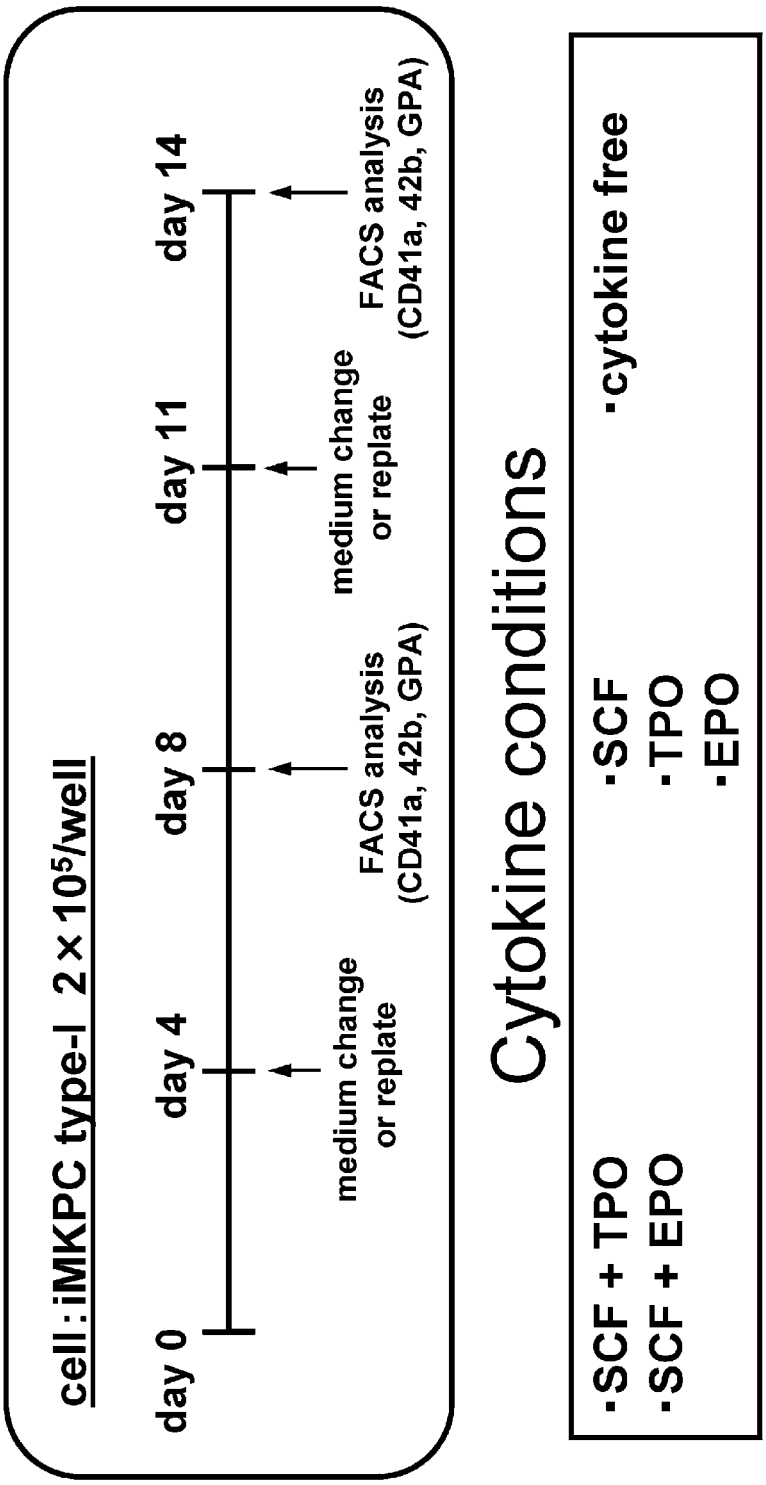
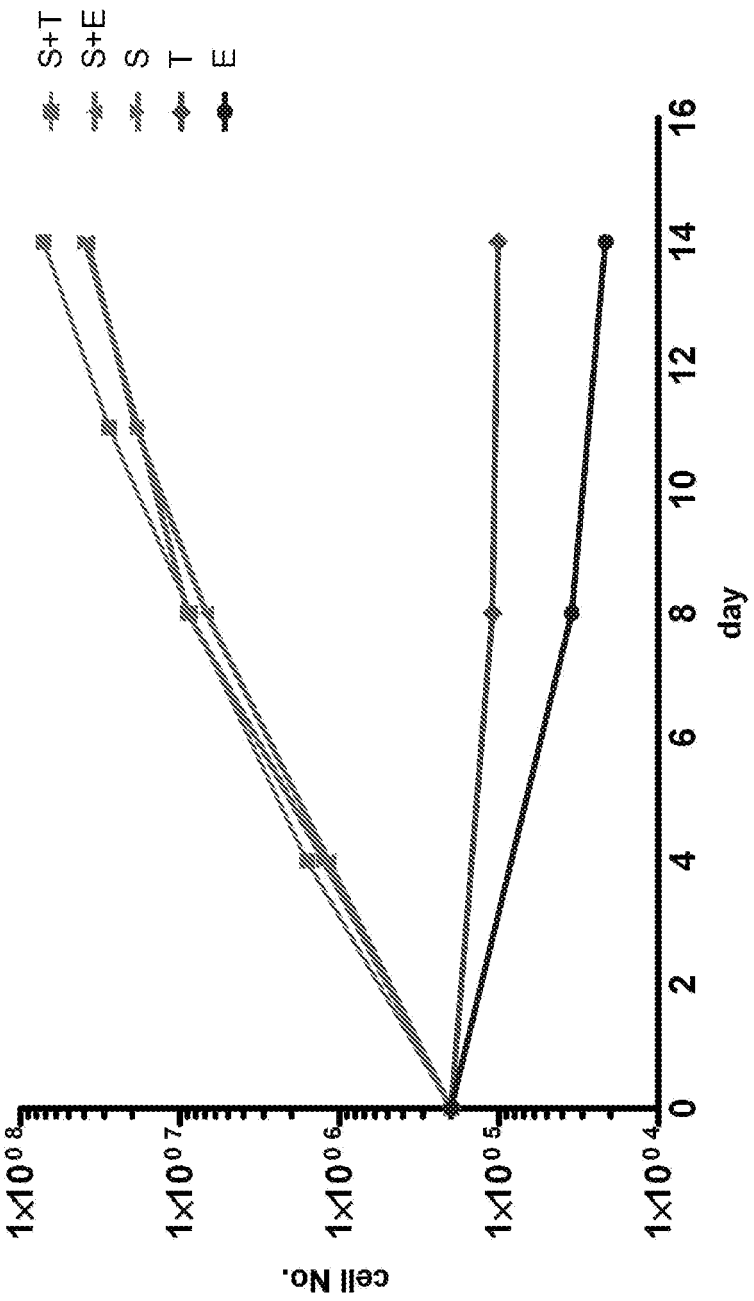
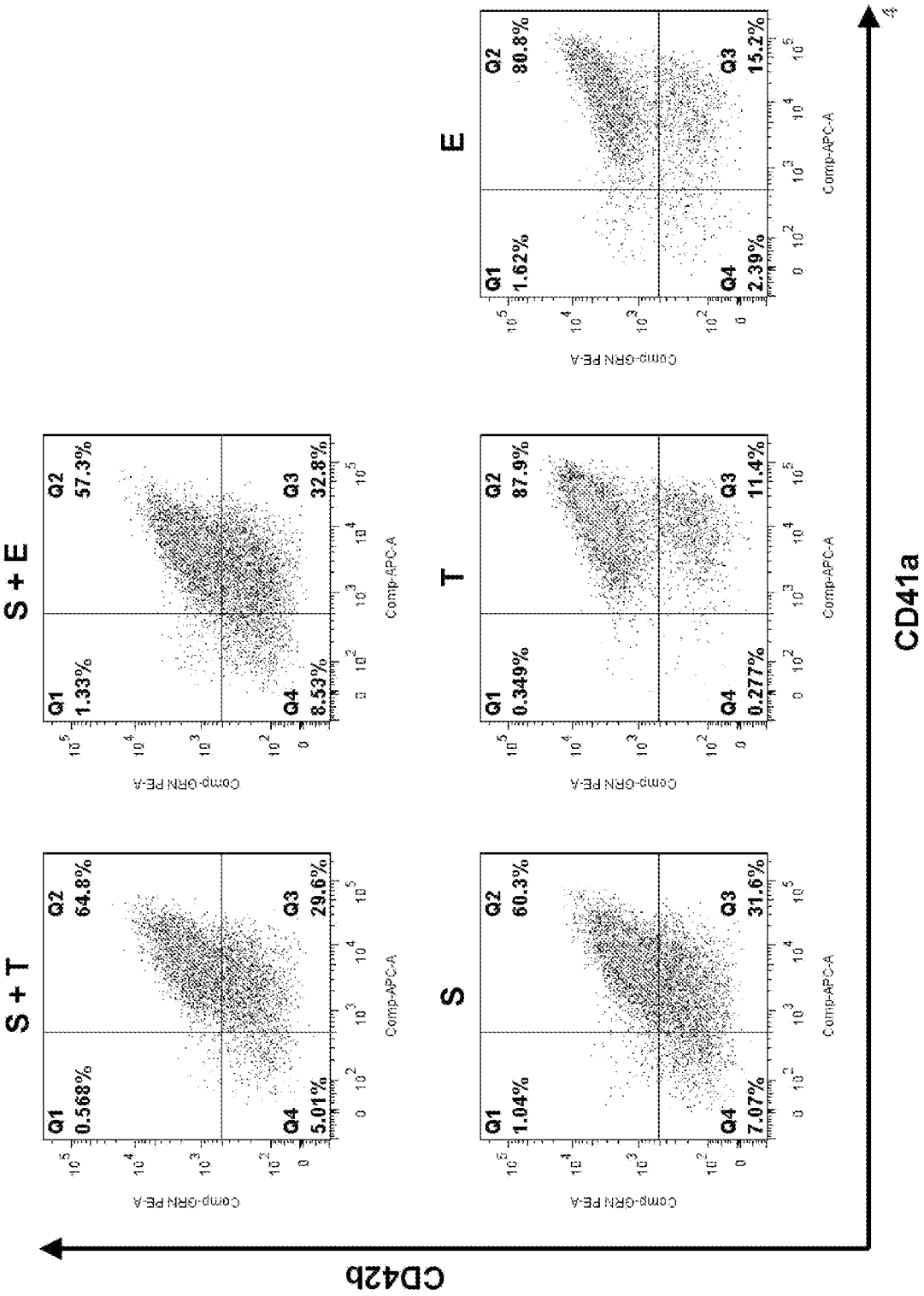
[図1A]


cell : iMKPC type-I 2×10⁵/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

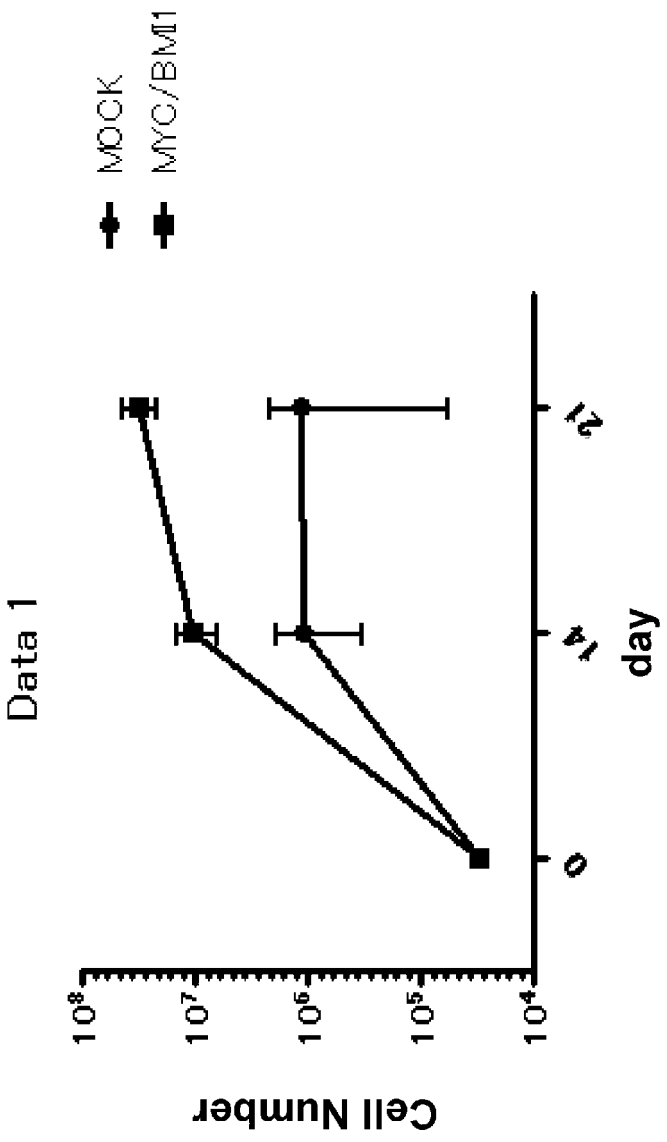
[図1B]



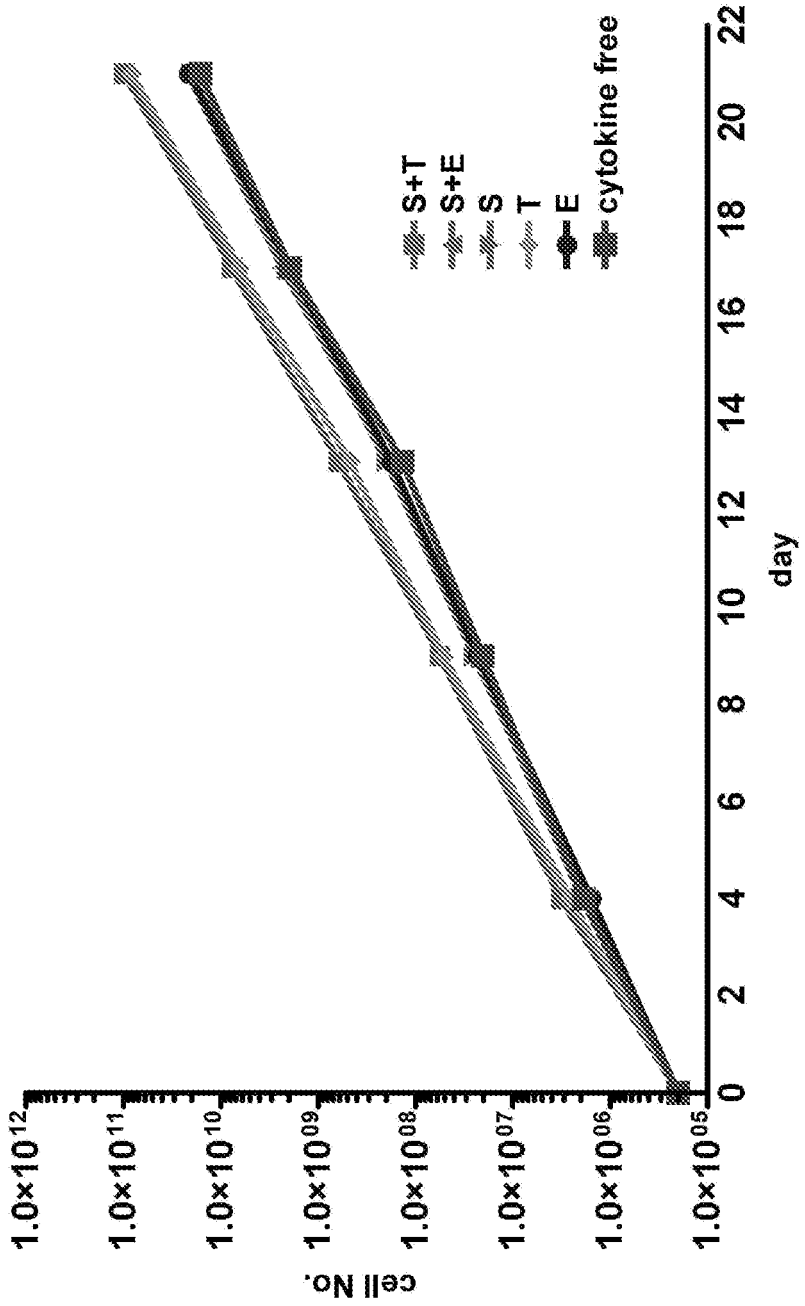
[図1C]


S + T
S
T
S + E
E
CD42b
CD41a
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 0.349%
Q2 87.9%
Q3 11.4%
Q4 0.277%
Q1 1.33%
Q2 57.3%
Q3 32.8%
Q4 8.53%
Q1 1.62%
Q2 80.8%
Q3 15.2%
Q4 2.39%
Comp-APC-A
Comp-GRN PE-A

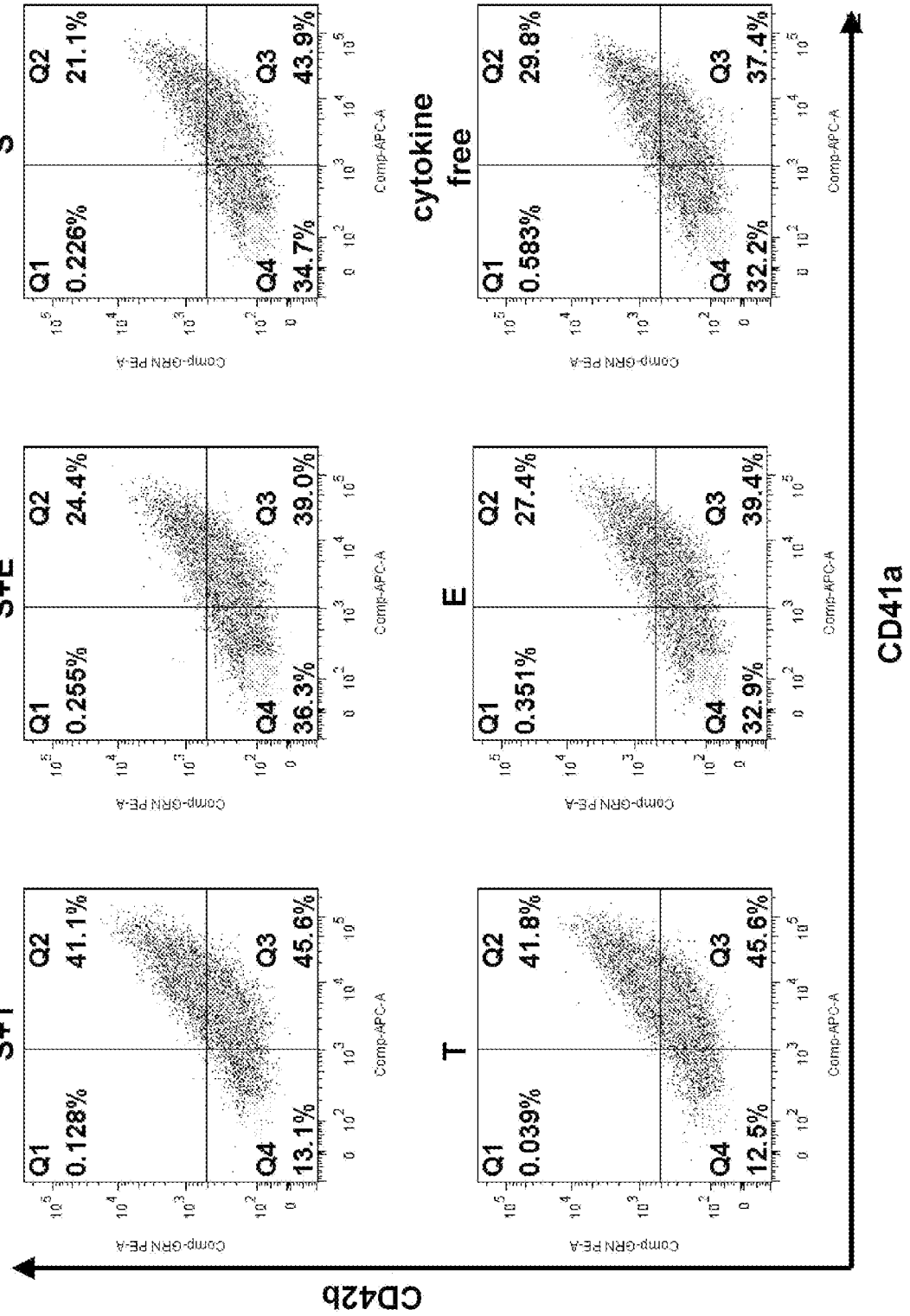
[図1D]



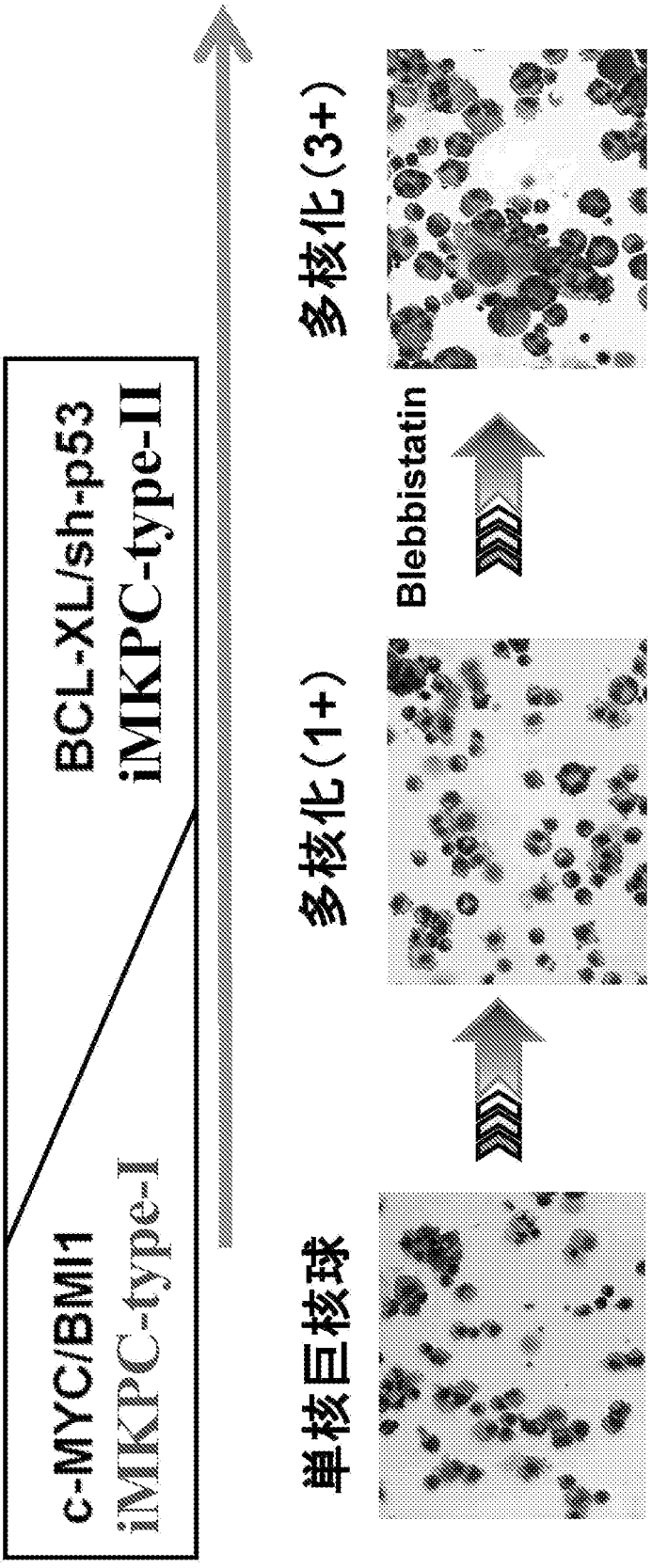
[図2A]


cell No.
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
0
2
4
6
8
10
12
14
16
18
20
22
day
S+T
S+E
S
T
E
cytokine free

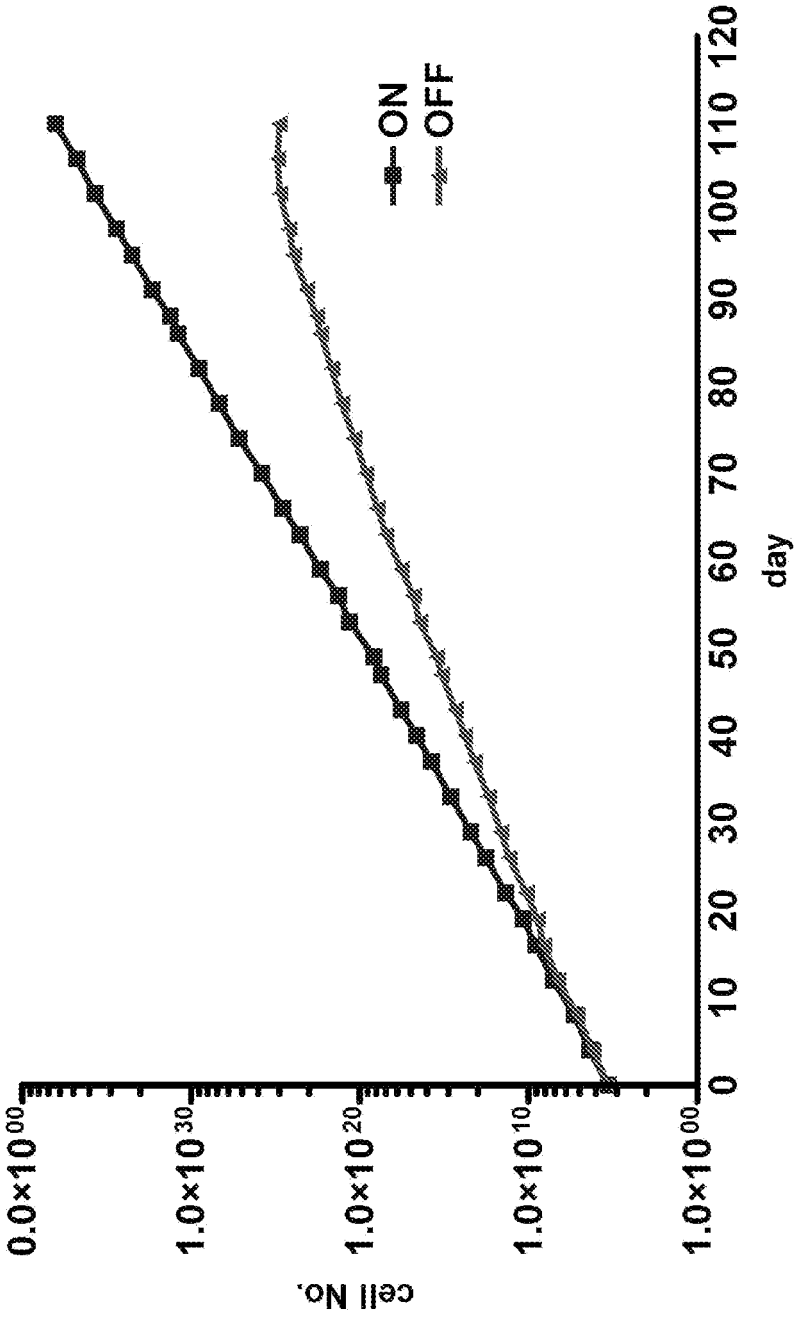
[図2B]


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%
Comp-GRN PE-A
Comp-APC-A
CD42b
CD41a

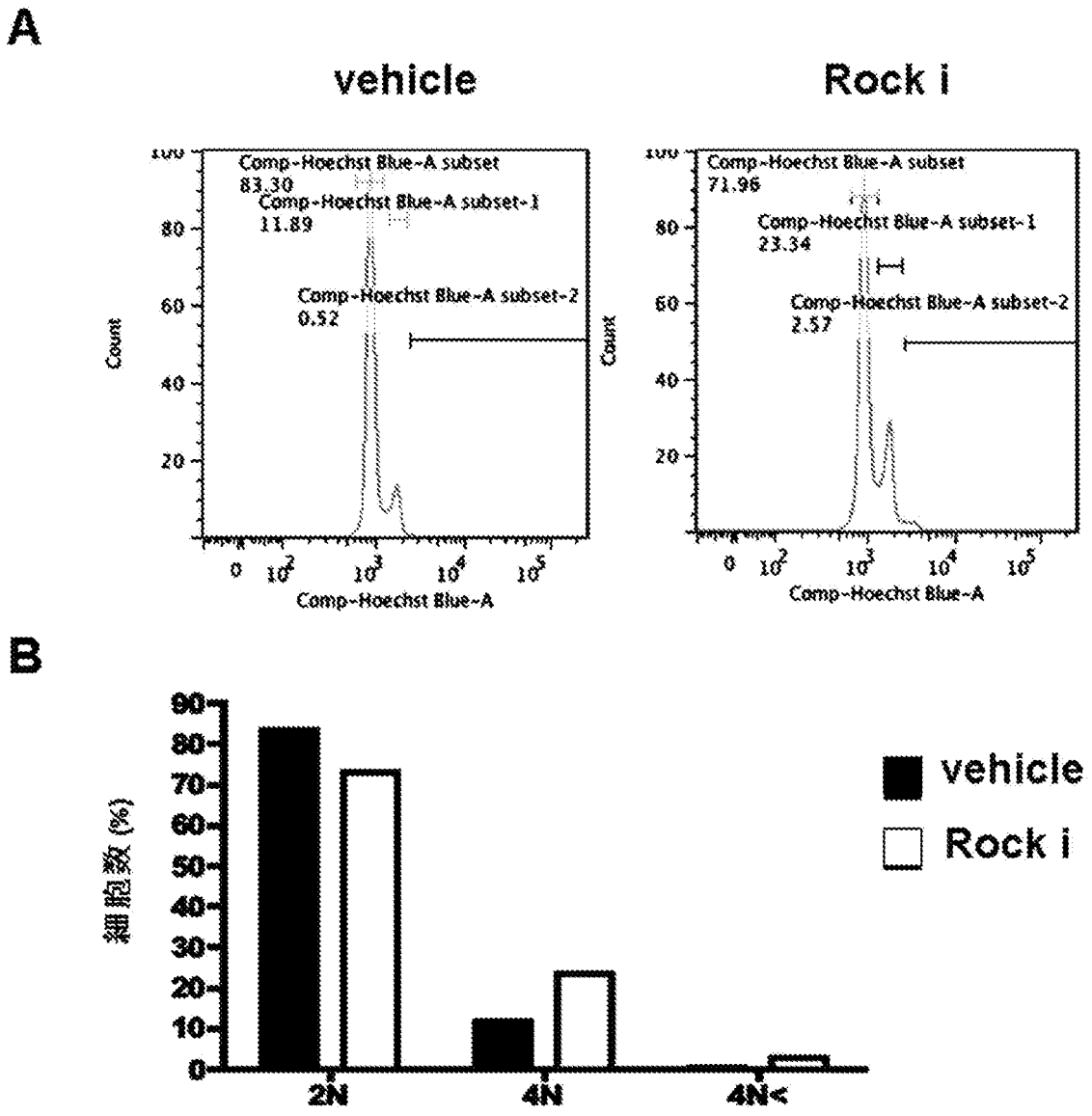
[図3]


c-MYC/BMI1
iMKPC-type-I
BCL-XL/sh-p53
iMKPC-type-II
単核巨核球
多核化(1+)
Blebbistatin
多核化(3+)

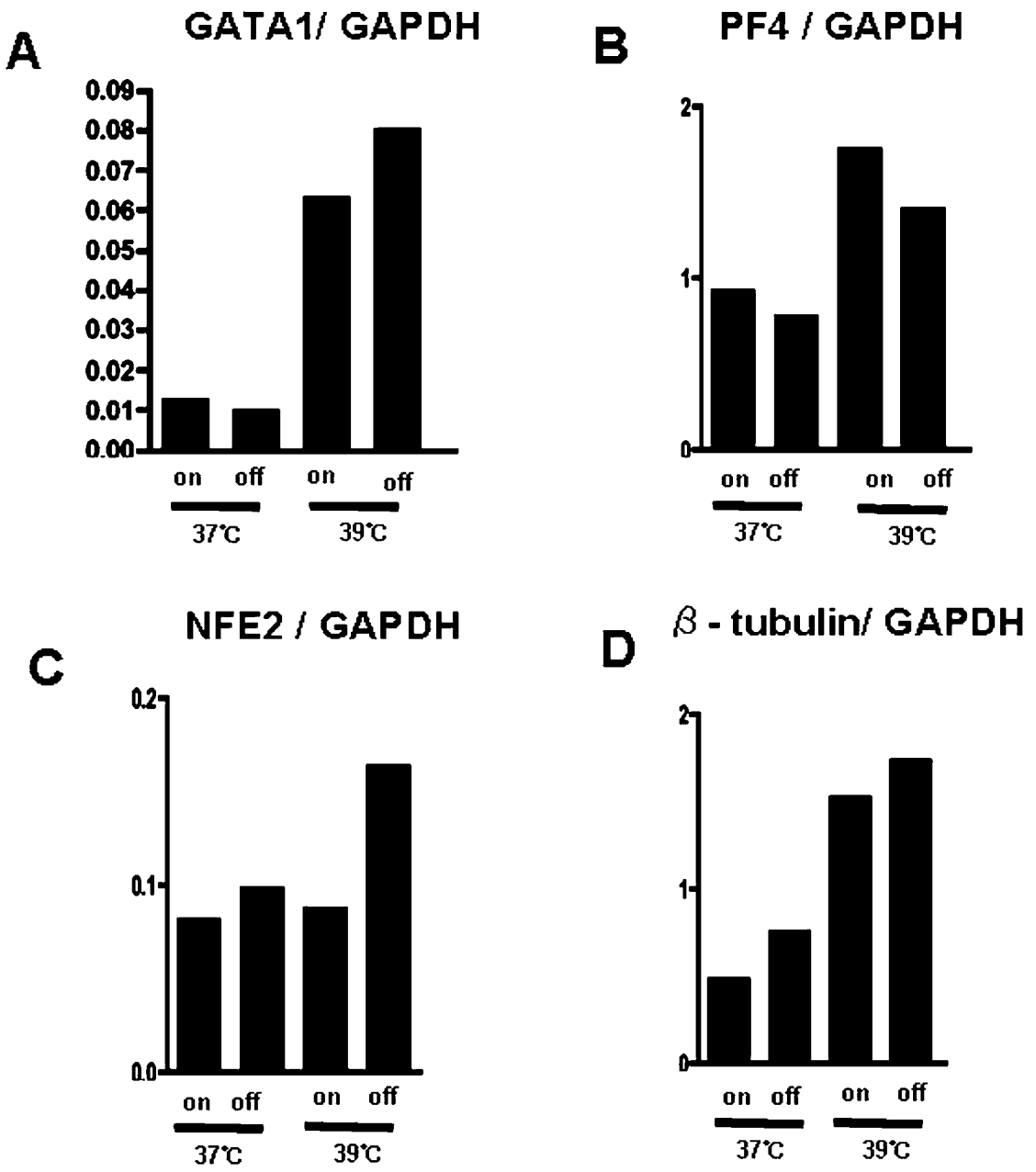
[図4]


ON
OFF
day
cell No.
0.0×10^00
1.0×10^30
1.0×10^20
1.0×10^10
1.0×10^00
0
10
20
30
40
50
60
70
80
90
100
110
120

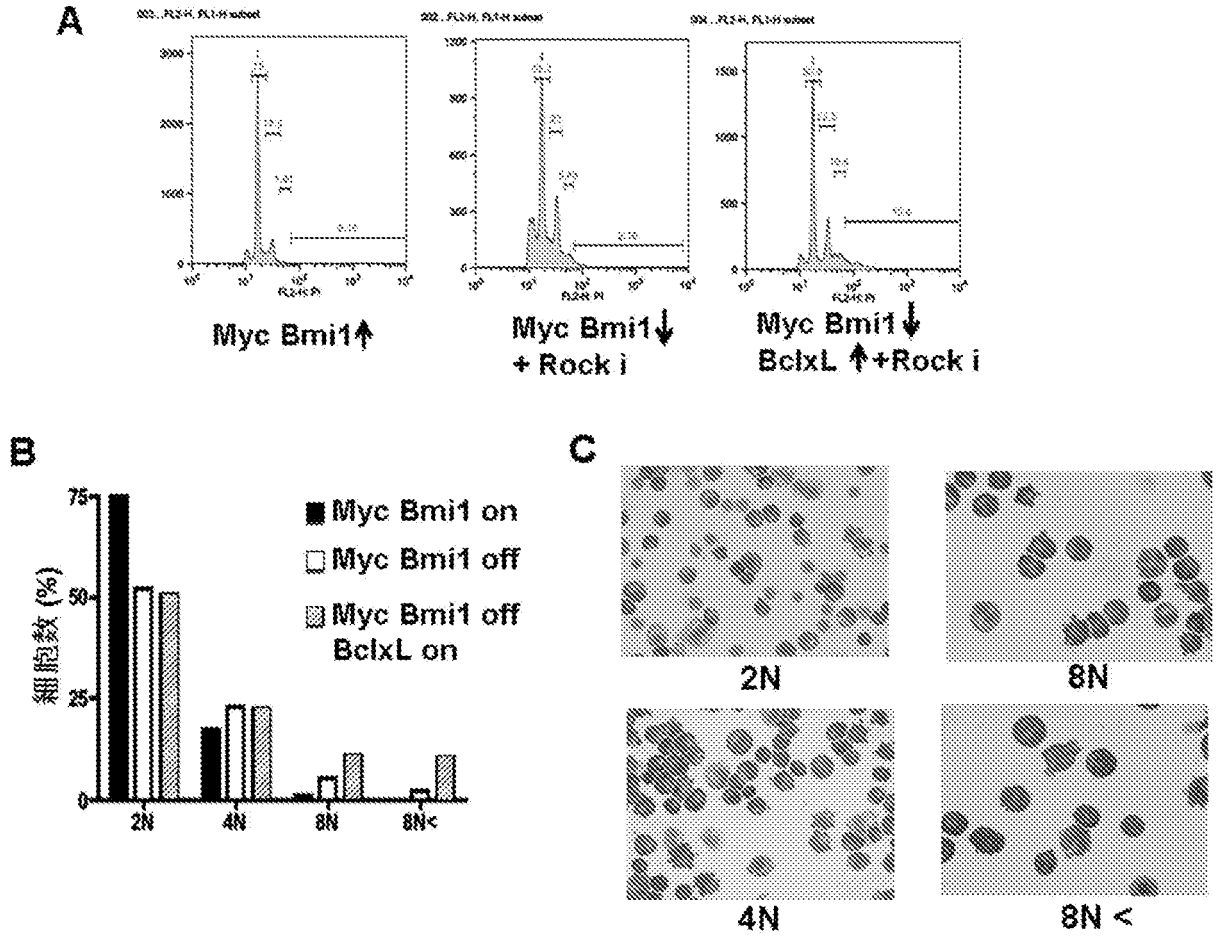
[図5]


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
細胞数 (%)
vehicle
Rock i
2N
4N
4N<

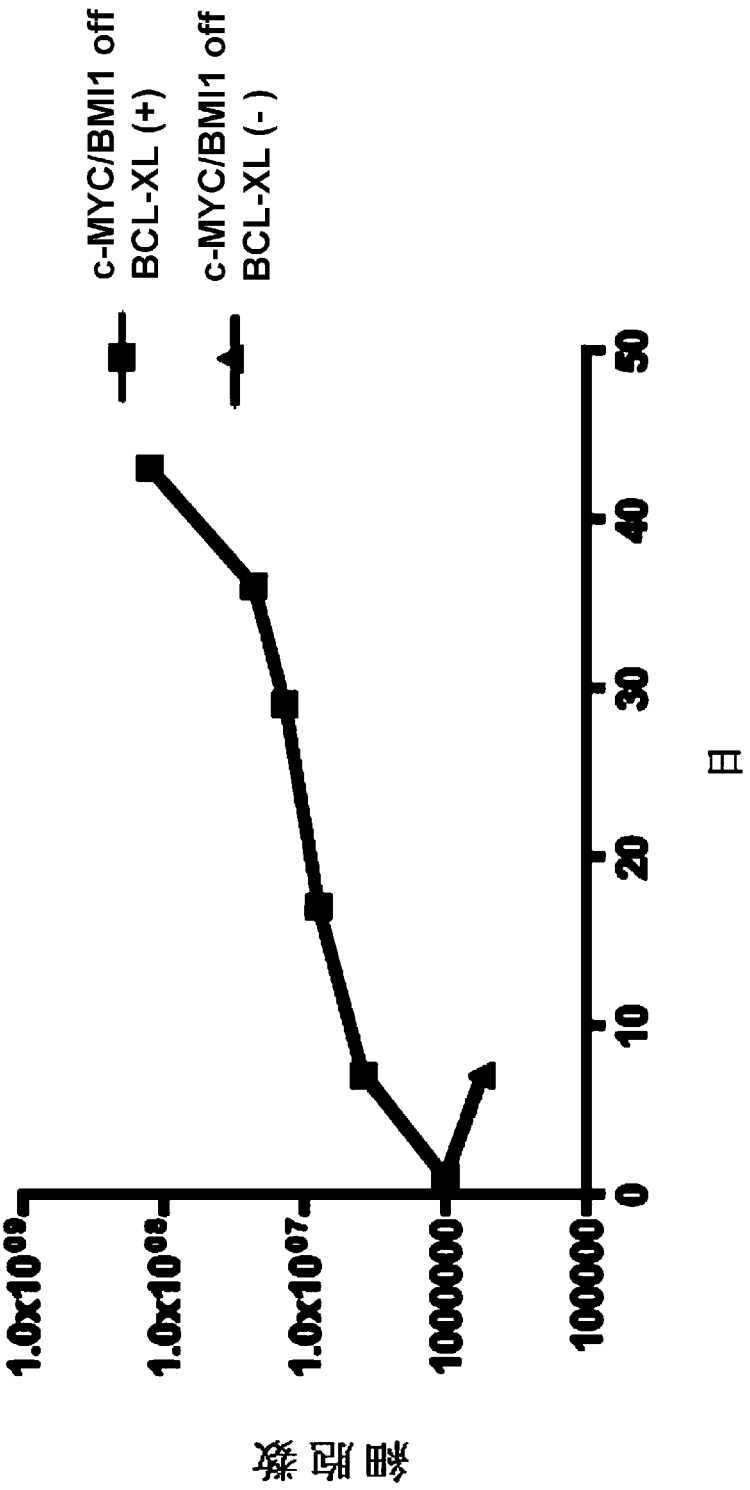
[図6]


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℃

[図7]

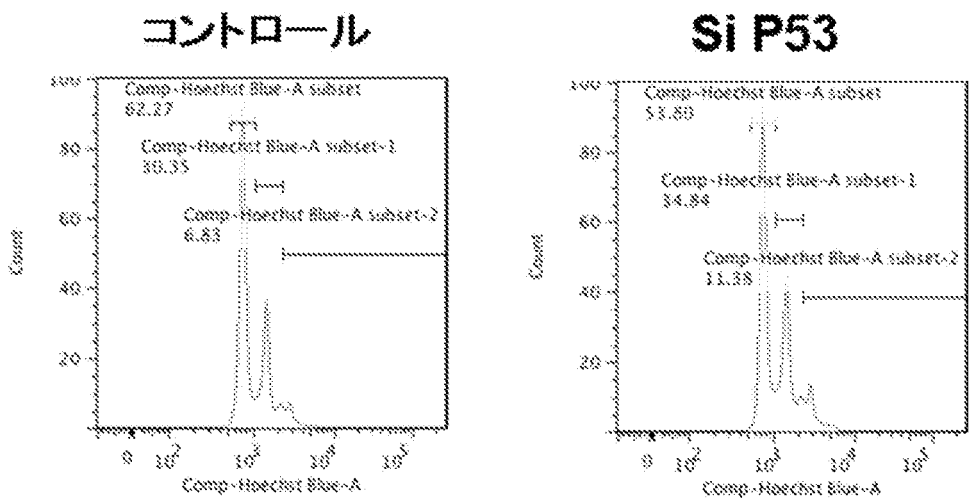

A
Myc Bmi1↑
Myc Bmi1↓
+ Rock i
Myc Bmi1↓
BclxL ↑+Rock i
B
細胞数 (%)
75
50
25
0
Myc Bmi1 on
Myc Bmi1 off
Myc Bmi1 off
BclxL on
2N
4N
8N
8N<
C
2N
8N
4N
8N <

[図8]

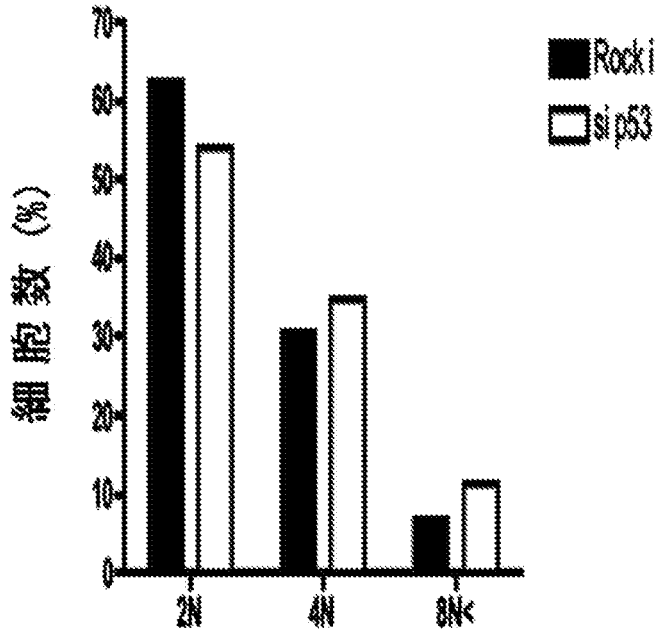

c-MYC/BMI1 off
BCL-XL (+)
c-MYC/BMI1 off
BCL-XL (-)
細胞数
1.0x10^9
1.0x10^8
1.0x10^7
1000000
100000
0
10
20
30
40
50
日

[図9]

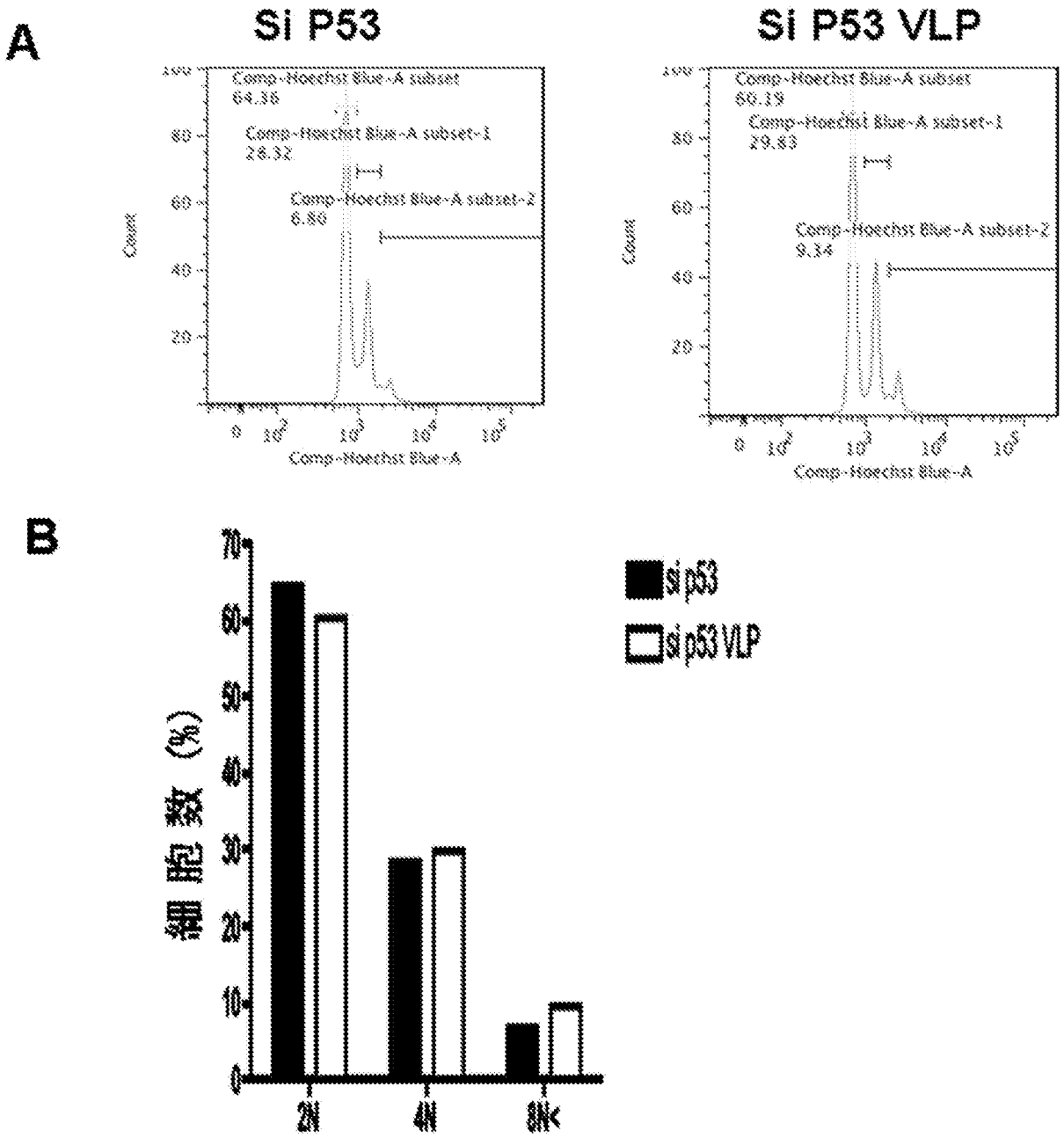
A


コントロール
Si P53
Comp-Hoechst Blue-A subset
62.27
Comp-Hoechst Blue-A subset-1
30.35
Comp-Hoechst Blue-A subset-2
6.83
Comp-Hoechst Blue-A subset
53.80
Comp-Hoechst Blue-A subset-1
34.84
Comp-Hoechst Blue-A subset-2
11.38
Count
Comp-Hoechst Blue-A

B

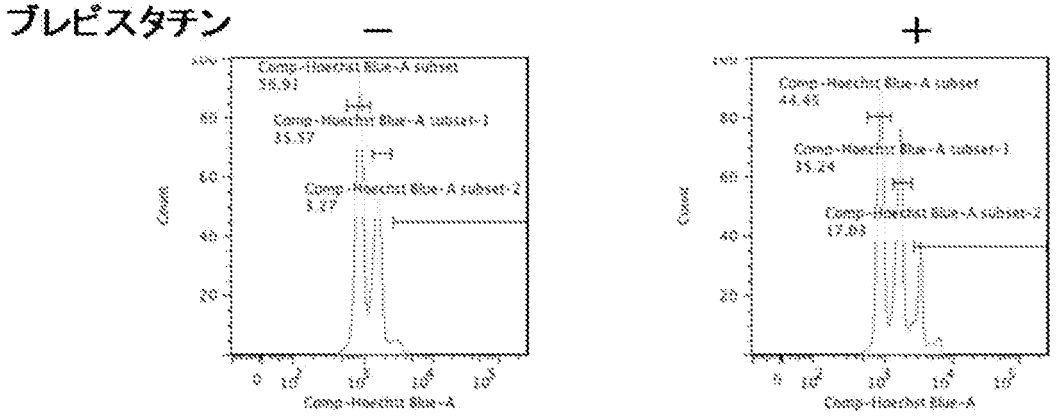

細胞数（%）
Rock i
si p53
2N
4N
8N<

[図10]

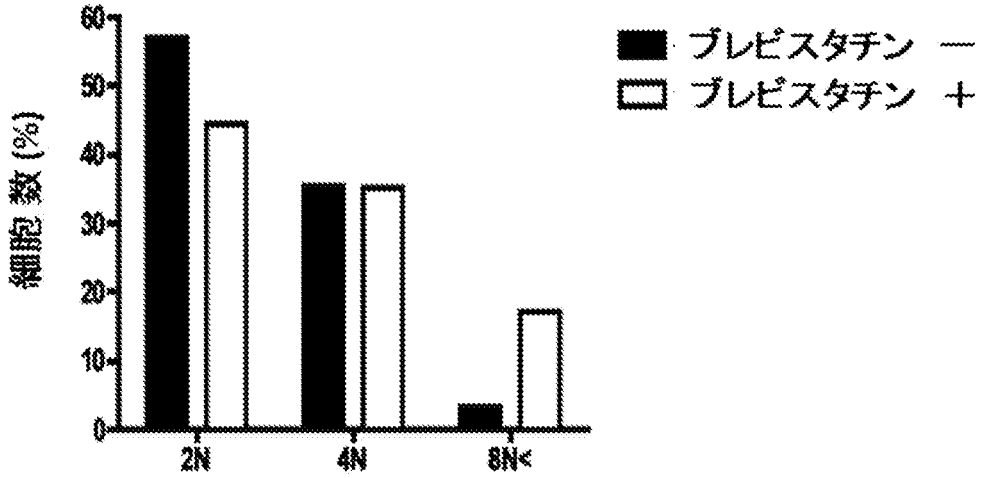

A
Si P53
Comp-Hoechst Blue-A subset
64.38
Comp-Hoechst Blue-A subset-1
28.32
Comp-Hoechst Blue-A subset-2
6.80
Count
Comp-Hoechst Blue-A
Si P53 VLP
Comp-Hoechst Blue-A subset
60.19
Comp-Hoechst Blue-A subset-1
29.83
Comp-Hoechst Blue-A subset-2
9.34
Count
Comp-Hoechst Blue-A
B
si p53
si p53 VLP
細胞数 (%)
2N
4N
8N<

[図11]

A

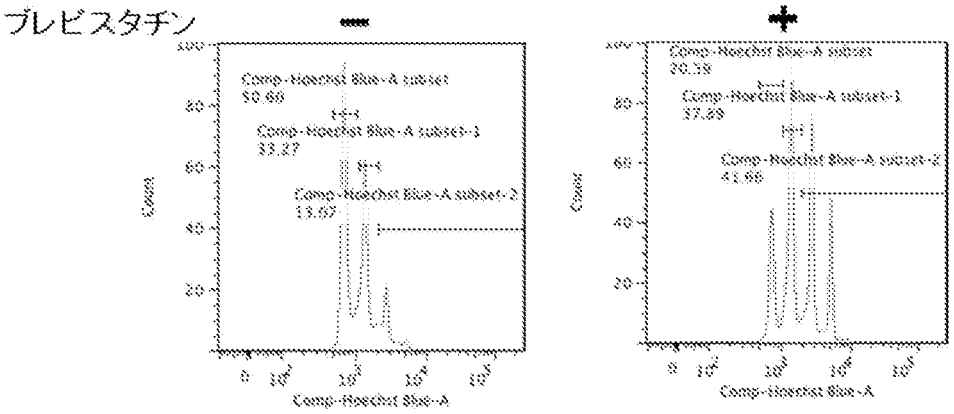

ブレビスタチン
—
+
Count
Comp-Hoechst Blue-A
Comp-Hoechst Blue-A subset
Comp-Hoechst Blue-A subset-1
Comp-Hoechst Blue-A subset-2

B

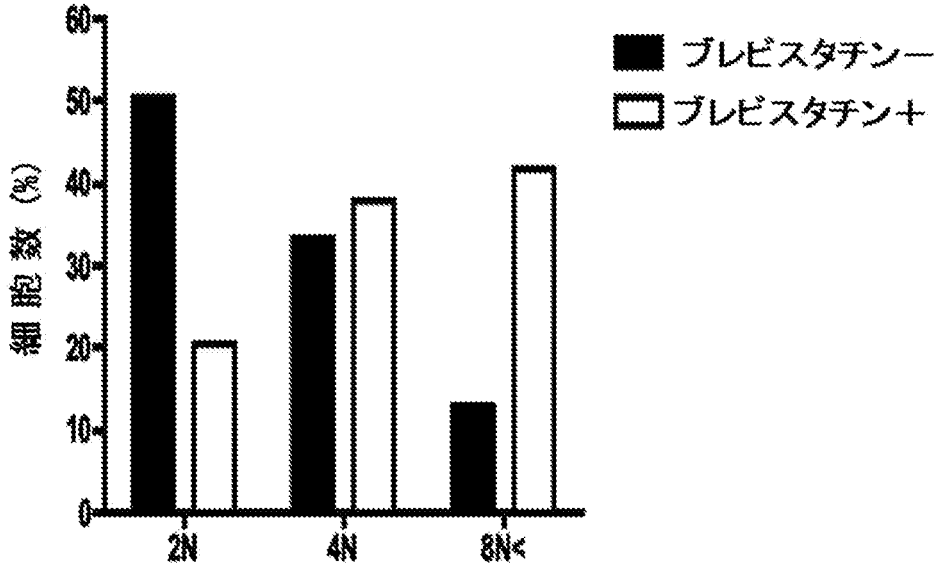

細胞数 (%)
0
10
20
30
40
50
60
2N
4N
8N<
ブレビスタチン —
ブレビスタチン +

[図12]

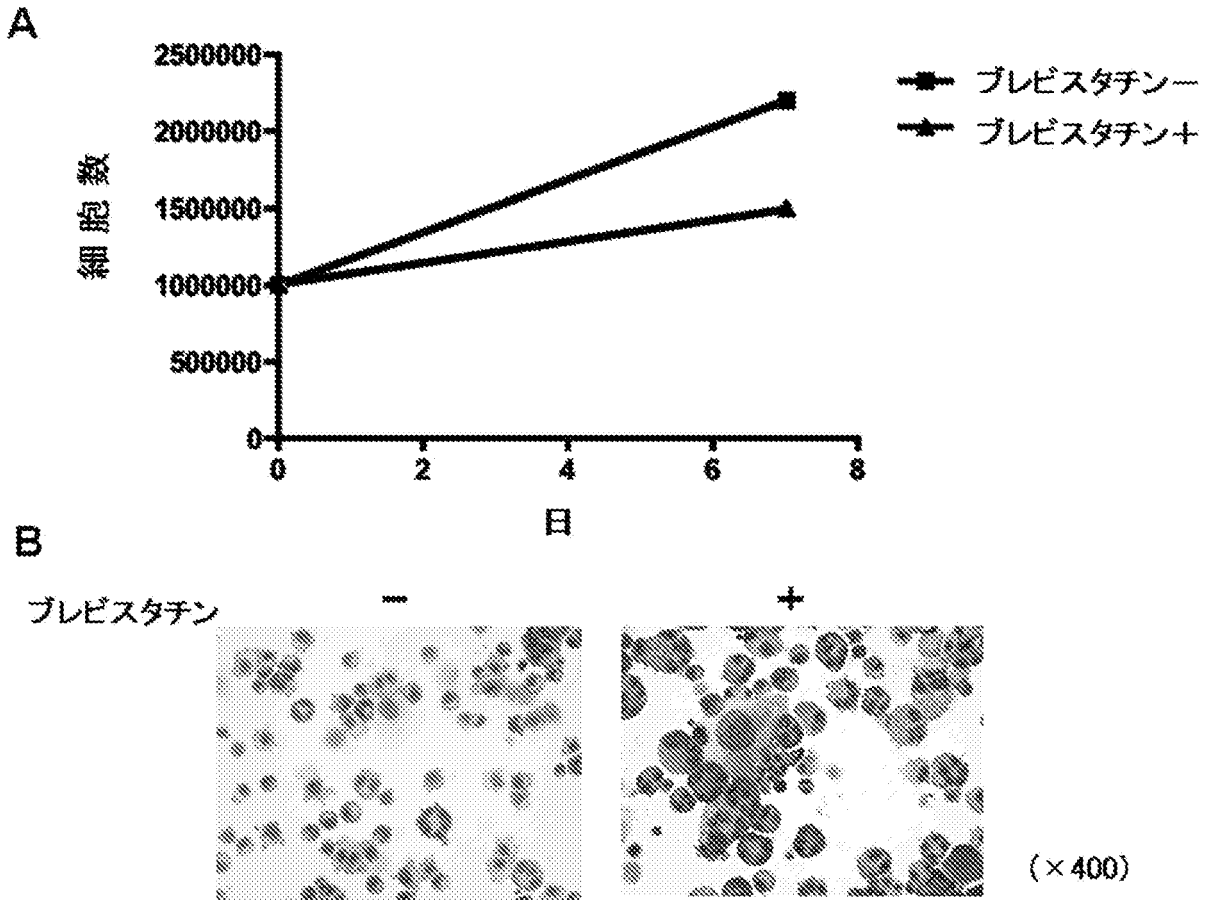
A


ブレビスタチン
−
+
Count
Comp-Hoechst Blue-A
Comp-Hoechst Blue-A subset
50.69
Comp-Hoechst Blue-A subset-1
33.27
Comp-Hoechst Blue-A subset-2
13.67
Comp-Hoechst Blue-A subset
30.78
Comp-Hoechst Blue-A subset-1
37.69
Comp-Hoechst Blue-A subset-2
41.66

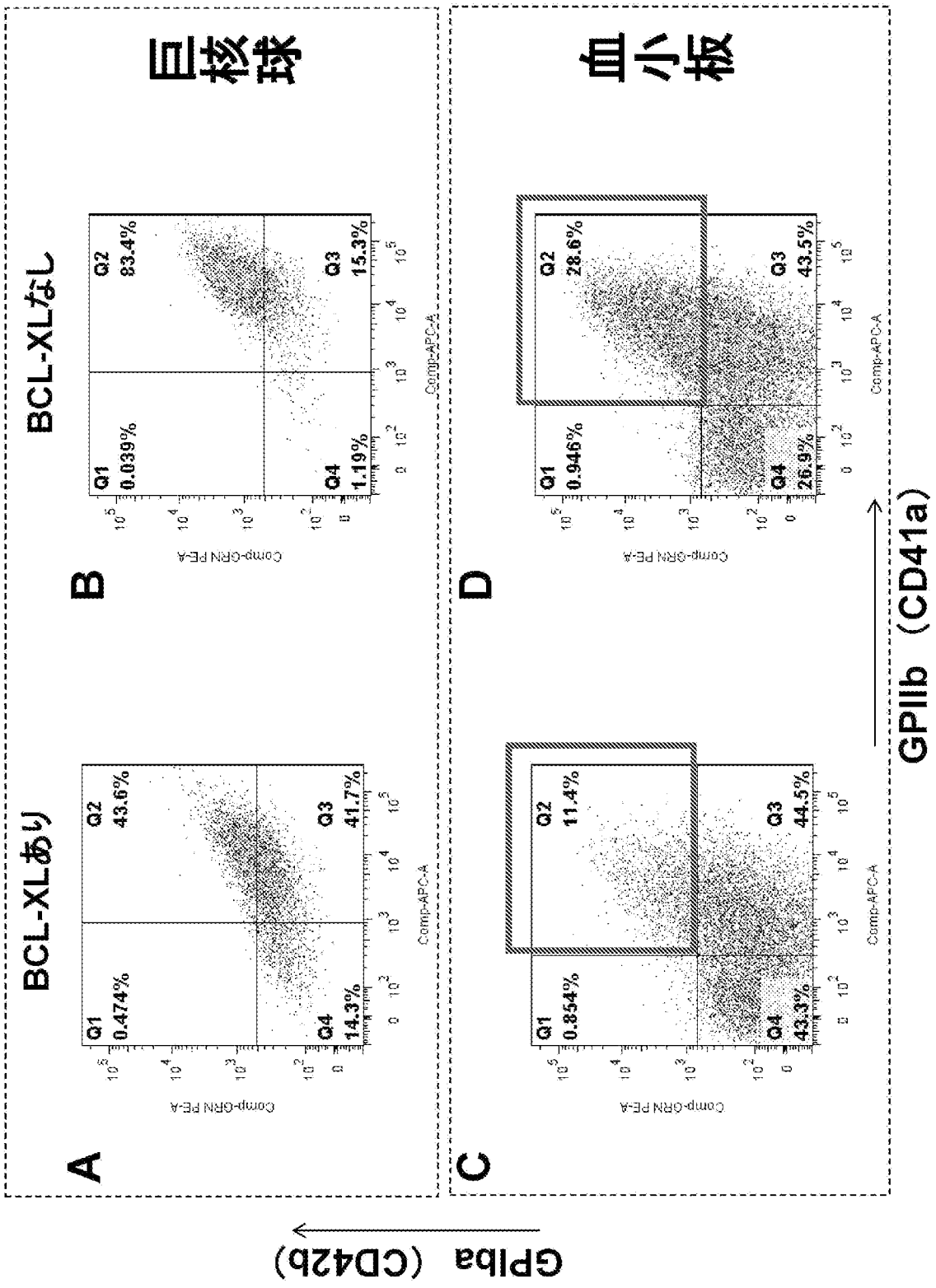
B


細胞数 (%)
60
50
40
30
20
10
0
2N
4N
8N<
ブレビスタチン−
ブレビスタチン+

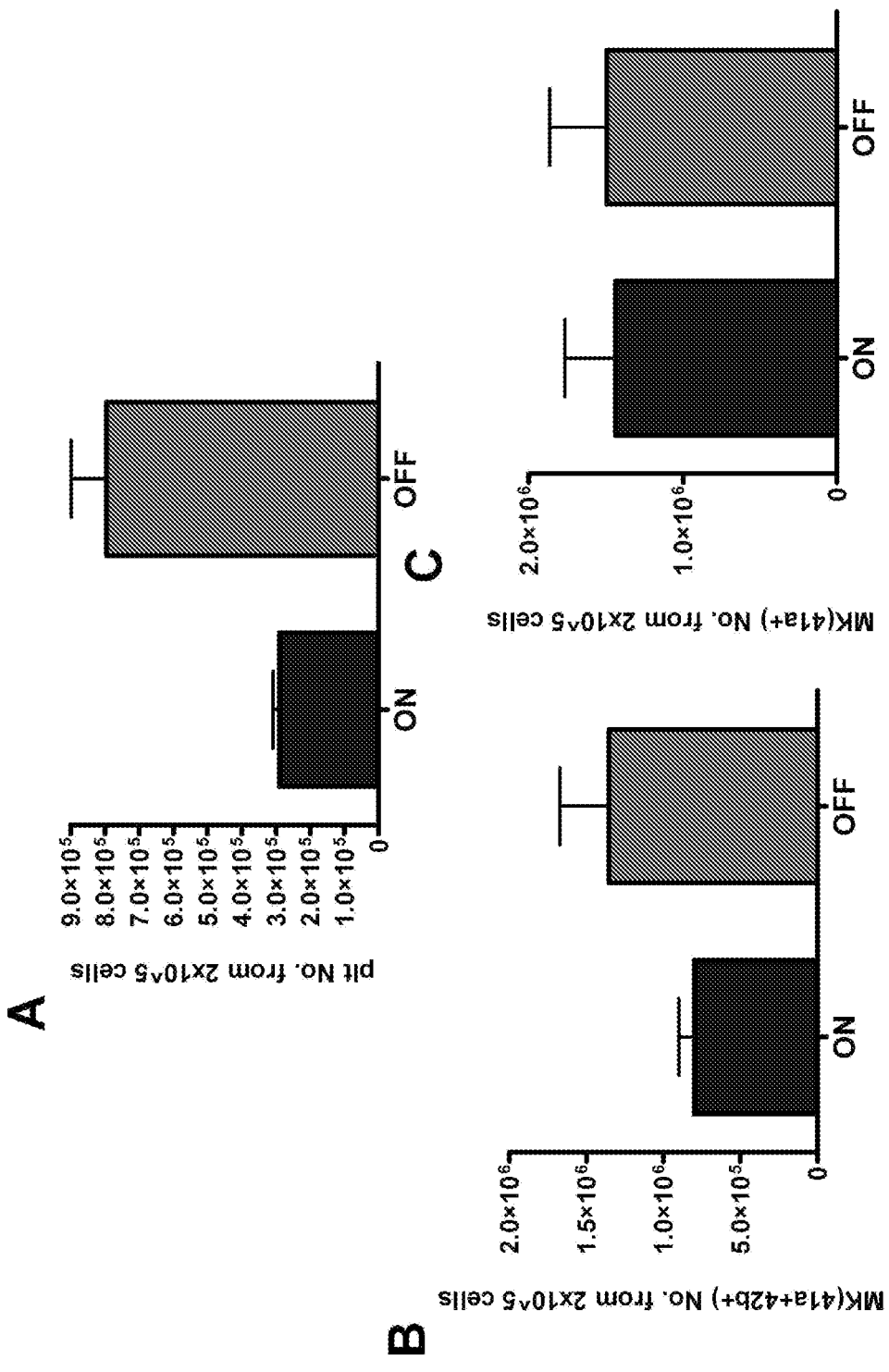
[図13]


A
2500000
2000000
1500000
1000000
500000
0
細胞数
0
2
4
6
8
日
ブレビスタチン−
ブレビスタチン+
B
ブレビスタチン
−
+
(×400)

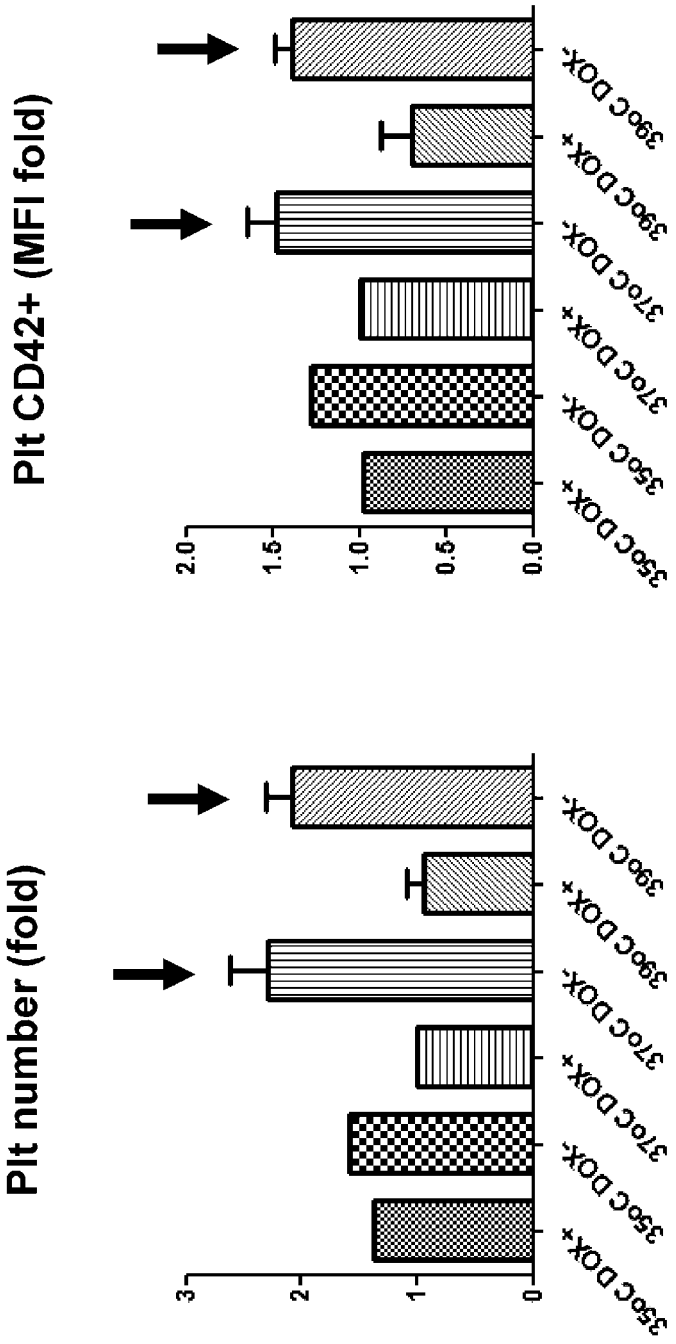
[図14]


巨核球
血小板
BCL-XLあり
BCL-XLなし
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%
Comp-ORN PE-A
Comp-APC-A
GPIIb (CD41a)
GPIba (CD42b)

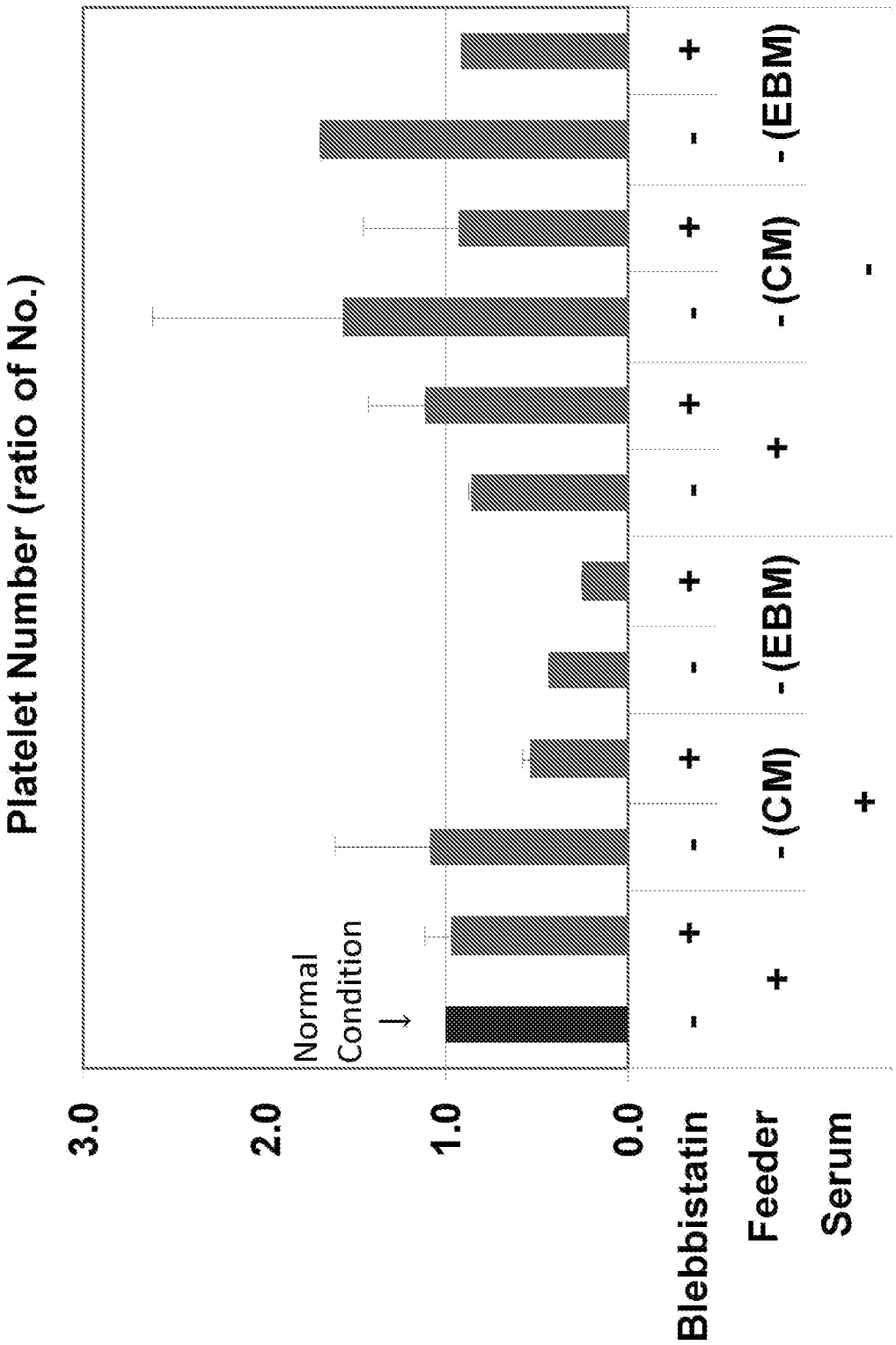
[図15]


A
plt No. from 2x10^5 cells
9.0×10^5
8.0×10^5
7.0×10^5
6.0×10^5
5.0×10^5
4.0×10^5
3.0×10^5
2.0×10^5
1.0×10^5
0
ON
OFF
B
MK(41a+42b+) No. from 2x10^5 cells
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0
ON
OFF
C
MK(41a+) No. from 2x10^5 cells
2.0×10^6
1.0×10^6
0
ON
OFF

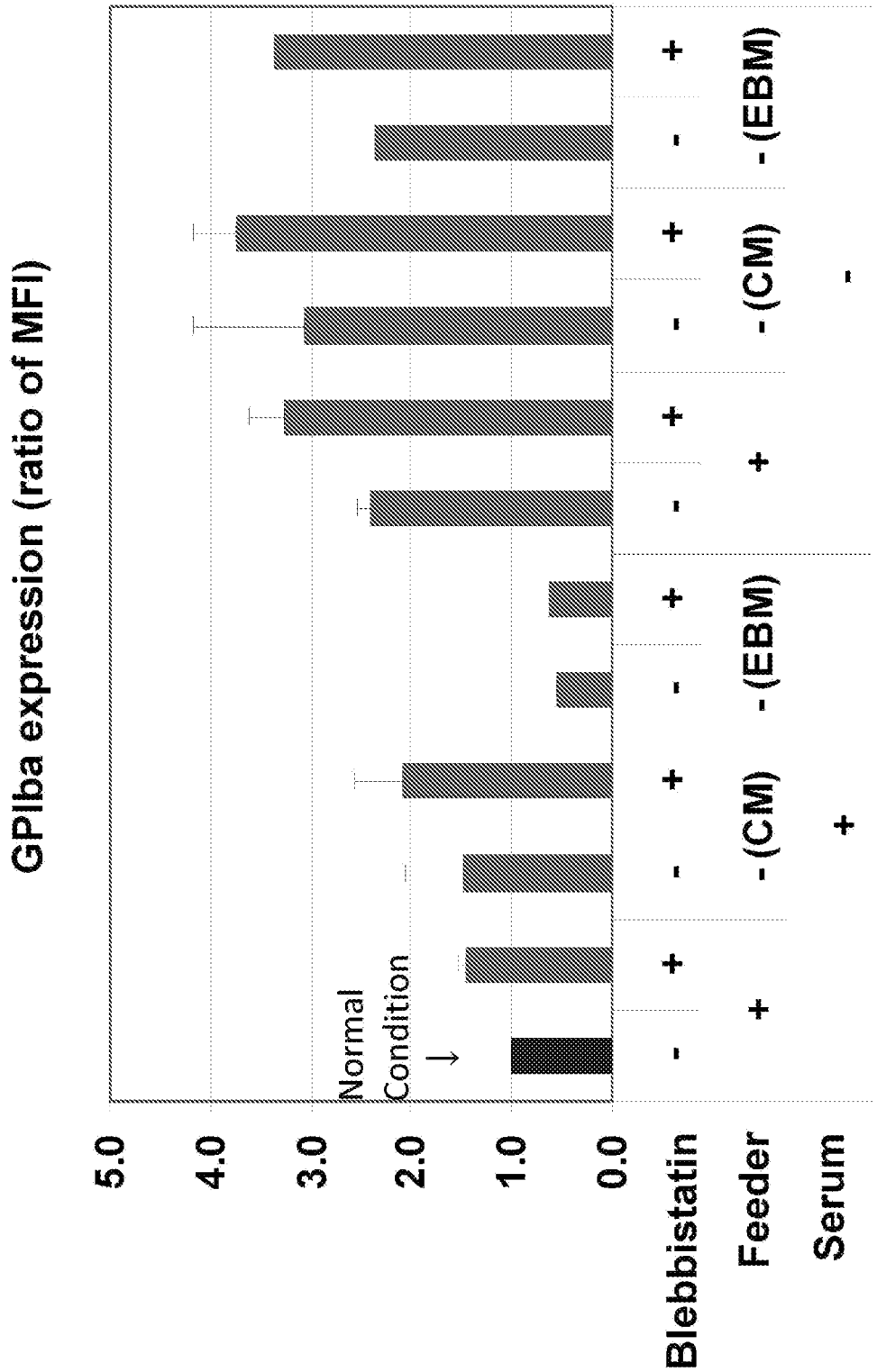
[図16]


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+

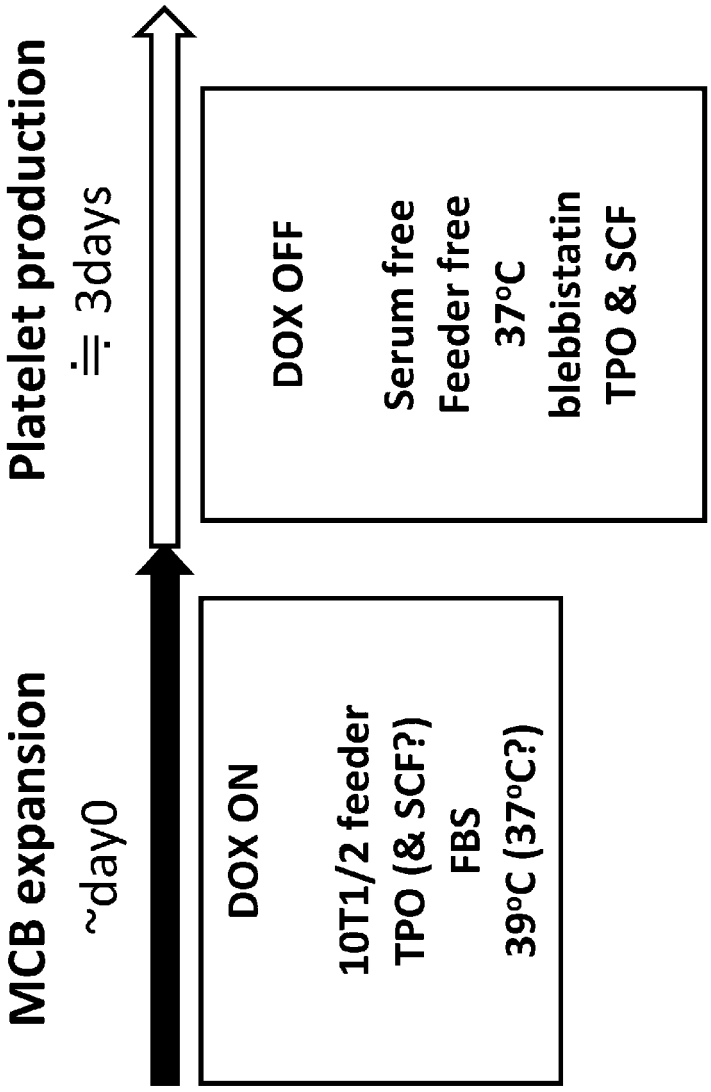
[図17]


Platelet Number (ratio of No.)
3.0
2.0
1.0
0.0
Normal Condition
Blebbistatin
Feeder
Serum
- + - + - + - + - + - +
+ + - - + + - - + + -
+ - + - + - + - + - +
- (CM) - (EBM) + - (CM) - (EBM)
+ -

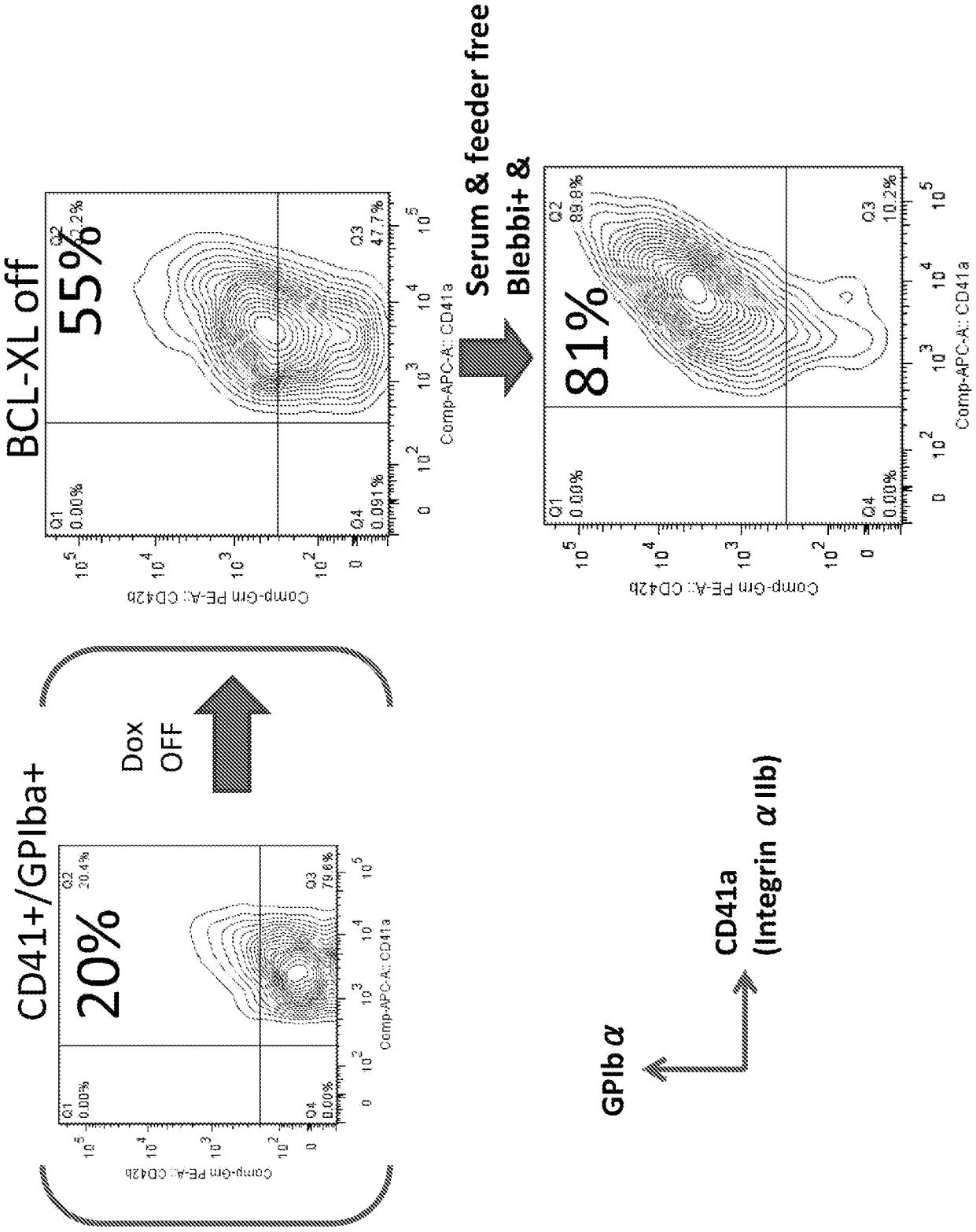
[図18]


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)
+
-

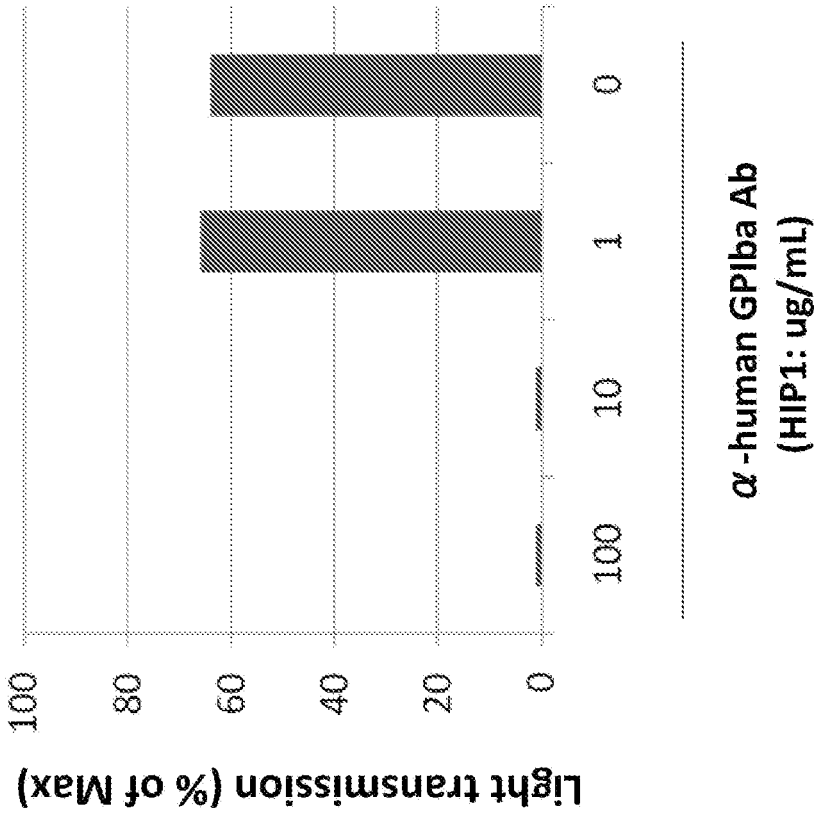
[図19]


MCB expansion
~day0
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

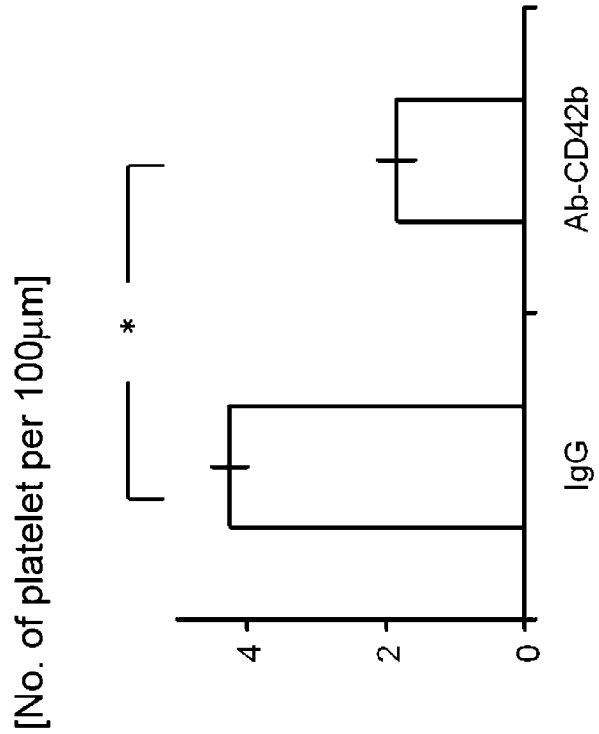
[図20]


CD41+/GPIba+
20%
Dox
OFF
BCL-XL off
55%
Serum & feeder free
Blebbi+ &
81%
Comp-Grn PE-A:: CD42b
Comp-APC-A:: CD41a
GPIb α
CD41a
(Integrin α IIb)

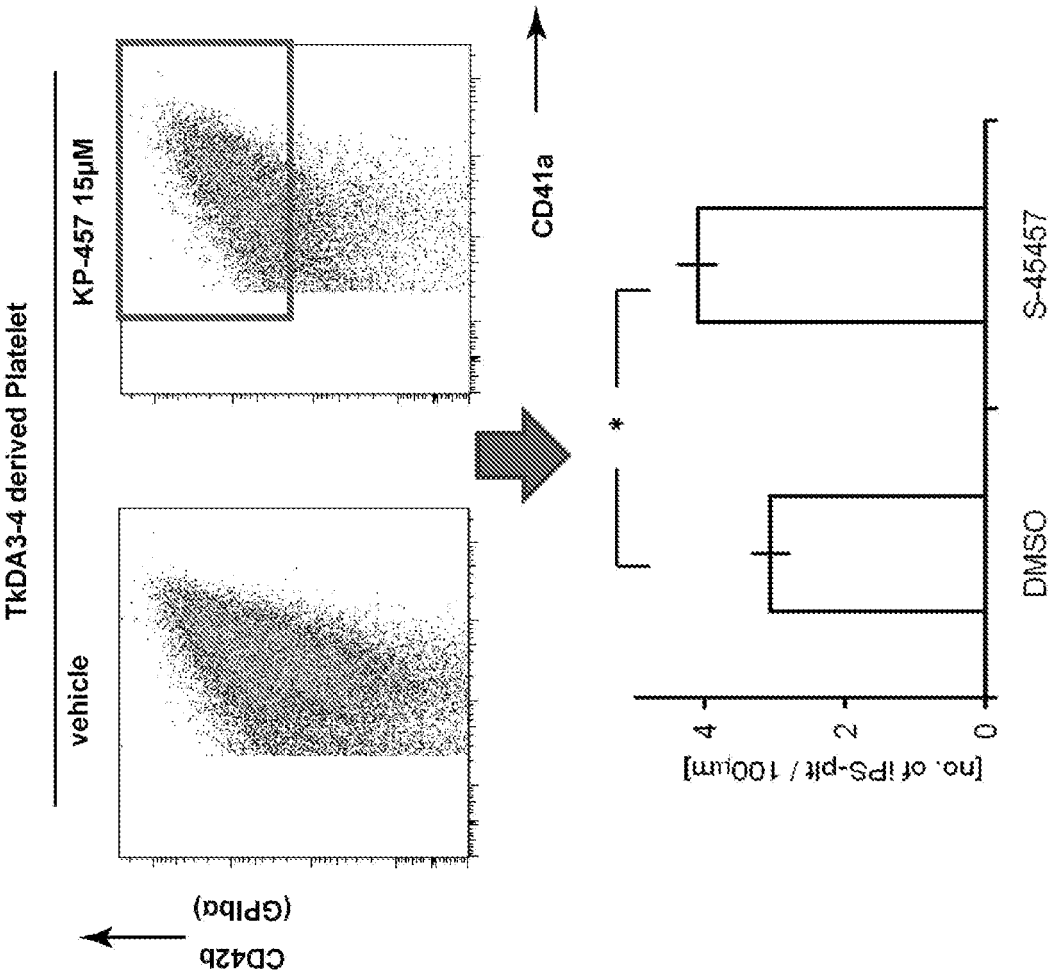
[図21]


Light transmission (% of Max)
100
80
60
40
20
0
0
1
10
100
α-human GPIba Ab
(HIP1: ug/mL)

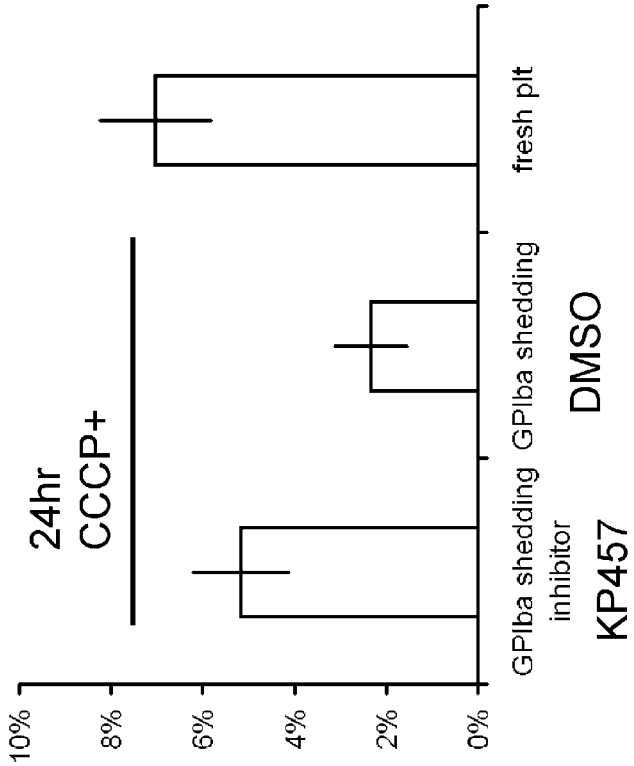
[図22]



[図23]


TkDA3-4 derived Platelet
vehicle
KP-457 15μM
CD42b
(GPIbα)
CD41a
*
S-45457
DMSO
0
2
4
[no. of PS-plt / 100μm]

[図24]


24hr
CCCP+
10%
8%
6%
4%
2%
0%
GPIba shedding inhibitor
KP457
GPIba shedding
DMSO
fresh plt