Antileukemic activity and mechanism of action of the novel PI3K and histone deacetylase dual inhibitor CUDC-907 in acute myeloid leukemia

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MATERIALS AND METHODS

Drugs

CUDC-907, MK-1775, LY2603618, SCH772984, GDC-0941, SAHA, KU-57788, and hydroxyurea (HU) were purchased from Selleck Chemicals (Houston, TX, USA). Z-VAD-FMK was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

AML cell lines were cultured as previously described.[1, 2] Mycoplasma testing was performed on a monthly basis using the PCR method.[3] MV4-11, THP-1, and U937 cell lines were purchased from the American Type Culture Collection (2006, 2014, 2002, respectively; Manassas, VA, USA). OCI-AML3 was purchased from the German Collection of Microorganisms and Cell Cultures (2011; DSMZ, Braunschweig, Germany). CMS and CTS were gifts from Dr. A Fuse from the National Institute of Infectious Diseases, Tokyo, Japan (2004). MOLM-13 was purchased from AddexBio (2012; San Diego, CA, USA). The cell lines were authenticated in 2017 at the Genomics Core at Karmanos Cancer Institute using the PowerPlex® 16 System from Promega (Madison, WI, USA). Normal bone marrow mononuclear cells (BMMNCs) were purchased from Lonza (Walkersville, MD, USA).

Clinical samples

AML blast samples were purified by standard Ficoll-Hypaque density centrifugation and cultured, as previously described.[1, 4]

MTT assays

MTT (Sigma-Aldrich) assays were performed as previously described.[5, 6] Patient samples were chosen solely based on sample availability.

Western blot analysis

Whole cell lysates were prepared by sonication in 10 mM Tris-Cl, pH 7.0, containing 1% SDS, protease inhibitors, and phosphatase inhibitors (Roche Diagnostics, Indianapolis, IN, USA). Whole cell lysates were subjected to SDS-polyacrylamide gel electrophoresis, electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Thermo Fisher Inc., Rockford, IL, USA) and immunoblotted with antibodies. Immunoreactive proteins were visualized using the Odyssey Infrared Imaging System (Li-Cor, Lincoln, NE, USA), as described by the manufacturer. Western blots were repeated at least three times and one representative blot is shown.

Anti-PARP, -Mcl-1, -Bcl-2, -Bcl-xL, -Bax, -β-actin, -ERK , -CHK1 (Proteintech, Chicago, IL, USA), -p-AKT (T308), -p-AKT (S473) (Affinity Biosciences, Zhenjiang, Jiangsu, China), -Bim, -p-Mcl-1(S159), -p-Mcl-1(T163), -Wee1, -RPA32(4E4), -p-CDC25C, -cf-Caspase 3, -c-Myc (Cell Signaling Technologies, Danvers, MA, USA), -Bak, -CDK1, -RRM1, -RRM2, -p-CDK1(Y15), -p-CDK2(Y15), -CDK2, -E2F1, -H4, -ac-Tubulin, -p-ERK, -AKT (Abcam, Cambridge, MA, USA), -ac-H4 or -γ-H2AX (Millipore, Billerica, MA, USA) antibodies were used for Western blot analysis.

Annexin V/PI staining

Experiments with AML cell lines were performed 3 independent times in triplicates, while patient sample experiments were performed once in triplicate due to limited sample.

shRNA knockdown of Bim and ectopic overexpression of Mcl-1, Bcl-xL, and c-Myc

The pMD-VSV-G and delta 8.2 plasmids were gifts from Dr. Dong at Tulane University. Bak, Bax, and non-target control (NTC) shRNA lentiviral vectors were purchased from Sigma-Aldrich. Red fluorescent protein (RFP), Bcl-xL, c-Myc, and Mcl-1 cDNA constructs were purchased from Thermo Fisher Scientific Biosciences (Lafayette, CO, USA). Lentivirus production and transduction were carried out as previously described.[7]

Alkaline comet assay

Approximately 50 comets per gel were scored using CometScore (TriTek Corp, Sumerduck, VA, USA). The median percent DNA in the tail was calculated and graphed ± SEM.

Chromatin fractionation

Chromatin fractionation was carried out as described previously.[4, 8]

Quantification of gene expression by real-time RT-PCR

Total RNA was extracted using TRIzol (Life Technologies), cDNAs were prepared, and then purified, as described previously.[1, 6, 9] Mcl-1 (Hs01050896_m1), Bim (Hs00708019_s1), CHK1 (Hs00967506_m1), and Wee1 (Hs01119384_g1) transcripts were quantitated using TaqMan probes (Life Technologies) and a LightCycler 480 real-time PCR machine (Roche Diagnostics), based on the manufacturer's instructions. RRM1 transcripts were quantified using forward (5'-ACTAAGCACCCTGACTATGCTATCC-3') and reverse (5'-CTTCCATCACATCACTGAACACTTT-3') primers and SYBR green, and the above-mentioned real-time PCR machine. c-Myc transcripts were quantified using forward (5'-GTGGTCTTCCCCTACCCTCT-3') and reverse (5'-CGAGGAGAGCAGAGAATCCG-3') primers. Real-time PCR results are expressed as means from three independent experiments and normalized to GAPDH transcripts measured by either TaqMan probe (Hs02786624_g1) or forward (5'-AGCCACATCGCTCAGACA-3') and reverse (5'-GCCCAATACGACCAAATCC-3') primers and SYBR green. Real-time PCR results are expressed as means from three independent experiments and normalized to GAPDH transcripts. Fold changes were calculated using the comparative Ct method.[10]

DNA-PK kinase assay

DNA-PK kinase activity was measured using the ADP-Glo Kinase Assay + DNA-PK Kinase Enzyme System (Promega, Madison, WI, USA).

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TITLES AND LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1. CUDC-907 treatment inhibits PI3K/Akt and HDACs in AML cell lines. (A) The structure of CUDC-907. (B&C) U937 (panel B) and MOLM-13 (panel C) cells were treated with variable concentrations of CUDC-907 for 3 hours. Whole cell lysates were subjected to Western blotting.

Figure S2. CUDC-907 treatment does not affect expression of Bcl-2, Bcl-xL, Bax, and Bak in AML cell lines. (A&B) MOLM-13 (panel A) and U937 (panel B) cells were treated with variable concentrations of CUDC-907 for 24 hours. Whole cell lysates were subjected to Western blotting.

Figure S3. Caspase-independent downregulation of Wee1, CHK1, and RRM1 by CUDC-907. MOLM-13 and U937 cells were treated with CUDC-907 in the absence or presence of the pan-caspase inhibitor Z-VAD-FMK for 24 h. Western blotting of whole cell lysates are shown. The fold changes for the densitometry measurements, normalized to β-actin and then compared to no drug control, are indicated below the corresponding blot.

Figure S4. CUDC-907 is more potent than the combination of PI3K inhibitor GDC-0941 and HDACi SAHA at inducing molecular changes and cell death in MOLM-13 AML cells. (A-B) MOLM-13 cells were treated with GDC-0941, SAHA, GDC + SAHA (1:1 ratio), or CUDC-907 for 24 h. The cells were subjected to Annexin V-FITC/PI staining and flow cytometry analyses (panel A). ***indicates $p<0.001$ compared to no drug control. $^{###}$indicates $p<0.001$ compared to single drug treatment. The EC_{50} values were calculated as concentration of CUDC-907 or both GDC-0941 and SAHA necessary to induce 50% Annexin V+ cells and are shown in panel B. (C) MOLM-13 cells were treated as in panel A. Whole cell lysates were subjected to Western blotting and probed with the indicated antibody. The fold changes for the densitometry measurements, normalized to β-actin and then compared to no drug control, are indicated below the corresponding blot.

Figure S5. Upregulation of Bim and downregulation of Mcl-1, CHK1, Wee1, and RRM1 coincide with induction of apoptosis in U937 AML cells. (A&B) U937 cells were treated with CUDC-907 for up to 24 hours. Whole cell lysates were subjected to Western blotting and probed with the indicated antibody. The fold changes for the densitometry measurements, normalized to

β-actin and then compared to no drug control, are indicated below the corresponding blot. Cells treated with 50 nM CUDC-907 for 24 hours were used as a positive control for γH2AX and cleaved caspase 3.

Figure S6. ERK inhibition downregulates Mcl-1 protein, both total and p-Mcl-1(T163), similar to CUDC-907 treatment. (A&B) MOLM-13 cells were treated with ERK-selective inhibitor SCH-772984, with or without the proteasome inhibitor MG-132, for 24 h. Whole cell lysates were subjected to Western blotting. The fold changes for the densitometry measurements, normalized to β-actin and then compared to no drug control, are indicated below the corresponding blot.

Figure S7. CUDC-907 treatment induces DNA damage independent of caspase. (A&B) U937 and MOLM-13 treated with CUDC-907 in the absence or presence of Z-VAD-FMK for 16 hours and then subjected to alkaline comet assay analysis. Representative images are shown. Data are graphed as mean percent DNA in the tail from 3 replicate gels ± SEM. ns indicates not significant.

Figure S8. CUDC-907 treatment does not significantly affect DNA-PK activity. Purified DNA-PK was treated with 0-100 nM CUDC-907 or 100 nM KU-57788 (positive control) for 1 hour, and then the treatments were subjected to the ADP-Glo Kinase Assay + DNA-PK Kinase Enzyme Assay. Data are graphed as mean DNA-PK activity from 3 replicates ± SEM.

Figure S9. Caspase-independent downregulation of c-Myc by CUDC-907 treatment in AML cells. (A) MOLM-13 and U937 cells were treated with CUDC-907 and Z-VAD-FMK, alone or combined, for 24 h. Whole cell lysates were subjected to Western blotting. (B) MOLM-13 cells were treated with GDC-0941, SAHA, GDC + SAHA, or CUDC-907 for 24 h. whole cell lysates were subjected to Western blotting and probed with the indicated antibody.

Figure S10. CUDC-907 downregulates FLT3 in MOLM-13 cells. MOLM-13 and U937 cells were treated with variable concentrations of CUDC-907 for 24 h and whole cell lysates were subjected to Western blotting. The fold changes for the densitometry measurements, normalized to β-actin and then compared to no drug control, are indicated below the corresponding blot.

Table S1. Characteristics and CUDC-907 sensitivity in AML cell lines

Cell line	Gender	Age (year)	Disease Status	FAB subtype	Cytogenetics	Gene fusion/mutation	CUDC-907 IC_{50} (nM)
MOLM-13	Male	20	At relapse	M5	Ins(11;9)(q23;p22p23)	FLT3-ITD, MLL-AF9	6.6
U937	Male	37	Refractory	M5	t(10;11)(p13;q14)	CALM-AF10	14.6
CTS	Female	13	At relapse	M1	t(6;11)(q27;q23)	MLL-AF6	73.7
MV4-11	Male	10	At diagnosis	M5	t(4;11)(q21;q23)	FLT3-ITD, MLL-AF4	12.4
CMS	Female	2	At relapse	M7			36.1
OCI-AML3	Male	57	At diagnosis	M5		NPM1	14.5
THP-1	male	1	At relapse	M5	t(9;11)(p21;q23)	MLL-AF9	39.9

Table S2. Patient characteristics and CUDC-907 sensitivity in primary AML patient samples

Patient	Gender	Age (year)	Disease status	FAB subtype	Cytogenetics	Blast purity (%)	Gene fusion/mutation	CUDC-907 IC_{50} (nM)
AML#1	Female	12	Newly diagnosed	M3	46, XX, t(15;17)(q22;q21)/46, XX	88.0		8.1
AML#2	male	59	Newly diagnosed	M2	46, XY	81.0	NPM-1	411.3
AML#3	Male	17	Newly diagnosed	M2	46, XY	68.5		207.2
AML#4	Female	76	Newly diagnosed	M5	46, XX	84.5	dupMLL, CEBPA	496.3
AML#5	Male	52	Newly diagnosed	M4	46, XY	96.0	DEK/CAN	710.2
AML#6	Male	65	Newly diagnosed	M5	47, XY, add(7q), -16, -17, +marx3	76.0		376.2
AML#7	Male	43	Newly diagnosed	M2	46, XY, t(8;21)(q22;q22)	48.0	AML1-ETO	623.1
AML#8	Male	50	Newly diagnosed	M2	45, X, -Y, t(8;21)(q22;q22), del(11q)	46.0	AML1-ETO	617.0
AML#9	Male	12	Newly diagnosed	M3	46, XY, t(15;17)(q22;q21)	92.5	PML-RARα	191.5
AML#10	Male	74	Newly diagnosed	M5	47, XY, +8	95.0	FLT-3 ITD, NPM-1, DNMT3A	92.5
AML#11	Male	19	Newly diagnosed	M2	45, X, -Y, t(8;21)(q22;q22), del(9q)	47.0	AML1-ETO	260.0
AML#12	Male	25	Newly diagnosed	M3	46, XY, t(15;17)(q22;q21)	94.0	PML-RARα	340.0
AML#13	Female	9	Newly diagnosed	M1	46, XX	93.5		375.2
AML#14	Female	50	Relapsed	M2	46, XX	81.0	CEBPA double mutation	606.0
AML#15	Female	7	Newly diagnosed	M4	46, XX, t(11;20)(p15;q11)/46, idem, del(9)(q22)	83.0		90.8
AML#16	Female	52	Newly diagnosed	M3	46, XX, t(15;17)(q22;q21)	90.0	PML-RARα	153.6
AML#17	Male	38	Newly diagnosed	M3	47, XY, add(1p), t(15;17)(q22;q21), +14	95.0	PML-RARα	651.4
AML#18	Female	51	Newly diagnosed	M5	46, XX	82.0		822.0
AML#19	Male	48	Newly diagnosed	M5	46, XY	42.0	IDH2, DNMT3A	27.5
AML#20	Female	77	Newly diagnosed	M4	46, XX	50.0		230.2
AML#21	Female	44	Newly diagnosed	M3	46, XX, t(15;17)(q22;q21)	89.0	PML-RARα	523.9
AML#22	Female	12	Newly diagnosed	M2	47, XX, +10	80.0	FLT-3 ITD, CEBPA	591.9
AML#23	Female	60	Newly diagnosed	M5	46, XX	69.5		658.7

Table S2. Patient characteristics and CUDC-907 sensitivity in primary AML patient samples (Continued)

Patient	Gender	Age (year)	Disease status	FAB subtype	Cytogenetics	Blast purity (%)	Gene mutation	CUDC-907 IC_{50} (nM)
AML#24	Female	65	Newly diagnosed	M5	46, XX	91.0	FLT-3 ITD, NPM-1	42.5
AML#25	Male	18	Newly diagnosed	M3	46, XY, t(15;17)(q22;q21)	95.0	PML-RARα	495.3
AML#26	Male	64	Newly diagnosed	M4	46, XY	69.0		313.3
AML#27	Male	59	Newly diagnosed	M2	46, XY	82.0	HOX11	276.8
AML#28	Male	75	Newly diagnosed	M4	46, XX,+8	91.0		374.3
AML#29	Female	54	Newly diagnosed	M5	46, XX	64.0	MLL-AF6	222.7
AML#30	Female	64	Newly diagnosed	M4	46, XX	91.0	dupMLL, FLT3-ITD, IDH1	46.4
AML#31	Male	8	Newly diagnosed	AML	46, XX, del(16)(q11)/46, ider, del(13)	68.5		57.4
AML#32	Female	74	Newly diagnosed	M1	46, XX	69.0		217.6
AML#33	Female	37	Newly diagnosed	M2	46, XX	74.0	CBFB-MYH11, FLT-3 ITD, C-Kit	140.7
AML#34	Male	44	Relapsed	M2	46, XY	72.0	CEBPA double mutation	123.0
AML#35	Female	28	Newly diagnosed	M3	46, XX	81.5	PML-RARα	435.4
AML#36	Male	4	Newly diagnosed	M2	46, XX, del(9)(q13)	50.5		56.4
AML#37	Female	67	Newly diagnosed	M5	46, XX	62.0		191.1
AML#38	Female	10	Newly diagnosed	AML	47, XY, -6, -10, del(11)(q21),t(12;18)(p13;q21), -13, +4mar/46, XY	91.0		105.5
AML#39	Male	48	Newly diagnosed	M2	46, XY	79.5	FLT-3 ITD	197.2

Table S2. Patient characteristics and CUDC-907 sensitivity in primary AML patient samples (Continued)

Patient	Gender	Age (year)	Disease status	FAB subtype	Cytogenetics	Blast purity (%)	Gene mutation	CUDC-907 IC_{50} (nM)
AML#40	Male	50	Newly diagnosed	M2	46, XY	88.0		196.5
AML#41	Male	8	newly diagnosed	M3	46, XY, t(15;17)(q22;q21)	88.0		259.6
AML#42	Male	76	Newly diagnosed	M4	46, XY	95.5		60.9
AML#43	Male	42	Newly diagnosed	M2	46, XY	59.5	CEBPA double mutation	165.4
AML#44	Female	46	Newly diagnosed	M2	46, XX	64.5		379.7
AML#45	Female	50	Newly diagnosed	M4	46, XX	91.5	FLT3-ITD，NPM-1，DNMT3A	146.5
AML#46	Male	55	Newly diagnosed	M2	46, XY	56.0	dupMLL	55.2
AML#47	Female	69	Newly diagnosed	M1	46, XX	91.5		153.2
AML#48	Female	59	Newly diagnosed	M4	46, XX	82.0	CEBPA double mutation	118.0
AML#49	Female	37	Newly diagnosed	M3	47, XX, +8, t(15;17)(q22;q21)	92.5	PML-RARα	183.3
AML#50	Male	72	Newly diagnosed	M5	46, XY	83.5	DNMT3A,NPM1,NRAS,SF3B1, TET2	340.7
AML#51	Female	66	Newly diagnosed	M4	46, XX	43.5		214.9
AML#52	Female	5	Newly diagnosed	M4	46, XX	49.0		149
AML#53	Female	23	Newly diagnosed	M4	46, XX	48.5	AML1-ETO, NRAS	1725
AML#54	Male	32	Newly diagnosed	M2	45, X, -Y, t(8;21)(q22;q22)	90.0		90.28
AML#55	Female	13	Newly diagnosed	M2	46, XY	66.0		55.41
AML#56	Male	65	Newly diagnosed	M4	46, XY	86.0	FLT3-ITD, DNMT3A, IDH2	281.8
AML#57	Male	55	Newly diagnosed	M3	46，X，-Y，t(15;17)(q22;q21)	93.0	PML-RARA	39.98

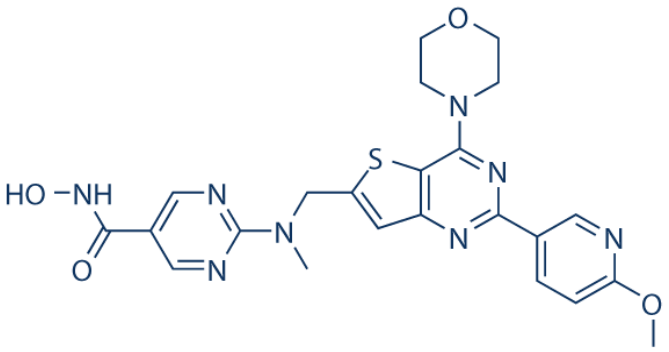
Table S2. Patient characteristics and CUDC-907 sensitivity in primary AML patient samples (Continued)

Patient	Gender	Age (year)	Disease status	FAB subtype	Cytogenetics	Blast purity (%)	Gene mutation	CUDC-907 IC_{50} (nM)
AML#58	Male	27	Newly diagnosed	M3	46, XY, t(15;17)(q22;q21)	94.0	FLT-3 ITD, PML-RARα	21.7
AML#59	Female	66	Relapsed	M4	45, XX，-7	48.0		73.63
AML#60	Female	44	Relapsed	M5	46, XX	84.5	NPM-1 FLT3-ITD/IDH2/DNMT3A	159.4
AML#61	Male	58	Newly diagnosed	M2	46, XY	63.5	CEBPαdouble mutation, DNMT3A, GATA2	263.4
AML#62	Male	46	Newly diagnosed	M1	46, XY	92.0	CEBPA double mutation	62.9
AML#63	Female	10	Newly diagnosed	M5	46, XX	97.0		31.63
AML#64	Female	60	Relapsed	M2	46, XX	69.0	CEBPA double mutation	355.6
AML#65	Male	40	Newly diagnosed	M2	46, XY	88.5	CEBPA double mutation	82.2
AML#66	Female	51	Relapsed	M5	46, XX	94.0	FLT-3 ITD, NPM1, DNMT3A	57.48
AML#67	Male	49	Relapsed	M2	46, XY	79.5	FLT-3 ITD, NPM1	145.8
AML#68	Male	24	Newly diagnosed	M5	46, XY	92.0		83.44
AML#69	Male	19	Newly diagnosed	M4	46, XY	63.0	CEBPA, c-Kit, NRAS, GATA2, Flt3 S451F	149.1
AML#70	Male	27	Newly diagnosed	M4	47, XY, +8	80.0	FLT-3 ITD	92.79
AML#71	Female	60	Relapsed	M2	46, XX	49.0	CEBPA mutation	414.3
AML#72	Male	66	Newly diagnosed	M5	46, XY	41.0		174
AML#73	Male	2	Newly diagnosed	M5	50, XY, +8, +11, +14, +19	66.4		ND
AML#74	Male	66	Newly diagnosed	M2	46, XY	58.0	CEBPA mutation	326.9
AML#75	Female	40	Newly diagnosed	M4	46, XY	53.5	NPM-1, IDH-1	1831
AML#76	Female	48	Newly diagnosed	M2	ND	61.0	ND	19.16
AML#77	Male	68	Newly diagnosed	M5	46, XY	96.5	FLT3-ITD，NPM1，DNMT3A	185.3
AML#78	Male	53	Relapsed	M2	ND	98	ND	351.7

Note: ND, not determined

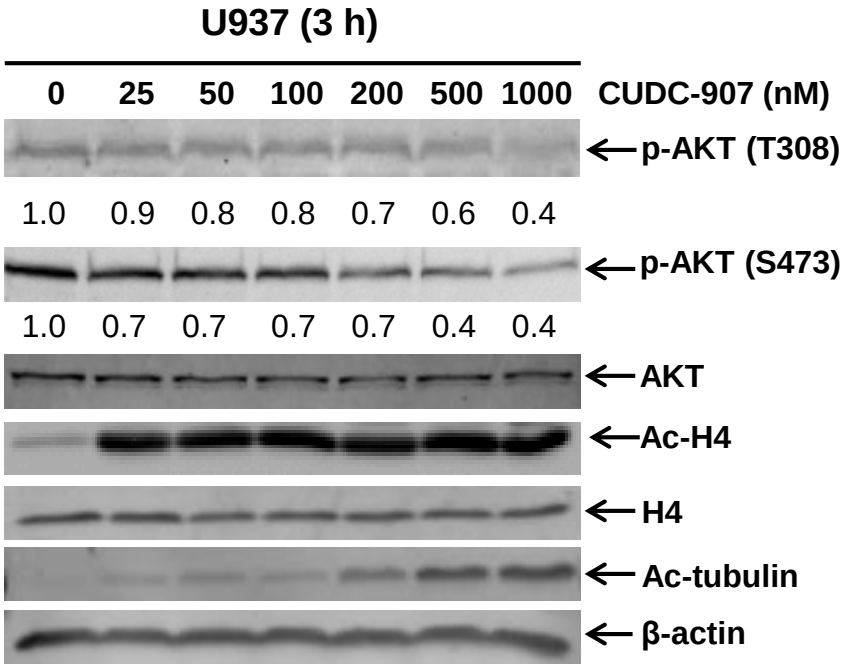
Figure S1

A



CUDC-907

B



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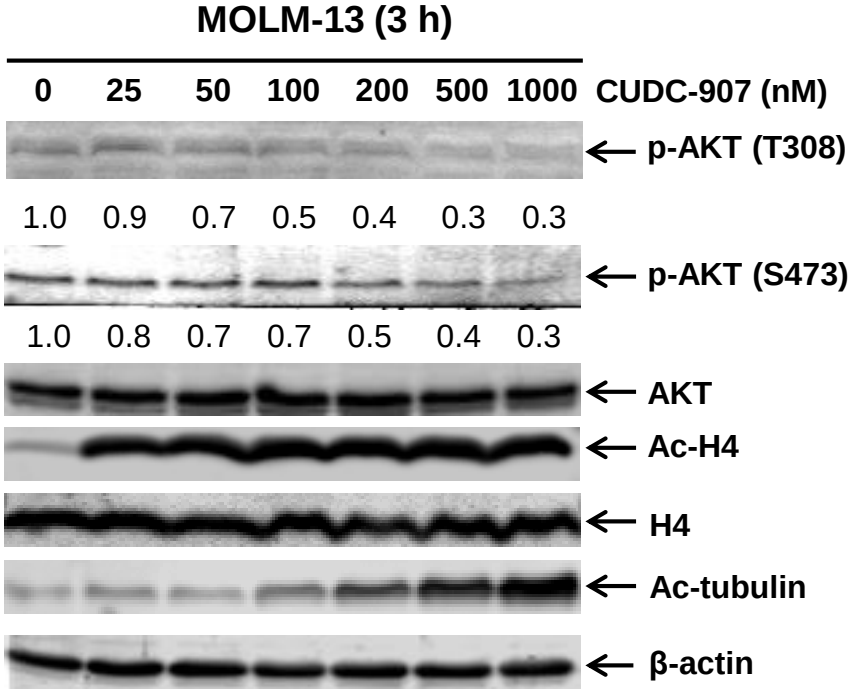
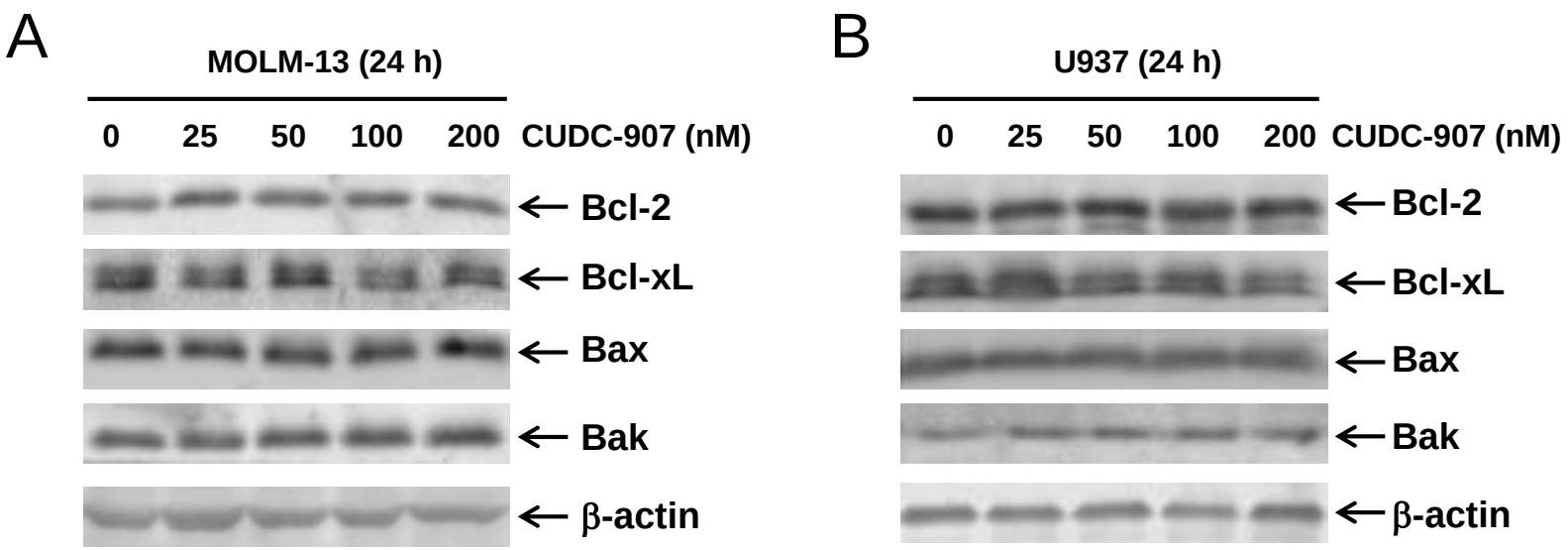


Figure S2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β-actin
B
U937 (24 h)
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100
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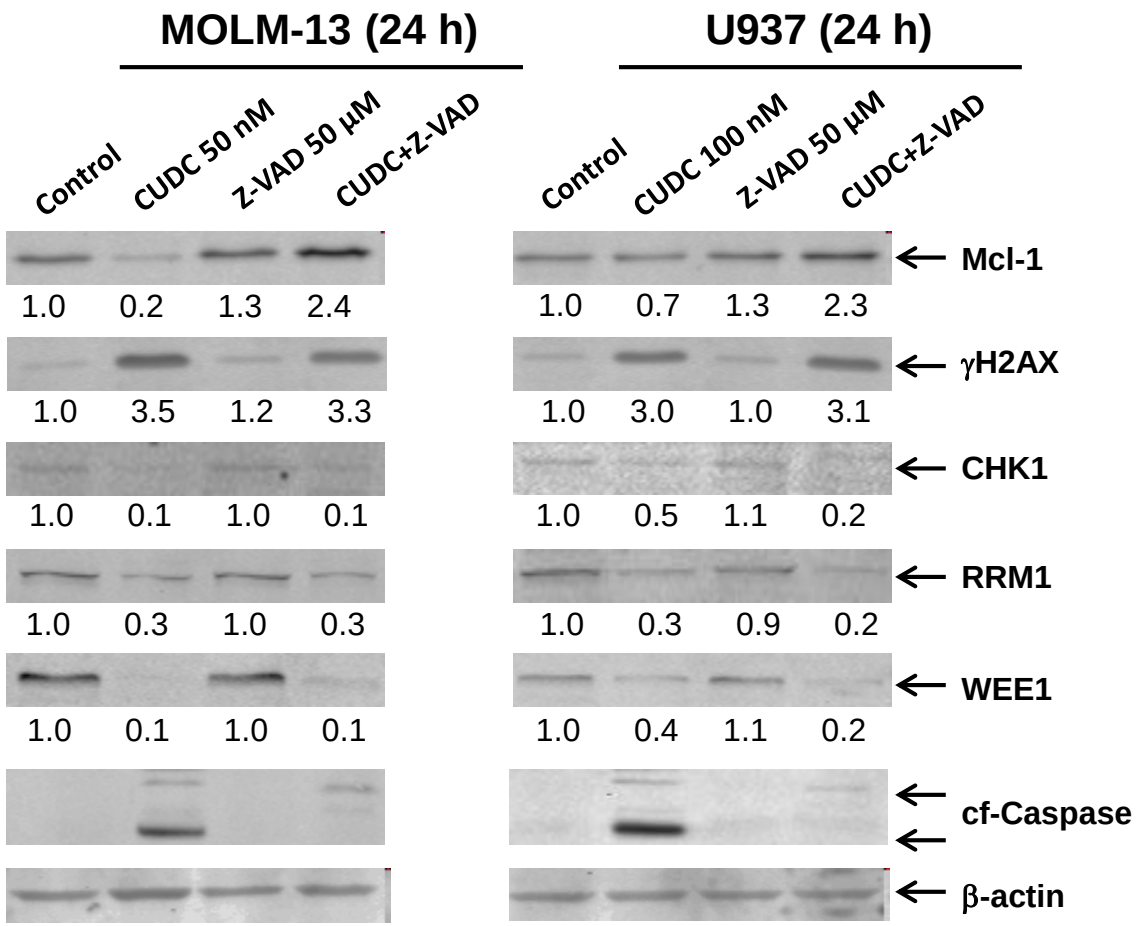
Figure S3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U937 (24 h)
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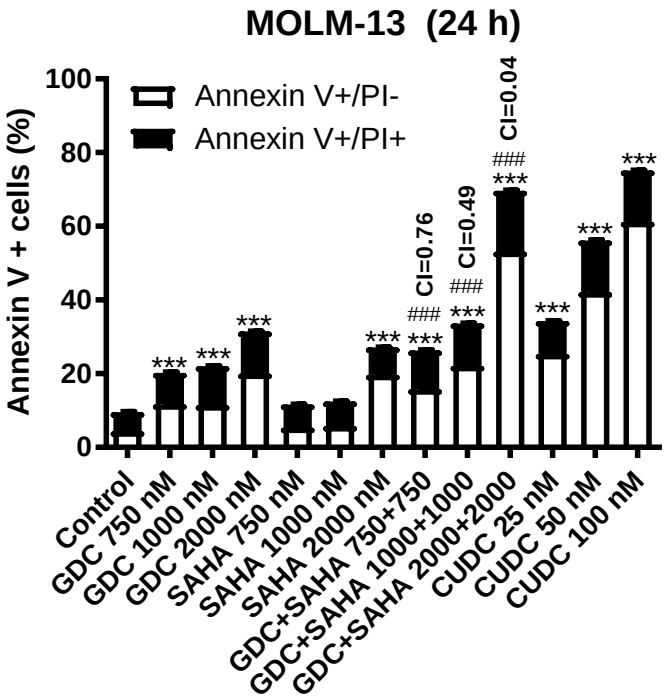
CUDC+Z-VAD
Control
CUDC 100 nM
Z-VAD 50 μM
CUDC+Z-VAD
Mcl-1
1.0 0.2 1.3 2.4
1.0 0.7 1.3 2.3
γH2AX
1.0 3.5 1.2 3.3
1.0 3.0 1.0 3.1
CHK1
1.0 0.1 1.0 0.1
1.0 0.5 1.1 0.2
RRM1
1.0 0.3 1.0 0.3
1.0 0.3 0.9 0.2
WEE1
1.0 0.1 1.0 0.1
1.0 0.4 1.1 0.2
cf-Caspase
β-actin

Figure S4

A



B

Inhibitor	EC50 (nM)
GDC+SAHA	1340
CUDC-907	42.4

C

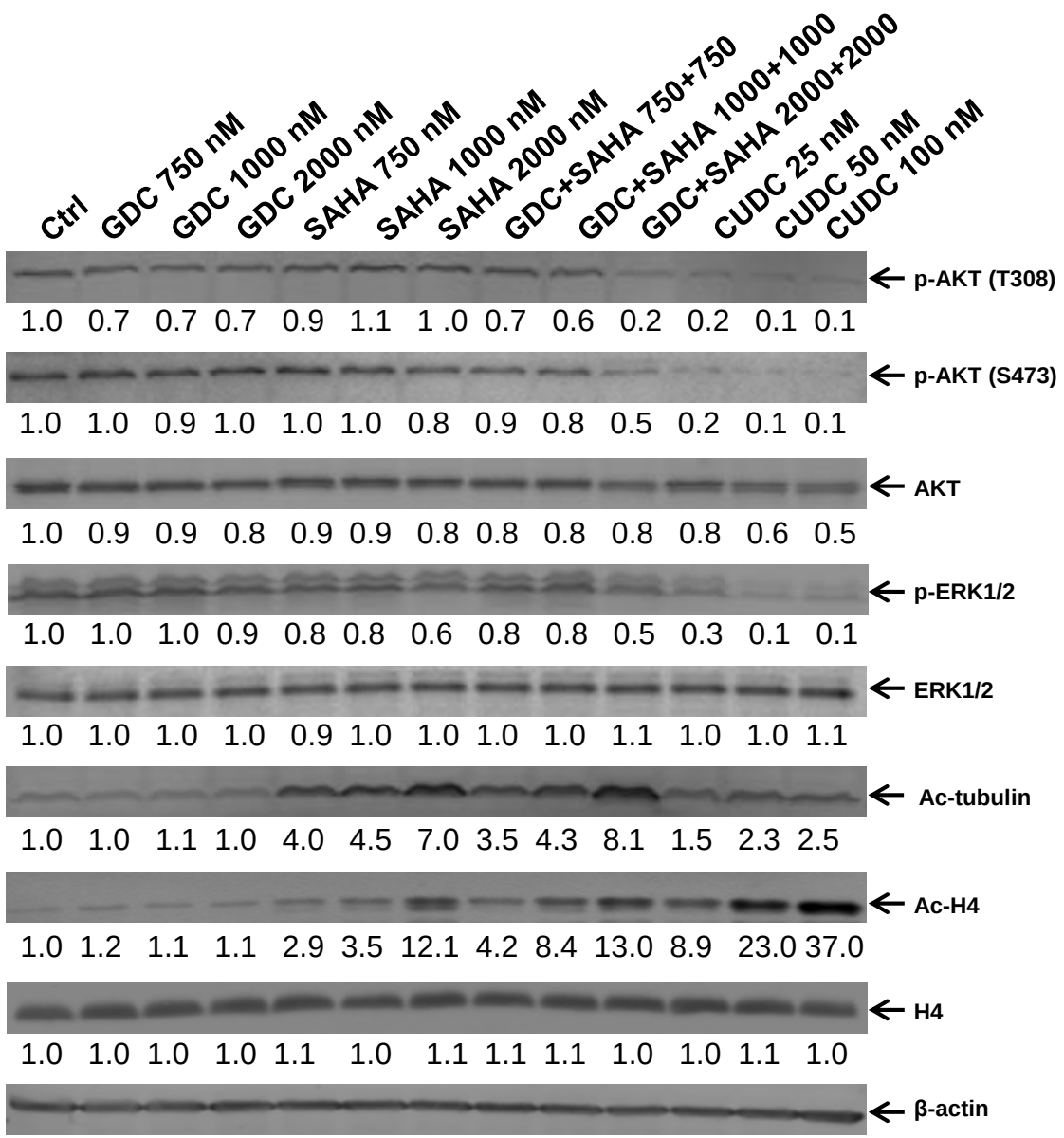


Figure S5A

A

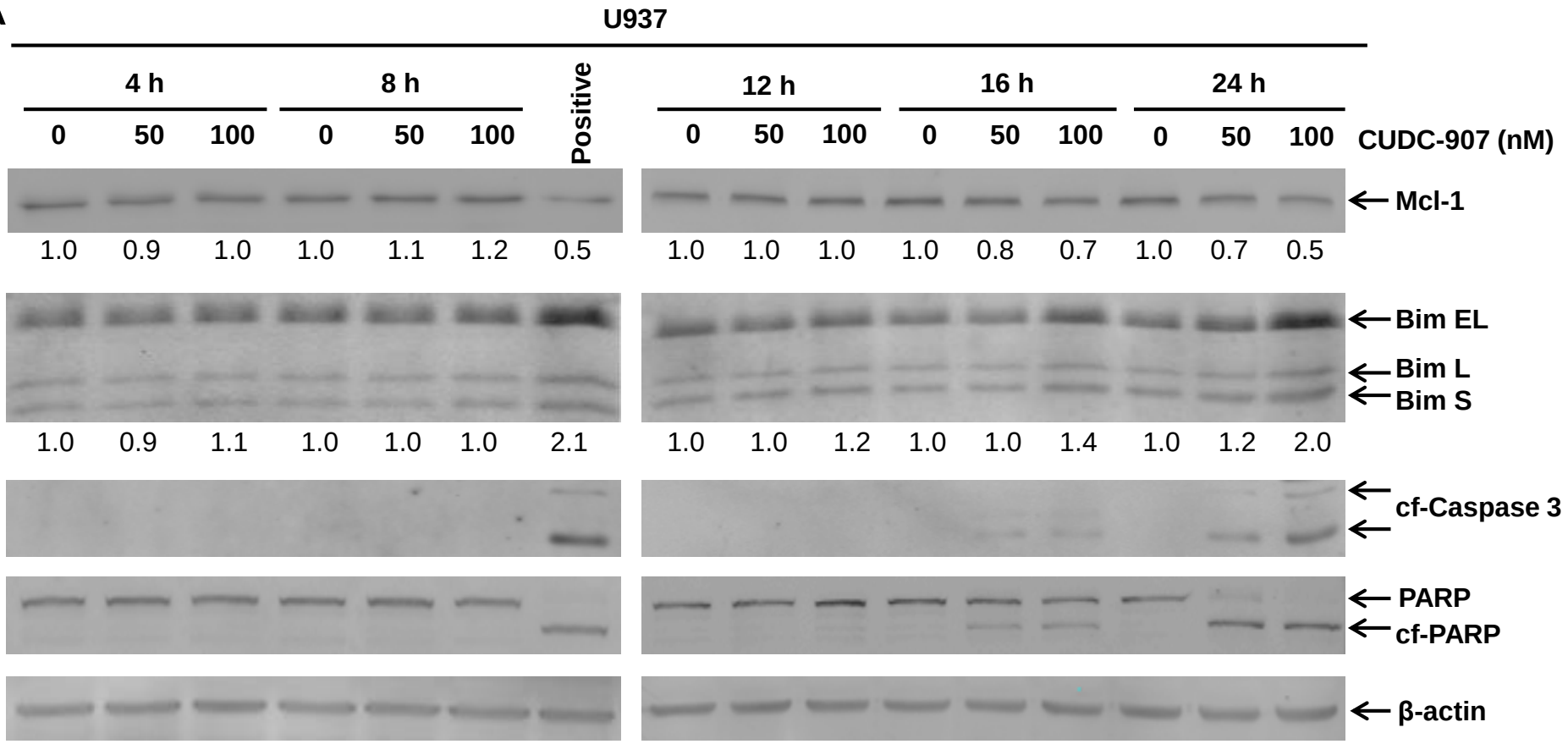

U937
4 h
8 h
Positive
12 h
16 h
24 h
0
50
100
0
50
100
0
50
100
0
50
100
0
50
100
CUDC-907 (nM)
Mcl-1
1.0 0.9 1.0 1.0 1.1 1.2 0.5 1.0 1.0 1.0 1.0 0.8 0.7 1.0 0.7 0.5
Bim EL
Bim L
Bim S
1.0 0.9 1.1 1.0 1.0 1.0 2.1 1.0 1.0 1.2 1.0 1.0 1.4 1.0 1.2 2.0
cf-Caspase 3
PARP
cf-PARP
β-actin

Figure S5B

B

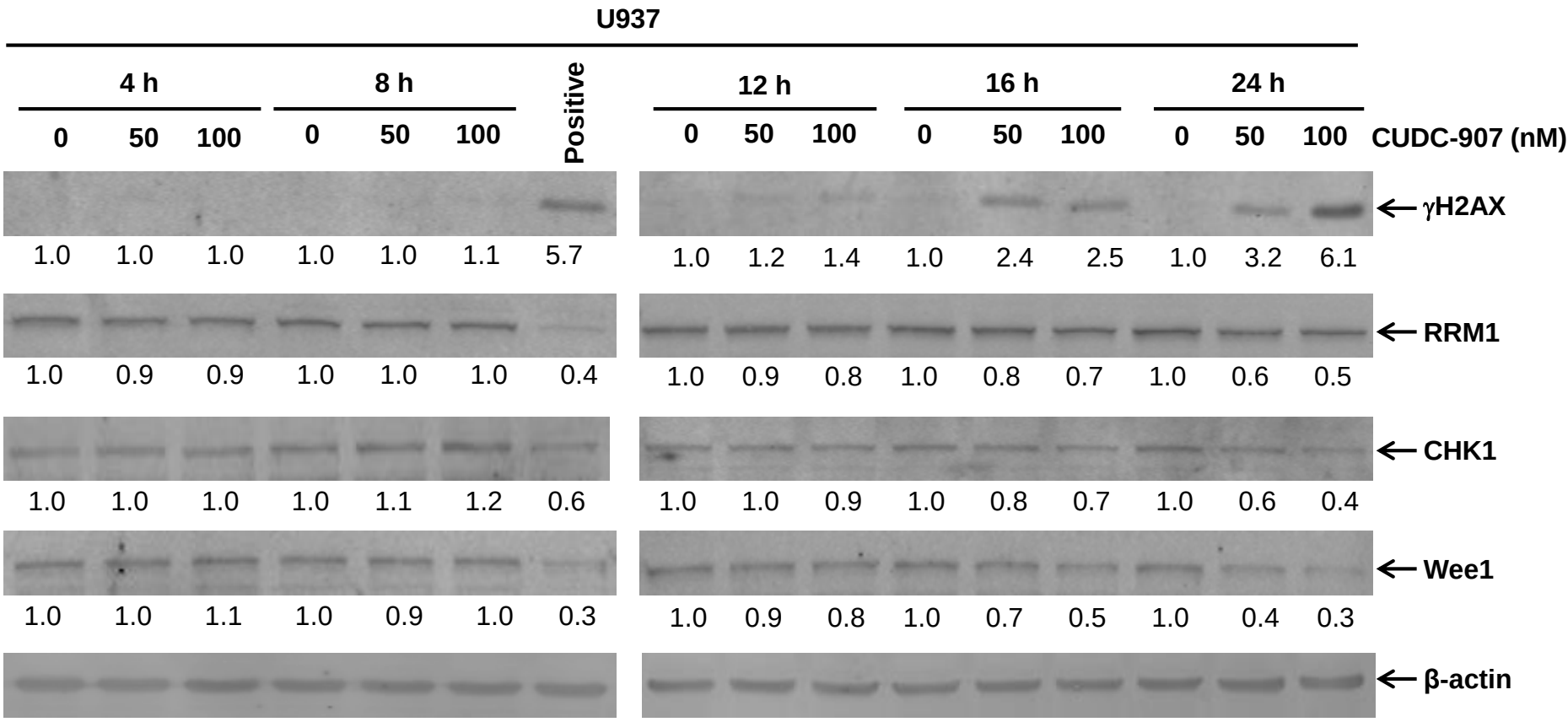

U937
4 h
8 h
Positive
12 h
16 h
24 h
0 50 100 0 50 100 0 50 100 0 50 100 0 50 100 0 50 100
CUDC-907 (nM)
γH2AX
1.0 1.0 1.0 1.0 1.0 1.1 5.7 1.0 1.2 1.4 1.0 2.4 2.5 1.0 3.2 6.1
RRM1
1.0 0.9 0.9 1.0 1.0 1.0 0.4 1.0 0.9 0.8 1.0 0.8 0.7 1.0 0.6 0.5
CHK1
1.0 1.0 1.0 1.0 1.1 1.2 0.6 1.0 1.0 0.9 1.0 0.8 0.7 1.0 0.6 0.4
Wee1
1.0 1.0 1.1 1.0 0.9 1.0 0.3 1.0 0.9 0.8 1.0 0.7 0.5 1.0 0.4 0.3
β-actin

Figure S6

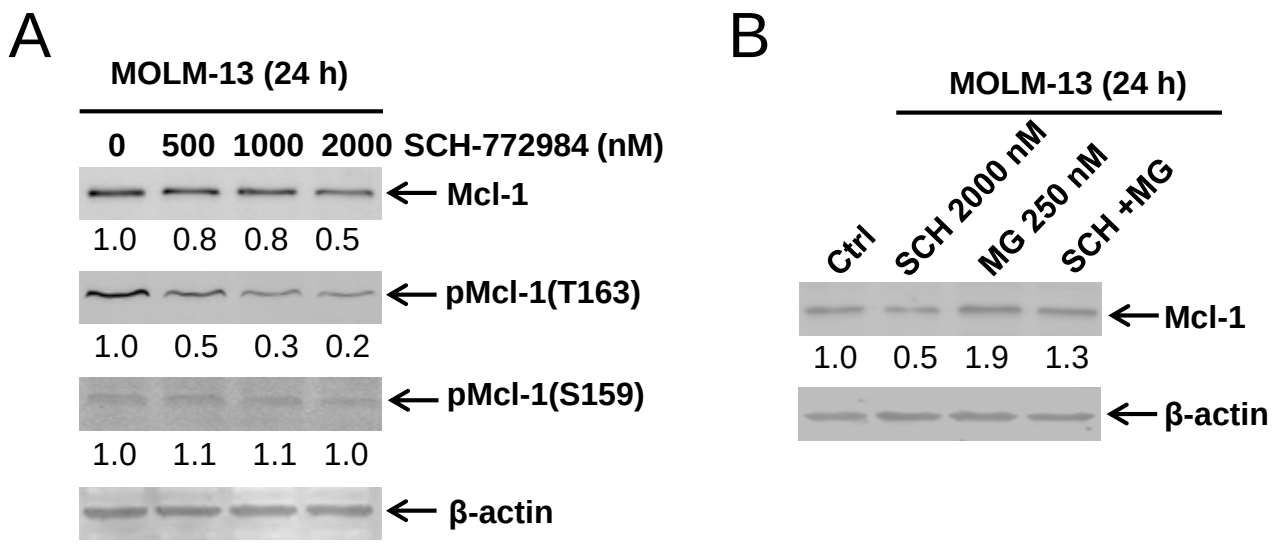

A
MOLM-13 (24 h)
0 500 1000 2000 SCH-772984 (nM)
Mcl-1
1.0 0.8 0.8 0.5
pMcl-1(T163)
1.0 0.5 0.3 0.2
pMcl-1(S159)
1.0 1.1 1.1 1.0
β-actin
B
MOLM-13 (24 h)
Ctrl
SCH 2000 nM
MG 250 nM
SCH +MG
Mcl-1
1.0 0.5 1.9 1.3
β-actin

Figure S7

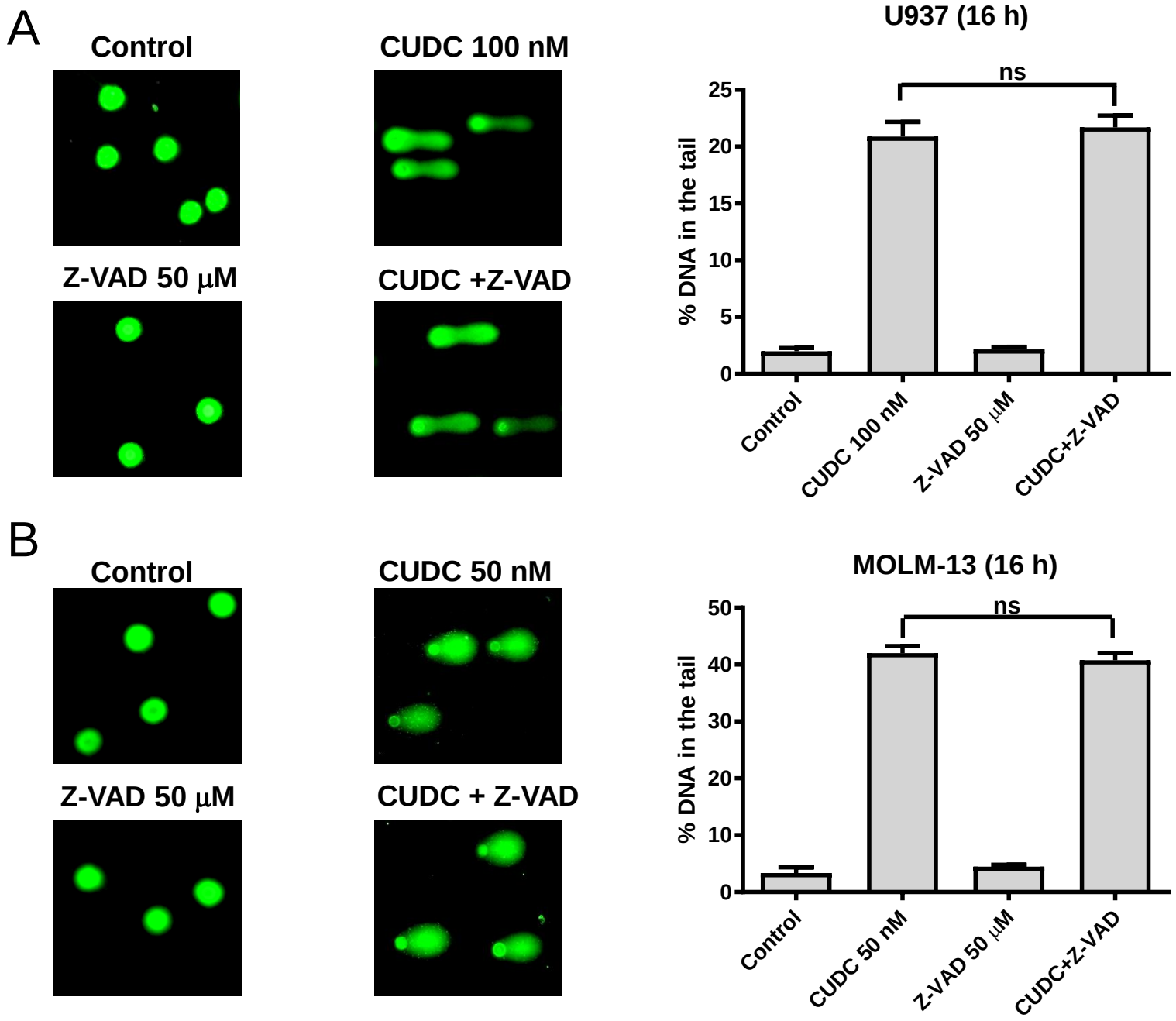

A
Control
CUDC 100 nM
Z-VAD 50 μM
CUDC +Z-VAD
U937 (16 h)
ns
% DNA in the tail
25
20
15
10
5
0
Control
CUDC 100 nM
Z-VAD 50 μM
CUDC+Z-VAD
B
Control
CUDC 50 nM
Z-VAD 50 μM
CUDC + Z-VAD
MOLM-13 (16 h)
ns
% DNA in the tail
50
40
30
20
10
0
Control
CUDC 50 nM
Z-VAD 50 μM
CUDC+Z-VAD

Figure S8

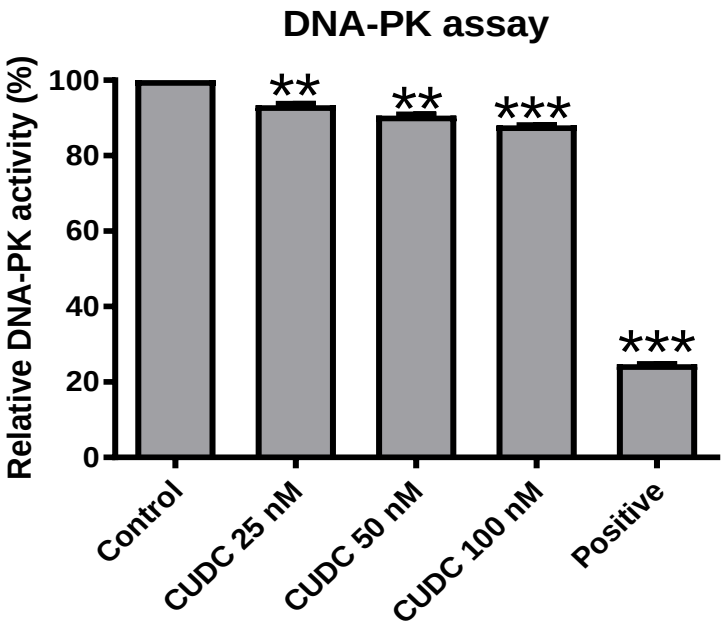

DNA-PK assay
Relative DNA-PK activity (%)
100
80
60
40
20
0
**
**
***
***
Control
CUDC 25 nM
CUDC 50 nM
CUDC 100 nM
Positive

Figure S9

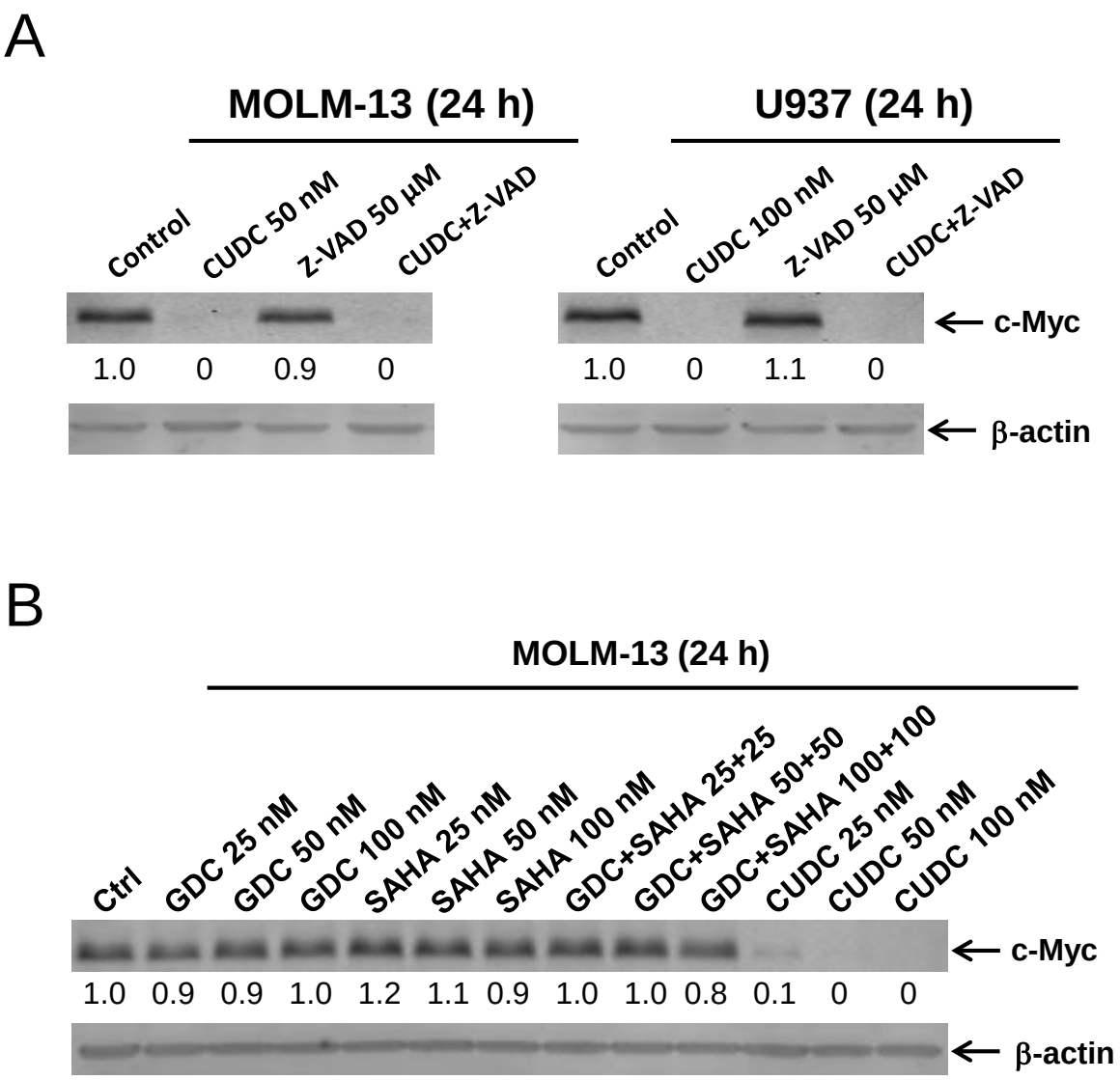
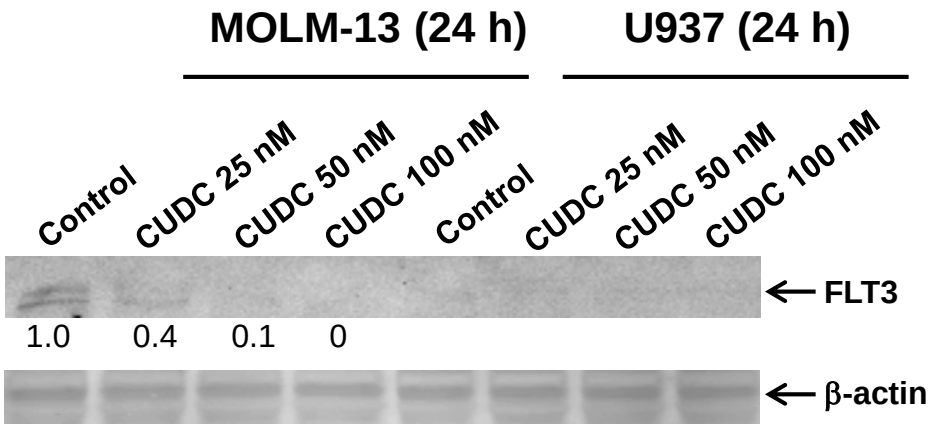

A
MOLM-13 (24 h)
U937 (24 h)
Control
CUDC 50 nM
Z-VAD 50 μM
CUDC+Z-VAD
Control
CUDC 100 nM
Z-VAD 50 μM
CUDC+Z-VAD
c-Myc
1.0 0 0.9 0
1.0 0 1.1 0
β-actin
B
MOLM-13 (24 h)
Ctrl
GDC 25 nM
GDC 50 nM
GDC 100 nM
SAHA 25 nM
SAHA 50 nM
SAHA 100 nM
GDC+SAHA 25+25
GDC+SAHA 50+50
GDC+SAHA 100+100
CUDC 25 nM
CUDC 50 nM
CUDC 100 nM
c-Myc
1.0 0.9 0.9 1.0 1.2 1.1 0.9 1.0 1.0 0.8 0.1 0 0
β-actin

Figure S10


MOLM-13 (24 h)
U937 (24 h)
Control
CUDC 25 nM
CUDC 50 nM
CUDC 100 nM
Control
CUDC 25 nM
CUDC 50 nM
CUDC 100 nM
FLT3
1.0
0.4
0.1
0
β-actin