FIG. 1A

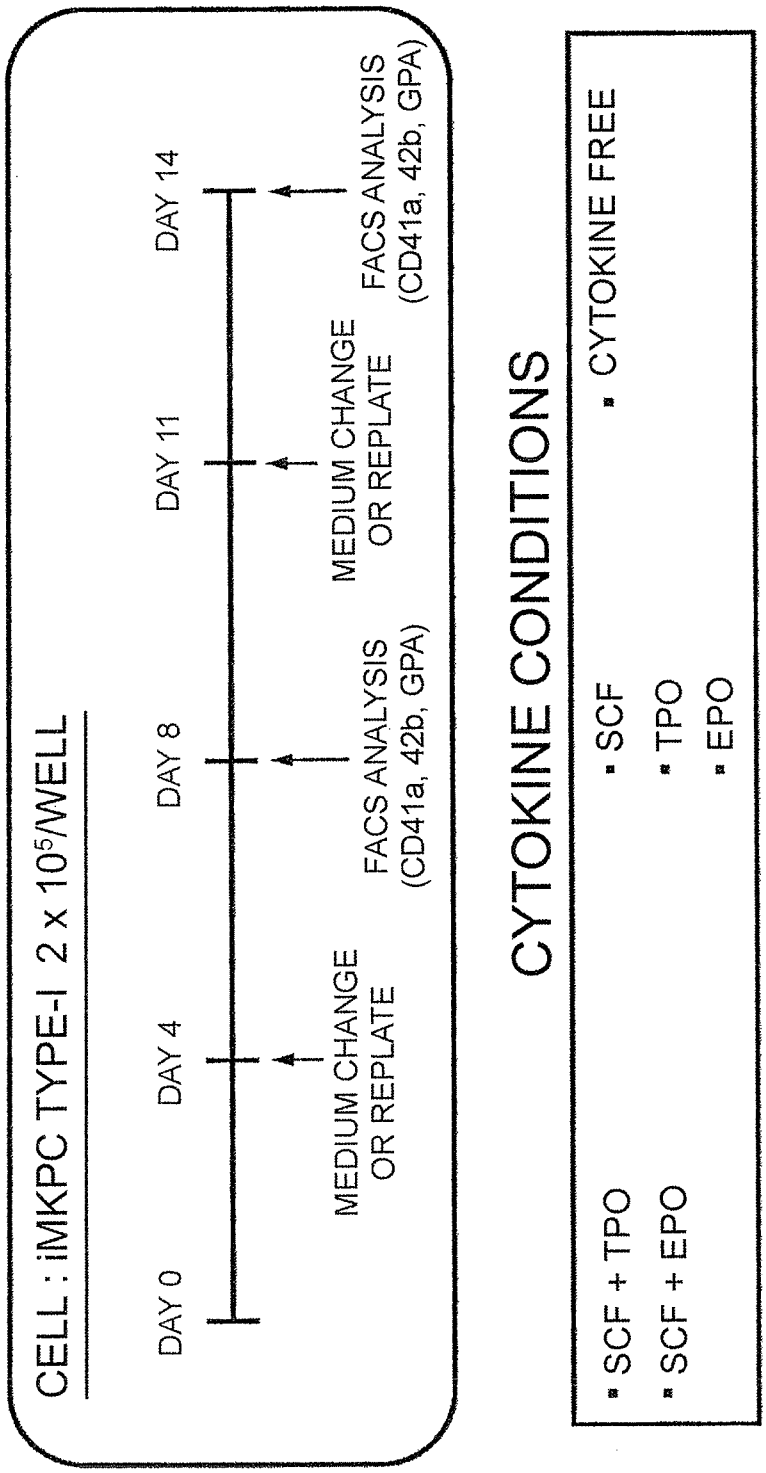

CELL : iMKPC TYPE-I 2 x 10⁵/WELL
DAY 0
DAY 4
DAY 8
DAY 11
DAY 14
MEDIUM CHANGE OR REPLATE
FACS ANALYSIS (CD41a, 42b, GPA)
MEDIUM CHANGE OR REPLATE
FACS ANALYSIS (CD41a, 42b, GPA)
CYTOKINE CONDITIONS
▪ SCF + TPO
▪ SCF + EPO
▪ SCF
▪ TPO
▪ EPO
▪ CYTOKINE FREE

FIG. 1B

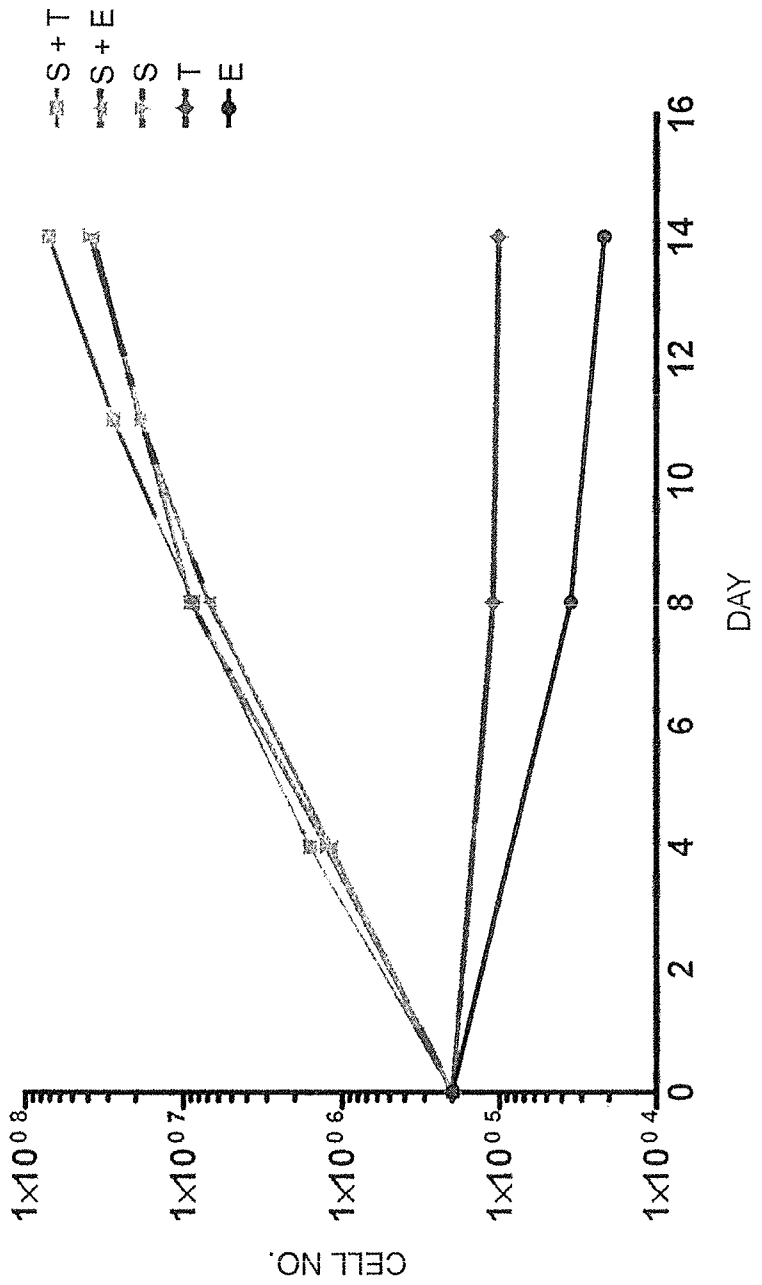


FIG. 1C

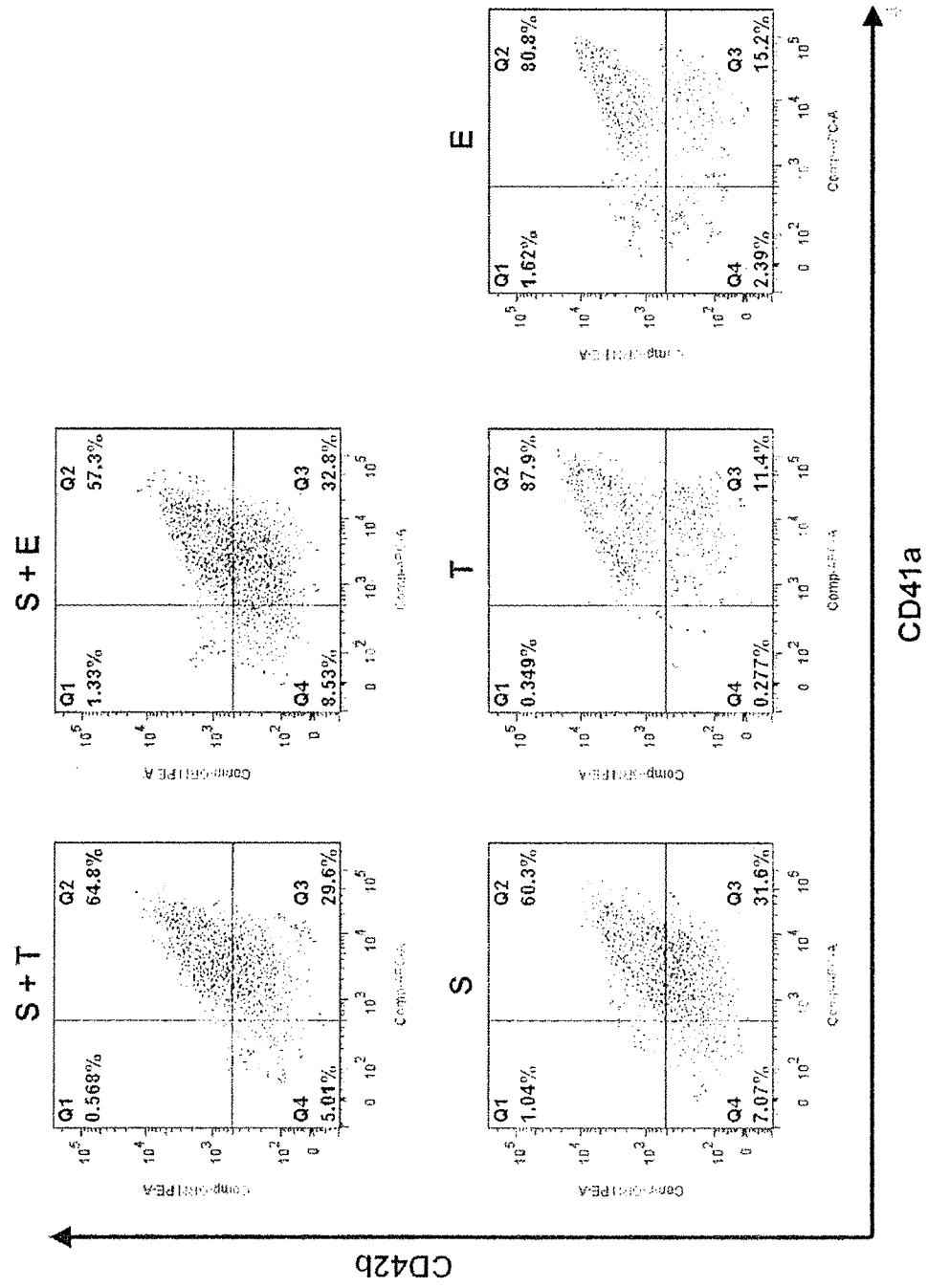

S + T
S
S + E
T
E
Q1
0.568%
Q2
64.8%
Q3
29.6%
Q4
5.01%
Q1
1.04%
Q2
60.3%
Q3
31.6%
Q4
7.07%
Q1
1.33%
Q2
57.3%
Q3
32.8%
Q4
8.53%
Q1
0.349%
Q2
87.9%
Q3
11.4%
Q4
0.277%
Q1
1.62%
Q2
80.8%
Q3
15.2%
Q4
2.39%
CD42b
CD41a

FIG. 1D

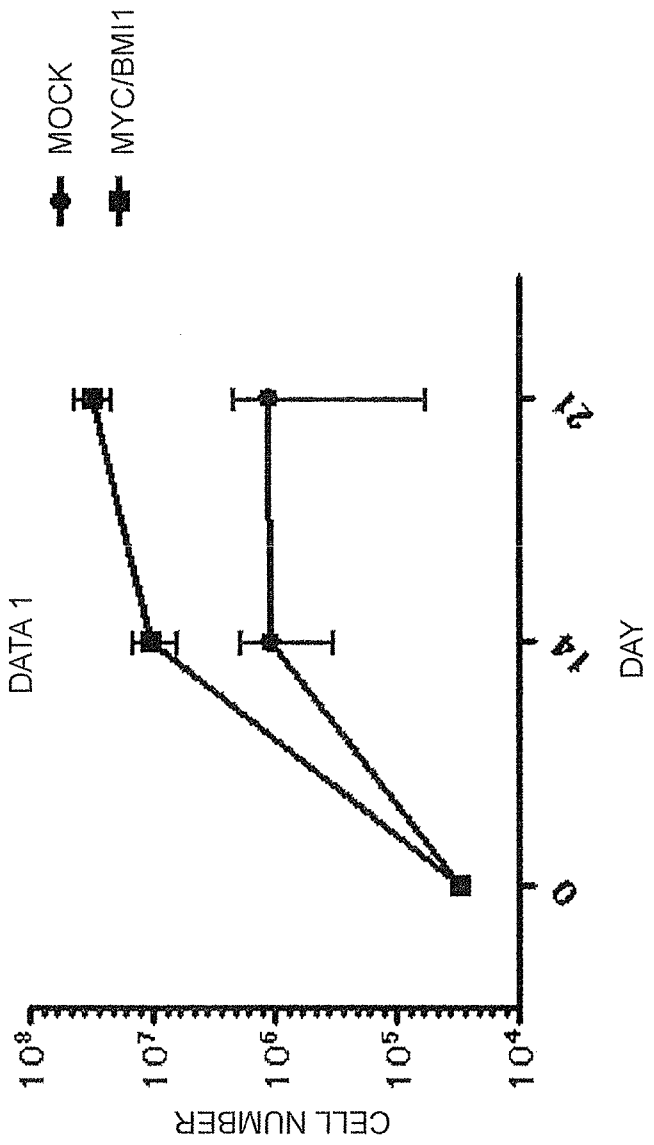


FIG. 2A

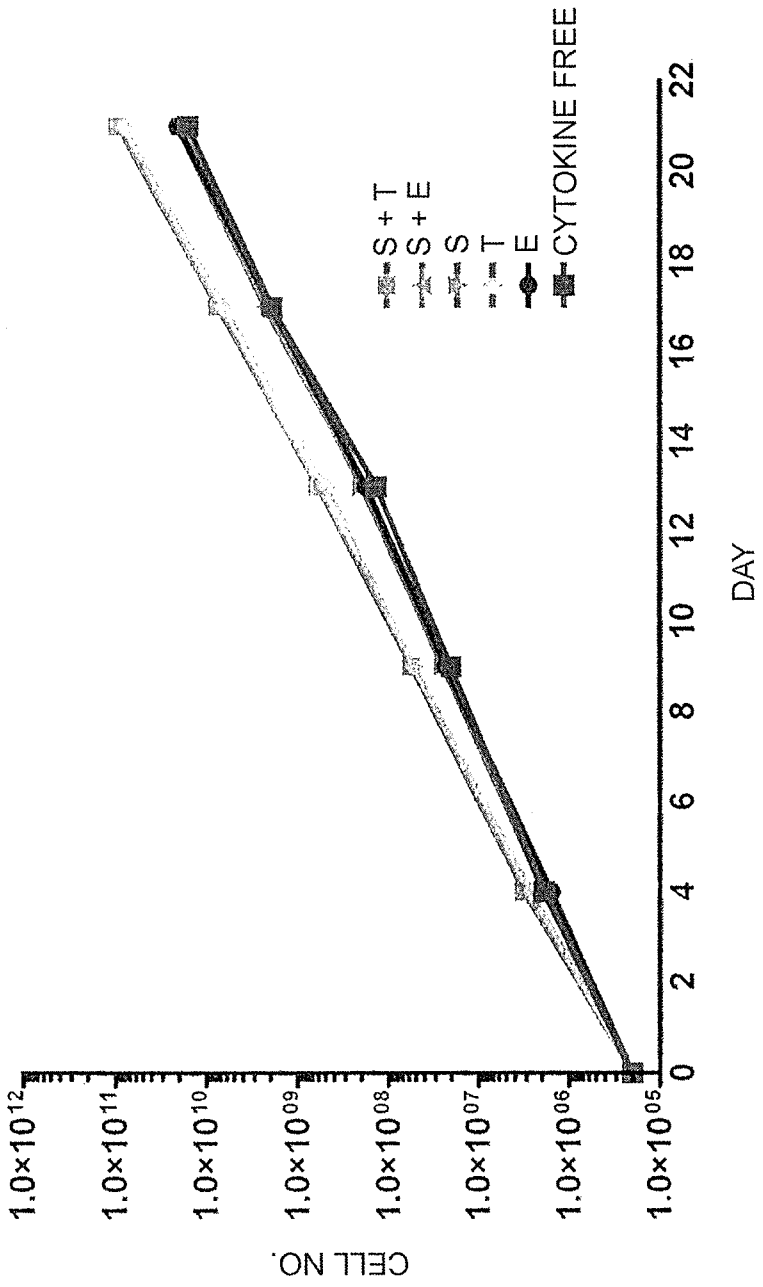

S + T
S + E
S
T
E
CYTOKINE FREE
1.0×10^12
1.0×10^11
1.0×10^10
1.0×10^09
1.0×10^08
1.0×10^07
1.0×10^06
1.0×10^05
CELL NO.
0
2
4
6
8
10
12
14
16
18
20
22
DAY

FIG. 2B

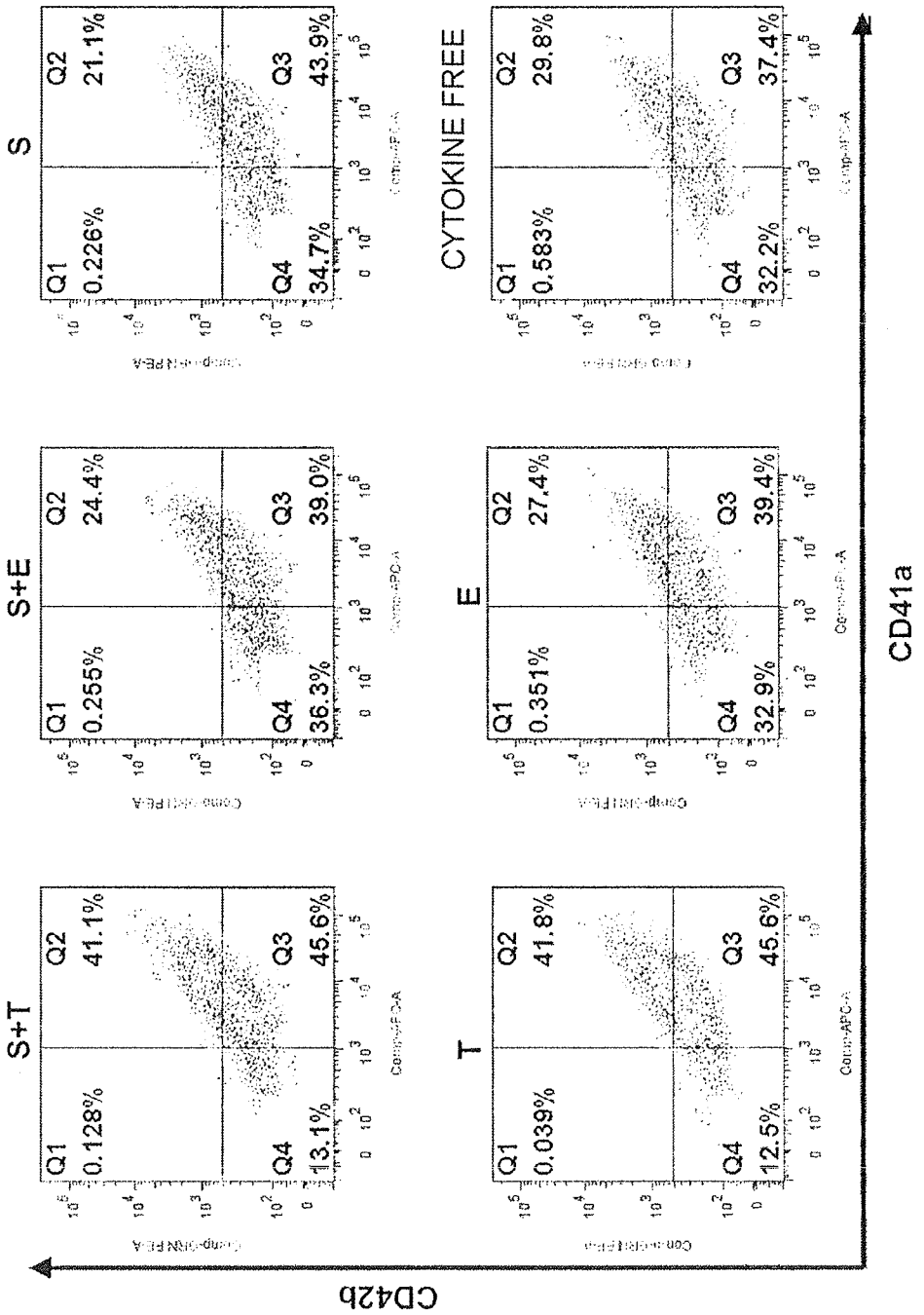

S
Q1
0.226%
Q2
21.1%
Q4
34.7%
Q3
43.9%
CYTOKINE FREE
Q1
0.583%
Q2
29.8%
Q4
32.2%
Q3
37.4%
S+E
Q1
0.255%
Q2
24.4%
Q4
36.3%
Q3
39.0%
E
Q1
0.351%
Q2
27.4%
Q4
32.9%
Q3
39.4%
S+T
Q1
0.128%
Q2
41.1%
Q4
13.1%
Q3
45.6%
T
Q1
0.039%
Q2
41.8%
Q4
12.5%
Q3
45.6%
CD42b
CD41a

FIG. 3

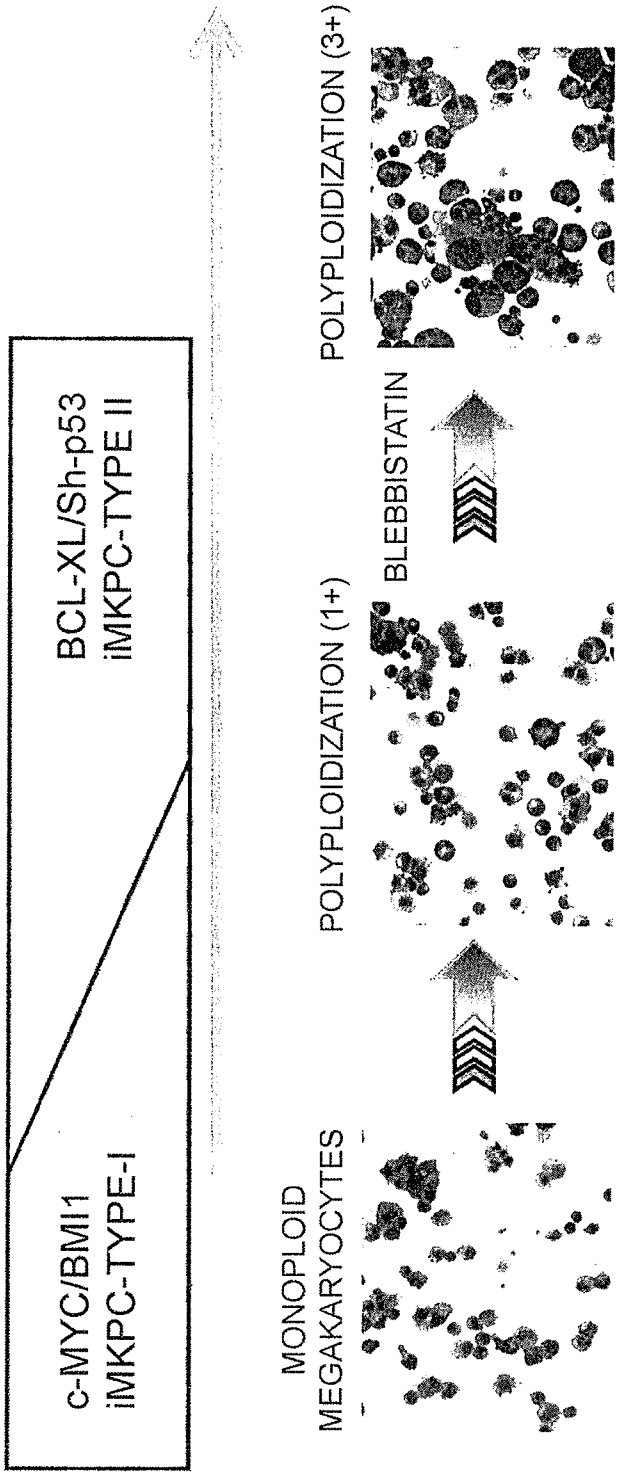

c-MYC/BMI1
iMKPC-TYPE-I
BCL-XL/Sh-p53
iMKPC-TYPE II
MONOPLOID
MEGAKARYOCYTES
POLYPLOIDIZATION (1+)
BLEBBISTATIN
POLYPLOIDIZATION (3+)

FIG. 4

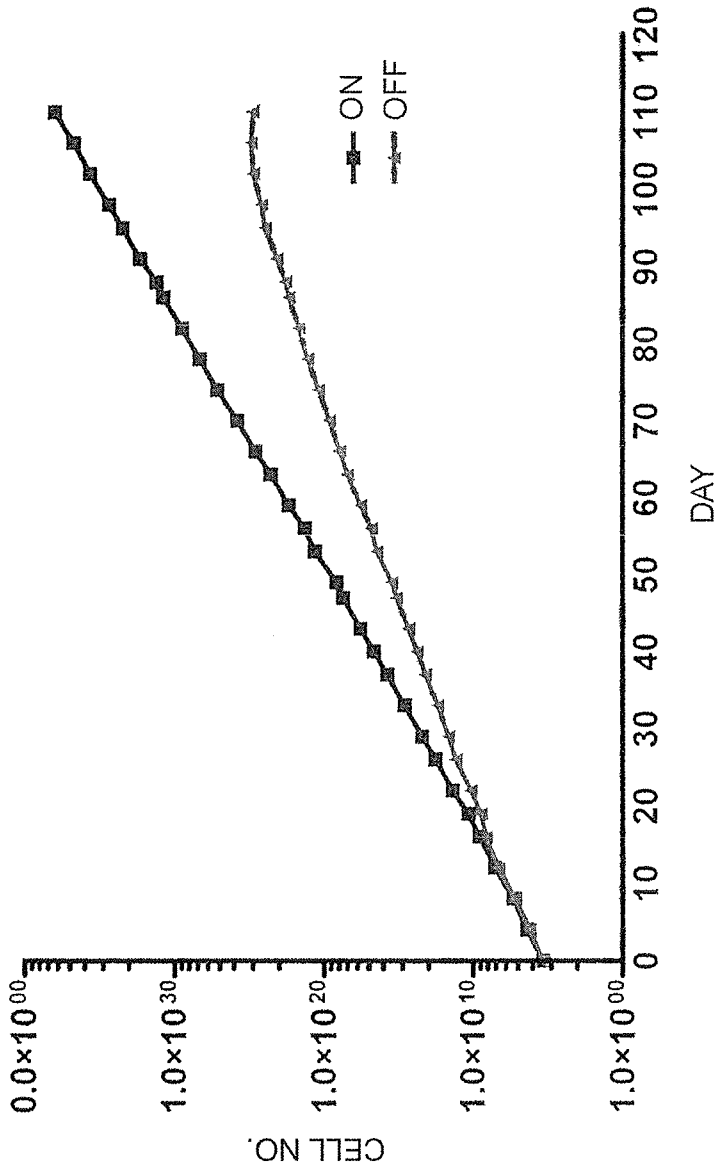
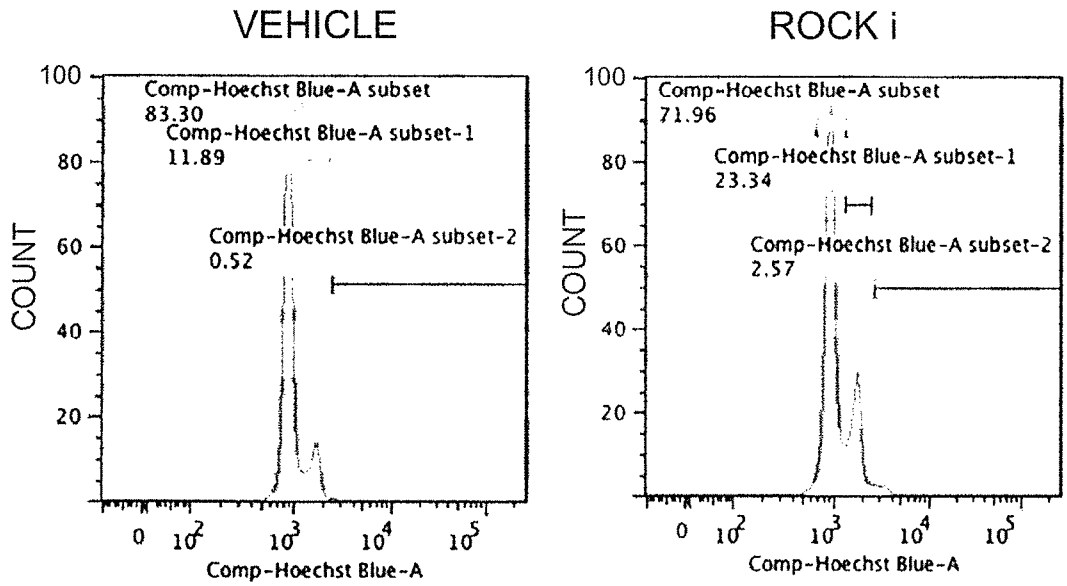

ON
OFF
0
10
20
30
40
50
60
70
80
90
100
110
120
DAY
1.0×10^00
1.0×10^10
1.0×10^20
1.0×10^30
0.0×10^00
CELL NO.

FIG. 5

A


VEHICLE
ROCK i
Comp-Hoechst Blue-A subset
83.30
Comp-Hoechst Blue-A subset-1
11.89
Comp-Hoechst Blue-A subset-2
0.52
Comp-Hoechst Blue-A subset
71.96
Comp-Hoechst Blue-A subset-1
23.34
Comp-Hoechst Blue-A subset-2
2.57
COUNT
Comp-Hoechst Blue-A

B

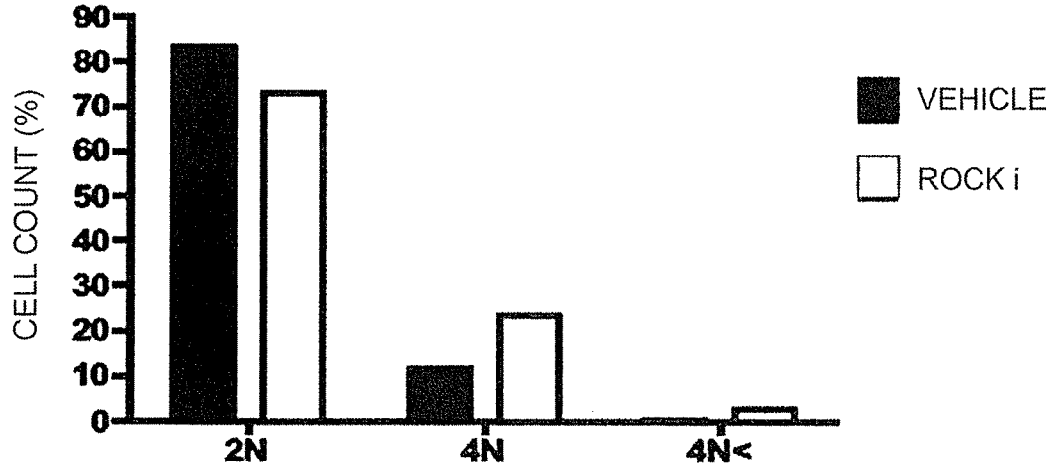

CELL COUNT (%)
2N
4N
4N<
VEHICLE
ROCK i

FIG. 6

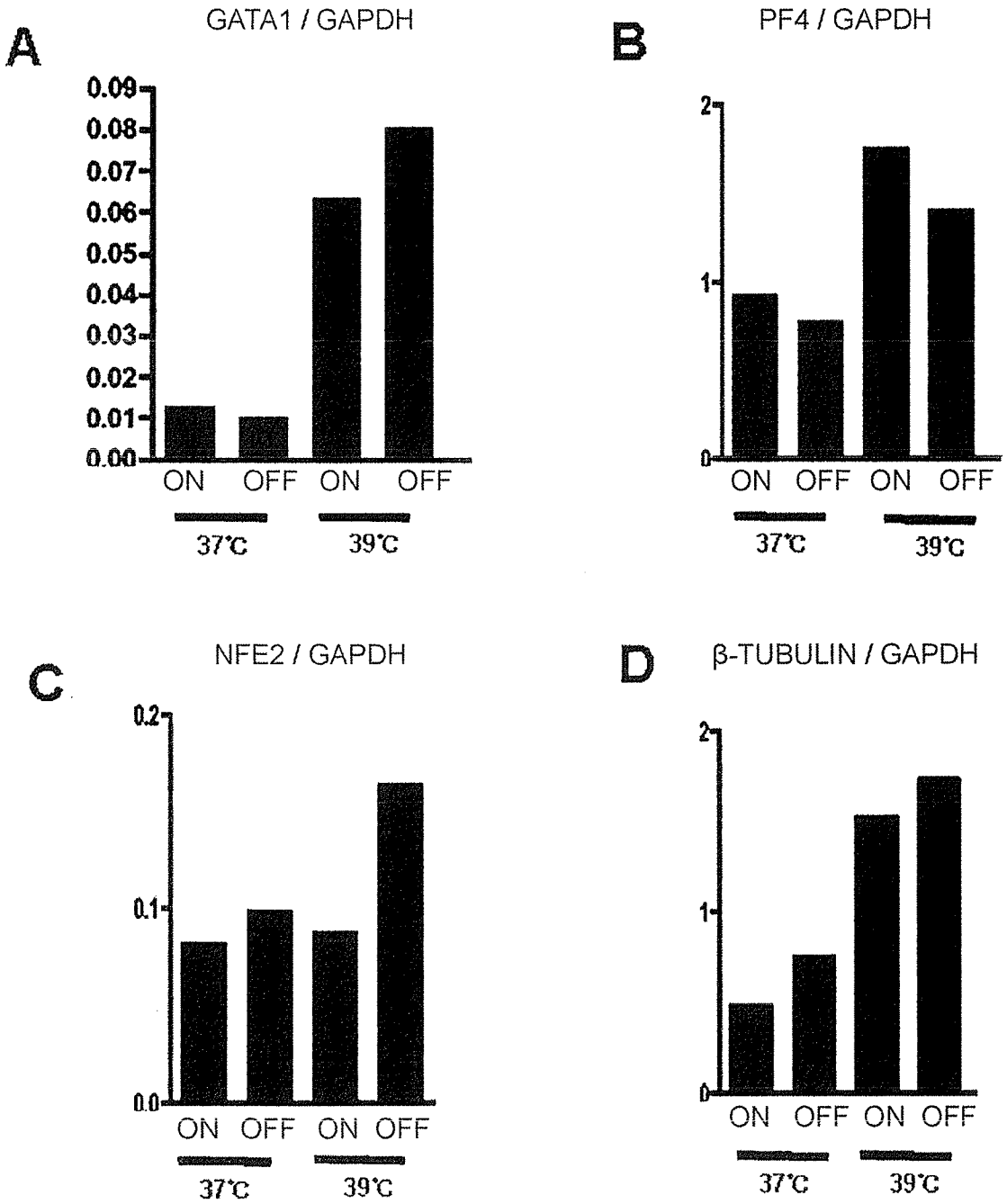

A
GATA1 / GAPDH
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
ON OFF ON OFF
37℃ 39℃
B
PF4 / GAPDH
2
1
0
ON OFF ON OFF
37℃ 39℃
C
NFE2 / GAPDH
0.2
0.1
0.0
ON OFF ON OFF
37℃ 39℃
D
β-TUBULIN / GAPDH
2
1
0
ON OFF ON OFF
37℃ 39℃

FIG. 7

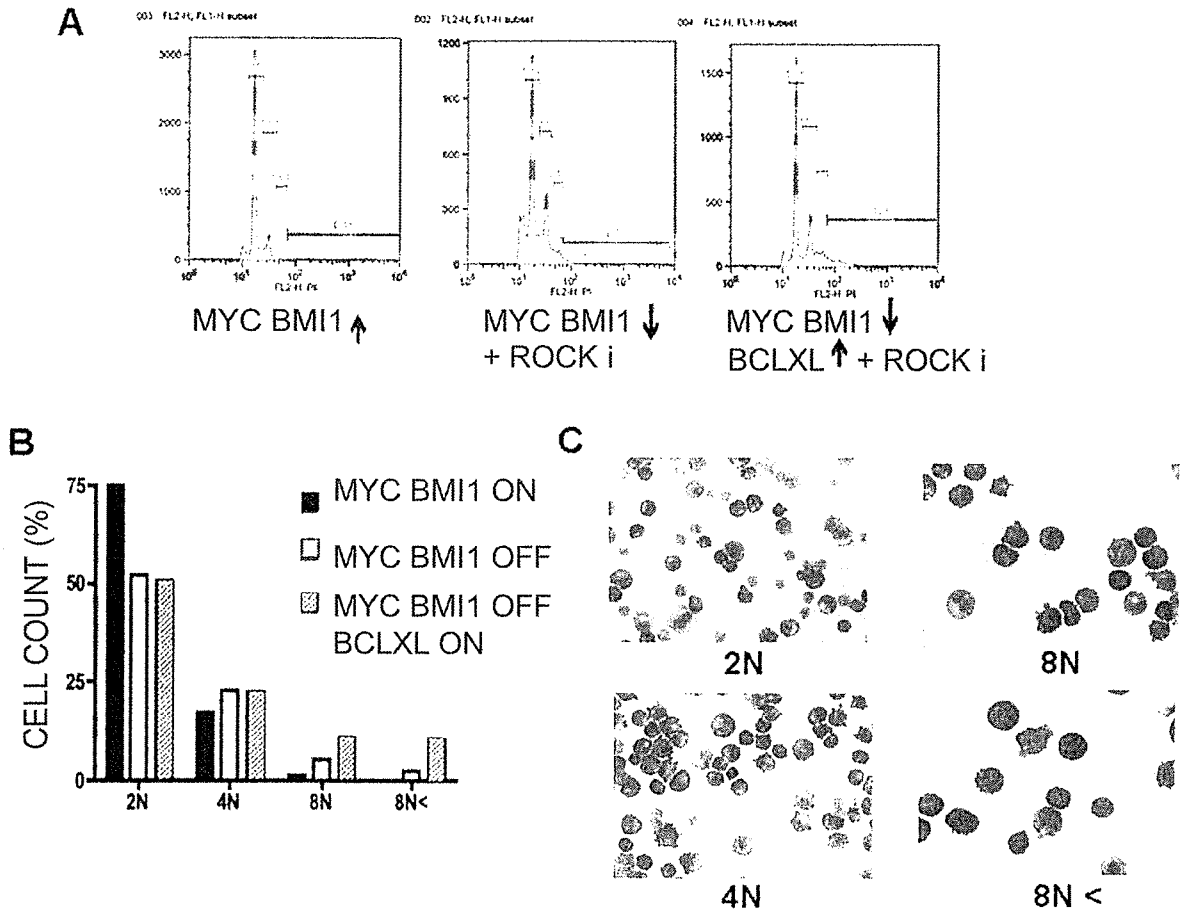

A
MYC BMI1 ↑
MYC BMI1 ↓
+ ROCK i
MYC BMI1 ↓
BCLXL ↑ + ROCK i
B
CELL COUNT (%)
75
50
25
0
2N
4N
8N
8N<
MYC BMI1 ON
MYC BMI1 OFF
MYC BMI1 OFF
BCLXL ON
C
2N
8N
4N
8N <

FIG. 8

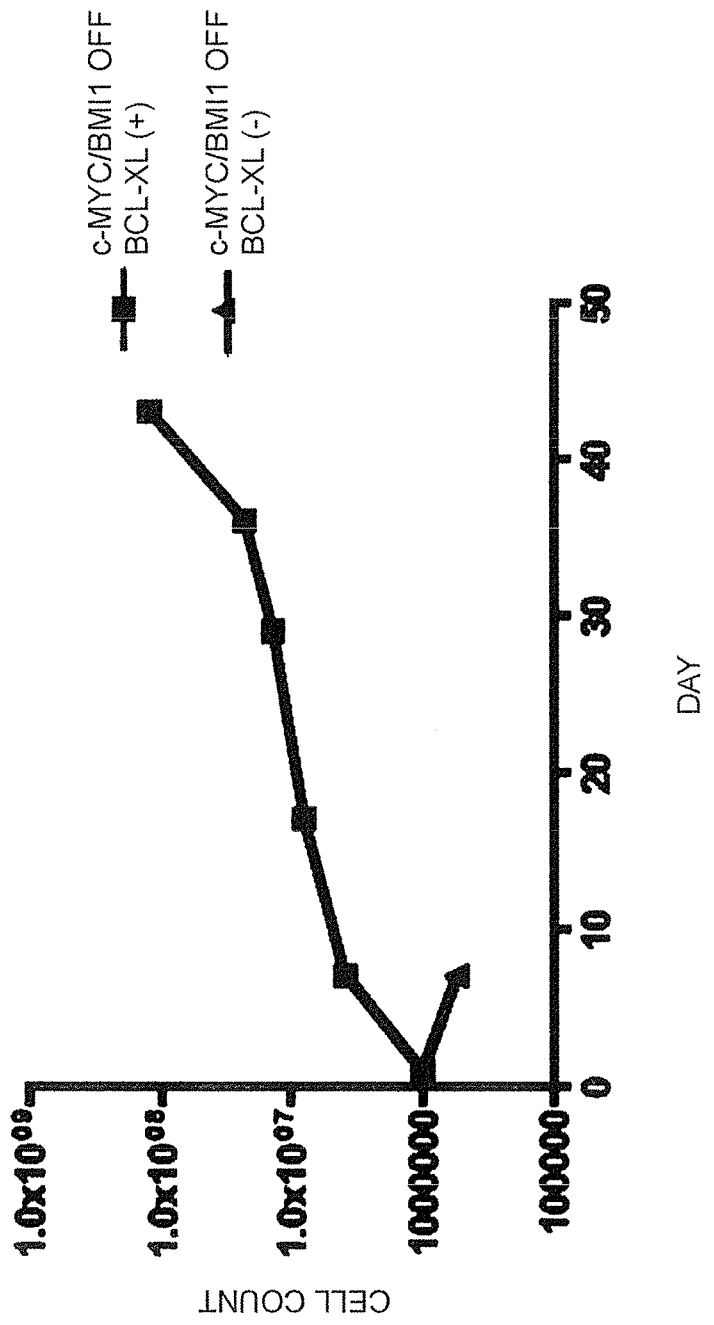

c-MYC/BMI1 OFF
BCL-XL (+)
c-MYC/BMI1 OFF
BCL-XL (-)
1.0x10^9
1.0x10^8
1.0x10^7
1000000
100000
CELL COUNT
0
10
20
30
40
50
DAY

FIG. 9

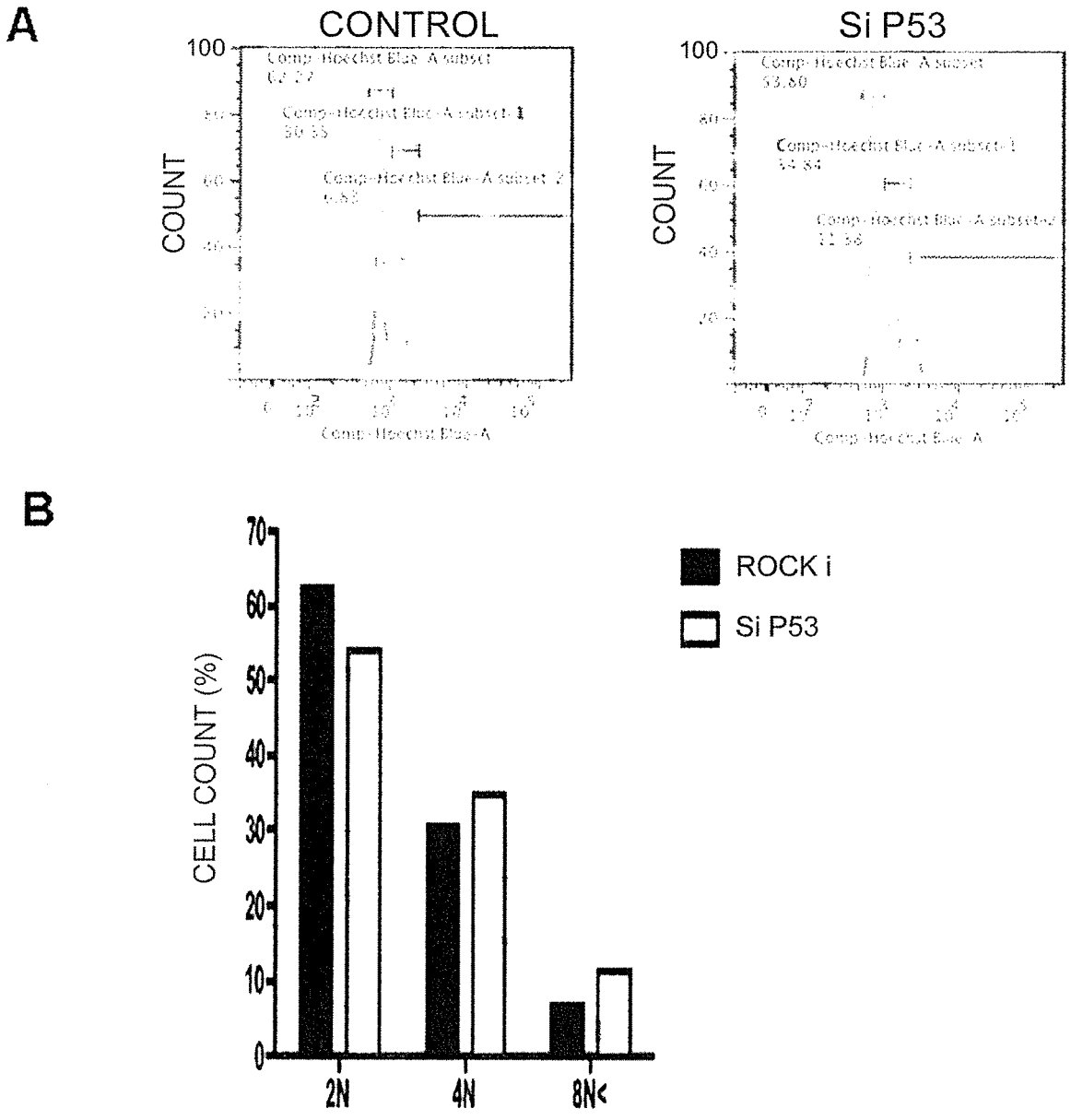

A
CONTROL
Si P53
COUNT
100
B
CELL COUNT (%)
70
60
50
40
30
20
10
0
2N
4N
8N<
ROCK i
Si P53

FIG. 10

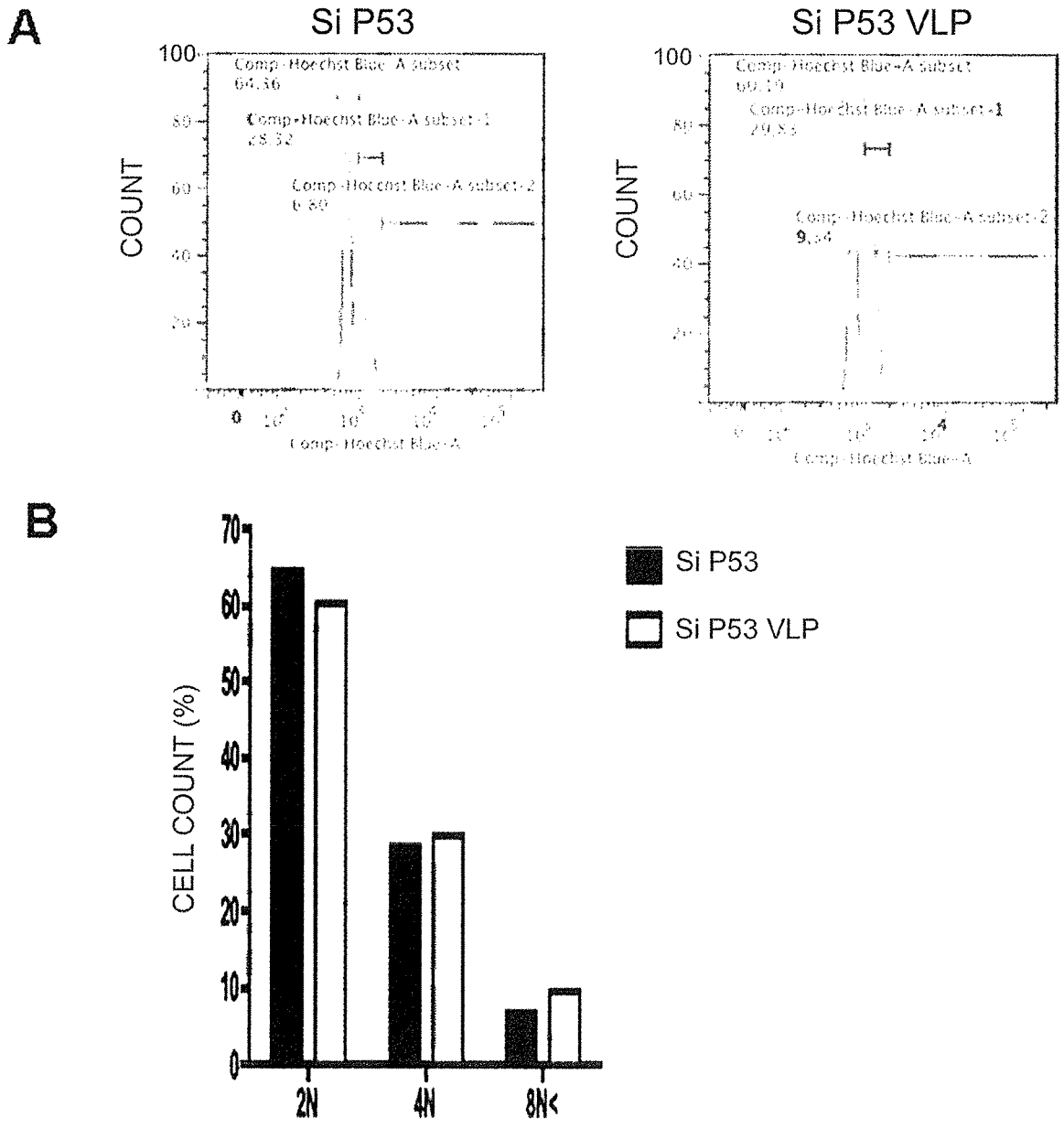
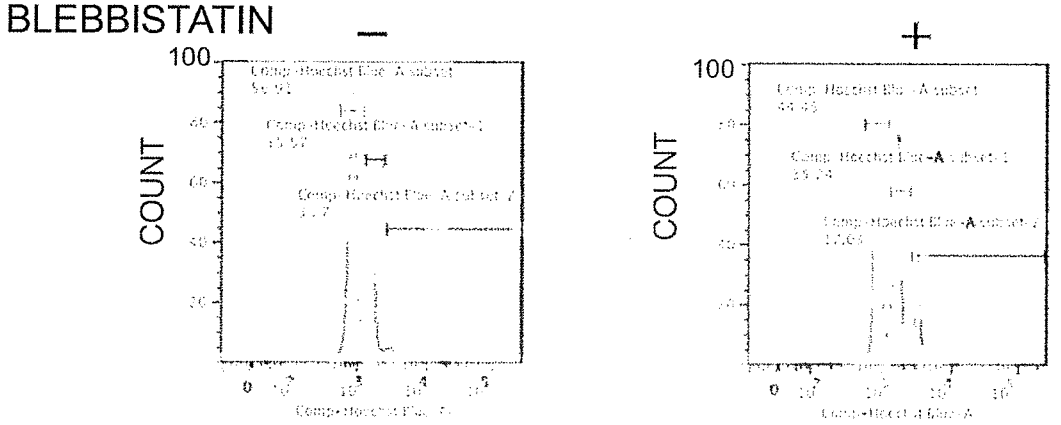

A
Si P53
Si P53 VLP
COUNT
100
80
60
40
20
0
Comp-Hoechst Blue-A
B
CELL COUNT (%)
70
60
50
40
30
20
10
0
2N
4N
8N<
Si P53
Si P53 VLP

FIG. 11

A


BLEBBISTATIN
—
+
100
COUNT
100
COUNT

B

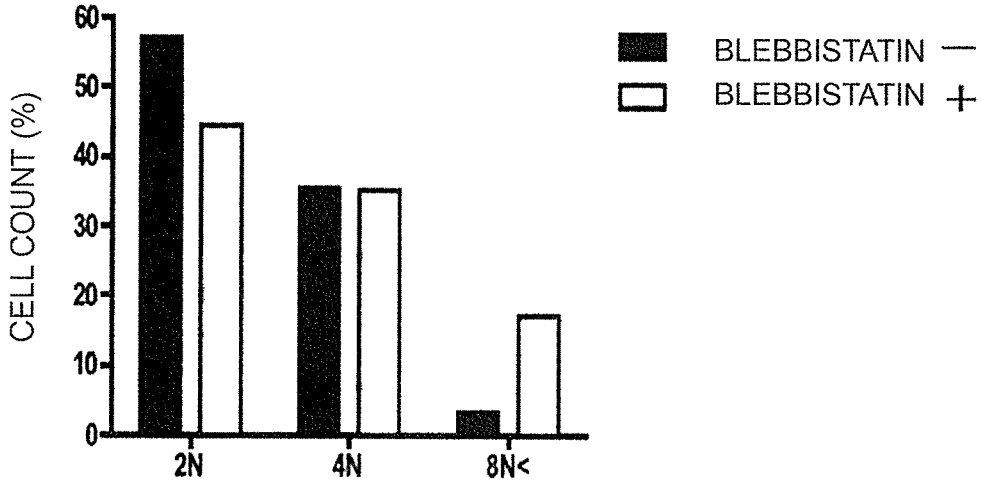

60
50
40
30
20
10
0
CELL COUNT (%)
2N
4N
8N<
BLEBBISTATIN —
BLEBBISTATIN +

FIG. 12

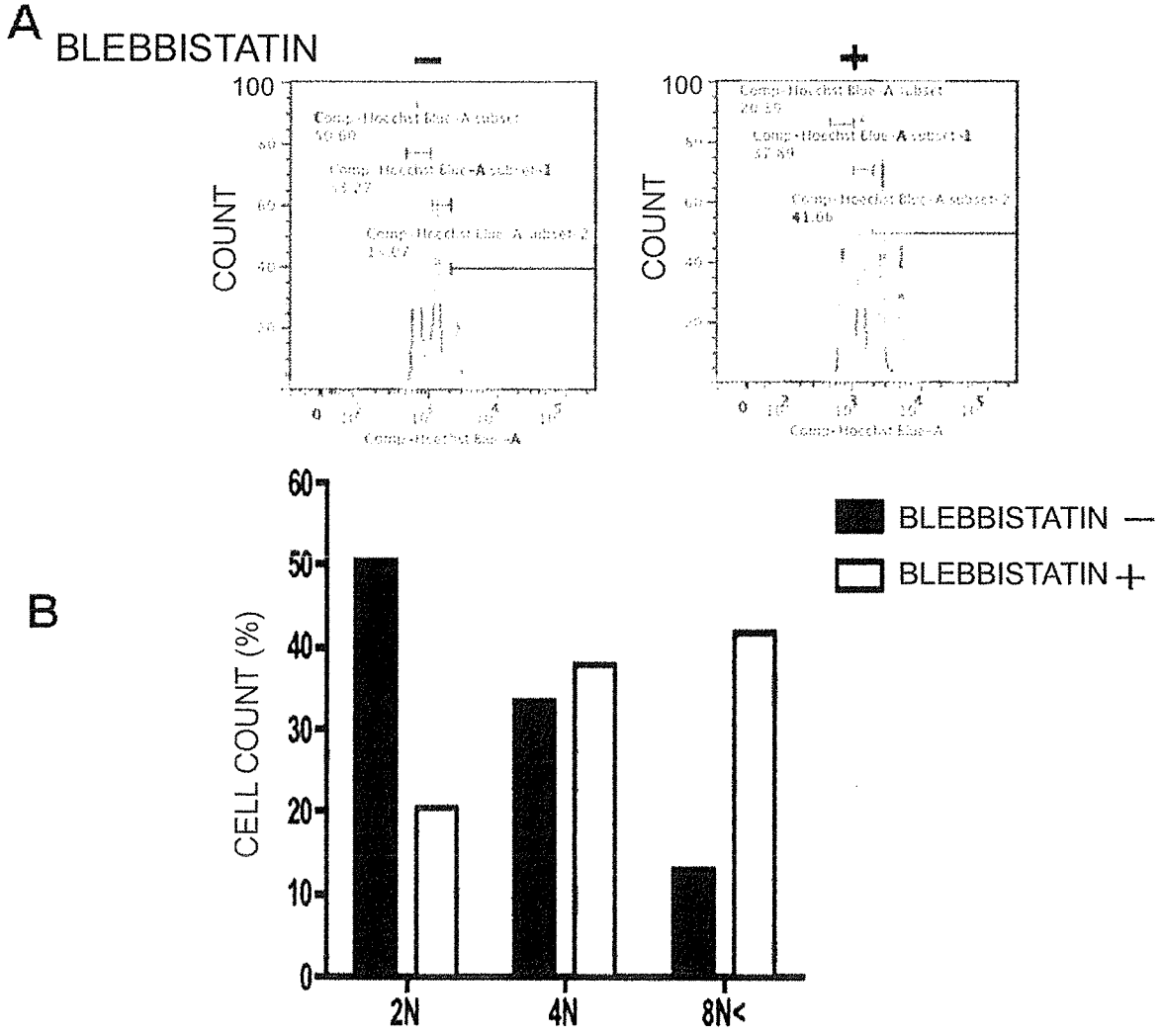

A
BLEBBISTATIN
—
+
100
COUNT
B
BLEBBISTATIN —
BLEBBISTATIN +
CELL COUNT (%)
60
50
40
30
20
10
0
2N
4N
8N<

FIG. 13

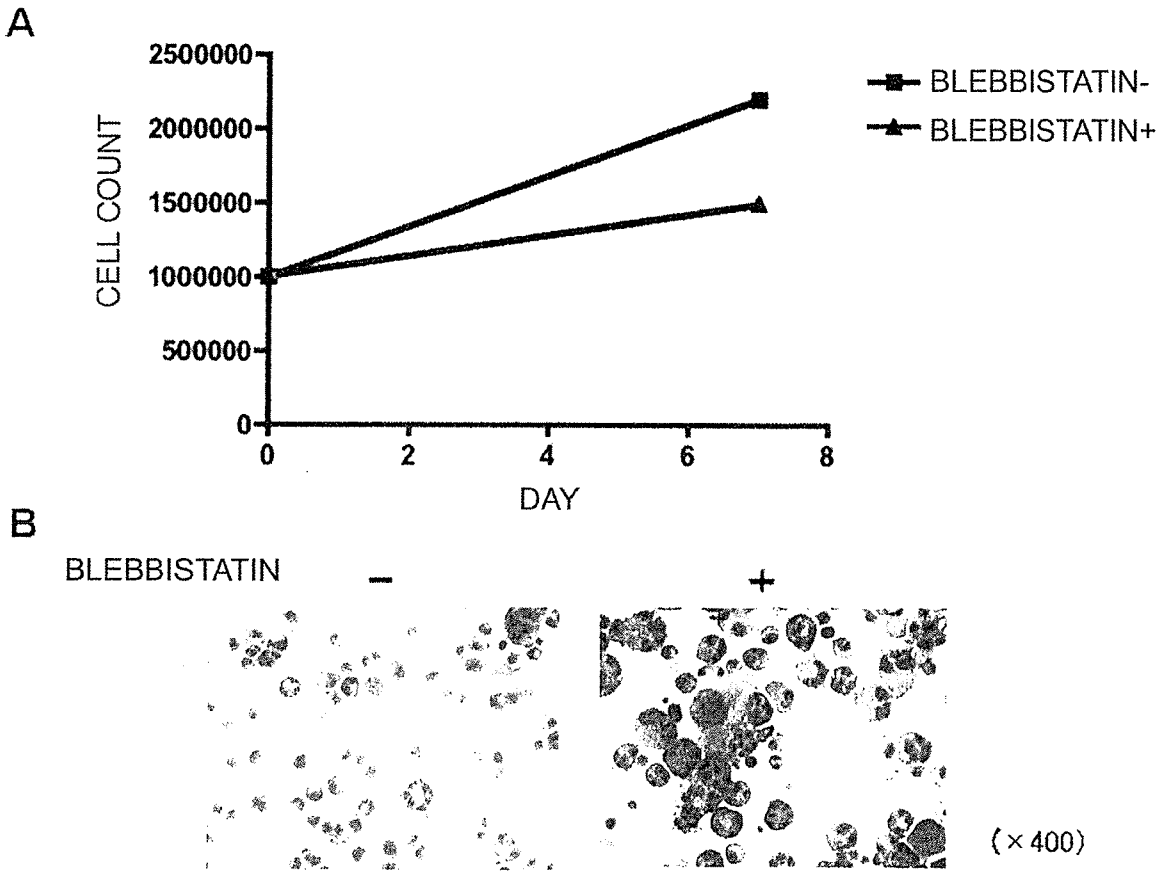

A
2500000
2000000
1500000
1000000
500000
0
CELL COUNT
0
2
4
6
8
DAY
BLEBBISTATIN-
BLEBBISTATIN+
B
BLEBBISTATIN
–
+
(×400)

FIG. 14

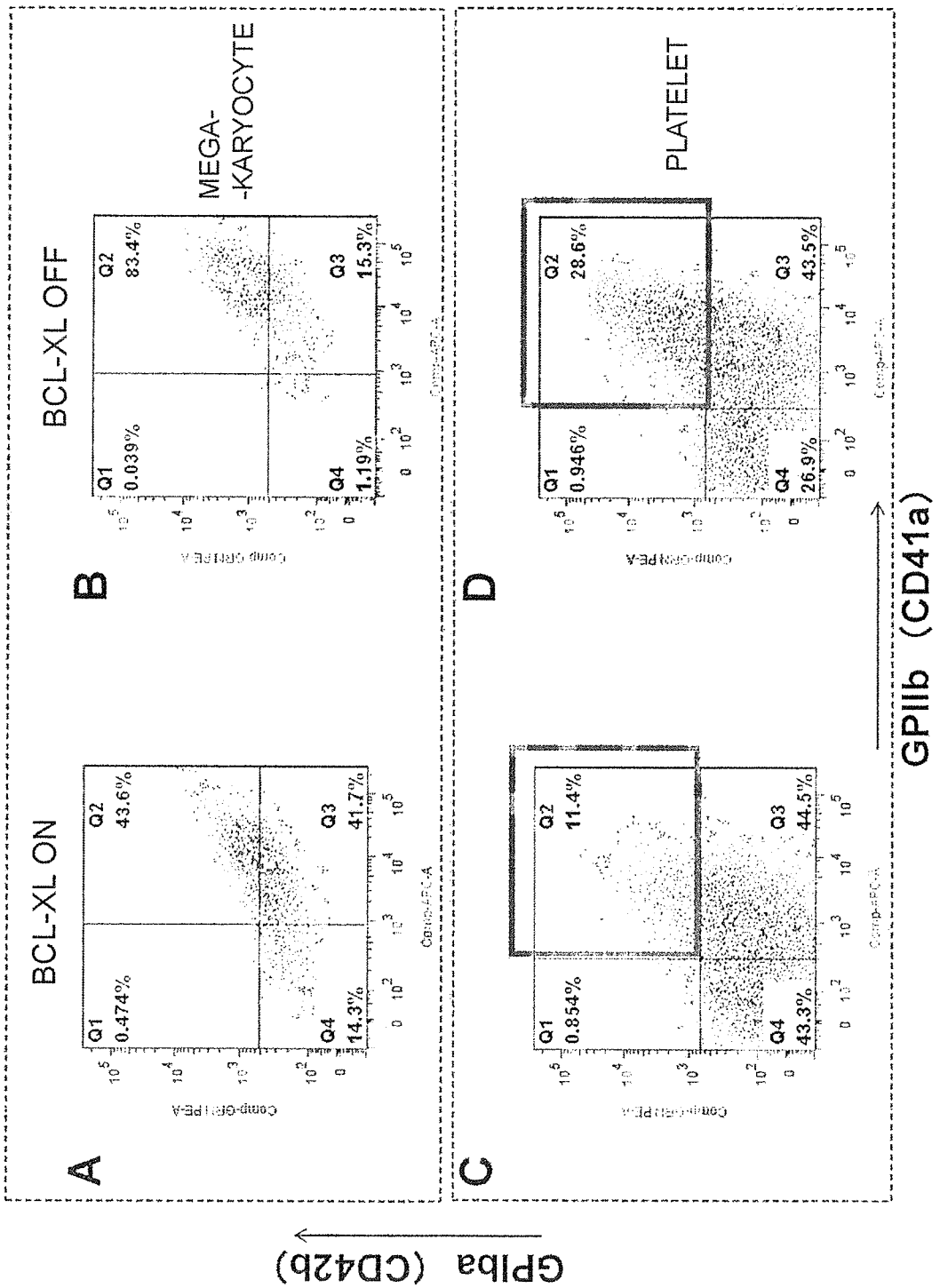

BCL-XL ON
BCL-XL OFF
MEGA-KARYOCYTE
PLATELET
A
B
C
D
Q1 0.474%
Q2 43.6%
Q3 41.7%
Q4 14.3%
Q1 0.039%
Q2 83.4%
Q3 15.3%
Q4 1.19%
Q1 0.854%
Q2 11.4%
Q3 44.5%
Q4 43.3%
Q1 0.946%
Q2 28.6%
Q3 43.5%
Q4 26.9%
GPIIb (CD41a)
GPIba (CD42b)

FIG. 15

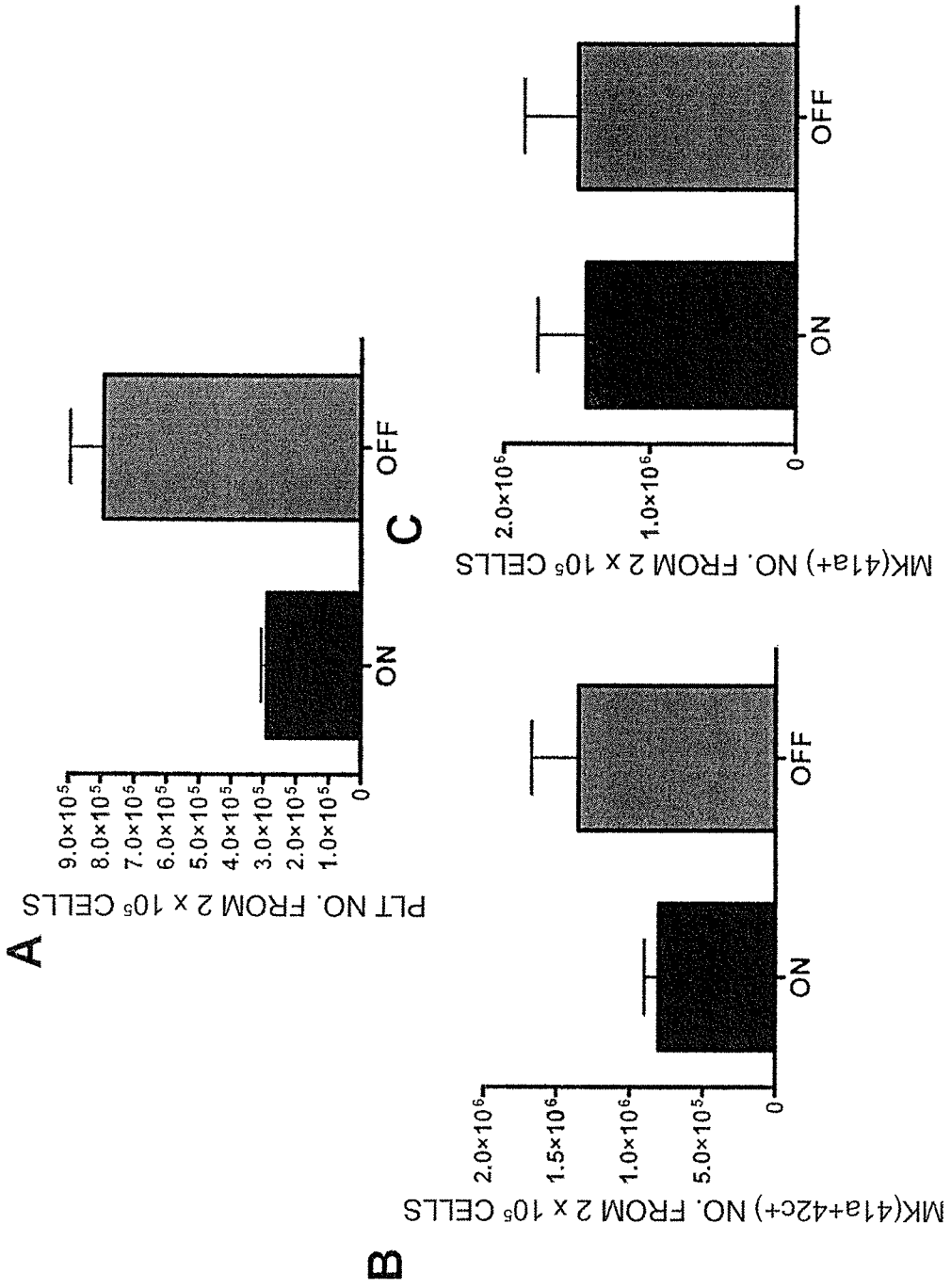

A
PLT NO. FROM 2 x 10⁵ CELLS
9.0×10⁵
8.0×10⁵
7.0×10⁵
6.0×10⁵
5.0×10⁵
4.0×10⁵
3.0×10⁵
2.0×10⁵
1.0×10⁵
0
ON
OFF
B
MK(41a+42c+) NO. FROM 2 x 10⁵ CELLS
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
ON
OFF
C
MK(41a+) NO. FROM 2 x 10⁵ CELLS
2.0×10⁶
1.0×10⁶
0
ON
OFF

FIG. 16

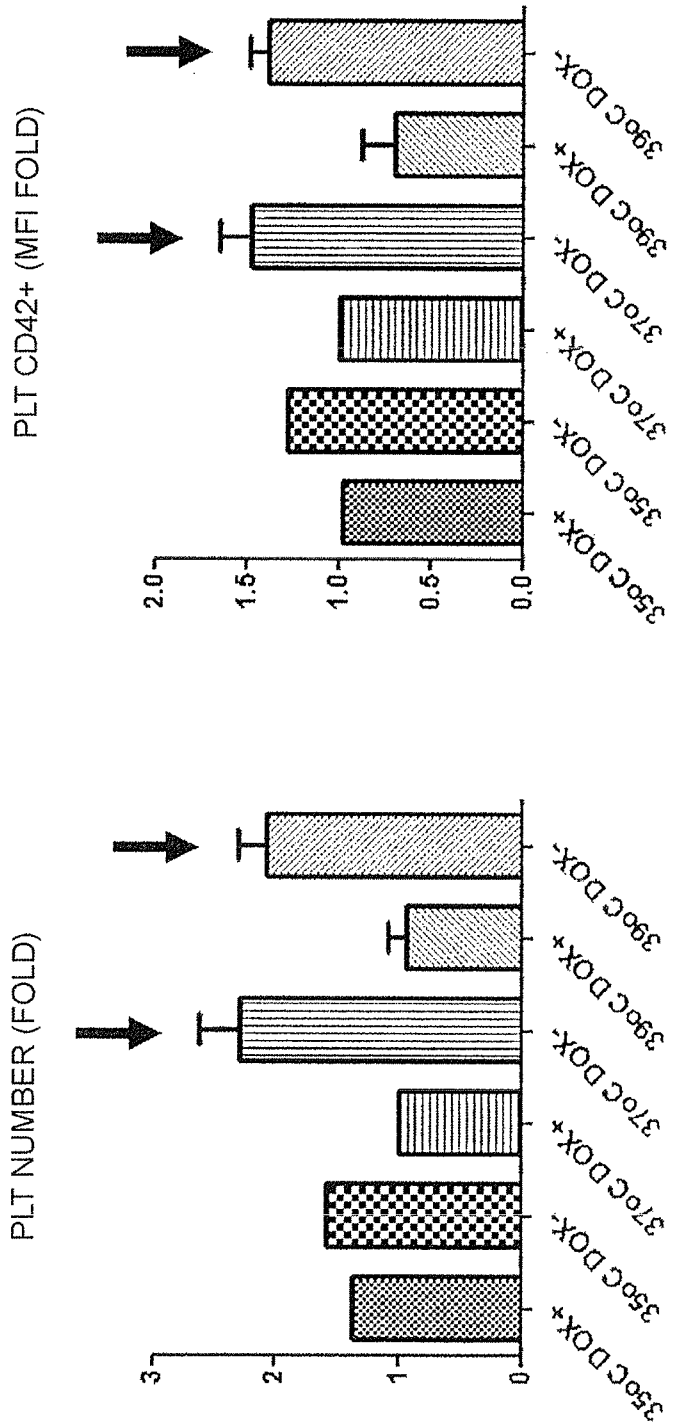

PLT CD42+ (MFI FOLD)
2.0
1.5
1.0
0.5
0.0
35oC DOX+
35oC DOX-
37oC DOX+
37oC DOX-
39oC DOX+
39oC DOX-
PLT NUMBER (FOLD)
3
2
1
0
35oC DOX+
35oC DOX-
37oC DOX+
37oC DOX-
39oC DOX+
39oC DOX-

FIG. 17

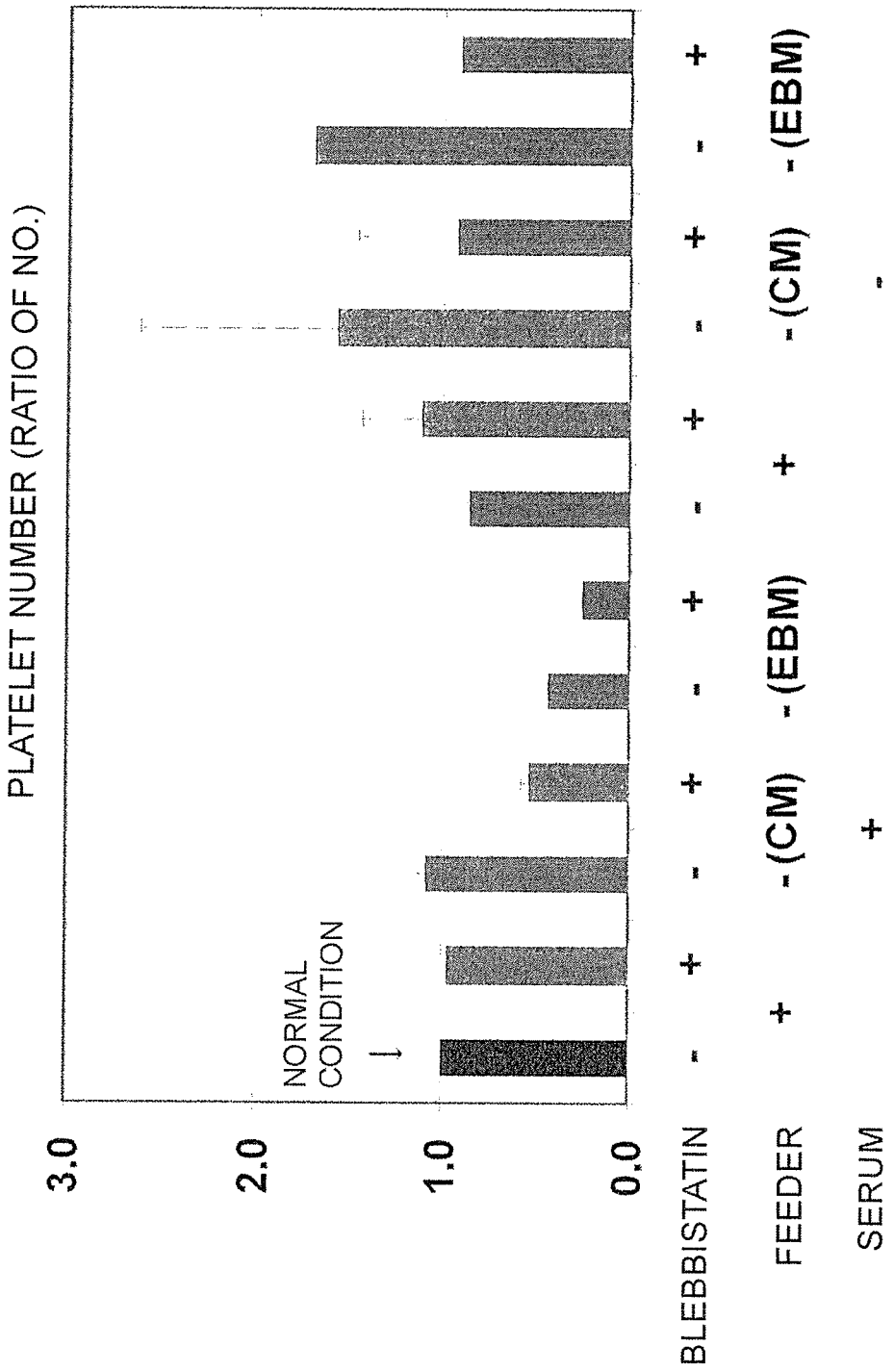

PLATELET NUMBER (RATIO OF NO.)
3.0
2.0
1.0
0.0
NORMAL CONDITION
BLEBBISTATIN
FEEDER
SERUM
- + - + - + - + - + - +
+ + - - + + - - + + - -
- - + + - - + + - - + +
- (CM) - (EBM) + - (CM) - (EBM)

FIG. 18

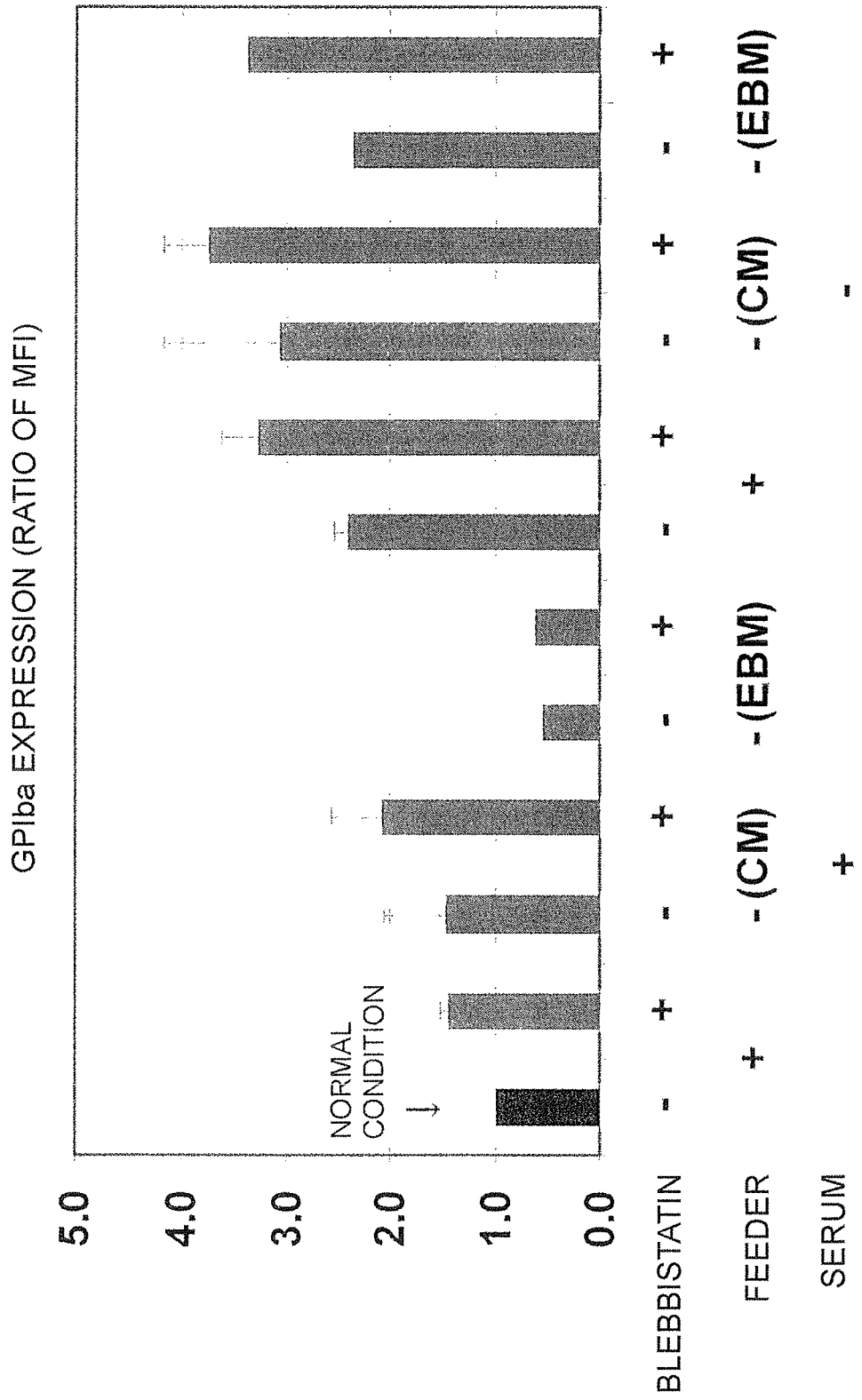

GPIba EXPRESSION (RATIO OF MFI)
5.0
4.0
3.0
2.0
1.0
0.0
NORMAL CONDITION
BLEBBISTATIN
FEEDER
SERUM
- + - + - + - + - + - +
+ + - - + + - - + + - -
- (CM) - (EBM) + - (CM) - (EBM)
+ -

FIG. 19

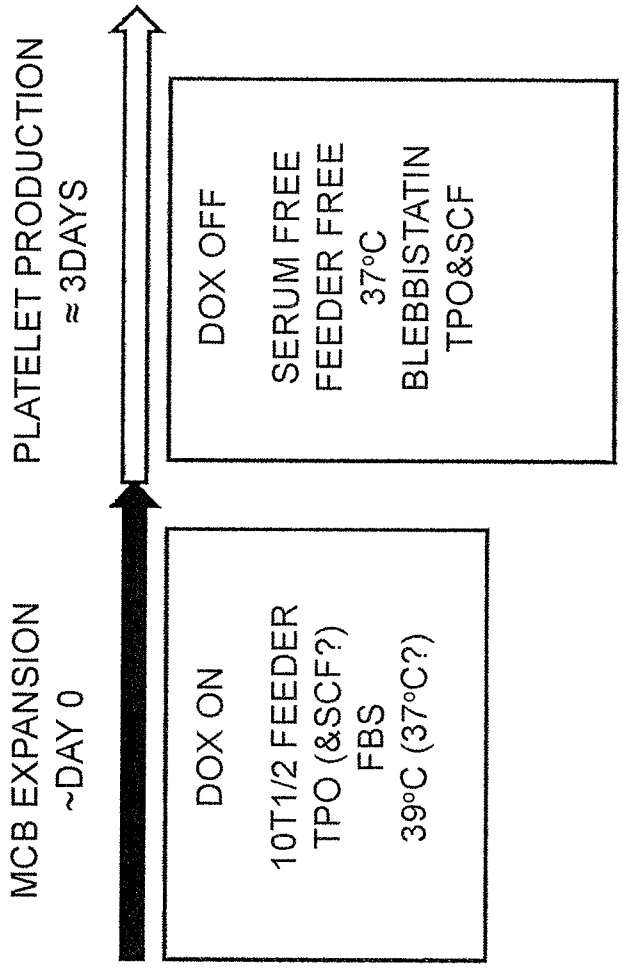

MCB EXPANSION
~DAY 0
DOX ON
10T1/2 FEEDER
TPO (&SCF?)
FBS
39°C (37°C?)
PLATELET PRODUCTION
≈ 3DAYS
DOX OFF
SERUM FREE
FEEDER FREE
37°C
BLEBBISTATIN
TPO&SCF

FIG. 20

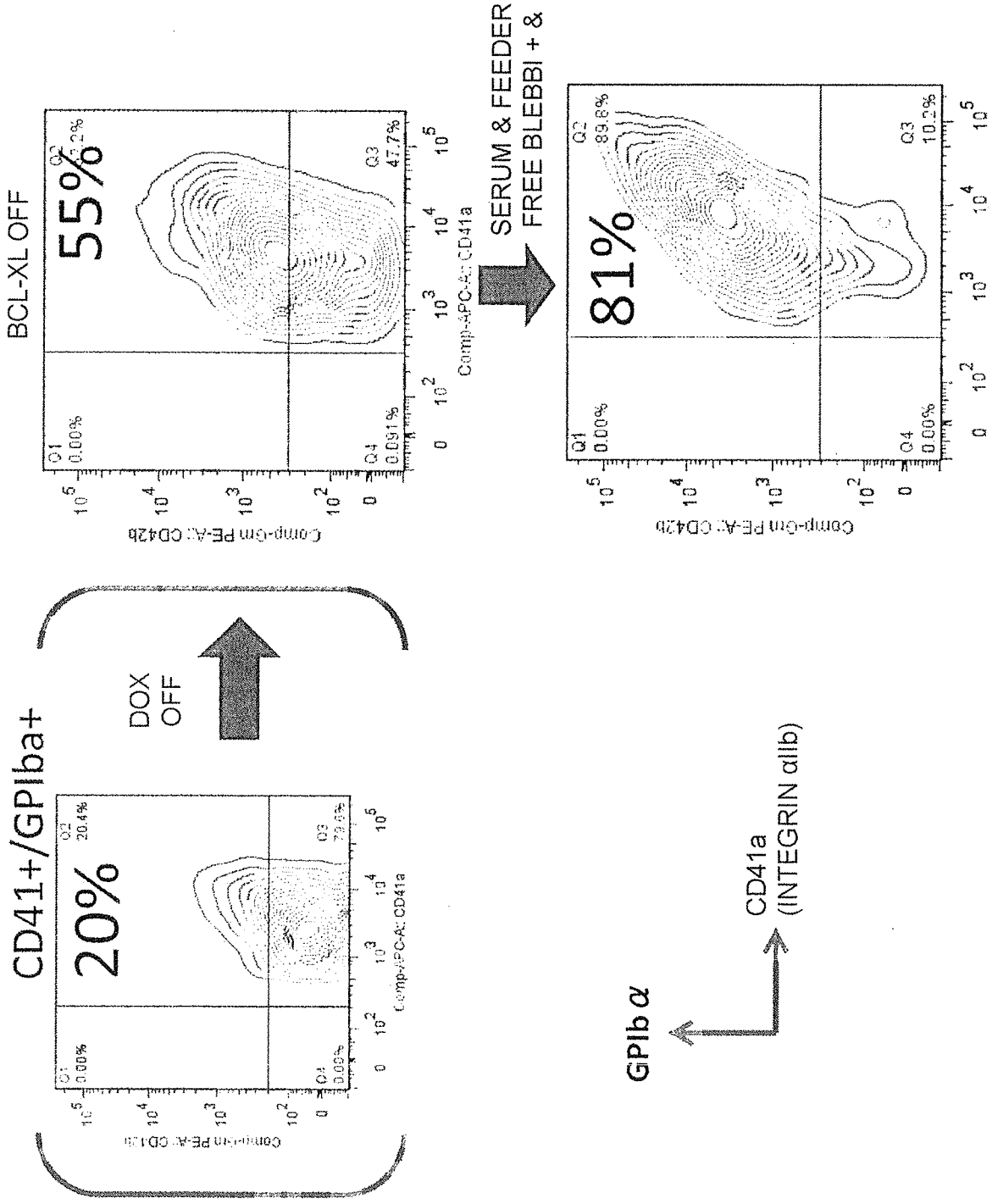

BCL-XL OFF
55%
SERUM & FEEDER FREE BLEBBI + &
81%
DOX OFF
CD41+/GPIba+
20%
GPIb α
CD41a (INTEGRIN αIIb)

FIG. 21

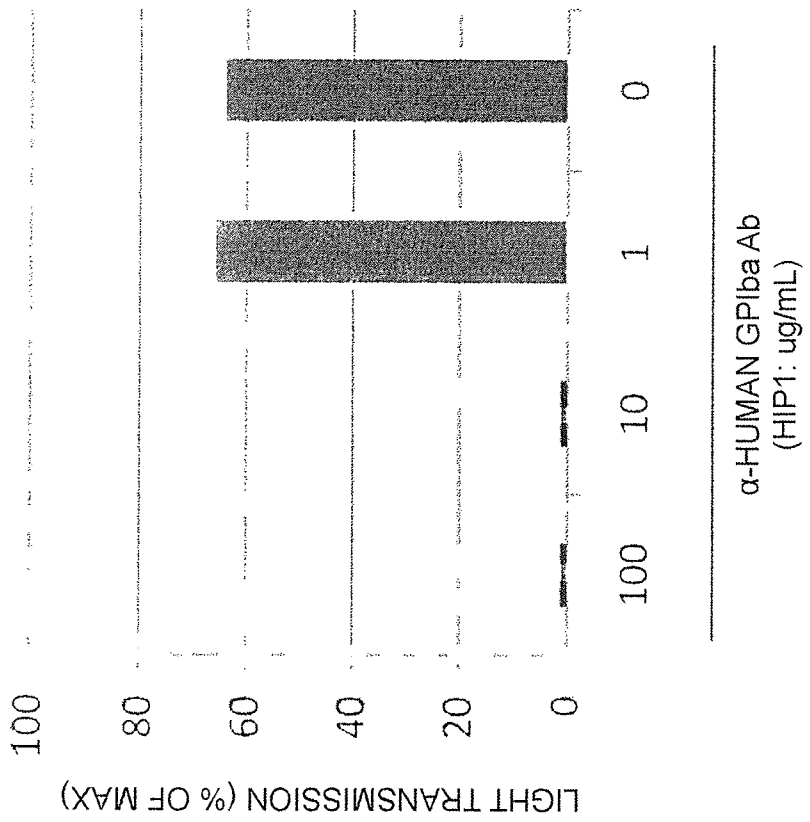

LIGHT TRANSMISSION (% OF MAX)
100
80
60
40
20
0
α-HUMAN GPIba Ab
(HIP1: ug/mL)
0
1
10
100

FIG. 22

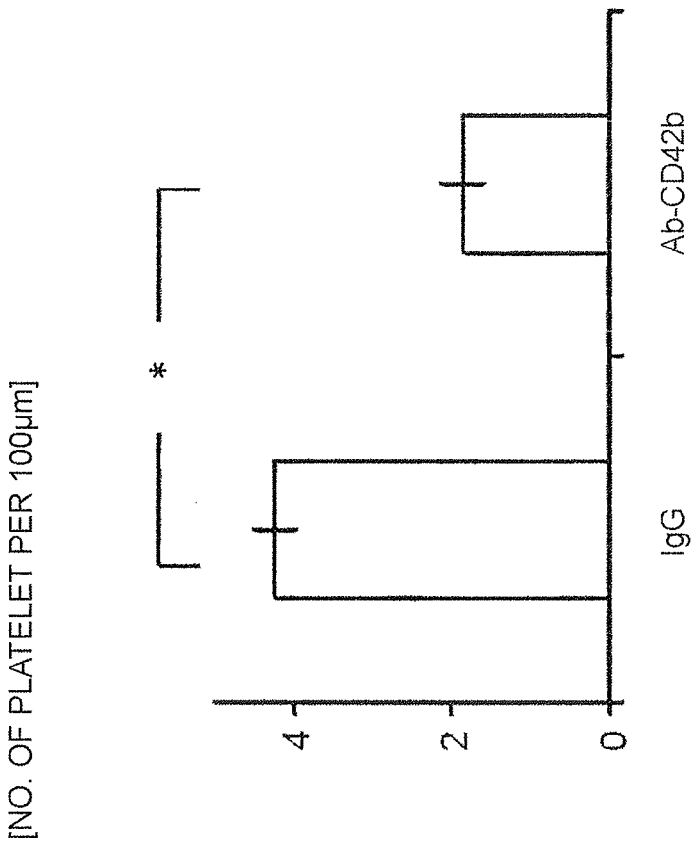


FIG. 23

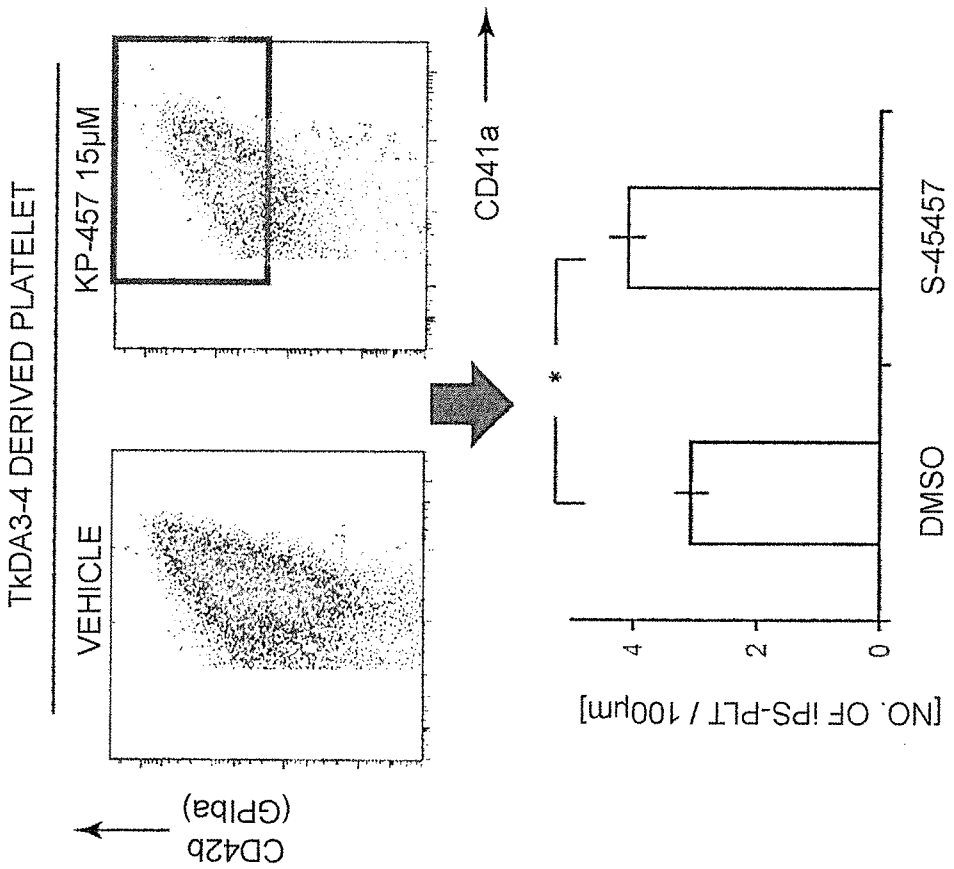
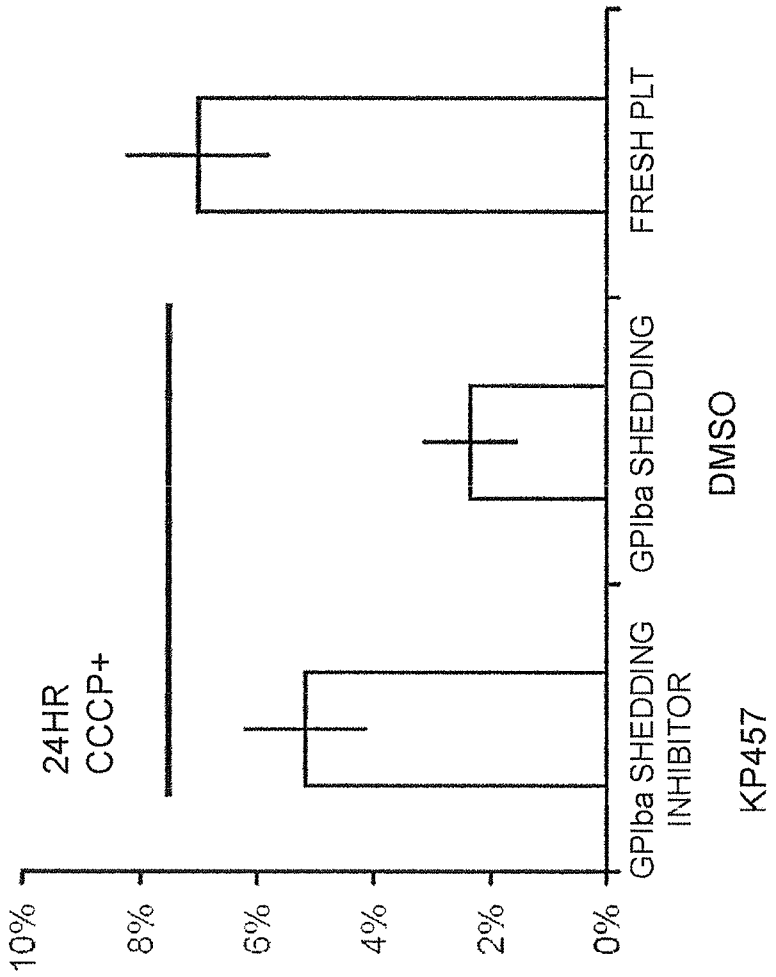

TkDA3-4 DERIVED PLATELET
KP-457 15μM
VEHICLE
CD42b
(GPIba)
CD41a
*
S-45457
DMSO
0
2
4
[NO. OF iPS-PLT / 100μm]

FIG. 24


10%
8%
6%
4%
2%
0%
24HR
CCCP+
GPIba SHEDDING
INHIBITOR
KP457
GPIba SHEDDING
DMSO
FRESH PLT