# Figure 1A

**Mosaic gp140 Env1 version 1 (mEnv) (SEQ ID NO: 1)**

MRVTGIRKNYQHLWRWGTMLLGILMICSAAGKLWVTVYYGVPV
WKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLE
NVTENFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLN
CTDDVRNVTNNATNTNSSWGEPMEKGEIKNCSFNITTSIRNKV
QKQYALFYKLDVVPIDNDSNNTNYRLISCNTSVITQACPKVSF
EPIPIHYCAPAGFAILKCNDKKFNGTGPCTNVSTVQCTHGIRP
VVSTQLLLNGSLAEEEVVIRSENFTNNAKTIMVQLNVSVEINC
TRPNNNTRKSIHIGPGRAFYTAGDIIGDIRQAHCNISRANWNN
TLRQIVEKLGKQFGNNKTIVFNHSSGGDPEIVMHSFNCGGEFF
YCNSTKLFNSTWTWNNSTWNNTKRSNDTEEHITLPCRIKQIIN
MWQEVGKAMYAPPIRGQIRCSSNITGLLLTRDGGNDTSGTEIF
RPGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVQSEKS
AVGIGAVFLGFLGAAGSTMGAASMTLTVQARLLLSGIVQQQNN
LLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWG
CSGKLICTTTVPWNASWSNKSLDKIWNNMTWMEWEREINNYTS
LIYTLIEESQNQQEKNEQELLELDKWASLWNWFDISNWLWGYI
PEAPRDGQAYVRKDGEWVLLSTFL

Boxed: signal/leader sequence
Underlined: gp120
Plain: gp41 ectodomain
___: T4-fibritin "foldon" trimerization domain

# Figure 1B

**Mosaic gp140 Env1 version 2 (mEnv+) (SEQ ID NO: 2)**

MRVRGIQRNCQHLWRWGTLILGMLMICSAAGKLWVTVYYGVPV
WKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLE
NVTENFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLN
CTDDVRNVTNNATNTNSSWGEPMEKGEIKNCSFNITTSIRNKV
QKQYALFYKLDVVPIDNDSNNTNYRLISCNTSVITQACPKVSF
EPIPIHYCAPAGFAILKCNDKKFNGTGPCTNVSTVQCTHGIRP
VVSTQLLLNGSLAEEEVVIRSENFTNNAKTIMVQLNVSVEINC
TRPNNNTRKSIHIGPGRAFYTAGDIIGDIRQAHCNISRANWNN
TLRQIVEKLGKQFGNNKTIVFNHSSGGDPEIVMHSFNCGGEFF
YCNSTKLFNSTWTWNNSTWNNTKRSNDTEEHITLPCRIKQIIN
MWQEVGKAMYAPPIRGQIRCSSNITGLLLTRDGGNDTSGTEIF
RPGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVQREKR
AVGIGAVFLGFLGAAGSTMGAASMTLTVQARLLLSGIVQQQNN
LLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWG
CSGKLICTTTVPWNASWSNKSLDKIWNNMTWMEWEREINNYTS
LIYTLIEESQNQQEKNEQELLELDKWASLWNWFDISNWLWYIK
SRIEGRGSGGYIPEAPRDGQAYVRKDGEWVLLSTFL

| | |
|---|---|
| Boxed: | signal/leader sequence |
| Underlined: | gp120 |
| Circled: | cleavage site mutations |
| Plain: | gp41 ectodomain |
| ____: | Factor Xa site |
| ____: | T4-fibritin "foldon" trimerization domain |

# Figure 1C

**clade C Env (cEnv) (SEQ ID NO: 3)**

MRVRGIQRNCQHLWRWGTLILGMLMICSAAENLWVGNMWVTVY
YGVPVWTDAKTTLFCASDTKAYDREVHNVWATHACVPTDPNPQ
EIVLENVTENFNMWKNDMVDQMHEDIISLWDQSLKPCVKLTPL
CVTLHCTNATFKNNVTNDMNKEIRNCSFNTTTEIRDKKQQGYA
LFYRPDIVLLKENRNNSNNSEYILINCNASTITQACPKVNFDP
IPIHYCAPAGYAILKCNNKTFSGKGPCNNVSTVQCTHGIKPVV
STQLLLNGSLAEKEIIIRSENLTDNVKTIIVHLNKSVEIVCTR
PNNNTRKSMRIGPGQTFYATGDIIGDIRQAYCNISGSKWNETL
KRVKEKLQENYNNNKTIKFAPSSGGDLEITTHSFNCRGEFFYC
NTTRLFNNNATEDETITLPCRIKQIINMWQGVGRAMYAPPIAG
NITCKSNITGLLLVRDGGEDNKTEEIFRPGGGNMKDNWRSELY
KYKVIELKPLGIAPTGAKERVVEREERAVGIGAVFLGFLGAAG
STMGAASLTLTVQARQLLSSIVQQQSNLLRAIEAQQHMLQLTV
WGIKQLQTRVLAIERYLKDQQLLGIWGCSGKLICTTNVPWNSS
WSNKSQTDIWNNMTWMEWDREISNYTDTIYRLLEDSQTQQEKN
EKDLLALDSWKNLWSWFDISNWLWYIKSRIEGRGSGGYIPEAP
RDGQAYVRKDGEWVLLSTFL

| | |
|---|---|
| Boxed: | signal/leader sequence |
| Underlined: | gp120 |
| Circled: | cleavage site mutations |
| Plain: | gp41 ectodomain |
| ____: | Factor Xa site |
| ____: | T4-fibritin "foldon" trimerization domain |

Figure 2

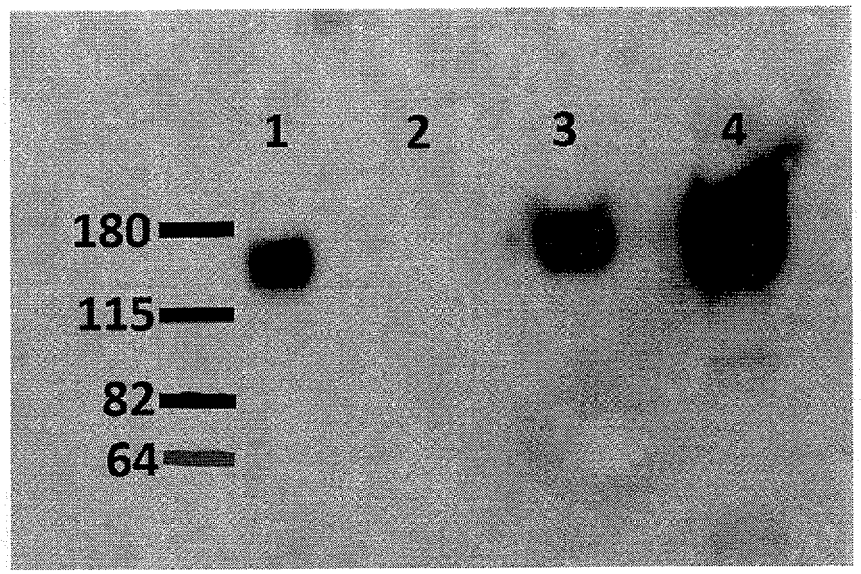


Figure 3

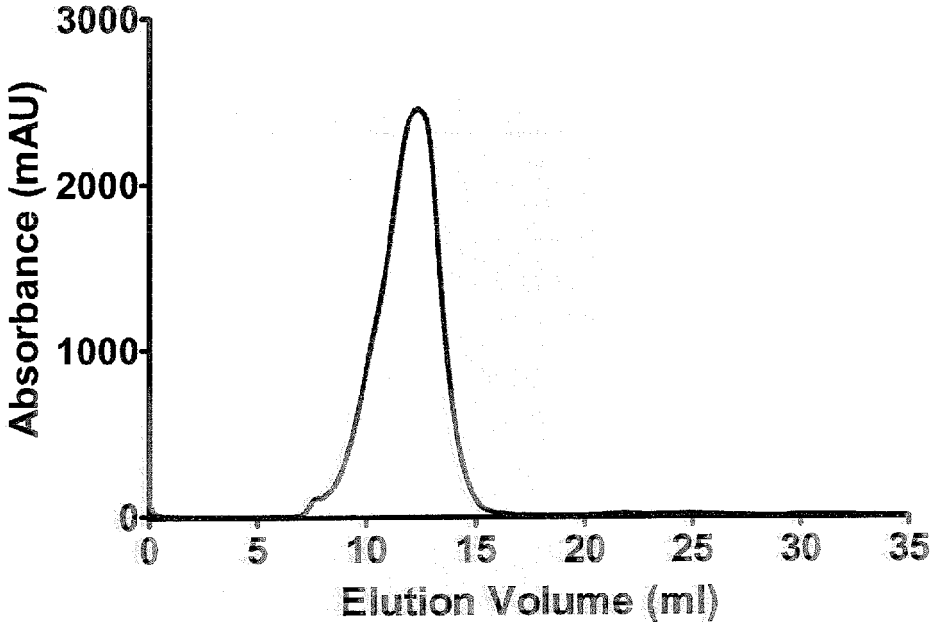
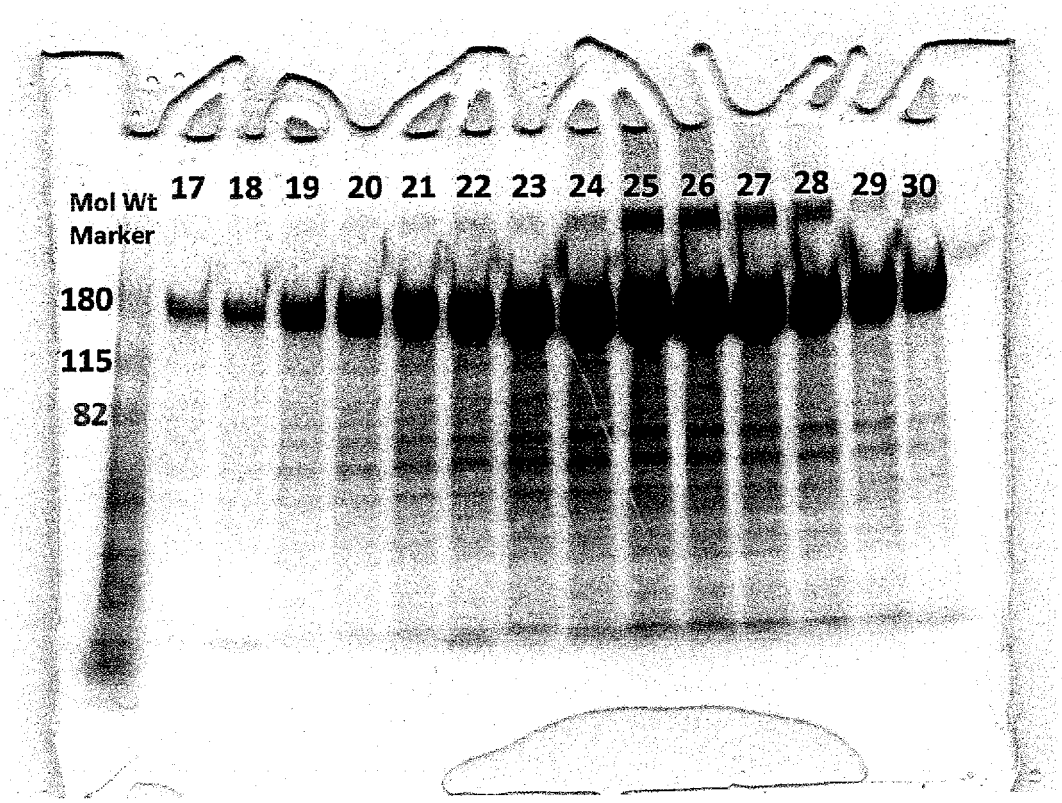
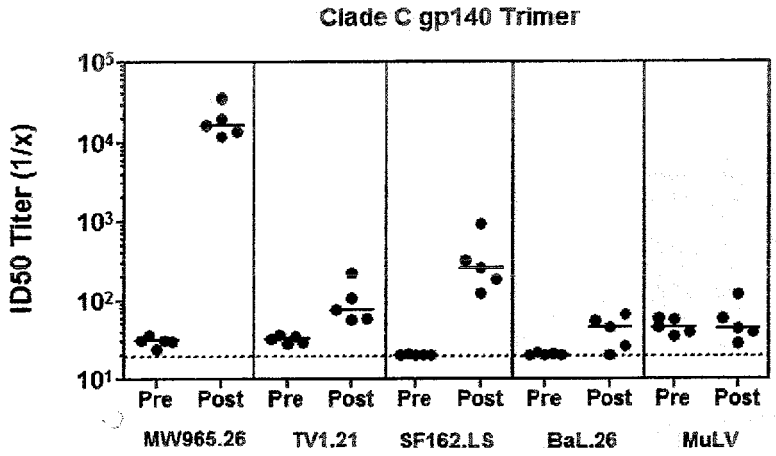

3000
2000
1000
0
Absorbance (mAU)
0
5
10
15
20
25
30
35
Elution Volume (ml)

Figure 4

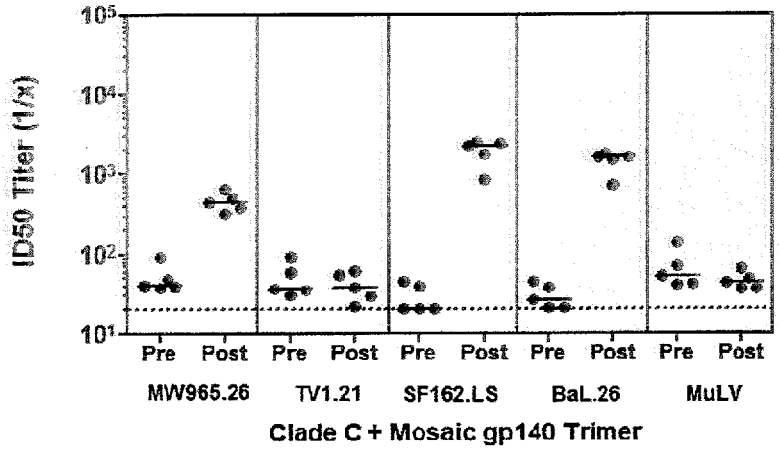


# Figures 5A-5C

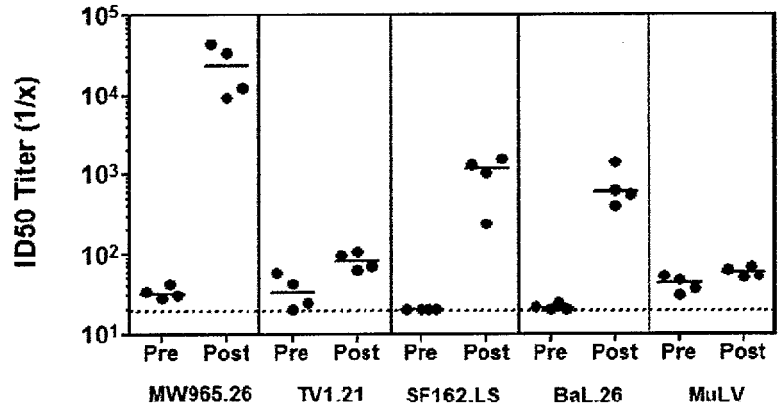
5A




5B



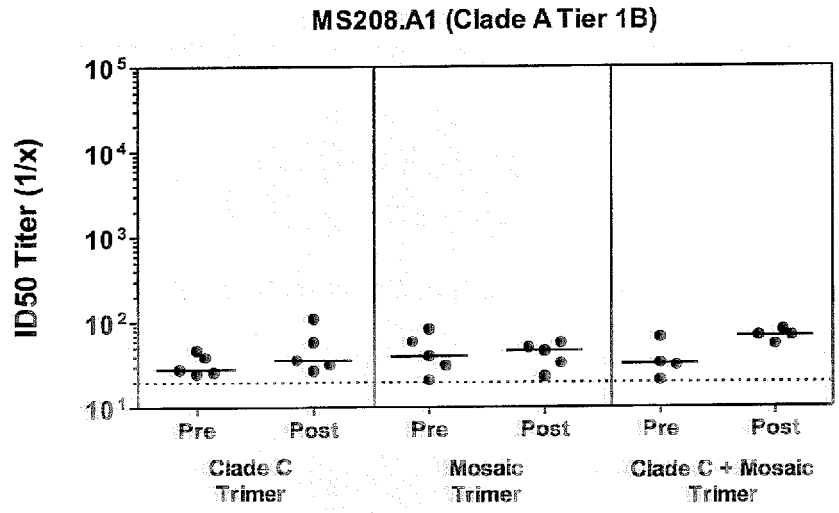

5C



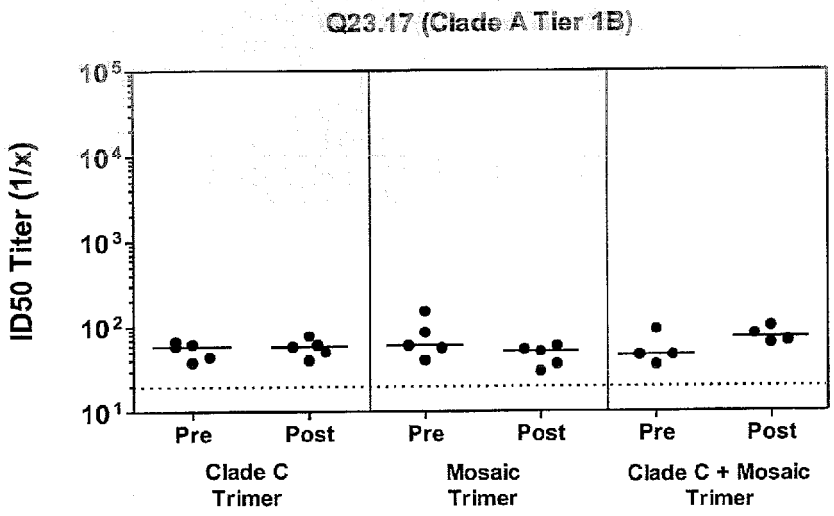
# Figures 6A-6B

## Clade A

6A



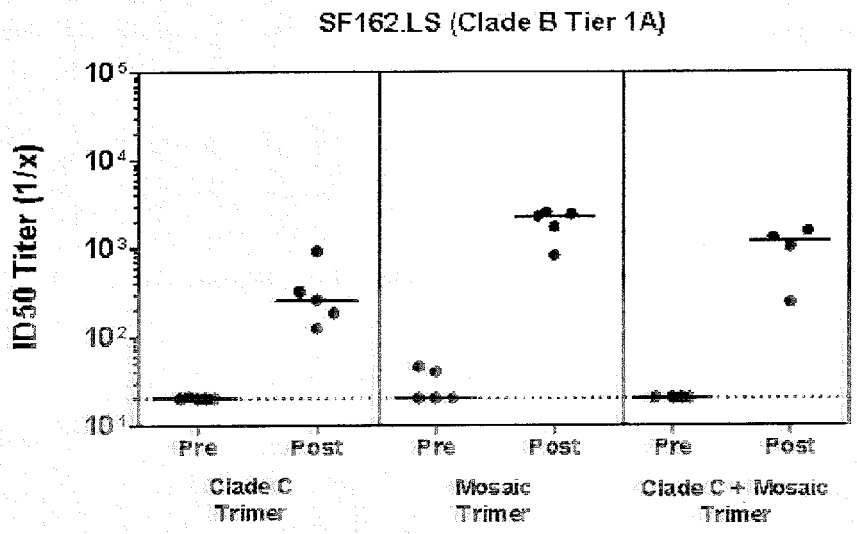

6B



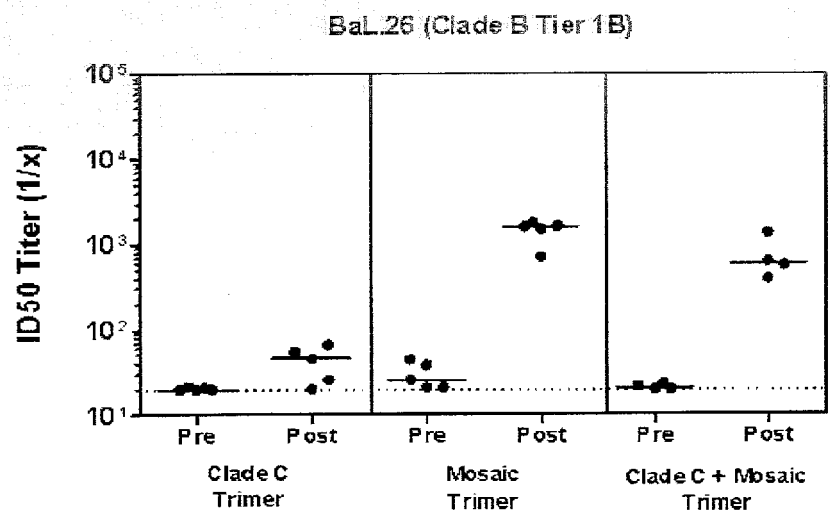
# Figures 7A-7B

## Clade B

7A



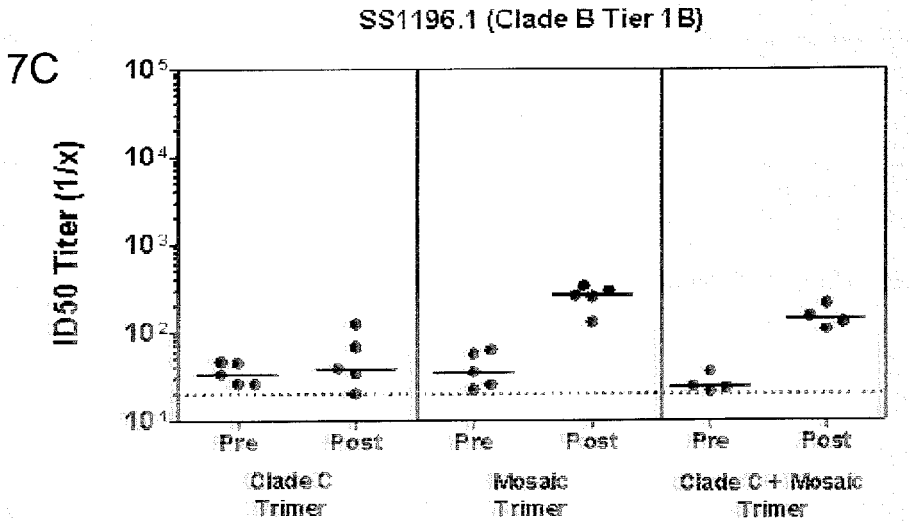

7B



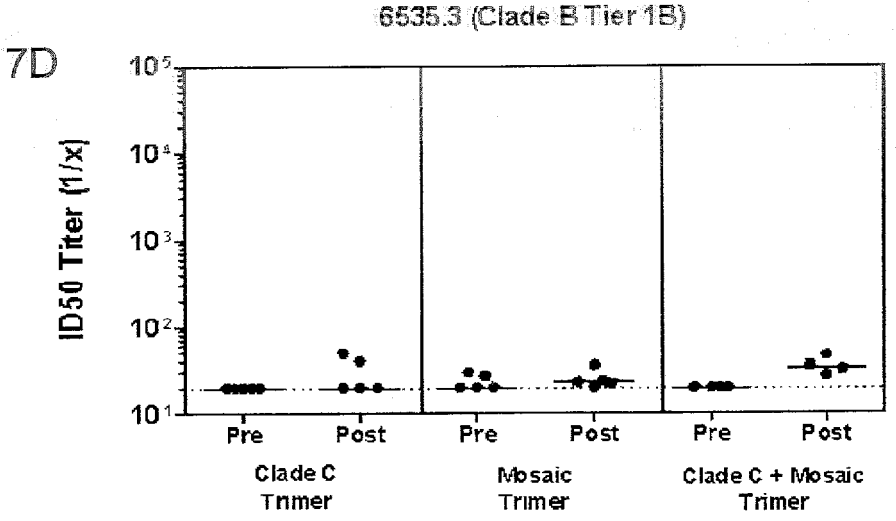
# Figures 7C-7D

## Clade B

7C



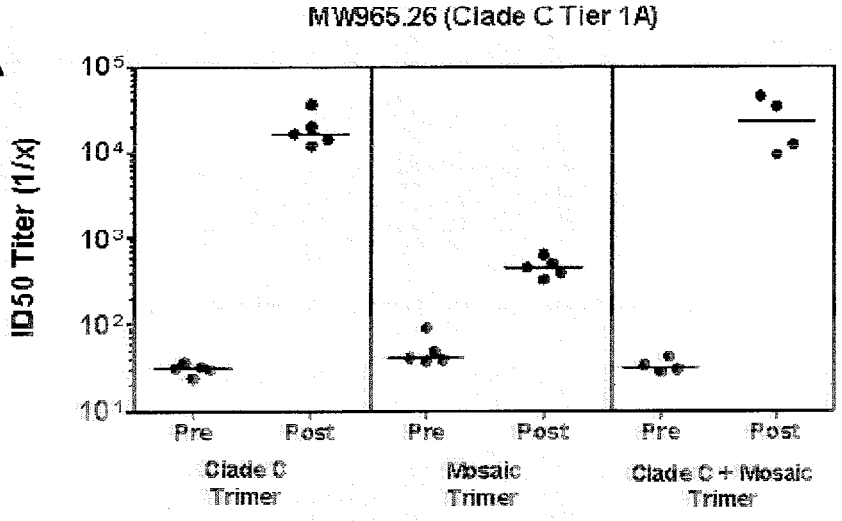

7D



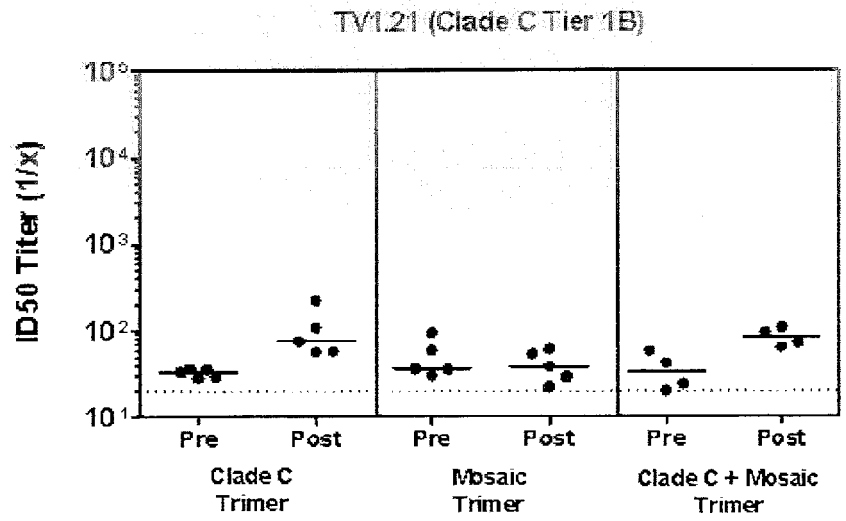
# Figures 8A-8B

## Clade C

8A



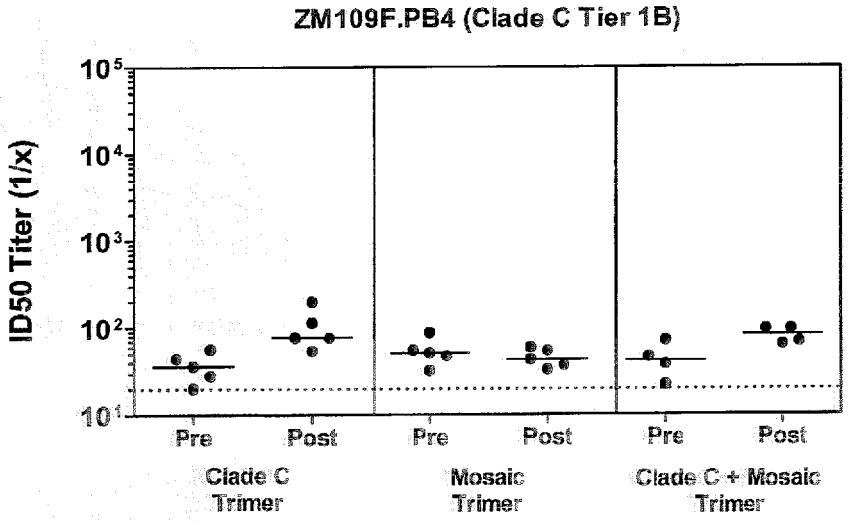

8B



# Figures 8C-8D

## Clade C

8C




8D

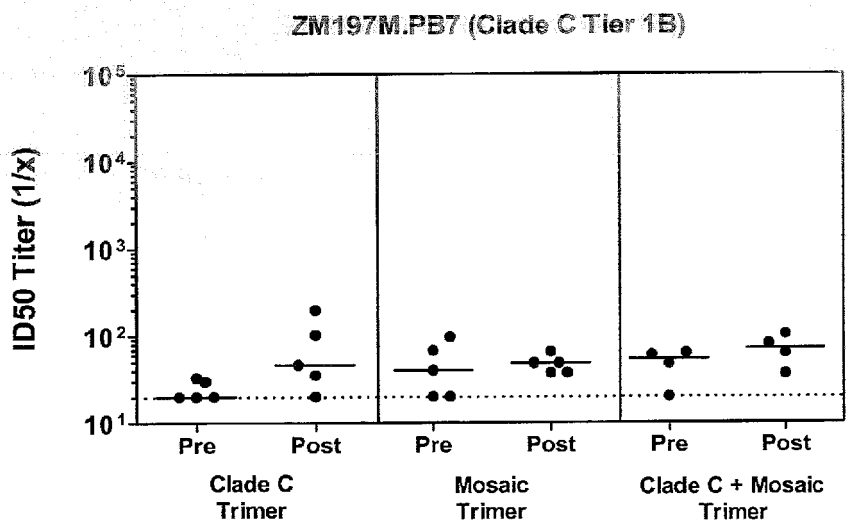


Figure 9

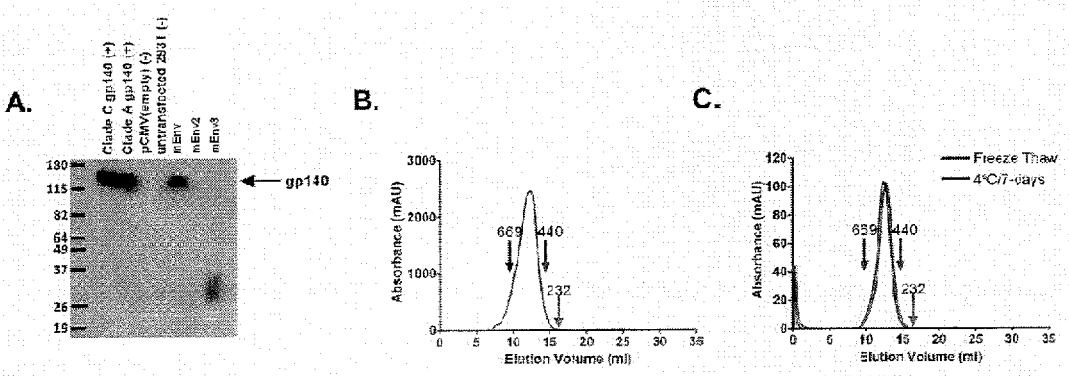

A.
Clade C gp140 (+)
Clade A gp140 (+)
pCMV(empty) (-)
untransfected 293T (-)
mEnv
mEnv2
mEnv3
180
115
82
64
49
37
26
19
gp140
B.
Absorbance (mAU)
3000
2000
1000
0
669
440
232
0 5 10 15 20 25 30 35
Elution Volume (ml)
C.
Freeze Thaw
4°C/7-days
Absorbance (mAU)
120
100
80
60
40
20
0
669
440
232
0 5 10 15 20 25 30 35
Elution Volume (ml)

Figure 10

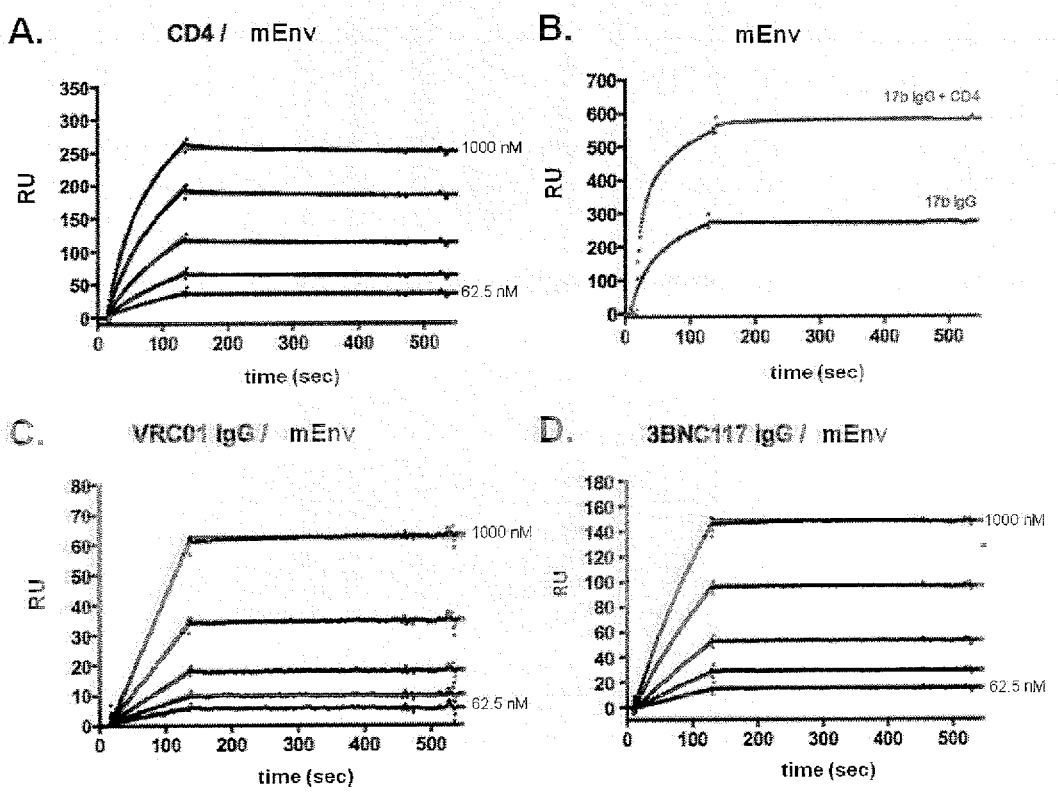
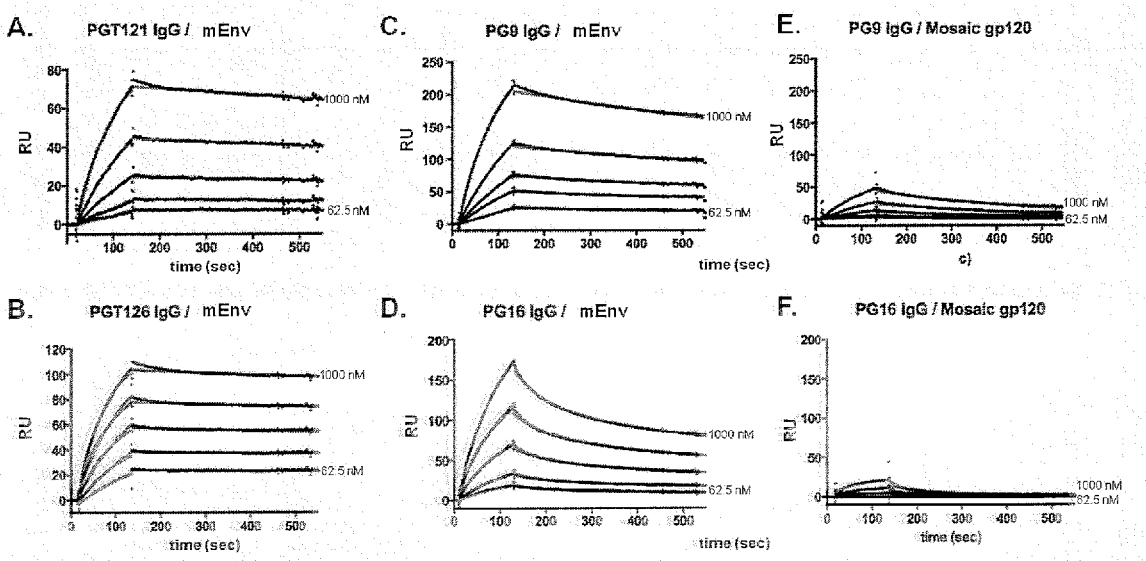

A.
CD4 / mEnv
RU
time (sec)
1000 nM
62.5 nM
B.
mEnv
17b IgG + CD4
17b IgG
RU
time (sec)
C.
VRC01 IgG / mEnv
RU
time (sec)
1000 nM
62.5 nM
D.
3BNC117 IgG / mEnv
RU
time (sec)
1000 nM
62.5 nM

Figure 11


A.
PGT121 IgG / mEnv
RU
time (sec)
1000 nM
62.5 nM
B.
PGT126 IgG / mEnv
C.
PG9 IgG / mEnv
D.
PG16 IgG / mEnv
E.
PG9 IgG / Mosaic gp120
F.
PG16 IgG / Mosaic gp120

# Figure 12

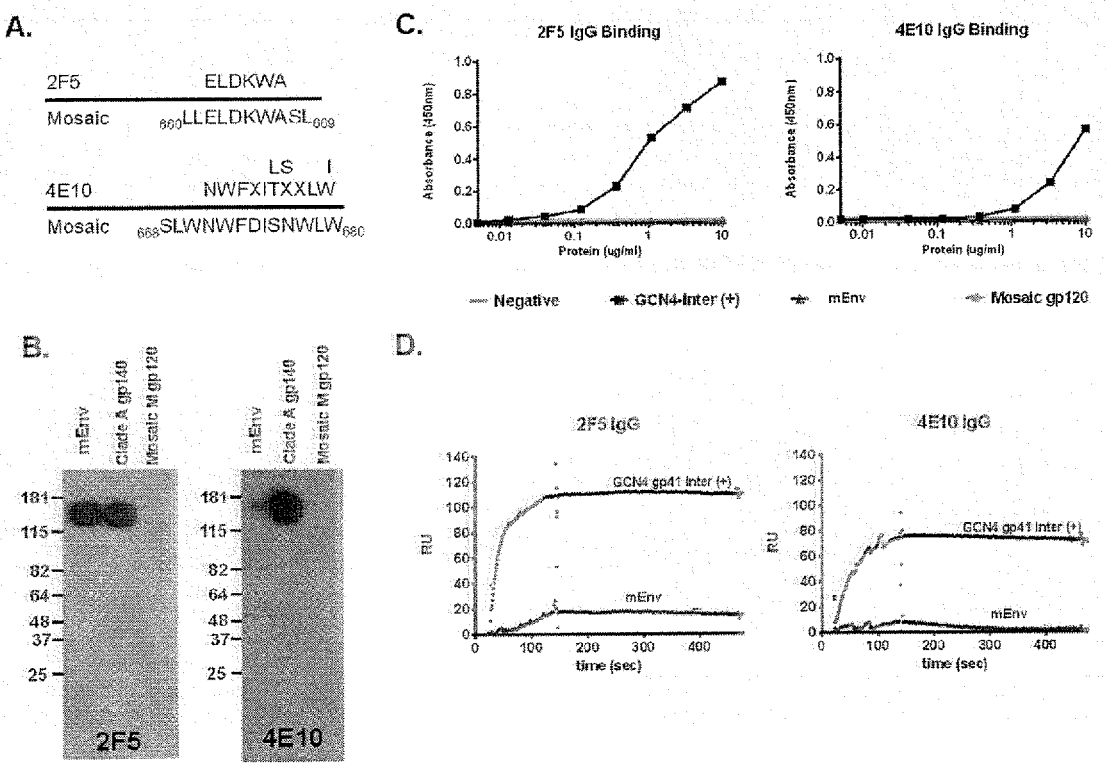

A.
2F5 ELDKWA
Mosaic 660LLELDKWASL669
LS I
4E10 NWFXITXXLW
Mosaic 668SLWNWFDISNWLW680
B.
mEnv
Clade A gp140
Mosaic M gp120
181
115
82
64
48
37
25
2F5
4E10
C.
2F5 IgG Binding
4E10 IgG Binding
Absorbance (450nm)
Protein (ug/ml)
Negative
GCN4-Inter (+)
mEnv
Mosaic gp120
D.
2F5 IgG
4E10 IgG
RU
GCN4 gp41 Inter (+)
mEnv
time (sec)

Figure 13

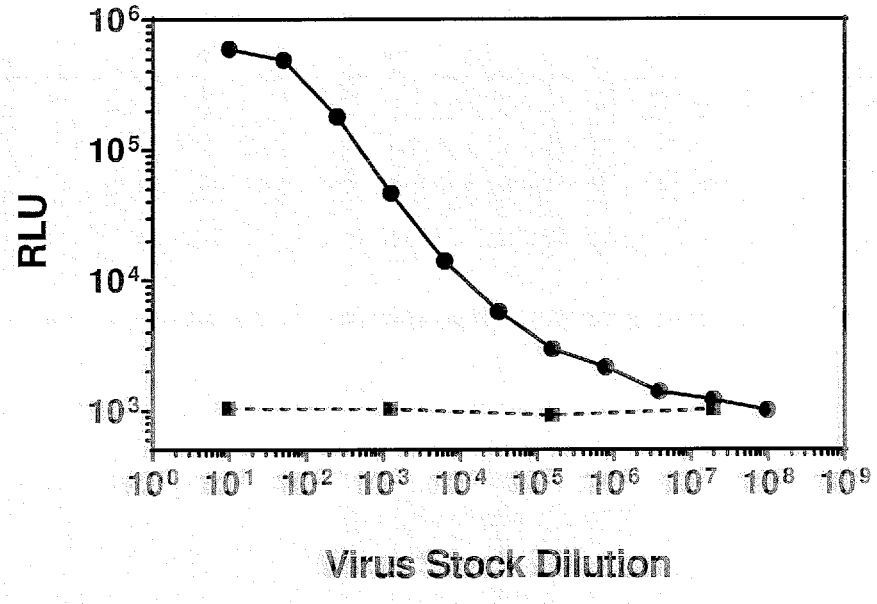

RLU
10^6
10^5
10^4
10^3
10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7 10^8 10^9
Virus Stock Dilution

Figure 14

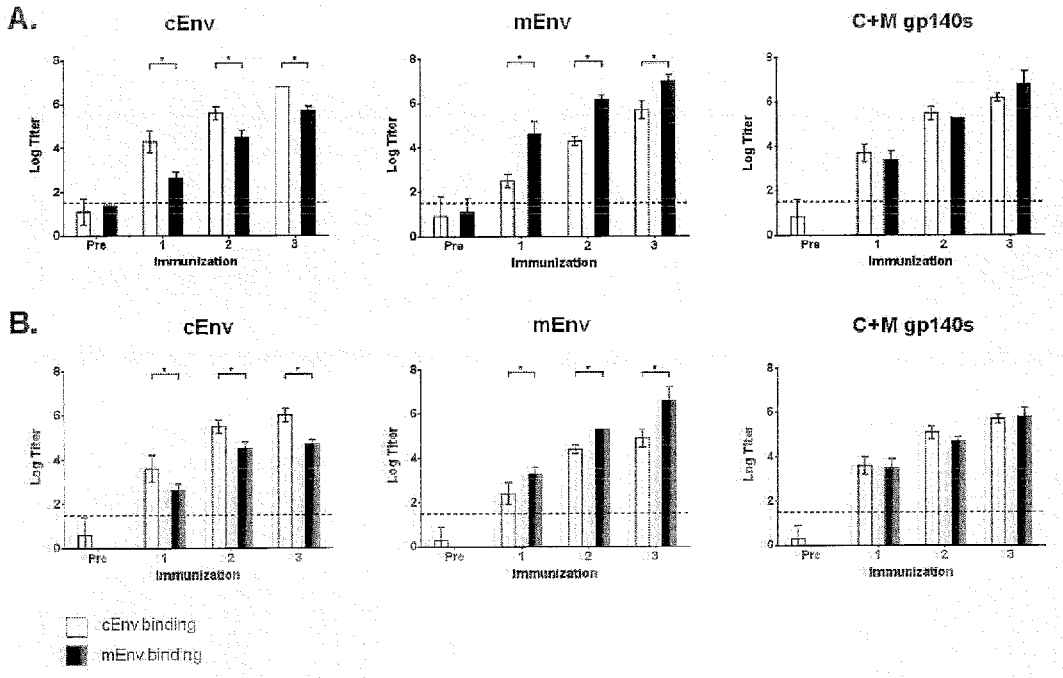

A.
cEnv
mEnv
C+M gp140s
B.
cEnv
mEnv
C+M gp140s
Log Titer
Immunization
Pre
1
2
3
cEnv binding
mEnv binding

Figure 15

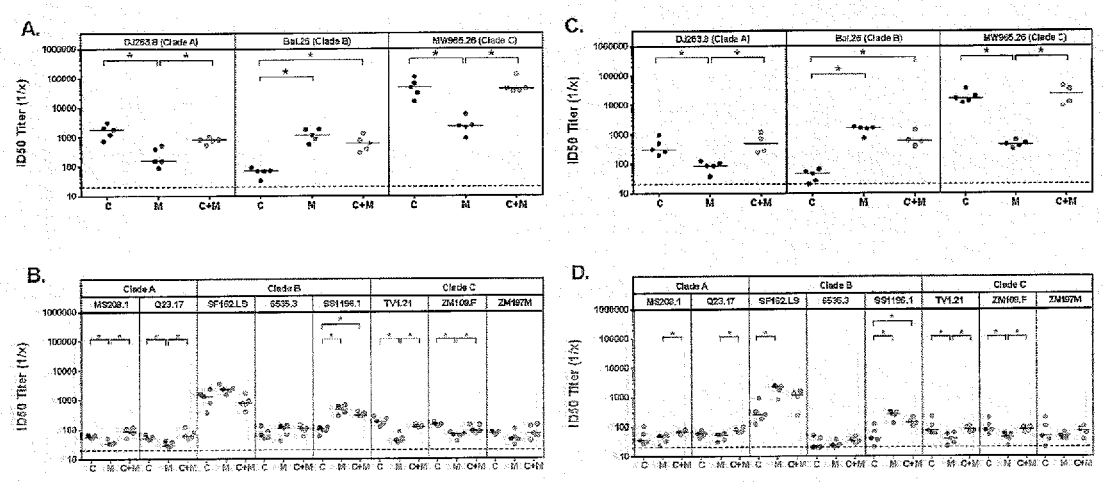

A.
DJ263.8 (Clade A)
BaL26 (Clade B)
MW965.26 (Clade C)
ID50 Titer (1/x)
C
M
C+M
B.
Clade A
Clade B
Clade C
MS208.1
Q23.17
SF162.LS
6535.3
SS1196.1
TV1.21
ZM109.F
ZM197M
C.
D.

Figure 16

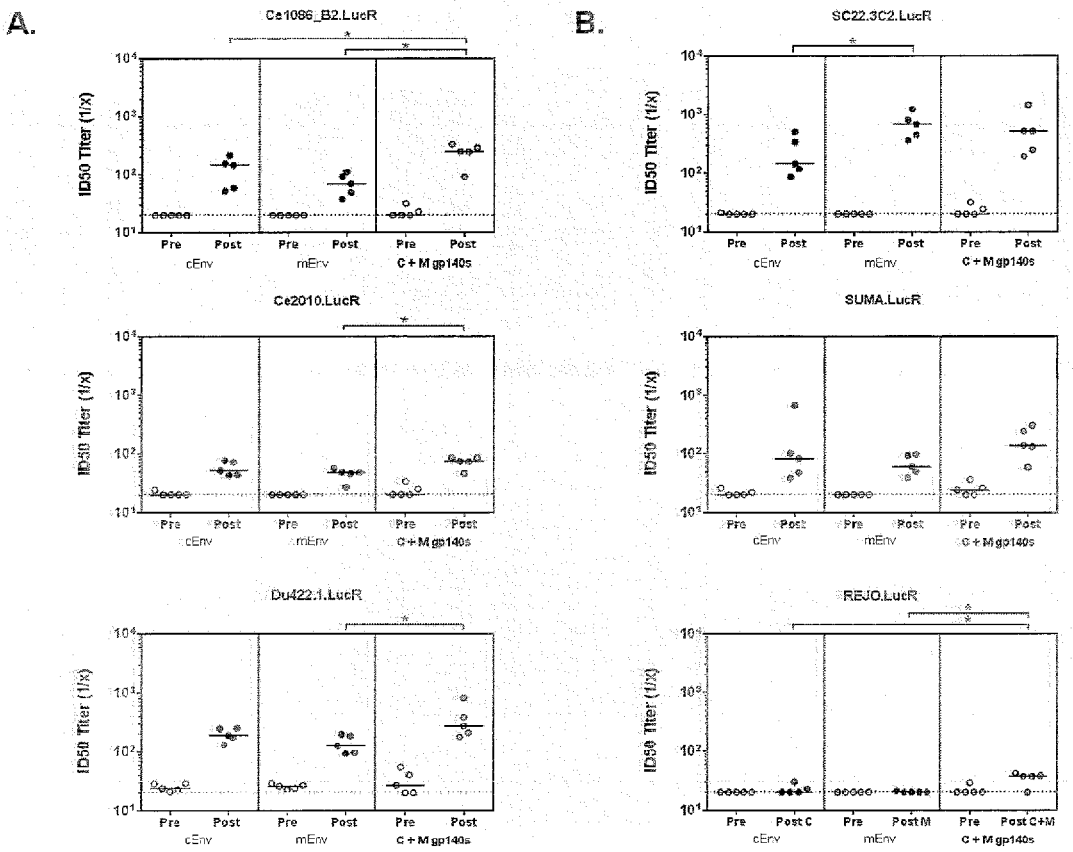

A.
B.
Ce1086_B2.LucR
SC22.3C2.LucR
Ce2010.LucR
SUMA.LucR
Du422.1.LucR
REJO.LucR
ID50 Titer (1/x)
Pre
Post
PostC
PostM
PostC+M
cEnv
mEnv
C+M gp140s

Figure 17

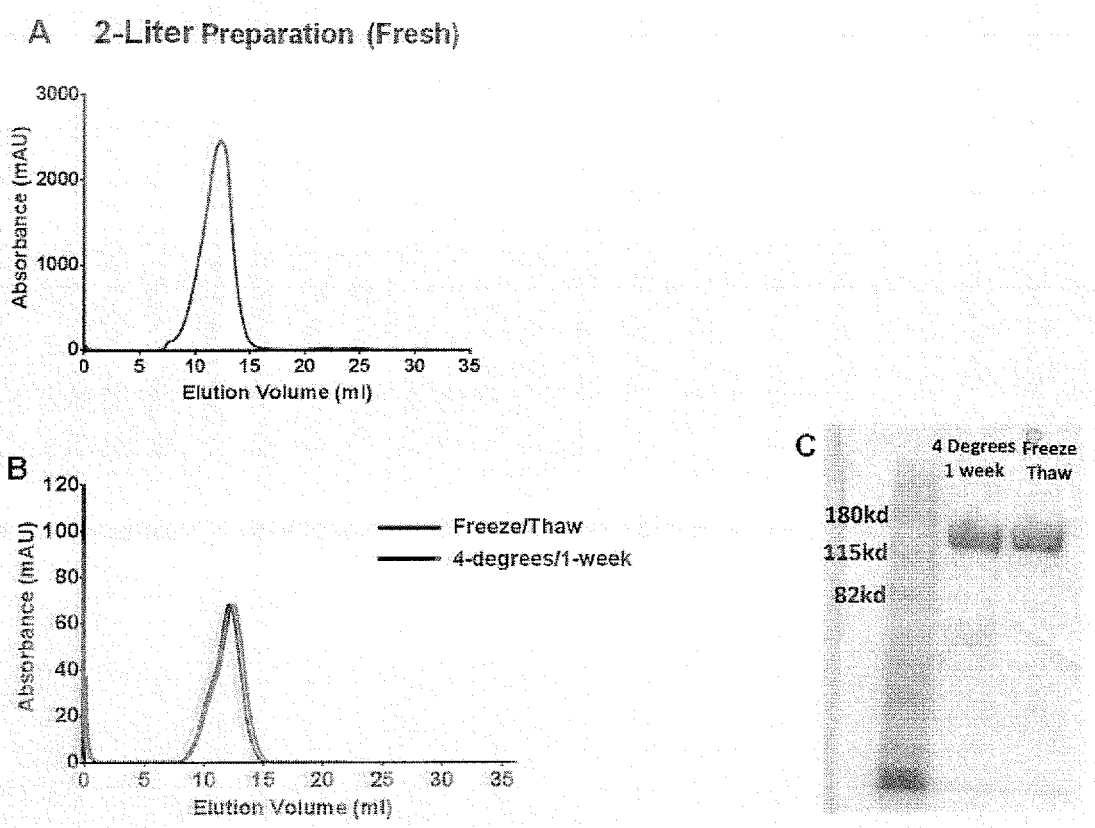

A 2-Liter Preparation (Fresh)
Absorbance (mAU)
3000
2000
1000
0
0 5 10 15 20 25 30 35
Elution Volume (ml)
B
Absorbance (mAU)
120
100
80
60
40
20
0
0 5 10 15 20 25 30 35
Elution Volume (ml)
Freeze/Thaw
4-degrees/1-week
C
4 Degrees
1 week
Freeze
Thaw
180kd
115kd
82kd

Figure 18

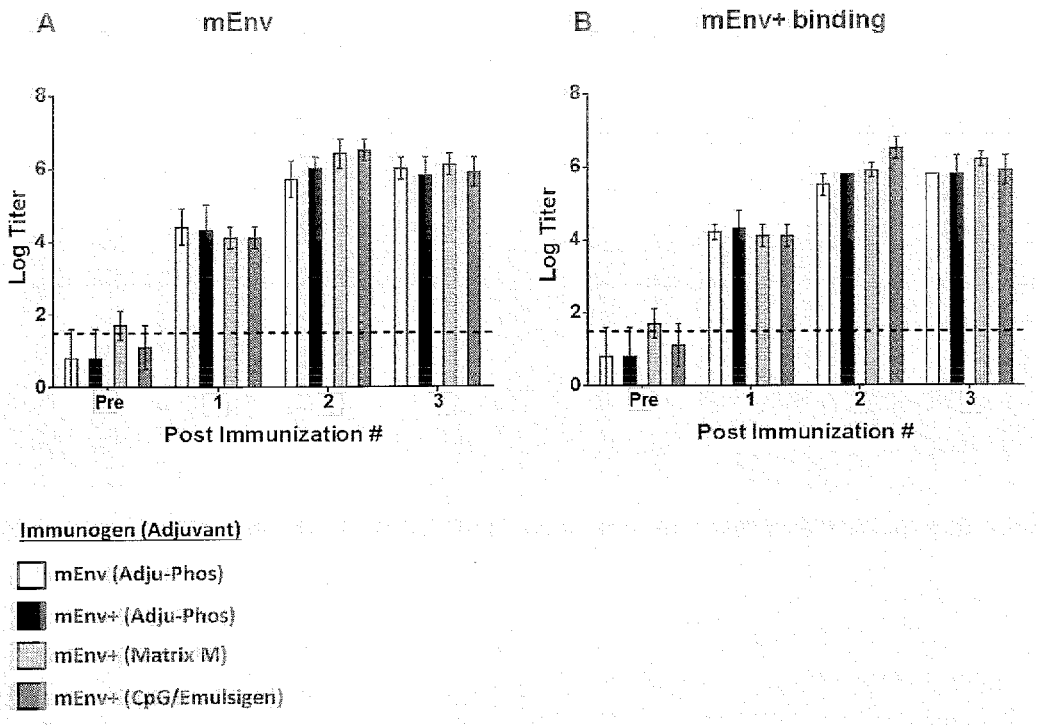
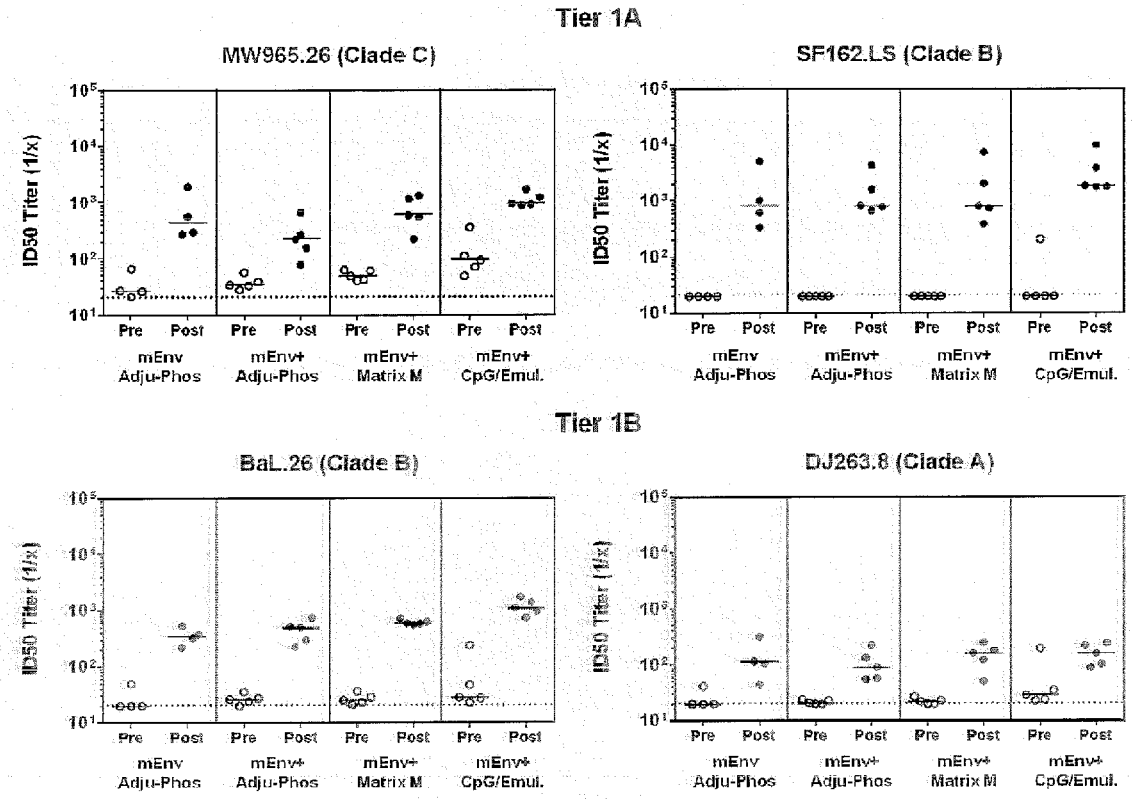

A
mEnv
B
mEnv+ binding
Log Titer
8
6
4
2
0
Pre
1
2
3
Post Immunization #
Immunogen (Adjuvant)
mEnv (Adju-Phos)
mEnv+ (Adju-Phos)
mEnv+ (Matrix M)
mEnv+ (CpG/Emulsigen)

Figure 19


Tier 1A
MW965.26 (Clade C)
SF162.LS (Clade B)
ID50 Titer (1/x)
Pre
Post
mEnv Adju-Phos
mEnv+ Adju-Phos
mEnv+ Matrix M
mEnv+ CpG/Emul.
Tier 1B
BaL.26 (Clade B)
DJ263.8 (Clade A)