DRAWINGS

Fig. 1

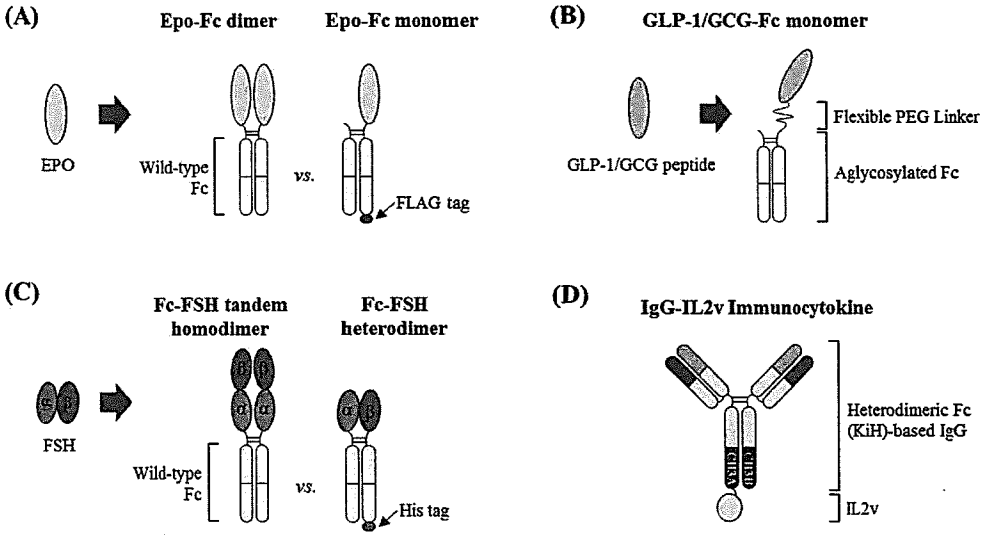


Fig. 2

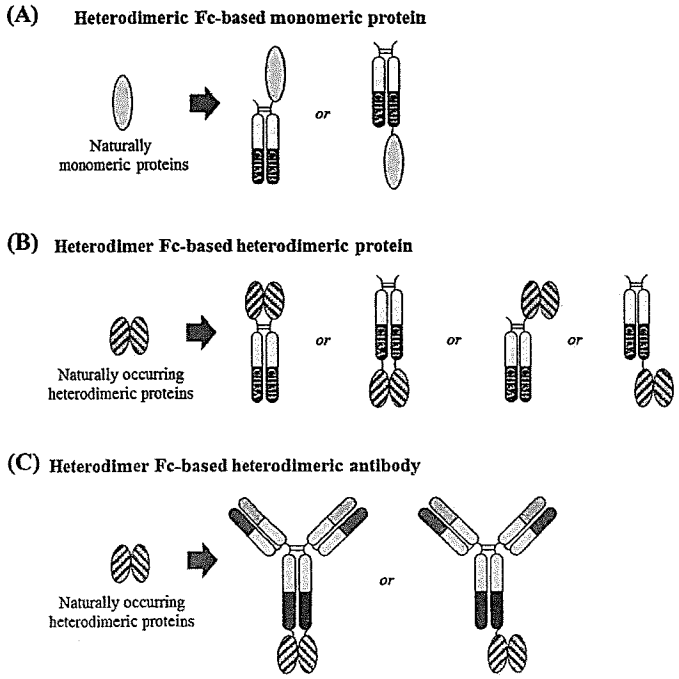


Fig. 3

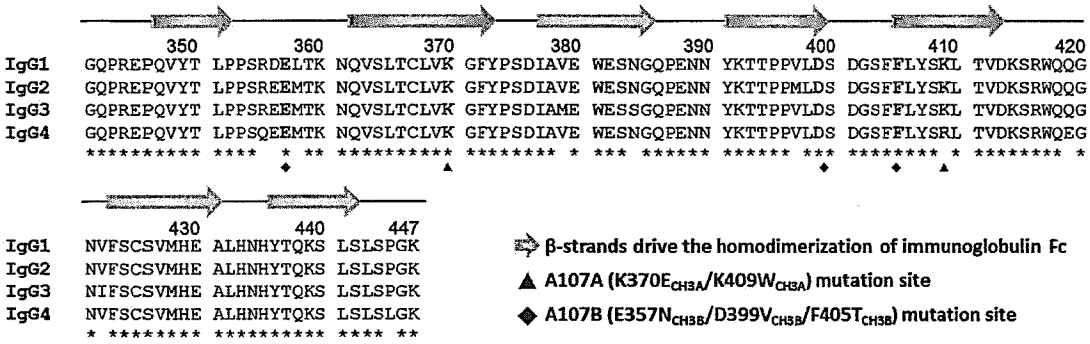

350 360 370 380 390 400 410 420
IgG1 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
IgG2 GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG
IgG3 GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAME WESSGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
IgG4 GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG
430 440 447
IgG1 NVFSCSVMHE ALHNHYTQKS LSLSPGK
IgG2 NVFSCSVMHE ALHNHYTQKS LSLSPGK
IgG3 NIFSCSVMHE ALHNHYTQKS LSLSPGK
IgG4 NVFSCSVMHE ALHNHYTQKS LSLSLGK
β-strands drive the homodimerization of immunoglobulin Fc
A107A (K370E$_{CH3A}$/K409W$_{CH3A}$) mutation site
A107B (E357N$_{CH3B}$/D399V$_{CH3B}$/F405T$_{CH3B}$) mutation site

Fig. 4

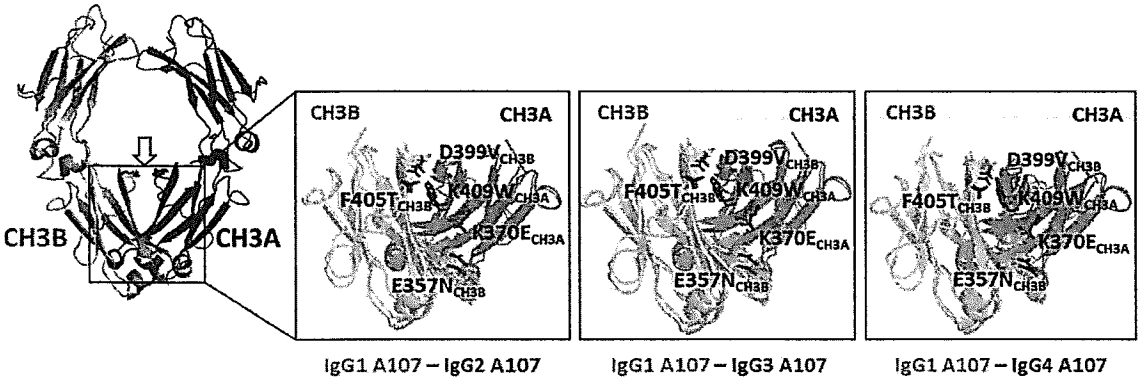

CH3B
CH3A
CH3B
CH3A
D399V$_{CH3B}$
F405T$_{CH3B}$
K409W$_{CH3A}$
K370E$_{CH3A}$
E357N$_{CH3B}$
IgG1 A107 – IgG2 A107
IgG1 A107 – IgG3 A107
IgG1 A107 – IgG4 A107

Fig. 5

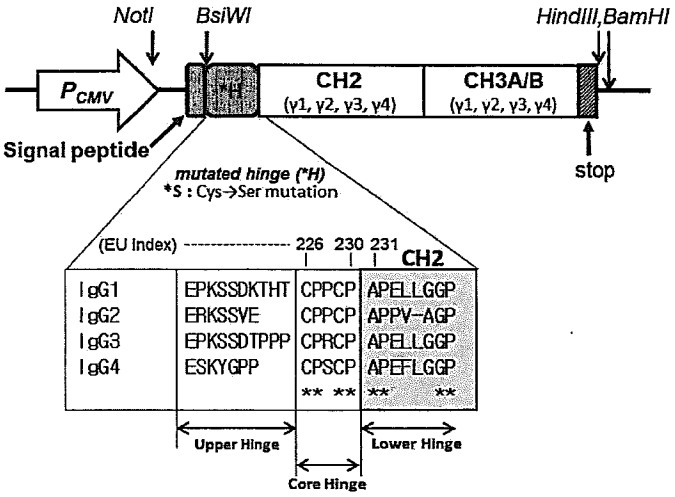

NotI
BsiWI
HindIII,BamHI
P_{CMV}
VH
CH2
(γ1, γ2, γ3, γ4)
CH3A/B
(γ1, γ2, γ3, γ4)
Signal peptide
stop
mutated hinge (*H)
*S : Cys→Ser mutation
(EU index) 226 230 231
CH2
IgG1 EPKSSDKTHT CPPCP APELLGGP
IgG2 ERKSSVE CPPCP APPV-AGP
IgG3 EPKSSDTPPP CPRCP APELLGGP
IgG4 ESKYGPP CPSCP APEFLGGP
** ** ** **
Upper Hinge
Lower Hinge
Core Hinge

Fig. 6

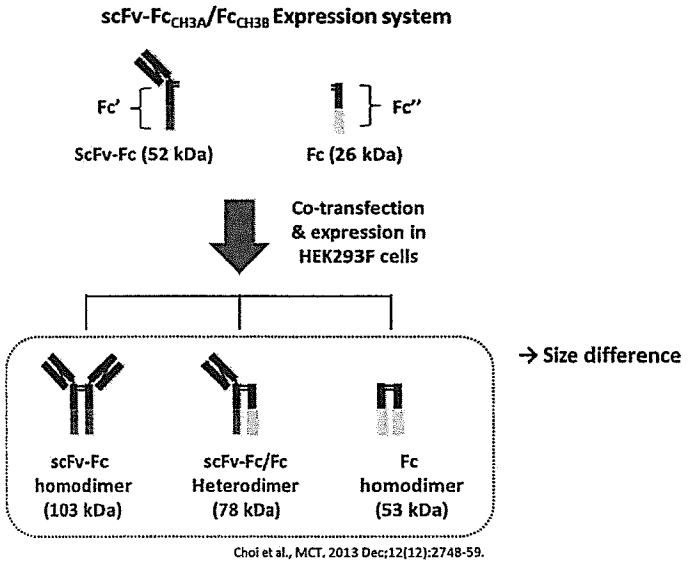

scFv-Fc_{CH3A}/Fc_{CH3B} Expression system
Fc'
Fc''
ScFv-Fc (52 kDa)
Fc (26 kDa)
Co-transfection
& expression in
HEK293F cells
→ Size difference
scFv-Fc
homodimer
(103 kDa)
scFv-Fc/Fc
Heterodimer
(78 kDa)
Fc
homodimer
(53 kDa)
Choi et al., MCT. 2013 Dec;12(12):2748-59.

Fig. 7

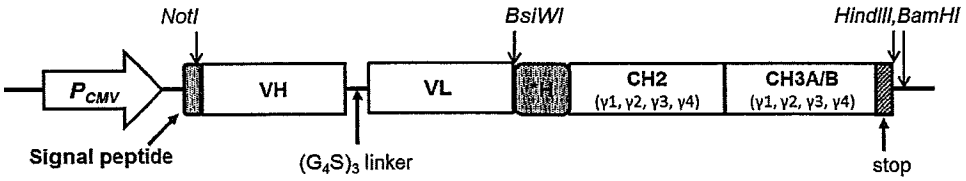

NotI
BsiWI
HindIII,BamHI
P_{CMV}
VH
VL
CH1
CH2
(γ1, γ2, γ3, γ4)
CH3A/B
(γ1, γ2, γ3, γ4)
Signal peptide
$(G_4S)_3$ linker
stop

Fig. 8

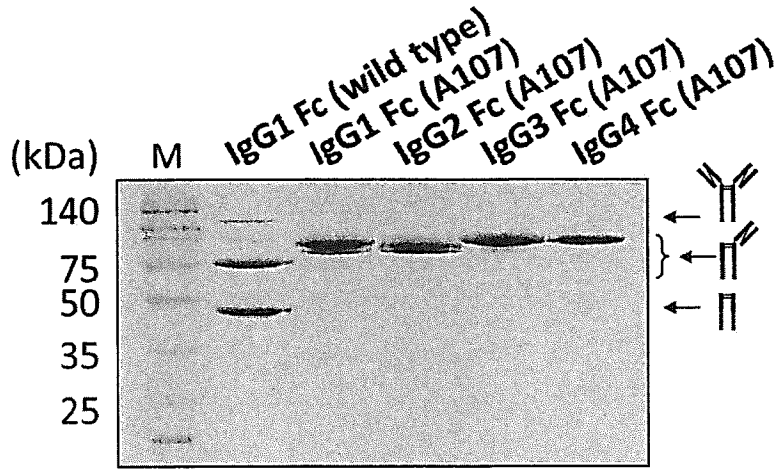

(kDa)
M
IgG1 Fc (wild type)
IgG1 Fc (A107)
IgG2 Fc (A107)
IgG3 Fc (A107)
IgG4 Fc (A107)
140
75
50
35
25

Fig. 9

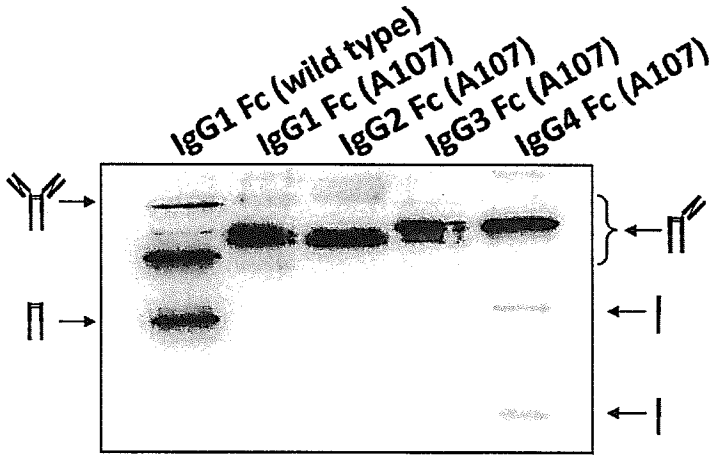

IgG1 Fc (wild type)
IgG1 Fc (A107)
IgG2 Fc (A107)
IgG3 Fc (A107)
IgG4 Fc (A107)

Fig. 10

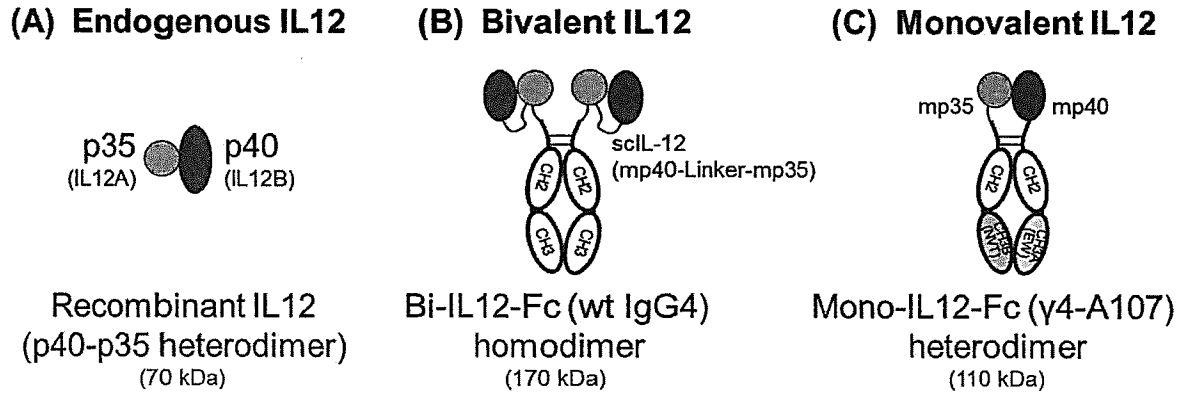

(A) Endogenous IL12
(B) Bivalent IL12
(C) Monovalent IL12
p35
(IL12A)
p40
(IL12B)
scIL-12
(mp40-Linker-mp35)
CH2
CH3
mp35
mp40
Recombinant IL12
(p40-p35 heterodimer)
(70 kDa)
Bi-IL12-Fc (wt IgG4)
homodimer
(170 kDa)
Mono-IL12-Fc (γ4-A107)
heterodimer
(110 kDa)

Fig. 11(A)

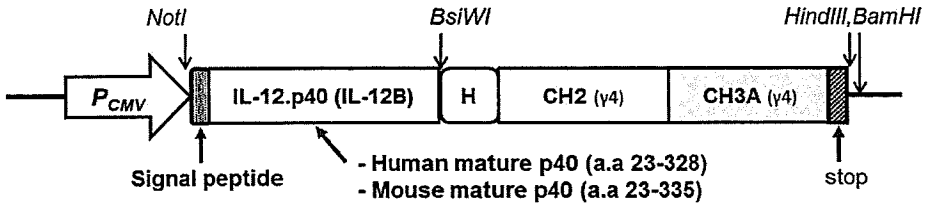


Fig. 11(B)

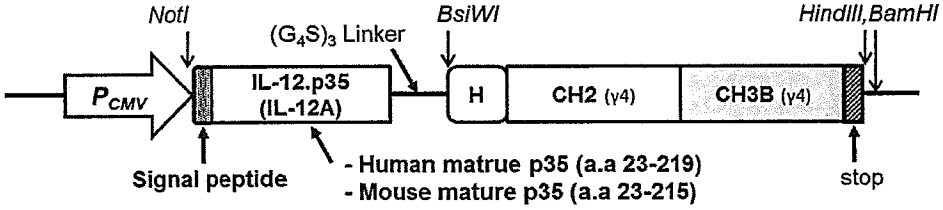


Fig. 12

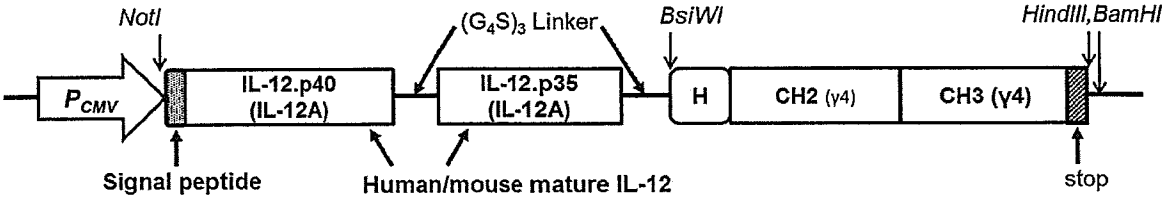


Fig. 13

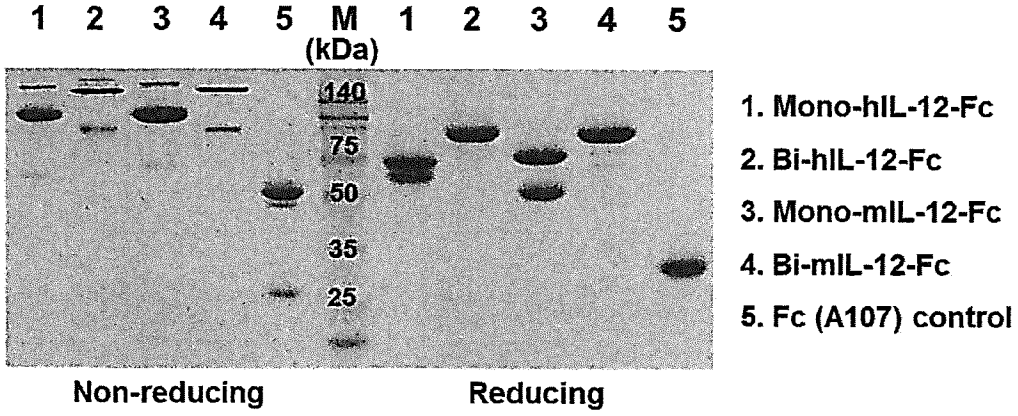

1 2 3 4 5 M 1 2 3 4 5
(kDa)
140
75
50
35
25
Non-reducing
Reducing
1. Mono-hIL-12-Fc
2. Bi-hIL-12-Fc
3. Mono-mIL-12-Fc
4. Bi-mIL-12-Fc
5. Fc (A107) control

Fig. 14

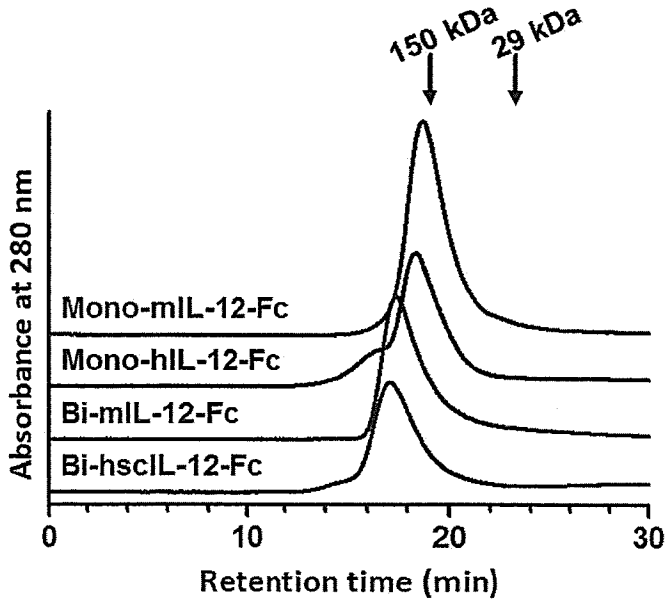

150 kDa
29 kDa
Absorbance at 280 nm
Mono-mIL-12-Fc
Mono-hIL-12-Fc
Bi-mIL-12-Fc
Bi-hscIL-12-Fc
0
10
20
30
Retention time (min)

Fig. 15

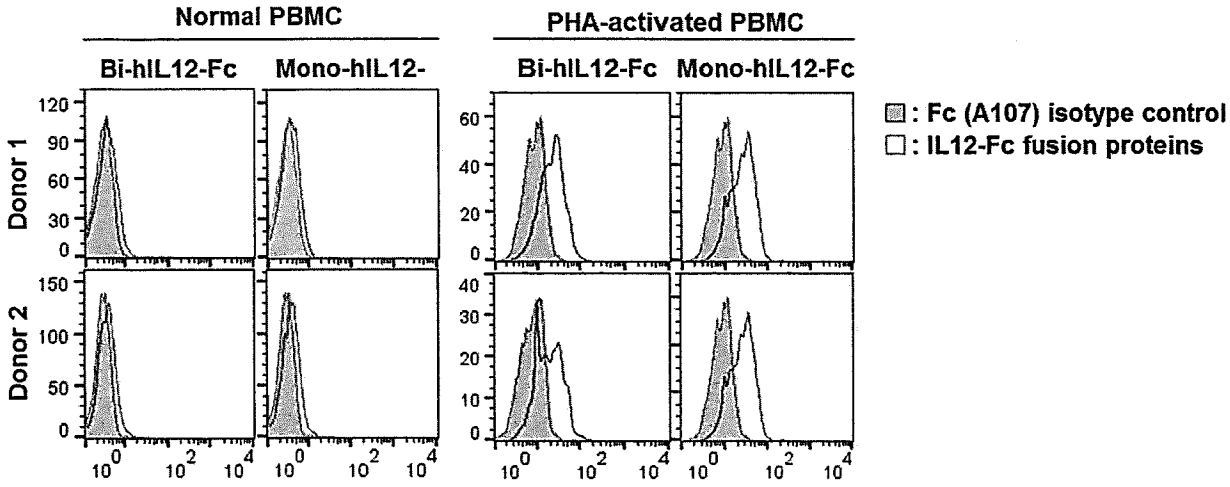

Normal PBMC
PHA-activated PBMC
Bi-hIL12-Fc
Mono-hIL12-
Bi-hIL12-Fc
Mono-hIL12-Fc
Donor 1
Donor 2
: Fc (A107) isotype control
: IL12-Fc fusion proteins

Fig. 16

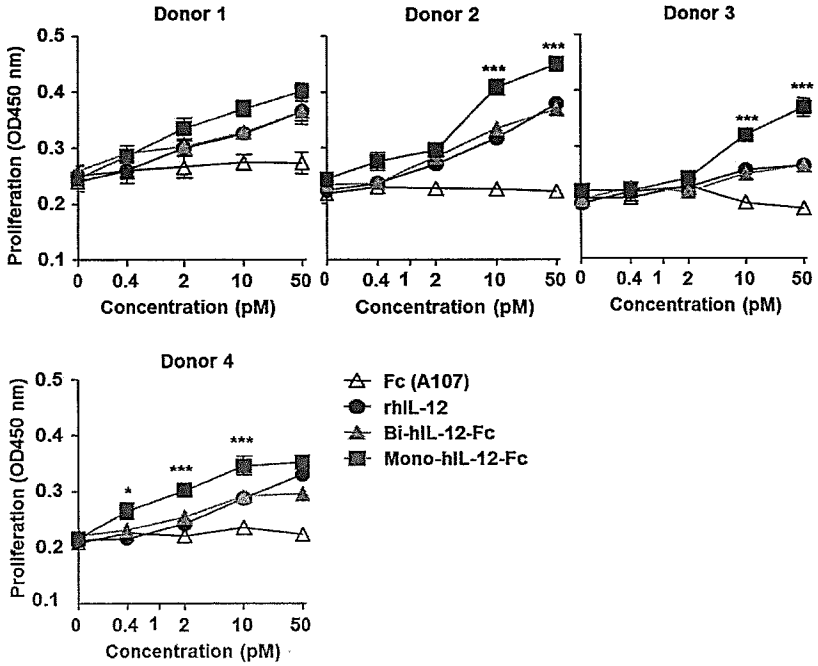

Donor 1
Donor 2
Donor 3
Donor 4
Proliferation (OD450 nm)
Concentration (pM)
Fc (A107)
rhIL-12
Bi-hIL-12-Fc
Mono-hIL-12-Fc

Fig. 17

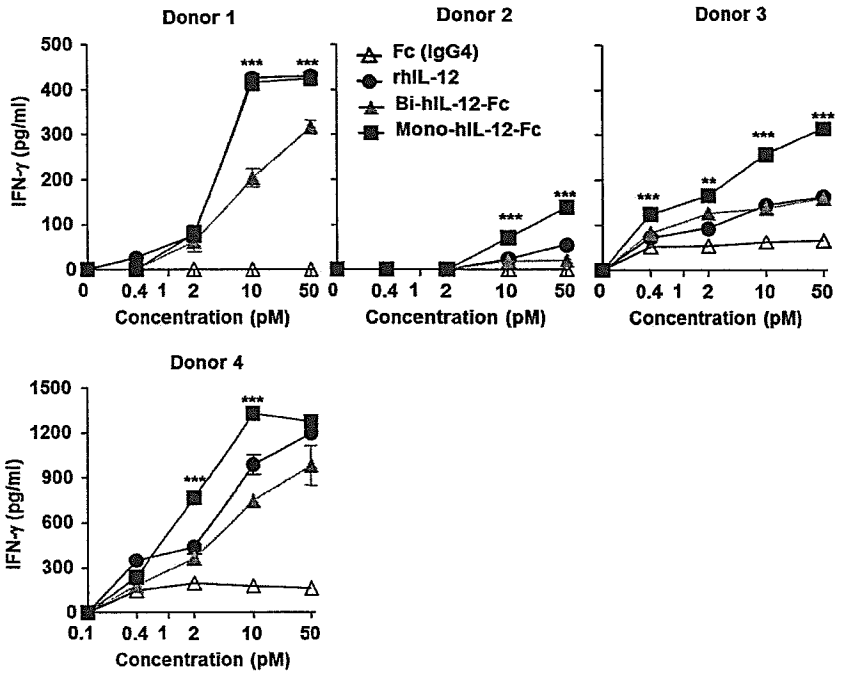

Donor 1
Donor 2
Donor 3
Donor 4
Fc (IgG4)
rhIL-12
Bi-hIL-12-Fc
Mono-hIL-12-Fc
IFN-γ (pg/ml)
Concentration (pM)
0
0.1
0.4
1
2
10
50
***
**

Fig. 18

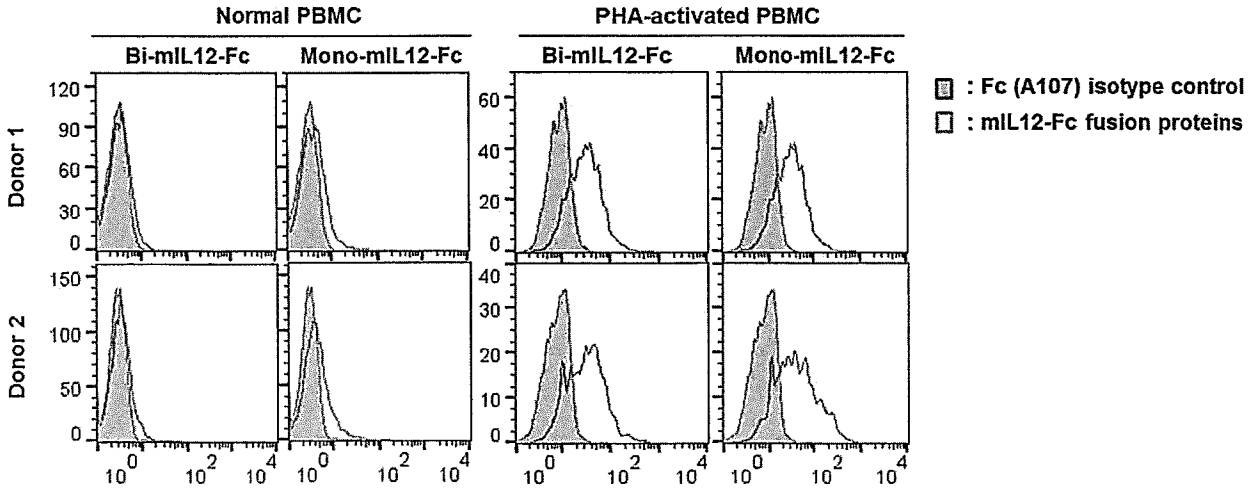

Normal PBMC
PHA-activated PBMC
Bi-mIL12-Fc
Mono-mIL12-Fc
Donor 1
Donor 2
: Fc (A107) isotype control
: mIL12-Fc fusion proteins

Fig. 19

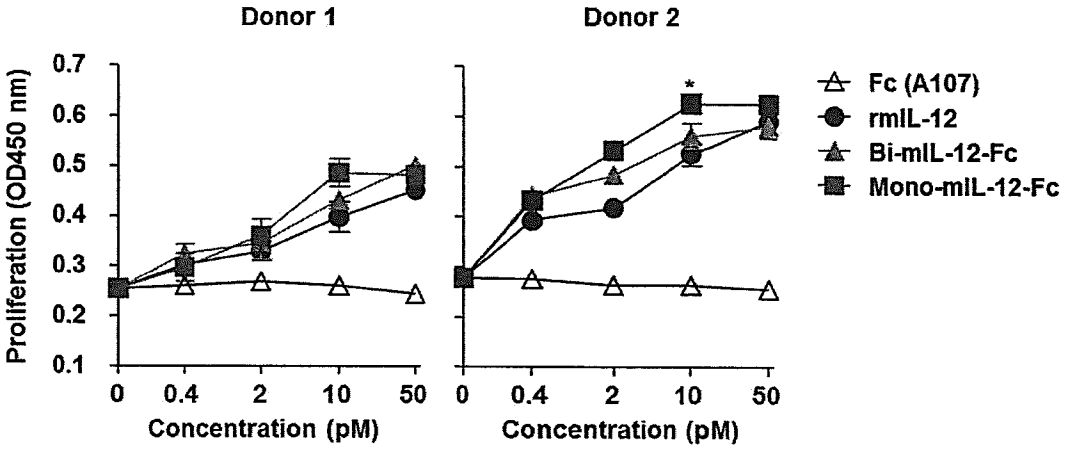


Fig. 20(A)

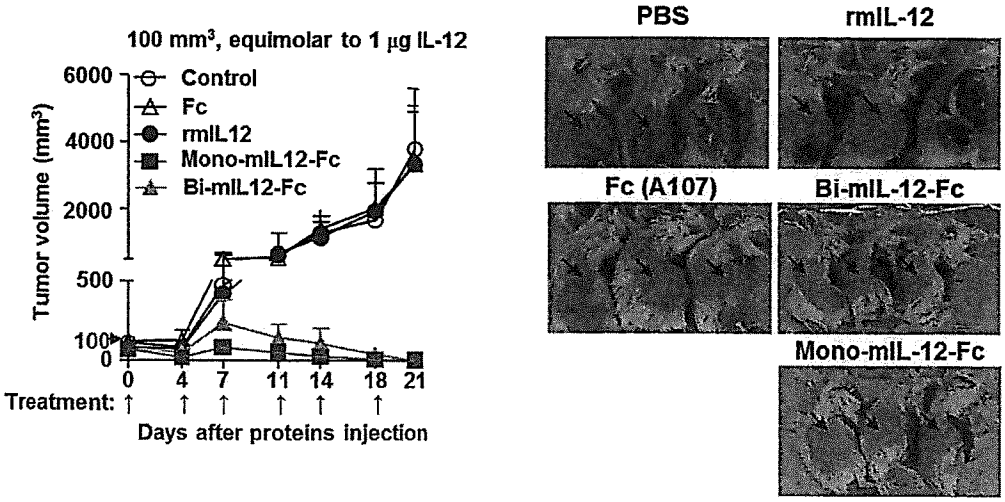


Fig. 20(B)

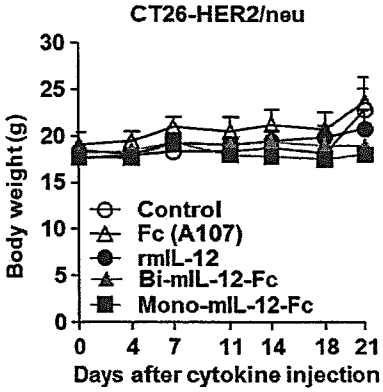


Fig. 21(A)

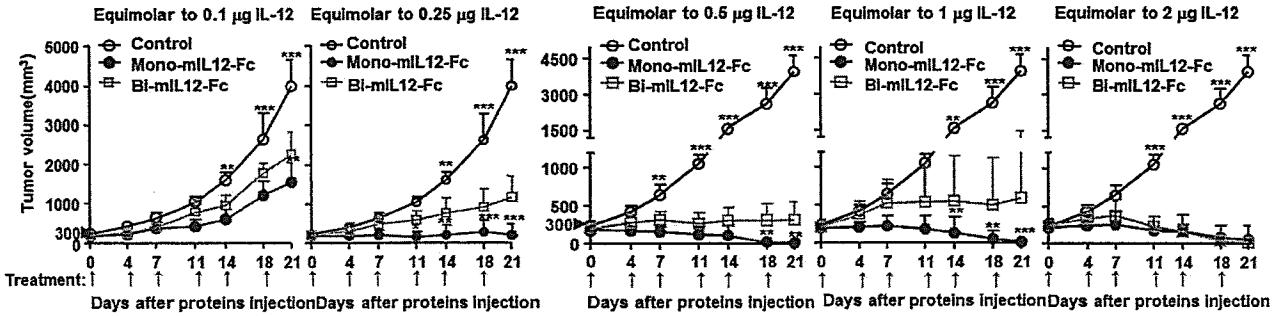


Fig. 21(B)

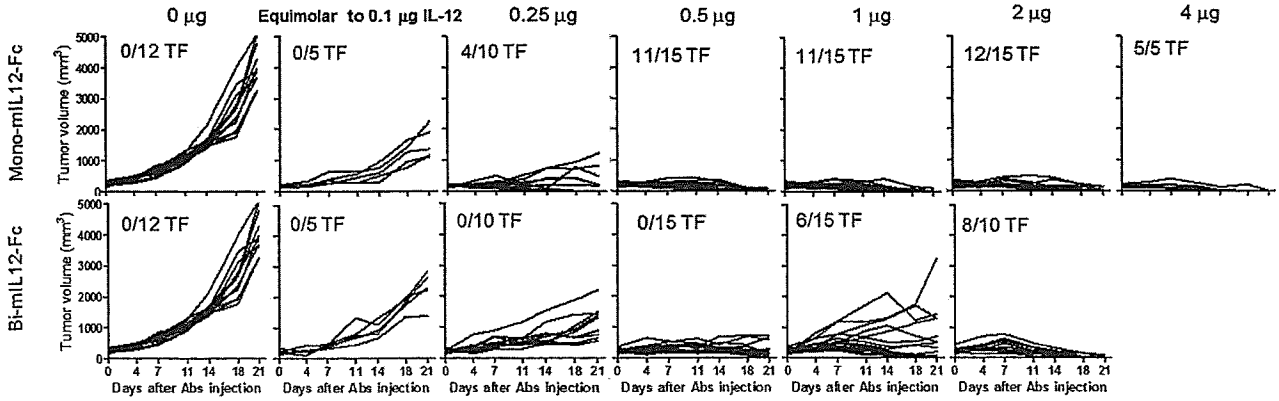


Fig. 21(C)

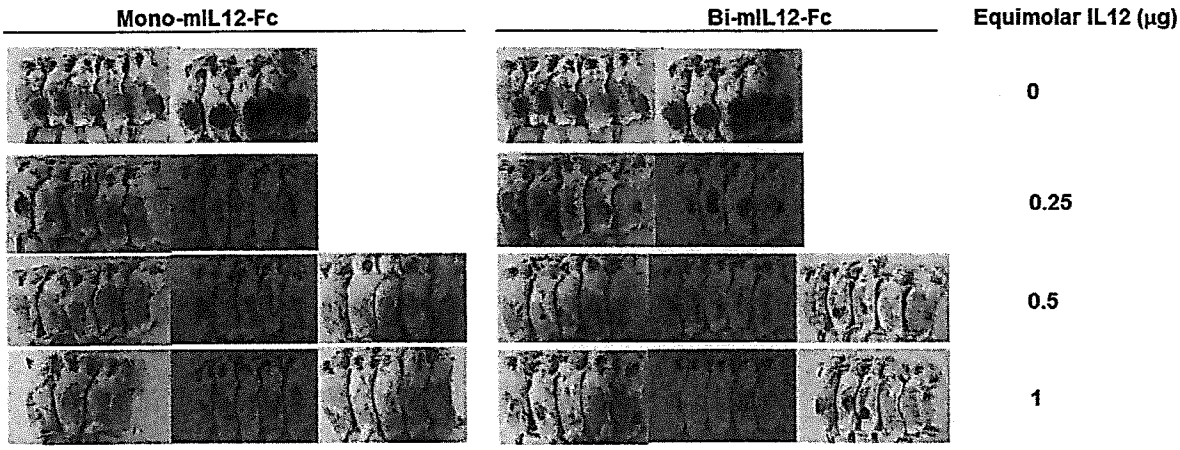


Fig. 21(D)

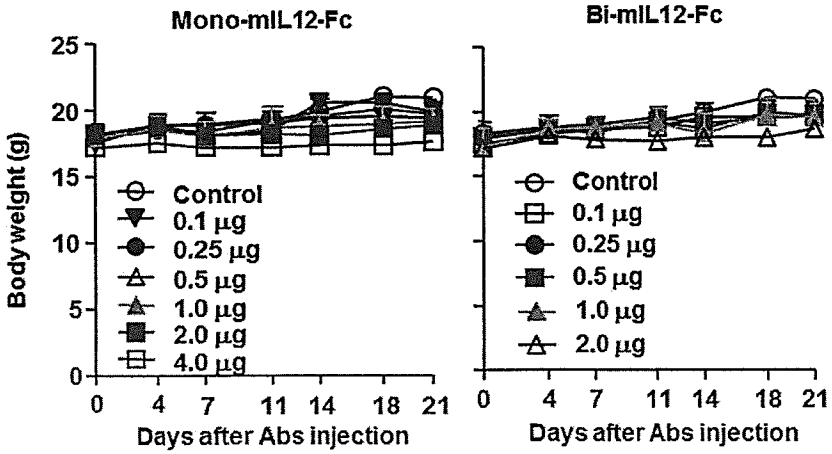


Fig. 21(E)

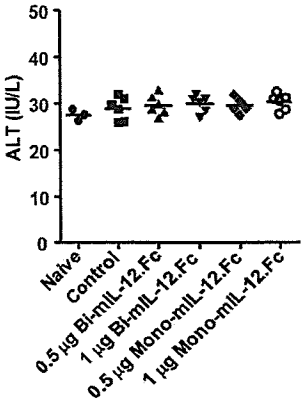


Fig. 22(A)

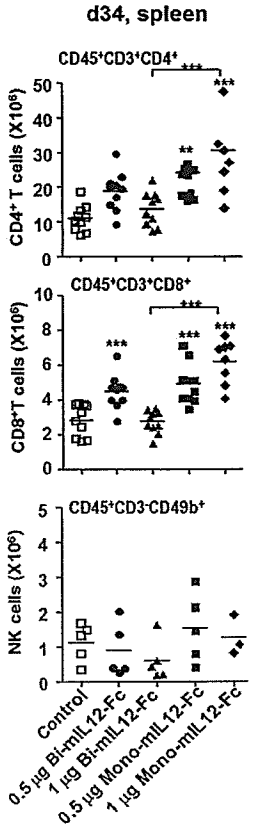


Fig. 22(B)

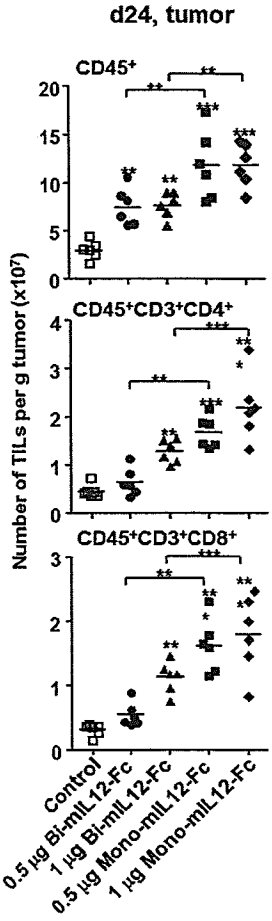


Fig. 23(A)

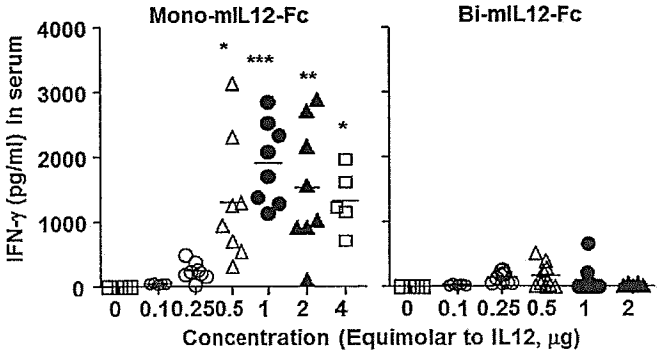


Fig. 23(B)

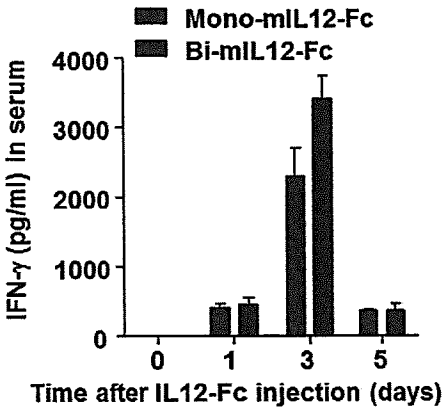


Fig. 23(C)

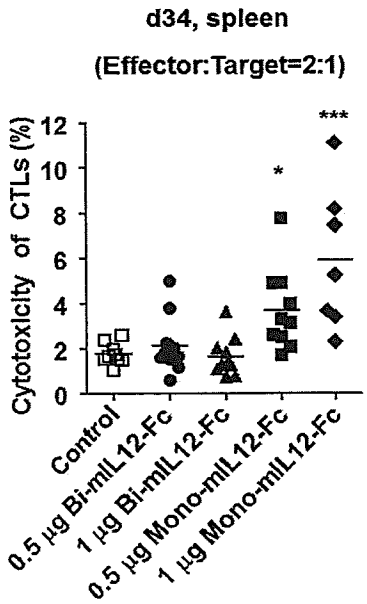


Fig. 23(D)

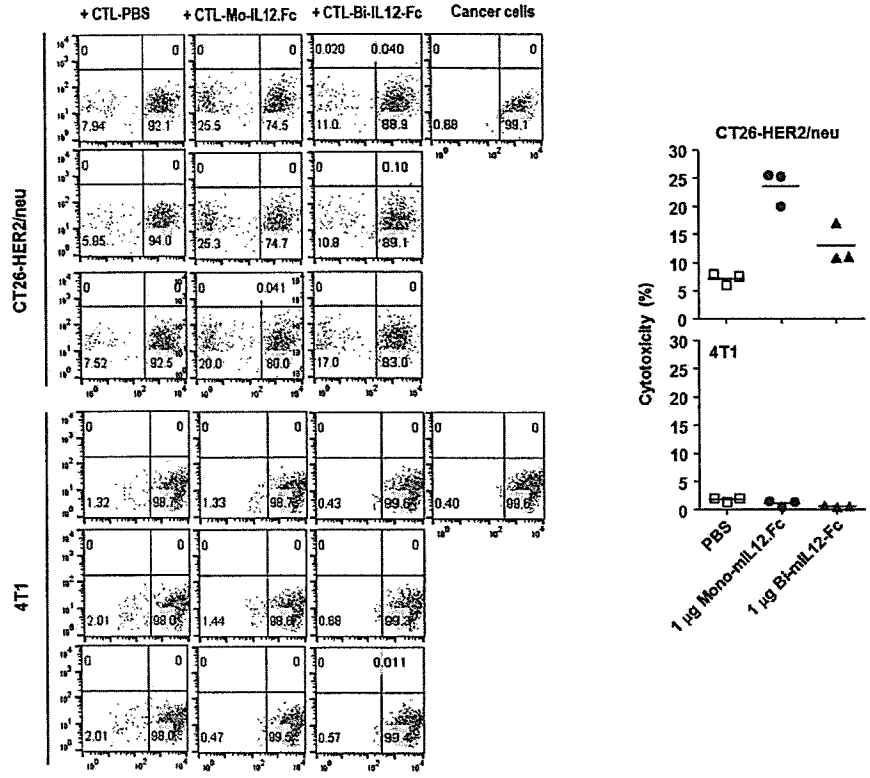


Fig. 23(E)

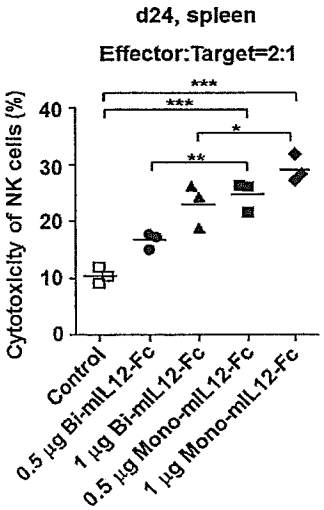


Fig. 24(A)

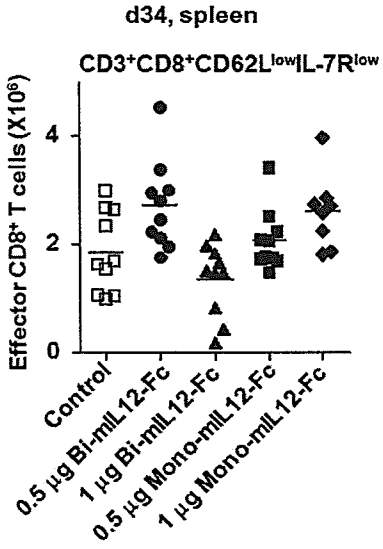


Fig. 24(B)

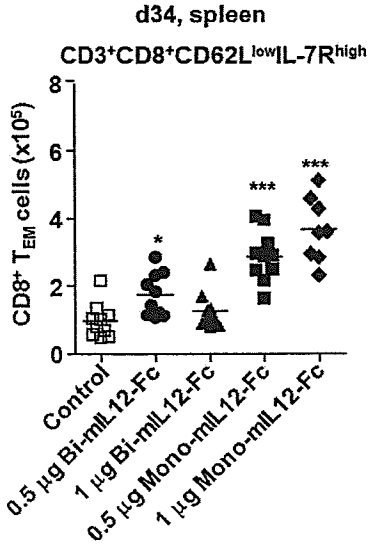


Fig. 24(C)

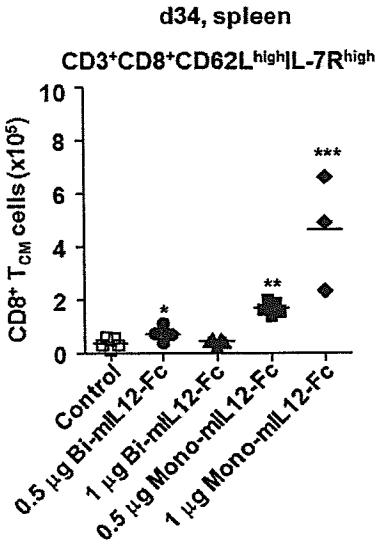


Fig. 24(D)

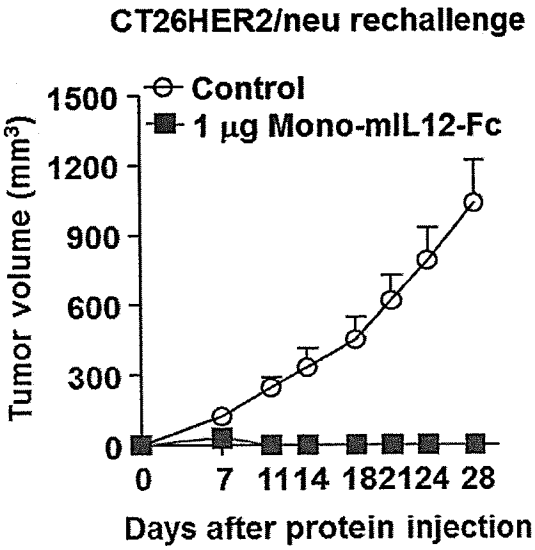


Fig. 24(E)

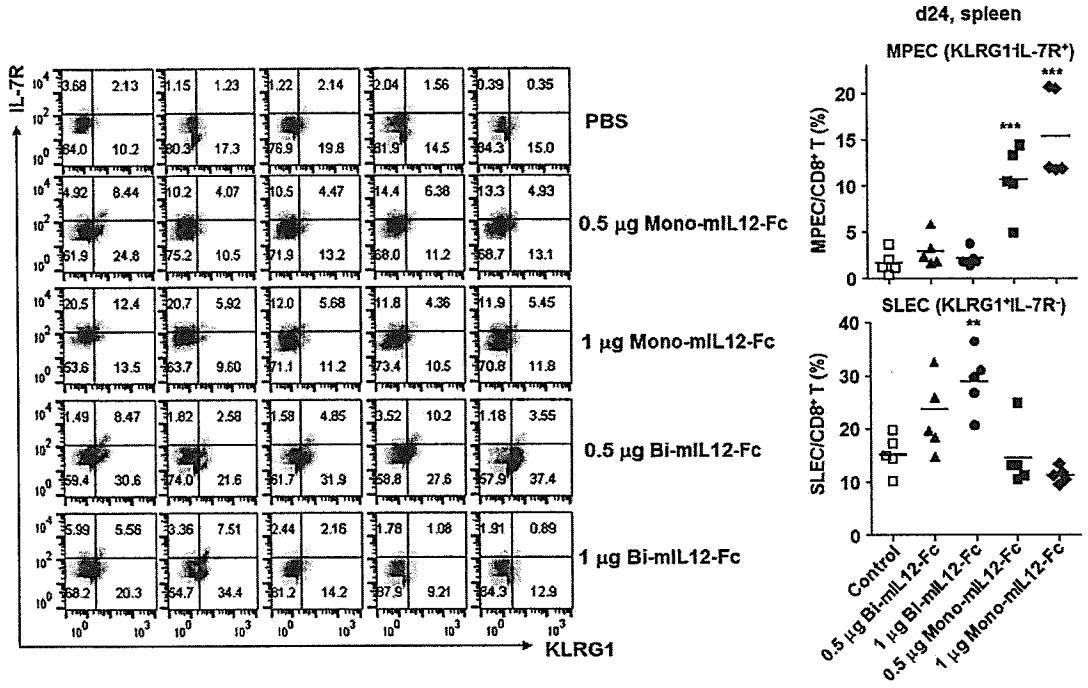


Fig. 25(A)

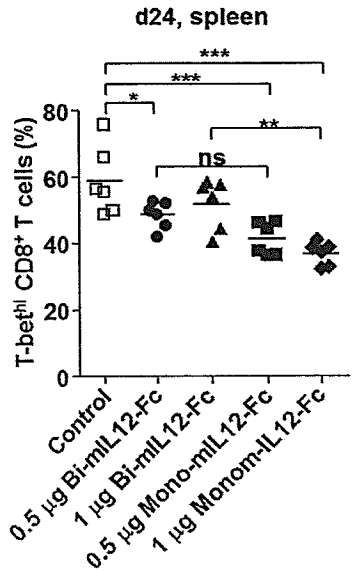


Fig. 25(B)

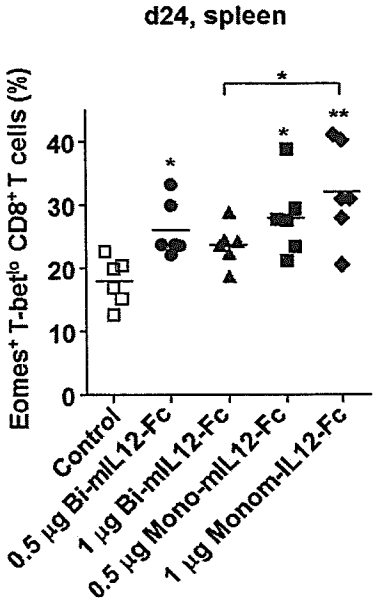


Fig. 25(C)

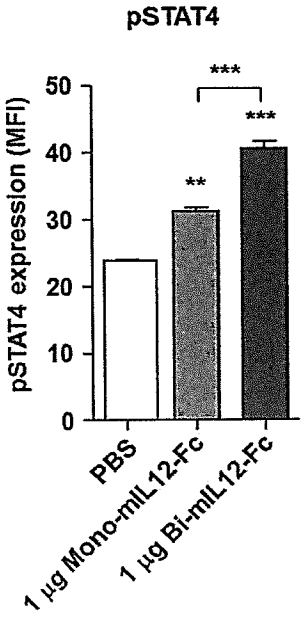


Fig. 25(D)

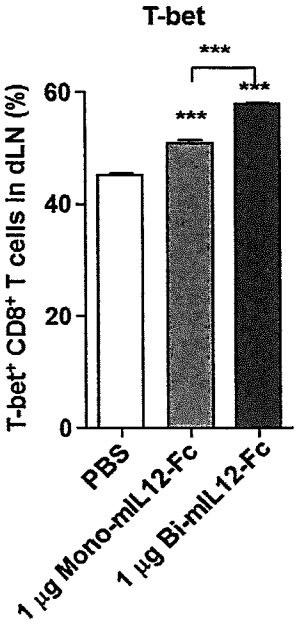
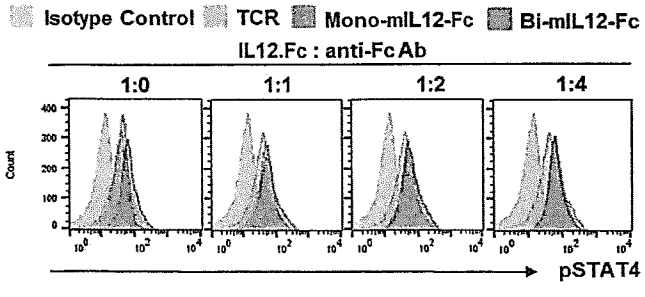


Fig. 25(E)



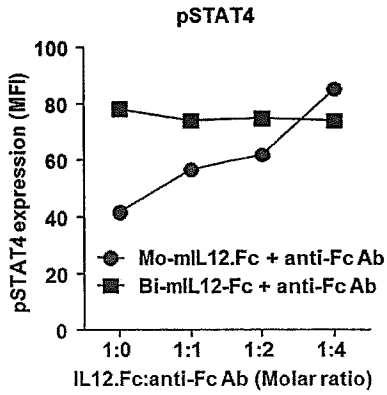


Fig. 25(F)

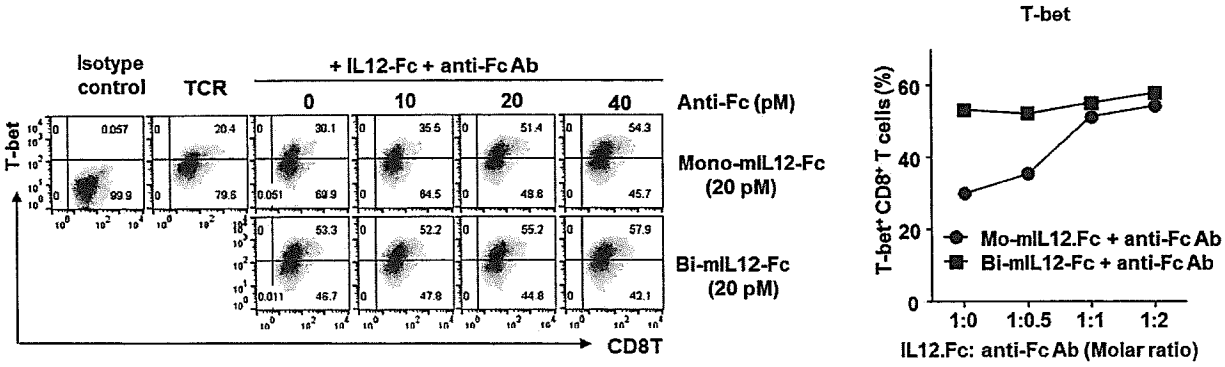


Fig. 26

