FIGURE 1A

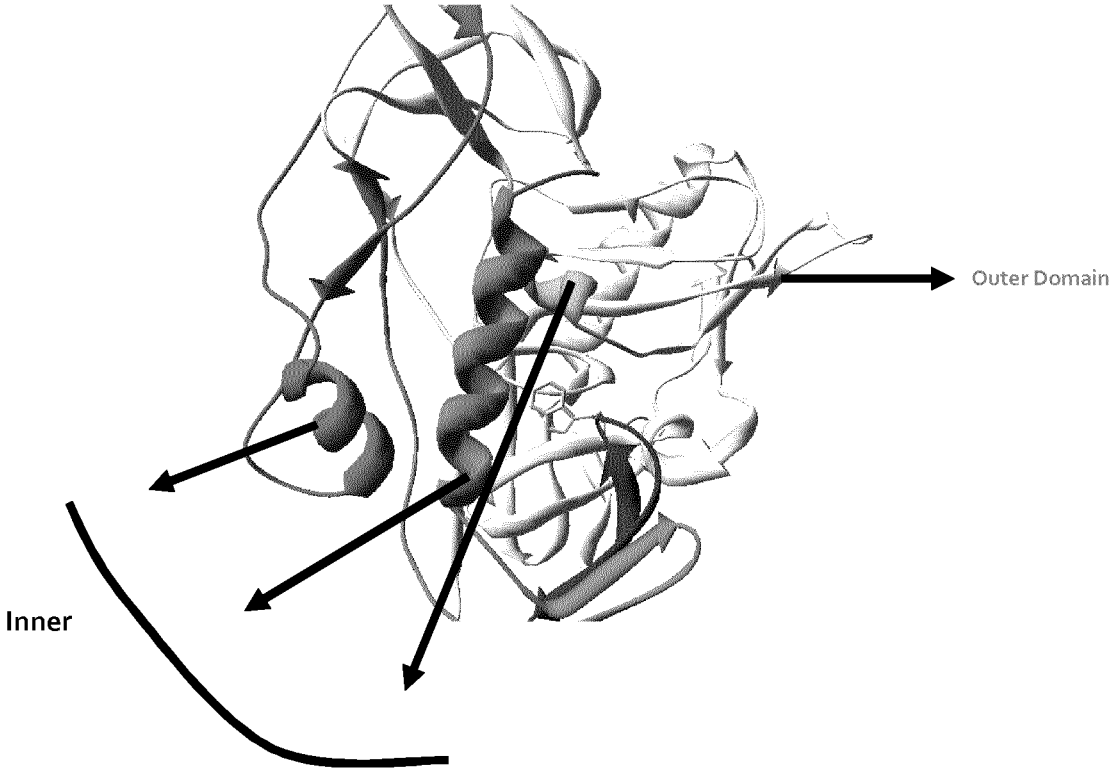


FIGURE 1B

MDAMKRGLCCVLLLCGAVFVSPSAVEKLWVTVYYGVPVWKE**ATTTLFCASDAKAYDTEVH**

NVWATHACVPTDPNPQEIVLENVTENFNM**WKNNMVEQMHEDIISLWDQSLKPCVKLT**PLC

VTLHCTNLKNATNTKSSNWKEMDRGEIKNCSFKVTTSIRNKMQKEYALFYKLDVVPIDND

NTSYKLINCNT**SVITQACPKVSFEPIPIHYCAPAGFAILKCND**KKFNGSGPCTNVSTV**QC**

THGIRPVVSTQLLLNGSLAEEGVVIRSENFTDNAKTIIVQLKESVEINCTRPNNNTRKSI

TIGPGRAFYATGDIIGDIRQAHCNISGEKWNNTLKQIVTKLQAQFGNKTIVFKQSSGGDP

EIVMHSFNCGGEFFYCNSTQLFNSTWNNTIGPNNTNGTITLPCRIKQIINRWQEVGKAMY

APPIRGQIRCSSNITGLLLTRDGGKEISNTTEIFRPGG**GDMRDNWRSELY**KYKVVKIEPL

GVAPTKAKRRVVQREKR

FIGURE 2

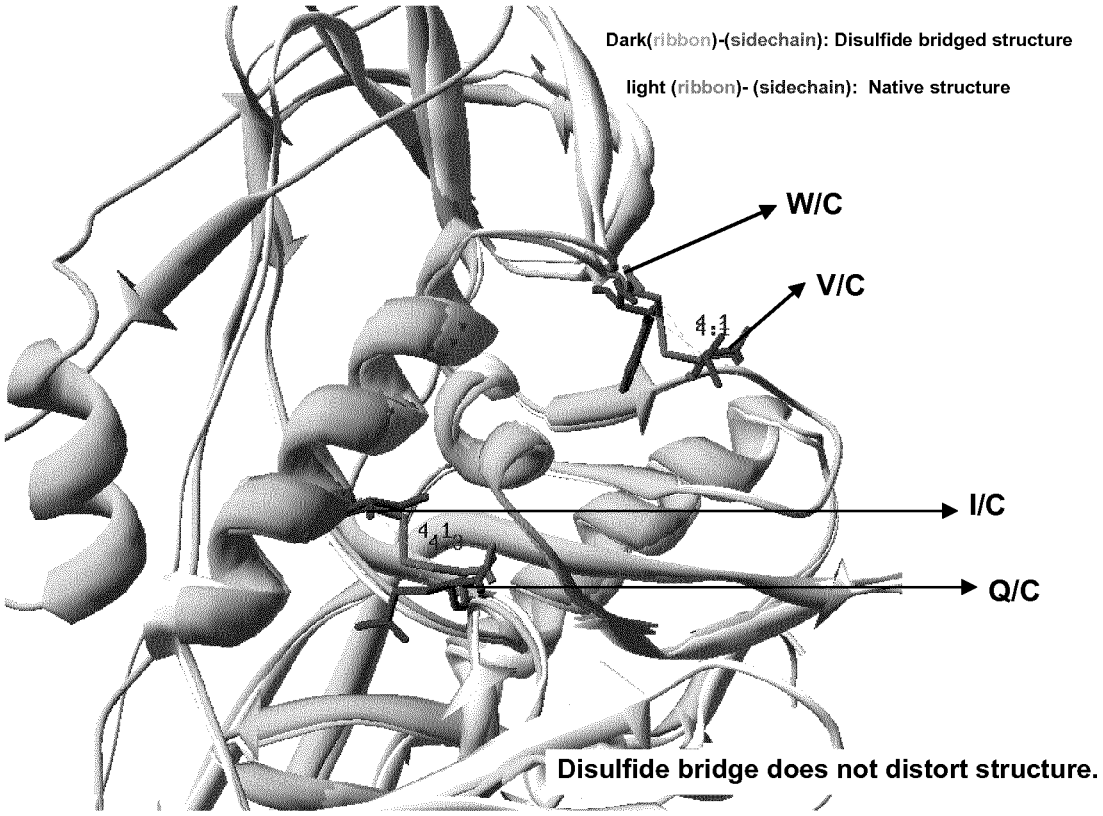

Dark(ribbon)-(sidechain): Disulfide bridged structure
light (ribbon)- (sidechain): Native structure
W/C
V/C
I/C
Q/C
Disulfide bridge does not distort structure.

FIGURE 3

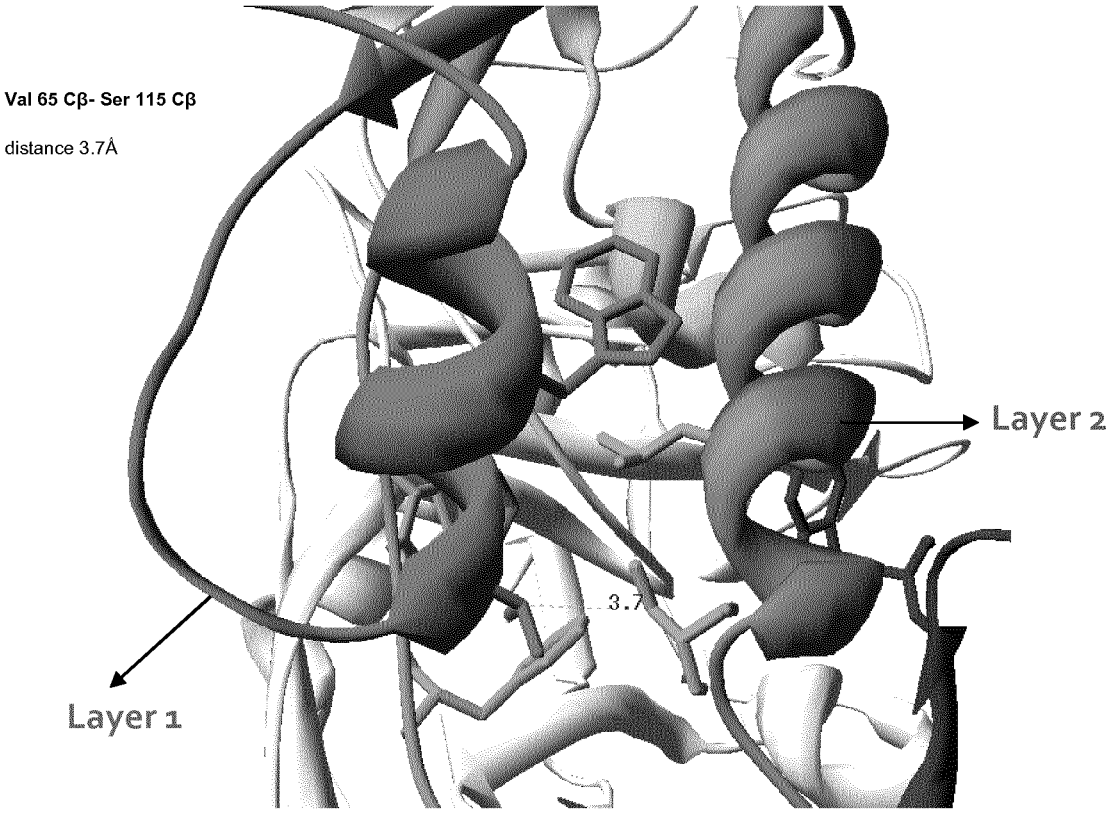


FIGURE 4

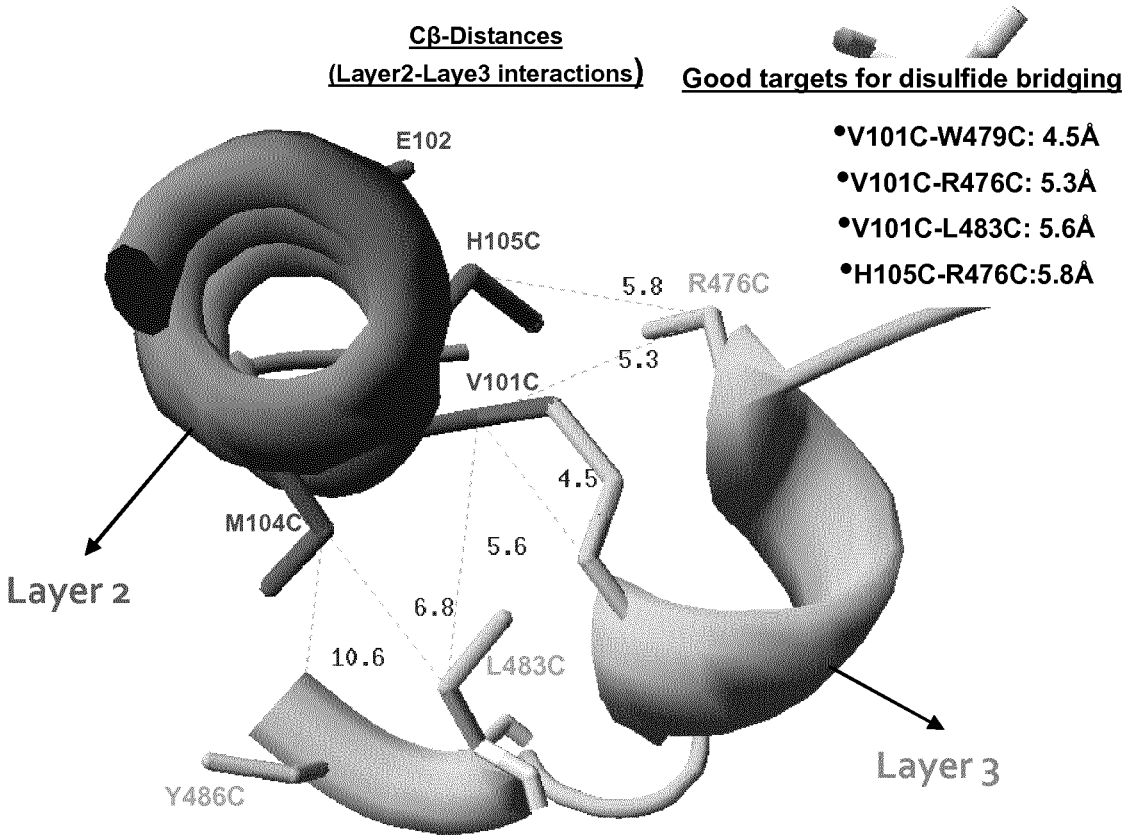
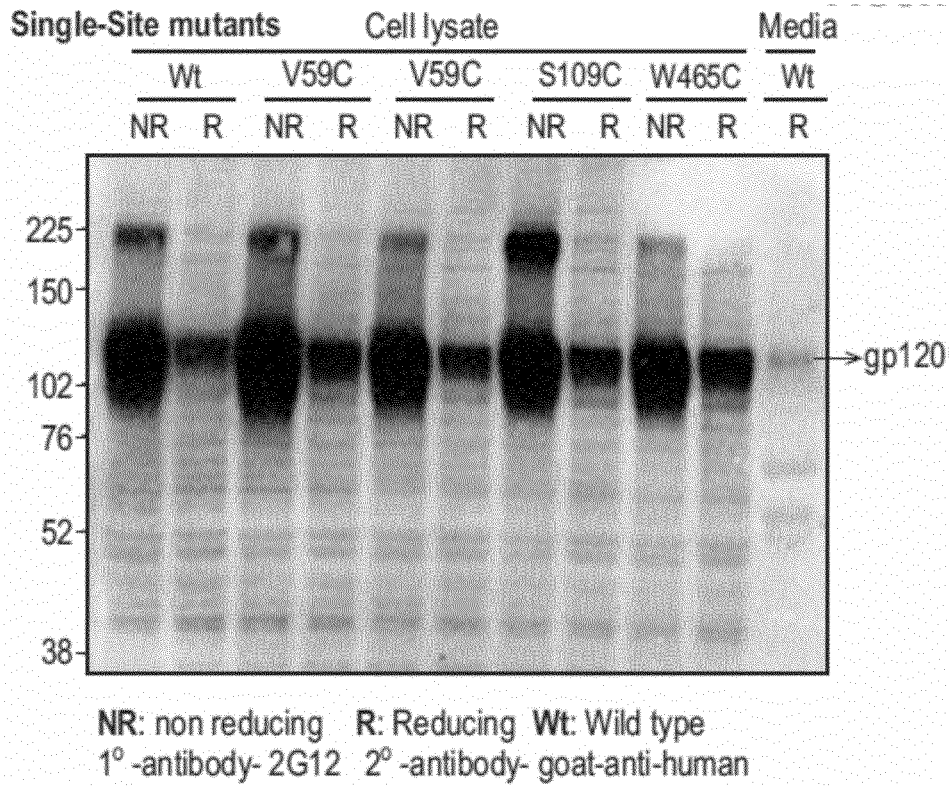

Cβ-Distances
(Layer2-Laye3 interactions)
Good targets for disulfide bridging
•V101C-W479C: 4.5Å
•V101C-R476C: 5.3Å
•V101C-L483C: 5.6Å
•H105C-R476C:5.8Å
E102
H105C
5.8
R476C
5.3
V101C
4.5
M104C
5.6
6.8
10.6
L483C
Y486C
Layer 2
Layer 3

FIGURE 5


V65C(V59C)
V101C(V95C)
H105C(H99C)
S115C(S109C)
W479C(W465C)
R476C(R462C) L483C(L469C)
SF162 MRVKGIRKN- YQHLWRGGTL LLGMLMICSA VEKLWVTVYY GVPVWKEATT TLFCASDAKA YDTEV
HxB2 MRVKEKYQHL WRWGWRWGTM LLGMLMICSA TEKLWVTVYY GVPVWKEATT TLFCASDAKA YDTEV
Consensus MRVK------ ----WR-GT- LLGMLMICSA -EKLWVTVYY GVPVWKEATT TLFCASDAKA YDTEV
SF162 HNVWA THACVPTDPN PQEIVLENVT ENFNMWKNNM VEQMHEDIIS LWDQSLKPCV KLTPLCVTLH
HxB2 HNVWA THAGVPTDPN PQEVVLVNVT ENFNMWKNDM VEQMHEDIIS LWDQSLKPCV KLTPLCVSLK
Consensus HNVWA THA-VPTDPN PQE-VL-NVT ENFNMWKN-M VEQMHEDIIS LWDQSLKPCV KLTPLCV-L-
SF162 CTNLKNATNT KSSNWKE-MD RGEIKNCSFK VTTSIRNKMQ KEYALFYKLD VVPIDNDNTS YKLIN
HxB2 CTDLKNDTNT NSSSGRMIME KGEIKNCSFN ISTSIRGKVQ KEYAFFYKLD IIPIDNDTTS YTLTS
Consensus CT-LKN-TNT -SS-----M- -GEIKNCSF- --TSIR-K-Q KEYA-FYKLD --PIDND-TS Y-L--
SF162 CNTSV ITQACPKVSF EPIPIHYCAP AGFAILKCND KKFNGSGPCT NVSTVQCTHG IRPVVSTQLL
HxB2 CNTSV ITQACPKVSF EPIPIHYCAP AGFAILKCNN KTFNGTGPCT NVSTVQCTHG IRPVVSTQLL
Consensus CNTSV ITQACPKVSF EPIPIHYCAP AGFAILKCN- K-FNG-GPCT NVSTVQCTHG IRPVVSTQLL
SF162 LNGSLAEEGV VIRSENFTDN AKTIIVQLKE SVEINCTRPN NNTRKSITI- -GPGRAFYAT GDIIG
HxB2 LNGSLAEEEV VIRSVNFTDN AKTIIVQLNT SVEINCTRPN NNTRKKIRIQ RGPGRAFVTI GKI-G
Consensus LNGSLAEE-V VIRS-NFTDN AKTIIVQL-- SVEINCTRPN NNTRK-I-I- -GPGRAF--- G-I-G
SF162 DIRQA HCNISGEKWN NTLKQIVTKL QAQFGN-KTI VFKQSSGGDP EIVMHSFNCG GEFFYCNSTQ
HxB2 NMRQA HCNISRAKWN ATLKQIASKL REQFGNNKTI IFKQSSGGDP EIVTHSFNCG GEFFYCNSTQ
Consensus --RQA HCNIS--KWN -TLKQI--KL --QFGN-KTI -FKQSSGGDP EIV-HSFNCG GEFFYCNSTQ
SF162 LFNSTWNN-- --TIGPNNTN G--TITLPCR IKQIINRWQE VGKAMYAPPI RGQIRCSSNI TGLLL
HxB2 LFNSTWFNST WSTEGSNNTE GSDTITLPCR IKQFINMWQE VGKAMYAPPI SGQIRCSSNI TGLLL
Consensus LFNSTW-N-- --T-G-NNT- G--TITLPCR IKQ-IN-WQE VGKAMYAPPI -GQIRCSSNI TGLLL
SF162 TRDGG KEISNTTEIF RPGGGDMRDN WRSELYKYKV VKIEPLGVAP TKAKRRVVQR EKR
HxB2 TRDGG NN-NNGSEIF RPGGGDMRDN WRSELYKYKV VKIEPLGVAP TKAKRRVVQR EKR
Consensus TRDGG ----N--EIF RPGGGDMRDN WRSELYKYKV VKIEPLGVAP TKAKRRVVQR EKR
Seq # in HxB2(Seq # in SF162)

FIGURE 6


Single-Site mutants
Cell lysate
Media
Wt
V59C
V59C
S109C
W465C
Wt
NR R NR R NR R NR R NR R R
225
150
102
76
52
38
gp120
NR: non reducing R: Reducing Wt: Wild type
1° -antibody- 2G12 2° -antibody- goat-anti-human

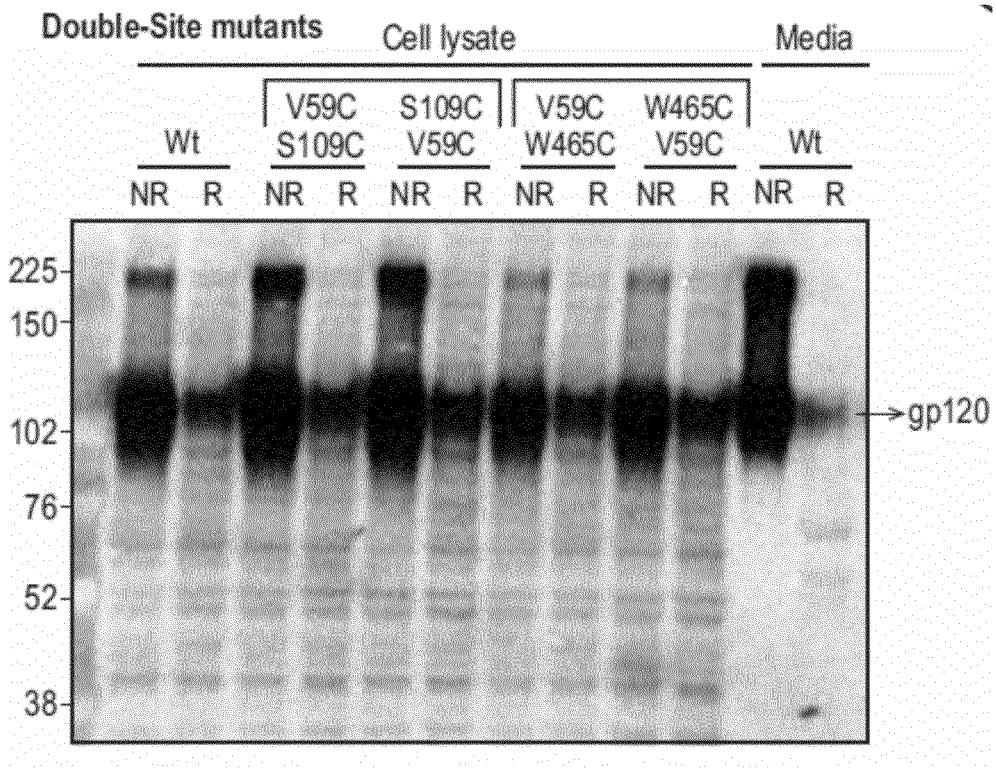

Double-Site mutants
Cell lysate
Media
Wt
V59C S109C
S109C V59C
V59C W465C
W465C V59C
Wt
NR R NR R NR R NR R NR R NR R
225
150
102
76
52
38
gp120

FIGURE 7

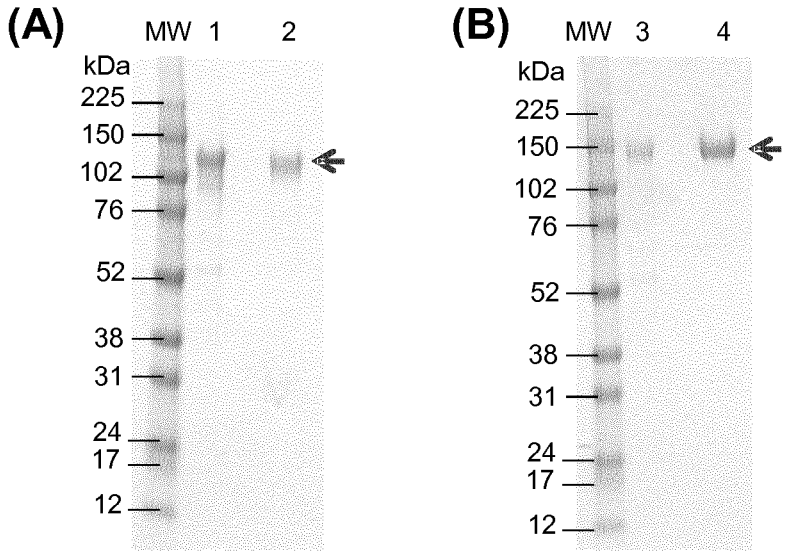

(A)
MW
1
2
kDa
225
150
102
76
52
38
31
24
17
12
(B)
MW
3
4
kDa
225
150
102
76
52
38
31
24
17
12

FIGURE 8

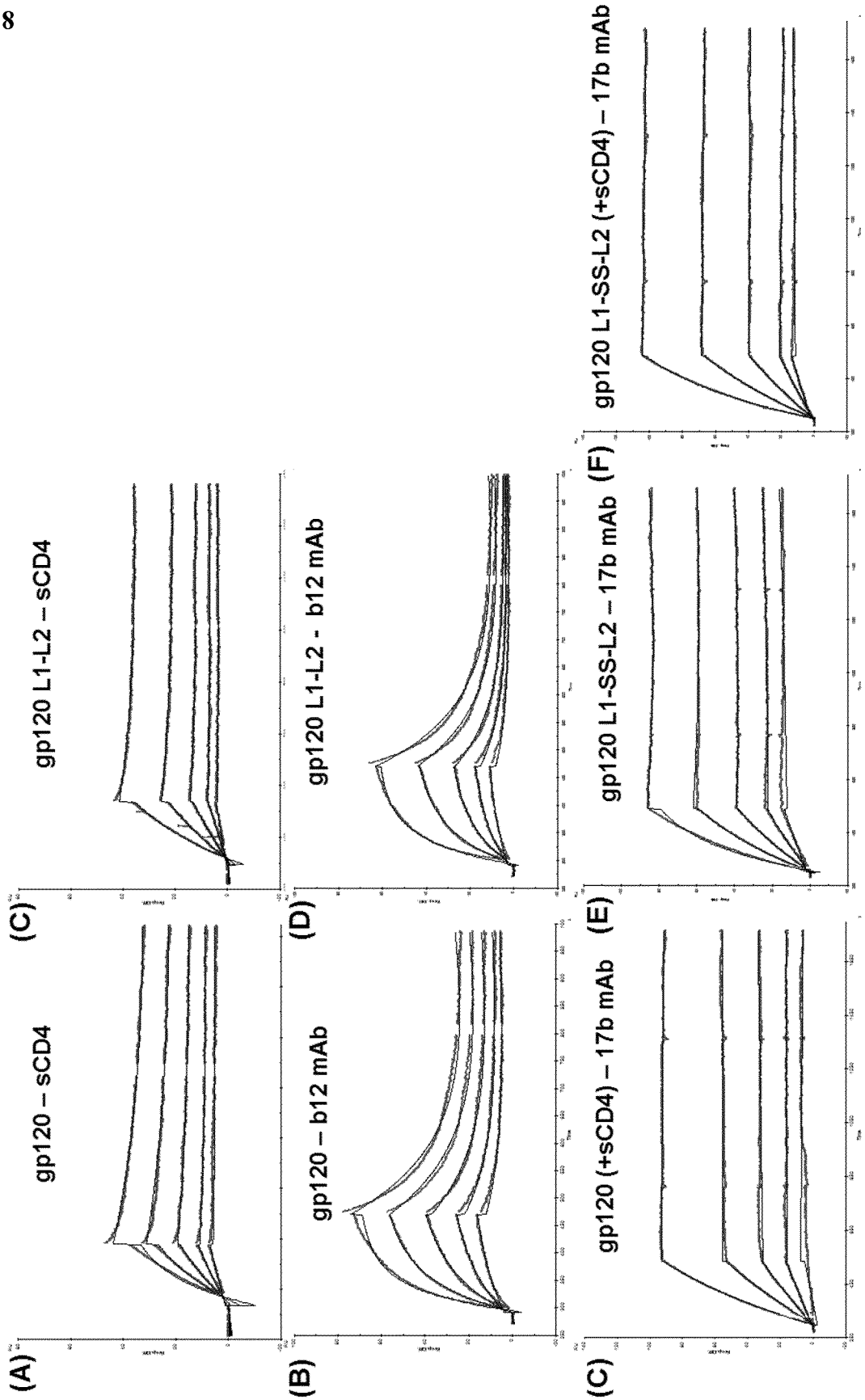
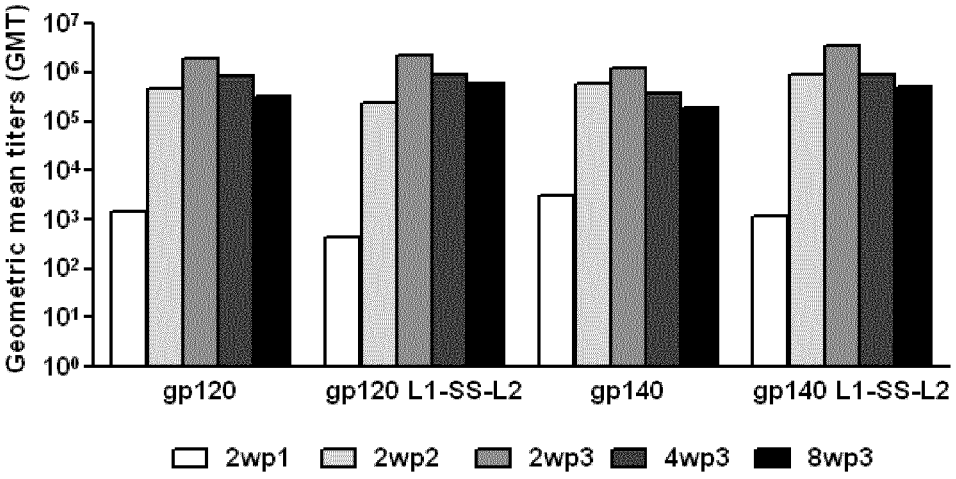

(A)
gp120 – sCD4
(B)
gp120 – b12 mAb
(C)
gp120 (+sCD4) – 17b mAb
(D)
gp120 L1-L2 – sCD4
gp120 L1-L2 - b12 mAb
(E)
gp120 L1-SS-L2 – 17b mAb
(F)
gp120 L1-SS-L2 (+sCD4) – 17b mAb

FIGURE 9

A


Geometric mean titers (GMT)
10⁷
10⁶
10⁵
10⁴
10³
10²
10¹
10⁰
gp120
gp120 L1-SS-L2
gp140
gp140 L1-SS-L2
2wp1
2wp2
2wp3
4wp3
8wp3

B

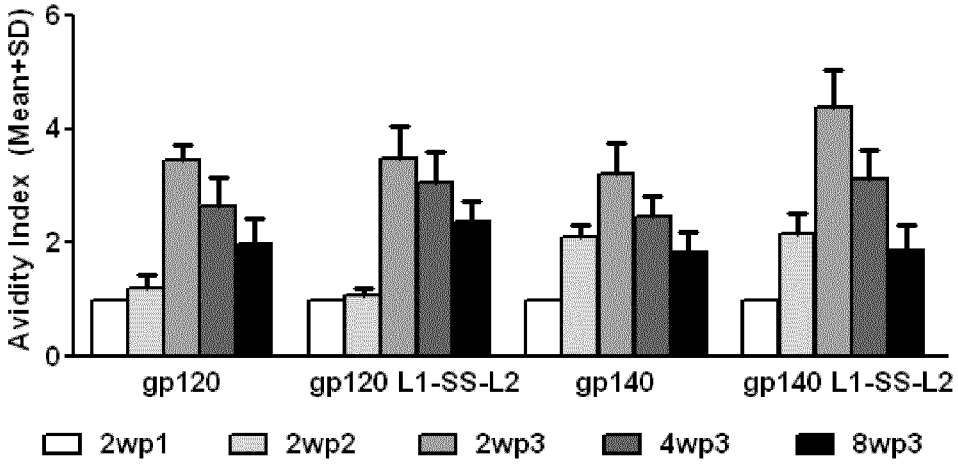
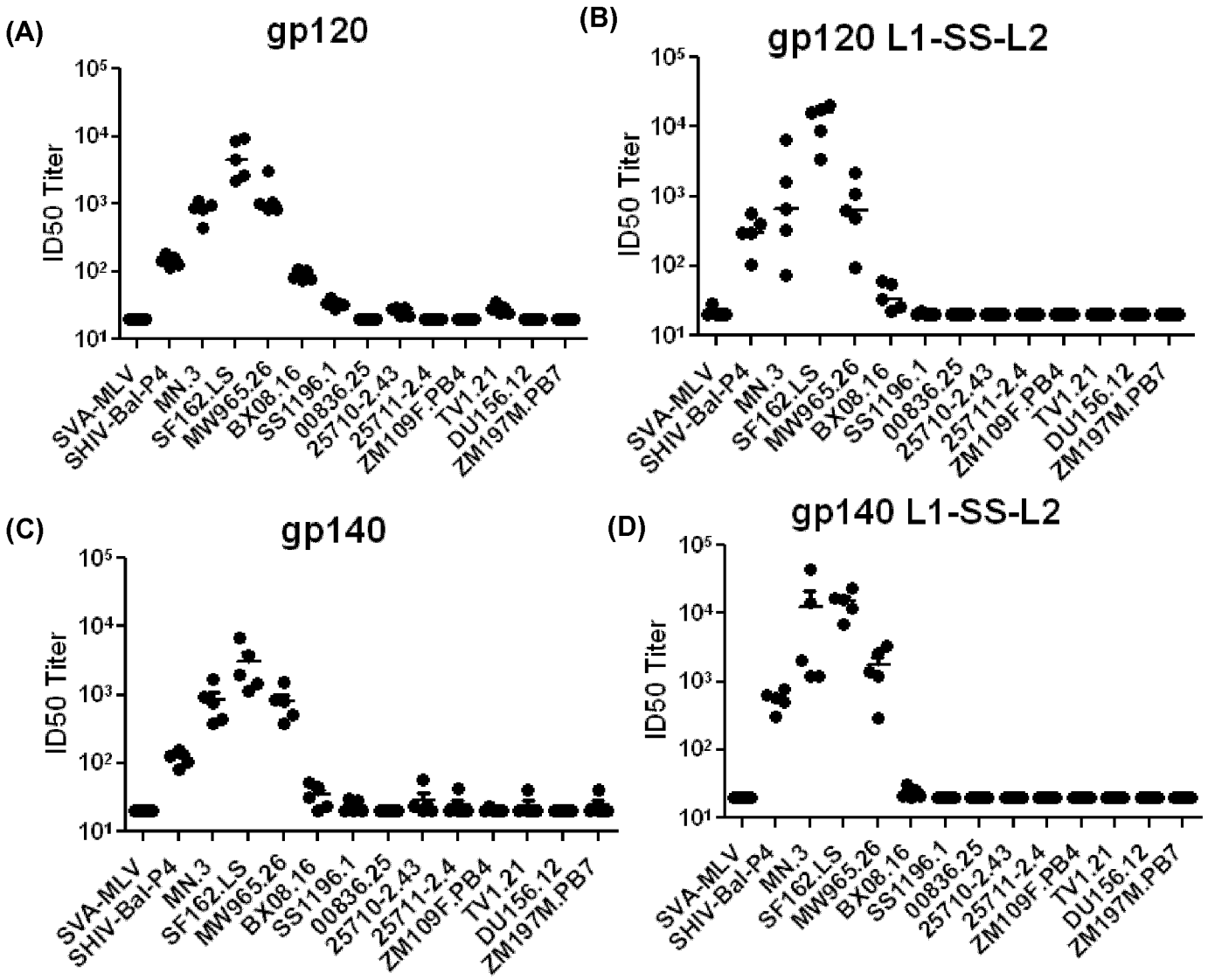

Avidity Index (Mean+SD)
6
4
2
0
gp120
gp120 L1-SS-L2
gp140
gp140 L1-SS-L2
2wp1
2wp2
2wp3
4wp3
8wp3

FIGURE 10


(A)
gp120
ID50 Titer
10^5
10^4
10^3
10^2
10^1
SVA-MLV
SHIV-BaI-P4
MN.3
SF162.LS
MW965.26
BX08.16
SS1196.1
00836.25
25710-2.43
25711-2.4
ZM109F.PB4
TV1.21
DU156.12
ZM197M.PB7
(B)
gp120 L1-SS-L2
ID50 Titer
10^5
10^4
10^3
10^2
10^1
SVA-MLV
SHIV-BaI-P4
MN.3
SF162.LS
MW965.26
BX08.16
SS1196.1
00836.25
25710-2.43
25711-2.4
ZM109F.PB4
TV1.21
DU156.12
ZM197M.PB7
(C)
gp140
ID50 Titer
10^5
10^4
10^3
10^2
10^1
SVA-MLV
SHIV-BaI-P4
MN.3
SF162.LS
MW965.26
BX08.16
SS1196.1
00836.25
25710-2.43
25711-2.4
ZM109F.PB4
TV1.21
DU156.12
ZM197M.PB7
(D)
gp140 L1-SS-L2
ID50 Titer
10^5
10^4
10^3
10^2
10^1
SVA-MLV
SHIV-BaI-P4
MN.3
SF162.LS
MW965.26
BX08.16
SS1196.1
00836.25
25710-2.43
25711-2.4
ZM109F.PB4
TV1.21
DU156.12
ZM197M.PB7

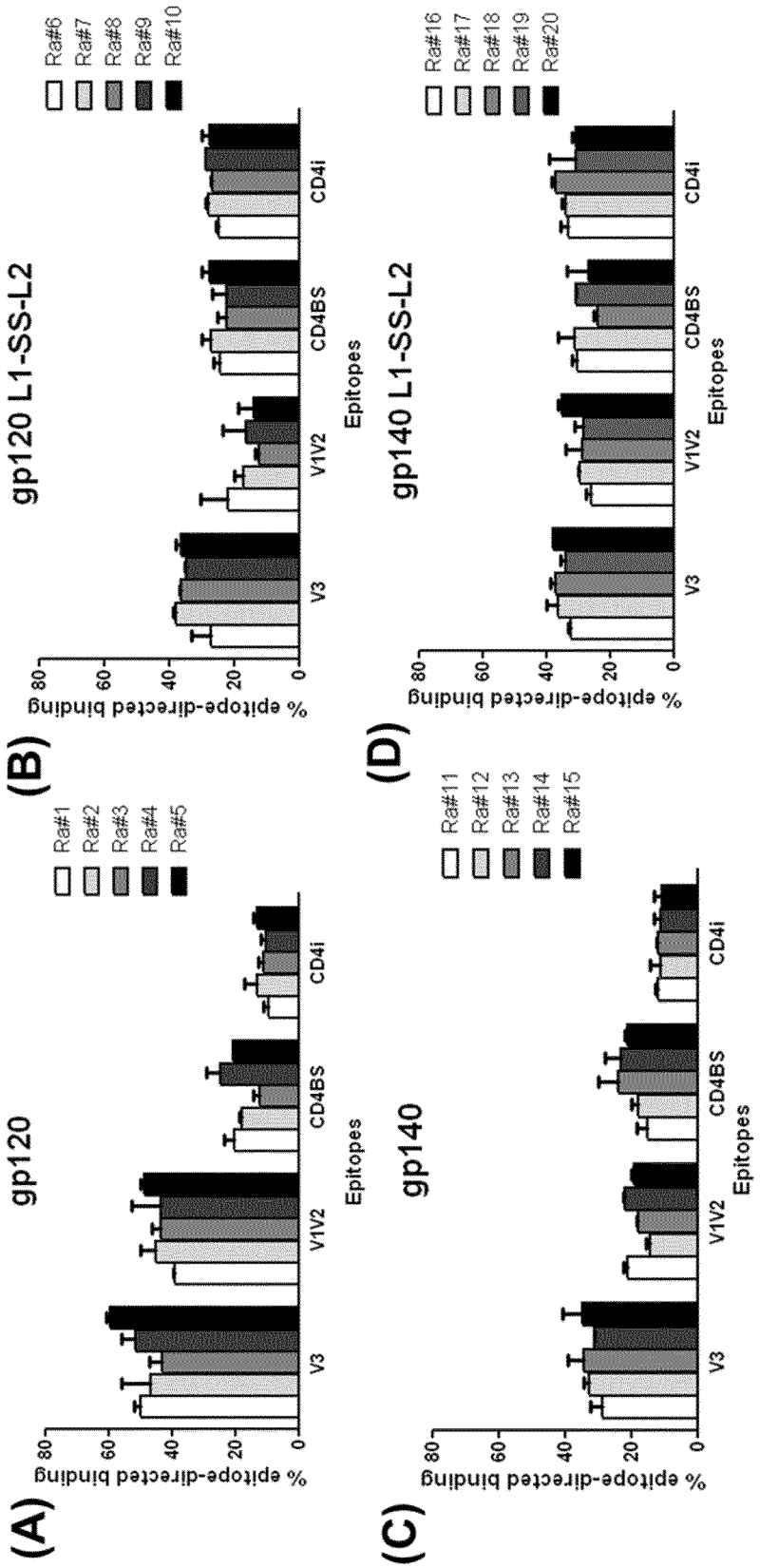

(A)
gp120
% epitope-directed binding
0
20
40
60
80
V3
V1V2
CD4BS
CD4i
Epitopes
Ra#1
Ra#2
Ra#3
Ra#4
Ra#5
(B)
gp120 L1-SS-L2
% epitope-directed binding
0
20
40
60
80
V3
V1V2
CD4BS
CD4i
Epitopes
Ra#6
Ra#7
Ra#8
Ra#9
Ra#10
(C)
gp140
% epitope-directed binding
0
20
40
60
80
V3
V1V2
CD4BS
CD4i
Epitopes
Ra#11
Ra#12
Ra#13
Ra#14
Ra#15
(D)
gp140 L1-SS-L2
% epitope-directed binding
0
20
40
60
80
V3
V1V2
CD4BS
CD4i
Epitopes
Ra#16
Ra#17
Ra#18
Ra#19
Ra#20

FIGURE 11