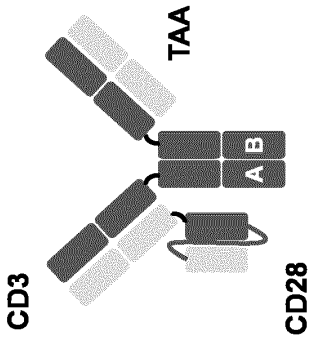


FIG. 1A

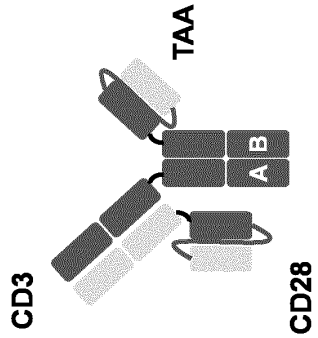


FIG. 1B

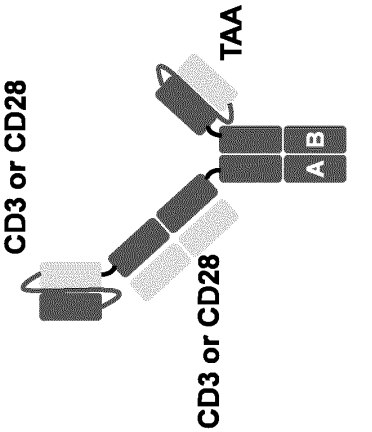


FIG. 1C

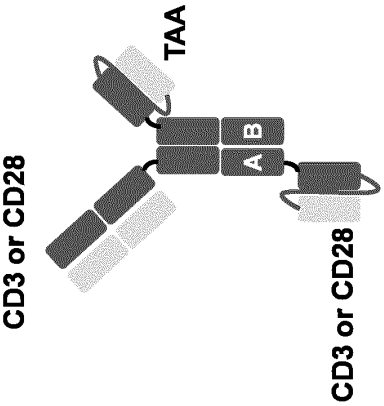


FIG. 1D

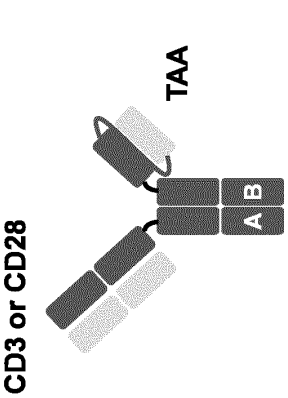


FIG. 1F

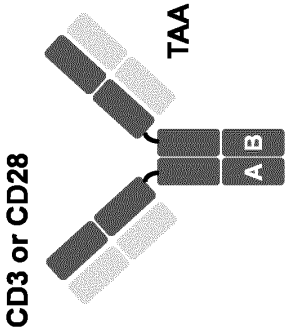


FIG. 1E

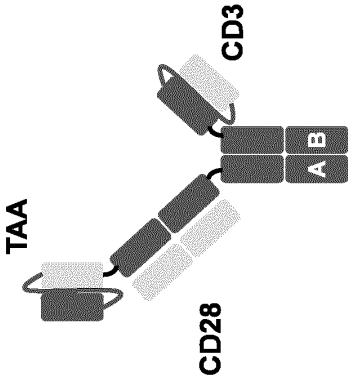


FIG. 1H

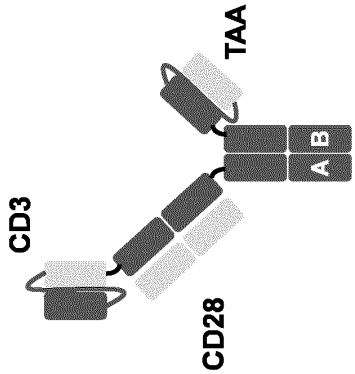


FIG. 1G

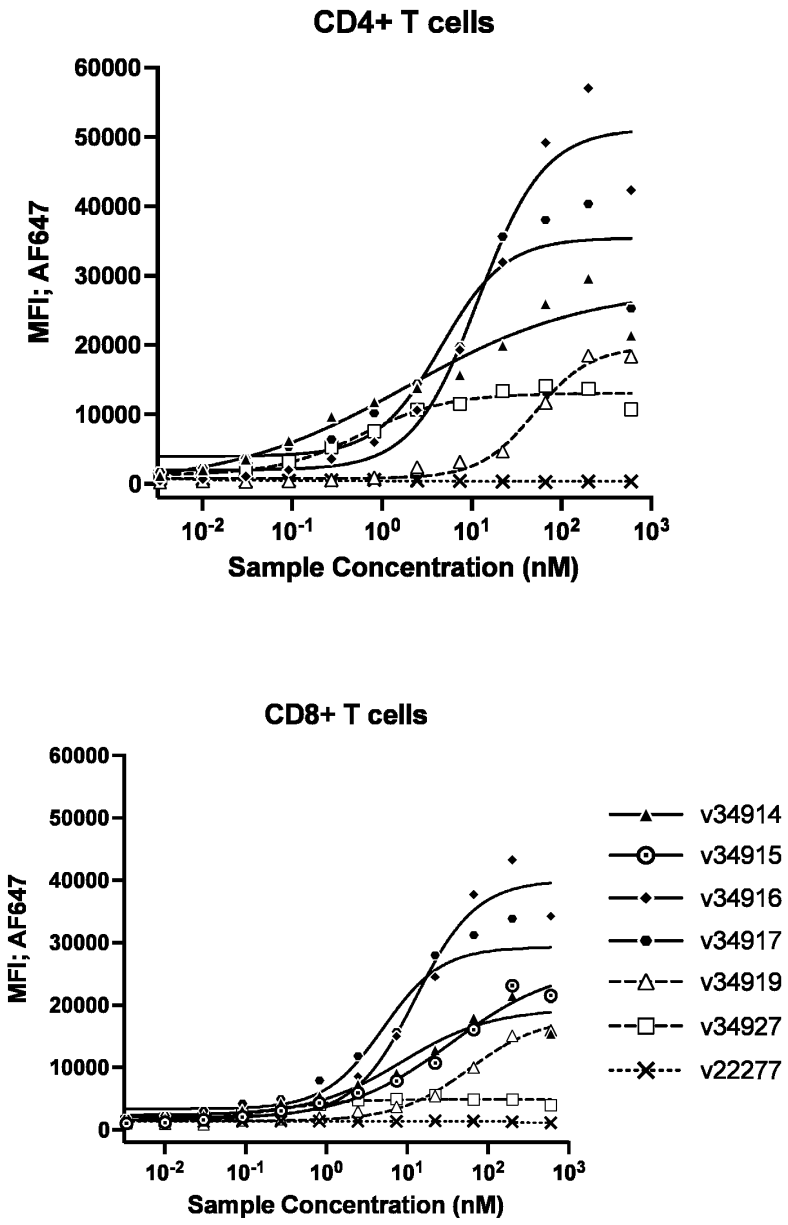

CD4+ T cells
MFI; AF647
60000
50000
40000
30000
20000
10000
0
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Sample Concentration (nM)
CD8+ T cells
MFI; AF647
60000
50000
40000
30000
20000
10000
0
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Sample Concentration (nM)
v34914
v34915
v34916
v34917
v34919
v34927
v22277

FIG. 2A

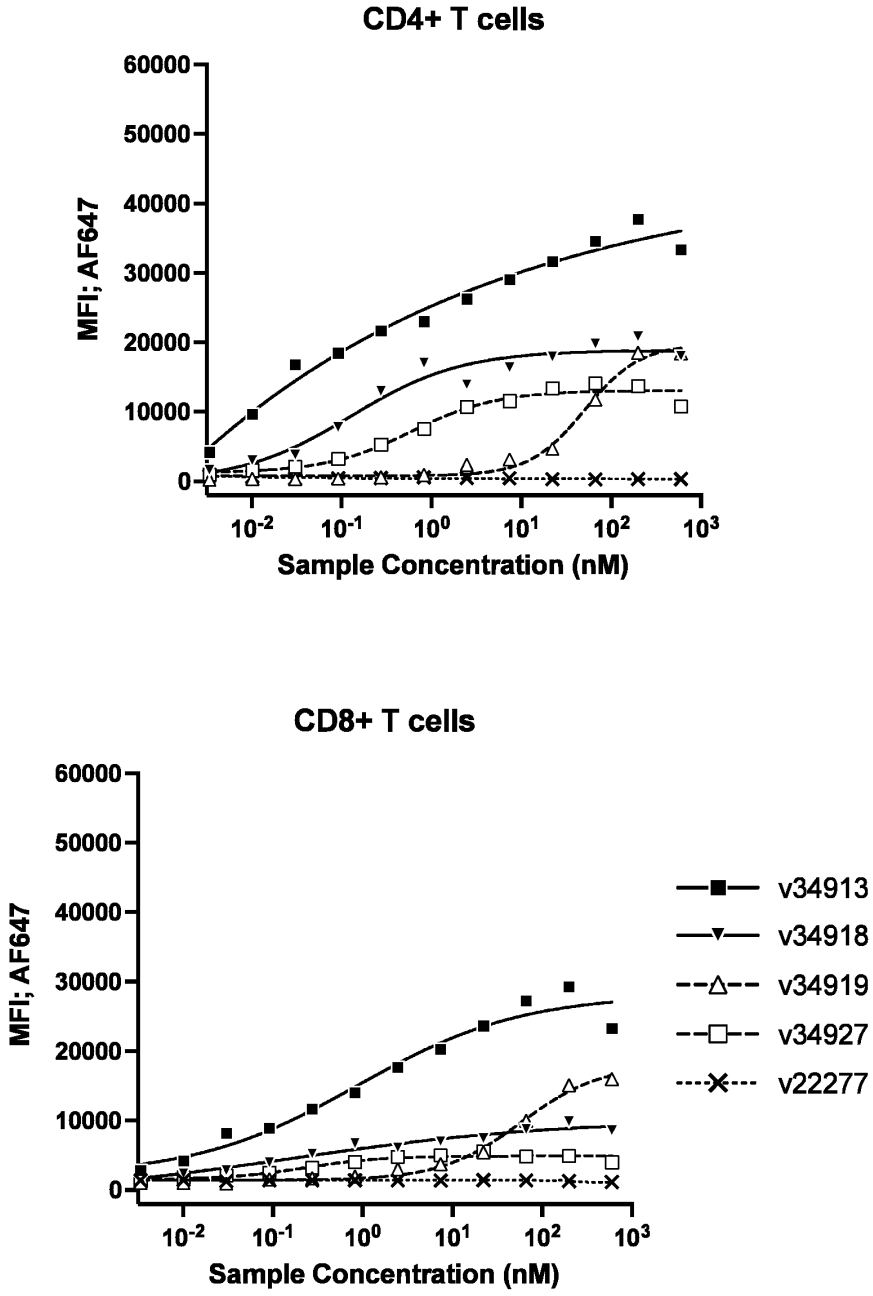

CD4+ T cells
MFI; AF647
60000
50000
40000
30000
20000
10000
0
10-2
10-1
100
101
102
103
Sample Concentration (nM)
CD8+ T cells
MFI; AF647
60000
50000
40000
30000
20000
10000
0
10-2
10-1
100
101
102
103
Sample Concentration (nM)
v34913
v34918
v34919
v34927
v22277

FIG. 2B

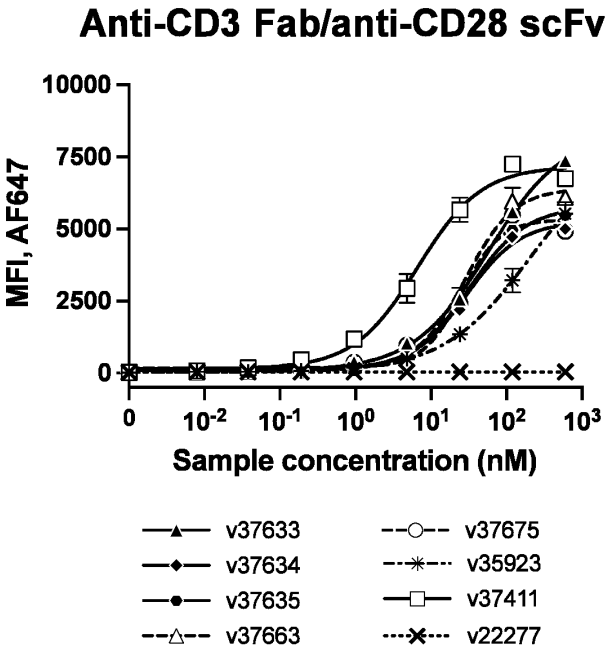

Anti-CD3 Fab/anti-CD28 scFv
MFI, AF647
10000
7500
5000
2500
0
0
10-2
10-1
100
101
102
103
Sample concentration (nM)
v37633
v37634
v37635
v37663
v37675
v35923
v37411
v22277

FIG. 3A

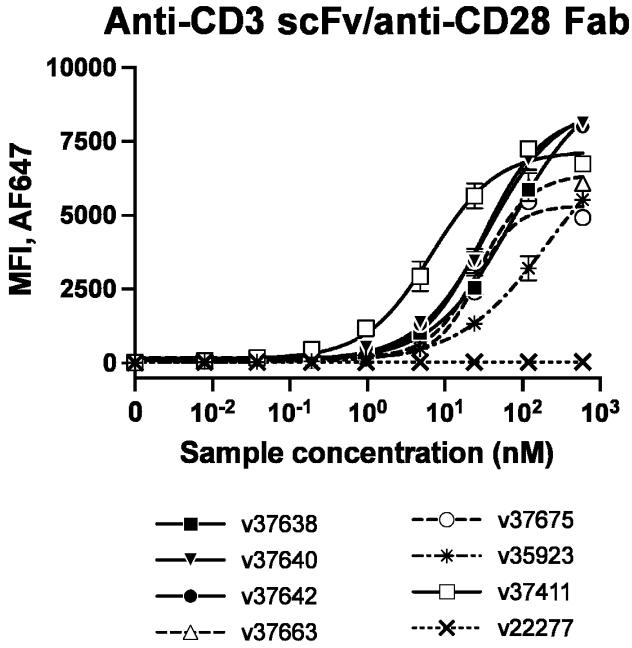

Anti-CD3 scFv/anti-CD28 Fab
MFI, AF647
10000
7500
5000
2500
0
0
10-2
10-1
100
101
102
103
Sample concentration (nM)
v37638
v37640
v37642
v37663
v37675
v35923
v37411
v22277

FIG. 3B

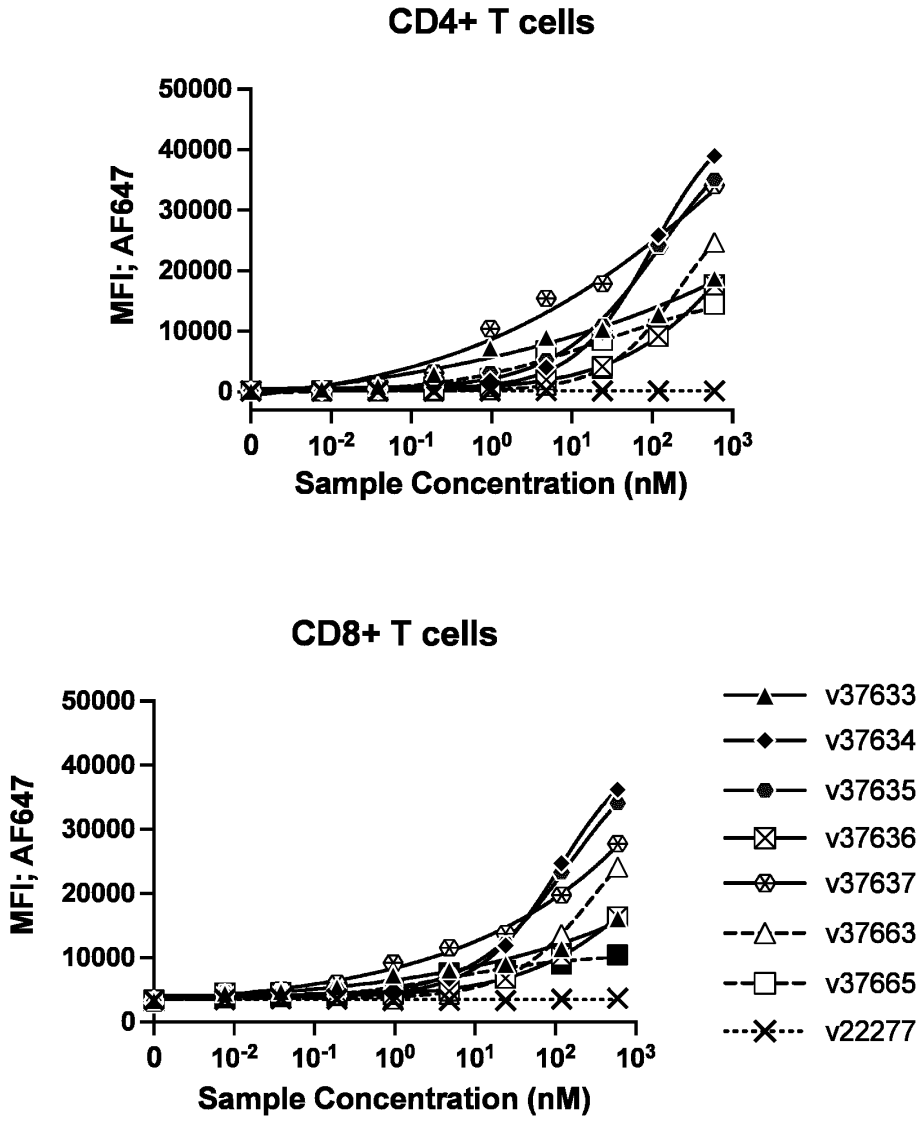

CD4+ T cells
MFI; AF647
50000
40000
30000
20000
10000
0
0
10-2
10-1
100
101
102
103
Sample Concentration (nM)
CD8+ T cells
MFI; AF647
50000
40000
30000
20000
10000
0
0
10-2
10-1
100
101
102
103
Sample Concentration (nM)
v37633
v37634
v37635
v37636
v37637
v37663
v37665
v22277

FIG. 4A

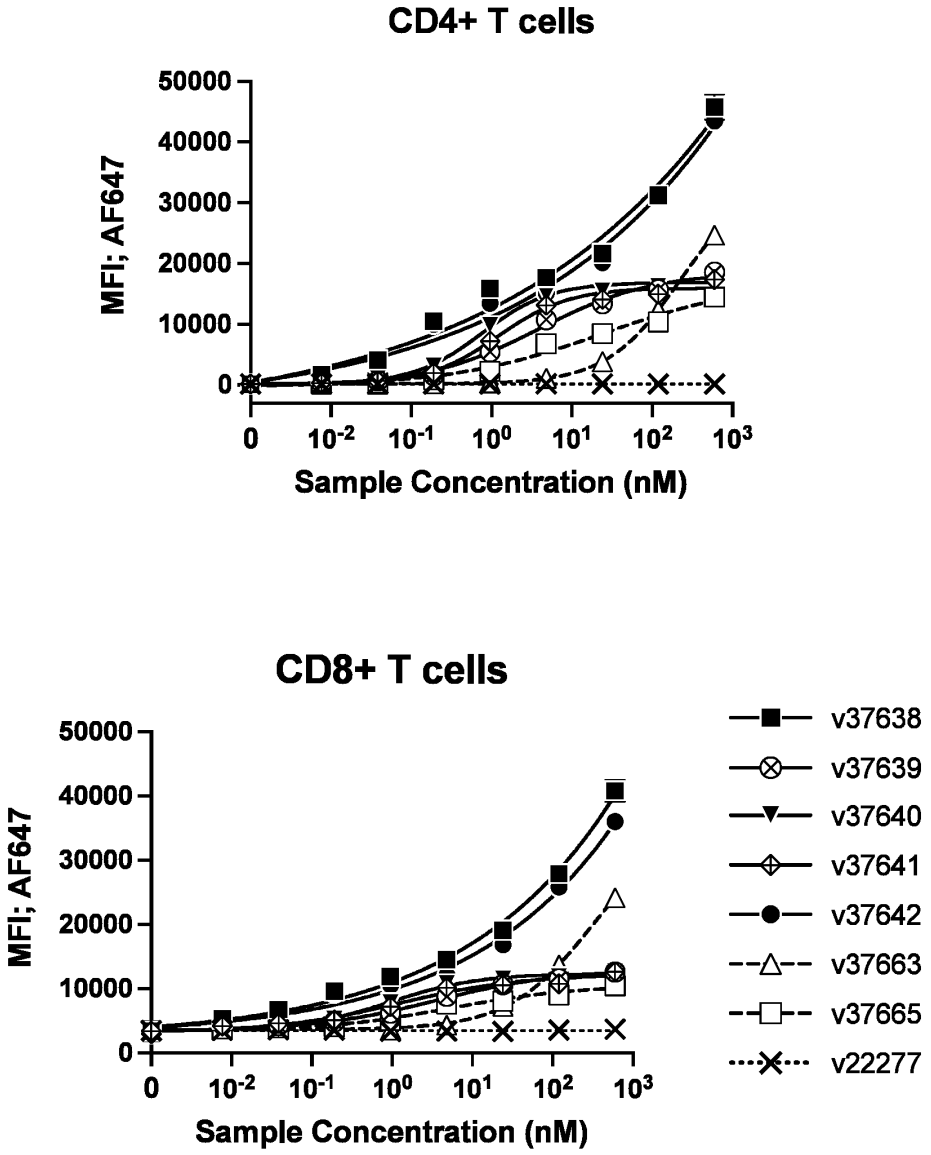

CD4+ T cells
MFI; AF647
50000
40000
30000
20000
10000
0
0
10-2
10-1
100
101
102
103
Sample Concentration (nM)
CD8+ T cells
MFI; AF647
50000
40000
30000
20000
10000
0
0
10-2
10-1
100
101
102
103
Sample Concentration (nM)
v37638
v37639
v37640
v37641
v37642
v37663
v37665
v22277

FIG. 4B

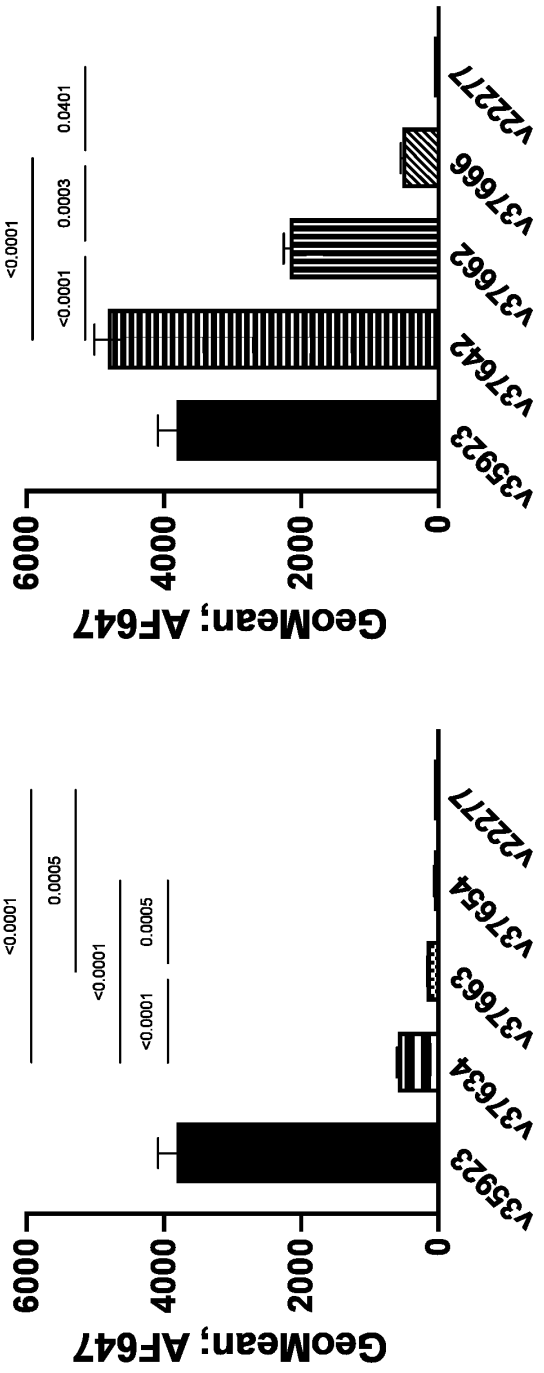

GeoMean; AF647
6000
4000
2000
0
V35923
V37634
V37663
V37654
V22277
<0.0001
0.0005
<0.0001
<0.0001
0.0005
GeoMean; AF647
6000
4000
2000
0
V35923
V37642
V37662
V37666
V22277
<0.0001
<0.0001
0.0003
0.0401

FIG. 5

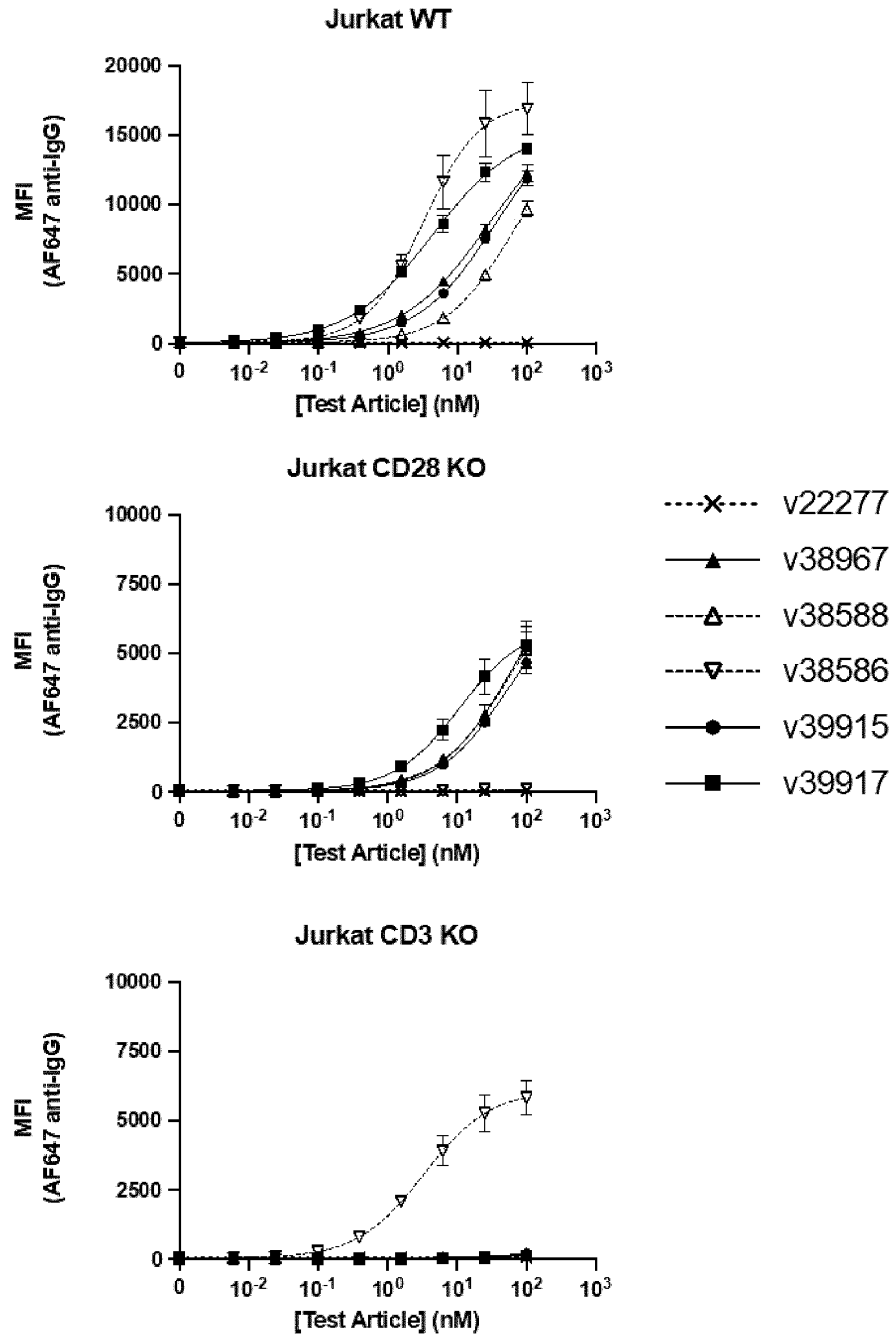

Jurkat WT
MFI (AF647 anti-IgG)
20000
15000
10000
5000
0
0
10-2
10-1
100
101
102
103
[Test Article] (nM)
Jurkat CD28 KO
MFI (AF647 anti-IgG)
10000
7500
5000
2500
0
[Test Article] (nM)
Jurkat CD3 KO
MFI (AF647 anti-IgG)
10000
7500
5000
2500
0
[Test Article] (nM)
v22277
v38967
v38588
v38586
v39915
v39917

FIG. 6

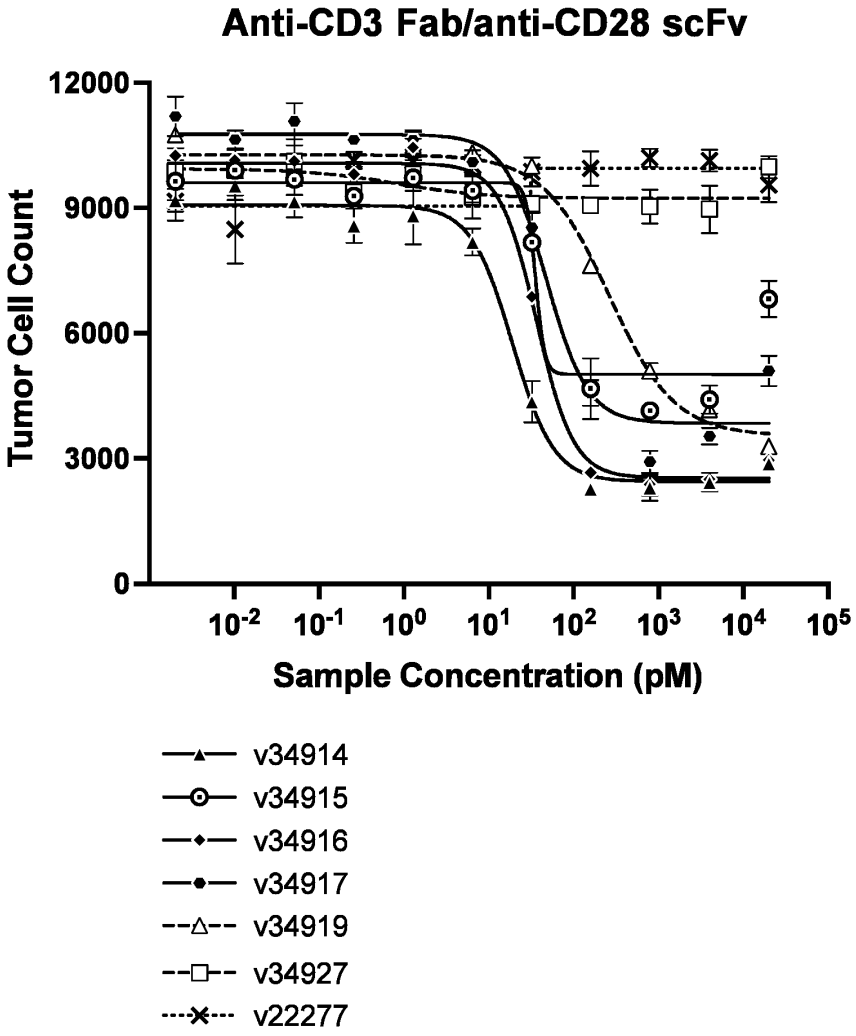

Anti-CD3 Fab/anti-CD28 scFv
Tumor Cell Count
12000
9000
6000
3000
0
10-2
10-1
100
101
102
103
104
105
Sample Concentration (pM)
v34914
v34915
v34916
v34917
v34919
v34927
v22277

FIG. 7A

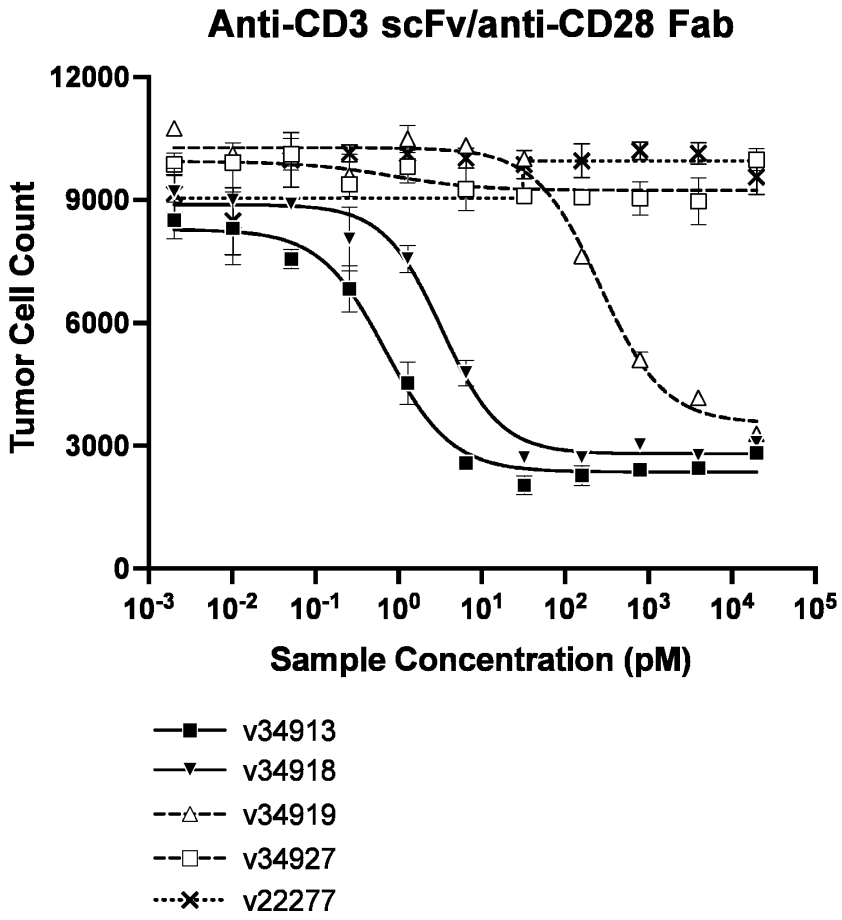

Anti-CD3 scFv/anti-CD28 Fab
Tumor Cell Count
12000
9000
6000
3000
0
10-3
10-2
10-1
100
101
102
103
104
105
Sample Concentration (pM)
v34913
v34918
v34919
v34927
v22277

FIG. 7B

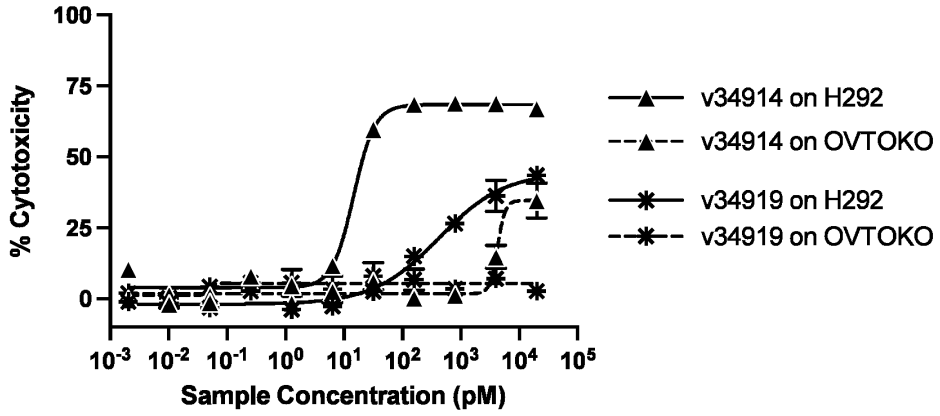

100
75
50
25
0
% Cytotoxicity
10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v34914 on H292
v34914 on OVTOKO
v34919 on H292
v34919 on OVTOKO

FIG. 8A

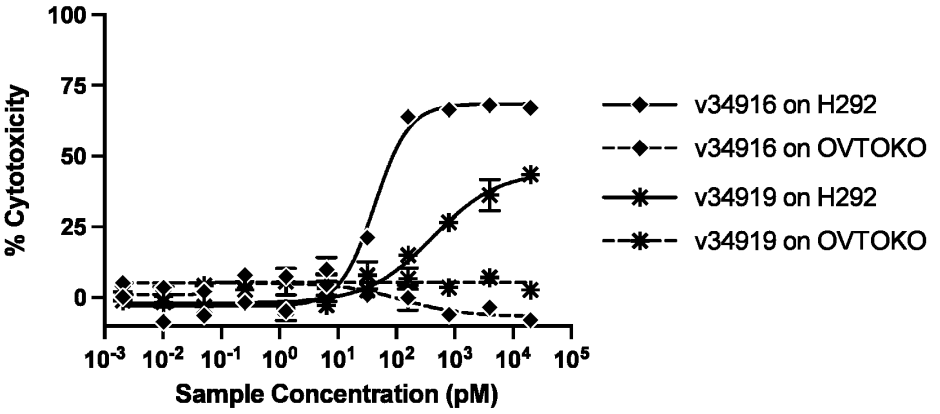

100
75
50
25
0
% Cytotoxicity
10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v34916 on H292
v34916 on OVTOKO
v34919 on H292
v34919 on OVTOKO

FIG. 8B

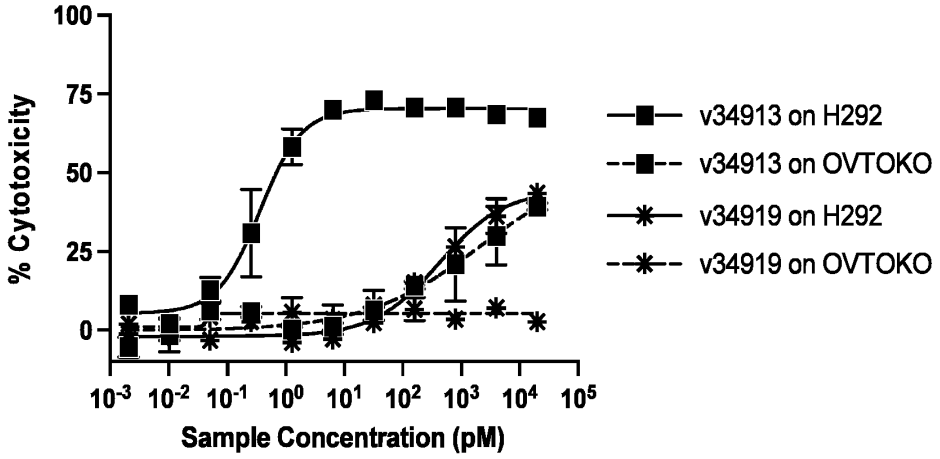

100
75
50
25
0
% Cytotoxicity
10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v34913 on H292
v34913 on OVTOKO
v34919 on H292
v34919 on OVTOKO

FIG. 8C

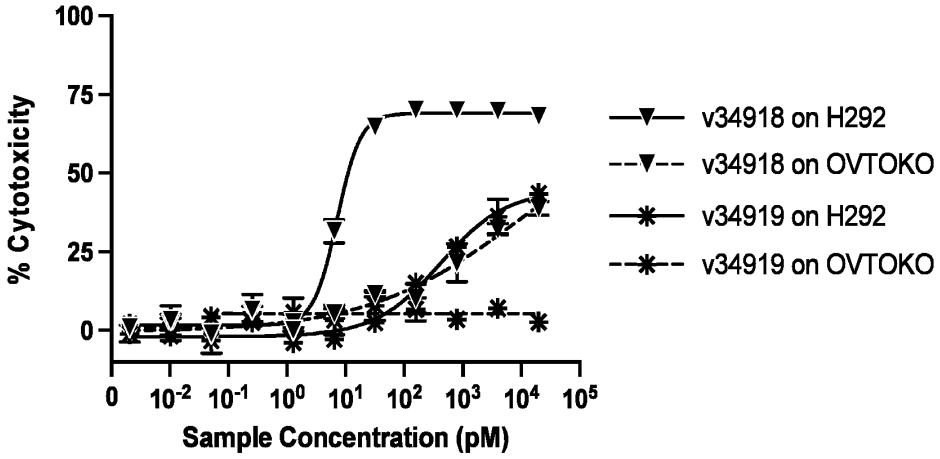

100
75
50
25
0
% Cytotoxicity
0 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v34918 on H292
v34918 on OVTOKO
v34919 on H292
v34919 on OVTOKO

FIG. 8D

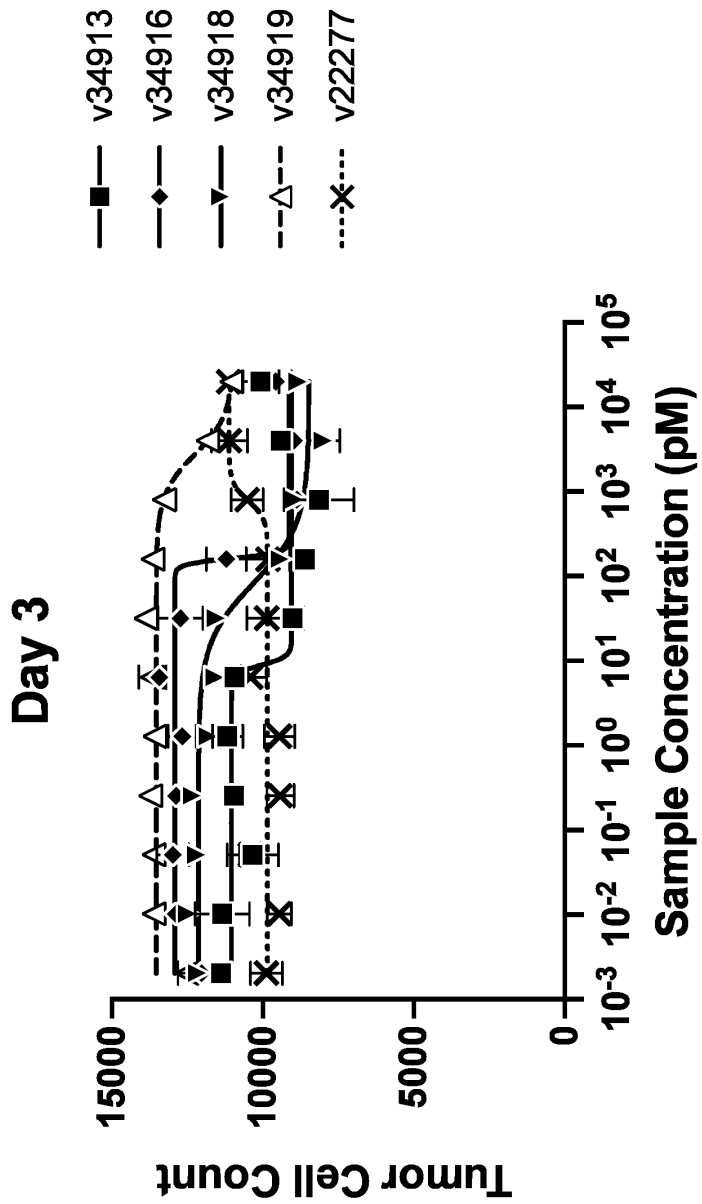

Day 3
v34913
v34916
v34918
v34919
v22277
Tumor Cell Count
15000
10000
5000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Sample Concentration (pM)

FIG. 9A

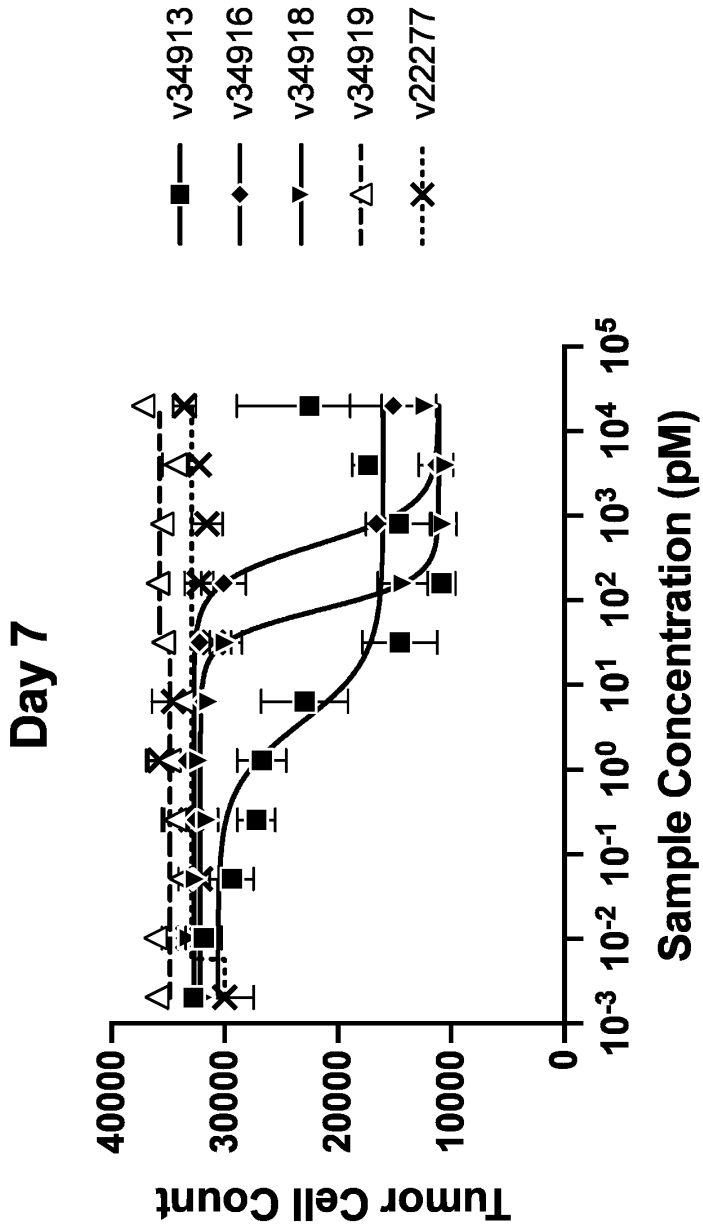

Day 7
Tumor Cell Count
40000
30000
20000
10000
0
Sample Concentration (pM)
10-3
10-2
10-1
100
101
102
103
104
105
v34913
v34916
v34918
v34919
v22277

FIG. 9B

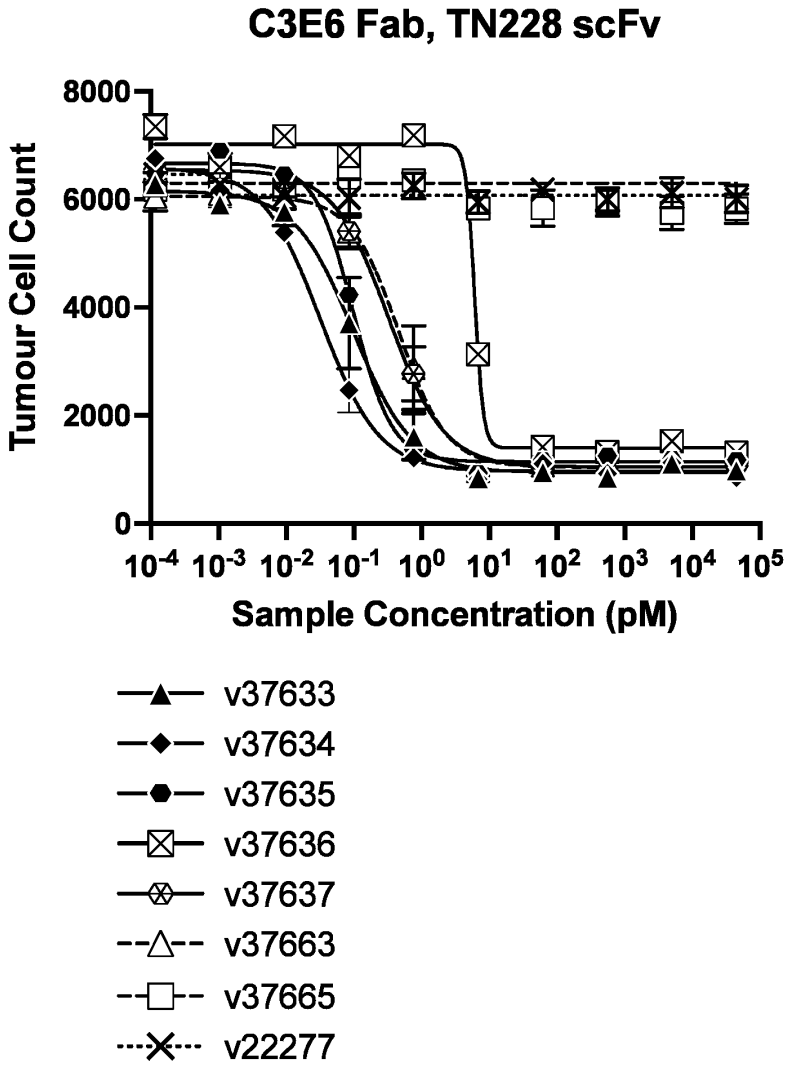

C3E6 Fab, TN228 scFv
Tumour Cell Count
8000
6000
4000
2000
0
10-4 10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v37633
v37634
v37635
v37636
v37637
v37663
v37665
v22277

FIG. 10A

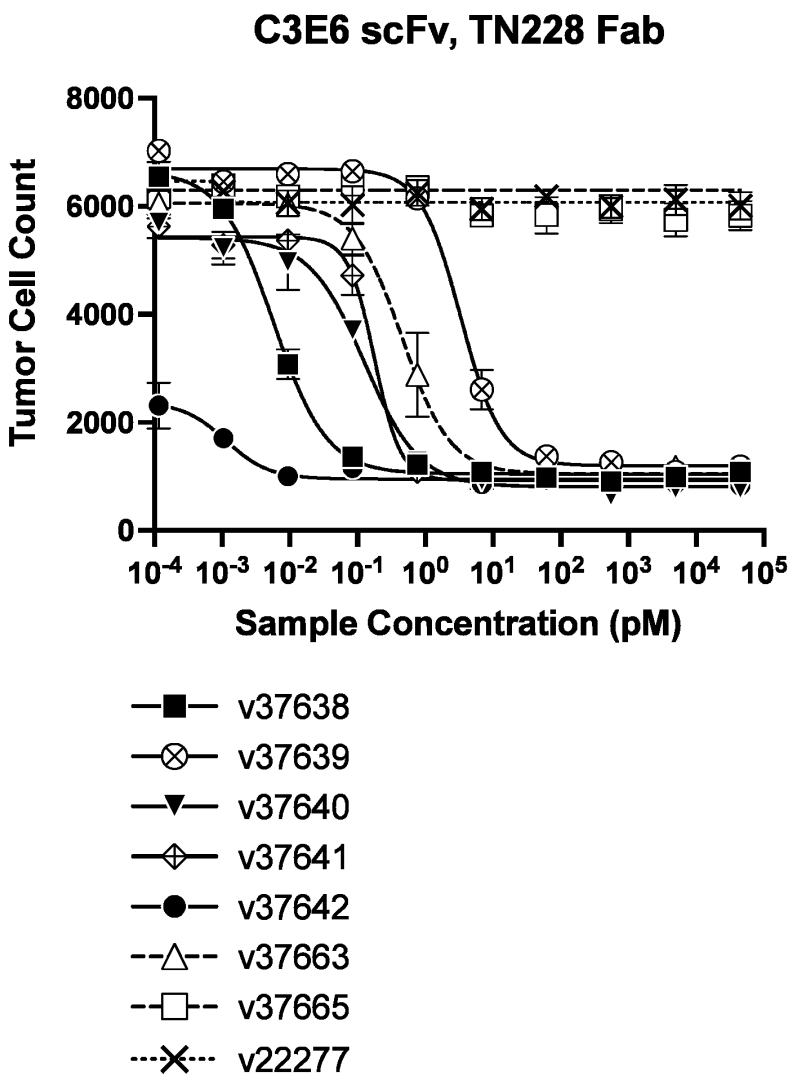

C3E6 scFv, TN228 Fab
Tumor Cell Count
8000
6000
4000
2000
0
10-4 10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v37638
v37639
v37640
v37641
v37642
v37663
v37665
v22277

FIG. 10B

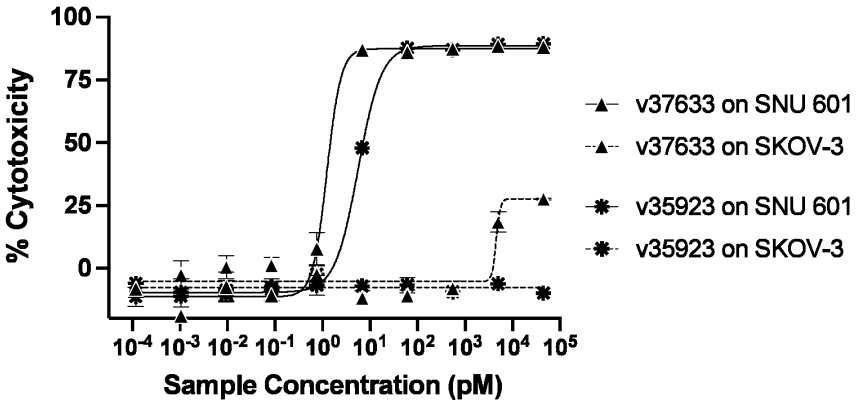


FIG. 11A

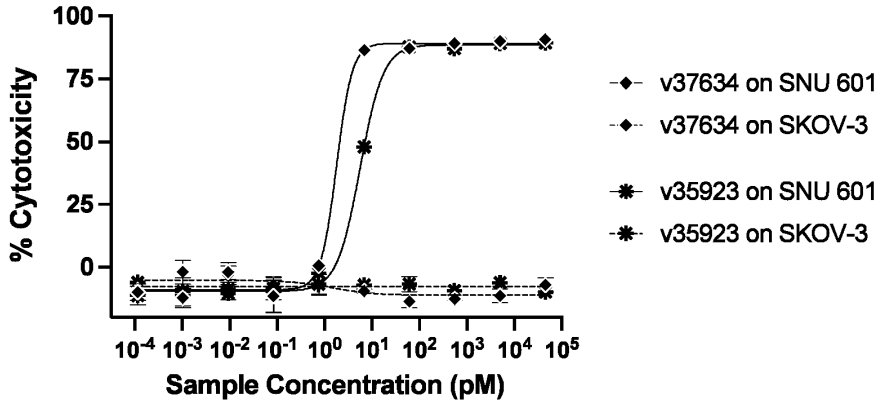


FIG. 11B

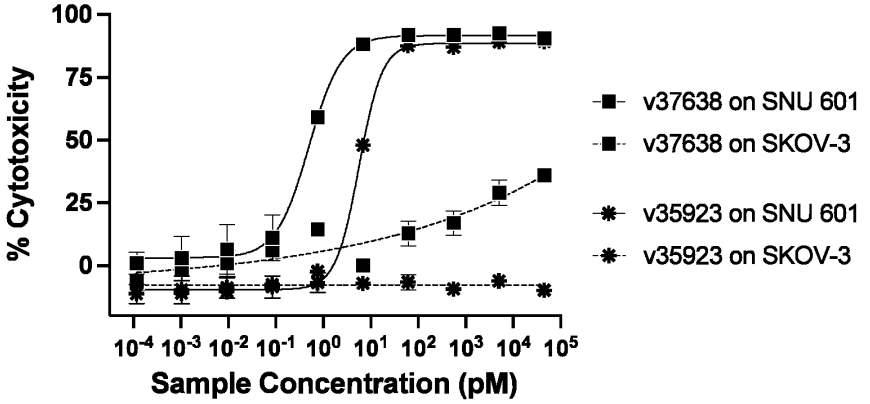


FIG. 11C

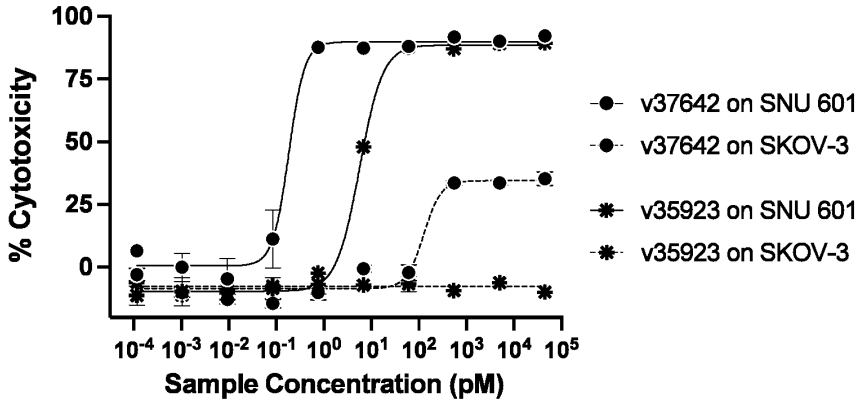


FIG. 11D

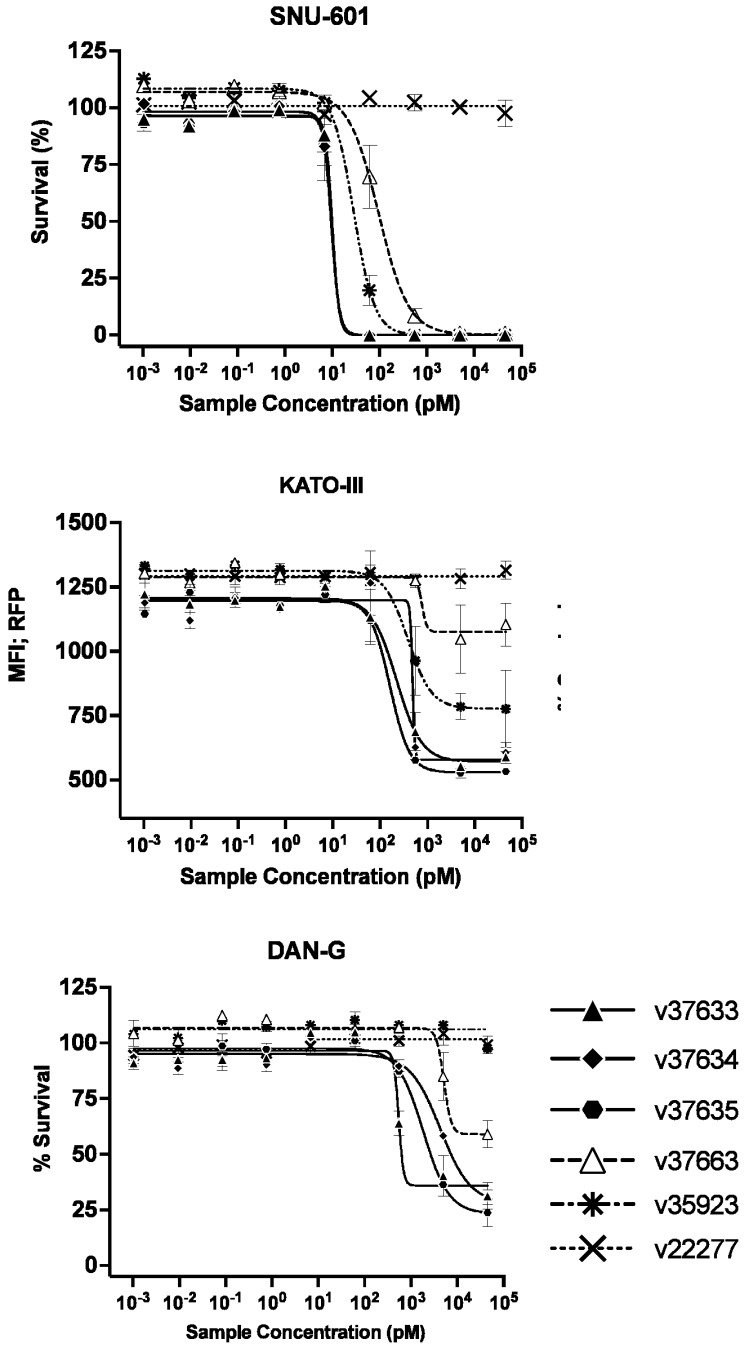

SNU-601
Survival (%)
125
100
75
50
25
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Sample Concentration (pM)
KATO-III
MFI; RFP
1500
1250
1000
750
500
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Sample Concentration (pM)
DAN-G
% Survival
125
100
75
50
25
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Sample Concentration (pM)
v37633
v37634
v37635
v37663
v35923
v22277

FIG. 12A

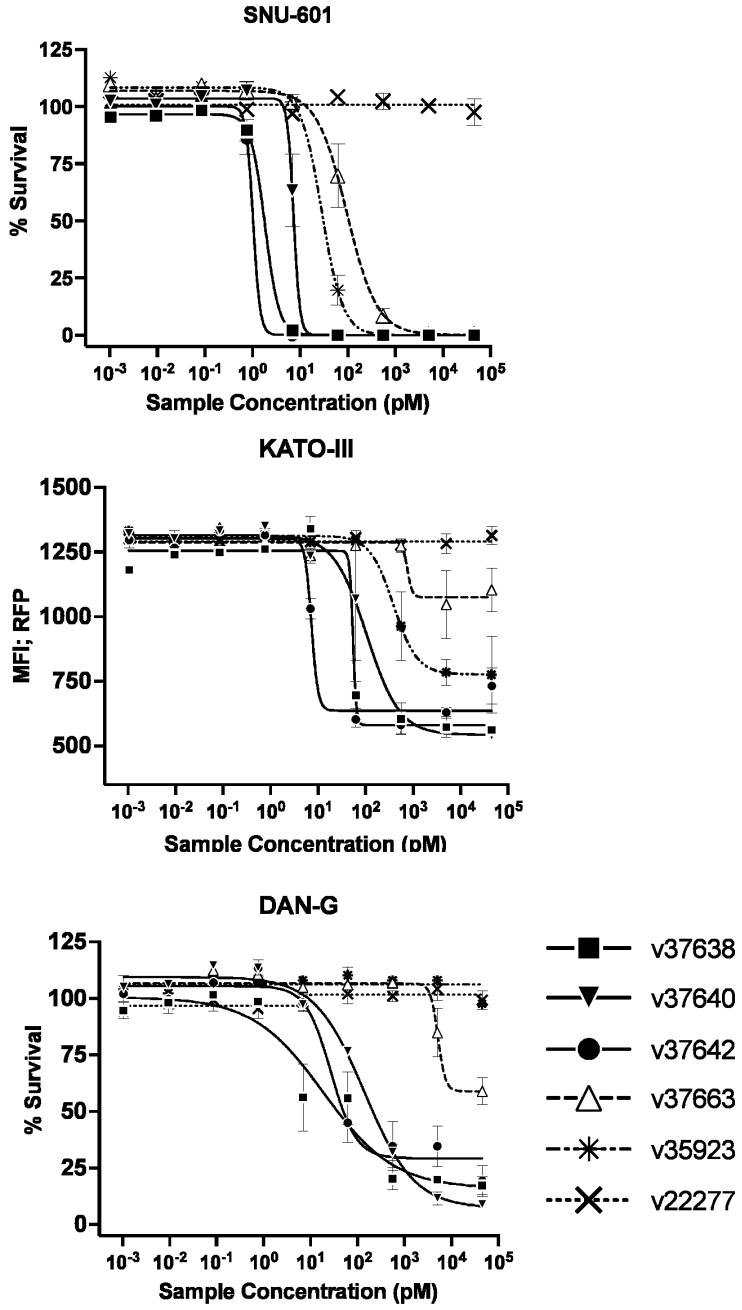

SNU-601
% Survival
125
100
75
50
25
0
10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
KATO-III
MFI; RFP
1500
1250
1000
750
500
10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
DAN-G
% Survival
125
100
75
50
25
0
10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v37638
v37640
v37642
v37663
v35923
v22277

FIG. 12B

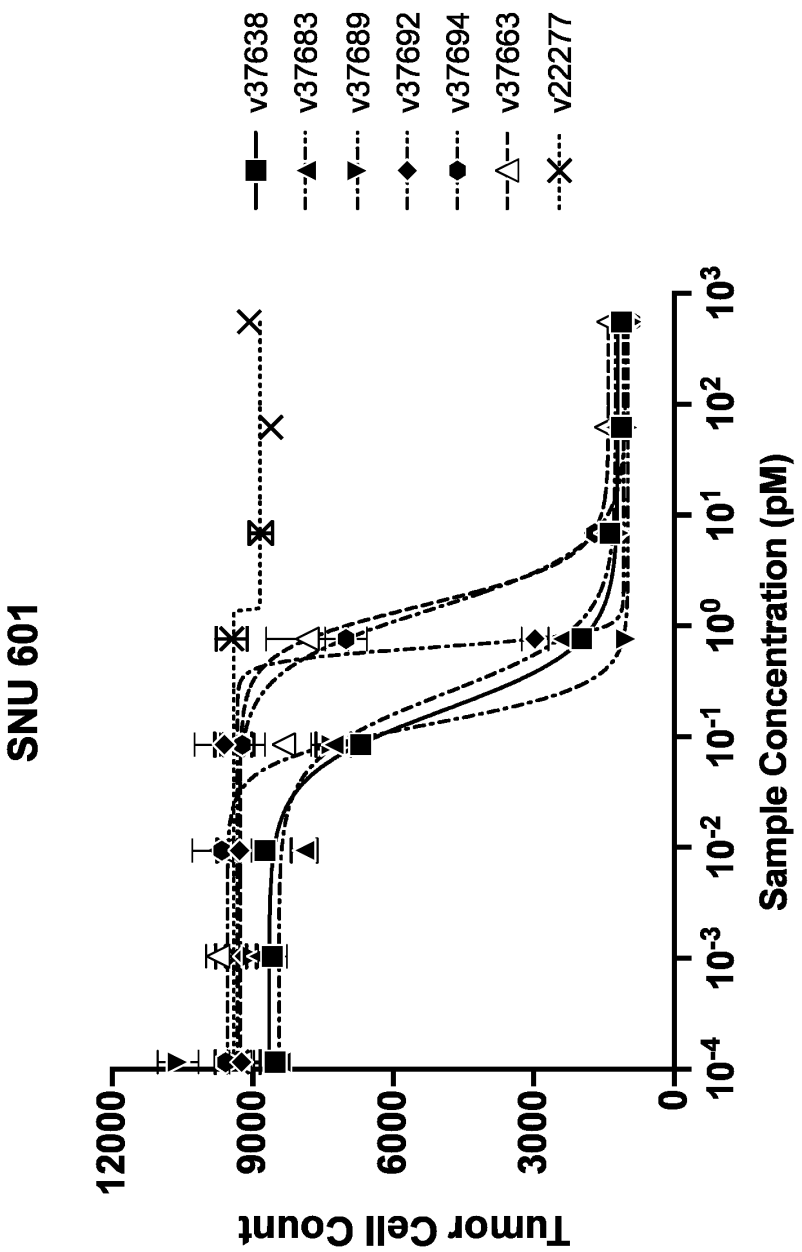

SNU 601
v37638
v37683
v37689
v37692
v37694
v37663
v22277
Tumor Cell Count
12000
9000
6000
3000
0
Sample Concentration (pM)
10^-4
10^-3
10^-2
10^-1
10^0
10^1
10^2
10^3

FIG. 13

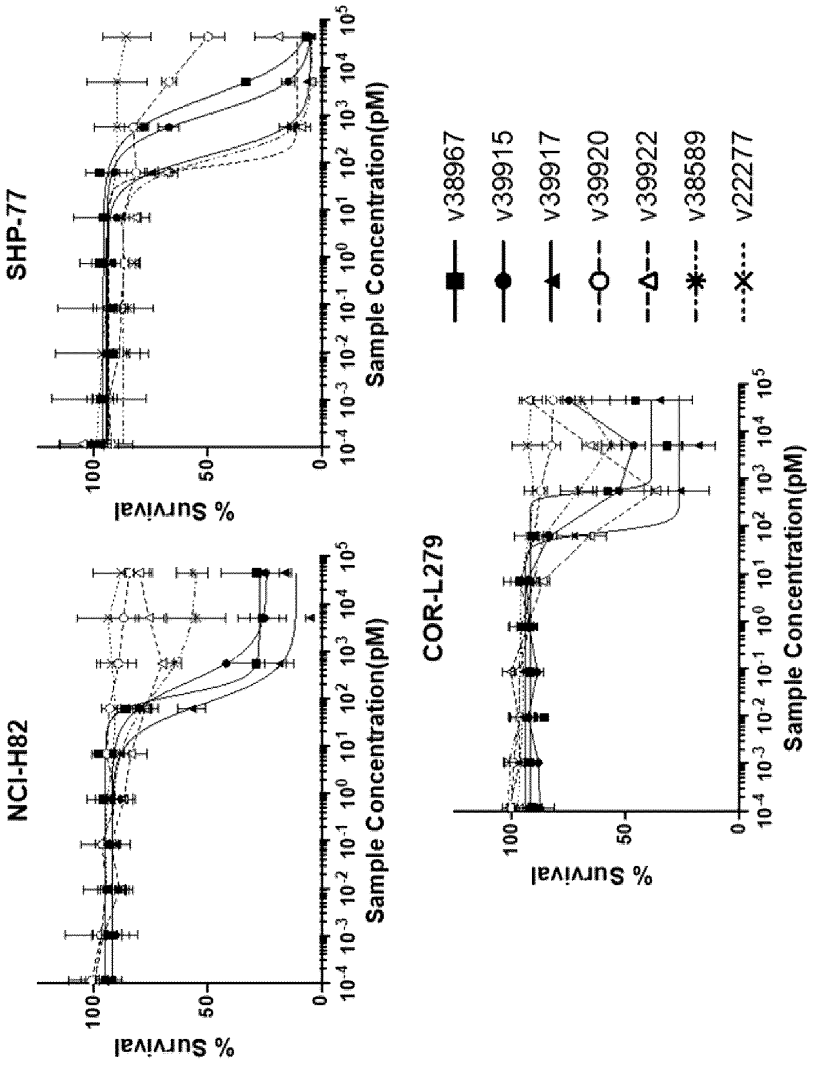

SHP-77
NCI-H82
COR-L279
% Survival
Sample Concentration(pM)
v38967
v39915
v39917
v39920
v39922
v38589
v22277

FIG. 14A

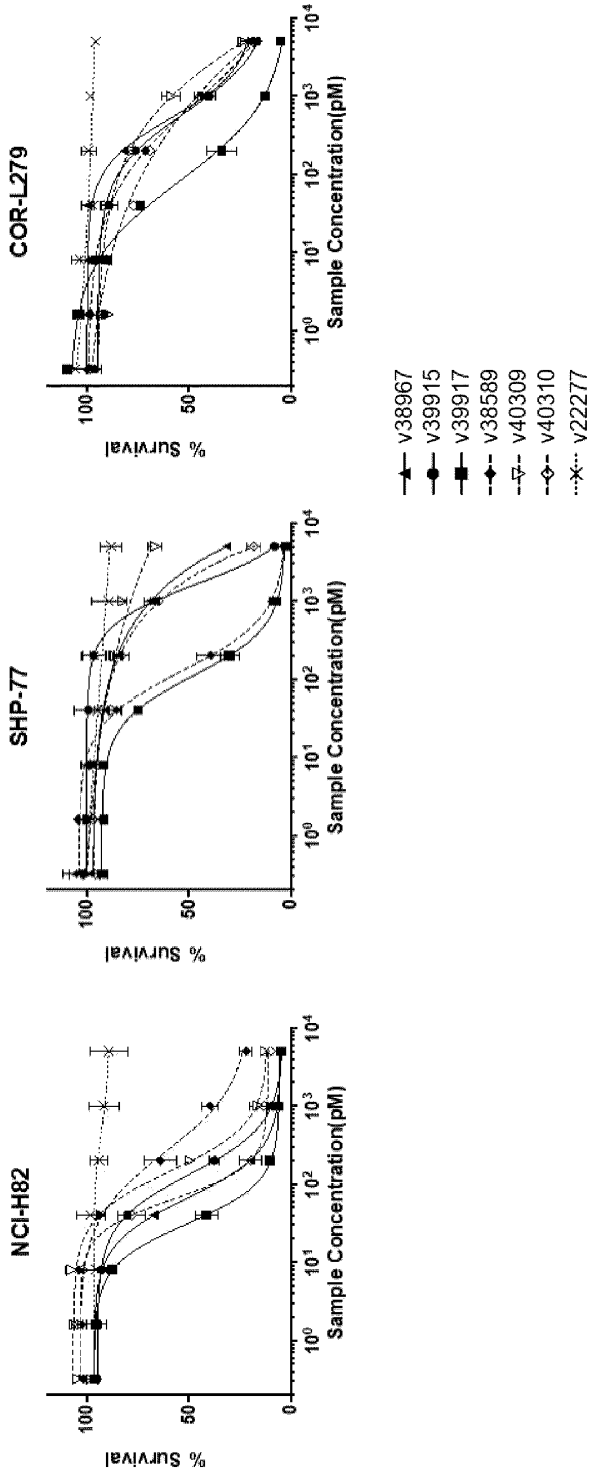

COR-L279
SHP-77
NCI-H82
% Survival
Sample Concentration(pM)
v38967
v39915
v39917
v38589
v40309
v40310
v22277

FIG. 14B

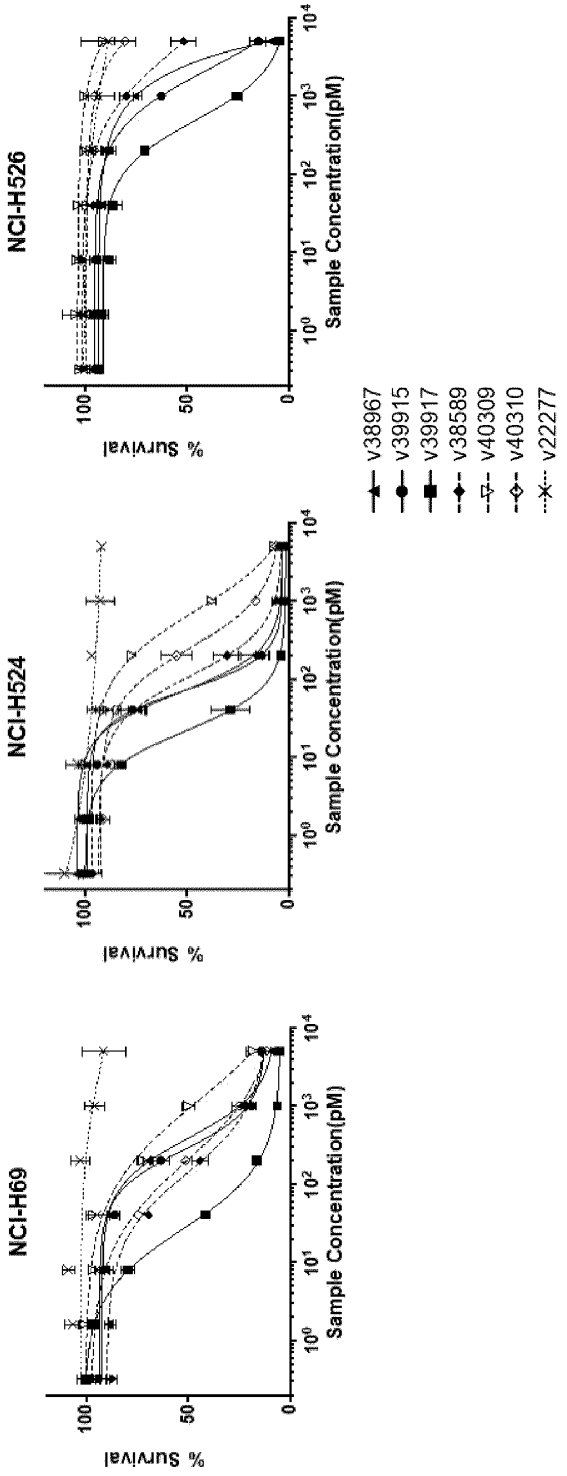

NCI-H69
NCI-H524
NCI-H526
% Survival
Sample Concentration(pM)
v38967
v39915
v39917
v38589
v40309
v40310
v22277

FIG. 14B (cont.)

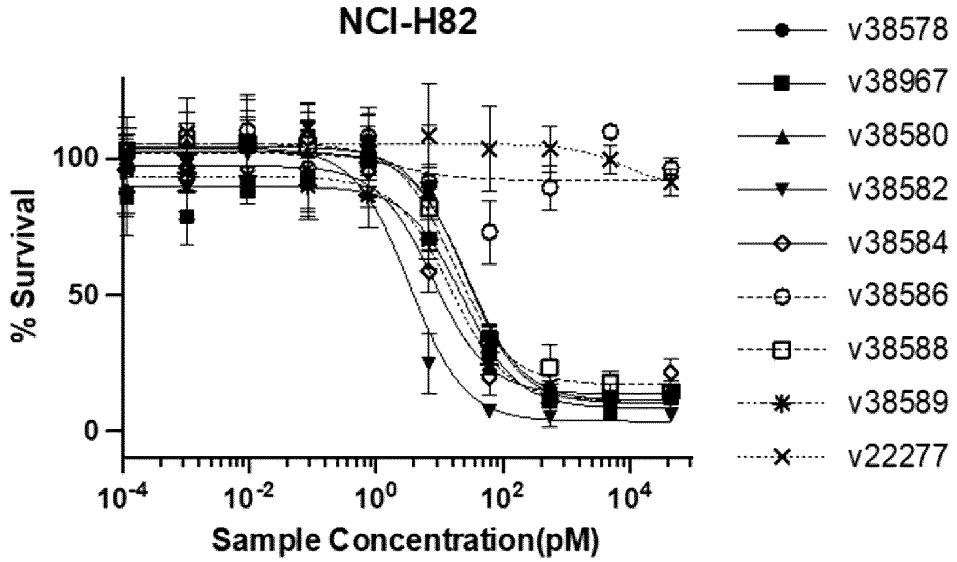


FIG. 14C

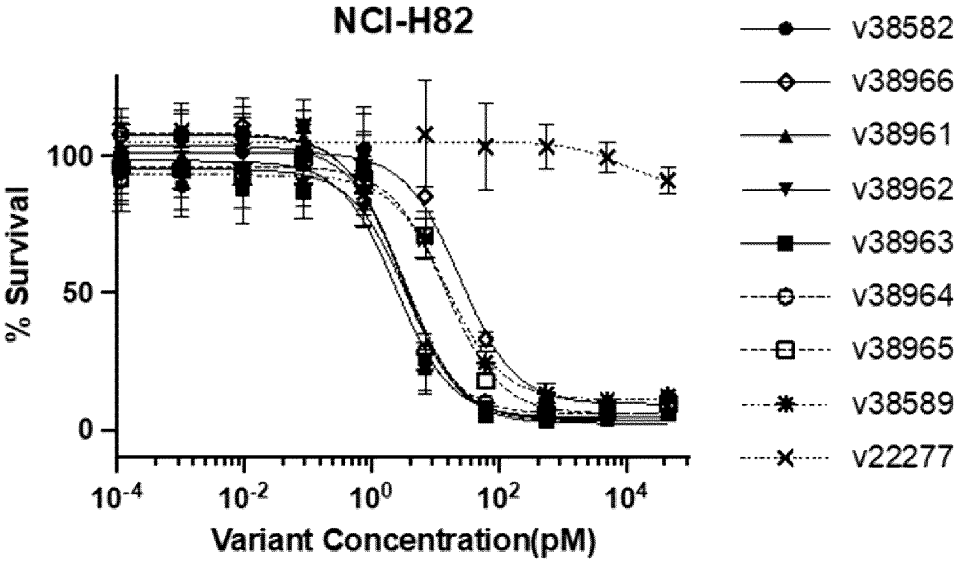


FIG. 14D

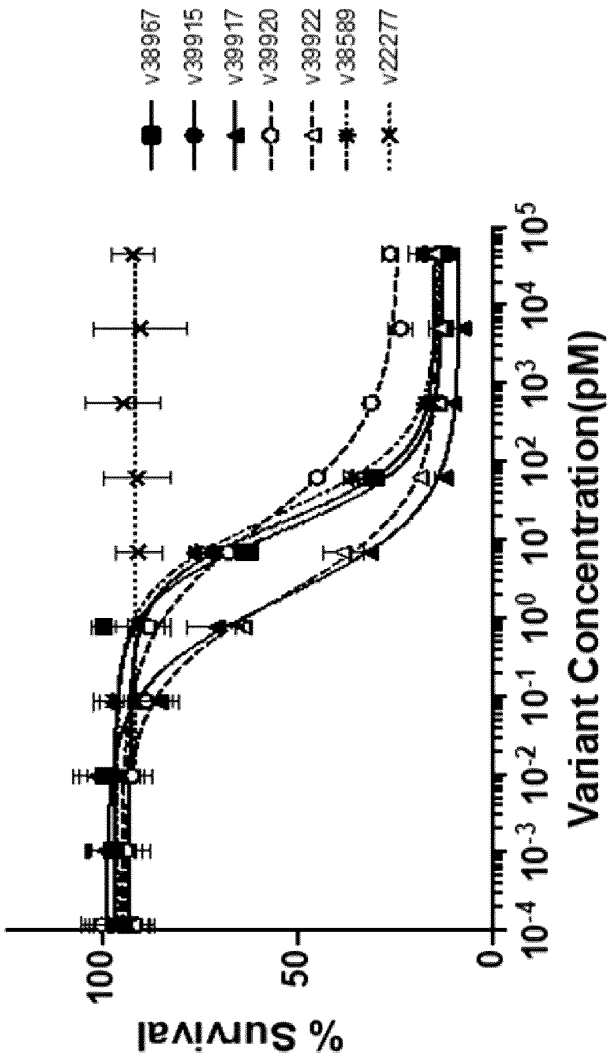

v38967
v39915
v39917
v39920
v39922
v38589
v22277
% Survival
100
50
0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Variant Concentration(pM)

FIG. 14E

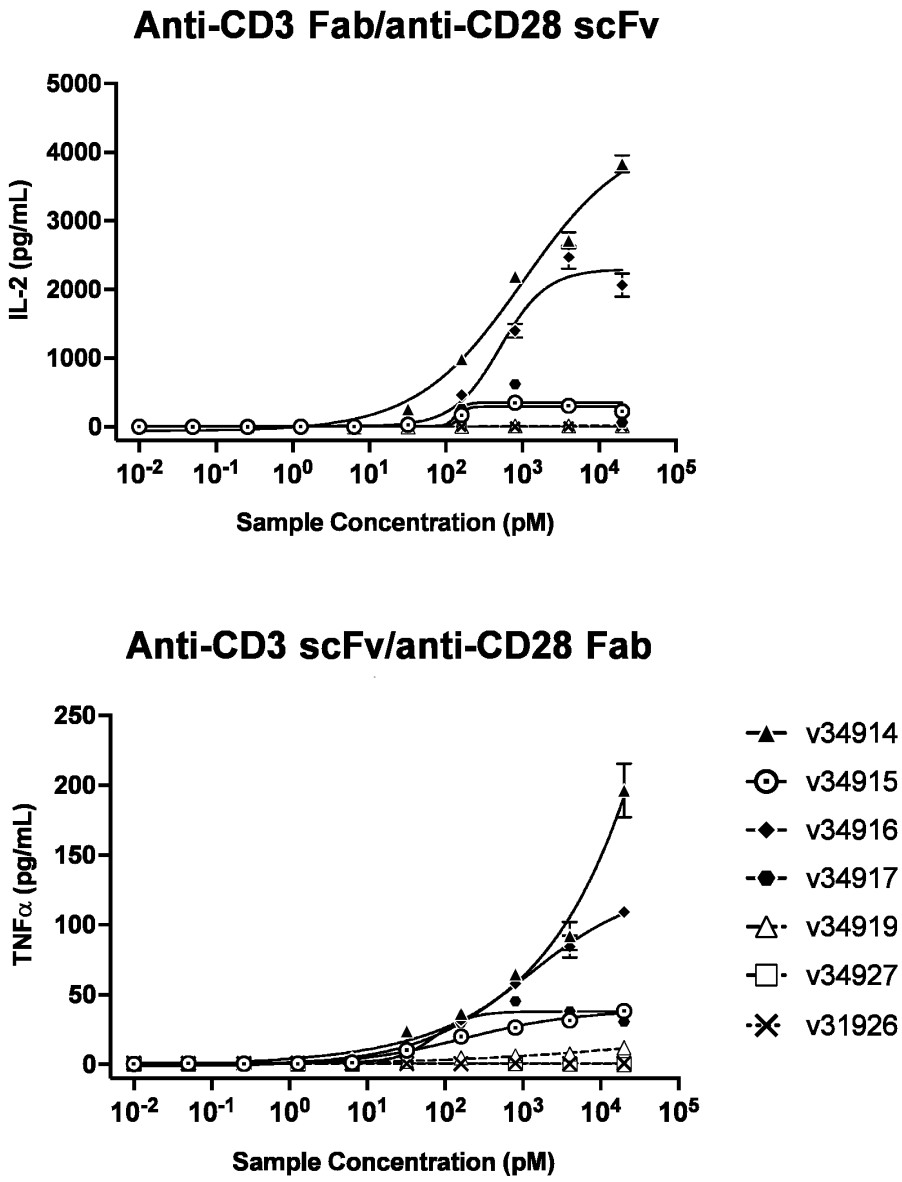

Anti-CD3 Fab/anti-CD28 scFv
IL-2 (pg/mL)
5000
4000
3000
2000
1000
0
10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
Anti-CD3 scFv/anti-CD28 Fab
TNFα (pg/mL)
250
200
150
100
50
0
10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v34914
v34915
v34916
v34917
v34919
v34927
v31926

FIG. 15A

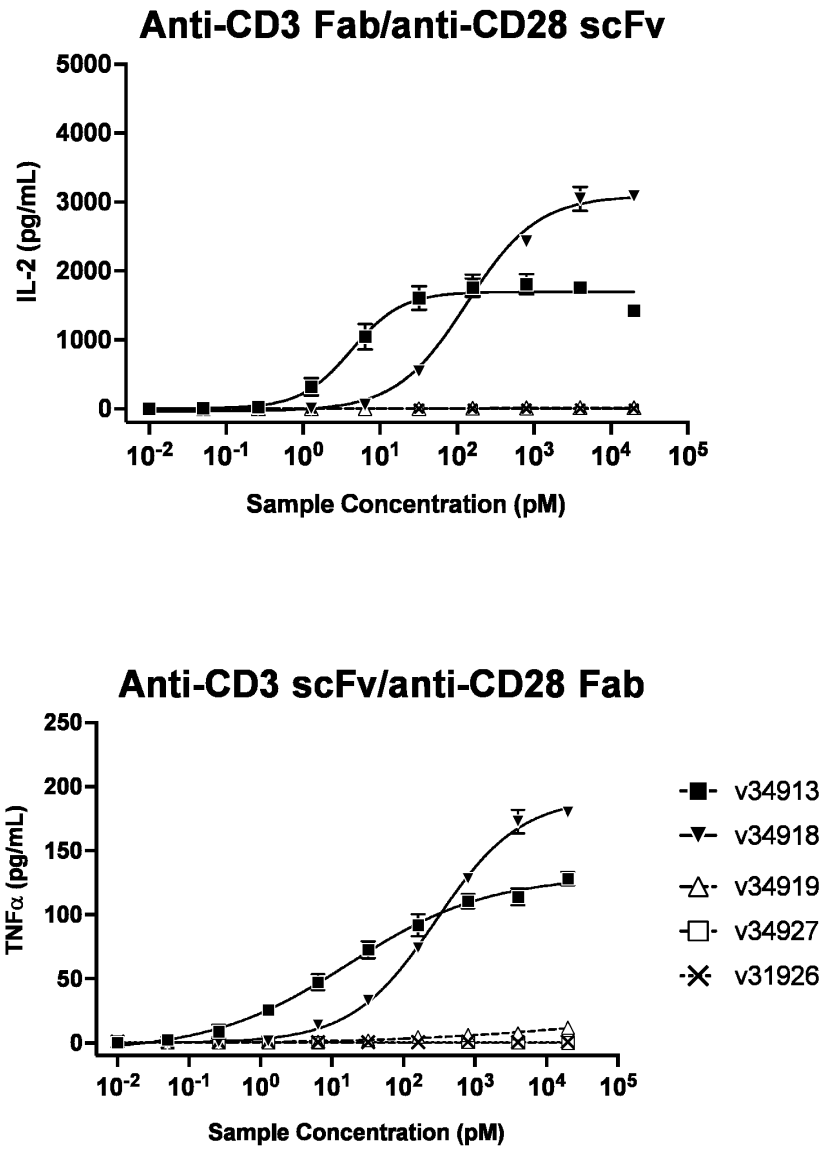

Anti-CD3 Fab/anti-CD28 scFv
IL-2 (pg/mL)
5000
4000
3000
2000
1000
0
10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
Anti-CD3 scFv/anti-CD28 Fab
TNFα (pg/mL)
250
200
150
100
50
0
10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v34913
v34918
v34919
v34927
v31926

FIG. 15B

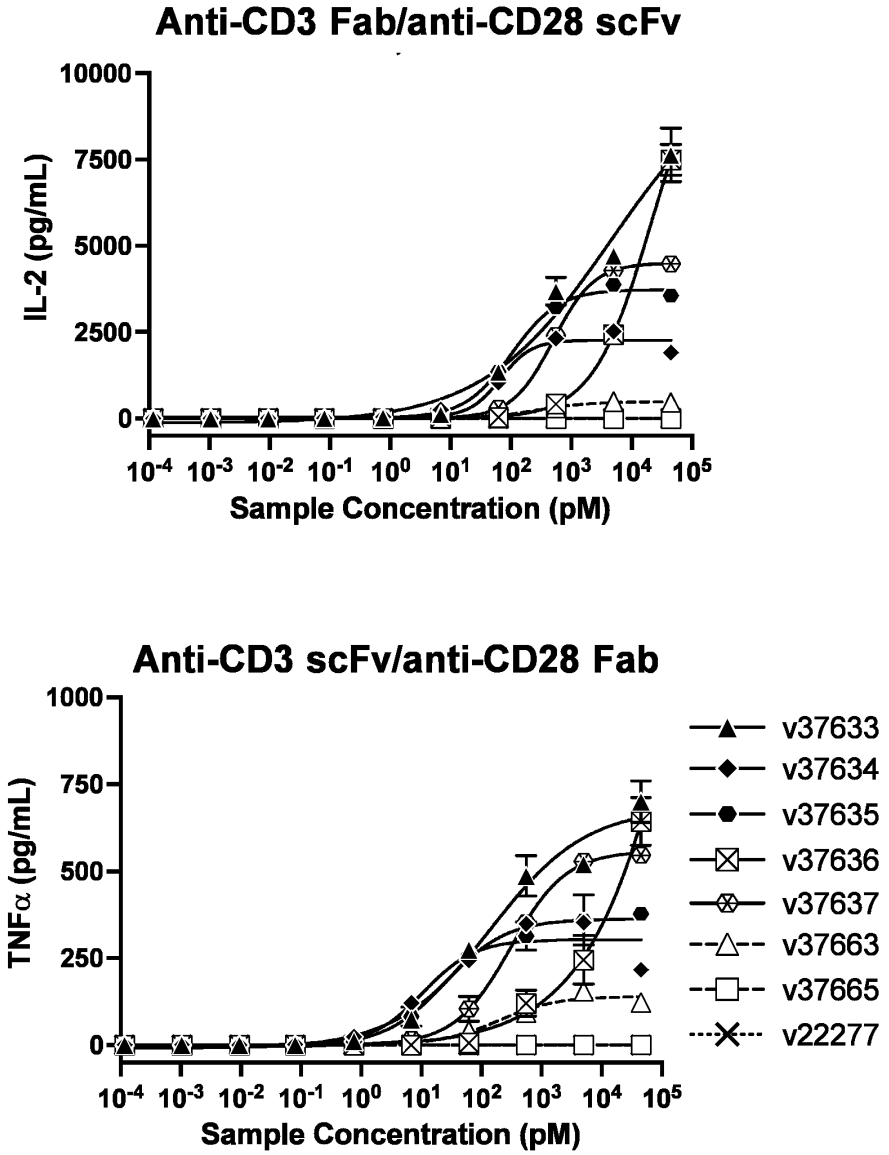

Anti-CD3 Fab/anti-CD28 scFv
IL-2 (pg/mL)
10000
7500
5000
2500
0
10-4 10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
Anti-CD3 scFv/anti-CD28 Fab
TNFα (pg/mL)
1000
750
500
250
0
10-4 10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v37633
v37634
v37635
v37636
v37637
v37663
v37665
v22277

FIG. 16A

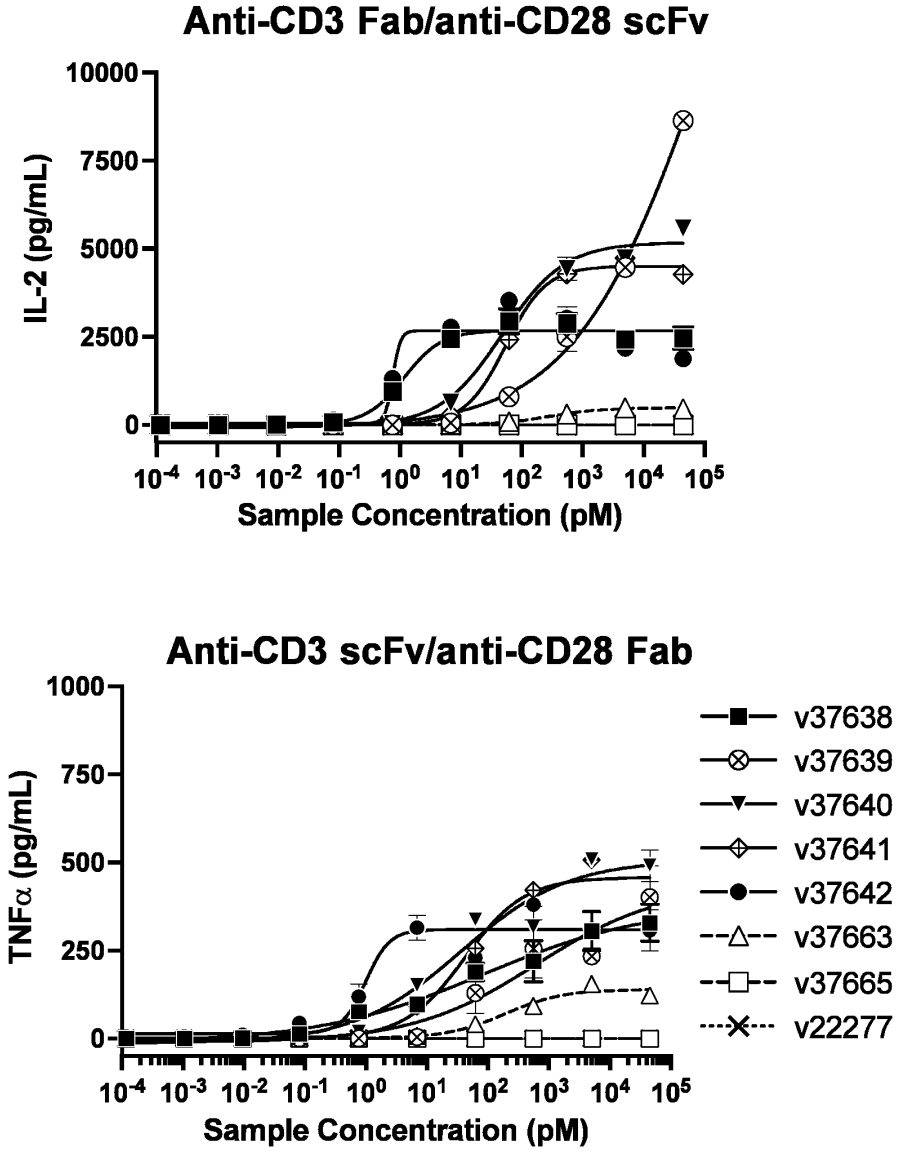

Anti-CD3 Fab/anti-CD28 scFv
IL-2 (pg/mL)
10000
7500
5000
2500
0
10^-4 10^-3 10^-2 10^-1 10^0 10^1 10^2 10^3 10^4 10^5
Sample Concentration (pM)
Anti-CD3 scFv/anti-CD28 Fab
TNFα (pg/mL)
1000
750
500
250
0
10^-4 10^-3 10^-2 10^-1 10^0 10^1 10^2 10^3 10^4 10^5
Sample Concentration (pM)
v37638
v37639
v37640
v37641
v37642
v37663
v37665
v22277

FIG. 16B

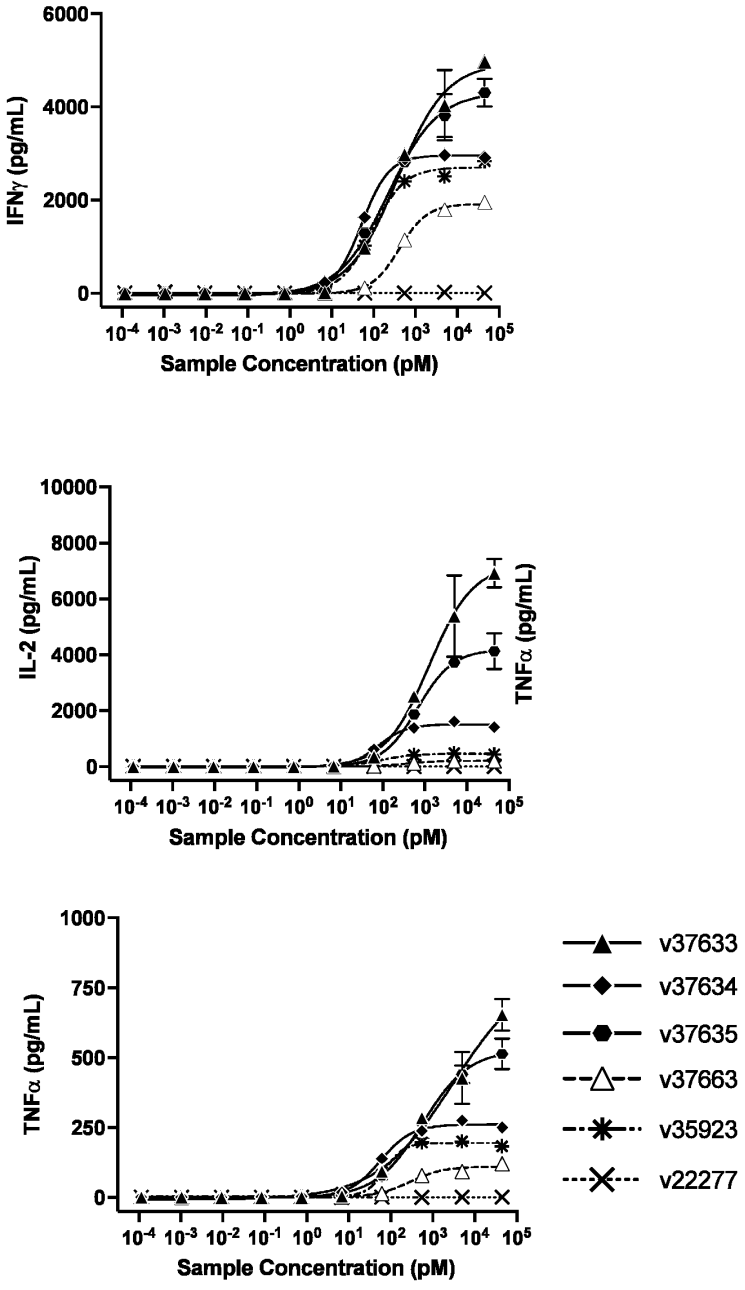

IFNγ (pg/mL)
6000
4000
2000
0
10-4 10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
IL-2 (pg/mL)
TNFα (pg/mL)
10000
8000
6000
4000
2000
0
Sample Concentration (pM)
TNFα (pg/mL)
1000
750
500
250
0
Sample Concentration (pM)
v37633
v37634
v37635
v37663
v35923
v22277

FIG. 17A

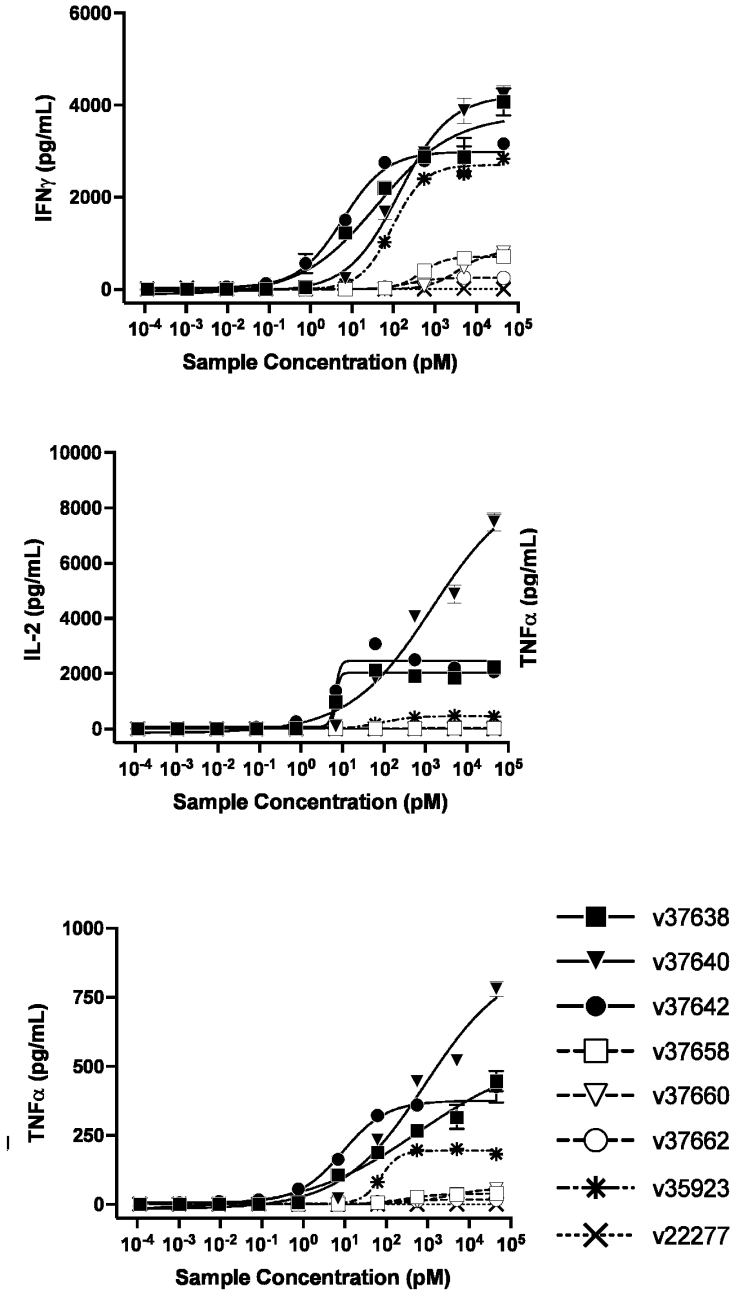

6000
4000
2000
0
IFNγ (pg/mL)
10-4 10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
10000
8000
6000
4000
2000
0
IL-2 (pg/mL)
TNFα (pg/mL)
10-4 10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
1000
750
500
250
0
TNFα (pg/mL)
10-4 10-3 10-2 10-1 100 101 102 103 104 105
Sample Concentration (pM)
v37638
v37640
v37642
v37658
v37660
v37662
v35923
v22277

FIG. 17B

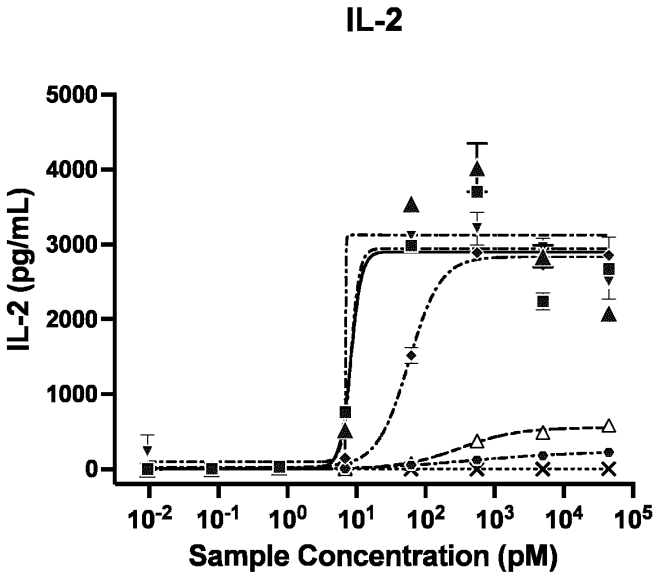


FIG. 18A

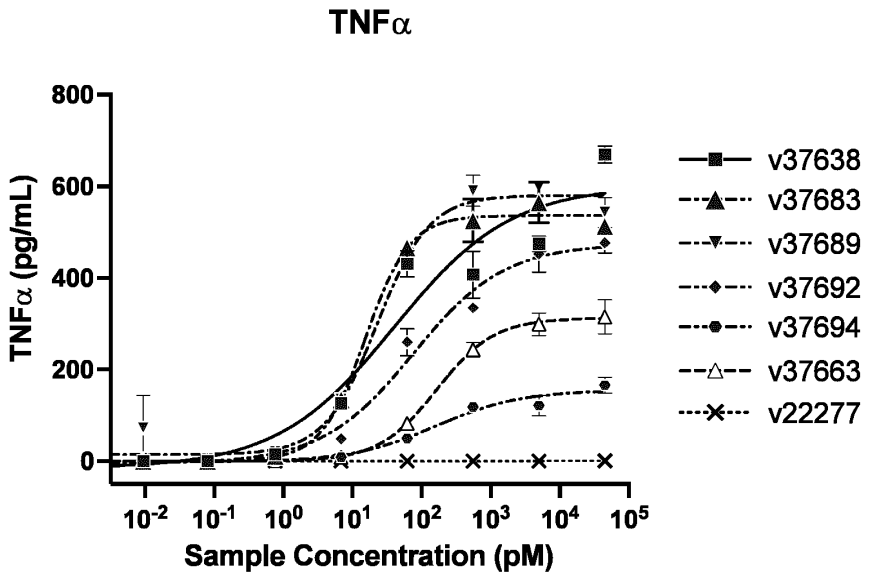


FIG. 18B

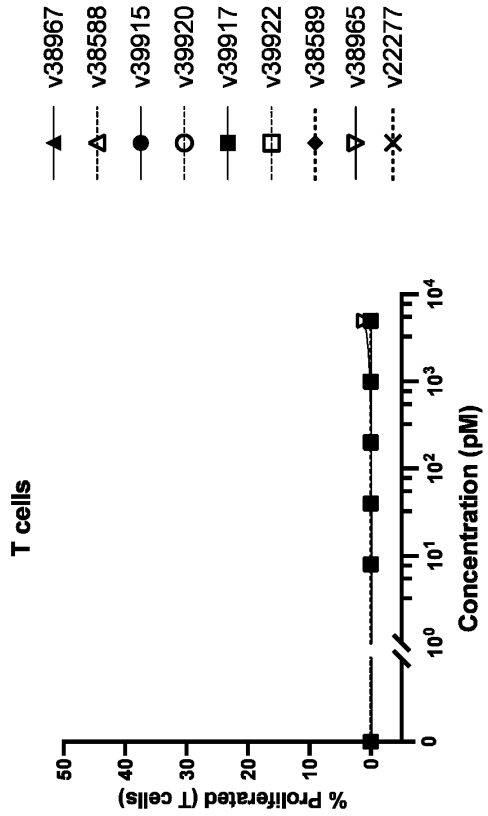


FIG. 18D

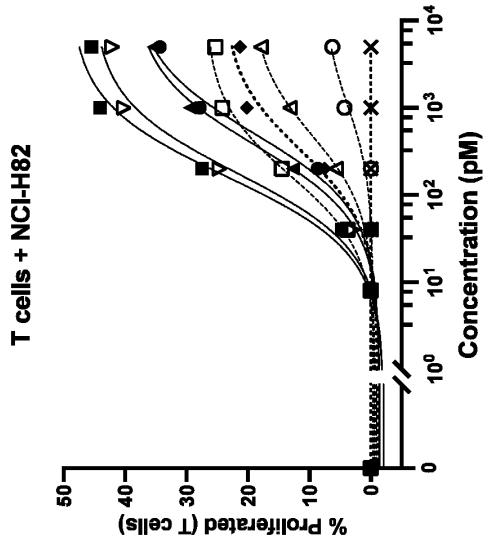


FIG. 18C

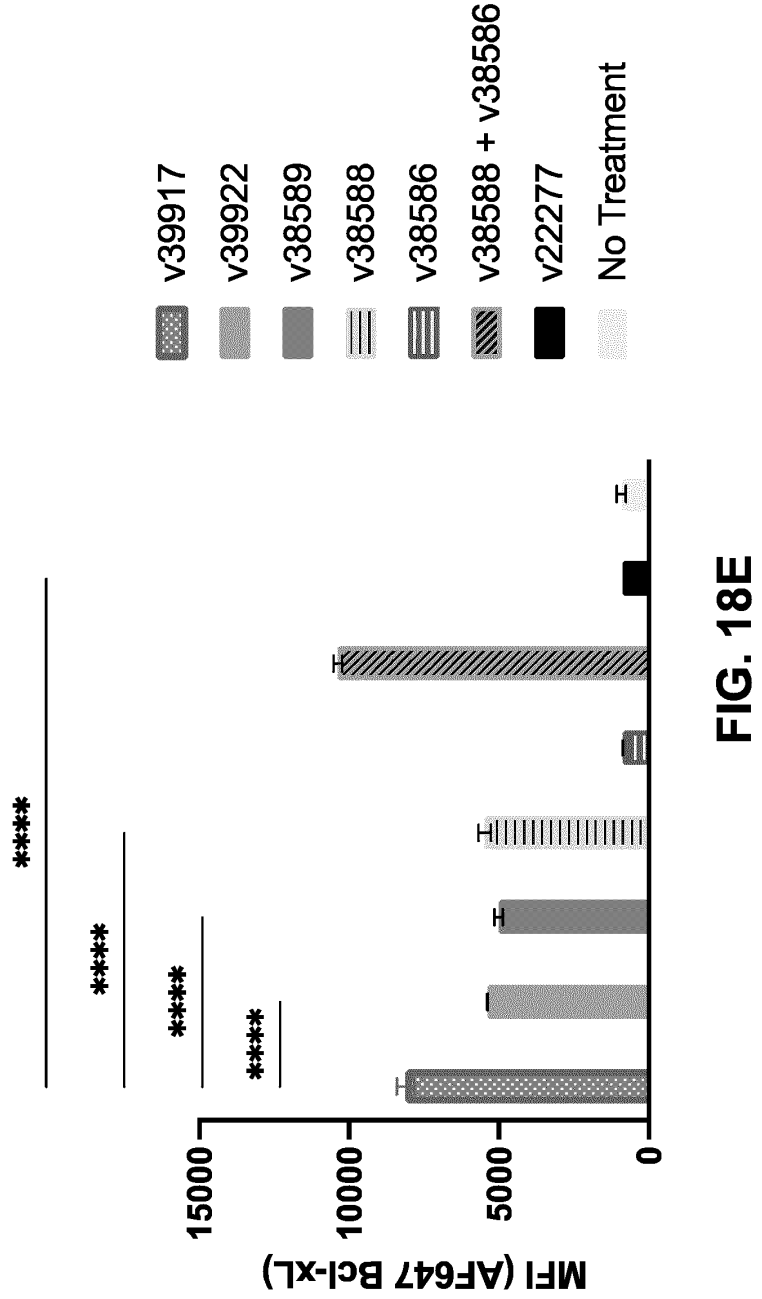

v39917
v39922
v38589
v38588
v38586
v38588 + v38586
v22277
No Treatment
MFI (AF647 Bcl-xL)
15000
10000
5000
0
*****
*****
*****
*****

FIG. 18E

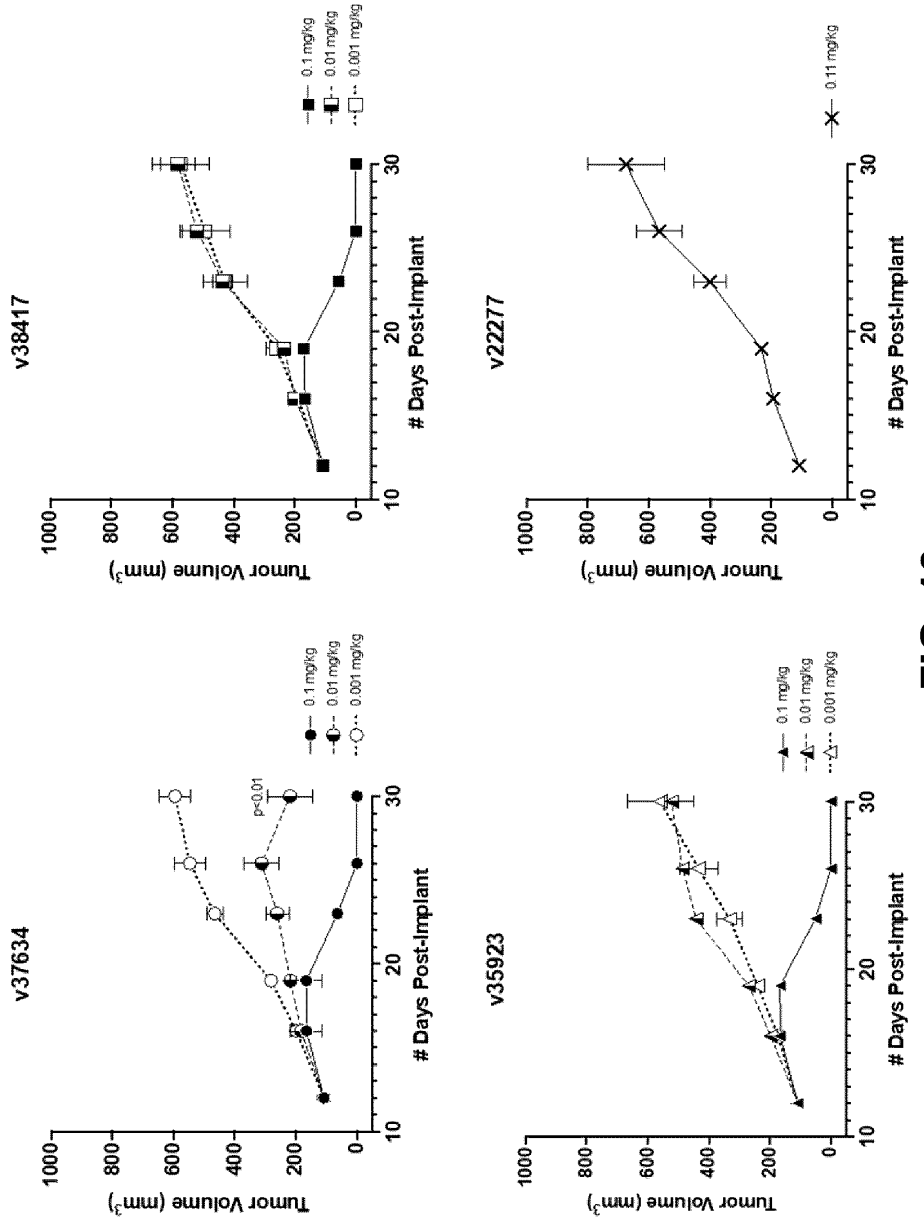

v38417
v22277
v37634
v35923
Tumor Volume (mm3)
Days Post-Implant
0.1 mg/kg
0.01 mg/kg
0.001 mg/kg
p<0.01

FIG. 19

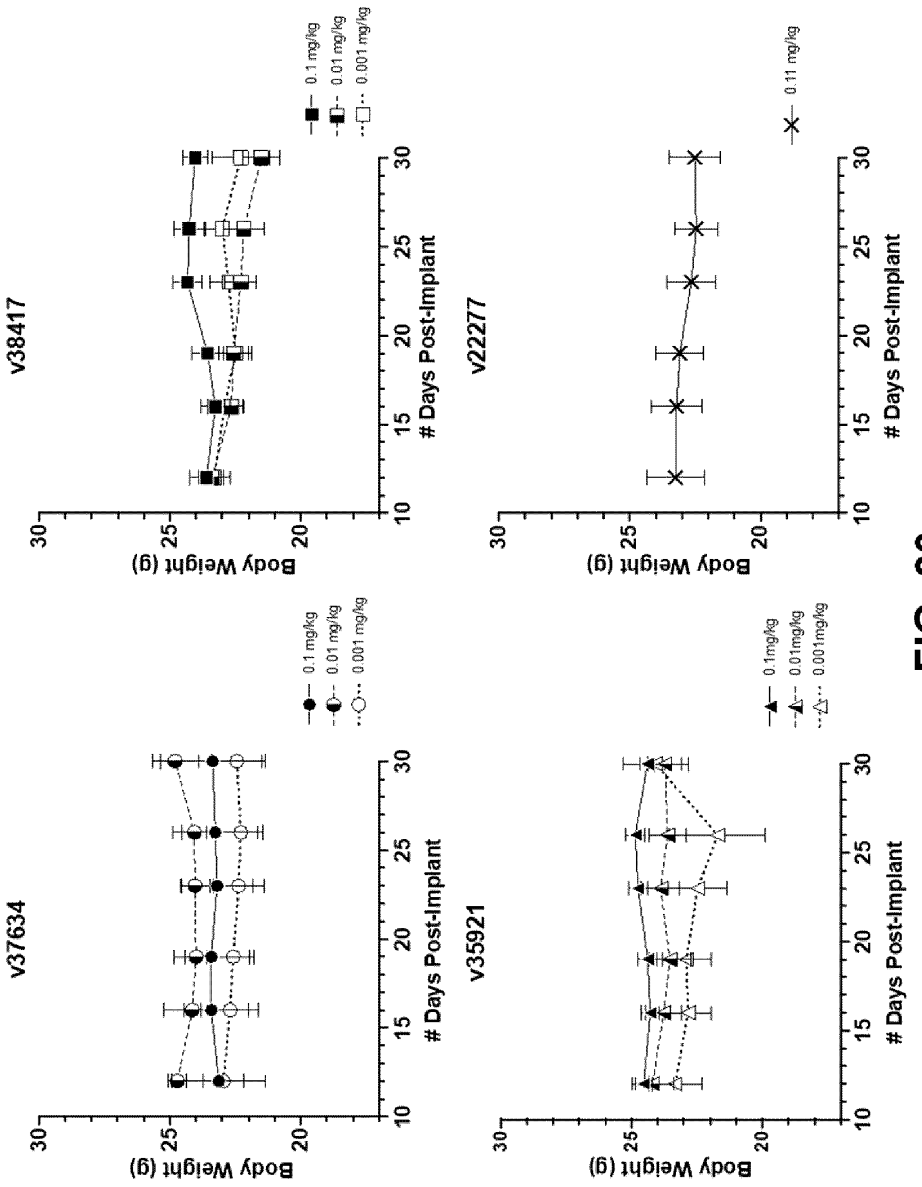

v38417
v22277
v37634
v35921
Body Weight (g)
Days Post-Implant
0.1 mg/kg
0.01 mg/kg
0.001 mg/kg
0.11 mg/kg
0.1mg/kg
0.01mg/kg
0.001mg/kg

FIG. 20

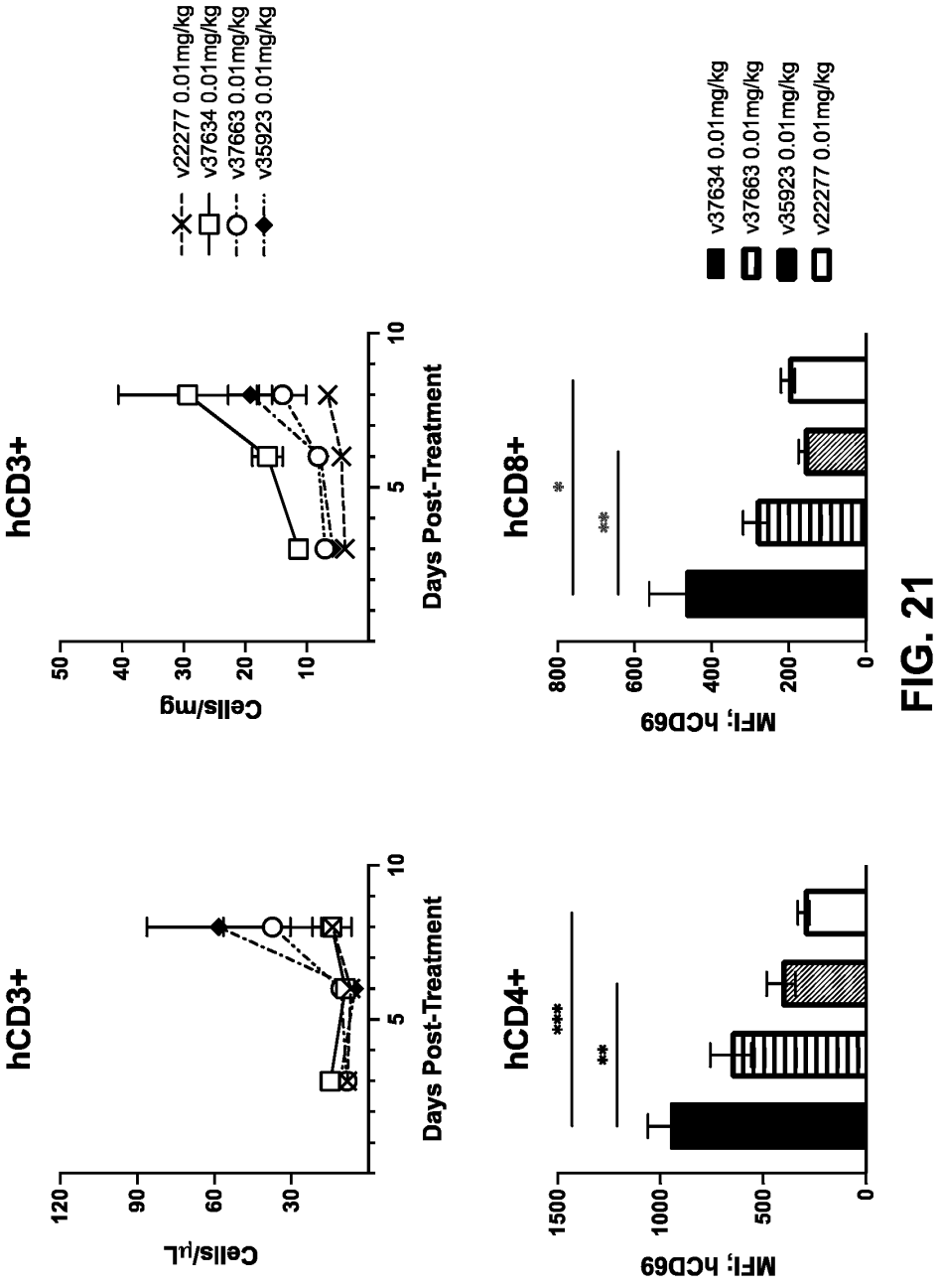

hCD3+
Cells/μL
Days Post-Treatment
hCD3+
Cells/mg
Days Post-Treatment
v22277 0.01mg/kg
v37634 0.01mg/kg
v37663 0.01mg/kg
v35923 0.01mg/kg
hCD4+
MFI; hCD69
***
**
hCD8+
MFI; hCD69
*
**
v37634 0.01mg/kg
v37663 0.01mg/kg
v35923 0.01mg/kg
v22277 0.01mg/kg

FIG. 21

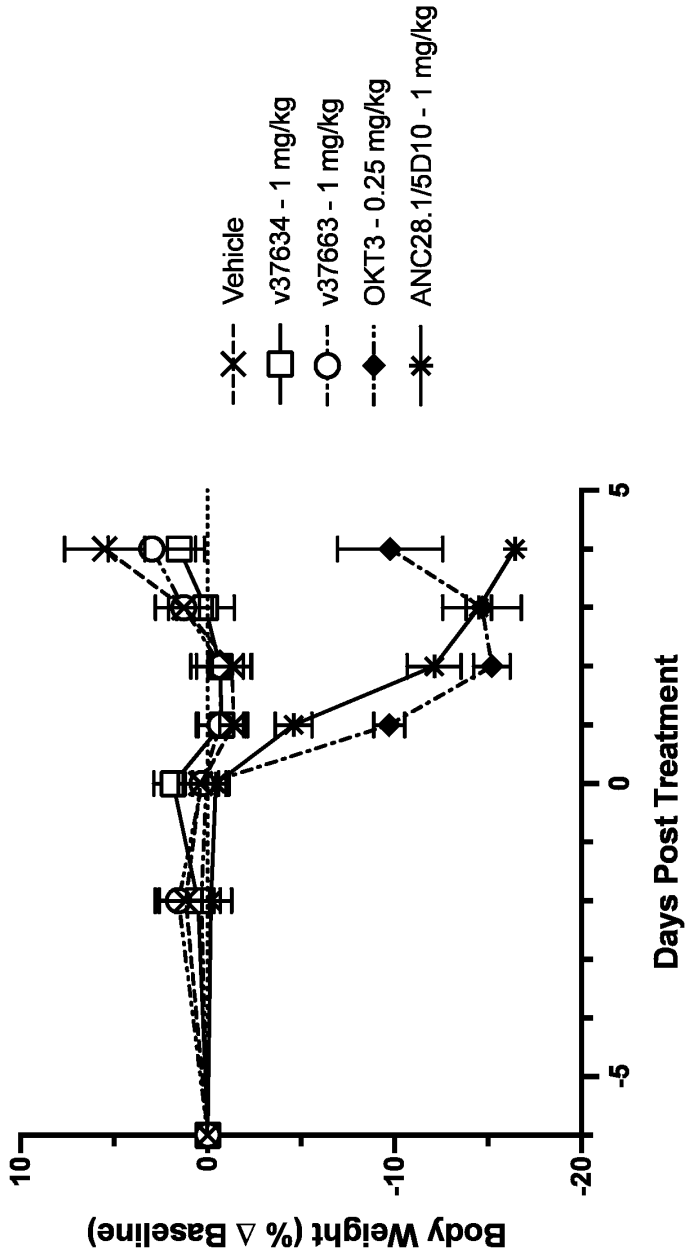

Vehicle
v37634 - 1 mg/kg
v37663 - 1 mg/kg
OKT3 - 0.25 mg/kg
ANC28.1/5D10 - 1 mg/kg
Body Weight (% ∆ Baseline)
10
0
-10
-20
-5
0
5
Days Post Treatment

FIG. 22

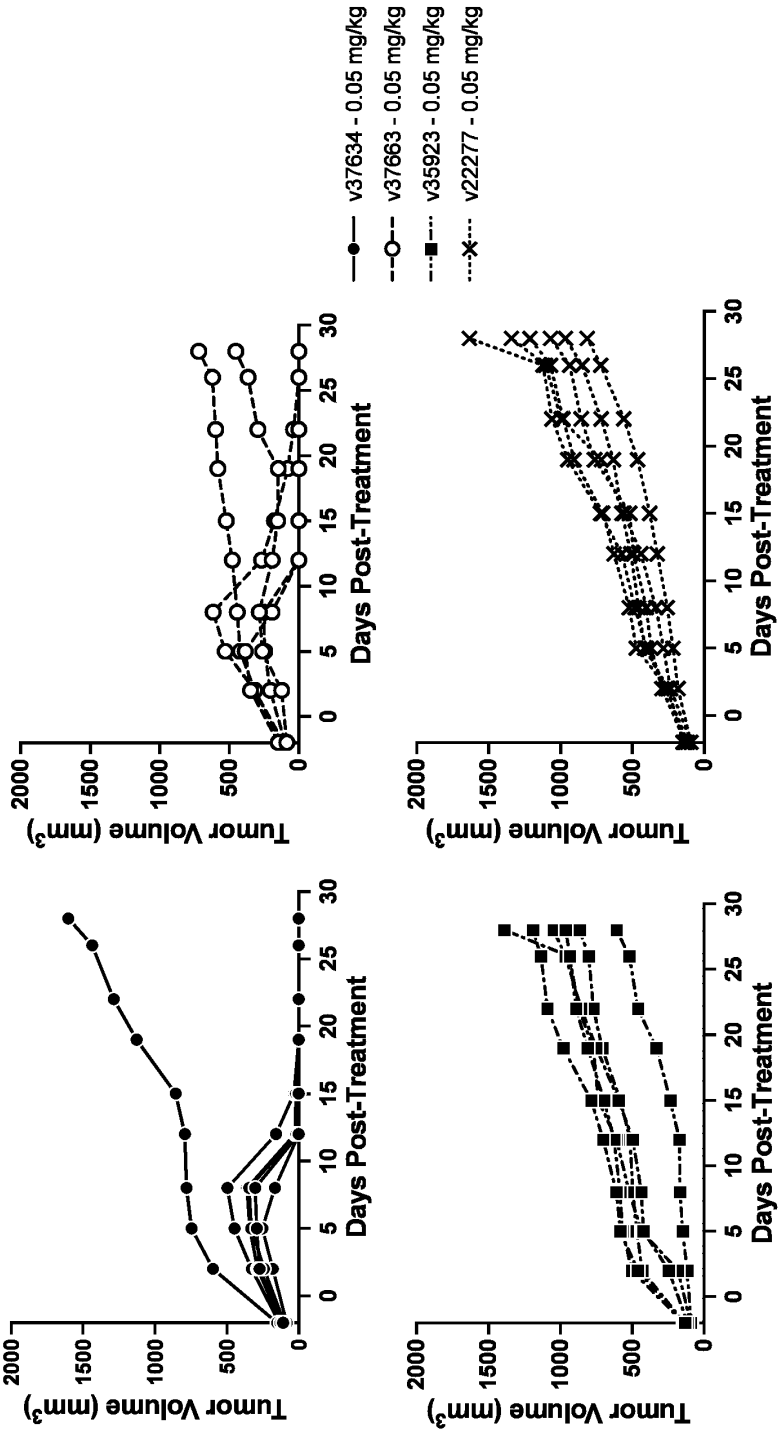

v37634 - 0.05 mg/kg
v37663 - 0.05 mg/kg
v35923 - 0.05 mg/kg
v22277 - 0.05 mg/kg
Tumor Volume (mm3)
Days Post-Treatment
0
500
1000
1500
2000
0
5
10
15
20
25
30

FIG. 23A

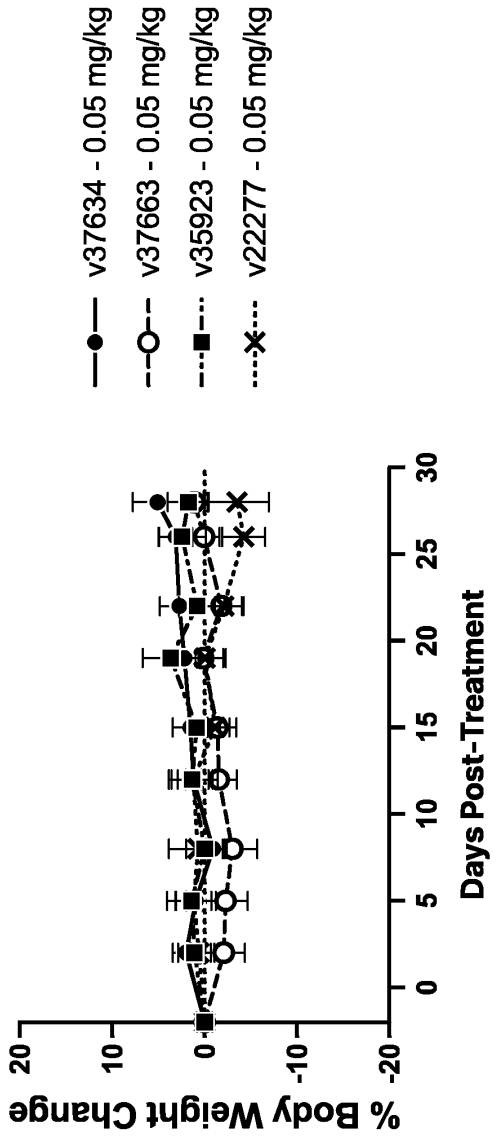

v37634 - 0.05 mg/kg
v37663 - 0.05 mg/kg
v35923 - 0.05 mg/kg
v22277 - 0.05 mg/kg
% Body Weight Change
20
10
0
-10
-20
0
5
10
15
20
25
30
Days Post-Treatment

FIG. 23B

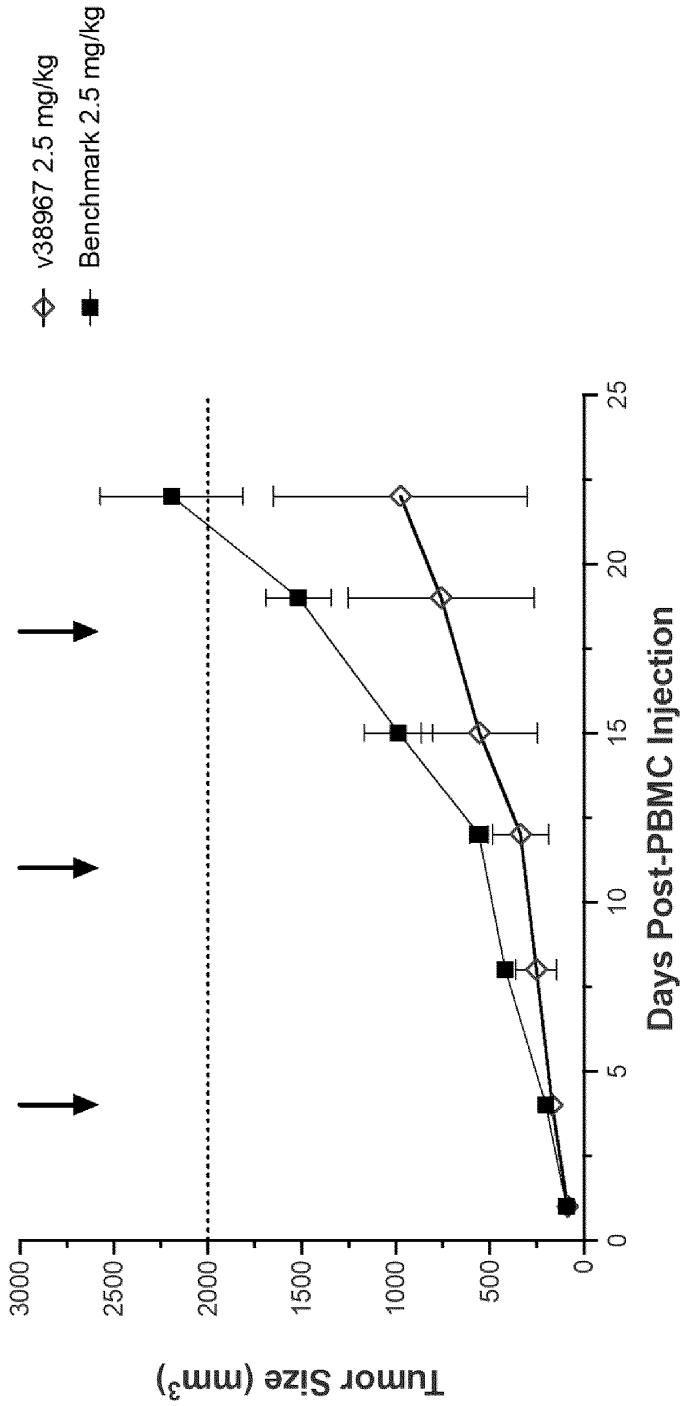

v38967 2.5 mg/kg
Benchmark 2.5 mg/kg
Tumor Size (mm³)
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
Days Post-PBMC Injection

FIG. 24

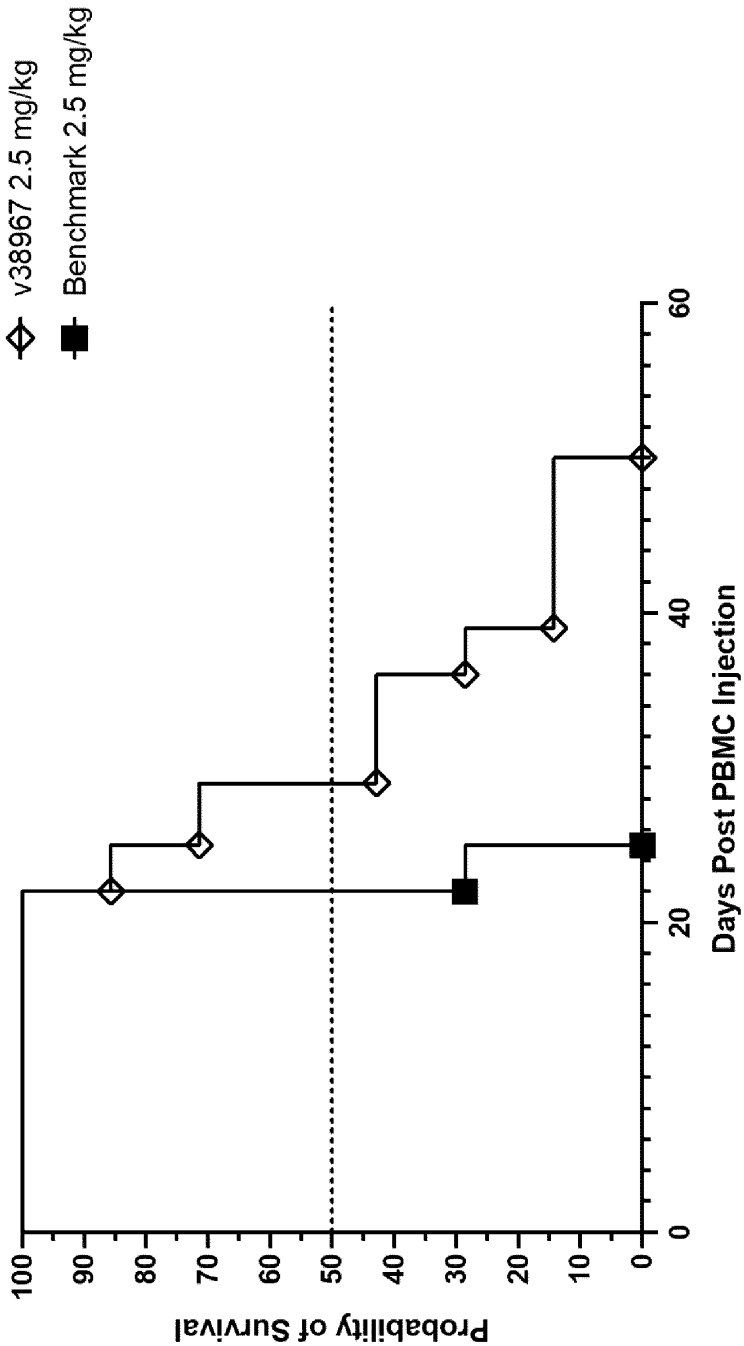

v38967 2.5 mg/kg
Benchmark 2.5 mg/kg
Probability of Survival
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
Days Post PBMC Injection

FIG. 25

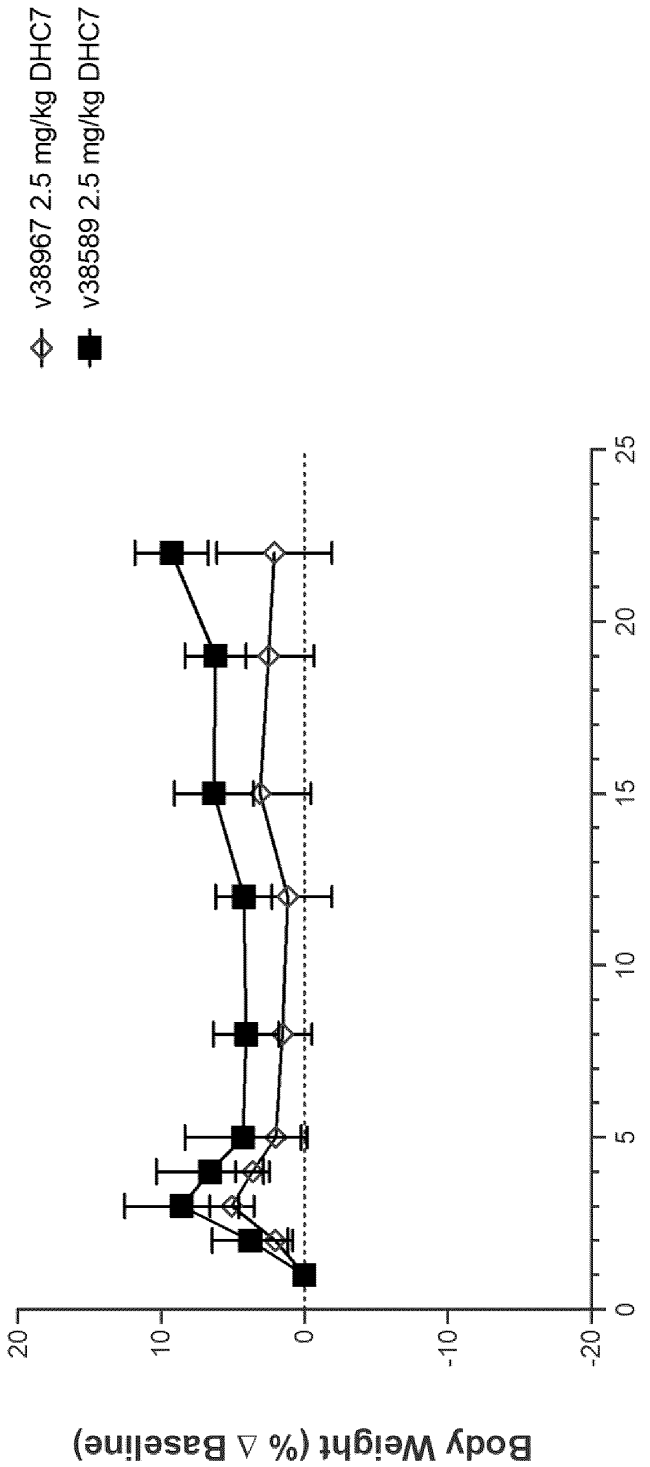

v38967 2.5 mg/kg DHC7
v38589 2.5 mg/kg DHC7
Body Weight (% Δ Baseline)
20
10
0
-10
-20
0
5
10
15
20
25
Days Post Treatment Start

FIG. 26

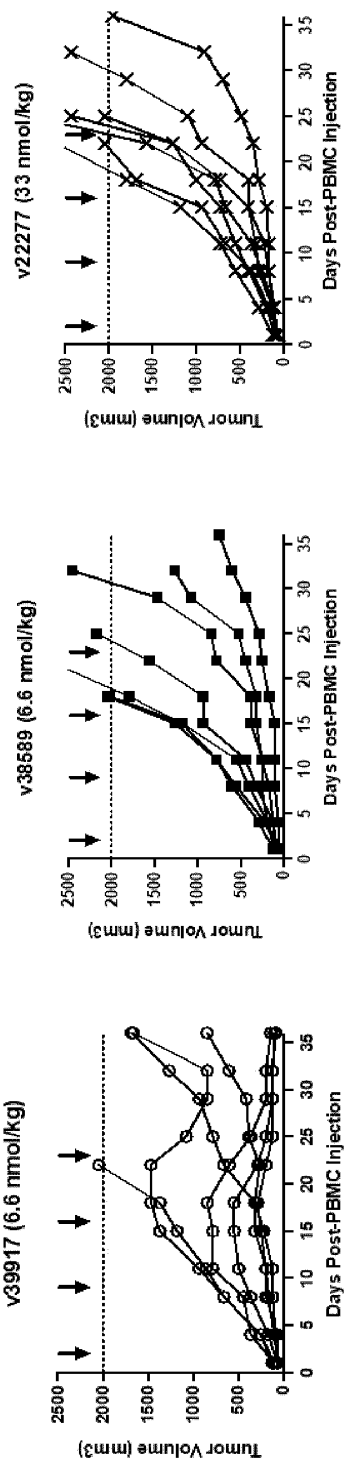

v39917 (6.6 nmol/kg)
v38589 (6.6 nmol/kg)
v22277 (33 nmol/kg)
Tumor Volume (mm3)
Days Post-PBMC Injection

FIG. 27

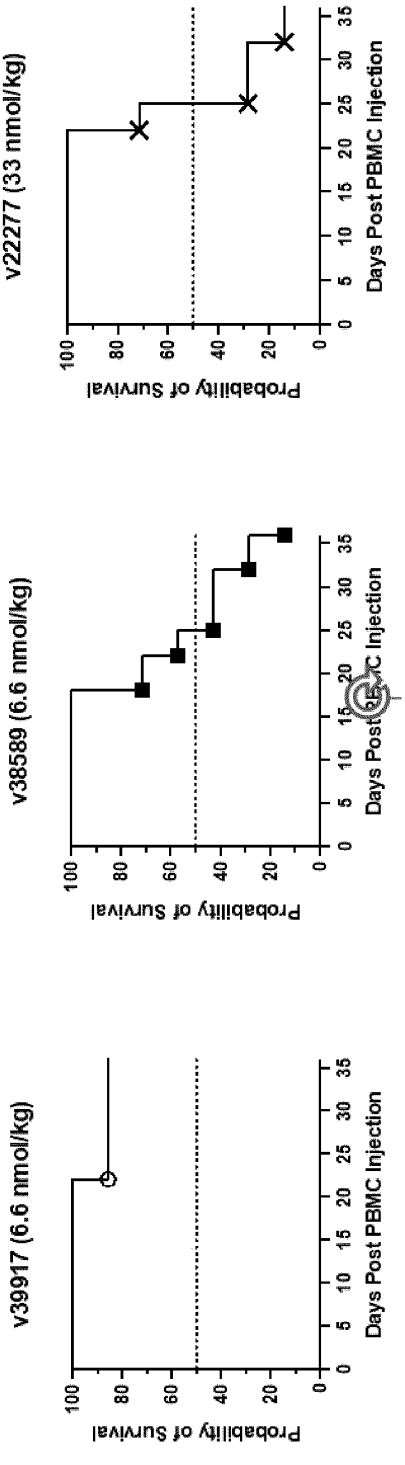

v39917 (6.6 nmol/kg)
v38589 (6.6 nmol/kg)
v22277 (33 nmol/kg)
Probability of Survival
Days Post PBMC Injection

FIG. 28

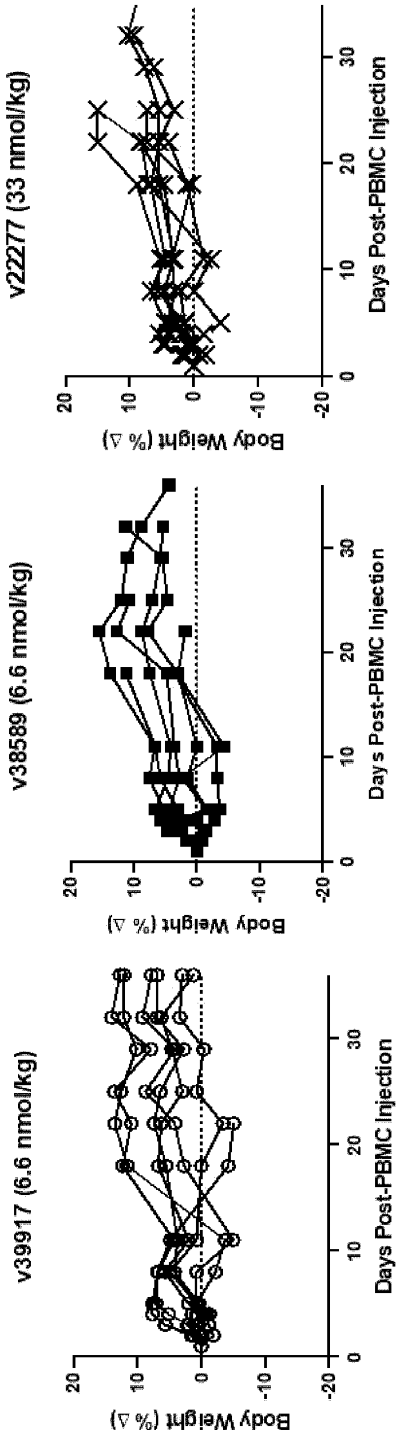

v39917 (6.6 nmol/kg)
v38589 (6.6 nmol/kg)
v22277 (33 nmol/kg)
Body Weight (% Δ)
Days Post-PBMC Injection
20
10
0
-10
-20
0
10
20
30

FIG. 29

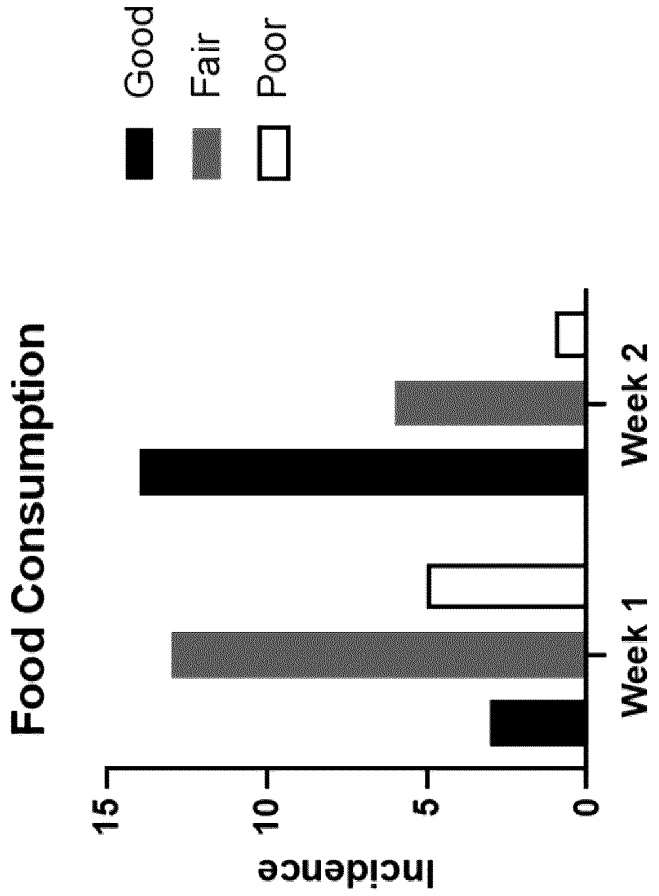

Food Consumption
Good
Fair
Poor
Incidence
15
10
5
0
Week 1
Week 2

FIG. 30

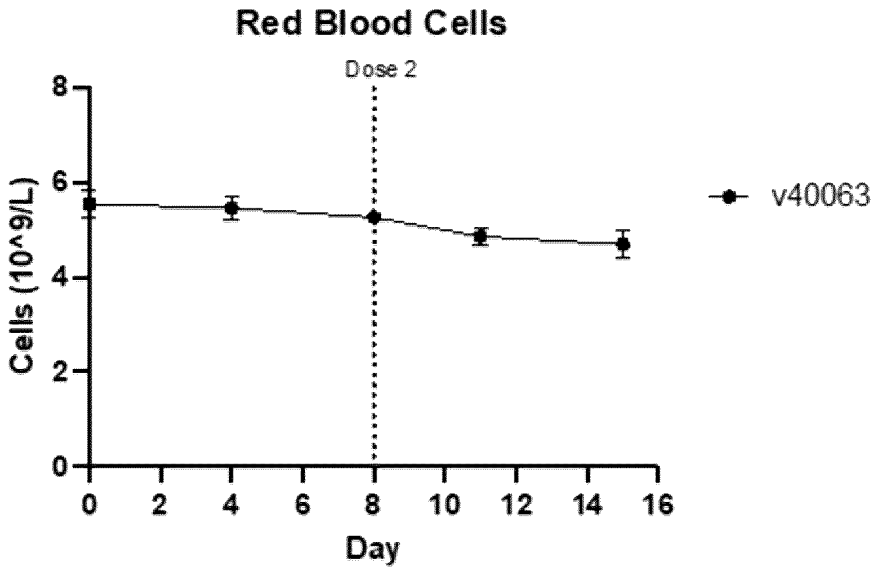


FIG. 31A

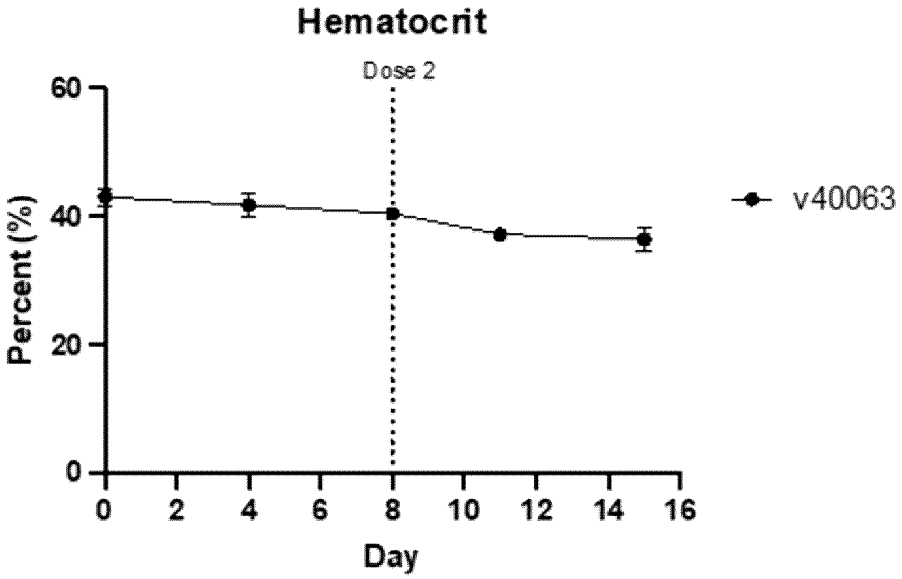


FIG. 31B

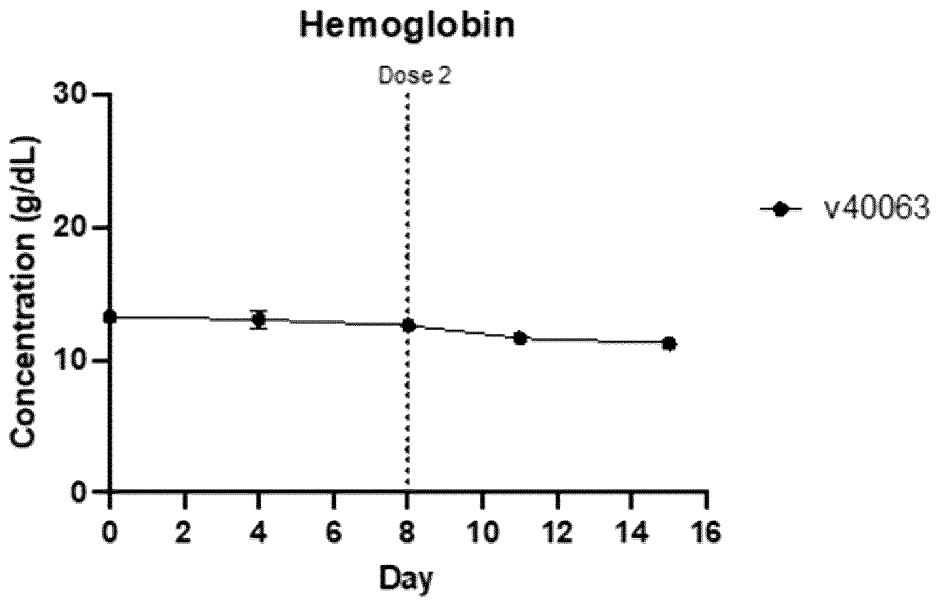

Hemoglobin
Dose 2
Concentration (g/dL)
30
20
10
0
0
2
4
6
8
10
12
14
16
Day
v40063

FIG. 31C

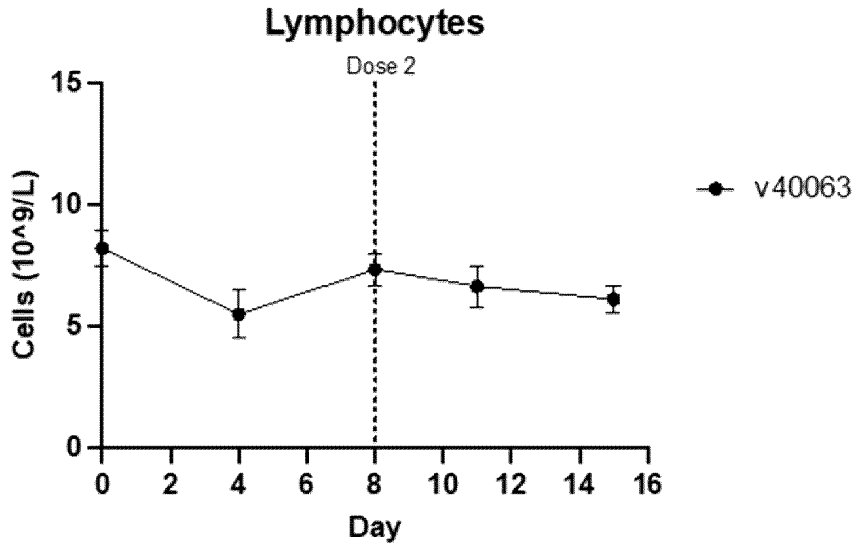


FIG. 32A

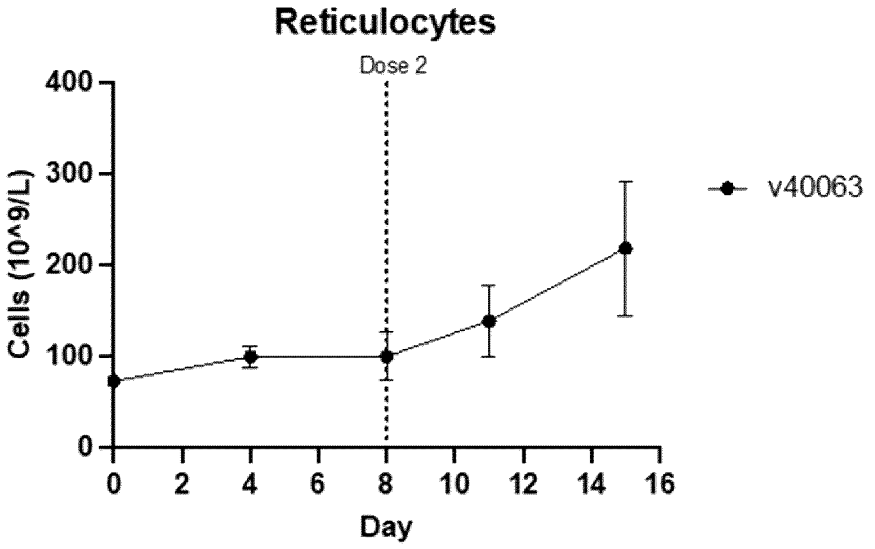


FIG. 32B

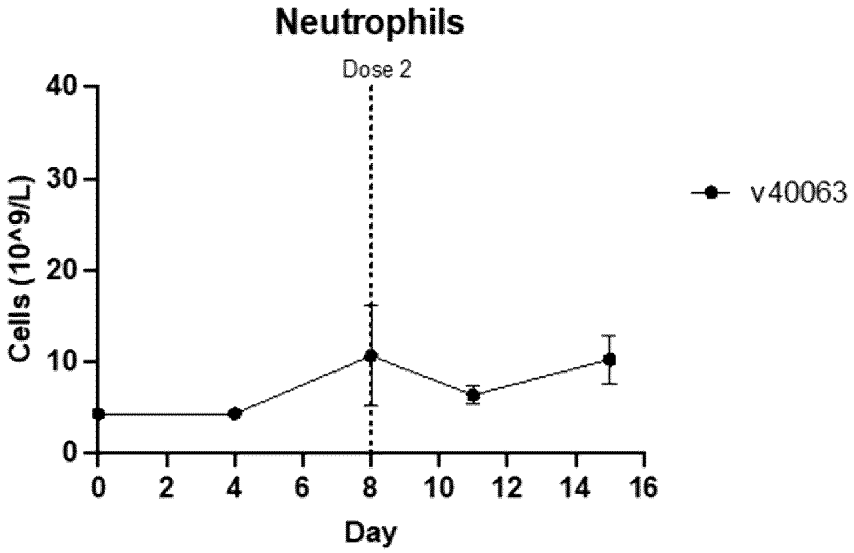


FIG. 32C

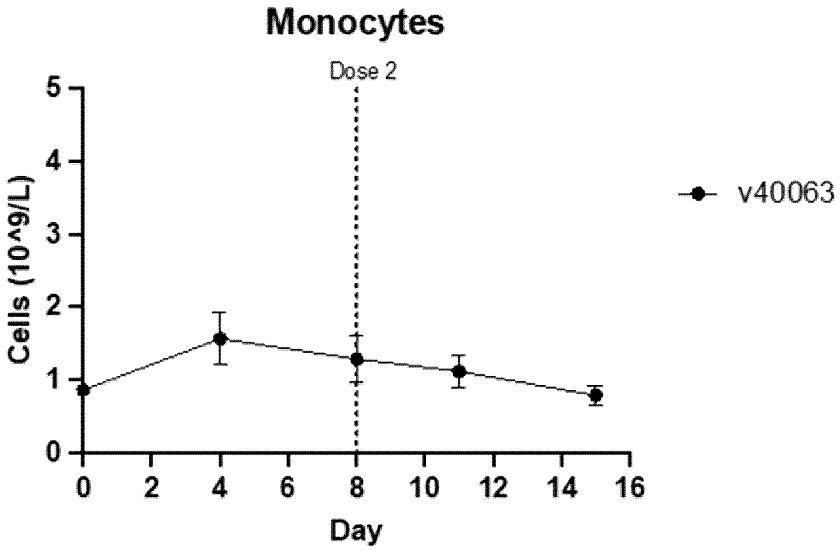


FIG. 32D

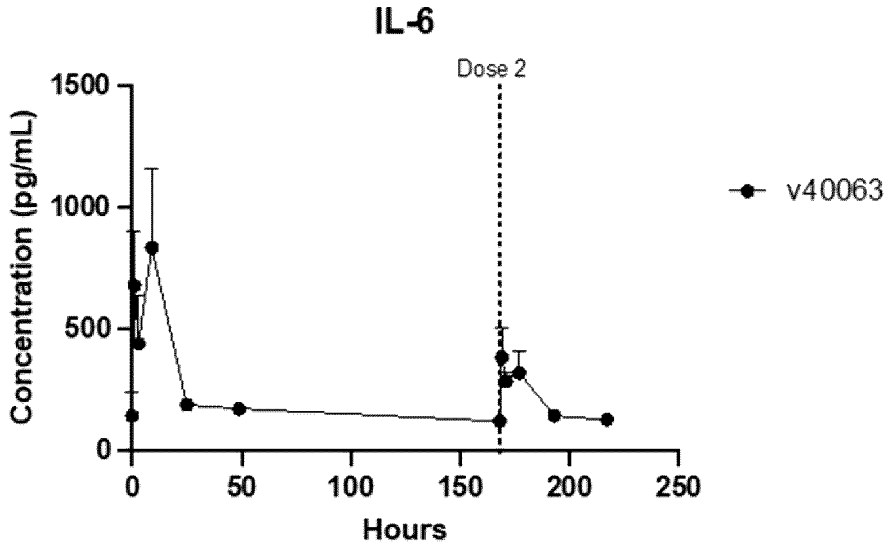


FIG. 33A

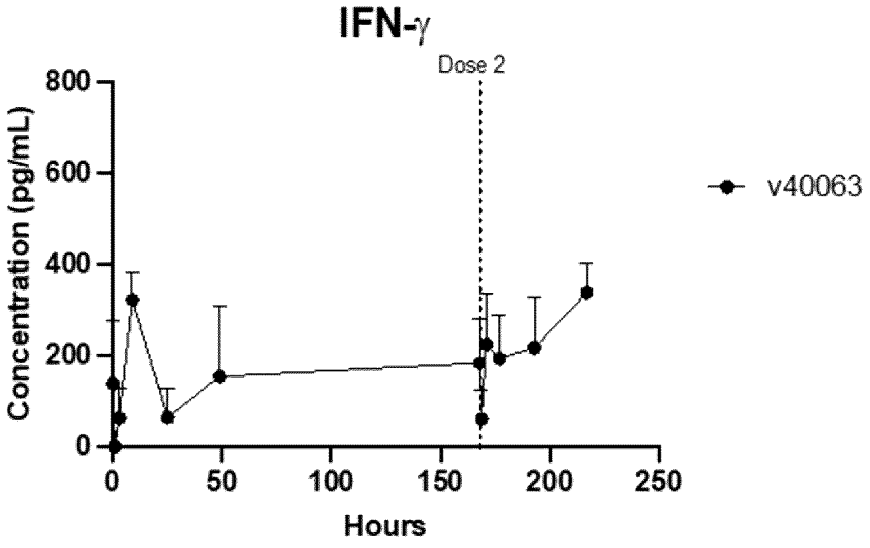


FIG. 33B

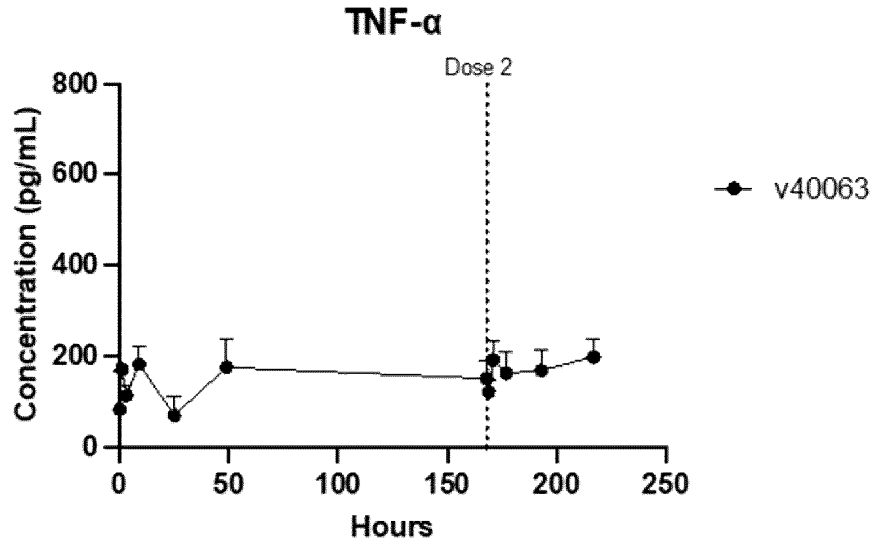


FIG. 33C

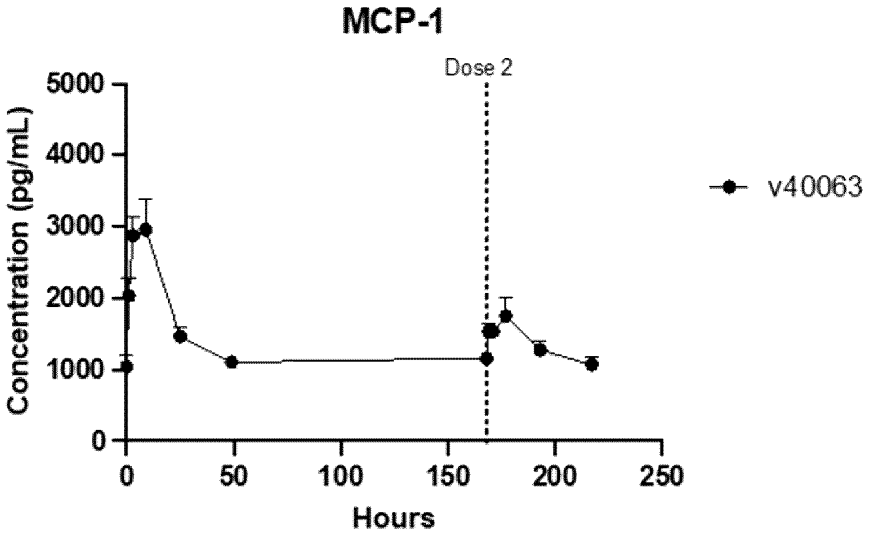


FIG. 33D

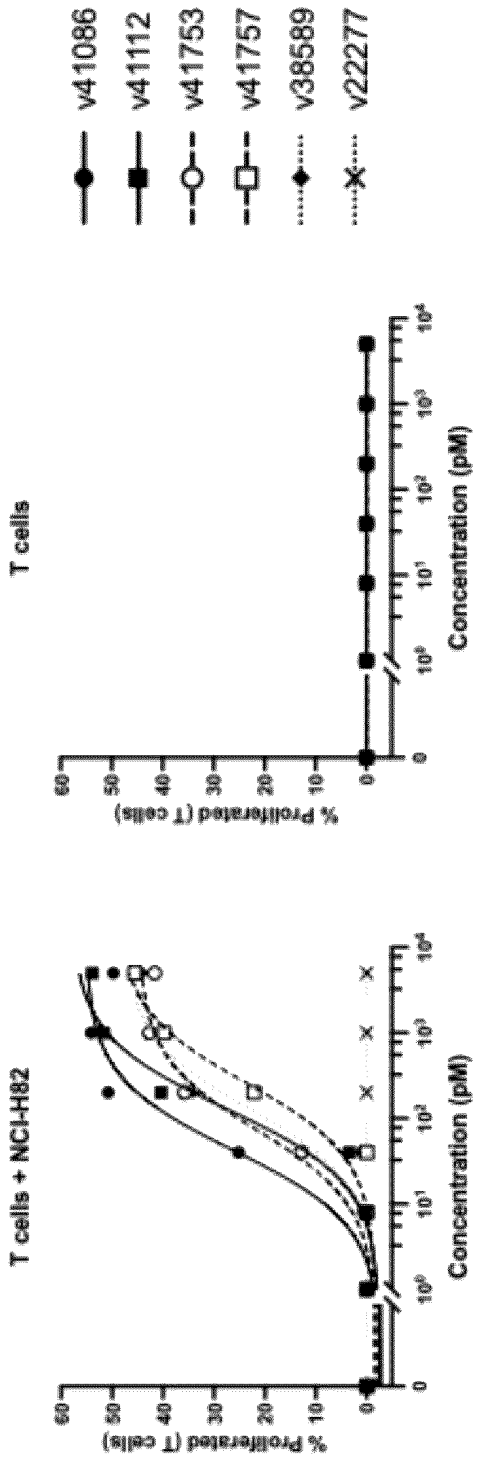

v41086
v41112
v41753
v41757
v38589
v22277
T cells
T cells + NCI-H82
% Proliferated (T cells)
Concentration (pM)
0
10
20
30
40
50
60
10⁰
10¹
10²
10³
10⁴

FIG. 34

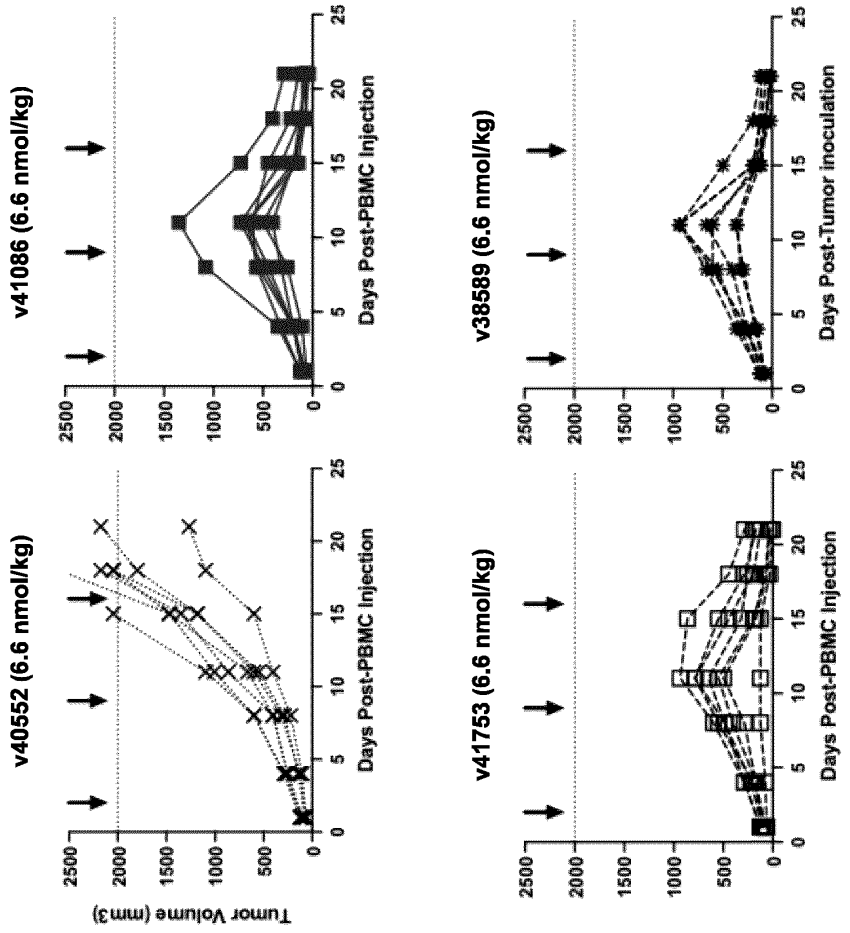

v40552 (6.6 nmol/kg)
v41086 (6.6 nmol/kg)
v41753 (6.6 nmol/kg)
v38589 (6.6 nmol/kg)
Tumor Volume (mm3)
Days Post-PBMC Injection
Days Post-Tumor inoculation
0
500
1000
1500
2000
2500
0
5
10
15
20
25

FIG. 35

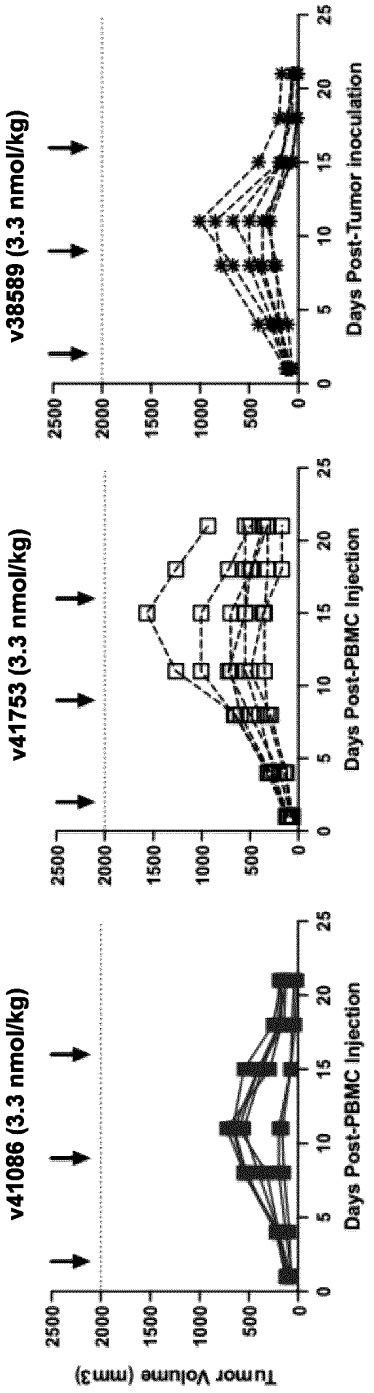

v41086 (3.3 nmol/kg)
v41753 (3.3 nmol/kg)
v38589 (3.3 nmol/kg)
Tumor Volume (mm3)
Days Post-PBMC Injection
Days Post-PBMC Injection
Days Post-Tumor inoculation

FIG. 35 (cont.)

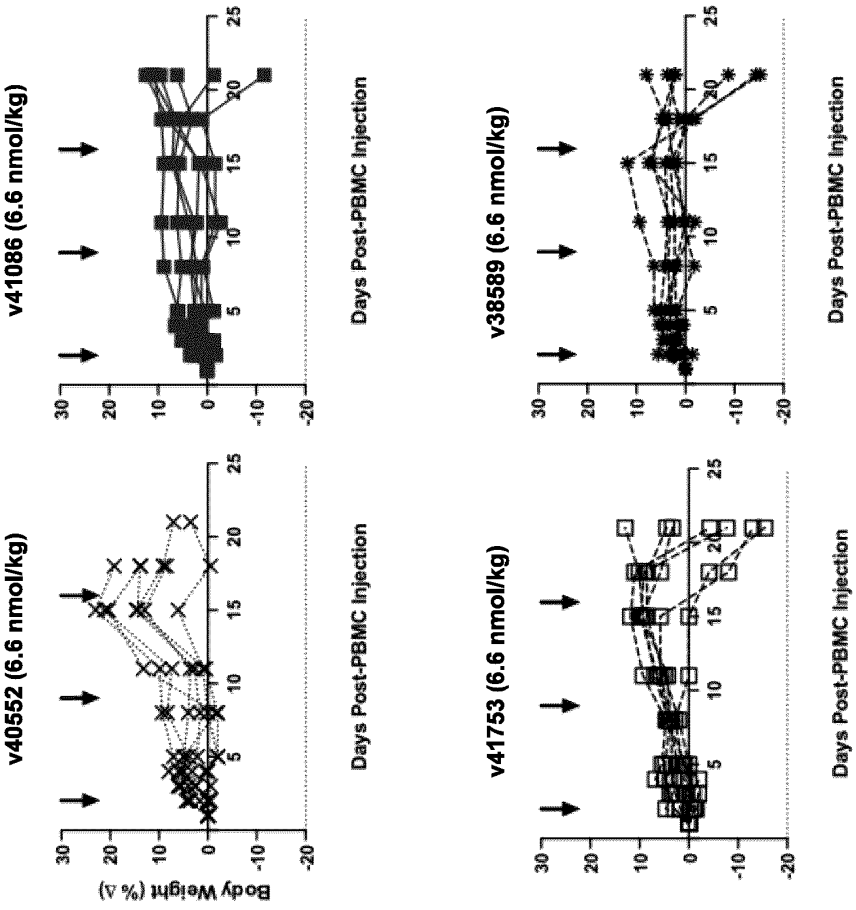

v41086 (6.6 nmol/kg)
v38589 (6.6 nmol/kg)
v40552 (6.6 nmol/kg)
v41753 (6.6 nmol/kg)
Body Weight (% Δ)
Days Post-PBMC Injection
30
20
10
0
-10
-20
5
10
15
20
25

FIG. 36

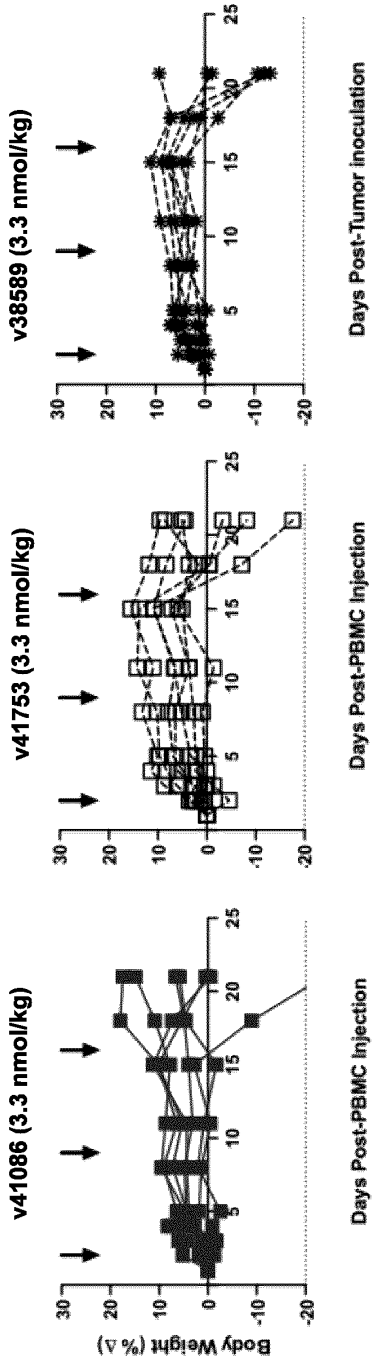

v41086 (3.3 nmol/kg)
v41753 (3.3 nmol/kg)
v38589 (3.3 nmol/kg)
Body Weight (% Δ)
Days Post-PBMC Injection
Days Post-PBMC Injection
Days Post-Tumor inoculation

FIG. 36 (cont.)

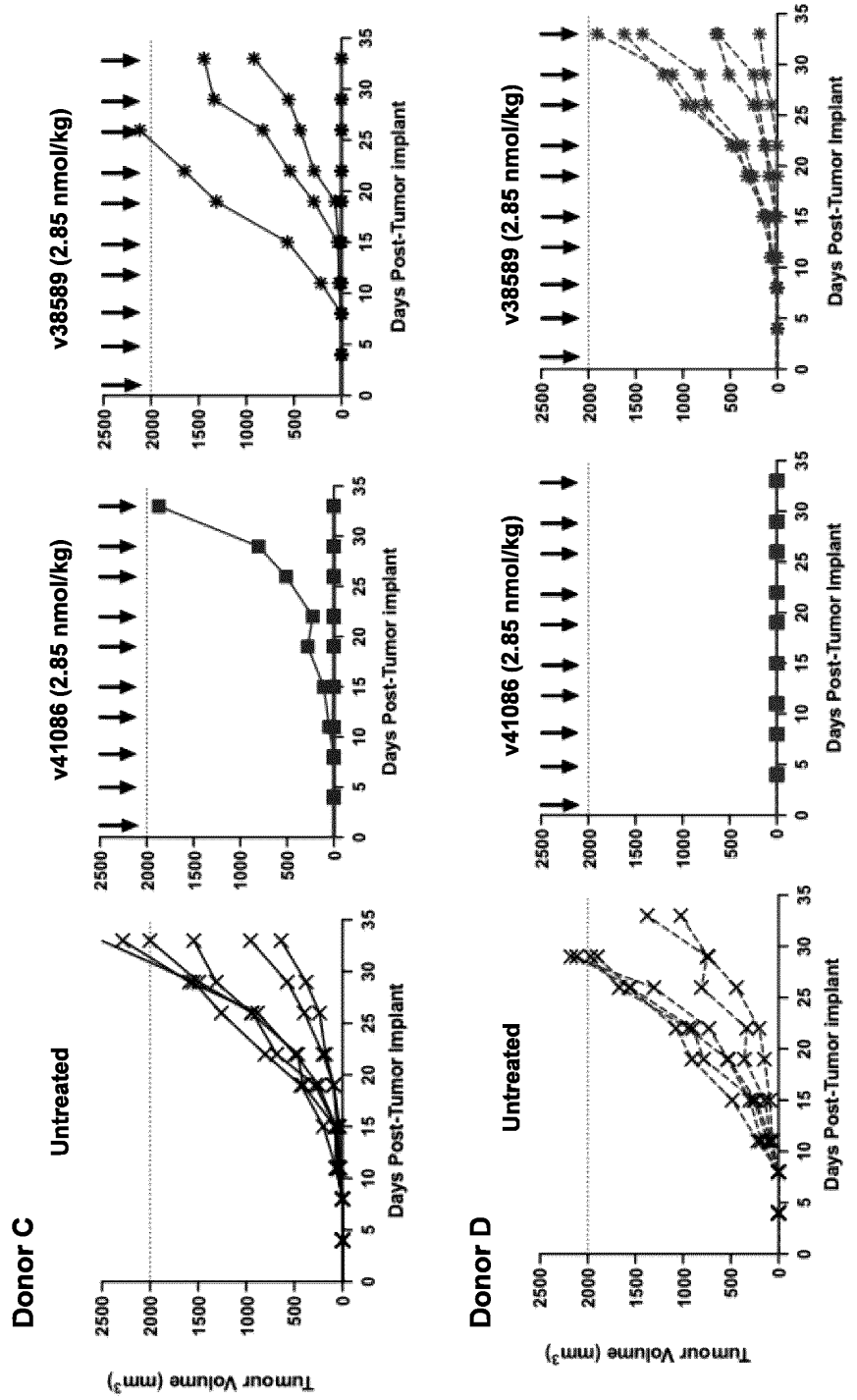

Donor C
Donor D
Untreated
v41086 (2.85 nmol/kg)
v38589 (2.85 nmol/kg)
Tumour Volume (mm³)
Days Post-Tumor implant
0
500
1000
1500
2000
2500
0
5
10
15
20
25
30
35

FIG. 37

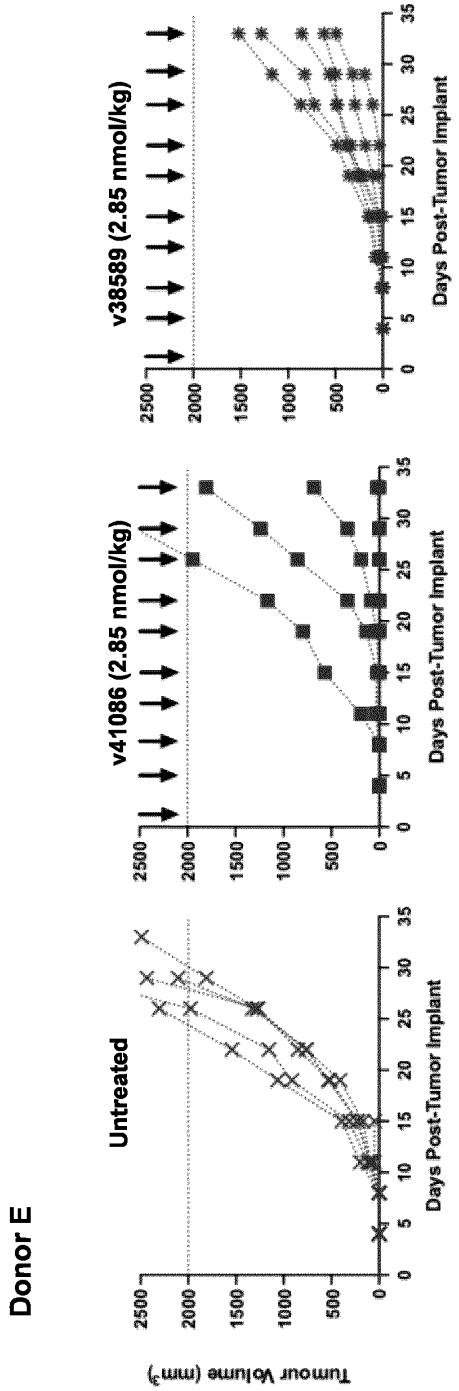

Donor E
Untreated
v41086 (2.85 nmol/kg)
v38589 (2.85 nmol/kg)
Tumour Volume (mm³)
Days Post-Tumor Implant
0
500
1000
1500
2000
2500
0
5
10
15
20
25
30
35

FIG. 37 (cont.)

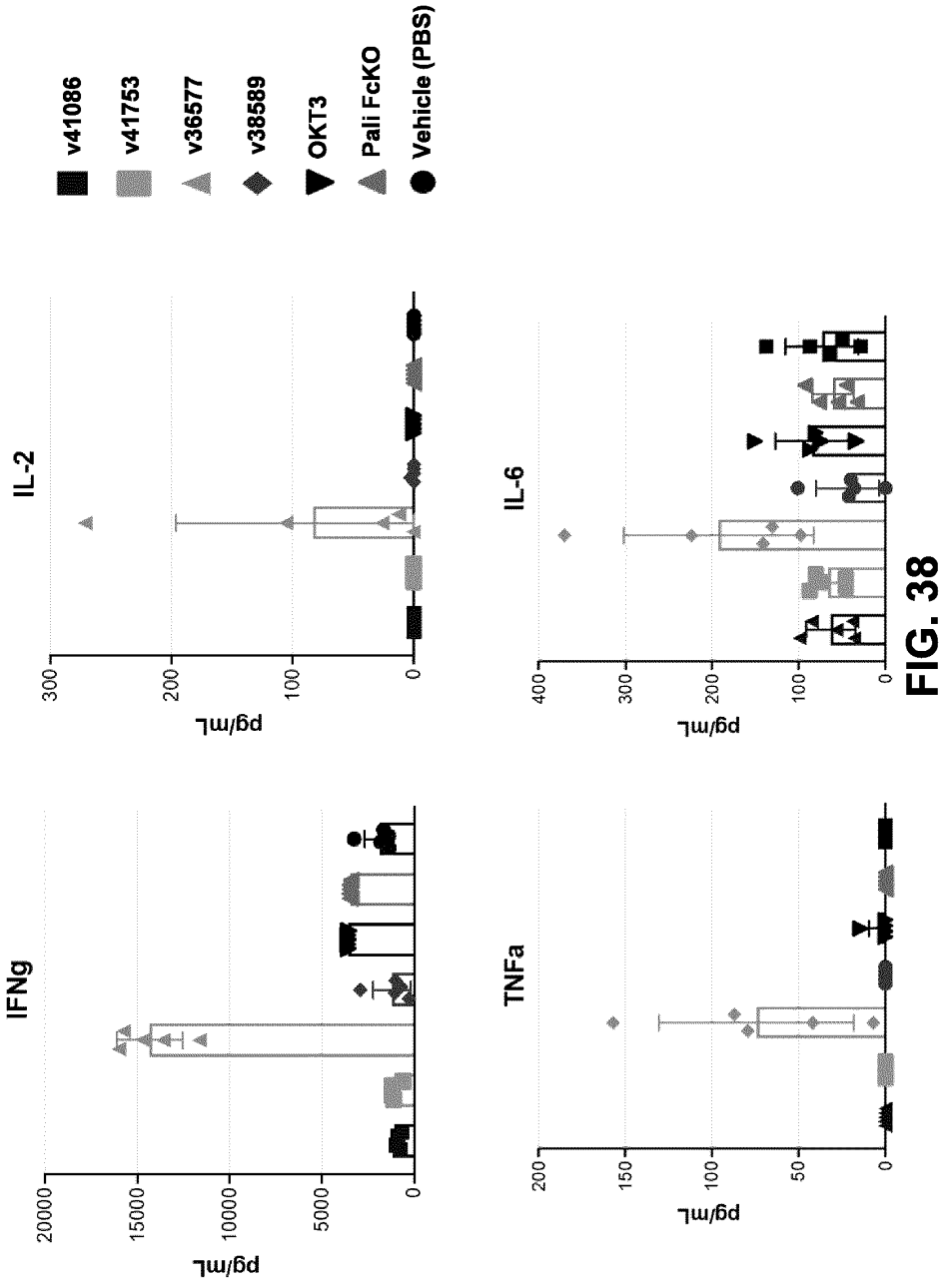

v41086
v41753
v36577
v38589
OKT3
Pali FcKO
Vehicle (PBS)
IL-2
IFNg
IL-6
TNFa
pg/mL
0
100
200
300
5000
10000
15000
20000
400
50
150

FIG. 38

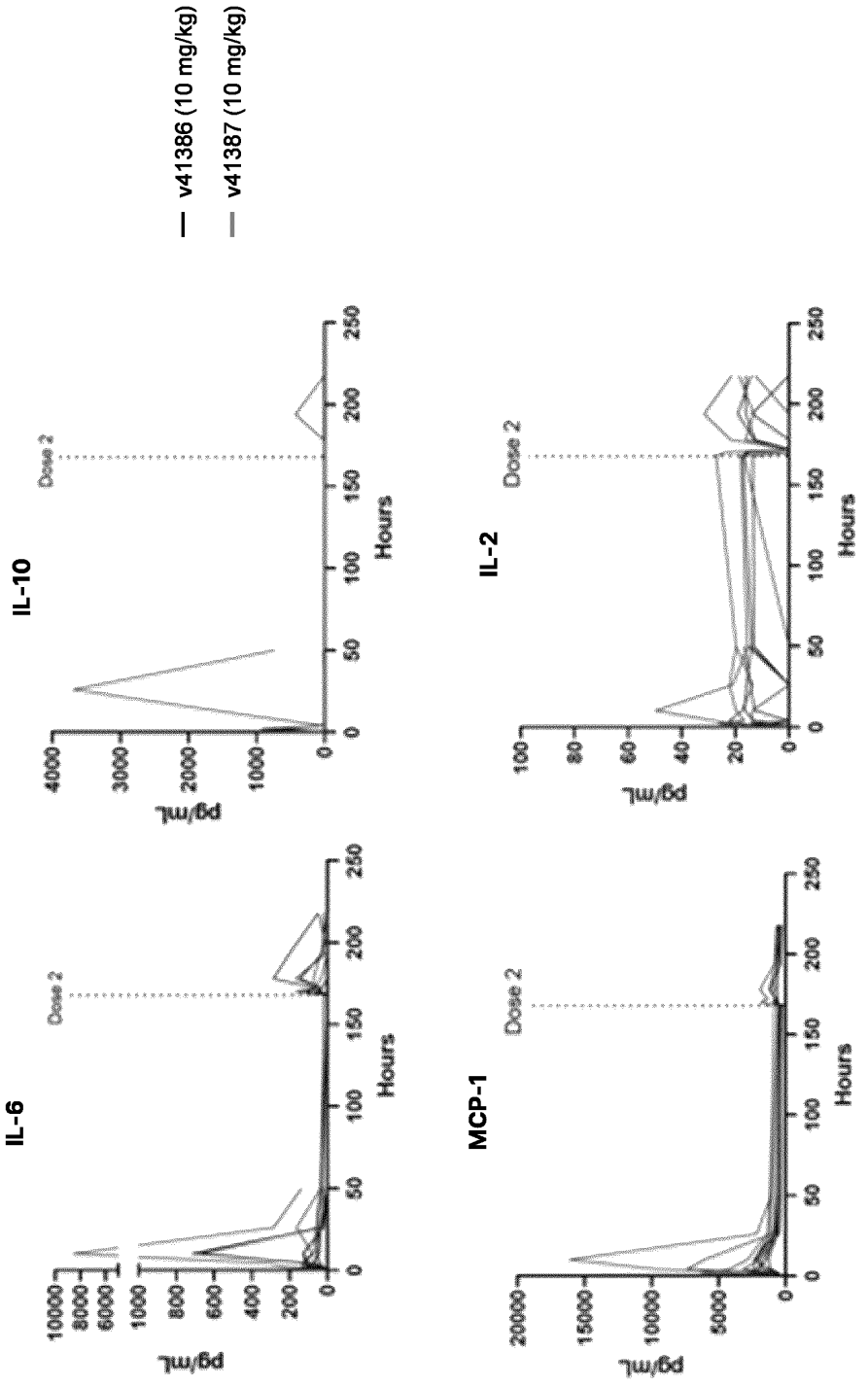

v41386 (10 mg/kg)
v41387 (10 mg/kg)
IL-10
IL-6
IL-2
MCP-1
pg/mL
Hours
Dose 2

FIG. 39

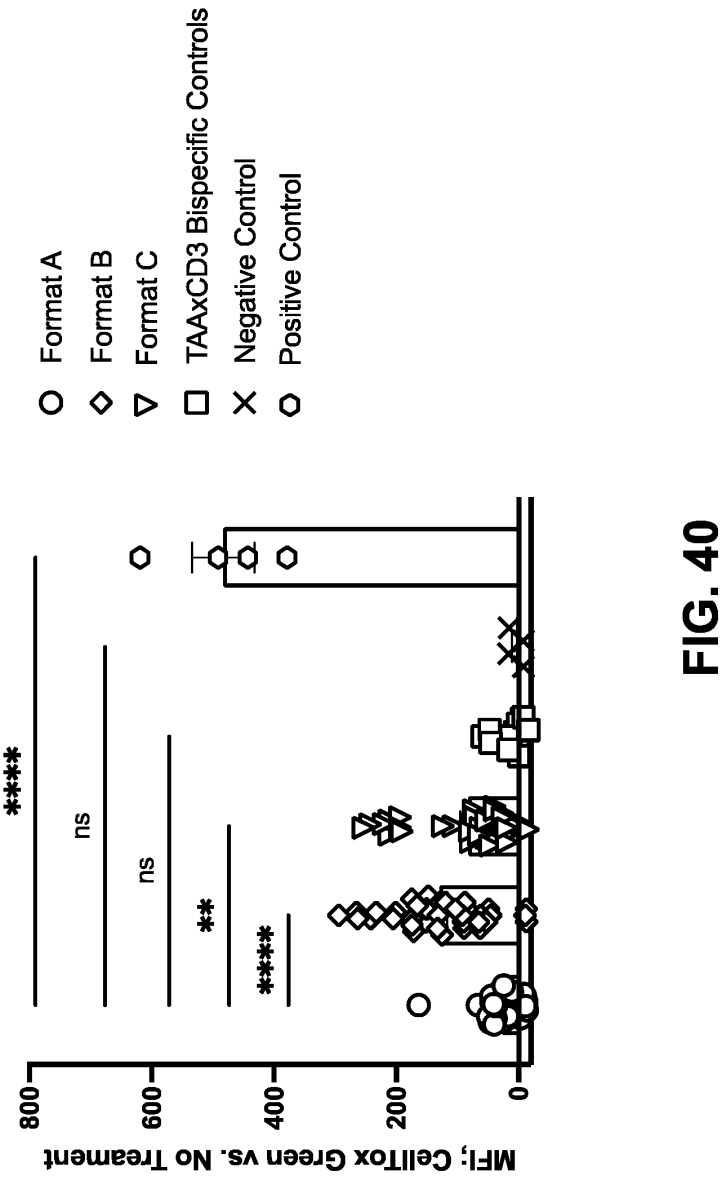

Format A
Format B
Format C
TAAxCD3 Bispecific Controls
Negative Control
Positive Control
MFI; CellTox Green vs. No Treament
800
600
400
200
0
****
ns
ns
**
****

FIG. 40

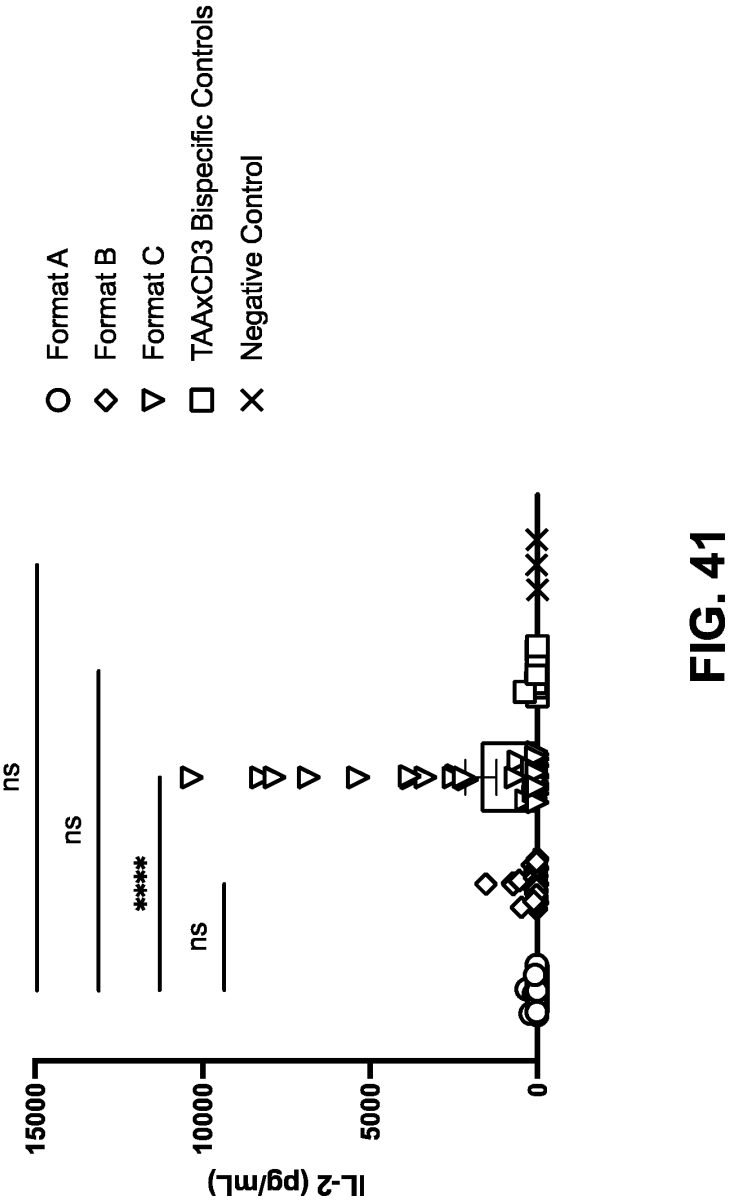

Format A
Format B
Format C
TAAxCD3 Bispecific Controls
Negative Control
ns
ns
****
ns
15000
10000
5000
0
IL-2 (pg/mL)

FIG. 41

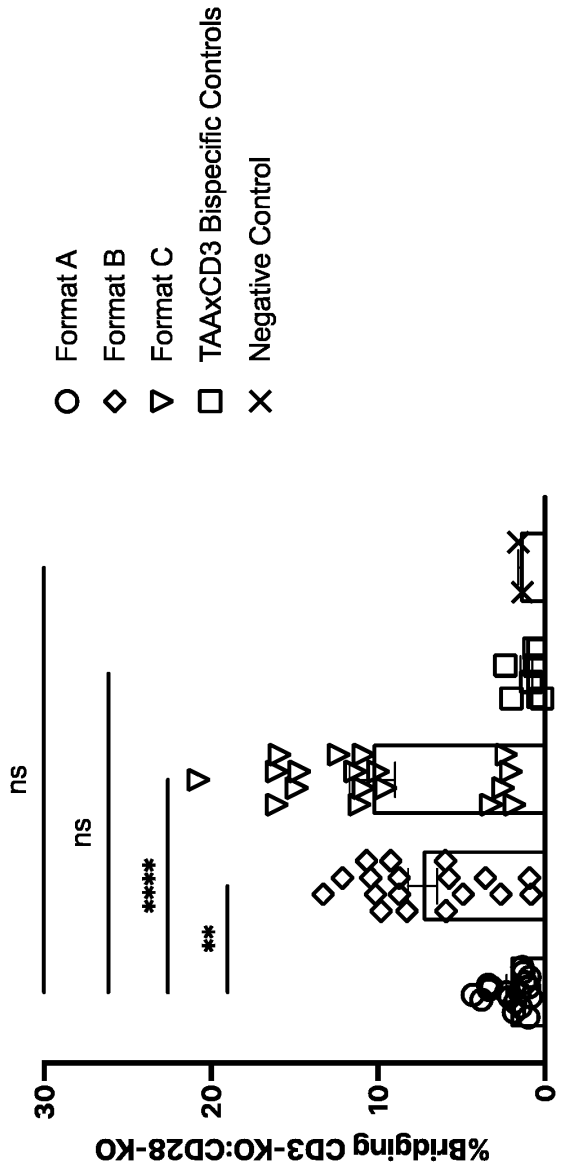

%Bridging CD3-KO:CD28-KO
0
10
20
30
ns
ns
****
**
Format A
Format B
Format C
TAAxCD3 Bispecific Controls
Negative Control

FIG. 42