Figure 1

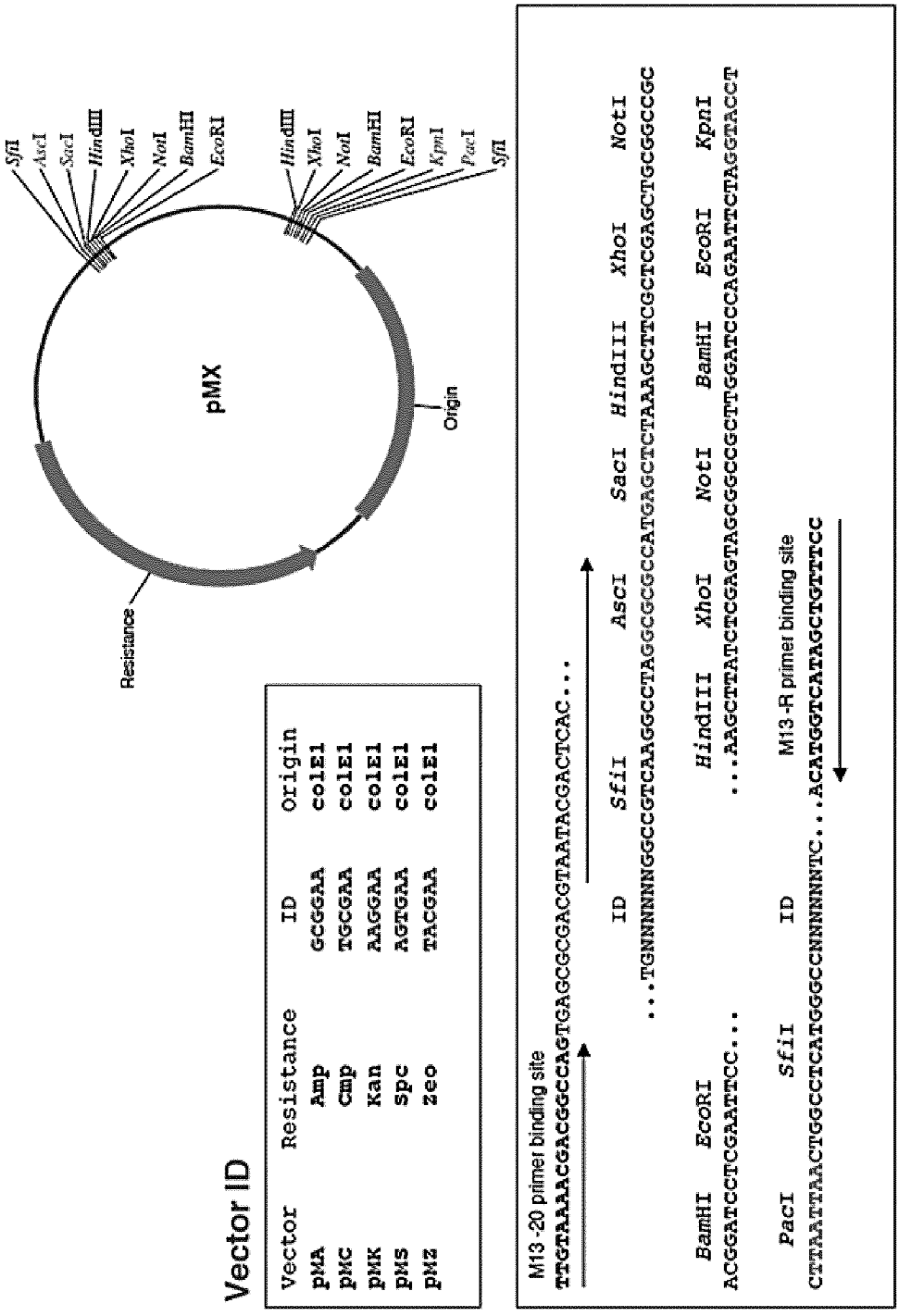

SfiI
AscI
SacI
HindIII
XhoI
NotI
BamHI
EcoRI
HindIII
XhoI
NotI
BamHI
EcoRI
KpnI
PacI
SfiI
pMX
Origin
Resistance
Vector ID
Vector Resistance ID Origin
pMA Amp GCGGAA colE1
pMC Cmp TGCGAA colE1
pMK Kan AAGGAA colE1
pMS Spc AGTGAA colE1
pMZ Zeo TACGAA colE1
M13 -20 primer binding site
TTGTAAAACGACGGCCAGTGAGCGCGACGTAATACGACTCAC...
ID SfiI AscI SacI HindIII XhoI NotI
...TGNNNNNNGGCCGTCAAGGCCTAGGCGCGCCATGAGCTCTAAAGCTTCGCTCGAGCTGCGGCCGC
BamHI EcoRI HindIII XhoI NotI BamHI EcoRI KpnI
ACGGATCCTCGAATTCC... ...AAGCTTATCTCGAGTAGCGGCCGCTTGGATCCCAGAATTCTAGGTACCT
PacI SfiI ID M13 -R primer binding site
CTTAATTAACTGGCCTCATGGGCCNNNNNNTC...ACATGGTCATAGCTGTTTCC

Figure 2

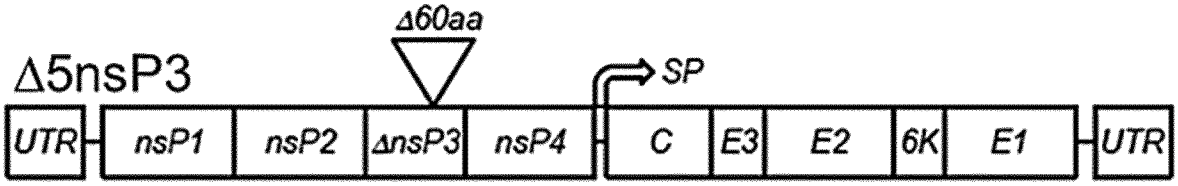


Figure 3

A.

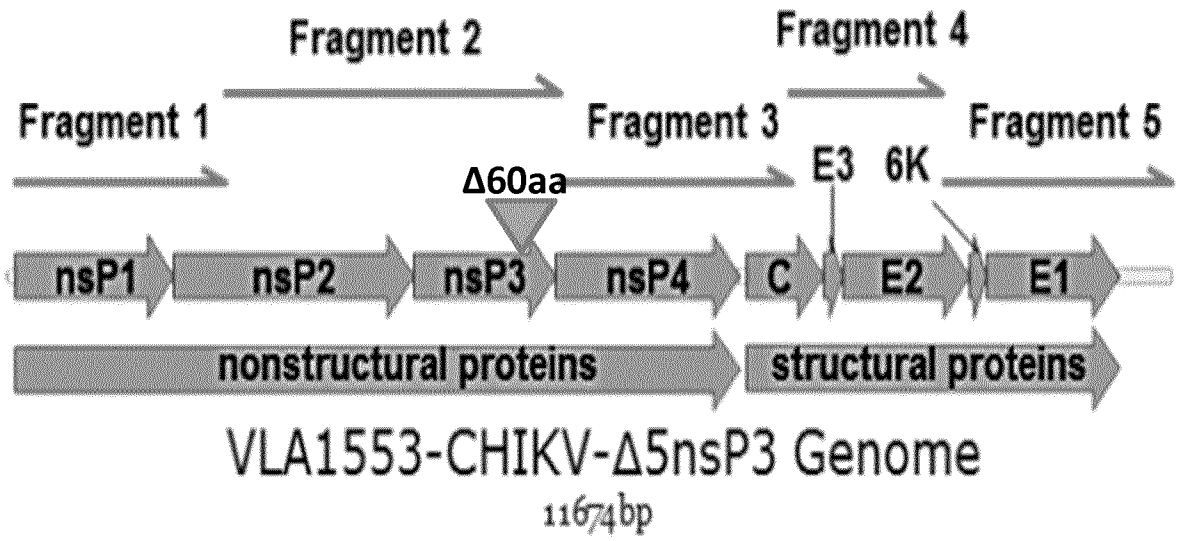

Fragment 2
Fragment 4
Fragment 1
Fragment 3
Fragment 5
Δ60aa
E3
6K
nsP1
nsP2
nsP3
nsP4
C
E2
E1
nonstructural proteins
structural proteins
VLA1553-CHIKV-Δ5nsP3 Genome
11674 bp

Figure 3 (continued)

B.

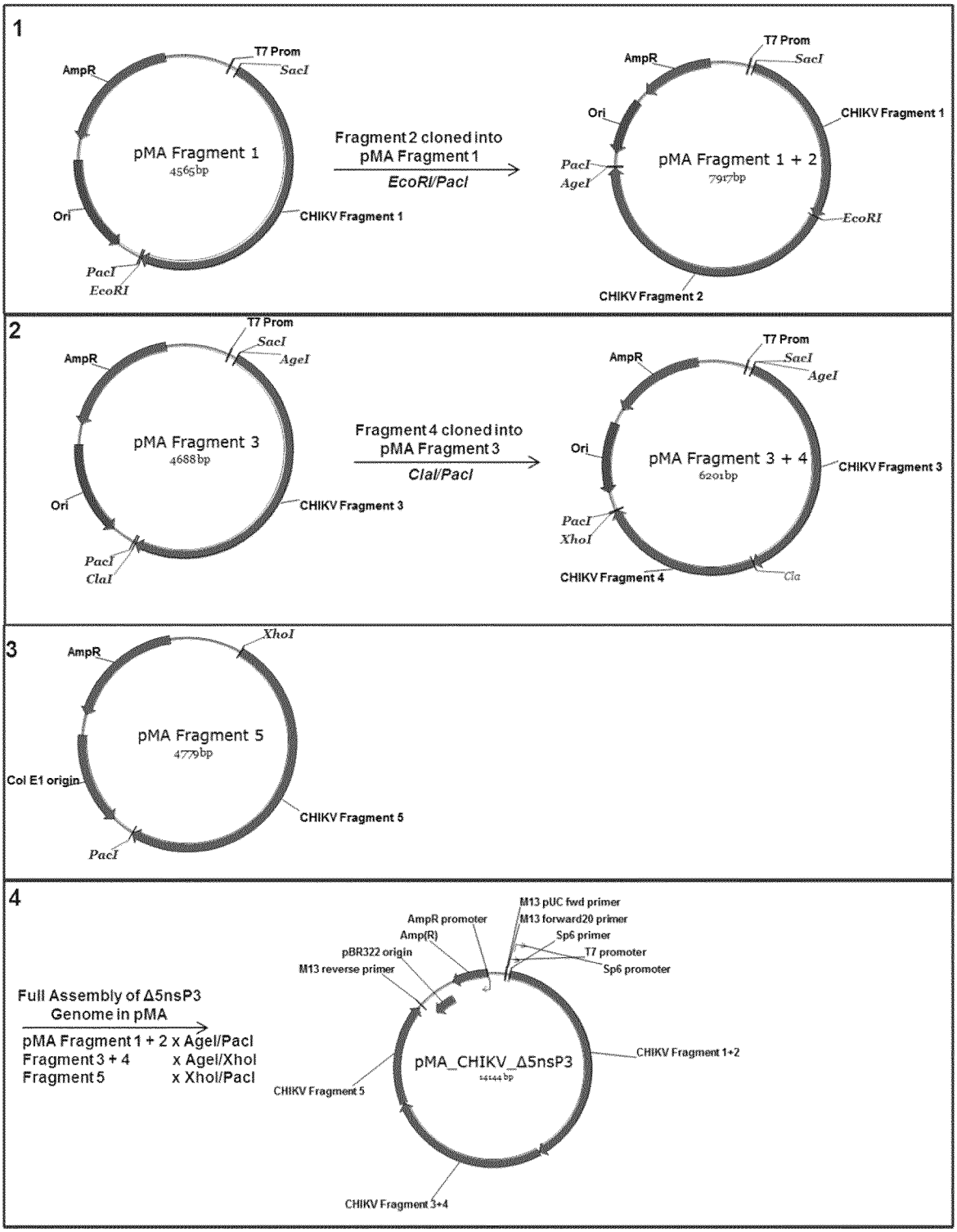
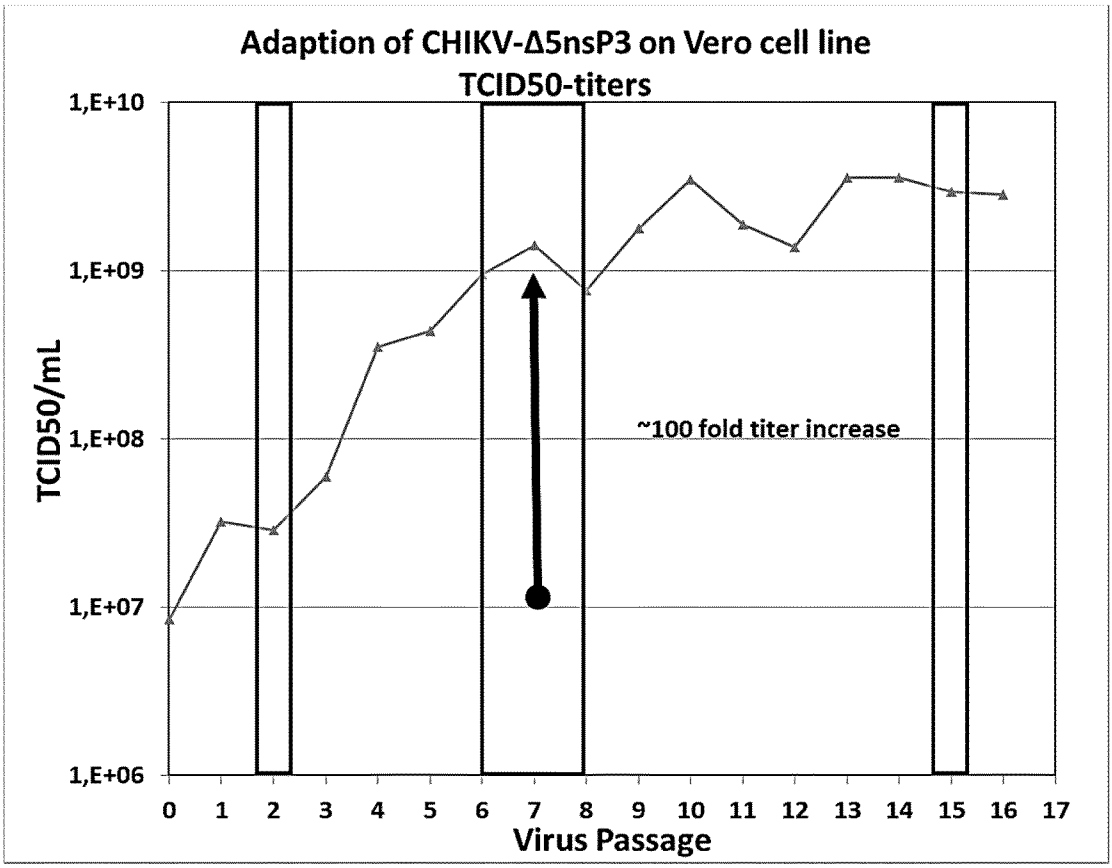


Figure 4

A.


Adaption of CHIKV-Δ5nsP3 on Vero cell line
TCID50-titers
1,E+10
1,E+09
1,E+08
1,E+07
1,E+06
TCID50/mL
~100 fold titer increase
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Virus Passage

B.

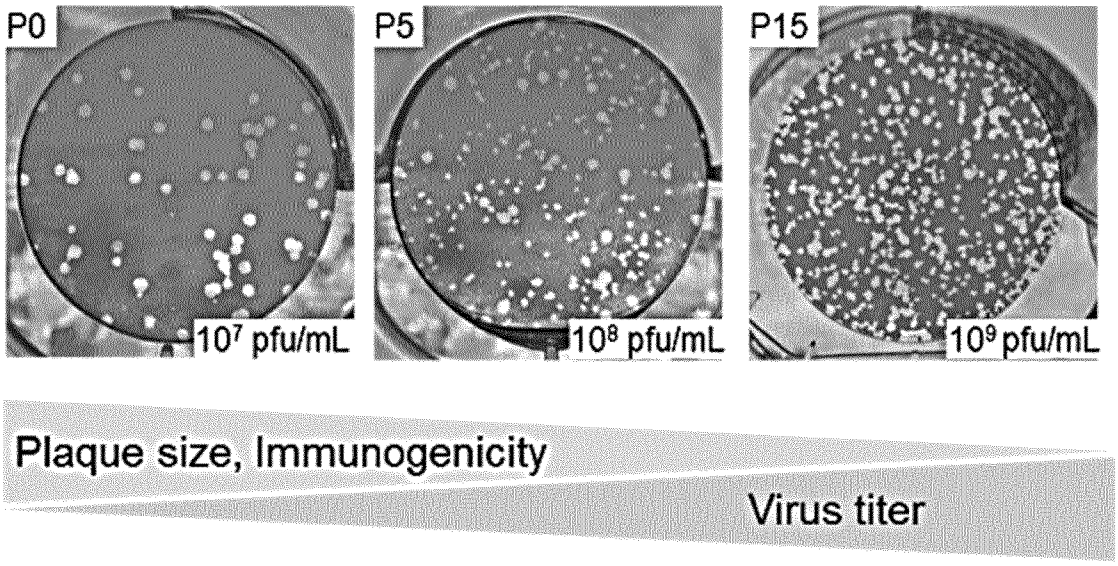

P0
10⁷ pfu/mL
P5
10⁸ pfu/mL
P15
10⁹ pfu/mL
Plaque size, Immunogenicity
Virus titer

Figure 4 (continued)

C.

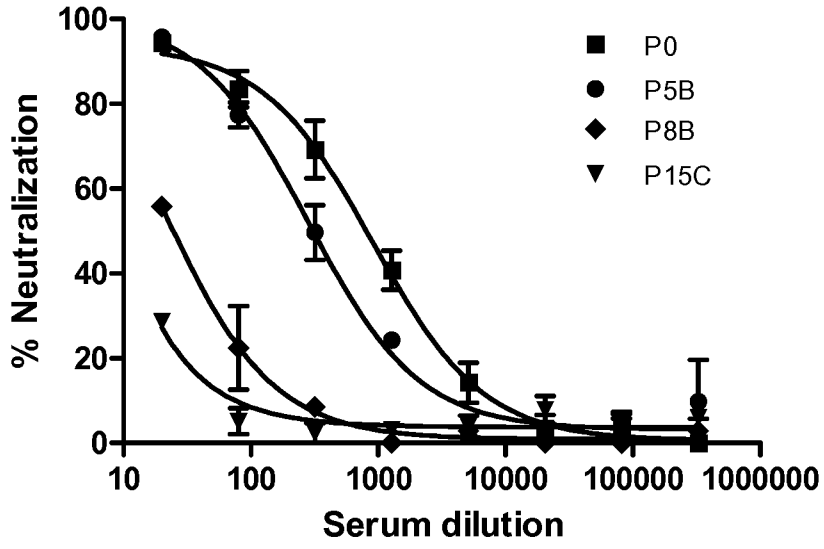
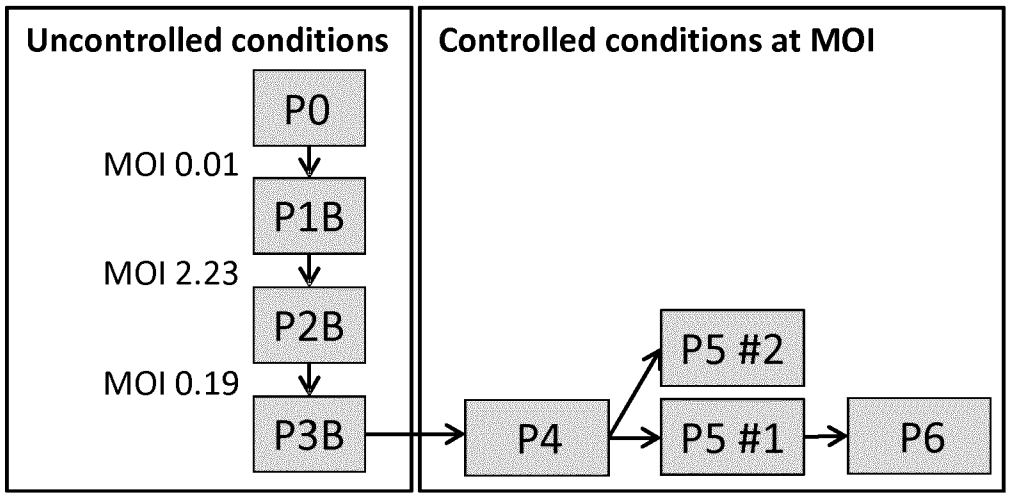


Figure 5

A.


Uncontrolled conditions
Controlled conditions at MOI
P0
MOI 0.01
P1B
MOI 2.23
P2B
MOI 0.19
P3B
P4
P5 #2
P5 #1
P6

B.

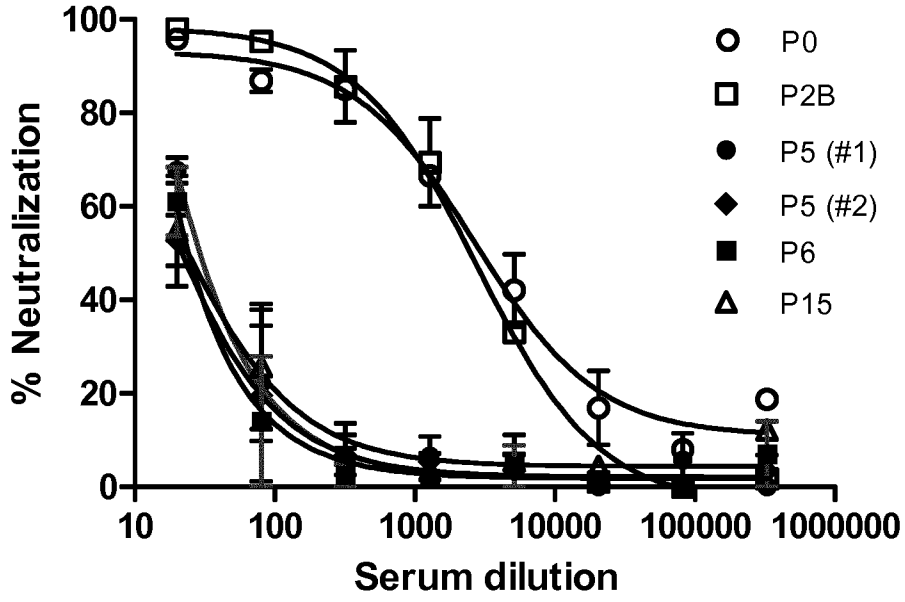
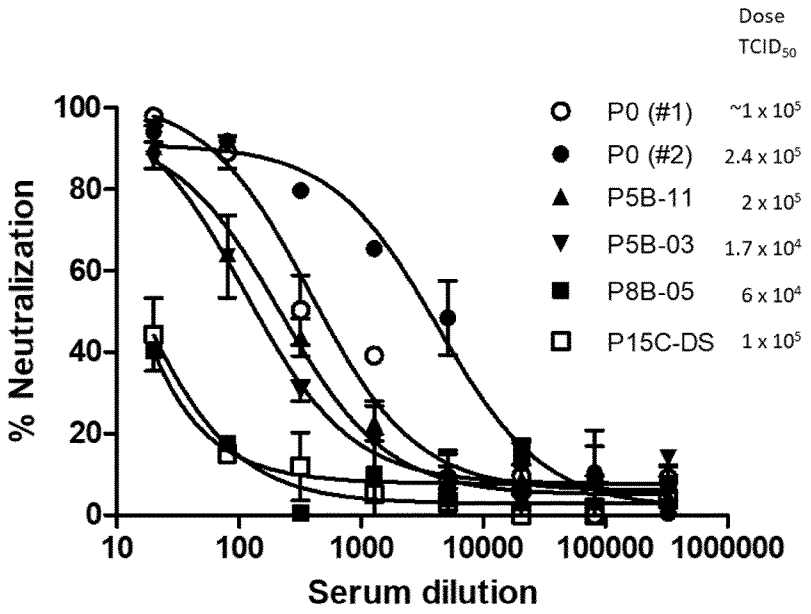

% Neutralization
Serum dilution
100
80
60
40
20
0
10
100
1000
10000
100000
1000000
P0
P2B
P5 (#1)
P5 (#2)
P6
P15

Figure 6

A.


Dose
TCID50
P0 (#1) ~1 x 10^5
P0 (#2) 2.4 x 10^5
P5B-11 2 x 10^5
P5B-03 1.7 x 10^4
P8B-05 6 x 10^4
P15C-DS 1 x 10^5
% Neutralization
100
80
60
40
20
0
10
100
1000
10000
100000
1000000
Serum dilution

B.

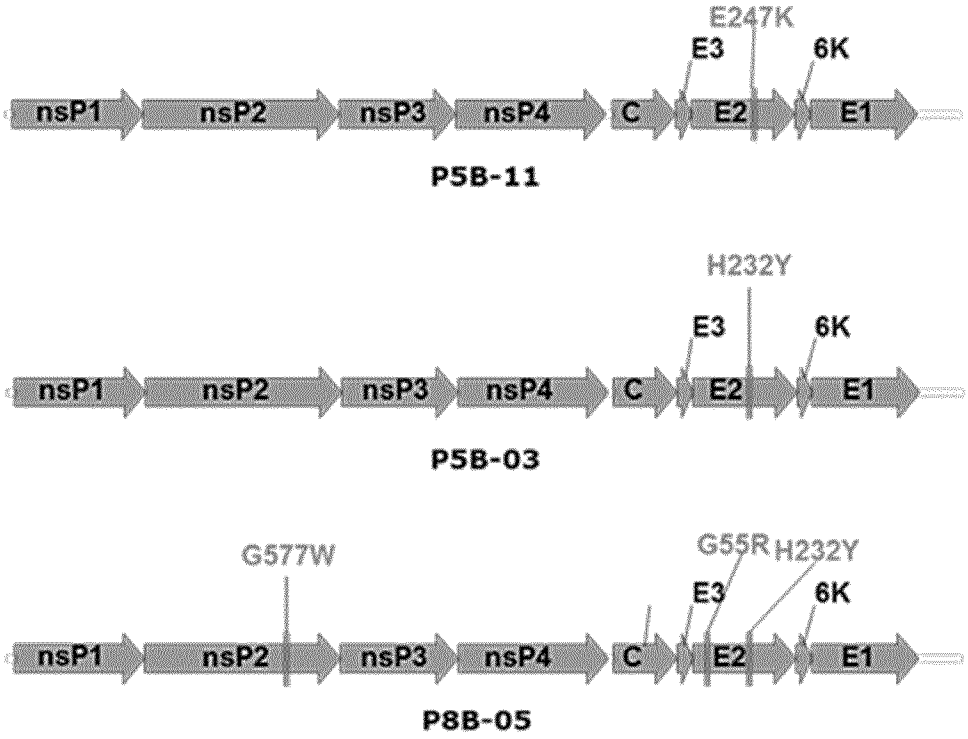
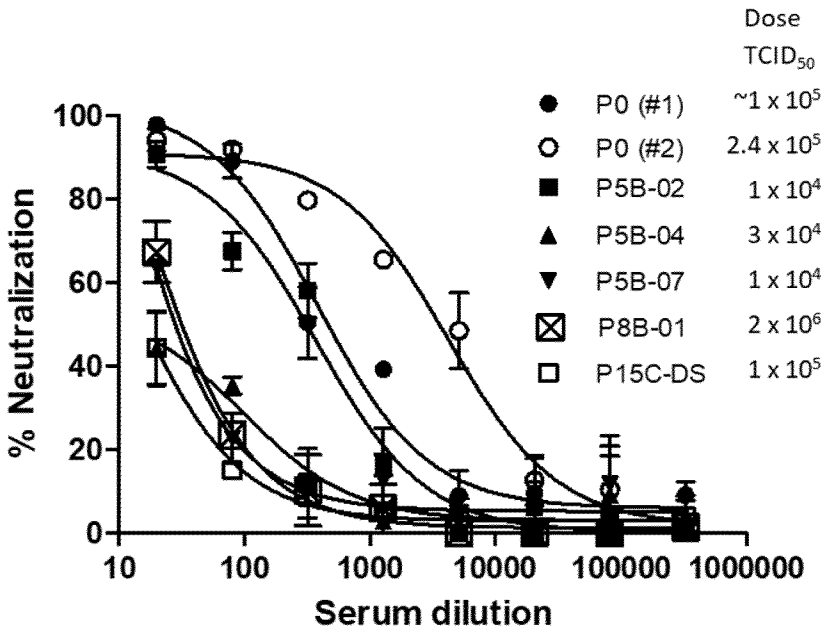

E247K
E3
6K
nsP1
nsP2
nsP3
nsP4
C
E2
E1
P5B-11
H232Y
E3
6K
nsP1
nsP2
nsP3
nsP4
C
E2
E1
P5B-03
G577W
G55R
H232Y
E3
6K
nsP1
nsP2
nsP3
nsP4
C
E2
E1
P8B-05

Figure 6 (continued)

C.



D.

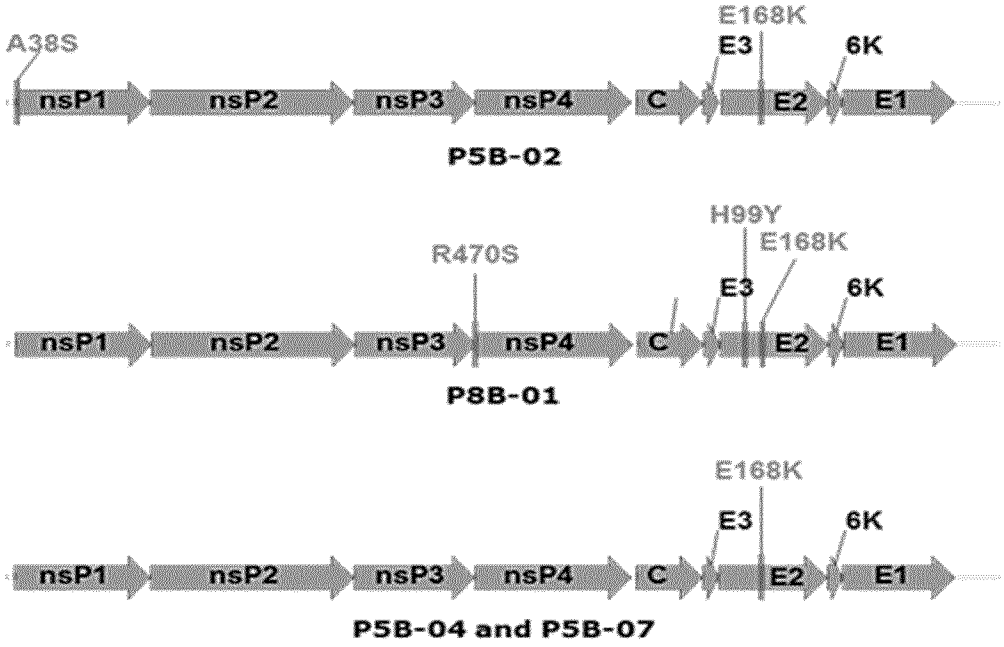


Figure 7

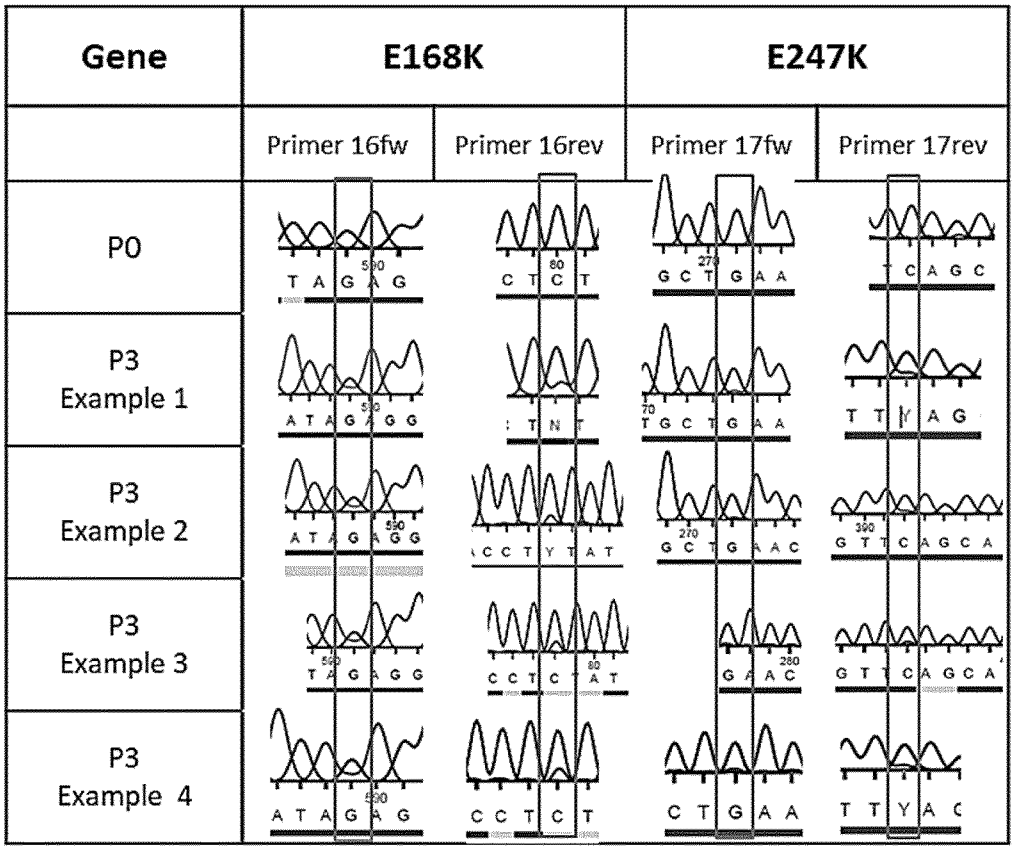
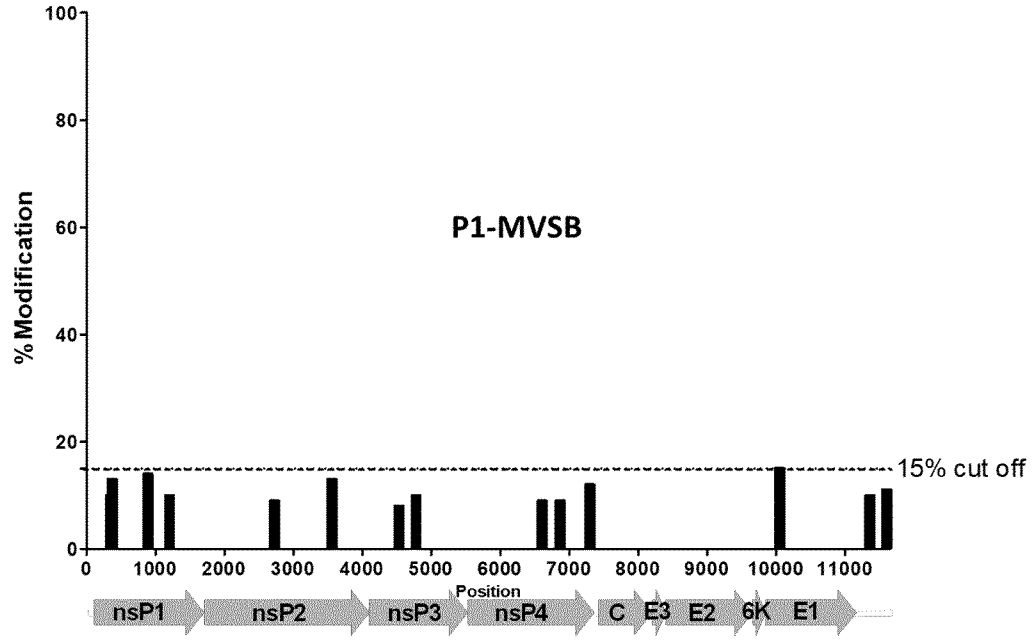

Gene
E168K
E247K
Primer 16fw
Primer 16rev
Primer 17fw
Primer 17rev
P0
P3 Example 1
P3 Example 2
P3 Example 3
P3 Example 4

Figure 8

A.


P1-MVSB
% Modification
100
80
60
40
20
0
15% cut off
0 1000 2000 3000 4000 5000 6000 7000 8000 9000 10000 11000
Position
nsP1 nsP2 nsP3 nsP4 C E3 E2 6K E1

B.

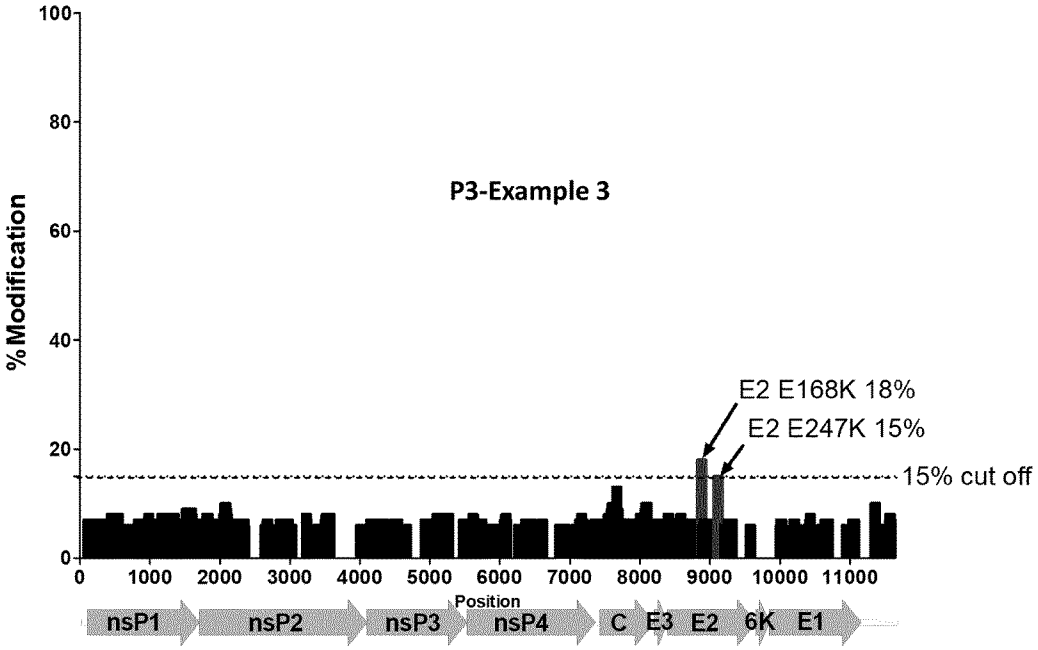
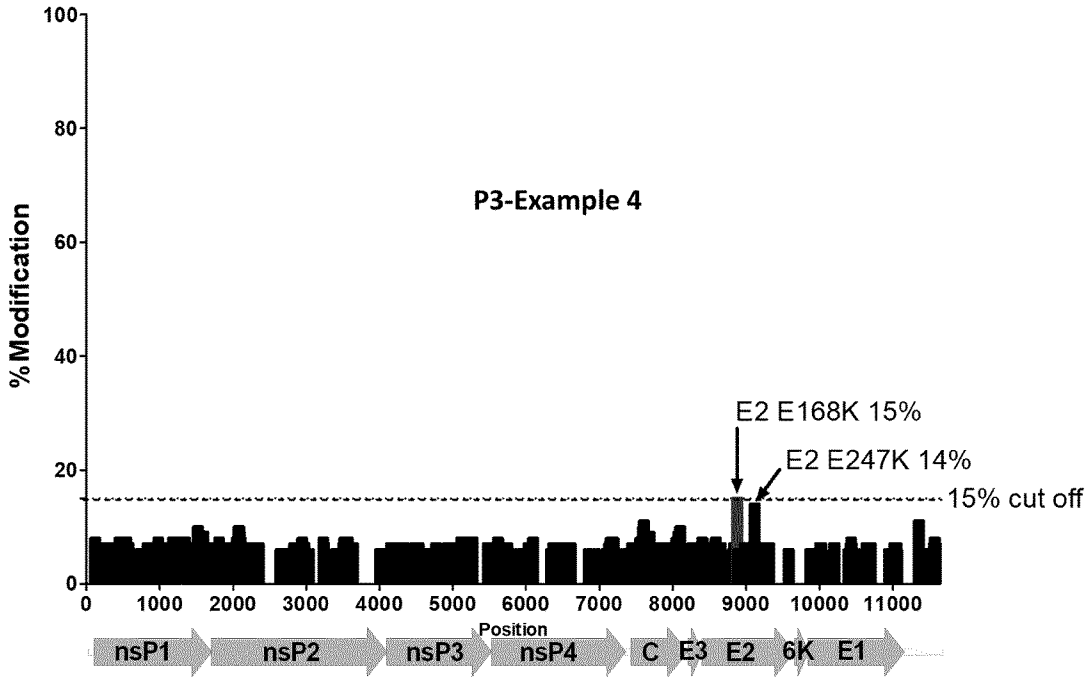

P3-Example 3
% Modification
100
80
60
40
20
0
E2 E168K 18%
E2 E247K 15%
15% cut off
0 1000 2000 3000 4000 5000 6000 7000 8000 9000 10000 11000
Position
nsP1 nsP2 nsP3 nsP4 C E3 E2 6K E1

Figure 8 (continued)

C.



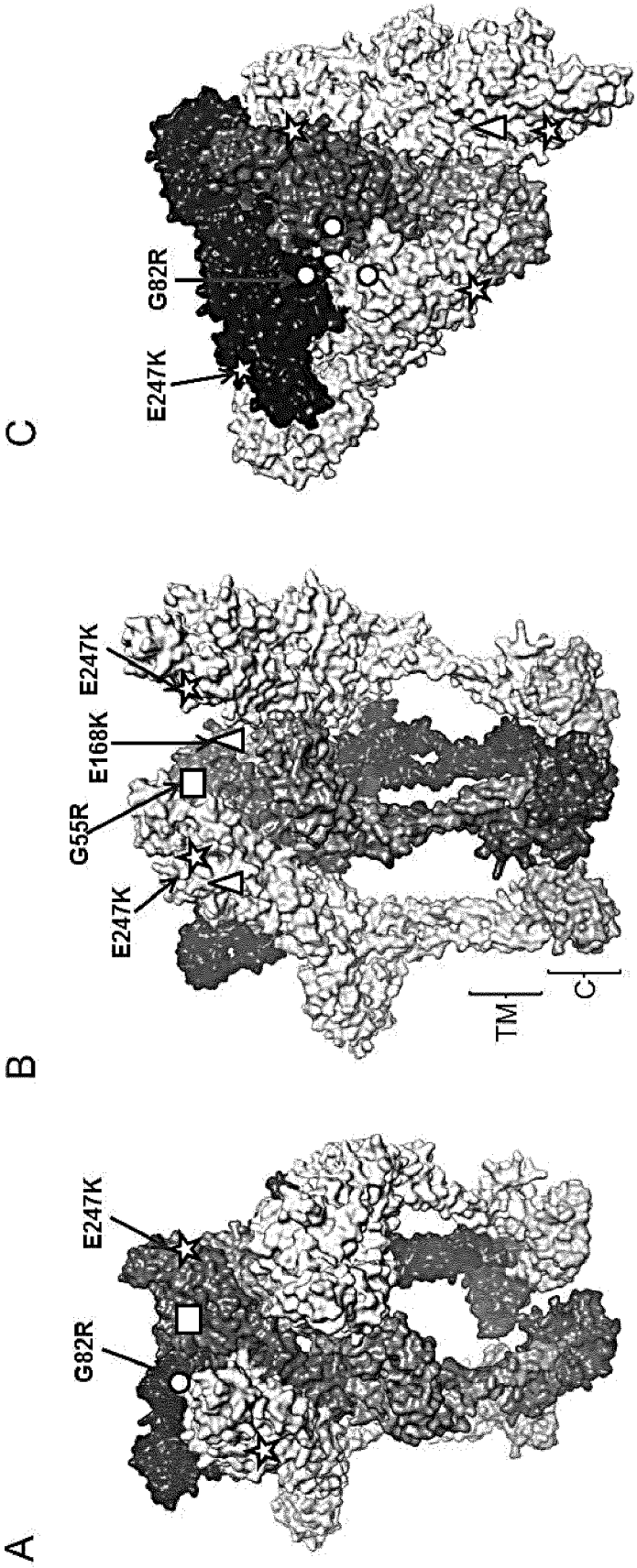


Figure 9

Figure 10

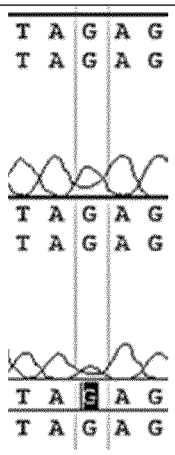
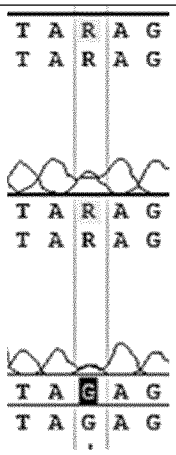
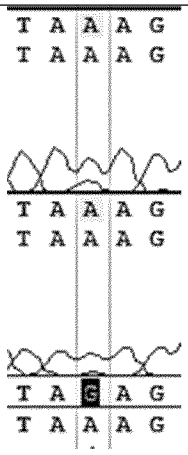
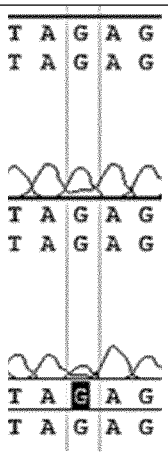
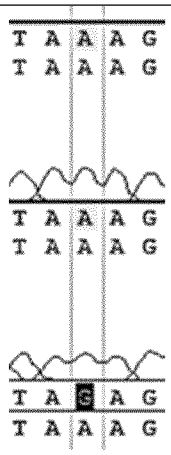
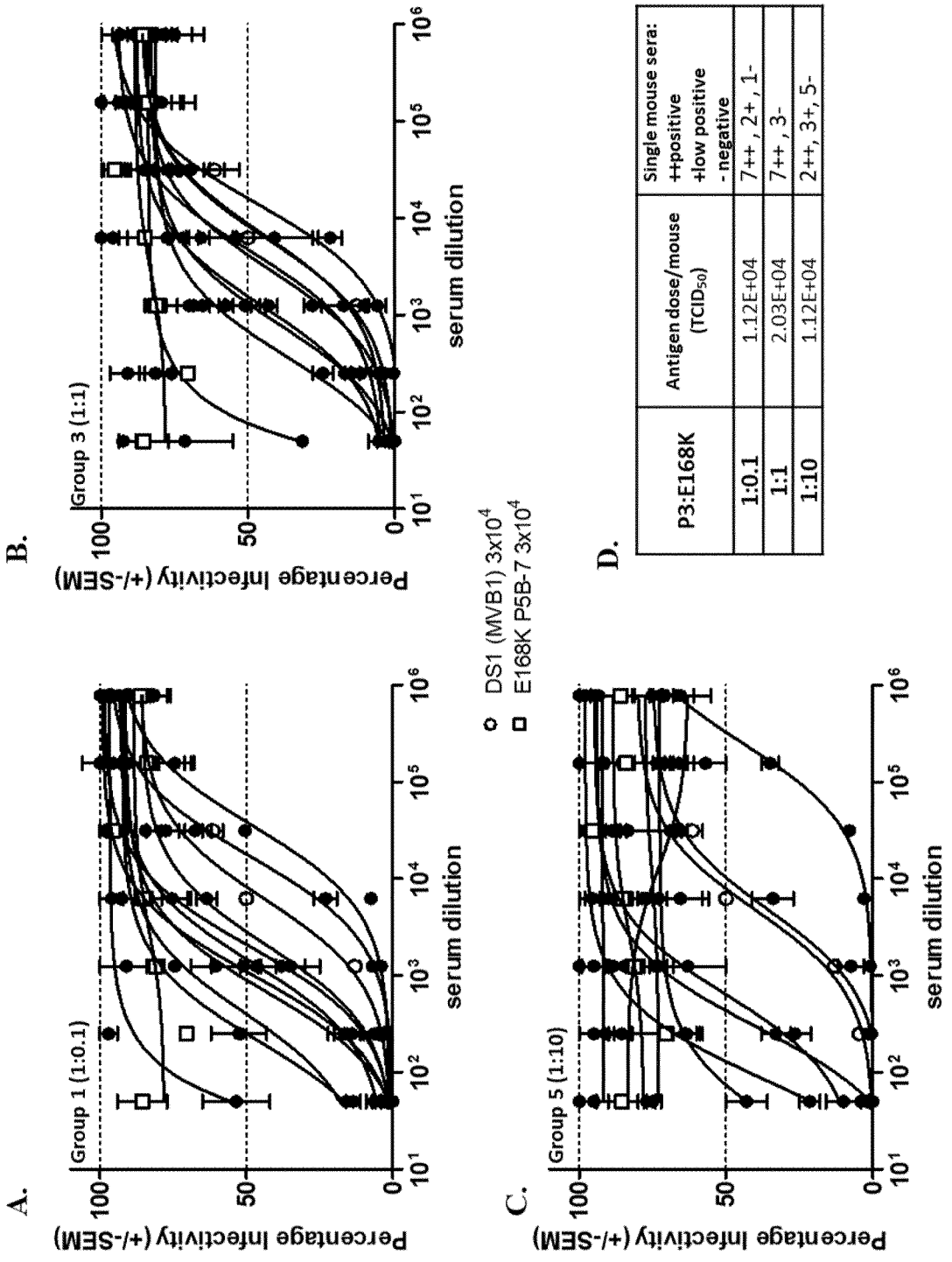
	Groups for subcutaneous immunization of C57Bl/6 mice				
	Group 1	**Group 3**	**Group 5**	**Group 6**	**Group 7**
Formulation ratio of P3 and P5B-07 (E168K)	**P3:E168K 1:0.1**	**P3:E168K 1:1**	**P3:E168K 1:10**	**P3**	**P5B-7**
Reference sequence	G	G	G	G	G
Heterogeneity	G>A	G=A	G<A	G>A	A
Chromatogram					

Figure 11



D.

P3:E168K	Antigen dose/mouse ($TCID_{50}$)	Single mouse sera: ++positive +low positive - negative
1:0.1	1.12E+04	7++ , 2+ , 1-
1:1	2.03E+04	7++ , 3-
1:10	1.12E+04	2++, 3+, 5-

Figure 12

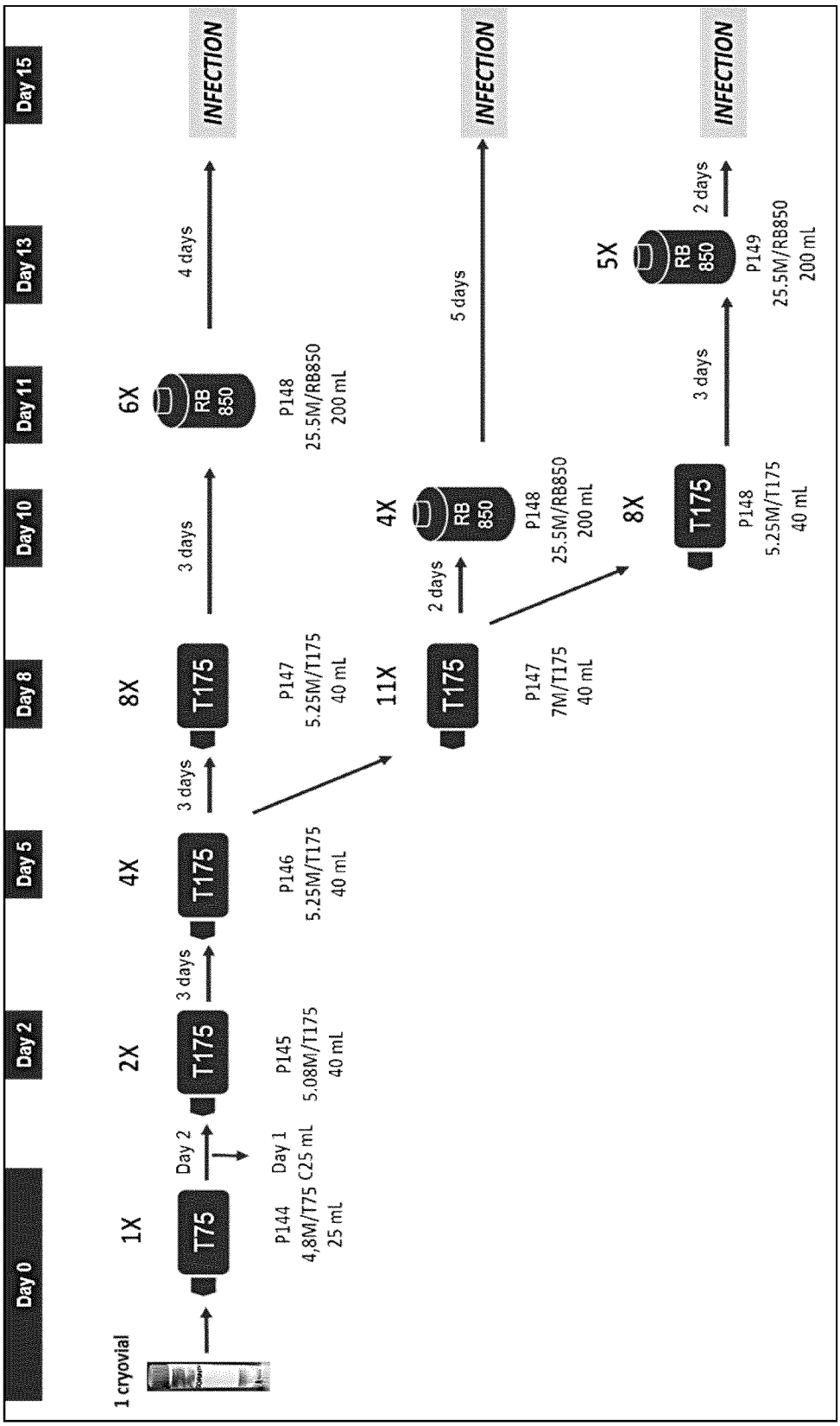
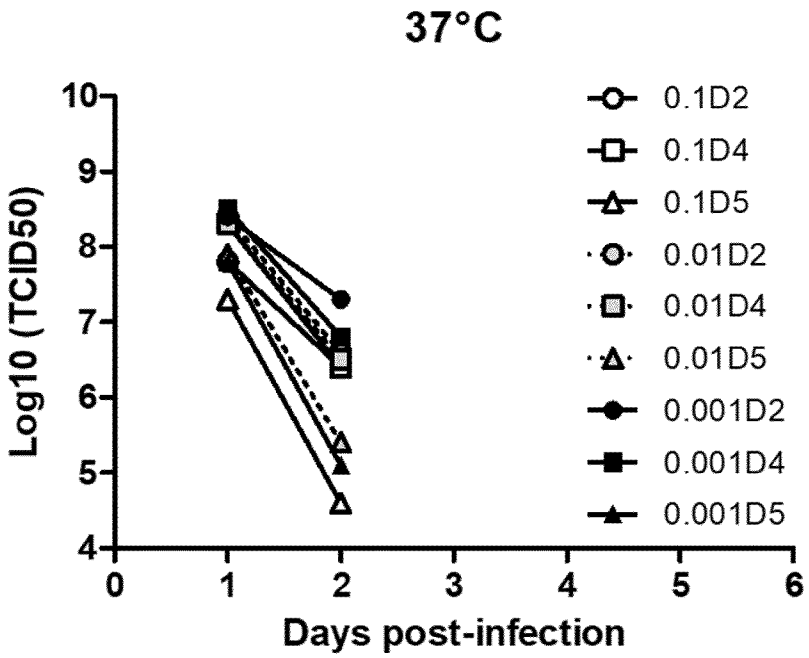

Day 0
Day 2
Day 5
Day 8
Day 10
Day 11
Day 13
Day 15
1 cryovial
1X
2X
4X
8X
11X
4X
8X
5X
6X
T75
T175
T175
T175
T175
T175
RB 850
RB 850
RB 850
Day 1
Day 2
3 days
3 days
3 days
2 days
5 days
3 days
2 days
4 days
INFECTION
INFECTION
INFECTION
P144 4,8M/T75 25 ml
P145 5.08M/T175 40 ml
P146 5.25M/T175 40 ml
P147 5.25M/T175 40 ml
P147 7M/T175 40 ml
P148 25.5M/RB850 200 ml
P148 25.5M/RB850 200 ml
P148 5.25M/T175 40 ml
P149 25.5M/RB850 200 ml

Figure 13

A.


37°C
Log10 (TCID50)
Days post-infection
0.1D2
0.1D4
0.1D5
0.01D2
0.01D4
0.01D5
0.001D2
0.001D4
0.001D5

B.

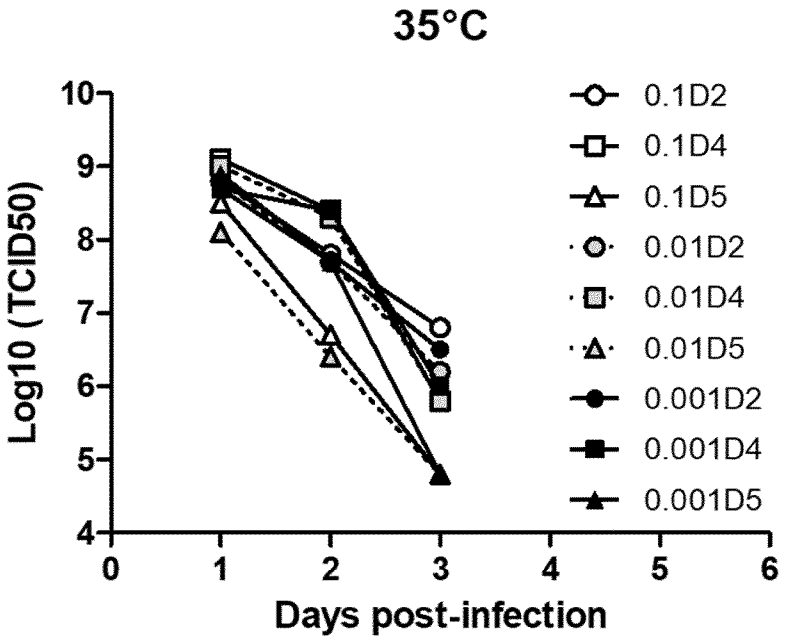
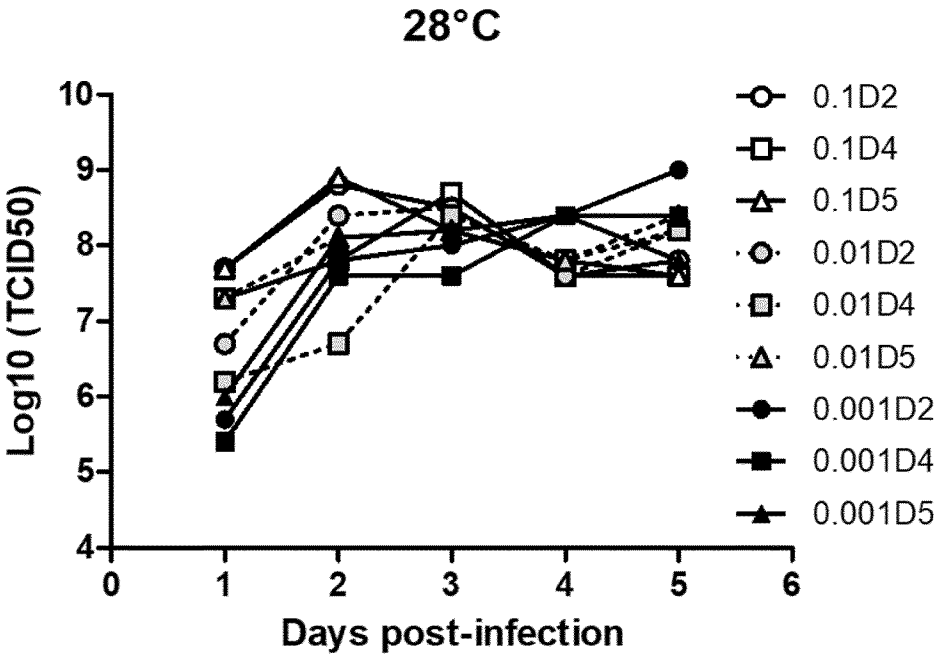

35°C
Log10 (TCID50)
Days post-infection
0.1D2
0.1D4
0.1D5
0.01D2
0.01D4
0.01D5
0.001D2
0.001D4
0.001D5

Figure 13 (continued)

C.



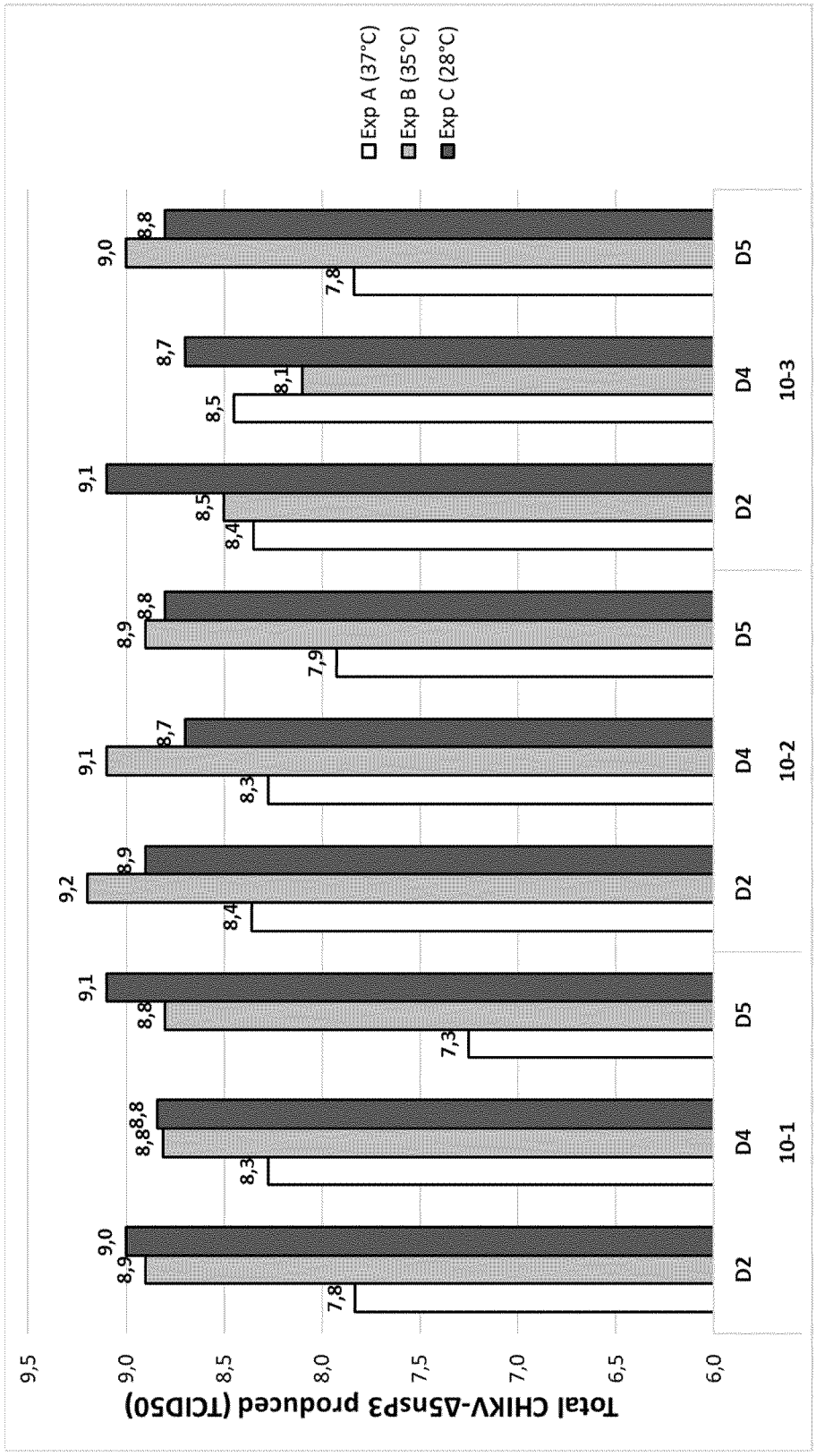

Exp A (37°C)
Exp B (35°C)
Exp C (28°C)
Total CHIKV-Δ5nsP3 produced (TCID50)
9,5
9,0
8,5
8,0
7,5
7,0
6,5
6,0
10-1
10-2
10-3
D2
D4
D5
9,0
8,9
7,8
8,8
8,8
8,3
9,1
8,8
7,3
9,2
8,9
8,4
9,1
8,7
8,3
8,9
8,8
7,9
9,1
8,5
8,4
8,7
8,1
8,5
9,0
8,8
7,8

Figure 14

Figure 15

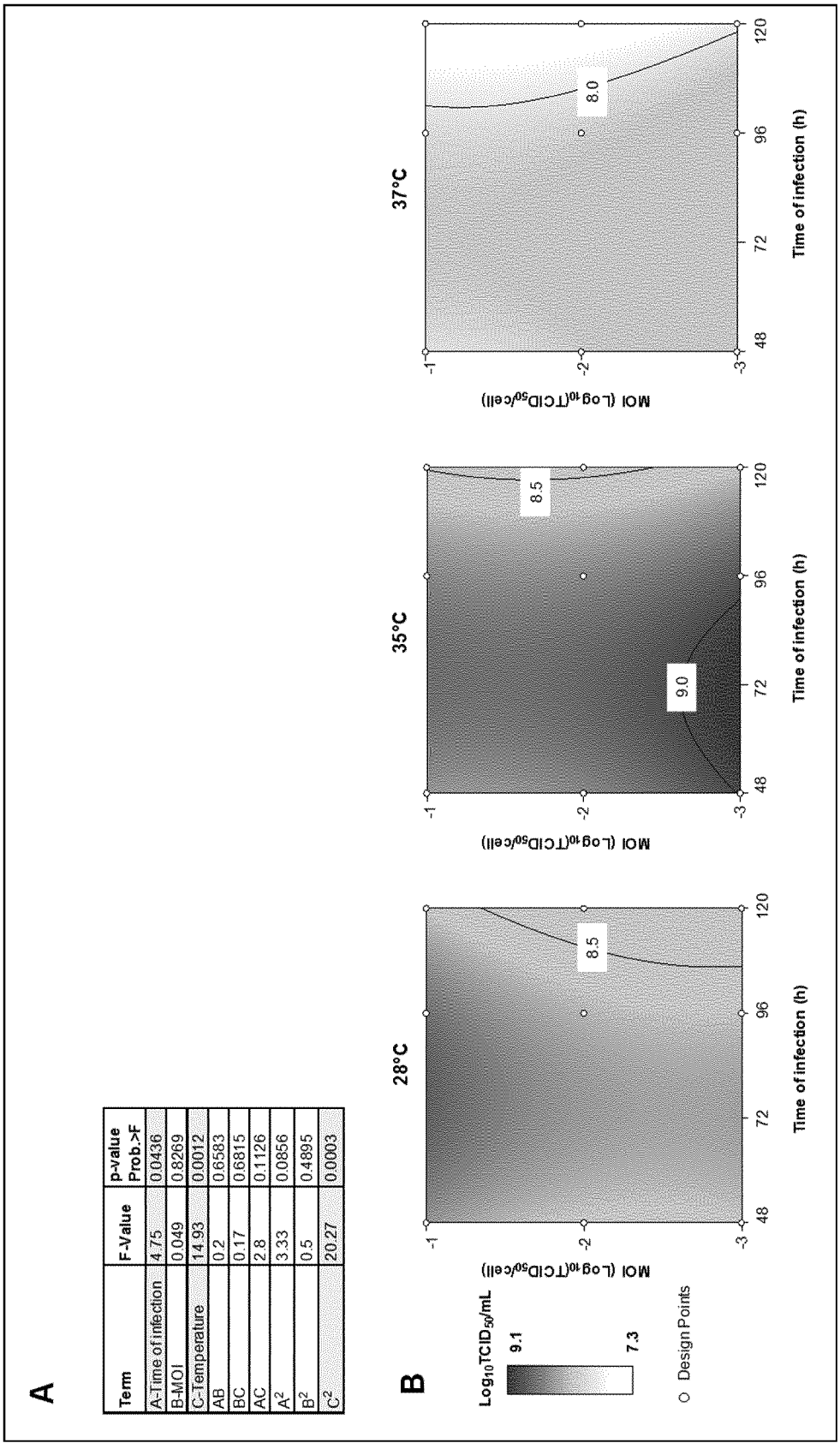
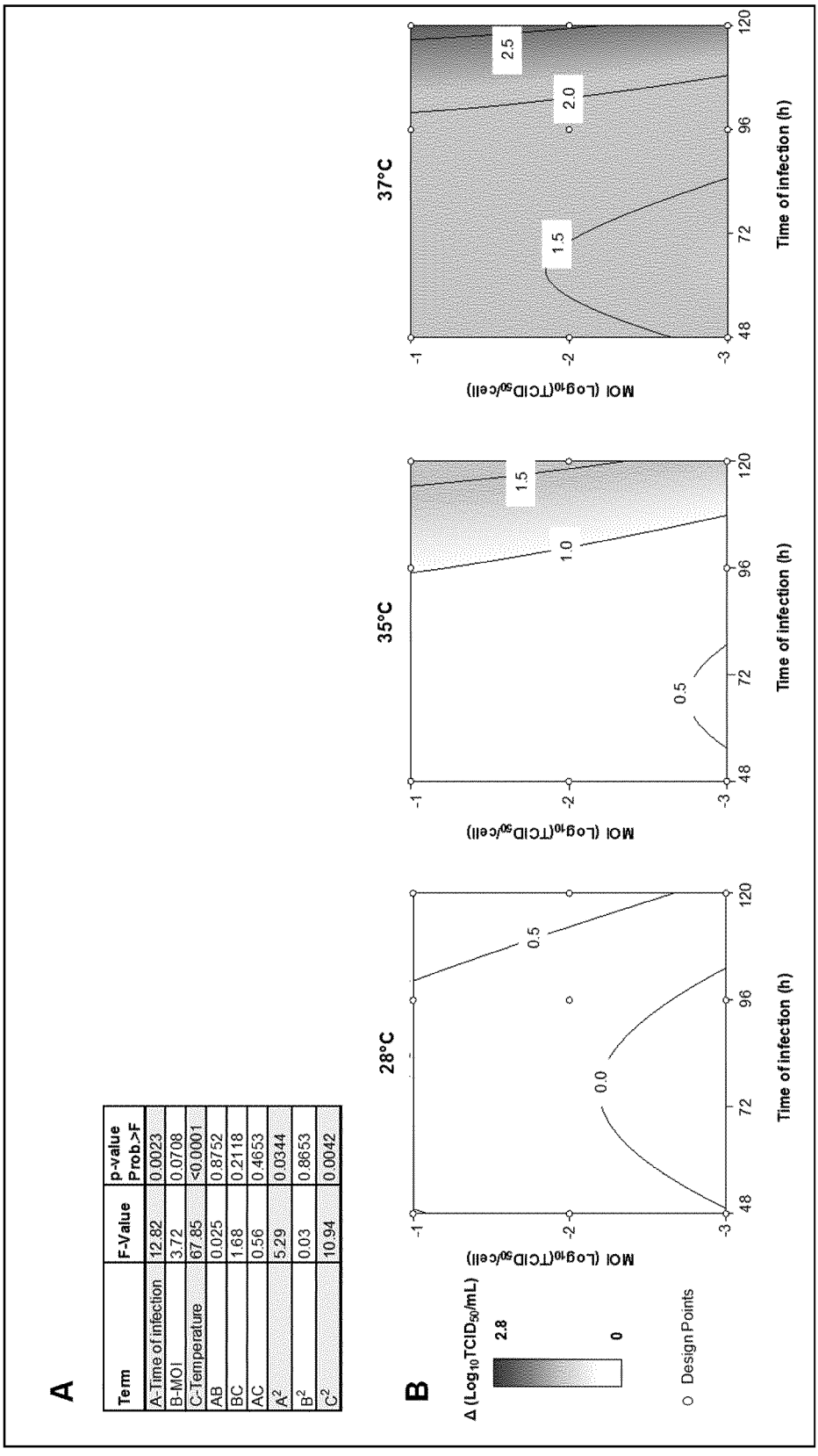

A
Term
F-Value
p-value Prob.>F
A-Time of infection
4.75
0.0436
B-MOI
0.049
0.8269
C-Temperature
14.93
0.0012
AB
0.2
0.6583
BC
0.17
0.6815
AC
2.8
0.1126
A²
3.33
0.0856
B²
0.5
0.4895
C²
20.27
0.0003
B
28°C
35°C
37°C
8.5
9.0
8.0
MOI (Log10(TCID50/cell))
Time of infection (h)
-1
-2
-3
48
72
96
120
Log10TCID50/mL
9.1
7.3
Design Points

Figure 16



Term	F-Value	p-value Prob.>F
A-Time of infection	12.82	0.0023
B-MOI	3.72	0.0708
C-Temperature	67.85	<0.0001
AB	0.025	0.8752
BC	1.68	0.2118
AC	0.56	0.4653
A^2	5.29	0.0344
B^2	0.03	0.8653
C^2	10.94	0.0042

Figure 17

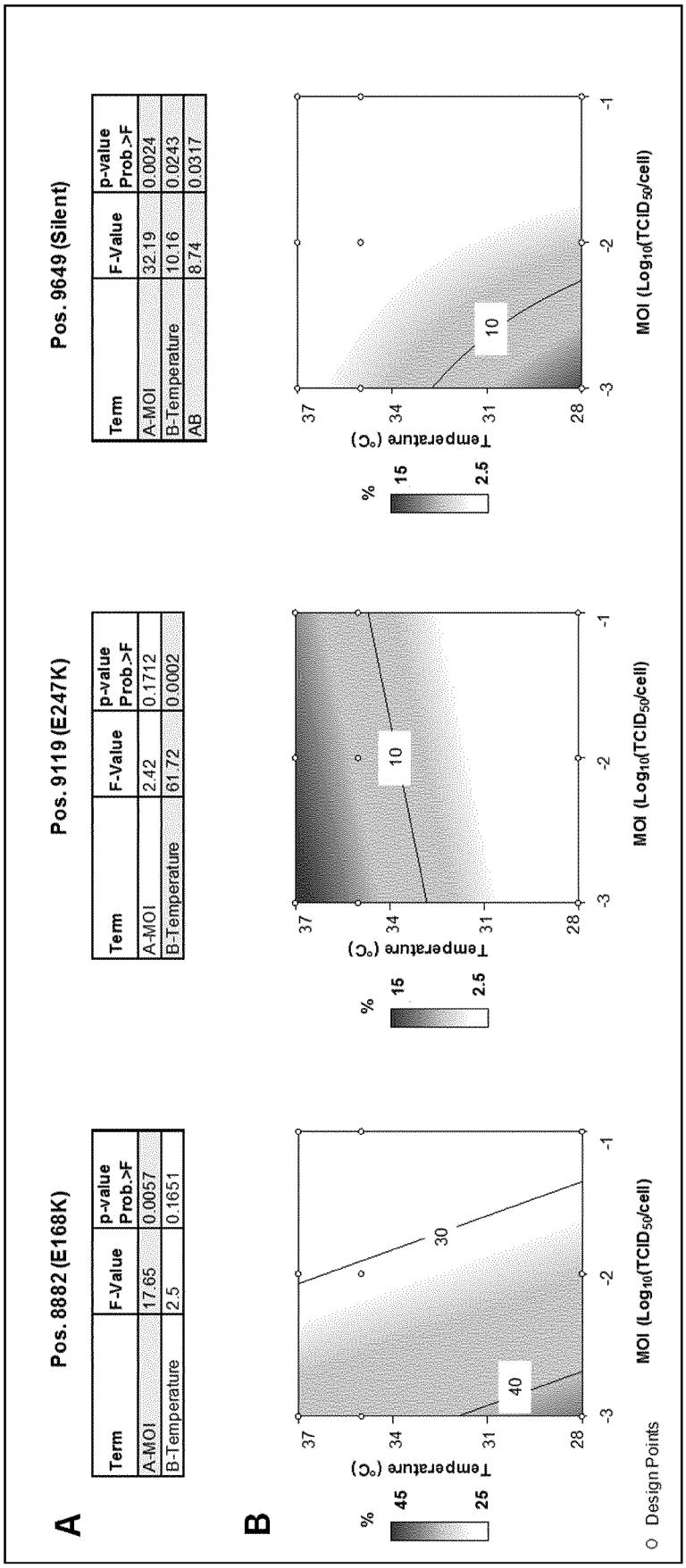
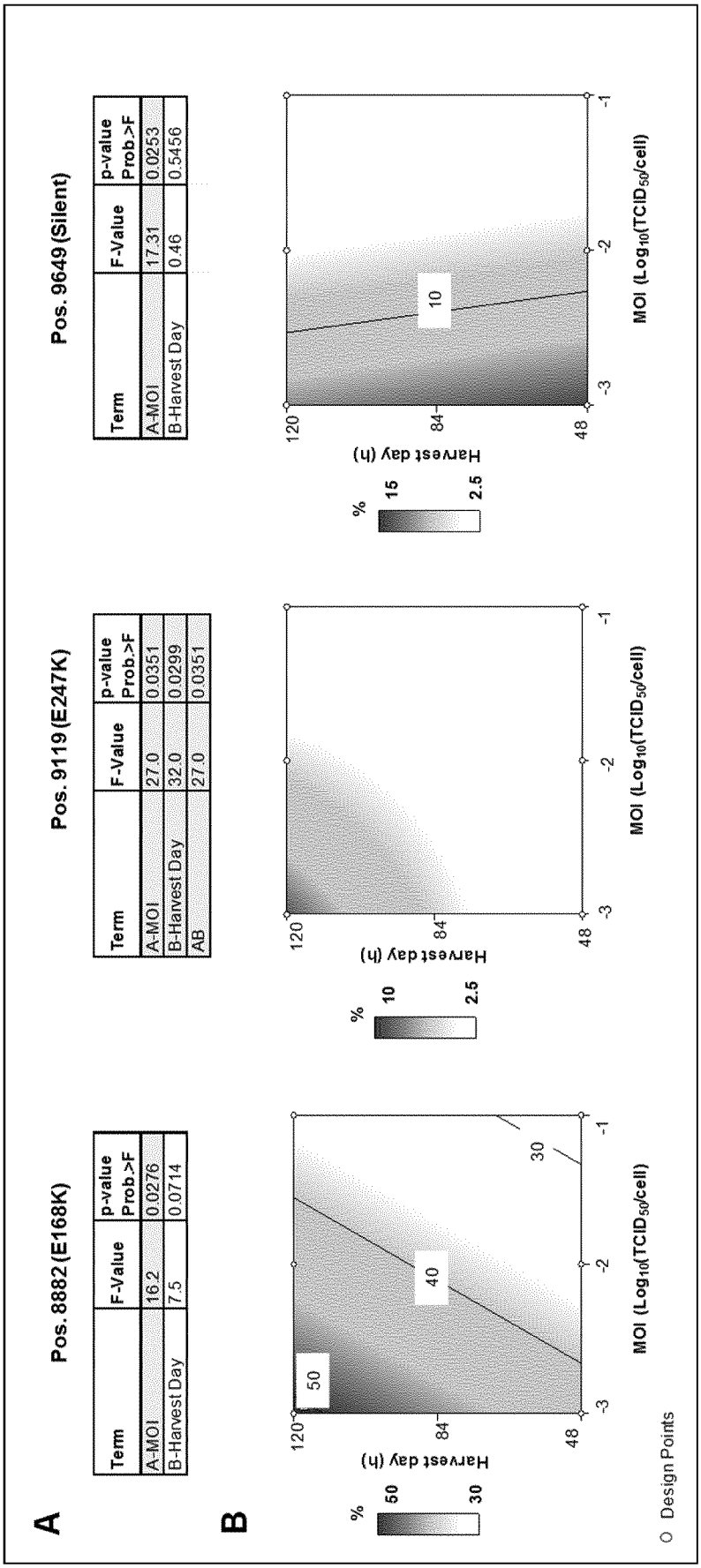

A
Pos. 8882 (E168K)
Term | F-Value | p-value Prob.>F
A-MOI | 17.65 | 0.0057
B-Temperature | 2.5 | 0.1651
Pos. 9119 (E247K)
Term | F-Value | p-value Prob.>F
A-MOI | 2.42 | 0.1712
B-Temperature | 61.72 | 0.0002
Pos. 9649 (Silent)
Term | F-Value | p-value Prob.>F
A-MOI | 32.19 | 0.0024
B-Temperature | 10.16 | 0.0243
AB | 8.74 | 0.0317
B
Temperature (°C)
MOI (Log10(TCID50/cell))
%
45
25
15
2.5
40
30
10
Design Points

Figure 18


A
Pos. 8882 (E168K)
Term | F-Value | p-value Prob.>F
A-MOI | 16.2 | 0.0276
B-Harvest Day | 7.5 | 0.0714
Pos. 9119 (E247K)
Term | F-Value | p-value Prob.>F
A-MOI | 27.0 | 0.0351
B-Harvest Day | 32.0 | 0.0299
AB | 27.0 | 0.0351
Pos. 9649 (Silent)
Term | F-Value | p-value Prob.>F
A-MOI | 17.31 | 0.0253
B-Harvest Day | 0.46 | 0.5456
B
Harvest day (h)
MOI (Log10(TCID50/cell))
%
50
30
40
10
2.5
15
120
84
48
-3
-2
-1
Design Points