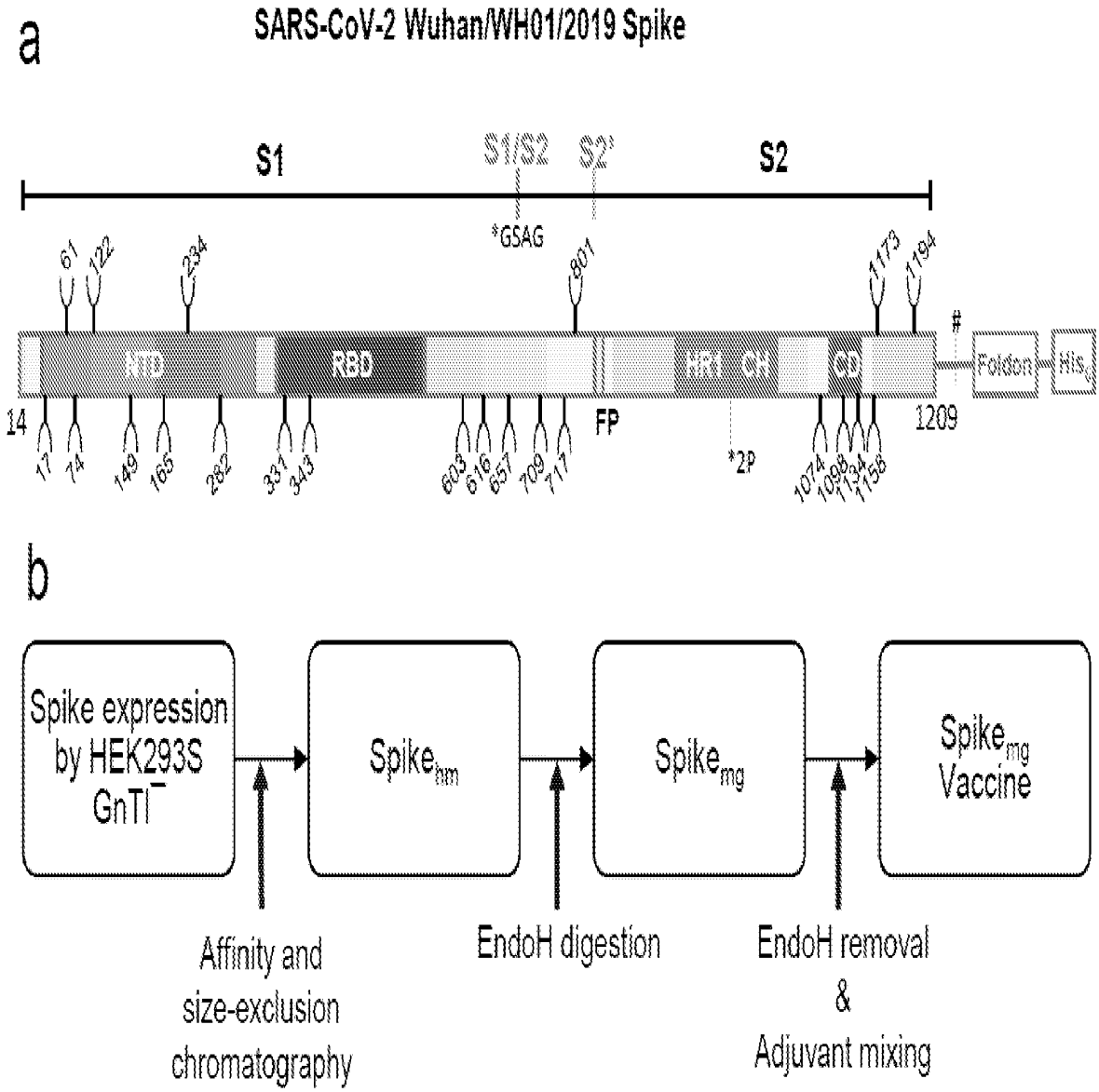

a
SARS-CoV-2 Wuhan/WH01/2019 Spike
S1
S1/S2
S2'
S2
*GSAG
61
122
234
801
1173
1194
#
NTD
RBD
HR1
CH
CD
Foldon
His6
14
1209
FP
*2P
17
74
149
165
282
331
343
603
616
657
709
717
1074
1098
1134
1158
b
Spike expression by HEK293S GnTI-
Spikehm
Spikemg
Spikemg Vaccine
Affinity and size-exclusion chromatography
EndoH digestion
EndoH removal & Adjuvant mixing

Fig. 1

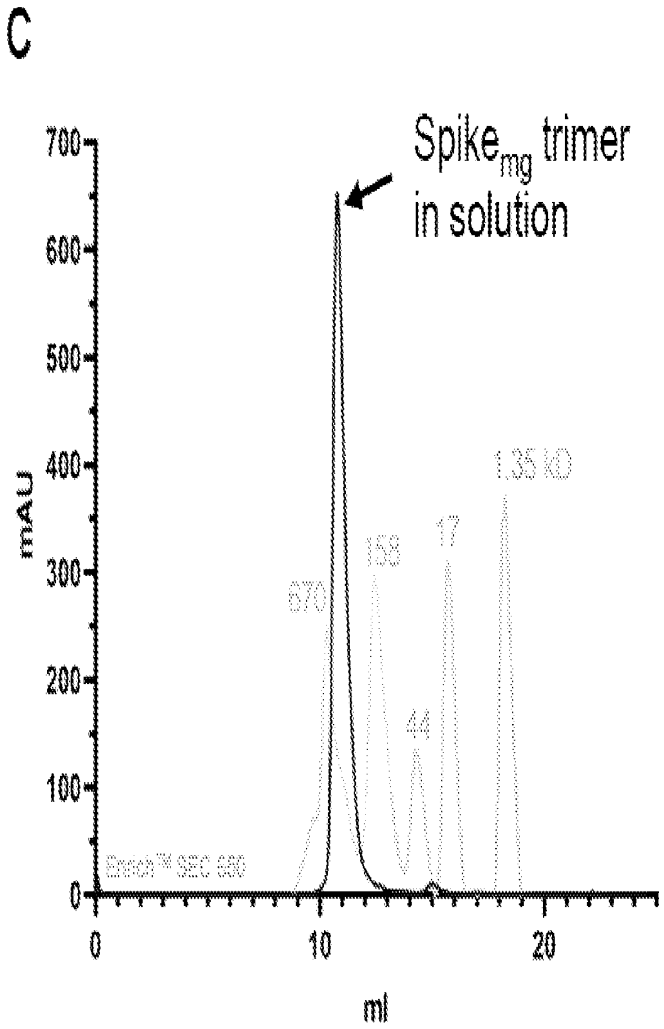

C
Spike$_{mg}$ trimer
in solution
mAU
ml
670
158
44
17
1.35 kD
ENrich™ SEC 650
0
100
200
300
400
500
600
700
0
10
20

Fig. 1

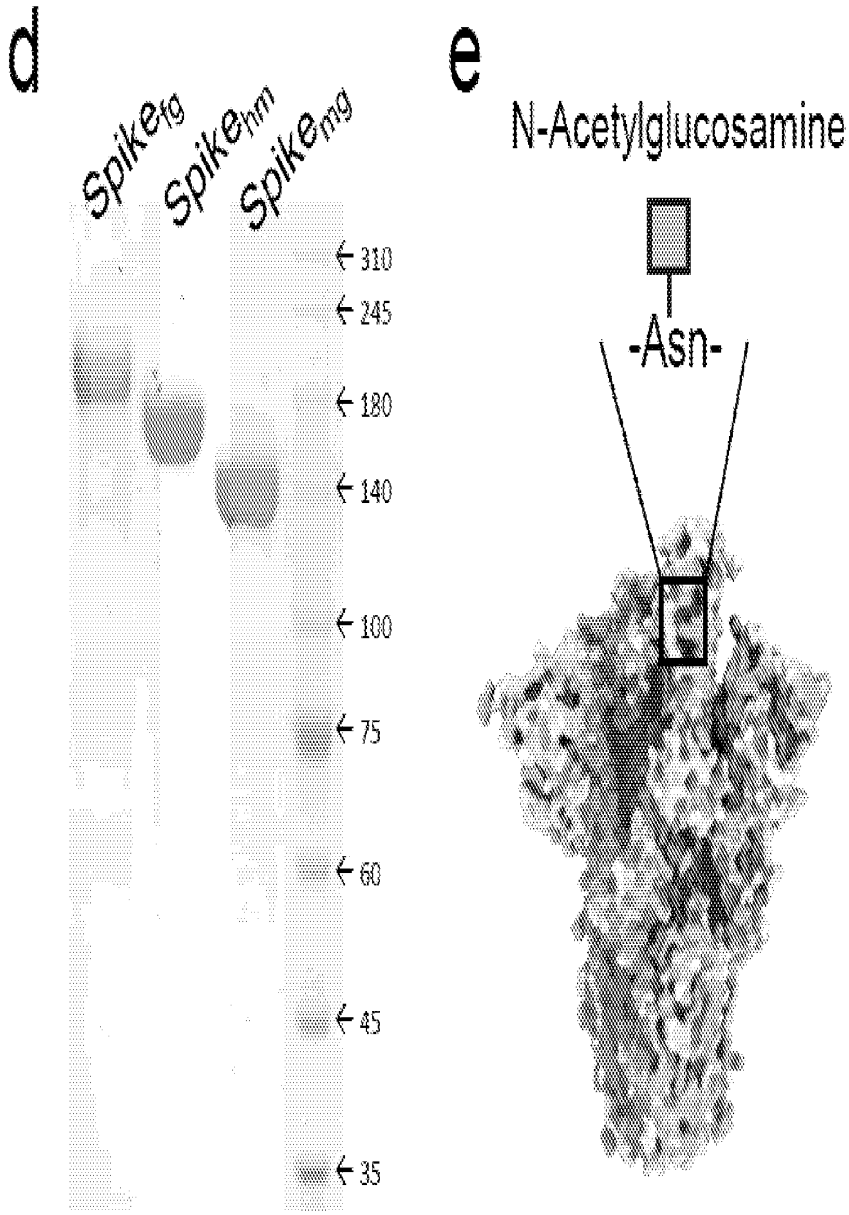

d
Spike$_{fg}$
Spike$_{hm}$
Spike$_{mg}$
310
245
180
140
100
75
60
45
35
e
N-Acetylglucosamine
-Asn-

Fig. 1

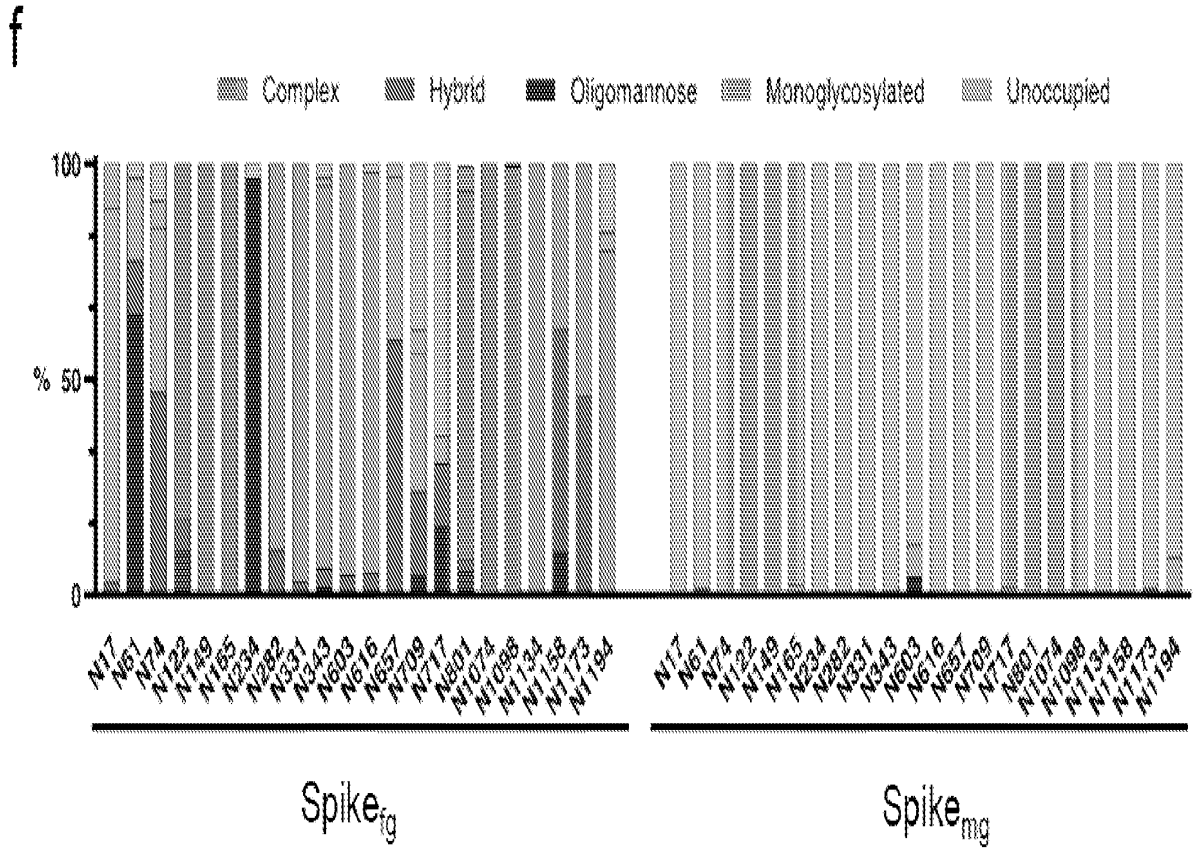

f
Complex
Hybrid
Oligomannose
Monoglycosylated
Unoccupied
100
% 50
0
N17
N61
N74
N122
N149
N165
N234
N262
N331
N343
N603
N616
N657
N709
N717
N801
N1074
N1098
N1134
N1158
N1173
N1194
Spike$_{fg}$
Spike$_{mg}$

Fig. 1

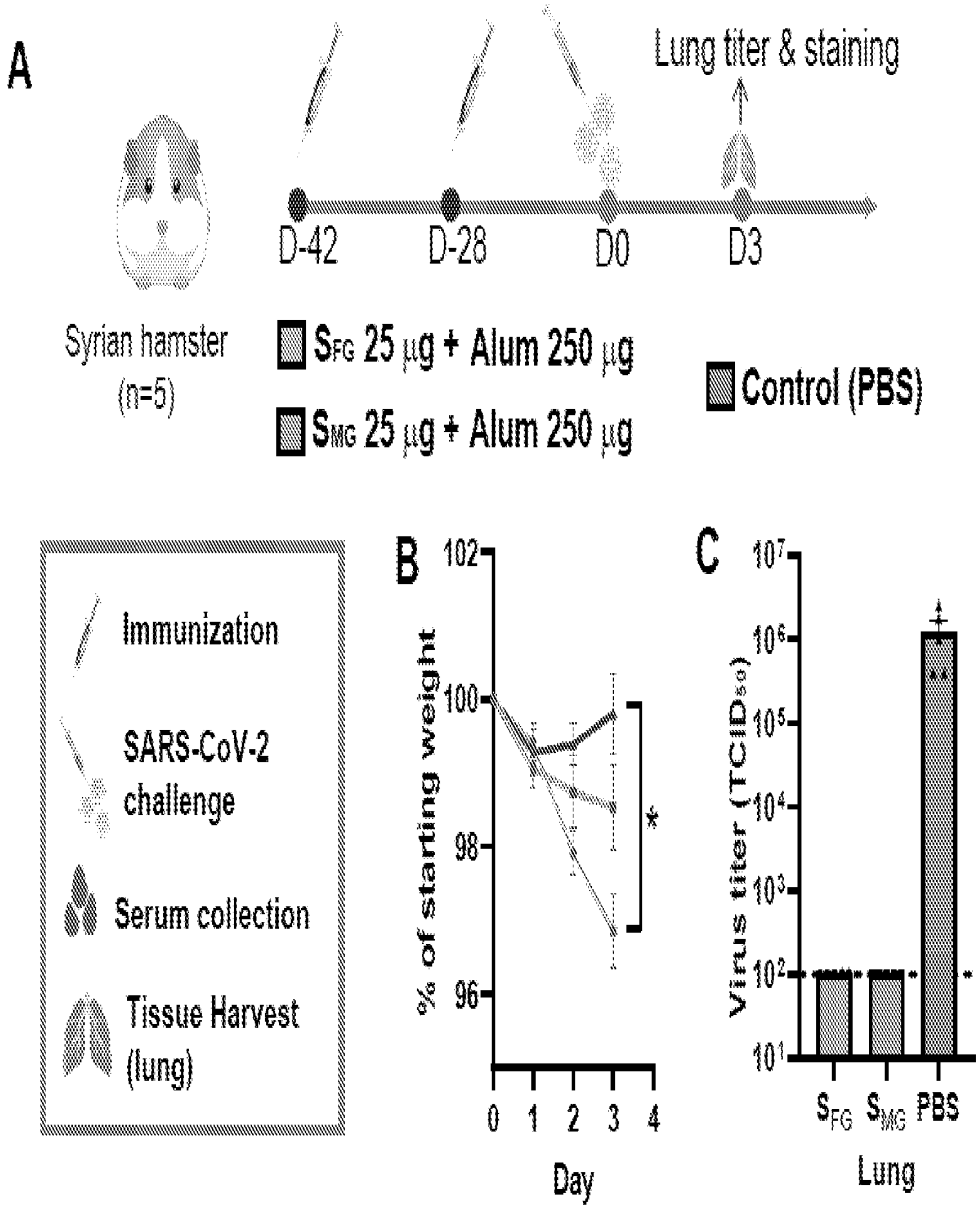

A
Lung titer & staining
D-42
D-28
D0
D3
Syrian hamster
(n=5)
SFG 25 μg + Alum 250 μg
SMG 25 μg + Alum 250 μg
Control (PBS)
Immunization
SARS-CoV-2
challenge
Serum collection
Tissue Harvest
(lung)
B
% of starting weight
Day
C
Virus titer (TCID50)
SFG SMG PBS
Lung

Fig. 2

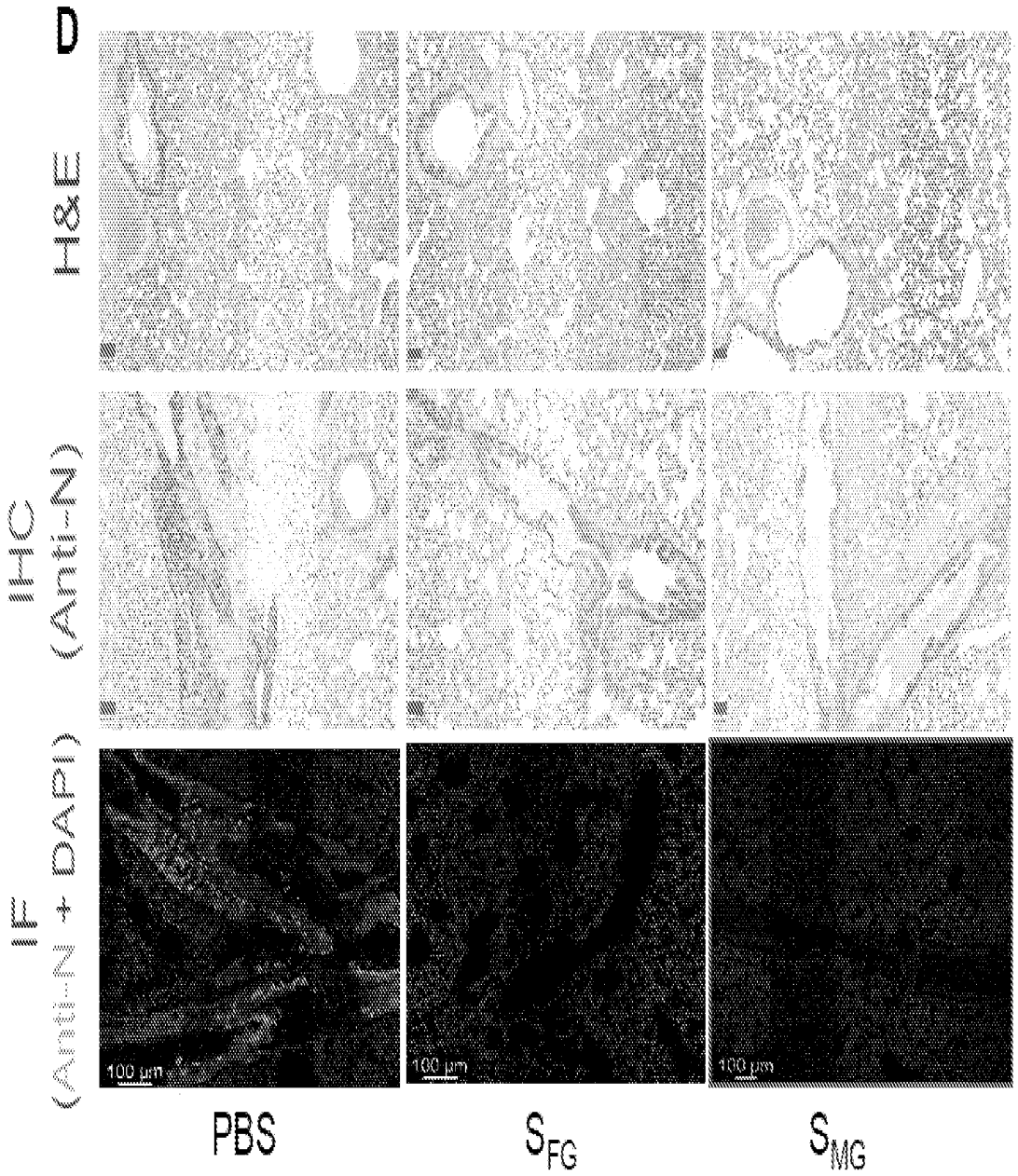


Fig. 2

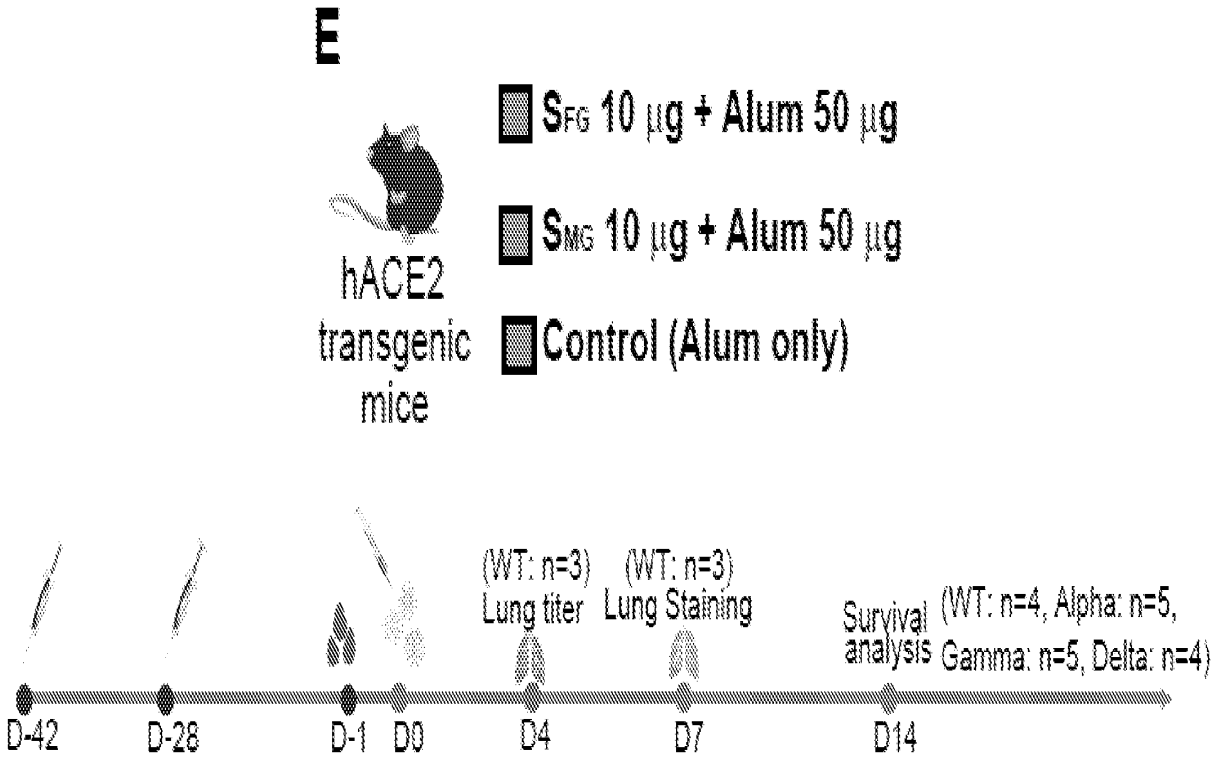

E
SFG 10 μg + Alum 50 μg
SMG 10 μg + Alum 50 μg
Control (Alum only)
hACE2 transgenic mice
(WT: n=3) Lung titer
(WT: n=3) Lung Staining
Survival analysis
(WT: n=4, Alpha: n=5, Gamma: n=5, Delta: n=4)
D-42
D-28
D-1
D0
D4
D7
D14

Fig. 2

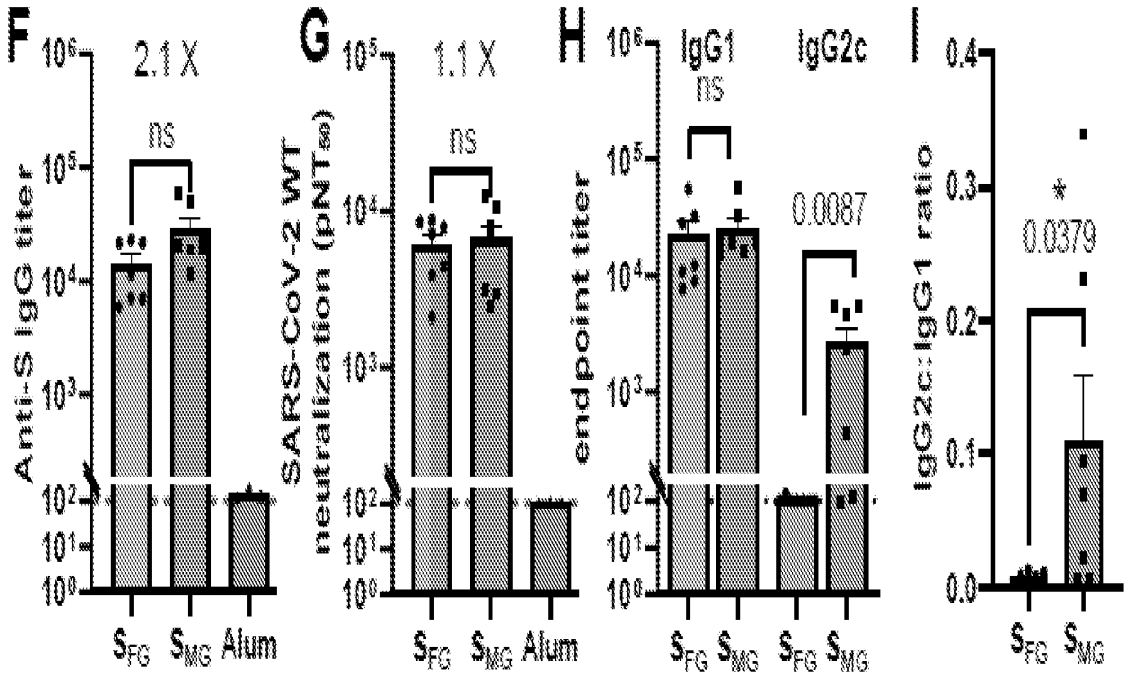

F
Anti-S IgG titer
2.1 X
ns
S_{FG} S_{MG} Alum
G
SARS-CoV-2 WT neutralization (pNT_{50})
1.1 X
ns
S_{FG} S_{MG} Alum
H
endpoint titer
IgG1
IgG2c
ns
0.0087
S_{FG} S_{MG} S_{FG} S_{MG}
I
IgG2c:IgG1 ratio
*
0.0379
S_{FG} S_{MG}

Fig. 2

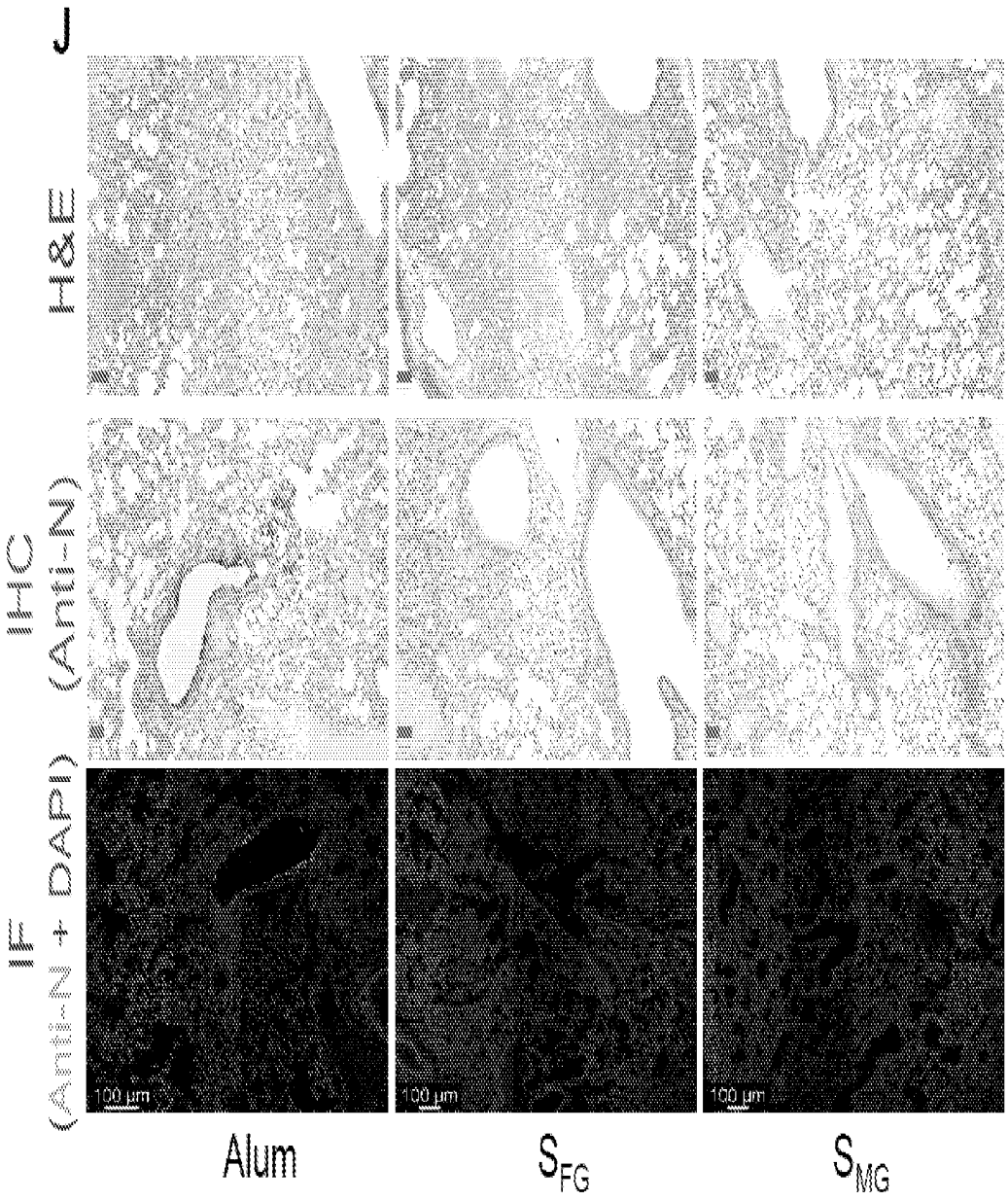


Fig. 2

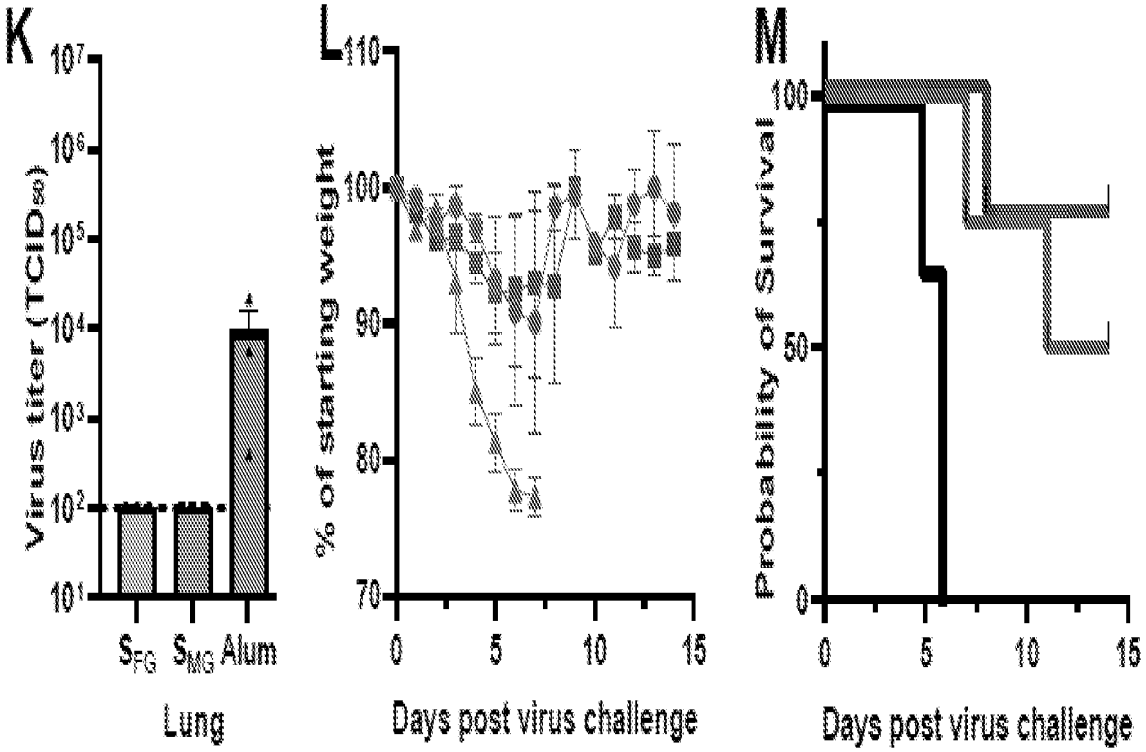

K
Virus titer (TCID50)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
S_{FG} S_{MG} Alum
Lung
L
% of starting weight
110
100
90
80
70
0 5 10 15
Days post virus challenge
M
Probability of Survival
100
50
0
0 5 10 15
Days post virus challenge

Fig. 2

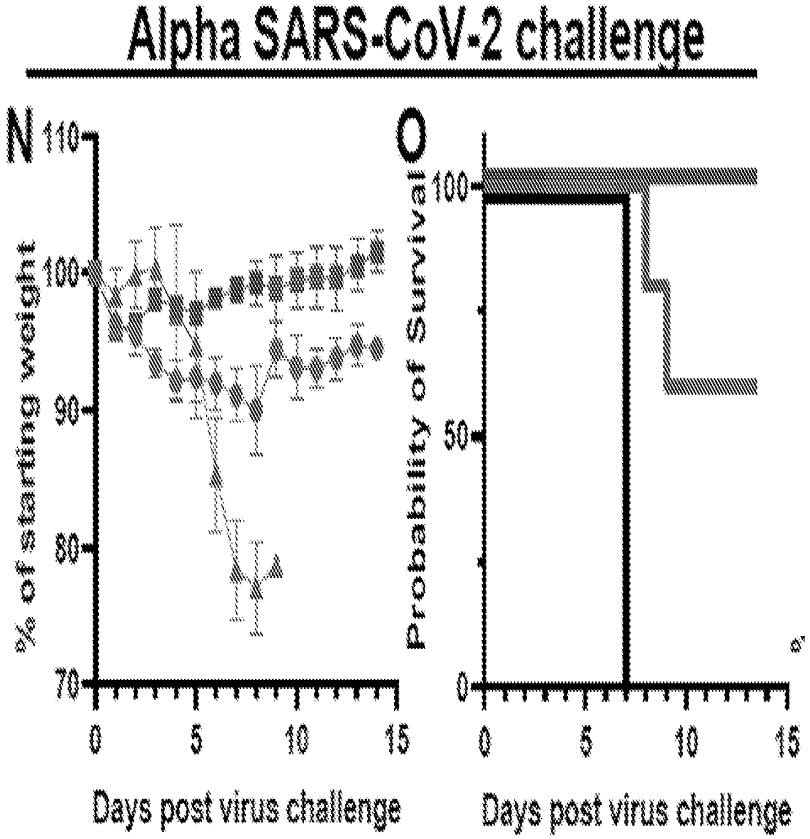

Alpha SARS-CoV-2 challenge
N
% of starting weight
110
100
90
80
70
0
5
10
15
Days post virus challenge
O
Probability of Survival
100
50
0
0
5
10
15
Days post virus challenge

Fig. 2

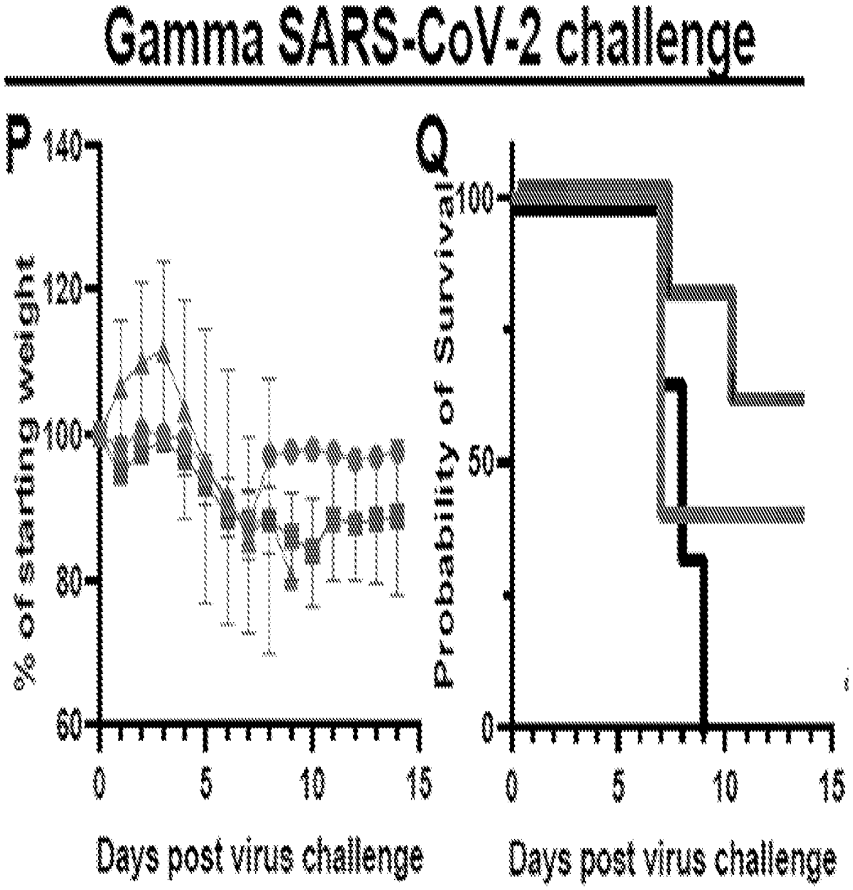

Gamma SARS-CoV-2 challenge
P
% of starting weight
140
120
100
80
60
0
5
10
15
Days post virus challenge
Q
Probability of Survival
100
50
0
0
5
10
15
Days post virus challenge

Fig. 2

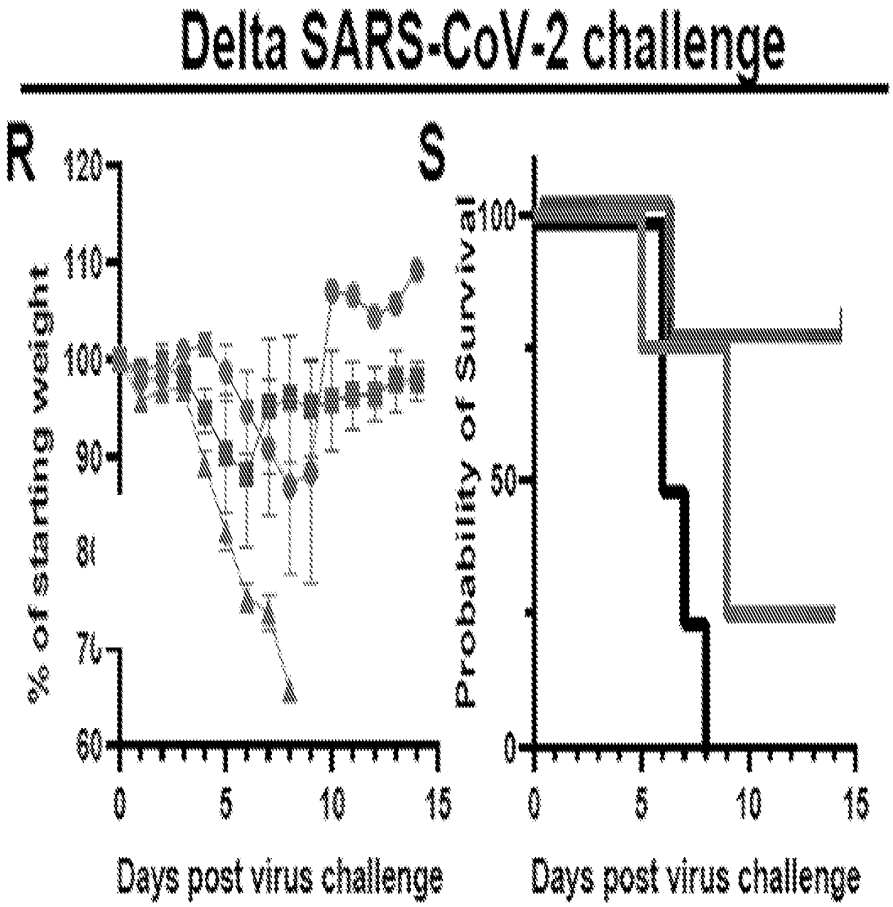

Delta SARS-CoV-2 challenge
R
% of starting weight
120
110
100
90
80
70
60
0
5
10
15
Days post virus challenge
S
Probability of Survival
100
50
0
0
5
10
15
Days post virus challenge

Fig. 2

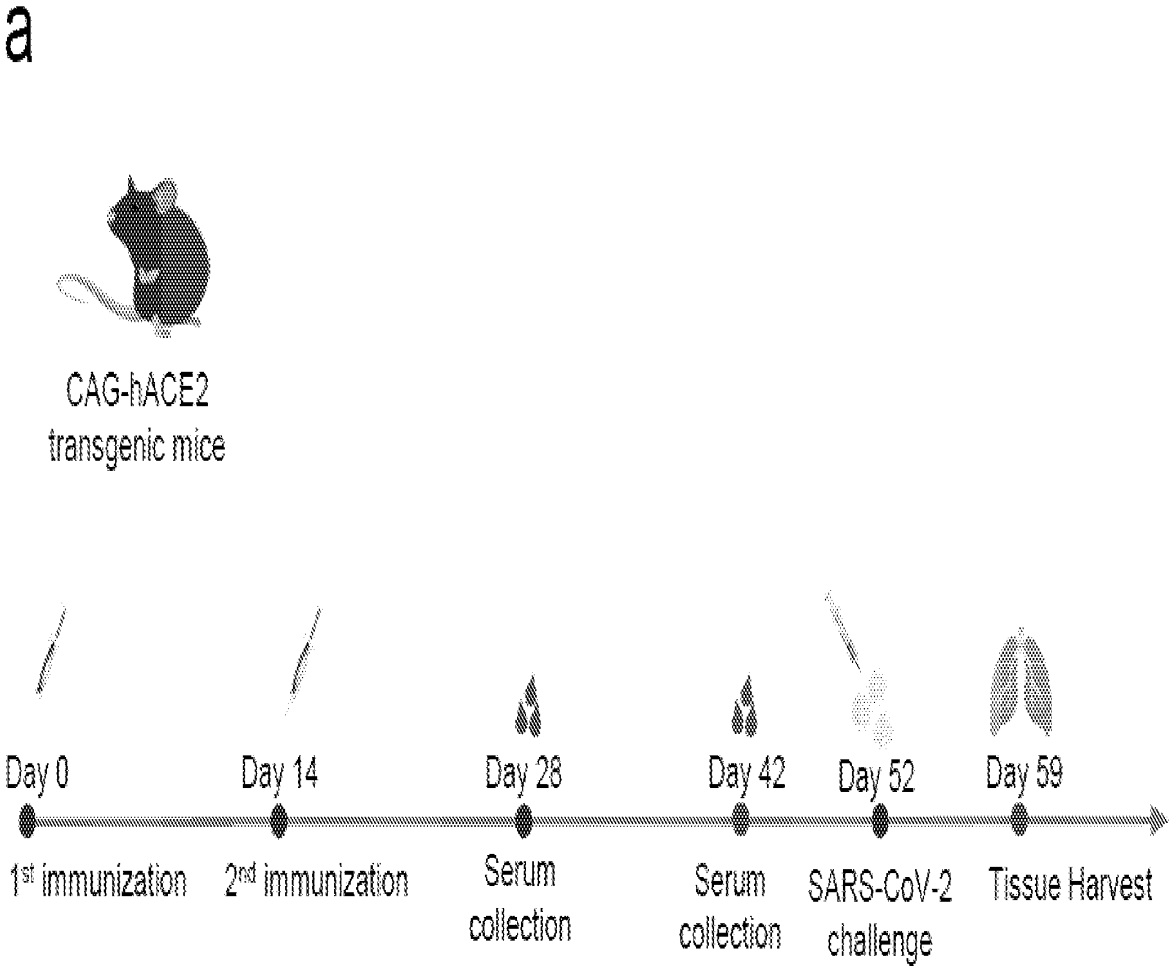

a
CAG-hACE2
transgenic mice
Day 0
Day 14
Day 28
Day 42
Day 52
Day 59
1st immunization
2nd immunization
Serum collection
Serum collection
SARS-CoV-2 challenge
Tissue Harvest

Fig. 3

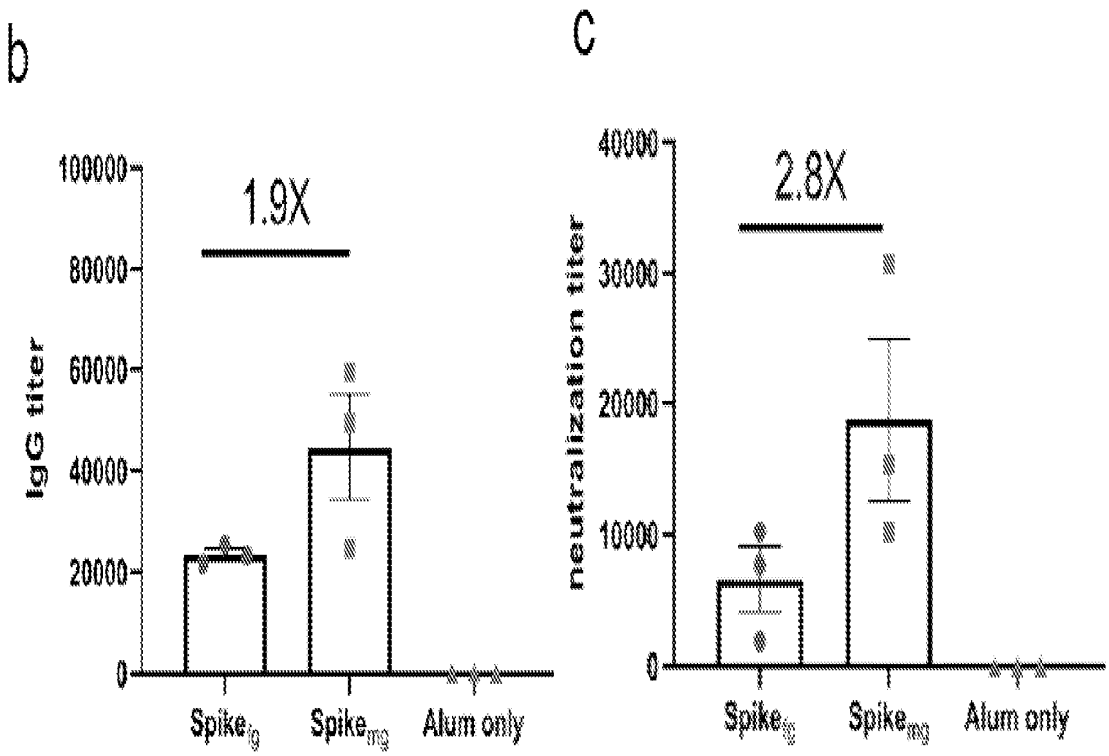

b
IgG titer
100000
80000
60000
40000
20000
0
1.9X
Spike
Spike
Alum only
c
neutralization titer
40000
30000
20000
10000
0
2.8X
Spike
Spike
Alum only

Fig. 3

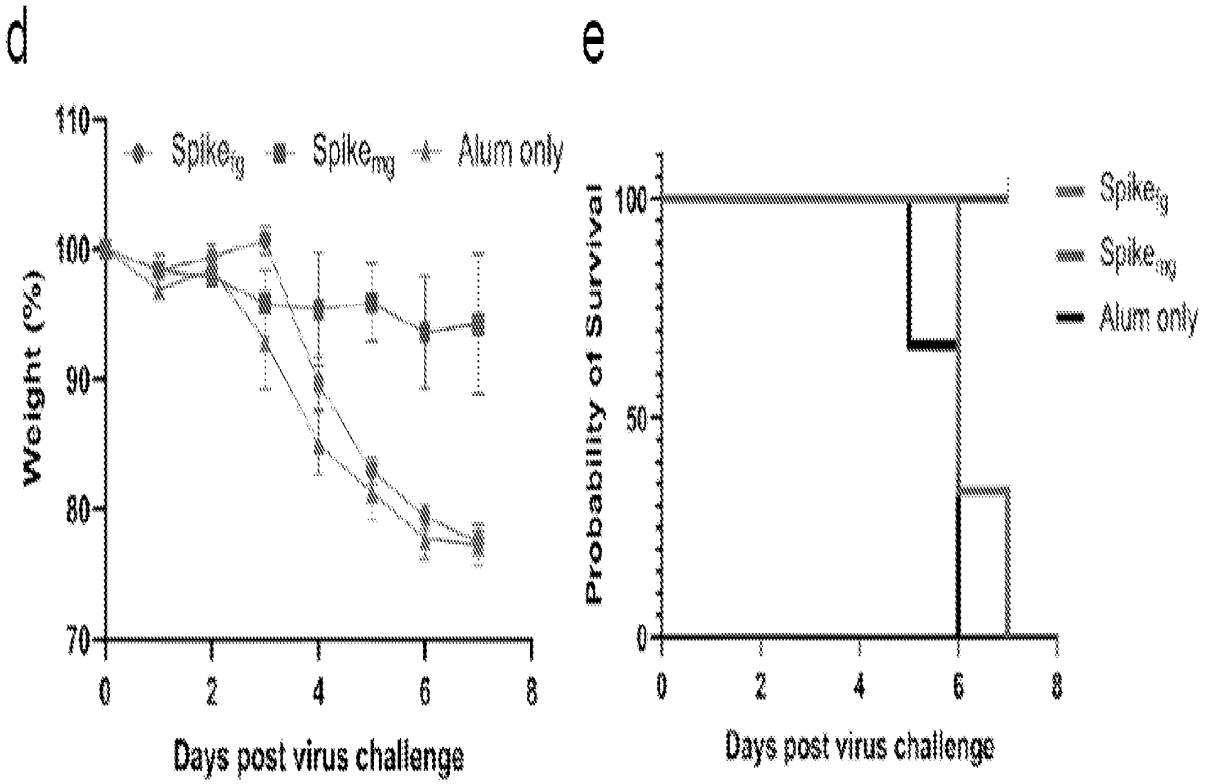

d
e
Spike$_{ig}$
Spike$_{mg}$
Alum only
Weight (%)
110
100
90
80
70
0
2
4
6
8
Days post virus challenge
Probability of Survival
100
50
0
Spike$_{ig}$
Spike$_{mg}$
Alum only
Days post virus challenge

Fig. 3

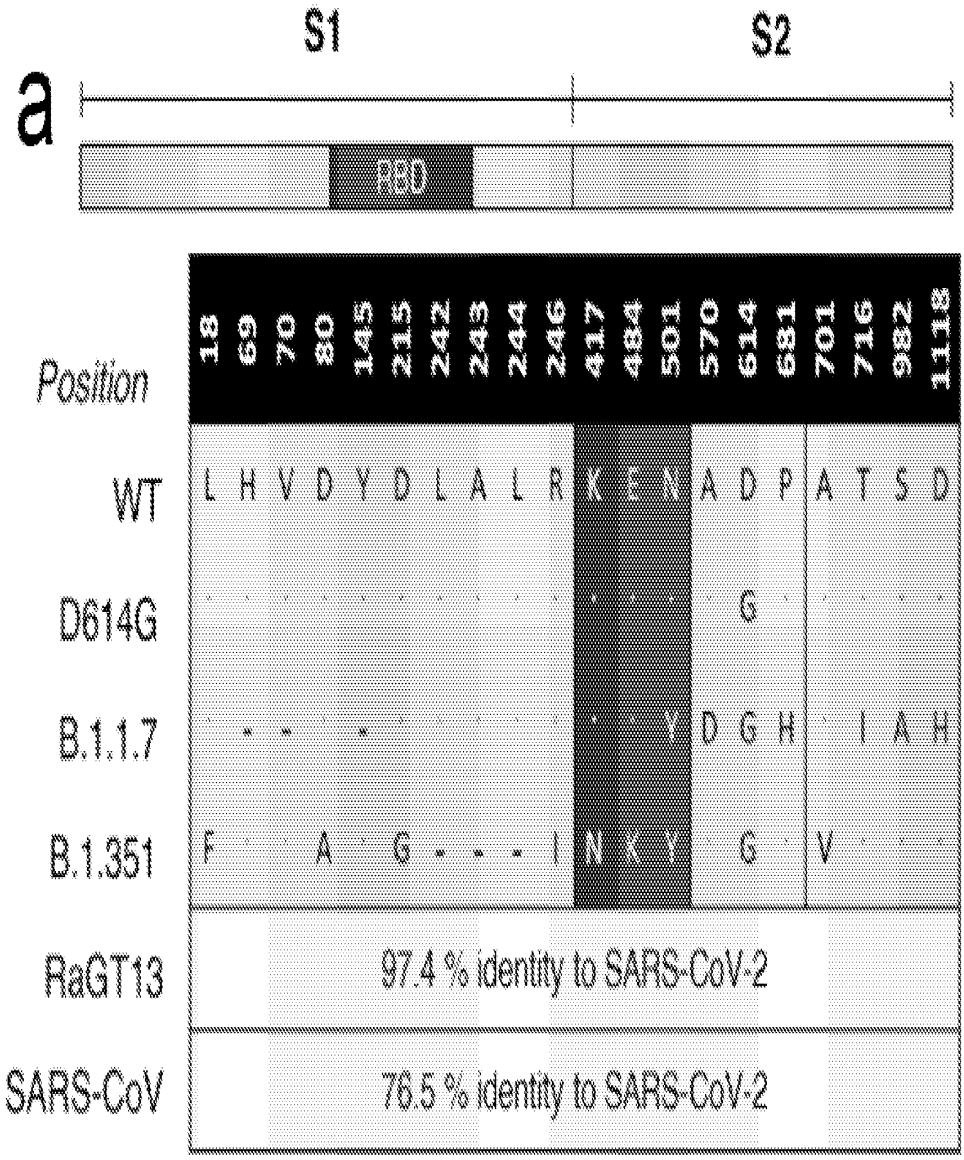

a
S1
S2
RBD
Position
18 69 70 80 145 215 242 243 244 246 417 484 501 570 614 681 701 716 982 1118
WT L H V D Y D L A L R K E N A D P A T S D
D614G G
B.1.1.7 - - - K N Y D G H I A H
B.1.351 F A G - - - I N K N Y G V
RaGT13 97.4 % identity to SARS-CoV-2
SARS-CoV 76.5 % identity to SARS-CoV-2

Fig. 4

b

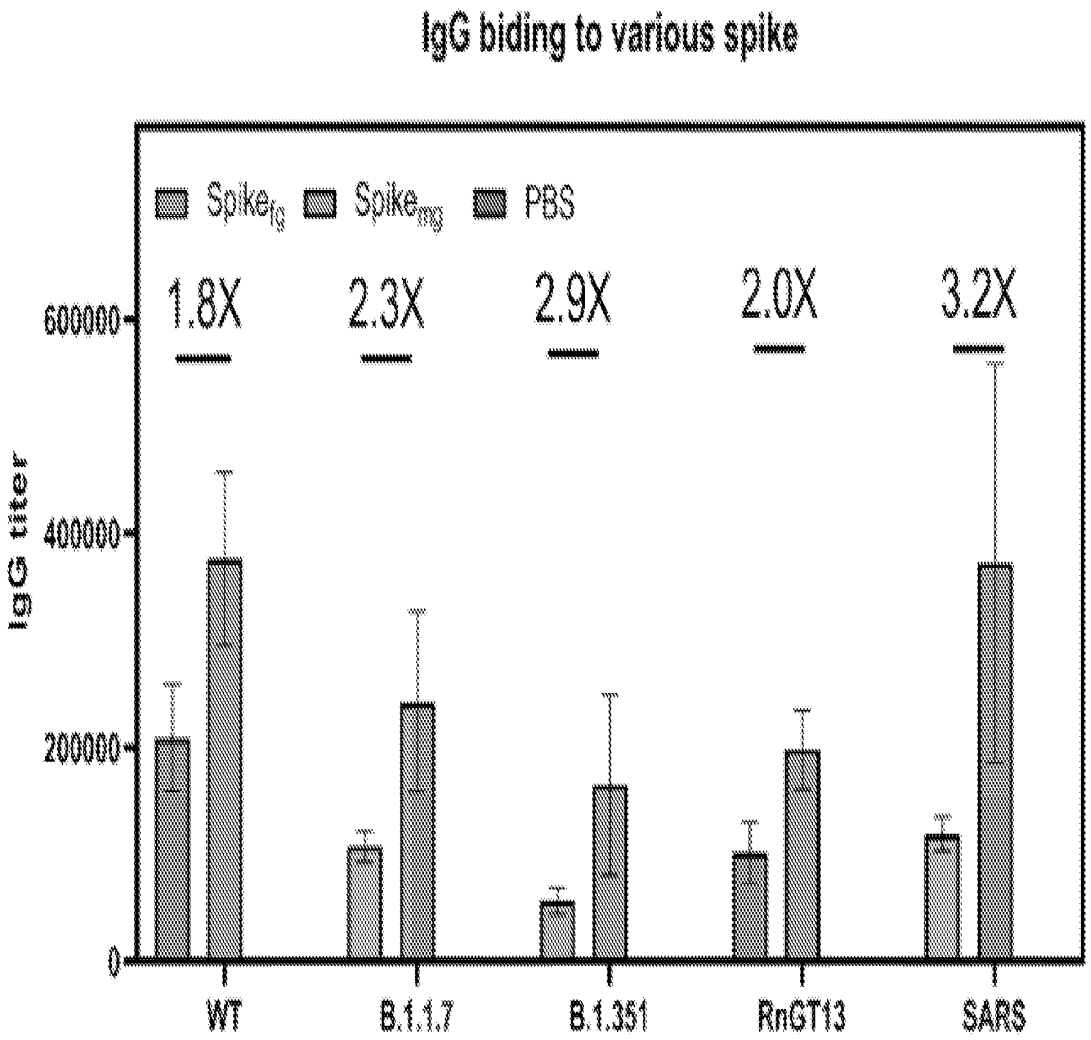

IgG biding to various spike
Spike$_{fg}$
Spike$_{mg}$
PBS
1.8X
2.3X
2.9X
2.0X
3.2X
IgG titer
600000
400000
200000
0
WT
B.1.1.7
B.1.351
RnGT13
SARS

Fig. 4

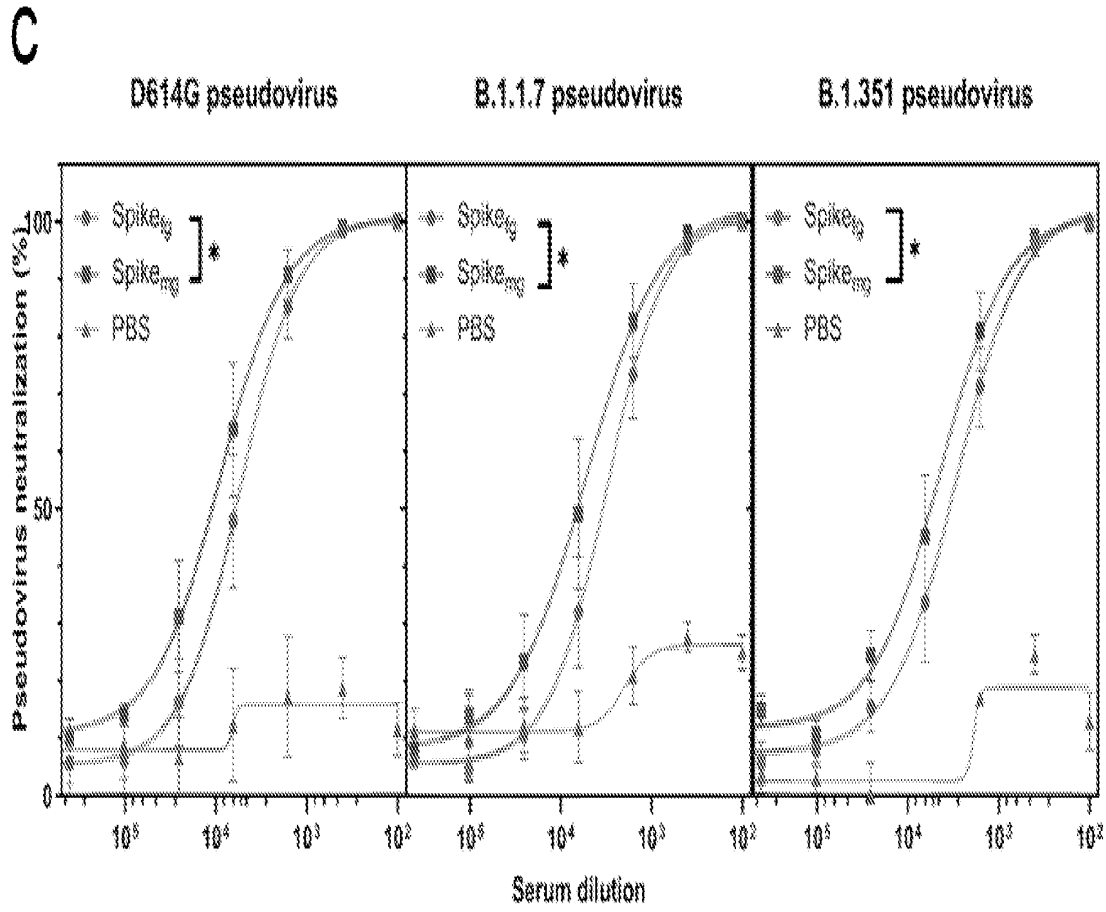

C
D614G pseudovirus
B.1.1.7 pseudovirus
B.1.351 pseudovirus
Spike$_{lg}$
Spike$_{mg}$
PBS
*
Pseudovirus neutralization (%)
100
50
0
10^5
10^4
10^3
10^2
Serum dilution

Fig. 4

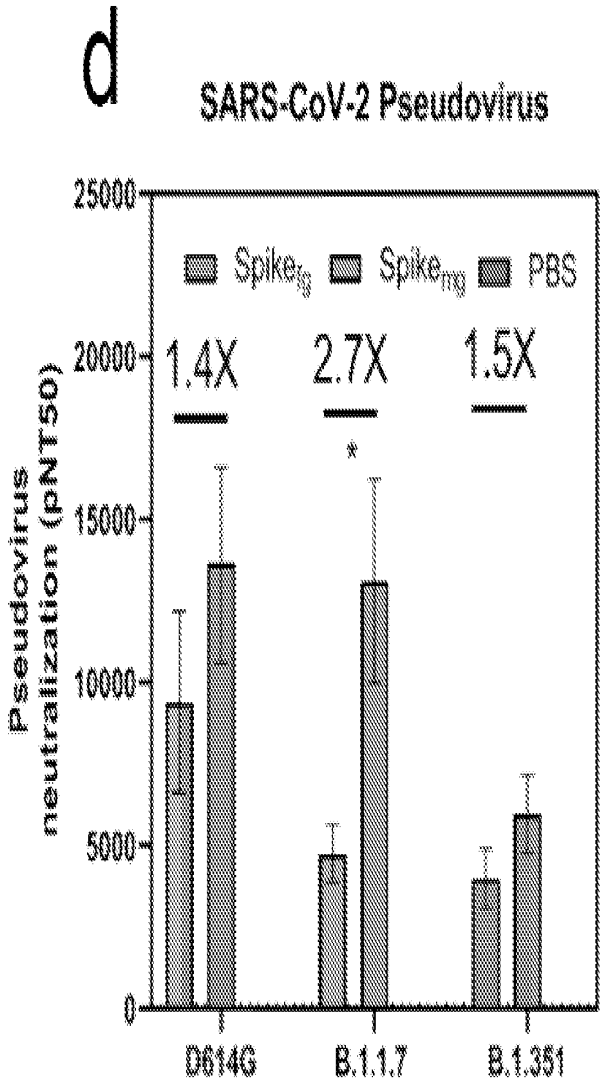

d
SARS-CoV-2 Pseudovirus
Pseudovirus neutralization (pNT50)
25000
20000
15000
10000
5000
0
Spike$_{tg}$
Spike$_{mg}$
PBS
1.4X
2.7X
1.5X
*
D614G
B.1.1.7
B.1.351

Fig. 4

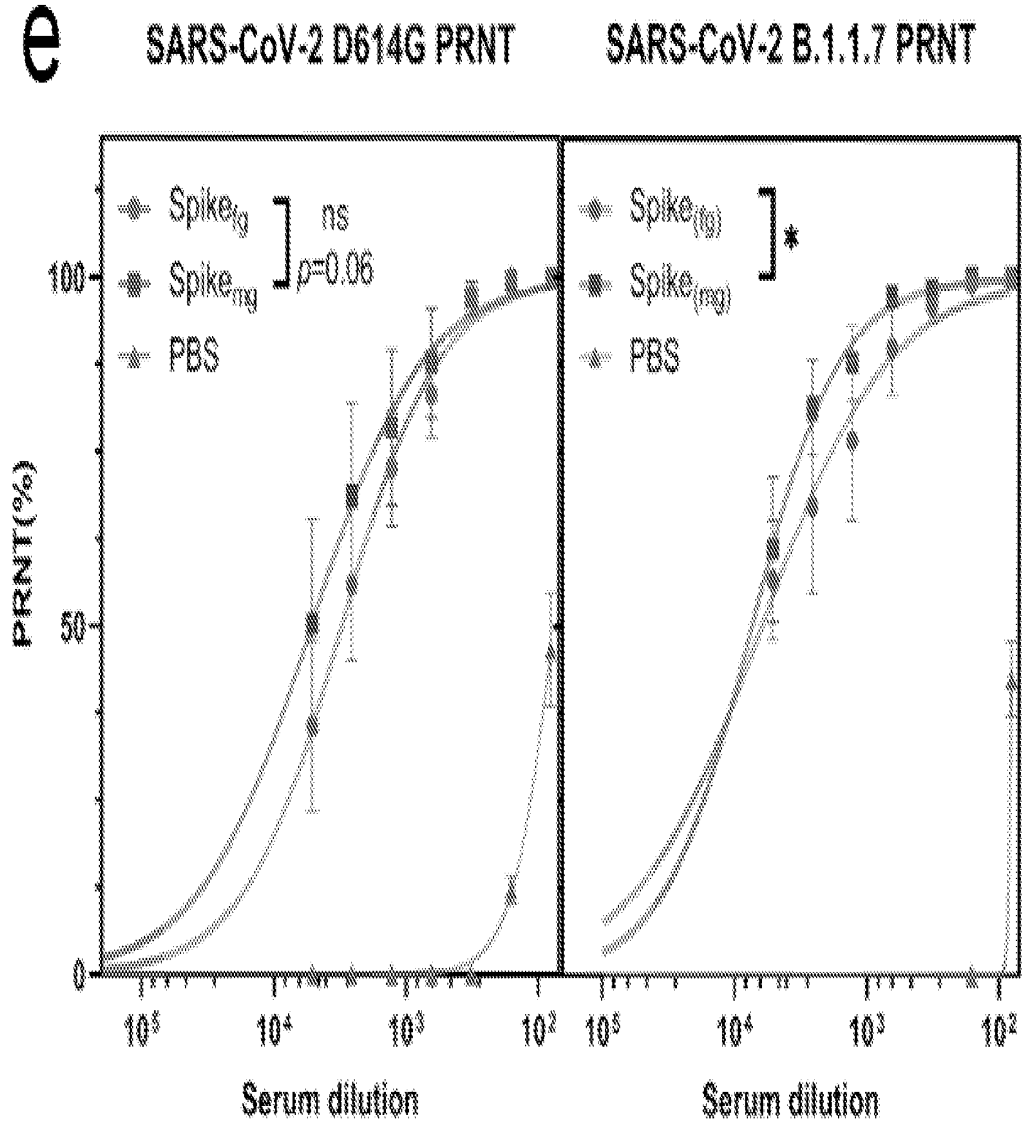

e
SARS-CoV-2 D614G PRNT
SARS-CoV-2 B.1.1.7 PRNT
Spike(tg)
Spike(mg)
PBS
ns
p=0.06
*
PRNT(%)
100
50
0
10^5
10^4
10^3
10^2
Serum dilution

Fig. 4

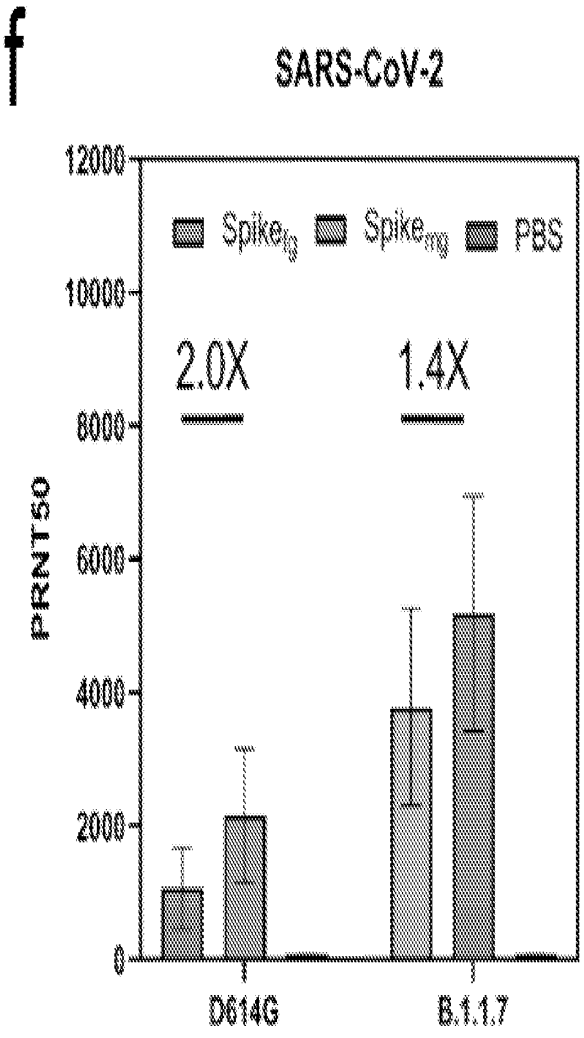

f
SARS-CoV-2
12000
10000
8000
6000
4000
2000
0
PRNT50
Spike$_{Ig}$
Spike$_{mg}$
PBS
2.0X
1.4X
D614G
B.1.1.7

Fig. 4

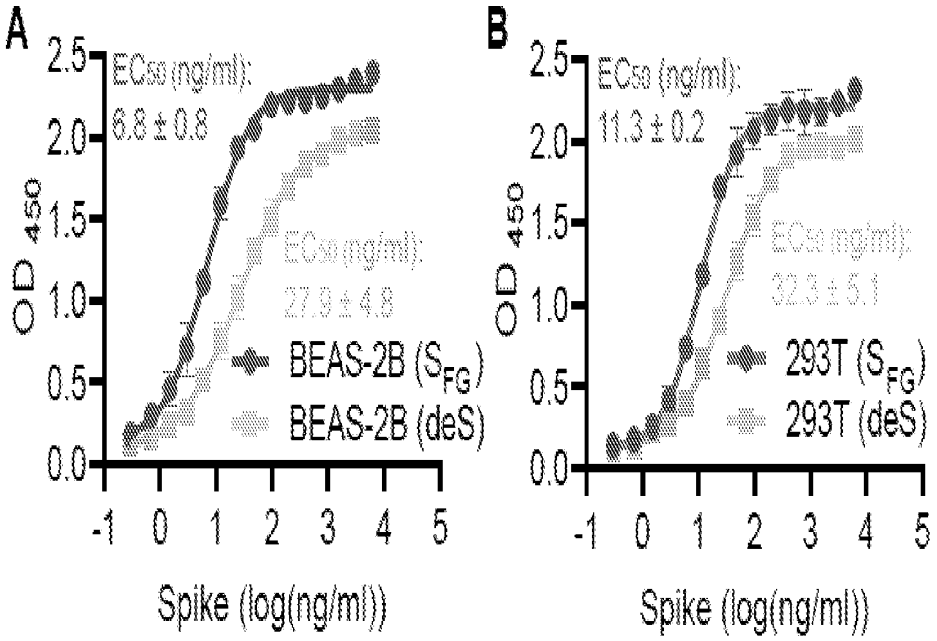

A
B
OD 450
Spike (log(ng/ml))
EC50 (ng/ml):
6.8 ± 0.8
27.9 ± 4.8
BEAS-2B (S_FG)
BEAS-2B (deS)
EC50 (ng/ml):
11.3 ± 0.2
32.3 ± 5.1
293T (S_FG)
293T (deS)

Fig. 5

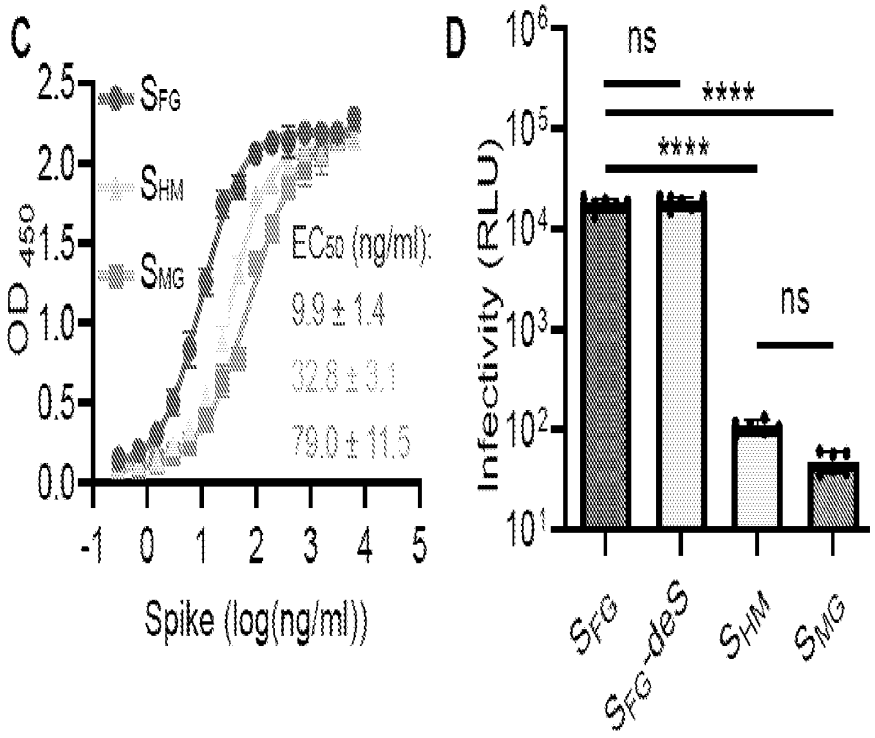

C
2.5
2.0
1.5
1.0
0.5
0.0
OD 450
SFG
SHM
SMG
EC50 (ng/ml):
9.9 ± 1.4
32.8 ± 3.1
79.0 ± 11.5
-1 0 1 2 3 4 5
Spike (log(ng/ml))
D
Infectivity (RLU)
ns
****
****
ns
SFG
SFG-deS
SHM
SMG

Fig. 5

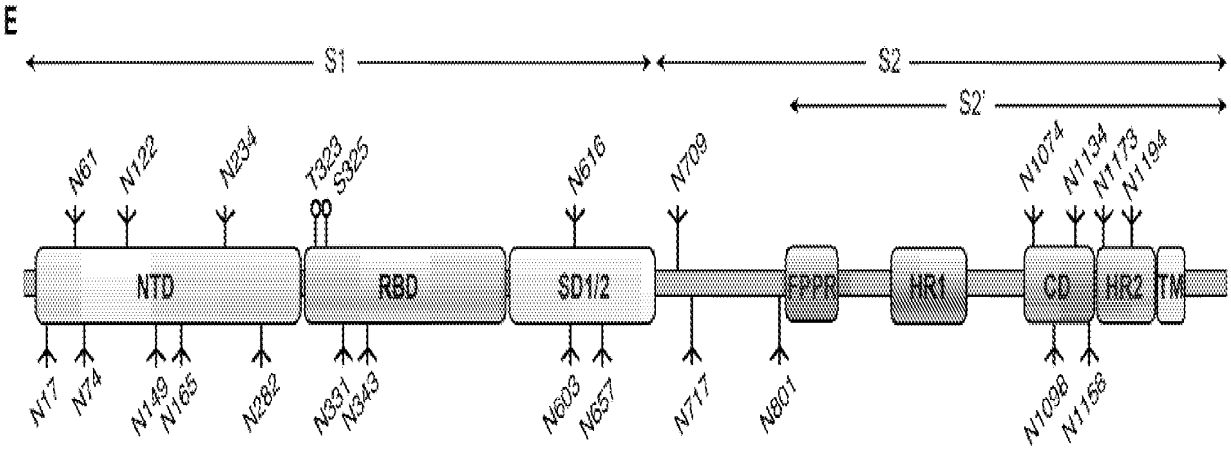

E
S1
S2
S2'
N61
N122
N234
T323
S325
N616
N709
N1074
N1134
N1173
N1194
NTD
RBD
SD1/2
FPPR
HR1
CD
HR2
TM
N17
N74
N149
N165
N282
N331
N343
N603
N657
N717
N801
N1098
N1158

Fig. 5

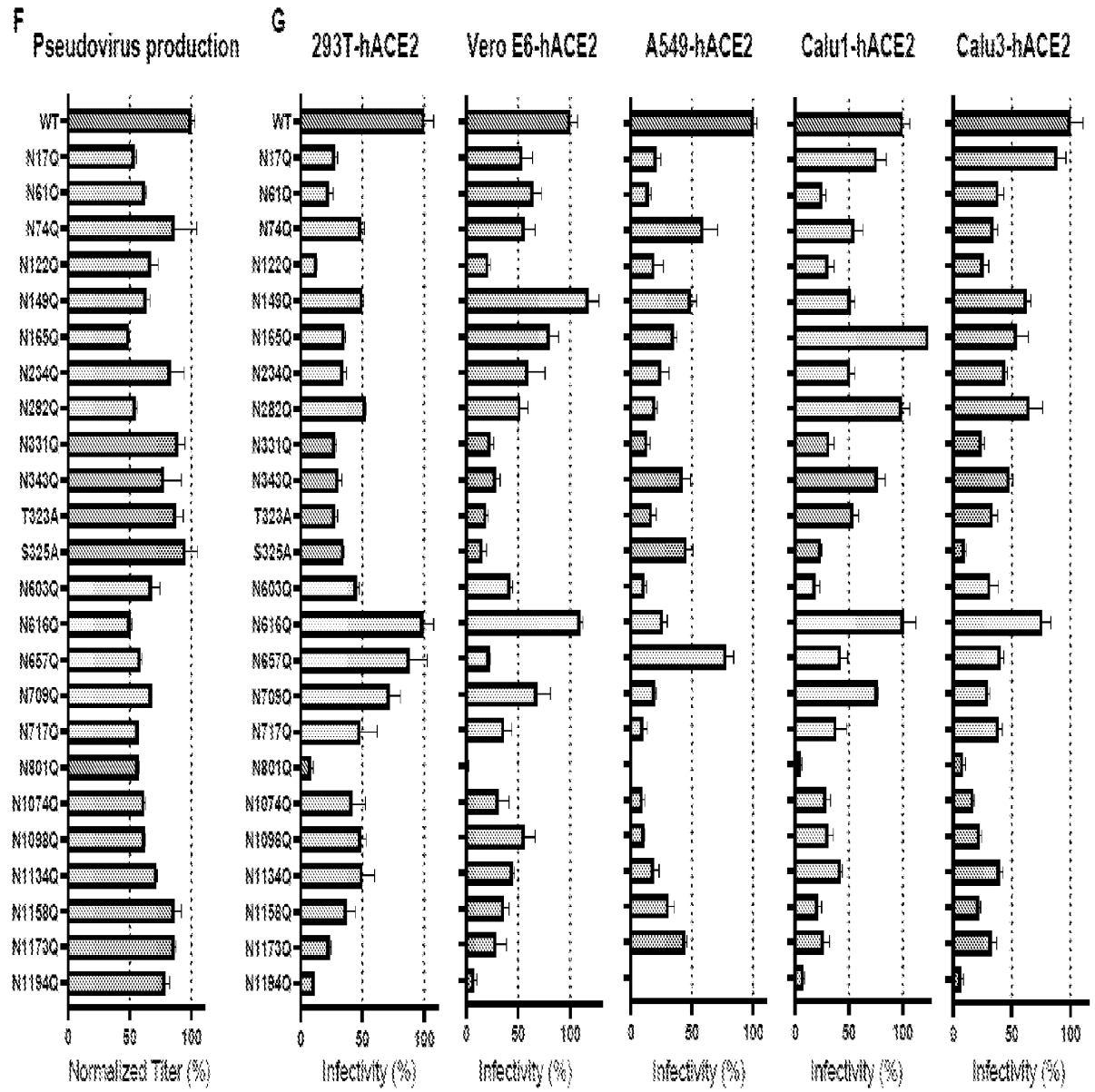

F
Pseudovirus production
WT
N17Q
N61Q
N74Q
N122Q
N149Q
N165Q
N234Q
N282Q
N331Q
N343Q
T323A
S325A
N603Q
N616Q
N657Q
N709Q
N717Q
N801Q
N1074Q
N1098Q
N1134Q
N1158Q
N1173Q
N1194Q
0
50
100
Normalized Titer (%)
G
293T-hACE2
Vero E6-hACE2
A549-hACE2
Calu1-hACE2
Calu3-hACE2
Infectivity (%)

Fig. 5

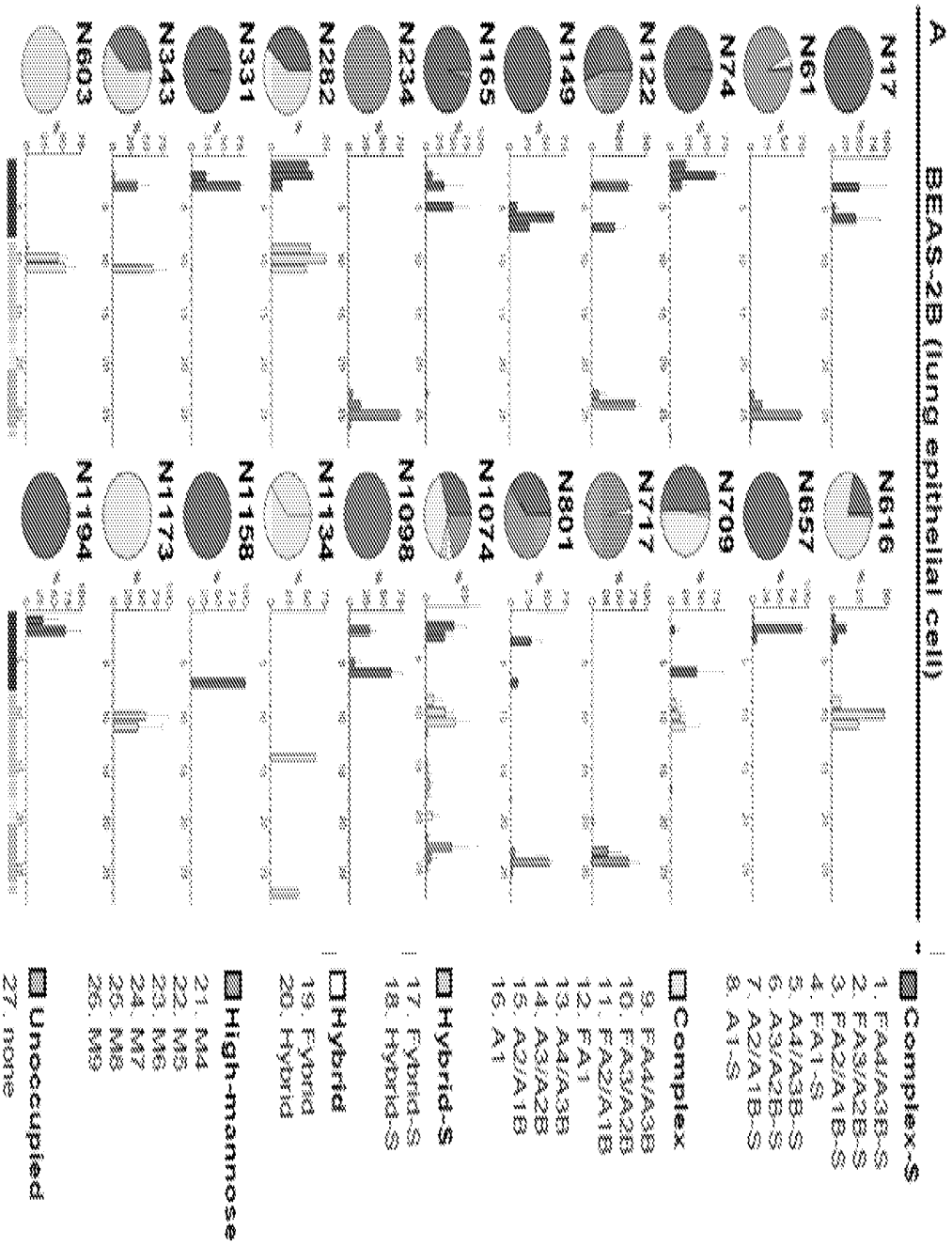

A
BEAS-2B (lung epithelial cell)
N17
N61
N74
N122
N149
N165
N234
N282
N331
N343
N603
N616
N657
N709
N717
N801
N1074
N1098
N1134
N1158
N1173
N1194
Complex-S
1. FA4/A3B-S
2. FA3/A2B-S
3. FA2/A1B-S
4. FA1-S
5. A4/A3B-S
6. A3/A2B-S
7. A2/A1B-S
8. A1-S
Complex
9. FA4/A3B
10. FA3/A2B
11. FA2/A1B
12. FA1
13. A4/A3B
14. A3/A2B
15. A2/A1B
16. A1
Hybrid-S
17. FyMid-S
18. Hybrid-S
Hybrid
19. FyMid
20. Hybrid
High-mannose
21. M4
22. M5
23. M6
24. M7
25. M8
26. M9
Unoccupied
27. none

Fig. 6

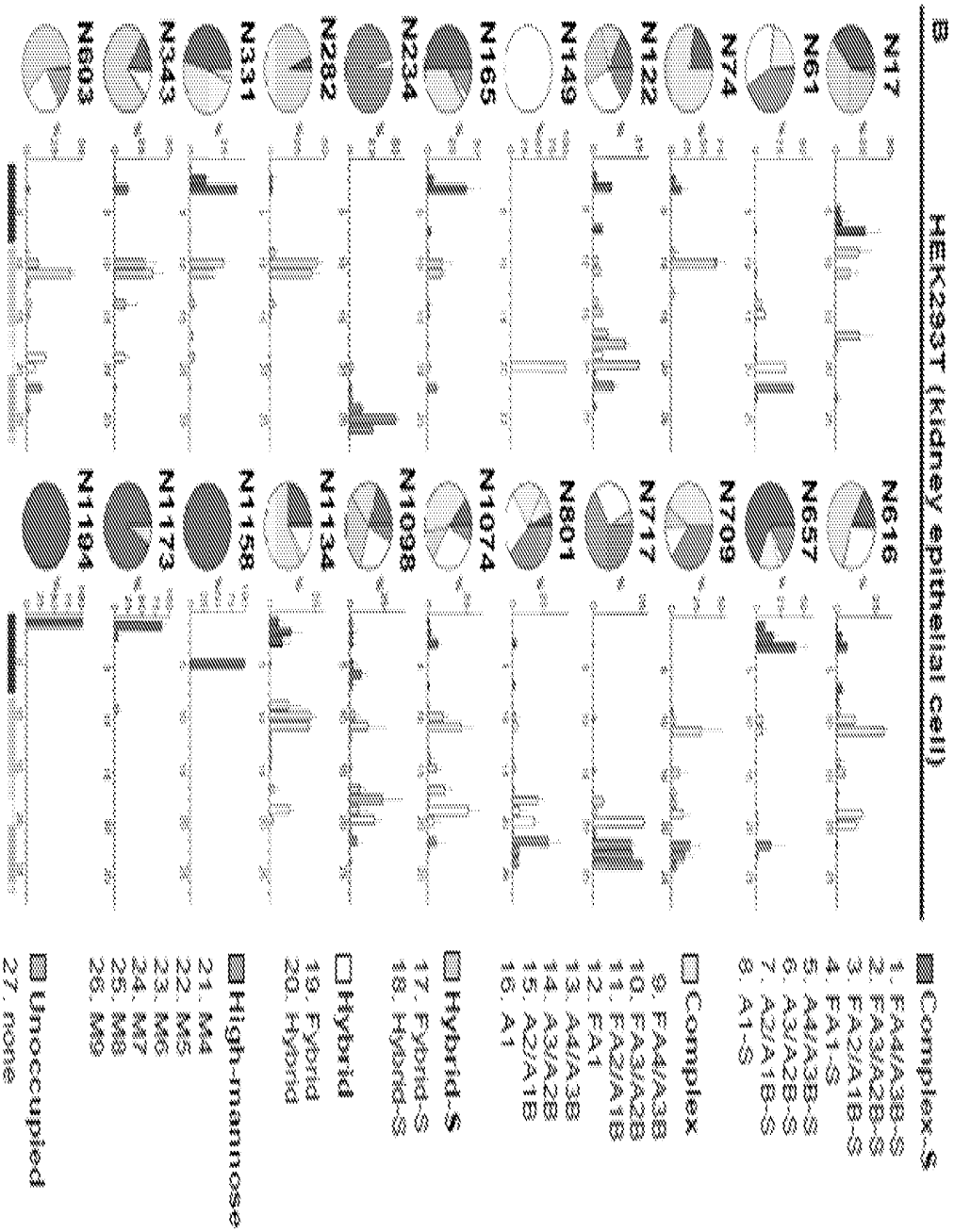

B
HEK293T (kidney epithelial cell)
N17
N61
N74
N122
N149
N165
N234
N282
N331
N343
N603
N616
N657
N709
N717
N801
N1074
N1098
N1134
N1158
N1173
N1194
Complex-S
1. FA4/A3B-S
2. FA3/A2B-S
3. FA2/A1B-S
4. FA1-S
5. A4/A3B-S
6. A3/A2B-S
7. A2/A1B-S
8. A1-S
Complex
9. FA4/A3B
10. FA3/A2B
11. FA2/A1B
12. FA1
13. A4/A3B
14. A3/A2B
15. A2/A1B
16. A1
Hybrid-S
17. Fybrid-S
18. Hybrid-S
Hybrid
19. Fybrid
20. Hybrid
High-mannose
21. M4
22. M5
23. M6
24. M7
25. M8
26. M9
Unoccupied
27. none

Fig. 6

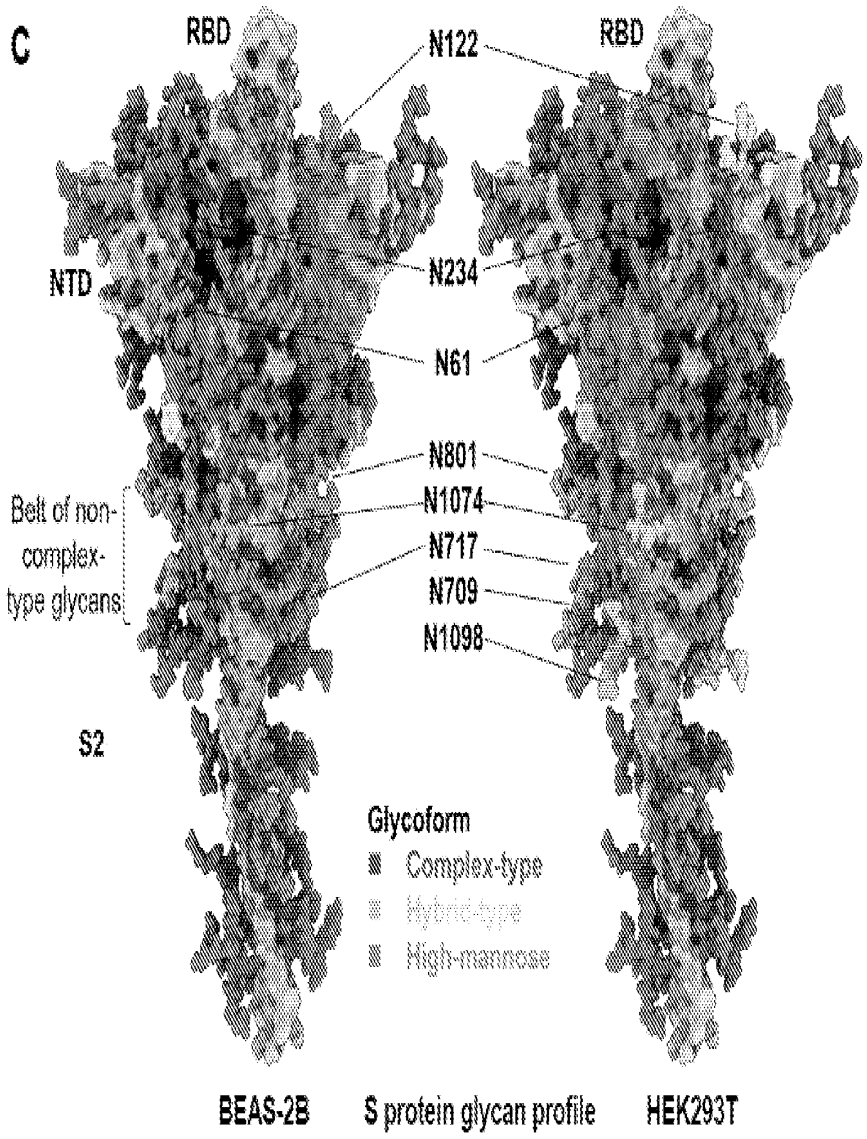

C
RBD
RBD
N122
NTD
N234
N61
N801
N1074
N717
N709
N1098
Belt of non-complex-type glycans
S2
Glycoform
Complex-type
Hybrid-type
High-mannose
BEAS-2B
S protein glycan profile
HEK293T

Fig. 6

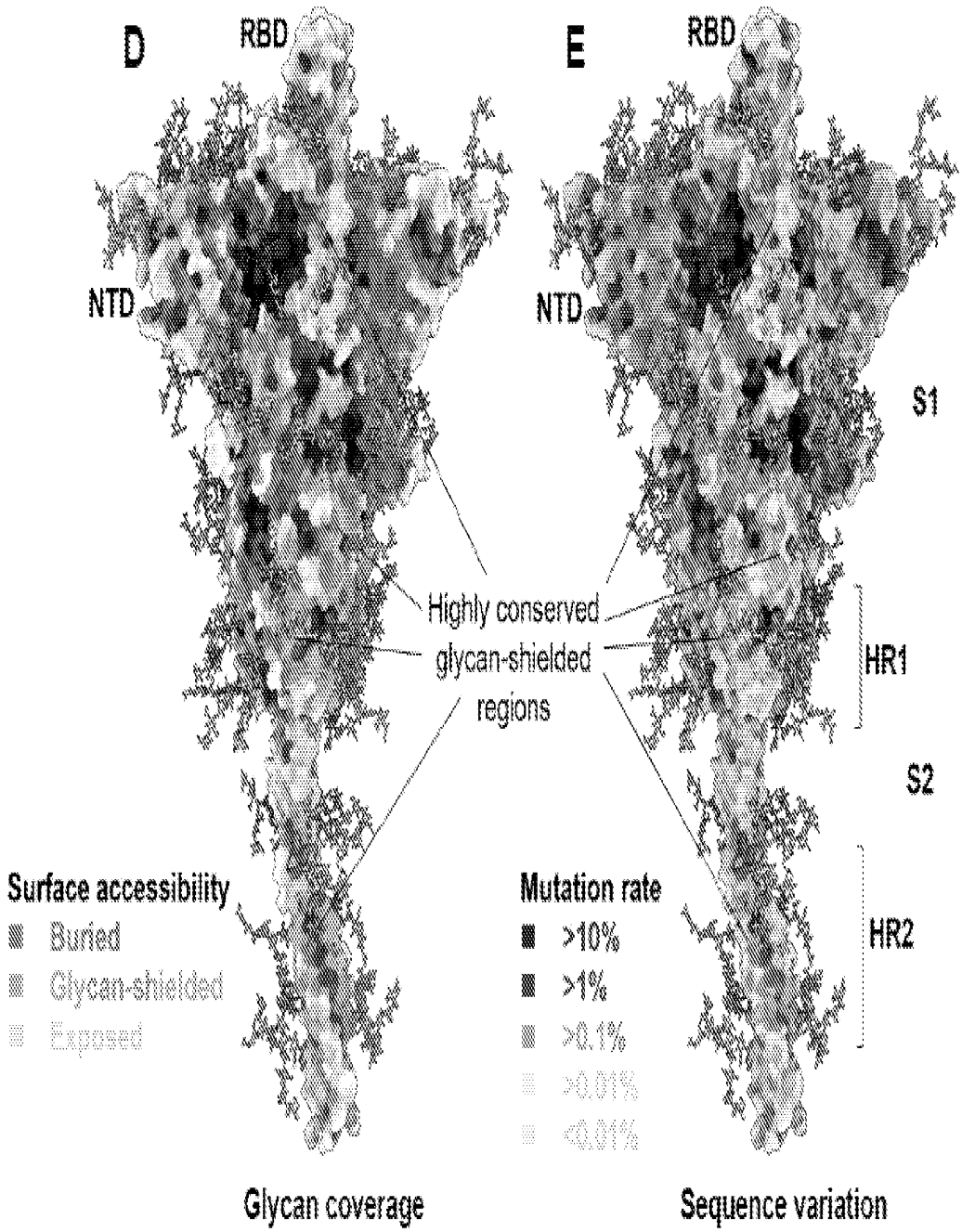

D
RBD
NTD
E
RBD
NTD
S1
Highly conserved
glycan-shielded
regions
HR1
S2
HR2
Surface accessibility
Buried
Glycan-shielded
Exposed
Mutation rate
>10%
>1%
>0.1%
>0.01%
<0.01%
Glycan coverage
Sequence variation

Fig. 6

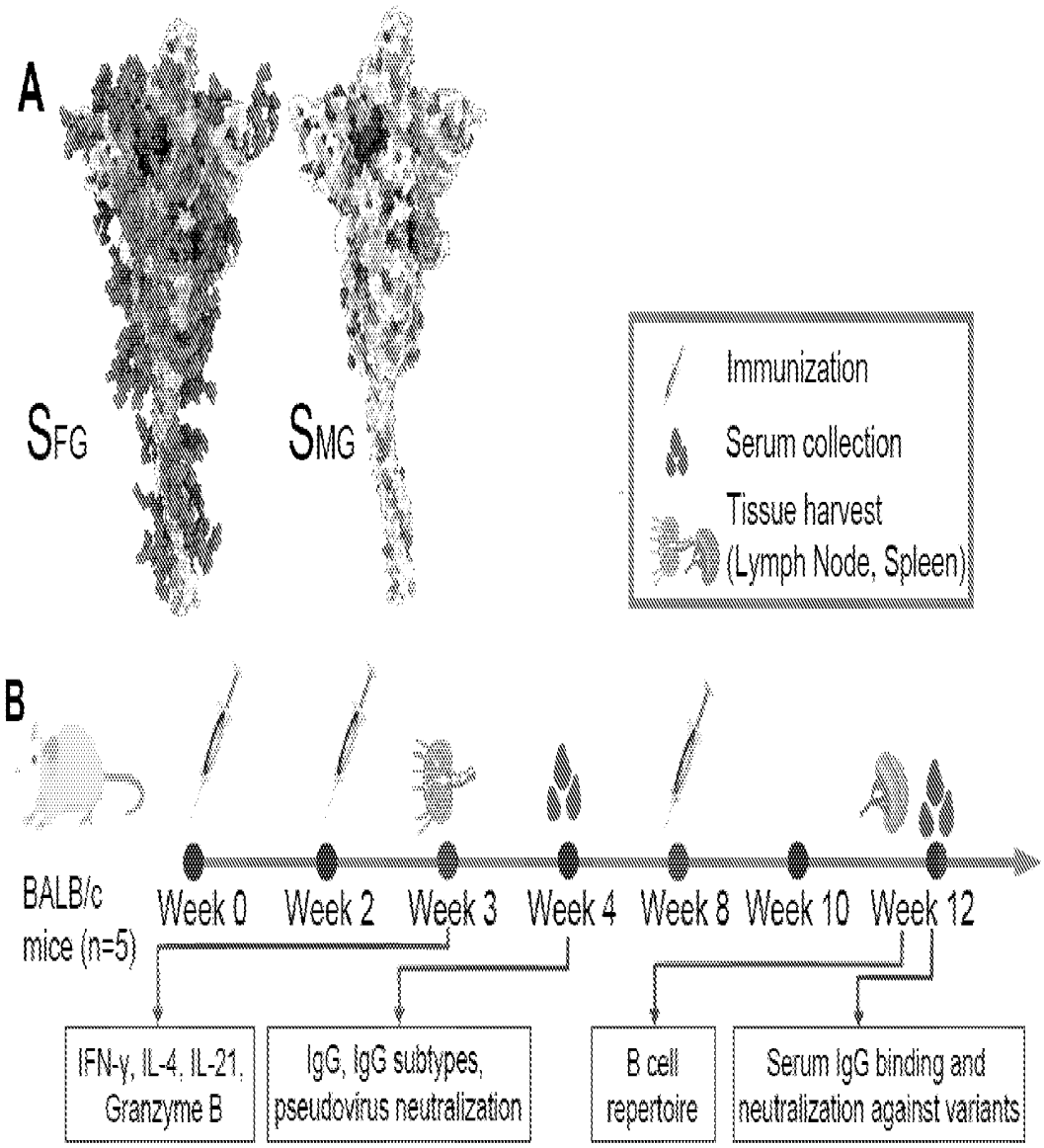

A
SFG
SMG
Immunization
Serum collection
Tissue harvest
(Lymph Node, Spleen)
B
BALB/c
mice (n=5)
Week 0
Week 2
Week 3
Week 4
Week 8
Week 10
Week 12
IFN-γ, IL-4, IL-21,
Granzyme B
IgG, IgG subtypes,
pseudovirus neutralization
B cell
repertoire
Serum IgG binding and
neutralization against variants

Fig. 7

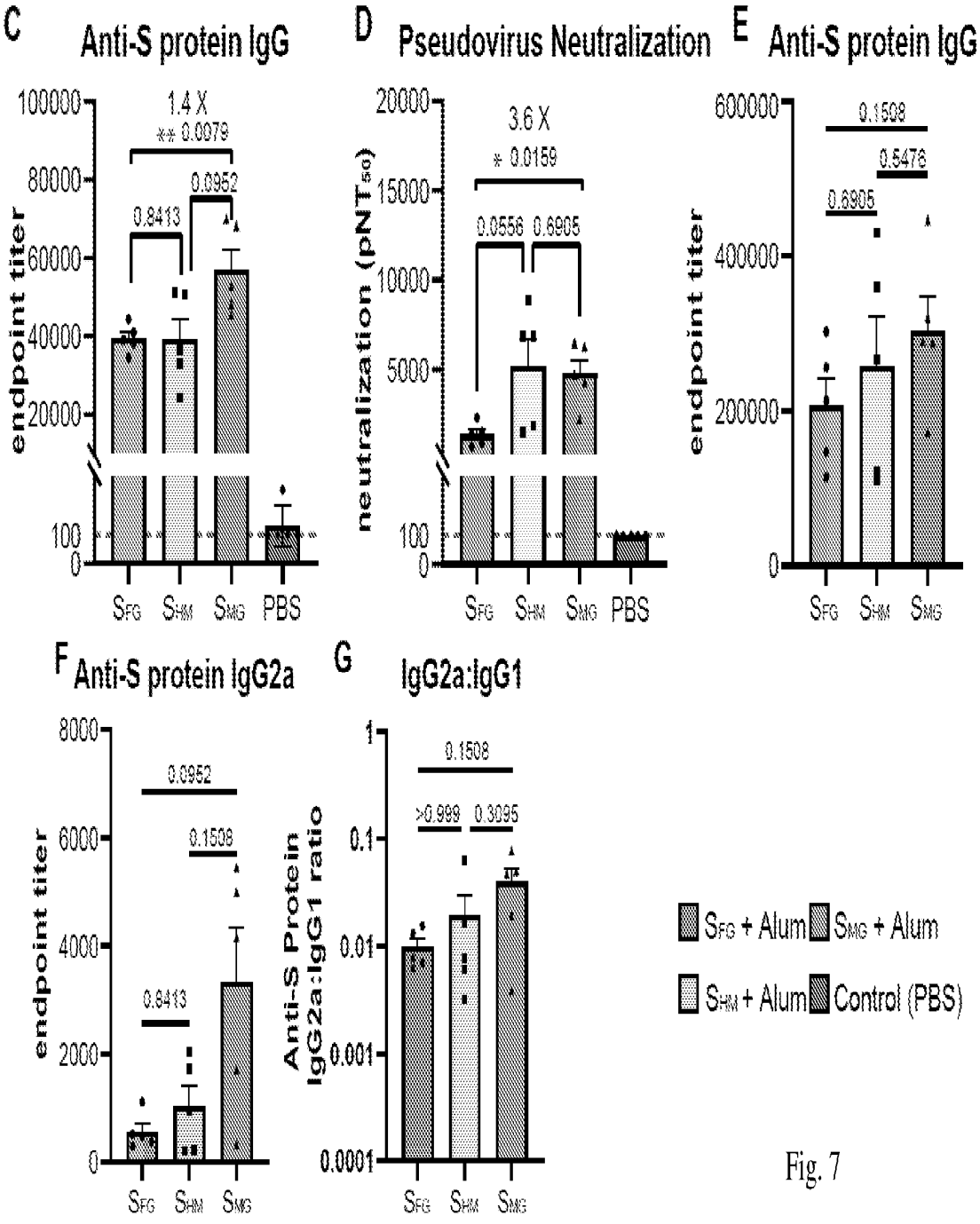

C
Anti-S protein IgG
endpoint titer
1.4 X
** 0.0079
0.0952
0.8413
SFG
SHM
SMG
PBS
D
Pseudovirus Neutralization
neutralization (pNT50)
3.6 X
* 0.0159
0.0556
0.6905
E
Anti-S protein IgG1
0.1508
0.5476
0.6905
F
Anti-S protein IgG2a
0.0952
0.1508
0.8413
G
IgG2a:IgG1
Anti-S Protein IgG2a:IgG1 ratio
0.1508
>0.999
0.3095
SFG + Alum
SMG + Alum
SHM + Alum
Control (PBS)

Fig. 7

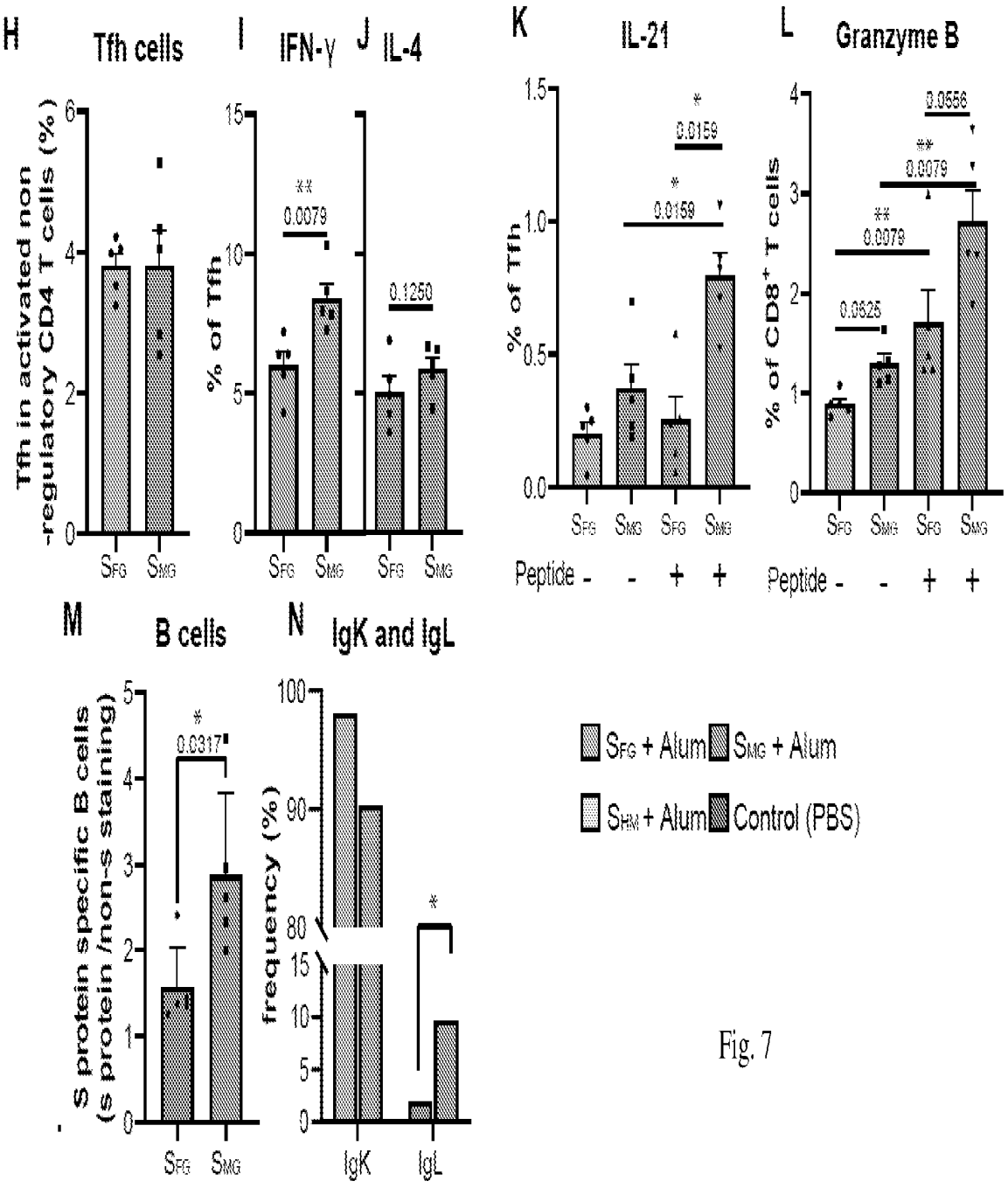

H
Tfh cells
Tfh in activated non -regulatory CD4 T cells (%)
I
IFN-γ
% of Tfh
J
IL-4
0.0079
0.1250
K
IL-21
% of Tfh
0.0159
0.0159
L
Granzyme B
% of CD8+ T cells
0.0625
0.0079
0.0079
0.0556
SFG
SMG
Peptide - - + +
M
B cells
S protein specific B cells (s protein /non-s staining)
0.0317
N
IgK and IgL
frequency (%)
IgK
IgL
SFG + Alum
SMG + Alum
SHM + Alum
Control (PBS)

Fig. 7

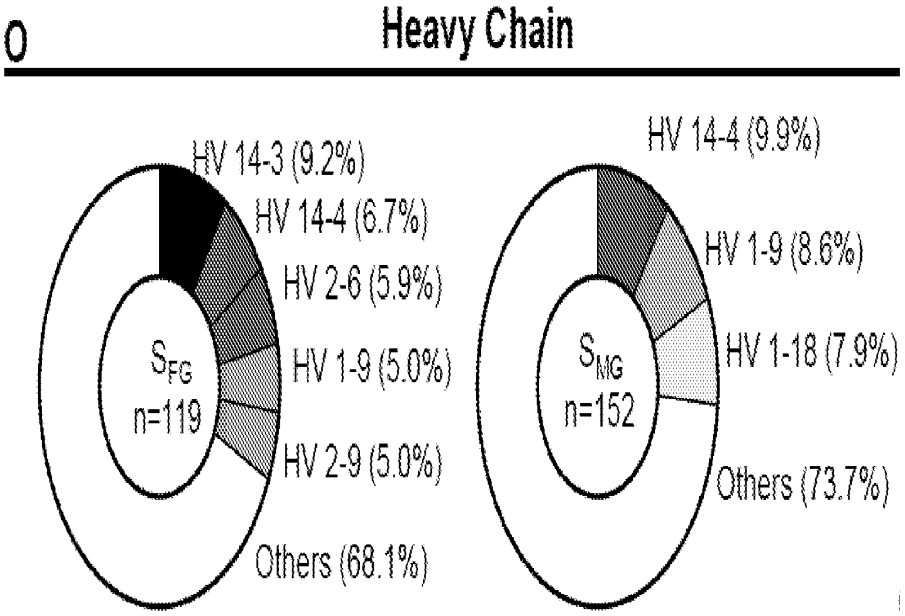
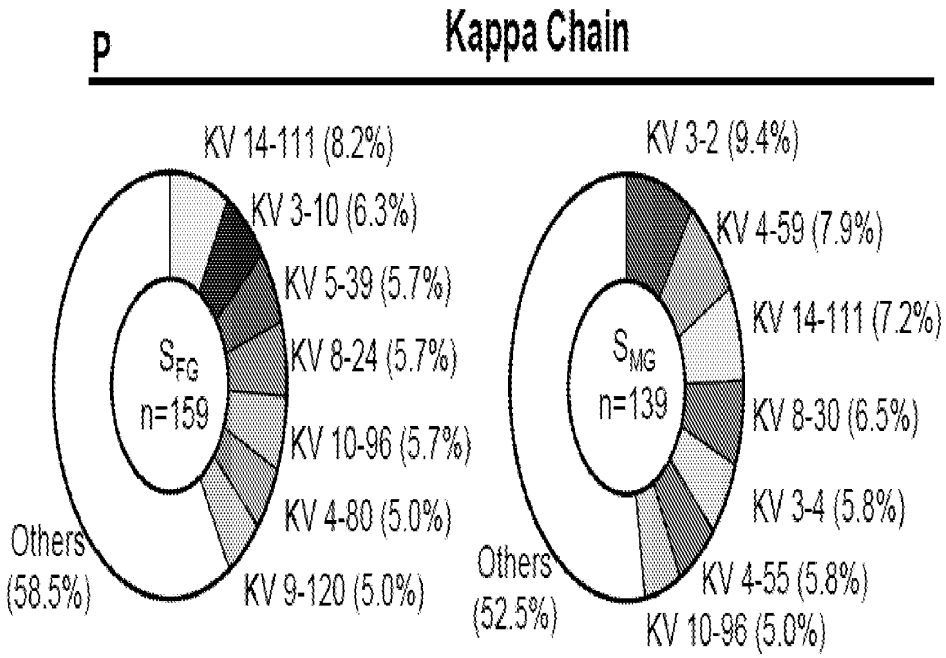

O
Heavy Chain
HV 14-3 (9.2%)
HV 14-4 (6.7%)
HV 2-6 (5.9%)
HV 1-9 (5.0%)
HV 2-9 (5.0%)
Others (68.1%)
S_FG
n=119
HV 14-4 (9.9%)
HV 1-9 (8.6%)
HV 1-18 (7.9%)
Others (73.7%)
S_MG
n=152

Fig. 7


P
Kappa Chain
KV 14-111 (8.2%)
KV 3-10 (6.3%)
KV 5-39 (5.7%)
KV 8-24 (5.7%)
KV 10-96 (5.7%)
KV 4-80 (5.0%)
KV 9-120 (5.0%)
Others
(58.5%)
S_FG
n=159
KV 3-2 (9.4%)
KV 4-59 (7.9%)
KV 14-111 (7.2%)
KV 8-30 (6.5%)
KV 3-4 (5.8%)
KV 4-55 (5.8%)
KV 10-96 (5.0%)
Others
(52.5%)
S_MG
n=139

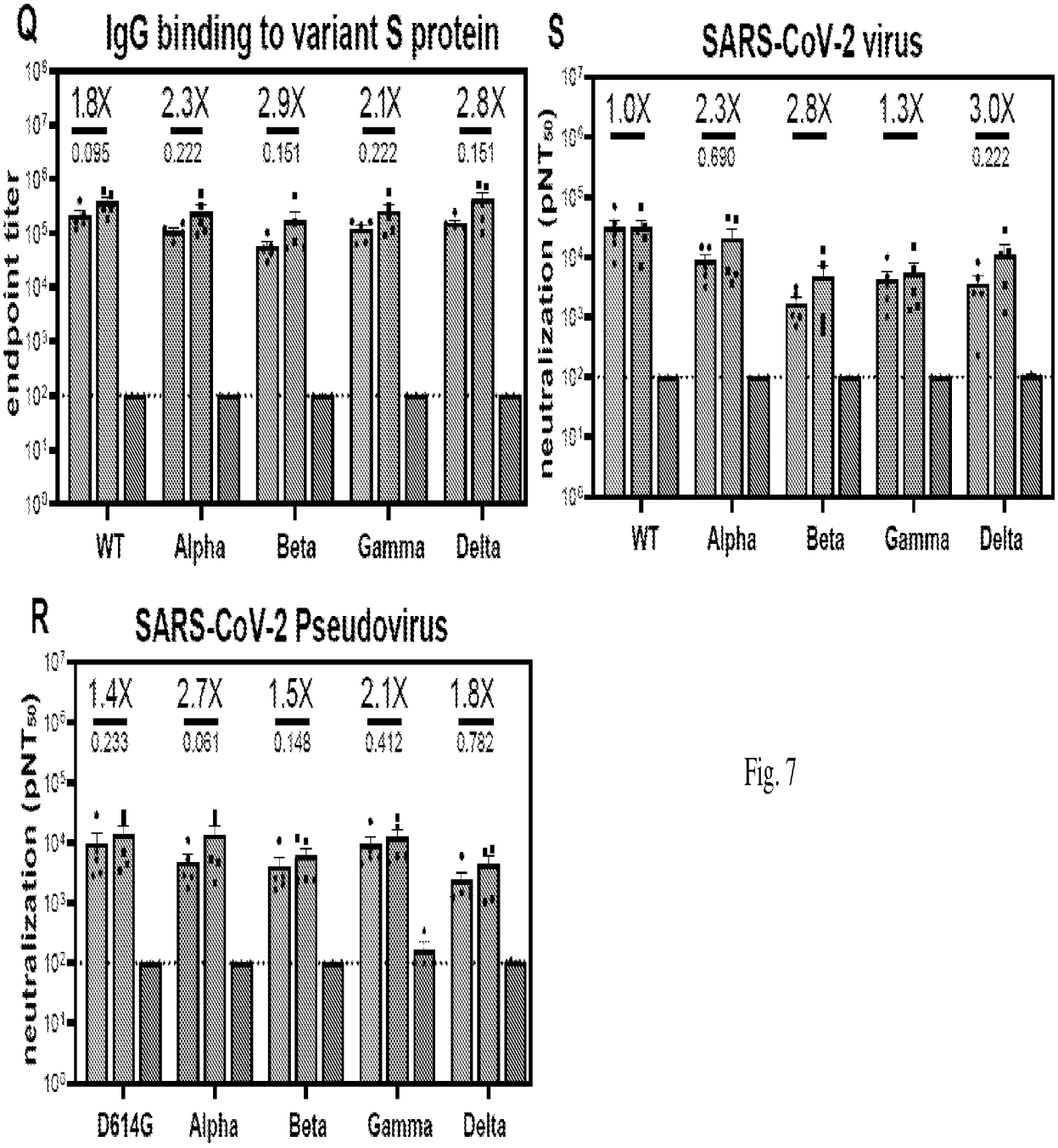

Q
IgG binding to variant S protein
endpoint titer
1.8X
2.3X
2.9X
2.1X
2.8X
0.095
0.222
0.151
0.222
0.151
WT
Alpha
Beta
Gamma
Delta
S
SARS-CoV-2 virus
neutralization (pNT50)
1.0X
2.3X
2.8X
1.3X
3.0X
0.690
0.222
WT
Alpha
Beta
Gamma
Delta
R
SARS-CoV-2 Pseudovirus
neutralization (pNT50)
1.4X
2.7X
1.5X
2.1X
1.8X
0.233
0.061
0.148
0.412
0.782
D614G
Alpha
Beta
Gamma
Delta

Fig. 7

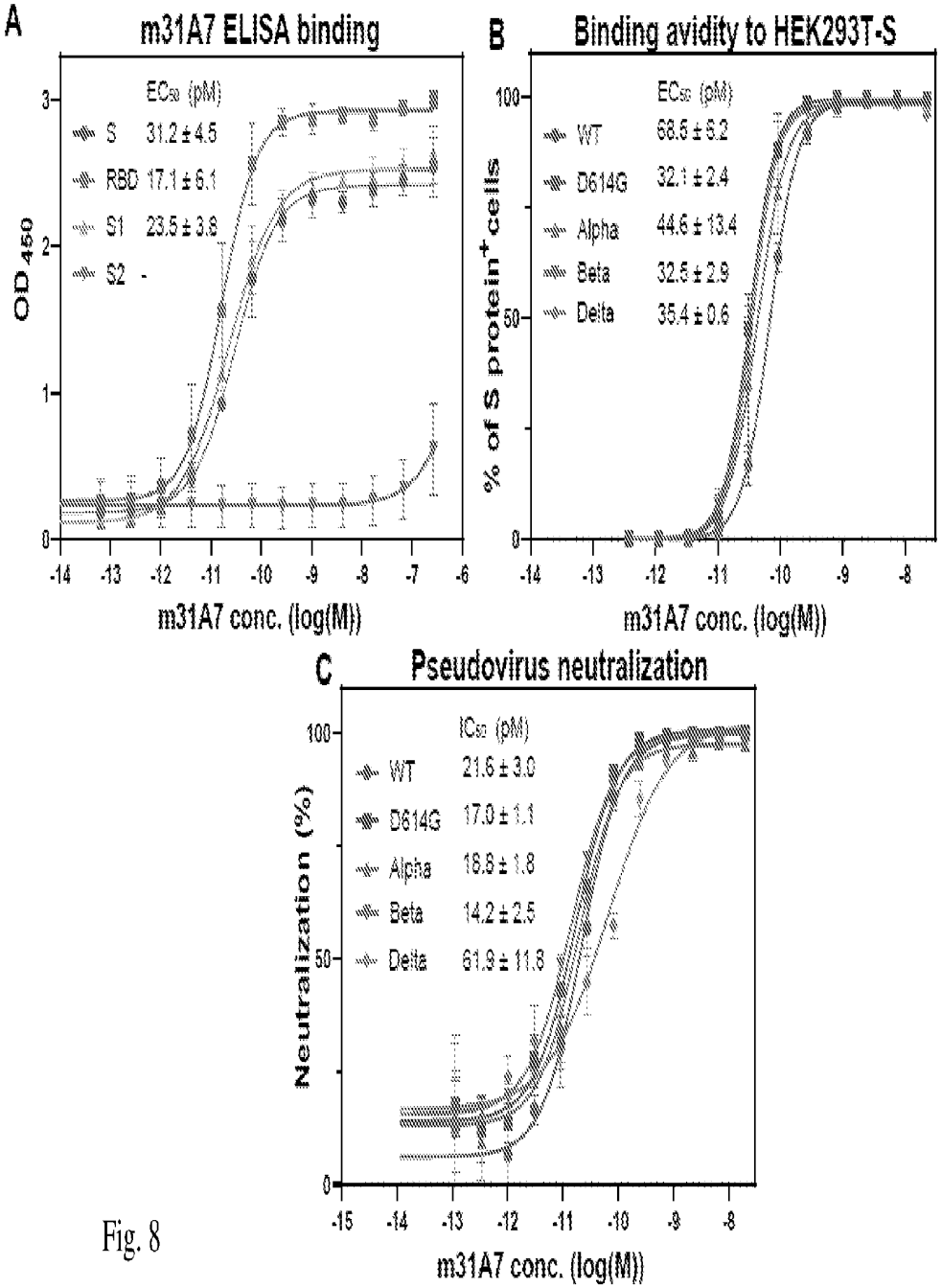

A
m31A7 ELISA binding
EC50 (pM)
S 31.2 ± 4.5
RBD 17.1 ± 6.1
S1 23.5 ± 3.8
S2 -
OD 450
m31A7 conc. (log(M))
B
Binding avidity to HEK293T-S
EC50 (pM)
WT 68.5 ± 6.2
D614G 32.1 ± 2.4
Alpha 44.6 ± 13.4
Beta 32.5 ± 2.9
Delta 35.4 ± 0.6
% of S protein+ cells
m31A7 conc. (log(M))
C
Pseudovirus neutralization
IC50 (pM)
WT 21.6 ± 3.0
D614G 17.0 ± 1.1
Alpha 18.8 ± 1.8
Beta 14.2 ± 2.5
Delta 61.9 ± 11.8
Neutralization (%)
m31A7 conc. (log(M))

Fig. 8

Fig. 8

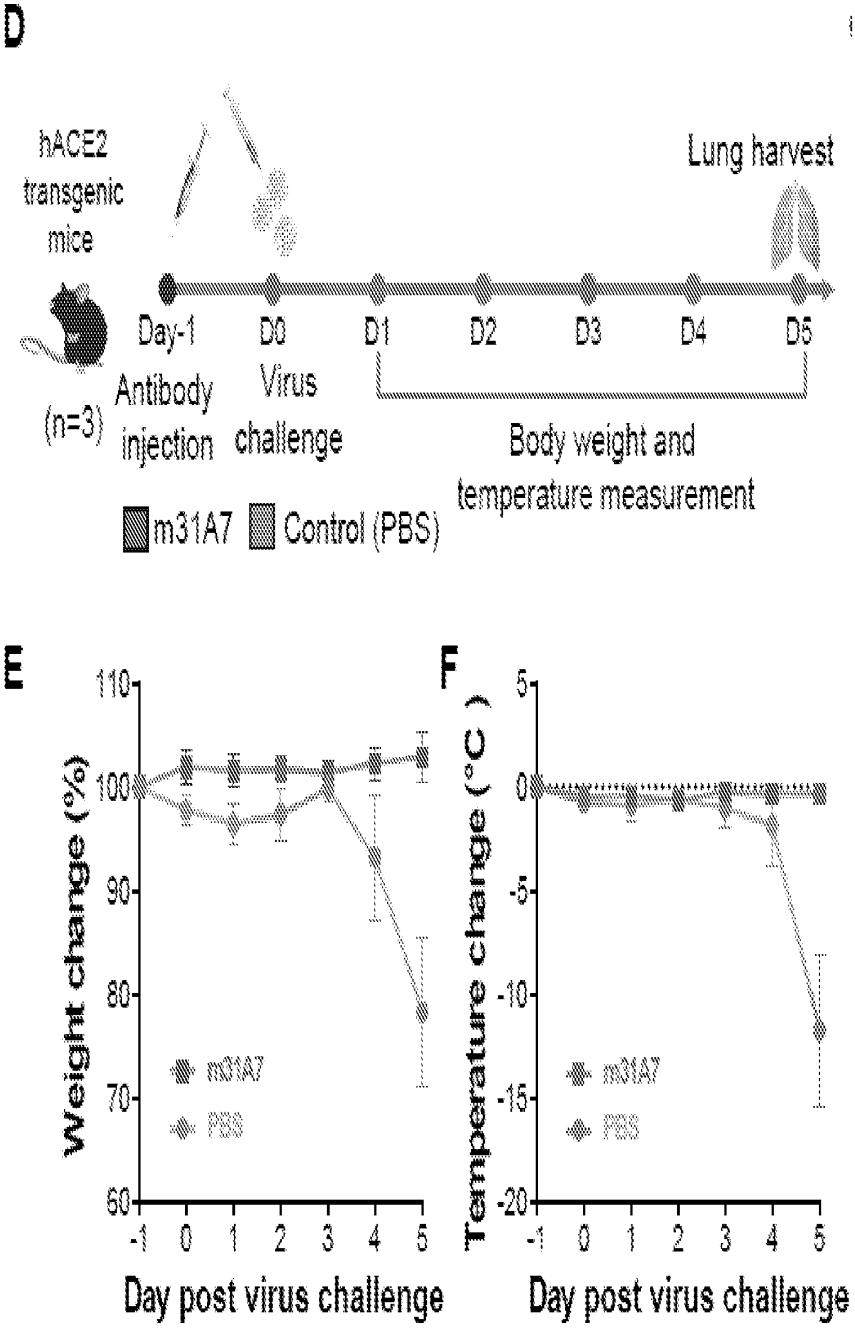

D
hACE2 transgenic mice
(n=3)
Day-1
Antibody injection
D0
Virus challenge
D1
D2
D3
D4
D5
Lung harvest
Body weight and temperature measurement
m31A7
Control (PBS)
E
Weight change (%)
110
100
90
80
70
60
m31A7
PBS
-1 0 1 2 3 4 5
Day post virus challenge
F
Temperature change (°C)
5
0
-5
-10
-15
-20
m31A7
PBS
-1 0 1 2 3 4 5
Day post virus challenge

Fig. 8

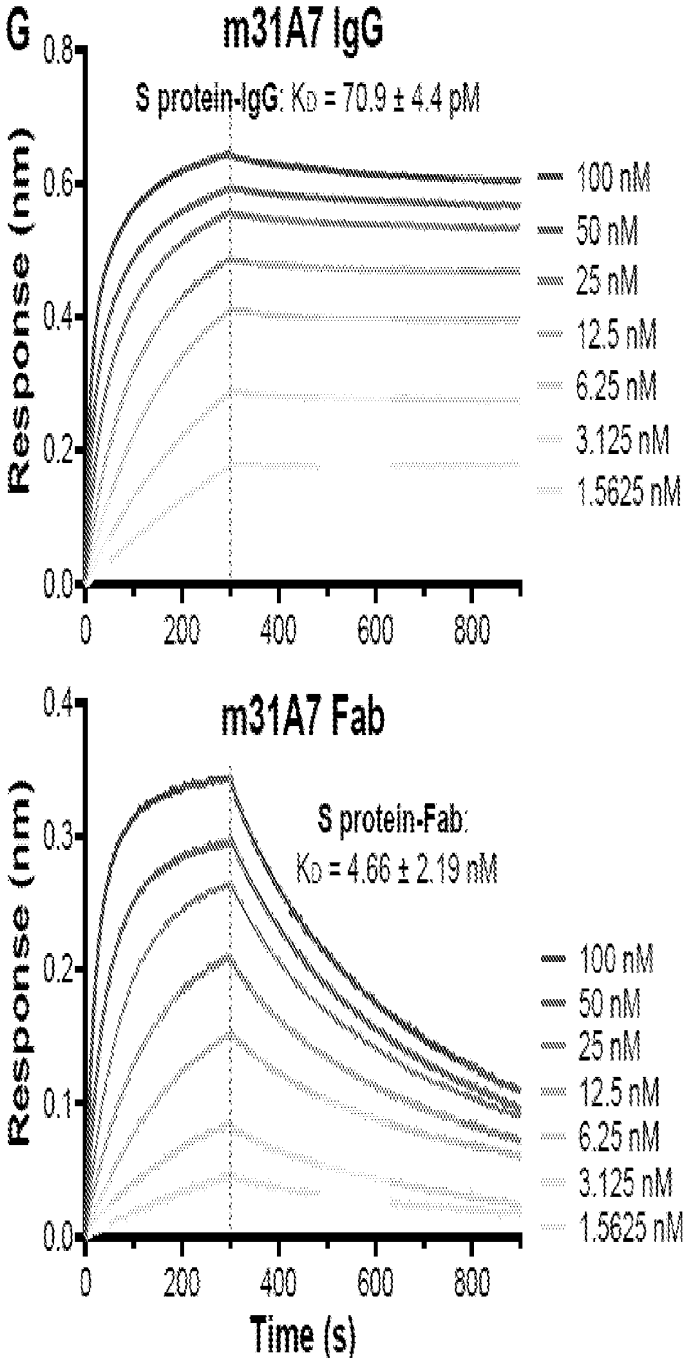
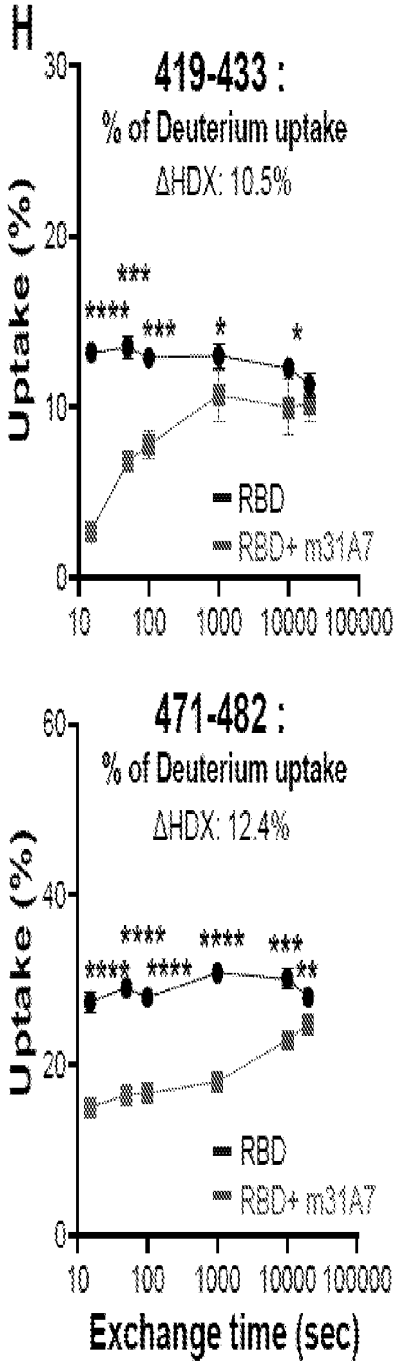

G
m31A7 IgG
S protein-IgG: KD = 70.9 ± 4.4 pM
Response (nm)
0.0
0.2
0.4
0.6
0.8
0
200
400
600
800
100 nM
50 nM
25 nM
12.5 nM
6.25 nM
3.125 nM
1.5625 nM
m31A7 Fab
S protein-Fab:
KD = 4.66 ± 2.19 nM
Response (nm)
0.0
0.1
0.2
0.3
0.4
0
200
400
600
800
Time (s)
100 nM
50 nM
25 nM
12.5 nM
6.25 nM
3.125 nM
1.5625 nM

Fig. 8


419-433 :
% of Deuterium uptake
ΔHDX: 10.5%
Uptake (%)
RBD
RBD+ m31A7
471-482 :
% of Deuterium uptake
ΔHDX: 12.4%
Uptake (%)
RBD
RBD+ m31A7
Exchange time (sec)

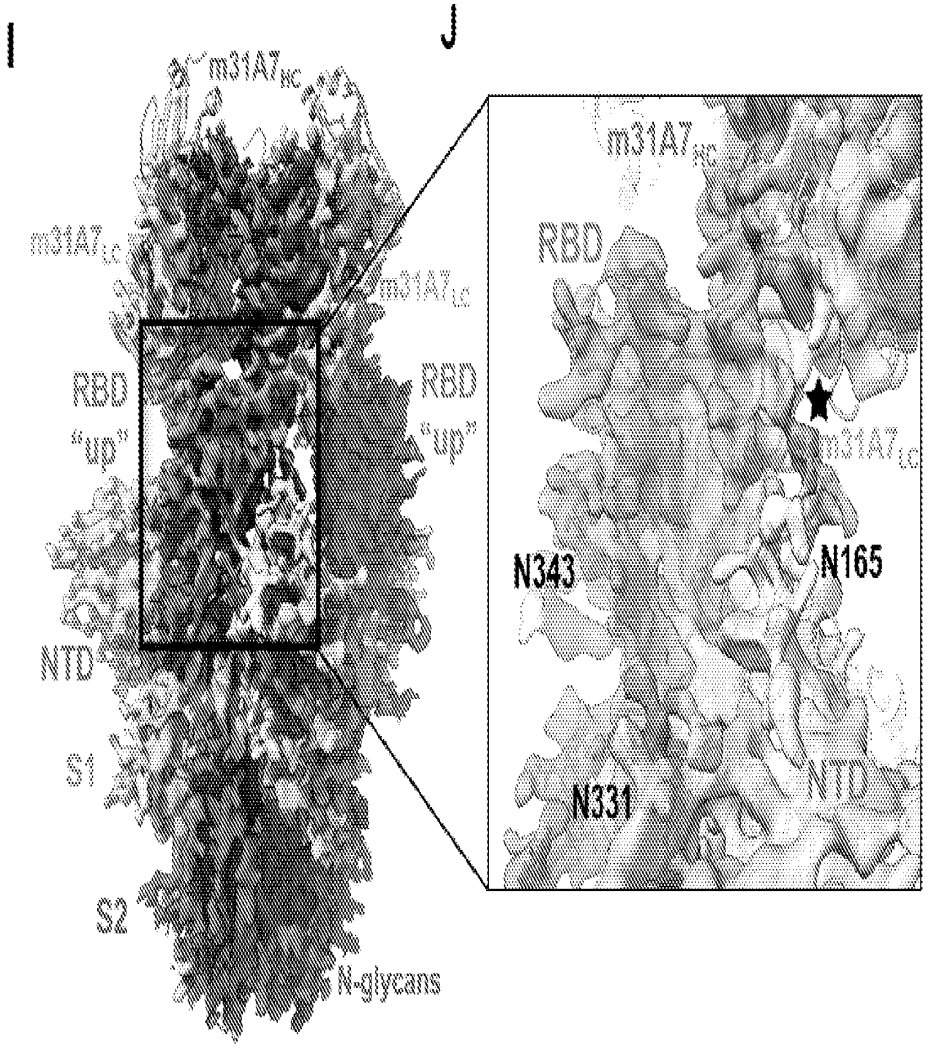

I
J
m31A7HC
m31A7LC
m31A7LC
RBD
"up"
RBD
"up"
NTD
S1
S2
N-glycans
m31A7HC
RBD
m31A7LC
N343
N165
N331
NTD

Fig. 8

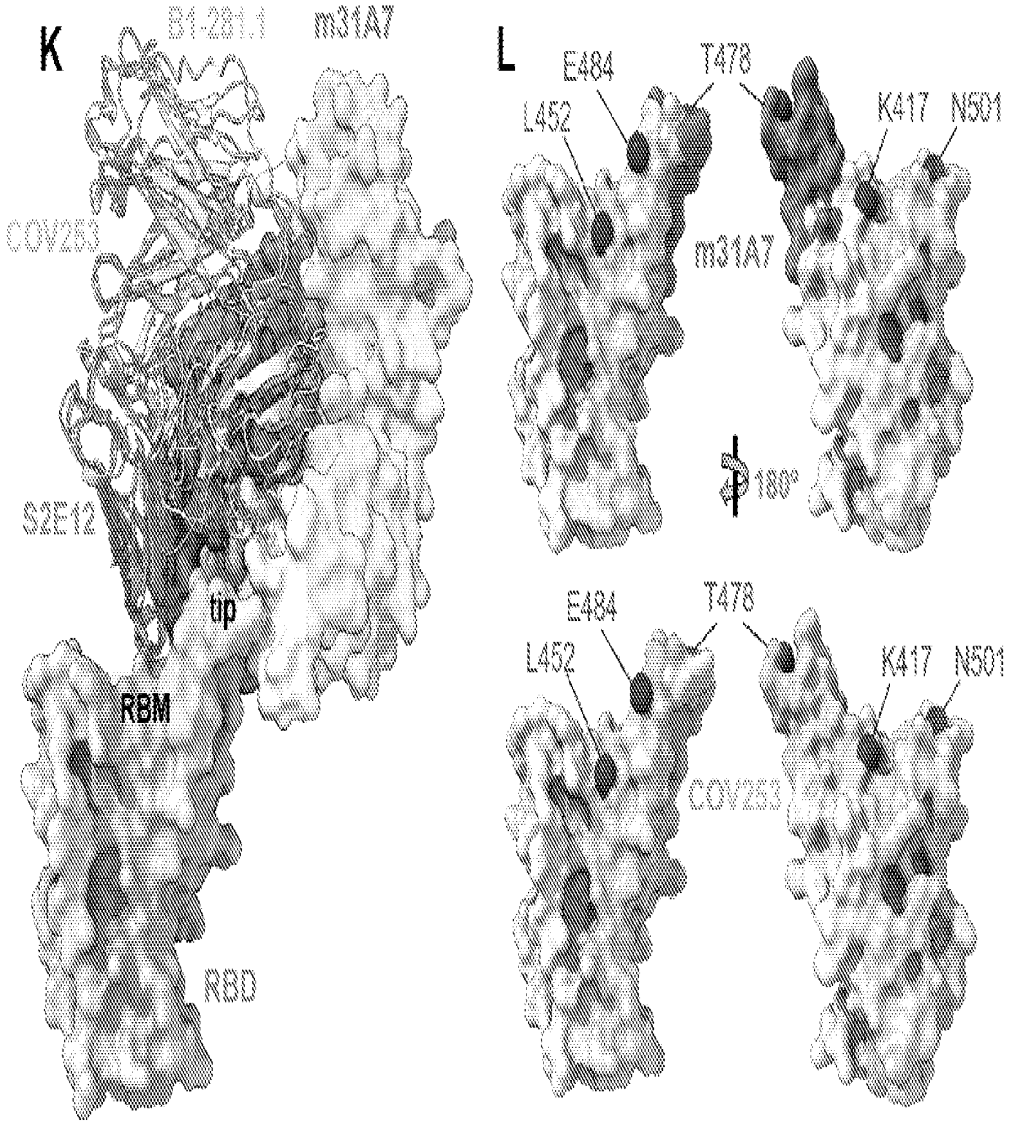

K
B1-28L1
m31A7
COV253
S2E12
tip
RBM
RBD
L
E484
T478
L452
K417
N501
m31A7
180°
E484
T478
L452
K417
N501
COV253

Fig. 8

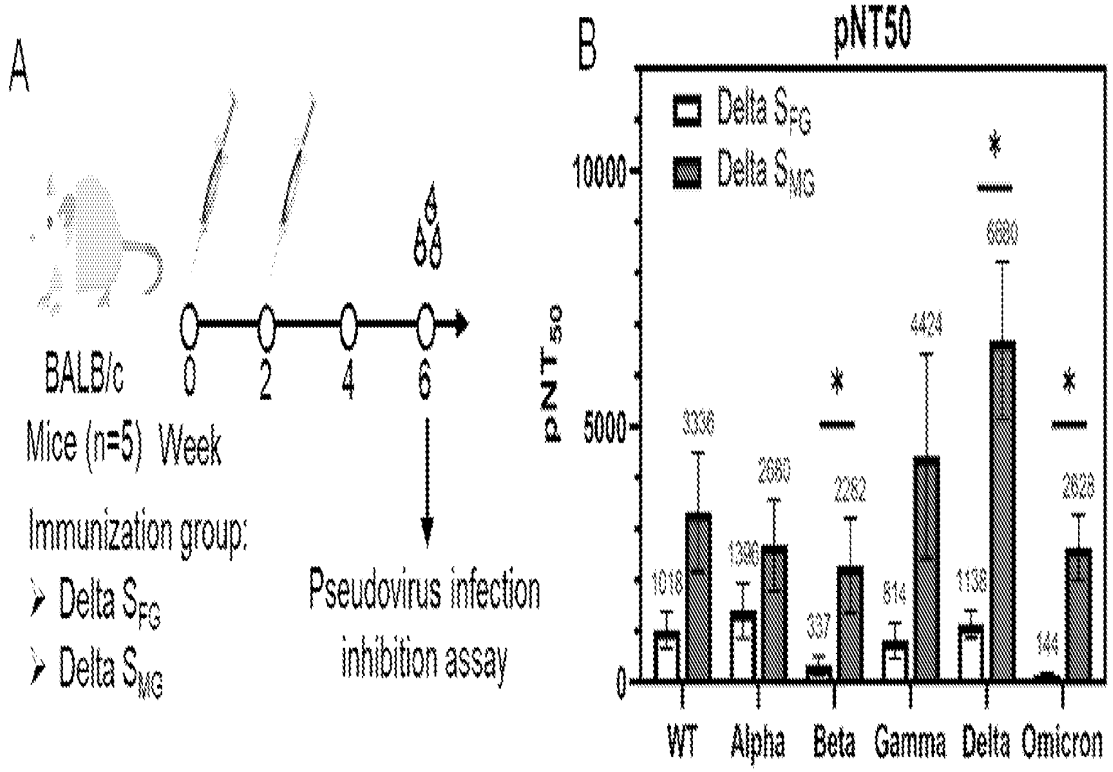

A
BALB/c
0
2
4
6
Mice (n=5) Week
Immunization group:
Delta S_{FG}
Delta S_{MG}
Pseudovirus infection inhibition assay
B
pNT50
Delta S_{FG}
Delta S_{MG}
pNT50
10000
5000
0
1018
3338
1306
2667
337
2262
614
4424
1138
6880
144
2623
WT
Alpha
Beta
Gamma
Delta
Omicron

Fig 9

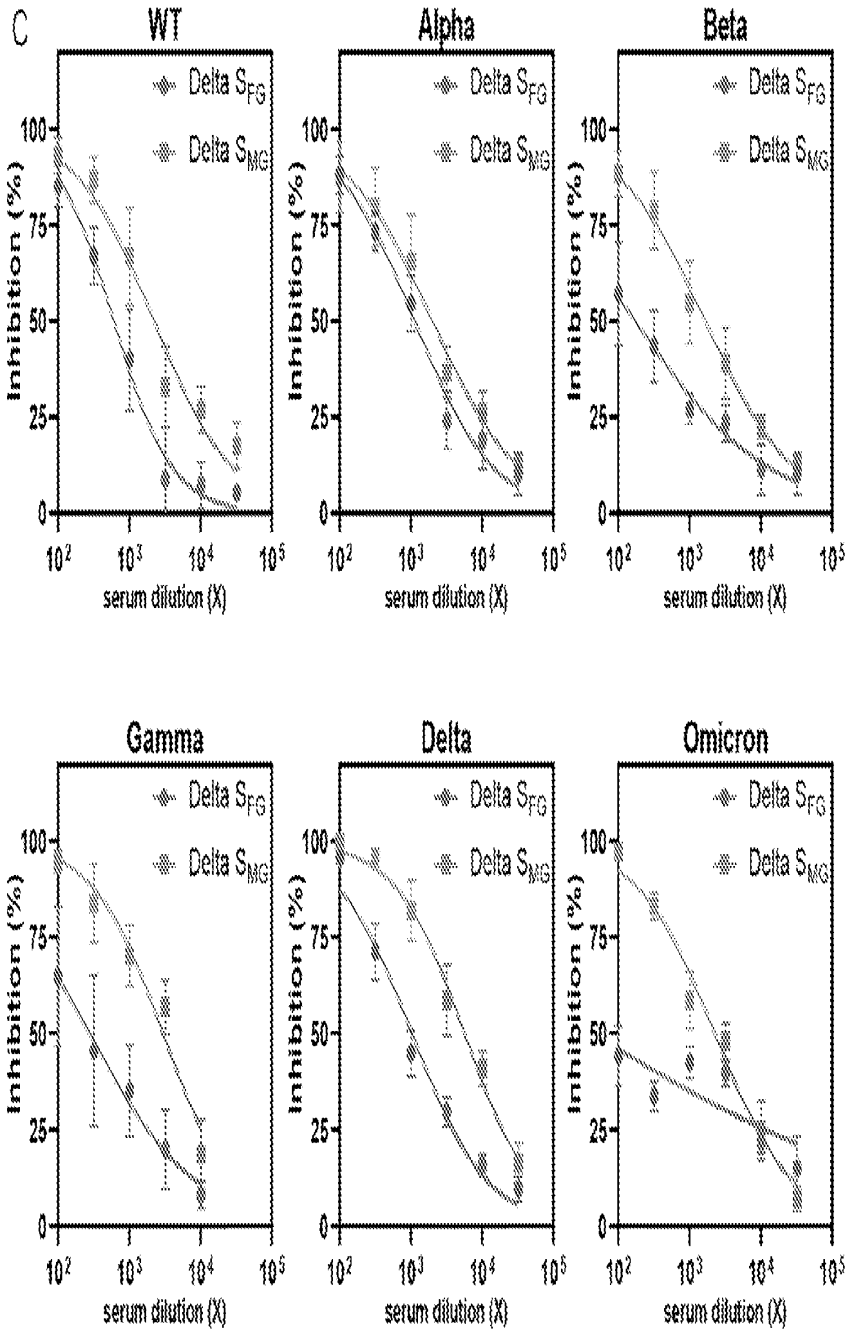

C
WT
Alpha
Beta
Gamma
Delta
Omicron
Delta S_{FG}
Delta S_{MG}
Inhibition (%)
serum dilution (X)

Fig 9

A

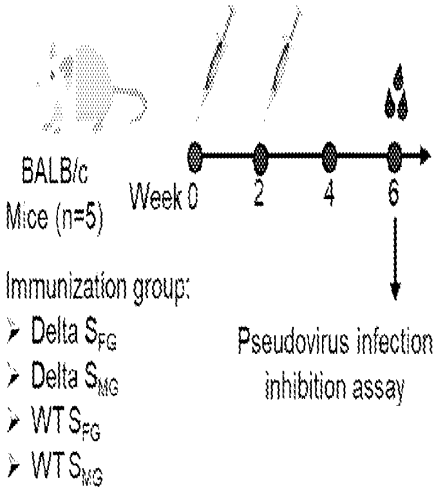
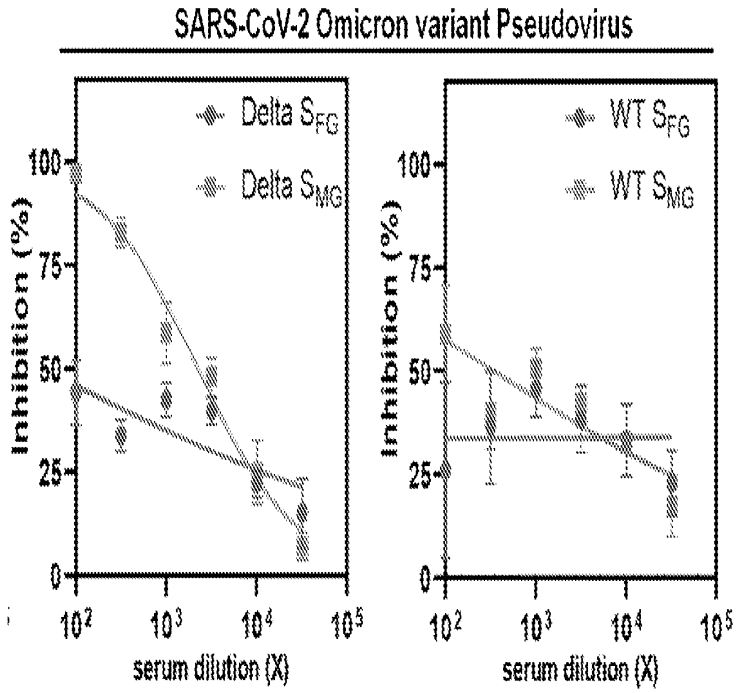

BALB/c
Mice (n=5)
Week 0
2
4
6
Immunization group:
Delta S_{FG}
Delta S_{MG}
WT S_{FG}
WT S_{MG}
Pseudovirus infection inhibition assay

Fig. 10


SARS-CoV-2 Omicron variant Pseudovirus
Delta S_{FG}
Delta S_{MG}
WT S_{FG}
WT S_{MG}
Inhibition (%)
serum dilution (X)

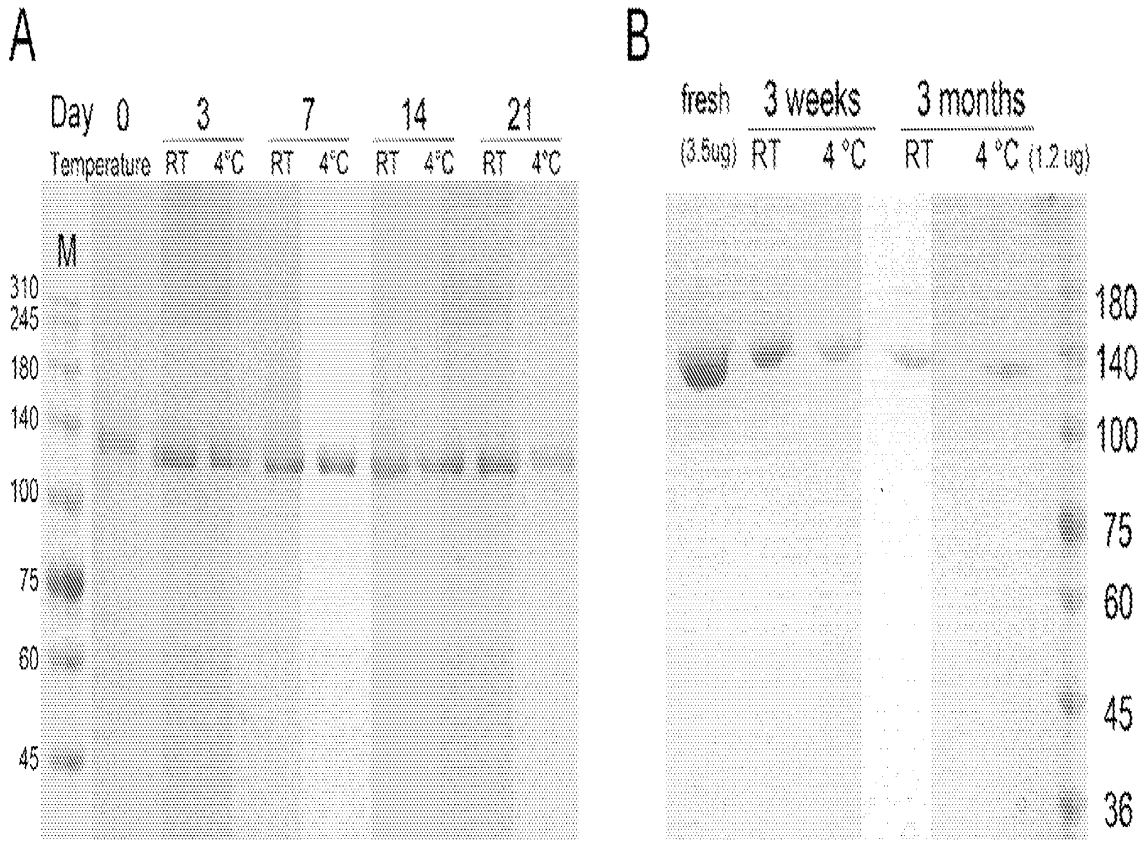

A
Day 0 3 7 14 21
Temperature RT 4°C RT 4°C RT 4°C RT 4°C
M
310
245
180
140
100
75
60
45
B
fresh 3 weeks 3 months
(3.5ug) RT 4 °C RT 4 °C (1.2 ug)
180
140
100
75
60
45
36

Fig. 11

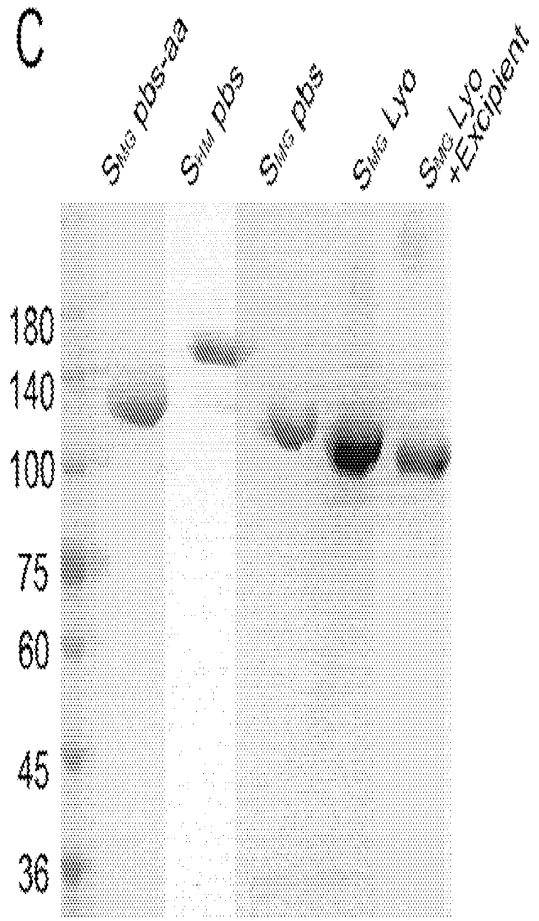

C
SMG pbs-aa
SHM pbs
SMG pbs
SMG Lyo
SMG Lyo +Excipient
180
140
100
75
60
45
36

Fig. 11

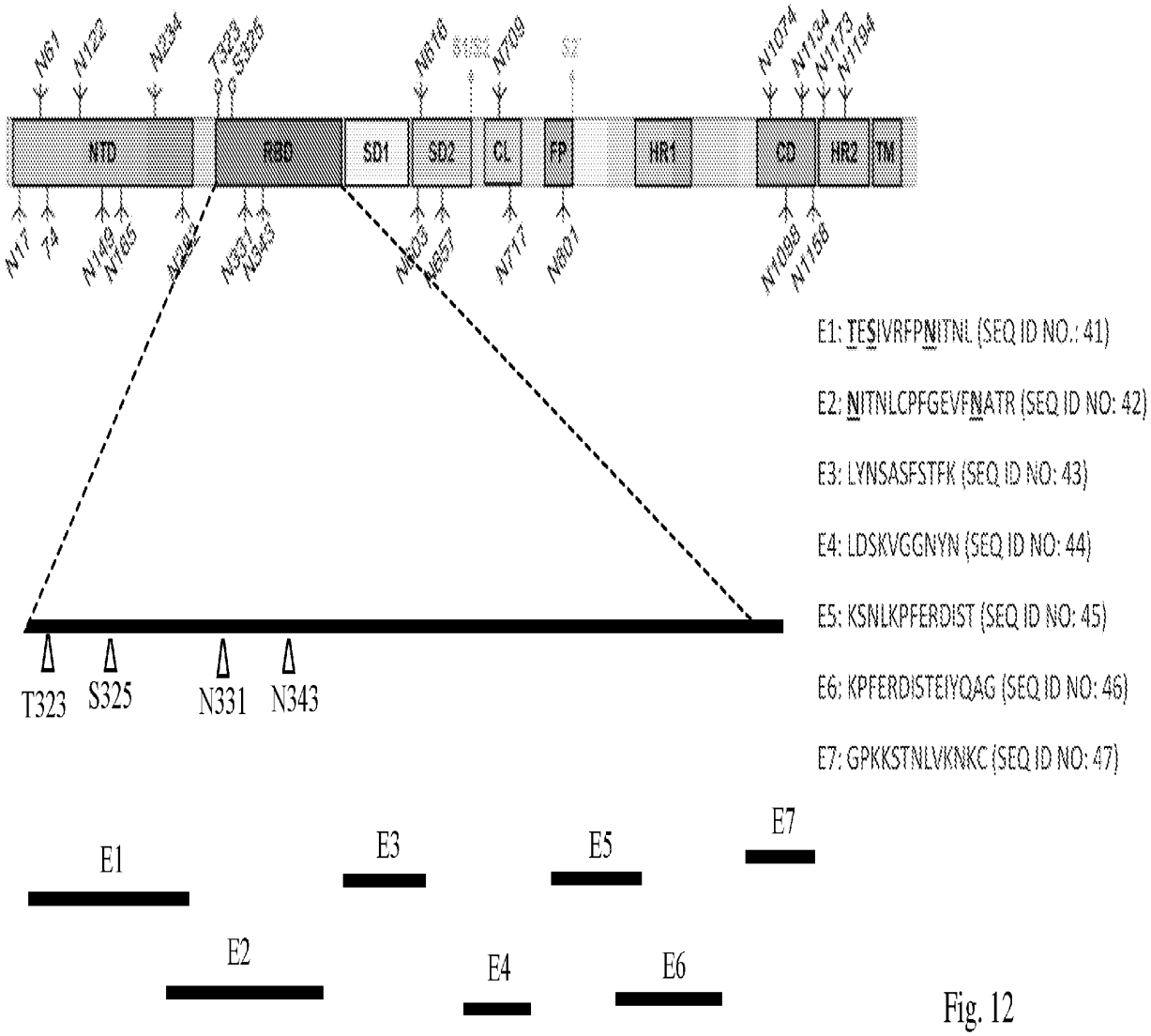

NTD
RBD
SD1
SD2
CL
FP
HR1
CD
HR2
TM
T323
S325
N331
N343
E1: TESIVRFPNITNL (SEQ ID NO.: 41)
E2: NITNLCPFGEVFNATR (SEQ ID NO: 42)
E3: LYNSASFSTFK (SEQ ID NO: 43)
E4: LDSKVGGNYN (SEQ ID NO: 44)
E5: KSNLKPFERDIST (SEQ ID NO: 45)
E6: KPFERDISTEIYQAG (SEQ ID NO: 46)
E7: GPKKSTNLVKNKC (SEQ ID NO: 47)
E1
E2
E3
E4
E5
E6
E7

Fig. 12

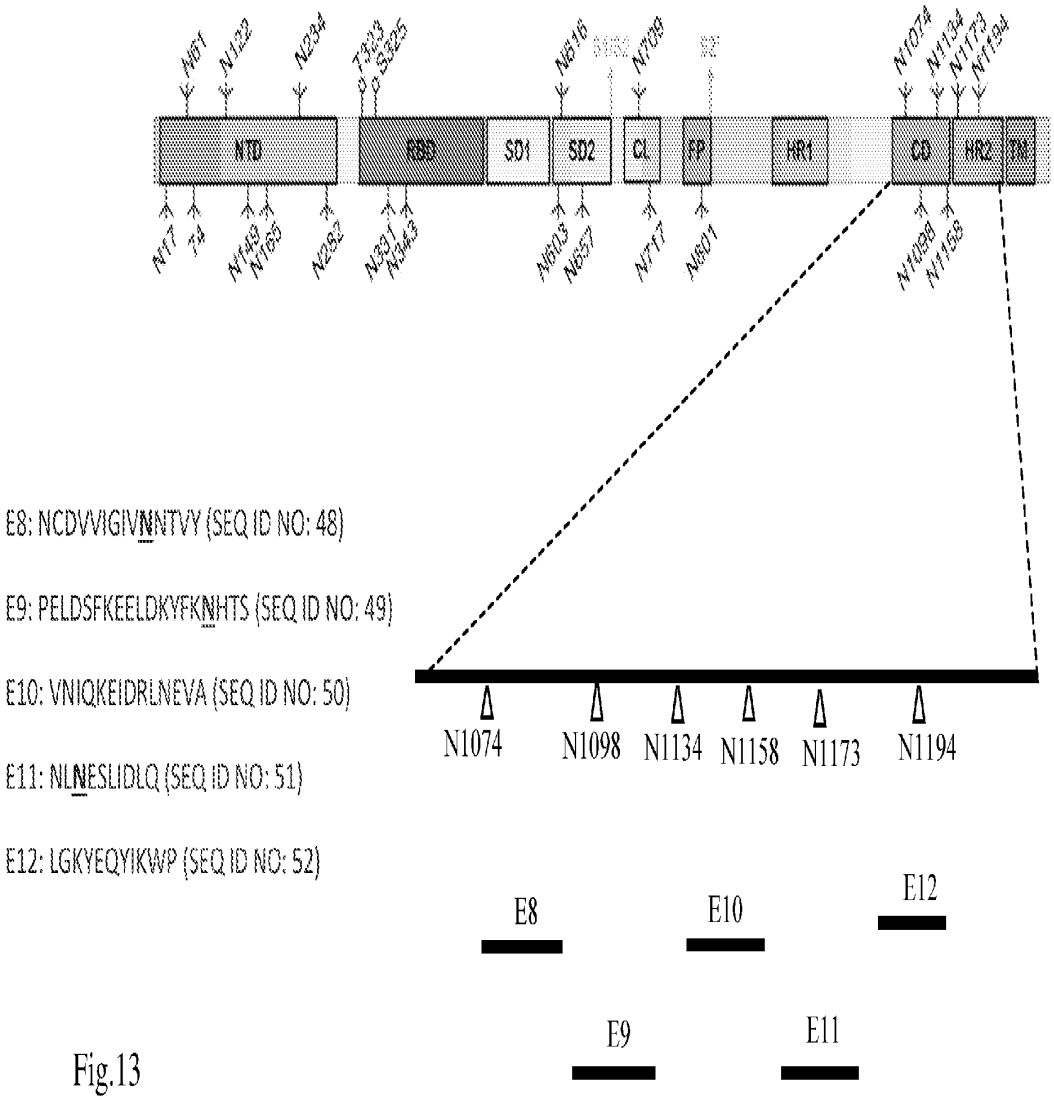

NTD
RBD
SD1
SD2
CL
FP
HR1
CD
HR2
TM
E8: NCDVVIGIVNNTVY (SEQ ID NO: 48)
E9: PELDSFKEELDKYFKNHTS (SEQ ID NO: 49)
E10: VNIQKEIDRLNEVA (SEQ ID NO: 50)
E11: NLNESLIDLQ (SEQ ID NO: 51)
E12: LGKYEQYIKWP (SEQ ID NO: 52)
N1074
N1098
N1134
N1158
N1173
N1194
E8
E10
E12
E9
E11

Fig.13