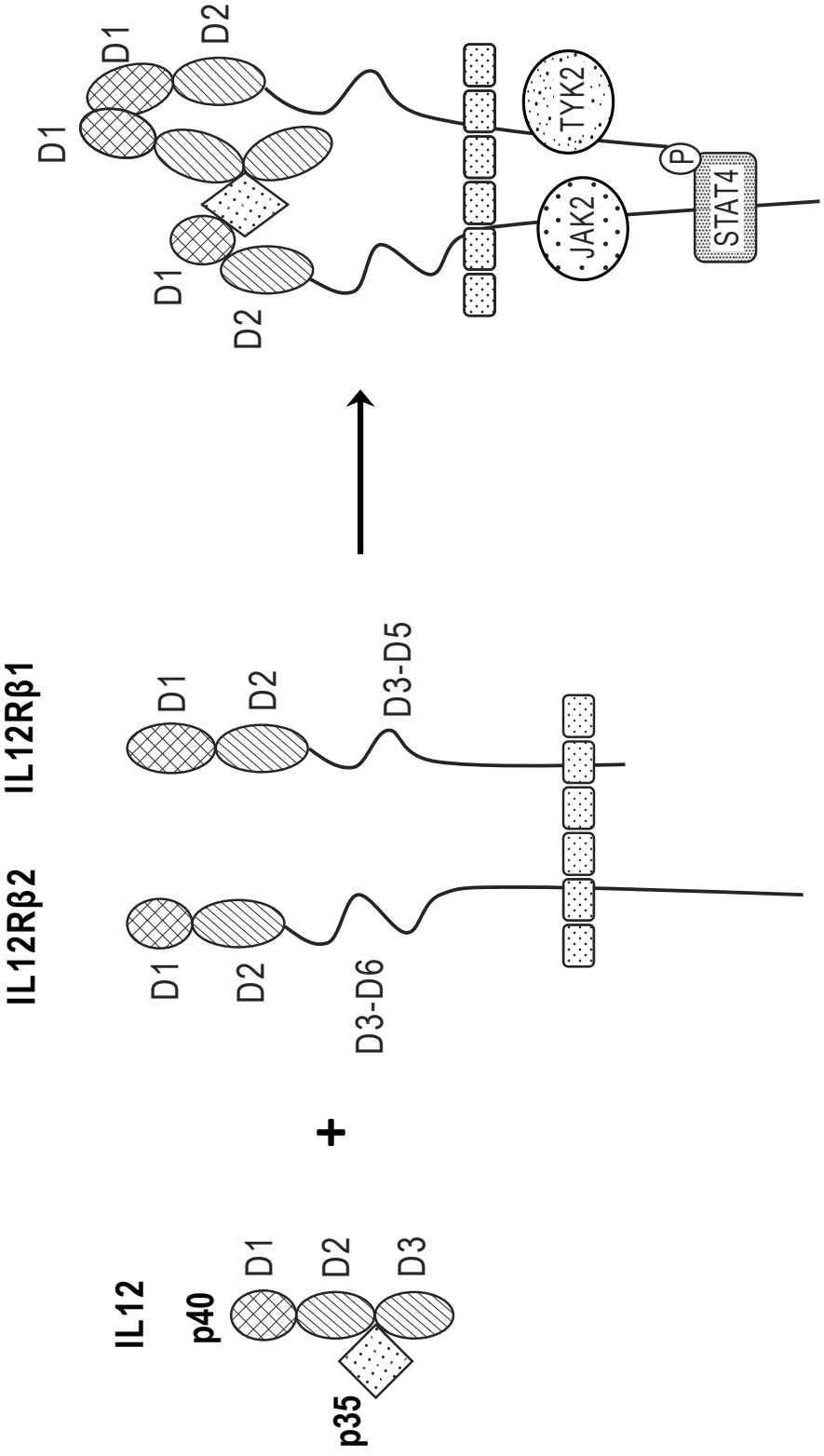

IL12
p40
p35
D1
D2
D3
+
IL12Rβ2
IL12Rβ1
D1
D2
D3-D6
D1
D2
D3-D5
D1
D2
D1
D2
D1
D2
TYK2
JAK2
P
STAT4

FIG. 1

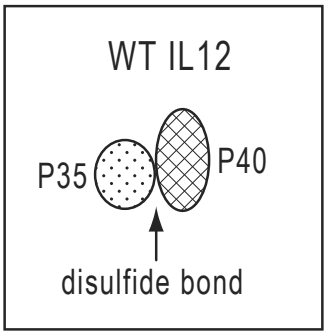


FIG. 2A

Monovalent:
IL12(p35xp40)-Fc

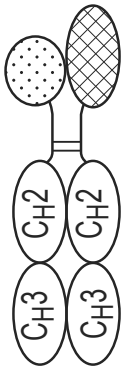


FIG. 2B

Monovalent:
IL12*(p35*xp40*)-Fc

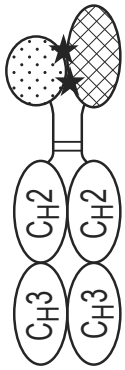


FIG. 2C

Monovalent:
Fc-IL12(p35xp40)

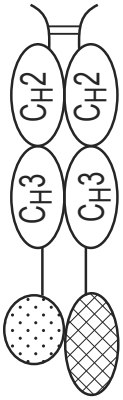


FIG. 2D

Monovalent:
Fc-IL12*(p35*xp40*)

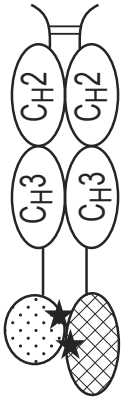


FIG. 2E

Monovalent:
Fc-p40-p35 x Fc

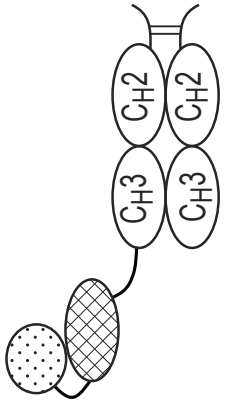


FIG. 2F

Monovalent:
Fc-IL12(p35-p40)

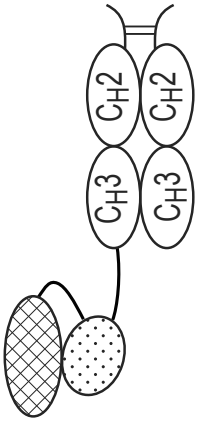


FIG. 2G

Bivalent:
IL12(p40-p35)-Fc

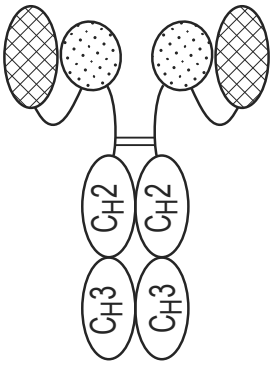


FIG. 2H

Bivalent:
IL12*(p40*-p35*)-Fc

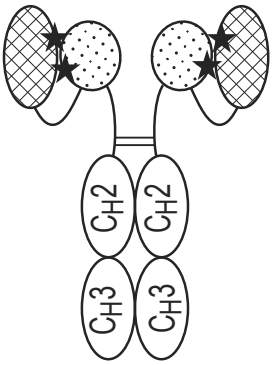


FIG. 2I

Bivalent:
IL12(p35-p40)-Fc

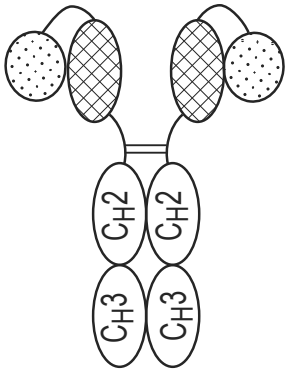


FIG. 2J

Bivalent:
Fc-IL12(p40-p35)

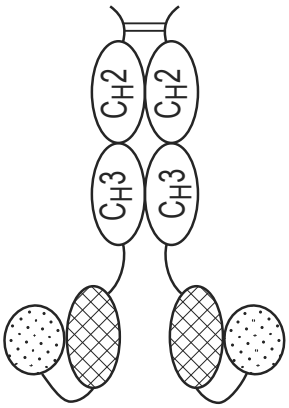


FIG. 2K

Bivalent:
Fc-IL12(p35-p40)

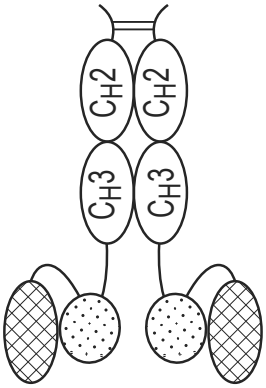


FIG. 2L

Bivalent:
Fc-IL12*(p40*-p35*)

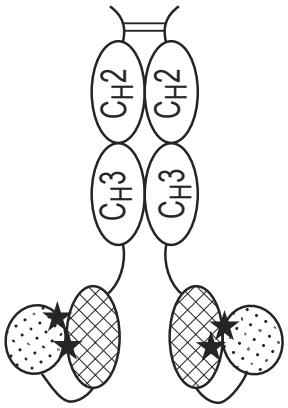


FIG. 2M

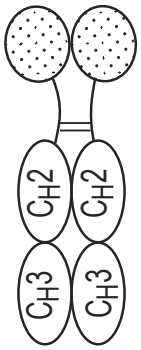


FIG. 2N

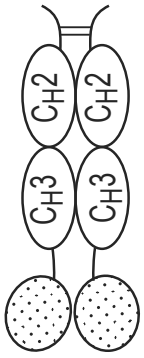


FIG. 2O

Monovalent:
Fc-p35 x Fc x p40

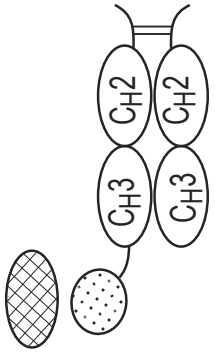


FIG. 2P

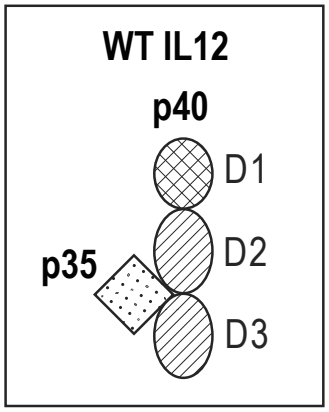


FIG. 3A

p40(mutein)-p35-Fc

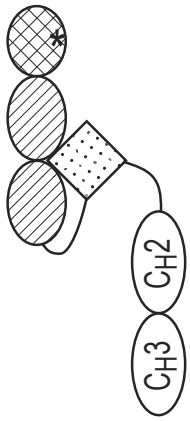


FIG. 3B

Fc-p40(mutein)-p35

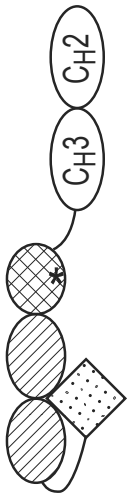


FIG. 3C

p40-p35(mutein)-Fc

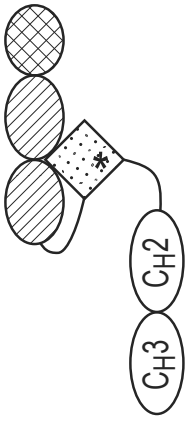


FIG. 3D

Fc-p40-p35(mutein)

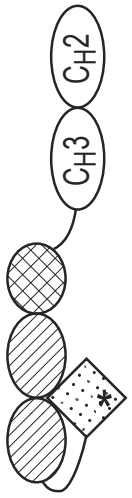


FIG. 3E

Fc-p40(mutein-p35
(mutein)

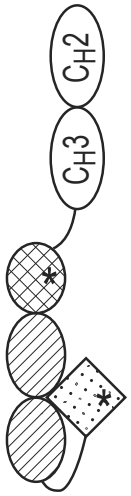


FIG. 3F

p40(D2-D3)-p35-Fc

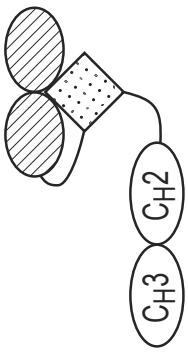


FIG. 3G

Fc-p40-(D2-D3)-p35

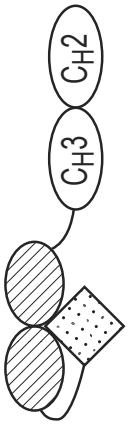


FIG. 3H

Fc-p35-p40(D2-D3)

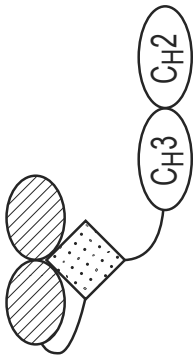


FIG. 3I

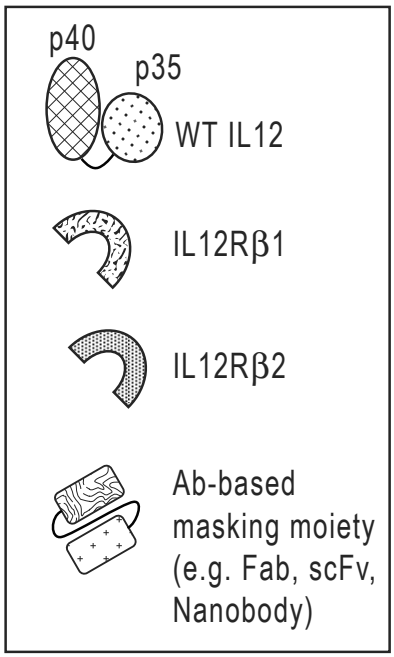


FIG. 4A

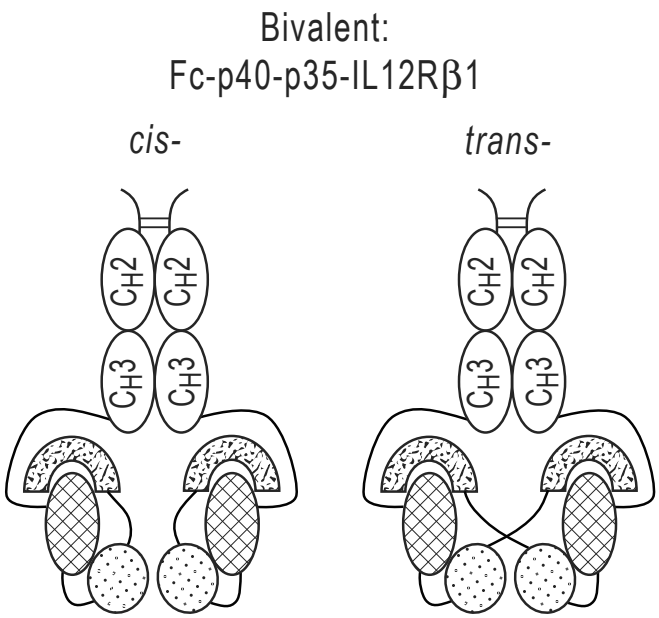


FIG. 4B

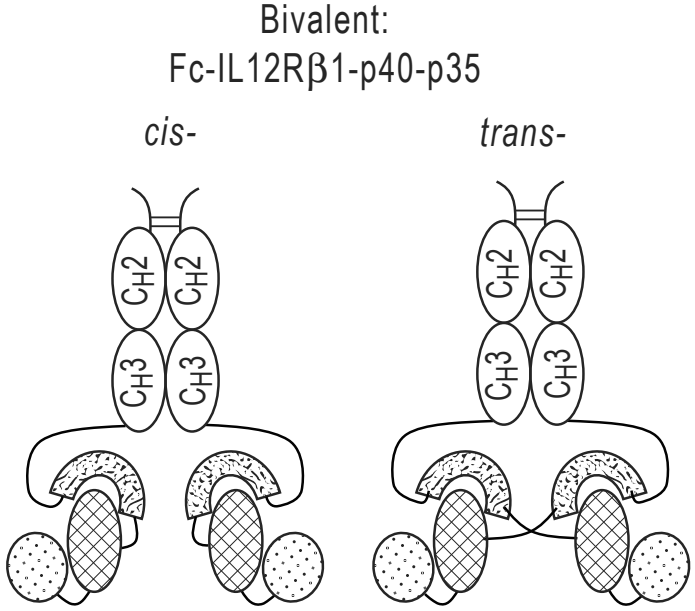


FIG. 4C

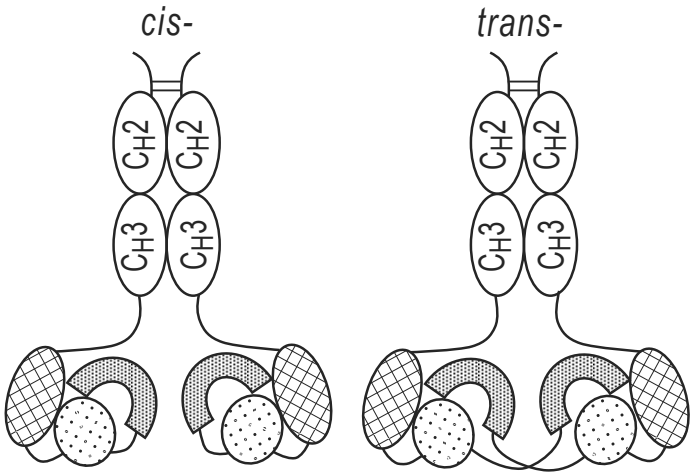


FIG. 4D

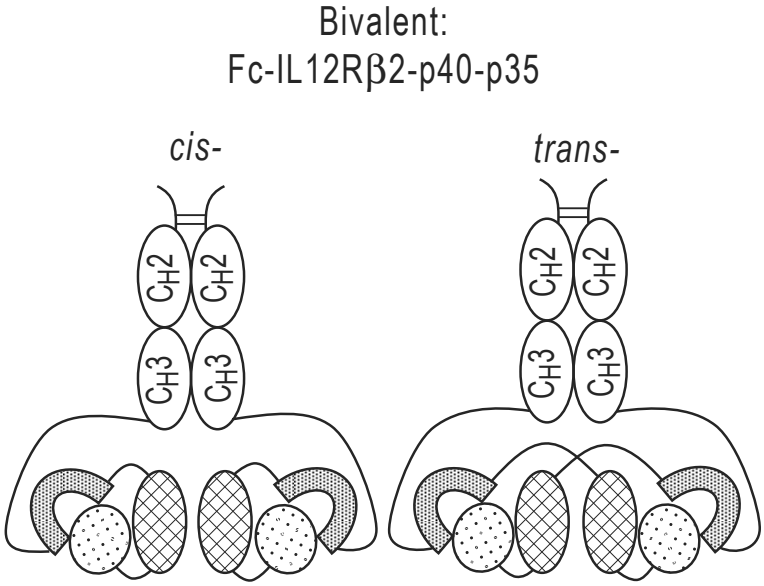


FIG. 4E

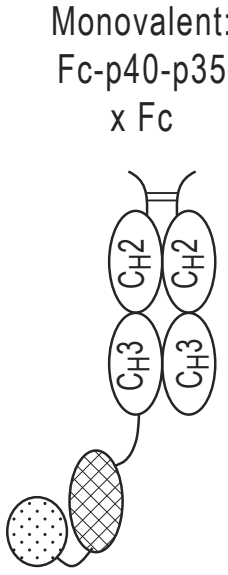


FIG. 4F

Monovalent:
Fc-p40-p35-IL12Rβ2
x Fc

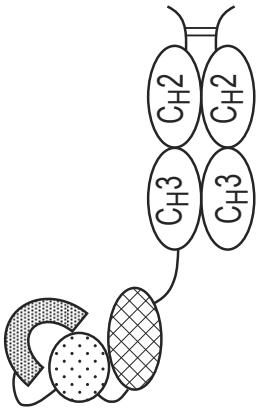


FIG. 4G

Monovalent:
Fc-p40-p35-IL12Rβ1
x Fc

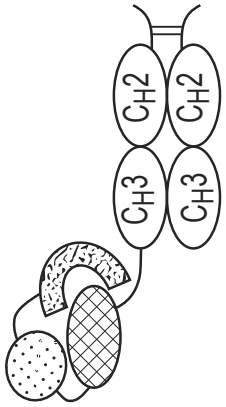


FIG. 4H

Monovalent:
Fc-IL12Rβ1-p40-p35
x Fc

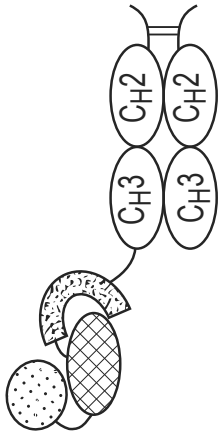


FIG. 4I

Monovalent:
Fc-p40-p35
x Fc-IL12Rβ1

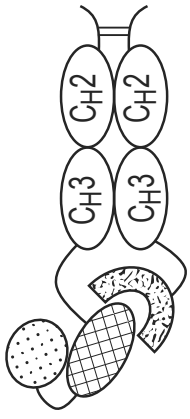


FIG. 4J

Monovalent:
Fc-p40-p35
x Fc-IL12Rβ2

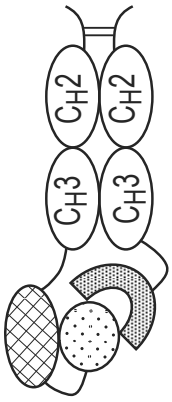


FIG. 4K

Monovalent:
Fc-p35-IL12Rβ2
x Fc-p40

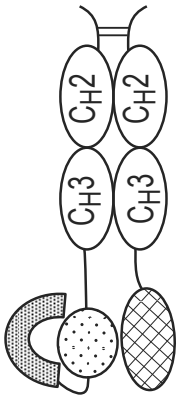


FIG. 4L

Monovalent:
Fc-p35
x Fc-p40-IL12Rβ1

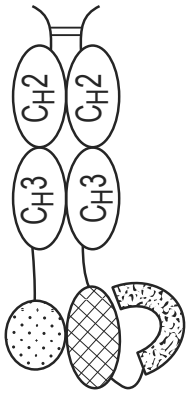


FIG. 4M

Monovalent:
Fc-IL12Rβ1-p40-p35
x Fc-IL12Rβ2

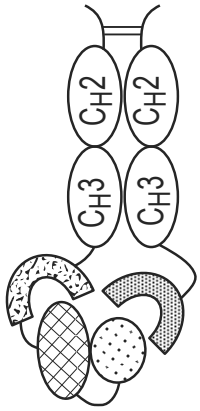


FIG. 4N

Monovalent:
Fc-p40-p35-αp40
x Fc

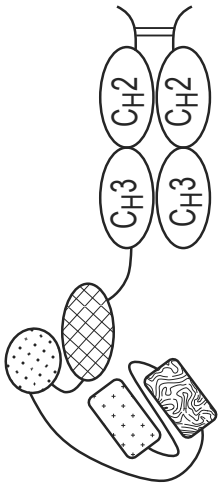


FIG. 4O

Monovalent:
Fc-p40-p35-αp35
x Fc

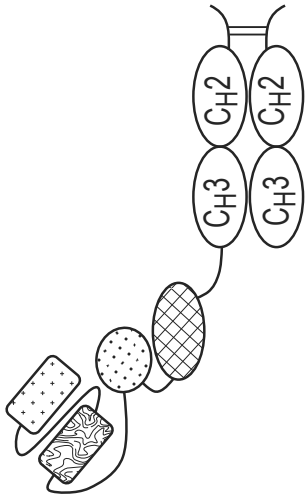


FIG. 4P

Monovalent:
Fc-p40-p35
x Fc-αp40

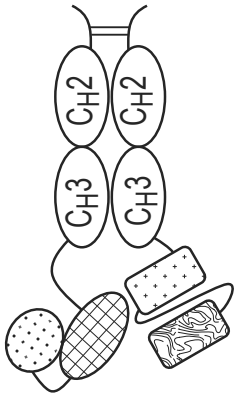


FIG. 4Q

Monovalent:
Fc-p40-p35
x Fc-αp35

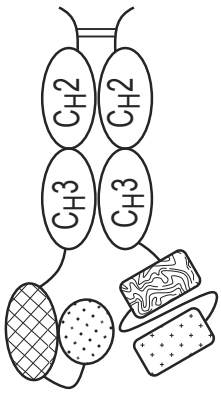


FIG. 4R

Monovalent:
Fc-p40(mutein)-p35
x Fc-IL12Rβ2

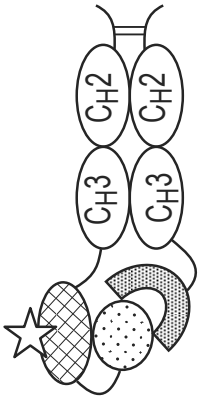


FIG. 4S

Monovalent:
Fc-IL12Rβ1-p40-p35(mutein)
x Fc

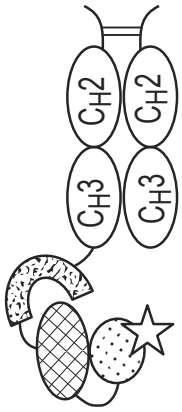


FIG. 4T

Monovalent:
Fc-p35
x Fc
x IL12Rβ1-p40

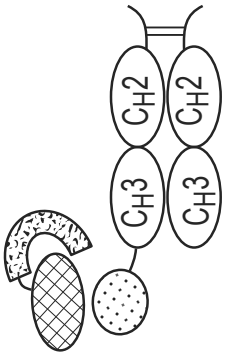


FIG. 4U

Monovalent:
Fc-p35
x Fc-IL12Rβ2
x p40

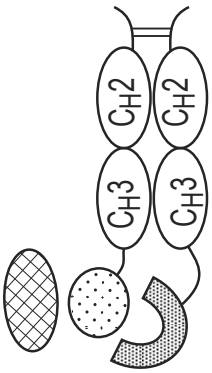


FIG. 4V

Monovalent:
Fc-p35
x Fc-IL12Rβ2
x IL12Rβ1-p40

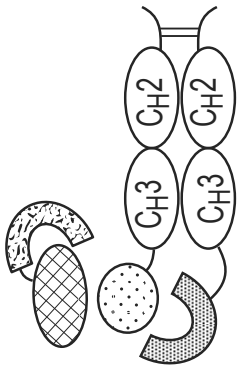


FIG. 4W

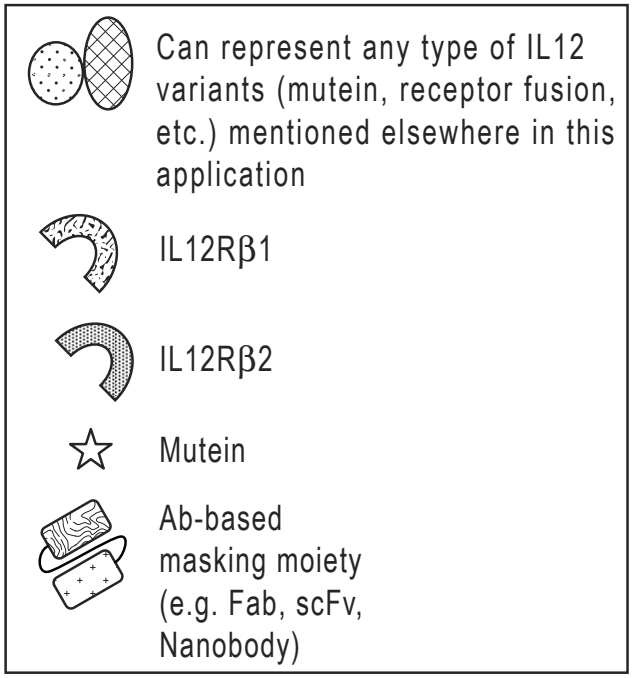


FIG. 5A

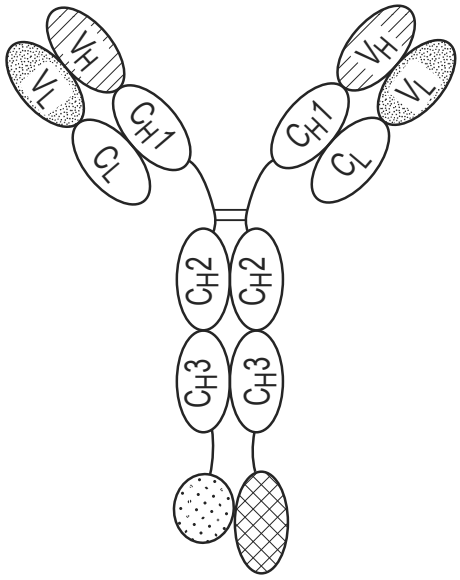


FIG. 5B

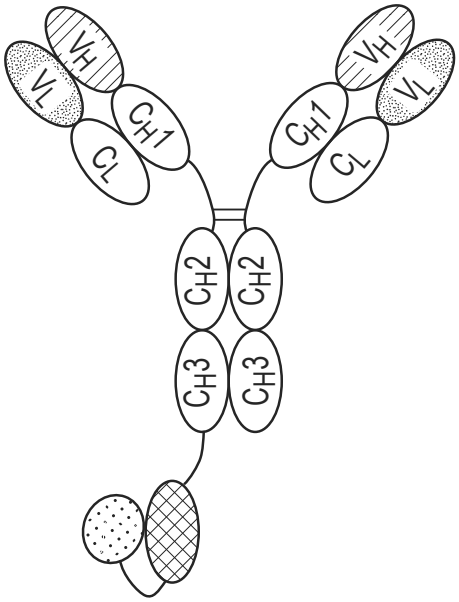


FIG. 5C

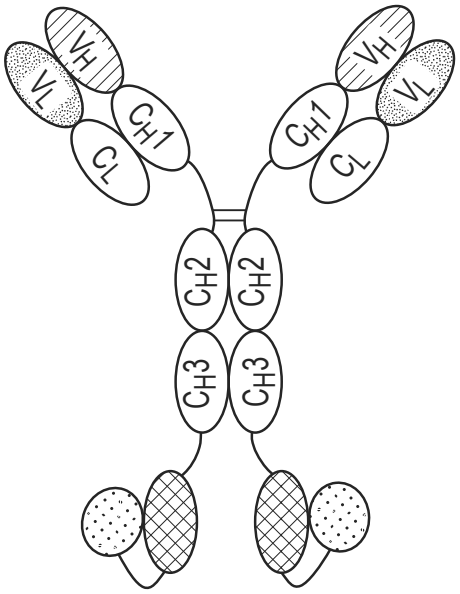


FIG. 5D

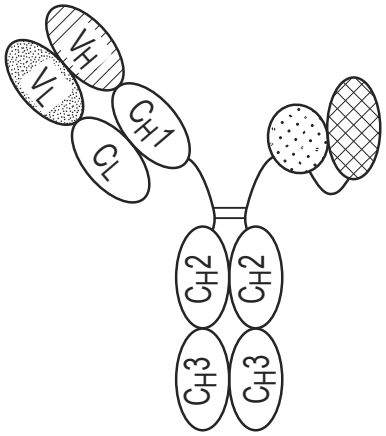


FIG. 5E

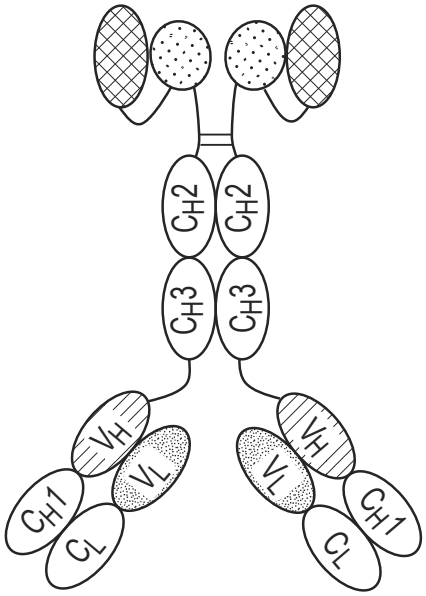


FIG. 5F

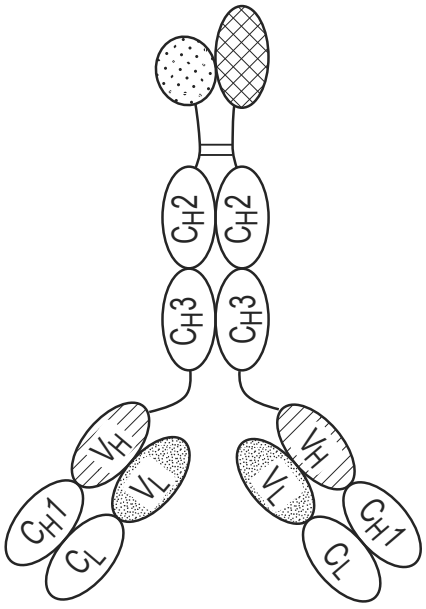


FIG. 5G

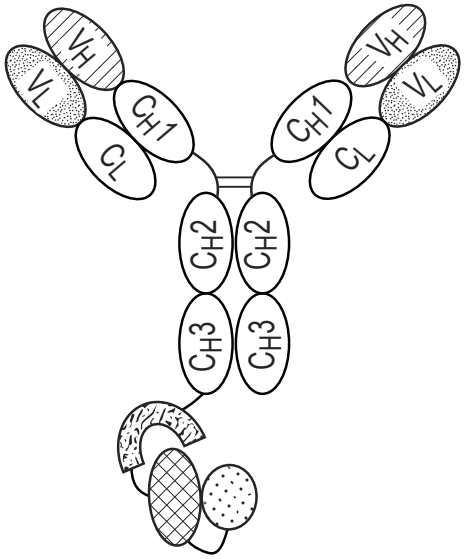


FIG. 5H

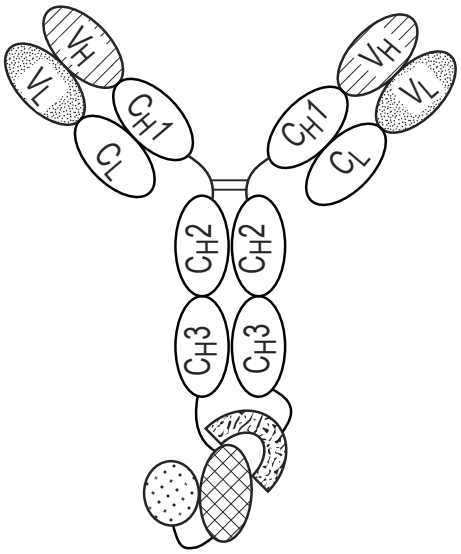


FIG. 5I

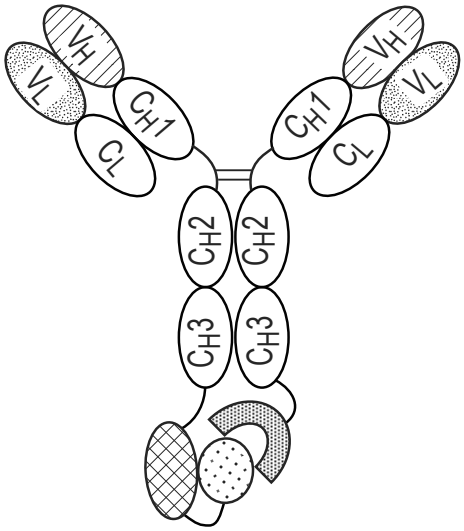


FIG. 5J

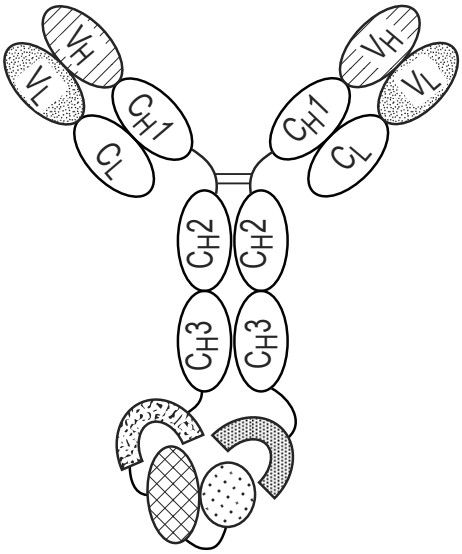


FIG. 5K

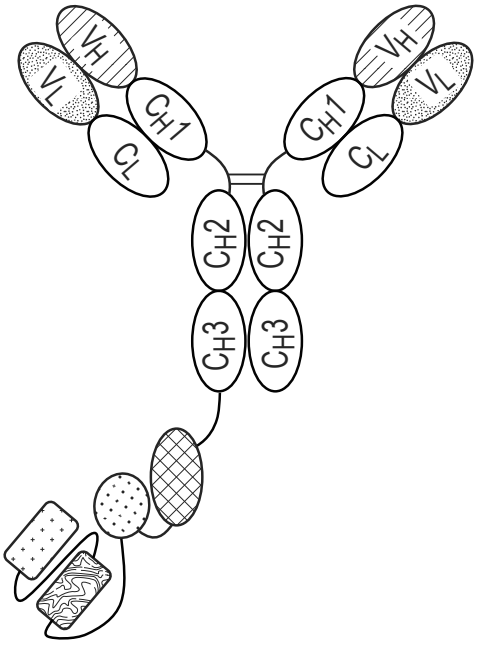


FIG. 5L

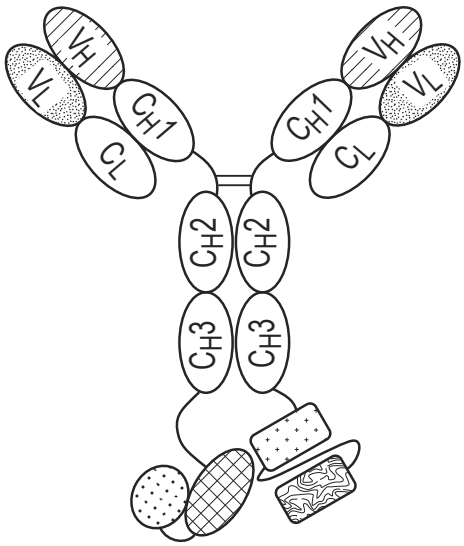


FIG. 5M

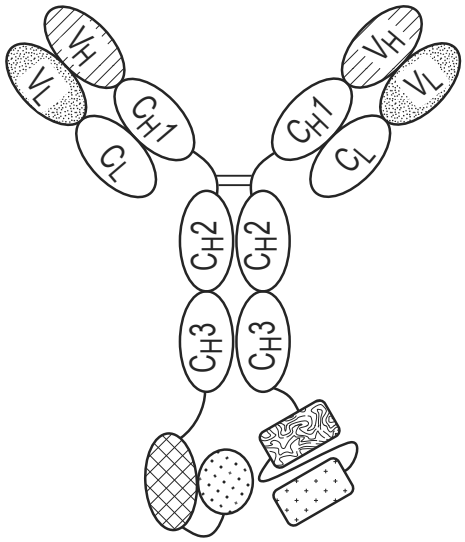


FIG. 5N

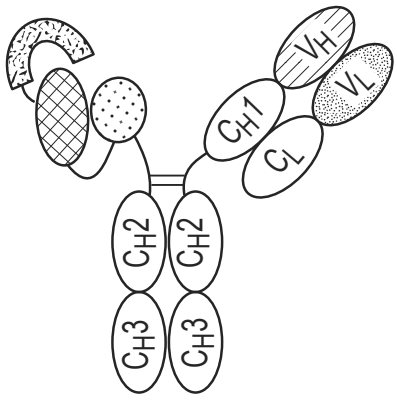


FIG. 5O

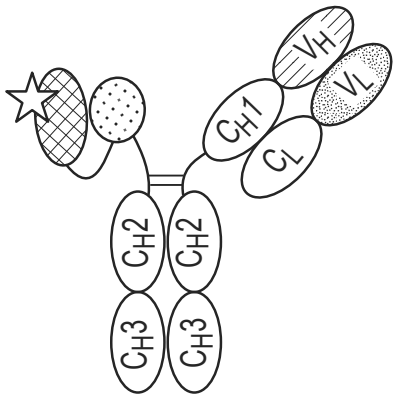


FIG. 5P

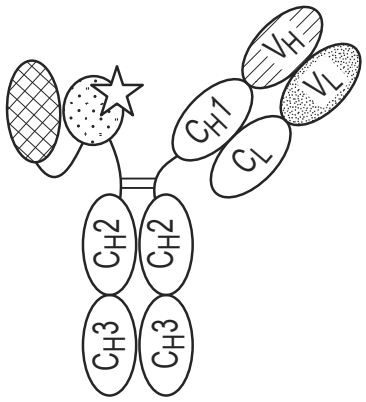


FIG. 5Q

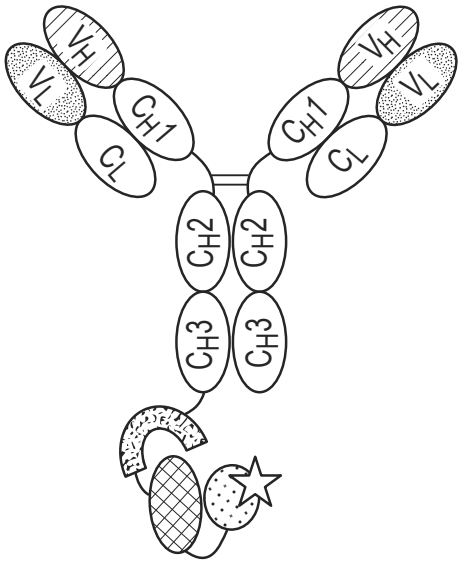


FIG. 5R

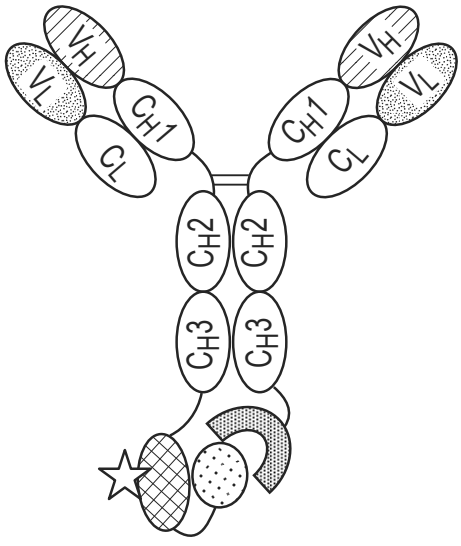


FIG. 5S

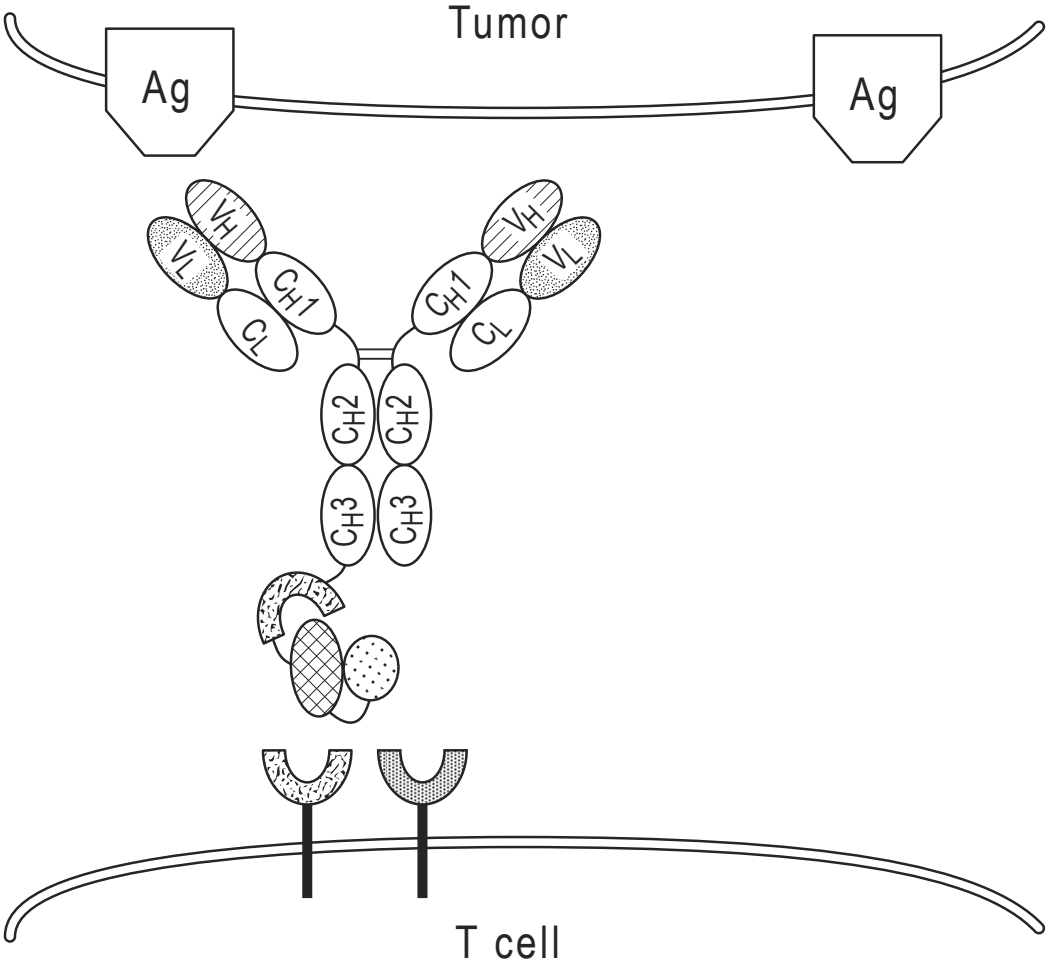

Tumor
Ag
Ag
VH
VL
CH1
CL
CH2
CH2
CH3
CH3
T cell

FIG. 5T

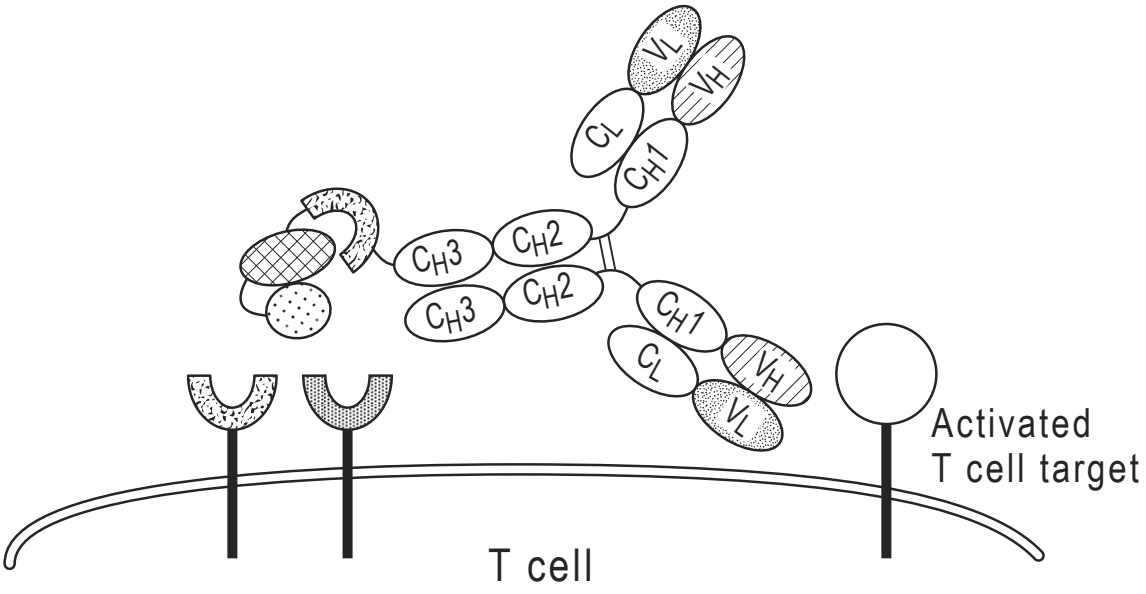

VL
VH
CL
CH1
CH3
CH2
CH3
CH2
CH1
CL
VH
VL
Activated
T cell target
T cell

FIG. 5U

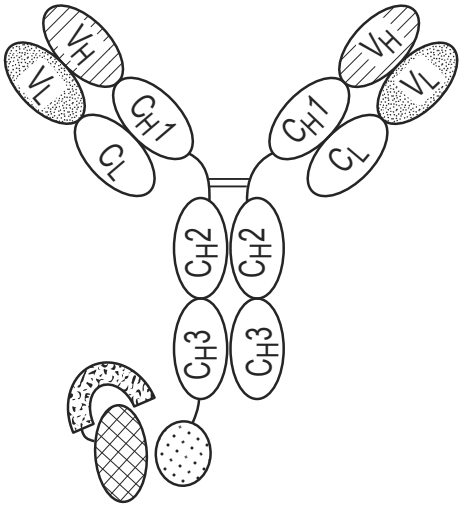


FIG. 5V

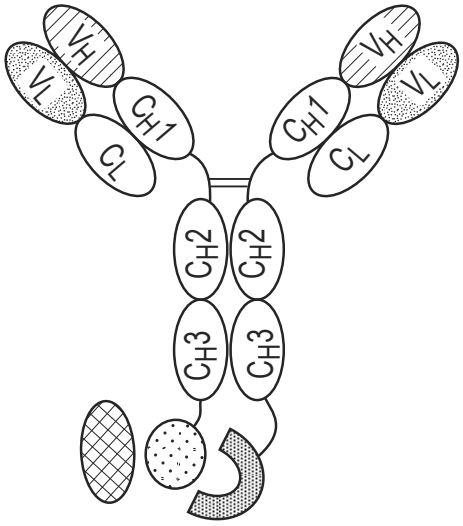


FIG. 5W

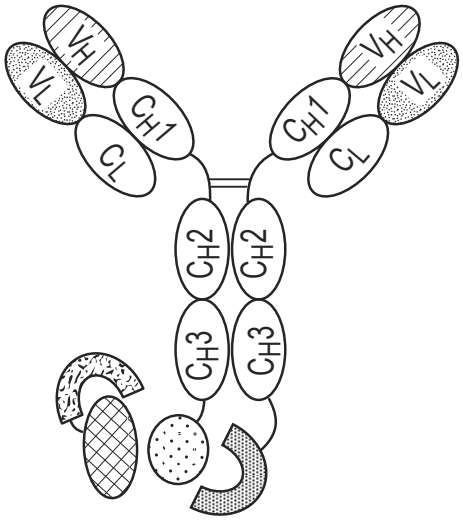


FIG. 5X

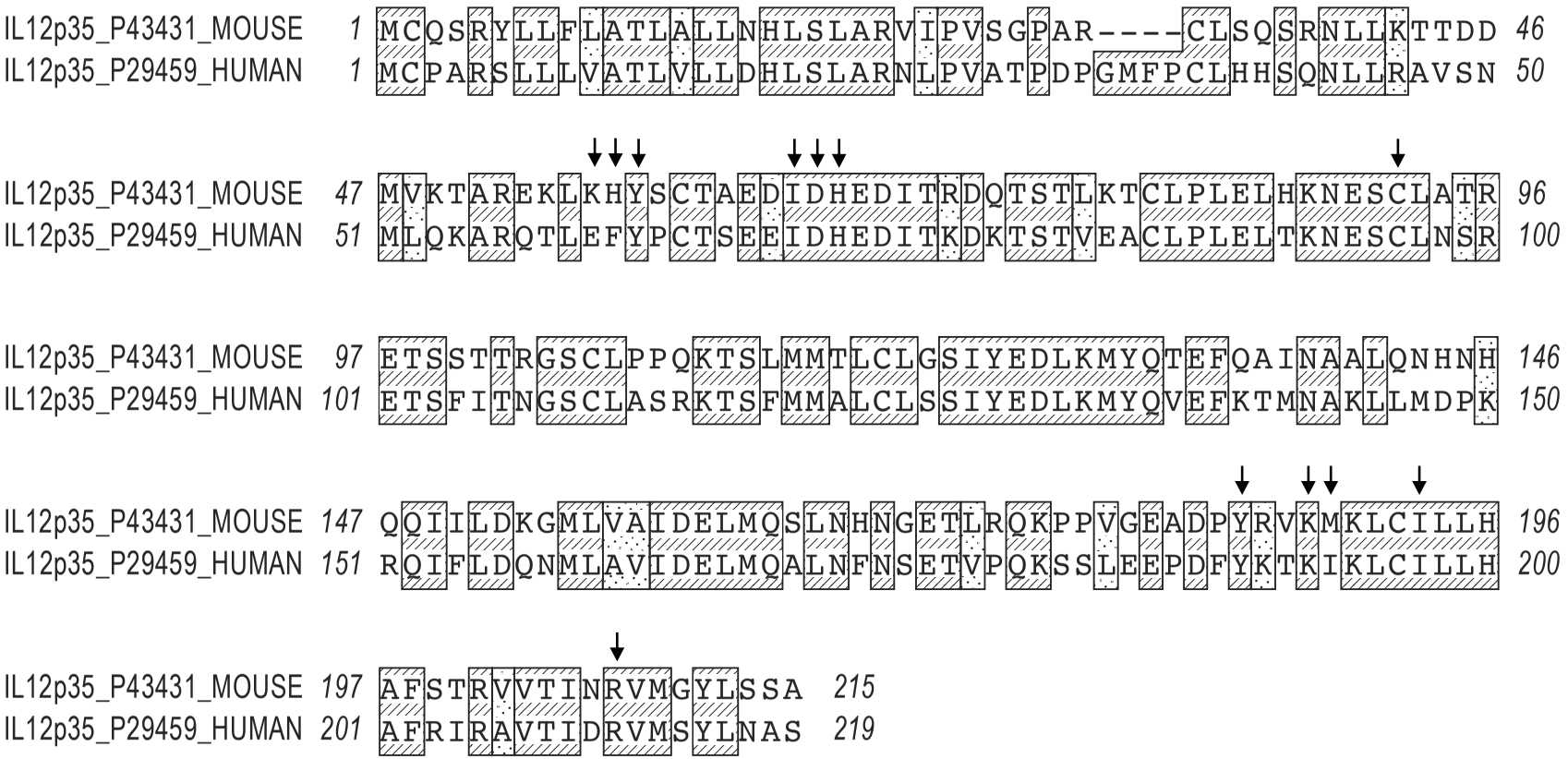

IL12p35_P43431_MOUSE 1 MCQSRYLLFLATLALLNHLSLARVIPVSGPAR----CLSQSRNLLKTTDD 46
IL12p35_P29459_HUMAN 1 MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMFPCLHHSQNLLRAVSN 50
IL12p35_P43431_MOUSE 47 MVKTAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATR 96
IL12p35_P29459_HUMAN 51 MLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSR 100
IL12p35_P43431_MOUSE 97 ETSSTTRGSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNH 146
IL12p35_P29459_HUMAN 101 ETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPK 150
IL12p35_P43431_MOUSE 147 QQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLH 196
IL12p35_P29459_HUMAN 151 RQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLH 200
IL12p35_P43431_MOUSE 197 AFSTRVVTINRVMGYLSSA 215
IL12p35_P29459_HUMAN 201 AFRIRAVTIDRVMSYLNAS 219

FIG. 6

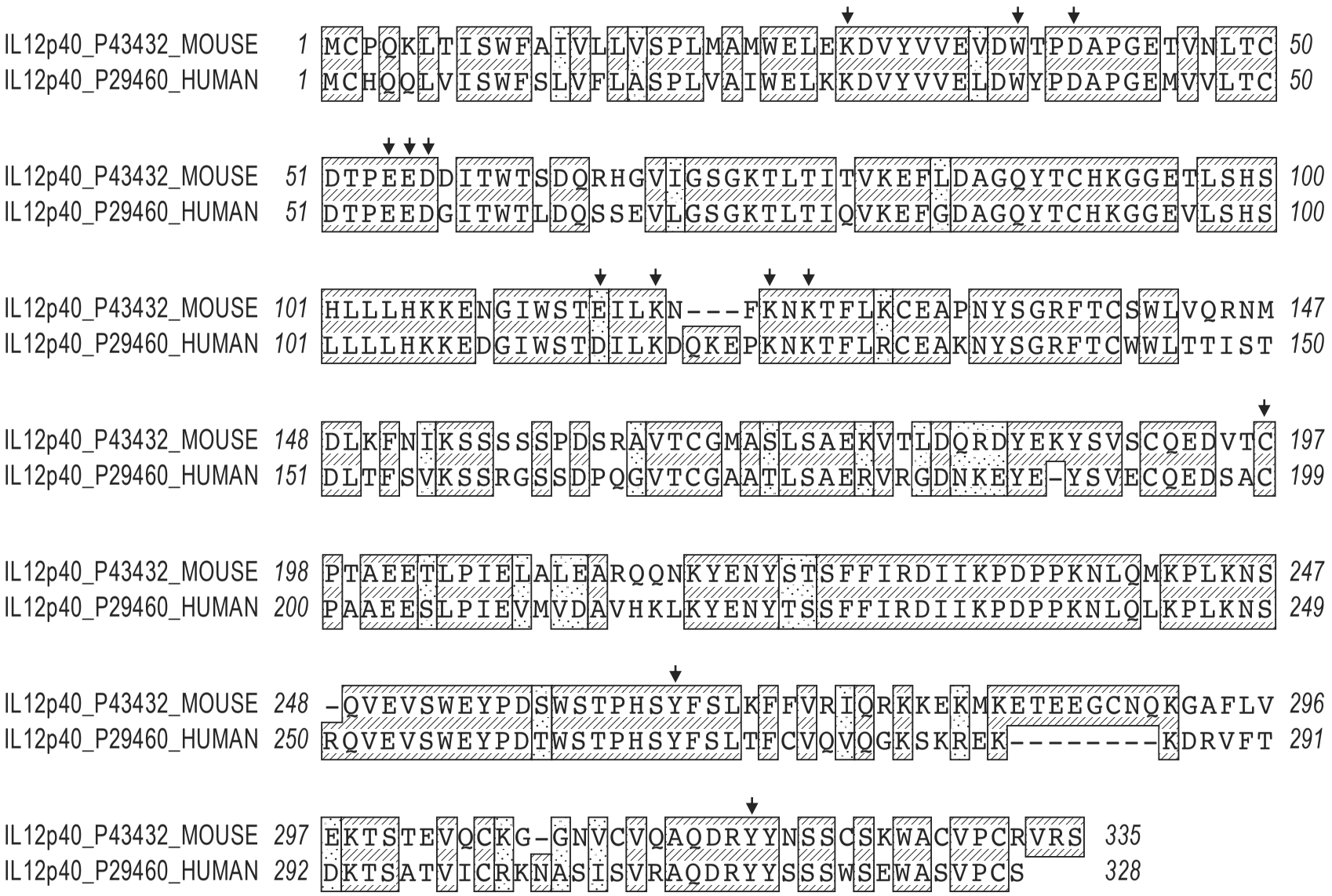

IL12p40_P43432_MOUSE 1 MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAPGETVNLTC 50
IL12p40_P29460_HUMAN 1 MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTC 50
IL12p40_P43432_MOUSE 51 DTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHS 100
IL12p40_P29460_HUMAN 51 DTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHS 100
IL12p40_P43432_MOUSE 101 HLLLHKKENGIWSTEILKN---FKNKTFLKCEAPNYSGRFTCSWLVQRNM 147
IL12p40_P29460_HUMAN 101 LLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTIST 150
IL12p40_P43432_MOUSE 148 DLKFNIKSSSSPDSRAVTCGMASLSAEKVTLDQRDYEKYSVSCQEDVTC 197
IL12p40_P29460_HUMAN 151 DLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYE-YSVECQEDSAC 199
IL12p40_P43432_MOUSE 198 PTAEETLPIELALEARQQNKYENYSTSFFIRDIIKPDPPKNLQMKPLKNS 247
IL12p40_P29460_HUMAN 200 PAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNS 249
IL12p40_P43432_MOUSE 248 -QVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLV 296
IL12p40_P29460_HUMAN 250 RQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREK--------KDRVFT 291
IL12p40_P43432_MOUSE 297 EKTSTEVQCKG-GNVCVQAQDRYYNSSCSKWACVPCRVRS 335
IL12p40_P29460_HUMAN 292 DKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS 328

FIG. 7


IL12p35 1 MCPARSLLLVATLVLLDHLSLARNLPVATP-DPGMFPCLHHSQNLLRAVSNMLQKARQTLEFY 62
IL23p19 1 MLG--SRAVMLLLLLPWTAQGRAV--PG-GSSP--AWTQCQQLSQKLCTLAWSAHPL 50
IL27p28 1 MGQTAGDLGWRLSLLLLPLLLVQAGVWGFPRP--PG--RPQLSLQELRREFTVSLHLARKLLSEV 61
IL-6 1 MNSFSTS-AFGPVAFSLGLLLVLPAAFPAPVP--PG-EDSKDVAAPHRQPLTSSERIDKQIRYIL 61
CNTF 1 MAFTEHSPLTPHRR------DLCSRSIWLARKIRSDLTALTESYVKH 41
CLCF1 1 MDLRAGD-SWGMLACLCTVLWHLPAVPALNRTGDPGPGPSIQKTYDLTRYLEHQLRSLAGTYLNY 64
M G .L .LL L . GDPG L R .. L . .
IL12p35 63 PCTSEEIDHEDITKDKTS---TVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCL 124
IL23p19 51 VGHMDLREEGDE-------------ETTNDVPHIQCGDGCDPQGLRDNSQFCLQRIHQGLIFYEK 102
IL27p28 62 RGQAHRFAESHLPG-----VNLYLLPLGEQLPDVSLTFQAWRRLSDPERLCFISTTLQPFHALLG 121
IL-6 62 DGISALRKETCKNSNMCESSKEALAENNLNLPKMAEKDGCFQSGFNEET--CLVKIITGLLEFEV 124
CNTF 42 QGLNKNIN----------------LDSADGMPVAS--TDQWSELTEAERLQENLQAYRTFHVLLA 88
CLCF1 65 LGPPFNEPDFNPPR----------LGAETLPRATVDLEVWRSLNDKLRLTQNYEAYSHLLCYLR 118
G E S L. LP.. . . L . . .
IL12p35 125 SSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALN----------FN 173
IL23p19 103 LLG----------SDIFTGEPSLLPDSPVGQLHASLLGLSQLLQPEG----------HHW 142
IL27p28 122 GLG-----TQGRWTNMERMQLWAMRLDLRDLQRHLRFQVLAAGFNLPEEEEEEEEEEEEEEKGLL 181
IL-6 125 YLEYLQ--NRFESSEEQARAVQMSTKVLIQFLQKKAKNLDAITTPD----------PTT 171
CNTF 89 RLLEDQQVHFTPTEGDFHQAIHTLLLQVAAFAYQIEELMILLEYKIP----------R 136
CLCF1 119 GLN------RQAATAELRRSLAHFCTSLQGLLGSIAGVMAALGYPLP----------QPL 162
L.Y Q . . .. .L .PEEEEEEEEEEEERK
IL12p35 174 SETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS 219
IL23p19 143 ETQQIPSLS--PSQPWQRLLLRFKILRSLQAFVAVAARVFAHGAATLSP 189
IL27p28 182 PGALGSALQGPAQVSWPQLLSTYRLLHSLELVLSRAVRELLLLSKAGHSVWPLGFPTLSPQP 243
IL-6 172 NASLLTKLQ--AQNQWLQDMTTHLILRSFKEFLQSSLRALRQM 212
CNTF 137 NEADGMPINVGDGGLFEKKLWGLKVLQELSQWTVRSIHDLRFISSHQTGIPARGSHYIANNKKM 200
CLCF1 163 PGTEPTWTPGPAHSDFLQKMDDFWLLKELQTWLWRSAKDFNRLKKKMQPPAAAVTLHLGAHGF 225
. . . . . .L. L .. .R . A G L M

FIG. 8

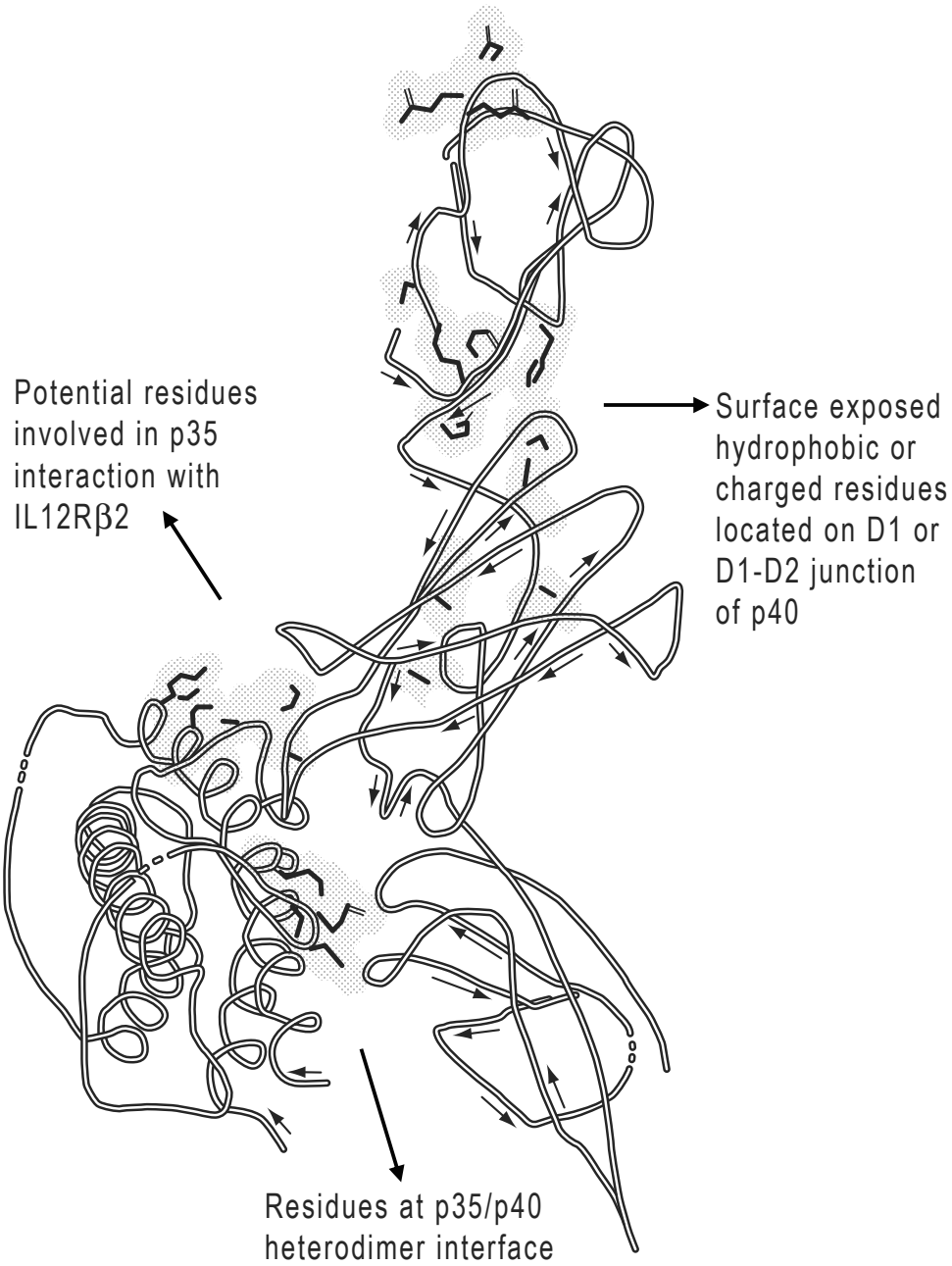

Potential residues involved in p35 interaction with IL12Rβ2
Surface exposed hydrophobic or charged residues located on D1 or D1-D2 junction of p40
Residues at p35/p40 heterodimer interface

FIG. 9

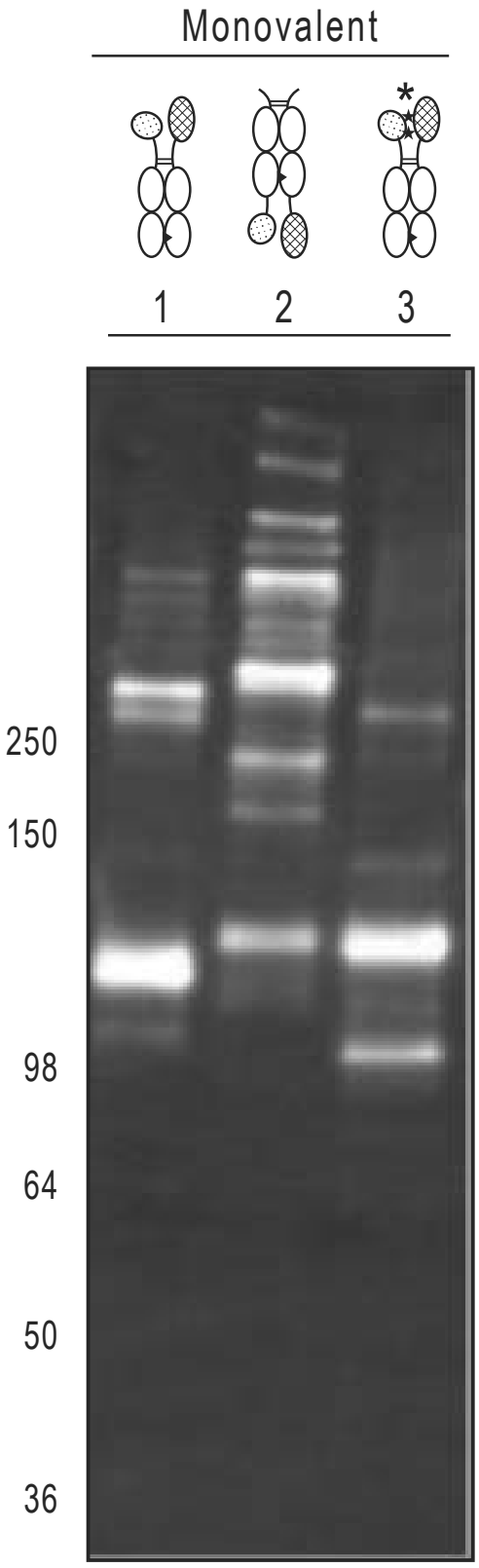
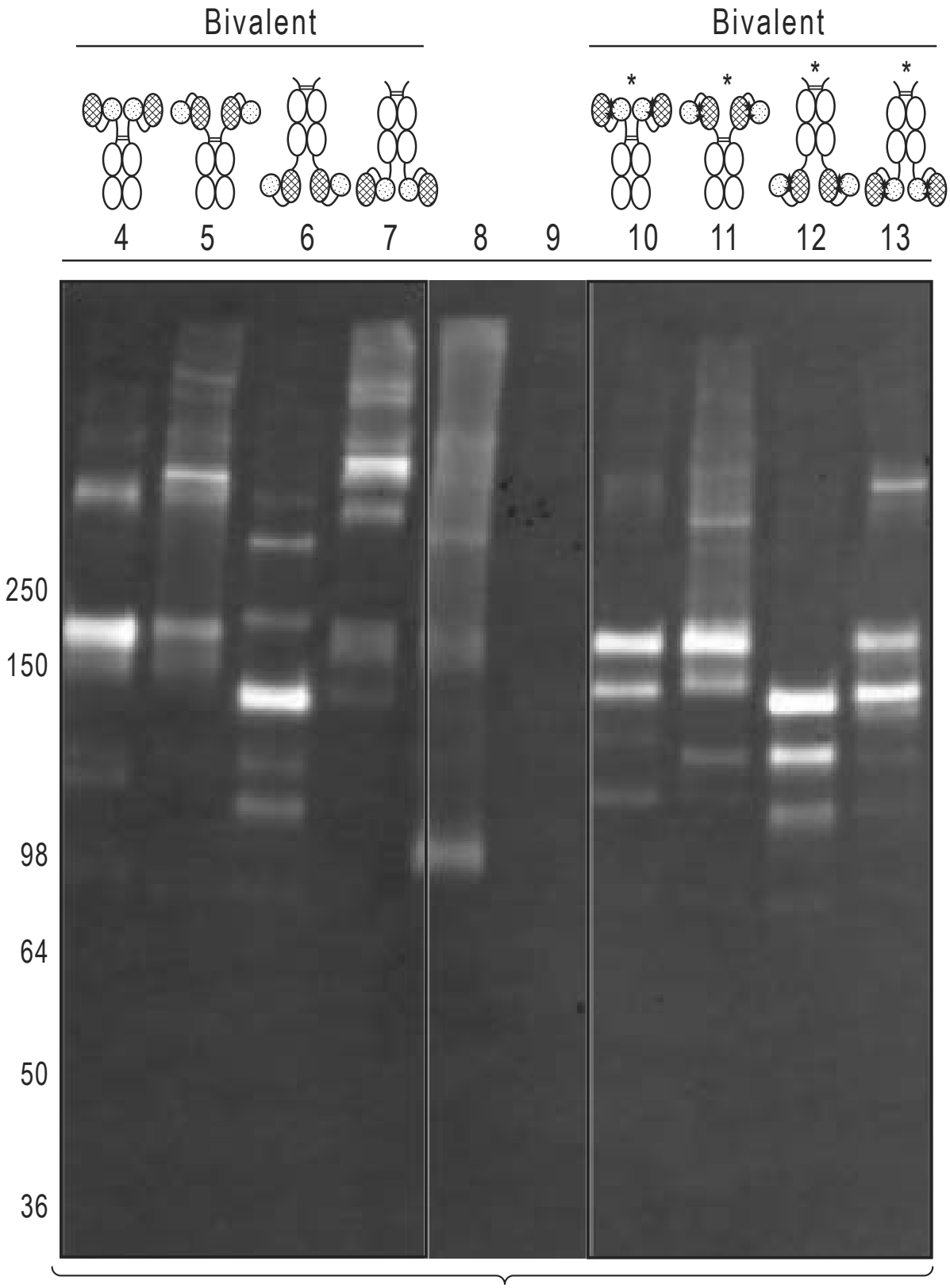


FIG. 10A



*Disulfide removal reduces HMW species in p35-p40 orientation on denaturing gel

FIG. 10B

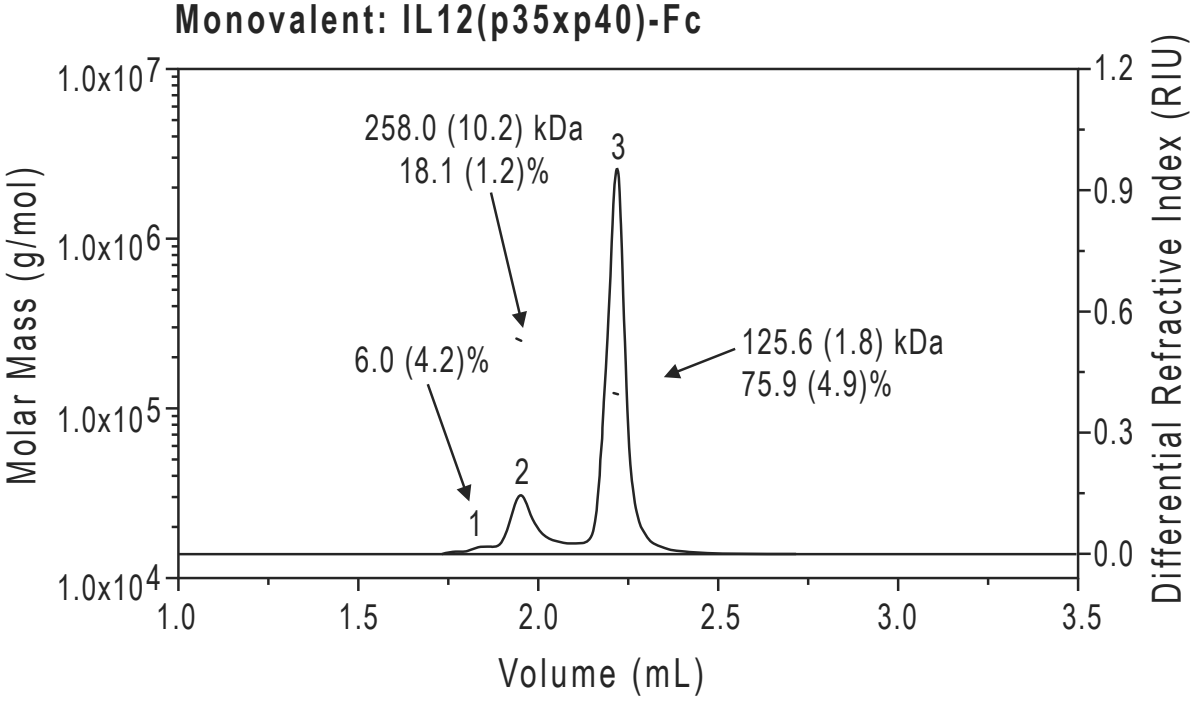

Monovalent: IL12(p35xp40)-Fc
Molar Mass (g/mol)
Differential Refractive Index (RIU)
Volume (mL)
258.0 (10.2) kDa
18.1 (1.2)%
6.0 (4.2)%
125.6 (1.8) kDa
75.9 (4.9)%
1
2
3

FIG. 11

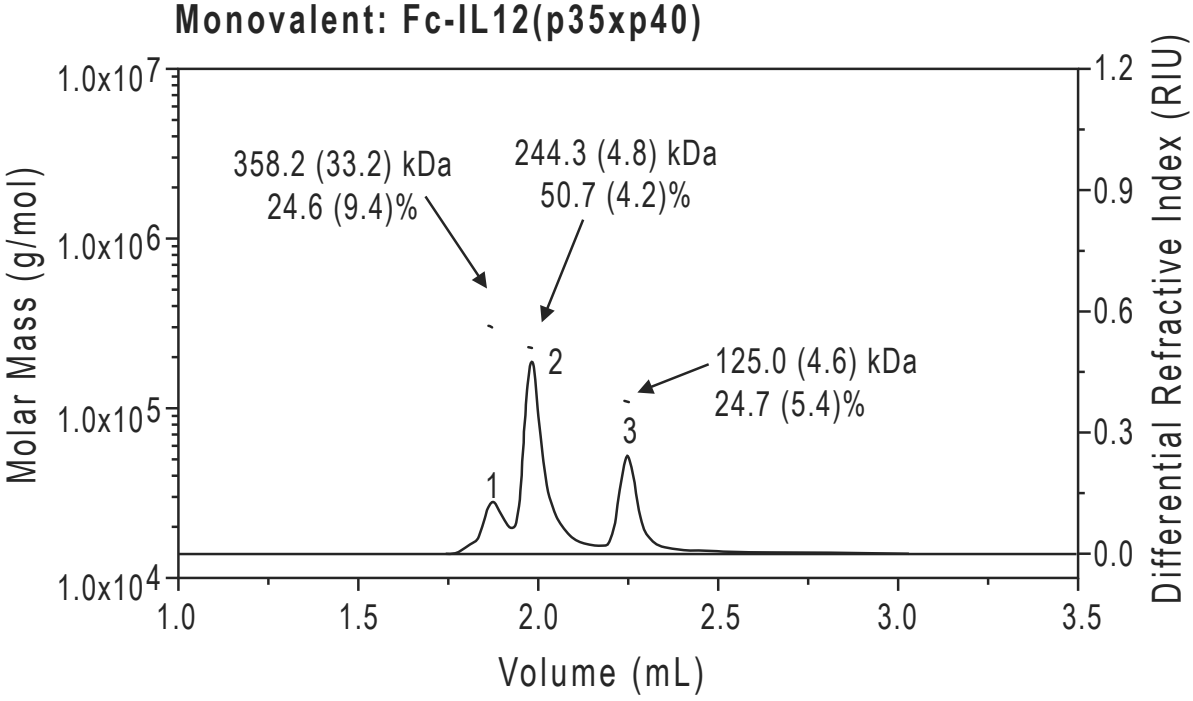

Monovalent: Fc-IL12(p35xp40)
Molar Mass (g/mol)
Differential Refractive Index (RIU)
Volume (mL)
358.2 (33.2) kDa
24.6 (9.4)%
244.3 (4.8) kDa
50.7 (4.2)%
125.0 (4.6) kDa
24.7 (5.4)%
1
2
3

FIG. 12

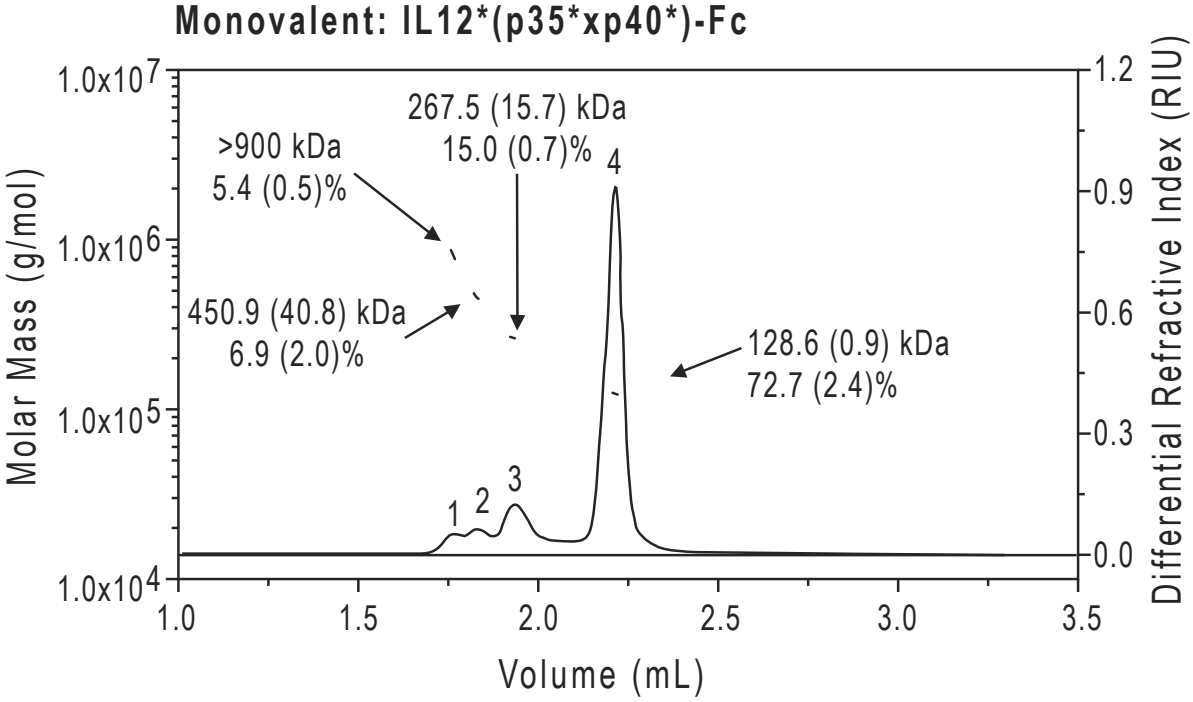

Monovalent: IL12*(p35*xp40*)-Fc
Molar Mass (g/mol)
Differential Refractive Index (RIU)
Volume (mL)
>900 kDa
5.4 (0.5)%
267.5 (15.7) kDa
15.0 (0.7)%
450.9 (40.8) kDa
6.9 (2.0)%
128.6 (0.9) kDa
72.7 (2.4)%
1
2
3
4

FIG. 13

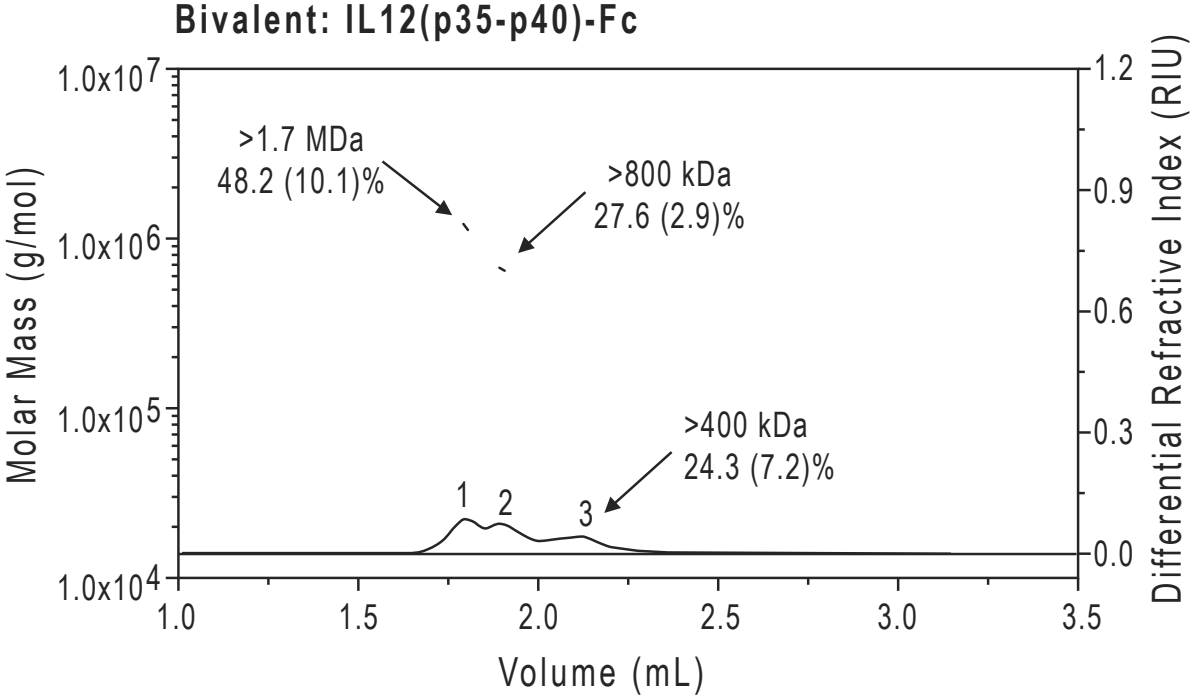

Bivalent: IL12(p35-p40)-Fc
Molar Mass (g/mol)
Differential Refractive Index (RIU)
Volume (mL)
>1.7 MDa
48.2 (10.1)%
>800 kDa
27.6 (2.9)%
>400 kDa
24.3 (7.2)%
1
2
3

FIG. 14

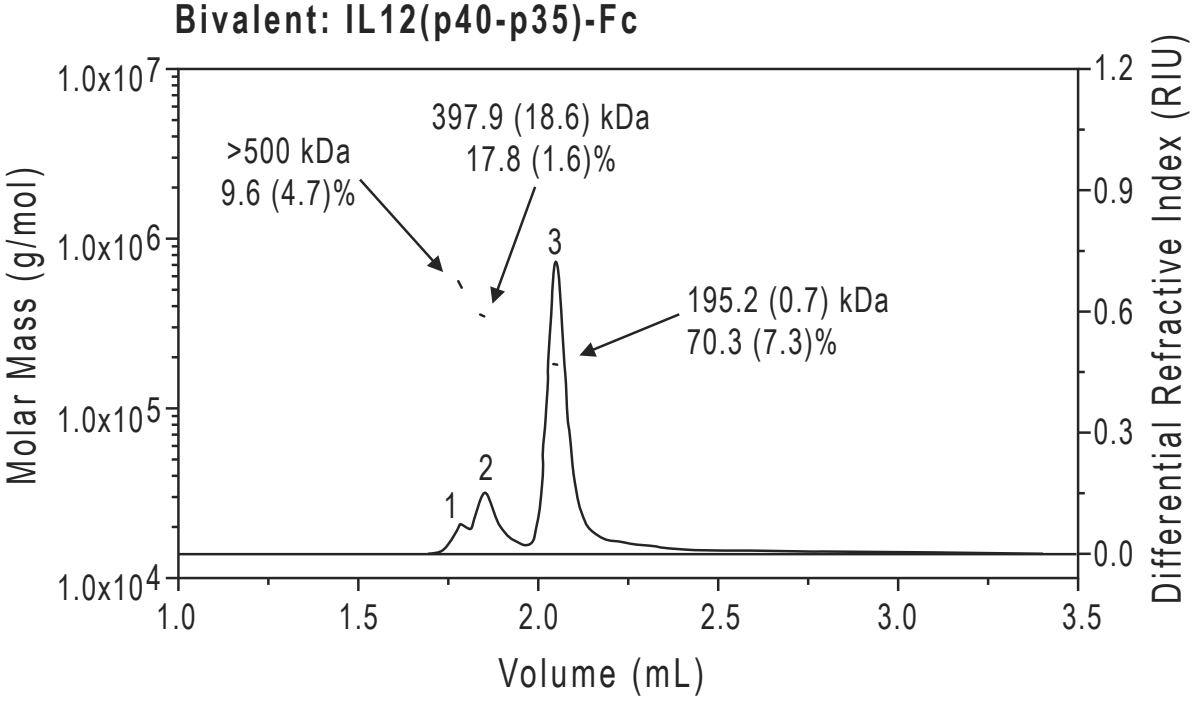

Bivalent: IL12(p40-p35)-Fc
>500 kDa
9.6 (4.7)%
397.9 (18.6) kDa
17.8 (1.6)%
195.2 (0.7) kDa
70.3 (7.3)%
1
2
3
Molar Mass (g/mol)
Differential Refractive Index (RIU)
Volume (mL)

FIG. 15

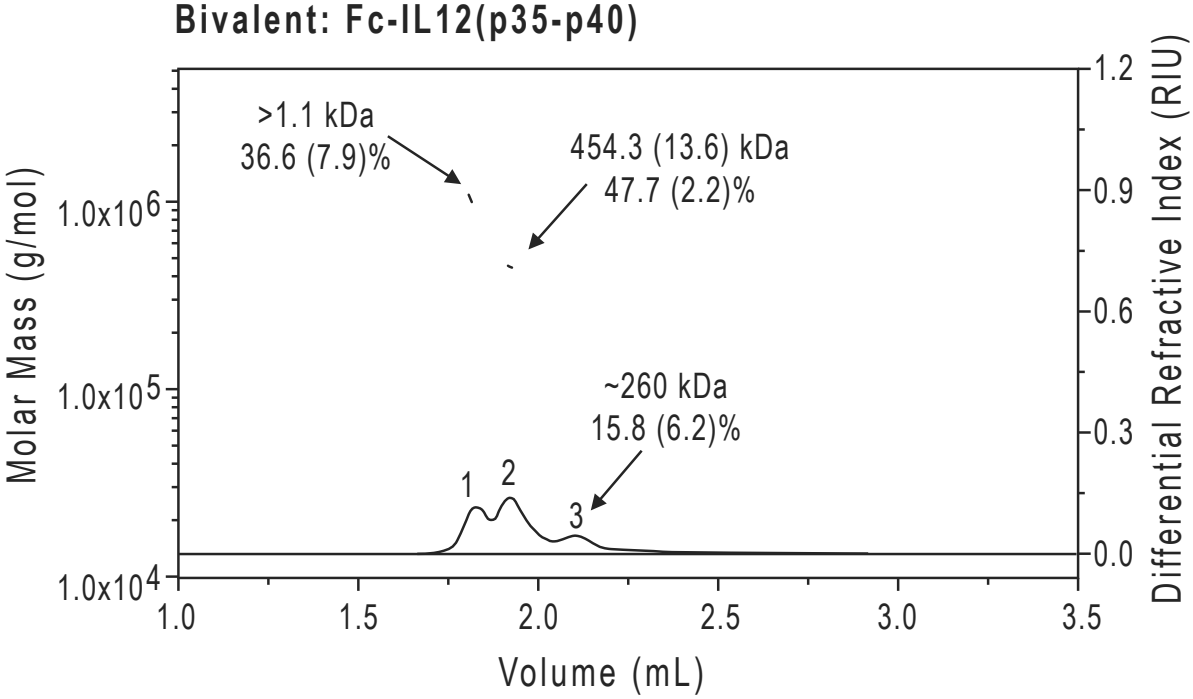

Bivalent: Fc-IL12(p35-p40)
>1.1 kDa
36.6 (7.9)%
454.3 (13.6) kDa
47.7 (2.2)%
~260 kDa
15.8 (6.2)%
1
2
3
Molar Mass (g/mol)
Differential Refractive Index (RIU)
Volume (mL)

FIG. 16

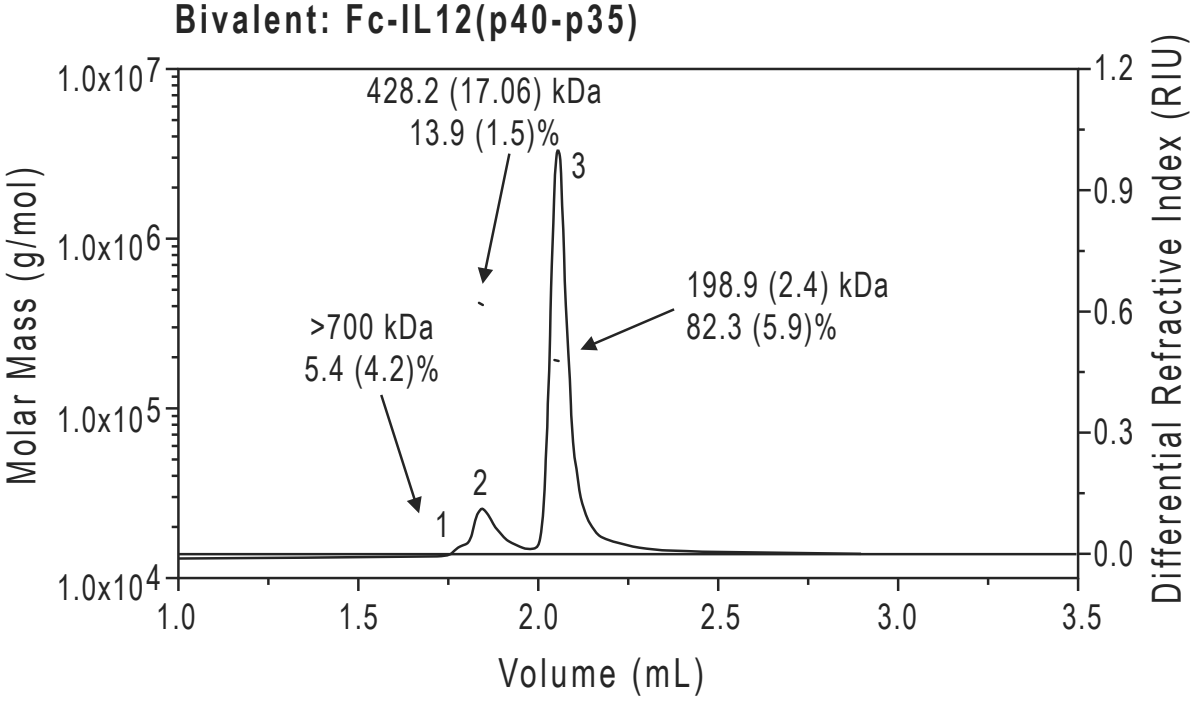

Bivalent: Fc-IL12(p40-p35)
428.2 (17.06) kDa
13.9 (1.5)%
198.9 (2.4) kDa
82.3 (5.9)%
>700 kDa
5.4 (4.2)%
1
2
3
Molar Mass (g/mol)
Differential Refractive Index (RIU)
Volume (mL)

FIG. 17

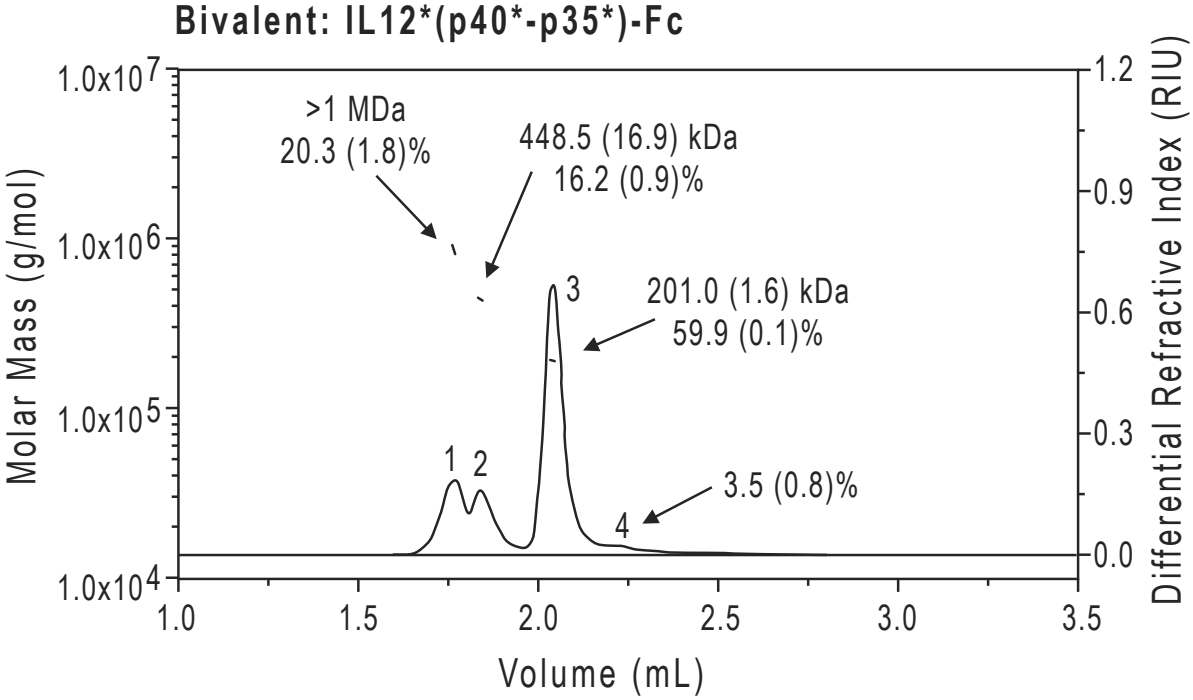

Bivalent: IL12*(p40*-p35*)-Fc
>1 MDa
20.3 (1.8)%
448.5 (16.9) kDa
16.2 (0.9)%
201.0 (1.6) kDa
59.9 (0.1)%
3.5 (0.8)%
1
2
3
4
Molar Mass (g/mol)
Differential Refractive Index (RIU)
Volume (mL)

FIG. 18

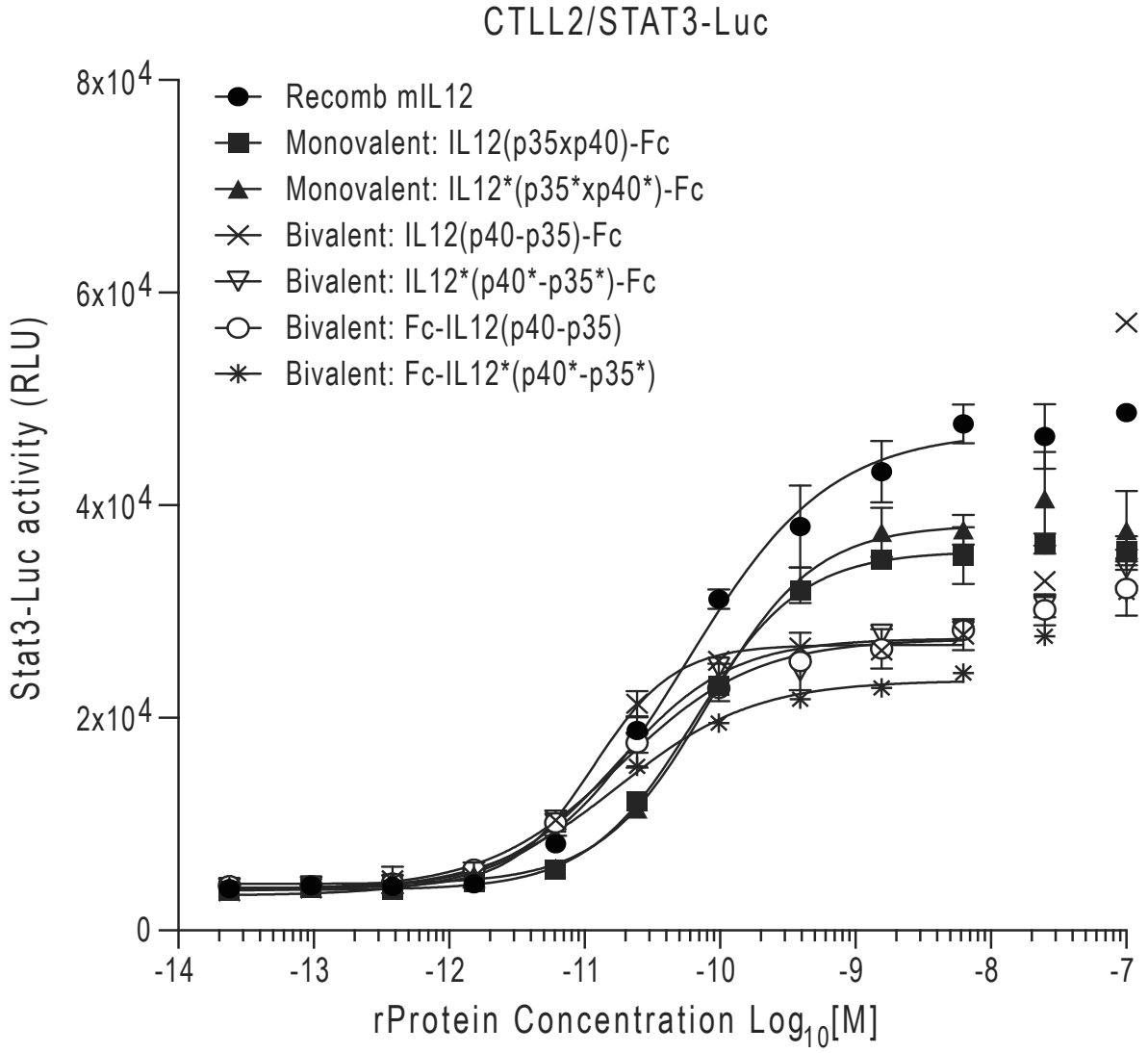

CTLL2/STAT3-Luc
Recomb mIL12
Monovalent: IL12(p35xp40)-Fc
Monovalent: IL12*(p35*xp40*)-Fc
Bivalent: IL12(p40-p35)-Fc
Bivalent: IL12*(p40*-p35*)-Fc
Bivalent: Fc-IL12(p40-p35)
Bivalent: Fc-IL12*(p40*-p35*)
Stat3-Luc activity (RLU)
8x10^4
6x10^4
4x10^4
2x10^4
0
-14
-13
-12
-11
-10
-9
-8
-7
rProtein Concentration Log10[M]

FIG. 19

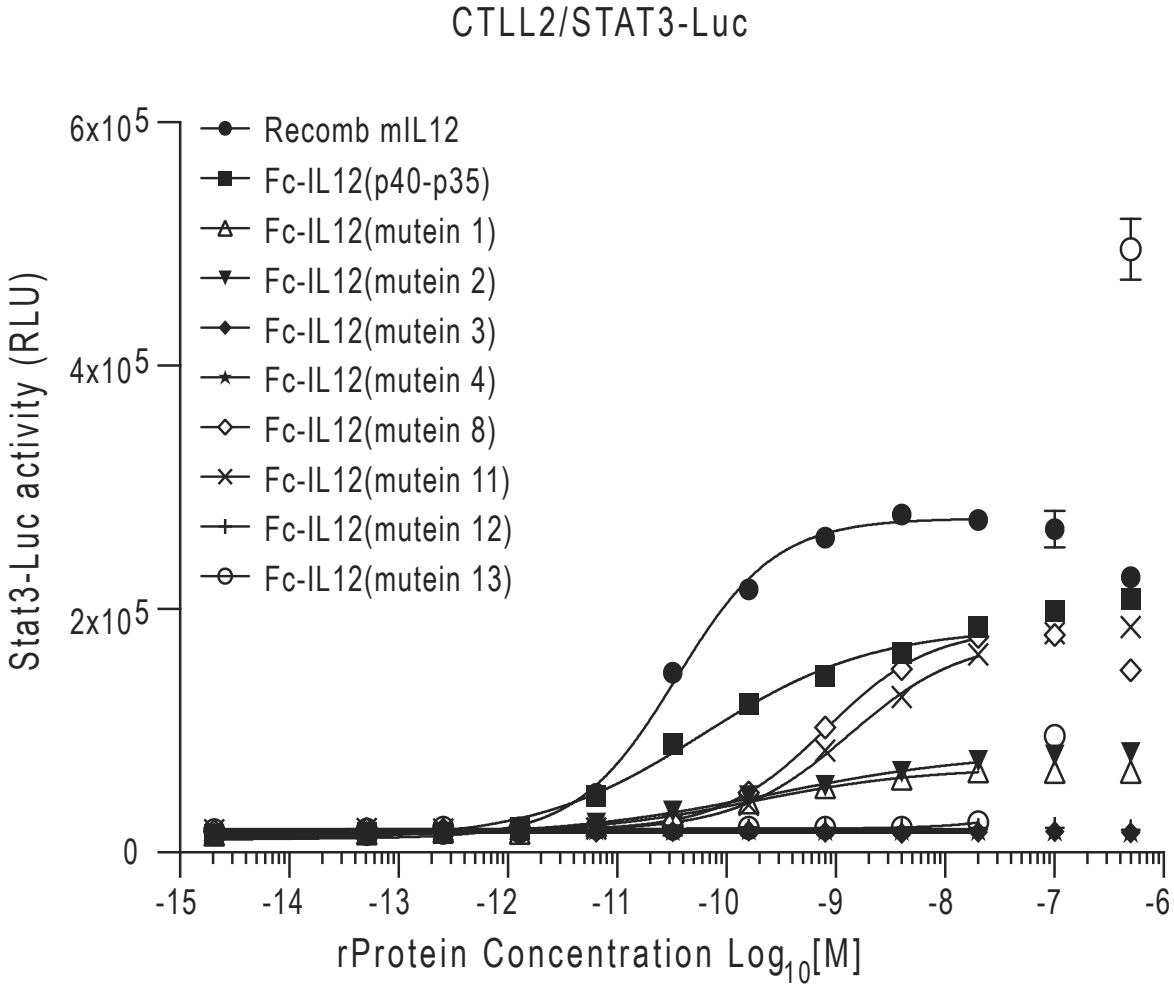

CTLL2/STAT3-Luc
Stat3-Luc activity (RLU)
6x10^5
4x10^5
2x10^5
0
Recomb mIL12
Fc-IL12(p40-p35)
Fc-IL12(mutein 1)
Fc-IL12(mutein 2)
Fc-IL12(mutein 3)
Fc-IL12(mutein 4)
Fc-IL12(mutein 8)
Fc-IL12(mutein 11)
Fc-IL12(mutein 12)
Fc-IL12(mutein 13)
-15
-14
-13
-12
-11
-10
-9
-8
-7
-6
rProtein Concentration Log10[M]

FIG. 20A

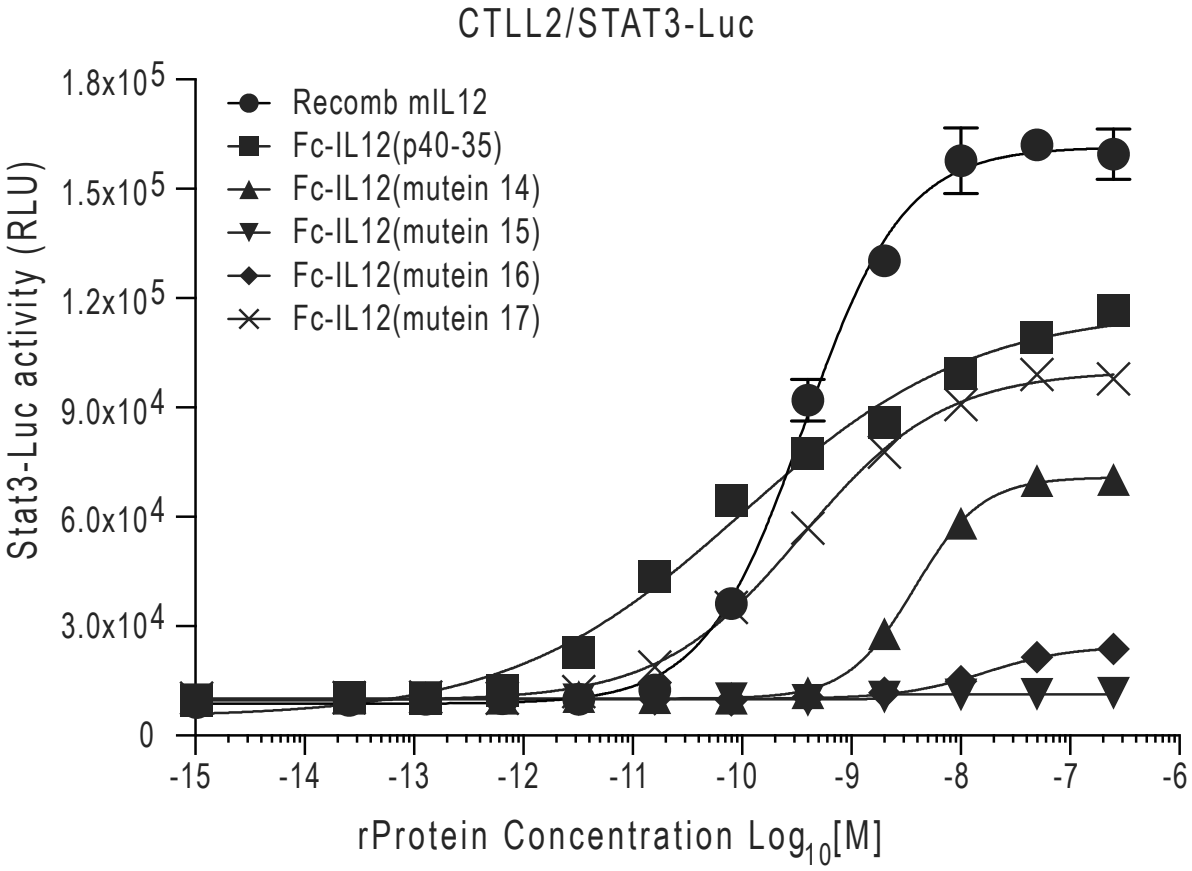


FIG. 20B

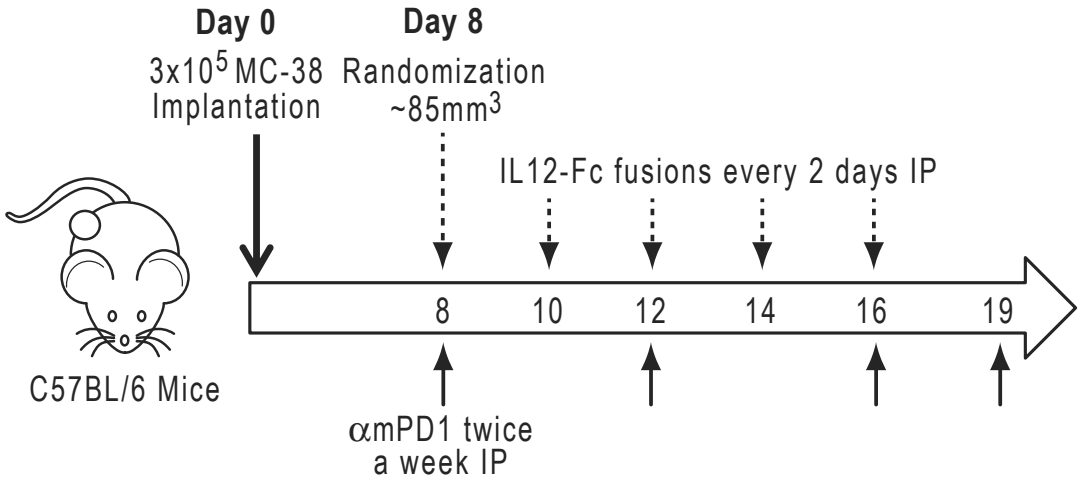


FIG. 21

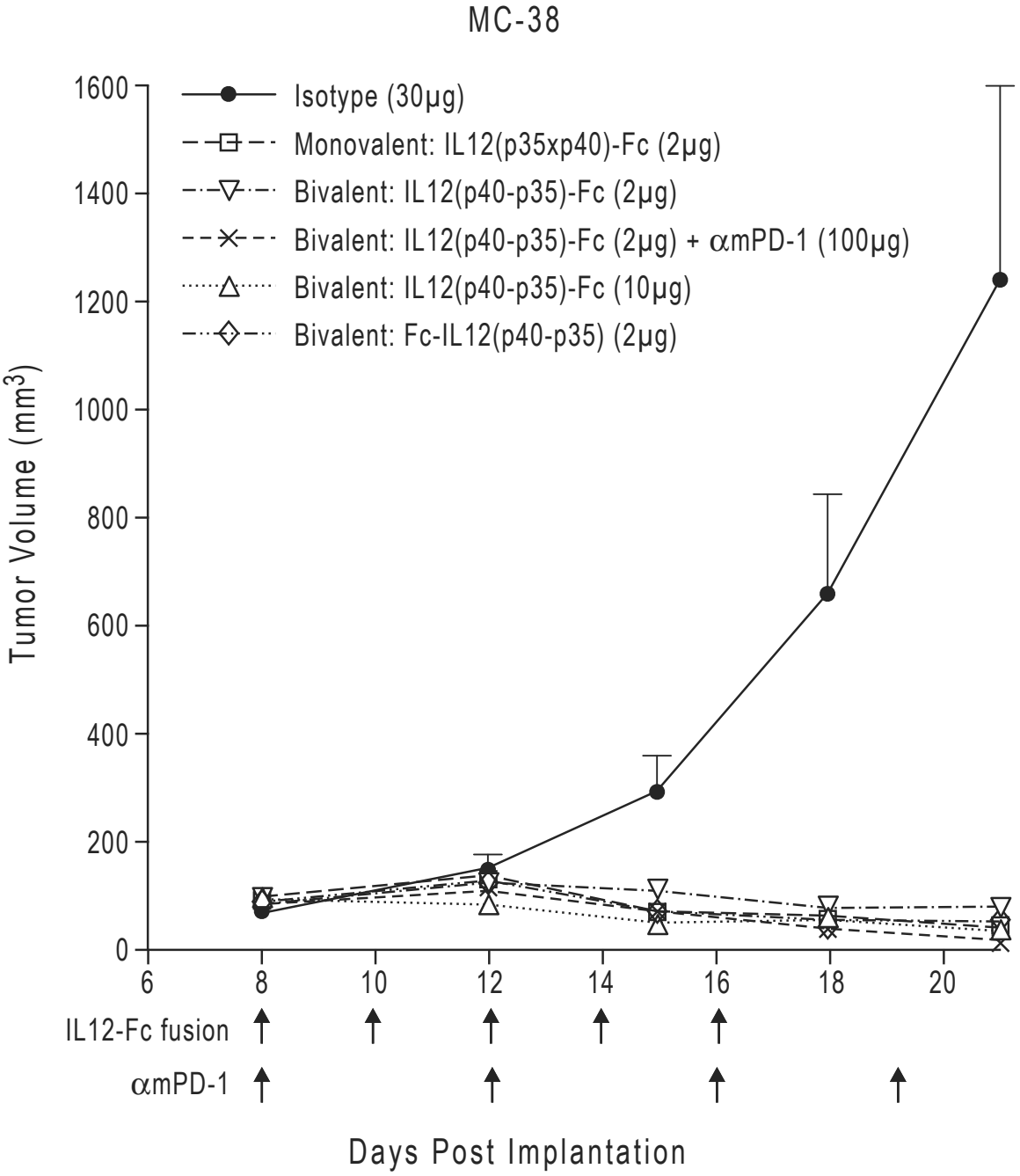

MC-38
Isotype (30µg)
Monovalent: IL12(p35xp40)-Fc (2µg)
Bivalent: IL12(p40-p35)-Fc (2µg)
Bivalent: IL12(p40-p35)-Fc (2µg) + αmPD-1 (100µg)
Bivalent: IL12(p40-p35)-Fc (10µg)
Bivalent: Fc-IL12(p40-p35) (2µg)
Tumor Volume (mm3)
1600
1400
1200
1000
800
600
400
200
0
6
8
10
12
14
16
18
20
IL12-Fc fusion
αmPD-1
Days Post Implantation

FIG. 22

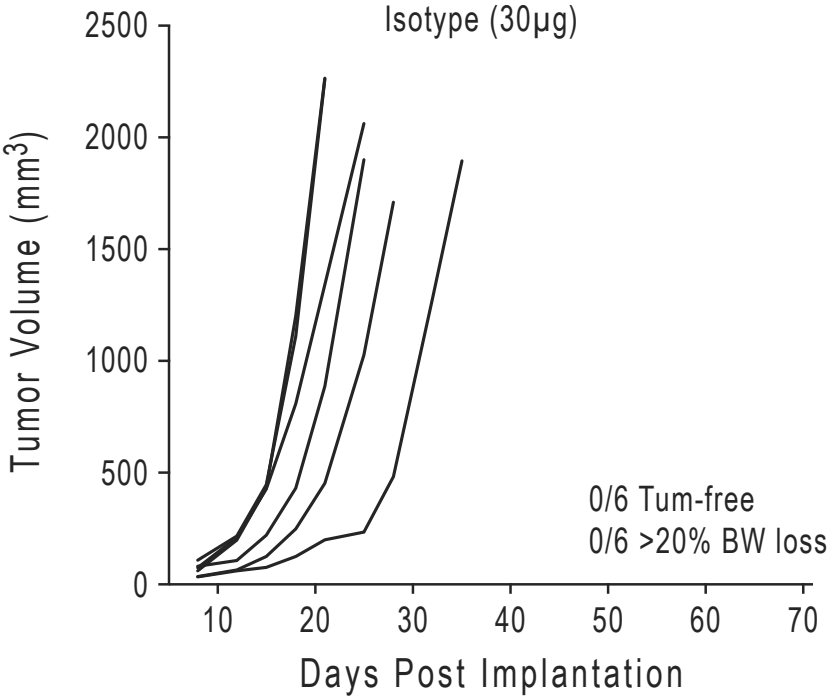


FIG. 23A

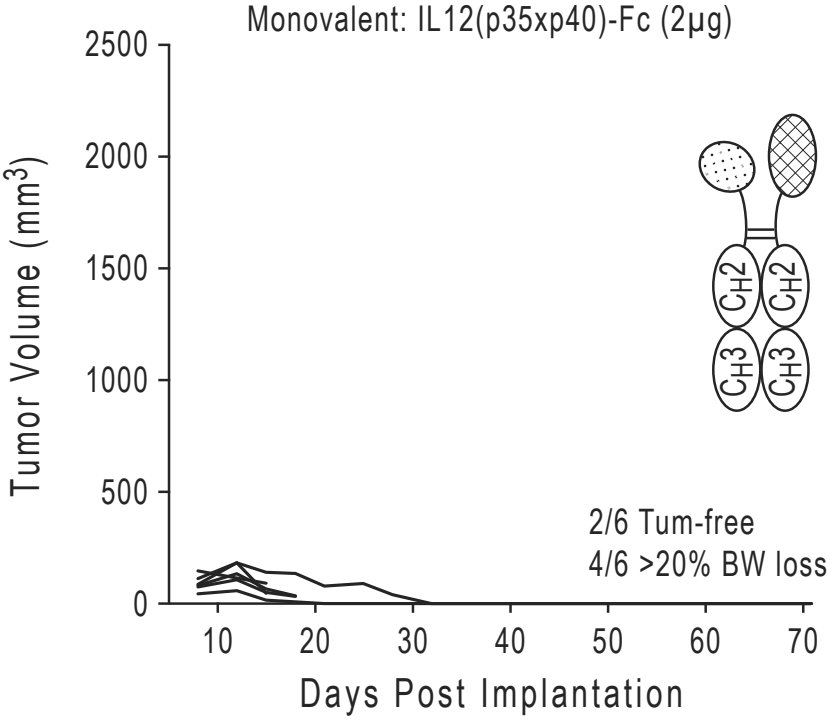


FIG. 23B

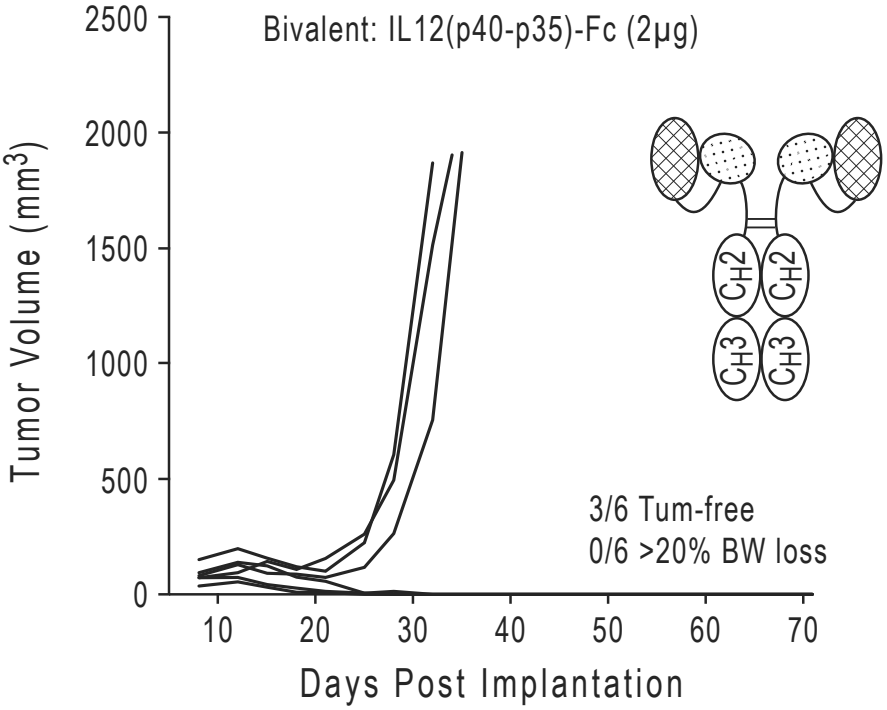


FIG. 23C

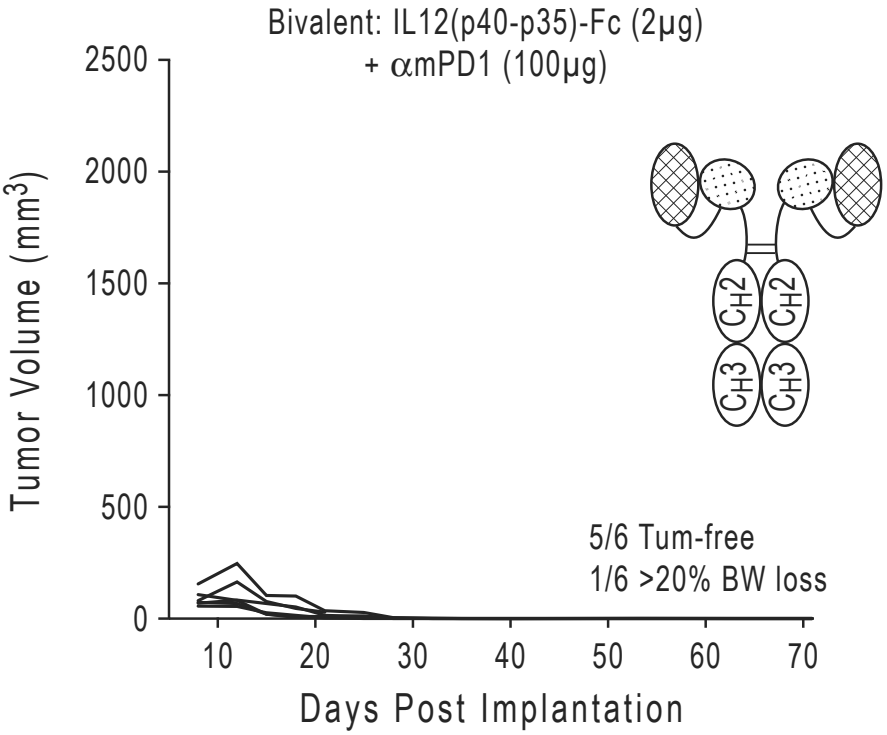


FIG. 23D

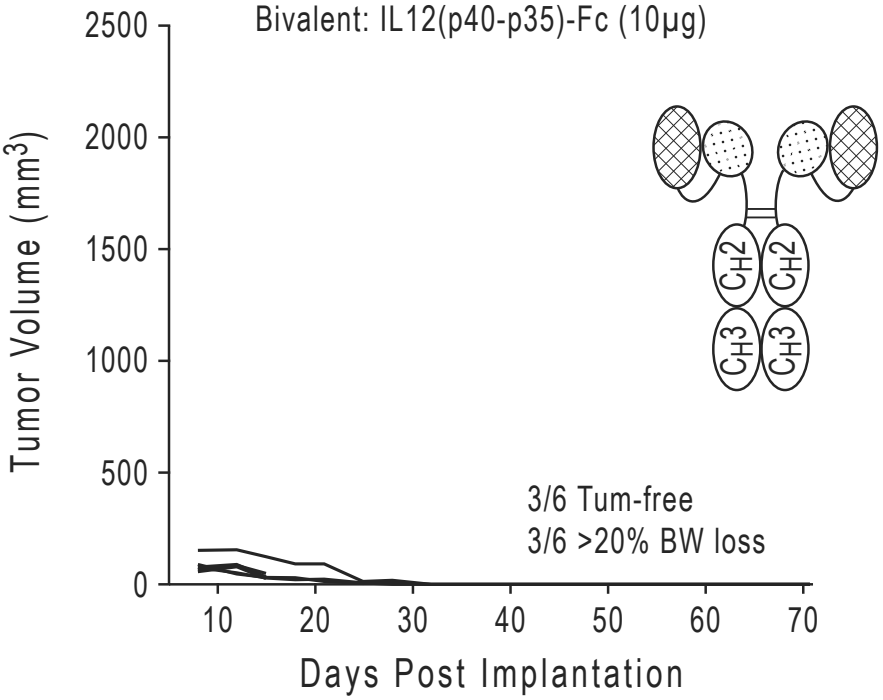


FIG. 23E

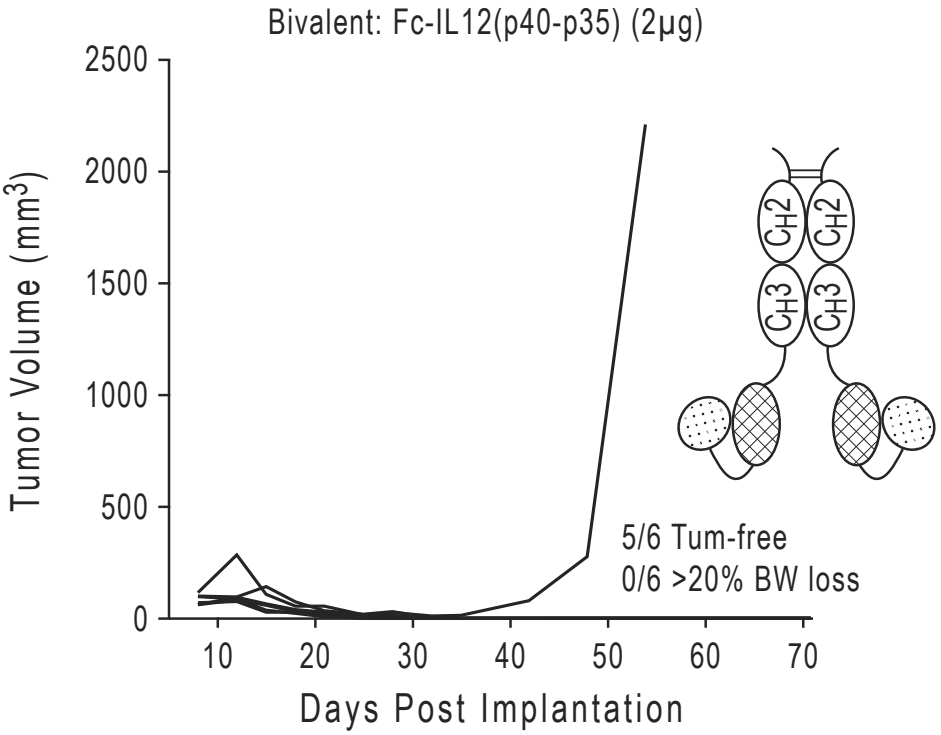


FIG. 23F

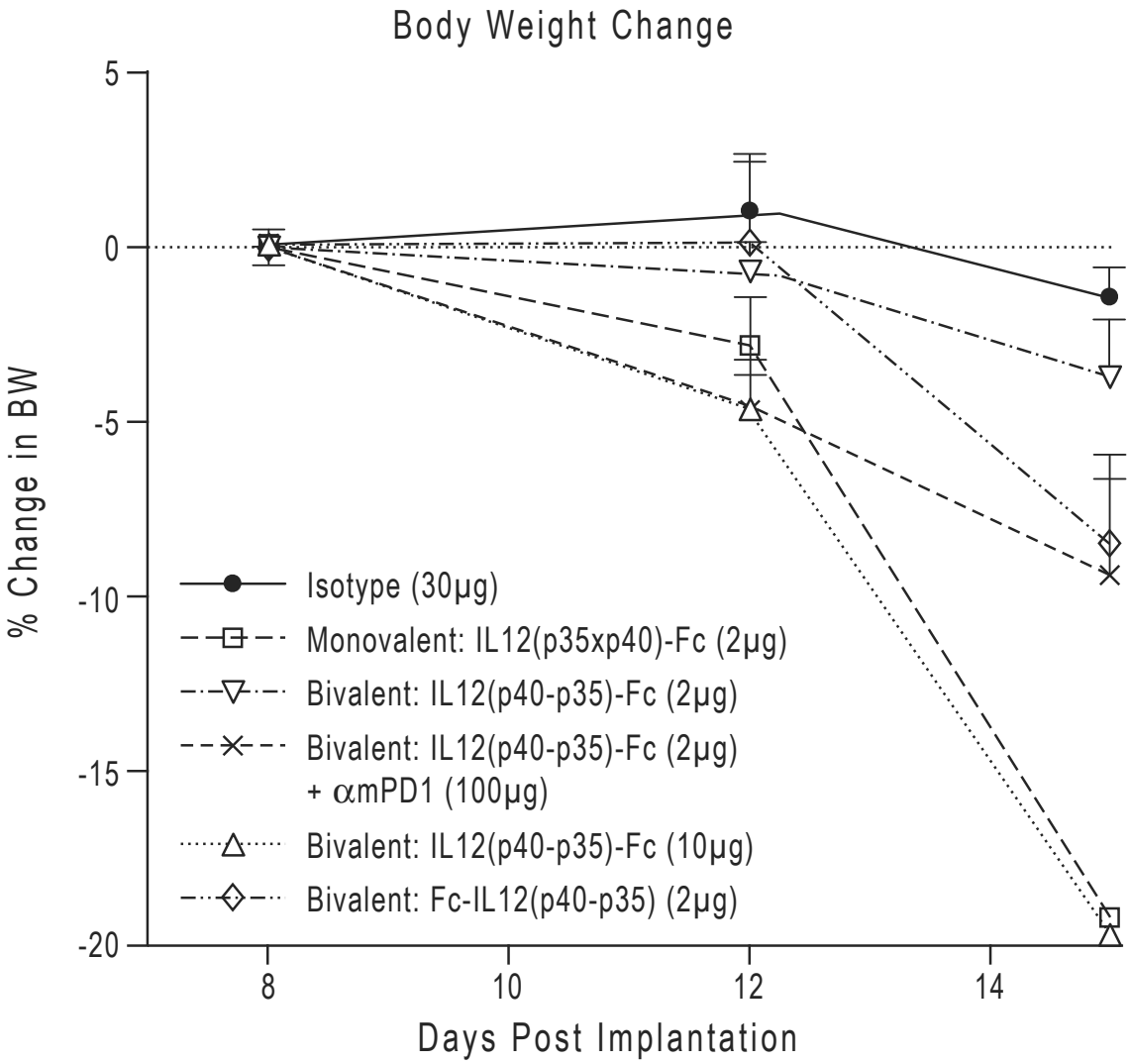

Body Weight Change
% Change in BW
5
0
-5
-10
-15
-20
8
10
12
14
Days Post Implantation
Isotype (30µg)
Monovalent: IL12(p35xp40)-Fc (2µg)
Bivalent: IL12(p40-p35)-Fc (2µg)
Bivalent: IL12(p40-p35)-Fc (2µg)
+ αmPD1 (100µg)
Bivalent: IL12(p40-p35)-Fc (10µg)
Bivalent: Fc-IL12(p40-p35) (2µg)

FIG. 24

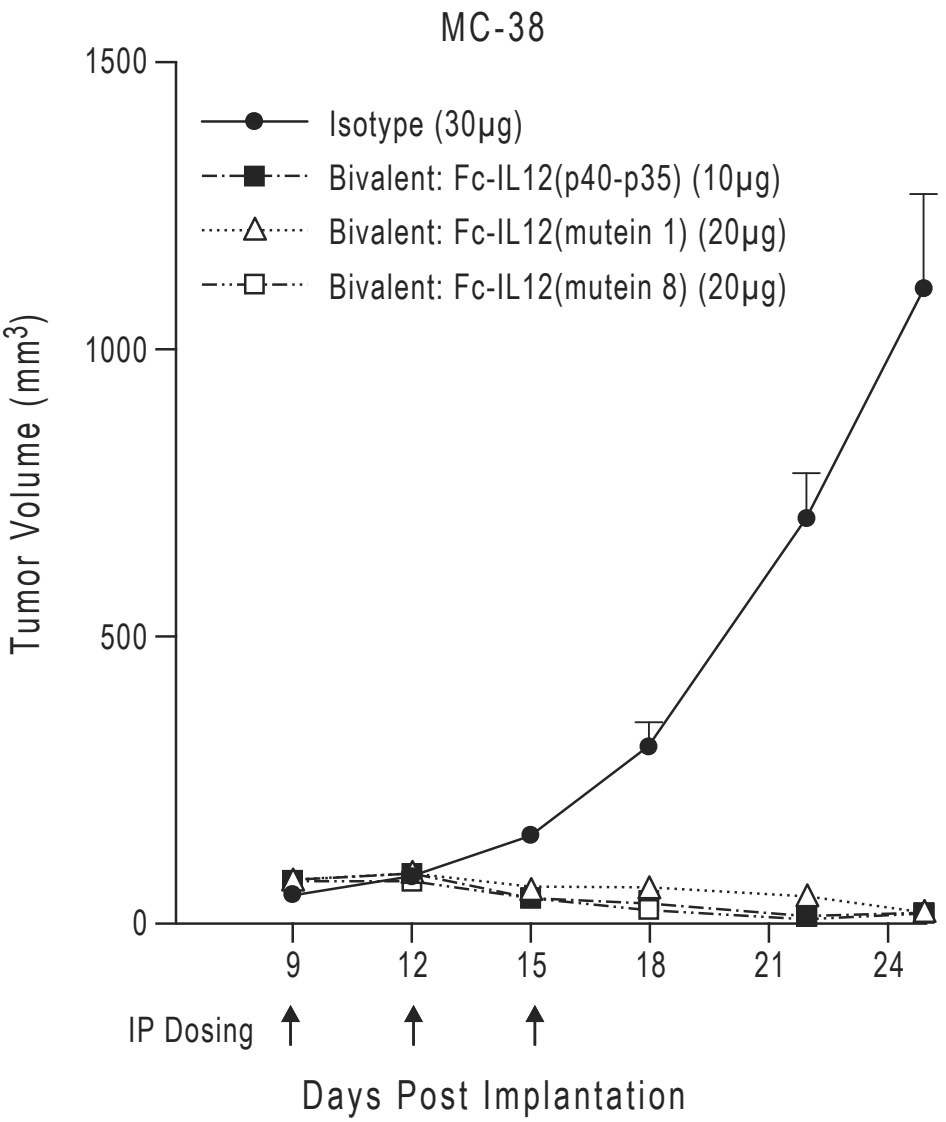

MC-38
1500
1000
500
0
Tumor Volume (mm3)
Isotype (30µg)
Bivalent: Fc-IL12(p40-p35) (10µg)
Bivalent: Fc-IL12(mutein 1) (20µg)
Bivalent: Fc-IL12(mutein 8) (20µg)
9
12
15
18
21
24
IP Dosing
Days Post Implantation

FIG. 25A

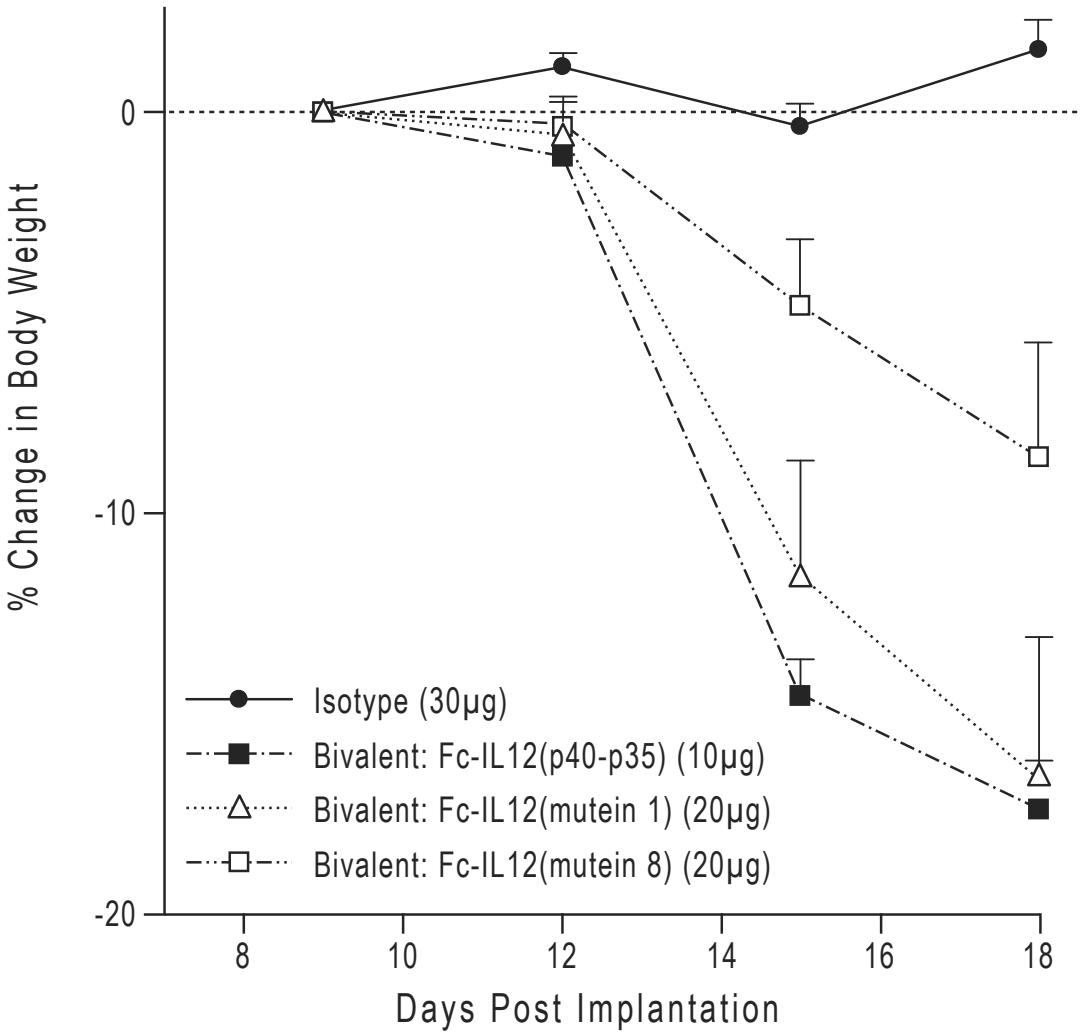

% Change in Body Weight
0
-10
-20
8
10
12
14
16
18
Days Post Implantation
Isotype (30µg)
Bivalent: Fc-IL12(p40-p35) (10µg)
Bivalent: Fc-IL12(mutein 1) (20µg)
Bivalent: Fc-IL12(mutein 8) (20µg)

FIG. 25B

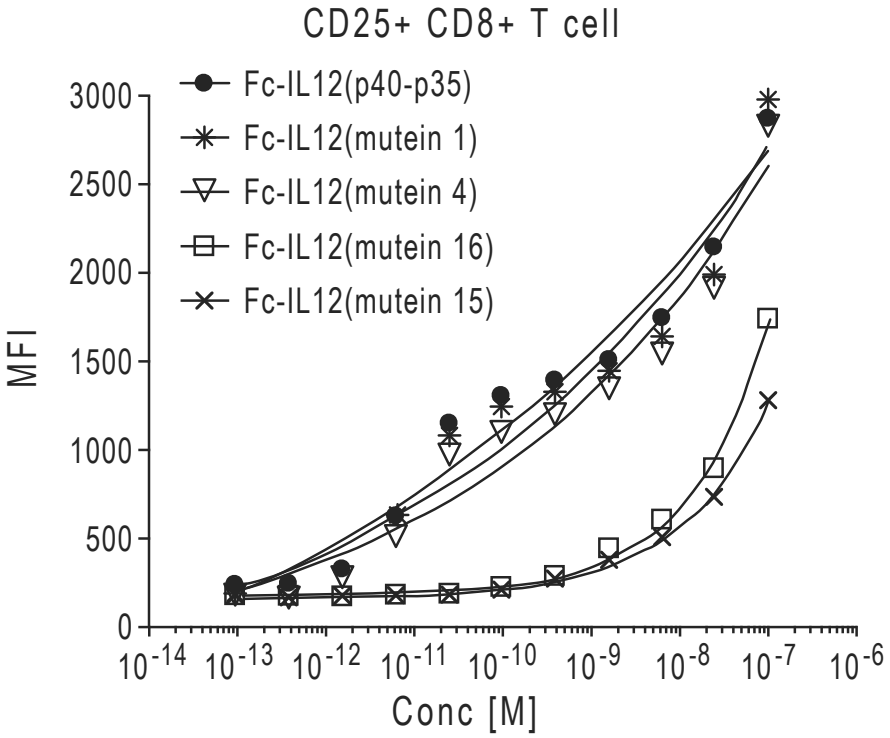


FIG. 26A

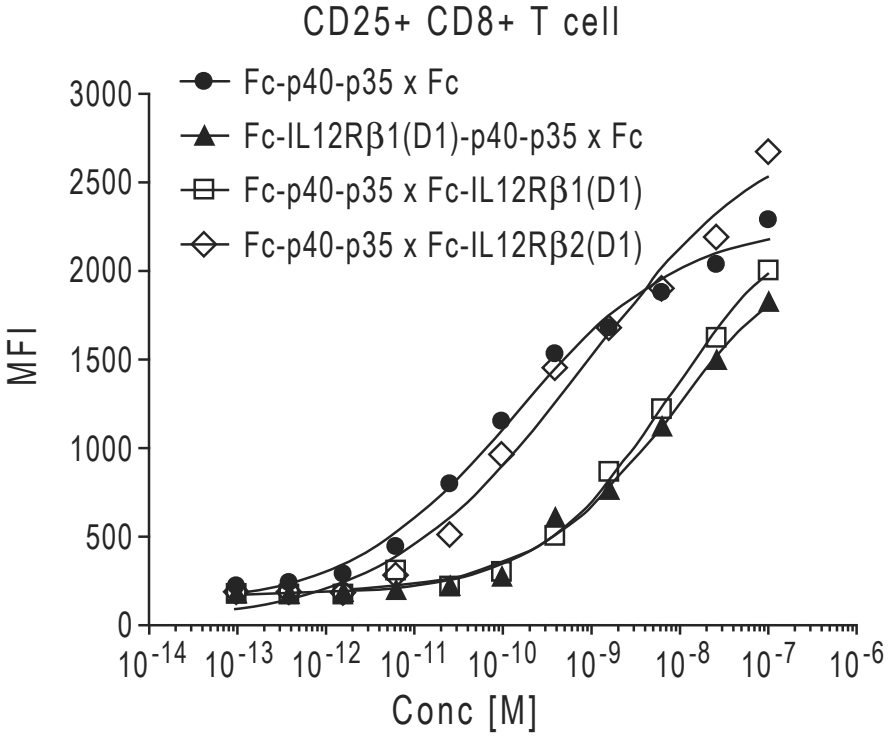


FIG. 26B

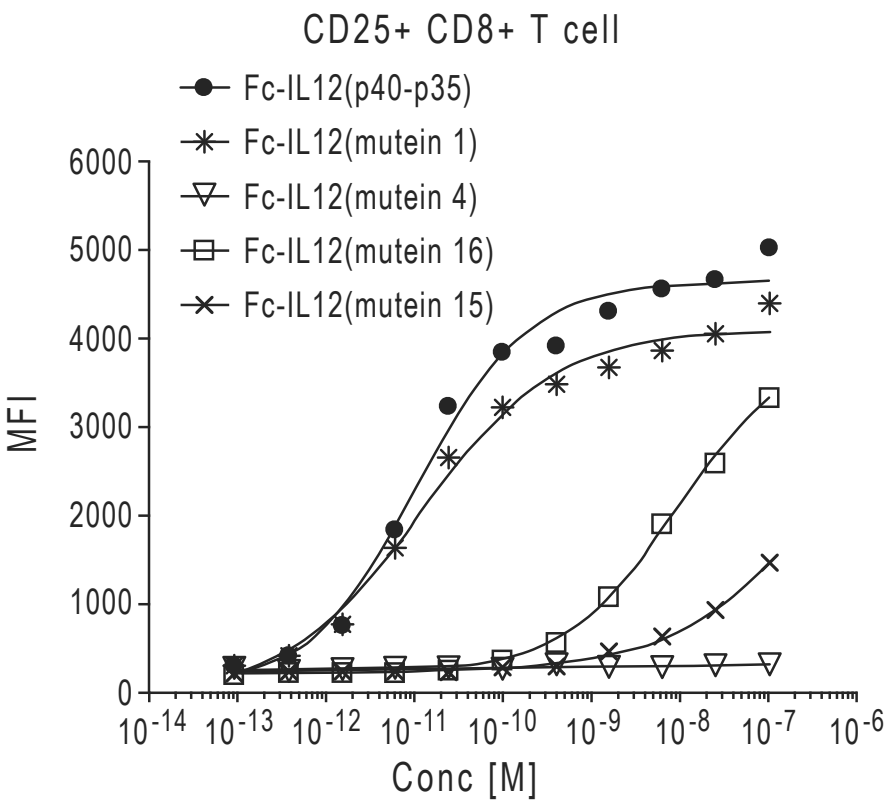


FIG. 27A

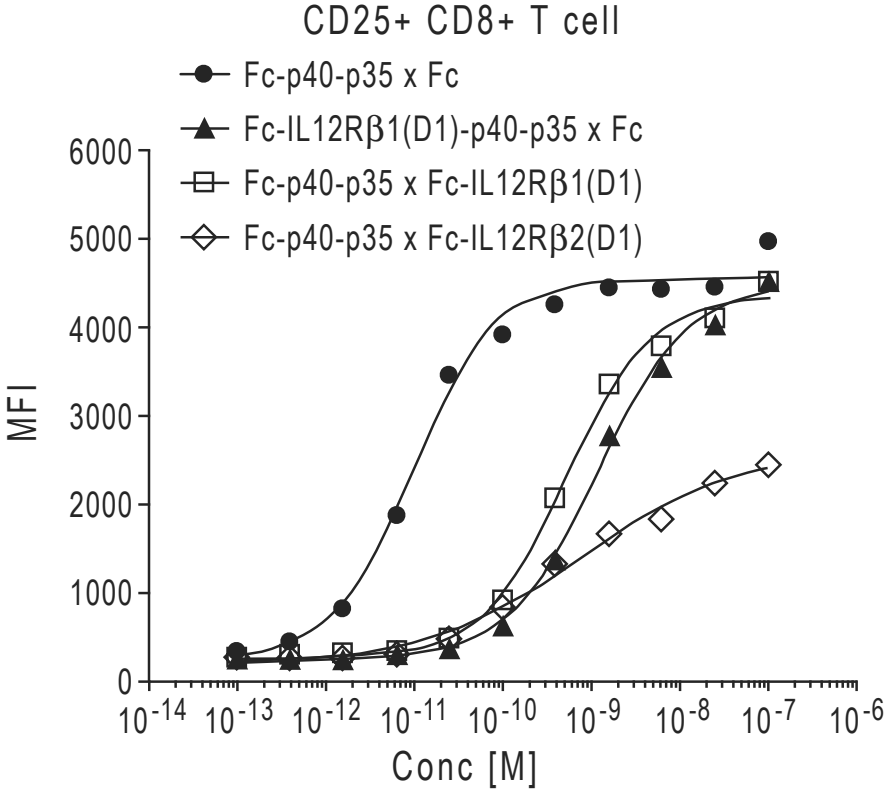


FIG. 27B

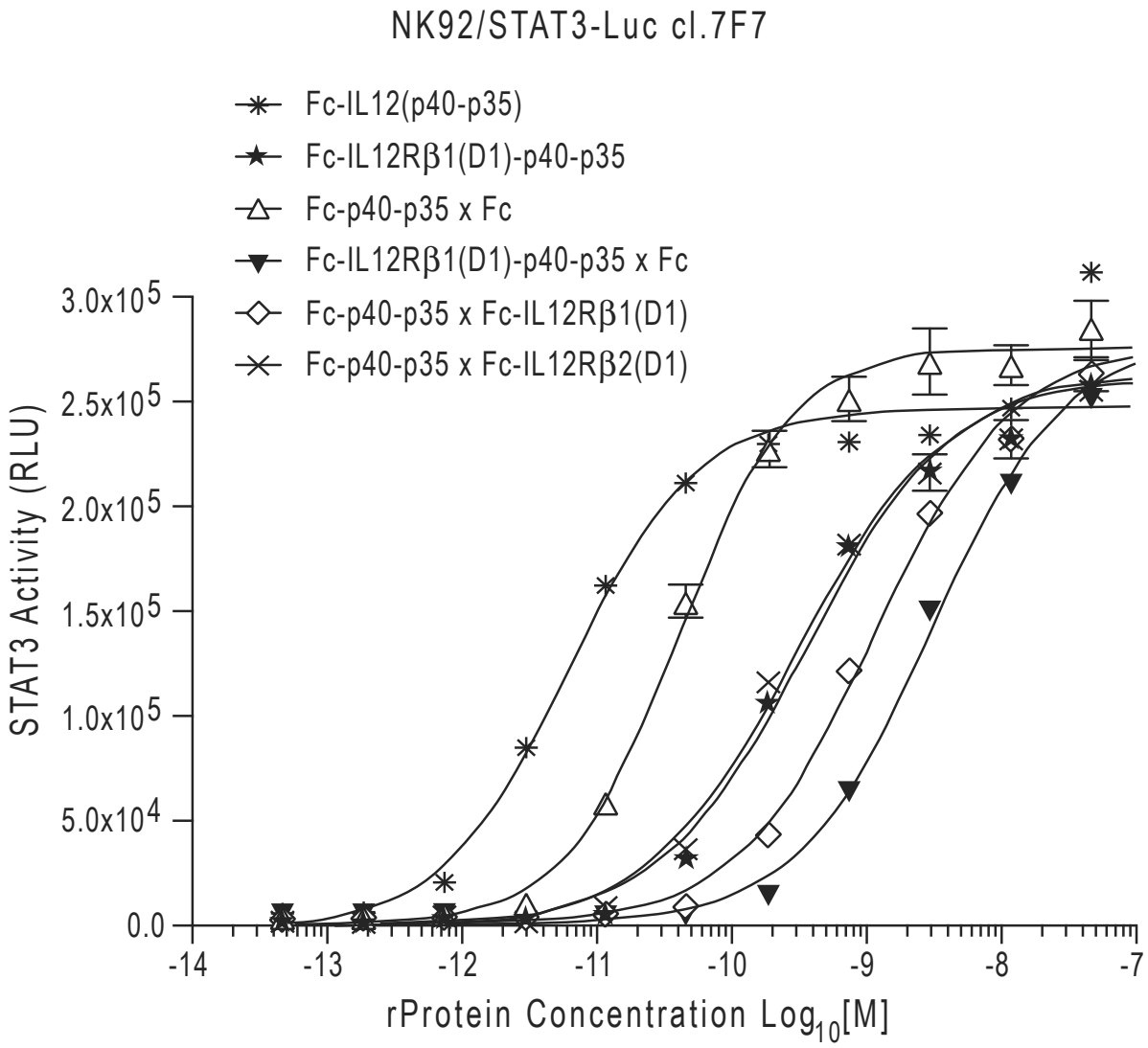

NK92/STAT3-Luc cl.7F7
Fc-IL12(p40-p35)
Fc-IL12Rβ1(D1)-p40-p35
Fc-p40-p35 x Fc
Fc-IL12Rβ1(D1)-p40-p35 x Fc
Fc-p40-p35 x Fc-IL12Rβ1(D1)
Fc-p40-p35 x Fc-IL12Rβ2(D1)
STAT3 Activity (RLU)
3.0x10^5
2.5x10^5
2.0x10^5
1.5x10^5
1.0x10^5
5.0x10^4
0.0
-14
-13
-12
-11
-10
-9
-8
-7
rProtein Concentration Log10[M]

FIG. 28

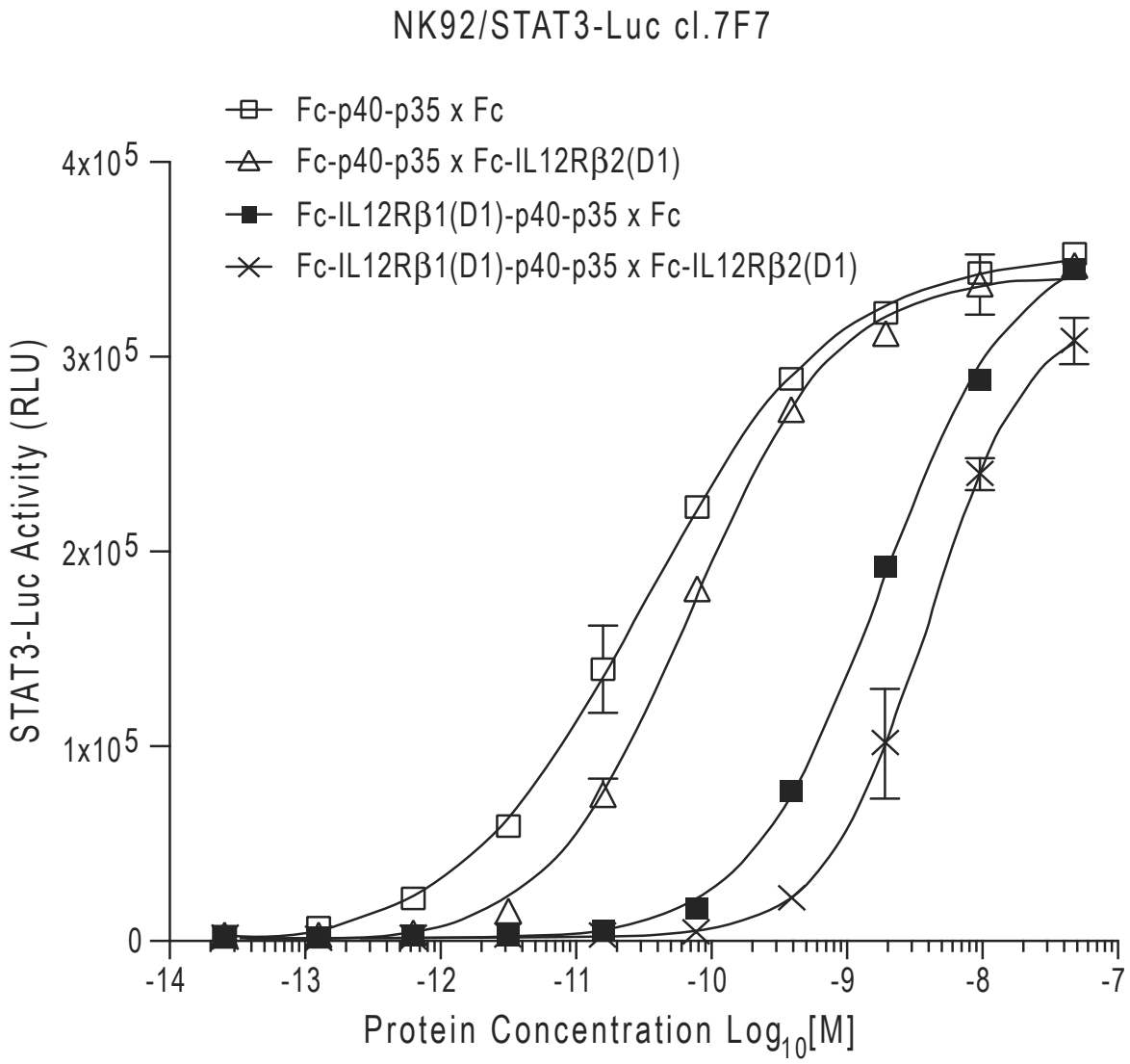

NK92/STAT3-Luc cl.7F7
Fc-p40-p35 x Fc
Fc-p40-p35 x Fc-IL12Rβ2(D1)
Fc-IL12Rβ1(D1)-p40-p35 x Fc
Fc-IL12Rβ1(D1)-p40-p35 x Fc-IL12Rβ2(D1)
STAT3-Luc Activity (RLU)
4x10^5
3x10^5
2x10^5
1x10^5
0
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration Log10[M]

FIG. 29

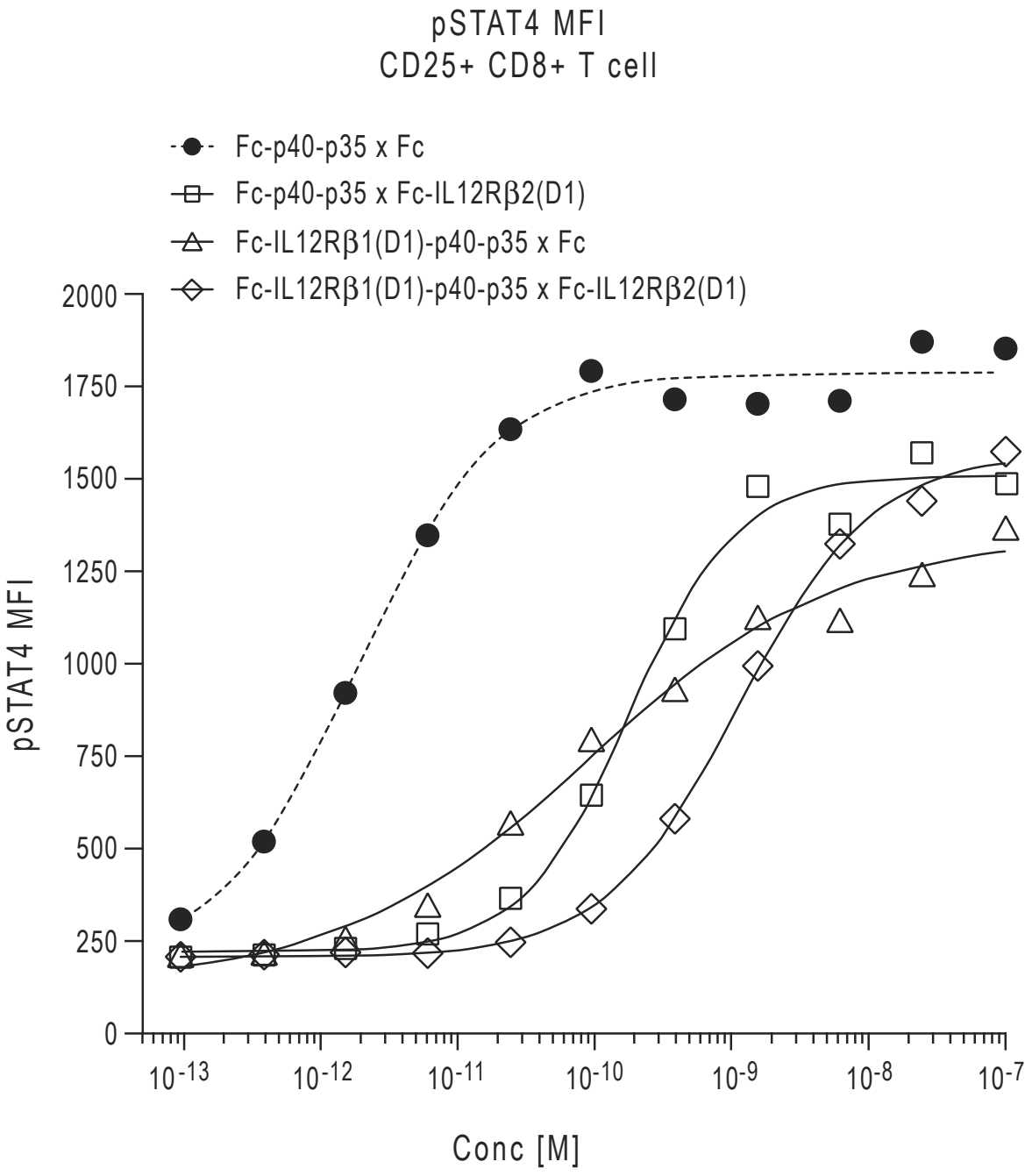

pSTAT4 MFI
CD25+ CD8+ T cell
Fc-p40-p35 x Fc
Fc-p40-p35 x Fc-IL12Rβ2(D1)
Fc-IL12Rβ1(D1)-p40-p35 x Fc
Fc-IL12Rβ1(D1)-p40-p35 x Fc-IL12Rβ2(D1)
pSTAT4 MFI
2000
1750
1500
1250
1000
750
500
250
0
10-13
10-12
10-11
10-10
10-9
10-8
10-7
Conc [M]

FIG. 30

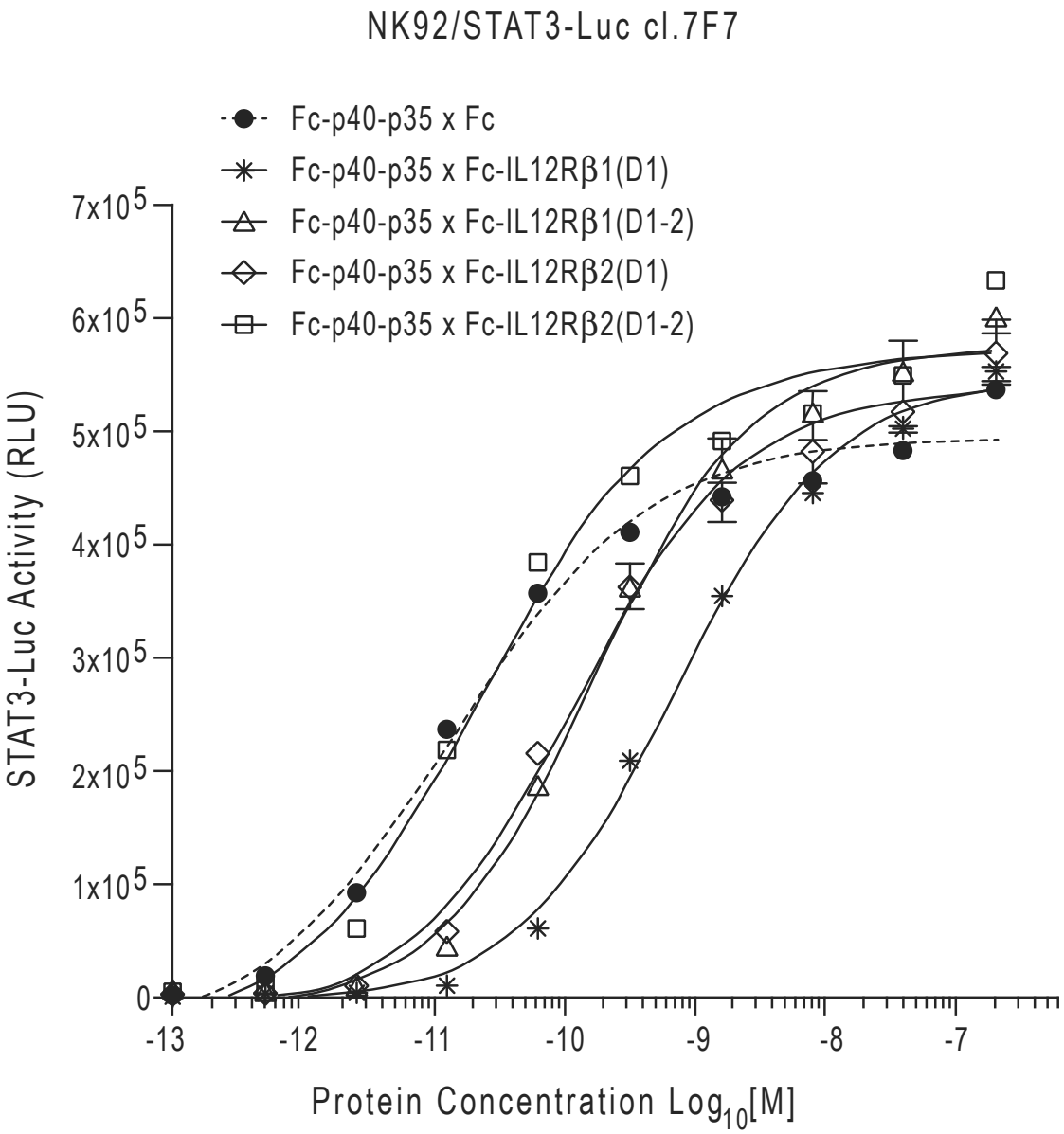

NK92/STAT3-Luc cl.7F7
Fc-p40-p35 x Fc
Fc-p40-p35 x Fc-IL12Rβ1(D1)
Fc-p40-p35 x Fc-IL12Rβ1(D1-2)
Fc-p40-p35 x Fc-IL12Rβ2(D1)
Fc-p40-p35 x Fc-IL12Rβ2(D1-2)
STAT3-Luc Activity (RLU)
7x10^5
6x10^5
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
-13
-12
-11
-10
-9
-8
-7
Protein Concentration Log10[M]

FIG. 31

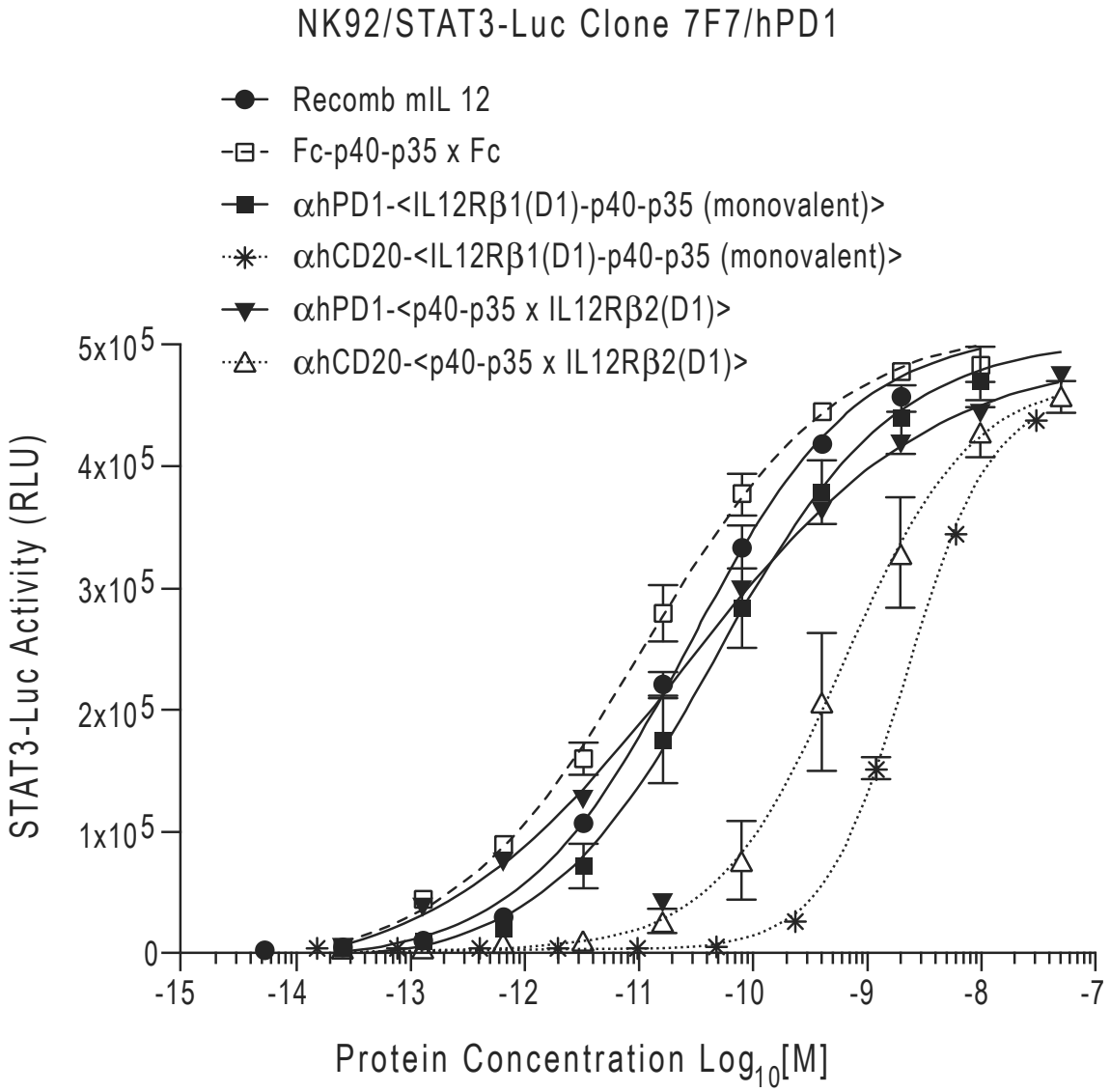

NK92/STAT3-Luc Clone 7F7/hPD1
Recomb mIL 12
Fc-p40-p35 x Fc
αhPD1-<IL12Rβ1(D1)-p40-p35 (monovalent)>
αhCD20-<IL12Rβ1(D1)-p40-p35 (monovalent)>
αhPD1-<p40-p35 x IL12Rβ2(D1)>
αhCD20-<p40-p35 x IL12Rβ2(D1)>
STAT3-Luc Activity (RLU)
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
-15
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration Log10[M]

FIG. 32

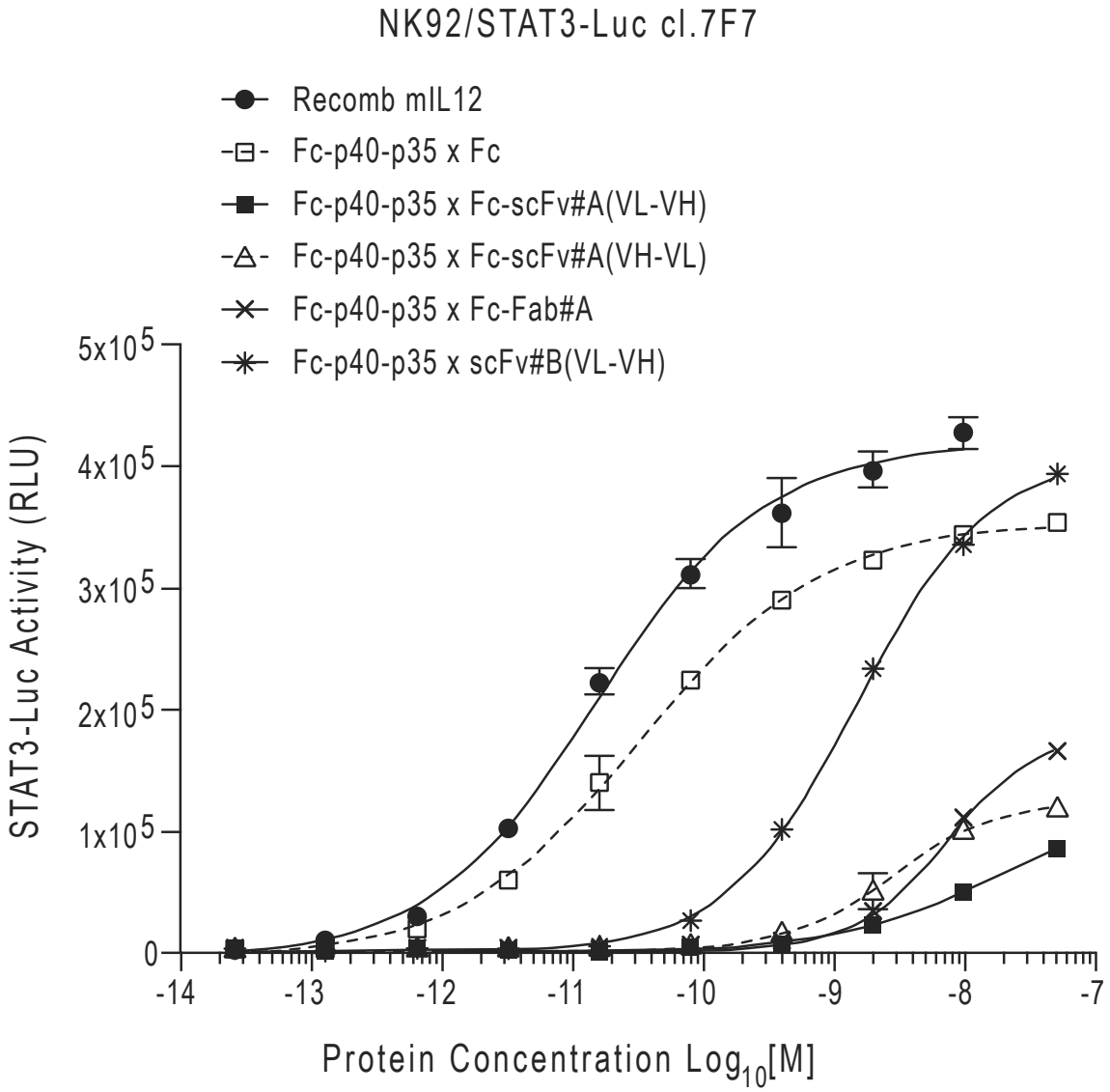

NK92/STAT3-Luc cl.7F7
Recomb mIL12
Fc-p40-p35 x Fc
Fc-p40-p35 x Fc-scFv#A(VL-VH)
Fc-p40-p35 x Fc-scFv#A(VH-VL)
Fc-p40-p35 x Fc-Fab#A
Fc-p40-p35 x scFv#B(VL-VH)
STAT3-Luc Activity (RLU)
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration Log10[M]

FIG. 33

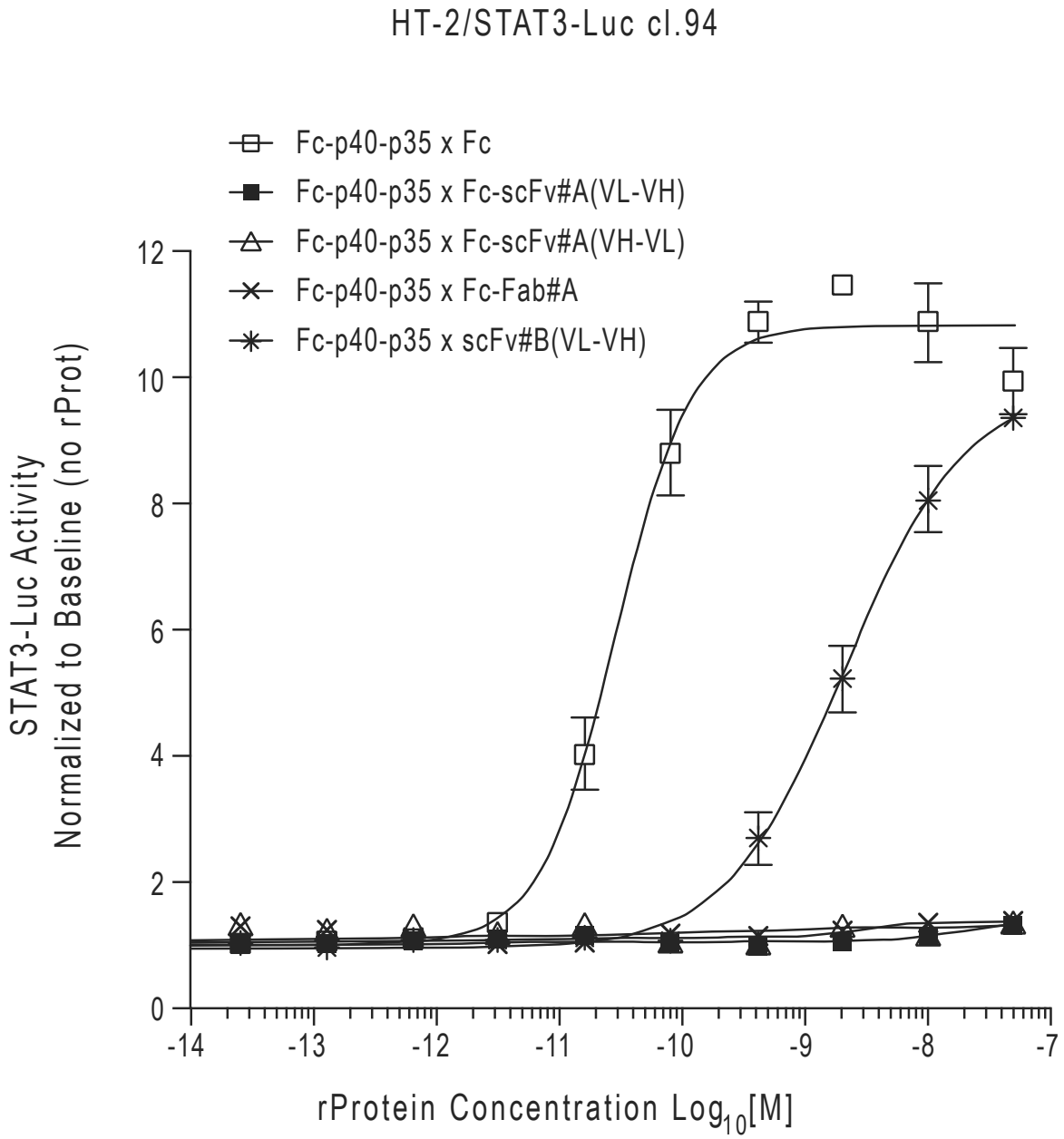

HT-2/STAT3-Luc cl.94
Fc-p40-p35 x Fc
Fc-p40-p35 x Fc-scFv#A(VL-VH)
Fc-p40-p35 x Fc-scFv#A(VH-VL)
Fc-p40-p35 x Fc-Fab#A
Fc-p40-p35 x scFv#B(VL-VH)
STAT3-Luc Activity
Normalized to Baseline (no rProt)
0
2
4
6
8
10
12
-14
-13
-12
-11
-10
-9
-8
-7
rProtein Concentration Log10[M]

FIG. 34

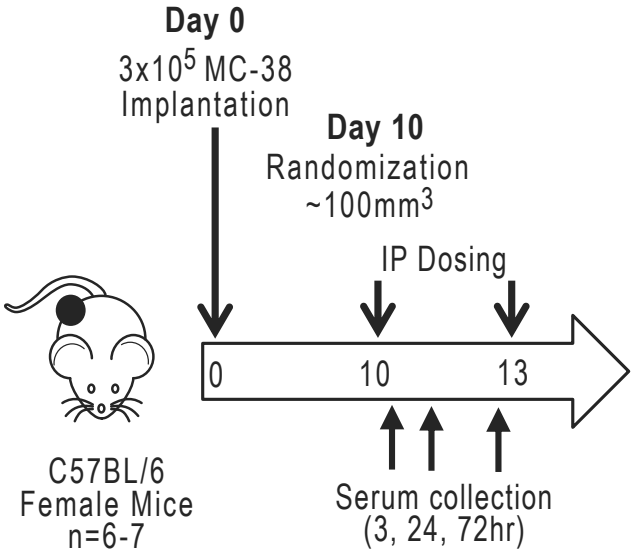


FIG. 35A

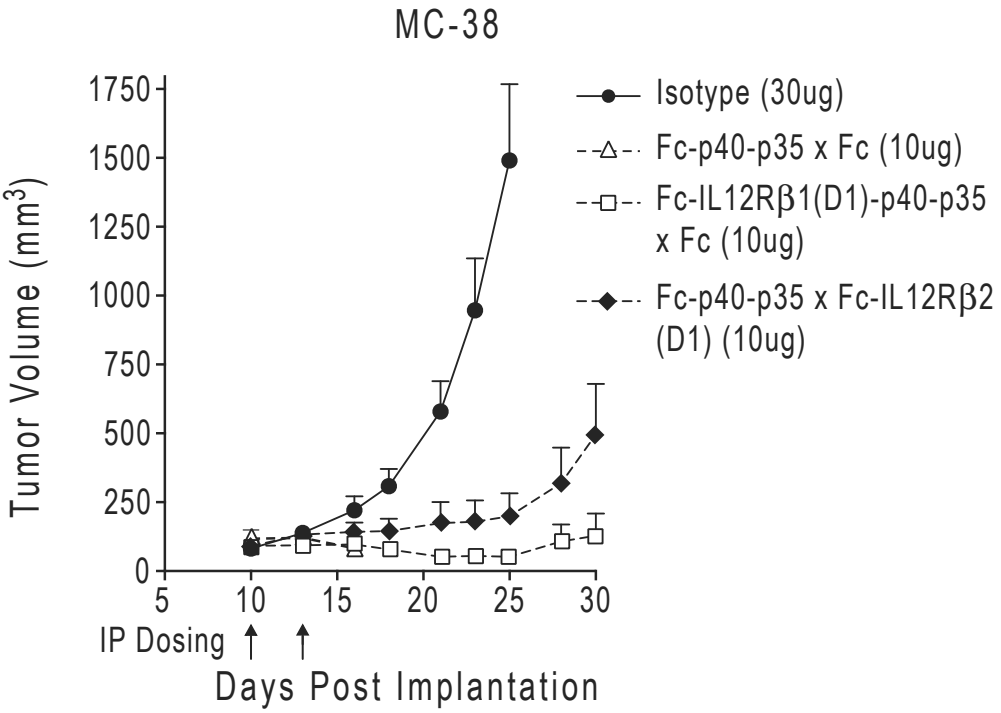


FIG. 35B

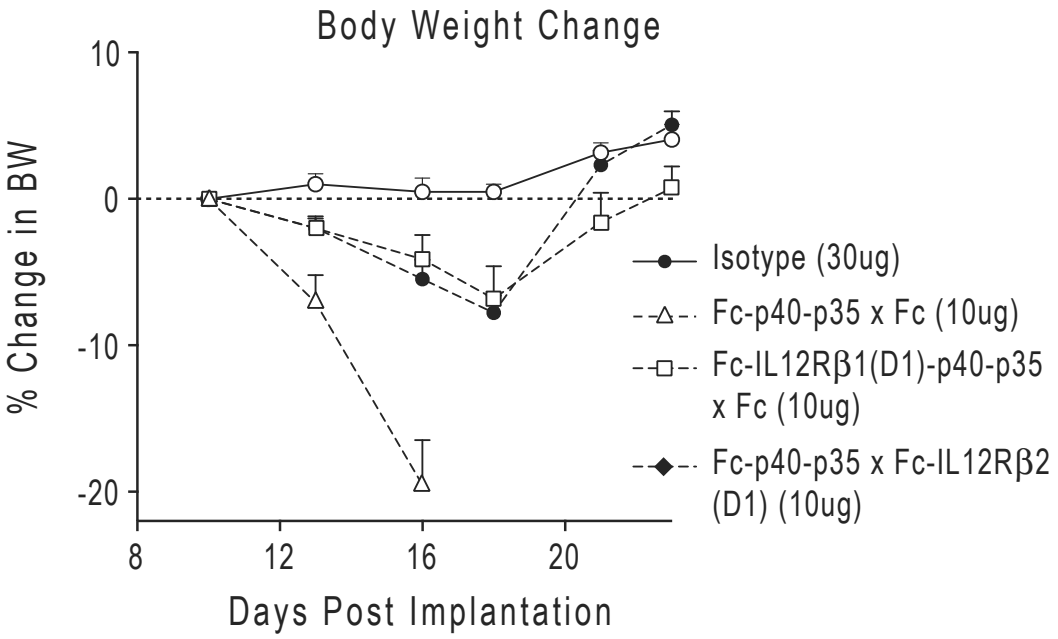


FIG. 35C

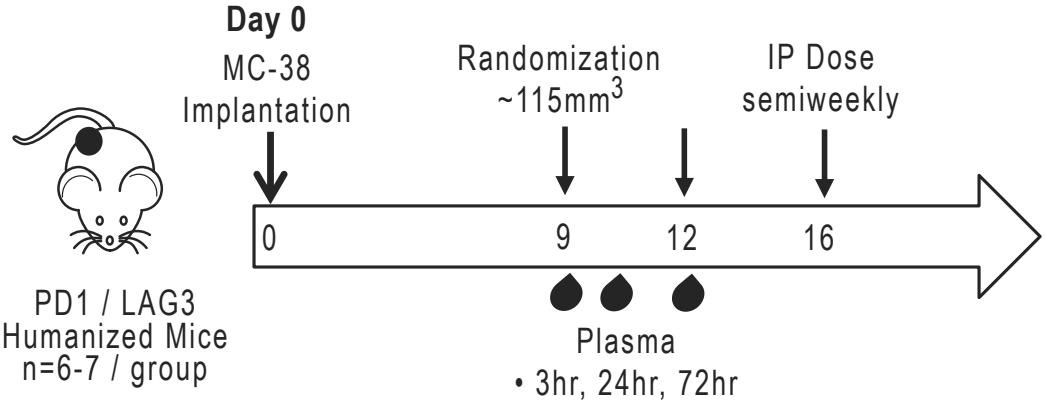


FIG. 36A

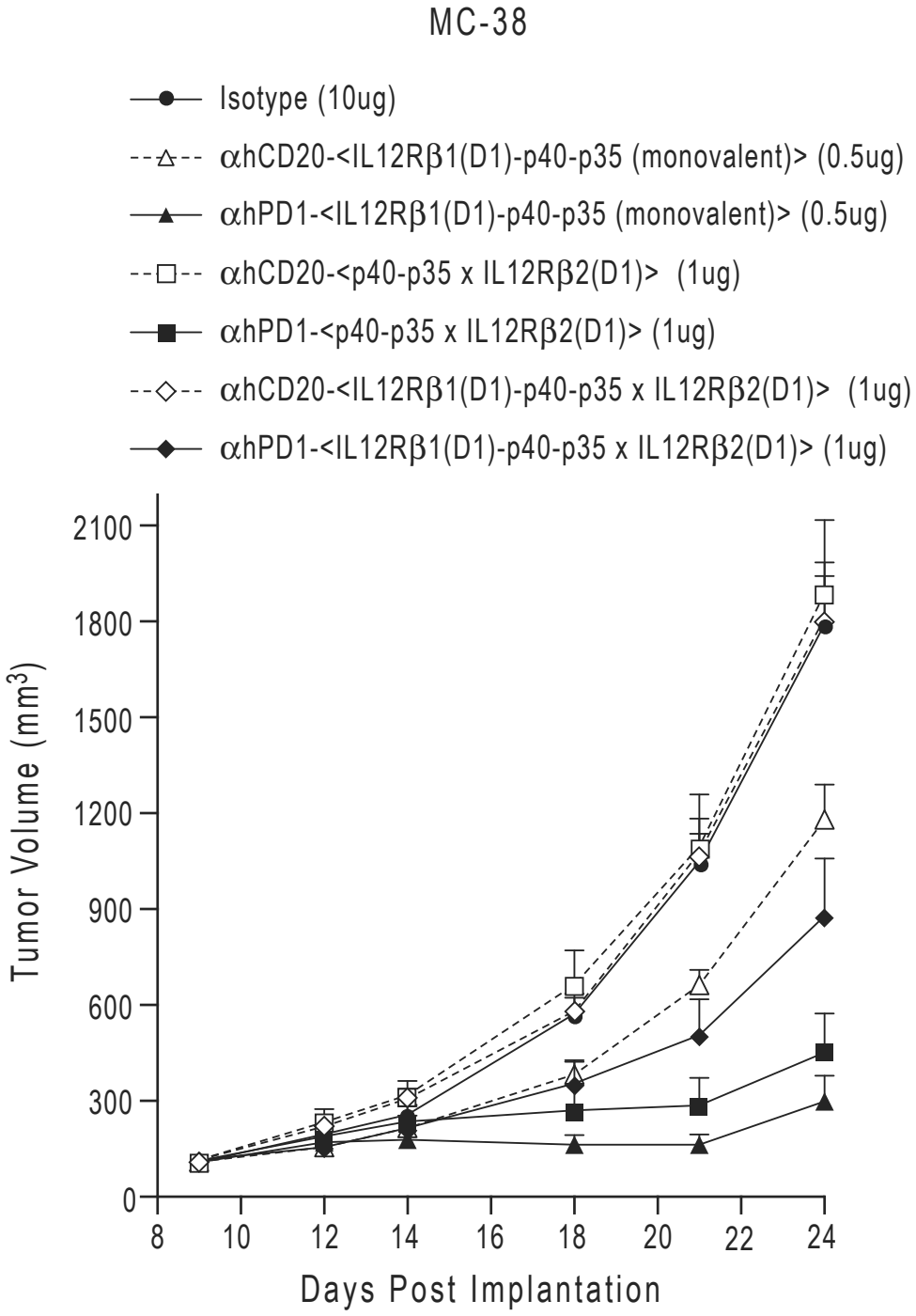

MC-38
Isotype (10ug)
αhCD20-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.5ug)
αhPD1-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.5ug)
αhCD20-<p40-p35 x IL12Rβ2(D1)> (1ug)
αhPD1-<p40-p35 x IL12Rβ2(D1)> (1ug)
αhCD20-<IL12Rβ1(D1)-p40-p35 x IL12Rβ2(D1)> (1ug)
αhPD1-<IL12Rβ1(D1)-p40-p35 x IL12Rβ2(D1)> (1ug)
Tumor Volume (mm3)
2100
1800
1500
1200
900
600
300
0
8
10
12
14
16
18
20
22
24
Days Post Implantation

FIG. 36B

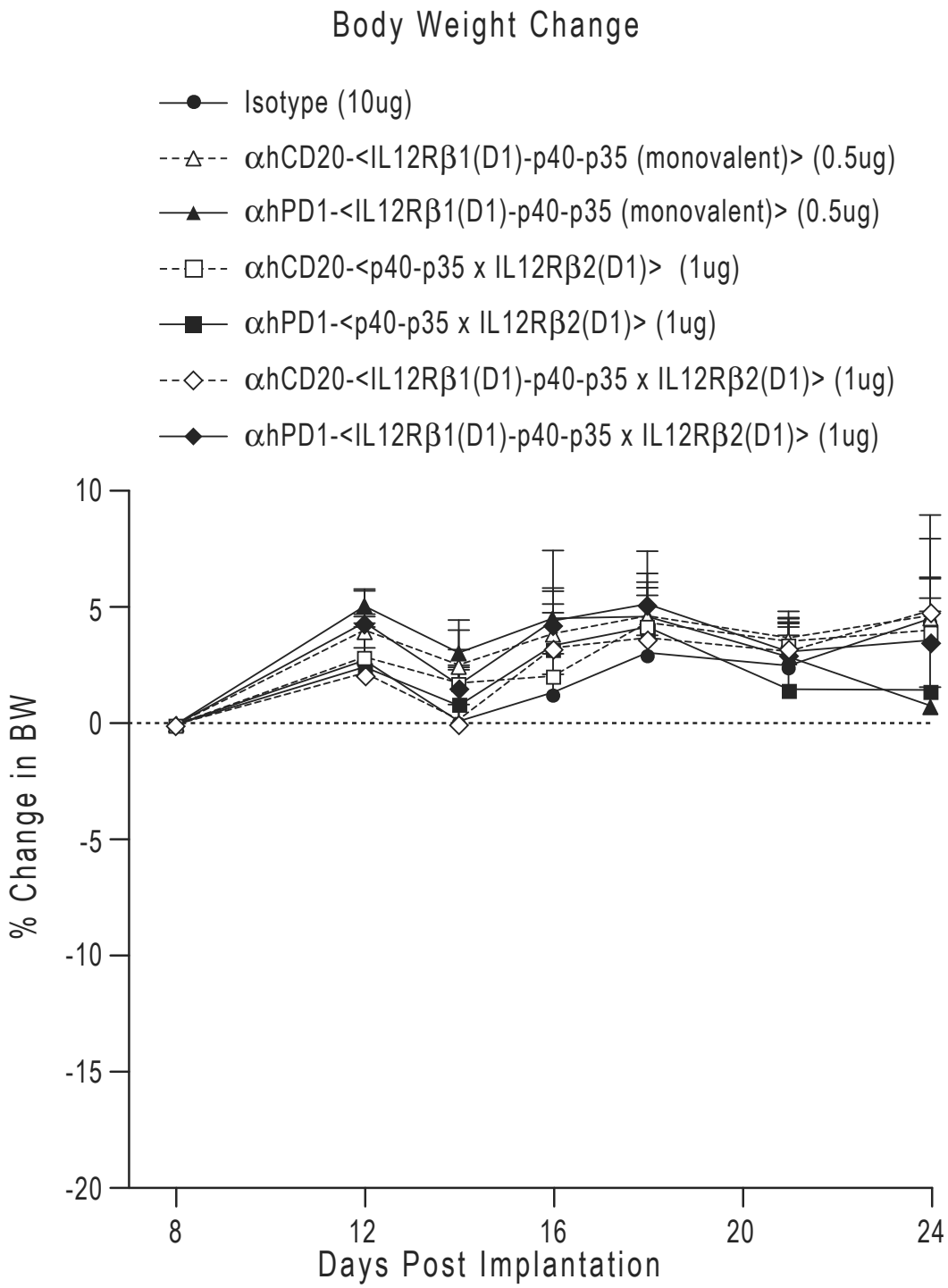

Body Weight Change
Isotype (10ug)
αhCD20-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.5ug)
αhPD1-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.5ug)
αhCD20-<p40-p35 x IL12Rβ2(D1)> (1ug)
αhPD1-<p40-p35 x IL12Rβ2(D1)> (1ug)
αhCD20-<IL12Rβ1(D1)-p40-p35 x IL12Rβ2(D1)> (1ug)
αhPD1-<IL12Rβ1(D1)-p40-p35 x IL12Rβ2(D1)> (1ug)
% Change in BW
10
5
0
-5
-10
-15
-20
8
12
16
20
24
Days Post Implantation

FIG. 36C

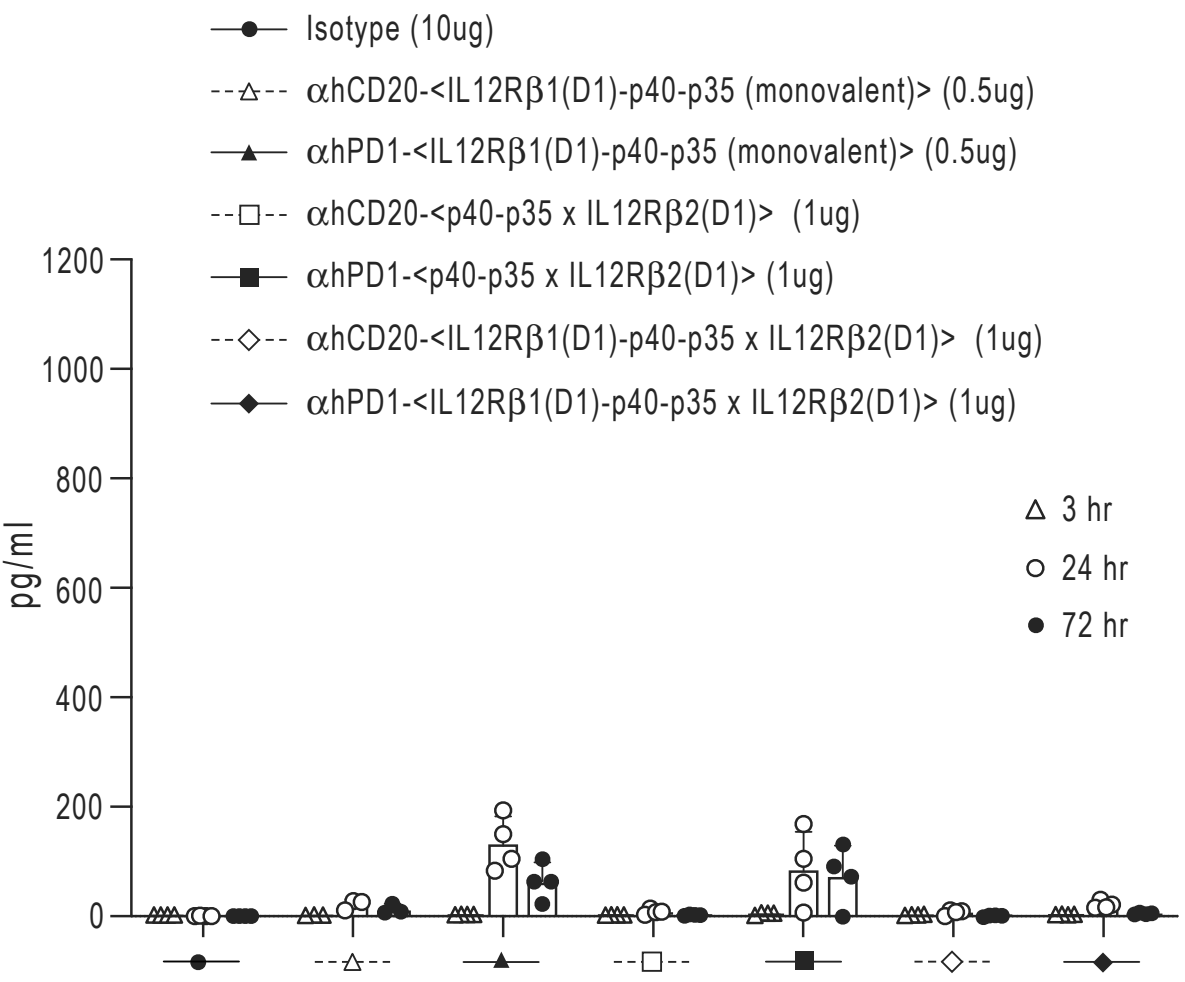

IFNγ
Isotype (10ug)
αhCD20-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.5ug)
αhPD1-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.5ug)
αhCD20-<p40-p35 x IL12Rβ2(D1)> (1ug)
αhPD1-<p40-p35 x IL12Rβ2(D1)> (1ug)
αhCD20-<IL12Rβ1(D1)-p40-p35 x IL12Rβ2(D1)> (1ug)
αhPD1-<IL12Rβ1(D1)-p40-p35 x IL12Rβ2(D1)> (1ug)
3 hr
24 hr
72 hr
pg/ml
1200
1000
800
600
400
200
0

FIG. 36D

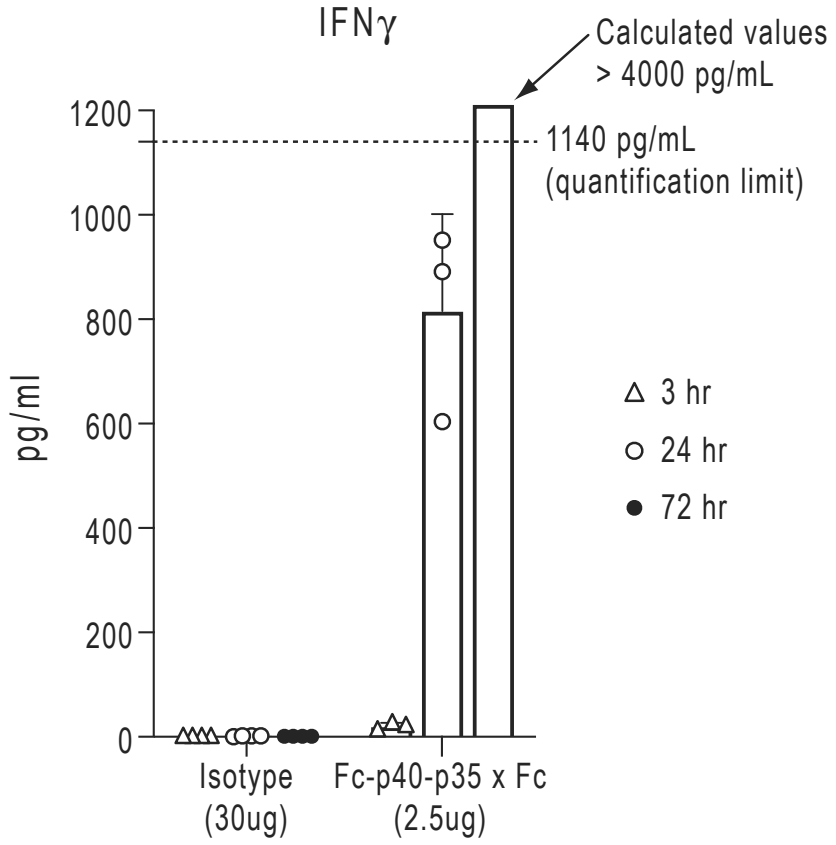


FIG. 36E

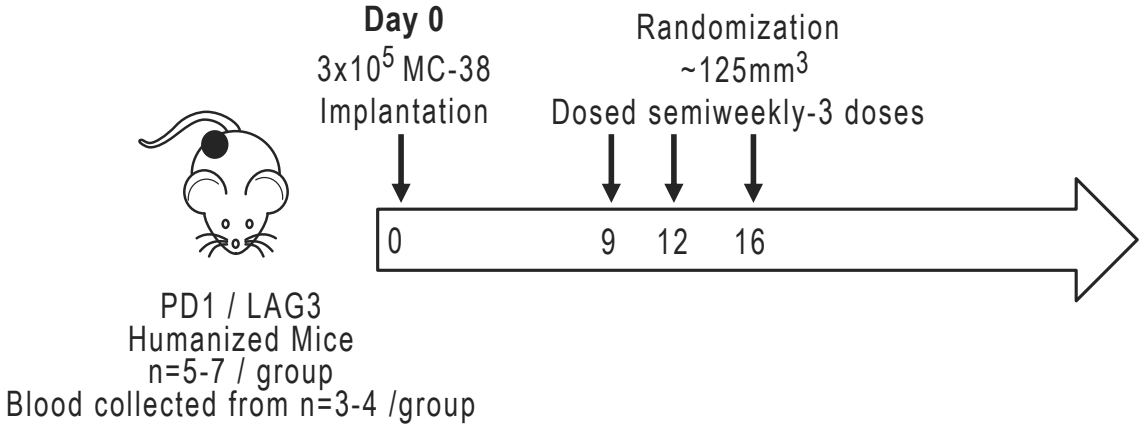


FIG. 37A

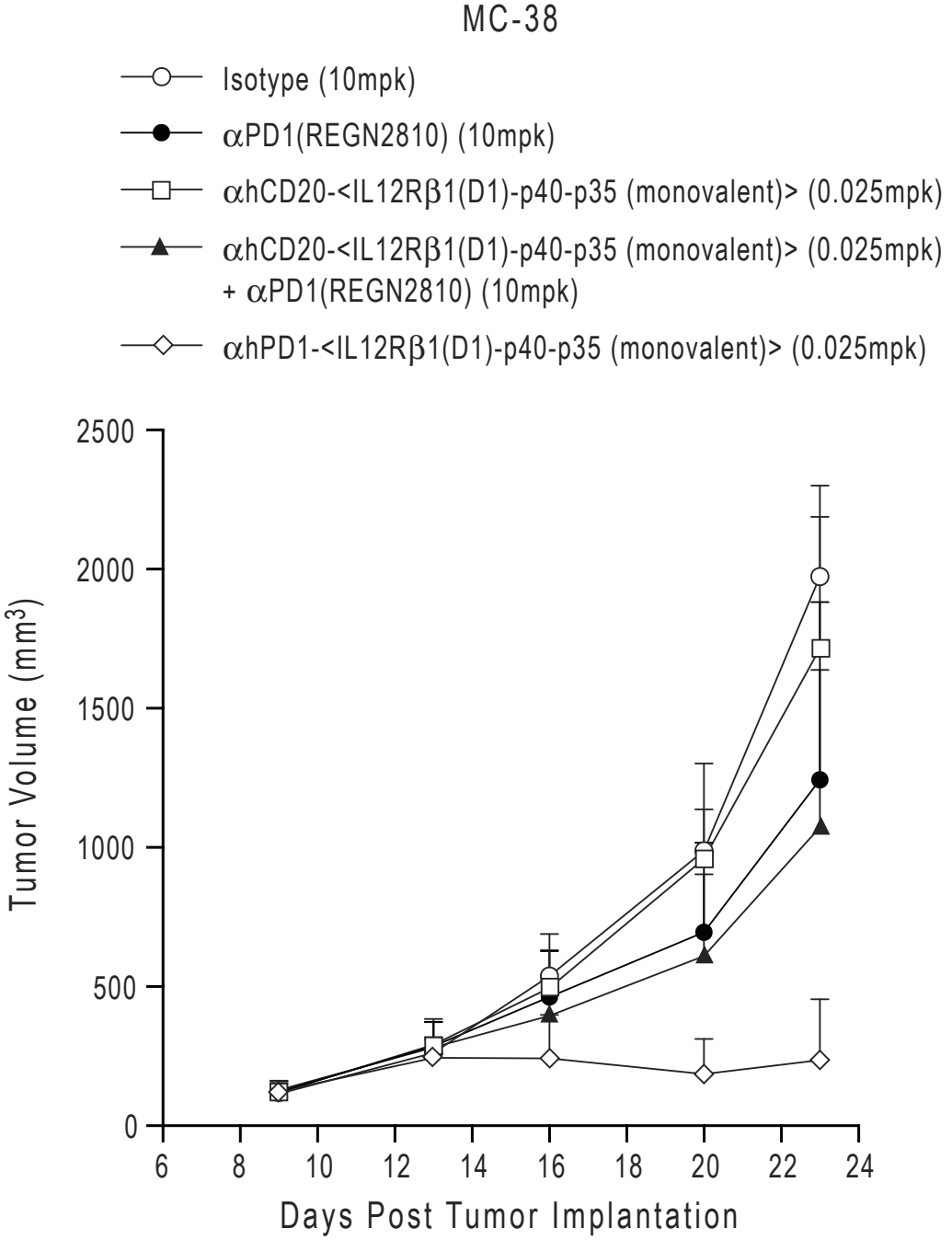

MC-38
Isotype (10mpk)
αPD1(REGN2810) (10mpk)
αhCD20-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.025mpk)
αhCD20-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.025mpk) + αPD1(REGN2810) (10mpk)
αhPD1-<IL12Rβ1(D1)-p40-p35 (monovalent)> (0.025mpk)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
6
8
10
12
14
16
18
20
22
24
Days Post Tumor Implantation

FIG. 37B

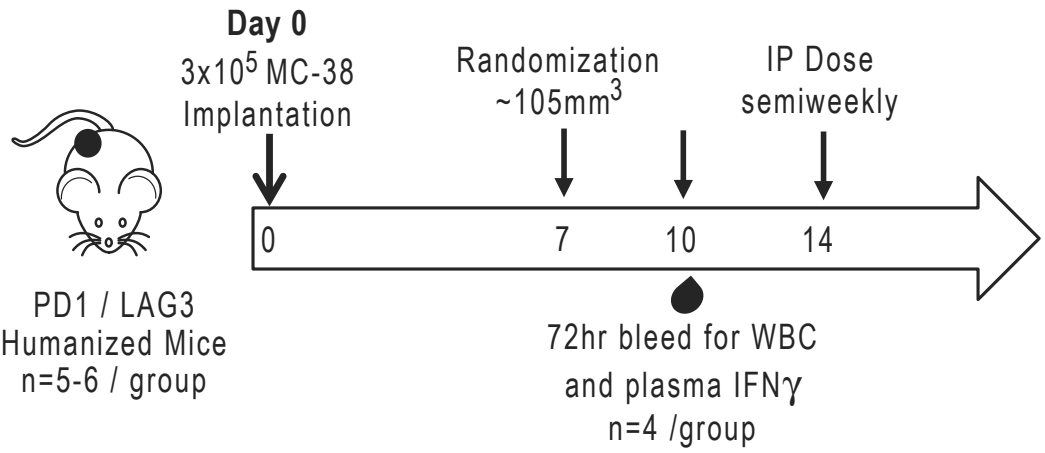


FIG. 38A

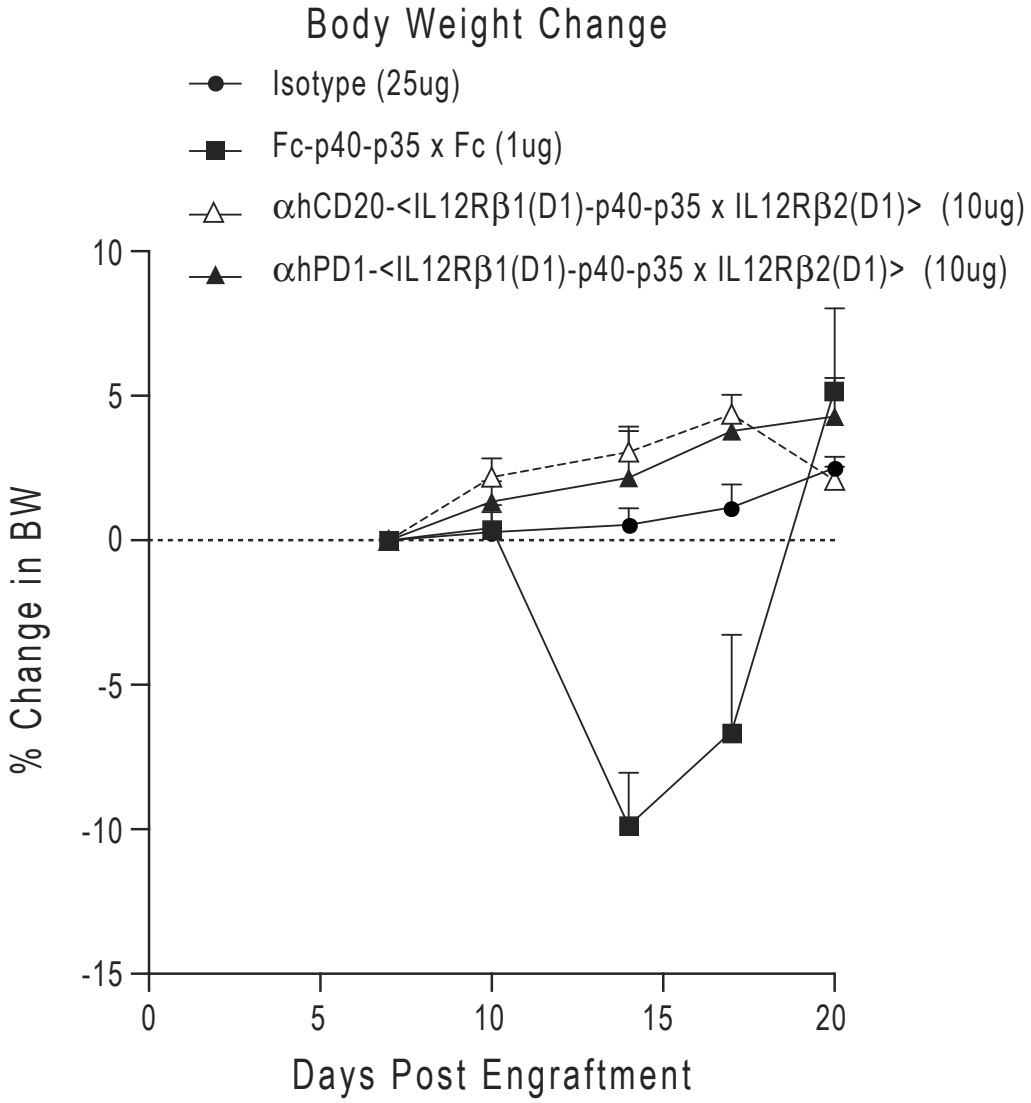


FIG. 38B-1

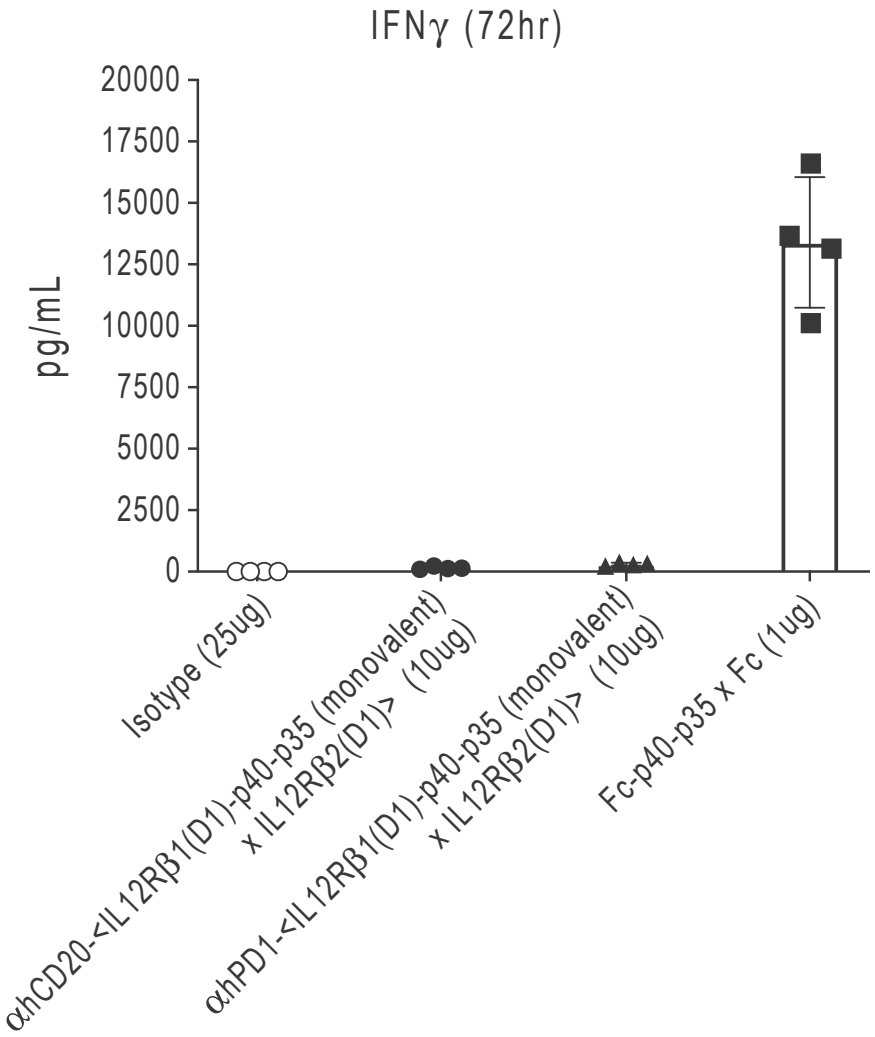

IFNγ (72hr)
pg/mL
20000
17500
15000
12500
10000
7500
5000
2500
0
Isotype (25ug)
αhCD20-<IL12Rβ1(D1)-p40-p35 (monovalent) x IL12Rβ2(D1)> (10ug)
αhPD1-<IL12Rβ1(D1)-p40-p35 (monovalent) x IL12Rβ2(D1)> (10ug)
Fc-p40-p35 x Fc (1ug)

FIG. 38B-2

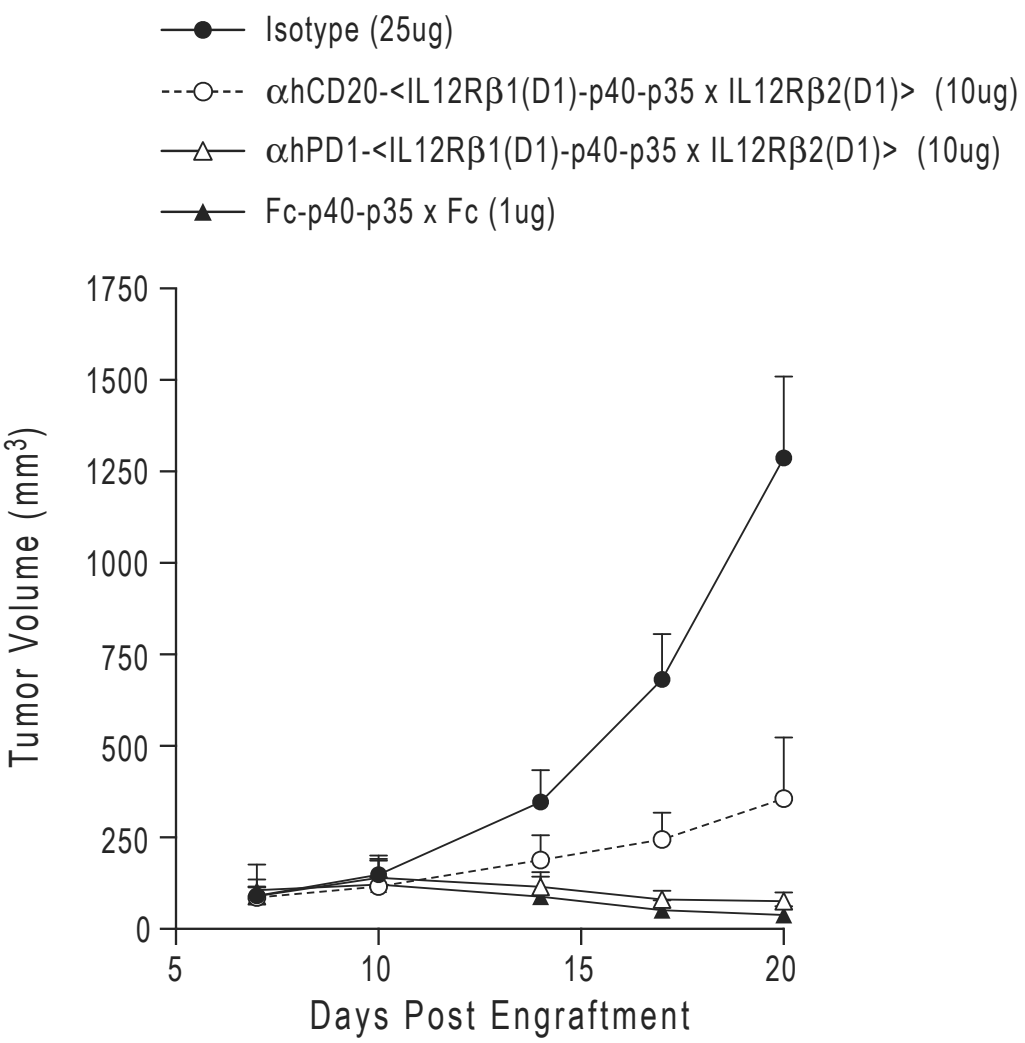

MC-38
Isotype (25ug)
αhCD20-<IL12Rβ1(D1)-p40-p35 x IL12Rβ2(D1)> (10ug)
αhPD1-<IL12Rβ1(D1)-p40-p35 x IL12Rβ2(D1)> (10ug)
Fc-p40-p35 x Fc (1ug)
Tumor Volume (mm3)
1750
1500
1250
1000
750
500
250
0
5
10
15
20
Days Post Engraftment

FIG. 38C-1

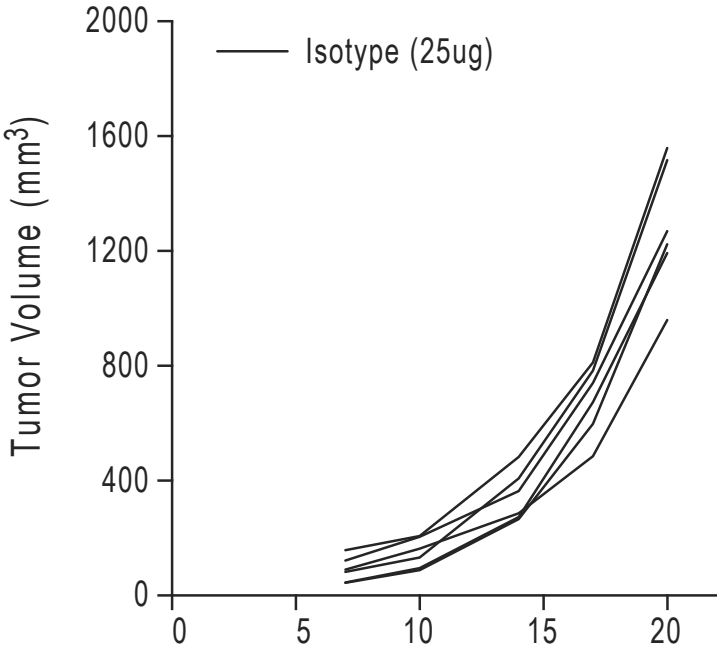


FIG. 38C-2

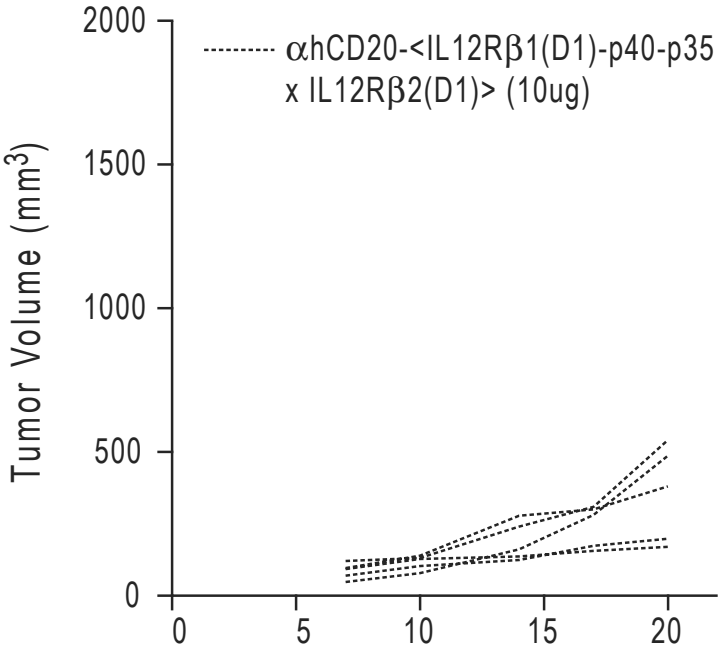


FIG. 38C-3

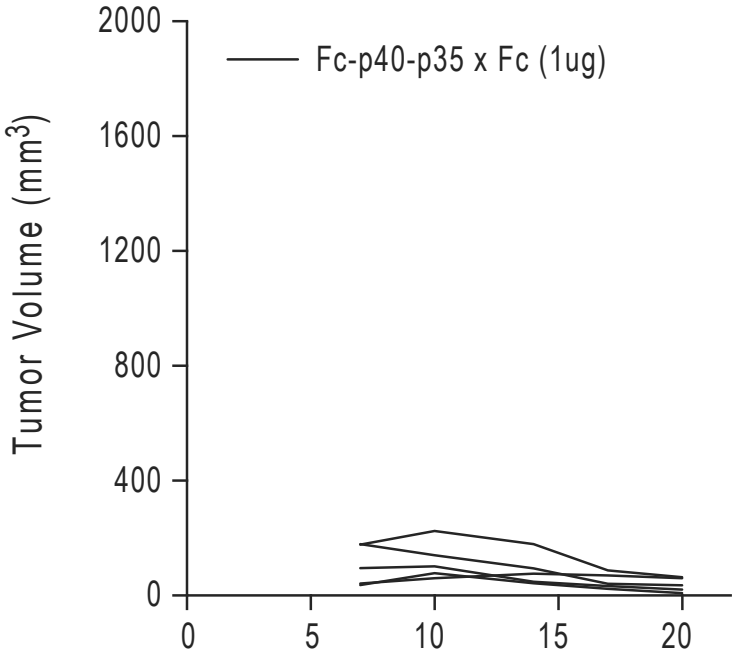


FIG. 38C-4

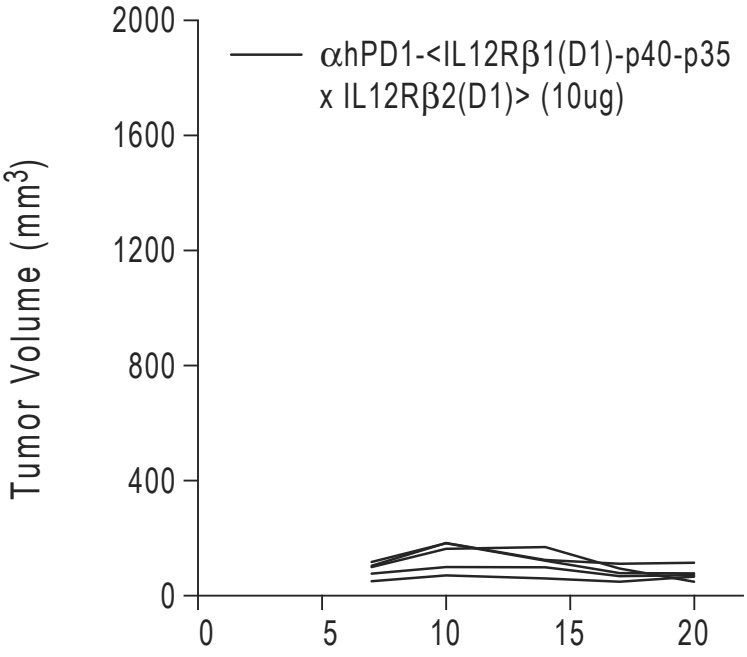


FIG. 38C-5

Monovalent:
Fc-p40-p35 x Fc-αp35Fab

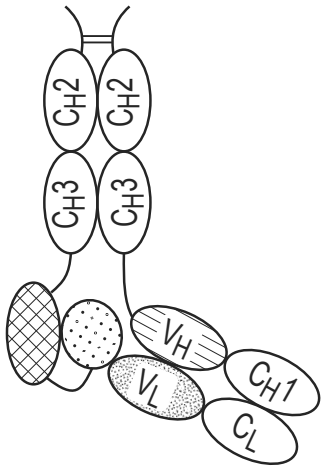


FIG. 39A

Monovalent:
Fc-p40-p35 x Fc-αp40Fab

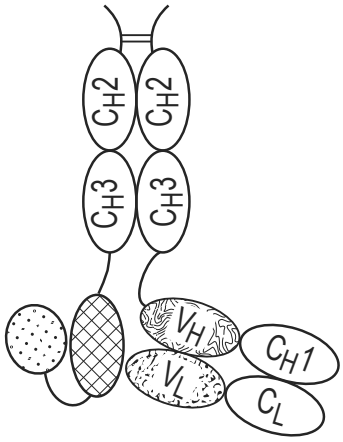


FIG. 39B

Monovalent:
Targeting Moiety-Fc-p40-p35
x Targeting Moiety-Fc-αp35Fab

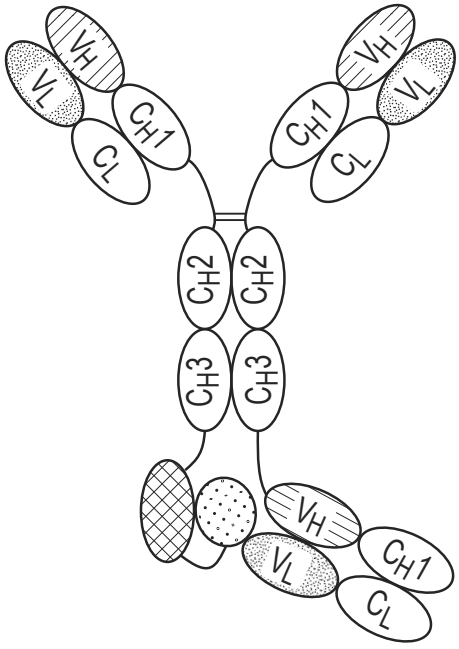


FIG. 39C

Monovalent:
Targeting Moiety-Fc-p40-p35
x Targeting Moiety-Fc-αp40Fab

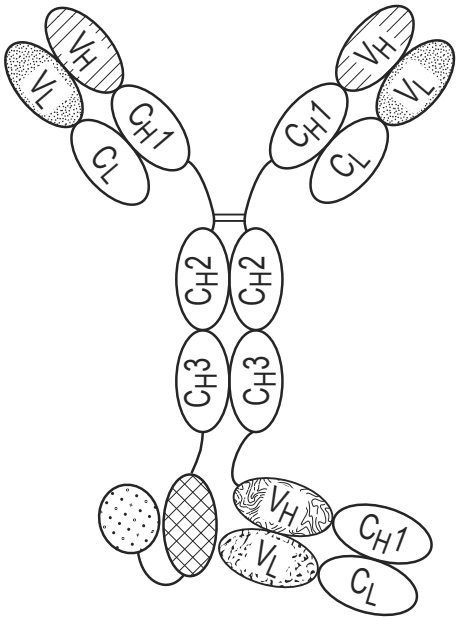


FIG. 39D

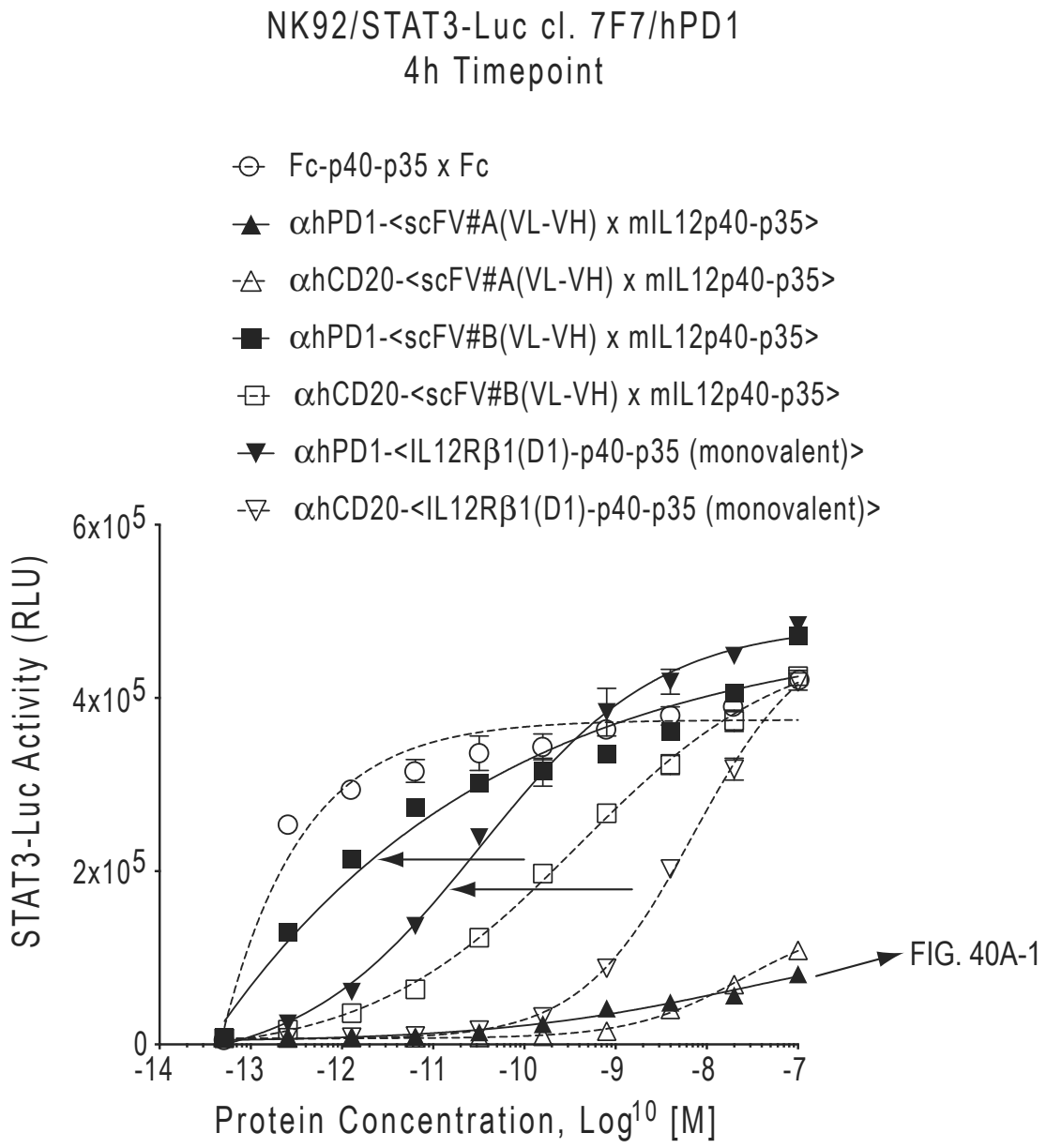

NK92/STAT3-Luc cl. 7F7/hPD1
4h Timepoint
Fc-p40-p35 x Fc
αhPD1-<scFV#A(VL-VH) x mIL12p40-p35>
αhCD20-<scFV#A(VL-VH) x mIL12p40-p35>
αhPD1-<scFV#B(VL-VH) x mIL12p40-p35>
αhCD20-<scFV#B(VL-VH) x mIL12p40-p35>
αhPD1-<IL12Rβ1(D1)-p40-p35 (monovalent)>
αhCD20-<IL12Rβ1(D1)-p40-p35 (monovalent)>
STAT3-Luc Activity (RLU)
6x10^5
4x10^5
2x10^5
0
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration, Log^10 [M]
FIG. 40A-1

FIG. 40A

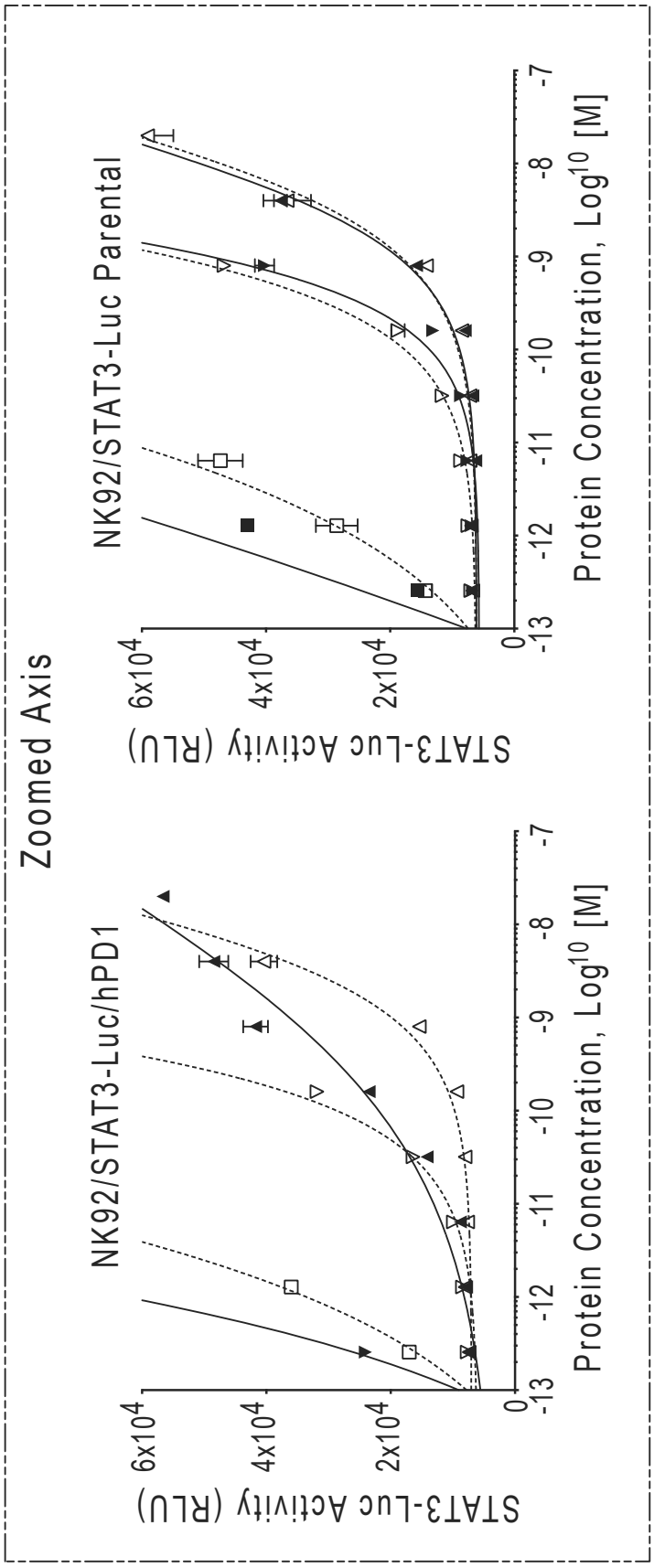

Zoomed Axis
NK92/STAT3-Luc/hPD1
NK92/STAT3-Luc Parental
STAT3-Luc Activity (RLU)
6x10^4
4x10^4
2x10^4
0
-13
-12
-11
-10
-9
-8
-7
Protein Concentration, Log10 [M]

FIG. 40A-1

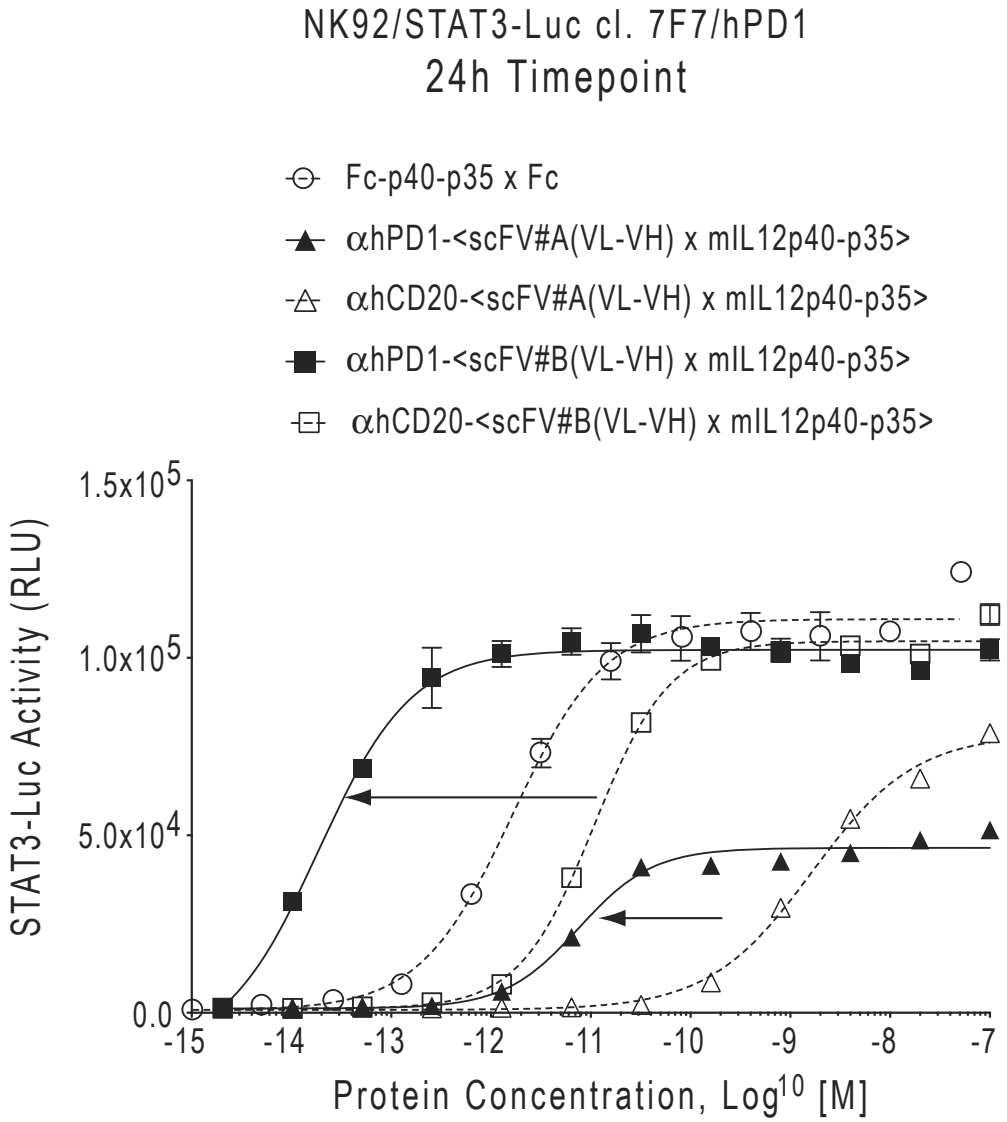

NK92/STAT3-Luc cl. 7F7/hPD1
24h Timepoint
Fc-p40-p35 x Fc
αhPD1-<scFV#A(VL-VH) x mIL12p40-p35>
αhCD20-<scFV#A(VL-VH) x mIL12p40-p35>
αhPD1-<scFV#B(VL-VH) x mIL12p40-p35>
αhCD20-<scFV#B(VL-VH) x mIL12p40-p35>
STAT3-Luc Activity (RLU)
1.5x10^5
1.0x10^5
5.0x10^4
0.0
-15
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration, Log10 [M]

FIG. 40B

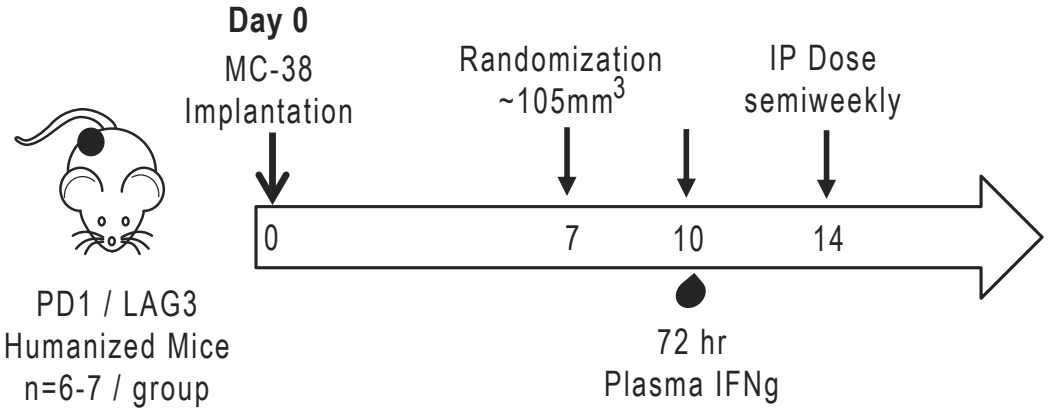


FIG. 41A

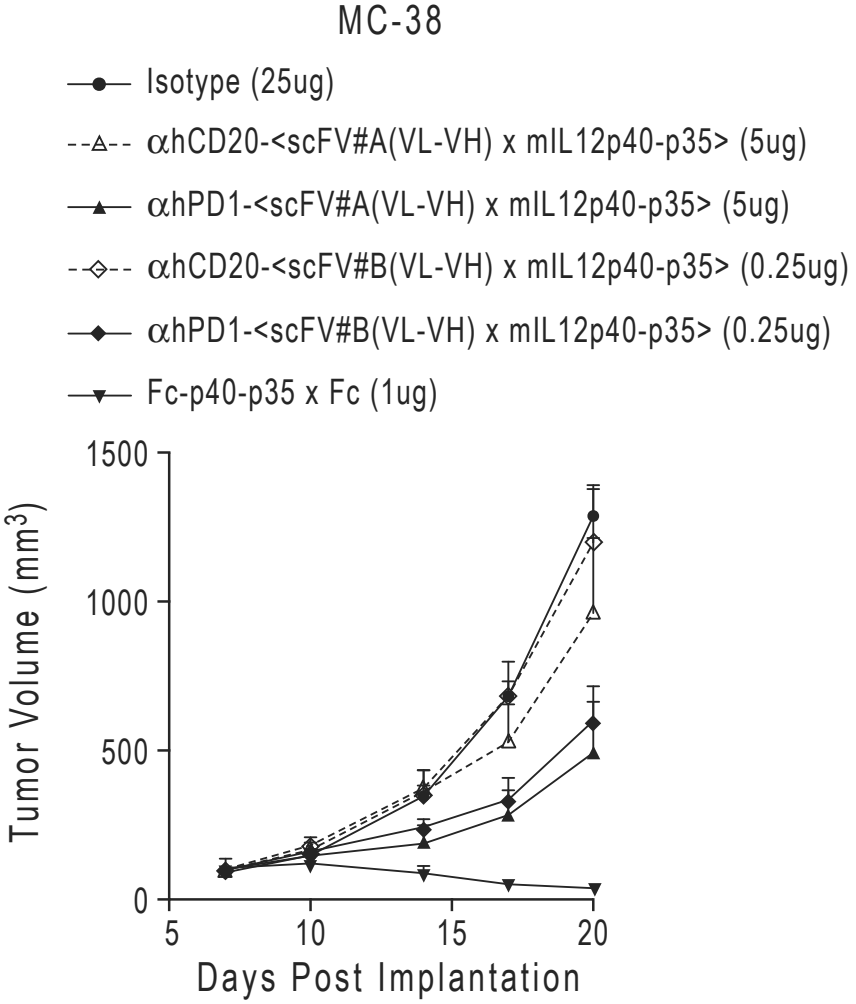


FIG. 41B

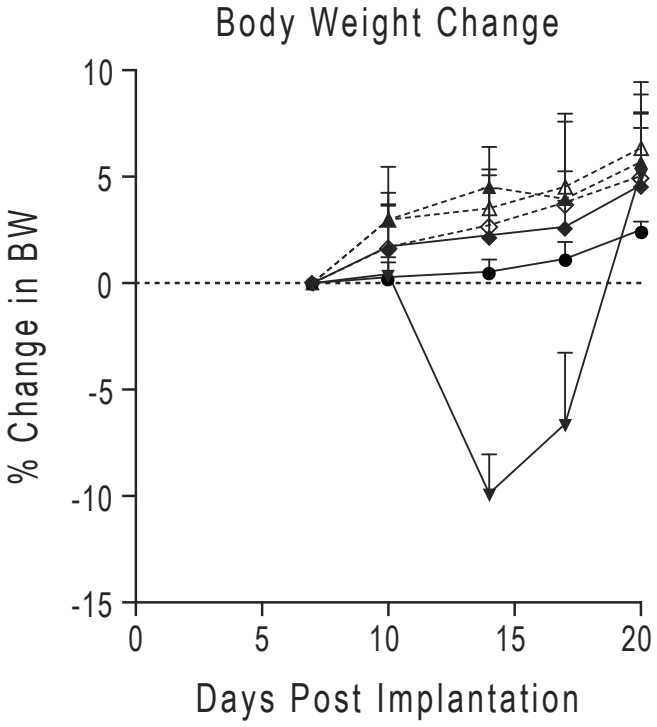

Body Weight Change
% Change in BW
10
5
0
-5
-10
-15
0
5
10
15
20
Days Post Implantation

FIG. 41C

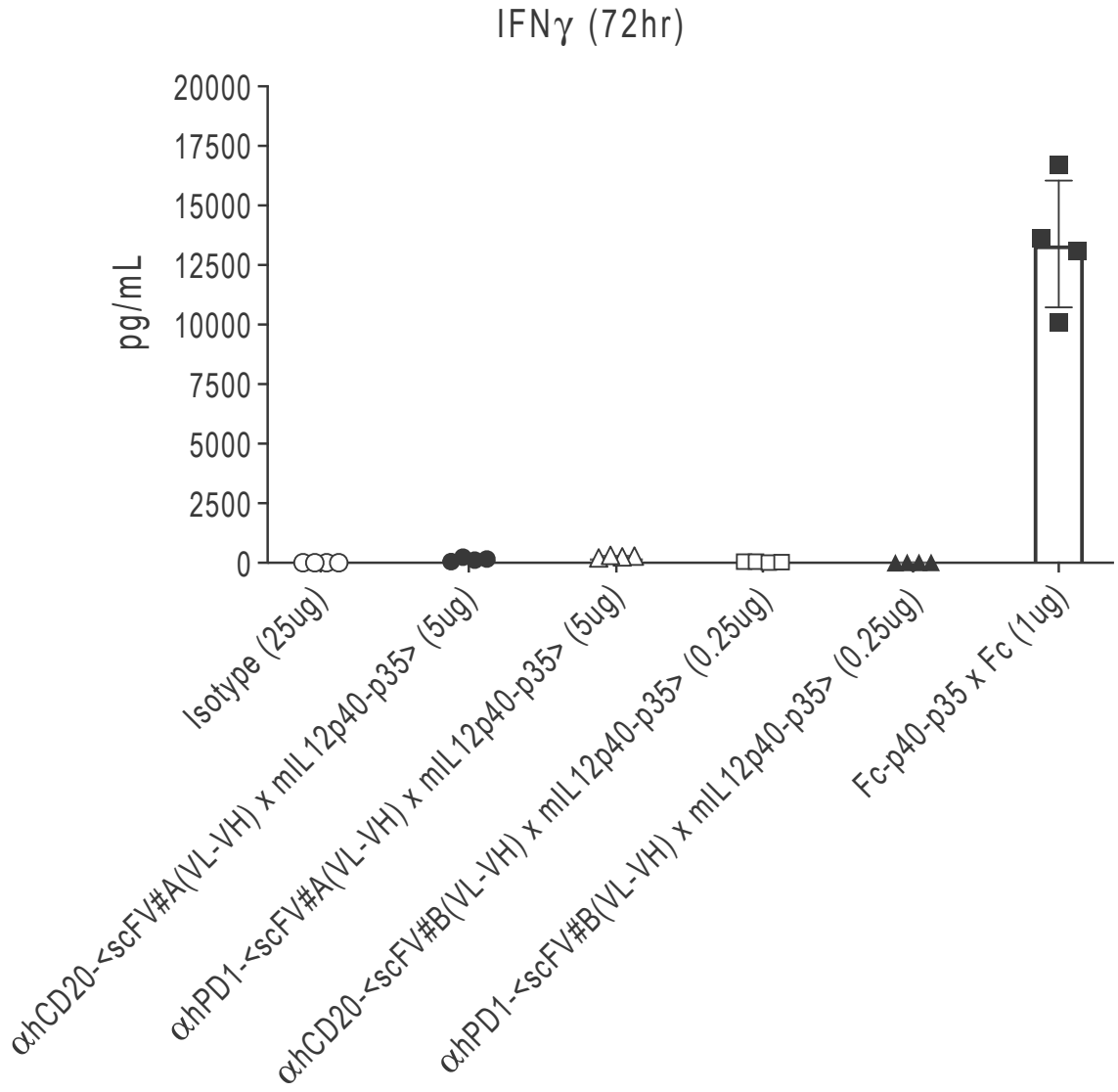

IFNγ (72hr)
pg/mL
20000
17500
15000
12500
10000
7500
5000
2500
0
Isotype (25ug)
αhCD20-<scFV#A(VL-VH) x mIL12p40-p35> (5ug)
αhPD1-<scFV#A(VL-VH) x mIL12p40-p35> (5ug)
αhCD20-<scFV#B(VL-VH) x mIL12p40-p35> (0.25ug)
αhPD1-<scFV#B(VL-VH) x mIL12p40-p35> (0.25ug)
Fc-p40-p35 x Fc (1ug)

FIG. 41D

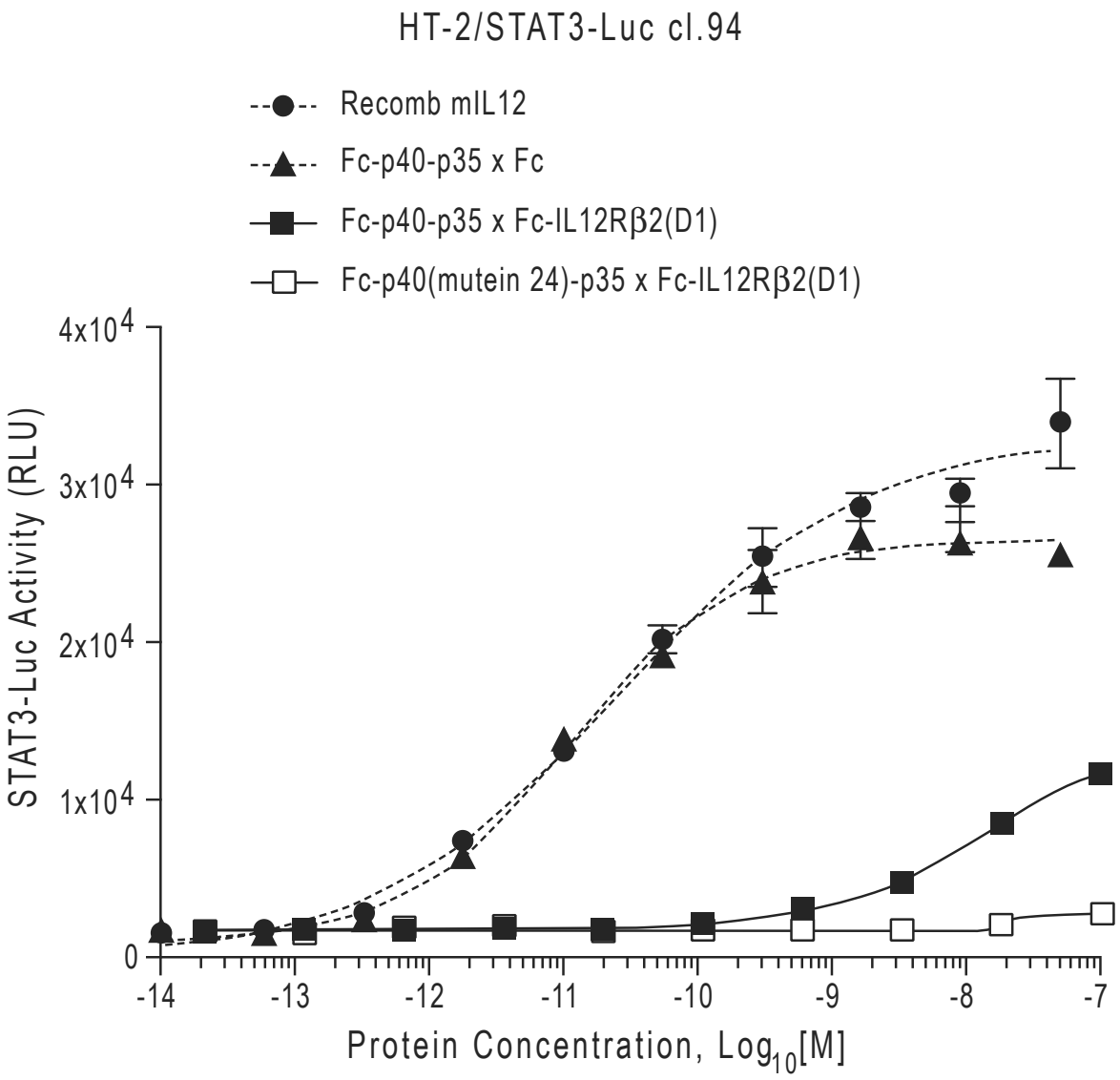

HT-2/STAT3-Luc cl.94
Recomb mIL12
Fc-p40-p35 x Fc
Fc-p40-p35 x Fc-IL12Rβ2(D1)
Fc-p40(mutein 24)-p35 x Fc-IL12Rβ2(D1)
STAT3-Luc Activity (RLU)
4x10^4
3x10^4
2x10^4
1x10^4
0
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration, Log10[M]

FIG. 42

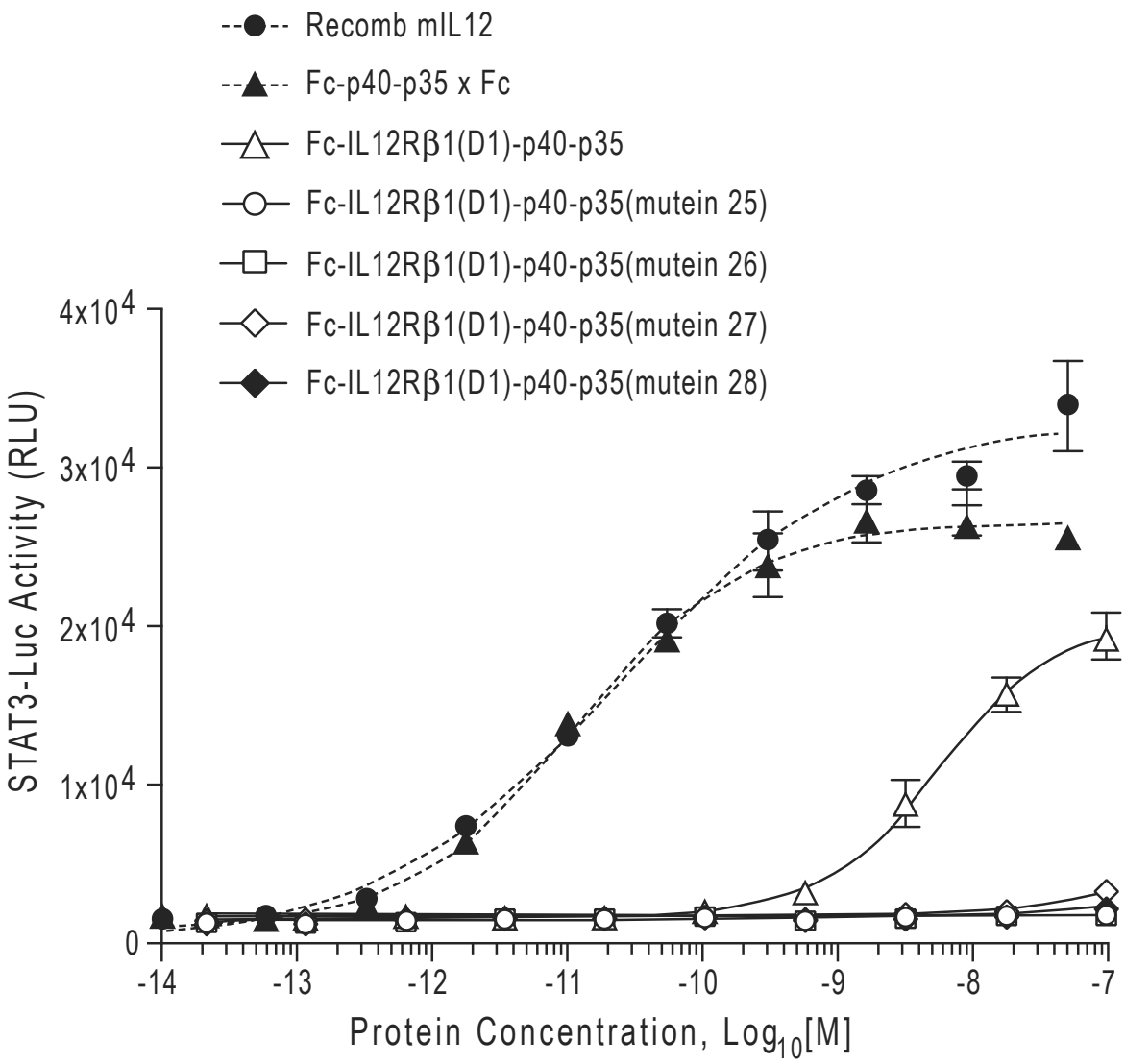

HT-2/STAT3-Luc cl.94
Recomb mIL12
Fc-p40-p35 x Fc
Fc-IL12Rβ1(D1)-p40-p35
Fc-IL12Rβ1(D1)-p40-p35(mutein 25)
Fc-IL12Rβ1(D1)-p40-p35(mutein 26)
Fc-IL12Rβ1(D1)-p40-p35(mutein 27)
Fc-IL12Rβ1(D1)-p40-p35(mutein 28)
STAT3-Luc Activity (RLU)
4x10^4
3x10^4
2x10^4
1x10^4
0
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration, Log10[M]

FIG. 43

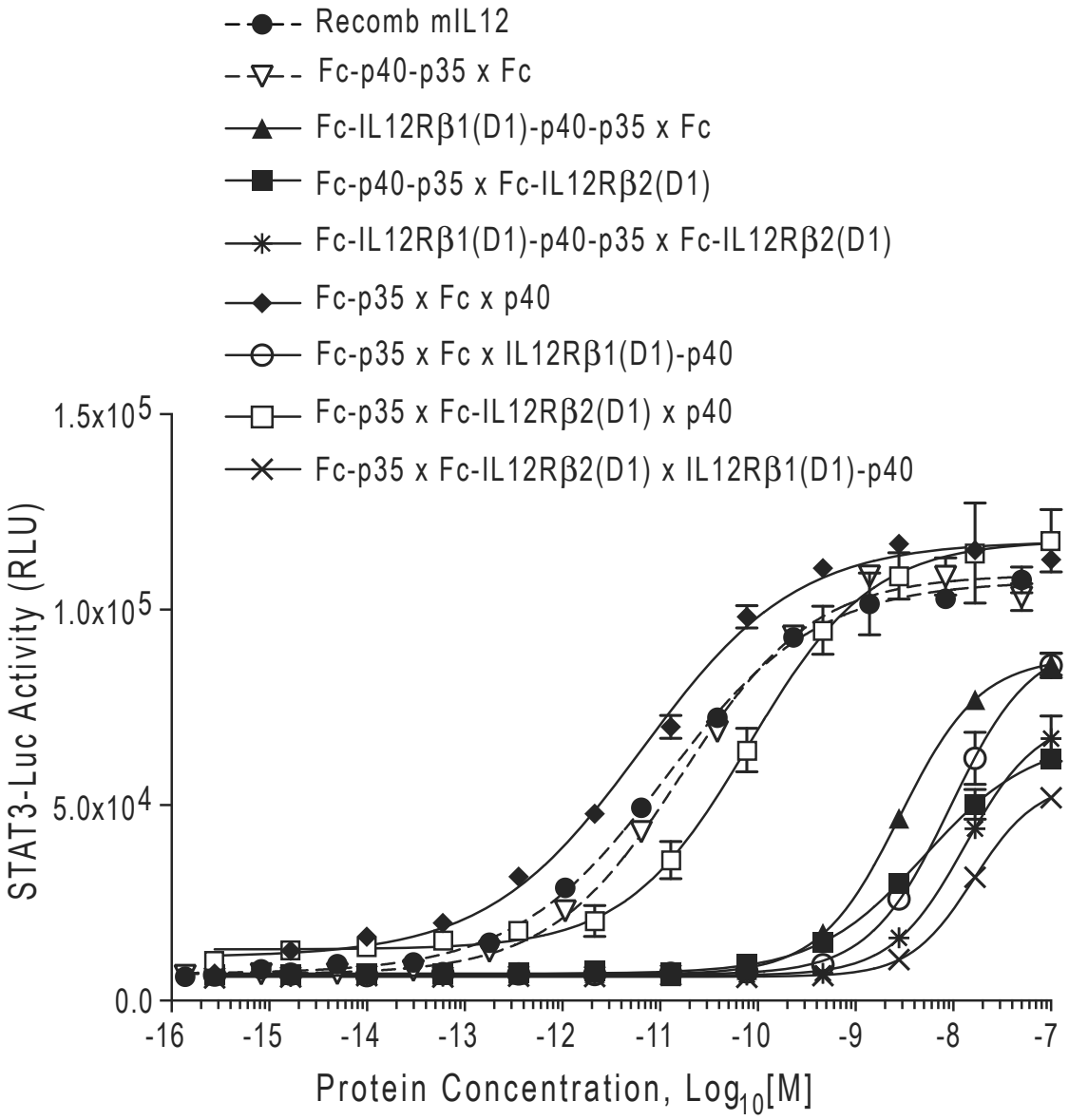

HT-2/STAT3-Luc cl.94
Recomb mIL12
Fc-p40-p35 x Fc
Fc-IL12Rβ1(D1)-p40-p35 x Fc
Fc-p40-p35 x Fc-IL12Rβ2(D1)
Fc-IL12Rβ1(D1)-p40-p35 x Fc-IL12Rβ2(D1)
Fc-p35 x Fc x p40
Fc-p35 x Fc x IL12Rβ1(D1)-p40
Fc-p35 x Fc-IL12Rβ2(D1) x p40
Fc-p35 x Fc-IL12Rβ2(D1) x IL12Rβ1(D1)-p40
STAT3-Luc Activity (RLU)
1.5x10^5
1.0x10^5
5.0x10^4
0.0
-16
-15
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration, Log10[M]

FIG. 44

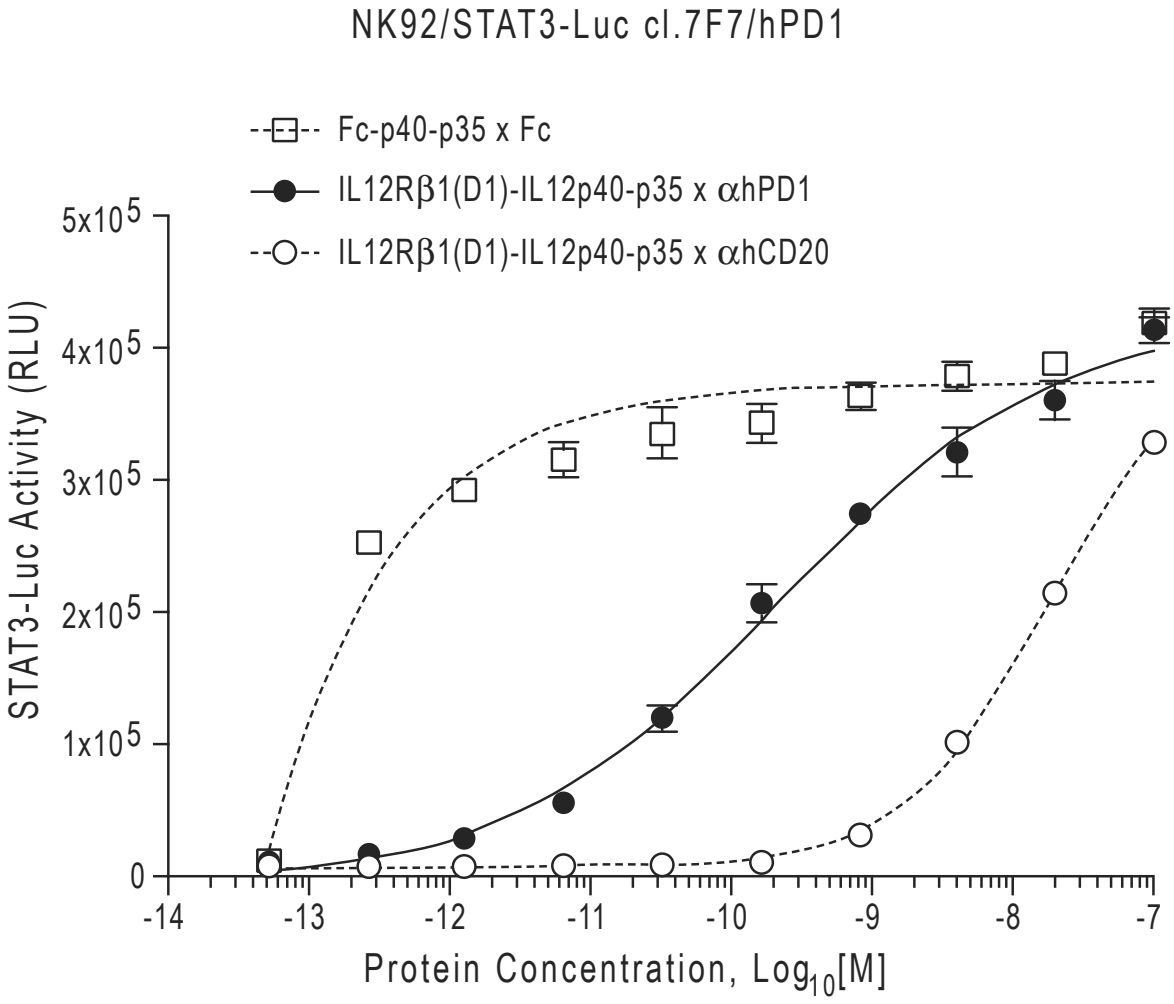

NK92/STAT3-Luc cl.7F7/hPD1
Fc-p40-p35 x Fc
IL12Rβ1(D1)-IL12p40-p35 x αhPD1
IL12Rβ1(D1)-IL12p40-p35 x αhCD20
STAT3-Luc Activity (RLU)
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
-14
-13
-12
-11
-10
-9
-8
-7
Protein Concentration, Log10[M]

FIG. 45

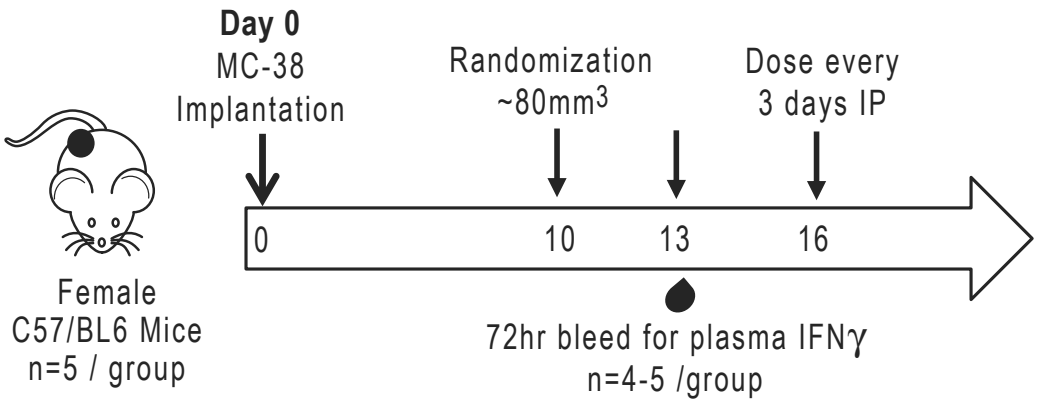


FIG. 46A

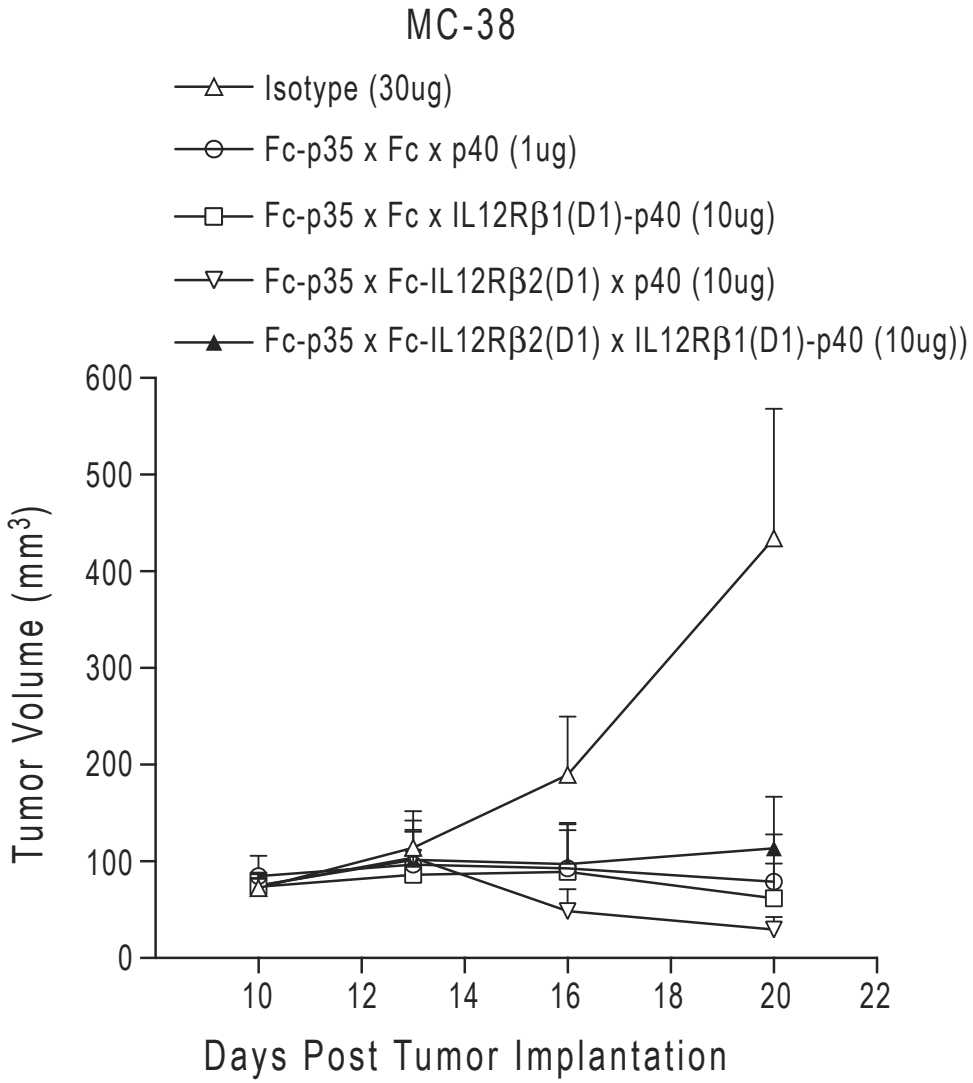


FIG. 46B

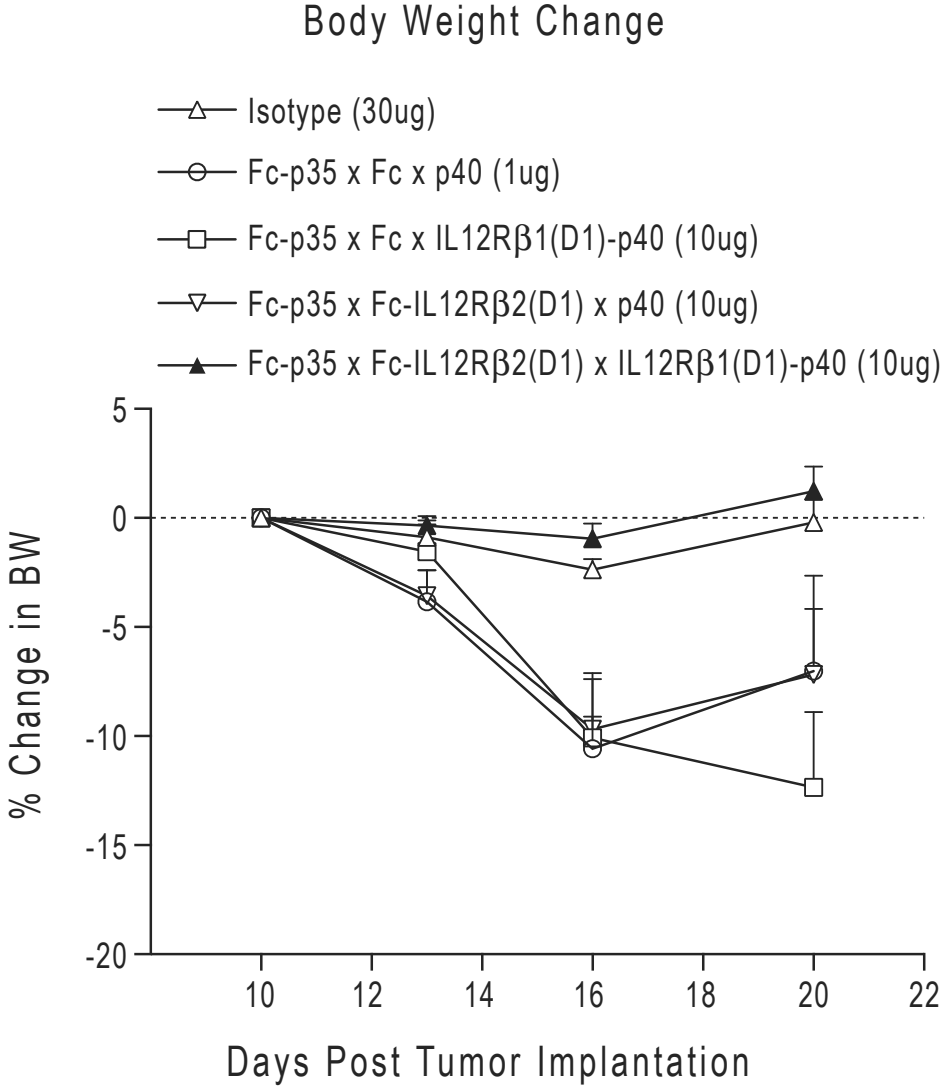

Body Weight Change
Isotype (30ug)
Fc-p35 x Fc x p40 (1ug)
Fc-p35 x Fc x IL12Rβ1(D1)-p40 (10ug)
Fc-p35 x Fc-IL12Rβ2(D1) x p40 (10ug)
Fc-p35 x Fc-IL12Rβ2(D1) x IL12Rβ1(D1)-p40 (10ug)
% Change in BW
5
0
-5
-10
-15
-20
10
12
14
16
18
20
22
Days Post Tumor Implantation

FIG. 46C

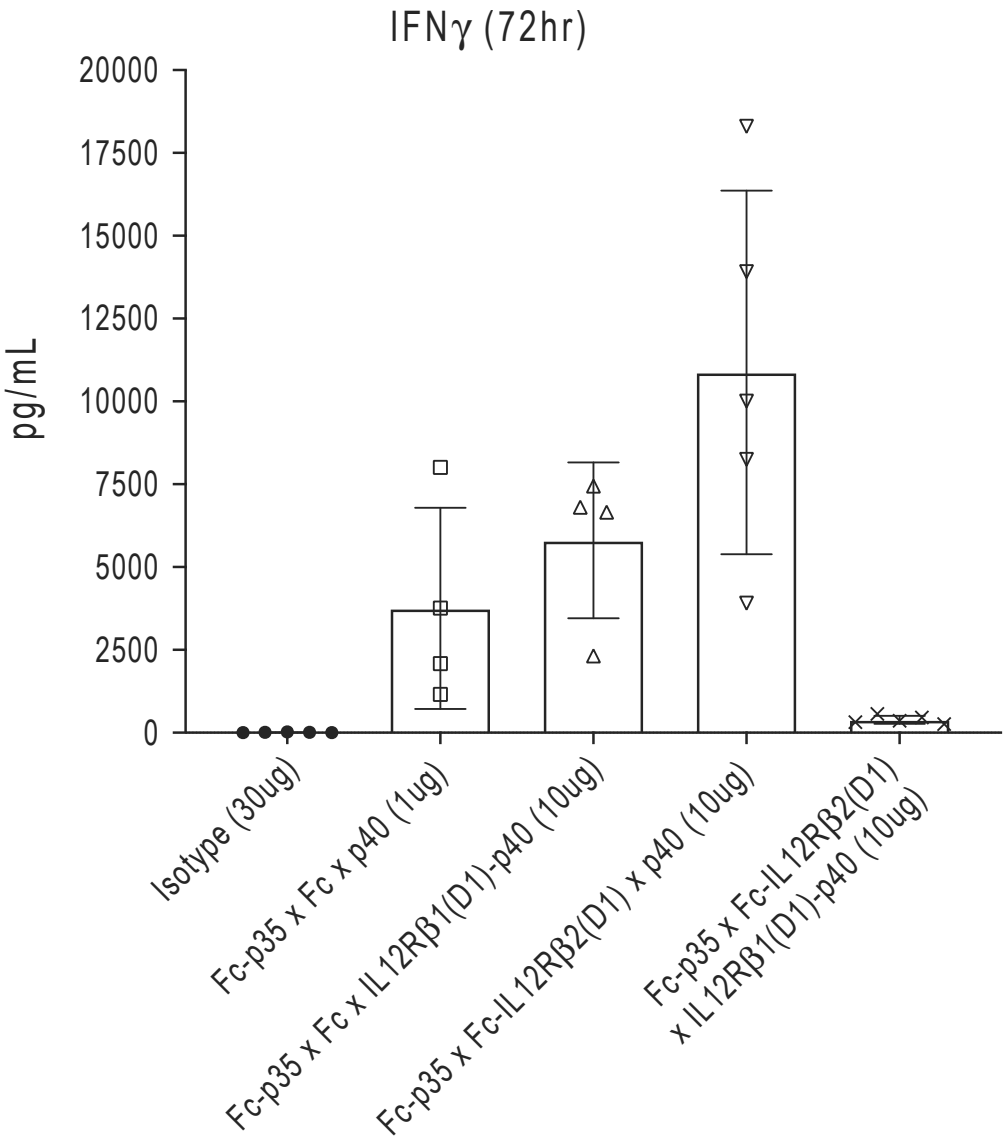

IFNγ (72hr)
pg/mL
20000
17500
15000
12500
10000
7500
5000
2500
0
Isotype (30ug)
Fc-p35 x Fc x p40 (1ug)
Fc-p35 x Fc x IL12Rβ1(D1)-p40 (10ug)
Fc-p35 x Fc-IL12Rβ2(D1) x p40 (10ug)
Fc-p35 x Fc-IL12Rβ2(D1) x IL12Rβ1(D1)-p40 (10ug)

FIG. 46D