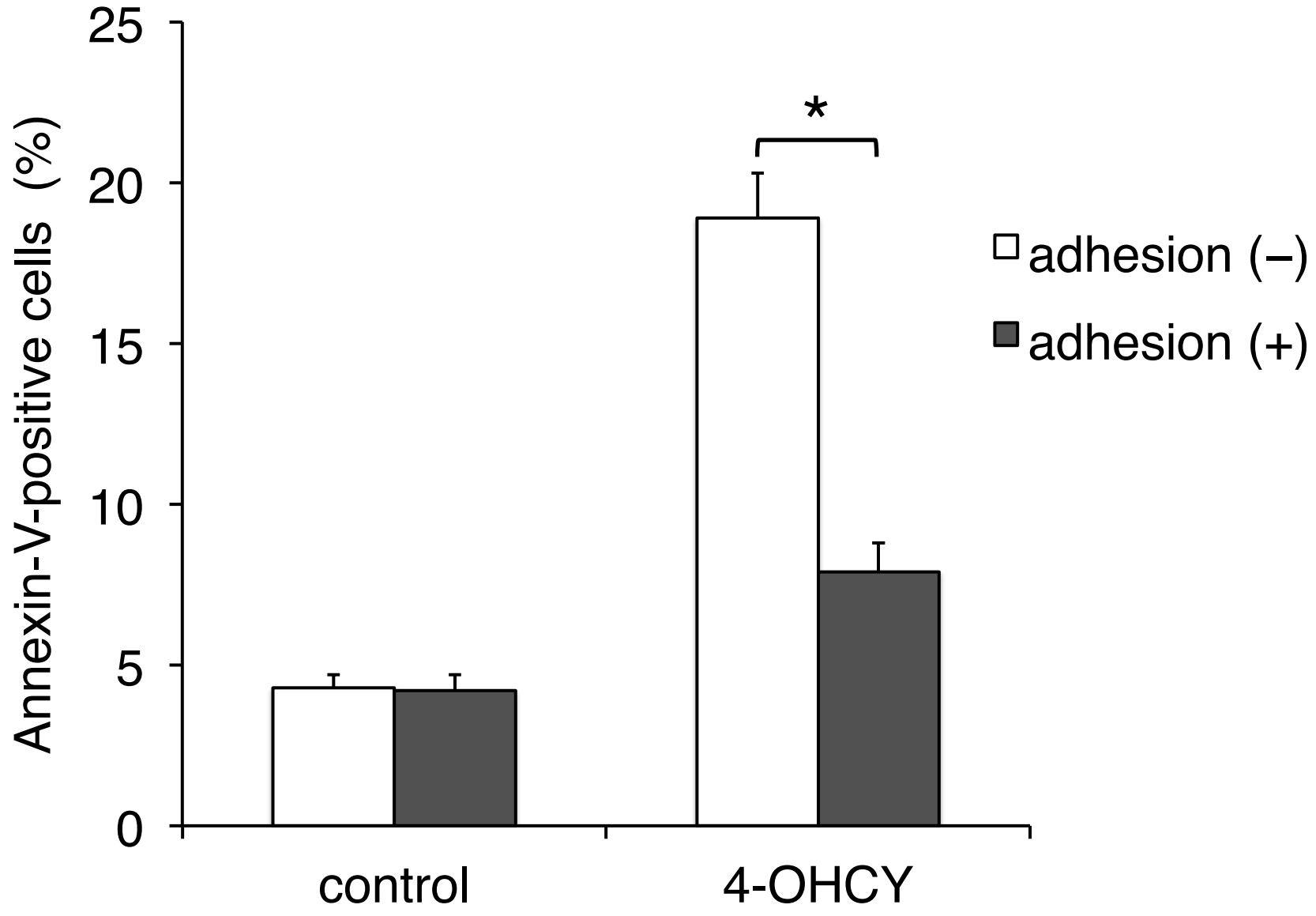
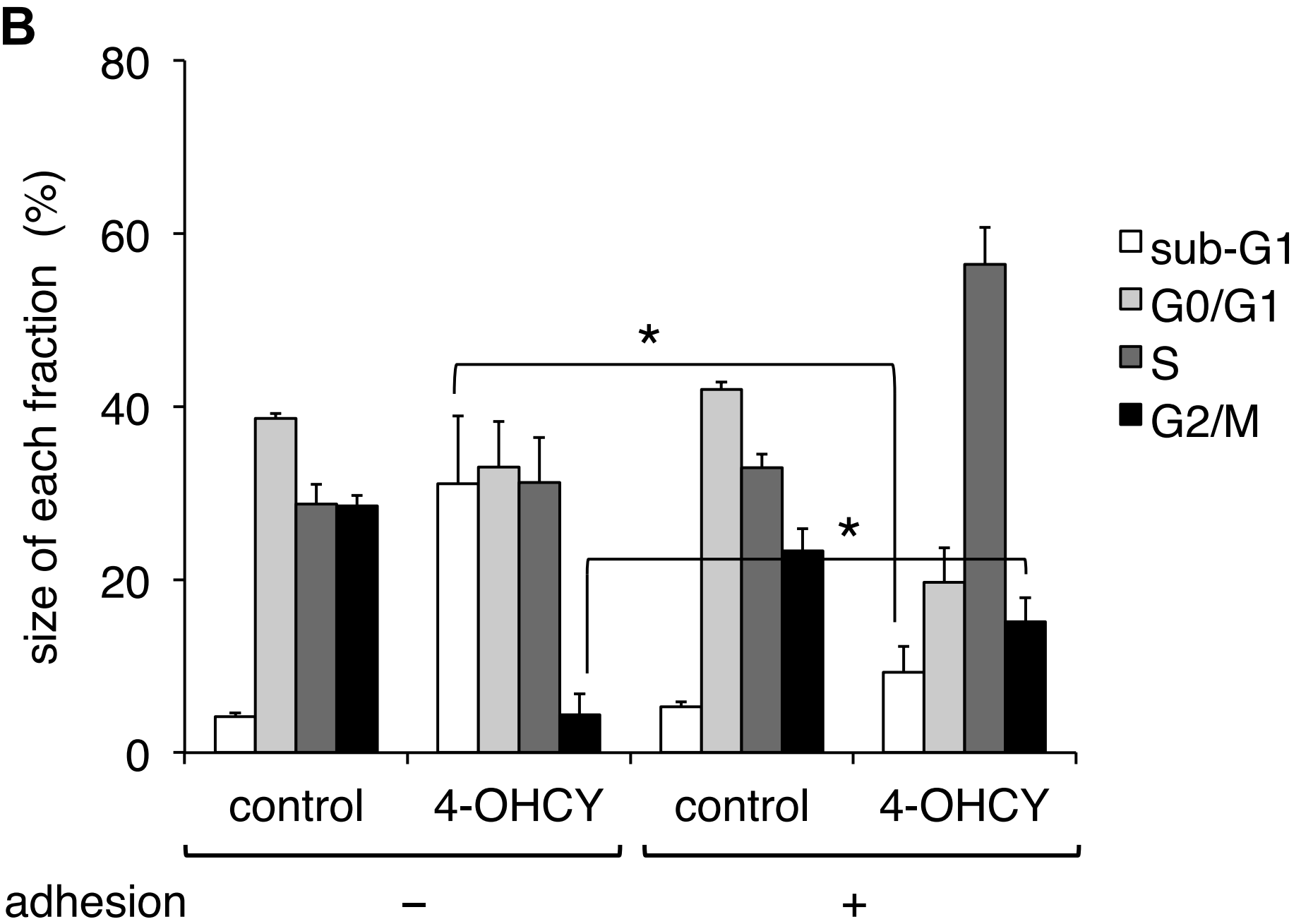
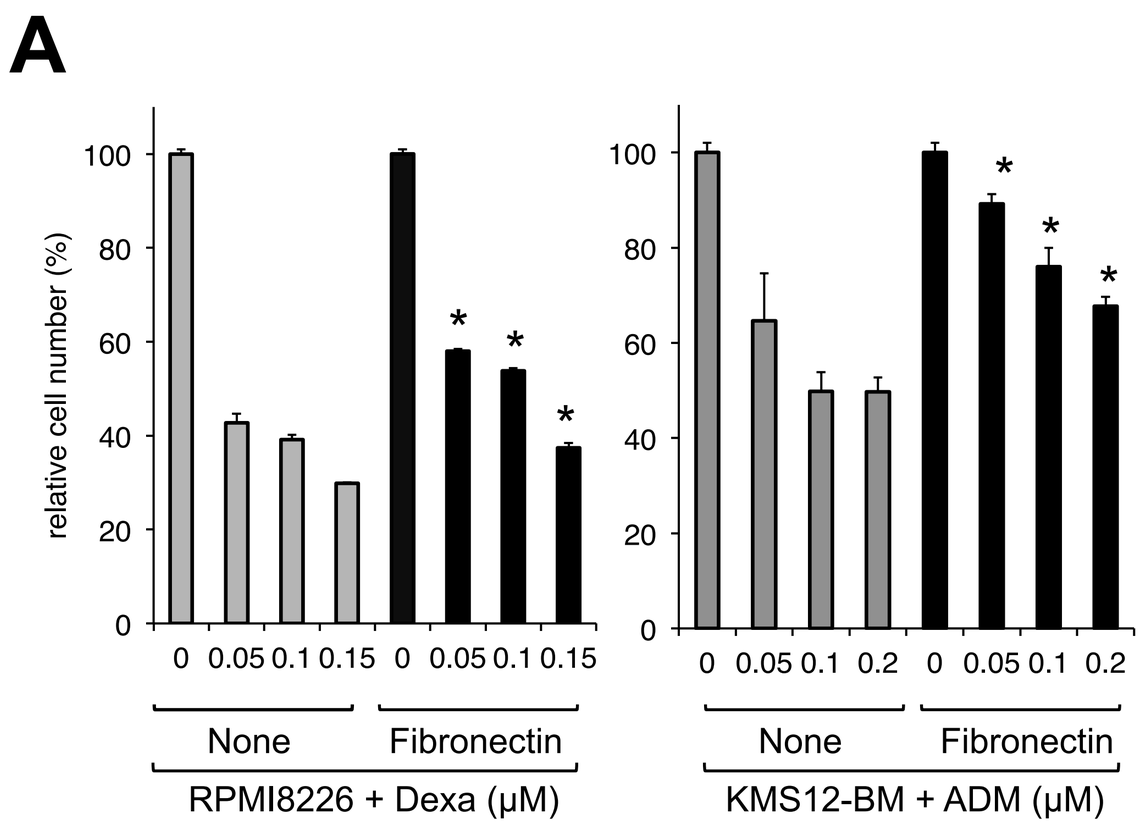
A

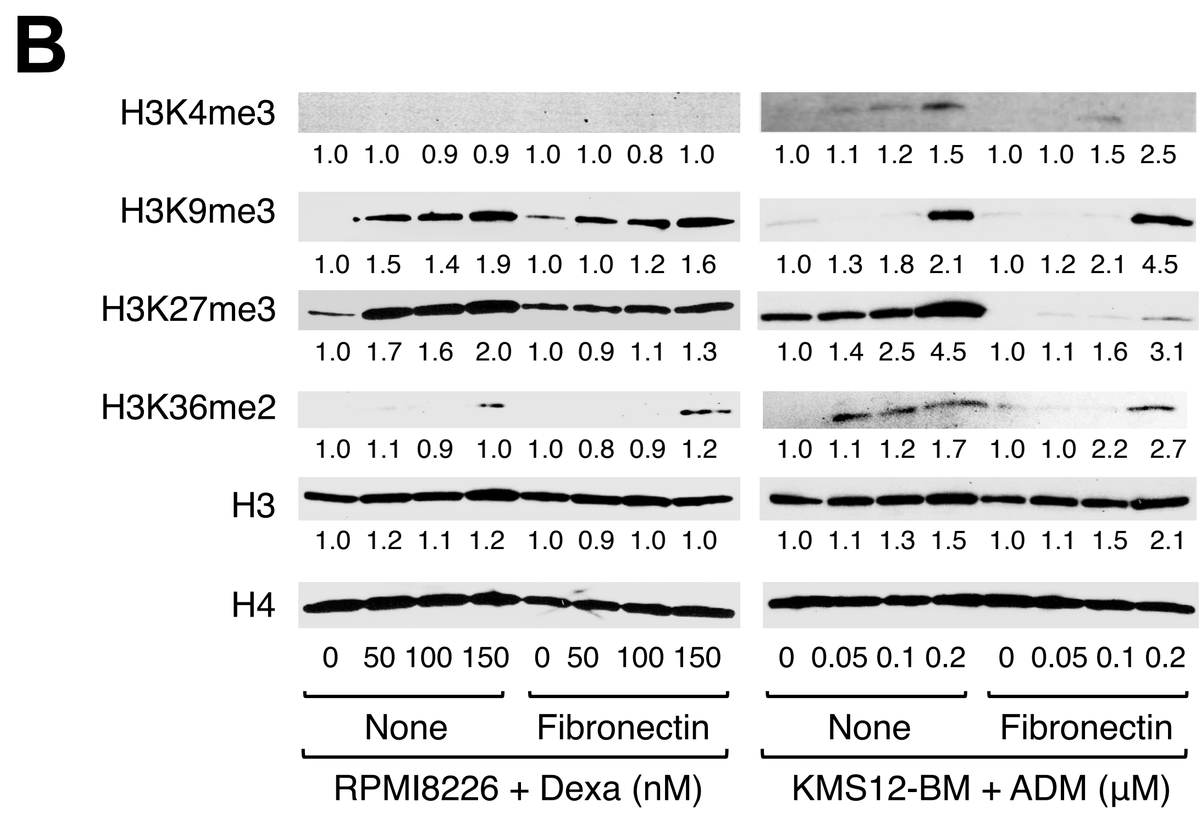

Annexin-V-positive cells (%)
25
20
15
10
5
0
*
adhesion (–)
adhesion (+)
control
4-OHCY

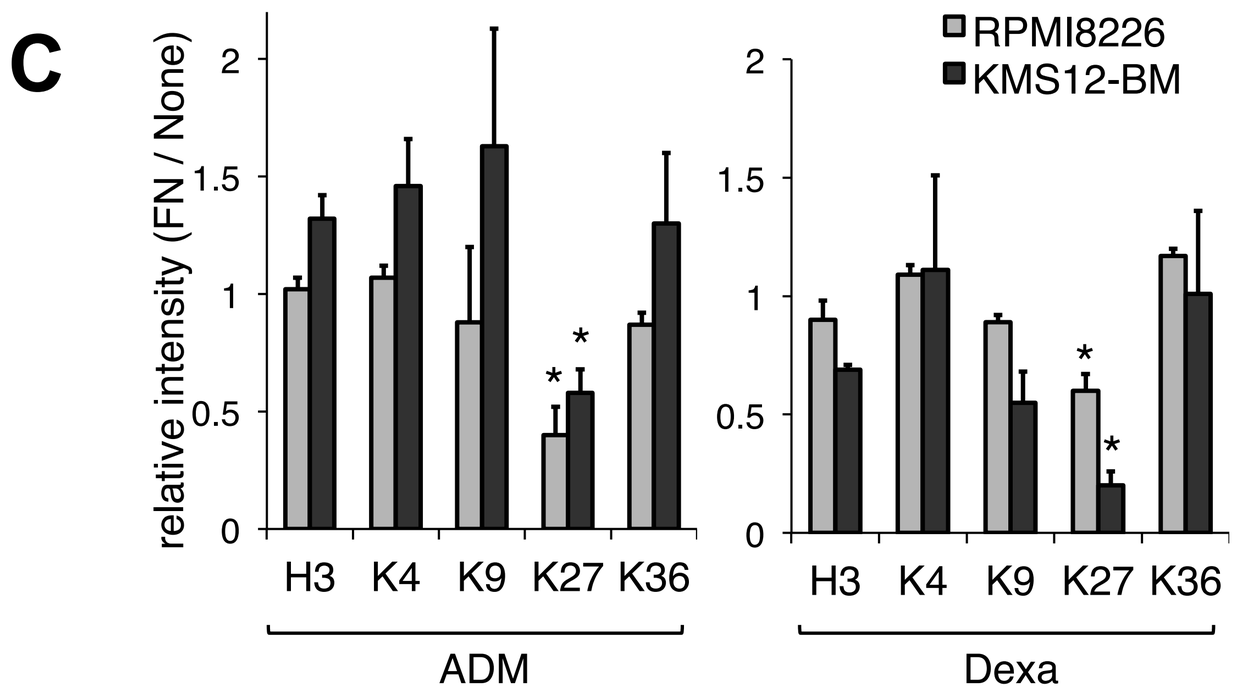
B

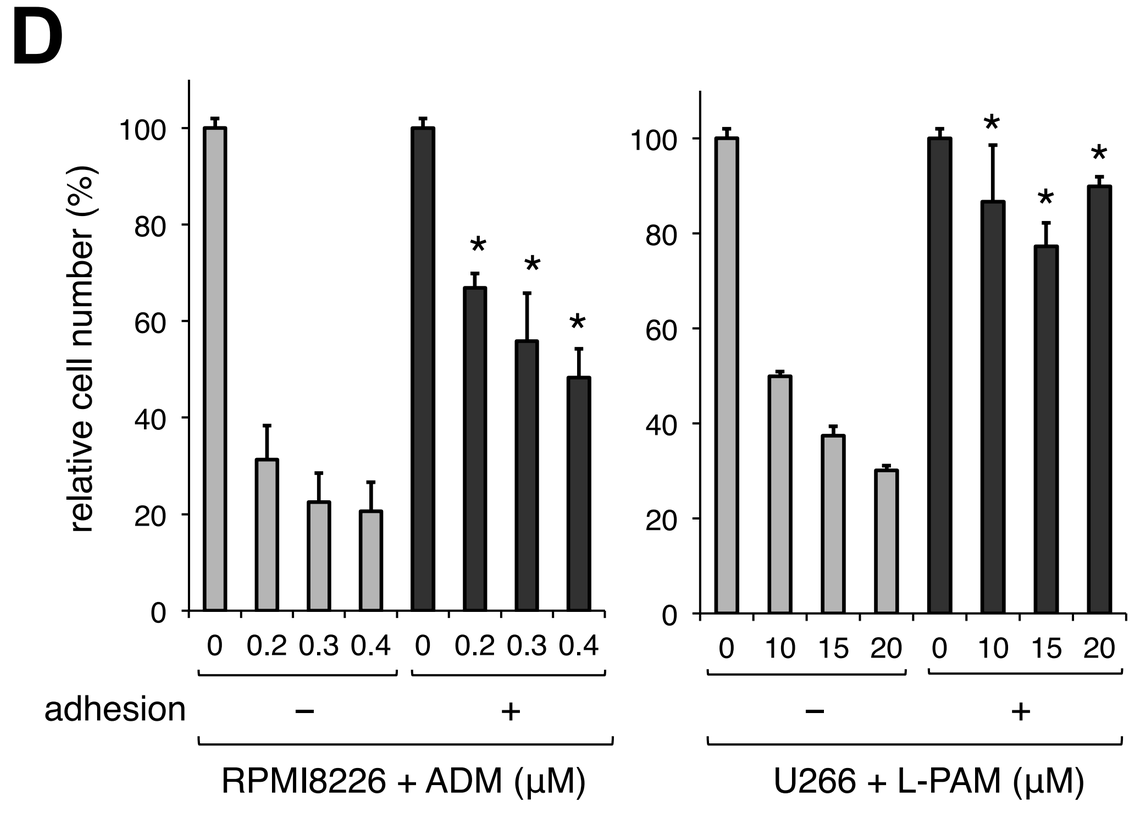

size of each fraction (%)
80
60
40
20
0
*
*
sub-G1
G0/G1
S
G2/M
control
4-OHCY
control
4-OHCY
adhesion
–
+

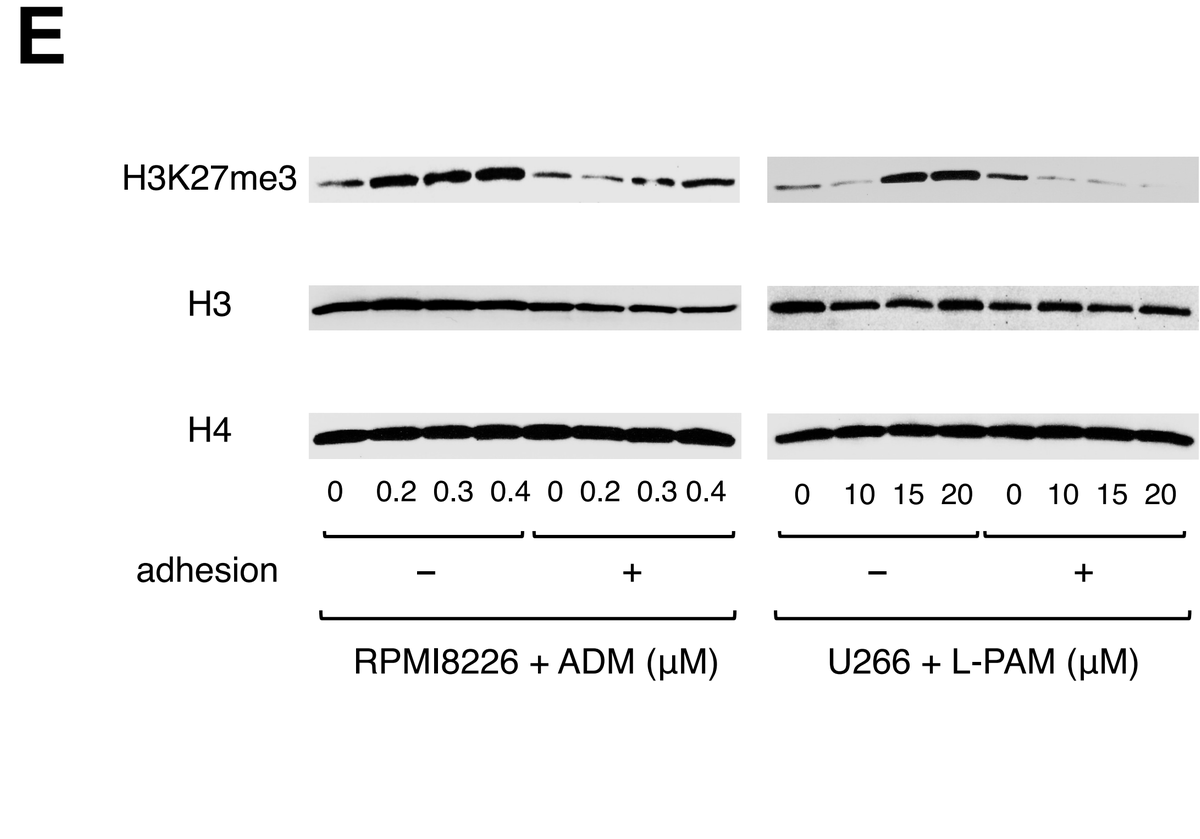
Supplemental Figure 1. RPMI8226 cells were cultured in the absence or presence of 4-OHCY in a cell culture insert with or without direct adhesion to UBE6T-7 cells. After 48 hours of culture, cells were stained with annexin-V/PE for apoptotic cell detection (A) and propidium iodide for cell cycle analysis (B). The size of the sub-G1, G0/G1, S, and G2/M fractions was calculated as a percentage by analyzing DNA histograms with the ModFitLT 2.0 program (Becton-Dickinson). The means ± S.D. (bars) of three independent experiments are shown. *P*-values were calculated by one-way ANOVA with the Student-Newman-Keuls multiple comparisons test. Asterisks indicate $P < 0.05$.

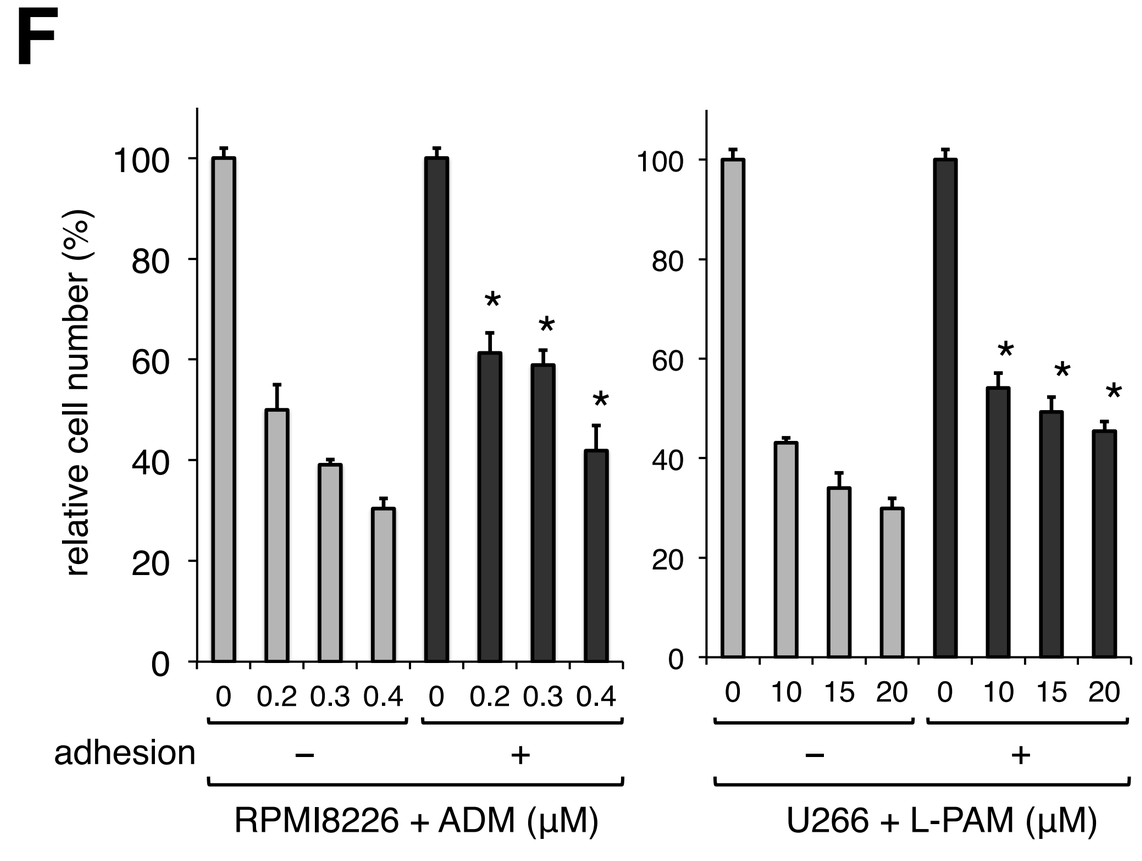

A
relative cell number (%)
100
80
60
40
20
0
0 0.05 0.1 0.15 0 0.05 0.1 0.15
None
Fibronectin
RPMI8226 + Dexa (μM)
0 0.05 0.1 0.2 0 0.05 0.1 0.2
None
Fibronectin
KMS12-BM + ADM (μM)

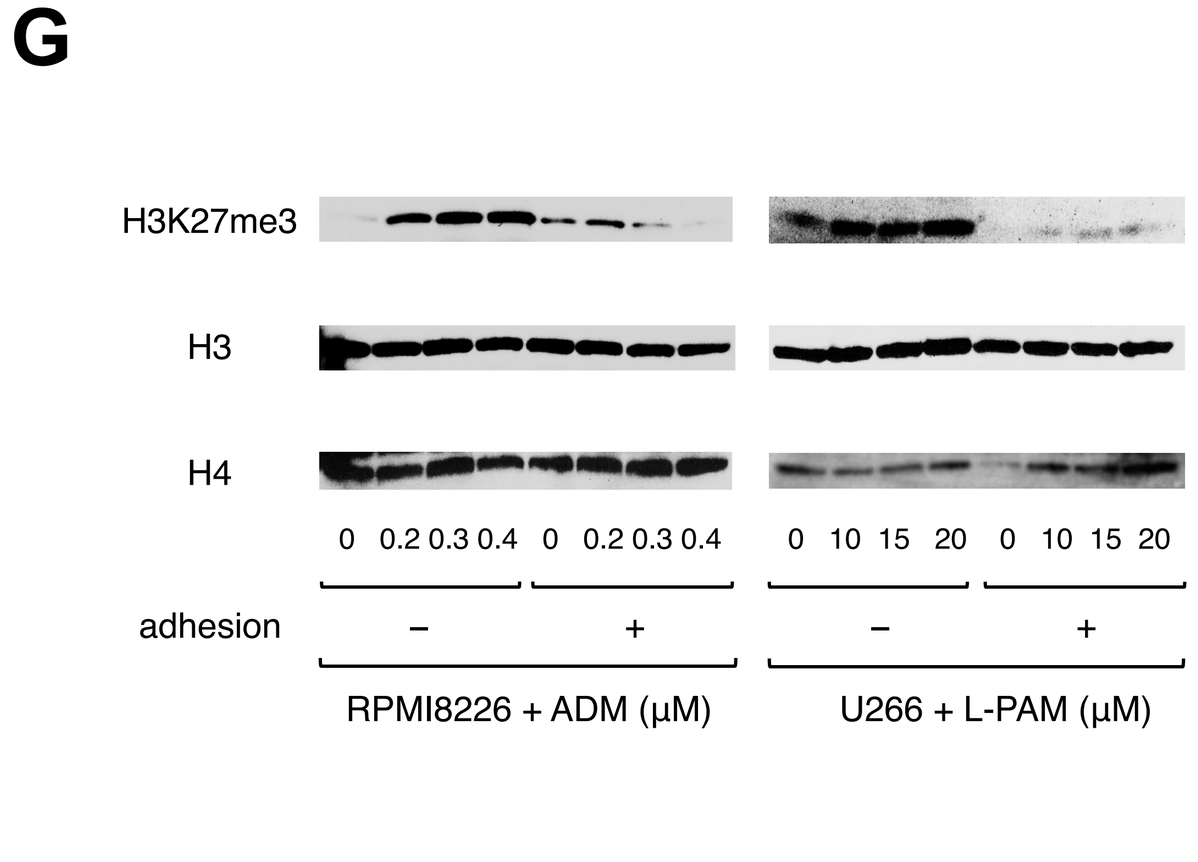

B
H3K4me3
1.0 1.0 0.9 0.9 1.0 1.0 0.8 1.0
1.0 1.1 1.2 1.5 1.0 1.0 1.5 2.5
H3K9me3
1.0 1.5 1.4 1.9 1.0 1.0 1.2 1.6
1.0 1.3 1.8 2.1 1.0 1.2 2.1 4.5
H3K27me3
1.0 1.7 1.6 2.0 1.0 0.9 1.1 1.3
1.0 1.4 2.5 4.5 1.0 1.1 1.6 3.1
H3K36me2
1.0 1.1 0.9 1.0 1.0 0.8 0.9 1.2
1.0 1.1 1.2 1.7 1.0 1.0 2.2 2.7
H3
1.0 1.2 1.1 1.2 1.0 0.9 1.0 1.0
1.0 1.1 1.3 1.5 1.0 1.1 1.5 2.1
H4
0 50 100 150 0 50 100 150
0 0.05 0.1 0.2 0 0.05 0.1 0.2
None
Fibronectin
None
Fibronectin
RPMI8226 + Dexa (nM)
KMS12-BM + ADM (μM)


C
relative intensity (FN / None)
2
1.5
1
0.5
0
RPMI8226
KMS12-BM
H3 K4 K9 K27 K36
H3 K4 K9 K27 K36
ADM
Dexa


D
relative cell number (%)
100
80
60
40
20
0
0 0.2 0.3 0.4 0 0.2 0.3 0.4
adhesion
–
+
RPMI8226 + ADM (μM)
0 10 15 20 0 10 15 20
–
+
U266 + L-PAM (μM)

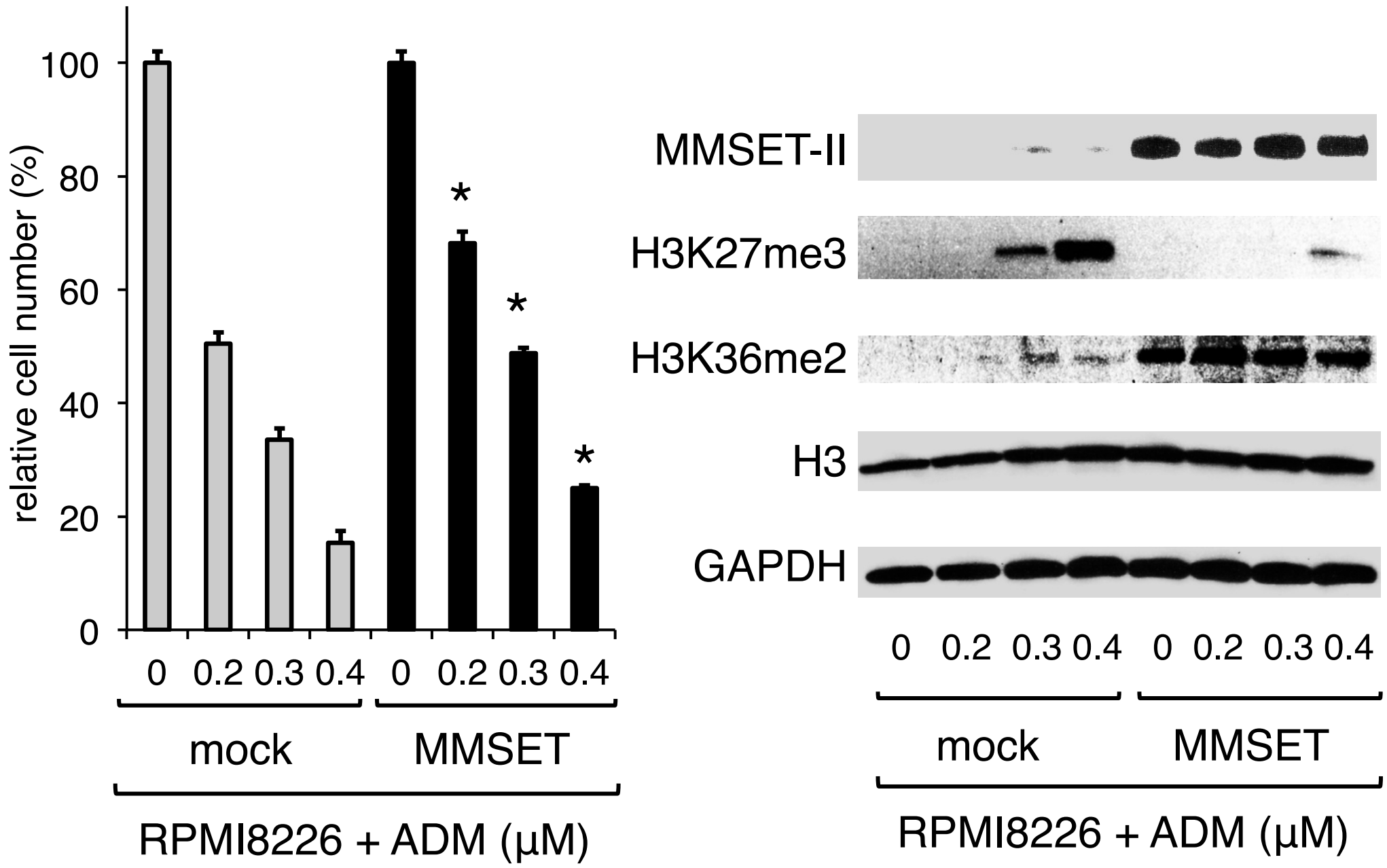

E
H3K27me3
H3
H4
0 0.2 0.3 0.4 0 0.2 0.3 0.4
0 10 15 20 0 10 15 20
adhesion
–
+
–
+
RPMI8226 + ADM (μM)
U266 + L-PAM (μM)


F
relative cell number (%)
100
80
60
40
20
0
0 0.2 0.3 0.4 0 0.2 0.3 0.4
adhesion
–
+
RPMI8226 + ADM (μM)
0 10 15 20 0 10 15 20
–
+
U266 + L-PAM (μM)

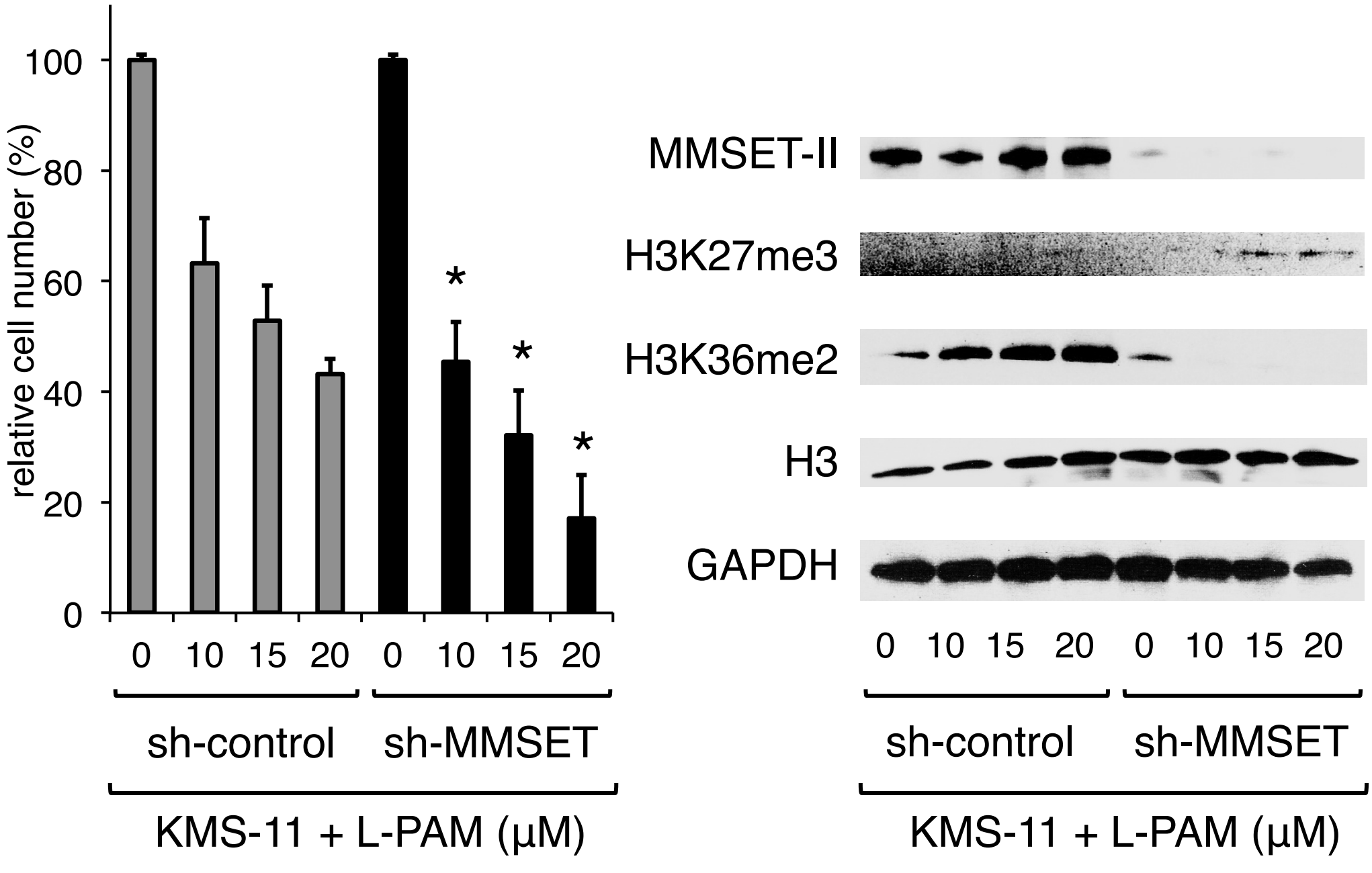

G
H3K27me3
H3
H4
0 0.2 0.3 0.4 0 0.2 0.3 0.4
0 10 15 20 0 10 15 20
adhesion
–
+
–
+
RPMI8226 + ADM (μM)
U266 + L-PAM (μM)

Supplemental Figure 2. [A] RPMI8226 and KMS12-BM cells were cultured in the absence or presence of either dexamethasone (Dexa) (left panel) or doxorubicin (ADM) (right panel) with or without fibronectin. After 72 hours of culture, cells were harvested and subjected to the MTT reduction assay for cell proliferation, which is expressed as a percentage of the values of corresponding non-adherent cells. The means ± S.D. (bars) of three independent experiments are shown (*P <0.05 determined by one-way ANOVA with the Student-Newman-Keuls multiple comparisons test). [B] Whole cell lysates were prepared after 48 hours for immunoblotting. The signal intensities of each band were quantified, normalized to those of the corresponding histone H3, and shown as relative values setting untreated controls at 1.0. [C] Relative intensities of fibronectin-positive (FN) against fibronectin-negative (None) samples at the dose of 0.2 μM ADM and 150 nM Dexa, respectively, in panel B. *P <0.05 against the value of histone H3 calculated by one-way ANOVA with the Student-Newman-Keuls multiple comparisons test (n=3). [D] RPMI8226 and U266 cells were cultured in the absence or presence of either ADM (left panel) or melphalan (L-PAM) (right panel) in a cell culture insert with or without direct adhesion to stroma-NK cells. Cells were harvested after 72 hours and subjected to the MTT reduction assay for cell proliferation. Asterisks indicate P <0.05 between adhesion (–) and (+) determined by one-way ANOVA with the Student-Newman-Keuls multiple comparisons test (n=3). [E] Whole cell lysates were prepared after 48 hours during the experiments described in panel D for immunoblotting. [F] The same experiments were performed with MM patient-derived MSCs instead of stroma-NK cells. [G] Whole cell lysates were prepared after 48 hours during the experiments described in panel F for immunoblotting.

A

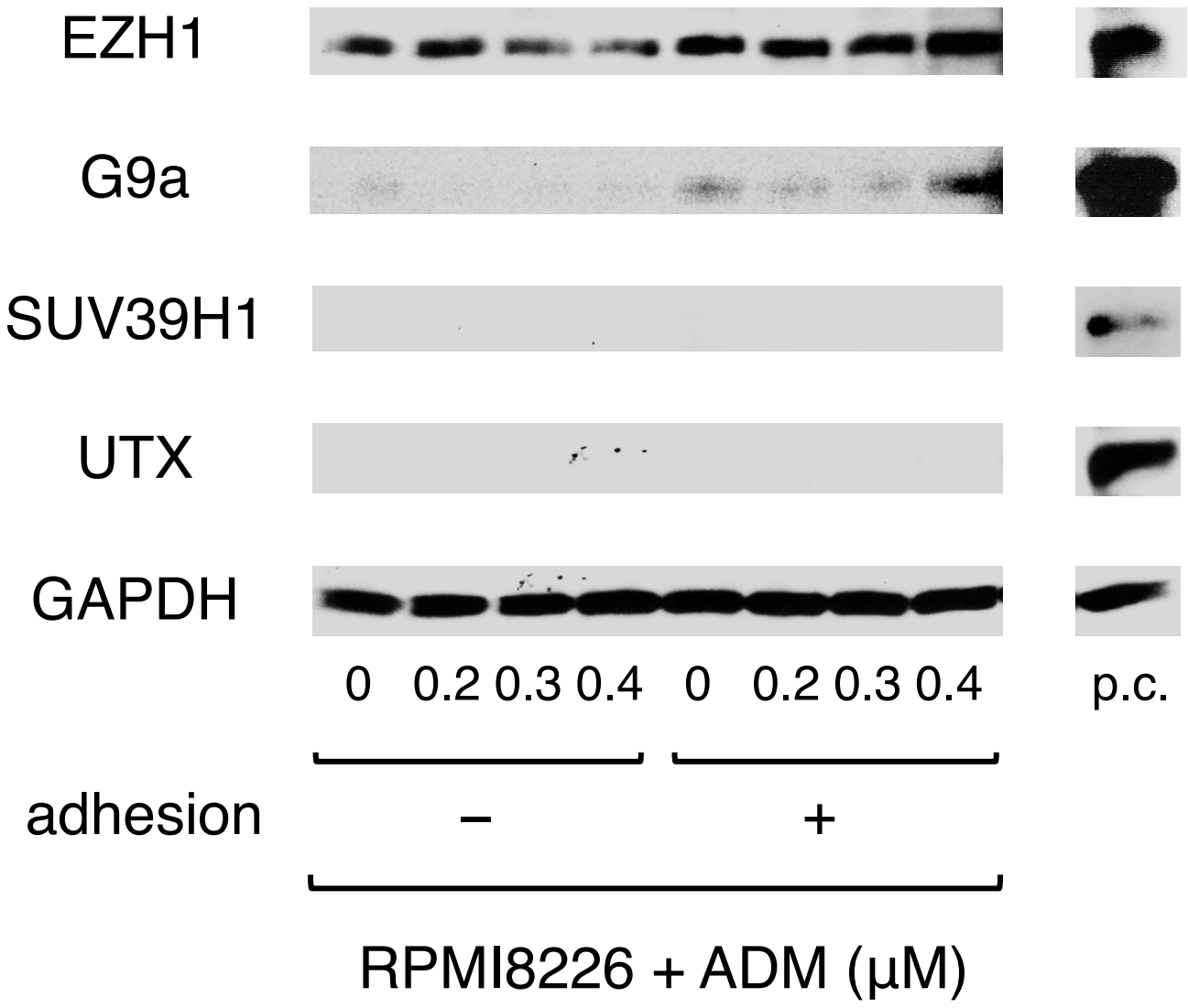

relative cell number (%)
100
80
60
40
20
0
*
*
*
0 0.2 0.3 0.4 0 0.2 0.3 0.4
mock MMSET
RPMI8226 + ADM (μM)
MMSET-II
H3K27me3
H3K36me2
H3
GAPDH
0 0.2 0.3 0.4 0 0.2 0.3 0.4
mock MMSET
RPMI8226 + ADM (μM)

B

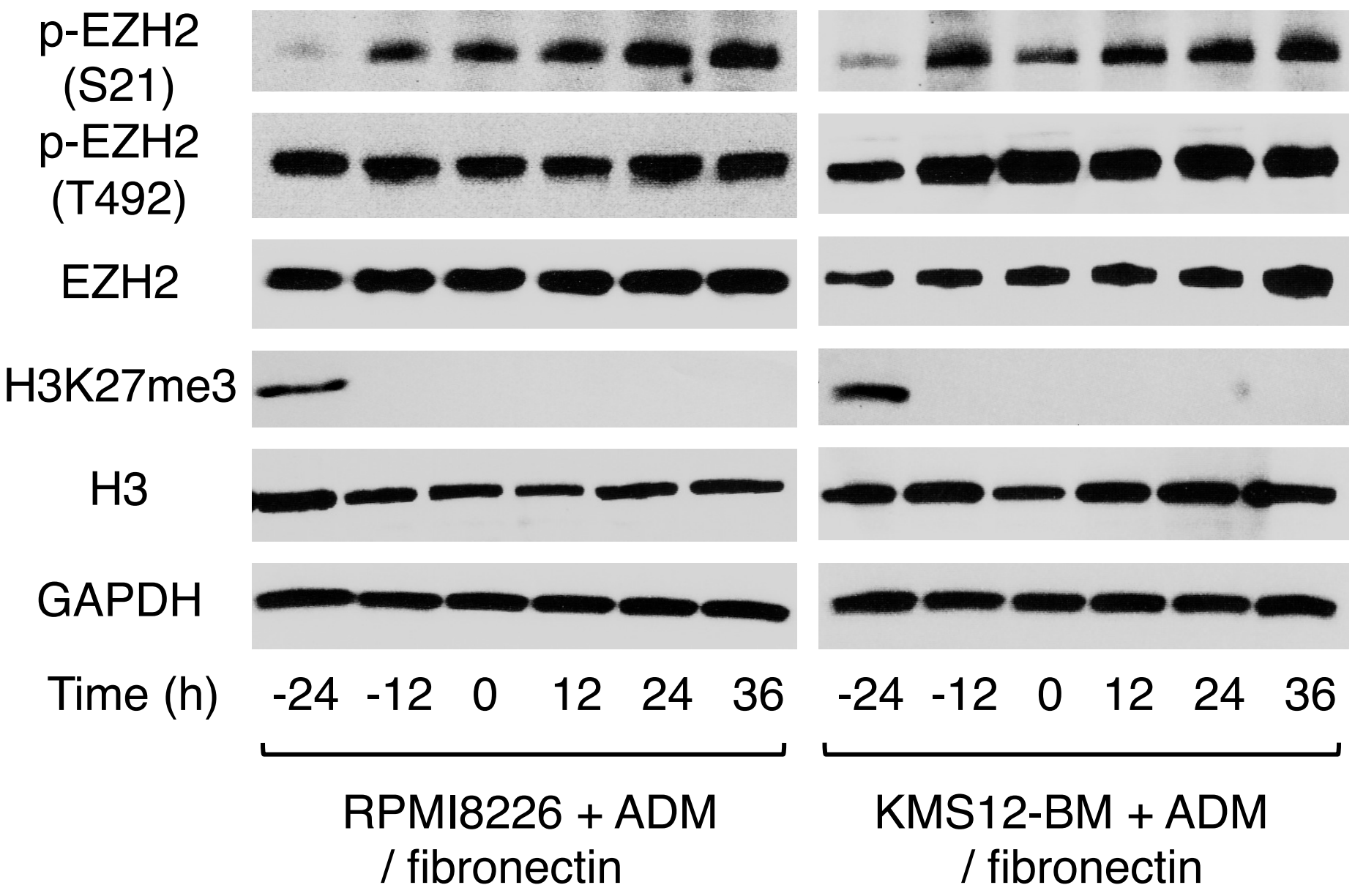

relative cell number (%)
100
80
60
40
20
0
*
*
*
0 10 15 20 0 10 15 20
sh-control sh-MMSET
KMS-11 + L-PAM (μM)
MMSET-II
H3K27me3
H3K36me2
H3
GAPDH
0 10 15 20 0 10 15 20
sh-control sh-MMSET
KMS-11 + L-PAM (μM)

Supplemental Figure 3. [A] RPMI8226 cells were transduced with either the CSII-VENUS (mock) or CSII-VENUS-MMSET (MMSET) vector and cultured in the absence or presence of doxorubicin (ADM) for 72 hours (left panel). Asterisks indicate $P < 0.05$ against the mock control determined by one-way ANOVA with the Student-Newman-Keuls multiple comparisons test (n=3). Whole cell lysates were prepared simultaneously for immunoblotting (right panel). [B] KMS-11 cells were transduced with either the pLL3.7-sh-control (sh-control) or pLL3.7-sh-MMSET (sh-MMSET) vector and cultured in the absence or presence of melphalan (L-PAM) for 72 hours (left panel). Asterisks indicate $P < 0.05$ against sh-control determined by one-way ANOVA with the Student-Newman-Keuls multiple comparisons test (n=3). Whole cell lysates were prepared simultaneously for immunoblotting (right panel).

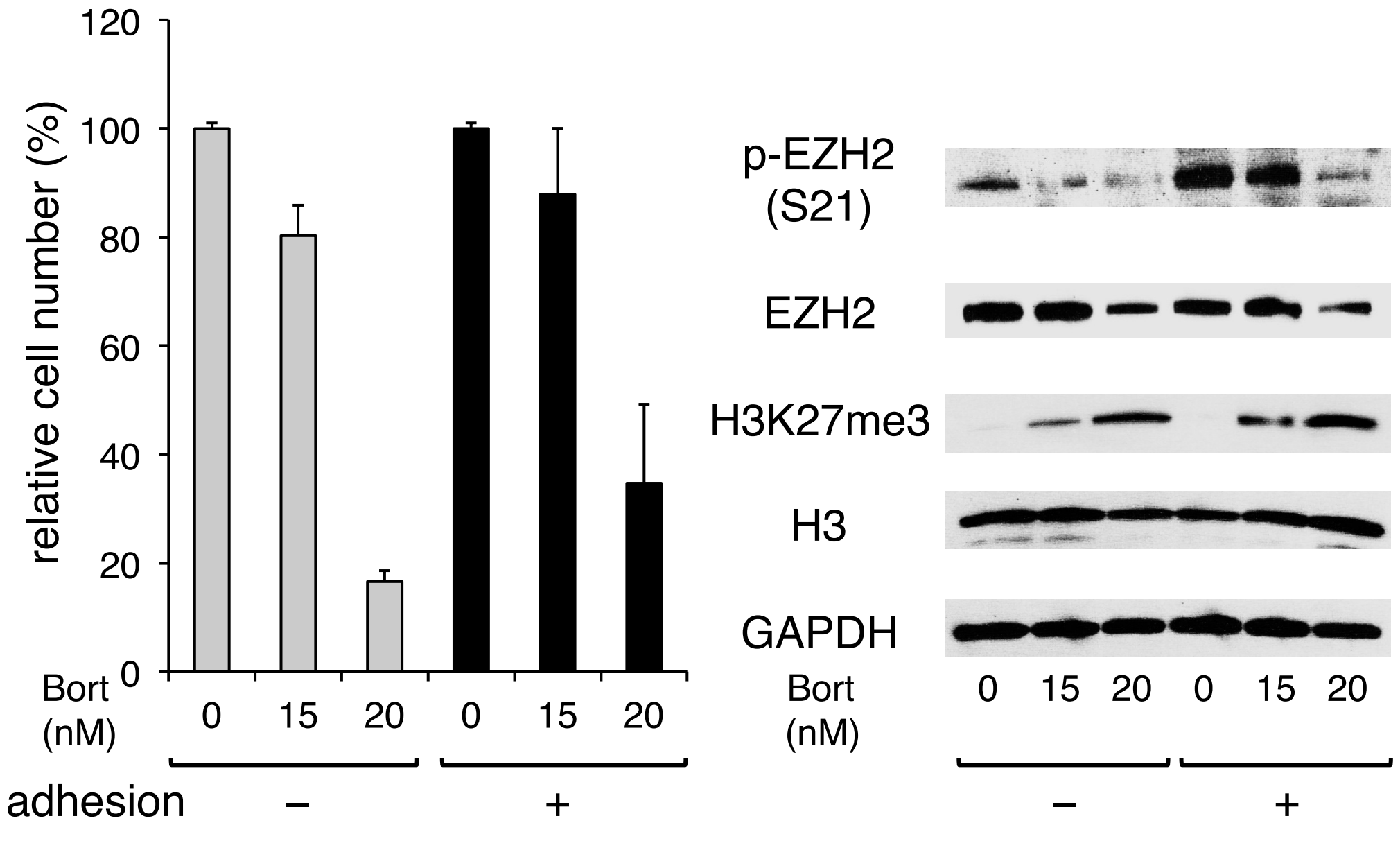
A


EZH1
G9a
SUV39H1
UTX
GAPDH
0 0.2 0.3 0.4 0 0.2 0.3 0.4
p.c.
adhesion – +
RPMI8226 + ADM (μM)

B

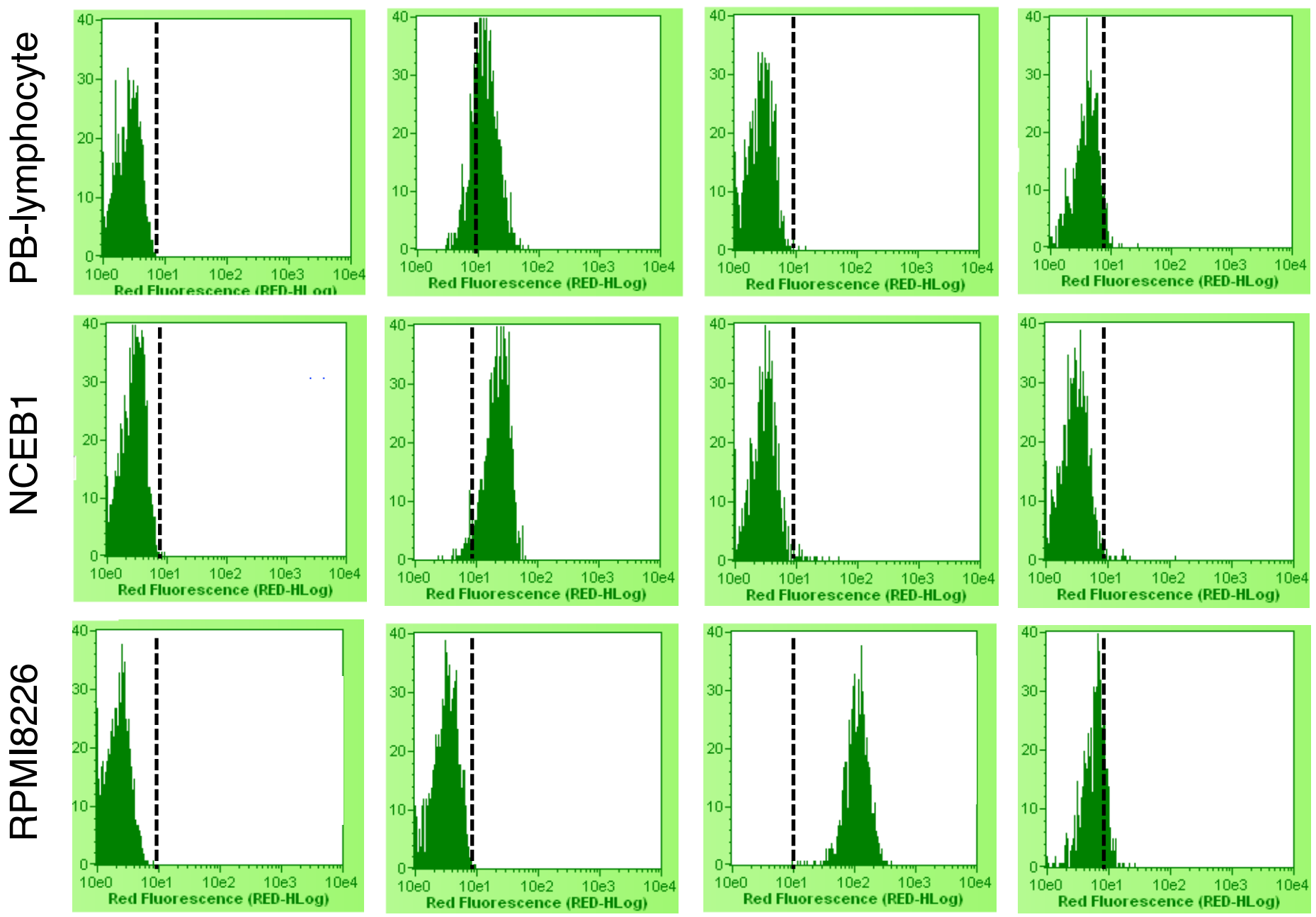

p-EZH2 (S21)
p-EZH2 (T492)
EZH2
H3K27me3
H3
GAPDH
Time (h) -24 -12 0 12 24 36 -24 -12 0 12 24 36
RPMI8226 + ADM / fibronectin
KMS12-BM + ADM / fibronectin

C

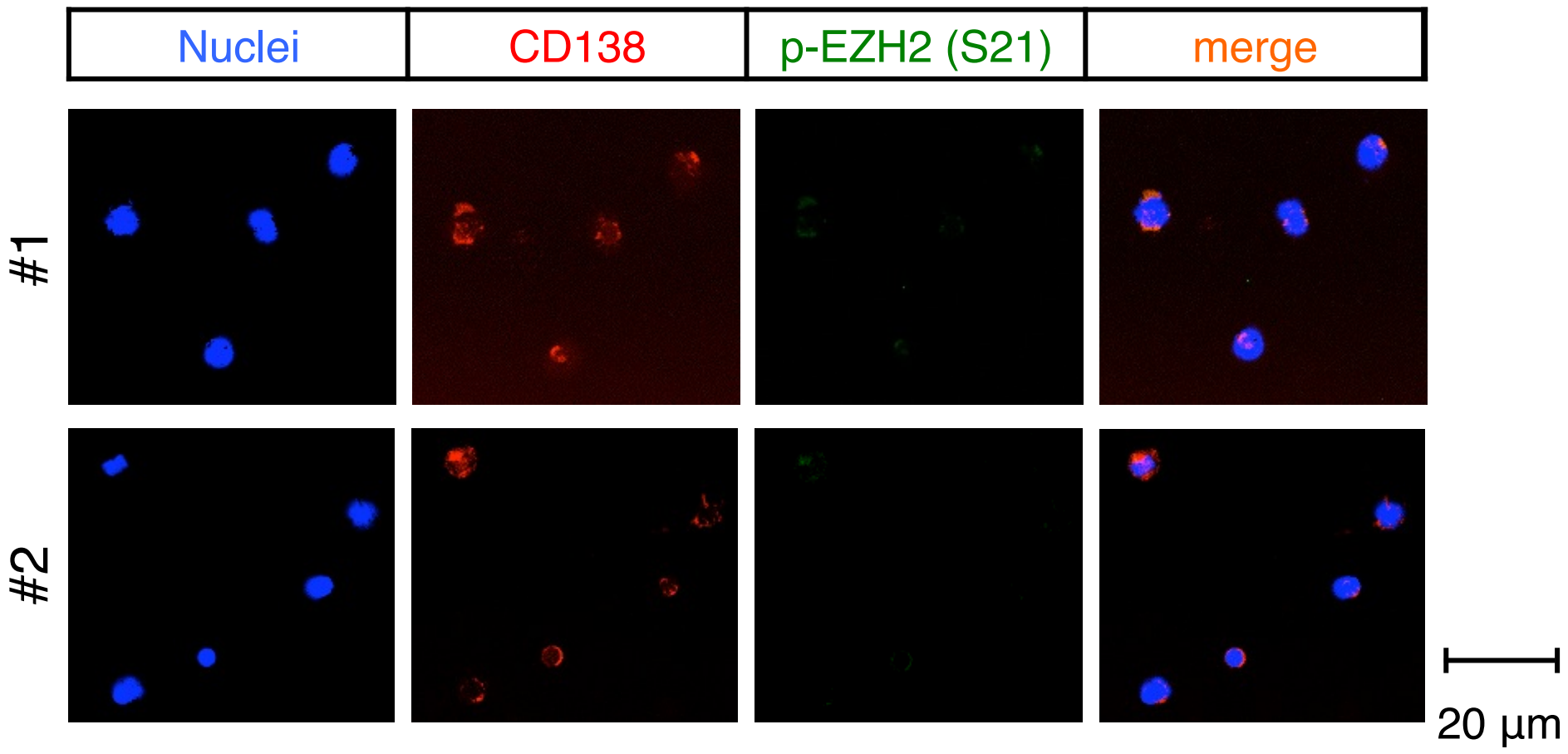

relative cell number (%)
120
100
80
60
40
20
0
Bort (nM)
0 15 20 0 15 20
adhesion – +
p-EZH2 (S21)
EZH2
H3K27me3
H3
GAPDH
Bort (nM)
0 15 20 0 15 20
– +

Supplemental Figure 4. [A] Whole cell lysates were prepared at 48 hours during the experiments described in Figure 2A for immunoblotting. We used HEK293T cells transduced with *G9a* and *SUV39H1* cDNA as positive controls (indicated as p.c. in the figure). [B] RPMI8226 and KMS12-BM cells were cultured in the presence of fibronectin for 24 hours, and further incubated for 36 hours after the addition of 0.4 μM doxorubicin (ADM) (T-0). Whole cell lysates were prepared at the indicated time points and subjected to immunoblotting. [C] KMS12-BM cells were cultured with or without direct adhesion to UBE6T-7 cells in the presence of the indicated concentrations of bortezomib (Bor). We performed the MTT reduction assay (left panel) and immunoblotting (right panel) after 24 hours. No significant difference in cell numbers between adhesion (–) and (+) conditions at the corresponding doses of bortezomib.

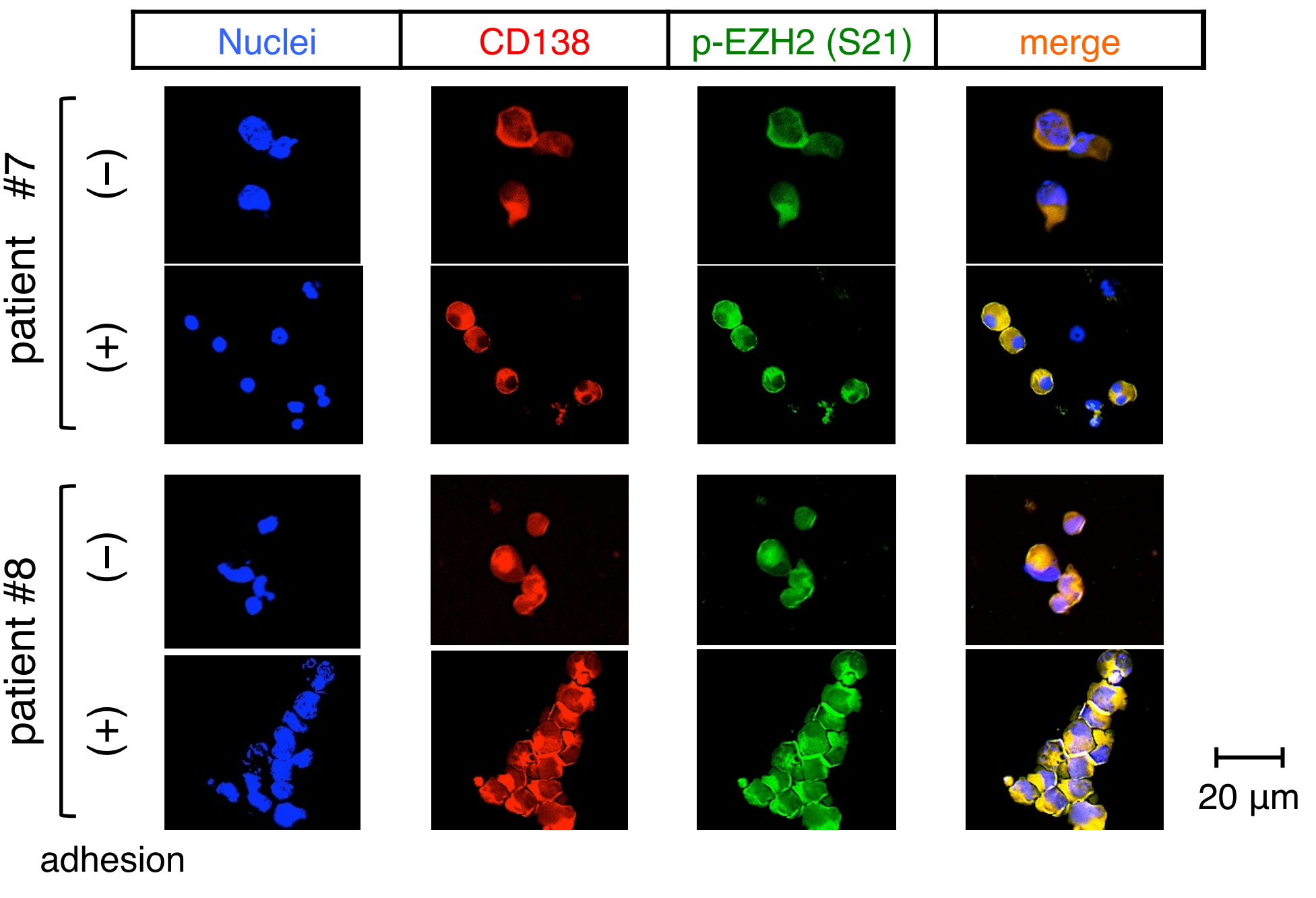
A


Iso
CD19
CD38
CD138
PB-lymphocyte
NCEB1
RPMI8226
Red Fluorescence (RED-HLog)

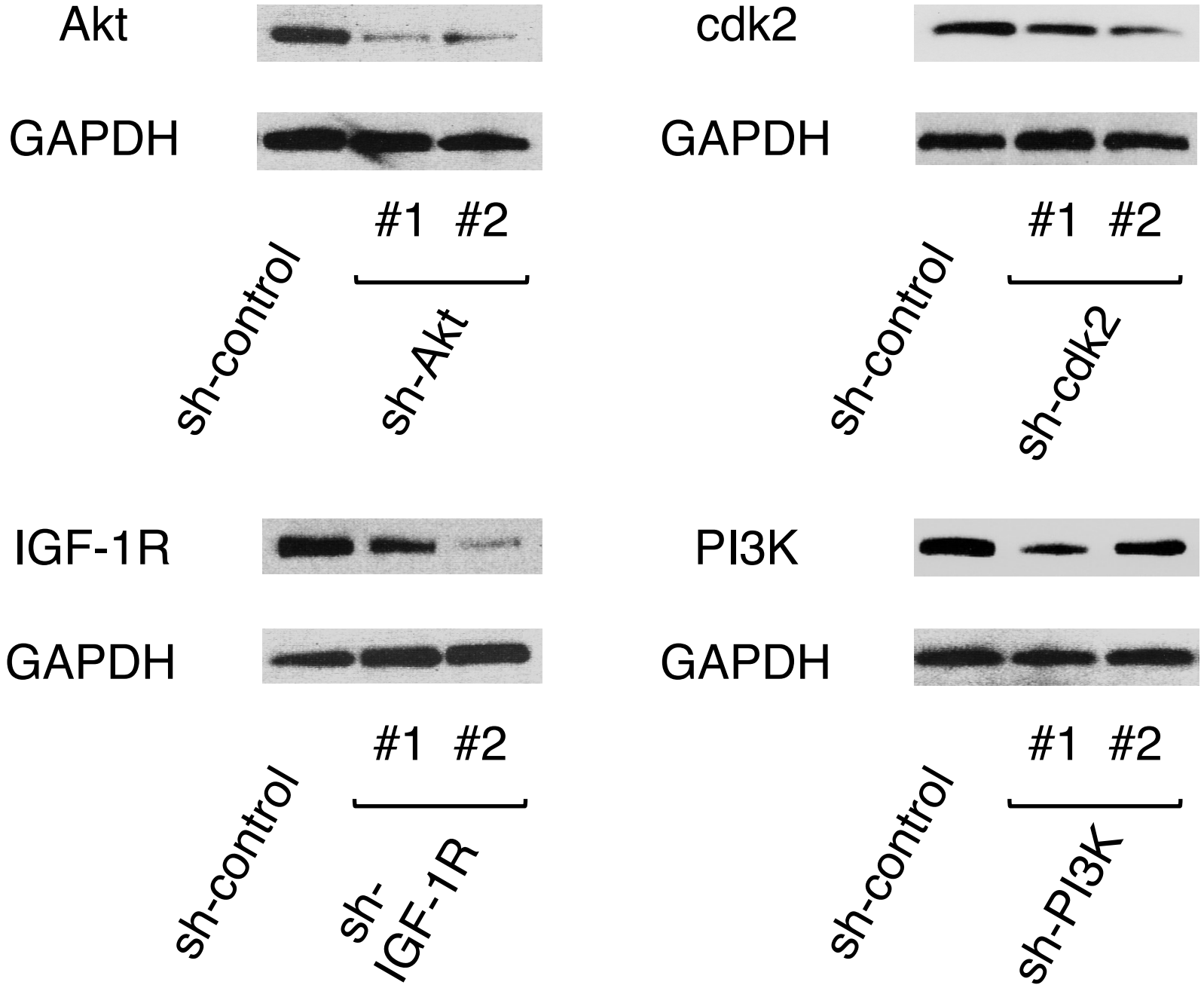
B


Nuclei
CD138
p-EZH2 (S21)
merge
#1
#2
20 μm

C

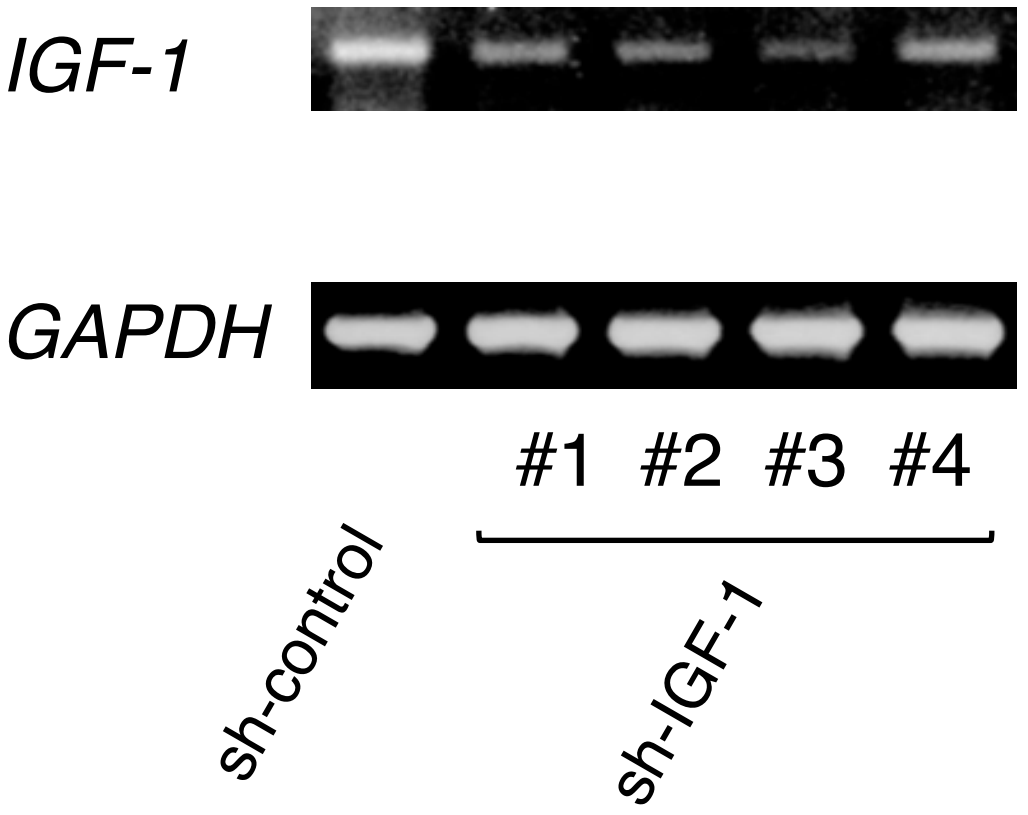

Nuclei
CD138
p-EZH2 (S21)
merge
patient #7
patient #8
(−)
(+)
20 μm
adhesion

Supplemental Figure 5. [A] Flow cytometric analysis of the expression of CD19, CD38 and CD138 on normal peripheral blood (PB) lymphocytes, the mantle cell lymphoma cell line NCEB1 and RPMI8226 cells. Iso: isotype-matched control. [B] We stained normal plasma cells from healthy volunteers with anti-phospho-EZH2 (S21), followed by Alexa Fluor 488-conjugated anti-rabbit IgG, and PE-conjugated anti-CD138 antibodies, which yield green and red fluorescence, respectively. [C] We cultured myeloma patient-derived BM mononuclear cells with (+) or without (–) BMSCs for 24 hours and stained with anti-phospho-EZH2 (S21), followed by Alexa Fluor 488-conjugated anti-rabbit IgG, and PE-conjugated anti-CD138 antibodies, which yield green and red fluorescence, respectively. Nuclei were counterstained with DAPI (blue). Scale bars indicate 20 μm.

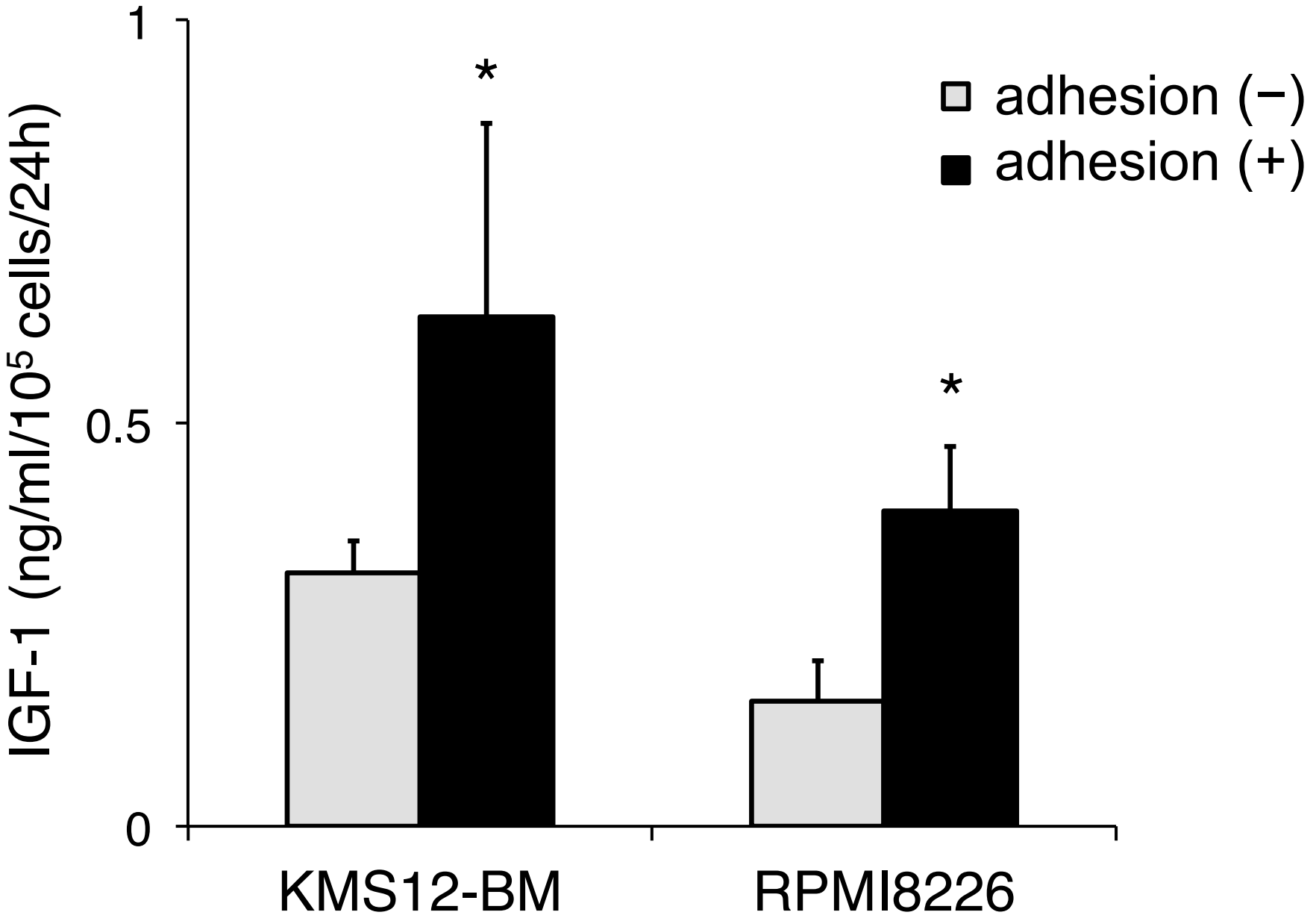

Akt
GAPDH
#1
#2
sh-control
sh-Akt
cdk2
GAPDH
#1
#2
sh-control
sh-cdk2
IGF-1R
GAPDH
#1
#2
sh-control
sh-IGF-1R
PI3K
GAPDH
#1
#2
sh-control
sh-PI3K

Supplemental Figure 6. RPMI8226 cells were transduced with pLL3.7-sh-Akt (#1 and #2), sh-cdk2 (#1 and #2), sh-IGF-1R (#1 and #2), sh-PI3K (#1 and #2) or sh-control (sh-control) lentiviral vector. After 48 hours of culture, whole cell lysates was isolated and subjected to immunoblotting. The membranes were reprobed with anti-GAPDH antibody to serve as a loading control.

A

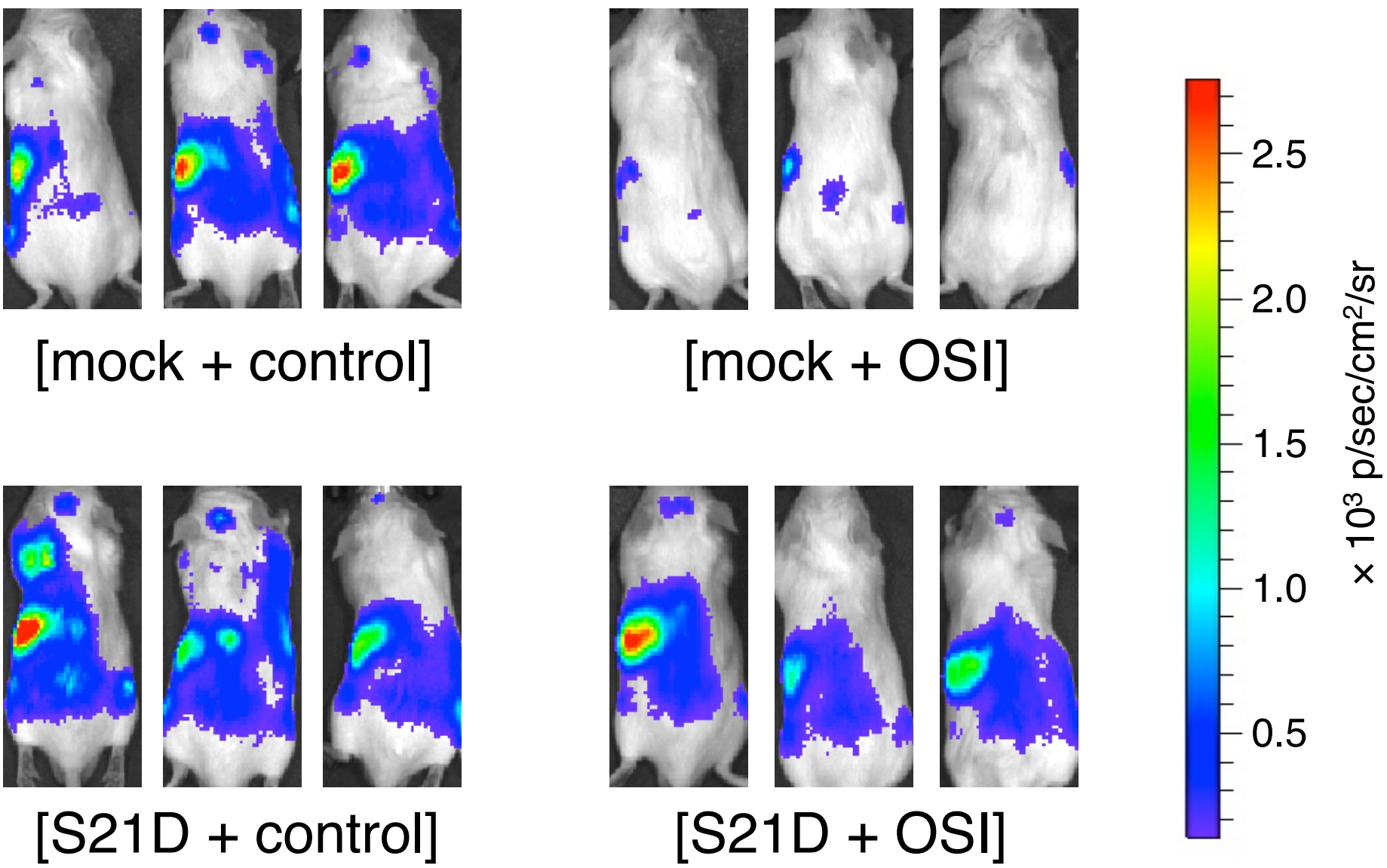

IGF-1
GAPDH
#1 #2 #3 #4
sh-control
sh-IGF-1

B

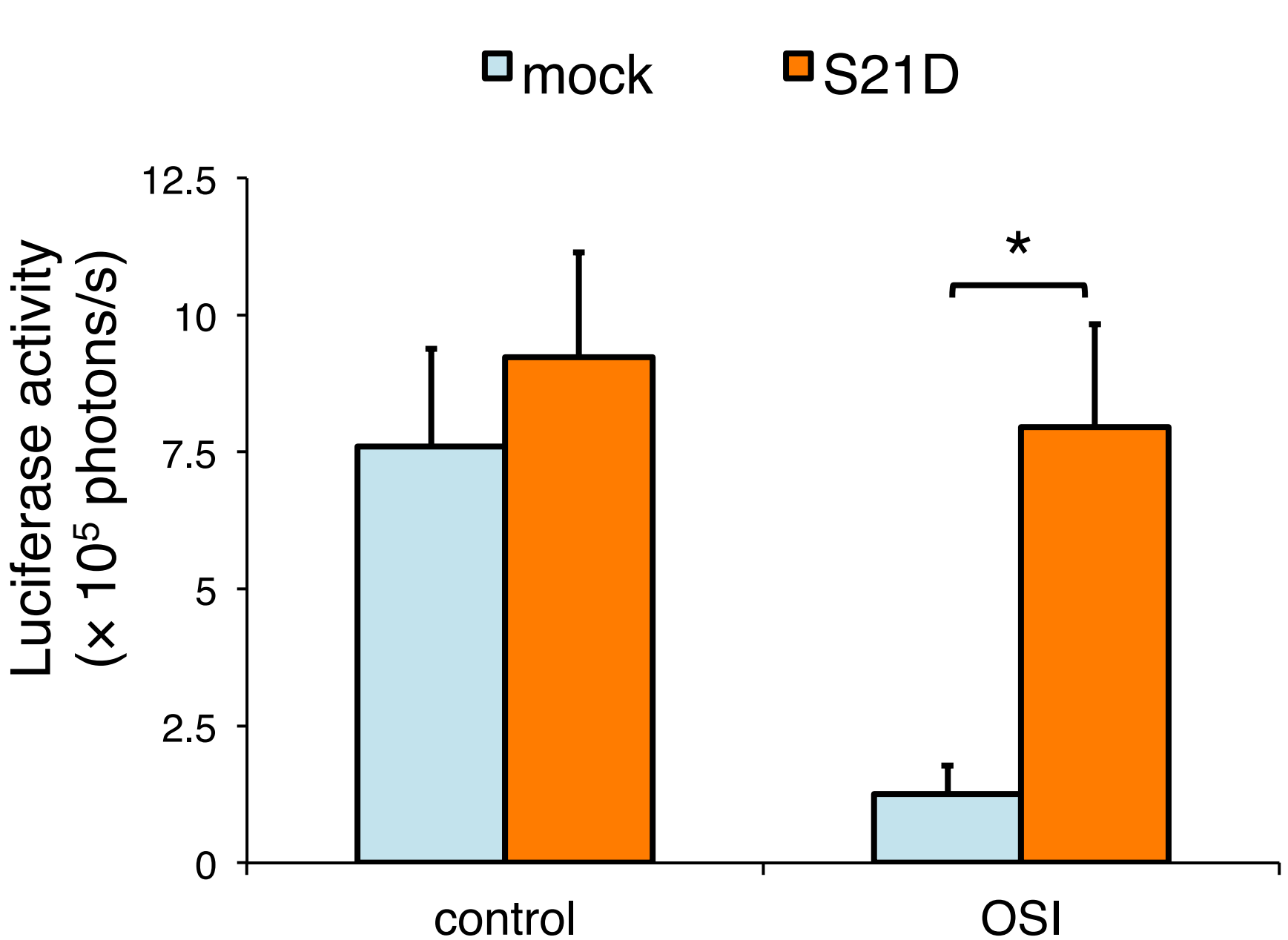

IGF-1 (ng/ml/10^5 cells/24h)
1
0.5
0
*
*
adhesion (−)
adhesion (+)
KMS12-BM
RPMI8226

Supplemental Figure 7. [A] RPMI8226 cells were transduced with either pLL3.7-sh-IGF-1 (#1~#4) or scrambled control (sh-control) lentiviral vector. After 48 hours of culture, total cellular RNA was isolated and subjected to semi-quantitative RT-PCR analysis for the expression of *IGF1* and *GAPDH* (internal control). The amplified products were visualized by ethidium bromide staining after 2% agarose gel electrophoresis. The results of suboptimal amplification cycles (35 cycles) are shown. Detailed information on primers, including sequences, corresponding nucleotide positions and PCR product sizes, is shown in Table S2. [B] KMS12-BM and RPMI8226 cells were cultured in a cell culture insert with or without adhesion to BMSCs for 48 hours, followed by additional 24 h-culture after removal of BMSCs. IGF-1 concentration in the culture medium was measured by a Human IGF-1 Quantikine ELISA Kit (R&D Systems). The means ± S.D. (bars) of three independent experiments are shown (*P <0.05 determined by one-way ANOVA with the Student-Newman-Keuls multiple comparisons test).

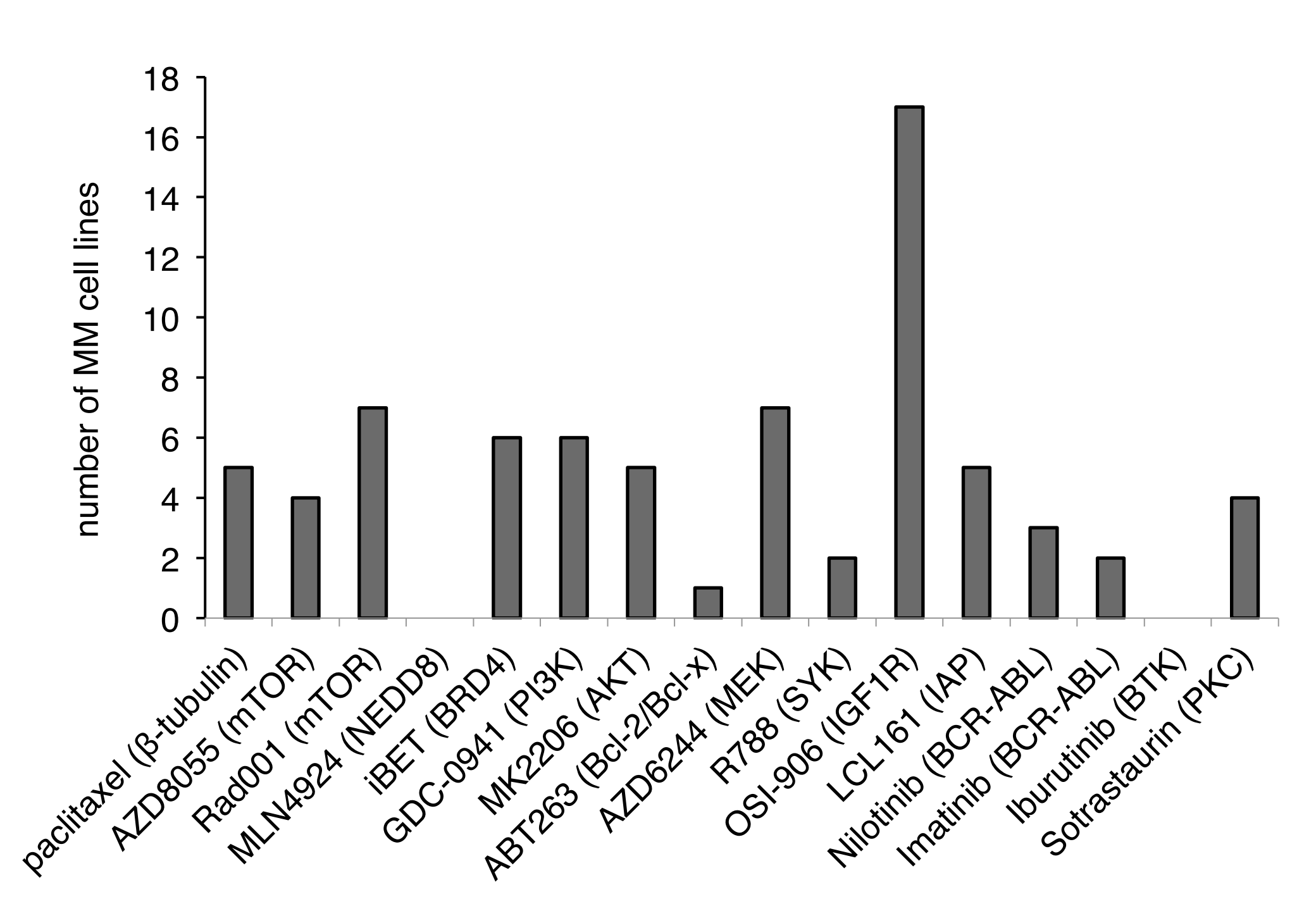
A


[mock + control]
[mock + OSI]
[S21D + control]
[S21D + OSI]
2.5
2.0
1.5
1.0
0.5
× 10³ p/sec/cm²/sr

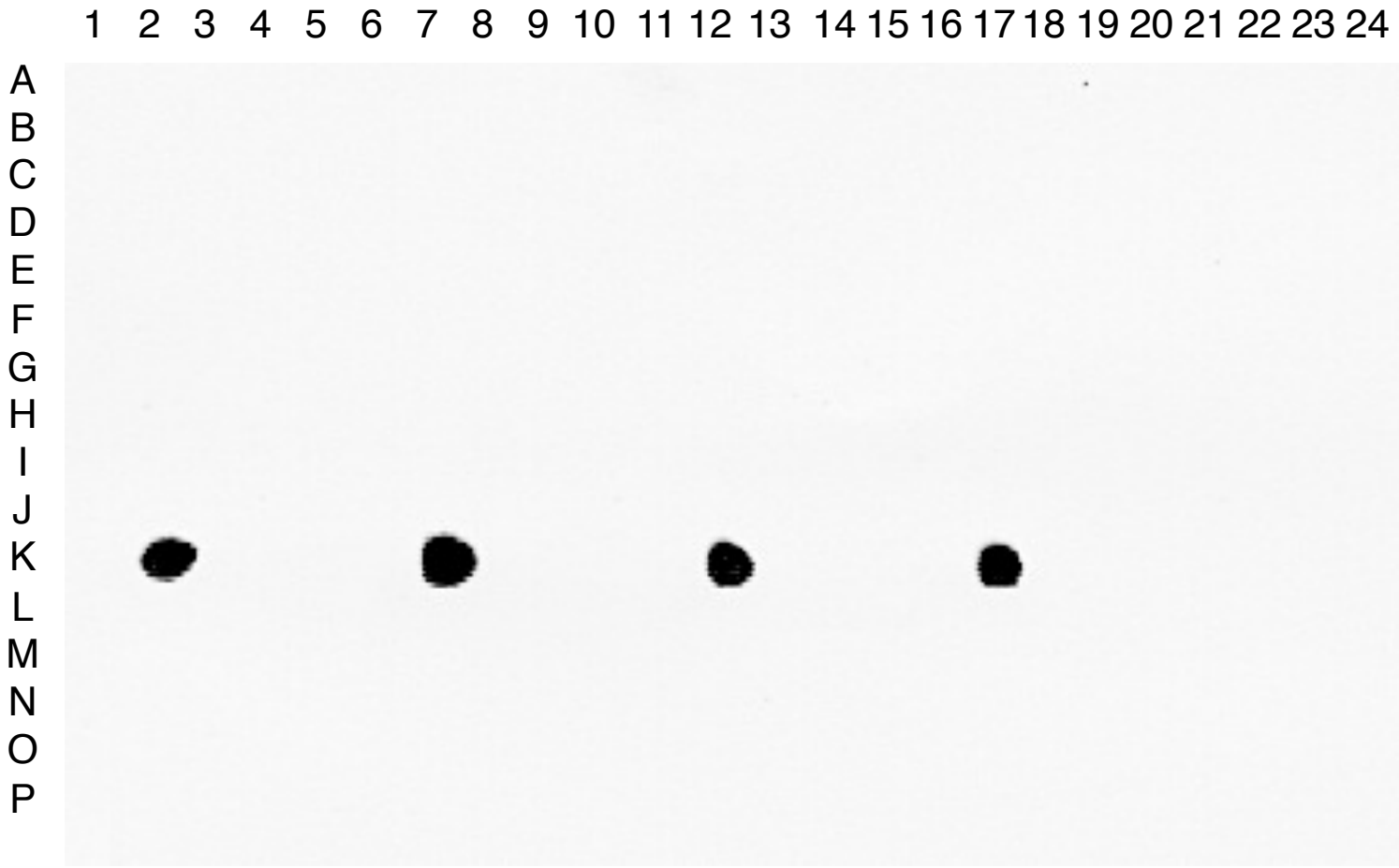
B


mock
S21D
Luciferase activity
(x 10⁵ photons/s)
12.5
10
7.5
5
2.5
0
*
control
OSI

Supplemental Figure 8. [A] MOPC315.BM.Luc cells were transduced with either the CSII-VENUS (mock) or CSII-VENUS carrying EZH2-S21D (S21D) lentiviral vector. We injected 3×10^5 transduced luciferase-expressing MOPC315.BM.Luc cells intravenously into Balb/c mice and treated them in two ways: the vehicle alone (control) and 30 mg/kg OSI-906 (OSI). Treatments were started 2 weeks after transplantation, defined as day 0. *In vivo* luciferase activity of the whole body was measured by the IVIS Imaging System on day 14. Photographs of all Balb/c mice on day 14 are shown (original magnification: ×2). [B] Quantitative data of *in vivo* bioluminescence imaging expressed as photon units (photons/s). Asterisks indicate $P < 0.05$ determined by one-way ANOVA with the Tukey's multiple comparison test (n=3).


number of MM cell lines
0
2
4
6
8
10
12
14
16
18
paclitaxel (β-tubulin)
AZD8055 (mTOR)
Rad001 (mTOR)
MLN4924 (NEDD8)
iBET (BRD4)
GDC-0941 (PI3K)
MK2206 (AKT)
ABT263 (Bcl-2/Bcl-x)
AZD6244 (MEK)
R788 (SYK)
OSI-906 (IGF1R)
LCL161 (IAP)
Nilotinib (BCR-ABL)
Imatinib (BCR-ABL)
Iburutinib (BTK)
Sotrastaurin (PKC)

Supplemental Figure 9. The number of MM cell lines ranked in the top 30 sensitive cell lines to each inhibitor (target molecules are indicated in parentheses). Data are modified from Rahal et al. (38).


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P

Supplemental Figure 10. A histone peptide array was subjected to immunoblotting with anti-histone H3K27me3 antibody (Cell Signaling Technology, #9733) according to the manufacturer's instruction to validate the specificity of the antibody. The array contains 59 different post-translational modifications, including acetylation, methylation, phosphorylation and citrullination, on N-terminal tails of histones H2A, H2B, H3 and H4 (Active Motif, #13001 and #13005). Signals were specifically detected at K2, K7, K12 and K17, on which histone H3 peptides with tri-methylation at lysine 27 (H3K27me3) were spotted. Designation of all spots is shown below.

Active Motif
MODified™ Histone Peptide Array*
Catalog Nos. 13001 & 13005

Peptide location	Peptide sequence	to array	name	Mod1	Mod2	Mod 3	Mod 4	N-terminus
A 1	A R T K Q T A R K S T G G K A P R K Q	▶	H3 1-19	unmod				free
A 2	A Rme2s T K Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s				free
A 3	A Rme2a T K Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a				free
A 4	A Cit T K Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2Citr				free
A 5	A R pT K Q T A R K S T G G K A P R K Q	▶	H3 1-19	T3P				free
A 6	A R T Kme1 Q T A R K S T G G K A P R K Q	▶	H3 1-19	K4me1				free
A 7	A R T Kme2 Q T A R K S T G G K A P R K Q	▶	H3 1-19	K4me2				free
A 8	A R T Kme3 Q T A R K S T G G K A P R K Q	▶	H3 1-19	K4me3				free
A 9	A R T Kac Q T A R K S T G G K A P R K Q	▶	H3 1-19	K4ac				free
A10	A R T K Q T A Rme2s K S T G G K A P R K Q	▶	H3 1-19	R8me2s				free
A11	A R T K Q T A Rme2a K S T G G K A P R K Q	▶	H3 1-19	R8me2a				free
A12	A R T K Q T A Cit K S T G G K A P R K Q	▶	H3 1-19	R8Citr				free
A13	A R T K Q T A R Kme1 S T G G K A P R K Q	▶	H3 1-19	K9me1				free
A14	A R T K Q T A R Kme2 S T G G K A P R K Q	▶	H3 1-19	K9me2				free
A15	A R T K Q T A R Kme3 S T G G K A P R K Q	▶	H3 1-19	K9me3				free
A16	A R T K Q T A R Kac S T G G K A P R K Q	▶	H3 1-19	K9ac				free
A17	A R T K Q T A R K pS T G G K A P R K Q	▶	H3 1-19	S10P				free
A18	A R T K Q T A R K S pT G G K A P R K Q	▶	H3 1-19	T11P				free
A19	A R T K Q T A R K S T G G Kac A P R K Q	▶	H3 1-19	K14ac				free
A20	A Rme2s pT K Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	T3P			free
A21	A Rme2s T Kme1 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me1			free
A22	A Rme2s T Kme2 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me2			free
A23	A Rme2s T Kme3 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me3			free
A24	A Rme2s T Kac Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac			free
B 1	A Rme2a pT K Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	T3P			free
B 2	A Rme2a T Kme1 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me1			free
B 3	A Rme2a T Kme2 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me2			free
B 4	A Rme2a T Kme3 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me3			free
B 5	A Rme2a T Kac Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac			free
B 6	A Cit pT K Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2Citr	T3P			free
B 7	A Cit T Kme1 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2Citr	K4me1			free
B 8	A Cit T Kme2 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2Citr	K4me2			free
B 9	A Cit T Kme3 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2Citr	K4me3			free
B10	A Cit T Kac Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2Citr	K4ac			free
B11	A R pT Kme1 Q T A R K S T G G K A P R K Q	▶	H3 1-19	T3P	K4me1			free
B12	A R pT Kme2 Q T A R K S T G G K A P R K Q	▶	H3 1-19	T3P	K4me2			free
B13	A R pT Kme3 Q T A R K S T G G K A P R K Q	▶	H3 1-19	T3P	K4me3			free
B14	A R pT Kac Q T A R K S T G G K A P R K Q	▶	H3 1-19	T3P	K4ac			free
B15	A Rme2s pT Kme1 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	T3P	K4me1		free
B16	A Rme2s pT Kme2 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	T3P	K4me2		free
B17	A Rme2s pT Kme3 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	T3P	K4me3		free
B18	A Rme2s pT Kac Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2s	T3P	K4ac		free
B19	A Rme2a pT Kme1 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	T3P	K4me1		free
B20	A Rme2a pT Kme2 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	T3P	K4me2		free
B21	A Rme2a pT Kme3 Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	T3P	K4me3		free
B22	A Rme2a pT Kac Q T A R K S T G G K A P R K Q	▶	H3 1-19	R2me2a	T3P	K4ac		free
B23	A R T K Q T A Rme2a Kme1 S T G G K A P R K Q	▶	H3 1-19	R8me2s	K9me1			free
B24	A R T K Q T A Rme2a Kme2 S T G G K A P R K Q	▶	H3 1-19	R8me2s	K9me2			free
C 1	A R T K Q T A Rme2a Kme3 S T G G K A P R K Q	▶	H3 1-19	R8me2s	K9me3			free
C 2	A R T K Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R8me2s	K9ac			free
C 3	A R T K Q T A Rme2a K pS T G G K A P R K Q	▶	H3 1-19	R8me2s	S10P			free
C 4	A R T K Q T A Rme2a K S pT G G K A P R K Q	▶	H3 1-19	R8me2s	T11P			free
C 5	A R T K Q T A Rme2a Kme1 S T G G K A P R K Q	▶	H3 1-19	R8me2a	K9me1			free
C 6	A R T K Q T A Rme2a Kme2 S T G G K A P R K Q	▶	H3 1-19	R8me2a	K9me2			free
C 7	A R T K Q T A Rme2a Kme3 S T G G K A P R K Q	▶	H3 1-19	R8me2a	K9me3			free
C 8	A R T K Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R8me2a	K9ac			free
C 9	A R T K Q T A Rme2a K pS T G G K A P R K Q	▶	H3 1-19	R8me2a	S10P			free
C10	A R T K Q T A Rme2a K S pT G G K A P R K Q	▶	H3 1-19	R8me2a	T11P			free
C11	A R T K Q T A Cit Kme1 S T G G K A P R K Q	▶	H3 1-19	R8Citr	K9me1			free
C12	A R T K Q T A Cit Kme2 S T G G K A P R K Q	▶	H3 1-19	R8Citr	K9me2			free
C13	A R T K Q T A Cit Kme3 S T G G K A P R K Q	▶	H3 1-19	R8Citr	K9me3			free
C14	A R T K Q T A Cit Kac S T G G K A P R K Q	▶	H3 1-19	R8Citr	K9ac			free
C15	A R T K Q T A Cit K pS T G G K A P R K Q	▶	H3 1-19	R8Citr	S10P			free
C16	A R T K Q T A Cit K S pT G G K A P R K Q	▶	H3 1-19	R8Citr	T11P			free
C17	A R T K Q T A R Kme1 pS T G G K A P R K Q	▶	H3 1-19	K9me1	S10P			free
C18	A R T K Q T A R Kme1 S pT G G K A P R K Q	▶	H3 1-19	K9me1	T11P			free
C19	A R T K Q T A R Kme1 S T G G Kac A P R K Q	▶	H3 1-19	K9me1	K14ac			free
C20	A R T K Q T A R Kme2 pS T G G K A P R K Q	▶	H3 1-19	K9me2	S10P			free
C21	A R T K Q T A R Kme2 S pT G G K A P R K Q	▶	H3 1-19	K9me2	T11P			free
C22	A R T K Q T A R Kme2 S T G G Kac A P R K Q	▶	H3 1-19	K9me2	K14ac			free
C23	A R T K Q T A R Kme3 pS T G G K A P R K Q	▶	H3 1-19	K9me3	S10P			free

Active Motif
MODified™ Histone Peptide Array*
Catalog Nos. 13001 & 13005

Peptide location	Peptide sequence	to array	name	Mod1	Mod2	Mod 3	Mod 4	N-terminus
C24	A R T K Q T A R Kme3 S pT G G K A P R K Q	▶	H3 1-19	K9me3	T11P			free
D 1	A R T K Q T A R Kme3 S T G G Kac A P R K Q	▶	H3 1-19	K9me3	K14ac			free
D 2	A R T K Q T A R Kac pS T G G K A P R K Q	▶	H3 1-19	K9ac	S10P			free
D 3	A R T K Q T A R Kac S pT G G K A P R K Q	▶	H3 1-19	K9ac	T11P			free
D 4	A R T K Q T A R Kac S T G G Kac A P R K Q	▶	H3 1-19	K9ac	K14ac			free
D 5	A R T K Q T A R K pS pT G G K A P R K Q	▶	H3 1-19	S10P	T11P			free
D 6	A R T K Q T A R K pS T G G Kac A P R K Q	▶	H3 1-19	S10P	K14ac			free
D 7	A R T K Q T A R K S pT G G Kac A P R K Q	▶	H3 1-19	T11P	K14ac			free
D 8	A R T K Q T A Rme2s Kme1 pS T G G K A P R K Q	▶	H3 1-19	R8me2s	K9me1	S10P		free
D 9	A R T K Q T A Rme2s Kme2 pS T G G K A P R K Q	▶	H3 1-19	R8me2s	K9me2	S10P		free
D10	A R T K Q T A Rme2s Kme3 pS T G G K A P R K Q	▶	H3 1-19	R8me2s	K9me3	S10P		free
D11	A R T K Q T A Rme2s Kac pS T G G K A P R K Q	▶	H3 1-19	R8me2s	K9ac	S10P		free
D12	A R T K Q T A Rme2s Kme1 S pT G G K A P R K Q	▶	H3 1-19	R8me2s	K9me1	T11P		free
D13	A R T K Q T A Rme2s Kme2 S pT G G K A P R K Q	▶	H3 1-19	R8me2s	K9me2	T11P		free
D14	A R T K Q T A Rme2s Kme3 S pT G G K A P R K Q	▶	H3 1-19	R8me2s	K9me3	T11P		free
D15	A R T K Q T A Rme2s Kac S pT G G K A P R K Q	▶	H3 1-19	R8me2s	K9ac	T11P		free
D16	A R T K Q T A Rme2a Kme1 pS T G G K A P R K Q	▶	H3 1-19	R8me2a	K9me1	S10P		free
D17	A R T K Q T A Rme2a Kme2 pS T G G K A P R K Q	▶	H3 1-19	R8me2a	K9me2	S10P		free
D18	A R T K Q T A Rme2a Kme3 pS T G G K A P R K Q	▶	H3 1-19	R8me2a	K9me3	S10P		free
D19	A R T K Q T A Rme2a Kac pS T G G K A P R K Q	▶	H3 1-19	R8me2a	K9ac	S10P		free
D20	A R T K Q T A Rme2a Kme1 S pT G G K A P R K Q	▶	H3 1-19	R8me2a	K9me1	T11P		free
D21	A R T K Q T A Rme2a Kme2 S pT G G K A P R K Q	▶	H3 1-19	R8me2a	K9me2	T11P		free
D22	A R T K Q T A Rme2a Kme3 S pT G G K A P R K Q	▶	H3 1-19	R8me2a	K9me3	T11P		free
D23	A R T K Q T A Rme2a Kac S pT G G K A P R K Q	▶	H3 1-19	R8me2a	K9ac	T11P		free
D24	A R T K Q T A Rme2a Kme1 pS pT G G K A P R K Q	▶	H3 1-19	R8me2a	K9me1	S10P	T11P	free
E 1	A R T K Q T A Rme2a Kme2 pS pT G G K A P R K Q	▶	H3 1-19	R8me2a	K9me2	S10P	T11P	free
E 2	A R T K Q T A Rme2a Kme3 pS pT G G K A P R K Q	▶	H3 1-19	R8me2a	K9me3	S10P	T11P	free
E 3	A R T K Q T A Rme2a Kac pS pT G G K A P R K Q	▶	H3 1-19	R8me2a	K9ac	S10P	T11P	free
E 4	A Rme2s T Kme1 Q T A Rme2s K S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me1	R8me2s		free
E 5	A Rme2s T Kme2 Q T A Rme2s K S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me2	R8me2s		free
E 6	A Rme2s T Kme3 Q T A Rme2s K S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me3	R8me2s		free
E 7	A Rme2s T Kac Q T A Rme2s K S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2s		free
E 8	A Rme2a T Kme1 Q T A Rme2a K S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me1	R8me2a		free
E 9	A Rme2a T Kme2 Q T A Rme2a K S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me2	R8me2a		free
E10	A Rme2a T Kme3 Q T A Rme2a K S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me3	R8me2a		free
E11	A Rme2a T Kac Q T A Rme2a K S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2a		free
E12	A Rme2s T Kme1 Q T A R Kme1 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me1	K9me1		free
E13	A Rme2s T Kme2 Q T A R Kme1 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me2	K9me1		free
E14	A Rme2s T Kme3 Q T A R Kme1 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me3	K9me1		free
E15	A Rme2s T Kac Q T A R Kme1 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	K9me1		free
E16	A Rme2a T Kme1 Q T A R Kme2 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me1	K9me2		free
E17	A Rme2a T Kme2 Q T A R Kme2 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me2	K9me2		free
E18	A Rme2a T Kme3 Q T A R Kme2 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me3	K9me2		free
E19	A Rme2a T Kac Q T A R Kme2 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	K9me2		free
E20	A Rme2s T Kme1 Q T A R Kme3 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me1	K9me3		free
E21	A Rme2s T Kme2 Q T A R Kme3 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me2	K9me3		free
E22	A Rme2s T Kme3 Q T A R Kme3 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me3	K9me3		free
E23	A Rme2s T Kac Q T A R Kme3 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	K9me3		free
E24	A Rme2a T Kme1 Q T A R Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me1	K9ac		free
F 1	A Rme2a T Kme2 Q T A R Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me2	K9ac		free
F 2	A Rme2a T Kme3 Q T A R Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me3	K9ac		free
F 3	A Rme2a T Kac Q T A R Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	K9ac		free
F 4	A R T Kme1 Q T A Rme2s Kme1 S T G G K A P R K Q	▶	H3 1-19	K4me1	R8me2s	K9me1		free
F 5	A R T Kme2 Q T A Rme2s Kme1 S T G G K A P R K Q	▶	H3 1-19	K4me2	R8me2s	K9me1		free
F 6	A R T Kme3 Q T A Rme2s Kme1 S T G G K A P R K Q	▶	H3 1-19	K4me3	R8me2s	K9me1		free
F 7	A R T Kac Q T A Rme2s Kme1 S T G G K A P R K Q	▶	H3 1-19	K4ac	R8me2s	K9me1		free
F 8	A R T Kme1 Q T A Rme2a Kme1 S T G G K A P R K Q	▶	H3 1-19	K4me1	R8me2a	K9me1		free
F 9	A R T Kme2 Q T A Rme2a Kme1 S T G G K A P R K Q	▶	H3 1-19	K4me2	R8me2a	K9me1		free
F10	A R T Kme3 Q T A Rme2a Kme1 S T G G K A P R K Q	▶	H3 1-19	K4me3	R8me2a	K9me1		free
F11	A R T Kac Q T A Rme2a Kme1 S T G G K A P R K Q	▶	H3 1-19	K4ac	R8me2a	K9me1		free
F12	A R T Kme1 Q T A Rme2s Kme2 S T G G K A P R K Q	▶	H3 1-19	K4me1	R8me2s	K9me2		free
F13	A R T Kme2 Q T A Rme2s Kme2 S T G G K A P R K Q	▶	H3 1-19	K4me2	R8me2s	K9me2		free
F14	A R T Kme3 Q T A Rme2s Kme2 S T G G K A P R K Q	▶	H3 1-19	K4me3	R8me2s	K9me2		free
F15	A R T Kac Q T A Rme2s Kme2 S T G G K A P R K Q	▶	H3 1-19	K4ac	R8me2s	K9me2		free
F16	A R T Kme1 Q T A Rme2a Kme2 S T G G K A P R K Q	▶	H3 1-19	K4me1	R8me2a	K9me2		free
F17	A R T Kme2 Q T A Rme2a Kme2 S T G G K A P R K Q	▶	H3 1-19	K4me2	R8me2a	K9me2		free
F18	A R T Kme3 Q T A Rme2a Kme2 S T G G K A P R K Q	▶	H3 1-19	K4me3	R8me2a	K9me2		free
F19	A R T Kac Q T A Rme2a Kme2 S T G G K A P R K Q	▶	H3 1-19	K4ac	R8me2a	K9me2		free
F20	A R T Kme1 Q T A Rme2s Kme3 S T G G K A P R K Q	▶	H3 1-19	K4me1	R8me2s	K9me3		free
F21	A R T Kme2 Q T A Rme2s Kme3 S T G G K A P R K Q	▶	H3 1-19	K4me2	R8me2s	K9me3		free
F22	A R T Kme3 Q T A Rme2s Kme3 S T G G K A P R K Q	▶	H3 1-19	K4me3	R8me2s	K9me3		free

Active Motif
MODified™ Histone Peptide Array*
Catalog Nos. 13001 & 13005

Peptide location	Peptide sequence	to array	name	Mod1	Mod2	Mod 3	Mod 4	N-terminus
F23	A R T Kac Q T A Rme2s Kme3 S T G G K A P R K Q	▶	H3 1-19	K4ac	R8me2s	K9me3		free
F24	A R T Kme1 Q T A Rme2a Kme3 S T G G K A P R K Q	▶	H3 1-19	K4me1	R8me2a	K9me3		free
G 1	A R T Kme2 Q T A Rme2a Kme3 S T G G K A P R K Q	▶	H3 1-19	K4me2	R8me2a	K9me3		free
G 2	A R T Kme3 Q T A Rme2a Kme3 S T G G K A P R K Q	▶	H3 1-19	K4me3	R8me2a	K9me3		free
G 3	A R T Kac Q T A Rme2a Kme3 S T G G K A P R K Q	▶	H3 1-19	K4ac	R8me2a	K9me3		free
G 4	A R T Kme1 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	K4me1	R8me2s	K9ac		free
G 5	A R T Kme2 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	K4me2	R8me2s	K9ac		free
G 6	A R T Kme3 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	K4me3	R8me2s	K9ac		free
G 7	A R T Kac Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	K4ac	R8me2s	K9ac		free
G 8	A R T Kme1 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	K4me1	R8me2a	K9ac		free
G 9	A R T Kme2 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	K4me2	R8me2a	K9ac		free
G10	A R T Kme3 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	K4me3	R8me2a	K9ac		free
G11	A R T Kac Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	K4ac	R8me2a	K9ac		free
G12	A Rme2s T Kme1 Q T A Rme2s Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me1	R8me2s	K9me1	free
G13	A Rme2s T Kme2 Q T A Rme2s Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me2	R8me2s	K9me1	free
G14	A Rme2s T Kme3 Q T A Rme2s Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me3	R8me2s	K9me1	free
G15	A Rme2s T Kac Q T A Rme2s Kme1 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2s	K9me1	free
G16	A Rme2a T Kme1 Q T A Rme2s Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me1	R8me2s	K9me1	free
G17	A Rme2a T Kme2 Q T A Rme2s Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me2	R8me2s	K9me1	free
G18	A Rme2a T Kme3 Q T A Rme2s Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me3	R8me2s	K9me1	free
G19	A Rme2a T Kac Q T A Rme2s Kme1 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2s	K9me1	free
G20	A Rme2s T Kme1 Q T A Rme2s Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me1	R8me2s	K9me2	free
G21	A Rme2s T Kme2 Q T A Rme2s Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me2	R8me2s	K9me2	free
G22	A Rme2s T Kme3 Q T A Rme2s Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me3	R8me2s	K9me2	free
G23	A Rme2s T Kac Q T A Rme2s Kme2 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2s	K9me2	free
G24	A Rme2a T Kme1 Q T A Rme2s Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me1	R8me2s	K9me2	free
H 1	A Rme2a T Kme2 Q T A Rme2s Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me2	R8me2s	K9me2	free
H 2	A Rme2a T Kme3 Q T A Rme2s Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me3	R8me2s	K9me2	free
H 3	A Rme2a T Kac Q T A Rme2s Kme2 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2s	K9me2	free
H 4	A Rme2s T Kme1 Q T A Rme2s Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me1	R8me2s	K9me3	free
H 5	A Rme2s T Kme2 Q T A Rme2s Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me2	R8me2s	K9me3	free
H 6	A Rme2s T Kme3 Q T A Rme2s Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me3	R8me2s	K9me3	free
H 7	A Rme2s T Kac Q T A Rme2s Kme3 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2s	K9me3	free
H 8	A Rme2a T Kme1 Q T A Rme2s Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me1	R8me2s	K9me3	free
H 9	A Rme2a T Kme2 Q T A Rme2s Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me2	R8me2s	K9me3	free
H10	A Rme2a T Kme3 Q T A Rme2s Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me3	R8me2s	K9me3	free
H11	A Rme2a T Kac Q T A Rme2s Kme3 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2s	K9me3	free
H12	A Rme2s T Kme1 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me1	R8me2s	K9ac	free
H13	A Rme2s T Kme2 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me2	R8me2s	K9ac	free
H14	A Rme2s T Kme3 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me3	R8me2s	K9ac	free
H15	A Rme2s T Kac Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2s	K9ac	free
H16	A Rme2a T Kme1 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me1	R8me2s	K9ac	free
H17	A Rme2a T Kme2 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me2	R8me2s	K9ac	free
H18	A Rme2a T Kme3 Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me3	R8me2s	K9ac	free
H19	A Rme2a T Kac Q T A Rme2s Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2s	K9ac	free
H20	A Rme2s T Kme1 Q T A Rme2a Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me1	R8me2a	K9me1	free
H21	A Rme2s T Kme2 Q T A Rme2a Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me2	R8me2a	K9me1	free
H22	A Rme2s T Kme3 Q T A Rme2a Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me3	R8me2a	K9me1	free
H23	A Rme2s T Kac Q T A Rme2a Kme1 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2a	K9me1	free
H24	A Rme2a T Kme1 Q T A Rme2a Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me1	R8me2a	K9me1	free
I 1	A Rme2a T Kme2 Q T A Rme2a Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me2	R8me2a	K9me1	free
I 2	A Rme2a T Kme3 Q T A Rme2a Kme1 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me3	R8me2a	K9me1	free
I 3	A Rme2a T Kac Q T A Rme2a Kme1 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2a	K9me1	free
I 4	A Rme2s T Kme1 Q T A Rme2a Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me1	R8me2a	K9me2	free
I 5	A Rme2s T Kme2 Q T A Rme2a Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me2	R8me2a	K9me2	free
I 6	A Rme2s T Kme3 Q T A Rme2a Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me3	R8me2a	K9me2	free
I 7	A Rme2s T Kac Q T A Rme2a Kme2 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2a	K9me2	free
I 8	A Rme2a T Kme1 Q T A Rme2a Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me1	R8me2a	K9me2	free
I 9	A Rme2a T Kme2 Q T A Rme2a Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me2	R8me2a	K9me2	free
I10	A Rme2a T Kme3 Q T A Rme2a Kme2 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me3	R8me2a	K9me2	free
I11	A Rme2a T Kac Q T A Rme2a Kme2 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2a	K9me2	free
I12	A Rme2s T Kme1 Q T A Rme2a Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me1	R8me2a	K9me3	free
I13	A Rme2s T Kme2 Q T A Rme2a Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me2	R8me2a	K9me3	free
I14	A Rme2s T Kme3 Q T A Rme2a Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2s	K4me3	R8me2a	K9me3	free
I15	A Rme2s T Kac Q T A Rme2a Kme3 S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2a	K9me3	free
I16	A Rme2a T Kme1 Q T A Rme2a Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me1	R8me2a	K9me3	free
I17	A Rme2a T Kme2 Q T A Rme2a Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me2	R8me2a	K9me3	free
I18	A Rme2a T Kme3 Q T A Rme2a Kme3 S T G G K A P R K (	▶	H3 1-19	R2me2a	K4me3	R8me2a	K9me3	free
I19	A Rme2a T Kac Q T A Rme2a Kme3 S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2a	K9me3	free
I20	A Rme2s T Kme1 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me1	R8me2a	K9ac	free
I21	A Rme2s T Kme2 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me2	R8me2a	K9ac	free

Active Motif
MODified™ Histone Peptide Array*
Catalog Nos. 13001 & 13005

Peptide location	Peptide sequence	to array	name	Mod1	Mod2	Mod 3	Mod 4	N-terminus
I22	A Rme2s T Kme3 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4me3	R8me2a	K9ac	free
I23	A Rme2s T Kac Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R2me2s	K4ac	R8me2a	K9ac	free
I24	A Rme2a T Kme1 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me1	R8me2a	K9ac	free
J 1	A Rme2a T Kme2 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me2	R8me2a	K9ac	free
J 2	A Rme2a T Kme3 Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4me3	R8me2a	K9ac	free
J 3	A Rme2a T Kac Q T A Rme2a Kac S T G G K A P R K Q	▶	H3 1-19	R2me2a	K4ac	R8me2a	K9ac	free
J 4	A R K S T G G K A P R K Q L A T K A A R	▶	H3 7-26	unmod				acetylated
J 5	A R K S T G G Kac A P R K Q L A T K A A R	▶	H3 7-26	K14ac				acetylated
J 6	A R K pS T G G Kac A P R K Q L A T K A A R	▶	H3 7-26	K14ac	S10P			acetylated
J 7	A R K S pT G G Kac A P R K Q L A T K A A R	▶	H3 7-26	K14ac	T11P			acetylated
J 8	A R K S T G G K A P Rme2s K Q L A T K A A R	▶	H3 7-26	R17me2s				acetylated
J 9	A R K S T G G K A P Rme2a K Q L A T K A A R	▶	H3 7-26	R17me2a				acetylated
J10	A R K S T G G K A P Cit K Q L A T K A A R	▶	H3 7-26	R17Citr				acetylated
J11	A R K S T G G K A P R Kac Q L A T K A A R	▶	H3 7-26	K18ac				acetylated
J12	A R K S T G G Kac A P Rme2s K Q L A T K A A R	▶	H3 7-26	K14ac	R17me2s			acetylated
J13	A R K S T G G Kac A P Rme2a K Q L A T K A A R	▶	H3 7-26	K14ac	R17me2a			acetylated
J14	A R K S T G G Kac A P R Kac Q L A T K A A R	▶	H3 7-26	K14ac	K18ac			acetylated
J15	A R K S T G G K A P Rme2s Kac Q L A T K A A R	▶	H3 7-26	R17me2s	K18ac			acetylated
J16	A R K S T G G K A P Rme2a Kac Q L A T K A A R	▶	H3 7-26	R17me2a	K18ac			acetylated
J17	A R K S T G G K A P Cit Kac Q L A T K A A R	▶	H3 7-26	R17Citr	K18ac			acetylated
J18	A R K S T G G Kac A P Rme2s Kac Q L A T K A A R	▶	H3 7-26	K14ac	R17me2s	K18ac		acetylated
J19	A R K S T G G Kac A P Rme2a Kac Q L A T K A A R	▶	H3 7-26	K14ac	R17me2a	K18ac		acetylated
J20	P R K Q L A T K A A R K S A P A T G G	▶	H3 16-35	unmod				acetylated
J21	P R K Q L A T K A A Rme2s K S A P A T G G	▶	H3 16-35	R26me2s				acetylated
J22	P R K Q L A T K A A Rme2a K S A P A T G G	▶	H3 16-35	R26me2a				acetylated
J23	P R K Q L A T K A A Cit K S A P A T G G	▶	H3 16-35	R26Citr				acetylated
J24	P R K Q L A T K A A R Kme1 S A P A T G G	▶	H3 16-35	K27me1				acetylated
K 1	P R K Q L A T K A A R Kme2 S A P A T G G	▶	H3 16-35	K27me2				acetylated
K 2	P R K Q L A T K A A R Kme3 S A P A T G G	▶	H3 16-35	K27me3				acetylated
K 3	P R K Q L A T K A A R Kac S A P A T G G	▶	H3 16-35	K27ac				acetylated
K 4	P R K Q L A T K A A R K pS A P A T G G	▶	H3 16-35	S28P				acetylated
K 5	P R K Q L A T K A A Rme2s Kme1 S A P A T G G	▶	H3 16-35	R26me2s	K27me1			acetylated
K 6	P R K Q L A T K A A Rme2s Kme2 S A P A T G G	▶	H3 16-35	R26me2s	K27me2			acetylated
K 7	P R K Q L A T K A A Rme2s Kme3 S A P A T G G	▶	H3 16-35	R26me2s	K27me3			acetylated
K 8	P R K Q L A T K A A Rme2s Kac S A P A T G G	▶	H3 16-35	R26me2s	K27ac			acetylated
K 9	P R K Q L A T K A A Rme2s K pS A P A T G G	▶	H3 16-35	R26me2s	S28P			acetylated
K10	P R K Q L A T K A A Rme2a Kme1 S A P A T G G	▶	H3 16-35	R26me2a	K27me1			acetylated
K11	P R K Q L A T K A A Rme2a Kme2 S A P A T G G	▶	H3 16-35	R26me2a	K27me2			acetylated
K12	P R K Q L A T K A A Rme2a Kme3 S A P A T G G	▶	H3 16-35	R26me2a	K27me3			acetylated
K13	P R K Q L A T K A A Rme2a Kac S A P A T G G	▶	H3 16-35	R26me2a	K27ac			acetylated
K14	P R K Q L A T K A A Rme2a K pS A P A T G G	▶	H3 16-35	R26me2a	S28P			acetylated
K15	P R K Q L A T K A A Cit Kme1 S A P A T G G	▶	H3 16-35	R26Citr	K27me1			acetylated
K16	P R K Q L A T K A A Cit Kme2 S A P A T G G	▶	H3 16-35	R26Citr	K27me2			acetylated
K17	P R K Q L A T K A A Cit Kme3 S A P A T G G	▶	H3 16-35	R26Citr	K27me3			acetylated
K18	P R K Q L A T K A A Cit K pS A P A T G G	▶	H3 16-35	R26Citr	S28P			acetylated
K19	P R K Q L A T K A A R Kme1 pS A P A T G G	▶	H3 16-35	K27me1	S28P			acetylated
K20	P R K Q L A T K A A R Kme2 pS A P A T G G	▶	H3 16-35	K27me2	S28P			acetylated
K21	P R K Q L A T K A A R Kme3 pS A P A T G G	▶	H3 16-35	K27me3	S28P			acetylated
K22	P R K Q L A T K A A R Kac pS A P A T G G	▶	H3 16-35	K27ac	S28P			acetylated
K23	P R K Q L A T K A A Rme2s Kme1 pS A P A T G G	▶	H3 16-35	R26me2s	K27me1	S28P		acetylated
K24	P R K Q L A T K A A Rme2s Kme2 pS A P A T G G	▶	H3 16-35	R26me2s	K27me2	S28P		acetylated
L 1	P R K Q L A T K A A Rme2s Kme3 pS A P A T G G	▶	H3 16-35	R26me2s	K27me3	S28P		acetylated
L 2	P R K Q L A T K A A Rme2s Kac pS A P A T G G	▶	H3 16-35	R26me2s	K27ac	S28P		acetylated
L 3	P R K Q L A T K A A Rme2a Kme1 pS A P A T G G	▶	H3 16-35	R26me2a	K27me1	S28P		acetylated
L 4	P R K Q L A T K A A Rme2a Kme2 pS A P A T G G	▶	H3 16-35	R26me2a	K27me2	S28P		acetylated
L 5	P R K Q L A T K A A Rme2a Kme3 pS A P A T G G	▶	H3 16-35	R26me2a	K27me3	S28P		acetylated
L 6	P R K Q L A T K A A Rme2a Kac pS A P A T G G	▶	H3 16-35	R26me2a	K27ac	S28P		acetylated
L 7	R K S A P A T G G V K K P H R Y R P G	▶	H3 26-45	unmod				acetylated
L 8	R K S A P A T G G V Kme1 K P H R Y R P G	▶	H3 26-45	K36me1				acetylated
L 9	R K S A P A T G G V Kme2 K P H R Y R P G	▶	H3 26-45	K36me2				acetylated
L10	R K S A P A T G G V Kme3 K P H R Y R P G	▶	H3 26-45	K36me3				acetylated
L11	R K S A P A T G G V Kac K P H R Y R P G	▶	H3 26-45	K36ac				acetylated
L12	S G R G K G G K G L G K G G A K R H R	▶	H4 1-19	unmod				free
L13	pS G R G K G G K G L G K G G A K R H R	▶	H4 1-19	S1P				free
L14	S G Rme2s G K G G K G L G K G G A K R H R	▶	H4 1-19	R3me2s				free
L15	S G Rme2a G K G G K G L G K G G A K R H R	▶	H4 1-19	R3me2a				free
L16	S G R G Kac G G K G L G K G G A K R H R	▶	H4 1-19	K5ac				free
L17	S G R G K G G Kac G L G K G G A K R H R	▶	H4 1-19	K8ac				free
L18	S G R G K G G K G L G Kac G G A K R H R	▶	H4 1-19	K12ac				free
L19	S G R G K G G K G L G K G G A Kac R H R	▶	H4 1-19	K16ac				free
L20	pS G Rme2s G K G G K G L G K G G A K R H R	▶	H4 1-19	S1P	R3me2s			free

Active Motif
MODified™ Histone Peptide Array*
Catalog Nos. 13001 & 13005

Peptide location	Peptide sequence	to array	name	Mod1	Mod2	Mod 3	Mod 4	N-terminus
L21	pS G Rme2a G K G G K G L G K G G A K R H R	▶	H4 1-19	S1P	R3me2a			free
L22	pS G R G Kac G G K G L G K G G A K R H R	▶	H4 1-19	S1P	K5ac			free
L23	S G Rme2s G Kac G G K G L G K G G A K R H R	▶	H4 1-19	R3me2s	K5ac			free
L24	S G Rme2s G K G G Kac G L G K G G A K R H R	▶	H4 1-19	R3me2s	K8ac			free
M 1	S G Rme2a G Kac G G K G L G K G G A K R H R	▶	H4 1-19	R3me2a	K5ac			free
M 2	S G Rme2a G K G G Kac G L G K G G A K R H R	▶	H4 1-19	R3me2a	K8ac			free
M 3	S G R G Kac G G Kac G L G K G G A K R H R	▶	H4 1-19	K5ac	K8ac			free
M 4	S G R G K G G Kac G L G Kac G G A K R H R	▶	H4 1-19	K8ac	K12ac			free
M 5	S G R G K G G Kac G L G K G G A Kac R H R	▶	H4 1-19	K8ac	K16ac			free
M 6	S G R G K G G K G L G Kac G G A Kac R H R	▶	H4 1-19	K12ac	K16ac			free
M 7	pS G Rme2s G Kac G G K G L G K G G A K R H R	▶	H4 1-19	S1P	R3me2s	K5ac		free
M 8	pS G Rme2a G Kac G G K G L G K G G A K R H R	▶	H4 1-19	S1P	R3me2a	K5ac		free
M 9	S G Rme2s G Kac G G Kac G L G K G G A K R H R	▶	H4 1-19	R3me2s	K5ac	K8ac		free
M10	S G Rme2a G Kac G G Kac G L G K G G A K R H R	▶	H4 1-19	R3me2a	K5ac	K8ac		free
M11	S G R G Kac G G Kac G L G Kac G G A K R H R	▶	H4 1-19	K5ac	K8ac	K12ac		free
M12	S G R G K G G Kac G L G Kac G G A Kac R H R	▶	H4 1-19	K8ac	K12ac	K16ac		free
M13	pS G Rme2s G Kac G G Kac G L G K G G A K R H R	▶	H4 1-19	S1P	R3me2s	K5ac	K8ac	free
M14	pS G Rme2a G Kac G G Kac G L G K G G A K R H R	▶	H4 1-19	S1P	R3me2a	K5ac	K8ac	free
M15	S G Rme2s G Kac G G Kac G L G Kac G G A K R H R	▶	H4 1-19	R3me2s	K5ac	K8ac	K12ac	free
M16	S G Rme2a G Kac G G Kac G L G Kac G G A K R H R	▶	H4 1-19	R3me2a	K5ac	K8ac	K12ac	free
M17	S G R G Kac G G Kac G L G Kac G G A Kac R H R	▶	H4 1-19	K5ac	K8ac	K12ac	K16ac	free
M18	G K G G A K R H R K V L R D N I Q G I T	▶	H4 11-30	unmod				acetylated
M19	G Kac G G A K R H R K V L R D N I Q G I T	▶	H4 11-30	K12ac				acetylated
M20	G K G G A Kac R H R K V L R D N I Q G I T	▶	H4 11-30	K16ac				acetylated
M21	G K G G A K Rme2s H R K V L R D N I Q G I T	▶	H4 11-30	R17me2s				acetylated
M22	G K G G A K Rme2a H R K V L R D N I Q G I T	▶	H4 11-30	R17me2a				acetylated
M23	G K G G A K R H Rme2s K V L R D N I Q G I T	▶	H4 11-30	R19me2s				acetylated
M24	G K G G A K R H Rme2a K V L R D N I Q G I T	▶	H4 11-30	R19me2a				acetylated
N 1	G K G G A K R H R Kme1 V L R D N I Q G I T	▶	H4 11-30	K20me1				acetylated
N 2	G K G G A K R H R Kme2 V L R D N I Q G I T	▶	H4 11-30	K20me2				acetylated
N 3	G K G G A K R H R Kme3 V L R D N I Q G I T	▶	H4 11-30	K20me3				acetylated
N 4	G K G G A K R H R Kac V L R D N I Q G I T	▶	H4 11-30	K20ac				acetylated
N 5	G K G G A K R H R K V L Rme2a D N I Q G I T	▶	H4 11-30	R24me2a				acetylated
N 6	G K G G A K R H R K V L Rme2s D N I Q G I T	▶	H4 11-30	R24me2s				acetylated
N 7	G Kac G G A Kac R H R K V L R D N I Q G I T	▶	H4 11-30	K12ac	K16ac			acetylated
N 8	G K G G A Kac Rme2s H R K V L R D N I Q G I T	▶	H4 11-30	K16ac	R17me2s			acetylated
N 9	G K G G A Kac Rme2a H R K V L R D N I Q G I T	▶	H4 11-30	K16ac	R17me2a			acetylated
N10	G K G G A Kac R H Rme2s K V L R D N I Q G I T	▶	H4 11-30	K16ac	R19me2s			acetylated
N11	G K G G A Kac R H Rme2a K V L R D N I Q G I T	▶	H4 11-30	K16ac	R19me2a			acetylated
N12	G K G G A Kac R H R Kme1 V L R D N I Q G I T	▶	H4 11-30	K16ac	K20me1			acetylated
N13	G K G G A Kac R H R Kme2 V L R D N I Q G I T	▶	H4 11-30	K16ac	K20me2			acetylated
N14	G K G G A Kac R H R Kme3 V L R D N I Q G I T	▶	H4 11-30	K16ac	K20me3			acetylated
N15	G K G G A Kac R H R Kac V L R D N I Q G I T	▶	H4 11-30	K16ac	K20ac			acetylated
N16	G Kac G G A Kac R H R Kme1 V L R D N I Q G I T	▶	H4 11-30	K12ac	K16ac	K20me1		acetylated
N17	G Kac G G A Kac R H R Kme2 V L R D N I Q G I T	▶	H4 11-30	K12ac	K16ac	K20me2		acetylated
N18	G Kac G G A Kac R H R Kme3 V L R D N I Q G I T	▶	H4 11-30	K12ac	K16ac	K20me3		acetylated
N19	G Kac G G A Kac R H R Kac V L R D N I Q G I T	▶	H4 11-30	K12ac	K16ac	K20ac		acetylated
N20	G K G G A K R H Rme2a Kme1 V L R D N I Q G I T	▶	H4 11-30	R19me2a	K20me1			acetylated
N21	G K G G A K R H Rme2a Kme2 V L R D N I Q G I T	▶	H4 11-30	R19me2a	K20me2			acetylated
N22	G K G G A K R H Rme2a Kme3 V L R D N I Q G I T	▶	H4 11-30	R19me2a	K20me3			acetylated
N23	G K G G A K R H Rme2a Kac V L R D N I Q G I T	▶	H4 11-30	R19me2a	K20ac			acetylated
N24	G K G G A K R H Rme2s Kme1 V L R D N I Q G I T	▶	H4 11-30	R19me2s	K20me1			acetylated
O 1	G K G G A K R H Rme2s Kme2 V L R D N I Q G I T	▶	H4 11-30	R19me2s	K20me2			acetylated
O 2	G K G G A K R H Rme2s Kme3 V L R D N I Q G I T	▶	H4 11-30	R19me2s	K20me3			acetylated
O 3	G K G G A K R H Rme2s Kac V L R D N I Q G I T	▶	H4 11-30	R19me2s	K20ac			acetylated
O 4	G K G G A K R H R Kme1 V L Rme2a D N I Q G I T	▶	H4 11-30	R24me2a	K20me1			acetylated
O 5	G K G G A K R H R Kme2 V L Rme2a D N I Q G I T	▶	H4 11-30	R24me2a	K20me2			acetylated
O 6	G K G G A K R H R Kme3 V L Rme2a D N I Q G I T	▶	H4 11-30	R24me2a	K20me3			acetylated
O 7	G K G G A K R H R Kac V L Rme2a D N I Q G I T	▶	H4 11-30	R24me2a	K20ac			acetylated
O 8	G K G G A K R H R Kme1 V L Rme2s D N I Q G I T	▶	H4 11-30	R24me2s	K20me1			acetylated
O 9	G K G G A K R H R Kme2 V L Rme2s D N I Q G I T	▶	H4 11-30	R24me2s	K20me2			acetylated
O10	G K G G A K R H R Kme3 V L Rme2s D N I Q G I T	▶	H4 11-30	R24me2s	K20me3			acetylated
O11	G K G G A K R H R Kac V L Rme2s D N I Q G I T	▶	H4 11-30	R24me2s	K20ac			acetylated
O12	S G R G K Q G G K A R A K A K S R S S	▶	H2a 1-19	unmod				free
O13	pS G R G K Q G G K A R A K A K S R S S	▶	H2a 1-19	S1P				free
O14	S G R G Kac Q G G K A R A K A K S R S S	▶	H2a 1-19	K5ac				free
O15	S G R G K Q G G Kac A R A K A K S R S S	▶	H2a 1-19	K9ac				free
O16	S G R G K Q G G K A R A Kac A K S R S S	▶	H2a 1-19	K13ac				free
O17	pS G R G Kac Q G G K A R A K A K S R S S	▶	H2a 1-19	S1P	K5ac			free
O18	pS G R G K Q G G Kac A R A K A K S R S S	▶	H2a 1-19	S1P	K9ac			free
O19	pS G R G K Q G G K A R A Kac A K S R S S	▶	H2a 1-19	S1P	K13ac			free

Active Motif
MODified™ Histone Peptide Array*
Catalog Nos. 13001 & 13005

Peptide location	Peptide sequence	to array	name	Mod1	Mod2	Mod 3	Mod 4	N-terminus
O20	S G R G Kac Q G G Kac A R A K A K S R S S	▶	H2a 1-19	K5ac	K9ac			free
O21	S G R G Kac Q G G K A R A Kac A K S R S S	▶	H2a 1-19	K5ac	K13ac			free
O22	S G R G K Q G G Kac A R A Kac A K S R S S	▶	H2a 1-19	K9ac	K13ac			free
O23	pS G R G Kac Q G G Kac A R A K A K S R S S	▶	H2a 1-19	S1P	K5ac	K9ac		free
O24	pS G R G Kac Q G G K A R A Kac A K S R S S	▶	H2a 1-19	S1P	K5ac	K13ac		free
P 1	pS G R G K Q G G Kac A R A Kac A K S R S S	▶	H2a 1-19	S1P	K9ac	K13ac		free
P 2	S G R G Kac Q G G Kac A R A Kac A K S R S S	▶	H2a 1-19	K5ac	K9ac	K13ac		free
P 3	pS G R G Kac Q G G Kac A R A Kac A K S R S S	▶	H2a 1-19	S1P	K5ac	K9ac	K13ac	free
P 4	P D P A K S A P A P K K G S K K A V T	▶	H2B 1-19	unmod				free
P 5	P D P A Kac S A P A P K K G S K K A V T	▶	H2B 1-19	K5ac				free
P 6	P D P A K S A P A P K Kac G S K K A V T	▶	H2B 1-19	K12ac				free
P 7	P D P A K S A P A P K K G pS K K A V T	▶	H2B 1-19	S14P				free
P 8	P D P A K S A P A P K K G S Kac K A V T	▶	H2B 1-19	K15ac				free
P 9	P D P A Kac S A P A P K Kac G S K K A V T	▶	H2B 1-19	K5ac	K12ac			free
P10	P D P A Kac S A P A P K K G pS K K A V T	▶	H2B 1-19	K5ac	S14P			free
P11	P D P A Kac S A P A P K K G S Kac K A V T	▶	H2B 1-19	K5ac	K15ac			free
P12	P D P A K S A P A P K Kac G pS K K A V T	▶	H2B 1-19	K12ac	S14P			free
P13	P D P A K S A P A P K Kac G S Kac K A V T	▶	H2B 1-19	K12ac	K15ac			free
P14	P D P A K S A P A P K K G pS Kac K A V T	▶	H2B 1-19	S14P	K15ac			free
P15	P D P A Kac S A P A P K Kac G pS K K A V T	▶	H2B 1-19	K5ac	K12ac	S14P		free
P16	P D P A Kac S A P A P K Kac G S Kac K A V T	▶	H2B 1-19	K5ac	K12ac	K15ac		free
P17	P D P A Kac S A P A P K K G pS Kac K A V T	▶	H2B 1-19	K5ac	S14P	K15ac		free
P18	P D P A K S A P A P K Kac G pS Kac K A V T	▶	H2B 1-19	K12ac	S14P	K15ac		free
P19	P D P A Kac S A P A P K Kac G pS Kac K A V T	▶	H2B 1-19	K5ac	K12ac	S14P	K15ac	free
P20	Bio A A N W S H P Q F E K A A	▶	n, control peptide					biotinylated
P21	E Q K L I S E E D L A	▶	c-myc tag					free
P22	HAc	▶	neg. contol					acetylated
P23	K Kme1 Kme2 Kme3 Kac R Rme2s R Rme2a R Cit K Kme1 I	▶	background 01					acetylated
P24	R Rme2s K Kme1 Kac R Rme2a Kme2 K Kme3 R Kme1 Rme2s	▶	background 02					acetylated

Supplemental Methods

Drugs

The drugs used in this study and their sources are 4-hydroxycyclophosphamide (4-OHCY; an active form of cyclophosphamide), melphalan (L-PAM), dexamethasone (Wako Biochemicals, Osaka, Japan), doxorubicin (ADM) (Meiji, Tokyo, Japan), PD0332991, AT7519, perifosin, PF-04691502, OSI-906, AG-1024, GSK-343 (Selleck Chemicals, Houston, TX), tranylcypromine hydrochloride (2-PCPA), GSK-J4 (R&D Systems, Minneapolis, MN), chaetocin (Enzo Life Science, Am Arbor, MI) and 3-deazaneplanocin A (DZNep) (Cayman Chemical, Am Arbor, MI). The kinase inhibitors listed in Supplemental Table 2 were supplied from the Screening Committee of Anticancer Drugs (SCADS; Tokyo, Japan). All drugs were dissolved in dimethyl sulfoxide at appropriate concentrations and used at a final dilution of 1/1000. The final concentration of dimethyl sulfoxide in the culture medium was <0.1%, a concentration that did not affect drug effects or cell growth *per se*.

Cells and cell culture

We used four *bone fide* human MM cell lines, KMS12-BM, RPMI8226, KMS-11 and U266, in this study (1). These cell lines were purchased from the Health Science Research Resources Bank (Osaka, Japan) and maintained in RPMI1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Sigma-Aldrich). We used human BM-derived stromal cell lines UBE6T-7 and stroma-NK, which were immortalized by transducing with a human telomerase catalytic protein subunit, as BMSCs. These cell lines were kindly provided by Dr. Akihiro Umezawa (National Research Institute for Child Health and Development, Tokyo, Japan) and Dr. Hirofumi Hamada (Sapporo Medical University, Sapporo, Japan), respectively, and maintained in DMEM medium (Sigma-Aldrich) supplemented with 10% FCS (2, 3). Primary bone marrow cells were isolated from MM patients at the time of diagnostic procedure and used with or without positive selection of MM cells using CD138 MicroBeads and MACS separation columns (Miltenyi Biotech, Gladbach, Germany). Normal plasma cells were isolated similarly from healthy volunteers (purchased from LONZA, Basel, Switzerland). Informed consent was obtained in accordance with the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of Jichi Medical University. Patient-derived MSCs were also isolated from the bone marrow of MM patients using the MesenCult® Proliferation Kit (Stem Cell Technologies, Vancouver, Canada) according to the manufacturer's instruction.

Flow cytometric analysis

To analyze cell surface antigens, cells were stained with specific monoclonal antibodies against CD90, CD105, CD106, CD19, CD38 and CD138, and analyzed using the BD-LSR (Becton Dickinson) and GUAVA (Merk Millipore, Darmstadt, Germany) flow cytometers as described previously (4). To assess cell death, cells were washed with phosphate-buffered saline (PBS), stained with PE-conjugated annexin-V (annexin-V/PE) (BioVision, Mountain View, CA), and subjected to flow cytometric analysis using the FACSaria flow cytometer (Becton Dickinson) (5). For detection of intracellular H3K27me3, cells were washed with PBS, permeabilized using the BD Cytofix/Cytoperm® Fixation/Permeabilization Kit (Becton Dickinson), followed by staining with Alexa 488-conjugated anti-H3K27me3 and 7-AAD for flow cytometric analysis. For cell cycle analysis, cells were washed with PBS and stained with Vindelov's solution (0.04 mg/ml propidium iodide in 5 mM Tris-HCl, 5 mM NaCl and 0.005% nonidet P-40) in preparation for flow cytometry with the BD-LSR/CellFIT system (Becton-Dickinson). The size of the sub-G1, G0/G1, S and G2/M fractions was calculated as a percentage by analyzing DNA histograms with the ModFitLT 2.0 program (Becton-Dickinson) (6).

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated from 1 to 10 × 10^4 cells, reverse-transcribed into cDNA using SuperScript reverse transcriptase and oligo(dT) primers (Invitrogen), and subjected to subsequent semi-quantitative RT-PCR. Detailed information on primers, including sequences, corresponding nucleotide positions, and PCR product sizes, is shown in Supplemental Table 1.

Cell proliferation assays

Cell proliferation was monitored by the MTT reduction assay with Cell Counting Kit (Wako Biochemicals). Cells were seeded in 96-well flat-bottomed microplates. Absorbance was measured at a wavelength of 450 nm using a microplate reader and expressed as a percentage of the value of the corresponding untreated cells (5).

Immunoblotting

Nuclear extracts and whole cell lysates were prepared using the Nuclear Extraction Kit (Cayman Chemical, Ann Arbor, MI, USA) or RIPA buffer supplemented with the inhibitor cocktails of phosphatases and proteases (Sigma), respectively (7). Immunoblotting was carried out according to the standard method using the antibodies against histones H3, H4, H3K4me3 (#9727), H3K27me2 (#9728), H3K27me3 (#9733). H3K36me2 (#9758), H3K36me3 (#9763), H3K79me2

(#9757), H4K20me2 (#9759), EZH1, EZH2, G9a, SUV39H1, UTX, JMJD3 (Cell Signaling Technology, Beverly, MA), H3K9me2 (#1220), MMSET (Abcam, Cambridge, UK), H3K4me2 (#07-030) (Millipore, Temecula, CA), H3K9me3 (#3916) (Active Motif), EZH2 phosphorylated at S21 (Bethyl Laboratories Inc, Montgomery, TX), EZH2 phosphorylated at T487 (Gene Tex Inc, Irvine, CA), CD138 and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) (5). We run the samples in duplicate to use one membrane for target molecules and the other for loading controls when target and control molecules are similar in size.

The isobologram of Chou and Talalay

The cytotoxic interaction between two drugs was evaluated using the method of Chou and Talalay (7, 8). We generated isobolograms using CompuSyn software according to the manufacturer's instructions (www.combosyn.com).

Construction and production of lentiviral expression vectors

We used the lentiviral short-hairpin RNA/short-interfering RNA (shRNA/siRNA) expression vector pLL3.7 for knockdown experiments. Oligonucleotides containing siRNA target sequences are shown in Supplemental Table 3. Scrambled sequences were used as controls. We used the lentiviral vector CSII-CMV-MCS-IRES-VENUS (kindly provided by Dr. Hiroyuki Miyoshi, RIKEN BioResource Center, Ibaraki, Japan) containing the coding region of *MMSET* or *EZH2-S21D* cDNA for gain-of-function experiments. To obtain *EZH2-S21D* cDNA, site directed mutagenesis was performed according to the manufacturer's protocol (TOYOBO, Tokyo, Japan). The serine-21 residue of EZH2 was replaced with aspartic acid using the following primers: sense, 5'-TGTCTCAGTCGCATGTACTCATCTTTTACACGCTTCCGCCAAC-3', antisense, 5'-GTTGGCGGAAGCGTGTAAAAGATGAGTACATGCGACTGAGACA-3' (9). These vectors were co-transfected into 293FT cells with packaging plasmids (Invitrogen, Carlsbad, CA) to produce infective lentiviruses in culture supernatants. Lentiviruses were then added to cell suspensions in the presence of 8 μg/ml polybrene and transduced for 24 hours as previously described (5).

Immunofluorescence staining

Cells were mounted onto glass slides using the Cytospin centrifuge (Shandon Scientific, Cheshire, England). Frozen sections of undecalcified femoral bone were prepared by the Kawamoto film method (10). Immunostaining was carried out as described previously (11) using an anti-phospho EZH2 (S21) antibody (Bethyl Laboratories) at a 1:1000 dilution, PE-conjugated

anti-human CD138 (#12-1389-42), PE-conjugated anti-mouse CD138 (#561070) (Becton Dickinson) and Alexa Fluor 488-conjugated anti-rabbit IgG (Invitrogen) at a 1:2000 dilution. Nuclei were counterstained with DAPI.

Global analysis of mRNA expression

RNA samples (50 ng) were T7-primed and reverse-transcribed, followed by T7 transcription in the presence of Cy3- or Cy5-CTP. Labeled cRNAs were hybridized with the SurePrint G3 Human Gene Expression 8x60K v2 Microarray (Agilent, Santa Clara, CA). The hybridized arrays were scanned on an Agilent G2565AA Scanner, and the scanned images were processed using the Agilent Feature Extraction Software (version 10.7.3.1). The data have been deposited in the MIAME-compliant GEO database under accession number GSE66466.

Chromatin immunoprecipitation assays

We used the ChIP-IT Chromatin Immunoprecipitation Kit (Active Motif) to perform chromatin immunoprecipitation assays. In brief, cells were fixed with 1% formaldehyde at 37 °C for 5 minutes, and sonicated to obtain chromatin suspensions. After centrifugation, supernatants were incubated with antibodies of interest at 4 °C overnight. The mixture was then incubated with protein A-agarose beads at 4 °C for 1 hour and centrifuged to collect the beads. DNA fragments bound to the beads were purified with vigorous washing and subjected to PCR. Detailed information on primers, including sequences, corresponding nucleotide positions, and PCR product sizes, is shown in Supplemental Table 4.

Supplemental References

1) Drexler HG, Matsuo Y, MacLeod, RA. Persistent use of false myeloma cell lines. Hum Cell 2003; 16: 101-105.

2) Kawano Y, Kobune M, Yamaguchi M, Nakamura K, Ito Y, Sasaki K, Takahashi S, Nakamura T, Chiba H, Sato T, et al. Ex vivo expansion of human umbilical cord hematopoietic progenitor cells using a coculture system with human telomerase catalytic subunit (hTERT)-transfected human stromal cells. Blood 2003; 101: 532-540.

3) Mori T, Kiyono T, Imabayashi H, Takeda Y, Tsuchiya K, Miyoshi S, Makino H, Matsumoto K, Saito H, Ogawa S, et al. Combination of hTERT and bmi-1, E6, or E7 induces prolongation of

the life span of bone marrow stromal cells from an elderly donor without affecting their neurogenic potential. Mol Cell Biol. 2005; 25: 5183-5195.

4) Mabuchi Y., Morikawa S, Harada S, Niibe K, Suzuki S, Renault-Mihara F, Houlihan DD, Akazawa C, Okano H, Matsuzaki Y. LNGFR(+)THY-1(+)VCAM-1(hi+) cells reveal functionally distinct subpopulations in mesenchymal stem cells. Stem Cell Repots 1, 152-165.

5) Kikuchi J, Wada T, Shimizu R, Izumi T, Akutsu M, Mitsunaga K, Noborio-Hatano K, Nobuyoshi M, Ozawa K, Kano Y, et al. Histone deacetylases are critical targets of bortezomib-induced cytotoxicity in multiple myeloma. Blood 2010; 116: 406-417.

6) Hiraoka N, Kikuchi J, Yamauchi T, Koyama D, Wada T, Uesawa M, Akutsu M, Mori S, Nakamura Y, Ueda T, et al. Purine analog-like properties of bendamustine underlie rapid activation of DNA damage response and synergistic effects with pyrimidine analogues in lymphoid malignancies. PLoS One 2014; 9: e90675.

7) Koyama D, Kikuchi J, Hiraoka N, Wada T, Kurosawa H, Chiba S, Furukawa Y. Proteasome inhibitors exert cytotoxicity and increase chemosensitivity via transcriptional repression of Notch1 in T-cell acute lymphoblastic leukemia. Leukemia 2014; 28: 1216-1226.

8) Chou TC. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res. 2010; 70: 440-446.

9) Cha T-L, Zhou BP, Xia W, Wu Y, Yang C-C, Chen C-T, Ping B, Otte AP, Hung M-C. Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. Science 2005; 310: 306-310.

10) Kawamoto T. Use of a new adhesive film for the preparation of multi-purpose fresh-frozen sections from hard tissues, whole-animals, insects and plants. Arch Histol Cytol. 2003; 66: 123-143.

11) Mitsunaga K, Kikuchi J, Wada T, Furukawa Y. Latexin Regulates the abundance of multiple cellular proteins in hematopoietic stem cells. J Cell Physiol. 2012; 227: 1138-1147.

Supplemental Table 1. Primers for semi-quantitative RT-PCR

Gene	Sequence (corresponding nucleotide positions)*	Product size
IL6	forward: 5'- AGGAGACTTGCCTGGTGAAA-3' (439-458)	180 bp
	reverse: 5'-CAGGGGTGGTTATTGCATCT-3' (599-618)	
SDF1	forward: 5'- CTAGTCAAGTGCGTCCACGA-3' (2636-2655)	221 bp
	reverse: 5'- GGACACACCACAGCACAAAC-3' (2837-2856)	
IGF1	forward: 5'- TGCCAATGTGGTGCTATTGT-3' (4337-4356)	169 bp
	reverse: 5'-GAAAGGTGGTGGTGGCTAGA-3' (4486-4505)	
Fibronectin	forward: 5'-CAGTGGGAGACCTCGAGAAG -3' (4457-4476)	235 bp
	reverse: 5'-CACTGTGACAGCAGGAGCAT-3' (4672-4691)	
BCL2	forward: 5'- TTGACAGAGGATCATGCTGTACTT-3' (173-196)	369 bp
	reverse: 5'- CATCACTATCTCCCGGTTATCG-3' (520-541)	
HIF1A	forward: 5'- TCCATGTGACCATGAGGAAA-3' (815-834)	251 bp
	reverse: 5'-CCAAGCAGGTCATAGGTGGT-3' (1046-1065)	
IRF4	forward: 5'-GACAACGCCTTACCCTTCG-3' (588-607)	271 bp
	reverse: 5'-GGCTTCGGCAGACCTTATG-3' (839-858)	
MYC	forward: 5'-AGCTTGTACCTGCAGGATCT -3' (905-924)	159 bp
	reverse: 5'-CGTCGAGGAGAGCAGAGAAT -3' (1044-1063)	
GAPDH	forward: 5'-CCACCCATGGCAAATTCCATGGCA-3' (211-234)	600 bp
	reverse: 5'-TCTAGACGGCAGGTCAGGTCCACC-3' (788-811)	

*The validity of each primer pair was verified using appropriate cell lines.

Supplemental Table 2. Kinase inhibitors used in the present study

Target	Compound*	Distributor	Dose (μM)
AKT	Akt Inhibitor IV	Calbiochem, La Jolla, CA	2
Aurora	Aurora kinase inhibitor III	Calbiochem, La Jolla, CA	1
CDK	Cdk1/2 inhibitor III	Calbiochem, La Jolla, CA	0.04
CDK	Cdk4 inhibitor	Calbiochem, La Jolla, CA	1
CK	D4476	Calbiochem, La Jolla, CA	1
GSK-3a	GSK-3 inhibitor IX	Calbiochem, La Jolla, CA	1
GSK-3b	1-Azakenpaullone	Calbiochem, La Jolla, CA	1
IGF-IR	AG1024	Calbiochem, La Jolla, CA	10
IKK	BMS-345541	Calbiochem, La Jolla, CA	1
Jak	JAK Inhibitor I	Calbiochem, La Jolla, CA	1
JNK	JNK inhibitor VIII	Calbiochem, La Jolla, CA	1
MAPK	ERK inhibitor II	Calbiochem, La Jolla, CA	1
MEK	U-0126	Wako Chemical, Osaka, Japan	1
PI3K	LY-294002	Cayman Chemical, Ann Arbor, MI	1
PKA	4-cyano-3-methylisoquinoline	Calbiochem, La Jolla, CA	1
PKC	Bisindolymaleimide I, HCl	Calbiochem, La Jolla, CA	1
Raf	RAF1 kinase inhibitor I	Calbiochem, La Jolla, CA	1
ROCK	H-1152	Calbiochem, La Jolla, CA	1
Src	PP1 analog	Calbiochem, La Jolla, CA	1
Syk	Syk inhibitor	Calbiochem, La Jolla, CA	1
TGF-ßRI	TGF-ßRI kinase inhibitor II	Calbiochem, La Jolla, CA	1
VEGFR	VEGFR receptor tyrosine kinase inhibitor II	Calbiochem, La Jolla, CA	1

*These compounds were provided by the Screening Committee of Anticancer Drugs (Tokyo, Japan).

Supplemental Table 3. Oligonucleotides containing siRNA target sequences

Gene	Sequence (sequences corresponding to siRNA are lowercased)
EZH2	Forward: 5'- T gagtactgtgggcaattta TTCAAGAGA taaattgcccacagtactc TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA gagtactgtgggcaattta TCTCTTGAA taaattgcccacagtactc A-3'
MMSET	Forward: 5'- T aatgtccttggcatcattg TTCAAGAGA caatgatgccaaggacatt TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA aatgtccttggcatcattg TCTCTTGAA caatgatgccaaggacatt A-3'
*AKT#*1	Forward: 5'- T acaaggacgggcacattaa TTCAAGAGA ttaatgtgcccgtccttgt TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA acaaggacgggcacattaa TCTCTTGAA ttaatgtgcccgtccttgt A-3'
*AKT#*2	Forward: 5'- T ttcttcctcagcatcaact TTCAAGAGA agttgatgctgaggaagaa TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA ttcttcctcagcatcaact TCTCTTGAA agttgatgctgaggaagaa A-3'
*CDK2#*1	Forward: 5'- T ggtggtggcgcttaagaaa TTCAAGAGA tttcttaagcgccaccacc TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA ggtggtggcgcttaagaaa TCTCTTGAA tttcttaagcgccaccacc A-3'
*CDK2#*2	Forward: 5'- T gctgccacaatgtttataa TTCAAGAGA ttataaacattgtggcagc TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA gctgccacaatgtttataa TCTCTTGAA ttataaacattgtggcagc A-3'
*IGF1R#*1	Forward: 5'- T cggcaacctgagttactac TTCAAGAGA gtagtaactcaggttgccg TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA cggcaacctgagttactac TCTCTTGAA gtagtaactcaggttgccg A-3'
*IGF1R#*2	Forward: 5'- T gtatgacgcgagatatcta TTCAAGAGA tagatatctcgcgtcatac TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA gtatgacgcgagatatcta TCTCTTGAA tagatatctcgcgtcatac A-3'
*PI3K#*1	Forward: 5'- T GCCAGTACCTCATGGATTA TTCAAGAGA taatccatgtggtactggc TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA GCCAGTACCTCATGGATTA TCTCTTGAA taatccatgtggtactggc A-3'

*PI3K#*2	Forward: 5'- T CAGTACCTCATGGATTAGT TTCAAGAGA ACTAATCCATGAGGTACTG TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA CAGTACCTCATGGATTAGT TCTCTTGAA ACTAATCCATGAGGTACTG A-3'
*IGF1#*1	Forward: 5'- T gcacgagttacctgttaaa TTCAAGAGA tttaacaggtaactcgtgc TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA gcacgagttacctgttaaa TCTCTTGAA tttaacaggtaactcgtgc A-3'
*IGF1#*2	Forward: 5'- T ggcatttgtaccaaatata TTCAAGAGA tatatttggtacaaatgcc TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA ggcatttgtaccaaatata TCTCTTGAA tatatttggtacaaatgcc A-3'
*IGF1#*3*	Forward: 5'- T ggttctgtggaataagata TTCAAGAGA tatcttattccacagaacc TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA ggttctgtggaataagata TCTCTTGAA tatcttattccacagaacc A-3'
*IGF1#*4	Forward: 5'- T gctttgcttcagaattaga TTCAAGAGA tctaattctgaagcaaagc TTTTTTC-3'
	Reverse: 5'- TCGAGAAAAAA gctttgcttcagaattaga TCTCTTGAA tctaattctgaagcaaagc A-3'

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*Among 4 sh-RNA against IGF-1, we used IGF-1#3 according to the results shown in Supplemental Figure 6.

Supplemental Table 4. Primers for ChIP assays

Gene	Sequence (corresponding nucleotide positions)*	Product size
IGF1 promoter	forward: 5'-TTGTCACCATGCCCAAAAAA-3' (–312 to –291)	202 bp
	reverse: 5'-TTGCGCAGGCTCTATCTGC-3' (–129 to –111)	
BCL2 promoter	forward: 5'- GGCTCAGAGGAGGGCTCTTT-3' (–395 to –374)	178 bp
	reverse: 5'- GTGCCTGTCCTCTTACTTCATTCTC-3' (–242 to –218)	
IRF4 promoter	forward: 5'-TCACCACTGCCAGCTGCTA-3' (–2836 to –2818)	62 bp
	reverse: 5'-AAACTCCGGATGGCCTCAT-3' (–2916 to –2898)	