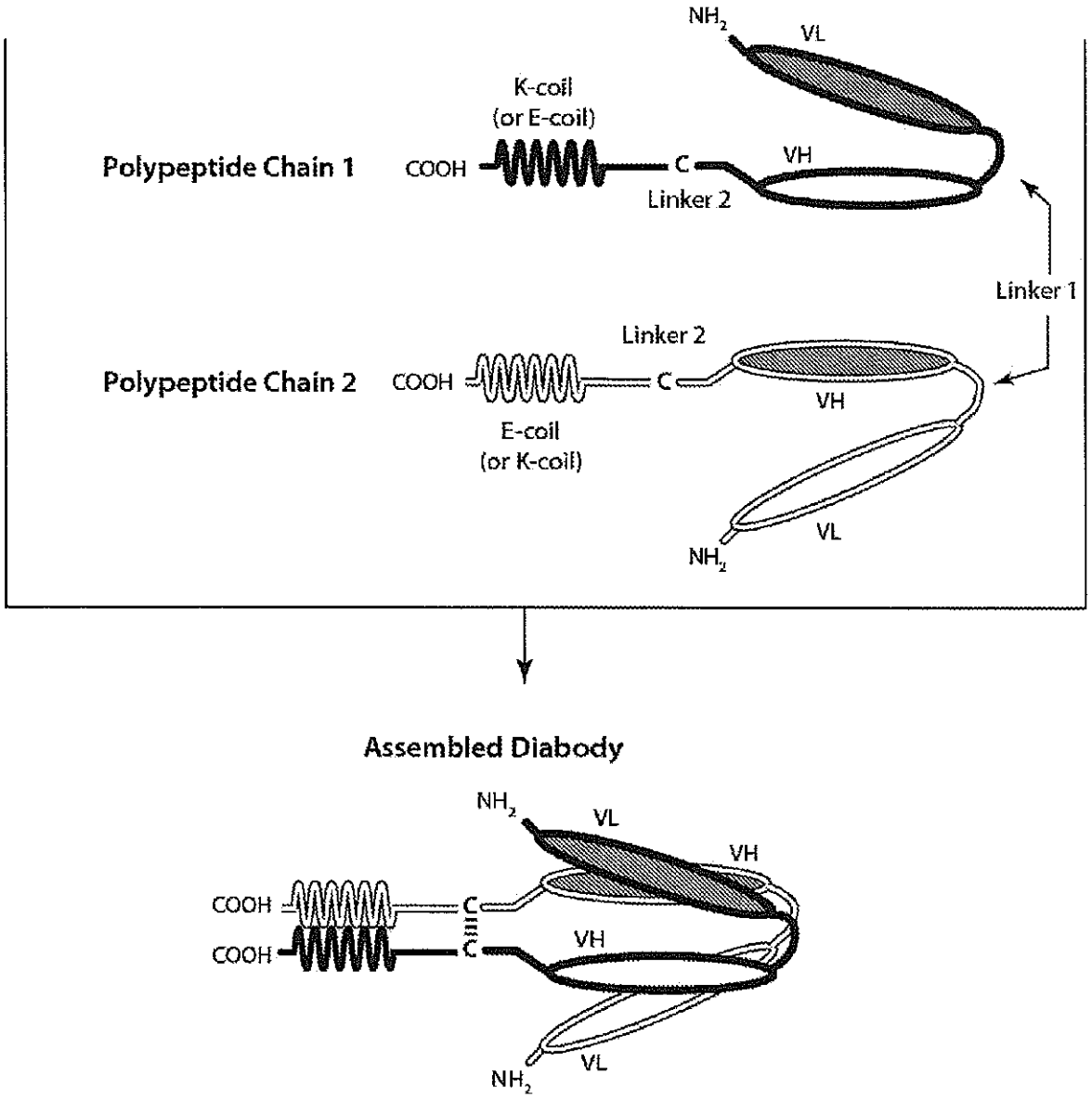
NH2
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
C
VH
Linker 2
Linker 1
Linker 2
Polypeptide Chain 2
COOH
C
VH
E-coil
(or K-coil)
VL
NH2
Assembled Diabody
NH2
VL
VH
COOH
C
COOH
C
VH
VL
NH2

Figure 1

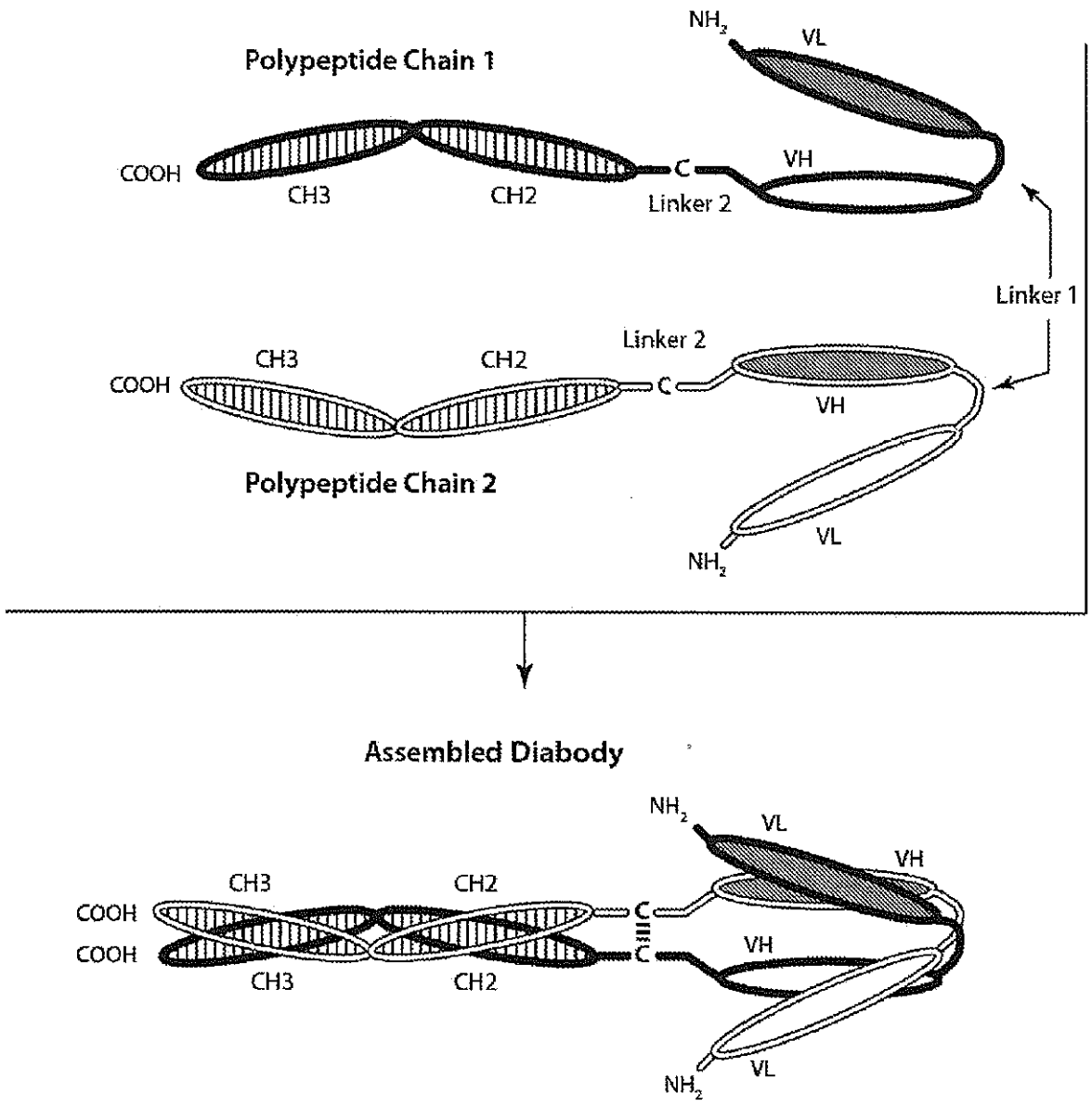
Polypeptide Chain 1
NH2
VL
COOH
CH3
CH2
C
VH
Linker 2
Linker 1
Linker 2
CH3
CH2
COOH
C
VH
Polypeptide Chain 2
VL
NH2
Assembled Diabody
NH2
VL
VH
CH3
CH2
COOH
COOH
C
C
VH
CH3
CH2
VL
NH2

Figure 2

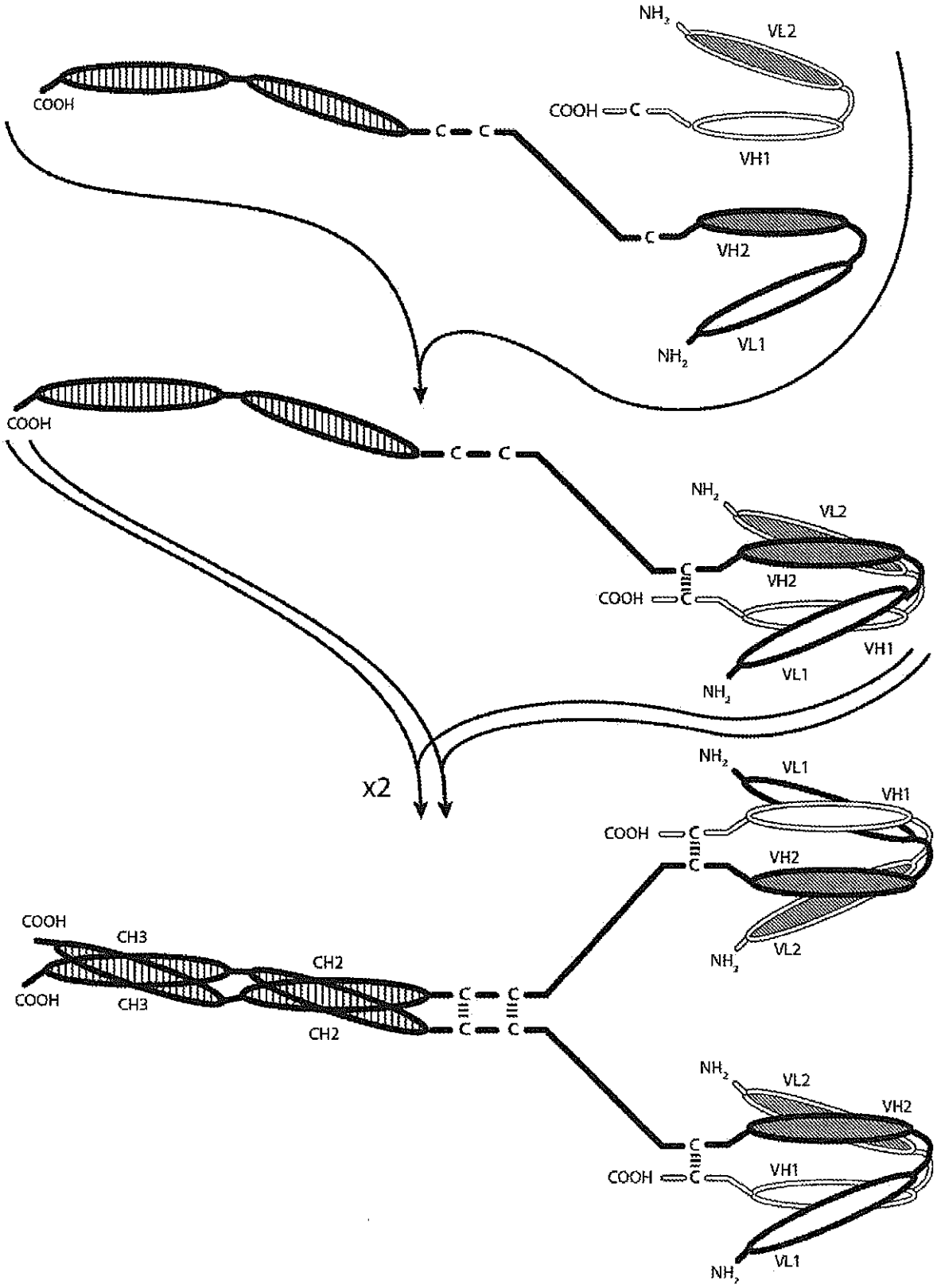
NH2
VL2
COOH
C
C
COOH
C
VH1
C
VH2
NH2
VL1
COOH
C
C
NH2
VL2
C
VH2
COOH
C
VH1
VL1
NH2
x2
NH2
VL1
VH1
COOH
C
C
VH2
NH2
VL2
COOH
CH3
CH2
COOH
CH3
CH2
C
C
C
C
NH2
VL2
VH2
C
C
COOH
VH1
NH2
VL1

Figure 3A

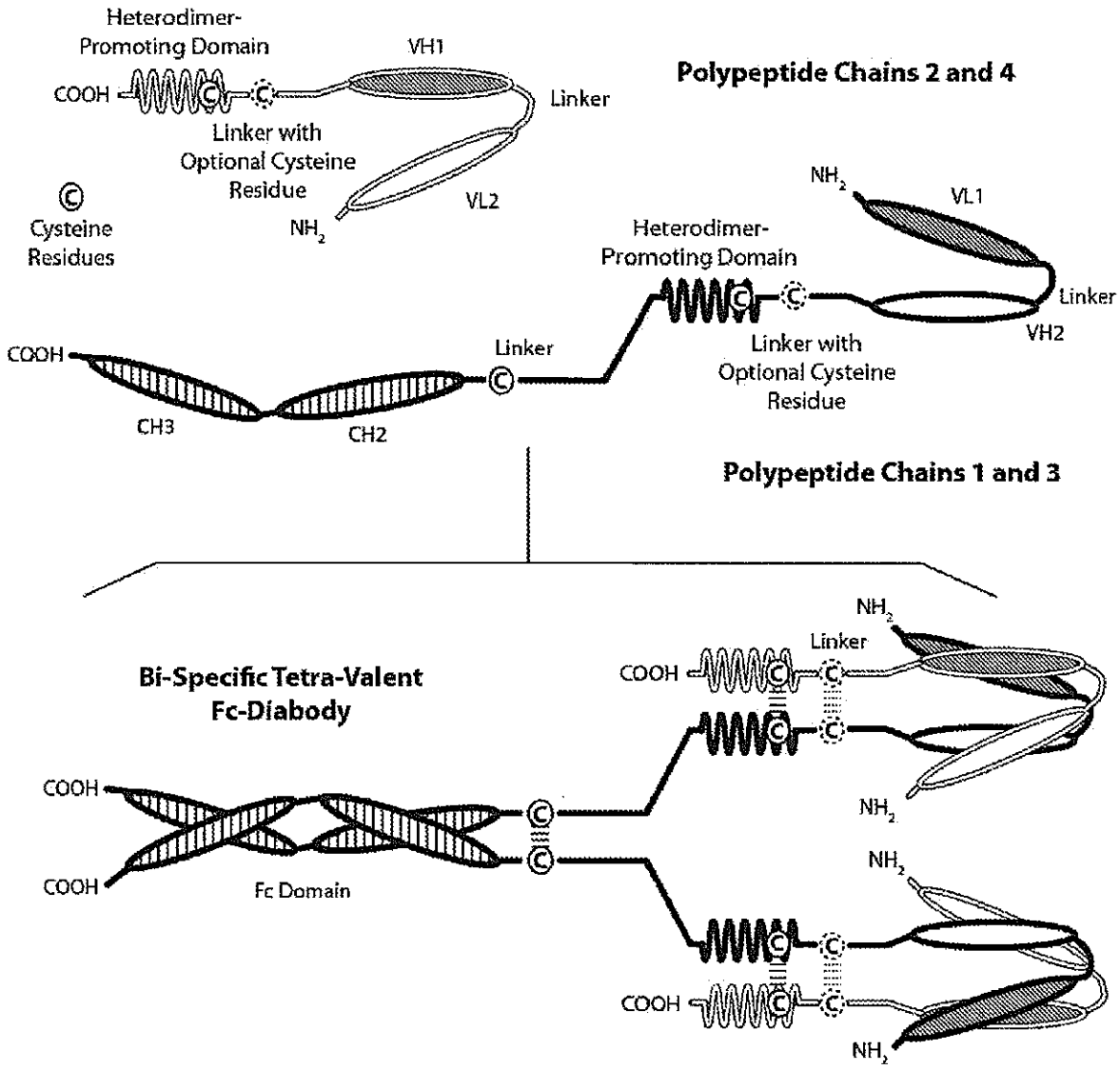
Heterodimer-
Promoting Domain
VH1
COOH
Linker
Linker with
Optional Cysteine
Residue
VL2
NH2
Cysteine
Residues
Polypeptide Chains 2 and 4
NH2
VL1
Heterodimer-
Promoting Domain
Linker
VH2
Linker with
Optional Cysteine
Residue
COOH
Linker
CH3
CH2
Polypeptide Chains 1 and 3
Bi-Specific Tetra-Valent
Fc-Diabody
NH2
Linker
COOH
COOH
Fc Domain
NH2
NH2
COOH
NH2

Figure 3B

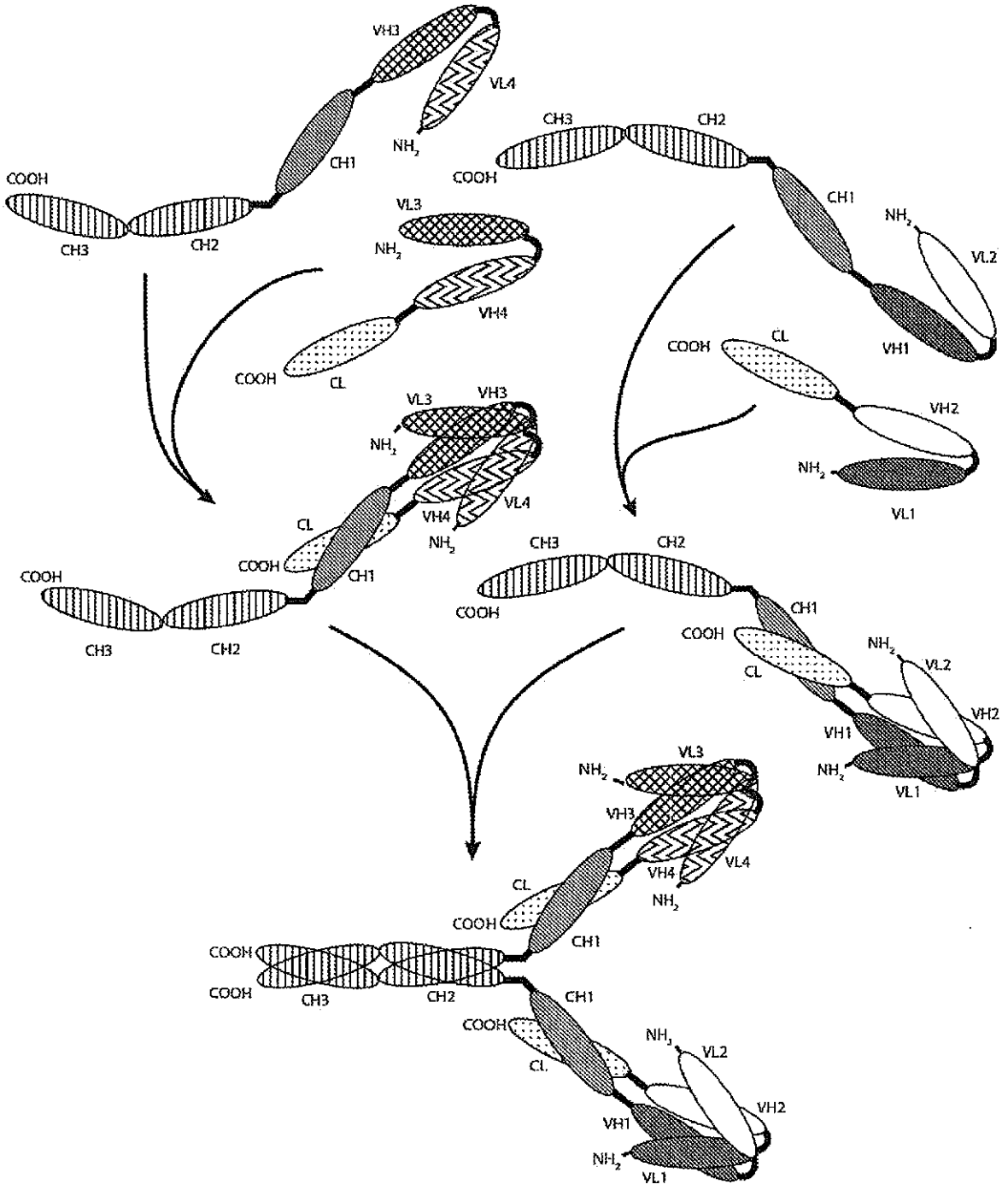
VH3
VL4
NH2
CH1
COOH
CH3
CH2
VL3
NH2
VH4
COOH
CL
CH3
CH2
CH1
NH2
VL2
VH1
COOH
CL
VH2
NH2
VL1
VL3
VH3
NH2
VH4
VL4
NH2
CL
COOH
CH1
COOH
CH3
CH2
CH3
CH2
COOH
CH1
COOH
CL
NH2
VL2
VH2
VH1
NH2
VL1
VL3
NH2
VH3
VH4
VL4
NH2
CL
COOH
CH1
COOH
COOH
CH3
CH2
CH1
COOH
CL
NH2
VL2
VH2
VH1
NH2
VL1

Figure 3C

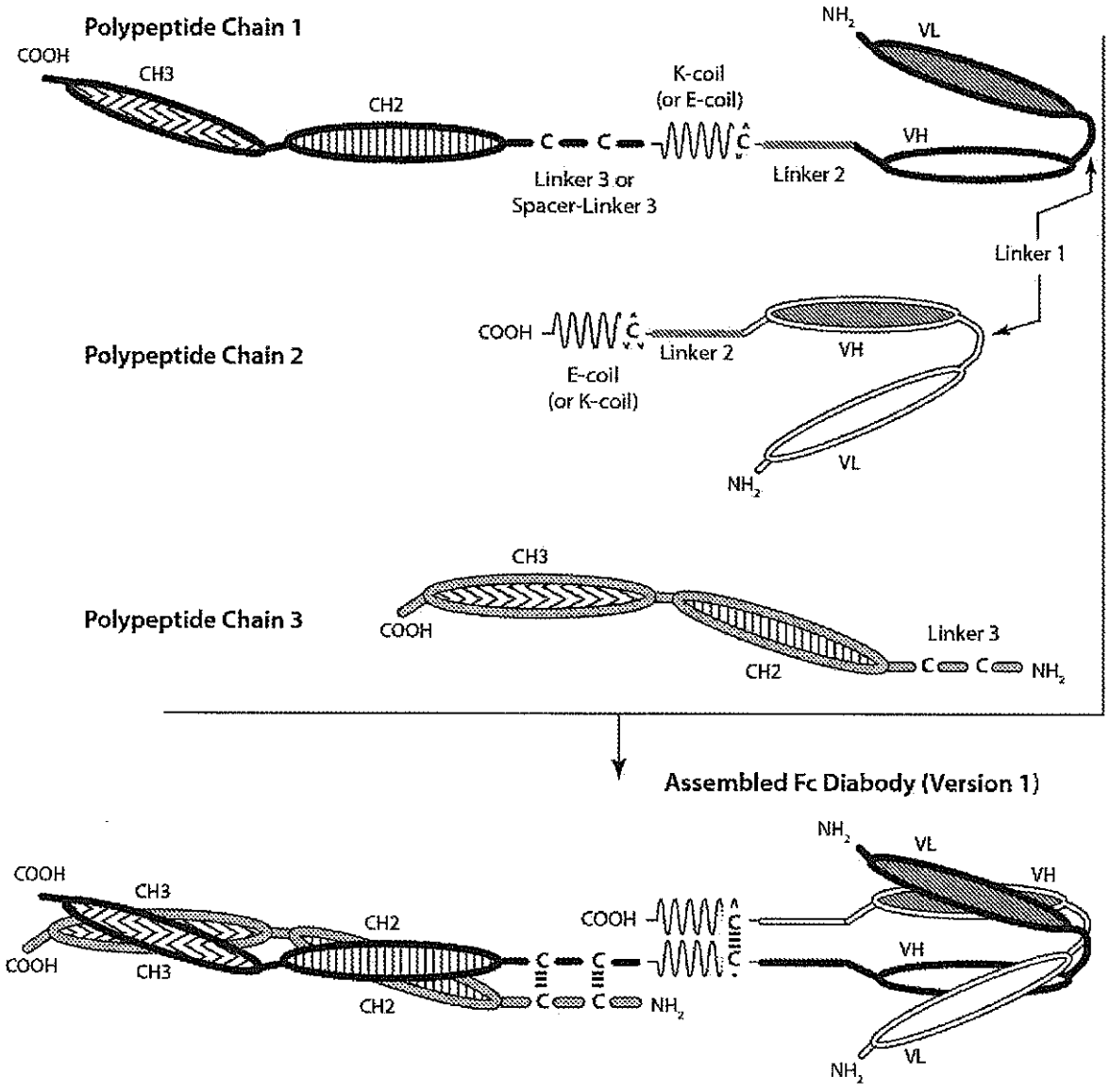
Polypeptide Chain 1
COOH
CH3
CH2
K-coil
(or E-coil)
Linker 3 or
Spacer-Linker 3
Linker 2
VH
VL
NH2
Linker 1
Polypeptide Chain 2
COOH
E-coil
(or K-coil)
Linker 2
VH
VL
NH2
Polypeptide Chain 3
CH3
COOH
CH2
Linker 3
NH2
Assembled Fc Diabody (Version 1)
COOH
CH3
CH3
COOH
CH2
CH2
NH2
VL
VH
VH
VL
NH2

Figure 4A

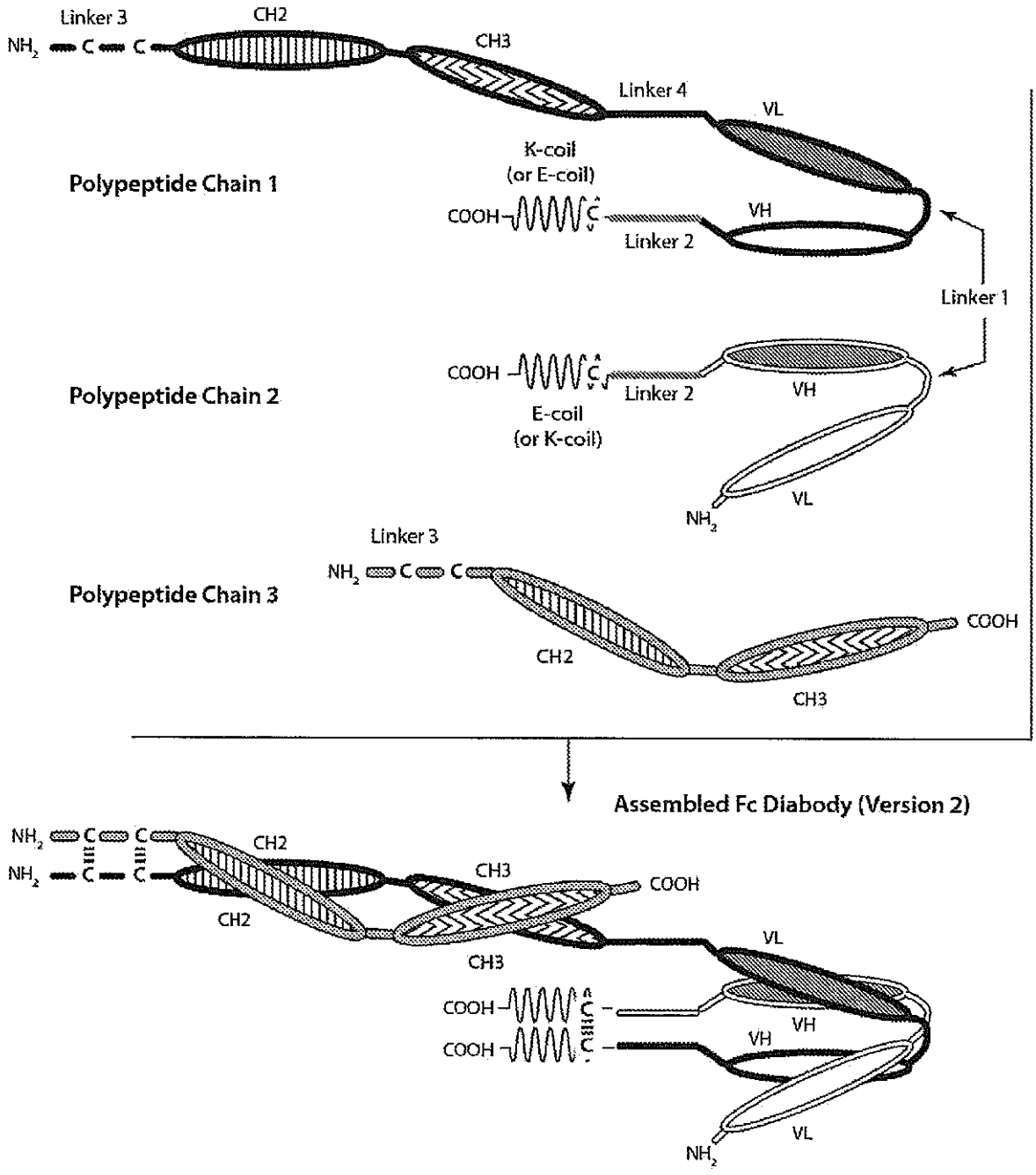
Linker 3
NH2
C
C
CH2
CH3
Linker 4
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
VH
Linker 2
Linker 1
Polypeptide Chain 2
COOH
Linker 2
VH
E-coil
(or K-coil)
VL
NH2
Linker 3
Polypeptide Chain 3
NH2
C
C
COOH
CH2
CH3
Assembled Fc Diabody (Version 2)
NH2
NH2
CH2
CH2
CH3
COOH
CH3
VL
COOH
VH
COOH
VH
VL
NH2

Figure 4B

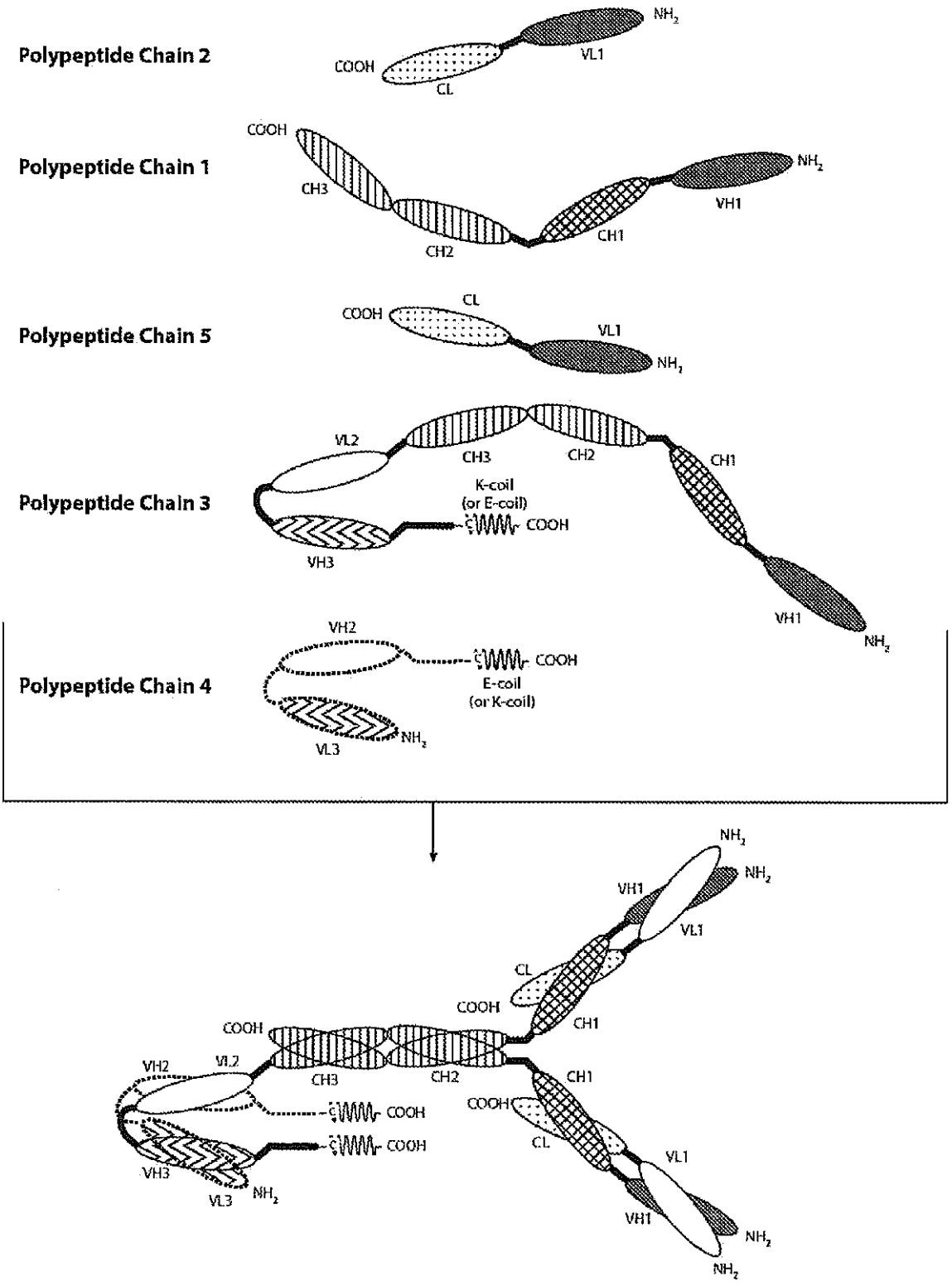
Polypeptide Chain 2
NH2
VL1
COOH
CL
Polypeptide Chain 1
COOH
CH3
NH2
VH1
CH2
CH1
Polypeptide Chain 5
CL
COOH
VL1
NH2
Polypeptide Chain 3
VL2
CH3
CH2
CH1
K-coil
(or E-coil)
COOH
VH3
VH1
NH2
Polypeptide Chain 4
VH2
COOH
E-coil
(or K-coil)
NH2
VL3
NH2
VH1
NH2
VL1
CL
COOH
CH1
COOH
VH2
VL2
CH3
CH2
CH1
COOH
COOH
CL
VL1
VH3
VL3
NH2
VH1
NH2
NH2

Figure 5

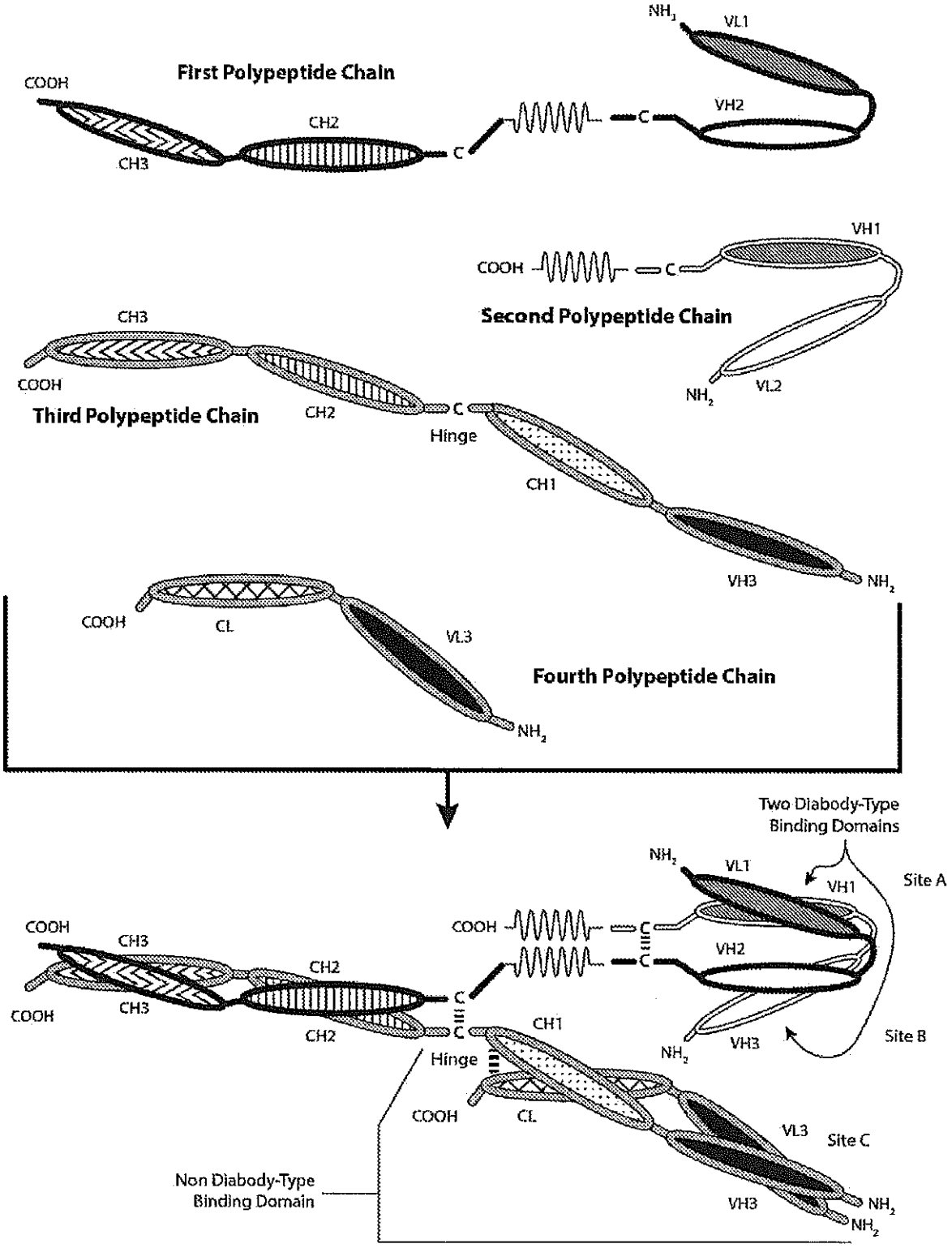
First Polypeptide Chain
COOH
CH2
CH3
C
C
NH2
VL1
VH2
Second Polypeptide Chain
COOH
C
VH1
NH2
VL2
Third Polypeptide Chain
CH3
COOH
CH2
C
Hinge
CH1
VH3
NH2
Fourth Polypeptide Chain
COOH
CL
VL3
NH2
Two Diabody-Type Binding Domains
Site A
Site B
Site C
Non Diabody-Type Binding Domain

Figure 6A

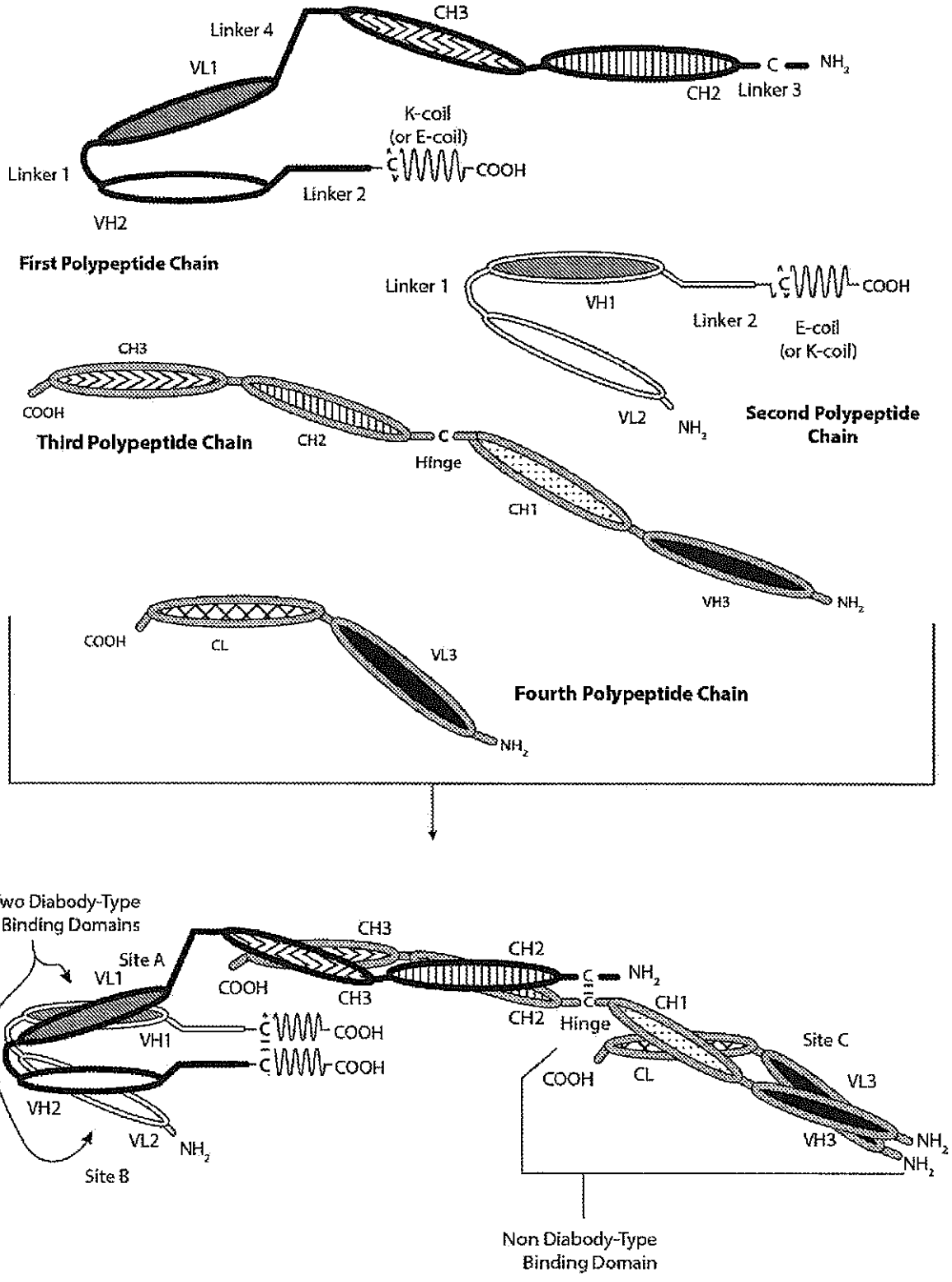
CH3
Linker 4
VL1
C
NH2
CH2
Linker 3
K-coil
(or E-coil)
Linker 1
COOH
Linker 2
VH2
First Polypeptide Chain
Linker 1
VH1
COOH
Linker 2
E-coil
(or K-coil)
CH3
VL2
NH2
Second Polypeptide Chain
COOH
Third Polypeptide Chain
CH2
C
Hinge
CH1
VH3
NH2
COOH
CL
VL3
Fourth Polypeptide Chain
NH2
Two Diabody-Type
Binding Domains
Site A
VL1
CH3
CH2
COOH
CH3
NH2
CH2
CH1
VH1
COOH
Hinge
Site C
COOH
CL
VL3
VH2
VH3
NH2
VL2
NH2
NH2
Site B
Non Diabody-Type
Binding Domain

Figure 6B

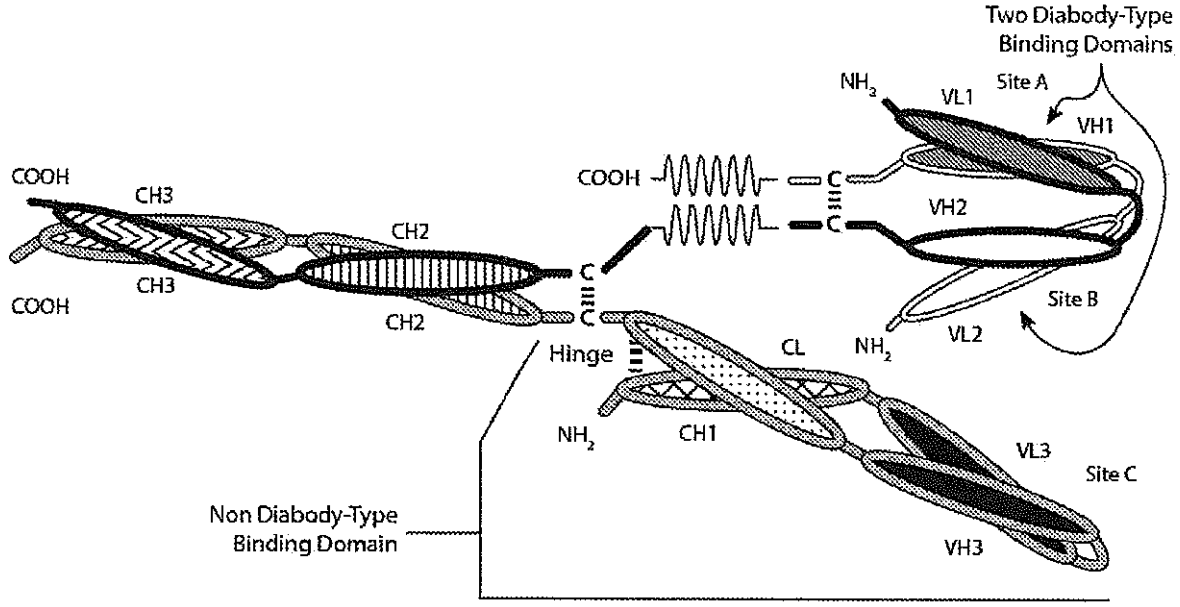

Figure 6C

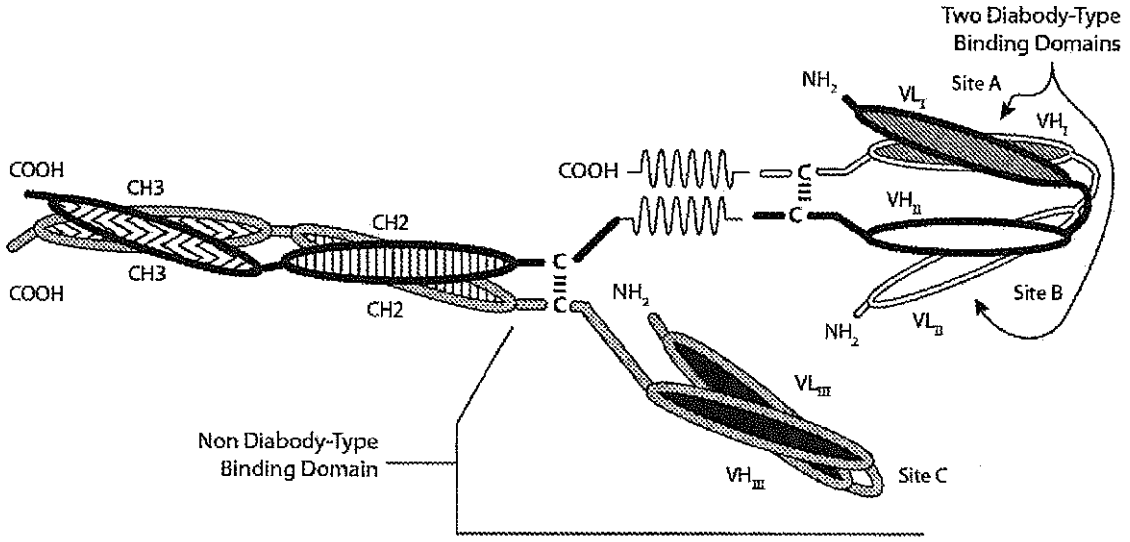

Figure 6D

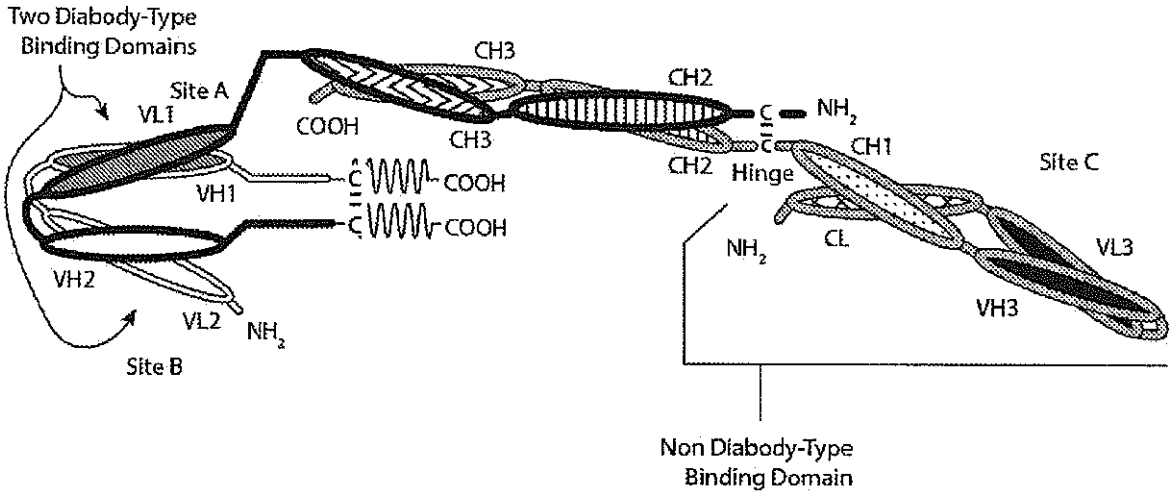

Figure 6E

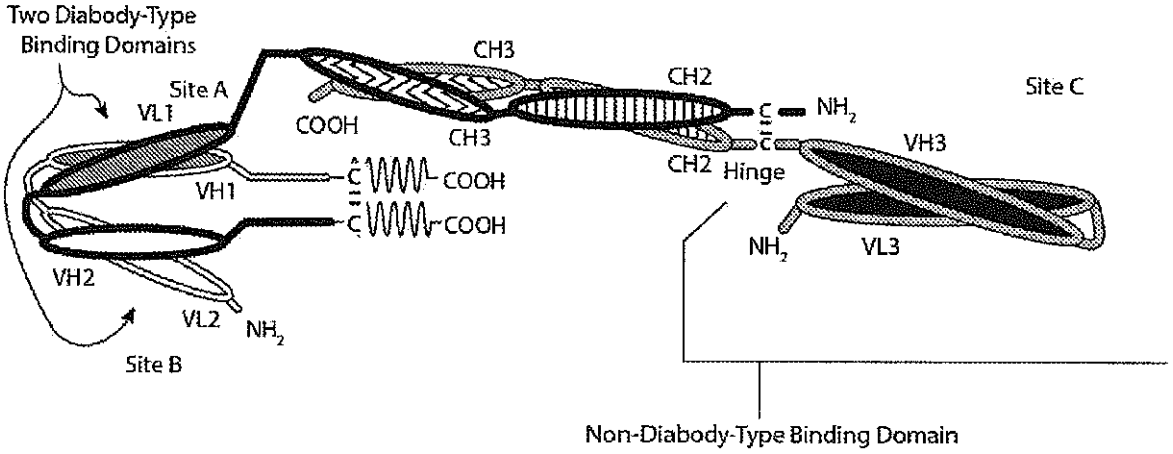

Figure 6F

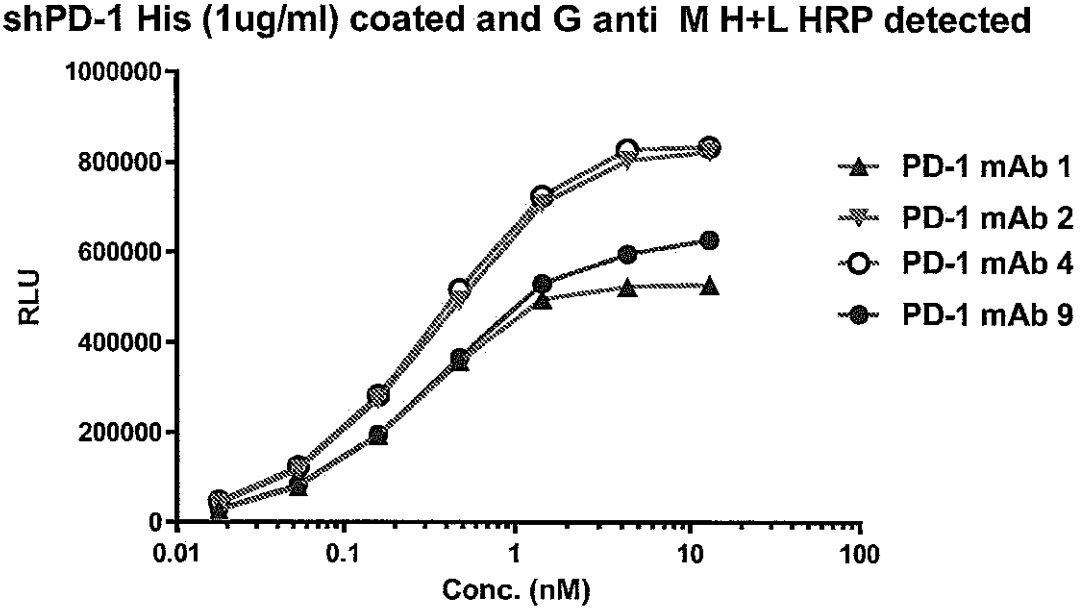

Figure 7A

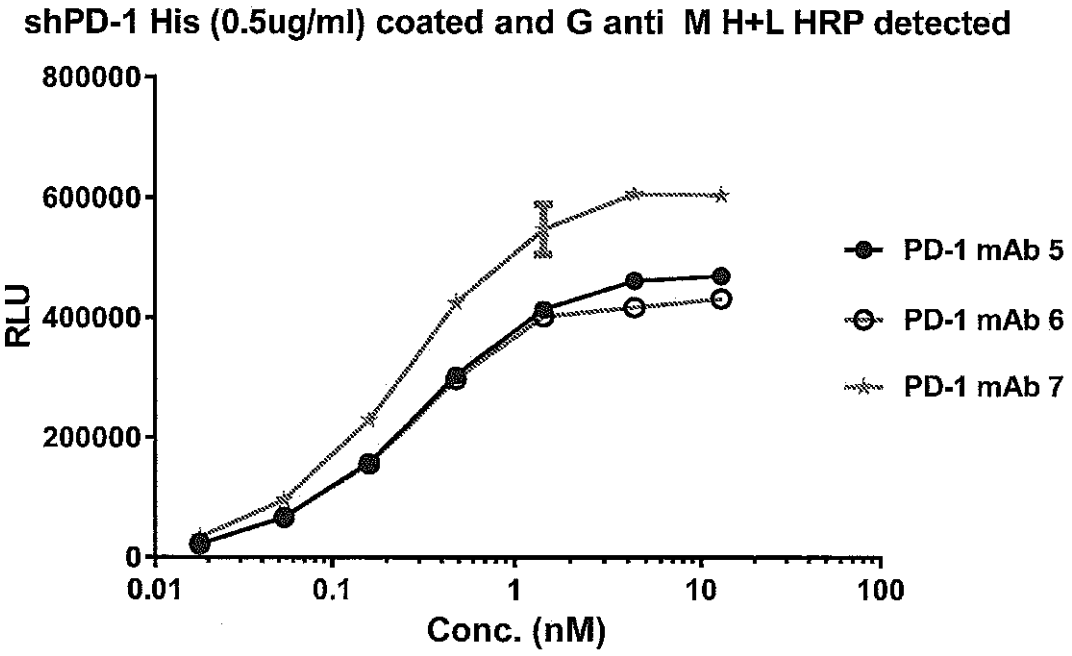

Figure 7B

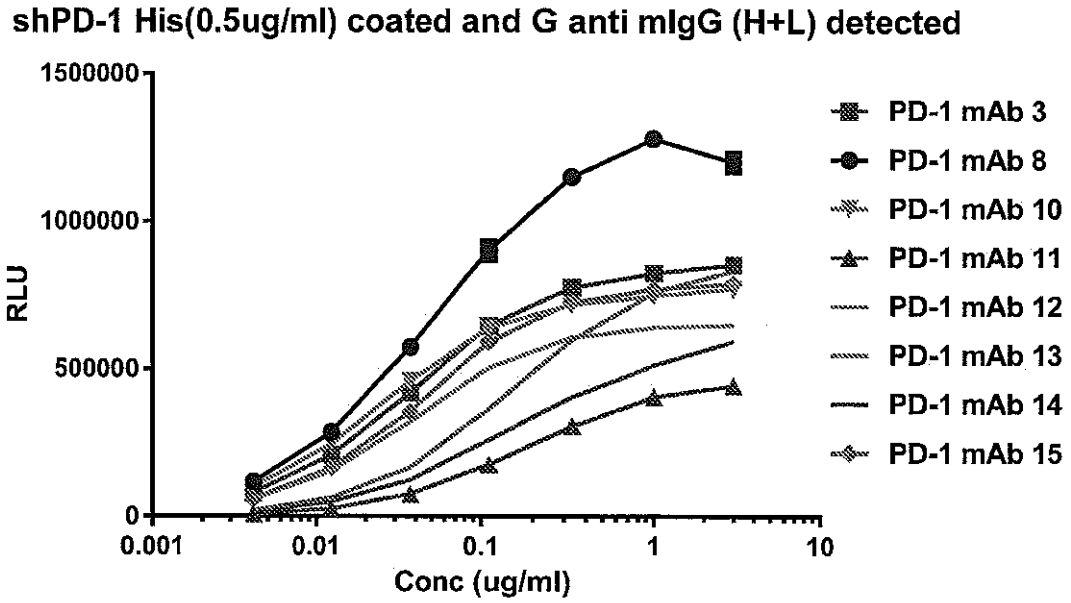

Figure 7C

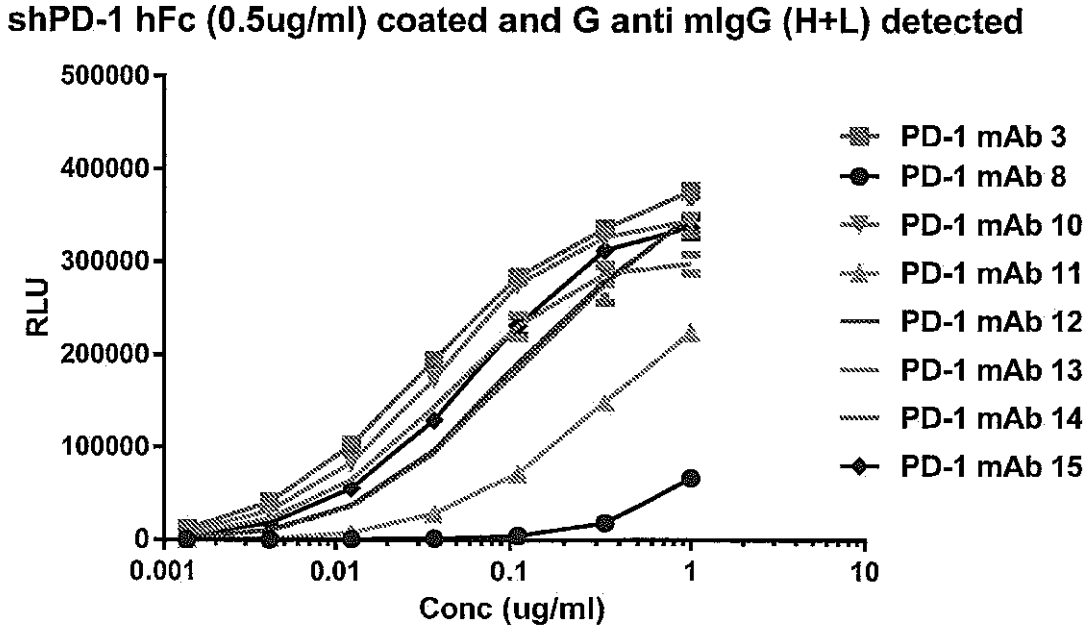

Figure 7D

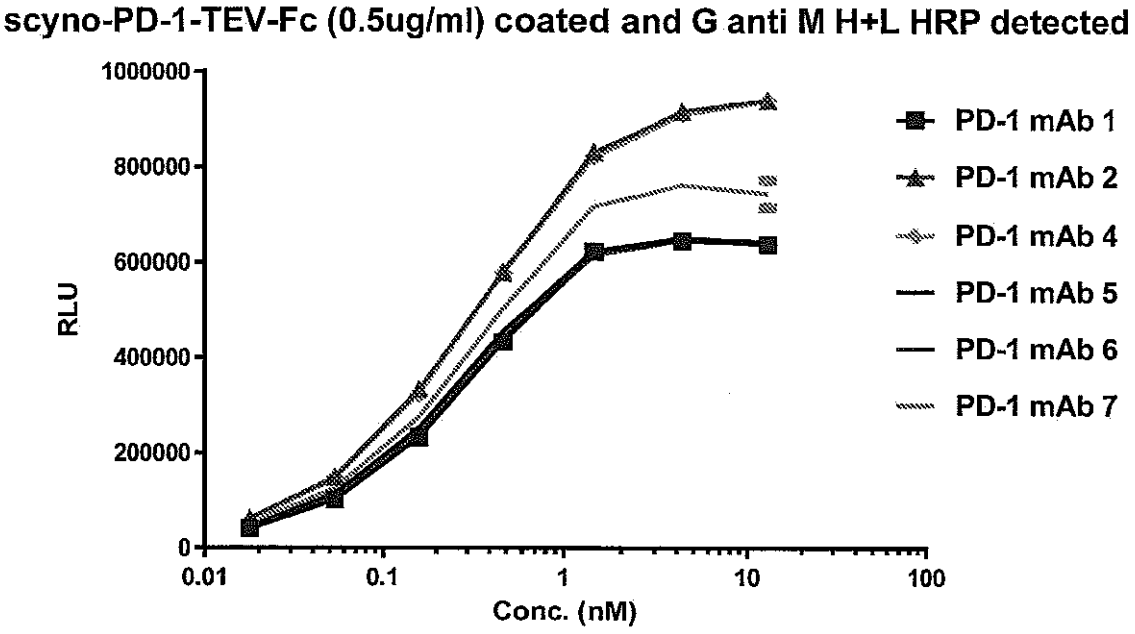

Figure 8A

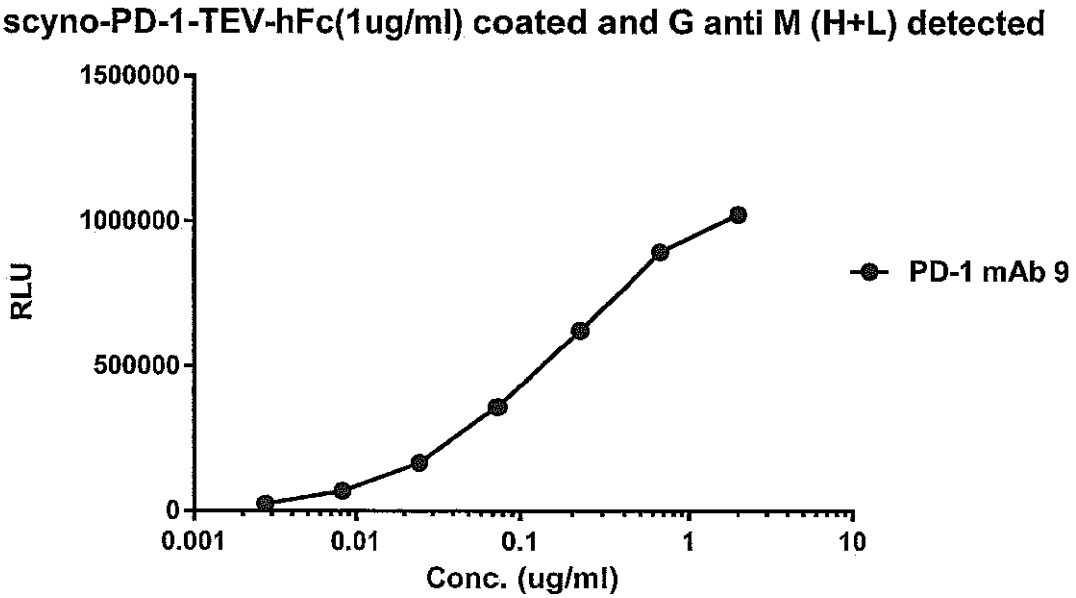

Figure 8B

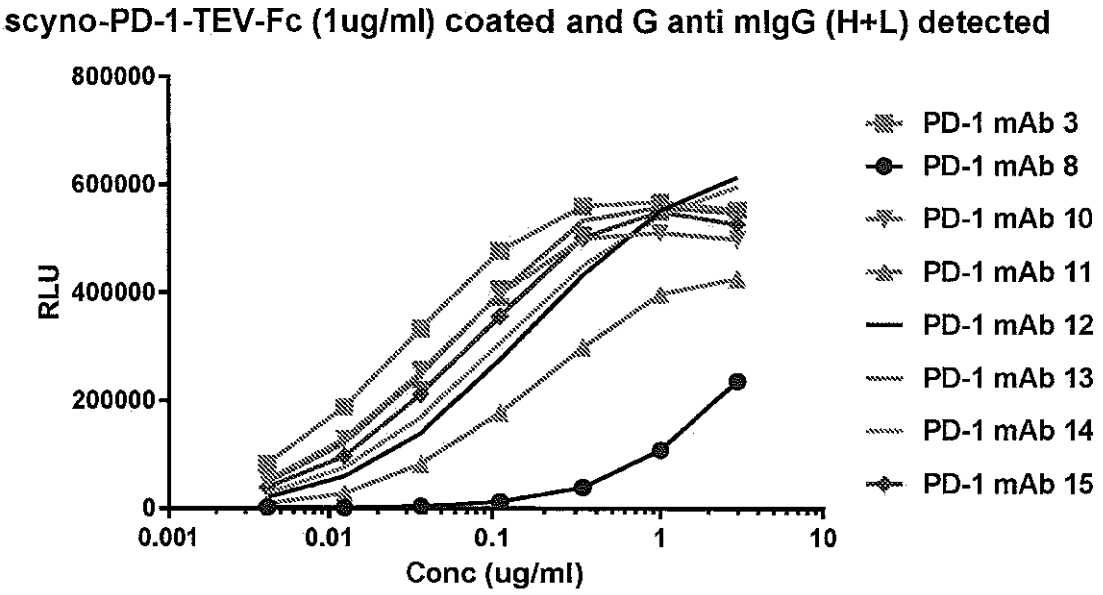
scyno-PD-1-TEV-Fc (1ug/ml) coated and G anti mIgG (H+L) detected
800000
600000
400000
200000
0
RLU
0.001
0.01
0.1
1
10
Conc (ug/ml)
PD-1 mAb 3
PD-1 mAb 8
PD-1 mAb 10
PD-1 mAb 11
PD-1 mAb 12
PD-1 mAb 13
PD-1 mAb 14
PD-1 mAb 15

Figure 8C

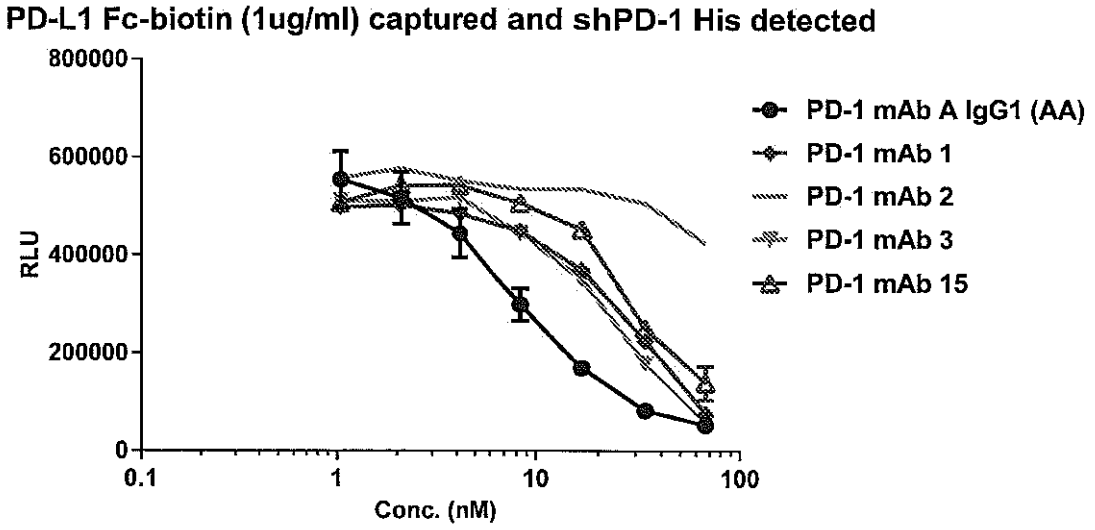

Figure 9A

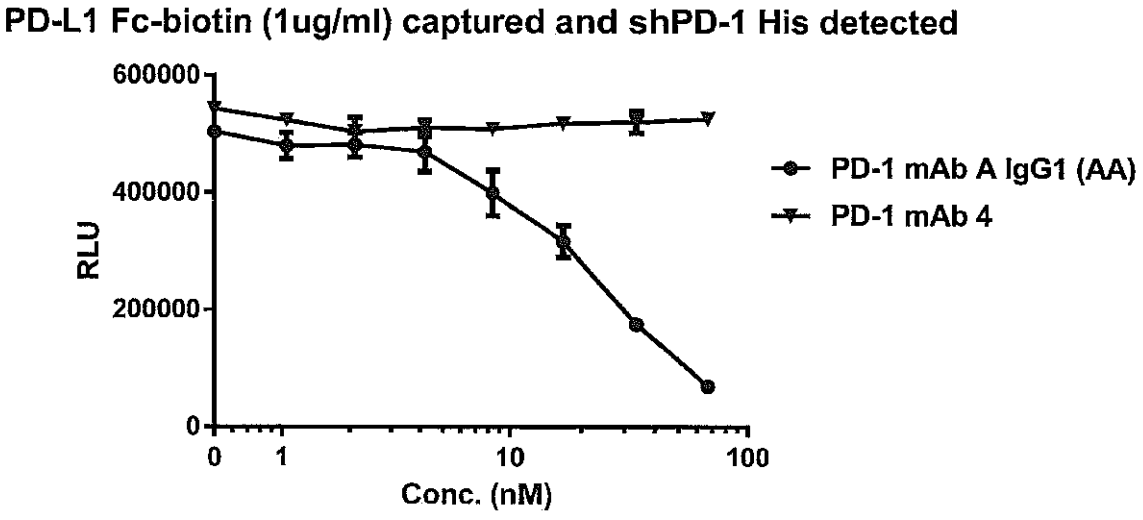

Figure 9B

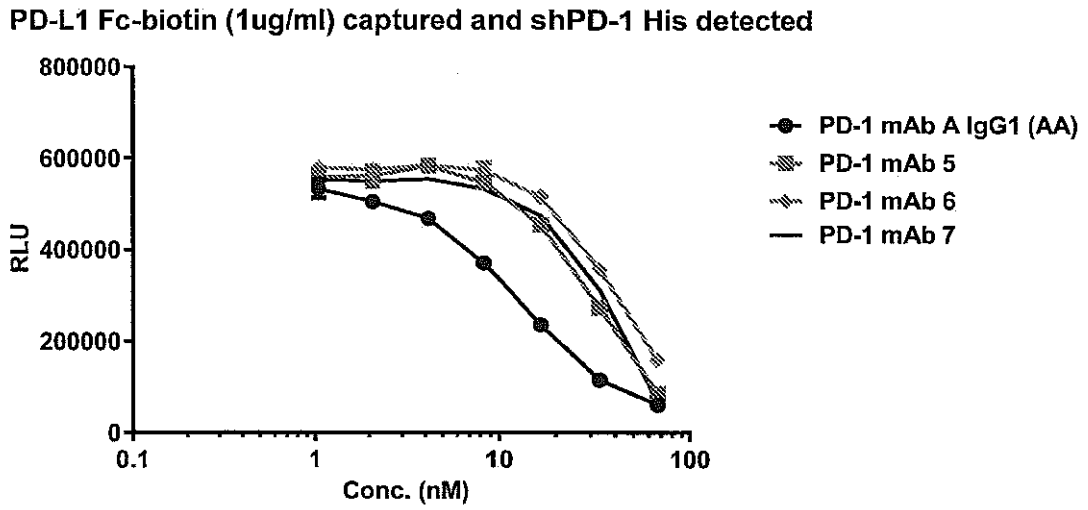

Figure 9C

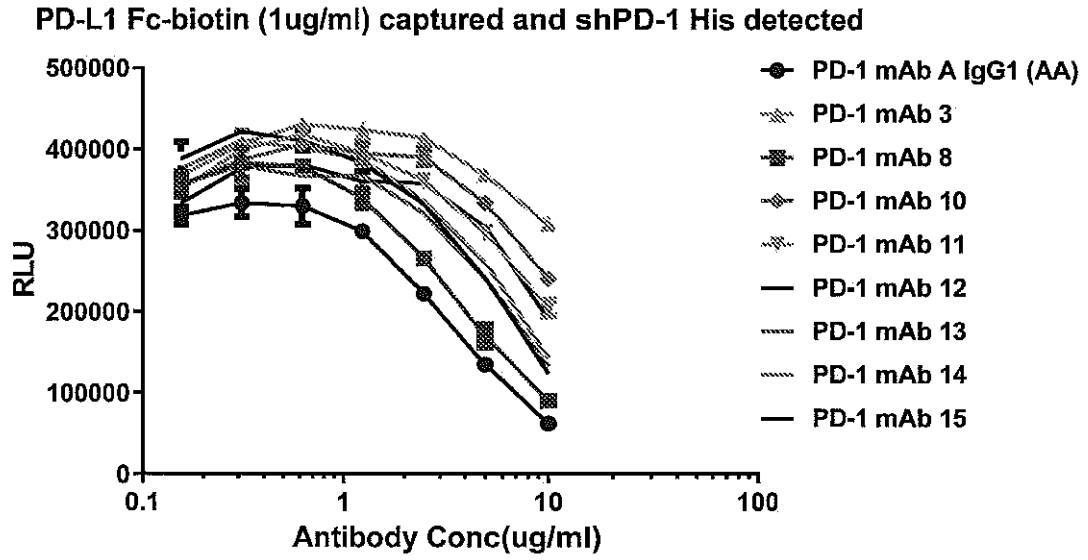

Figure 9D

Normal Tissues

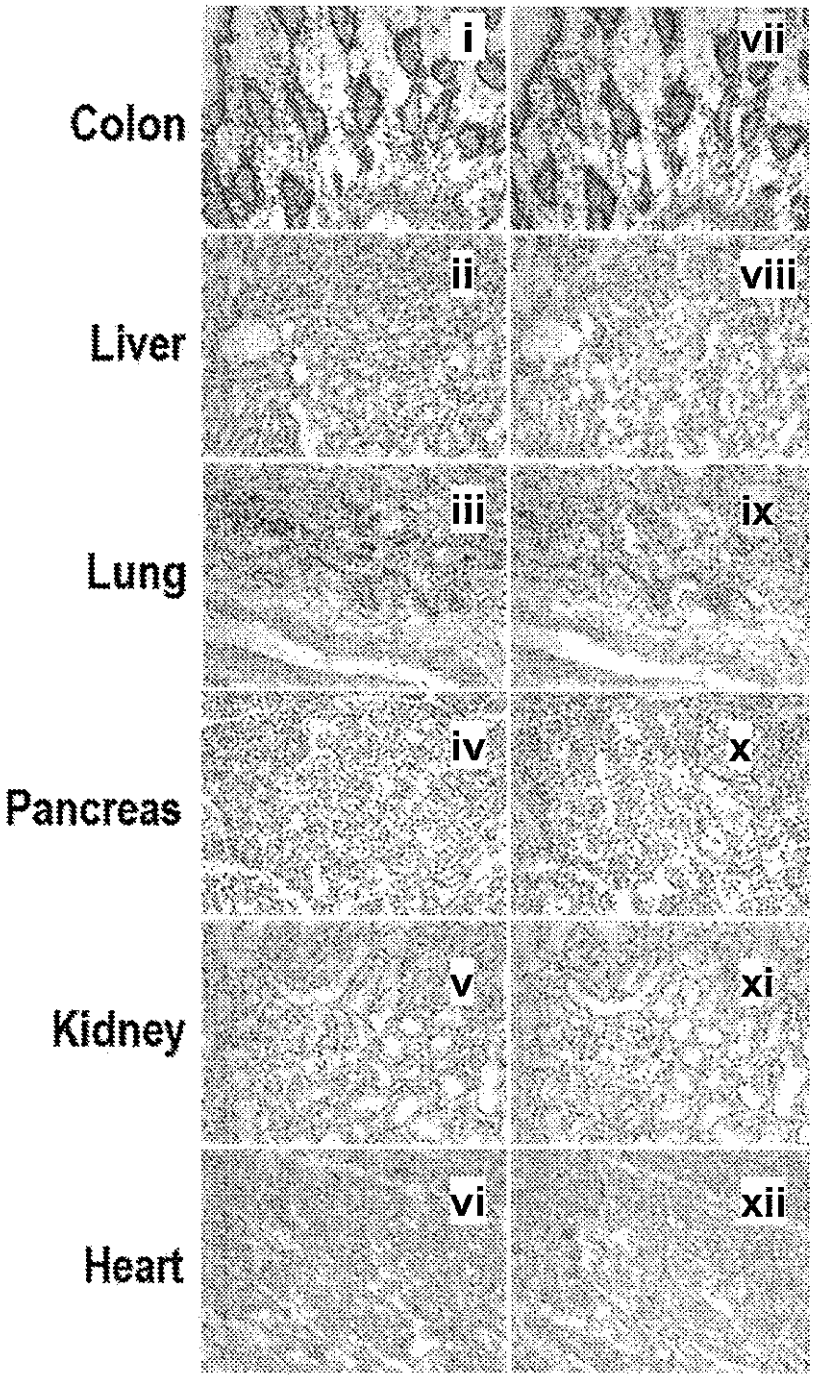

Figure 10A

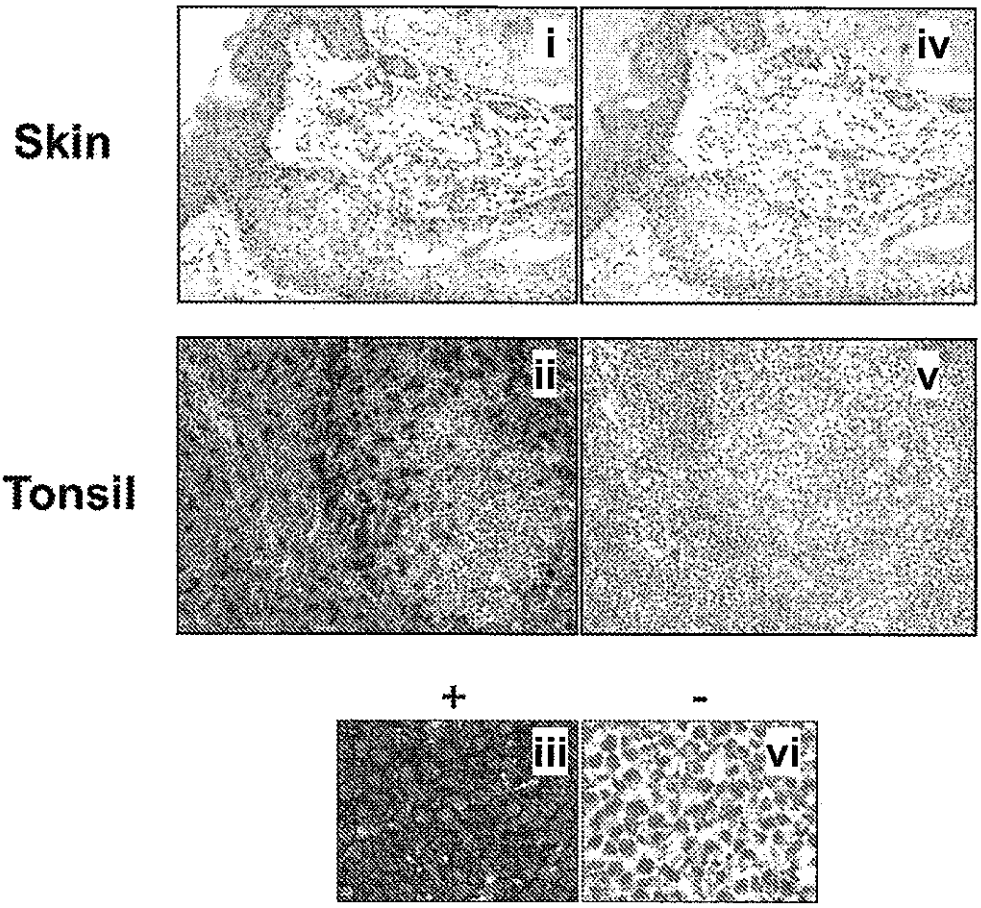
Skin
Tonsil
i
iv
ii
v
+
-
iii
vi
PD-1 mAb 7
0.313 µg/ml
Isotype Control
0.313 µg/ml

Figure 10B

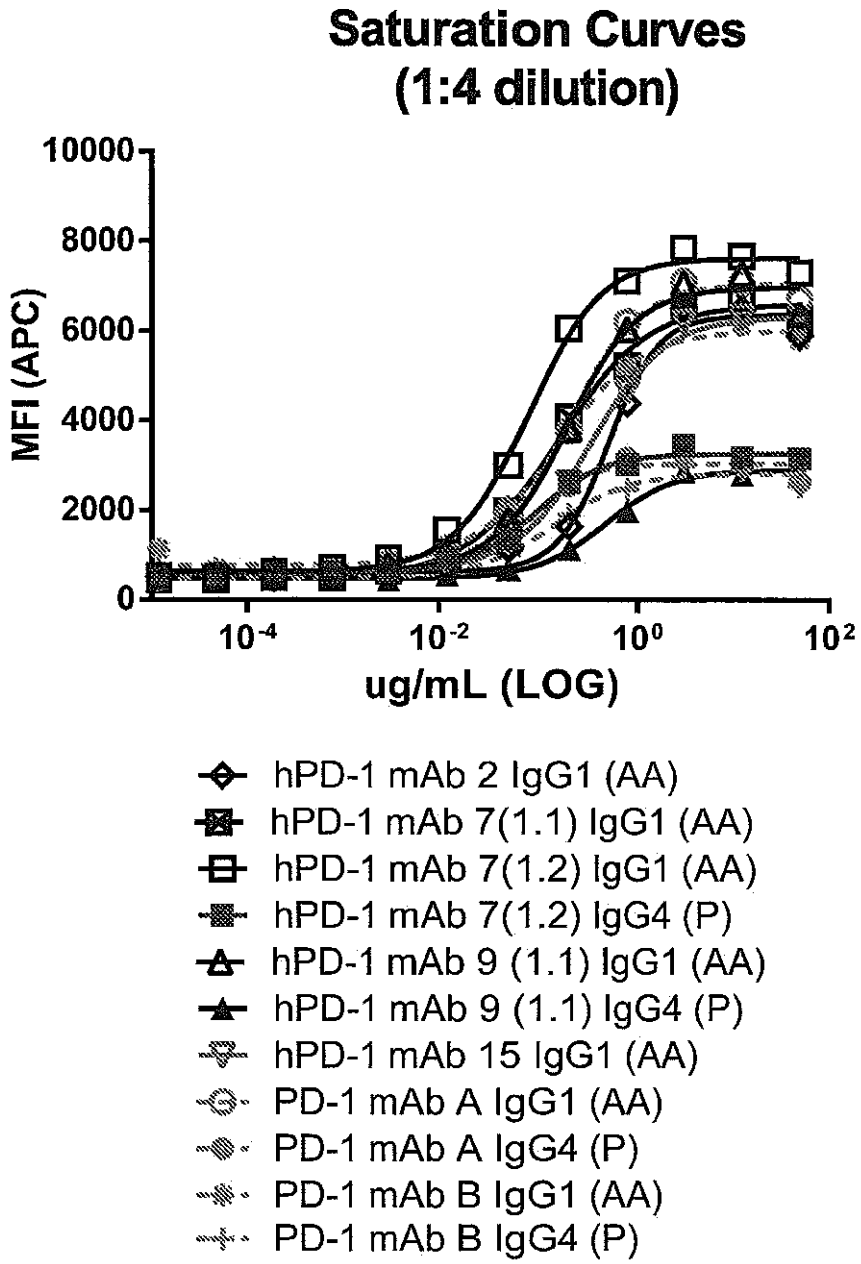
Saturation Curves
(1:4 dilution)
10000
8000
6000
4000
2000
0
MFI (APC)
10-4
10-2
100
102
ug/mL (LOG)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1 (AA)
PD-1 mAb A IgG4 (P)
PD-1 mAb B IgG1 (AA)
PD-1 mAb B IgG4 (P)

Figure 11

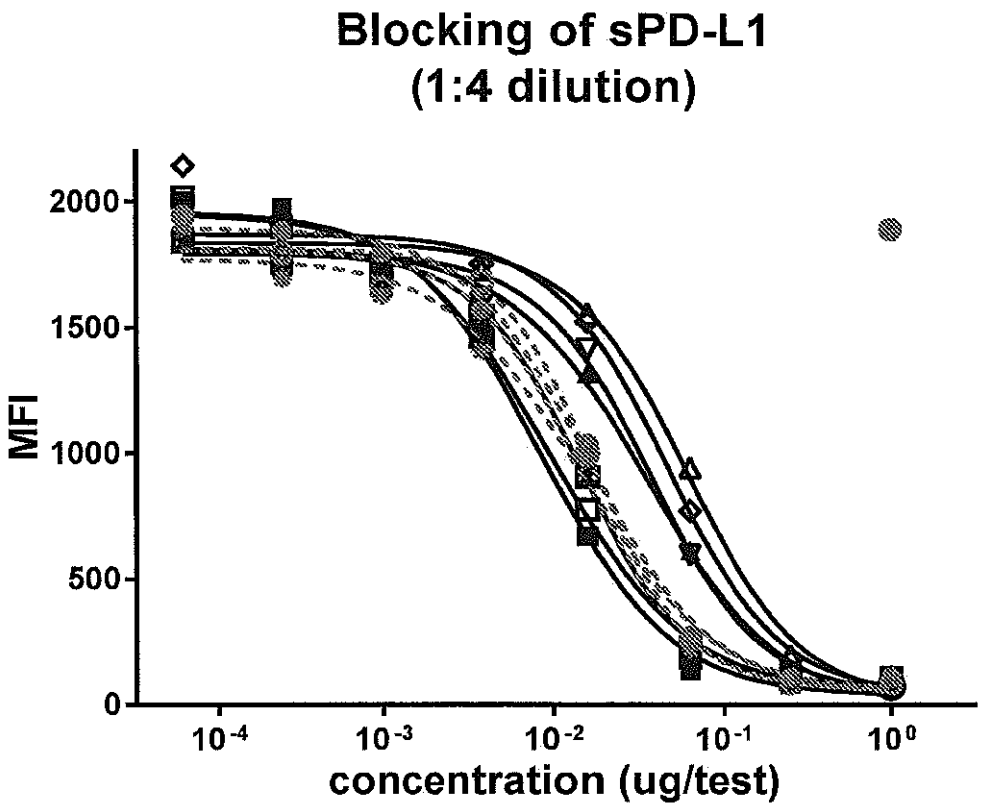
Blocking of sPD-L1
(1:4 dilution)
MFI
2000
1500
1000
500
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
concentration (ug/test)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1(AA)
PD-1 mAb A IgG4 (P)
PD-1 mAb B IgG1 (AA)
PD-1 mAb B IgG4 (P)
PDL1 only
Unstained

Figure 12A

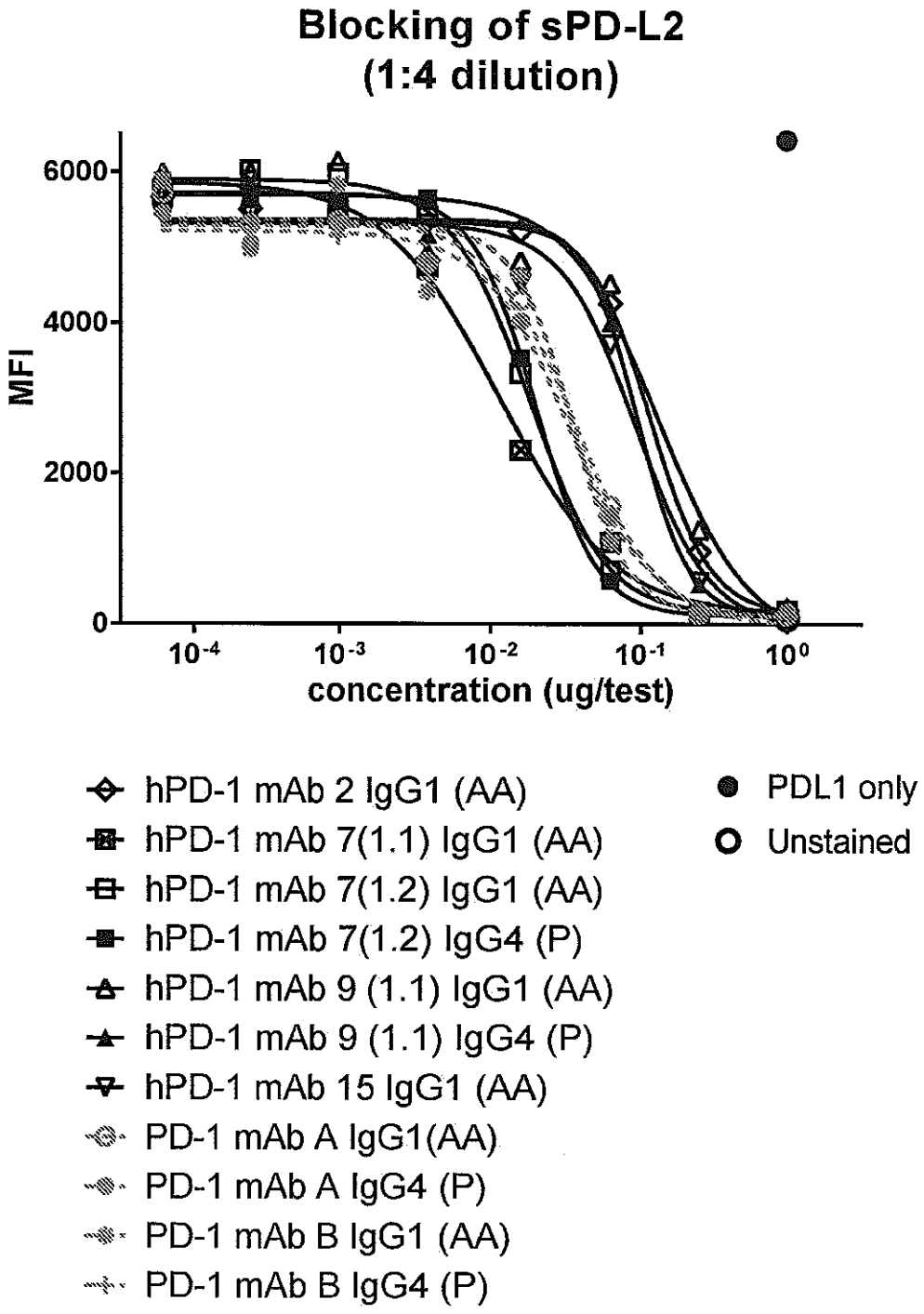
Blocking of sPD-L2
(1:4 dilution)
6000
4000
2000
0
MFI
10-4
10-3
10-2
10-1
100
concentration (ug/test)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1(AA)
PD-1 mAb A IgG4 (P)
PD-1 mAb B IgG1 (AA)
PD-1 mAb B IgG4 (P)
PDL1 only
Unstained

Figure 12B

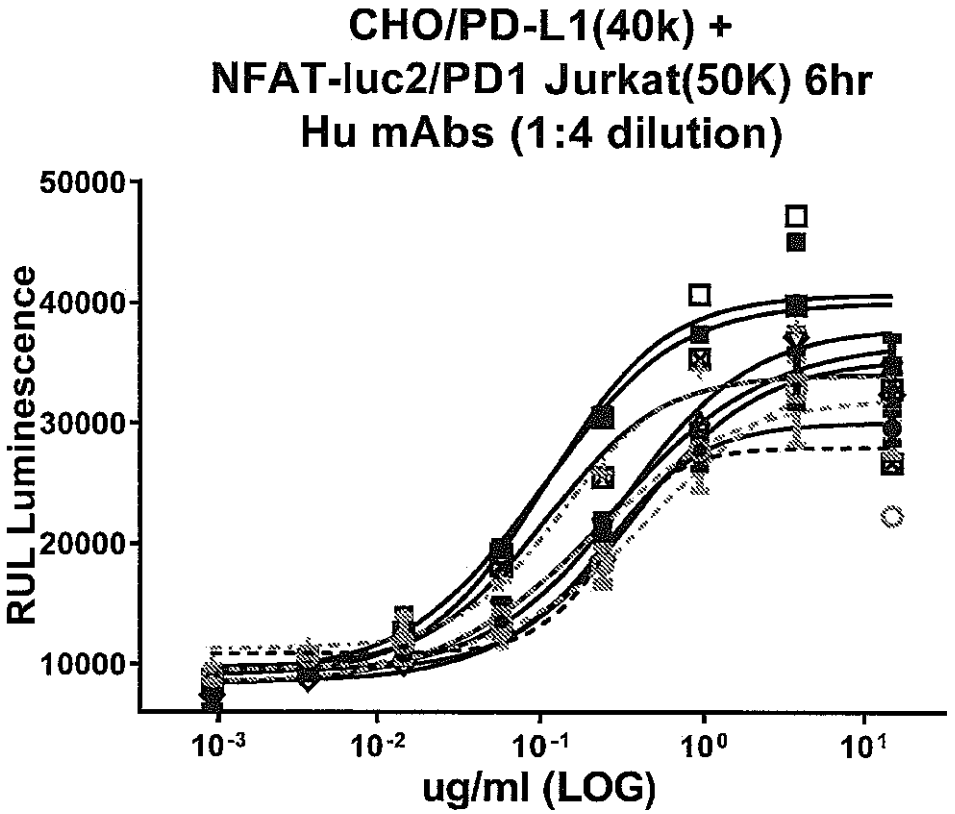
CHO/PD-L1(40k) +
NFAT-luc2/PD1 Jurkat(50K) 6hr
Hu mAbs (1:4 dilution)
RUL Luminescence
50000
40000
30000
20000
10000
10-3
10-2
10-1
100
101
ug/ml (LOG)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1(AA)
PD-1 mAb A IgG4 (P)
PD-1 mAb B IgG1 (AA)
PD-1 mAb B IgG4 (P)

Figure 13

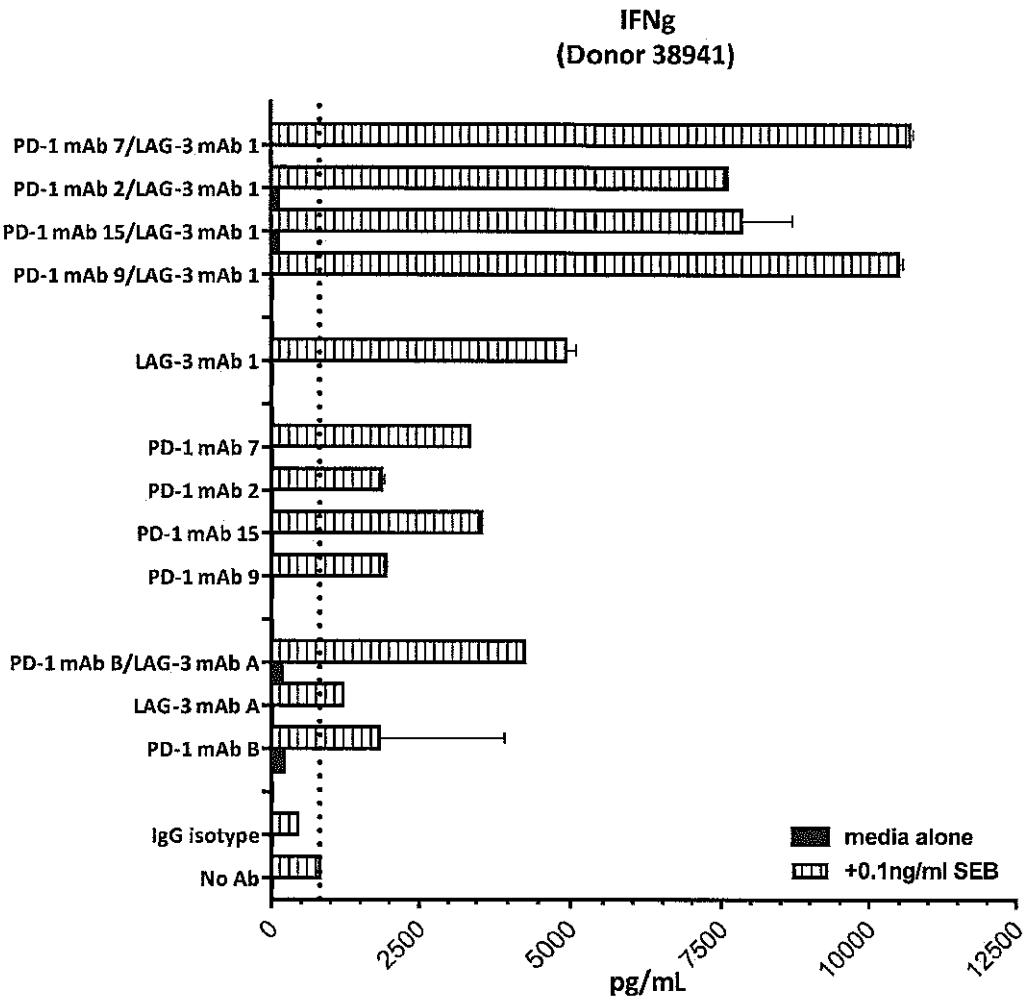
IFNg
(Donor 38941)
PD-1 mAb 7/LAG-3 mAb 1
PD-1 mAb 2/LAG-3 mAb 1
PD-1 mAb 15/LAG-3 mAb 1
PD-1 mAb 9/LAG-3 mAb 1
LAG-3 mAb 1
PD-1 mAb 7
PD-1 mAb 2
PD-1 mAb 15
PD-1 mAb 9
PD-1 mAb B/LAG-3 mAb A
LAG-3 mAb A
PD-1 mAb B
IgG isotype
No Ab
media alone
+0.1ng/ml SEB
0
2500
5000
7500
10000
12500
pg/mL

Figure 14

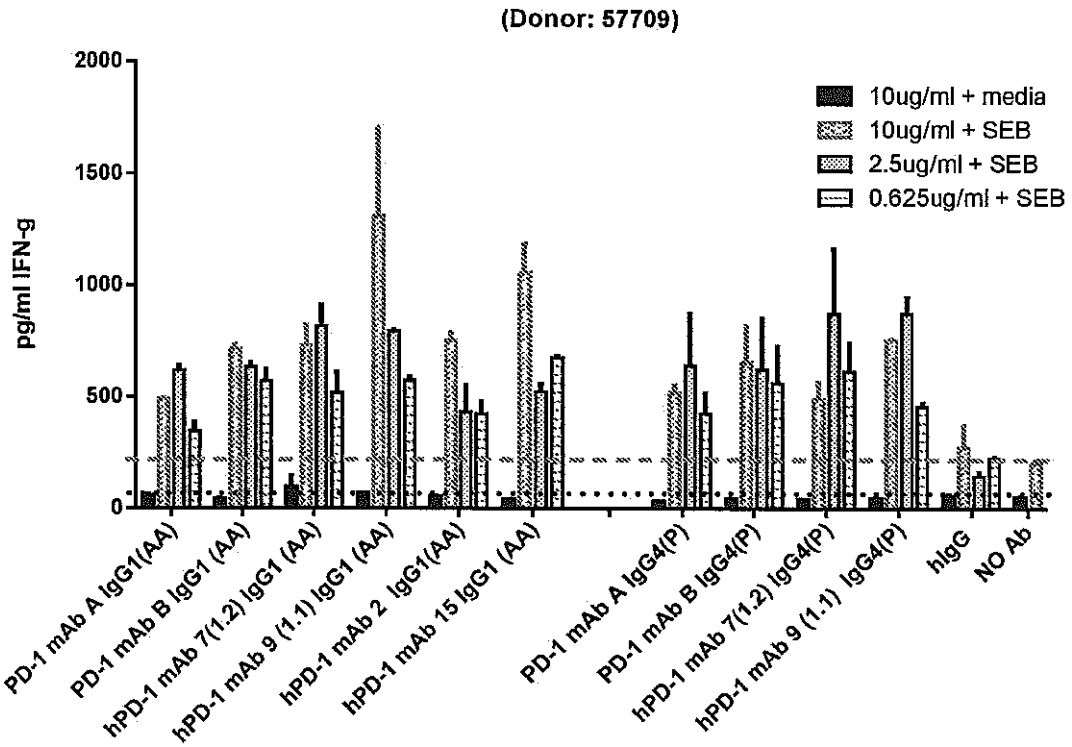
(Donor: 57709)
2000
1500
1000
500
0
pg/ml IFN-g
10ug/ml + media
10ug/ml + SEB
2.5ug/ml + SEB
0.625ug/ml + SEB
PD-1 mAb A IgG1(AA)
PD-1 mAb B IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 2 IgG1(AA)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG4(P)
PD-1 mAb B IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
hPD-1 mAb 9 (1.1) IgG4(P)
hIgG
NO Ab

Figure 15A

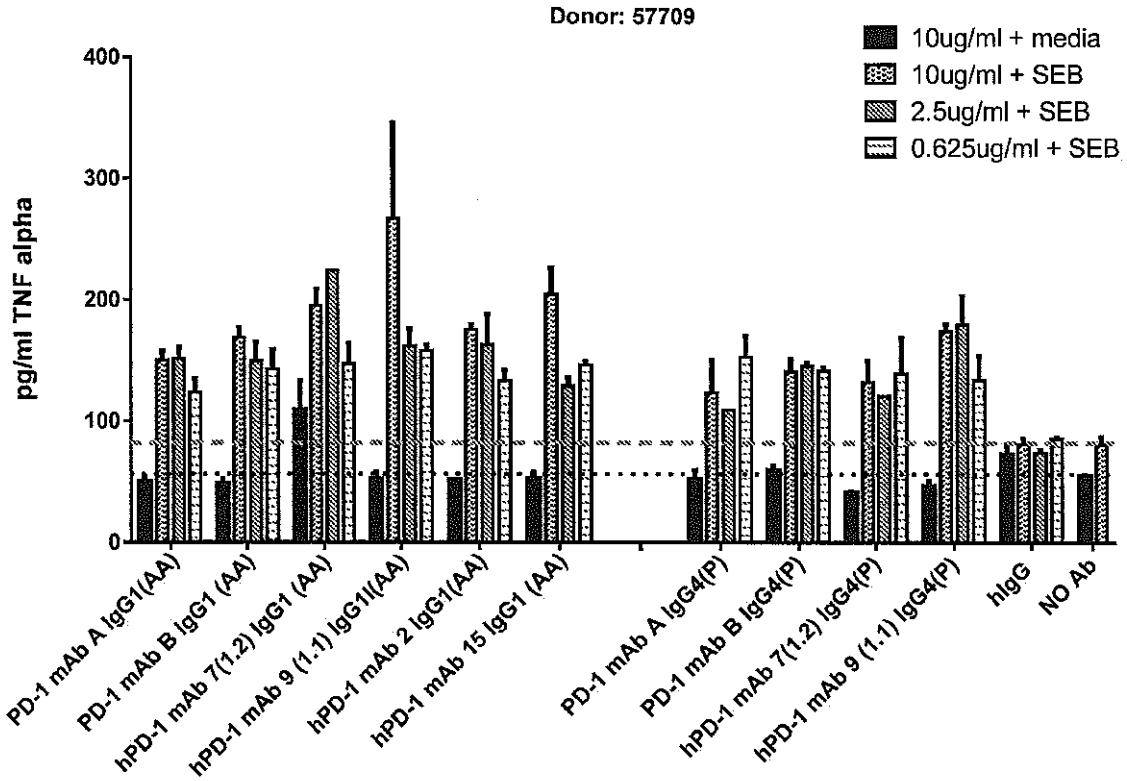
Donor: 57709
10ug/ml + media
10ug/ml + SEB
2.5ug/ml + SEB
0.625ug/ml + SEB
pg/ml TNF alpha
400
300
200
100
0
PD-1 mAb A IgG1(AA)
PD-1 mAb B igG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG1l(AA)
hPD-1 mAb 2 IgG1(AA)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG4(P)
PD-1 mAb B IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
hPD-1 mAb 9 (1.1) IgG4(P)
hIgG
NO Ab

Figure 15B

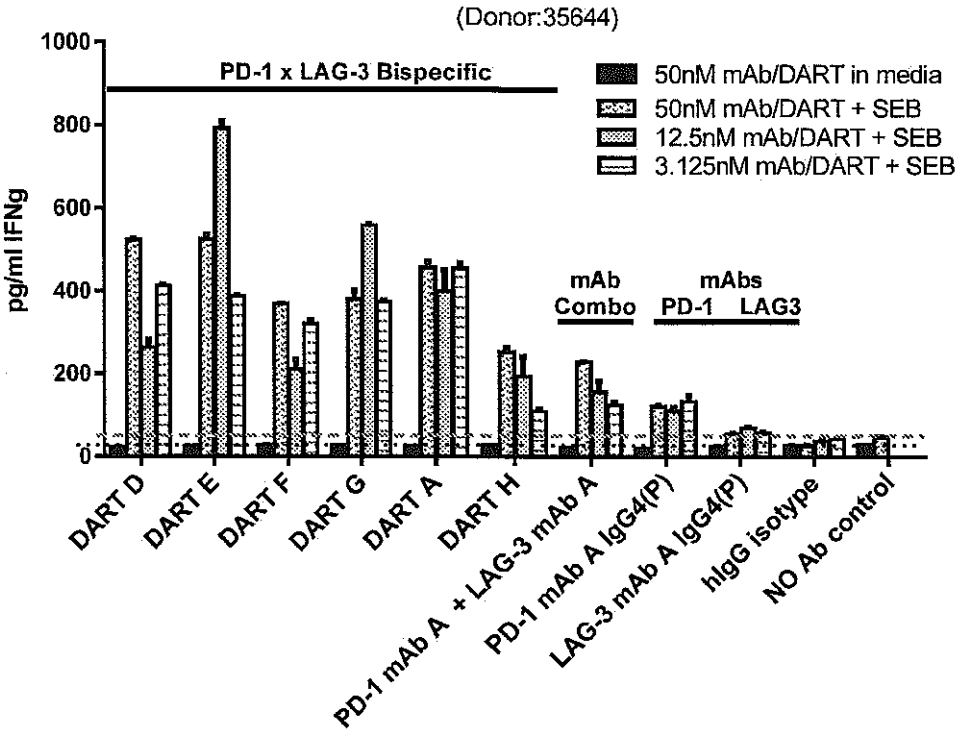

Figure 16A

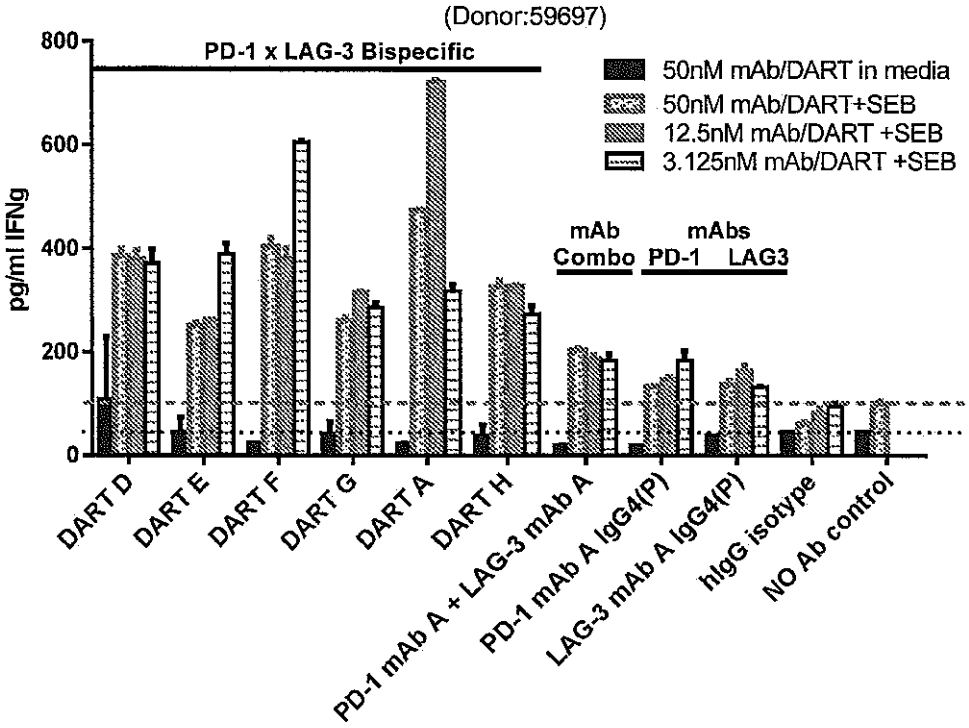

Figure 16B

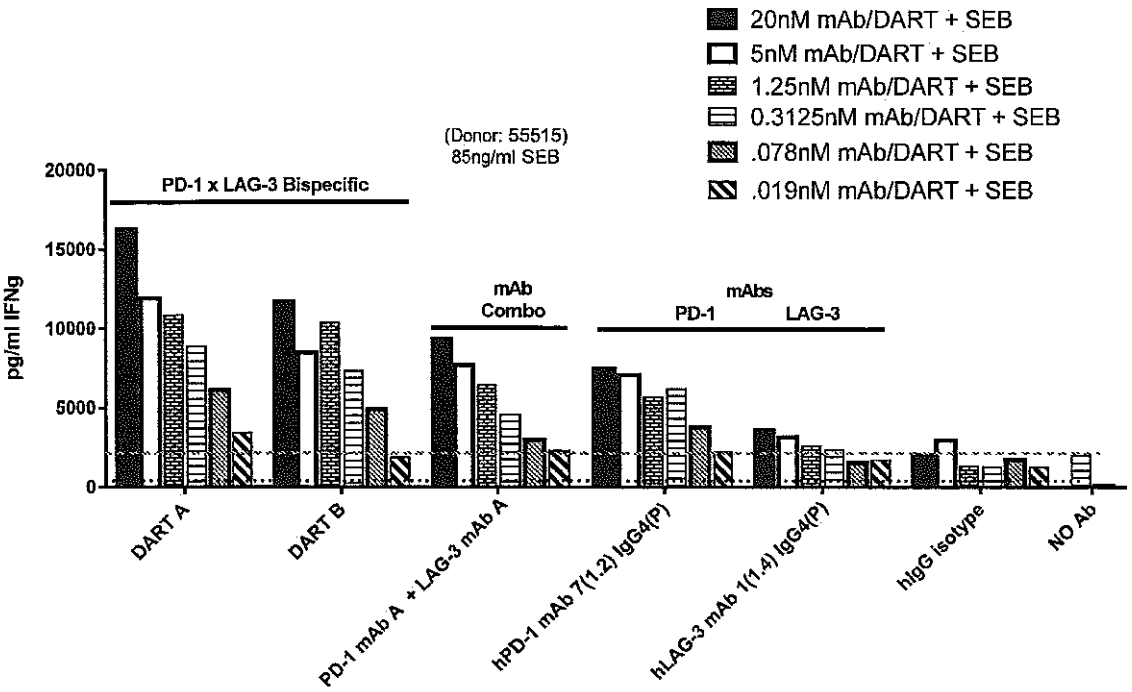

Figure 17A

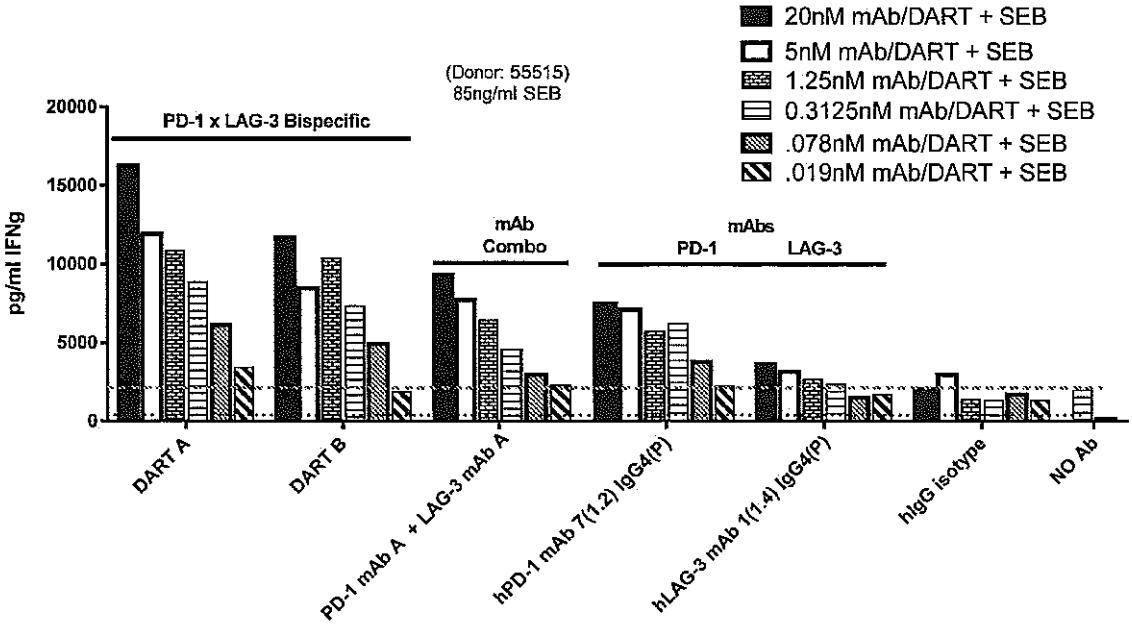

Figure 17B

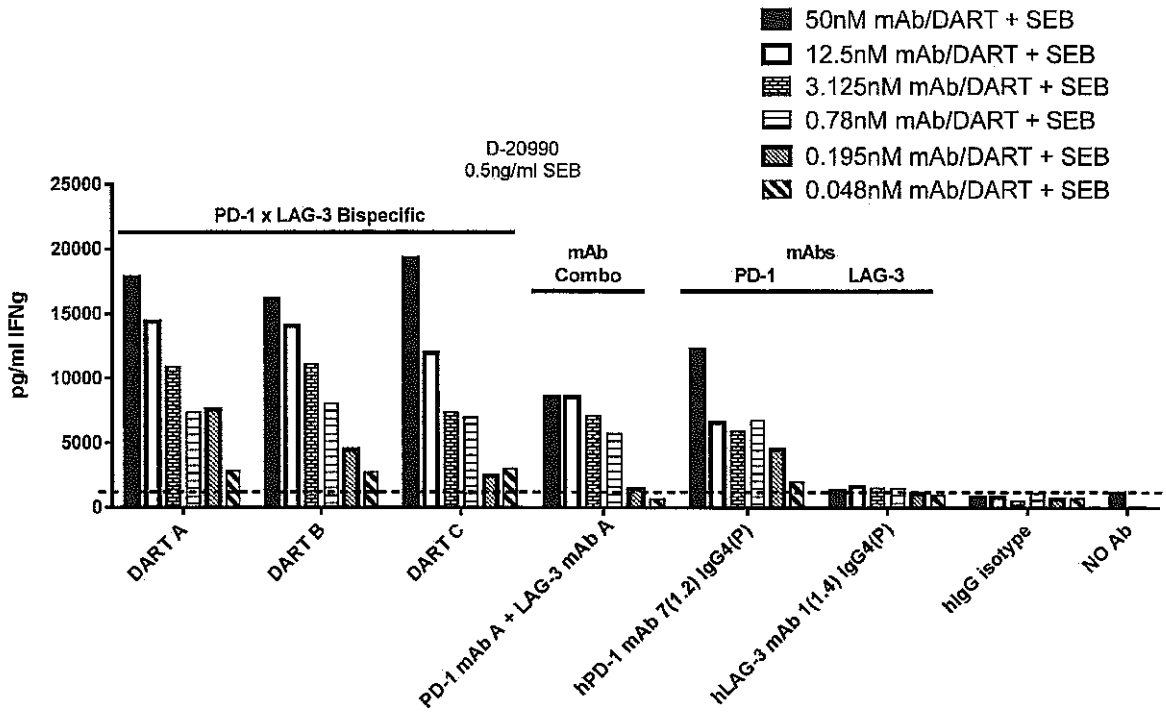
50nM mAb/DART + SEB
12.5nM mAb/DART + SEB
3.125nM mAb/DART + SEB
0.78nM mAb/DART + SEB
0.195nM mAb/DART + SEB
0.048nM mAb/DART + SEB
D-20990
0.5ng/ml SEB
PD-1 x LAG-3 Bispecific
mAb
Combo
mAbs
PD-1
LAG-3
pg/ml IFNg
25000
20000
15000
10000
5000
0
DART A
DART B
DART C
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2) IgG4(P)
hLAG-3 mAb 1(1.4) IgG4(P)
hIgG isotype
NO Ab

Figure 18A

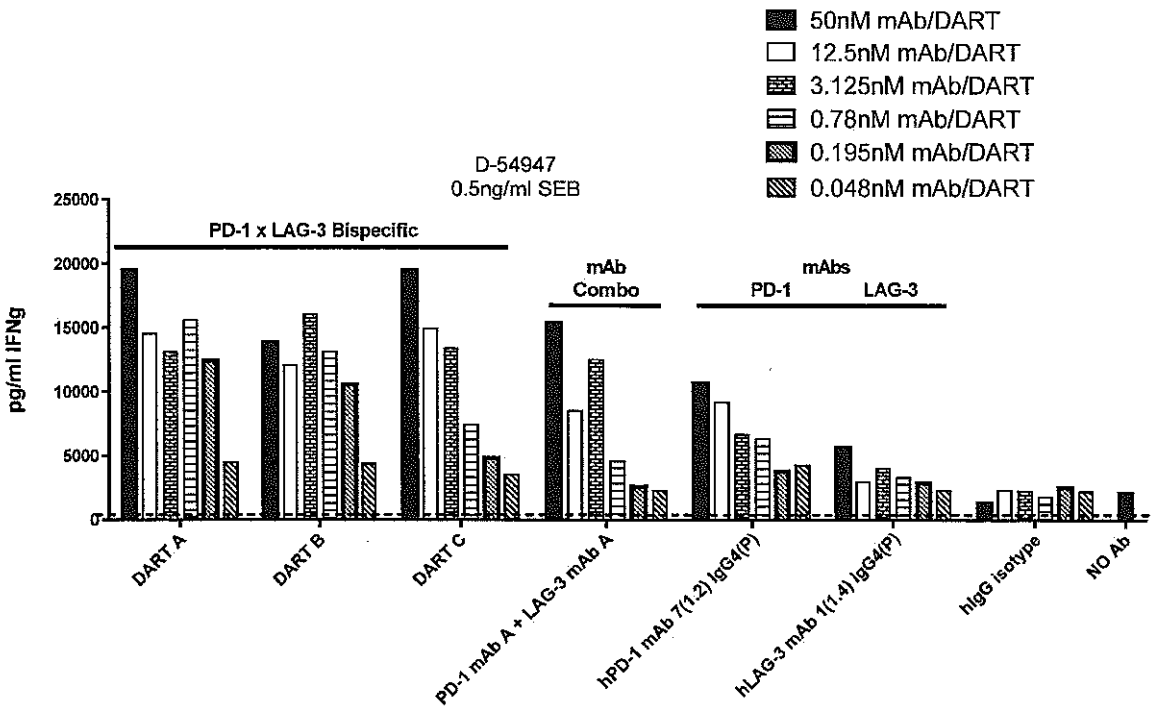
50nM mAb/DART
12.5nM mAb/DART
3.125nM mAb/DART
0.78nM mAb/DART
0.195nM mAb/DART
0.048nM mAb/DART
D-54947
0.5ng/ml SEB
PD-1 x LAG-3 Bispecific
mAb Combo
mAbs
PD-1
LAG-3
pg/ml IFNg
25000
20000
15000
10000
5000
0
DART A
DART B
DART C
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2) IgG4(P)
hLAG-3 mAb 1(1.4) IgG4(P)
hIgG isotype
NO Ab

Figure 18B

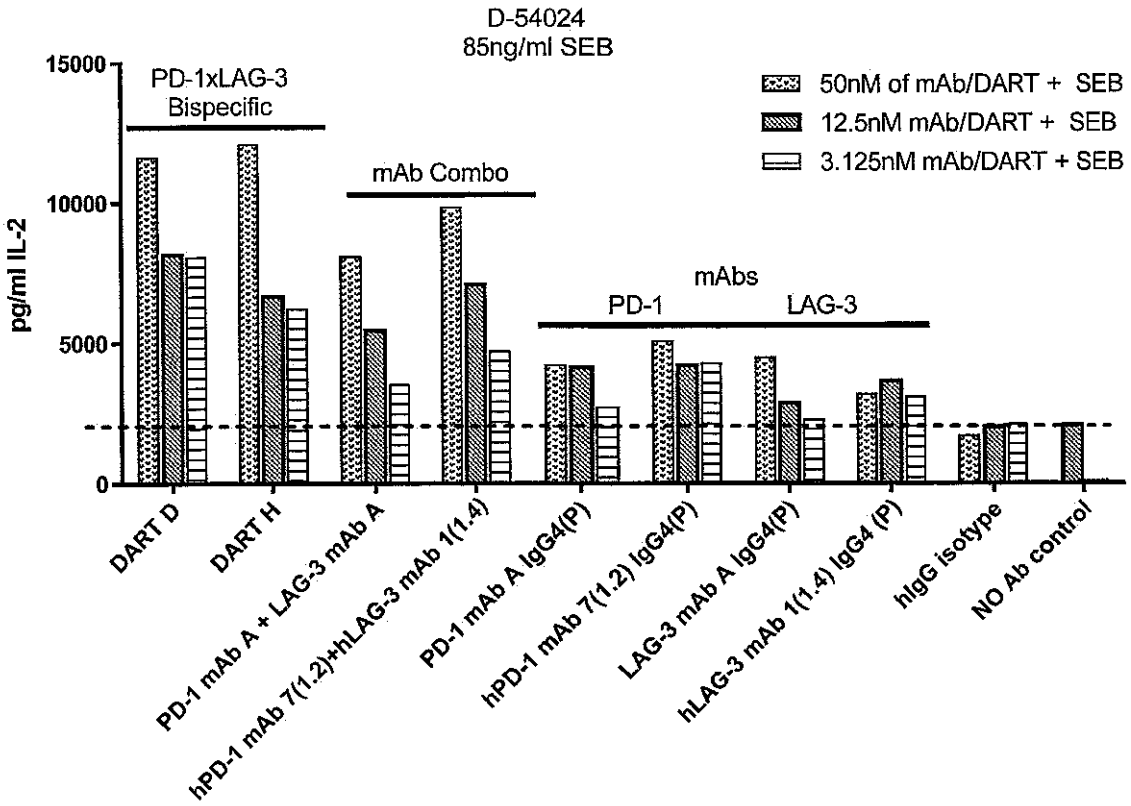
D-54024
85ng/ml SEB
50nM of mAb/DART + SEB
12.5nM mAb/DART + SEB
3.125nM mAb/DART + SEB
pg/ml IL-2
15000
10000
5000
0
PD-1xLAG-3 Bispecific
mAb Combo
mAbs
PD-1
LAG-3
DART D
DART H
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2)+hLAG-3 mAb 1(1.4)
PD-1 mAb A IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
LAG-3 mAb A IgG4(P)
hLAG-3 mAb 1(1.4) IgG4 (P)
hIgG isotype
NO Ab control

Figure 19

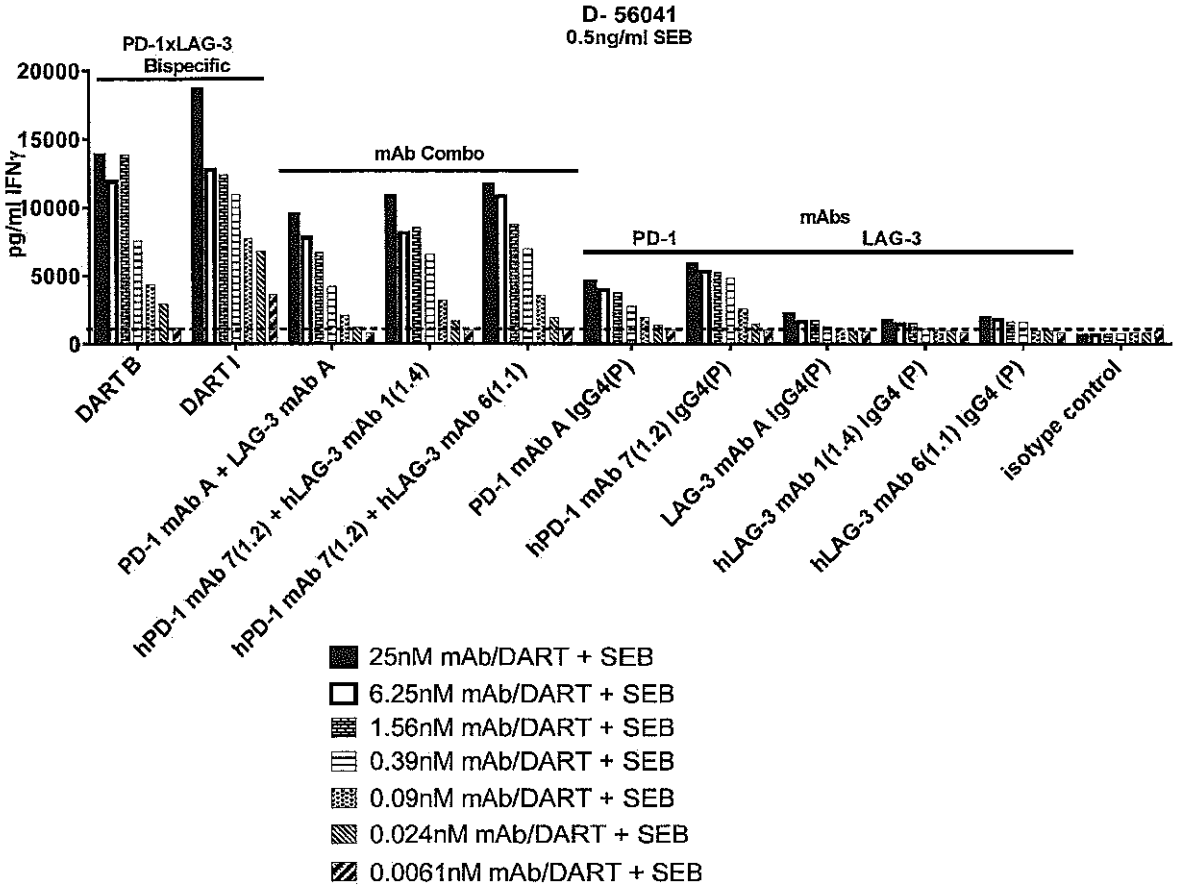
D- 56041
0.5ng/ml SEB
PD-1xLAG-3
Bispecific
mAb Combo
mAbs
PD-1
LAG-3
pg/ml IFNγ
20000
15000
10000
5000
0
DART B
DART I
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2) + hLAG-3 mAb 1(1.4)
hPD-1 mAb 7(1.2) + hLAG-3 mAb 6(1.1)
PD-1 mAb A IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
LAG-3 mAb A IgG4(P)
hLAG-3 mAb 1(1.4) IgG4 (P)
hLAG-3 mAb 6(1.1) IgG4 (P)
isotype control
25nM mAb/DART + SEB
6.25nM mAb/DART + SEB
1.56nM mAb/DART + SEB
0.39nM mAb/DART + SEB
0.09nM mAb/DART + SEB
0.024nM mAb/DART + SEB
0.0061nM mAb/DART + SEB

Figure 20

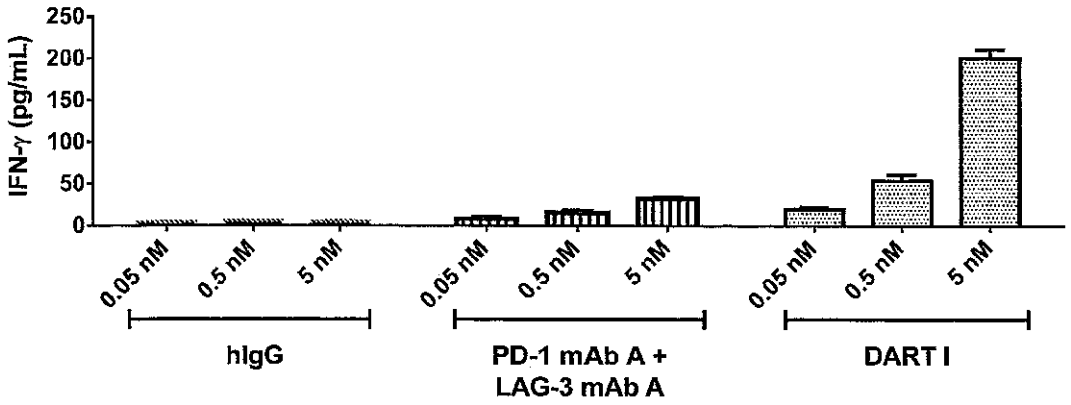

Figure 21A

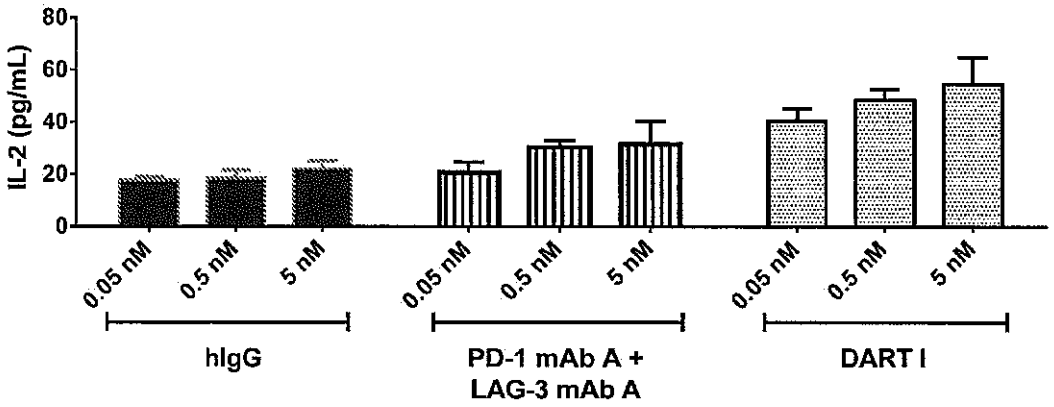

Figure 21B

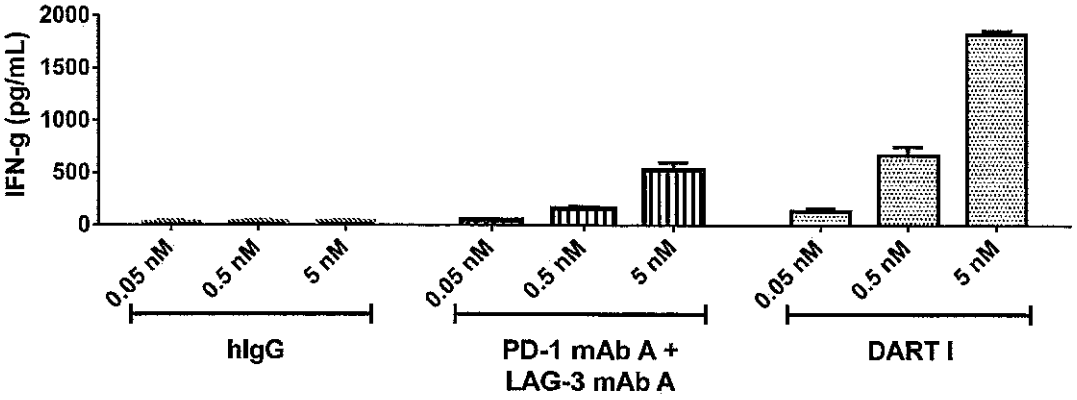

Figure 21C

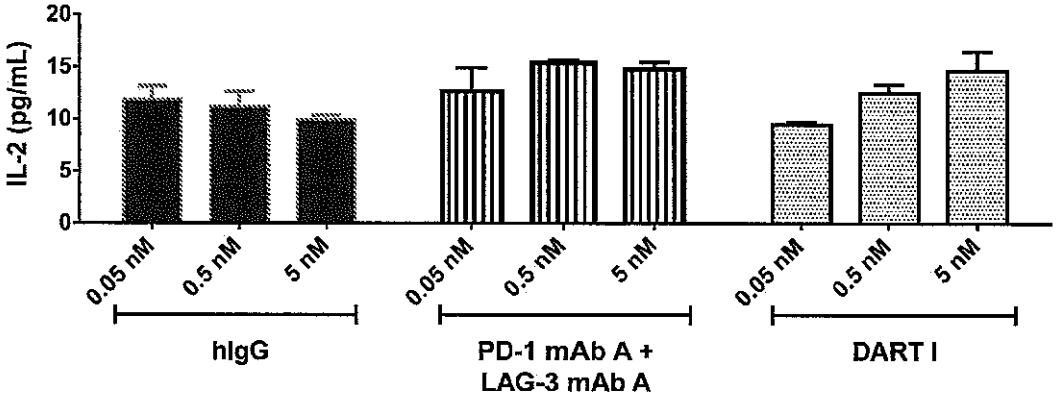

Figure 21D

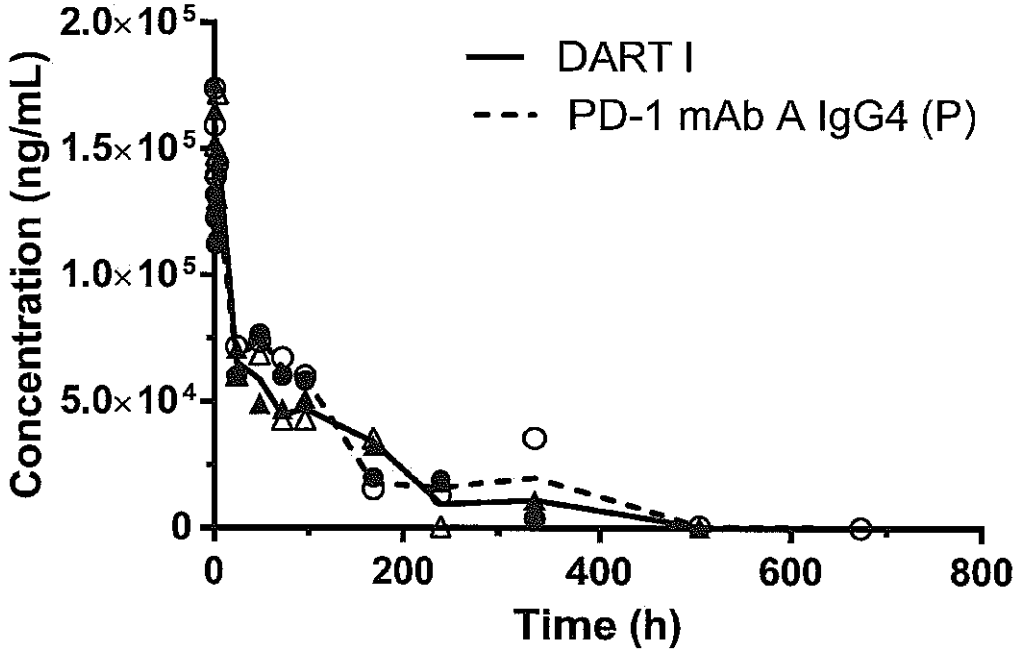
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0
Concentration (ng/mL)
DART I
PD-1 mAb A IgG4 (P)
0
200
400
600
800
Time (h)

Figure 22

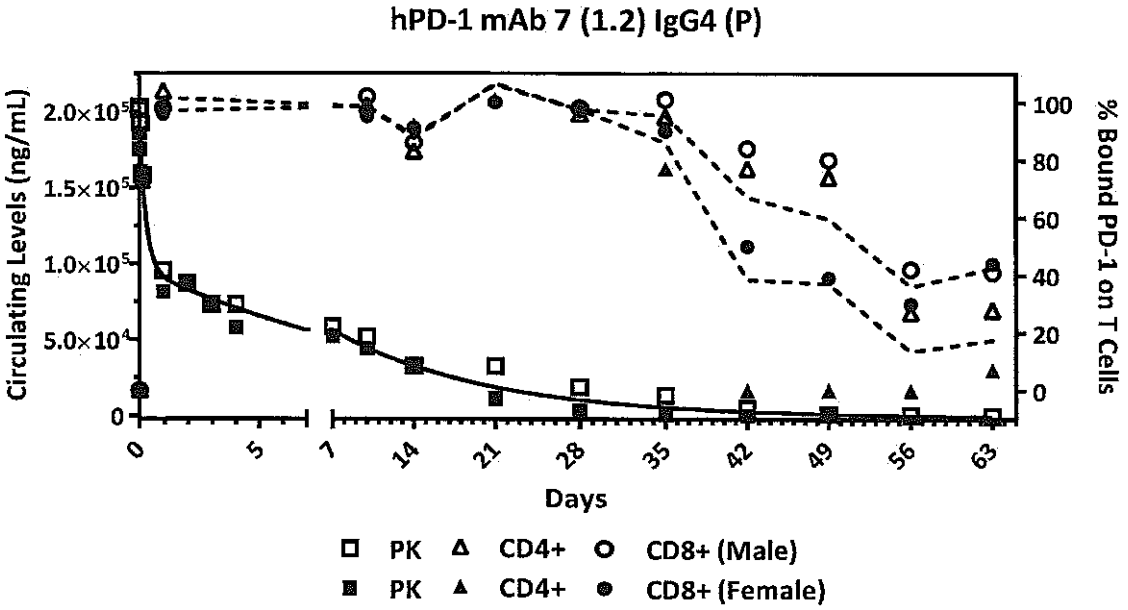

Figure 23A

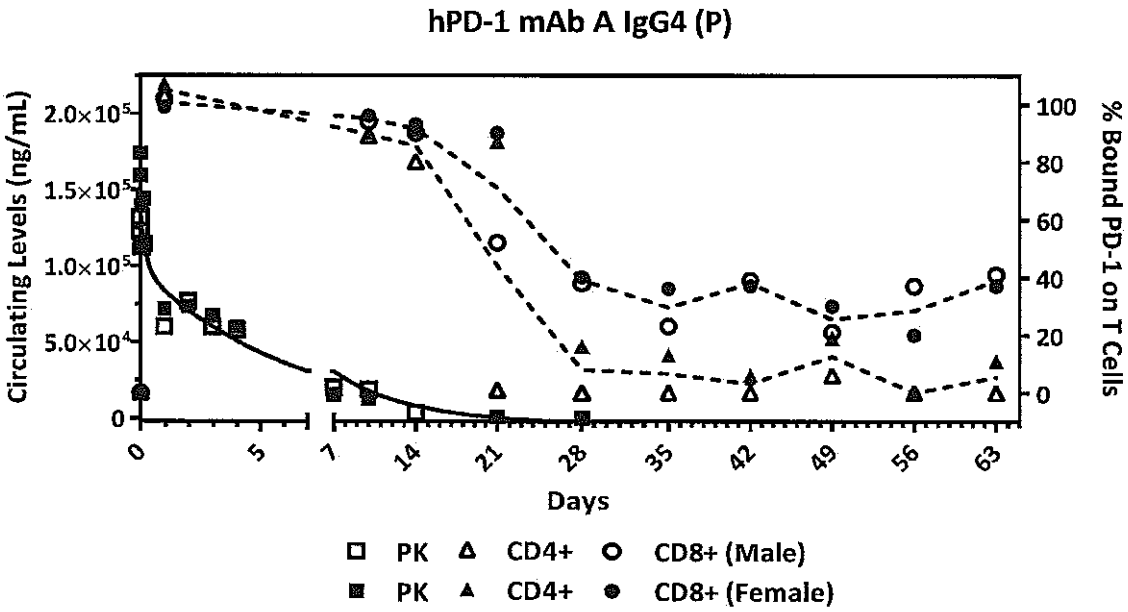

Figure 23B

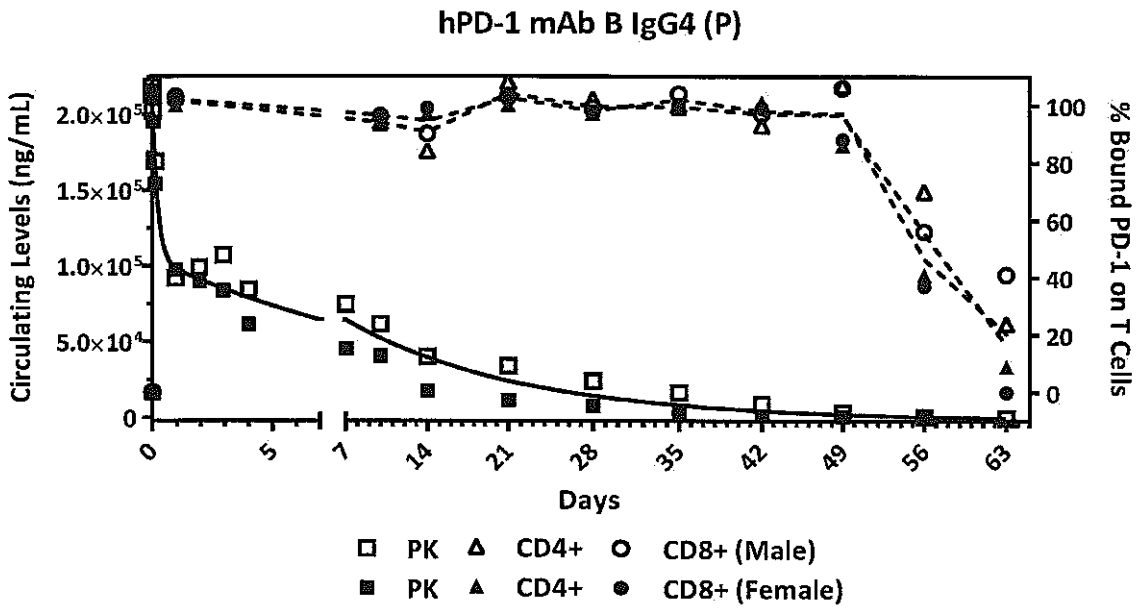
hPD-1 mAb B IgG4 (P)
Circulating Levels (ng/mL)
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0
% Bound PD-1 on T Cells
100
80
60
40
20
0
0
5
7
14
21
28
35
42
49
56
63
Days
PK
CD4+
CD8+ (Male)
PK
CD4+
CD8+ (Female)

Figure 23C