FIG. 1

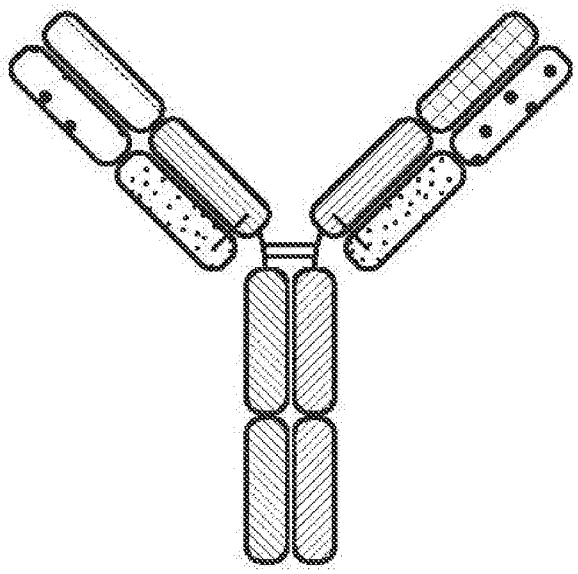


FIG. 2

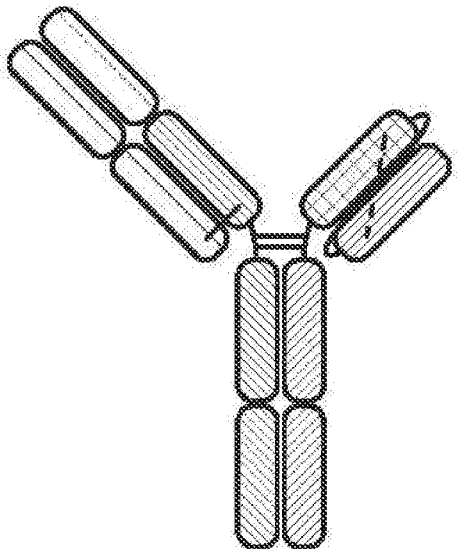


FIG. 3

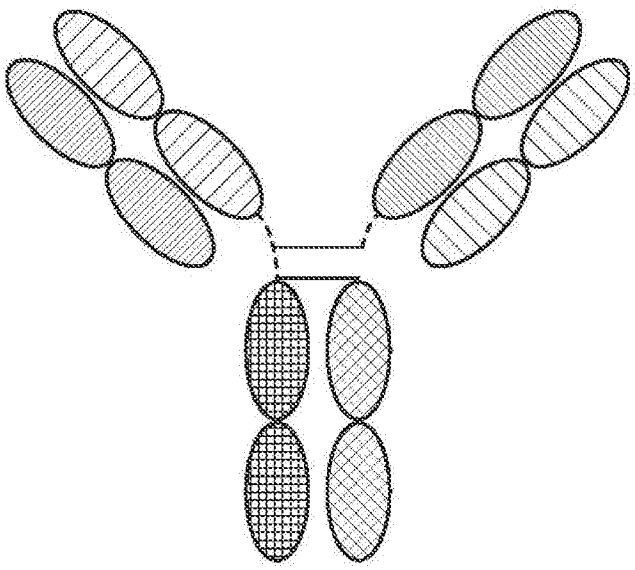


FIG. 4

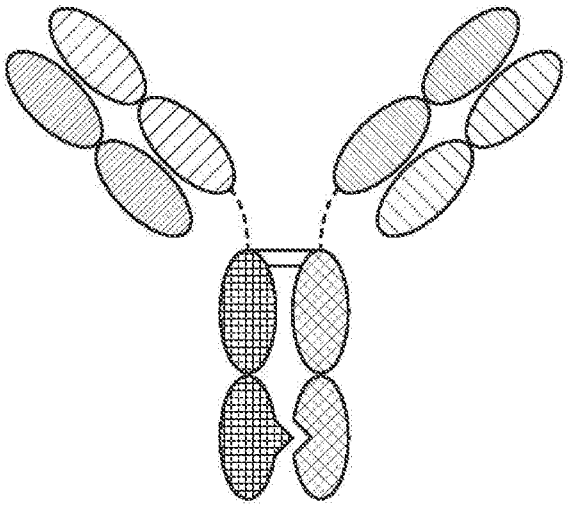


FIG. 5

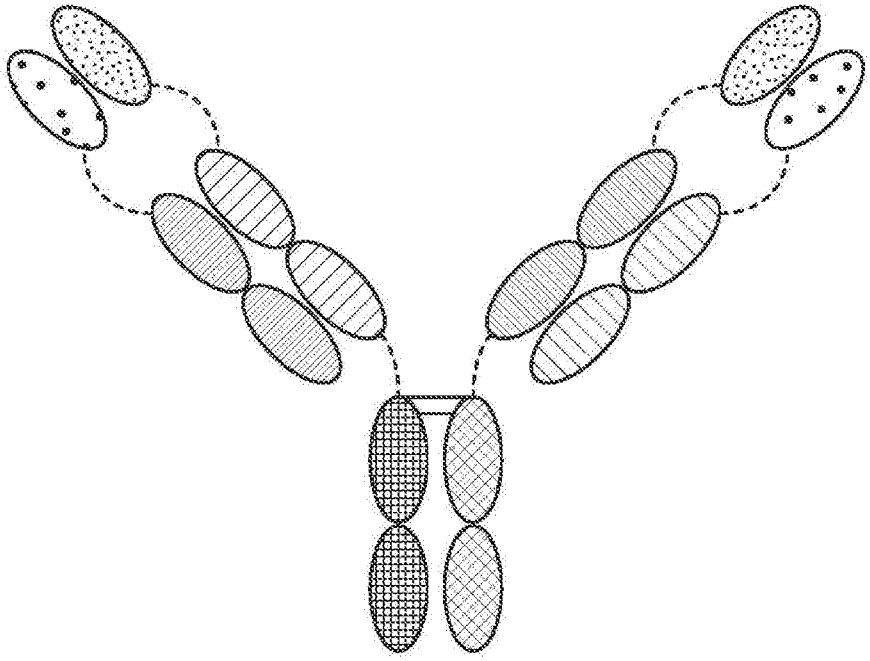


FIG. 6

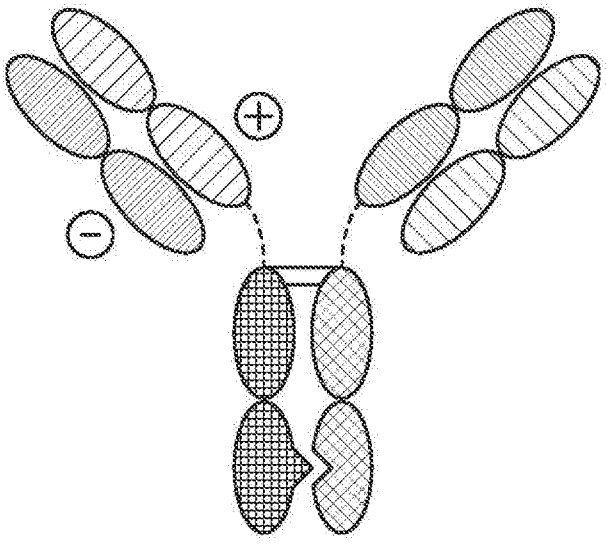


FIG. 7

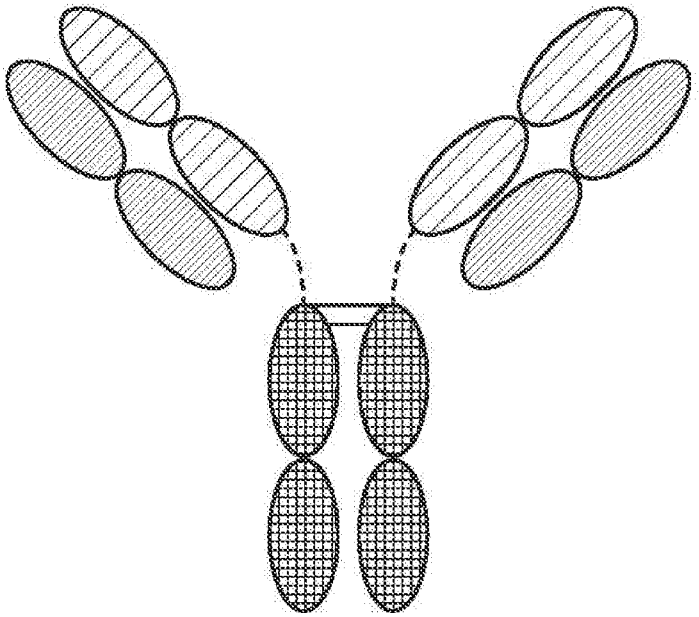


FIG. 8

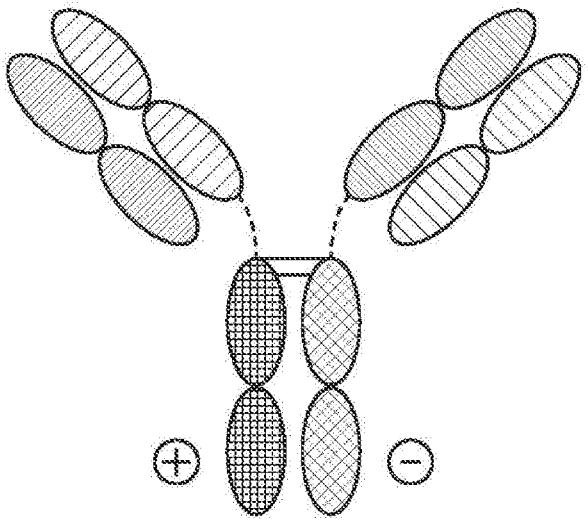


FIG. 9

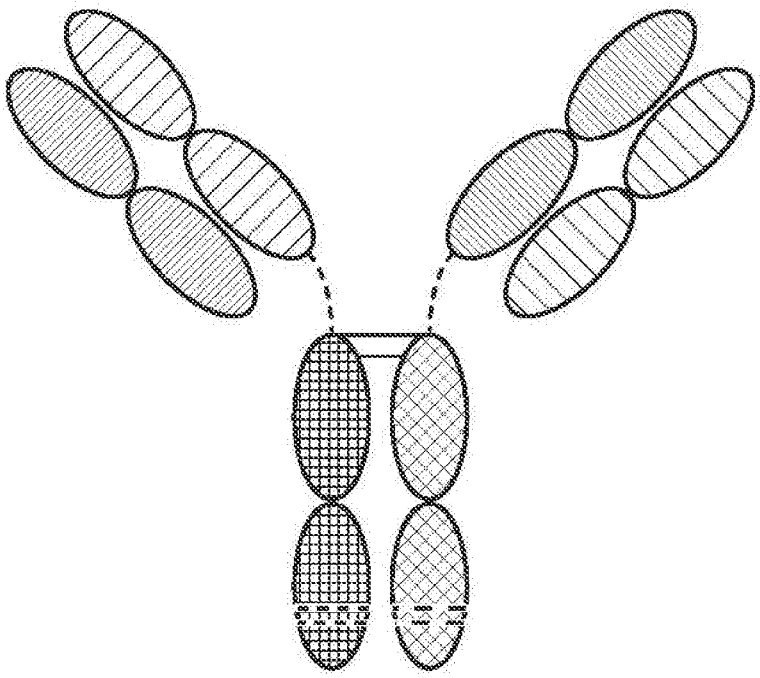


FIG. 10

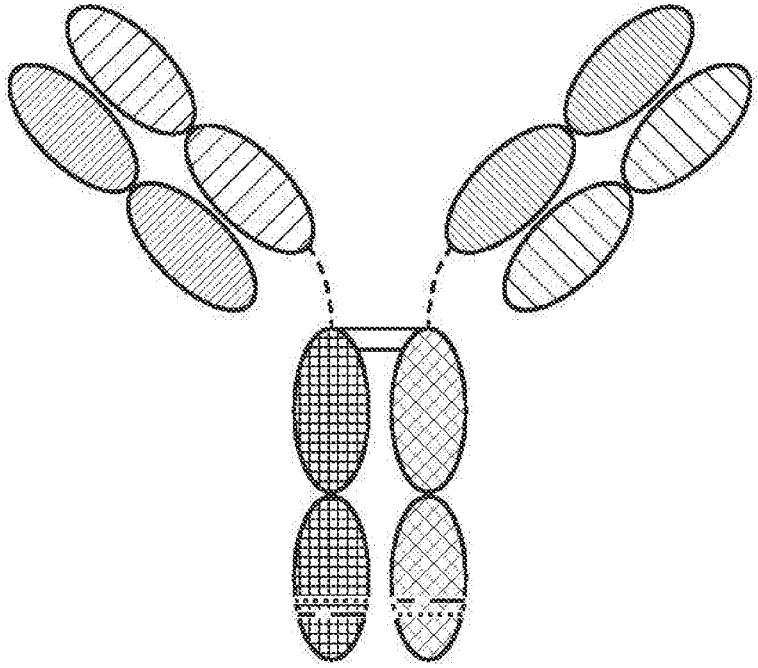


FIG. 11

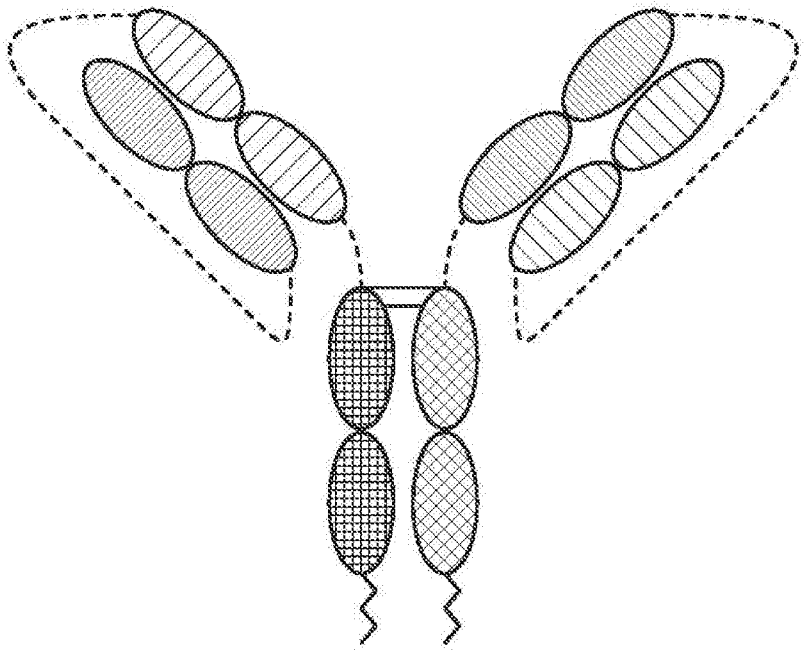


FIG. 12

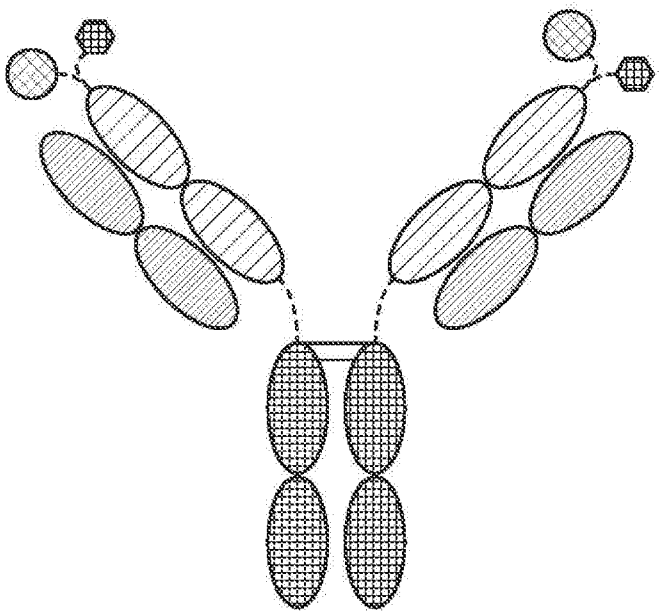


FIG. 13A

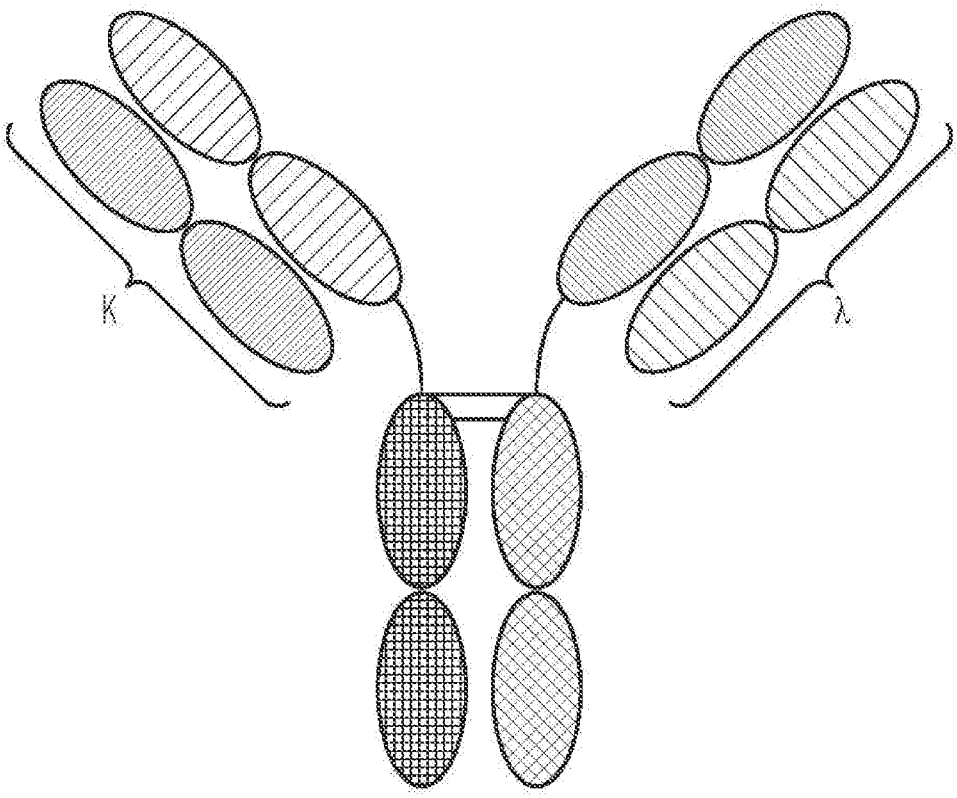


FIG. 13B

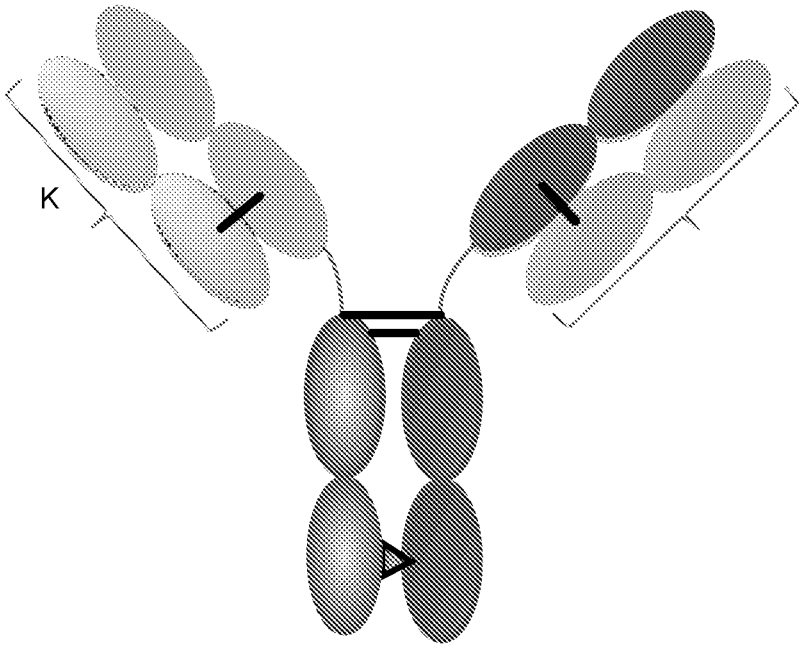


FIG. 14

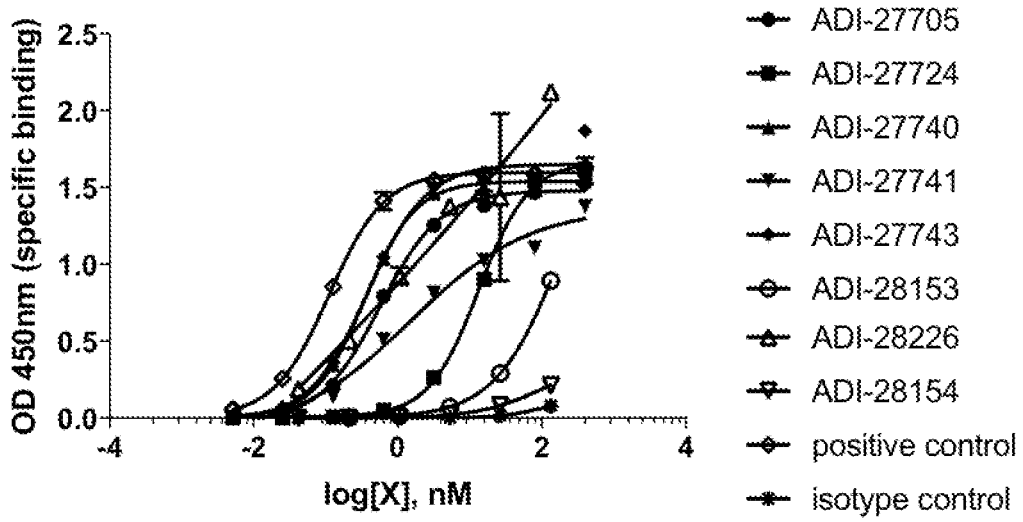

OD 450nm (specific binding)
2.5
2.0
1.5
1.0
0.5
0.0
-4
-2
0
2
4
log[X], nM
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
positive control
isotype control

FIG. 15

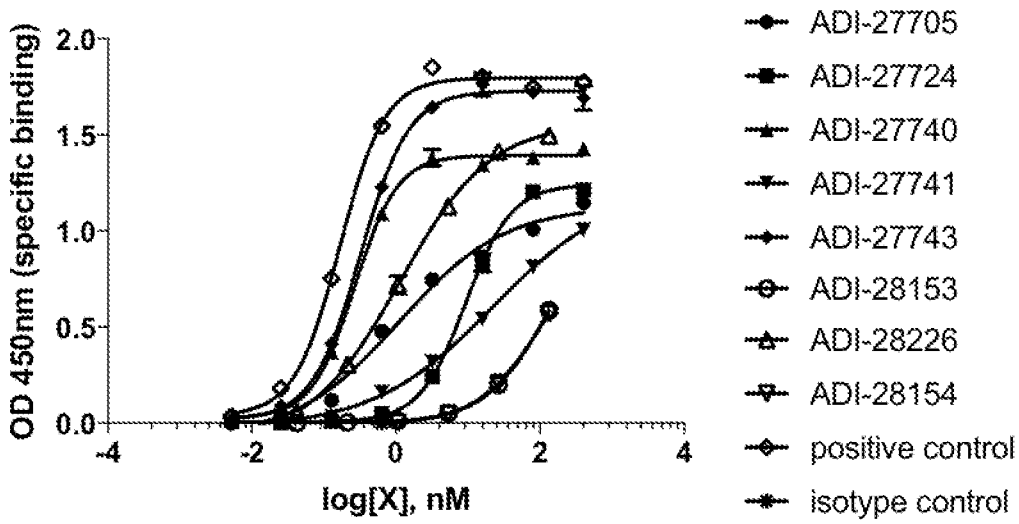

OD 450nm (specific binding)
2.0
1.5
1.0
0.5
0.0
-4
-2
0
2
4
log[X], nM
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
positive control
isotype control

FIG. 16

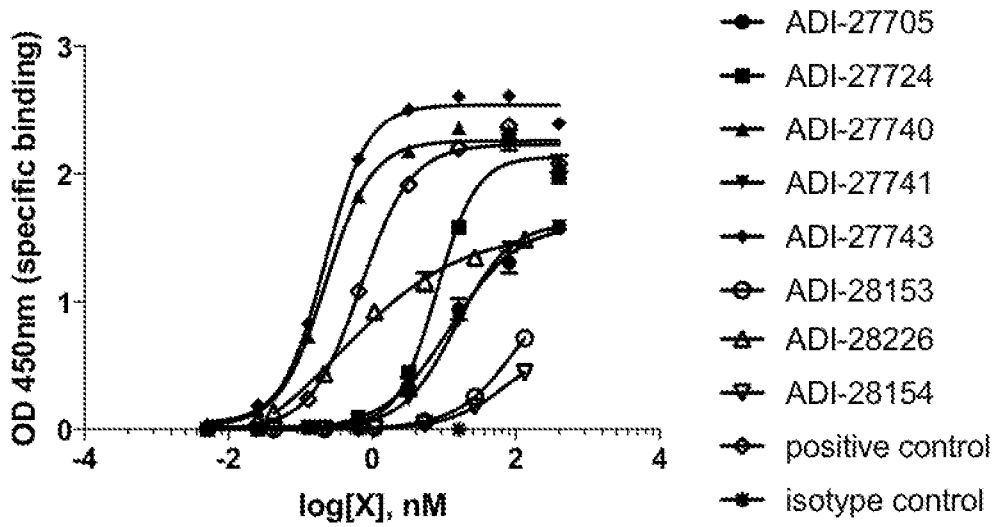

OD 450nm (specific binding)
3
2
1
0
-4
-2
0
2
4
log[X], nM
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
positive control
isotype control

FIG. 17

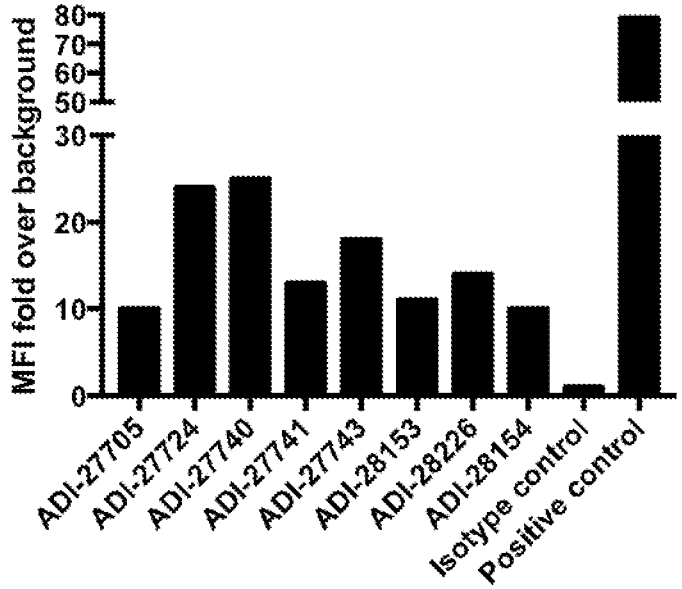

MFI fold over background
80
70
60
50
30
20
10
0
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 18

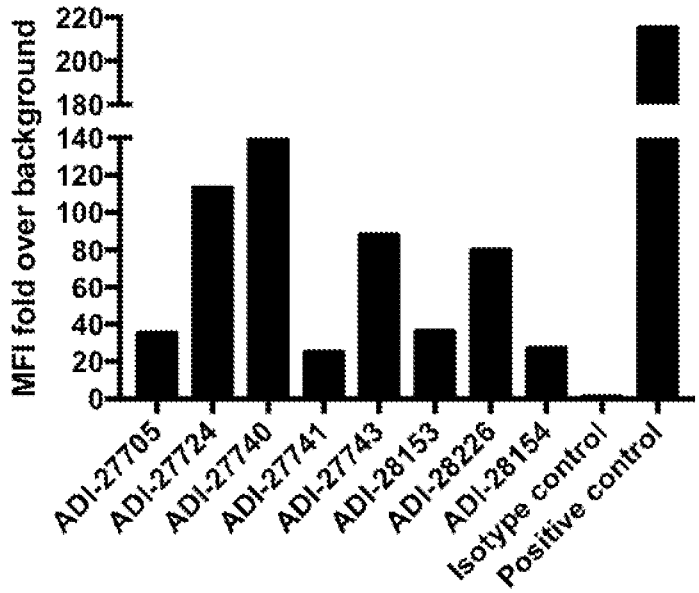

MFI fold over background
220
200
180
140
120
100
80
60
40
20
0
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 19

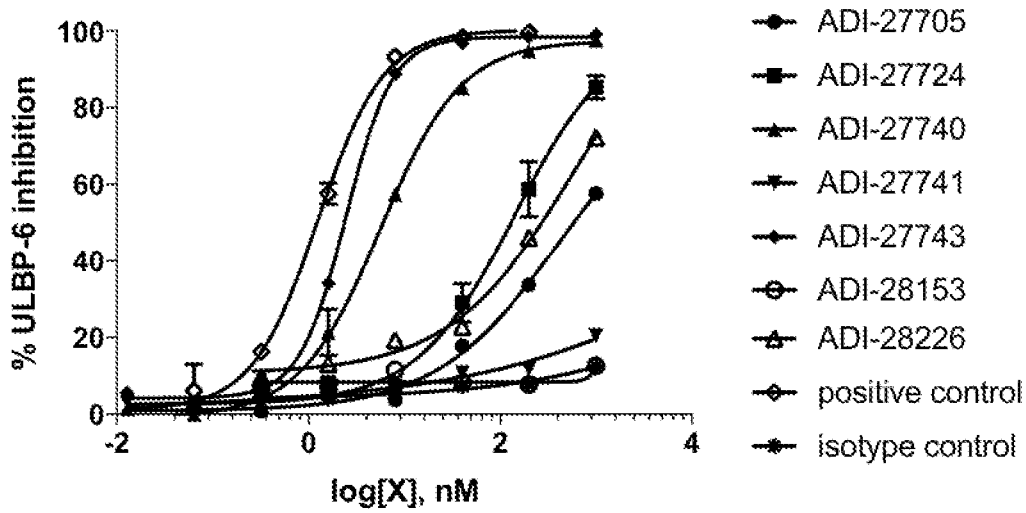

% ULBP-6 inhibition
100
80
60
40
20
0
-2
0
2
4
log[X], nM
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
positive control
isotype control

FIG. 20

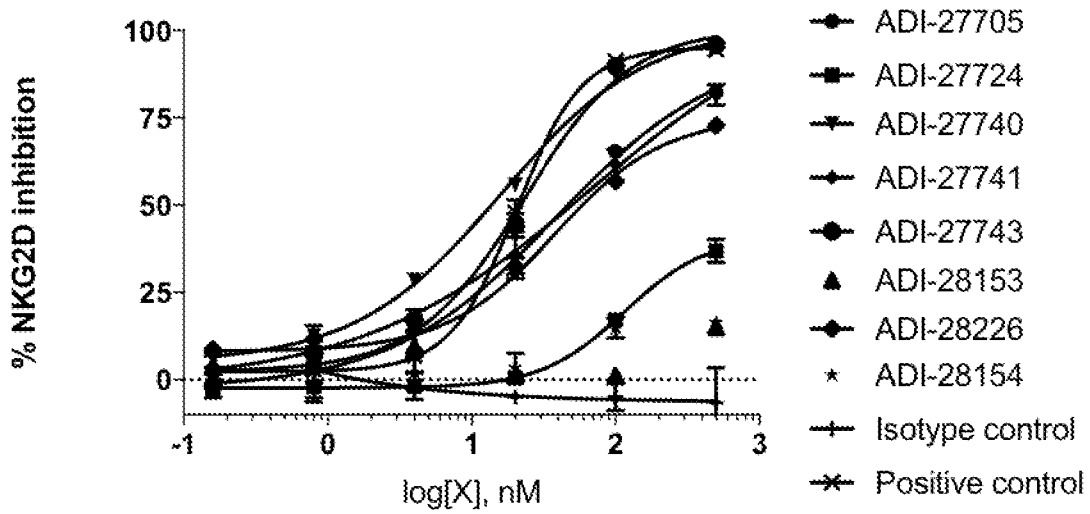

% NKG2D inhibition
100
75
50
25
0
-1
0
1
2
3
log[X], nM
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 21

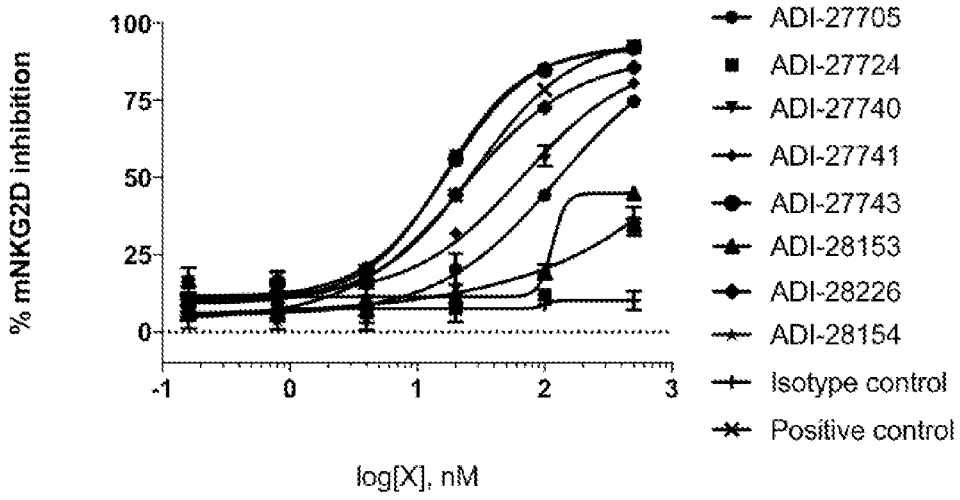

% mNKG2D inhibition
100
75
50
25
0
-1
0
1
2
3
log[X], nM
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 22

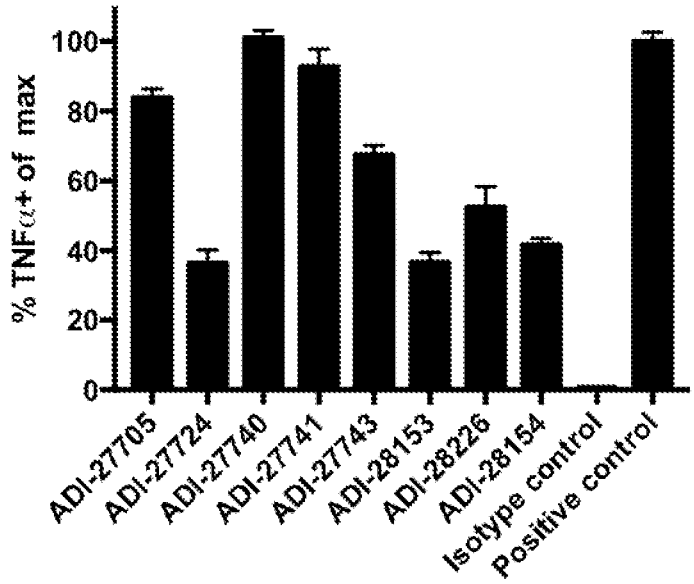

% TNFα+ of max
100
80
60
40
20
0
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 23

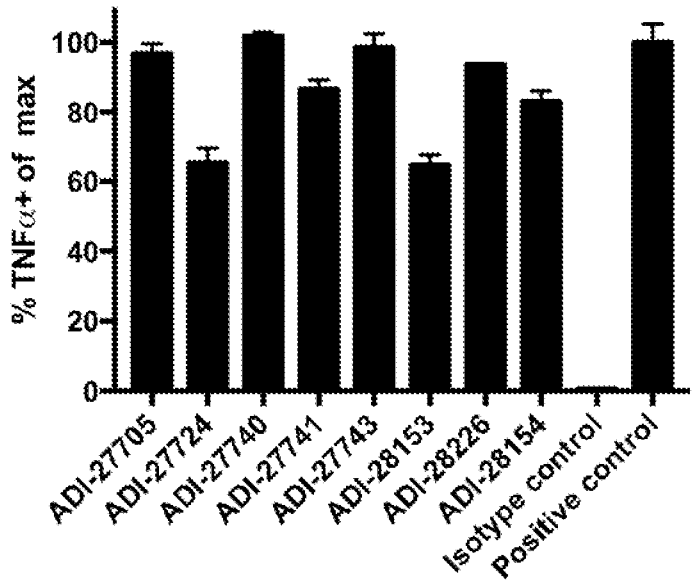

% TNFα+ of max
100
80
60
40
20
0
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 24

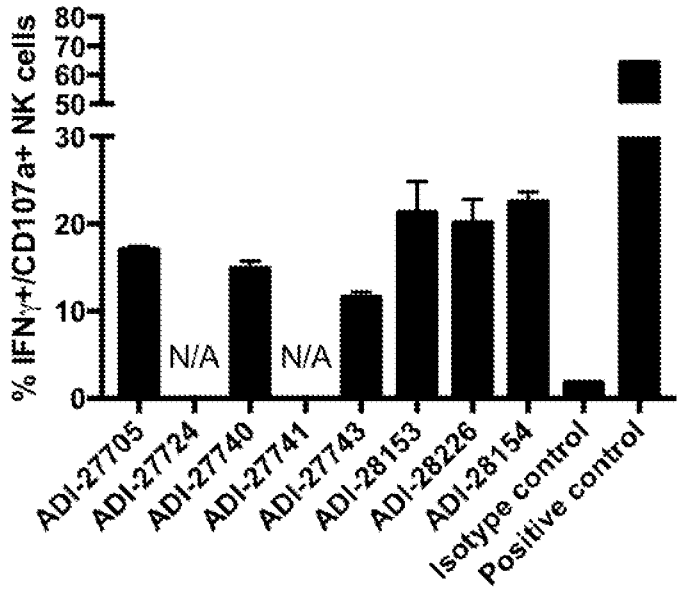

% IFNγ+/CD107a+ NK cells
80
70
60
50
30
20
10
0
N/A
N/A
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 25

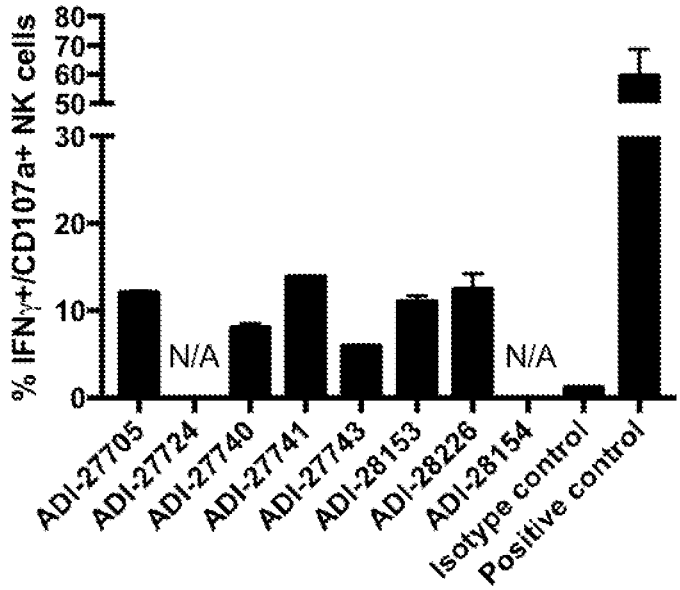

% IFNγ+/CD107a+ NK cells
80
70
60
50
30
20
10
0
N/A
N/A
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 26

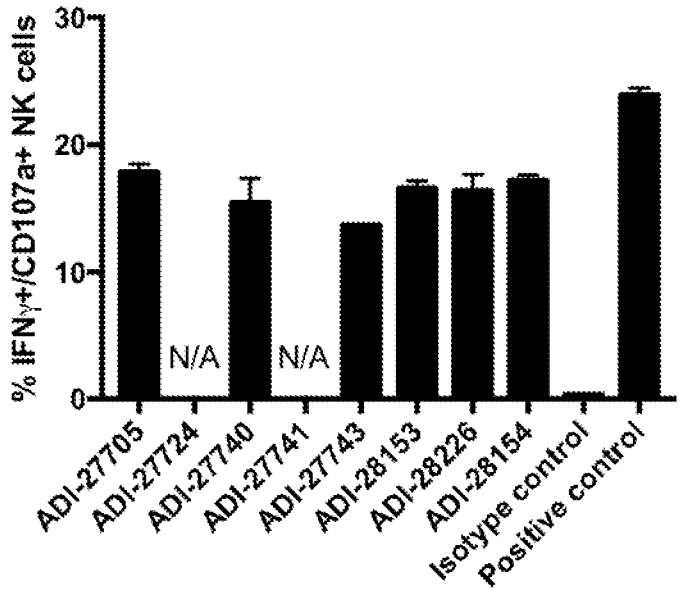

% IFNγ+/CD107a+ NK cells
30
20
10
0
N/A
N/A
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 27

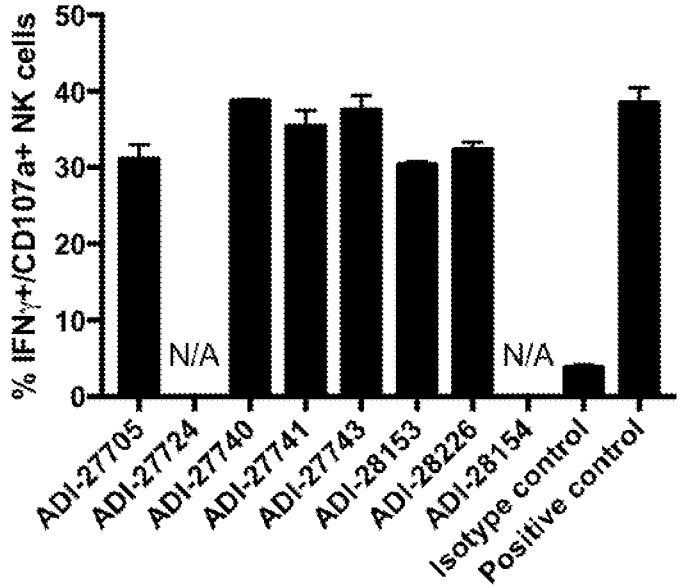

% IFNγ+/CD107a+ NK cells
50
40
30
20
10
0
N/A
N/A
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 28

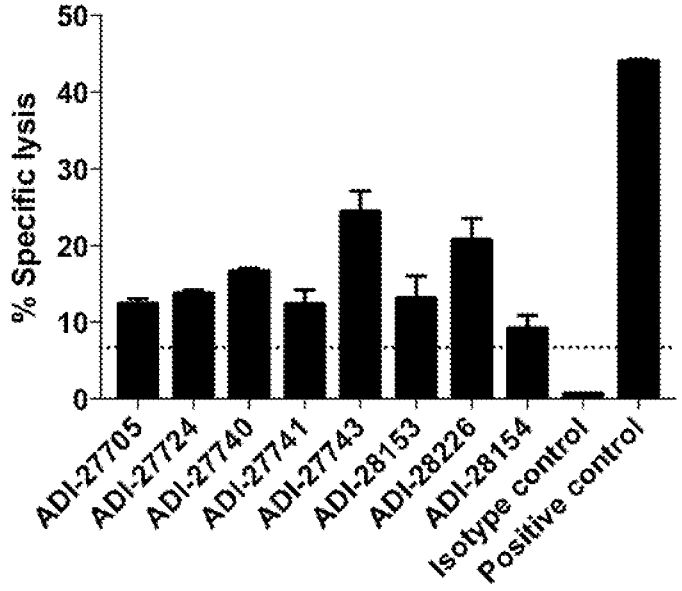

% Specific lysis
50
40
30
20
10
0
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154
Isotype control
Positive control

FIG. 29

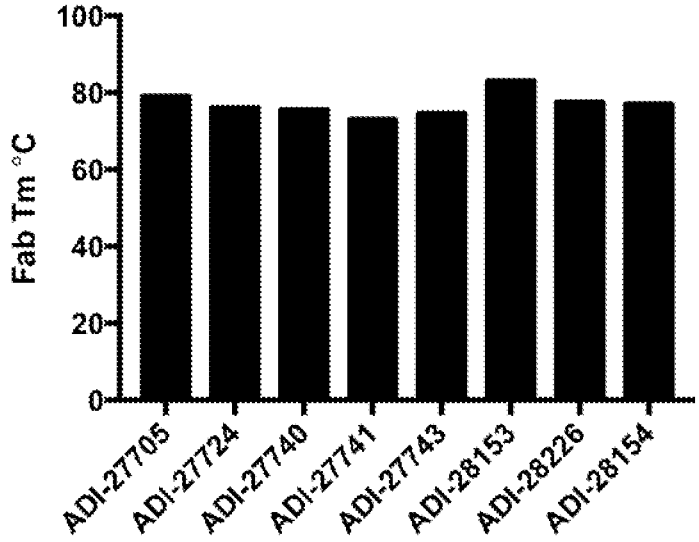

Fab Tm °C
100
80
60
40
20
0
ADI-27705
ADI-27724
ADI-27740
ADI-27741
ADI-27743
ADI-28153
ADI-28226
ADI-28154

FIG. 30

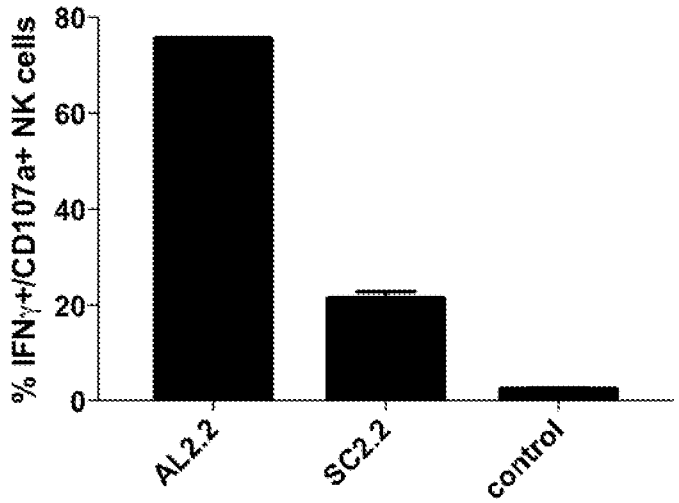

% IFNγ+/CD107a+ NK cells
80
60
40
20
0
AL2.2
SC2.2
control

FIG. 31

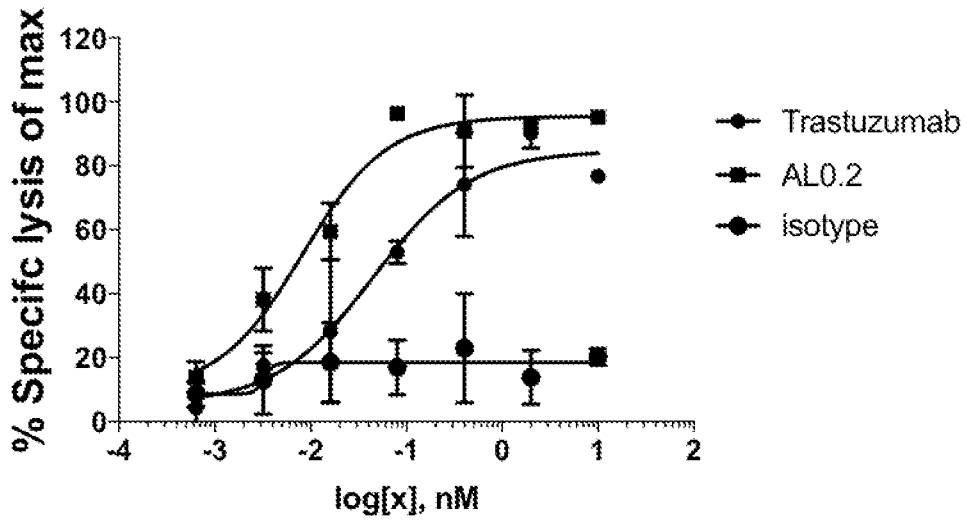

% Specifc lysis of max
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
log[x], nM
Trastuzumab
AL0.2
isotype

FIG. 32

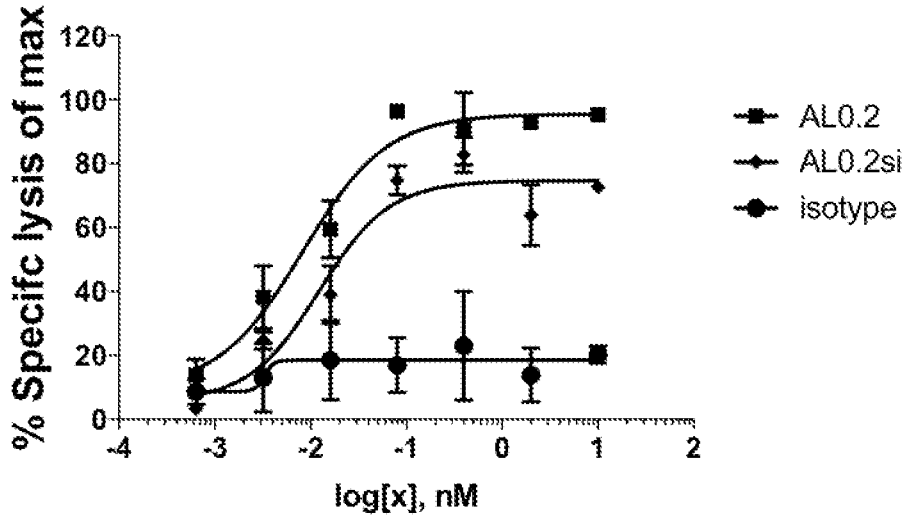

% Specifc lysis of max
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
log[x], nM
AL0.2
AL0.2si
isotype

FIG. 33

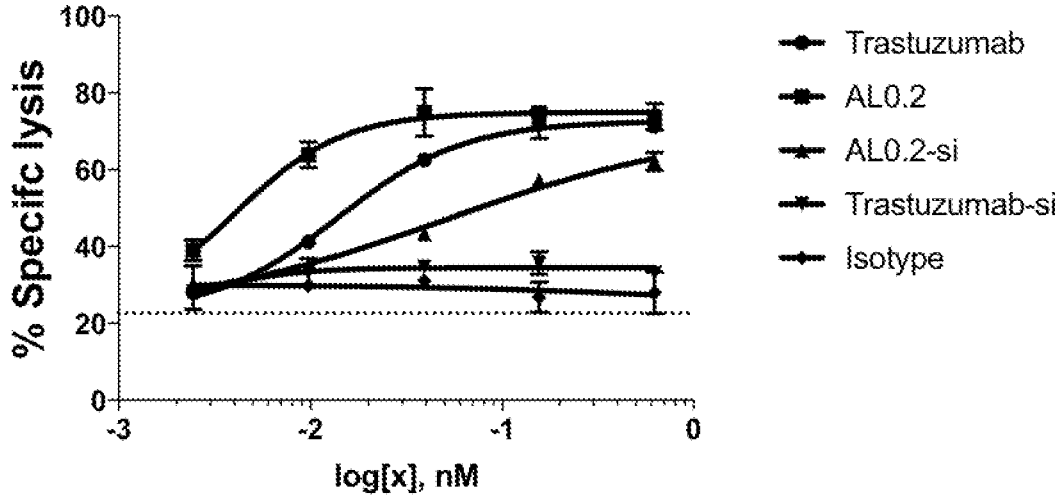

% Specifc lysis
100
80
60
40
20
0
-3
-2
-1
0
log[x], nM
Trastuzumab
AL0.2
AL0.2-si
Trastuzumab-si
Isotype

FIG. 34

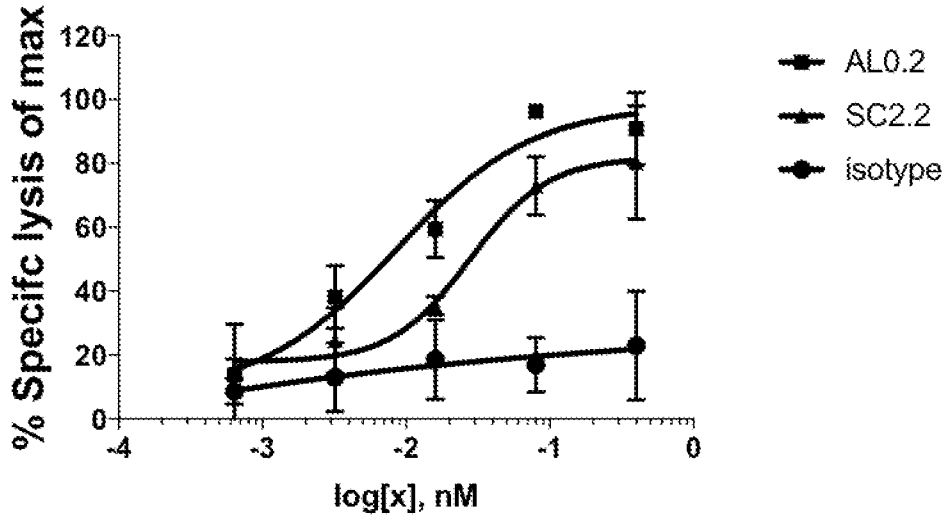

% Specifc lysis of max
120
100
80
60
40
20
0
-4
-3
-2
-1
0
log[x], nM
AL0.2
SC2.2
isotype

FIG. 35

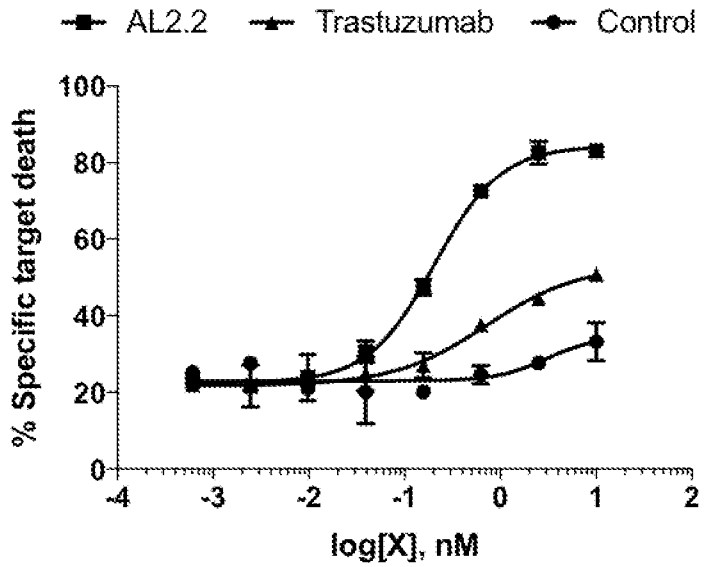

AL2.2
Trastuzumab
Control
% Specific target death
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
log[X], nM

FIG. 36

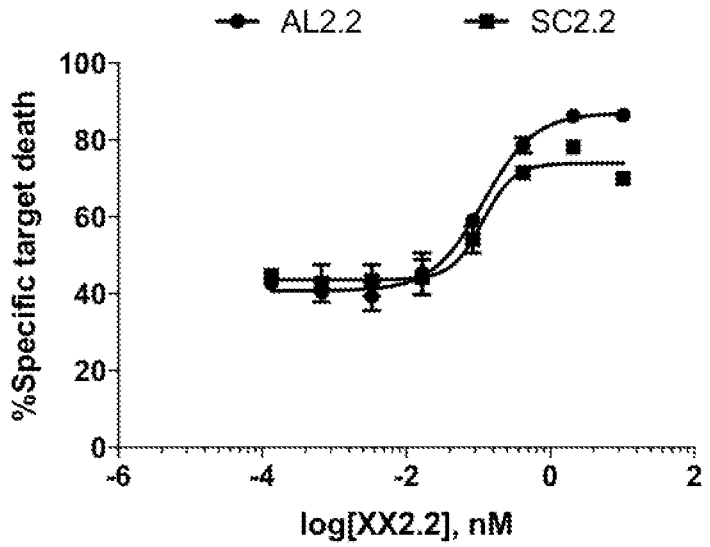

AL2.2
SC2.2
%Specific target death
100
80
60
40
20
0
-6
-4
-2
0
2
log[XX2.2], nM

FIG. 37

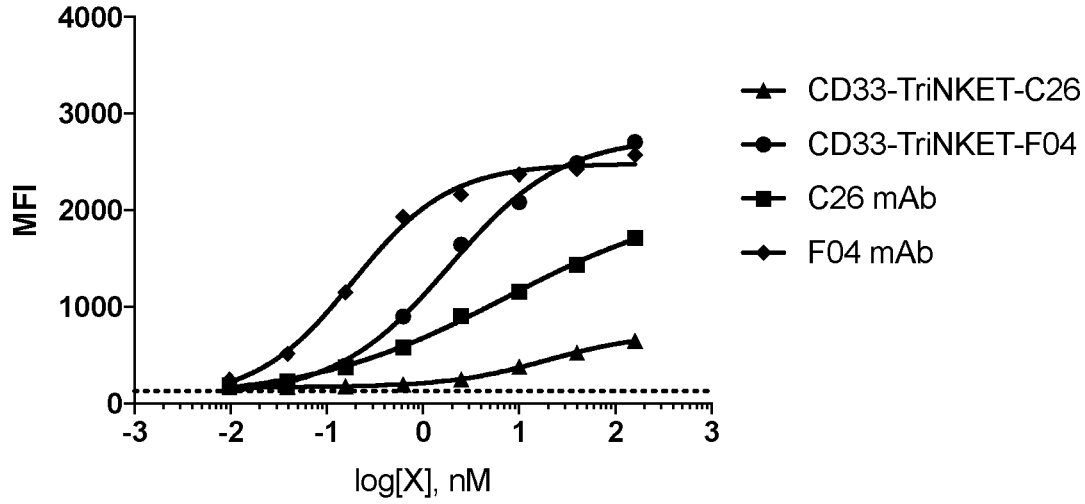

MFI
4000
3000
2000
1000
0
-3
-2
-1
0
1
2
3
log[X], nM
CD33-TriNKET-C26
CD33-TriNKET-F04
C26 mAb
F04 mAb

FIG. 38

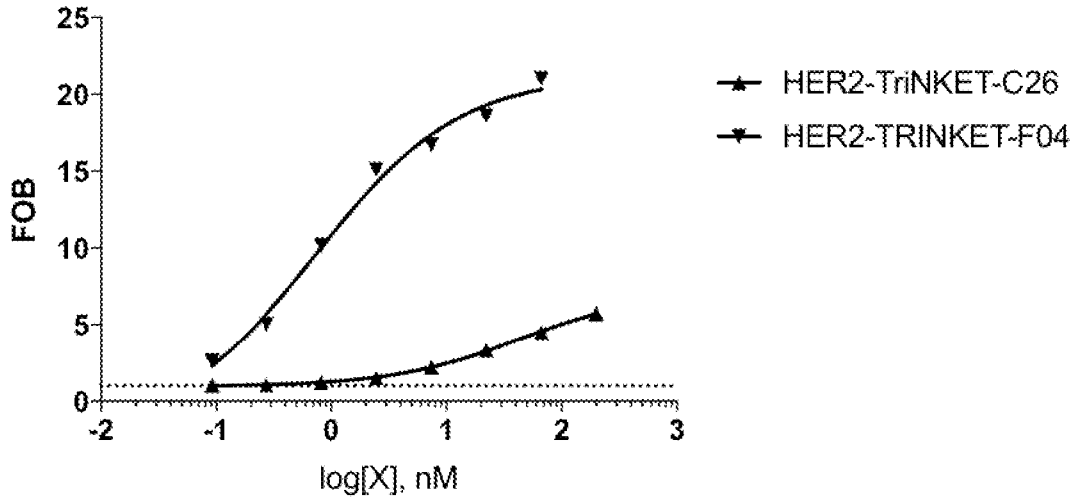

25
20
15
10
5
0
FOB
-2
-1
0
1
2
3
log[X], nM
HER2-TriNKET-C26
HER2-TRINKET-F04

FIG. 39

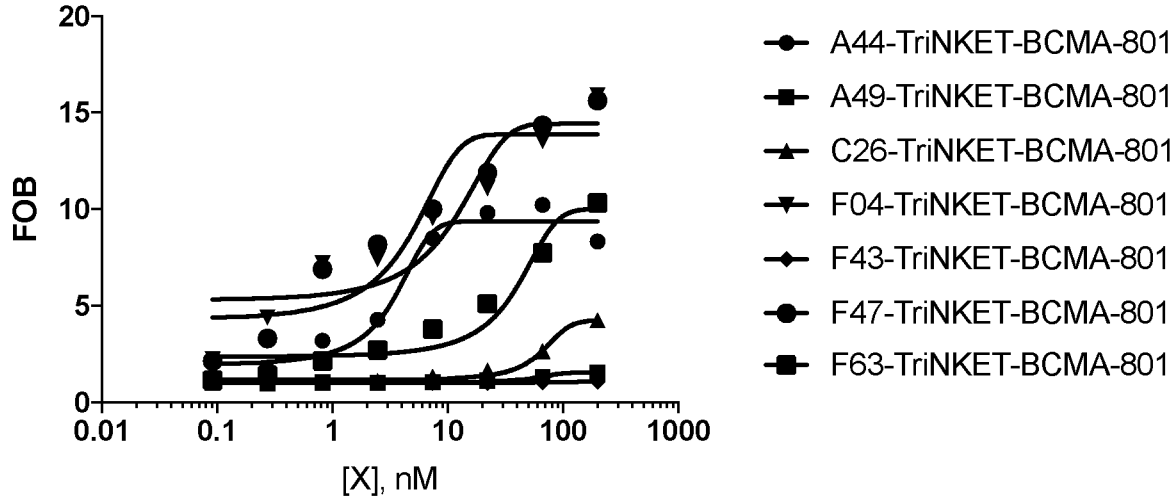
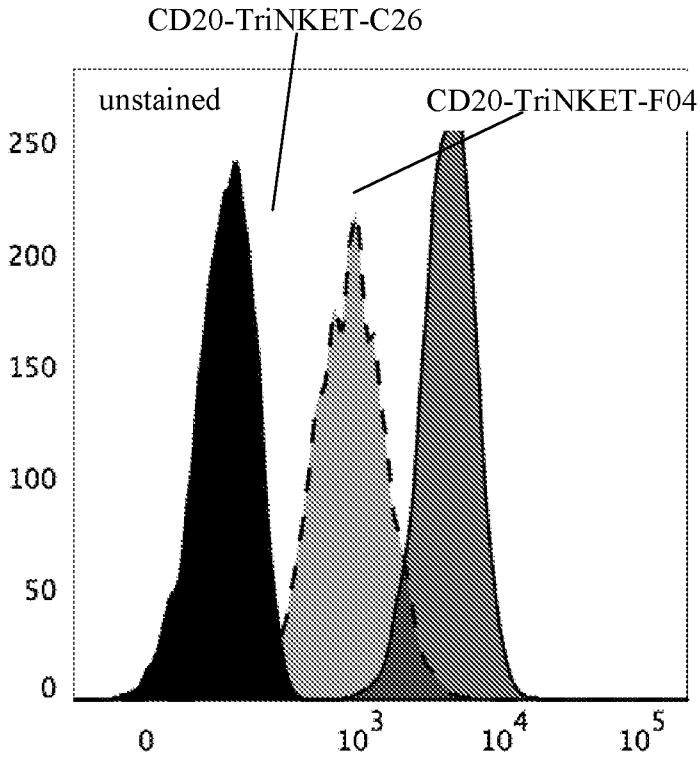

20
15
10
5
0
FOB
0.01
0.1
1
10
100
1000
[X], nM
A44-TriNKET-BCMA-801
A49-TriNKET-BCMA-801
C26-TriNKET-BCMA-801
F04-TriNKET-BCMA-801
F43-TriNKET-BCMA-801
F47-TriNKET-BCMA-801
F63-TriNKET-BCMA-801

FIG. 40



Fluorescence measured by flow cytometry

FIG. 41

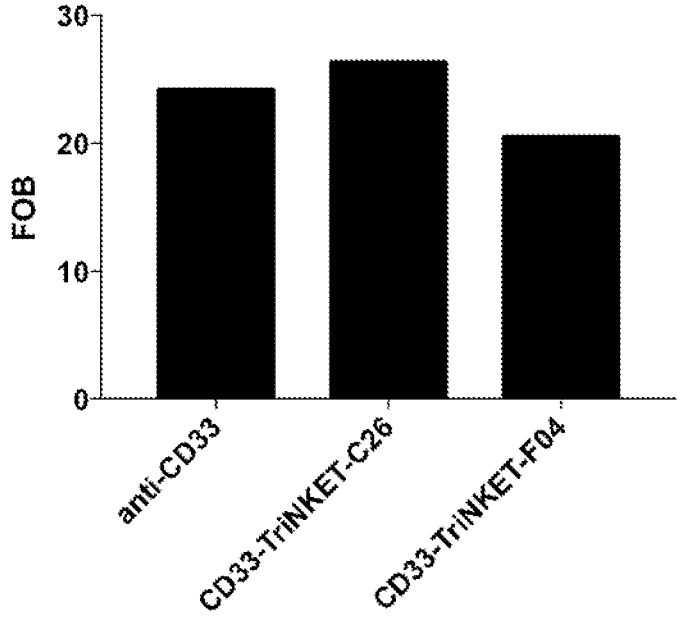

30
20
10
0
FOB
anti-CD33
CD33-TriNKET-C26
CD33-TriNKET-F04

FIG. 42

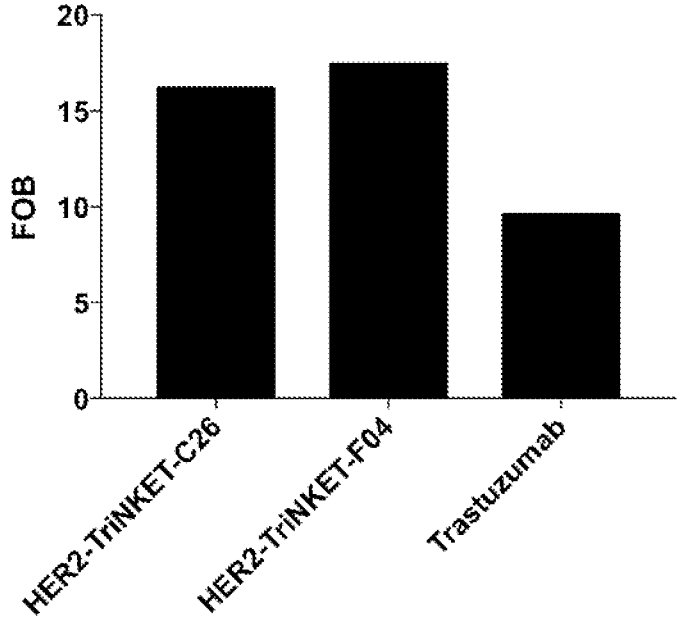

20
15
10
5
0
FOB
HER2-TriNKET-C26
HER2-TriNKET-F04
Trastuzumab

FIG. 43

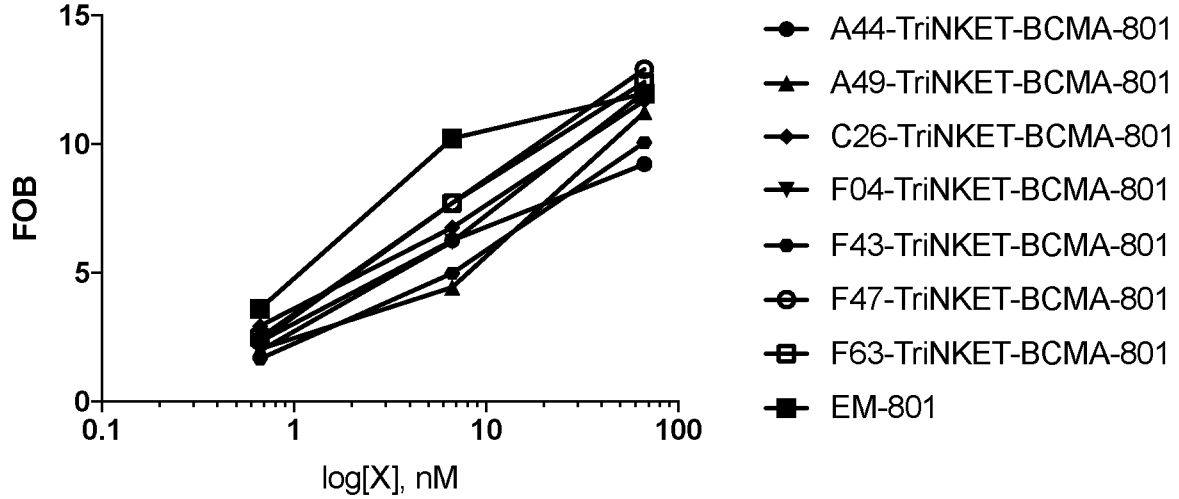
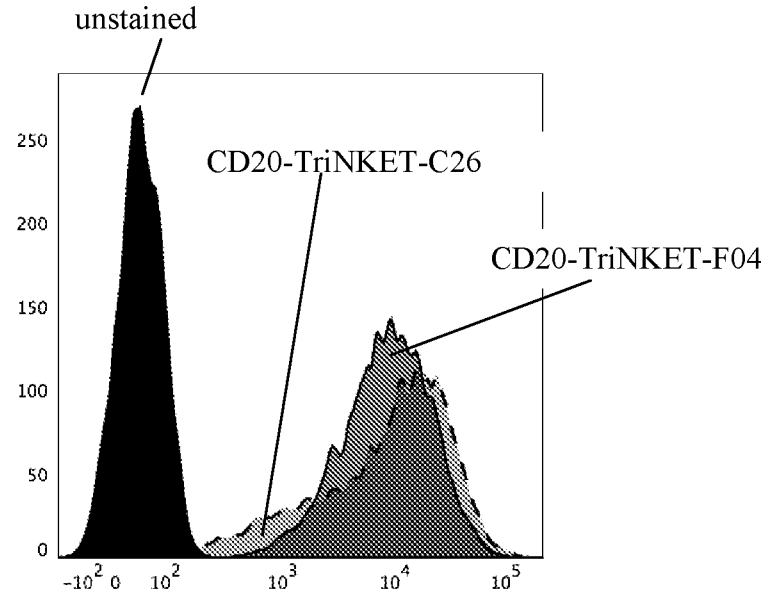


FIG. 44



Fluorescence measured by flow cytometry

FIG. 45A

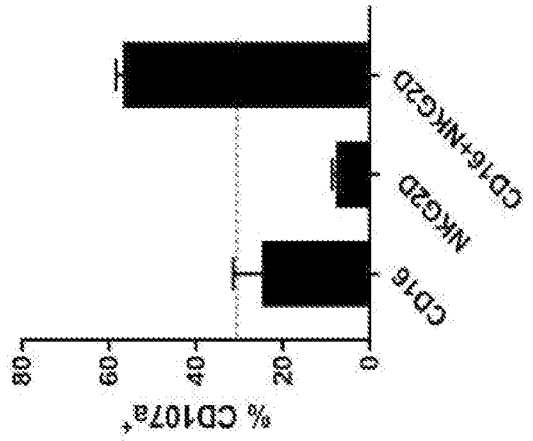


FIG. 45B

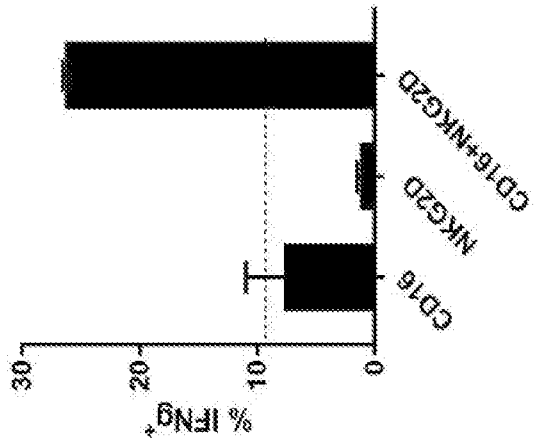


FIG. 45C

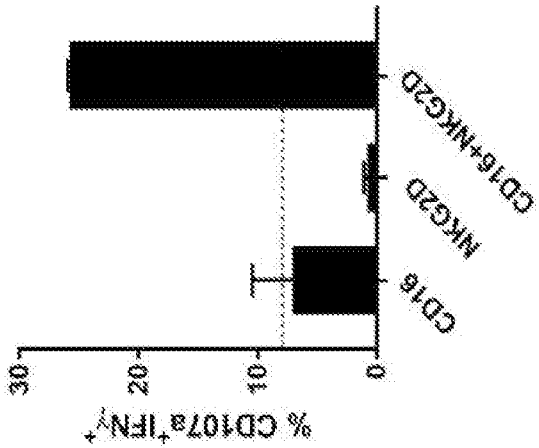


FIG. 46

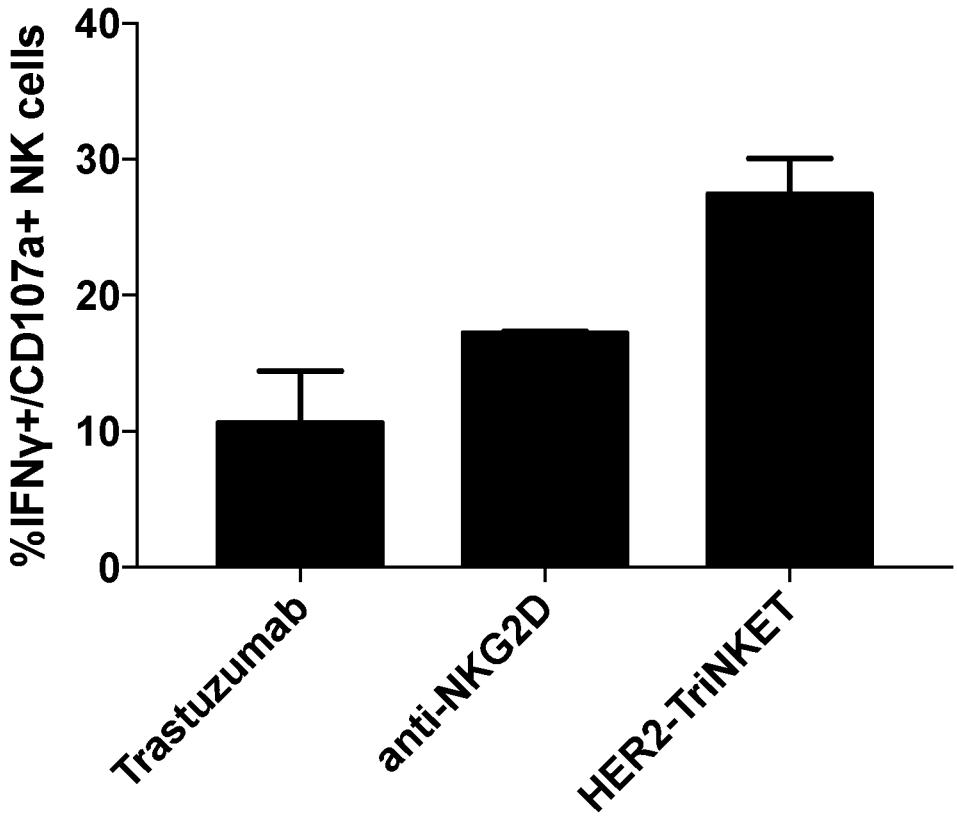

%IFNγ+/CD107a+ NK cells
40
30
20
10
0
Trastuzumab
anti-NKG2D
HER2-TriNKET

FIG. 47A

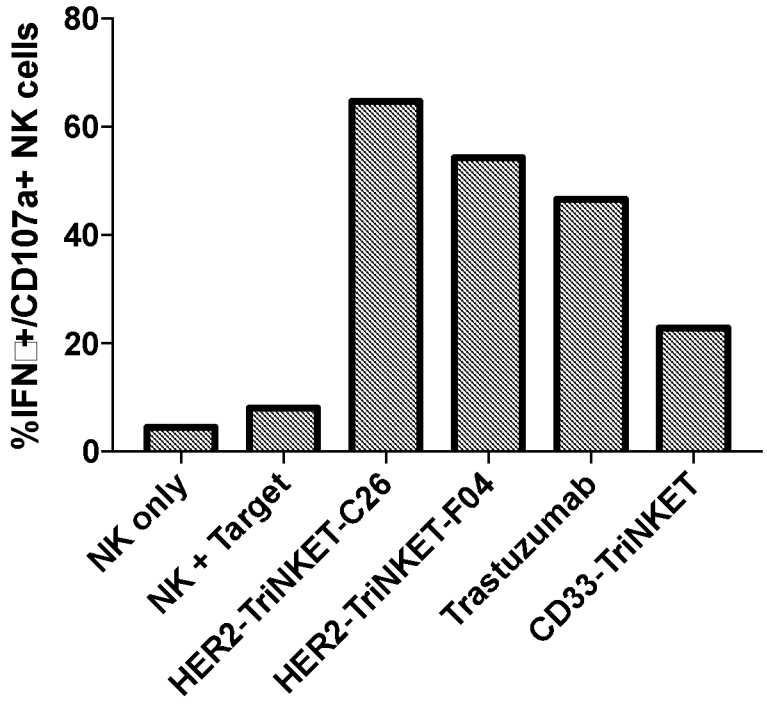


FIG. 47B

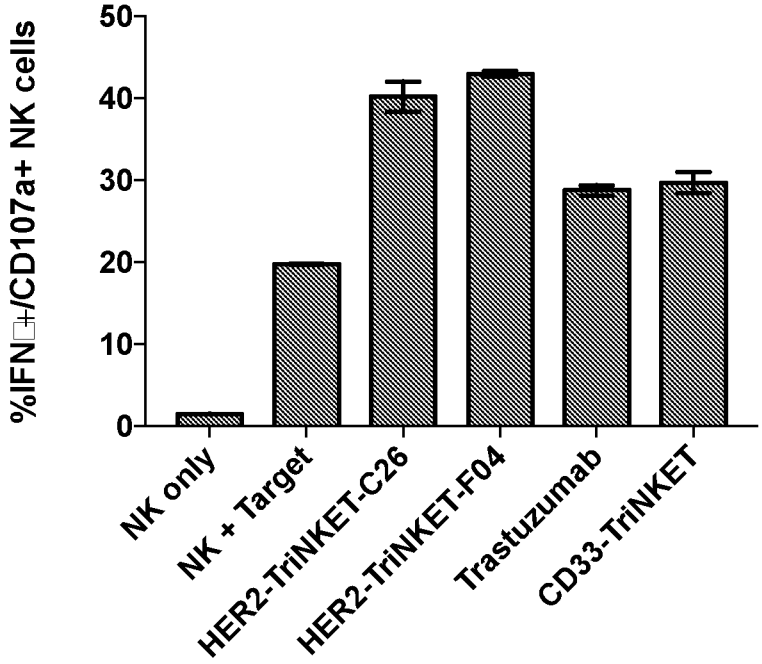


FIG. 47C

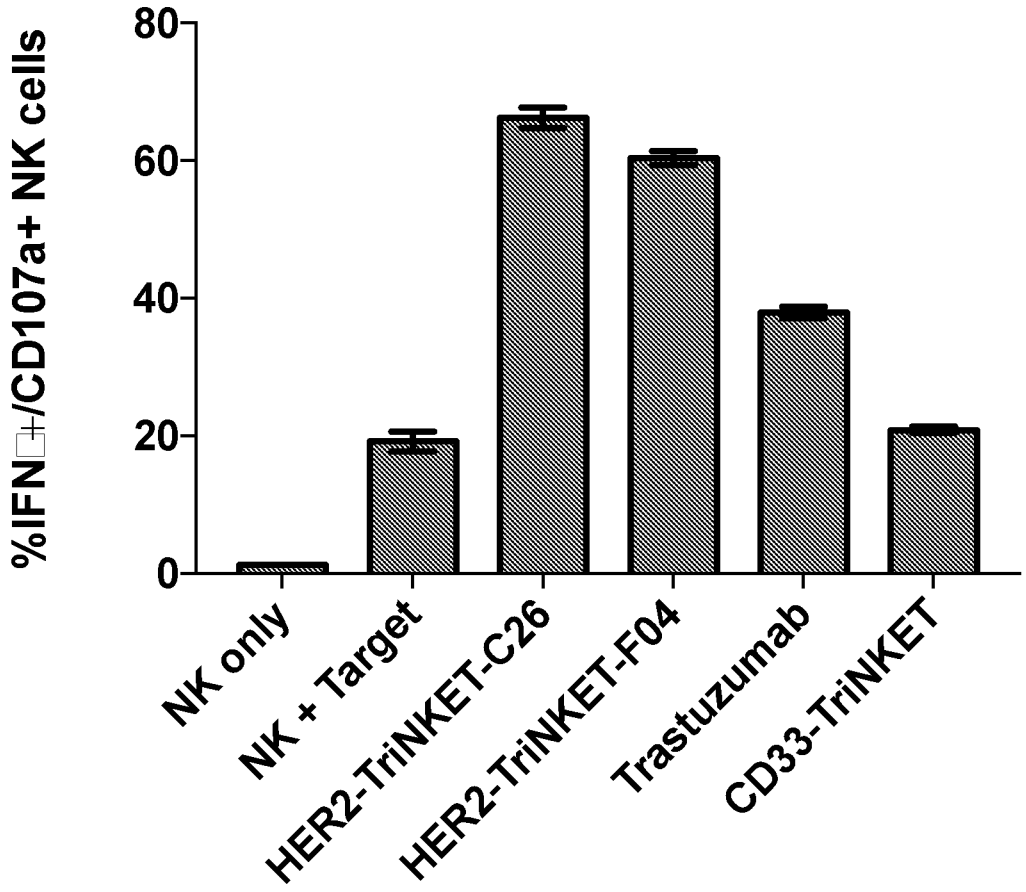

%IFN□+/CD107a+ NK cells
80
60
40
20
0
NK only
NK + Target
HER2-TriNKET-C26
HER2-TriNKET-F04
Trastuzumab
CD33-TriNKET

FIG. 48A

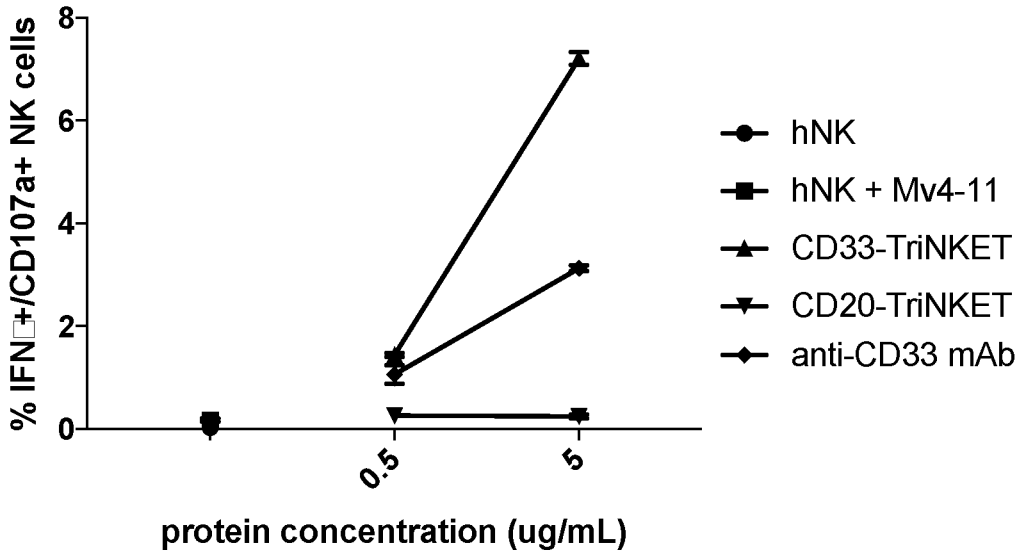


FIG. 48B

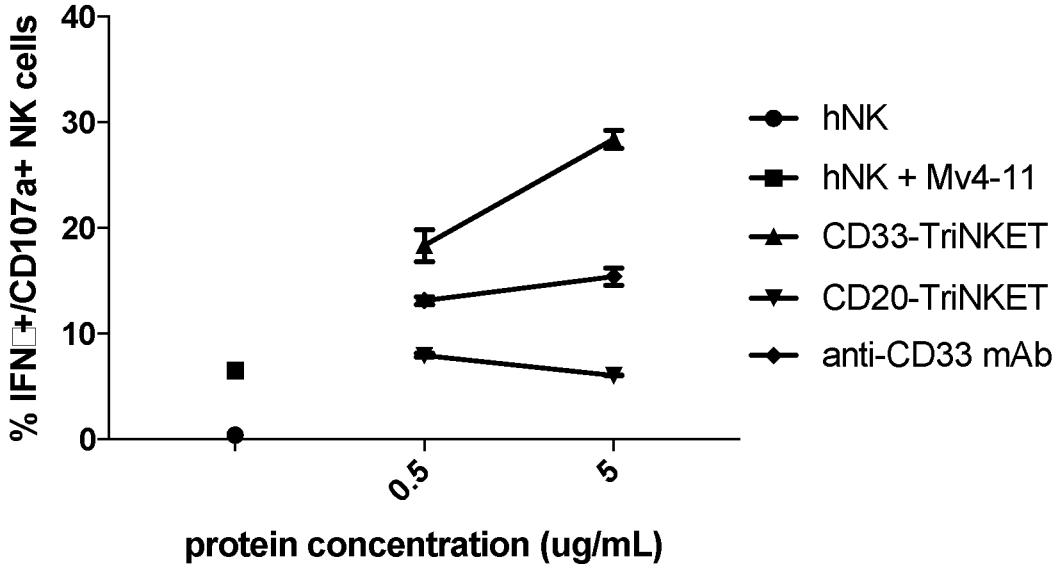


FIG. 49A

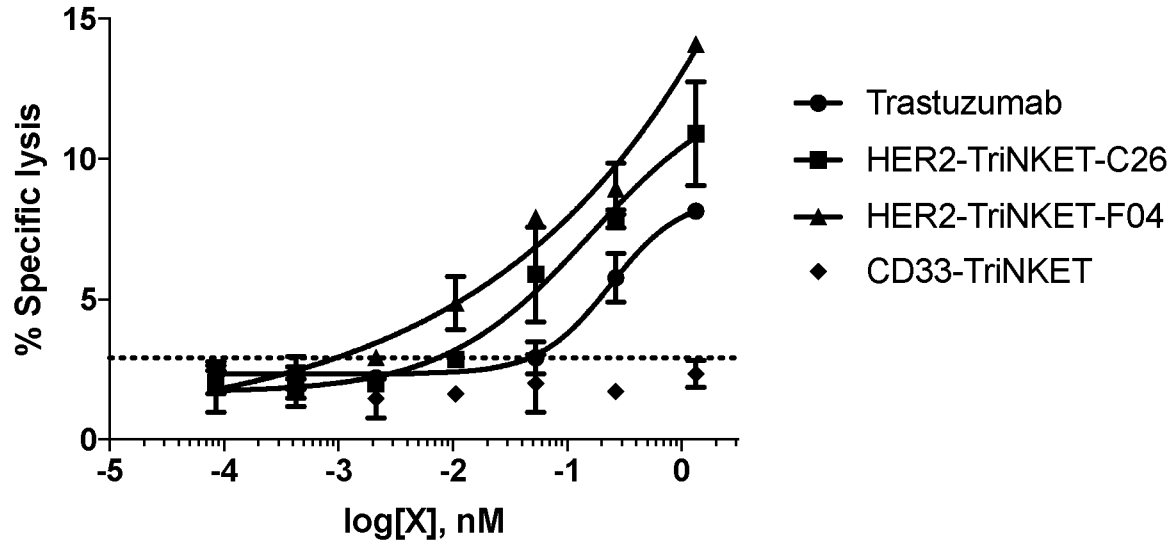


FIG. 49B

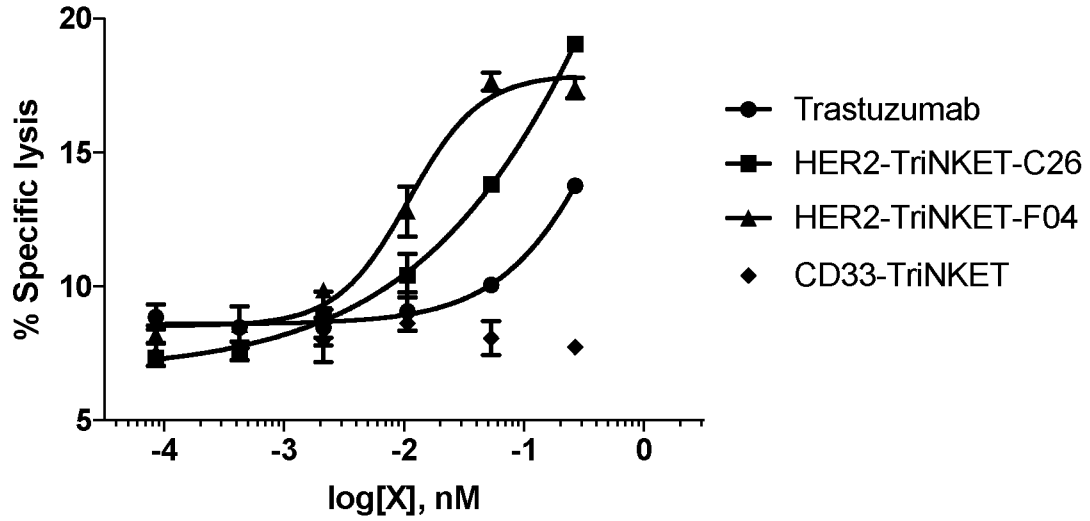


FIG. 50A

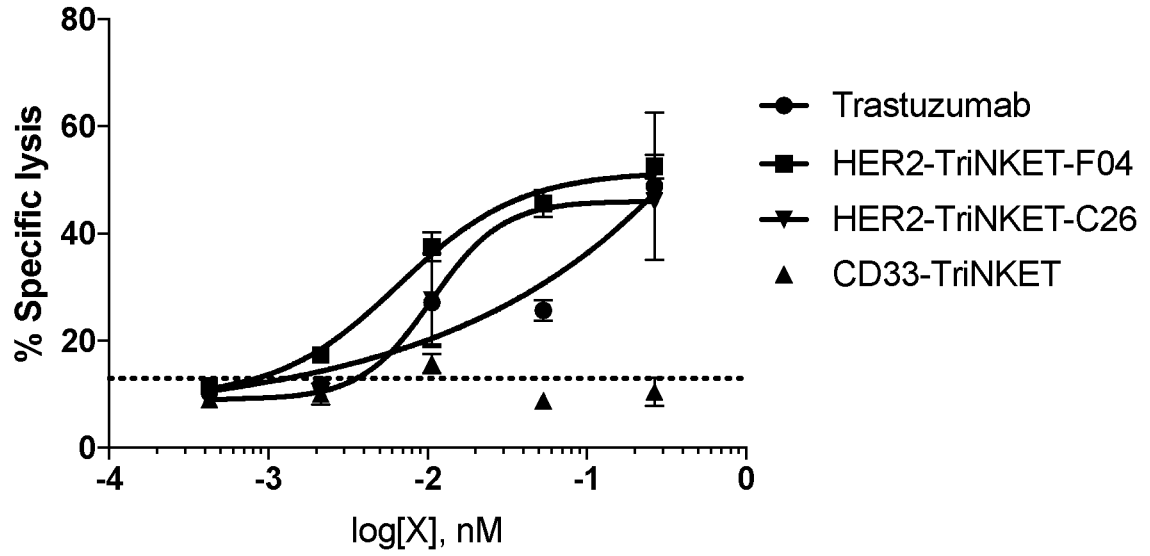
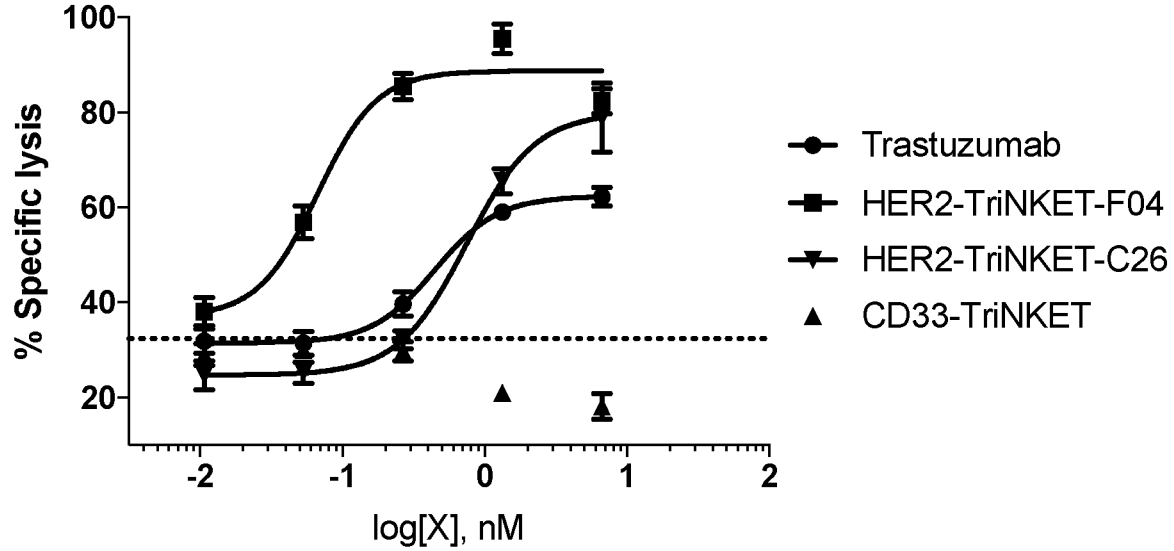


FIG. 50B



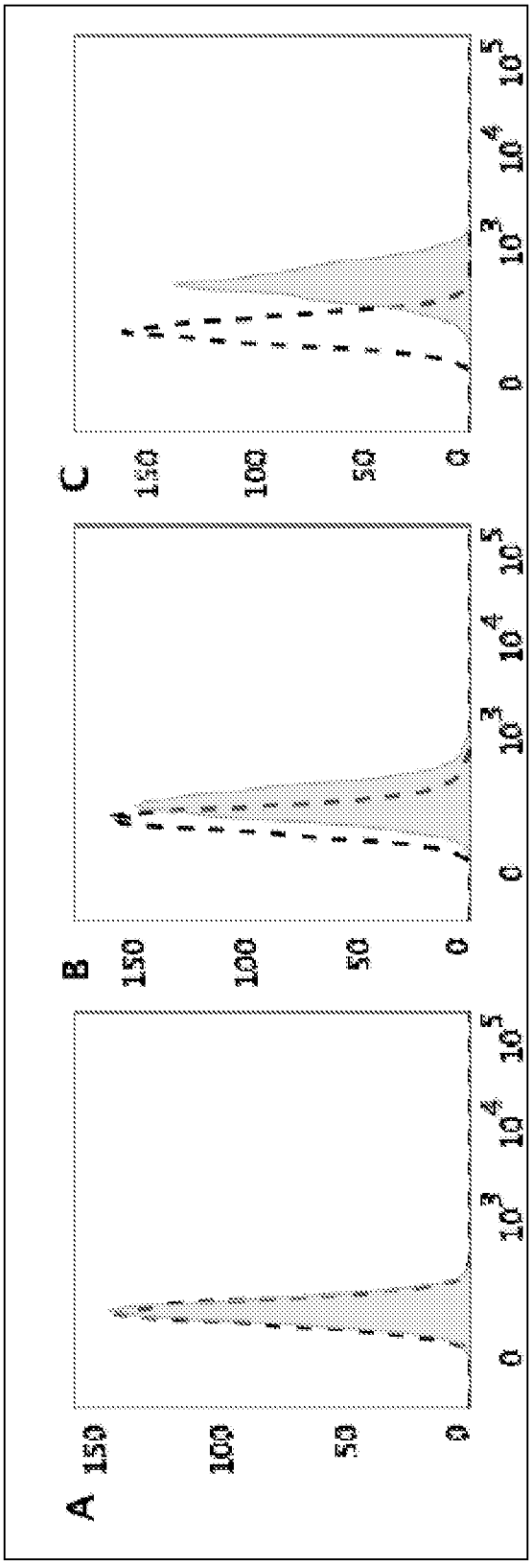


FIG. 51A FIG. 51B FIG. 51C

FIG. 52A

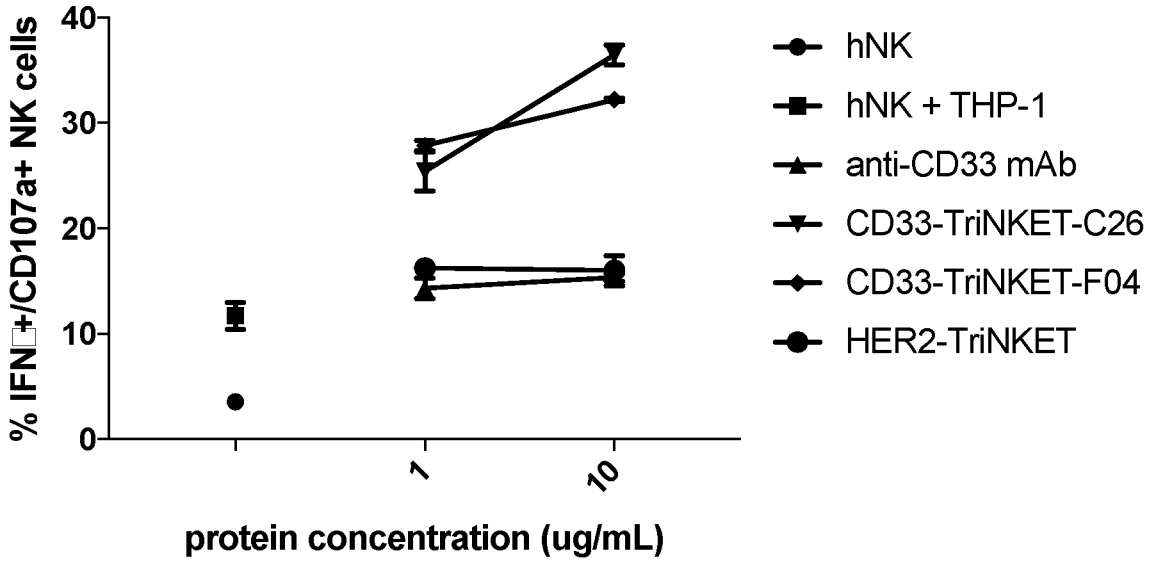


FIG. 52B

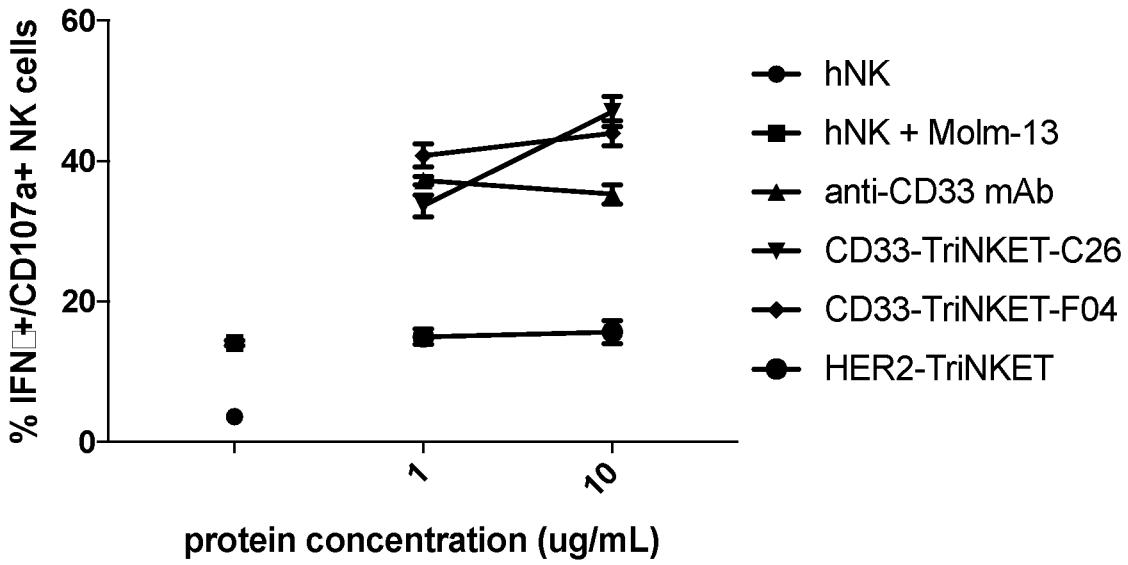


FIG. 53A

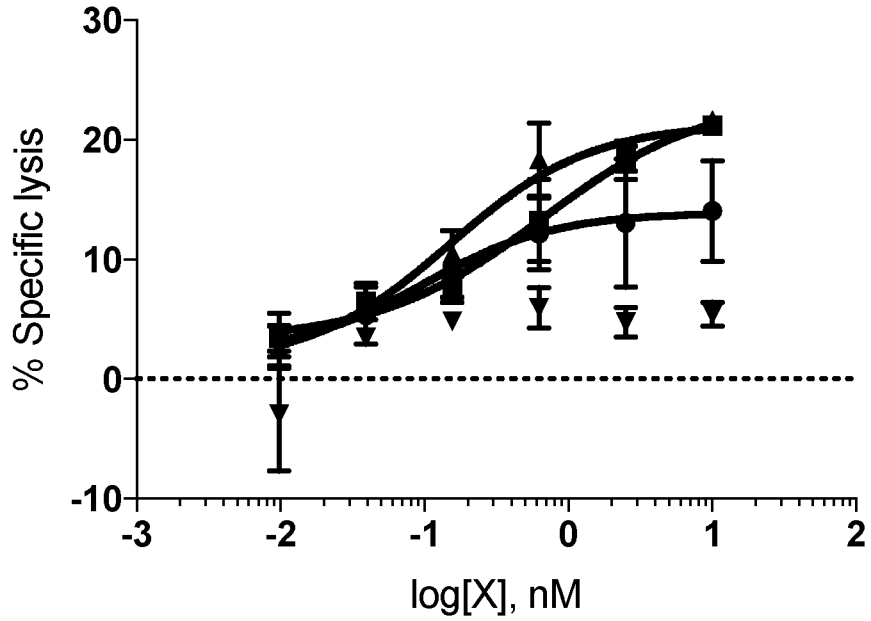


FIG. 53B

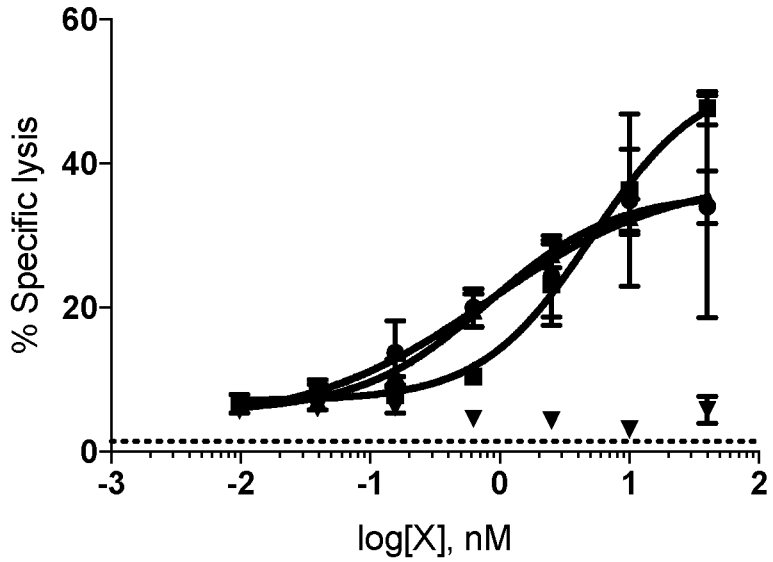


FIG. 53C

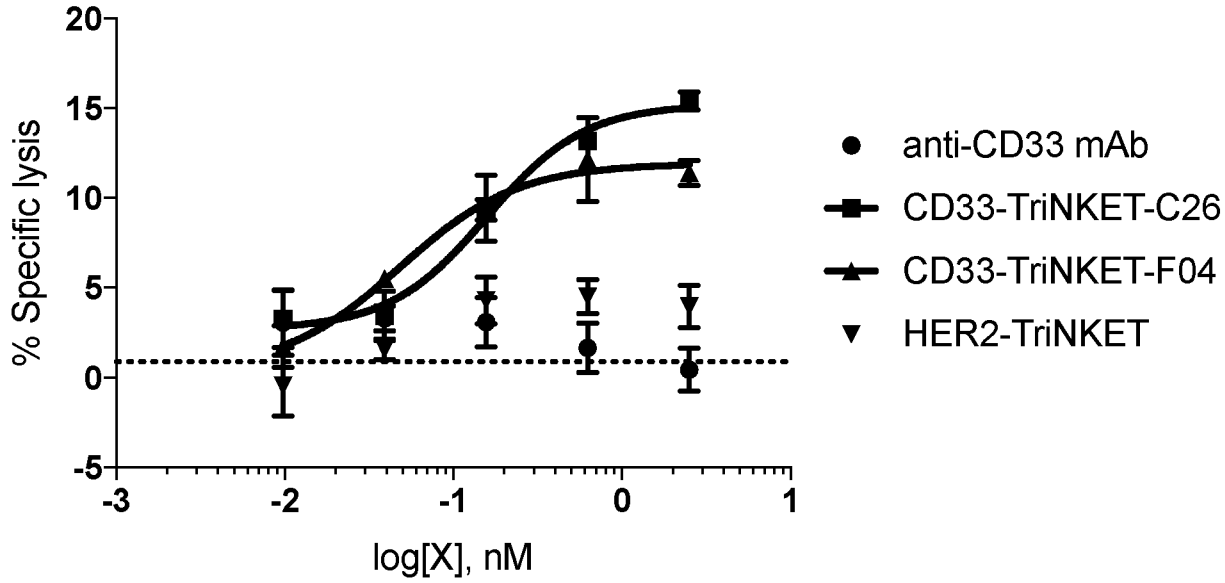

20
15
10
5
0
-5
% Specific lysis
-3
-2
-1
0
1
log[X], nM
anti-CD33 mAb
CD33-TriNKET-C26
CD33-TriNKET-F04
HER2-TriNKET

FIG. 54A

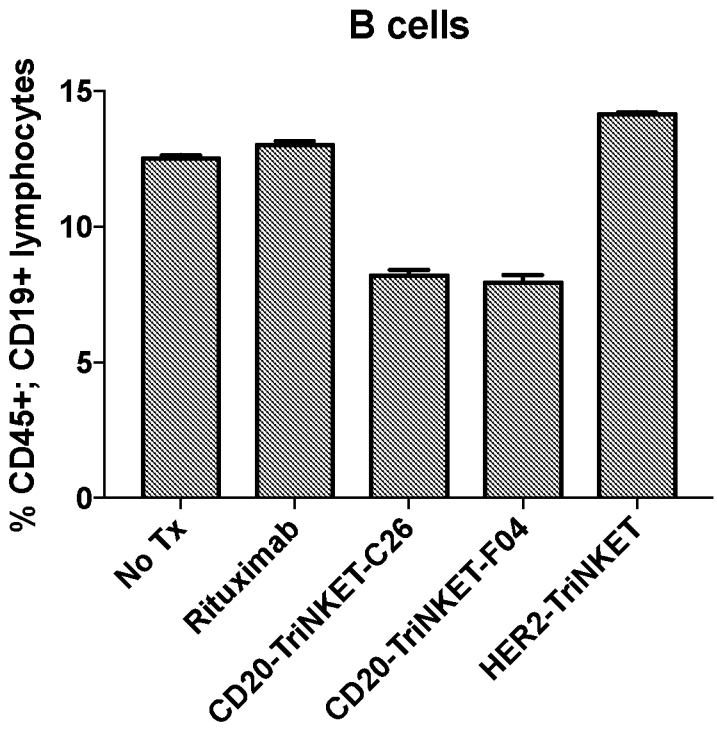


FIG. 54B

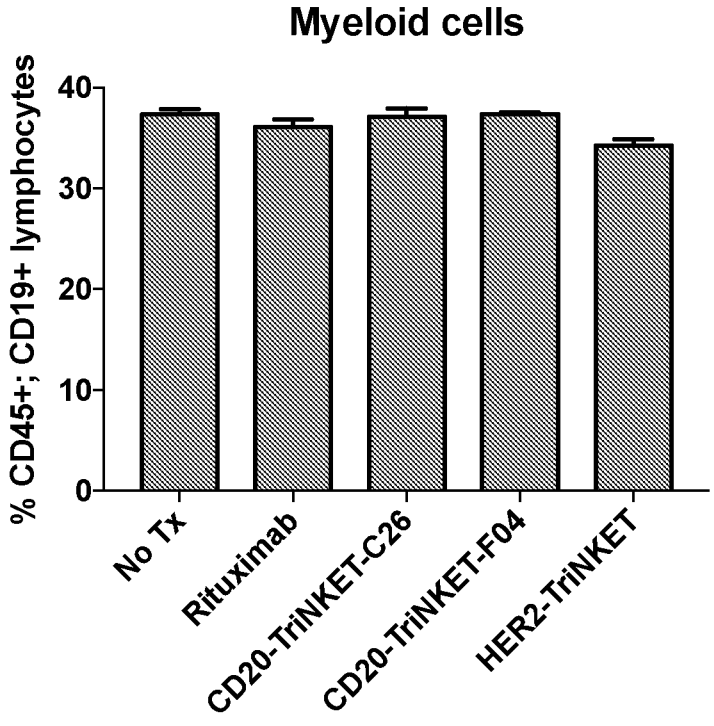


FIG. 54C

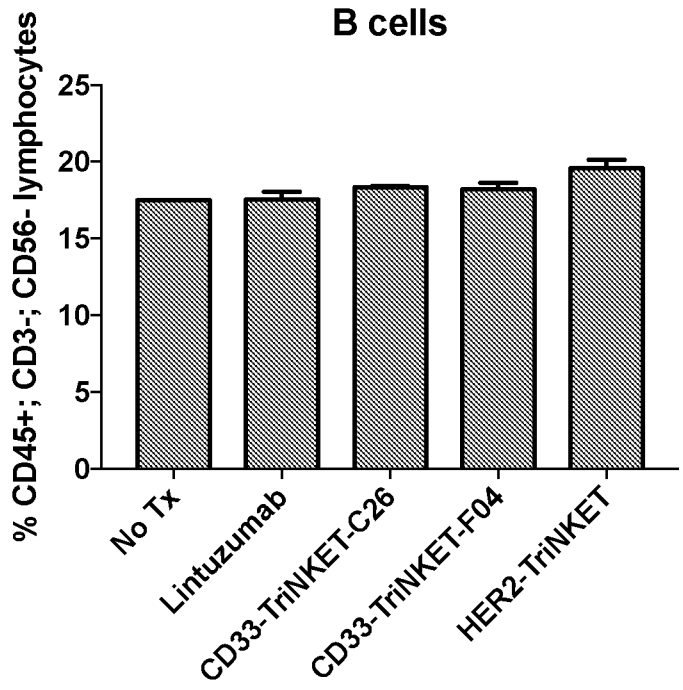


FIG. 54D

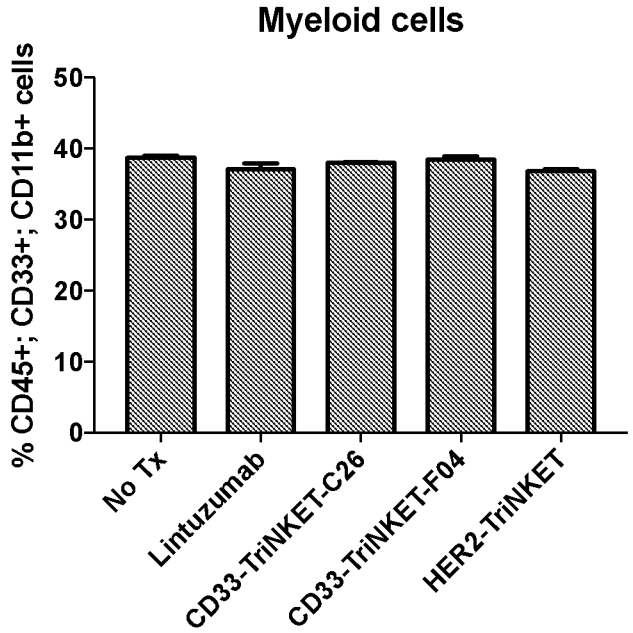
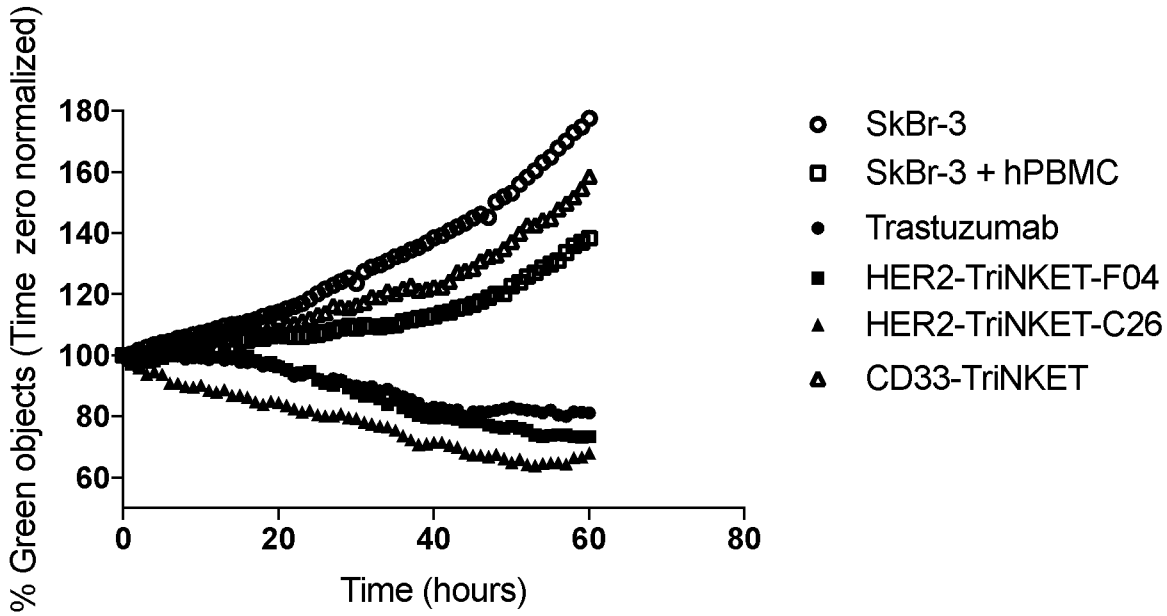


FIG. 55


% Green objects (Time zero normalized)
180
160
140
120
100
80
60
0
20
40
60
80
Time (hours)
SkBr-3
SkBr-3 + hPBMC
Trastuzumab
HER2-TriNKET-F04
HER2-TriNKET-C26
CD33-TriNKET

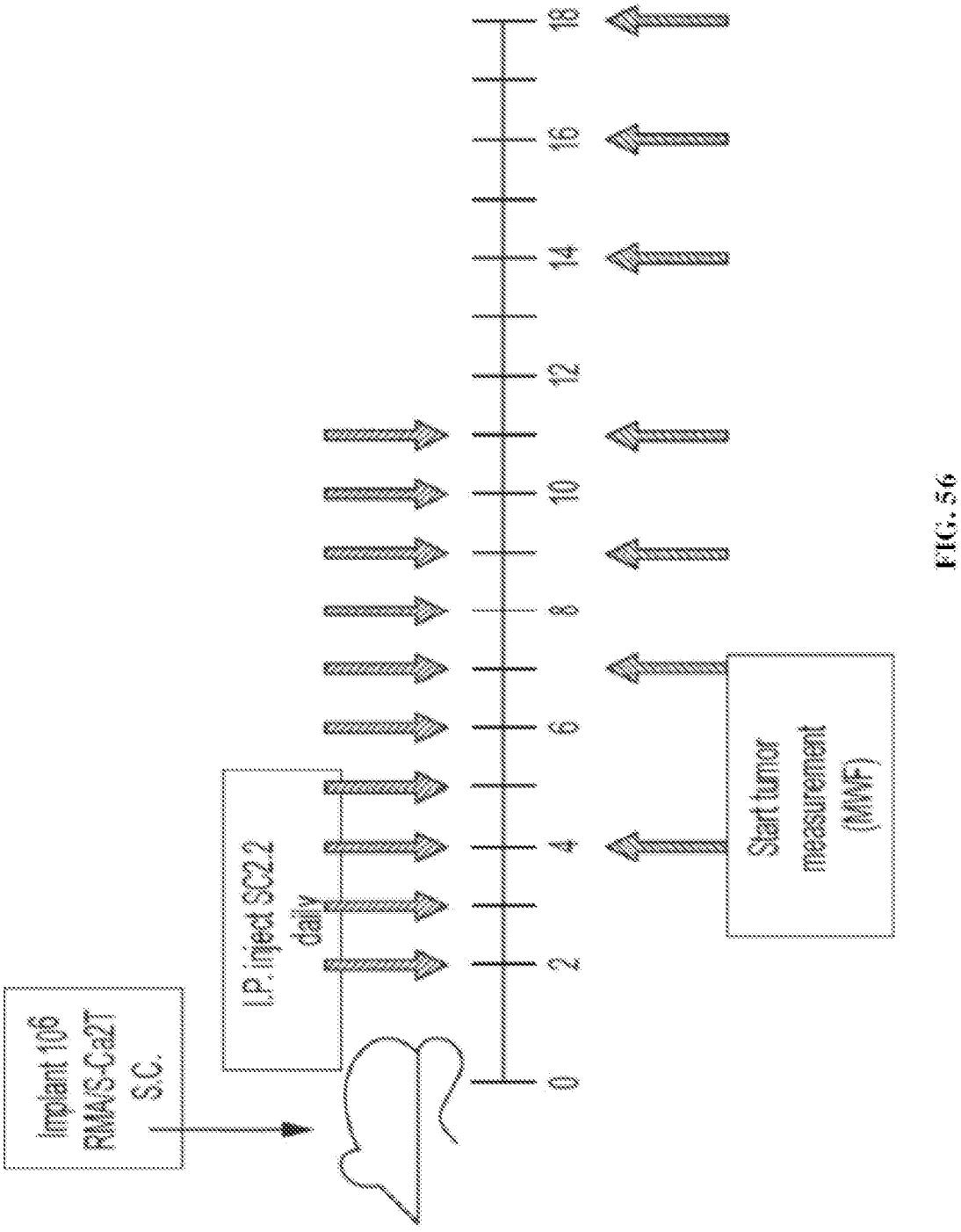

Implant 10^6
RMAS-Ca2T
S.C.
I.P. inject SC22
daily
Start tumor
measurement
(MMF)
0
2
4
6
8
10
12
14
16
18

FIG. 56

FIG. 57

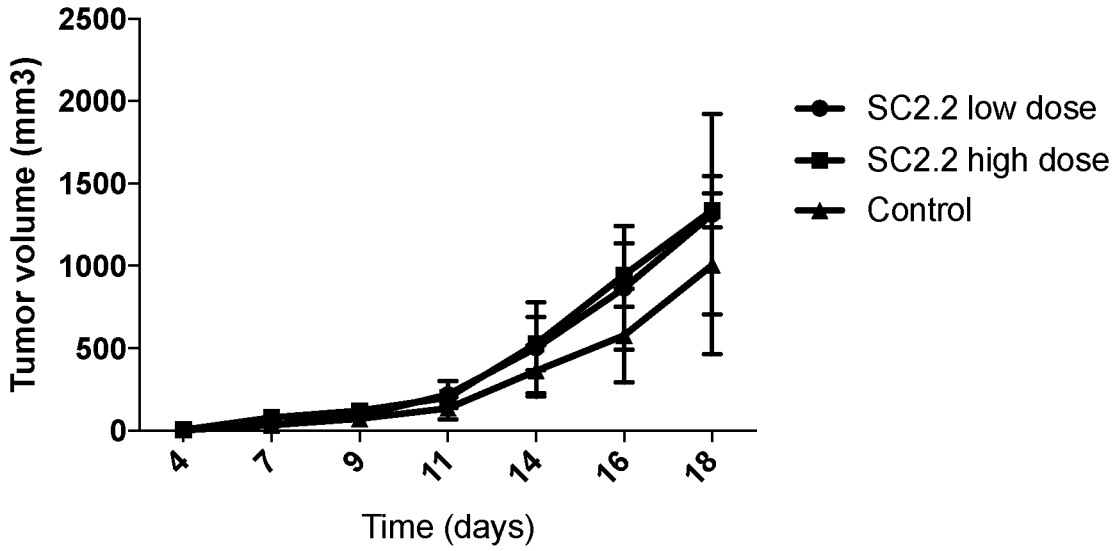

2500
2000
1500
1000
500
0
Tumor volume (mm3)
4
7
9
11
14
16
18
Time (days)
SC2.2 low dose
SC2.2 high dose
Control

FIG. 58A

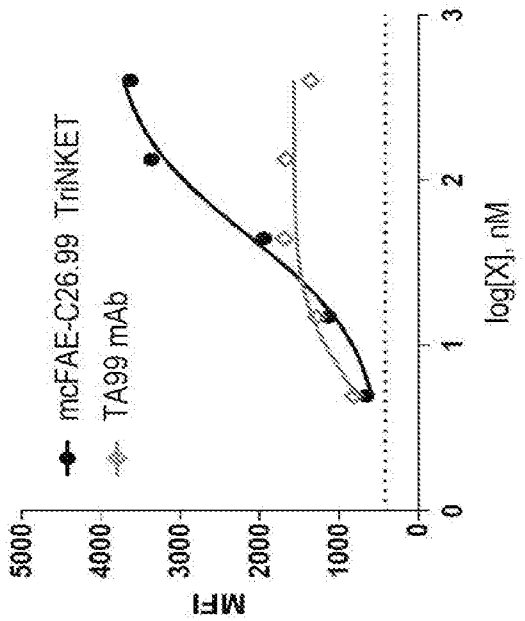
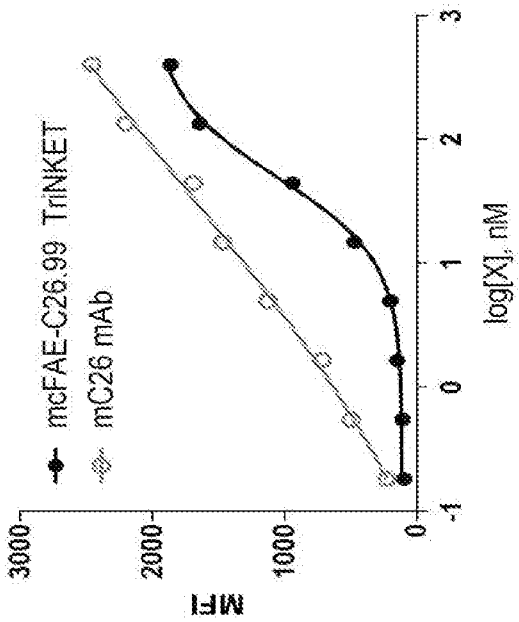


FIG. 58B



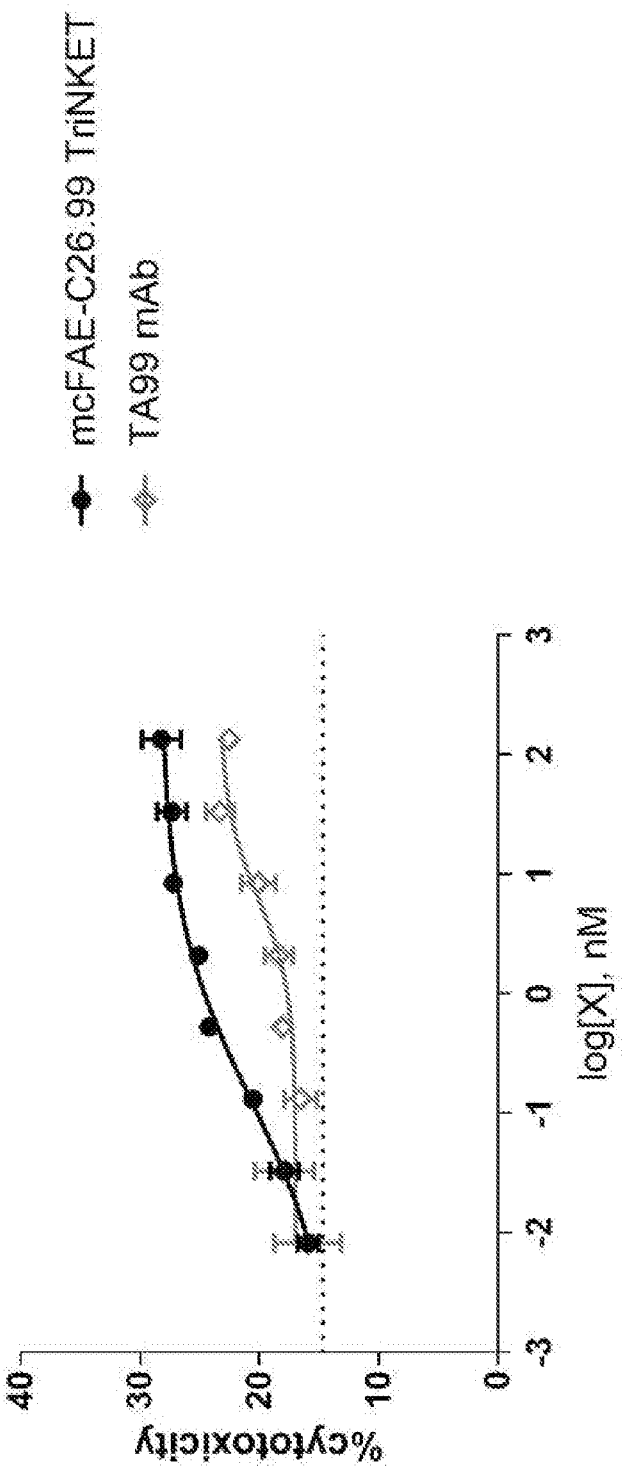

mcFAE-C26.99 TriNKET
TA99 mAb
%cytotoxicity
40
30
20
10
0
-3
-2
-1
0
1
2
3
log[X], nM

FIG. 59

FIG. 60A

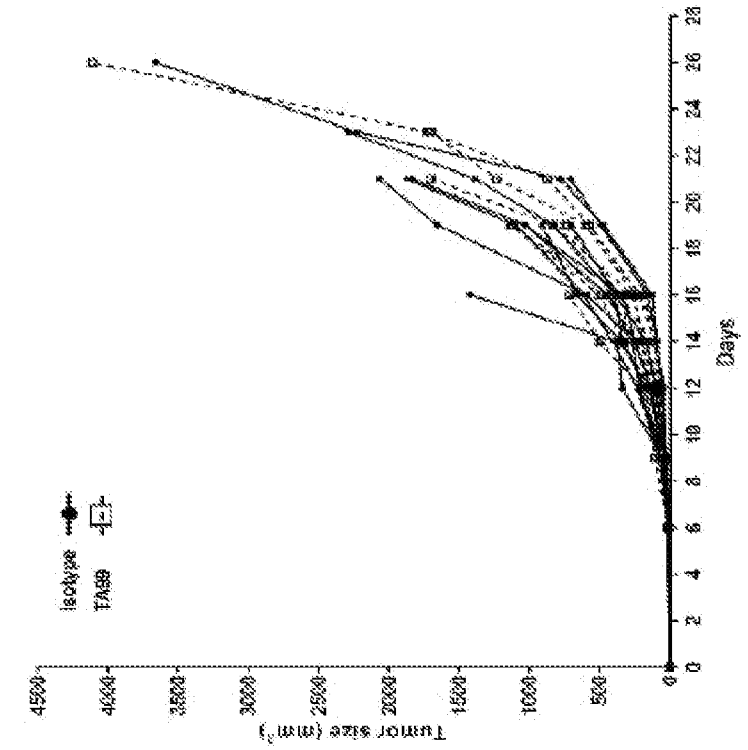


FIG. 60B

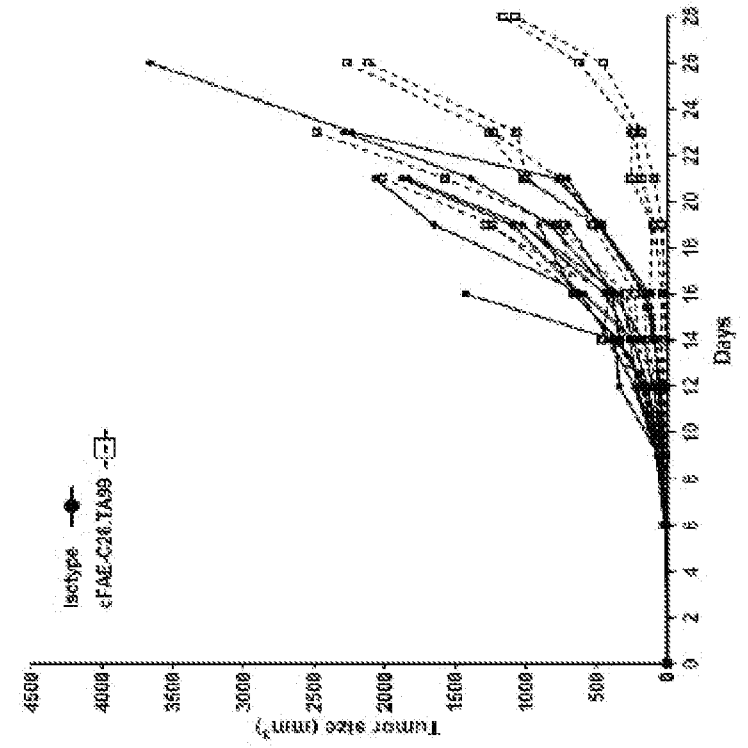


FIG. 61B

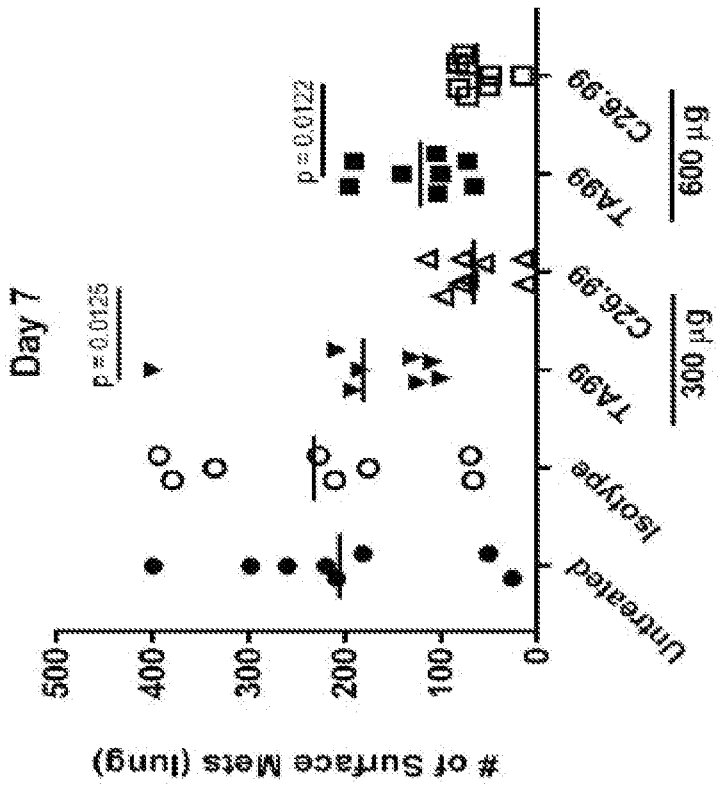


FIG. 61A

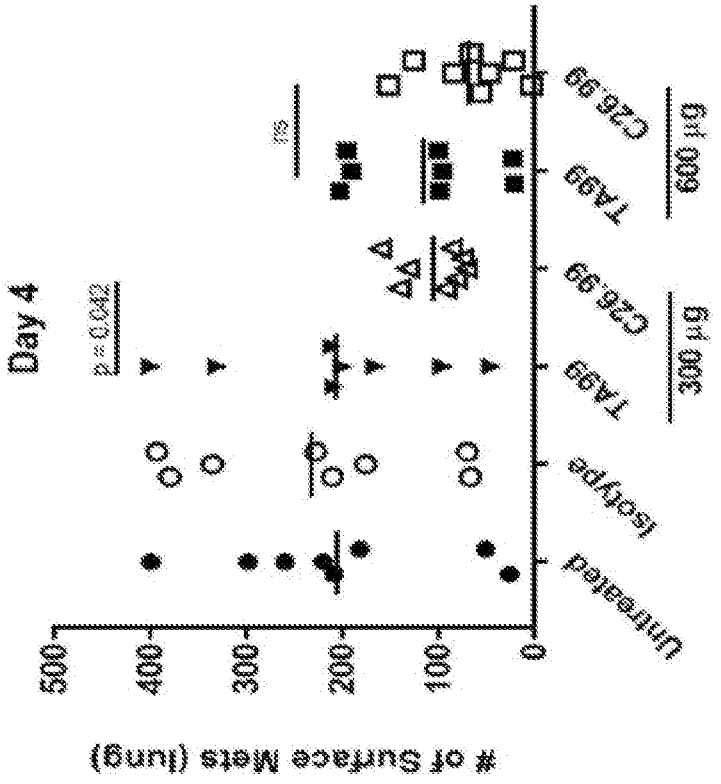


FIG. 62

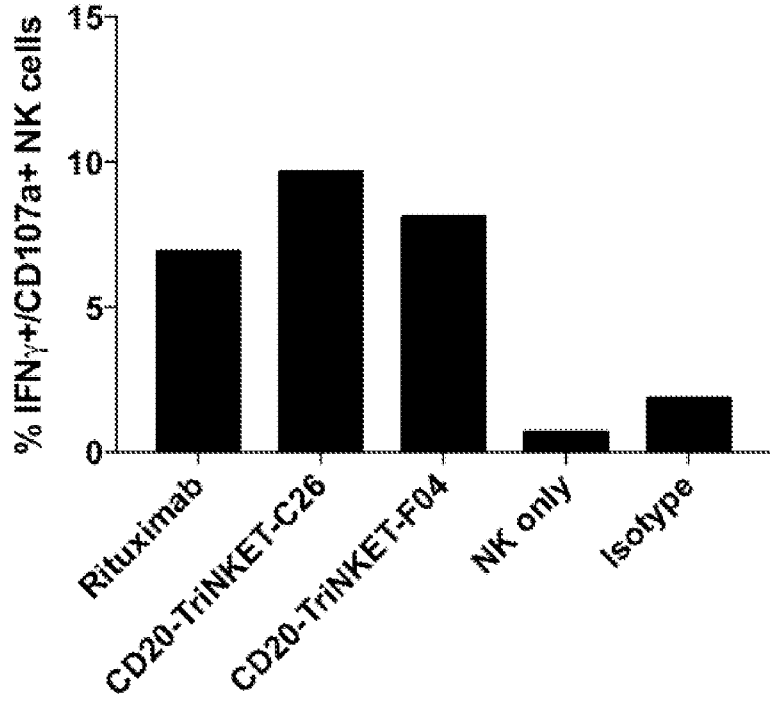

% IFNγ+/CD107a+ NK cells
15
10
5
0
Rituximab
CD20-TriNKET-C26
CD20-TriNKET-F04
NK only
Isotype

FIG. 63

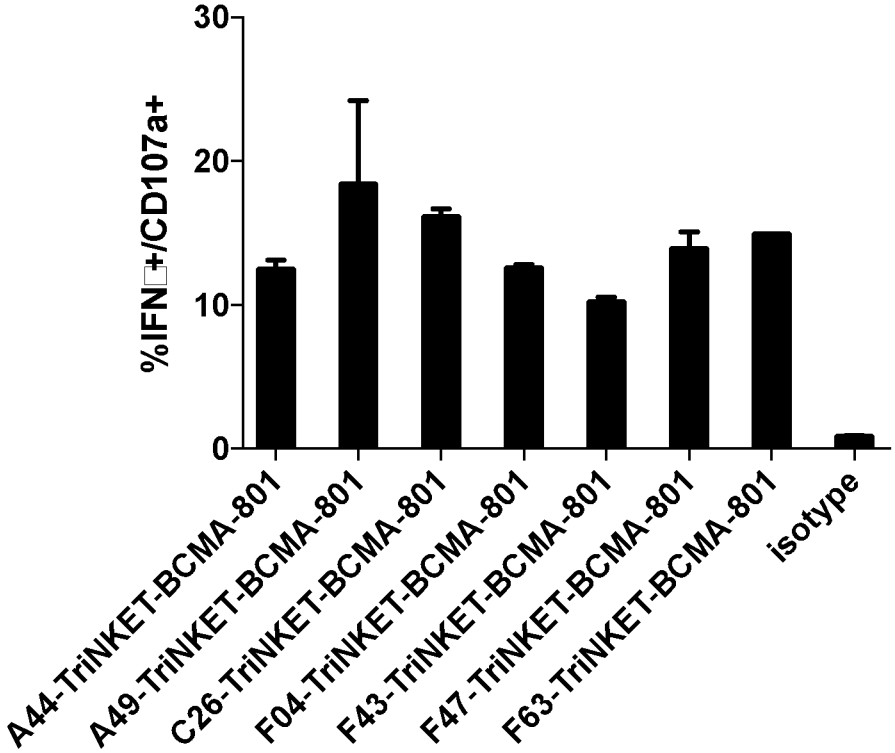

%IFN□+/CD107a+
30
20
10
0
A44-TriNKET-BCMA-801
A49-TriNKET-BCMA-801
C26-TriNKET-BCMA-801
F04-TriNKET-BCMA-801
F43-TriNKET-BCMA-801
F47-TriNKET-BCMA-801
F63-TriNKET-BCMA-801
isotype

FIG. 64

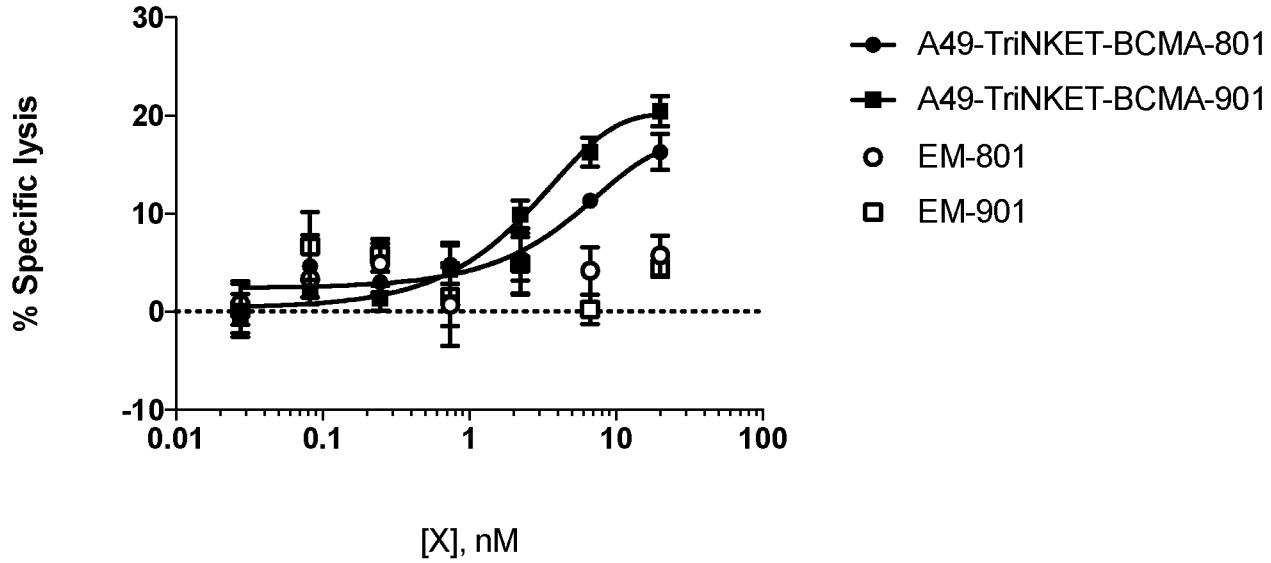

30
20
10
0
-10
0.01
0.1
1
10
100
% Specific lysis
[X], nM
A49-TriNKET-BCMA-801
A49-TriNKET-BCMA-901
EM-801
EM-901

FIG. 65

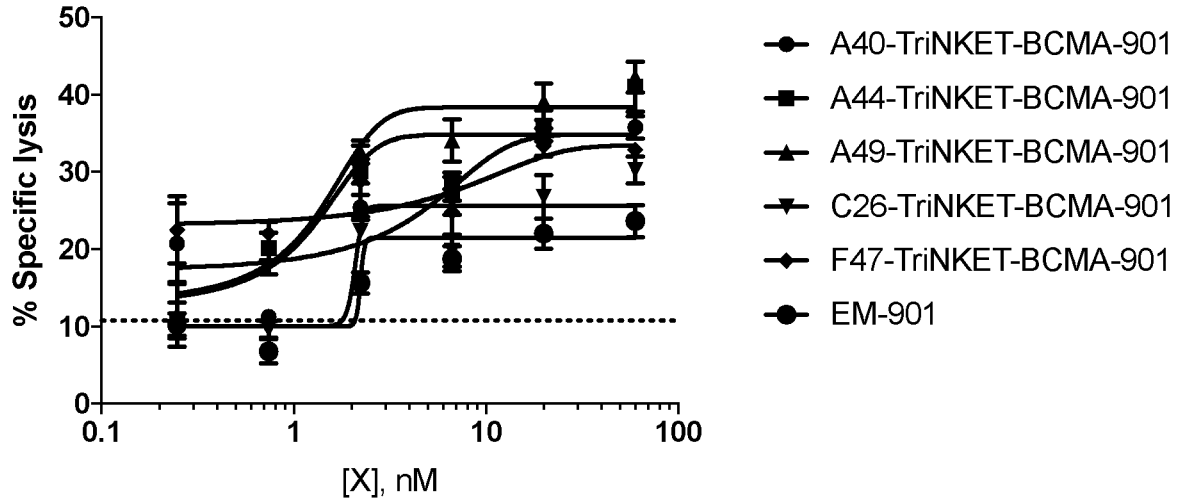

50
40
30
20
10
0
0.1
1
10
100
% Specific lysis
[X], nM
A40-TriNKET-BCMA-901
A44-TriNKET-BCMA-901
A49-TriNKET-BCMA-901
C26-TriNKET-BCMA-901
F47-TriNKET-BCMA-901
EM-901

FIG. 66

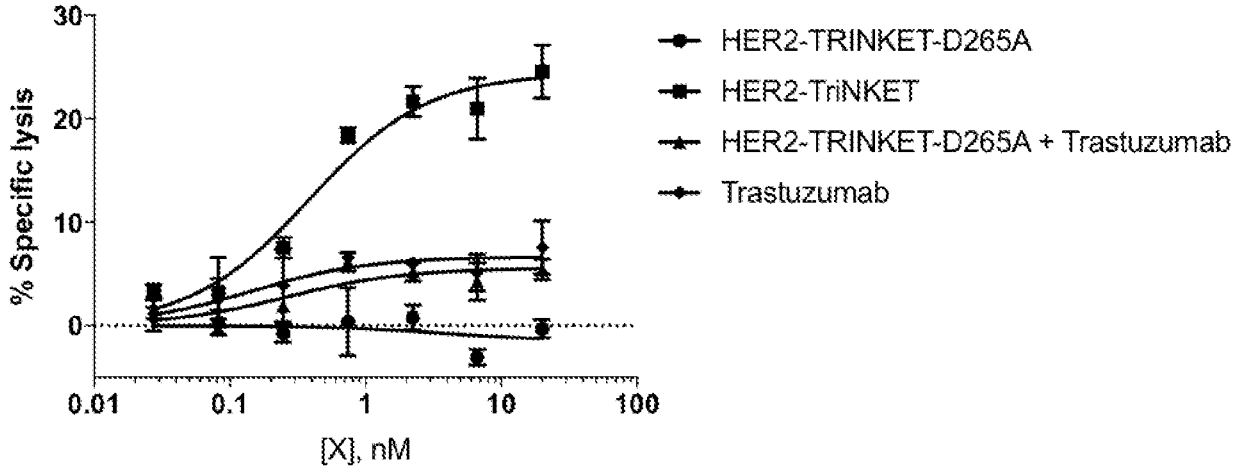

30
20
10
0
% Specific lysis
0.01
0.1
1
10
100
[X], nM
HER2-TRINKET-D265A
HER2-TriNKET
HER2-TRINKET-D265A + Trastuzumab
Trastuzumab

FIG. 67

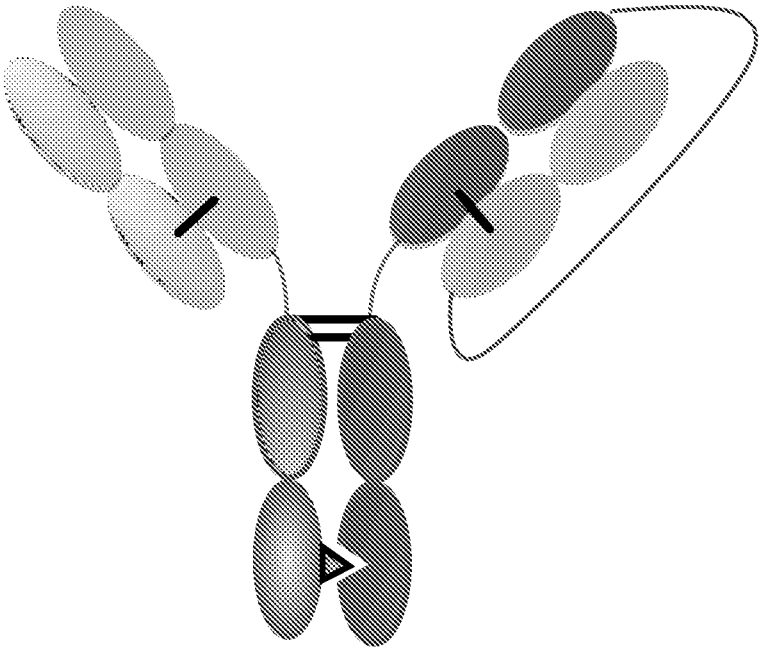


FIG. 68

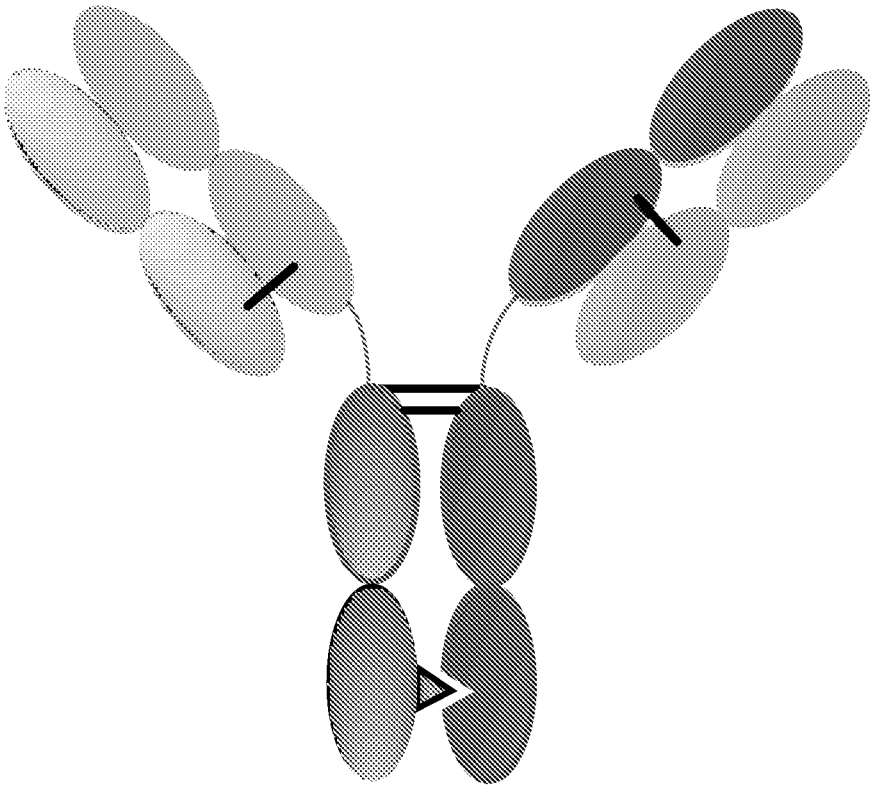


FIG. 69

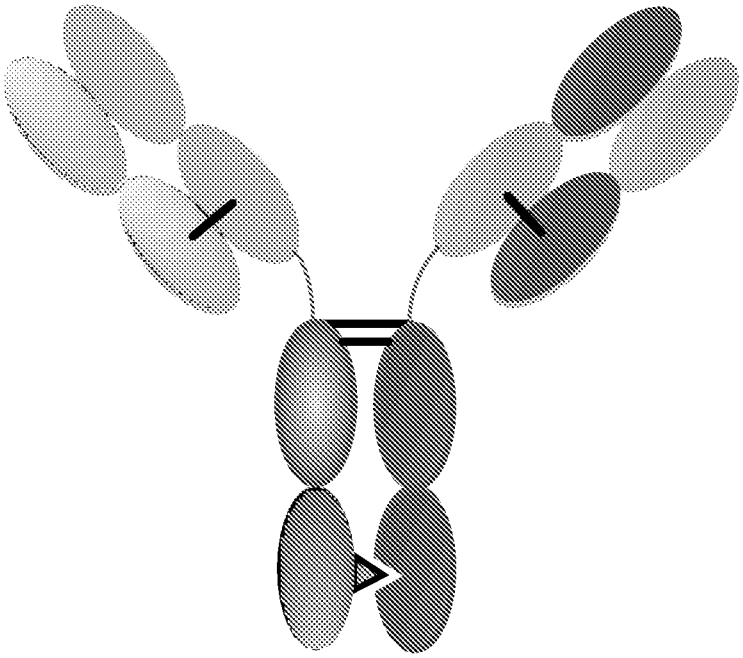


FIG. 70

