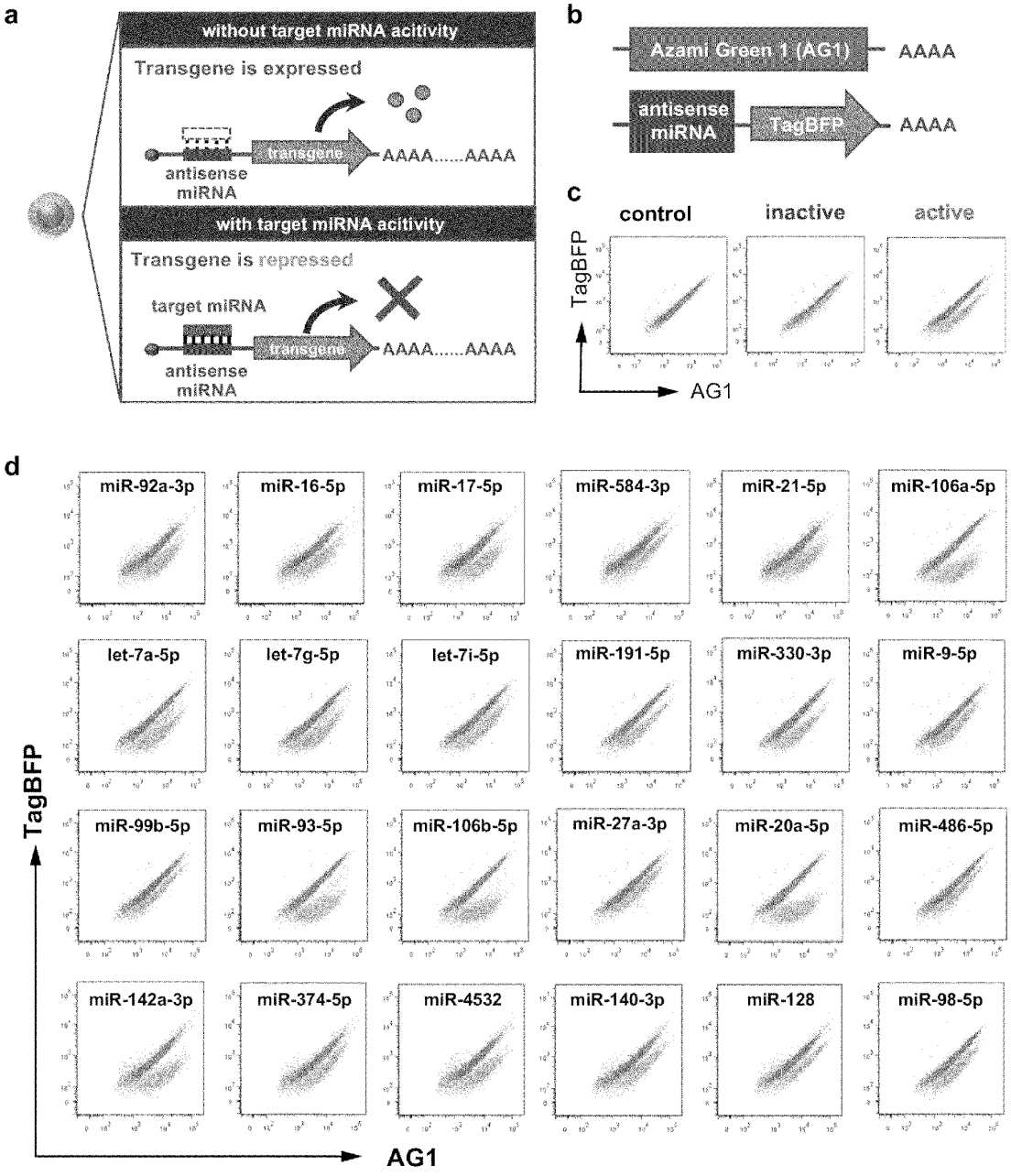
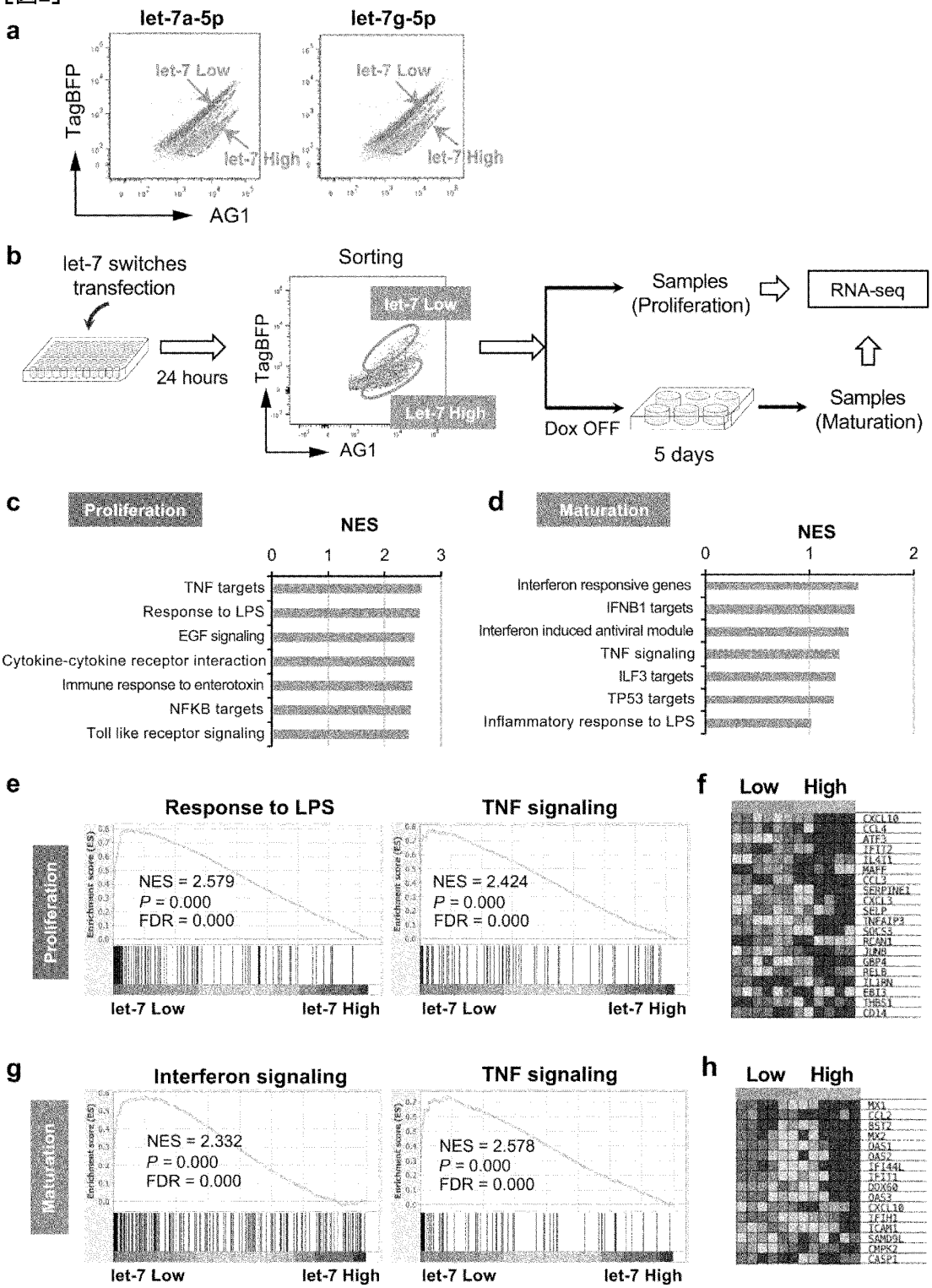
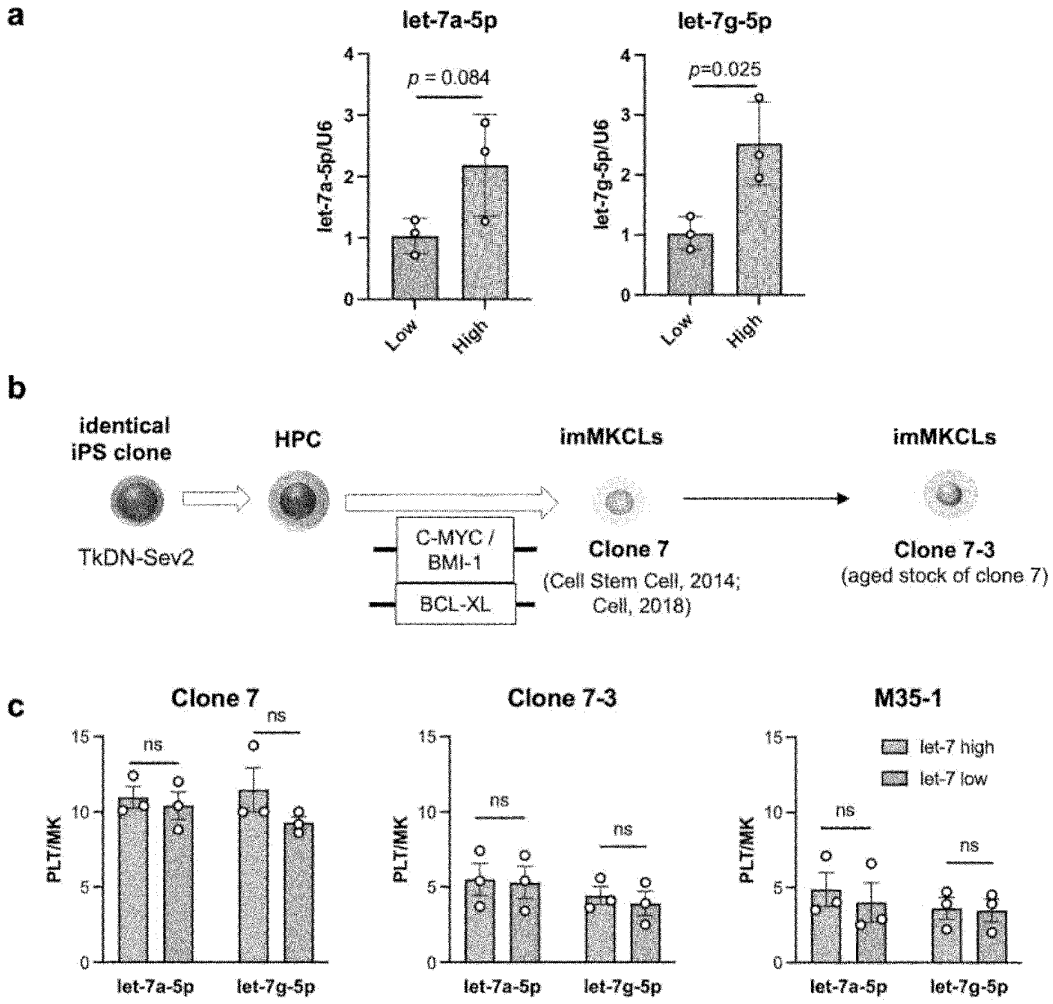
[図1]


a
without target miRNA acitivity
Transgene is expressed
antisense miRNA
transgene
AAAA.....AAAA
with target miRNA acitivity
Transgene is repressed
target miRNA
antisense miRNA
transgene
AAAA.....AAAA
b
Azami Green 1 (AG1)
AAAA
antisense miRNA
TagBFP
AAAA
c
control
inactive
active
TagBFP
AG1
d
miR-92a-3p
miR-16-5p
miR-17-5p
miR-584-3p
miR-21-5p
miR-106a-5p
let-7a-5p
let-7g-5p
let-7i-5p
miR-191-5p
miR-330-3p
miR-9-5p
miR-99b-5p
miR-93-5p
miR-106b-5p
miR-27a-3p
miR-20a-5p
miR-486-5p
miR-142a-3p
miR-374-5p
miR-4532
miR-140-3p
miR-128
miR-98-5p
TagBFP
AG1

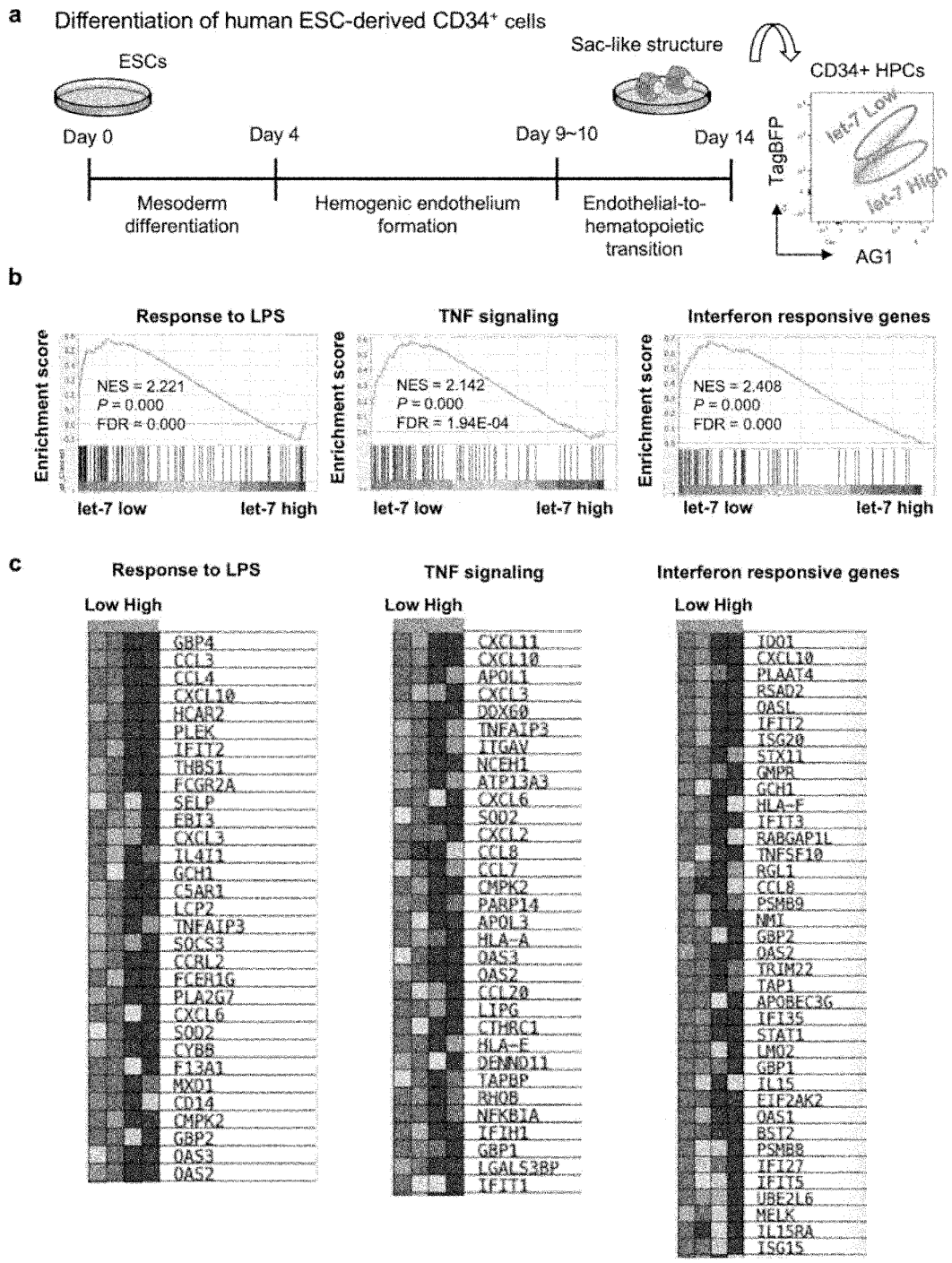
[図2]


a
let-7a-5p
let-7g-5p
let-7 Low
let-7 High
TagBFP
AG1
b
let-7 switches transfection
24 hours
Sorting
Let-7 Low
Let-7 High
Samples (Proliferation)
RNA-seq
Dox OFF
5 days
Samples (Maturation)
c
Proliferation
NES
TNF targets
Response to LPS
EGF signaling
Cytokine-cytokine receptor interaction
Immune response to enterotoxin
NFKB targets
Toll like receptor signaling
d
Maturation
NES
Interferon responsive genes
IFNB1 targets
Interferon induced antiviral module
TNF signaling
ILF3 targets
TP53 targets
Inflammatory response to LPS
e
Response to LPS
TNF signaling
Proliferation
NES = 2.579
P = 0.000
FDR = 0.000
NES = 2.424
P = 0.000
FDR = 0.000
let-7 Low
let-7 High
f
Low
High
g
Interferon signaling
TNF signaling
Maturation
NES = 2.332
P = 0.000
FDR = 0.000
NES = 2.578
P = 0.000
FDR = 0.000
h

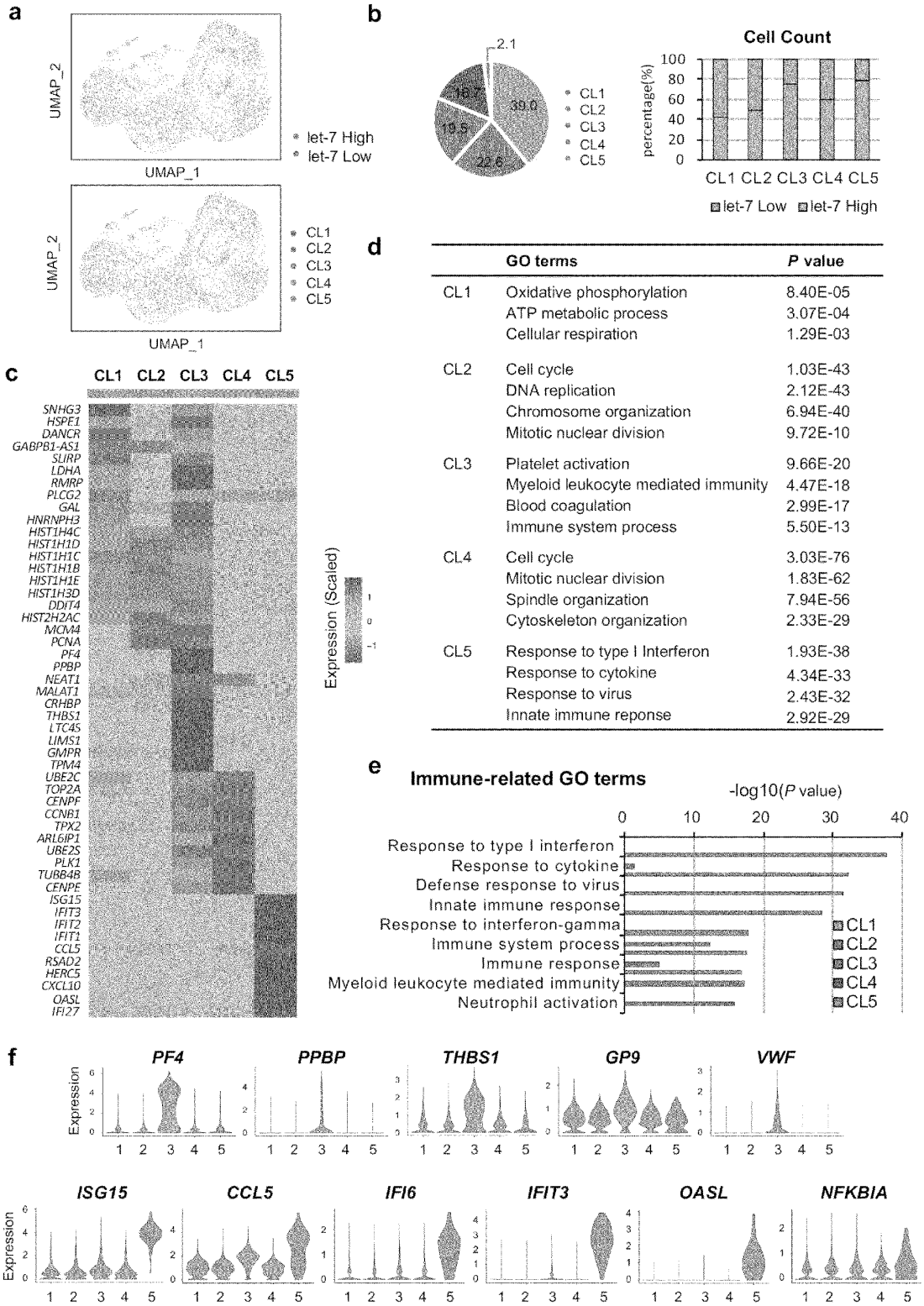
[図3]


a
let-7a-5p
let-7g-5p
p = 0.084
p=0.025
let-7a-5p/U6
let-7g-5p/U6
Low
High
b
identical iPS clone
HPC
imMKCLs
imMKCLs
TkDN-Sev2
C-MYC / BMI-1
BCL-XL
Clone 7
(Cell Stem Cell, 2014; Cell, 2018)
Clone 7-3
(aged stock of clone 7)
c
Clone 7
Clone 7-3
M35-1
let-7 high
let-7 low
ns
PLT/MK
let-7a-5p
let-7g-5p

[図4]

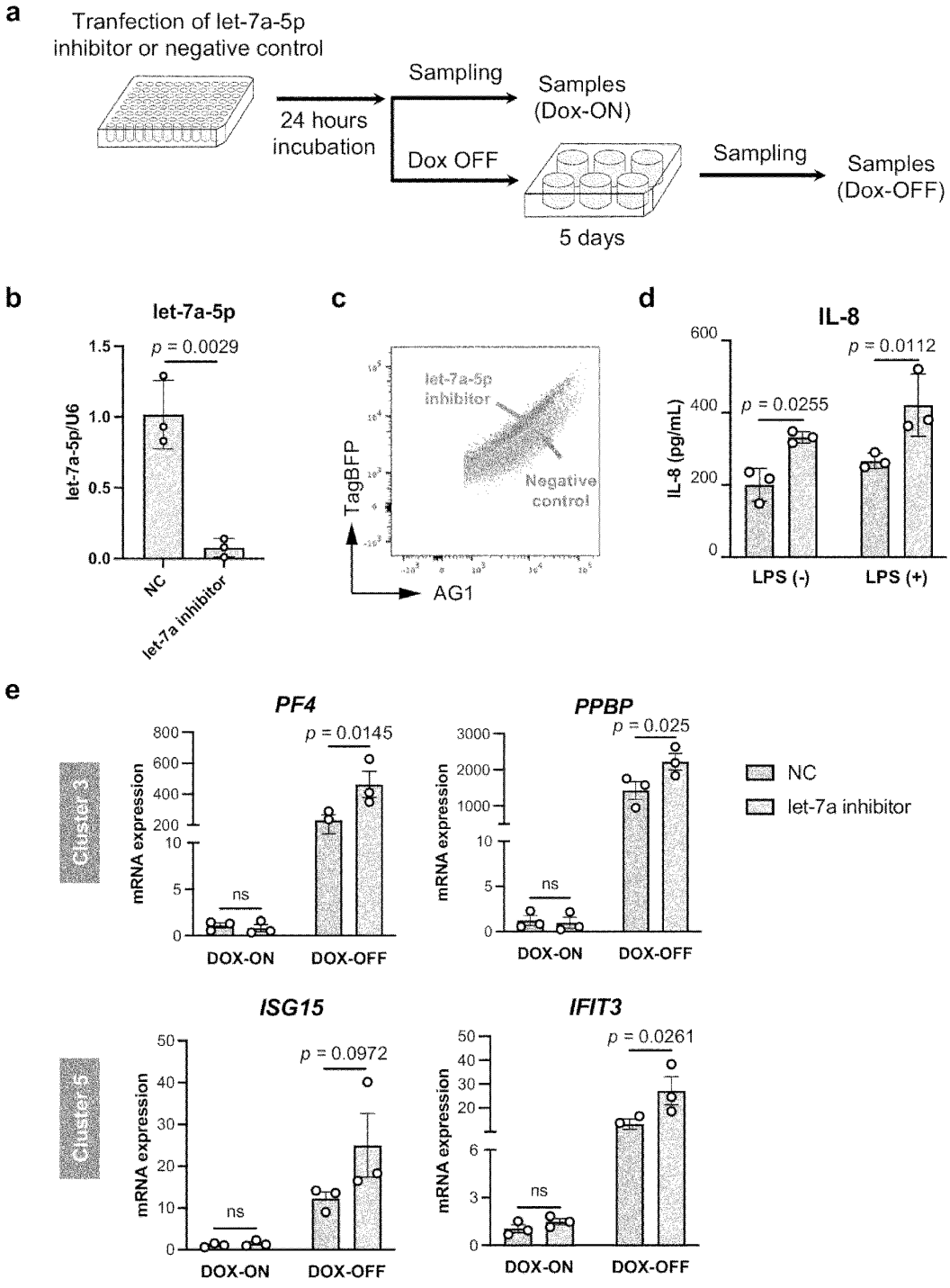

a
Differentiation of human ESC-derived CD34+ cells
ESCs
Sac-like structure
CD34+ HPCs
Day 0
Day 4
Day 9~10
Day 14
Mesoderm differentiation
Hemogenic endothelium formation
Endothelial-to-hematopoietic transition
let-7 Low
let-7 High
TagBFP
AG1
b
Response to LPS
TNF signaling
Interferon responsive genes
Enrichment score
NES = 2.221
P = 0.000
FDR = 0.000
NES = 2.142
P = 0.000
FDR = 1.94E-04
NES = 2.408
P = 0.000
FDR = 0.000
let-7 low
let-7 high
c
Response to LPS
TNF signaling
Interferon responsive genes
Low High
GBP4
CCL3
CCL4
CXCL10
HCAR2
PLEK
IFIT2
THBS1
FCGR2A
SELP
EBI3
CXCL3
IL4I1
GCH1
C5AR1
LCP2
TNFAIP3
SOCS3
CCRL2
FCER1G
PLA2G7
CXCL6
SOD2
CYBB
F13A1
MXD1
CD14
CMPK2
GBP2
OAS3
OAS2
CXCL11
CXCL10
APOL1
CXCL3
DDX60
TNFAIP3
ITGAV
NCEH1
ATP13A3
CXCL6
SOD2
CXCL2
CCL8
CCL7
CMPK2
PARP14
APOL3
HLA-A
OAS3
OAS2
CCL20
LIPG
CTHRC1
HLA-F
DENND1I
TAPBP
RHOB
NFKBIA
IFIH1
GBP1
LGALS3BP
IFIT1
IDO1
CXCL10
PLAAT4
RSAD2
OASL
IFIT2
ISG20
SIX1I
GMPR
GCH1
HLA-F
IFIT3
RABGAP1L
TNFSF10
RGL1
CCL8
PSMB9
NMI
GBP2
OAS2
TRIM22
TAP1
APOBEC3G
IFI35
STAT1
LMO2
GBP1
IL15
EIF2AK2
OAS1
BST2
PSMB8
IFI27
IFIT5
UBE2L6
MELK
IL15RA
ISG15

[図5]

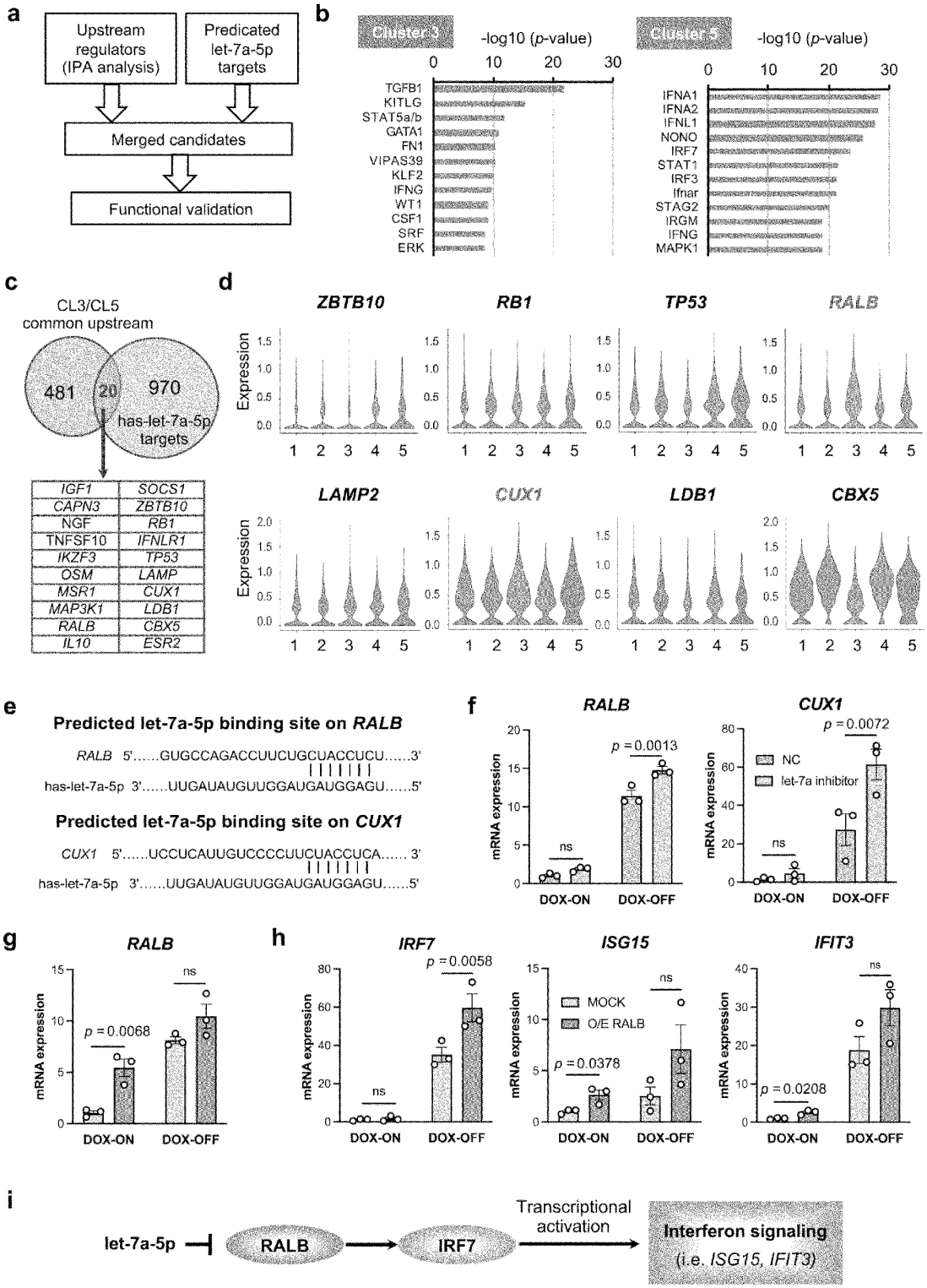


	GO terms	*P* value
CL1	Oxidative phosphorylation	8.40E-05
	ATP metabolic process	3.07E-04
	Cellular respiration	1.29E-03
CL2	Cell cycle	1.03E-43
	DNA replication	2.12E-43
	Chromosome organization	6.94E-40
	Mitotic nuclear division	9.72E-10
CL3	Platelet activation	9.66E-20
	Myeloid leukocyte mediated immunity	4.47E-18
	Blood coagulation	2.99E-17
	Immune system process	5.50E-13
CL4	Cell cycle	3.03E-76
	Mitotic nuclear division	1.83E-62
	Spindle organization	7.94E-56
	Cytoskeleton organization	2.33E-29
CL5	Response to type I Interferon	1.93E-38
	Response to cytokine	4.34E-33
	Response to virus	2.43E-32
	Innate immune reponse	2.92E-29

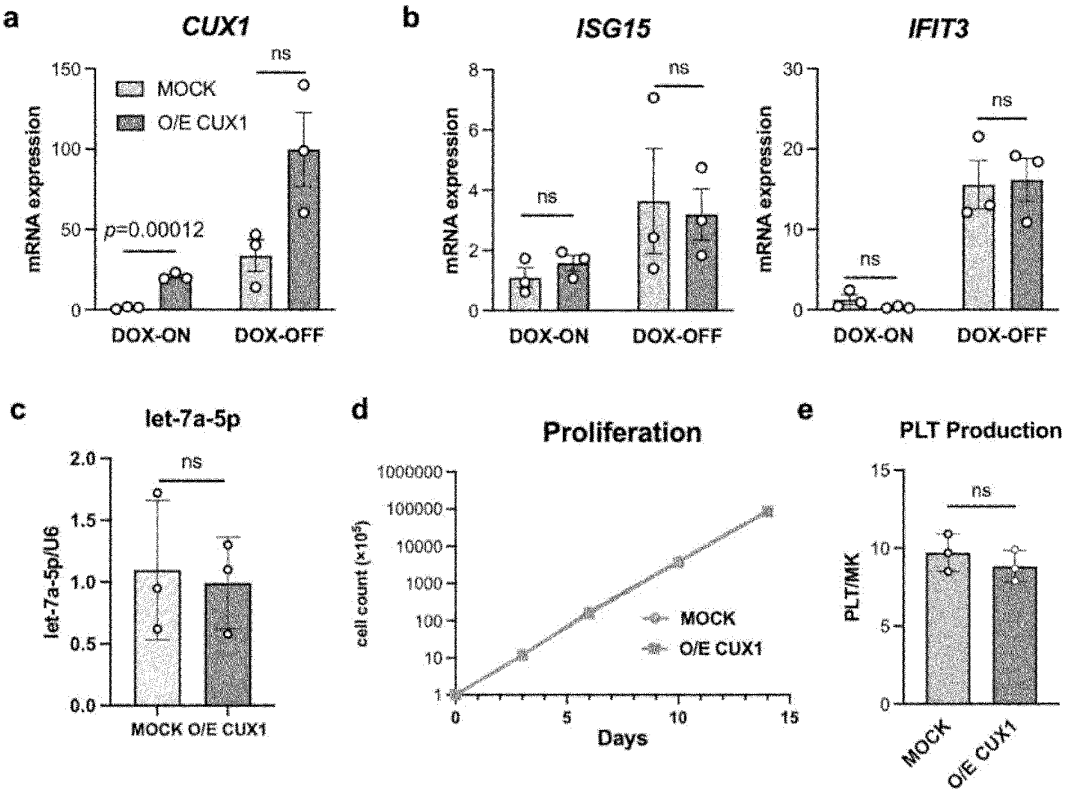
[図6]


a
Tranfection of let-7a-5p inhibitor or negative control
24 hours incubation
Sampling
Samples (Dox-ON)
Dox OFF
5 days
Sampling
Samples (Dox-OFF)
b
let-7a-5p
p = 0.0029
let-7a-5p/U6
NC
let-7a inhibitor
c
let-7a-5p inhibitor
Negative control
TagBFP
AG1
d
IL-8
p = 0.0112
p = 0.0255
IL-8 (pg/mL)
LPS (-)
LPS (+)
e
PF4
p = 0.0145
PPBP
p = 0.025
Cluster 3
mRNA expression
ns
DOX-ON
DOX-OFF
NC
let-7a inhibitor
ISG15
p = 0.0972
IFIT3
p = 0.0261
Cluster 5

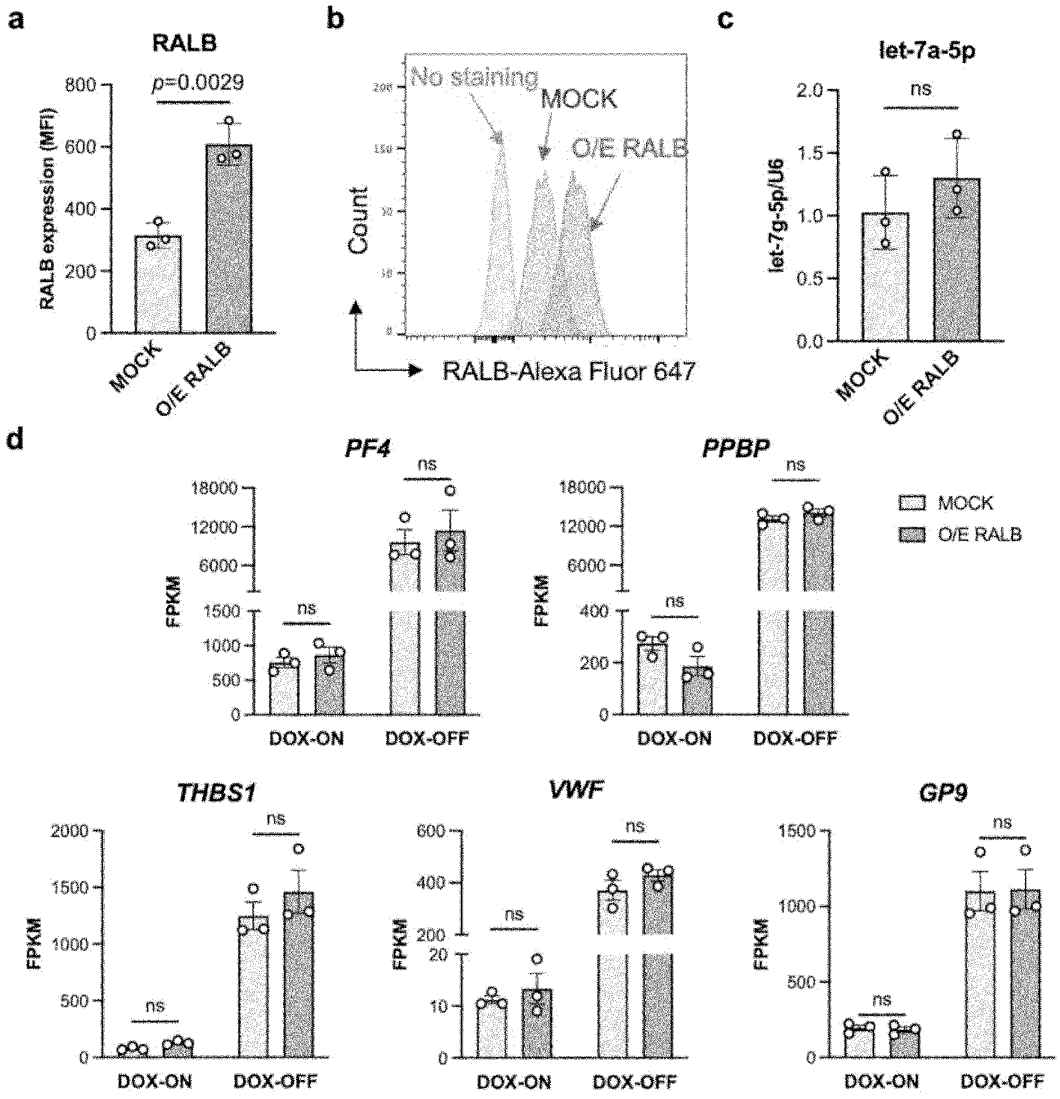
[図7]


a
Upstream regulators (IPA analysis)
Predicated let-7a-5p targets
Merged candidates
Functional validation
b
Cluster 3
-log10 (p-value)
TGFB1
KITLG
STAT5a/b
GATA1
FN1
VIPAS39
KLF2
IFNG
WT1
CSF1
SRF
ERK
Cluster 5
-log10 (p-value)
IFNA1
IFNA2
IFNL1
NONO
IRF7
STAT1
IRF3
Ifnar
STAG2
IRGM
IFNG
MAPK1
c
CL3/CL5 common upstream
481
20
970
has-let-7a-5p targets
IGF1 SOCS1
CAPN3 ZBTB10
NGF RB1
TNFSF10 IFNLR1
IKZF3 TP53
OSM LAMP
MSR1 CUX1
MAP3K1 LDB1
RALB CBX5
IL10 ESR2
d
ZBTB10
RB1
TP53
RALB
LAMP2
CUX1
LDB1
CBX5
Expression
e
Predicted let-7a-5p binding site on RALB
RALB 5'.....GUGCCAGACCUUCUGCUACCUCU.....3'
has-let-7a-5p 3'.....UUGAUAUGUUGGAUGAUGGAGU.....5'
Predicted let-7a-5p binding site on CUX1
CUX1 5'.....UCCUCAUUGUCCCCUUCUACCUCA..... 3'
has-let-7a-5p 3'.....UUGAUAUGUUGGAUGAUGGAGU.....5'
f
RALB
p = 0.0013
ns
CUX1
p = 0.0072
NC
let-7a inhibitor
mRNA expression
DOX-ON
DOX-OFF
g
RALB
p = 0.0068
ns
h
IRF7
p = 0.0058
ISG15
MOCK
O/E RALB
p = 0.0378
IFIT3
p = 0.0208
i
let-7a-5p
RALB
IRF7
Transcriptional activation
Interferon signaling
(i.e. ISG15, IFIT3)

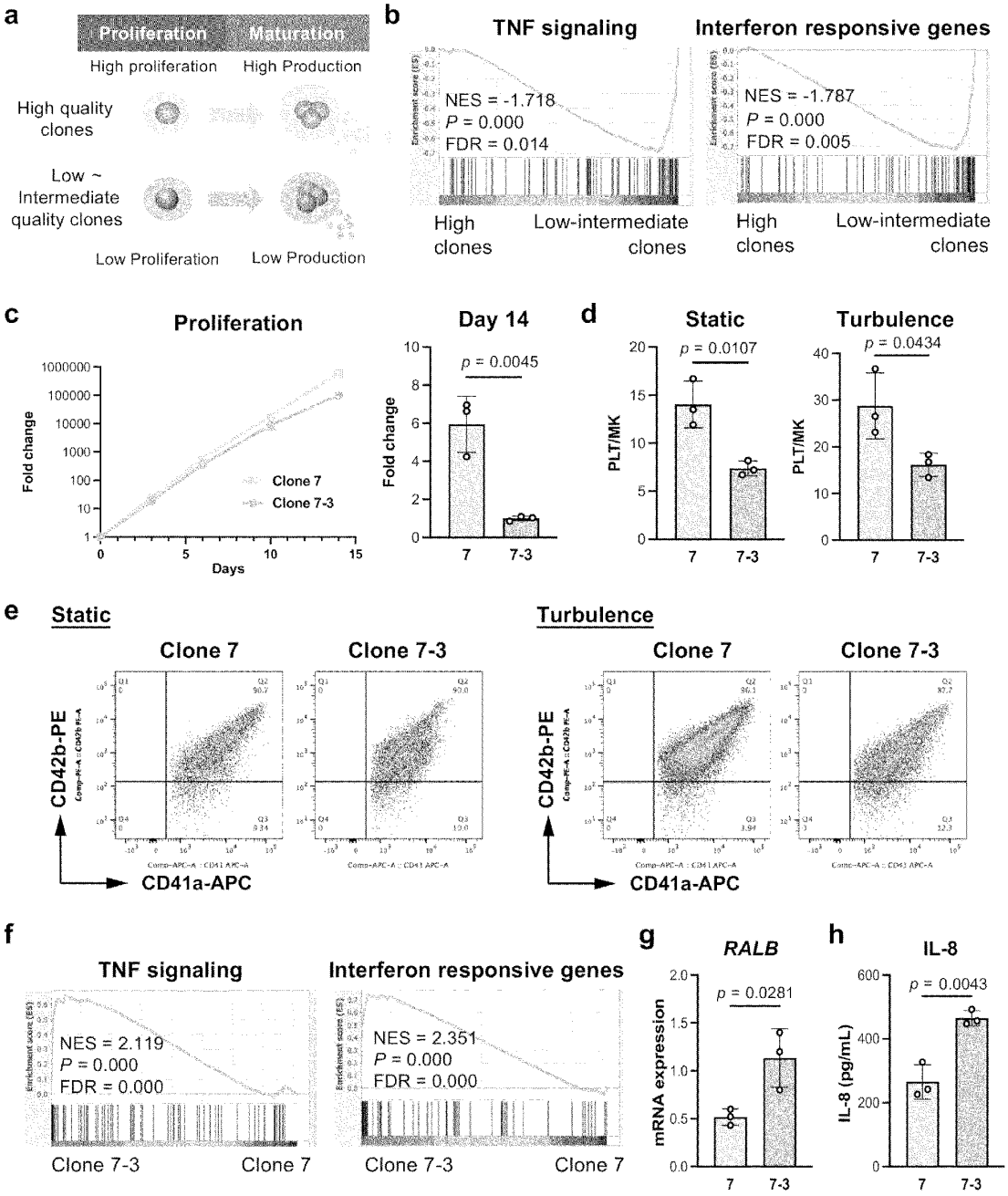
[図8]


a
CUX1
mRNA expression
MOCK
O/E CUX1
p=0.00012
ns
DOX-ON
DOX-OFF
b
ISG15
IFIT3
c
let-7a-5p
let-7a-5p/U6
MOCK O/E CUX1
d
Proliferation
cell count (×10^5)
Days
e
PLT Production
PLT/MK

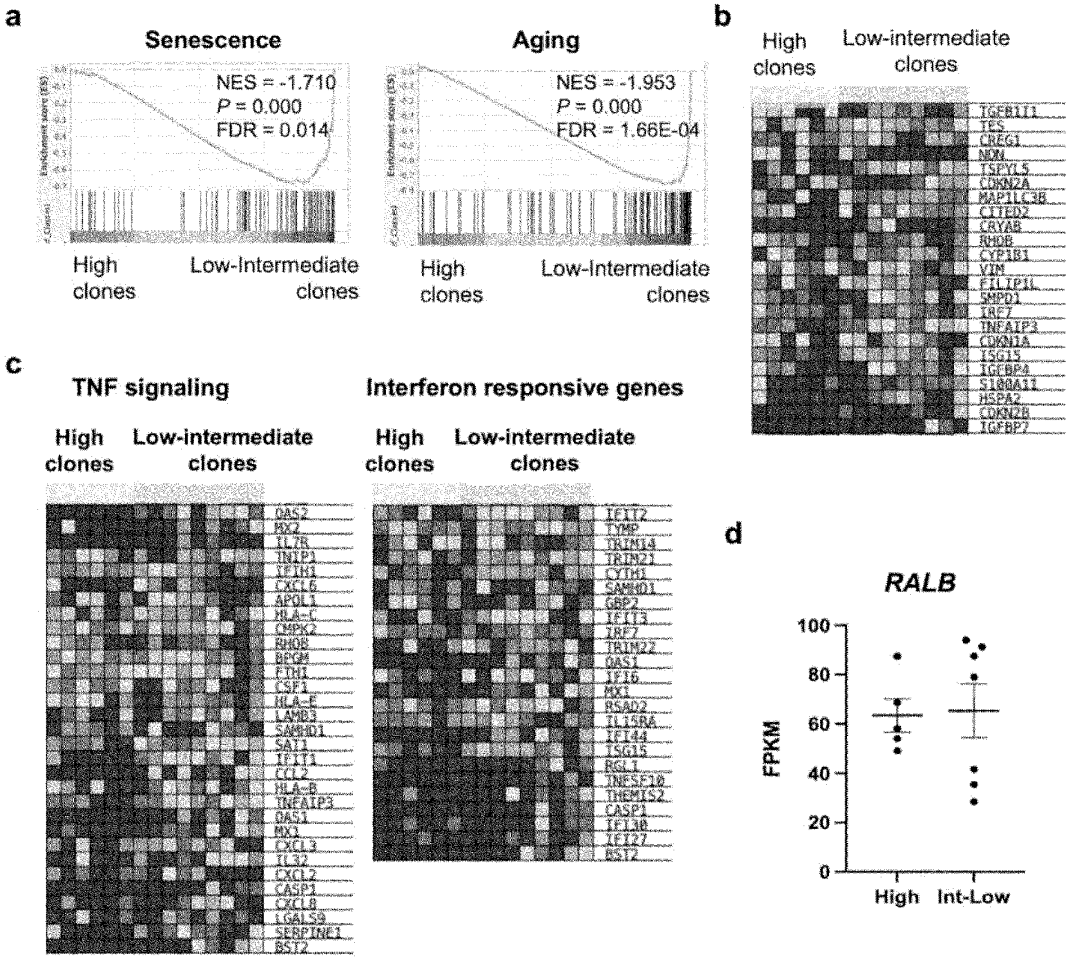
[図9]


a
RALB
p=0.0029
RALB expression (MFI)
800
600
400
200
0
MOCK
O/E RALB
b
No staining
MOCK
O/E RALB
Count
RALB-Alexa Fluor 647
c
let-7a-5p
ns
let-7g-5p/U6
2.0
1.5
1.0
0.5
0.0
MOCK
O/E RALB
d
PF4
PPBP
THBS1
VWF
GP9
FPKM
ns
DOX-ON
DOX-OFF
MOCK
O/E RALB

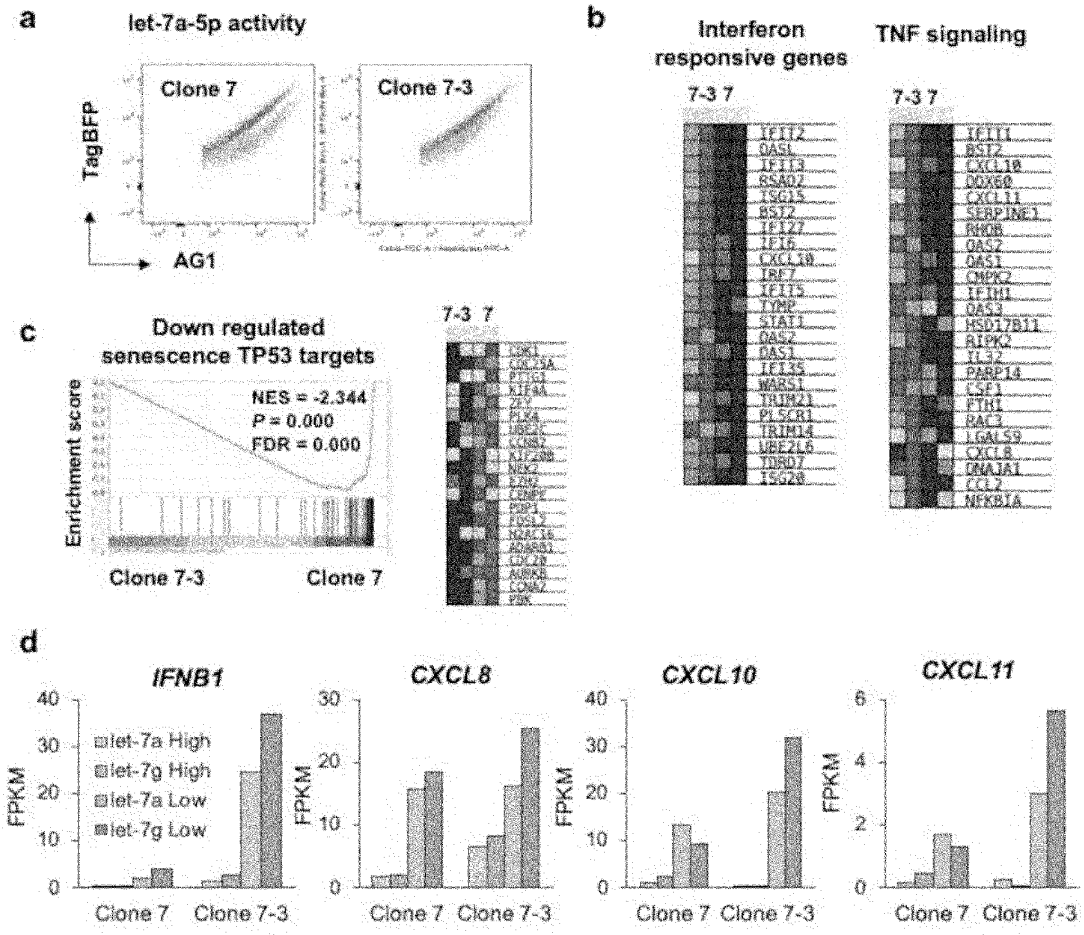
[図10]


a
Proliferation
Maturation
High proliferation
High Production
High quality clones
Low ~ Intermediate quality clones
Low Proliferation
Low Production
b
TNF signaling
NES = -1.718
P = 0.000
FDR = 0.014
High clones
Low-intermediate clones
Interferon responsive genes
NES = -1.787
P = 0.000
FDR = 0.005
High clones
Low-intermediate clones
c
Proliferation
Fold change
Clone 7
Clone 7-3
Days
Day 14
p = 0.0045
Fold change
7
7-3
d
Static
p = 0.0107
PLT/MK
Turbulence
p = 0.0434
PLT/MK
e
Static
Turbulence
Clone 7
Clone 7-3
CD42b-PE
CD41a-APC
f
TNF signaling
NES = 2.119
P = 0.000
FDR = 0.000
Clone 7-3
Clone 7
Interferon responsive genes
NES = 2.351
P = 0.000
FDR = 0.000
Clone 7-3
Clone 7
g
RALB
p = 0.0281
mRNA expression
h
IL-8
p = 0.0043
IL-8 (pg/mL)

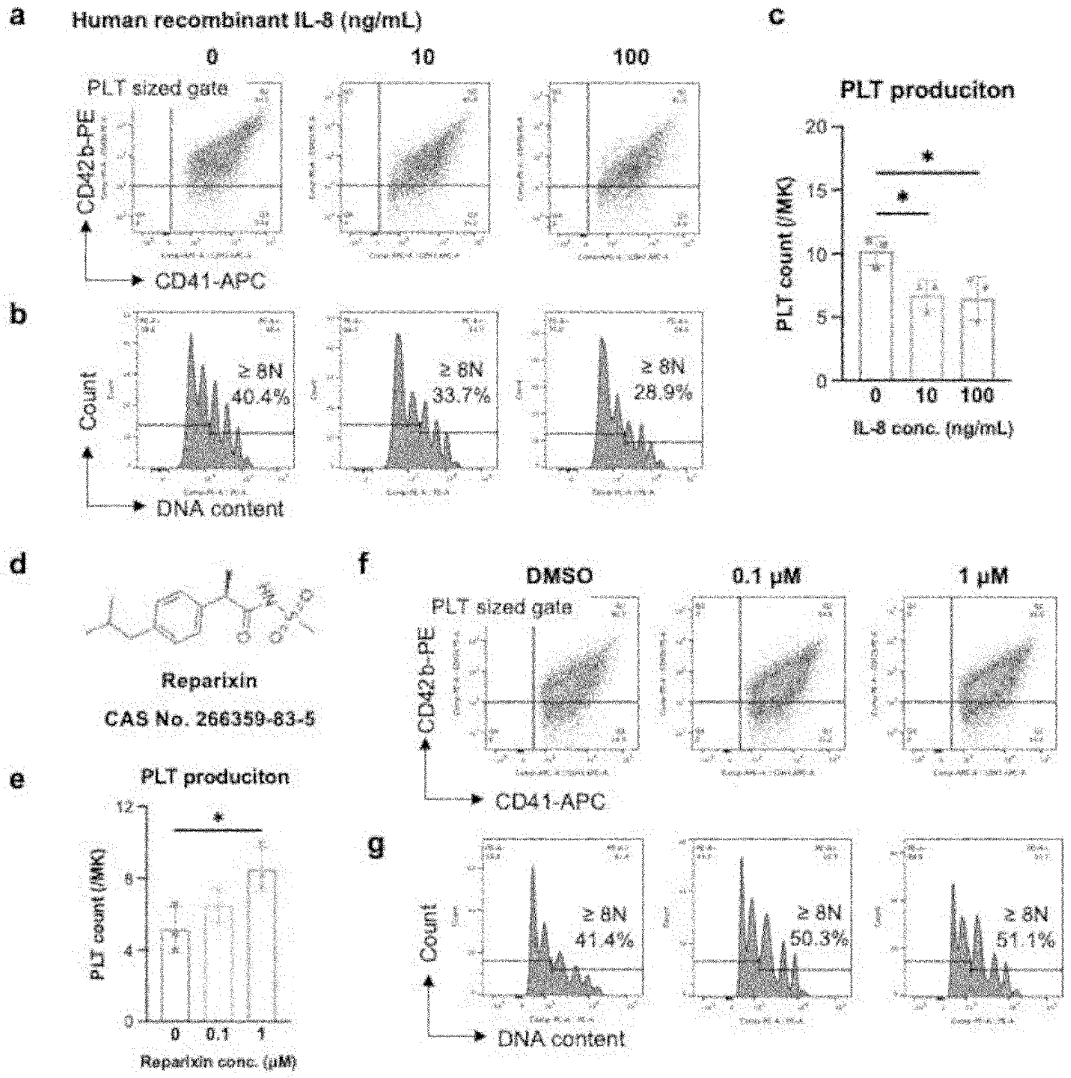
[図11]


a
Senescence
NES = -1.710
P = 0.000
FDR = 0.014
High clones
Low-Intermediate clones
Aging
NES = -1.953
P = 0.000
FDR = 1.66E-04
High clones
Low-Intermediate clones
b
High clones
Low-intermediate clones
TGFB1I1
TES
CREG1
NKN
TSPYL5
CDKN2A
MAP1LC3B
CITED2
CRYAB
RHOB
CYP1B1
VIM
FILIP1L
SMPD1
IRF7
TNFAIP3
CDKN1A
ISG15
IGFBP4
S100A11
HSPA2
CDKN2B
IGFBP7
c
TNF signaling
High clones
Low-intermediate clones
DAS2
MX2
IL7R
IN1P1
IFIH1
CXCL6
APOL1
HLA-C
CMPK2
RHOB
BPGM
FTH1
CSF1
HLA-E
LAMB3
SAMHD1
SAT1
IFIT1
CCL2
HLA-B
TNFAIP3
DAS1
MX1
CXCL3
IL32
CXCL2
CASP1
CXCL8
LGALS9
SERPINE1
BST2
Interferon responsive genes
High clones
Low-intermediate clones
IFIT2
TYMP
TRIM14
TRIM21
CYTH1
SAMHD1
GBP2
IFIT3
IRF7
TRIM22
OAS1
IFI6
MX1
RSAD2
IL15RA
IFI44
ISG15
RGL1
TNFSF10
THEMIS2
CASP1
IFI30
IFI27
BST2
d
RALB
FPKM
100
80
60
40
20
0
High
Int-Low

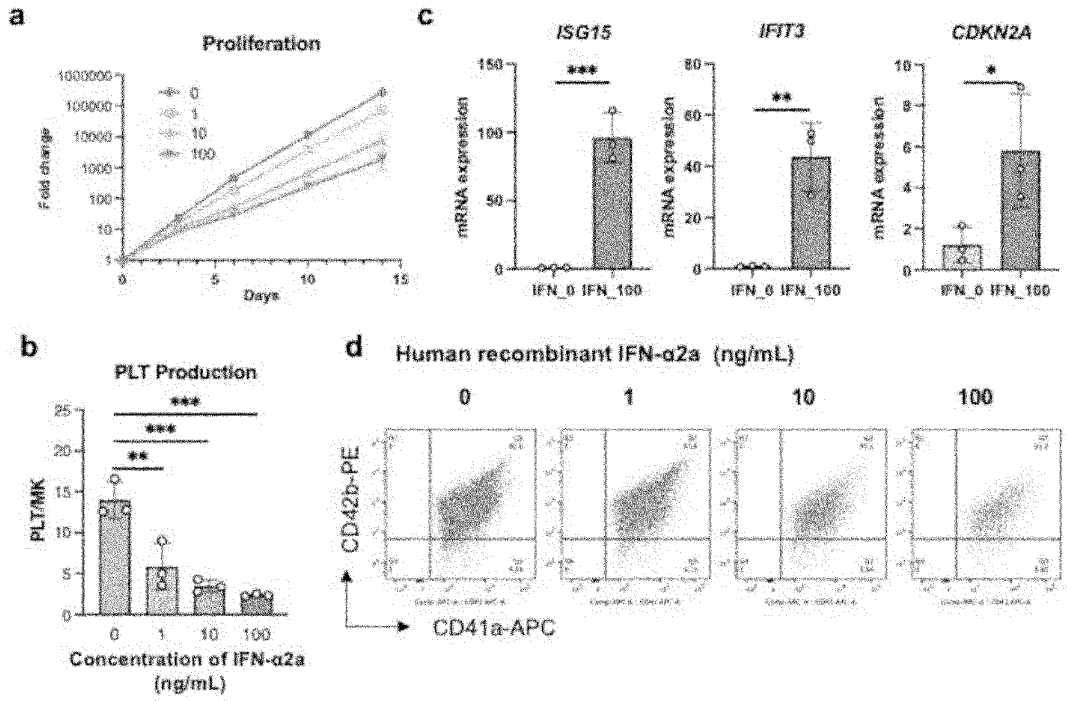
[図12]


a
let-7a-5p activity
Clone 7
Clone 7-3
TagBFP
AG1
b
Interferon responsive genes
TNF signaling
7-3 7
c
Down regulated senescence TP53 targets
NES = -2.344
P = 0.000
FDR = 0.000
Enrichment score
Clone 7-3
Clone 7
7-3 7
d
IFNB1
CXCL8
CXCL10
CXCL11
FPKM
let-7a High
let-7g High
let-7a Low
let-7g Low
Clone 7
Clone 7-3

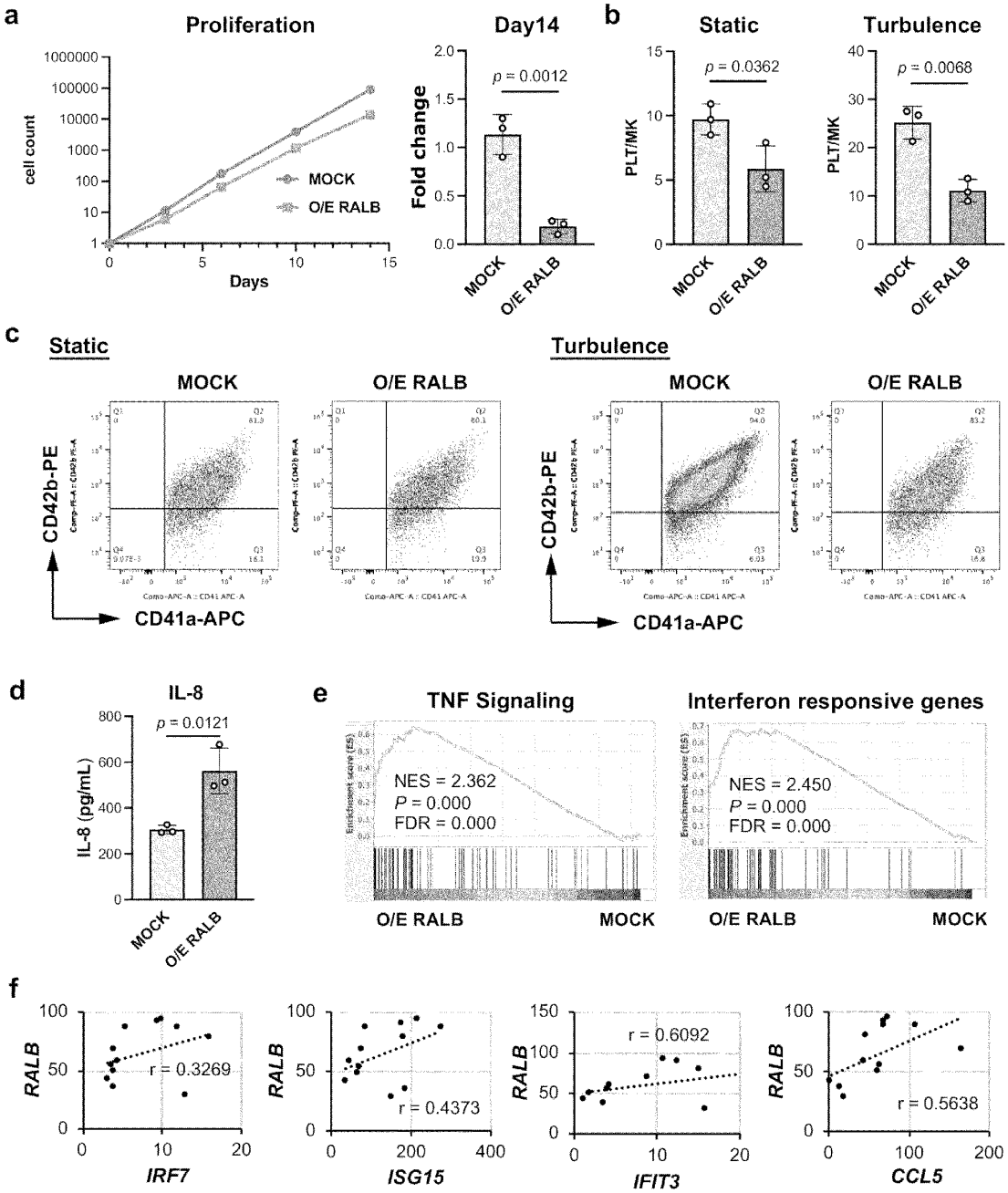
[図13]


a
Human recombinant IL-8 (ng/mL)
0
10
100
PLT sized gate
CD42b-PE
CD41-APC
b
Count
≥ 8N
40.4%
≥ 8N
33.7%
≥ 8N
28.9%
DNA content
c
PLT produciton
PLT count (/MK)
IL-8 conc. (ng/mL)
d
Reparixin
CAS No. 266359-83-5
e
PLT produciton
PLT count (/MK)
Reparixin conc. (μM)
f
DMSO
0.1 μM
1 μM
PLT sized gate
CD42b-PE
CD41-APC
g
Count
≥ 8N
41.4%
≥ 8N
50.3%
≥ 8N
51.1%
DNA content

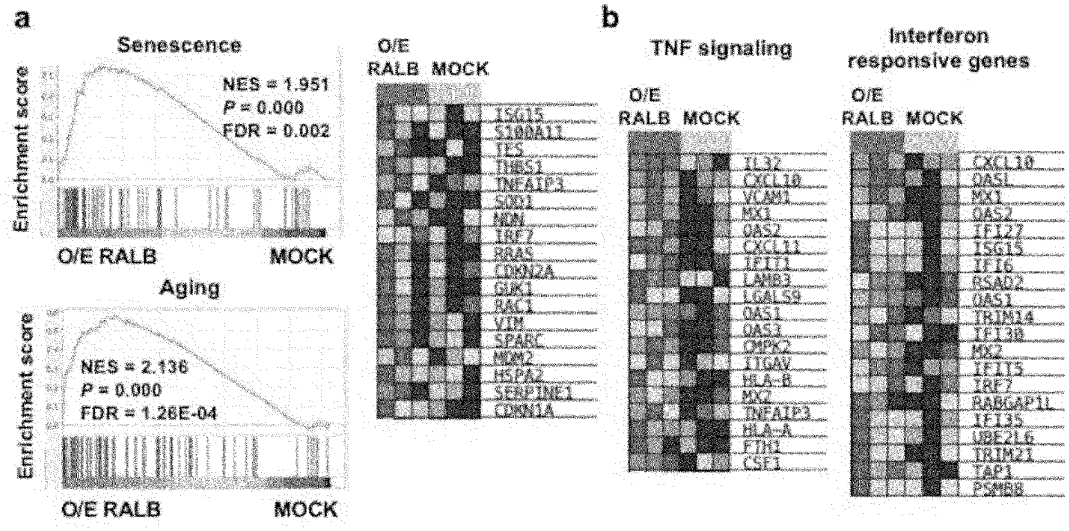
[図14]


a
Proliferation
Fold change
Days
0
1
10
100
b
PLT Production
PLT/MK
Concentration of IFN-α2a (ng/mL)
c
ISG15
IFIT3
CDKN2A
mRNA expression
IFN_0
IFN_100
d
Human recombinant IFN-α2a (ng/mL)
CD42b-PE
CD41a-APC

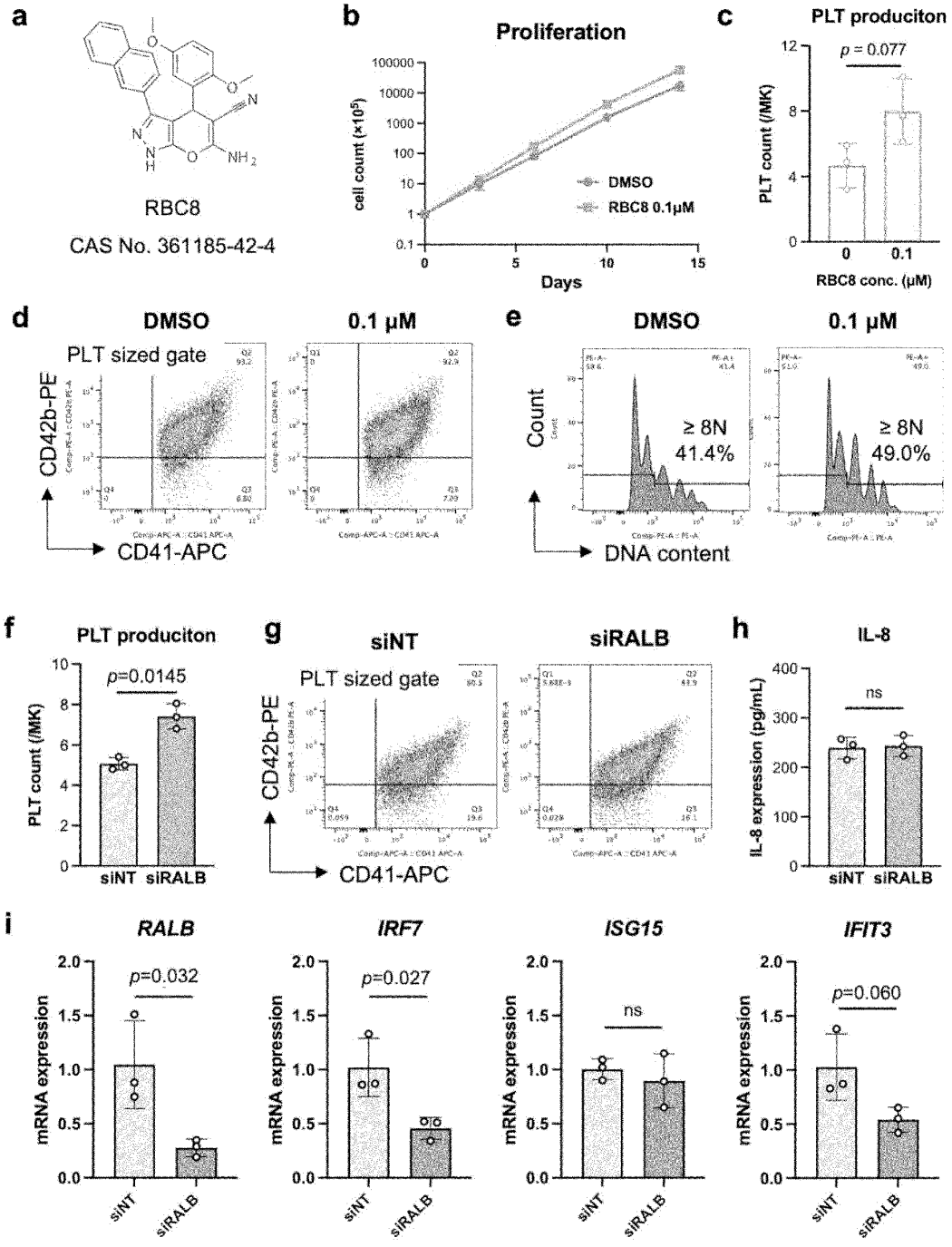
[図15]


a
Proliferation
cell count
Days
MOCK
O/E RALB
Day14
Fold change
p = 0.0012
b
Static
Turbulence
PLT/MK
p = 0.0362
p = 0.0068
c
Static
Turbulence
MOCK
O/E RALB
CD42b-PE
CD41a-APC
d
IL-8
IL-8 (pg/mL)
p = 0.0121
e
TNF Signaling
Interferon responsive genes
NES = 2.362
P = 0.000
FDR = 0.000
NES = 2.450
P = 0.000
FDR = 0.000
f
RALB
IRF7
r = 0.3269
ISG15
r = 0.4373
IFIT3
r = 0.6092
CCL5
r = 0.5638

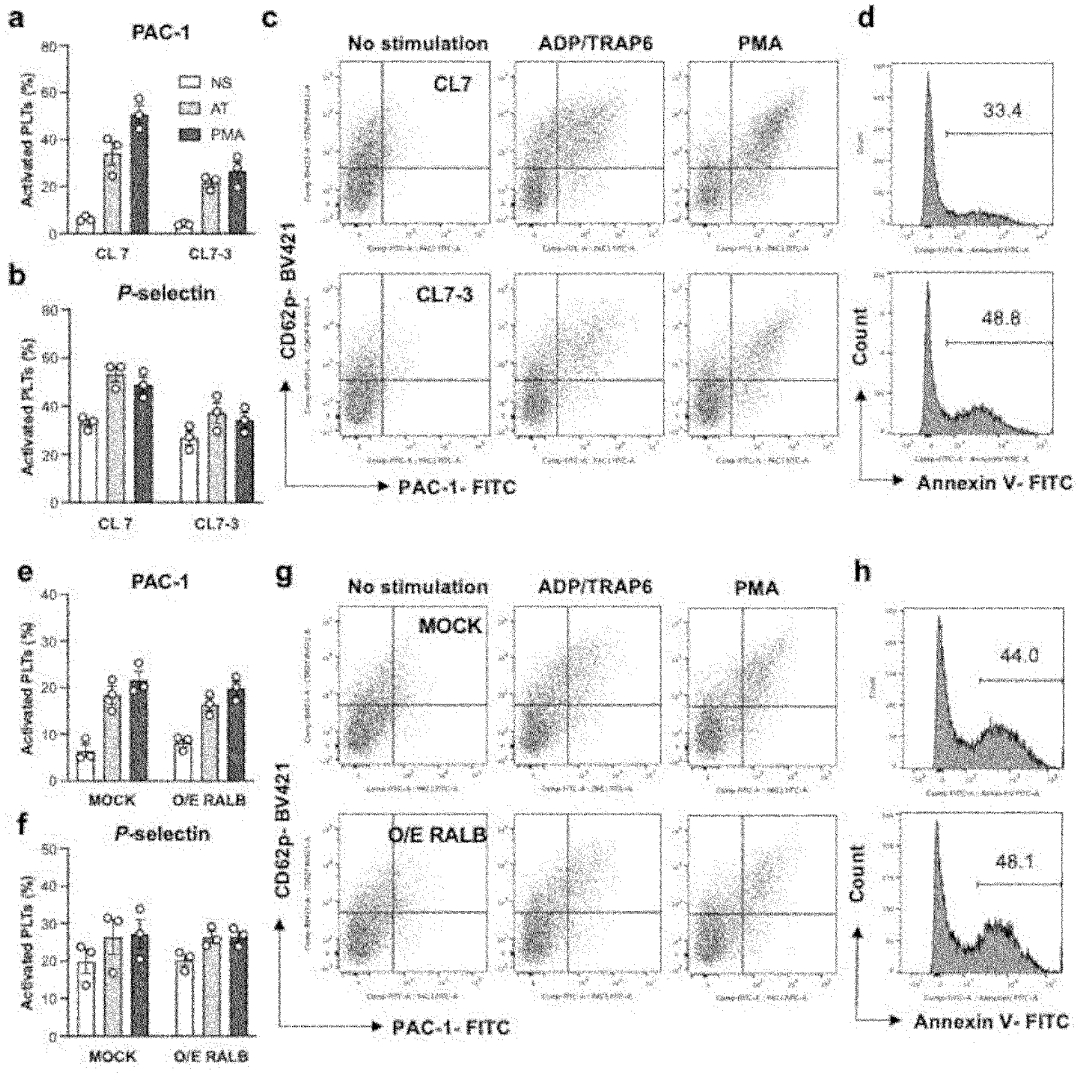
[図16]


a
Senescence
Enrichment score
NES = 1.951
P = 0.000
FDR = 0.002
O/E RALB
MOCK
Aging
Enrichment score
NES = 2.136
P = 0.000
FDR = 1.26E-04
O/E RALB
MOCK
O/E
RALB MOCK
ISG15
S100A11
IFS
THBS1
TNFAIP3
SOD1
MDM
IRF7
RRAS
CDKN2A
GUK1
RAC1
VIM
SPARC
MDM2
HSPA2
SERPINE1
CDKN1A
b
TNF signaling
O/E
RALB MOCK
IL32
CXCL10
VCAM1
MX1
OAS2
CXCL11
IFIT1
LAMB3
LGALS9
OAS1
OAS3
CMPK2
ITGAV
HLA-B
MX2
TNFAIP3
HLA-A
F7H1
CSF1
Interferon
responsive genes
O/E
RALB MOCK
CXCL10
OASL
MX1
OAS2
IFI27
ISG15
IFI6
RSAD2
OAS1
TRIM14
IFI3B
MX2
IFIT3
IRF7
RABGAP1L
IFI35
UBE2L6
TRIM21
TAP1
PSMB8

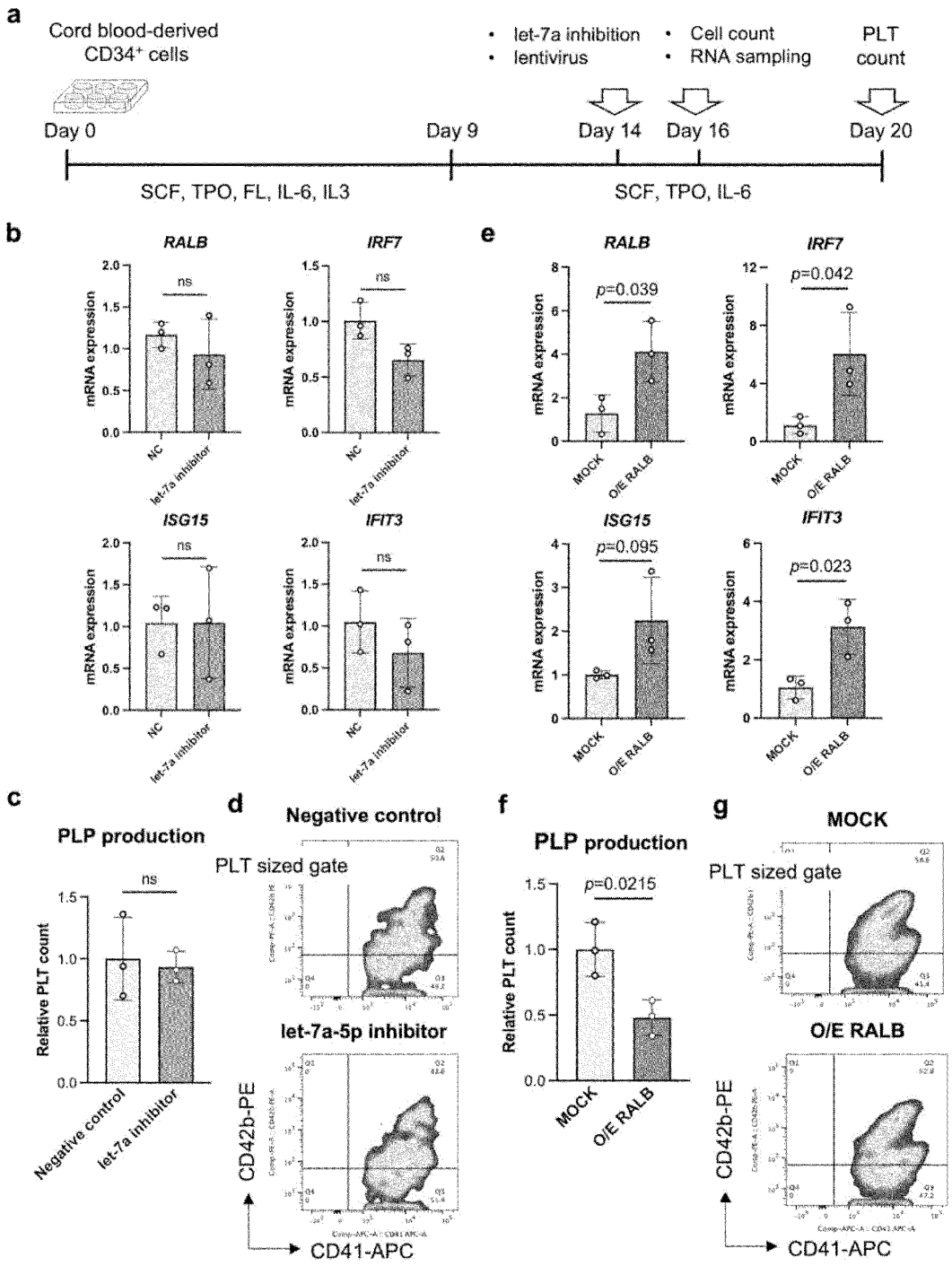
[図17]


a
RBC8
CAS No. 361185-42-4
b
Proliferation
cell count (×10⁵)
DMSO
RBC8 0.1μM
Days
c
PLT produciton
p = 0.077
PLT count (/MK)
RBC8 conc. (μM)
d
DMSO
0.1 μM
PLT sized gate
CD42b-PE
CD41-APC
e
DMSO
0.1 μM
Count
≥ 8N
41.4%
≥ 8N
49.0%
DNA content
f
PLT produciton
p=0.0145
PLT count (/MK)
siNT siRALB
g
siNT
siRALB
PLT sized gate
CD42b-PE
CD41-APC
h
IL-8
ns
IL-8 expression (pg/mL)
siNT siRALB
i
RALB
p=0.032
IRF7
p=0.027
ISG15
ns
IFIT3
p=0.060
mRNA expression
siNT
siRALB

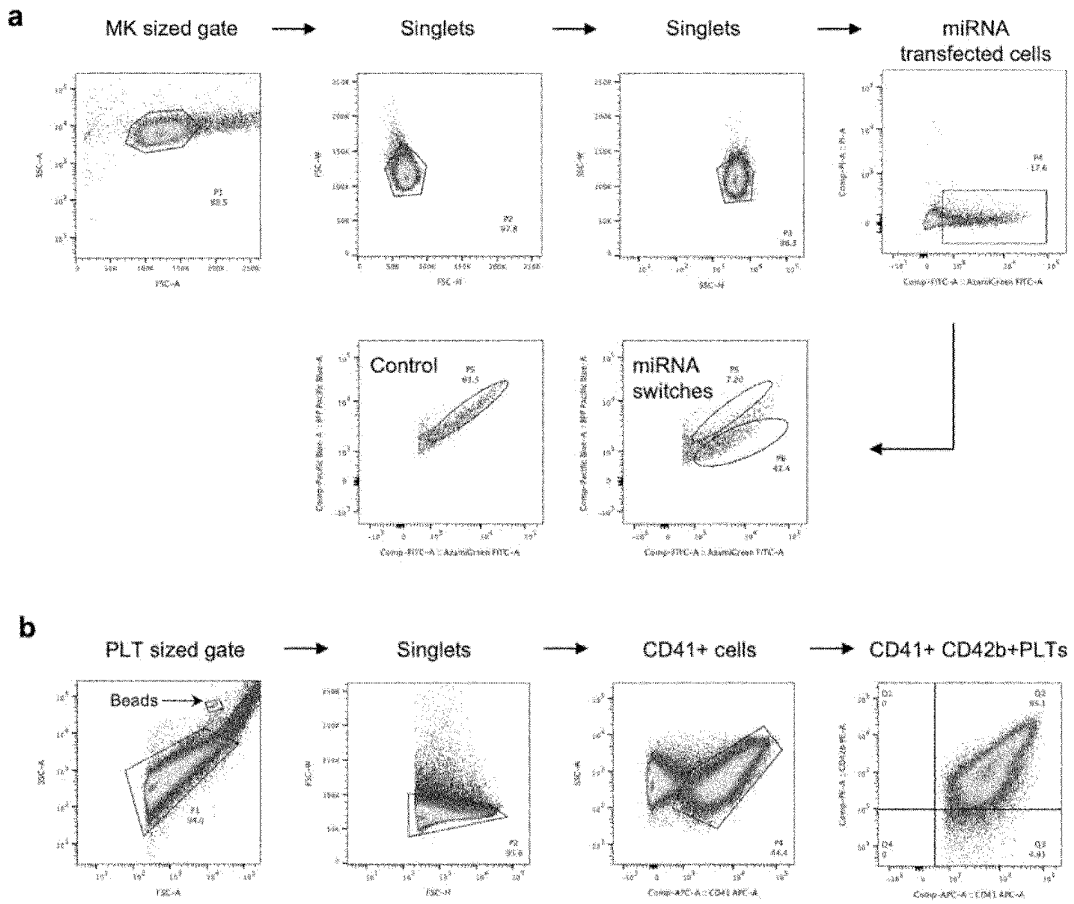
[図18]


a
PAC-1
Activated PLTs (%)
NS
AT
PMA
CL7
CL7-3
b
P-selectin
c
No stimulation
ADP/TRAP6
PMA
CL7
CL7-3
CD62p- BV421
PAC-1- FITC
d
33.4
48.8
Count
Annexin V- FITC
e
PAC-1
MOCK
O/E RALB
f
P-selectin
g
h
44.0
48.1

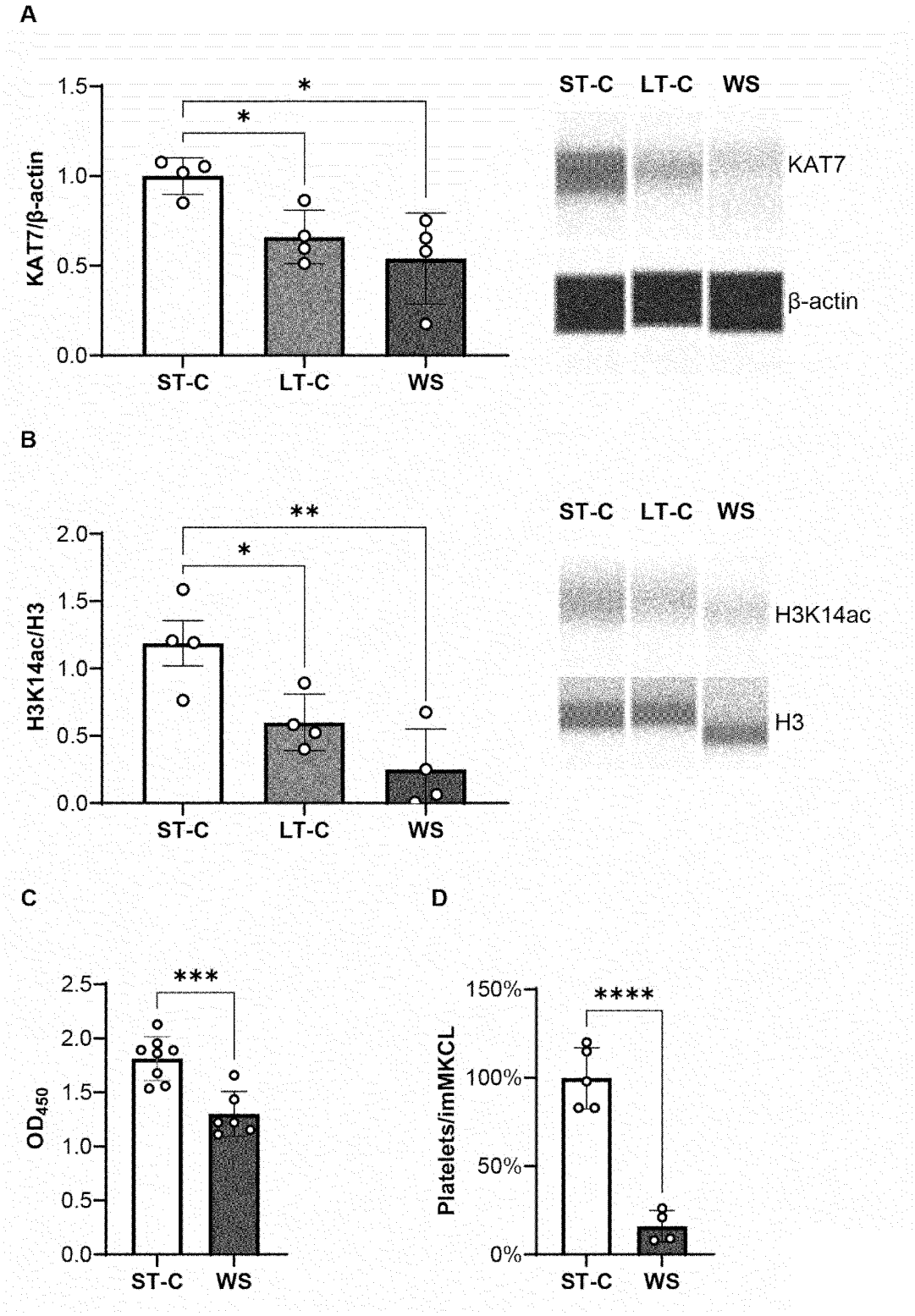
[図19]


a
Cord blood-derived CD34+ cells
• let-7a inhibition
• lentivirus
• Cell count
• RNA sampling
PLT count
Day 0
Day 9
Day 14
Day 16
Day 20
SCF, TPO, FL, IL-6, IL3
SCF, TPO, IL-6
b
RALB
IRF7
ISG15
IFIT3
mRNA expression
ns
NC
let-7a inhibitor
e
RALB
IRF7
ISG15
IFIT3
p=0.039
p=0.042
p=0.095
p=0.023
MOCK
O/E RALB
c
PLP production
Relative PLT count
ns
Negative control
let-7a inhibitor
d
Negative control
PLT sized gate
let-7a-5p inhibitor
CD42b-PE
CD41-APC
f
PLP production
p=0.0215
MOCK
O/E RALB
g
MOCK
PLT sized gate
O/E RALB
CD42b-PE
CD41-APC

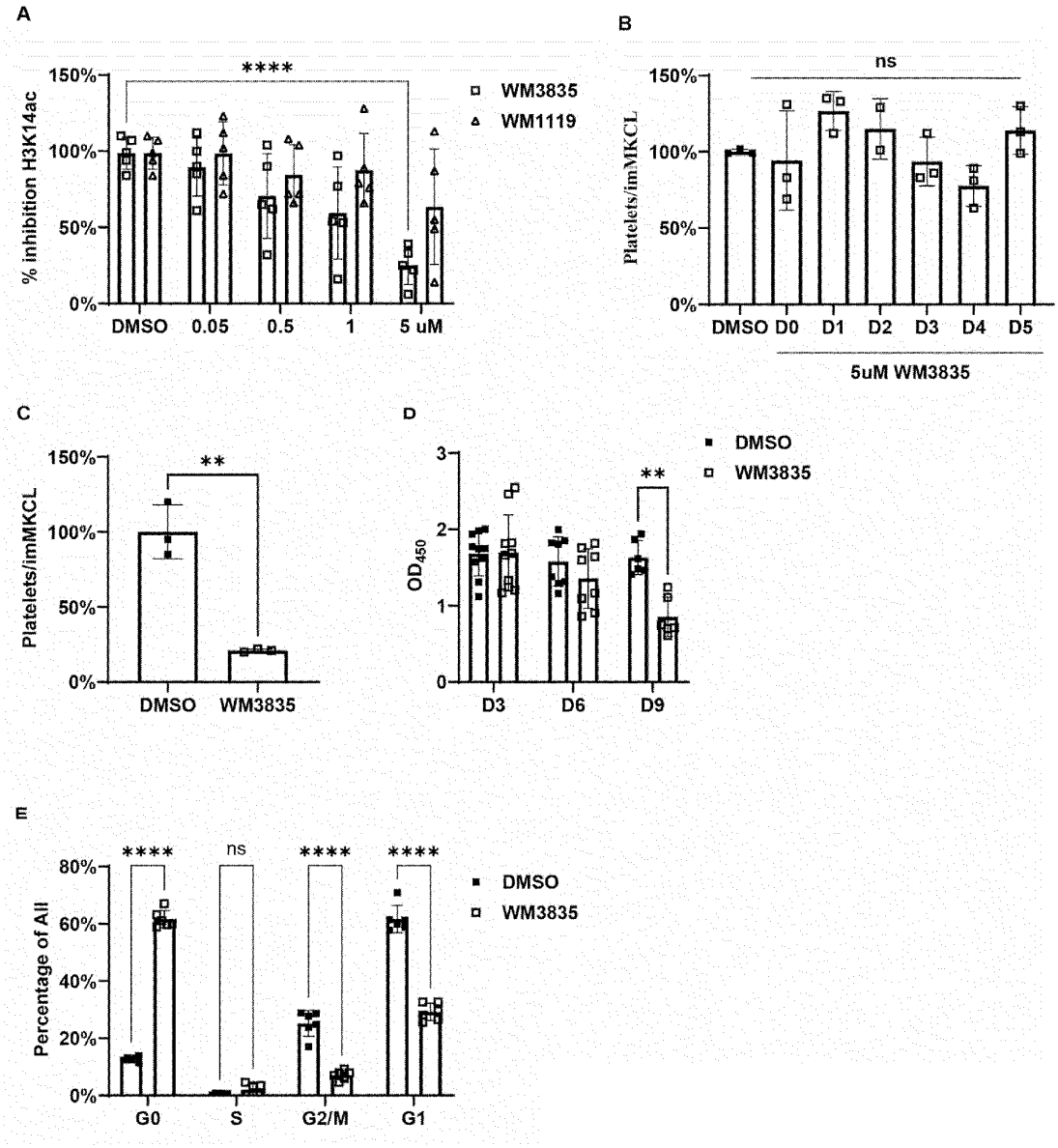
[図20]


a
MK sized gate
Singlets
Singlets
miRNA transfected cells
Control
miRNA switches
b
PLT sized gate
Singlets
CD41+ cells
CD41+ CD42b+PLTs
Beads

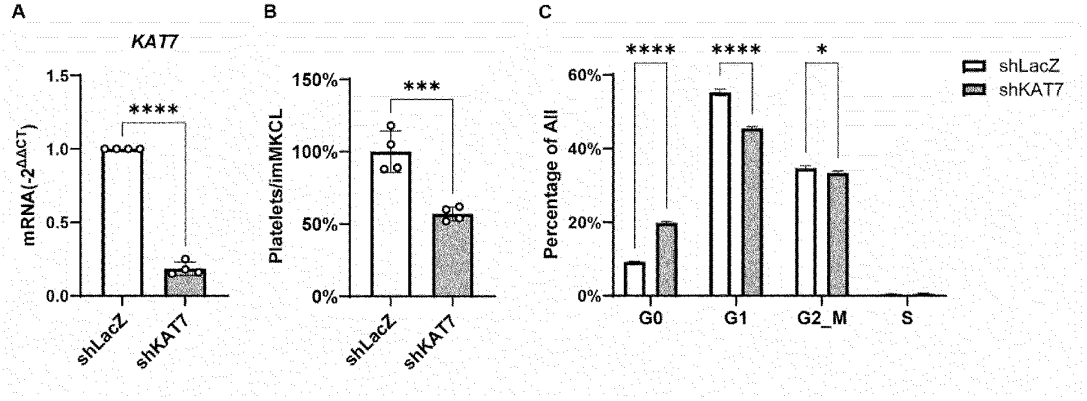
[図21]


A
KAT7/β-actin
1.5
1.0
0.5
0.0
*
*
ST-C
LT-C
WS
ST-C LT-C WS
KAT7
β-actin
B
H3K14ac/H3
2.0
1.5
1.0
0.5
0.0
**
*
ST-C
LT-C
WS
ST-C LT-C WS
H3K14ac
H3
C
OD450
2.5
2.0
1.5
1.0
0.5
0.0
***
ST-C
WS
D
Platelets/imMKCL
150%
100%
50%
0%
****
ST-C
WS

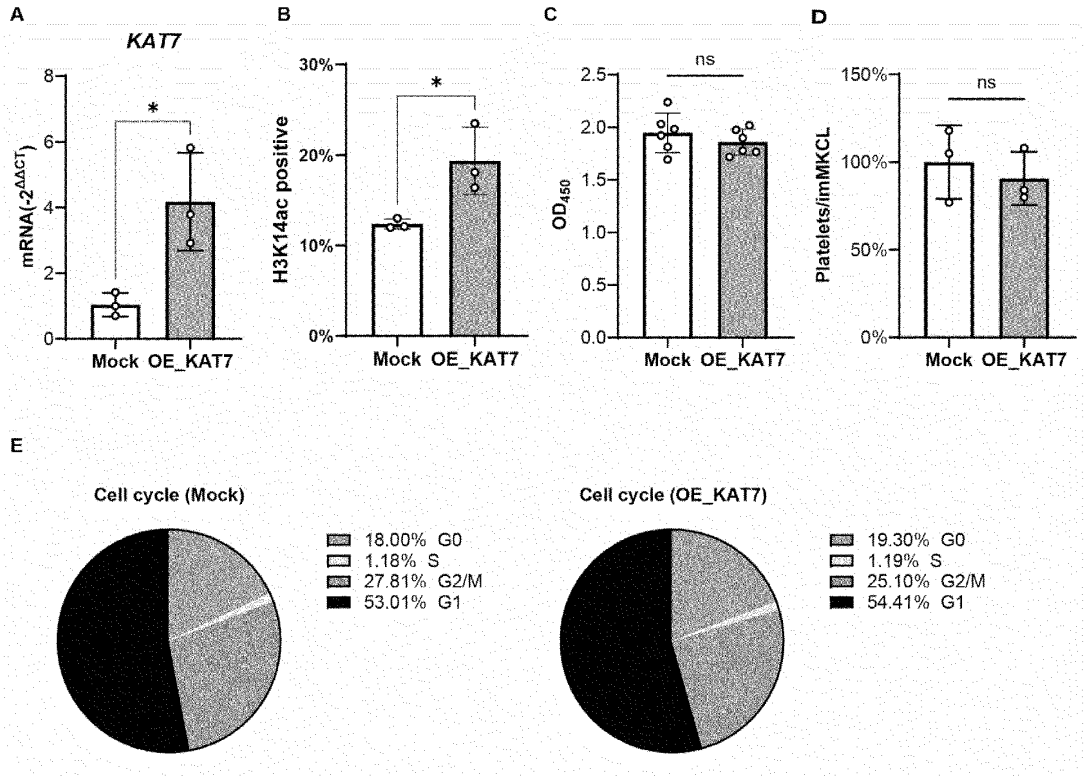
[図22]


A
% inhibition H3K14ac
150%
100%
50%
0%
****
DMSO
0.05
0.5
1
5 uM
WM3835
WM1119
B
Platelets/imMKCL
ns
DMSO D0 D1 D2 D3 D4 D5
5uM WM3835
C
Platelets/imMKCL
**
DMSO
WM3835
D
OD450
**
D3
D6
D9
DMSO
WM3835
E
Percentage of All
80%
60%
40%
20%
0%
****
ns
****
****
G0
S
G2/M
G1
DMSO
WM3835

[図23]


A
KAT7
mRNA(-2ΔΔCT)
1.5
1.0
0.5
0.0
****
shLacZ
shKAT7
B
Platelets/imMKCL
150%
100%
50%
0%
***
shLacZ
shKAT7
C
Percentage of All
60%
40%
20%
0%
****
****
*
G0
G1
G2_M
S
shLacZ
shKAT7

[図24]


A
KAT7
mRNA(-2^ΔΔCT)
*
Mock OE_KAT7
B
H3K14ac positive
*
Mock OE_KAT7
C
OD450
ns
Mock OE_KAT7
D
Platelets/imMKCL
ns
Mock OE_KAT7
E
Cell cycle (Mock)
18.00% G0
1.18% S
27.81% G2/M
53.01% G1
Cell cycle (OE_KAT7)
19.30% G0
1.19% S
25.10% G2/M
54.41% G1