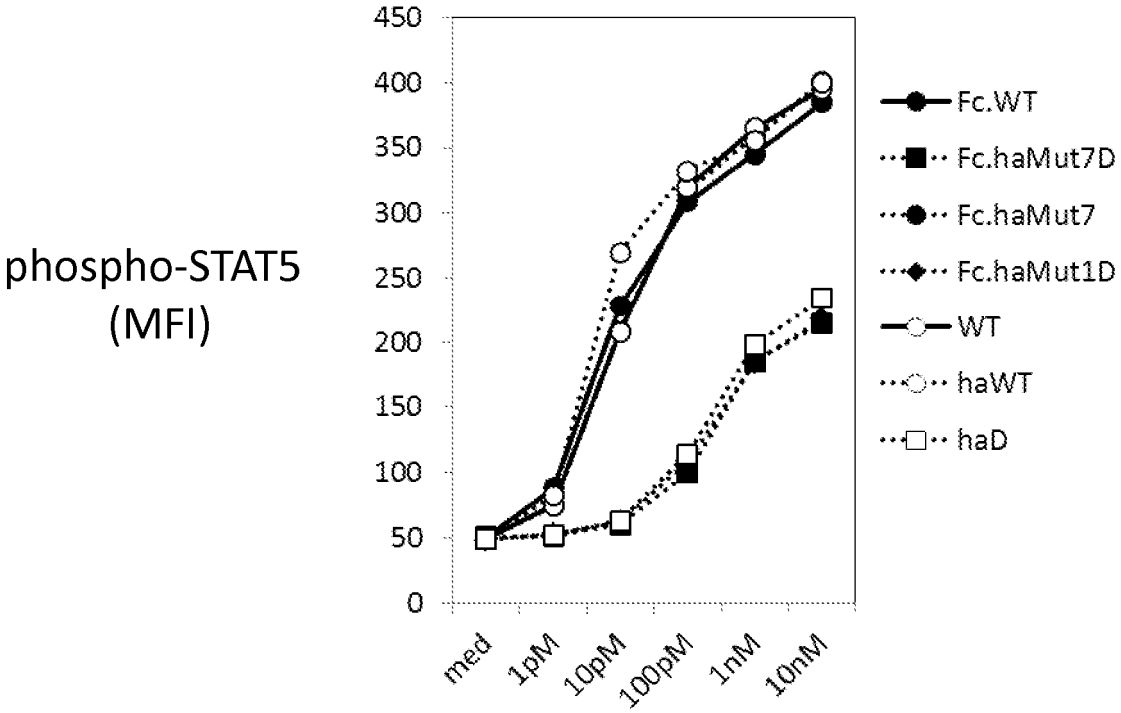

phospho-STAT5
(MFI)
450
400
350
300
250
200
150
100
50
0
med
1pM
10pM
100pM
1nM
10nM
Fc.WT
Fc.haMut7D
Fc.haMut7
Fc.haMut1D
WT
haWT
haD

FIG. 1

FIG. 2A

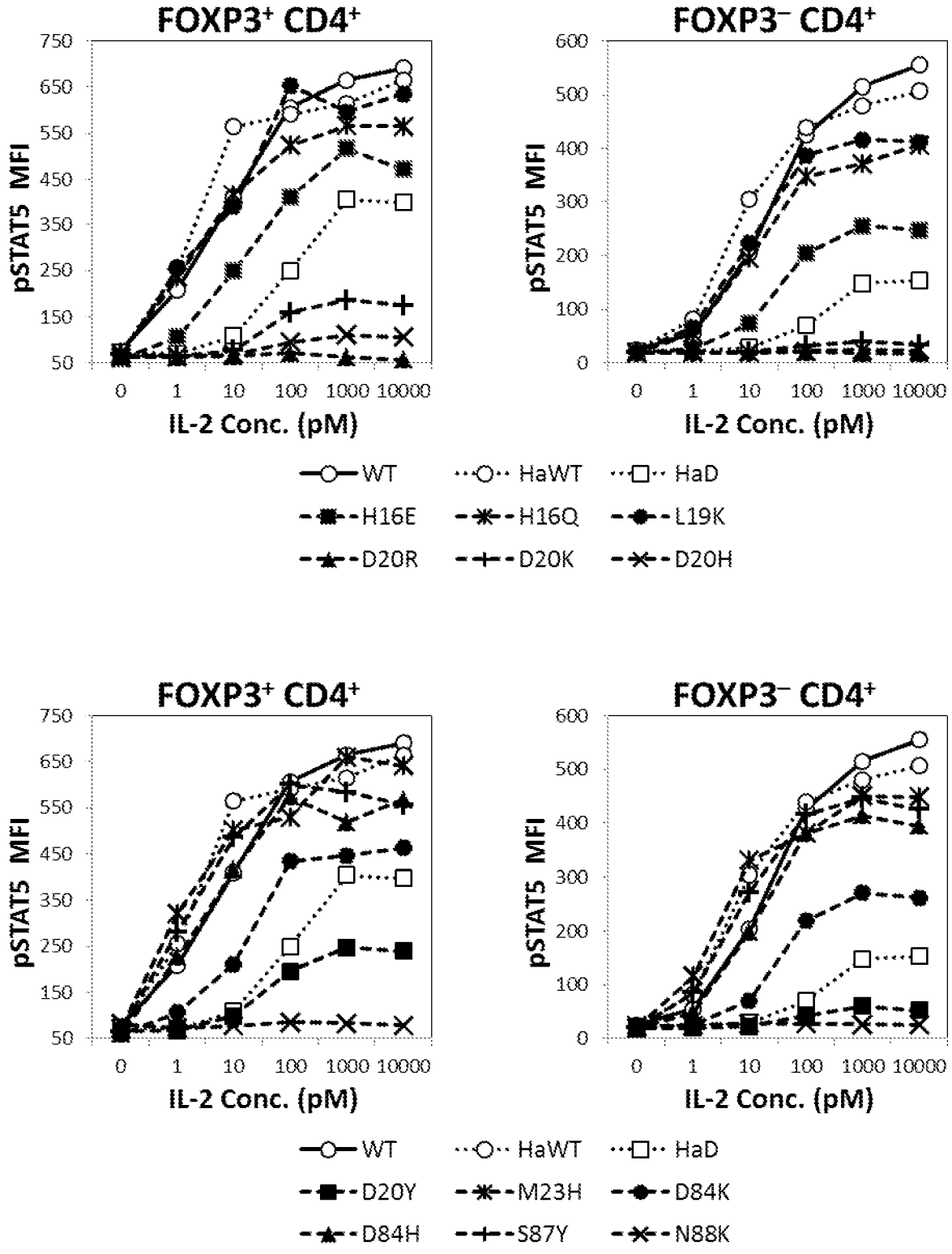
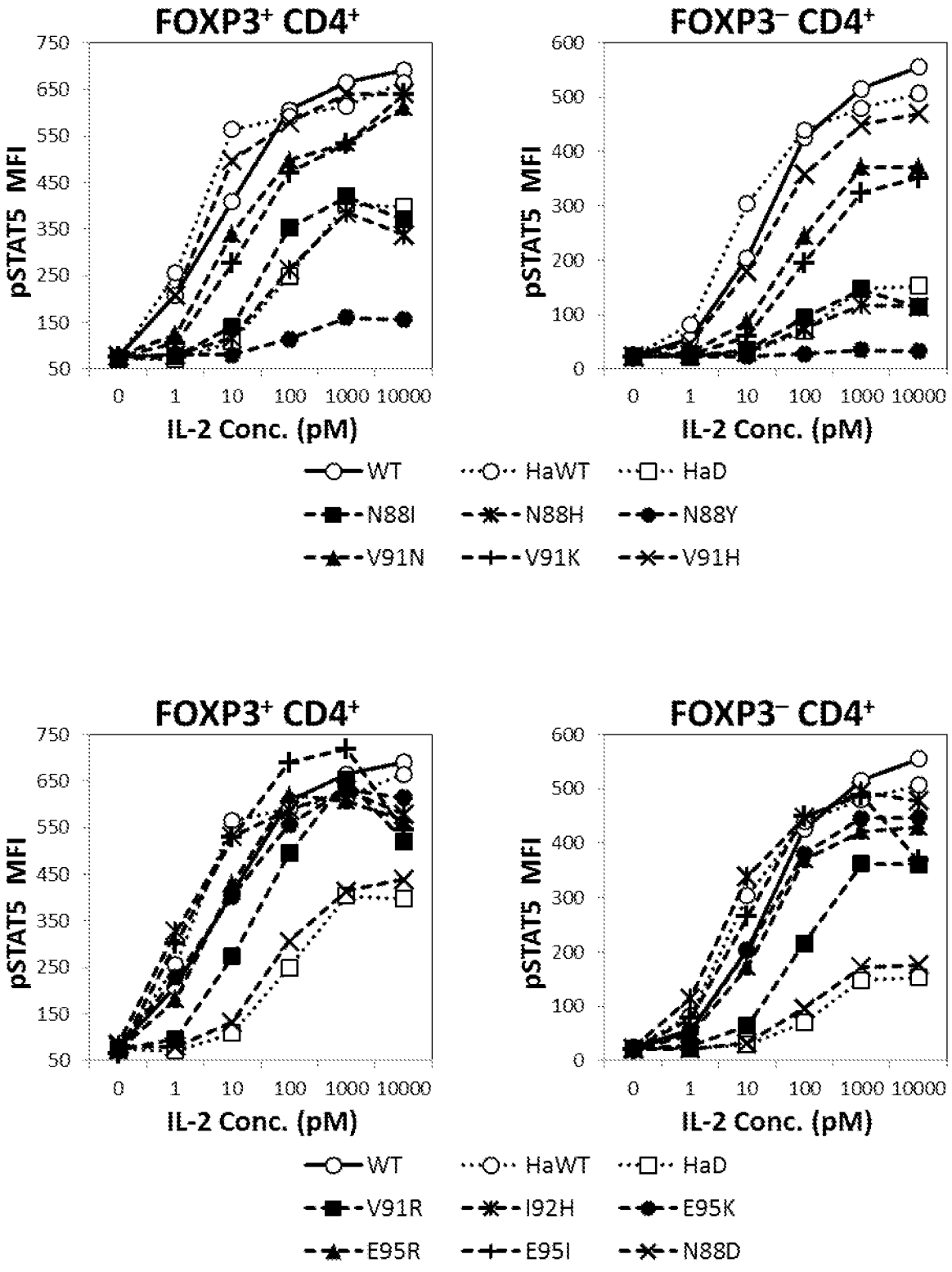

FOXP3+ CD4+
pSTAT5 MFI
750
650
550
450
350
250
150
50
0 1 10 100 1000 10000
IL-2 Conc. (pM)
FOXP3− CD4+
pSTAT5 MFI
600
500
400
300
200
100
0
0 1 10 100 1000 10000
IL-2 Conc. (pM)
WT
HaWT
HaD
H16E
H16Q
L19K
D20R
D20K
D20H
FOXP3+ CD4+
pSTAT5 MFI
750
650
550
450
350
250
150
50
0 1 10 100 1000 10000
IL-2 Conc. (pM)
FOXP3− CD4+
pSTAT5 MFI
600
500
400
300
200
100
0
0 1 10 100 1000 10000
IL-2 Conc. (pM)
WT
HaWT
HaD
D20Y
M23H
D84K
D84H
S87Y
N88K

FIG. 2B


FOXP3+ CD4+
pSTAT5 MFI
IL-2 Conc. (pM)
FOXP3− CD4+
WT
HaWT
HaD
N88I
N88H
N88Y
V91N
V91K
V91H
V91R
I92H
E95K
E95R
E95I
N88D

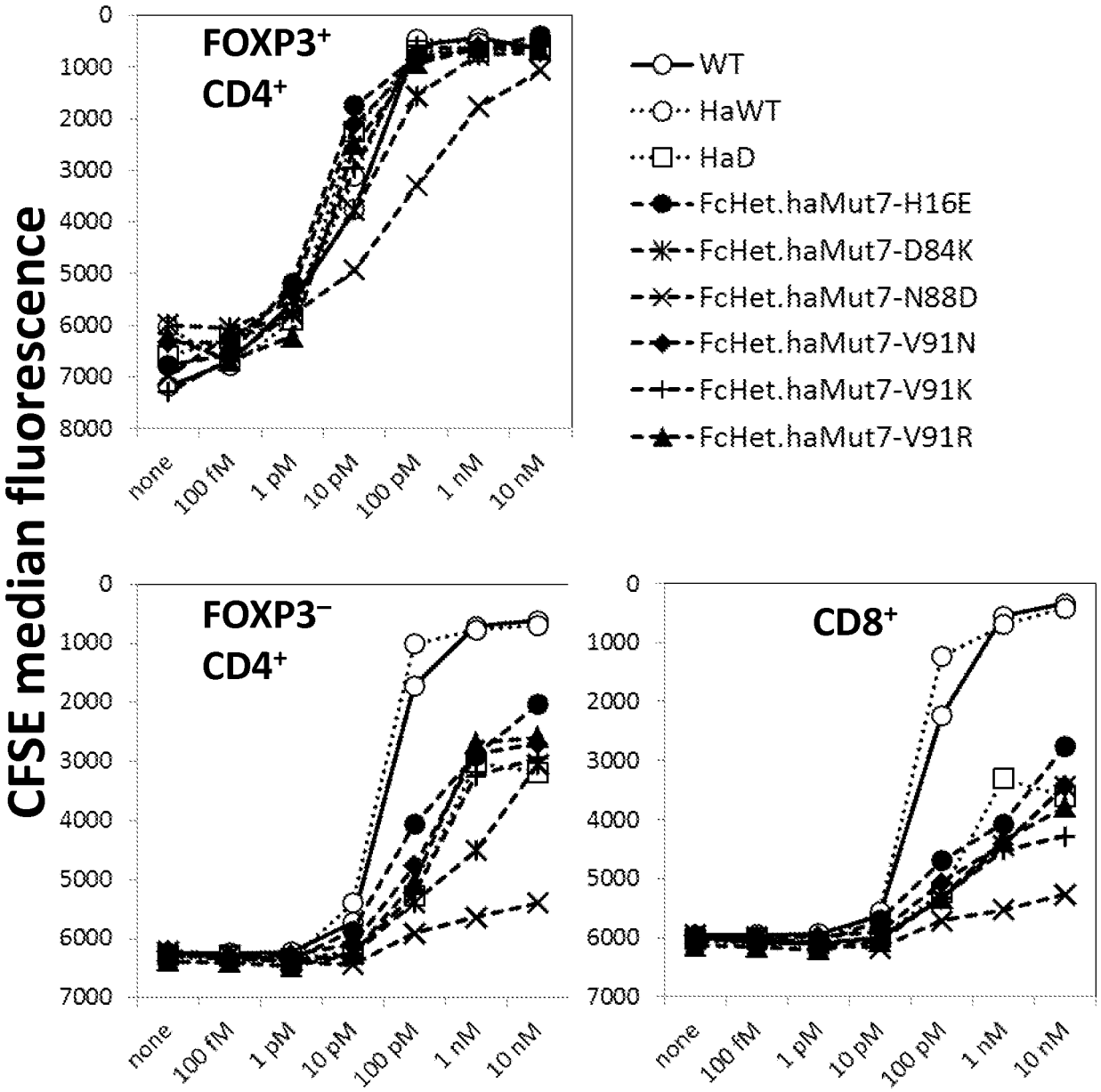

CFSE median fluorescence
FOXP3+ CD4+
FOXP3− CD4+
CD8+
WT
HaWT
HaD
FcHet.haMut7-H16E
FcHet.haMut7-D84K
FcHet.haMut7-N88D
FcHet.haMut7-V91N
FcHet.haMut7-V91K
FcHet.haMut7-V91R
0
1000
2000
3000
4000
5000
6000
7000
8000
none
100 fM
1 pM
10 pM
100 pM
1 nM
10 nM

FIG. 3

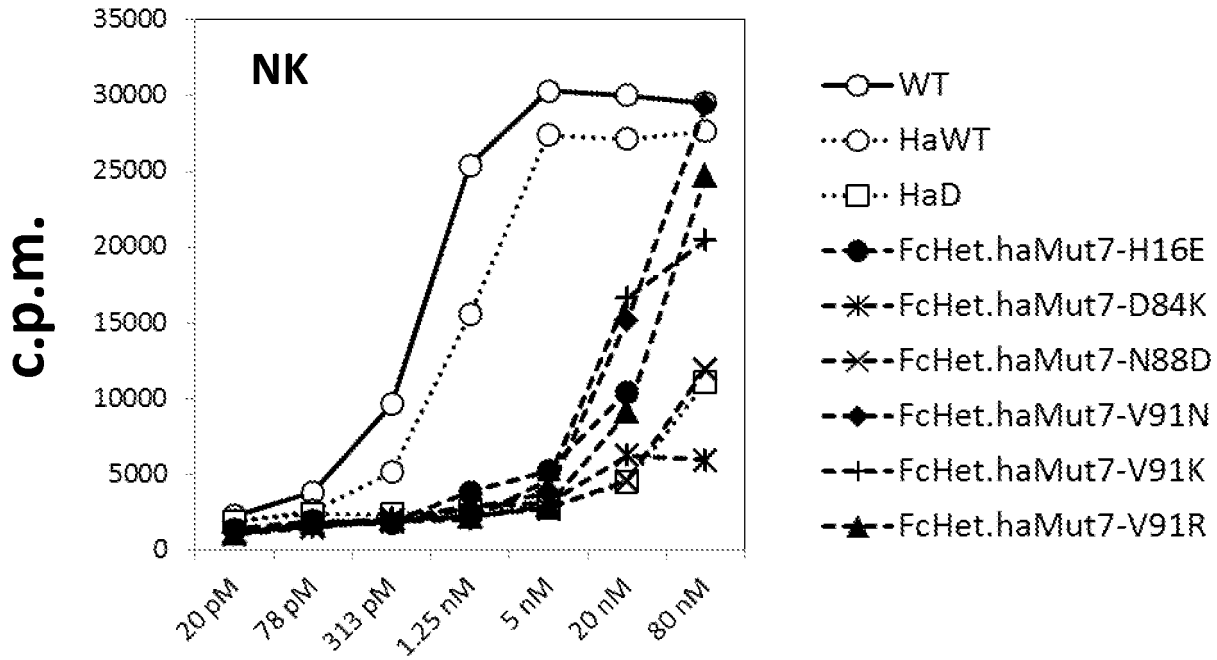

NK
c.p.m.
35000
30000
25000
20000
15000
10000
5000
0
20 pM
78 pM
313 pM
1.25 nM
5 nM
20 nM
80 nM
WT
HaWT
HaD
FcHet.haMut7-H16E
FcHet.haMut7-D84K
FcHet.haMut7-N88D
FcHet.haMut7-V91N
FcHet.haMut7-V91K
FcHet.haMut7-V91R

FIG. 4

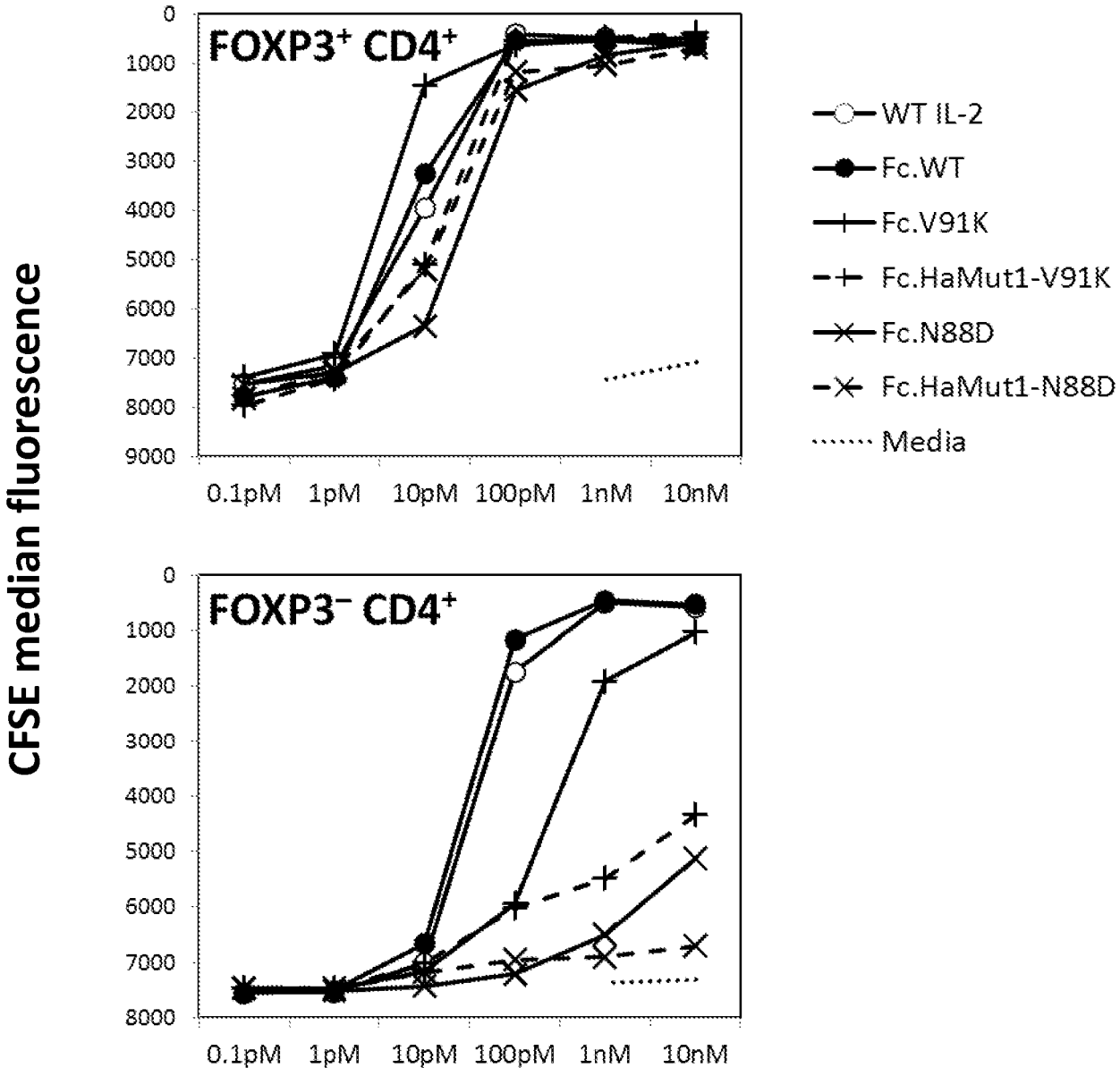

CFSE median fluorescence
FOXP3+ CD4+
FOXP3− CD4+
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
0.1pM
1pM
10pM
100pM
1nM
10nM
WT IL-2
Fc.WT
Fc.V91K
Fc.HaMut1-V91K
Fc.N88D
Fc.HaMut1-N88D
Media

FIG. 5

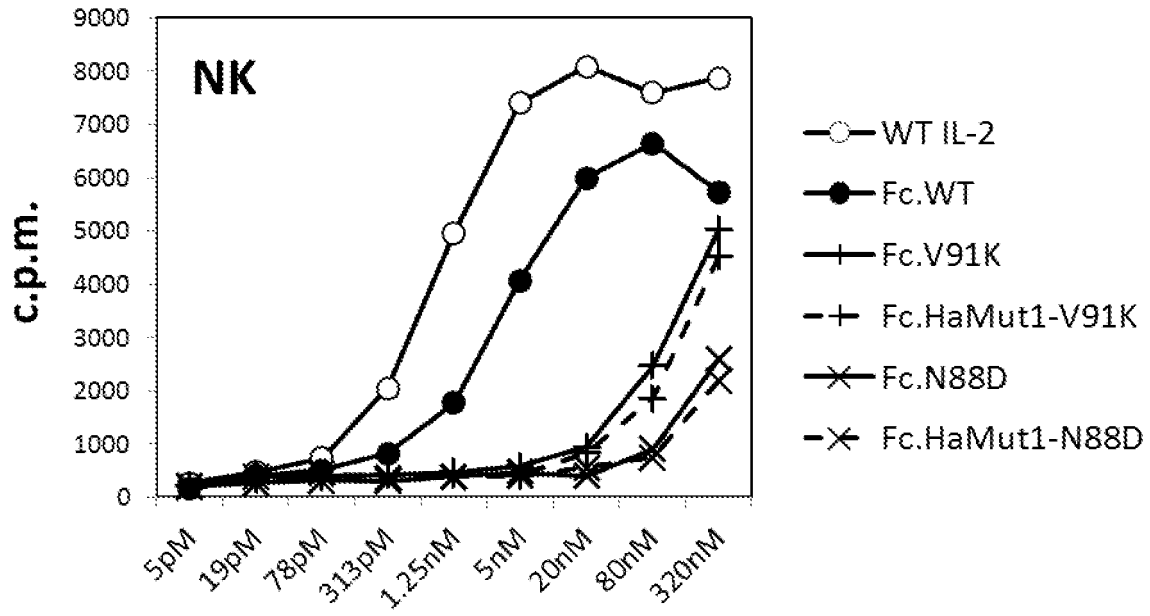

NK
c.p.m.
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
5pM
19pM
78pM
313pM
1.25nM
5nM
20nM
80nM
320nM
WT IL-2
Fc.WT
Fc.V91K
Fc.HaMut1-V91K
Fc.N88D
Fc.HaMut1-N88D

FIG. 6

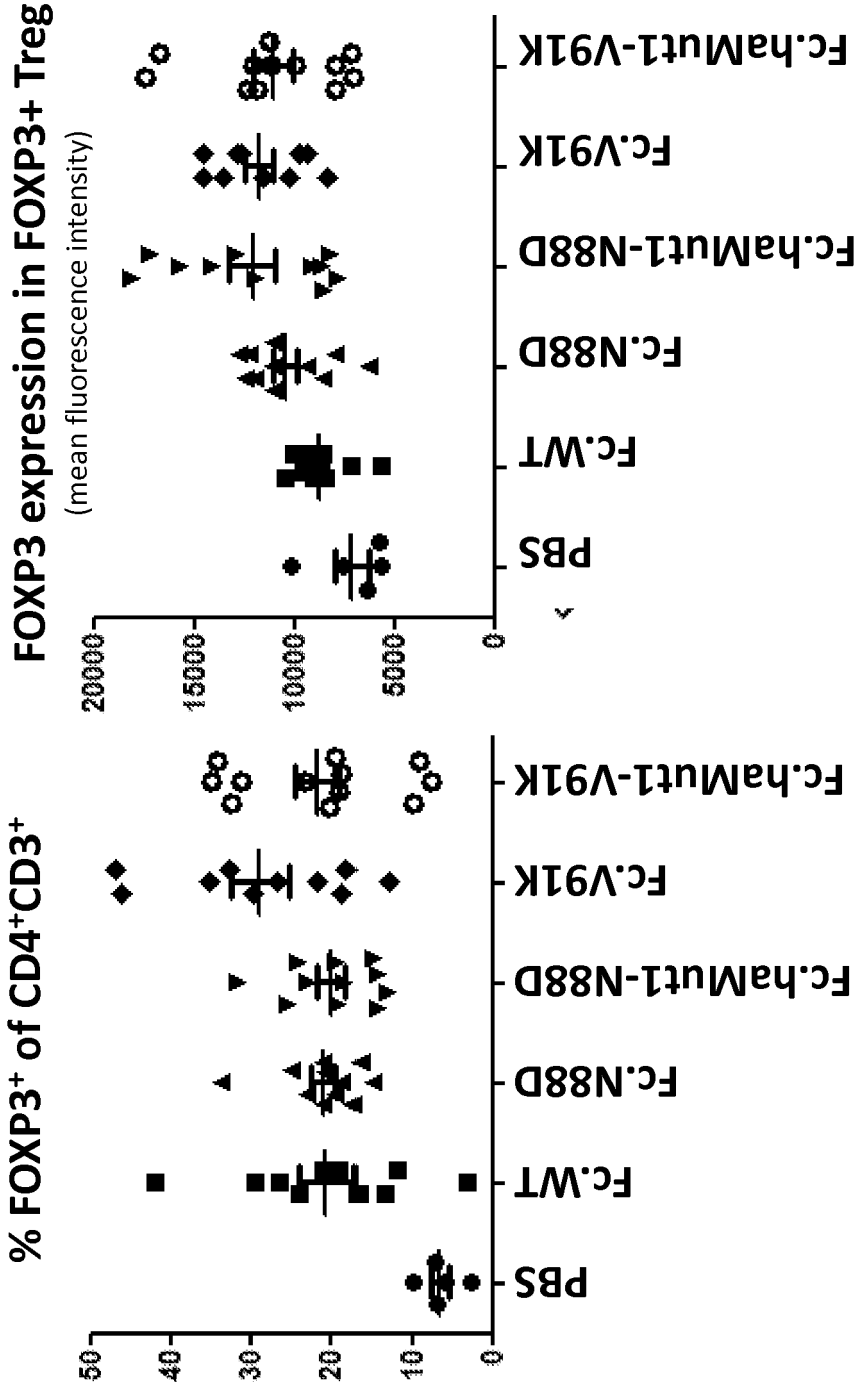

% FOXP3+ of CD4+CD3+
50
40
30
20
10
0
PBS
Fc.WT
Fc.N88D
Fc.haMut1-N88D
Fc.V91K
Fc.haMut1-V91K
FOXP3 expression in FOXP3+ Treg
(mean fluorescence intensity)
20000
15000
10000
5000
0
PBS
Fc.WT
Fc.N88D
Fc.haMut1-N88D
Fc.V91K
Fc.haMut1-V91K

FIG. 7A

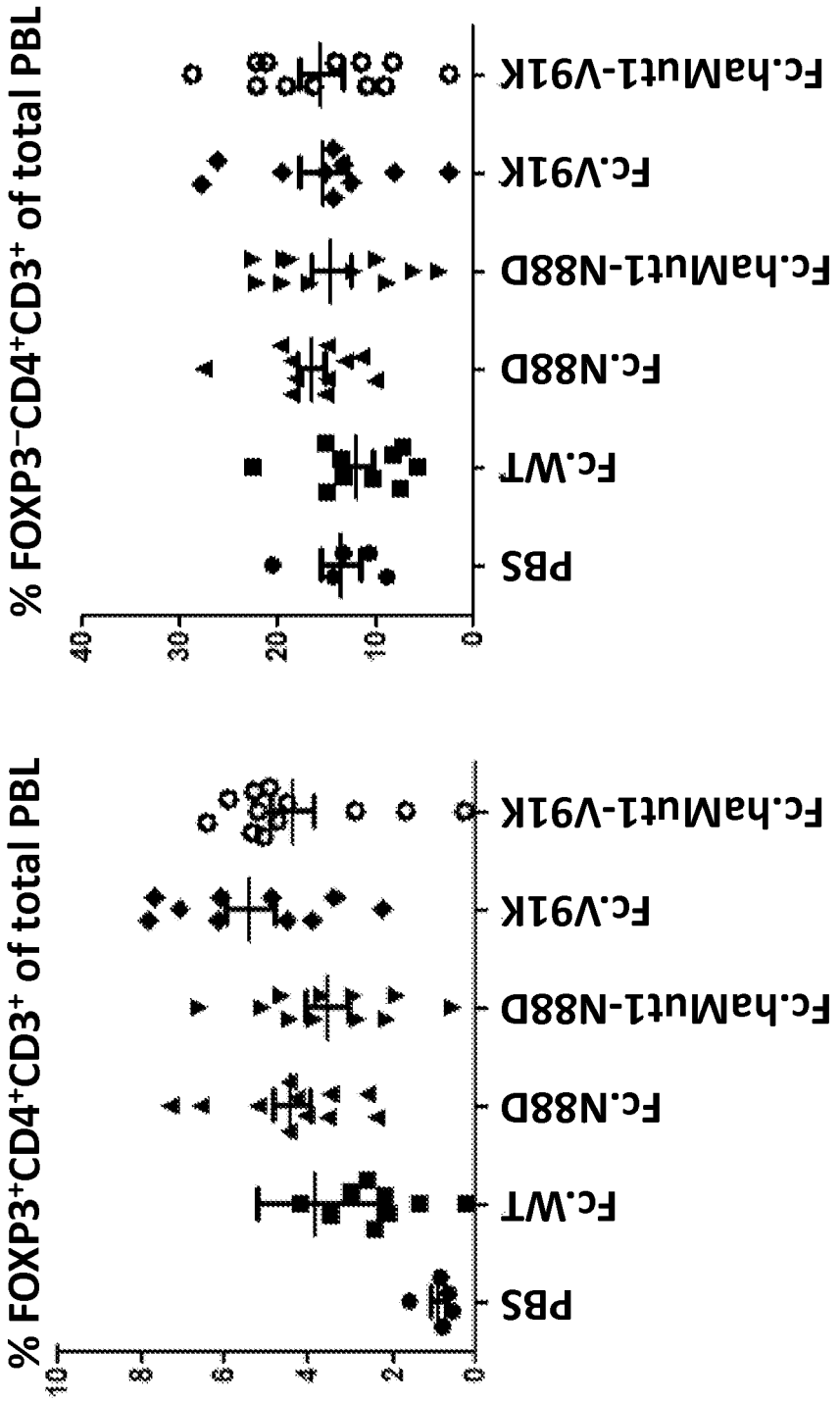

% FOXP3+CD4+CD3+ of total PBL
0
2
4
6
8
10
PBS
Fc.WT
Fc.N88D
Fc.haMut1-N88D
Fc.V91K
Fc.haMut1-V91K
% FOXP3−CD4+CD3+ of total PBL
0
10
20
30
40
PBS
Fc.WT
Fc.N88D
Fc.haMut1-N88D
Fc.V91K
Fc.haMut1-V91K

FIG. 7B

FIG. 8

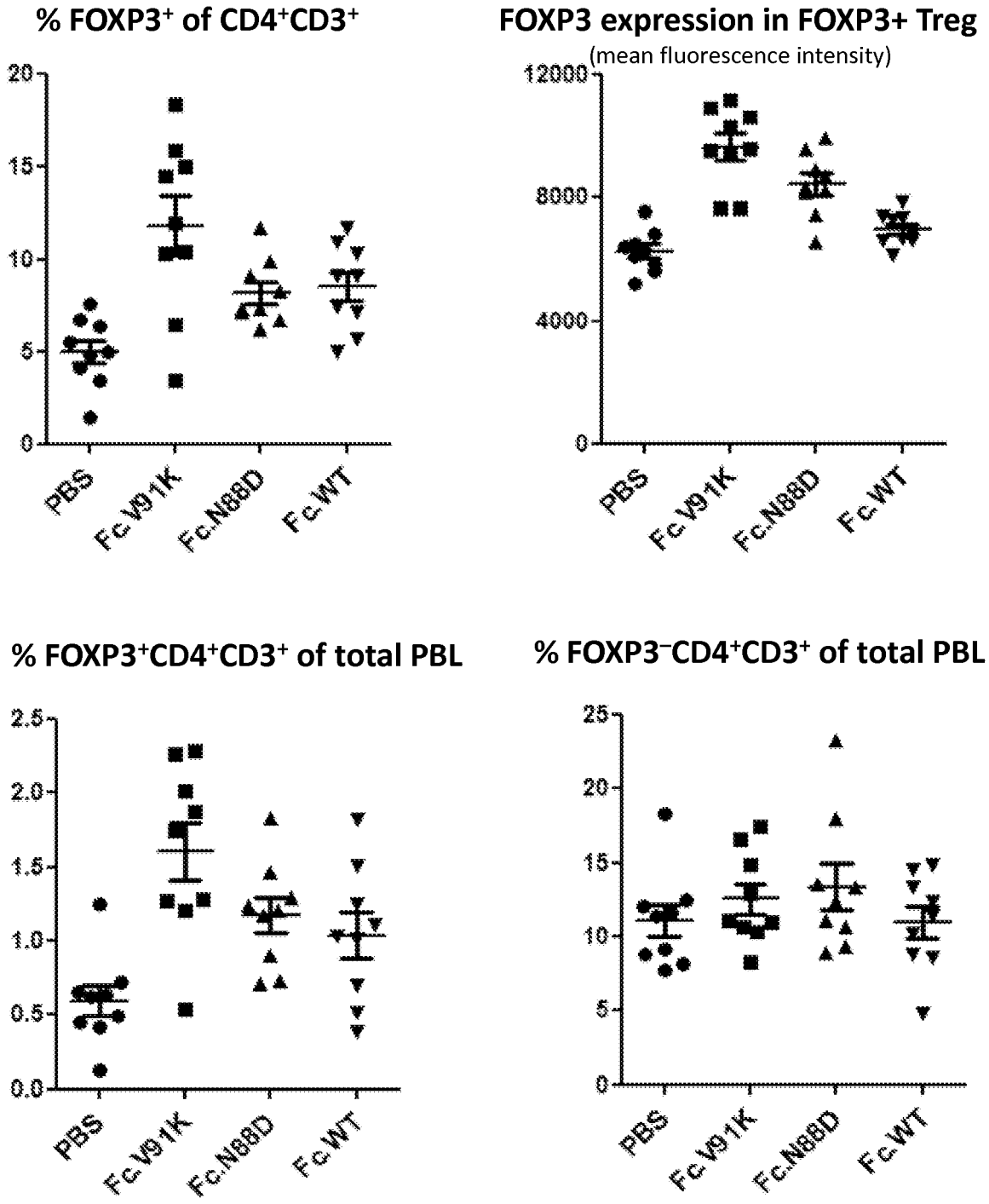

% FOXP3+ of CD4+CD3+
20
15
10
5
0
PBS
Fc.V91K
Fc.N88D
Fc.WT
FOXP3 expression in FOXP3+ Treg
(mean fluorescence intensity)
12000
8000
4000
0
PBS
Fc.V91K
Fc.N88D
Fc.WT
% FOXP3+CD4+CD3+ of total PBL
2.5
2.0
1.5
1.0
0.5
0.0
PBS
Fc.V91K
Fc.N88D
Fc.WT
% FOXP3−CD4+CD3+ of total PBL
25
20
15
10
5
0
PBS
Fc.V91K
Fc.N88D
Fc.WT

FIG. 9A

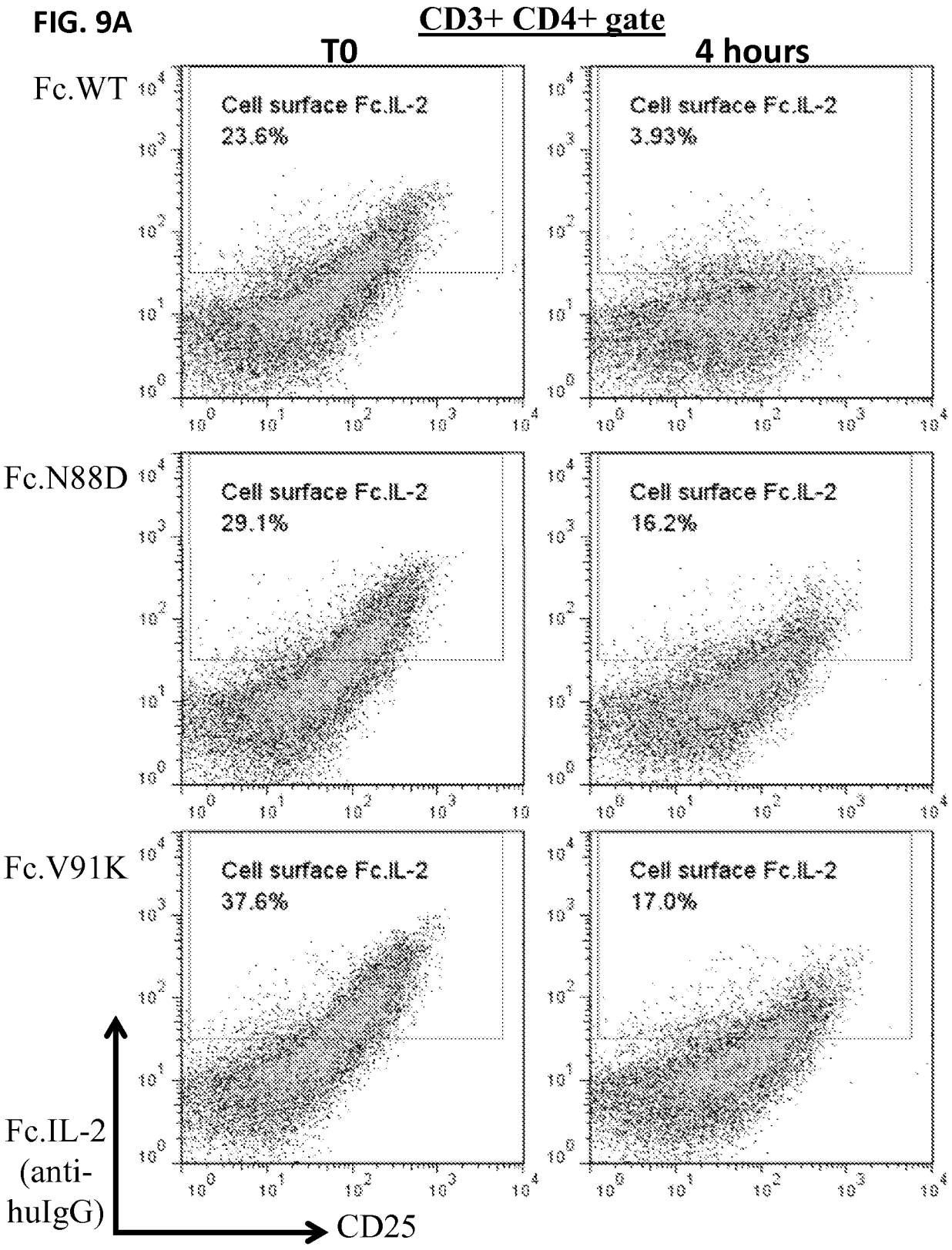

CD3+ CD4+ gate
T0
4 hours
Fc.WT
Fc.N88D
Fc.V91K
Cell surface Fc.IL-2
23.6%
Cell surface Fc.IL-2
3.93%
Cell surface Fc.IL-2
29.1%
Cell surface Fc.IL-2
16.2%
Cell surface Fc.IL-2
37.6%
Cell surface Fc.IL-2
17.0%
Fc.IL-2 (anti-huIgG)
CD25

FIG. 9B

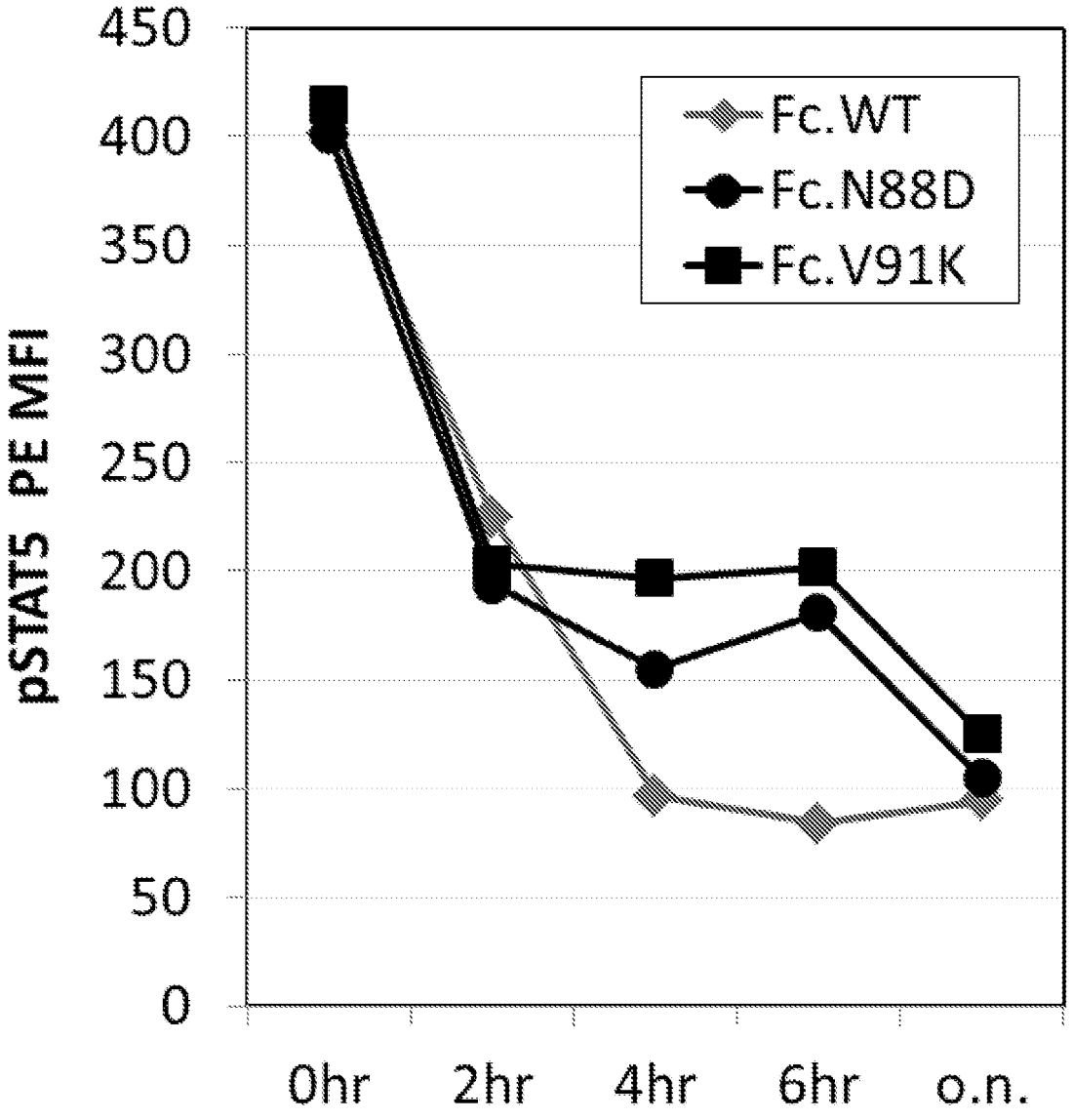
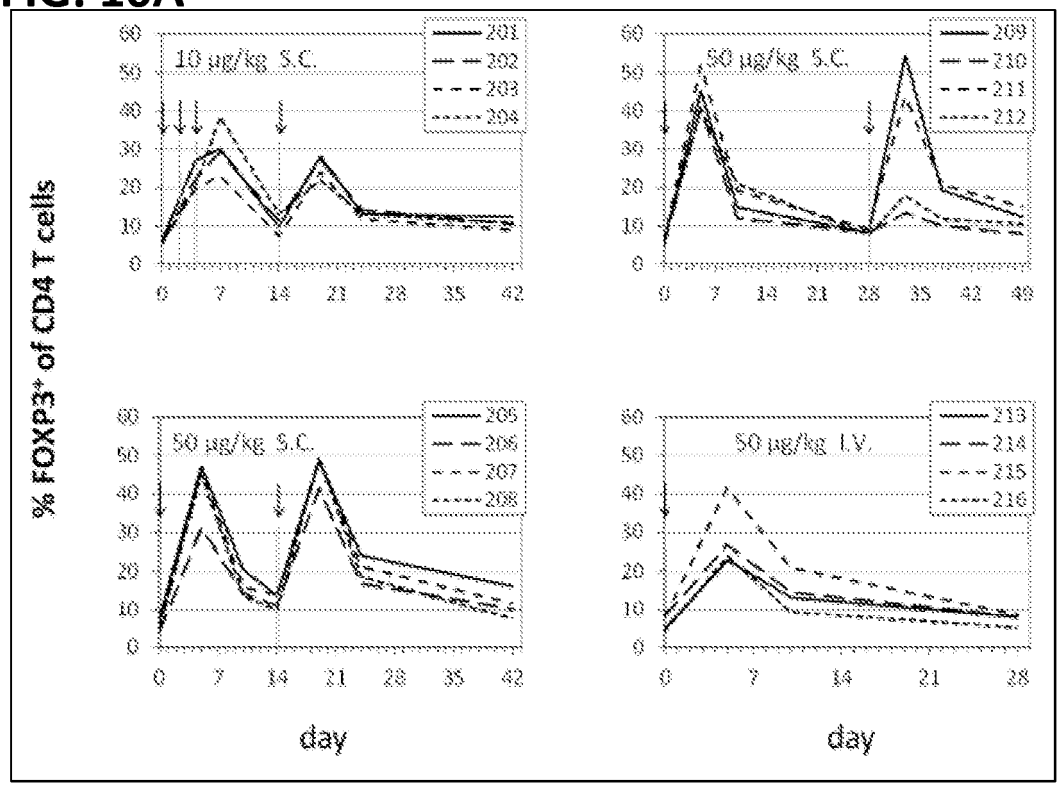

450
400
350
300
250
200
150
100
50
0
pSTAT5 PE MFI
Fc.WT
Fc.N88D
Fc.V91K
0hr
2hr
4hr
6hr
o.n.

FIG. 10A


% FOXP3+ of CD4 T cells
10 µg/kg S.C.
50 µg/kg S.C.
50 µg/kg S.C.
50 µg/kg I.V.
201
202
203
204
209
210
211
212
205
206
207
208
213
214
215
216
day
day

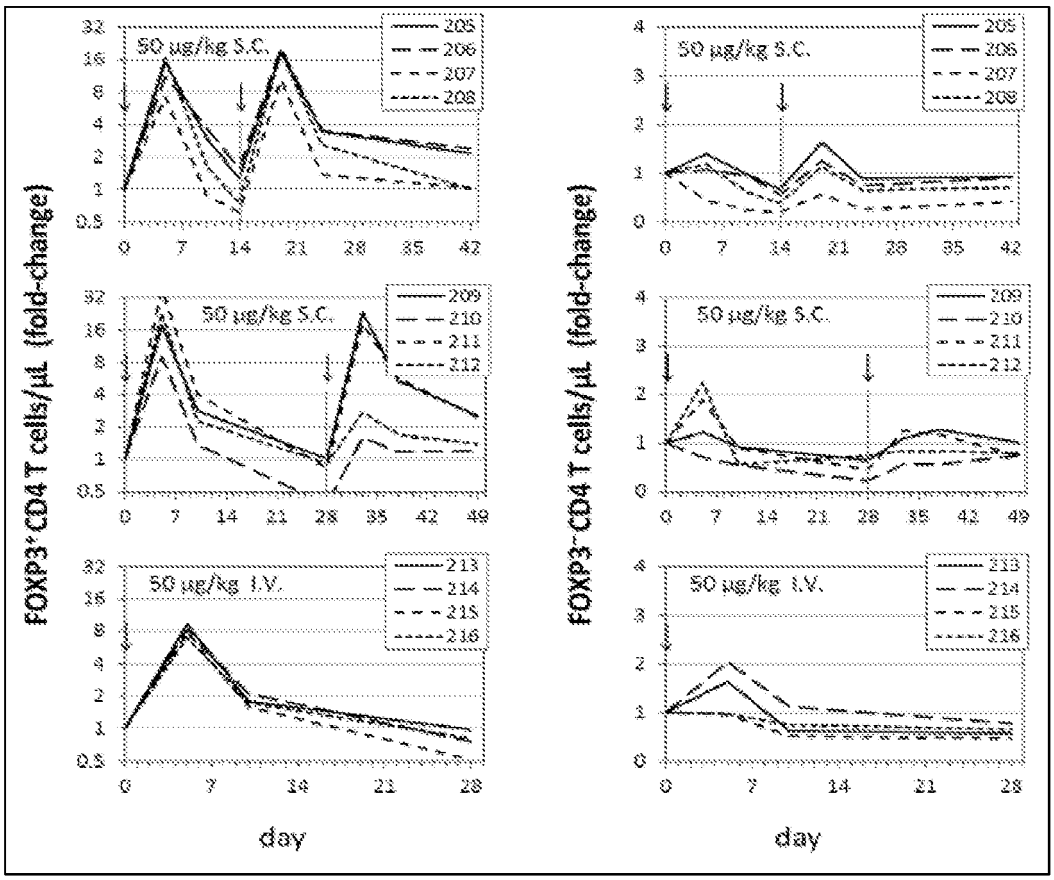

FOXP3+CD4 T cells/µL (fold-change)
FOXP3-CD4 T cells/µL (fold-change)
50 µg/kg S.C.
50 µg/kg S.C.
50 µg/kg I.V.
50 µg/kg S.C.
50 µg/kg S.C.
50 µg/kg I.V.
205
206
207
208
209
210
211
212
213
214
215
216
day
day

FIG. 10B

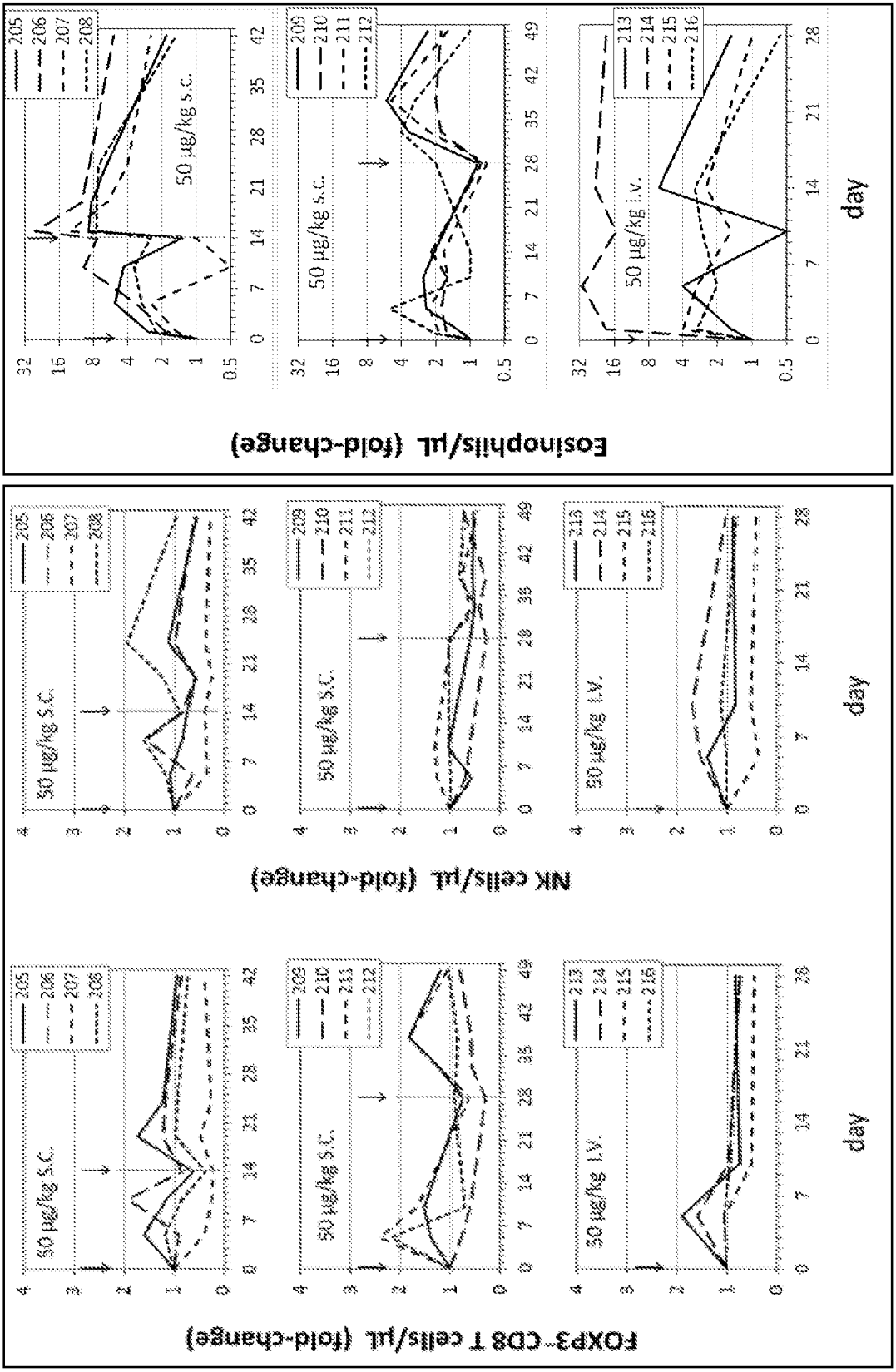
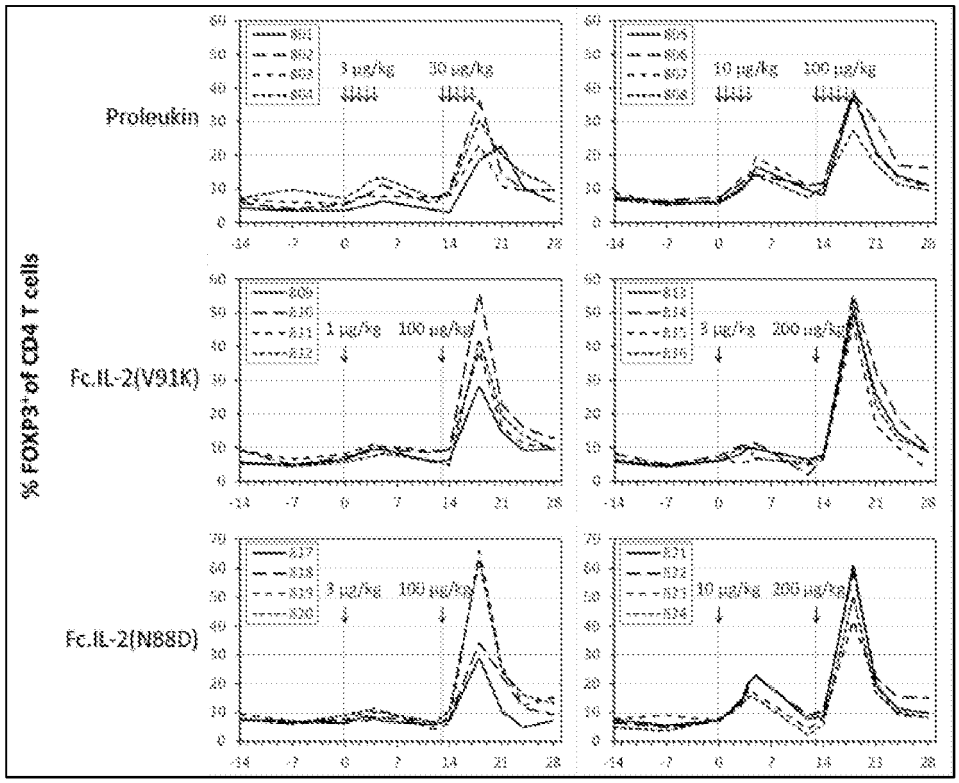

Eosinophils/μL (fold-change)
205
206
207
208
209
210
211
212
213
214
215
216
50 μg/kg s.c.
50 μg/kg i.v.
day
NK cells/μL (fold-change)
FOXP3- CD8 T cells/μL (fold-change)

FIG. 11A


% FOXP3+ of CD4 T cells
Proleukin
Fc.IL-2(V91K)
Fc.IL-2(N88D)
3 µg/kg
30 µg/kg
10 µg/kg
100 µg/kg
1 µg/kg
200 µg/kg
30 µg/kg
300 µg/kg

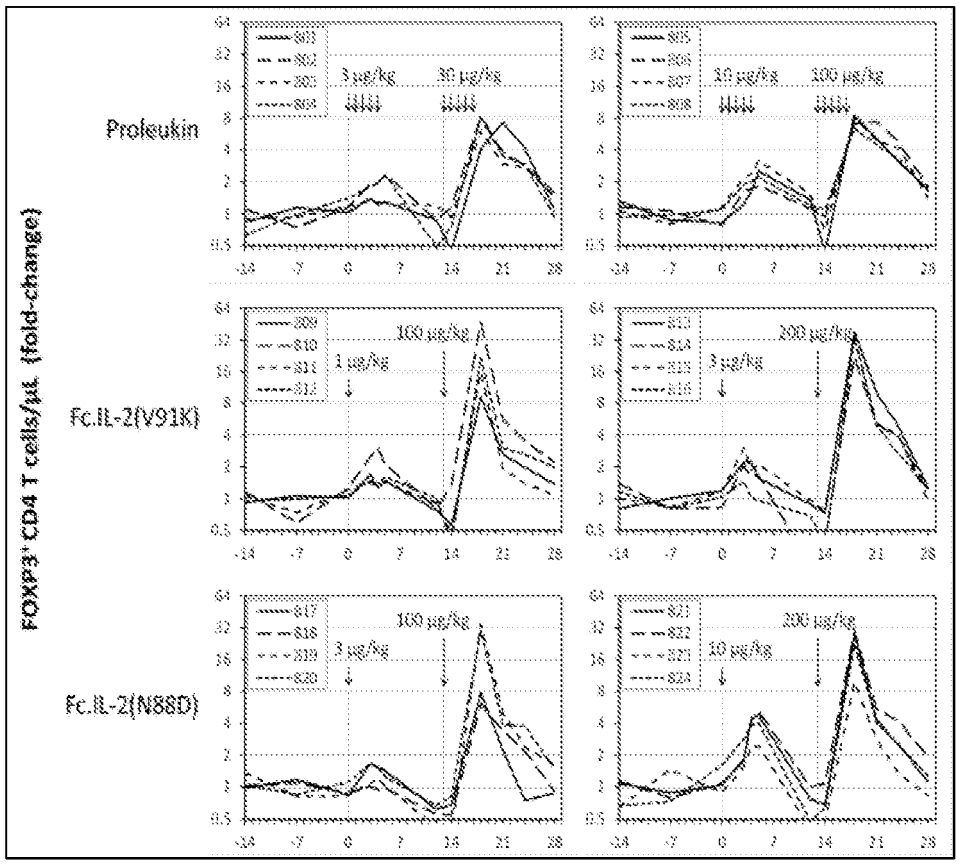

FOXP3+ CD4 T cells/µL (fold-change)
Proleukin
Fc.IL-2(V91K)
Fc.IL-2(N88D)
3 µg/kg
30 µg/kg
10 µg/kg
100 µg/kg
1 µg/kg
200 µg/kg
300 µg/kg

FIG. 11B

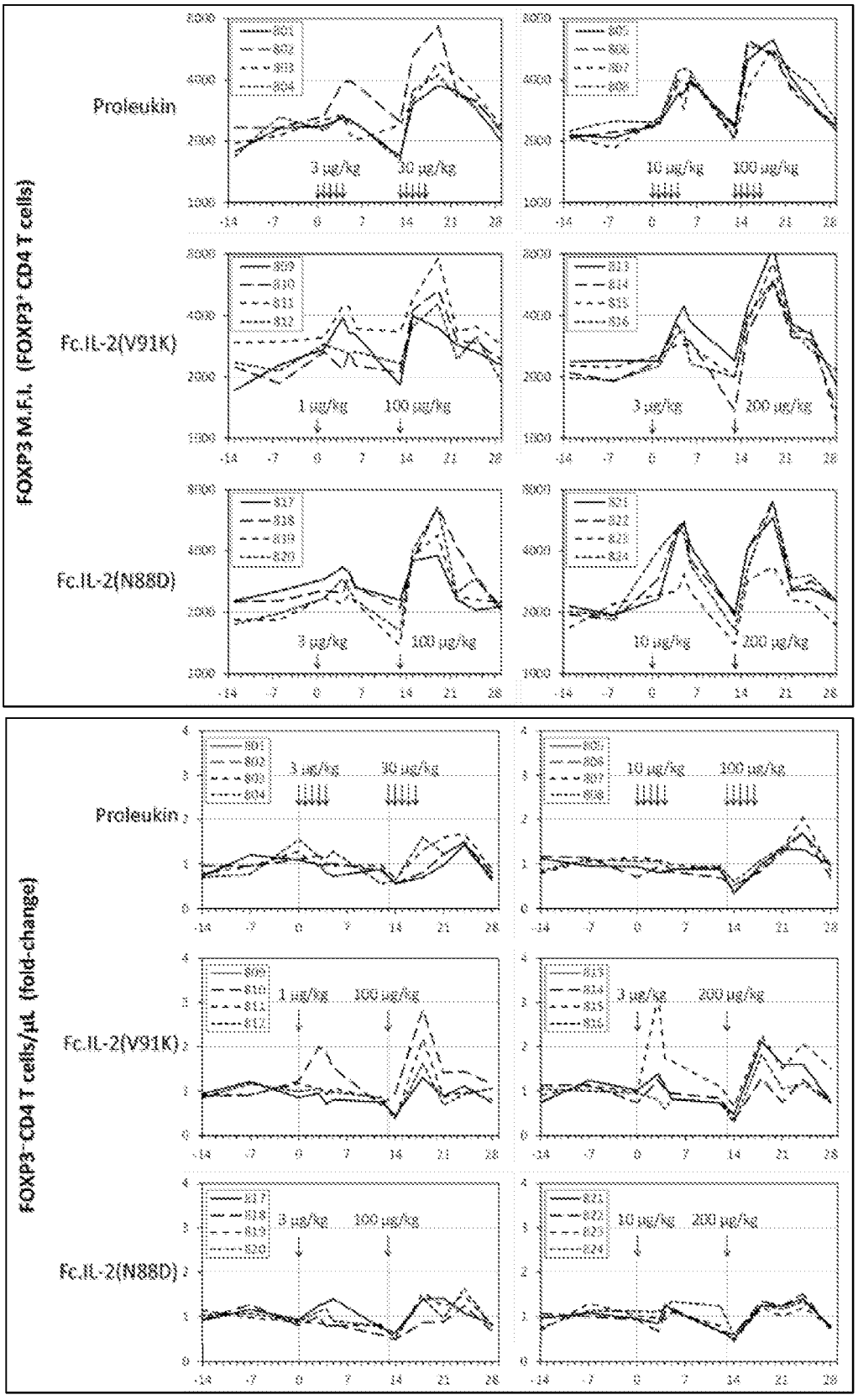

FOXP3 M.F.I. (FOXP3+ CD4 T cells)
FOXP3+CD4 T cells/μL (fold-change)
Proleukin
Fc.IL-2(V91K)
Fc.IL-2(N88D)
3 μg/kg
30 μg/kg
10 μg/kg
100 μg/kg
1 μg/kg
200 μg/kg

FIG. 11C

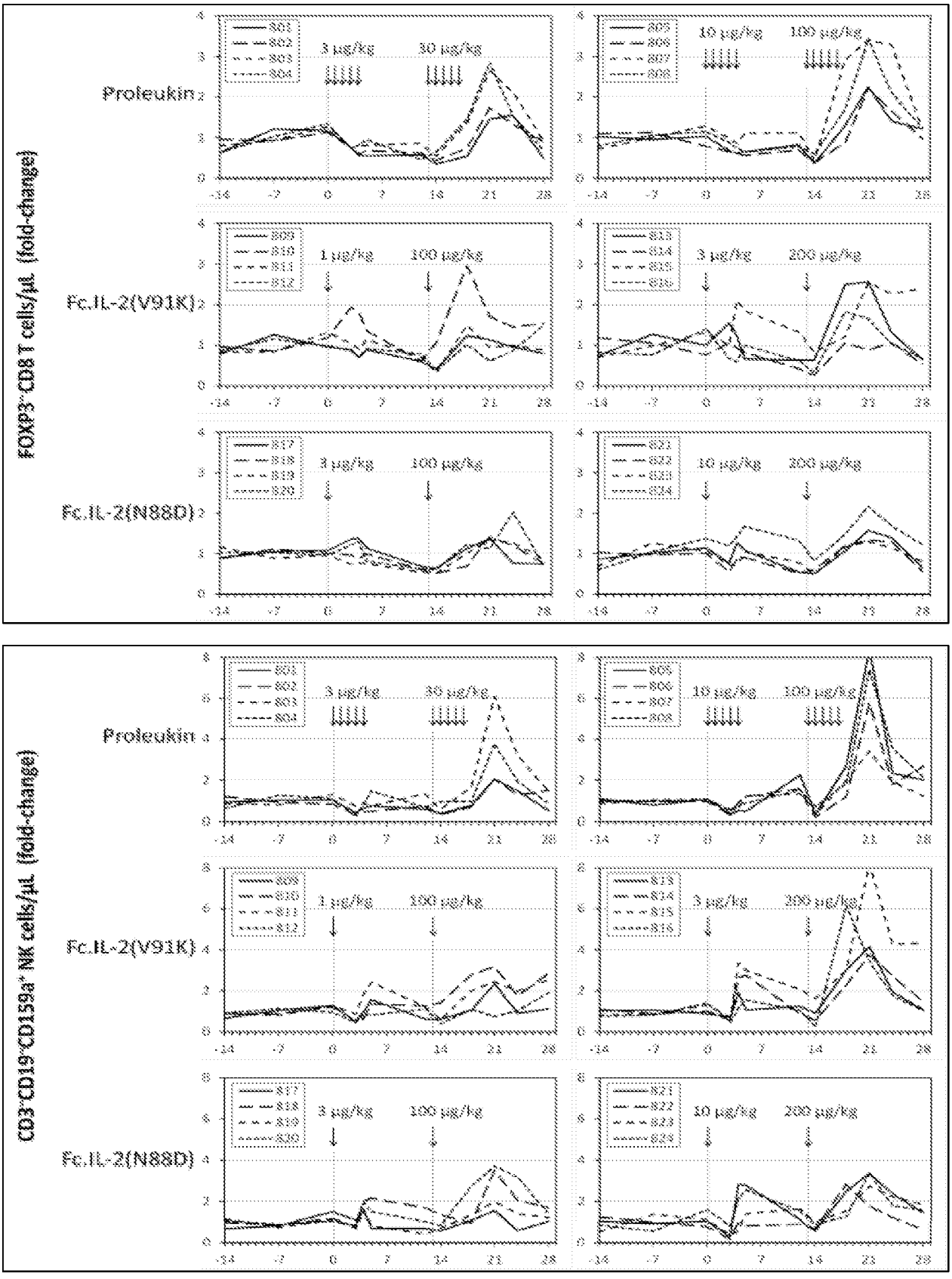

FOXP3⁻CD8 T cells/μL (fold-change)
Proleukin
Fc.IL-2(V91K)
Fc.IL-2(N88D)
3 μg/kg
30 μg/kg
10 μg/kg
100 μg/kg
1 μg/kg
100 μg/kg
3 μg/kg
200 μg/kg
3 μg/kg
100 μg/kg
10 μg/kg
200 μg/kg
CD3⁻CD19⁻CD159a⁺ NK cells/μL (fold-change)
Proleukin
Fc.IL-2(V91K)
Fc.IL-2(N88D)
3 μg/kg
30 μg/kg
10 μg/kg
100 μg/kg
1 μg/kg
100 μg/kg
3 μg/kg
200 μg/kg
3 μg/kg
100 μg/kg
10 μg/kg
200 μg/kg

FIG. 11D

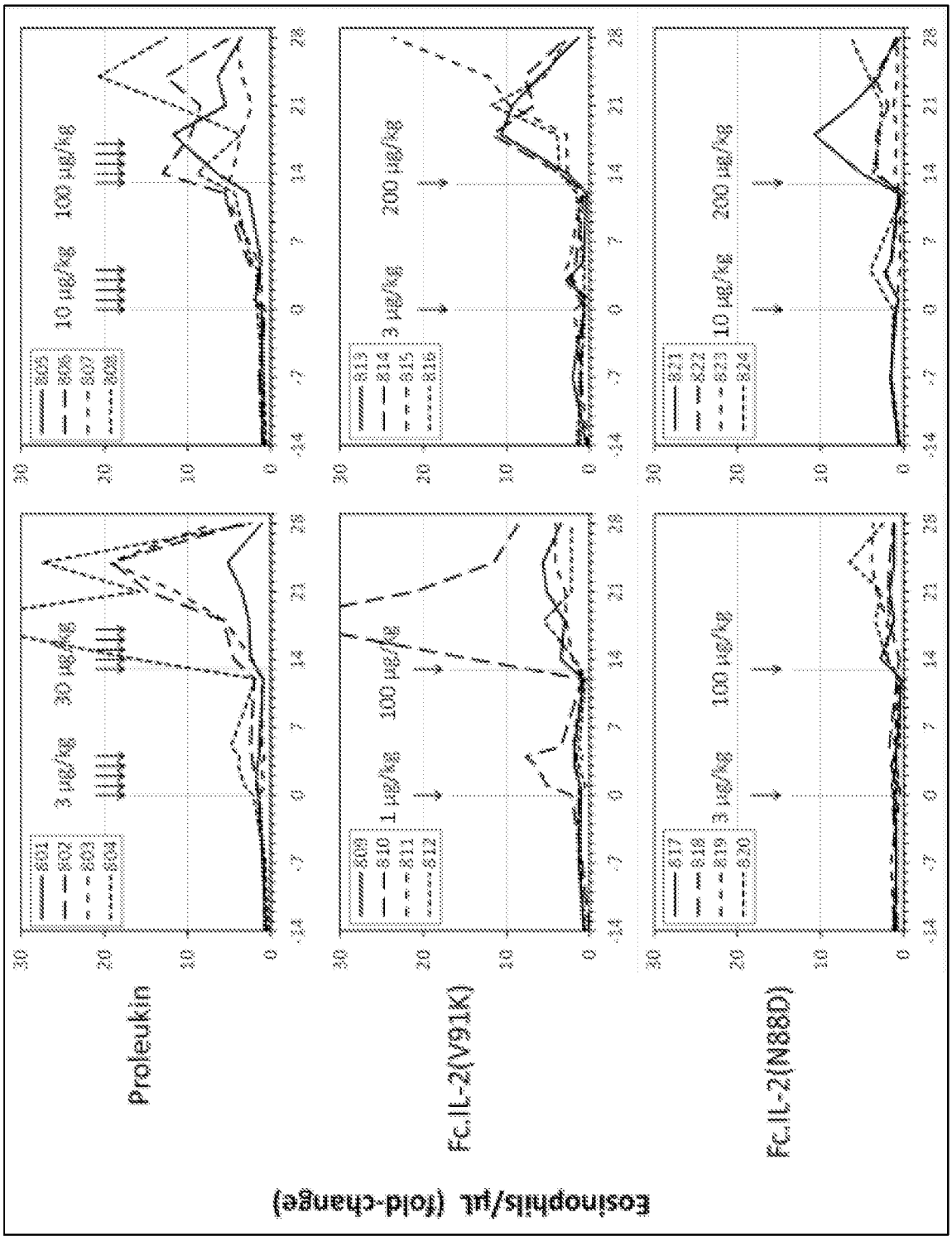

Proleukin
Fc.IL-2(V91K)
Fc.IL-2(N88D)
eosinophils/μL (fold-change)
801
802
803
804
3 μg/kg
30 μg/kg
809
810
811
812
1 μg/kg
100 μg/kg
817
818
819
820
3 μg/kg
100 μg/kg
805
806
807
808
10 μg/kg
100 μg/kg
813
814
815
816
3 μg/kg
200 μg/kg
821
822
823
824
10 μg/kg
200 μg/kg

FIG. 11E

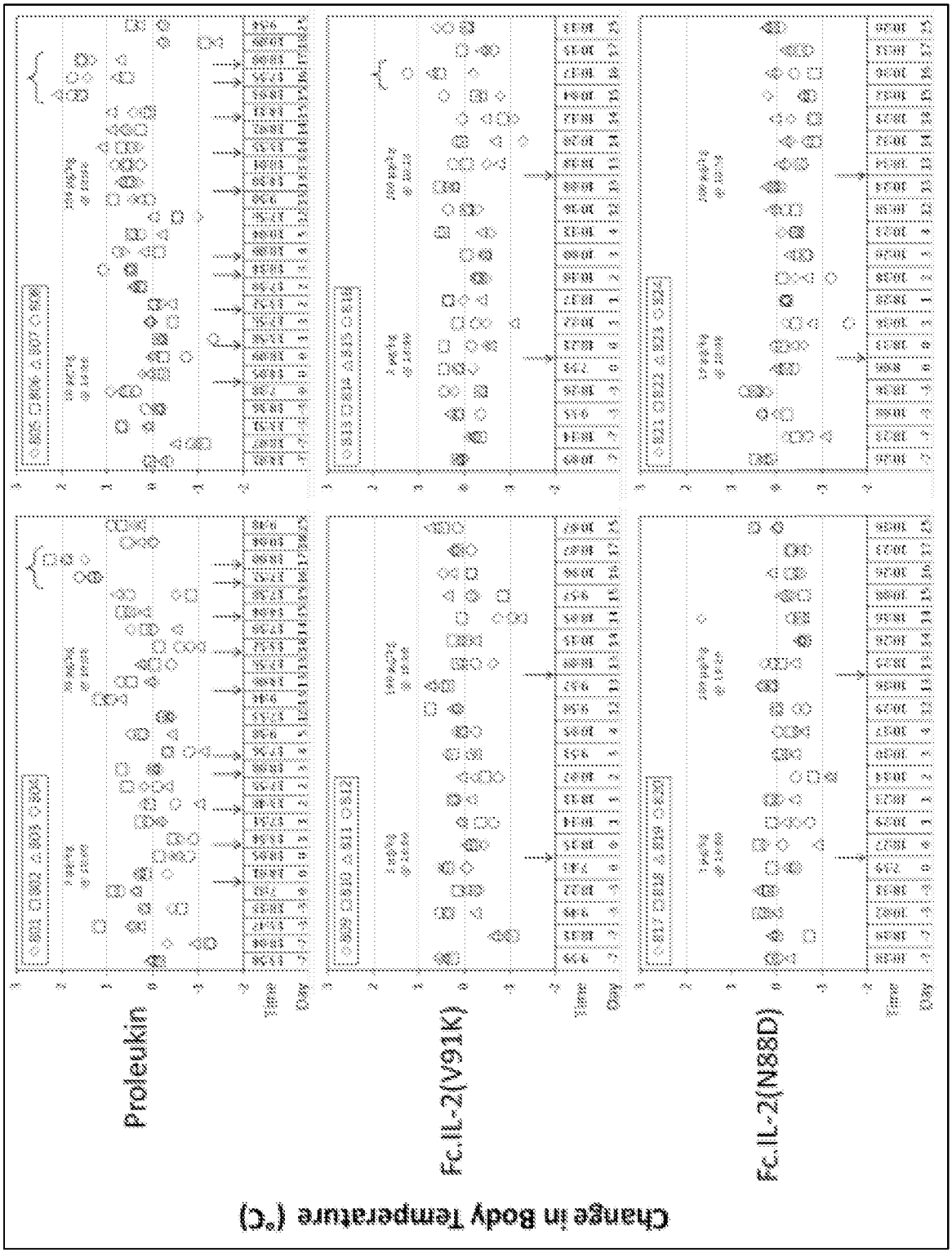
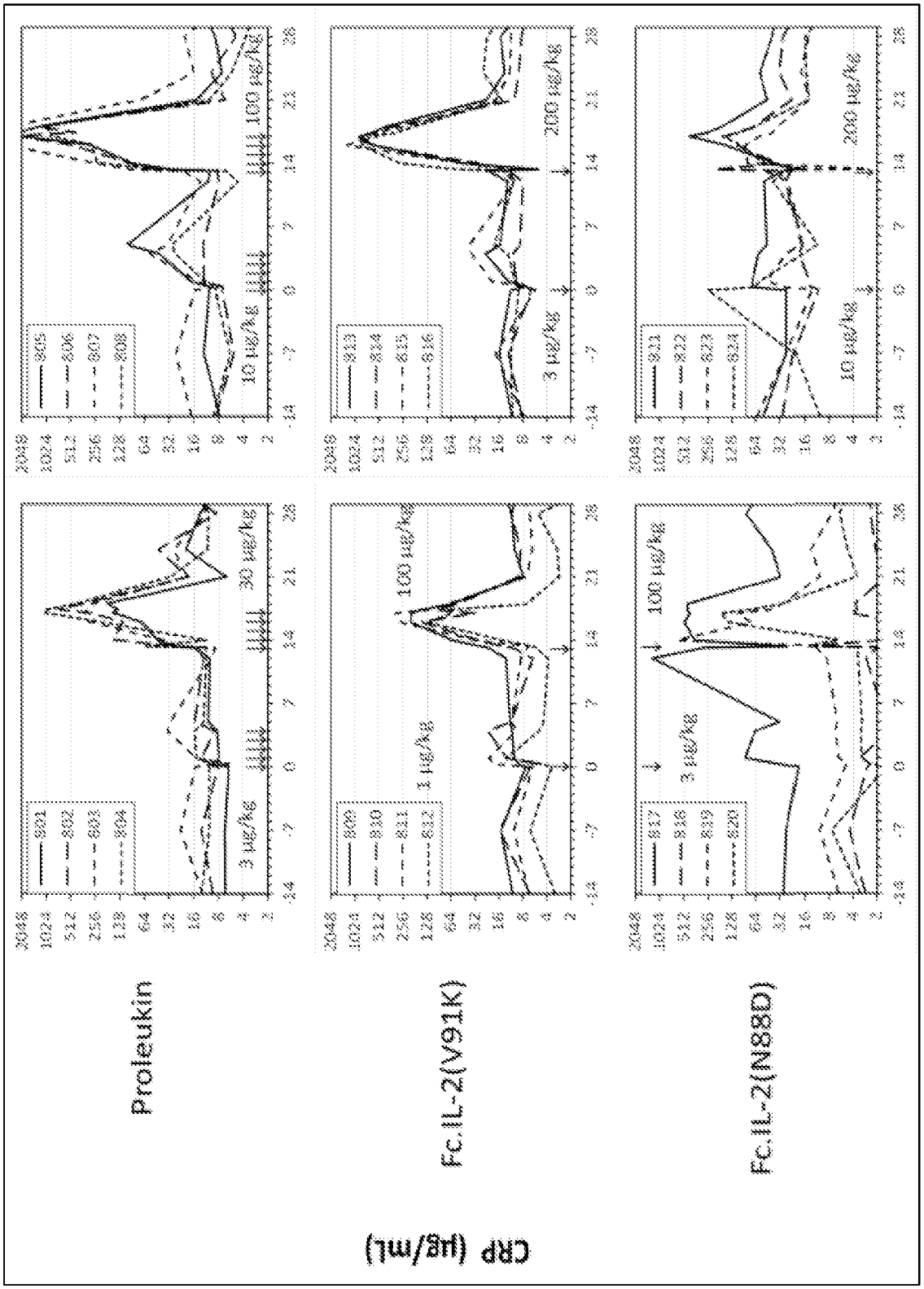

Change in Body Temperature (°C)
Proleukin
Fc.IL-2(V91K)
Fc.IL-2(N88D)

FIG. 11F


CRP (μg/mL)
Proleukin
Fc.IL-2(V91K)
Fc.IL-2(N88D)
3 μg/kg
10 μg/kg
30 μg/kg
1 μg/kg
100 μg/kg
200 μg/kg

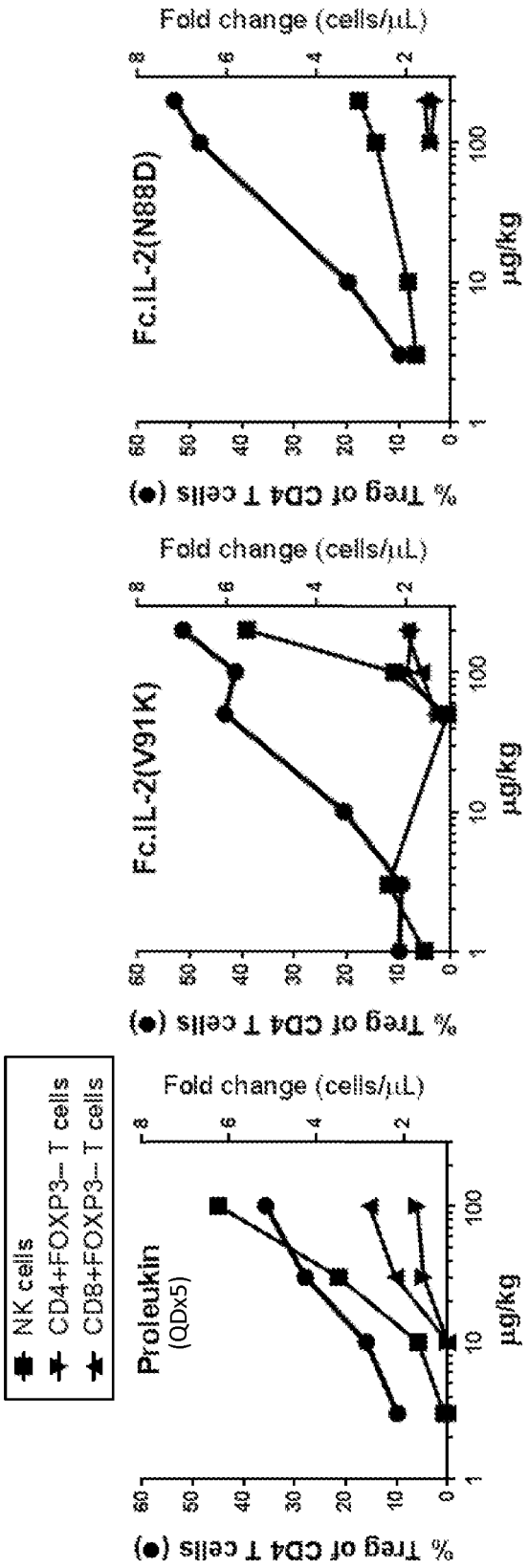


FIG. 12A

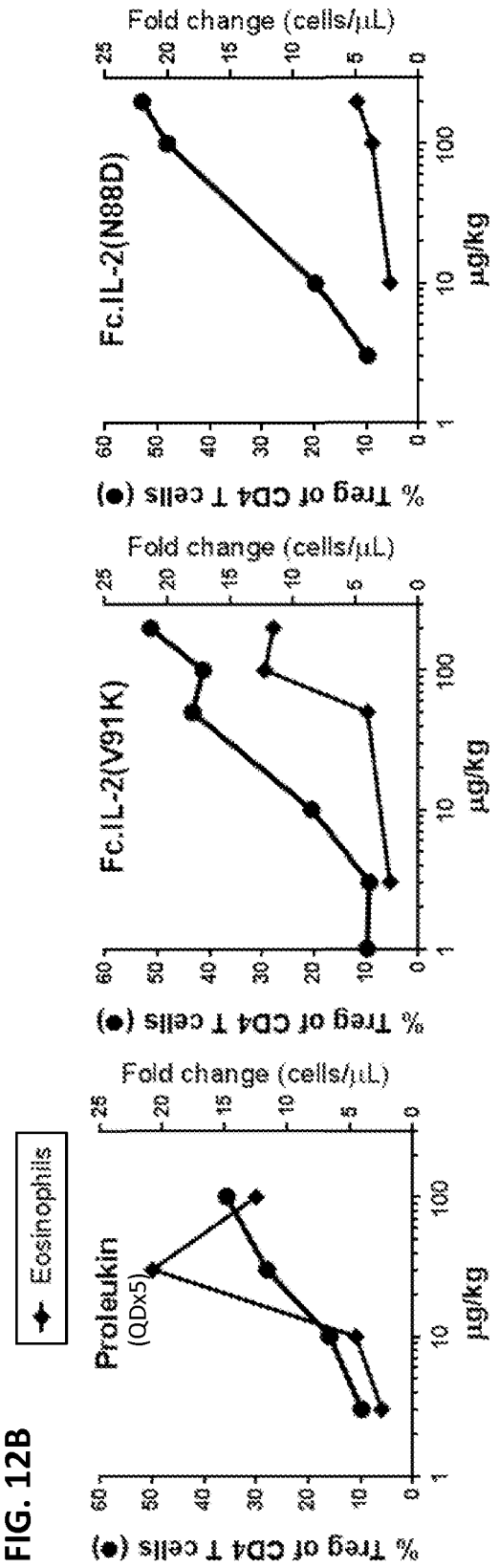


FIG. 12B

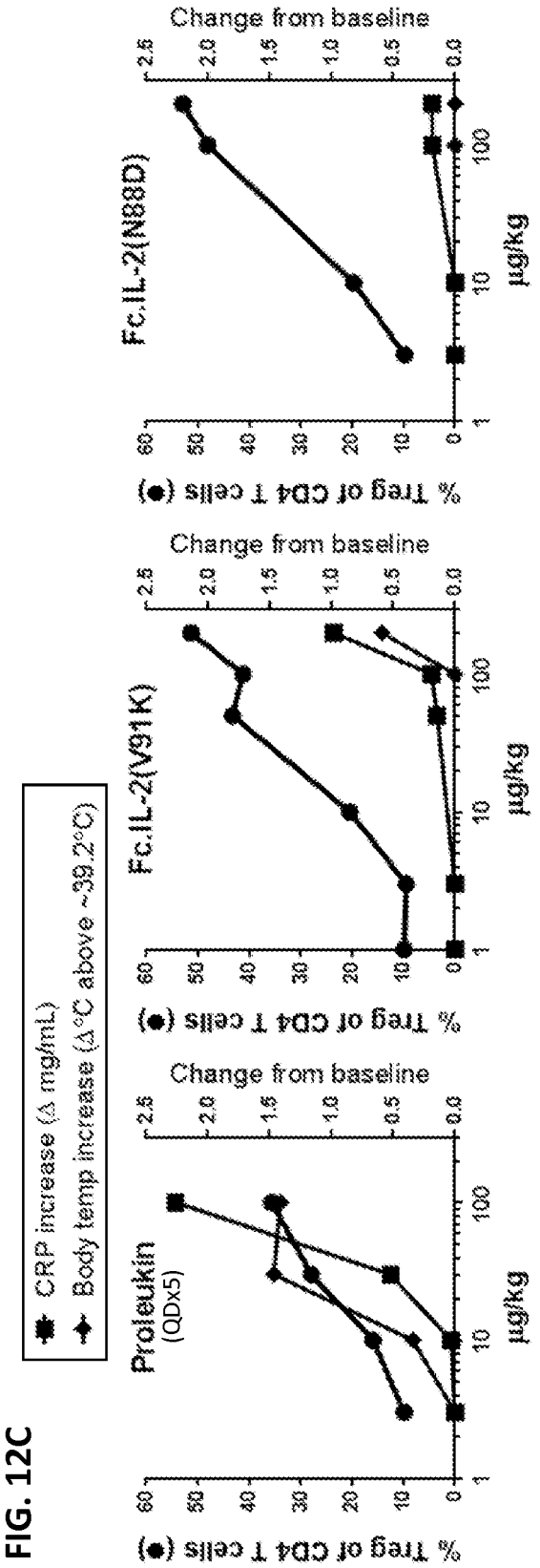


FIG. 12C

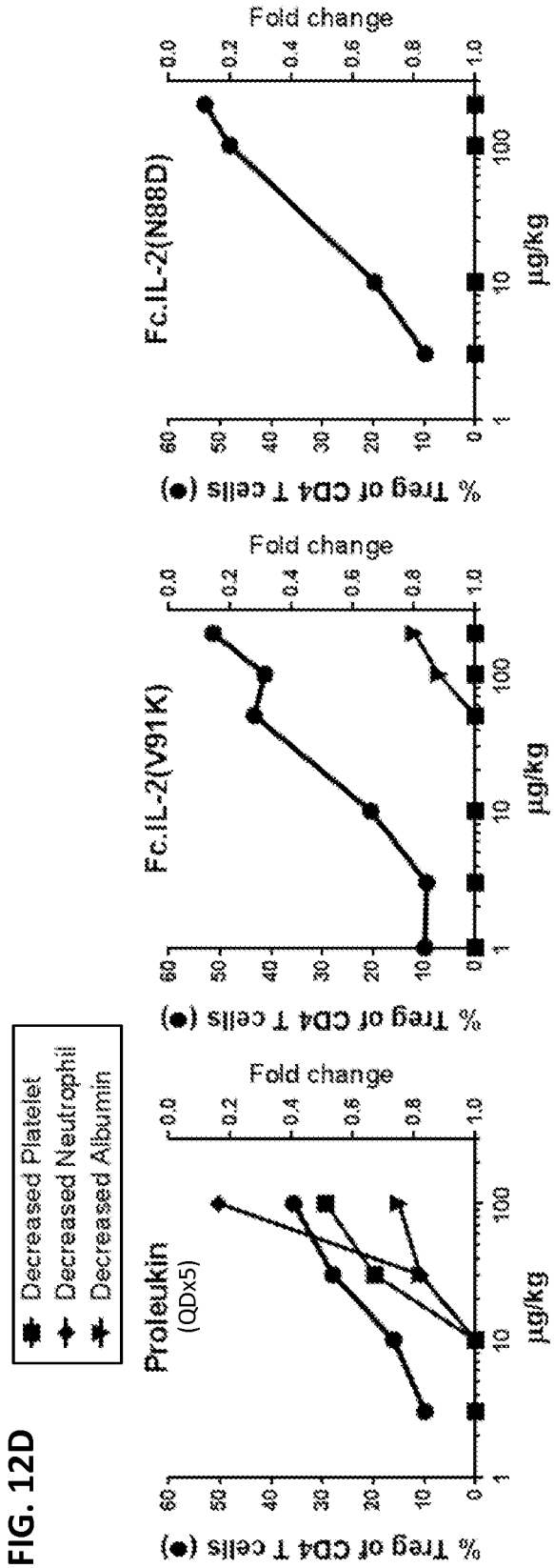
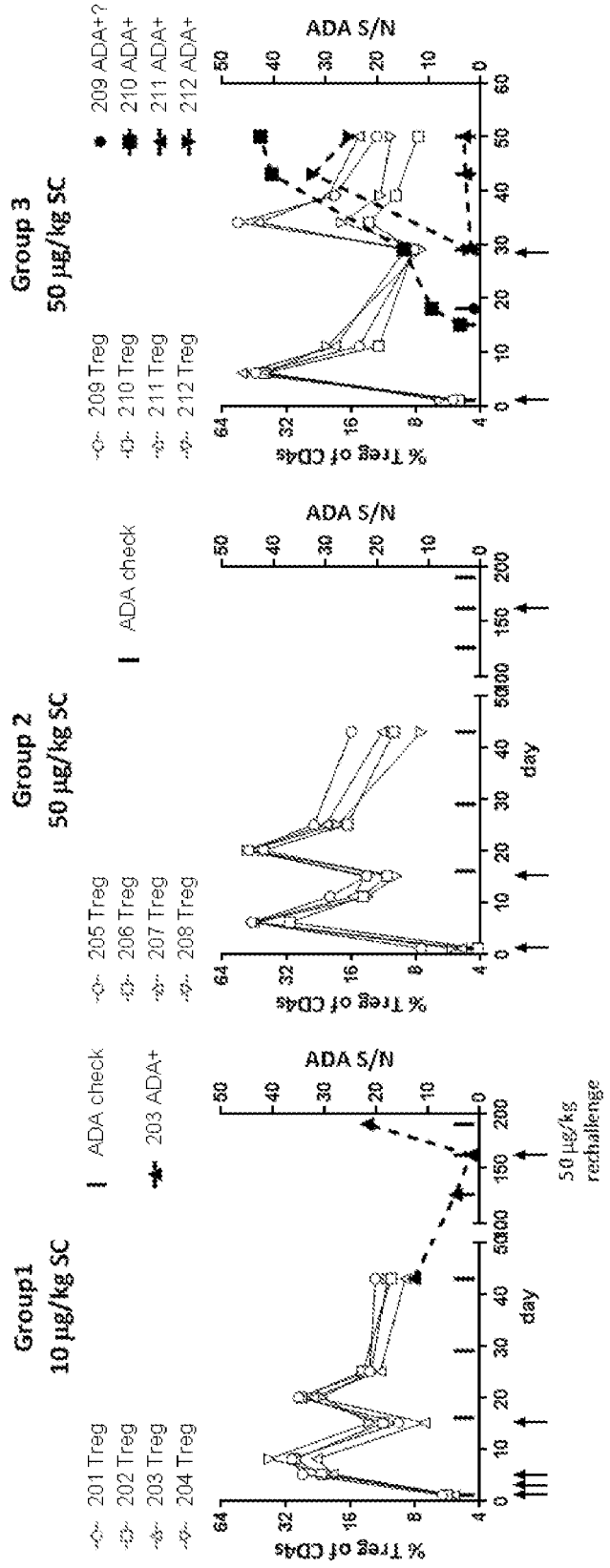


FIG. 12D

FIG. 13


Group1
10 µg/kg SC
Group 2
50 µg/kg SC
Group 3
50 µg/kg SC
201 Treg
202 Treg
203 Treg
204 Treg
205 Treg
206 Treg
207 Treg
208 Treg
209 Treg
210 Treg
211 Treg
212 Treg
ADA check
203 ADA+
209 ADA+?
210 ADA+
211 ADA+
212 ADA+
% Treg of CD4s
ADA S/N
day
50 µg/kg rechallenge