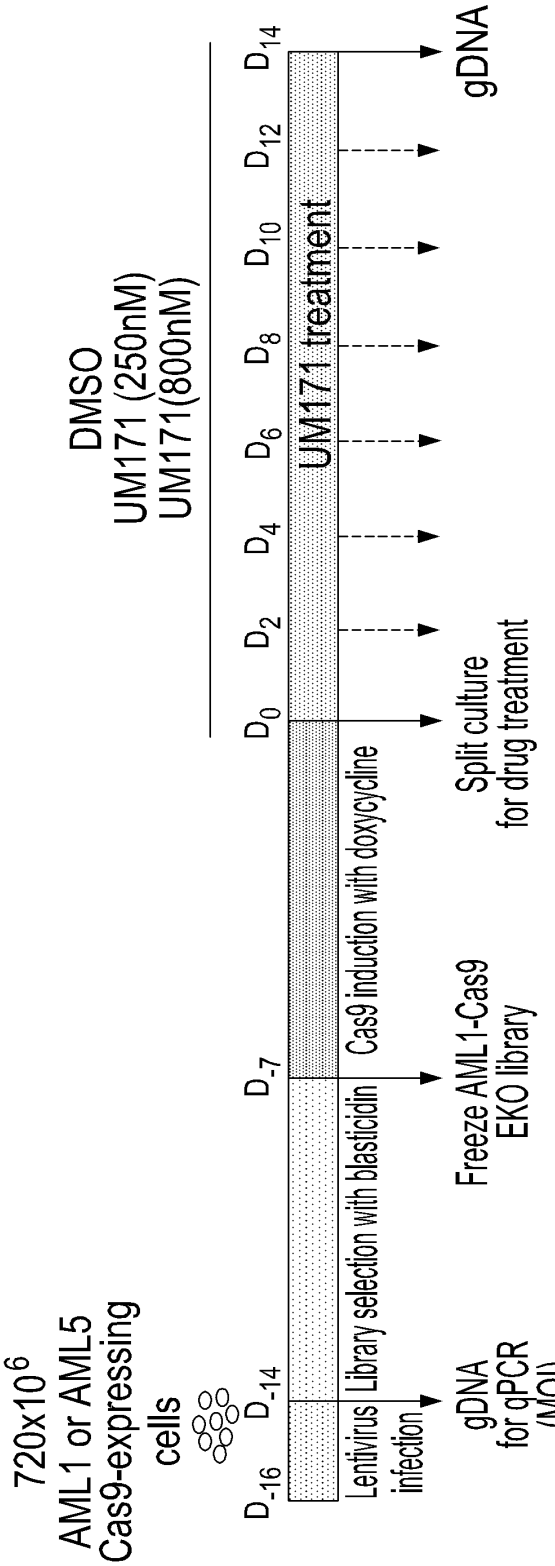
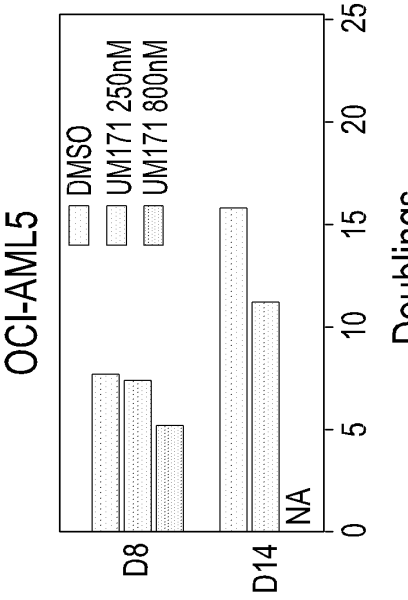

720x10^6
AML1 or AML5
Cas9-expressing
cells
D-16
D-14
D-7
D0
D2
D4
D6
D8
D10
D12
D14
Lentivirus infection
Library selection with blasticidin
Cas9 induction with doxycycline
UM171 treatment
gDNA
for qPCR
(MOI)
Freeze AML1-Cas9
EKO library
Split culture
for drug treatment
DMSO
UM171 (250nM)
UM171(800nM)
gDNA

Fig. 1A


OCI-AML5
DMSO
UM171 250nM
UM171 800nM
D8
D14
NA
0
5
10
15
20
25
Doublings

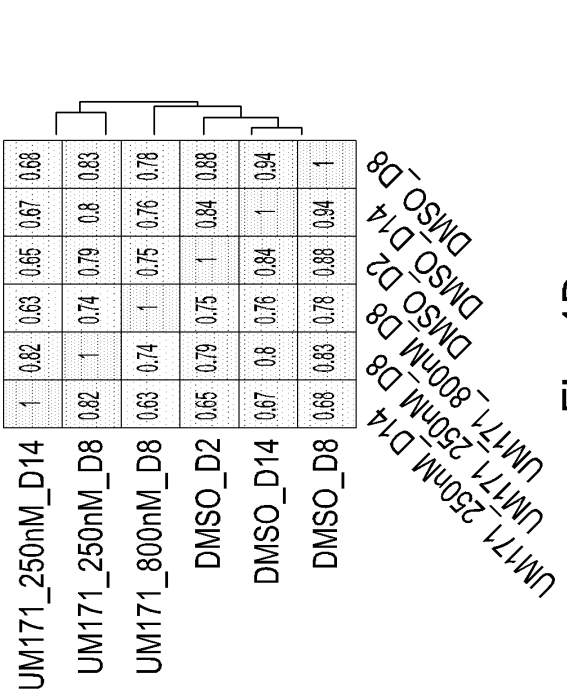
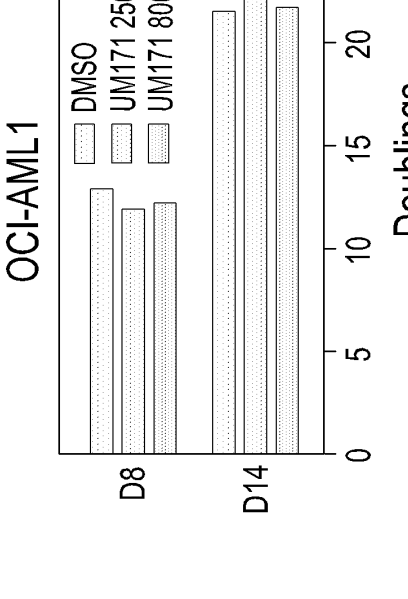

OCI-AML5
UM171_250nM_D14
UM171_250nM_D8
UM171_800nM_D8
DMSO_D2
DMSO_D14
DMSO_D8
1 0.82 0.63 0.65 0.67 0.68
0.82 1 0.74 0.79 0.8 0.83
0.63 0.74 1 0.75 0.76 0.78
0.65 0.79 0.75 1 0.84 0.88
0.67 0.8 0.76 0.84 1 0.94
0.68 0.83 0.78 0.88 0.94 1
UM171_250nM_D14
UM171_250nM_D8
UM171_800nM_D8
DMSO_D2
DMSO_D14
DMSO_D8

Fig. 1B


OCI-AML1
DMSO
UM171 250nM
UM171 800nM
D8
D14
0
5
10
15
20
25
Doublings

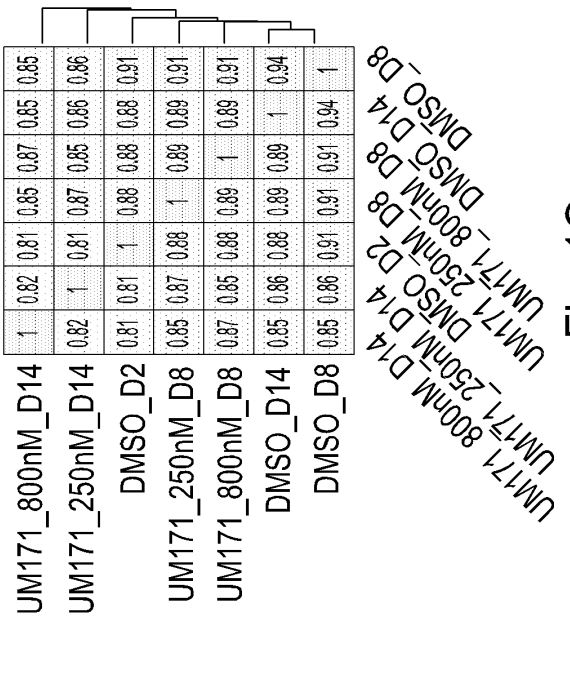

OCI-AML1
UM171_800nM_D14
UM171_250nM_D14
DMSO_D2
UM171_250nM_D8
UM171_800nM_D8
DMSO_D14
DMSO_D8
1 0.82 0.81 0.85 0.87 0.85
0.82 1 0.81 0.87 0.85 0.86
0.81 0.81 1 0.88 0.88 0.91
0.85 0.87 0.88 1 0.89 0.91
0.87 0.85 0.88 0.89 1 0.91
0.85 0.86 0.89 0.89 1 0.94
0.85 0.86 0.91 0.91 0.94 1
UM171_800nM_D14
UM171_250nM_D14
UM171_250nM_D8
UM171_800nM_D8
DMSO_D2
DMSO_D14
DMSO_D8

Fig. 1C

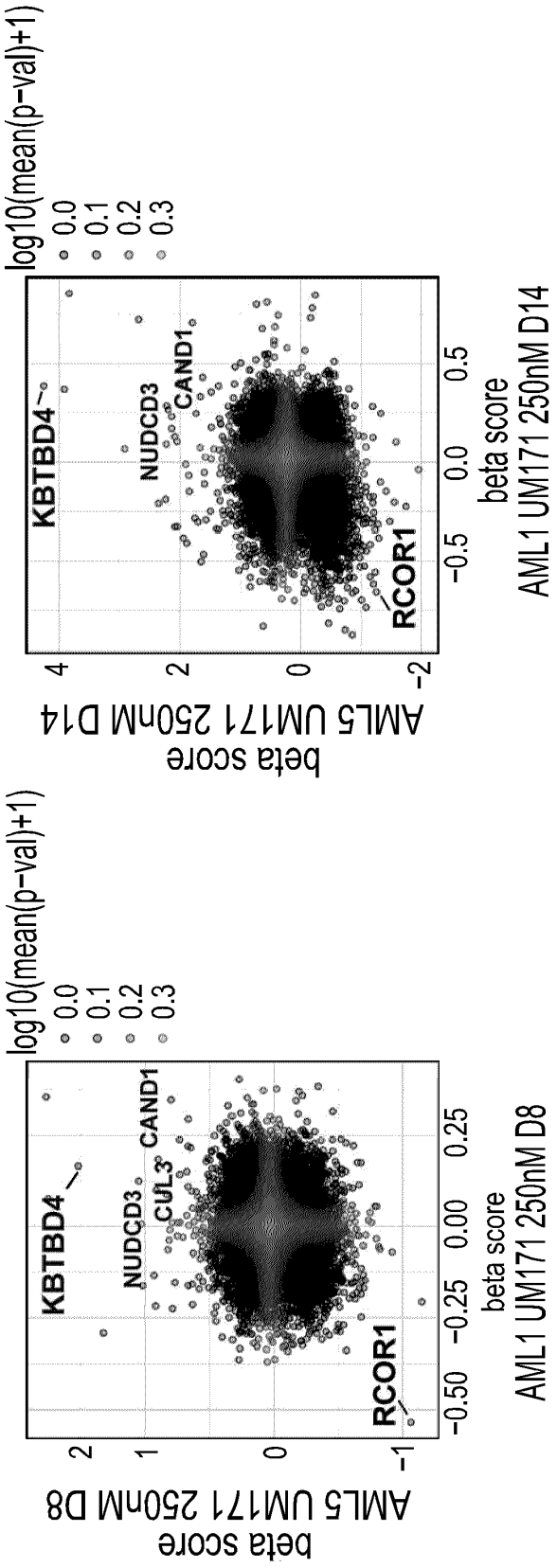

log10(mean(p−val)+1)
0.0
0.1
0.2
0.3
KBTBD4
NUDCD3
CAND1
CUL3
RCOR1
beta score
AML1 UM171 250nM D8
AML5 UM171 250nM D8
beta score
AML1 UM171 250nM D14
AML5 UM171 250nM D14

Fig. 1D

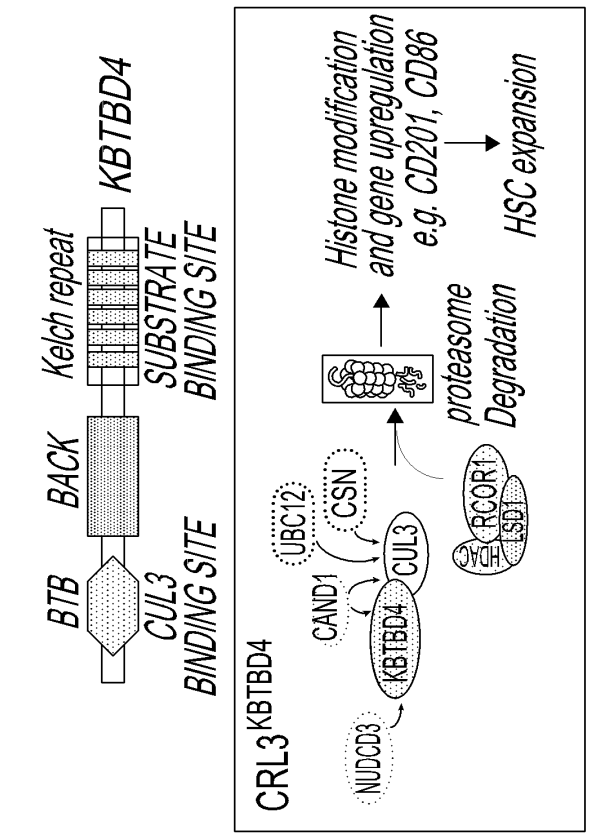

KBTBD4
Kelch repeat
BACK
BTB
SUBSTRATE
BINDING SITE
CUL3
BINDING SITE
CRL3KBTBD4
UBC12
CSN
CAND1
CUL3
KBTBD4
NUDCD3
RBX1
RCOR1
LSD1
proteasome
Degradation
Histone modification
and gene upregulation
e.g. CD201, CD86
HSC expansion

Fig. 2B

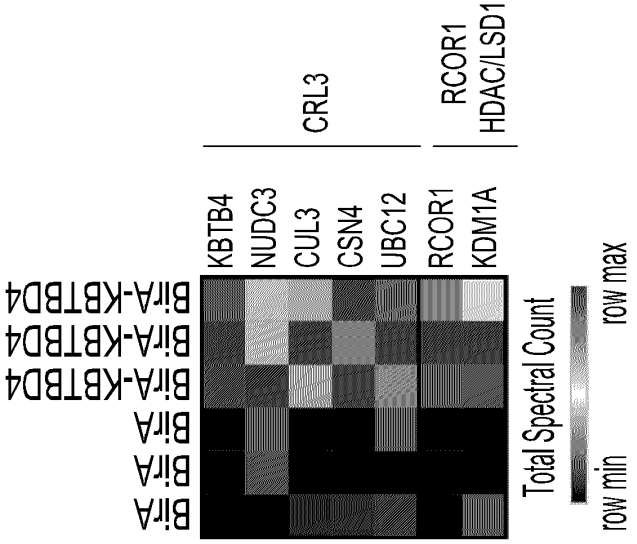

BirA
BirA
BirA
BirA-KBTBD4
BirA-KBTBD4
BirA-KBTBD4
KBTB4
NUDC3
CUL3
CSN4
UBC12
RCOR1
KDM1A
CRL3
RCOR1
HDAC/LSD1
Total Spectral Count
row min
row max

Fig. 2A

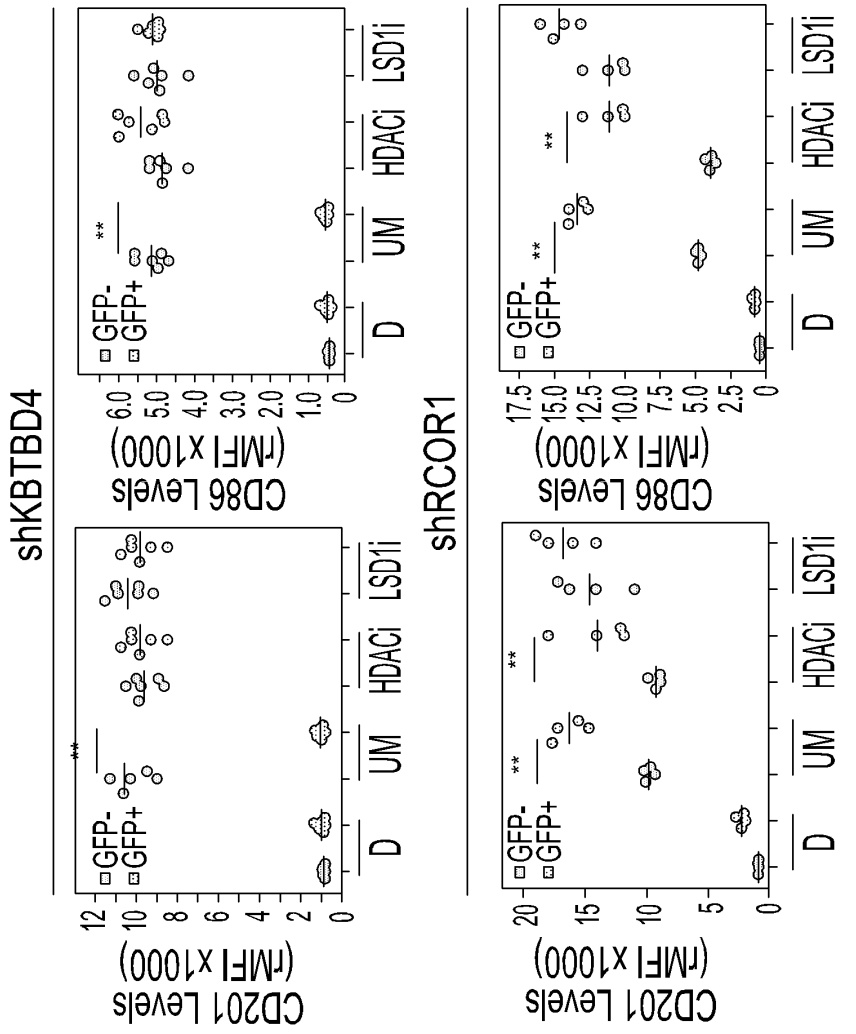

shKBTBD4
shRCOR1
CD201 Levels (rMFI x1000)
CD86 Levels (rMFI x1000)
GFP-
GFP+
D
UM
HDACi
LSD1i

Fig. 2D

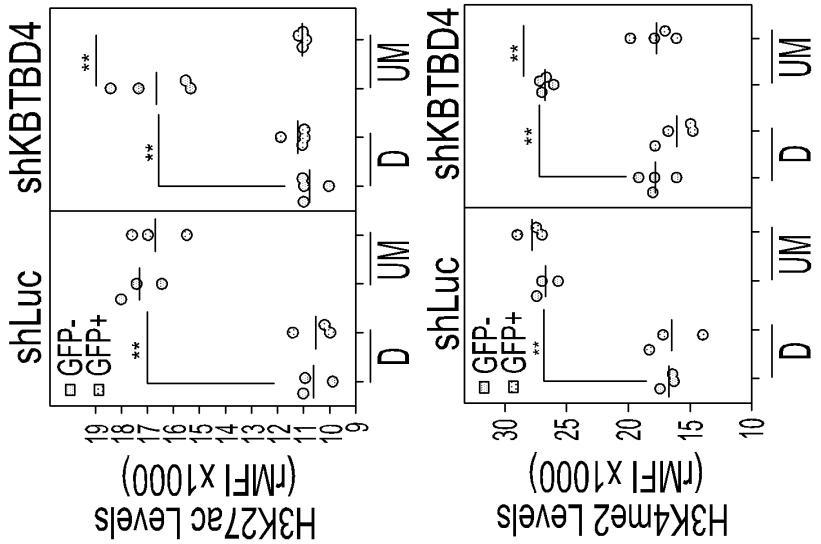

shLuc
shKBTBD4
H3K27ac Levels (rMFI x1000)
H3K4me2 Levels (rMFI x1000)
GFP-
GFP+
D
UM

Fig. 2C

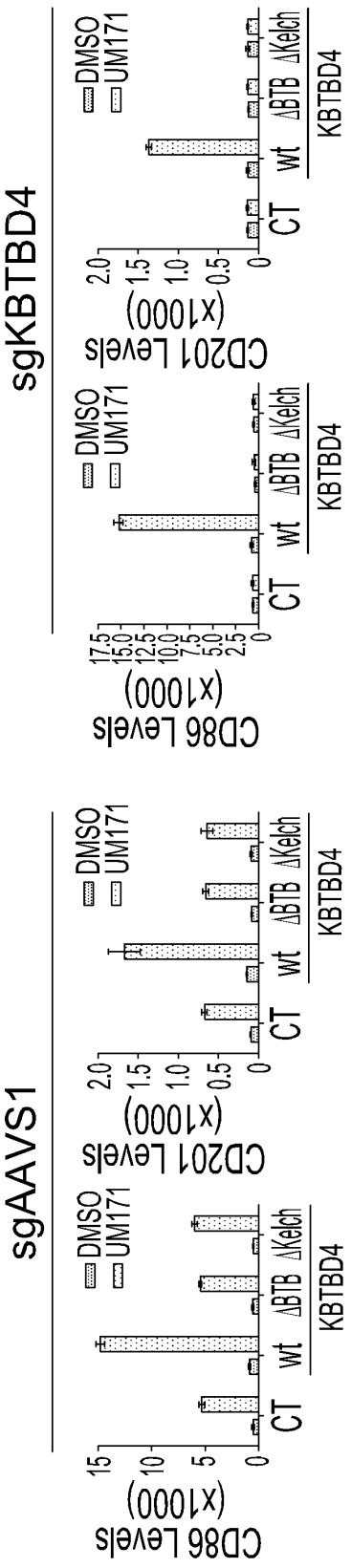

sgKBTBD4
CD86 Levels (x1000)
CD201 Levels (x1000)
DMSO
UM171
CT
wt
ΔBTB
ΔKelch
KBTBD4
sgAAVS1

Fig. 2E

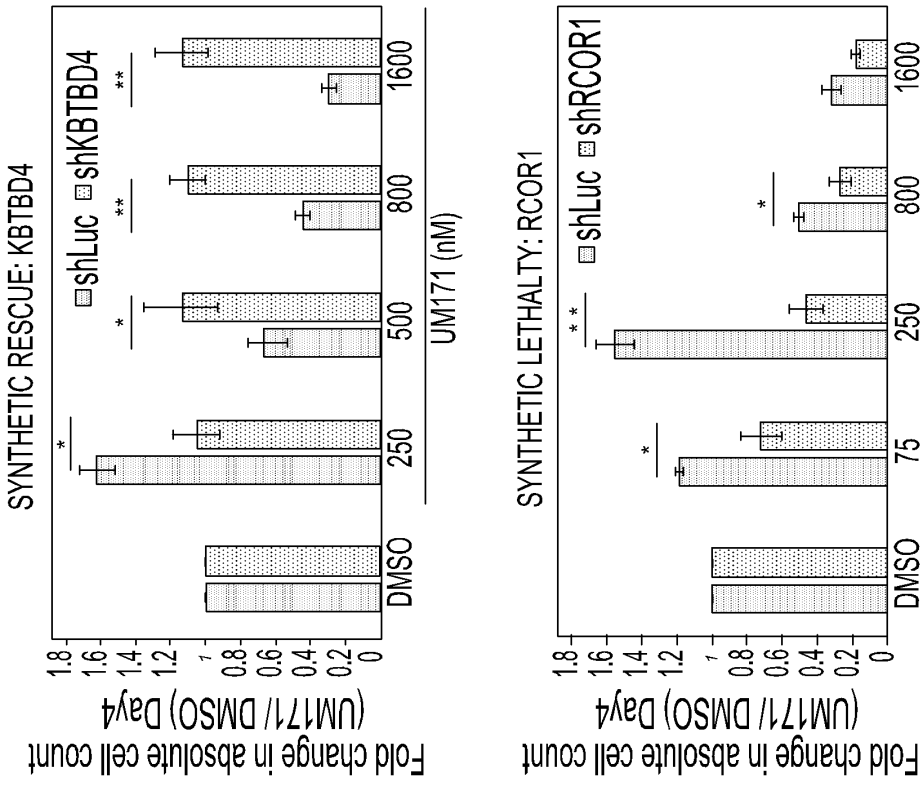

SYNTHETIC RESCUE: KBTBD4
shLuc
shKBTBD4
Fold change in absolute cell count
(UM171/ DMSO) Day4
UM171 (nM)
DMSO
250
500
800
1600
SYNTHETIC LETHALTY: RCOR1
shLuc
shRCOR1
UM171 (nM)
DMSO
75
250
800
1600

Fig. 3A

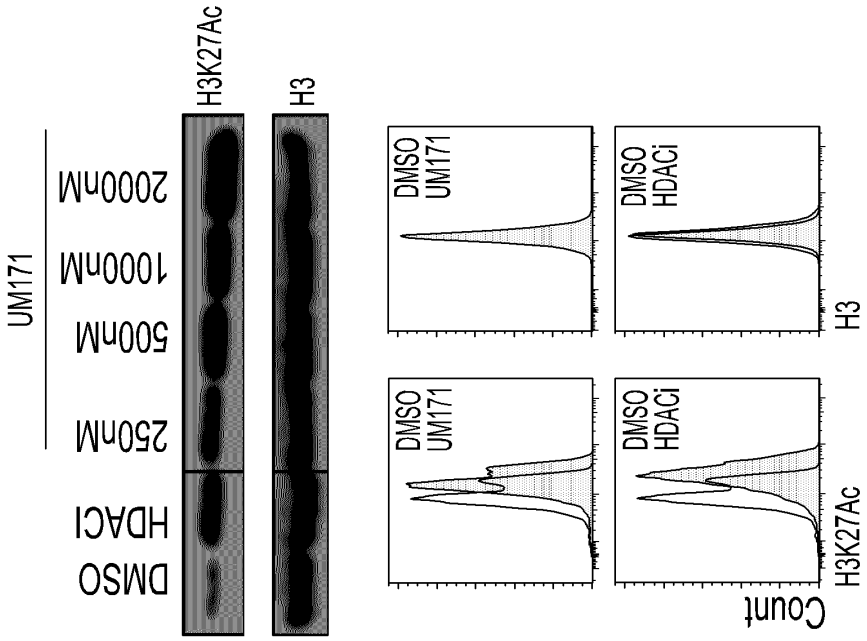

UM171
DMSO
HDACi
250nM
500nM
1000nM
2000nM
H3K27Ac
H3
DMSO
UM171
DMSO
HDACi
Count
H3K27Ac
H3

Fig. 3B

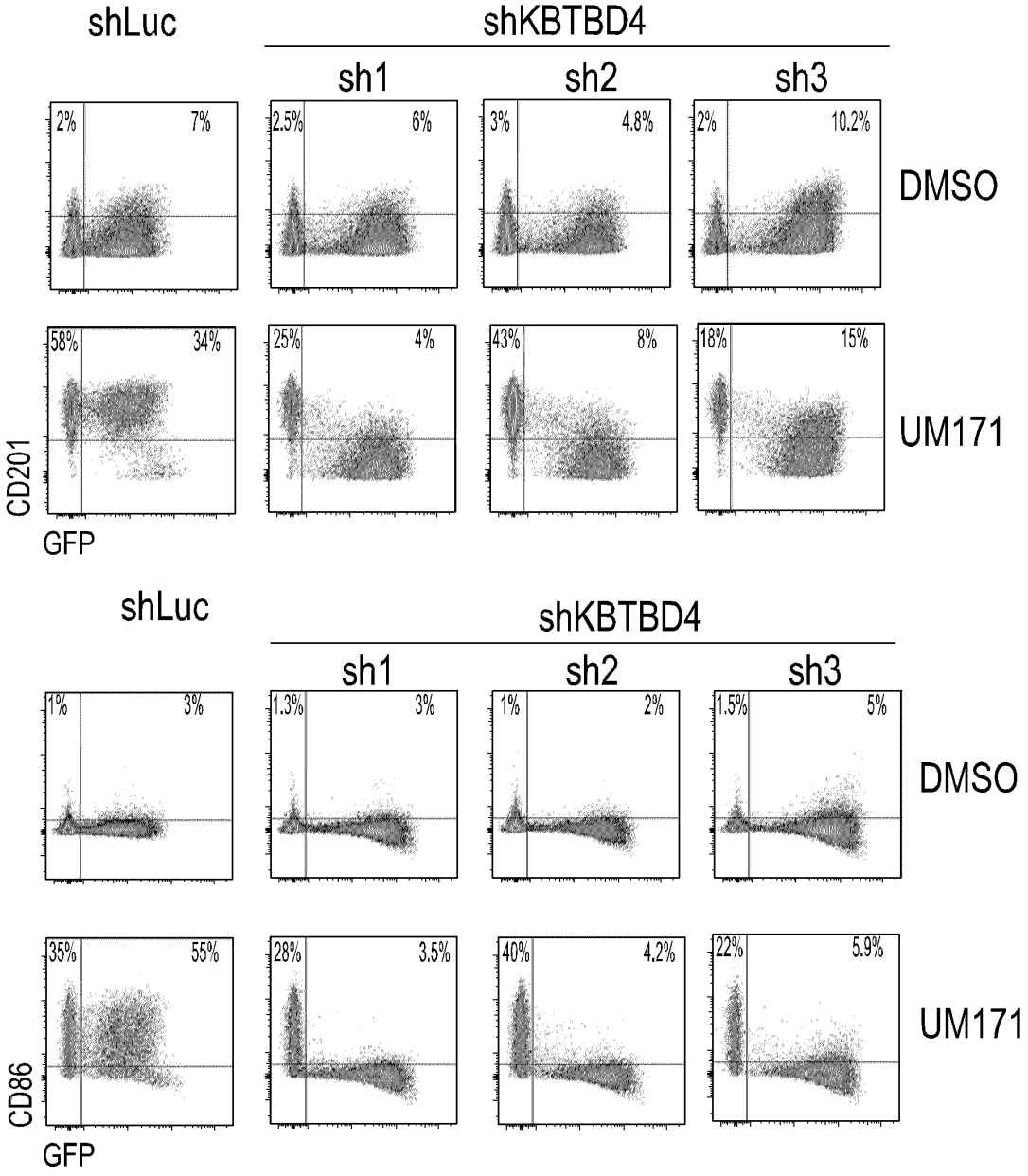

shLuc
shKBTBD4
sh1
sh2
sh3
2%
7%
2.5%
6%
3%
4.8%
2%
10.2%
DMSO
58%
34%
25%
4%
43%
8%
18%
15%
UM171
CD201
GFP
shLuc
shKBTBD4
sh1
sh2
sh3
1%
3%
1.3%
3%
1%
2%
1.5%
5%
DMSO
35%
55%
28%
3.5%
40%
4.2%
22%
5.9%
UM171
CD86
GFP

Fig. 3C

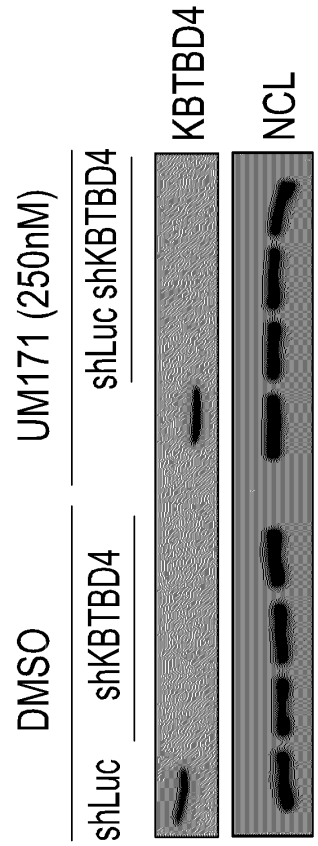


Fig. 3D

Fig. 3E

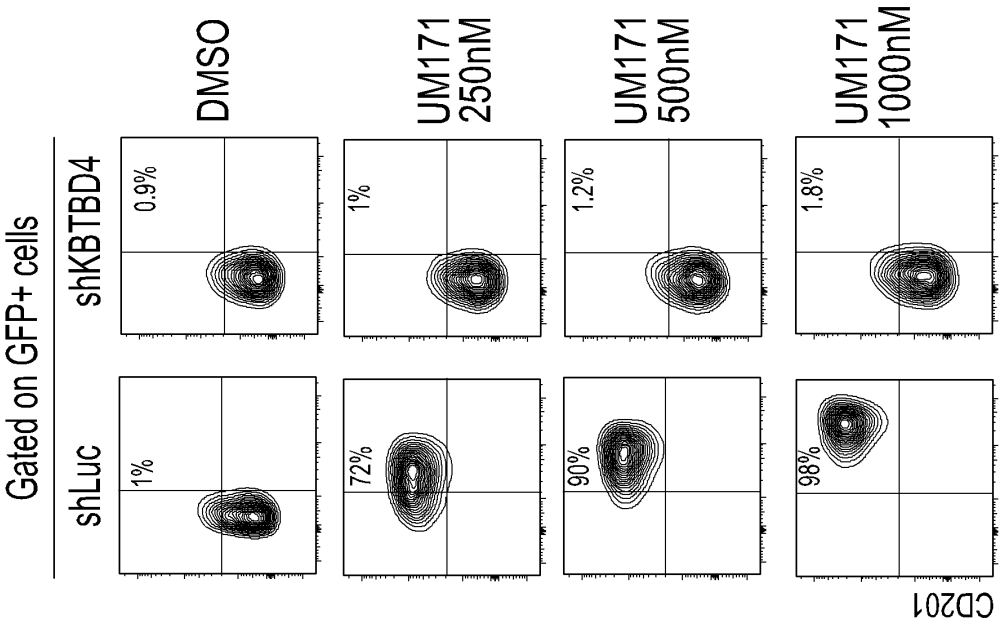


Fig. 3F

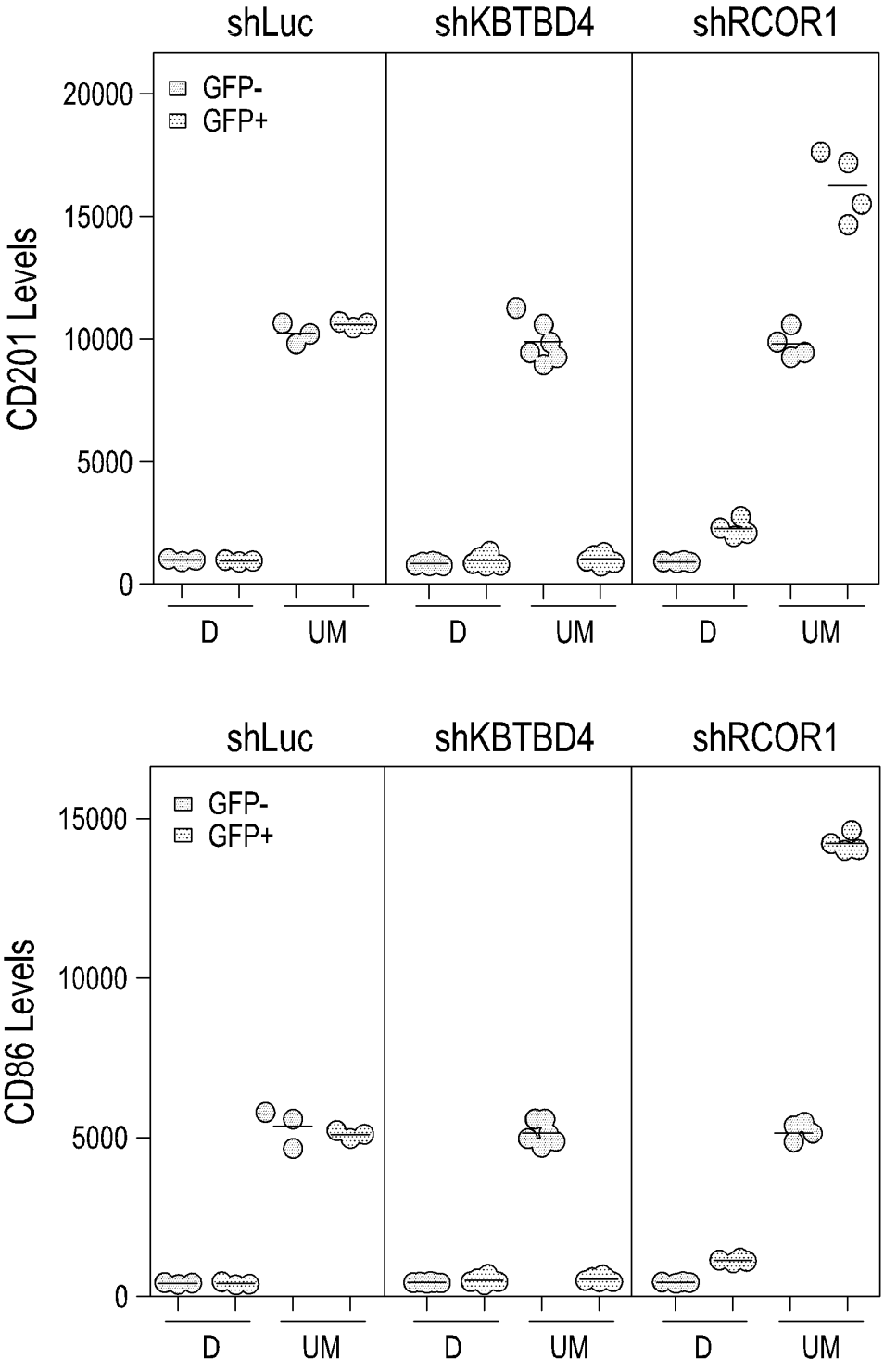

shLuc
shKBTBD4
shRCOR1
GFP-
GFP+
CD201 Levels
20000
15000
10000
5000
0
D
UM
CD86 Levels

Fig. 3G

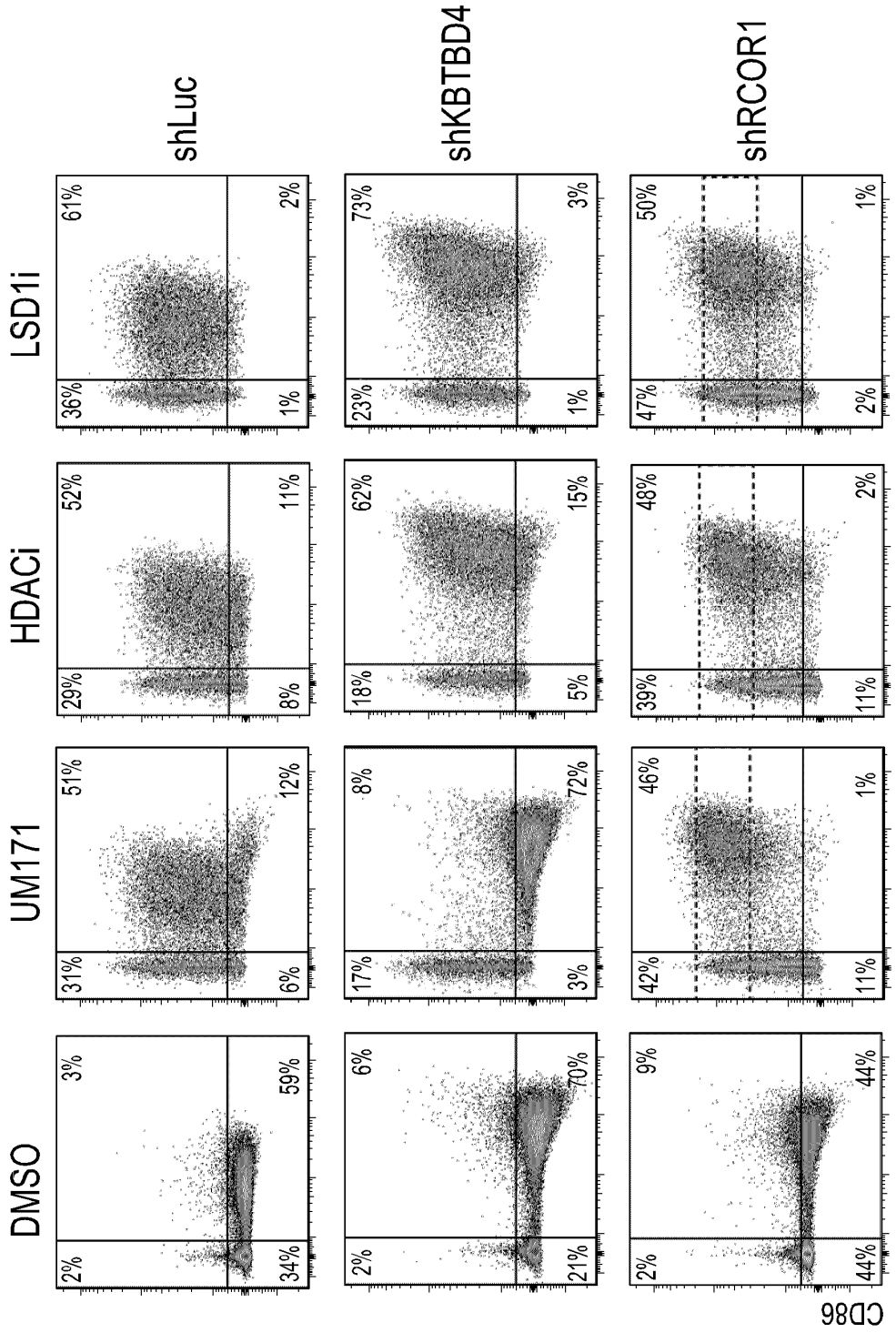

shLuc
shKBTBD4
shRCOR1
LSD1i
HDACi
UM171
DMSO
CD86
GFP
2%
34%
59%
3%
31%
6%
12%
51%
29%
8%
11%
52%
36%
1%
2%
61%
2%
21%
70%
6%
17%
3%
72%
8%
18%
5%
15%
62%
23%
1%
3%
73%
42%
11%
1%
46%
39%
11%
2%
48%
47%
2%
1%
50%
2%
44%
44%
9%

Fig. 3H

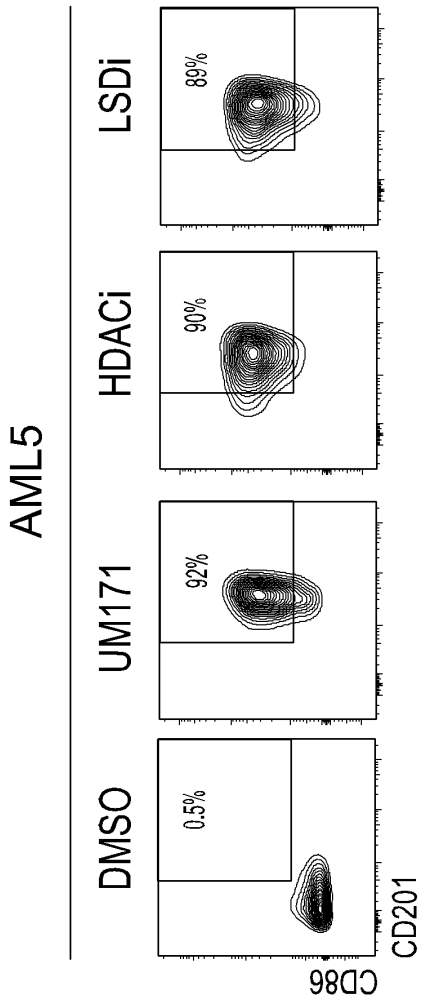

AML5
DMSO
UM171
HDACi
LSDi
0.5%
92%
90%
89%
CD86
CD201

Fig. 4A

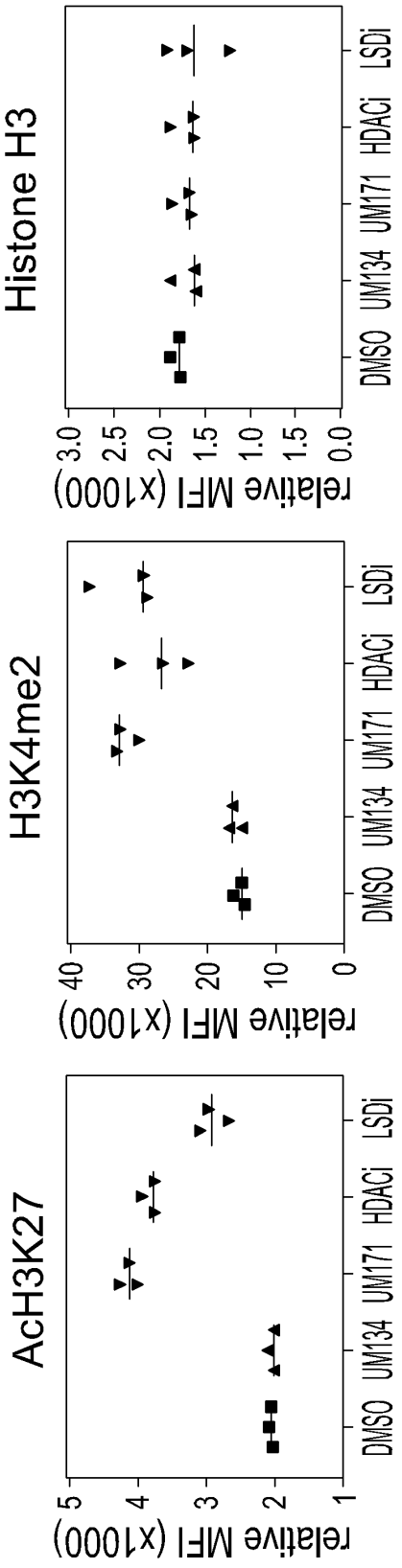

AcH3K27
H3K4me2
Histone H3
relative MFI (x1000)
DMSO UM134 UM171 HDACi LSDi

Fig. 4B

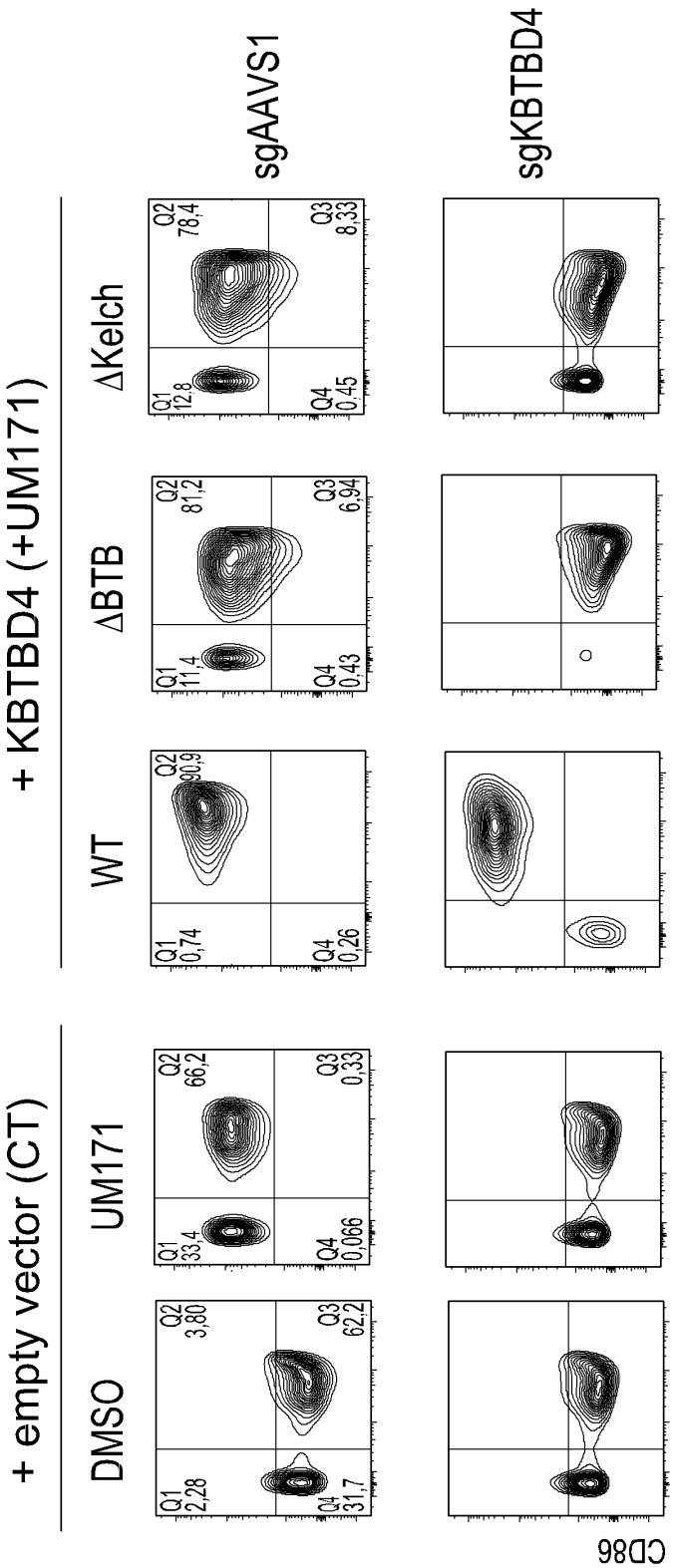

+ empty vector (CT)
+ KBTBD4 (+UM171)
DMSO
UM171
WT
ΔBTB
ΔKelch
sgAAVS1
sgKBTBD4
Q1 2,28
Q2 3,80
Q3 62,2
Q4 31,7
Q1 33,4
Q2 66,2
Q3 0,33
Q4 0,066
Q1 0,74
Q2 90,9
Q4 0,26
Q1 11,4
Q2 81,2
Q3 6,94
Q4 0,43
Q1 12,8
Q2 78,4
Q3 8,33
Q4 0,45
CD86
GFP

Fig. 5

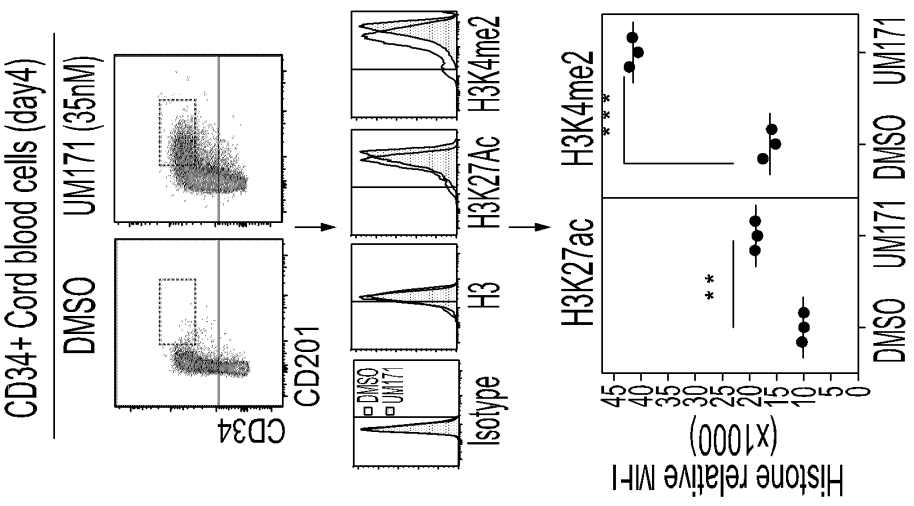


Fig. 6A

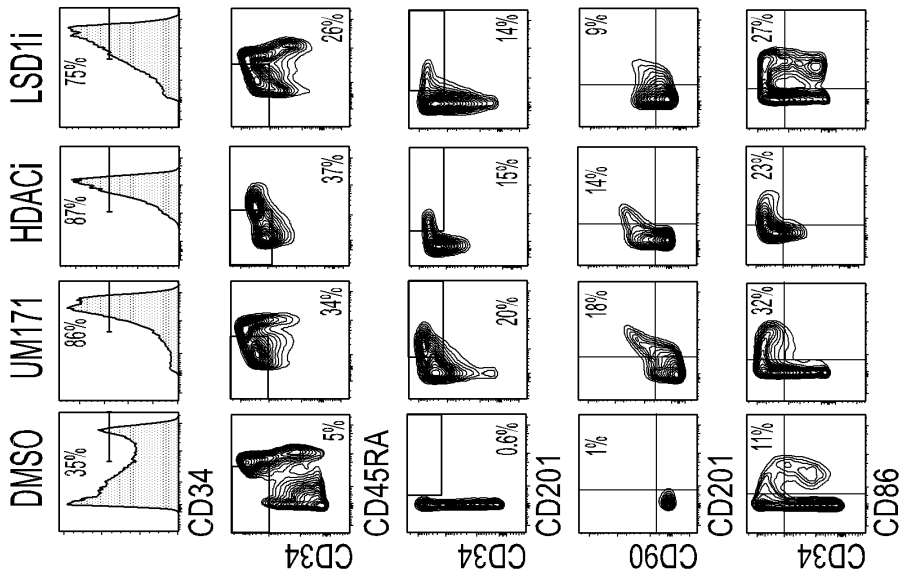


Fig. 6B

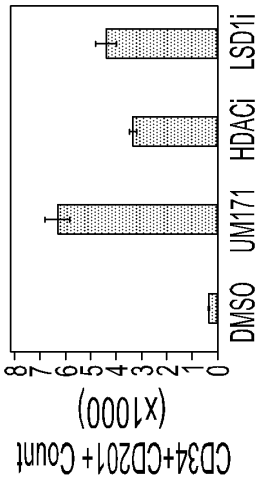


Fig. 6C

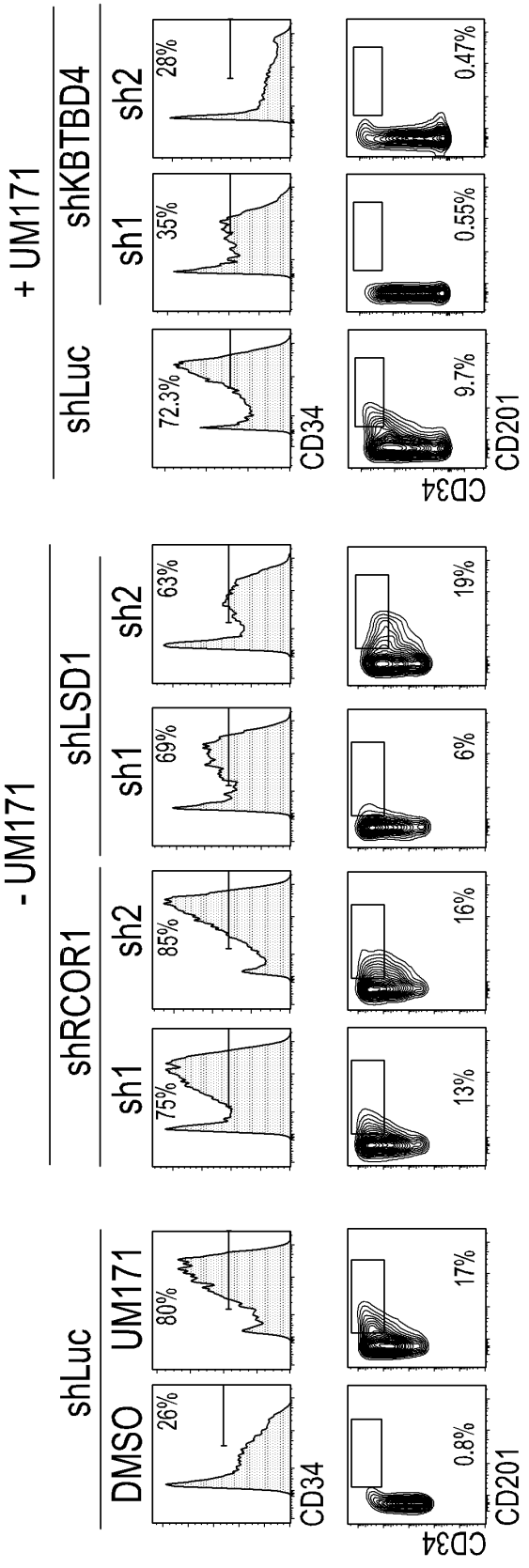
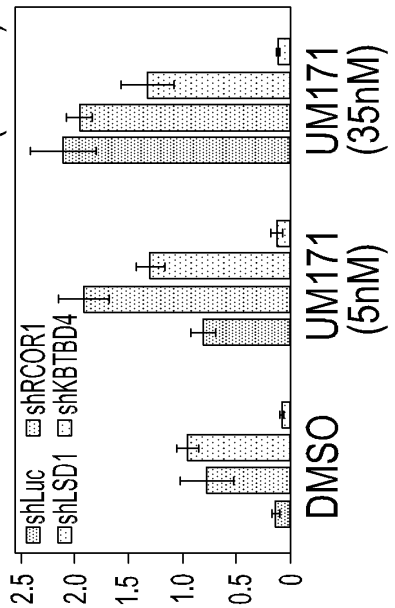

- UM171
+ UM171
shLuc
DMSO
UM171
shRCOR1
shLSD1
shKBTBD4
sh1
sh2
26%
80%
75%
85%
69%
63%
72.3%
35%
28%
0.8%
17%
13%
16%
6%
19%
9.7%
0.55%
0.47%
CD34
CD201

Fig. 6D


CD34+CD201+ Count (x1000)
shLuc
shRCOR1
shLSD1
shKBTBD4
2.5
2.0
1.5
1.0
0.5
0
DMSO
UM171 (5nM)
UM171 (35nM)

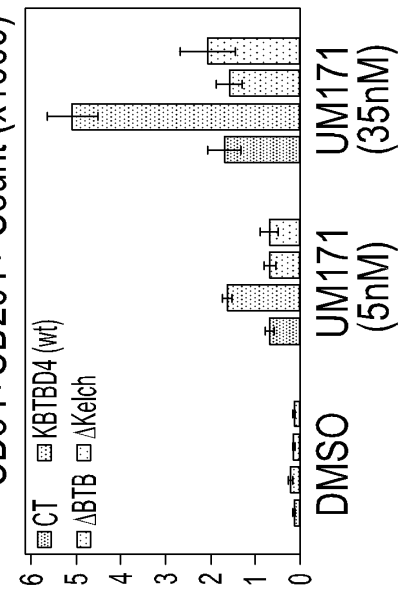

CD34+CD201+ Count (x1000)
CT
KBTBD4 (wt)
ΔBTB
ΔKelch
6
5
4
3
2
1
0
DMSO
UM171 (5nM)
UM171 (35nM)

Fig. 6F

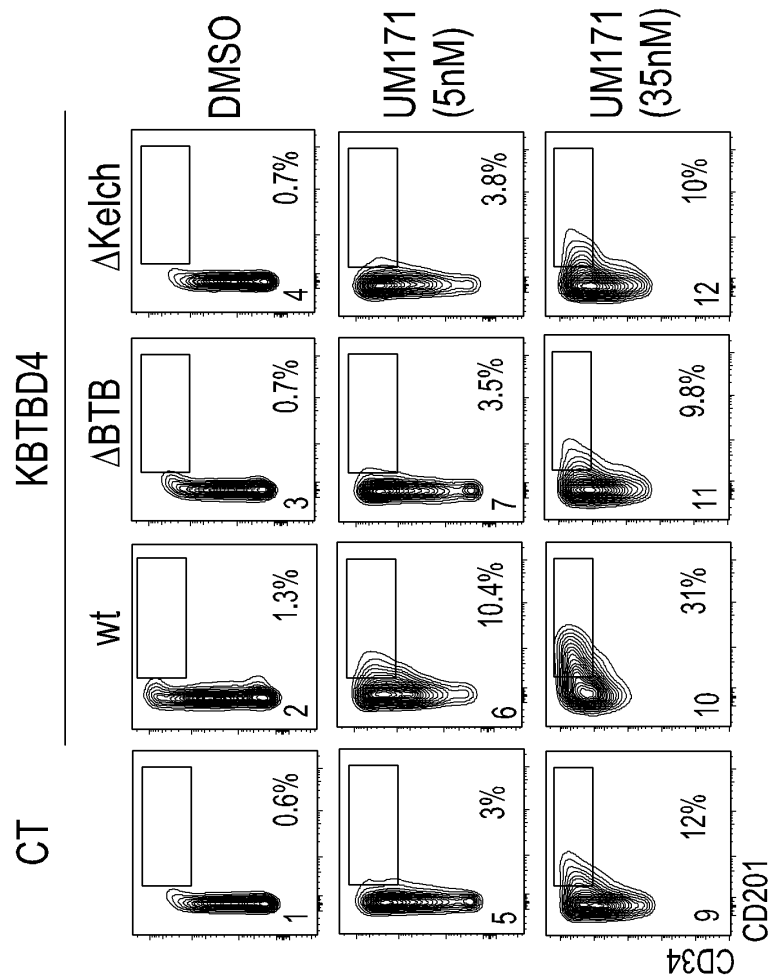

CT
KBTBD4
wt
ΔBTB
ΔKelch
DMSO
UM171 (5nM)
UM171 (35nM)
0.6%
1.3%
0.7%
0.7%
3%
10.4%
3.5%
3.8%
12%
31%
9.8%
10%
1
2
3
4
5
6
7
9
10
11
12
CD201
CD34

Fig. 6E

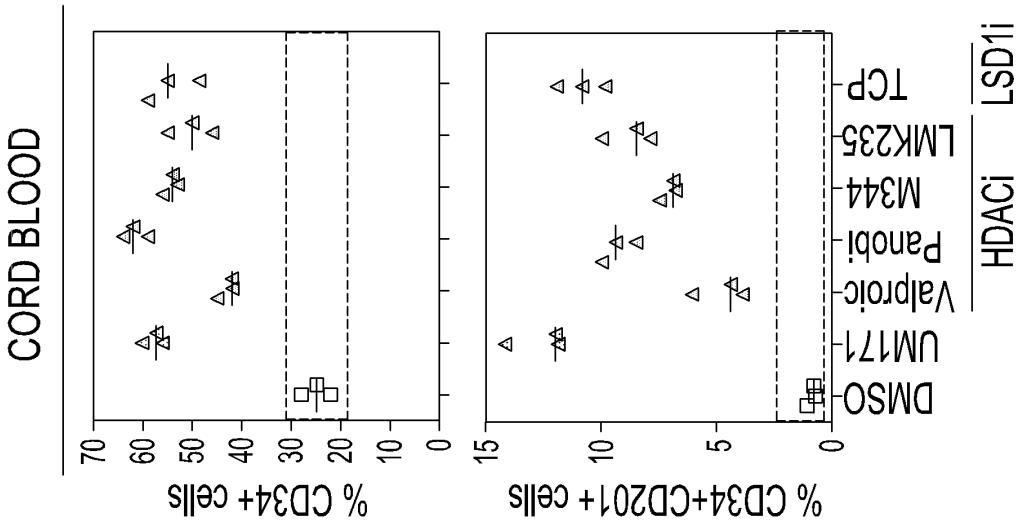

CORD BLOOD
% CD34+ cells
% CD34+CD201+ cells
DMSO
UM171
Valproic
Panobi
M344
LMK235
TCP
HDACi
LSD1i

Fig. 7A

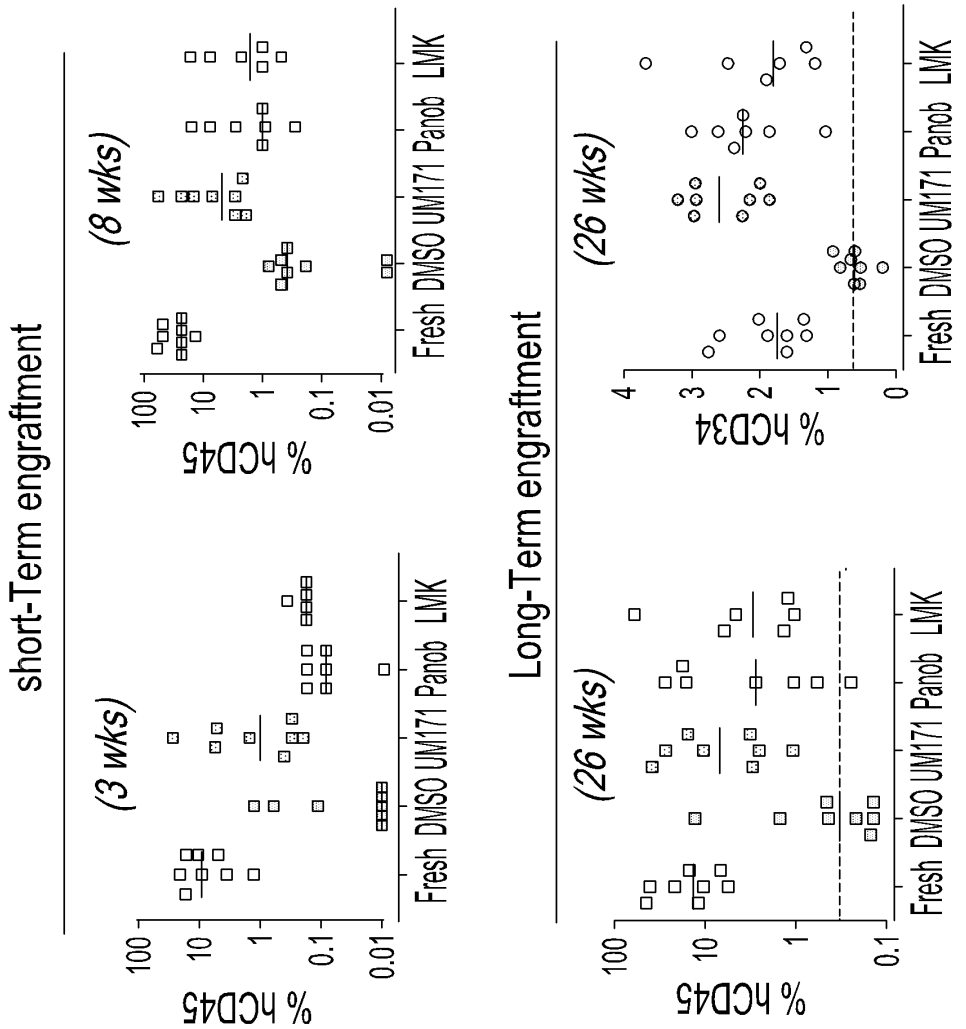

short-Term engraftment
(3 wks)
(8 wks)
% hCD45
Fresh DMSO UM171 Panob LMK
Long-Term engraftment
(26 wks)
(26 wks)
% hCD45
% hCD34
Fresh DMSO UM171 Panob LMK

Fig. 7B

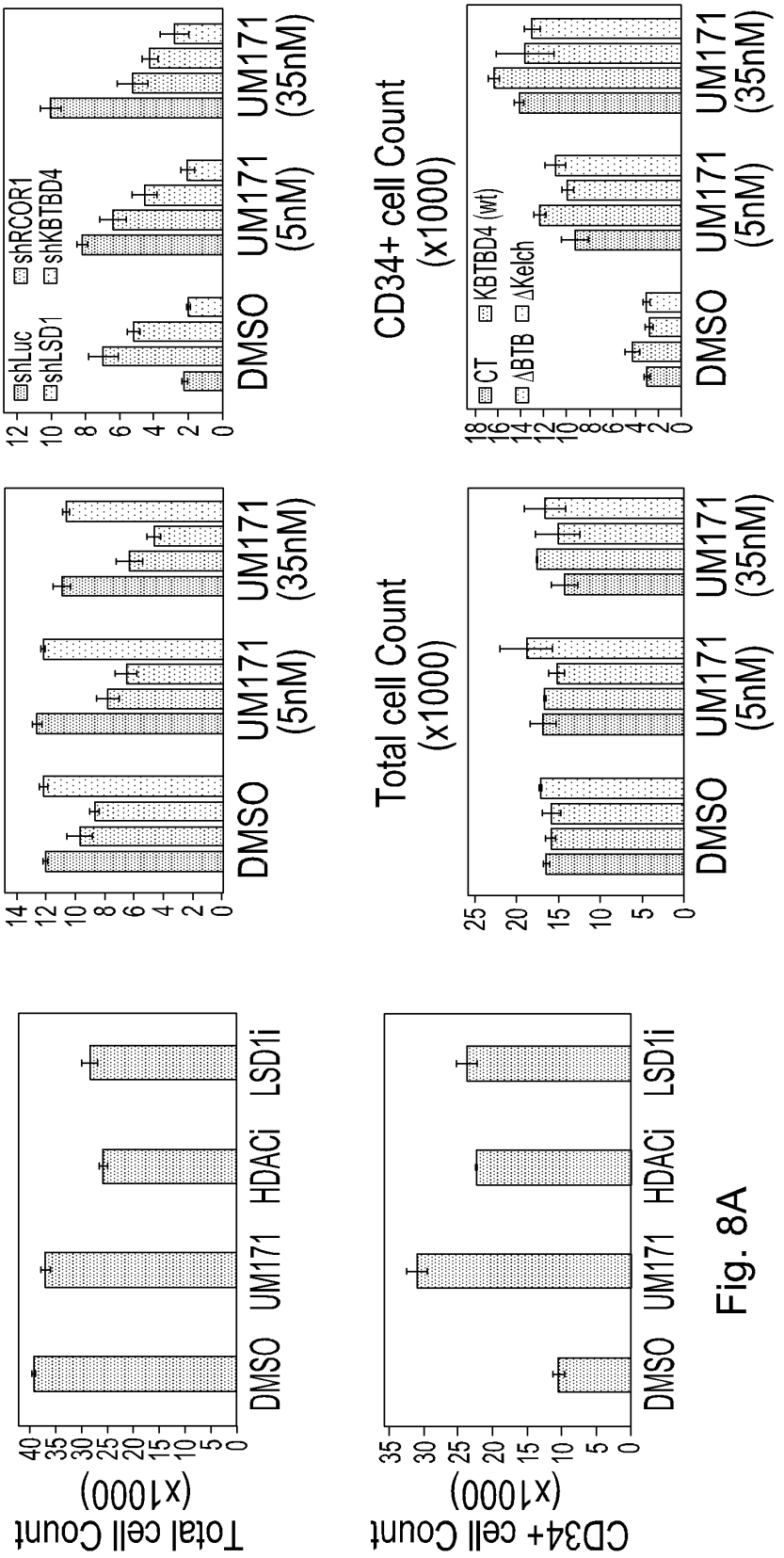

Total cell Count (x1000)
DMSO
UM171
HDACi
LSD1i
CD34+ cell Count (x1000)
DMSO
UM171
HDACi
LSD1i
Total cell Count (x1000)
shLuc
shLSD1
shRCOR1
shKBTBD4
DMSO
UM171 (5nM)
UM171 (35nM)
CD34+ cell Count (x1000)
shLuc
shLSD1
shRCOR1
shKBTBD4
DMSO
UM171 (5nM)
UM171 (35nM)
Total cell Count (x1000)
CT
KBTBD4 (wt)
ΔBTB
ΔKelch
DMSO
UM171 (5nM)
UM171 (35nM)
CD34+ cell Count (x1000)
CT
KBTBD4 (wt)
ΔBTB
ΔKelch
DMSO
UM171 (5nM)
UM171 (35nM)

Fig. 8A

Fig. 8B

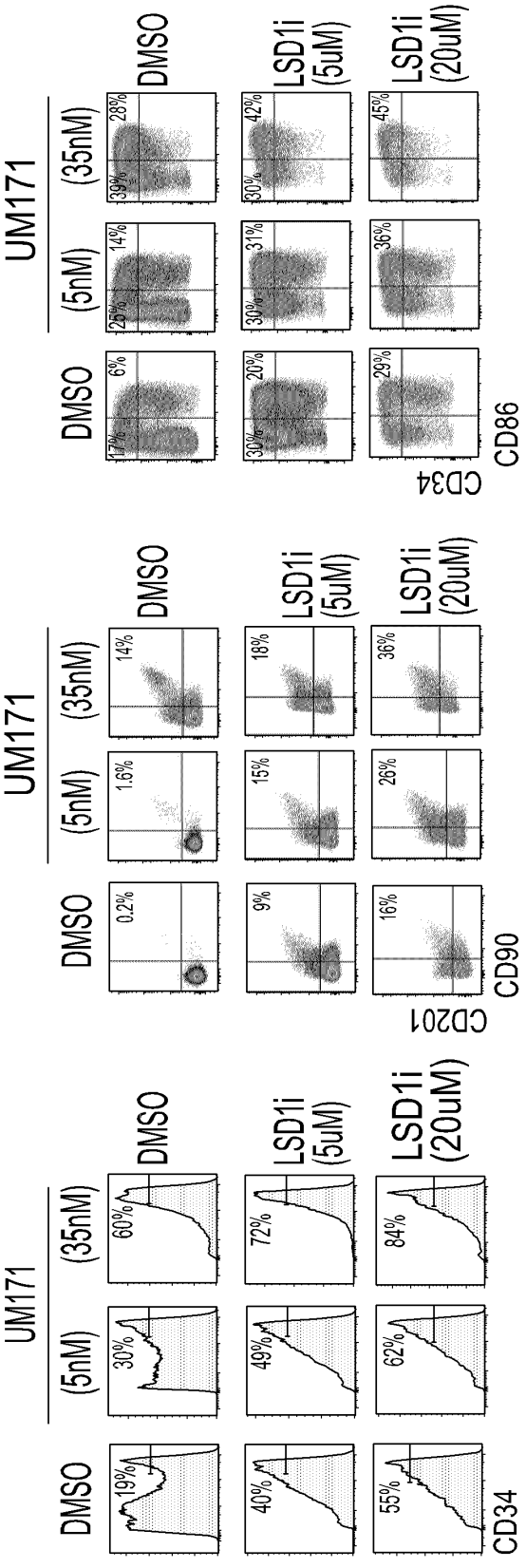

UM171
DMSO
(5nM)
(35nM)
DMSO
LSD1i (5uM)
LSD1i (20uM)
CD34
CD86
CD201
CD90
19%
30%
60%
40%
49%
72%
55%
62%
84%
0.2%
1.6%
14%
9%
15%
18%
16%
26%
36%
6%
14%
28%
20%
31%
42%
29%
36%
45%

Fig. 8C

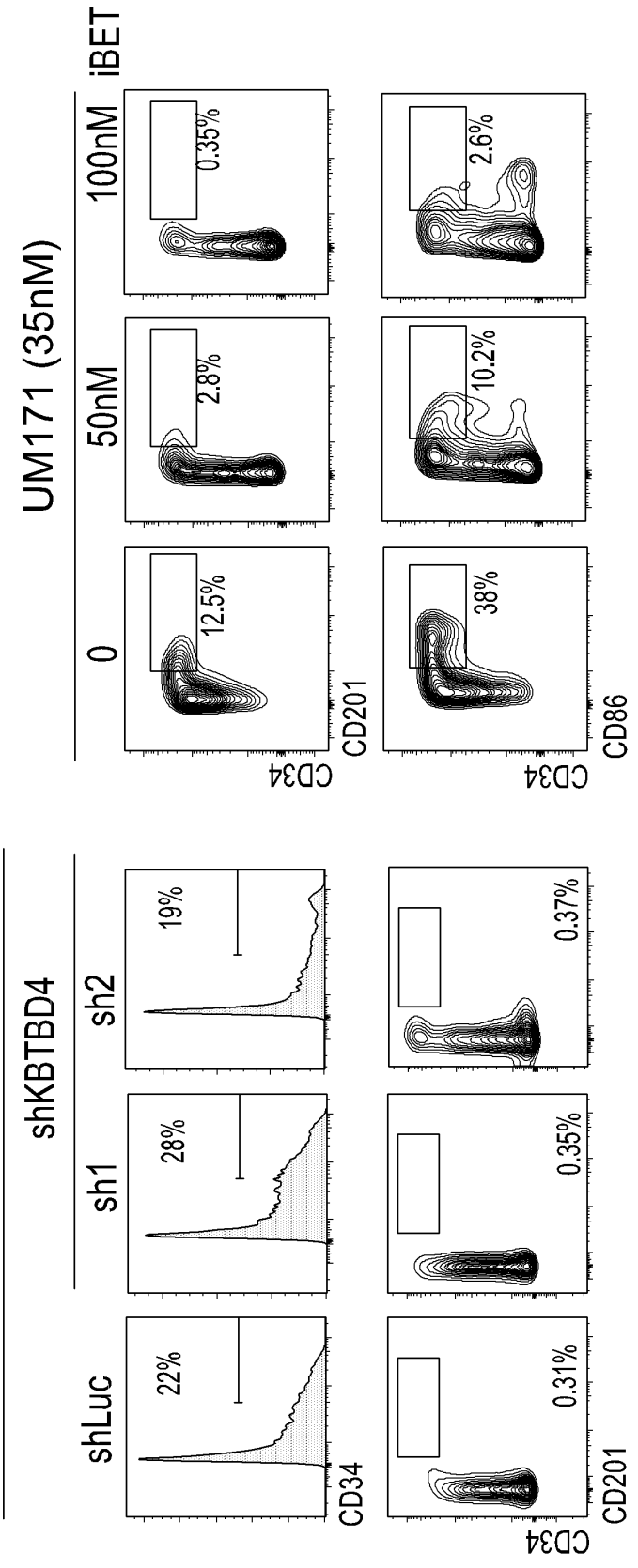

DMSO
shKBTBD4
shLuc
sh1
sh2
22%
28%
19%
0.31%
0.35%
0.37%
CD34
CD201
UM171 (35nM)
0
50nM
100nM
iBET
12.5%
2.8%
0.35%
38%
10.2%
2.6%
CD34
CD201
CD86

Fig. 8D

Fig. 9

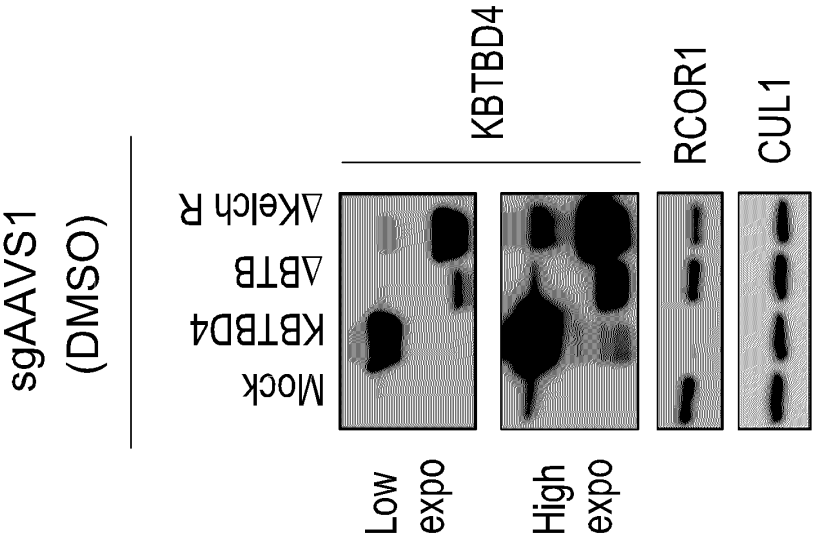


Fig. 11A

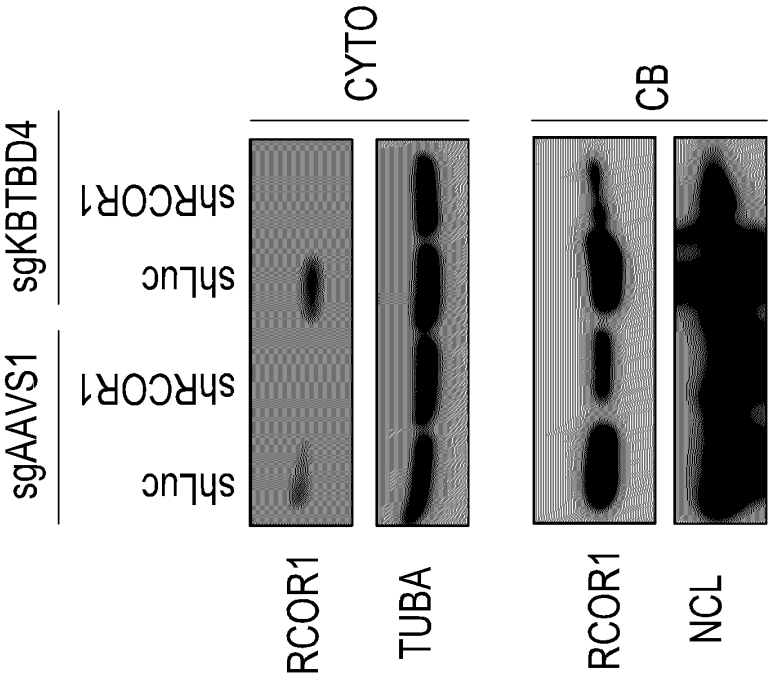
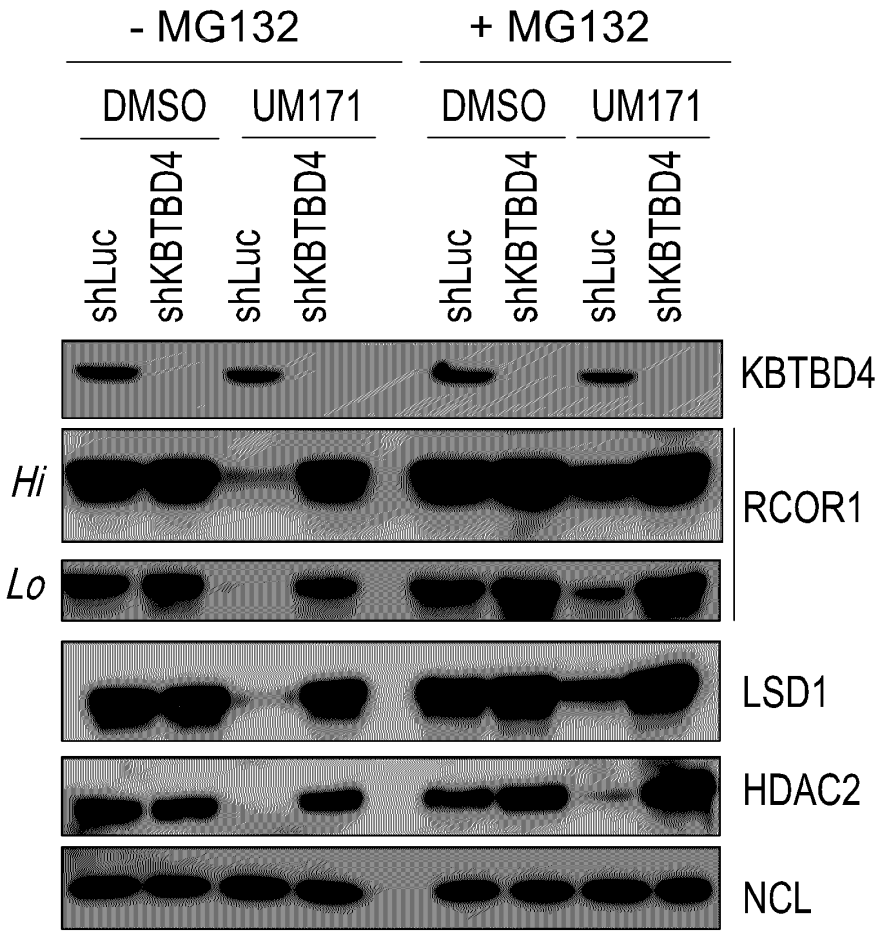


Fig. 10


- MG132
+ MG132
DMSO
UM171
DMSO
UM171
shLuc
shKBTBD4
shLuc
shKBTBD4
shLuc
shKBTBD4
shLuc
shKBTBD4
KBTBD4
Hi
Lo
RCOR1
LSD1
HDAC2
NCL

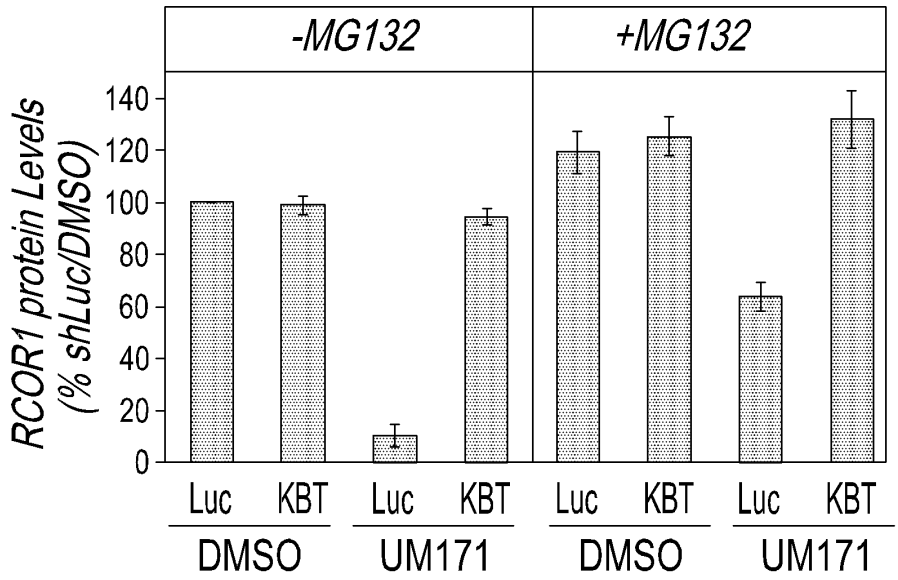
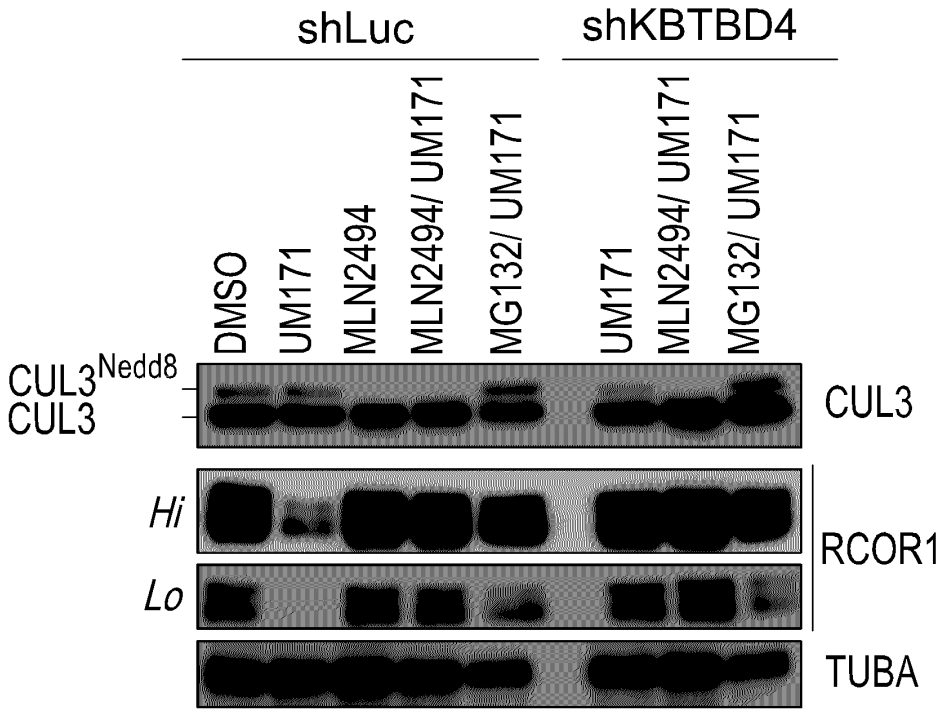

-MG132
+MG132
RCOR1 protein Levels
(% shLuc/DMSO)
140
120
100
80
60
40
20
0
Luc
KBT
Luc
KBT
Luc
KBT
Luc
KBT
DMSO
UM171
DMSO
UM171

Fig. 11B


shLuc
shKBTBD4
DMSO
UM171
MLN2494
MLN2494/ UM171
MG132/ UM171
UM171
MLN2494/ UM171
MG132/ UM171
CUL3Nedd8
CUL3
CUL3
Hi
Lo
RCOR1
TUBA

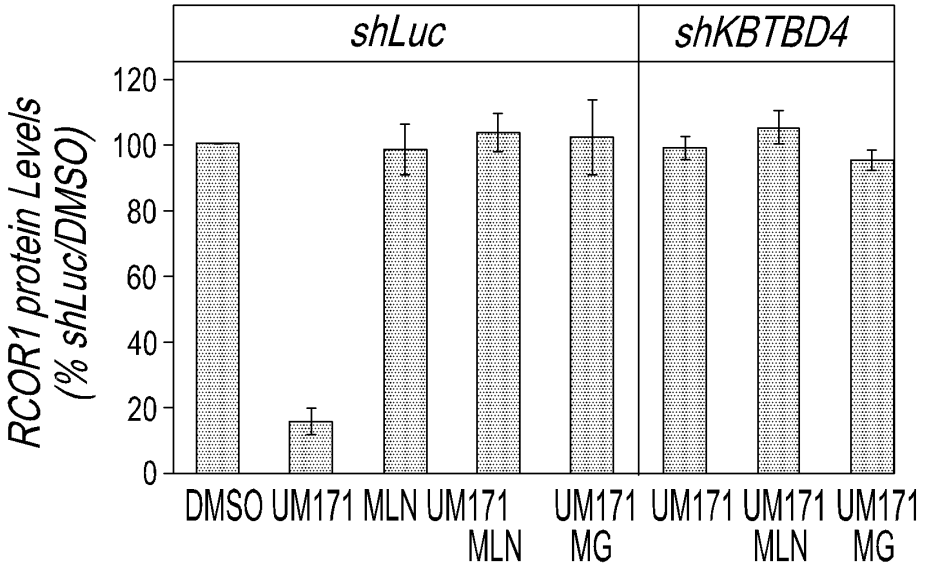
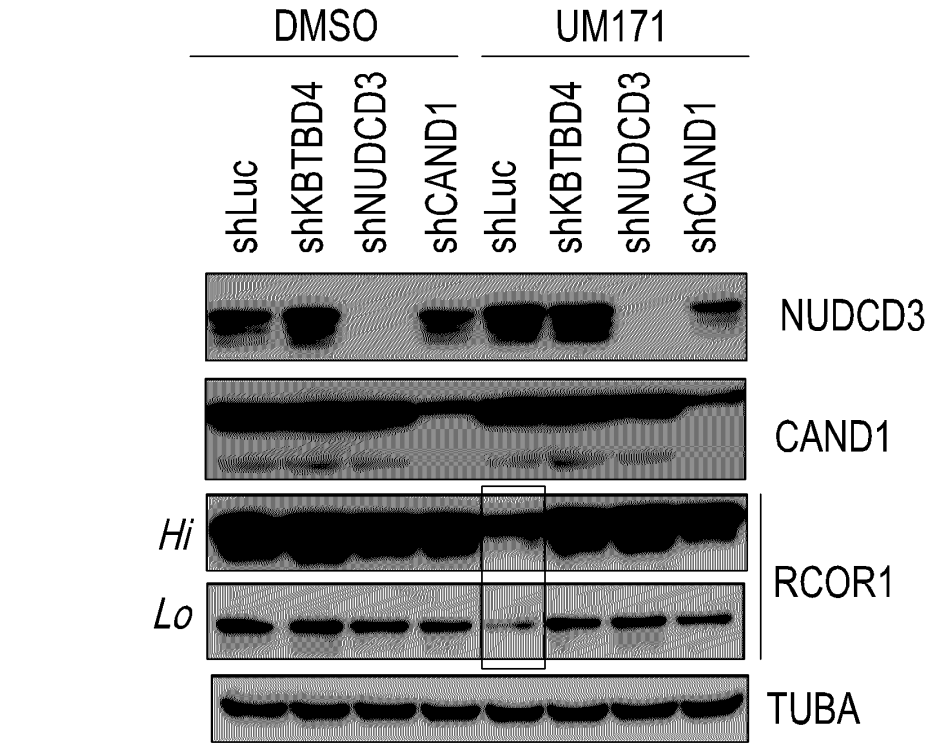

shLuc
shKBTBD4
RCOR1 protein Levels
(% shLuc/DMSO)
120
100
80
60
40
20
0
DMSO
UM171
MLN
UM171 MLN
UM171 MG
UM171
UM171 MLN
UM171 MG

Fig. 11C


DMSO
UM171
shLuc
shKBTBD4
shNUDCD3
shCAND1
shLuc
shKBTBD4
shNUDCD3
shCAND1
NUDCD3
CAND1
Hi
Lo
RCOR1
TUBA

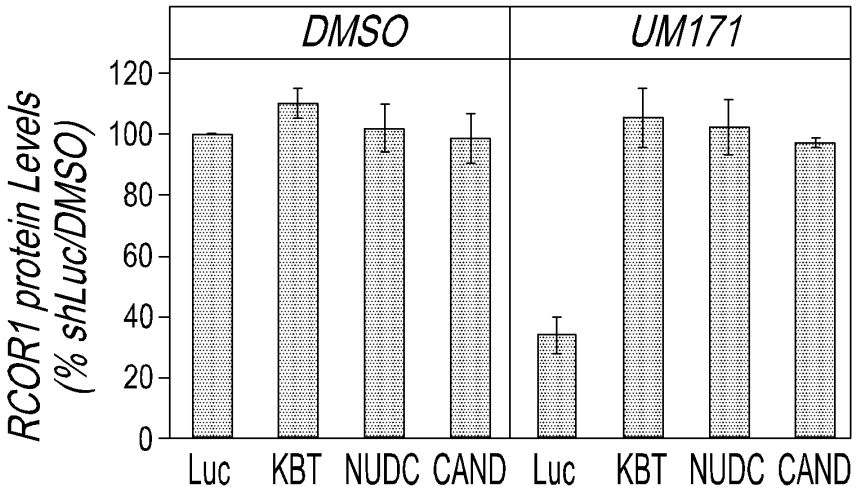

DMSO
UM171
RCOR1 protein Levels
(% shLuc/DMSO)
120
100
80
60
40
20
0
Luc
KBT
NUDC
CAND
Luc
KBT
NUDC
CAND

Fig. 11D

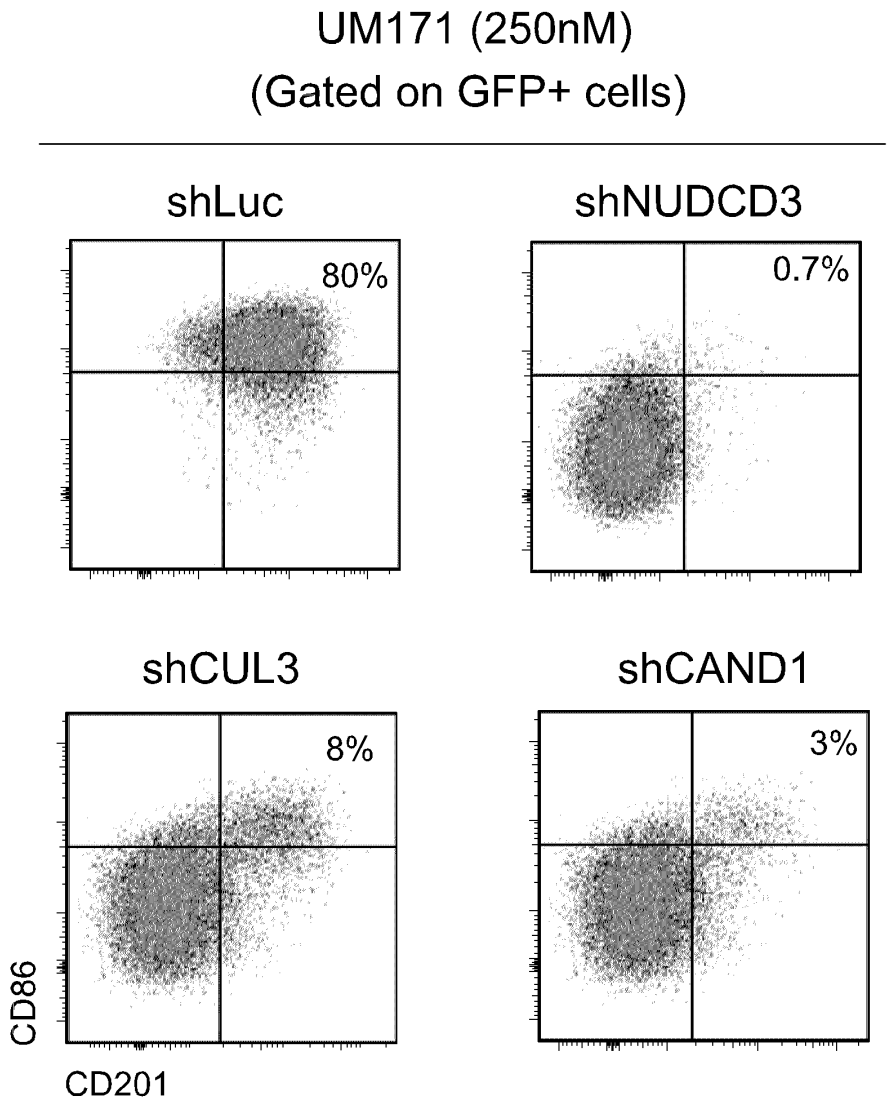

UM171 (250nM)
(Gated on GFP+ cells)
shLuc
80%
shNUDCD3
0.7%
shCUL3
8%
shCAND1
3%
CD86
CD201

Fig. 11E

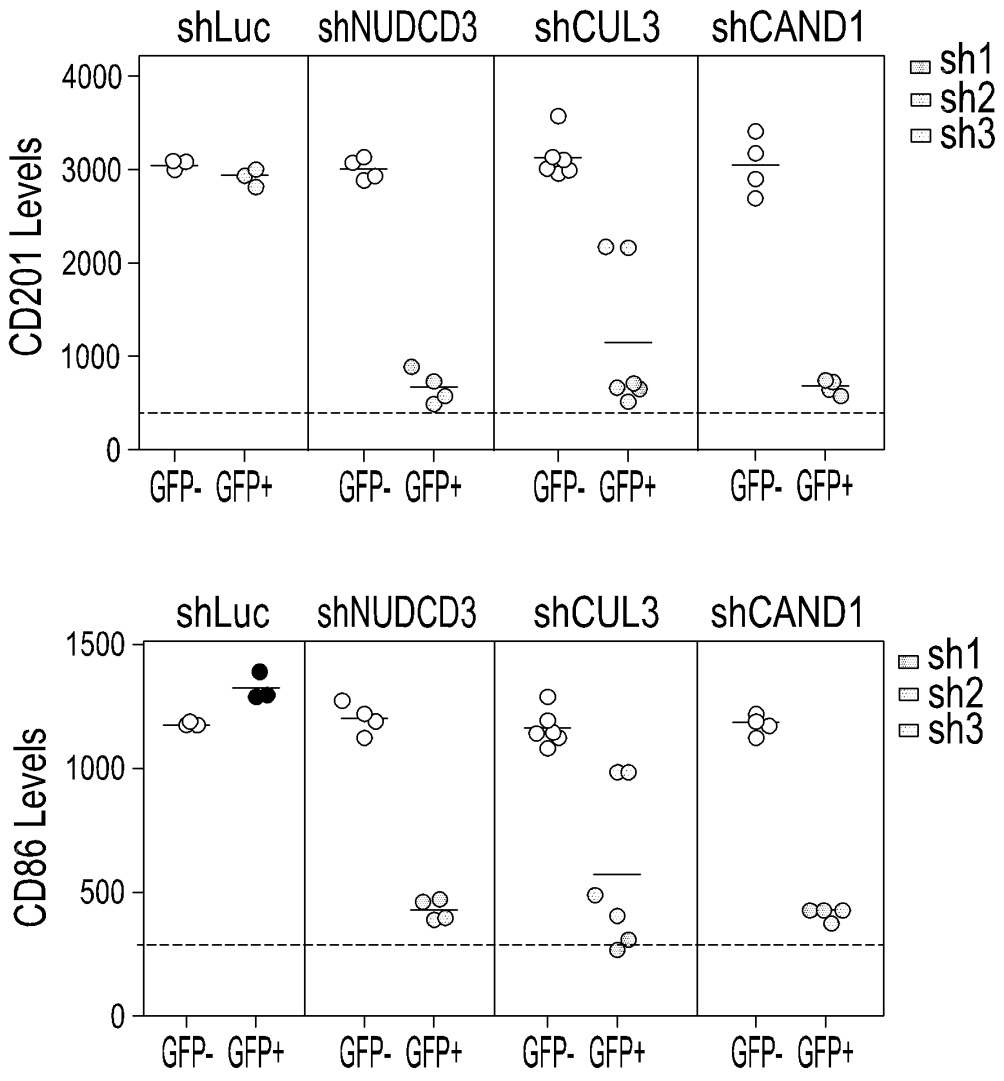

shLuc
shNUDCD3
shCUL3
shCAND1
CD201 Levels
4000
3000
2000
1000
0
GFP- GFP+
sh1
sh2
sh3
CD86 Levels
1500
1000
500
0

Fig. 11F

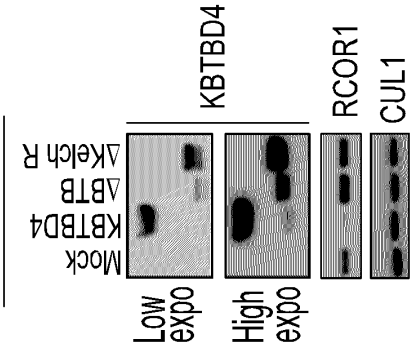


Fig. 12D

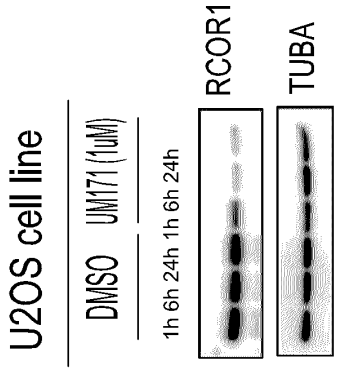


Fig. 12C

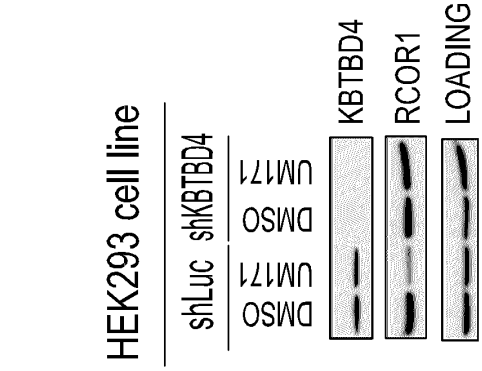


Fig. 12B

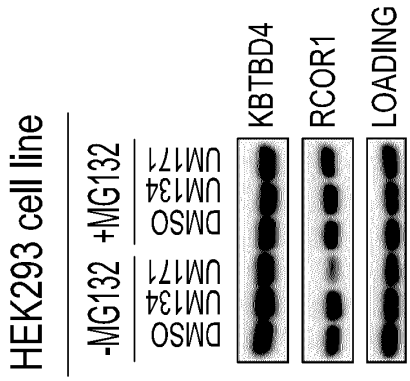


Fig. 12A

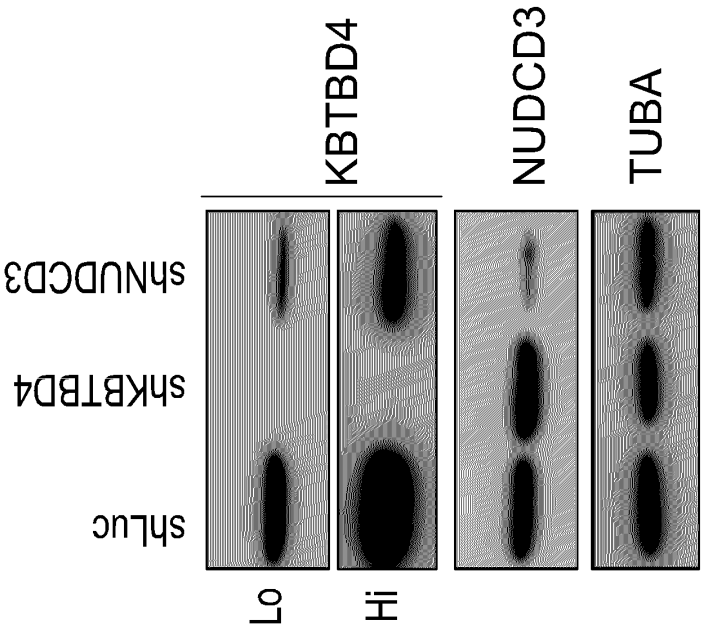


Fig. 13B

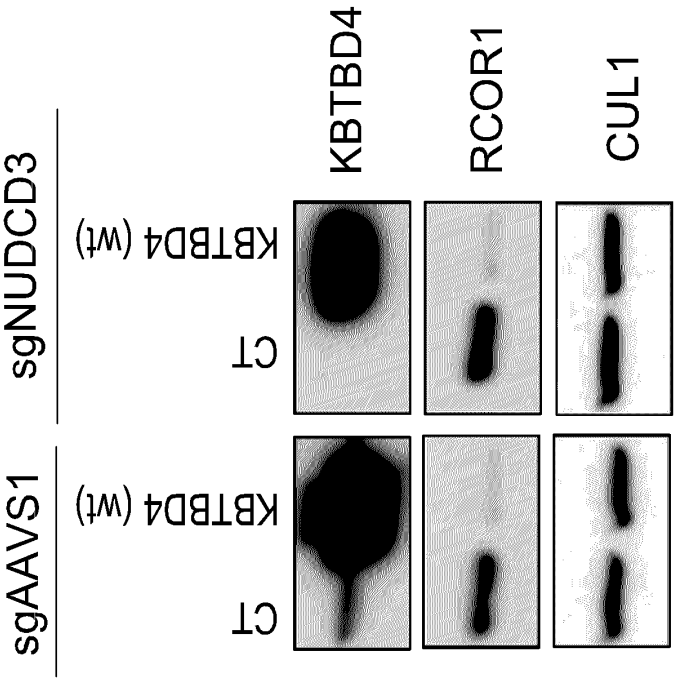


Fig. 13A

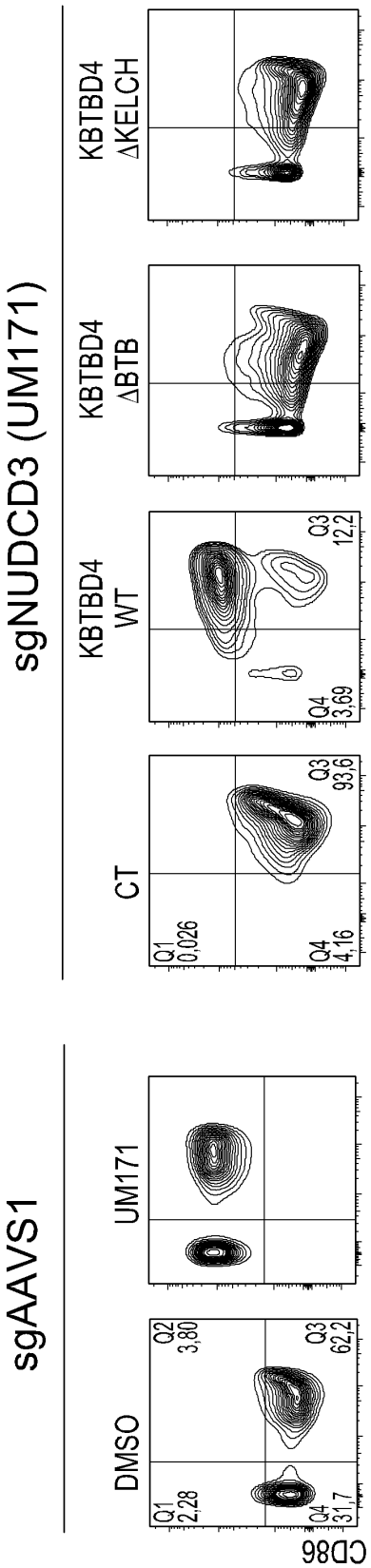

sgAAVS1
sgNUDCD3 (UM171)
DMSO
UM171
CT
KBTBD4 WT
KBTBD4 ΔBTB
KBTBD4 ΔKELCH
Q1 2,28
Q2 3,80
Q3 62,2
Q4 31,7
Q1 0,026
Q3 93,6
Q4 4,16
Q3 12,2
Q4 3,69
CD86
GFP

Fig. 13C

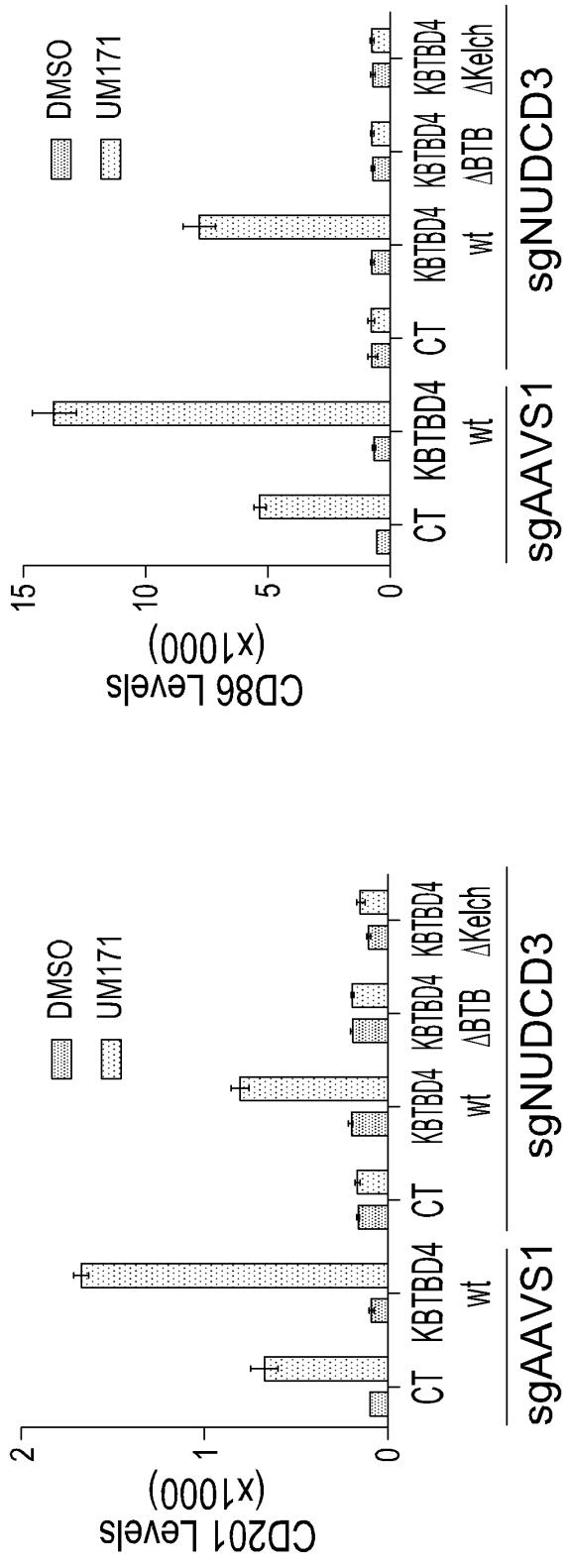

CD86 Levels (x1000)
15
10
5
0
DMSO
UM171
CT
KBTBD4
CT
KBTBD4
KBTBD4
KBTBD4
wt
wt
ΔBTB
ΔKelch
sgAAVS1
sgNUDCD3
CD201 Levels (x1000)
2
1
0

Fig. 13D

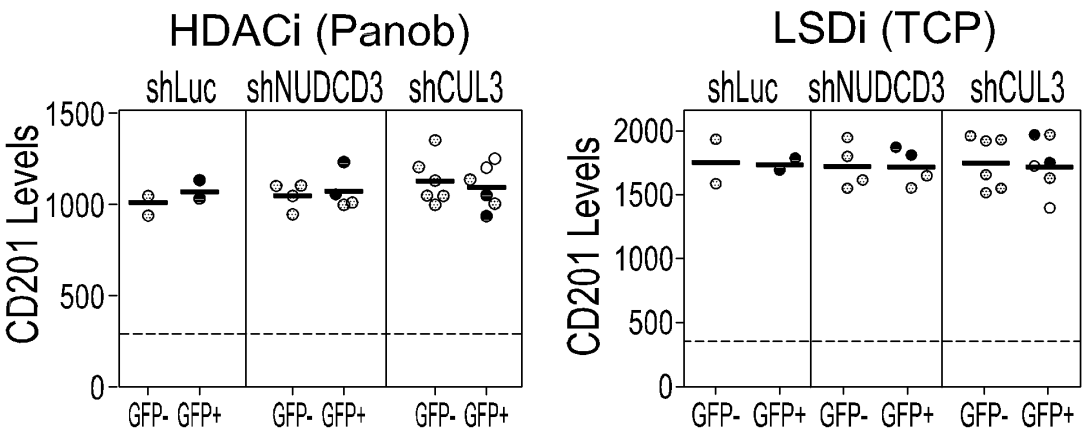


Fig. 14

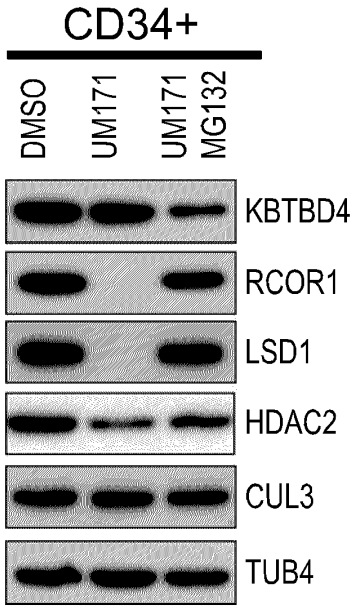


Fig. 15A

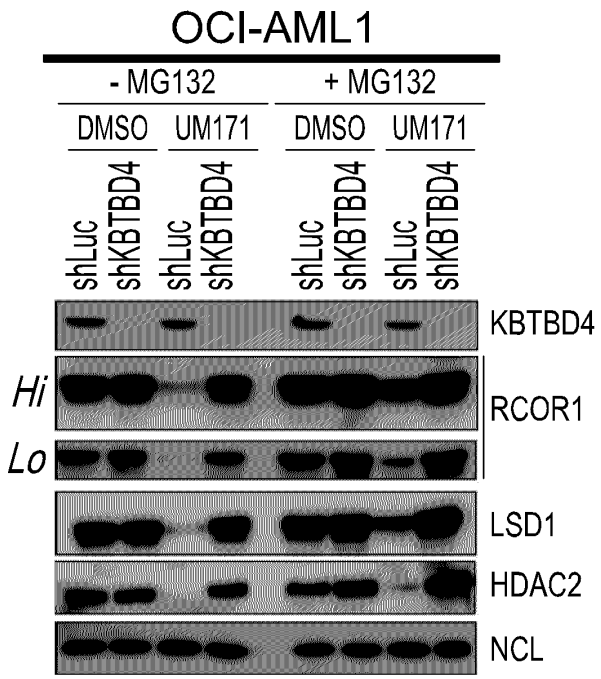


Fig. 15B

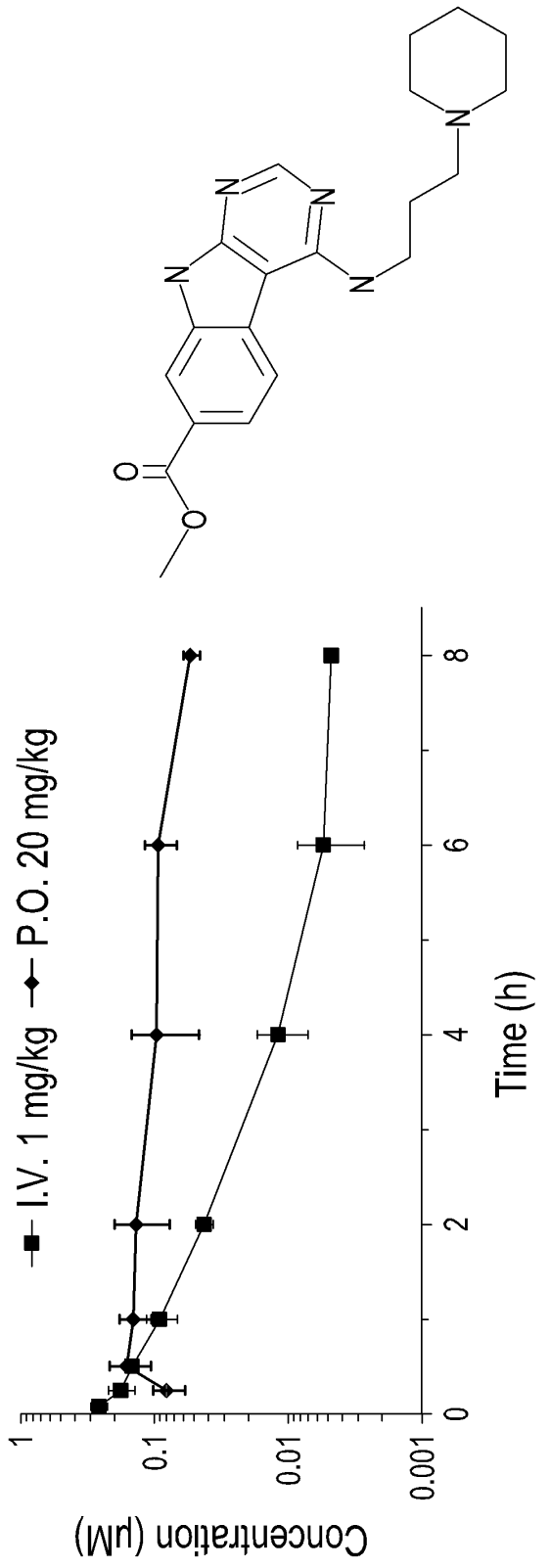

I.V. 1 mg/kg
P.O. 20 mg/kg
Concentration (μM)
1
0.1
0.01
0.001
0
2
4
6
8
Time (h)
N
N
N
N
O
O

Fig. 16

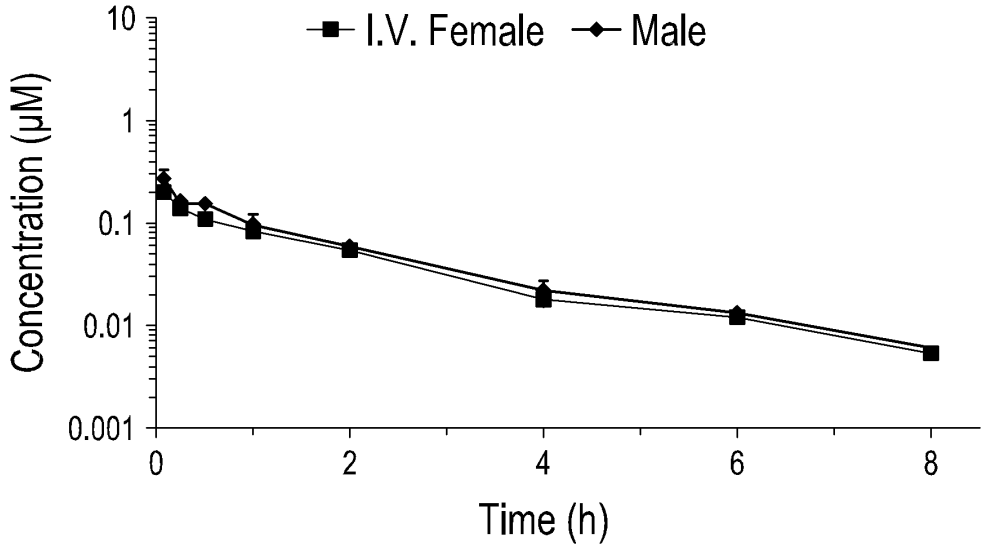


Fig. 17A

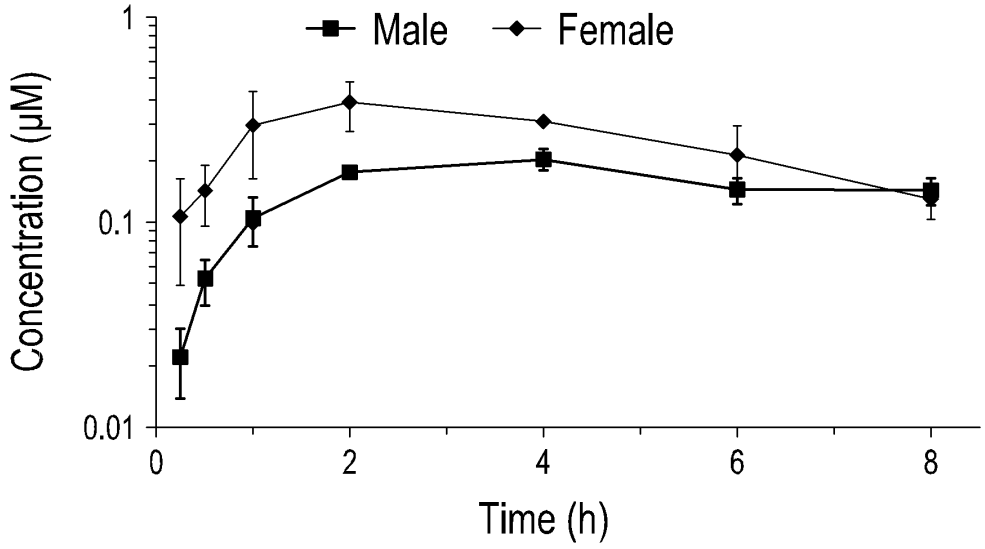


Fig. 17B