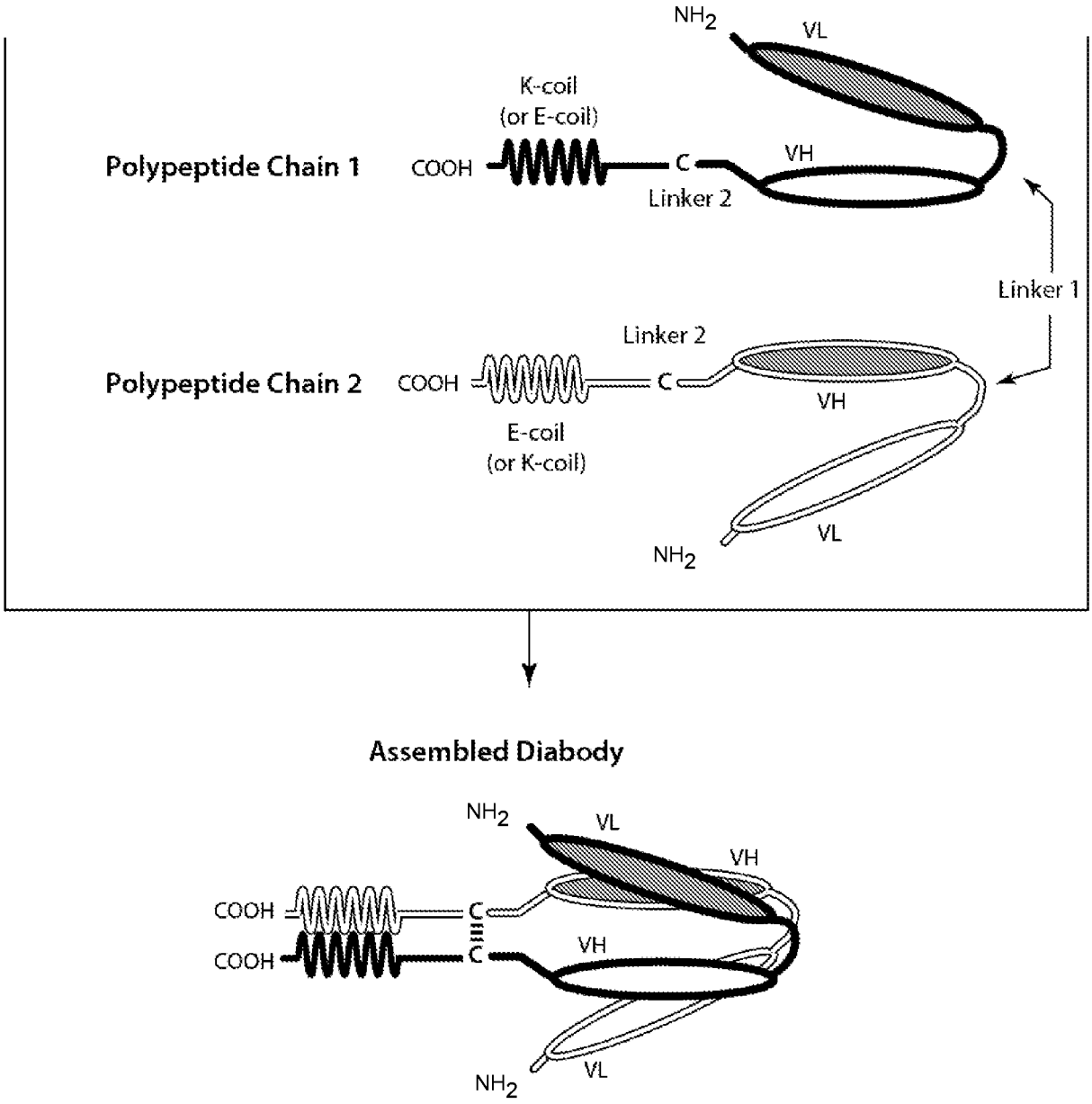

NH2
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
C
VH
Linker 2
Linker 1
Linker 2
Polypeptide Chain 2
COOH
C
VH
E-coil
(or K-coil)
VL
NH2
Assembled Diabody
NH2
VL
VH
COOH
C
COOH
C
VH
VL
NH2

Figure 1

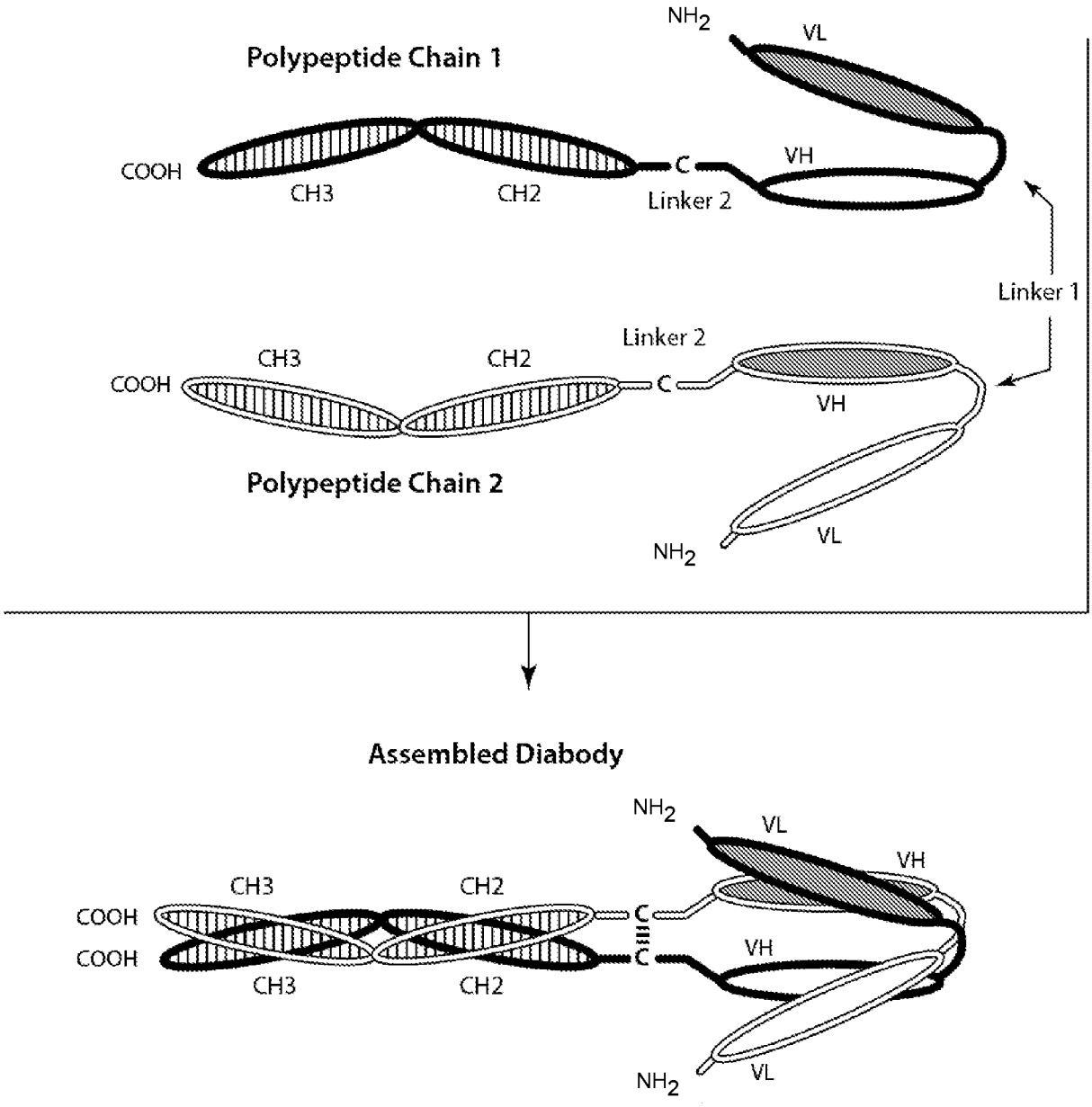

Polypeptide Chain 1
NH2
VL
VH
COOH
CH3
CH2
C
Linker 2
Linker 1
Linker 2
CH3
CH2
COOH
C
VH
Polypeptide Chain 2
NH2
VL
Assembled Diabody
NH2
VL
VH
CH3
CH2
COOH
COOH
C
C
VH
CH3
CH2
NH2
VL

Figure 2

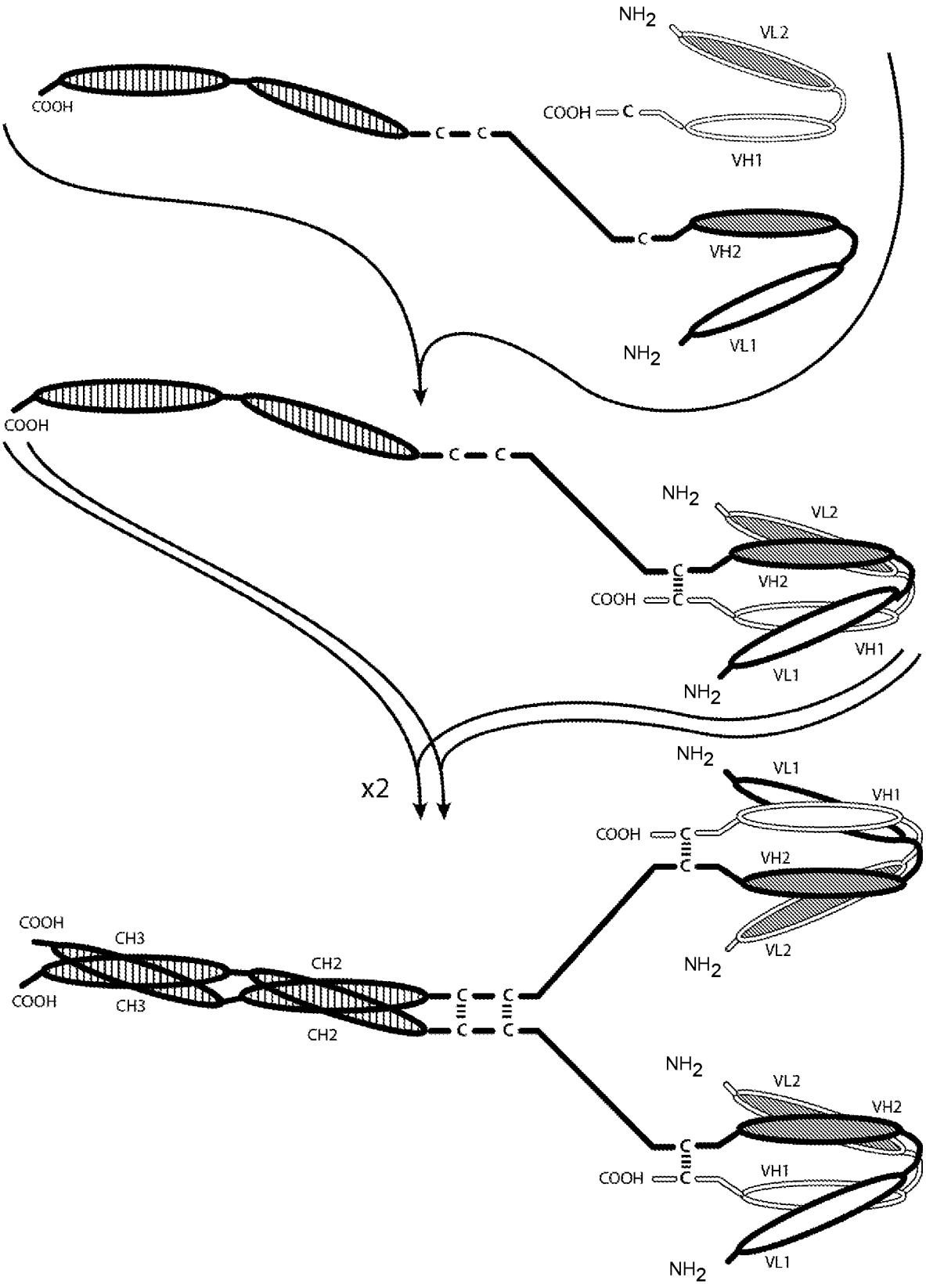

NH2
VL2
COOH
COOH
C
VH1
C
VH2
NH2
VL1
COOH
C
C
NH2
VL2
C
VH2
COOH
C
VH1
VL1
NH2
x2
NH2
VL1
VH1
COOH
C
VH2
C
NH2
VL2
COOH
CH3
CH2
COOH
CH3
CH2
NH2
VL2
VH2
C
COOH
C
VH1
NH2
VL1

Figure 3A

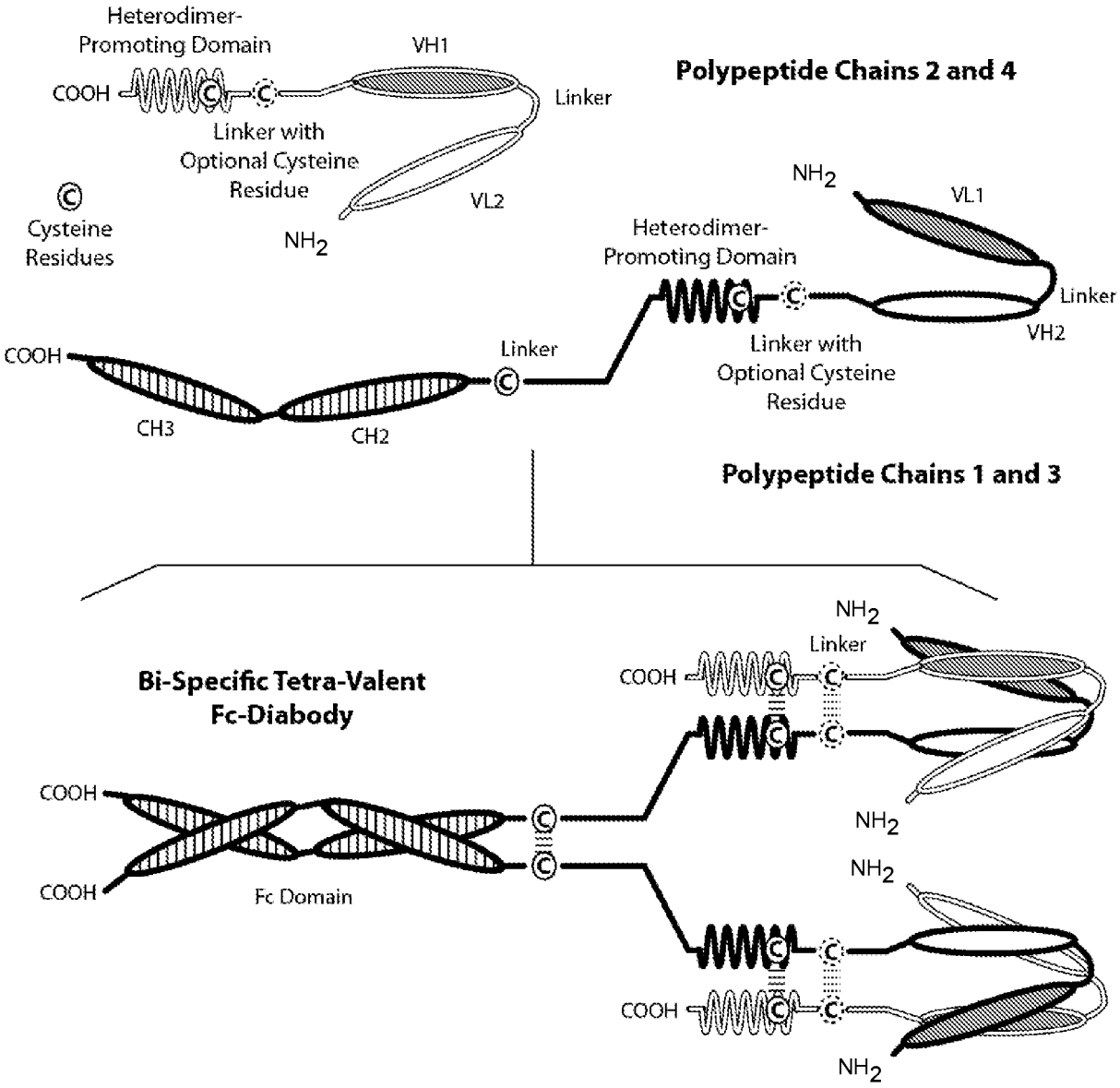

Heterodimer-
Promoting Domain
VH1
COOH
Linker
Linker with
Optional Cysteine
Residue
VL2
NH2
Cysteine
Residues
Polypeptide Chains 2 and 4
NH2
VL1
Heterodimer-
Promoting Domain
Linker
VH2
Linker with
Optional Cysteine
Residue
COOH
Linker
CH3
CH2
Polypeptide Chains 1 and 3
Bi-Specific Tetra-Valent
Fc-Diabody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

Figure 3B

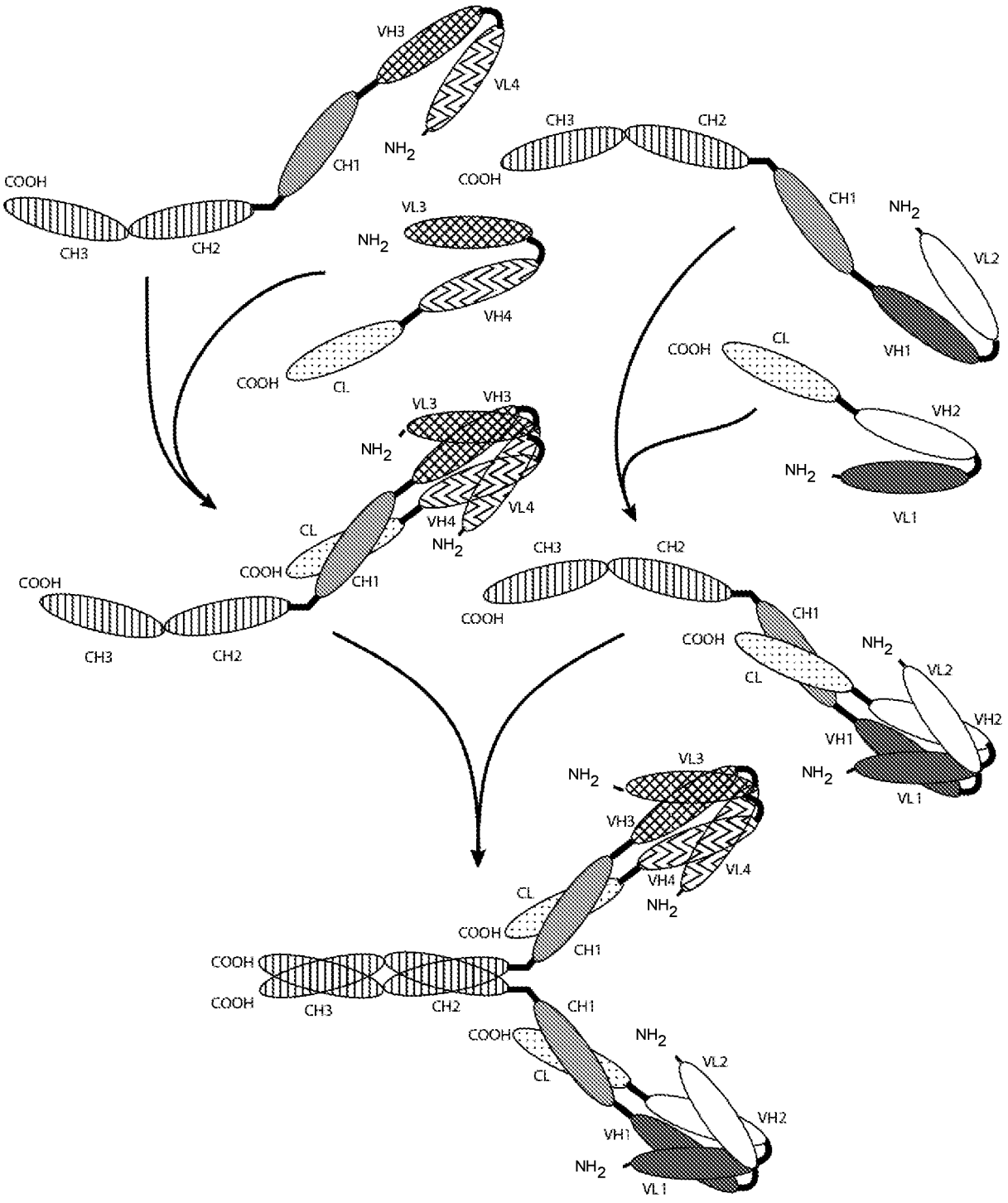

VH3
VL4
NH2
CH1
COOH
CH3
CH2
VL3
NH2
VH4
COOH
CL
CH3
CH2
CH1
NH2
VL2
COOH
CL
VH1
VH2
NH2
VL1
VL3
VH3
NH2
VH4
VL4
NH2
CL
COOH
CH1
COOH
CH3
CH2
CH3
CH2
COOH
CH1
COOH
CL
NH2
VL2
VH2
VH1
NH2
VL1
VL3
NH2
VH3
VH4
VL4
NH2
CL
COOH
CH1
COOH
COOH
CH3
CH2
CH1
COOH
CL
NH2
VL2
VH2
VH1
NH2
VL1

Figure 3C

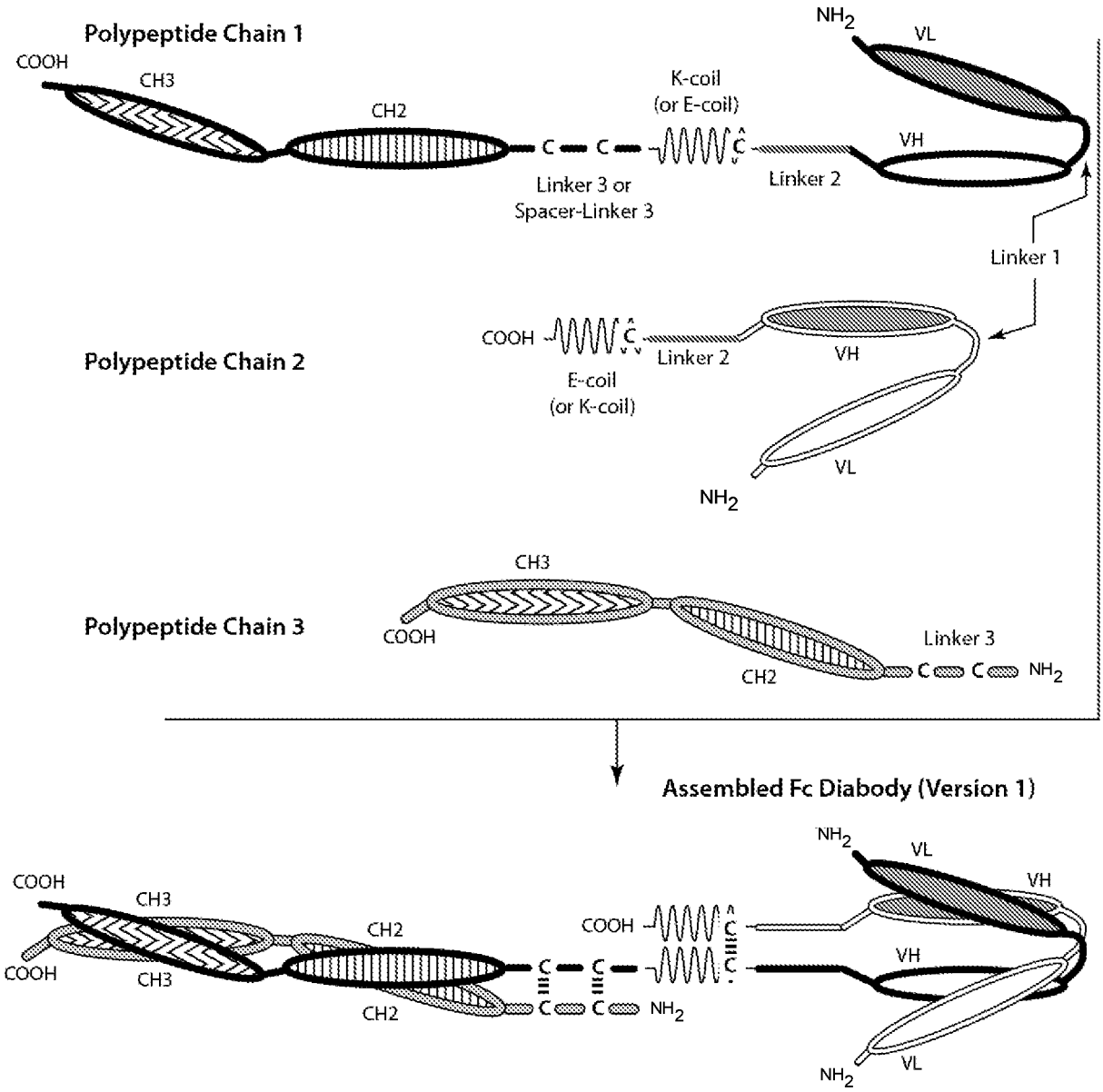

Polypeptide Chain 1
COOH
CH3
CH2
K-coil
(or E-coil)
NH2
VL
VH
Linker 3 or
Spacer-Linker 3
Linker 2
Linker 1
Polypeptide Chain 2
COOH
Linker 2
VH
E-coil
(or K-coil)
VL
NH2
Polypeptide Chain 3
CH3
COOH
Linker 3
CH2
NH2
Assembled Fc Diabody (Version 1)
NH2
VL
VH
COOH
CH3
CH2
COOH
CH3
VH
CH2
NH2
NH2
VL

Figure 4A

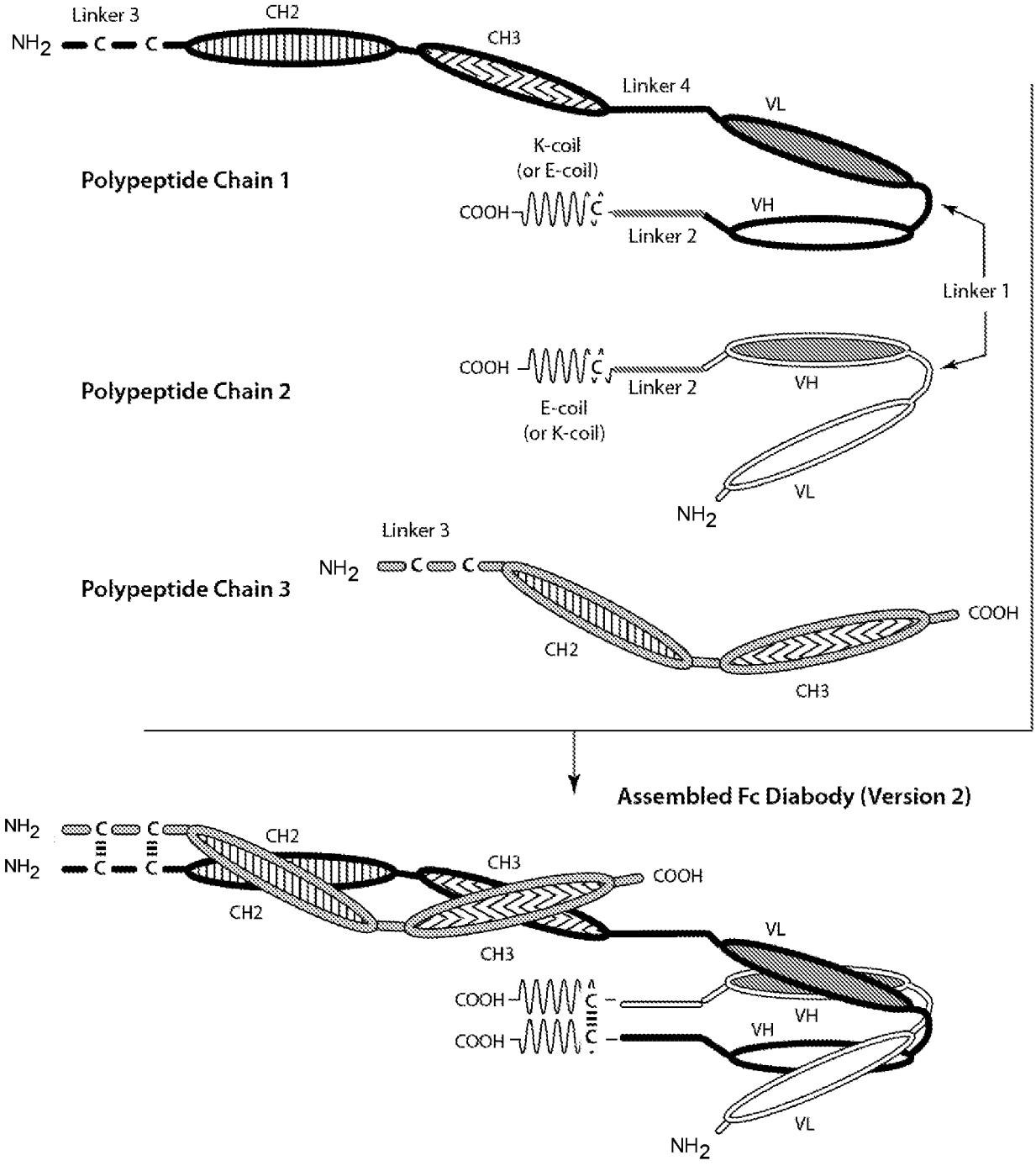

Linker 3
CH2
NH2
C
C
CH3
Linker 4
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
VH
Linker 2
Linker 1
Polypeptide Chain 2
COOH
Linker 2
VH
E-coil
(or K-coil)
VL
NH2
Linker 3
Polypeptide Chain 3
NH2
COOH
CH2
CH3
Assembled Fc Diabody (Version 2)
NH2
NH2
CH2
CH3
COOH
CH2
VL
CH3
COOH
VH
COOH
VH
VL
NH2

Figure 4B

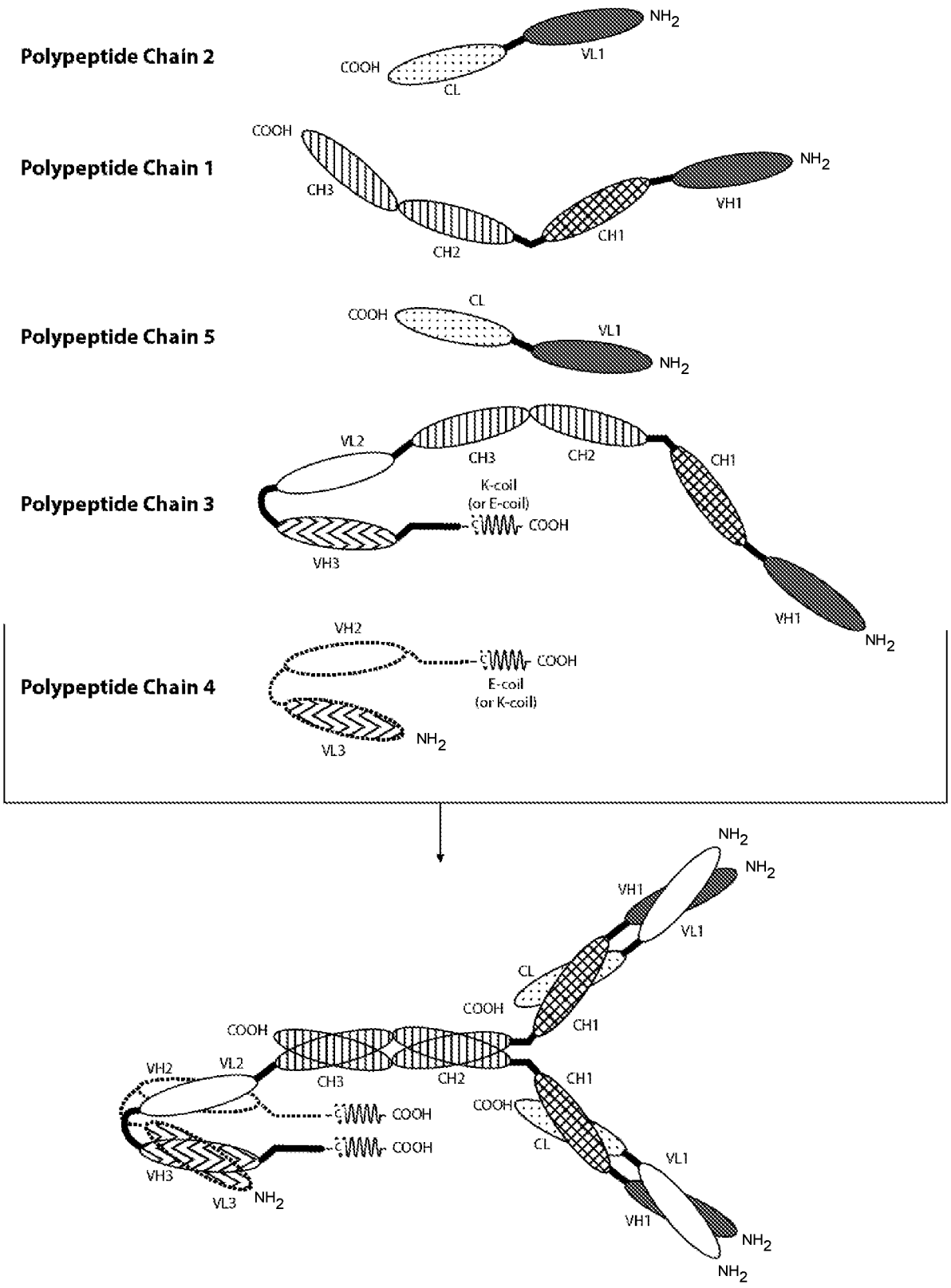

Polypeptide Chain 2
NH2
VL1
COOH
CL
Polypeptide Chain 1
COOH
CH3
NH2
VH1
CH2
CH1
Polypeptide Chain 5
CL
COOH
VL1
NH2
Polypeptide Chain 3
VL2
CH3
CH2
CH1
K-coil
(or E-coil)
COOH
VH3
VH1
NH2
Polypeptide Chain 4
VH2
COOH
E-coil
(or K-coil)
VL3
NH2
NH2
NH2
VH1
VL1
CL
COOH
CH1
COOH
VH2
VL2
CH3
CH2
CH1
COOH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

Figure 5

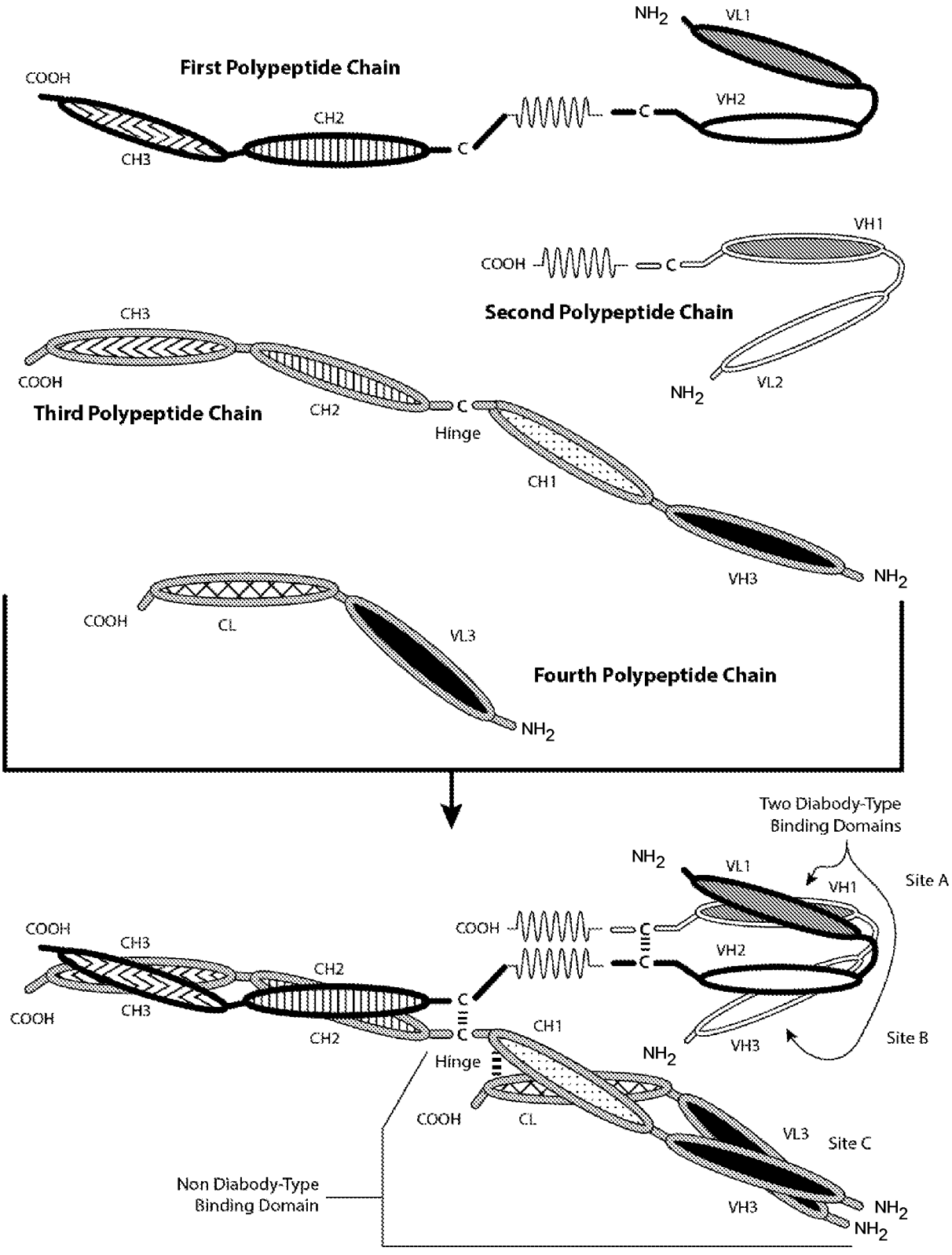

First Polypeptide Chain
COOH
CH3
CH2
C
C
NH2
VL1
VH2
Second Polypeptide Chain
COOH
C
VH1
NH2
VL2
Third Polypeptide Chain
COOH
CH3
CH2
C
Hinge
CH1
VH3
NH2
Fourth Polypeptide Chain
COOH
CL
VL3
NH2
Two Diabody-Type Binding Domains
Site A
Site B
Site C
Non Diabody-Type Binding Domain

Figure 6A

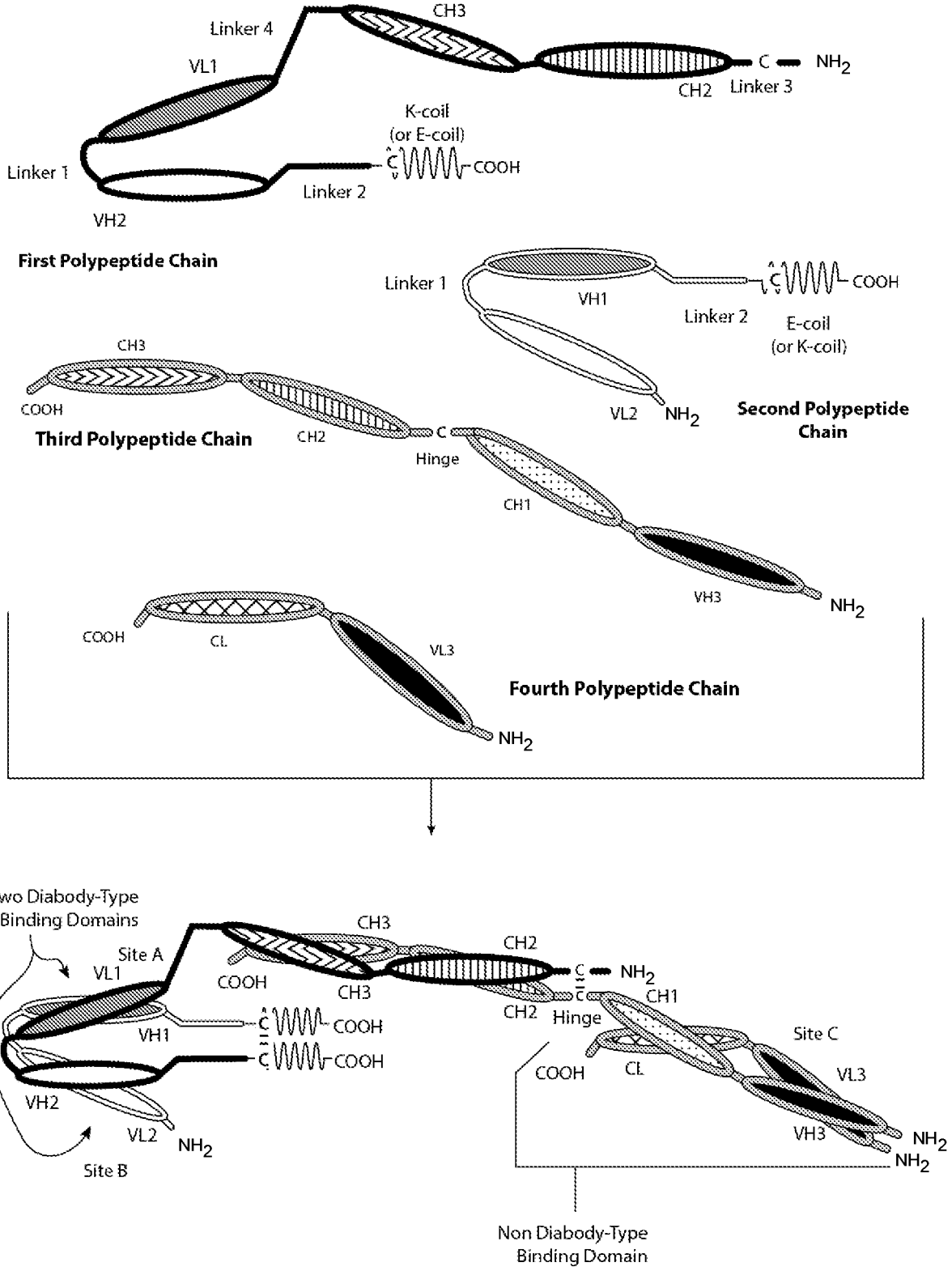

Linker 4
CH3
VL1
C
NH2
CH2
Linker 3
K-coil
(or E-coil)
Linker 1
COOH
Linker 2
VH2
First Polypeptide Chain
Linker 1
VH1
COOH
Linker 2
E-coil
(or K-coil)
CH3
COOH
VL2
NH2
Second Polypeptide Chain
Third Polypeptide Chain
CH2
C
Hinge
CH1
VH3
NH2
COOH
CL
VL3
Fourth Polypeptide Chain
NH2
Two Diabody-Type
Binding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

Figure 6B

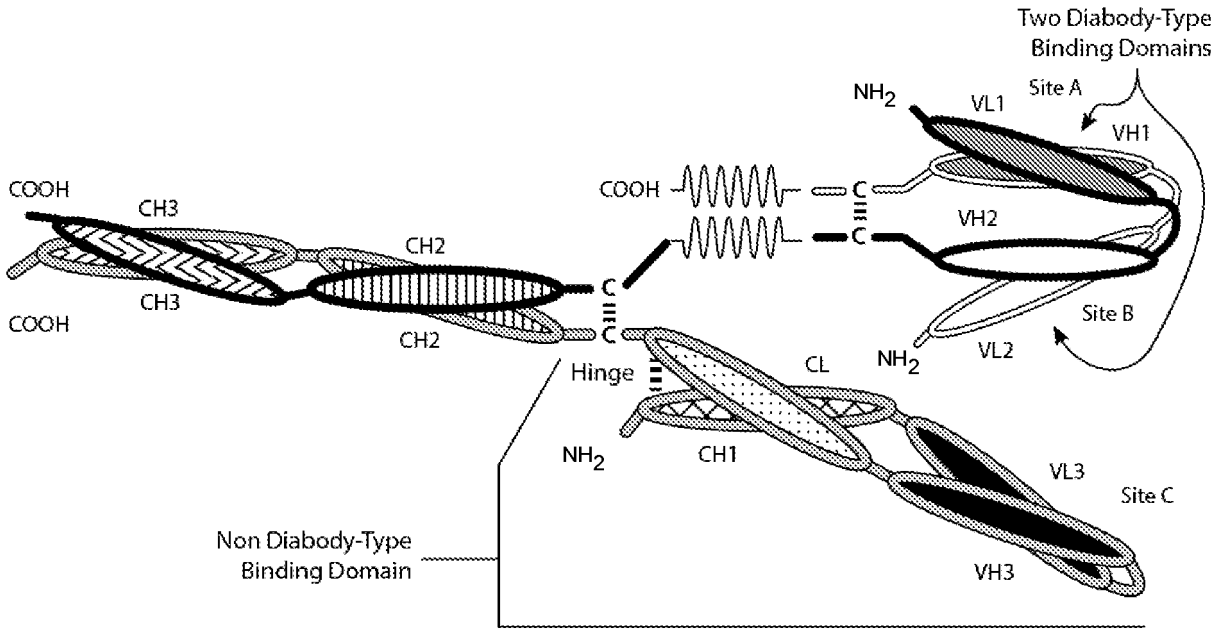


Figure 6C

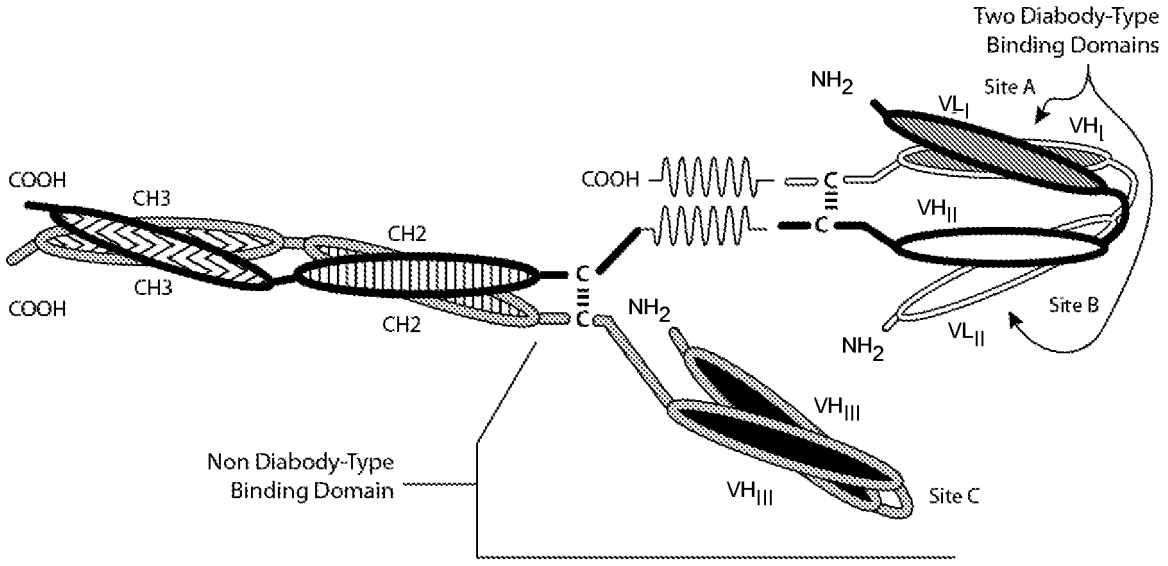


Figure 6D

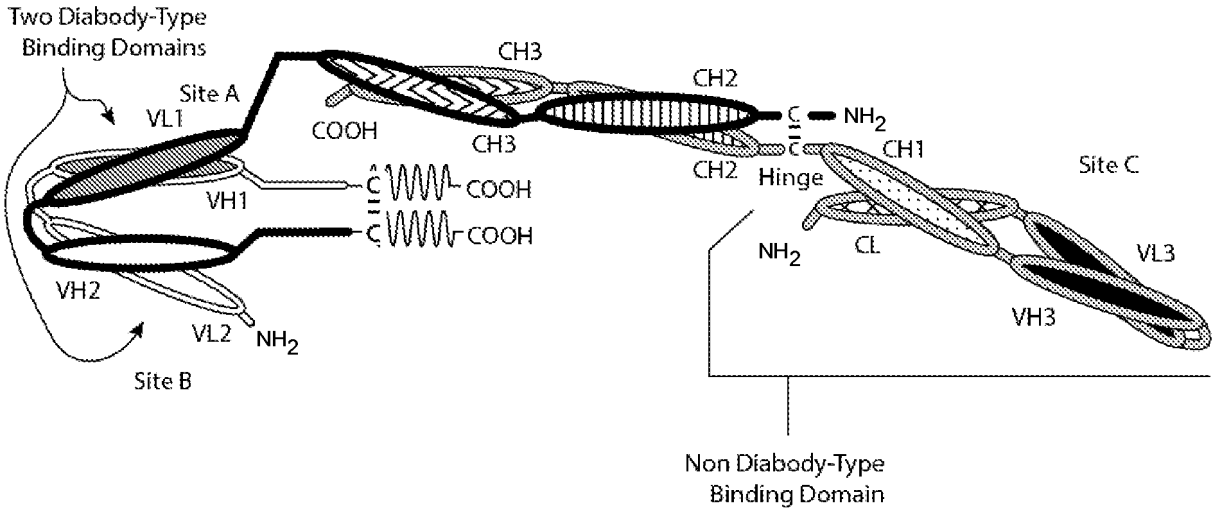


Figure 6E

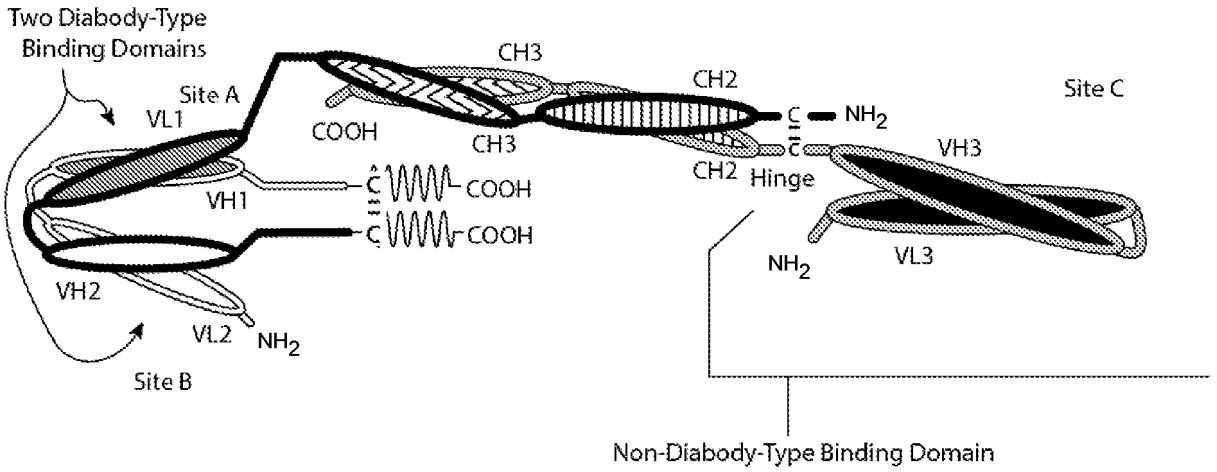


Figure 6F

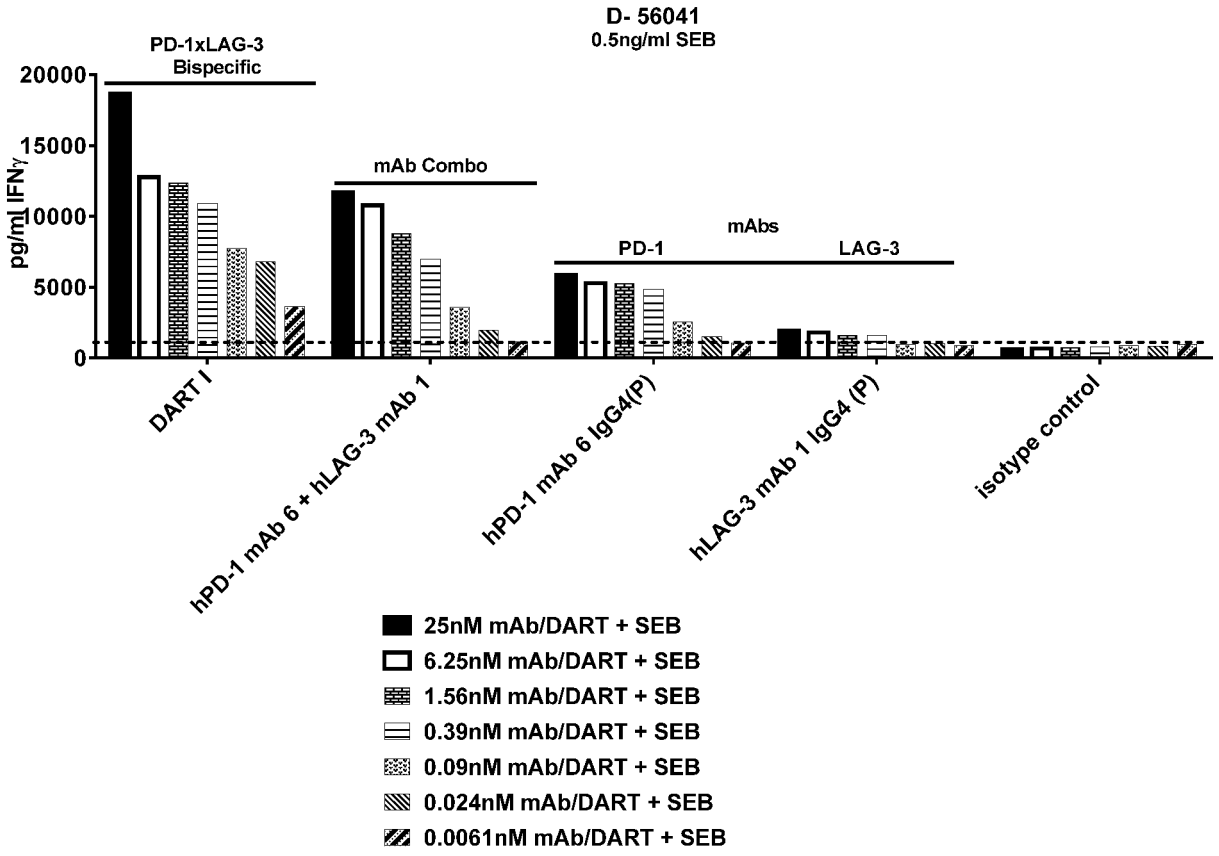

D- 56041
0.5ng/ml SEB
PD-1xLAG-3
Bispecific
mAb Combo
mAbs
PD-1
LAG-3
pg/ml IFNγ
20000
15000
10000
5000
0
DART I
hPD-1 mAb 6 + hLAG-3 mAb 1
hPD-1 mAb 6 IgG4(P)
hLAG-3 mAb 1 IgG4 (P)
isotype control
25nM mAb/DART + SEB
6.25nM mAb/DART + SEB
1.56nM mAb/DART + SEB
0.39nM mAb/DART + SEB
0.09nM mAb/DART +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

Figure 7

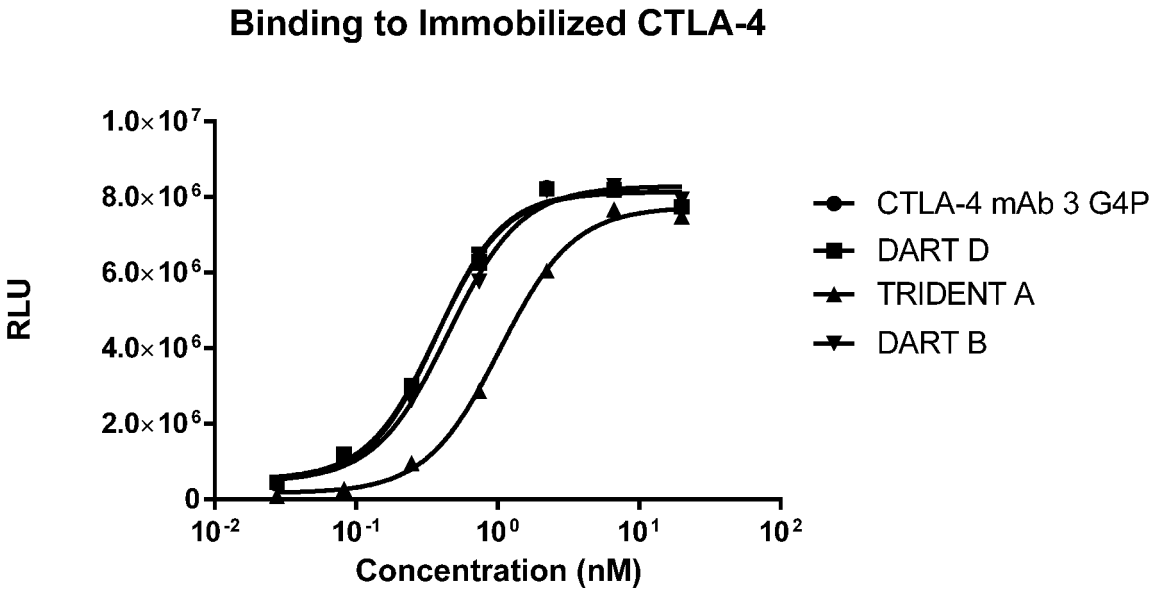


Figure 8A

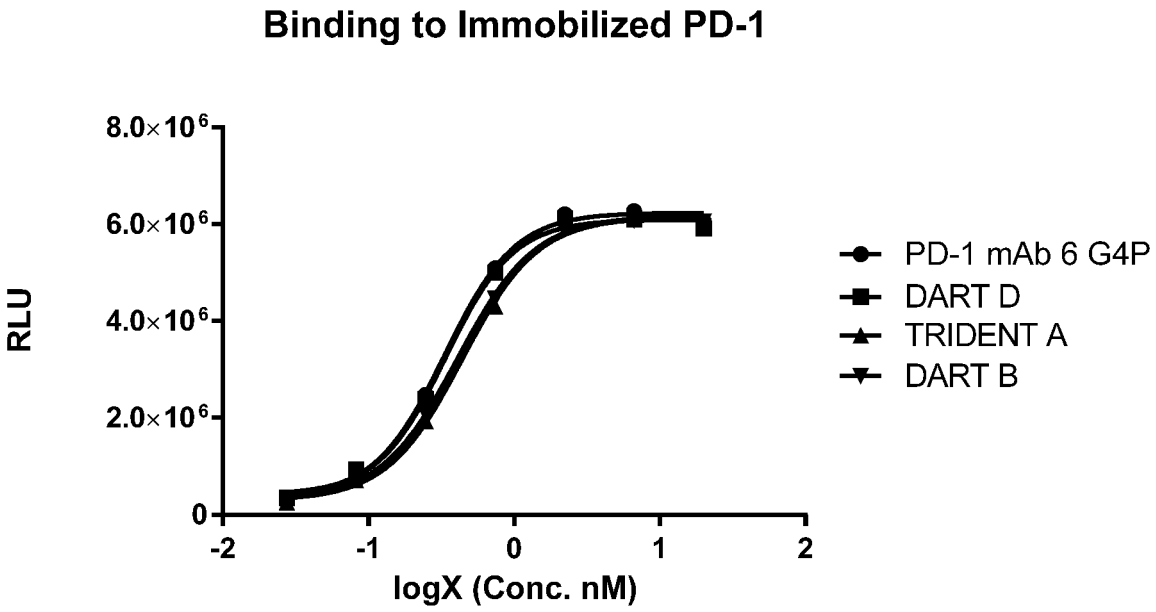


Figure 8B

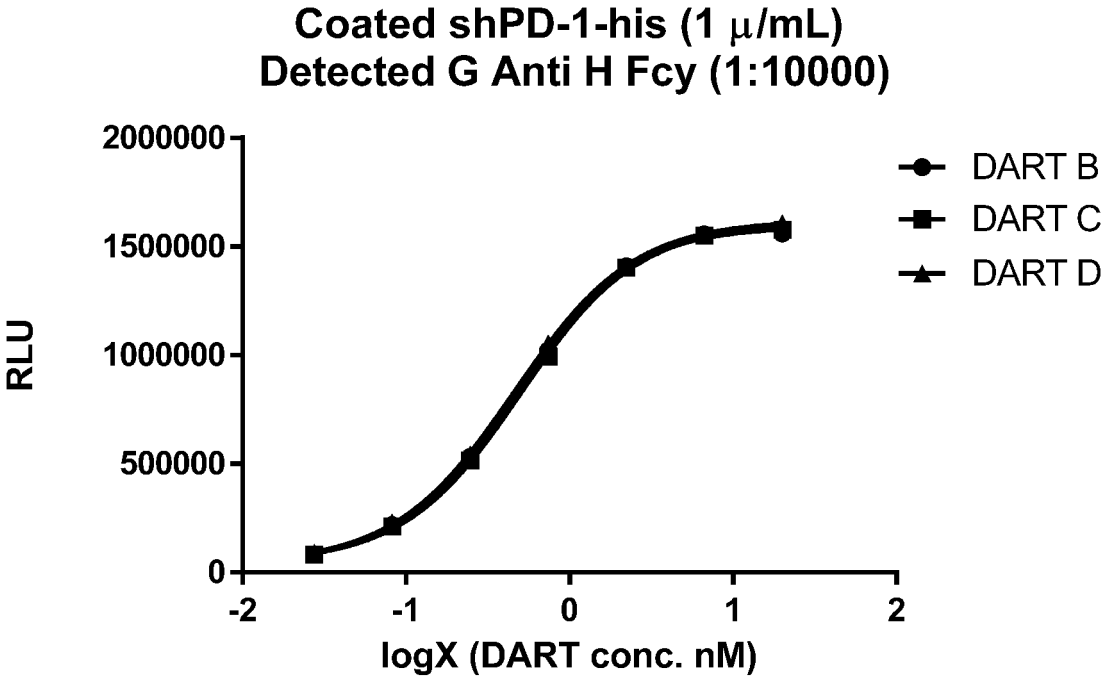


Figure 8C

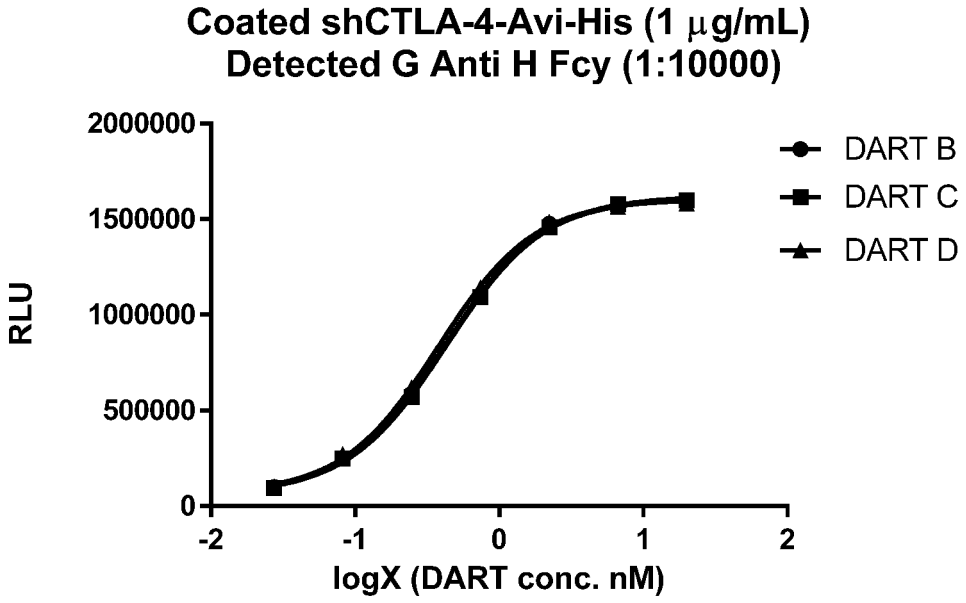


Figure 8D

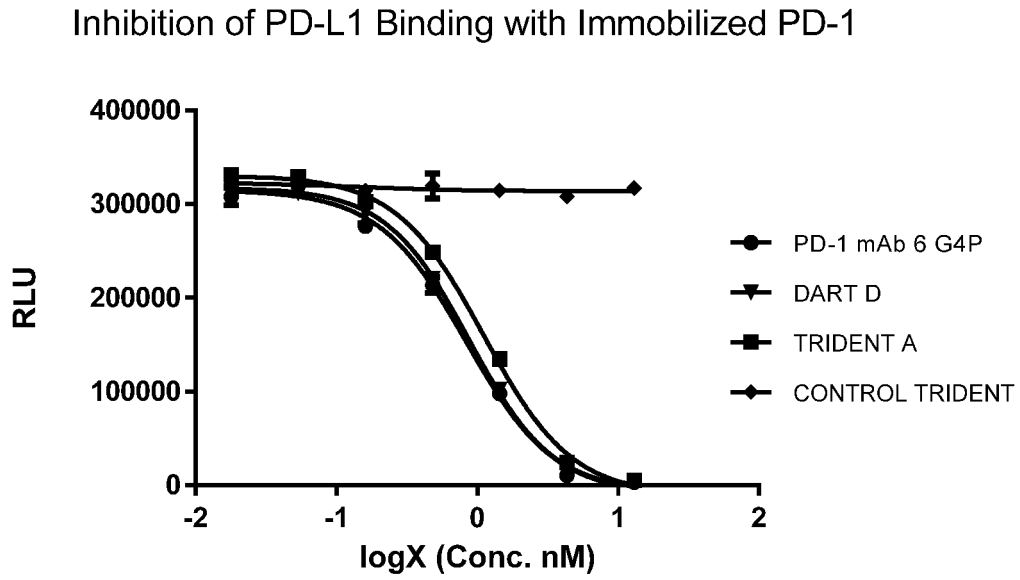


Figure 9A

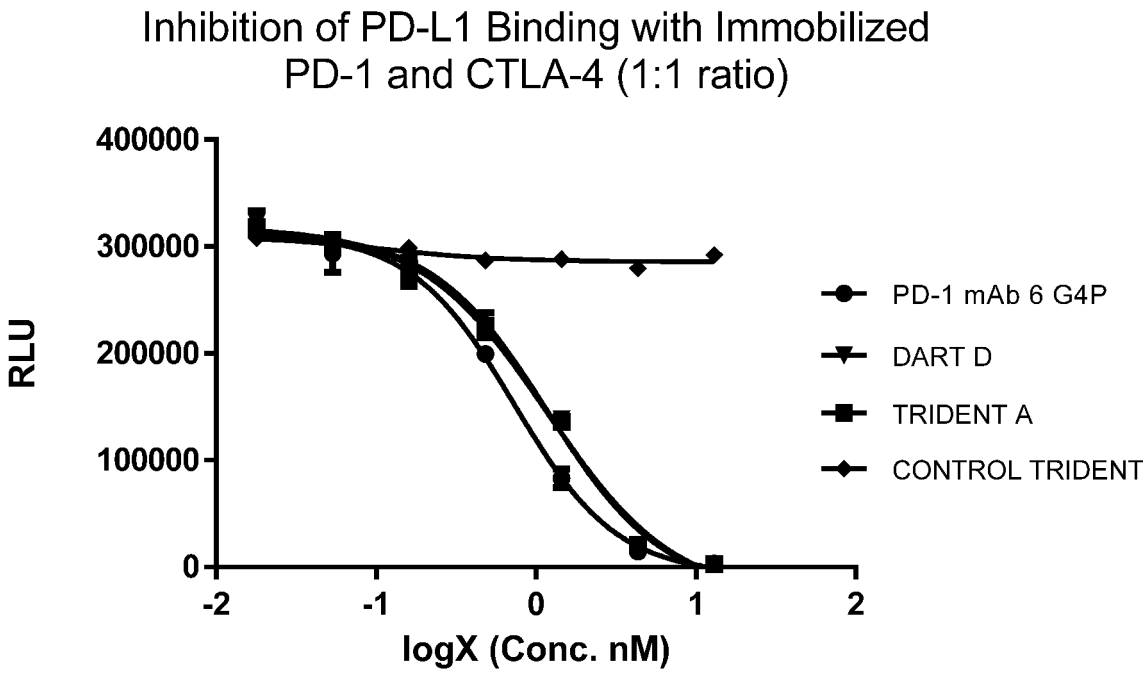


Figure 9B

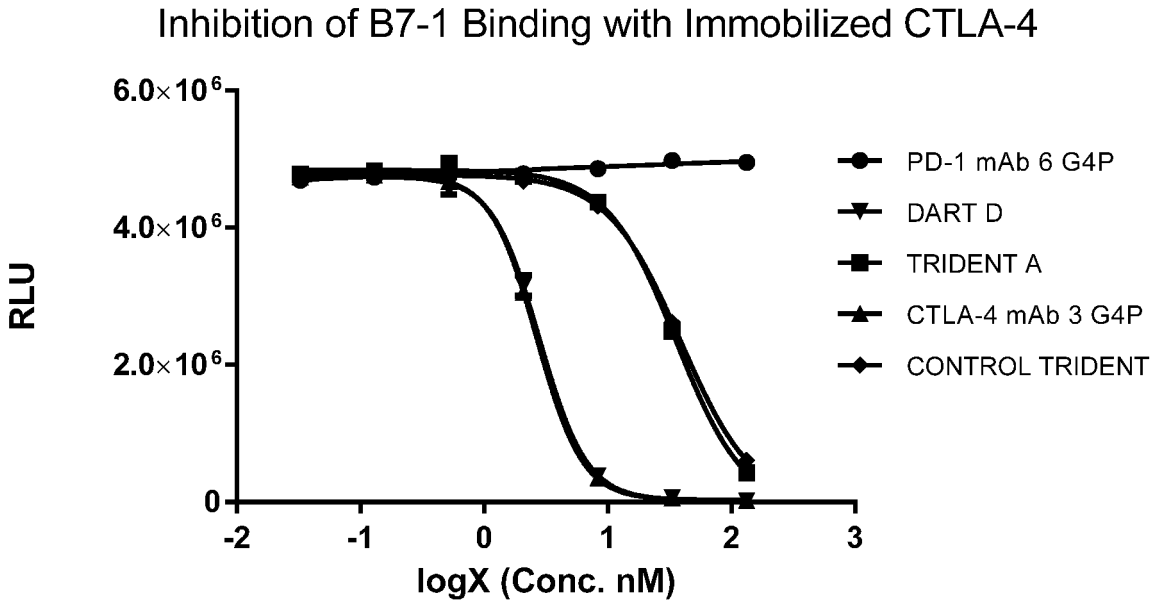


Figure 9C

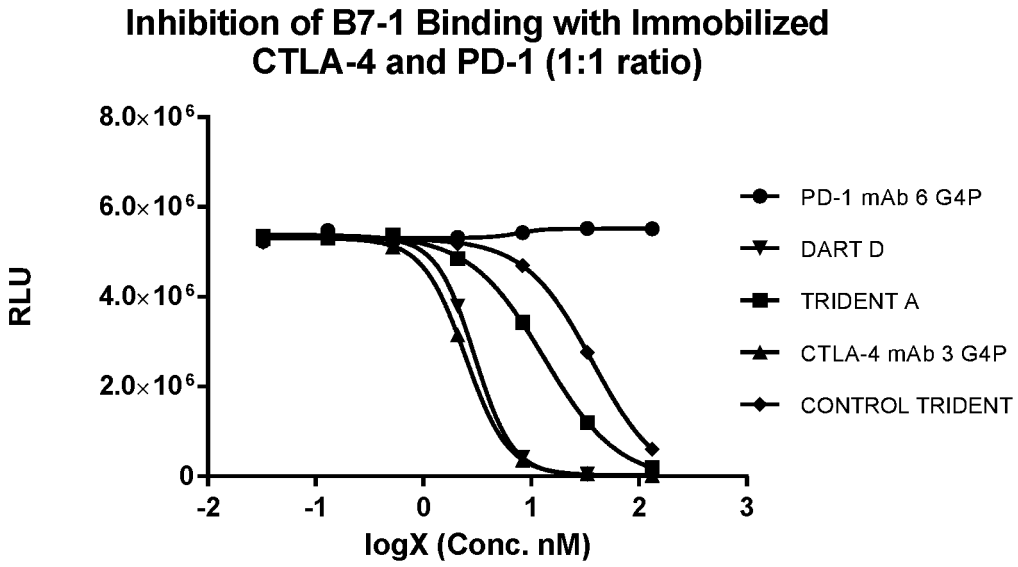


Figure 9D

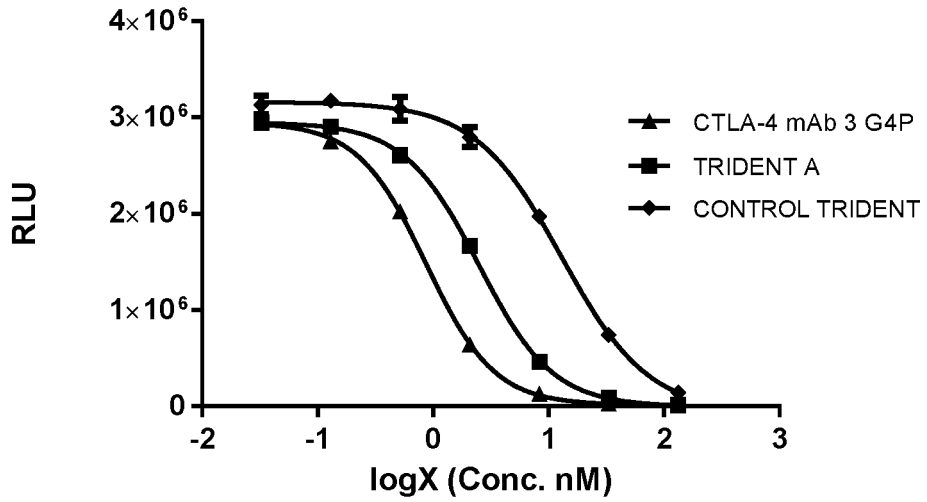

Inhibition of B7-1 Binding with Immobilized CTLA-4 and PD-1 (1:4 ratio)
4×10⁶
3×10⁶
2×10⁶
1×10⁶
0
RLU
-2
-1
0
1
2
3
logX (Conc. nM)
CTLA-4 mAb 3 G4P
TRIDENT A
CONTROL TRIDENT

Figure 9E

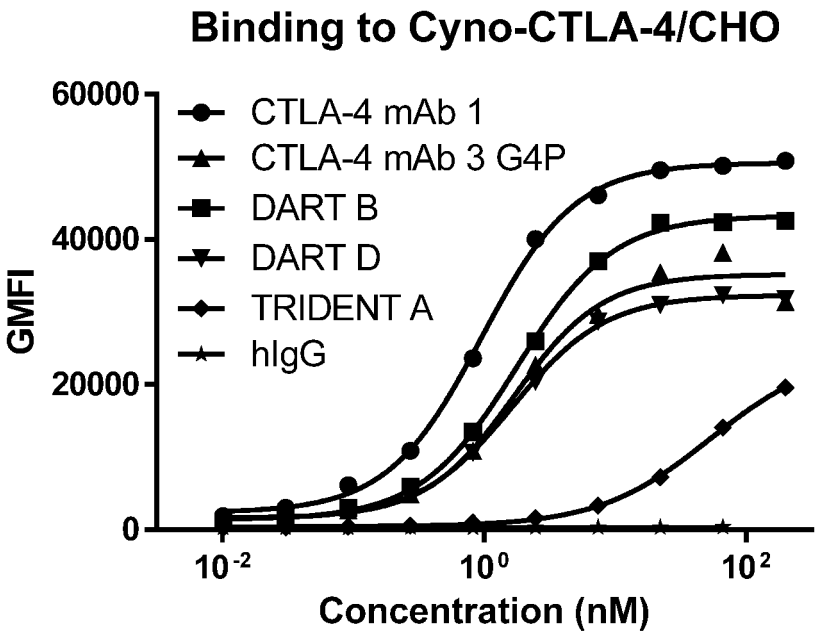


Figure 10A

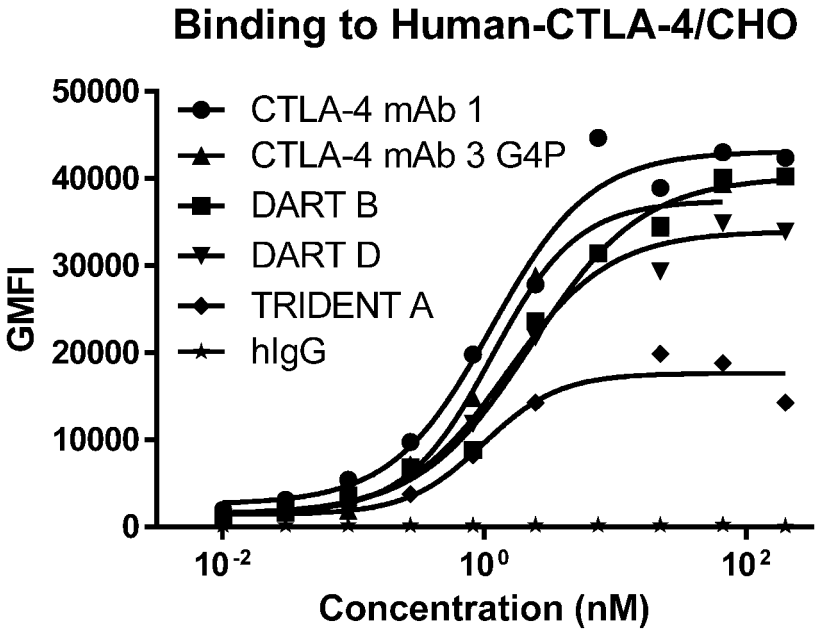


Figure 10B

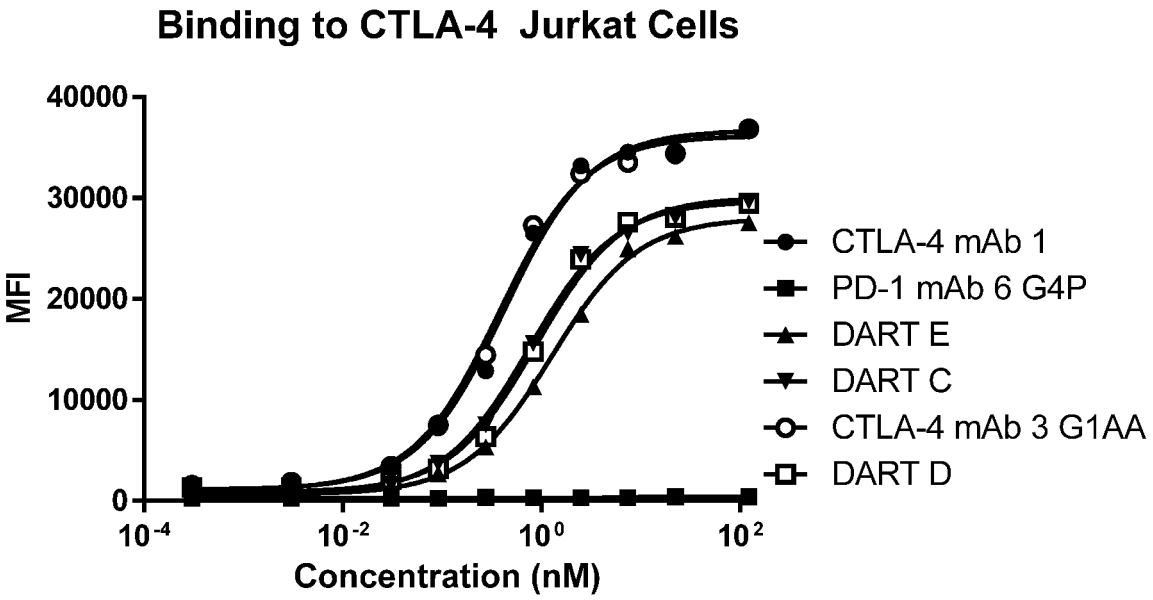


Figure 11A

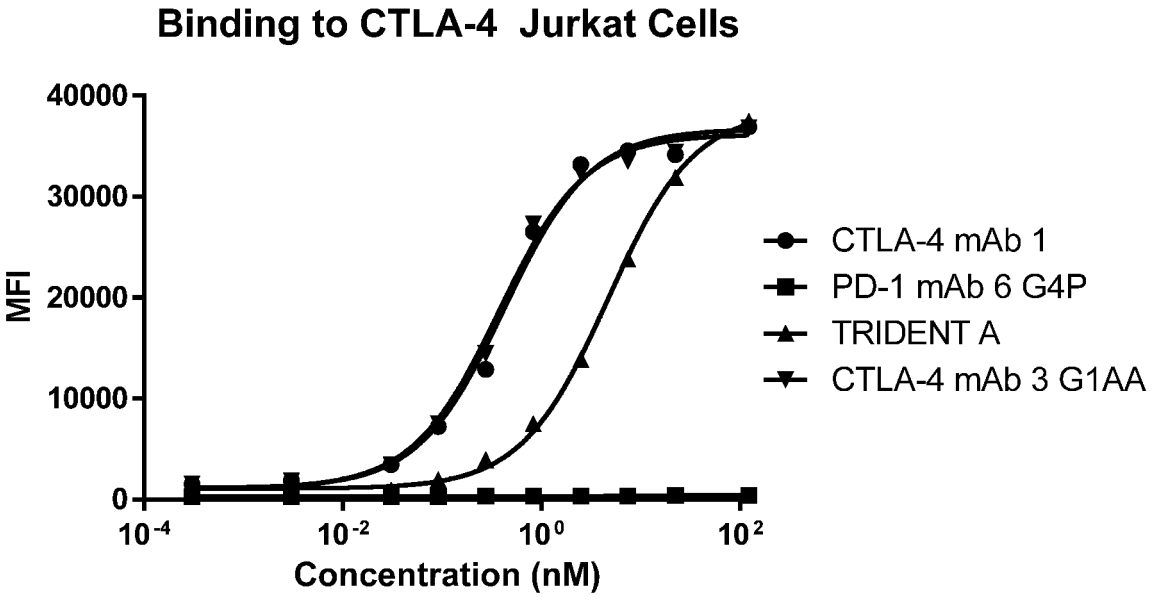
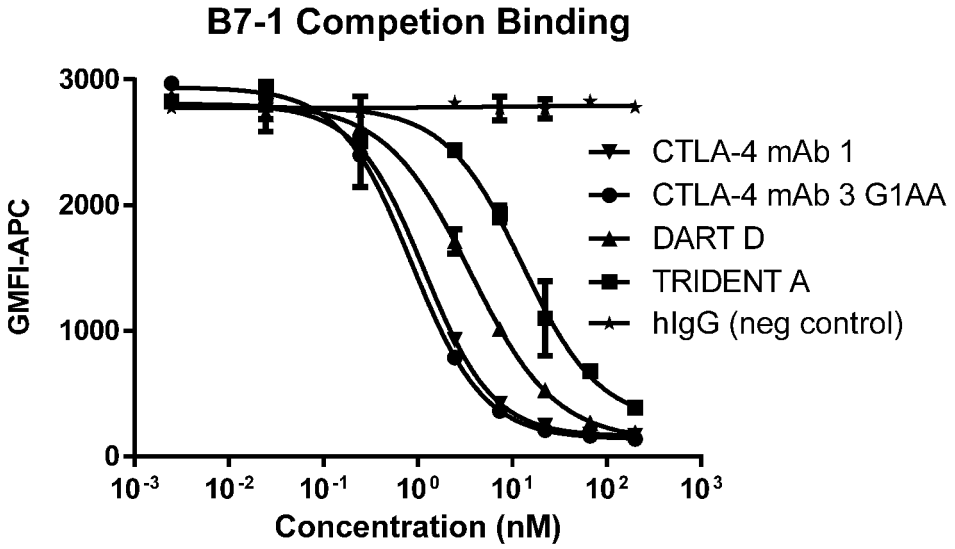
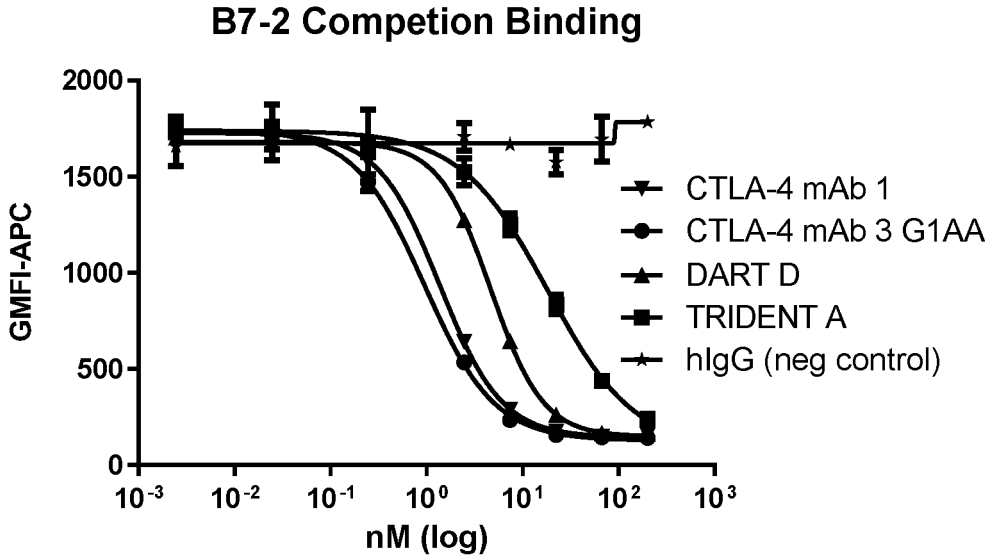


Figure 11B



B7-1: 10 ng/ml

Figure 12A



B7-2: 116.8 ng/ml

Figure 12B

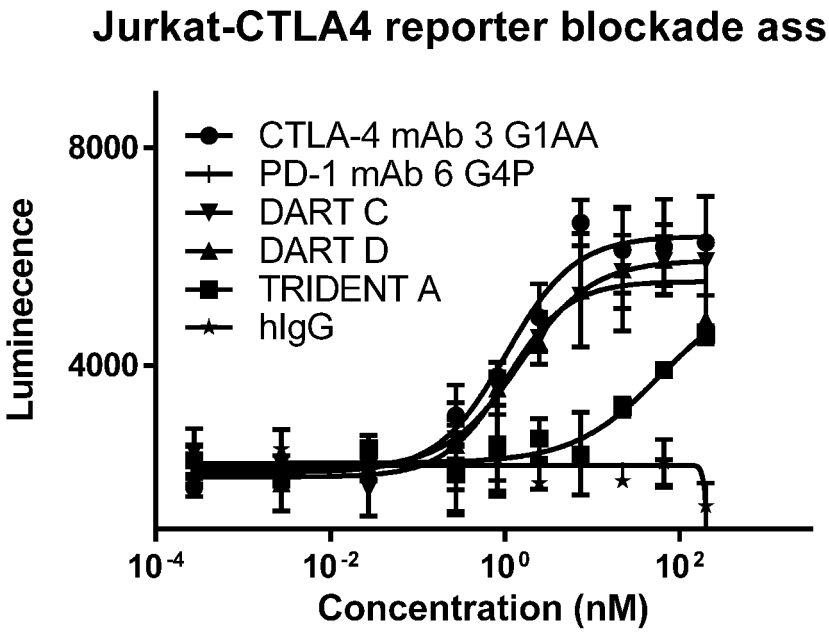


Figure 13

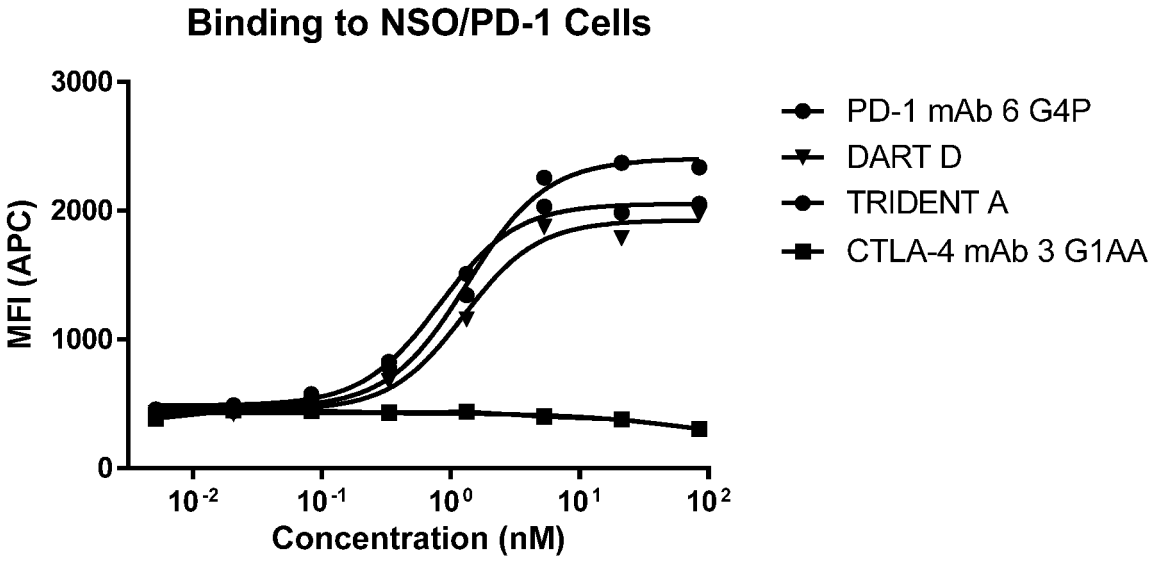


Figure 14

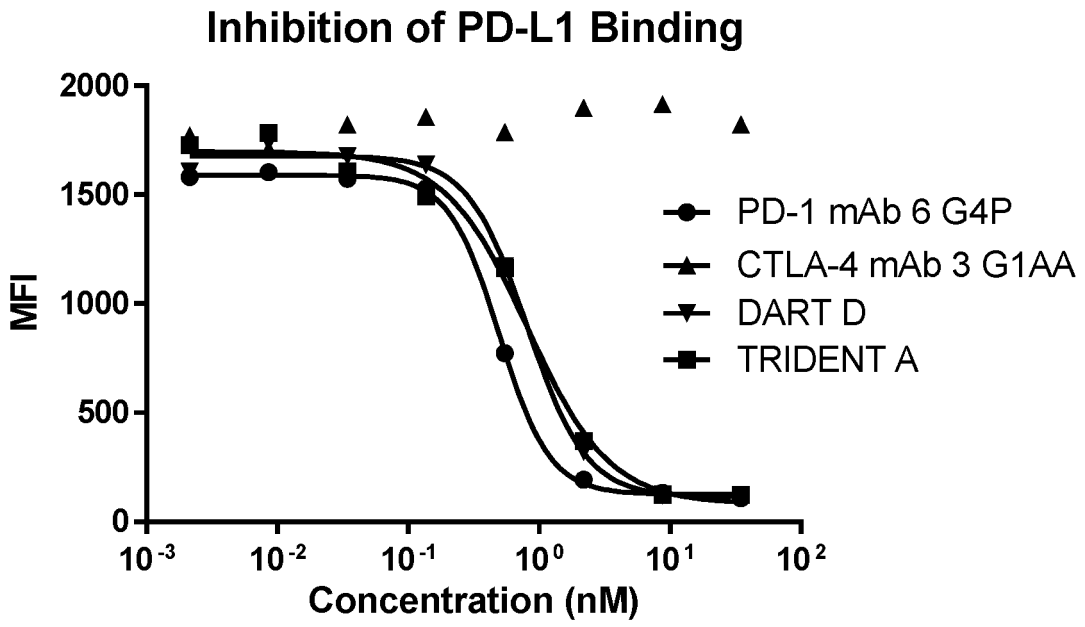


Figure 15A

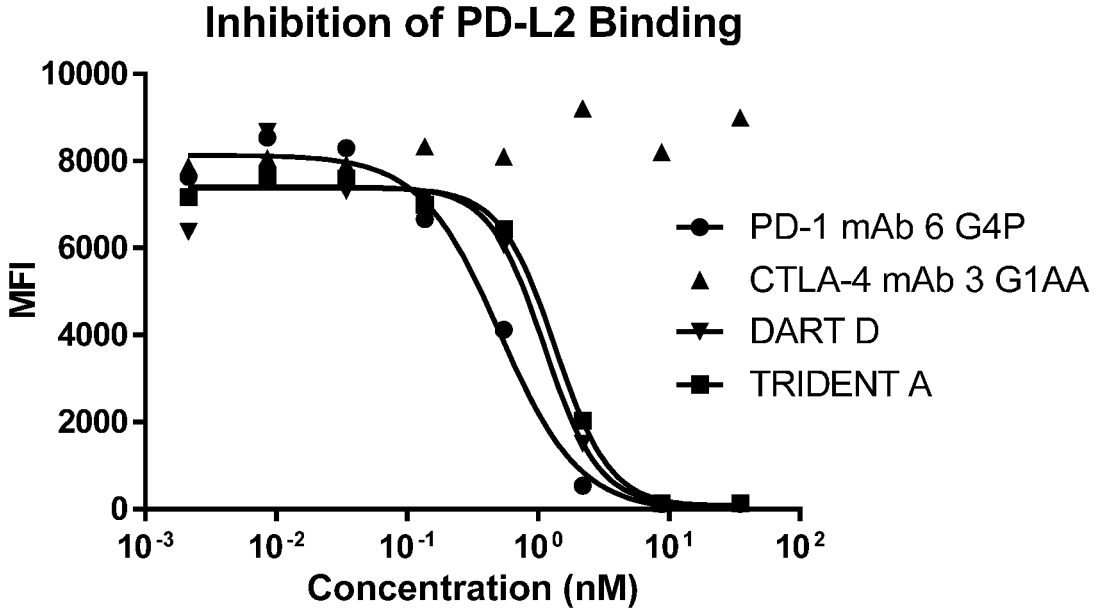


Figure 15B

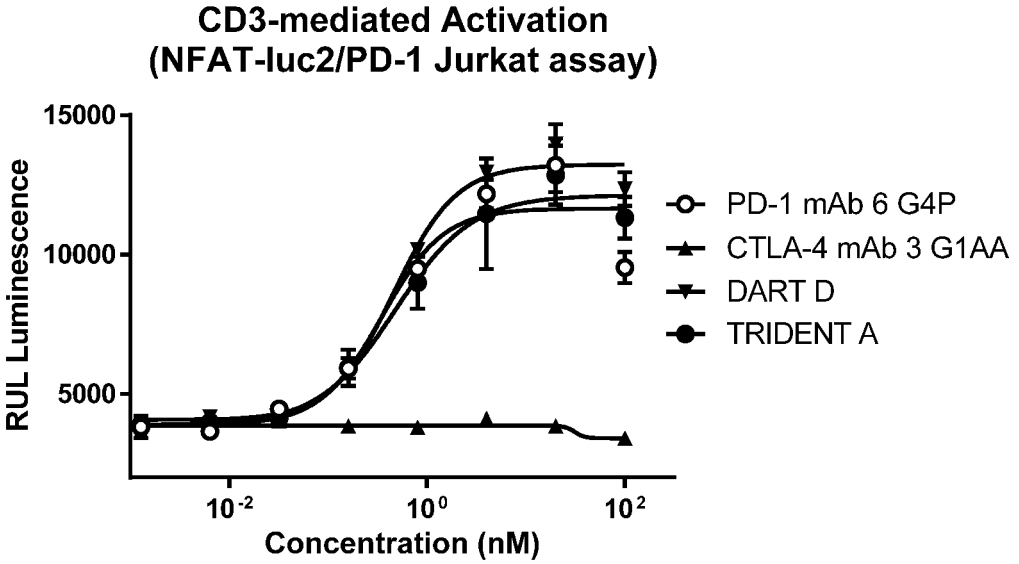


Figure 16

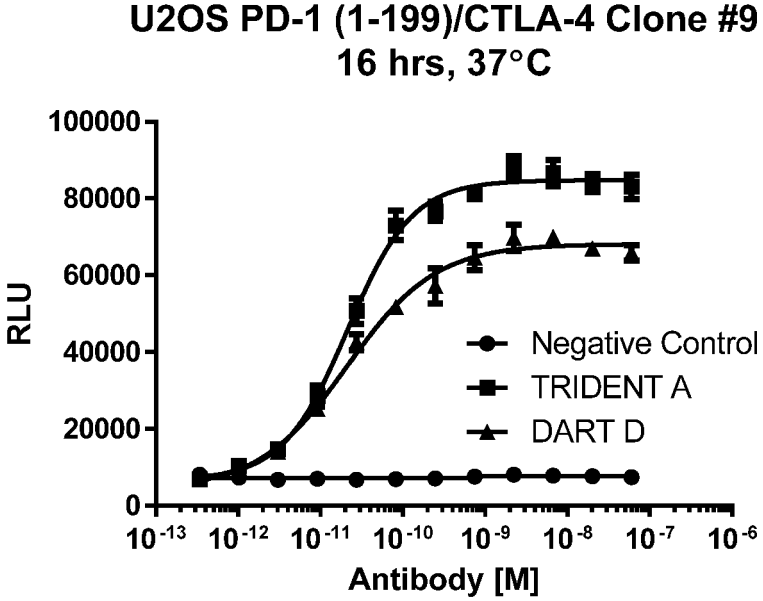
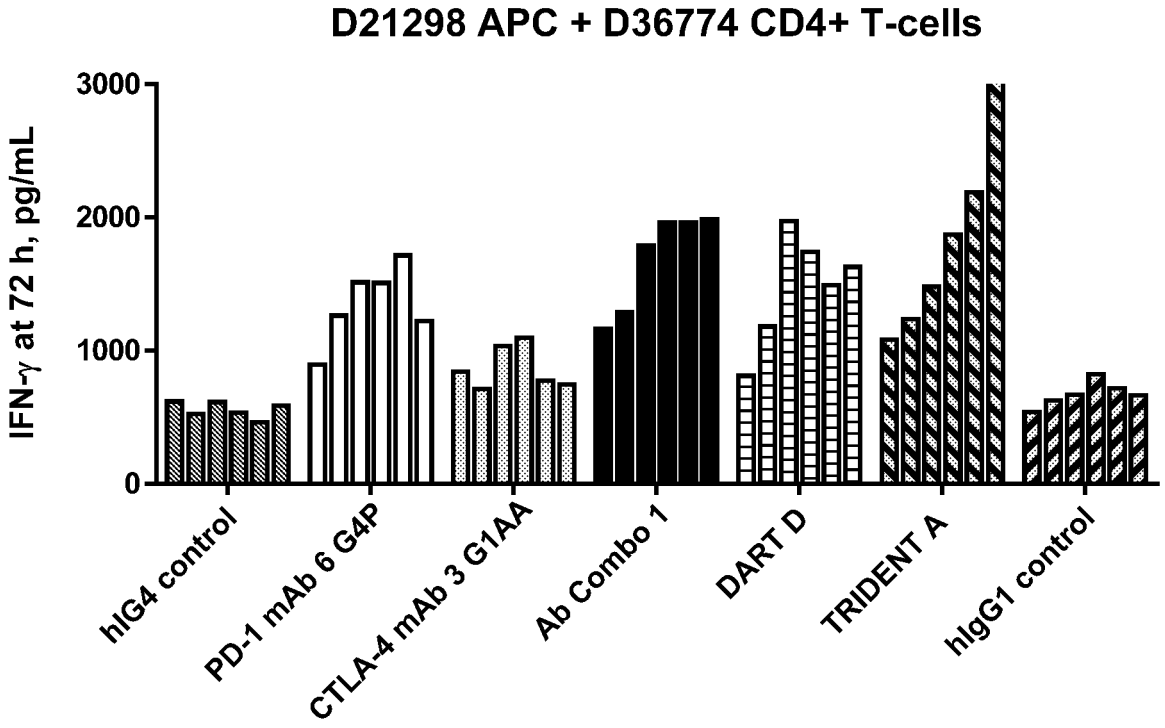


Figure 17



Concentration (nM): | 0.016 | 0.08 | 0.4 | 2| 10 | 50 |

Figure 18

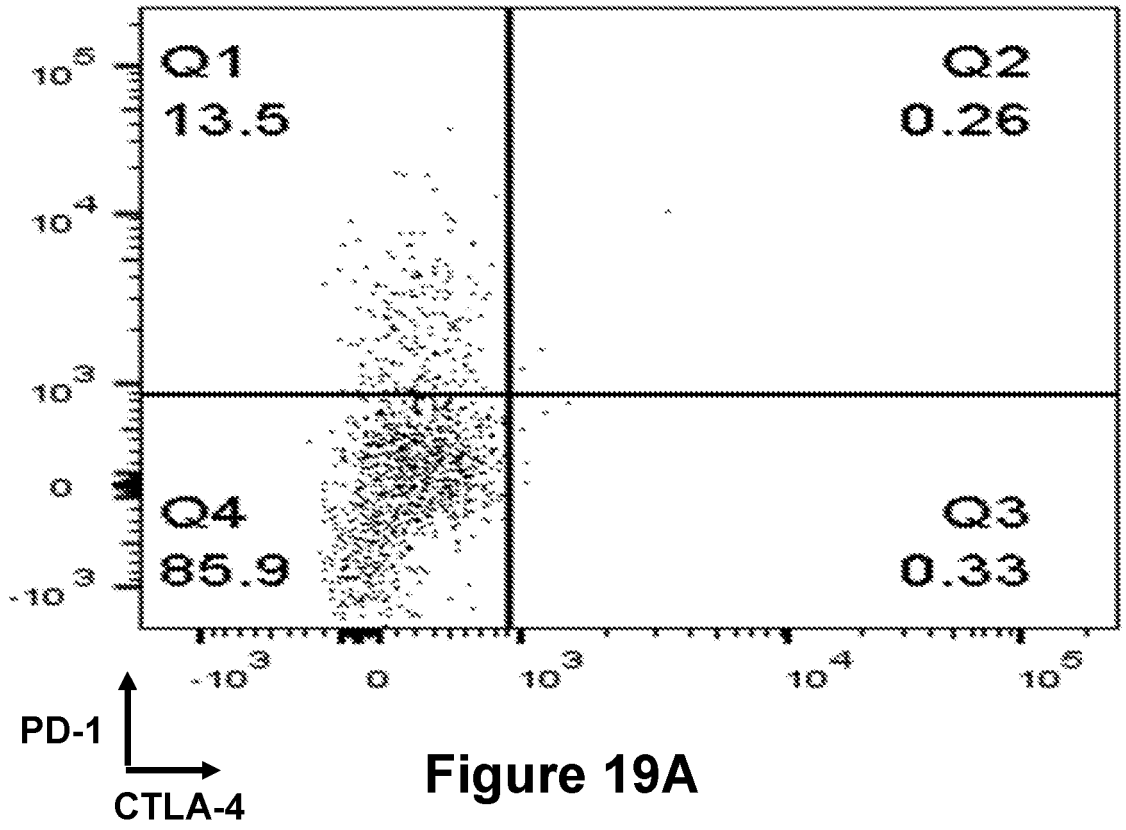


Figure 19A

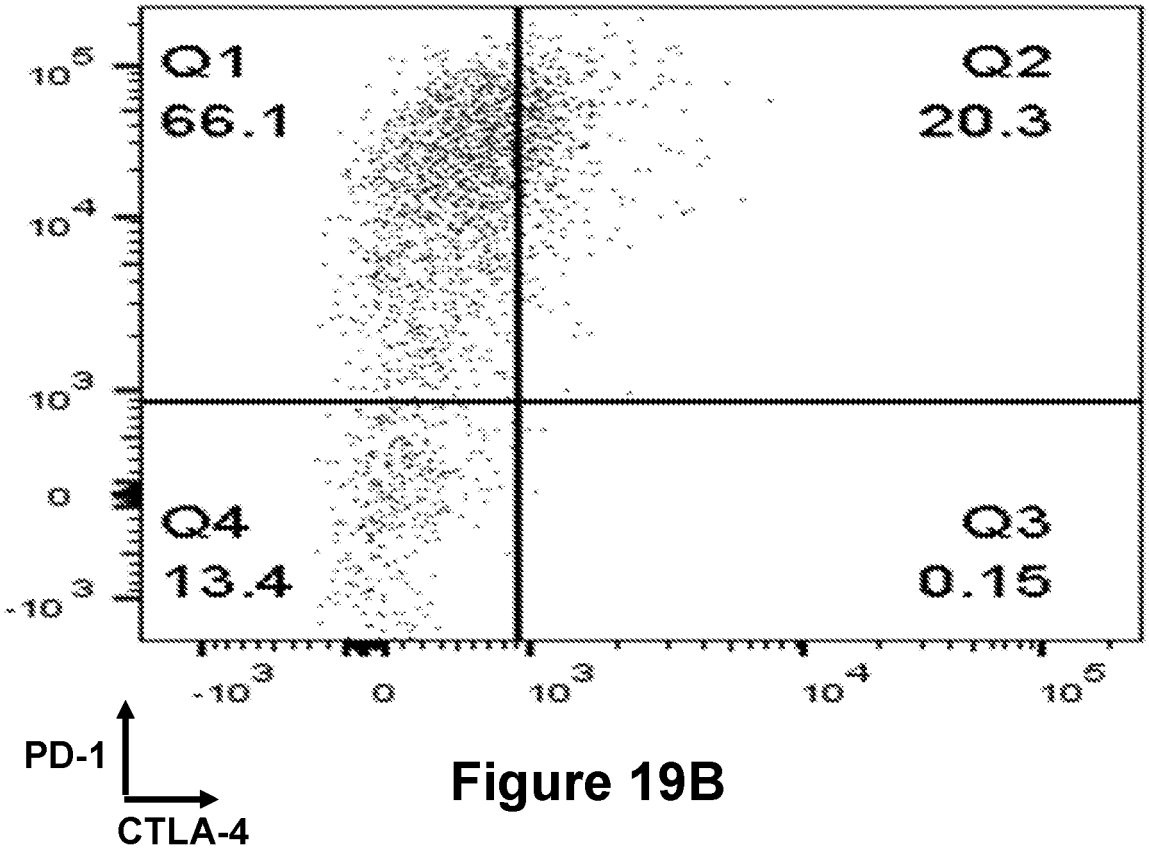


Figure 19B

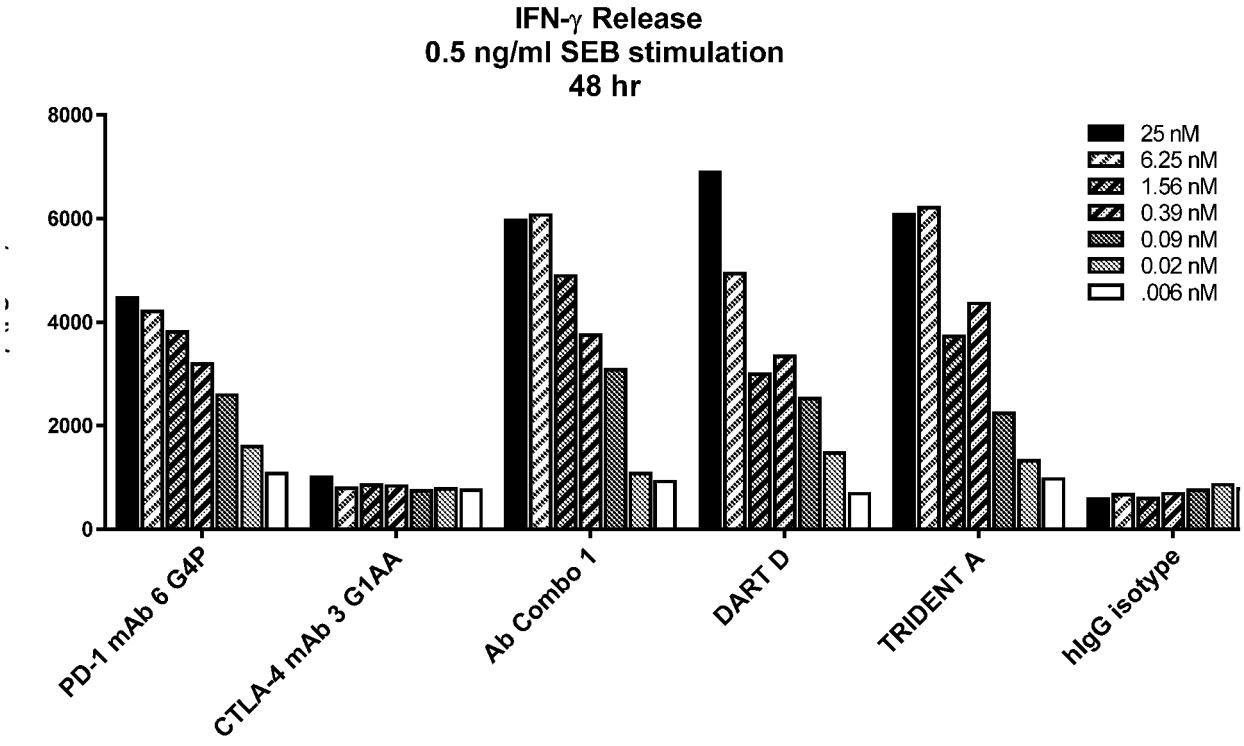

IFN-γ Release
0.5 ng/ml SEB stimulation
48 hr
8000
6000
4000
2000
0
25 nM
6.25 nM
1.56 nM
0.39 nM
0.09 nM
0.02 nM
.006 nM
PD-1 mAb 6 G4P
CTLA-4 mAb 3 G1AA
Ab Combo 1
DART D
TRIDENT A
hIgG isotype

Figure 19C

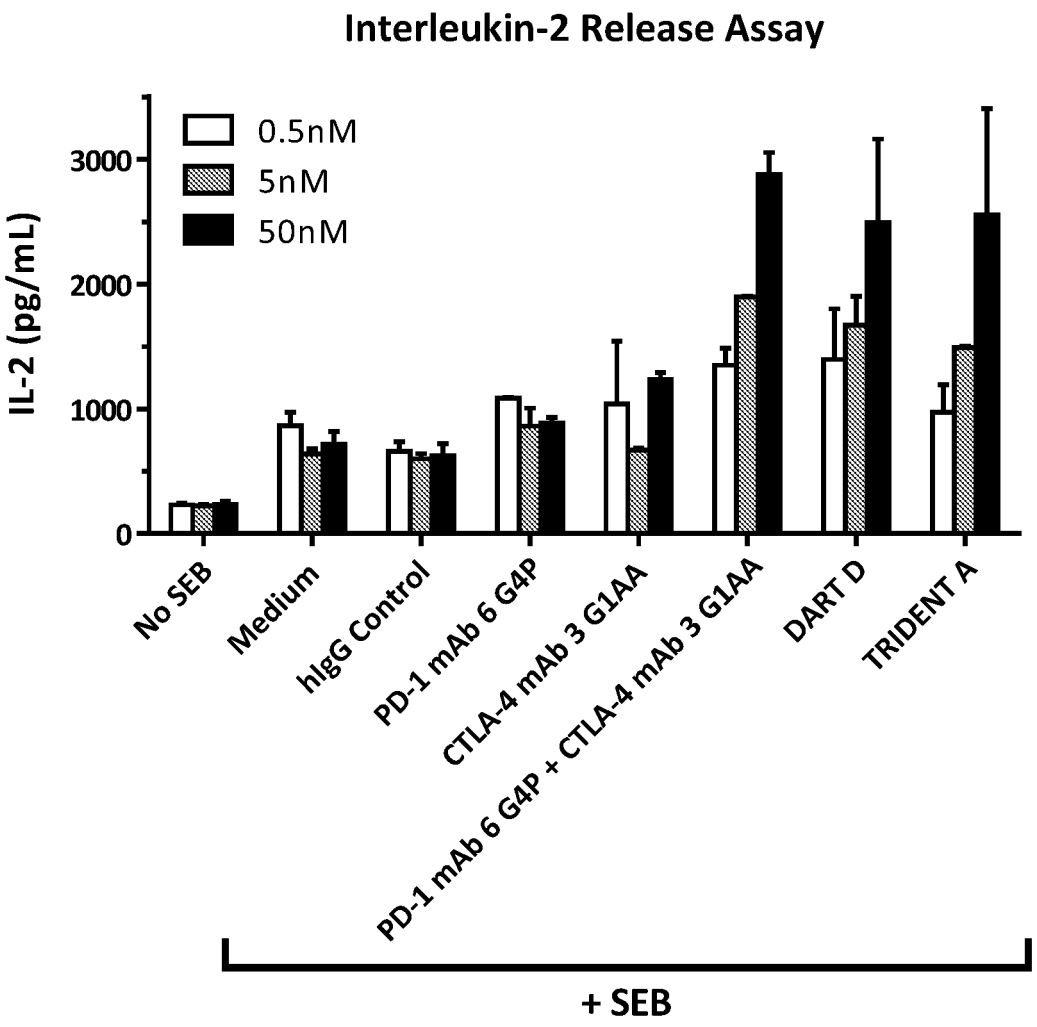

Interleukin-2 Release Assay
0.5nM
5nM
50nM
IL-2 (pg/mL)
3000
2000
1000
0
No SEB
Medium
hIgG Control
PD-1 mAb 6 G4P
CTLA-4 mAb 3 G1AA
PD-1 mAb 6 G4P + CTLA-4 mAb 3 G1AA
DART D
TRIDENT A
+ SEB

Figure 19D

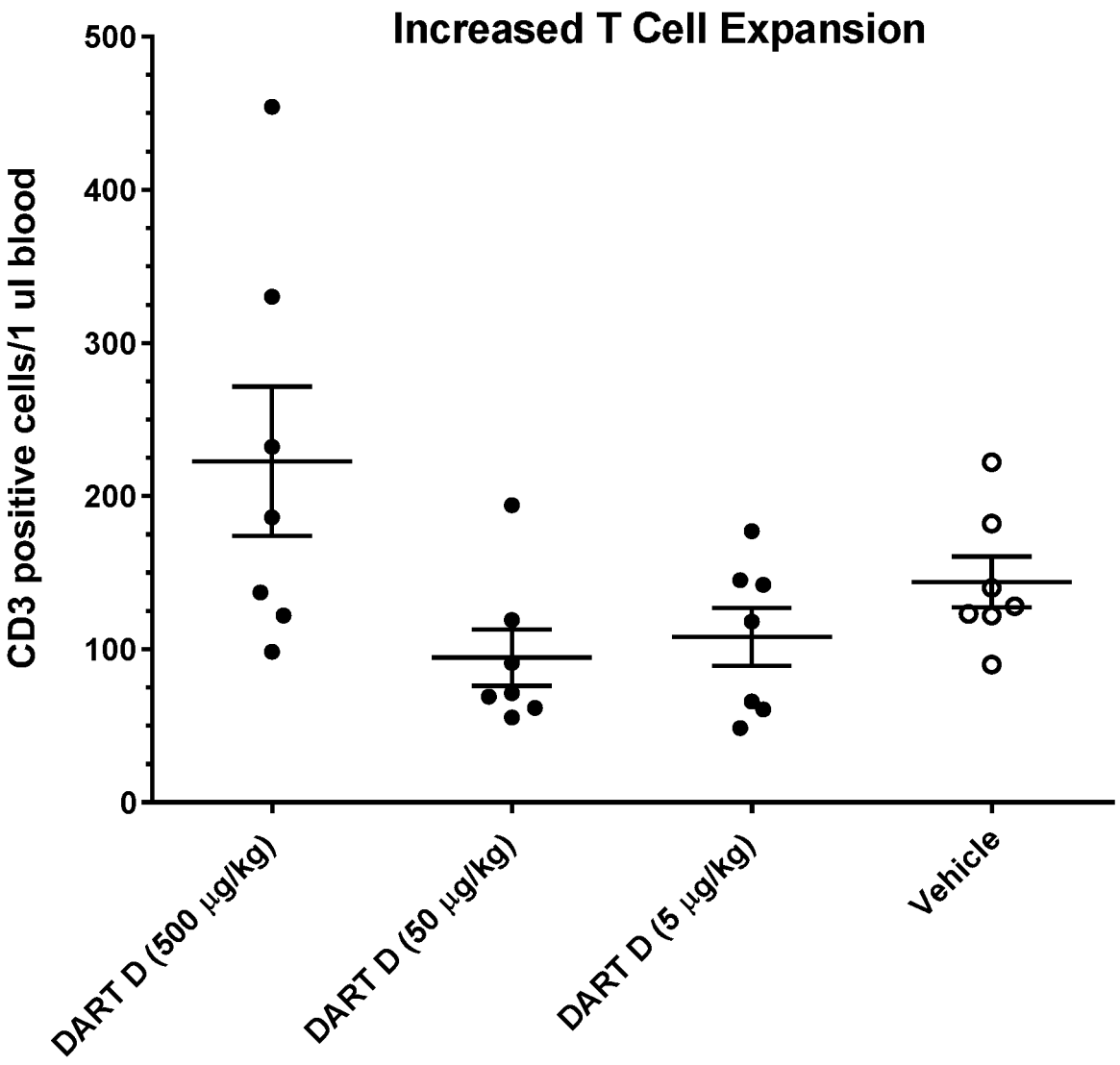

Increased T Cell Expansion
CD3 positive cells/1 ul blood
500
400
300
200
100
0
DART D (500 μg/kg)
DART D (50 μg/kg)
DART D (5 μg/kg)
Vehicle

Figure 20A

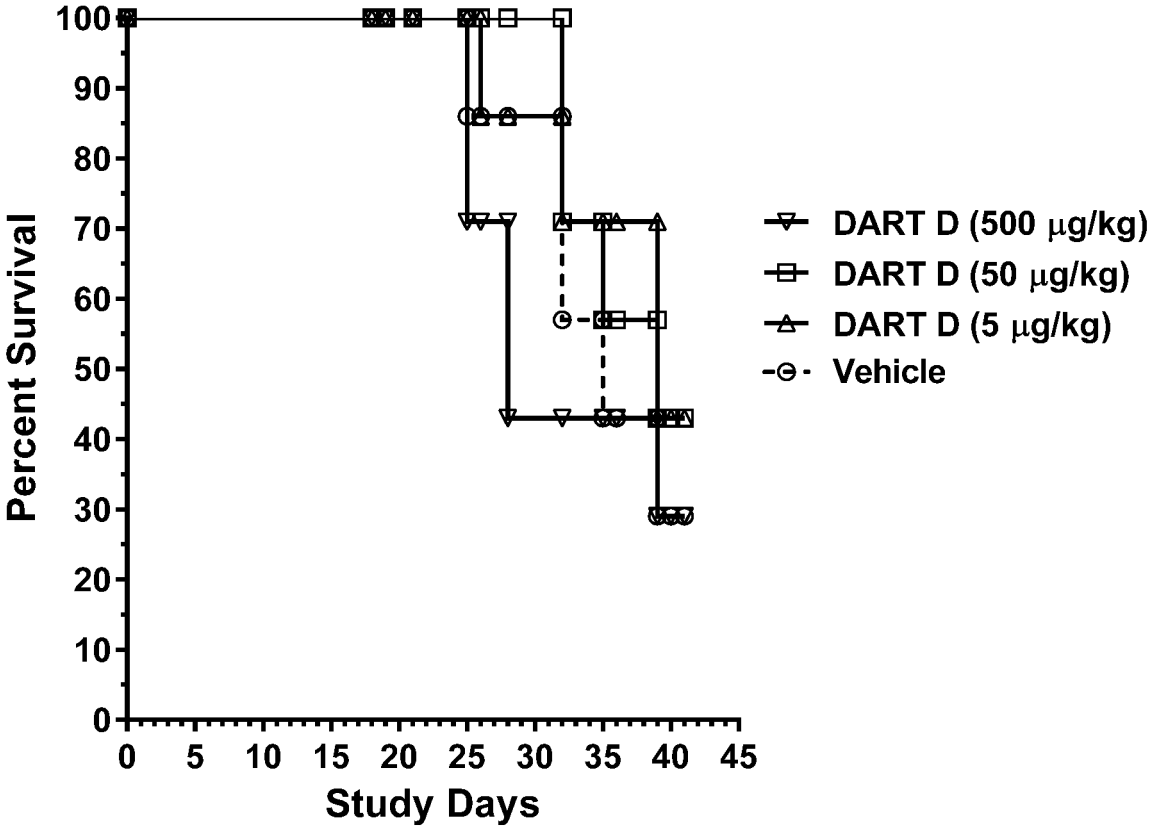

Percent Survival
100
90
80
70
60
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40 45
Study Days
DART D (500 μg/kg)
DART D (50 μg/kg)
DART D (5 μg/kg)
Vehicle

Figure 20B

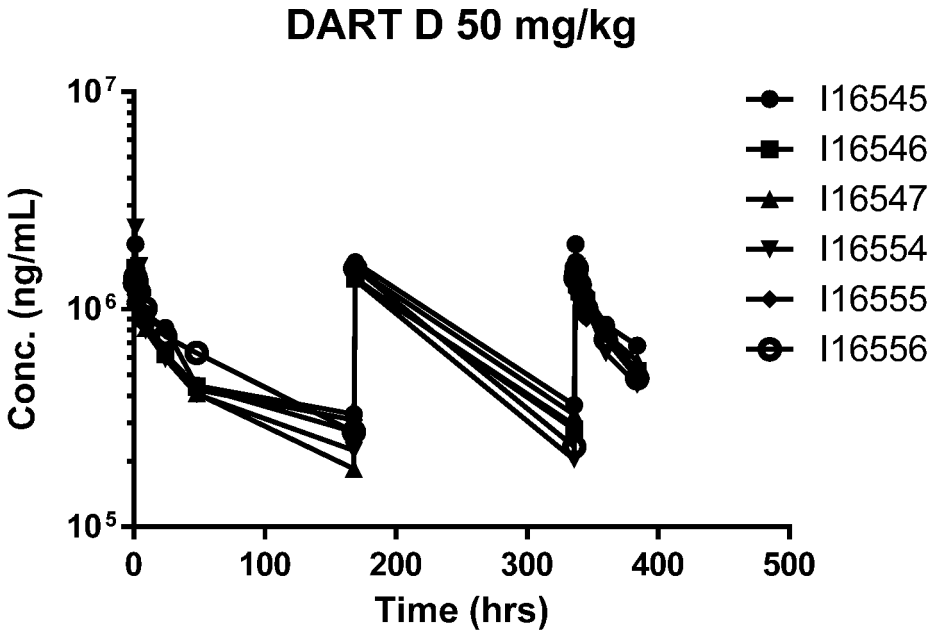


Figure 21A

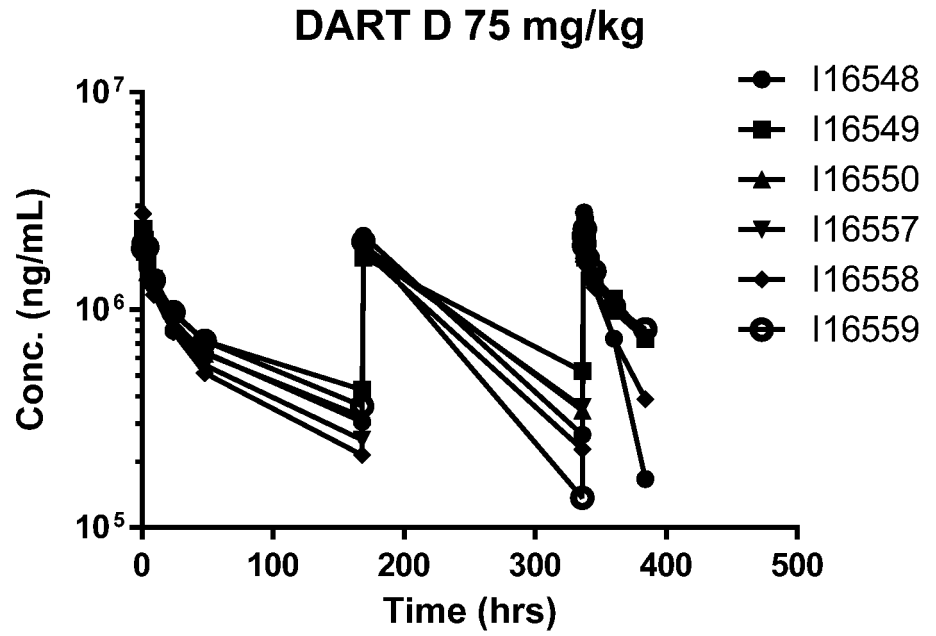


Figure 21B

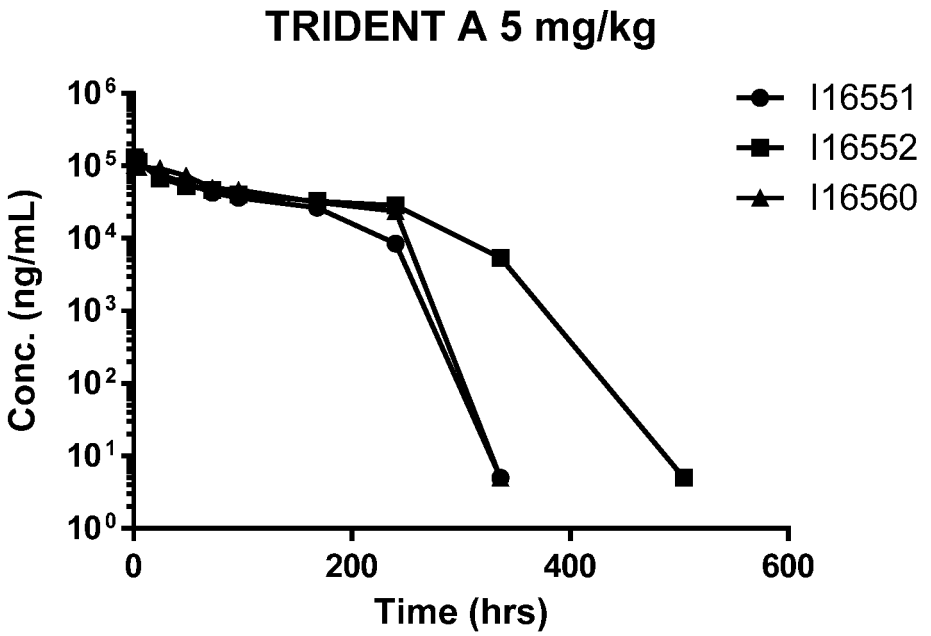

TRIDENT A 5 mg/kg
Conc. (ng/mL)
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
200
400
600
Time (hrs)
I16551
I16552
I16560

Figure 21C

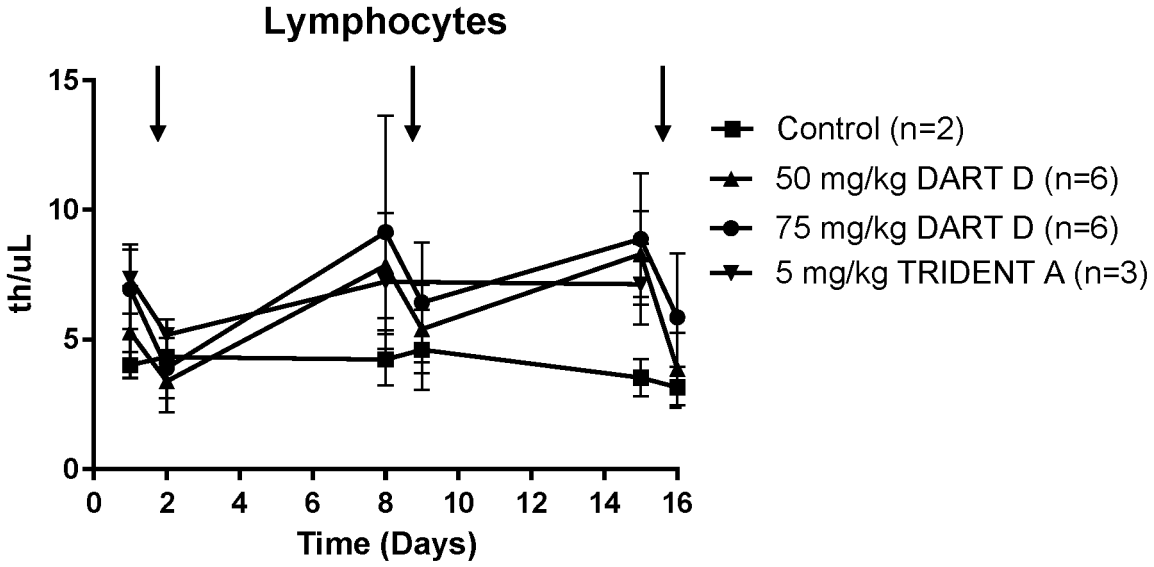


Figure 22A

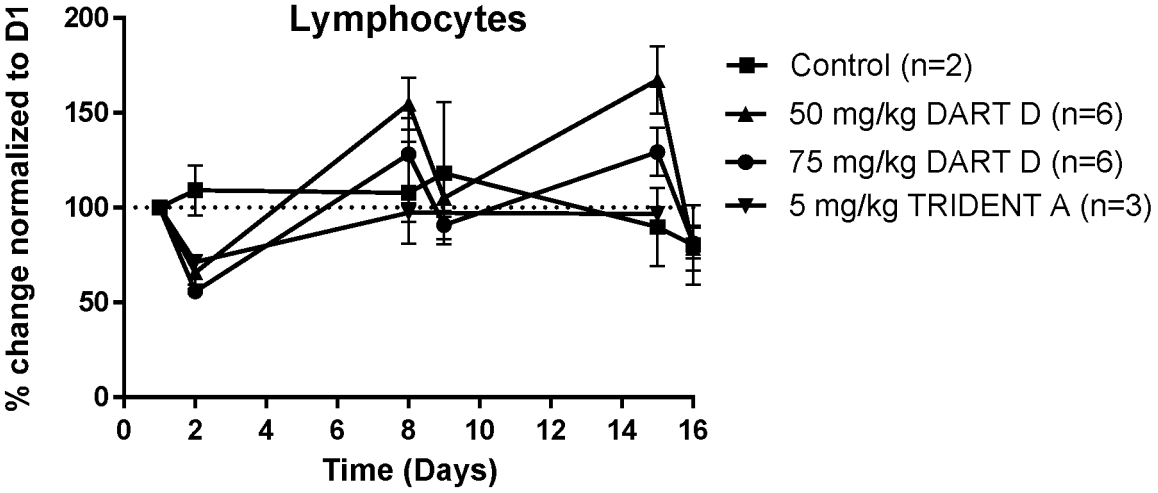


Figure 22B

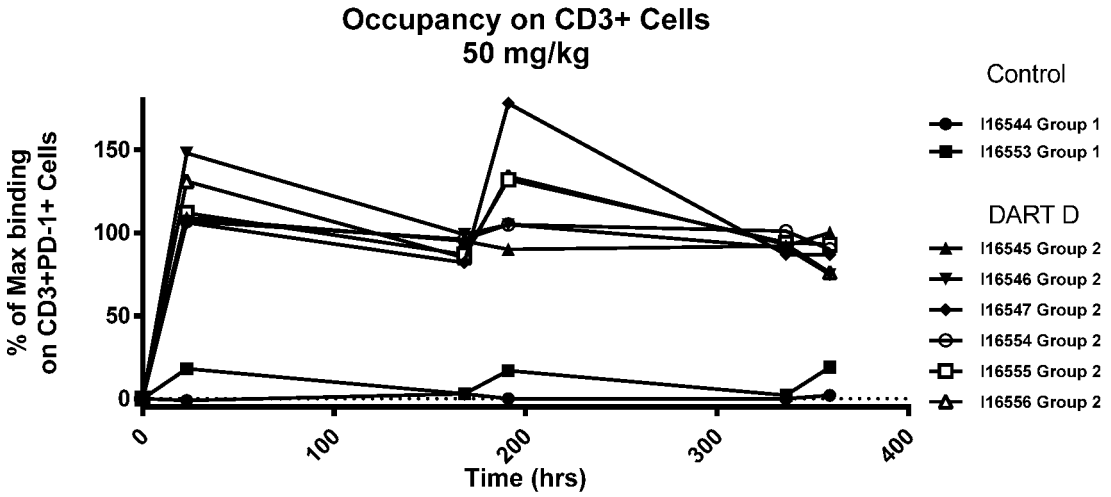


Figure 23A

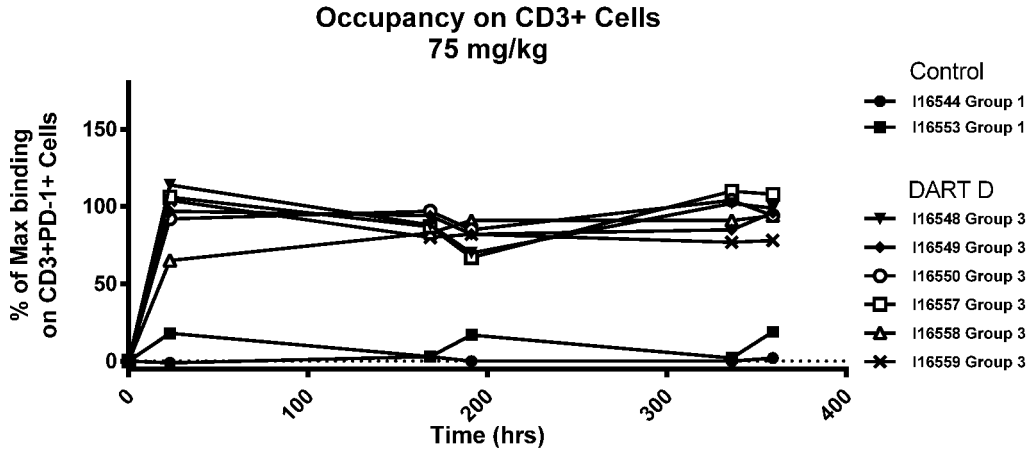


Figure 23B

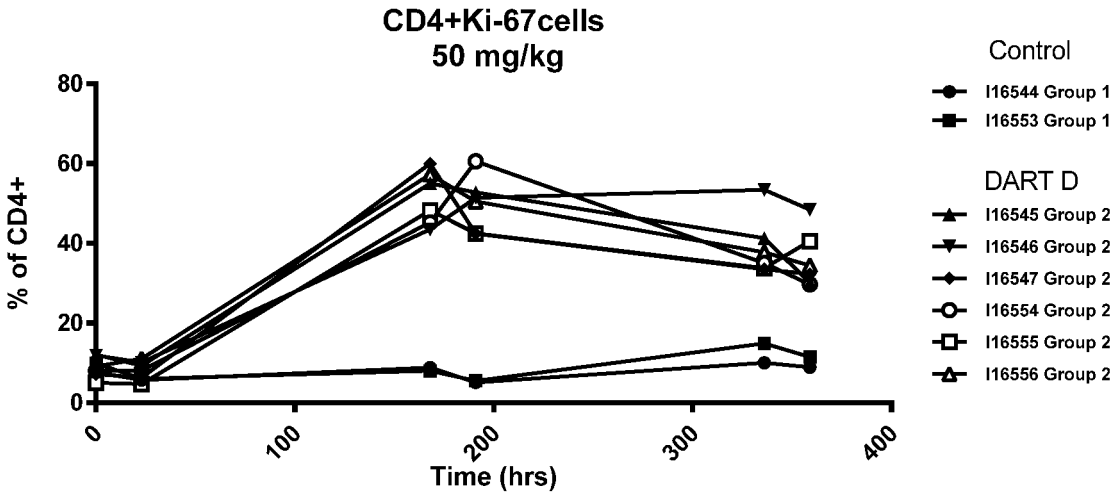


Figure 24A

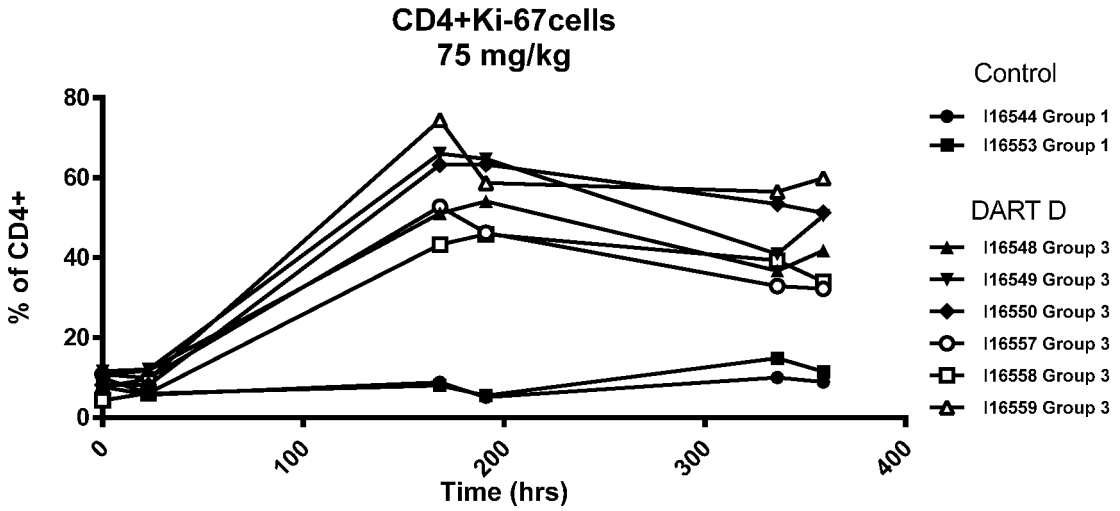


Figure 24B