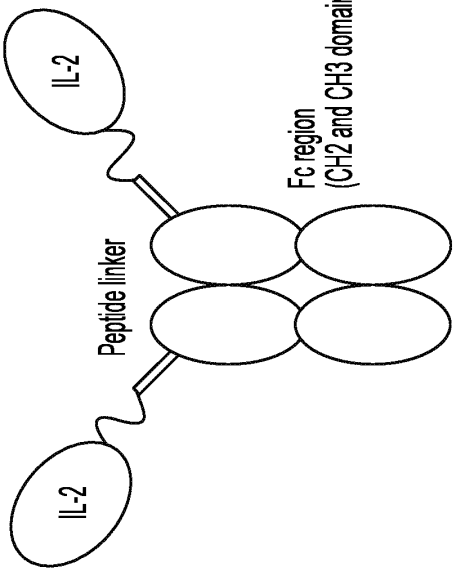

IL-2
Peptide linker
IL-2
Fc region
(CH2 and CH3 domains)

FIG. 1A

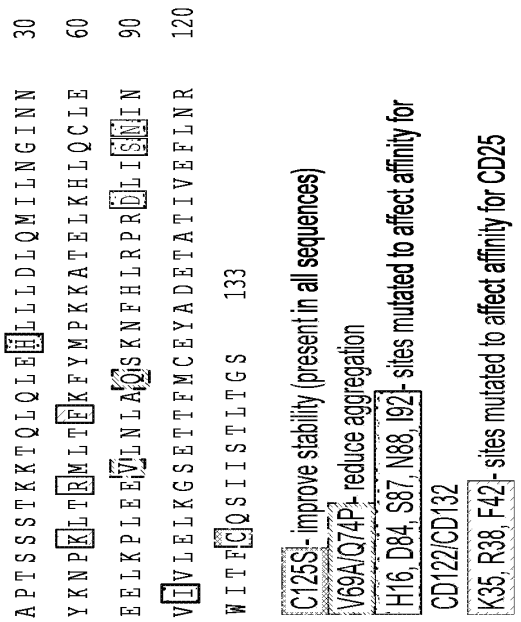

APTSSSTKKTQLQLEHLLLDLQMILNGINN 30
YKNPKLTRMLTFKFYMPKKATELKHLQCLE 60
EELKPLEEVLNLAQSKNFHLRPRDLISNIN 90
VIVLELKGSETTFMCEYADETATIVEFLNR 120
WITFCQSIISTLTGS 133
C125S- improve stability (present in all sequences)
V69A/Q74P- reduce aggregation
H16, D84, S87, N88, I92- sites mutated to affect affinity for CD122/CD132
K35, R38, F42- sites mutated to affect affinity for CD25

FIG. 1B

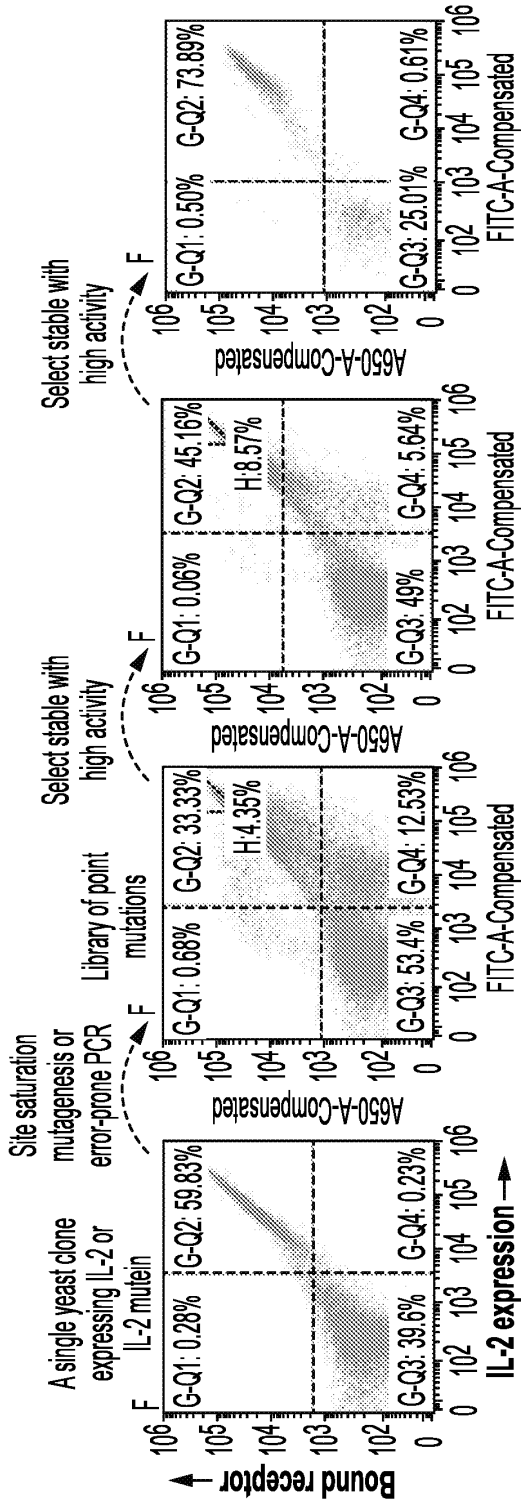

A single yeast clone expressing IL-2 or IL-2 mutein
Site saturation mutagenesis or error-prone PCR
Library of point mutations
Select stable with high activity
Select stable with high activity
Bound receptor
IL-2 expression
Incubated with CD25
A650-A-Compensated
FITC-A-Compensated
G-Q1: 0.28%
G-Q2: 59.83%
G-Q3: 39.6%
G-Q4: 0.23%
G-Q1: 0.68%
G-Q2: 33.33%
H:4.35%
G-Q3: 53.4%
G-Q4: 12.53%
G-Q1: 0.06%
G-Q2: 45.16%
H:8.57%
G-Q3: 49%
G-Q4: 5.64%
G-Q1: 0.50%
G-Q2: 73.89%
G-Q3: 25.01%
G-Q4: 0.61%

FIG. 2

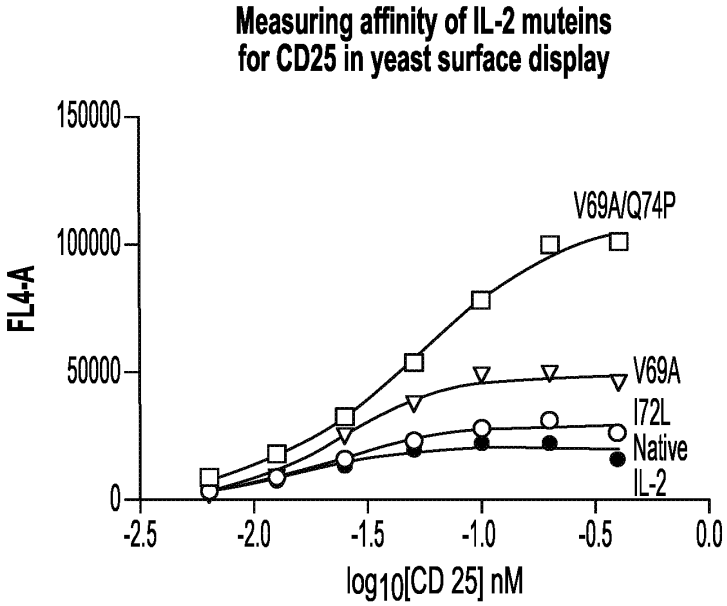

Measuring affinity of IL-2 muteins
for CD25 in yeast surface display
150000
100000
50000
0
FL4-A
V69A/Q74P
V69A
I72L
Native
IL-2
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
log10[CD 25] nM

FIG. 3A

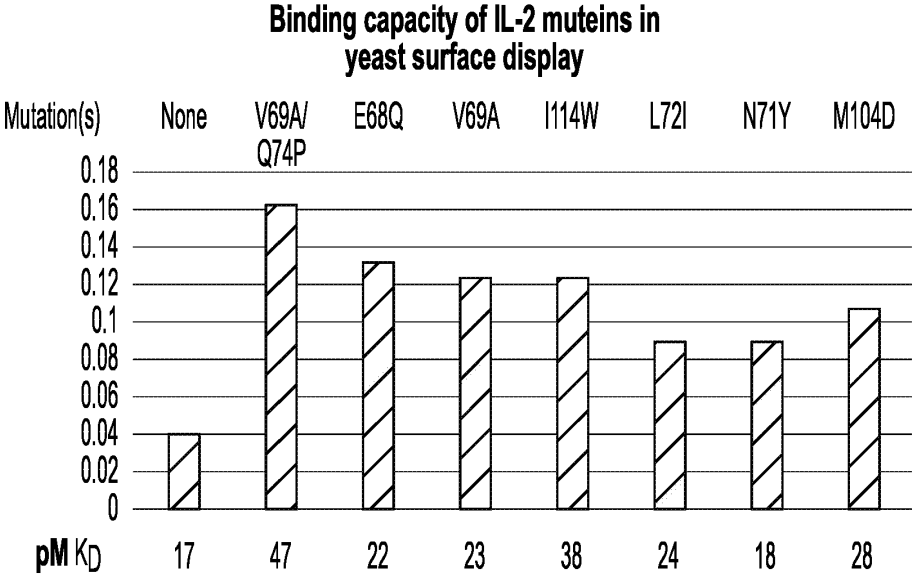

Binding capacity of IL-2 muteins in
yeast surface display
Mutation(s)
None
V69A/
Q74P
E68Q
V69A
I114W
L72I
N71Y
M104D
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
pM KD
17
47
22
23
38
24
18
28

FIG. 3B

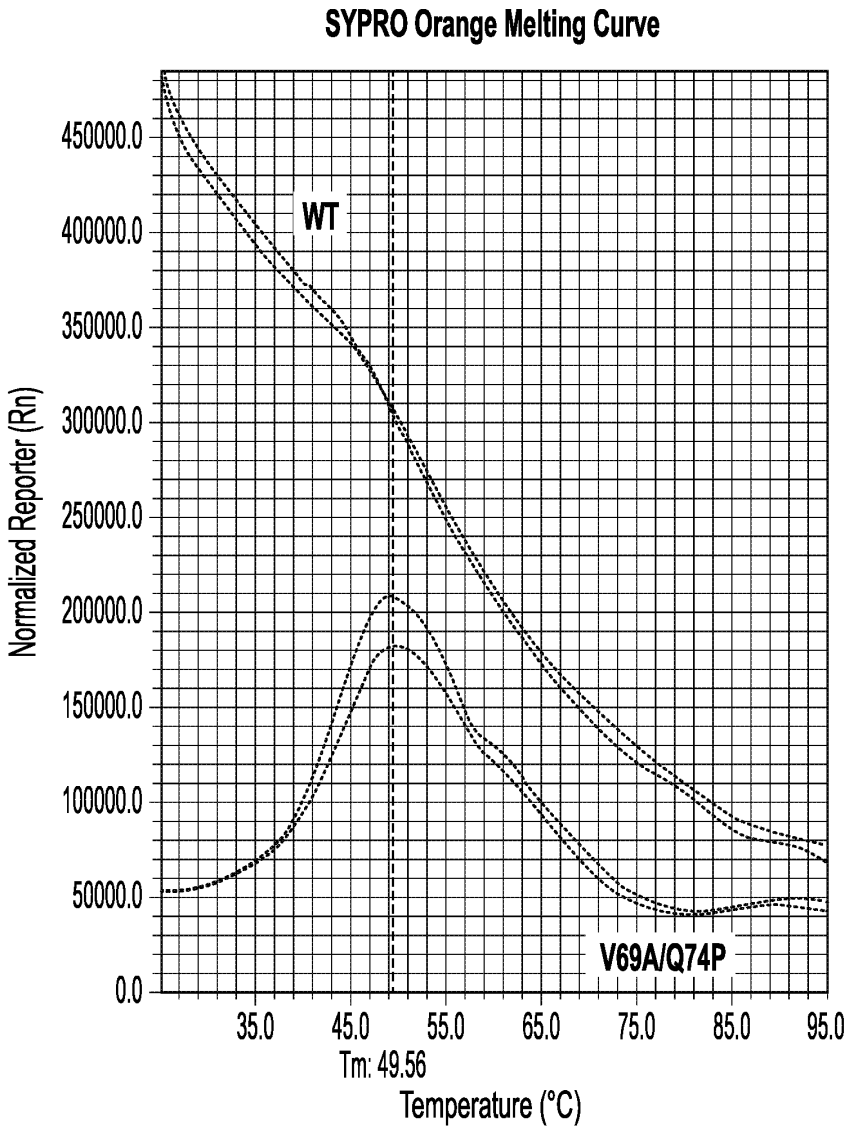

SYPRO Orange Melting Curve
Normalized Reporter (Rn)
450000.0
400000.0
350000.0
300000.0
250000.0
200000.0
150000.0
100000.0
50000.0
0.0
WT
V69A/Q74P
35.0
45.0
55.0
65.0
75.0
85.0
95.0
Tm: 49.56
Temperature (°C)

FIG. 4A

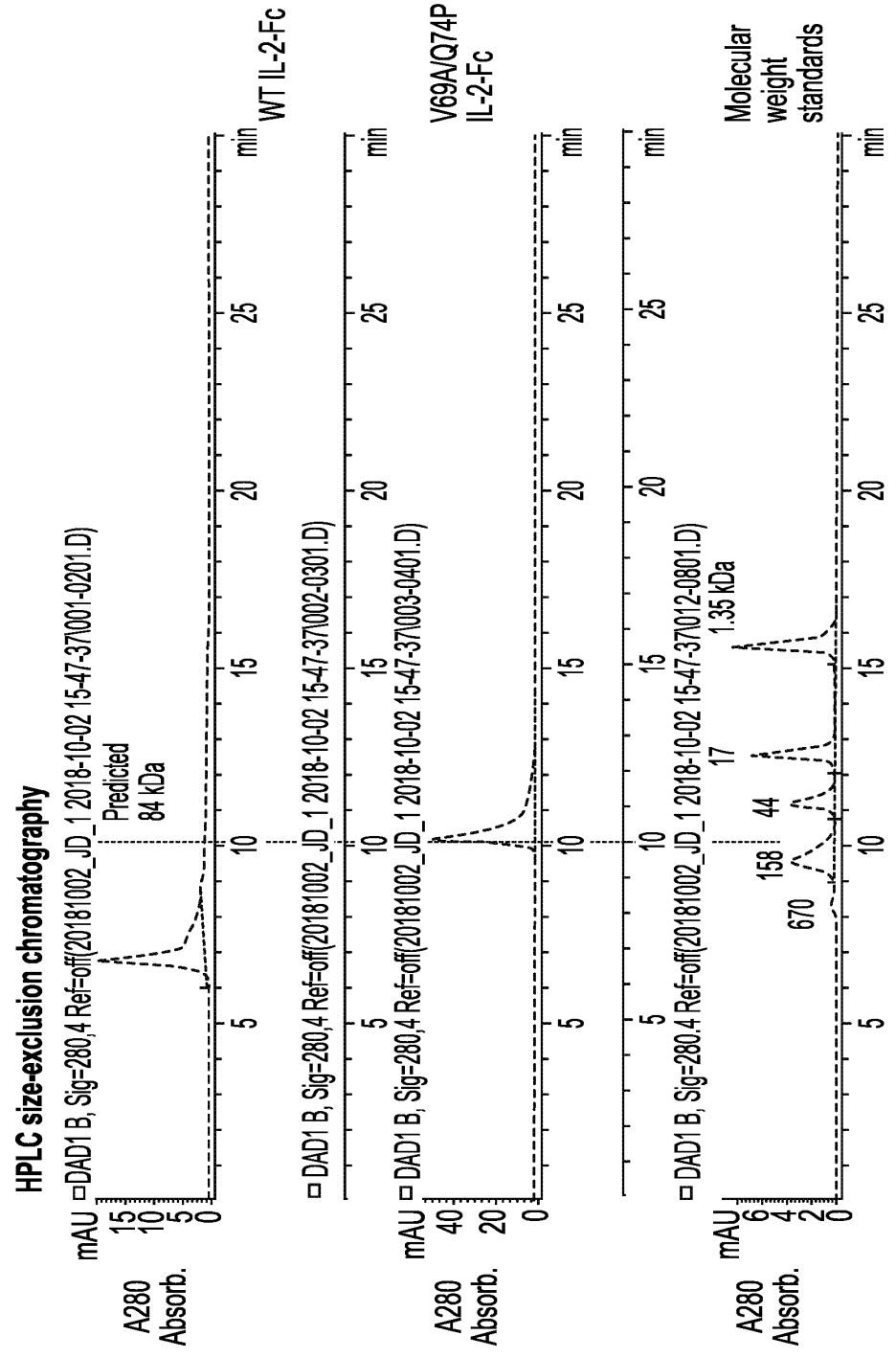

HPLC size-exclusion chromatography
DAD1 B, Sig=280,4 Ref=off(20181002_JD_1 2018-10-02 15-47-37\001-0201.D)
mAU
A280 Absorb.
Predicted 84 kDa
WT IL-2-Fc
DAD1 B, Sig=280,4 Ref=off(20181002_JD_1 2018-10-02 15-47-37\002-0301.D)
V69A/Q74P IL-2-Fc
DAD1 B, Sig=280,4 Ref=off(20181002_JD_1 2018-10-02 15-47-37\003-0401.D)
Molecular weight standards
DAD1 B, Sig=280,4 Ref=off(20181002_JD_1 2018-10-02 15-47-37\012-0801.D)
670
158
44
17
1.35 kDa
min
Time (min)

FIG. 4B

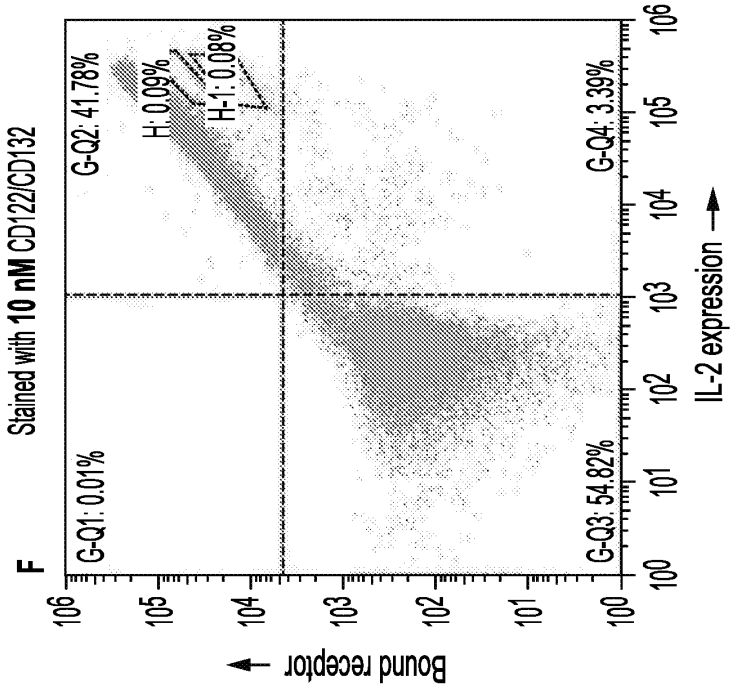

F
Stained with 10 nM CD122/CD132
G-Q1: 0.01%
G-Q2: 41.78%
H: 0.09%
H-1: 0.08%
G-Q4: 3.39%
G-Q3: 54.82%
Bound receptor
IL-2 expression

FIG. 5A

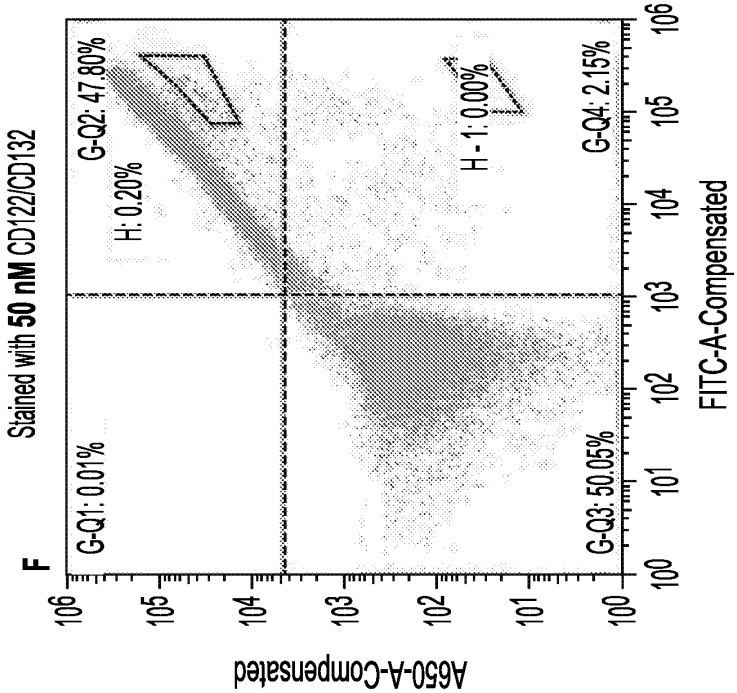

F
Stained with 50 nM CD122/CD132
G-Q1: 0.01%
G-Q2: 47.80%
H: 0.20%
H - 1: 0.00%
G-Q4: 2.15%
G-Q3: 50.05%
A650-A-Compensated
FITC-A-Compensated

FIG. 5B

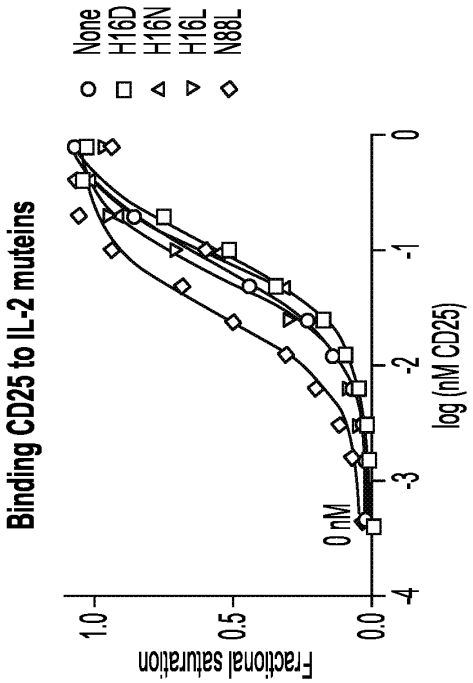

Binding CD25 to IL-2 muteins
None
H16D
H16N
H16L
N88L
Fractional saturation
1.0
0.5
0.0
0 nM
-4
-3
-2
-1
0
log (nM CD25)

FIG. 6B

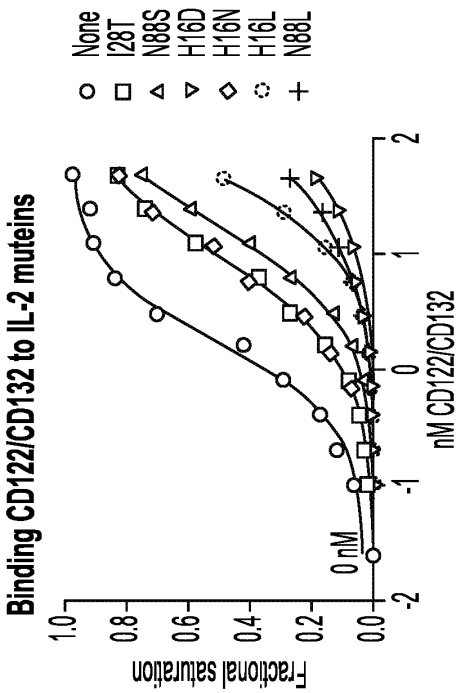

Binding CD122/CD132 to IL-2 muteins
None
I28T
N88S
H16D
H16N
H16L
N88L
Fractional saturation
1.0
0.8
0.6
0.4
0.2
0.0
0 nM
-2
-1
0
1
2
nM CD122/CD132

FIG. 6A

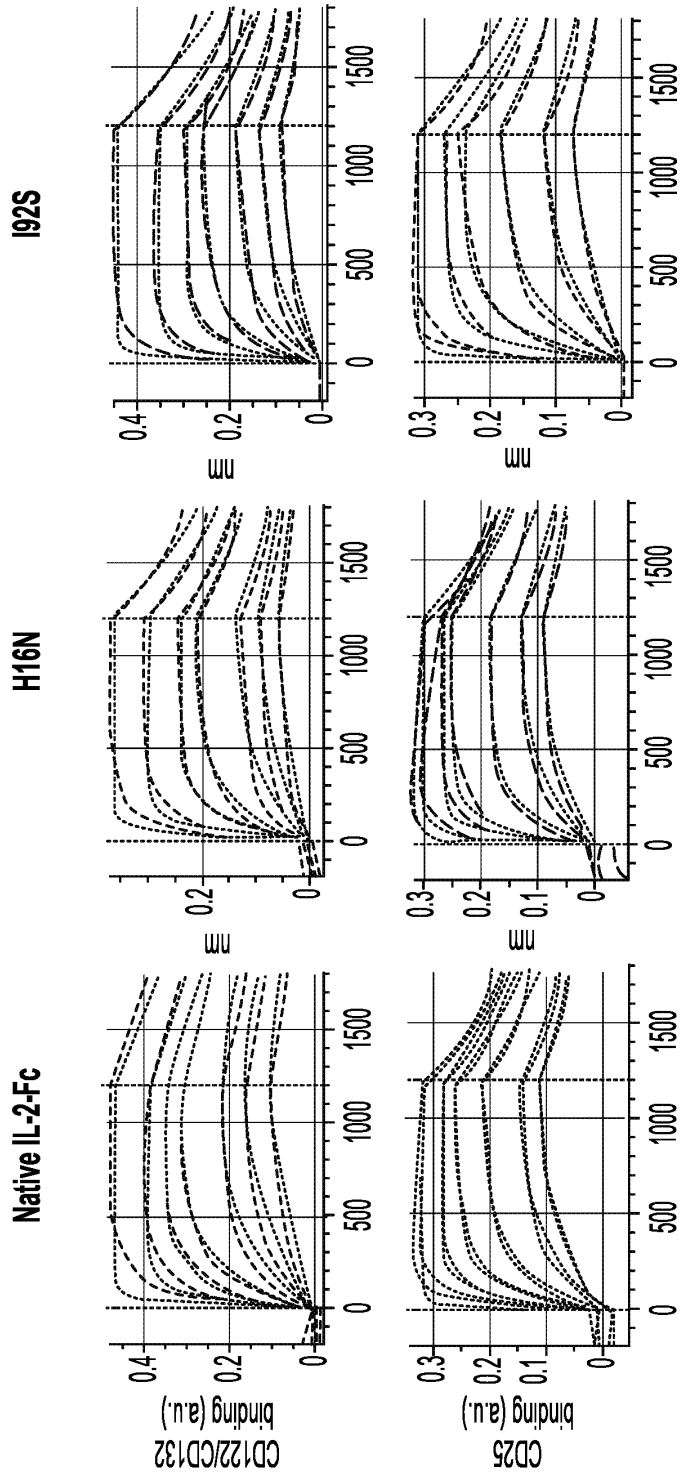

Native IL-2-Fc
H16N
I92S
CD122/CD132 binding (a.u.)
CD25 binding (a.u.)
nm
Time (s)

FIG. 7

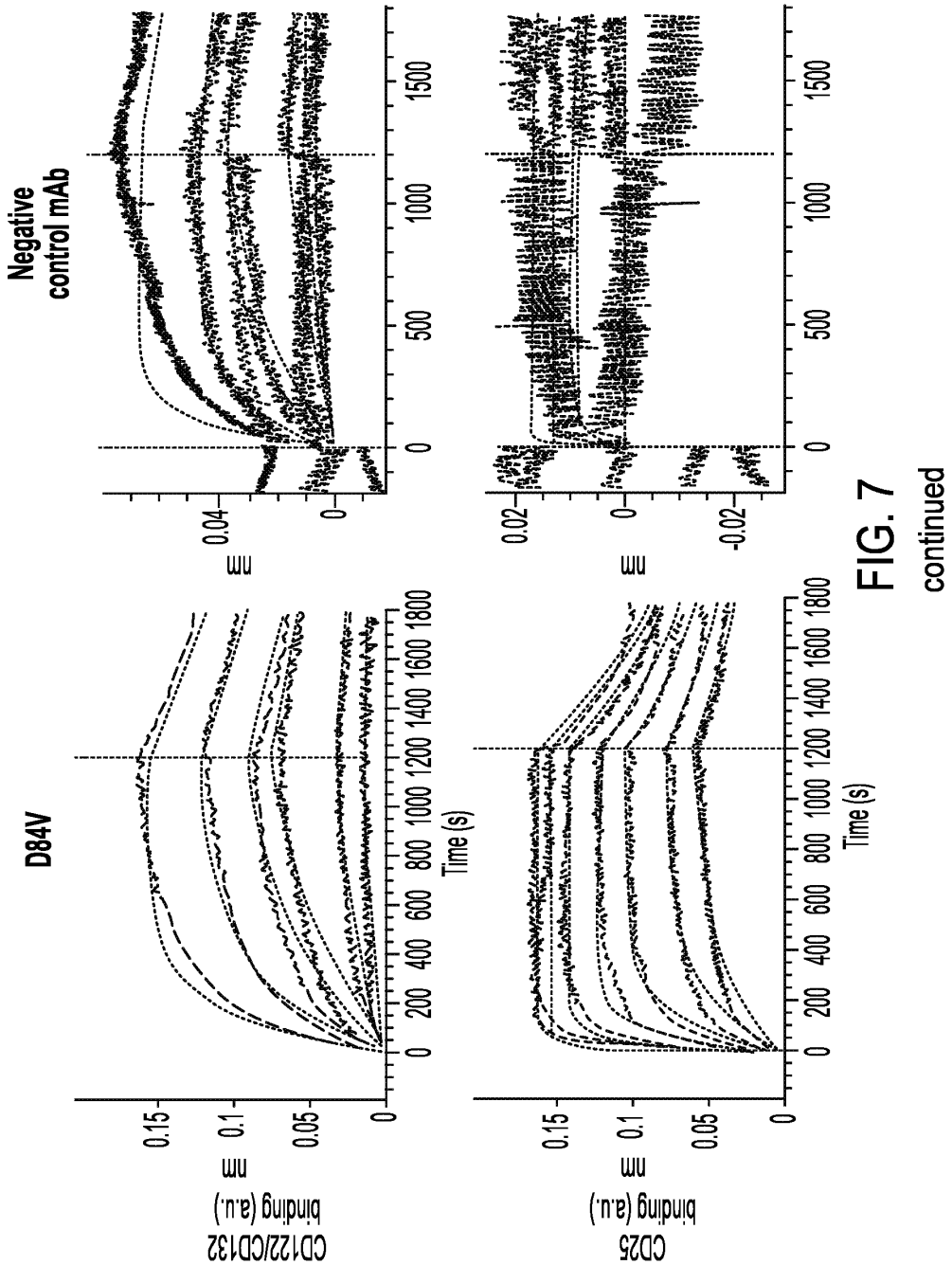


FIG. 7
continued

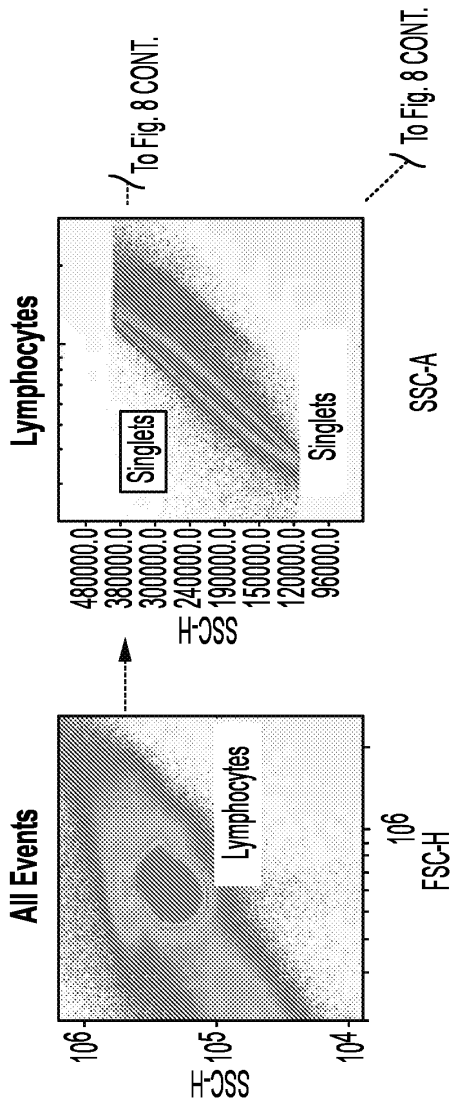

All Events
Lymphocytes
SSC-H
FSC-H
Lymphocytes
Singlets
Singlets
SSC-A
SSC-H
To Fig. 8 CONT.
To Fig. 8 CONT.

FIG. 8

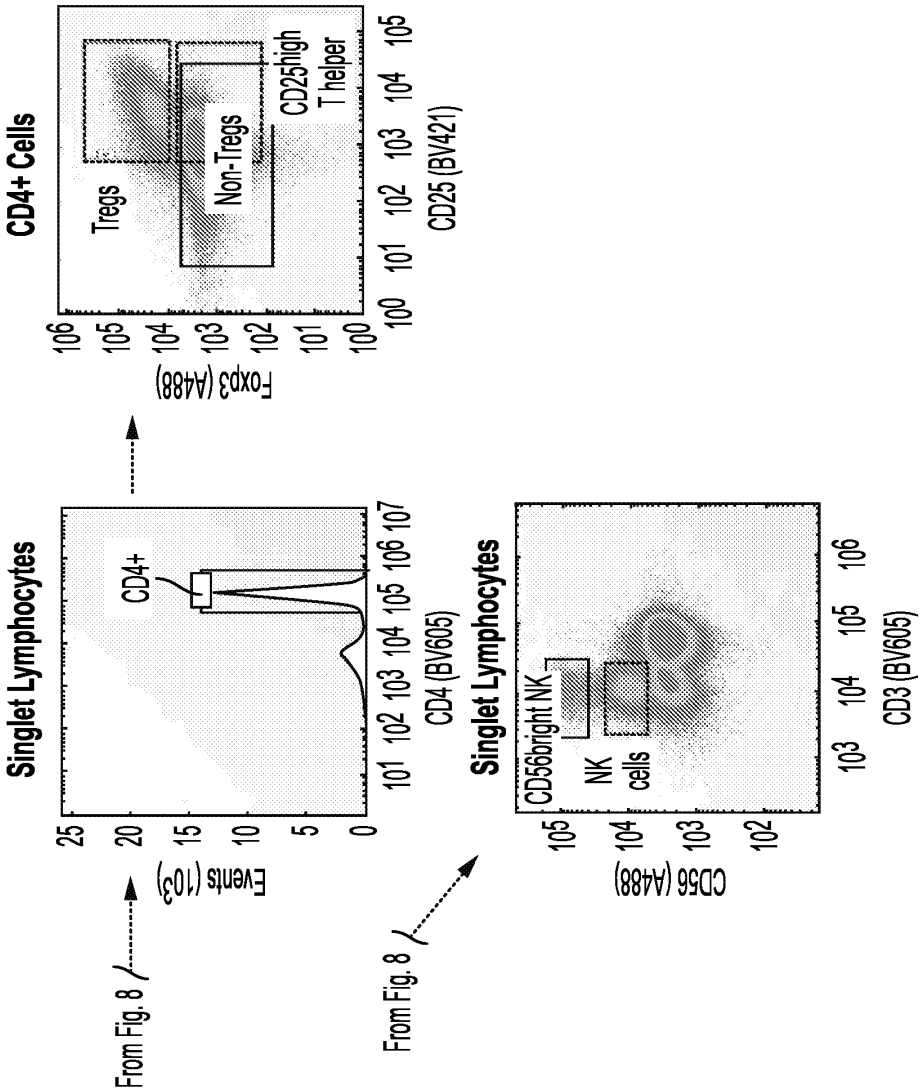


FIG. 8
continued

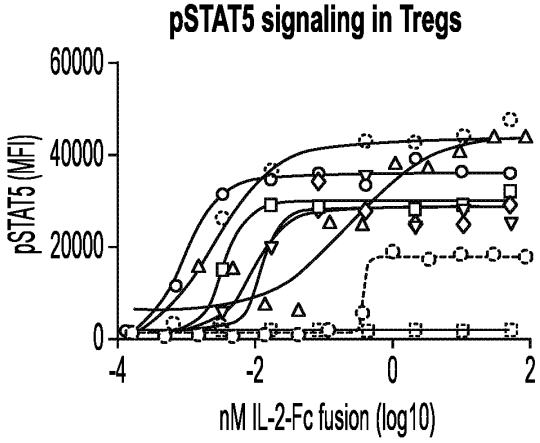


FIG. 9A

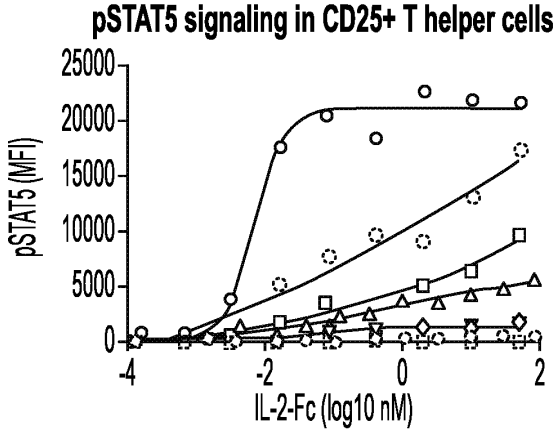


FIG. 9B

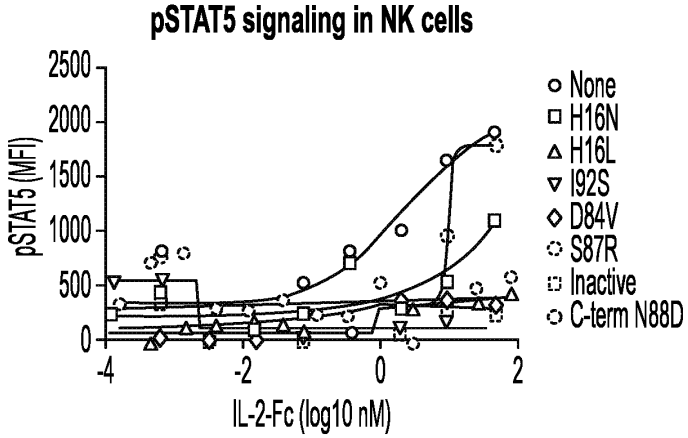


FIG. 9C

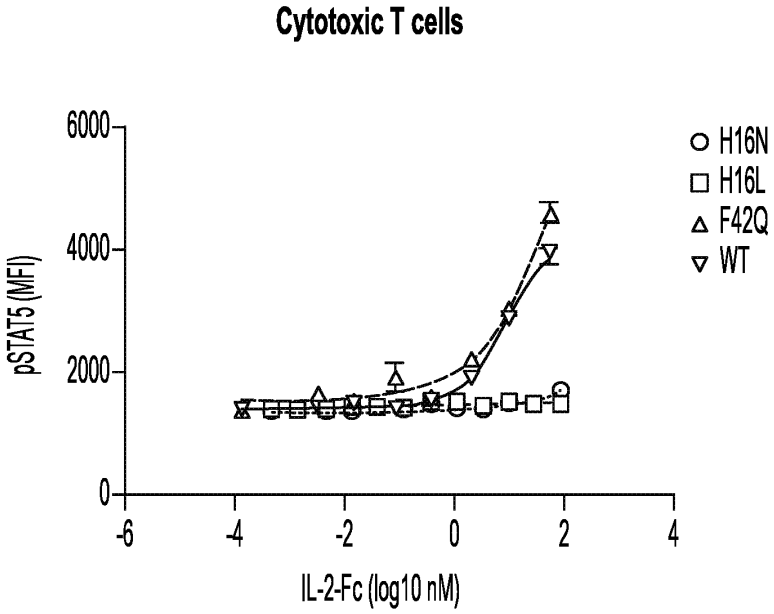

Cytotoxic T cells
6000
4000
2000
0
pSTAT5 (MFI)
-6
-4
-2
0
2
4
IL-2-Fc (log10 nM)
H16N
H16L
F42Q
WT

FIG. 9D

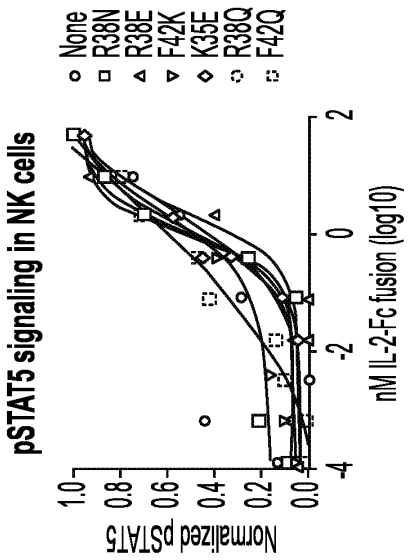


FIG. 10C

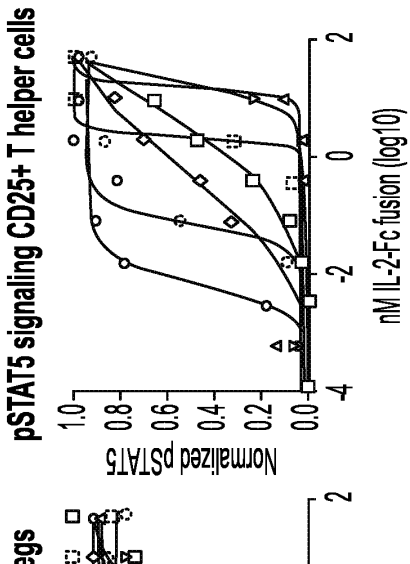


FIG. 10B

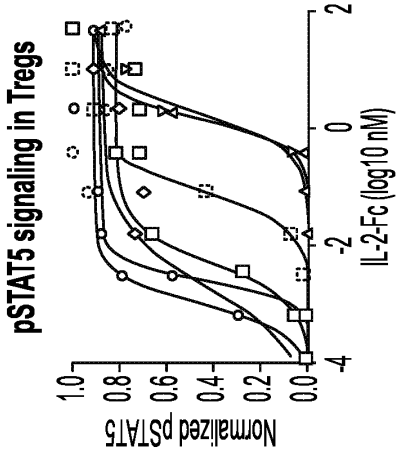


FIG. 10A

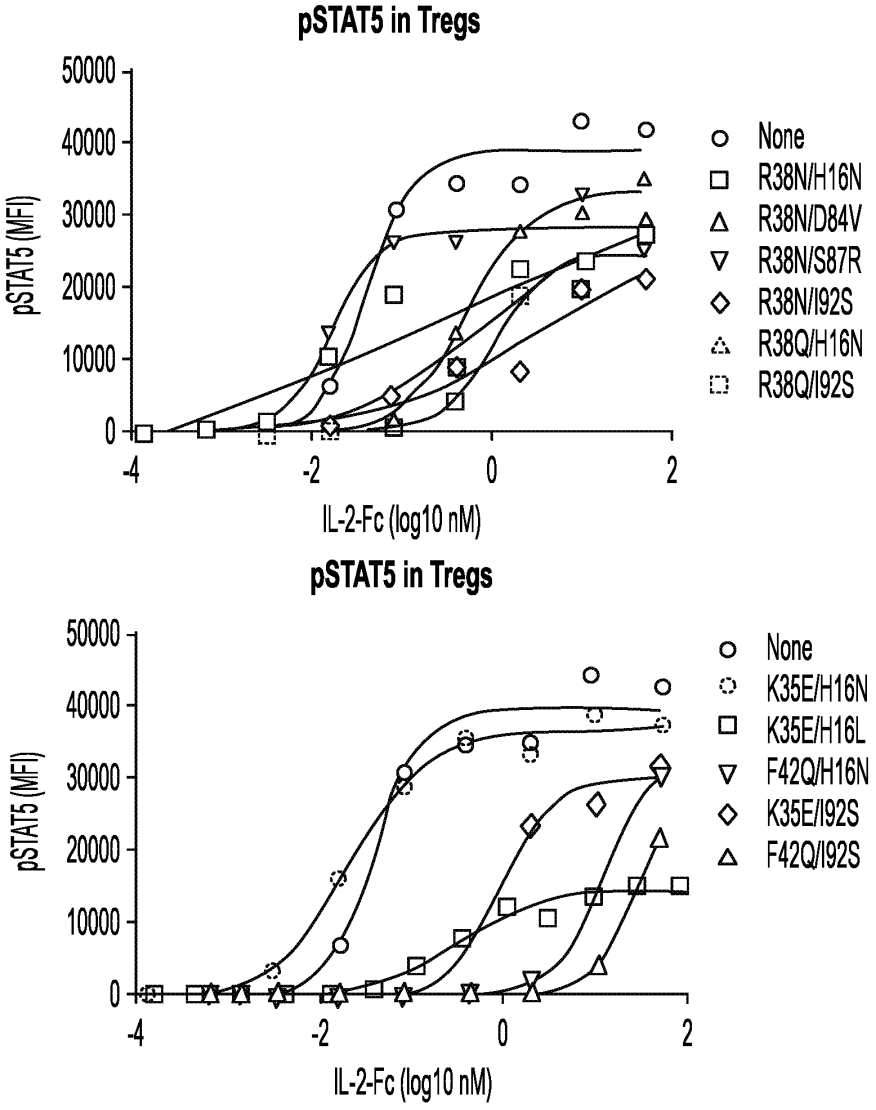

pSTAT5 in Tregs
pSTAT5 (MFI)
50000
40000
30000
20000
10000
0
-4
-2
0
2
IL-2-Fc (log10 nM)
None
R38N/H16N
R38N/D84V
R38N/S87R
R38N/I92S
R38Q/H16N
R38Q/I92S
pSTAT5 in Tregs
pSTAT5 (MFI)
50000
40000
30000
20000
10000
0
-4
-2
0
2
IL-2-Fc (log10 nM)
None
K35E/H16N
K35E/H16L
F42Q/H16N
K35E/I92S
F42Q/I92S

FIG. 11A

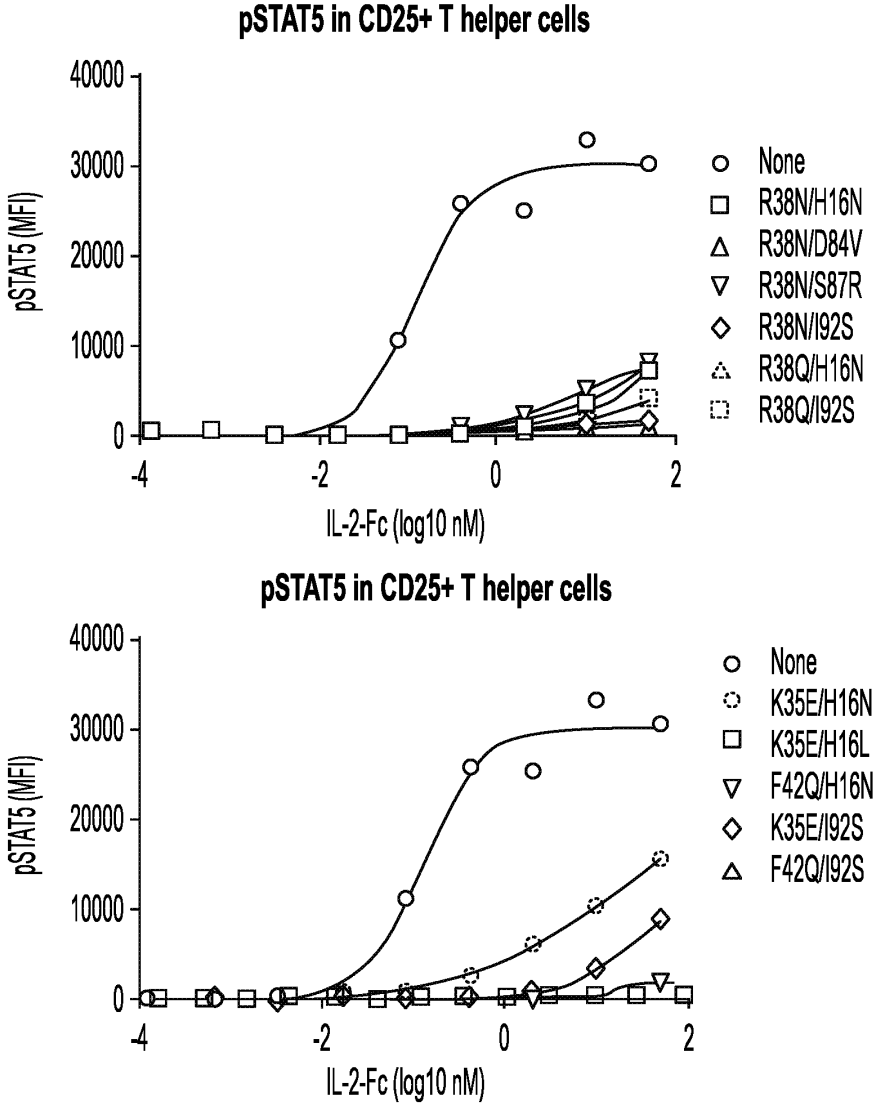

pSTAT5 in CD25+ T helper cells
pSTAT5 (MFI)
40000
30000
20000
10000
0
-4
-2
0
2
IL-2-Fc (log10 nM)
None
R38N/H16N
R38N/D84V
R38N/S87R
R38N/I92S
R38Q/H16N
R38Q/I92S
pSTAT5 in CD25+ T helper cells
pSTAT5 (MFI)
40000
30000
20000
10000
0
-4
-2
0
2
IL-2-Fc (log10 nM)
None
K35E/H16N
K35E/H16L
F42Q/H16N
K35E/I92S
F42Q/I92S

FIG. 11B

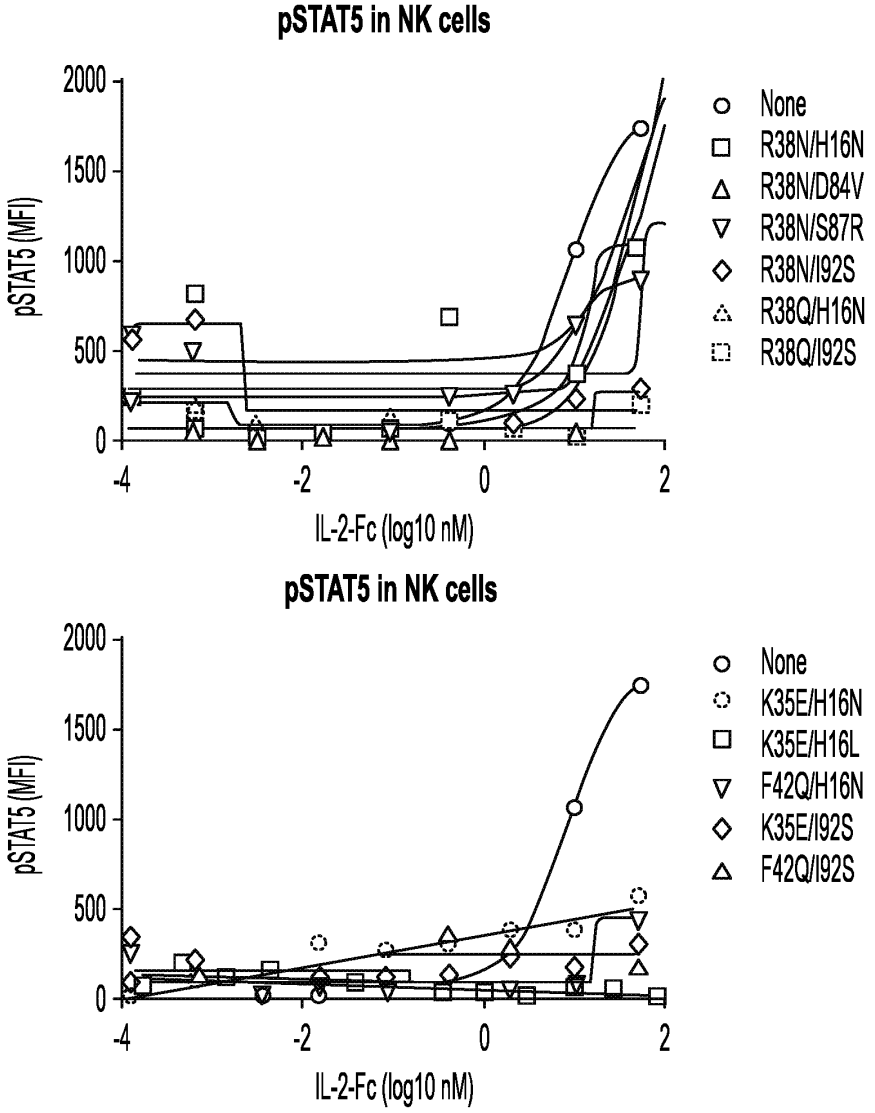

pSTAT5 in NK cells
pSTAT5 (MFI)
2000
1500
1000
500
0
-4
-2
0
2
IL-2-Fc (log10 nM)
None
R38N/H16N
R38N/D84V
R38N/S87R
R38N/I92S
R38Q/H16N
R38Q/I92S
pSTAT5 in NK cells
pSTAT5 (MFI)
2000
1500
1000
500
0
-4
-2
0
2
IL-2-Fc (log10 nM)
None
K35E/H16N
K35E/H16L
F42Q/H16N
K35E/I92S
F42Q/I92S

FIG. 11C

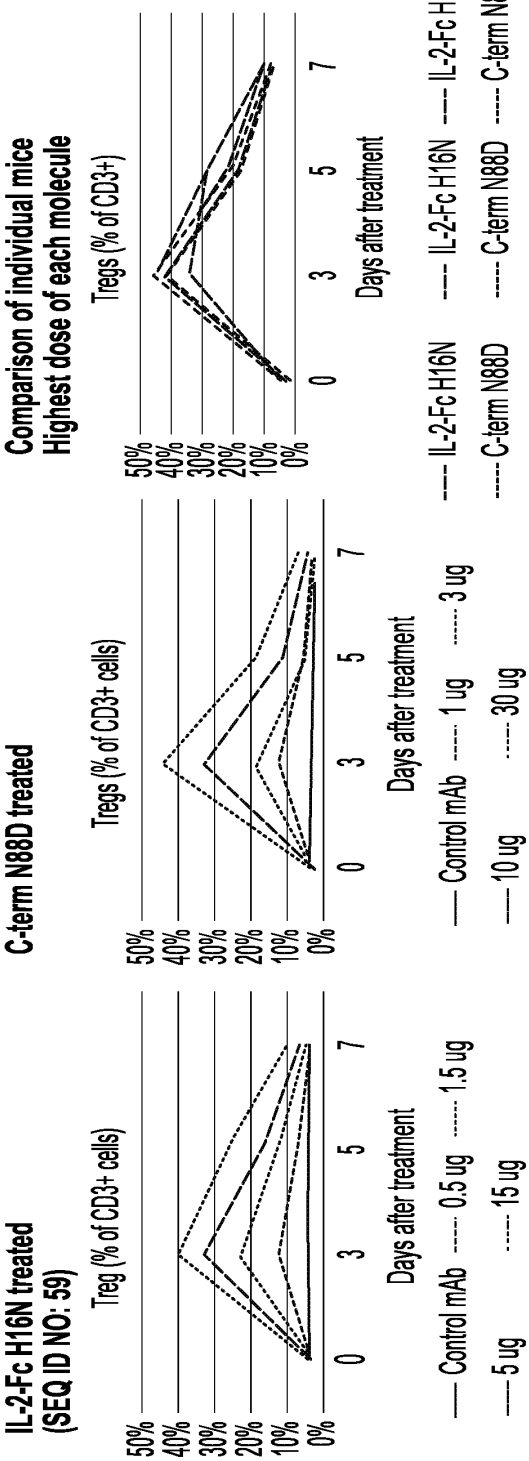


FIG. 12A

FIG. 12B

FIG. 12C

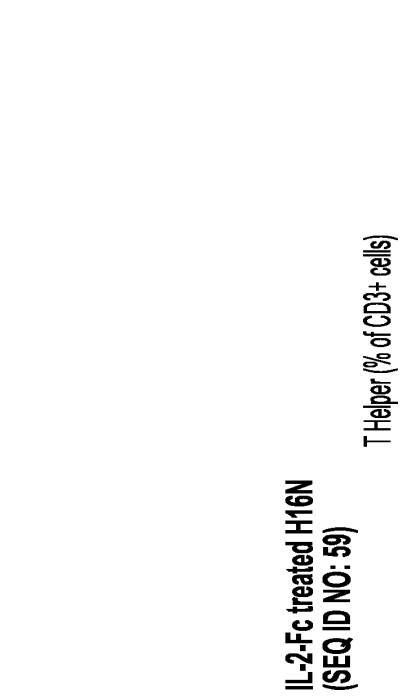


FIG. 13A

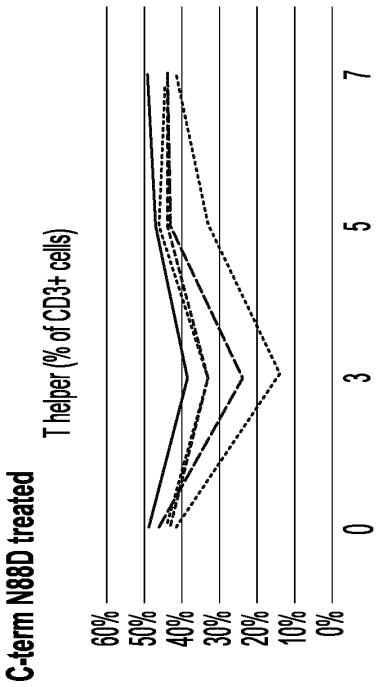


FIG. 13B

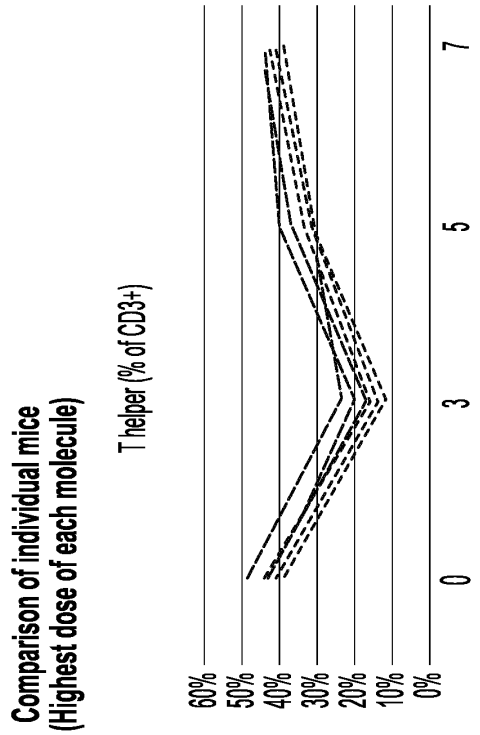

Comparison of individual mice
(Highest dose of each molecule)
T helper (% of CD3+)
60%
50%
40%
30%
20%
10%
0%
0
3
5
7
Days after treatment
---- IL-2-Fc H16N
---- IL-2-Fc H16N
---- IL-2-Fc H16N
----- C-term N88D
----- C-term N88D
----- C-term N88D

FIG. 13C

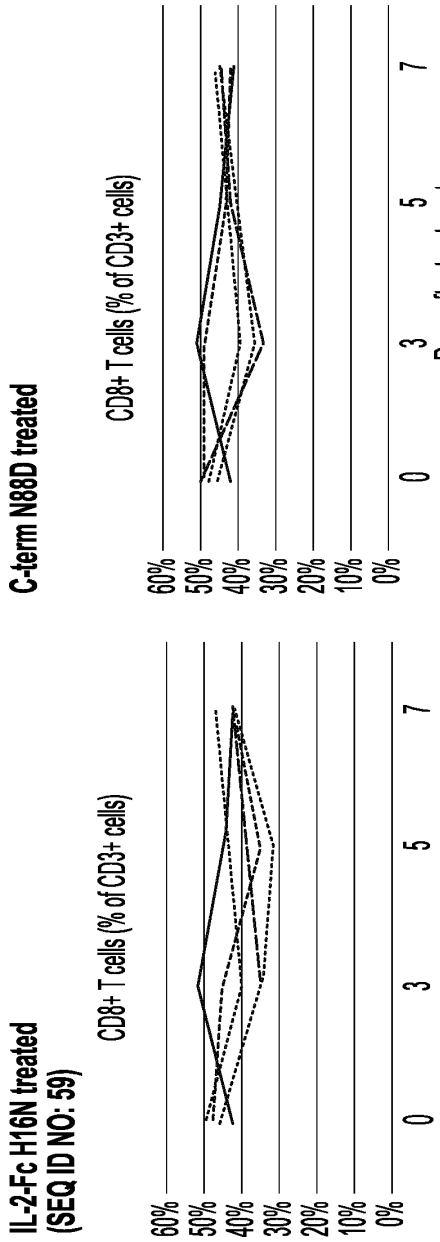


FIG. 14A

FIG. 14B

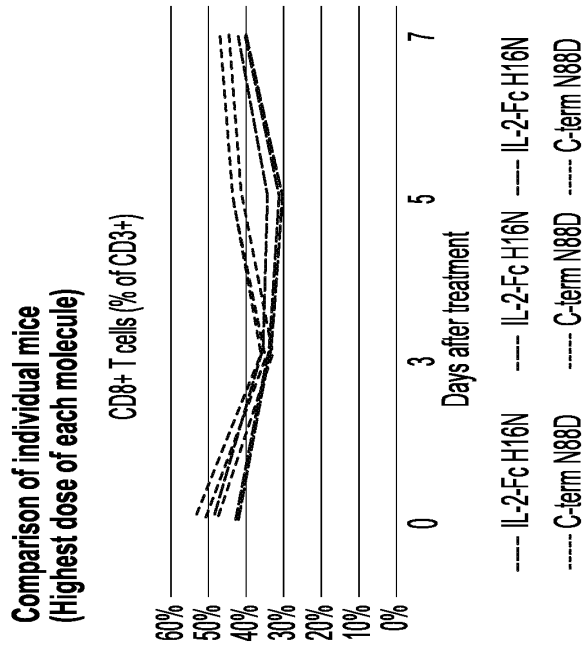

Comparison of individual mice
(Highest dose of each molecule)
CD8+ T cells (% of CD3+)
60%
50%
40%
30%
20%
10%
0%
0
3
5
7
Days after treatment
---- IL-2-Fc H16N
---- IL-2-Fc H16N
---- IL-2-Fc H16N
---- C-term N88D
---- C-term N88D
---- C-term N88D

FIG. 14C

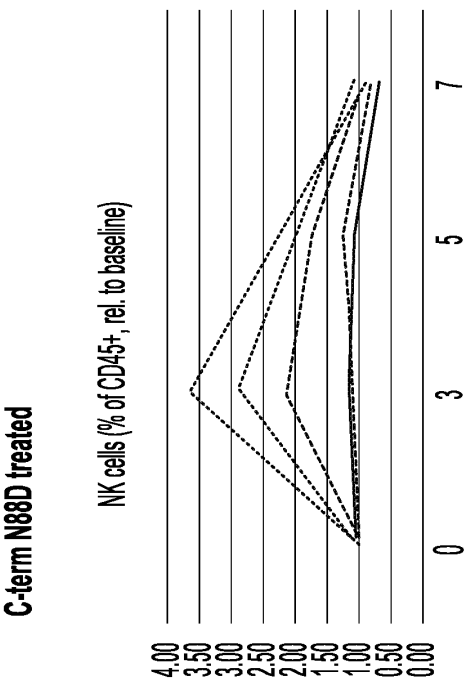


FIG. 15B

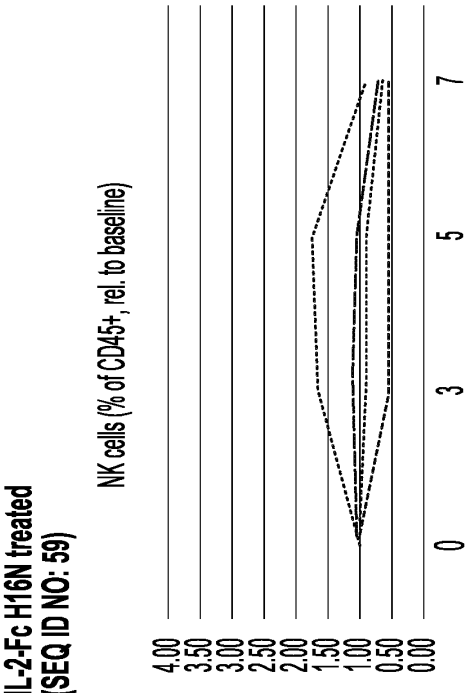


FIG. 15A

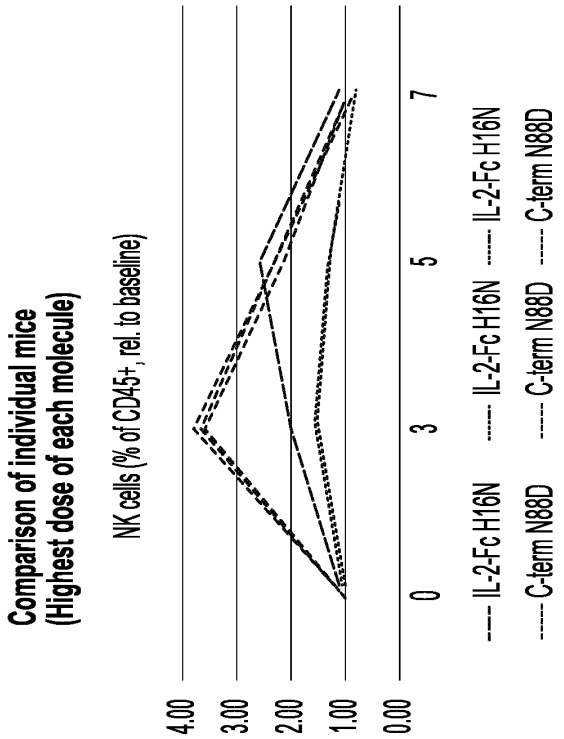

Comparison of individual mice
(Highest dose of each molecule)
NK cells (% of CD45+, rel. to baseline)
4.00
3.00
2.00
1.00
0.00
0
3
5
7
IL-2-Fc H16N
IL-2-Fc H16N
IL-2-Fc H16N
C-term N88D
C-term N88D
C-term N88D

FIG. 15C

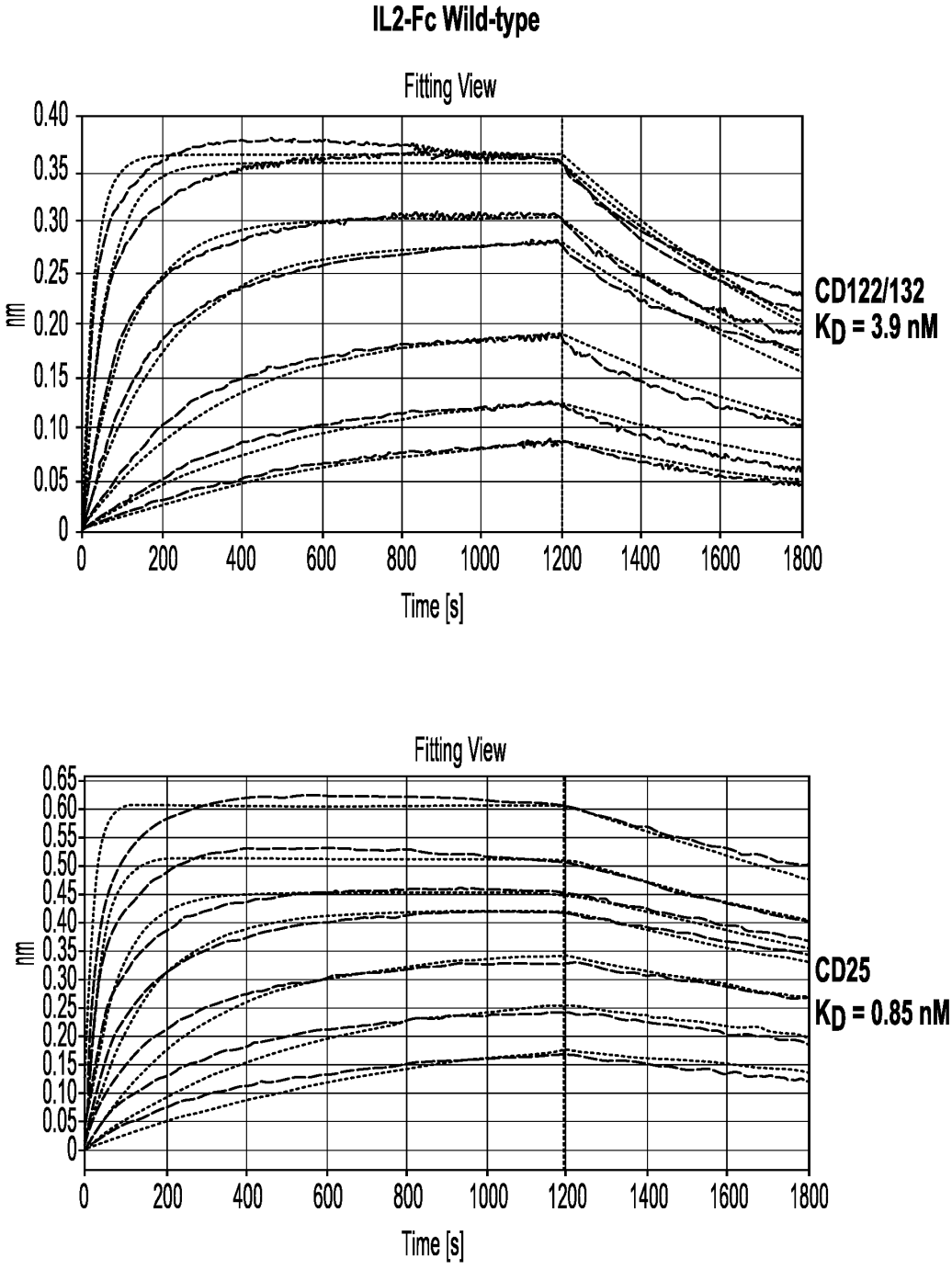

IL2-Fc Wild-type
Fitting View
nm
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time [s]
CD122/132
KD = 3.9 nM
Fitting View
nm
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time [s]
CD25
KD = 0.85 nM

FIG. 16A

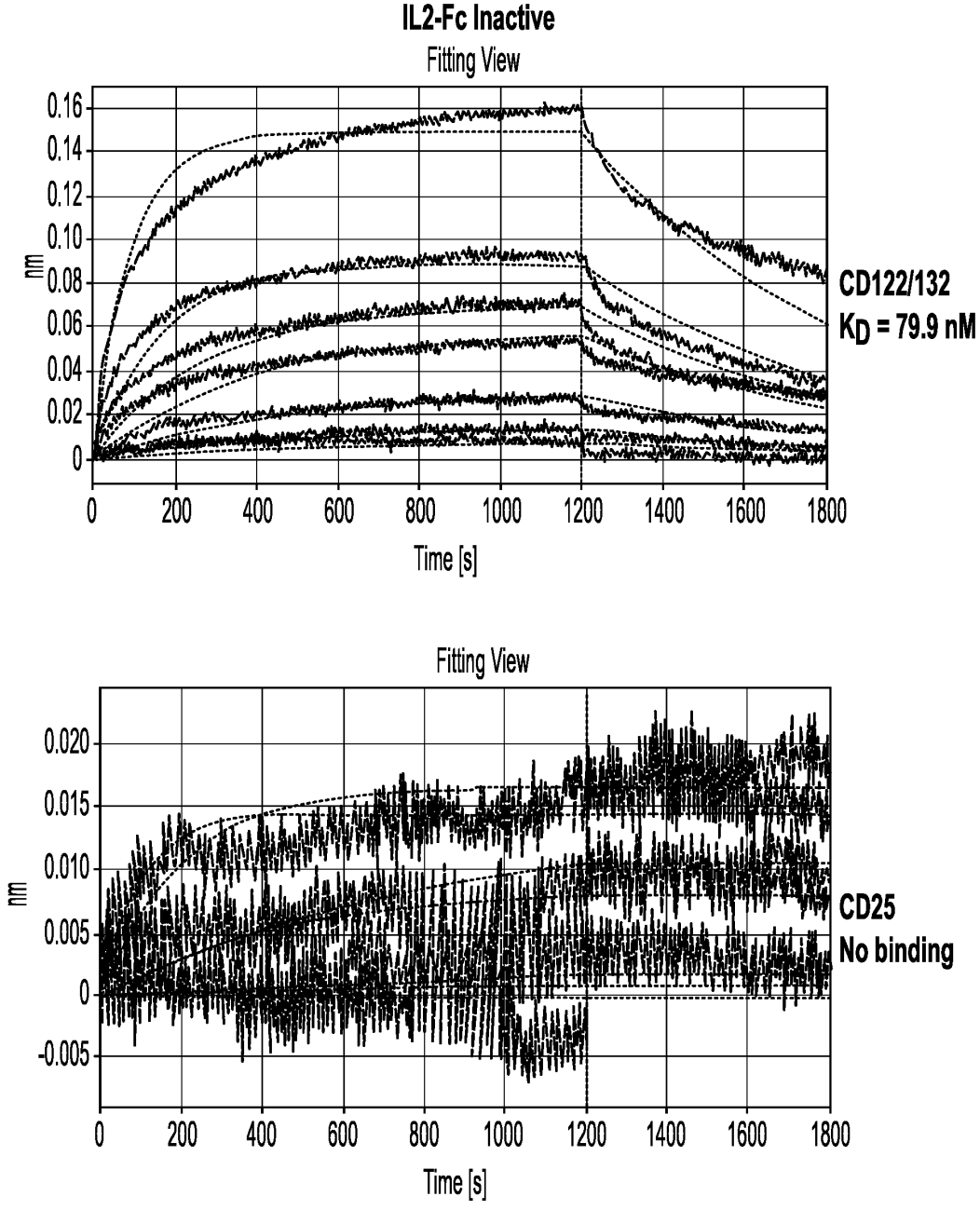

IL2-Fc Inactive
Fitting View
nm
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time [s]
CD122/132
KD = 79.9 nM
Fitting View
nm
0.020
0.015
0.010
0.005
0
-0.005
0
200
400
600
800
1000
1200
1400
1600
1800
Time [s]
CD25
No binding

FIG. 16B

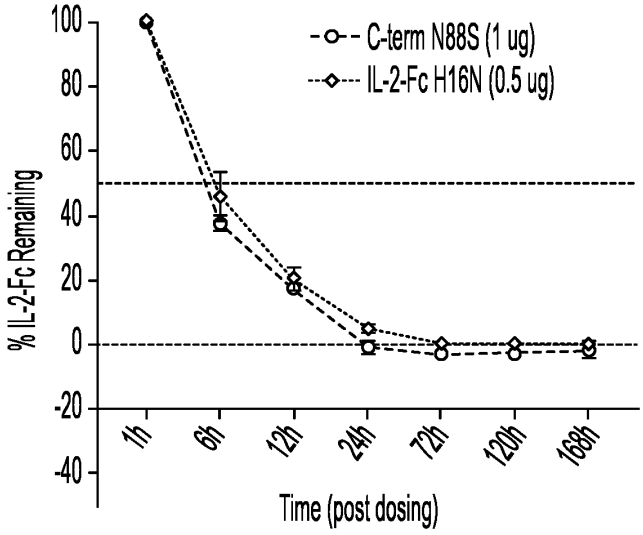


FIG. 17A

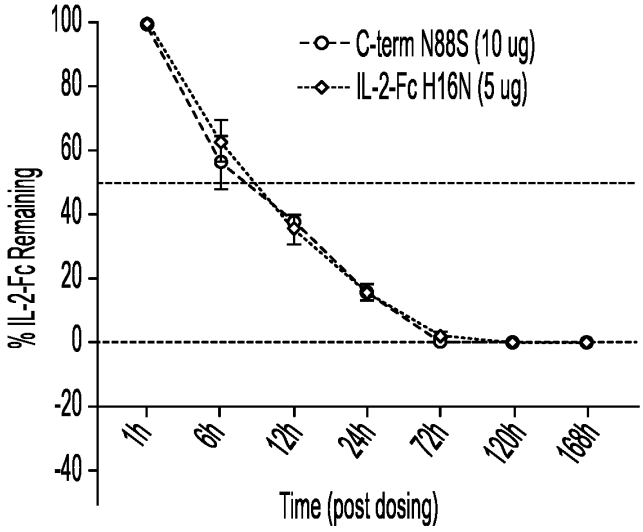


FIG. 17B

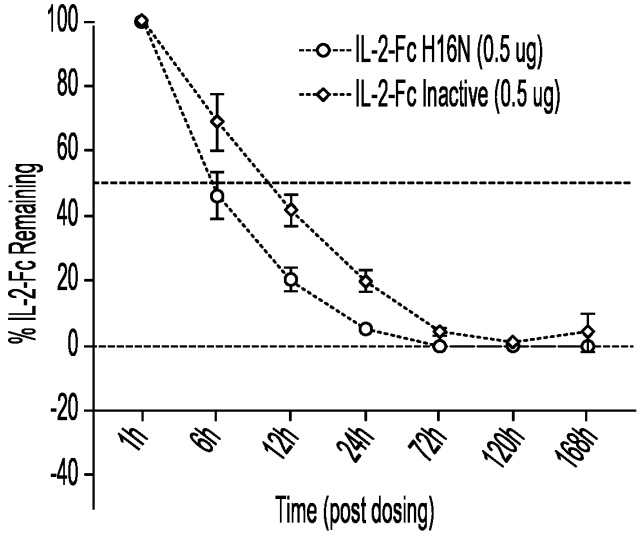


FIG. 17C

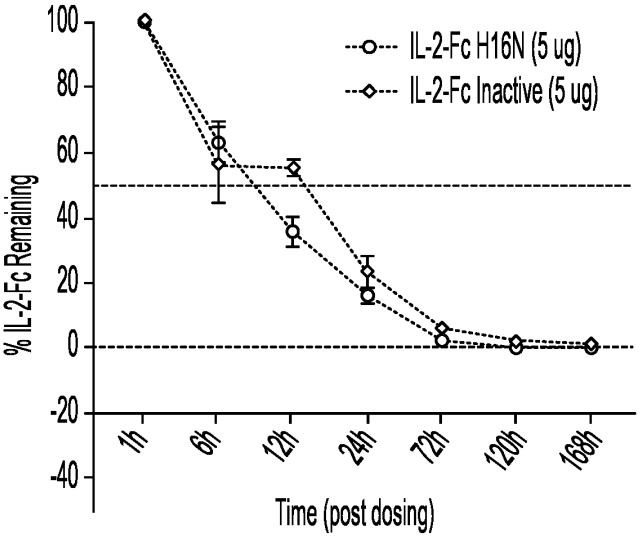


FIG. 17D

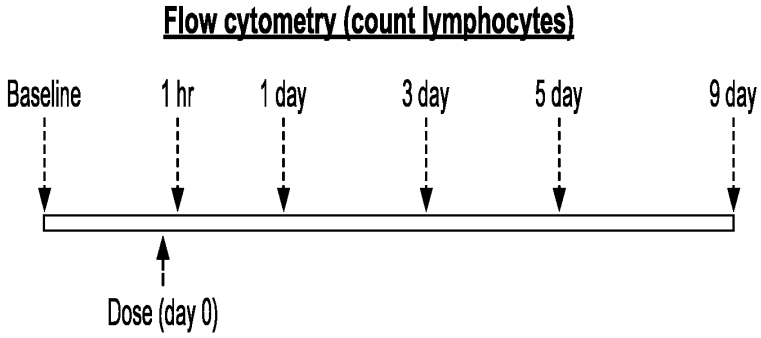

Flow cytometry (count lymphocytes)
Baseline
1 hr
1 day
3 day
5 day
9 day
Dose (day 0)

FIG. 18A

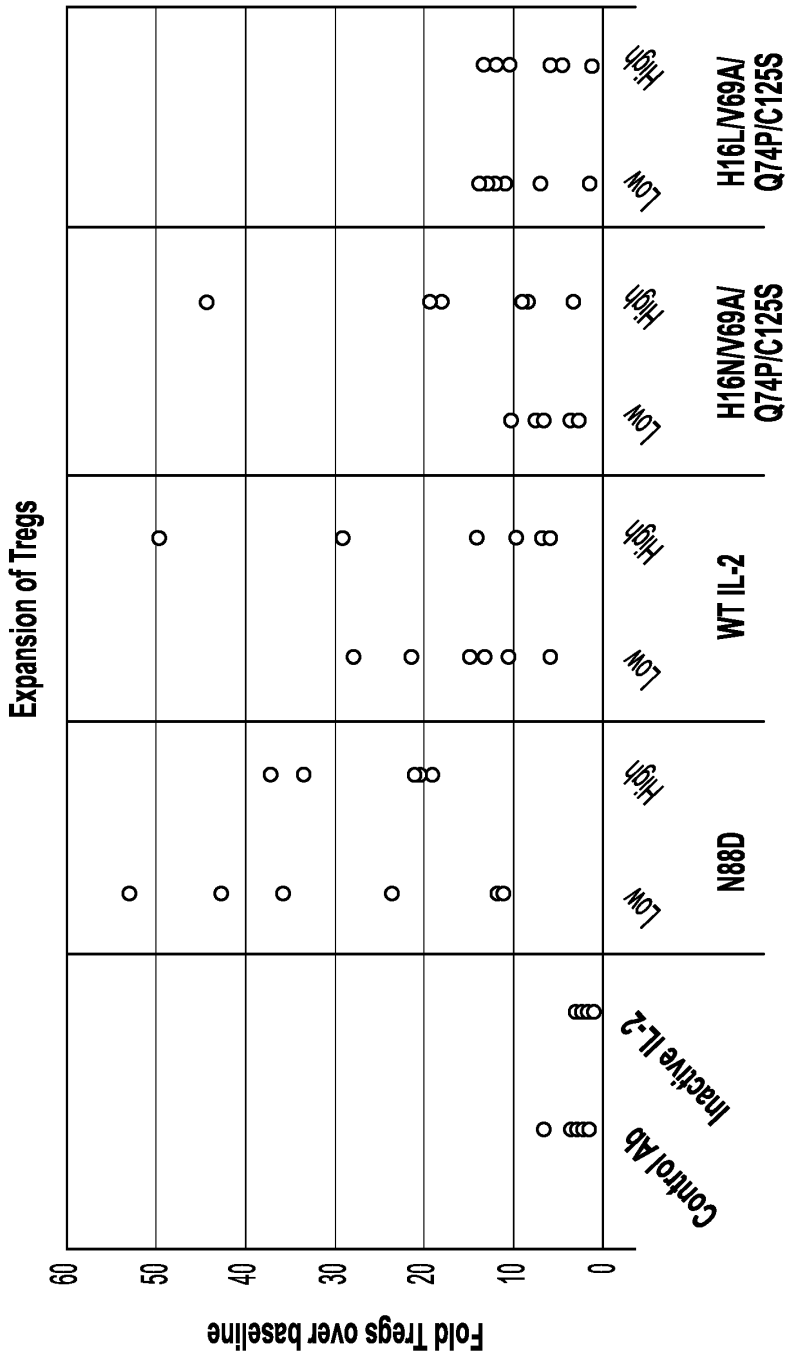

Expansion of Tregs
Fold Tregs over baseline
0
10
20
30
40
50
60
Control Ab
Inactive IL-2
N88D
WT IL-2
H16N/V69A/ Q74P/C125S
H16L/V69A/ Q74P/C125S
Low
High

FIG. 18B

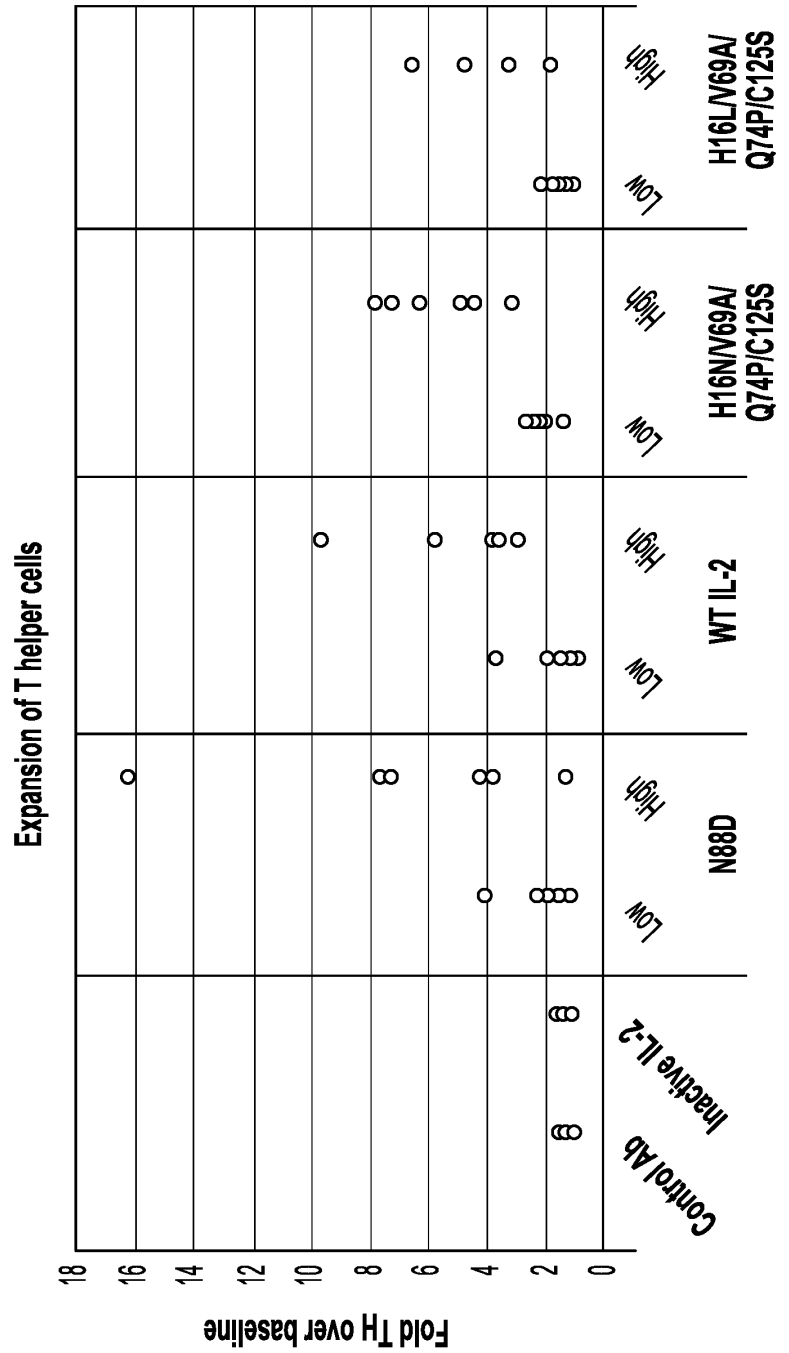

Expansion of T helper cells
Fold TH over baseline
0
2
4
6
8
10
12
14
16
18
Control Ab
Inactive IL-2
N88D
WT IL-2
H16N/V69A/ Q74P/C125S
H16L/V69A/ Q74P/C125S
Low
High

FIG. 18C

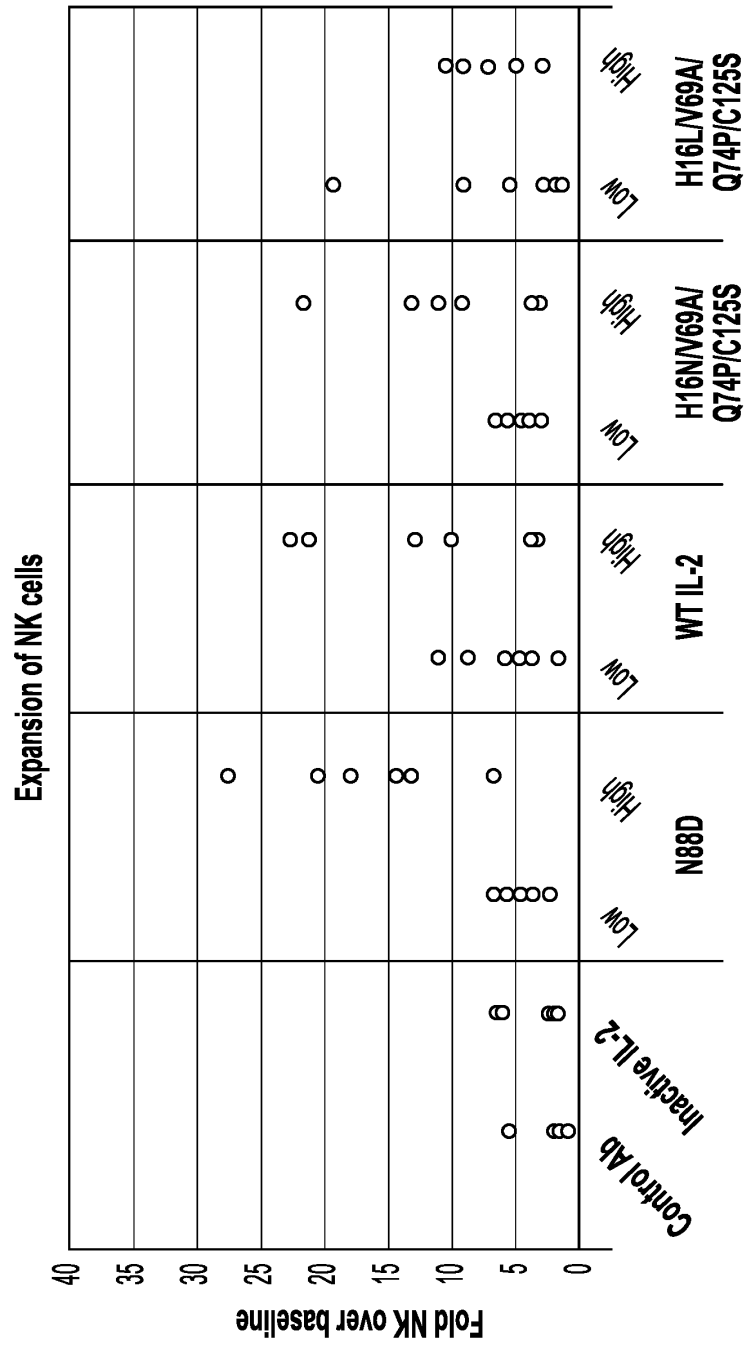

Expansion of NK cells
Fold NK over baseline
0
5
10
15
20
25
30
35
40
Control Ab
Inactive IL-2
N88D
WT IL-2
H16N/V69A/ Q74P/C125S
H16L/V69A/ Q74P/C125S
Low
High

FIG. 18D

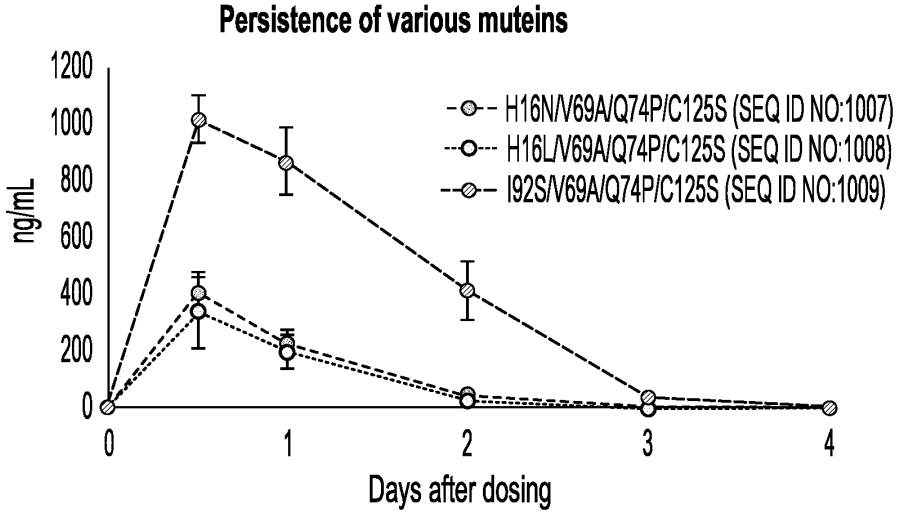


FIG. 19A

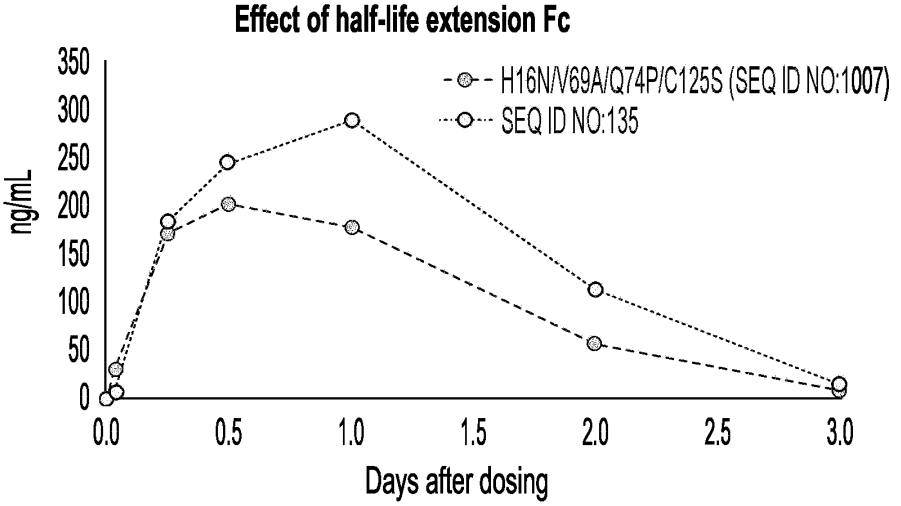


FIG. 19B

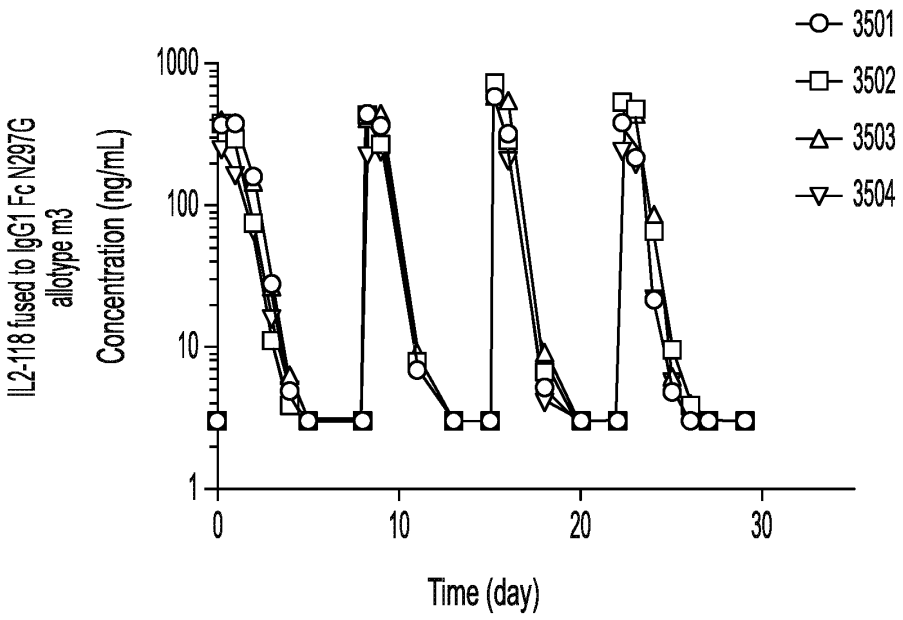

IL2-118 fused to IgG1 Fc N297G allotype m3
Concentration (ng/mL)
1000
100
10
1
0
10
20
30
Time (day)
3501
3502
3503
3504

FIG. 20

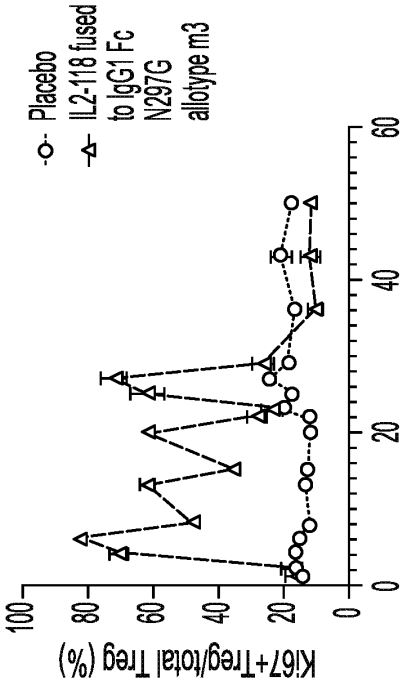


FIG. 21B

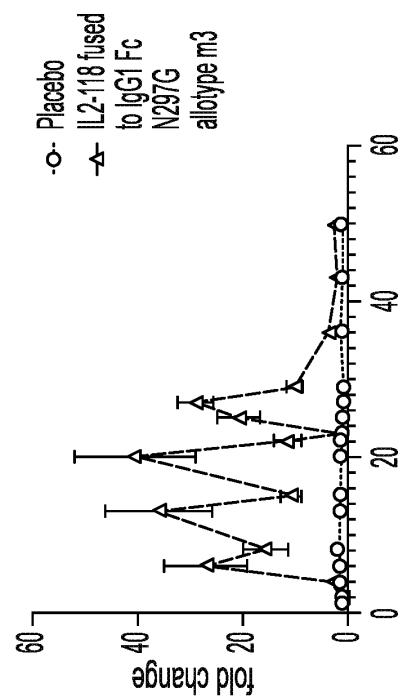


FIG. 21A

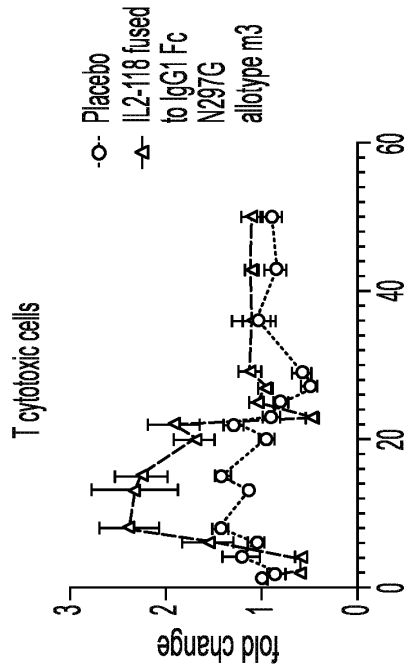


FIG. 22B

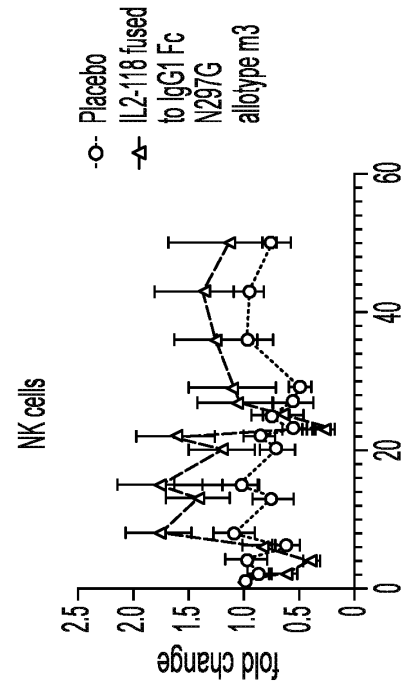


FIG. 22A

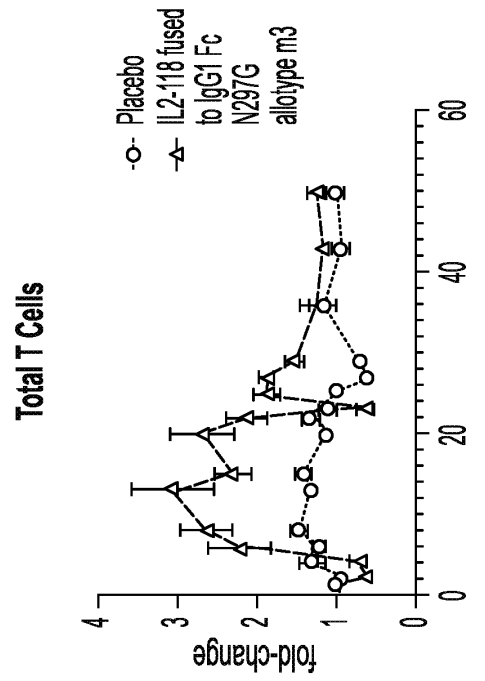


FIG. 22D

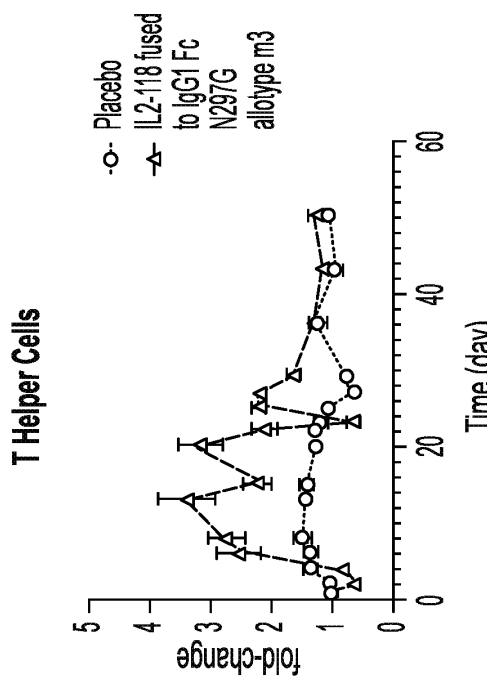


FIG. 22C

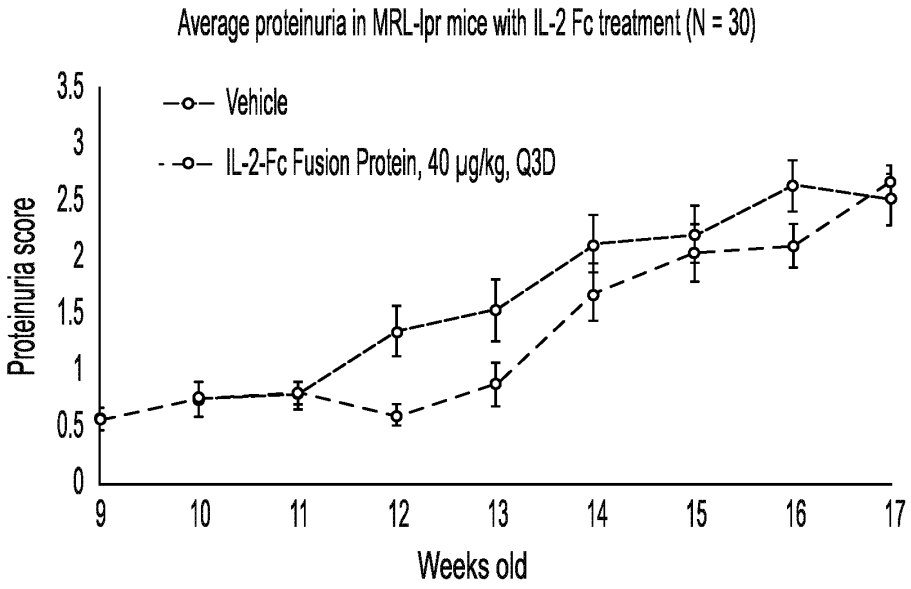


FIG. 23A

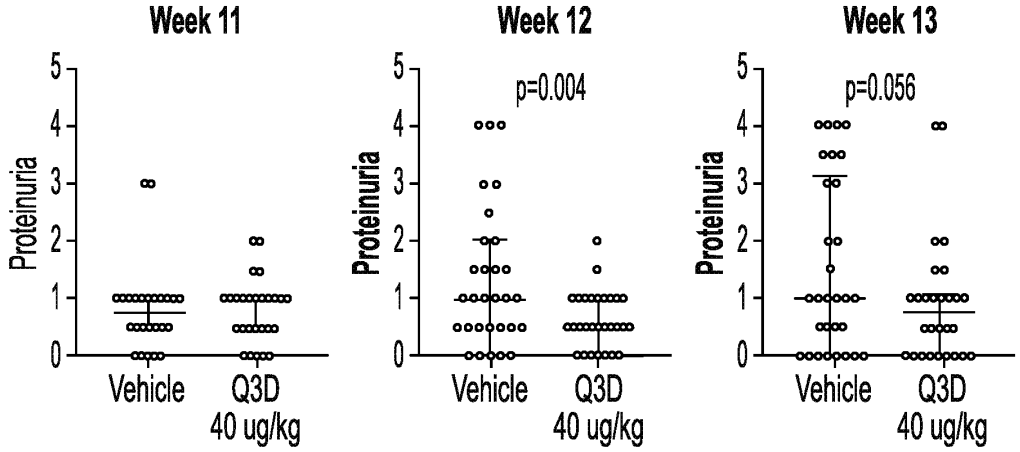


FIG. 23B

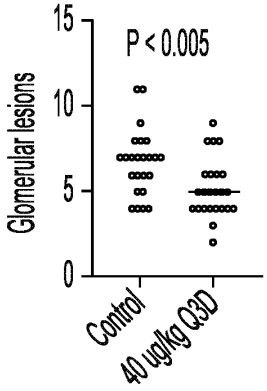


FIG. 23C

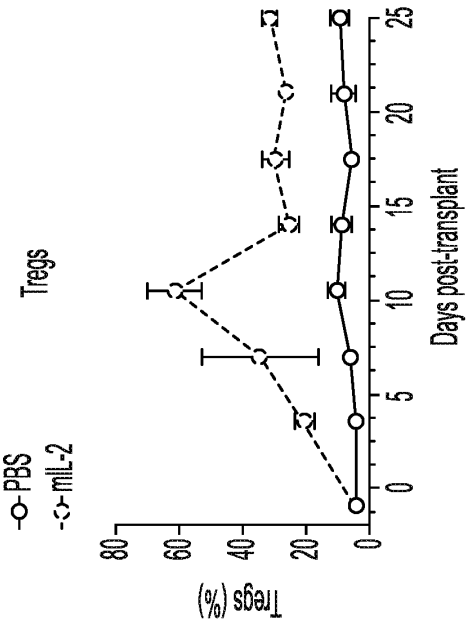


FIG. 24B

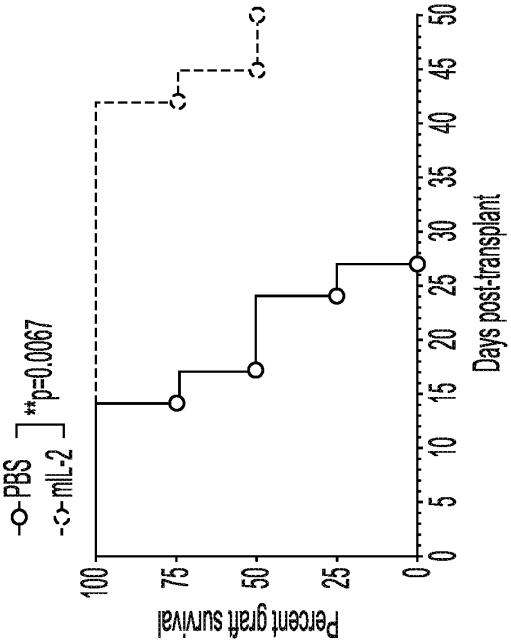


FIG. 24A

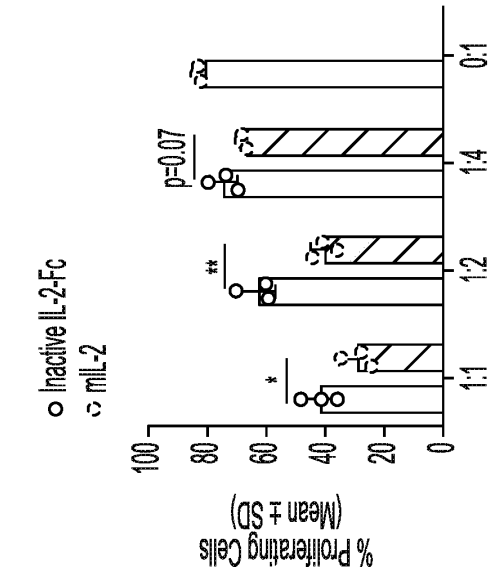


FIG. 24D

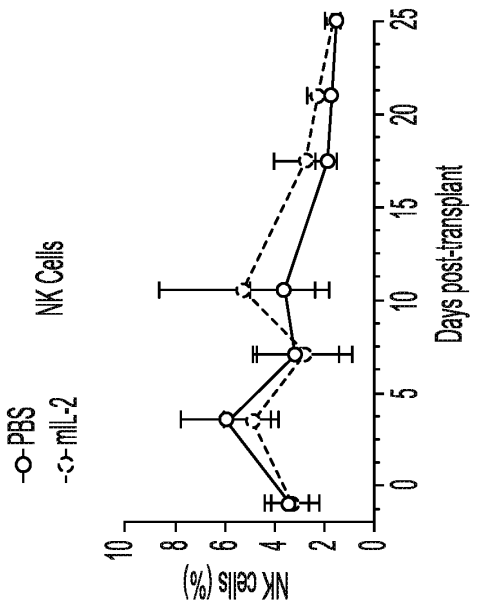


FIG. 24C

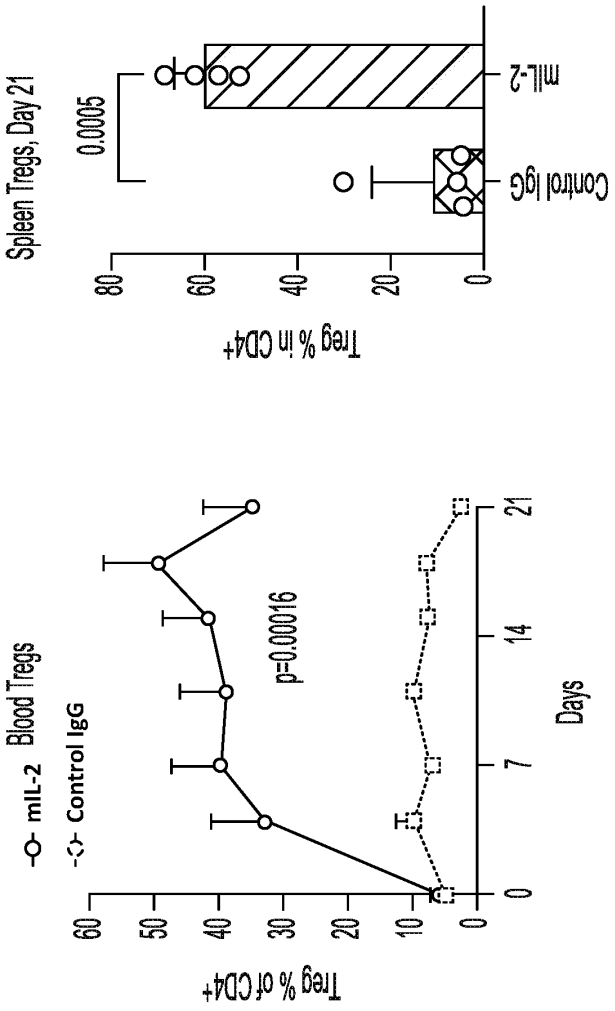

Spleen Tregs, Day 21
0.0005
mIL-2
Control IgG
Treg % in CD4+
0
20
40
60
80
Blood Tregs
mIL-2
Control IgG
p=0.00016
Treg % of CD4+
Days
0
7
14
21
10
30
50

FIG. 24E

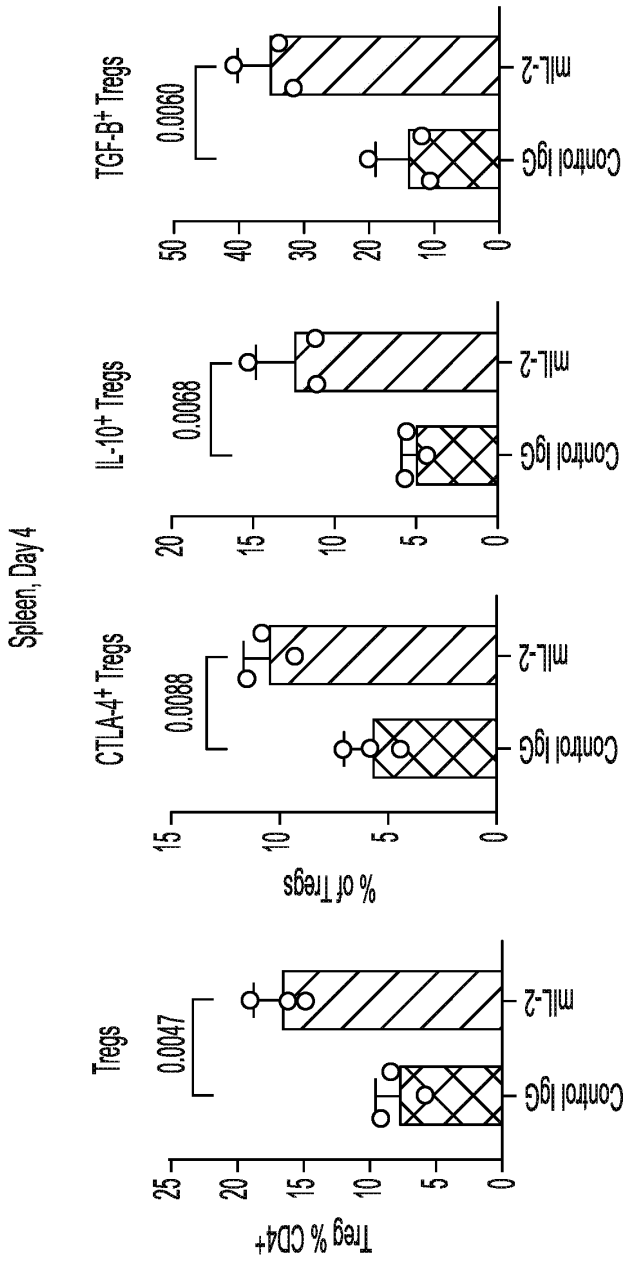

Spleen, Day 4
Treg % CD4+
Tregs
0.0047
Control IgG
mIL-2
% of Tregs
CTLA-4+ Tregs
0.0088
IL-10+ Tregs
0.0068
TGF-B+ Tregs
0.0060

FIG. 24F

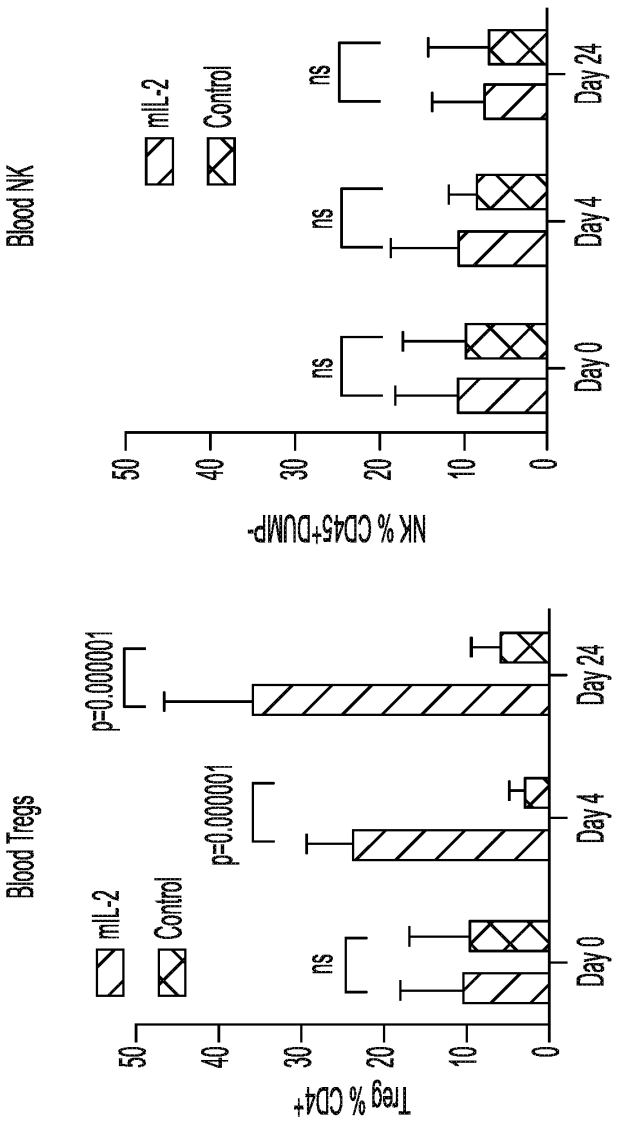

Blood NK
mIL-2
Control
NK % CD45+DUMP-
50
40
30
20
10
0
ns
ns
ns
Day 0
Day 4
Day 24
Blood Tregs
mIL-2
Control
Treg % CD4+
50
40
30
20
10
0
ns
p=0.000001
p=0.000001
Day 0
Day 4
Day 24

FIG. 24G

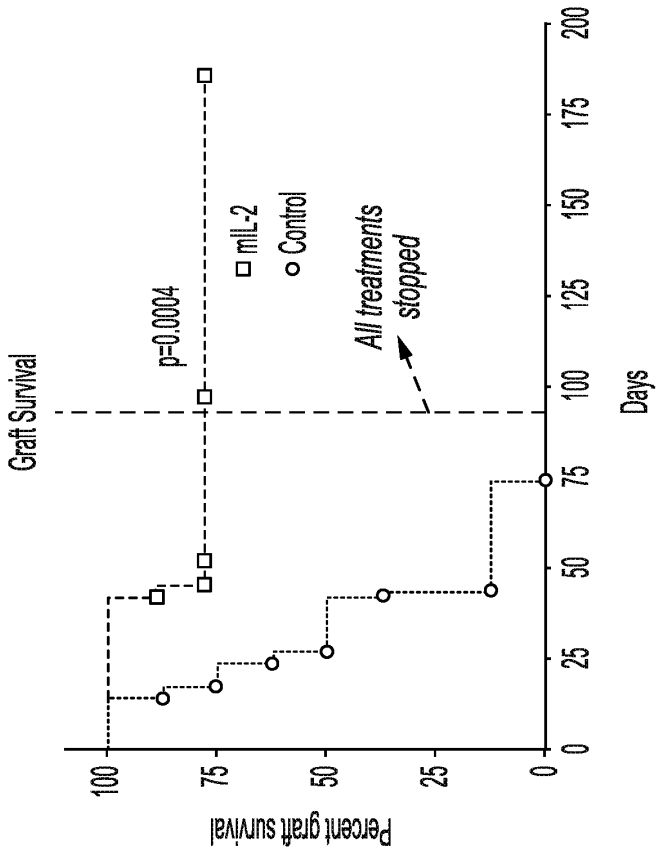

Graft Survival
p=0.0004
mIL-2
Control
All treatments stopped
Percent graft survival
Days
100
75
50
25
0
0
25
50
75
100
125
150
175
200

FIG. 24H

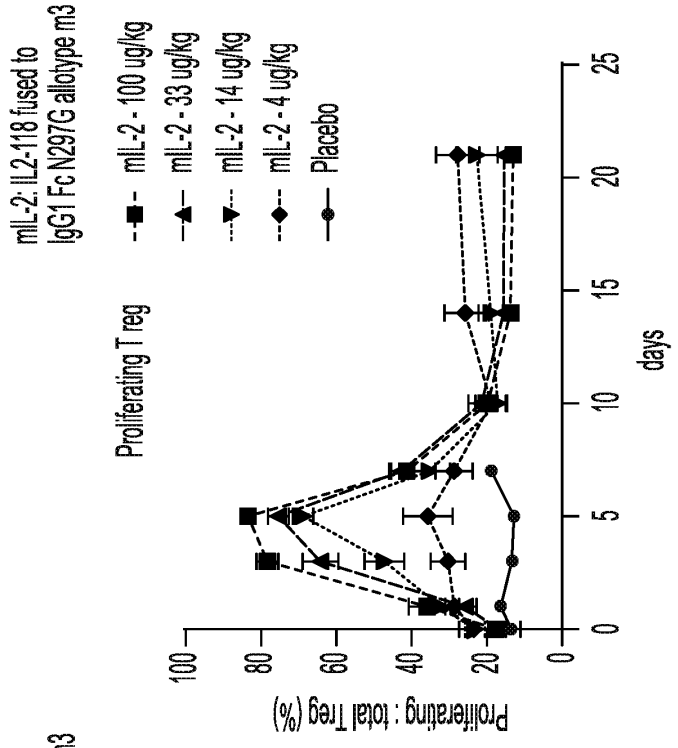


FIG. 25B

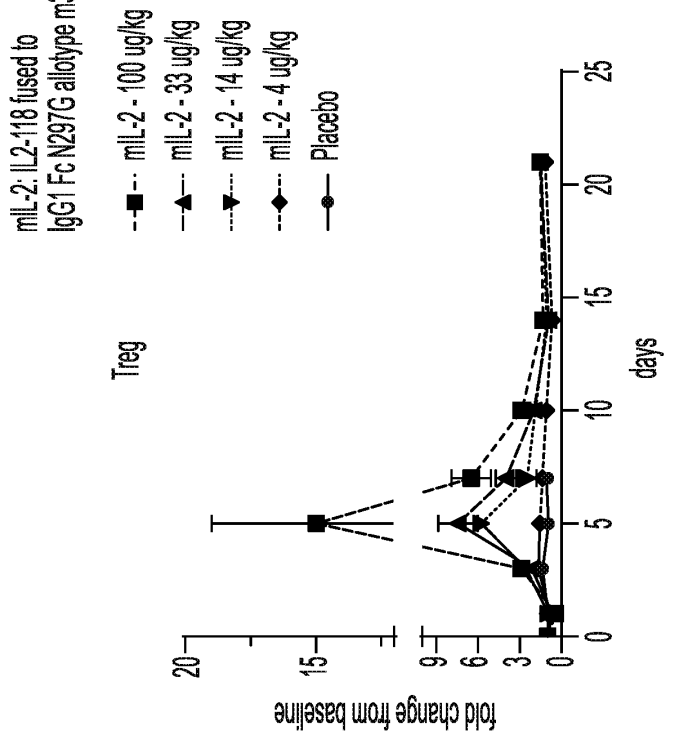


FIG. 25A

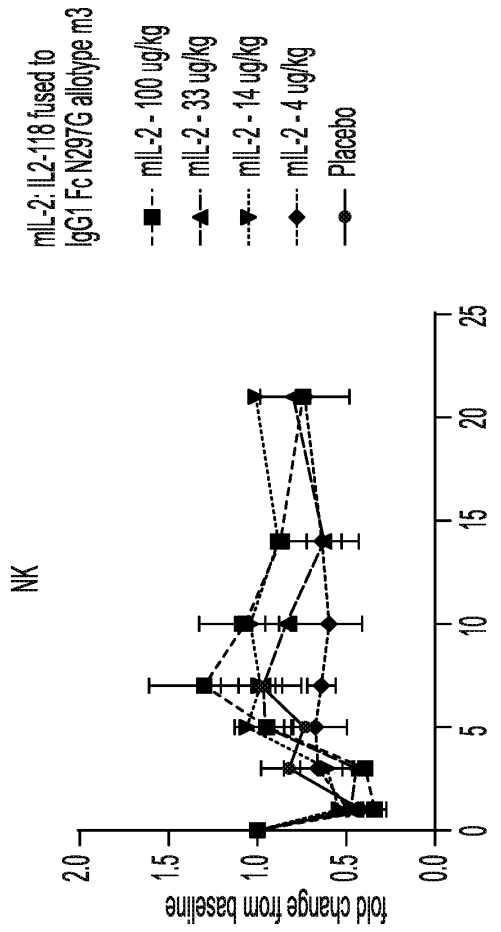

NK
mIL-2: IL2-118 fused to IgG1 Fc N297G allotype m3
mIL-2 - 100 ug/kg
mIL-2 - 33 ug/kg
mIL-2 - 14 ug/kg
mIL-2 - 4 ug/kg
Placebo
fold change from baseline
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
days

FIG. 25C

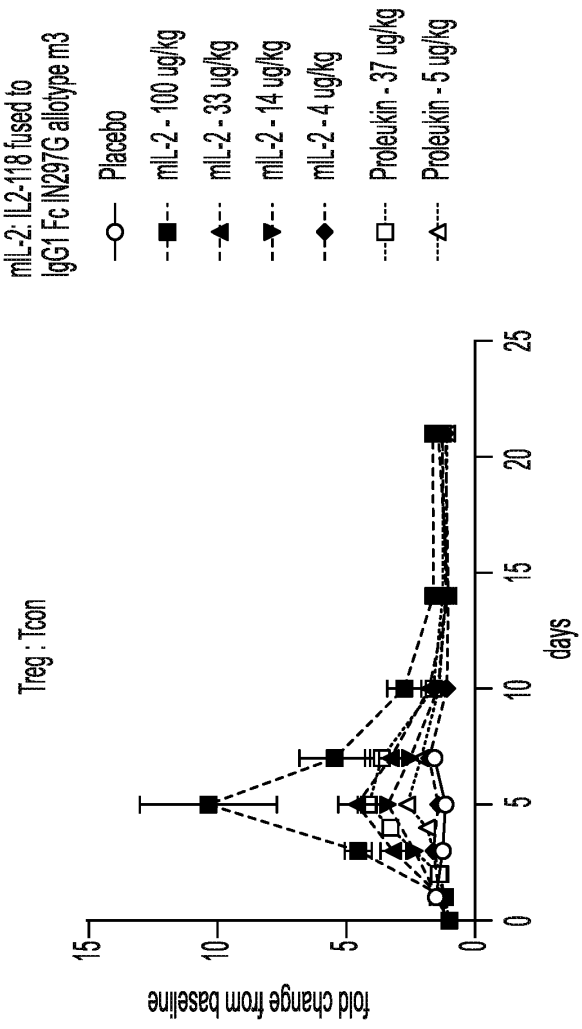

mIL-2: IL2-118 fused to IgG1 Fc IN297G allotype m3
Placebo
mIL-2 - 100 ug/kg
mIL-2 - 33 ug/kg
mIL-2 - 14 ug/kg
mIL-2 - 4 ug/kg
Proleukin - 37 ug/kg
Proleukin - 5 ug/kg
Treg : Tcon
fold change from baseline
0
5
10
15
0
5
10
15
20
25
days

FIG. 26A

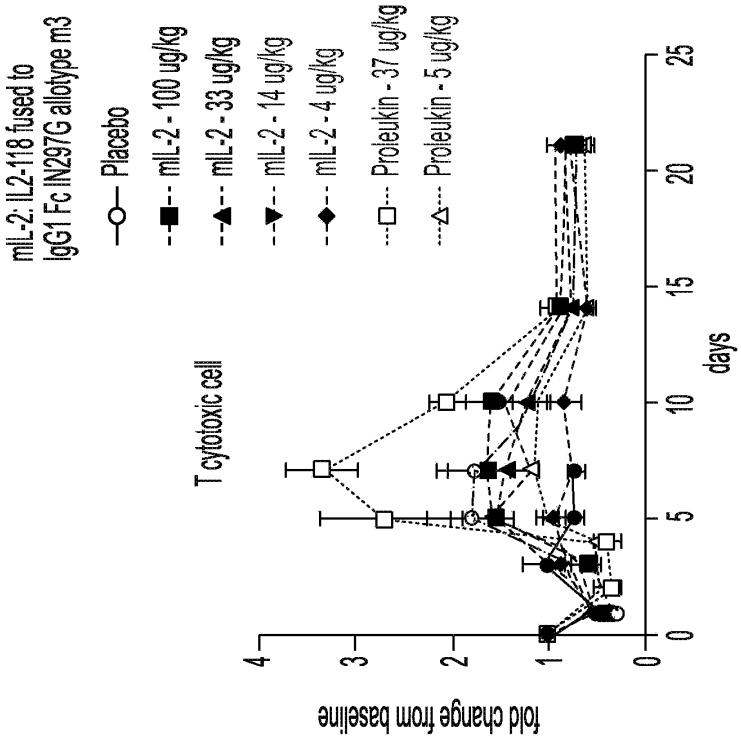


FIG. 26C

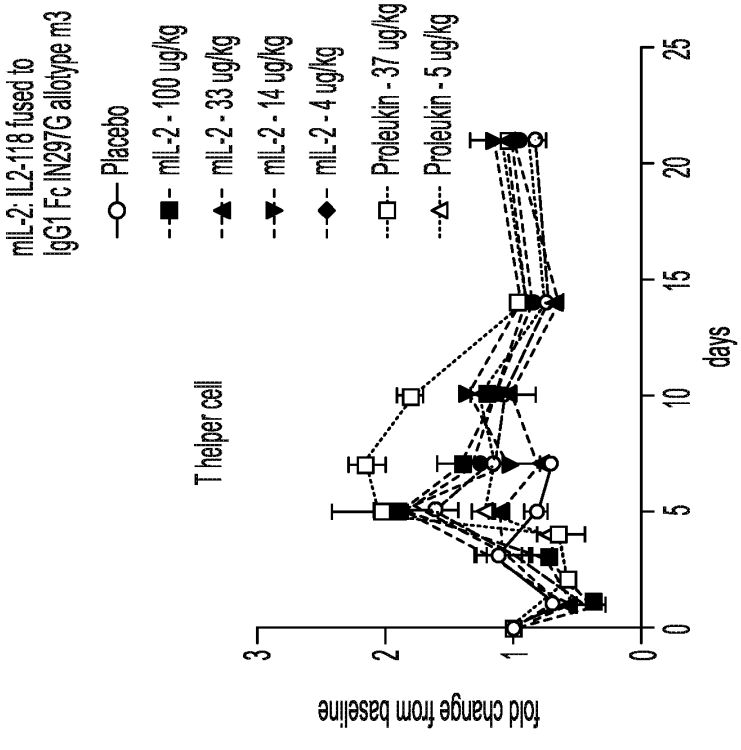


FIG. 26B

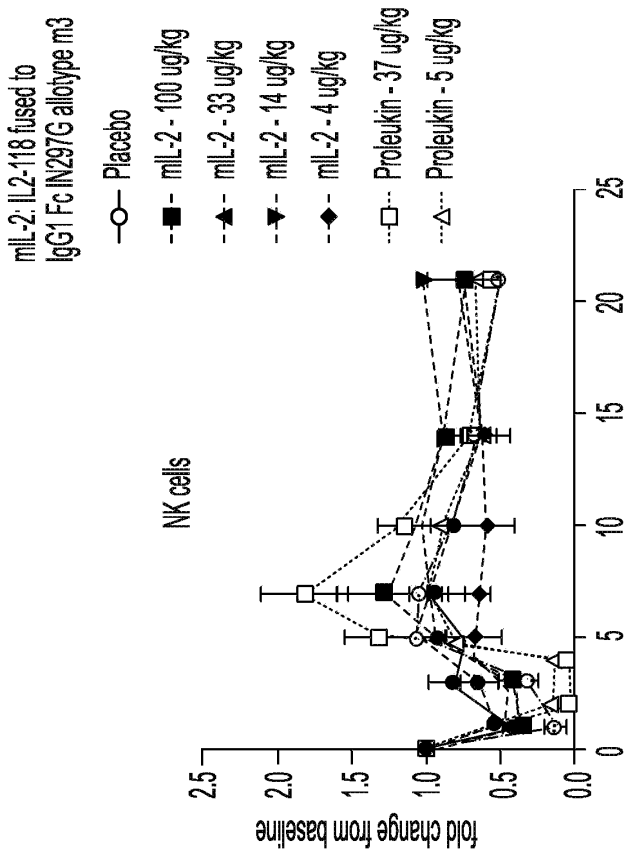

mIL-2: IL2-118 fused to IgG1 Fc IN297G allotype m3
Placebo
mIL-2 - 100 ug/kg
mIL-2 - 33 ug/kg
mIL-2 - 14 ug/kg
mIL-2 - 4 ug/kg
Proleukin - 37 ug/kg
Proleukin - 5 ug/kg
NK cells
fold change from baseline
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25

FIG. 26D

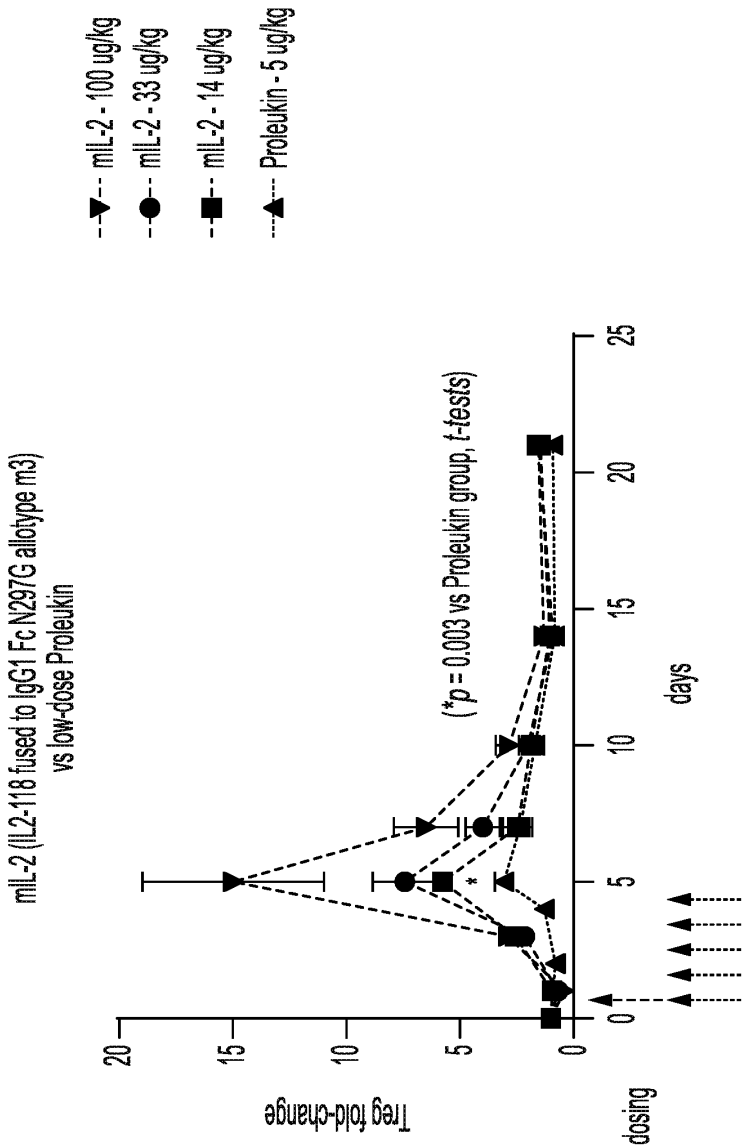

mIL-2 (IL2-118 fused to IgG1 Fc N297G allotype m3)
vs low-dose Proleukin
mIL-2 - 100 ug/kg
mIL-2 - 33 ug/kg
mIL-2 - 14 ug/kg
Proleukin - 5 ug/kg
(*p = 0.003 vs Proleukin group, t-tests)
Treg fold-change
days
dosing
0
5
10
15
20
25

FIG. 27

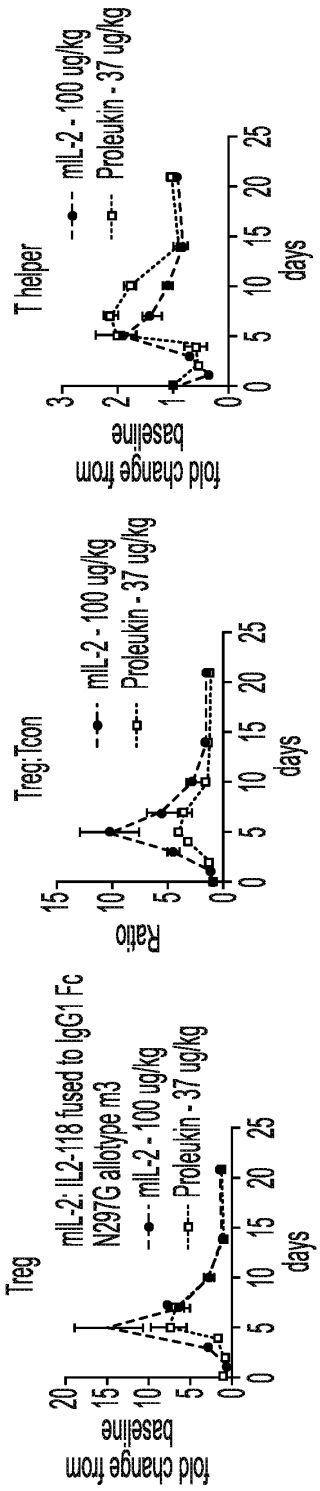


FIG. 28A

FIG. 28B

FIG. 28C

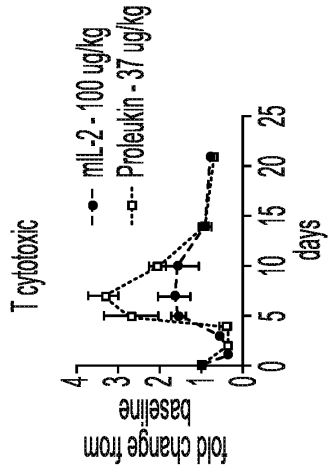


FIG. 28D

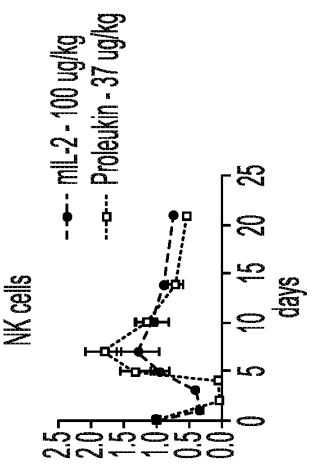


FIG. 28E

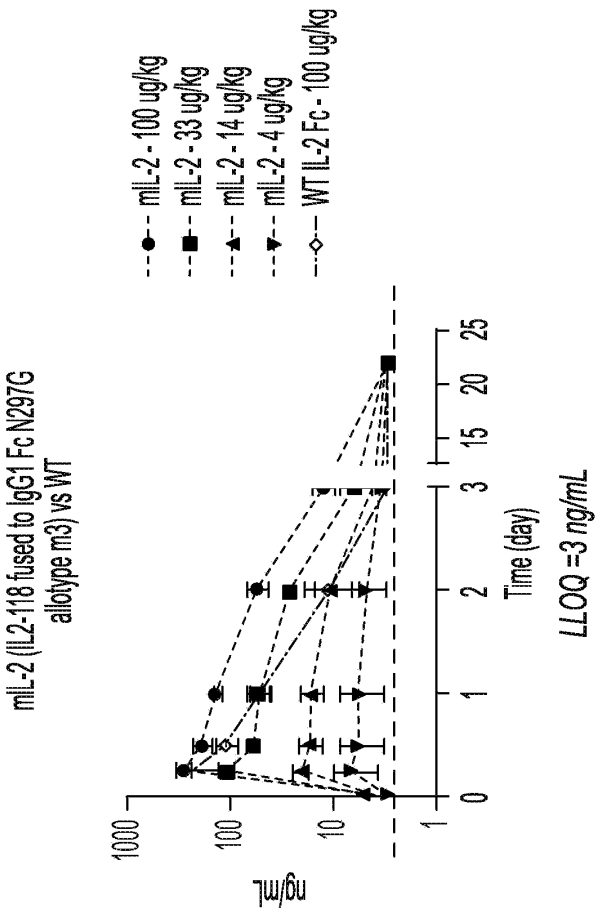

mIL-2 (IL2-118 fused to IgG1 Fc N297G allotype m3) vs WT
mIL-2 - 100 ug/kg
mIL-2 - 33 ug/kg
mIL-2 - 14 ug/kg
mIL-2 - 4 ug/kg
WT IL-2 Fc - 100 ug/kg
ng/mL
1000
100
10
1
0
1
2
3
15
20
25
Time (day)
LLOQ =3 ng/mL

FIG. 29A

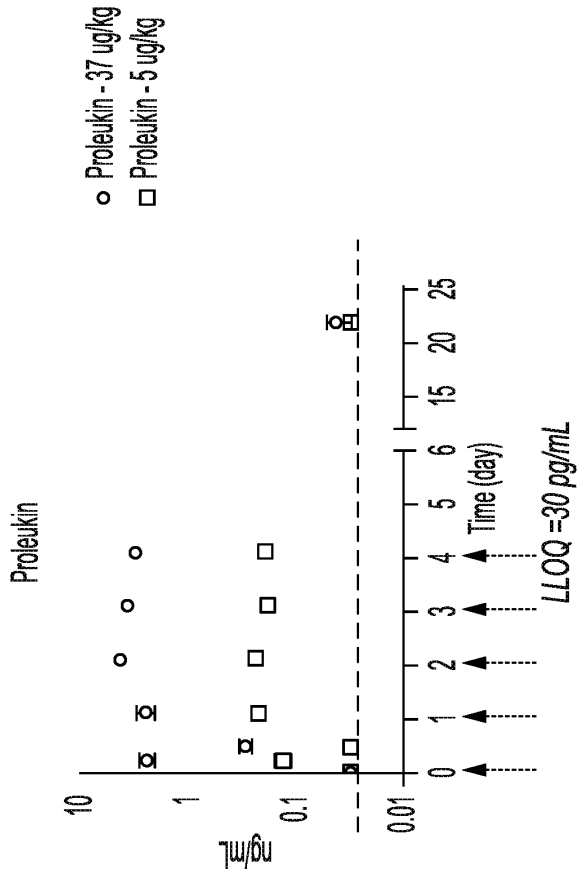

Proleukin - 37 ug/kg
Proleukin - 5 ug/kg
Proleukin
ng/mL
10
1
0.1
0.01
0
1
2
3
4
5
6
15
20
25
Time (day)
LLOQ =30 pg/mL

FIG. 29B