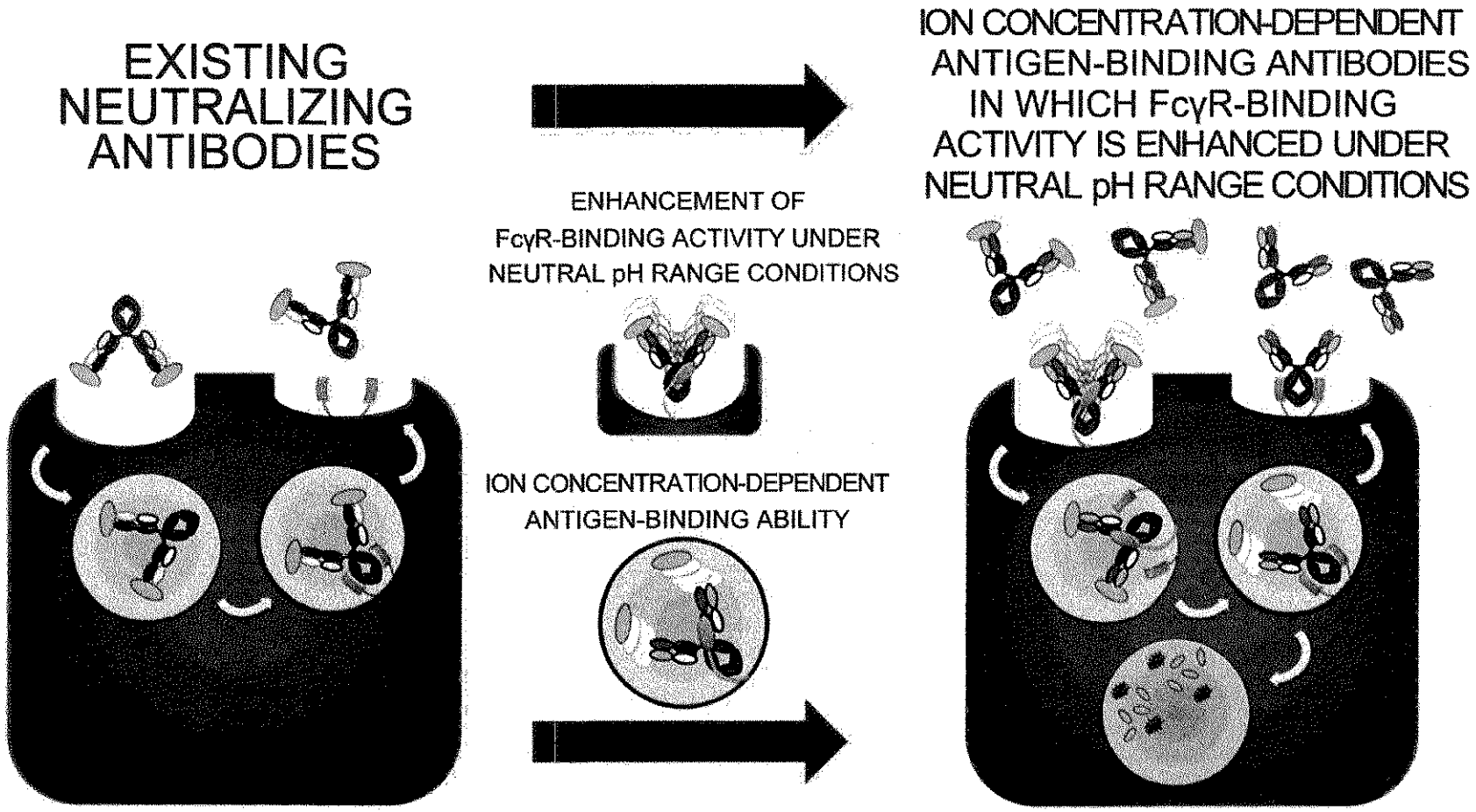

EXISTING NEUTRALIZING ANTIBODIES
ENHANCEMENT OF FcγR-BINDING ACTIVITY UNDER NEUTRAL pH RANGE CONDITIONS
ION CONCENTRATION-DEPENDENT ANTIGEN-BINDING ABILITY
ION CONCENTRATION-DEPENDENT ANTIGEN-BINDING ANTIBODIES IN WHICH FcγR-BINDING ACTIVITY IS ENHANCED UNDER NEUTRAL pH RANGE CONDITIONS

FIG. 1

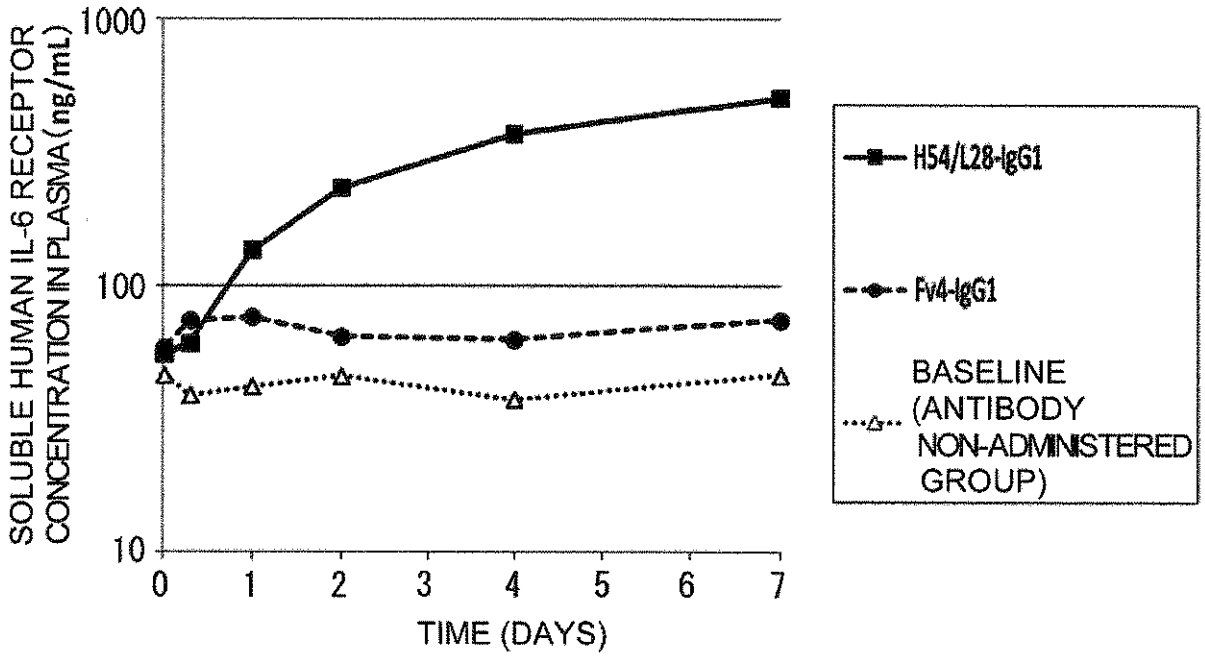

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
0
1
2
3
4
5
6
7
TIME (DAYS)
H54/L28-IgG1
Fv4-IgG1
BASELINE (ANTIBODY NON-ADMINISTERED GROUP)

FIG. 2

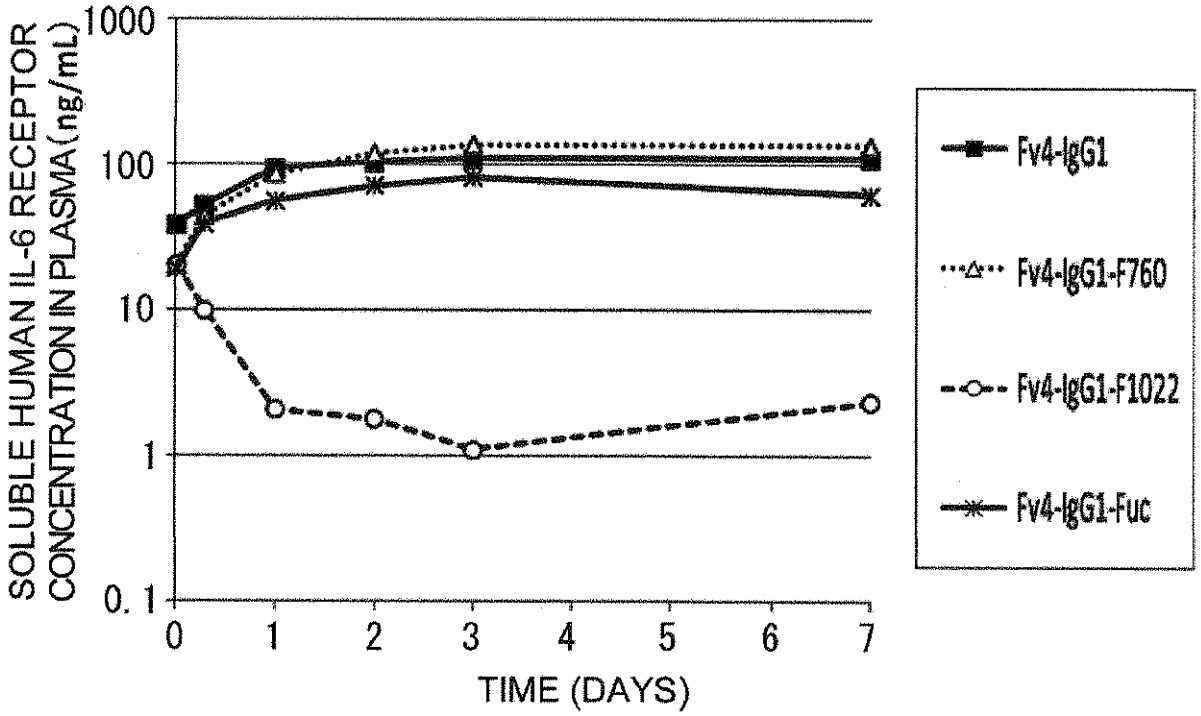

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA(ng/mL)
1000
100
10
1
0.1
0
1
2
3
4
5
6
7
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F760
Fv4-IgG1-F1022
Fv4-IgG1-Fuc

FIG. 3

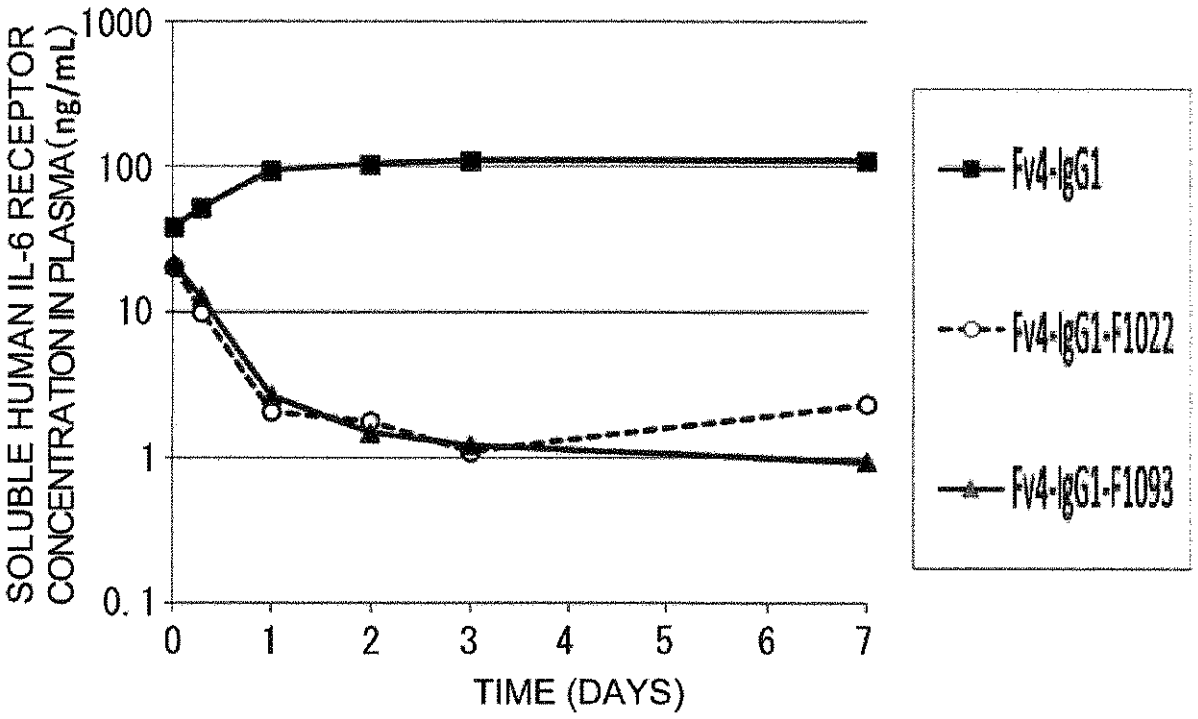

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA(ng/mL)
1000
100
10
1
0.1
0
1
2
3
4
5
6
7
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1022
Fv4-IgG1-F1093

FIG. 4

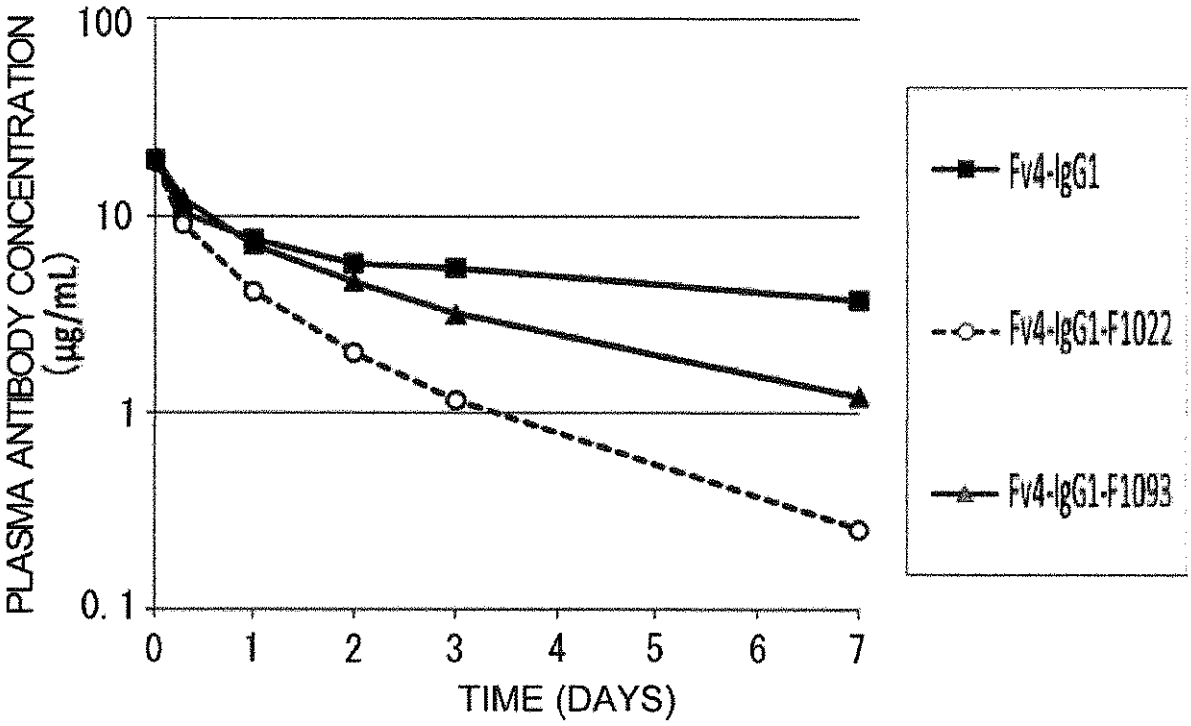

PLASMA ANTIBODY CONCENTRATION (μg/mL)
100
10
1
0.1
0
1
2
3
4
5
6
7
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1022
Fv4-IgG1-F1093

FIG. 5

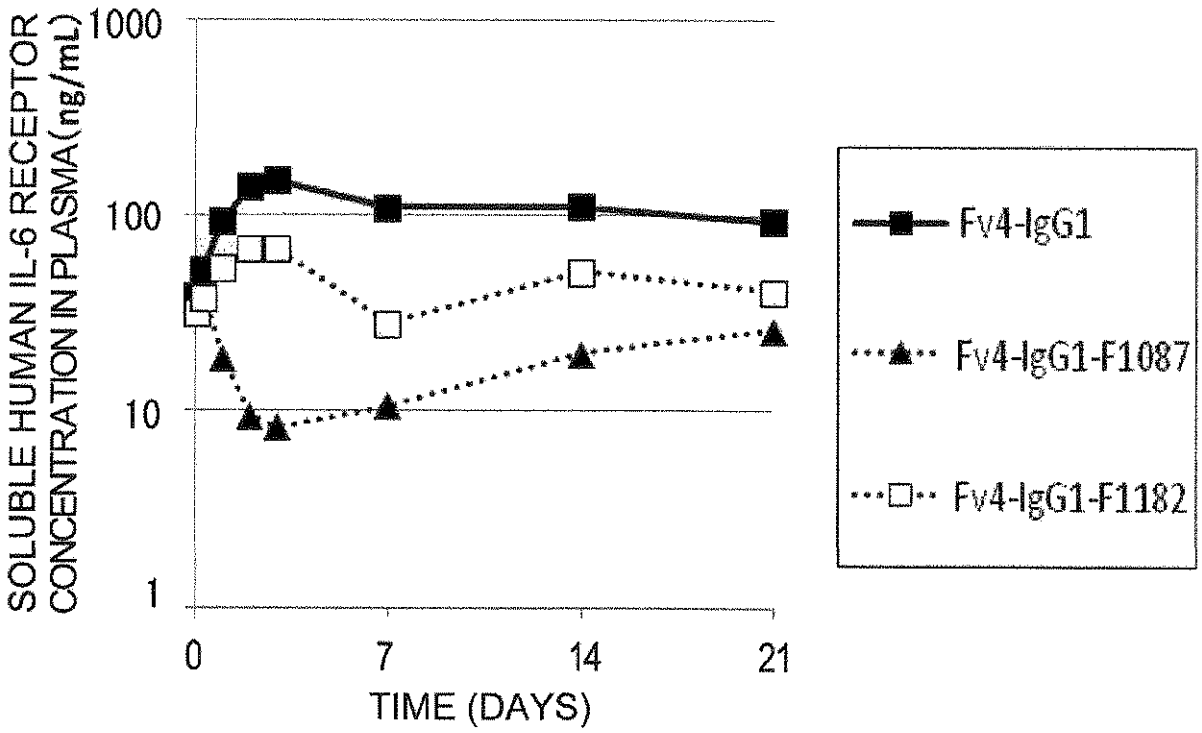

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA(ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1182

FIG. 6

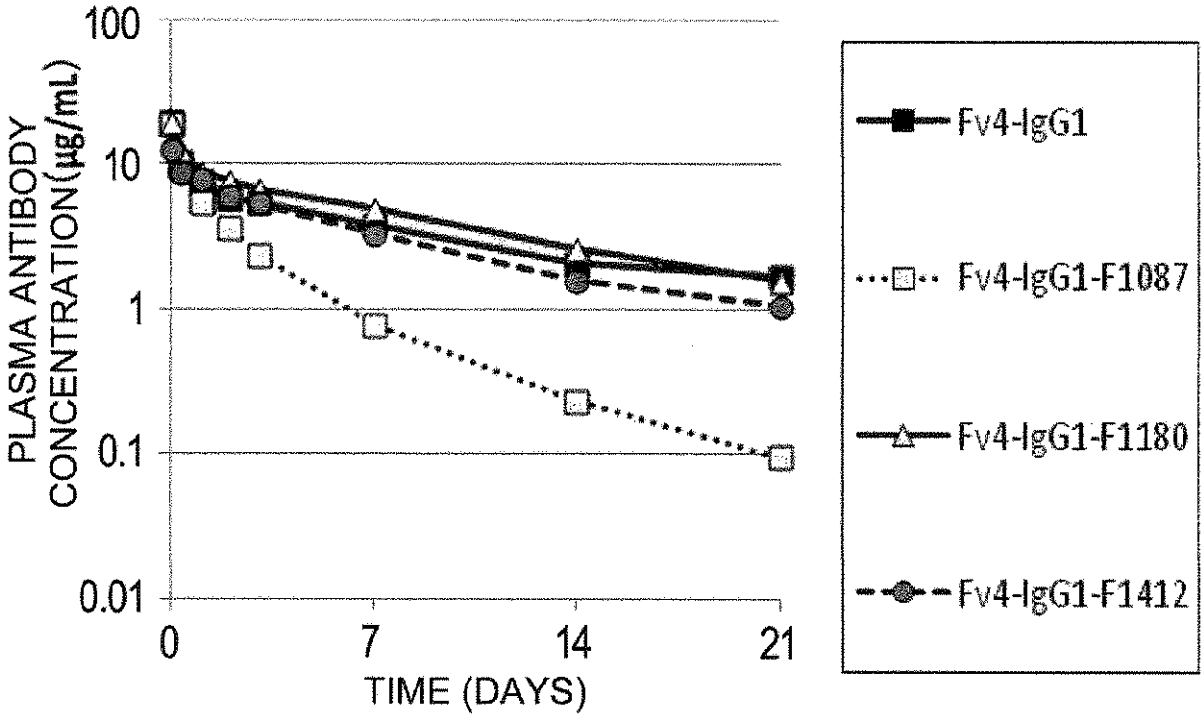

100
10
1
0.1
0.01
PLASMA ANTIBODY CONCENTRATION(μg/mL)
0
7
14
21
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1180
Fv4-IgG1-F1412

FIG. 7

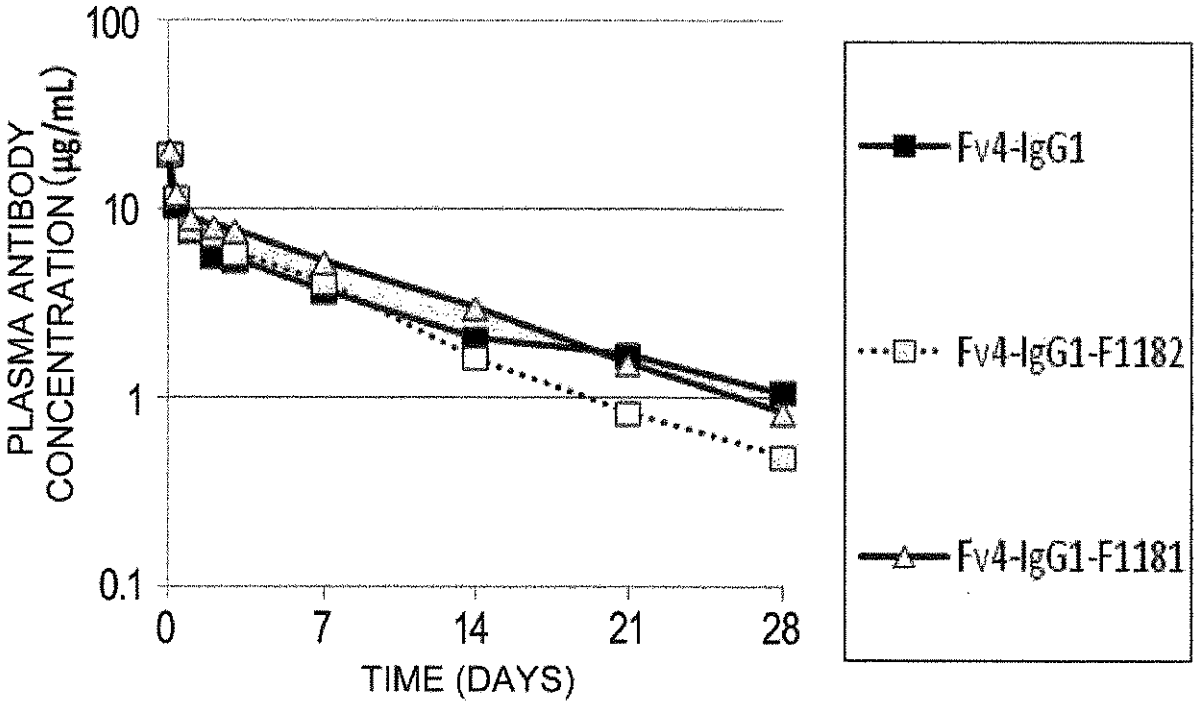

100
10
1
0.1
PLASMA ANTIBODY CONCENTRATION (μg/mL)
0
7
14
21
28
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1182
Fv4-IgG1-F1181

FIG. 8

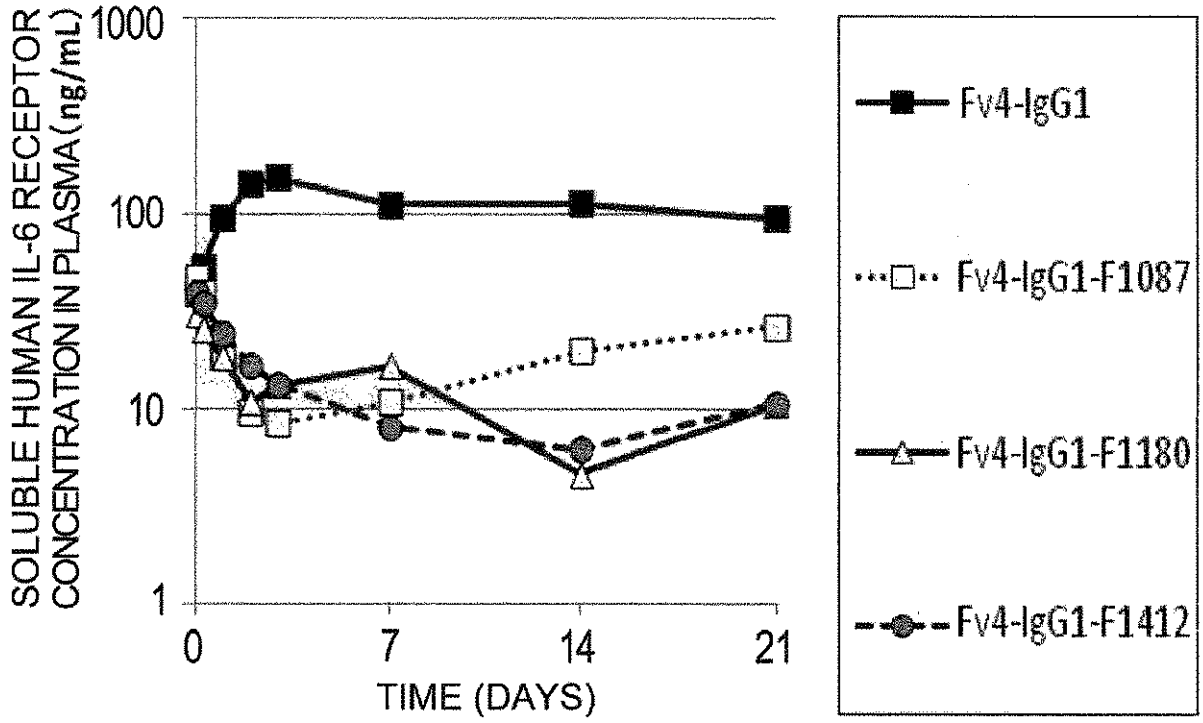

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1180
Fv4-IgG1-F1412

FIG. 9

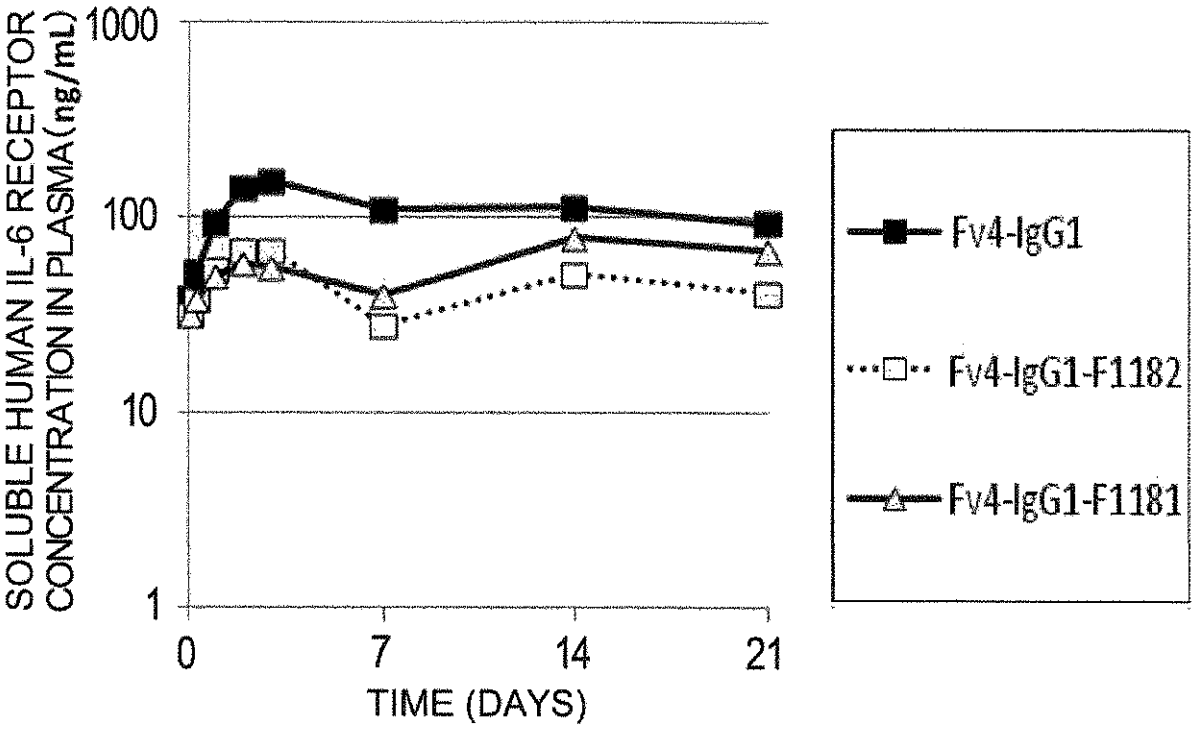

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA(ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1182
Fv4-IgG1-F1181

FIG. 10

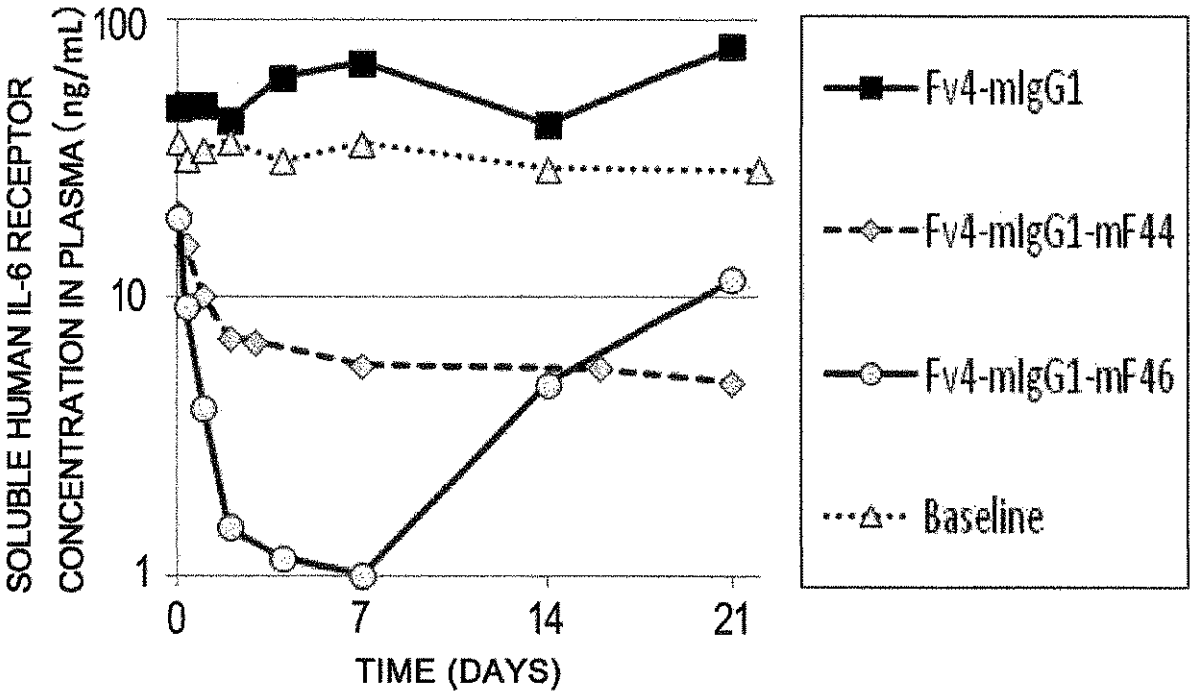

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
Baseline

FIG. 11

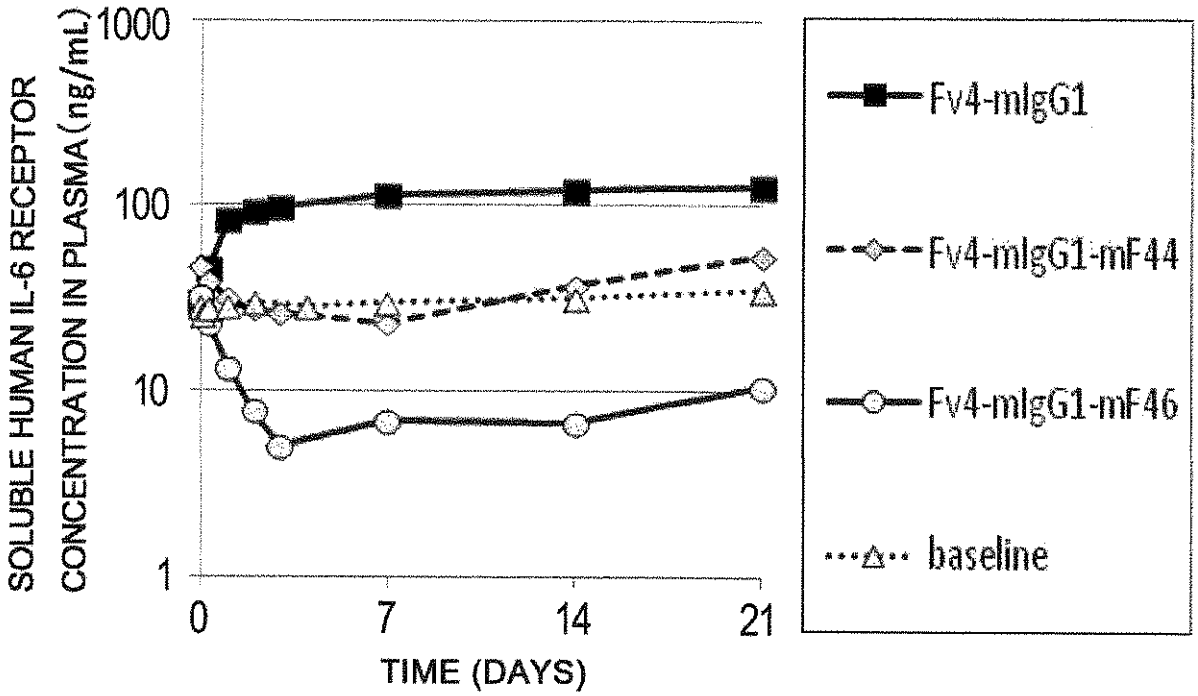

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
baseline

FIG. 12

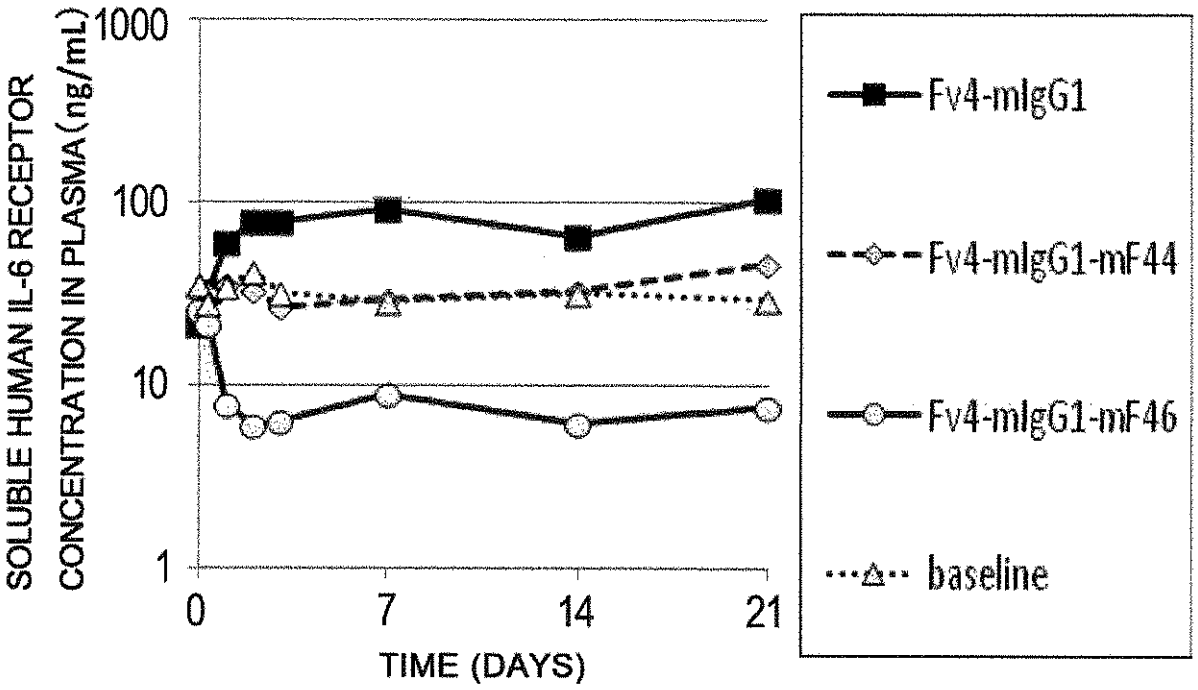

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
baseline

FIG. 13

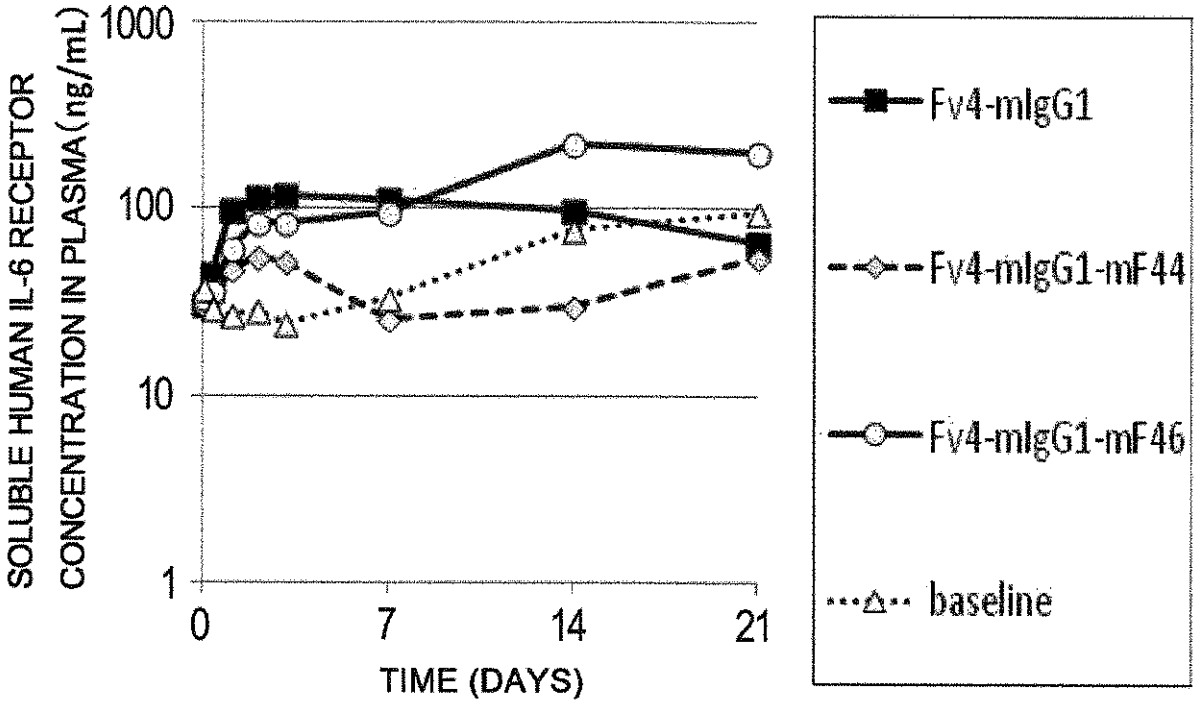

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA(ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
baseline

FIG. 14

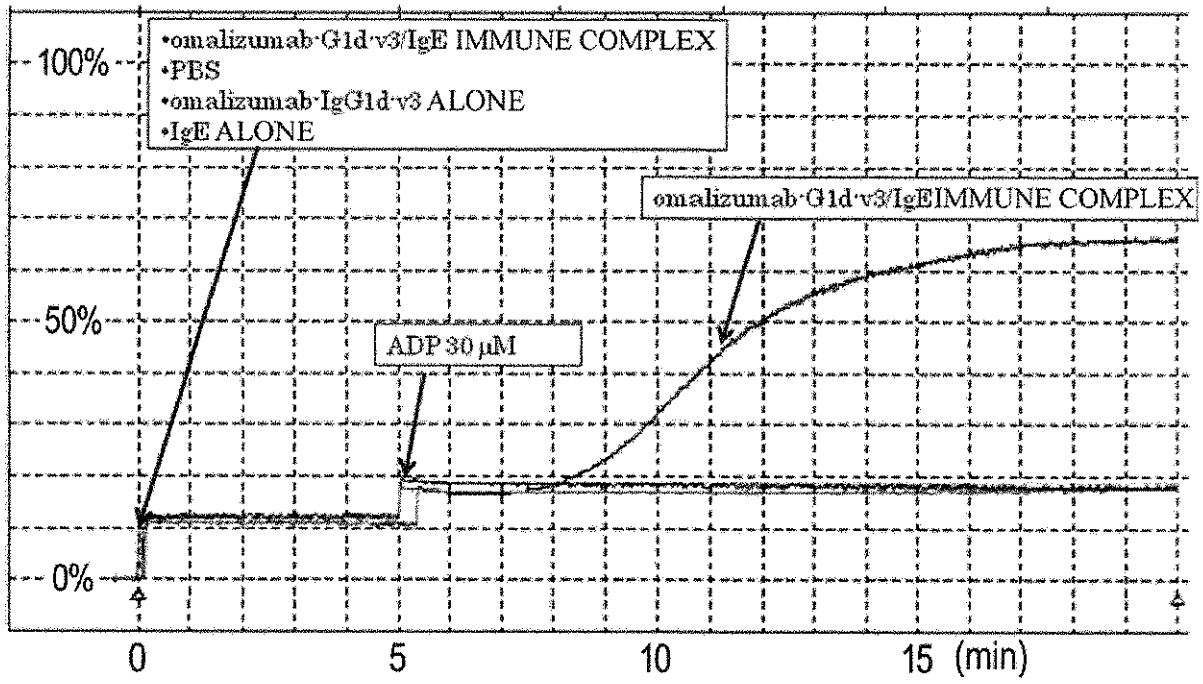

•omalizumab-G1d-v3/IgE IMMUNE COMPLEX
•PBS
•omalizumab-IgG1d-v3 ALONE
•IgE ALONE
omalizumab-G1d-v3/IgEIMMUNE COMPLEX
ADP 30 μM
100%
50%
0%
0
5
10
15 (min)

FIG. 15

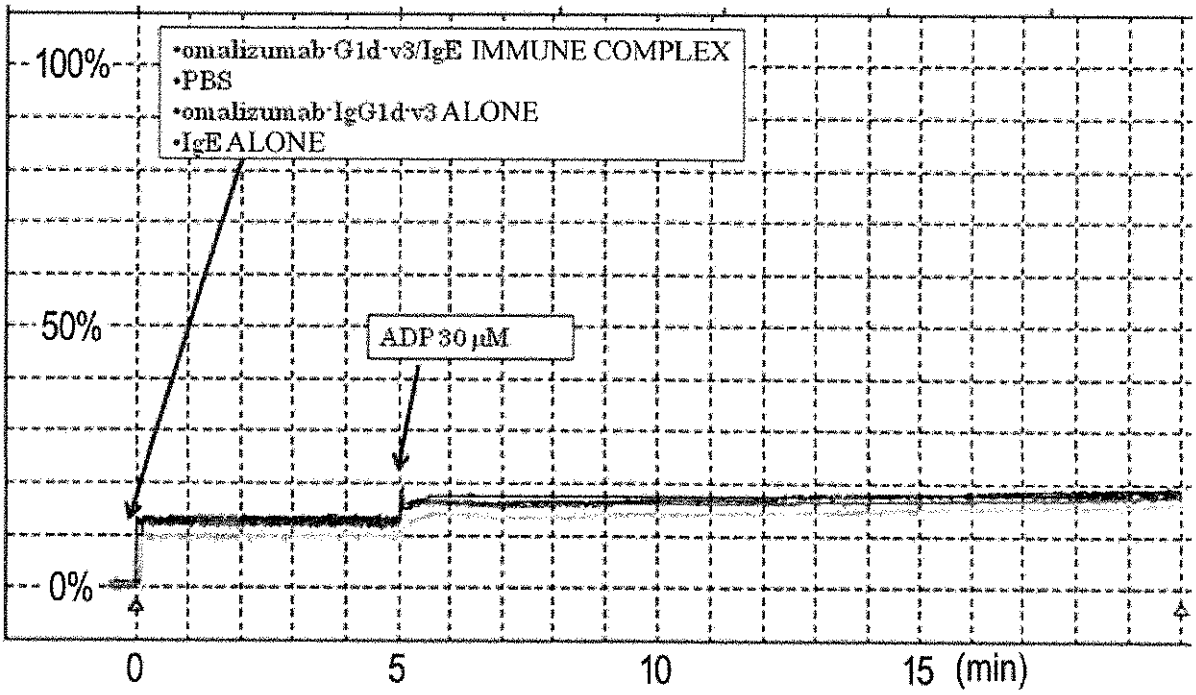

•omalizumab-G1d-v3/IgE IMMUNE COMPLEX
•PBS
•omalizumab-IgG1d-v3 ALONE
•IgE ALONE
100%
50%
0%
ADP 30 μM
0
5
10
15 (min)

FIG. 16

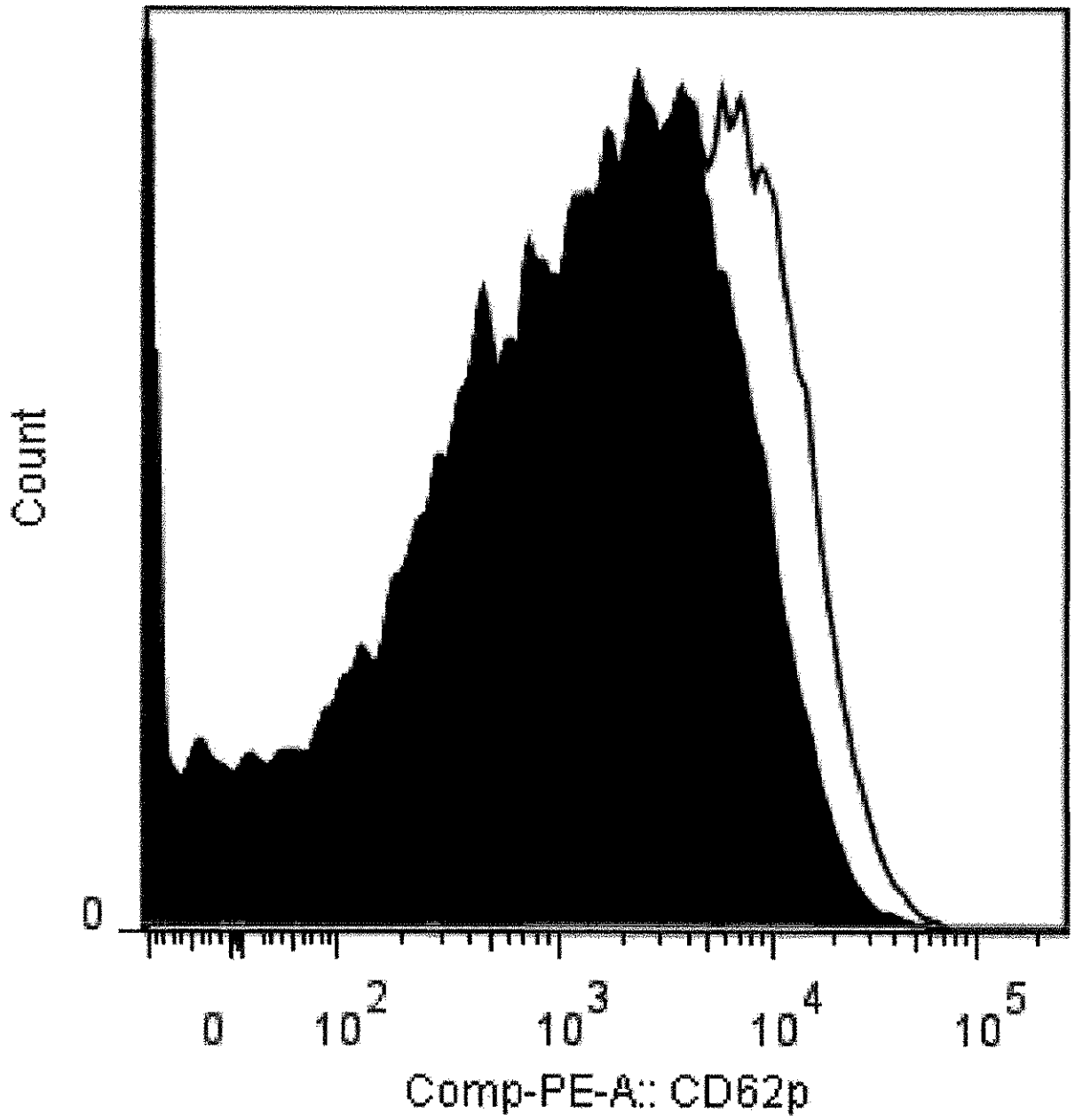


FIG. 17

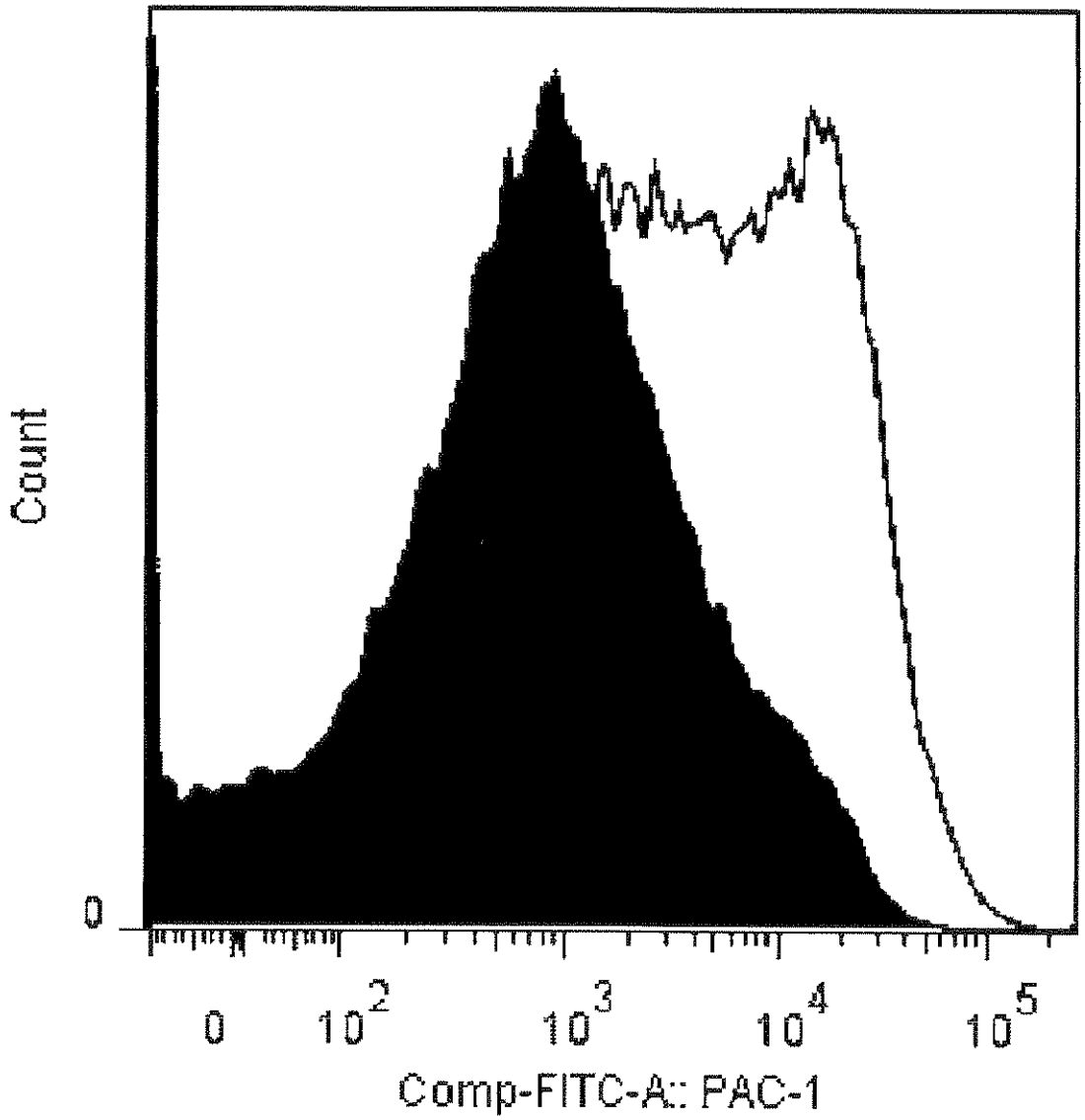


FIG. 18

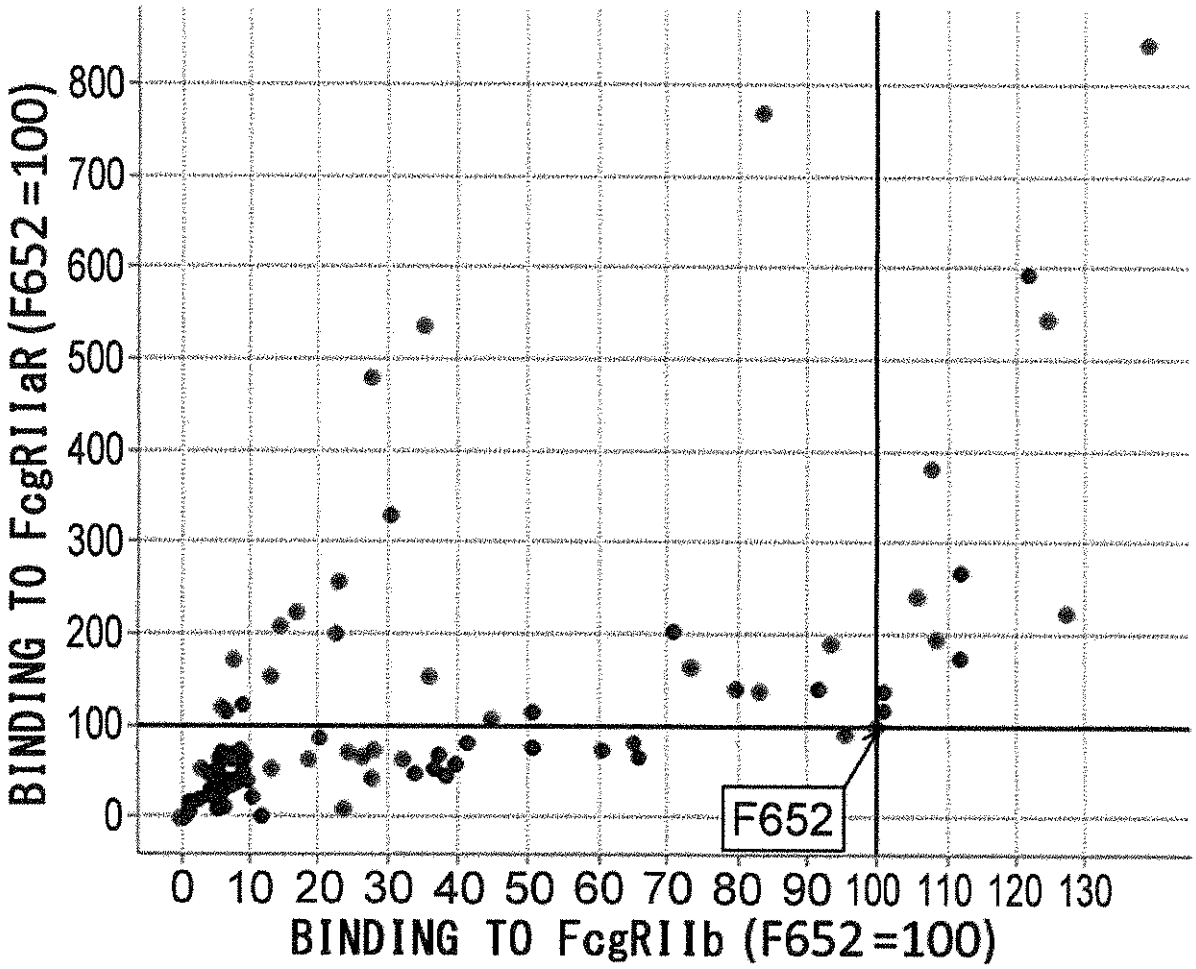

BINDING TO FcgRIIaR (F652=100)
800
700
600
500
400
300
200
100
0
F652
0 10 20 30 40 50 60 70 80 90 100 110 120 130
BINDING TO FcgRIIb (F652=100)

FIG. 19

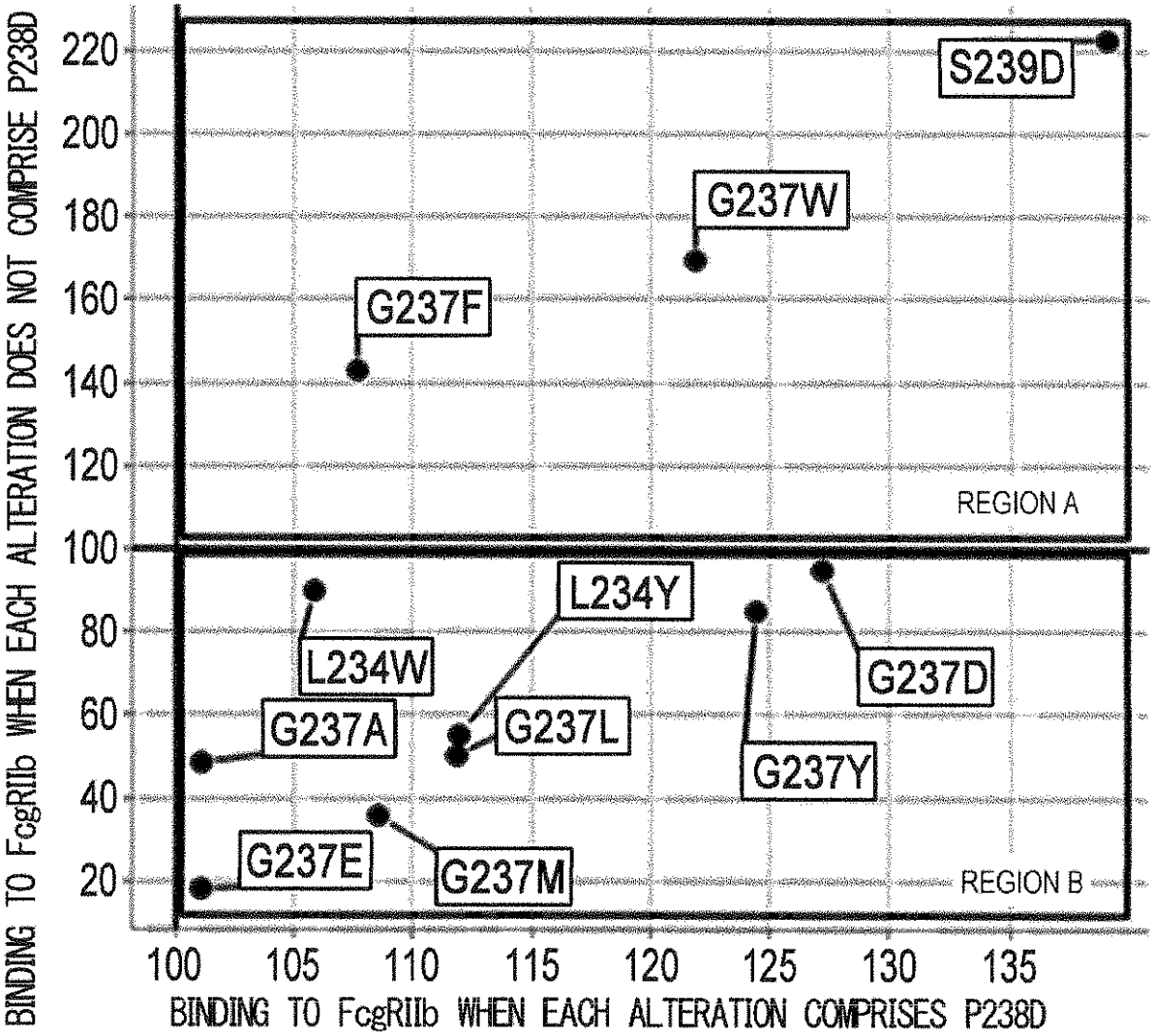

BINDING TO FcgRIIb WHEN EACH ALTERATION DOES NOT COMPRISE P238D
220
200
180
160
140
120
100
80
60
40
20
S239D
G237W
G237F
REGION A
L234Y
L234W
G237D
G237A
G237L
G237Y
G237E
G237M
REGION B
100
105
110
115
120
125
130
135
BINDING TO FcgRIIb WHEN EACH ALTERATION COMPRISES P238D

FIG. 20

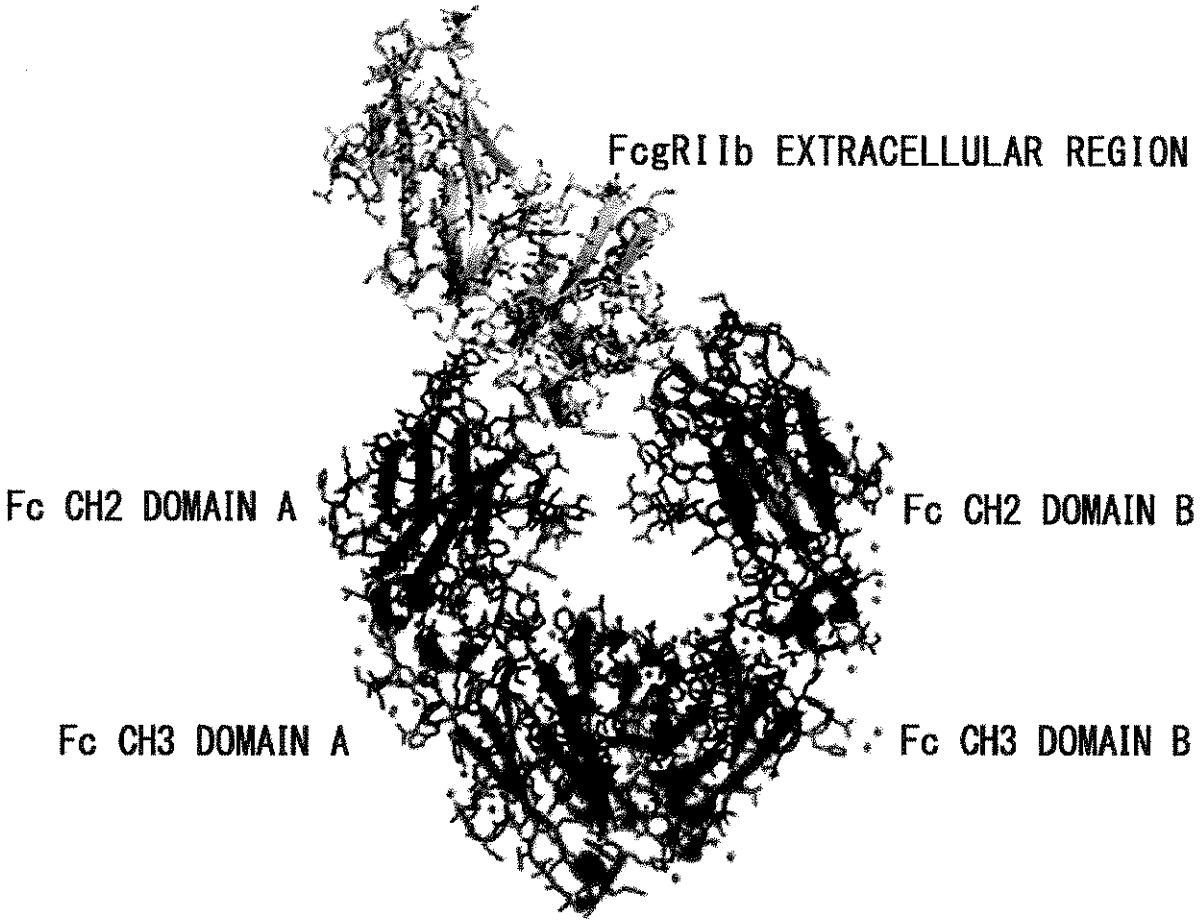
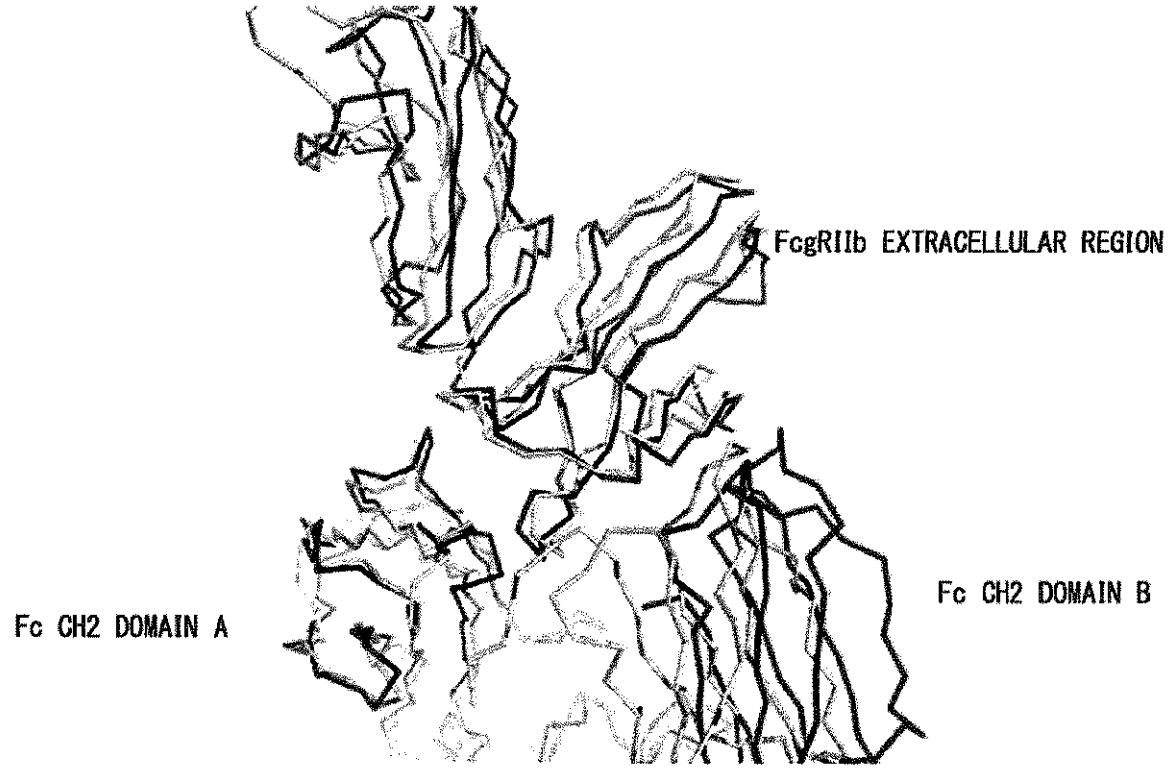
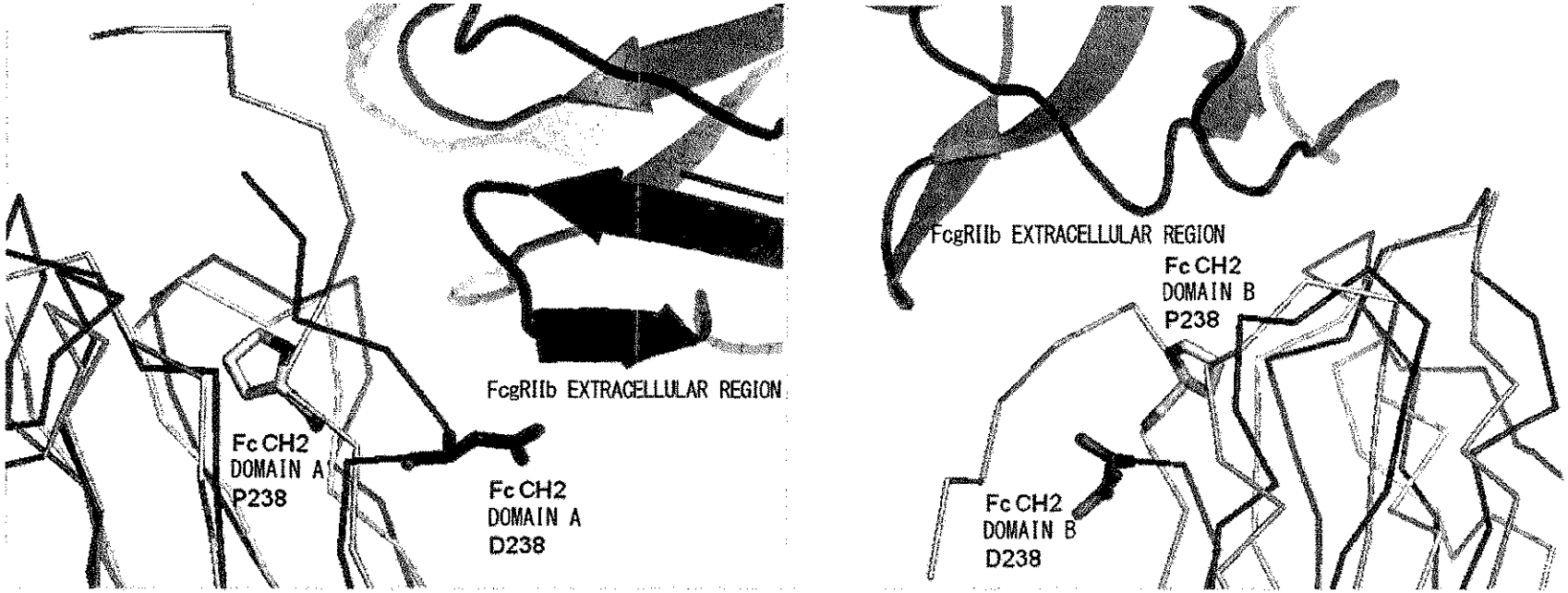

FcgRIIb EXTRACELLULAR REGION
Fc CH2 DOMAIN A
Fc CH2 DOMAIN B
Fc CH3 DOMAIN A
Fc CH3 DOMAIN B

FIG. 21



BLACK CRYSTAL STRUCTURE OF Fc(P238D)/FcγRIIb EXTRACELLULAR REGION COMPLEX
GRAY MODEL STRUCTURE OF Fc(WT)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 22



BLACK CRYSTAL STRUCTURE OF Fc(P238D)/FcγRIIb EXTRACELLULAR REGION COMPLEX

GRAY MODEL STRUCTURE OF Fc(WT)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 23

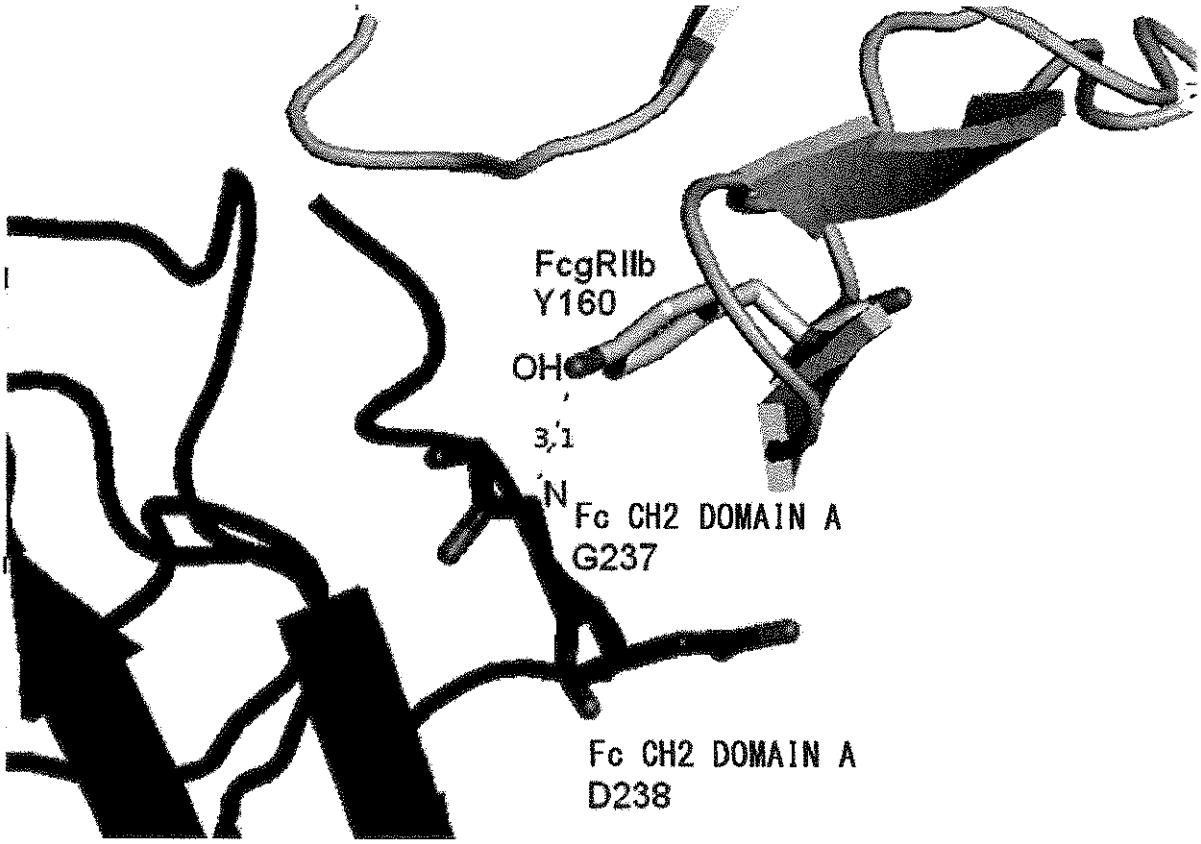


FIG. 24

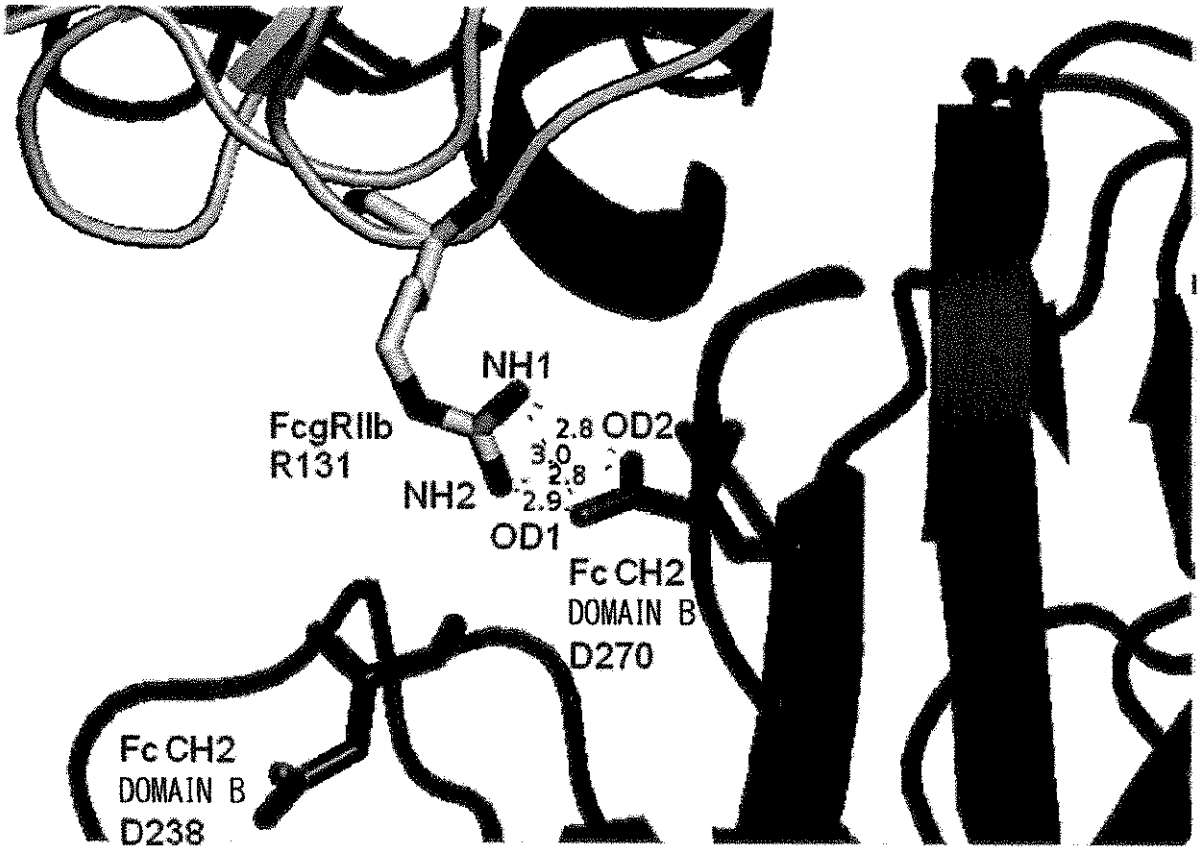

NH1
FcgRIIb
R131
2.8
OD2
3.0
2.8
NH2
2.9
OD1
Fc CH2
DOMAIN B
D270
Fc CH2
DOMAIN B
D238

FIG. 25

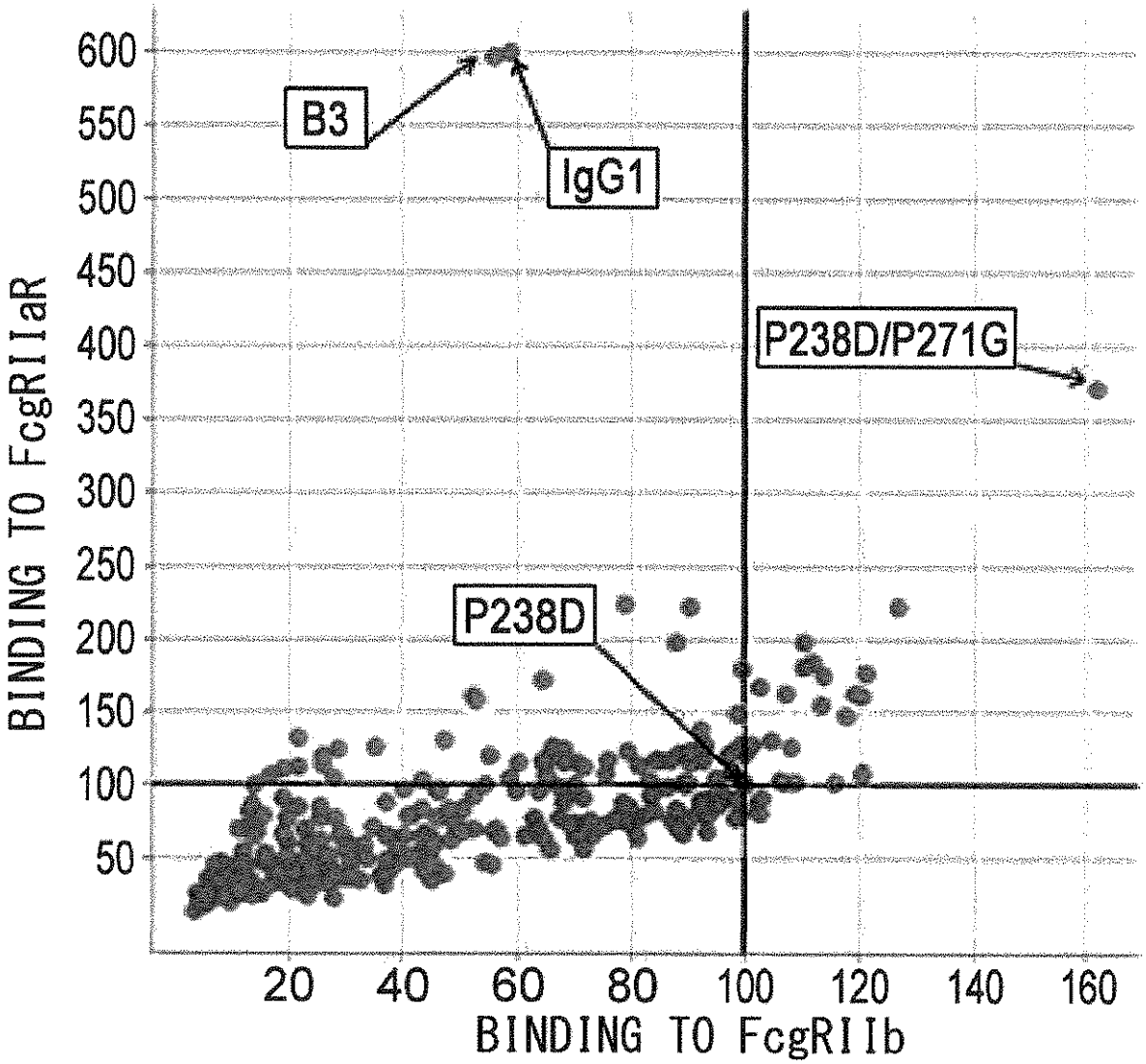

600
550
500
450
400
350
300
250
200
150
100
50
BINDING TO FcgRIIaR
B3
IgG1
P238D/P271G
P238D
20
40
60
80
100
120
140
160
BINDING TO FcgRIIb

FIG. 26

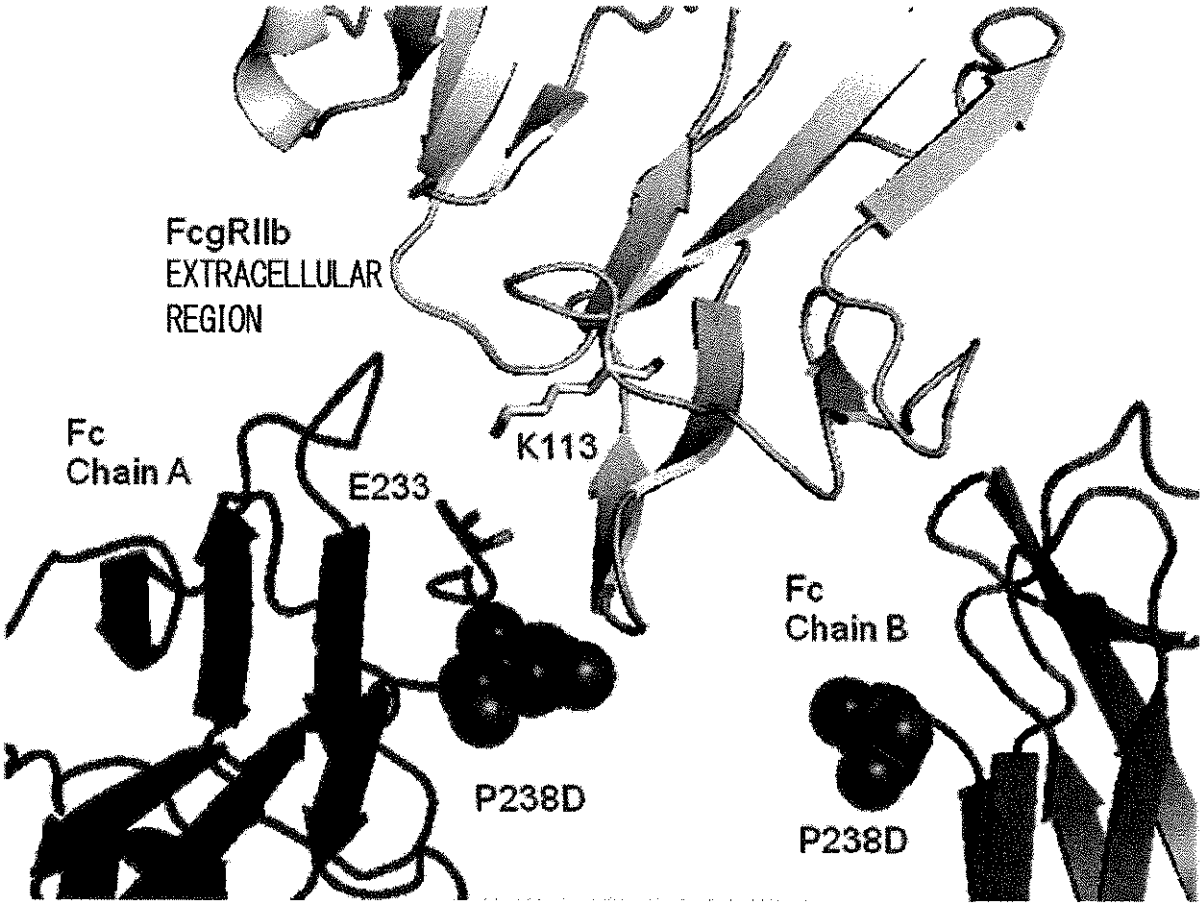

FcgRIIb
EXTRACELLULAR
REGION
Fc
Chain A
E233
K113
Fc
Chain B
P238D
P238D

FIG. 27

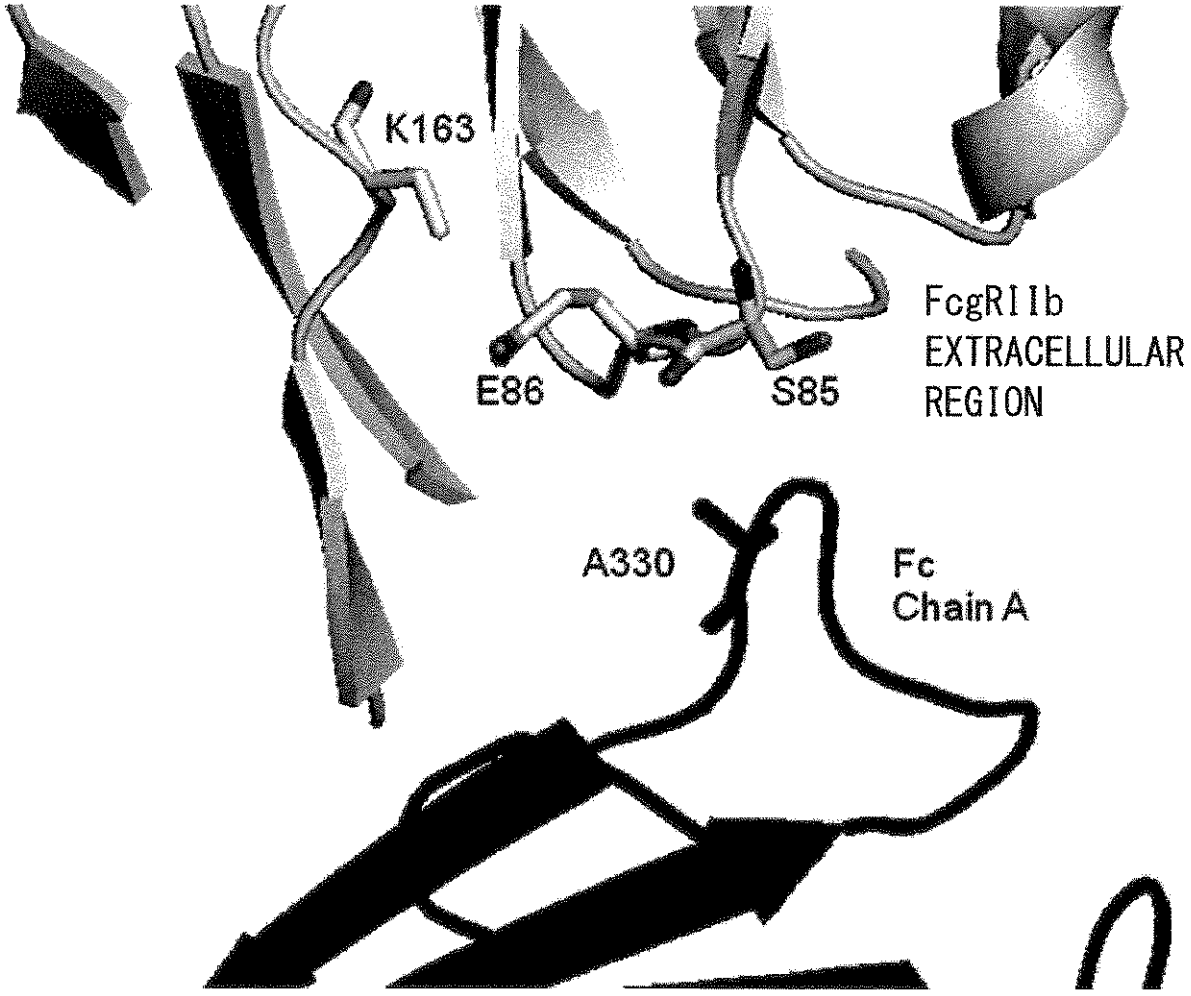
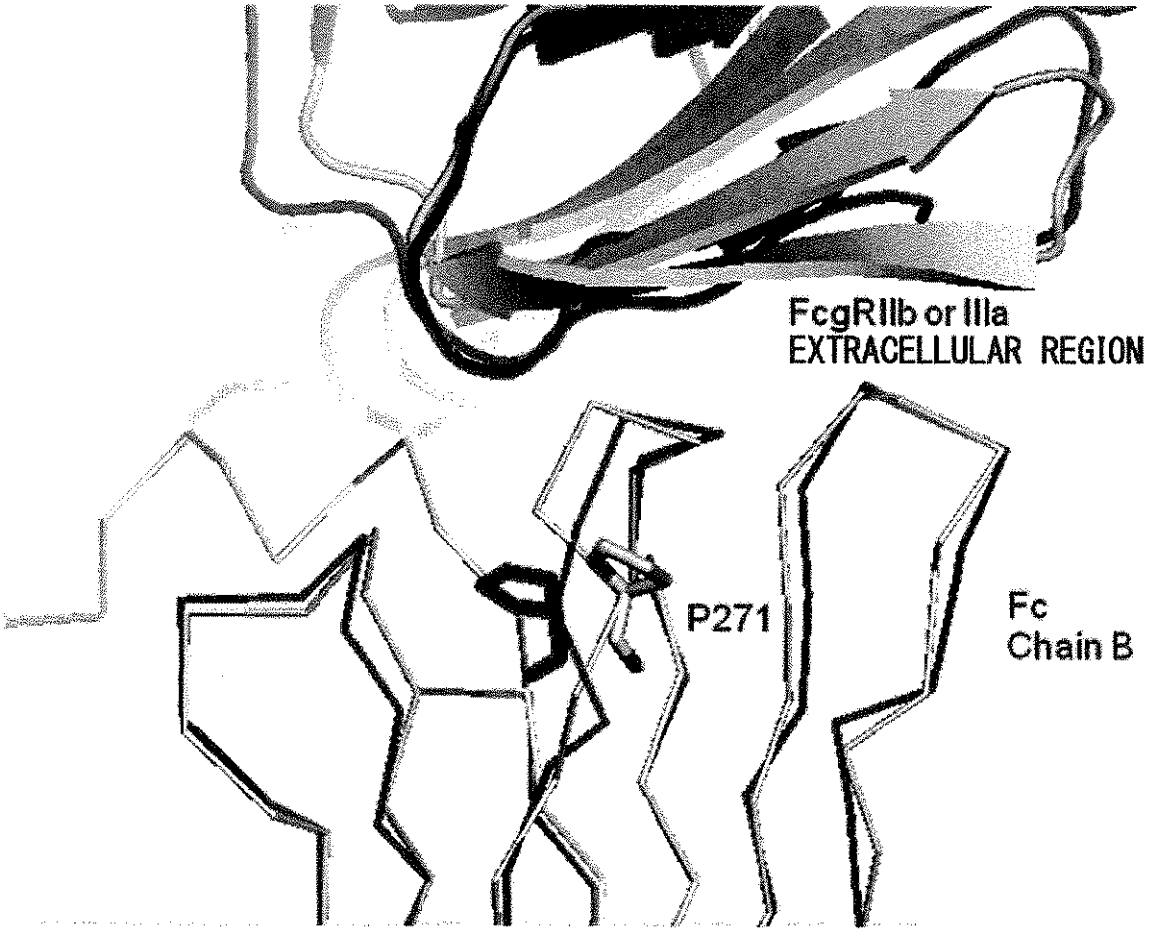


FIG. 28



BLACK CRYSTAL STRUCTURE OF Fc(P238D)/FcγRIIb EXTRACELLULAR REGION COMPLEX
GRAY CRYSTAL STRUCTURE OF Fc(WT)/FcγRIIIa EXTRACELLULAR REGION COMPLEX (PDB code: 3SGJ)

FIG. 29

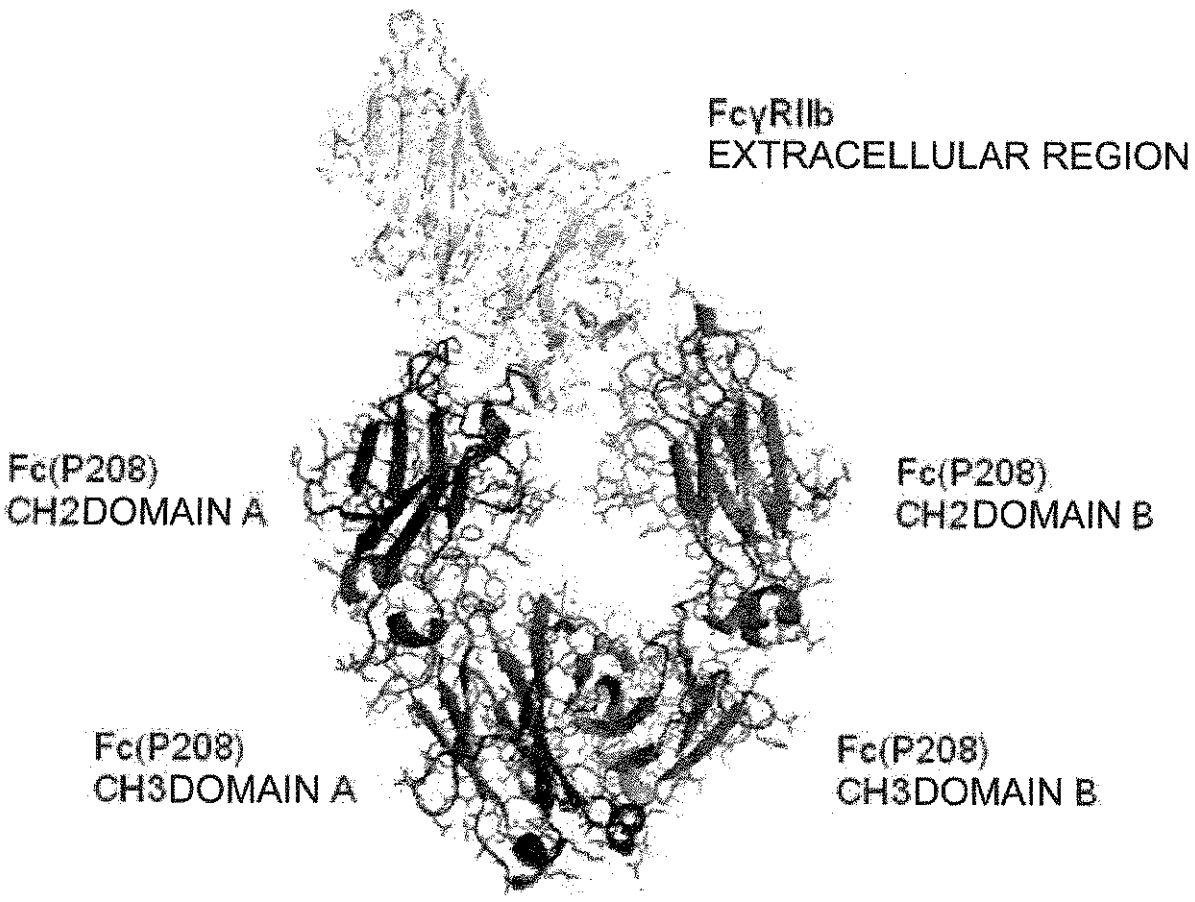
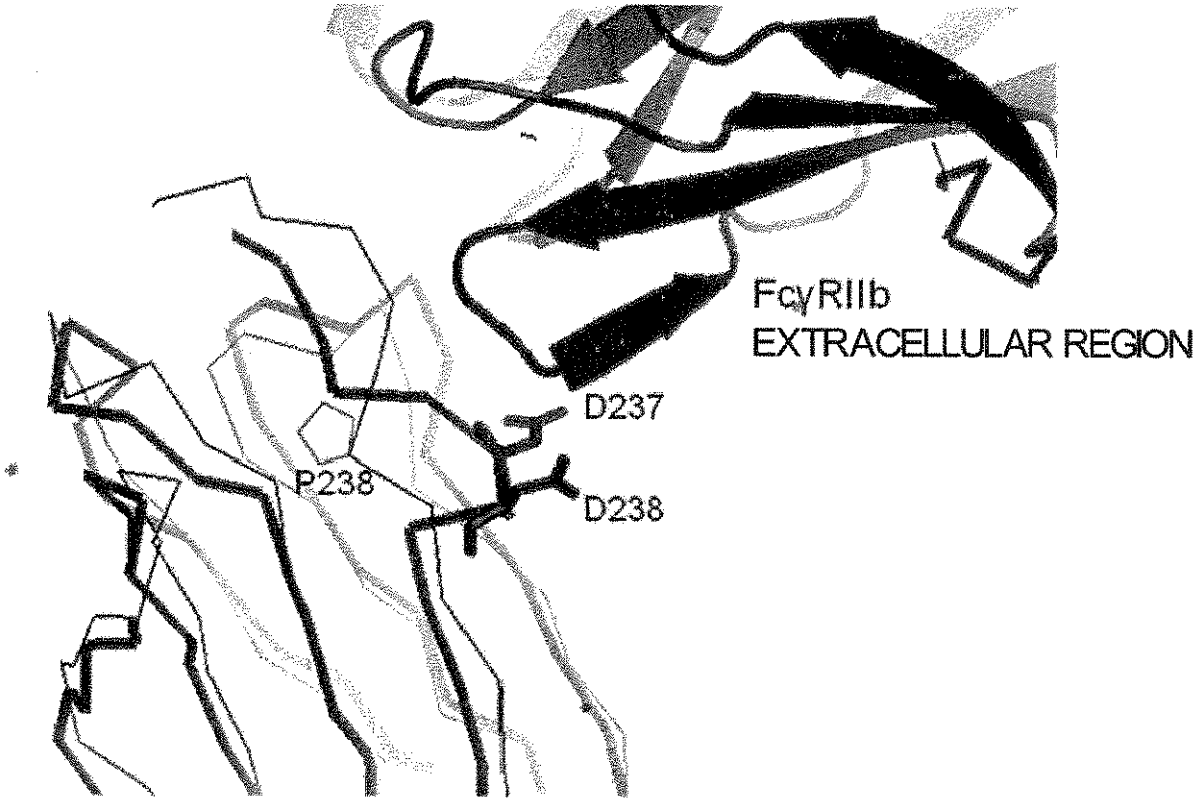

FcγRIIb
EXTRACELLULAR REGION
Fc(P208)
CH2DOMAIN A
Fc(P208)
CH2DOMAIN B
Fc(P208)
CH3DOMAIN A
Fc(P208)
CH3DOMAIN B

FIG. 30



THIN LINE: Fc(Wt) CH2DOMAIN A
HEAVY LINE: Fc(P208) CH2DOMAIN A

FIG. 31

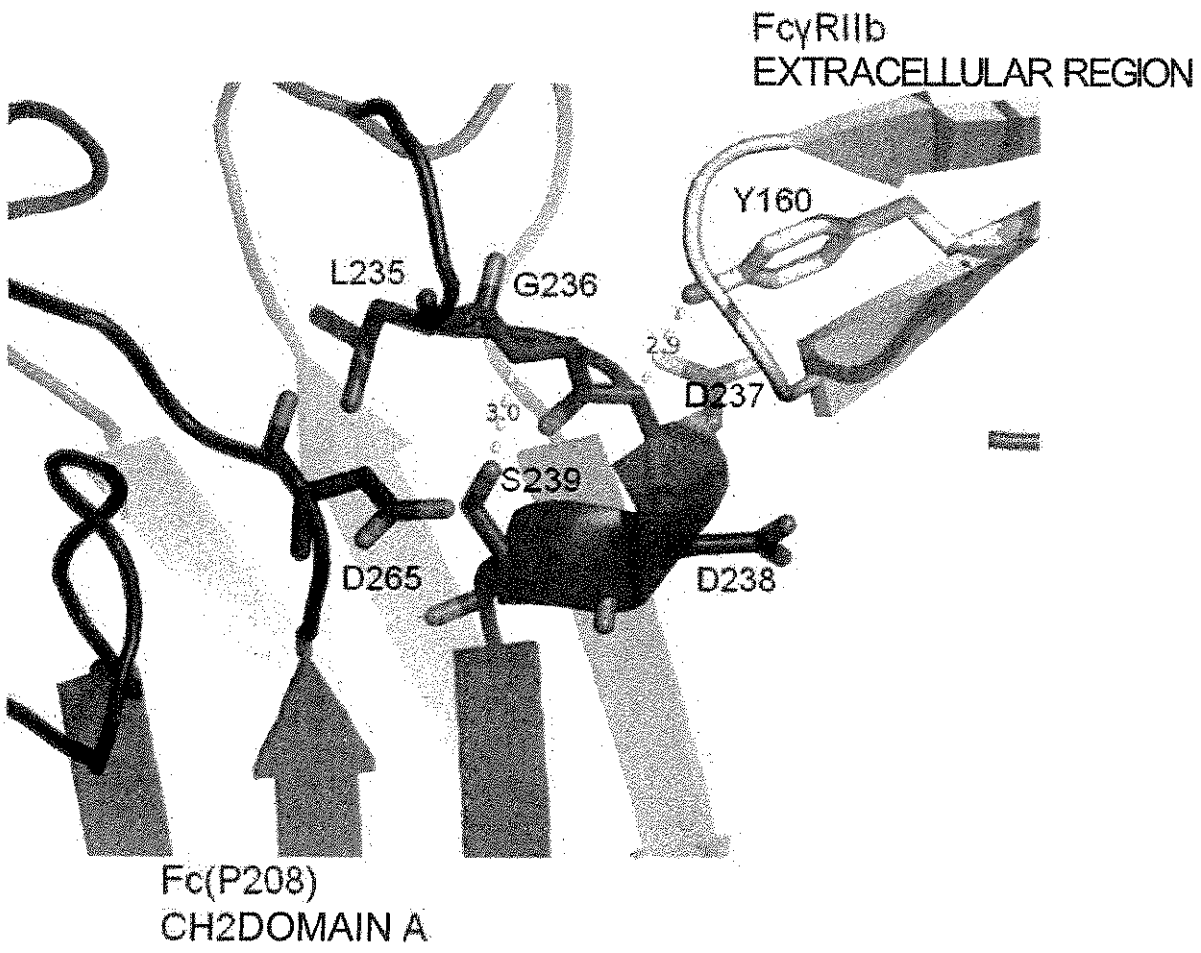

FcγRIIb
EXTRACELLULAR REGION
Y160
L235
G236
2.9
D237
3.0
S239
D265
D238
Fc(P208)
CH2DOMAIN A

FIG. 32

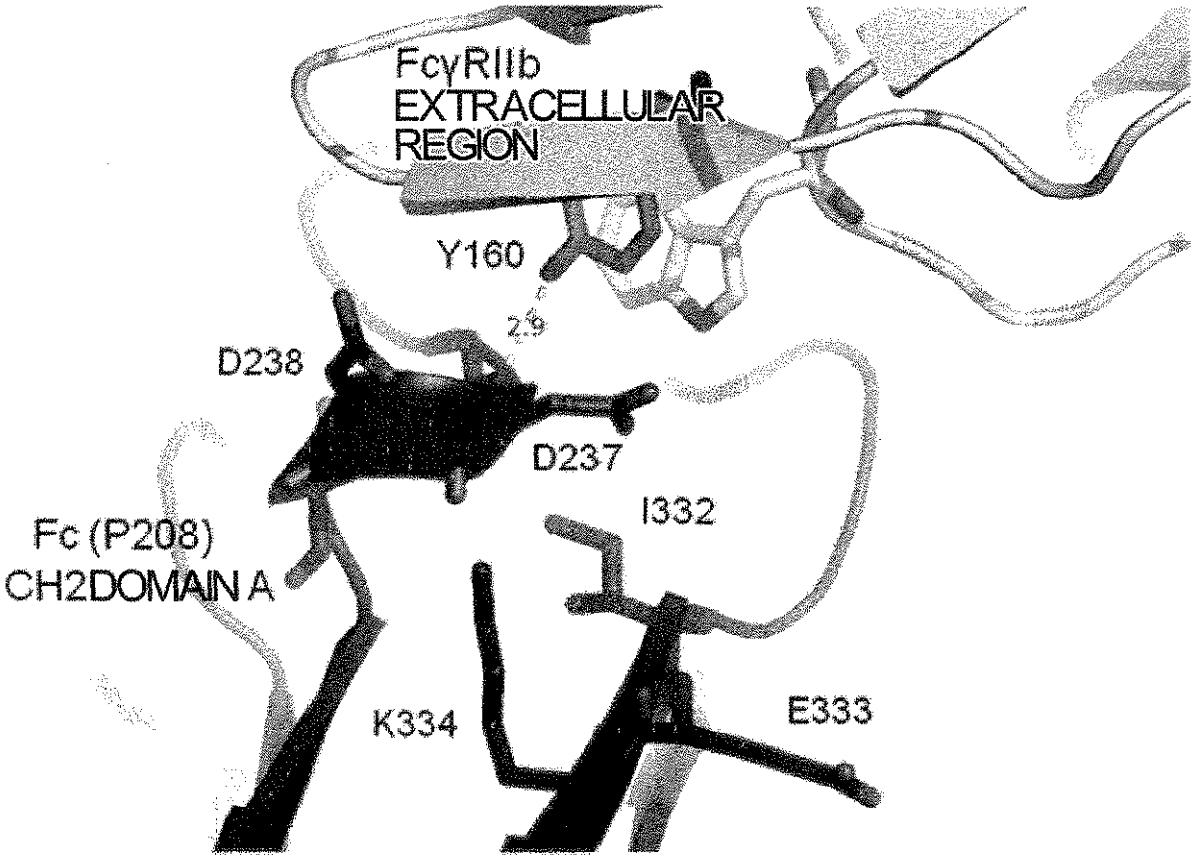
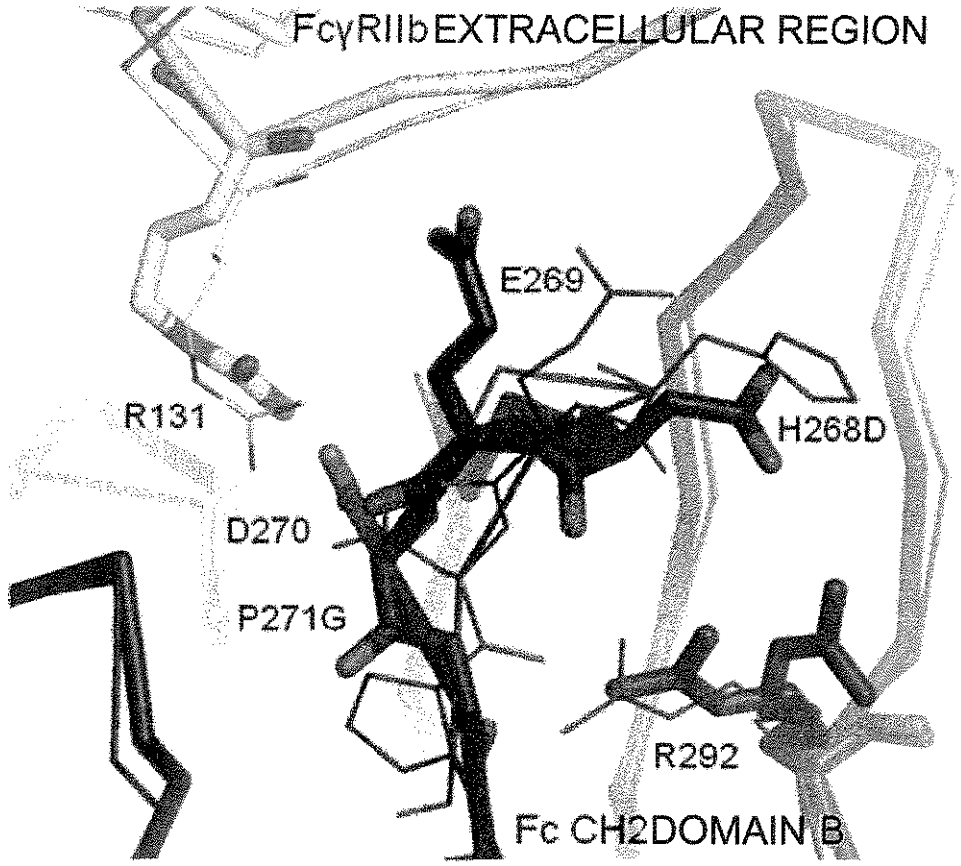
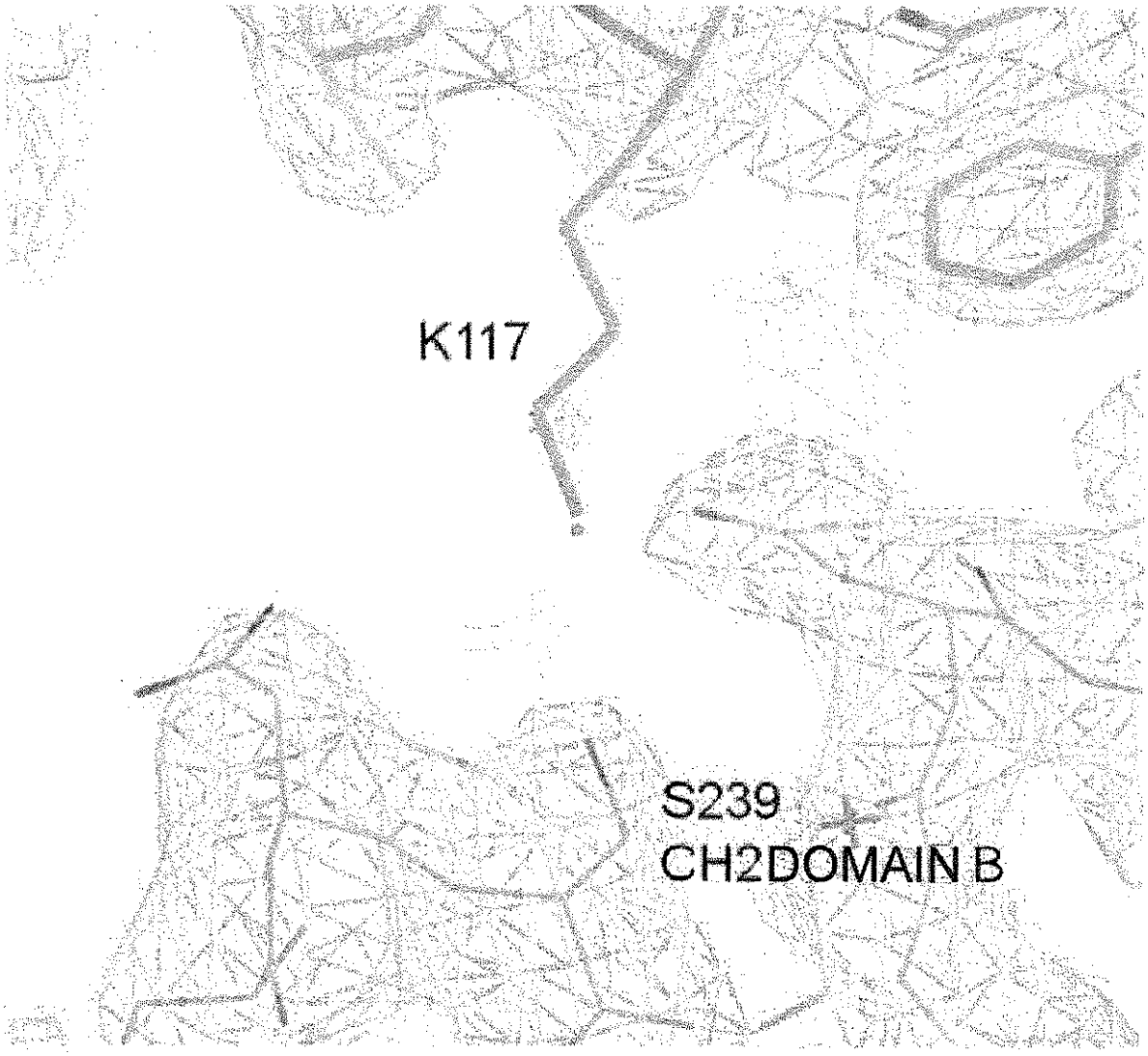

FcγRIIb
EXTRACELLULAR
REGION
Y160
2.9
D238
D237
I332
Fc (P208)
CH2DOMAIN A
K334
E333

FIG. 33



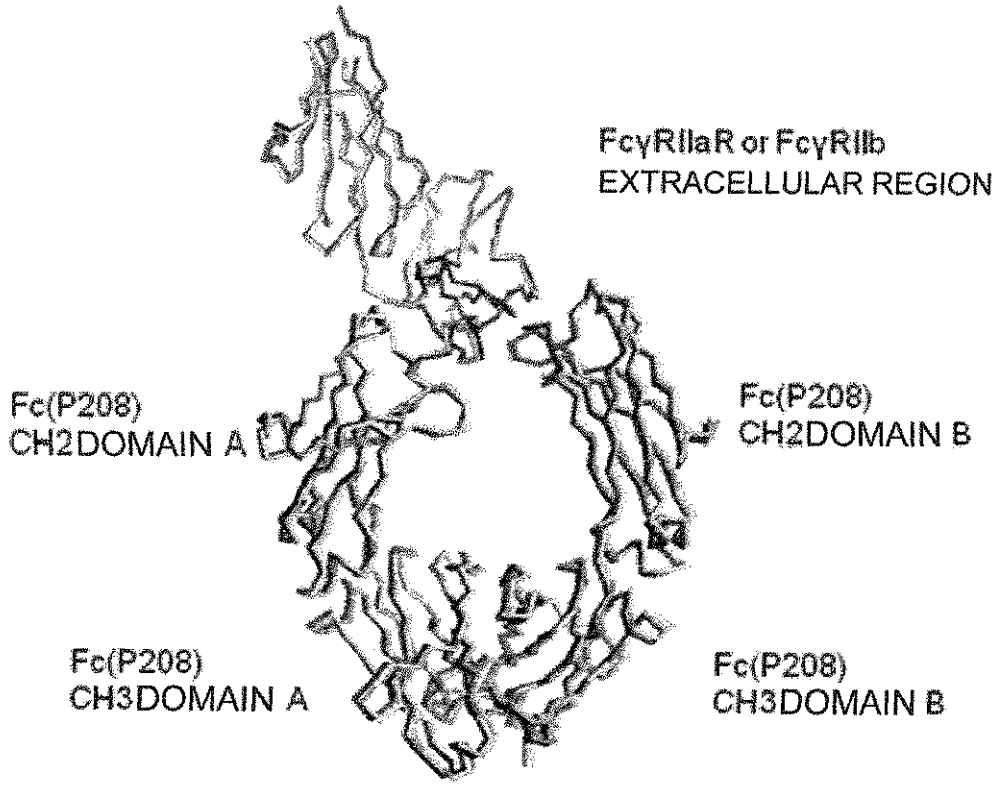
THIN LINE: X-RAY CRYSTAL STRUCTURE OF Fc(P238D)/FcγRIIb EXTRACELLULAR REGION COMPLEX
HEAVY LINE: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 34



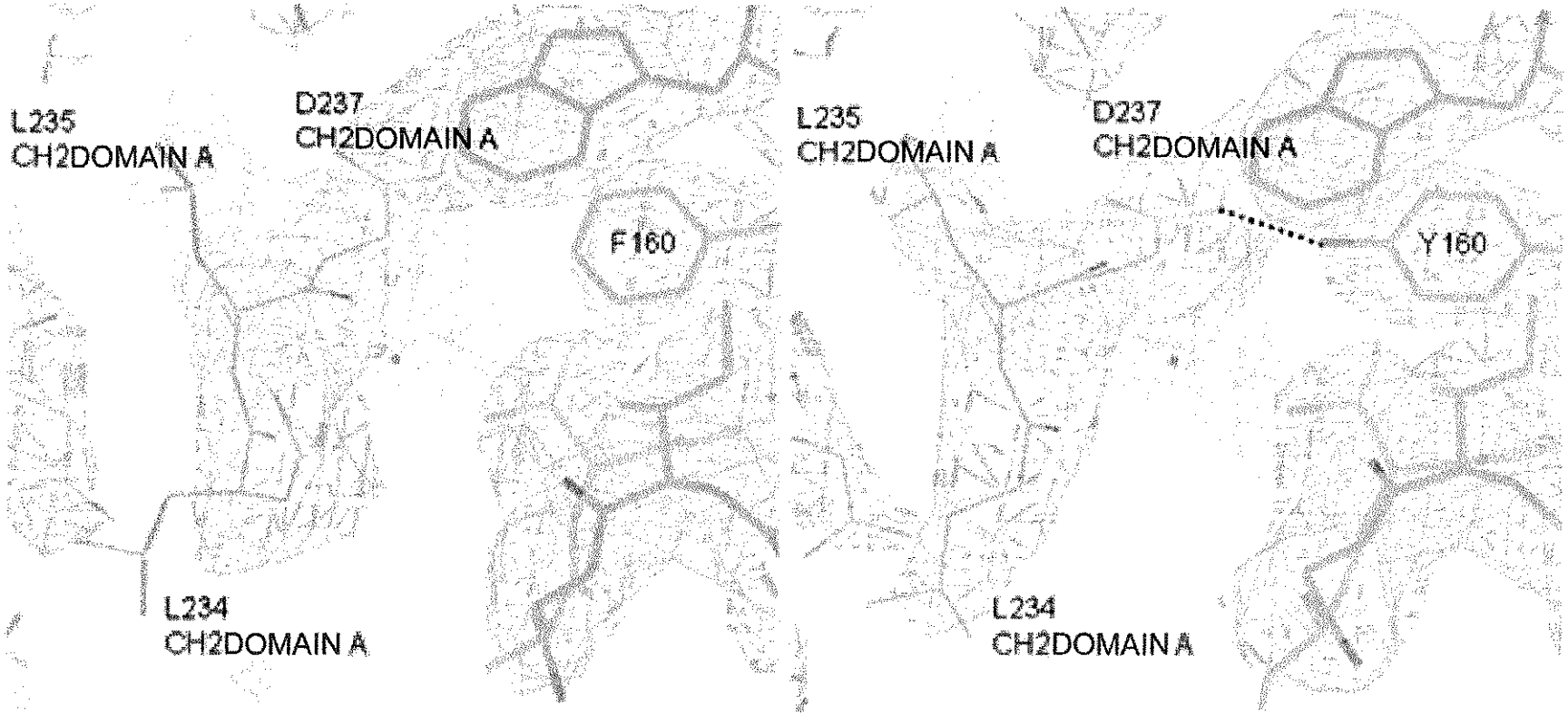
THIN LINE : Fc(P208)
HEAVY LINE : FcγRIIb EXTRACELLULAR REGION

FIG. 35



BLACK: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIaR EXTRACELLULAR REGION COMPLEX
GRAY: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 36



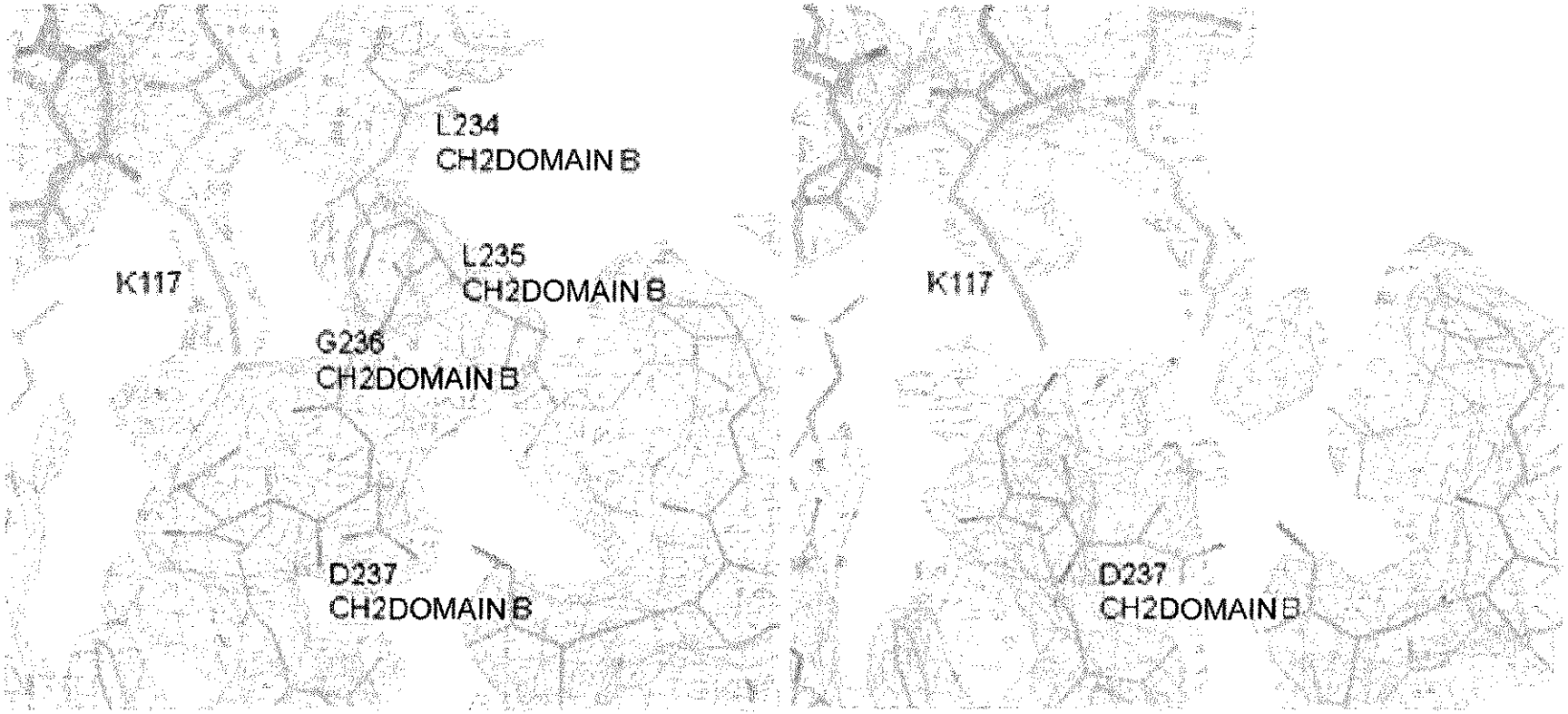
THIN LINE: Fc(P208)

HEAVY LINE: FcγRIIaR or FcγRIIb EXTRACELLULAR REGION

LEFT: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIaR EXTRACELLULAR REGION COMPLEX

RIGHT: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 37



THIN LINE: Fc(P208)

HEAVY LINE: FcγRIIaR or FcγRIIb EXTRACELLULAR REGION

LEFT: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIaR EXTRACELLULAR REGION COMPLEX

RIGHT: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 38

```
G1d  118 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
G4d  118 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS

G1d  208 NTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
G4d  208 NTKVDKRVESK---YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN

G1d  298 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
G4d  298 STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP

G1d  388 ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP (SEQ ID NO: 80)
G4d  388 ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL (SEQ ID NO: 81)
```

FIG. 39

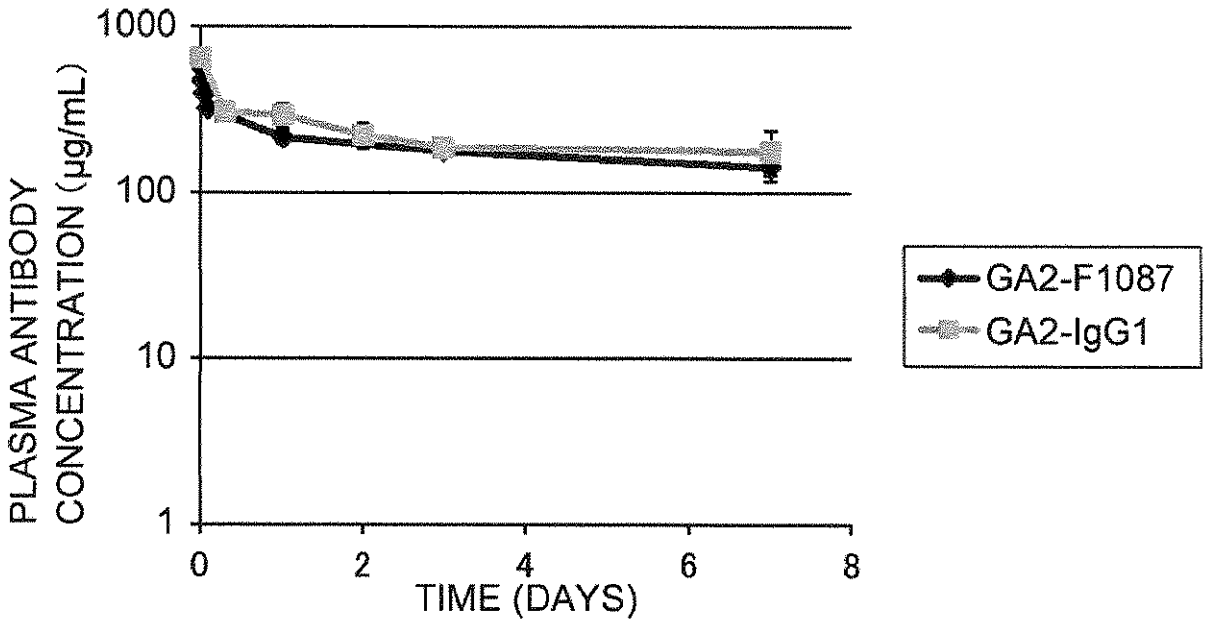

1000
100
10
1
PLASMA ANTIBODY CONCENTRATION (μg/mL)
0
2
4
6
8
TIME (DAYS)
GA2-F1087
GA2-IgG1

FIG. 40

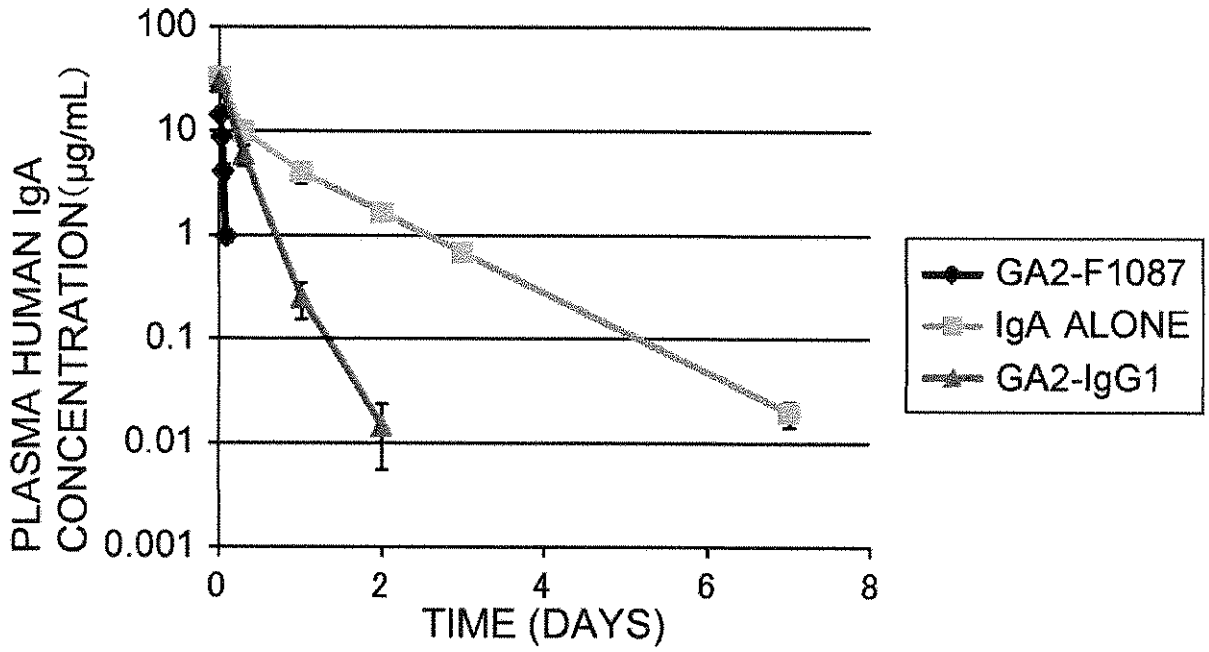

100
10
1
0.1
0.01
0.001
PLASMA HUMAN IgA CONCENTRATION(μg/mL)
0
2
4
6
8
TIME (DAYS)
GA2-F1087
IgA ALONE
GA2-IgG1

FIG. 41

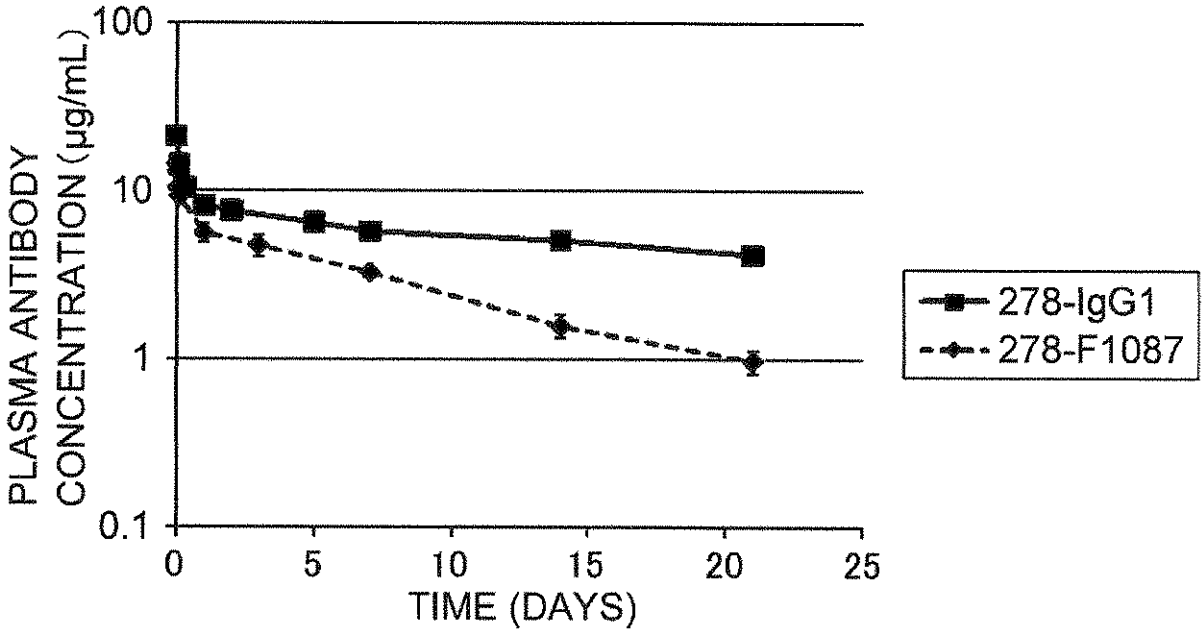

PLASMA ANTIBODY CONCENTRATION (μg/mL)
100
10
1
0.1
0
5
10
15
20
25
TIME (DAYS)
278-IgG1
278-F1087

FIG. 42

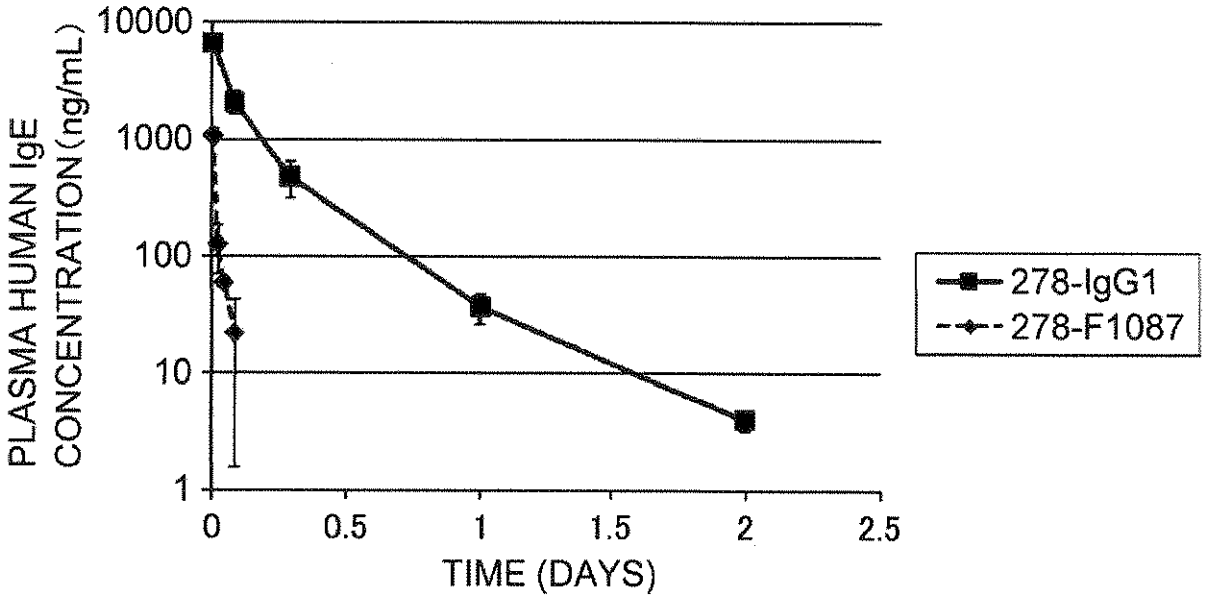

PLASMA HUMAN IgE CONCENTRATION (ng/mL)
10000
1000
100
10
1
0
0.5
1
1.5
2
2.5
TIME (DAYS)
278-IgG1
278-F1087

FIG. 43

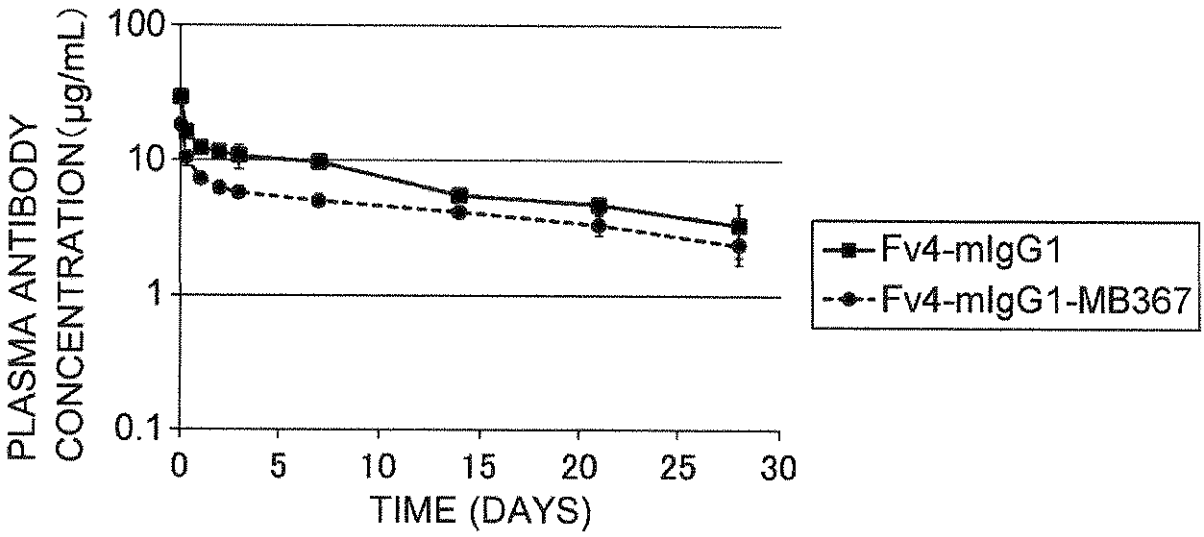

PLASMA ANTIBODY CONCENTRATION (μg/mL)
100
10
1
0.1
0
5
10
15
20
25
30
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-MB367

FIG. 44

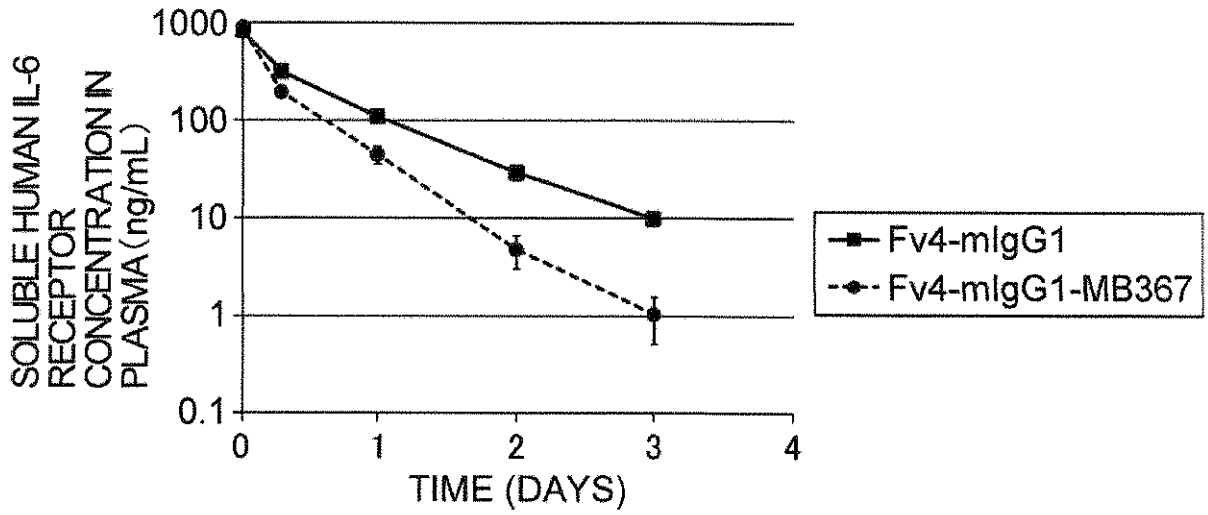

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0.1
0
1
2
3
4
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-MB367

FIG. 45