Figure 1

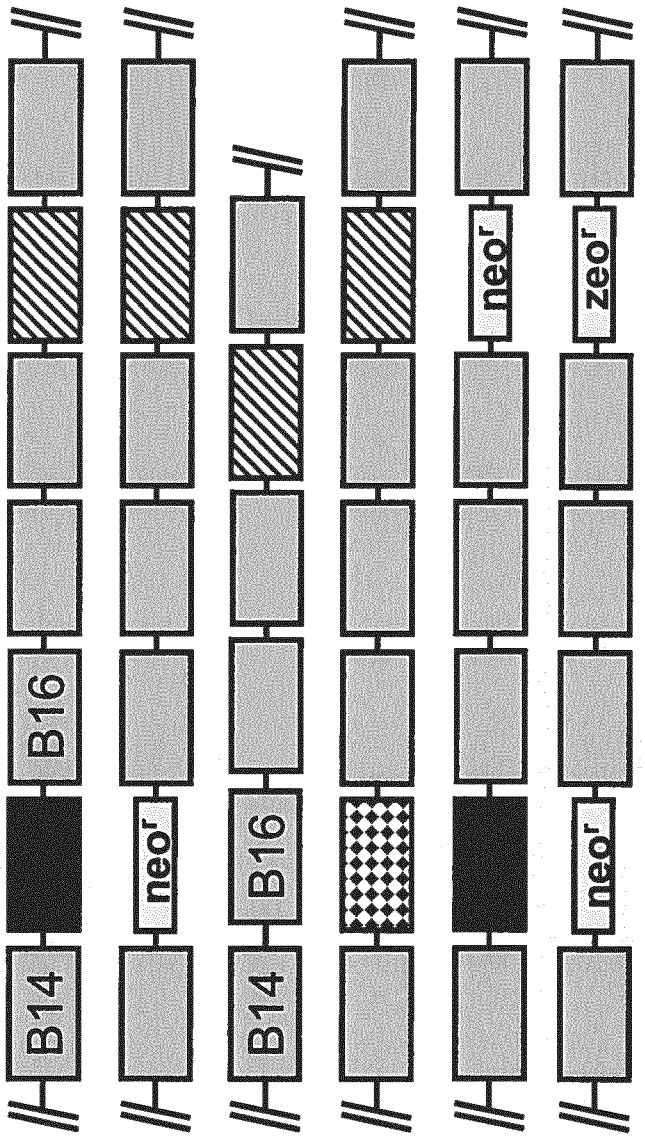

B15R
B19R
B14
B16
neo^r
zeo^r
CVA
CVA-dsneo-ΔB15
CVA-ΔB15
CVA-B15MVA
CVA-ΔB19
CVA-dsneo-ΔB15/B19

Figure 2

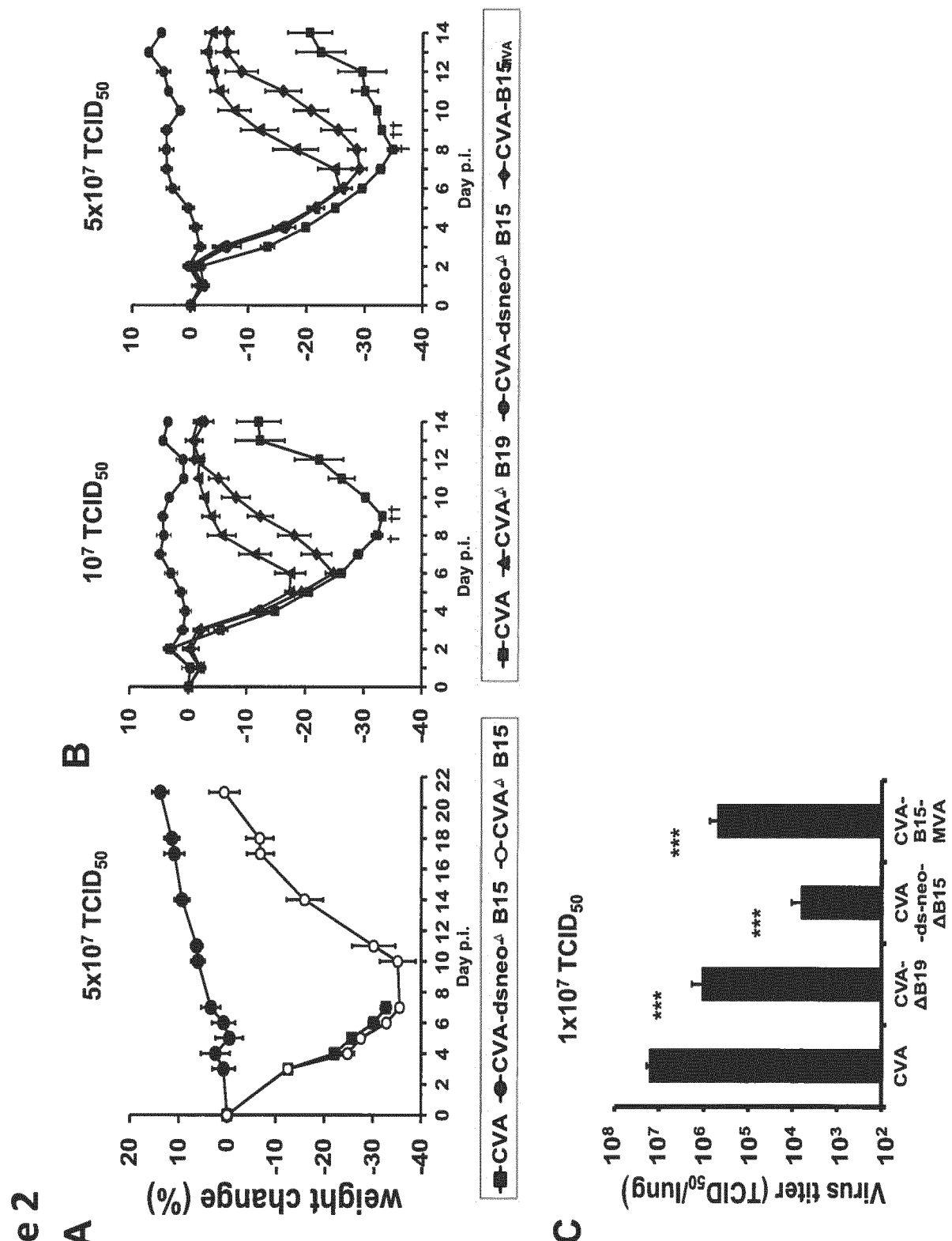

A
B
C
5x10^7 TCID50
10^7 TCID50
1x10^7 TCID50
weight change (%)
Day p.i.
CVA
CVA-dsneo^Δ B15
CVA^Δ B15
CVA^Δ B19
CVA-dsneo^Δ B19
CVA-B15^MVA
Virus titer (TCID50/lung)
***
CVA
CVA-ΔB19
CVA -ds-neo-ΔB15
CVA-B15-MVA

Figure 3

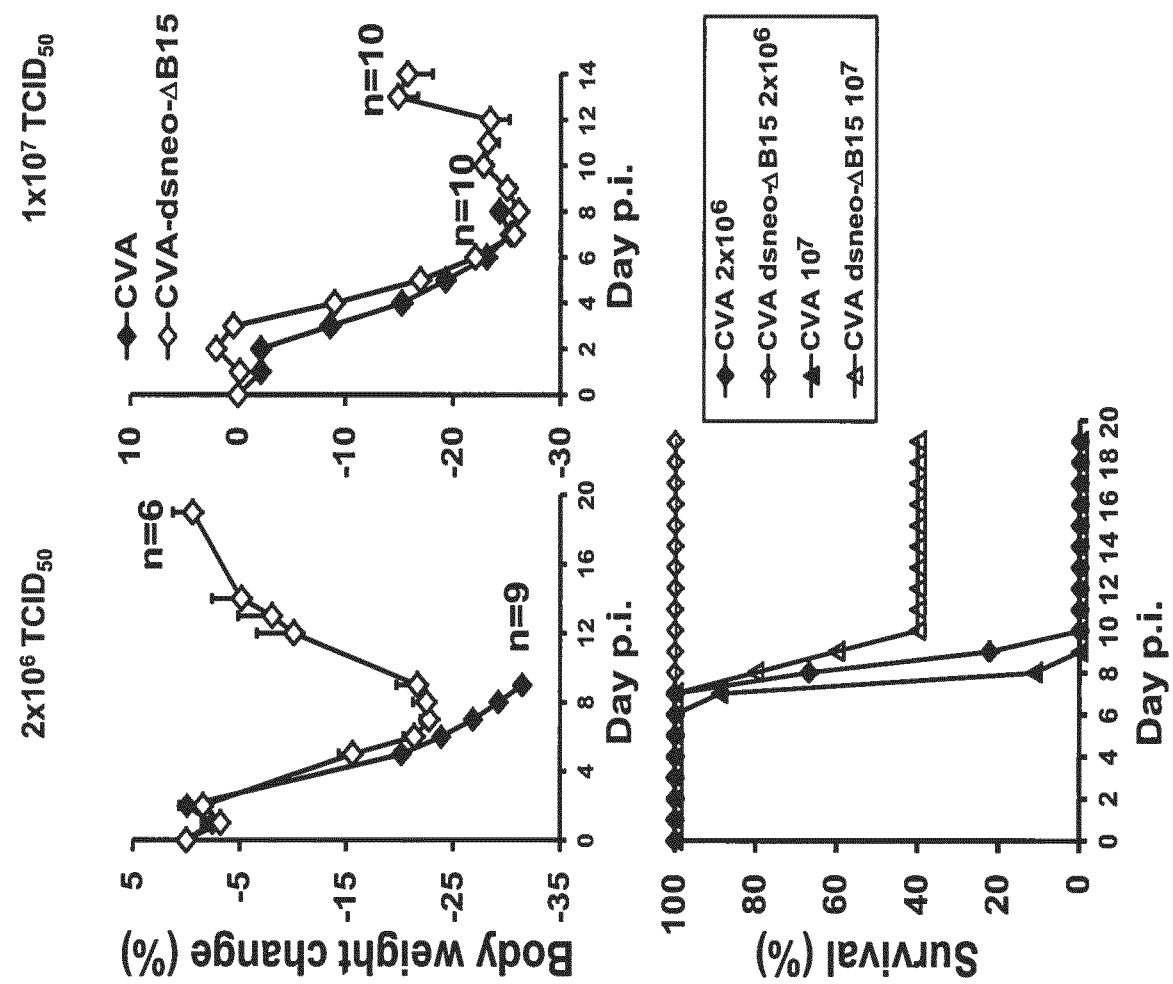
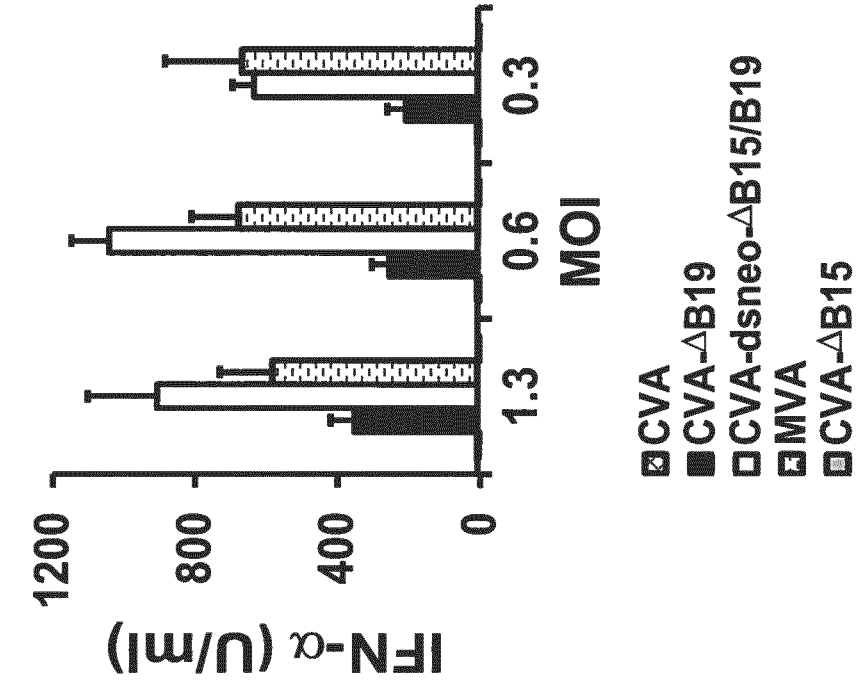

A
2x10⁶ TCID50
1x10⁷ TCID50
CVA
CVA-dsneo-ΔB15
n=6
n=9
n=10
n=10
Body weight change (%)
Day p.i.
B
CVA 2x10⁶
CVA dsneo-ΔB15 2x10⁶
CVA 10⁷
CVA dsneo-ΔB15 10⁷
Survival (%)
Day p.i.

Figure 4


IFN-α (U/ml)
1200
800
400
0
1.3
0.6
0.3
MOI
CVA
CVA-Δ B19
CVA-dsneo-Δ B15/B19
MVA
CVA-Δ B15

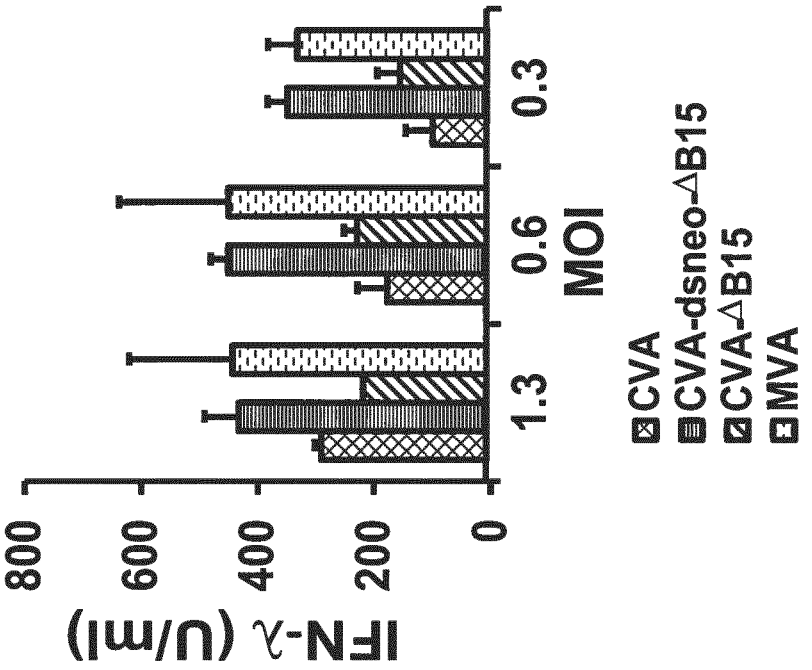
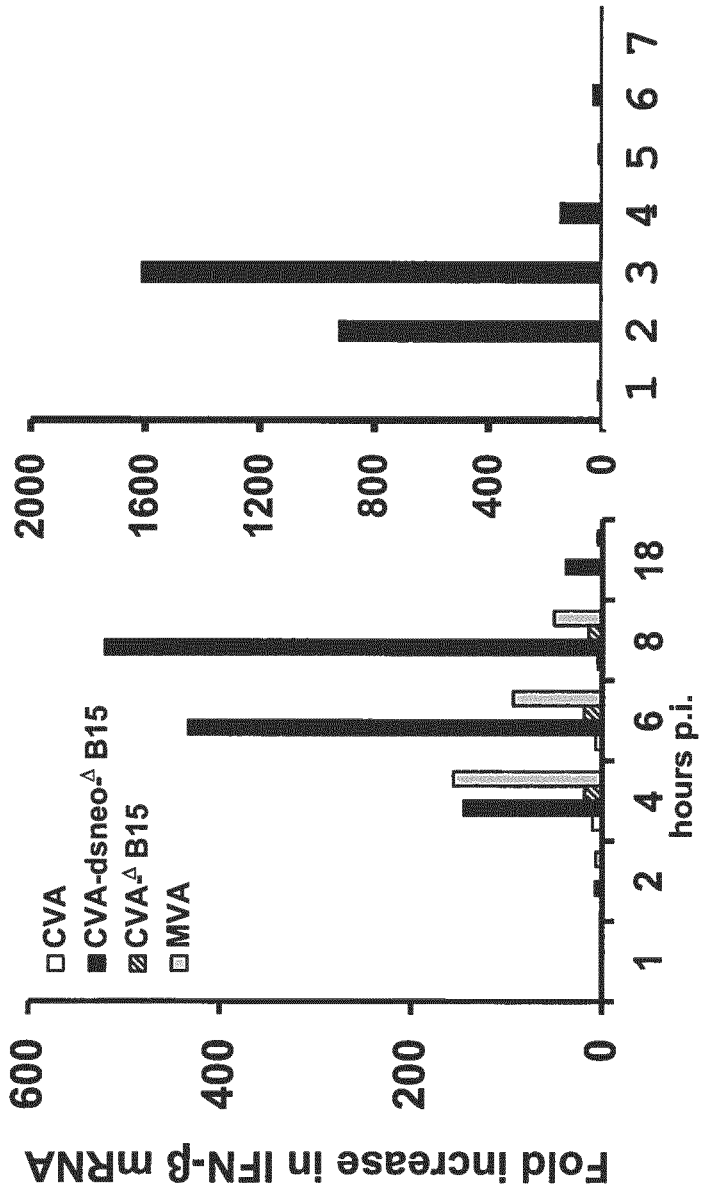

IFN-λ (U/ml)
800
600
400
200
0
1.3
0.6
0.3
MOI
CVA
CVA-dsneo-Δ B15
CVA-Δ B15
MVA

Figure 5


A
B
Fold increase in IFN-β mRNA
600
400
200
0
2000
1600
1200
800
400
0
1 2 4 6 8 18
1 2 3 4 5 6 7
hours p.i.
CVA
CVA-dsneo.Δ B15
CVA.Δ B15
MVA

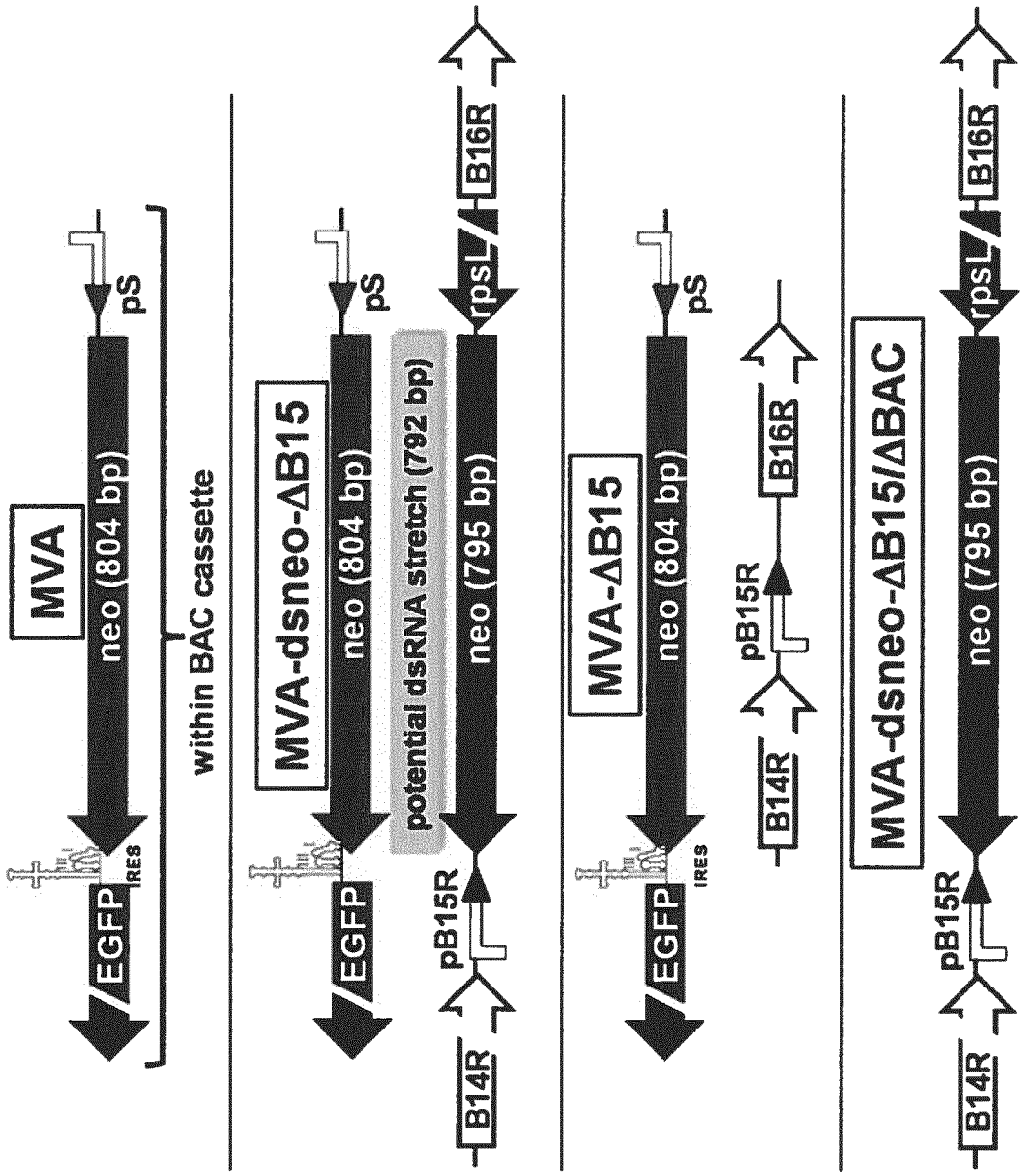

MVA
pS
neo (804 bp)
EGFP
IRES
within BAC cassette
MVA-dsneo-ΔB15
pS
neo (804 bp)
potential dsRNA stretch (792 bp)
neo (795 bp)
B16R
pB15R
B14R
EGFP
MVA-ΔB15
pS
neo (804 bp)
EGFP
IRES
pB15R
B16R
B14R
MVA-dsneo-ΔB15/ΔBAC
pS
neo (795 bp)
B16R
pB15R
B14R

Figure 6A

Figure 6B

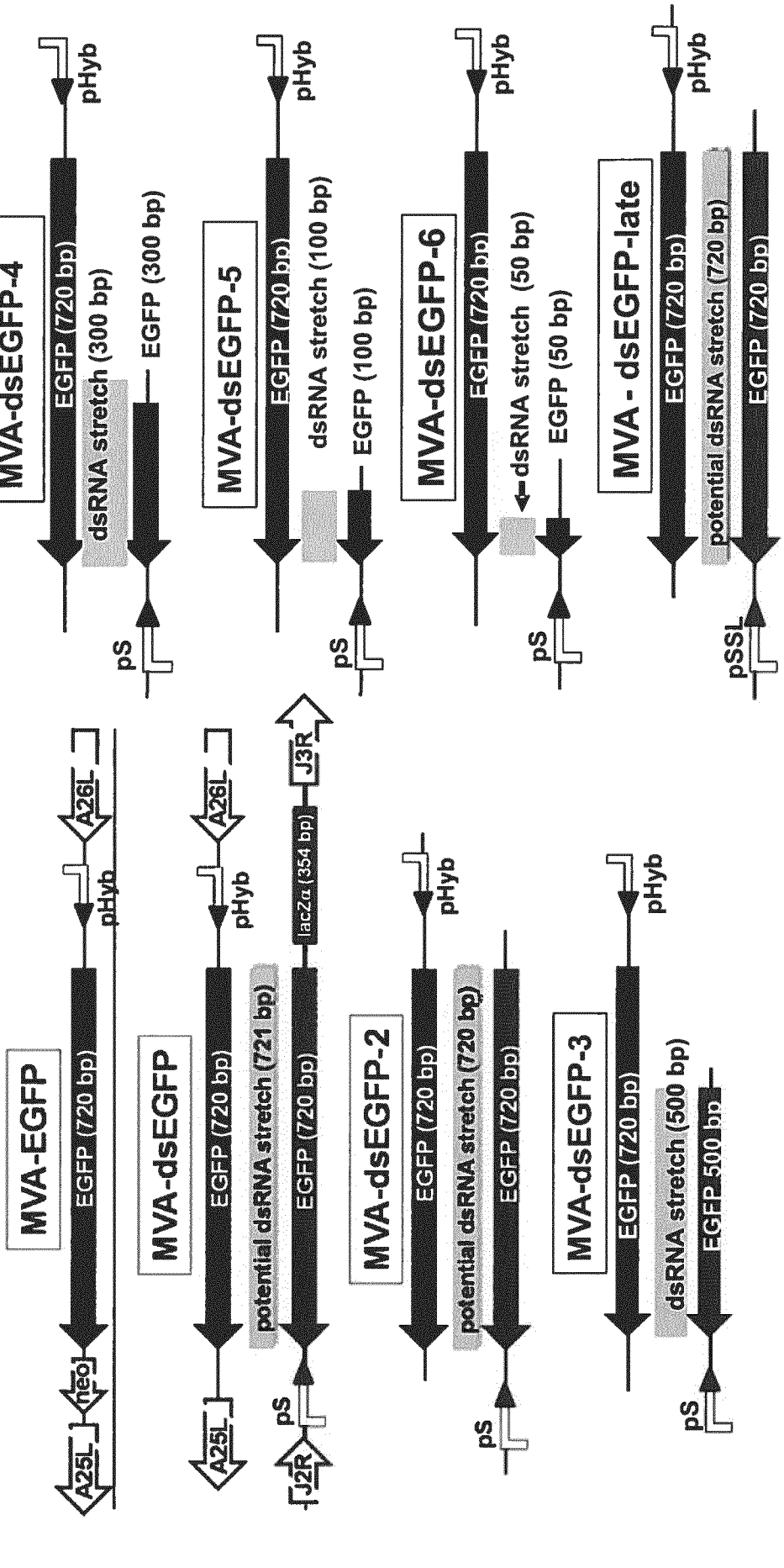
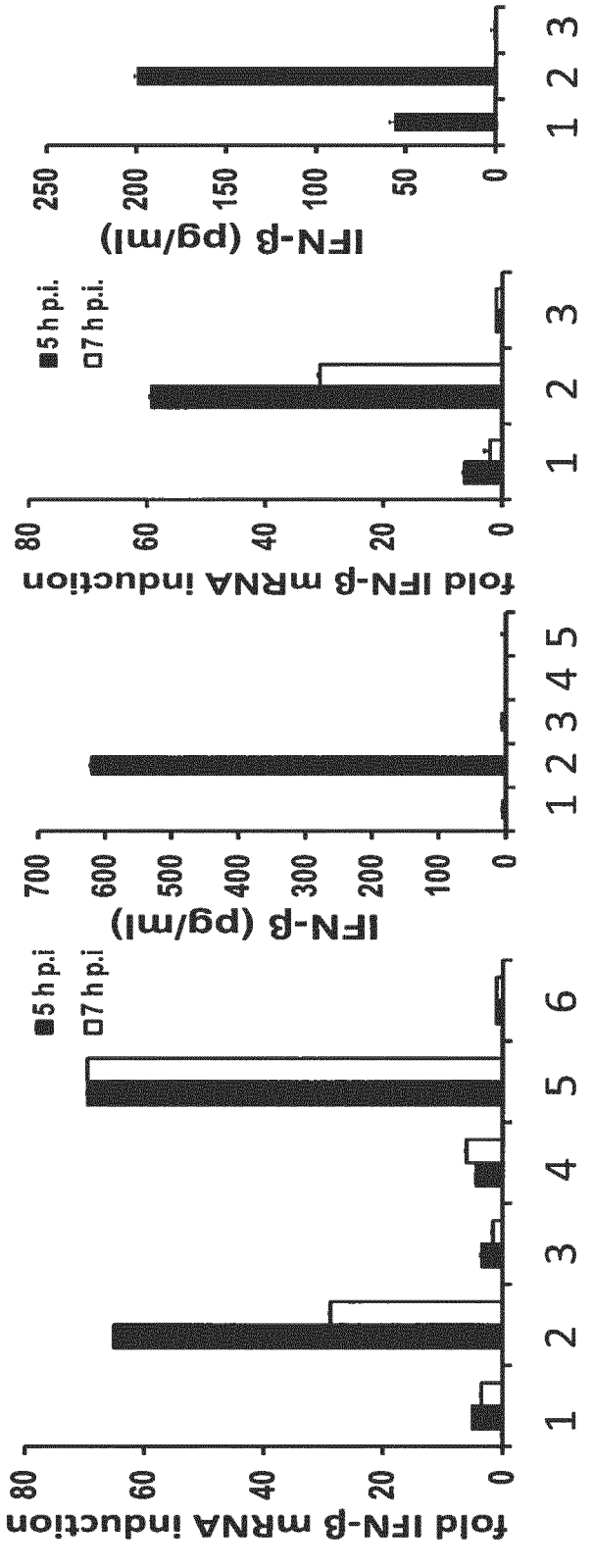

MVA-dsEGFP-4
pHyb
EGFP (720 bp)
dsRNA stretch (300 bp)
EGFP (300 bp)
pS
MVA-dsEGFP-5
pHyb
EGFP (720 bp)
dsRNA stretch (100 bp)
EGFP (100 bp)
pS
MVA-dsEGFP-6
pHyb
EGFP (720 bp)
dsRNA stretch (50 bp)
EGFP (50 bp)
pS
MVA - dsEGFP-late
pHyb
EGFP (720 bp)
potential dsRNA stretch (720 bp)
EGFP (720 bp)
pSSL
MVA-EGFP
A26L
pHyb
EGFP (720 bp)
neo
A25L
A25L
MVA-dsEGFP
A26L
pHyb
EGFP (720 bp)
potential dsRNA stretch (721 bp)
EGFP (720 bp)
lacZα (354 bp)
J3R
pS
J2R
MVA-dsEGFP-2
pHyb
EGFP (720 bp)
potential dsRNA stretch (720 bp)
EGFP (720 bp)
pS
MVA-dsEGFP-3
pHyb
EGFP (720 bp)
dsRNA stretch (500 bp)
EGFP 500 bp
pS

Figure 7



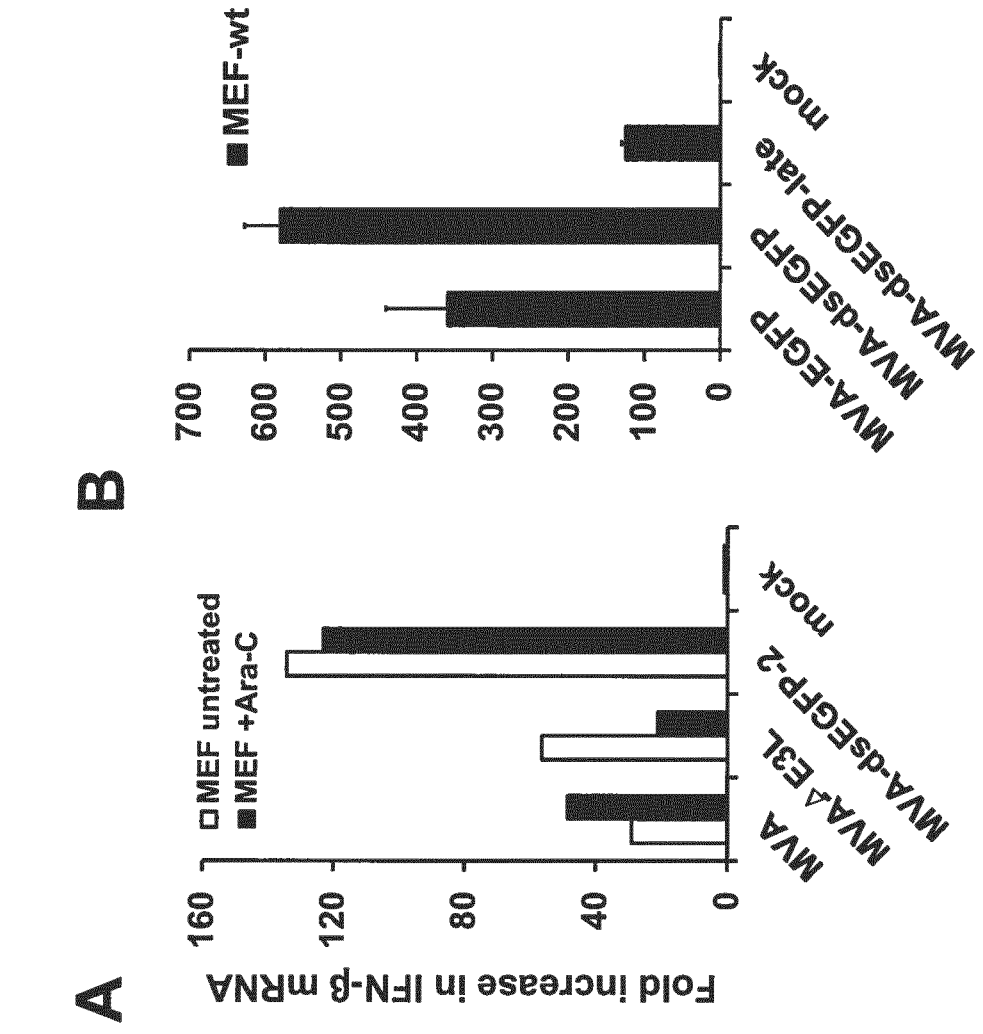

A
Fold increase in IFN-β mRNA
160
120
80
40
0
MEF untreated
MEF +Ara-C
MVA
MVAΔE3L
MVA-dsEGFP-2
mock
B
700
600
500
400
300
200
100
0
MEF-wt
MVA-EGFP
MVA-dsEGFP
MVA-dsEGFP-late
mock

Figure 8

Figure 9

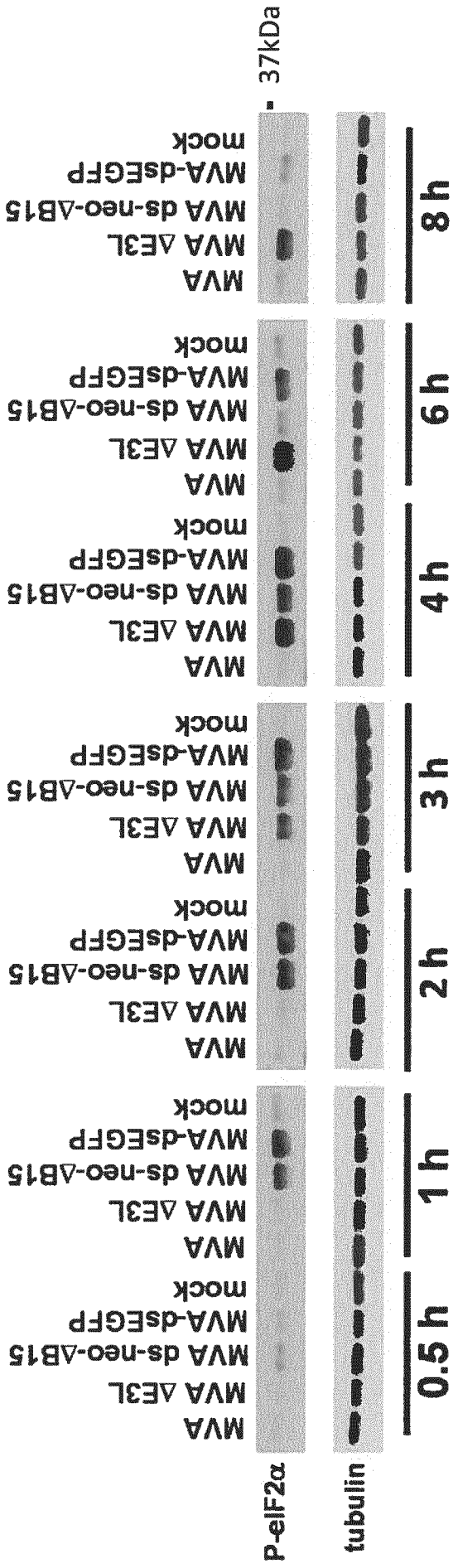

37kDa
P-eIF2α
tubulin
0.5 h
1 h
2 h
3 h
4 h
6 h
8 h
MVA
MVA ΔE3L
MVA ds-neo-ΔB15
MVA-dsEGFP
mock

Figure 10

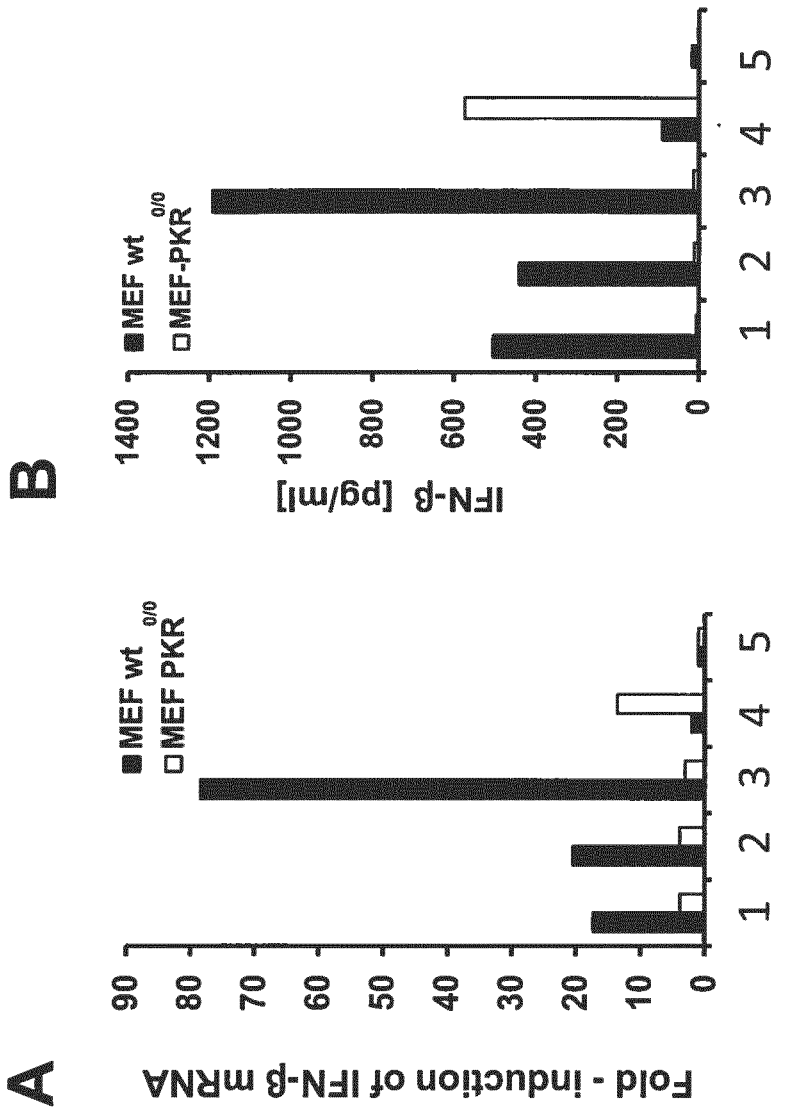

A
Fold - induction of IFN-β mRNA
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5
MEF wt
MEF PKR 0/0
B
IFN-β [pg/ml]
1400
1200
1000
800
600
400
200
0
1 2 3 4 5
MEF wt
MEF-PKR 0/0

Figure 11

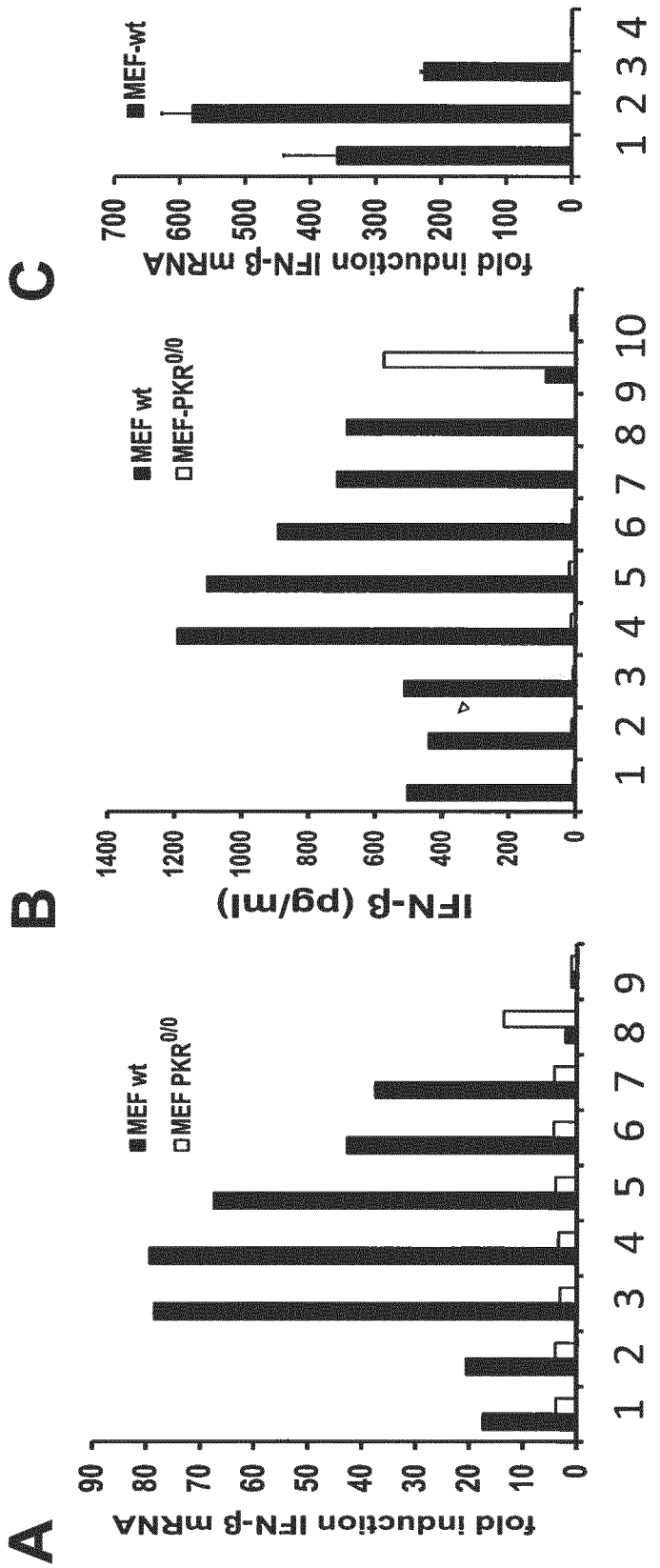
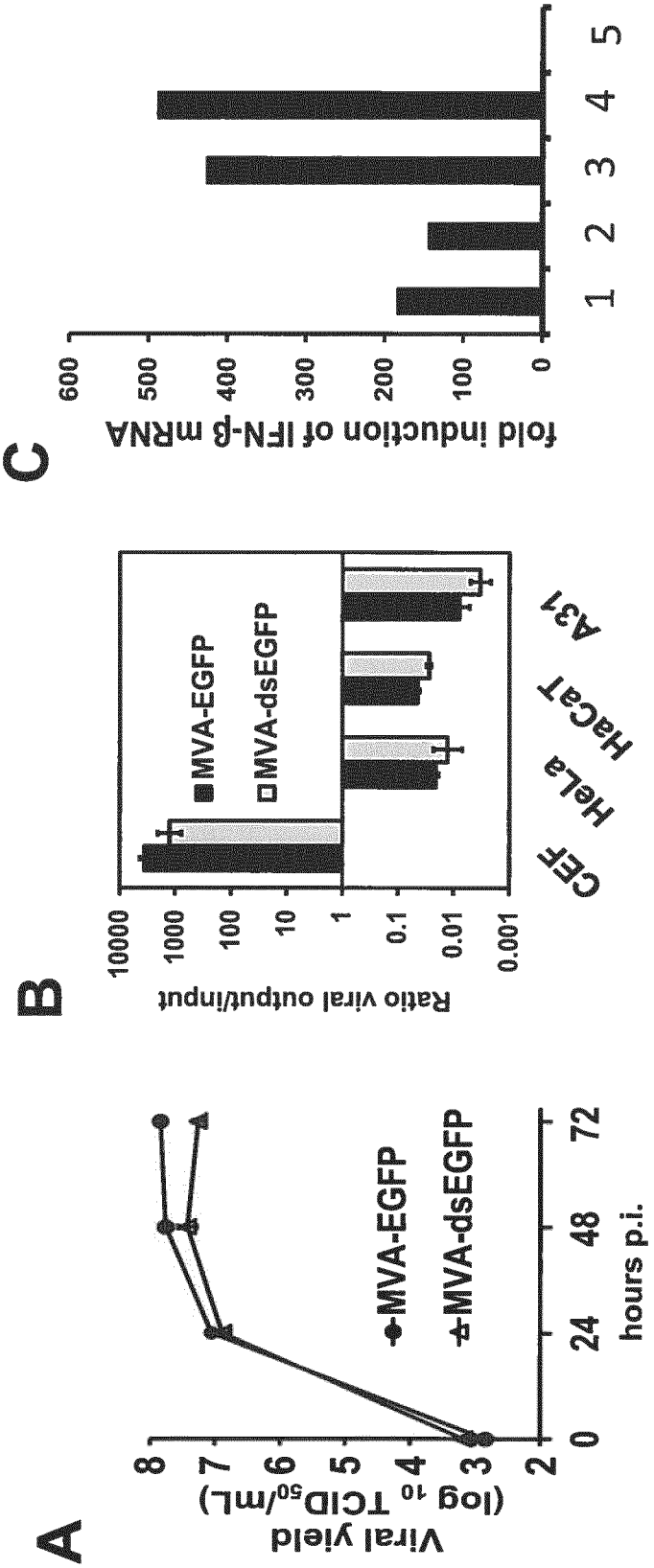

A
fold induction IFN-β mRNA
0
10
20
30
40
50
60
70
80
90
MEF wt
MEF PKR0/0
1 2 3 4 5 6 7 8 9
B
IFN-β (pg/ml)
0
200
400
600
800
1000
1200
1400
MEF wt
MEF-PKR0/0
1 2 3 4 5 6 7 8 9 10
C
fold induction IFN-β mRNA
0
100
200
300
400
500
600
700
MEF-wt
1 2 3 4

Figure 12


A
Viral yield
(log 10 TCID50/mL)
8
7
6
5
4
3
2
0
24
48
72
hours p.i.
MVA-EGFP
MVA-dsEGFP
B
Ratio viral output/input
10000
1000
100
10
1
0.1
0.01
0.001
MVA-EGFP
MVA-dsEGFP
CEF
HeLa
HaCaT
A31
C
fold induction of IFN-β mRNA
600
500
400
300
200
100
0
1
2
3
4
5

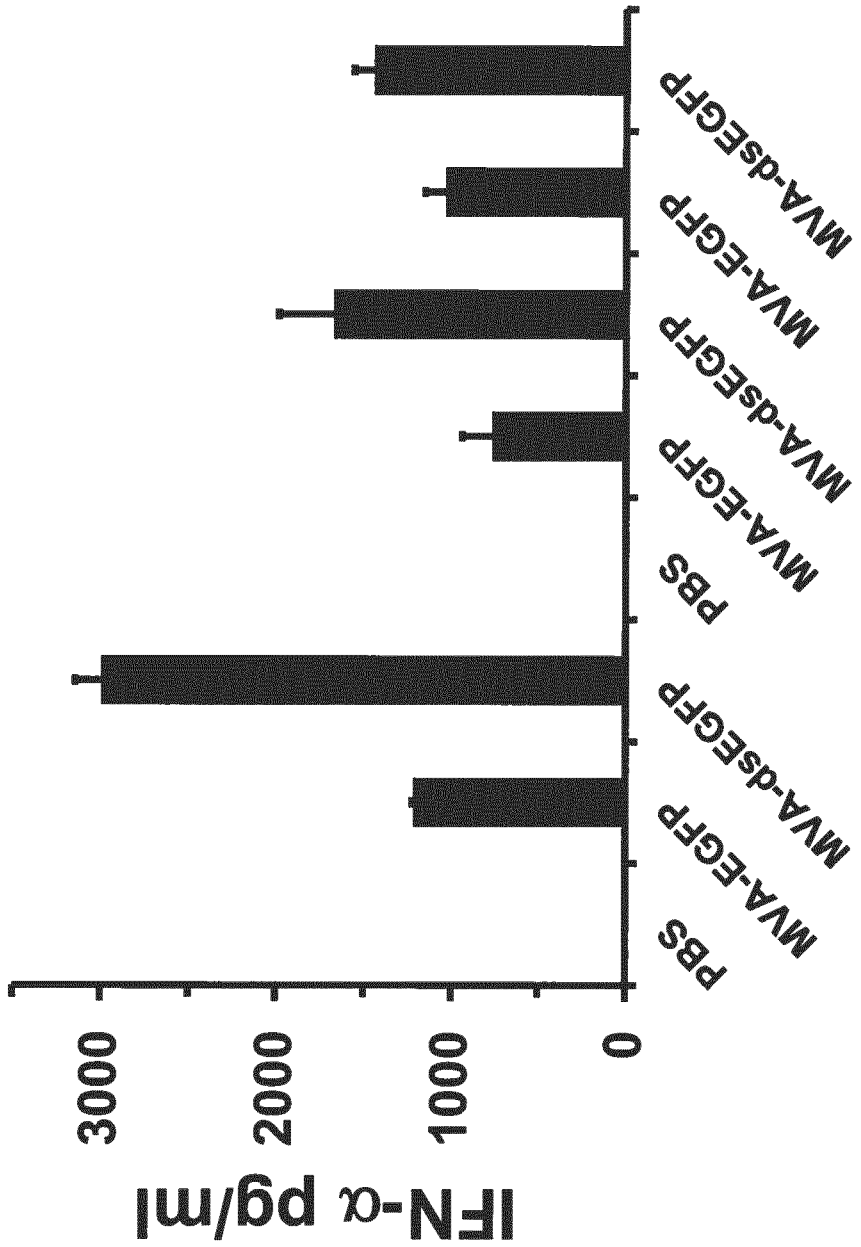

IFN-α pg/ml
3000
2000
1000
0
PBS
MVA-EGFP
MVA-dsEGFP
PBS
MVA-EGFP
MVA-dsEGFP
MVA-EGFP
MVA-dsEGFP

Figure 13A

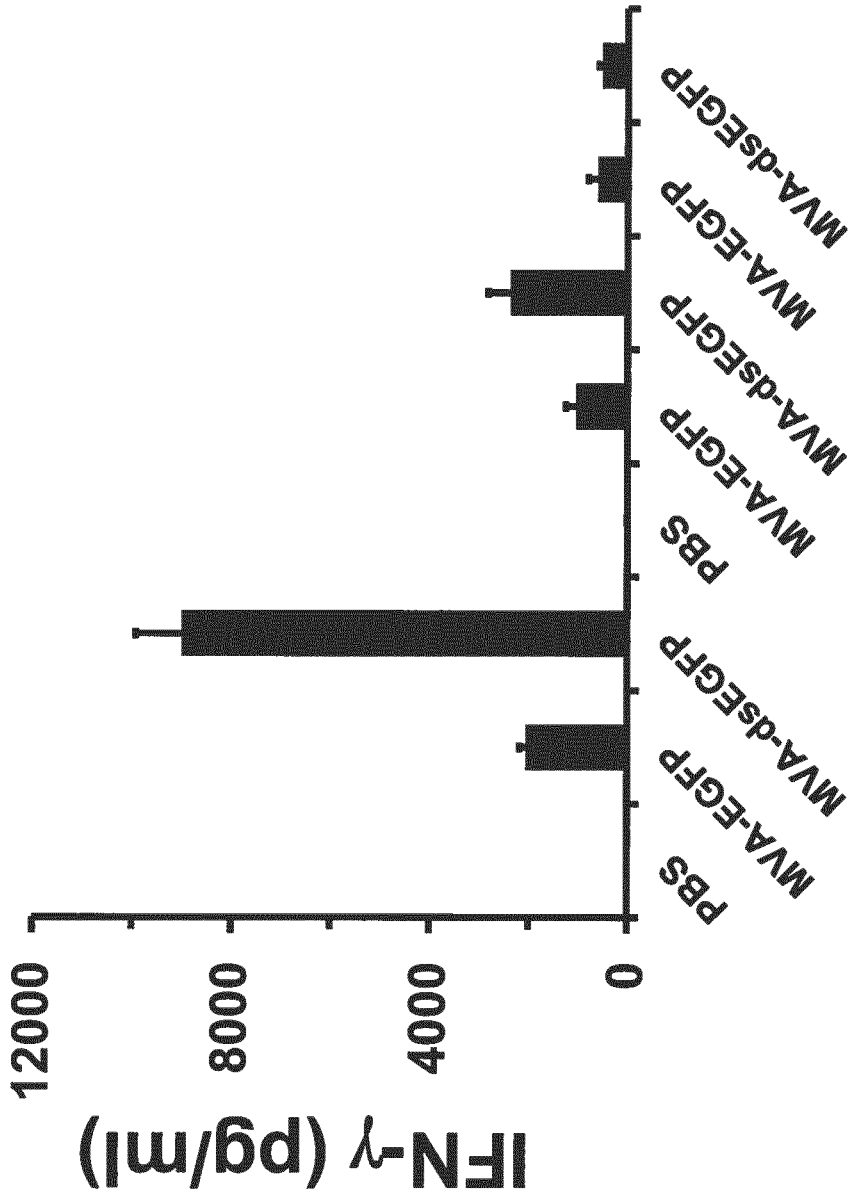

IFN-γ (pg/ml)
12000
8000
4000
0
PBS
MVA-EGFP
MVA-dsEGFP
PBS
MVA-EGFP
MVA-dsEGFP
MVA-EGFP
MVA-dsEGFP

Figure 13B

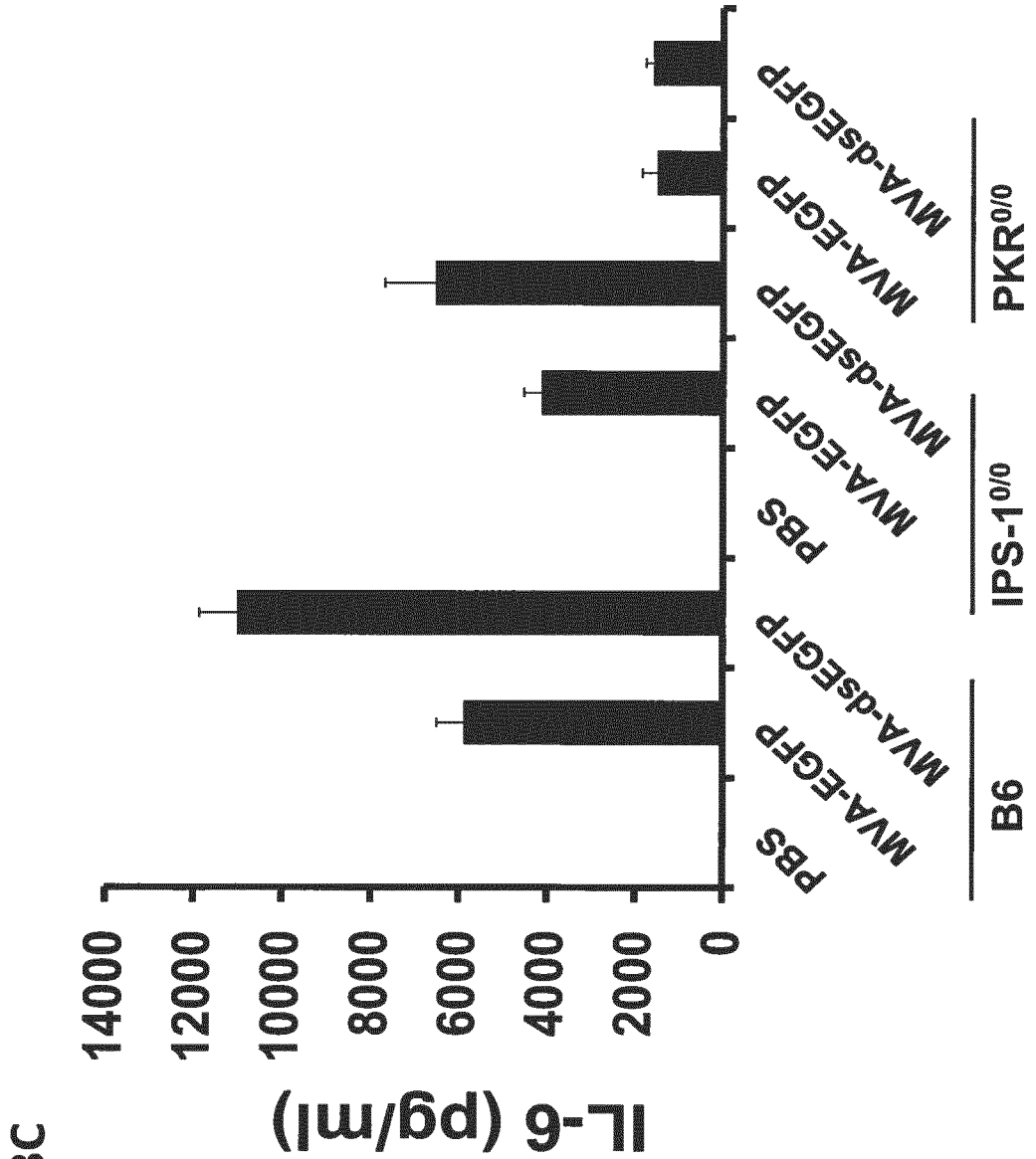

IL-6 (pg/ml)
14000
12000
10000
8000
6000
4000
2000
0
PBS
MVA-EGFP
MVA-dsEGFP
PBS
MVA-EGFP
MVA-dsEGFP
PBS
MVA-EGFP
MVA-dsEGFP
B6
IPS-1$^{0/0}$
PKR$^{0/0}$

Figure 13C

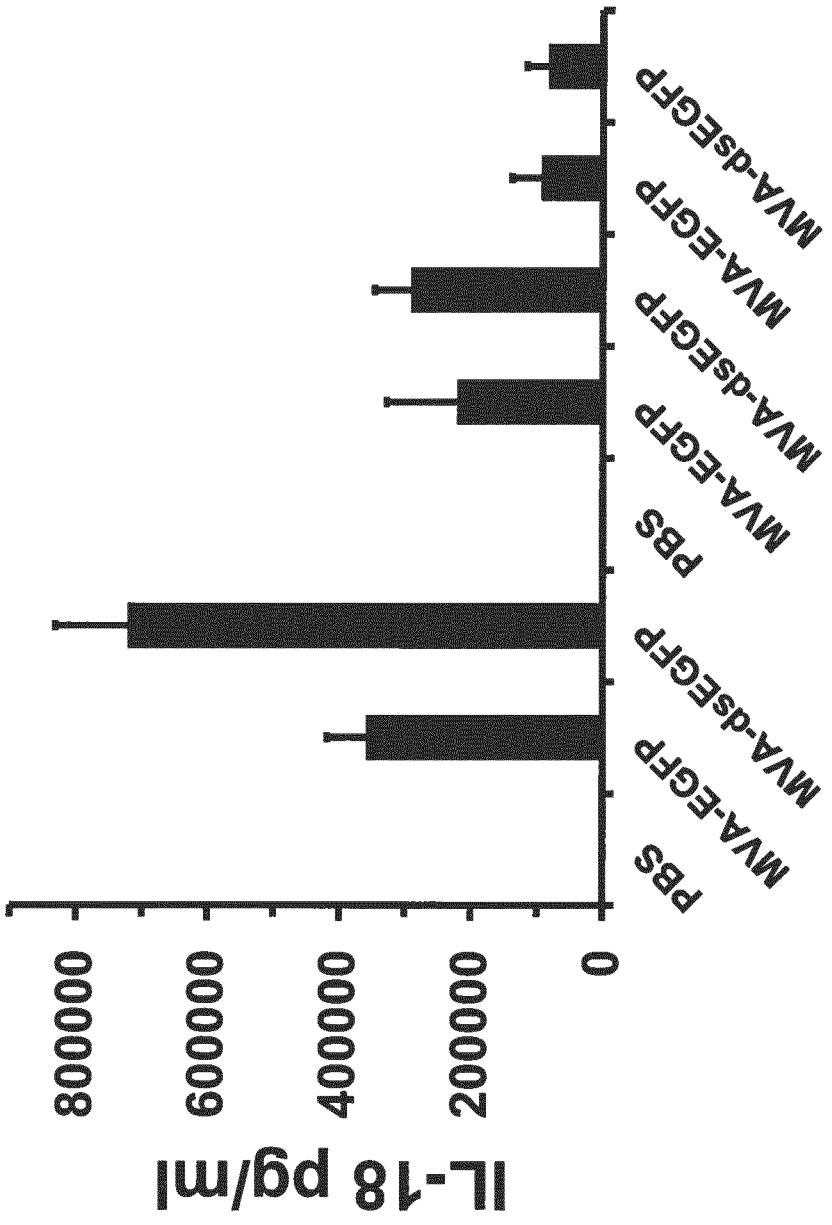

IL-18 pg/ml
800000
600000
400000
200000
0
PBS
MVA-EGFP
MVA-dsEGFP
PBS
MVA-EGFP
MVA-dsEGFP
MVA-EGFP
MVA-dsEGFP

Figure 13D

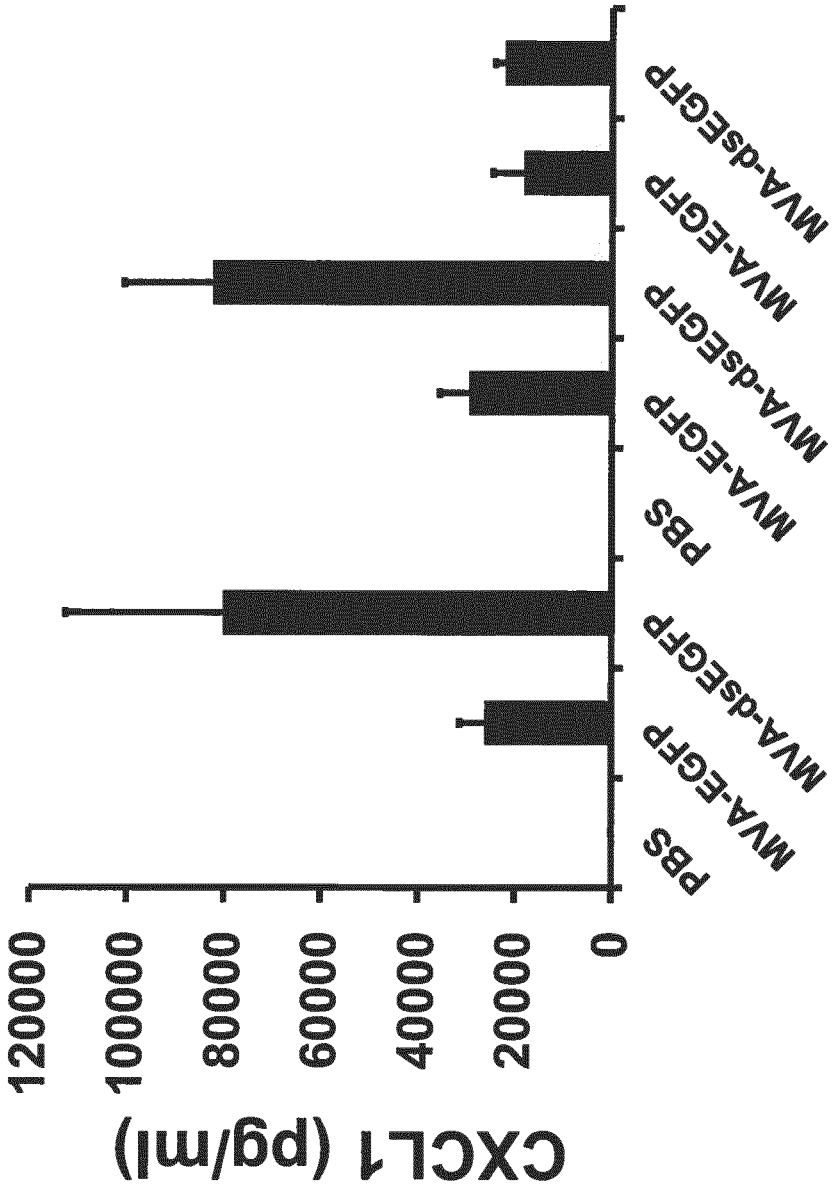

CXCL1 (pg/ml)
120000
100000
80000
60000
40000
20000
0
PBS
MVA-EGFP
MVA-dsEGFP
PBS
MVA-EGFP
MVA-dsEGFP
MVA-EGFP
MVA-dsEGFP

Figure 13E

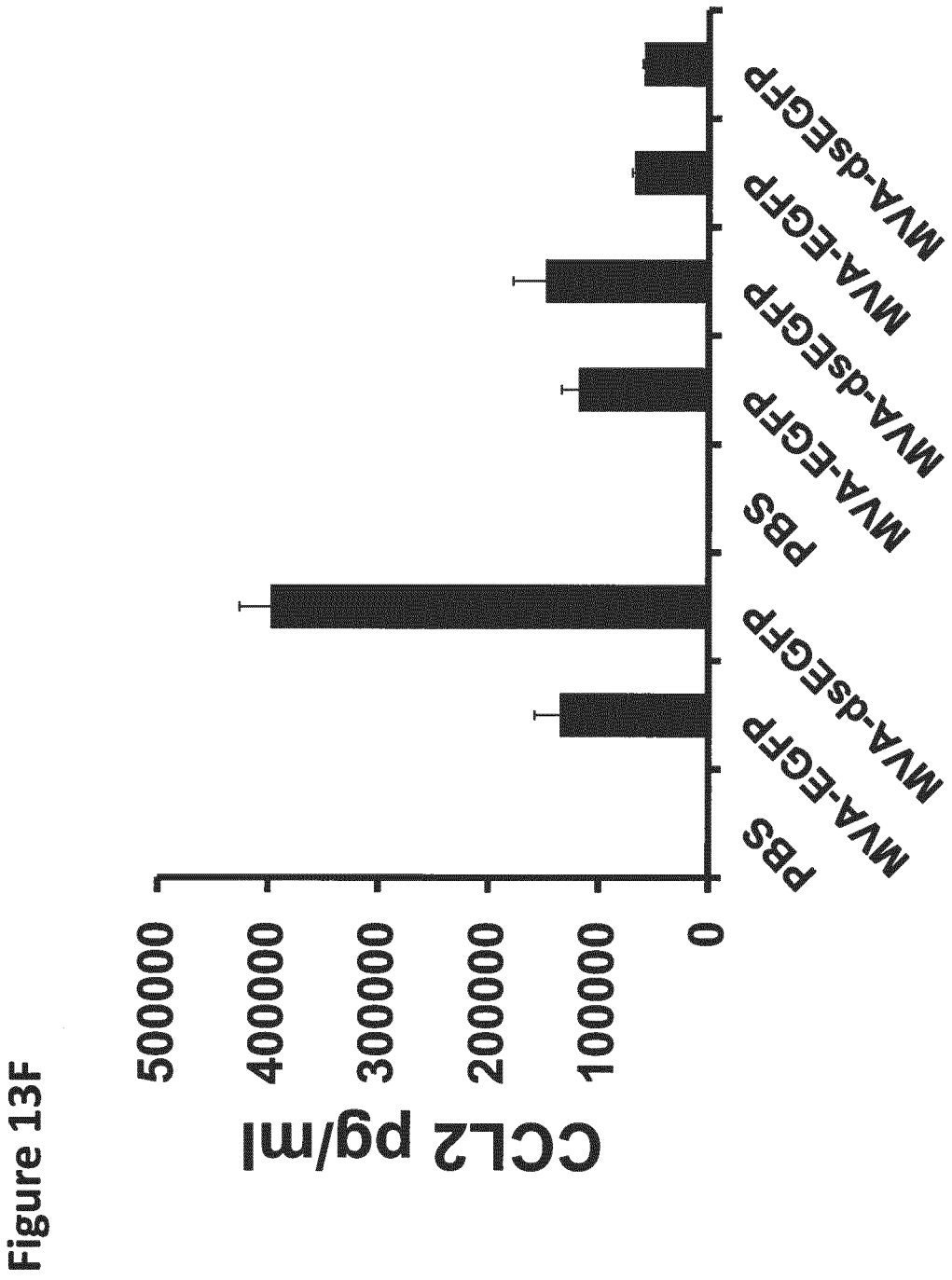

CCL2 pg/ml
500000
400000
300000
200000
100000
0
PBS
MVA-EGFP
MVA-dsEGFP
PBS
MVA-EGFP
MVA-dsEGFP
MVA-EGFP
MVA-dsEGFP

Figure 13F

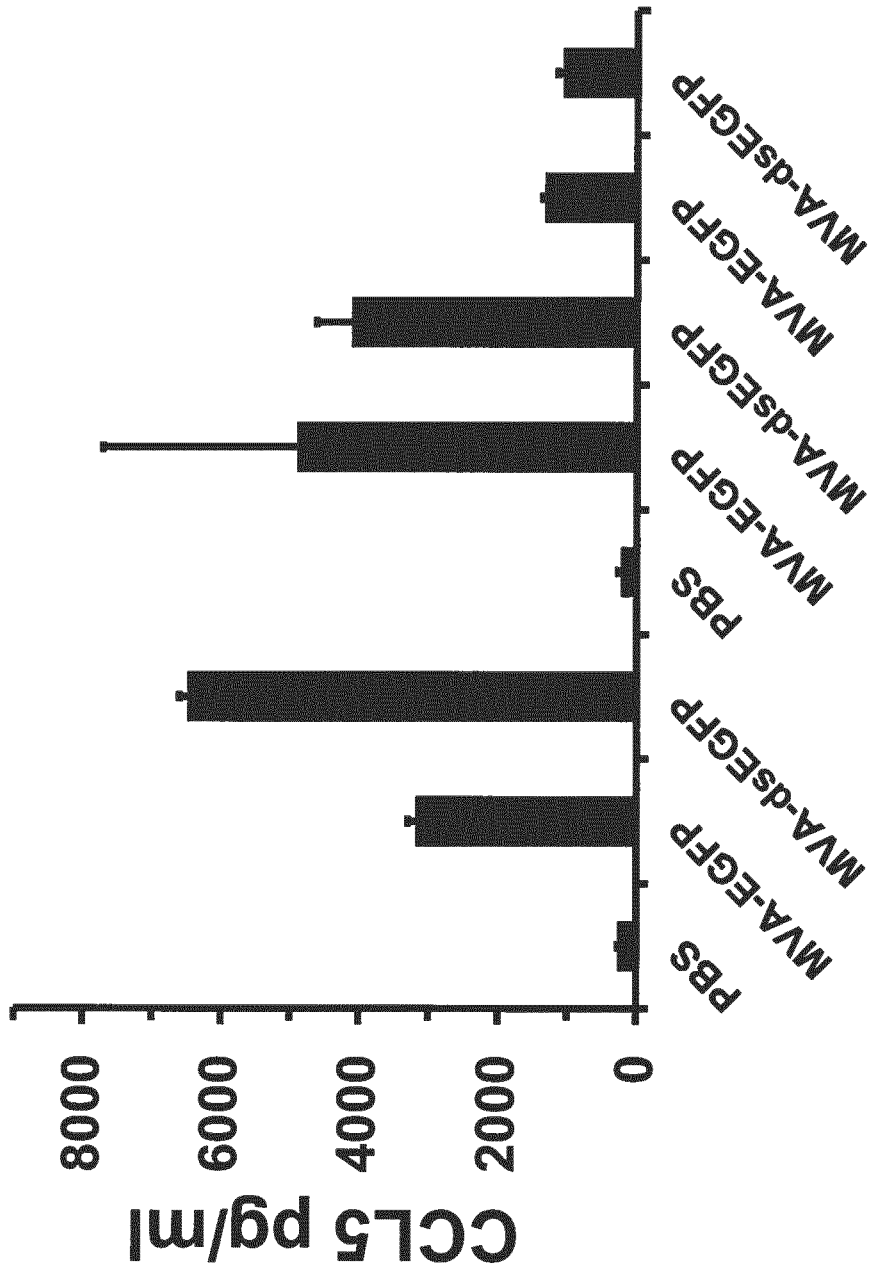

CCL5 pg/ml
8000
6000
4000
2000
0
PBS
MVA-EGFP
MVA-dsEGFP
PBS
MVA-EGFP
MVA-dsEGFP
MVA-EGFP
MVA-dsEGFP

Figure 13G

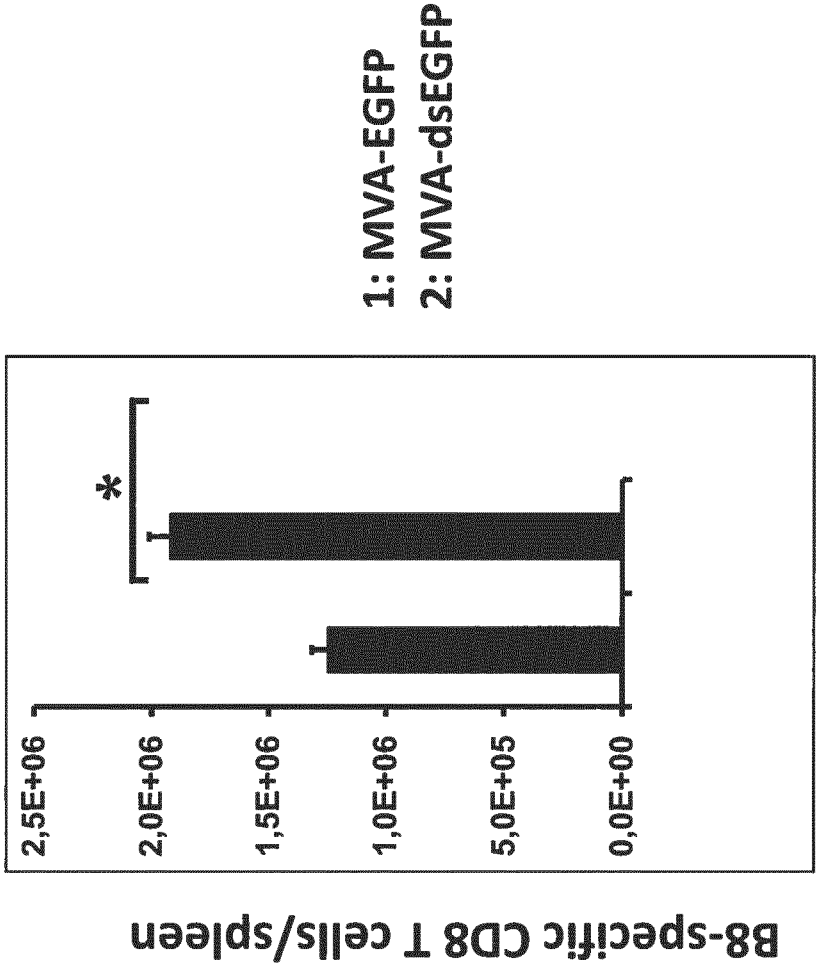

1: MVA-EGFP
2: MVA-dsEGFP
B8-specific CD8 T cells/spleen
2,5E+06
2,0E+06
1,5E+06
1,0E+06
5,0E+05
0,0E+00
*

Figure 14

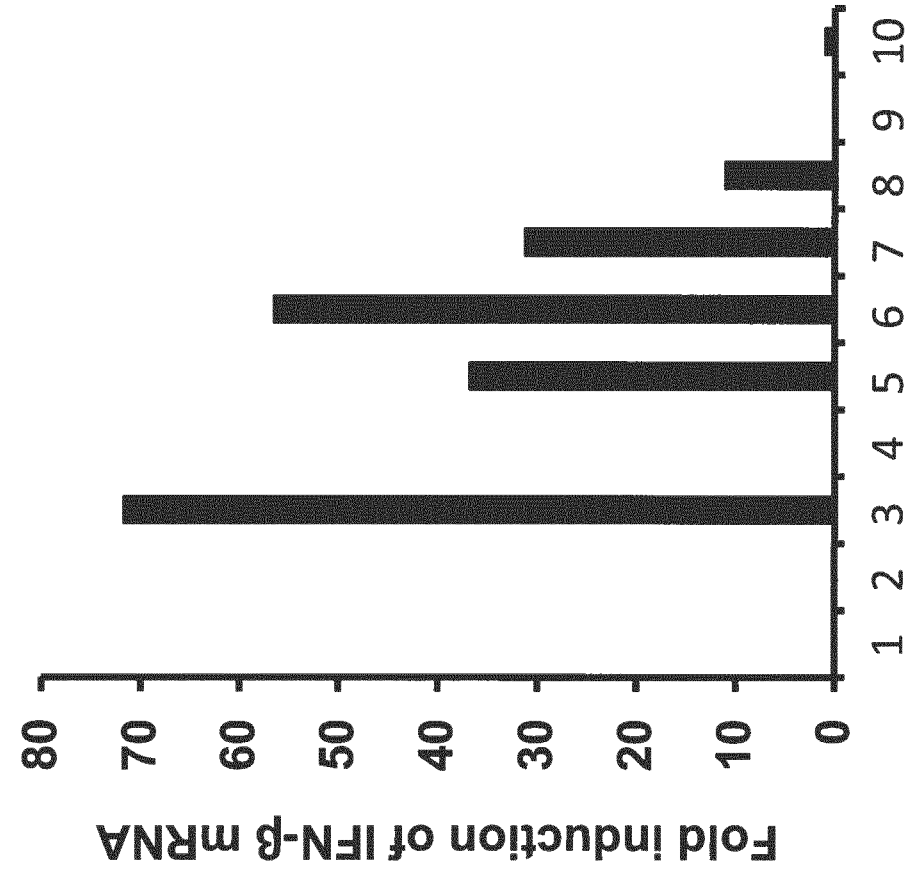

Fold induction of IFN-β mRNA
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9
10

Figure 15

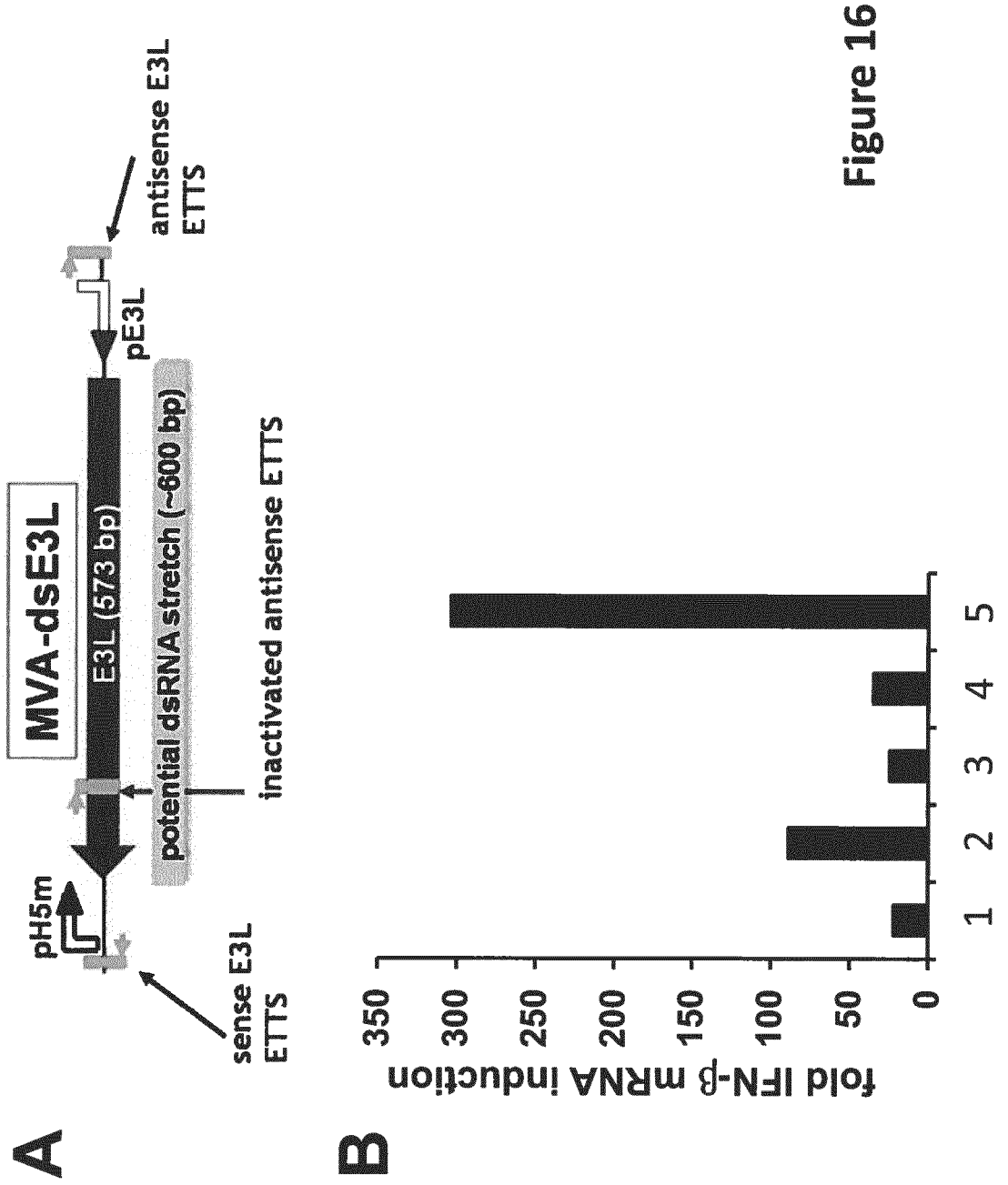

A
MVA-dsE3L
pH5m
E3L (573 bp)
pE3L
potential dsRNA stretch (~600 bp)
inactivated antisense ETTS
sense E3L ETTS
antisense E3L ETTS
B
fold IFN-β mRNA induction
0
50
100
150
200
250
300
350
1
2
3
4
5

Figure 16

Figure 17A

```
Monkeypox          --MMKMKMMVRIYFVSL----SLLLFHSYAI---DIENEITEFFN---KMRDTLPAKDSK
Vaccinia-WR        ---MTMKMMVHIYFVSL----LLLLFHSYAI---DIENEITEFFN---KMRDTLPAKDSK
Vaccinia-Copenhagen ---MTMKMMVHIYFVSL----LLLLFHSYAI---DIENEITEFFN---KMRDTLPAKDSK
CVA                -MKMTMKMMVHIYFVSL----LLLLFHSYAI---DIENEITEFFN---KMRDTLPAKDSK
Ectromelia         MMKMTMKMMVRIYFVSLSLSLSLLLFHSYAI---DIENEITEFFN---KMRDTLPAKDSK
Cowpox             -MKMTMKMMVHIYFVSLSLSLSLLLFHSYAI---DIENEITEFFN---KMKDTLPAKDSK
Camelpox           -MKMTMKMMVHIYFVSLS--LSLLLFHSYAI---DIENEITDFFN---KMKDILPTKDSK
Swinepox           --MISIK----KYN-------ILLFTISFIYCSADND--IDSLYE---GYKEFLDPKLKQ
Tanapox            -----MK----ITY--------IILLICKEIICDNSGDDMYDYIANGNIDYLKTIDNDIIN
                         :*          :*:: .     . :   : :     . :  .   :

Monkeypox          WLNPVCMFGGTMNDMAALGEPFSAKCPPIEDSLLSHRYK---DYVVKWERLEKNRRRQVS
Vaccinia-WR        WLNPACMFGGTMNDIAALGEPFSAKCPPIEDSLLSHRYK---DYVVKWERLEKNRRRQVS
Vaccinia-Copenhagen WLNPACMFGGTMNDIAALGEPFSAKCPPIEDSLLSHRYK---DYVVKWERLEKNRRRQVS
CVA                WLNPACMFGGTMNDIAALGEPFSAKCPPIEDSLLSHRYK---DYVVKWERLEKNRRRQVS
Ectromelia         WLNPSCMFGGTMNDMAALGEPFSAKCPPIEDSLLSHRYNDK-DNVVNWEKIGKTRR--PL
Cowpox             WLNPACMFGGTMNDMAAIGEPFSAKCPPIEDSLLSHRYKDK-DNVVNWEKIGKTRR--PL
Camelpox           WLNPACMFGGTTNDMAAIGEPFSAKCPPIEDSLLSHRYKNK-DNVVNWEKIGKTKR--PL
Swinepox           FLNDNCTYRGYRDFFLYNEEPANIKCPLLNDILLRQKYH---NYTILWKKLGERSSR--L
Tanapox            LVNKNCSFREIKTTLAKENEVLMLKCPQLDNYILPWKYMNRSEYTVTWKNISNSTEY--N
                     :*  * :     :    *    *** ::: :*  :*    : .: *:.: :
```

Figure 17B

```
Monkeypox           NKRVKHGDLWIANYTSKFSNRRYLCTVTTKNGDCVQGVVR-SHVWKPSSCIPKTYELGTY
Vaccinia-WR         NKRVKHGDLWIANYTSKFSNRRYLCTVTTKNGDCVQGIVR-SHIRKPPSCIPKTYELGTH
Vaccinia-Copenhagen NKRVKHGDLWIANYTSKFSNRRYLCTVTTKNGDCVQGIVR-SHIKKPPSCIPKTYELGTH
CVA                 NKRVKHGDLWIANYTSKFSNRRYLCTVTTKNGDCVQGIVR-SHIKKPPSCIPKTYELGTH
Ectromelia          NRRVKNGDLWIANYTSNDSHRRYLCTVTTKNGDCVQGIVR-SHIRKPPSCIPETYELGTH
Cowpox              NRRVKNGDLWIANYTSNDSRRRYLCTVITKNGDCIQGIVR-SHVRKPSSCIPEIYELGTH
Camelpox            NRRVKNGDLWIANYTSNDSRRRYLCTAITKNGDCIQGIIR-SHVRKPSSCIPEIYELGTH
Swinepox            LNTHGSIFLDFFPYKSELRGSVYECMIILNN-TCDQFILKLNDIRSNPVCYHNDYK----
Tanapox             NTRIENNMLMFFPFYNLQAGSKYLCTVSTNK-SCDQSVVIVKKSFYSNNCMLSEAK----
                              * :  : . .     * *    ::  * * :: ..     *   .  :

Monkeypox           DKYGIDLYCGILYAKHYNNITWYKDNKEINIDDFKYSQAGK--ELIIHNPELEDSGRYDC
Vaccinia-WR         DKYGIDLYCGILYAKHYNNITWYKDNKEINIDDIKYSQTGK--ELIIHNPELEDSGRYDC
Vaccinia-Copenhagen DKYGIDLYCGILYAKHYNNITWYKDNKEINIDDIKYSQTGK--KLIIHNPELEDSGRYNC
CVA                 DKYGIDLYCGILYAKHYNNITWYKDNKEINIDDIKYSQTGK--KLIIHNPELEDSGRYNC
Ectromelia          DKYGIDLYCGILYAKHYNNITWYKNNQELIIDGTKYSQSGQ--NLIIHNPELEDSGRYDC
Cowpox              DKYGIDLYCGIIYAKHYNNITWYKDNKEINIDDIKYSQTGK--ELIIHNPALEDSGRYDC
Camelpox            DKYGIDLYCGIIYAKHYNNITWYKDNKEINIDDIKYSQTGK--ELIIHNPALEDSGRYDC
Swinepox            VHTNIEIFCNVINL-QYDYITWYKNNSEIIIDGYKYSNQSR--RLLVYNTTYNDSGIYYC
Tanapox             ENDNFEIYCGILHA-KYNTIKWFKEEKEITN-NYKYYTKLGGYVKGINNVTYSDSGKYVC
                     .  :::::* ::   :*: *.*:*::.*:     **        : *   .*** * *
```

Figure 17C

```
Monkeypox           YVHYDDVRIKNDIVVSRCKILTVIPSQDHRFKLILDPKINVTI--GEPANITCSAVSTSL
Vaccinia-WR         YVHYDDVRIKNDIVVSRCKILTVIPSQDHRFKLILDPKINVTI--GEPANITCTAVSTSL
Vaccinia-Copenhagen YVHYDDVRIKNDIVVSRCKILTVIPSQDHRFKLILDPKINVTI--GEPANITCTAVSTSL
CVA                 YVHYDDVKIKNDIVVSRCKILTVIPSQDHRFKLILDPKINVTI--GEPANITCTAVSTSL
Ectromelia          YVHYDDVRIKNDIVVSRCKILTVIPSQDHRFKLILDPKINVTI--GEPANITCTAVSTSL
Cowpox              YVHYDDVRIKNDIVVSRCKILTVIPSQDHRFKLILDPKINVTI--GEPANITCTAVSTSL
Camelpox            YVHYDDVRIKNDIVVSRCKILTVTPSQDHRFKLILDPKINVTI--GEPANITCTAVSTSL
Swinepox            NAYTTH--GKNTYISRRCSSVSIHSHSYYDFYIEHINNITYIDPDSENTQIYCKA-ISYS
Tanapox             EGYYIDVLKNITYTAKRCVNLTVIPNTYYDFFIVDIPNVTYAKNNKKLE-VNCTSFVDIN
                       :  .   :      **   :::    :  * :     ::.      :   : *.:

Monkeypox           FVDDVLIEWKNPS-GWIIGLDFGVYSI-----LTSRGGITEATLYFENVTEEYIGNTYTC
Vaccinia-WR         LIDDVLIEWENPS-GWLIGFDFDVYSV-----LTSRGGITEATLYFENVTEEYIGNTYKC
Vaccinia-Copenhagen LIDDVLIEWENPS-GWLIGFDFDVYSV-----LTSRGGITEATLYFENVTEEYIGNTYKC
CVA                 LIDDVLIEWENPS-GWLIGFDFDVYSV-----LTSRGGITEATLYFENVTEEYIGNTYKC
Ectromelia          LVDDVLIDWENPS-GWIIGLDFGVYSI-----LTSSGGITEATLYFENVTEEYIGNTYTC
Cowpox              LVDDVLIEWENPS-GWLIGFDFDVYSV-----LTSRGGITEATLYFENVTEEYIGNTYKC
Camelpox            LVDDVLIEWENPS-GWLIGFDFDVYSV-----LTSRGGITEATLYFENVTEEYIGNTYKC
Swinepox            NSSYILIYWEDEYGGYIYDNGI--YQYDNITLIGNEKVYMSILVLEKSAYYRYVVNNTFTC
Tanapox             SYDYILLTSWLY--NGLYLPLGVRIYQLYSTDIFFENFIYRTSTLVFENVDISDDNKTFEC
                       . :*  *    *      .  *.      :  .      :   :..      :*: *
```

Figure 17D

```
Monkeypox           RGHNYYFDKTLTTTVVLE--
Vaccinia-WR         RGHNYYFEKTLTTTVVLE--
Vaccinia-Copenhagen RGHNYYFEKTLTTTVVLE--
CVA                 RGHNYYFEKTLTTTVVLE--
Ectromelia          RGHNYYFDKTLTTTVVLE--
Cowpox              RGHNYYFEKTLTTTVVLE--
Camelpox            RGHNYYFEKTLTTTVVLE--
Swinepox            LATSVYVEKKTTTTLVIKKT
Tanapox             EALSVTLKKIKYTTIKVEK-
                      . .  ..*   **: ::
```