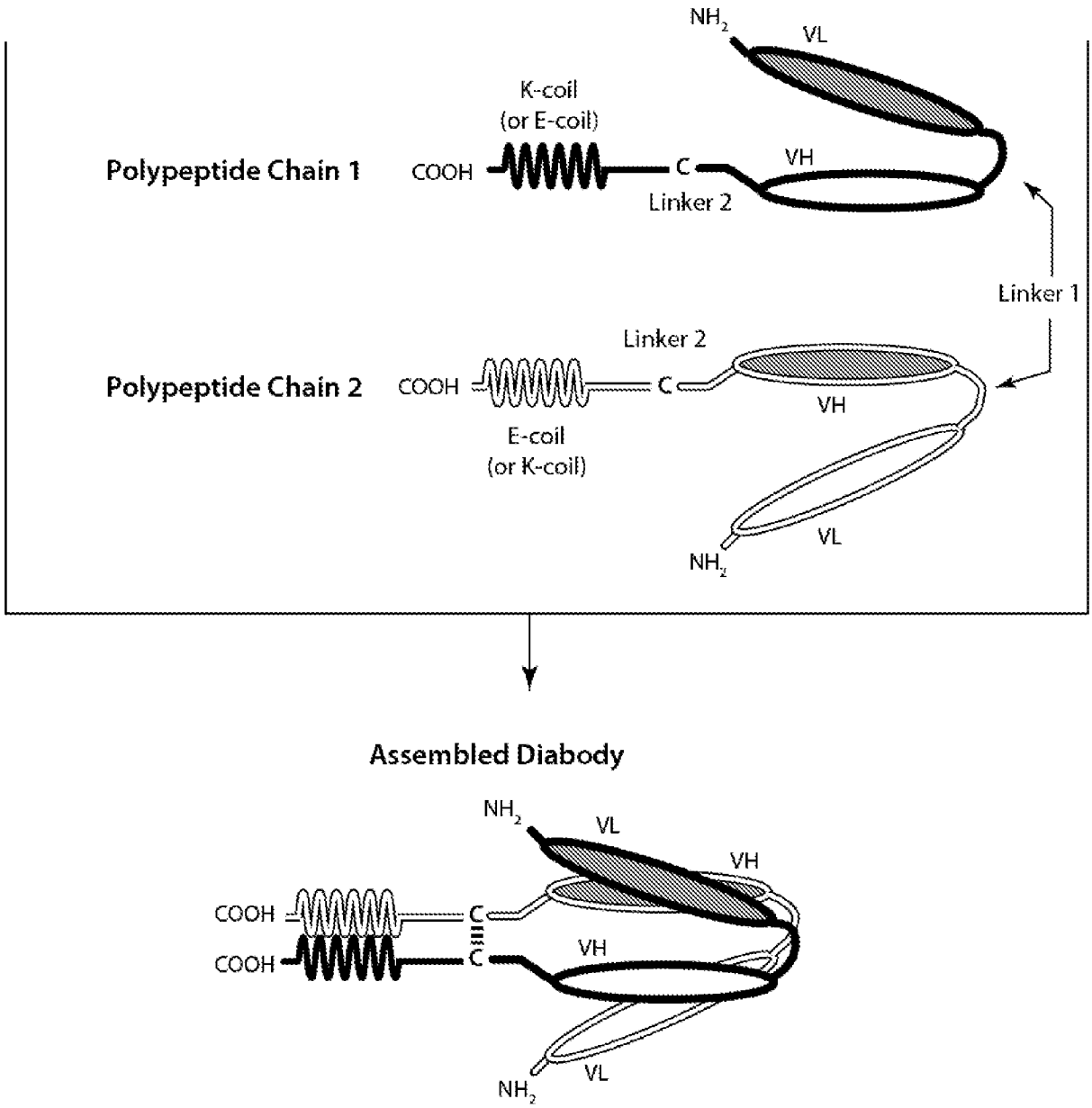
NH2
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
C
VH
Linker 2
Linker 1
Linker 2
Polypeptide Chain 2
COOH
C
VH
E-coil
(or K-coil)
VL
NH2
Assembled Diabody
NH2
VL
VH
COOH
C
COOH
C
VH
VL
NH2

Figure 1

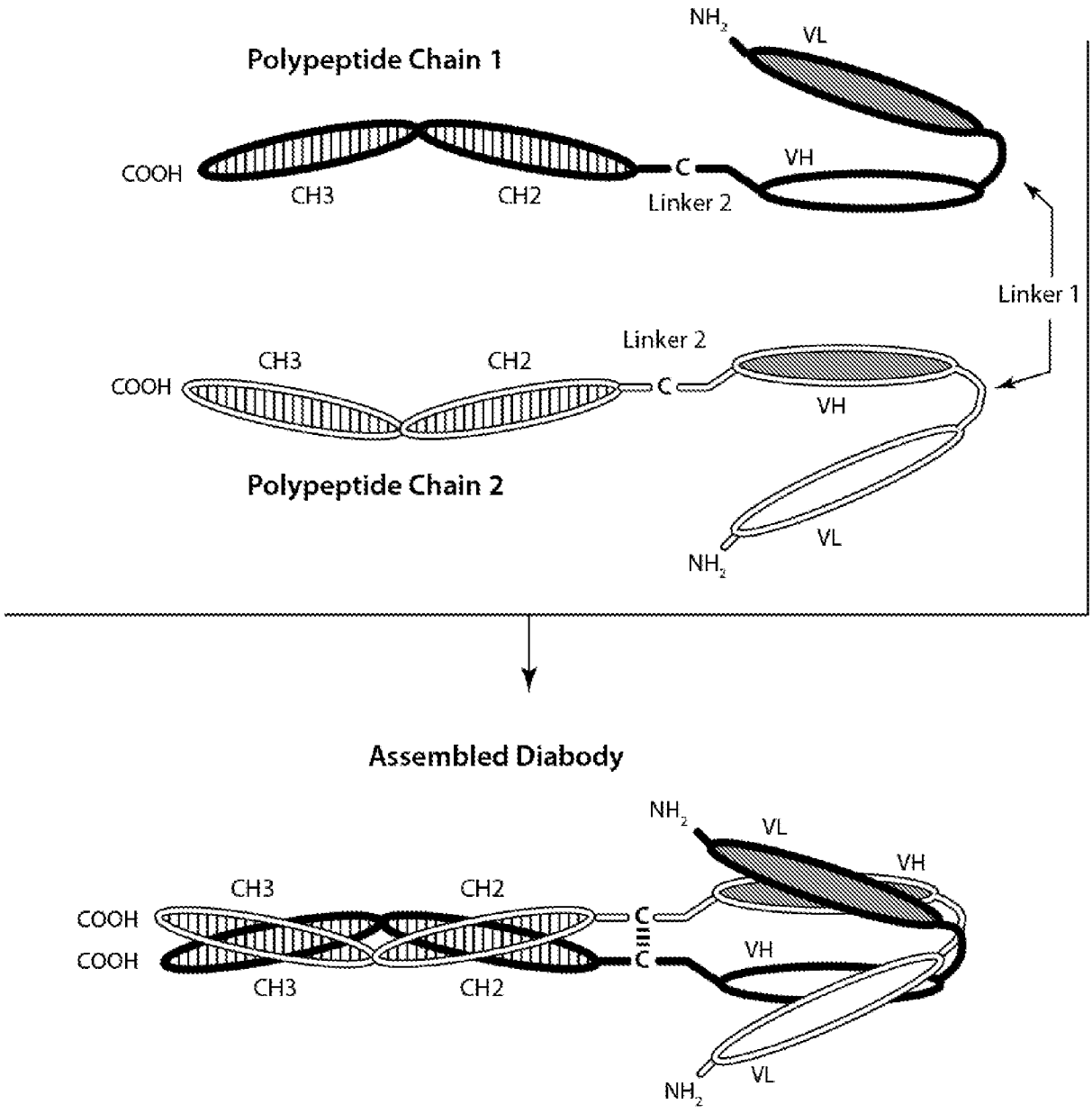
Polypeptide Chain 1
NH2
VL
COOH
CH3
CH2
C
Linker 2
VH
Linker 1
Linker 2
CH3
CH2
COOH
C
VH
Polypeptide Chain 2
VL
NH2
Assembled Diabody
NH2
VL
VH
CH3
CH2
COOH
COOH
C
C
VH
CH3
CH2
VL
NH2

Figure 2

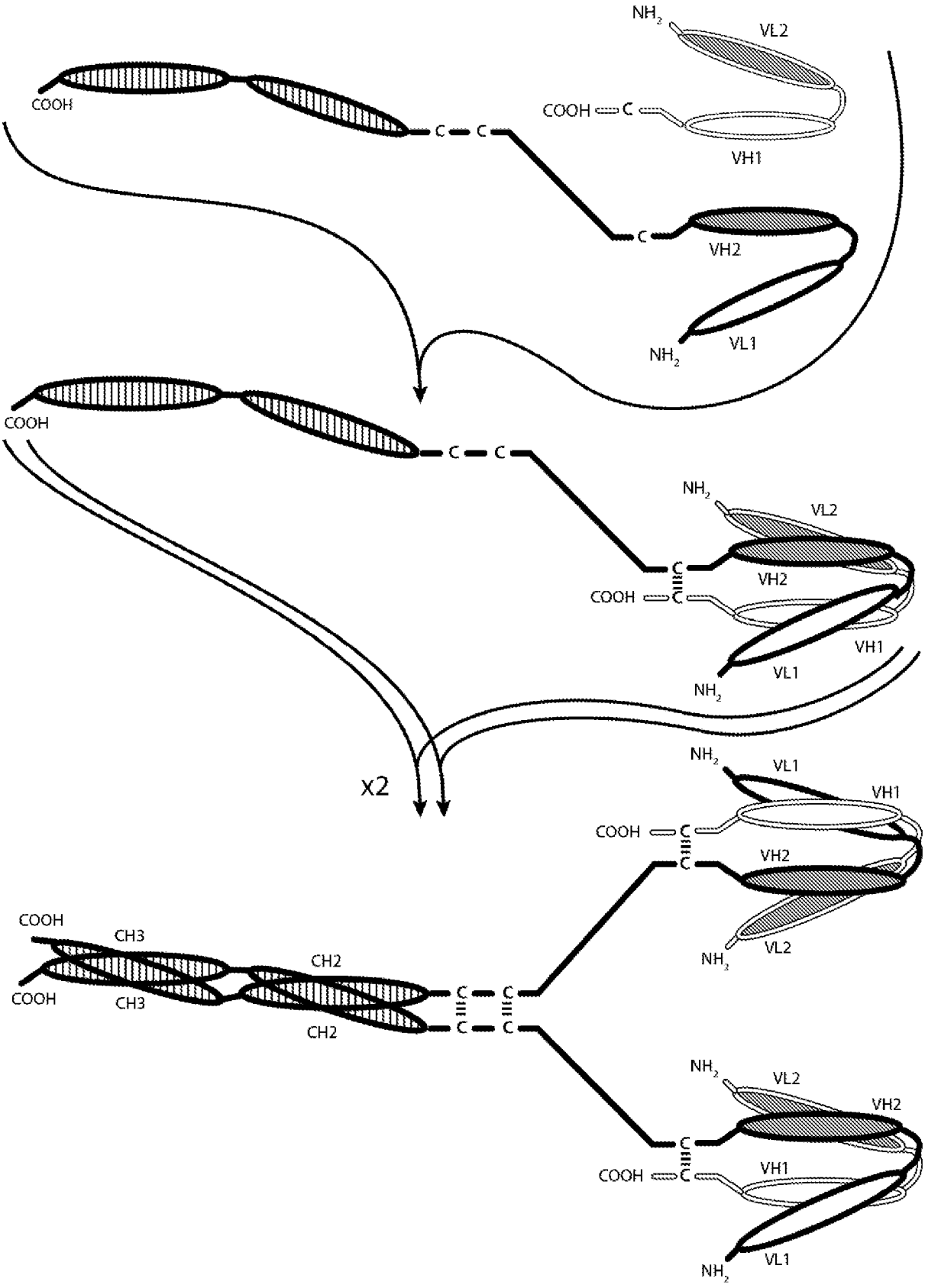
NH2
VL2
COOH
C
C
COOH
C
VH1
C
VH2
NH2
VL1
COOH
C
C
NH2
VL2
VH2
COOH
C
C
VH1
NH2
VL1
x2
NH2
VL1
VH1
COOH
C
C
VH2
NH2
VL2
COOH
CH3
CH2
COOH
CH3
CH2
C
C
C
C
NH2
VL2
VH2
COOH
C
C
VH1
NH2
VL1

Figure 3A

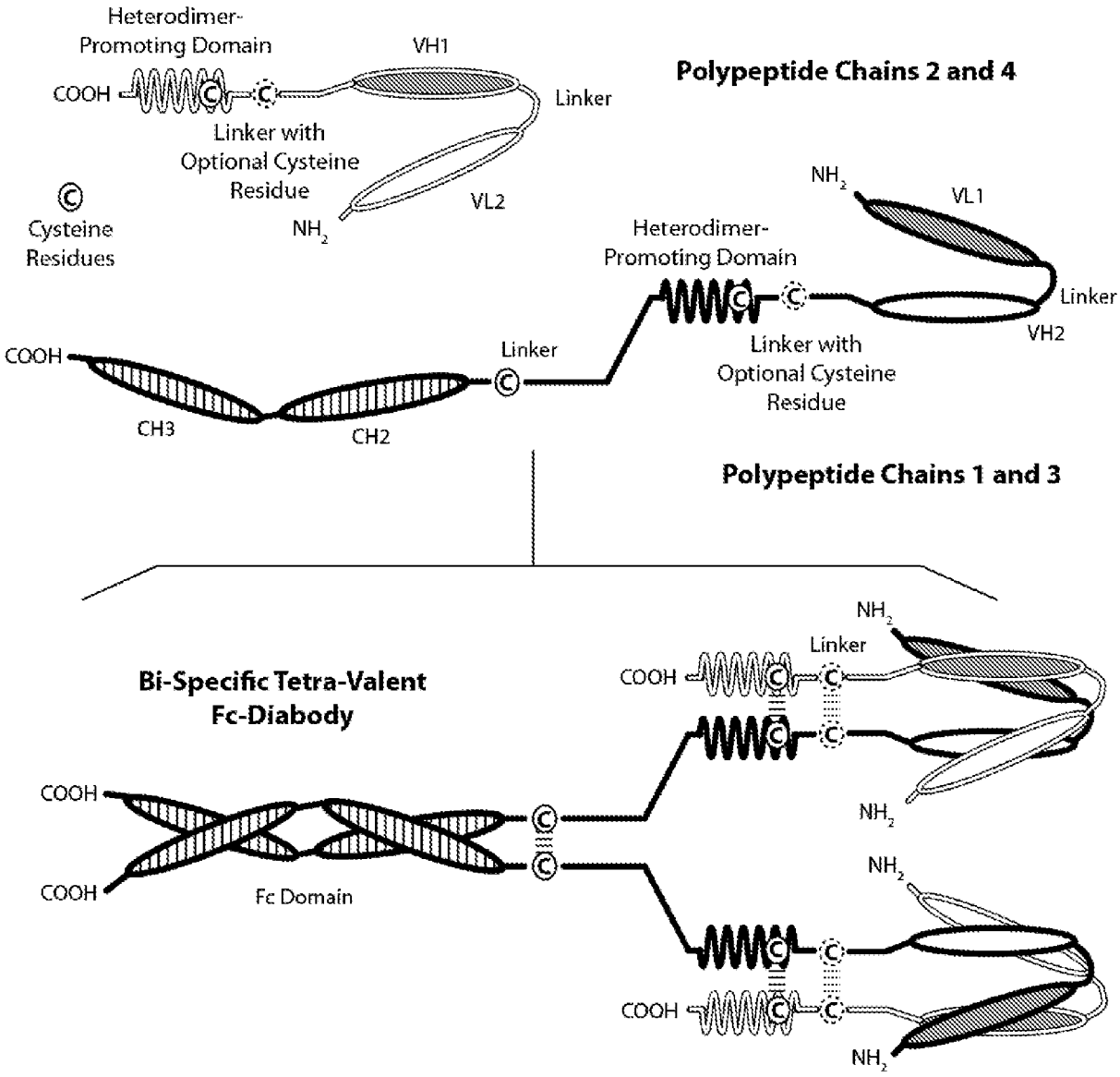
Heterodimer-
Promoting Domain
VH1
COOH
Linker
Linker with
Optional Cysteine
Residue
VL2
NH2
Cysteine
Residues
Polypeptide Chains 2 and 4
NH2
VL1
Heterodimer-
Promoting Domain
Linker
VH2
Linker with
Optional Cysteine
Residue
COOH
Linker
CH3
CH2
Polypeptide Chains 1 and 3
Bi-Specific Tetra-Valent
Fc-Diabody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

Figure 3B

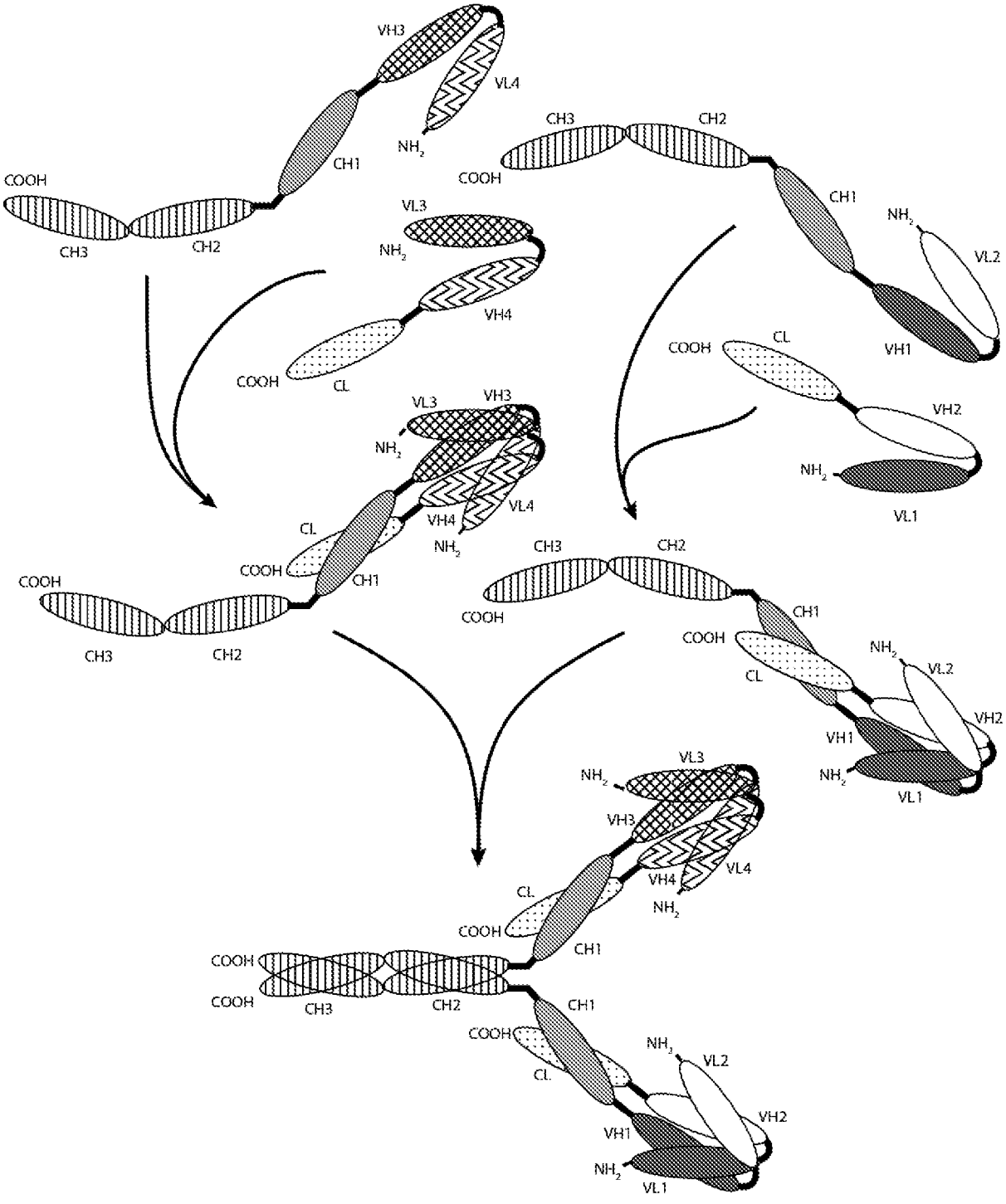

Figure 3C

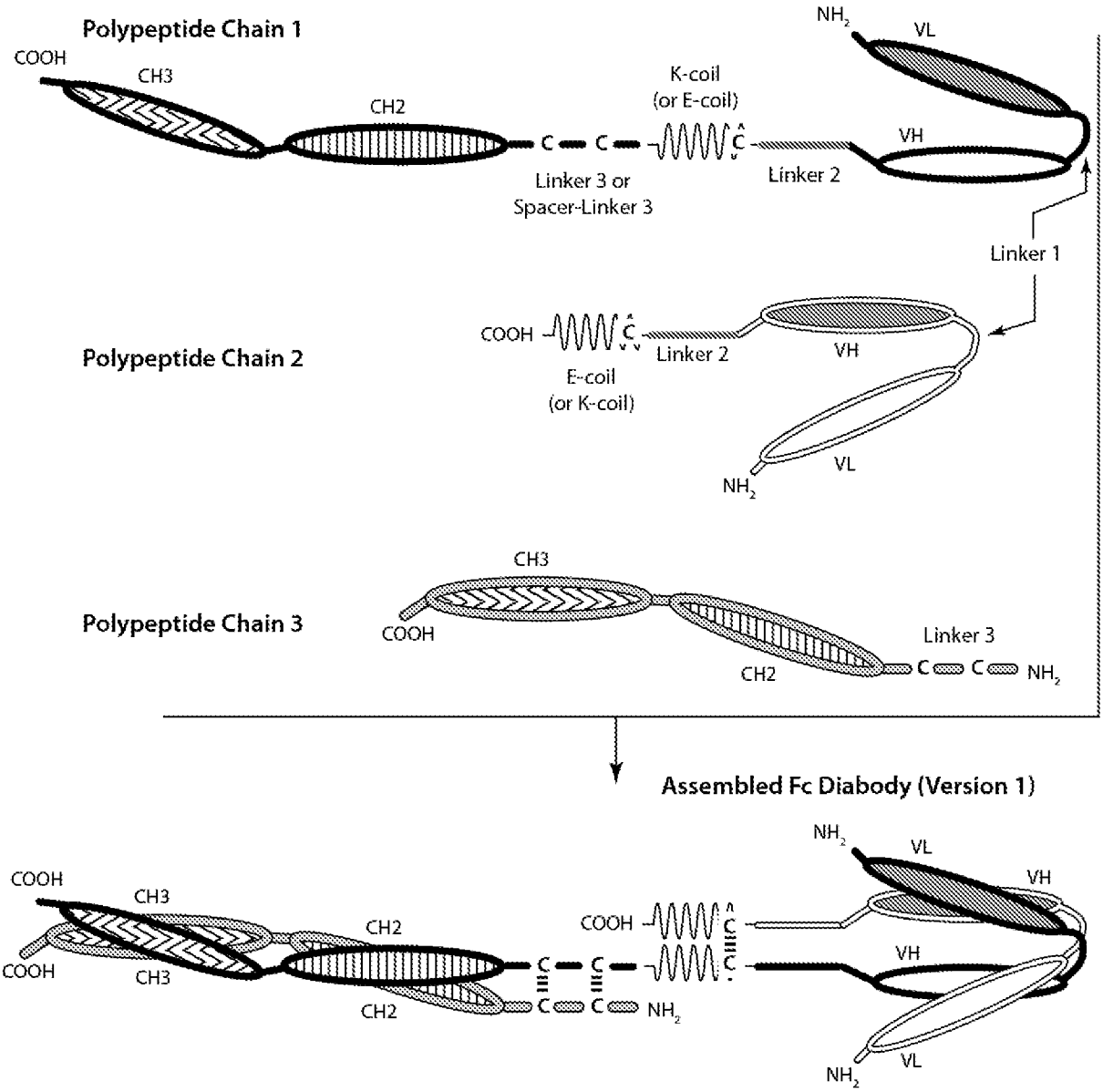
Polypeptide Chain 1
COOH
CH3
CH2
K-coil
(or E-coil)
NH2
VL
VH
Linker 3 or
Spacer-Linker 3
Linker 2
Linker 1
Polypeptide Chain 2
COOH
E-coil
(or K-coil)
Linker 2
VH
VL
NH2
Polypeptide Chain 3
CH3
COOH
Linker 3
CH2
NH2
Assembled Fc Diabody (Version 1)
COOH
CH3
CH2
COOH
CH3
CH2
NH2
NH2
VL
VH
COOH
VH
NH2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

Figure 4A

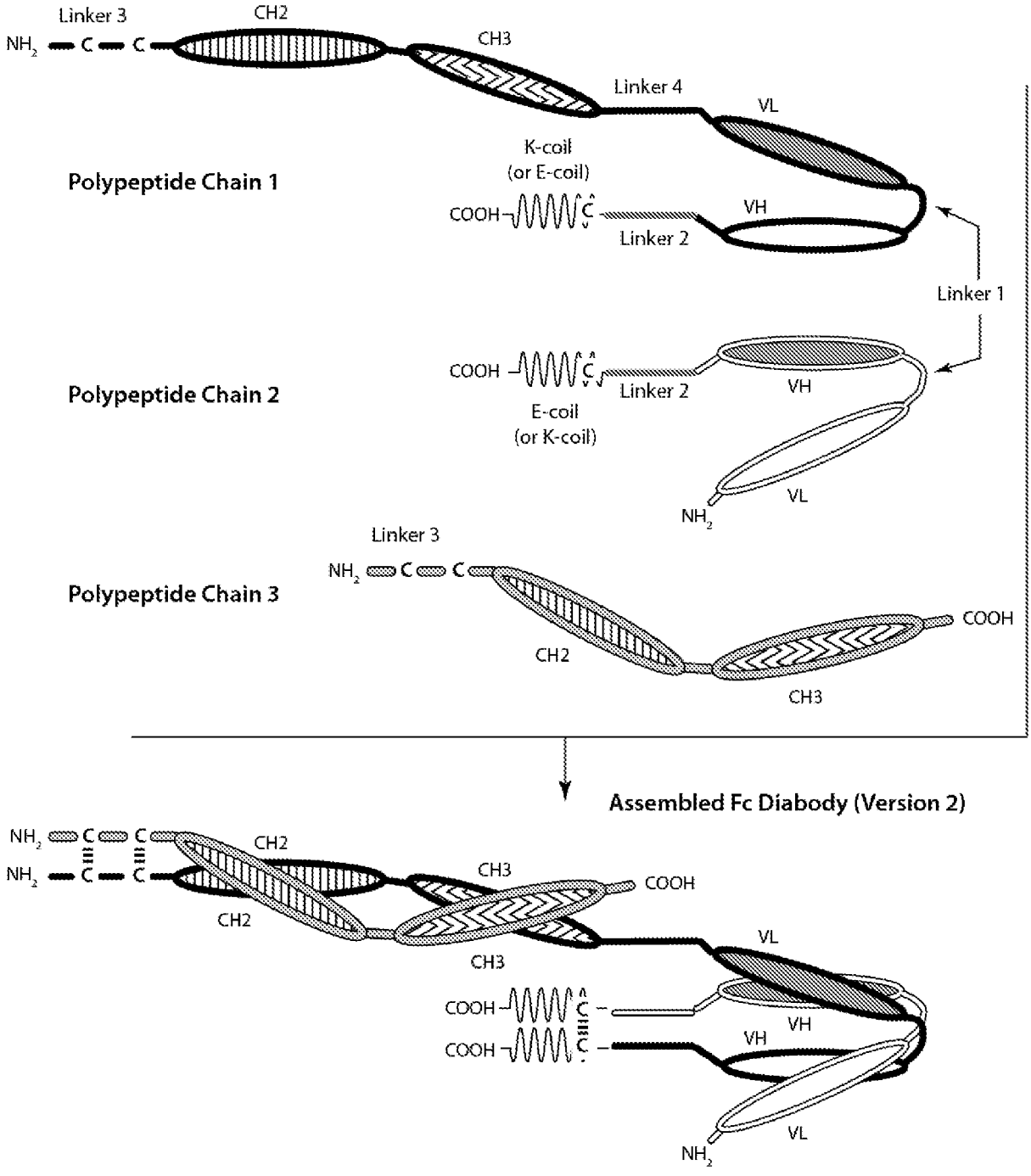
Linker 3
CH2
NH2
C
C
CH3
Linker 4
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
VH
Linker 2
Linker 1
Polypeptide Chain 2
COOH
Linker 2
VH
E-coil
(or K-coil)
VL
NH2
Linker 3
Polypeptide Chain 3
NH2
CH2
COOH
CH3
Assembled Fc Diabody (Version 2)
NH2
CH2
CH3
COOH
CH2
VL
CH3
COOH
VH
COOH
VH
VL
NH2

Figure 4B

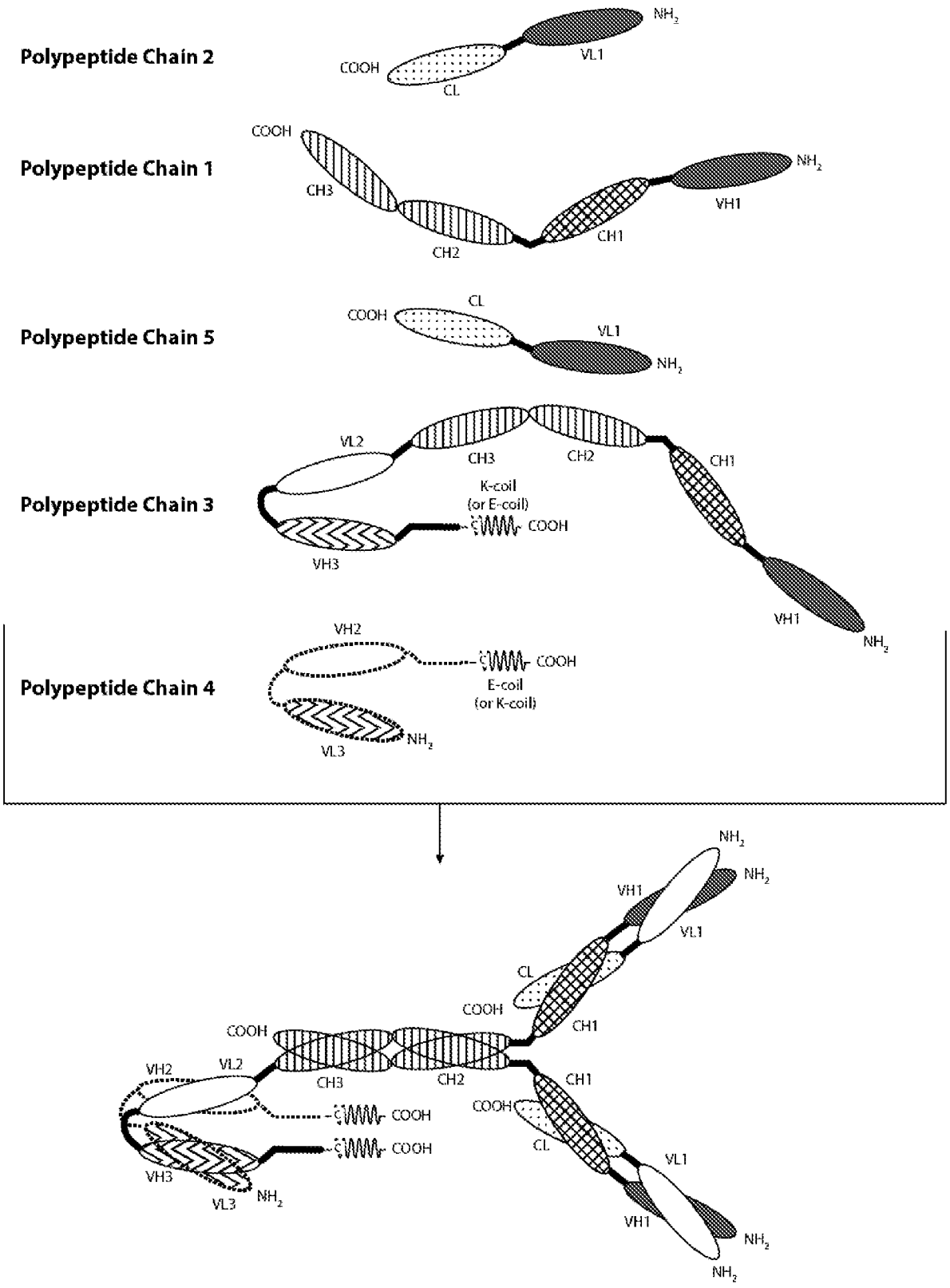
Polypeptide Chain 2
NH2
VL1
COOH
CL
Polypeptide Chain 1
COOH
CH3
CH2
CH1
VH1
NH2
Polypeptide Chain 5
CL
COOH
VL1
NH2
Polypeptide Chain 3
VL2
CH3
CH2
CH1
K-coil
(or E-coil)
COOH
VH3
VH1
NH2
Polypeptide Chain 4
VH2
COOH
E-coil
(or K-coil)
VL3
NH2
NH2
NH2
VH1
VL1
CL
COOH
CH1
COOH
VH2
VL2
CH3
CH2
CH1
COOH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

Figure 5

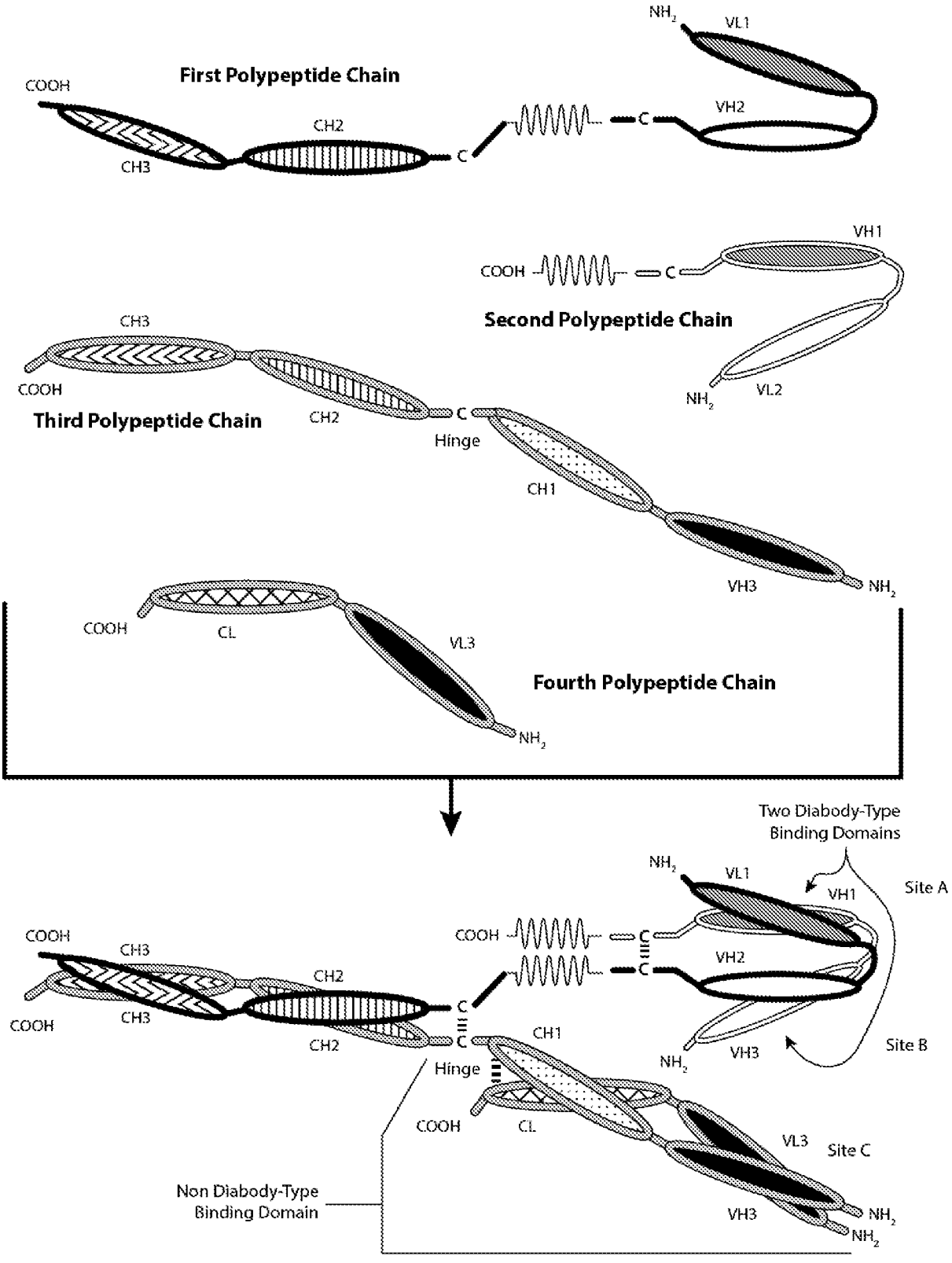
First Polypeptide Chain
COOH
CH2
CH3
C
NH2
VL1
VH2
Second Polypeptide Chain
VH1
COOH
C
NH2
VL2
Third Polypeptide Chain
CH3
COOH
CH2
C
Hinge
CH1
VH3
NH2
Fourth Polypeptide Chain
COOH
CL
VL3
NH2
Two Diabody-Type Binding Domains
Site A
Site B
Site C
Non Diabody-Type Binding Domain

Figure 6A

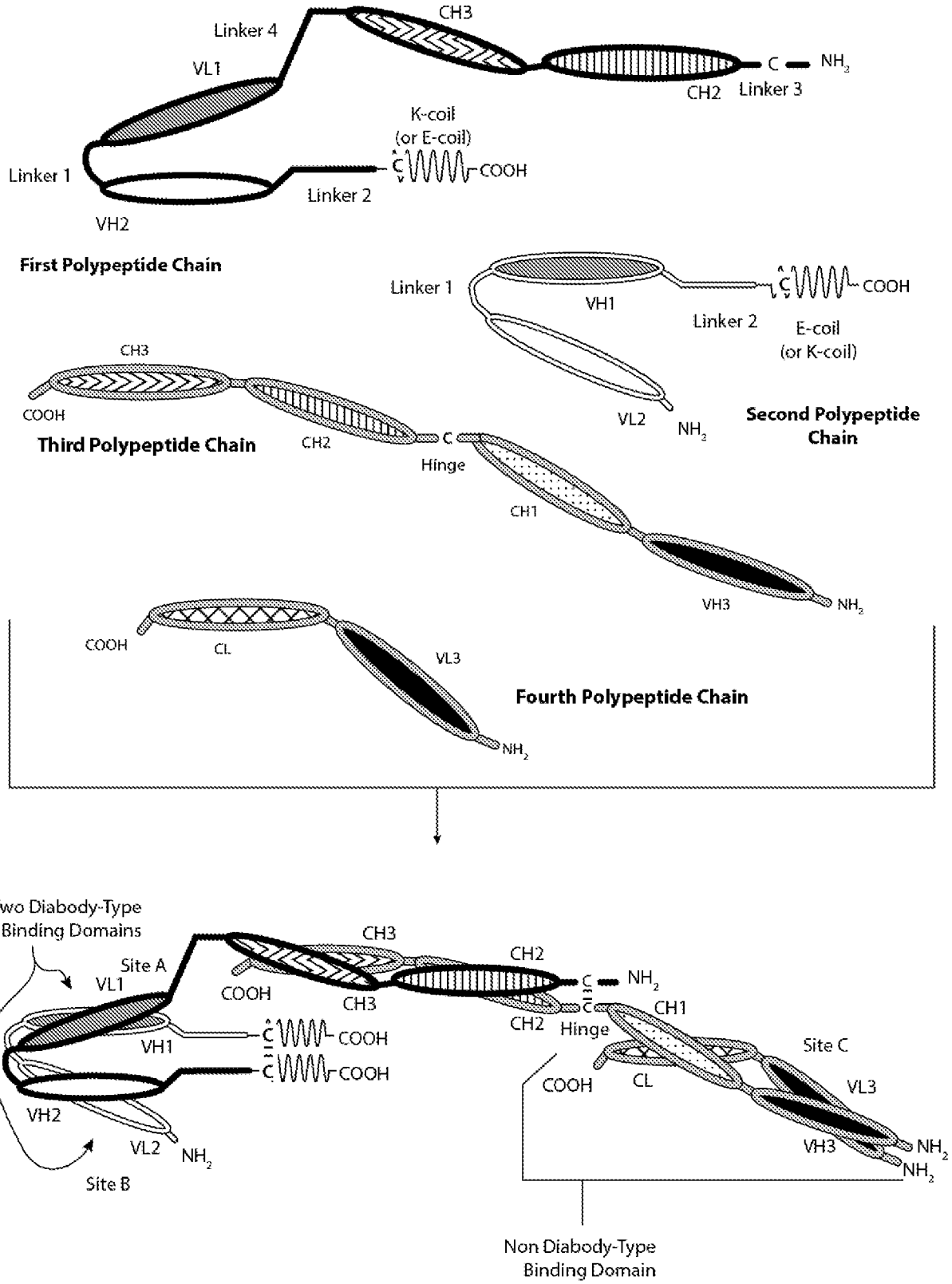
CH3
Linker 4
VL1
C
NH2
CH2
Linker 3
K-coil
(or E-coil)
Linker 1
COOH
Linker 2
VH2
First Polypeptide Chain
Linker 1
VH1
COOH
Linker 2
E-coil
(or K-coil)
CH3
VL2
NH2
Second Polypeptide
Chain
COOH
Third Polypeptide Chain
CH2
C
Hinge
CH1
VH3
NH2
COOH
CL
VL3
Fourth Polypeptide Chain
NH2
Two Diabody-Type
Binding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

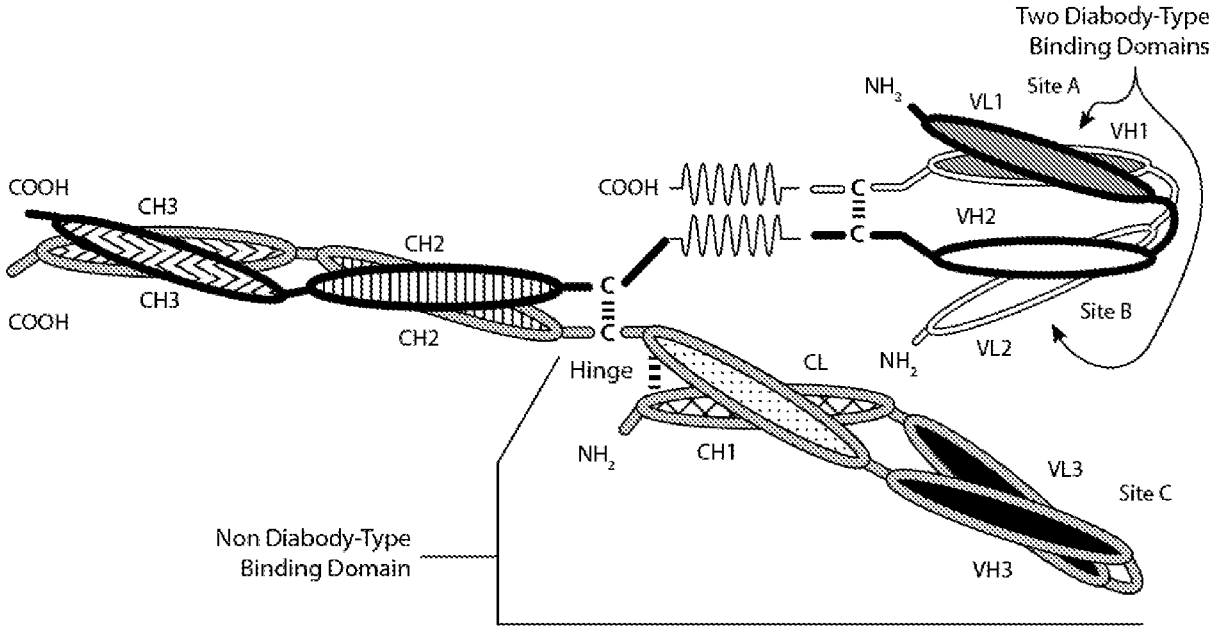

Figure 6C

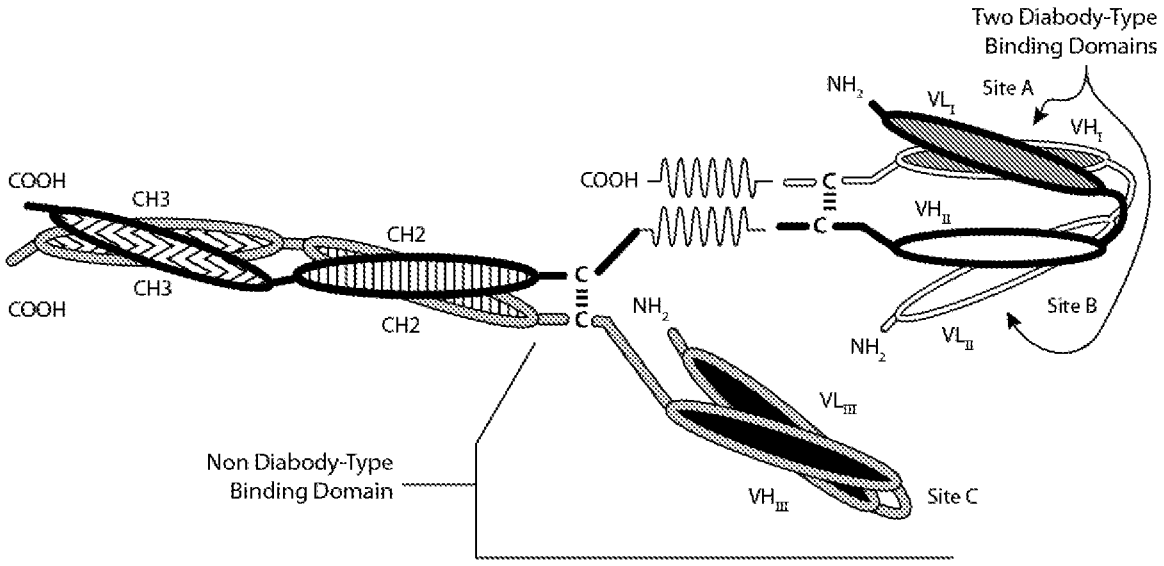

Figure 6D

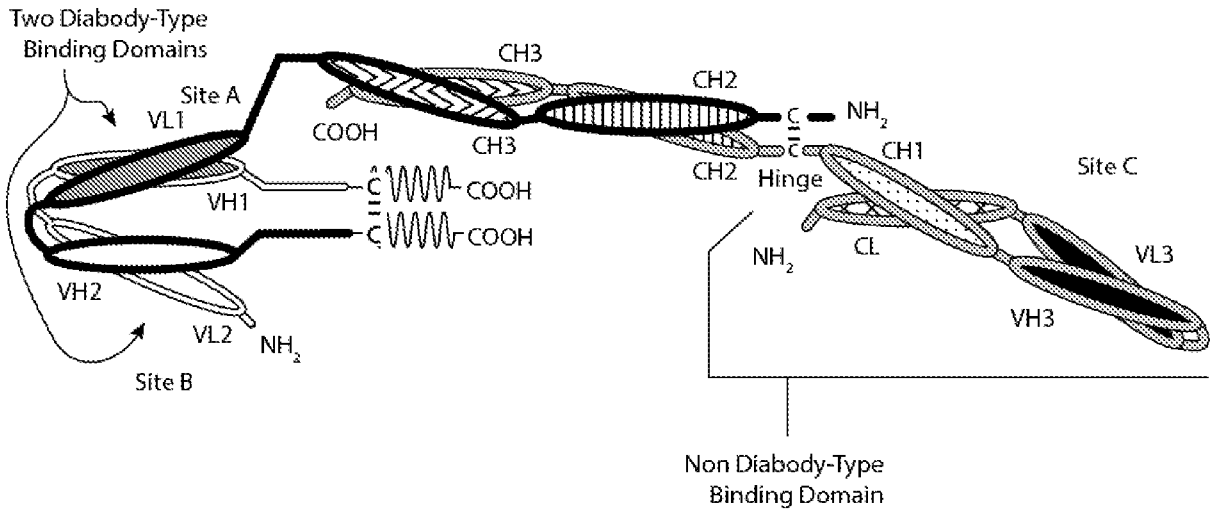

Figure 6E

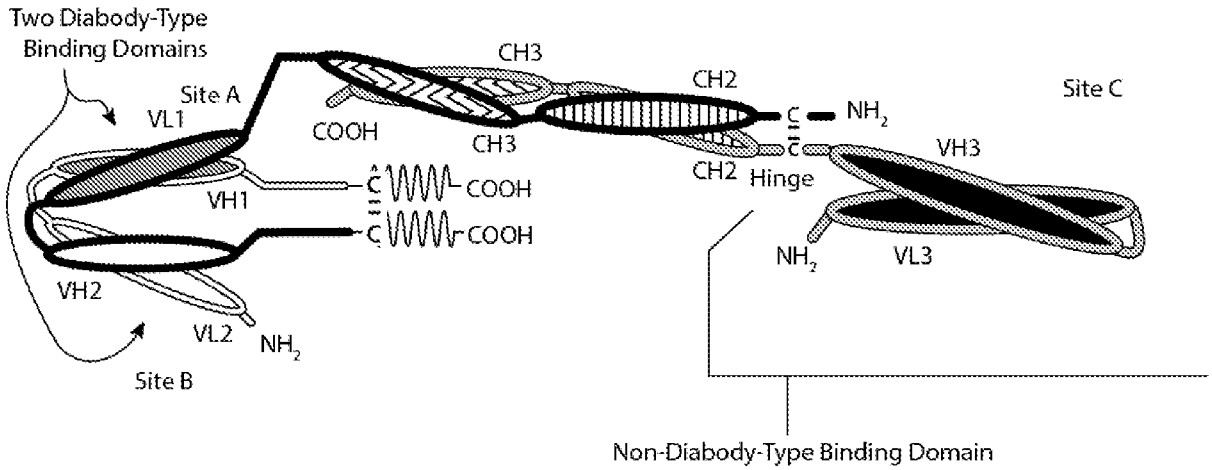

Figure 6F

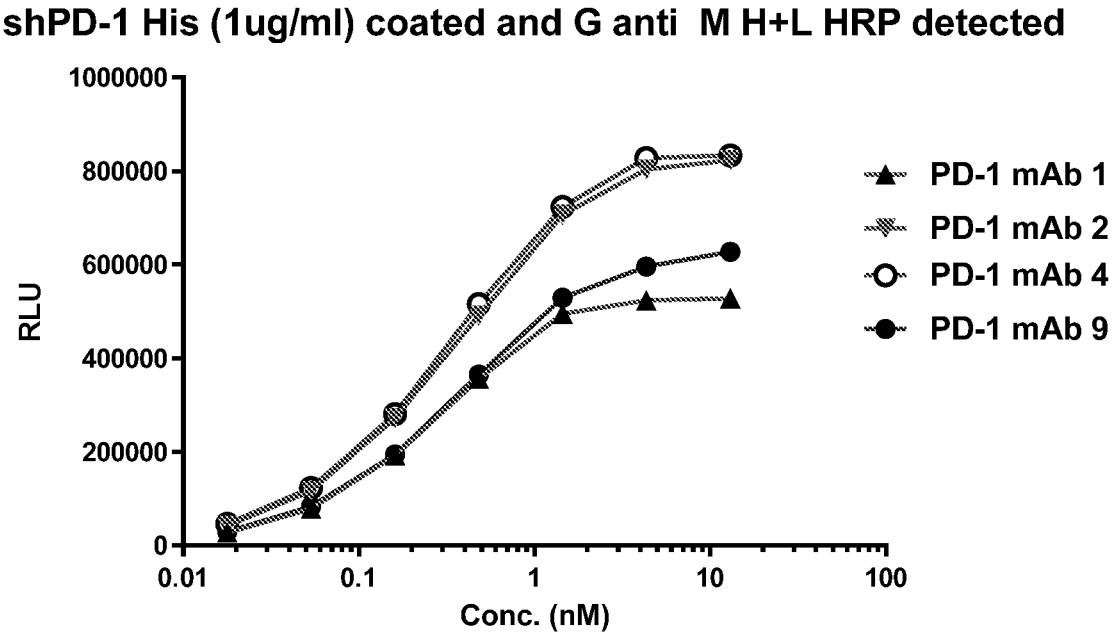

Figure 7A

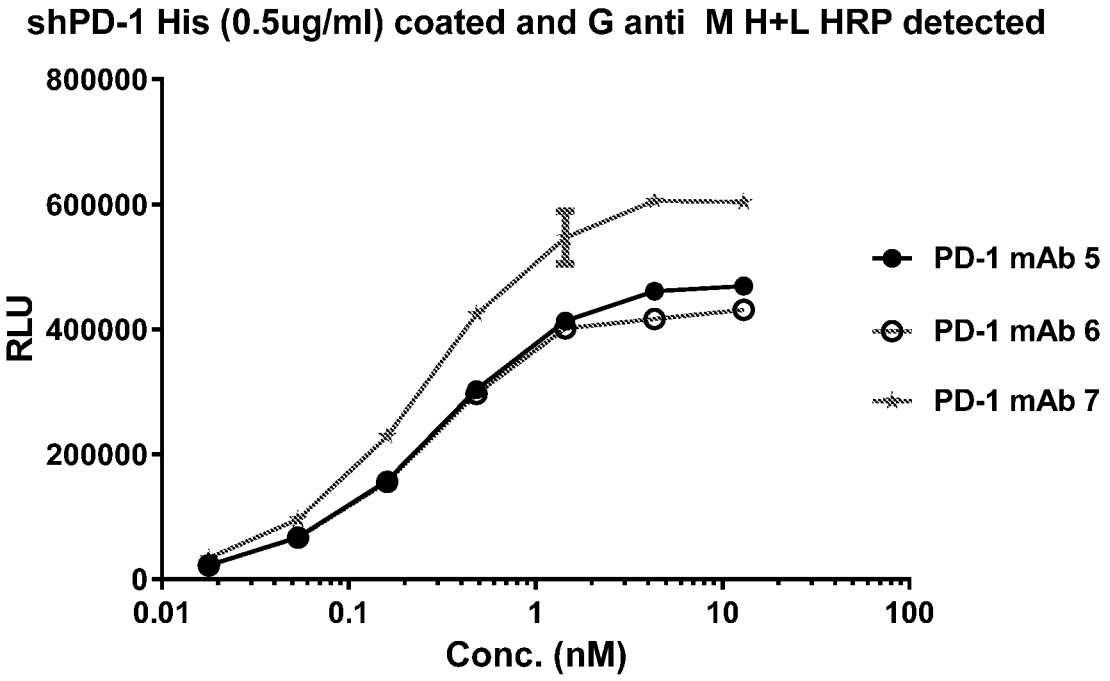

Figure 7B

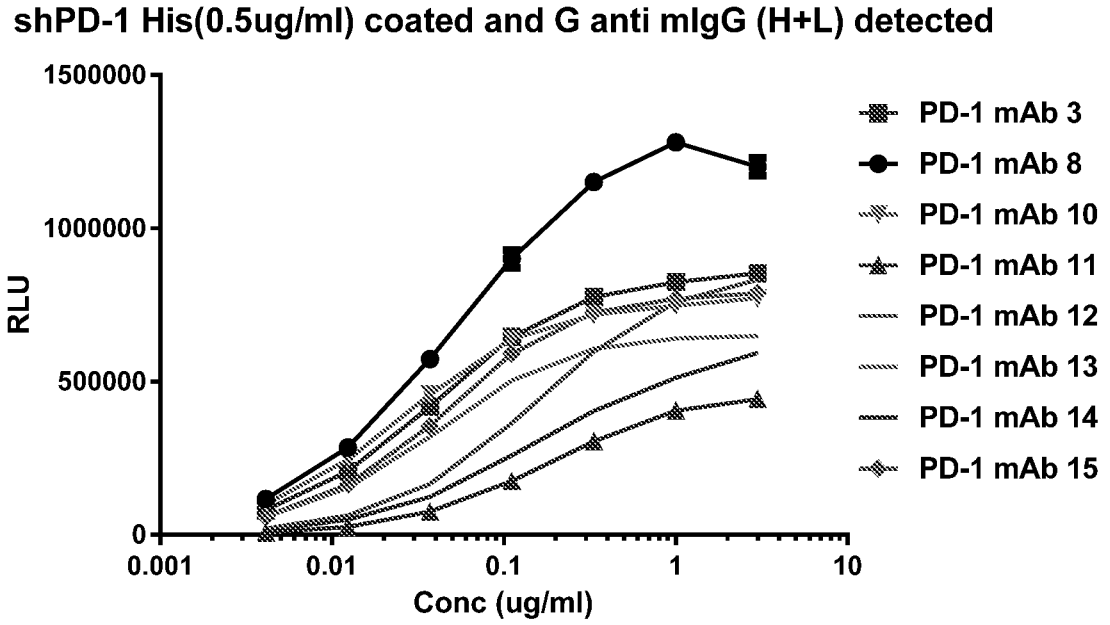

Figure 7C

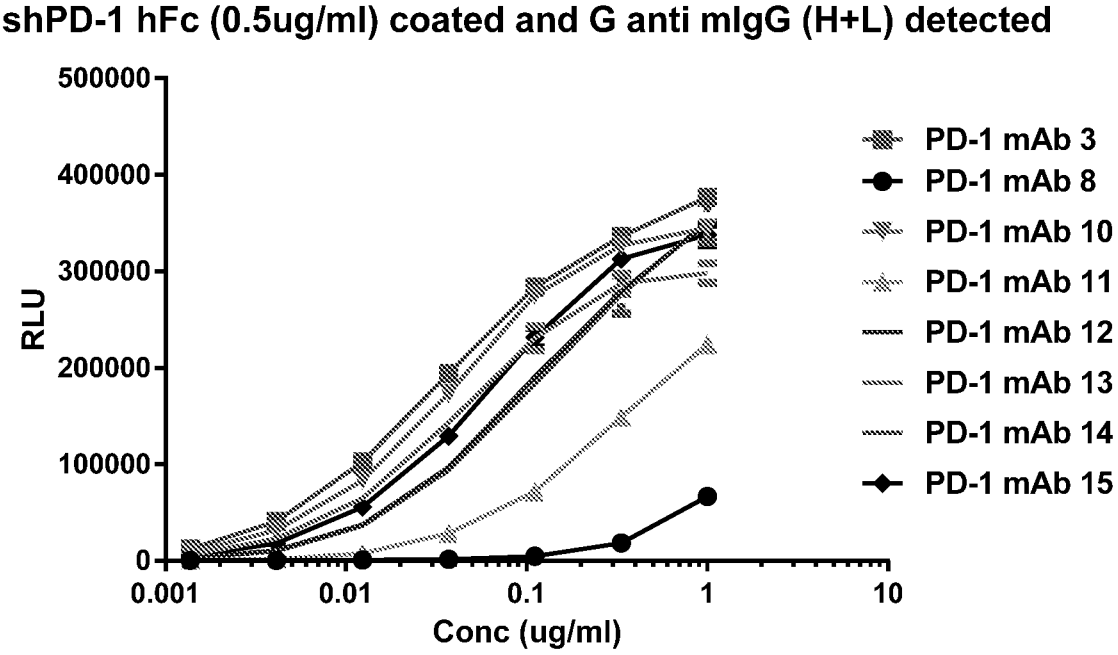

Figure 7D

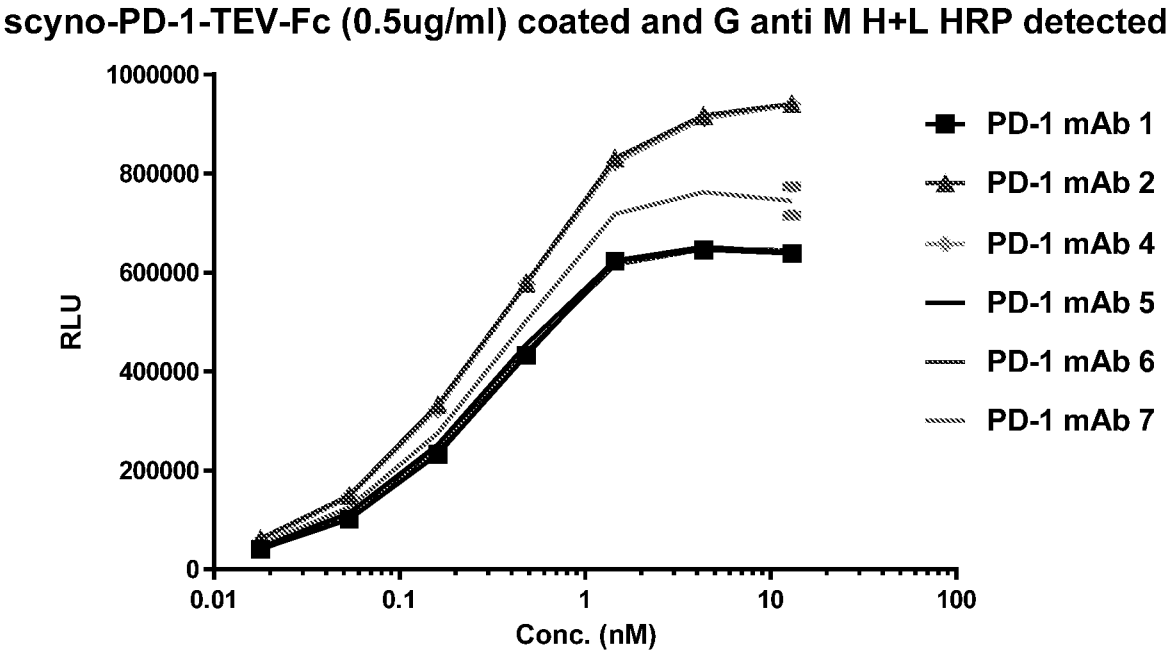

Figure 8A

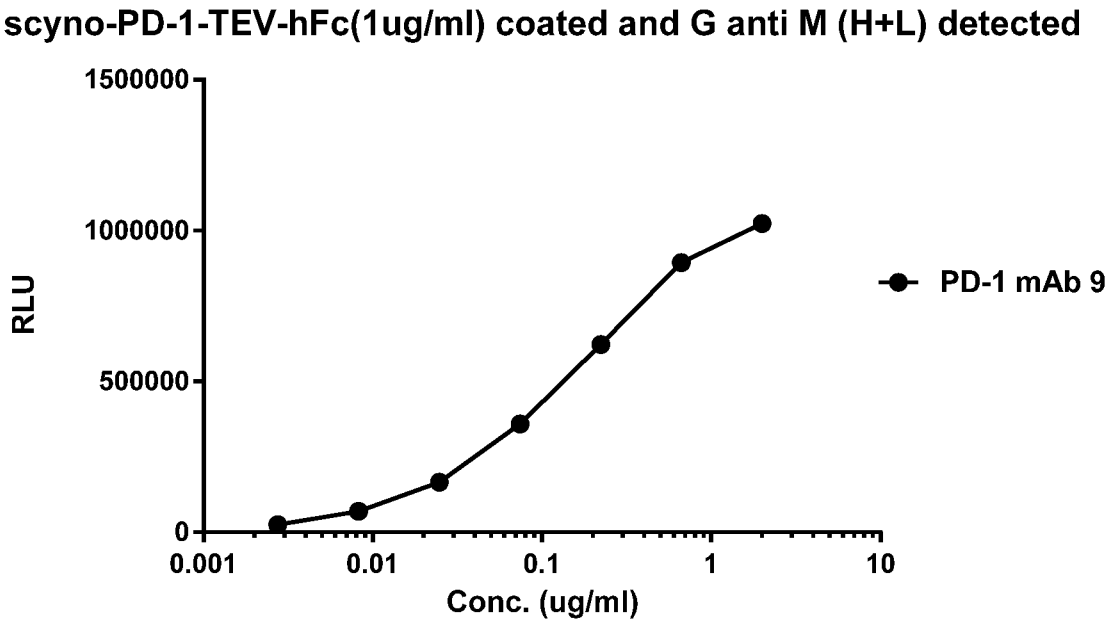

Figure 8B

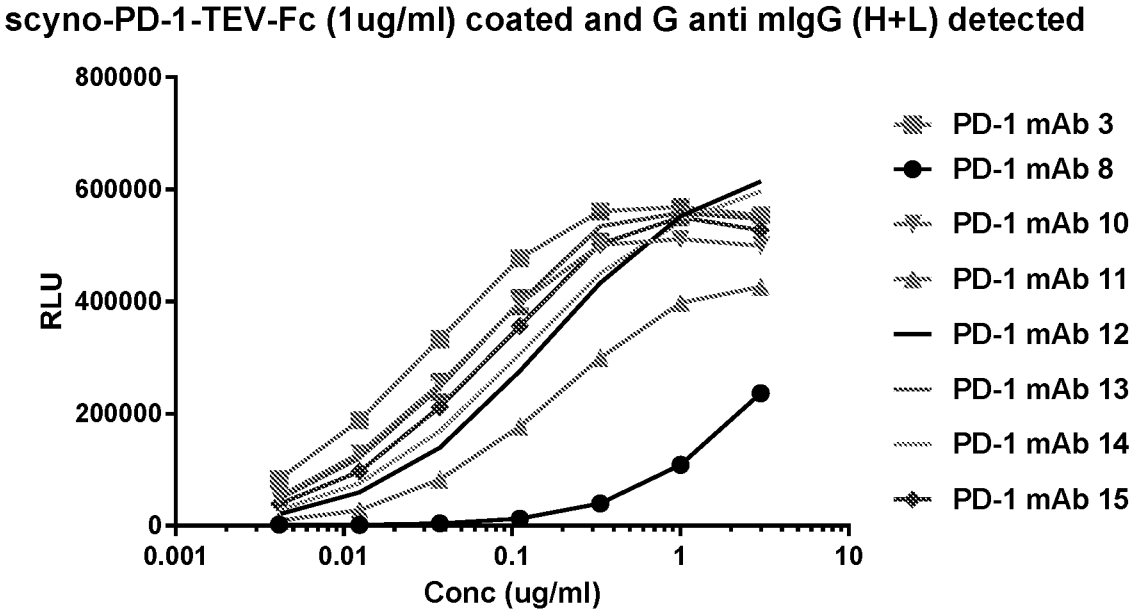
scyno-PD-1-TEV-Fc (1ug/ml) coated and G anti mIgG (H+L) detected
800000
600000
400000
200000
0
RLU
0.001
0.01
0.1
1
10
Conc (ug/ml)
PD-1 mAb 3
PD-1 mAb 8
PD-1 mAb 10
PD-1 mAb 11
PD-1 mAb 12
PD-1 mAb 13
PD-1 mAb 14
PD-1 mAb 15

Figure 8C

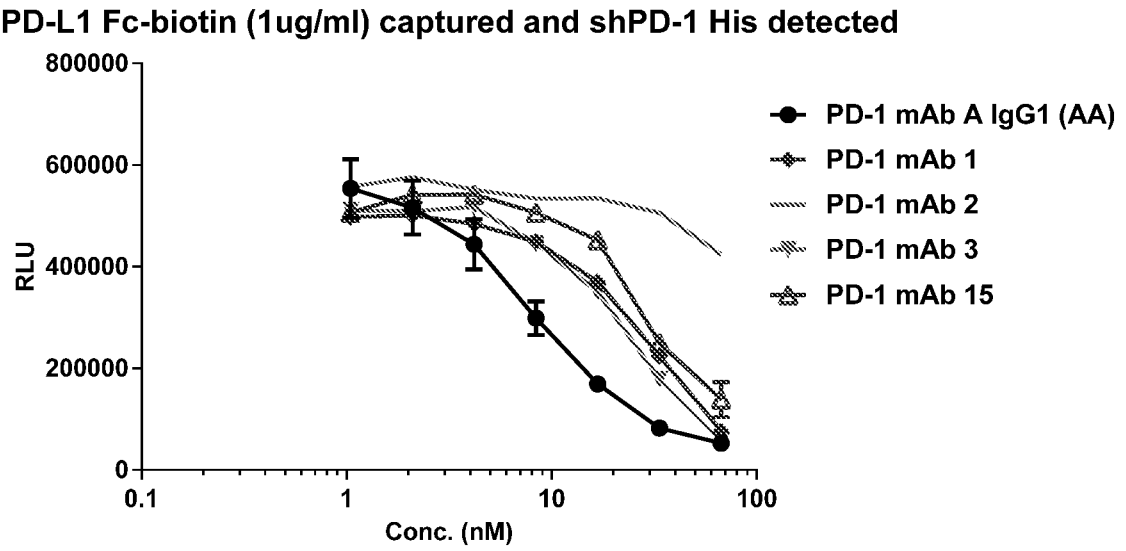

Figure 9A

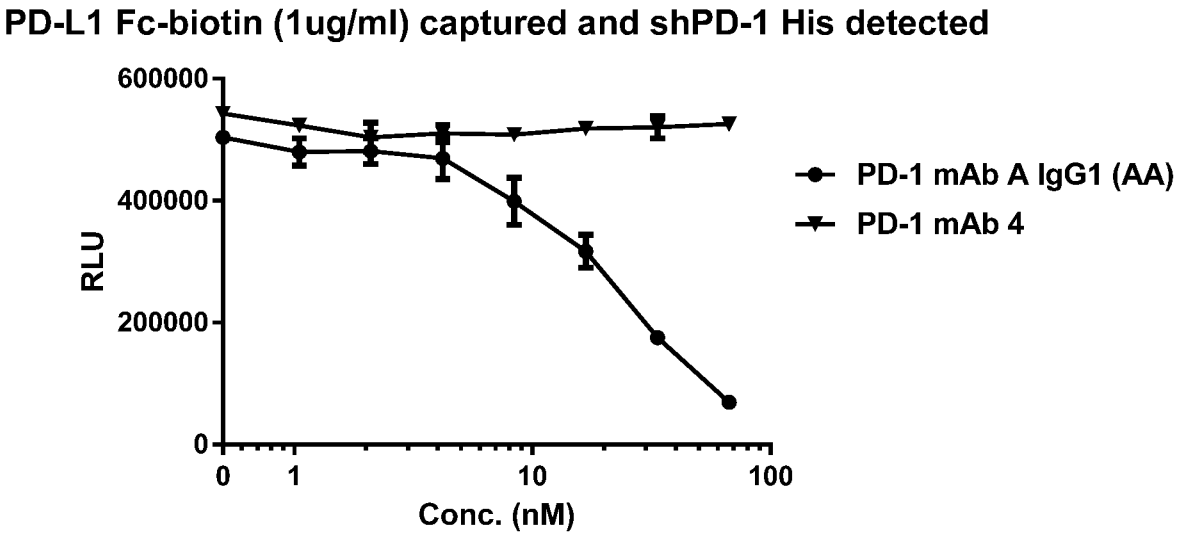

Figure 9B

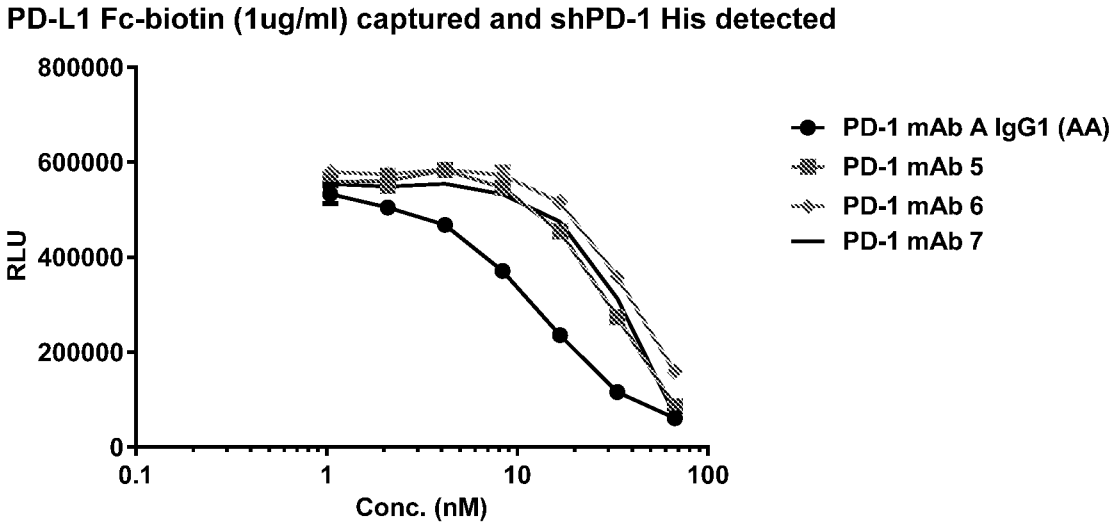

Figure 9C

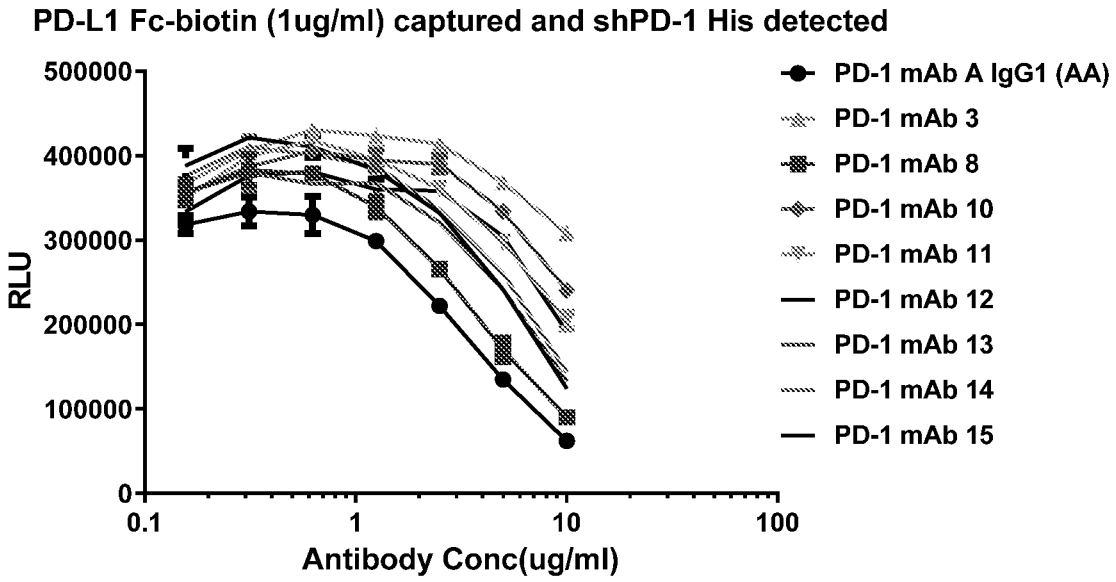

Figure 9D

Normal Tissues

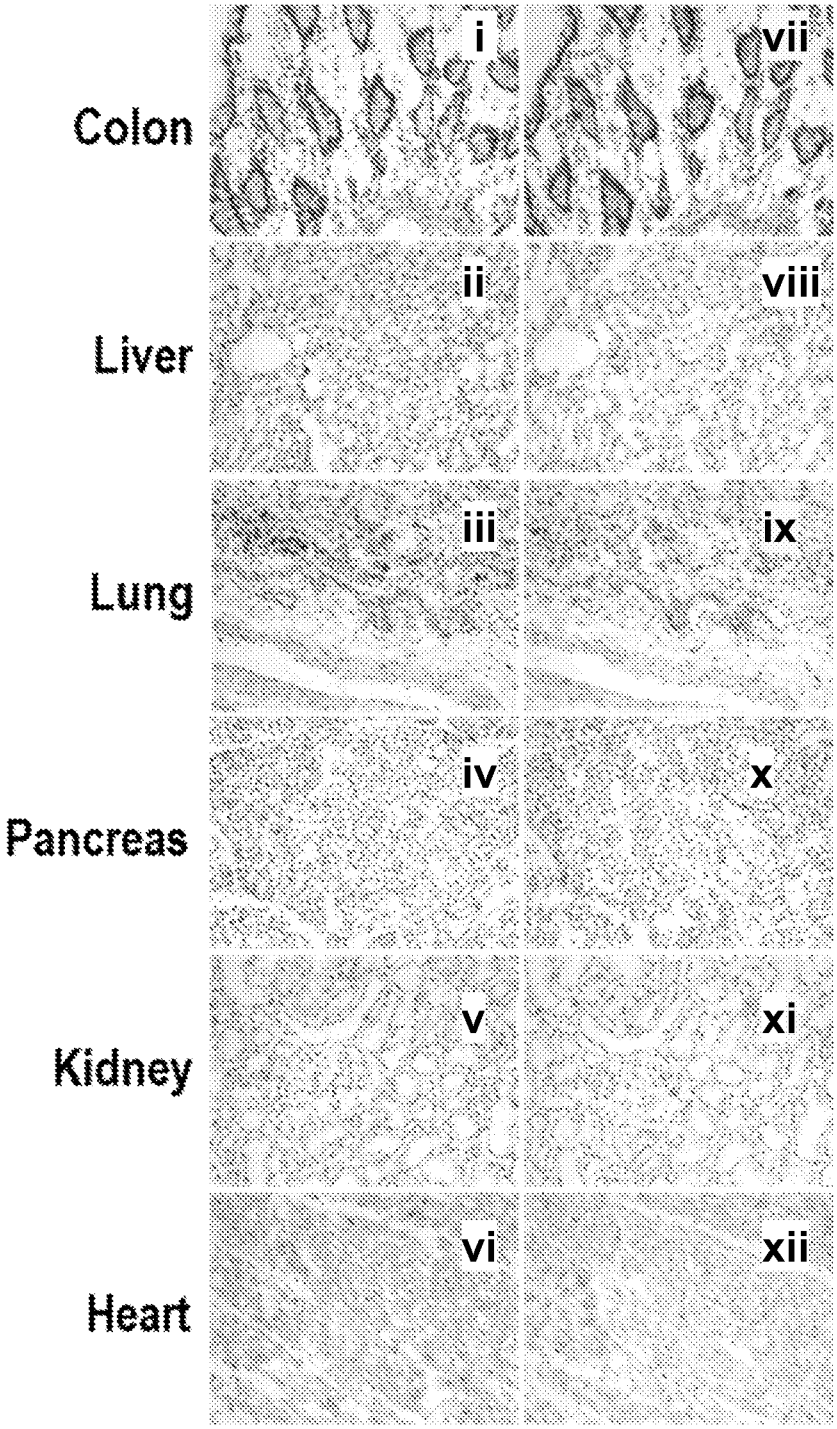

Figure 10A

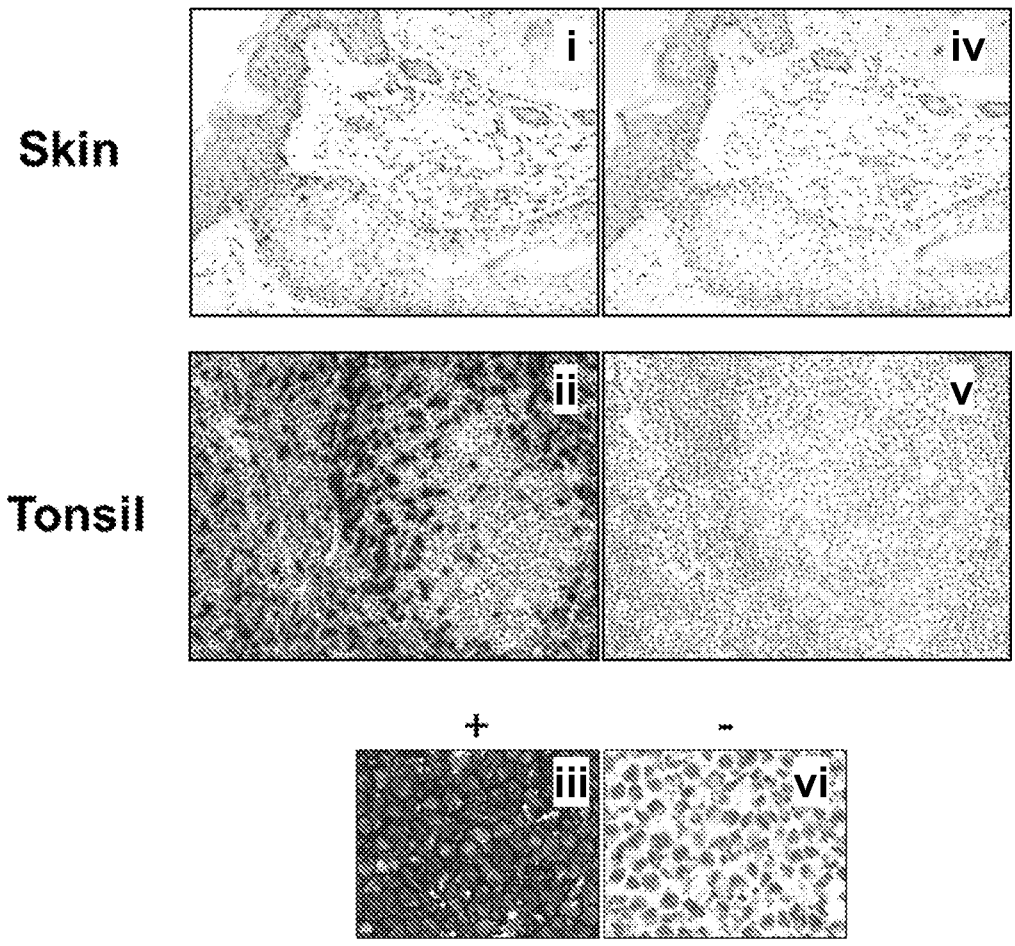
Skin
Tonsil
i
iv
ii
v
+
-
iii
vi
PD-1 mAb 7
0.313 µg/ml
Isotype Control
0.313 µg/ml

Figure 10B

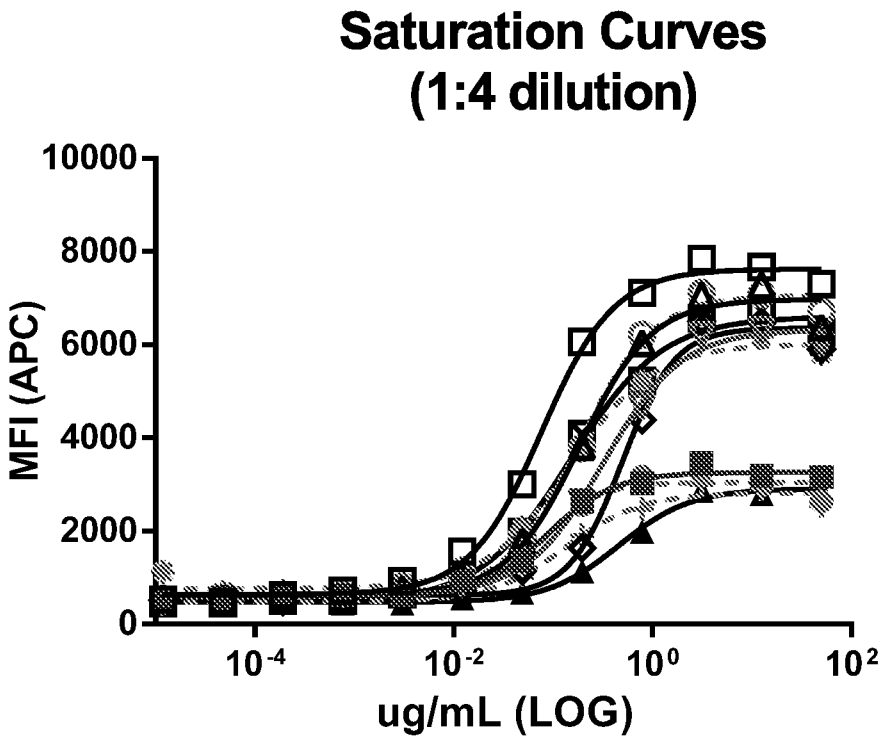
Saturation Curves
(1:4 dilution)
10000
8000
6000
4000
2000
0
MFI (APC)
10-4
10-2
100
102
ug/mL (LOG)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1 (AA)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

Figure 11

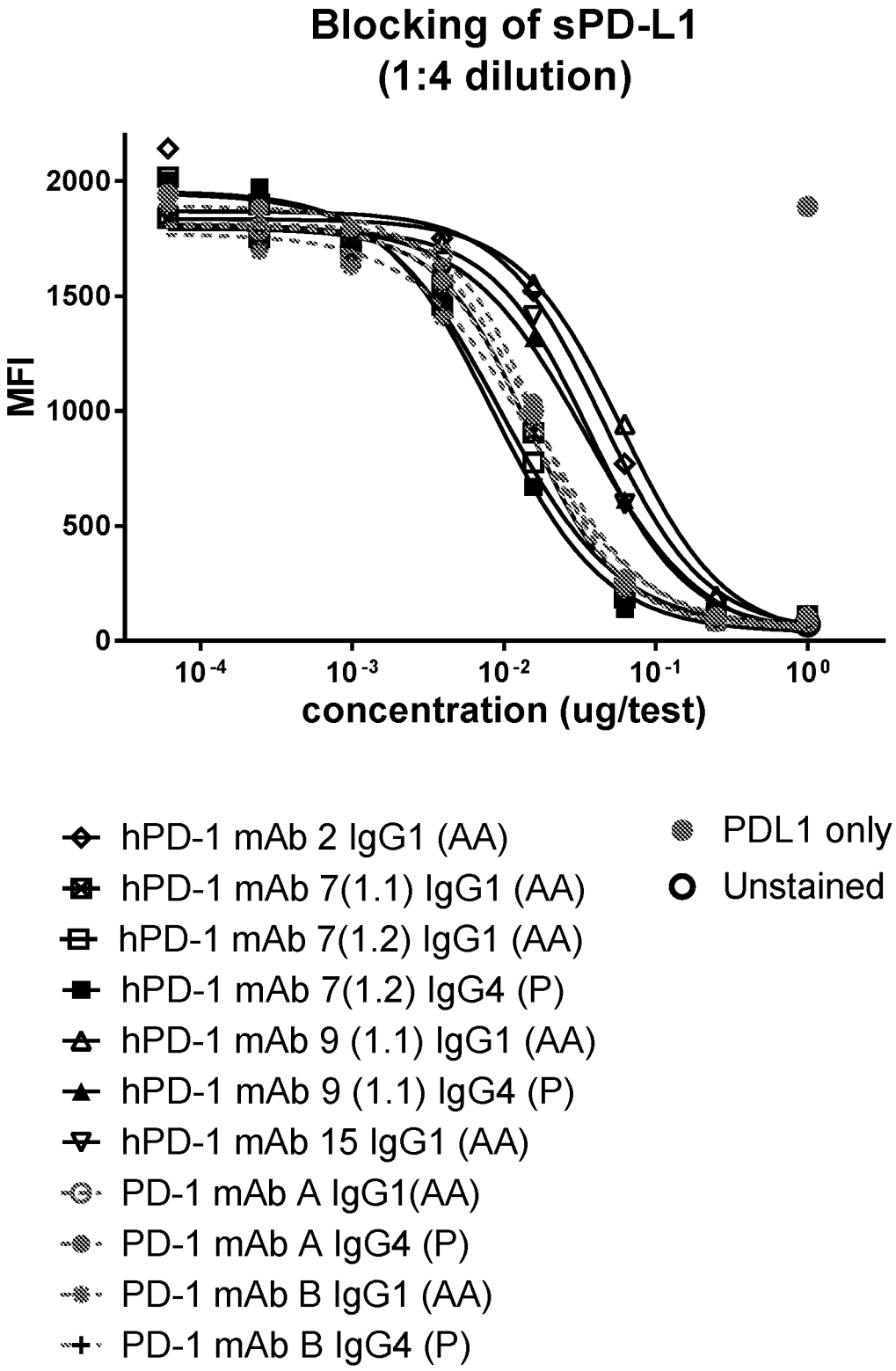
Blocking of sPD-L1
(1:4 dilution)
MFI
2000
1500
1000
500
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
concentration (ug/test)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)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

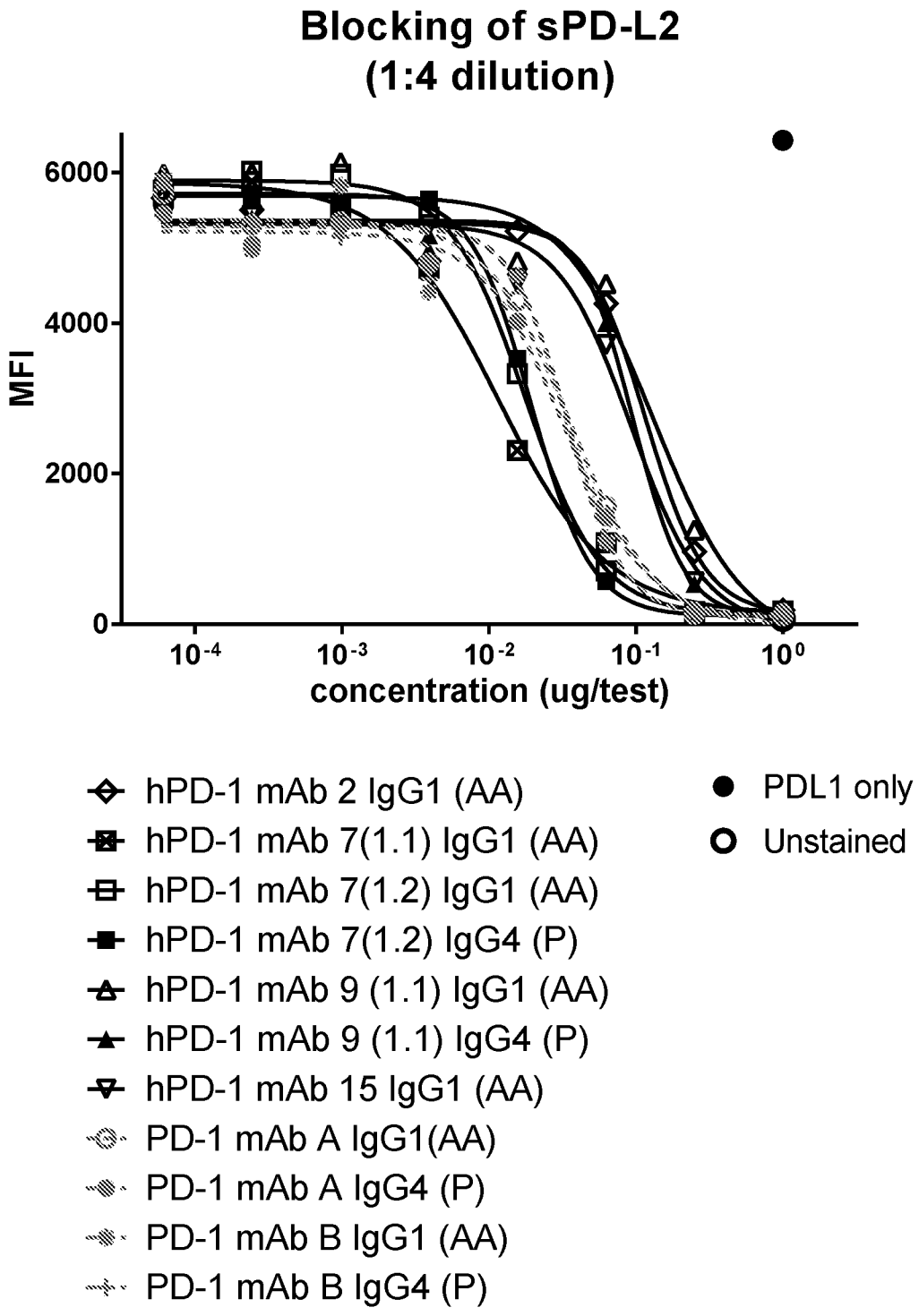
Blocking of sPD-L2
(1:4 dilution)
6000
4000
2000
0
MFI
10-4
10-3
10-2
10-1
100
concentration (ug/test)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4 (P)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG1(AA)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

Figure 12B

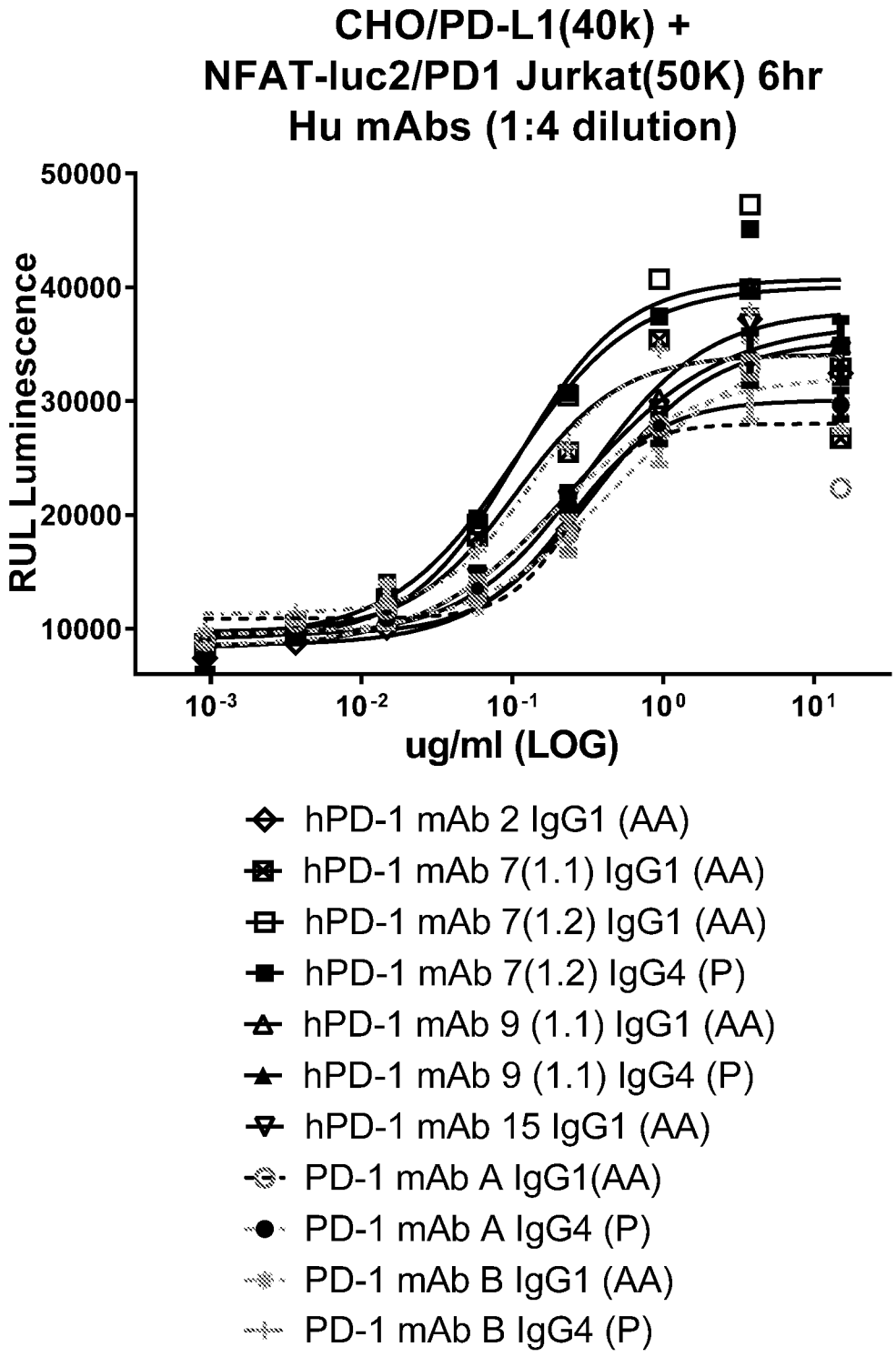
CHO/PD-L1(40k) +
NFAT-luc2/PD1 Jurkat(50K) 6hr
Hu mAbs (1:4 dilution)
RUL Luminescence
50000
40000
30000
20000
10000
10-3
10-2
10-1
100
101
ug/ml (LOG)
hPD-1 mAb 2 IgG1 (AA)
hPD-1 mAb 7(1.1) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 7(1.2) IgG4 (P)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG4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

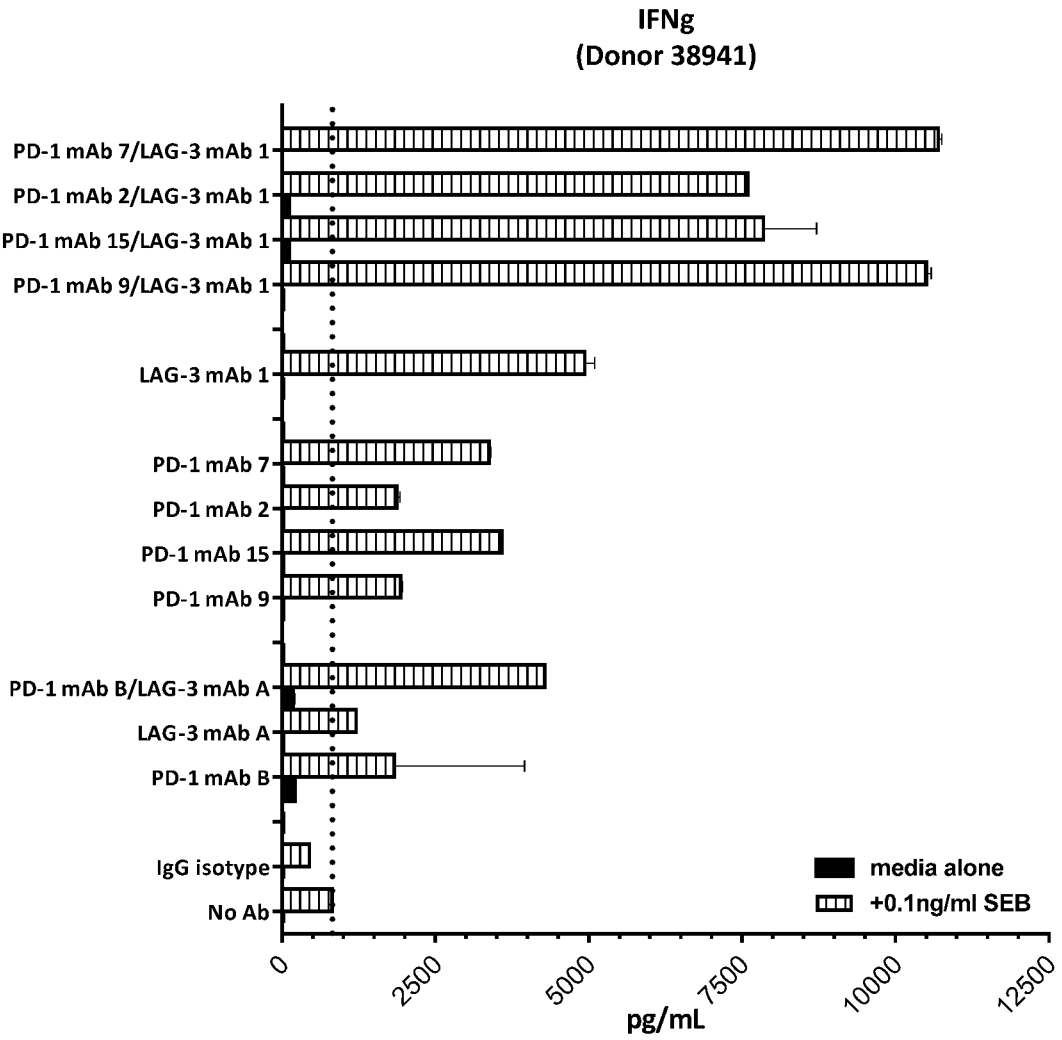
IFNg
(Donor 38941)
PD-1 mAb 7/LAG-3 mAb 1
PD-1 mAb 2/LAG-3 mAb 1
PD-1 mAb 15/LAG-3 mAb 1
PD-1 mAb 9/LAG-3 mAb 1
LAG-3 mAb 1
PD-1 mAb 7
PD-1 mAb 2
PD-1 mAb 15
PD-1 mAb 9
PD-1 mAb B/LAG-3 mAb A
LAG-3 mAb A
PD-1 mAb B
IgG isotype
No Ab
media alone
+0.1ng/ml SEB
0
2500
5000
7500
10000
12500
pg/mL

Figure 14

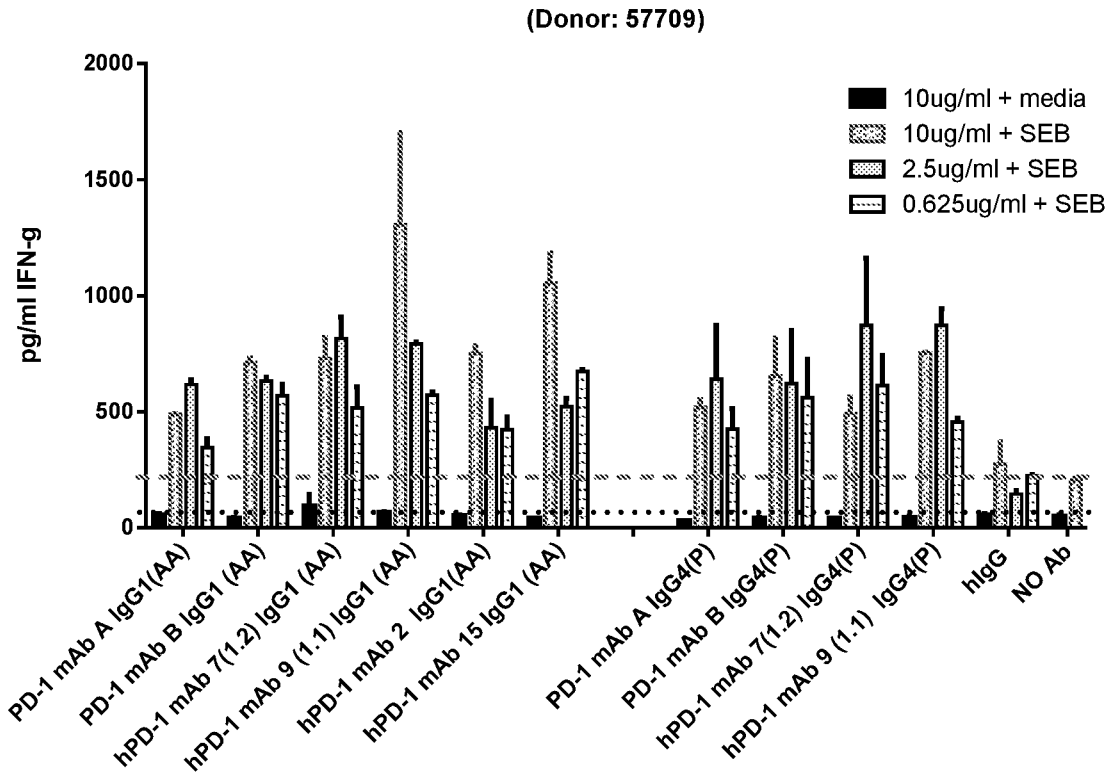
(Donor: 57709)
pg/ml IFN-g
2000
1500
1000
500
0
10ug/ml + media
10ug/ml + SEB
2.5ug/ml + SEB
0.625ug/ml + SEB
PD-1 mAb A IgG1(AA)
PD-1 mAb B IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG1 (AA)
hPD-1 mAb 2 IgG1(AA)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG4(P)
PD-1 mAb B IgG4(P)
hPD-1 mAb 7(1.2)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

Figure 15A

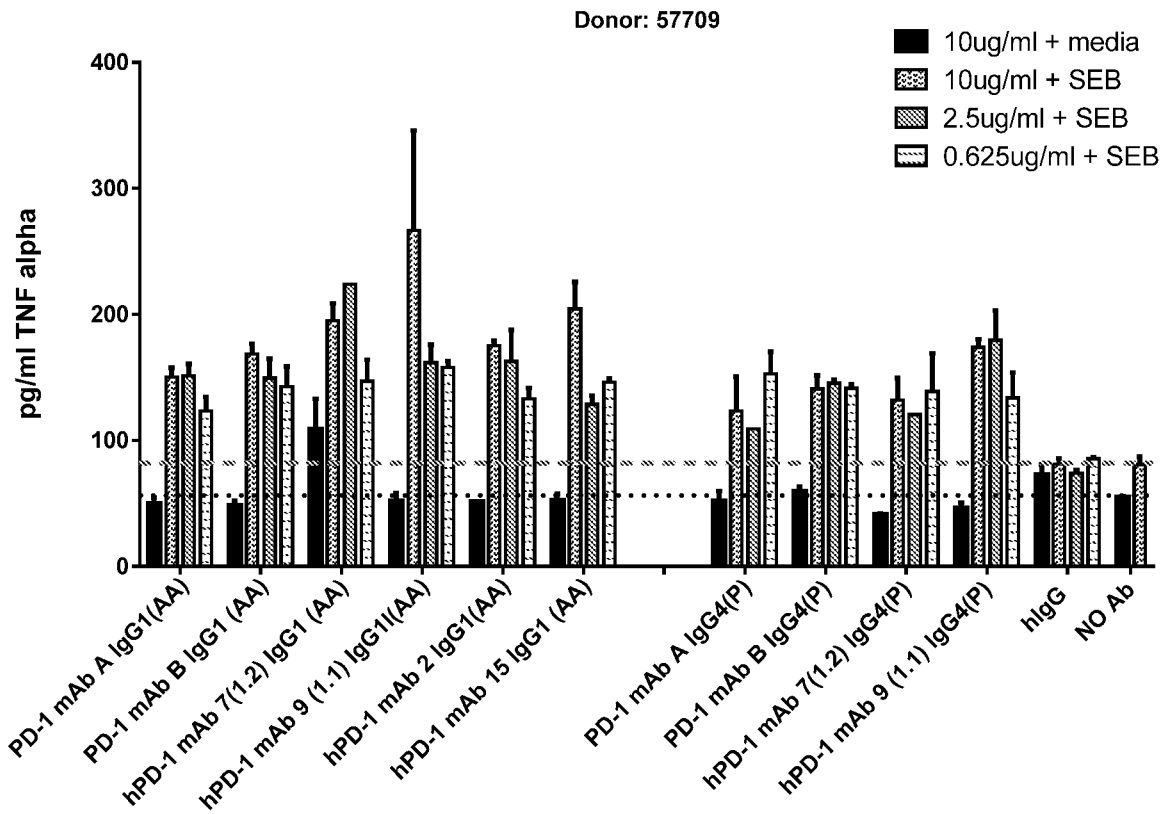
Donor: 57709
10ug/ml + media
10ug/ml + SEB
2.5ug/ml + SEB
0.625ug/ml + SEB
pg/ml TNF alpha
400
300
200
100
0
PD-1 mAb A IgG1(AA)
PD-1 mAb B IgG1 (AA)
hPD-1 mAb 7(1.2) IgG1 (AA)
hPD-1 mAb 9 (1.1) IgG1l(AA)
hPD-1 mAb 2 IgG1(AA)
hPD-1 mAb 15 IgG1 (AA)
PD-1 mAb A IgG4(P)
PD-1 mAb B IgG4(P)
hPD-1 mAb 7(1.2)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

Figure 15B

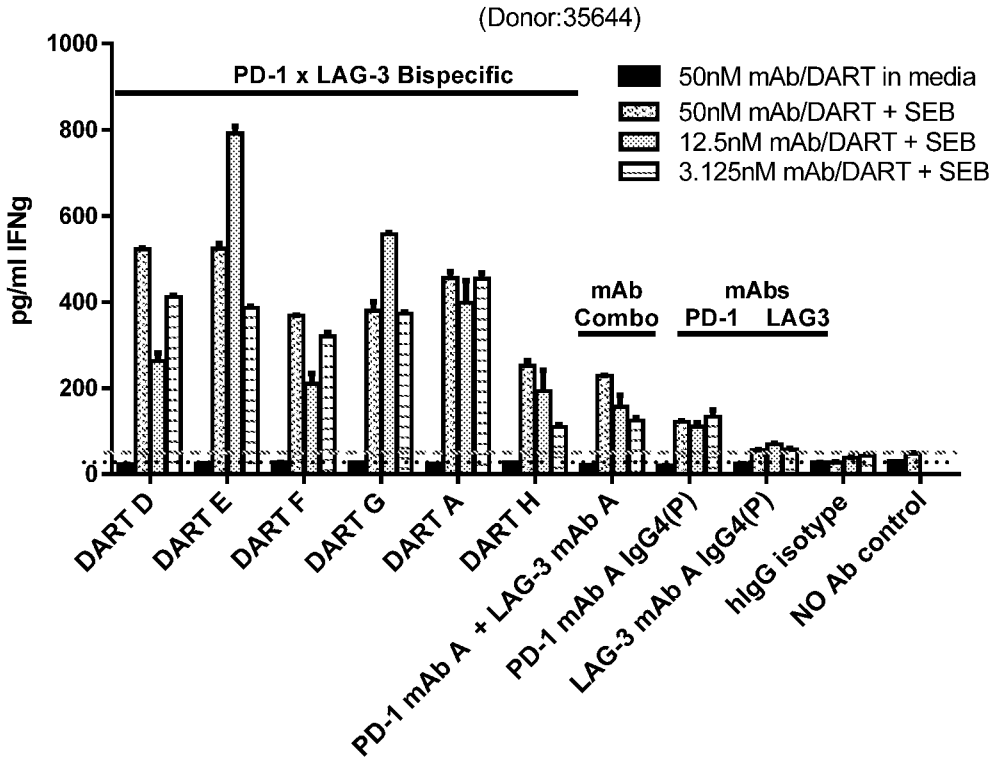

Figure 16A

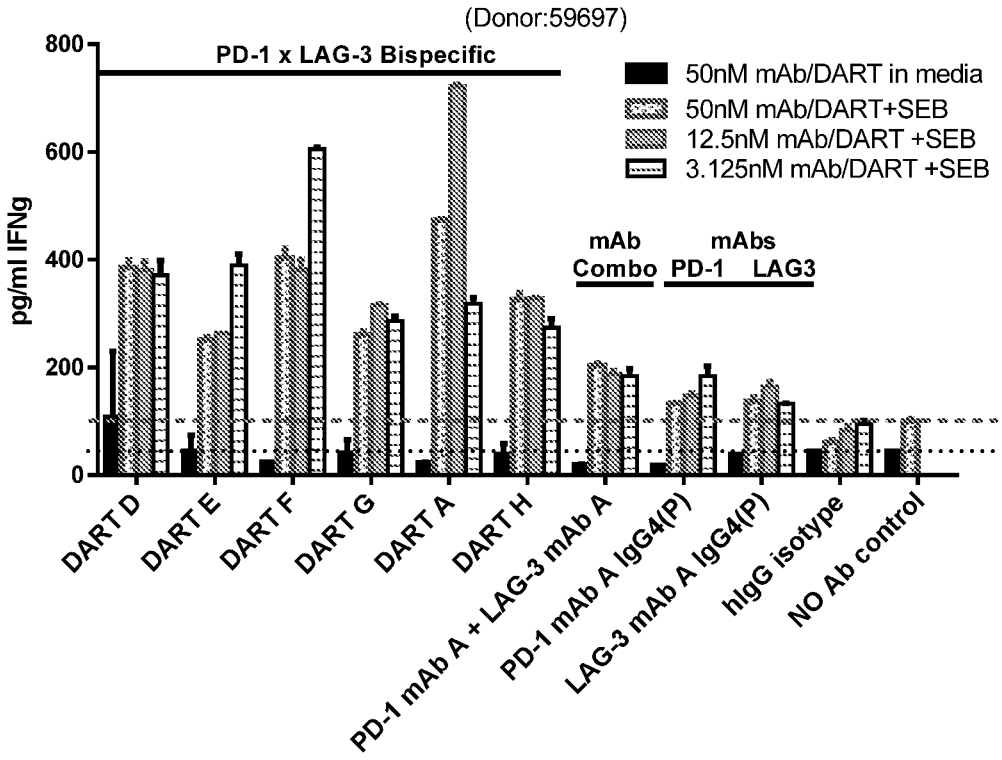

Figure 16B

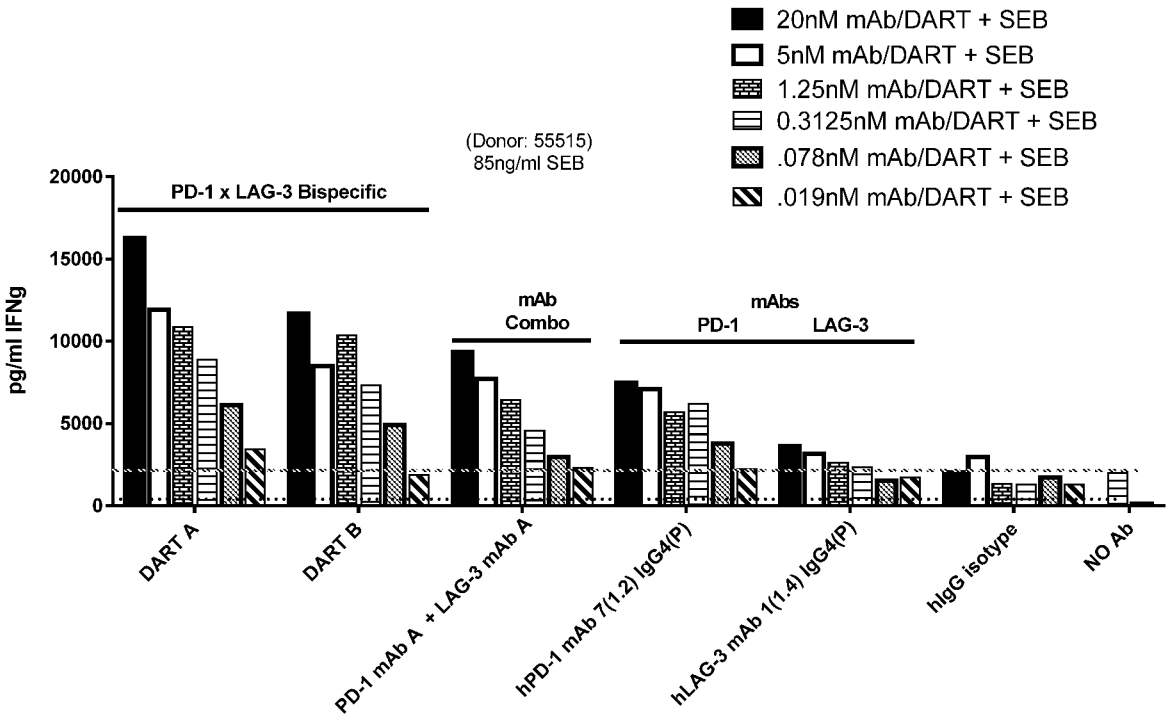

Figure 17A

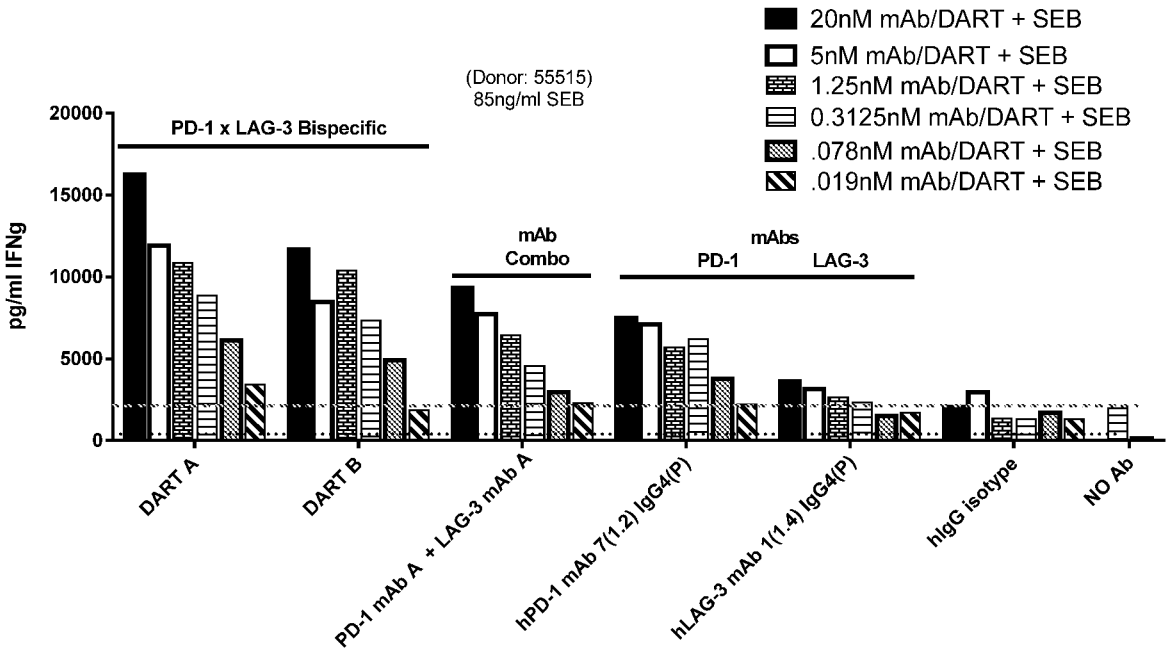

Figure 17B

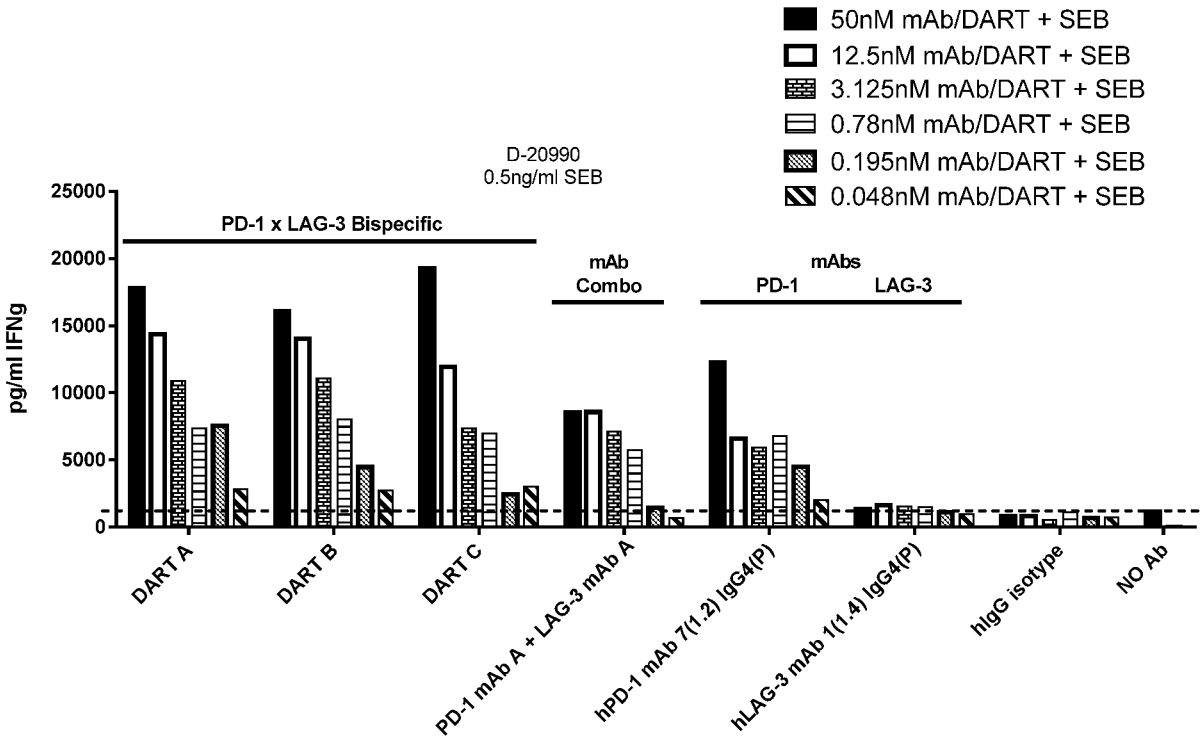
50nM mAb/DART + SEB
12.5nM mAb/DART + SEB
3.125nM mAb/DART + SEB
0.78nM mAb/DART + SEB
0.195nM mAb/DART + SEB
0.048nM mAb/DART + SEB
D-20990
0.5ng/ml SEB
PD-1 x LAG-3 Bispecific
mAb Combo
mAbs
PD-1
LAG-3
pg/ml IFNg
25000
20000
15000
10000
5000
0
DART A
DART B
DART C
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb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

Figure 18A

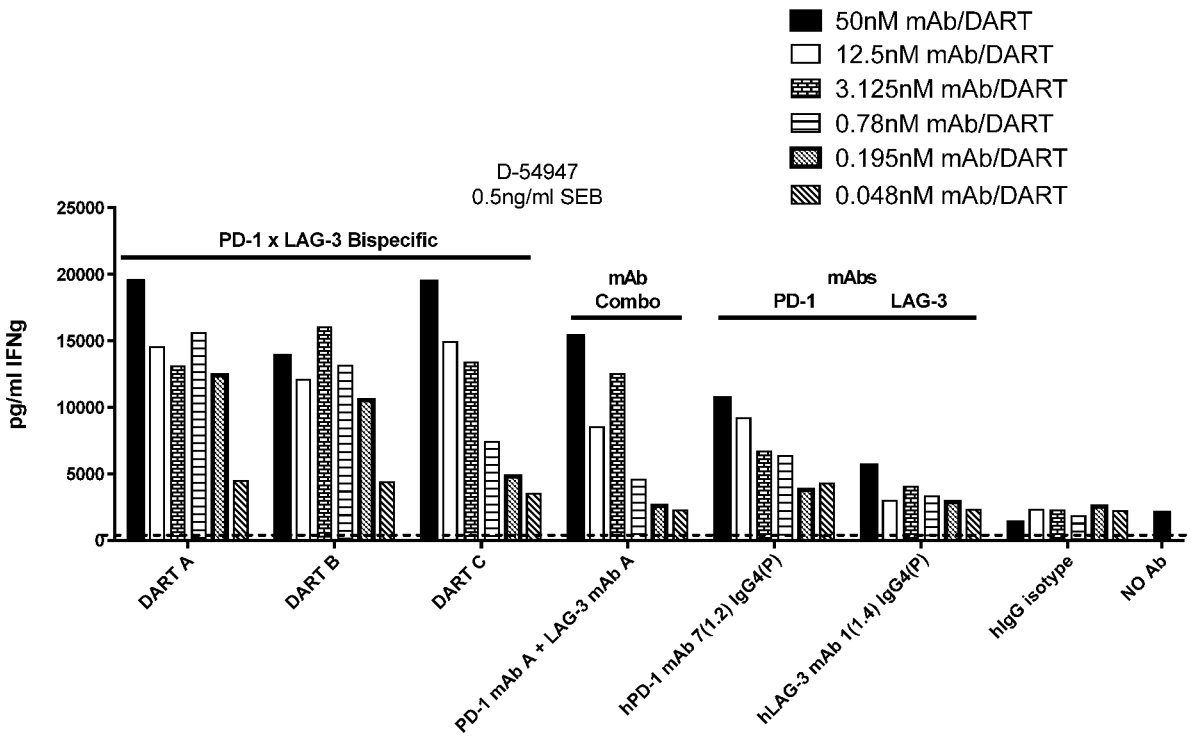
50nM mAb/DART
12.5nM mAb/DART
3.125nM mAb/DART
0.78nM mAb/DART
0.195nM mAb/DART
0.048nM mAb/DART
D-54947
0.5ng/ml SEB
PD-1 x LAG-3 Bispecific
mAb Combo
mAbs
PD-1
LAG-3
pg/ml IFNg
25000
20000
15000
10000
5000
0
DART A
DART B
DART C
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2) IgG4(P)
hLAG-3 mAb 1(1.4) IgG4(P)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

Figure 18B

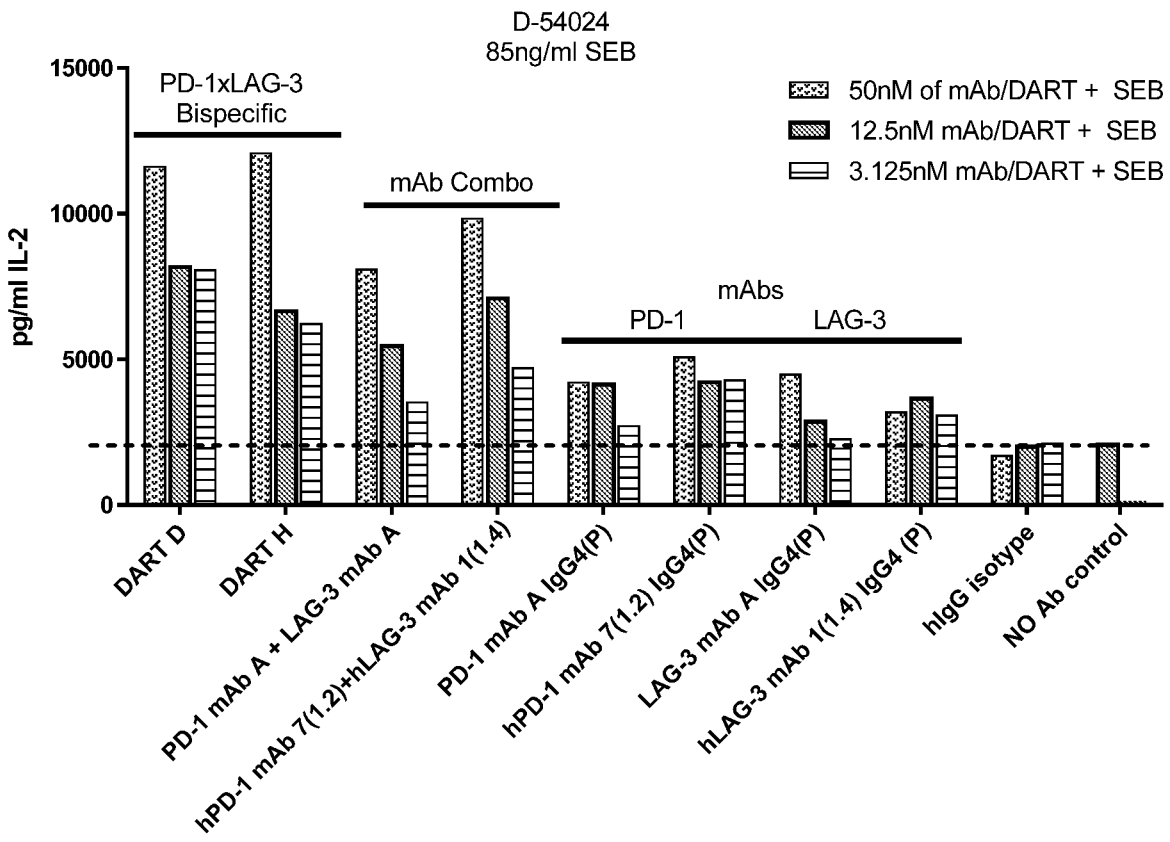
D-54024
85ng/ml SEB
50nM of mAb/DART + SEB
12.5nM mAb/DART + SEB
3.125nM mAb/DART + SEB
pg/ml IL-2
15000
10000
5000
0
PD-1xLAG-3 Bispecific
mAb Combo
mAbs
PD-1
LAG-3
DART D
DART H
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2)+hLAG-3 mAb 1(1.4)
PD-1 mAb A IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
LAG-3 mAb A IgG4(P)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

Figure 19

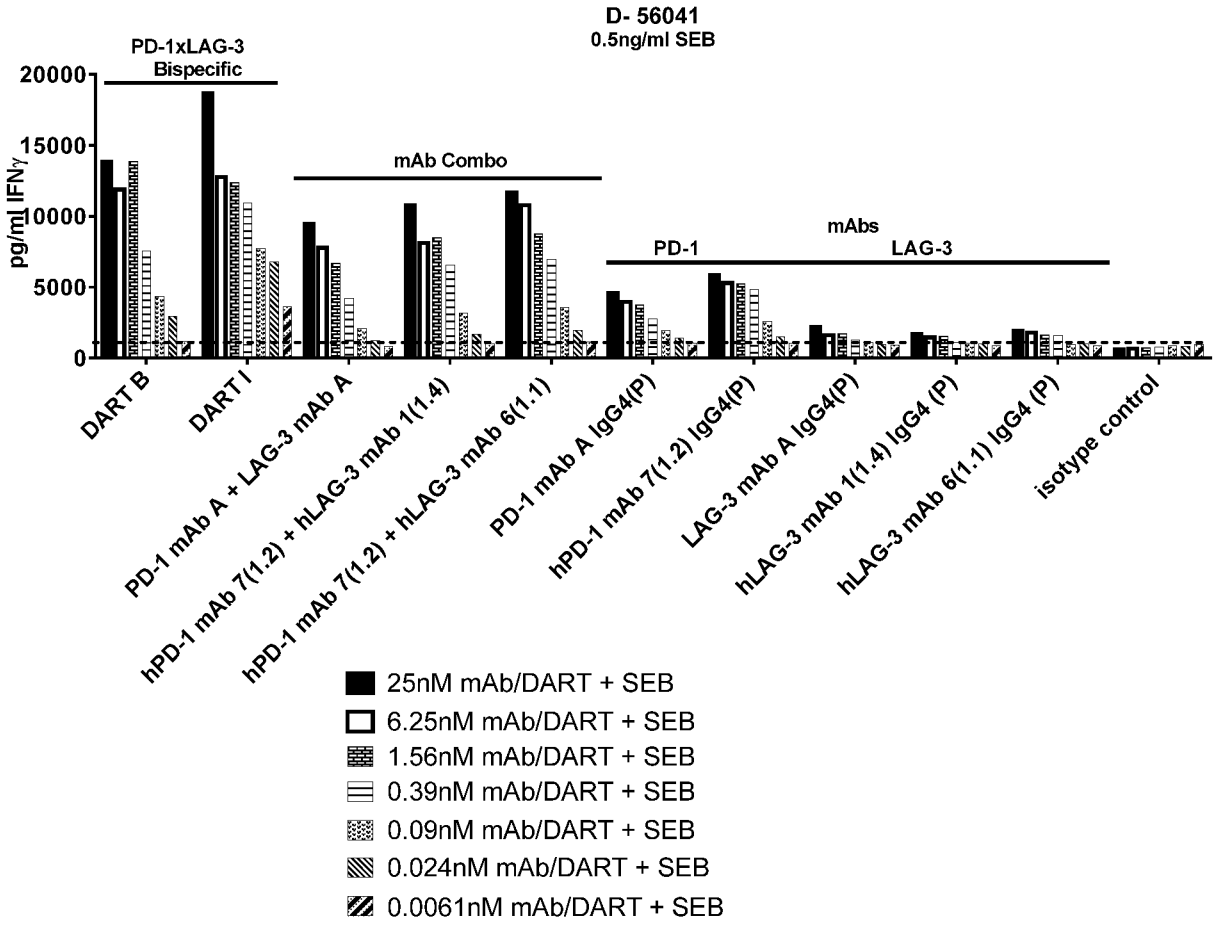
D- 56041
0.5ng/ml SEB
PD-1xLAG-3
Bispecific
mAb Combo
mAbs
PD-1
LAG-3
pg/ml IFNγ
20000
15000
10000
5000
0
DART B
DART I
PD-1 mAb A + LAG-3 mAb A
hPD-1 mAb 7(1.2) + hLAG-3 mAb 1(1.4)
hPD-1 mAb 7(1.2) + hLAG-3 mAb 6(1.1)
PD-1 mAb A IgG4(P)
hPD-1 mAb 7(1.2) IgG4(P)
LAG-3 mAb A IgG4(P)
hLAG-3 mAb 1(1.4)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

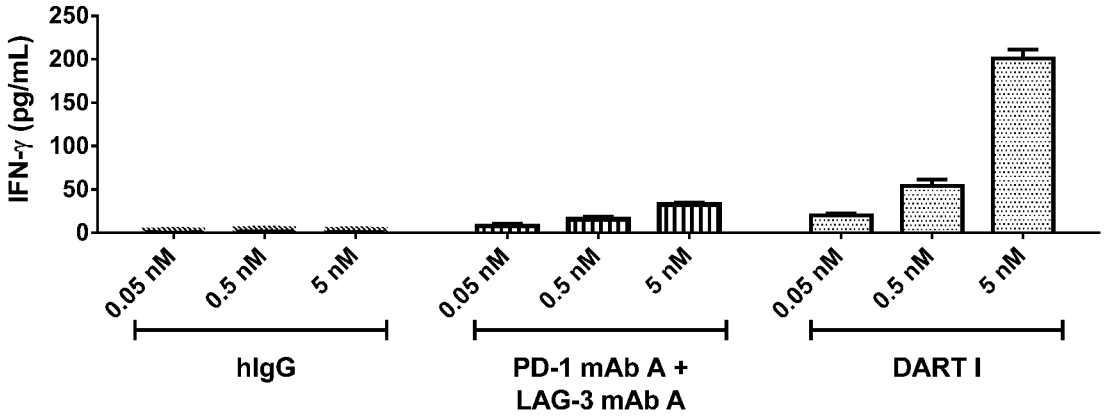

Figure 21A

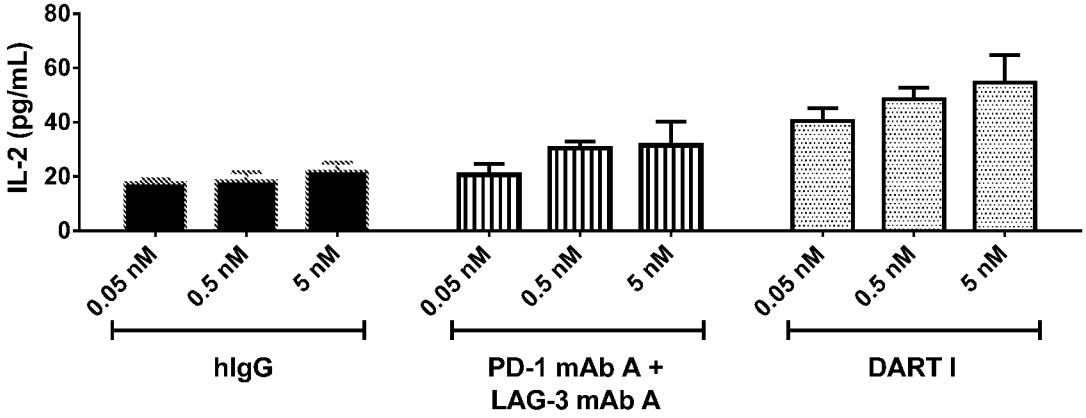

Figure 21B

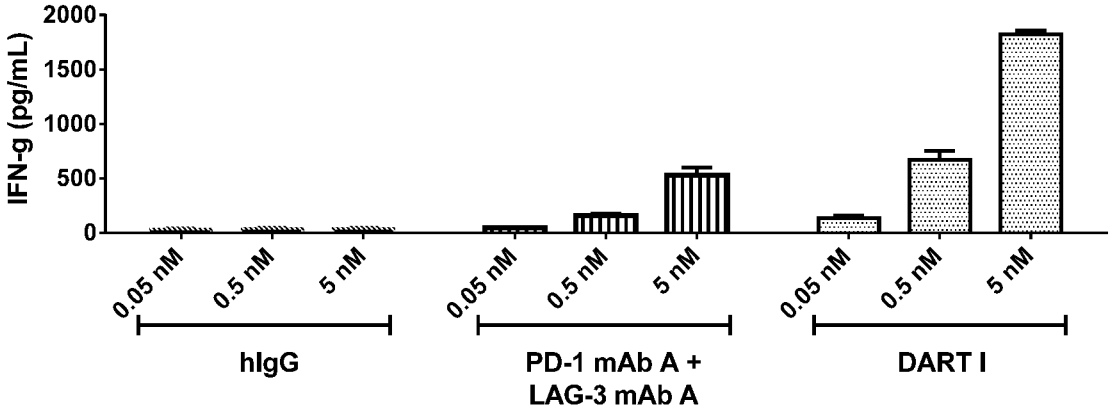

Figure 21C

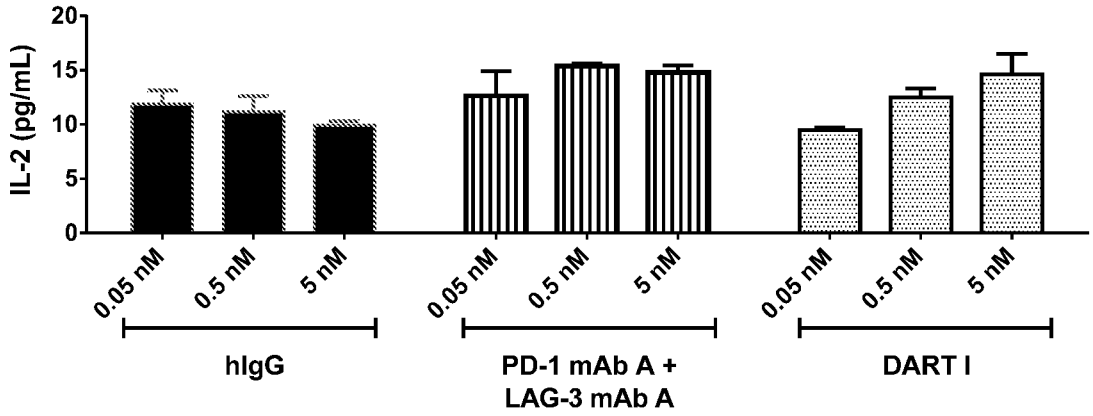

Figure 21D

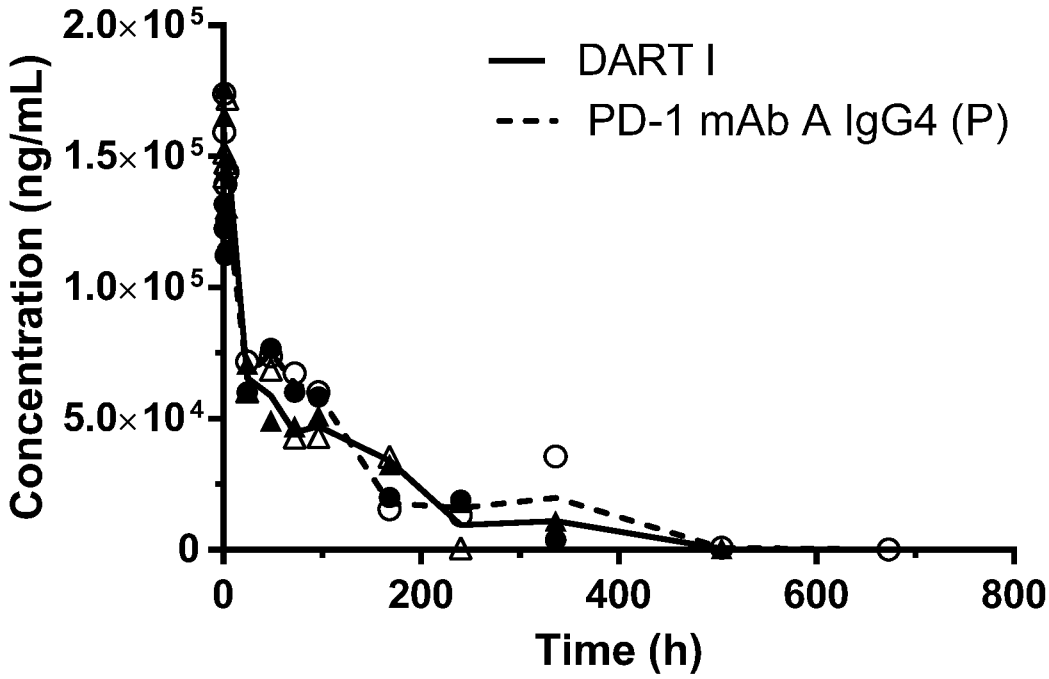
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0
Concentration (ng/mL)
0
200
400
600
800
Time (h)
DART I
PD-1 mAb A IgG4 (P)

Figure 22

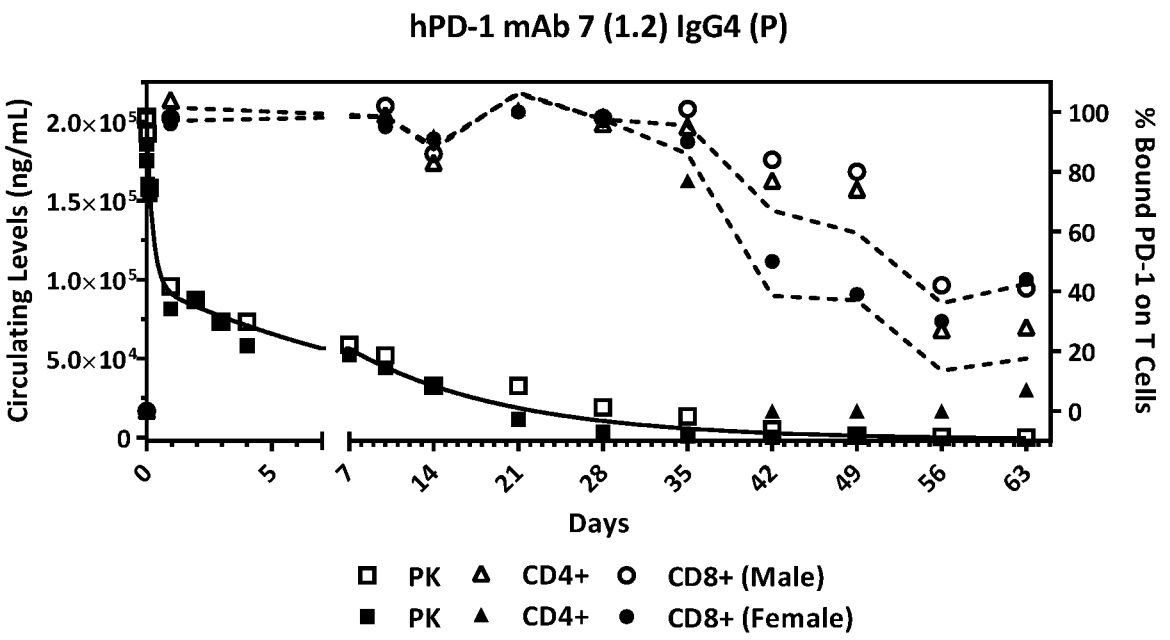

Figure 23A

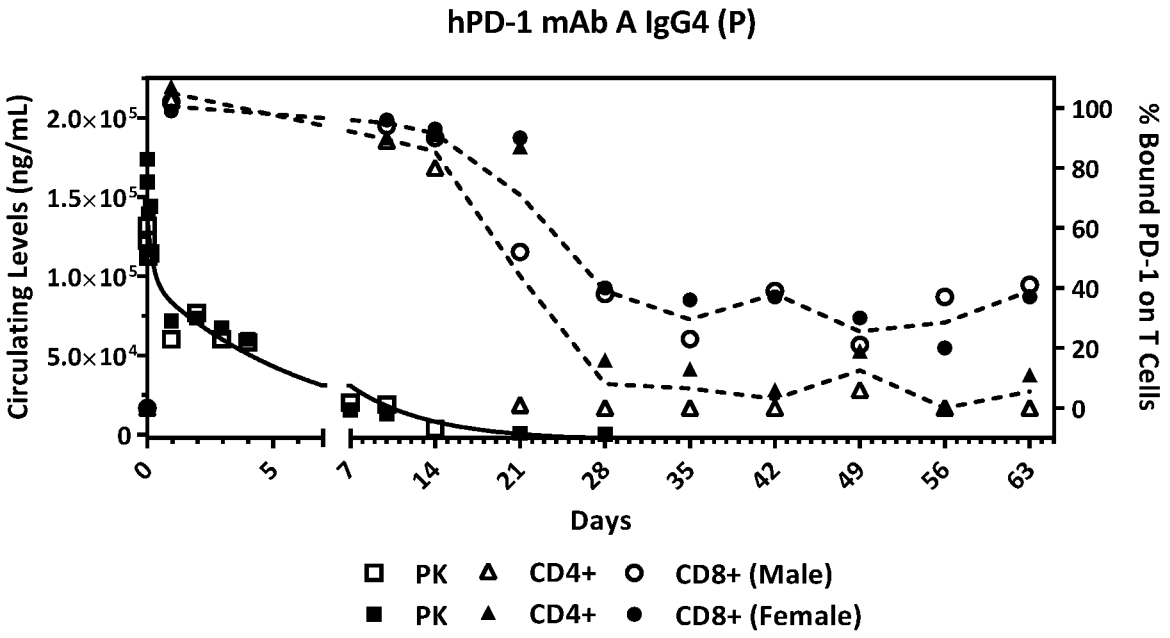

Figure 23B

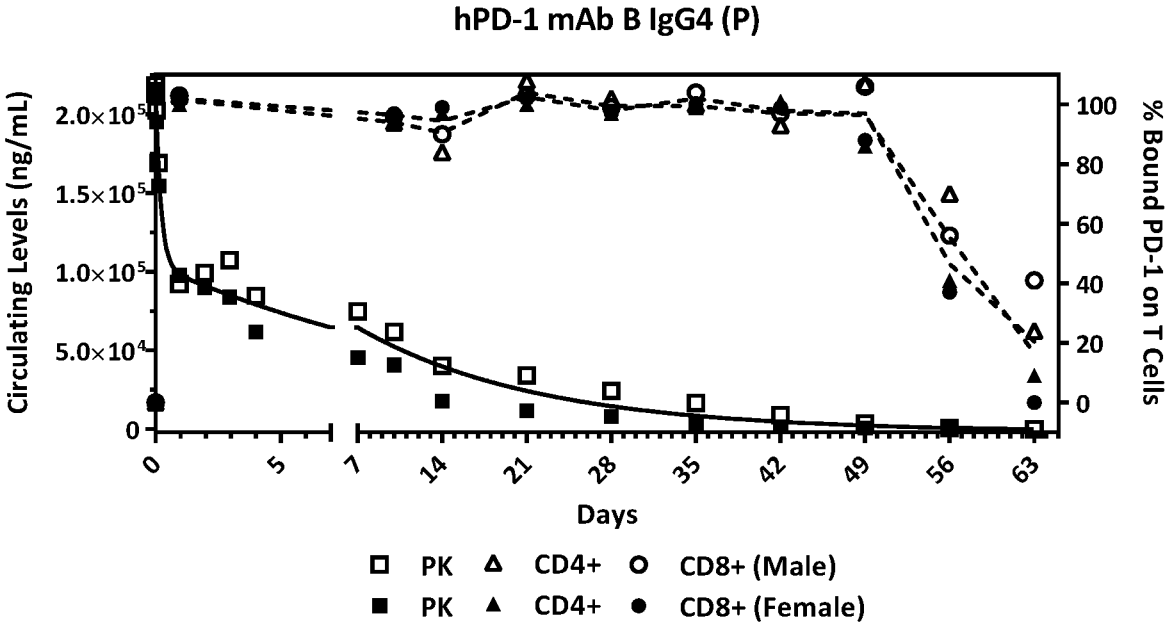
hPD-1 mAb B IgG4 (P)
Circulating Levels (ng/mL)
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0
% Bound PD-1 on T Cells
100
80
60
40
20
0
0
5
7
14
21
28
35
42
49
56
63
Days
PK
CD4+
CD8+ (Male)
PK
CD4+
CD8+ (Female)

Figure 23C