995 (A3)	M	6.0	040085 (B3)	M	6.5	040203 (C3)	M	5.1	041399 (D3)	M	4.8
041911 (A4)	M	4.3	031519 (B4)	M	5.0	031632 (C4)	F	6.2	041902 (D4)	F	6.1

	040669 M 5.5 (B5)	041811 M 5.1 (C5)	041659 M 4.5 (D5)
	040830 F 4.8 (B6)	041962 F 4.5 (C6)	041326 F 6.0 (D6)

Table S6 Vaccinated animals sacrificed at 2 DPI

Vac1		**Vac2**		**Vac3**		**Vac4**	
Animal code	**Gender**	**Animal code**	**Gender**	**Animal code**	**Gender**	**Animal code**	**Gender**
041870 (A1)	F	040888 (B1)	F	041057 (C1)	M	041642 (D1)	F
031860 (A2)	F	040390 (B2)	F	030874 (C2)	F	030177 (D2)	M
		040085 (B3)	M	040203 (C3)	M	041399 (D3)	M

Table S7 Vaccinated animals sacrificed at 6 DPI

Vac1		**Vac2**		**Vac3**		**Vac4**	
Animal code	**Gender**	**Animal code**	**Gender**	**Animal code**	**Gender**	**Animal code**	**Gender**
040995 (A3)	M	041519 (B4)	M	031632 (C4)	F	041902 (D4)	F
041911 (A4)	M	040669	M	041811	M	041659	M

		(B5)		(C5)		(D5)	
		040830 (B6)	F	041962 (C6)	F	041326 (D6)	F

Table S8 Pathologic classification of the severity of the lung damage in SARS-CoV-infected rhesus macaques

Grade	**Pathologic classification of the severity of the lung damage in SARS-CoV-infected rhesus macaques**
–	normal macaque lung section without SARS-CoV infection
±	minor inflammation, slight alveolar septal broadening and sparse monocyte infiltration
I	apparent inflammation, hemorrhage in septa, elastic fibers of alveolar wall distorted as shown by silver staining
II	early symptoms of acute diffuse alveolar damage (DAD), alveolar septal broadening with increasing infiltration of inflammatory cells
III	typical symptoms of acute DAD, extensive exudation and septal broadening, shrinkage of alveoli caused by pressure, restricted fusion of thick septa, ruptured elastic fibers of alveoli, variably filled with protein-rich edema fluid, fibrin, erythrocytes, cellular debris, and a moderate number of inflammatory cells in alveolar cavities
IV	severe acute DAD, massive cell infiltration and alveolar shrinking, sheets of septal fusion, necrotic lesions at the hemorrhagic septa and massive cell numbers in alveolar cavities

Table S9 Monkeys treated by mAb43-3-14 were sacrificed at 2 DPI or 6 DPI

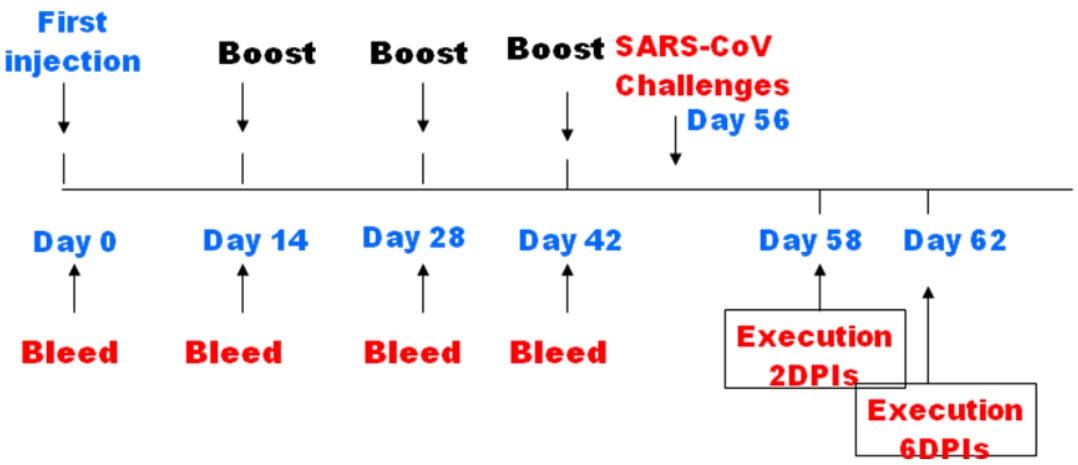
	Control group(0.9% NaCl)			**0.2mg/kg group**			**1.8mg/kg group**		
	Group	**Animal code**	**Body weight(kg)**	**Group**	**Animal code**	**Body weight(kg)**	**Group**	**Animal code**	**Body weight(kg)**
2 DPI	A1	061920	3.3	B1	041986	4.2	D1	051848	3.85
	A2	050426	4.1	B2	040654	3.8	D2	051976	3.9
	A3	051926	3.6	B3	060512	3.15	D3	060578	3.3
6 DPI	A4	060090	3.5	B4	051192	4.1	D4	060060	3.7
	A5	051804	3.9	B5	051820	3.6	D5	040478	3.8
	A6	050104	4.0	B6	051282	3.8	D6	051144	4.1

Table S10 The conditions for preparation of multiple antigen peptides

MAP\Conditions	**Lysine core/peptide amount**	**Reaction solvent**	**Reaction pH/Reaction time**	**Reaction monitoring gradient**	**Preparation condition**	**Molecular weight (Da, calculated/ observed)**	**Amount (Yield)**	**Purity**
MAP-$S_{604–625}$	0.4 mg (0.42 mmol)/5.1 mg (2.00 mmol)	CH_3CN/H_2O (25/75, v/v)	7.8 (saturated Na_2CO_3 adjusting), 2 h	0–25% CH_3CN for 8 min, 25–45% CH_3CN for 50 min	Vydac (208TP510), 0–25% CH_3CN for 5 min, 25–40% CH_3CN for 50 min	10655.83/10656.1072	2.1 mg (40%)	98%
MAP-$S_{597–625}$	0.35 mg (0.37 mmol)/5.0 mg (1.54	CH_3CN/H_2O (50/50, v/v)	8.5 (saturated Na_2CO_3 adjusting), 5 h	0–25% CH_3CN for 8 min, 25–45% CH_3CN for	Vydac (214TP1022), 0–25% CH_3CN for 5	13581.83/13587.2338	2.5 mg (50%)	95%

	mmol)			50 min	min, 25–40% CH_3CN for 50 min			
MAP-S$_{471–503}$	0.30 mg (0.31 mmol)/5.0 mg (1.29 mmol)	CH_3CN/H_2O/DMSO (40/50/10, v/v/v)	8.5 (saturated Na_2CO_3 adjusting), overnight, or ultrasonication	0–40% CH_3CN for 8 min, 40–55% CH_3CN for 50 min	Vydac (214TP1022), 0–25% CH_3CN for 5 min, 25–40% CH_3CN for 50 min	16084.23/16091.4292	1.5 mg (30%)	96%
MAP-S$_{1164–1191}$	0.35 mg (0.37 mmol)/5.0 mg (1.54 mmol)	CH_3CN/H_2O (25/75, v/v)	7.8 (saturated Na_2CO_3 adjusting), 2 h	0–25% CH_3CN for 8 min, 25–45% CH_3CN for 50 min	Vydac (208TP510), 0–35% CH_3CN for 5 min, 35–45% CH_3CN for 50 min	14389.03/14392.1516	2.1 mg (42%)	98%

Chart S1. The protocol of immunization of rhesus monkeys



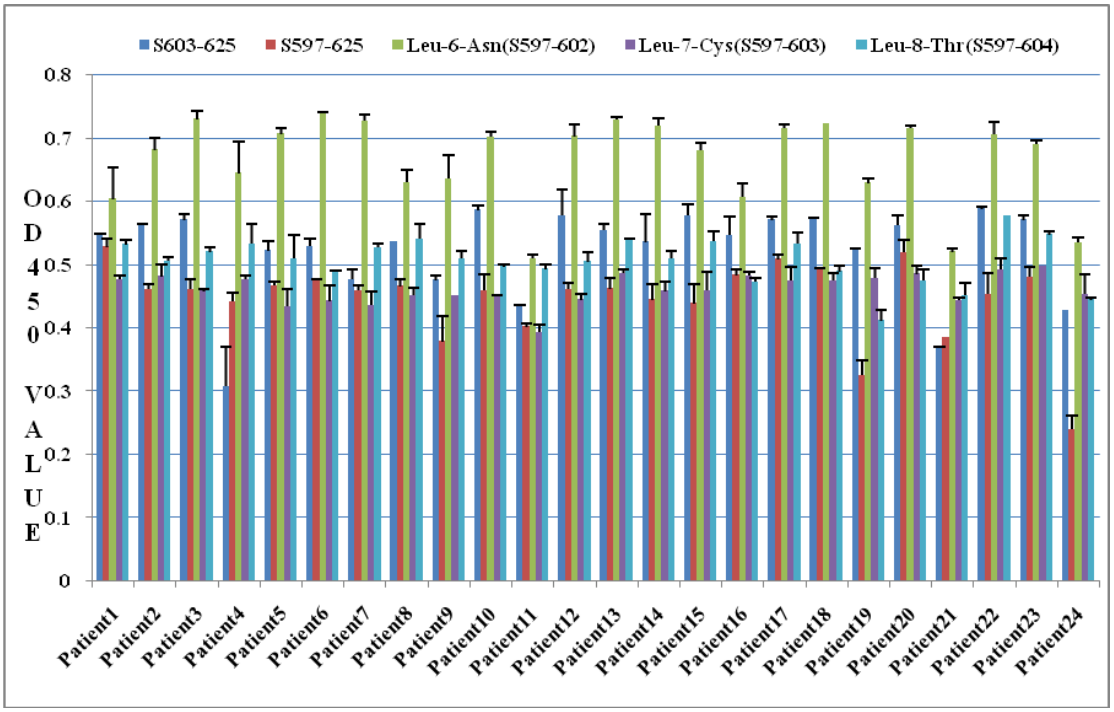


Fig. S1 The serologic reactivity of peptides with the human IgG of convalescent SARS patients.

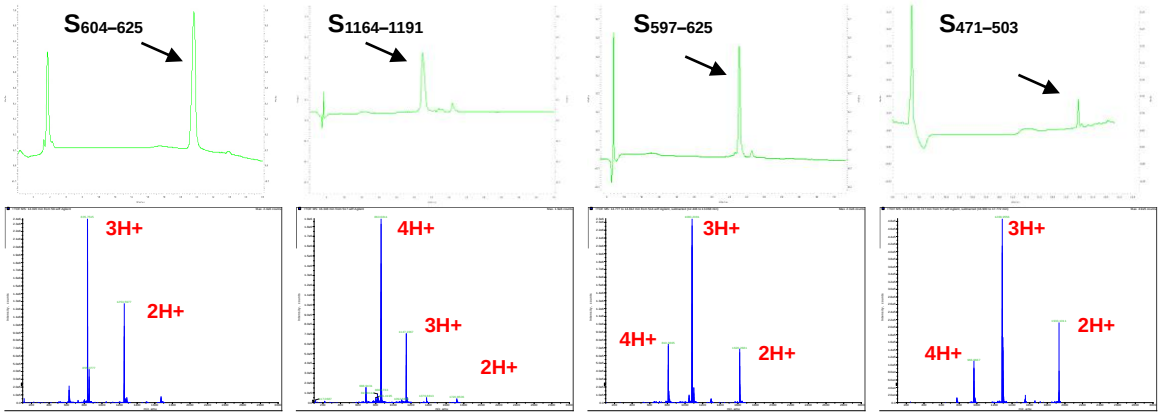


Fig. S2 Characterization of $S_{604–625}$, $S_{1164–1191}$, $S_{597–625}$ and $S_{471–503}$. RP–HPLC profiles and MS data.

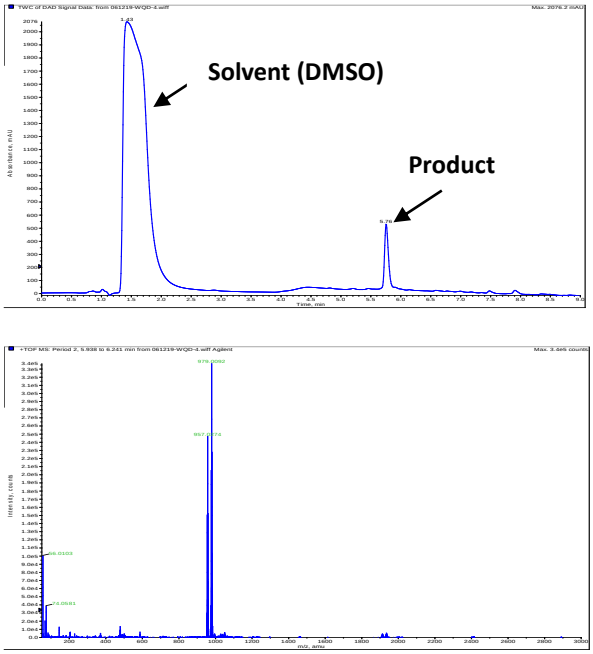


Fig. S3 Characterization of lysine core by RP–HPLC analysis and mass spectrum.

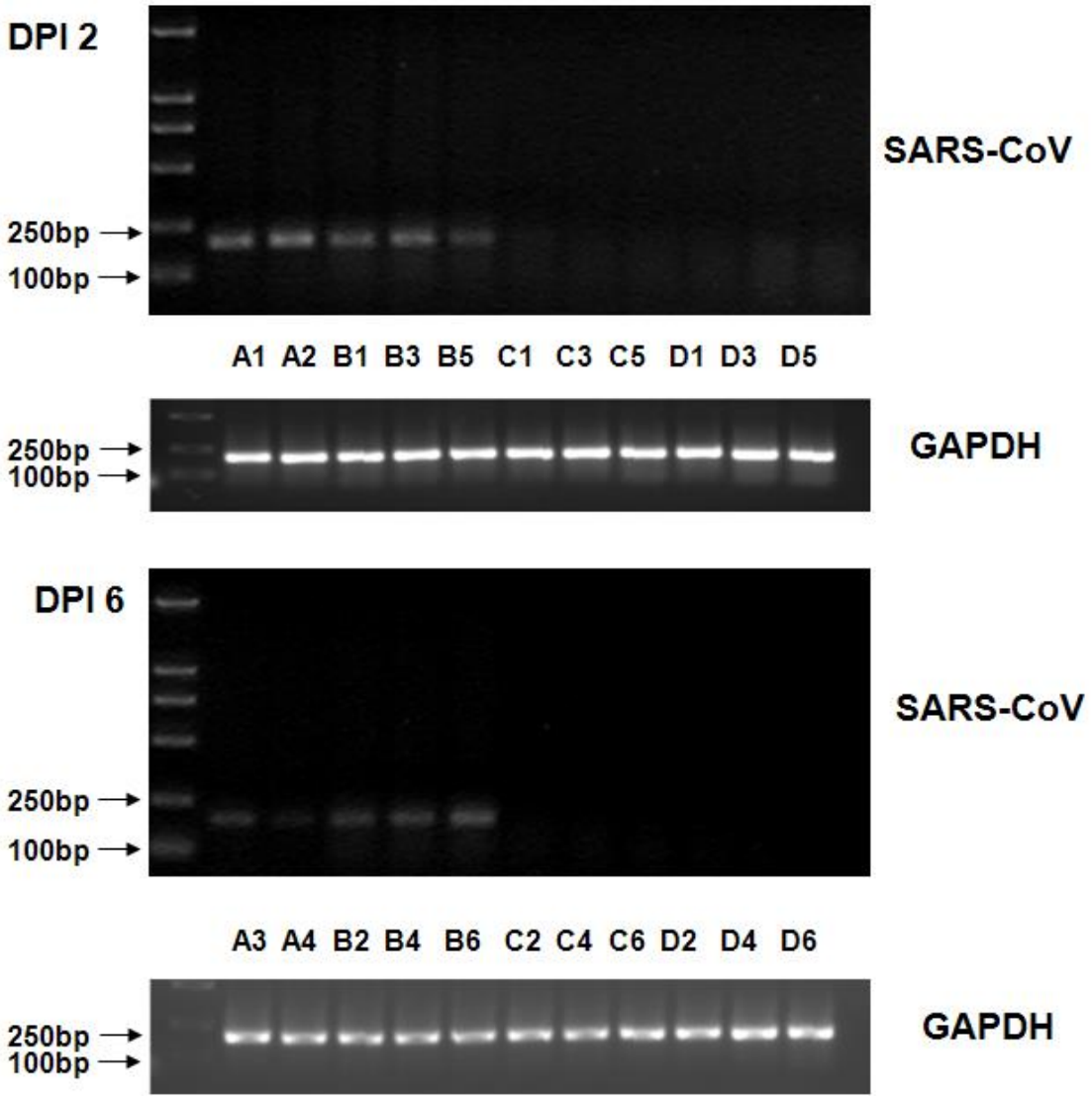


Fig. S4 Lung tissue viral burden analyzed by RT-PCR (A1-A4: Vac1 group, B1-B6: Vac 2 group, C1-C6: Vac 3 group, D1-D6: Vac 4 group)

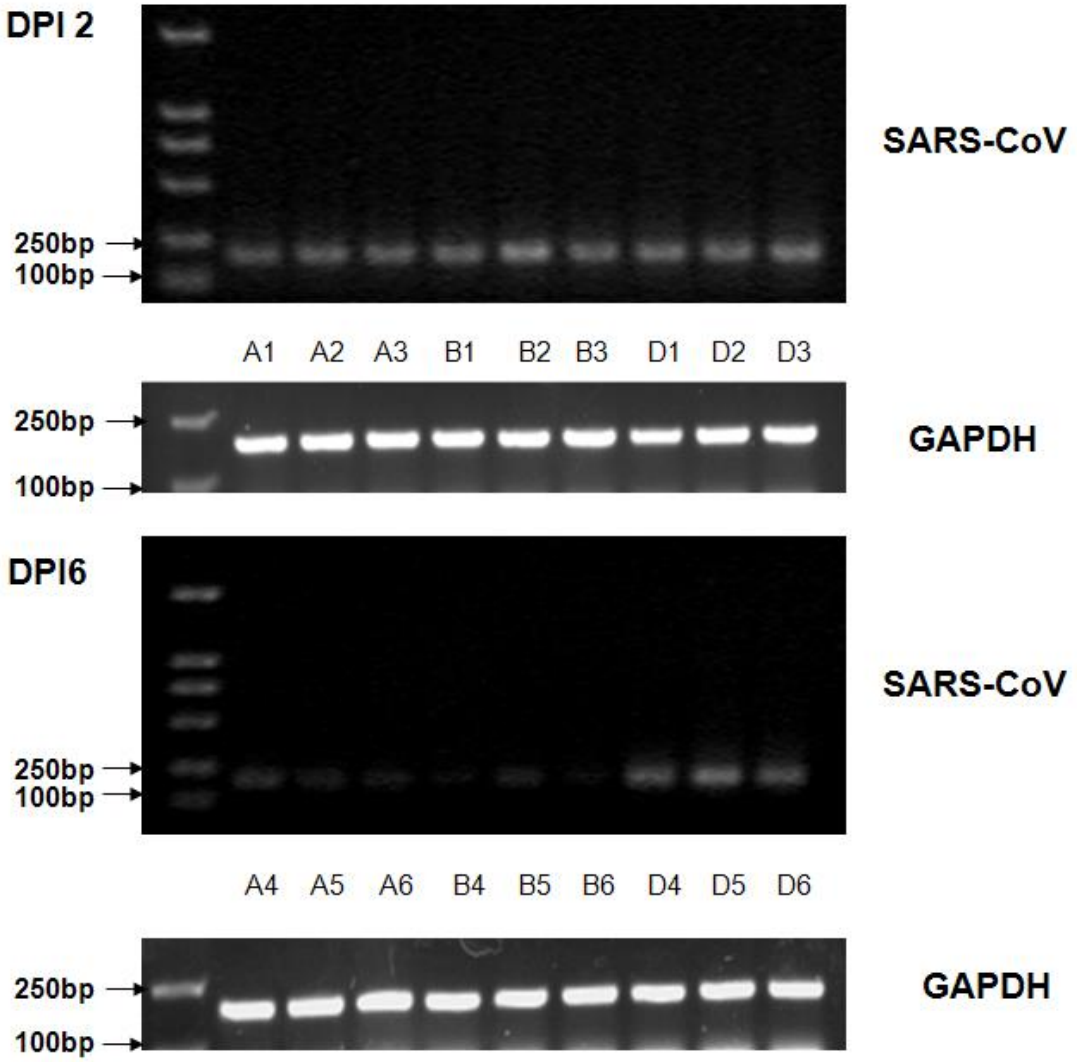


Fig. S5 Lung tissue viral burden analyzed by RT-PCR (A1-A6: 0.9% NaCl group, B1-B6: 0.2mg/kg group, C1-C6: 1.8mg/kg group)