FIG. 1

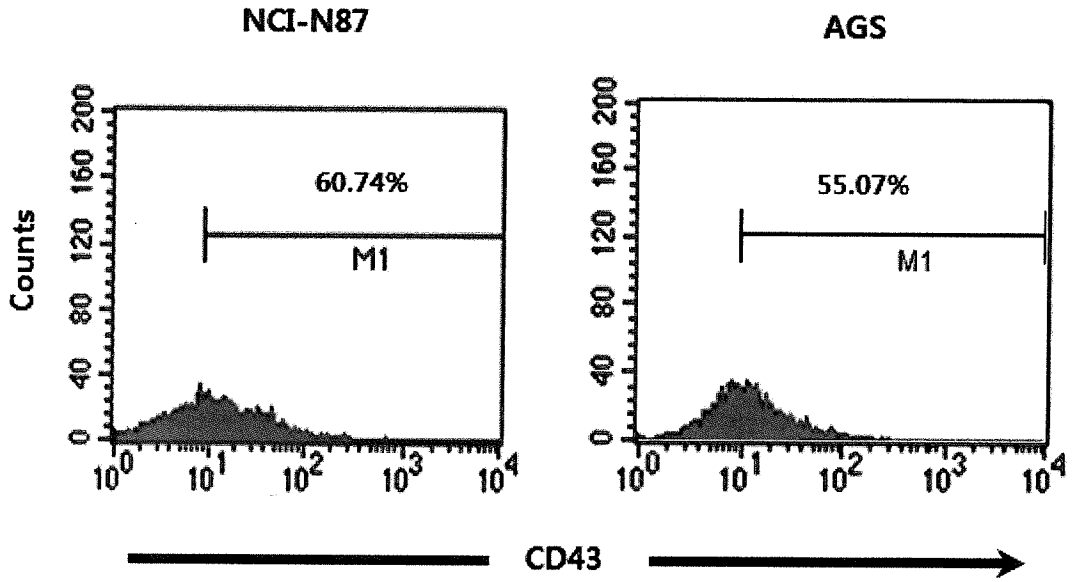

NCI-N87
AGS
Counts
60.74%
55.07%
M1
M1
0
40
80
120
160
200
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
CD43

FIG. 2a

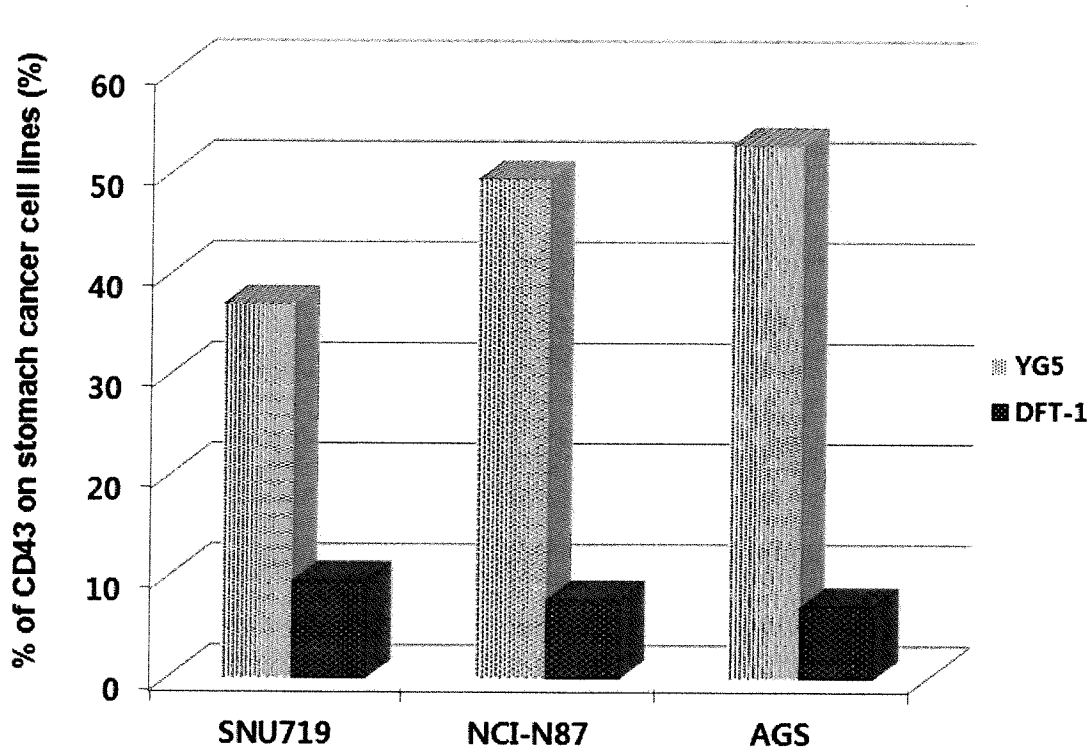

% of CD43 on stomach cancer cell lines (%)
60
50
40
30
20
10
0
SNU719
NCI-N87
AGS
YG5
DFT-1

FIG. 2b

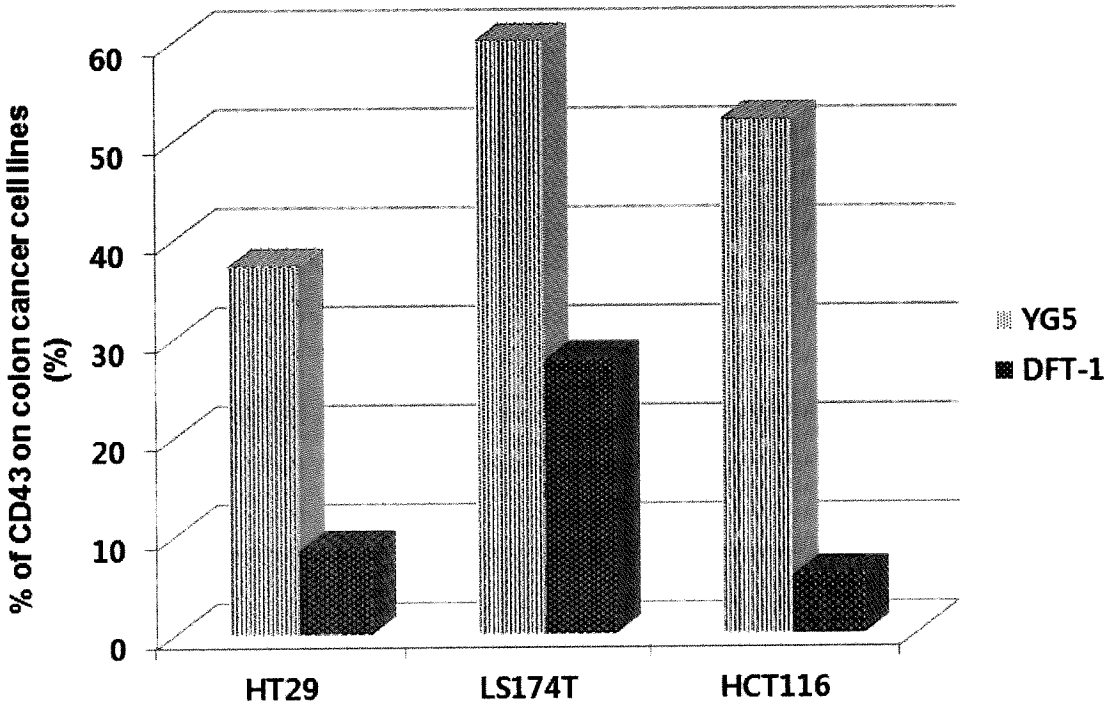

% of CD43 on colon cancer cell lines (%)
60
50
40
30
20
10
0
HT29
LS174T
HCT116
YG5
DFT-1

FIG. 2c

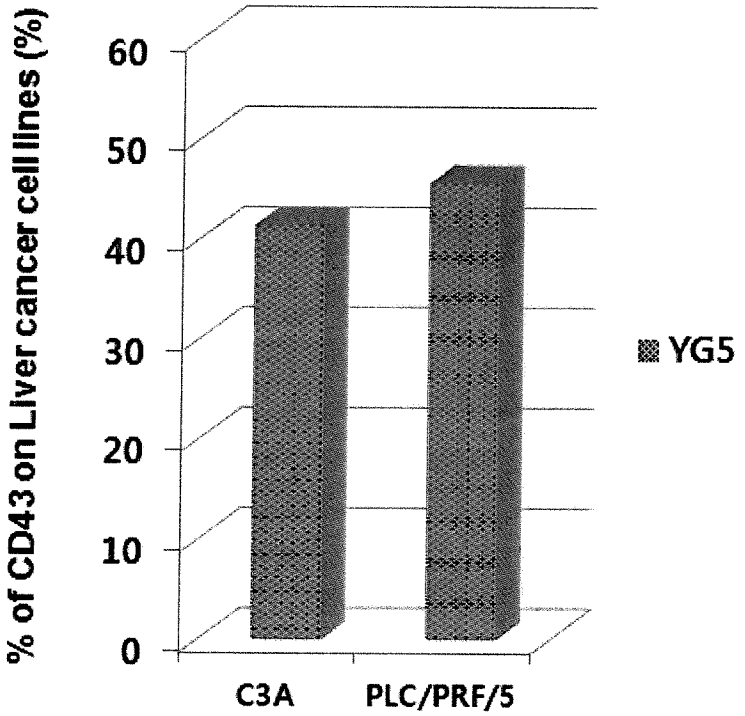

% of CD43 on Liver cancer cell lines (%)
60
50
40
30
20
10
0
C3A
PLC/PRF/5
YG5

FIG. 3

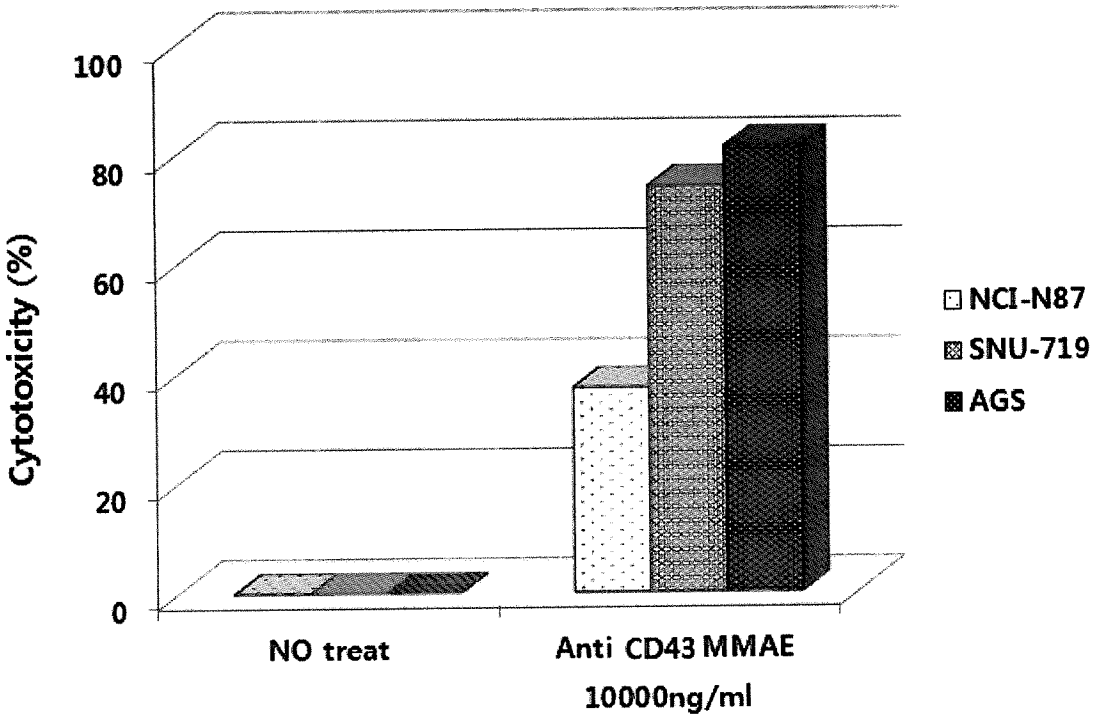

Cytotoxicity (%)
100
80
60
40
20
0
NO treat
Anti CD43 MMAE
10000ng/ml
NCI-N87
SNU-719
AGS

FIG. 4

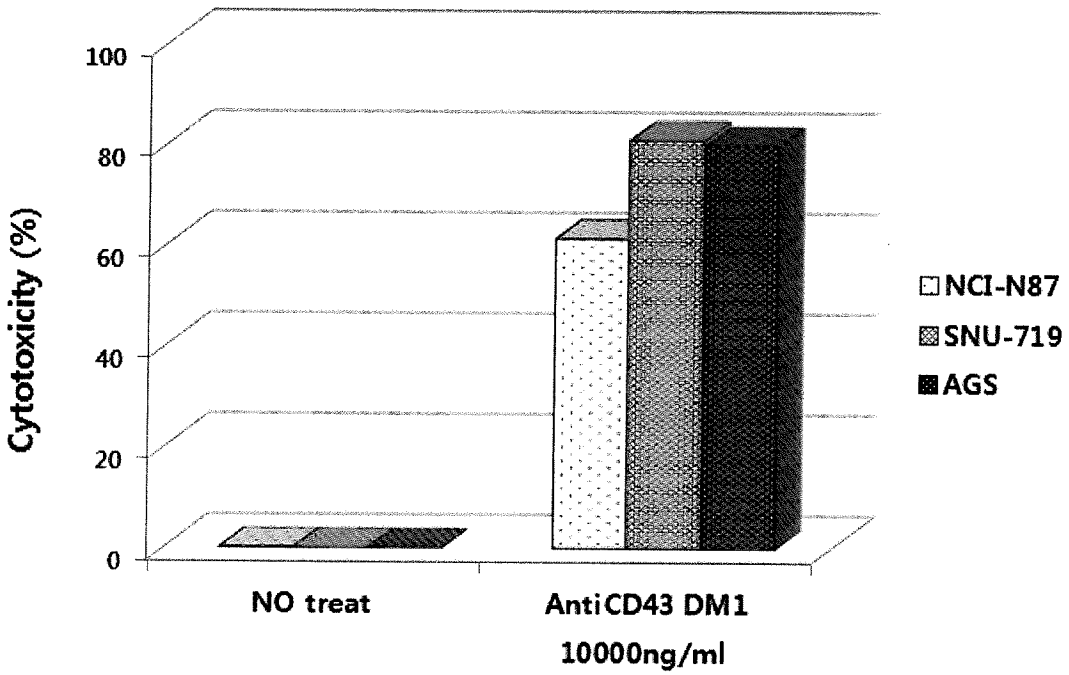

100
80
60
40
20
0
Cytotoxicity (%)
NO treat
AntiCD43 DM1
10000ng/ml
NCI-N87
SNU-719
AGS

FIG. 5

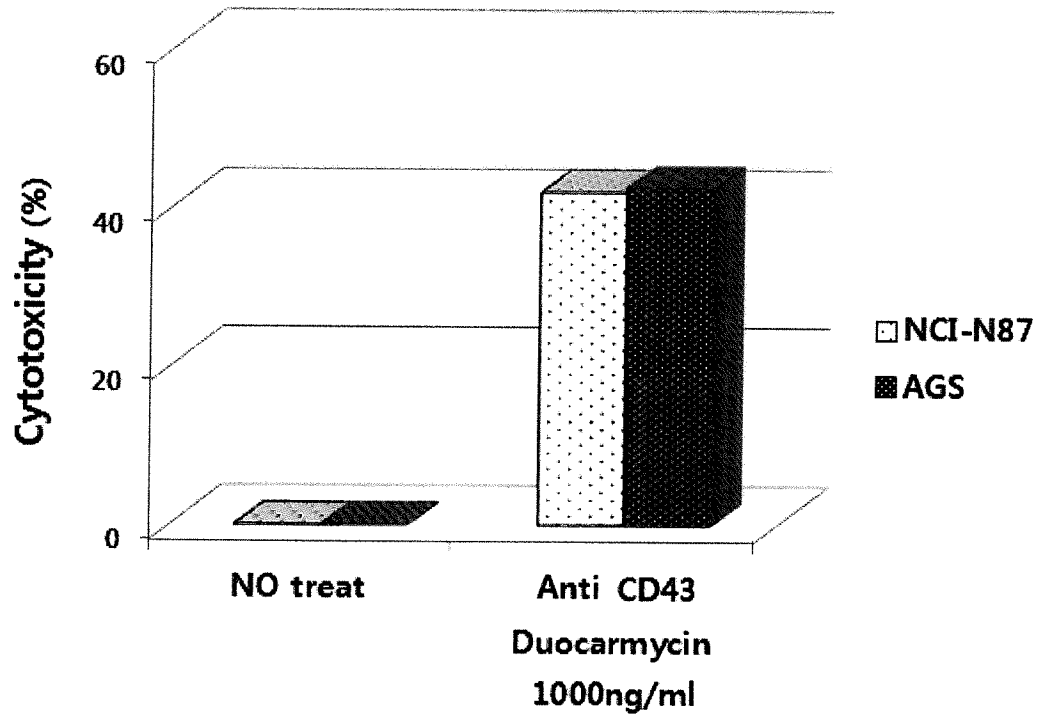

60
40
20
0
Cytotoxicity (%)
NO treat
Anti CD43
Duocarmycin
1000ng/ml
NCI-N87
AGS

FIG. 6

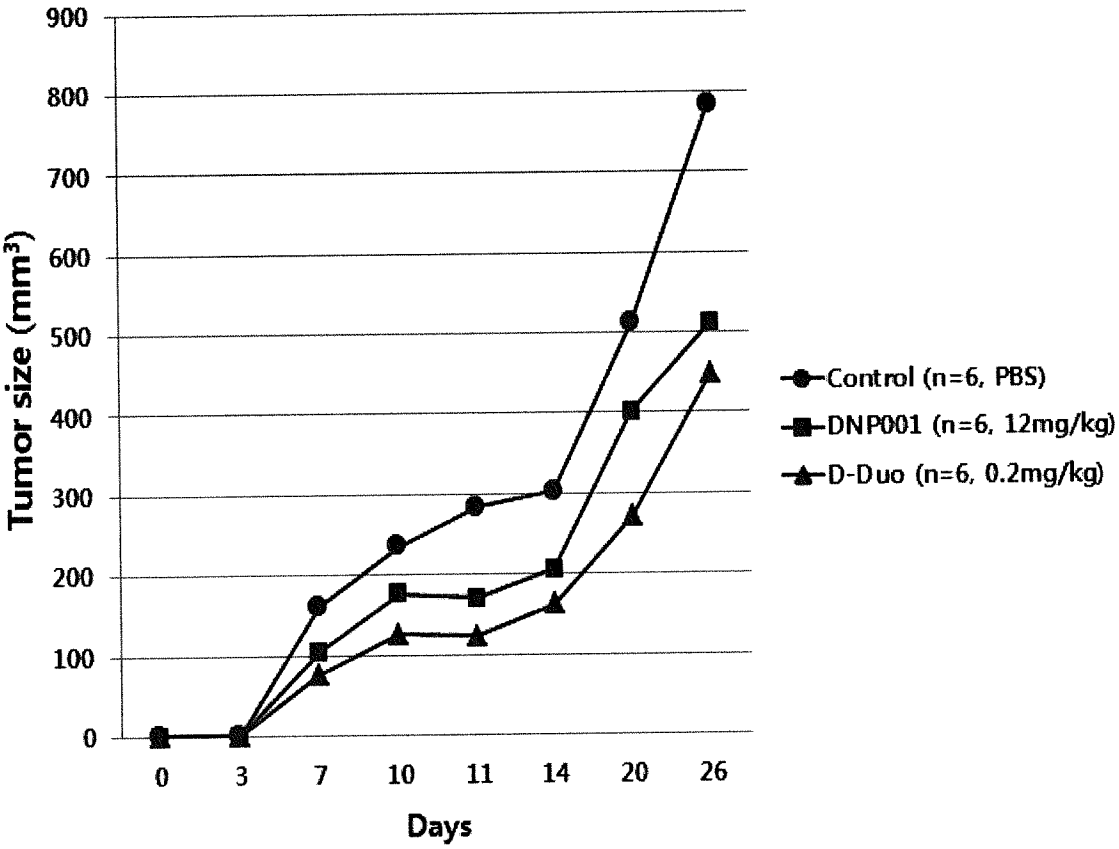

900
800
700
600
500
400
300
200
100
0
Tumor size (mm³)
0
3
7
10
11
14
20
26
Days
Control (n=6, PBS)
DNP001 (n=6, 12mg/kg)
D-Duo (n=6, 0.2mg/kg)

FIG. 7

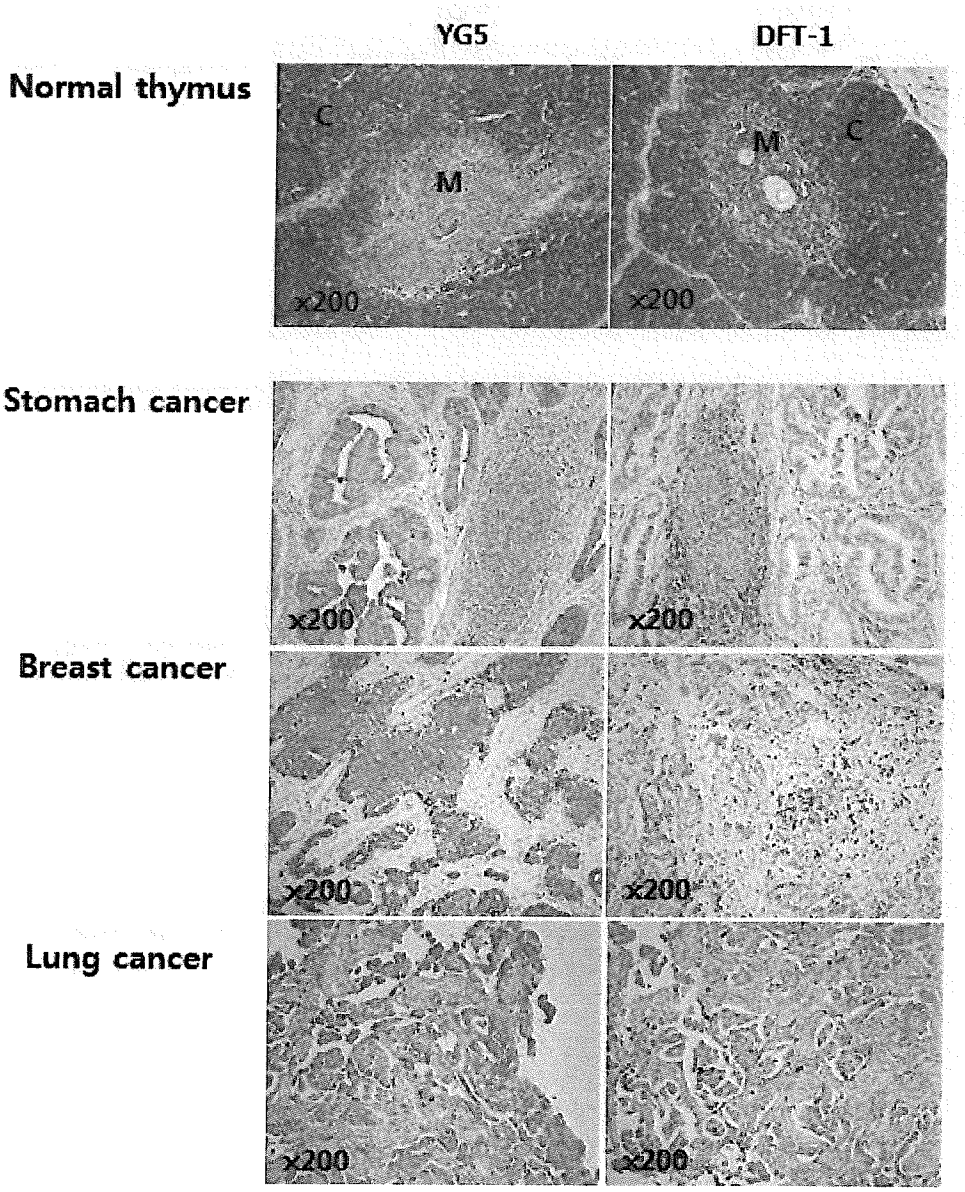

YG5
DFT-1
Normal thymus
C
M
M
C
x200
x200
Stomach cancer
x200
x200
Breast cancer
x200
x200
Lung cancer
x200
x200

FIG. 8

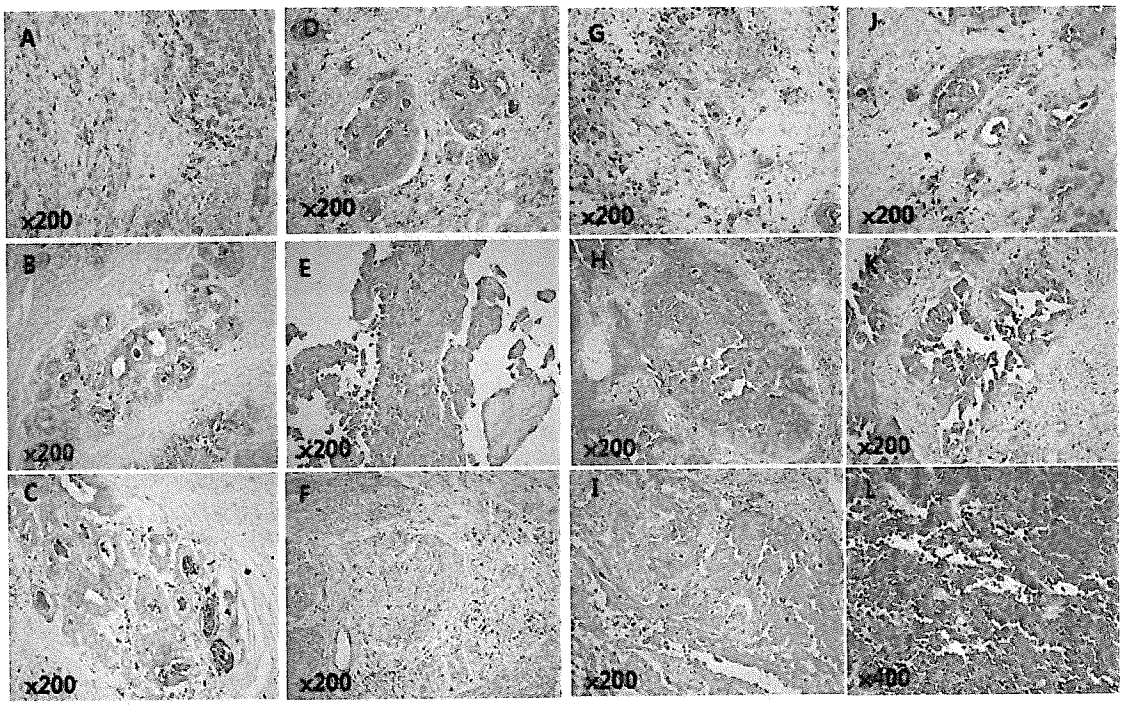

A
x200
D
x200
G
x200
J
x200
B
x200
E
x200
H
x200
K
x200
C
x200
F
x200
I
x200
L
x400

FIG. 9

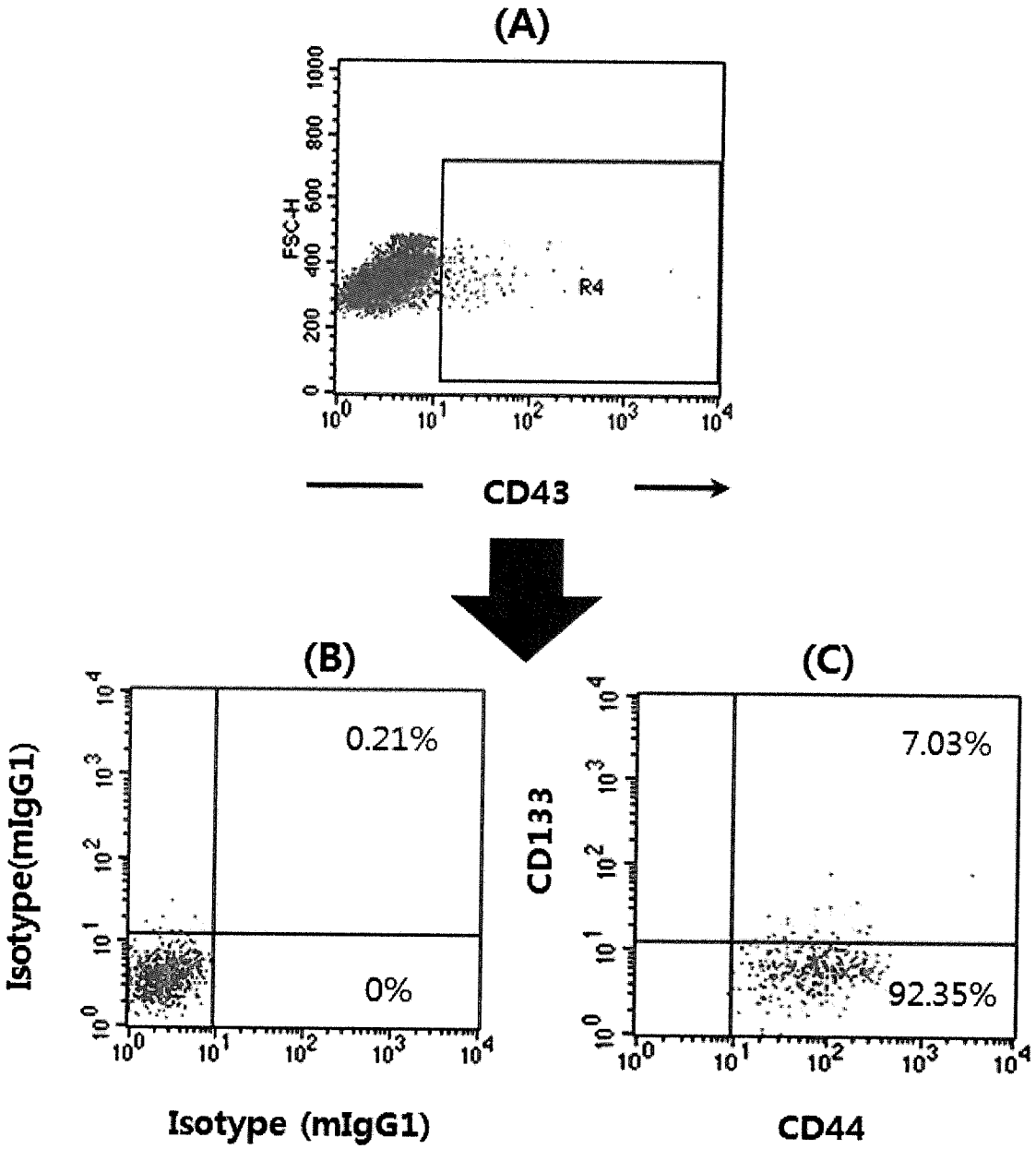

(A)
FSC-H
R4
CD43
(B)
0.21%
0%
Isotype(mIgG1)
Isotype (mIgG1)
(C)
7.03%
92.35%
CD133
CD44

FIG. 10

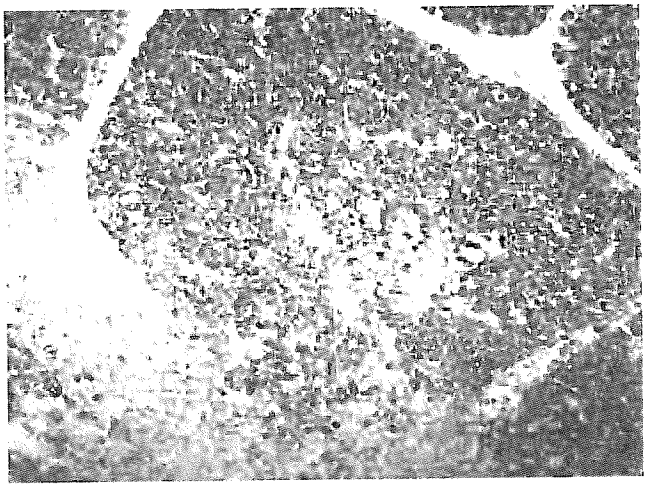


FIG. 11

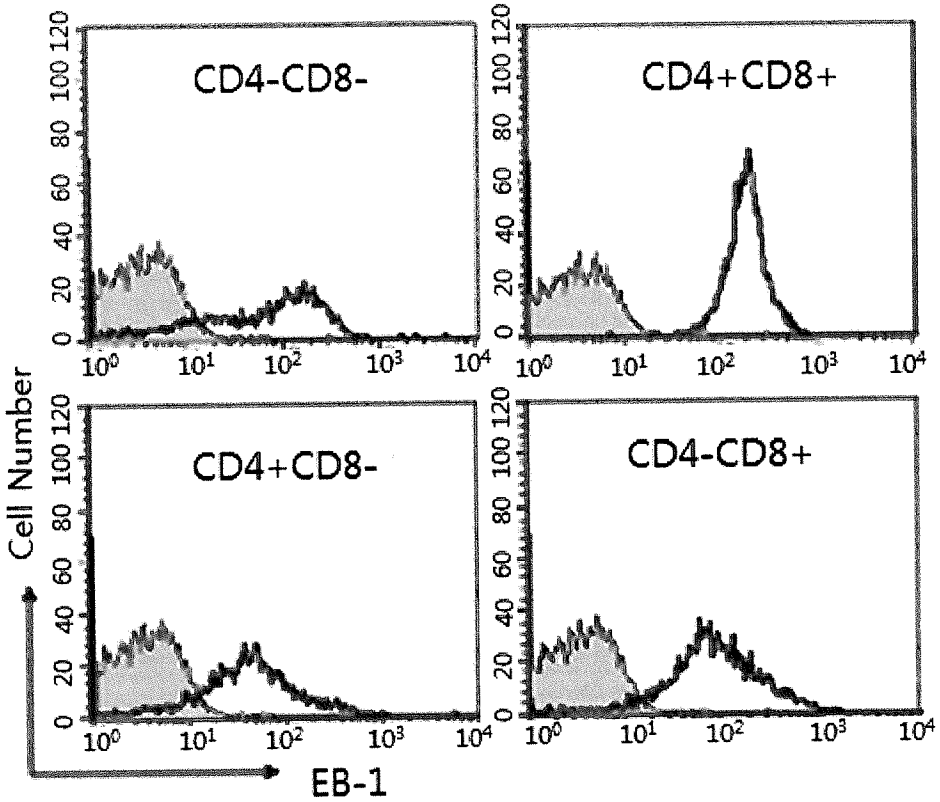

CD4-CD8-
CD4+CD8+
CD4+CD8-
CD4-CD8+
Cell Number
EB-1
0
20
40
60
80
100
120
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$

FIG. 12

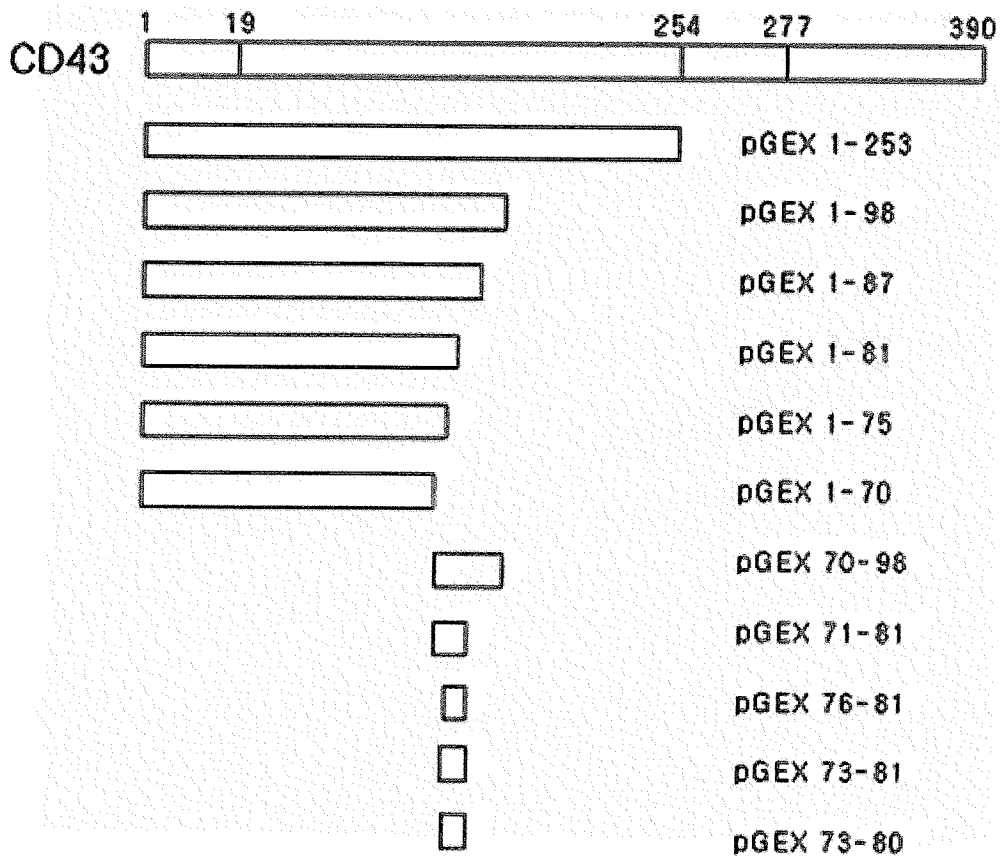

CD43
1
19
254
277
390
pGEX 1-253
pGEX 1-98
pGEX 1-87
pGEX 1-81
pGEX 1-75
pGEX 1-70
pGEX 70-98
pGEX 71-81
pGEX 76-81
pGEX 73-81
pGEX 73-80

FIG. 13

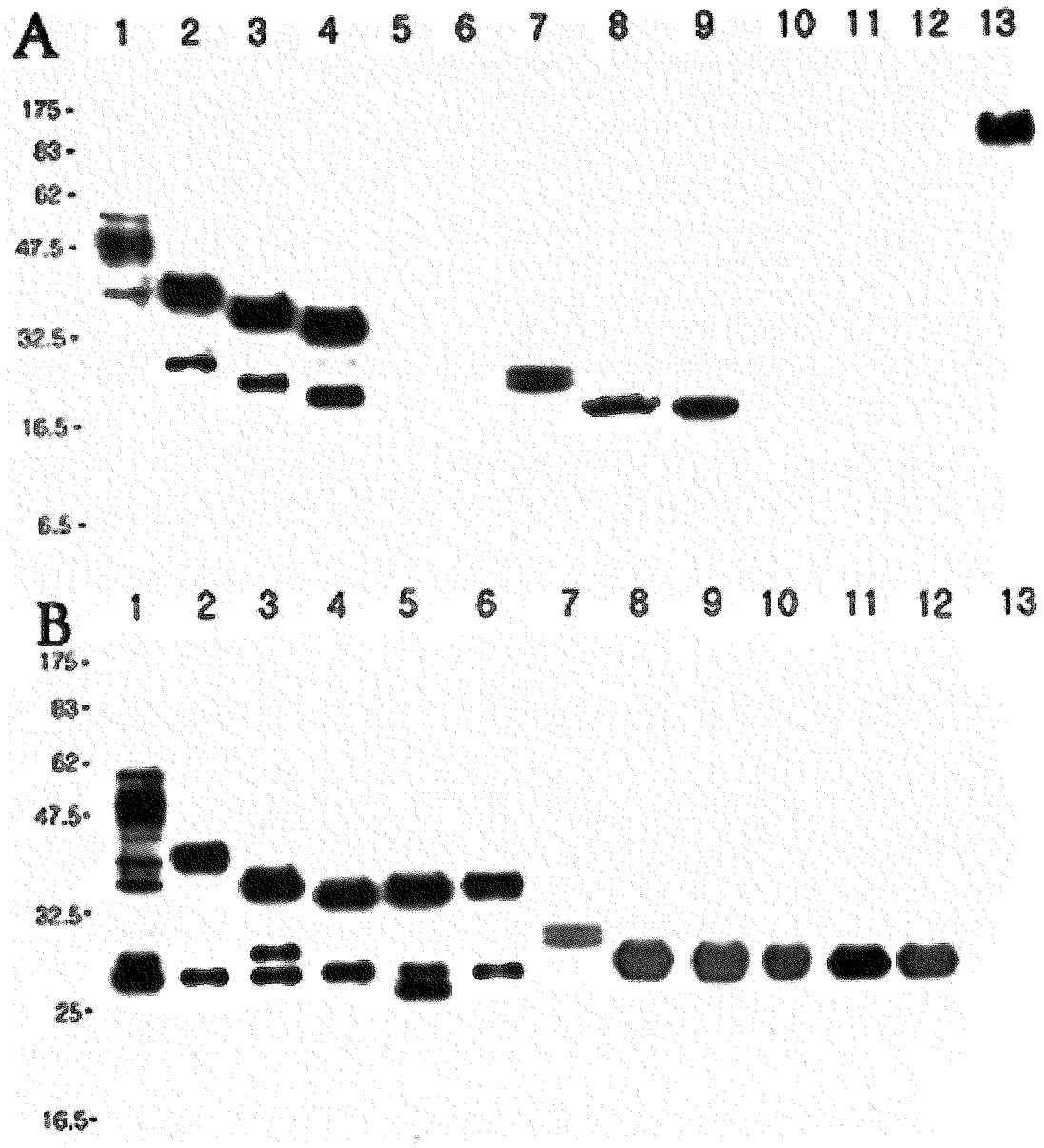

A
1 2 3 4 5 6 7 8 9 10 11 12 13
175-
83-
62-
47.5-
32.5-
16.5-
6.5-
B
1 2 3 4 5 6 7 8 9 10 11 12 13
175-
83-
62-
47.5-
32.5-
25-
16.5-

FIG. 14

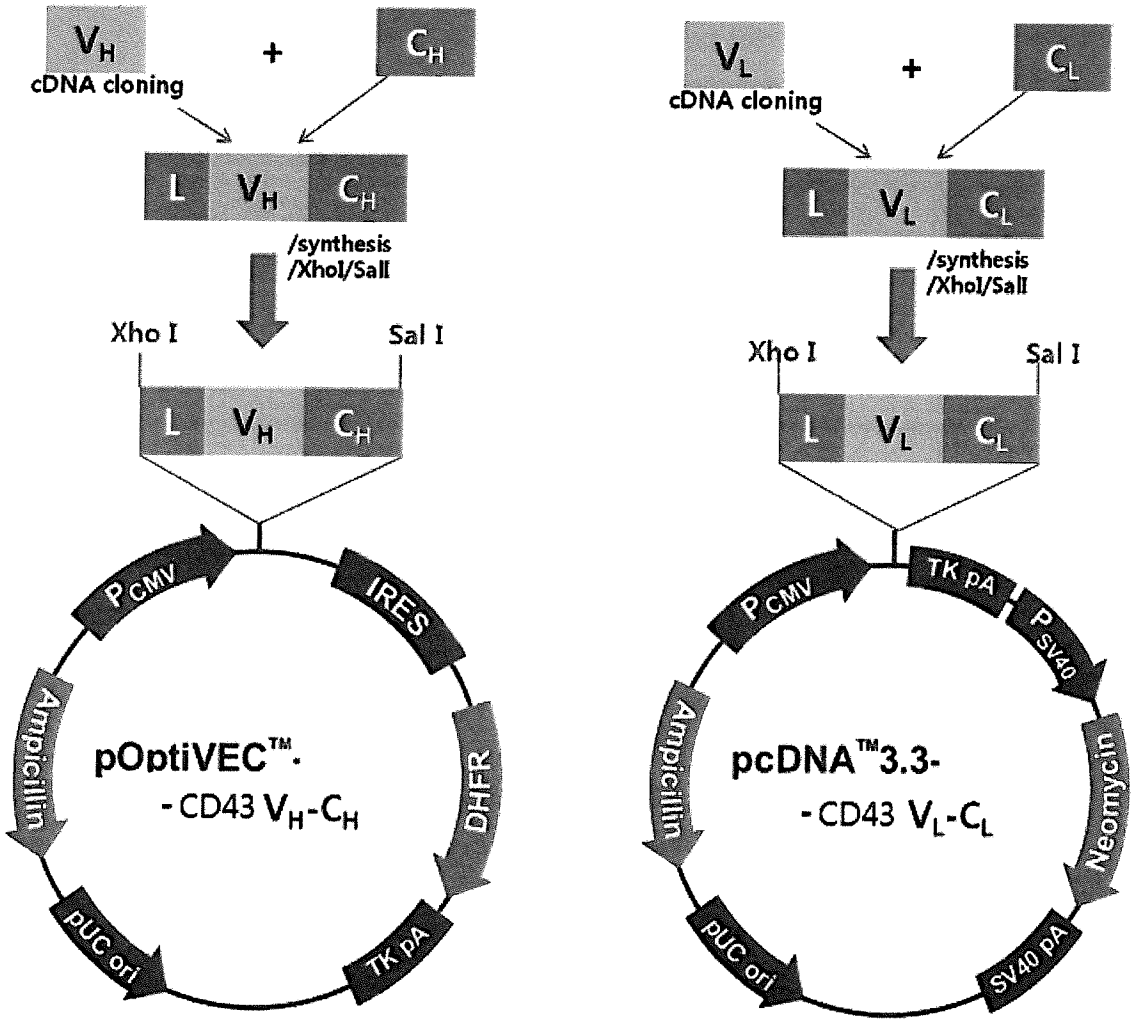

V_H
cDNA cloning
+
C_H
L V_H C_H
/synthesis
/XhoI/SalI
Xho I
Sal I
L V_H C_H
P CMV
IRES
Ampicillin
pOptiVEC™-
- CD43 V_H-C_H
DHFR
pUC ori
TK pA
V_L
cDNA cloning
+
C_L
L V_L C_L
/synthesis
/XhoI/SalI
Xho I
Sal I
L V_L C_L
P CMV
TK pA
P SV40
Ampicillin
pcDNA™3.3-
- CD43 V_L-C_L
Neomycin
pUC ori
SV40 pA

FIG. 15

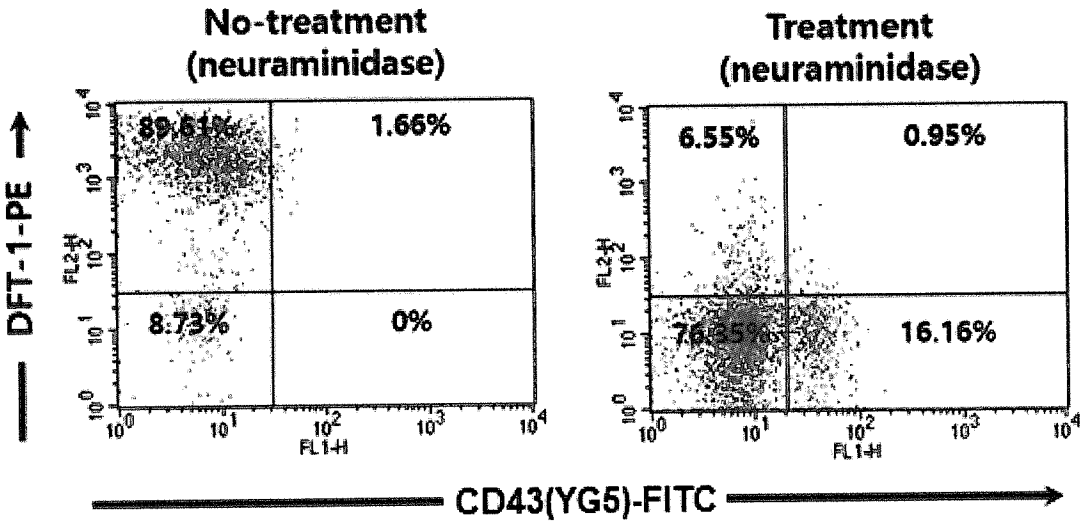

No-treatment
(neuraminidase)
Treatment
(neuraminidase)
89.61%
1.66%
8.73%
0%
6.55%
0.95%
76.35%
16.16%
DFT-1-PE
CD43(YG5)-FITC
FL2-H
FL1-H

FIG. 16

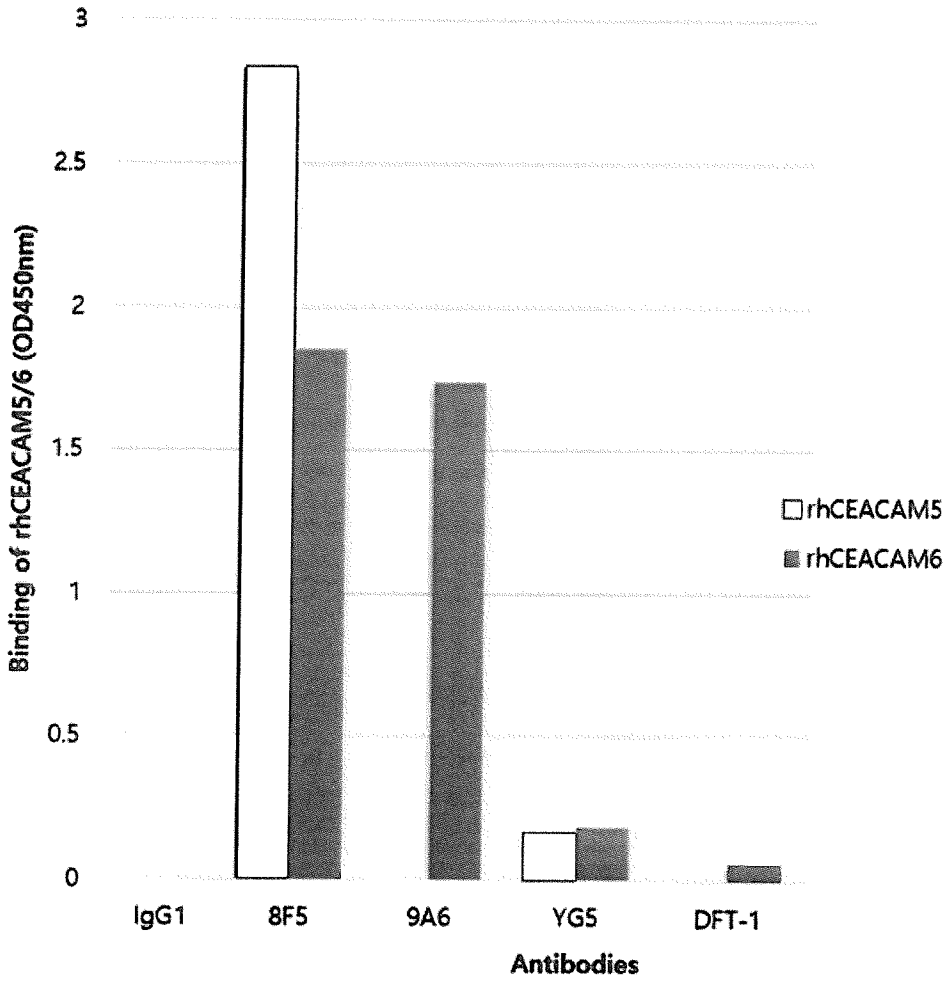

3
2.5
2
1.5
1
0.5
0
Binding of rhCEACAM5/6 (OD450nm)
rhCEACAM5
rhCEACAM6
IgG1
8F5
9A6
YG5
DFT-1
Antibodies

FIG. 17a

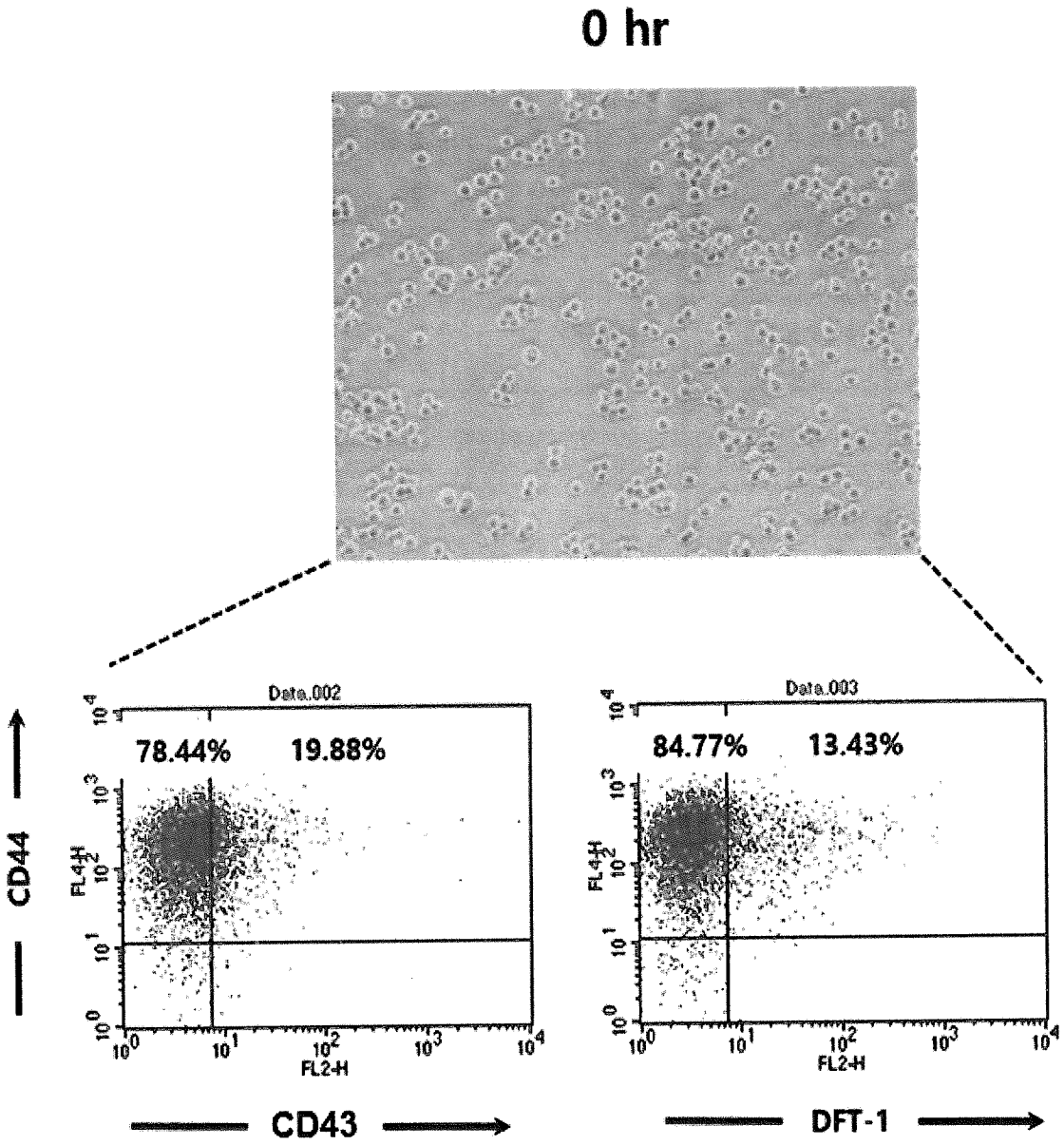

0 hr
Data.002
78.44%
19.88%
CD44
FL4-H
FL2-H
CD43
Data.003
84.77%
13.43%
FL4-H
FL2-H
DFT-1

FIG. 17b

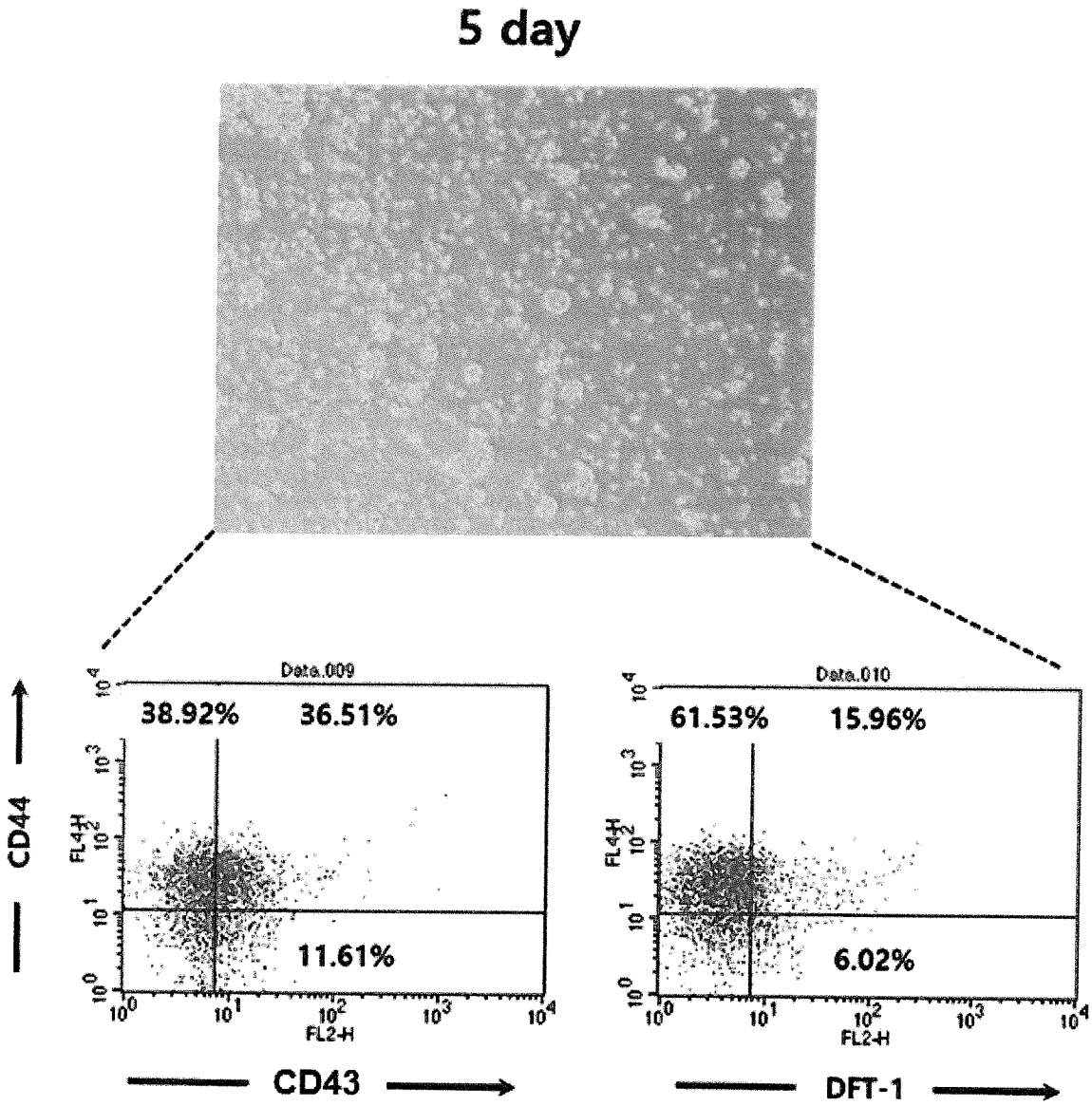

5 day
Data.009
38.92%
36.51%
11.61%
FL4-H
FL2-H
CD44
CD43
Data.010
61.53%
15.96%
6.02%
FL4-H
FL2-H
DFT-1

FIG. 18

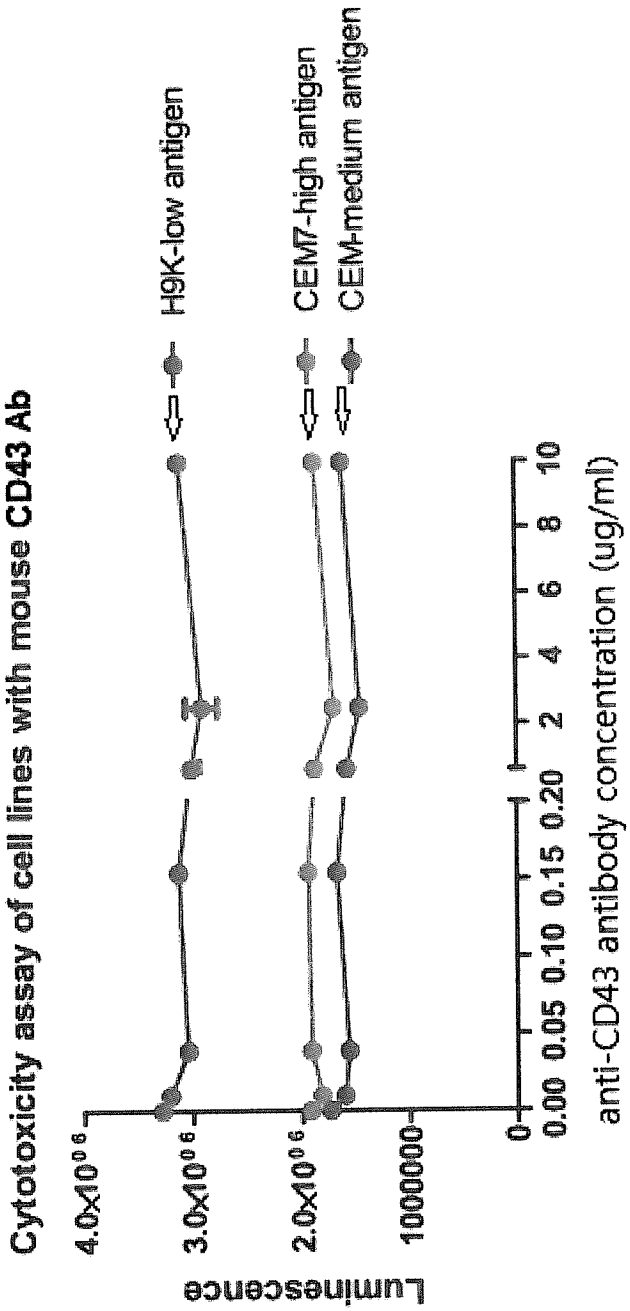

Cytotoxicity assay of cell lines with mouse CD43 Ab
H9K-low antigen
CEM7-high antigen
CEM-medium antigen
Luminescence
4.0x10^6
3.0x10^6
2.0x10^6
1000000
0
0.00 0.05 0.10 0.15 0.20 2 4 6 8 10
anti-CD43 antibody concentration (ug/ml)

FIG. 19

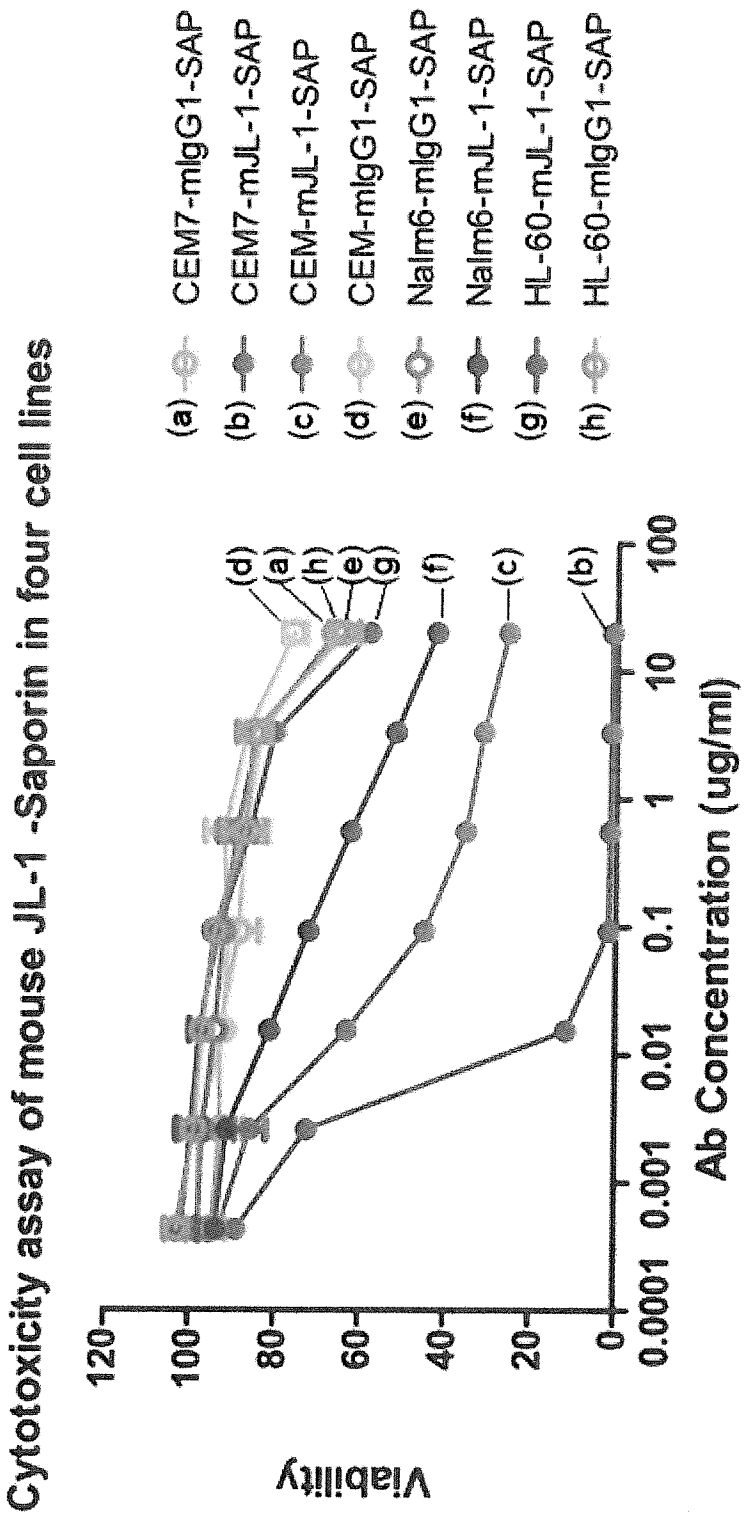

Cytotoxicity assay of mouse JL-1 -Saporin in four cell lines
(a) CEM7-mIgG1-SAP
(b) CEM7-mJL-1-SAP
(c) CEM-mJL-1-SAP
(d) CEM-mIgG1-SAP
(e) Nalm6-mIgG1-SAP
(f) Nalm6-mJL-1-SAP
(g) HL-60-mJL-1-SAP
(h) HL-60-mIgG1-SAP
Viability
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
Ab Concentration (ug/ml)
(d)
(a)
(h)
(e)
(g)
(f)
(c)
(b)

FIG. 20

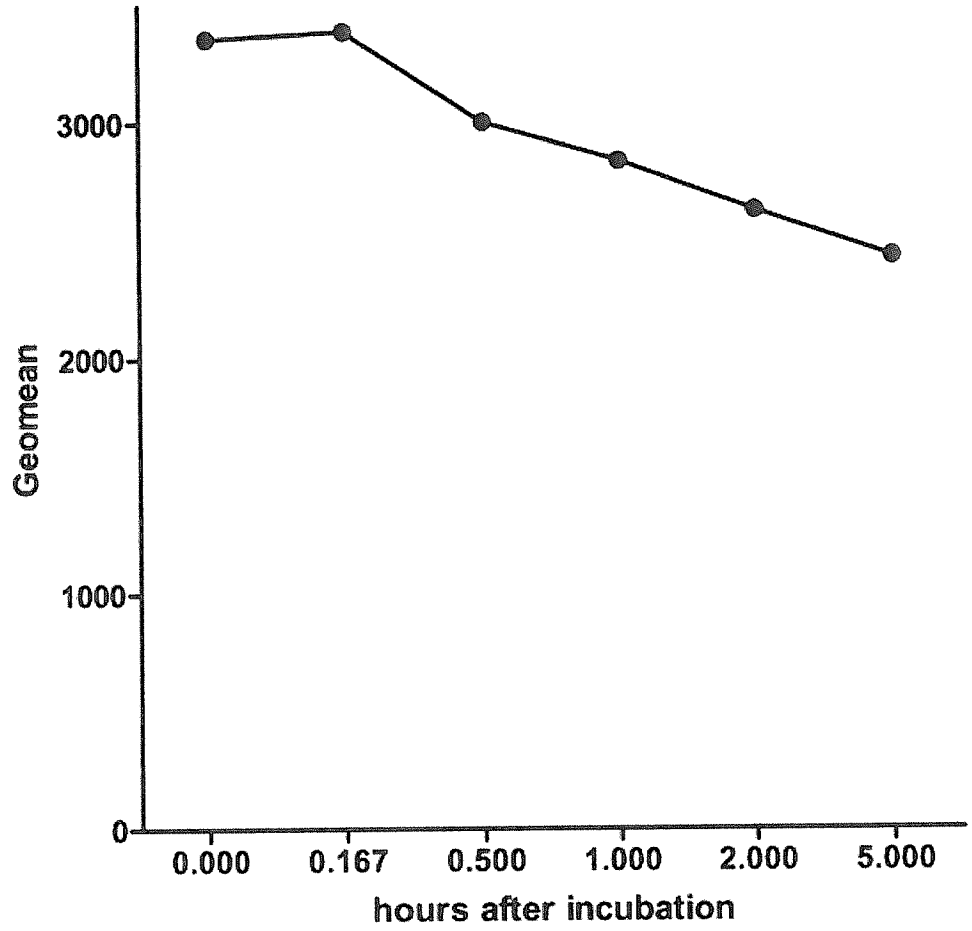

3000
2000
1000
0
Geomean
0.000
0.167
0.500
1.000
2.000
5.000
hours after incubation

FIG. 21

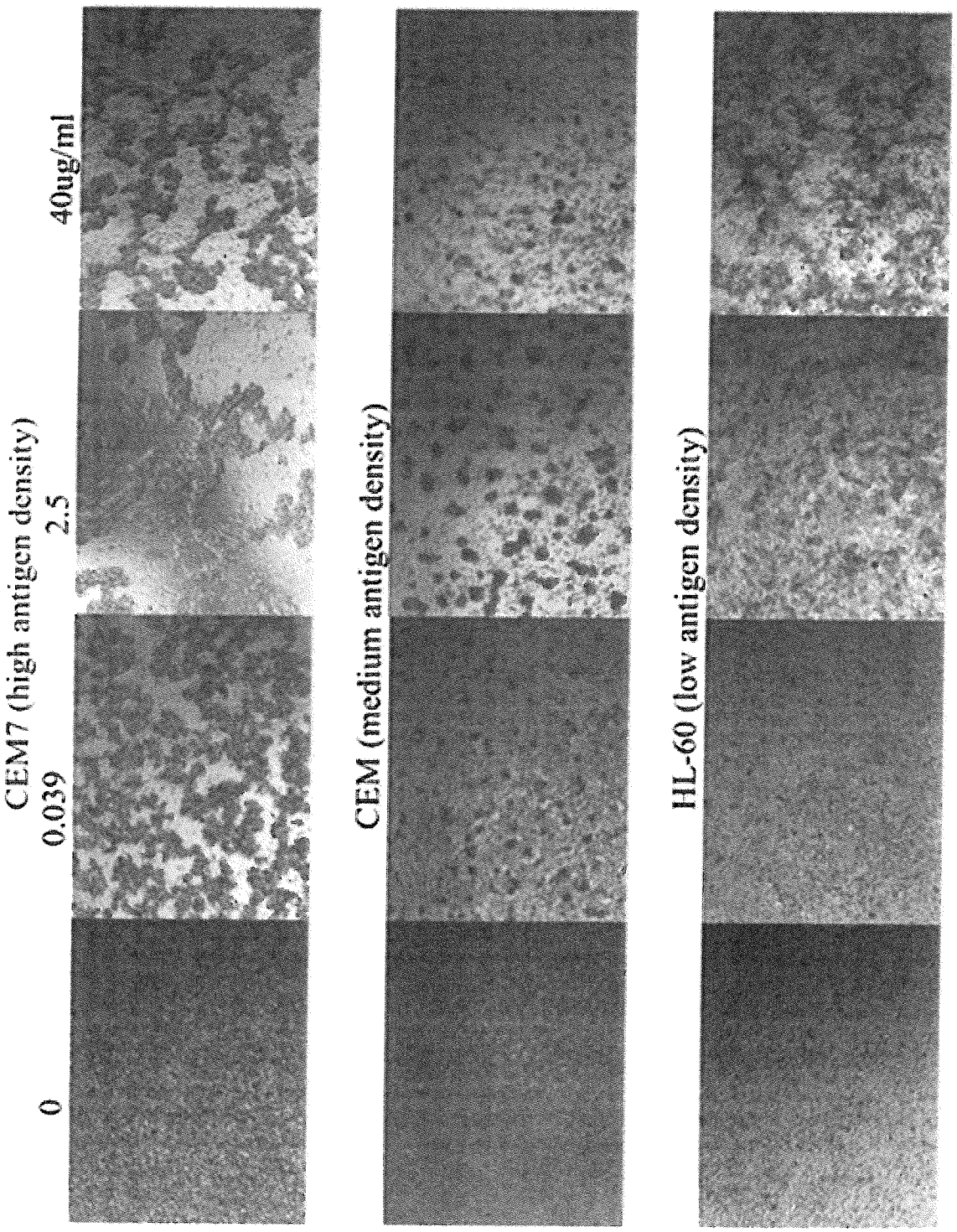

CEM7 (high antigen density)
0
0.039
2.5
40ug/ml
CEM (medium antigen density)
HL-60 (low antigen density)

FIG. 22

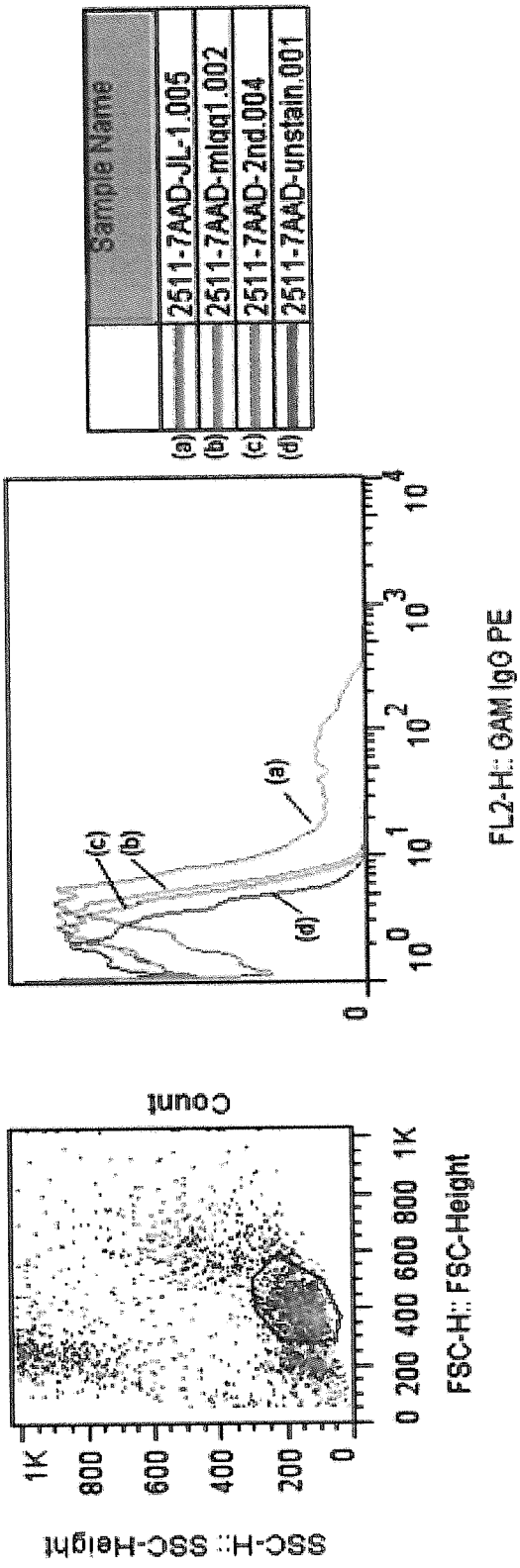

Sample Name
2511-7AAD-JL-1.005
2511-7AAD-mlgg1.002
2511-7AAD-2nd.004
2511-7AAD-unstain.001
(a)
(b)
(c)
(d)
FL2-H:: GAM IgG PE
SSC-H:: SSC-Height
Count
FSC-H:: FSC-Height

FIG. 23

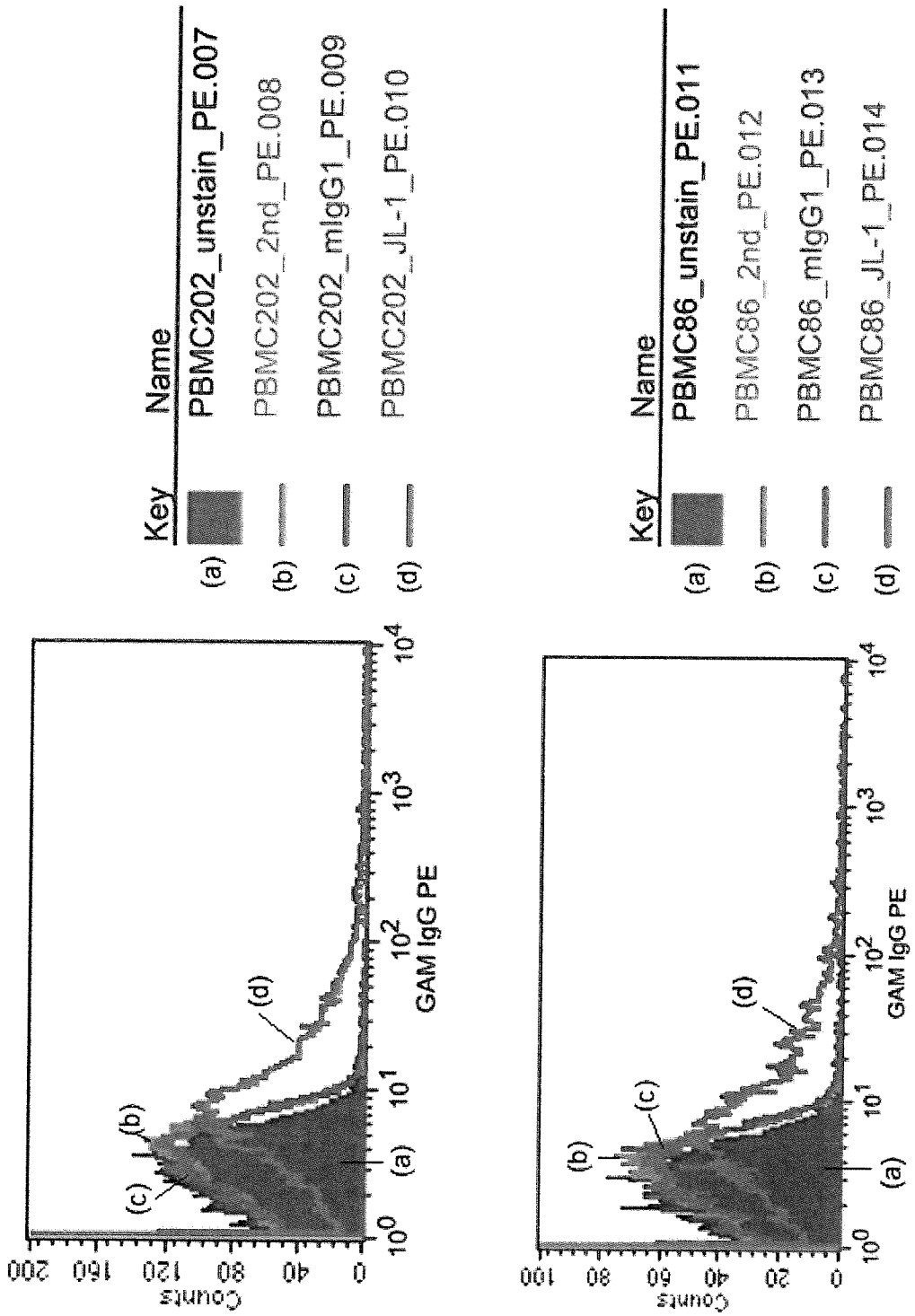

Key
Name
(a)
PBMC202_unstain_PE.007
(b)
PBMC202_2nd_PE.008
(c)
PBMC202_mIgG1_PE.009
(d)
PBMC202_JL-1_PE.010
Counts
GAM IgG PE
Key
Name
(a)
PBMC86_unstain_PE.011
(b)
PBMC86_2nd_PE.012
(c)
PBMC86_mIgG1_PE.013
(d)
PBMC86_JL-1_PE.014
Counts
GAM IgG PE

FIG. 24

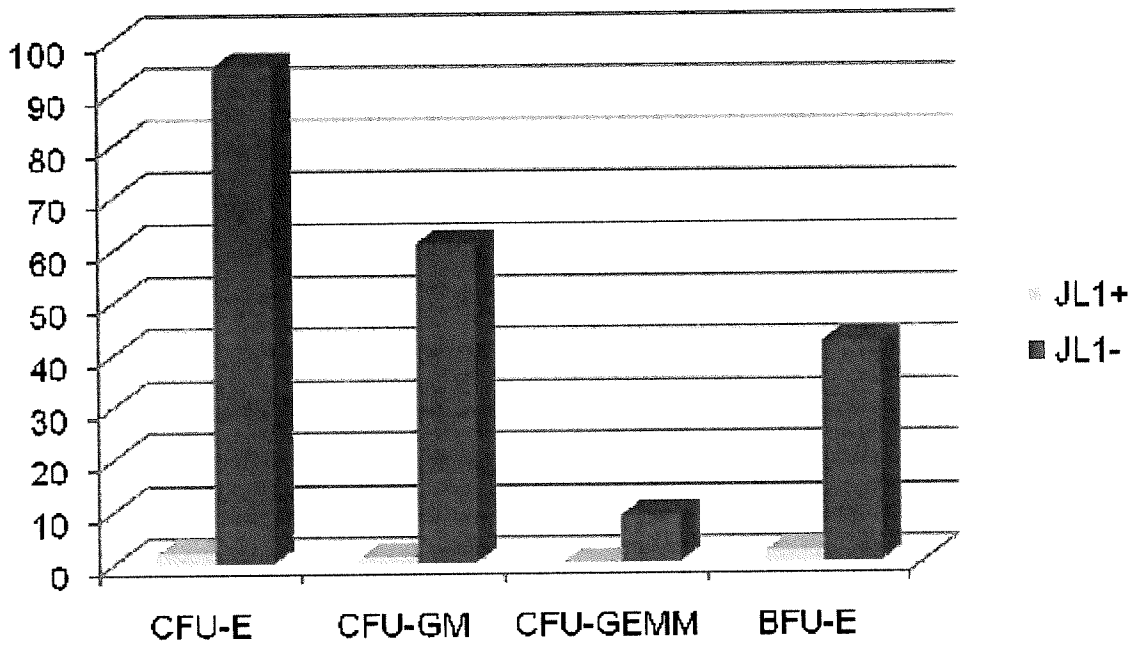


FIG. 25

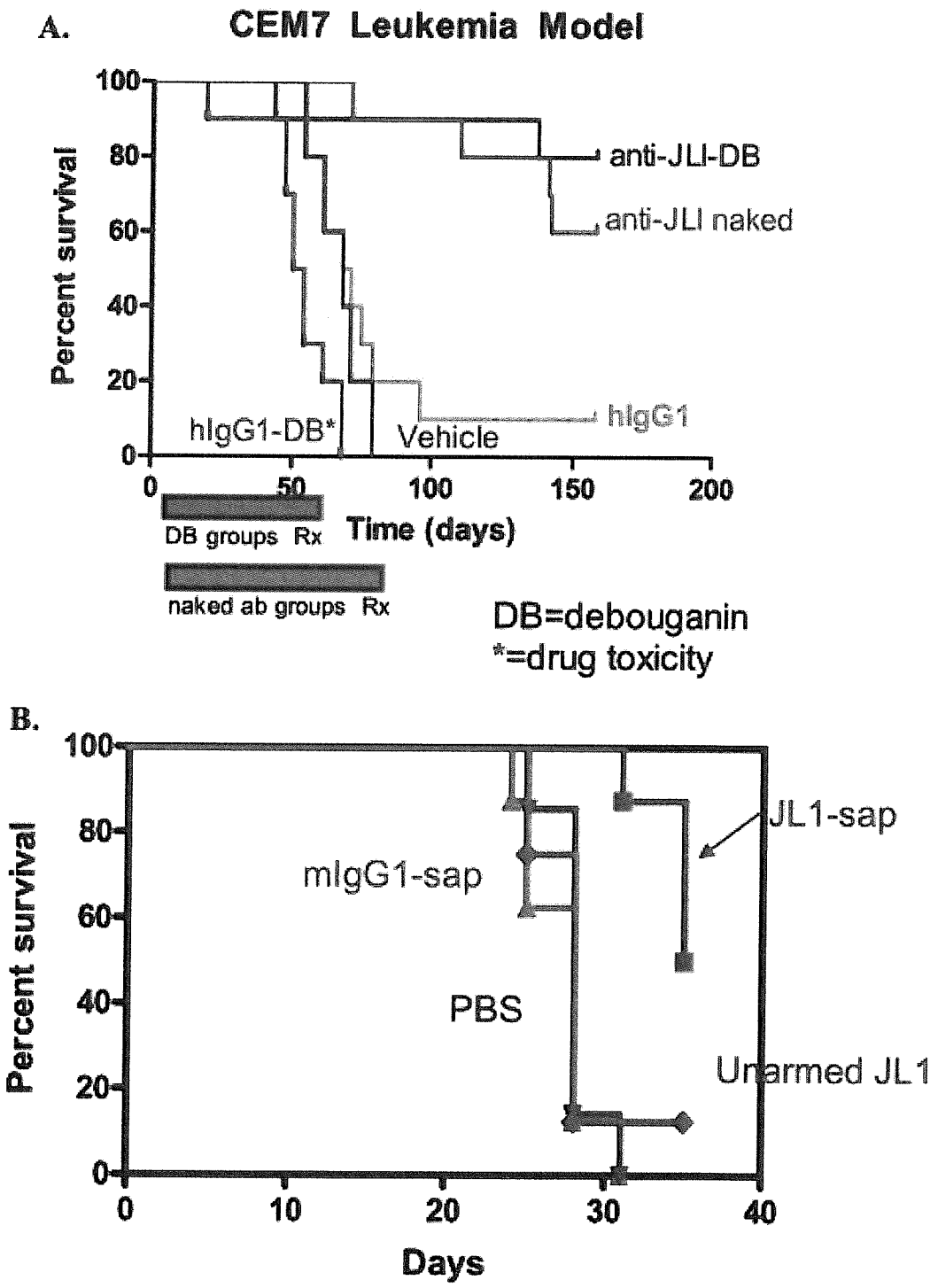

A.
CEM7 Leukemia Model
Percent survival
100
80
60
40
20
0
anti-JLI-DB
anti-JLI naked
hIgG1
hIgG1-DB*
Vehicle
0
50
100
150
200
DB groups Rx
Time (days)
naked ab groups Rx
DB=debouganin
*=drug toxicity
B.
Percent survival
100
80
60
40
20
0
mIgG1-sap
JL1-sap
PBS
Unarmed JL1
0
10
20
30
40
Days

FIG. 26

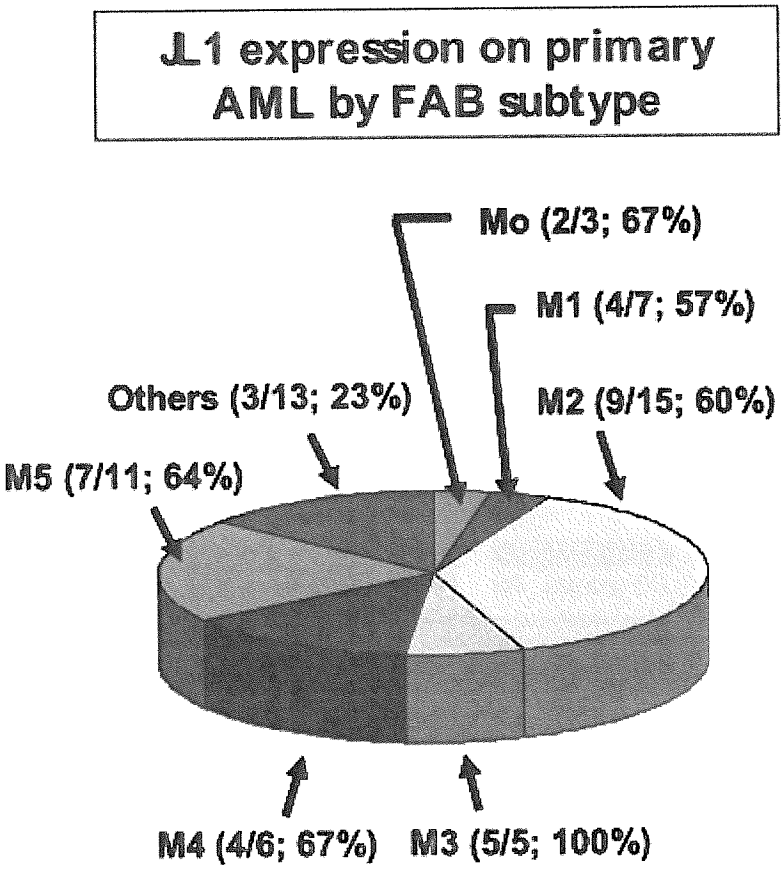
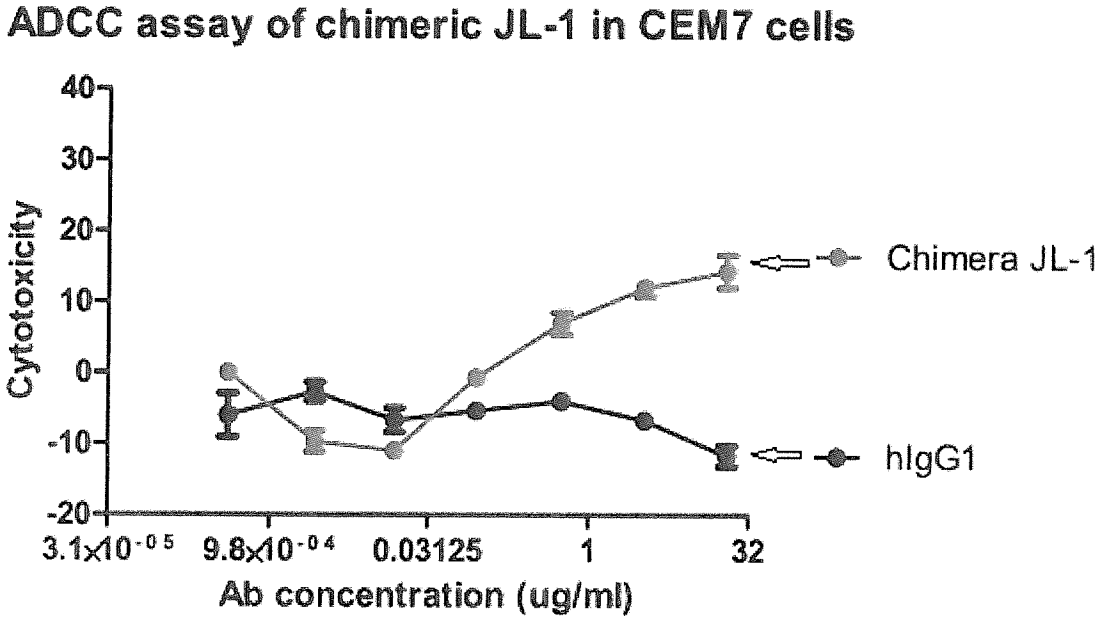

JL1 expression on primary AML by FAB subtype
Mo (2/3; 67%)
M1 (4/7; 57%)
M2 (9/15; 60%)
Others (3/13; 23%)
M5 (7/11; 64%)
M4 (4/6; 67%)
M3 (5/5; 100%)

FIG. 27

A.


ADCC assay of chimeric JL-1 in CEM7 cells
Cytotoxicity
40
30
20
10
0
-10
-20
3.1x10-05
9.8x10-04
0.03125
1
32
Ab concentration (ug/ml)
Chimera JL-1
hIgG1

B.

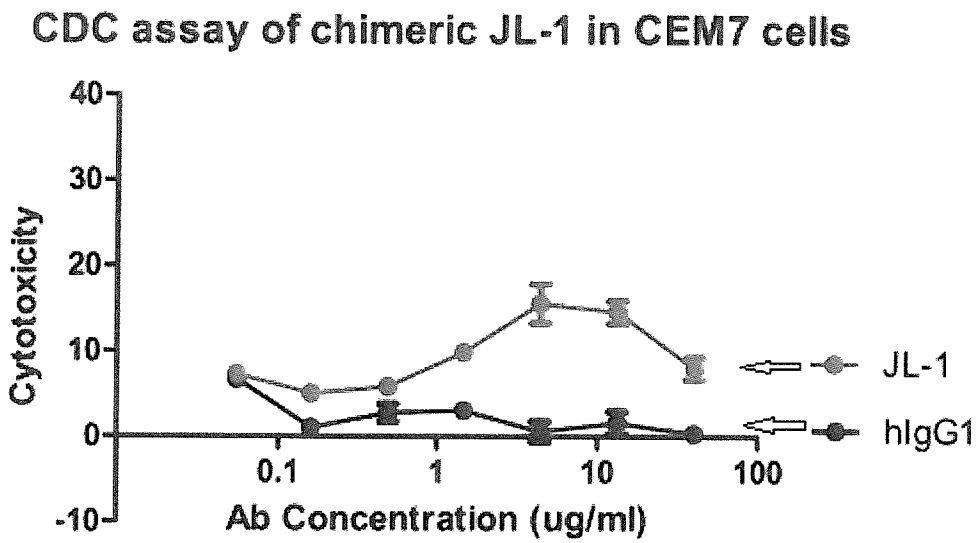
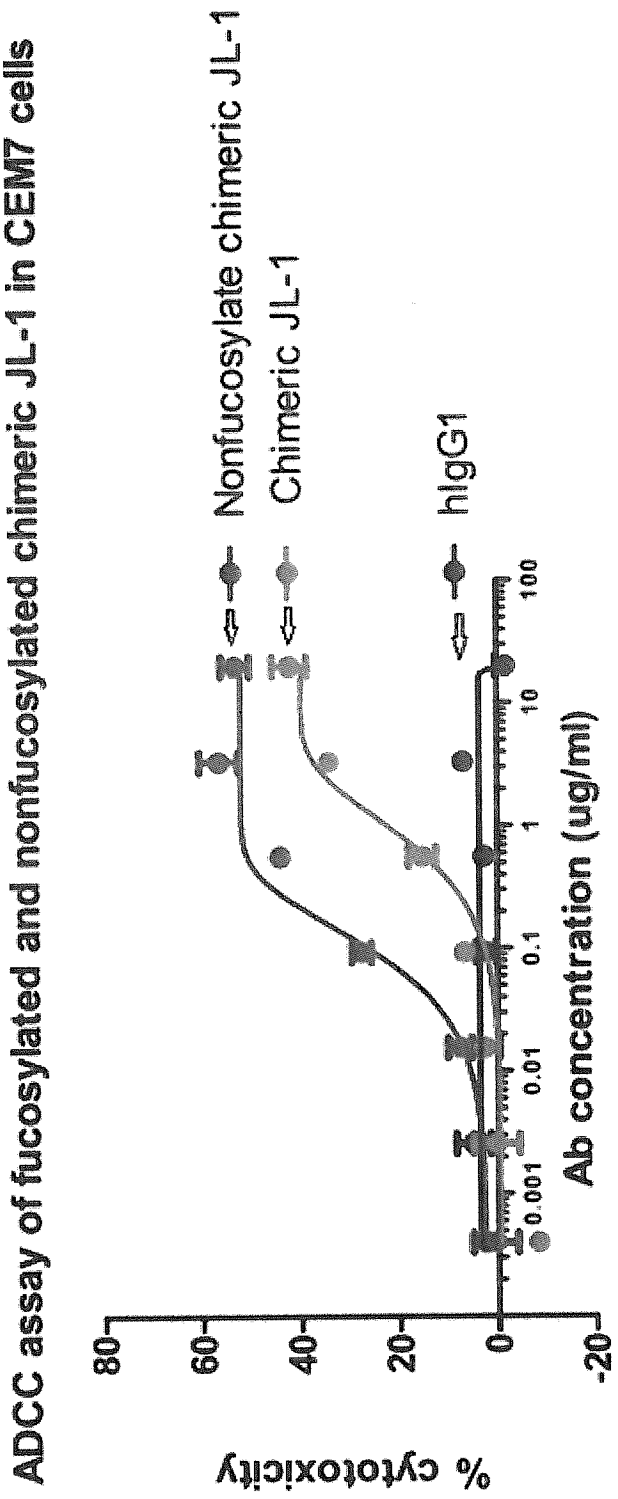

CDC assay of chimeric JL-1 in CEM7 cells
Cytotoxicity
40
30
20
10
0
-10
0.1
1
10
100
Ab Concentration (ug/ml)
JL-1
hIgG1

FIG. 28




| | hIgG1 | Nonfucosylate chimeric JL-1 | Chimeric JL-1 |
|---|---|---|---|
| EC50 | | 0.1093 | 2.828 |

FIG. 29

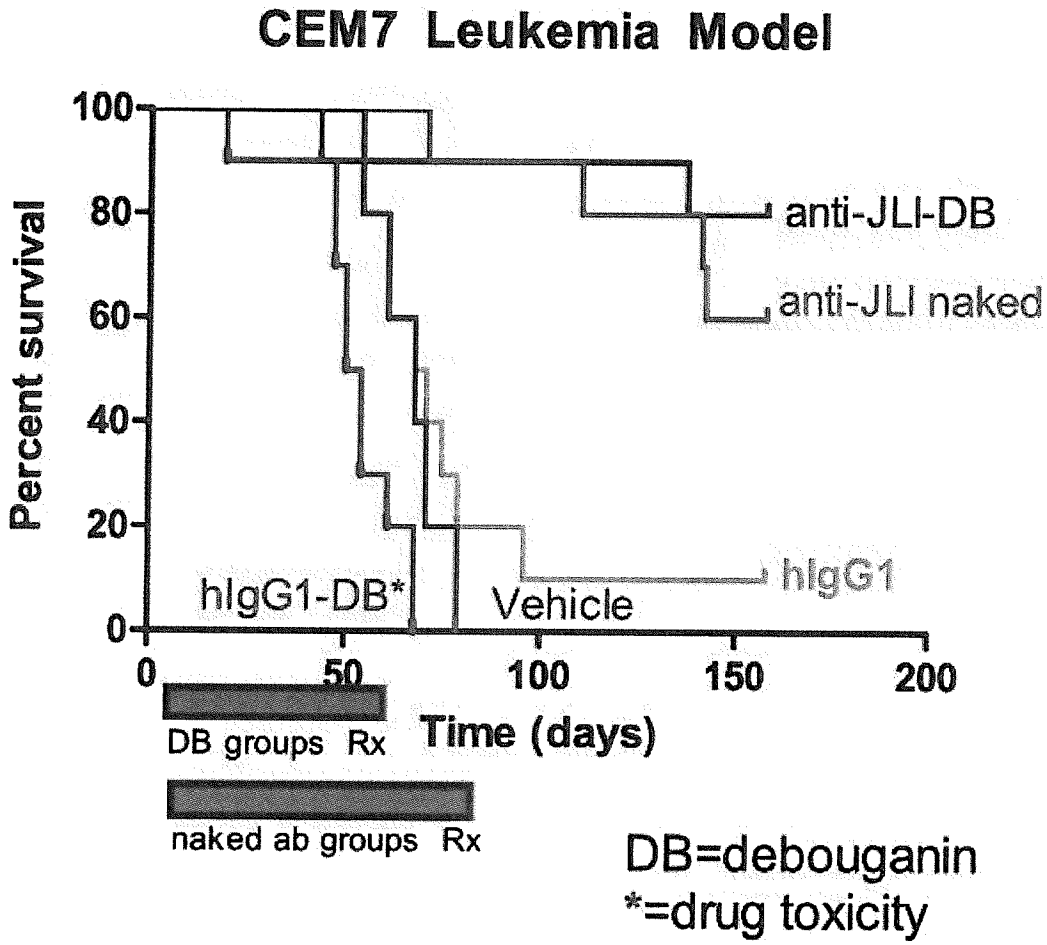

CEM7 Leukemia Model
Percent survival
100
80
60
40
20
0
0
50
100
150
200
Time (days)
anti-JLI-DB
anti-JLI naked
hIgG1
hIgG1-DB*
Vehicle
DB groups Rx
naked ab groups Rx
DB=debouganin
*=drug toxicity

FIG. 30

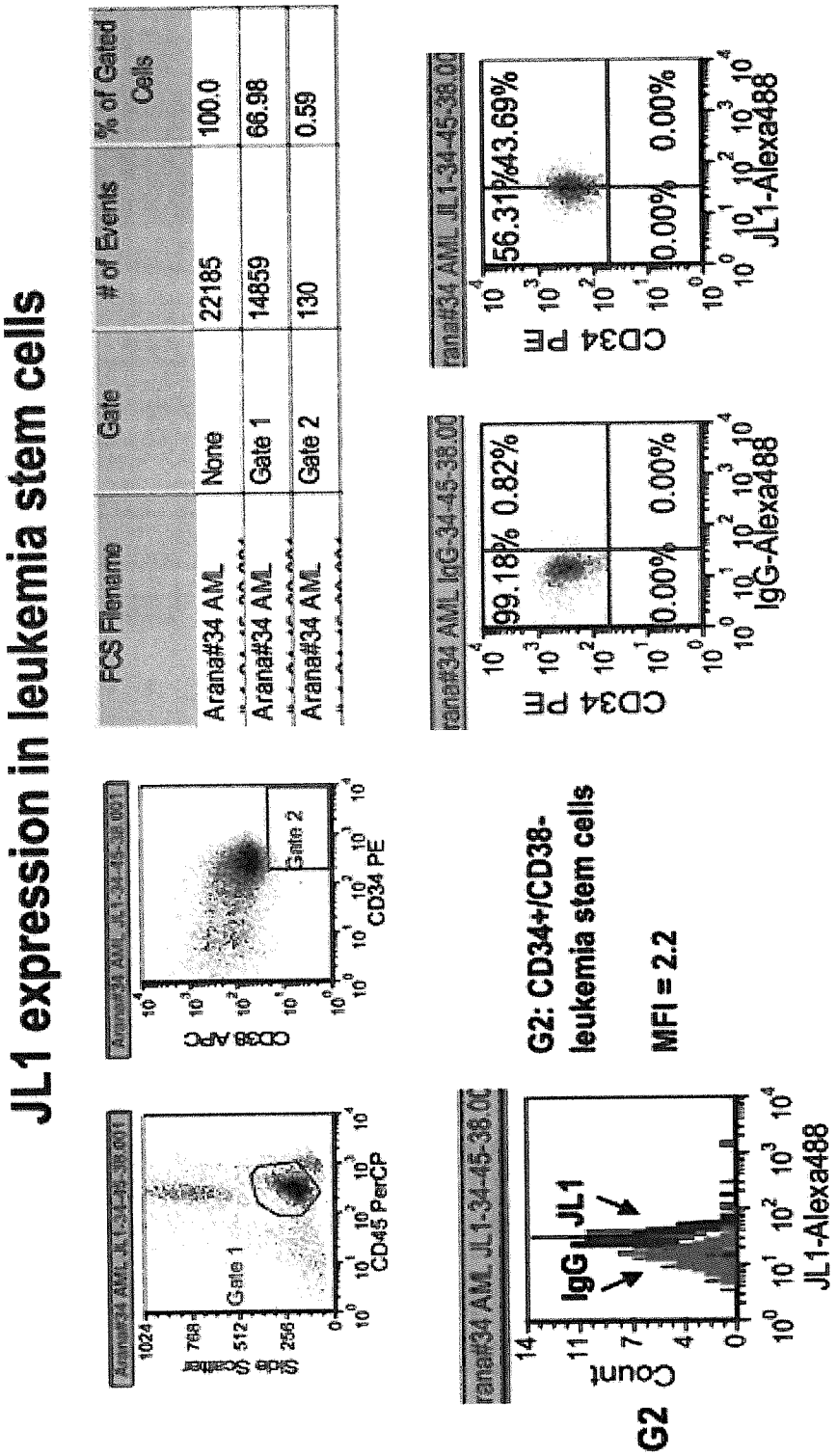

JL1 expression in leukemia stem cells
FCS Filename
Gate
# of Events
% of Gated Cells
Arana#34 AML
None
22185
100.0
Arana#34 AML
Gate 1
14859
66.98
Arana#34 AML
Gate 2
130
0.59
CD38 APC
CD34 PE
Gate 2
CD45 PerCP
Gate 1
G2
G2: CD34+/CD38- leukemia stem cells
MFI = 2.2
IgG1
JL1
Count
JL1-Alexa488
Arana#34 AML IgG-34-45-38.00
99.18%
0.82%
0.00%
0.00%
IgG-Alexa488
Arana#34 AML JL1-34-45-38.00
56.31%
43.69%
0.00%
0.00%
JL1-Alexa488
G2

FIG. 31

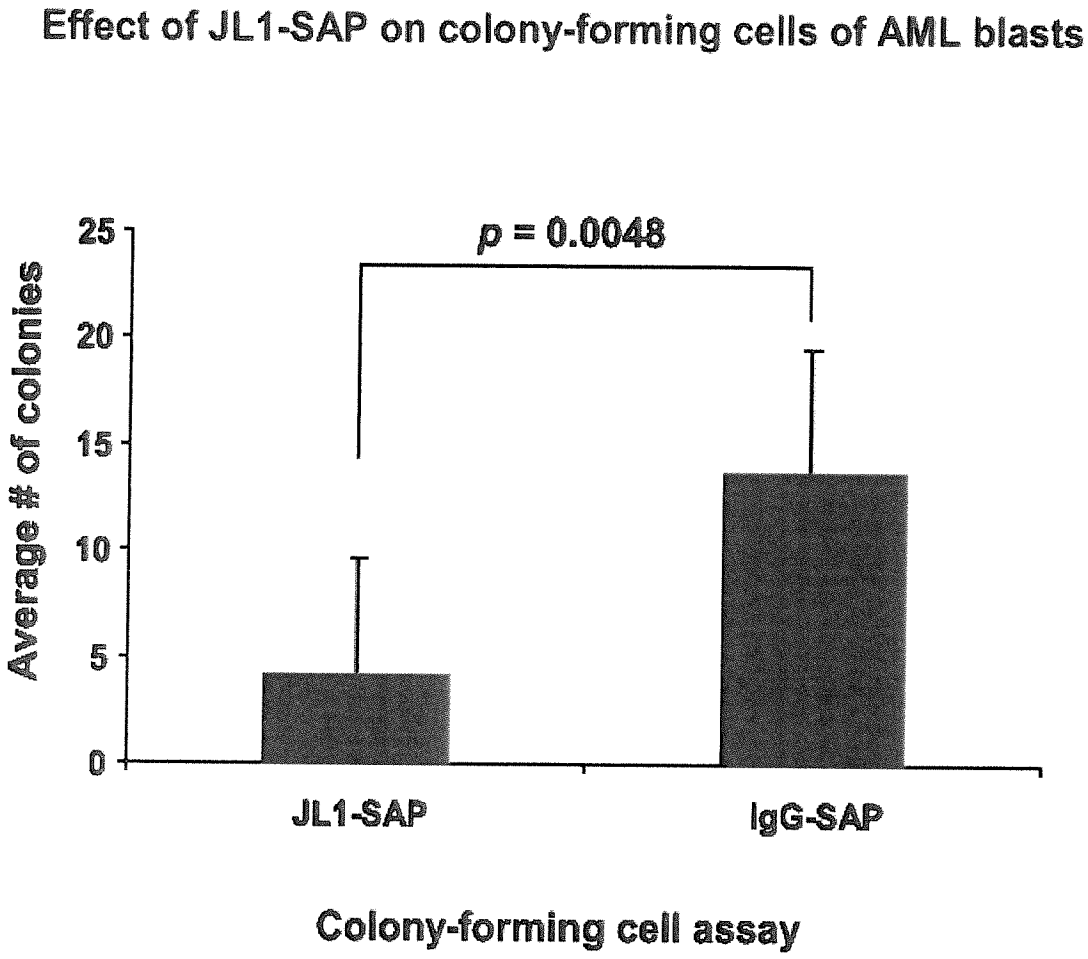
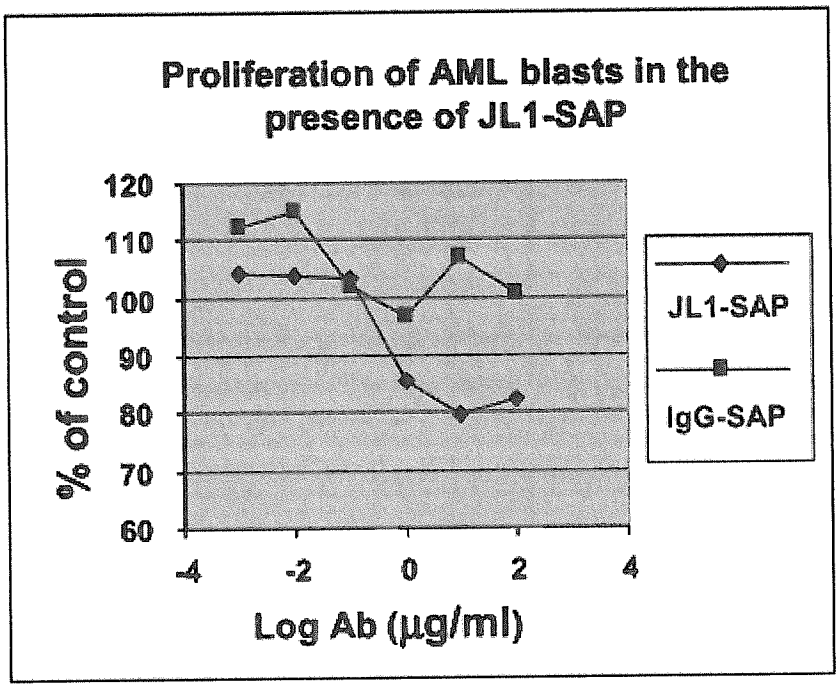

Effect of JL1-SAP on colony-forming cells of AML blasts
p = 0.0048
Average # of colonies
25
20
15
10
5
0
JL1-SAP
IgG-SAP
Colony-forming cell assay

FIG. 32

A.


Proliferation of AML blasts in the presence of JL1-SAP
% of control
120
110
100
90
80
70
60
-4
-2
0
2
4
Log Ab (µg/ml)
JL1-SAP
IgG-SAP

B.

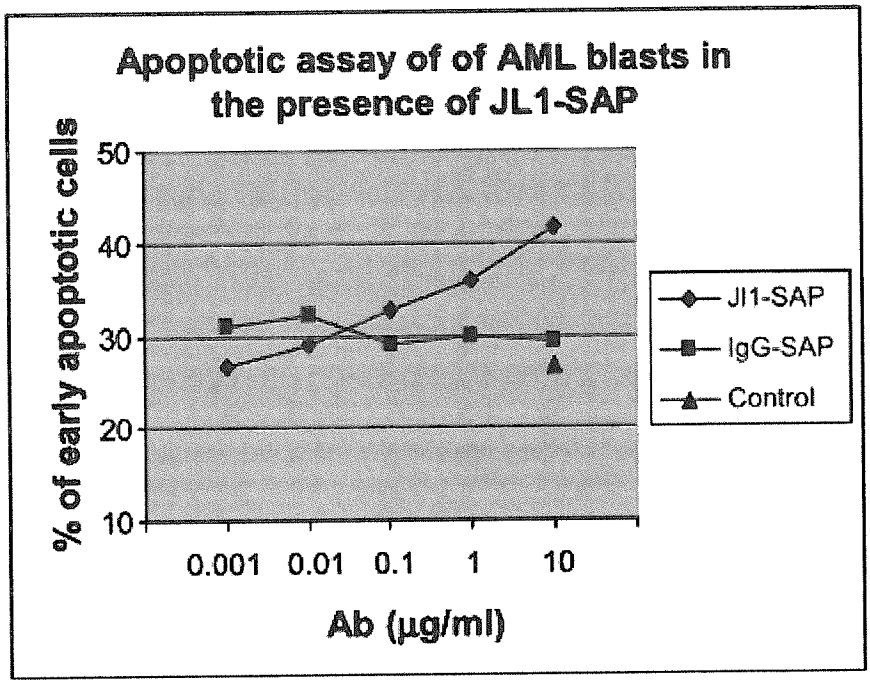

Apoptotic assay of of AML blasts in the presence of JL1-SAP
% of early apoptotic cells
50
40
30
20
10
0.001
0.01
0.1
1
10
Ab (µg/ml)
Jl1-SAP
IgG-SAP
Control

FIG. 33

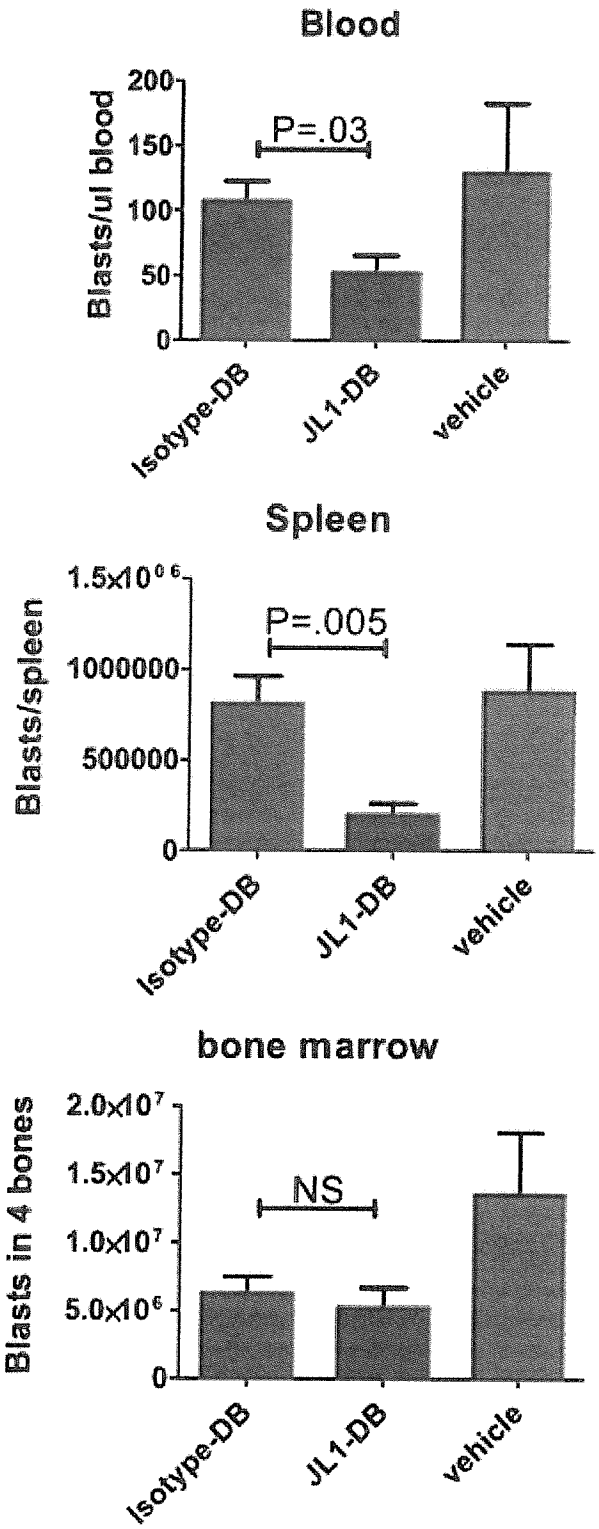
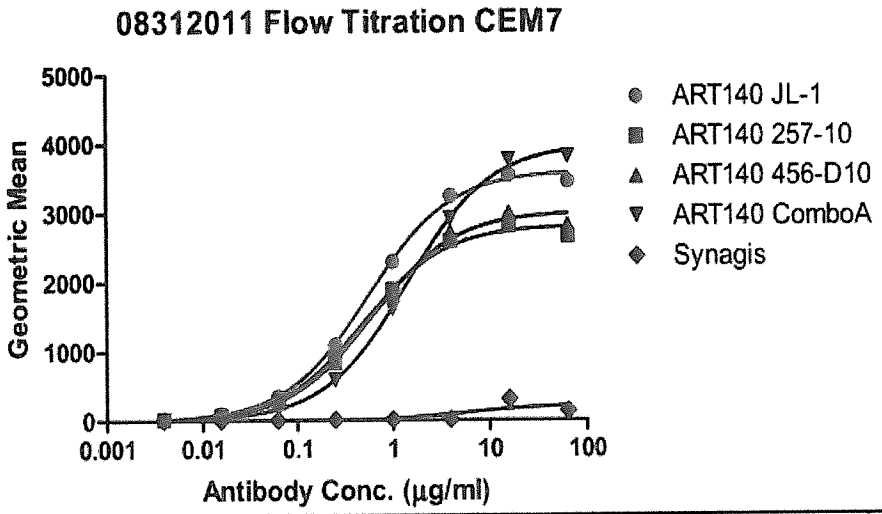
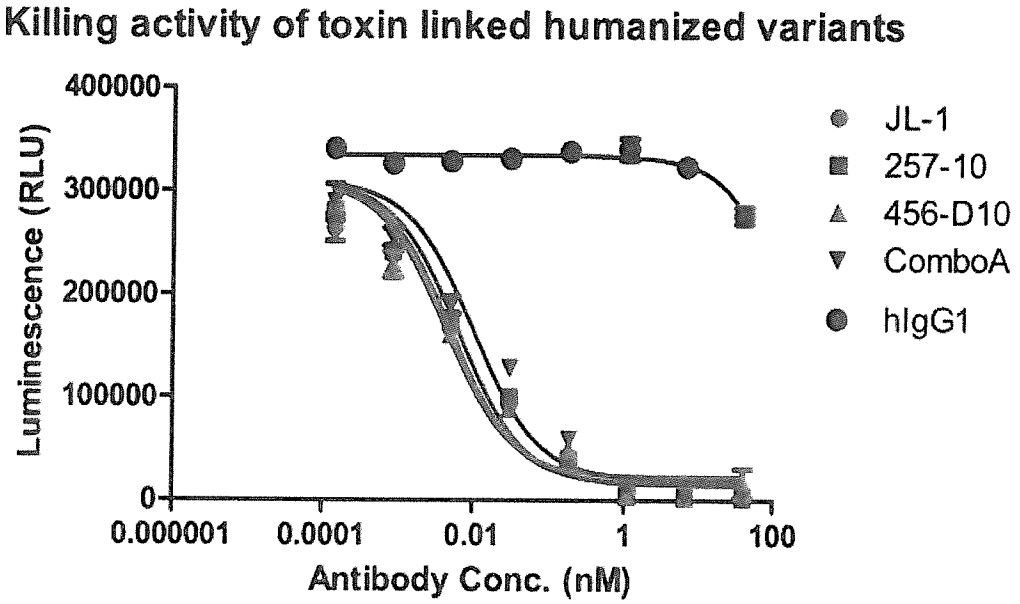

Blood
Blasts/ul blood
200
150
100
50
0
P=.03
Isotype-DB
JL1-DB
vehicle
Spleen
Blasts/spleen
1.5x10^6
1000000
500000
0
P=.005
Isotype-DB
JL1-DB
vehicle
bone marrow
Blasts in 4 bones
2.0x10^7
1.5x10^7
1.0x10^7
5.0x10^6
0
NS
Isotype-DB
JL1-DB
vehicle

FIG. 34




| | ART140 JL-1 | ART140 257-10 | ART140 456-D10 | ART140 ComboA | Synagis |
|---|---|---|---|---|---|
| EC50 | 0.5549 | 0.4841 | 0.6144 | 1.443 | 5.885 |

FIG. 35




| | JL-1 | 257-10 | 456-D10 | ComboA | hIgG1 |
|---|---|---|---|---|---|
| IC50 | 0.005186 | 0.006470 | 0.004285 | 0.01010 | ~ 3796 |

FIG. 36

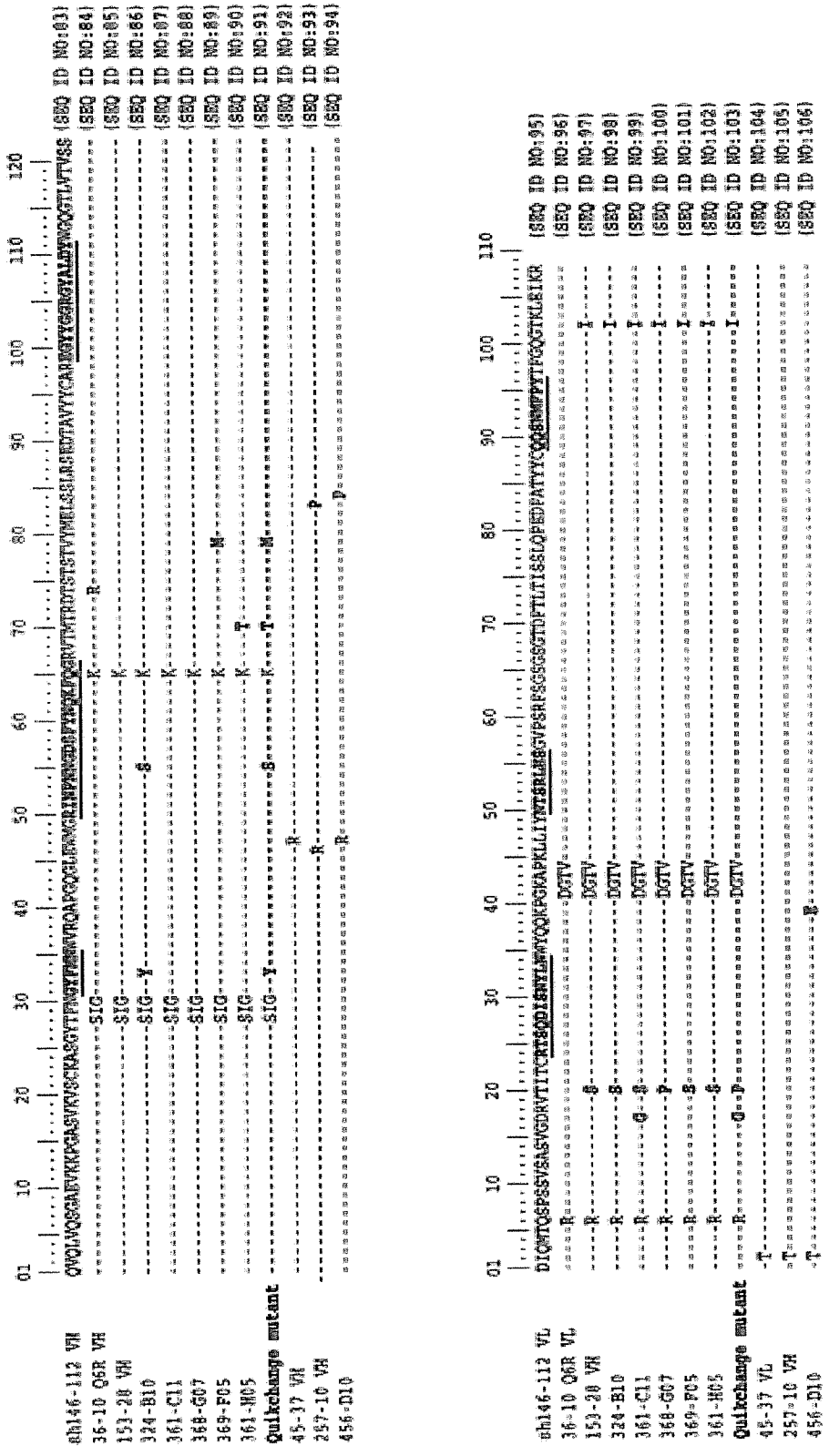


FIG. 37a

A.

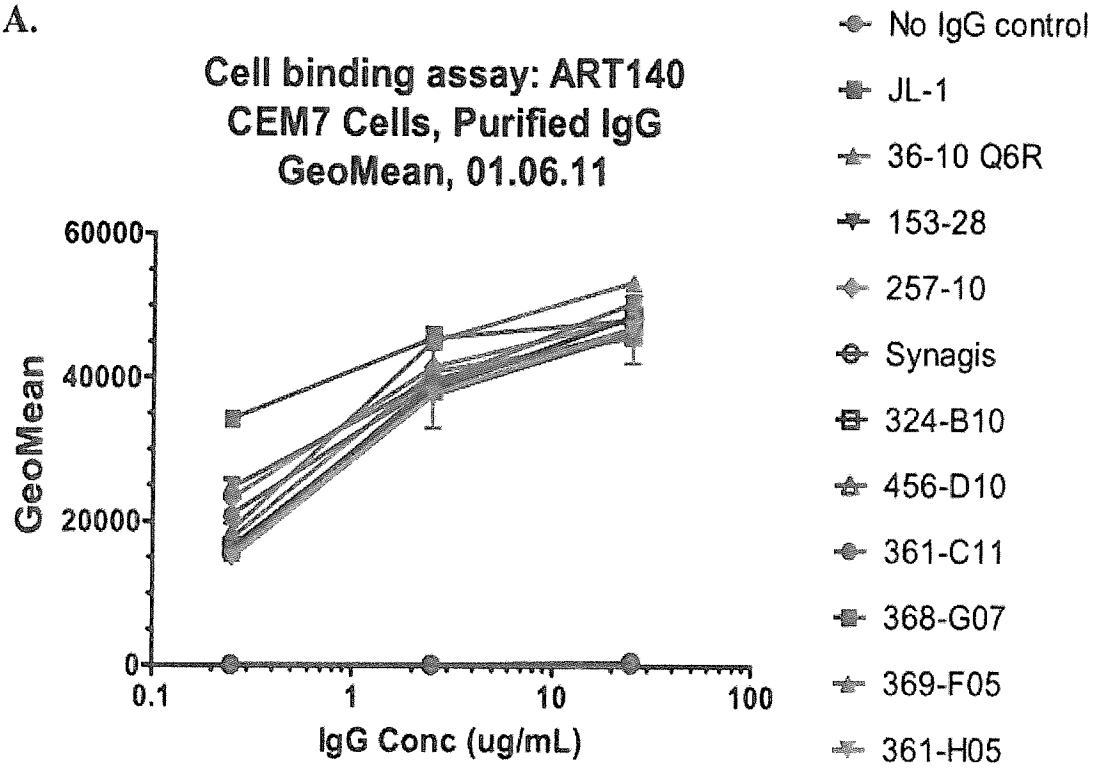

Cell binding assay: ART140
CEM7 Cells, Purified IgG
GeoMean, 01.06.11
GeoMean
60000
40000
20000
0
0.1
1
10
100
IgG Conc (ug/mL)
No IgG control
JL-1
36-10 Q6R
153-28
257-10
Synagis
324-B10
456-D10
361-C11
368-G07
369-F05
361-H05

FIG. 37b

B.

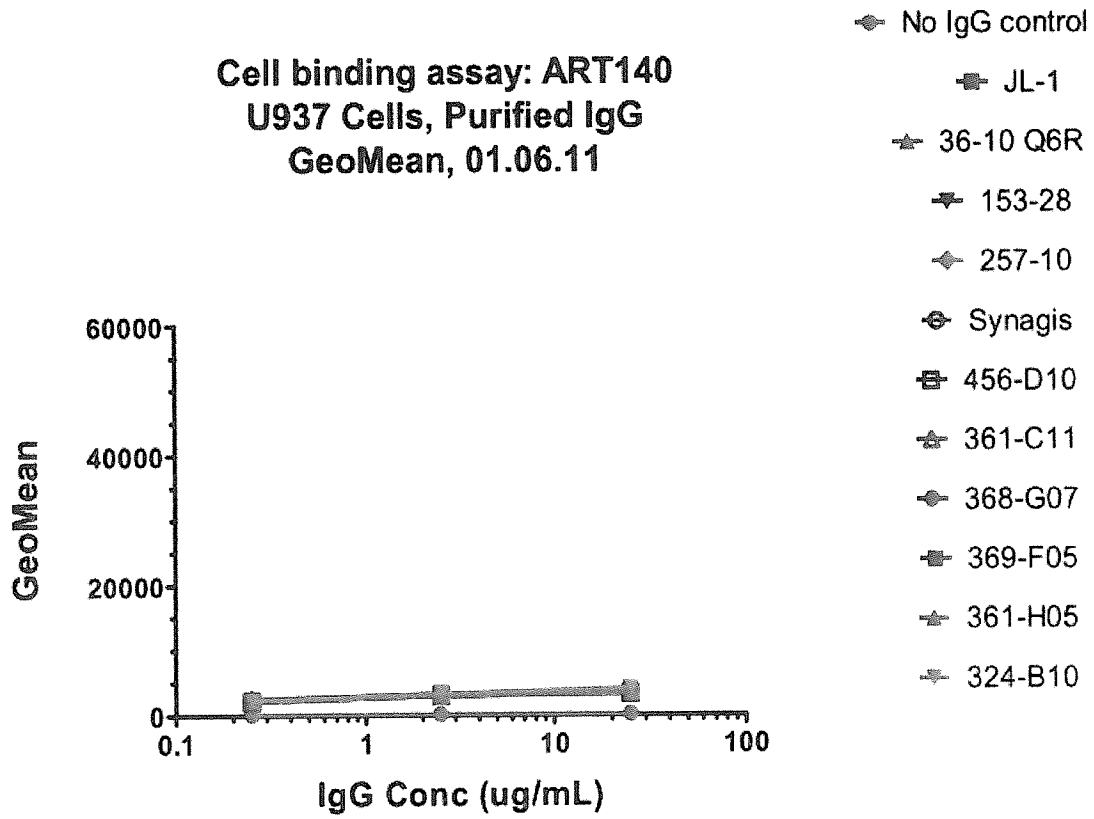

Cell binding assay: ART140
U937 Cells, Purified IgG
GeoMean, 01.06.11
GeoMean
60000
40000
20000
0
0.1
1
10
100
IgG Conc (ug/mL)
No IgG control
JL-1
36-10 Q6R
153-28
257-10
Synagis
456-D10
361-C11
368-G07
369-F05
361-H05
324-B10

FIG. 38a

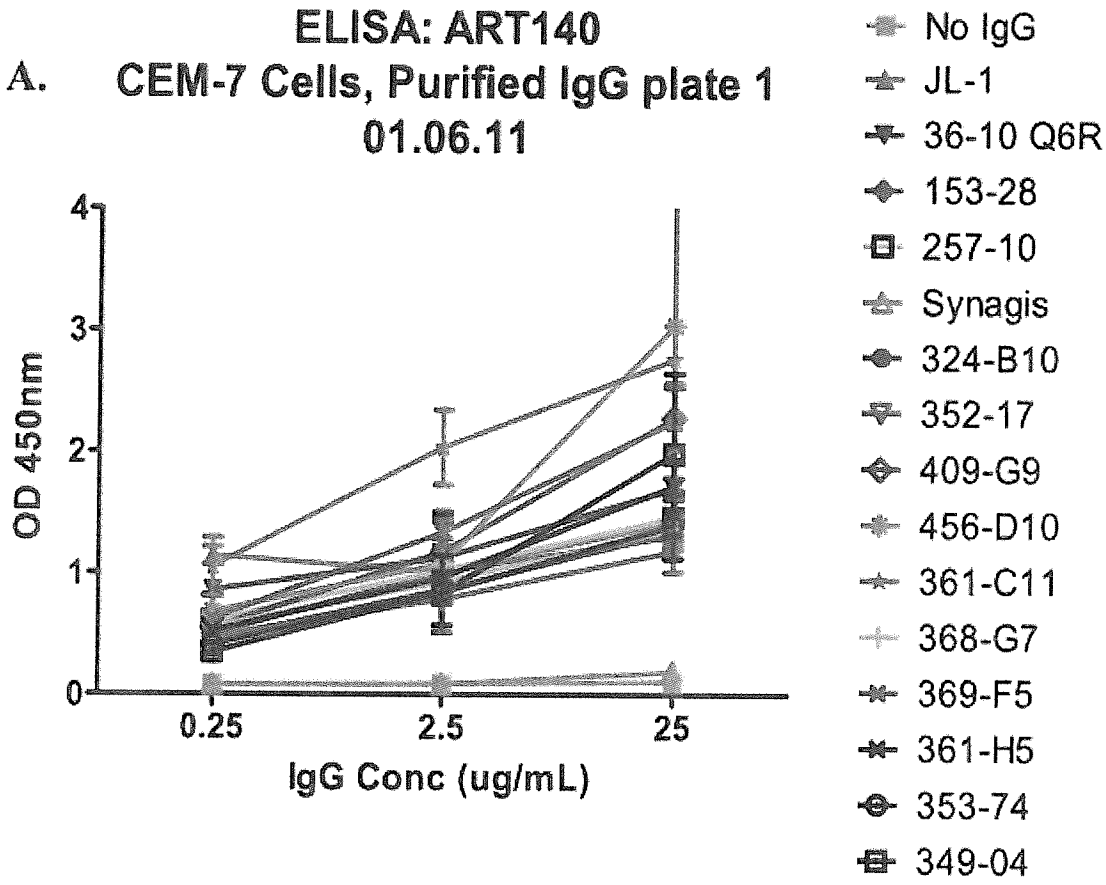

A.
ELISA: ART140
CEM-7 Cells, Purified IgG plate 1
01.06.11
OD 450nm
0
1
2
3
4
0.25
2.5
25
IgG Conc (ug/mL)
No IgG
JL-1
36-10 Q6R
153-28
257-10
Synagis
324-B10
352-17
409-G9
456-D10
361-C11
368-G7
369-F5
361-H5
353-74
349-04

FIG. 38b

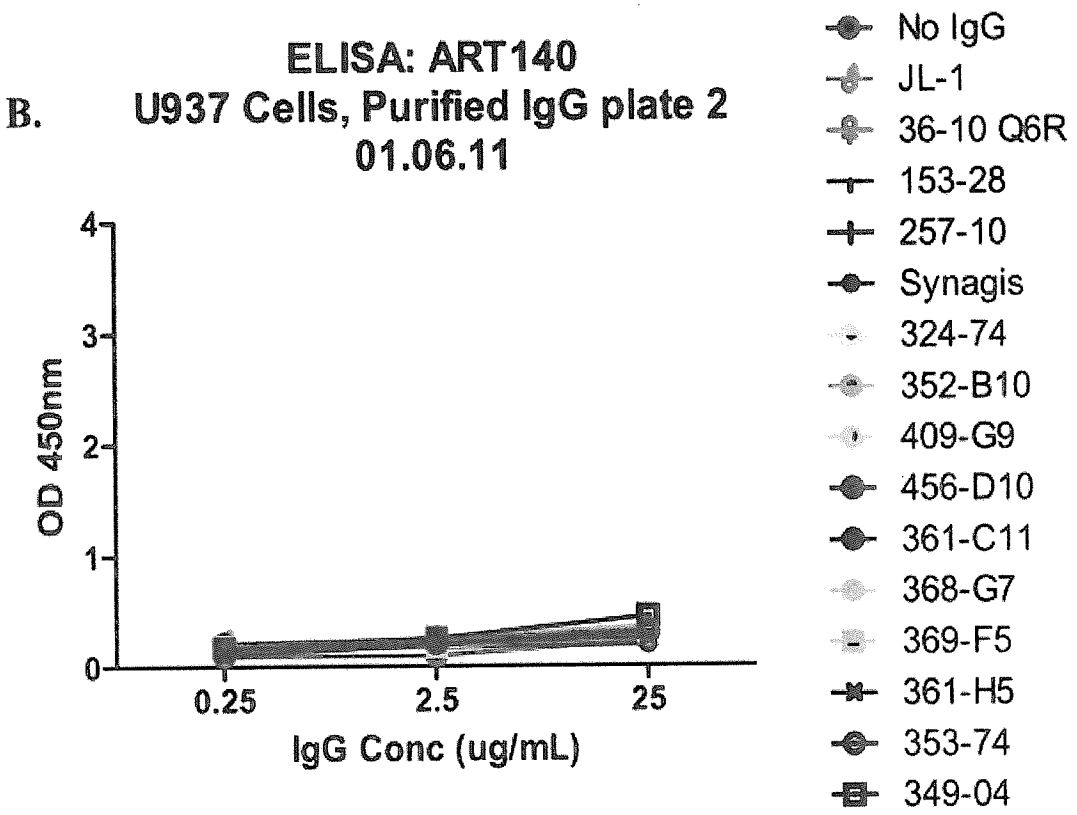

B.
ELISA: ART140
U937 Cells, Purified IgG plate 2
01.06.11
OD 450nm
0
1
2
3
4
0.25
2.5
25
IgG Conc (ug/mL)
No IgG
JL-1
36-10 Q6R
153-28
257-10
Synagis
324-74
352-B10
409-G9
456-D10
361-C11
368-G7
369-F5
361-H5
353-74
349-04

FIG. 39a

A.

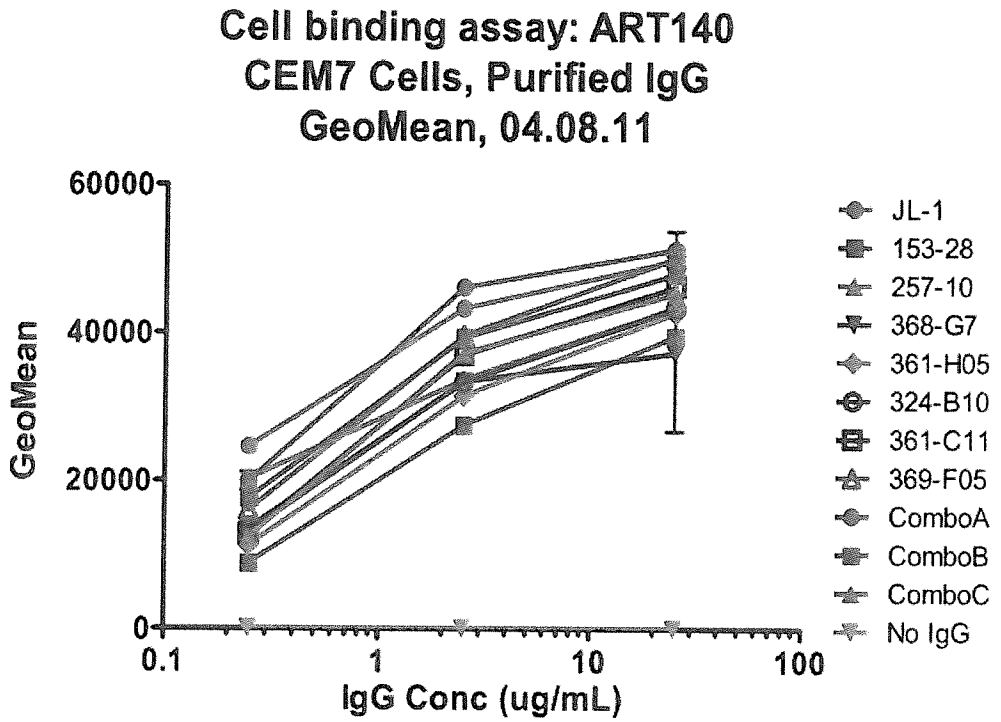



FIG. 39b

B.

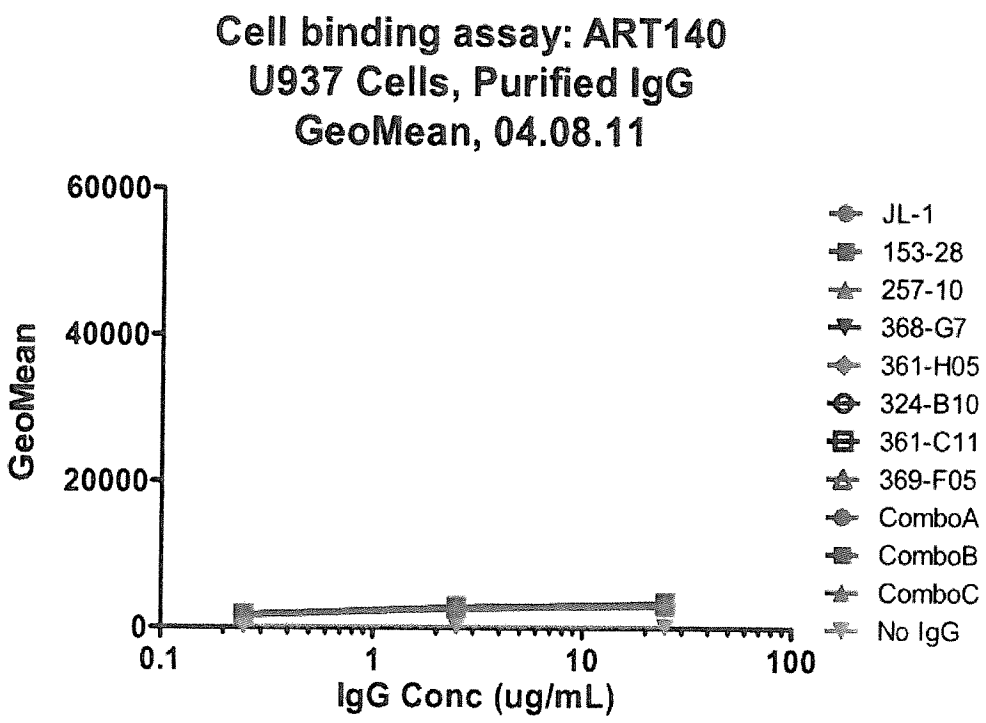
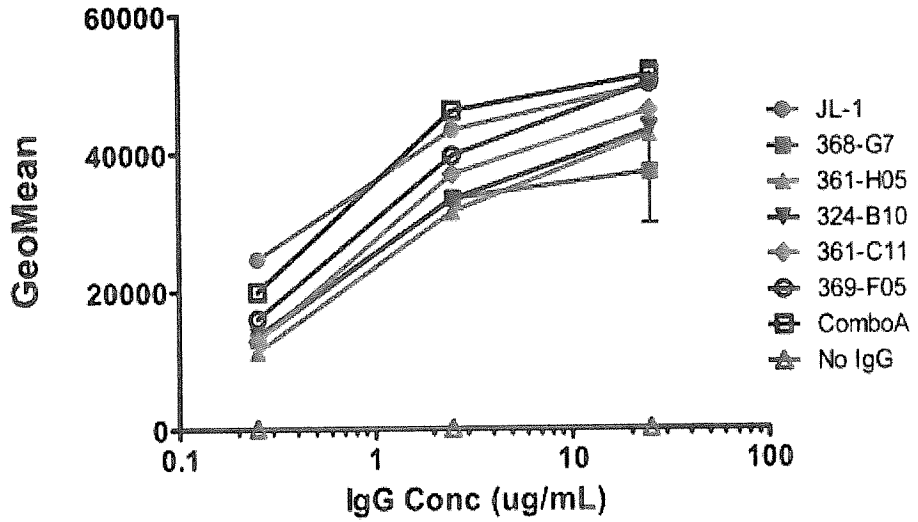


FIG. 40a

A.

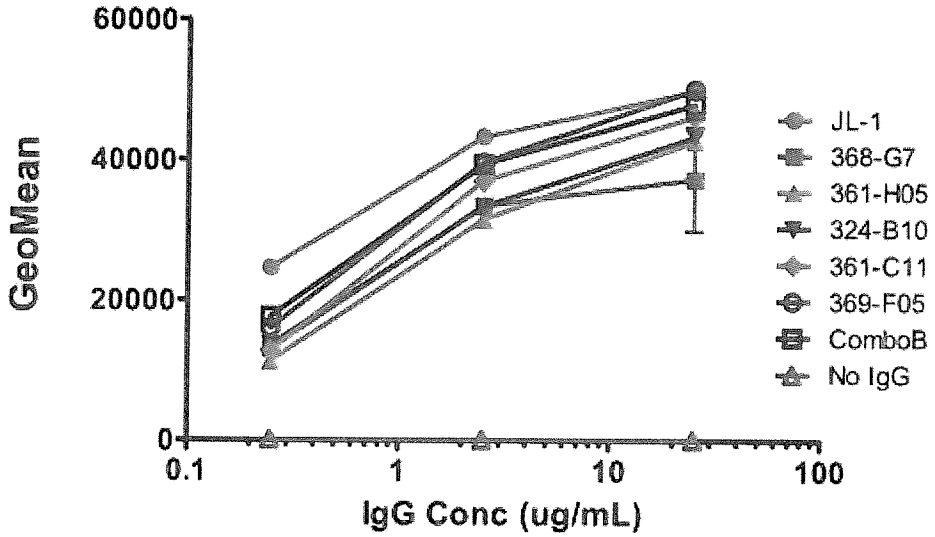
**Cell binding assay: ART140**
**CEM7 Cells, Purified IgG GeoMean, 04.08.11**
**Combined quikchange mutant Vs Round 3 Parental frameworks**




ComboA = quikchange HC&LC
ComboB = quikchange HC + 153-28 LC
ComboC = 153-28 HC + quikchange LC

FIG. 40b

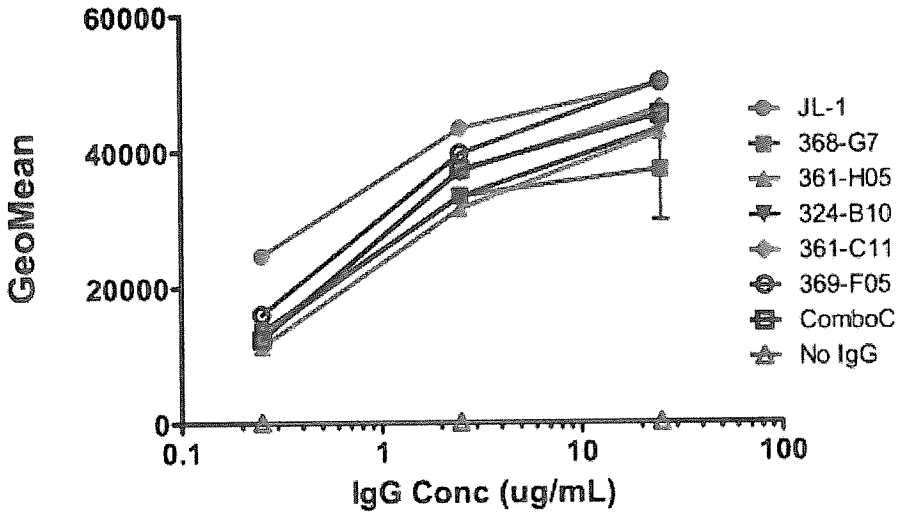
**B. Cell binding assay: ART140**
**CEM7 Cells, Purified IgG GeoMean, 04.08.11**
**Quikchange HC + 153-28 LC Vs Round 3 parental frameworks**




ComboA = quikchange HC&LC
ComboB = quikchange HC + 153-28 LC
ComboC = 153-28 HC + quikchange LC

FIG. 40c

C. Cell binding assay: ART140
CEM7 Cells, Purified IgG GeoMean, 04.08.11
153-28 HC + Quikchange LC Vs Round 3 parental frameworks




ComboA = quikchange HC&LC
ComboB = quikchange HC + 153-28 LC
ComboC = 153-28 HC + quikchange LC

FIG. 41a

A.

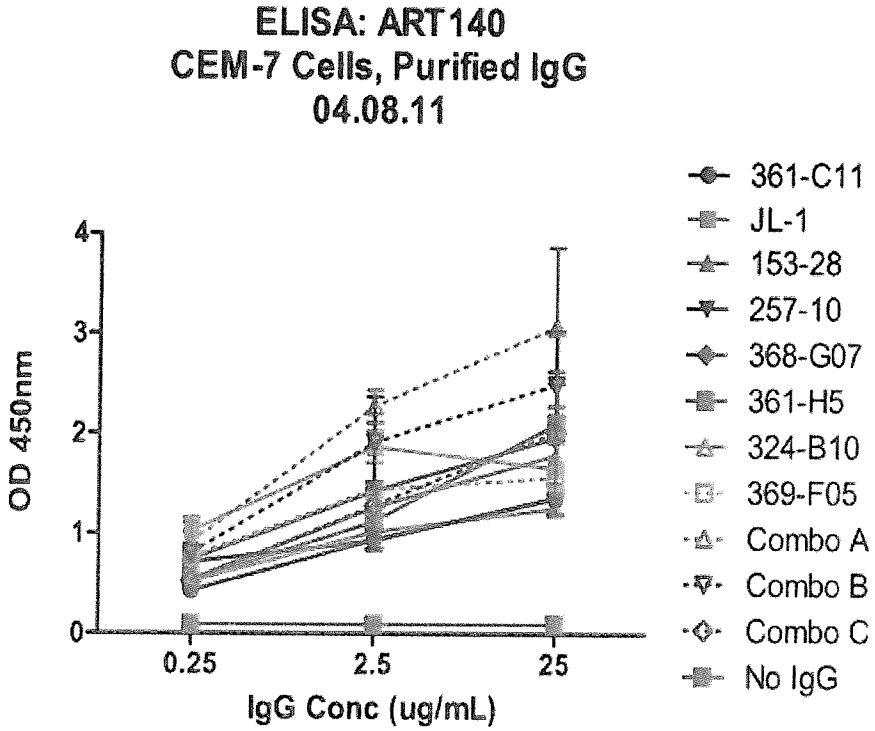



FIG. 41b

B.

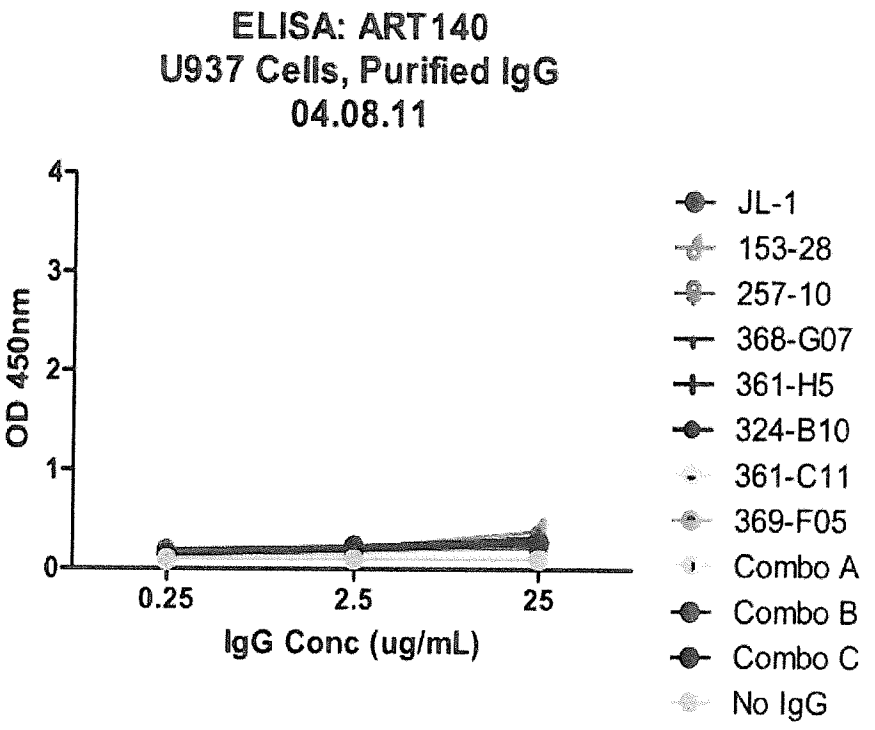


FIG. 41c

C.

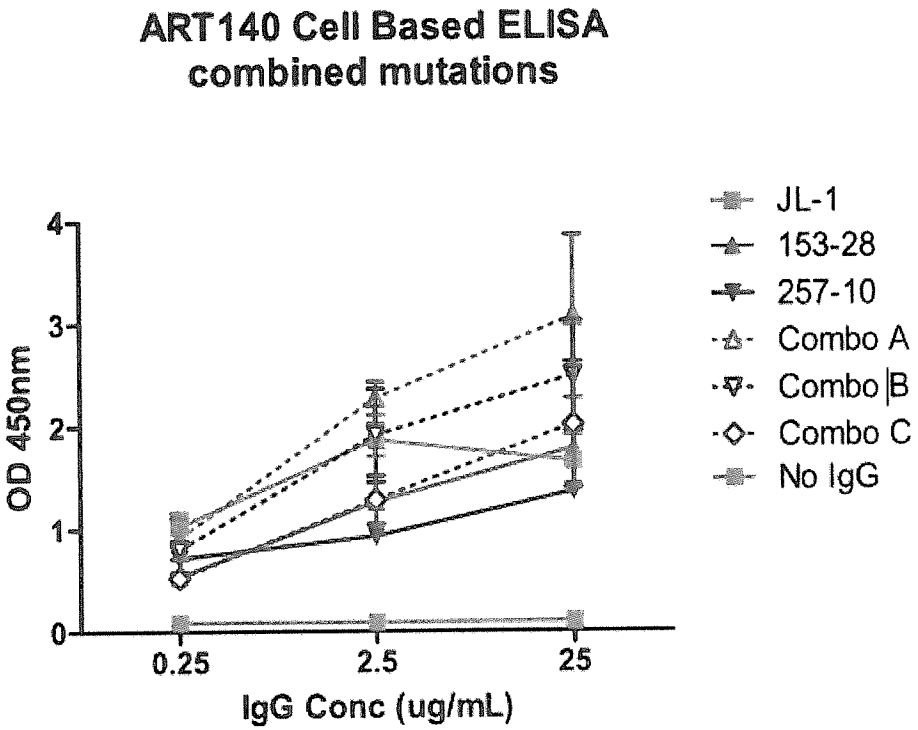



FIG. 42

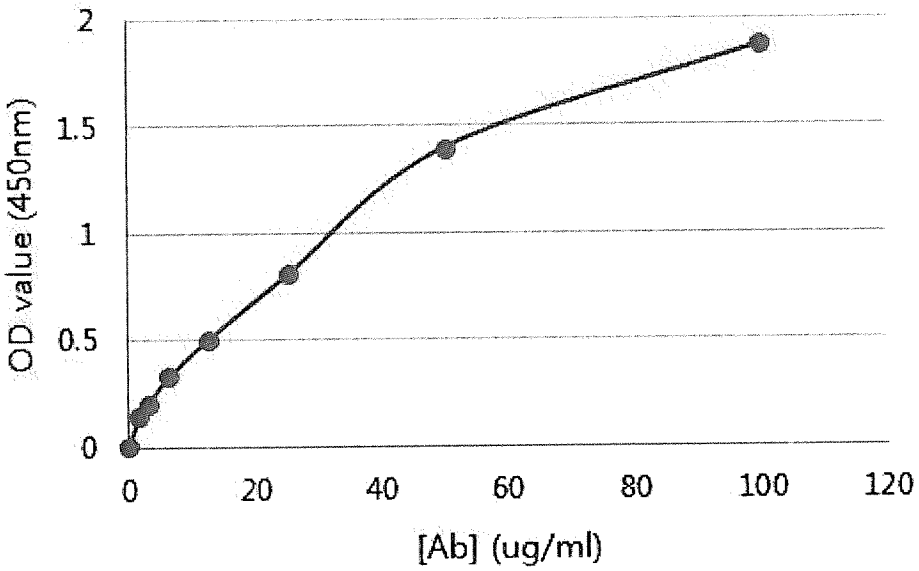


FIG. 43

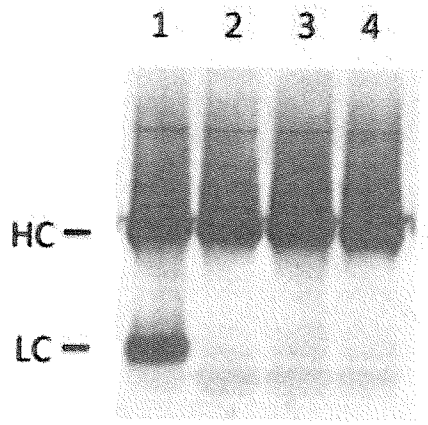
huJL-1 257-10 Vk

```
1-------10--------20--------30--------40--------50--------60
DTQMTQSPSSVSASVGDRVTITCRTSQDISNYLNWYQQKPGKAPKLLIYNTSRLHSGVPS

--------70--------80--------90-------100----107
RFSGSGSGTDFTLTISSLQPEDFATYYCQQSNMFPYTFGQGTKLEIK
```

huJL-1 257-10 VH

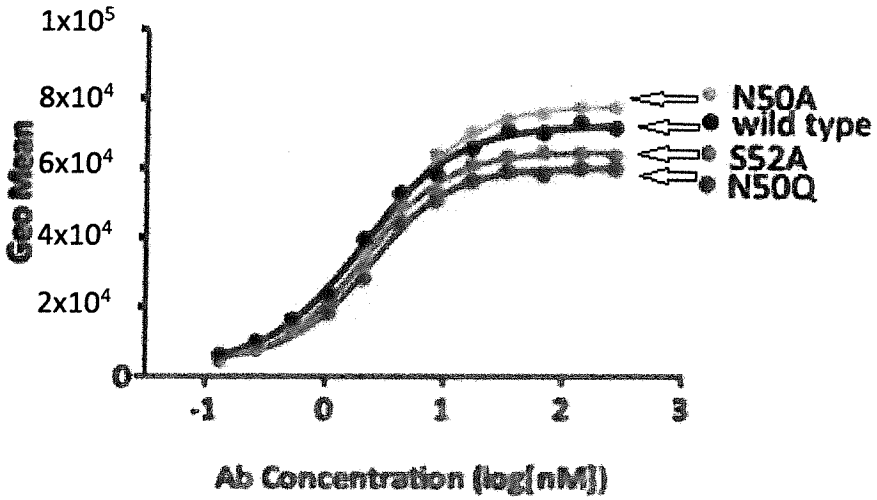
```
1-------10--------20--------30--------40--------50--------60
QVQLVQSGAEVKKPGASVKVSCKASGYTFNGYFMNWVRQAPGQGLERMGRINPNNGDSFY

--------70--------80--------90-------100-------110-------120
NQKFQGRVTMTRDTSTSTVYMELPSLRSEDTAVYYCAREGYYGGRGYALDYWGQGTLVTV

122
 SS
```

FIG. 44




1: wild type, 2: N50A, 3: N50Q, 4: S52A

FIG. 45




| | WT | S52A | N50Q | N50A |
|---|---|---|---|---|
| EC50 | 2.052 | 2.134 | 2.393 | 2.843 |

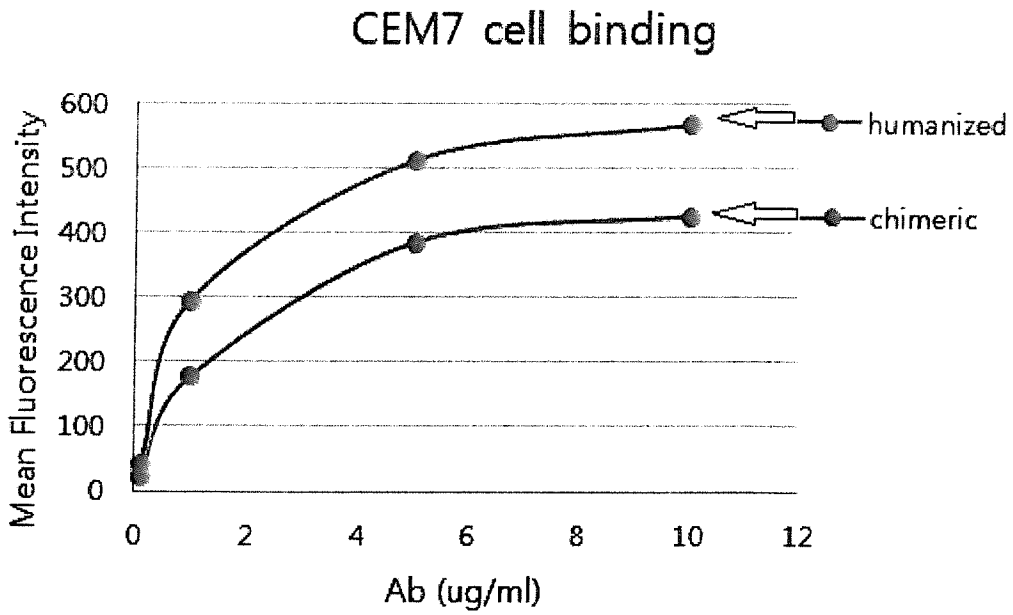



FIG. 46