FIGURE 1

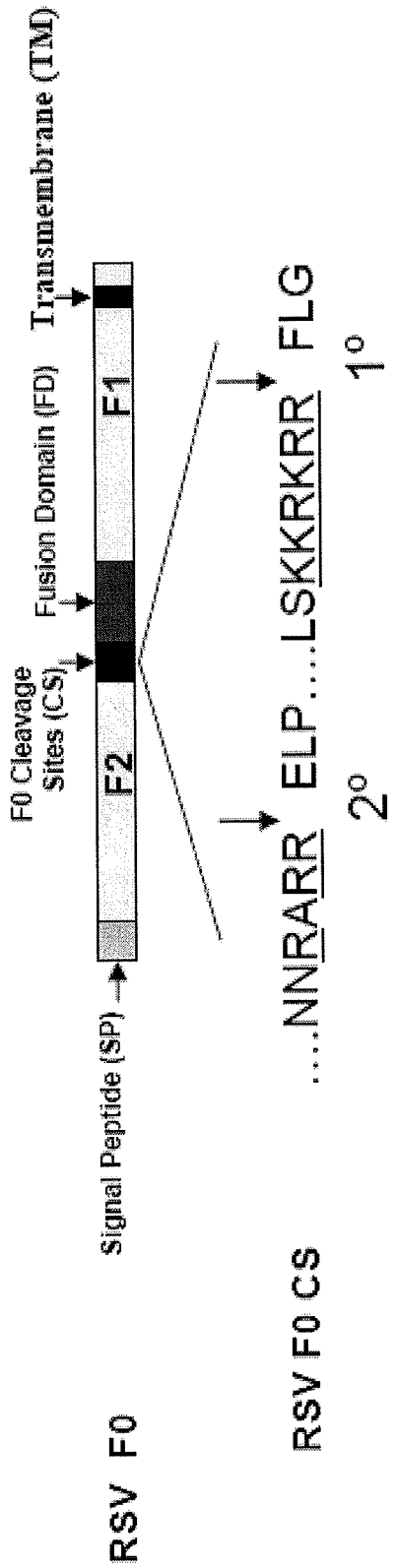

Transmembrane (TM)
Fusion Domain (FD)
F0 Cleavage Sites (CS)
F1
F2
Signal Peptide (SP)
RSV F0
RSV F0 CS
....NNRARR
ELP....LSKKRKRR
FLG
2°
1°

FIGURE 2

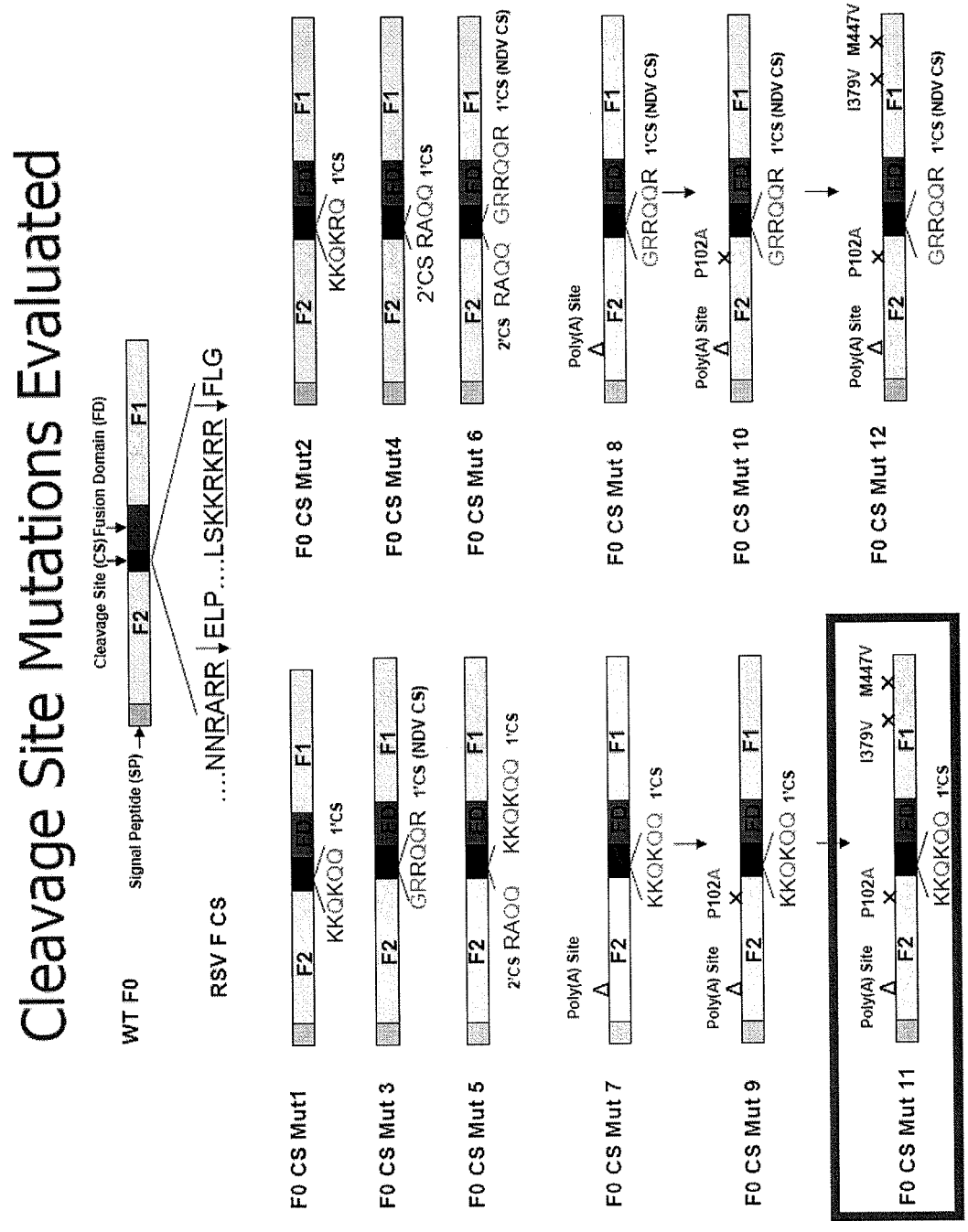

Cleavage Site Mutations Evaluated
Cleavage Site (CS)
Fusion Domain (FD)
Signal Peptide (SP)
F2
F1
WT F0
RSV F CS
.....NNRARR↓ELP.....LSKKRKRR↓FLG
F0 CS Mut1
KKQKQQ 1°CS
F0 CS Mut2
KKQKRQ 1°CS
F0 CS Mut3
GRRQQR 1°CS (NDV CS)
F0 CS Mut4
2°CS RAQQ 1°CS
F0 CS Mut5
2°CS RAQQ KKQKQQ 1°CS
F0 CS Mut6
2°CS RAQQ GRRQQR 1°CS (NDV CS)
Poly(A) Site
F0 CS Mut7
F0 CS Mut8
P102A
F0 CS Mut9
F0 CS Mut10
I379V
M447V
F0 CS Mut11
F0 CS Mut12

FIGURE 3

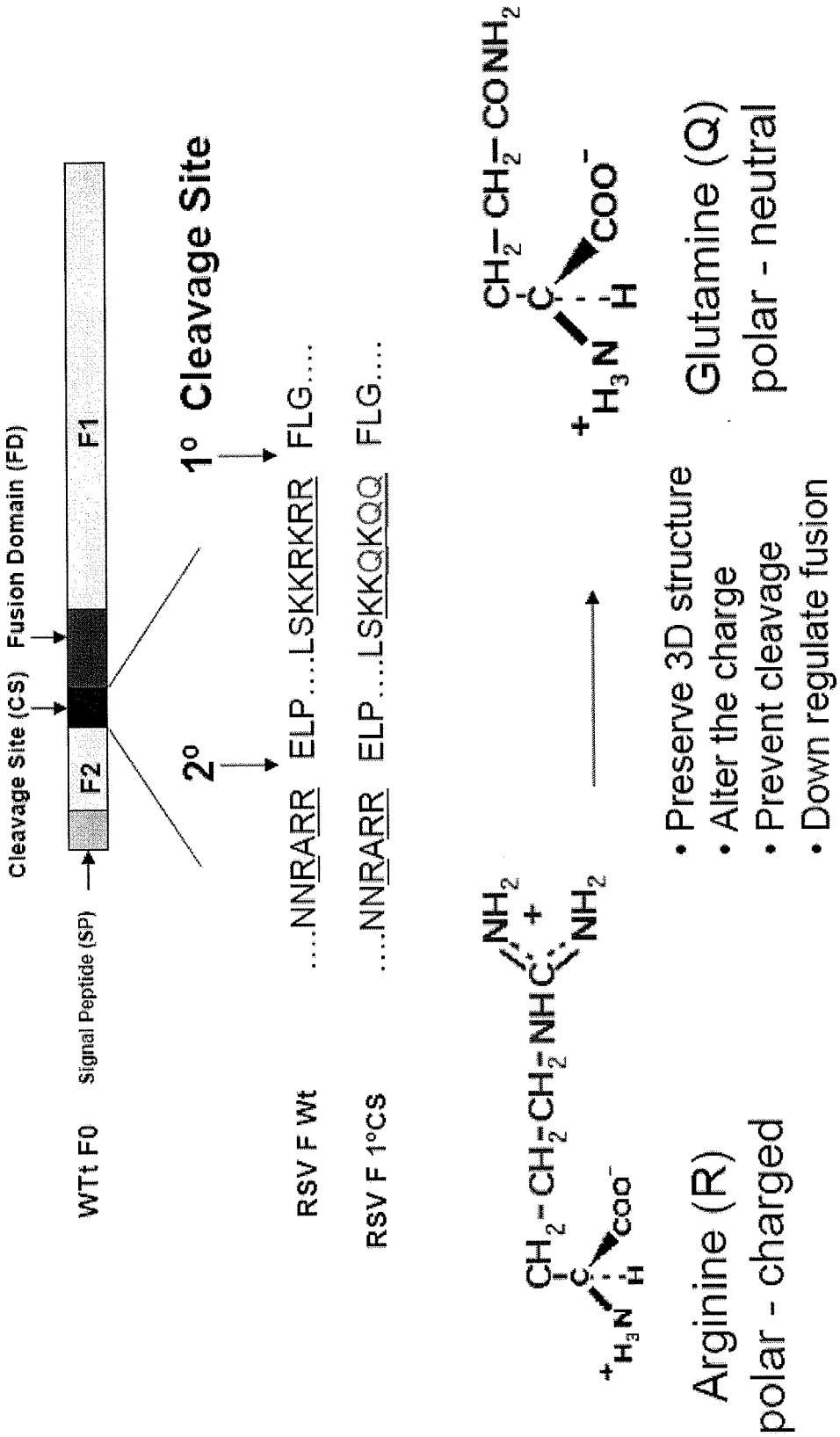
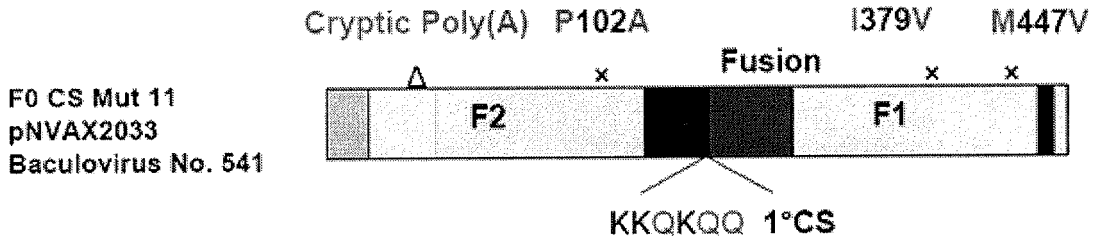

Cleavage Site (CS)
Fusion Domain (FD)
Signal Peptide (SP)
F2
F1
WTt F0
1° Cleavage Site
2°
RSV F Wt
.....NNRARR ELP....LSKKRKRR FLG....
RSV F 1°CS
.....NNRARR ELP....LSKKQKQQ FLG....
Arginine (R)
polar - charged
Glutamine (Q)
polar - neutral
• Preserve 3D structure
• Alter the charge
• Prevent cleavage
• Down regulate fusion

FIGURE 4

**Modified RSV F0 (GeneBank Assession AAB59858): pNVAX 2023 clone**

MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT
GWYTSVITIE LSNIKENKCN GTDAKVKLIK QELDKYKNAV TELQLLMQST
PATNNRARRE LPRFMNYTLN NAKKTNVTLS KKQKQQFLGF LLGVGSAIAS
GVAVSKVLHL EGEVNKIKSA LLSTNKAVVS LSNGVSVLTS KVLDLKNYID
KQLLPIVNKQ SCSISNIETV IEFQQKNNRL LEITREFSVN AGVTTPVSTY
MLTNSELLSL INDMPITNDQ KKLMSNNVQI VRQQSYSIMS IIKEEVLAYV
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRTDRG WYCDNAGSVS
FFPQAETCKV QSNRVFCDTM NSLTLPSEVN LCNVDIFNPK YDCKIMTSKT
DVSSSVITSL GAIVSCYGKT KCTASNKNRG IIKTFSNGCD YVSNKGVDTV
SVGNTLYYVN KQEGKSLYVK GEPIINFYDP LVFPSDEFDA SISQVNEKIN
QSLAFIRKSD ELLHNVNAGK STTNIMITTI IIVIIVILLS LIAVGLLLYC
KARSTPVTLS KDQLSGINNI AFSN

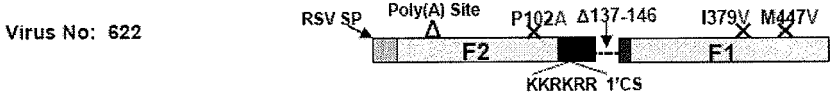
2° Cleavage Site  1° Cleavage Site  Fusion domain




F0 CS Mut 11
pNVAX2033
Baculovirus No. 541

**FIGURE 5**

# Partial deletion (Δ137-146) of F fusion domain




```
Δ137-146    MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIE 60
WT F0       MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIE 60
            ************************************************************

Δ137-146    LSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLN 120
WT F0       LSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLN 120
            ************************************************************

Δ137-146    NAKKTNVTLSKKRKRR----------AIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVS 170
WT F0       NAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVS 180
            ****************          **********************************

Δ137-146    LSNGVSVLTSKVLDLKNHIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLEITREFSVN 230
WT F0       LSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLEITREFSVN 240
            ****************:*******************************************

Δ137-146    AGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYV 290
WT F0       AGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYV 300
            ************************************************************

Δ137-146    VQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKV 350
WT F0       VQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKV 360
            ************************************************************

Δ137-146    QSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKT 410
WT F0       QSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKT 420
            ************************************************************

Δ137-146    KCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDP 470
WT F0       KCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDP 480
            ************************************************************

Δ137-146    LVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNIMITTIIIVIIVILLS 530
WT F0       LVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNIMITTIIIVIIVILLS 540
            ************************************************************

Δ137-146    LIAVGLLLYCKARSTPVTLSKDQLSGINNIAFSN- 564
WT F0       LIAVGLLLYCKARSTPVTLSKDQLSGINNIAFSN- 574
            ********************************
```

FIGURE 6

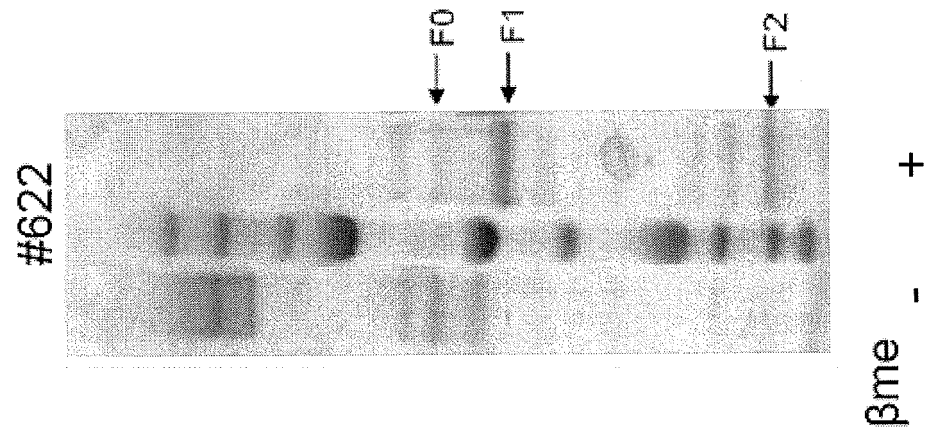
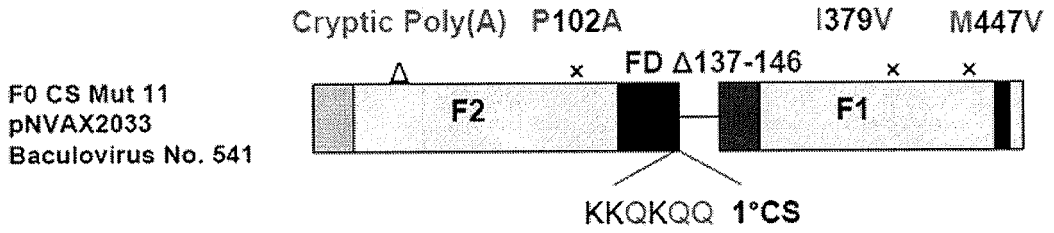


FIGURE 7

# BV #683 Modified RSV F0




**Codon optimized**
**1° cleavage site inactivation (3 conservative aa changes)**
**Cryptic poly(A) in F2 removed**
**3 aa corrections to GenBank sequence**
**Deletion of N-terminal half of the F1 fusion domain**

FIGURE 8

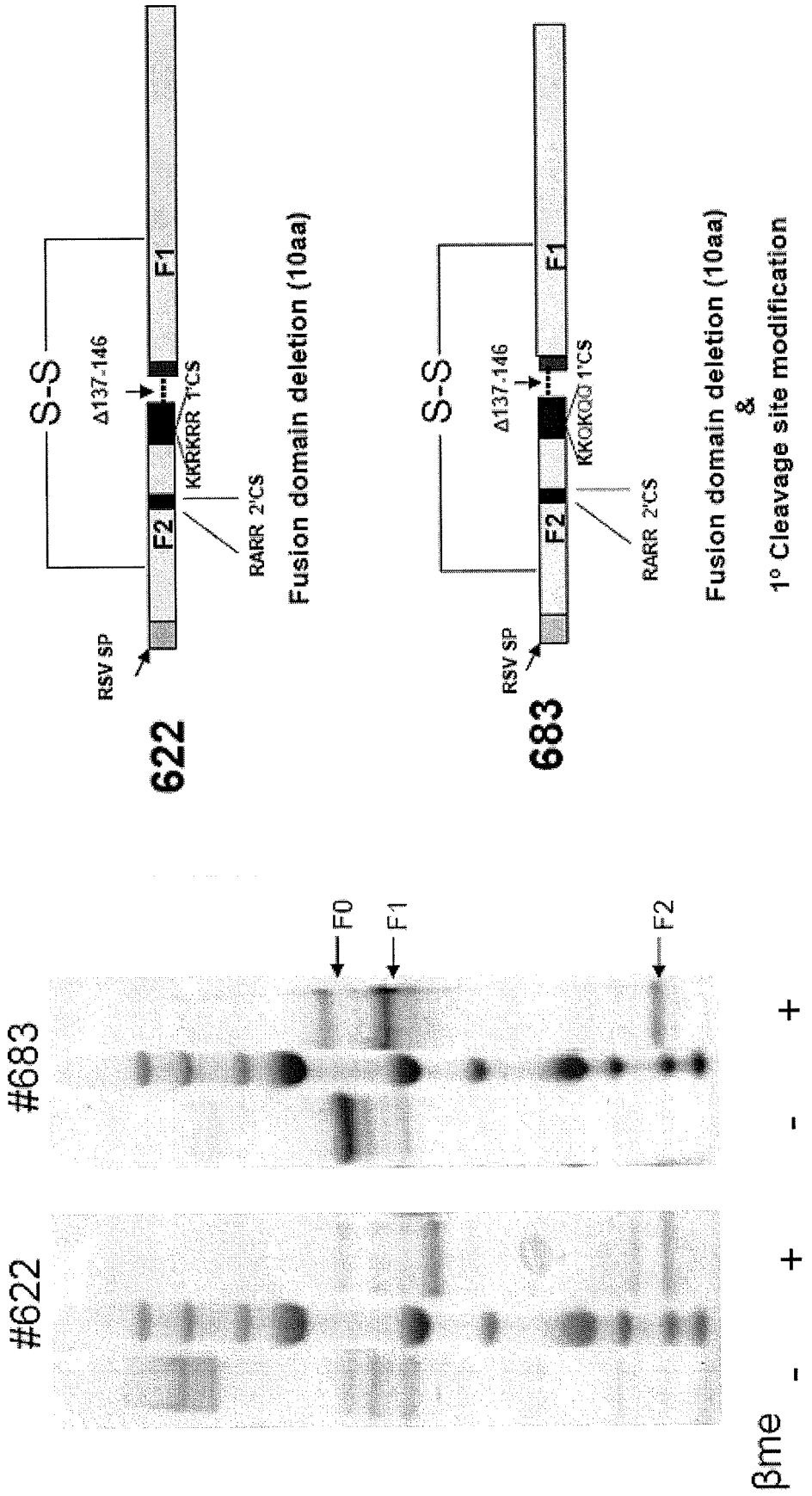

S-S
Δ137-146
F1
F2
RSV SP
KRRKRR 1°CS
RARR 2°CS
Fusion domain deletion (10aa)
622
S-S
Δ137-146
F1
F2
RSV SP
KKQKQQ 1°CS
RARR 2°CS
Fusion domain deletion (10aa)
&
1° Cleavage site modification
683
F0
F1
F2
#683
#622
βme - + - +

FIGURE 9

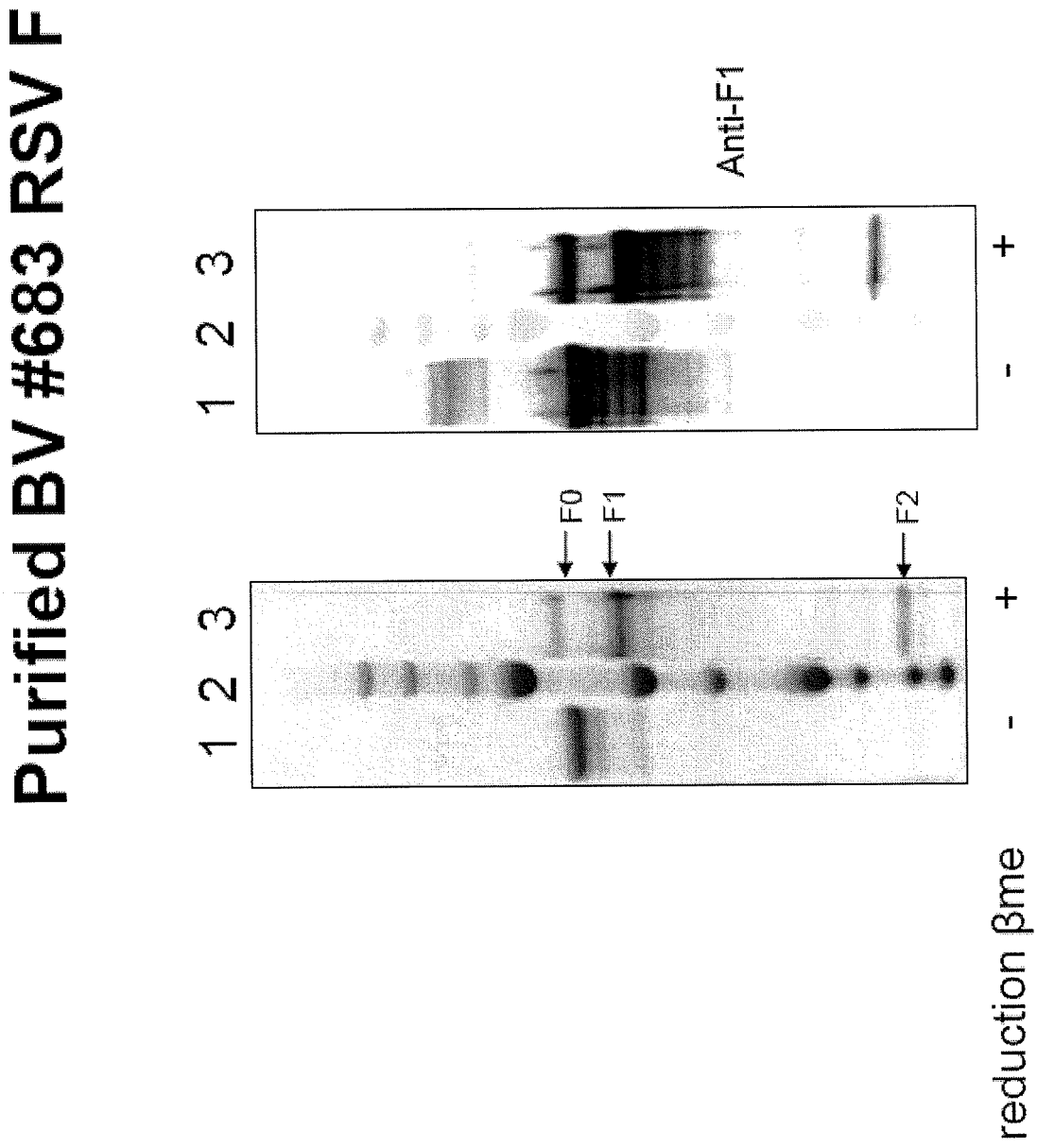


FIGURE 10

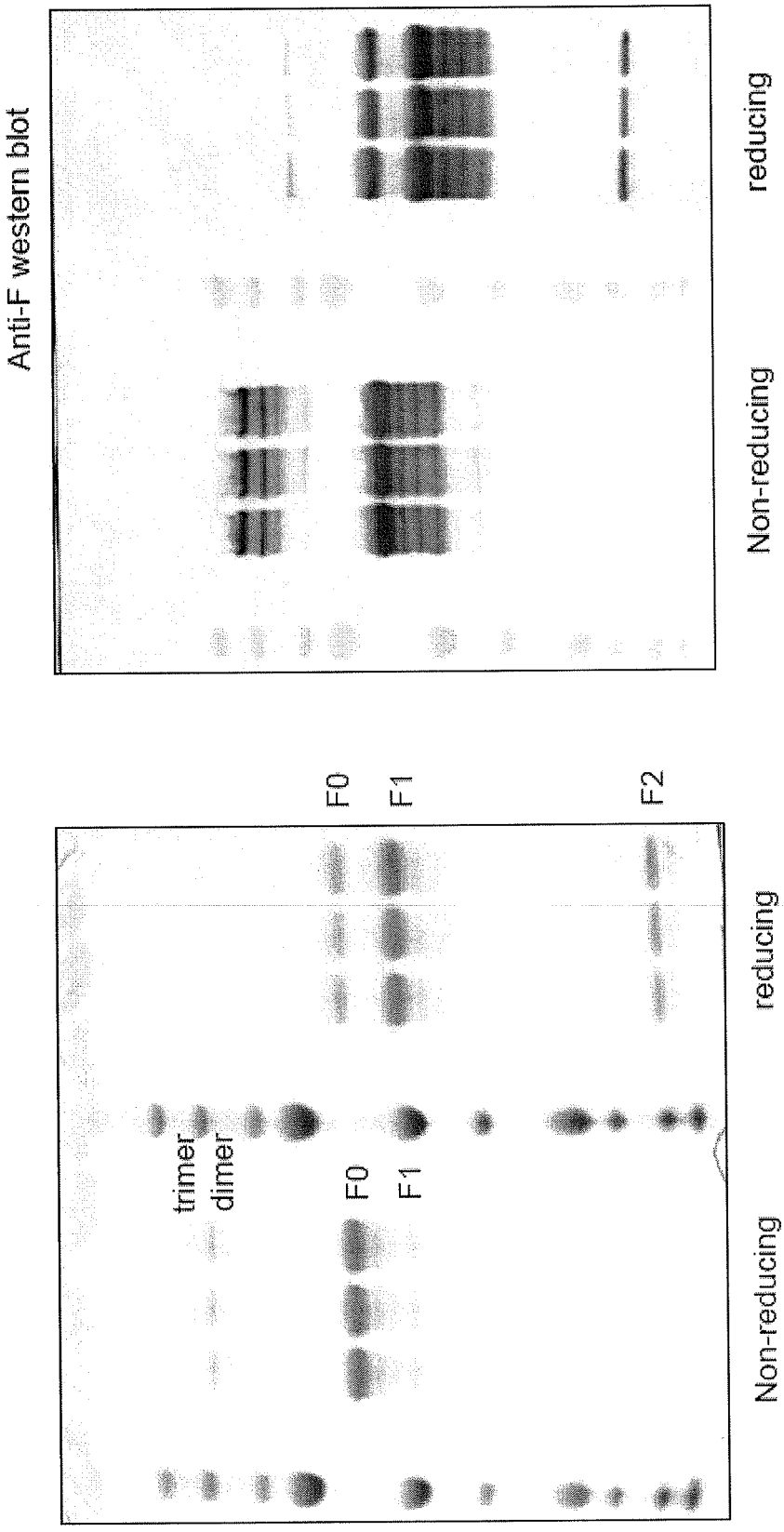

Anti-F western blot
reducing
Non-reducing
F0
F1
F2
trimer
dimer
F0
F1
reducing
Non-reducing
Purity >98% scanning densitometry

FIGURE 11

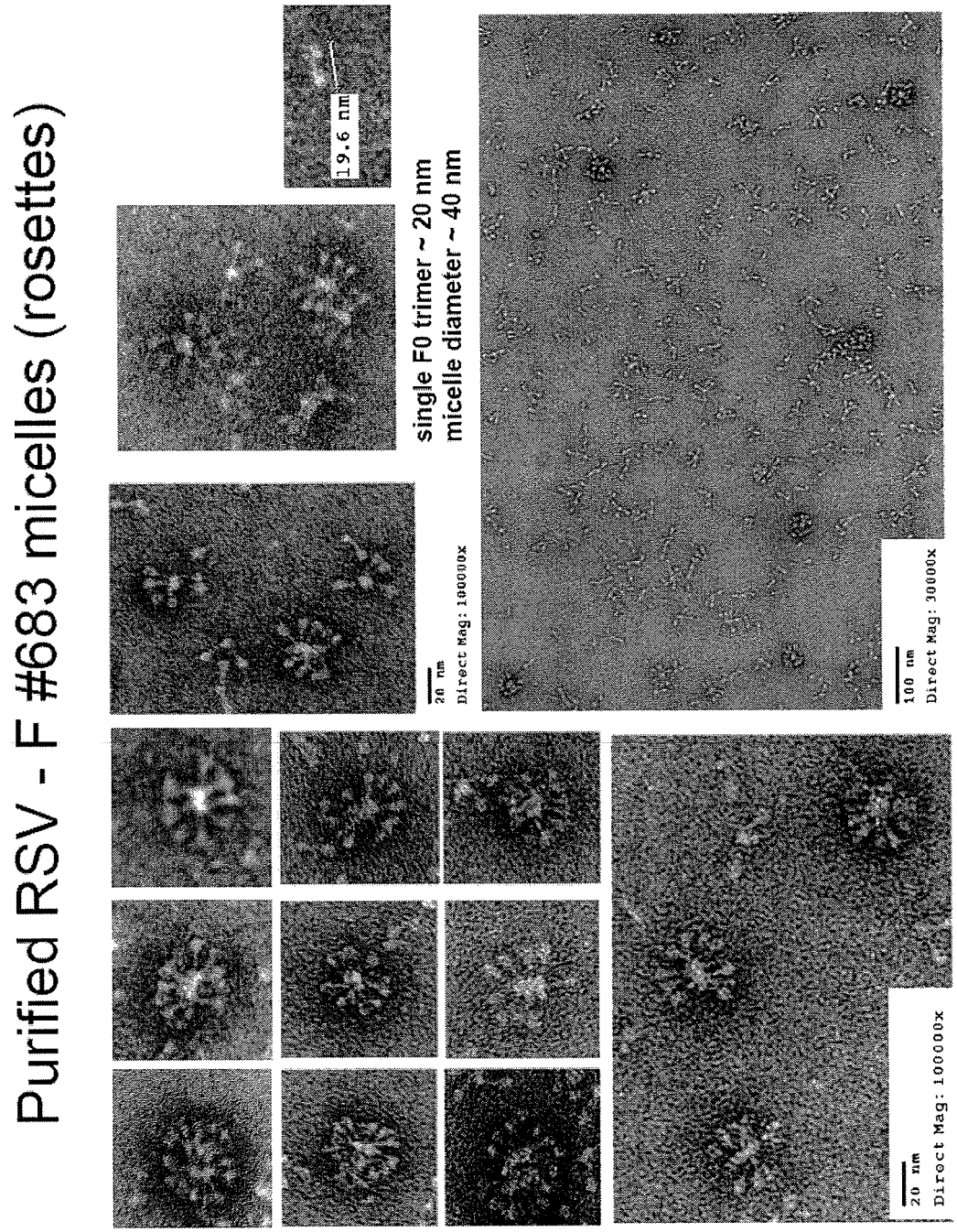

Purified RSV - F #683 micelles (rosettes)
19.6 nm
single F0 trimer ~ 20 nm
micelle diameter ~ 40 nm
20 nm
Direct Mag: 100000x
100 nm
Direct Mag: 30000x
20 nm
Direct Mag: 100000x

FIGURE 12

Particle size analysis shows RSV 683 F0 forms 40 nm particles

Results

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 40.2 | Peak 1: | 91.3 | 98.4 | 155 |
| PdI: 0.383 | Peak 2: | 4390 | 1.6 | 938 |
| Intercept: 0.823 | Peak 3: | 0.00 | 0.0 | 0.00 |

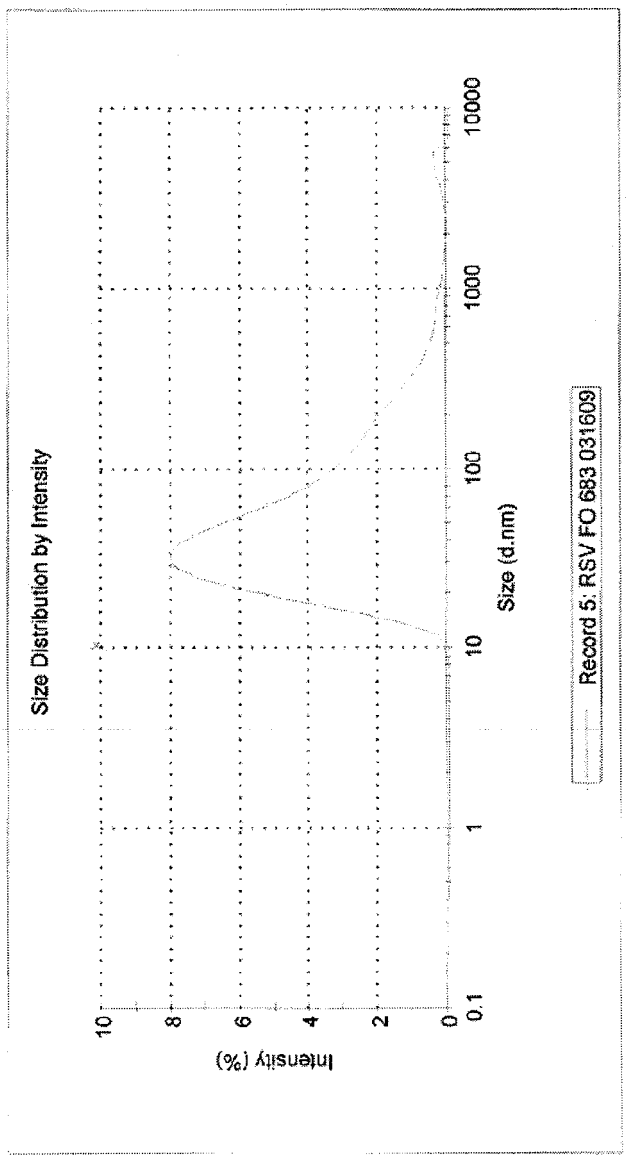


FIGURE 13

# Modified RSF F: BV #622 and #623

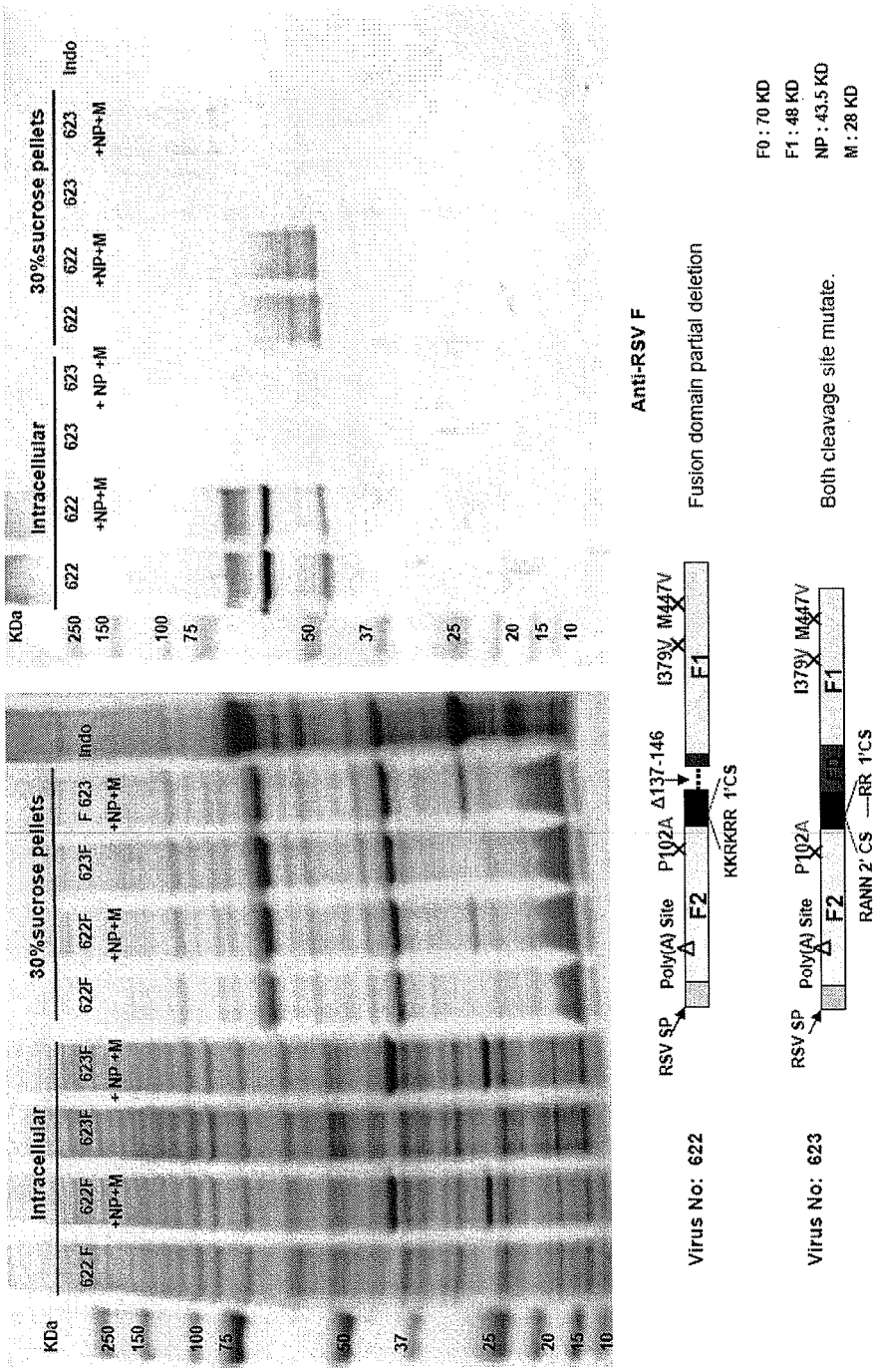


FIGURE 14

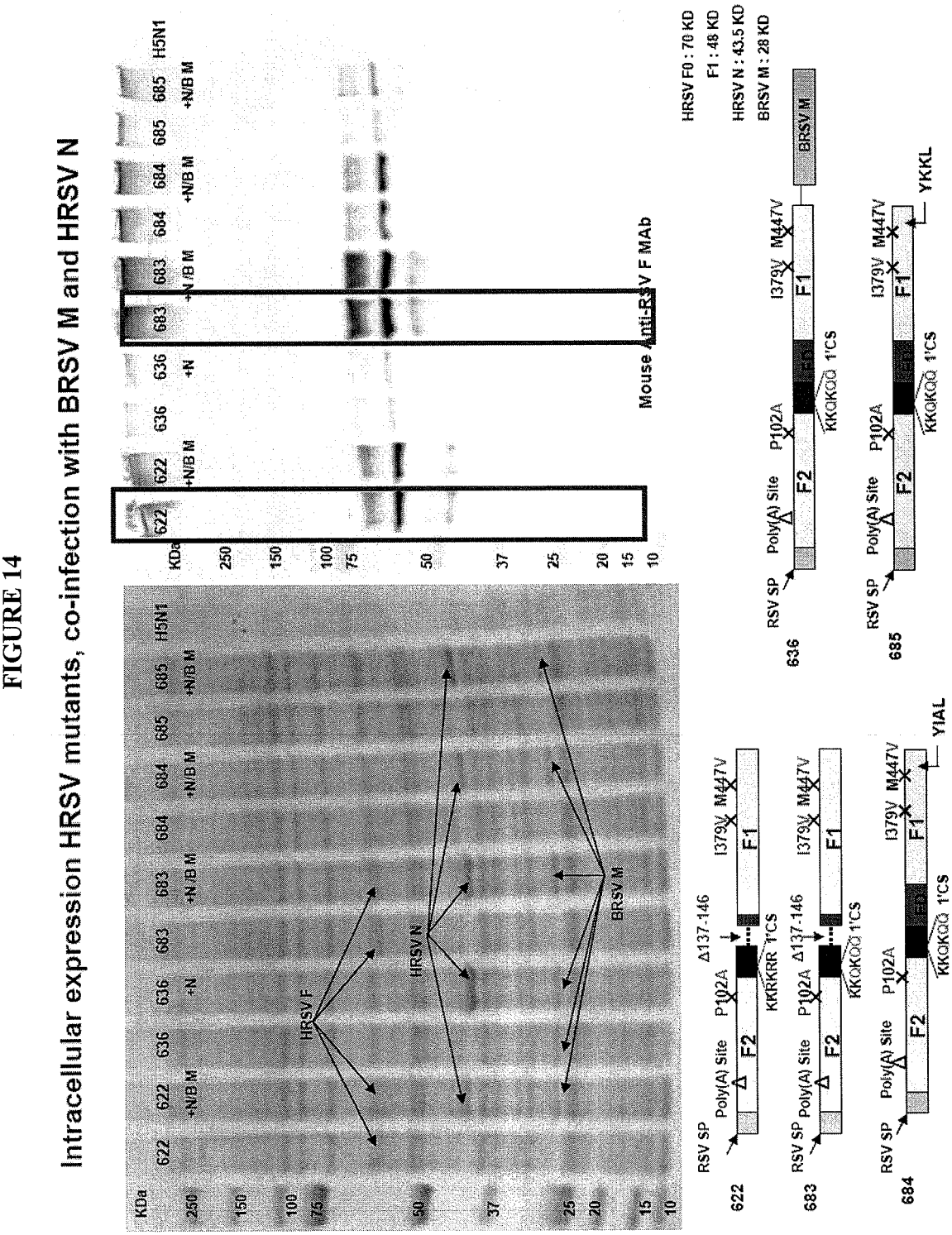
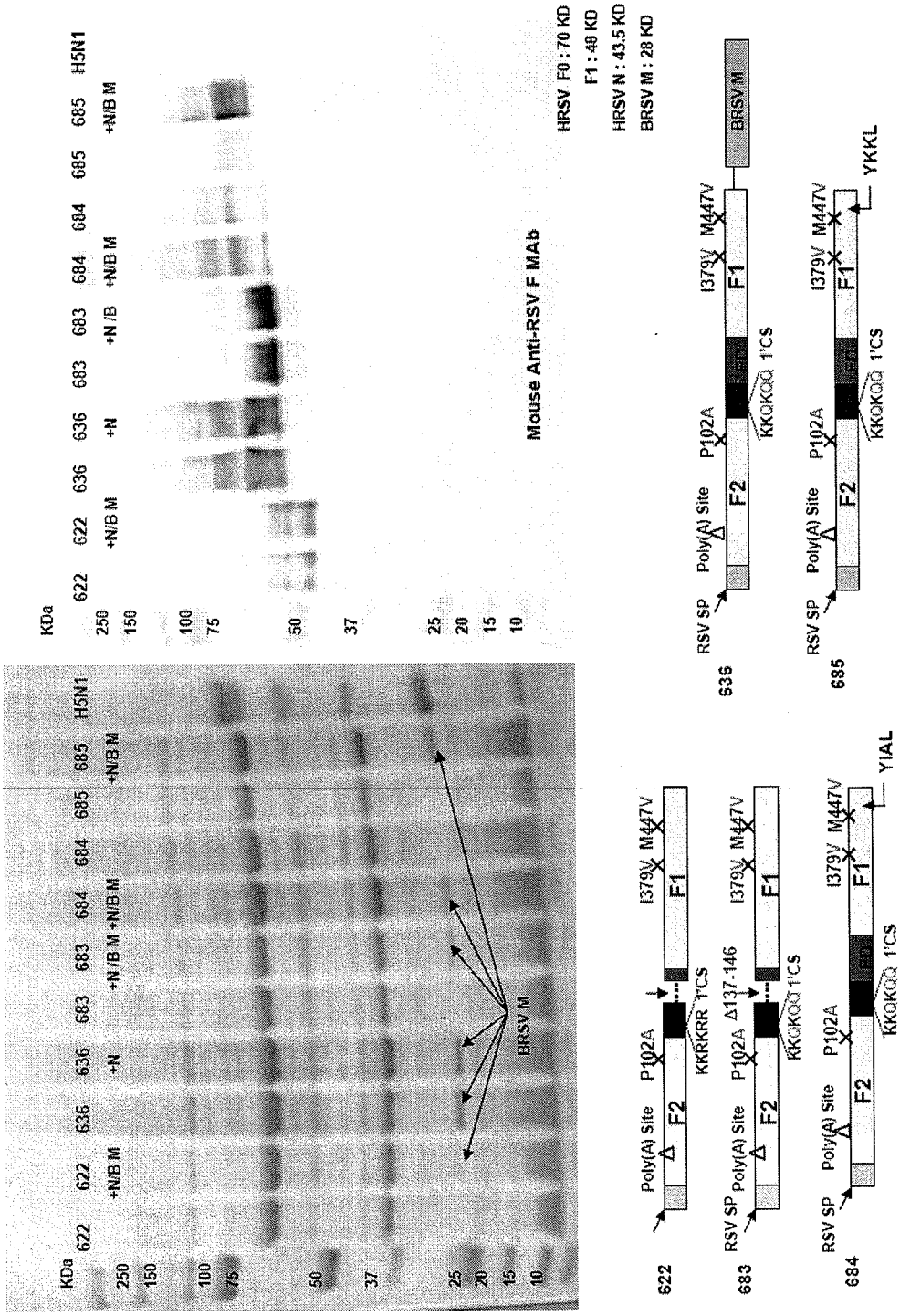
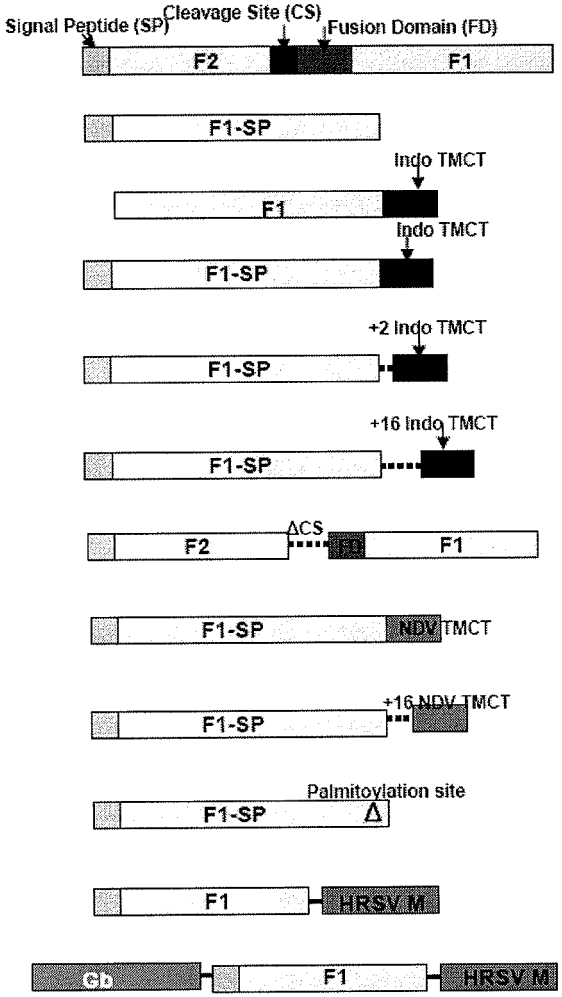

Intracellular expression HRSV mutants, co-infection with BRSV M and HRSV N
KDa
622
636
683
684
685
H5N1
+N/B M
+N
+N /B M
HRSV F
HRSV N
BRSV M
Mouse Anti-RSV F MAb
HRSV F0 : 70 KD
F1 : 48 KD
HRSV N : 43.5 KD
BRSV M : 28 KD
RSV SP
Poly(A) Site
F2
F1
P102A
Δ137-146
KKRKRR 1'CS
KKQKQG 1'CS
I379V
M447V
YIAL
YKKL

FIGURE 15

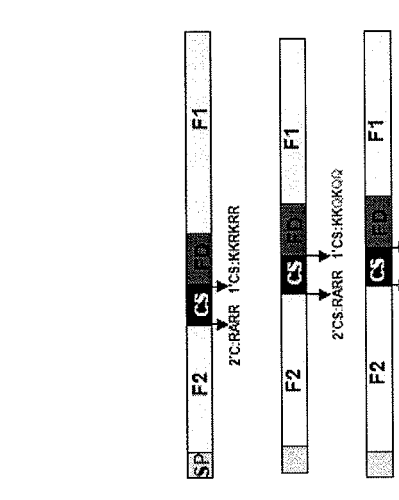

30% sucrose VLPs HRSV mutants, co-infection with BRSV M and HRSV N
KDa
622
636
683
684
685
H5N1
+N/B M
+N
+N /B
+N /B M
BRSV M
Mouse Anti-RSV F MAb
HRSV F0 : 70 KD
F1 : 48 KD
HRSV N : 43.5 KD
BRSV M : 28 KD
RSV SP
Poly(A) Site
F2
F1
P102A
I379V
M447V
KKRKRR 1'CS
KKQKQQ 1'CS
Δ137-146
YIAL
YKKL
622
683
684
636
685

# FIGURE 16a



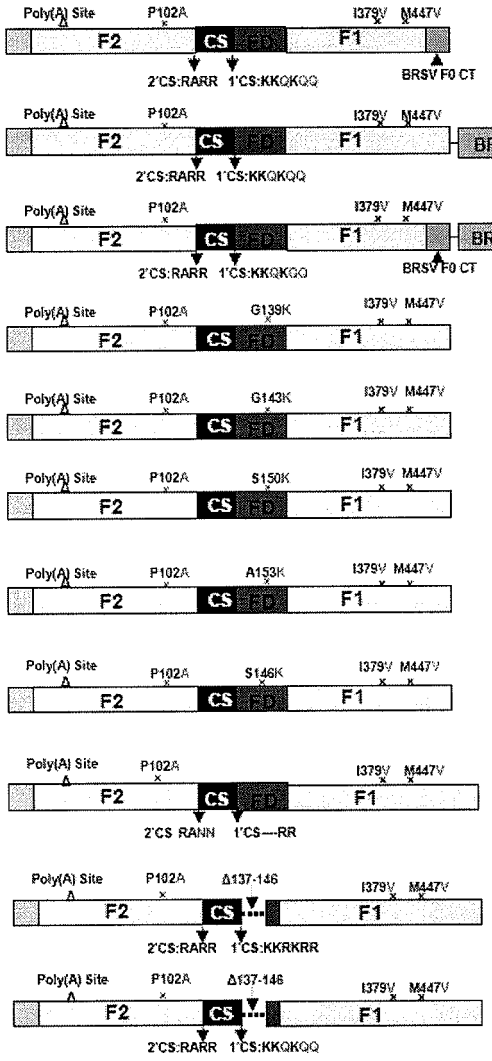

| Clone Name | Description | WB/Coomassie | Conclusion |
|---|---|---|---|
| WT HRSV F0 | Genebank | + / - | express really poor |
| F1-SP | ΔF2 | ++++ / ++++ | express good but insoluble, might be misfolded |
| F1 w/ Indo TMCT | ΔF2, Chimeric F1 No SP | +++ / +++ | express good but insoluble, might be misfolded |
| F1-SP w/ Indo TMCT | ΔF2,Chimeric F1 w Indo TMCT | +++ / +++ | express good but insoluble, might be misfolded |
| F1-SP w/ Indo | ΔF2,Chimeric F1 with TMCT+2AA | +++ / +++ | express good but insoluble, might be misfolded |
| F1-SP w/ Indo | Δ F2,Chimeric F1 with TMCT+16 AA | +++ / +++ | express good but insoluble, might be misfolded |
| F0 Δ CS | CS(KKRKRR) replace with GASGAG | - / - | No protein express |
| F1-SP w/ | Chimeric F1 with NDV HA NDV TMCT | +++ / +++ | express good but doesn't assemble with NDV M well |
| F1-SP w/NDV TMCT+16AA | Chimeric F1 with NDV HA TMCT+ upstream 16AA | +++ / +++ | express good but doesn't assemble with NDV M well |
| F1-SP ΔPalm site | Delete Palmitoylation site in F1, improve the solubility of F1 | +++ / +++ | express good but still insoluble |
| MF1 tandem | F1+ HRSV M | F1+++, M+++ / F1-, M+ | both F1 and M express well in tandem, but VLP yield is poor. M expression inhibited when co-infection with F1 |
| GbMF1 tandem | F1+ HRSV M+ Gb | F1++, M++/F1-, M+ | both F1 and M express better in triple tandem than co-infection, but VLP yield is very poor. |

# FIGURE 16b




| Clone Name | Description | WB/Coomassie | Conclusion |
|---|---|---|---|
| WT HRSV F0 | Genebank, intact cleavage sites | + / - | express really poor |
| HRSV F0 triple mutation | 1' CS mutate to KKQKQQ | + / - | 1' CS mutation improve expression a little |
| HRSV F0 double mutation | 1' CS mutate to KKQKRQ | + / - | 1' CS mutation doesn't improve expression |
| HRSV F0 with NDV CS | 1' CS mutate to NDV CS (GRRQQR) | + / - | 1' CS mutation doesn't improve expression |
| HRSV F0 with 2' CS mutation | 2'CS mutate to RAQQ | ++ / - | 2' CS mutation improve expression a little |
| HRSV F0(fixed) WT | Found errors in WT F0 derived from genebank, remove ploy(A)sitebut change P to A, I to V and M to V | ++ / - | Error fixed F0 express better than genebank F0, but still doesn't express well, can't be detected by blue stained gel |
| HRSV F0 (fixed) WT with 1'CS mutation(541) | mutate 1'CS to KKQKQQ | +++ / - | 1'CS mutate to KKQKQQ improve F0 expression, can assemble with BRSV M, HRSV N to form VLP, yield poor and rBV titerlow, need to improve |
| HRSV F0 (fixed) WT with 1'CS mutation | mutate 1'CS to NDV CS | + / - | 1'CS mutate to NDV CS doesn't improve F0 expression |
| HRSV F0 (fixed) with 1'CS and 2'CS mutation | mutate both 1'CS and 2'CS | - / - | No F0 expression |
| HRSV F0(fixed) ΔCS | delete entire CS | ++ / - | doesn't express well |
| HRSV F0 (fixed) w/ ΔCS BV SP, Indo TMCT+16AA | delete CS, replace HRSV SP with BV SP, swap HRSV TMCT with Indo HA TMCT,+ upstream 16AA | ++/- | VLP assemble efficient with Indo M1 poor |

# FIGURE 16c




| Clone Name | Description | WB/Coomassie | Conclusion |
|---|---|---|---|
| HRSV F0 (541) W/ BRSV F0 CT | HRSV F0 CT replaced by BRSV F0 CT | +++/- | Intracellular level is similar to 541, but inhibit BRSV M when co-infection. VLP yield is not as good as 541 |
| HRSV F0(541)+BRSV M | double genes tandem | F+++, M+++/ F-, M++ | Both intracellular and VLP yield is better than 541 co-infection with BRSV M |
| HRSV F0 (541) W/BRSV F0 CT+BRSV M | double genes tandem | F+++, M+++ F-, M++ | Both intracellular and VLP yield is better than co-infection, inhibition of BRSV M solved |
| HRSV F0 FD(G139K) | introduce G139K Point mutation (G139K) into Fusion domian | ++/ - | FD(G139K) doesn't improve F0 expression |
| HRSV F0 FD(G143K) | introduce G143K Point mutation (G139K) into Fusion domian | ++/ - | FD(G143K) doesn't improve F0 expression |
| HRSV F0 FD(S150K) | introduce S150K Point mutation (G139K) into Fusion domian | ++/ - | FD(S150K) doesn't improve F0 expression |
| HRSV F0 FD(S153K) | introduce S153K Point mutation (G139K) into Fusion domian | ++/ - | FD(S153K) doesn't improve F0 expression |
| HRSV F0 FD(S146K) | introduce S146K Point mutation (G139K) into Fusion domian | ++/ - | FD(S146K) doesn't improve F0 expression |
| HRSV F0 CS mut | delete 4AA in 1'CS, change 2'CS from RARR to RANN | ++/ - | very poor F0 expression |
| HRSV F0 CS mut (622) | F0 Fusion domain partial deletion(10AA) Intact Cleavage Site | ++++/ +++ | excellent F0 intracellular expression, strong band on coomassie gel |
| HRSV F0 CS mut (683) | F0 Fusion domain partial deletion(10AA) Intact Cleavage Site | ++++/ ++++ | excellent F0 intracellular expression, strong band on coomassie gel |

FIGURE 16d

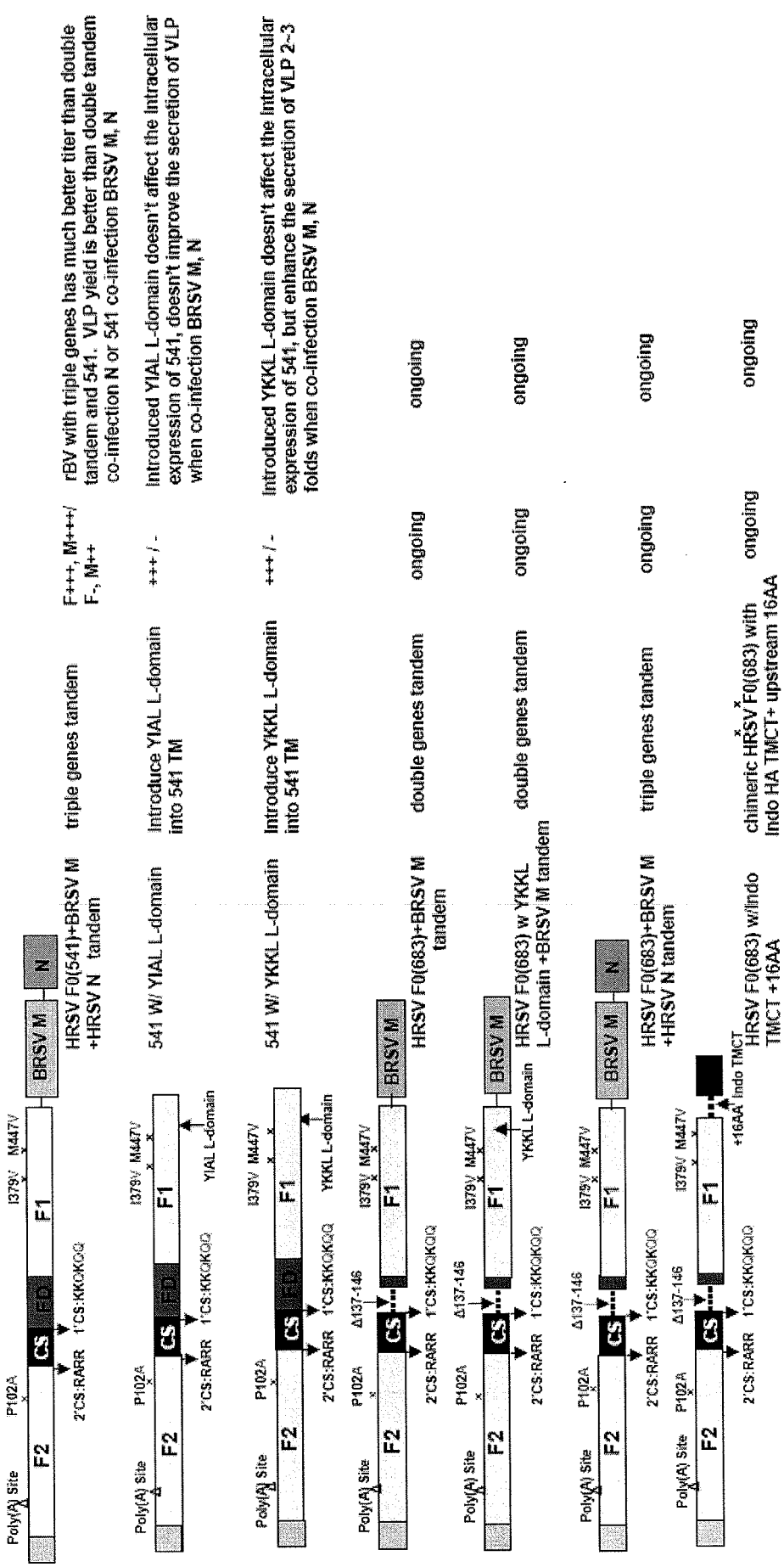

Clone Name
Description
WB/Coomassie
Conclusion
Poly(A) Site
P102A
I379V
M447V
F2
CS
FD
F1
BRSV M
N
2'CS:RARR
1'CS:KKQKQQ
HRSV F0(541)+BRSV M +HRSV N tandem
triple genes tandem
F+++, M+++/ F-, M++
rBV with triple genes has much better titer than double tandem and 541. VLP yield is better than double tandem co-infection N or 541 co-infection BRSV M, N
541 W/ YIAL L-domain
Introduce YIAL L-domain into 541 TM
+++ / -
Introduced YIAL L-domain doesn't affect the intracellular expression of 541, doesn't improve the secretion of VLP when co-infection BRSV M, N
YIAL L-domain
541 W/ YKKL L-domain
Introduce YKKL L-domain into 541 TM
+++ / -
Introduced YKKL L-domain doesn't affect the intracellular expression of 541, but enhance the secretion of VLP 2~3 folds when co-infection BRSV M, N
YKKL L-domain
Δ137-146
HRSV F0(683)+BRSV M tandem
double genes tandem
ongoing
ongoing
HRSV F0(683) w YKKL L-domain +BRSV M tandem
double genes tandem
ongoing
ongoing
HRSV F0(683)+BRSV M +HRSV N tandem
triple genes tandem
ongoing
ongoing
+16AA Indo TMCT
HRSV F0(683) w/indo TMCT +16AA
chimeric HRSV F0(683) with Indo HA TMCT+ upstream 16AA
ongoing
ongoing

FIGURE 17

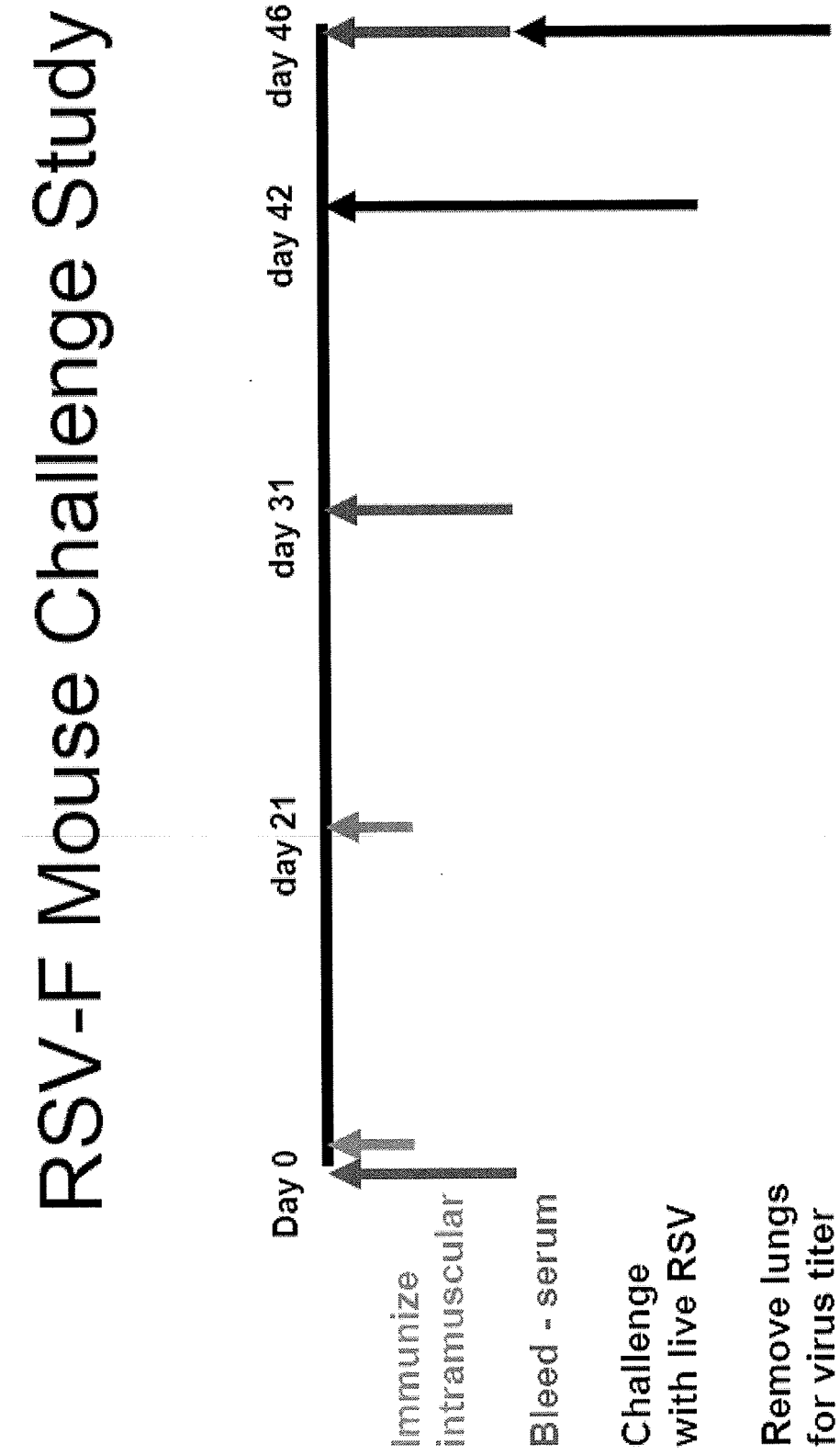

RSV-F Mouse Challenge Study
Day 0
day 21
day 31
day 42
day 46
Immunize intramuscular
Bleed - serum
Challenge with live RSV
Remove lungs for virus titer

FIGURE 18

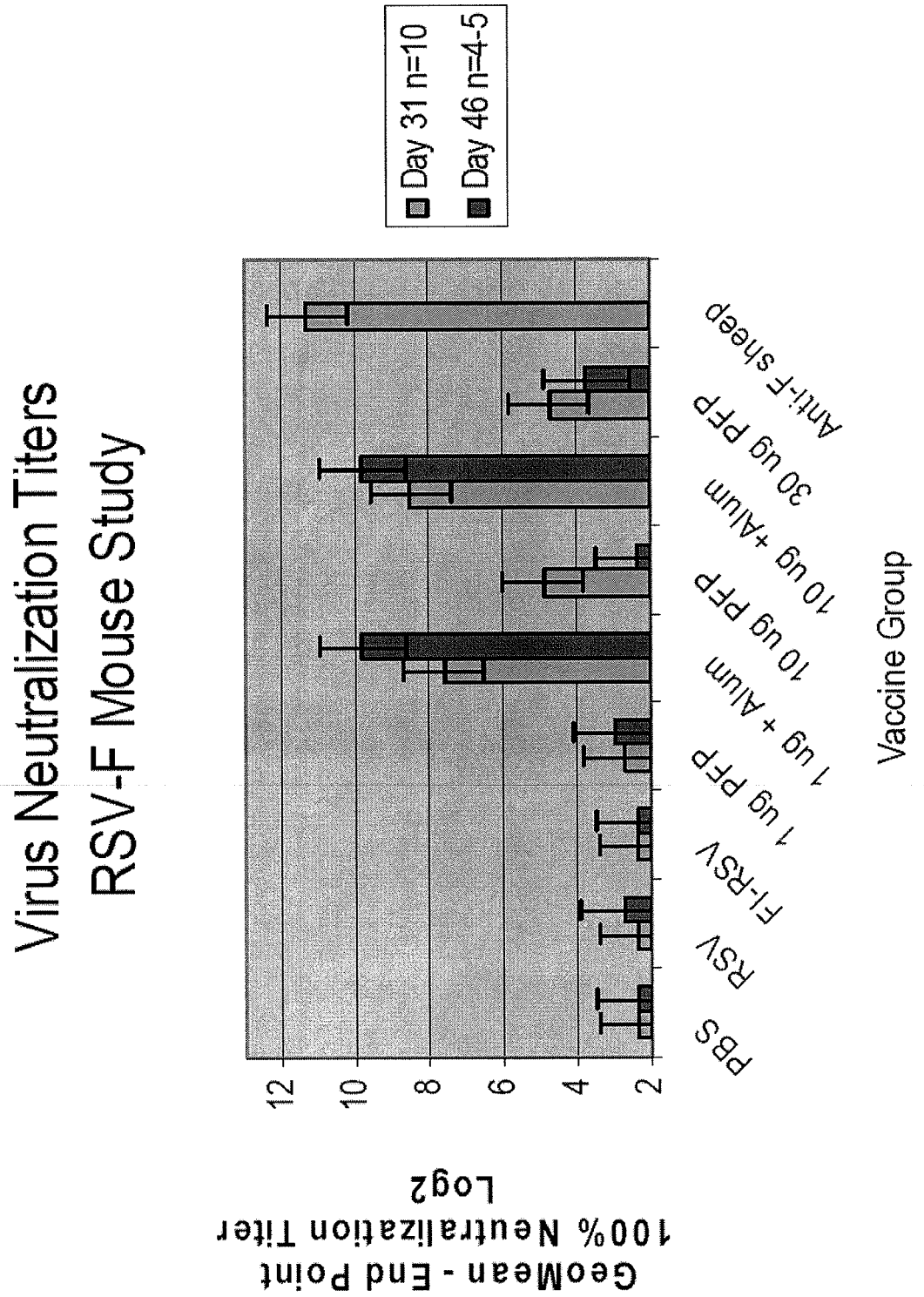

Virus Neutralization Titers
RSV-F Mouse Study
Day 31 n=10
Day 46 n=4-5
GeoMean - End Point
100% Neutralization Titer
Log2
12
10
8
6
4
2
PBS
RSV
Fl-RSV
1 ug PFP
1 ug + Alum
10 ug PFP
10 ug +Alum
30 ug PFP
Anti-F sheep
Vaccine Group

FIGURE 19

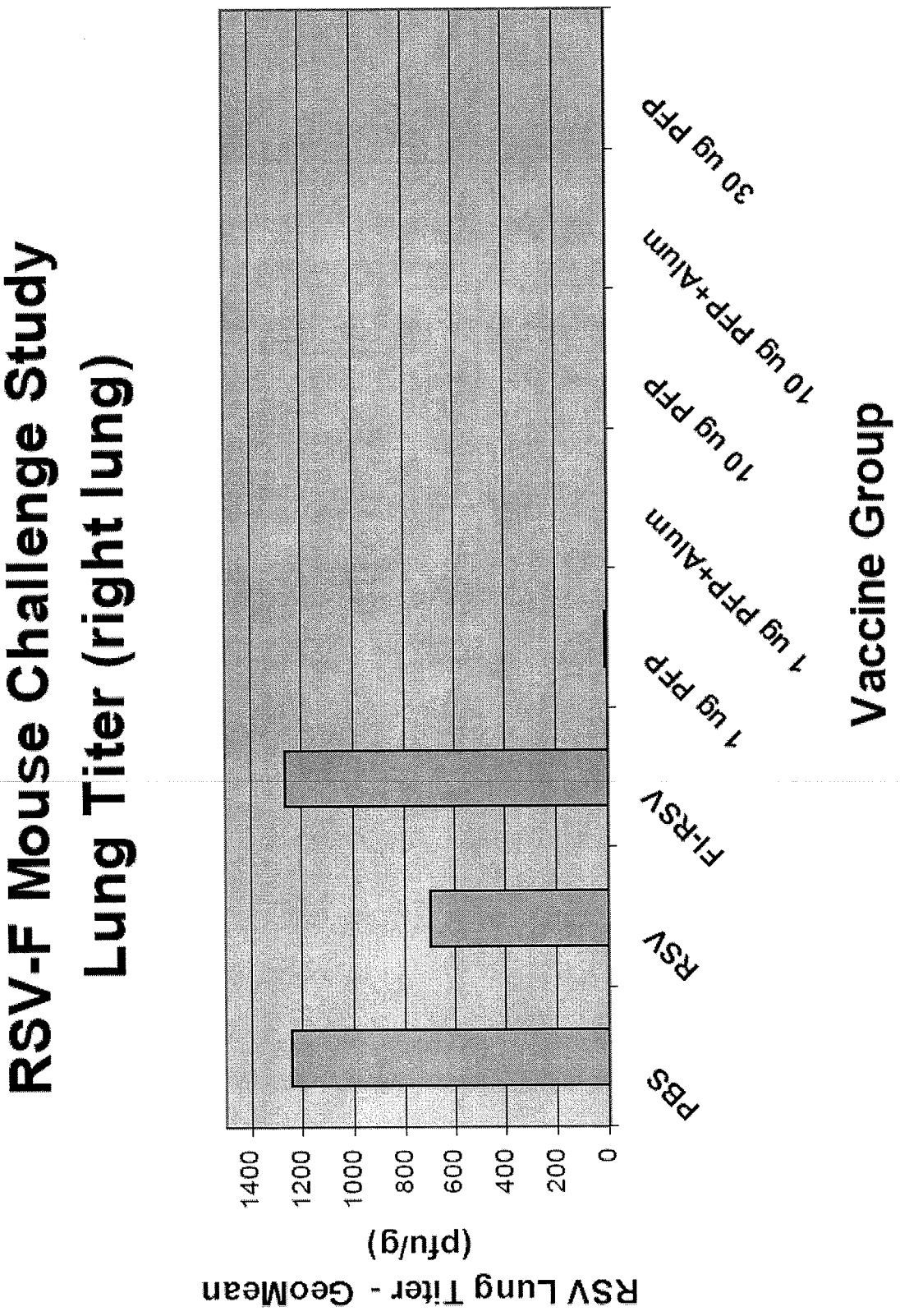

RSV-F Mouse Challenge Study
Lung Titer (right lung)
RSV Lung Titer - GeoMean (pfu/g)
1400
1200
1000
800
600
400
200
0
PBS
RSV
Fl-RSV
1 ug PFP
1 ug PFP+Alum
10 ug PFP
10 ug PFP+Alum
30 ug PFP
Vaccine Group

FIGURE 20

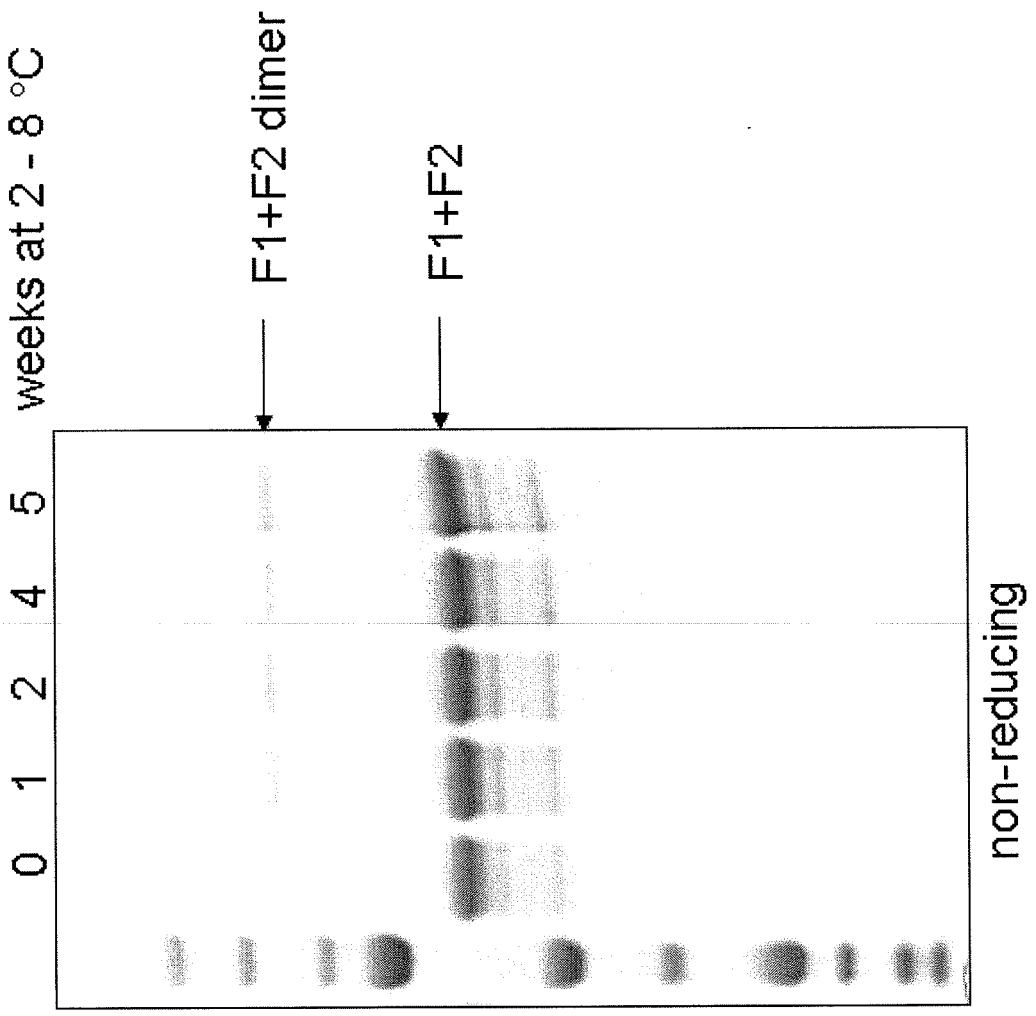
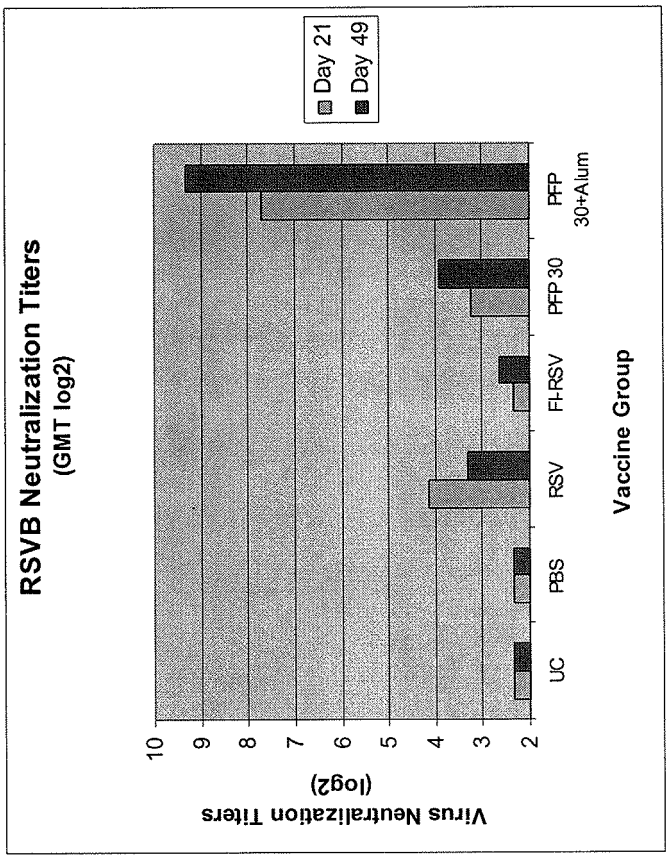


FIGURE 21


RSVB Neutralization Titers
(GMT log2)
Day 21
Day 49
Virus Neutralization Titers
(log2)
10
9
8
7
6
5
4
3
2
UC
PBS
RSV
FI-RSV
PFP 30
PFP 30+Alum
Vaccine Group

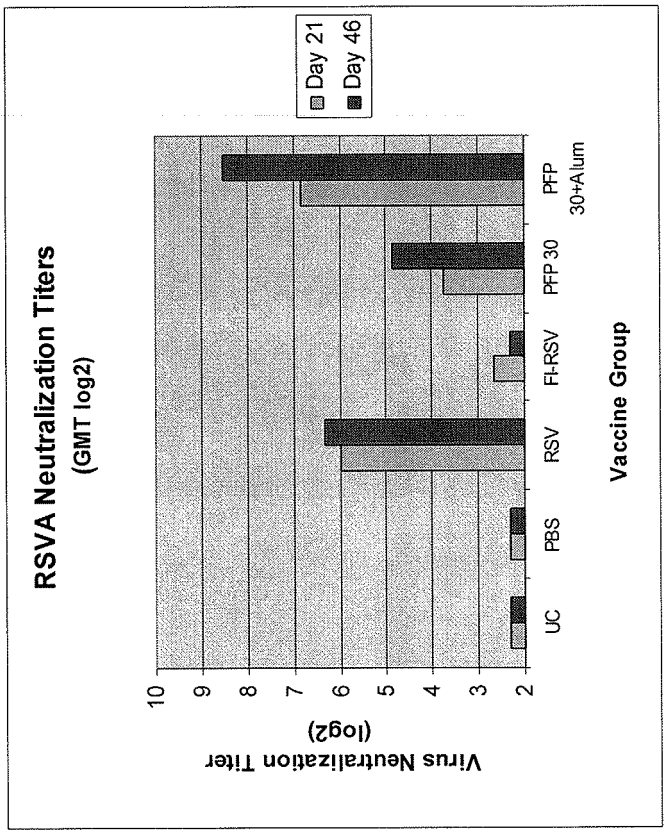
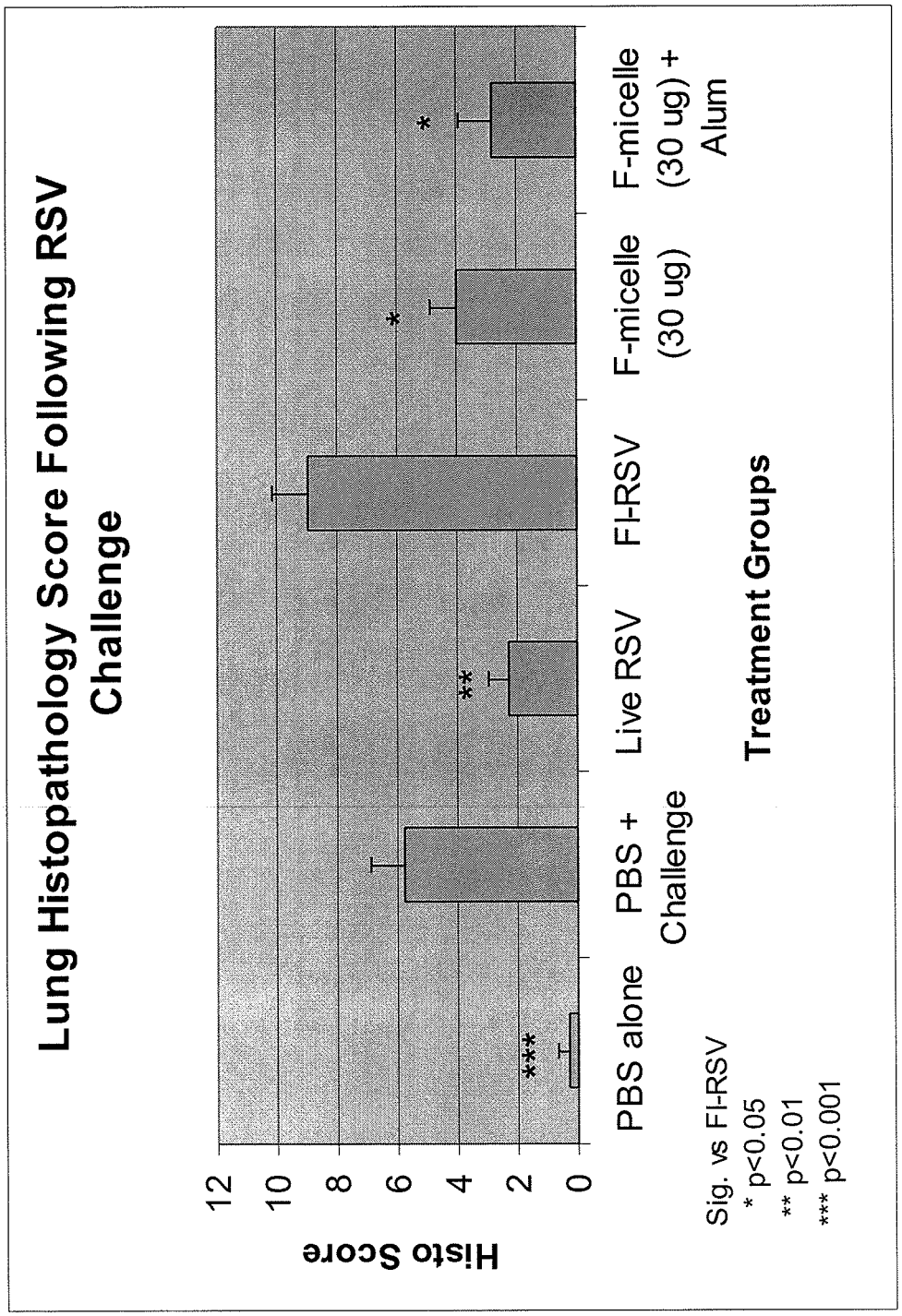

RSVA Neutralization Titers
(GMT log2)
Day 21
Day 46
Virus Neutralization Titer
(log2)
10
9
8
7
6
5
4
3
2
UC
PBS
RSV
FI-RSV
PFP 30
PFP 30+Alum
Vaccine Group

FIGURE 22


Lung Histopathology Score Following RSV Challenge
Histo Score
0
2
4
6
8
10
12
PBS alone
PBS + Challenge
Live RSV
FI-RSV
F-micelle (30 ug)
F-micelle (30 ug) + Alum
Treatment Groups
Sig. vs FI-RSV
* p<0.05
** p<0.01
*** p<0.001