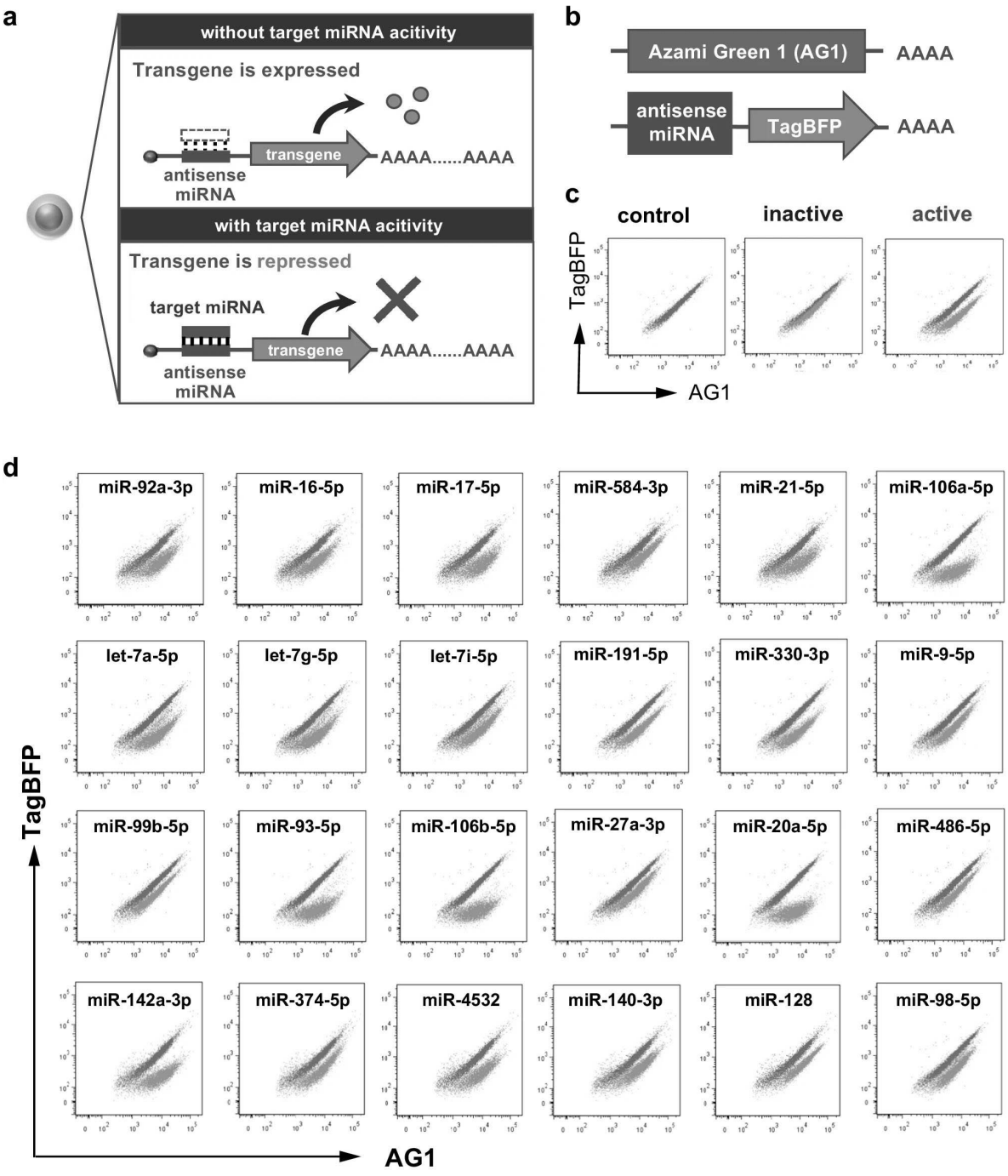
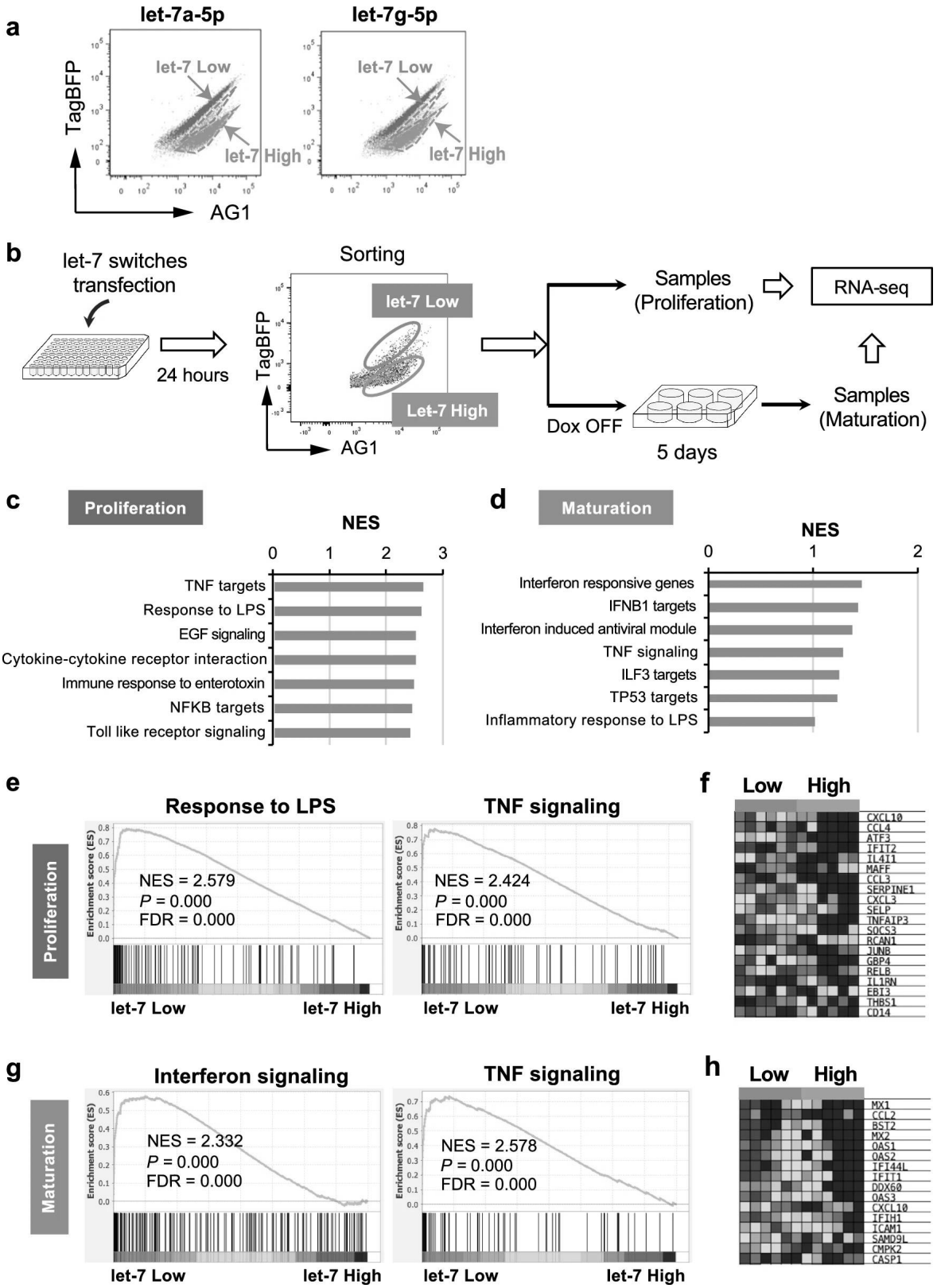
DRAWINGS

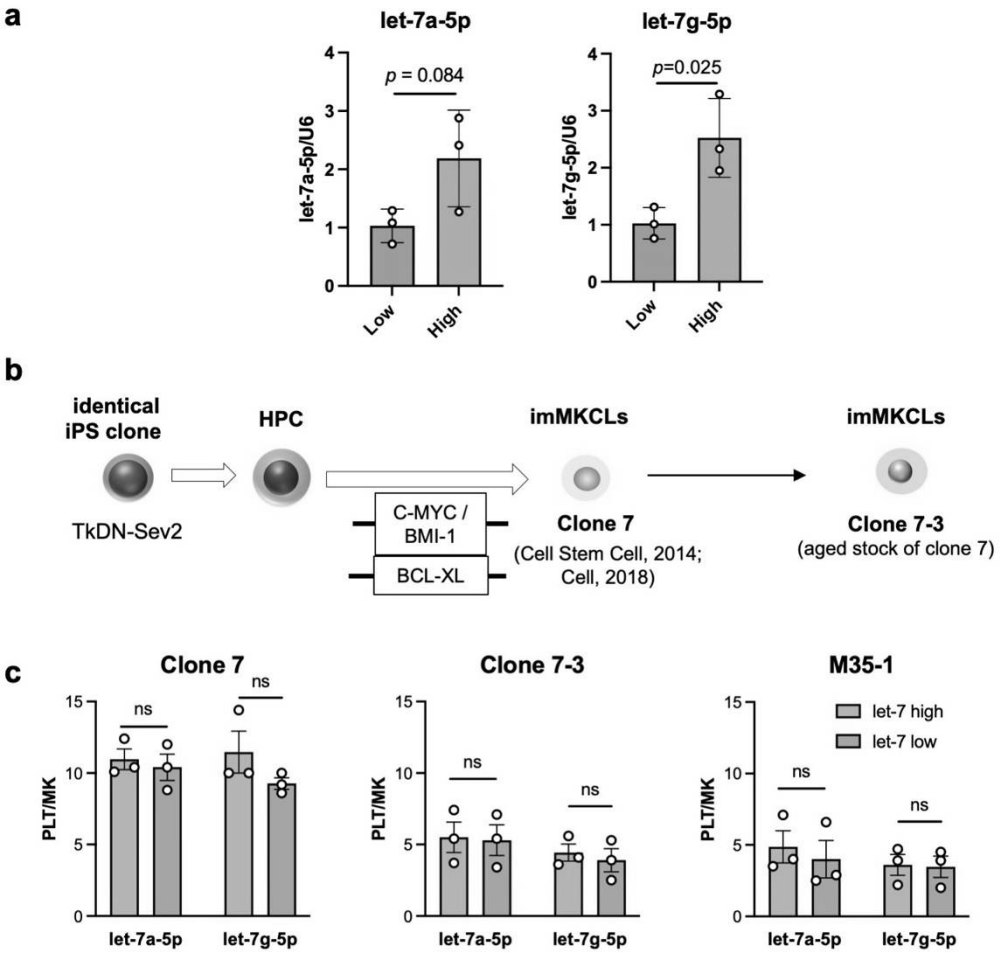
[Fig. 1]



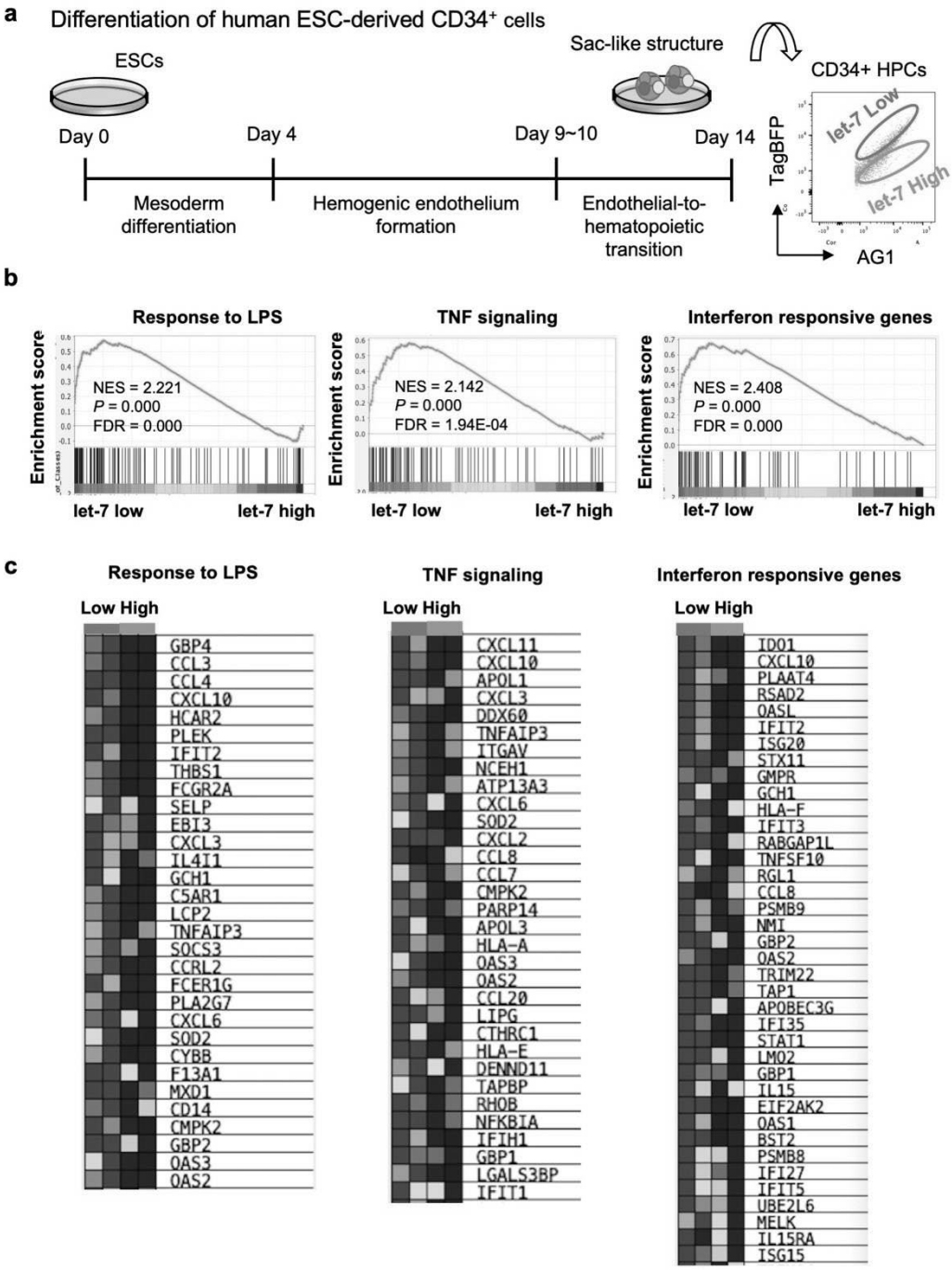
[Fig. 2]


a
let-7a-5p
let-7g-5p
TagBFP
AG1
let-7 Low
let-7 High
b
let-7 switches transfection
24 hours
Sorting
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
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
Enrichment score (ES)
NES = 2.579
P = 0.000
FDR = 0.000
NES = 2.424
P = 0.000
FDR = 0.000
f
Low
High
CXCL10
CCL4
ATF3
IFIT2
IL4I1
MAFF
CCL3
SERPINE1
CXCL3
SELP
TNFAIP3
SOCS3
RCAN1
JUNB
GBP4
RELB
IL1RN
EBI3
THBS1
CD14
g
Interferon signaling
TNF signaling
NES = 2.332
P = 0.000
FDR = 0.000
NES = 2.578
P = 0.000
FDR = 0.000
h
Low
High
MX1
CCL2
BST2
MX2
OAS1
OAS2
IFI44L
IFI1
DDX60
OAS3
CXCL10
IFIH1
ICAM1
SAMD9L
CMPK2
CASP1

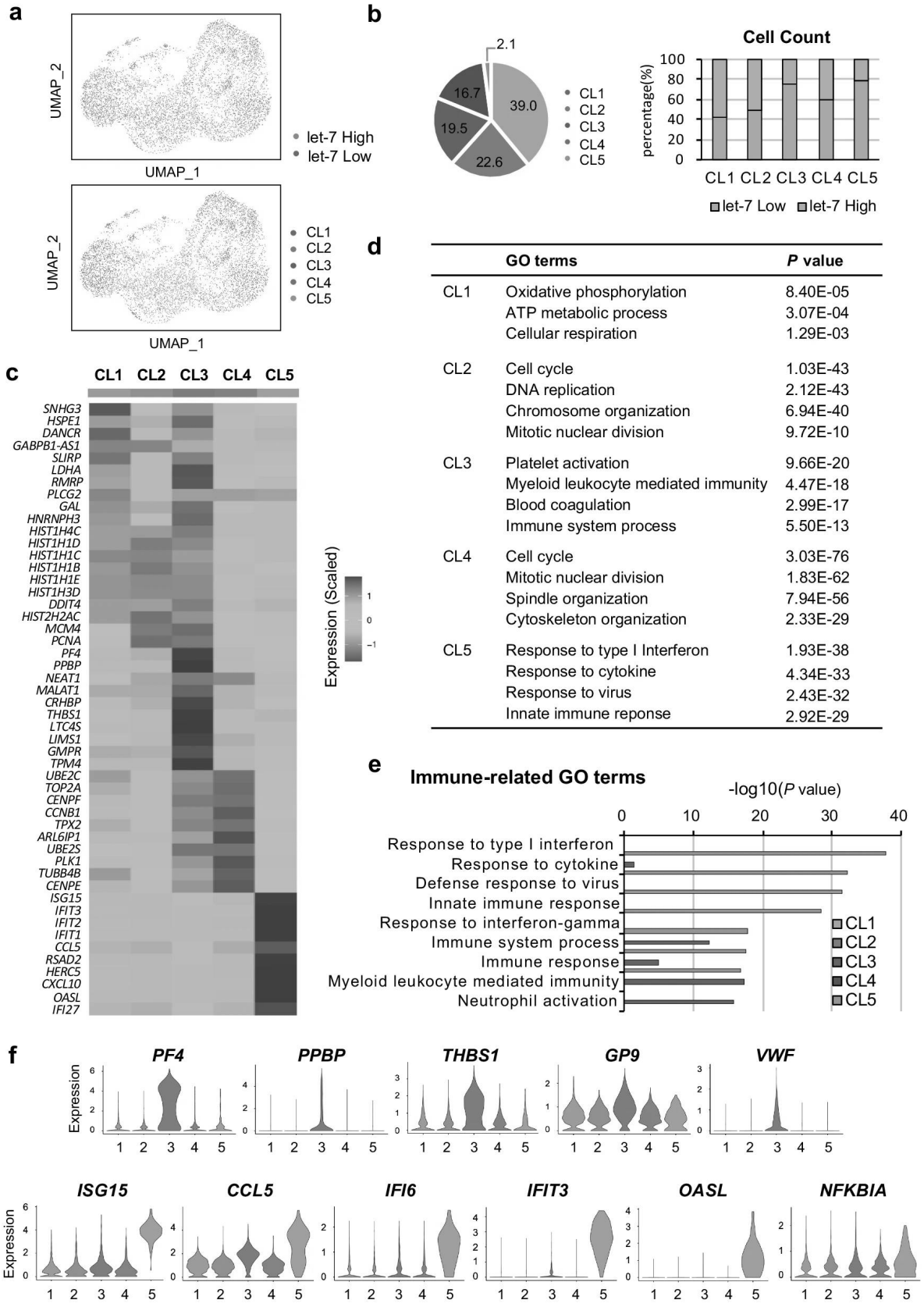
[Fig. 3]


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
PLT/MK
ns
let-7a-5p
let-7g-5p

[Fig. 4]

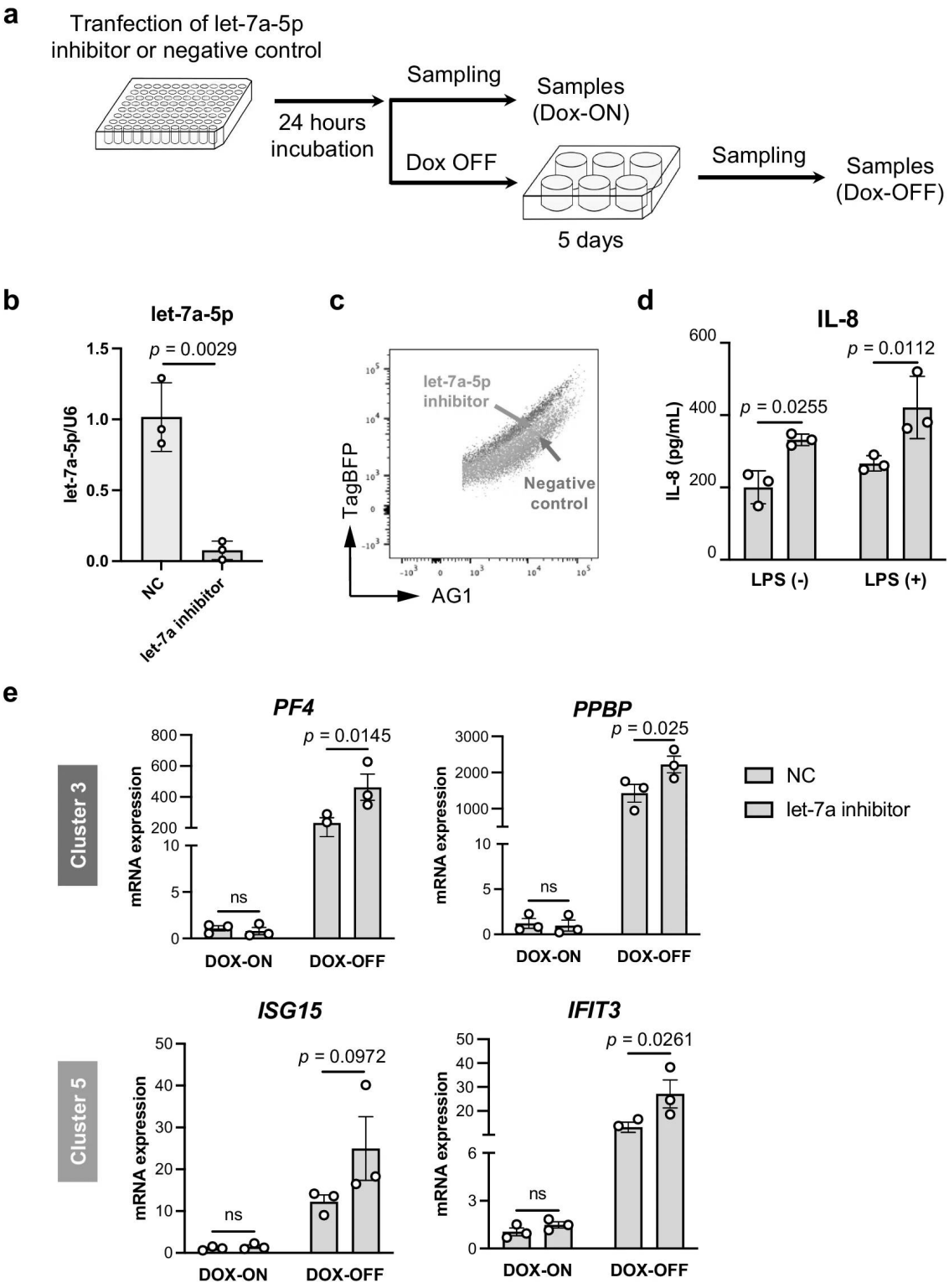

a
Differentiation of human ESC-derived CD34+ cells
ESCs
Day 0
Day 4
Day 9~10
Day 14
Sac-like structure
CD34+ HPCs
let-7 Low
let-7 High
TagBFP
AG1
Mesoderm differentiation
Hemogenic endothelium formation
Endothelial-to-hematopoietic transition
b
Response to LPS
TNF signaling
Interferon responsive genes
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
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
TNF signaling
Low High
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-E
DENND11
TAPBP
RHOB
NFKBIA
IFIH1
GBP1
LGALS3BP
IFIT1
Interferon responsive genes
Low High
IDO1
CXCL10
PLAAT4
RSAD2
OASL
IFIT2
ISG20
STX11
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

[Fig. 5]

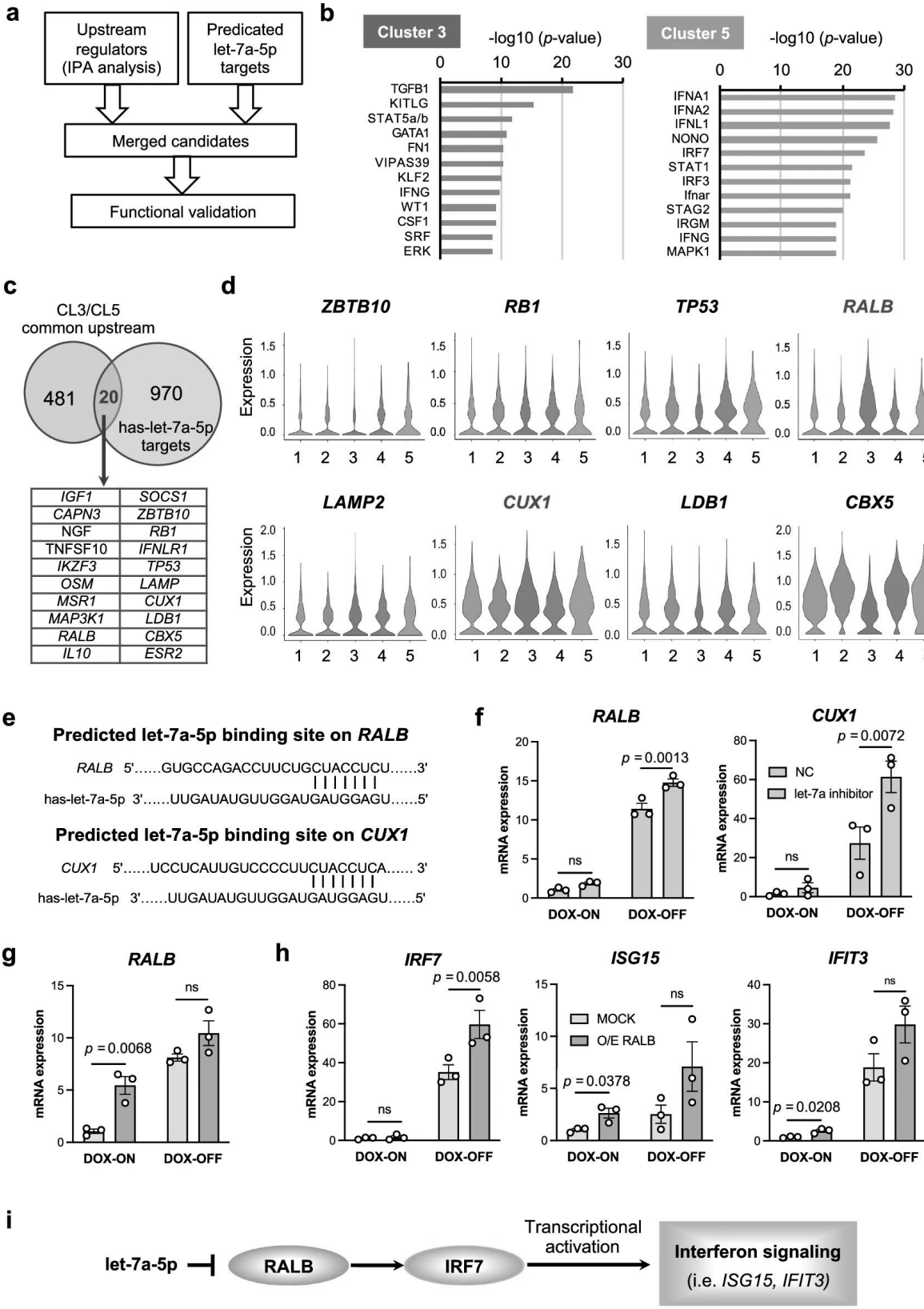


	GO terms	*P* value
CL1	Oxidative phosphorylation	8.40E-05
	ATP metabolic process	3.07E-04
	Cellular respiration	1.29E-03
CL2	Cell cycle	1.03E-43
	DNA replication	2.12E-43
	Chromosome organization	6.94E-40
	Mitotic nuclear division	9.72E-10
CL3	Platelet activation	9.66E-20
	Myeloid leukocyte mediated 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

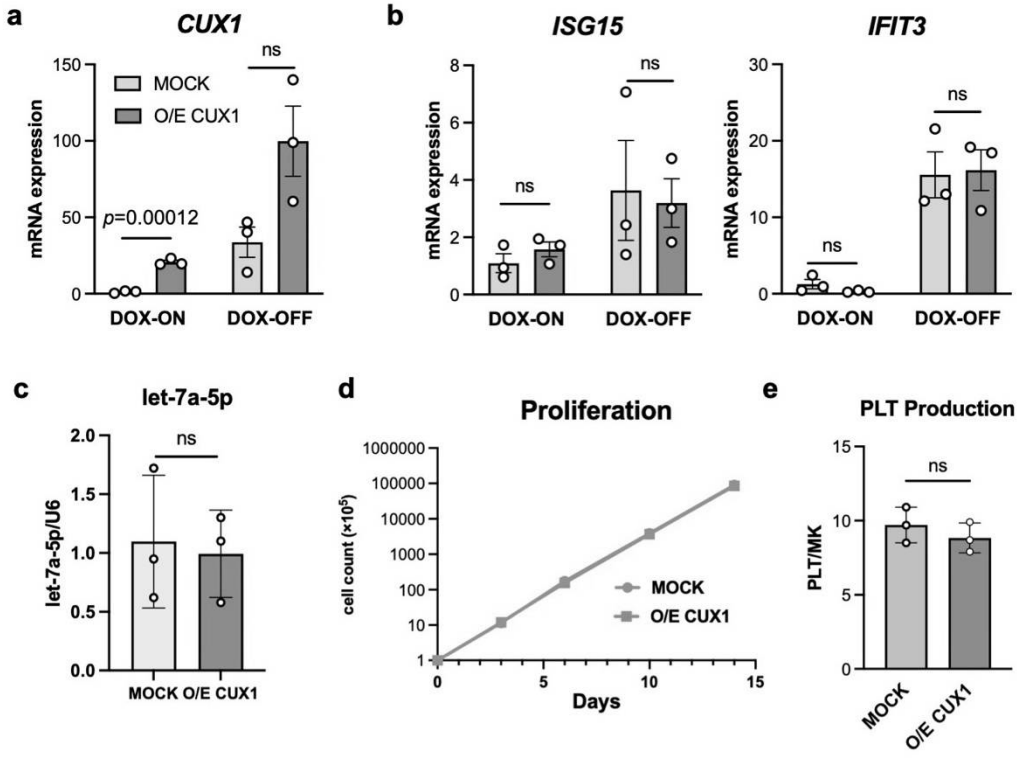
[Fig. 6]


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
ISG15
p = 0.0972
IFIT3
p = 0.0261
mRNA expression
ns
DOX-ON
DOX-OFF
Cluster 3
Cluster 5
NC
let-7a inhibitor

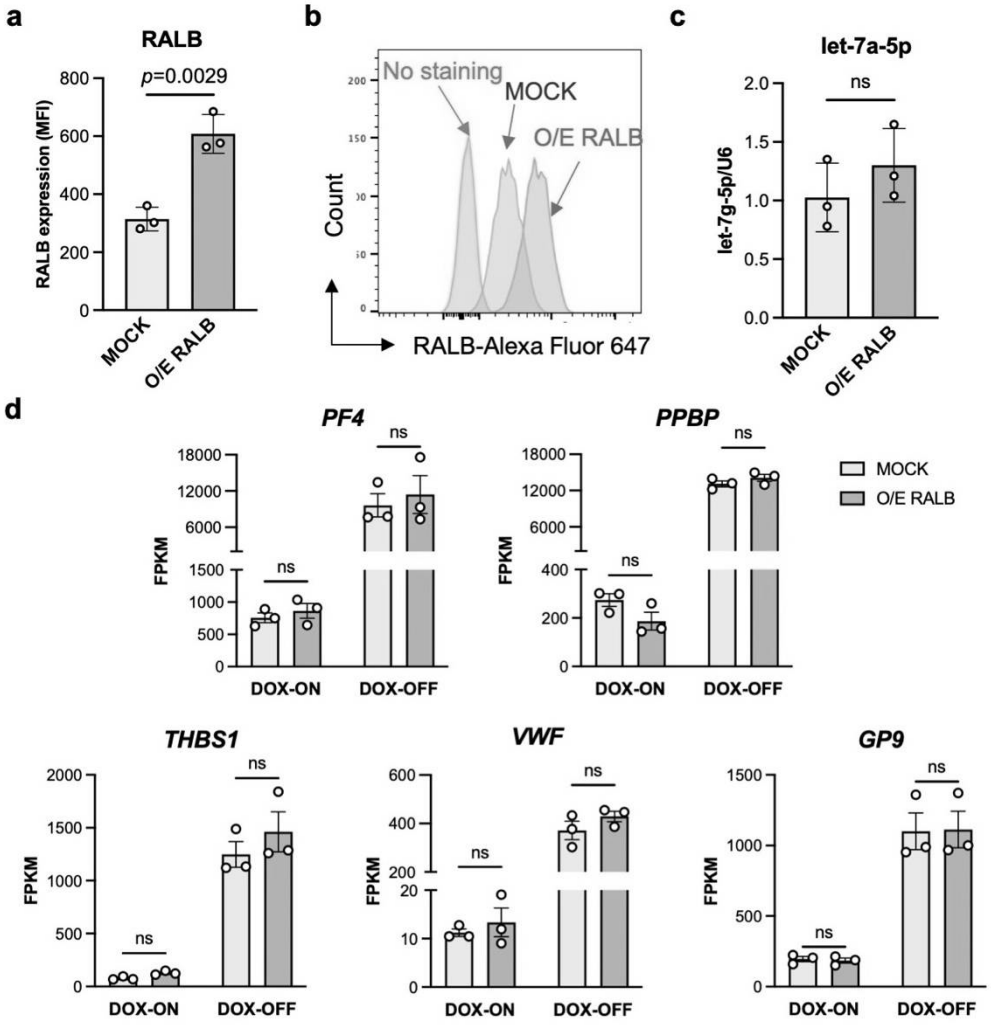
[Fig. 7]


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)

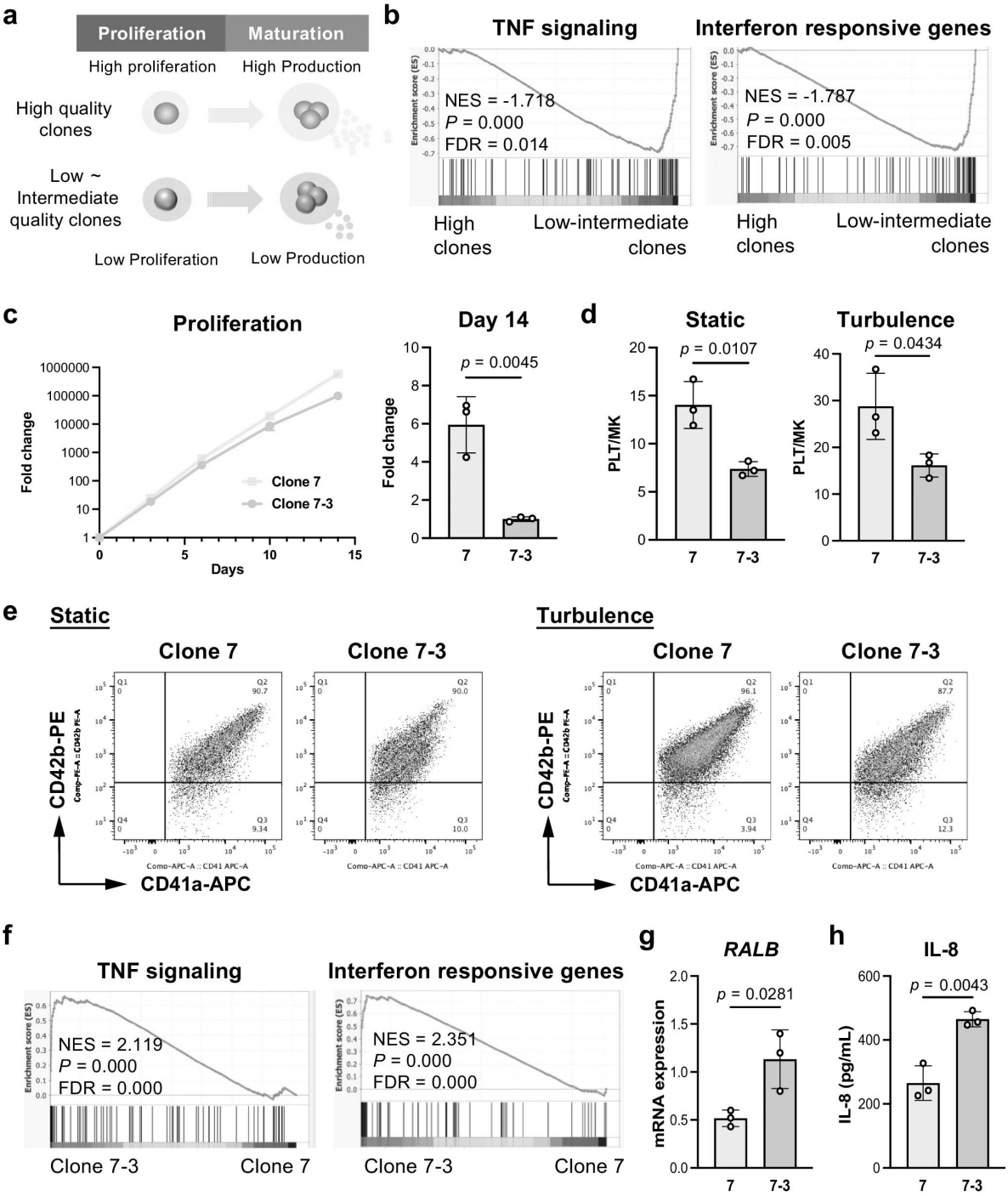
[Fig. 8]


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

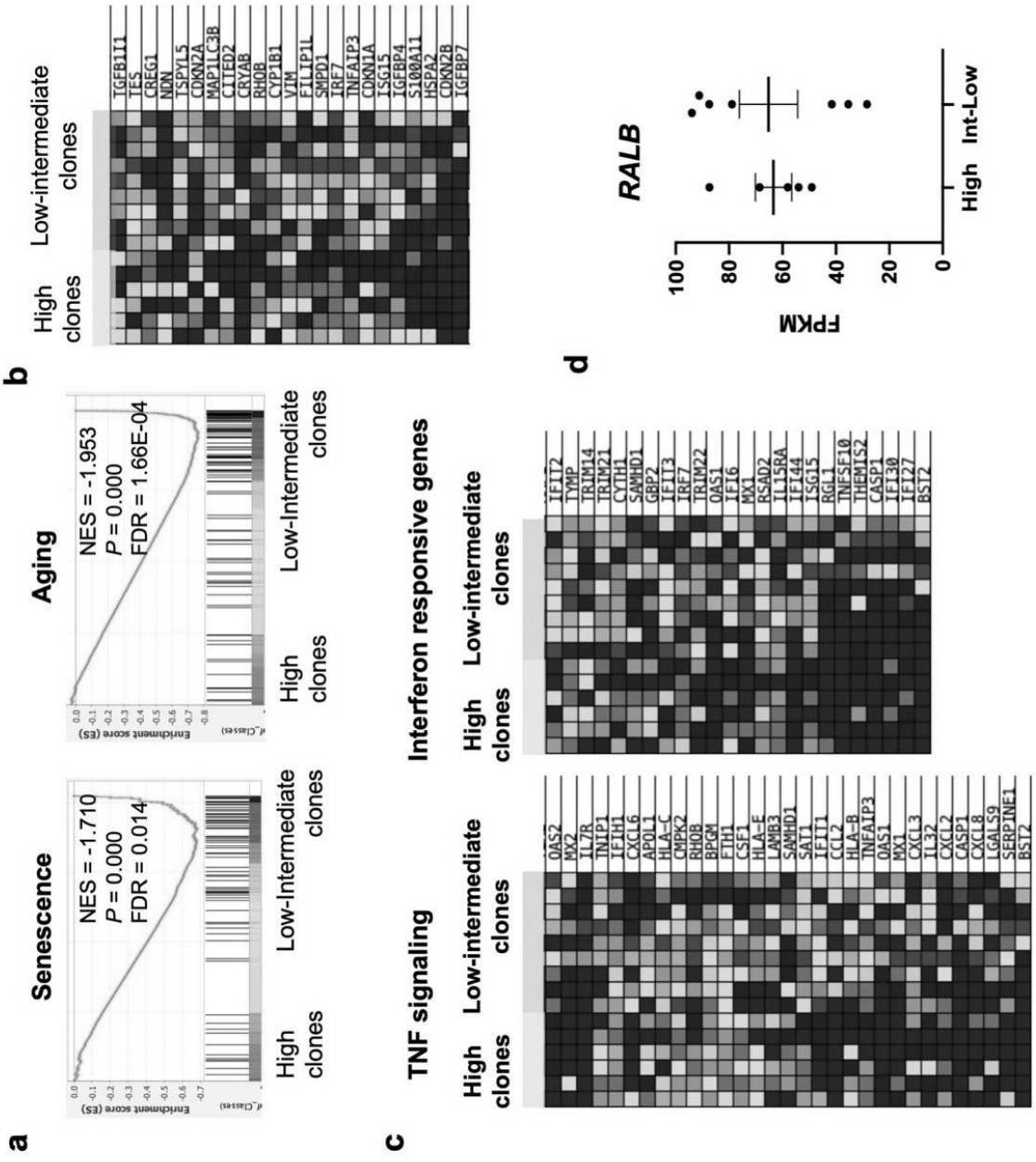
[Fig. 9]


a
RALB
p=0.0029
RALB expression (MFI)
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
MOCK
O/E RALB
d
PF4
PPBP
THBS1
VWF
GP9
FPKM
ns
DOX-ON
DOX-OFF
MOCK
O/E RALB

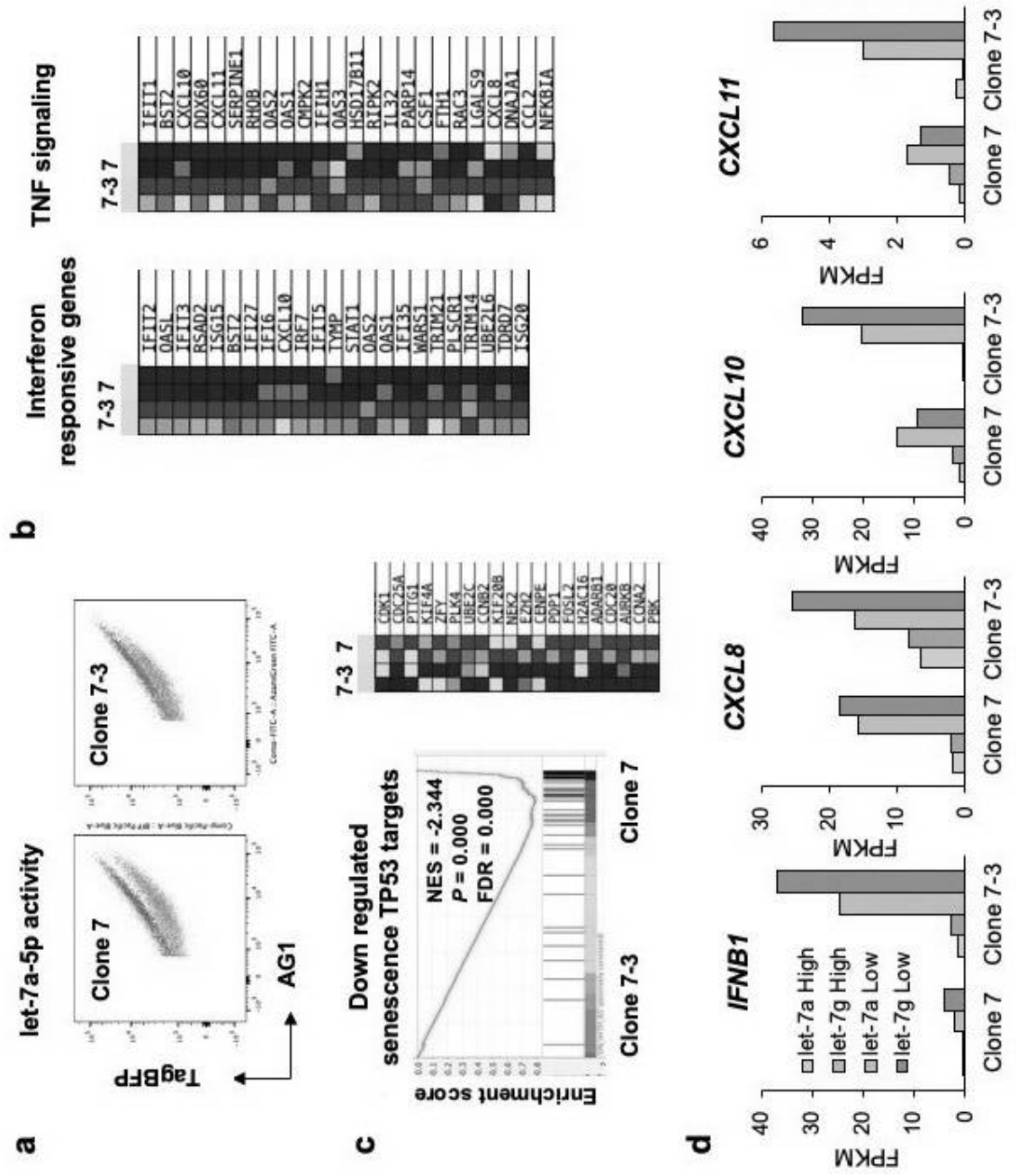
[Fig. 10]


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
Days
Clone 7
Clone 7-3
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
Clone 7
Clone 7-3
CD42b-PE
CD41a-APC
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)

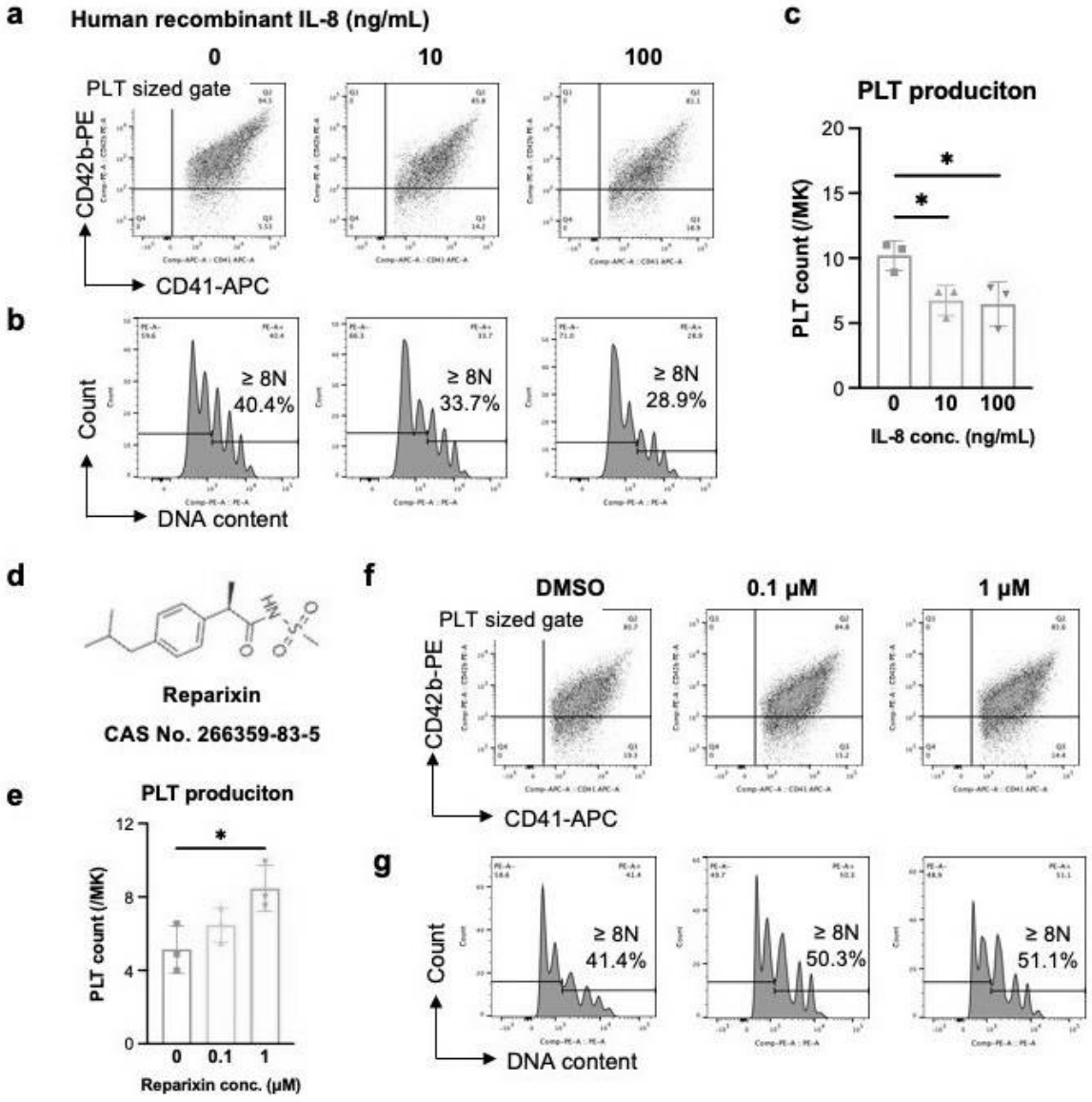
[Fig. 11]


a
Senescence
NES = -1.710
P = 0.000
FDR = 0.014
Enrichment score (ES)
High clones
Low-Intermediate clones
Aging
NES = -1.953
P = 0.000
FDR = 1.66E-04
Enrichment score (ES)
High clones
Low-Intermediate clones
b
High clones
Low-intermediate clones
TGFB1I1
TES
CREG1
NDN
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
OAS2
MX2
IL7R
TNIP1
IFIH1
CXCL6
APOL1
HLA-C
CMPK2
RHOB
BPGM
FTH1
CSF1
HLA-E
LAMB3
SAMHD1
SAT1
IFIT1
CCL2
HLA-B
TNFAIP3
OAS1
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
IFIT27
BST2
d
RALB
FPKM
100
80
60
40
20
0
High
Int-Low

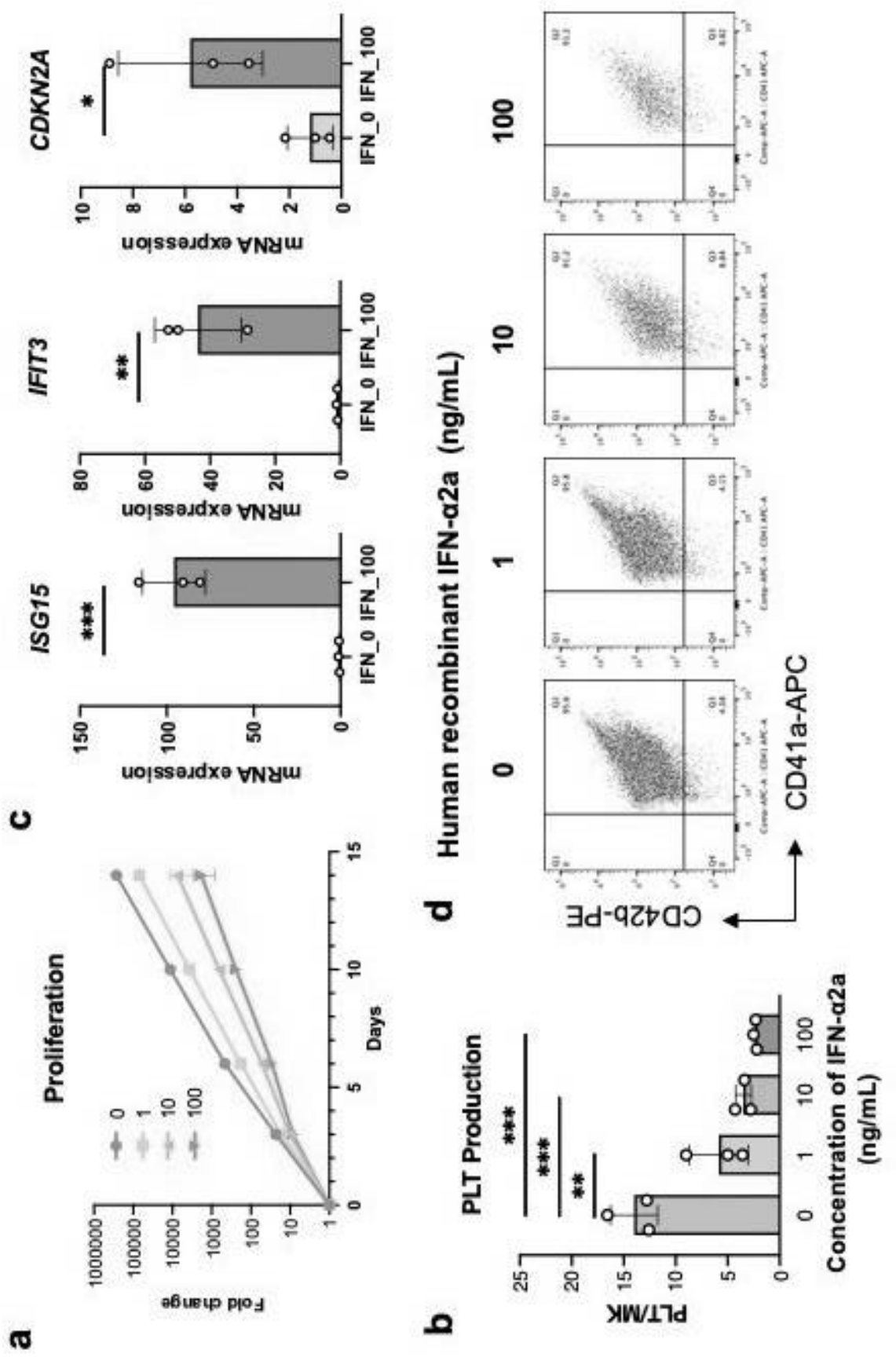
[Fig. 12]


a
let-7a-5p activity
Clone 7
Clone 7-3
TagBFP
AG1
b
Interferon responsive genes
7-3 7
IFIT2
OASL
IFIT3
RSAD2
ISG15
BST2
IFI27
IFI6
CXCL10
IRF7
IFIT5
TYMP
STAT1
OAS2
OAS1
IFI35
WARS1
TRIM21
PLSCR1
TRIM14
UBE2L6
TDRD7
ISG20
TNF signaling
7-3 7
IFIT1
BST2
CXCL10
DDX60
CXCL11
SERPINE1
RHOB
OAS2
OAS1
CMPK2
IFIH1
OAS3
HSD17B11
RIPK2
IL32
PARP14
CSF1
FTH1
RAC3
LGALS9
CXCL8
DNAJA1
CCL2
NFKBIA
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
CDK1
CDC25A
PTTG1
KIF4A
ZFY
PLK4
UBE2C
CCNB2
KIF20B
NEK2
EZH2
CENPE
PDP1
FOSL2
H2AC16
AOAH81
CDC20
AURKB
CCNA2
PBK
d
IFNB1
CXCL8
CXCL10
CXCL11
FPKM
let-7a High
let-7g High
let-7a Low
let-7g Low
Clone 7
Clone 7-3

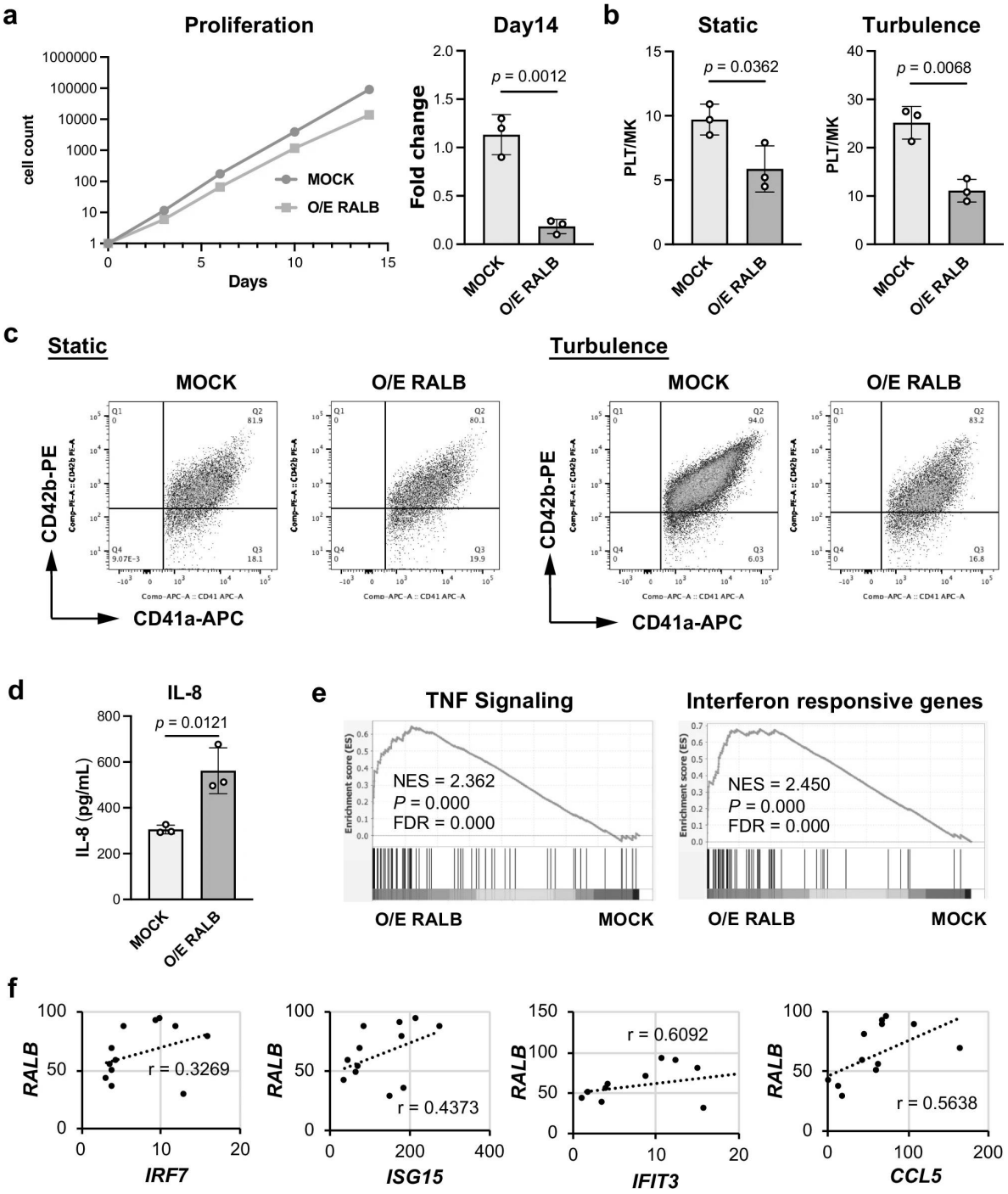
[Fig. 13]


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

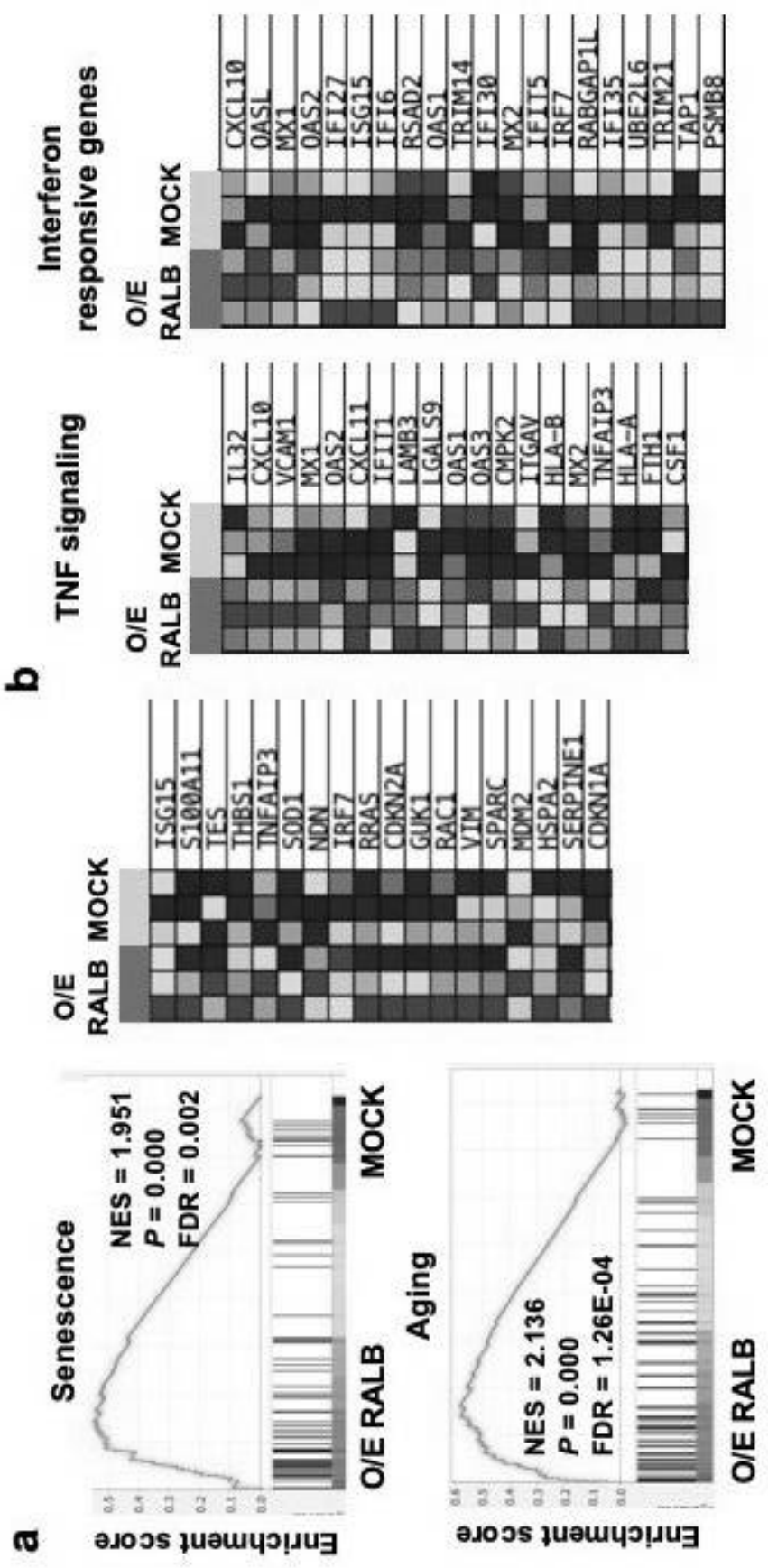
[Fig. 14]


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

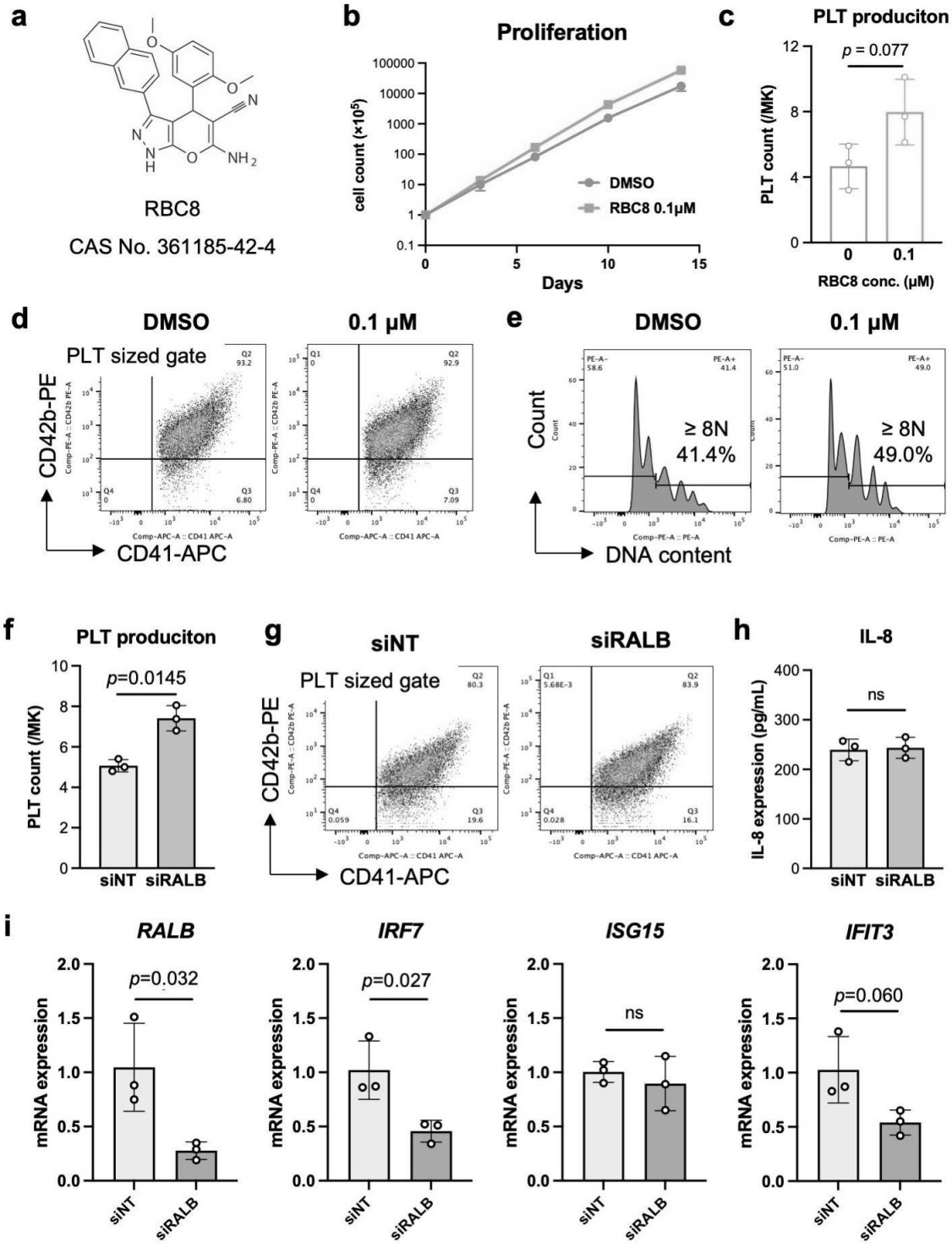
[Fig. 15]


a
Proliferation
cell count
Days
MOCK
O/E RALB
Day14
Fold change
p = 0.0012
b
Static
PLT/MK
p = 0.0362
Turbulence
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
NES = 2.362
P = 0.000
FDR = 0.000
Interferon responsive genes
NES = 2.450
P = 0.000
FDR = 0.000
Enrichment score (ES)
f
RALB
IRF7
r = 0.3269
ISG15
r = 0.4373
IFIT3
r = 0.6092
CCL5
r = 0.5638

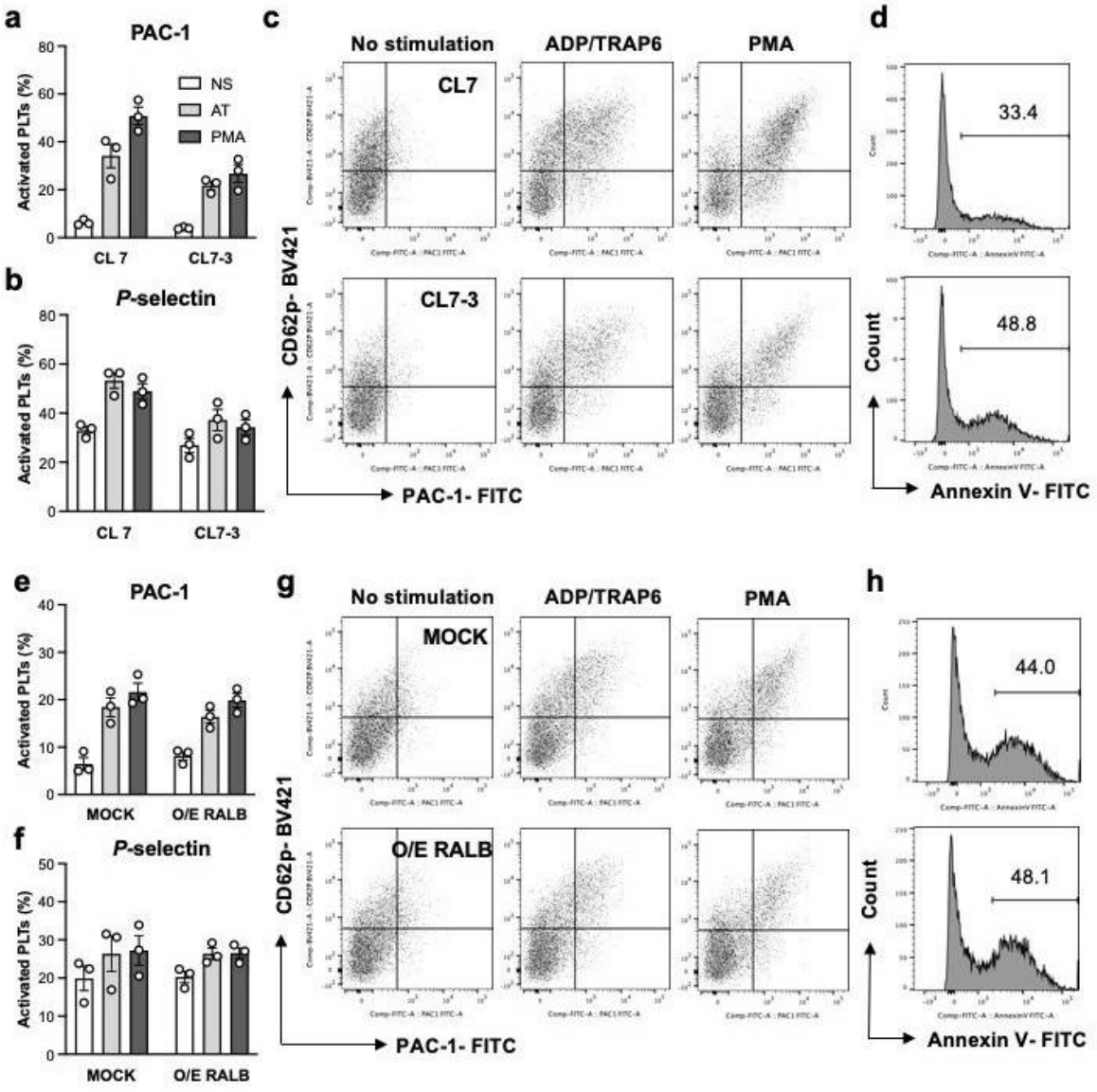
[Fig. 16]


a
Senescence
NES = 1.951
P = 0.000
FDR = 0.002
Enrichment score
O/E RALB
MOCK
Aging
NES = 2.136
P = 0.000
FDR = 1.26E-04
Enrichment score
O/E RALB
MOCK
b
O/E RALB MOCK
ISG15
S100A11
TES
THBS1
TNFAIP3
SOD1
NDN
IRF7
RRAS
CDKN2A
GUK1
RAC1
VIM
SPARC
MDM2
HSPA2
SERPINE1
CDKN1A
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
FTH1
CSF1
Interferon responsive genes
O/E RALB MOCK
CXCL10
OASL
MX1
OAS2
IFI27
ISG15
IFI6
RSAD2
OAS1
TRIM14
IFI30
MX2
IFIT5
IRF7
RABGAP1L
IFI35
UBE2L6
TRIM21
TAP1
PSMB8

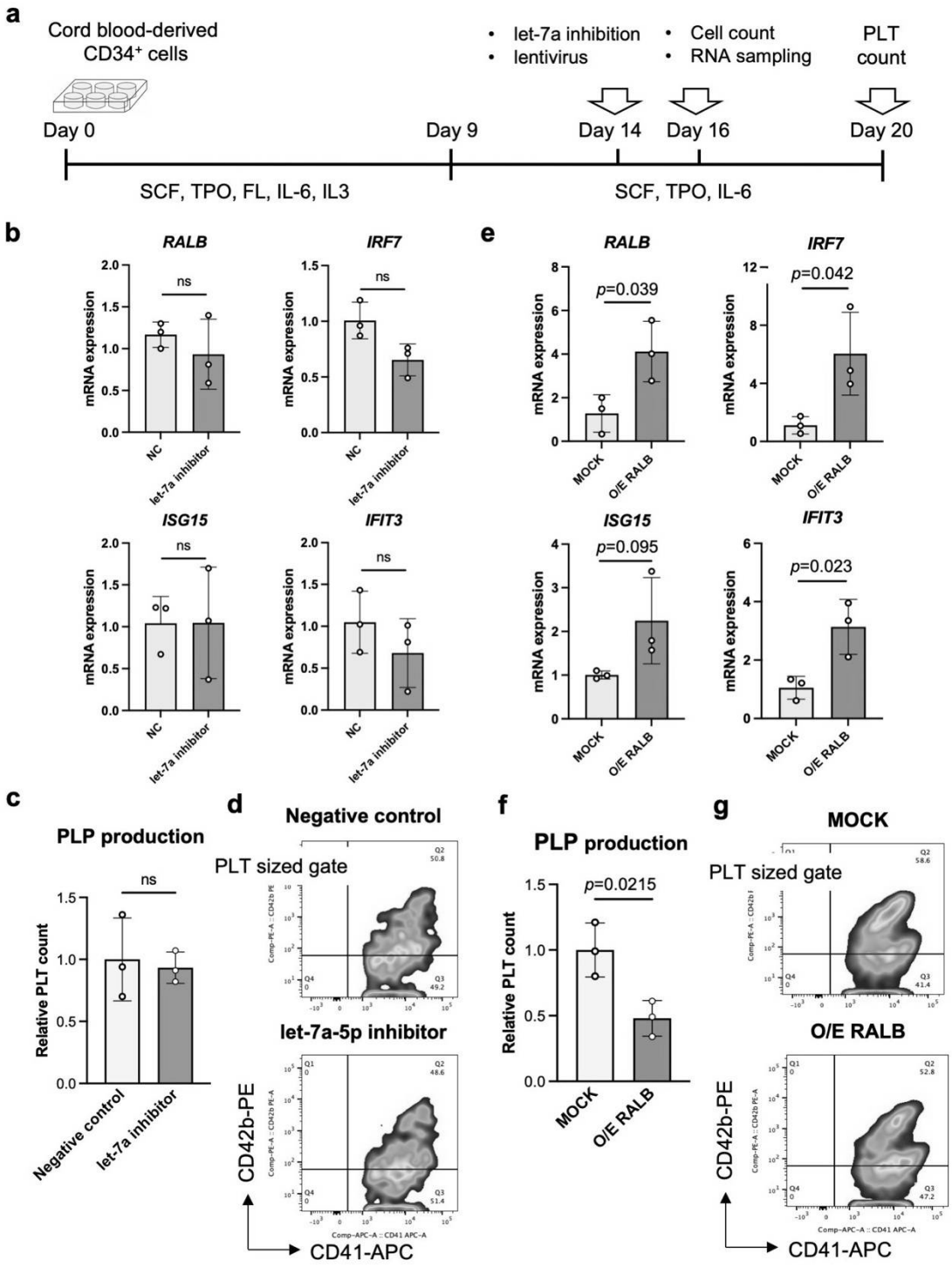
[Fig. 17]


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

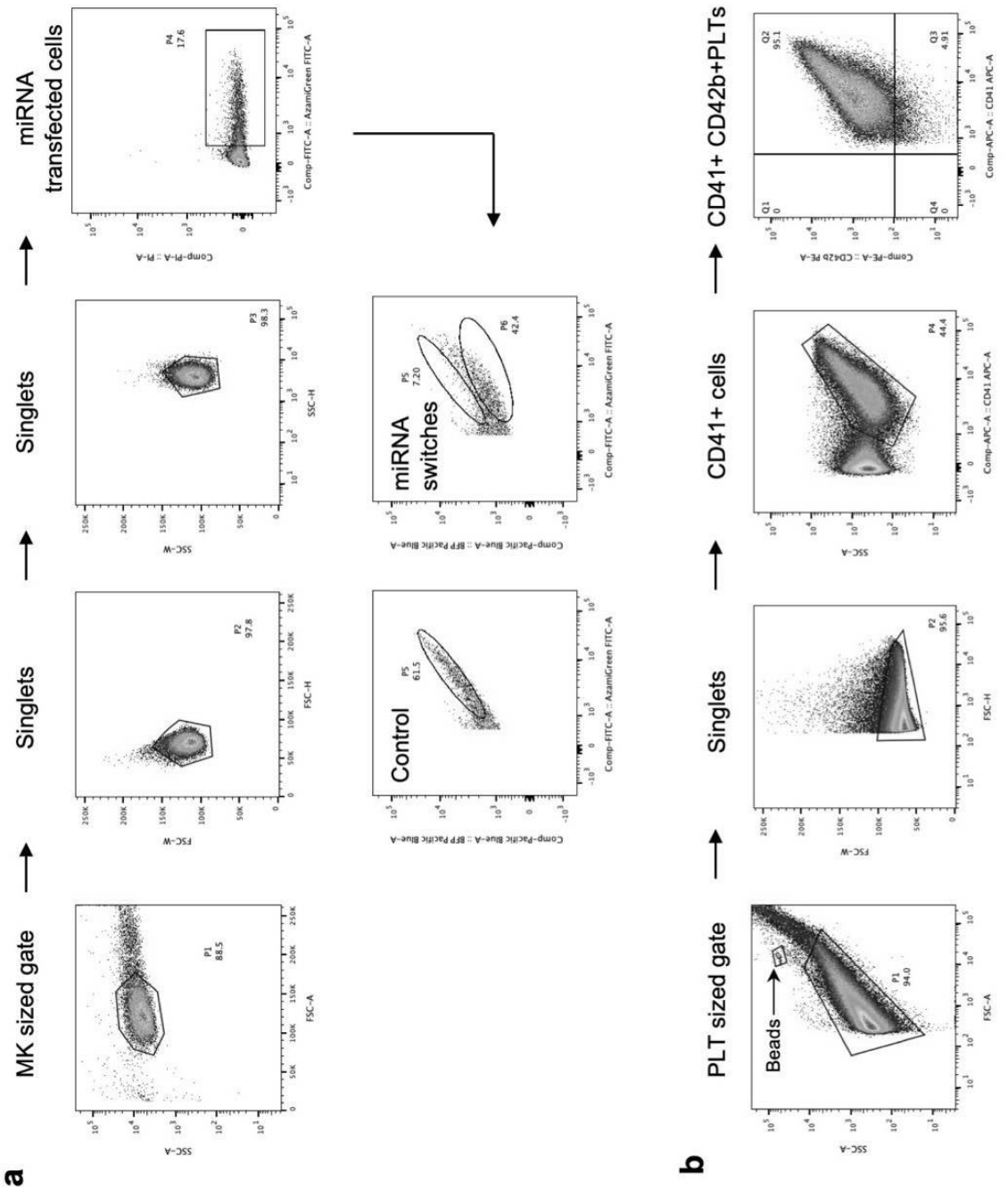
[Fig. 18]


a
PAC-1
Activated PLTs (%)
NS
AT
PMA
CL 7
CL7-3
b
P-selectin
Activated PLTs (%)
CL 7
CL7-3
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
Activated PLTs (%)
MOCK
O/E RALB
f
P-selectin
Activated PLTs (%)
MOCK
O/E RALB
g
No stimulation
ADP/TRAP6
PMA
MOCK
O/E RALB
CD62p- BV421
PAC-1- FITC
h
44.0
48.1
Count
Annexin V- FITC

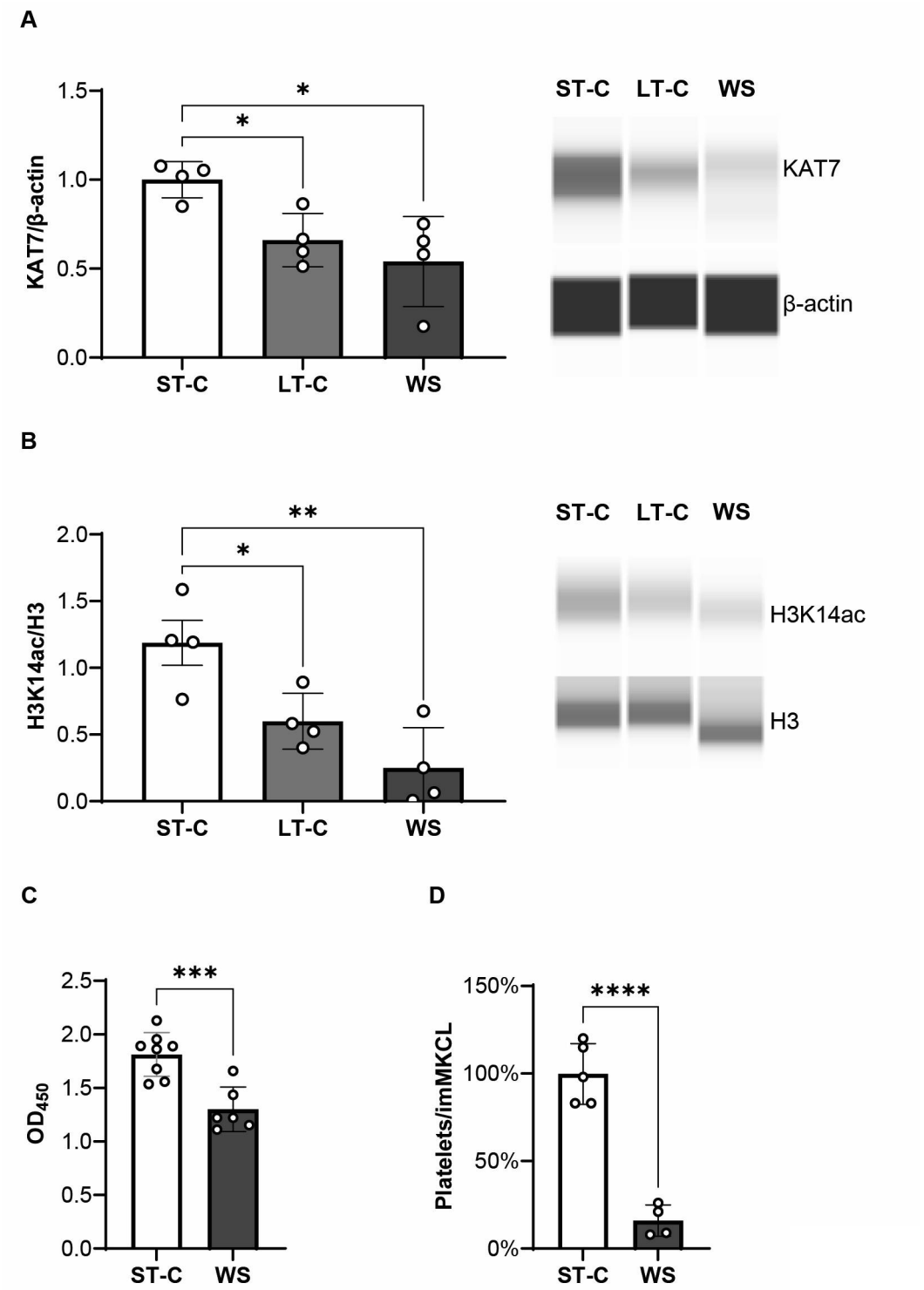
[Fig. 19]


a
Cord blood-derived CD34+ cells
Day 0
Day 9
Day 14
Day 16
Day 20
let-7a inhibition
lentivirus
Cell count
RNA sampling
PLT count
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
Relative PLT count
MOCK
O/E RALB
g
MOCK
PLT sized gate
O/E RALB
CD42b-PE
CD41-APC

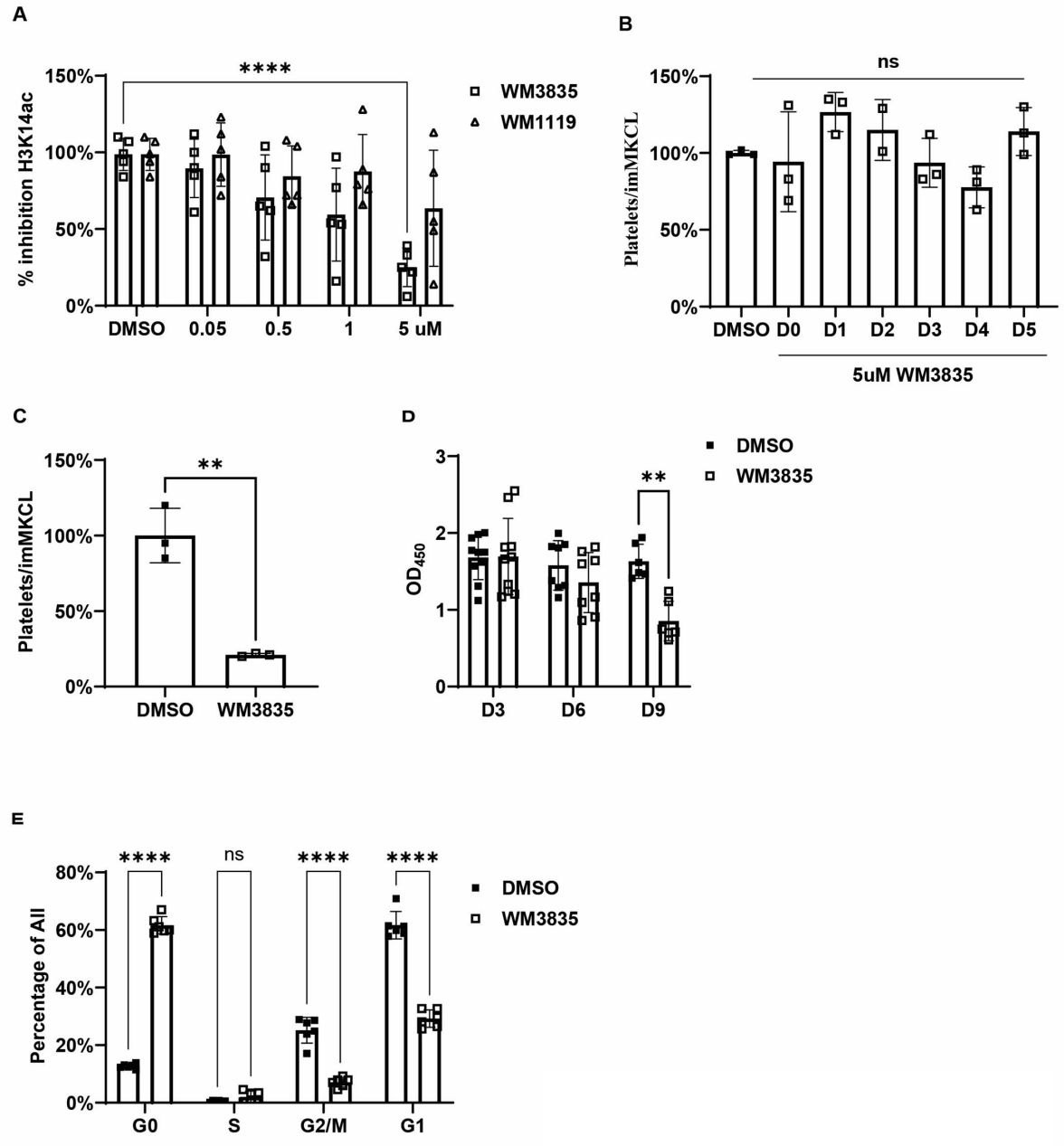
[Fig. 20]


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

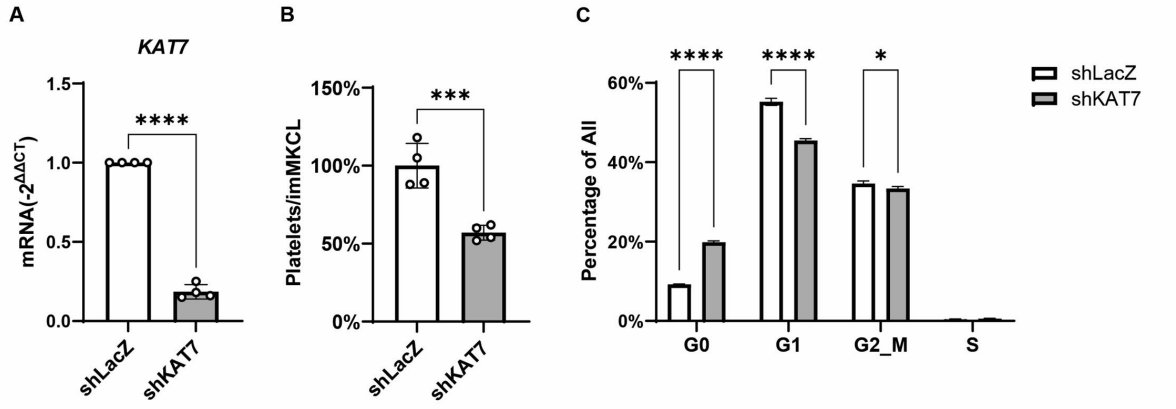
[Fig. 21]


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

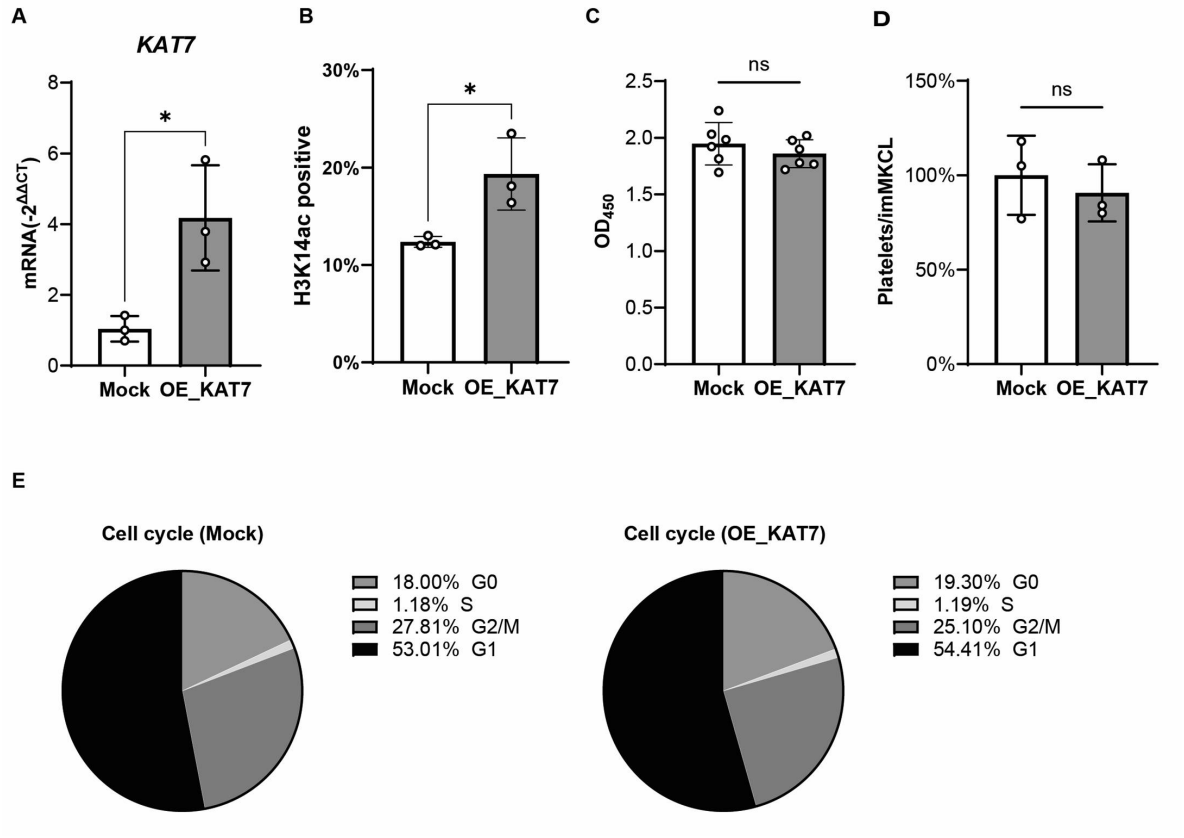
[Fig. 22]


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

[Fig. 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

[Fig. 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