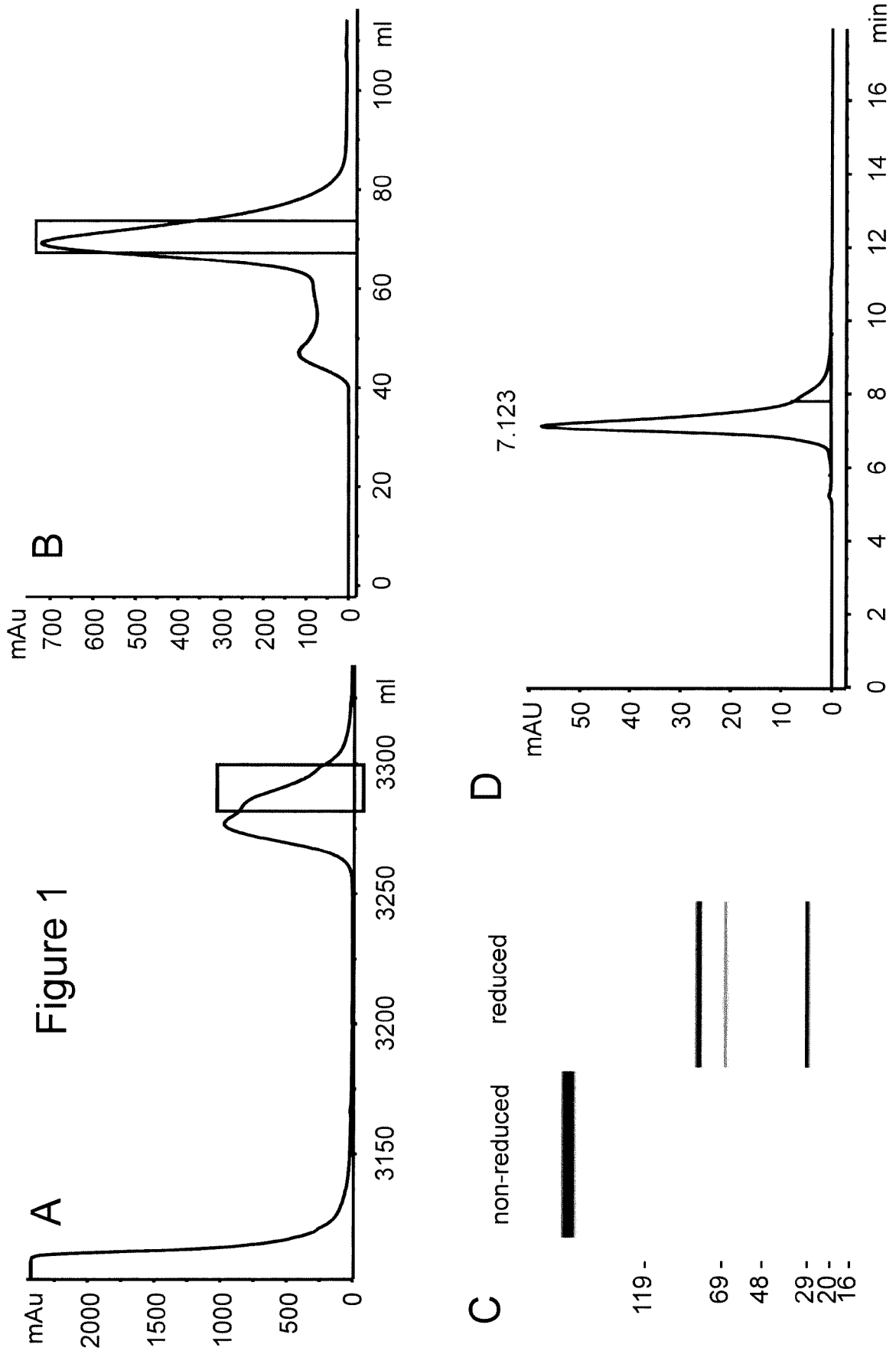

A
mAu
2000
1500
1000
500
0
3150
3200
3250
3300
ml
B
mAu
700
600
500
400
300
200
100
0
0
20
40
60
80
100
ml
C
non-reduced
reduced
119 -
69 -
48 -
29 -
20 -
16 -
D
7.123
mAU
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
min

Figure 1

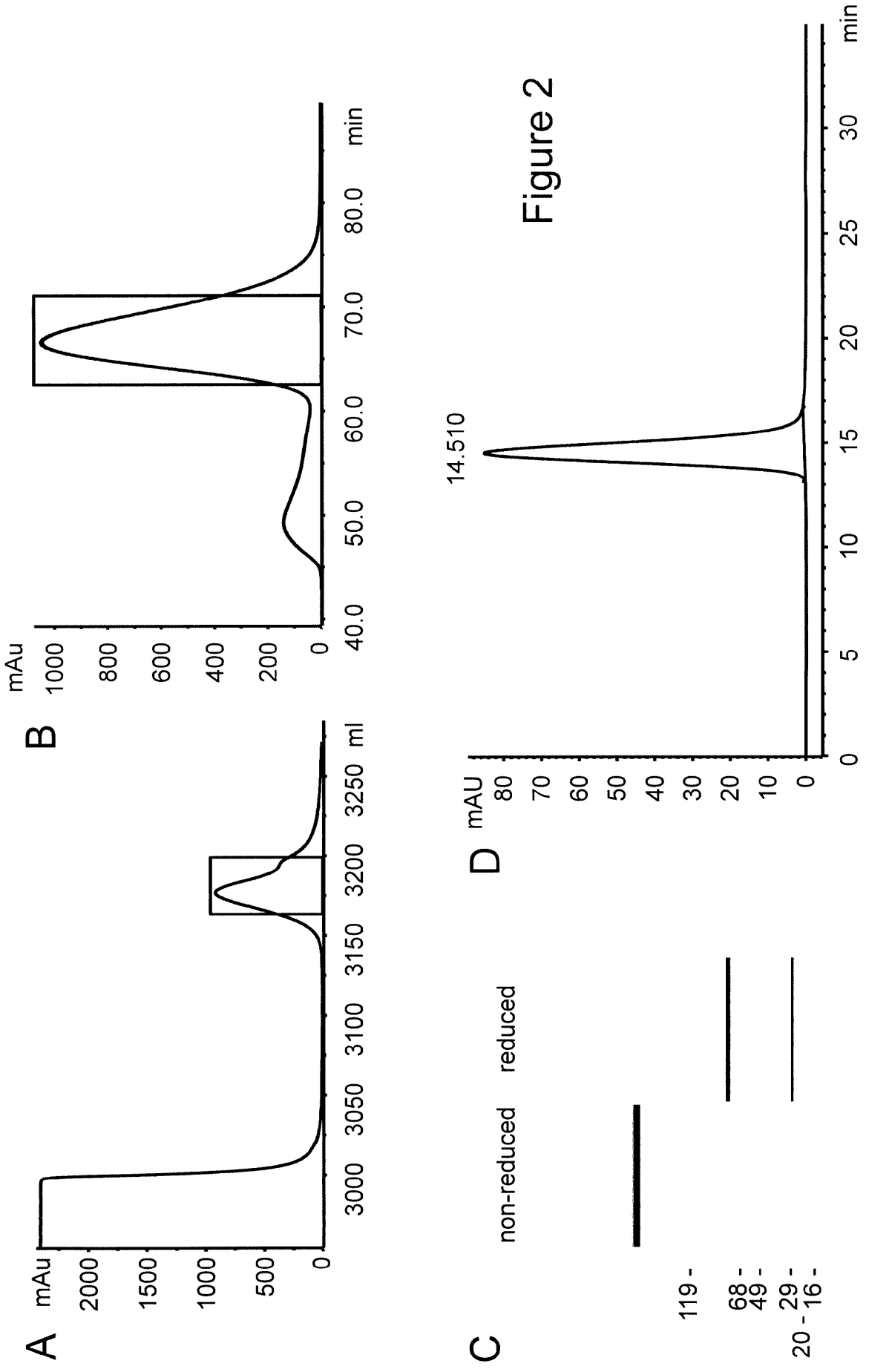
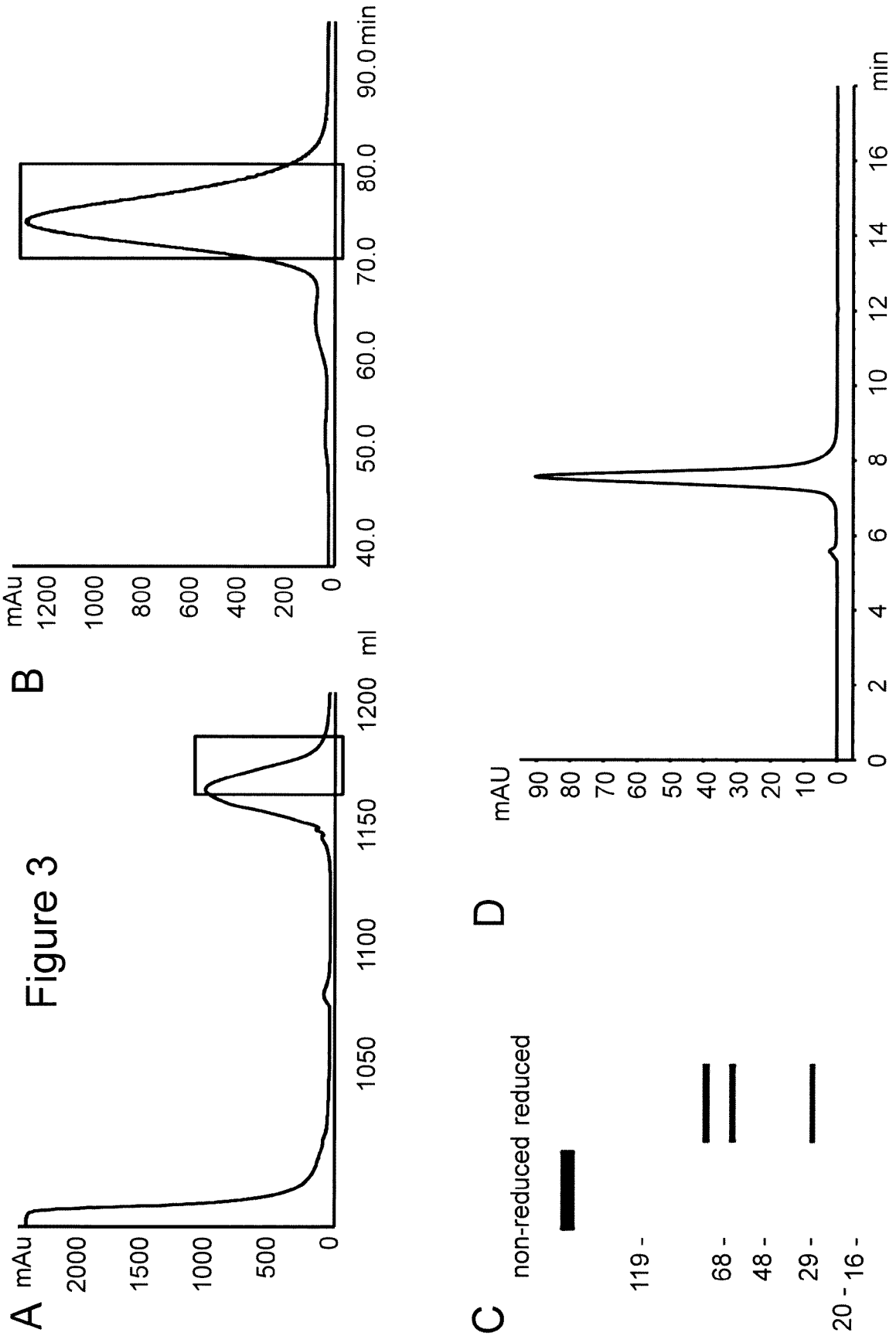

A
mAu
2000
1500
1000
500
0
3000
3050
3100
3150
3200
3250
ml
B
mAu
1000
800
600
400
200
0
40.0
50.0
60.0
70.0
80.0
min
C
non-reduced
reduced
119 -
68 -
49 -
29 -
20 -
16 -
D
mAU
80
70
60
50
40
30
20
10
0
14.510
0
5
10
15
20
25
30
min

Figure 2

Figure 3


A
mAu
2000
1500
1000
500
0
1050
1100
1150
1200
ml
B
mAu
1200
1000
800
600
400
200
0
40.0
50.0
60.0
70.0
80.0
90.0 min
C
non-reduced reduced
119 -
68 -
48 -
29 -
20 - 16 -
D
mAU
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
min

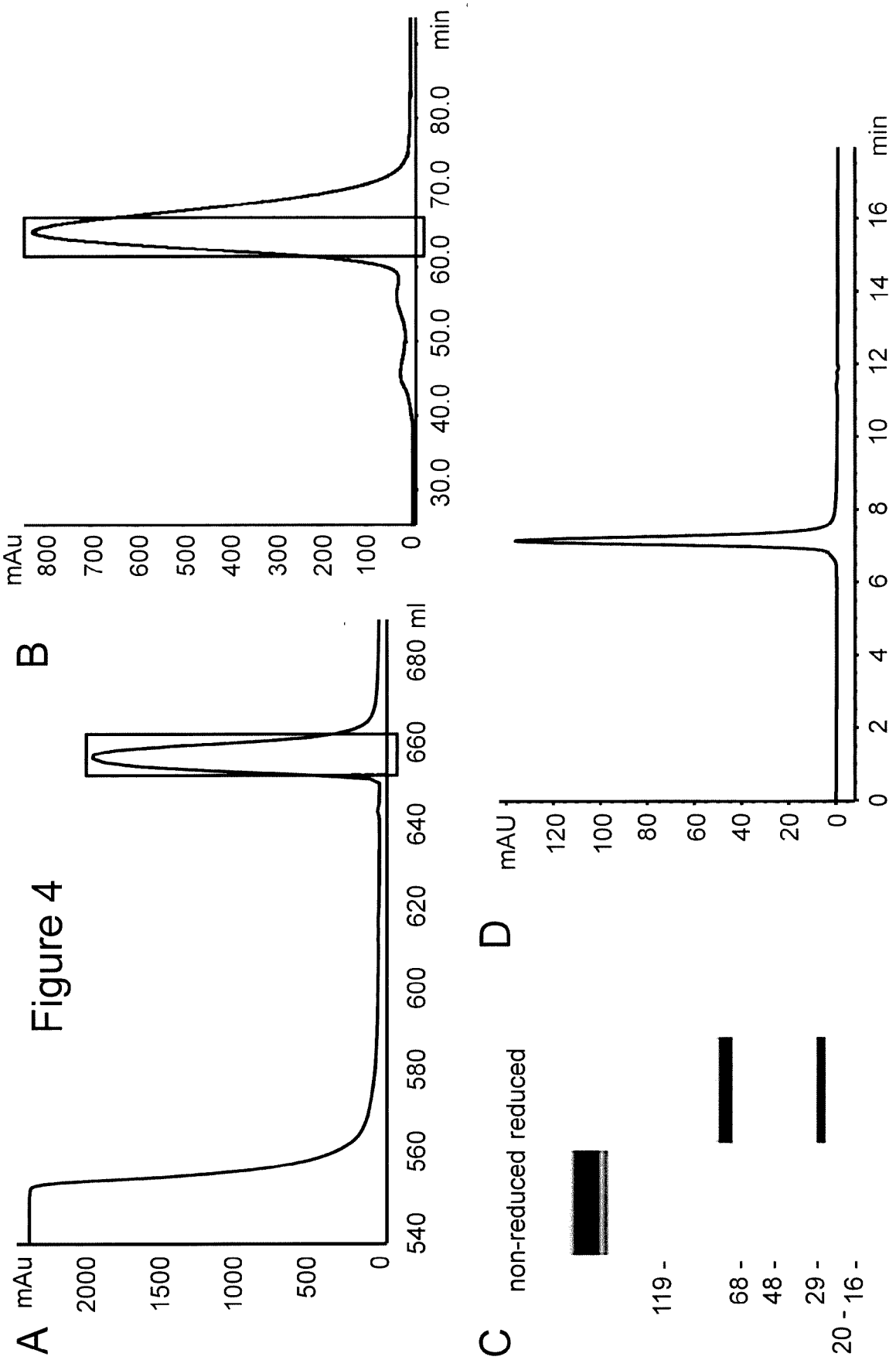
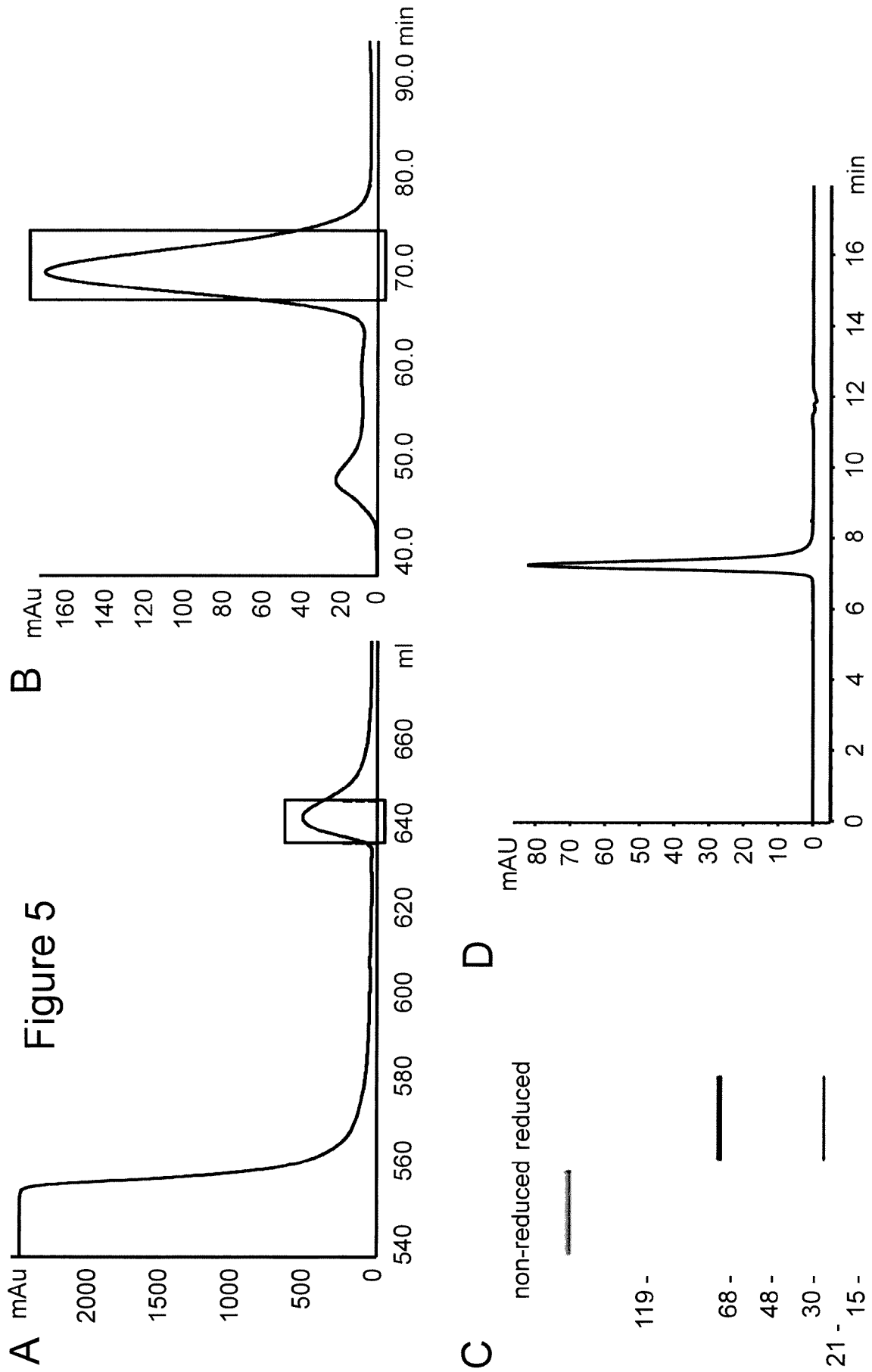

A
mAu
2000
1500
1000
500
0
540
560
580
600
620
640
660
680 ml
B
mAu
800
700
600
500
400
300
200
100
0
30.0
40.0
50.0
60.0
70.0
80.0
min
C
non-reduced reduced
119 -
68 -
48 -
29 -
20 - 16 -
D
mAU
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
min

Figure 4

Figure 5


A
mAu
2000
1500
1000
500
0
540
560
580
600
620
640
660
ml
B
mAu
160
140
120
100
80
60
40
20
0
40.0
50.0
60.0
70.0
80.0
90.0 min
C
non-reduced reduced
119 -
68 -
48 -
30 -
21 -
15 -
D
mAU
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
min

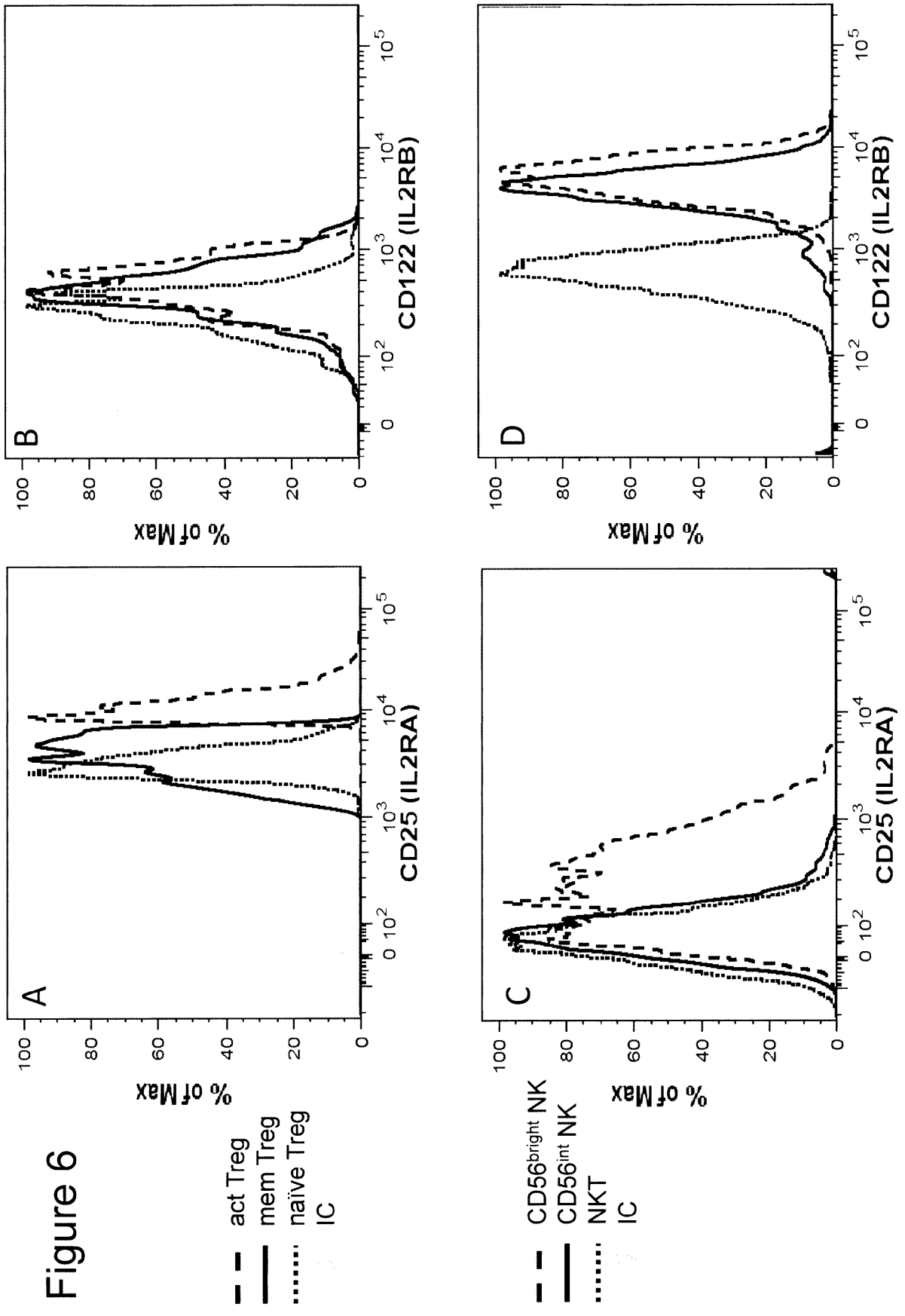

A
B
C
D
% of Max
CD25 (IL2RA)
CD122 (IL2RB)
act Treg
mem Treg
naïve Treg
IC
CD56bright NK
CD56int NK
NKT
IC

Figure 6

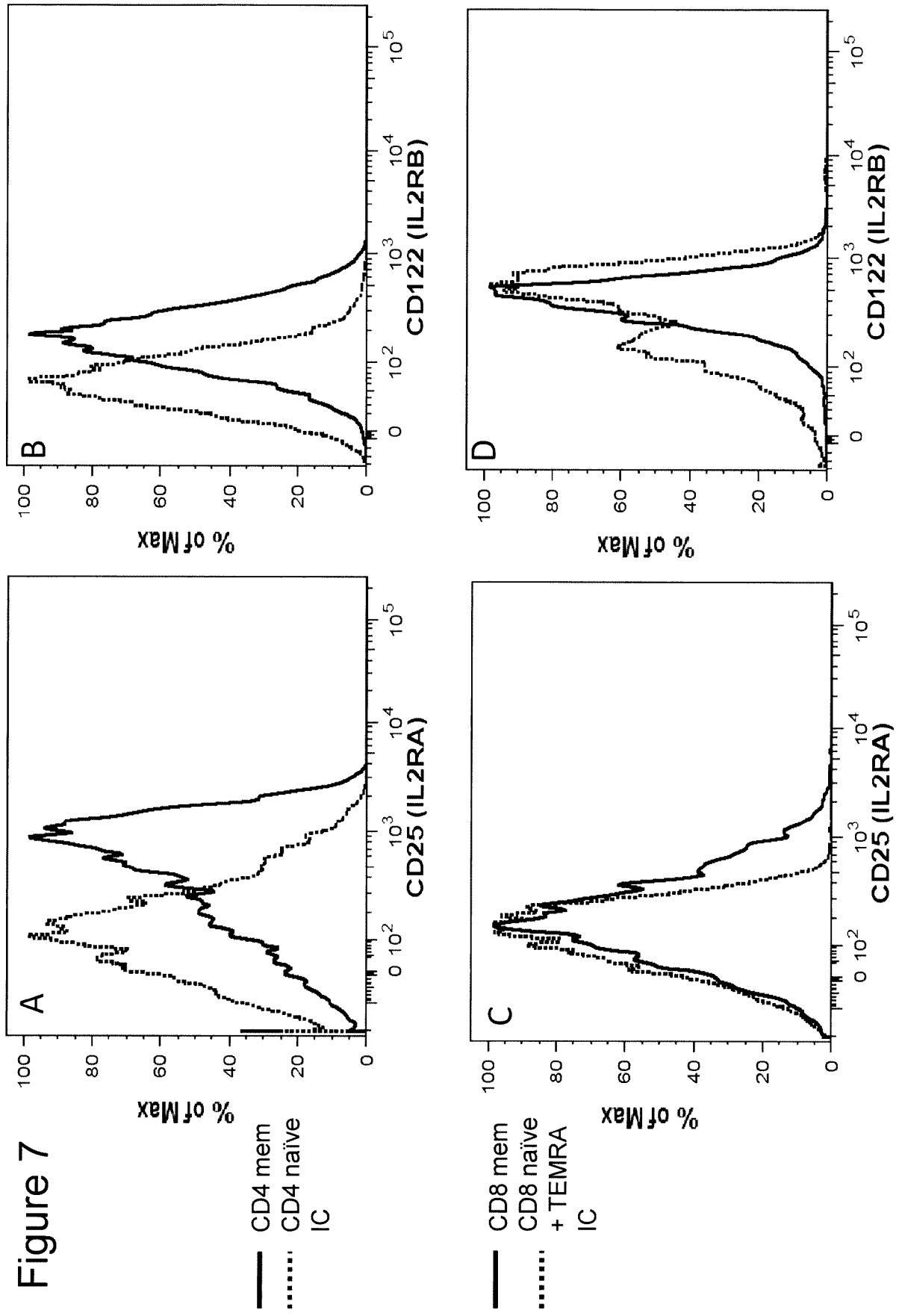
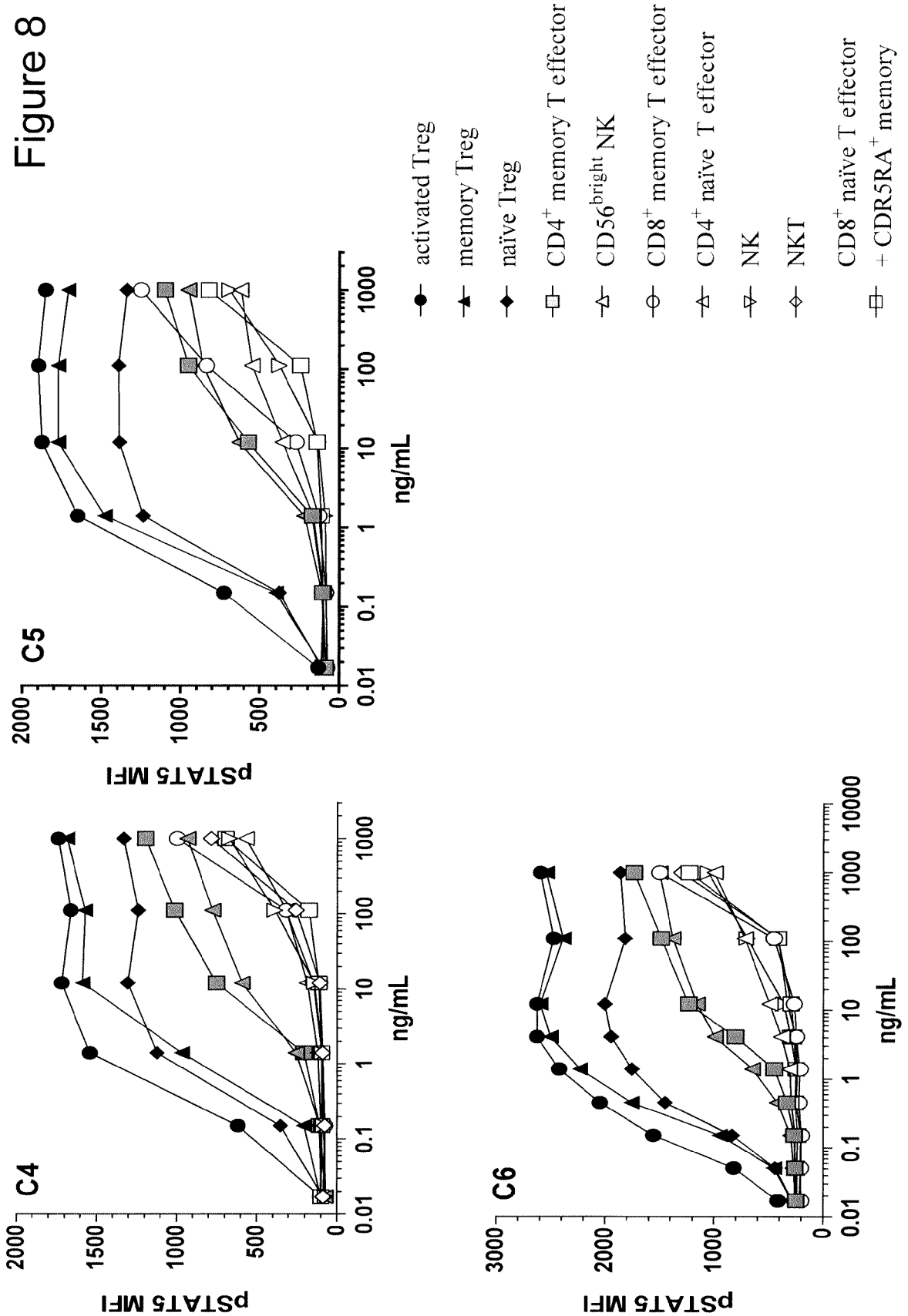

A
B
C
D
% of Max
CD25 (IL2RA)
CD122 (IL2RB)
CD4 mem
CD4 naïve
IC
CD8 mem
CD8 naïve
+ TEMRA
IC

Figure 7

Figure 8


C5
C4
C6
pSTAT5 MFI
ng/mL
0
500
1000
1500
2000
3000
0.01
0.1
1
10
100
1000
10000
activated Treg
memory Treg
naïve Treg
CD4+ memory T effector
CD56bright NK
CD8+ memory T effector
CD4+ naïve T effector
NK
NKT
CD8+ naïve T effector
+ CDR5RA+ memory

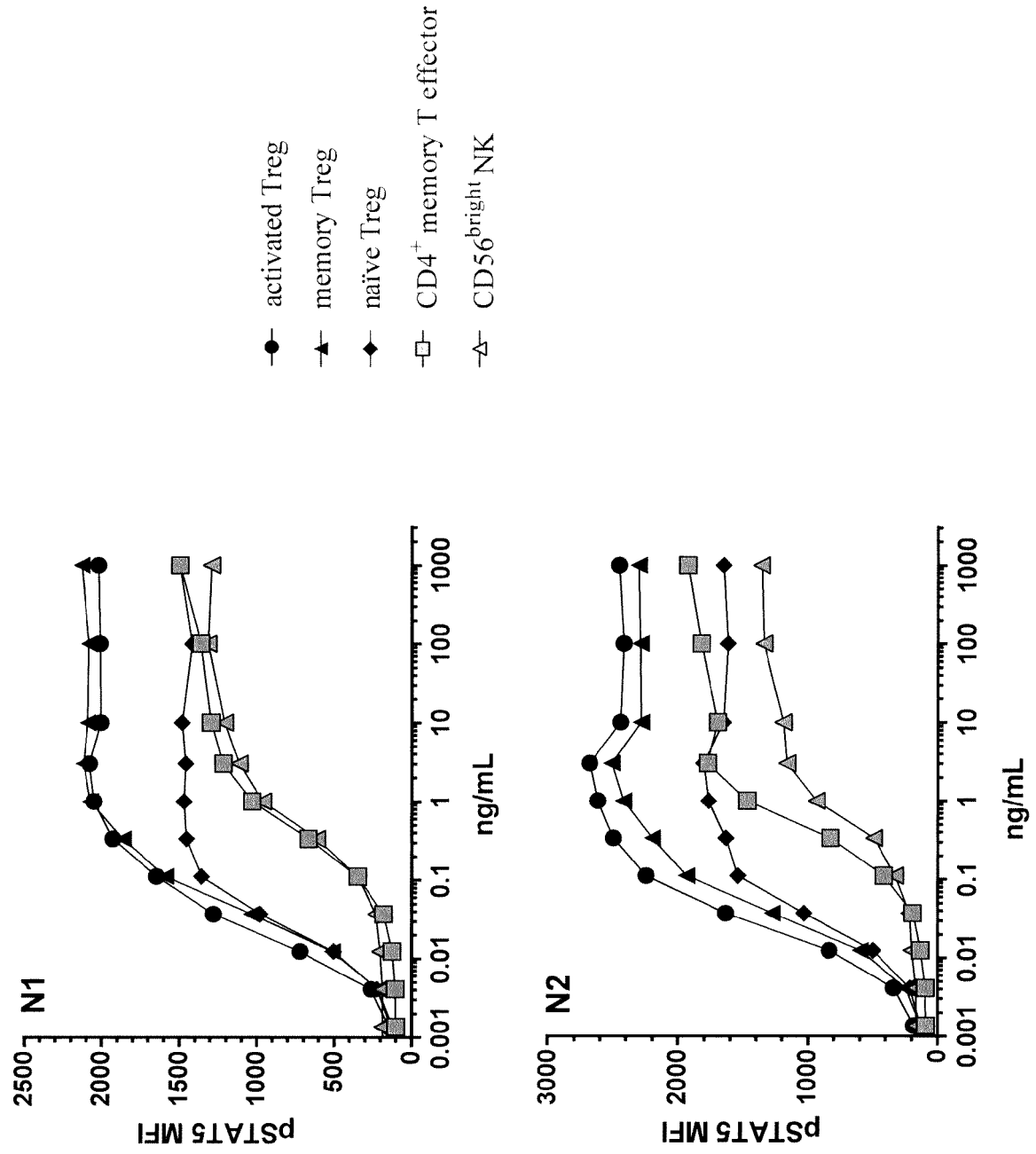

activated Treg
memory Treg
naïve Treg
CD4+ memory T effector
CD56bright NK
N1
N2
pSTAT5 MFI
ng/mL
0
500
1000
1500
2000
2500
3000
0.001
0.01
0.1
1
10
100
1000

Figure 9

Figure 9

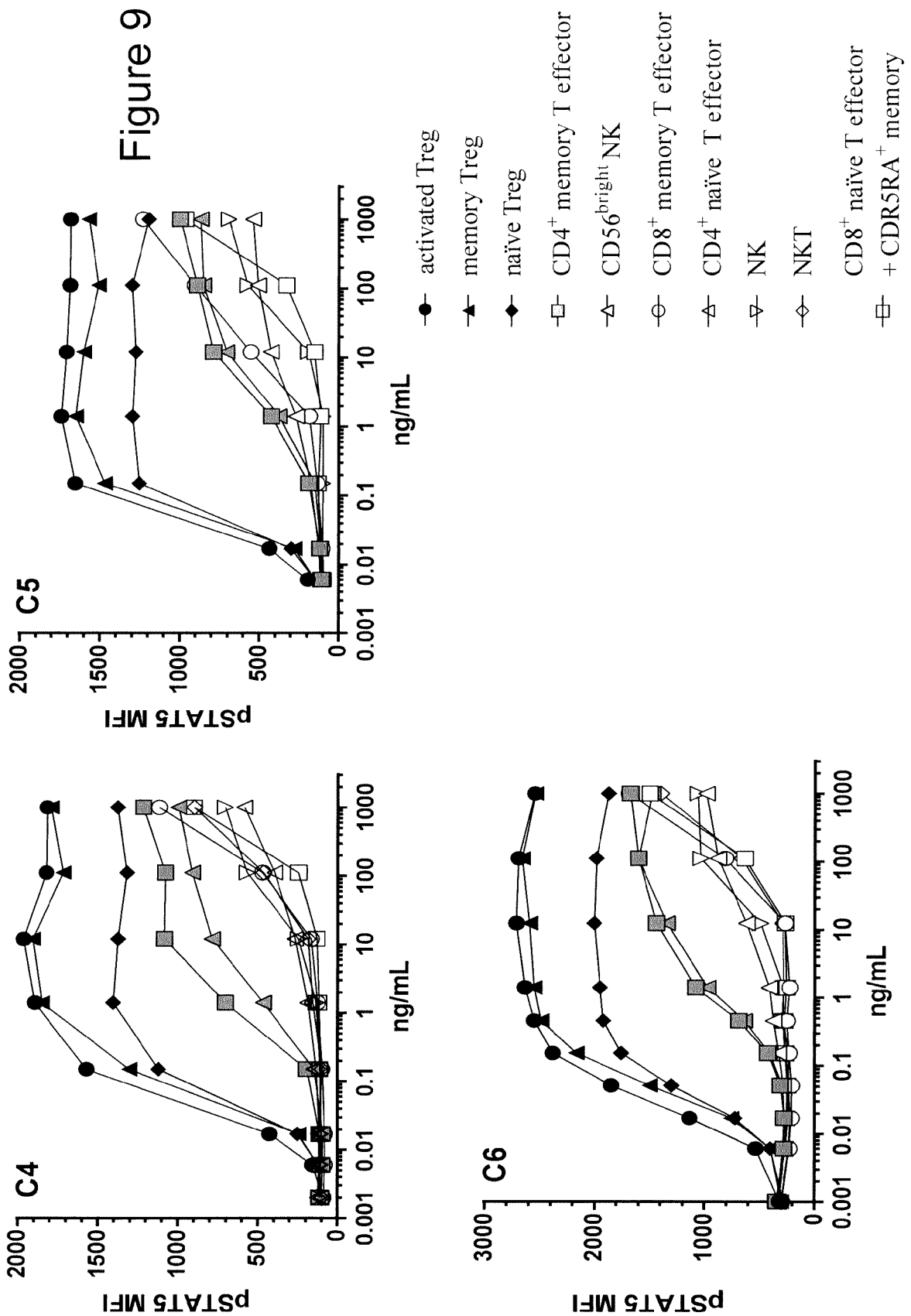

C5
C4
C6
pSTAT5 MFI
ng/mL
0
500
1000
1500
2000
3000
0.001
0.01
0.1
1
10
100
1000
activated Treg
memory Treg
naïve Treg
CD4+ memory T effector
CD56bright NK
CD8+ memory T effector
CD4+ naïve T effector
NK
NKT
CD8+ naïve T effector
+ CDR5RA+ memory

Figure 10

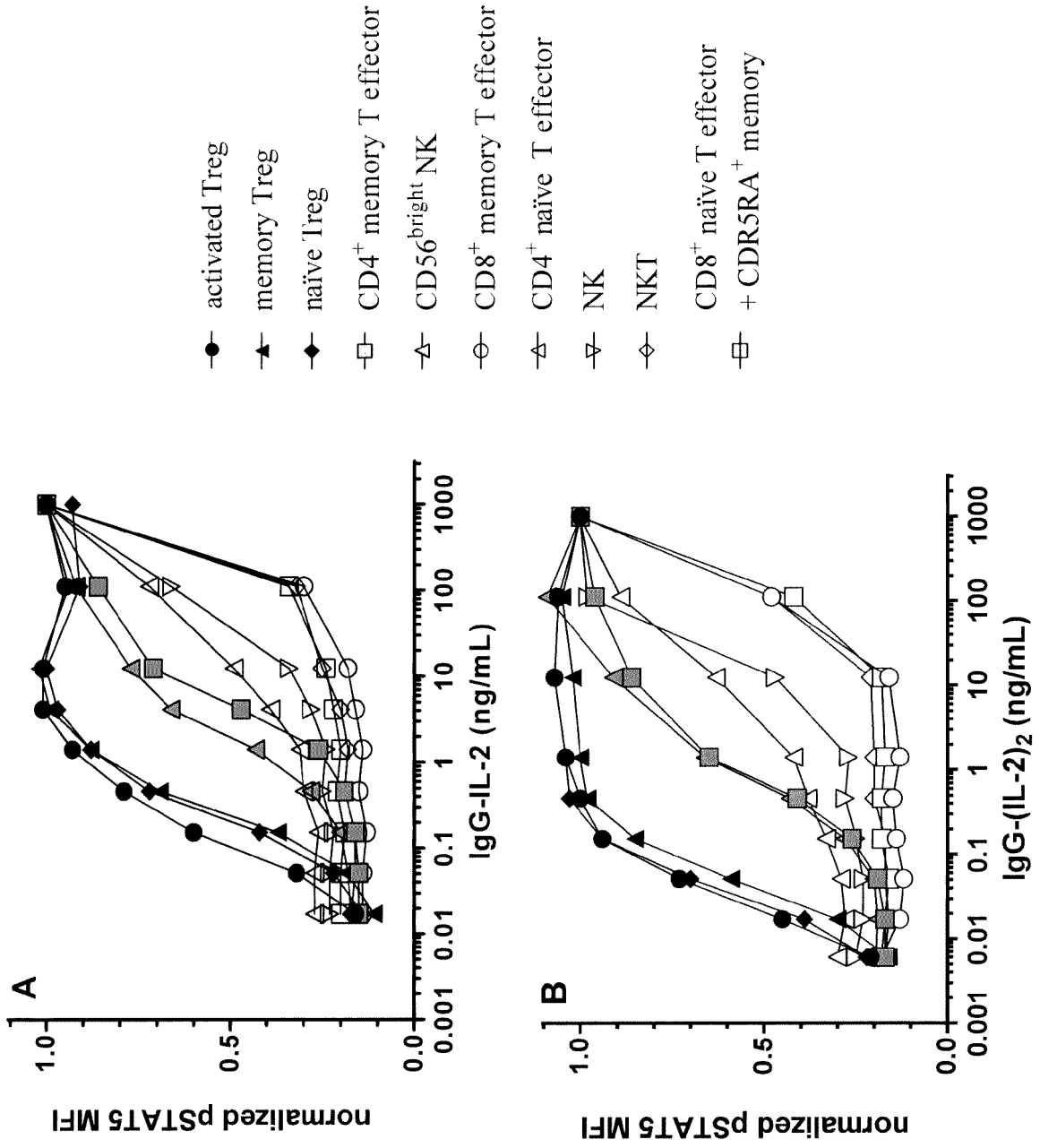

activated Treg
memory Treg
naïve Treg
CD4+ memory T effector
CD56bright NK
CD8+ memory T effector
CD4+ naïve T effector
NK
NKT
CD8+ naïve T effector
+ CDR5RA+ memory
A
B
normalized pSTAT5 MFI
IgG-IL-2 (ng/mL)
IgG-(IL-2)2 (ng/mL)
0.0
0.5
1.0
0.001
0.01
0.1
1
10
100
1000

Figure 11

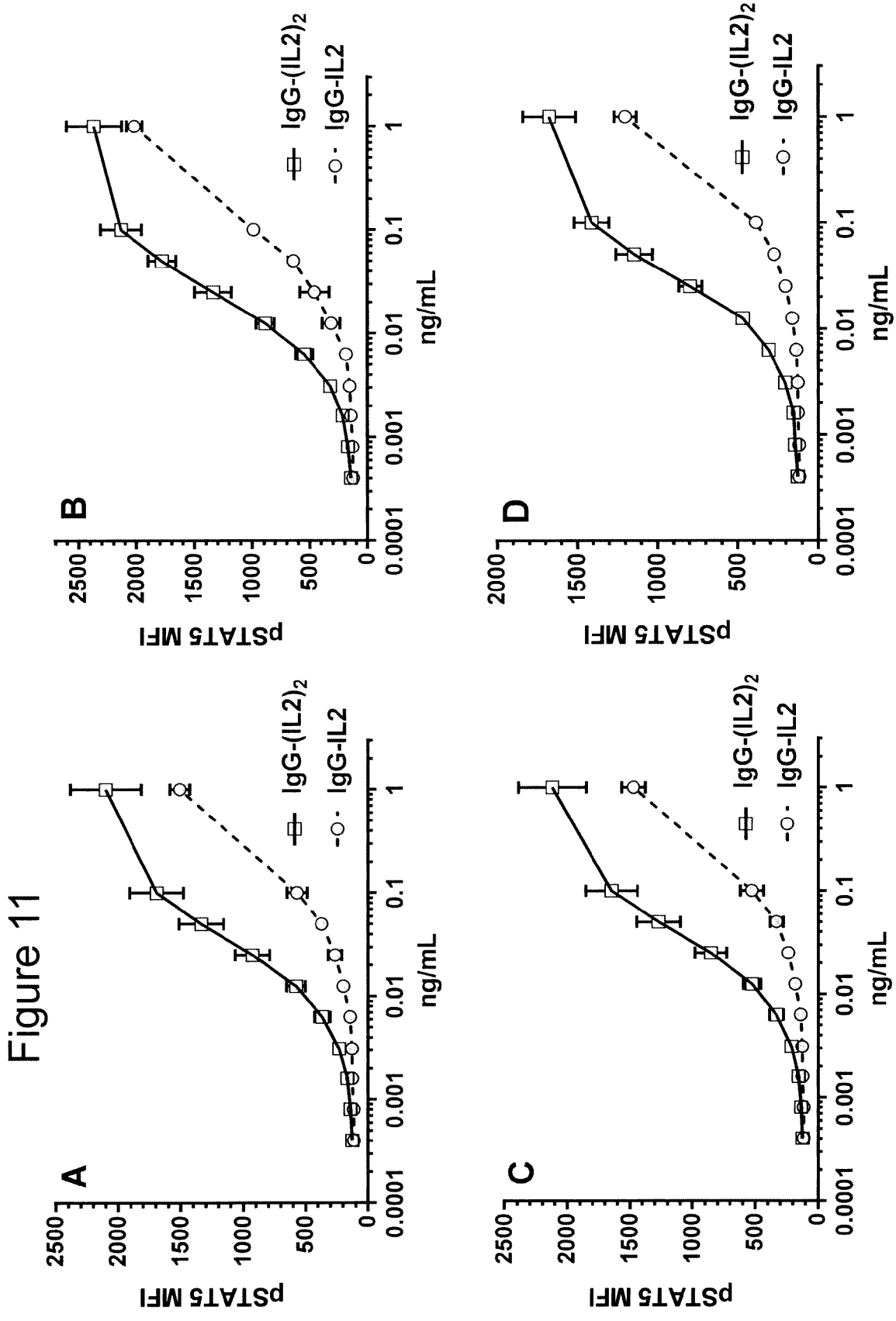
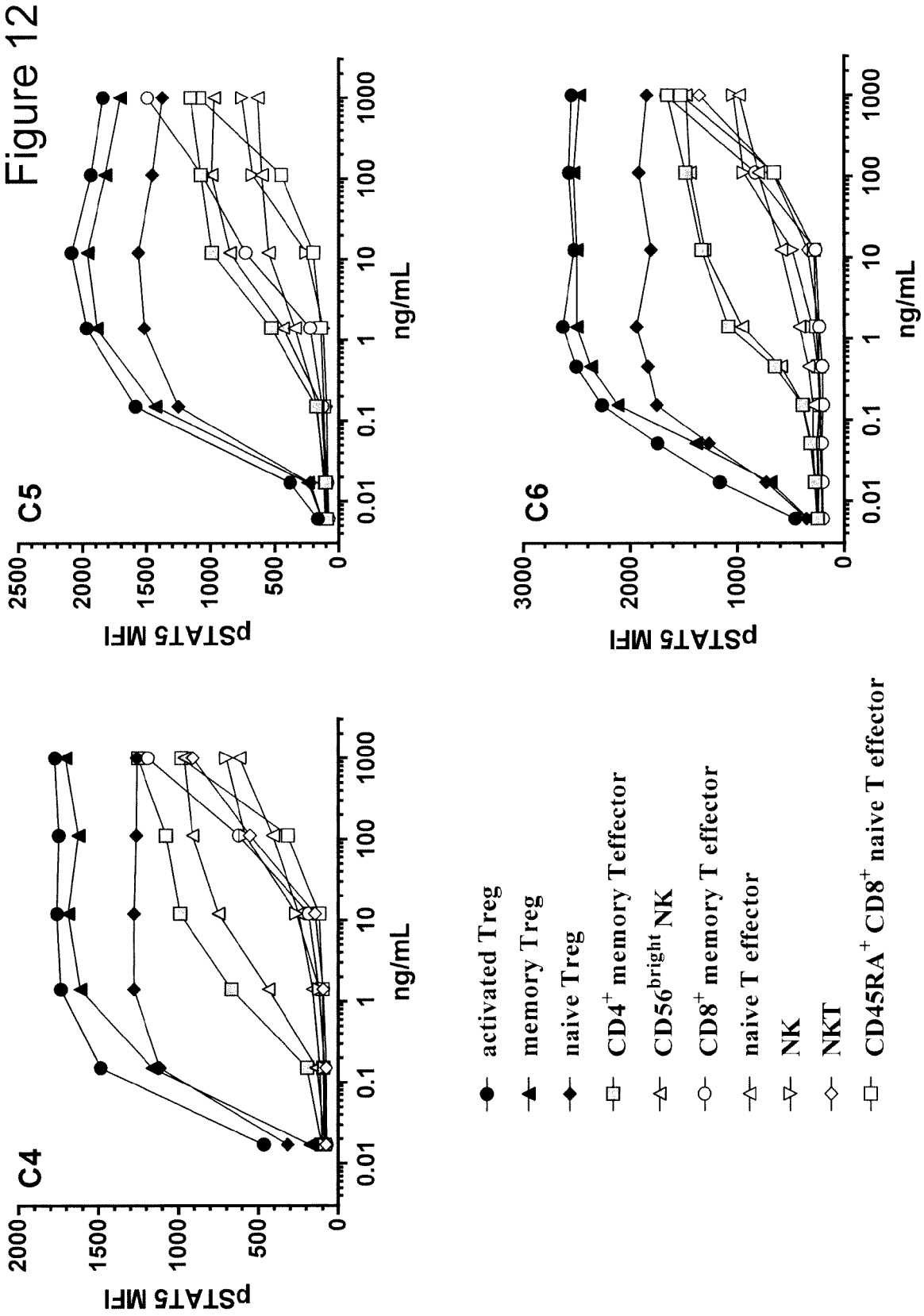

A
B
C
D
pSTAT5 MFI
ng/mL
IgG-(IL2)2
IgG-IL2
2500
2000
1500
1000
500
0
0.0001
0.001
0.01
0.1
1

Figure 12


C4
C5
C6
pSTAT5 MFI
ng/mL
0.01
0.1
1
10
100
1000
activated Treg
memory Treg
naive Treg
CD4+ memory Teffector
CD56bright NK
CD8+ memory T effector
naive T effector
NK
NKT
CD45RA+ CD8+ naive T effector

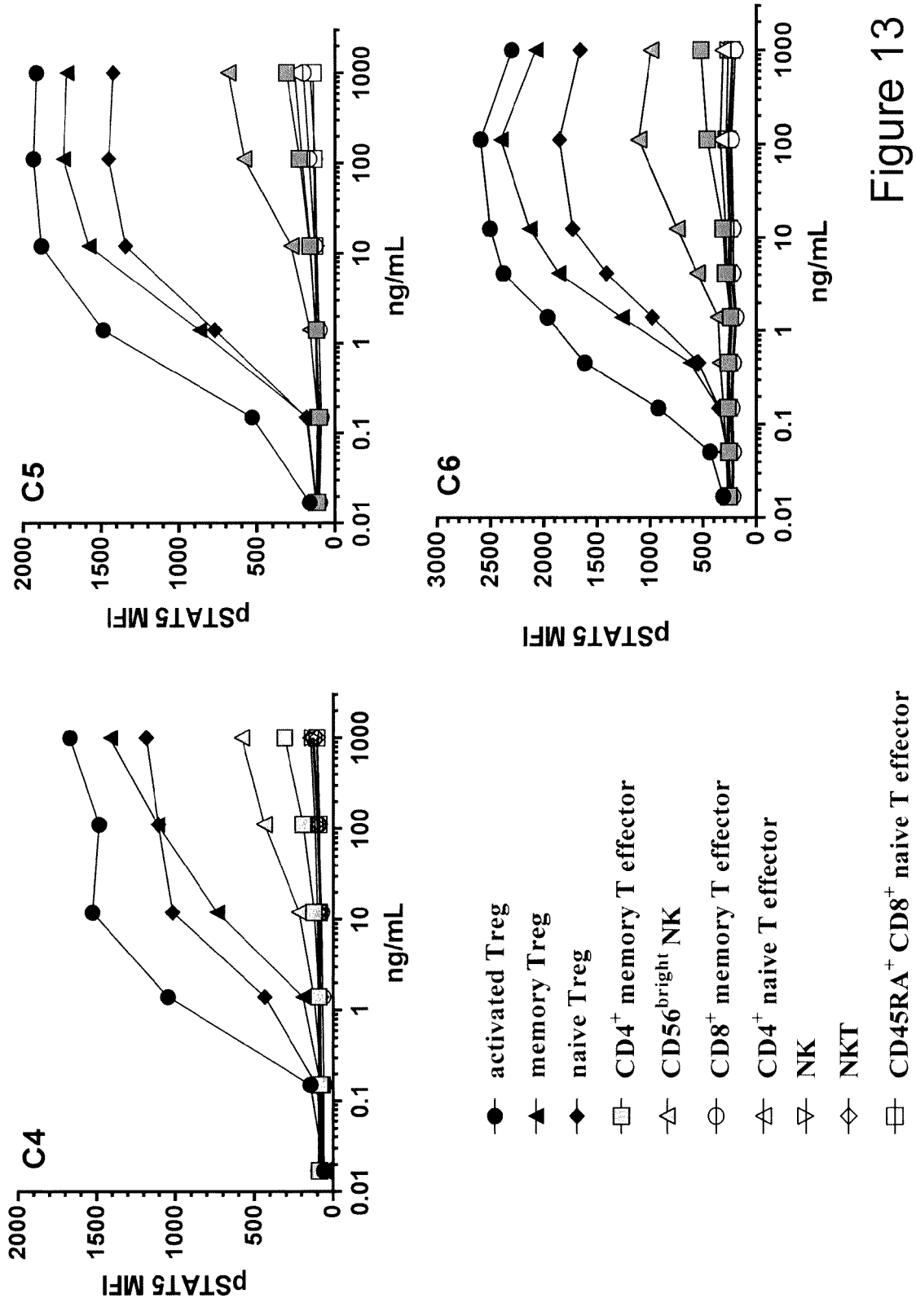

C4
C5
C6
pSTAT5 MFI
ng/mL
0
500
1000
1500
2000
2500
3000
0.01
0.1
1
10
100
1000
activated Treg
memory Treg
naive Treg
CD4+ memory T effector
CD56bright NK
CD8+ memory T effector
CD4+ naive T effector
NK
NKT
CD45RA+ CD8+ naive T effector

Figure 13

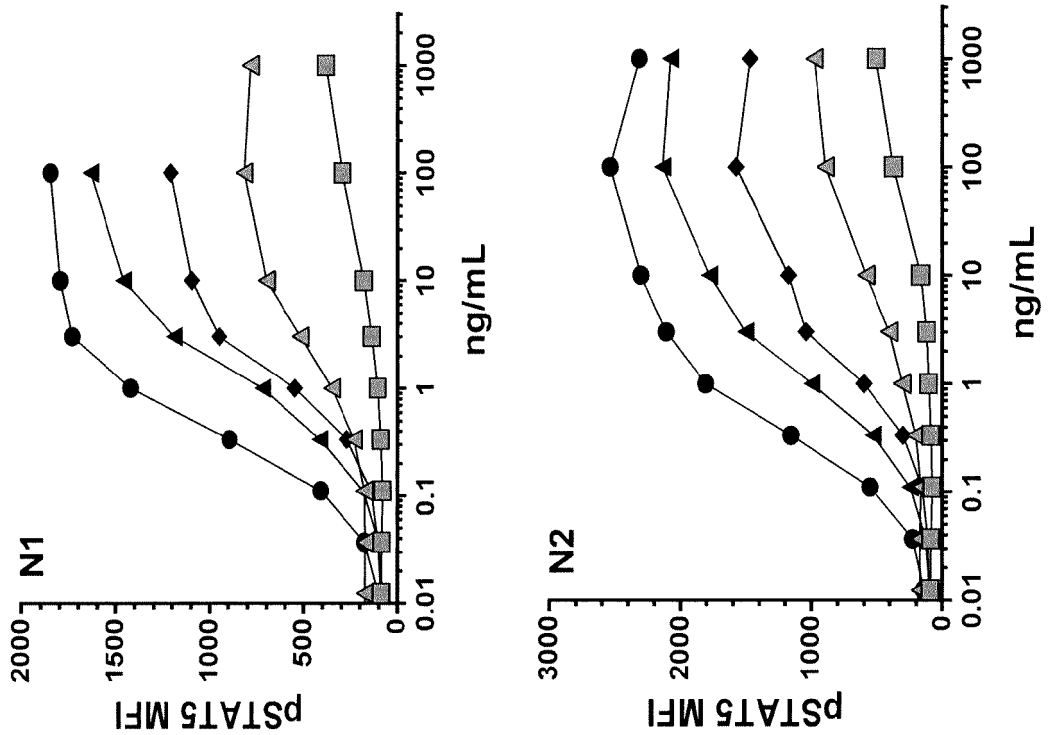

N1
pSTAT5 MFI
2000
1500
1000
500
0
0.01
0.1
1
10
100
1000
ng/mL
N2
pSTAT5 MFI
3000
2000
1000
0
0.01
0.1
1
10
100
1000
ng/mL
activated Treg
memory Treg
naive Treg
CD4+ memory T effector
CD56bright NK

Figure 13

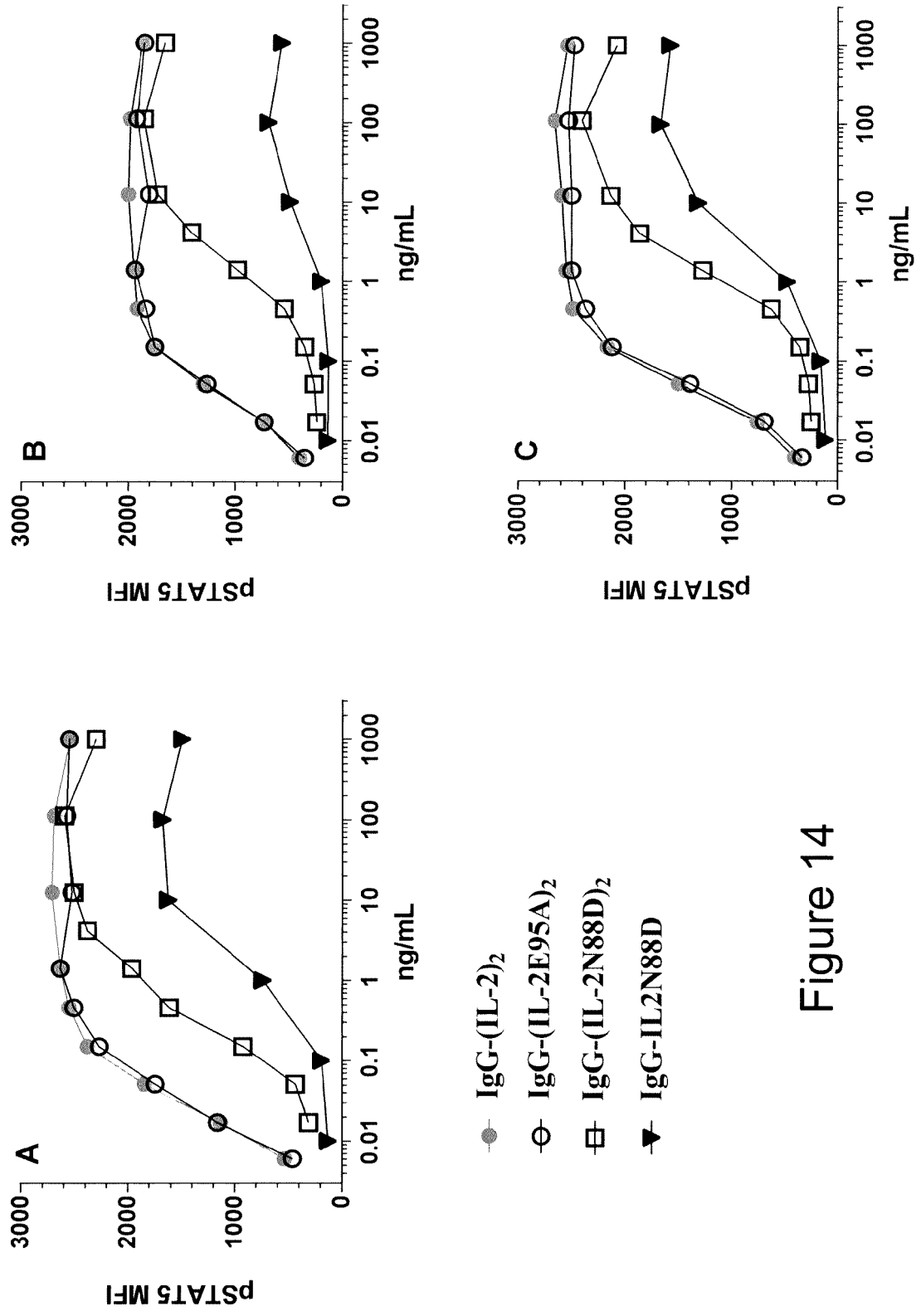

A
B
C
pSTAT5 MFI
ng/mL
0
1000
2000
3000
0.01
0.1
1
10
100
1000
IgG-(IL-2)2
IgG-(IL-2E95A)2
IgG-(IL-2N88D)2
IgG-IL2N88D

Figure 14

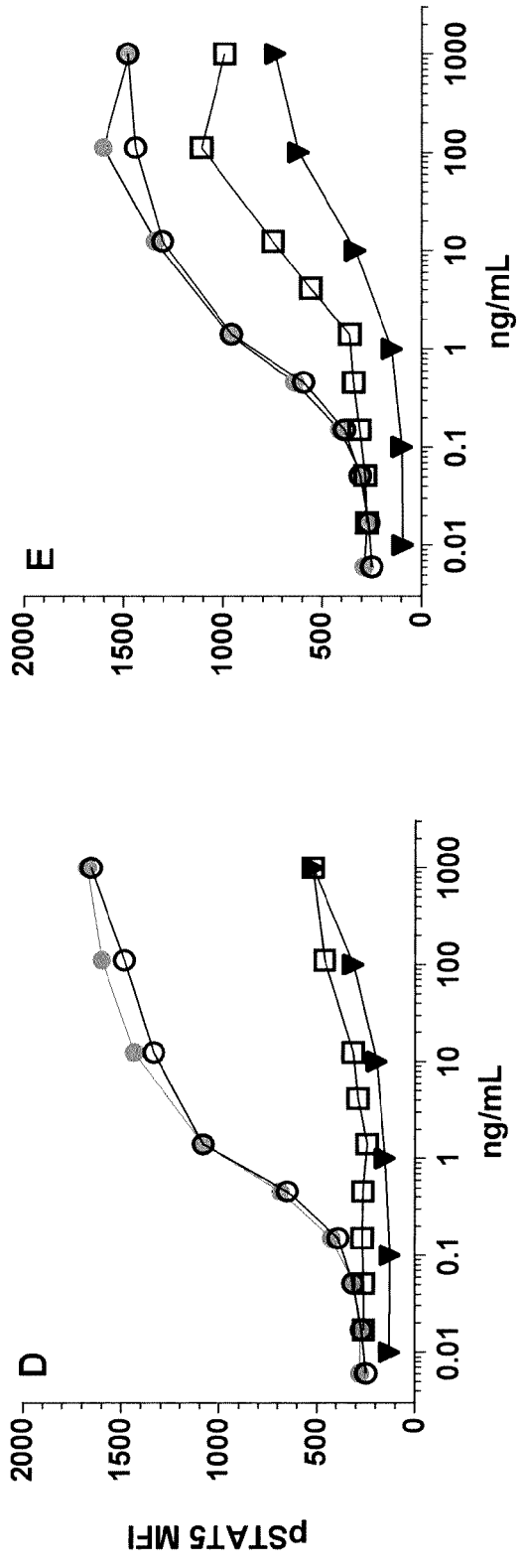

D
E
pSTAT5 MFI
2000
1500
1000
500
0
0.01
0.1
1
10
100
1000
ng/mL
IgG-(IL-2)2
IgG-(IL-2E95A)2
IgG-(IL-2N88D)2
IgG-IL2N88D

Figure 14

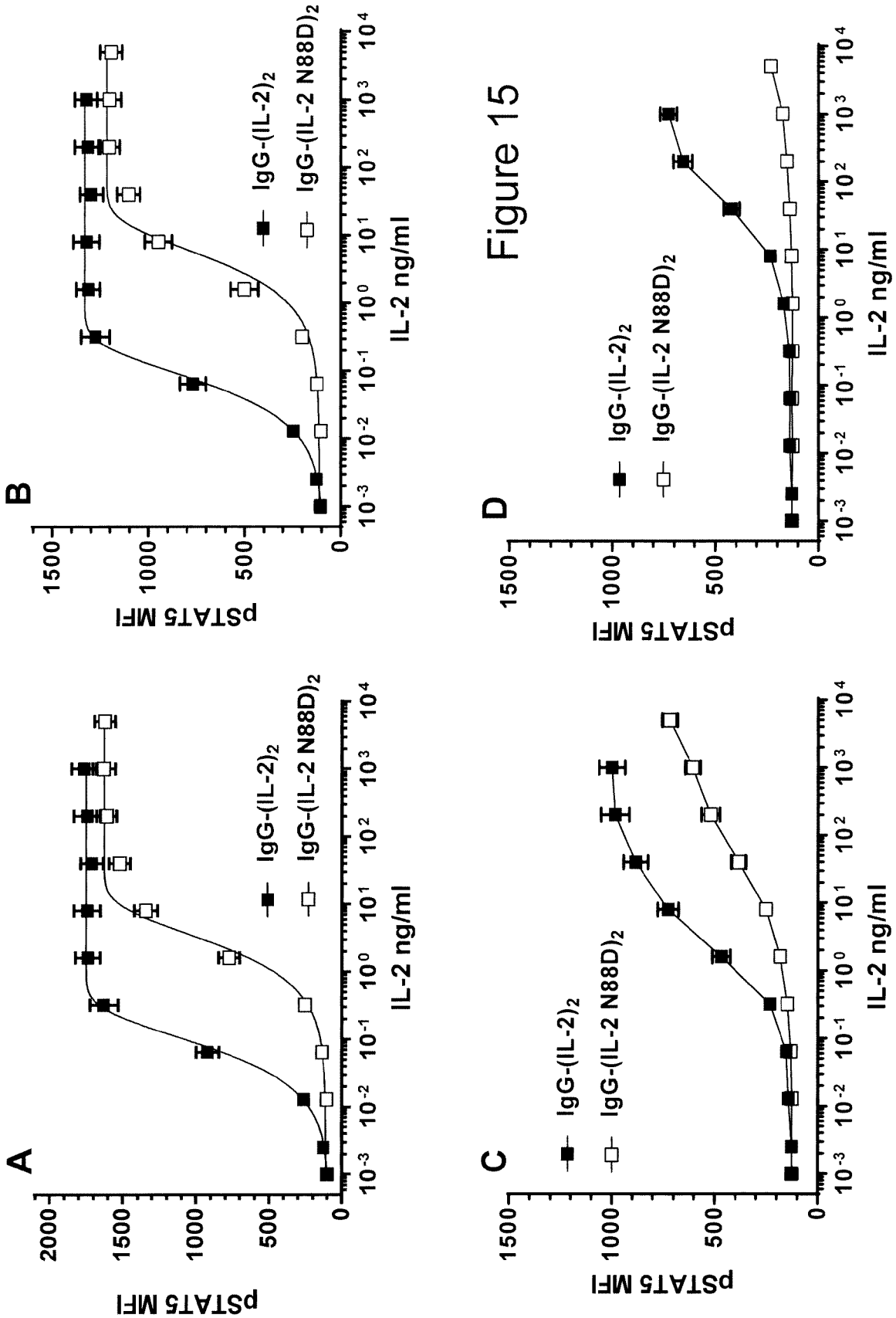


Figure 15

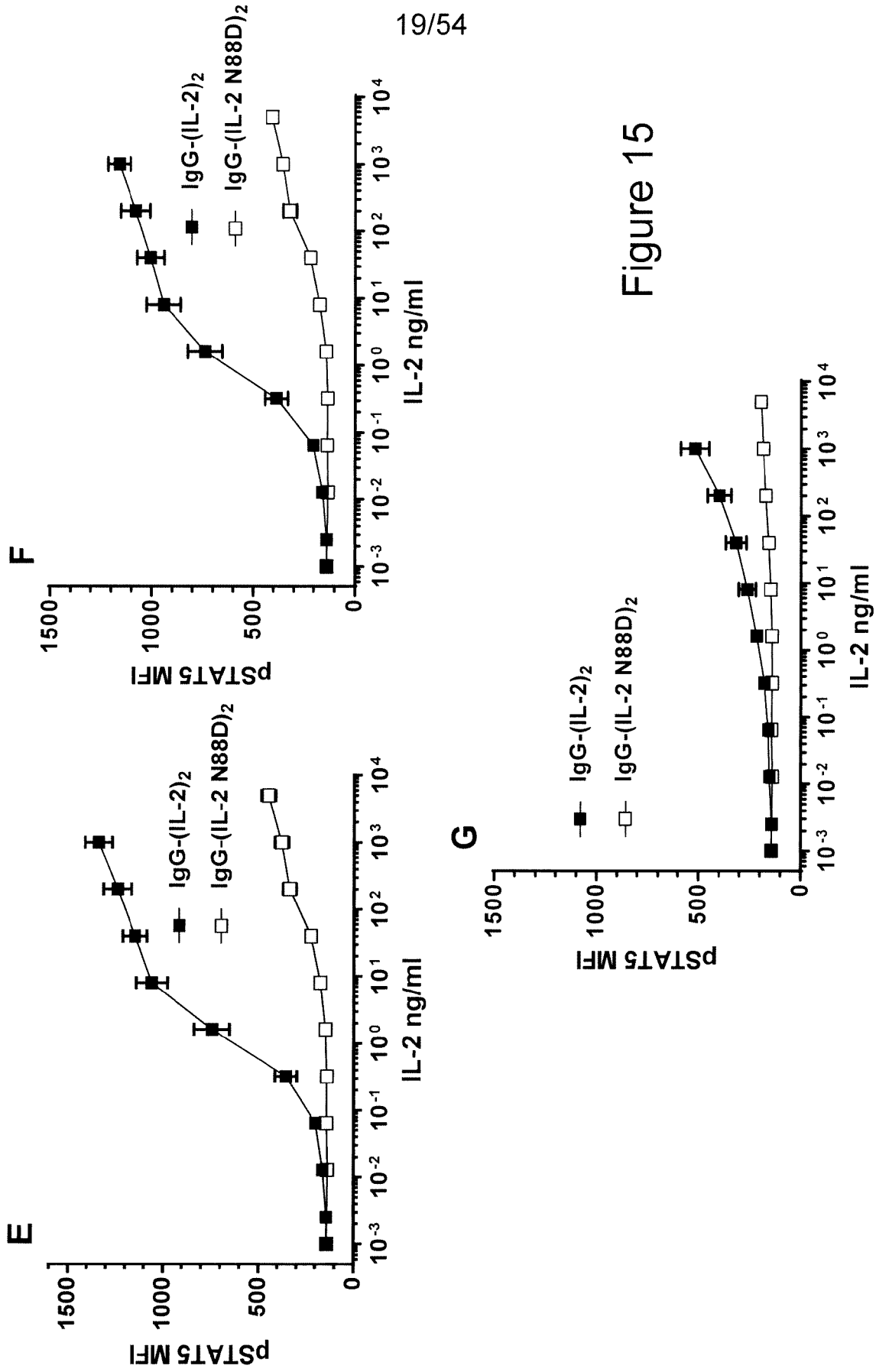
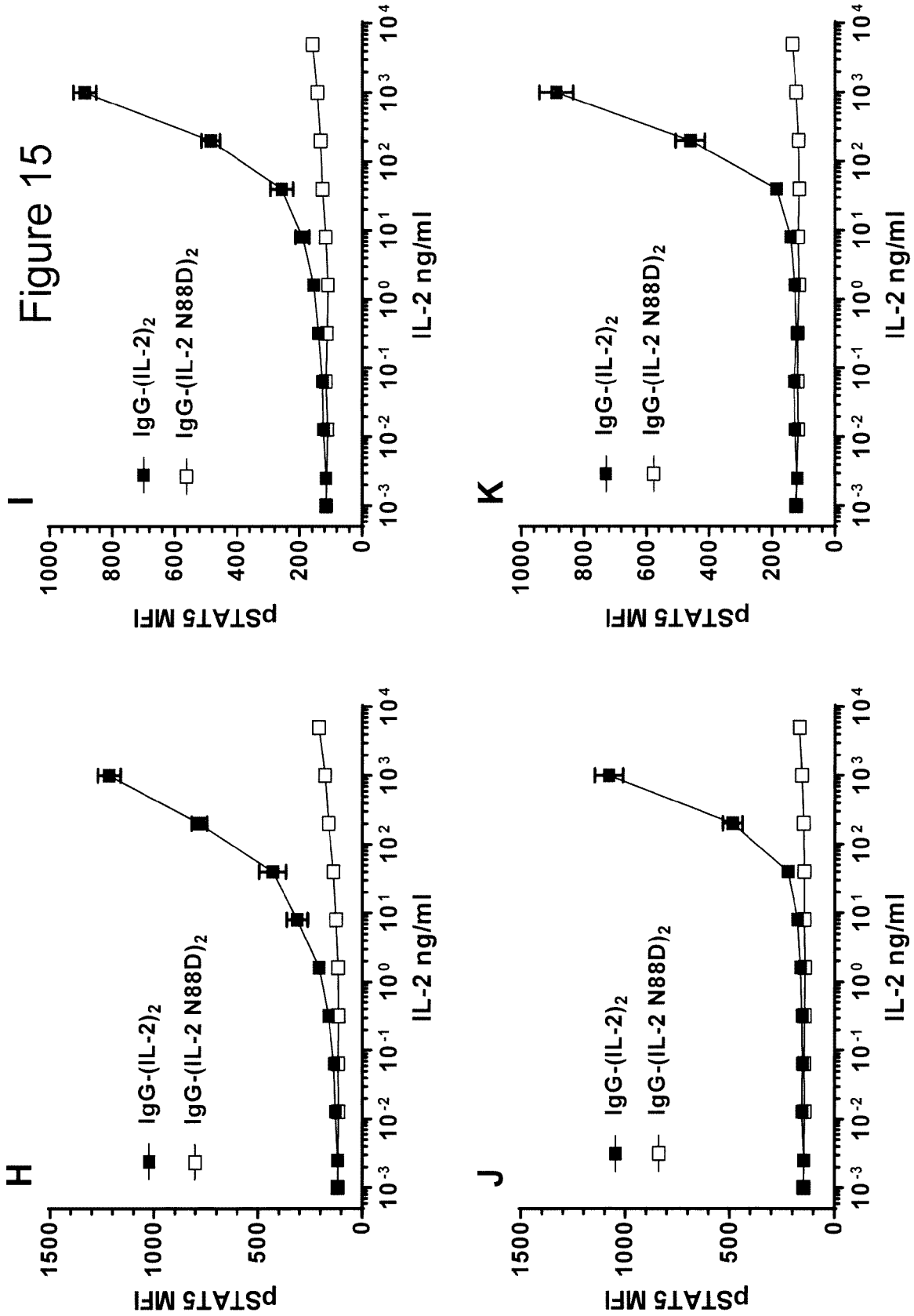

E
F
G
pSTAT5 MFI
IL-2 ng/ml
IgG-(IL-2)2
IgG-(IL-2 N88D)2
0
500
1000
1500
10-3
10-2
10-1
100
101
102
103
104

Figure 15

Figure 15



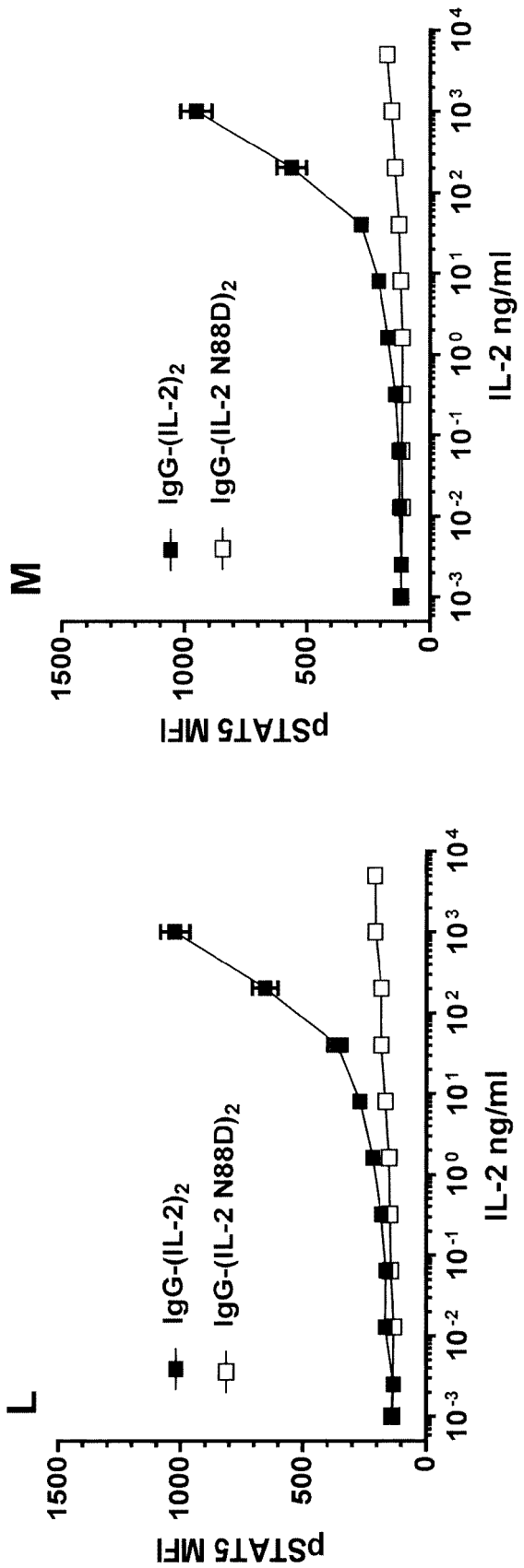

L
M
pSTAT5 MFI
1500
1000
500
0
IgG-(IL-2)2
IgG-(IL-2 N88D)2
10-3 10-2 10-1 100 101 102 103 104
IL-2 ng/ml

Figure 15

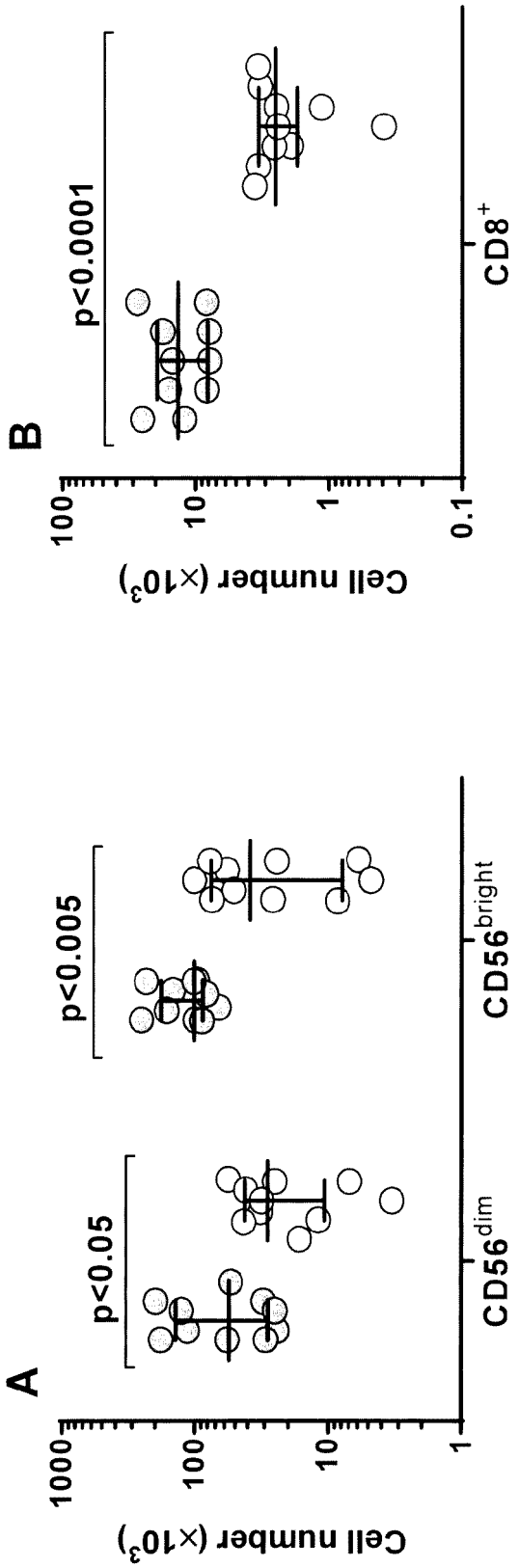

A
Cell number (×10³)
1000
100
10
1
p<0.05
p<0.005
CD56dim
CD56bright
B
Cell number (×10³)
100
10
1
0.1
p<0.0001
CD8+

Figure 16

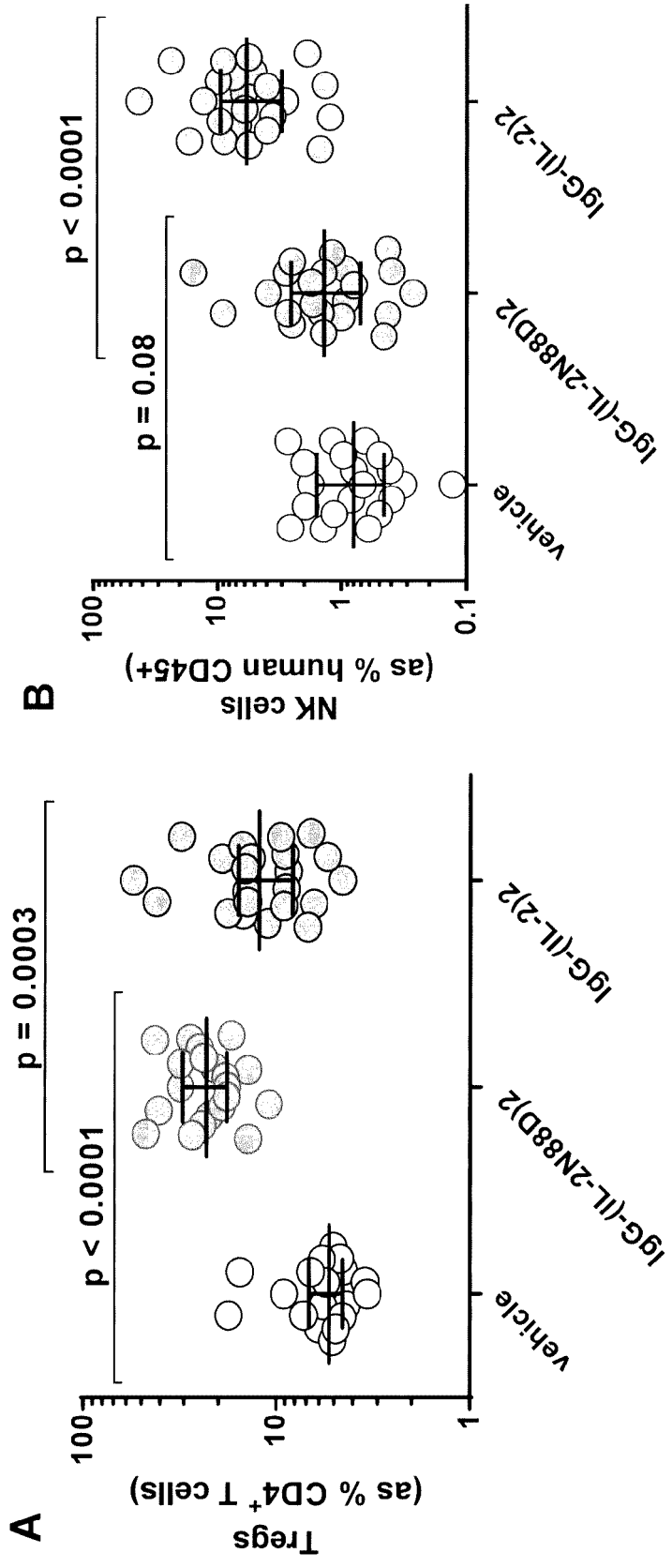

A
Tregs
(as % CD4+ T cells)
100
10
1
p < 0.0001
p = 0.0003
Vehicle
IgG-(IL-2)2
IgG-(IL-2N88D)2
B
NK cells
(as % human CD45+)
100
10
1
0.1
p = 0.08
p < 0.0001
Vehicle
IgG-(IL-2N88D)2
IgG-(IL-2)2

Figure 17

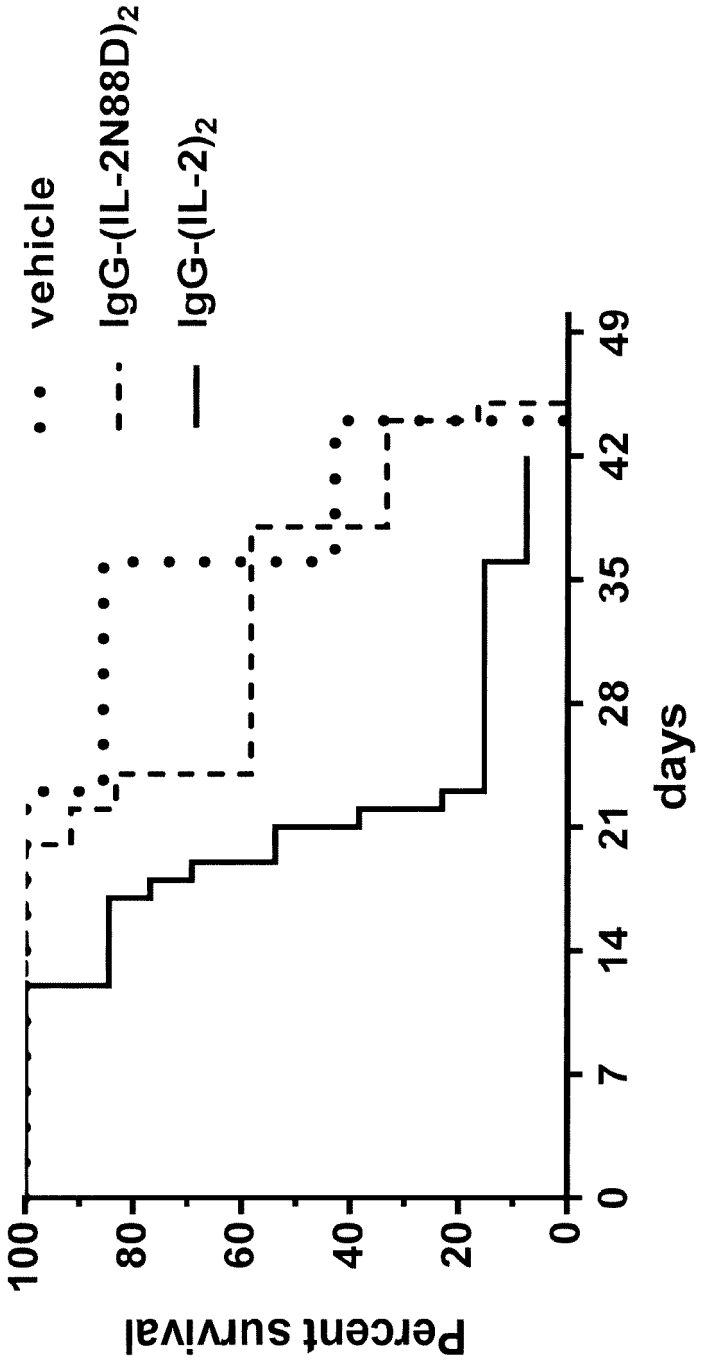

vehicle
IgG-(IL-2N88D)2
IgG-(IL-2)2
Percent survival
100
80
60
40
20
0
0
7
14
21
28
35
42
49
days

Figure 18

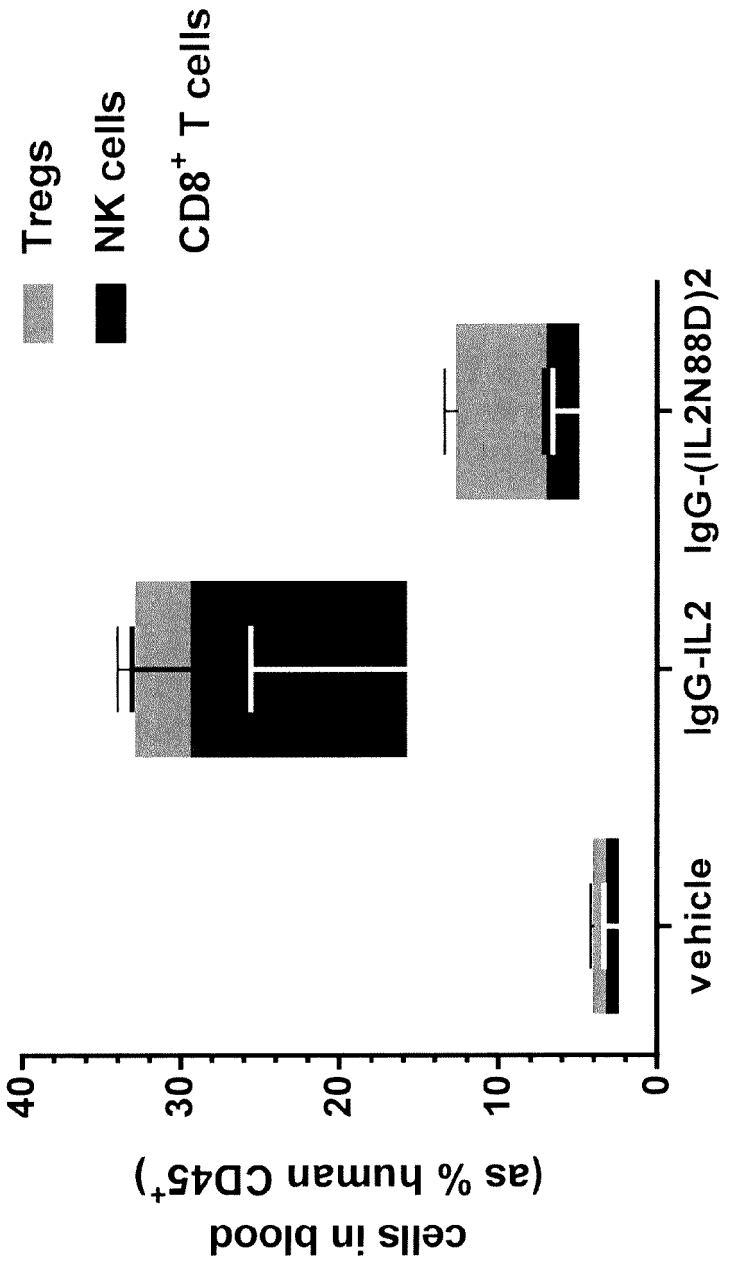

Tregs
NK cells
CD8+ T cells
cells in blood
(as % human CD45+)
40
30
20
10
0
vehicle
IgG-IL2
IgG-(IL2N88D)2

Figure 19

Figure 20

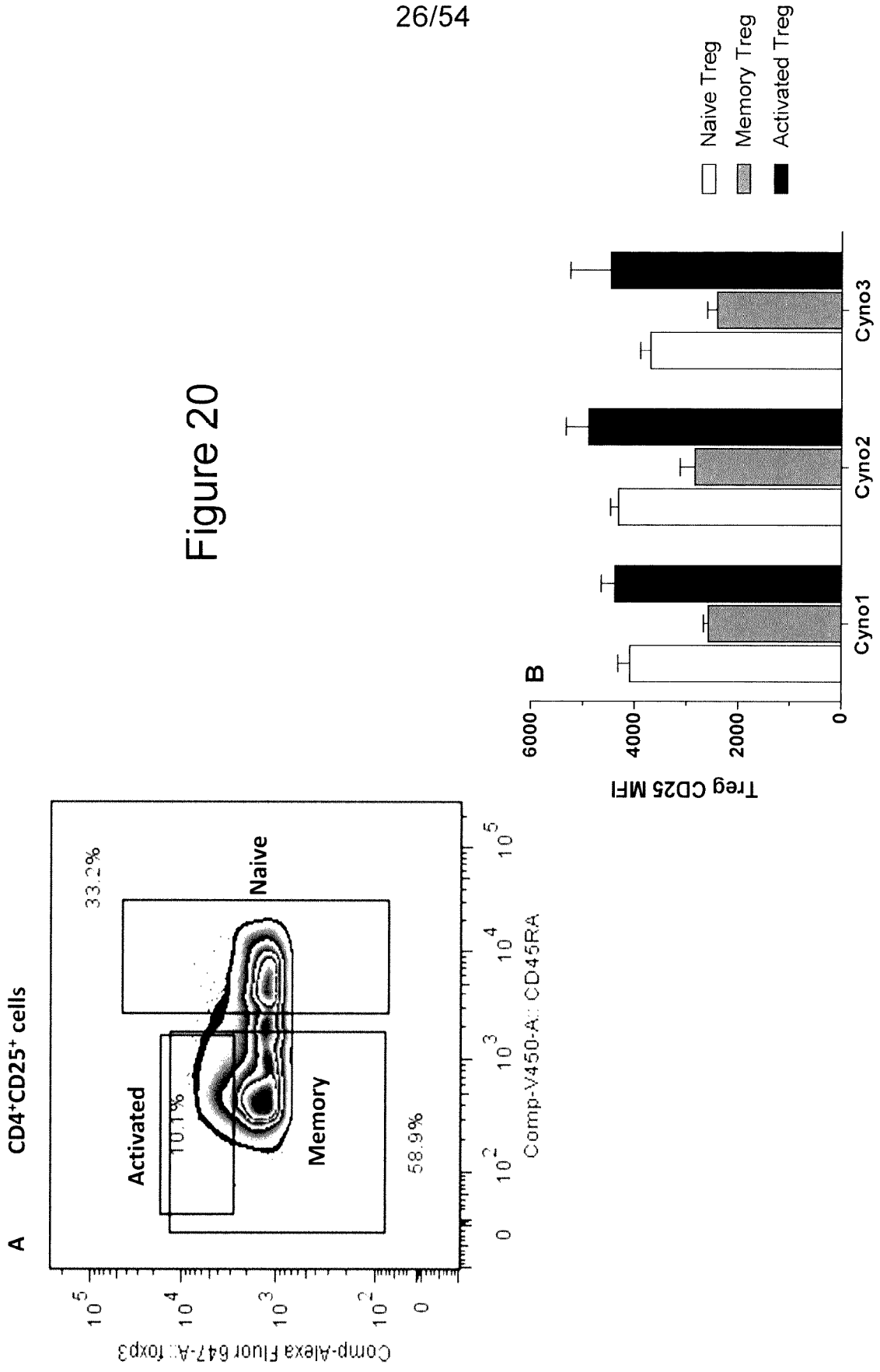
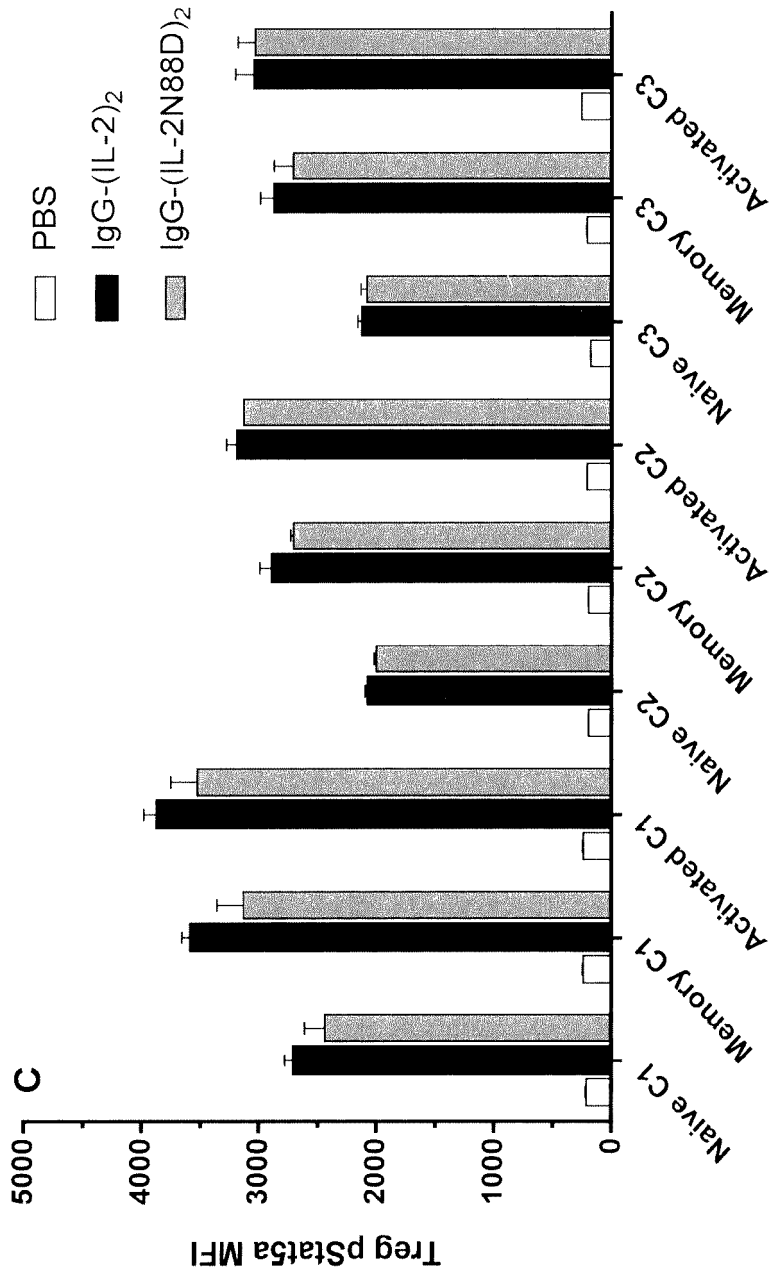

A
CD4+CD25+ cells
Activated
Naive
Memory
33.2%
10.1%
58.9%
Comp-Alexa Fluor 647-A:: foxp3
Comp-V450-A:: CD45RA
B
Treg CD25 MFI
Naive Treg
Memory Treg
Activated Treg
Cyno1
Cyno2
Cyno3

Figure 20


C
Treg pStat5a MFI
5000
4000
3000
2000
1000
0
PBS
IgG-(IL-2)2
IgG-(IL-2N88D)2
Naive C1
Memory C1
Activated C1
Naive C2
Memory C2
Activated C2
Naive C3
Memory C3
Activated C3

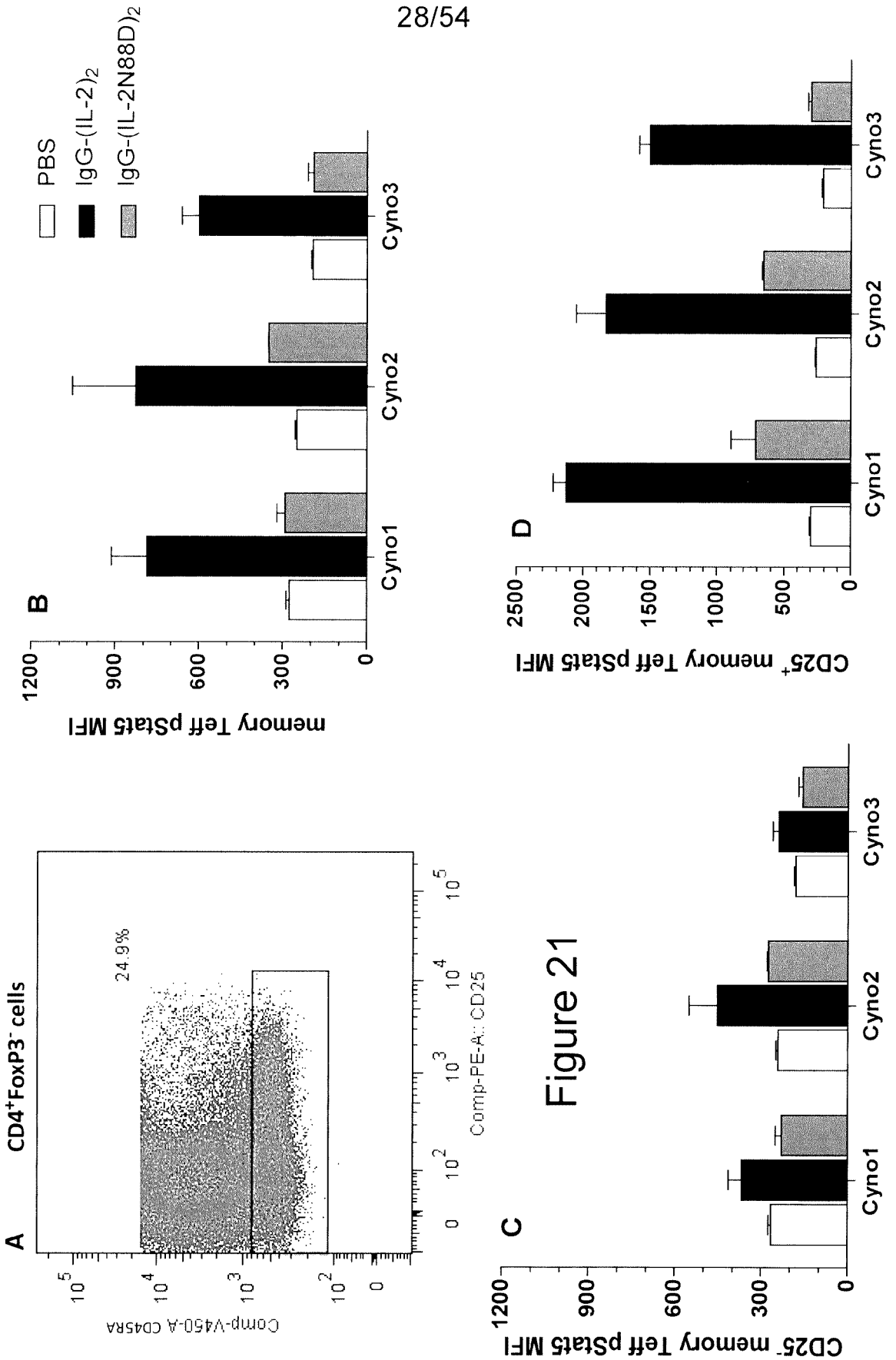

A
CD4+FoxP3- cells
24.9%
Comp-V450-A CD45RA
Comp-PE-A:: CD25
B
memory Teff pStat5 MFI
PBS
IgG-(IL-2)2
IgG-(IL-2N88D)2
Cyno1
Cyno2
Cyno3
C
CD25- memory Teff pStat5 MFI
D
CD25+ memory Teff pStat5 MFI

Figure 21

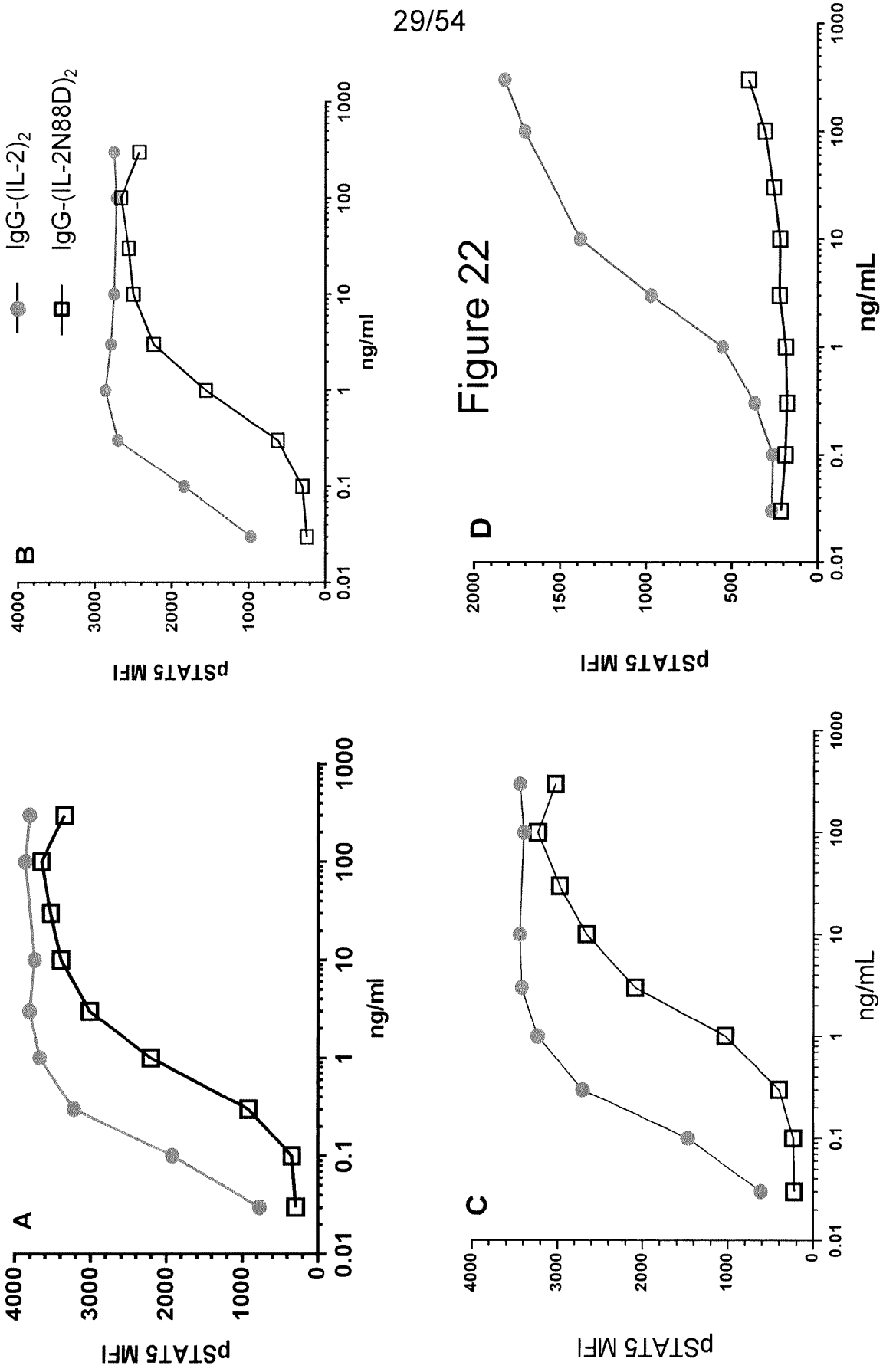

A
B
C
D
pSTAT5 MFI
ng/ml
ng/mL
IgG-(IL-2)2
IgG-(IL-2N88D)2
0
0.01
0.1
1
10
100
1000
500
1000
1500
2000
3000
4000

Figure 22

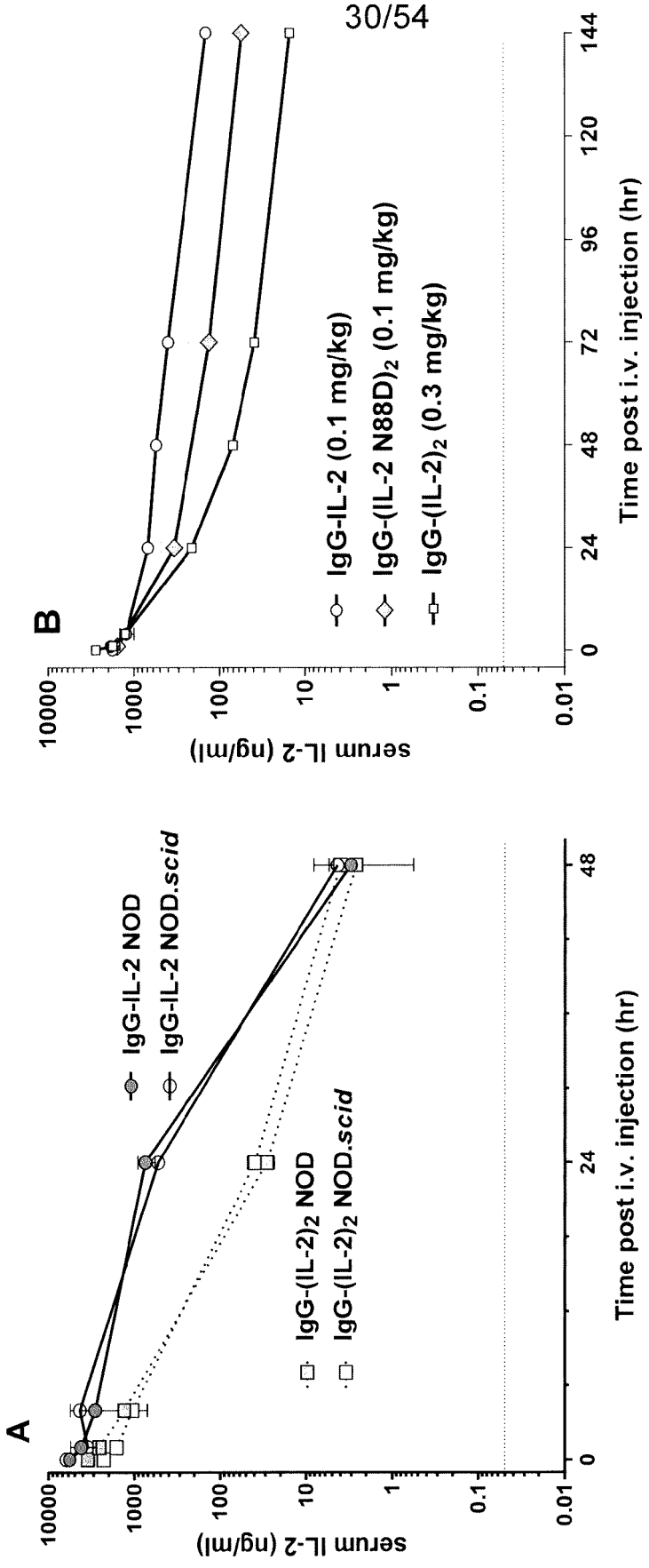

A
B
serum IL-2 (ng/ml)
Time post i.v. injection (hr)
IgG-IL-2 NOD
IgG-IL-2 NOD.scid
IgG-(IL-2)2 NOD
IgG-(IL-2)2 NOD.scid
IgG-IL-2 (0.1 mg/kg)
IgG-(IL-2 N88D)2 (0.1 mg/kg)
IgG-(IL-2)2 (0.3 mg/kg)

Figure 23

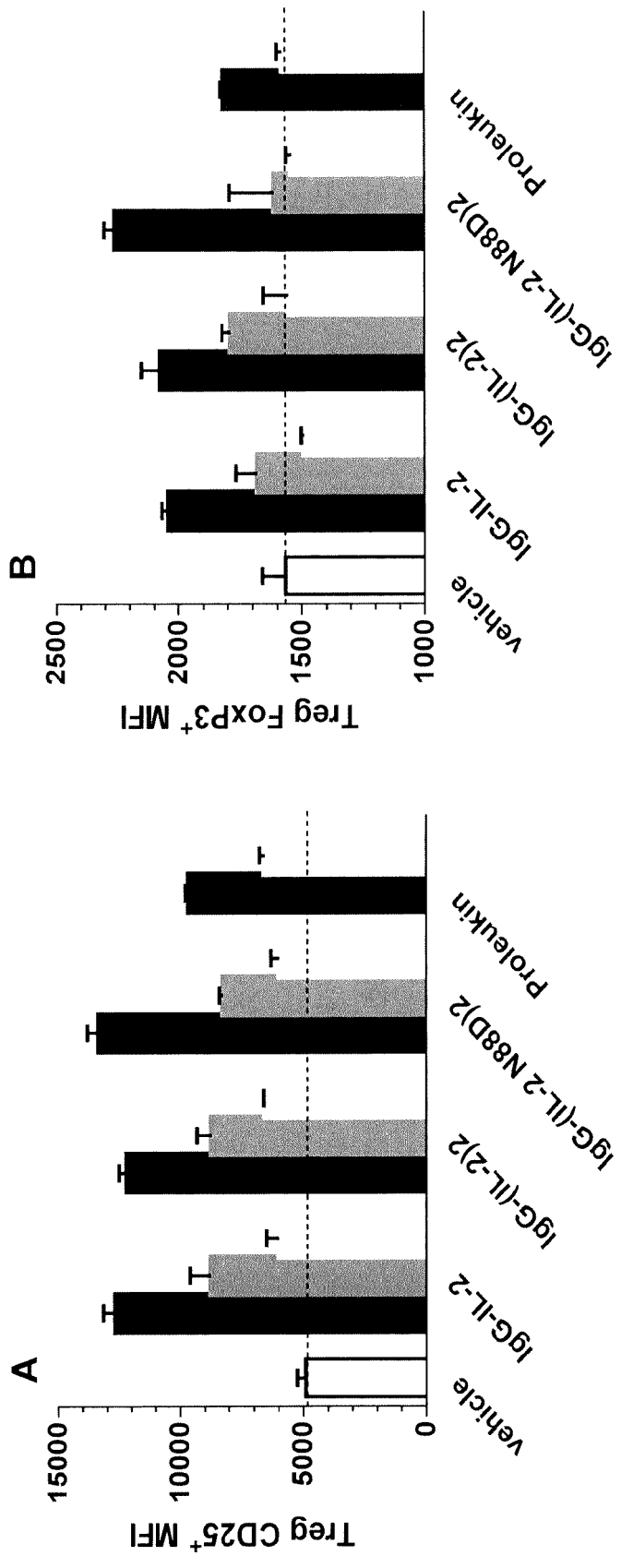

A
Treg CD25+ MFI
15000
10000
5000
0
Vehicle
IgG-IL-2
IgG-(IL-2)2
IgG-(IL-2 N88D)2
Proleukin
B
Treg FoxP3+ MFI
2500
2000
1500
1000
Vehicle
IgG-IL-2
IgG-(IL-2)2
IgG-(IL-2 N88D)2
Proleukin

Figure 24

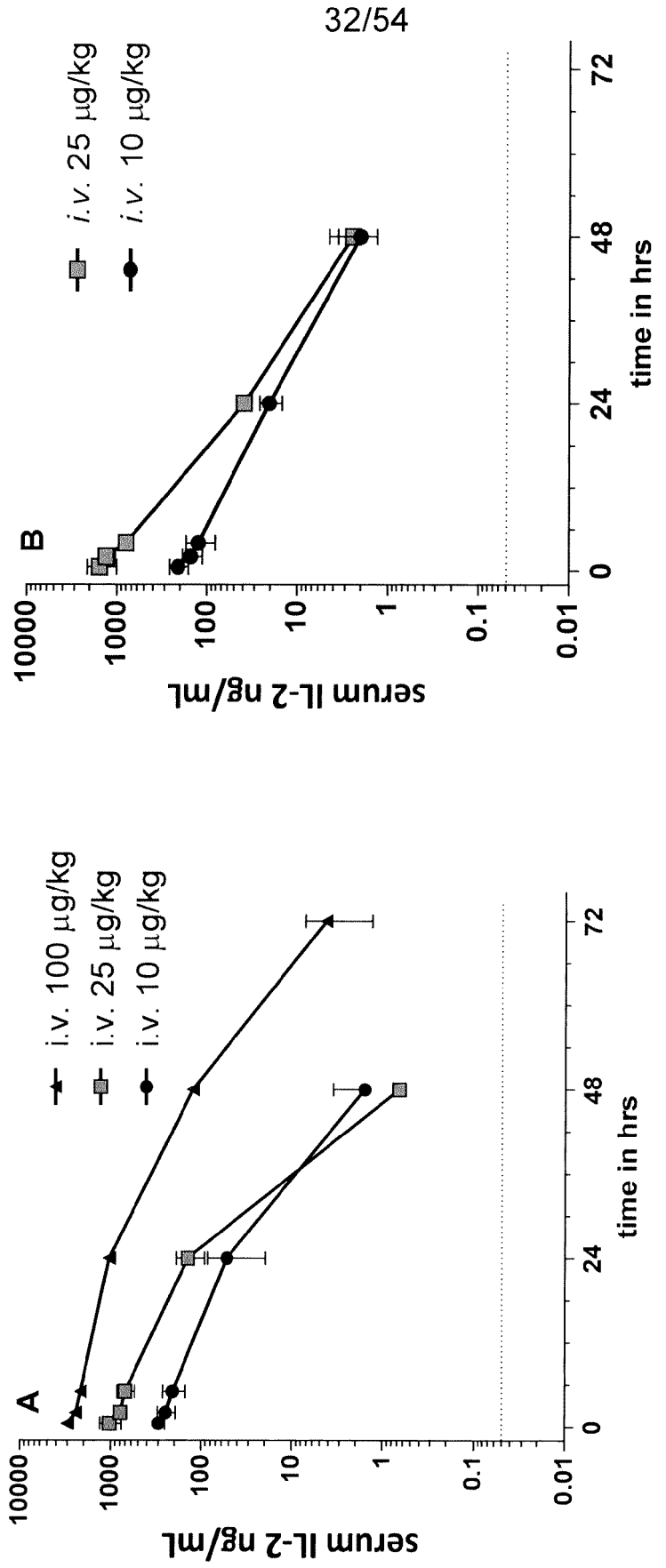

A
i.v. 100 µg/kg
i.v. 25 µg/kg
i.v. 10 µg/kg
serum IL-2 ng/mL
10000
1000
100
10
1
0.1
0.01
0
24
48
72
time in hrs
B
i.v. 25 µg/kg
i.v. 10 µg/kg
serum IL-2 ng/mL
10000
1000
100
10
1
0.1
0.01
0
24
48
72
time in hrs

Figure 25

Figure 26

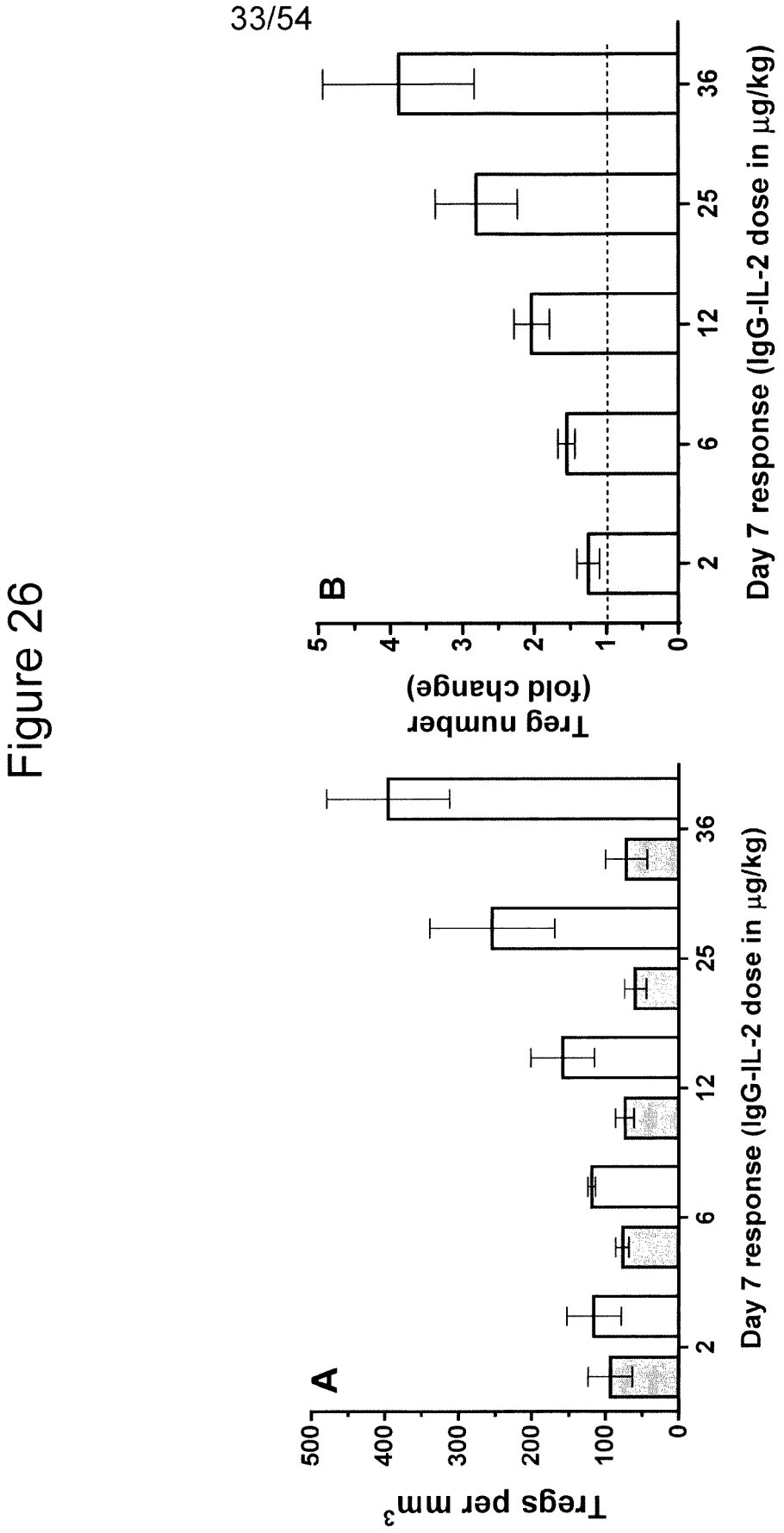
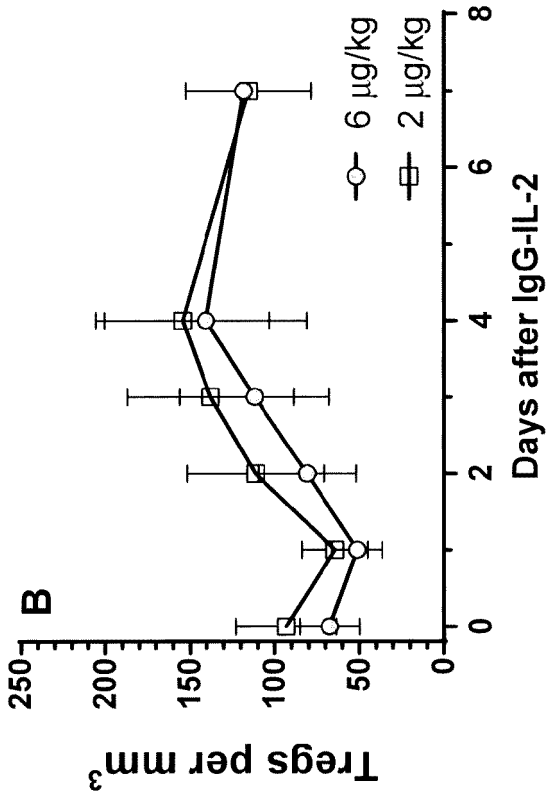
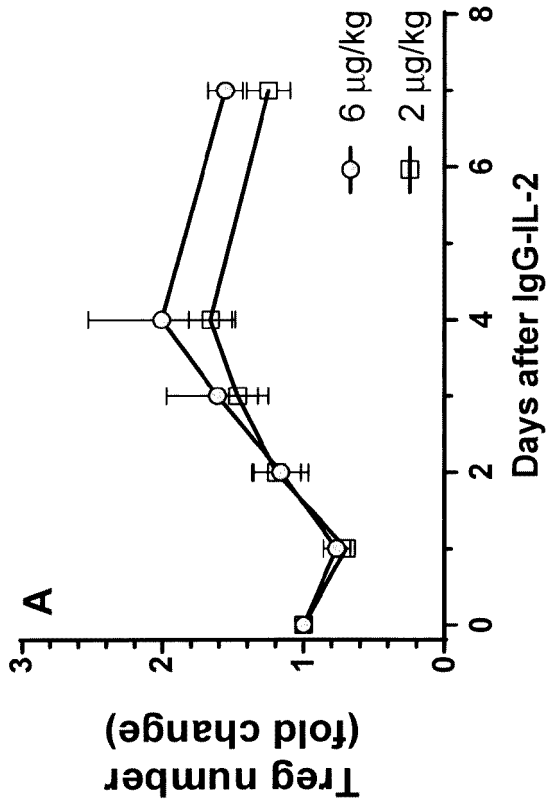

A
Tregs per mm³
500
400
300
200
100
0
2
6
12
25
36
Day 7 response (IgG-IL-2 dose in µg/kg)
B
Treg number
(fold change)
5
4
3
2
1
0
2
6
12
25
36
Day 7 response (IgG-IL-2 dose in µg/kg)

Figure 27


B
Tregs per mm³
250
200
150
100
50
0
0
2
4
6
8
Days after IgG-IL-2
6 µg/kg
2 µg/kg


A
Treg number
(fold change)
3
2
1
0
0
2
4
6
8
Days after IgG-IL-2
6 µg/kg
2 µg/kg

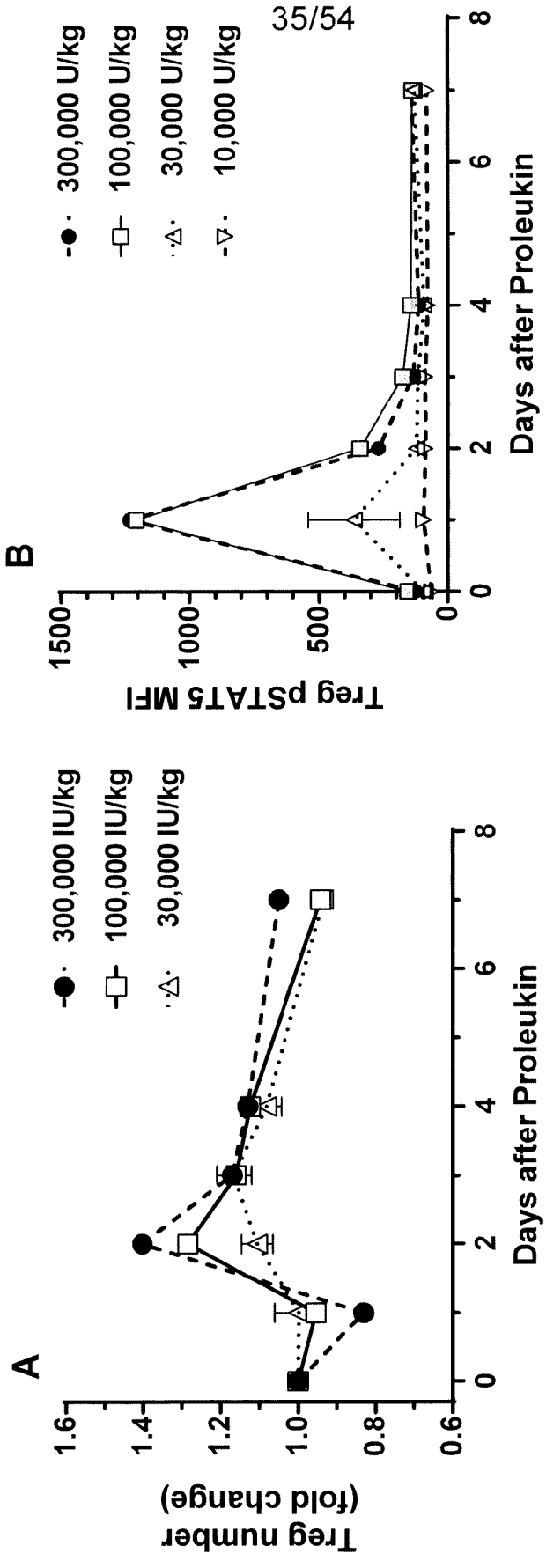

A
Treg number (fold change)
1.6
1.4
1.2
1.0
0.8
0.6
0
2
4
6
8
Days after Proleukin
300,000 IU/kg
100,000 IU/kg
30,000 IU/kg
B
Treg pSTAT5 MFI
1500
1000
500
0
0
2
4
6
8
Days after Proleukin
300,000 U/kg
100,000 U/kg
30,000 U/kg
10,000 U/kg

Figure 28

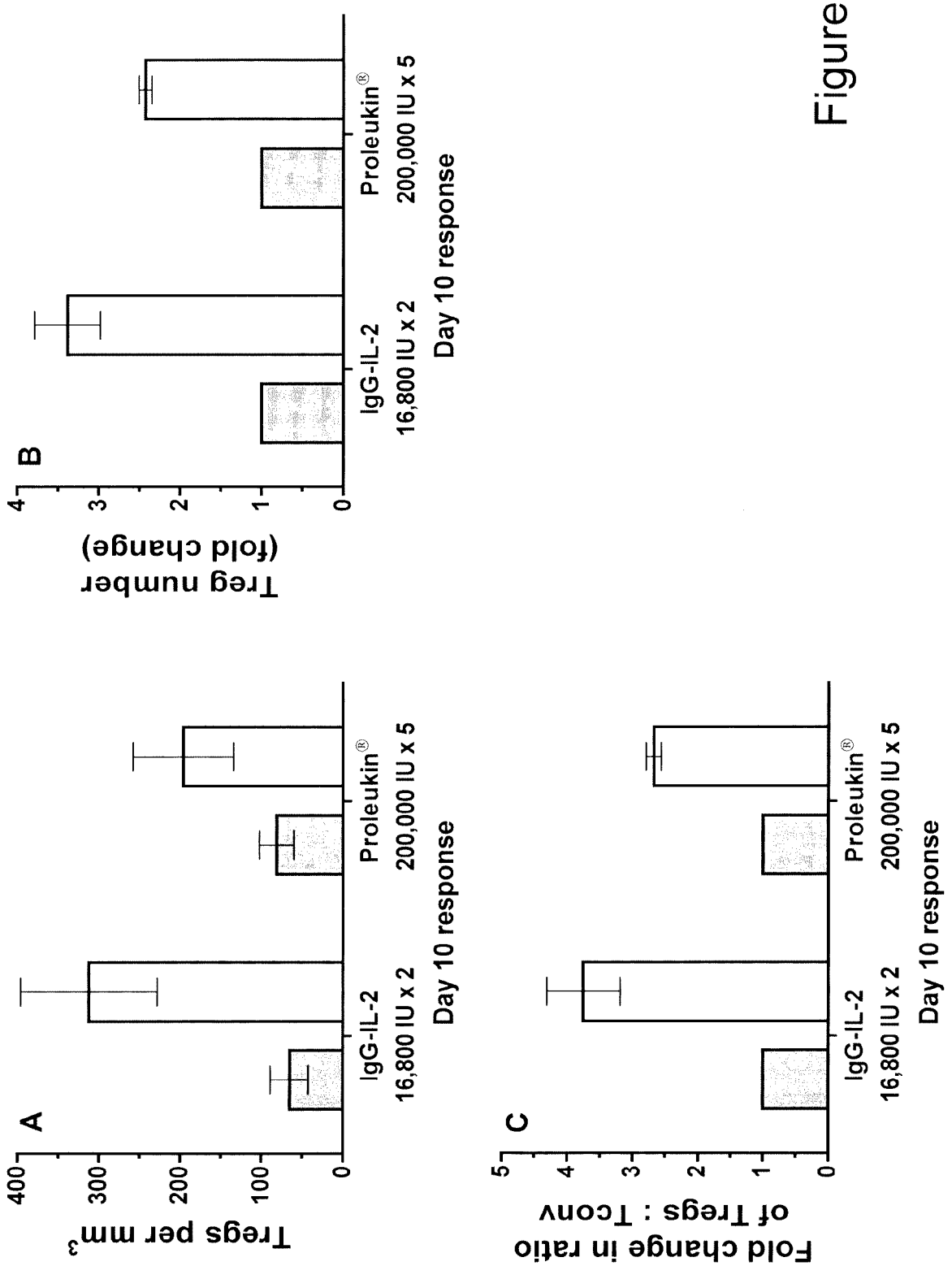

A
Tregs per mm3
400
300
200
100
0
IgG-IL-2
16,800 IU x 2
Proleukin®
200,000 IU x 5
Day 10 response
B
Treg number
(fold change)
4
3
2
1
0
IgG-IL-2
16,800 IU x 2
Proleukin®
200,000 IU x 5
Day 10 response
C
Fold change in ratio
of Tregs : Tconv
5
4
3
2
1
0
IgG-IL-2
16,800 IU x 2
Proleukin®
200,000 IU x 5
Day 10 response

Figure 29

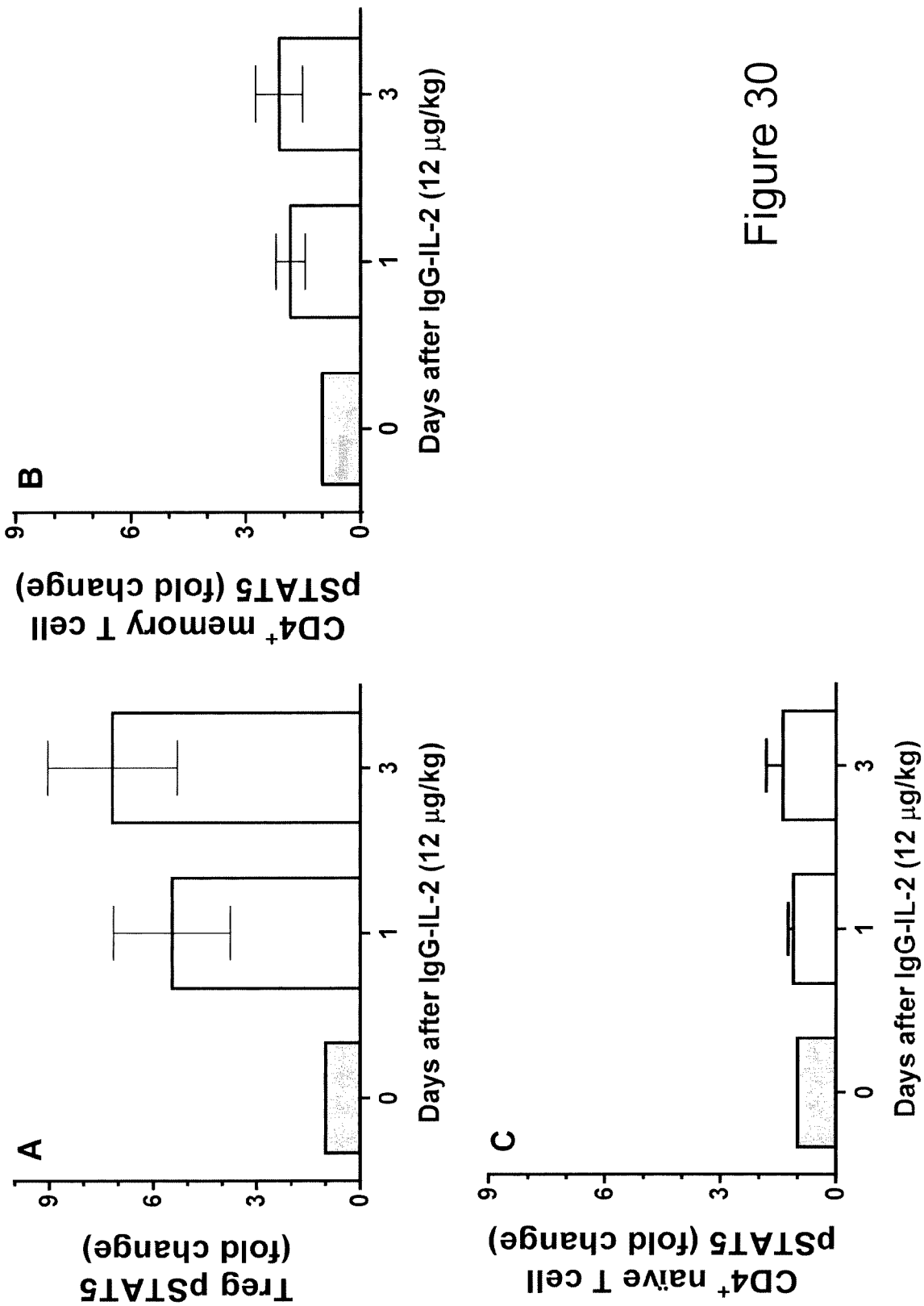

A
Treg pSTAT5
(fold change)
0
3
6
9
0
1
3
Days after IgG-IL-2 (12 μg/kg)
B
CD4+ memory T cell
pSTAT5 (fold change)
0
3
6
9
0
1
3
Days after IgG-IL-2 (12 μg/kg)
C
CD4+ naïve T cell
pSTAT5 (fold change)
0
3
6
9
0
1
3
Days after IgG-IL-2 (12 μg/kg)

Figure 30

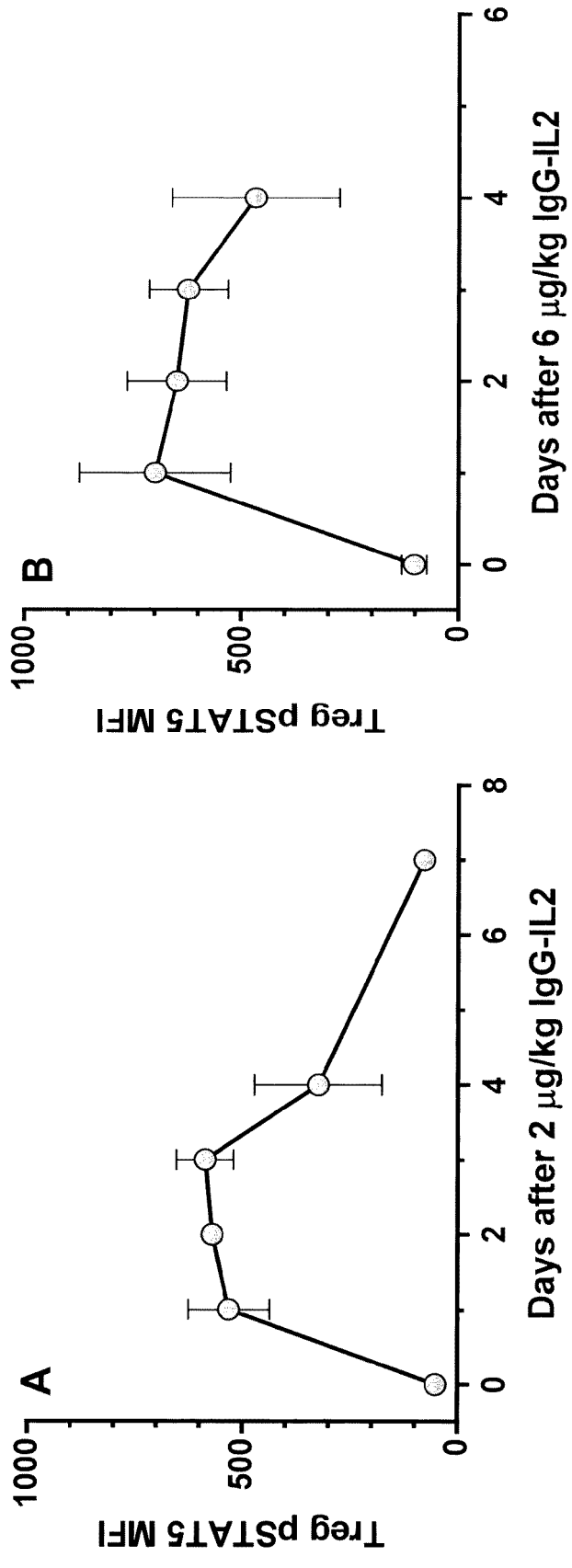
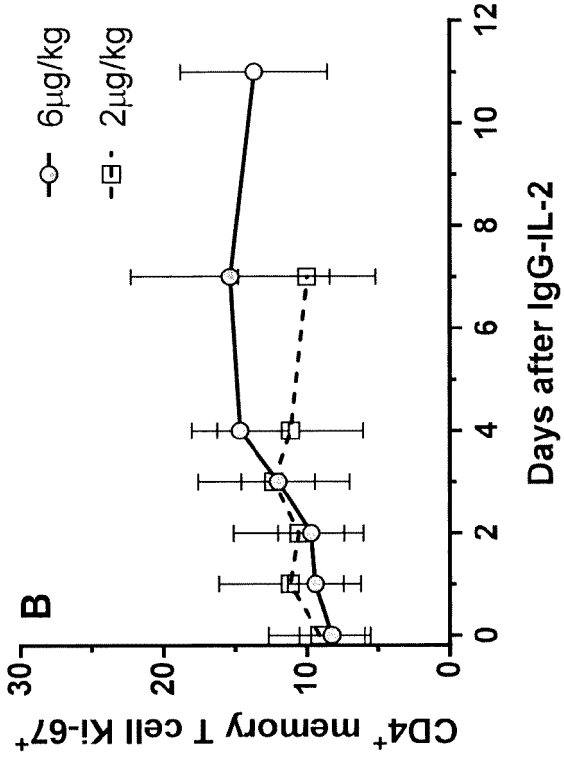
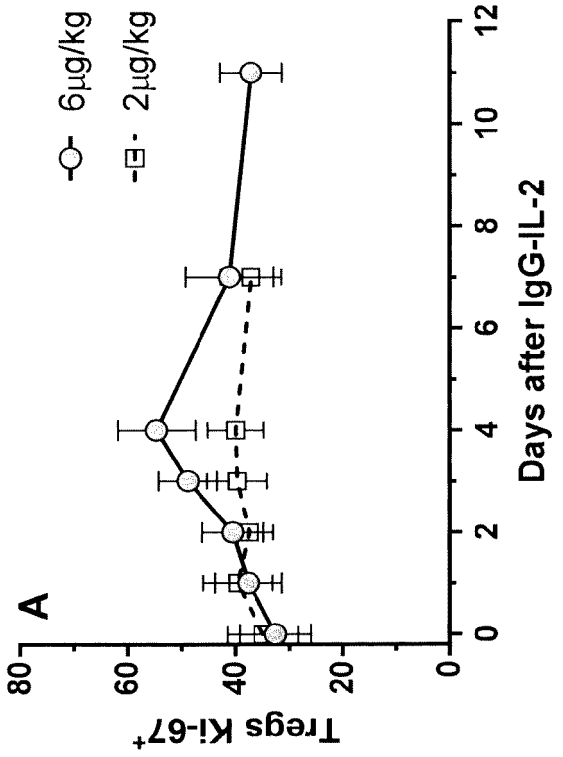

A
B
Treg pSTAT5 MFI
1000
500
0
0
2
4
6
8
Days after 2 µg/kg IgG-IL2
Days after 6 µg/kg IgG-IL2

Figure 31


B
6µg/kg
2µg/kg
CD4+ memory T cell Ki-67+
Days after IgG-IL-2
0
10
20
30
0
2
4
6
8
10
12


A
6µg/kg
2µg/kg
Tregs Ki-67+
Days after IgG-IL-2
0
20
40
60
80
0
2
4
6
8
10
12

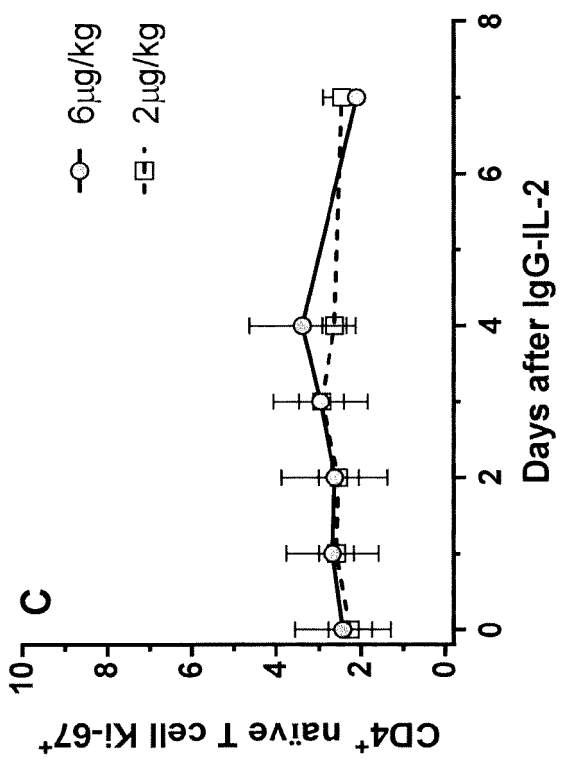

C
6µg/kg
2µg/kg
CD4+ naïve T cell Ki-67+
Days after IgG-IL-2
0
2
4
6
8
10
0
2
4
6
8

Figure 32

Figure 33

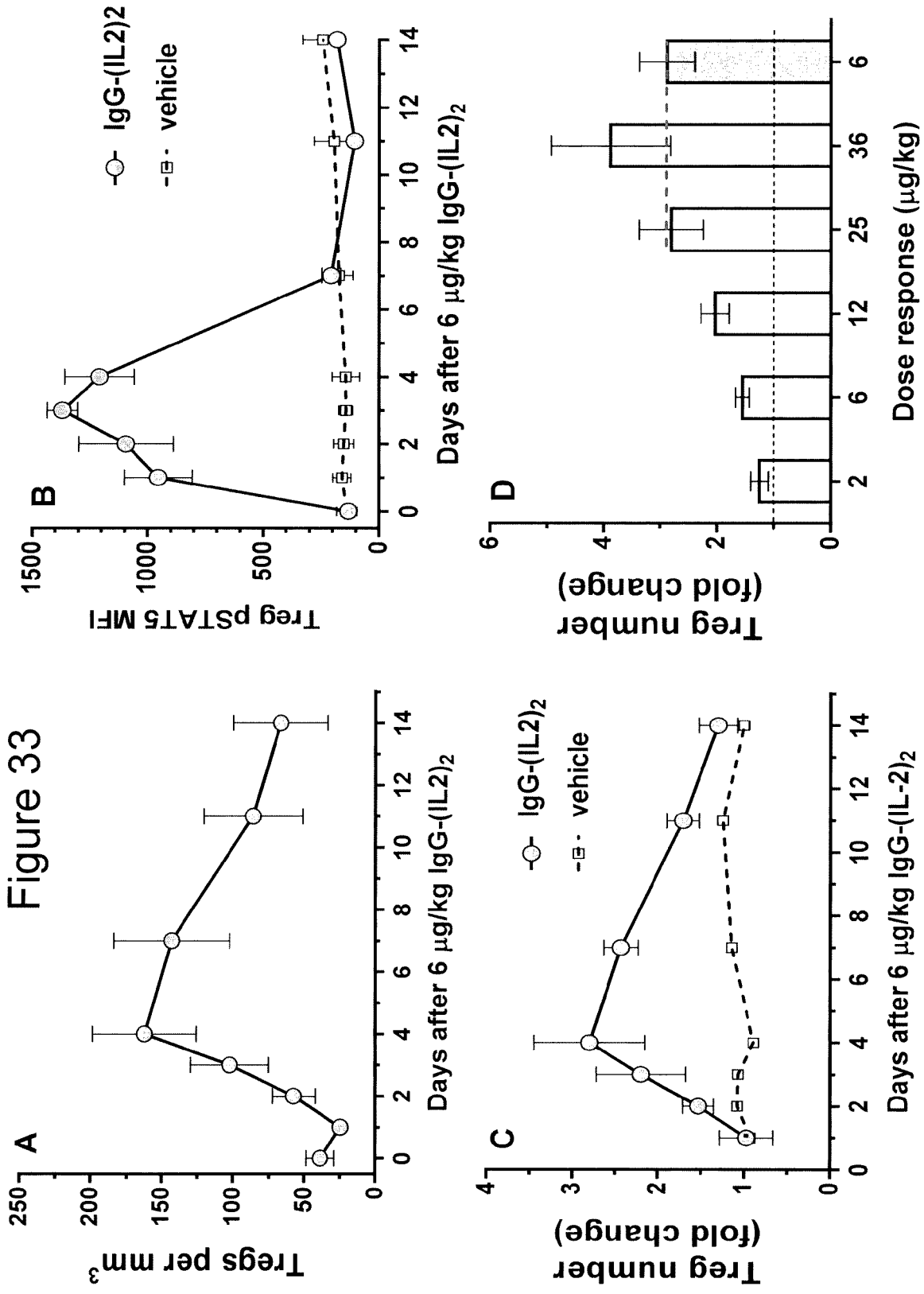
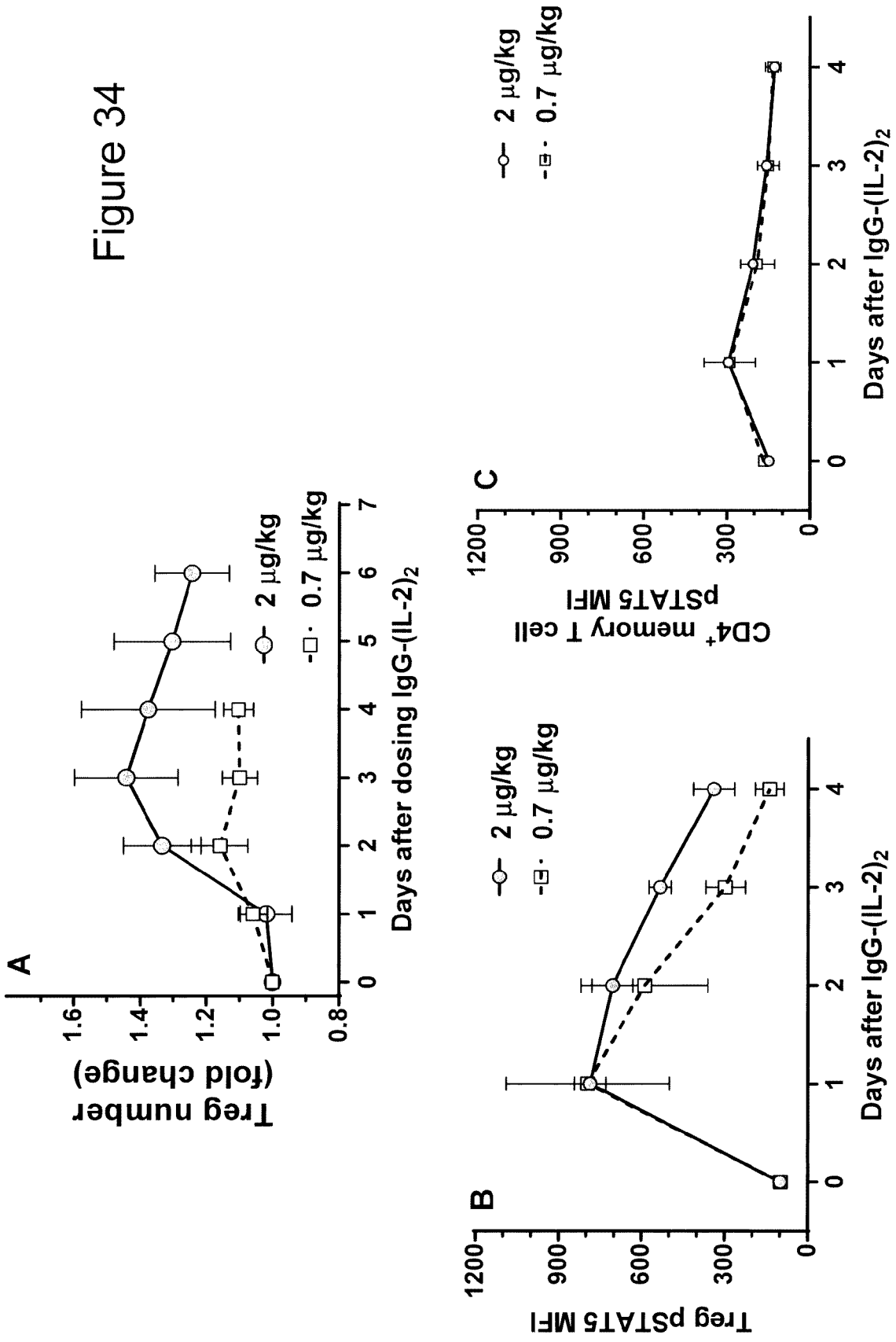

A
Tregs per mm3
Days after 6 µg/kg IgG-(IL2)2
B
Treg pSTAT5 MFI
IgG-(IL2)2
vehicle
Days after 6 µg/kg IgG-(IL2)2
C
Treg number (fold change)
IgG-(IL2)2
vehicle
Days after 6 µg/kg IgG-(IL-2)2
D
Treg number (fold change)
Dose response (µg/kg)

Figure 34


A
Treg number (fold change)
1.6
1.4
1.2
1.0
0.8
0
1
2
3
4
5
6
7
Days after dosing IgG-(IL-2)2
2 µg/kg
0.7 µg/kg
B
Treg pSTAT5 MFI
1200
900
600
300
0
Days after IgG-(IL-2)2
C
CD4+ memory T cell pSTAT5 MFI

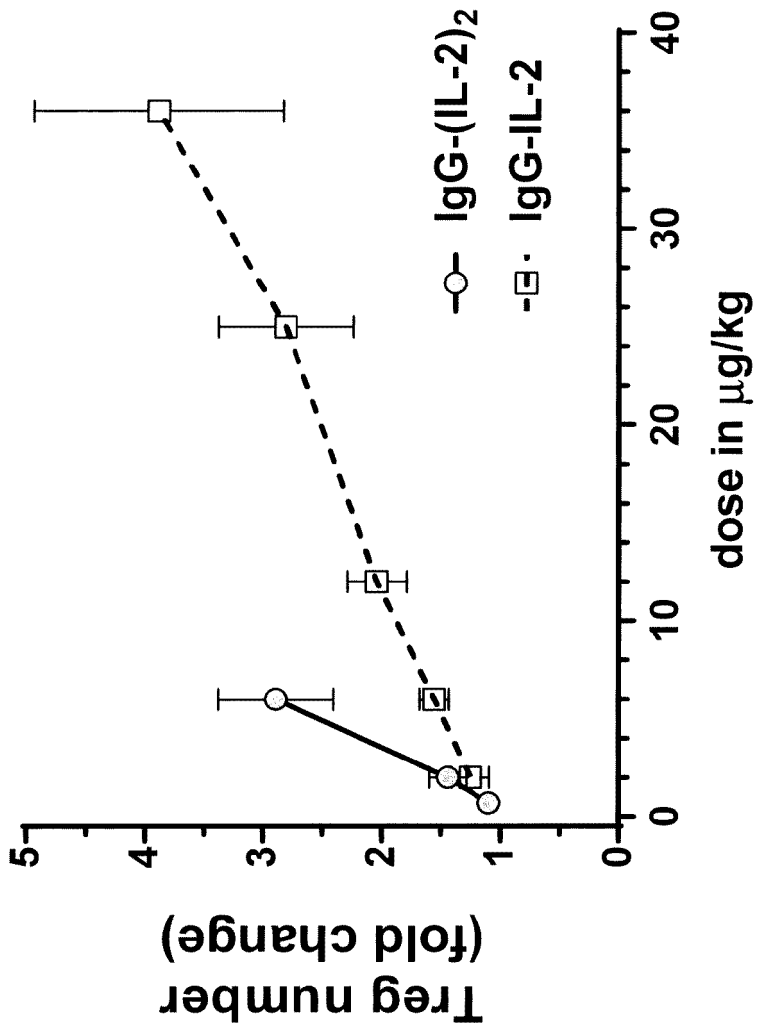
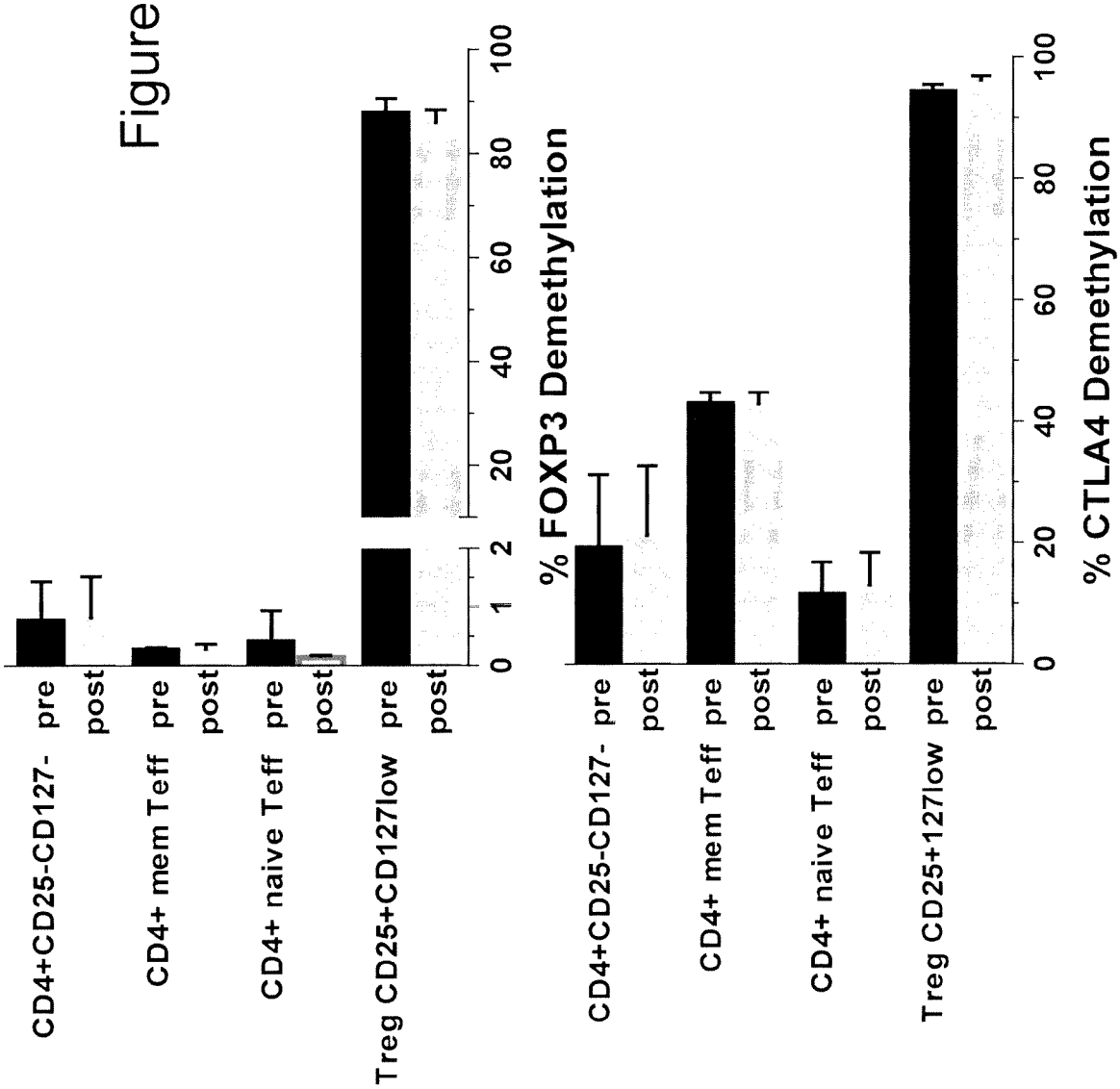

Treg number
(fold change)
5
4
3
2
1
0
0
10
20
30
40
dose in µg/kg
IgG-(IL-2)2
IgG-IL-2

Figure 35

Figure 36


% FOXP3 Demethylation
% CTLA4 Demethylation
0
1
2
20
40
60
80
100
CD4+CD25-CD127- pre
post
CD4+ mem Teff pre
post
CD4+ naive Teff pre
post
Treg CD25+CD127low pre
post
Treg CD25+127low pre

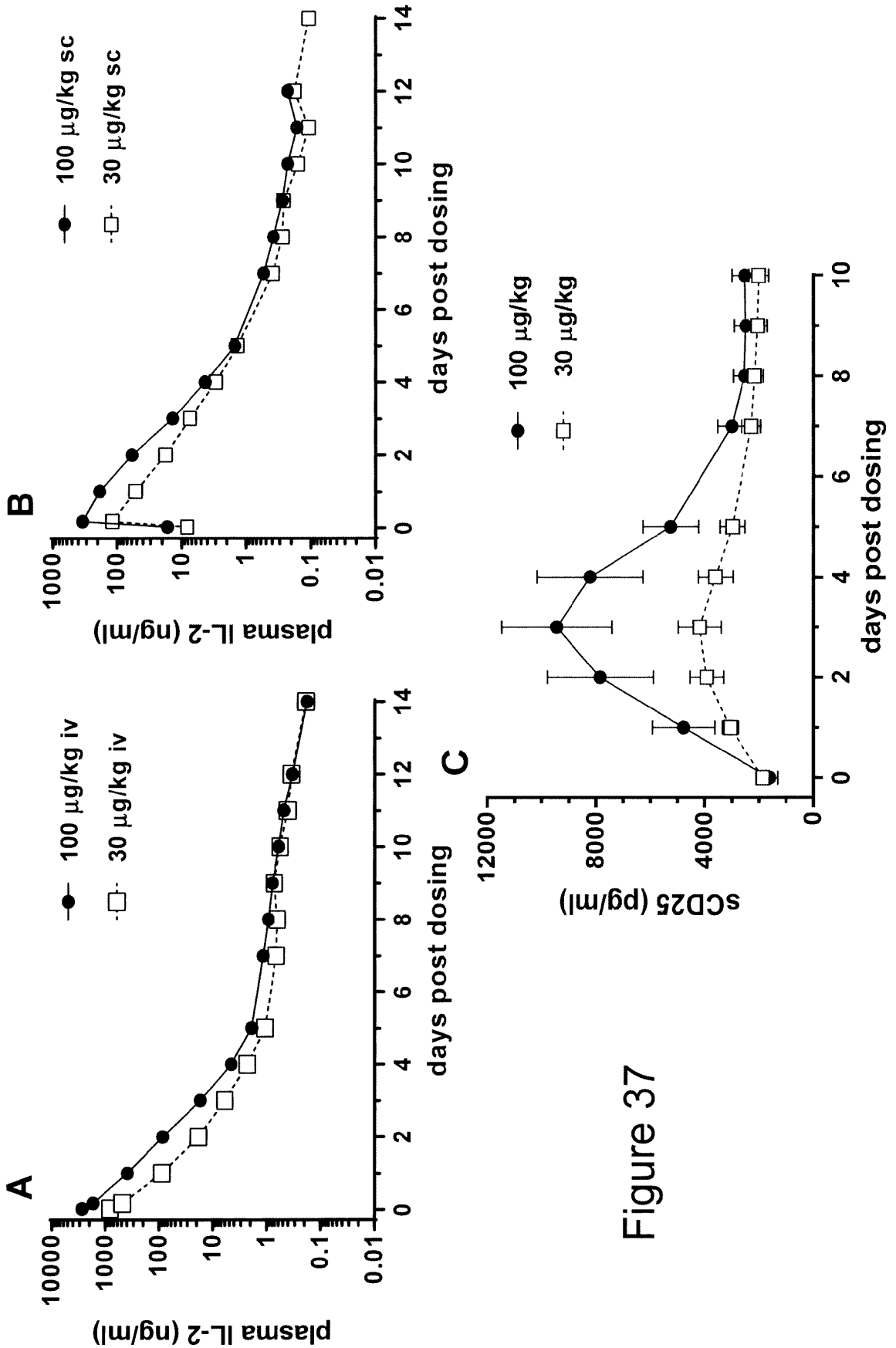

A
plasma IL-2 (ng/ml)
10000
1000
100
10
1
0.1
0.01
0
2
4
6
8
10
12
14
days post dosing
100 µg/kg iv
30 µg/kg iv
B
plasma IL-2 (ng/ml)
1000
100
10
1
0.1
0.01
0
2
4
6
8
10
12
14
days post dosing
100 µg/kg sc
30 µg/kg sc
C
sCD25 (pg/ml)
12000
8000
4000
0
0
2
4
6
8
10
days post dosing
100 µg/kg
30 µg/kg

Figure 37

Figure 38

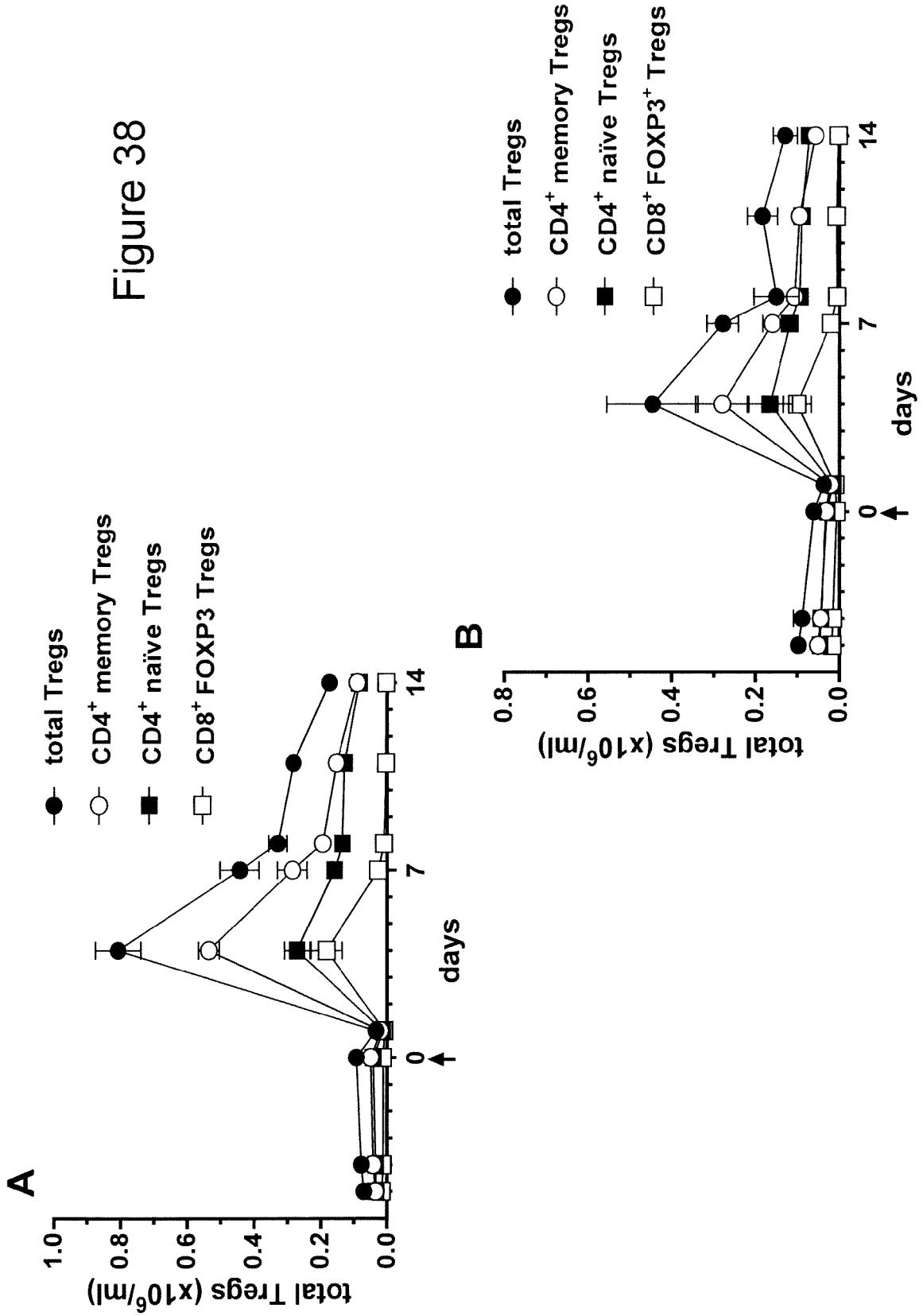
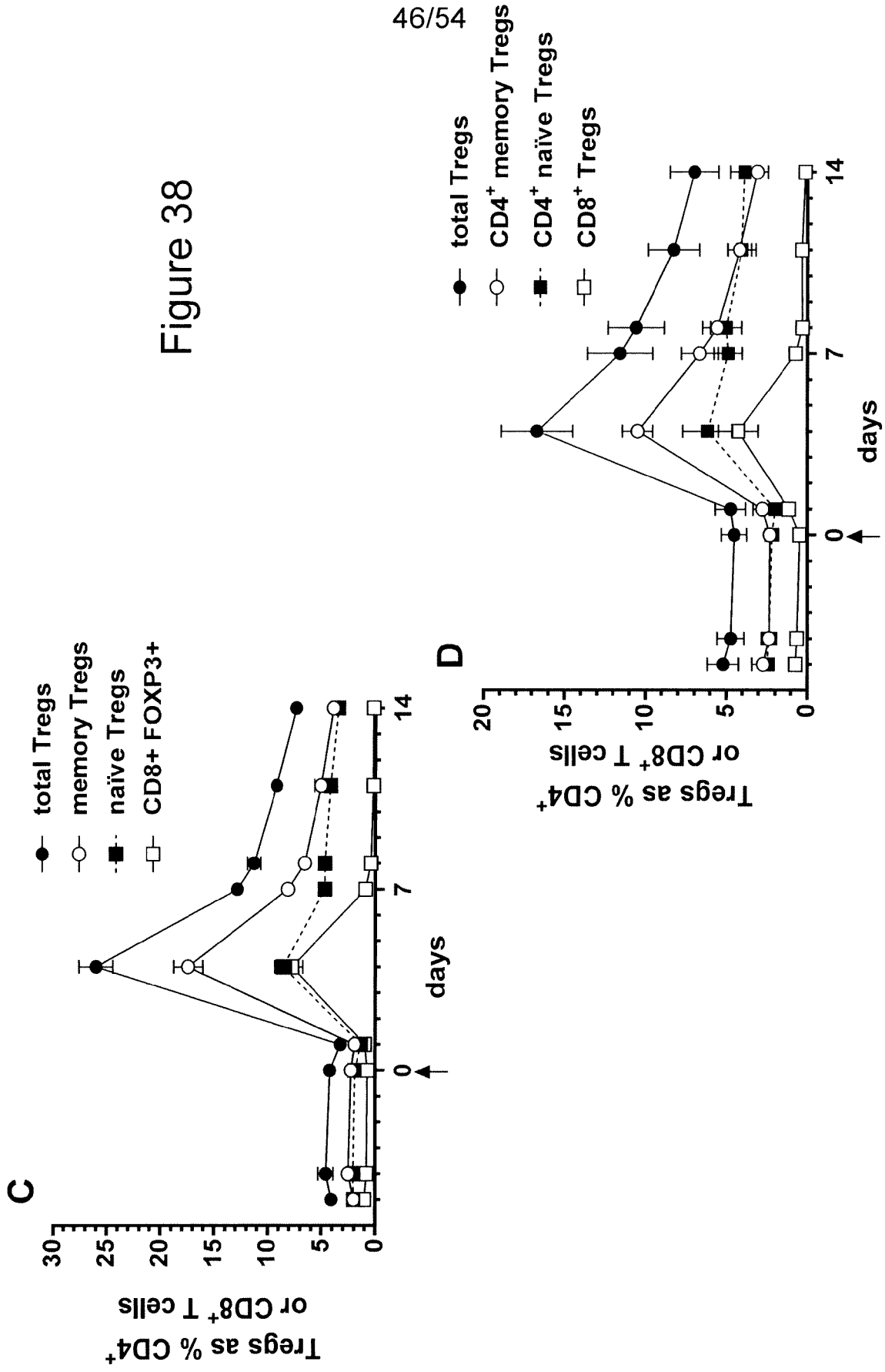

A
B
total Tregs
CD4+ memory Tregs
CD4+ naïve Tregs
CD8+ FOXP3 Tregs
total Tregs (x10^6/ml)
days
0
7
14
0.0
0.2
0.4
0.6
0.8
1.0

Figure 38


C
Tregs as % CD4+
or CD8+ T cells
30
25
20
15
10
5
0
0
7
14
days
total Tregs
memory Tregs
naïve Tregs
CD8+ FOXP3+
D
Tregs as % CD4+
or CD8+ T cells
20
15
10
5
0
0
7
14
days
total Tregs
CD4+ memory Tregs
CD4+ naïve Tregs
CD8+ Tregs

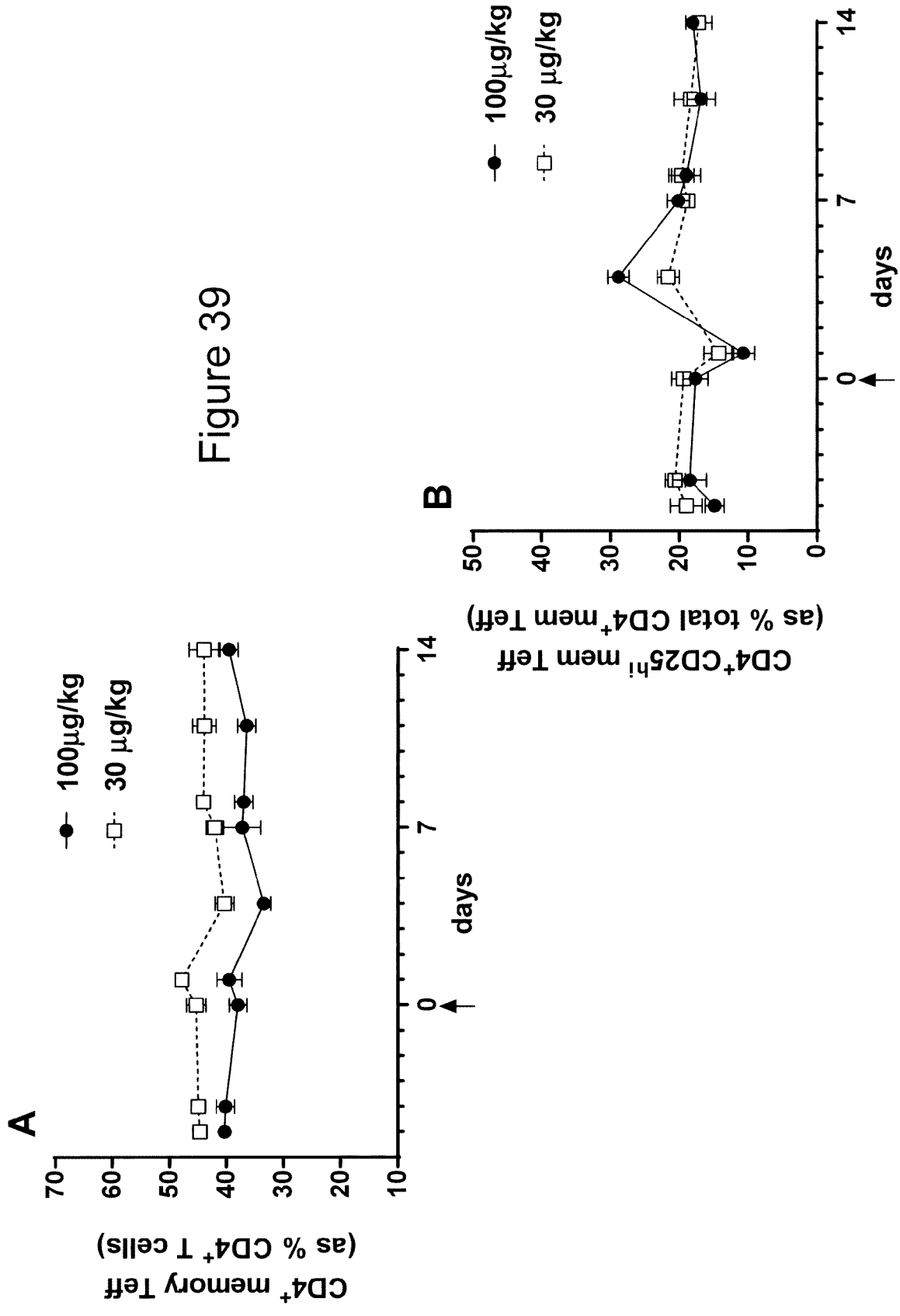

A
CD4+ memory Teff
(as % CD4+ T cells)
70
60
50
40
30
20
10
0
7
14
days
100µg/kg
30 µg/kg
B
CD4+CD25hi mem Teff
(as % total CD4+mem Teff)
50
40
30
20
10
0
0
7
14
days
100µg/kg
30 µg/kg

Figure 39

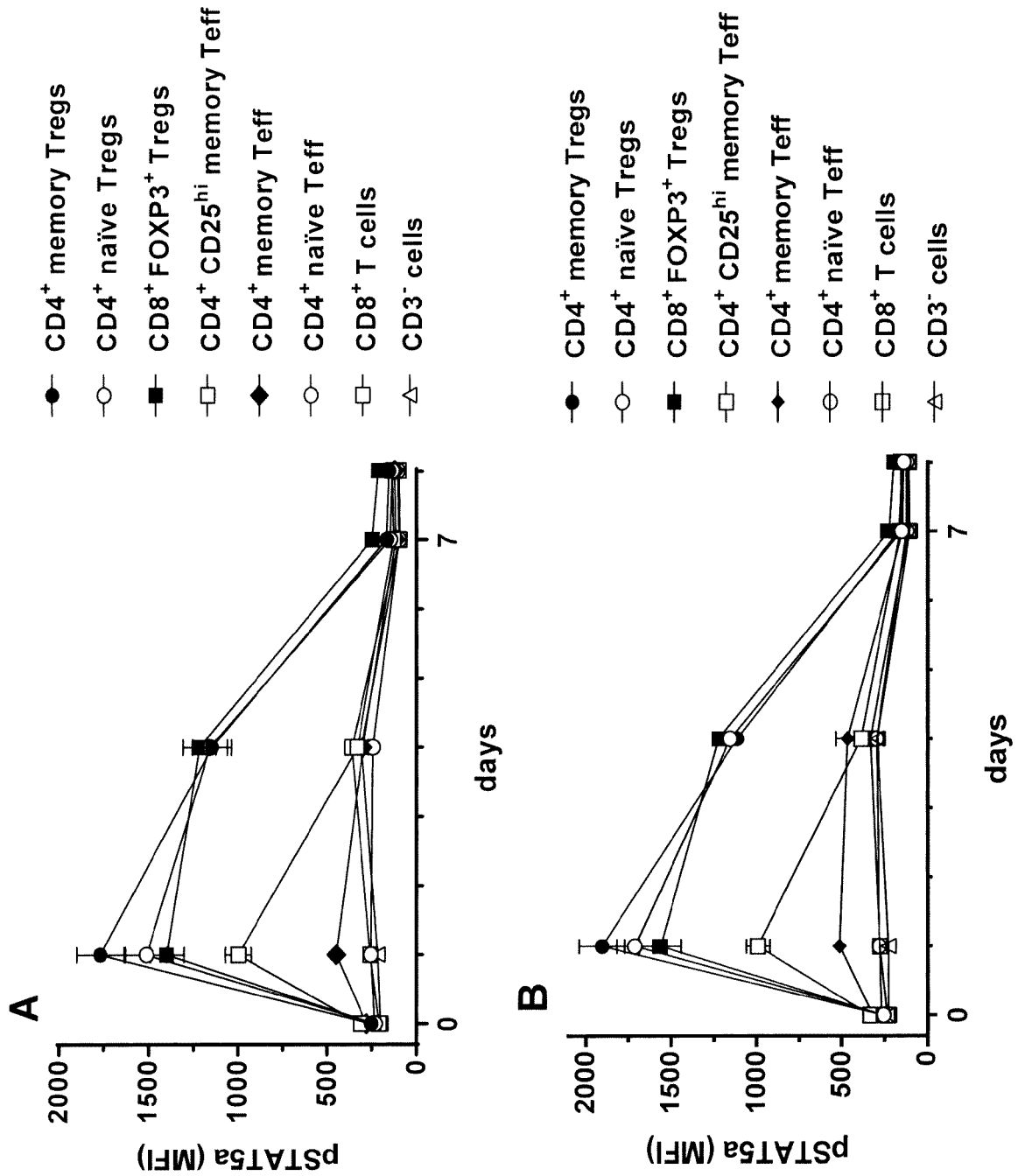

A
B
CD4+ memory Tregs
CD4+ naïve Tregs
CD8+ FOXP3+ Tregs
CD4+ CD25hi memory Teff
CD4+ memory Teff
CD4+ naïve Teff
CD8+ T cells
CD3- cells
pSTAT5a (MFI)
2000
1500
1000
500
0
0
7
days

Figure 40

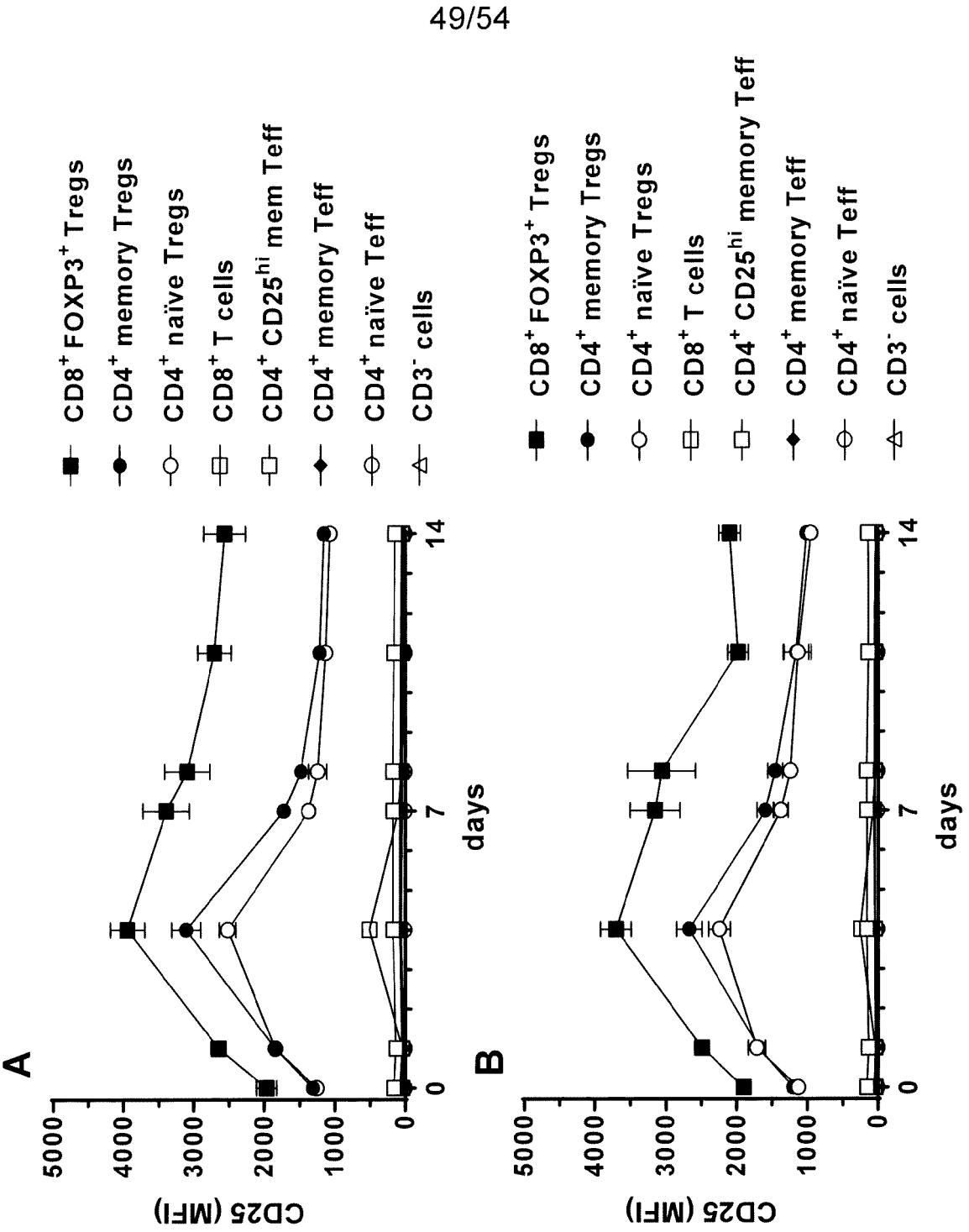

A
B
CD25 (MFI)
5000
4000
3000
2000
1000
0
0
7
14
days
CD8+ FOXP3+ Tregs
CD4+ memory Tregs
CD4+ naïve Tregs
CD8+ T cells
CD4+ CD25hi mem Teff
CD4+ memory Teff
CD4+ naïve Teff
CD3- cells
CD4+ CD25hi memory Teff

Figure 41

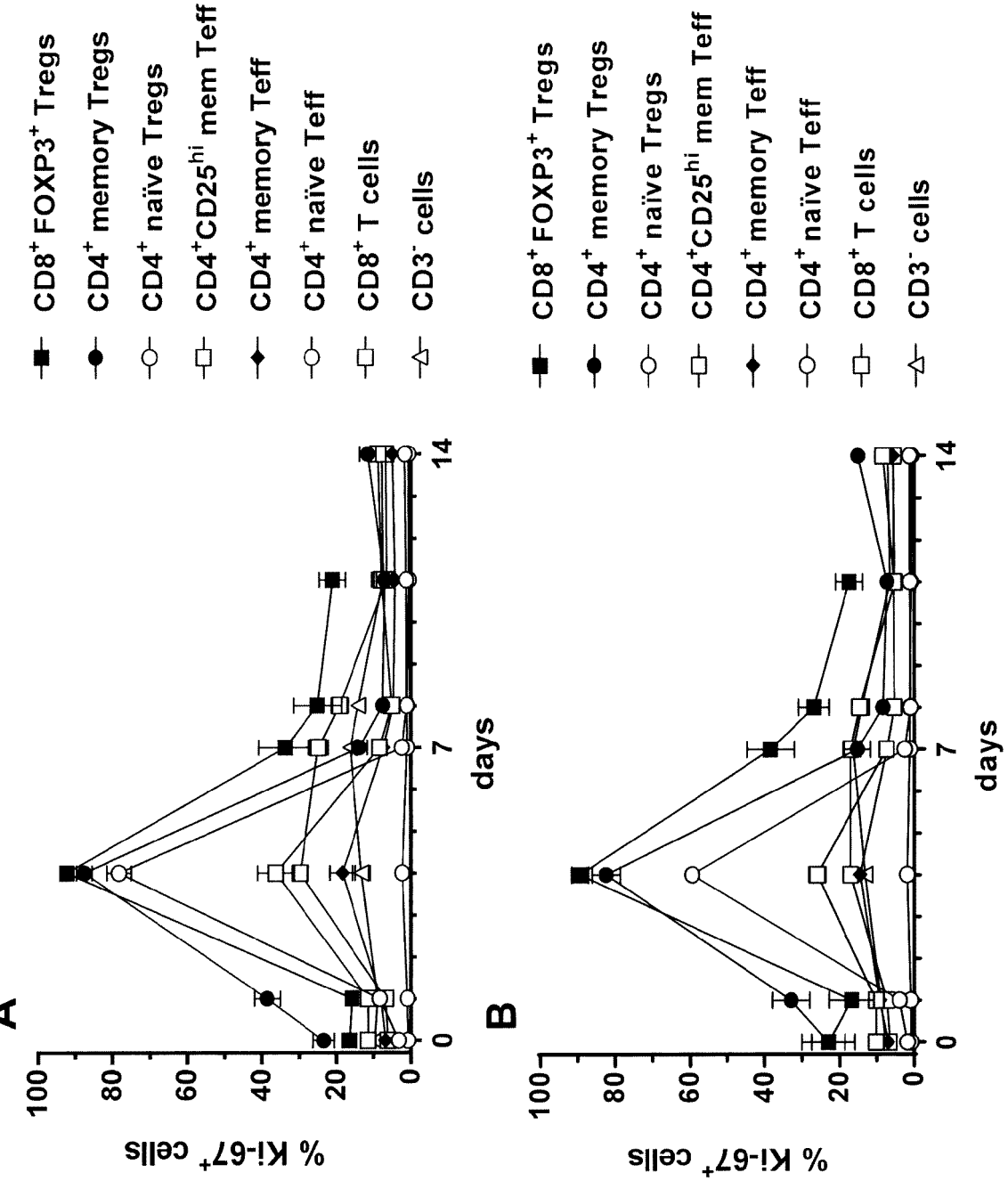

A
B
CD8+ FOXP3+ Tregs
CD4+ memory Tregs
CD4+ naïve Tregs
CD4+CD25hi mem Teff
CD4+ memory Teff
CD4+ naïve Teff
CD8+ T cells
CD3- cells
% Ki-67+ cells
days
0
7
14
20
40
60
80
100

Figure 42

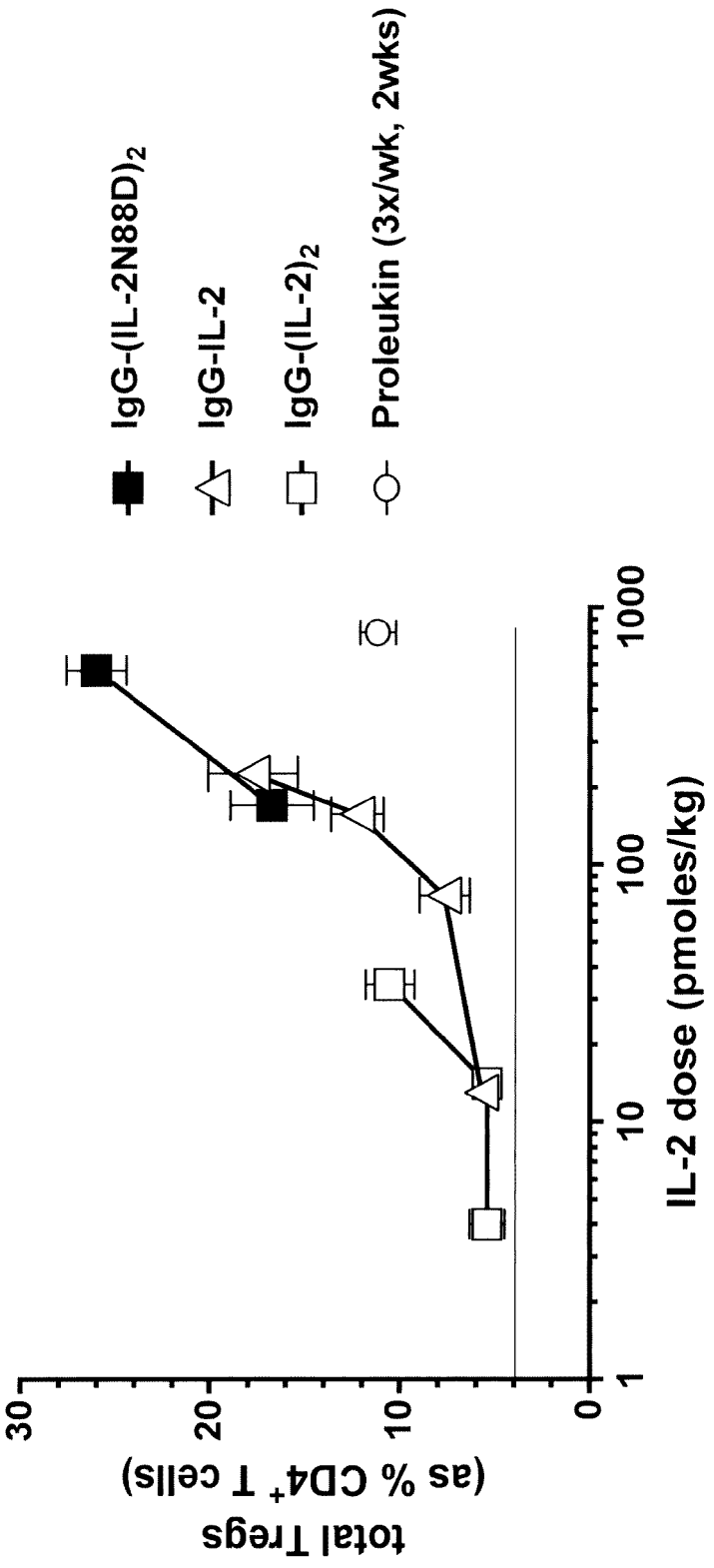

IgG-(IL-2N88D)2
IgG-IL-2
IgG-(IL-2)2
Proleukin (3x/wk, 2wks)
total Tregs
(as % CD4+ T cells)
30
20
10
0
1
10
100
1000
IL-2 dose (pmoles/kg)

Figure 43

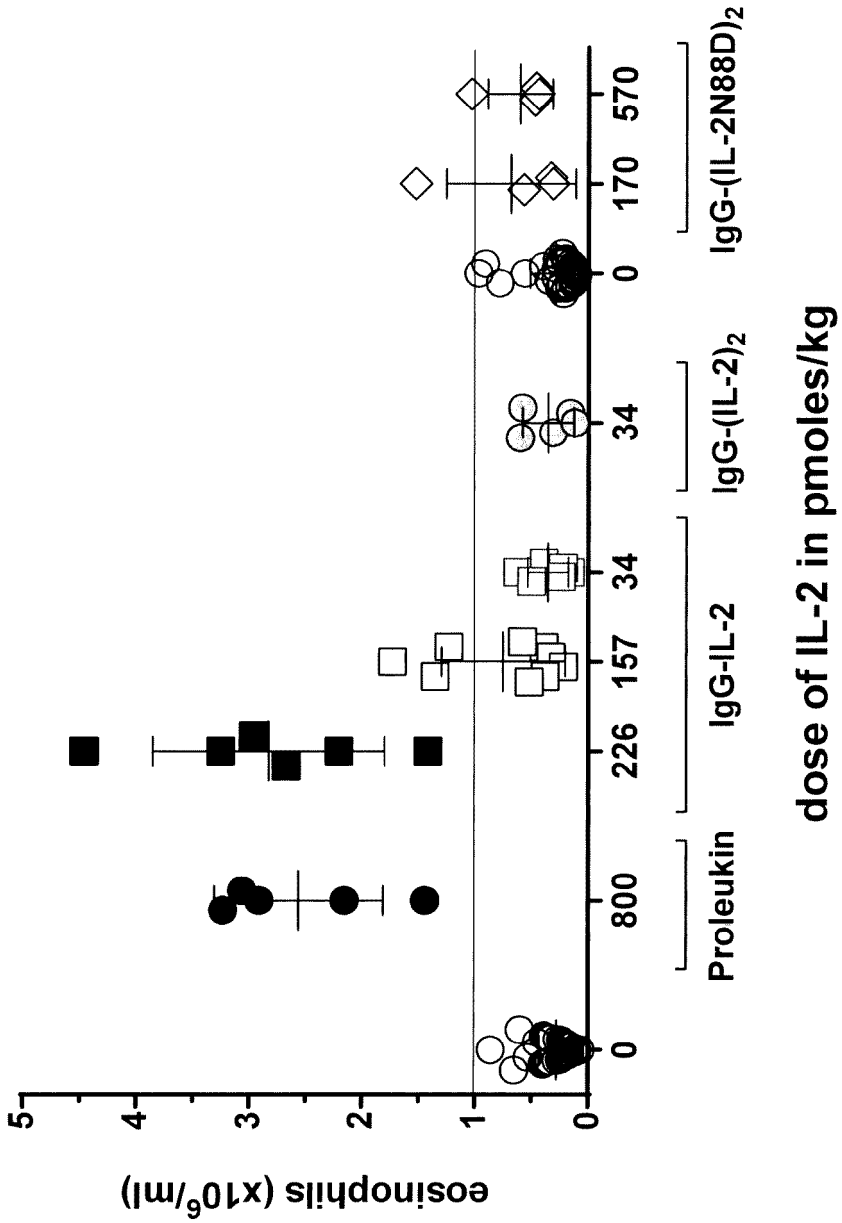

eosinophils (x10^6/ml)
0
1
2
3
4
5
0
800
226
157
34
0
34
170
570
Proleukin
IgG-IL-2
IgG-(IL-2)$_2$
IgG-(IL-2N88D)$_2$
dose of IL-2 in pmoles/kg

Figure 44

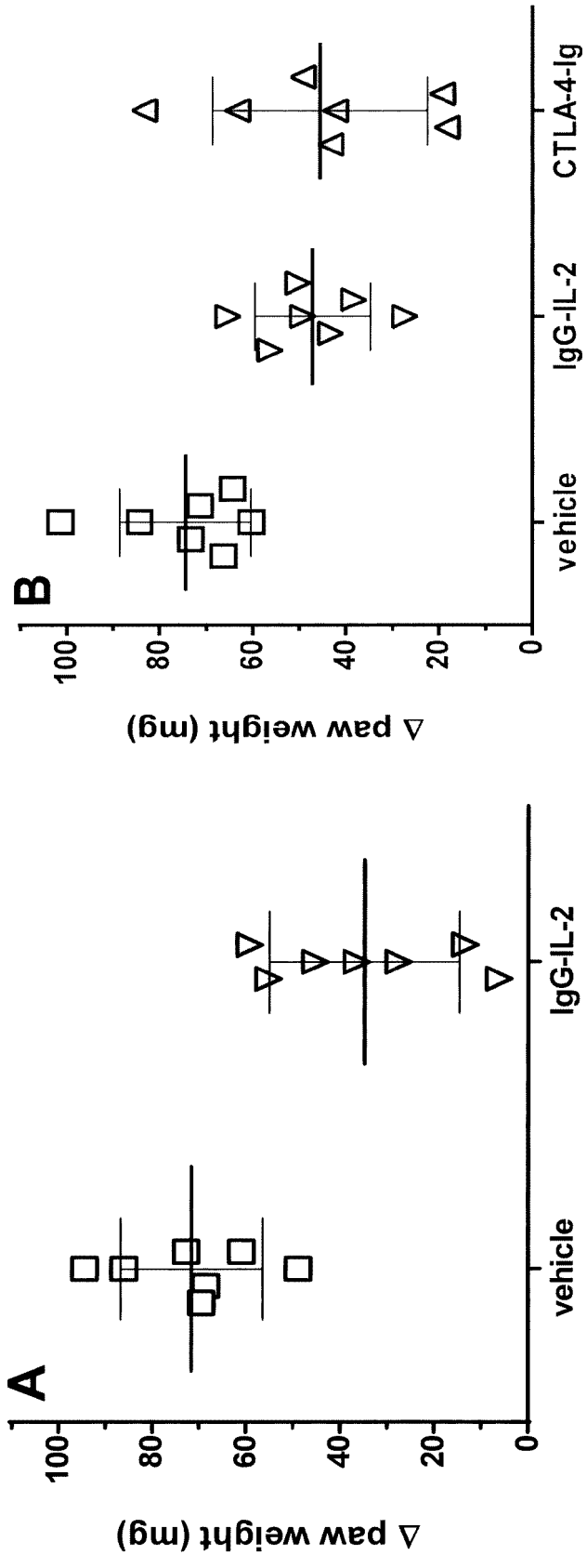


Figure 45

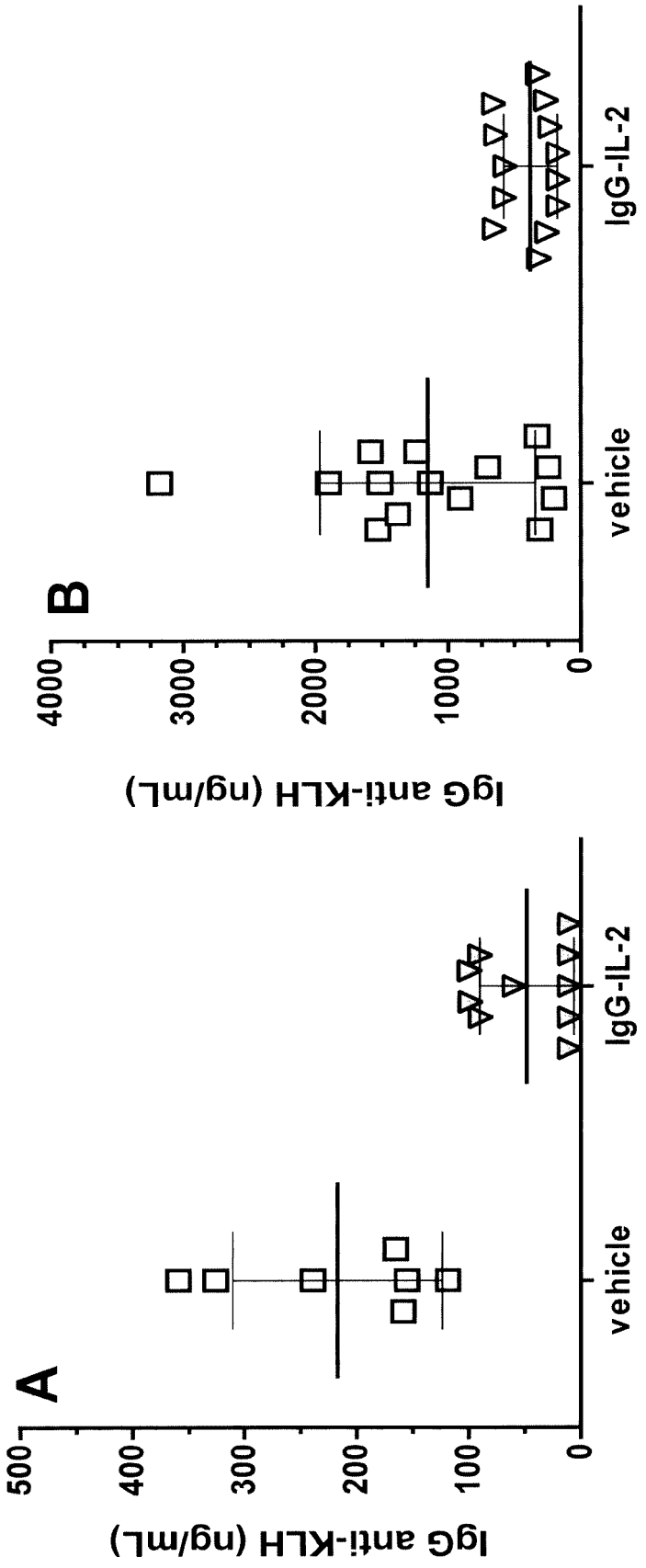

A
B
IgG anti-KLH (ng/mL)
0
100
200
300
400
500
1000
2000
3000
4000
vehicle
IgG-IL-2

Figure 46