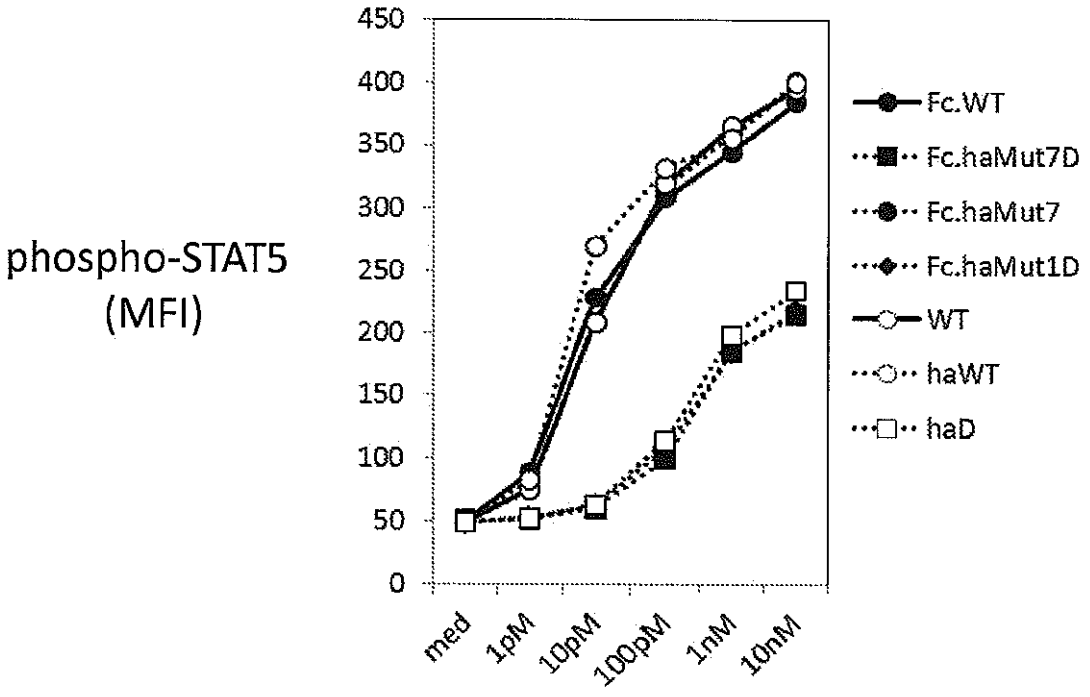
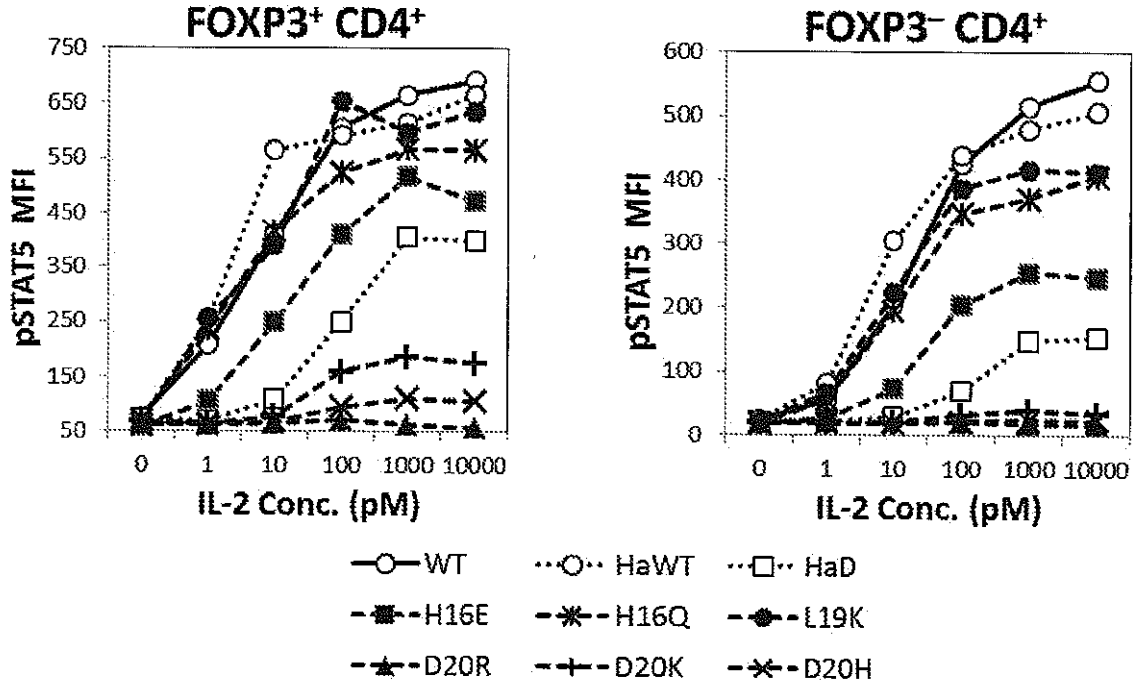

phospho-STAT5
(MFI)
450
400
350
300
250
200
150
100
50
0
med
1pM
10pM
100pM
1nM
10nM
Fc.WT
Fc.haMut7D
Fc.haMut7
Fc.haMut1D
WT
haWT
haD

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

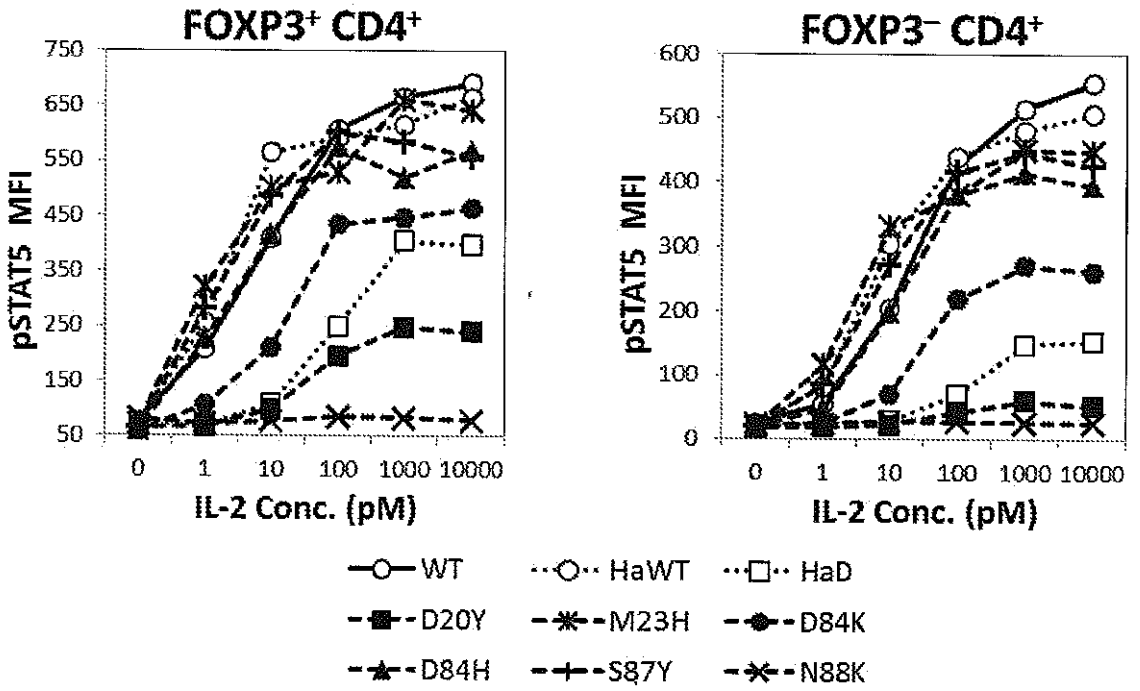
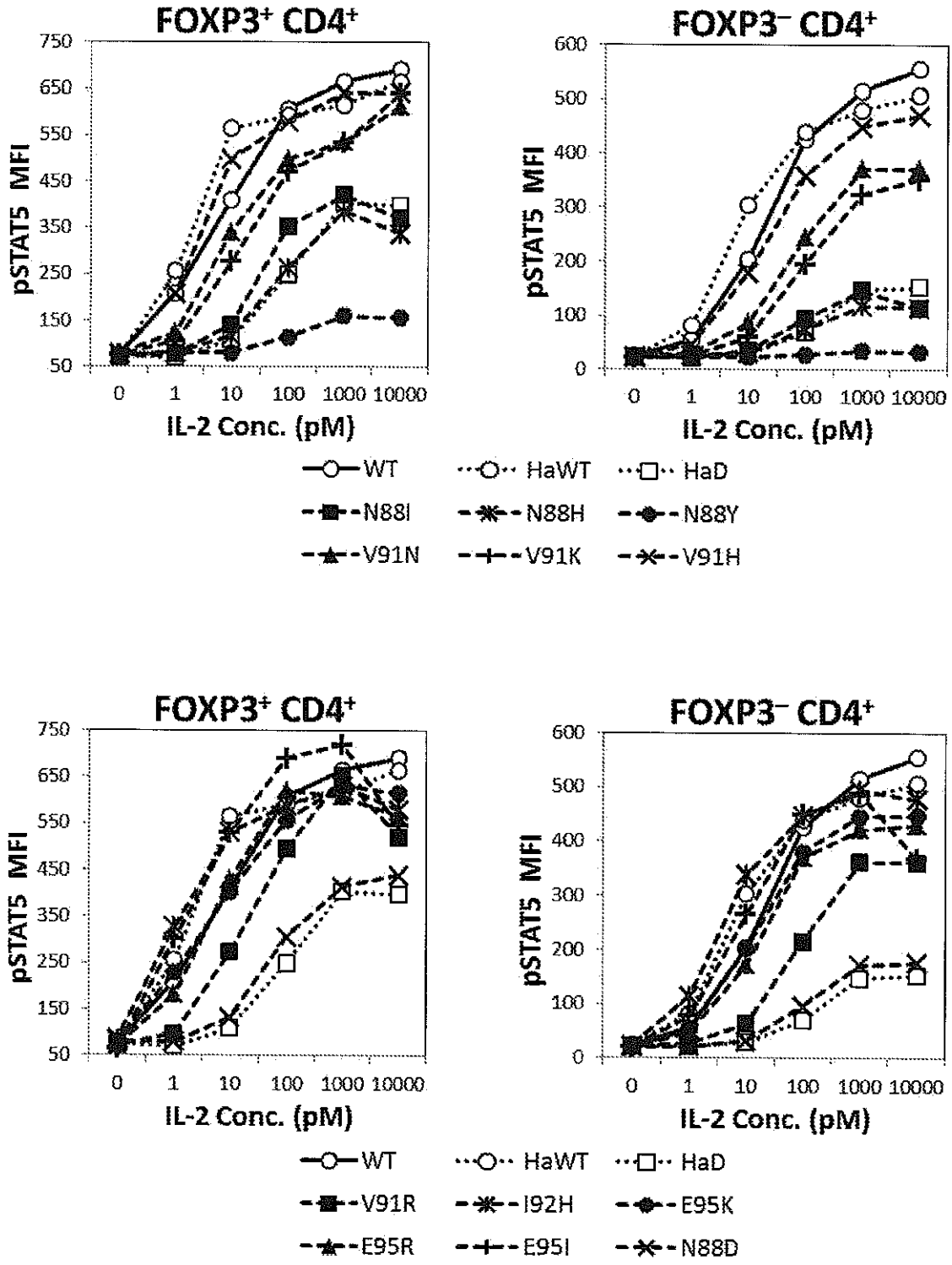

FOXP3+ CD4+
pSTAT5 MFI
750
650
550
450
350
250
150
50
0 1 10 100 1000 10000
IL-2 Conc. (pM)
FOXP3− CD4+
pSTAT5 MFI
600
500
400
300
200
100
0
0 1 10 100 1000 10000
IL-2 Conc. (pM)
WT
HaWT
HaD
D20Y
M23H
D84K
D84H
S87Y
N88K

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

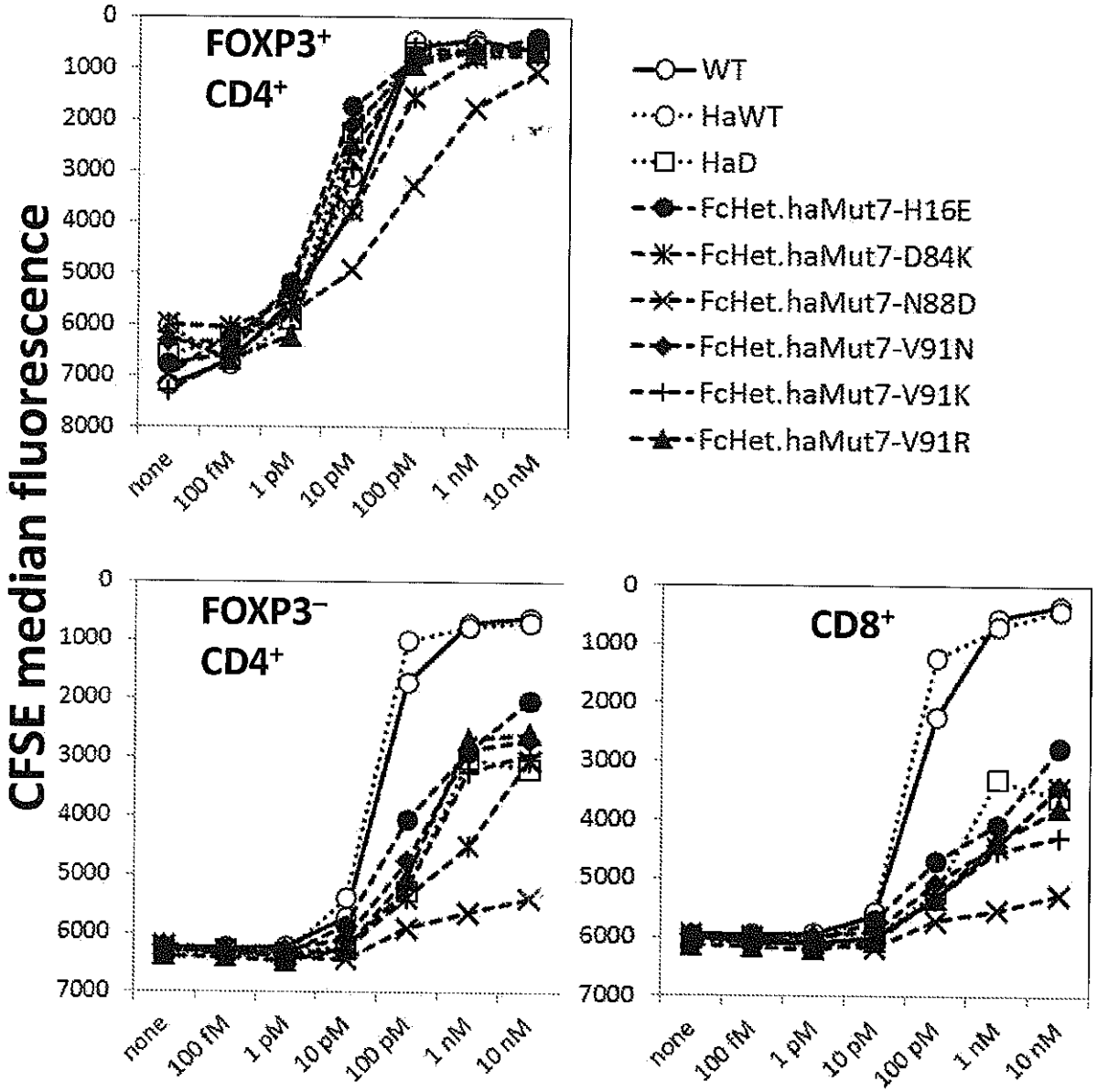

CFSE median fluorescence
FOXP3+ CD4+
FOXP3− CD4+
CD8+
0
1000
2000
3000
4000
5000
6000
7000
8000
none
100 fM
1 pM
10 pM
100 pM
1 nM
10 nM
WT
HaWT
HaD
FcHet.haMut7-H16E
FcHet.haMut7-D84K
FcHet.haMut7-N88D
FcHet.haMut7-V91N
FcHet.haMut7-V91K
FcHet.haMut7-V91R

FIG. 3

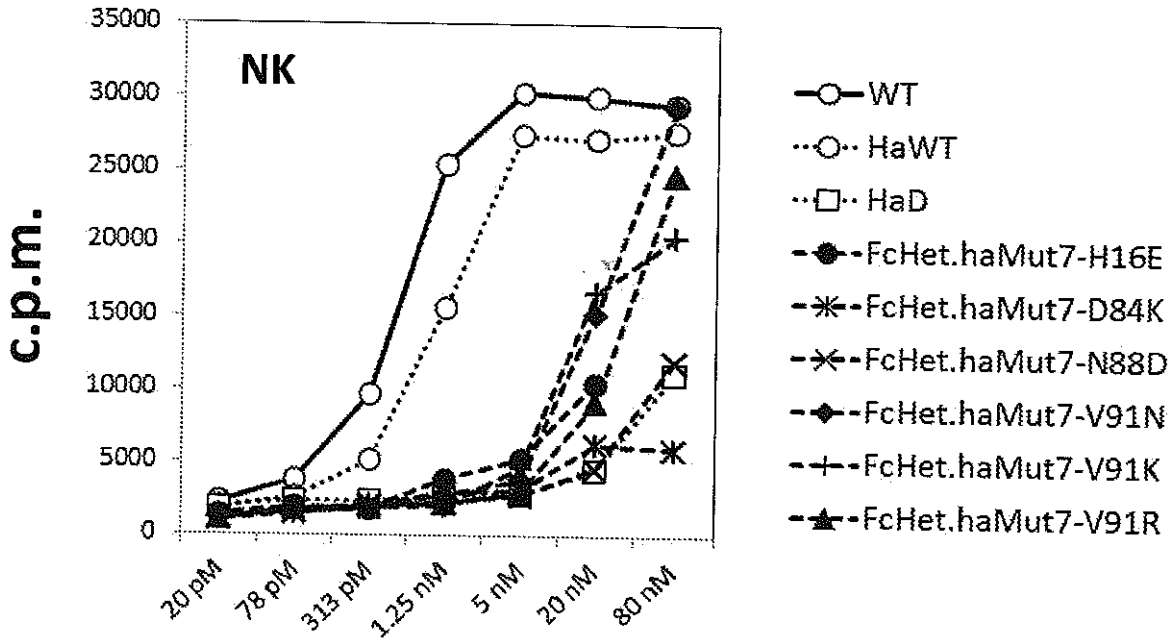

NK
c.p.m.
35000
30000
25000
20000
15000
10000
5000
0
20 pM
78 pM
313 pM
1.25 nM
5 nM
20 nM
80 nM
WT
HaWT
HaD
FcHet.haMut7-H16E
FcHet.haMut7-D84K
FcHet.haMut7-N88D
FcHet.haMut7-V91N
FcHet.haMut7-V91K
FcHet.haMut7-V91R

FIG. 4

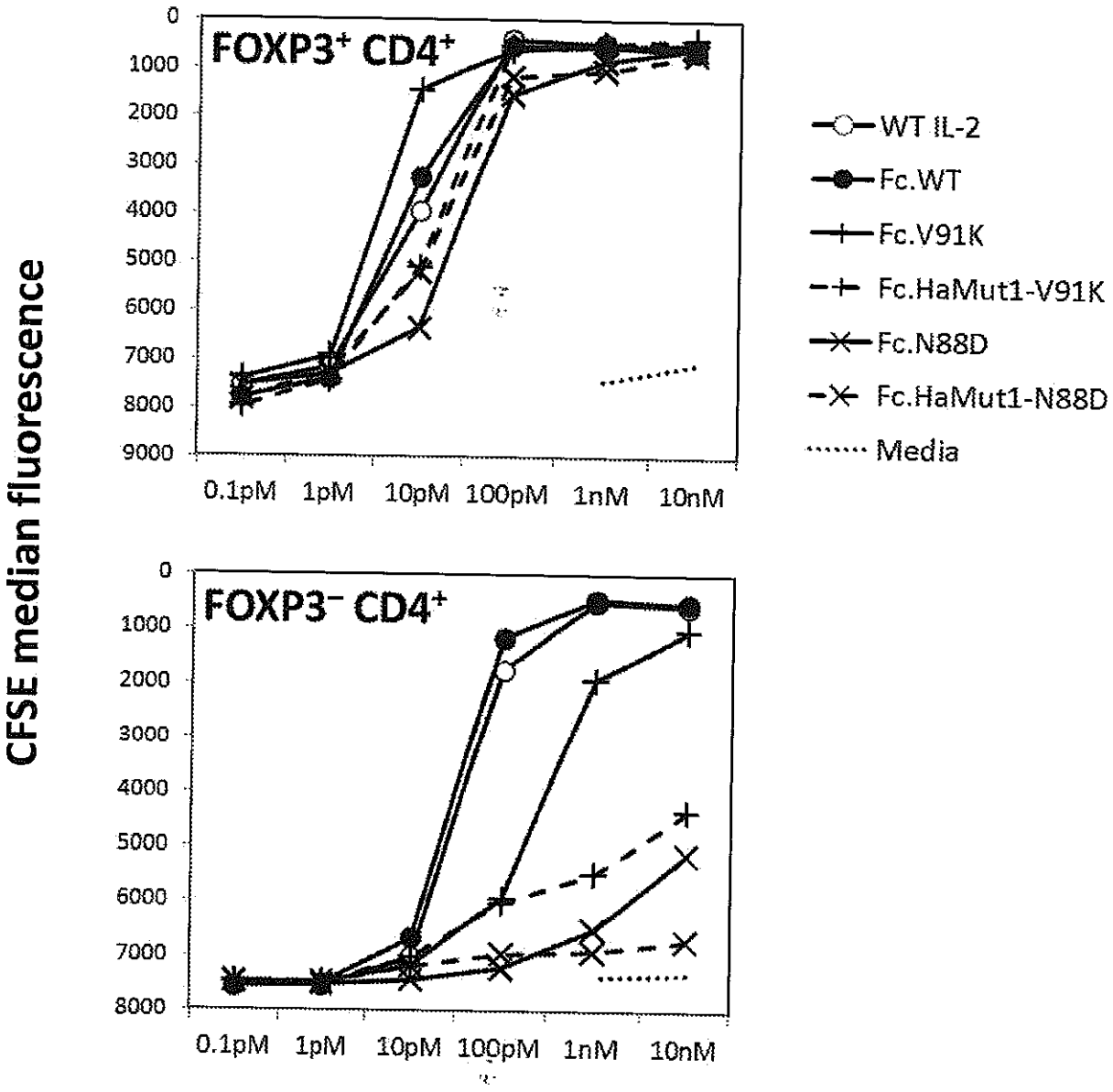

CFSE median fluorescence
FOXP3+ CD4+
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
0.1pM
1pM
10pM
100pM
1nM
10nM
FOXP3− CD4+
WT IL-2
Fc.WT
Fc.V91K
Fc.HaMut1-V91K
Fc.N88D
Fc.HaMut1-N88D
Media

FIG. 5

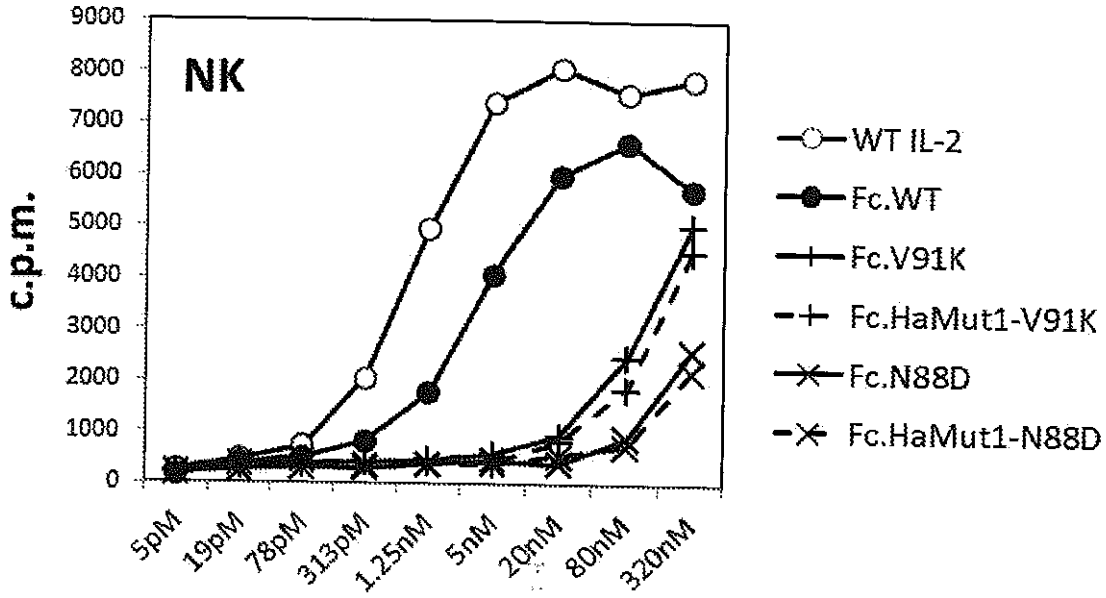

NK
c.p.m.
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
5pM
19pM
78pM
313pM
1.25nM
5nM
20nM
80nM
320nM
WT IL-2
Fc.WT
Fc.V91K
Fc.HaMut1-V91K
Fc.N88D
Fc.HaMut1-N88D

FIG. 6

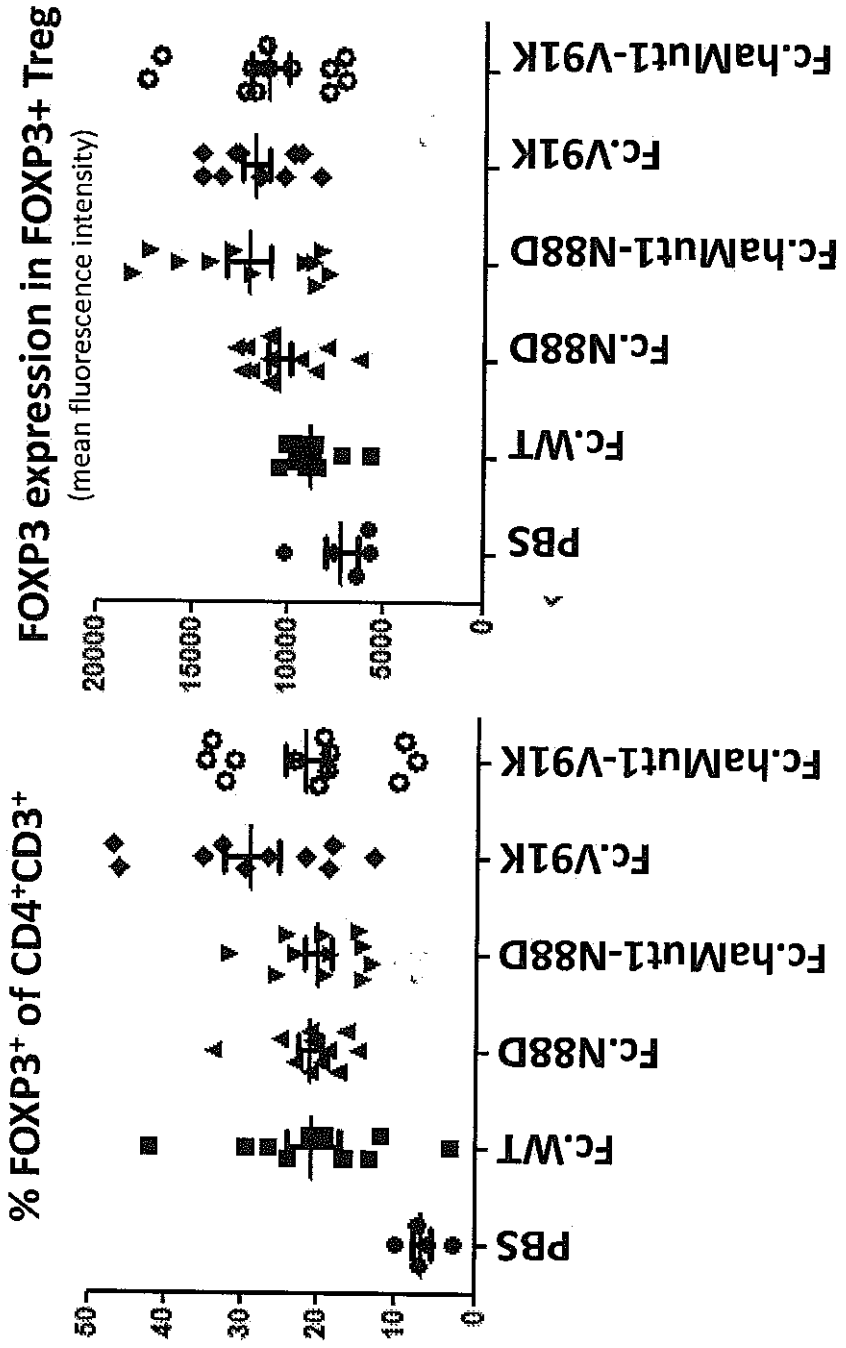

% FOXP3+ of CD4+CD3+
50
40
30
20
10
0
PBS
Fc.WT
Fc.N88D
Fc.haMut1-N88D
Fc.V91K
Fc.haMut1-V91K
FOXP3 expression in FOXP3+ Treg
(mean fluorescence intensity)
20000
15000
10000
5000
0
PBS
Fc.WT
Fc.N88D
Fc.haMut1-N88D
Fc.V91K
Fc.haMut1-V91K

FIG. 7A

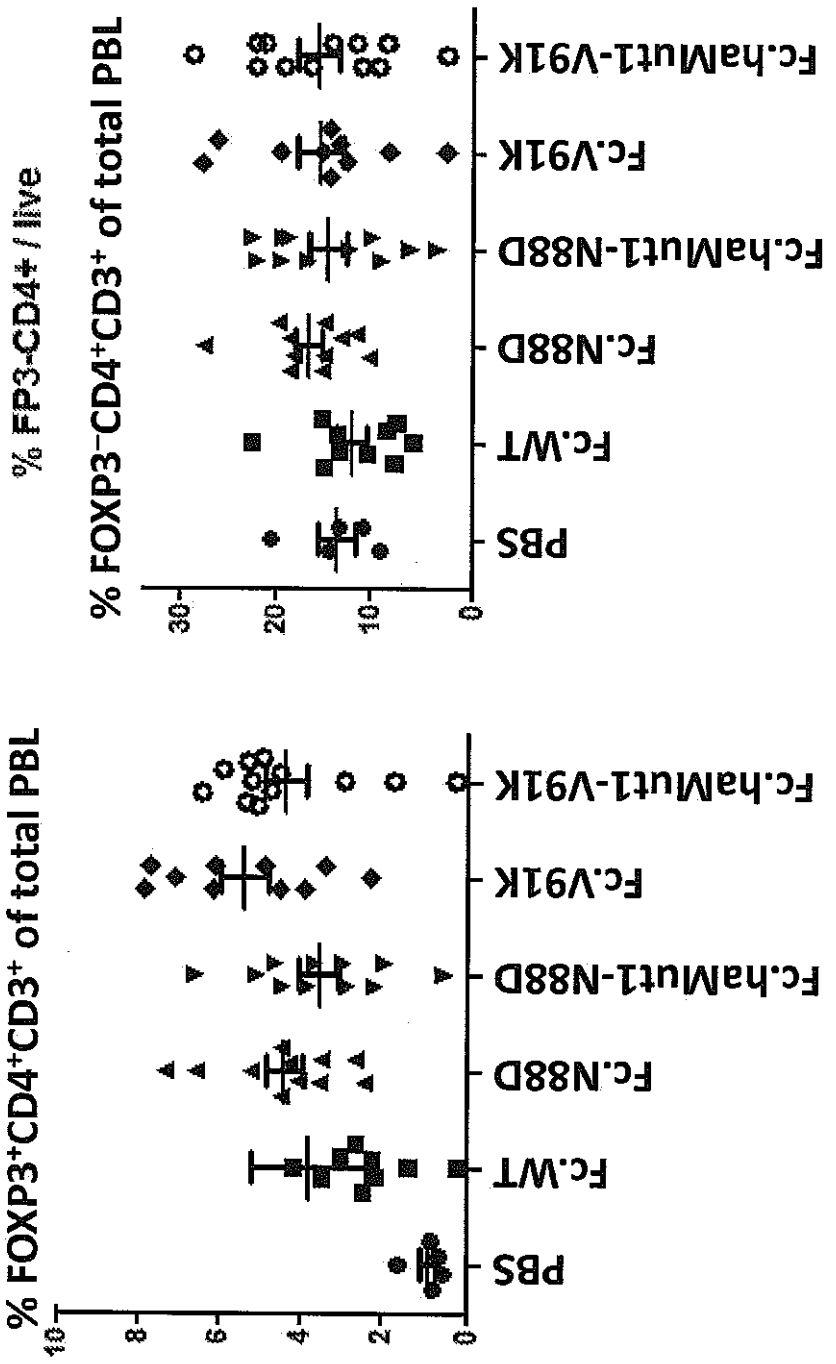

% FP3-CD4+ / live
% FOXP3⁻CD4⁺CD3⁺ of total PBL
30
20
10
0
PBS
Fc.WT
Fc.N88D
Fc.haMut1-N88D
Fc.V91K
Fc.haMut1-V91K
% FOXP3⁺CD4⁺CD3⁺ of total PBL
10
8
6
4
2
0
PBS
Fc.WT
Fc.N88D
Fc.haMut1-N88D
Fc.V91K
Fc.haMut1-V91K

FIG. 7B

FIG. 8

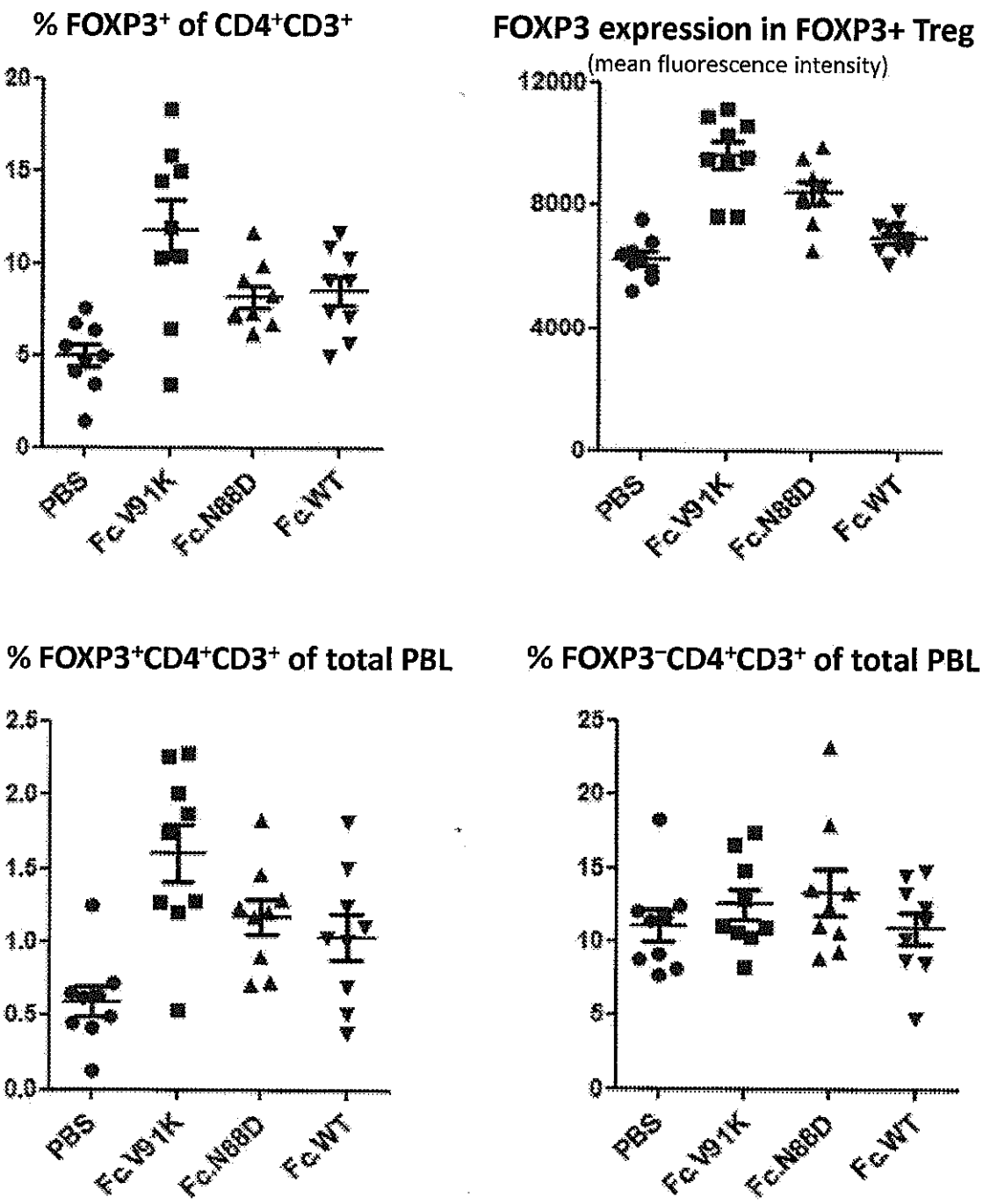
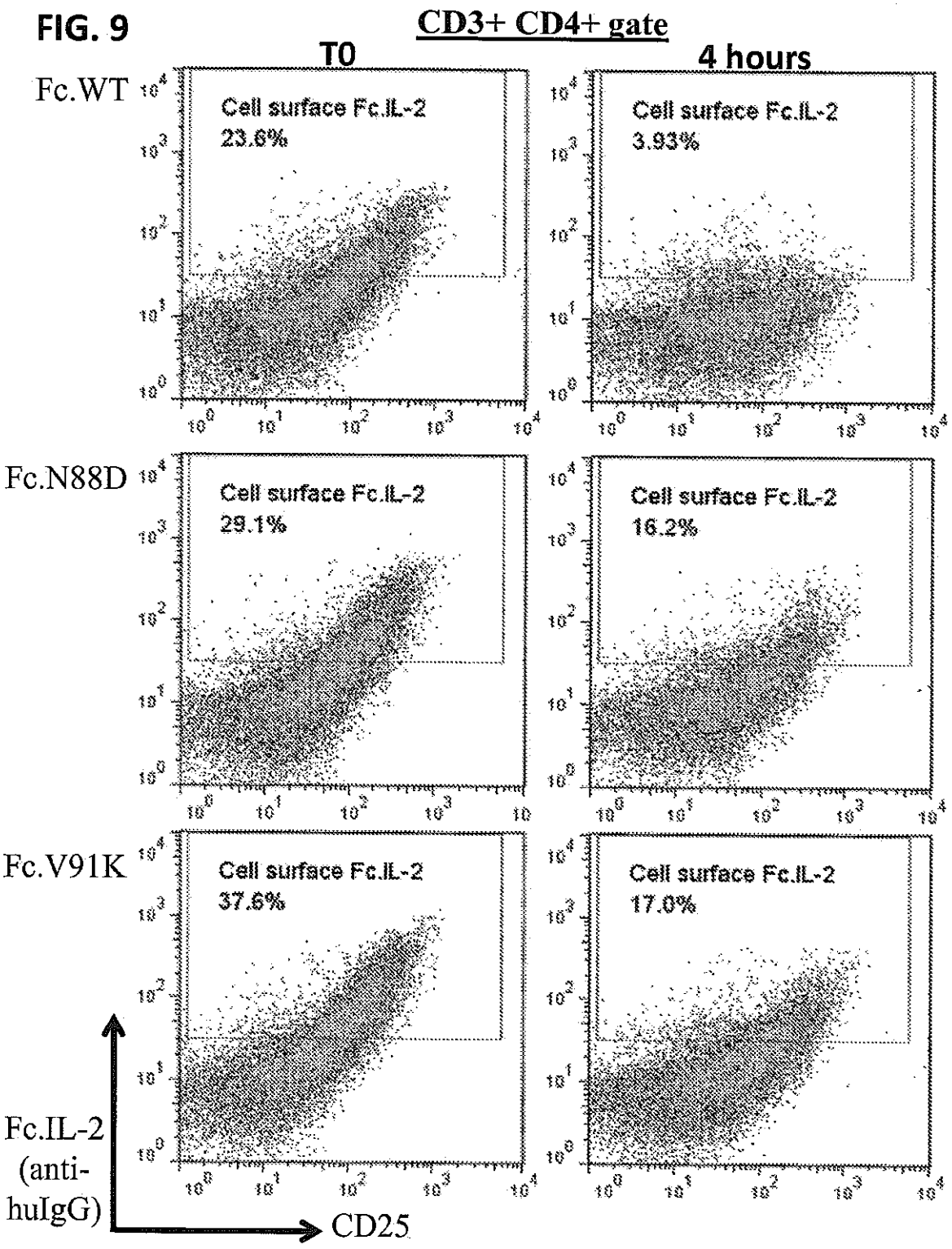

% FOXP3+ of CD4+CD3+
20
15
10
5
0
PBS
Fc.V91K
Fc.N88D
Fc.WT
FOXP3 expression in FOXP3+ Treg
(mean fluorescence intensity)
12000
8000
4000
0
PBS
Fc.V91K
Fc.N88D
Fc.WT
% FOXP3+CD4+CD3+ of total PBL
2.5
2.0
1.5
1.0
0.5
0.0
PBS
Fc.V91K
Fc.N88D
Fc.WT
% FOXP3-CD4+CD3+ of total PBL
25
20
15
10
5
0
PBS
Fc.V91K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

FIG. 9


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