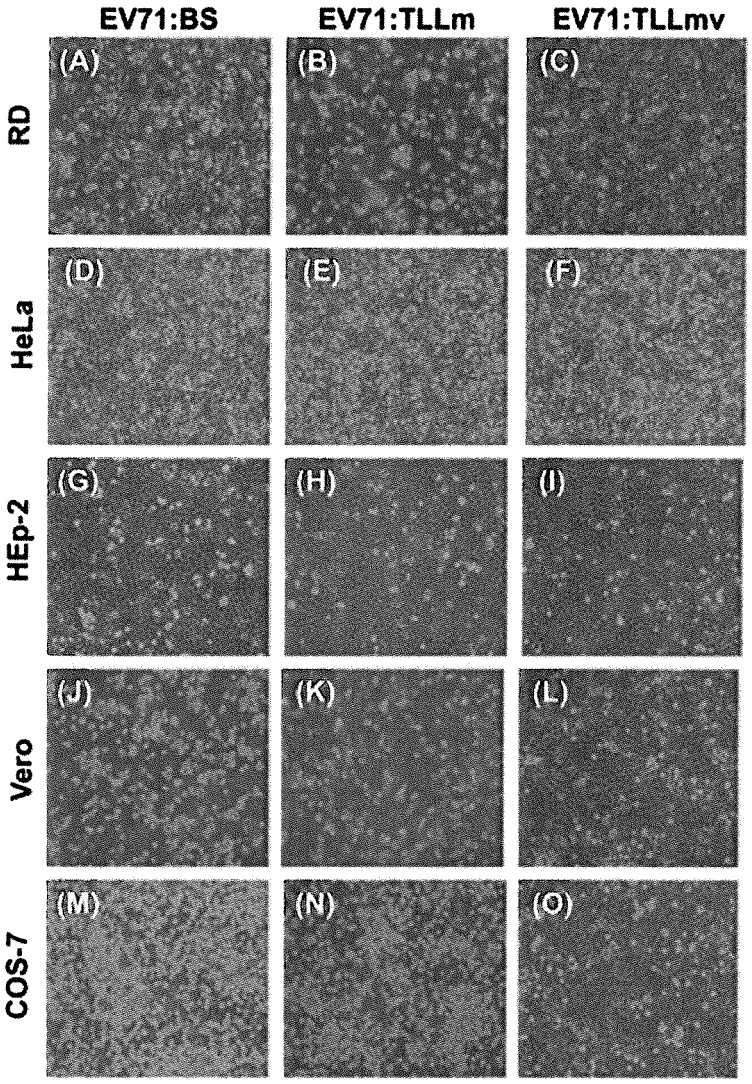

EV71:BS
EV71:TLLm
EV71:TLLmv
RD
HeLa
HEp-2
Vero
COS-7
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
(N)
(O)

Figure 1

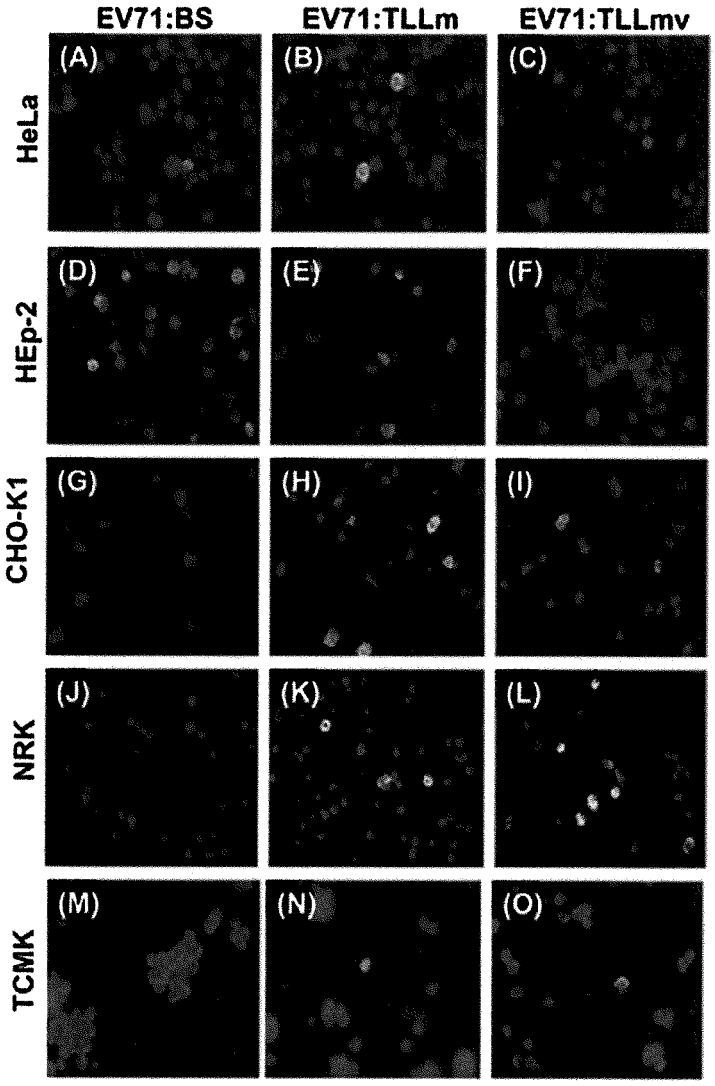

EV71:BS
EV71:TLLm
EV71:TLLmv
HeLa
HEp-2
CHO-K1
NRK
TCMK
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
(N)
(O)

Figure 2

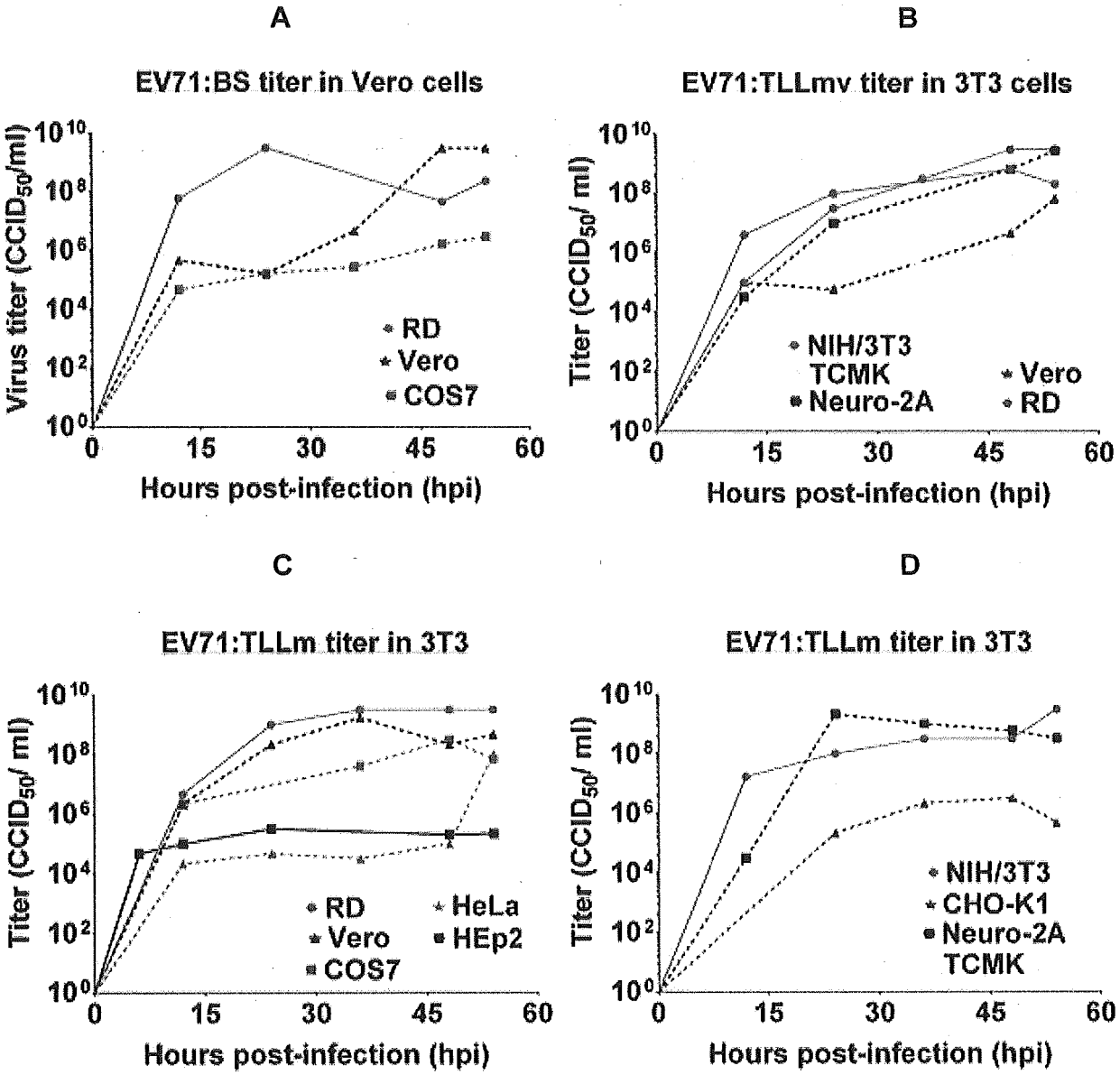

A
EV71:BS titer in Vero cells
Virus titer (CCID50/ml)
Hours post-infection (hpi)
RD
Vero
COS7
B
EV71:TLLmv titer in 3T3 cells
Titer (CCID50/ ml)
Hours post-infection (hpi)
NIH/3T3
TCMK
Neuro-2A
Vero
RD
C
EV71:TLLm titer in 3T3
Titer (CCID50/ ml)
Hours post-infection (hpi)
RD
Vero
COS7
HeLa
HEp2
D
EV71:TLLm titer in 3T3
Titer (CCID50/ ml)
Hours post-infection (hpi)
NIH/3T3
CHO-K1
Neuro-2A
TCMK

Figure 3

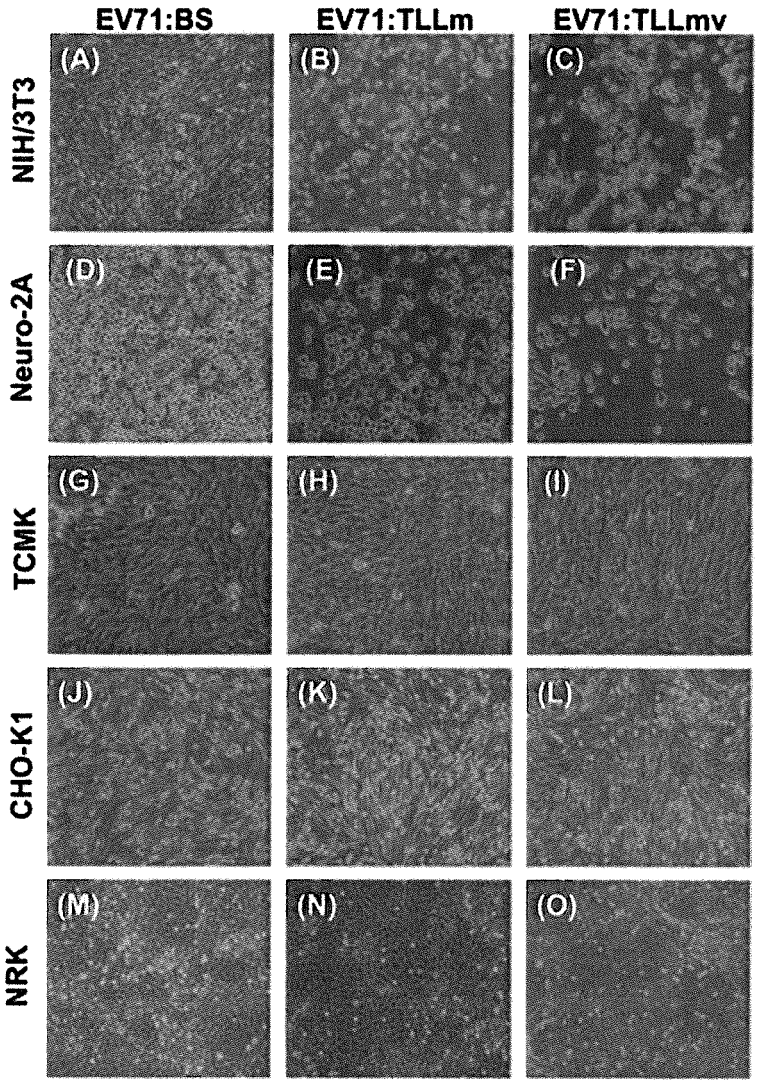

EV71:BS
EV71:TLLm
EV71:TLLmv
NIH/3T3
Neuro-2A
TCMK
CHO-K1
NRK
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
(N)
(O)

Figure 4

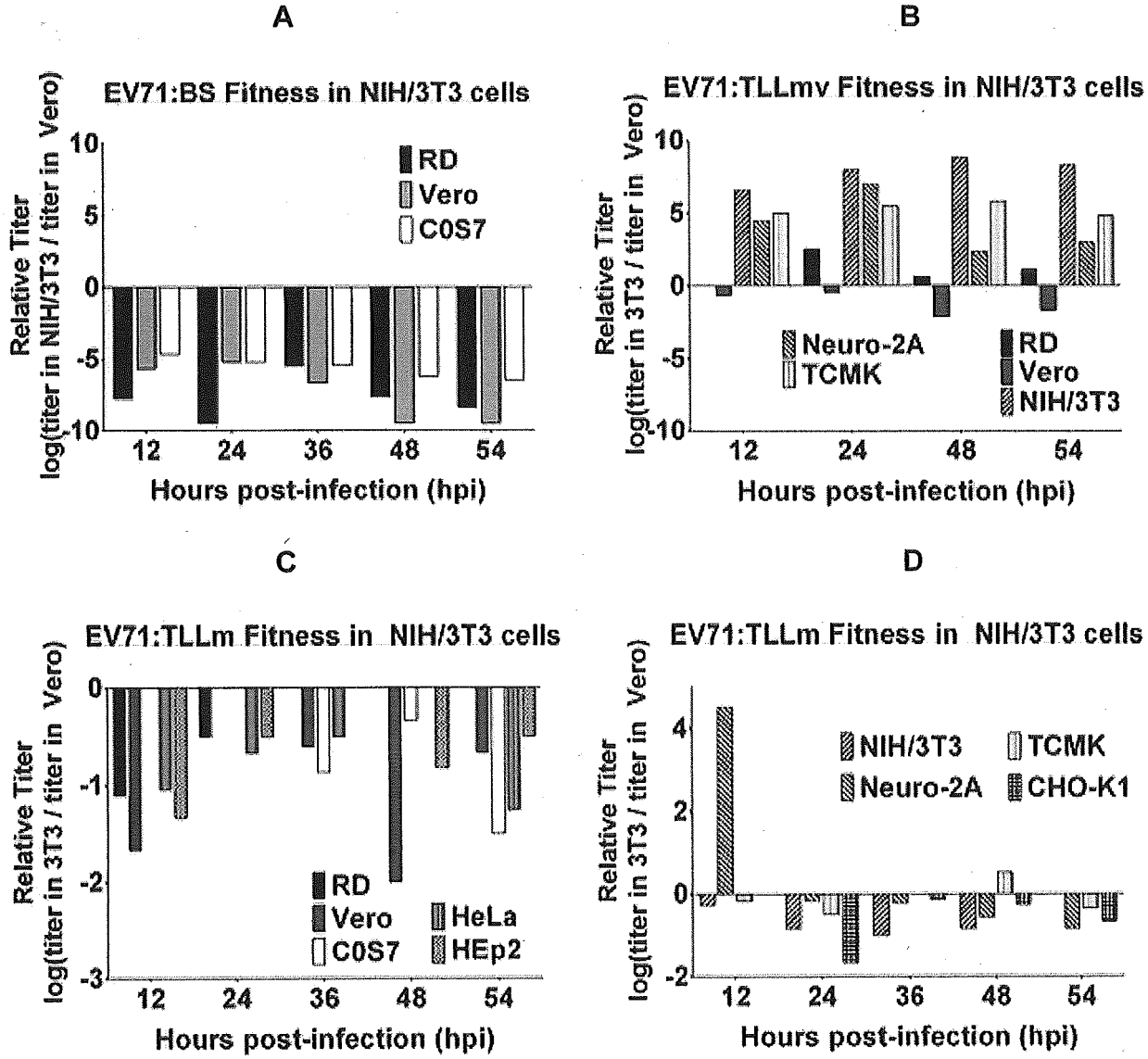

A
EV71:BS Fitness in NIH/3T3 cells
Relative Titer
log(titer in NIH/3T3 / titer in Vero)
10
5
0
-5
-10
RD
Vero
C0S7
12
24
36
48
54
Hours post-infection (hpi)
B
EV71:TLLmv Fitness in NIH/3T3 cells
Relative Titer
log(titer in 3T3 / titer in Vero)
10
5
0
-5
-10
Neuro-2A
TCMK
RD
Vero
NIH/3T3
12
24
48
54
Hours post-infection (hpi)
C
EV71:TLLm Fitness in NIH/3T3 cells
Relative Titer
log(titer in 3T3 / titer in Vero)
0
-1
-2
-3
RD
Vero
C0S7
HeLa
HEp2
12
24
36
48
54
Hours post-infection (hpi)
D
EV71:TLLm Fitness in NIH/3T3 cells
Relative Titer
log(titer in 3T3 / titer in Vero)
4
2
0
-2
NIH/3T3
Neuro-2A
TCMK
CHO-K1
12
24
36
48
54
Hours post-infection (hpi)

Figure 5

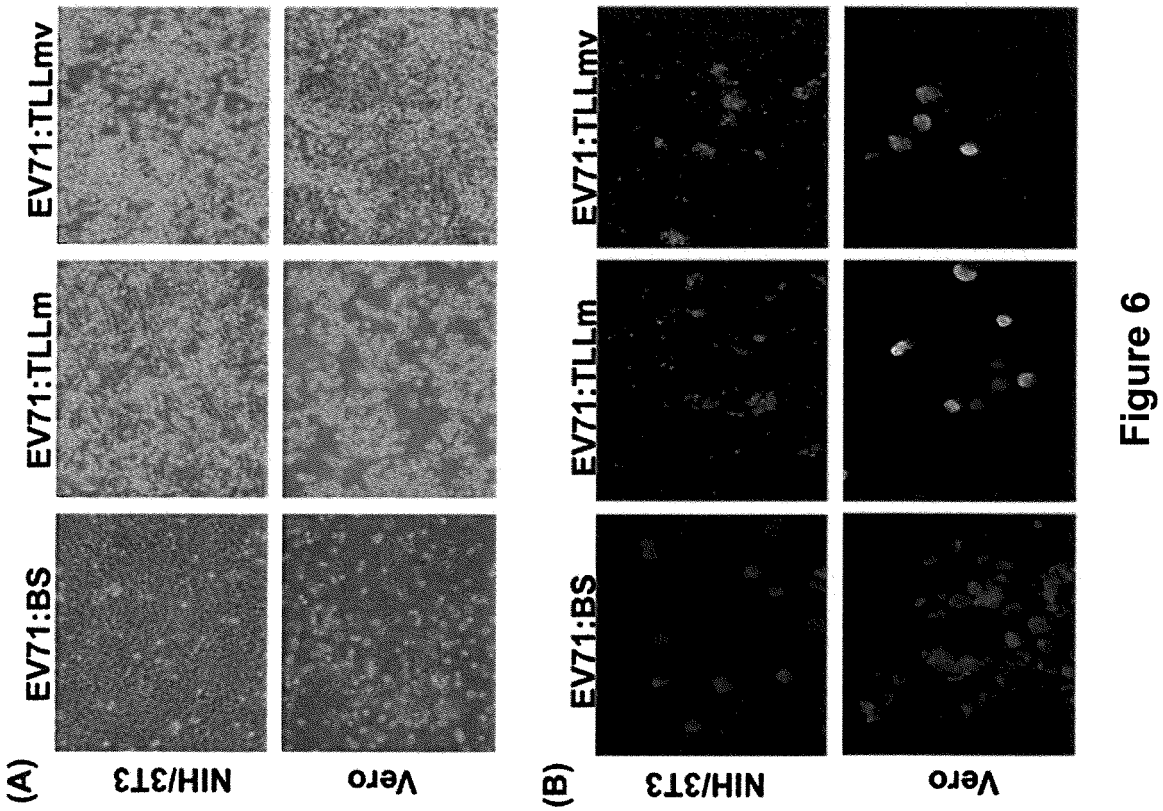

(A)
NIH/3T3
Vero
EV71:BS
EV71:TLLm
EV71:TLLmv
(B)
NIH/3T3
Vero
EV71:BS
EV71:TLLm
EV71:TLLmv

Figure 6

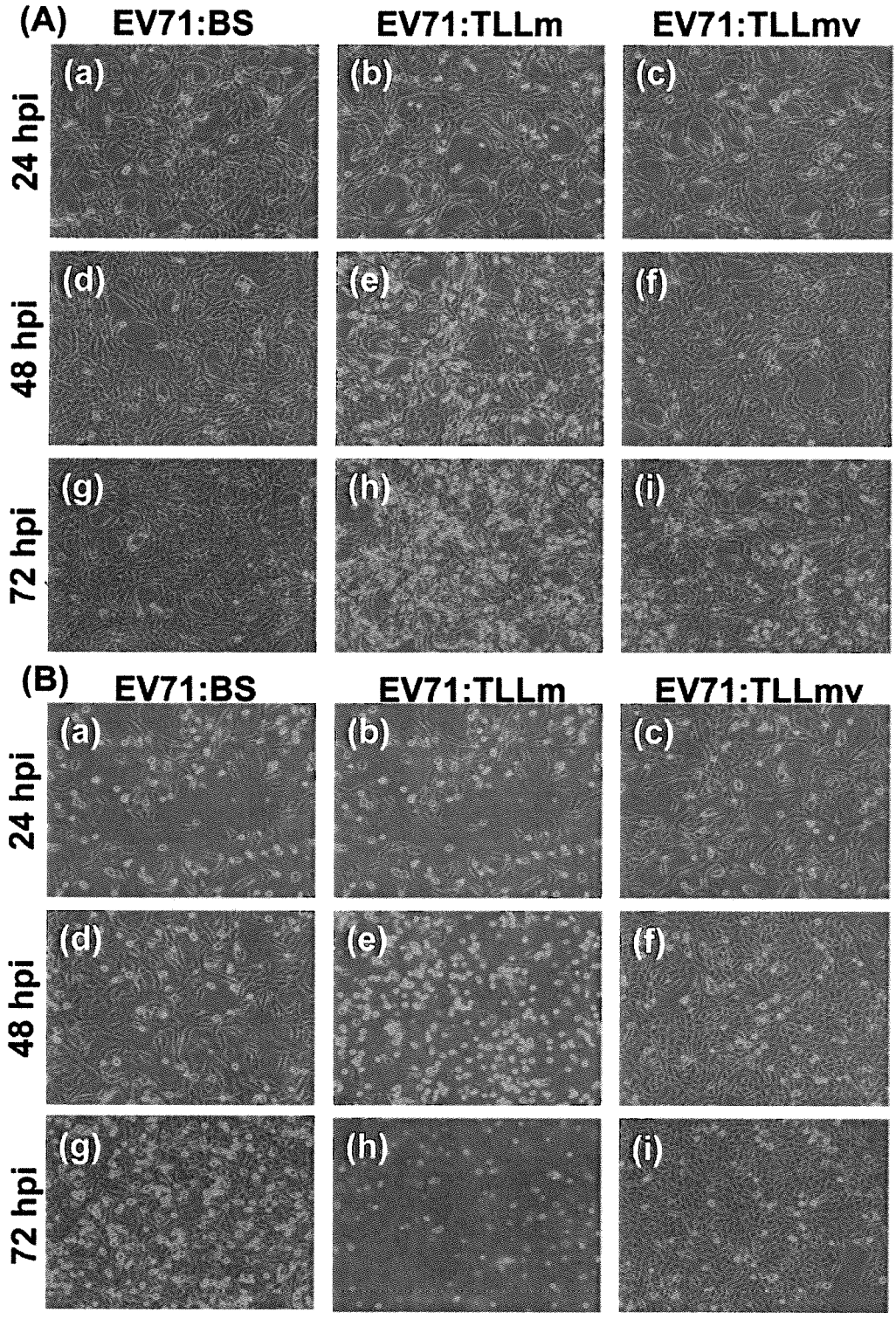

(A)
EV71:BS
EV71:TLLm
EV71:TLLmv
24 hpi
48 hpi
72 hpi
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(B)
EV71:BS
EV71:TLLm
EV71:TLLmv
24 hpi
48 hpi
72 hpi
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)

Figure 7

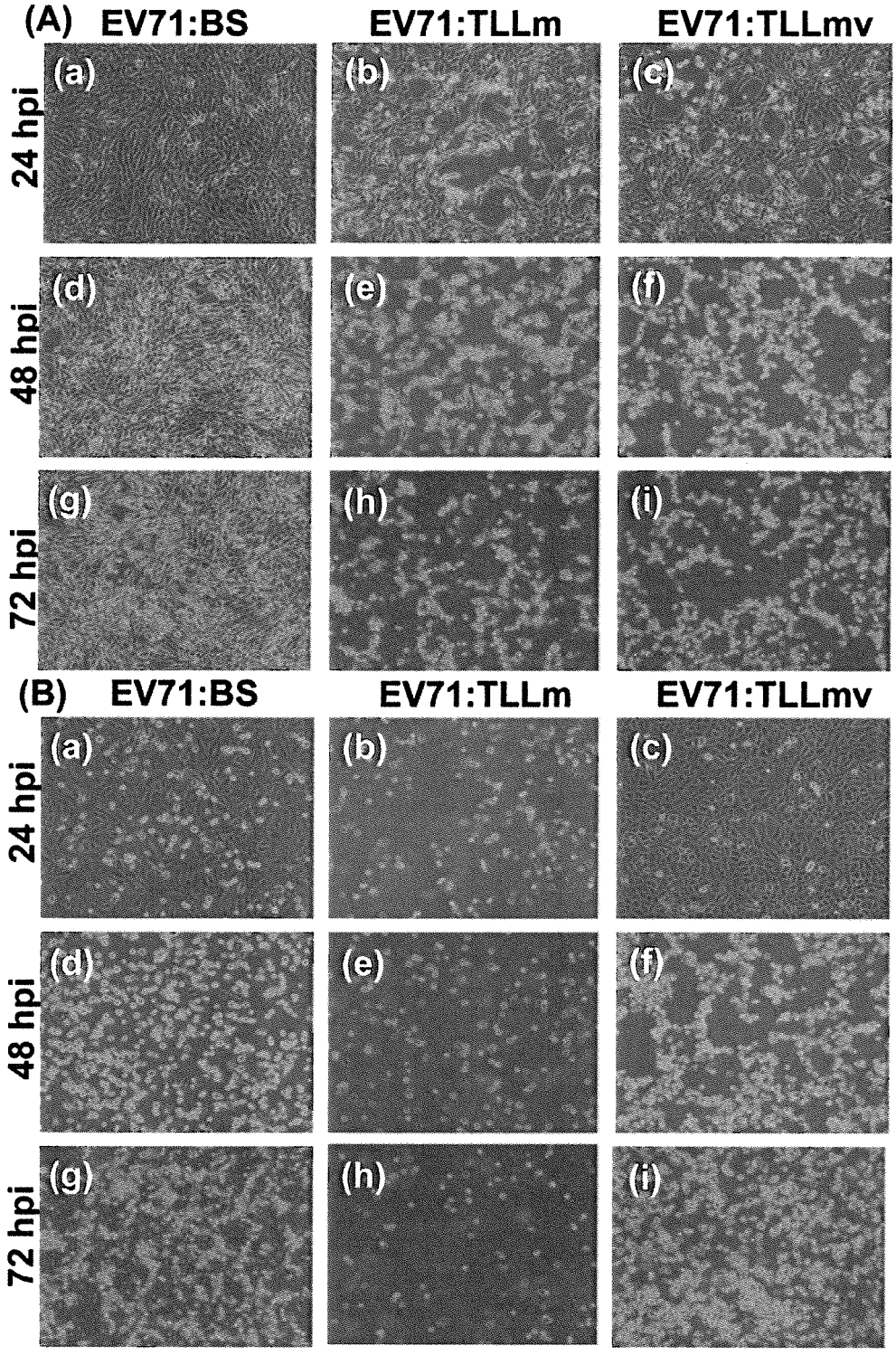

(A)
EV71:BS
EV71:TLLm
EV71:TLLmv
24 hpi
48 hpi
72 hpi
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(B)
EV71:BS
EV71:TLLm
EV71:TLLmv
24 hpi
48 hpi
72 hpi
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)

Figure 8

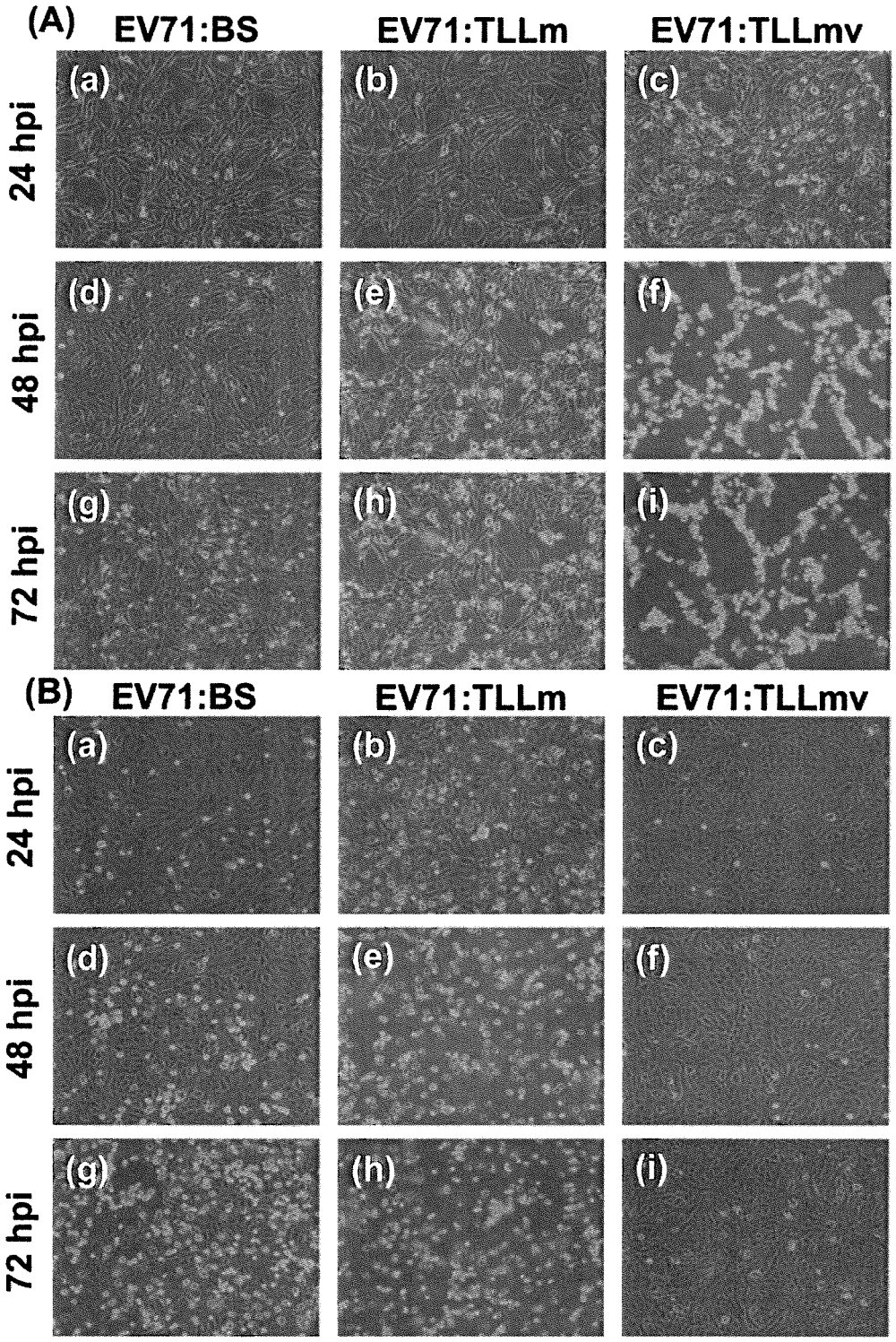

(A)
EV71:BS
EV71:TLLm
EV71:TLLmv
24 hpi
48 hpi
72 hpi
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(B)
EV71:BS
EV71:TLLm
EV71:TLLmv
24 hpi
48 hpi
72 hpi
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)

Figure 9

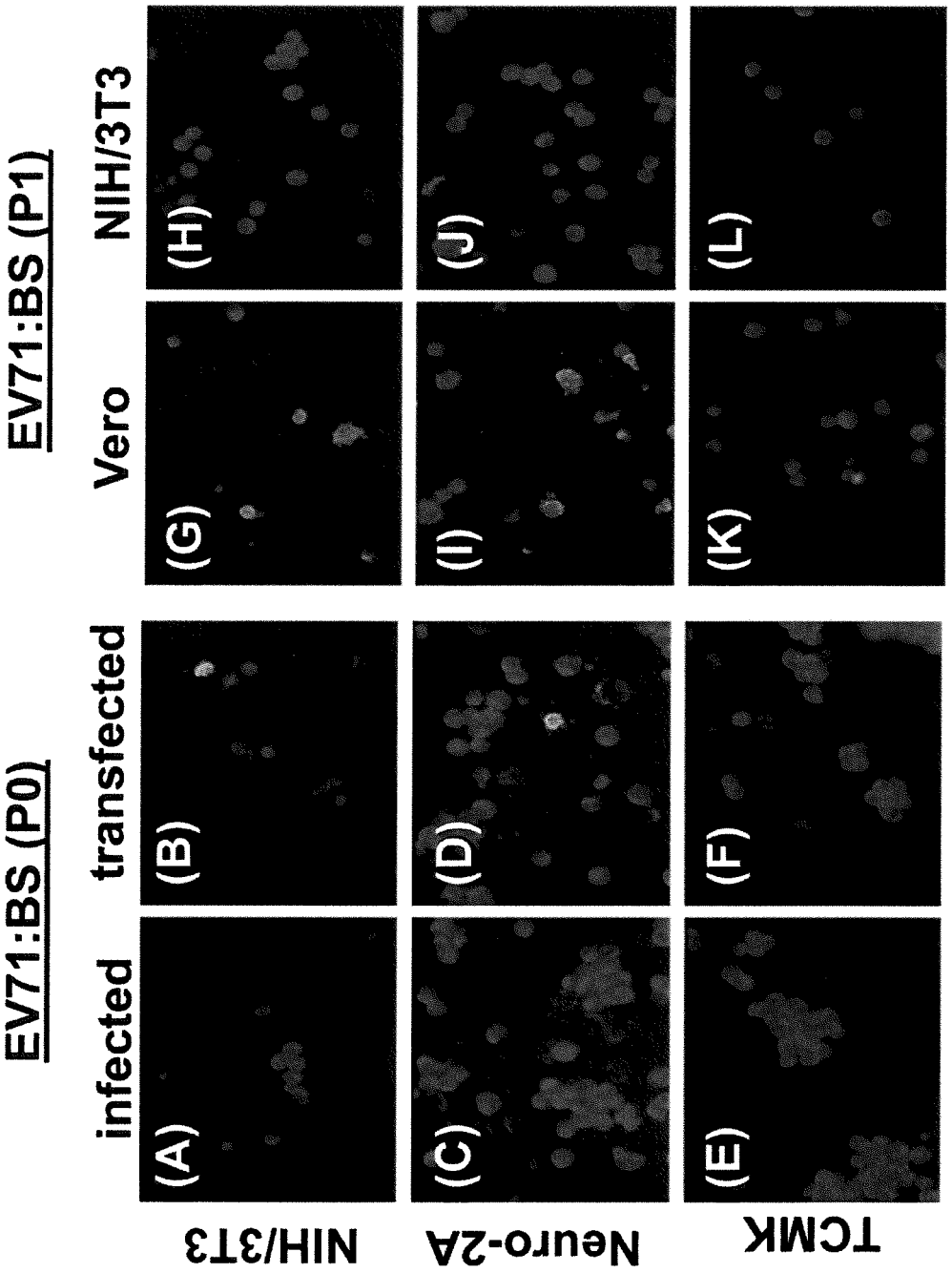

EV71:BS (P0)
infected
transfected
EV71:BS (P1)
Vero
NIH/3T3
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
NIH/3T3
Neuro-2A
TCMK

Figure 10

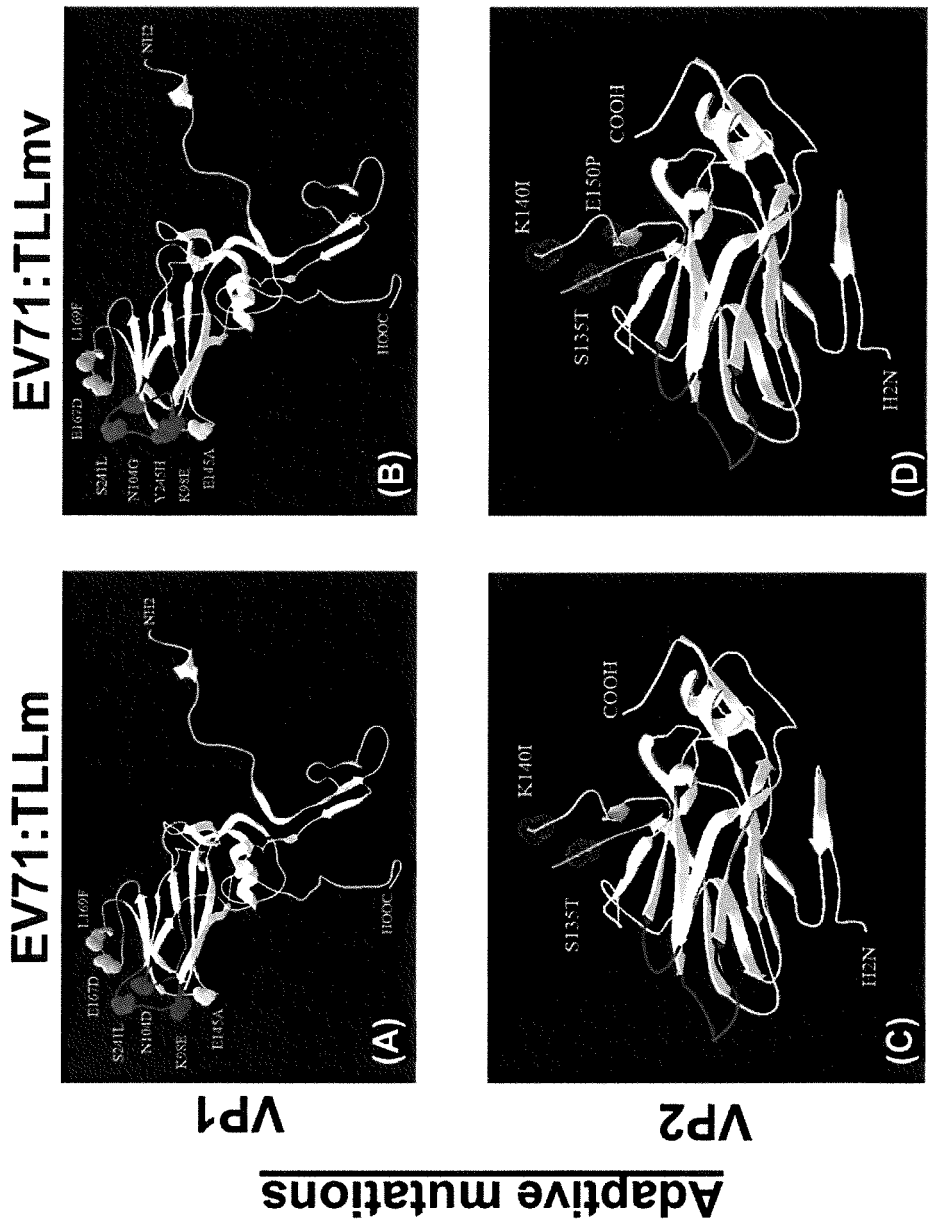

EV71:TLLm
EV71:TLLmv
VP1
VP2
Adaptive mutations
(A)
(B)
(C)
(D)
E167D
L169F
S241L
N104G
K98E
E145A
V245I
K140I
S135T
E150P
COOH
HEN
NH2
HOOC

Figure 11

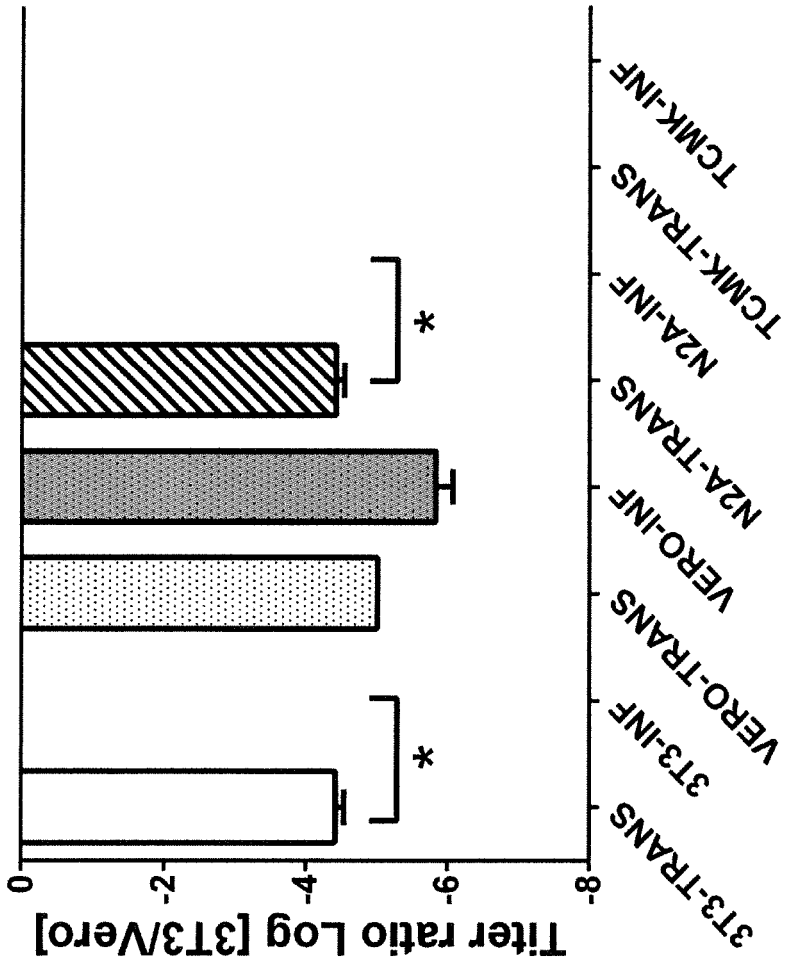

Titer ratio Log [3T3/Vero]
0
-2
-4
-6
-8
*
*
3T3-TRANS
3T3-INF
VERO-TRANS
VERO-INF
N2A-TRANS
N2A-INF
TCMK-TRANS
TCMK-INF

Figure 12

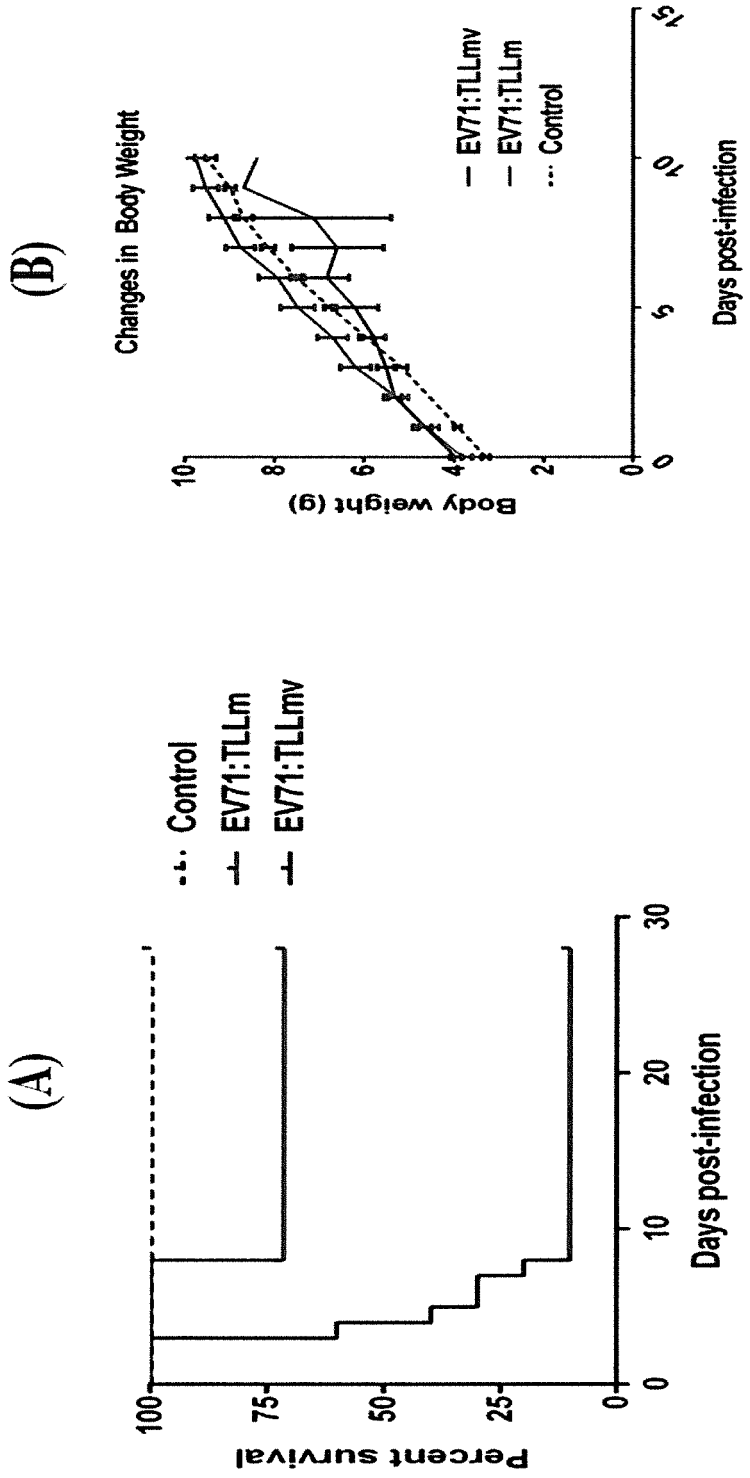
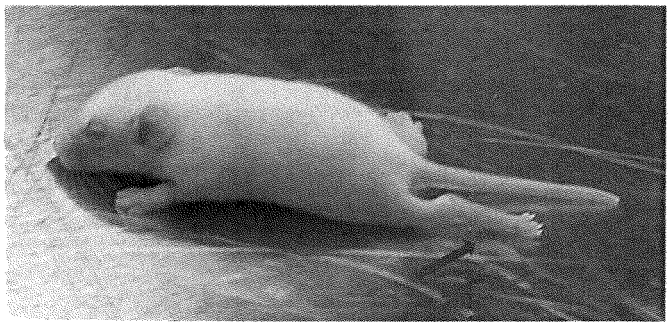
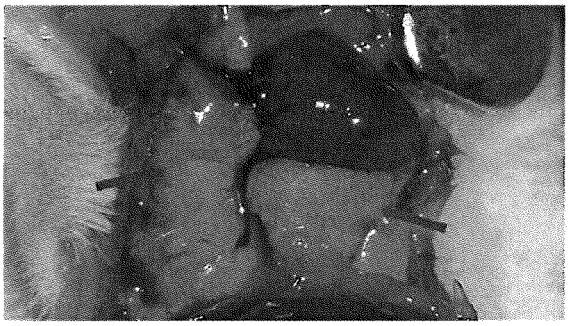

(A)
Control
EV71:TLLm
EV71:TLLmv
Percent survival
100
75
50
25
0
0
10
20
30
Days post-infection
(B)
Changes in Body Weight
EV71:TLLmv
EV71:TLLm
Control
Body weight (g)
10
8
6
4
2
0
0
5
10
15
Days post-infection

Figure 13

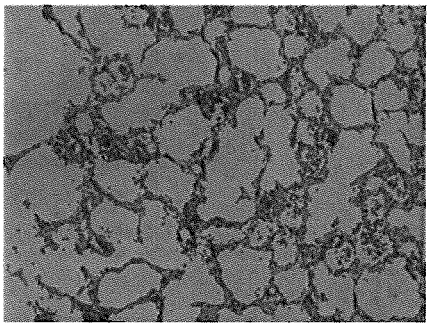
(A)



(B)



(C)



(D)

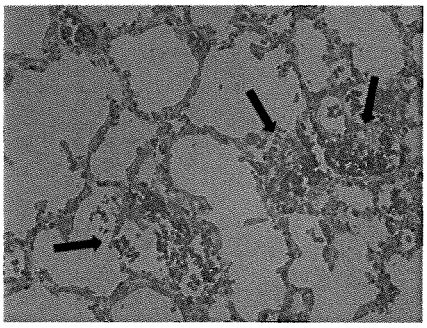


Figure 14

Figure 15

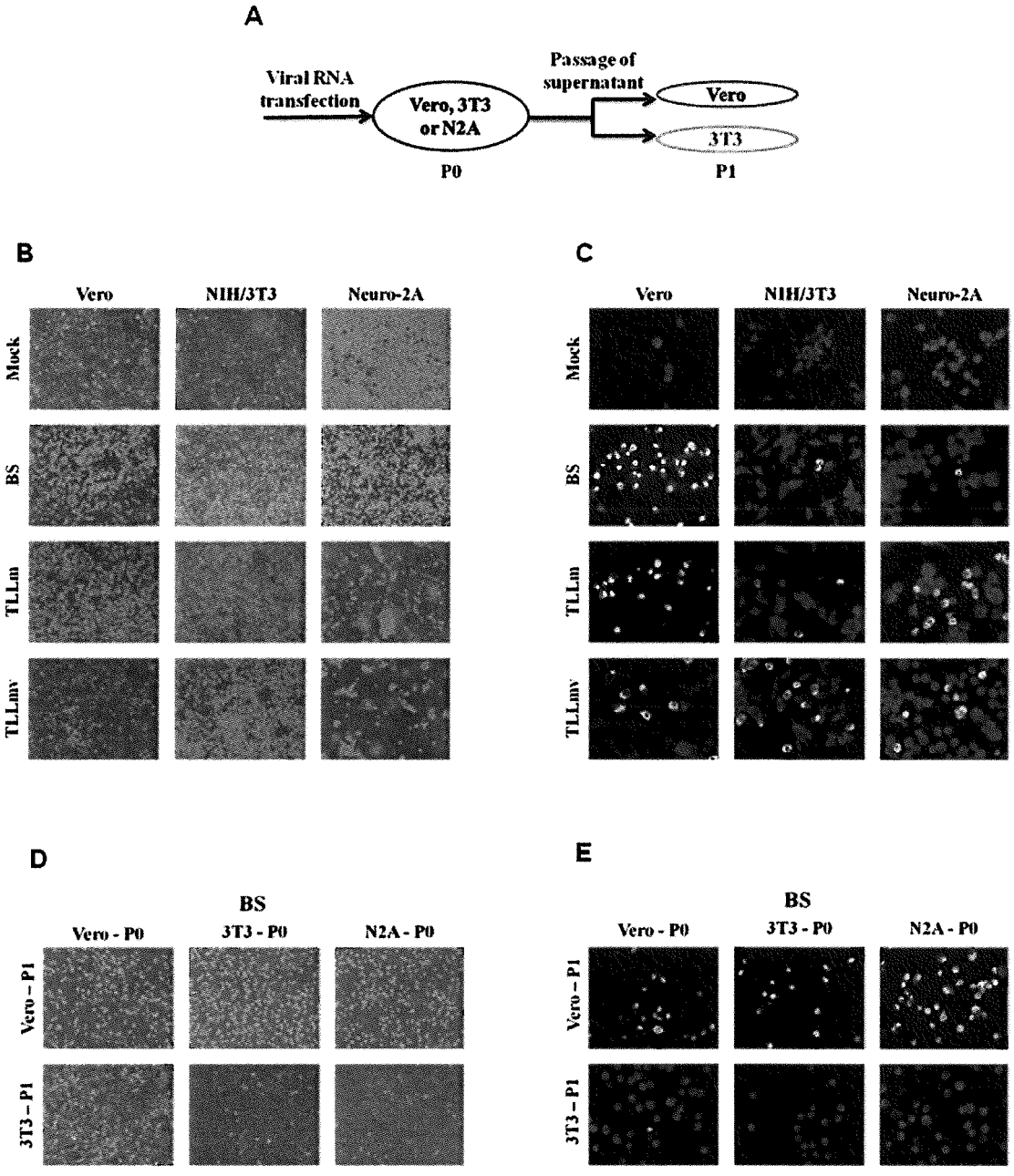

A
Viral RNA transfection
Vero, 3T3 or N2A
P0
Passage of supernatant
Vero
3T3
P1
B
Vero
NIH/3T3
Neuro-2A
Mock
BS
TLLm
TLLmv
C
Vero
NIH/3T3
Neuro-2A
Mock
BS
TLLm
TLLmv
D
BS
Vero - P0
3T3 - P0
N2A - P0
Vero – P1
3T3 – P1
E
BS
Vero - P0
3T3 - P0
N2A - P0
Vero – P1
3T3 – P1

Figure 16

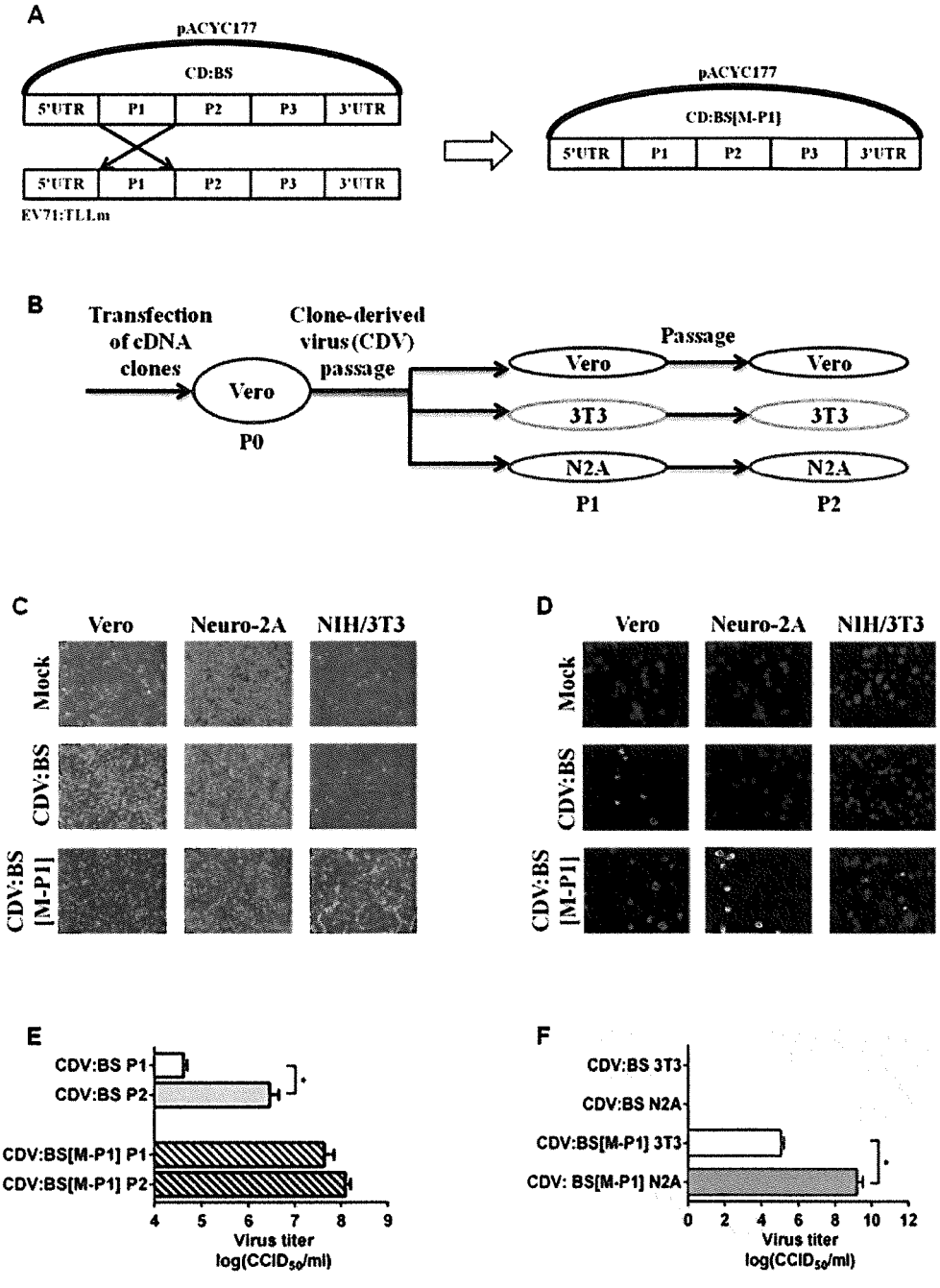

A
pACYC177
CD:BS
5'UTR
P1
P2
P3
3'UTR
EV71:TLLm
pACYC177
CD:BS[M-P1]
B
Transfection of cDNA clones
Vero
P0
Clone-derived virus (CDV) passage
Passage
Vero
3T3
N2A
P1
P2
C
Vero
Neuro-2A
NIH/3T3
Mock
CDV:BS
CDV:BS [M-P1]
D
E
CDV:BS P1
CDV:BS P2
CDV:BS[M-P1] P1
CDV:BS[M-P1] P2
Virus titer log(CCID50/ml)
F
CDV:BS 3T3
CDV:BS N2A
CDV:BS[M-P1] 3T3
CDV: BS[M-P1] N2A
Virus titer log(CCID50/ml)

Figure 17

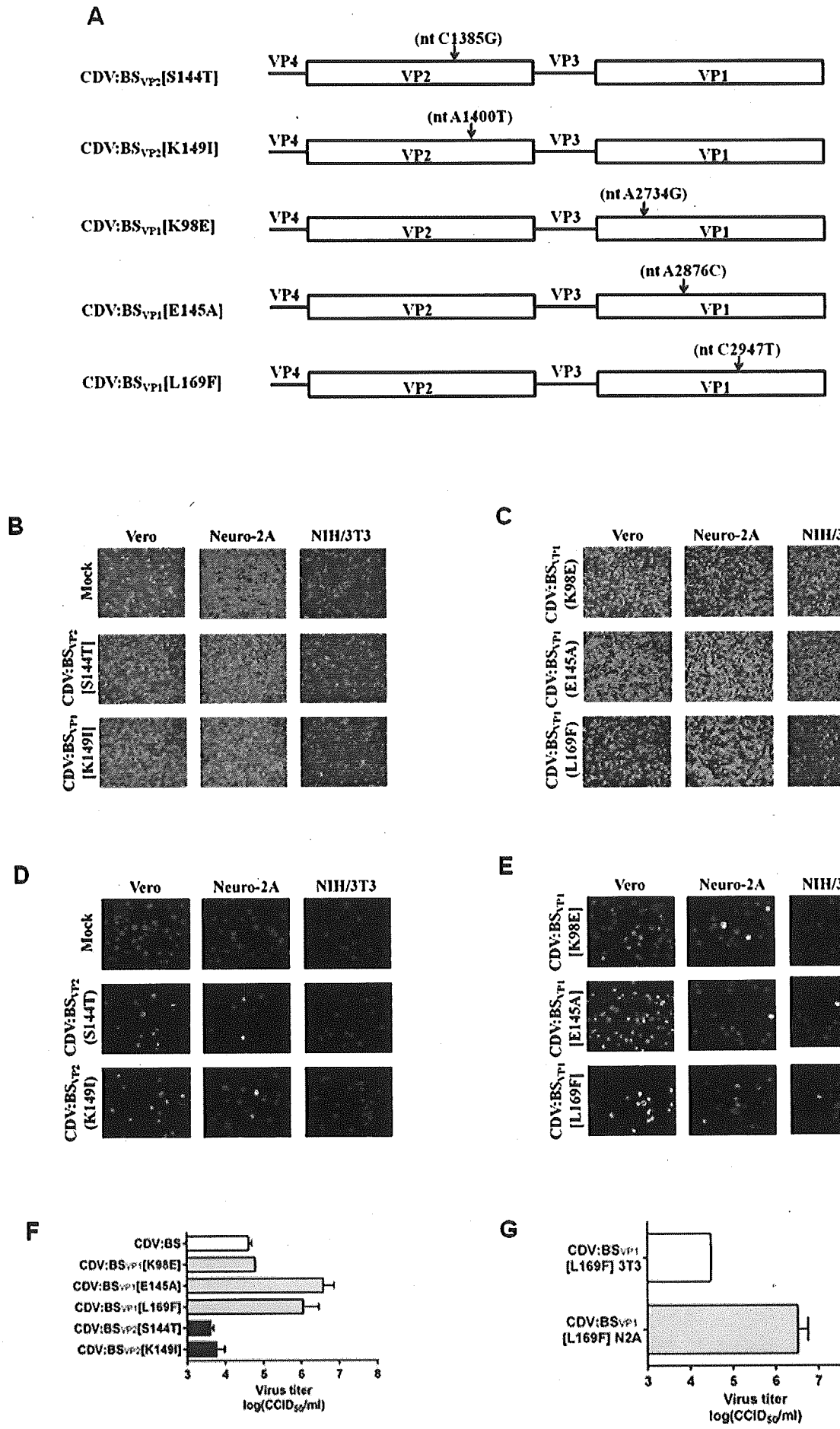

A
CDV:BS$_{VP2}$[S144T]
CDV:BS$_{VP2}$[K149I]
CDV:BS$_{VP1}$[K98E]
CDV:BS$_{VP1}$[E145A]
CDV:BS$_{VP1}$[L169F]
(nt C1385G)
(nt A1400T)
(nt A2734G)
(nt A2876C)
(nt C2947T)
VP4
VP2
VP3
VP1
B
C
D
E
Vero
Neuro-2A
NIH/3T3
Mock
CDV:BS$_{VP2}$ (S144T)
CDV:BS$_{VP2}$ (K149I)
CDV:BS$_{VP1}$ (K98E)
CDV:BS$_{VP1}$ (E145A)
CDV:BS$_{VP1}$ (L169F)
F
CDV:BS
CDV:BS$_{VP1}$[K98E]
CDV:BS$_{VP1}$[E145A]
CDV:BS$_{VP1}$[L169F]
CDV:BS$_{VP2}$[S144T]
CDV:BS$_{VP2}$[K149I]
3 4 5 6 7 8
Virus titer log(CCID$_{50}$/ml)
G
CDV:BS$_{VP1}$ [L169F] 3T3
CDV:BS$_{VP1}$ [L169F] N2A
3 4 5 6 7 8
Virus titer log(CCID$_{50}$/ml)

Figure 18

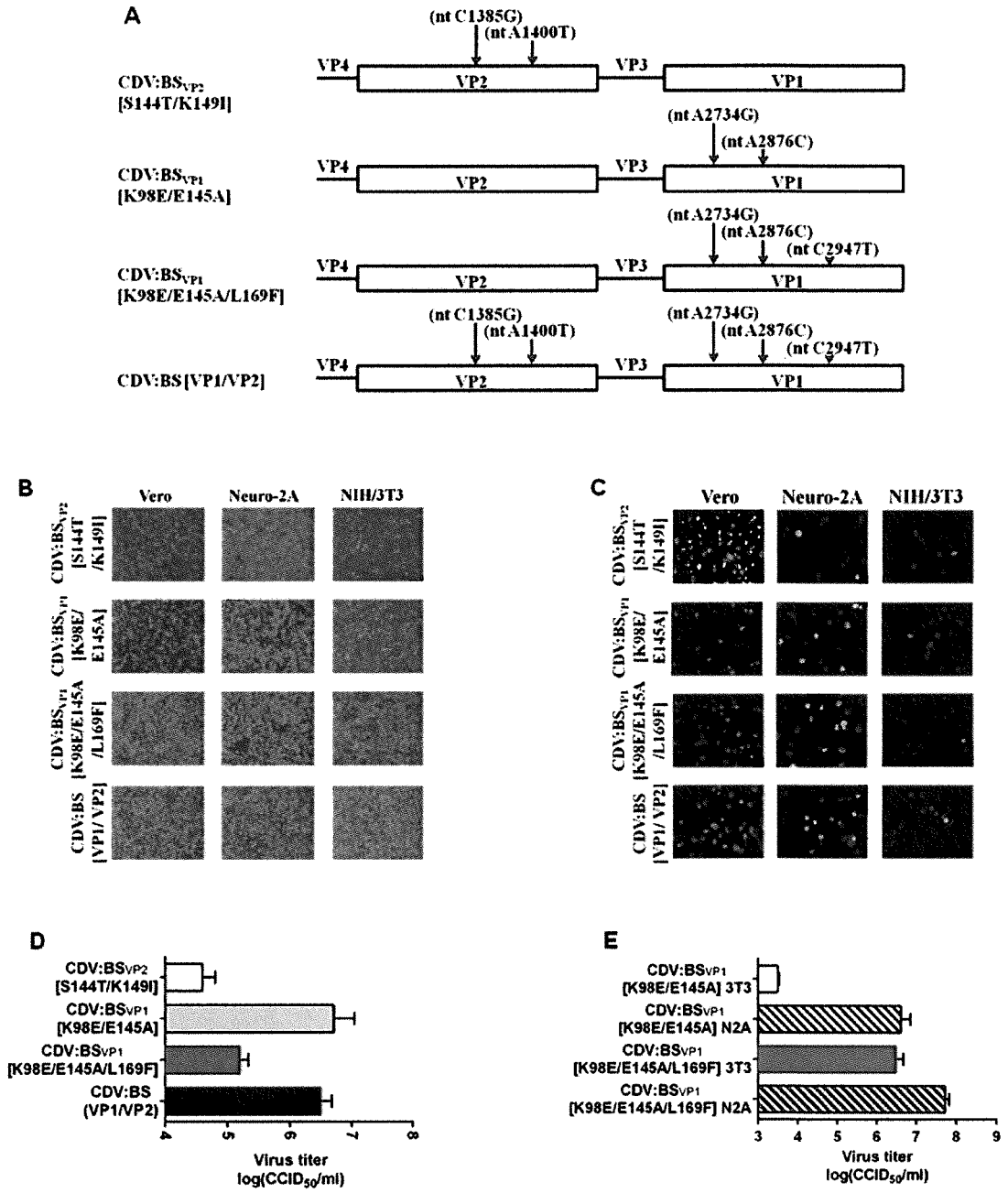

A
(nt C1385G)
(nt A1400T)
CDV:BS$_{VP2}$ [S144T/K149I]
VP4
VP2
VP3
VP1
(nt A2734G)
(nt A2876C)
CDV:BS$_{VP1}$ [K98E/E145A]
(nt C2947T)
CDV:BS$_{VP1}$ [K98E/E145A/L169F]
CDV:BS [VP1/VP2]
B
Vero
Neuro-2A
NIH/3T3
C
D
CDV:BS$_{VP2}$ [S144T/K149I]
CDV:BS$_{VP1}$ [K98E/E145A]
CDV:BS$_{VP1}$ [K98E/E145A/L169F]
CDV:BS (VP1/VP2)
Virus titer log(CCID$_{50}$/ml)
E
CDV:BS$_{VP1}$ [K98E/E145A] 3T3
CDV:BS$_{VP1}$ [K98E/E145A] N2A
CDV:BS$_{VP1}$ [K98E/E145A/L169F] 3T3
CDV:BS$_{VP1}$ [K98E/E145A/L169F] N2A

Figure 19

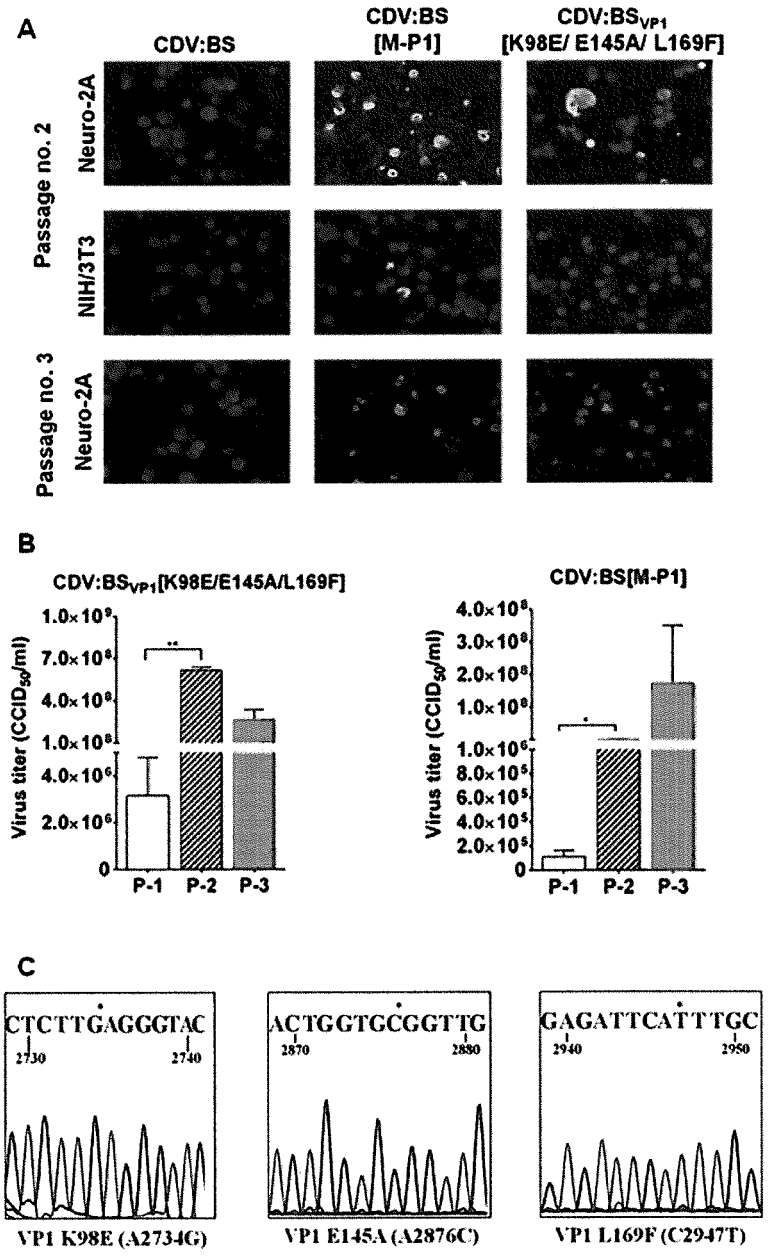
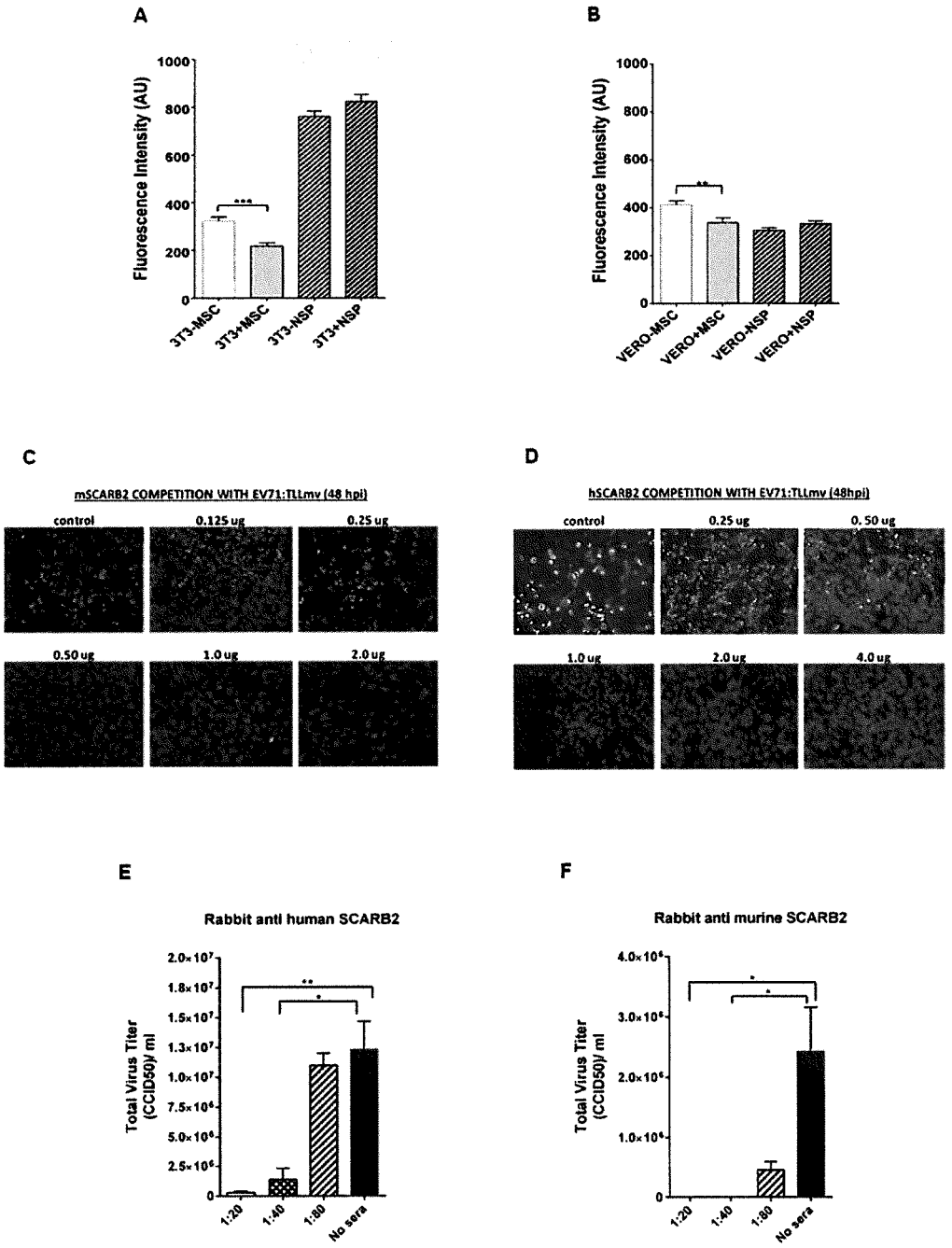

A
CDV:BS
CDV:BS [M-P1]
CDV:BS$_{VP1}$ [K98E/ E145A/ L169F]
Passage no. 2
Neuro-2A
NIH/3T3
Passage no. 3
Neuro-2A
B
CDV:BS$_{VP1}$[K98E/E145A/L169F]
CDV:BS[M-P1]
Virus titer (CCID$_{50}$/ml)
P-1
P-2
P-3
C
CTCTTGAGGGTAC
2730
2740
ACTGGTGCGGTTG
2870
2880
GAGATTCATTTGC
2940
2950
VP1 K98E (A2734G)
VP1 E145A (A2876C)
VP1 L169F (C2947T)

Figure 20


A
Fluorescence Intensity (AU)
1000
800
600
400
200
0
***
3T3-MSC
3T3+MSC
3T3-NSP
3T3+NSP
B
Fluorescence Intensity (AU)
1000
800
600
400
200
0
**
VERO-MSC
VERO+MSC
VERO-NSP
VERO+NSP
C
mSCARB2 COMPETITION WITH EV71:TLLmv (48 hpi)
control
0.125 ug
0.25 ug
0.50 ug
1.0 ug
2.0 ug
D
hSCARB2 COMPETITION WITH EV71:TLLmv (48hpi)
control
0.25 ug
0. 50 ug
1.0 ug
2.0 ug
4.0 ug
E
Rabbit anti human SCARB2
Total Virus Titer (CCID50)/ ml
2.0×10⁷
1.8×10⁷
1.5×10⁷
1.3×10⁷
1.0×10⁷
7.5×10⁶
5.0×10⁶
2.5×10⁶
0
**
*
1:20
1:40
1:80
No sera
F
Rabbit anti murine SCARB2
Total Virus Titer (CCID50)/ ml
4.0×10⁴
3.0×10⁴
2.0×10⁴
1.0×10⁴
0
*
*
1:20
1:40
1:80
No sera

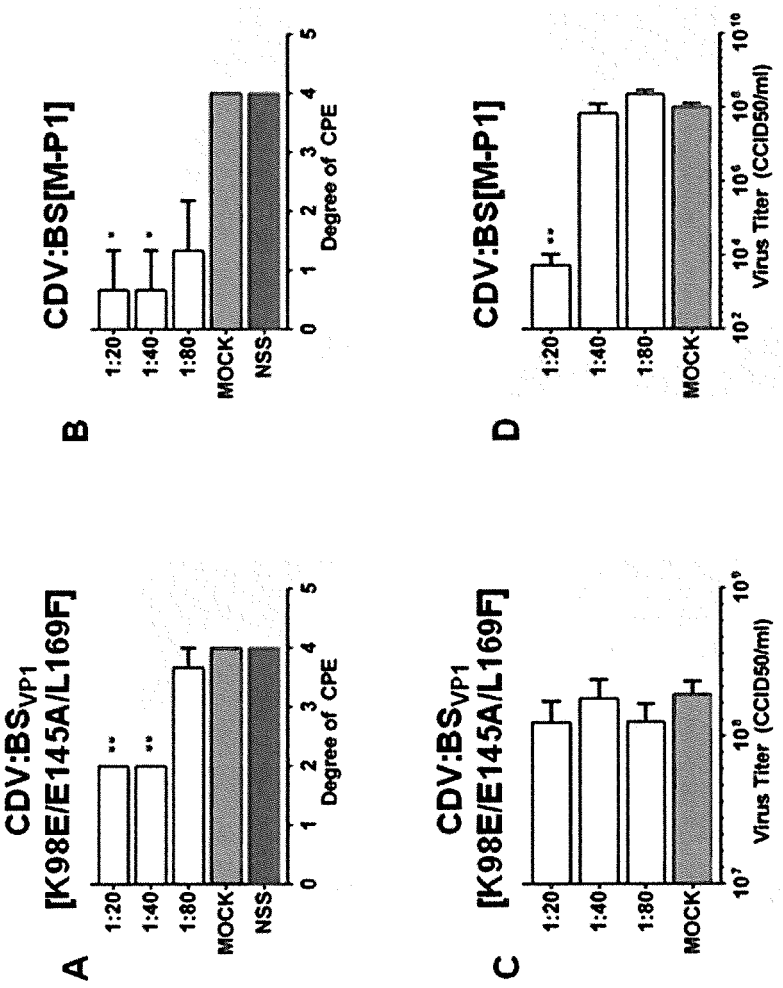

A
CDV:BS$_{VP1}$ [K98E/E145A/L169F]
Degree of CPE
1:20
1:40
1:80
MOCK
NSS
B
CDV:BS[M-P1]
Degree of CPE
C
CDV:BS$_{VP1}$ [K98E/E145A/L169F]
Virus Titer (CCID50/ml)
D
CDV:BS[M-P1]
Virus Titer (CCID50/ml)

Figure 21

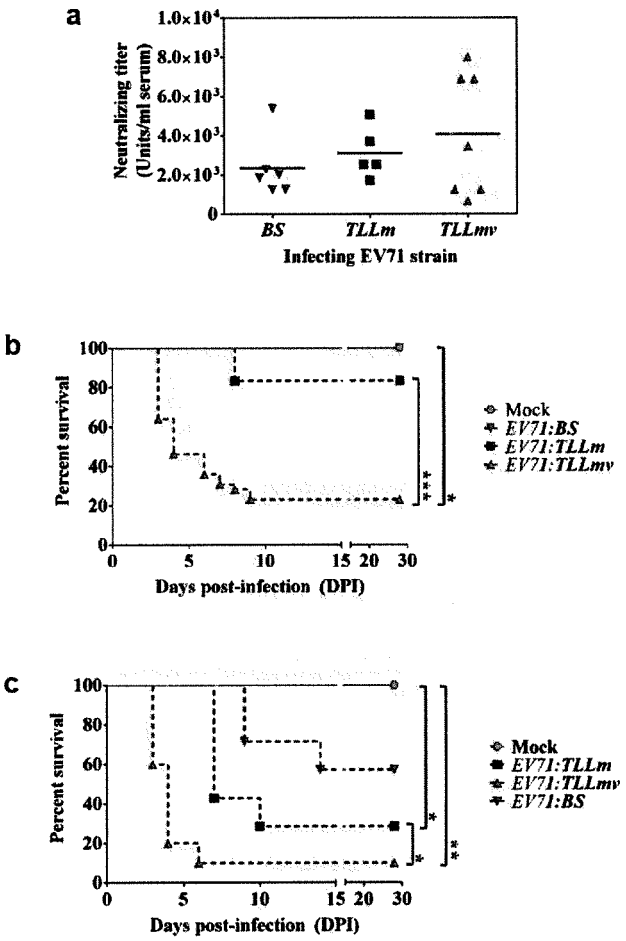

a
Neutralizing titer (Units/ml serum)
1.0×10⁴
8.0×10³
6.0×10³
4.0×10³
2.0×10³
0
BS
TLLm
TLLmv
Infecting EV71 strain
b
Percent survival
100
80
60
40
20
0
0
5
10
15 20 30
Days post-infection (DPI)
Mock
EV71:BS
EV71:TLLm
EV71:TLLmv
***
*
c
Percent survival
100
80
60
40
20
0
0
5
10
15 20 30
Days post-infection (DPI)
Mock
EV71:TLLm
EV71:TLLmv
EV71:BS
*
*
**

Figure 22

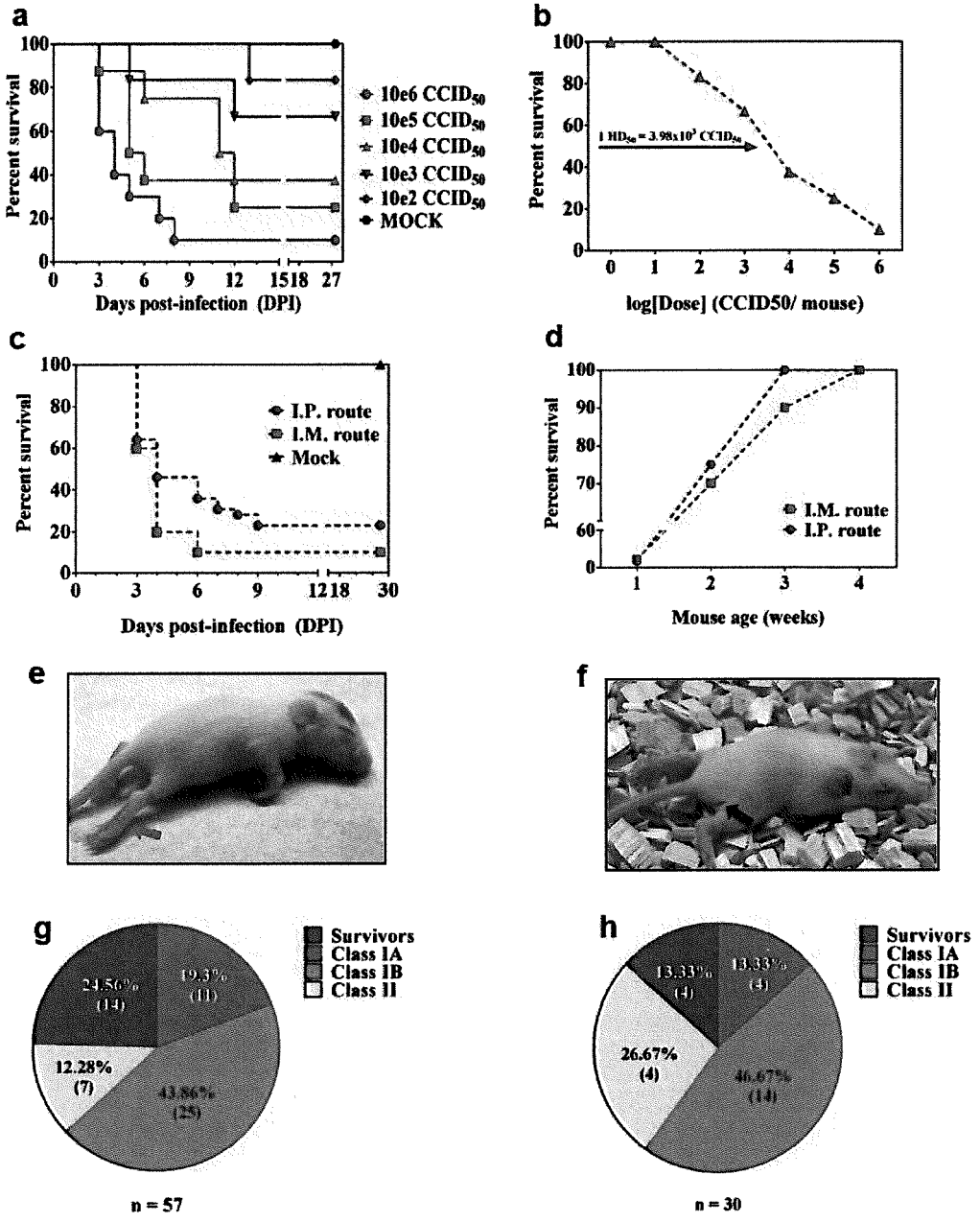

a
Percent survival
Days post-infection (DPI)
10e6 CCID50
10e5 CCID50
10e4 CCID50
10e3 CCID50
10e2 CCID50
MOCK
b
Percent survival
log[Dose] (CCID50/ mouse)
c
Percent survival
Days post-infection (DPI)
I.P. route
I.M. route
Mock
d
Percent survival
Mouse age (weeks)
I.M. route
I.P. route
e
f
g
Survivors
Class IA
Class IB
Class II
24.56% (14)
19.3% (11)
12.28% (7)
43.86% (25)
n = 57
h
Survivors
Class IA
Class IB
Class II
13.33% (4)
13.33% (4)
26.67% (4)
46.67% (14)
n = 30

Figure 23

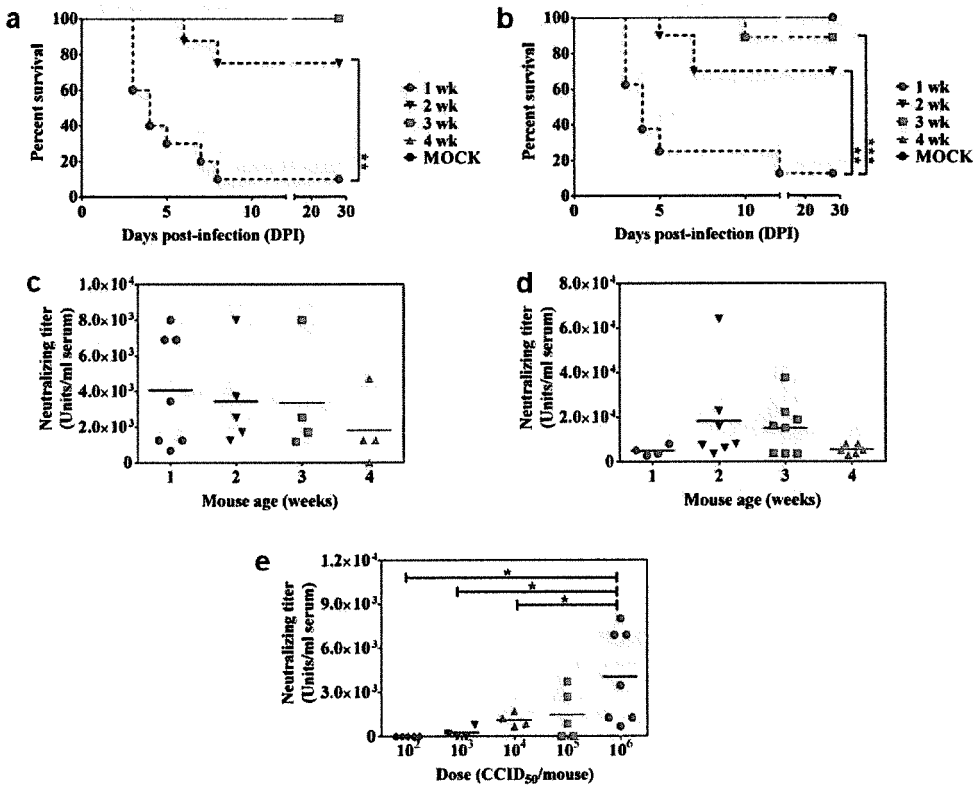

a
Percent survival
Days post-infection (DPI)
1 wk
2 wk
3 wk
4 wk
MOCK
b
Percent survival
Days post-infection (DPI)
1 wk
2 wk
3 wk
4 wk
MOCK
c
Neutralizing titer (Units/ml serum)
Mouse age (weeks)
d
Neutralizing titer (Units/ml serum)
Mouse age (weeks)
e
Neutralizing titer (Units/ml serum)
Dose (CCID50/mouse)

Figure 24

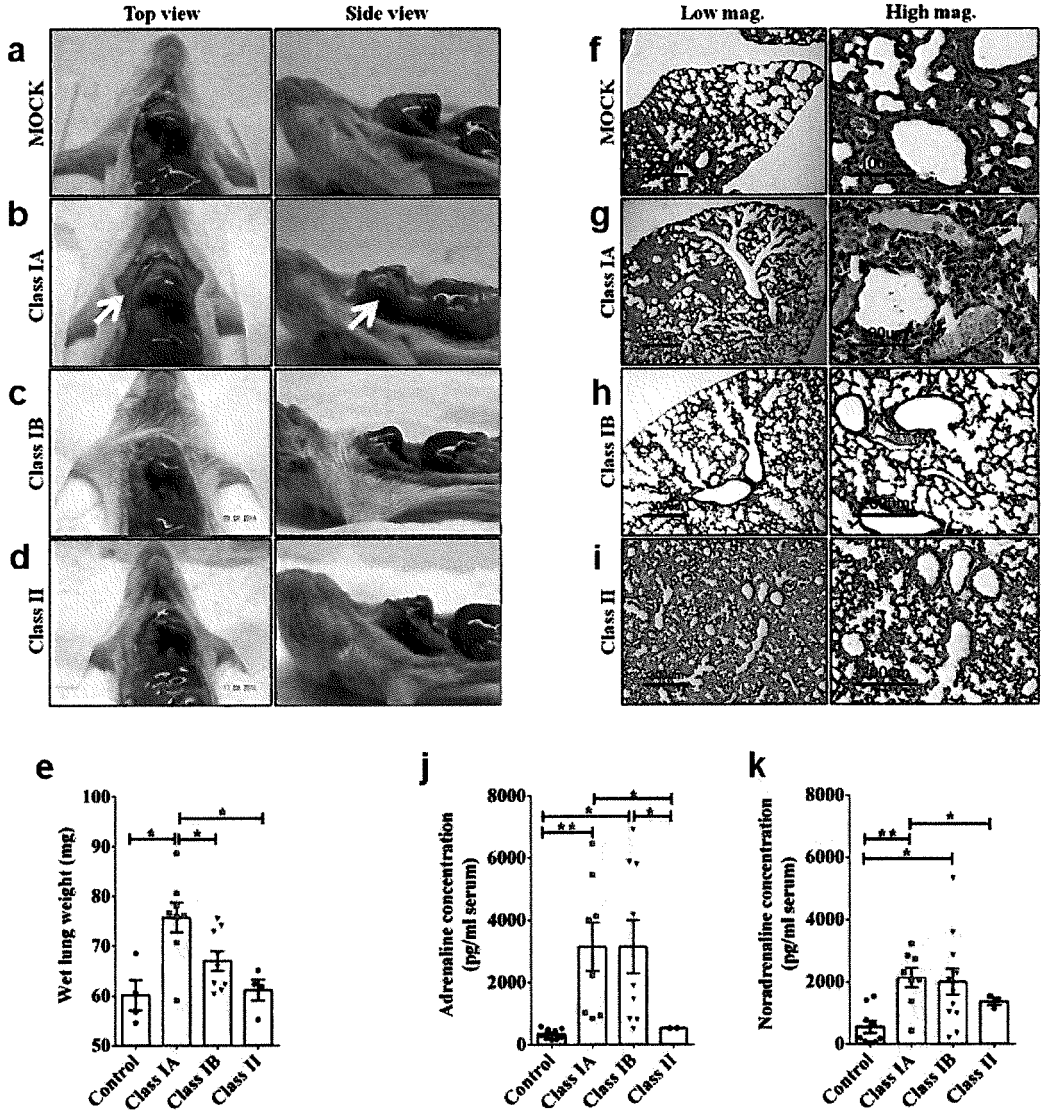

Top view
Side view
Low mag.
High mag.
a
MOCK
b
Class IA
c
Class IB
d
Class II
f
MOCK
g
Class IA
h
Class IB
i
Class II
e
Wet lung weight (mg)
100
90
80
70
60
50
Control
Class IA
Class IB
Class II
j
Adrenaline concentration (pg/ml serum)
8000
6000
4000
2000
0
Control
Class IA
Class IB
Class II
k
Noradrenaline concentration (pg/ml serum)
8000
6000
4000
2000
0
Control
Class IA
Class IB
Class II

Figure 25

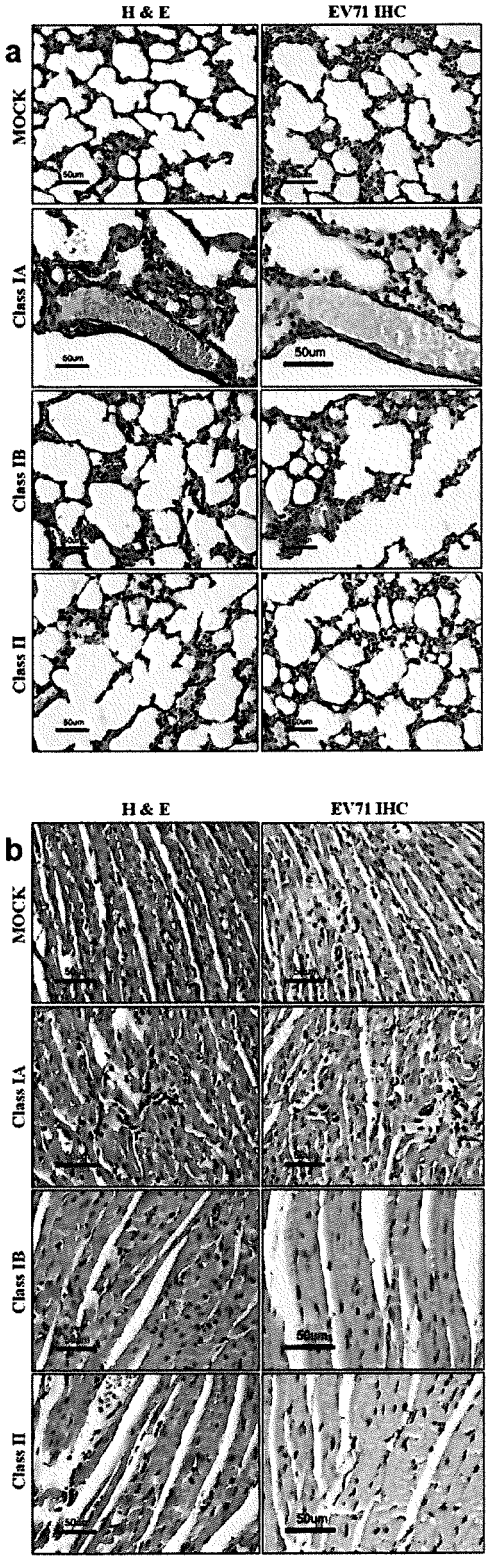

H & E
EV71 IHC
a
MOCK
Class IA
Class IB
Class II
50um
H & E
EV71 IHC
b
MOCK
Class IA
Class IB
Class II
50um

Figure 26

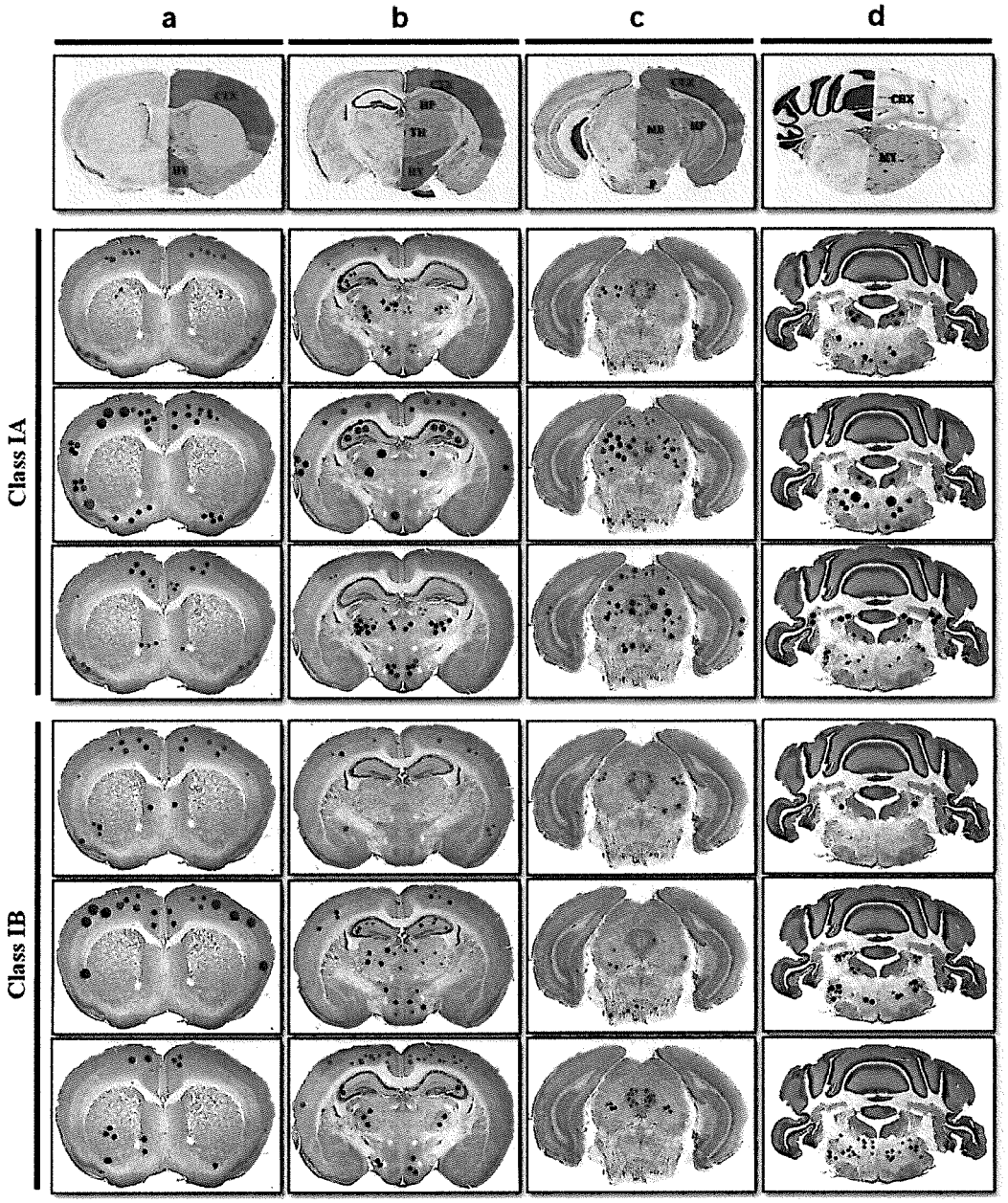


Figure 27

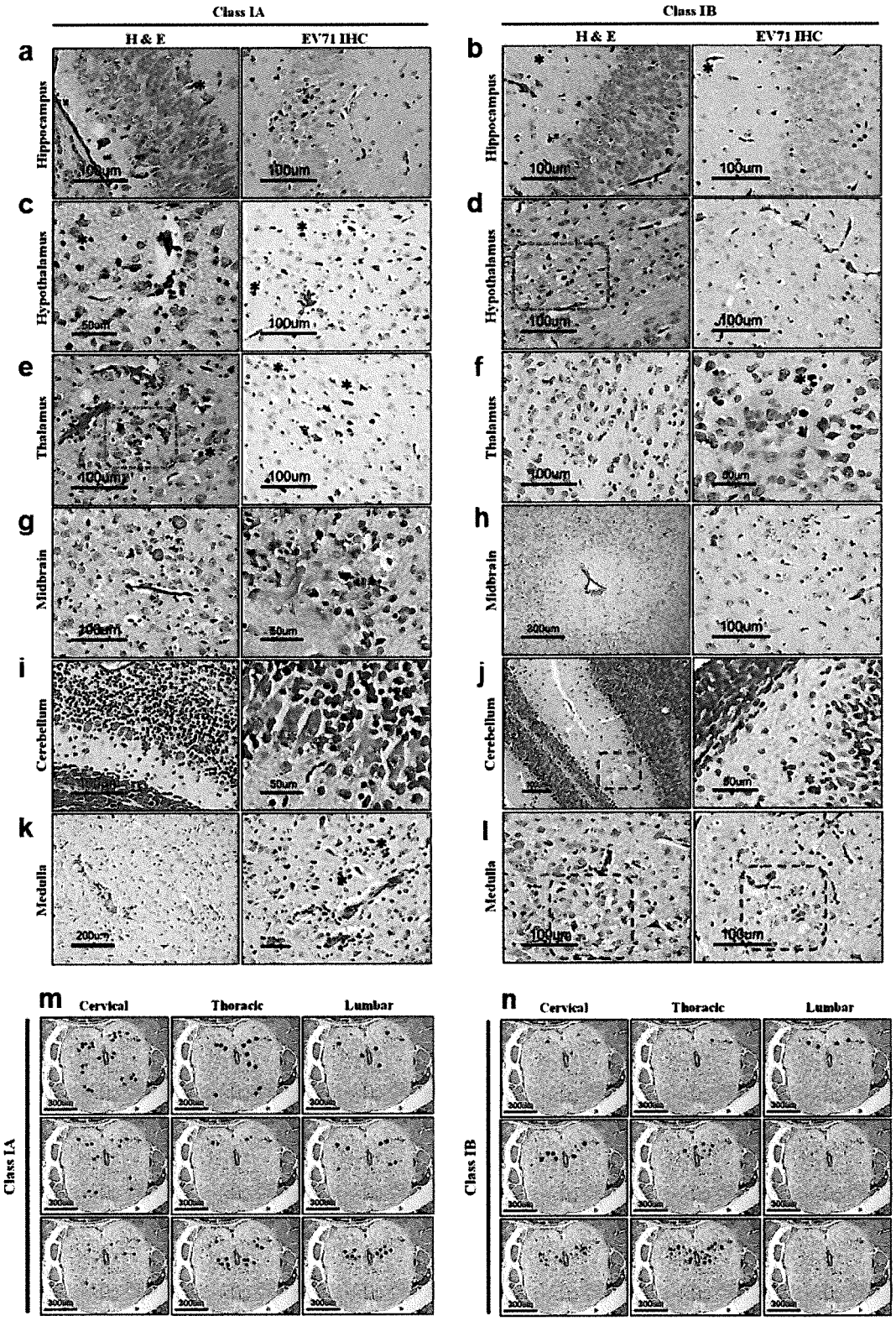

Class IA
Class IB
H & E
EV71 IHC
a
b
c
d
e
f
g
h
i
j
k
l
Hippocampus
Hypothalamus
Thalamus
Midbrain
Cerebellum
Medulla
m
n
Cervical
Thoracic
Lumbar
Class IA
Class IB

Figure 28

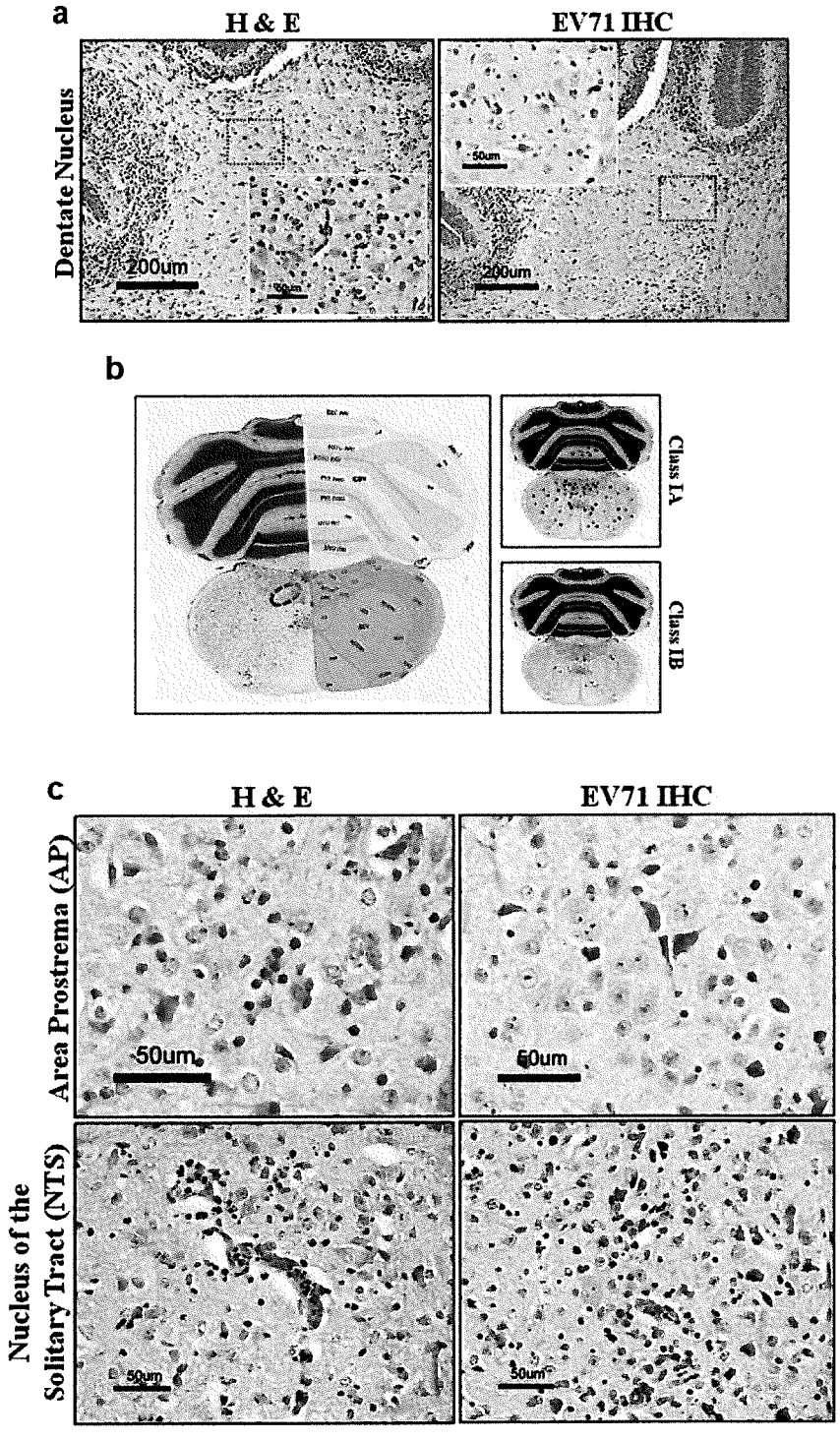

a
H & E
EV71 IHC
Dentate Nucleus
200um
50um
200um
b
Class I A
Class II B
c
H & E
EV71 IHC
Area Prostrema (AP)
50um
60um
Nucleus of the Solitary Tract (NTS)
50um
50um

Figure 29

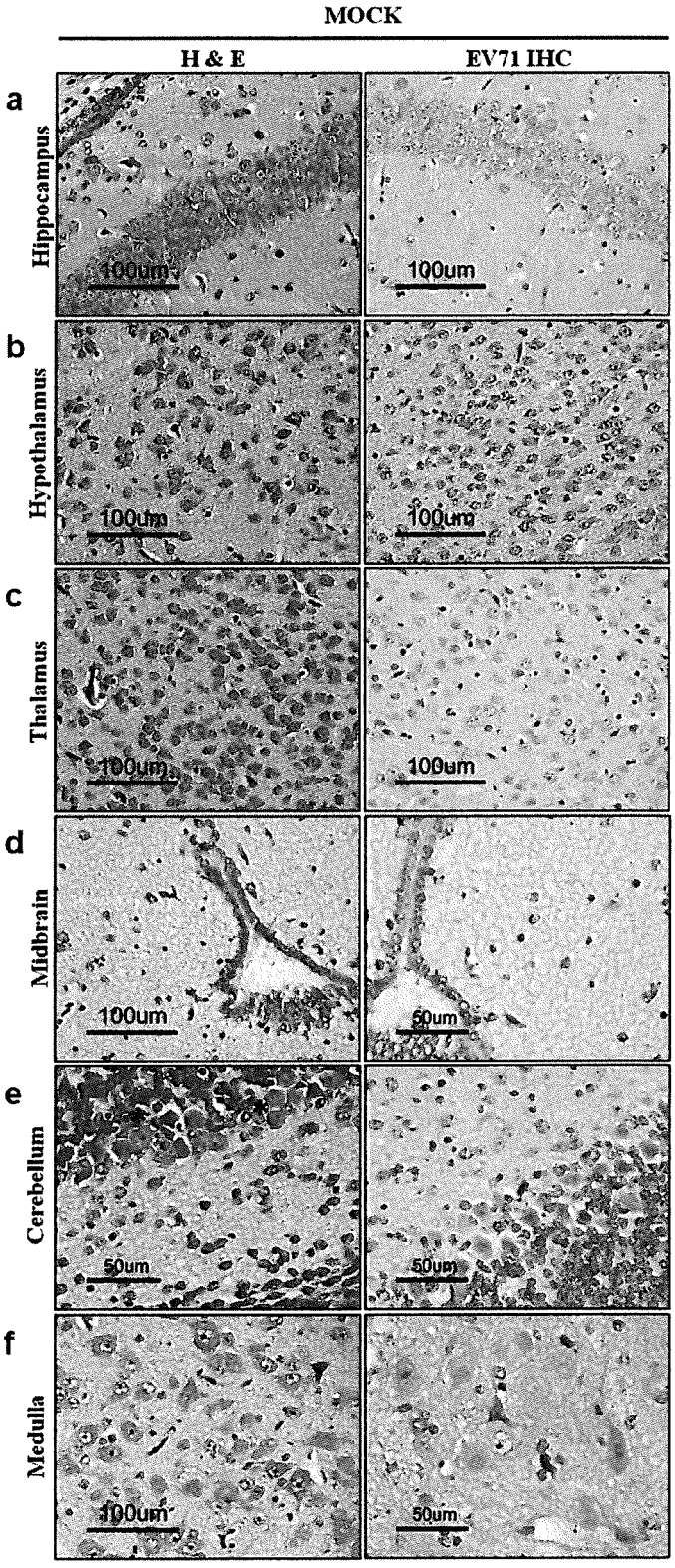

MOCK
H & E
EV71 IHC
a
Hippocampus
100um
100um
b
Hypothalamus
100um
100um
c
Thalamus
100um
100um
d
Midbrain
100um
50um
e
Cerebellum
50um
50um
f
Medulla
100um
50um

Figure 30

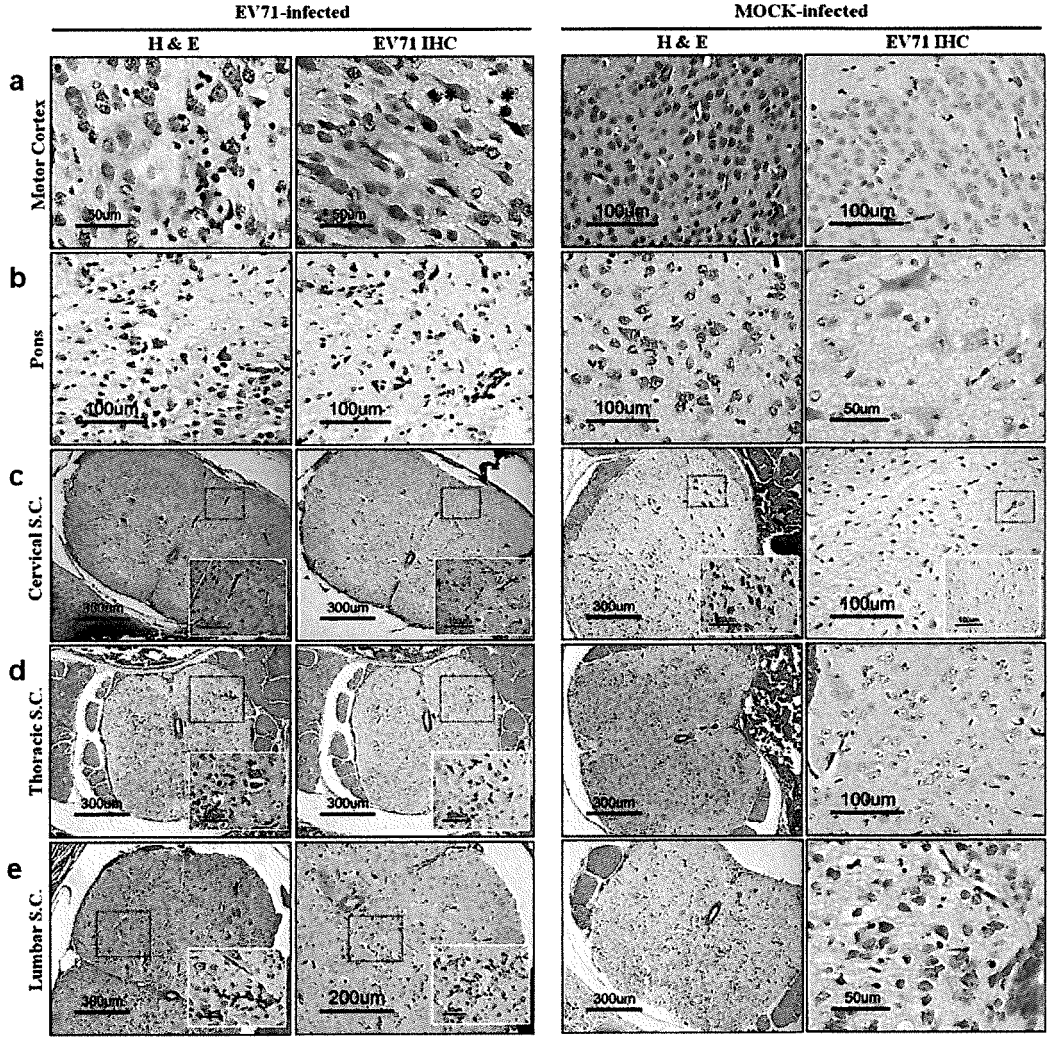

EV71-infected
MOCK-infected
H & E
EV71 IHC
H & E
EV71 IHC
a
Motor Cortex
b
Pons
c
Cervical S.C.
d
Thoracic S.C.
e
Lumbar S.C.
100um
50um
300um
200um

Figure 31