Figure 1

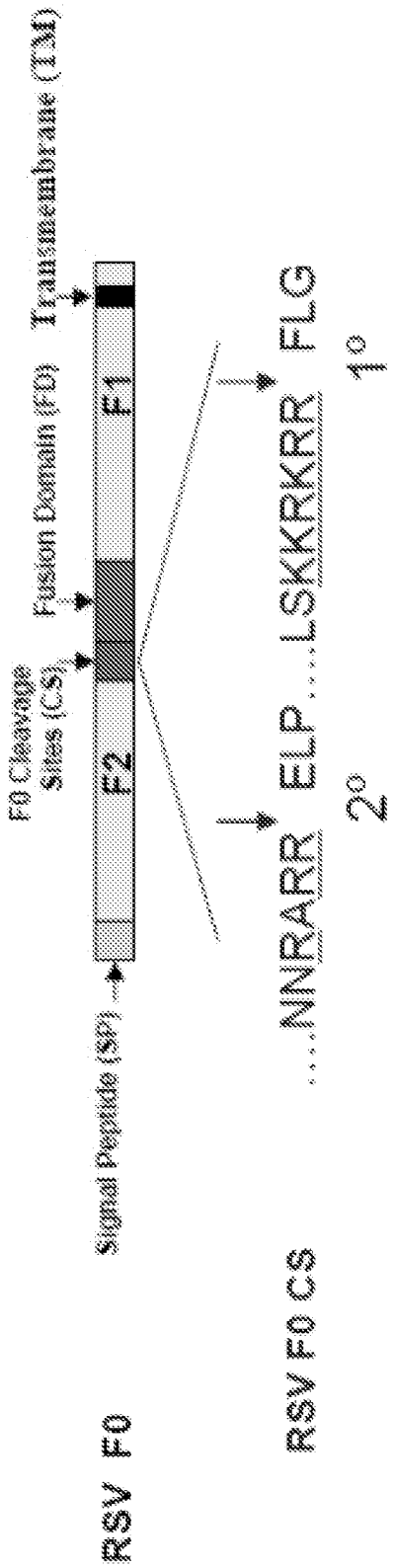
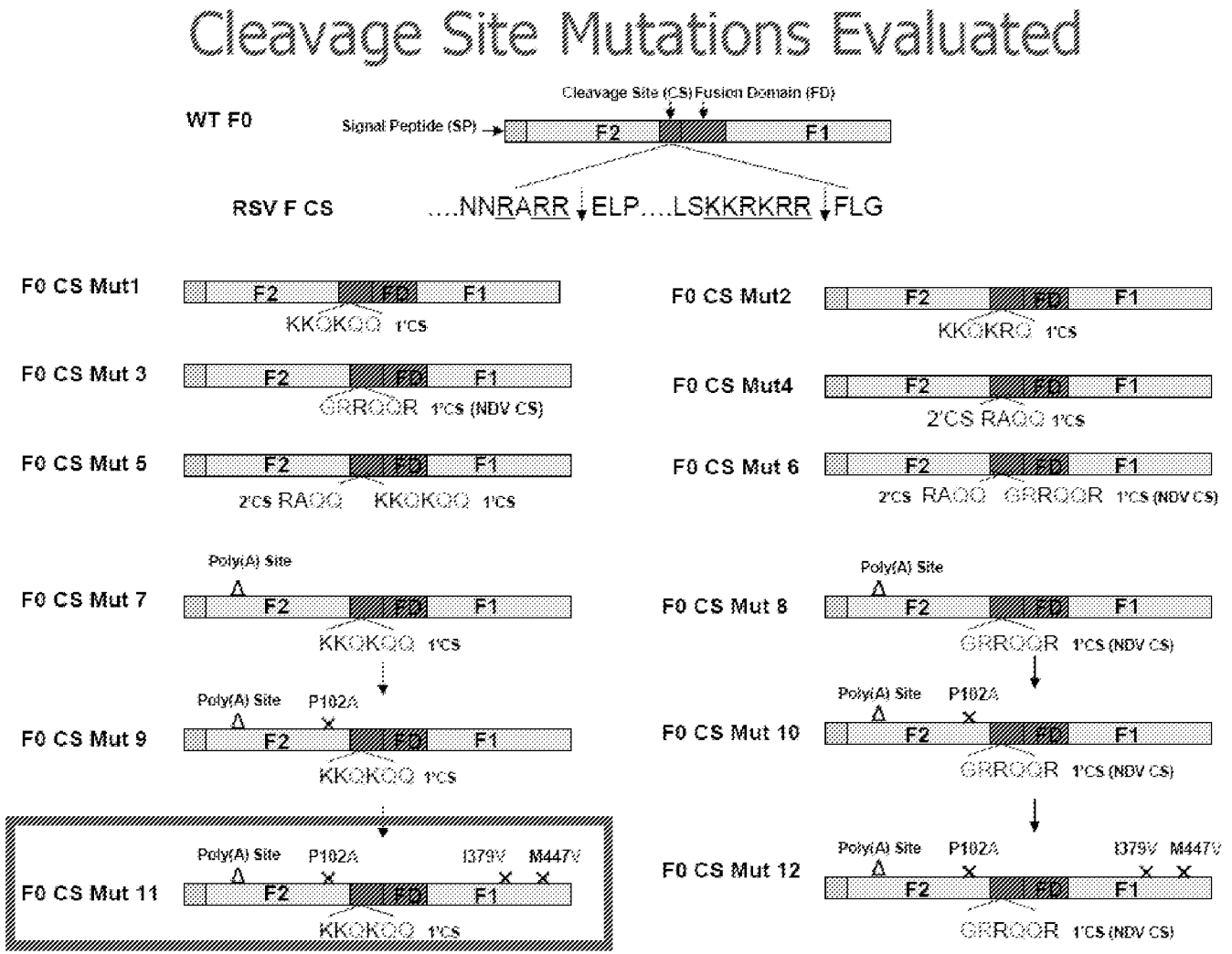

Transmembrane (TM)
Fusion Domain (FD)
F0 Cleavage Sites (CS)
F1
F2
Signal Peptide (SP)
RSV F0
RSV F0 CS
....NNRARR
ELP....LSKKRKRR
FLG
2°
1°

Figure 2

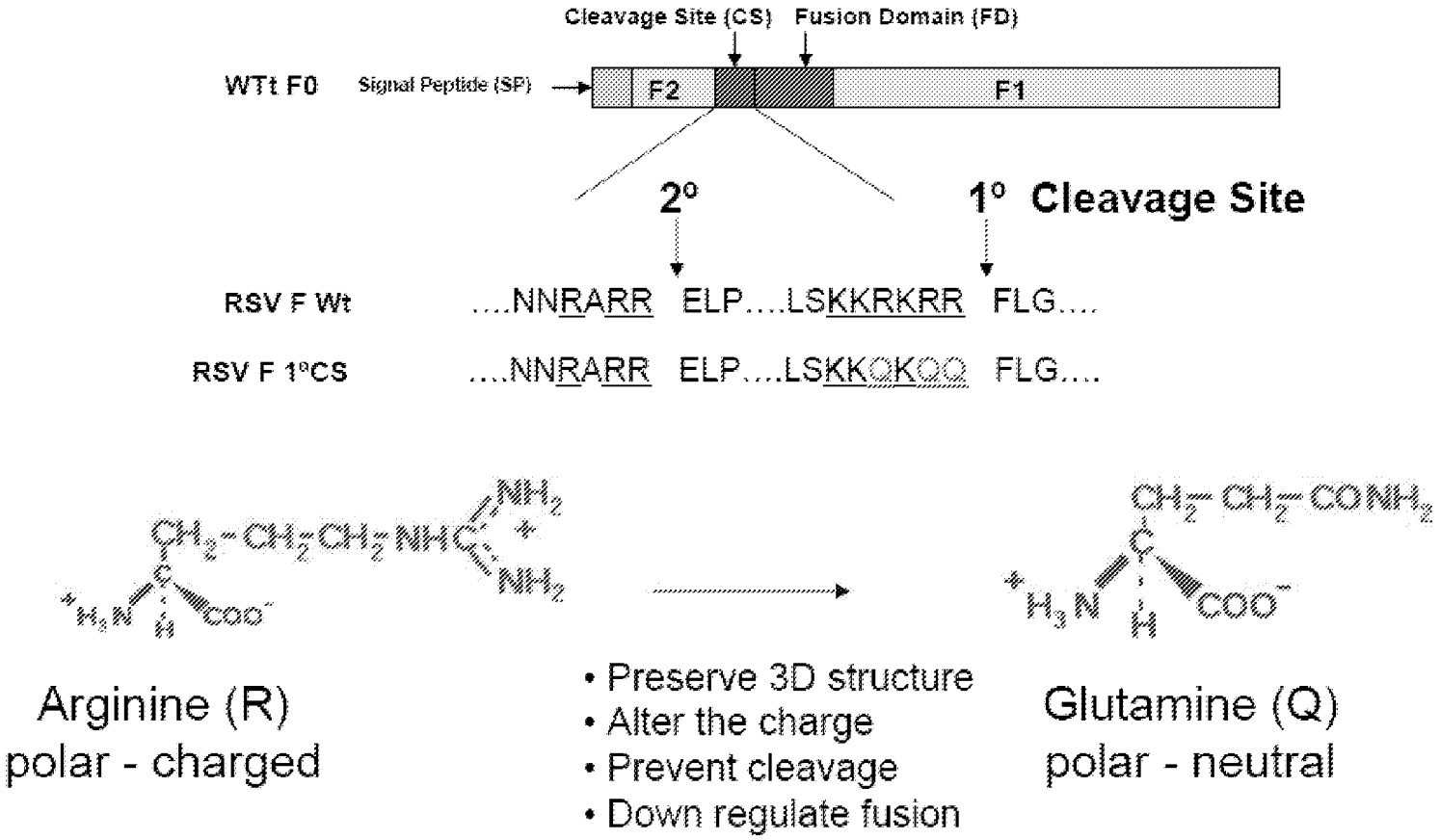


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Figure 3

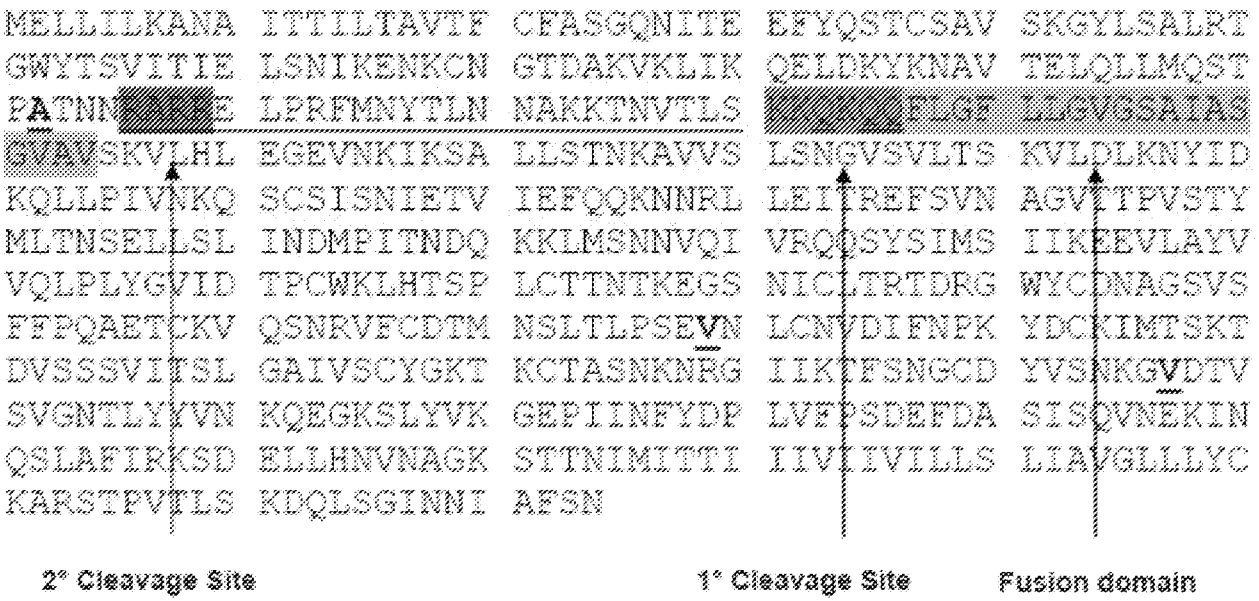


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Figure 4

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



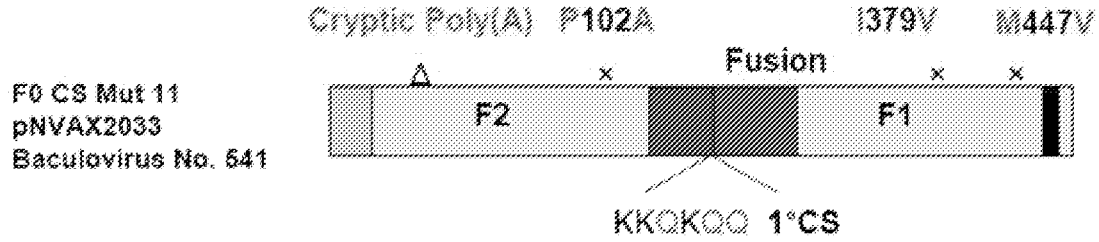
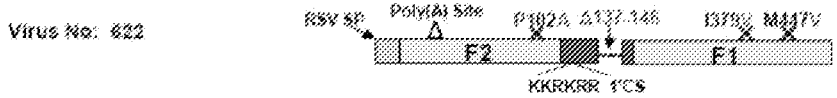


Figure 5

Partial deletion (Δ137-146) of F fusion domain




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

Δ137-146        LSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLN 120
WT FO           LSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLN 120
                ************************************************************

Δ137-146        NAKKTNVTLSKKRKRR----------AIASGIAVSKVLHLEGEVNKIKSALLSTNKAVV 170
WT FO           NAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVV 180
                ****************          ************************************

Δ137-146        LSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLEITREFSVN 530
WT FO           LSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLEITREFSVN 540
                ****************;***********************************************

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

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

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

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

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

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

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Figure 6

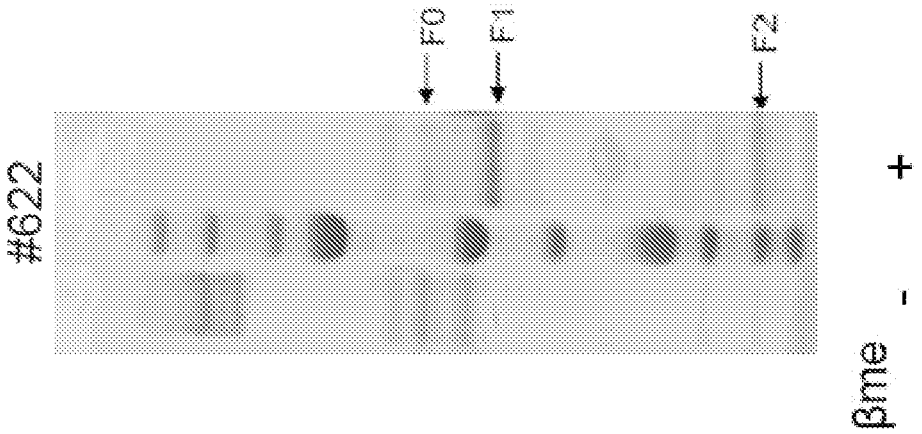
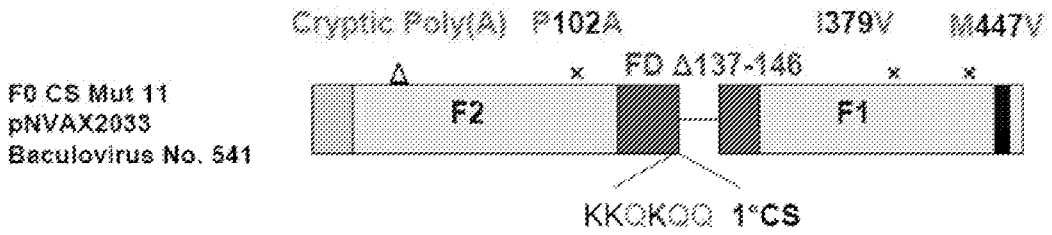


Figure 7A

BV #683 Modified RSV F0



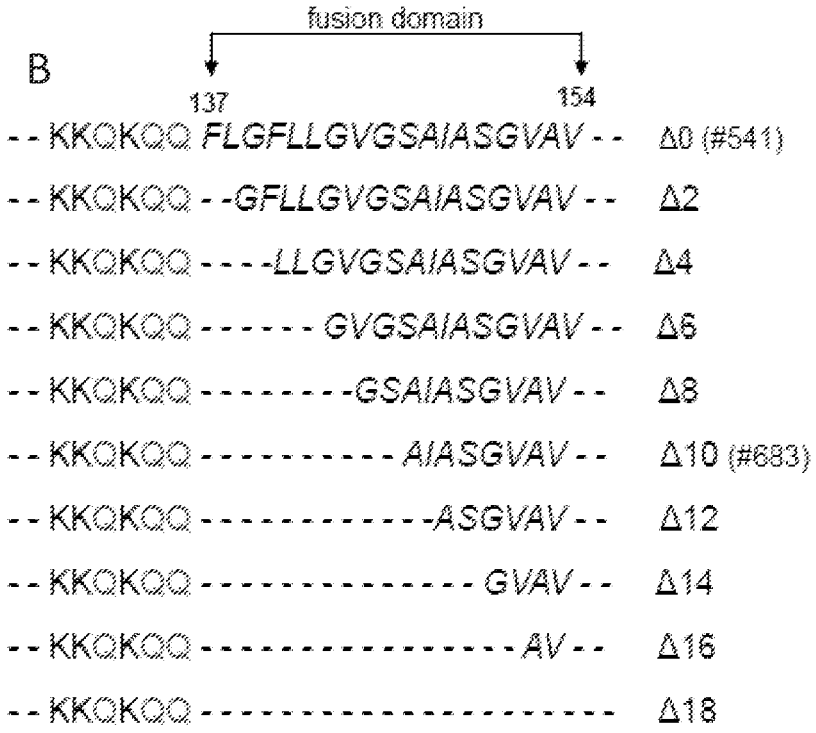
Codon optimized
1° cleavage site inactivation (3 conservative aa changes)
Cryptic poly(A) in F2 removed
3 aa corrections to GenBank sequence
Deletion of N-terminal half of the F1 fusion domain

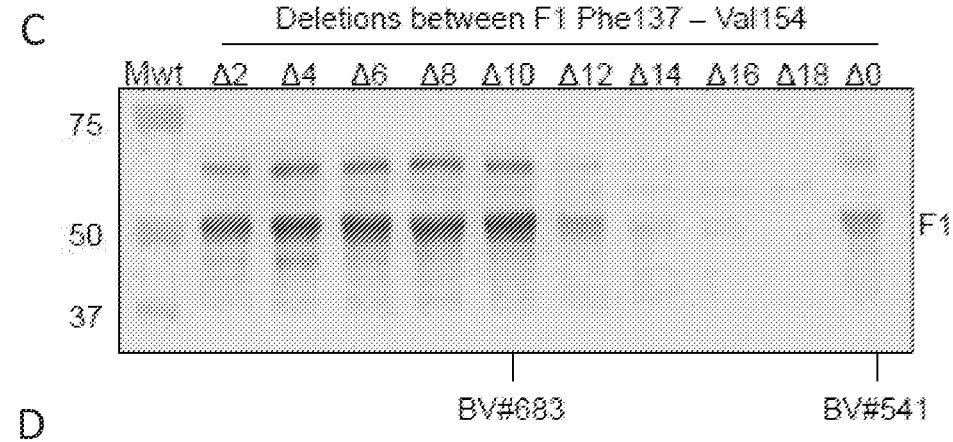
WO 2013/049342 PCT/US2012/057546

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PCT/US2012/057546

Figure 7B, 7C, 7D





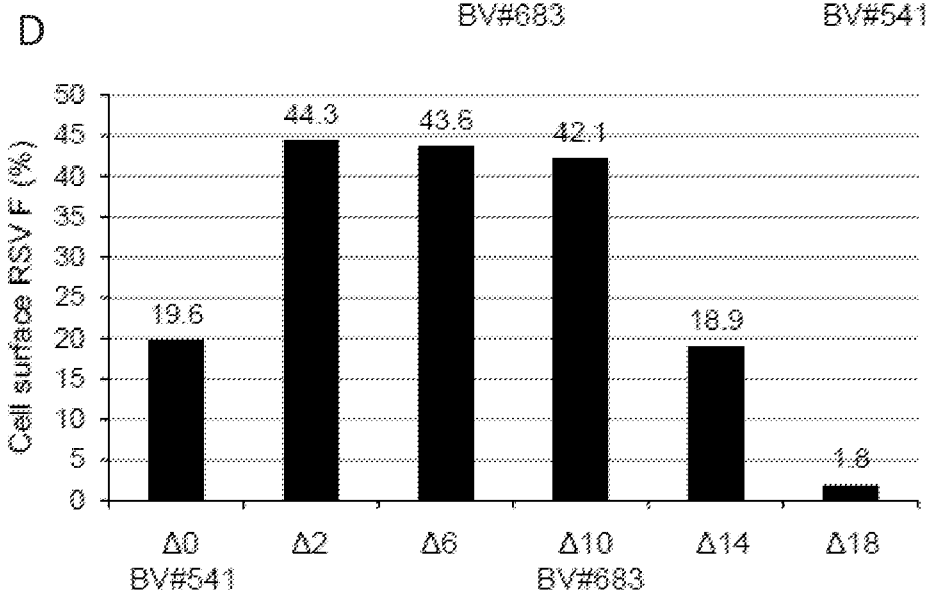


Figure 8

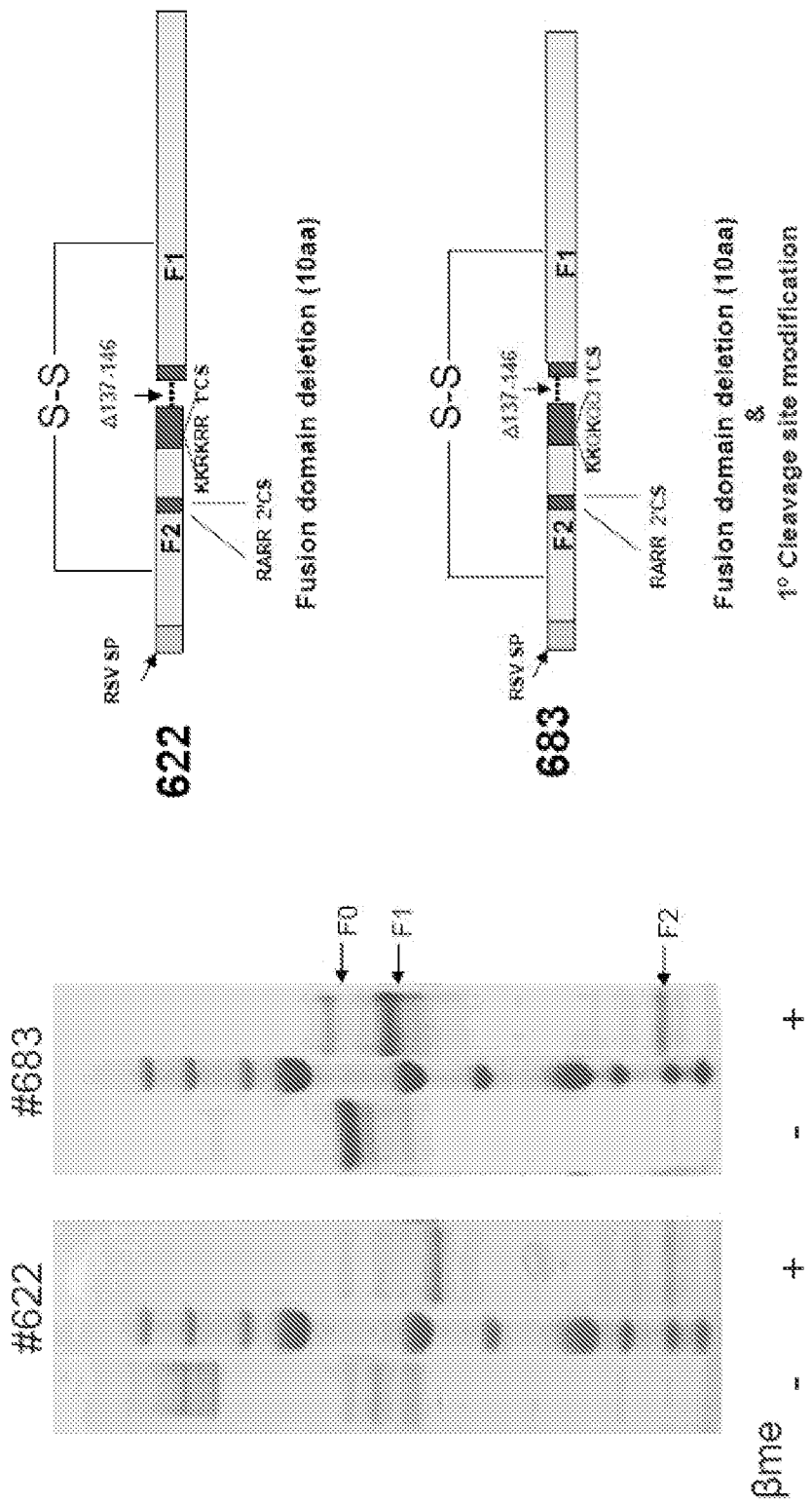

S-S
Δ137-146
F1
F2
KKGKKRR
RARR
RSV SP
622
Fusion domain deletion (10aa)
S-S
Δ137-146
F1
F2
RARR
RSV SP
683
Fusion domain deletion (10aa)
&
1° Cleavage site modification
#683
#622
F0
F1
F2
βme - + - +

Figure 9

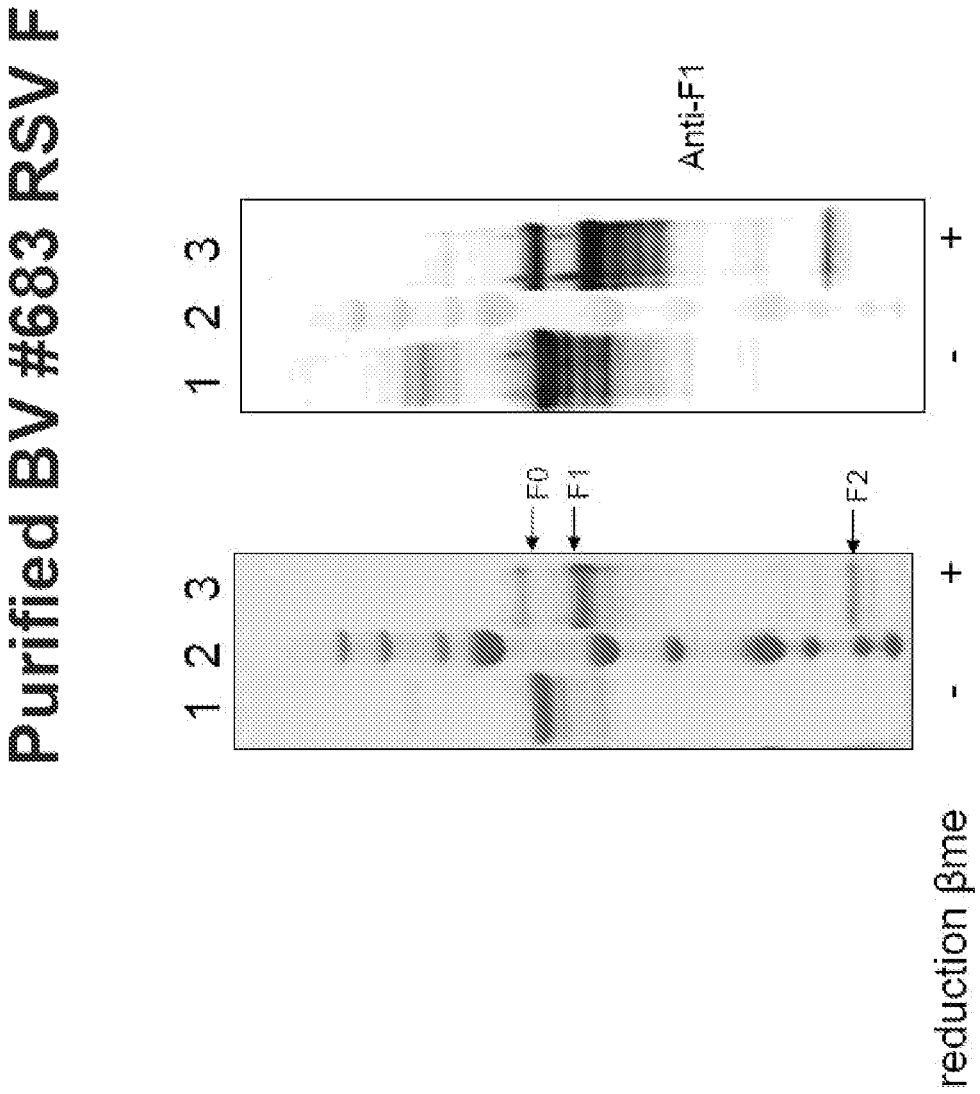
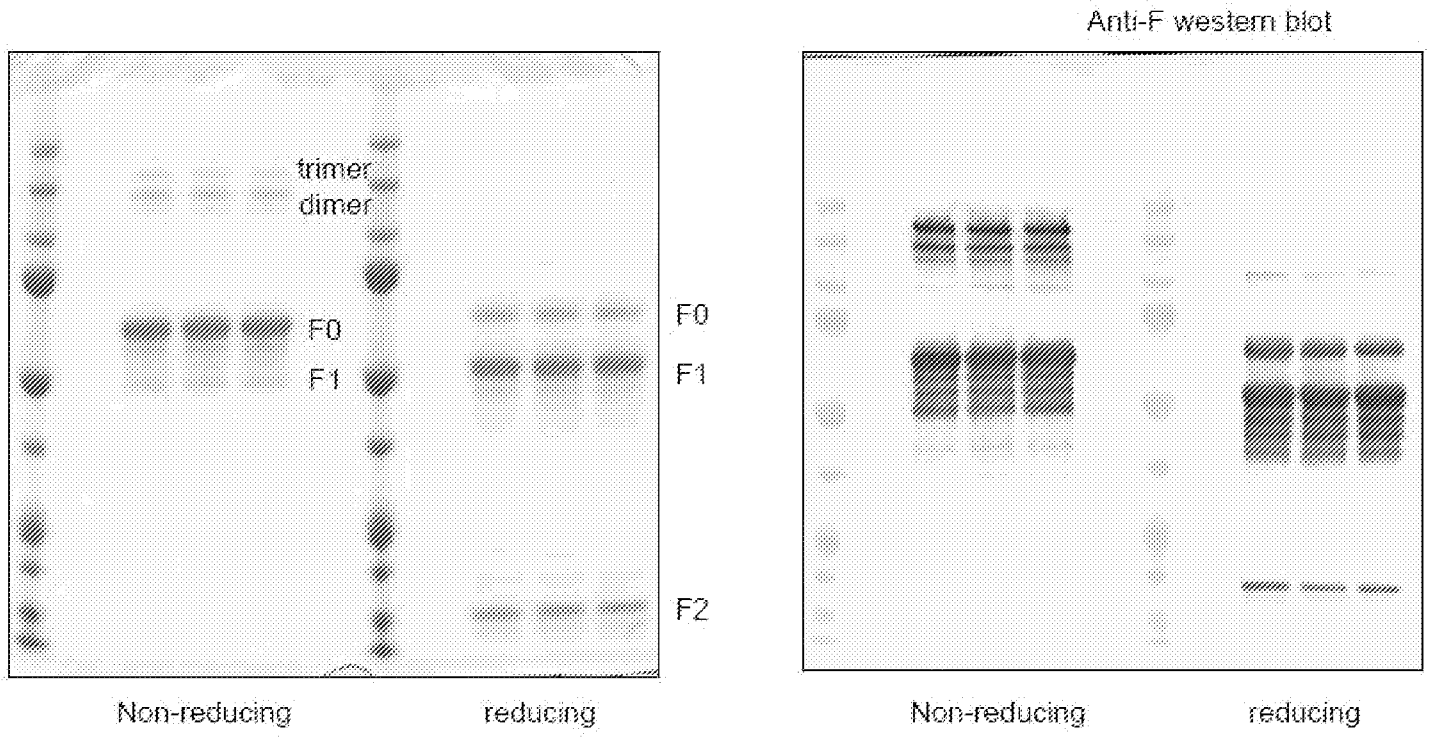


Figure 10



Purity >98% scanning densitometry

Figure 11

Purified RSV - F #683 micelles (rosettes)

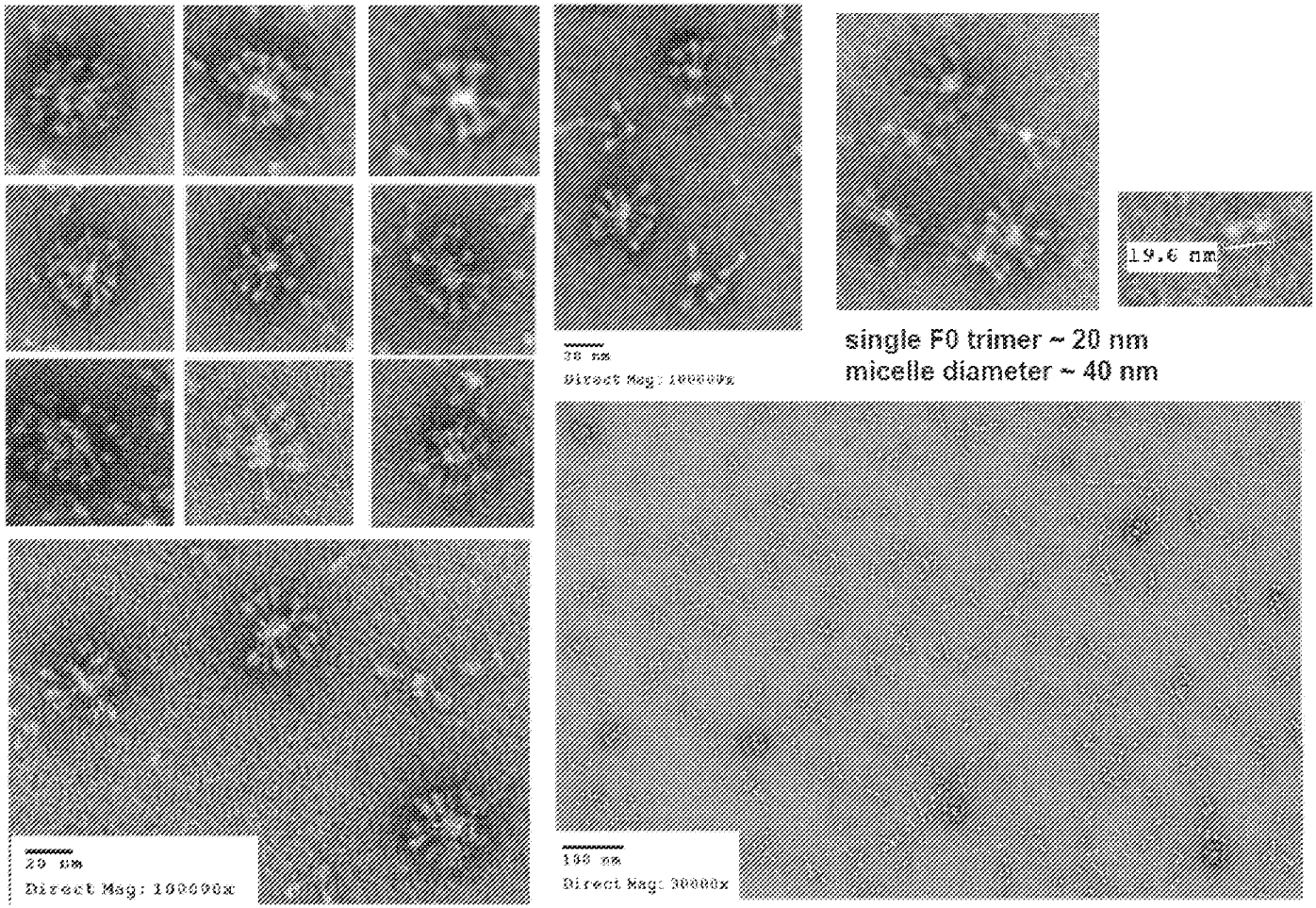
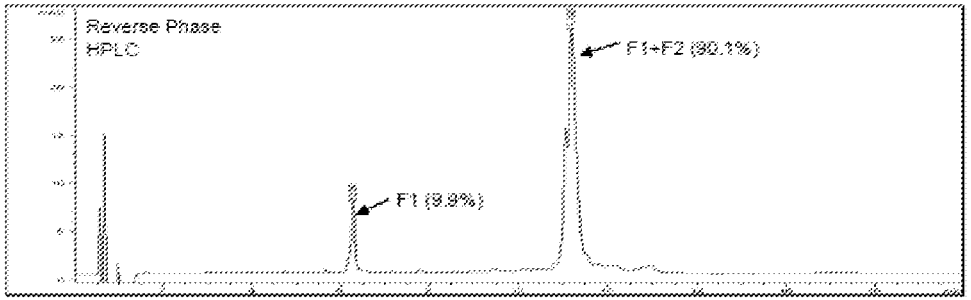
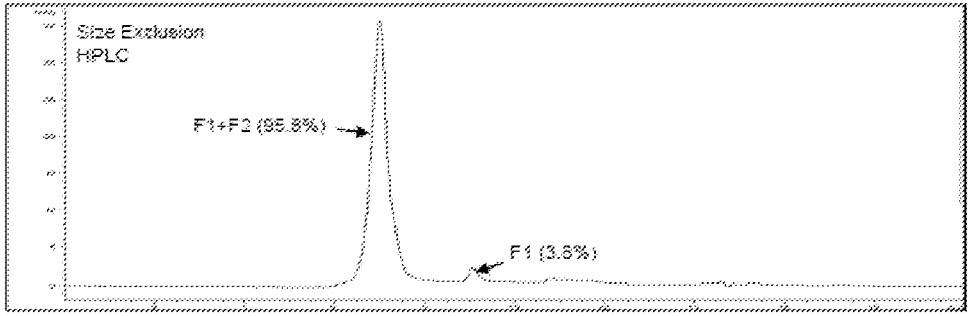


Figure 12A, 12B, 12C

A



B



Particle size analysis shows RSV 683 F0 forms 40 nm particles

C

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	40.0	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

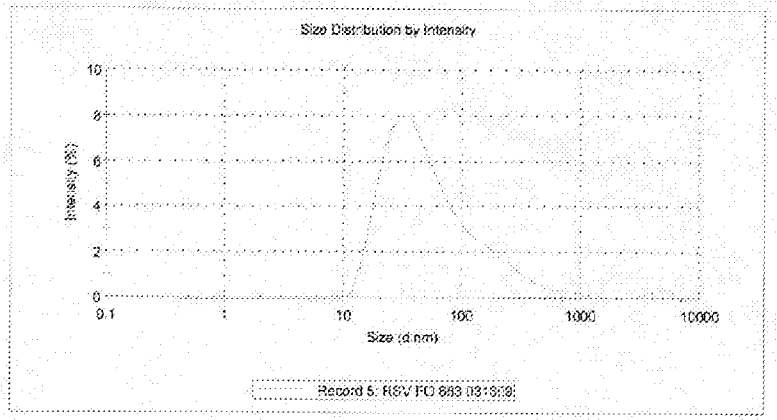
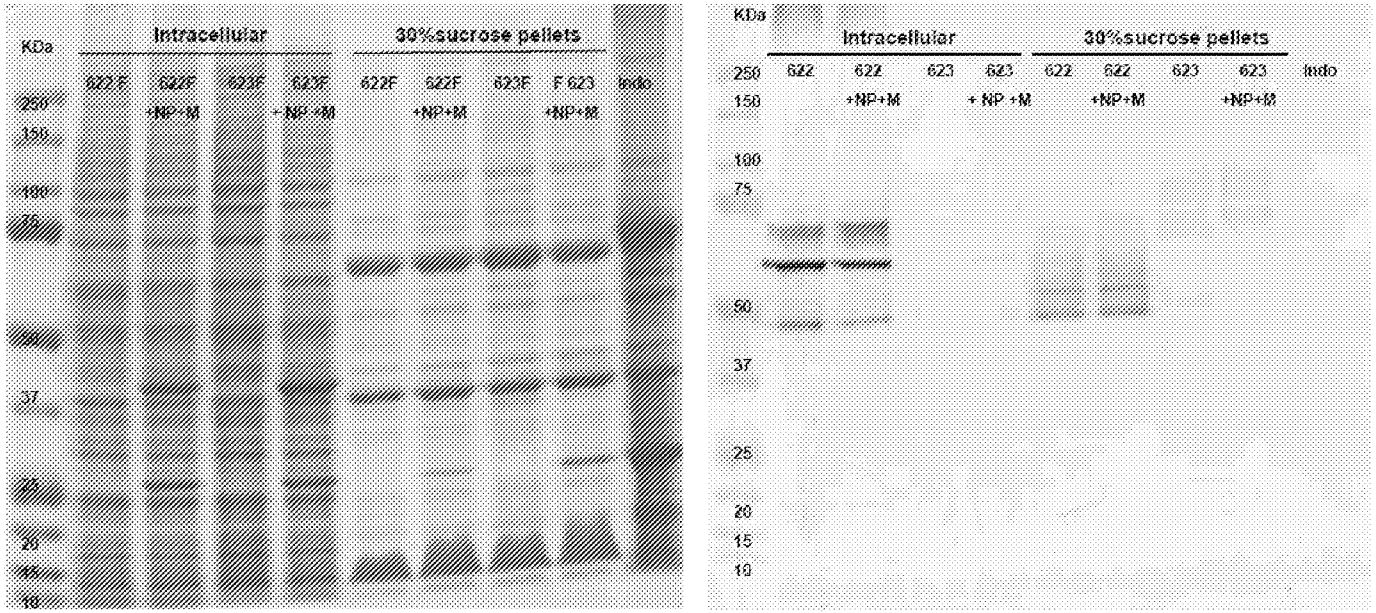
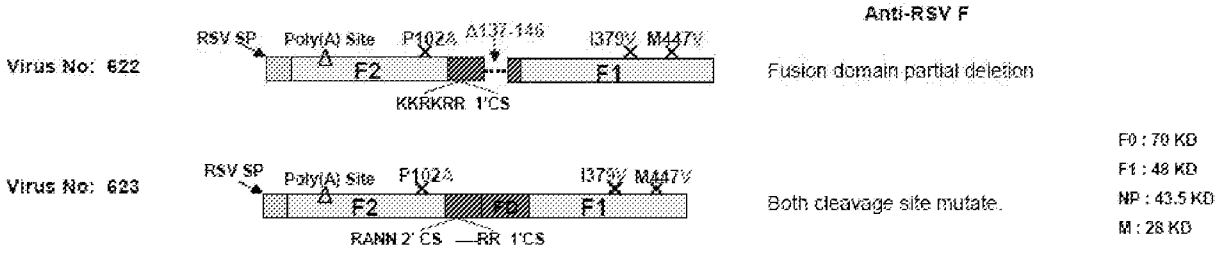


Figure 13

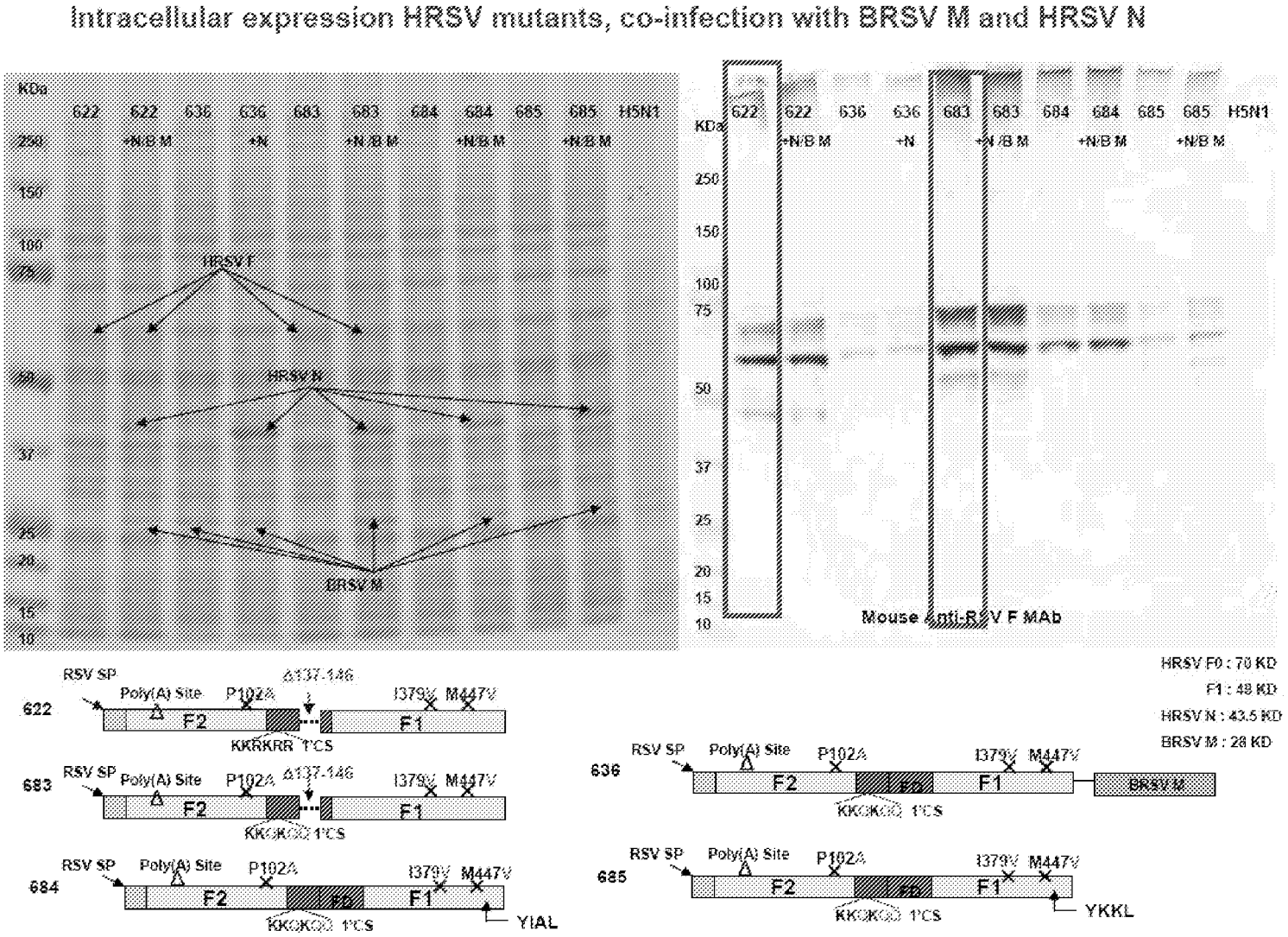
Modified RSF F: BV #622 and #623





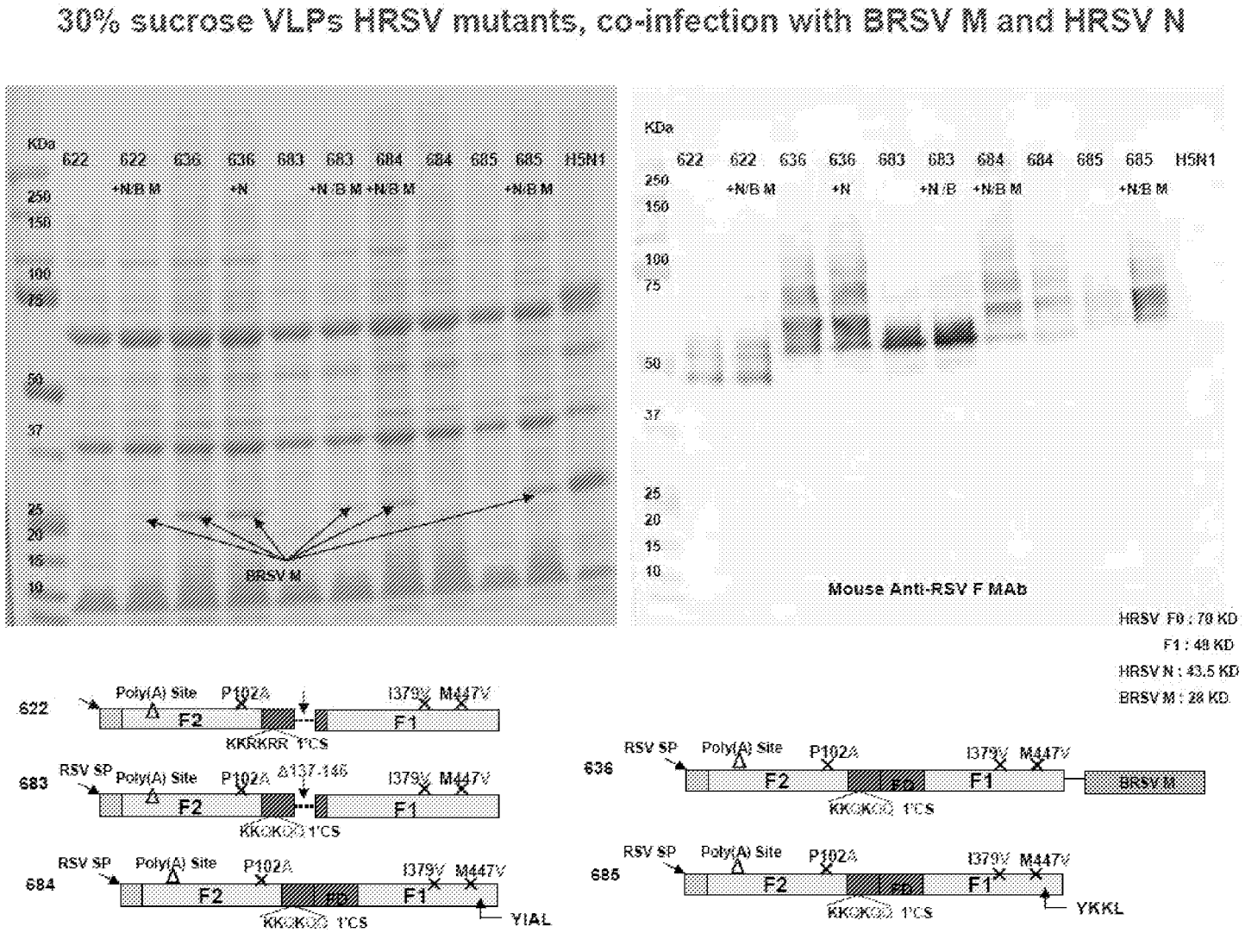
WO 2013/049342 PCT/US2012/057546

Figure 14



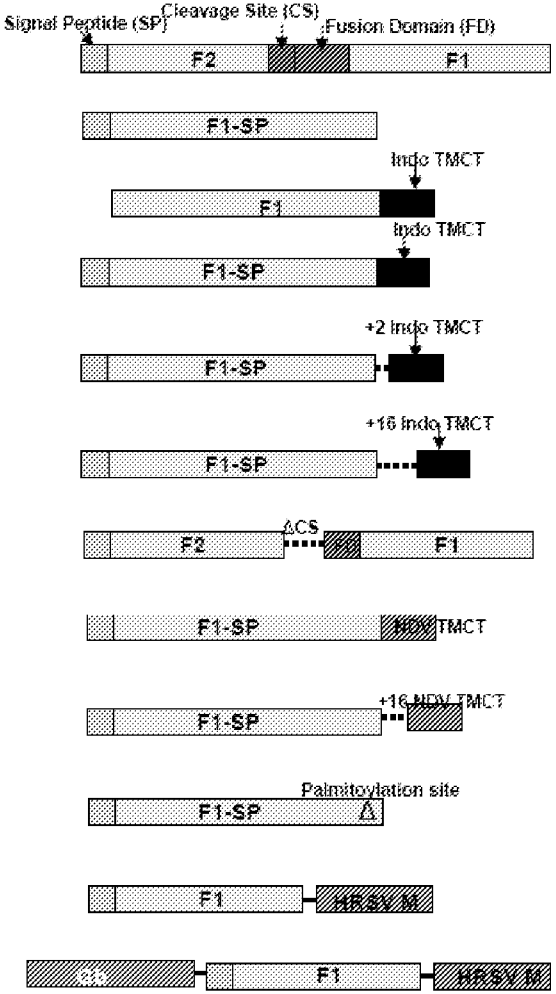
WO 2013/049342 PCT/US2012/057546

Figure 15



WO 2013/049342 PCT/US2012/057546

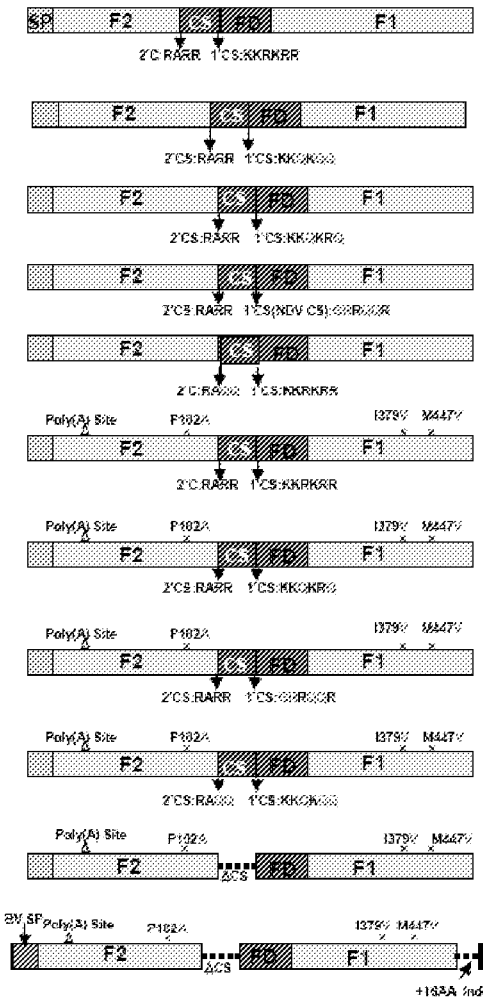
Figure 16A



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/ NDV TMCT	Chimeric F1 with NDV HA	+++ / +++	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.

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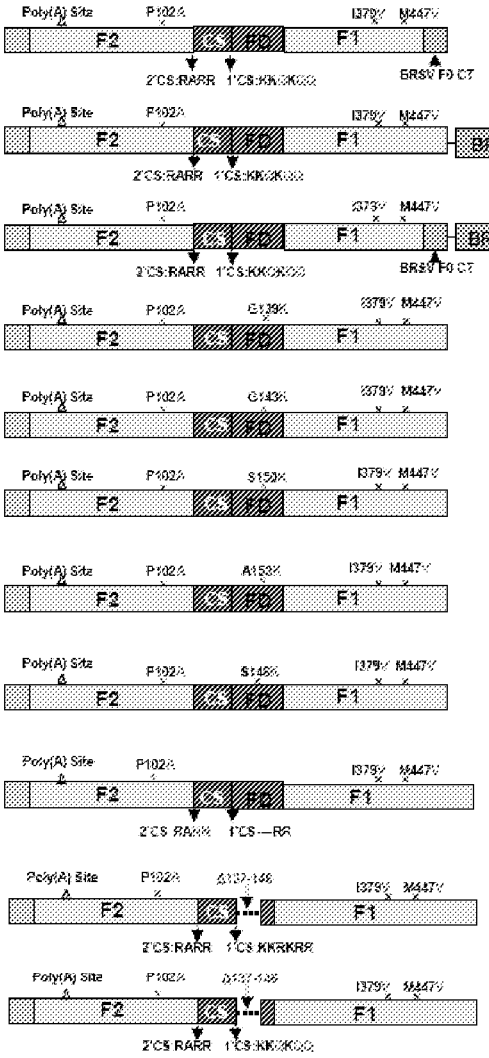
Figure 16B



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 (GRRQCR)	+ / -	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)site\but 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

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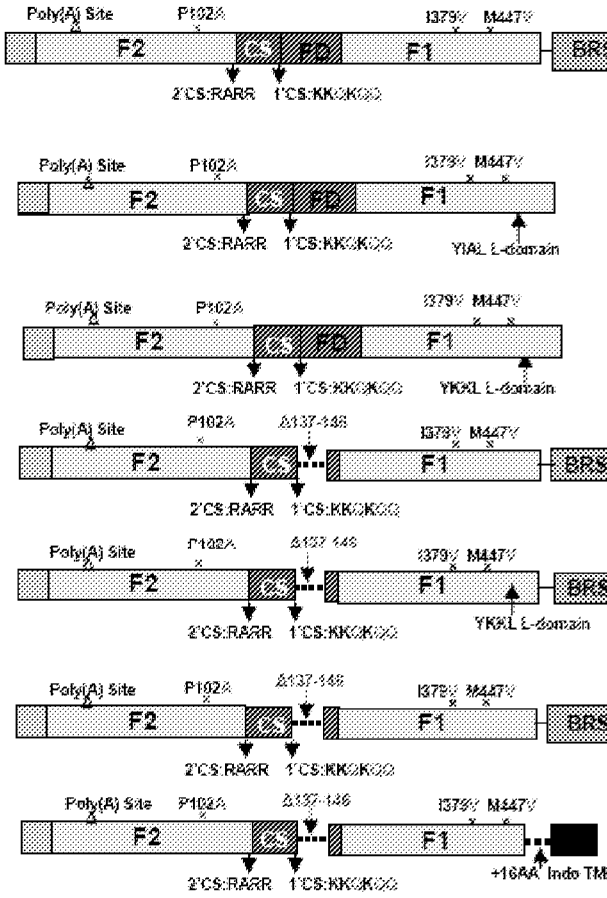
Figure 16C



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(G139X)	introduce G139X Point mutation (G139K) into Fusion domian	++/ -	FD(G139X) doesn't improve F0 expression
HRSV F0 FD(G143X)	introduce G143X Point mutation (G139K) into Fusion domian	++/ -	FD(G143X) doesn't improve F0 expression
HRSV F0 FD(S150X)	introduce S150X Point mutation (G139K) into Fusion domian	++/ -	FD(S150X) doesn't improve F0 expression
HRSV F0 FD(S153X)	introduce S153X Point mutation (G139K) into Fusion domian	++/ -	FD(S153X) doesn't improve F0 expression
HRSV F0 FD(S146X)	introduce S146X Point mutation (G139K) into Fusion domian	++/ -	FD(S146X) doesn't improve F0 expression
HRSV F0 CS mut	delete 4AA in 1'CS, change 2'CS from RARR to RAXX	++/ -	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

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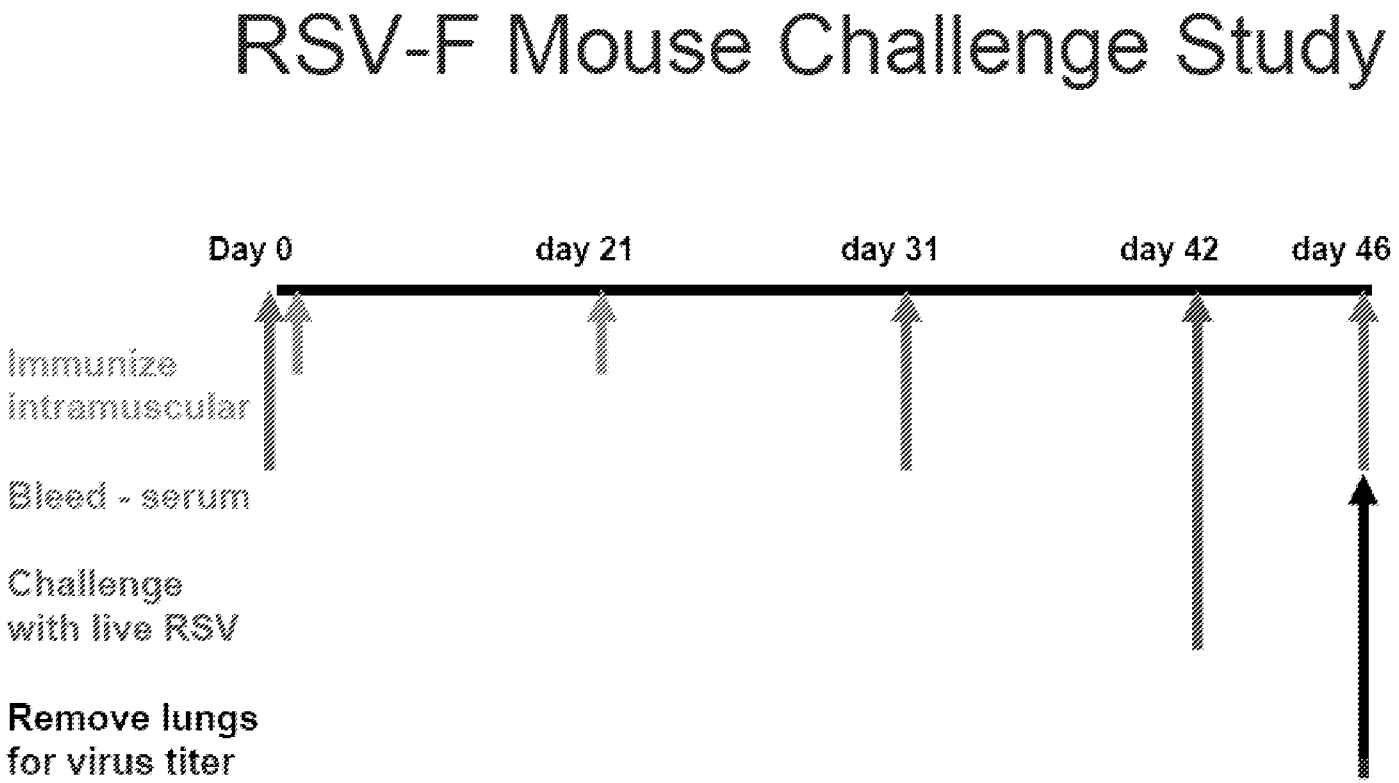
Figure 16D



Clone Name	Description	WB/Coomassie	Conclusion
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
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
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
HRSV F0(683) w/indo TMCT +16AA	chimeric HRSV F0(683) with Indo HA TMCT+ upstream 16AA	ongoing	ongoing

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Figure 17



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Figure 18

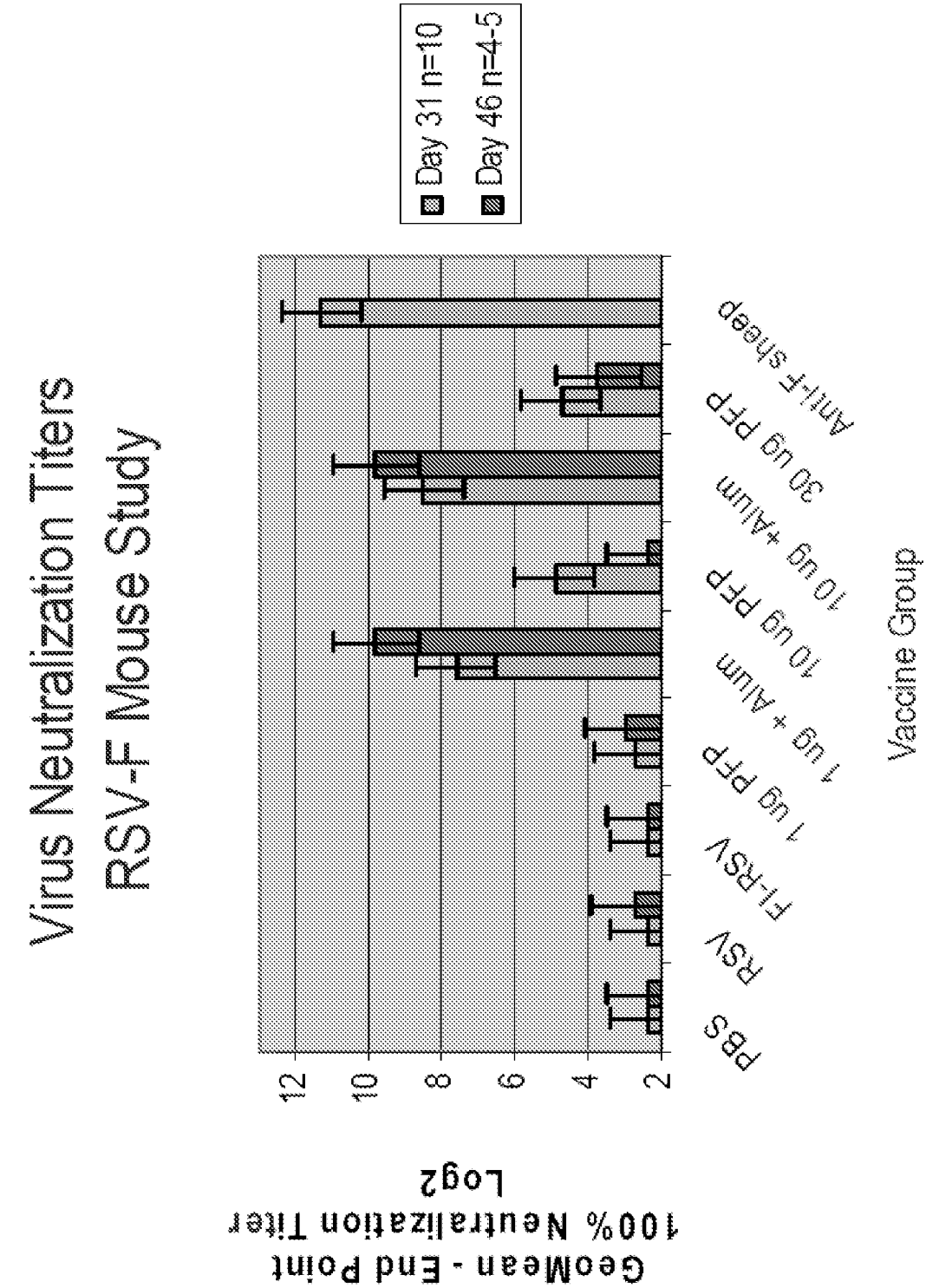

Virus Neutralization Titers
RSV-F Mouse Study
Day 31 n=10
Day 46 n=4-5
GeoMean - End Point
100% Neutralization Titer
Log2
12
10
8
6
4
2
PBS
RSV
FI-RSV
1 ug PFP
1 ug + Alum
10 ug PFP
10 ug +Alum
30 ug PFP
Anti-F sheep
Vaccine Group

Figure 19

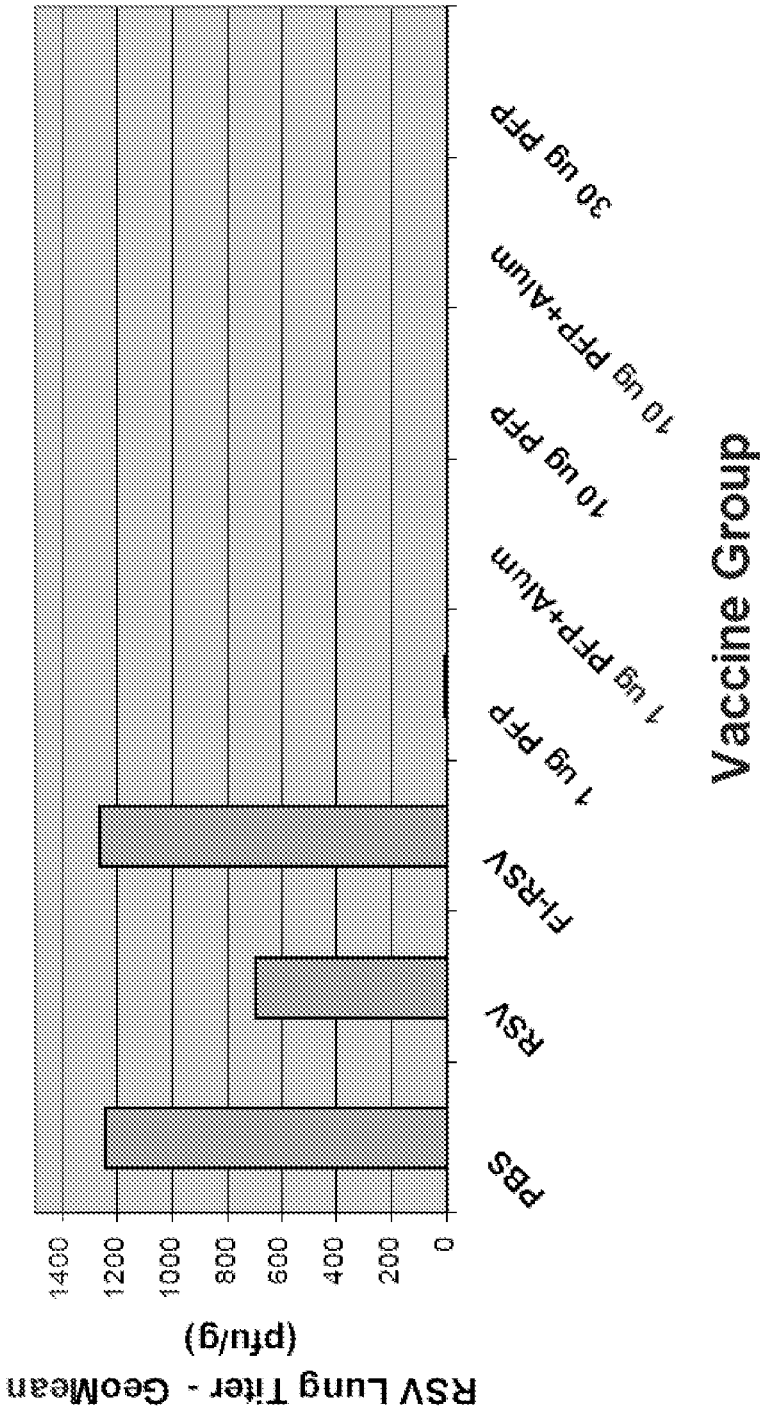

RSV-F Mouse Challenge Study
Lung Titer (right lung)
RSV Lung Titer - GeoMean (pfu/g)
1400
1200
1000
800
600
400
200
0
PBS
RSV
Fl-RSV
1 ug PFP
1 ug PFP+Alum
10 ug PFP
10 ug PFP+Alum
30 ug PFP
Vaccine Group

Figure 20

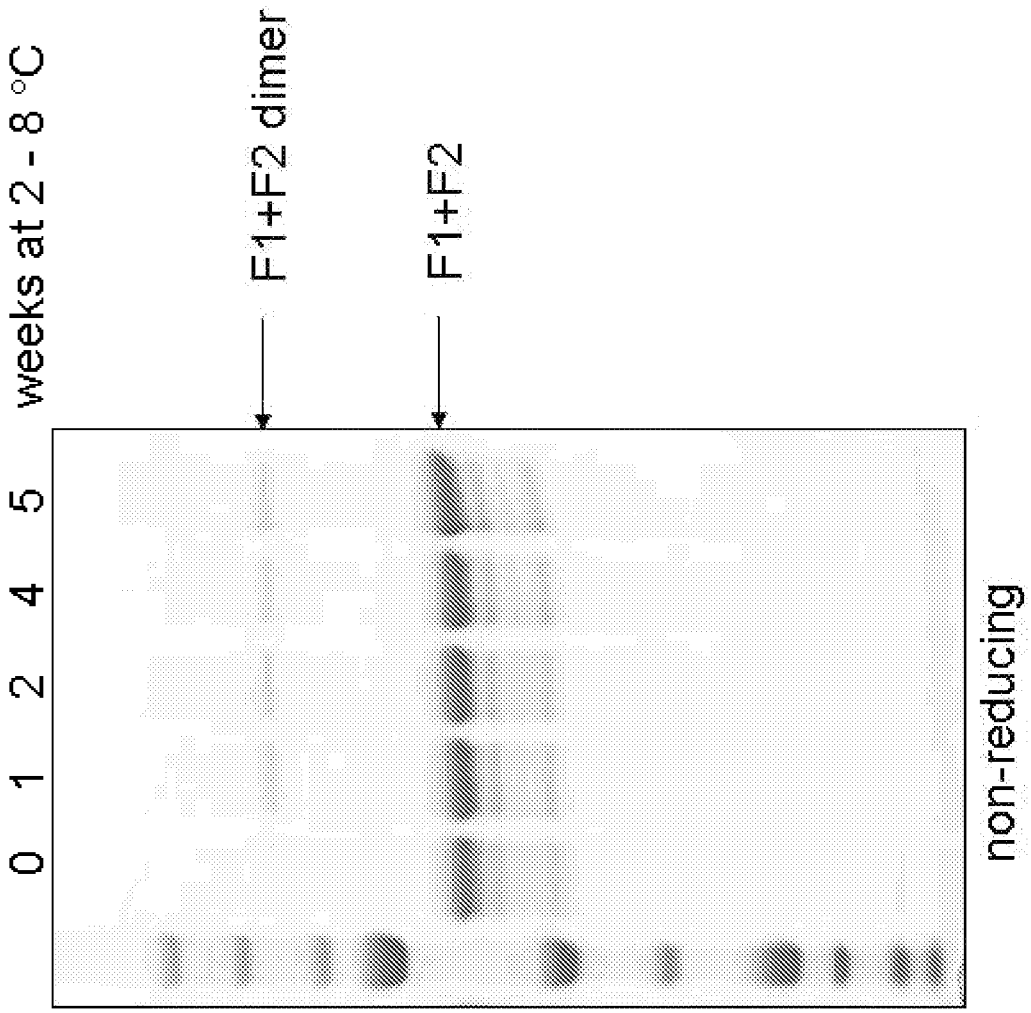
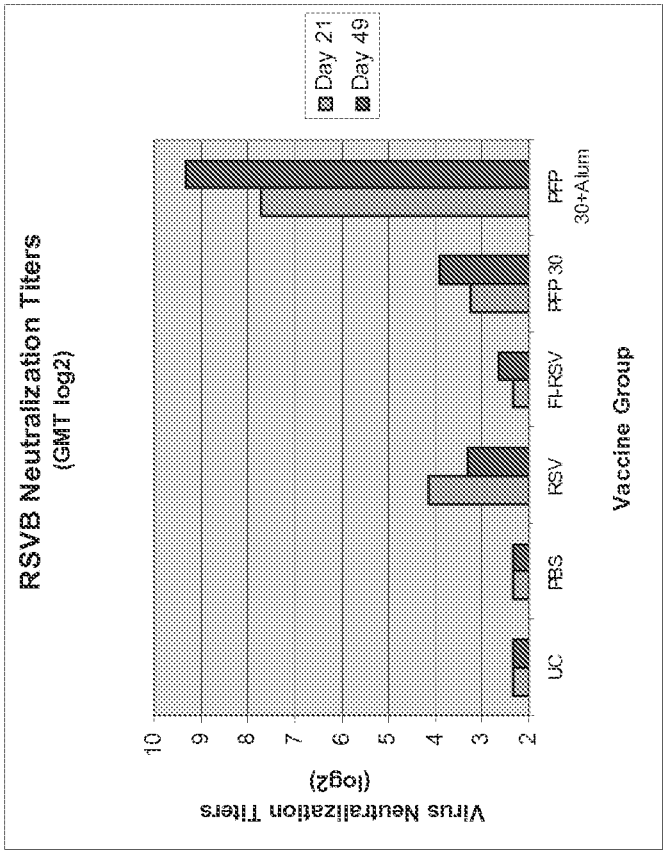


Figure 21


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

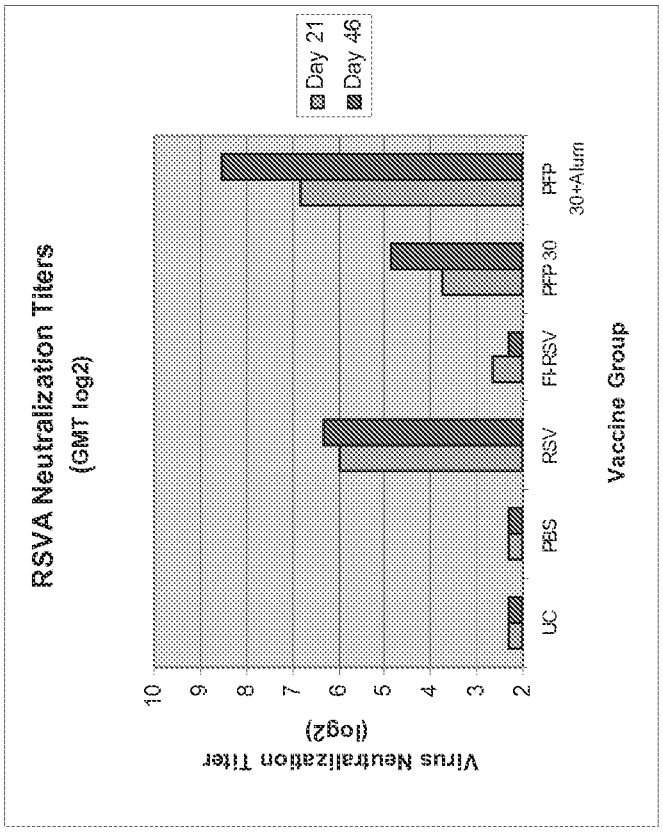

RSVA Neutralization Titers
(GMT log2)
Virus Neutralization Titer
(log2)
10
9
8
7
6
5
4
3
2
Day 21
Day 46
UC
PBS
RSV
FI-RSV
PFP 30
PFP 30+Alum
Vaccine Group

Figure 22

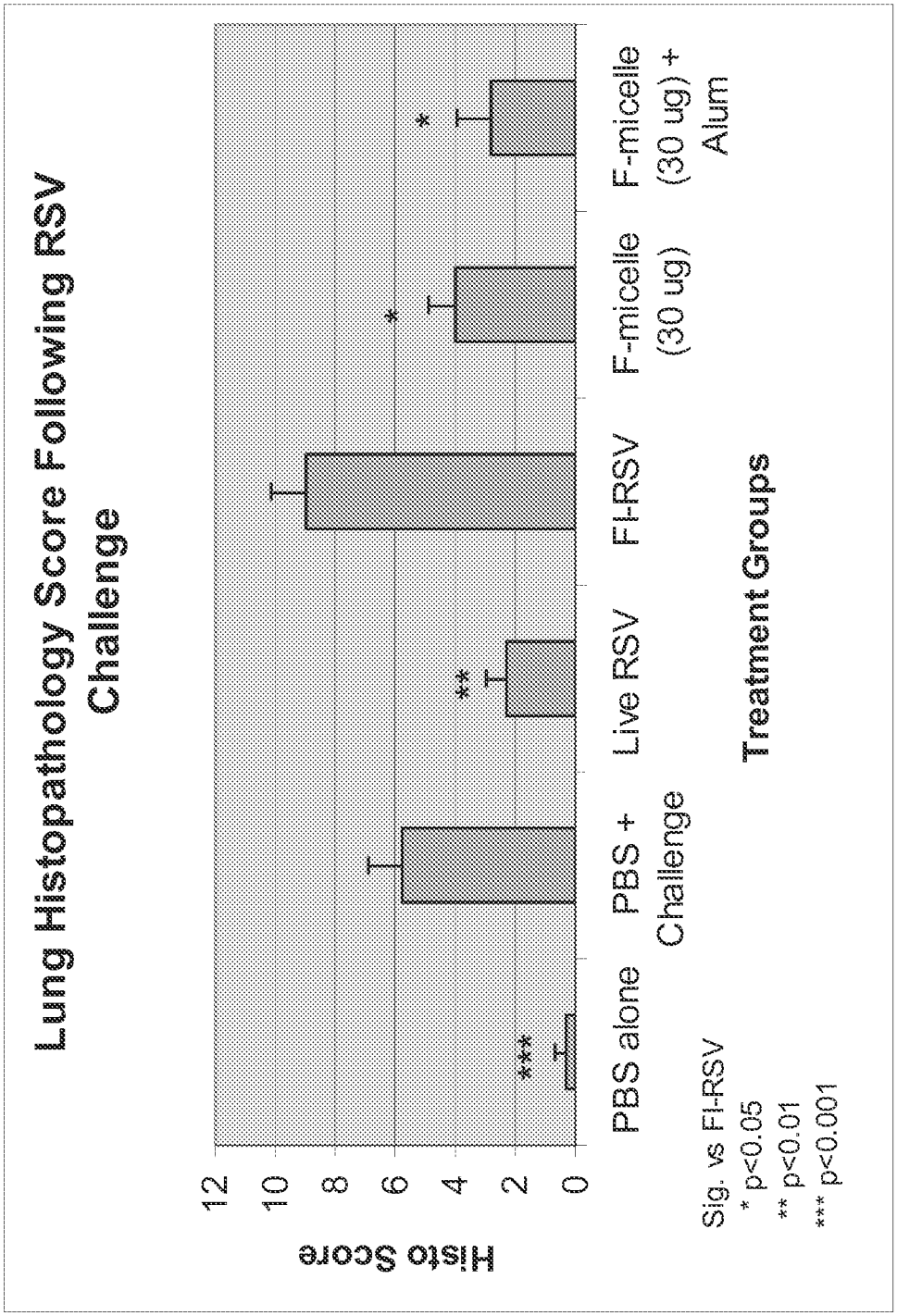

Lung Histopathology Score Following RSV Challenge
Histo Score
0
2
4
6
8
10
12
***
**
*
*
PBS alone
PBS + Challenge
Live RSV
FI-RSV
F-micelle (30 ug)
F-micelle (30 ug) + Alum
Treatment Groups
Sig. vs FI-RSV
* p<0.05
** p<0.01
*** p<0.001

Figure 23

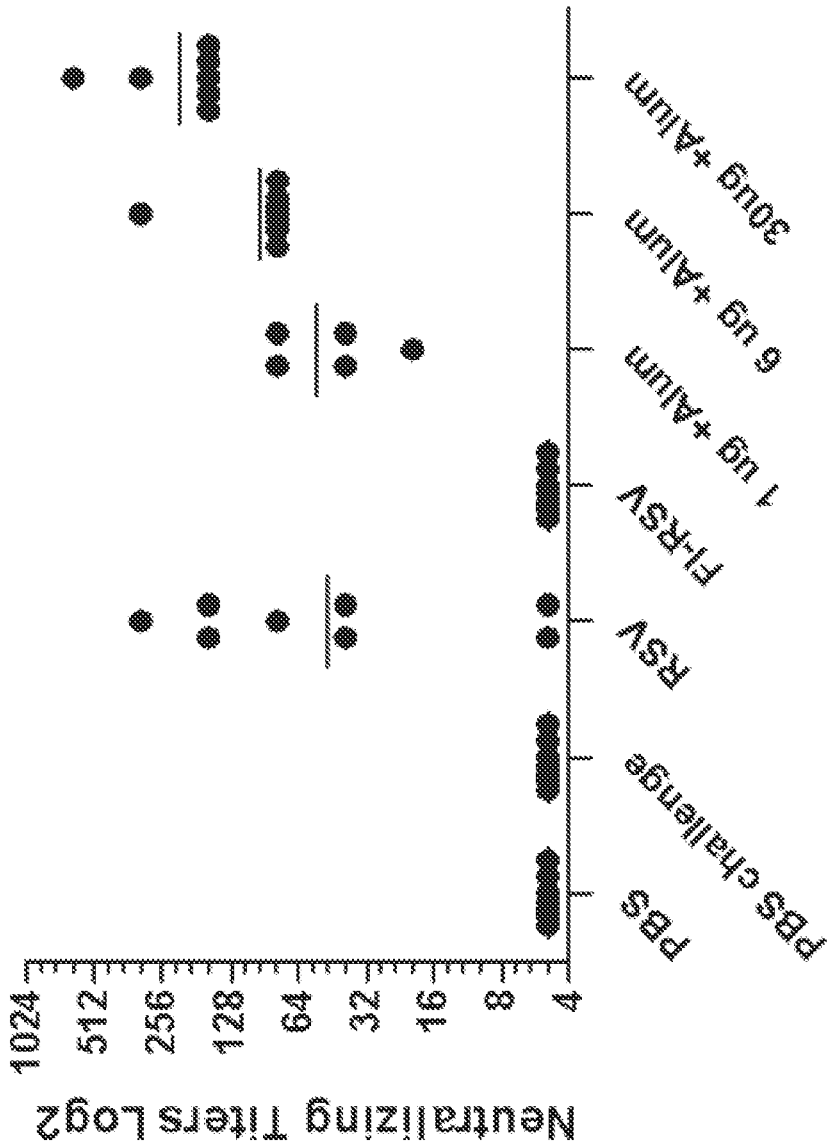

Neutralizing Titers Log2
1024
512
256
128
64
32
16
8
4
PBS
PBS challenge
RSV
FI-RSV
1 ug +Alum
6 ug +Alum
30ug +Alum

Figure 24

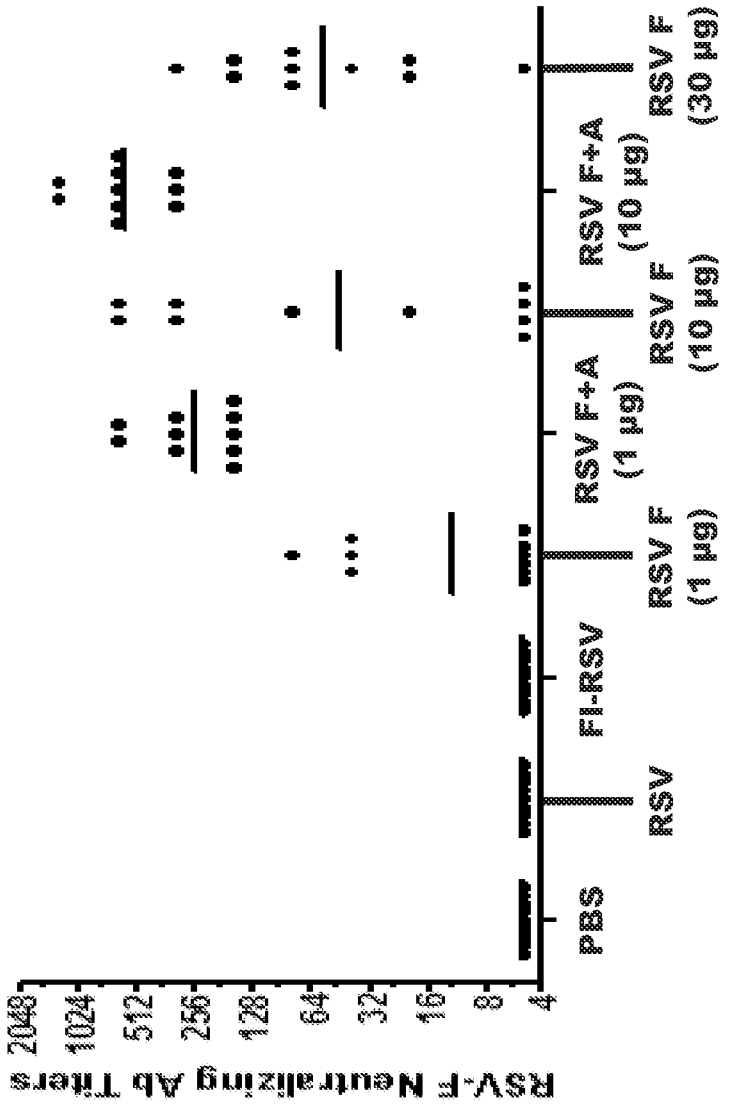

RSV-F Neutralizing Ab Titers
2048
1024
512
256
128
64
32
16
8
4
PBS
RSV
FI-RSV
RSV F
(1 µg)
RSV F+A
(1 µg)
RSV F
(10 µg)
RSV F+A
(10 µg)
RSV F
(30 µg)

Figure 25

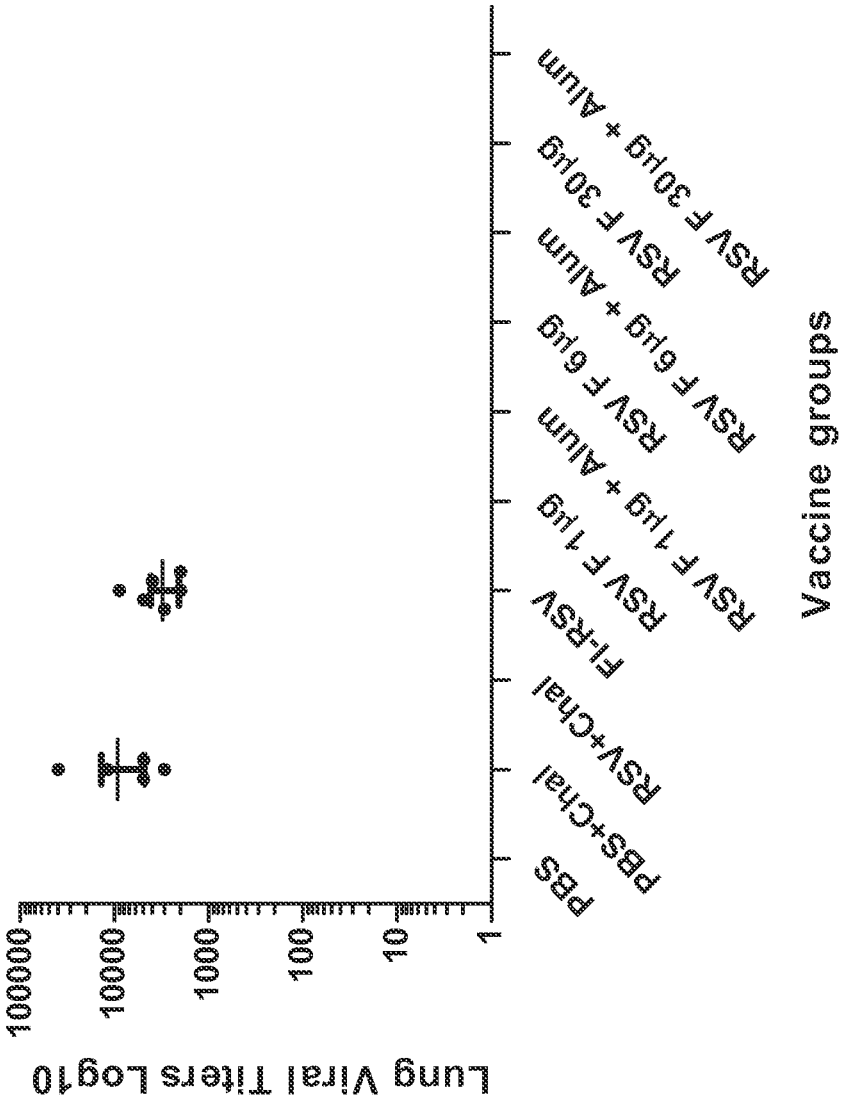

Lung Viral Titers Log10
100000
10000
1000
100
10
1
PBS
PBS+Chal
RSV+Chal
FI-RSV
RSV F 1µg
RSV F 1µg + Alum
RSV F 6µg
RSV F 6µg + Alum
RSV F 30µg
RSV F 30µg + Alum
Vaccine groups

Figure 26A, 26B

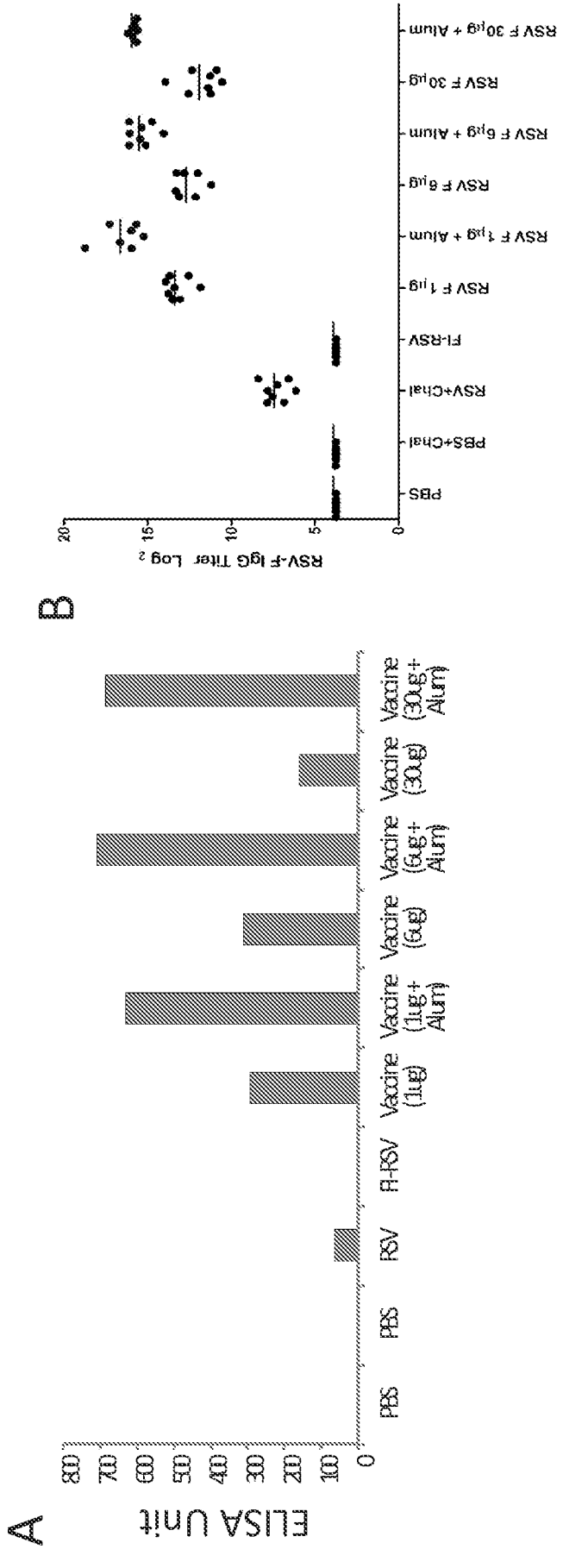


Figure 26C, 26D

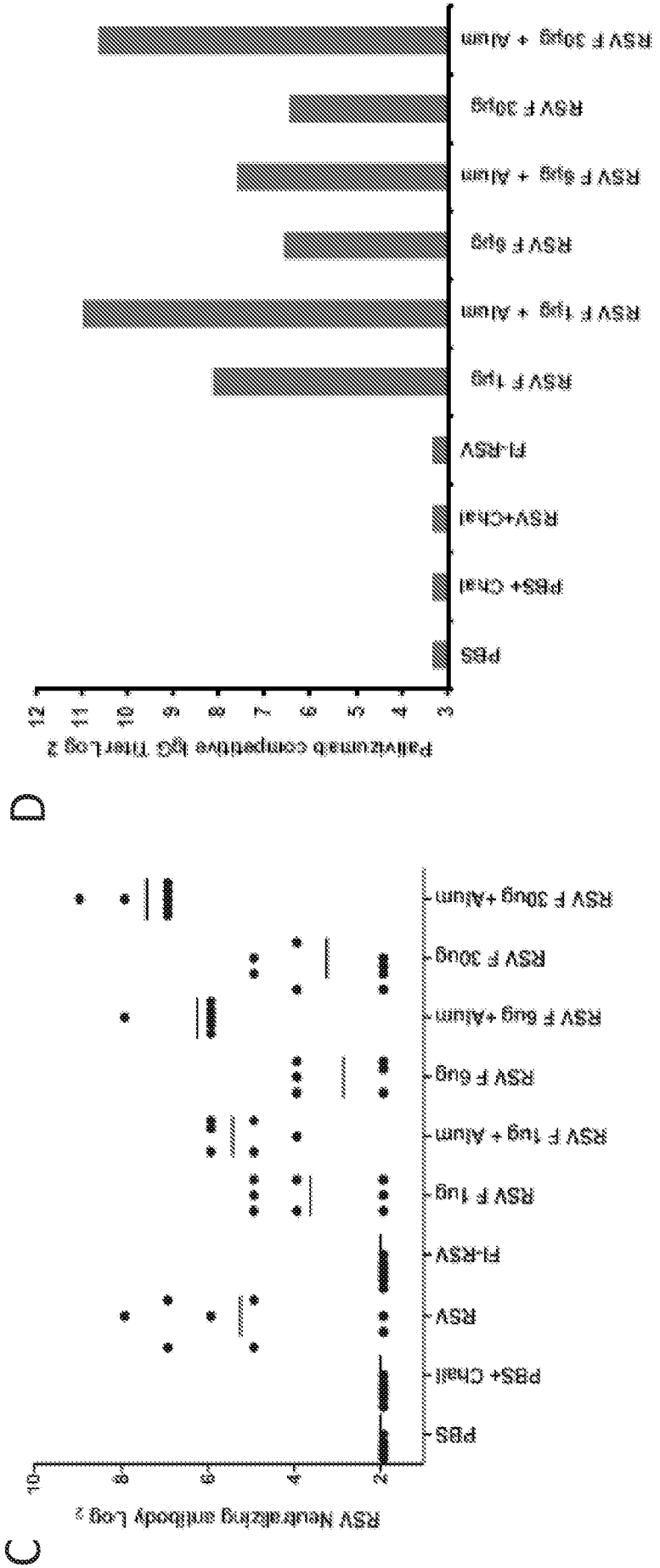


Figure 27

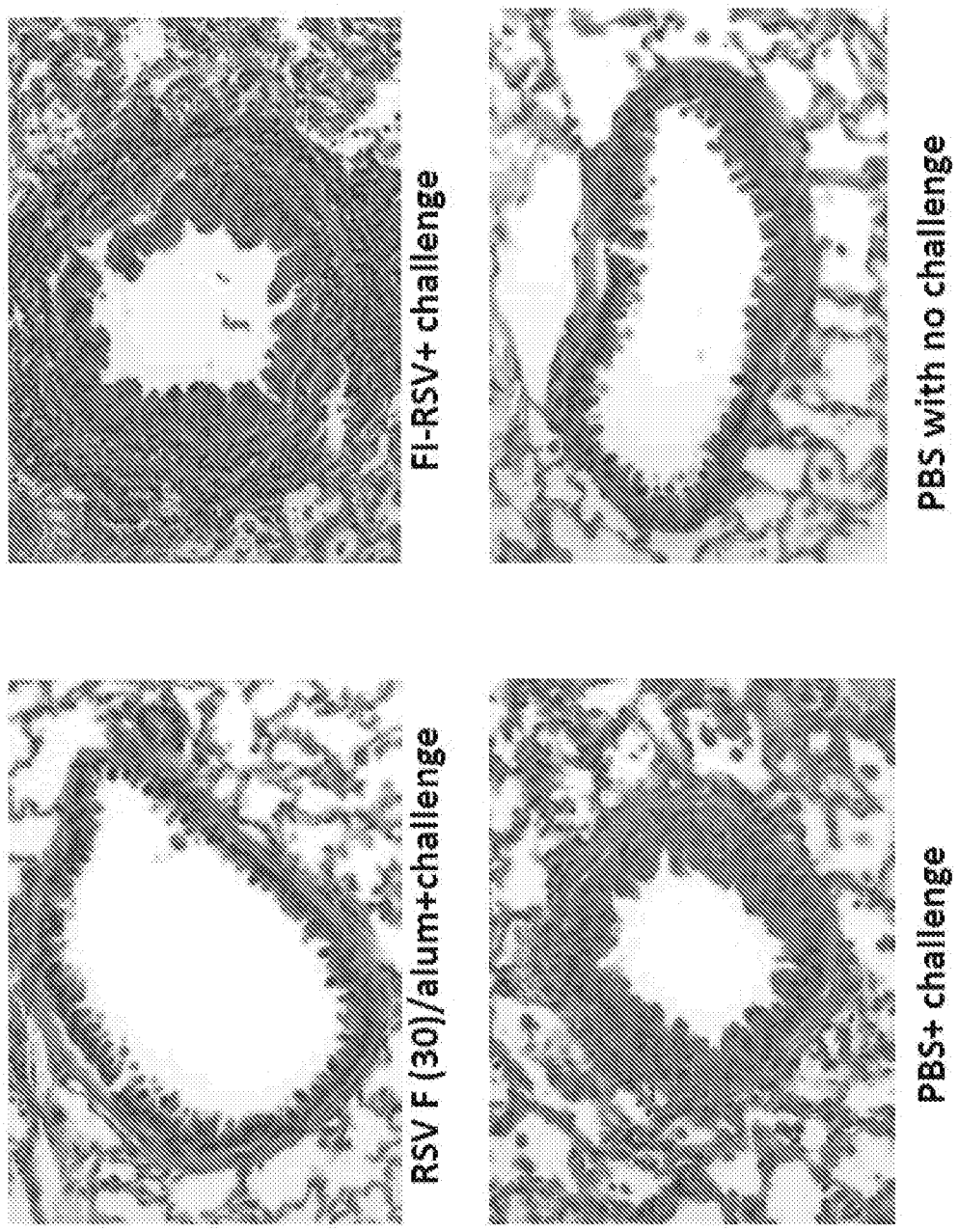

FI-RSV+ challenge
PBS with no challenge
RSV F (30)/alum+challenge
PBS+ challenge

Figure 28

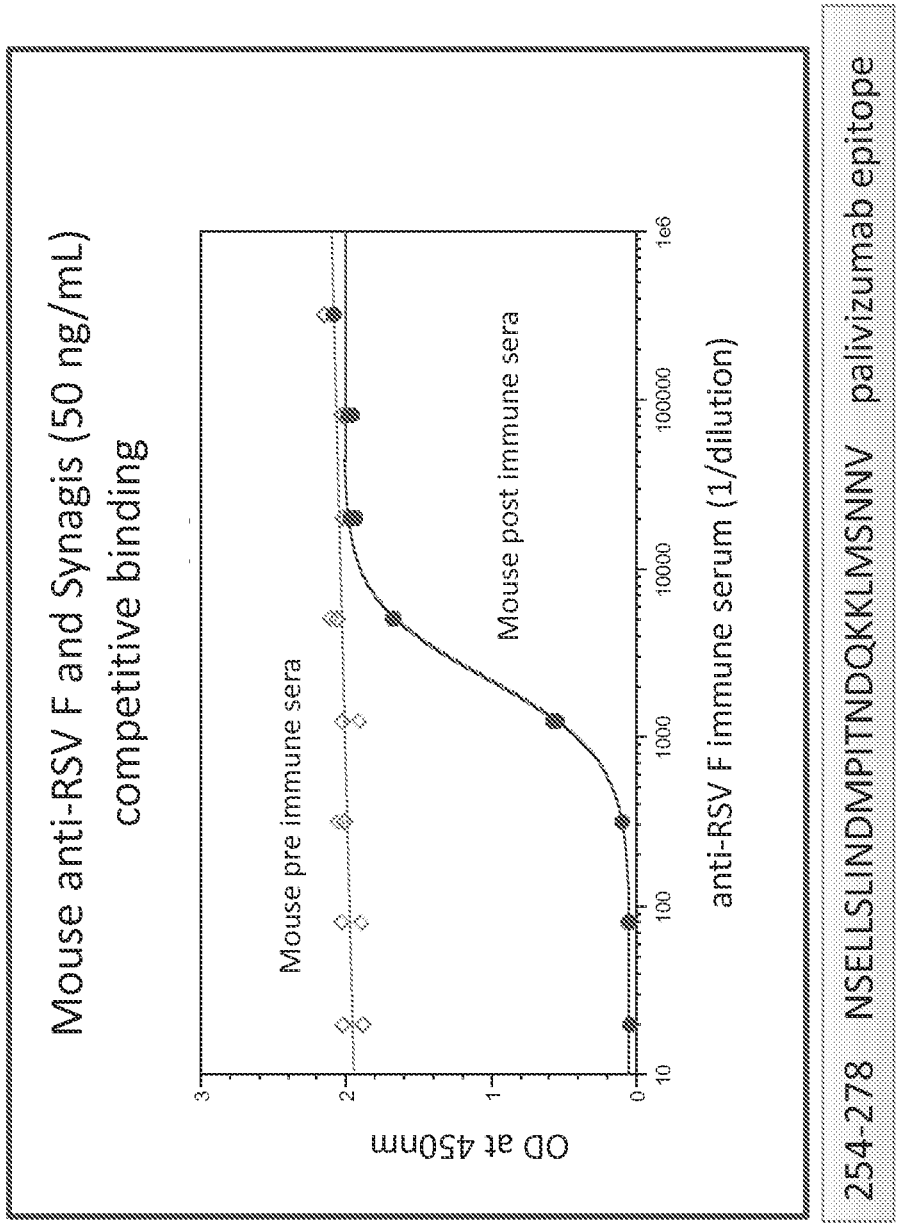

Mouse anti-RSV F and Synagis (50 ng/mL)
competitive binding
Mouse pre immune sera
Mouse post immune sera
OD at 450nm
anti-RSV F immune serum (1/dilution)
254-278 NSELLSLINDMPITNDQKKLMSNNV palivizumab epitope

Figure 29

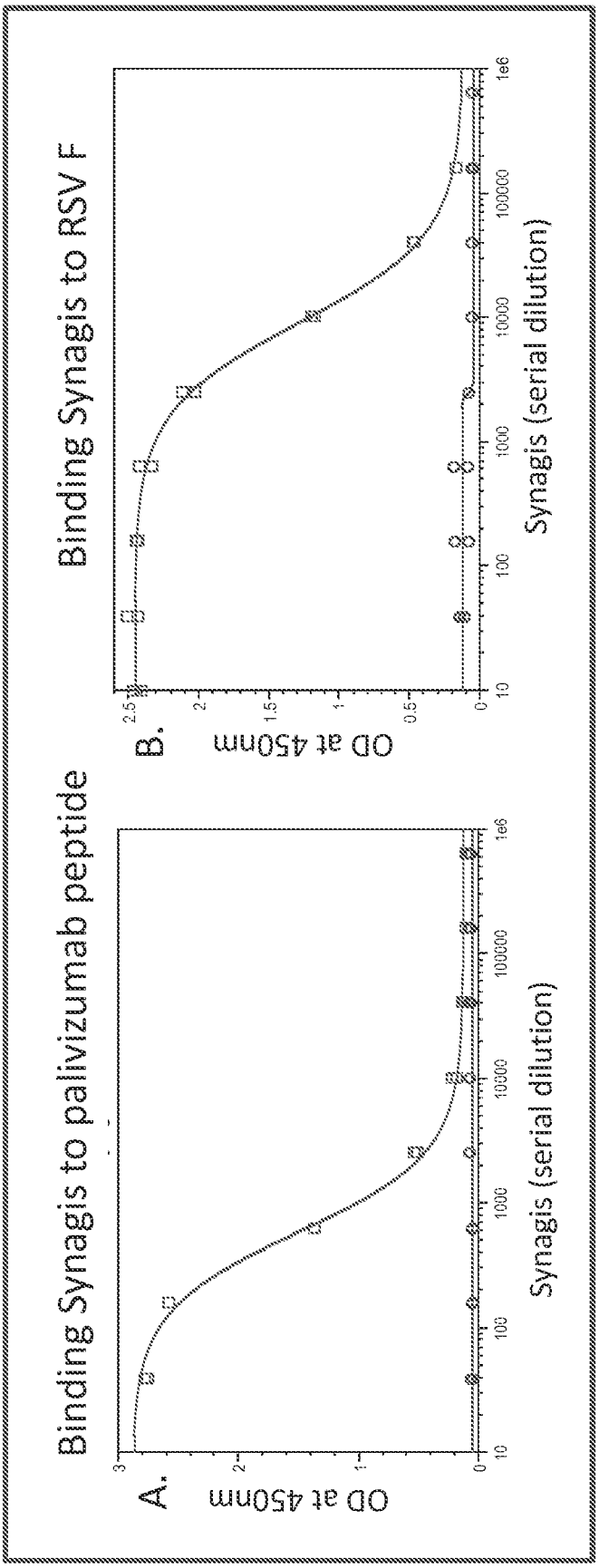
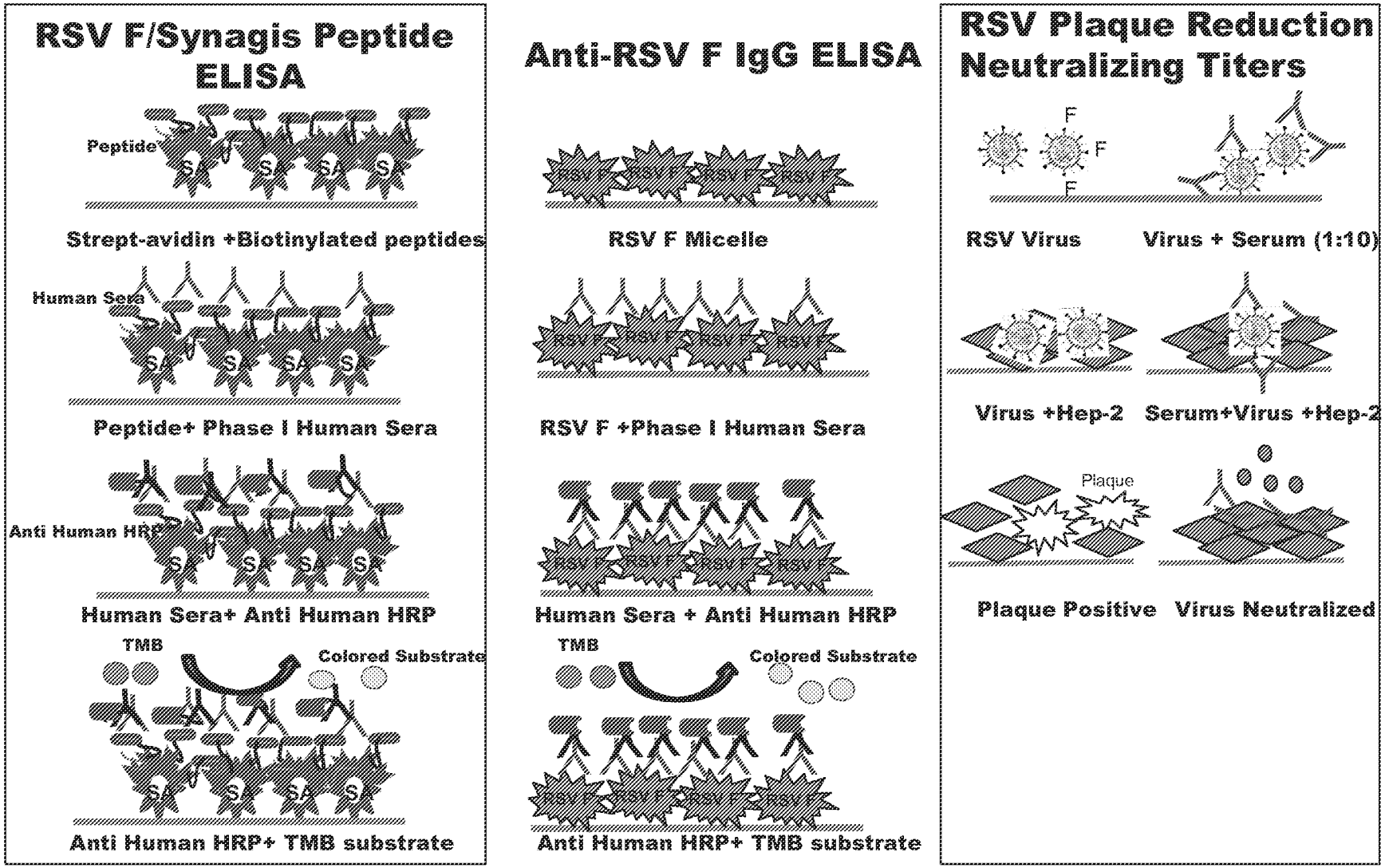

A.
Binding Synagis to palivizumab peptide
OD at 450nm
Synagis (serial dilution)
B.
Binding Synagis to RSV F
OD at 450nm
Synagis (serial dilution)

Figure 30



WO 2013/049342 PCT/US2012/057546

Figure 31

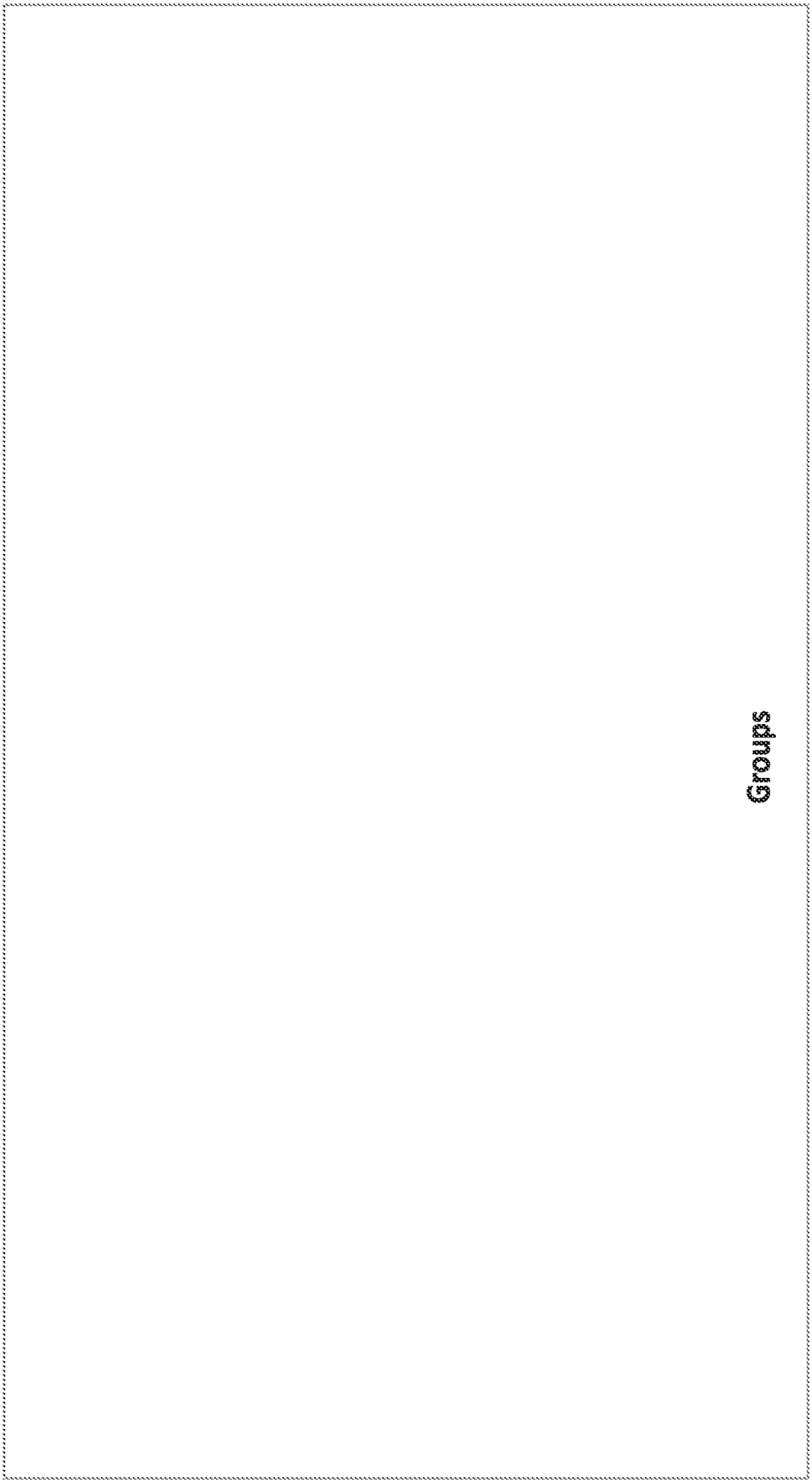


Figure 32

Day 30	585	3627	2618	4695	4554	6795	5652
Day 60	601	5913	4224	6521	4079	9256	5249

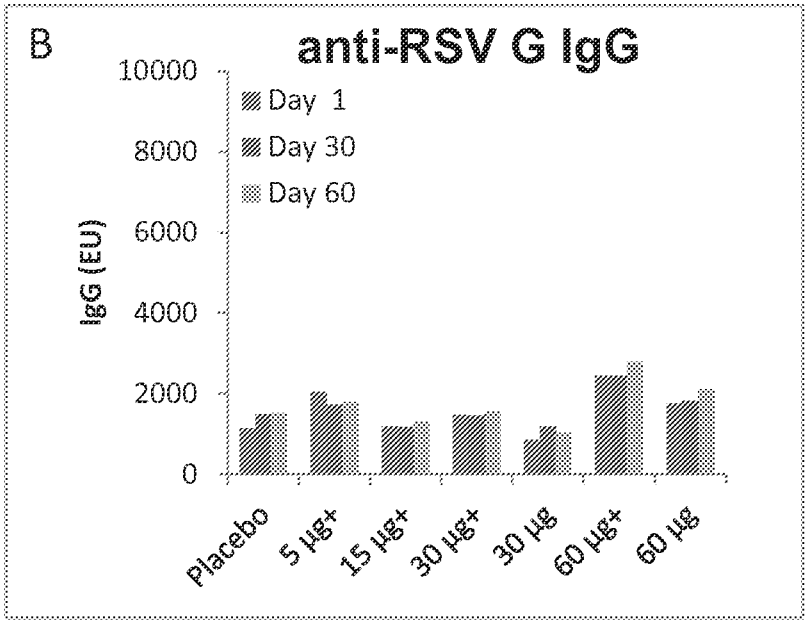


Figure 33

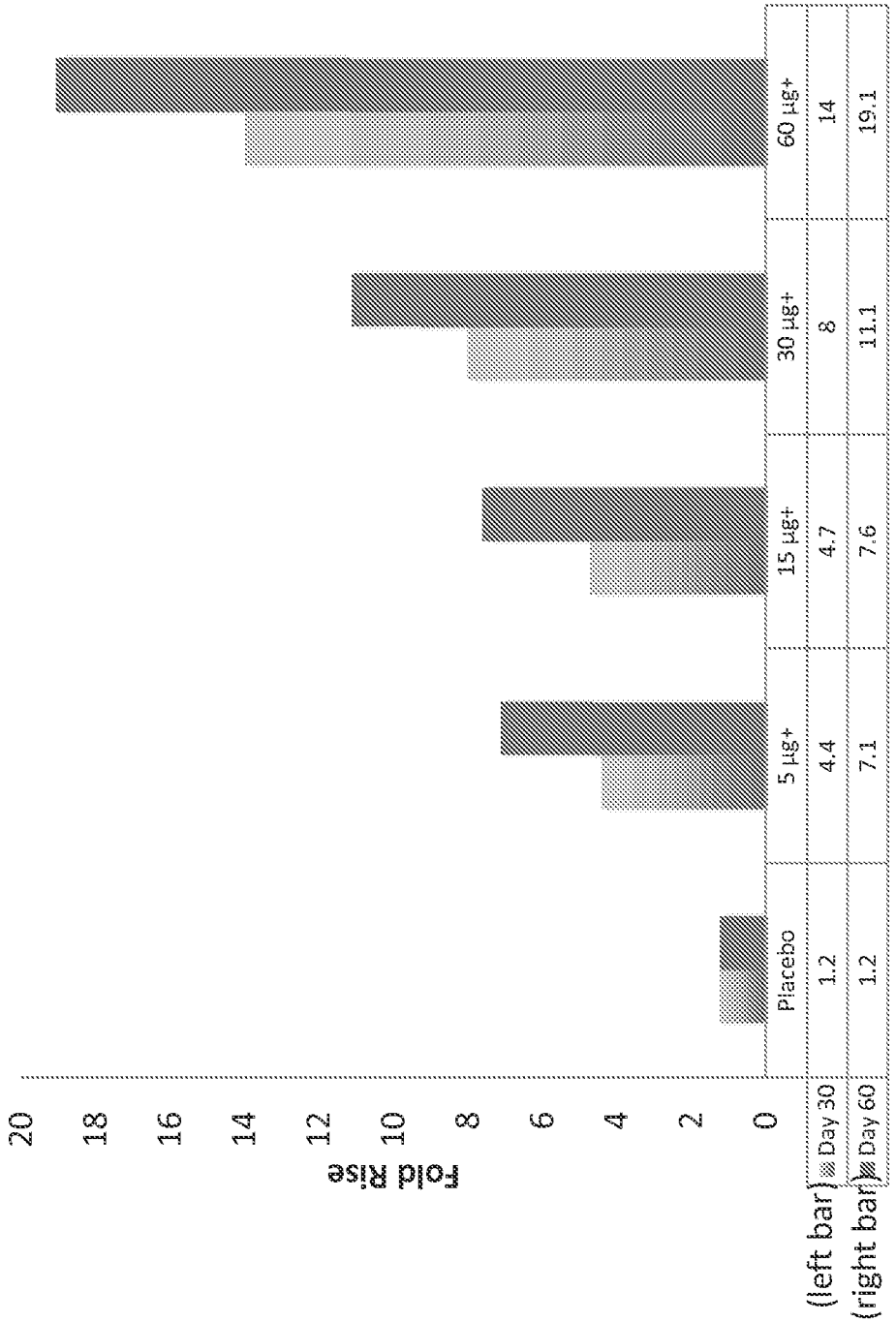

Fold Rise
20
18
16
14
12
10
8
6
4
2
0
Placebo
5 µg+
15 µg+
30 µg+
60 µg+
(left bar) Day 30
1.2
4.4
4.7
8
14
(right bar) Day 60
1.2
7.1
7.6
11.1
19.1

Figure 34

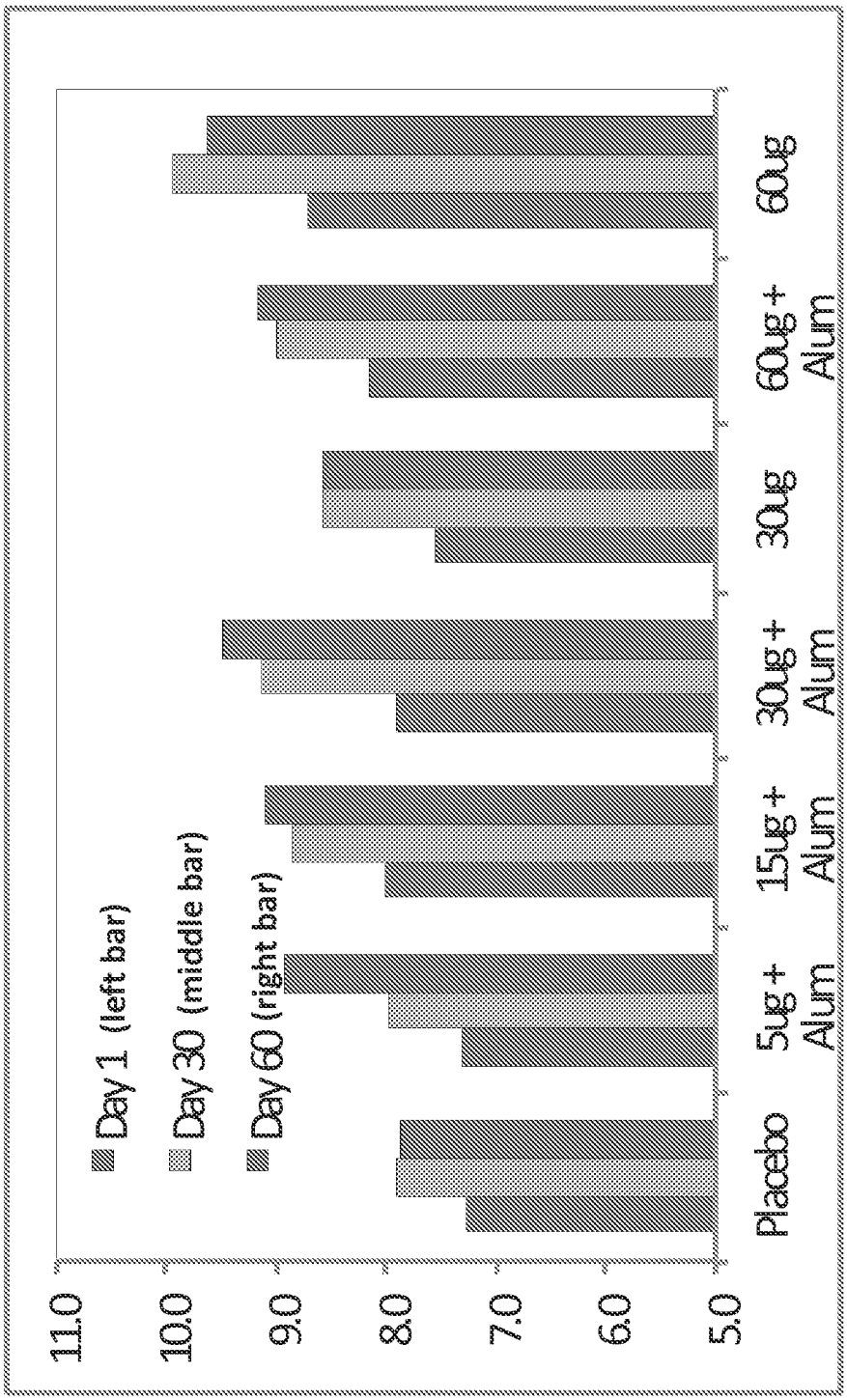

11.0
10.0
9.0
8.0
7.0
6.0
5.0
Day 1 (left bar)
Day 30 (middle bar)
Day 60 (right bar)
Placebo
5ug + Alum
15ug + Alum
30ug + Alum
30ug
60ug + Alum
60ug

Figure 35

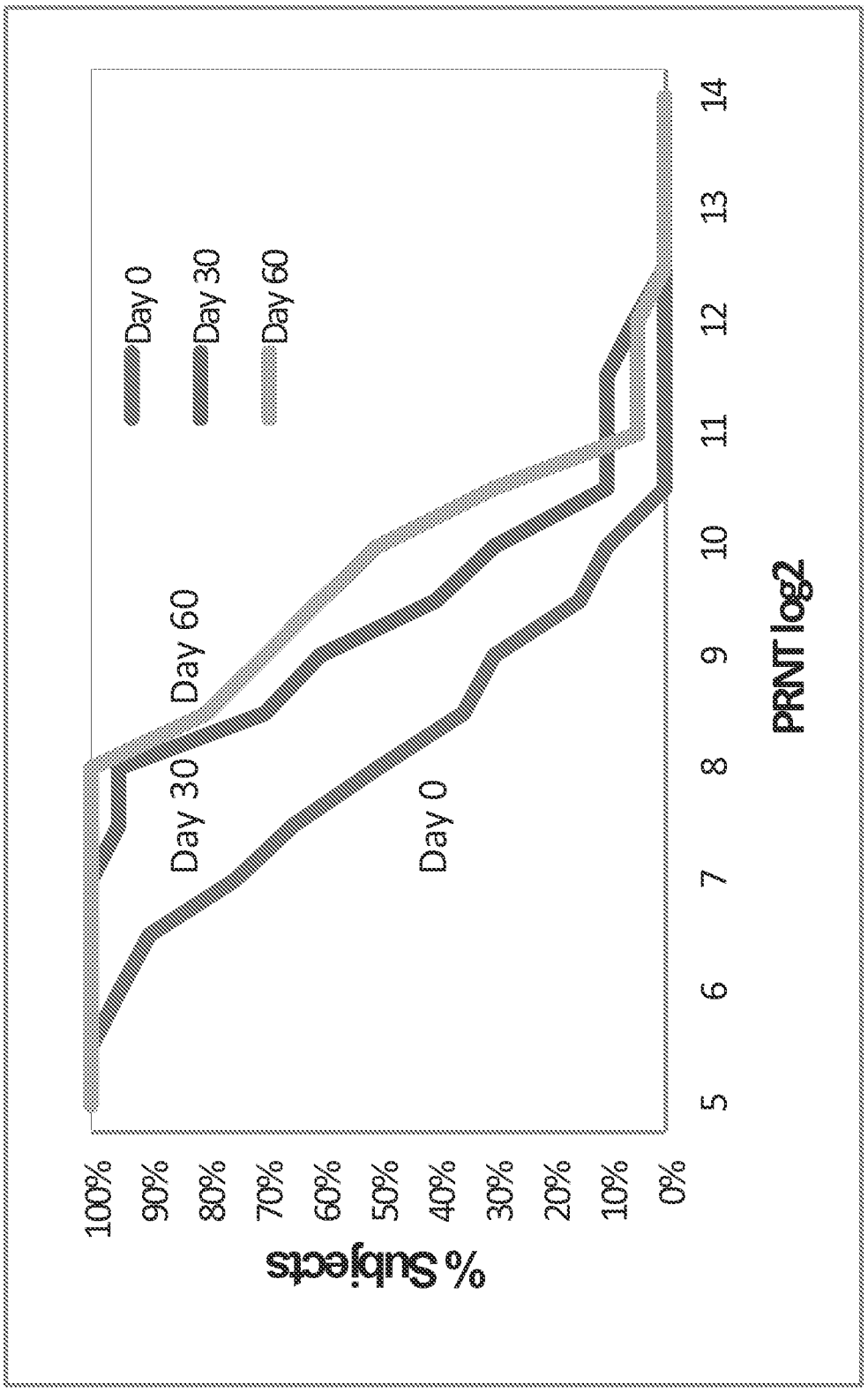

Day 0
Day 30
Day 60
Day 60
Day 30
Day 0
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Subjects
5
6
7
8
9
10
11
12
13
14
PRNT log2

Figure 36

A.

Antibody	Antigen	K-On	K-Off	KD
Palivizumab	100nM RSV F	2.40 E+5	2.50 E-5	1.00 E-10
BEI Ref	100nM RSV F	2.38E+5	5.67 E-7	2.37 E-12
BEI Ref (High)	100nM RSV F	2.10E+5	1.74 E-8	8.27 E-14

B.

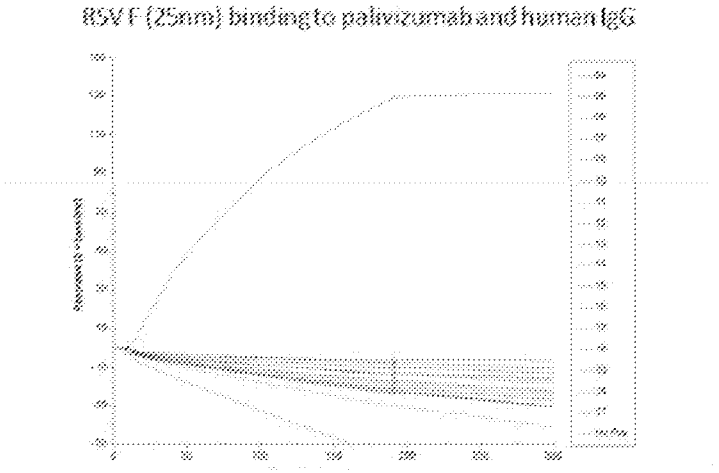
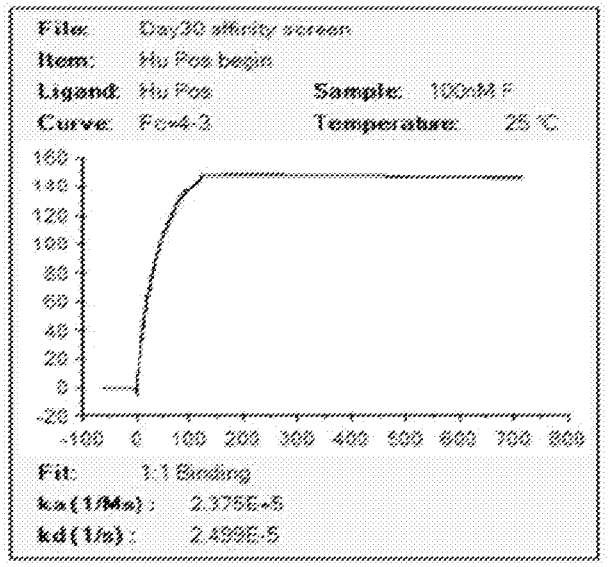
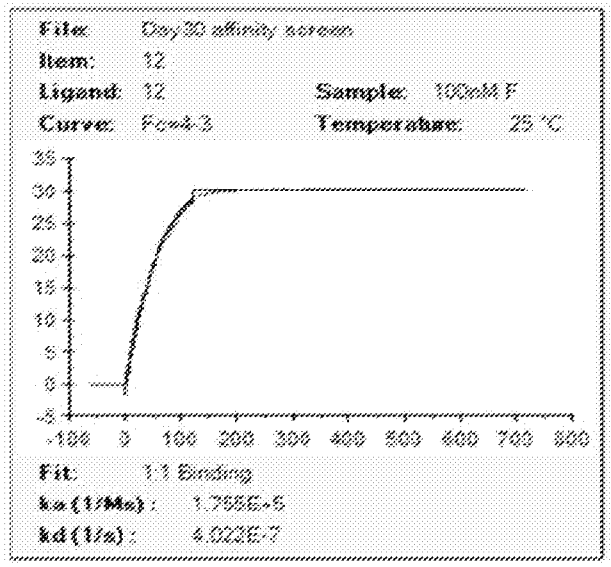


Figure 37



Palivizumab

KD = 0.95E-10



Vaccinee day 30; ID 1112

KD = 2.29E-12

Figure 38

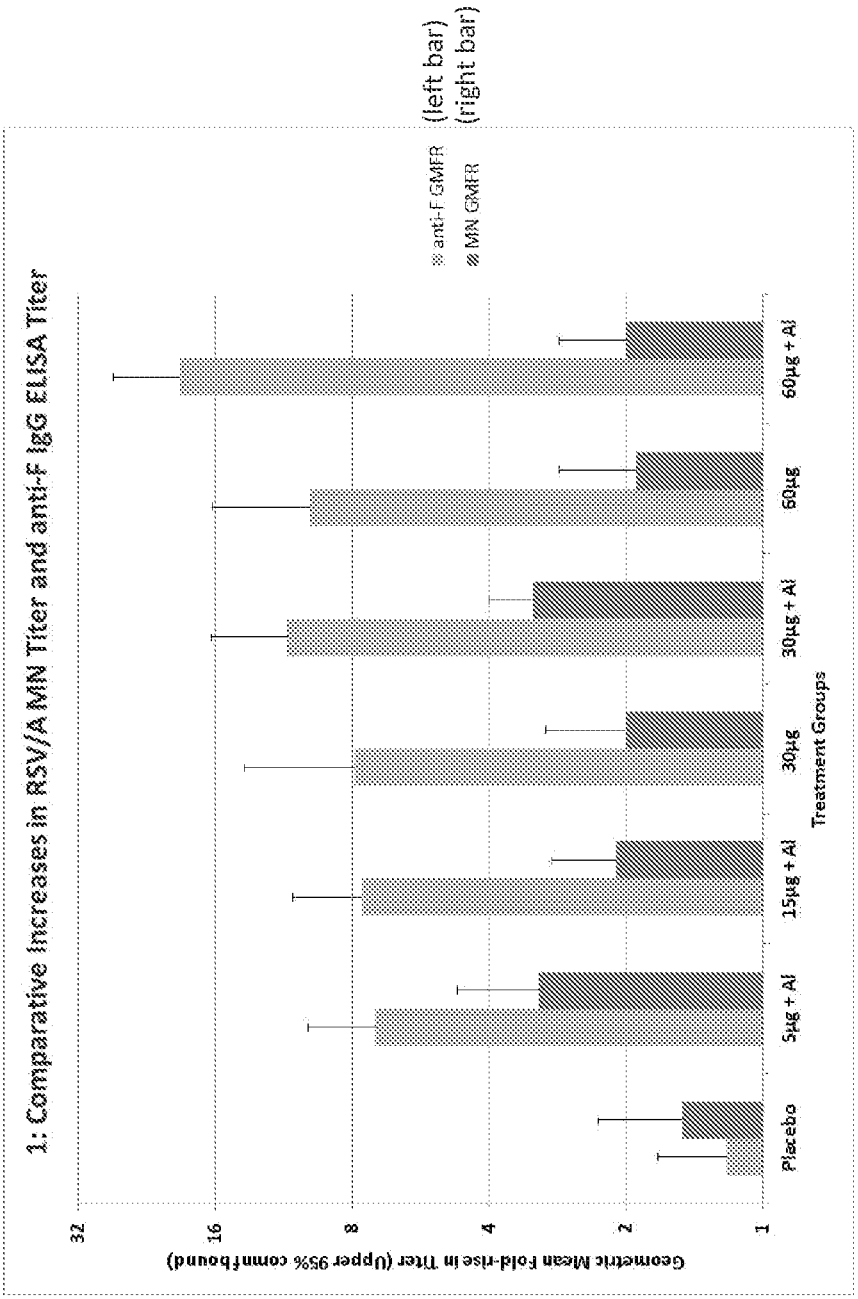

1: Comparative Increases in RSV/A MN Titer and anti-F IgG ELISA Titer
anti-F GMFR (left bar)
MN GMFR (right bar)
Geometric Mean Fold-rise in Titer (Upper 95% confbound)
32
16
8
4
2
1
Placebo
5µg + Al
15µg + Al
30µg
30µg + Al
60µg
60µg + Al
Treatment Groups

Figure 39

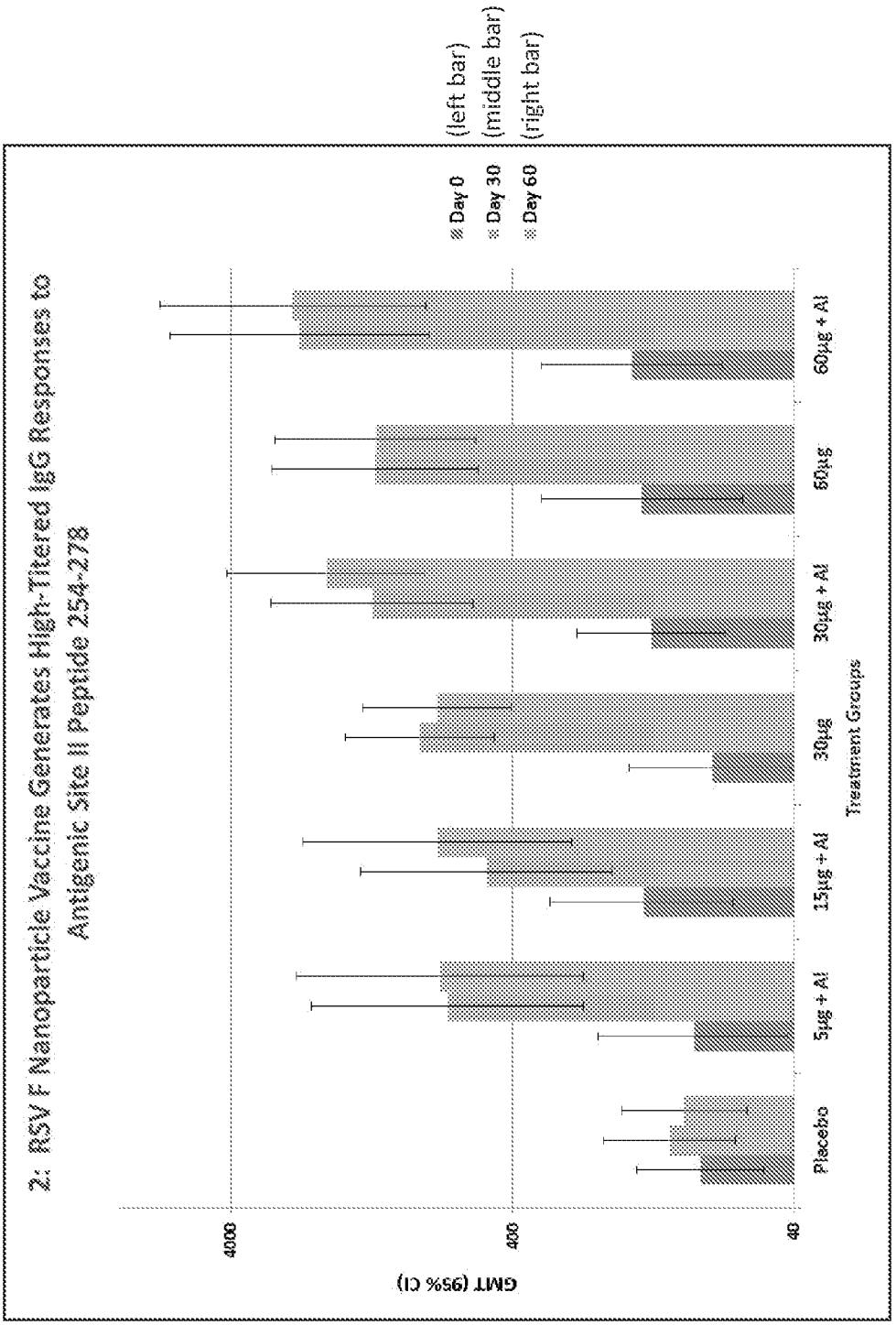

2: RSV F Nanoparticle Vaccine Generates High-Titered IgG Responses to Antigenic Site II Peptide 254-278
Day 0 (left bar)
Day 30 (middle bar)
Day 60 (right bar)
GMT (95% CI)
4000
400
40
Placebo
5µg + Al
15µg + Al
30µg
30µg + Al
60µg
60µg + Al
Treatment Groups

Figure 40A

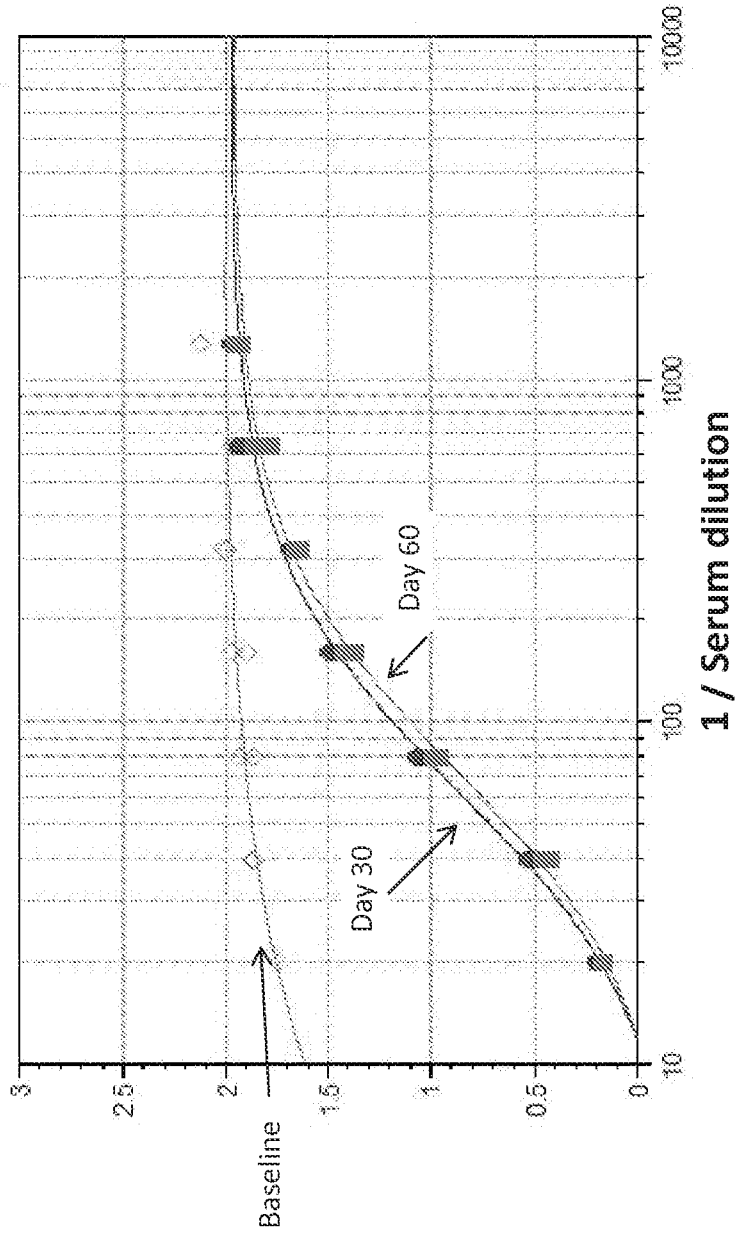

Baseline
Day 30
Day 60
1 / Serum dilution
0
0.5
1
1.5
2
2.5
3
10
100
1000
10000

Figure 40B

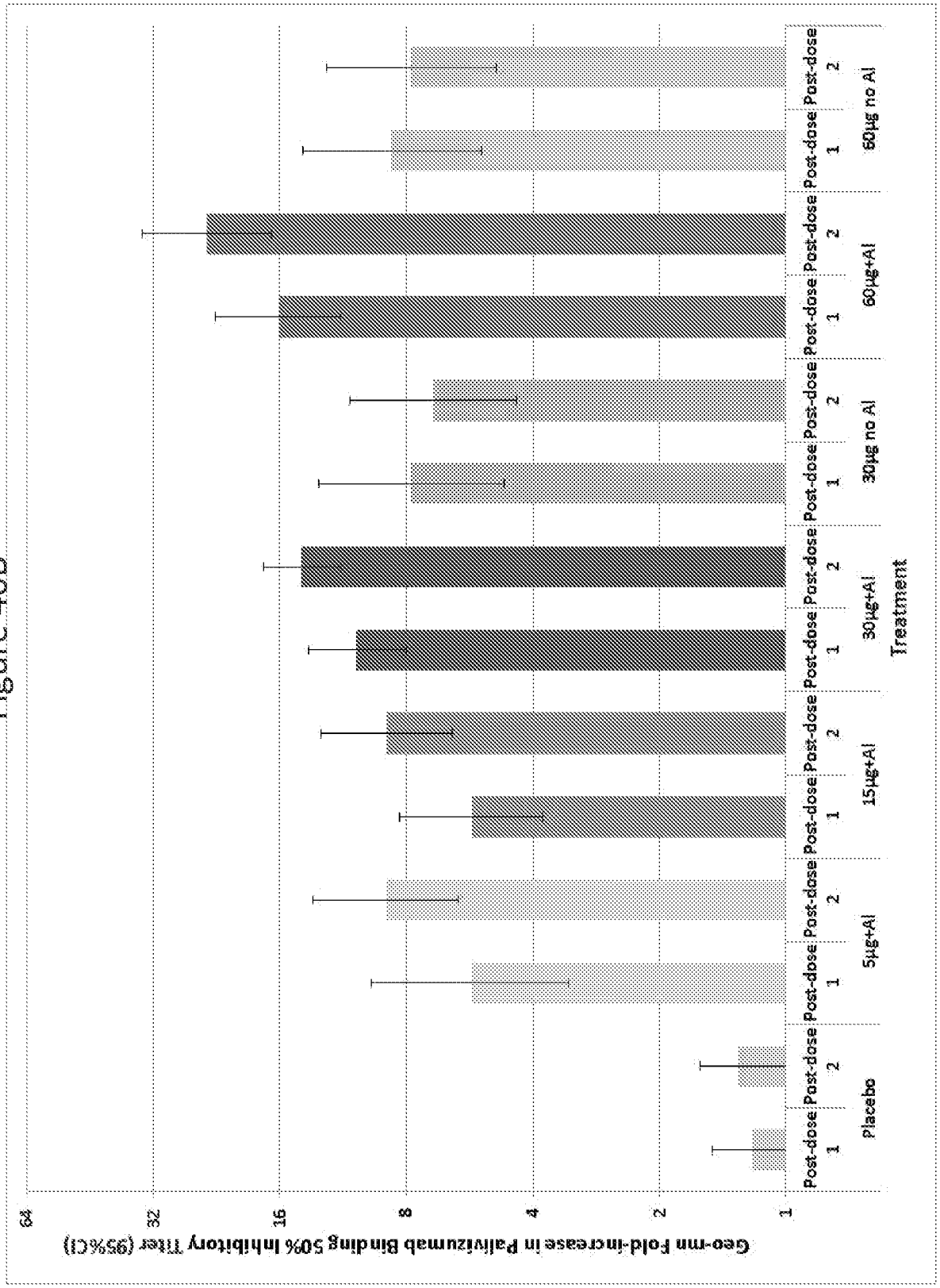
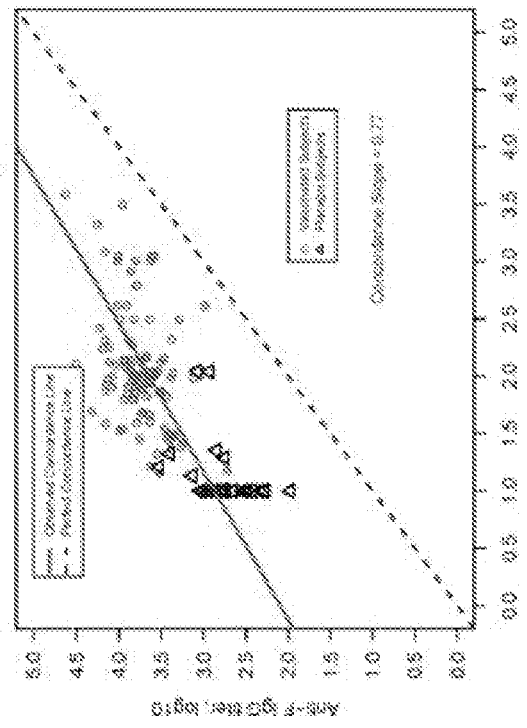

Geo-mn Fold-increase in Palivizumab Binding 50% Inhibitory Titer (95%CI)
64
32
16
8
4
2
1
Post-dose 1
Post-dose 2
Placebo
5µg+Al
15µg+Al
30µg+Al
30µg no Al
60µg+Al
60µg no Al
Treatment

Figure 41

Induction of anti-F Antibodies Post-Vaccination Correlates with the Presence of Palivizumab-Competing Activity



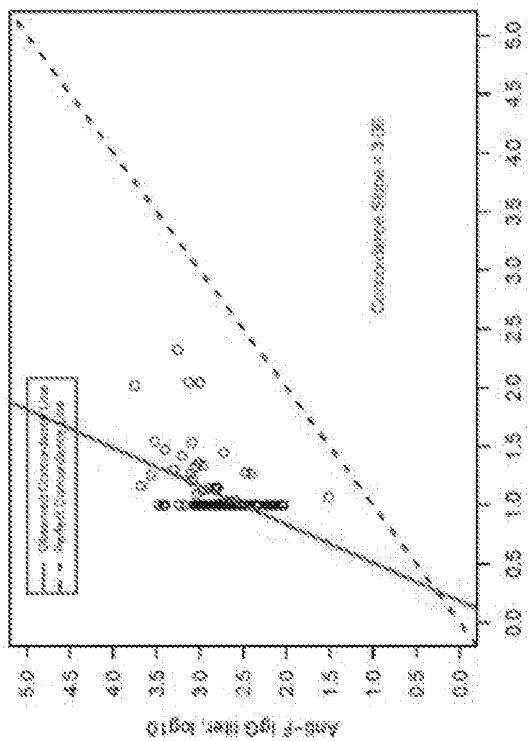
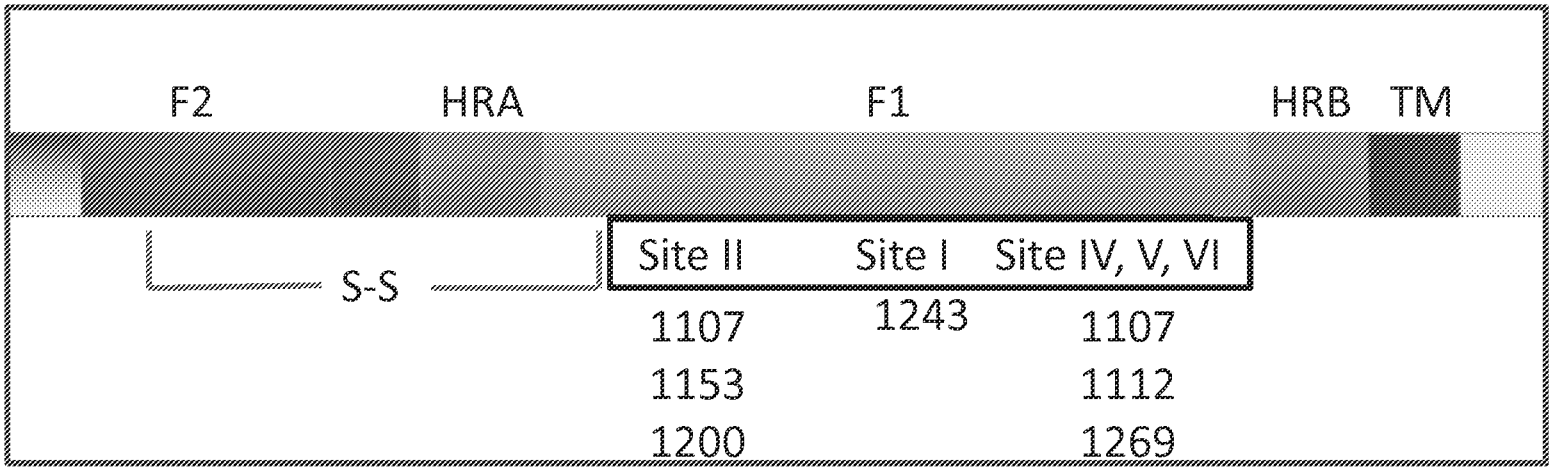


Figure 42



RSV F Binding Titers in ELISA UNITS (EU)			
Mab ID	Site A	Site B	Site C
1107*	6.5 E+04	6.5 E+04	
1153	1.3 E+04		
1200	8.8 E+05		
1243			1.0E+05
1112		4.5E+06	
1269		1.7E+06	

WO 2013/049342 PCT/US2012/057546

Figure 43

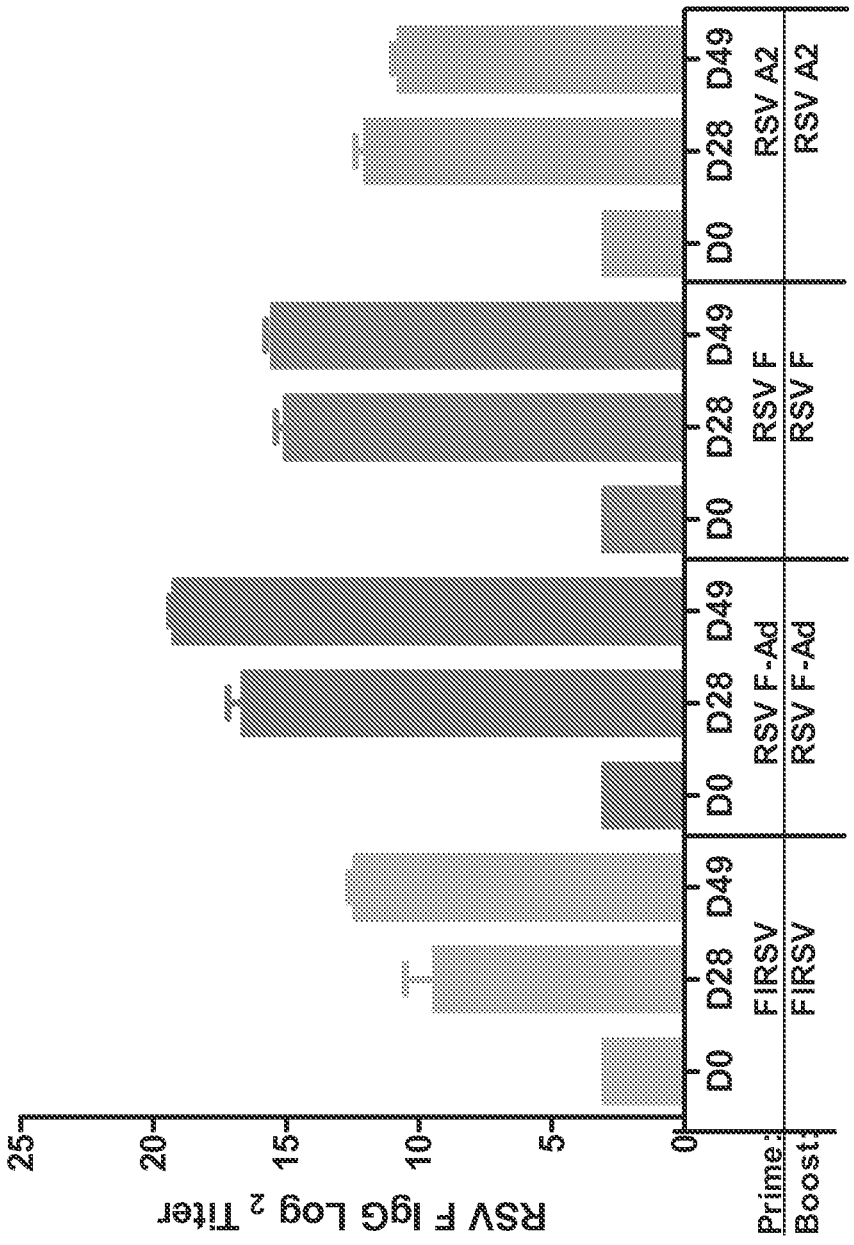

RSV F IgG Log 2 Titer
25
20
15
10
5
0
Prime:
Boost:
D0
D28
D49
F1RSV
F1RSV
RSV F-Ad
RSV F-Ad
RSV F
RSV F
RSV A2
RSV A2

Figure 44

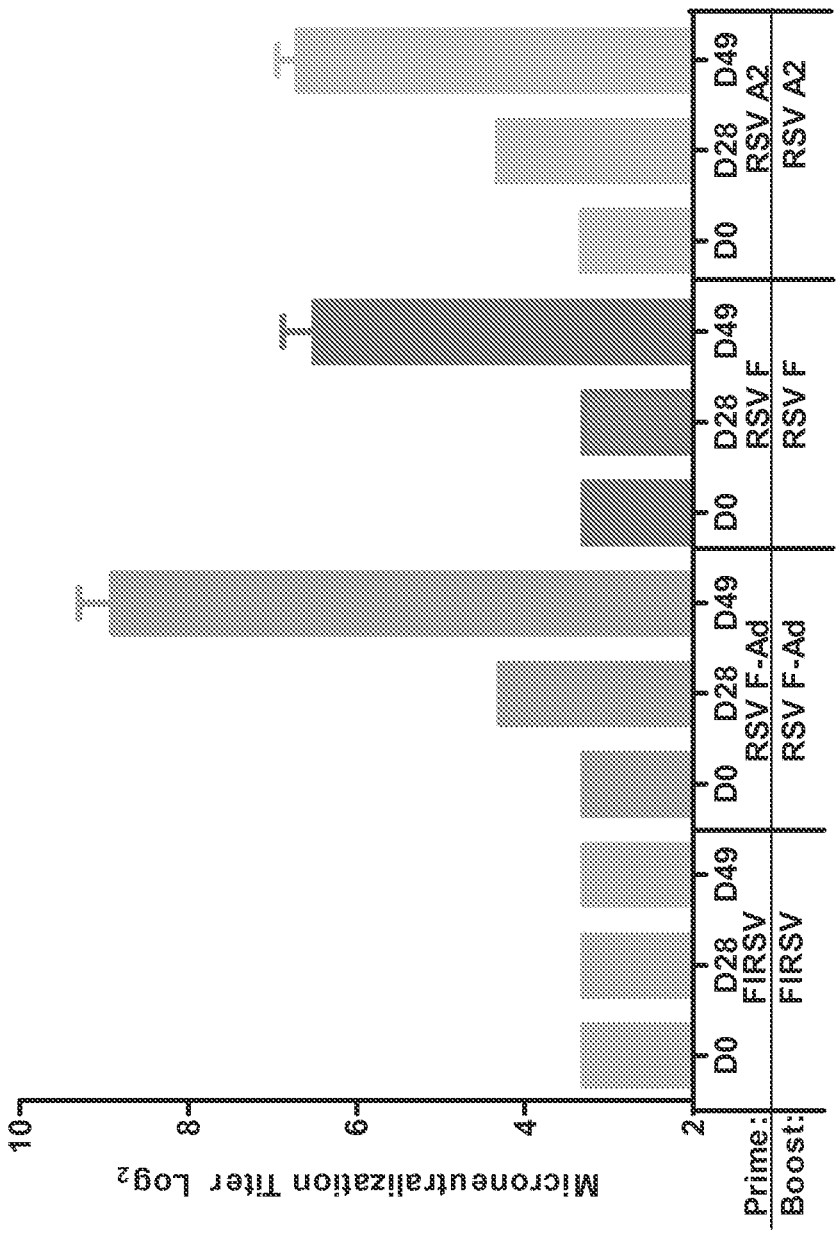

Microneutralization Titer Log2
10
8
6
4
2
Prime:
Boost:
D0
D28
D49
FIRSV
FIRSV
D0
D28
D49
RSV F-Ad
RSV F-Ad
D0
D28
D49
RSV F
RSV F
D0
D28
D49
RSV A2
RSV A2

Figure 45

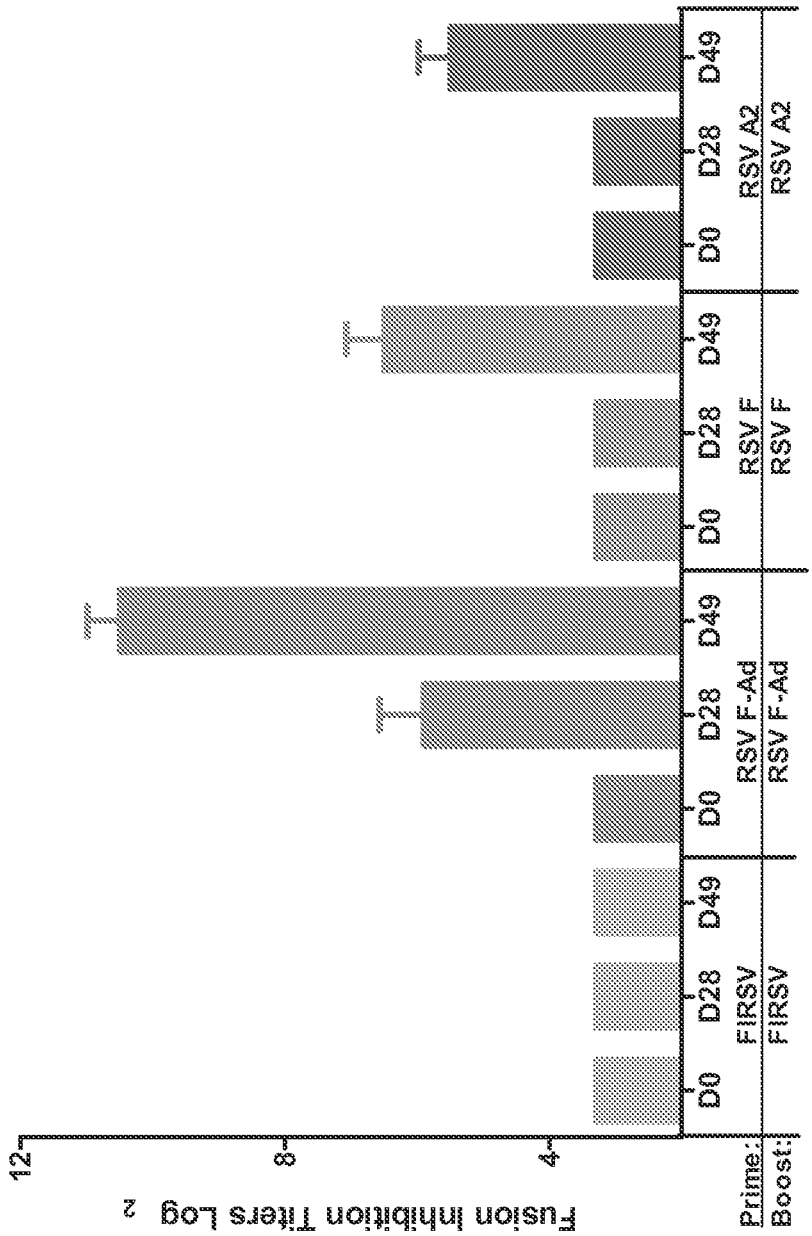

Fusion Inhibition Titers Log 2
12
8
4
Prime:
Boost:
FIRSV
FIRSV
RSV F-Ad
RSV F-Ad
RSV F
RSV F
RSV A2
RSV A2
D0
D28
D49

Figure 46

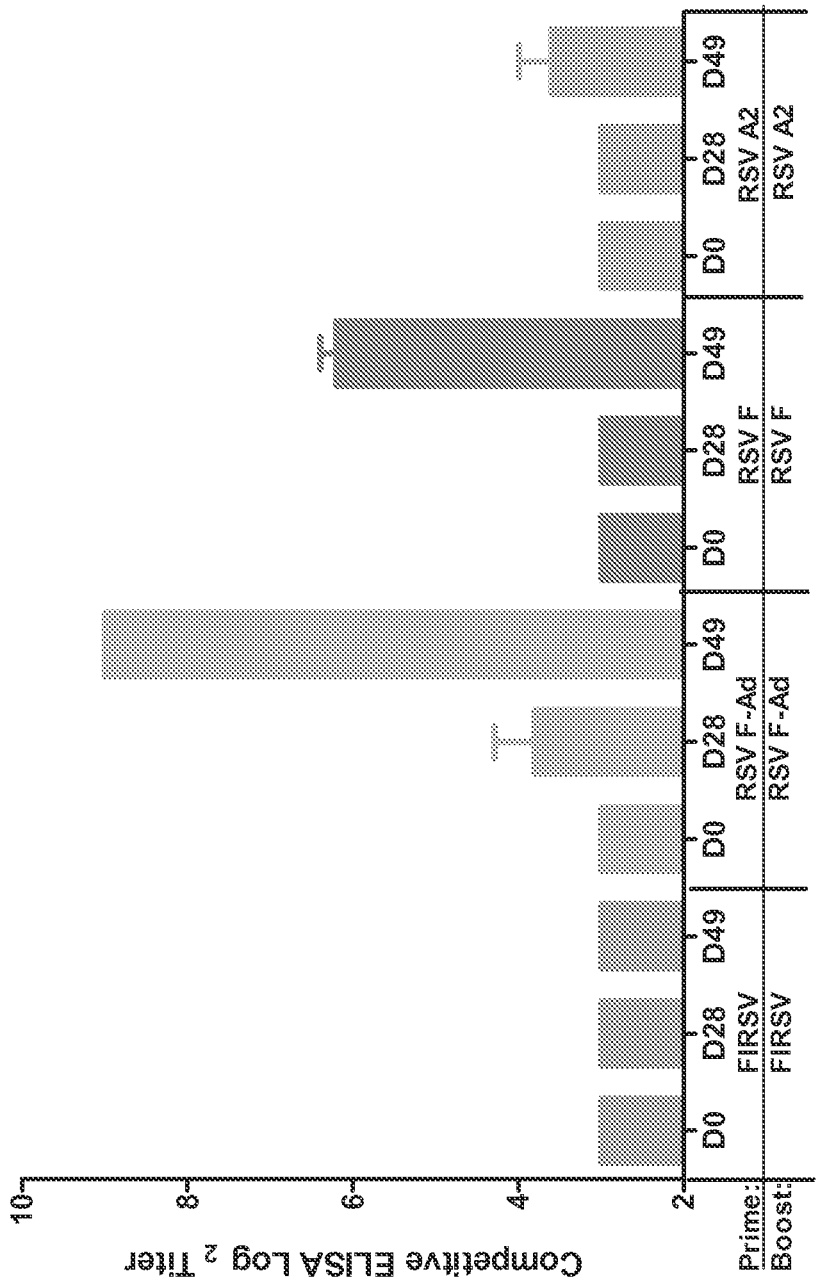

Competitive ELISA Log 2 Titer
10
8
6
4
2
Prime:
Boost:
D0
D28
D49
FIRSV
FIRSV
D0
D28
D49
RSV F-Ad
RSV F-Ad
D0
D28
D49
RSV F
RSV F
D0
D28
D49
RSV A2
RSV A2

Figure 47

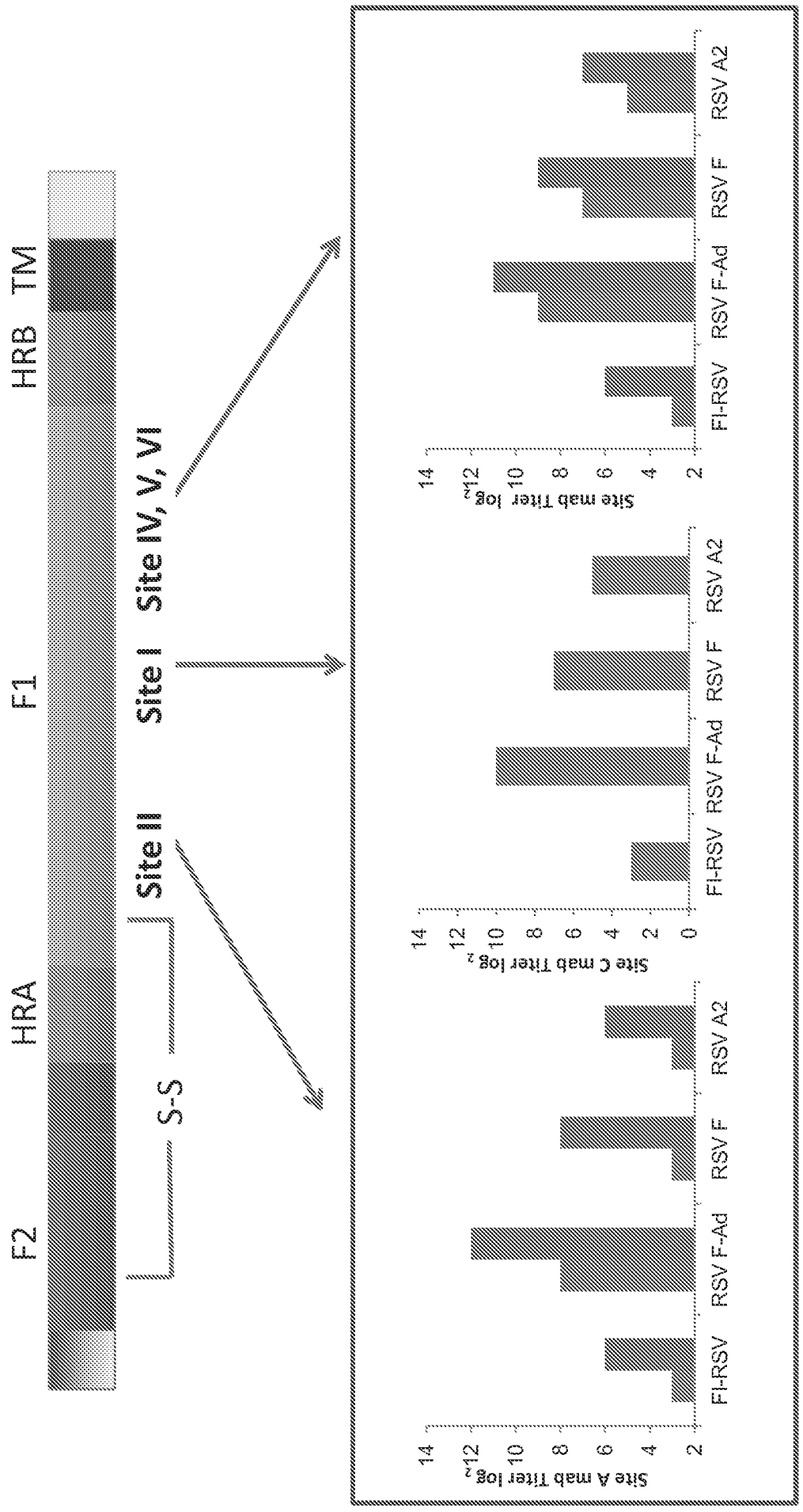
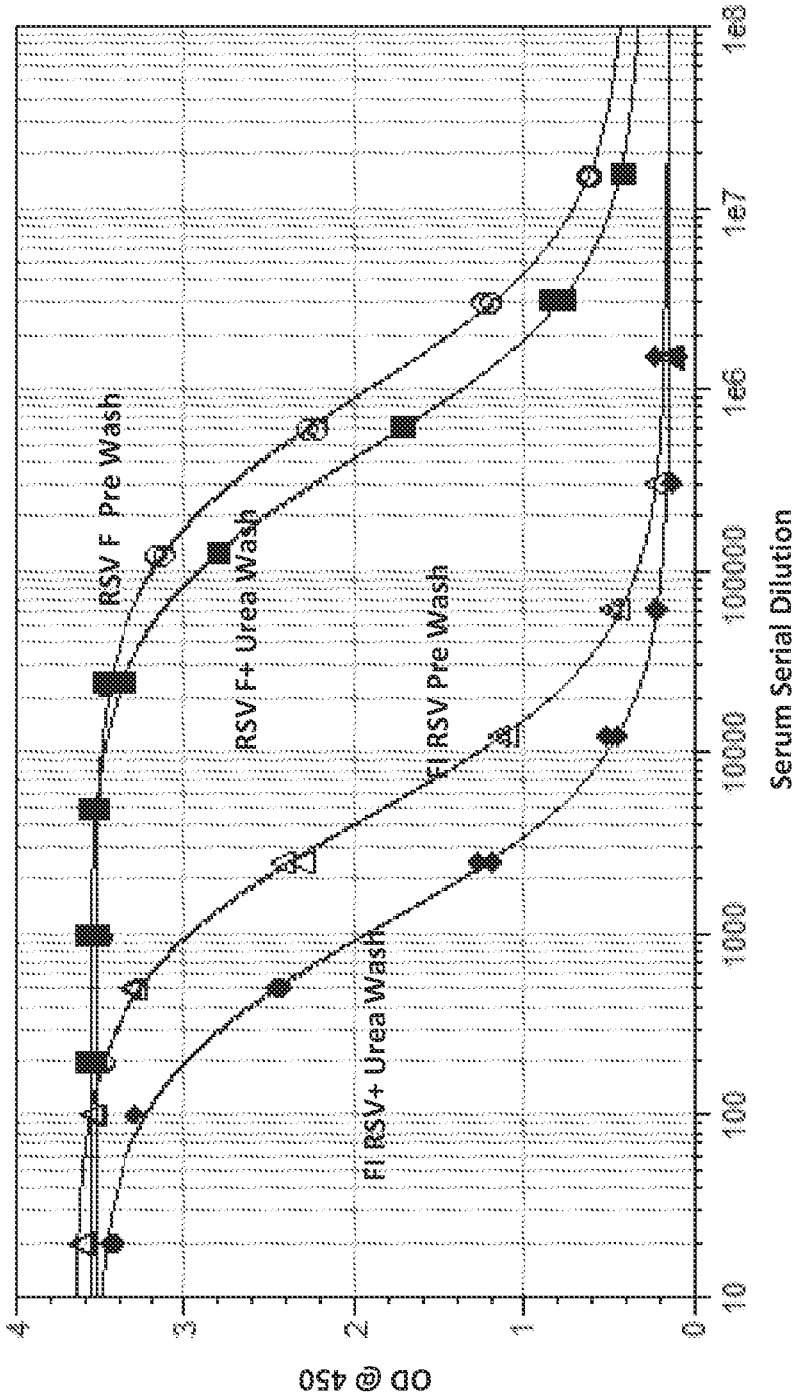

F2
HRA
F1
HRB
TM
S-S
Site II
Site I
Site IV, V, VI
Site A mab Titer log2
Site C mab Titer log2
Site mab Titer log2
14
12
10
8
6
4
2
0
Fl-RSV
RSV F-Ad
RSV F
RSV A2

Figure 48


OD @ 450
0
1
2
3
4
10
100
1000
10000
100000
1e6
1e7
1e8
Serum Serial Dilution
RSV F Pre Wash
RSV F+ Urea Wash
FI RSV Pre Wash
FI RSV+ Urea Wash