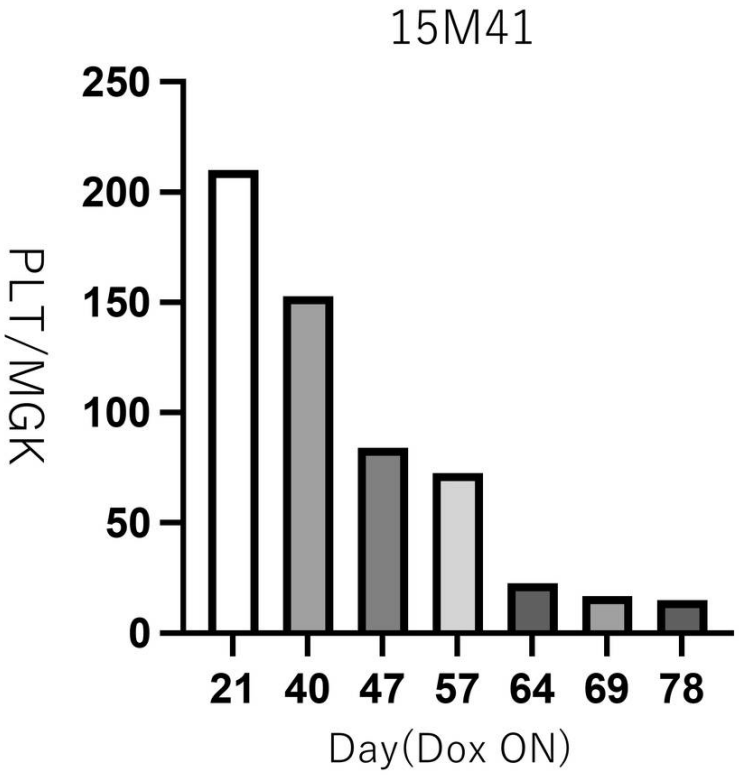
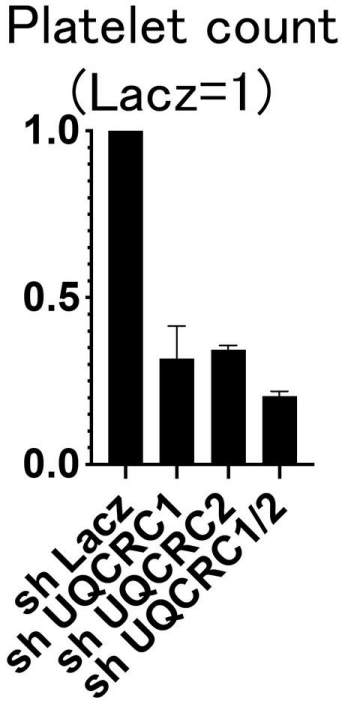
DRAWINGS

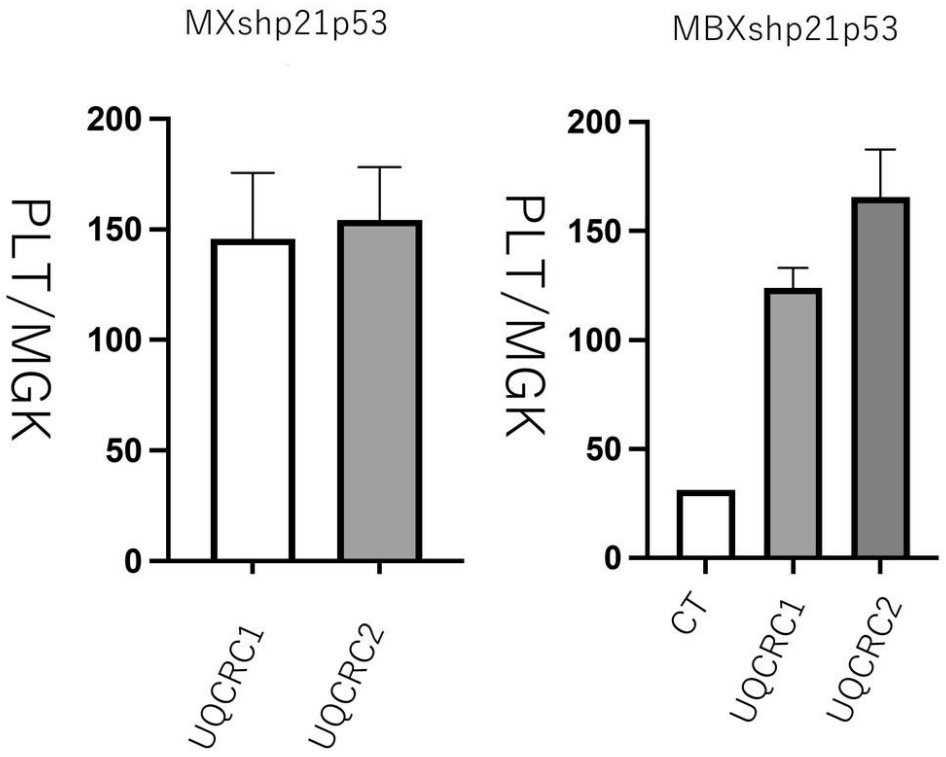
[Fig. 1]



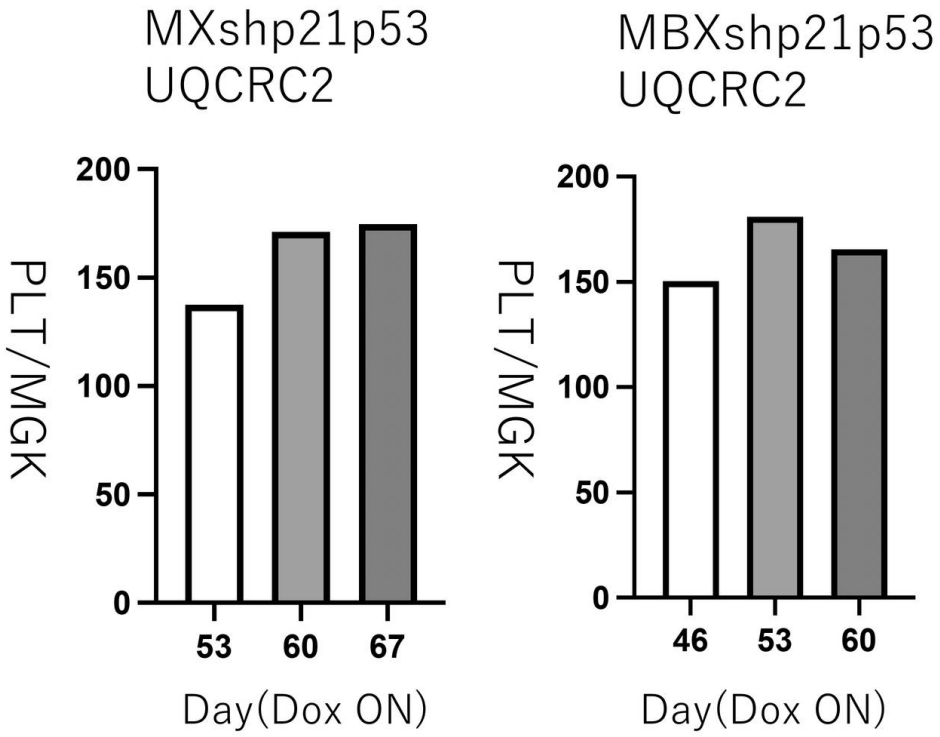
[Fig. 2]



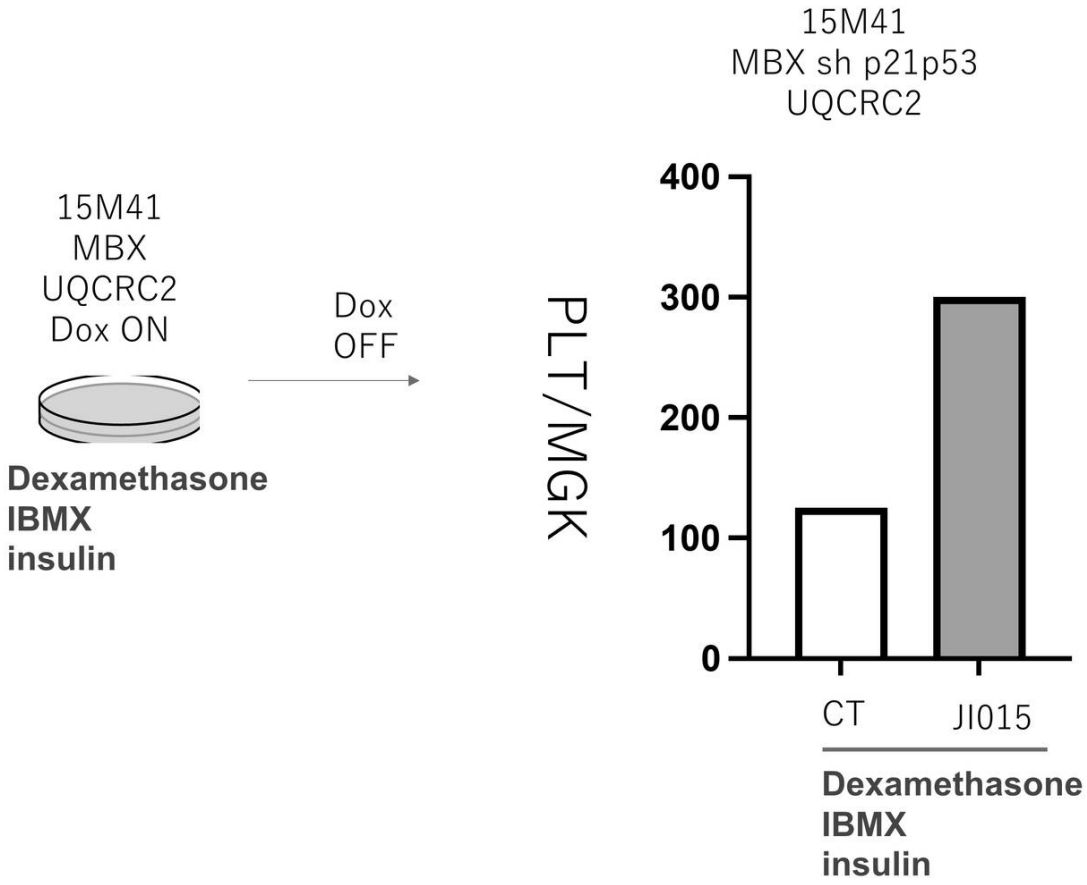
[Fig. 3]


MXshp21p53
MBXshp21p53
PLT/MGK
200
150
100
50
0
UQCRC1
UQCRC2
CT
UQCRC1
UQCRC2

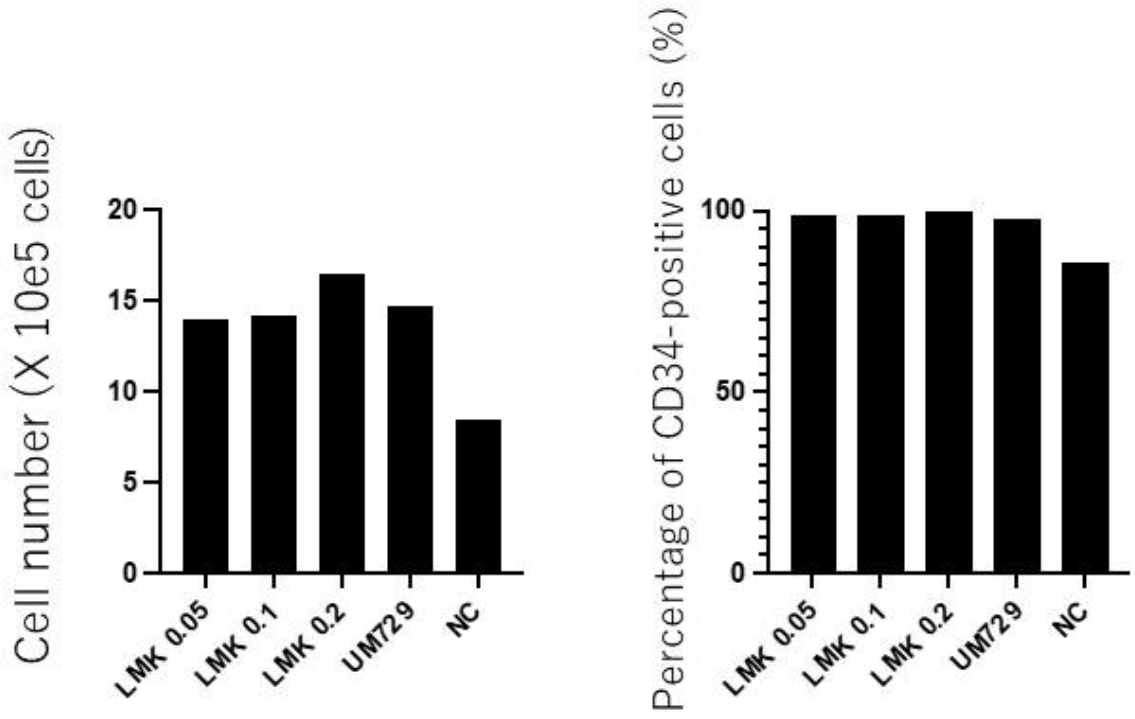
[Fig. 4]


MXshp21p53
UQCRC2
MBXshp21p53
UQCRC2
PLT/MGK
200
150
100
50
0
53
60
67
46
53
60
Day(Dox ON)
Day(Dox ON)

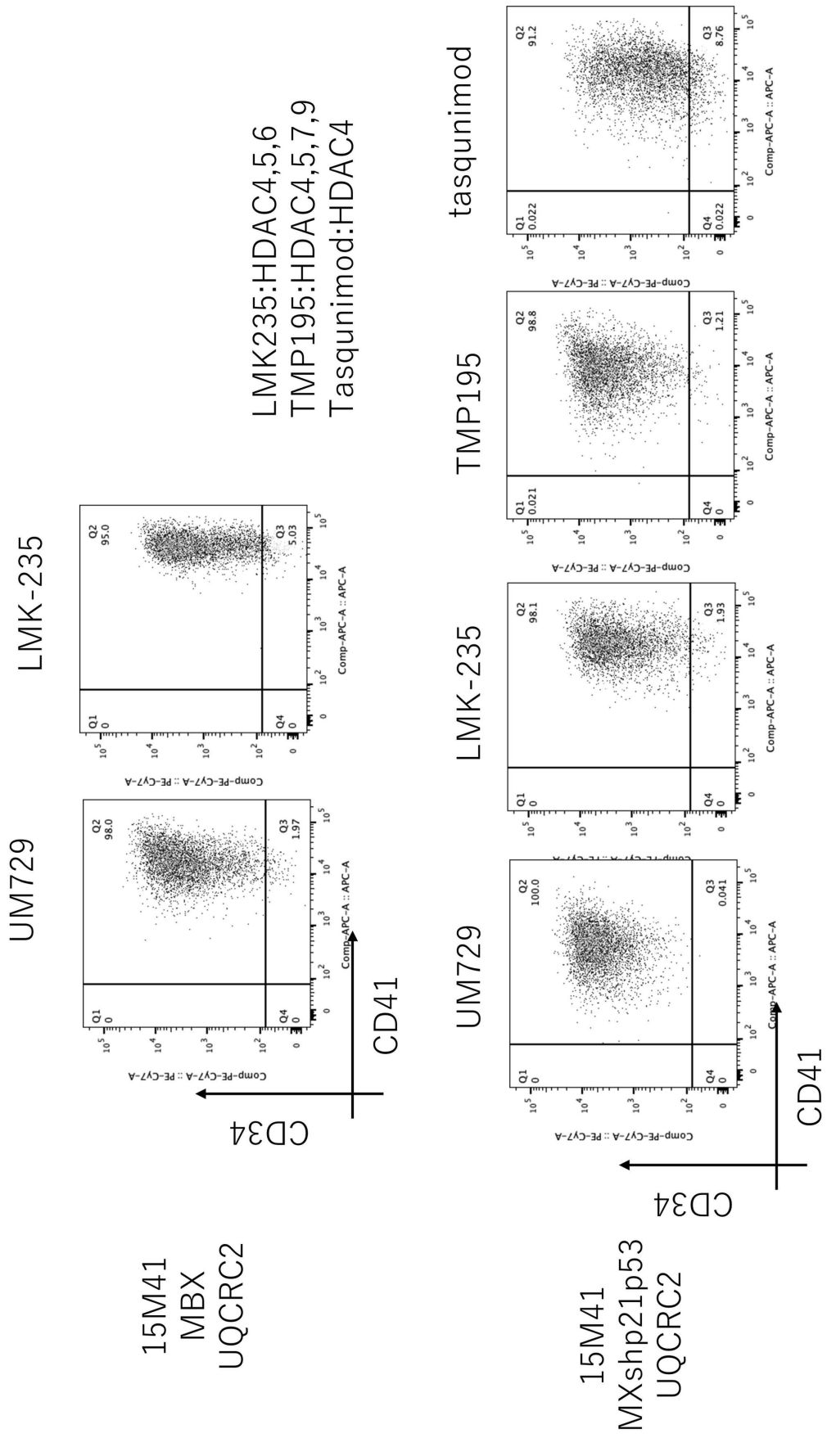
[Fig. 5]


15M41
MBX
UQCRC2
Dox ON
Dox
OFF
Dexamethasone
IBMX
insulin
15M41
MBX sh p21p53
UQCRC2
PLT/MGK
400
300
200
100
0
CT
JI015
Dexamethasone
IBMX
insulin

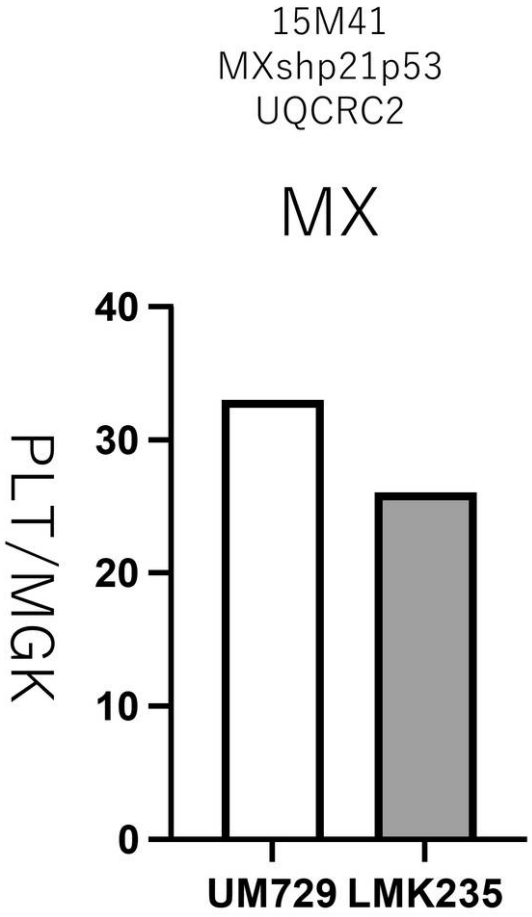
[Fig. 6]


Cell number (X 10e5 cells)
20
15
10
5
0
LMK 0.05
LMK 0.1
LMK 0.2
UM729
NC
Percentage of CD34-positive cells (%)
100
50
0
LMK 0.05
LMK 0.1
LMK 0.2
UM729
NC

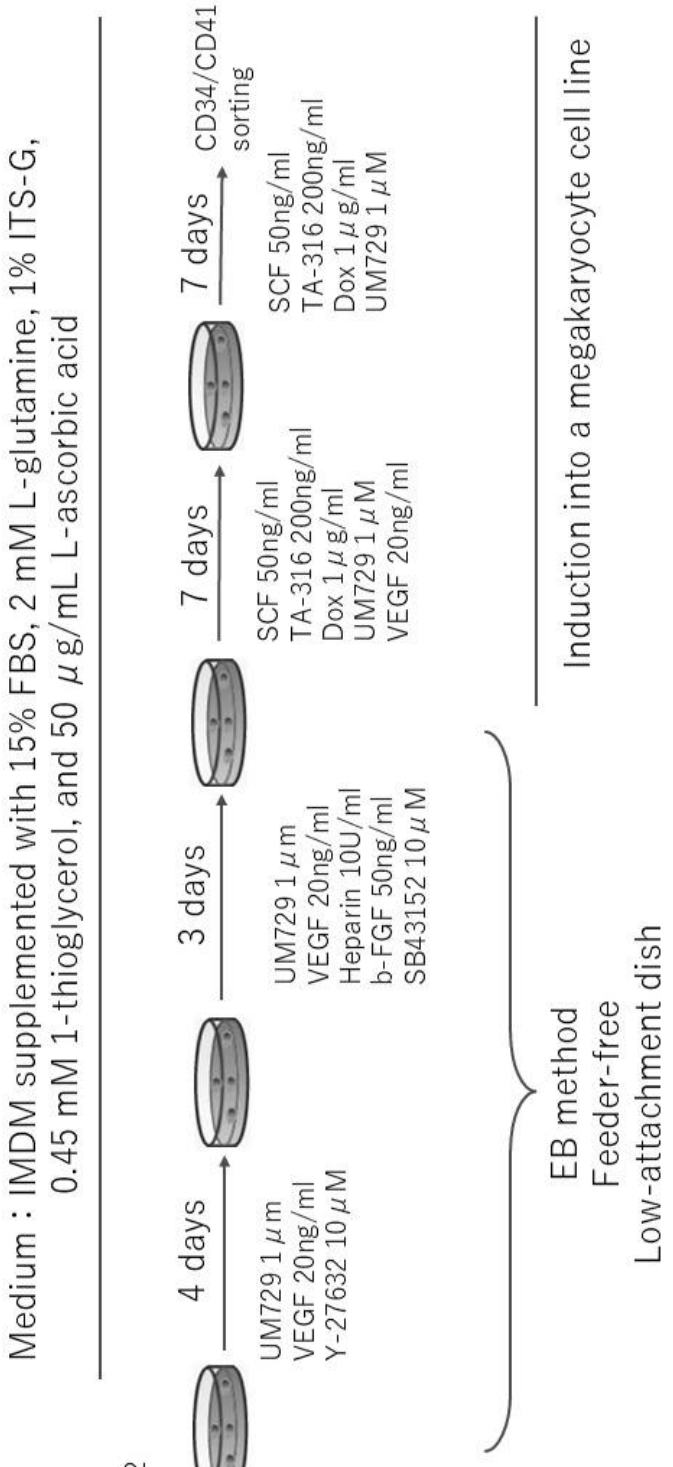
[Fig. 7]


15M41
MBX
UQCRC2
UM729
LMK-235
LMK235:HDAC4,5,6
TMP195:HDAC4,5,7,9
Tasqunimod:HDAC4
CD34
CD41
15M41
MXshp21p53
UQCRC2
UM729
LMK-235
TMP195
tasqunimod
CD34
CD41

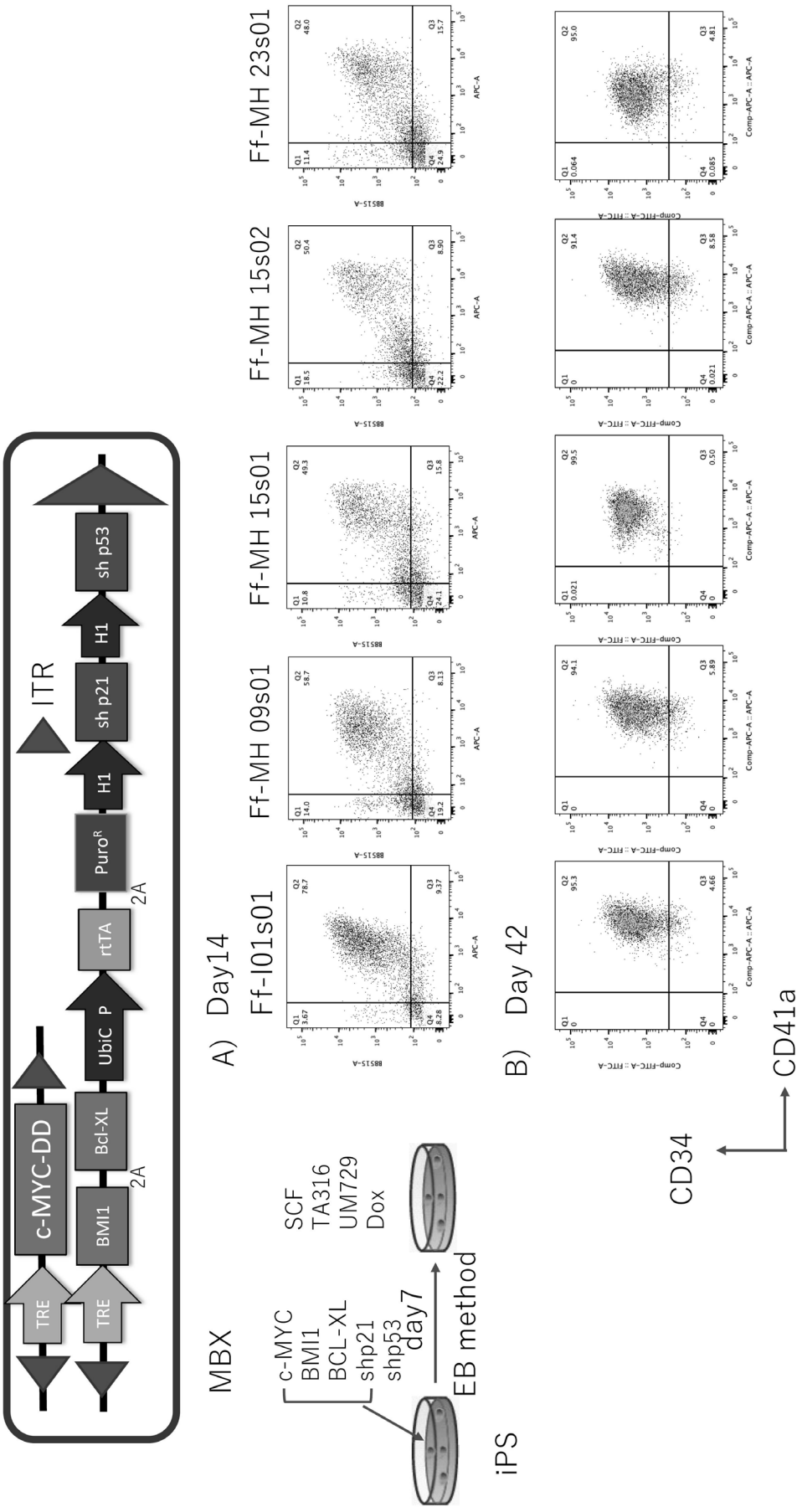
[Fig. 8]



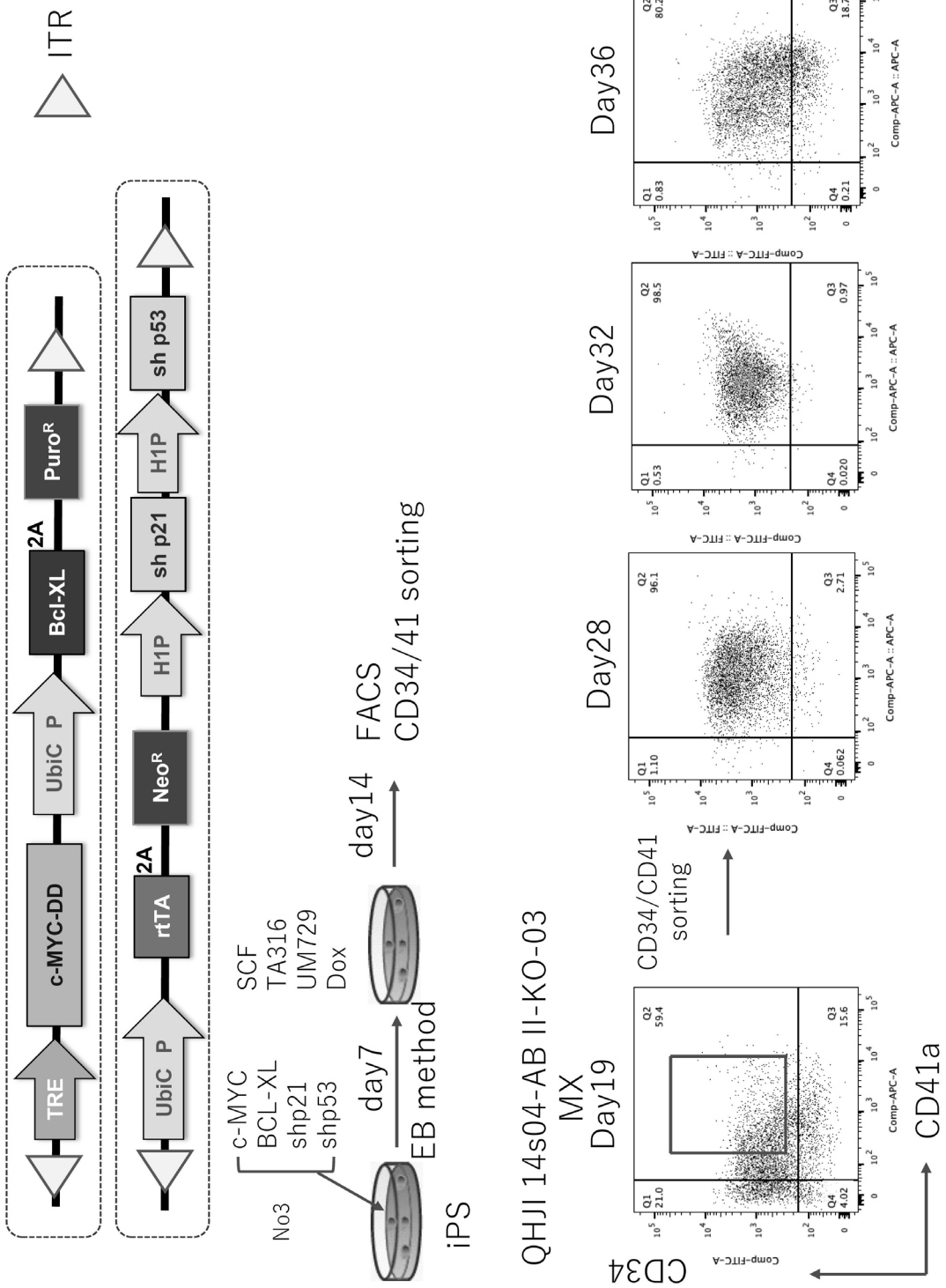
[Fig. 9]


Medium : IMDM supplemented with 15% FBS, 2 mM L-glutamine, 1% ITS-G,
0.45 mM 1-thioglycerol, and 50 μg/mL L-ascorbic acid
Medium : AK02
iPS
3 days
CHIR 3 μM
UM729 1 μM
Y-27632 10 μM
4 days
UM729 1 μm
VEGF 20ng/ml
Y-27632 10 μM
3 days
UM729 1 μm
VEGF 20ng/ml
Heparin 10U/ml
b-FGF 50ng/ml
SB43152 10 μM
7 days
SCF 50ng/ml
TA-316 200ng/ml
Dox 1 μg/ml
UM729 1 μM
VEGF 20ng/ml
7 days
CD34/CD41
sorting
SCF 50ng/ml
TA-316 200ng/ml
Dox 1 μg/ml
UM729 1 μM
EB method
Feeder-free
Low-attachment dish
Induction into a megakaryocyte cell line

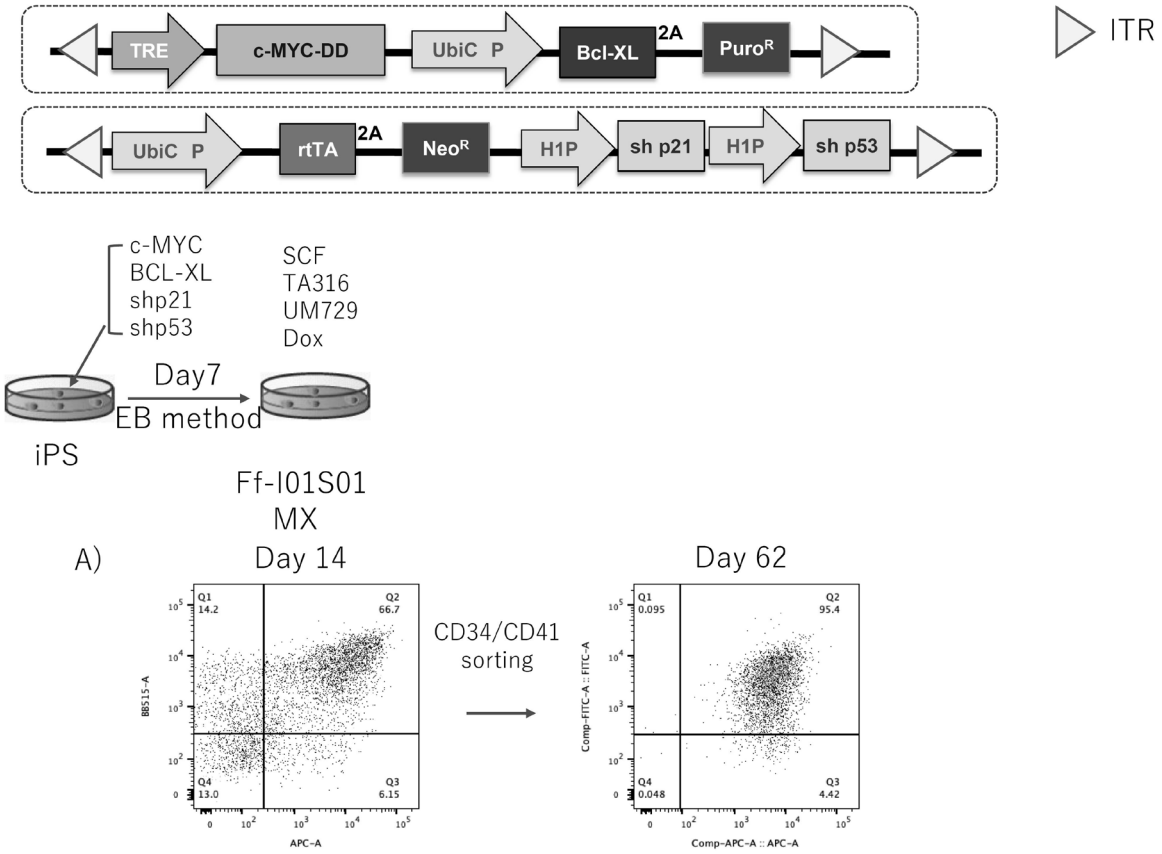
[Fig. 10]


MBX
TRE
TRE
c-MYC-DD
BMI1
2A
Bcl-XL
UbiC P
rtTA
2A
Puro^R
H1
sh p21
H1
sh p53
ITR
c-MYC
BMI1
BCL-XL
shp21
shp53
day7
EB method
SCF
TA316
UM729
Dox
iPS
A) Day14
Ff-I01s01
Ff-MH 09s01
Ff-MH 15s01
Ff-MH 15s02
Ff-MH 23s01
B) Day 42
CD34
CD41a

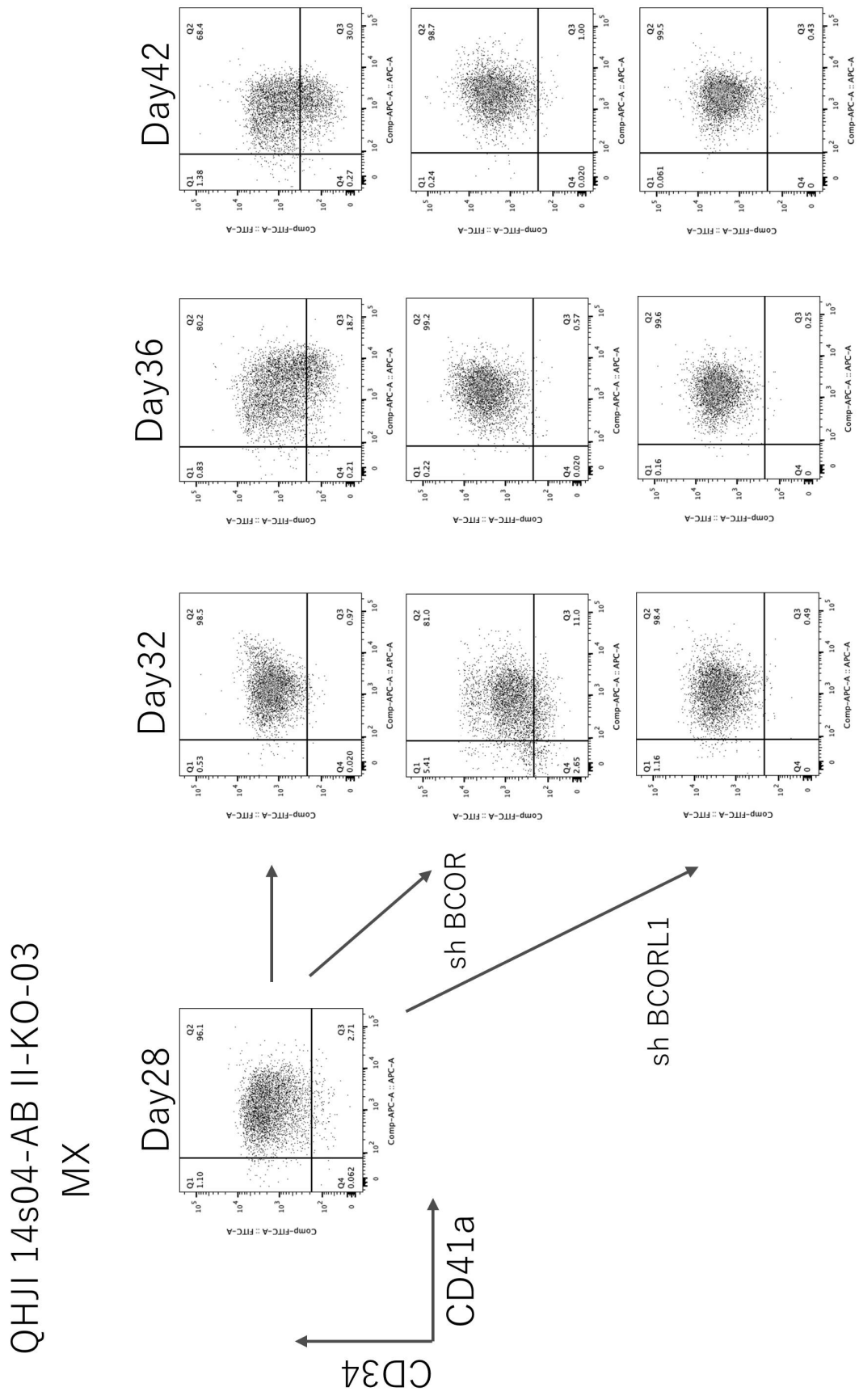
[Fig. 11]


ITR
TRE
c-MYC-DD
UbiC P
2A
Bcl-XL
Puro^R
UbiC P
rtTA
2A
Neo^R
H1P
sh p21
H1P
sh p53
No3
iPS
c-MYC
BCL-XL
shp21
shp53
SCF
TA316
UM729
Dox
day7
EB method
day14
FACS
CD34/41 sorting
QHJI 14s04-AB II-KO-03
MX
Day19
CD34/CD41
sorting
CD34
CD41a
Day28
Day32
Day36
Day42

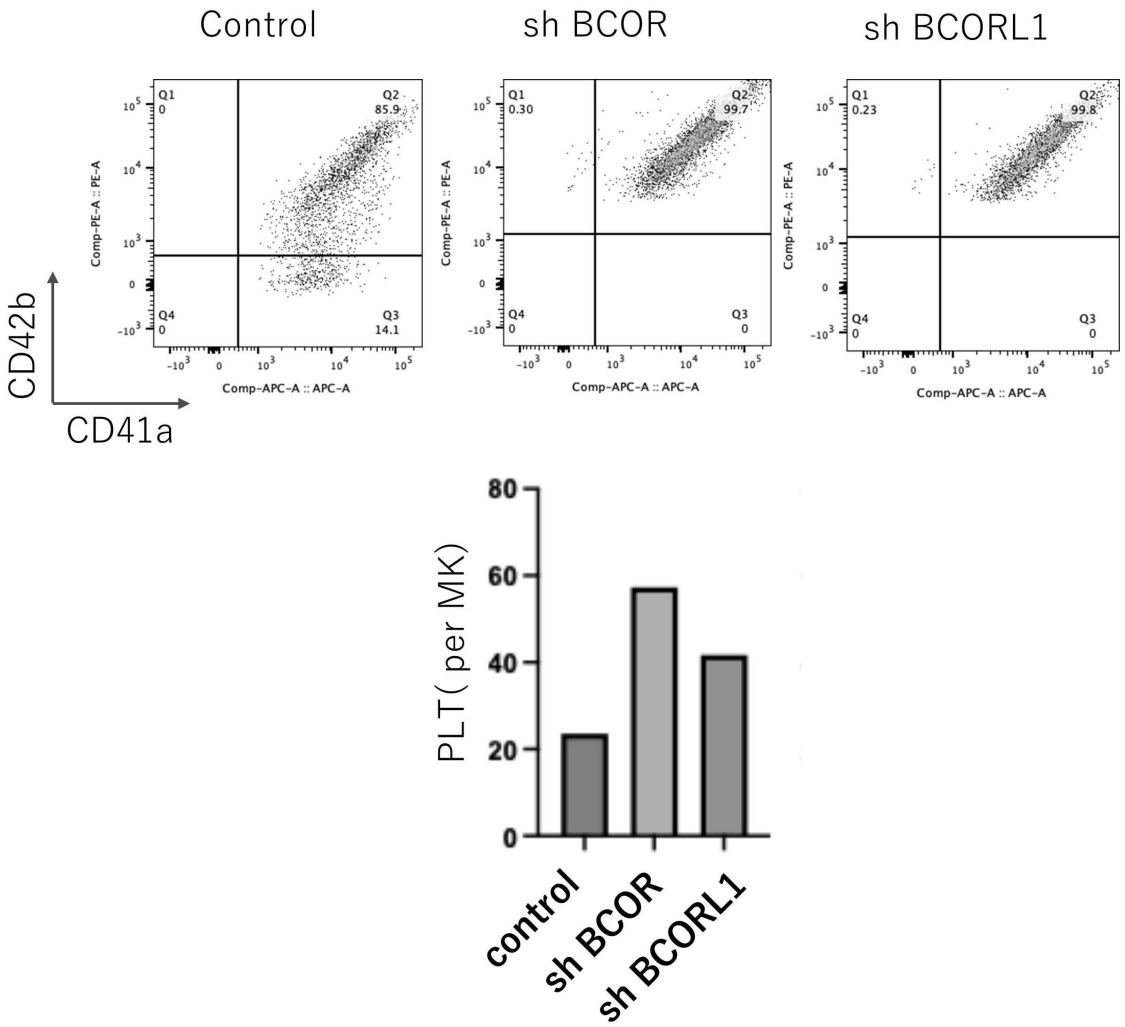
[Fig. 12]


TRE
c-MYC-DD
UbiC P
Bcl-XL
2A
Puro^R
ITR
UbiC P
rtTA
2A
Neo^R
H1P
sh p21
H1P
sh p53
c-MYC
BCL-XL
shp21
shp53
SCF
TA316
UM729
Dox
Day7
EB method
iPS
Ff-I01S01
MX
A)
Day 14
Q1
14.2
Q2
66.7
Q4
13.0
Q3
6.15
BB515-A
APC-A
CD34/CD41
sorting
Day 62
Q1
0.095
Q2
95.4
Q4
0.048
Q3
4.42
Comp-FITC-A :: FITC-A
Comp-APC-A :: APC-A

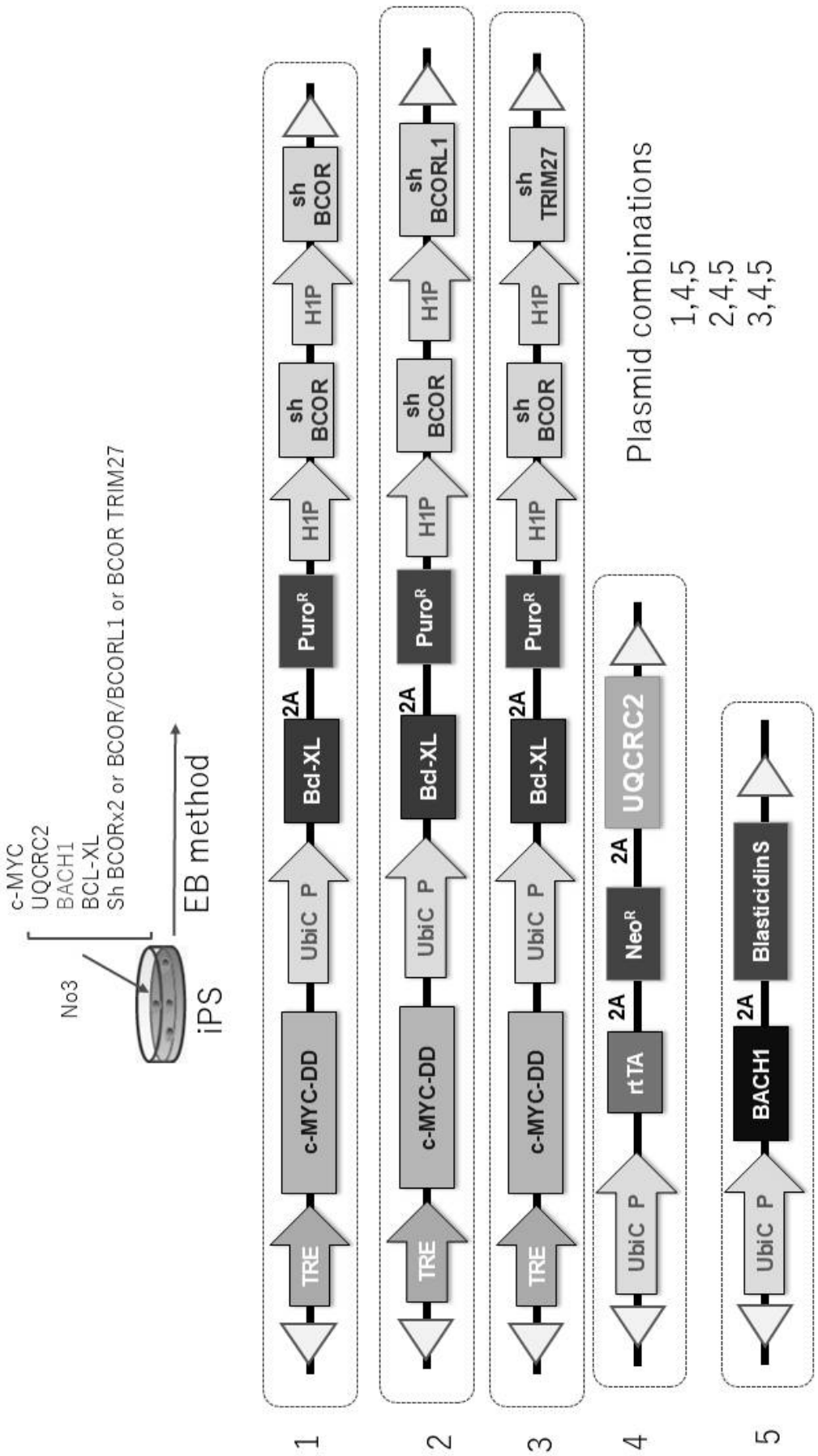
[Fig. 13]


QHJI 14s04-AB II-KO-03
MX
Day28
Day32
Day36
Day42
sh BCOR
sh BCORL1
CD34
CD41a

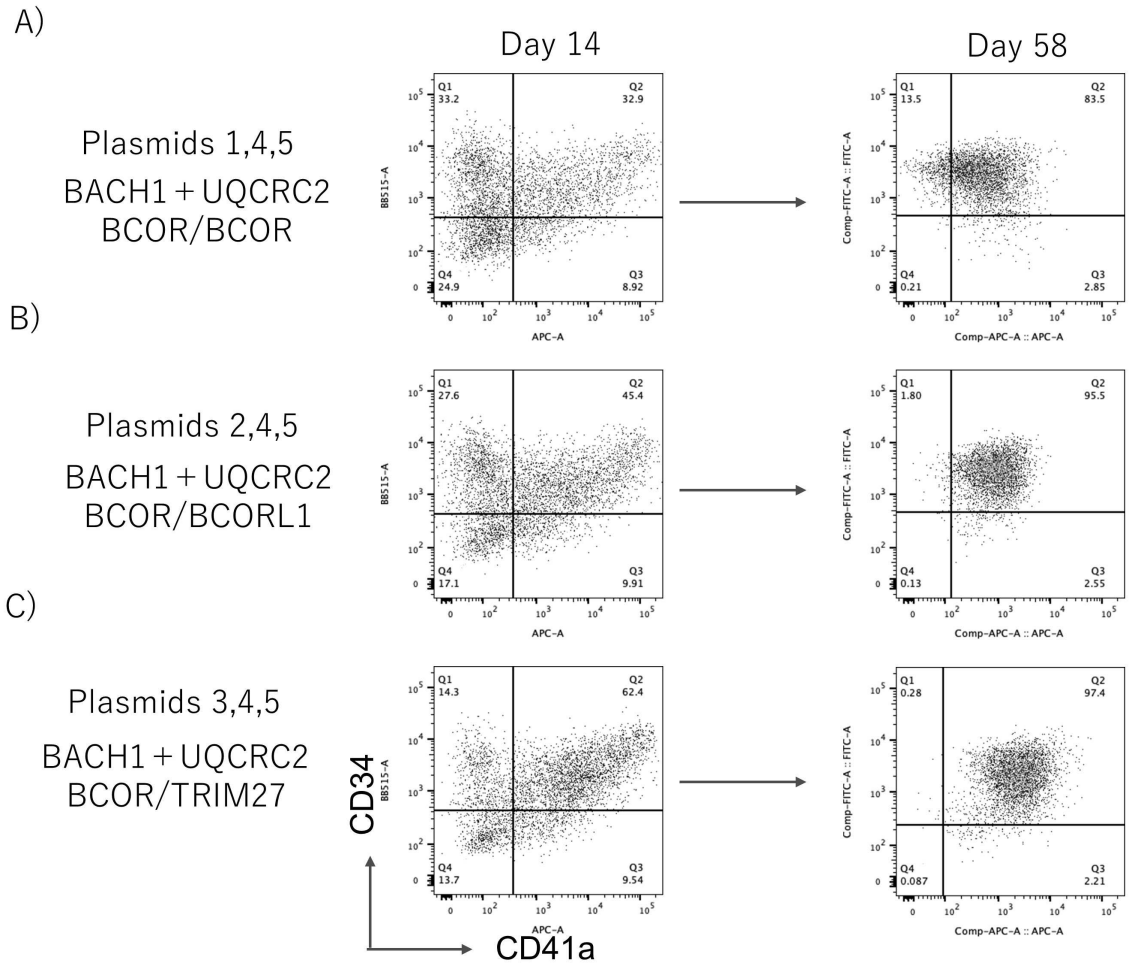
[Fig. 14]


Control
sh BCOR
sh BCORL1
CD42b
CD41a
Q1
0
Q2
85.9
Q4
0
Q3
14.1
Q1
0.30
Q2
99.7
Q4
0
Q3
0
Q1
0.23
Q2
99.8
Q4
0
Q3
0
Comp-PE-A :: PE-A
Comp-APC-A :: APC-A
PLT(per MK)
80
60
40
20
0
control
sh BCOR
sh BCORL1

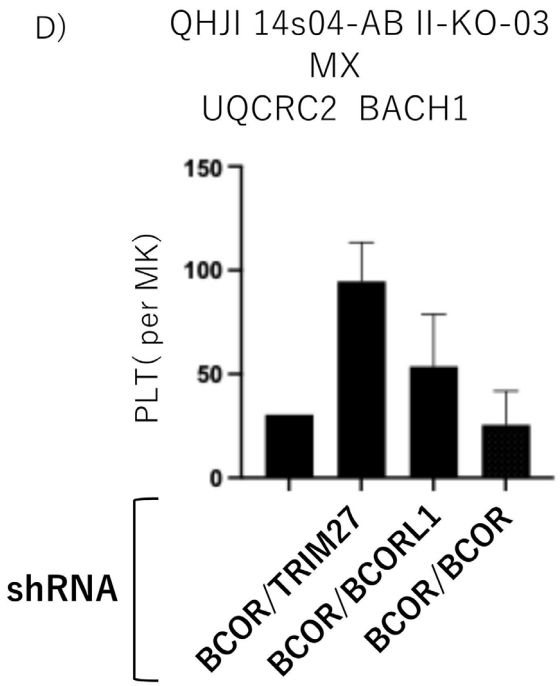
[Fig. 15]


c-MYC
UQCRC2
BACH1
BCL-XL
Sh BCORx2 or BCOR/BCORL1 or BCOR TRIM27
No3
iPS
EB method
TRE
c-MYC-DD
UbiC P
2A
Bcl-XL
Puro^R
H1P
sh BCOR
sh BCORL1
sh TRIM27
rtTA
Neo^R
UQCRC2
BACH1
BlasticidinS
Plasmid combinations
1,4,5
2,4,5
3,4,5
1
2
3
4
5

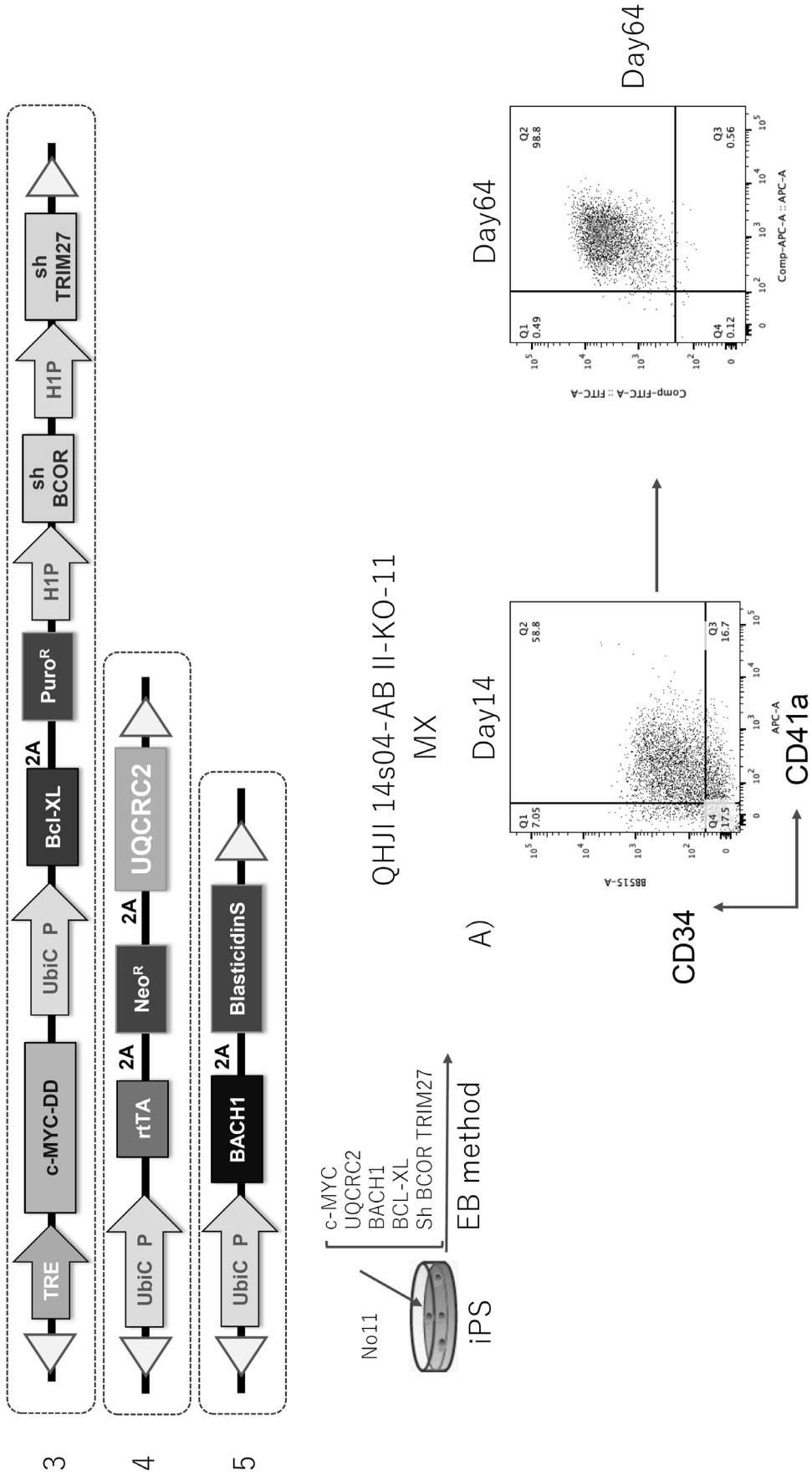
[Fig. 16-1]



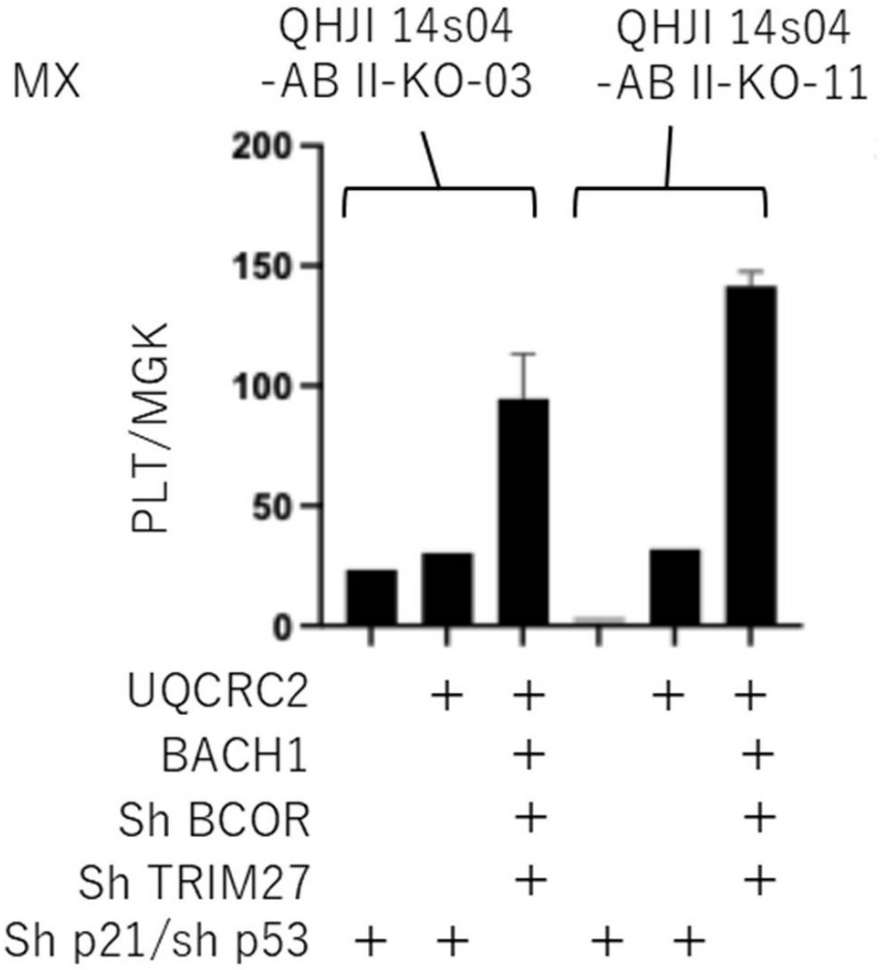
[Fig. 16-2]



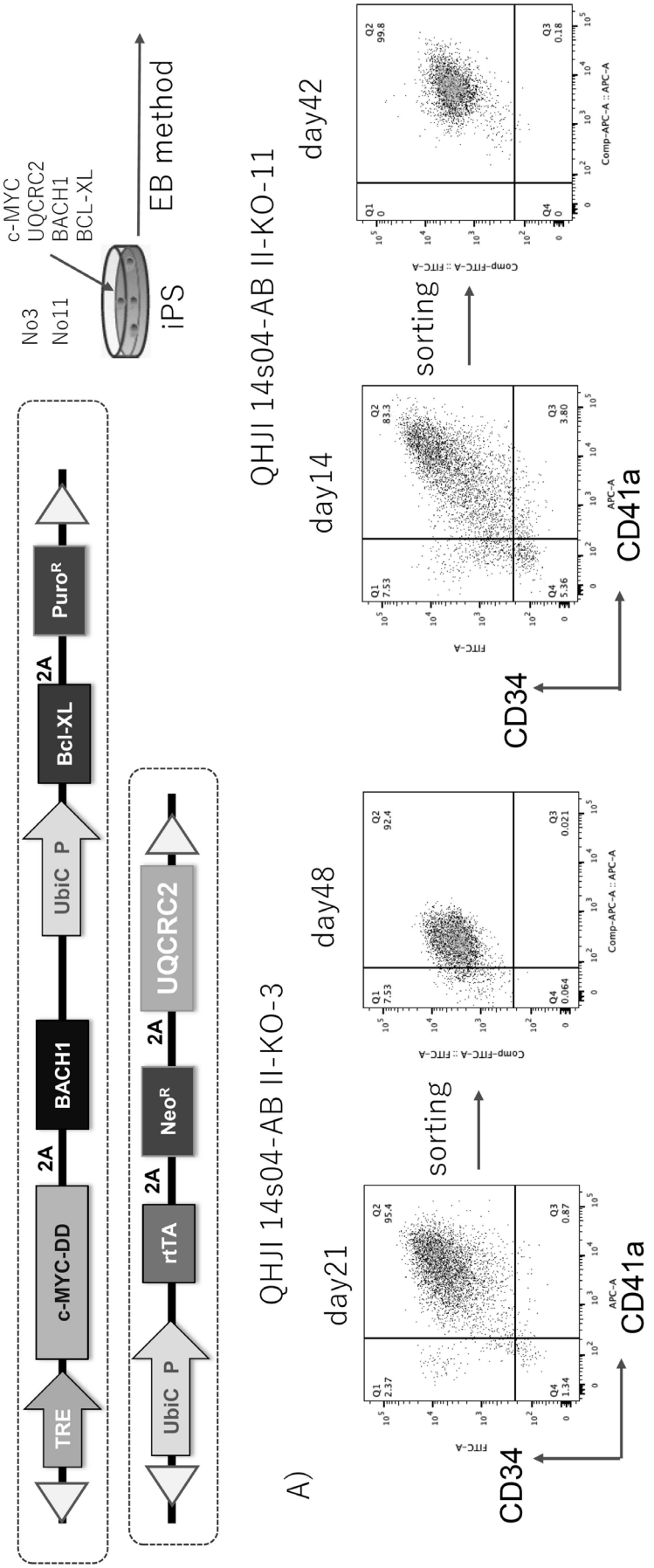
[Fig. 17-1]


3
TRE
c-MYC-DD
UbiC P
Bcl-XL
2A
Puro^R
H1P
sh BCOR
H1P
sh TRIM27
4
UbiC P
rtTA
2A
Neo^R
2A
UQCRC2
5
UbiC P
BACH1
2A
BlasticidinS
No11
iPS
c-MYC
UQCRC2
BACH1
BCL-XL
Sh BCOR TRIM27
EB method
QHJI 14s04-AB II-KO-11
MX
A)
Day14
Day64
Day64
CD34
CD41a
APC-A
Comp-APC-A :: APC-A
Comp-FITC-A :: FITC-A
Q1 7.05
Q2 58.8
Q3 16.7
Q4 17.5
Q1 0.49
Q2 98.8
Q3 0.56
Q4 0.12

[Fig. 17-2]

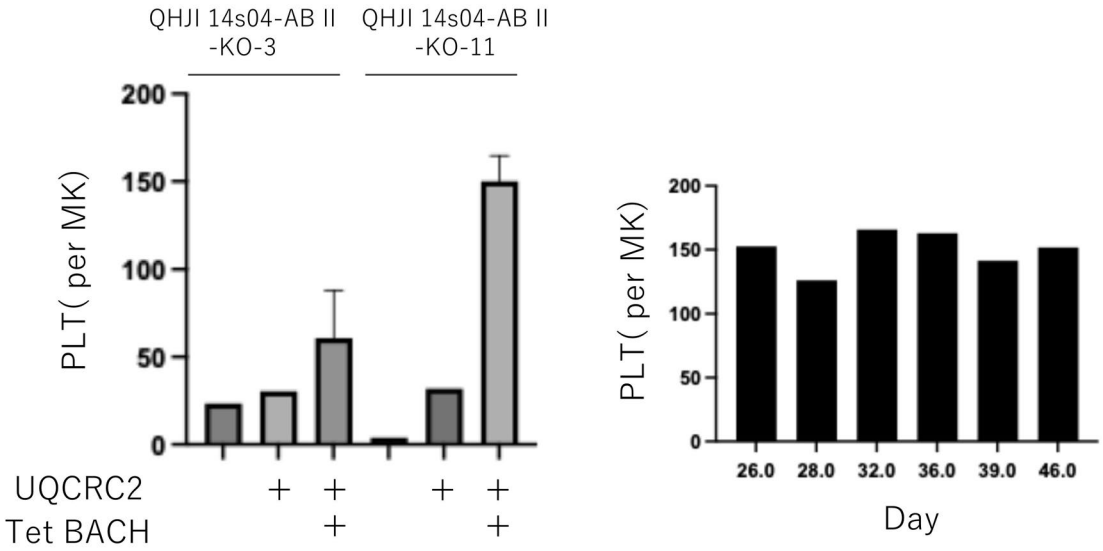

MX
QHJI 14s04
-AB II-KO-03
QHJI 14s04
-AB II-KO-11
PLT/MGK
200
150
100
50
0
UQCRC2
BACH1
Sh BCOR
Sh TRIM27
Sh p21/sh p53

[Fig. 18-1]

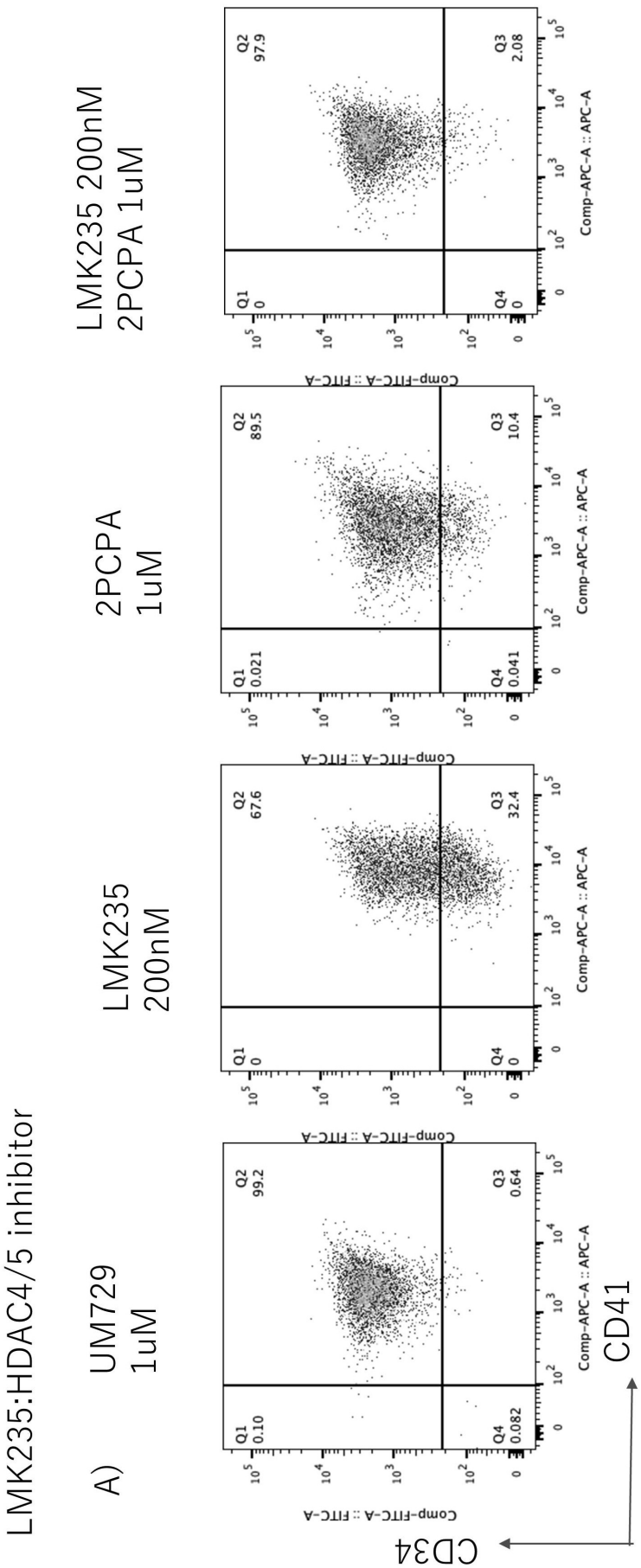

c-MYC
UQCRC2
BACH1
BCL-XL
EB method
No3
No11
iPS
TRE
c-MYC-DD
2A
BACH1
UbiC P
Bcl-XL
PuroR
UbiC P
rtTA
NeoR
UQCRC2
A)
QHJI 14s04-AB II-KO-3
QHJI 14s04-AB II-KO-11
day21
day48
day14
day42
sorting
CD34
CD41a

[Fig. 18-2]

B)


QHJI 14s04-AB II -KO-3
QHJI 14s04-AB II -KO-11
PLT(per MK)
200
150
100
50
0
UQCRC2
Tet BACH
+
+
+
+
+
+
PLT(per MK)
200
150
100
50
0
26.0
28.0
32.0
36.0
39.0
46.0
Day

[Fig. 19]


LMK235:HDAC4/5 inhibitor
A)
UM729 1uM
LMK235 200nM
2PCPA 1uM
LMK235 200nM 2PCPA 1uM
Q1 0.10
Q2 99.2
Q3 0.64
Q4 0.082
Q1 0
Q2 67.6
Q3 32.4
Q4 0
Q1 0.021
Q2 89.5
Q3 10.4
Q4 0.041
Q1 0
Q2 97.9
Q3 2.08
Q4 0
Comp-FITC-A :: FITC-A
Comp-APC-A :: APC-A
CD34
CD41