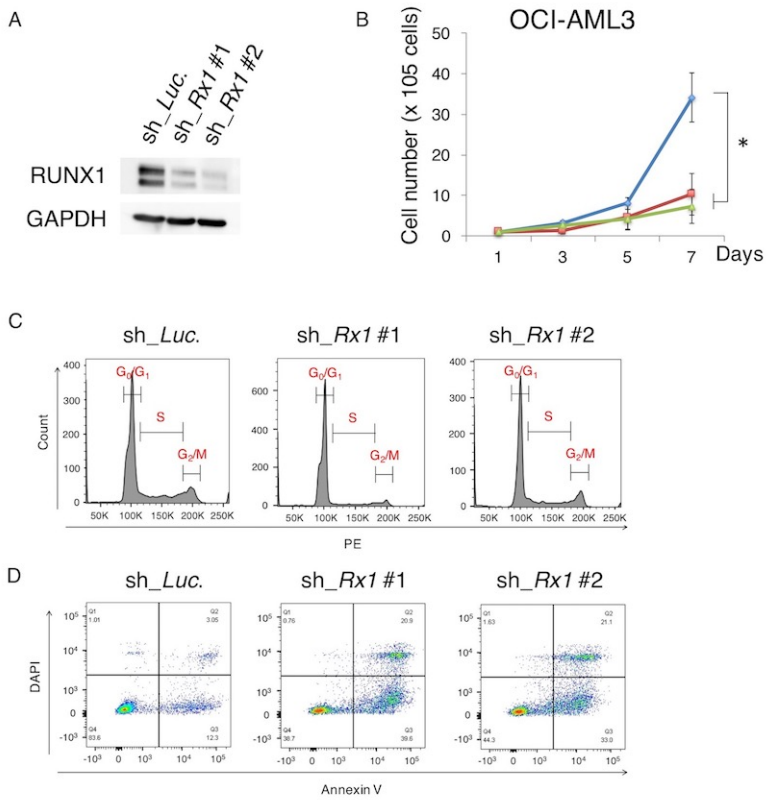
Supplemental Data

Anti-tumor potency of RUNX cluster regulation with “gene switch”.

Supplemental Figures



Supplemental Figure 1

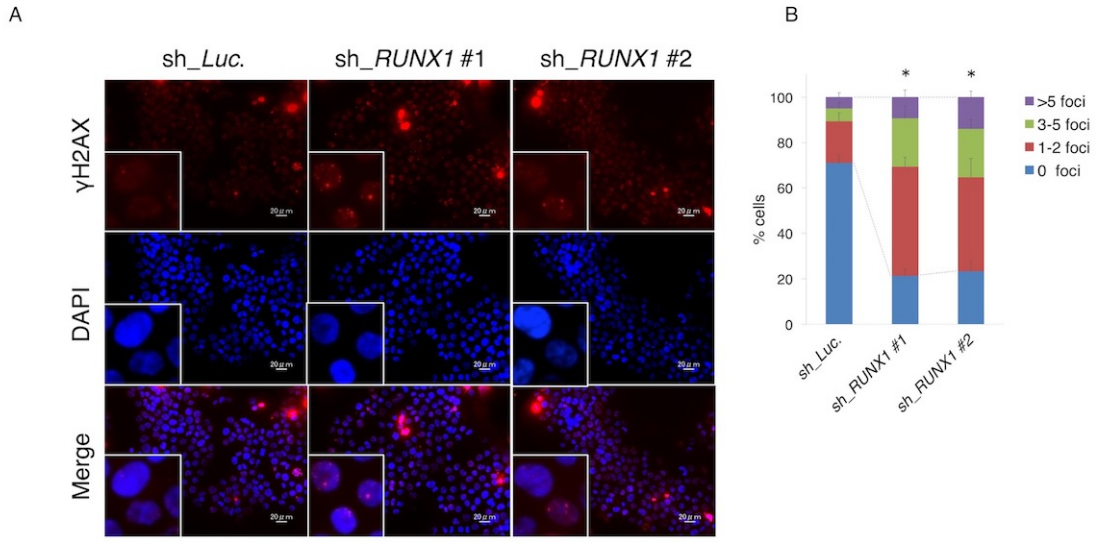
(A) Immunoblotting of RUNX1 and GAPDH in MV4-11 cells transduced with control (sh_*Luc*.) or *RUNX1* shRNAs (sh_*Rx1*#1 and sh_*Rx1*#2). Cells were treated with 3 μM doxycycline for 48 hours.

(B) Growth curve of OCI-AML3 cells transduced with control or *RUNX1* shRNAs in the presence of 3 μM of doxycycline (n = 3).

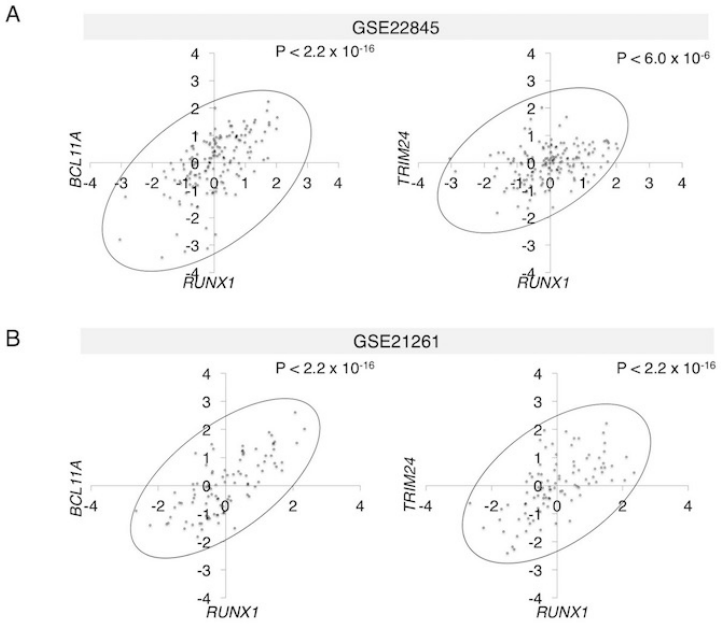
(C) Representative figures of cell cycle status determined in MV4-11 cells transduced with control or *RUNX1* shRNAs. Cells were treated with 3 μM doxycycline for 48 hours.

(D) Representative figures of apoptosis status determined in MV4-11 cells transduced with control or *RUNX1* shRNAs. Cells were treated with 3μM doxycycline for 48 hours.

Data are mean±SEM values. * $P < 0.05$, by two-tailed Student's *t* test.



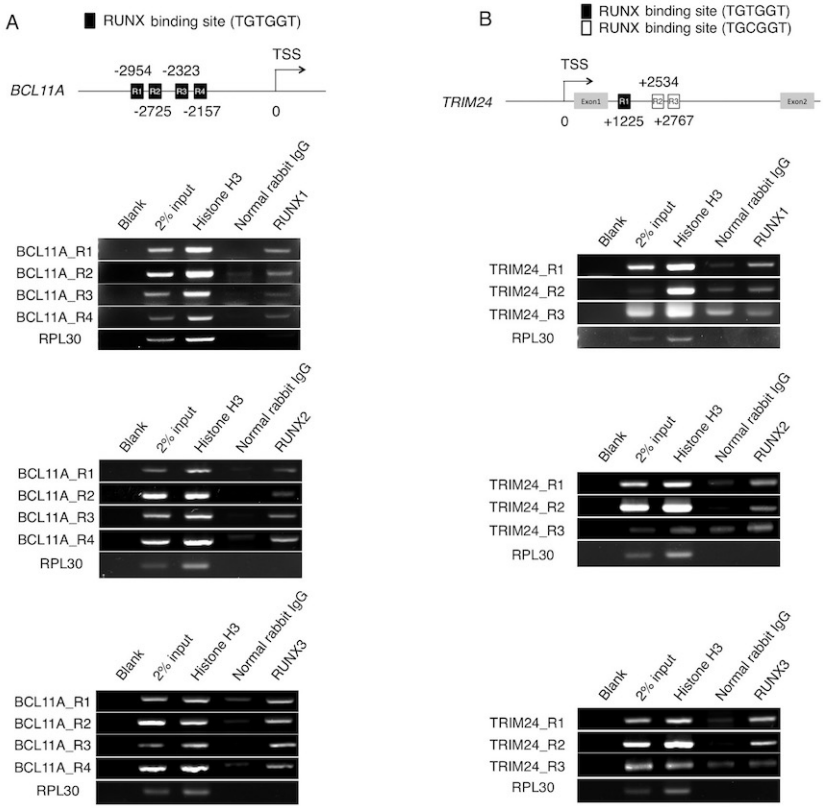
Supplemental Figure 2
(A and B) γ-H2AX assay detecting DNA repair dynamics following RUNX1 knockdown. MV4-11 cells were transduced with control (sh_*Luc*.) or *RUNX1* shRNAs (sh_*Rx1*#1 and sh_*Rx1*#2) and treated with 3 μM doxycycline for 48 hours. (A) Immunofluorescence image of γ-H2AX foci formation. (B) Formation of foci was counted in 100 randomly sampled cells from each slide (n = 3).
Data are mean±SEM values. * $P < 0.05$, by two-tailed Student's *t* test.



Supplemental Figure 3

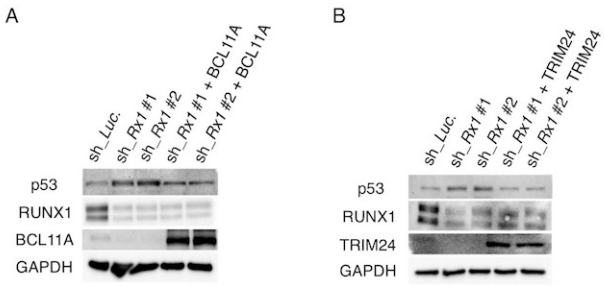
(A) Positive-correlation between *RUNX1* and *BCL11A* expressions or between *RUNX1* and *TRIM24* expressions in a AML patients cohort (GSE22845, n = 154). P values were calculated by Spearman's correlation.

(B) Positive-correlation between *RUNX1* and *BCL11A* expressions or between *RUNX1* and *TRIM24* expressions in a AML patients cohort (GSE21261, n = 96). P values were calculated by Spearman's correlation.



Supplemental Figure 4

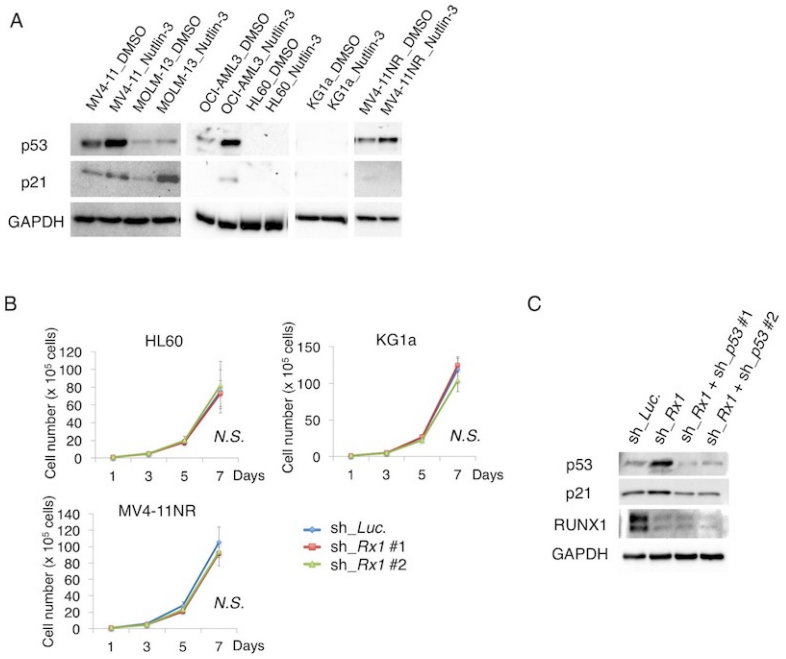
(A and B) ChIP analysis in MV4-11 cells using anti-RUNX1, anti-RUNX2, anti-RUNX3 antibodies, an isotope-matched control IgG and anti-Histone H3 antibody. ChIP products were amplified by PCR to determine abundance of the indicated amplicons. (A) Schematic illustration of promoters and the corresponding PCR results of *BCL11A*. (B) Schematic illustration of promoters and the corresponding PCR results of *TRIM24*.



Supplemental Figure 5

(A) Immunoblotting of p53, RUNX1, BCL11A and GAPDH in MV4-11 cells transduced with control or *RUNX1* shRNAs with or without *BCL11A* expressions. Cells were treated with 3μM doxycycline for 48 hours.

(B) Immunoblotting of p53, RUNX1, TRIM24 and GAPDH in MV4-11 cells transduced with control or *RUNX1* shRNAs with or without *TRIM24* expressions. Cells were treated with 3μM doxycycline for 48 hours.



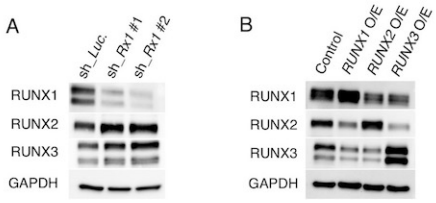
Supplemental Figure 6

(A) Immunoblotting of p53, p21 and GAPDH in AML cells (MV4-11, MOLM-13, OCI-AML3, HL60, KG1a and MV4-11NR cells) treated either with DMSO or 1 μM Nutlin-3 for 48 hours.

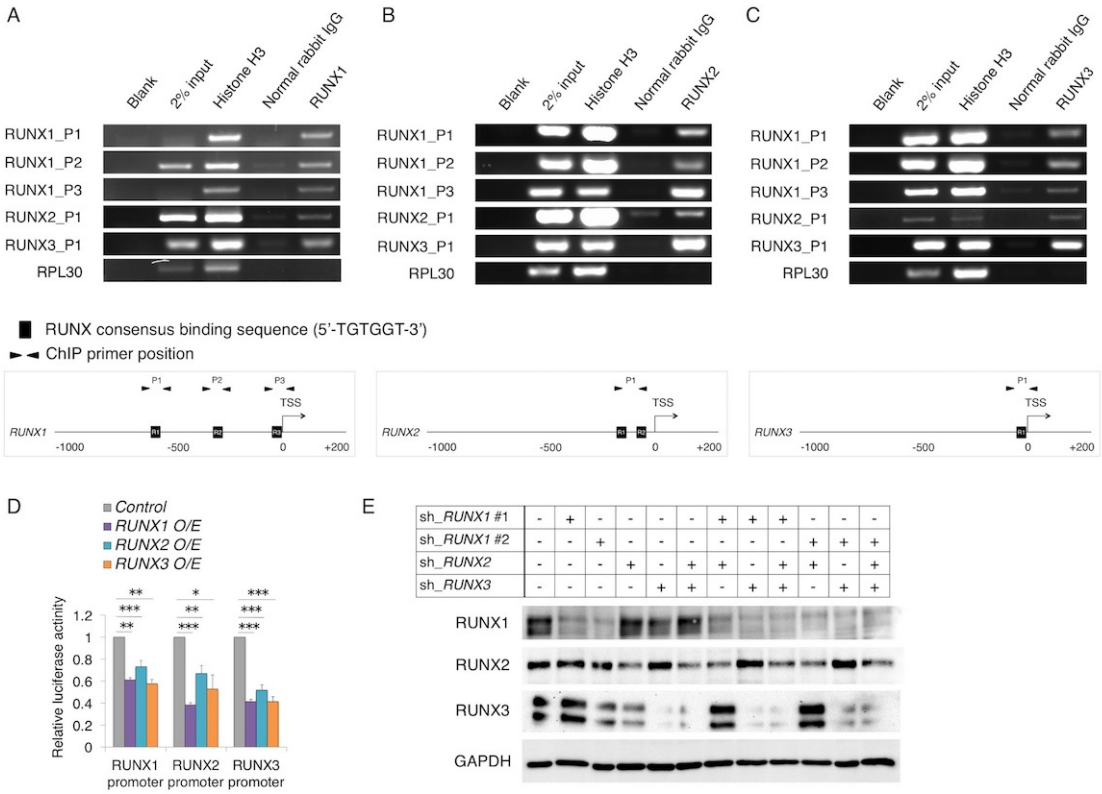
(B) Growth curves of HL60, KG1a and MV4-11NR cells transduced with control or *RUNX1* shRNAs. Cells were cultured with 3μM doxycycline (n = 3).

(C) Immunoblotting of p53, p21, RUNX1 and GAPDH in MV4-11 cells transduced with control or *RUNX1* shRNAs with or without expressions of shRNAs targeting *p53* (sh_*p53* #1 and sh_*p53* #2). Cells were treated with 3μM doxycycline for 48 hours.

Data are mean±SEM values. *N.S.* Not significant, by two-tailed Student's *t* test.



Supplemental Figure 7
(A) Induction of RUNX2 and RUNX3 in the absence of RUNX1. MV4-11 cells transduced with control (sh_*Luc*.) or with *RUNX1* shRNAs (sh_*Rx1* #1 and sh_*Rx1* #2) were cultured in the presence of 3 μM of doxycycline. Forty-eight hours after treatment, cell lysates were prepared and subjected to immunoblotting.
(B) Forced expression of RUNX1, RUNX2 or RUNX3 in MV4-11 cells. MV4-11 cells were transduced with the indicated lentivirus vectors and then incubated with 3μM doxycycline. Forty-eight hours after treatment, cell lysates were processed for immunoblotting.



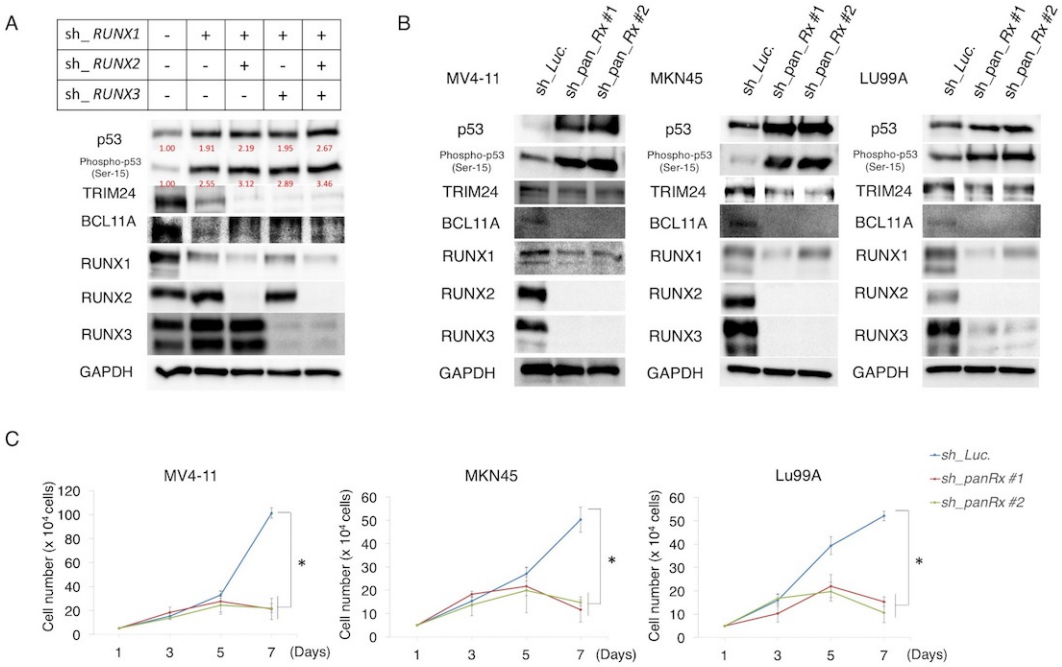
Supplemental Figure 8

(A-C) ChIP analysis in MV4-11 cells using anti-RUNX1, anti-RUNX2 or anti-RUNX3 antibodies, an isotope-matched control IgG and anti-Histone H3 antibody. ChIP products were amplified by PCR to determine abundance of the indicated amplicons. Schematic illustrations show proximal promoters of *RUNX1*, *RUNX2* and *RUNX3*. (A) PCR results with anti-RUNX1 antibody. (B) PCR results with anti-RUNX2 antibody. (C) PCR results with anti-RUNX3 antibody.

(D) Luciferase reporter assay of *RUNX1*, *RUNX2* and *RUNX3* promoters. MV4-11 cells were transduced with the indicated lentivirus vectors as well as with luciferase reporter plasmids and then incubated with 3μM doxycycline. Forty-eight hours after treatment, relative luciferase activity was determined (n = 3).

(E) Immunoblotting of RUNX1, RUNX2, RUNX3 and GAPDH in MV4-11 cells transduced with control or series of shRNAs targeting *RUNX1*, *RUNX2* and *RUNX3*. Cells were treated with 3μM doxycycline for 48 hours.

Data are mean±SEM values. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, by two-tailed Student's *t* test.



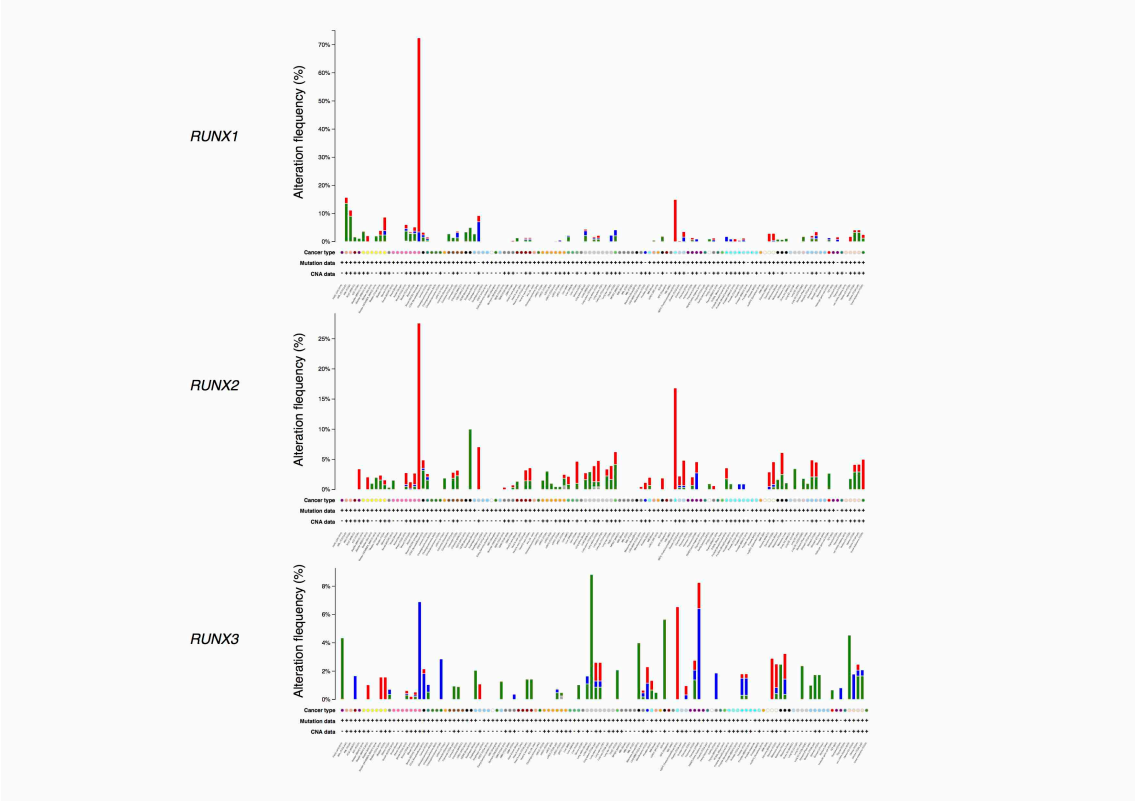
Supplemental Figure 9

(A) Immunoblotting of p53, phosphor-p53 (Ser-15), TRIM24, BCL11A, RUNX1, RUNX2, RUNX3 and GAPDH in MV4-11 cells transduced with control or series of shRNAs targeting *RUNX1*, *RUNX2* and *RUNX3*. Cells were treated with 3μM doxycycline for 48 hours.

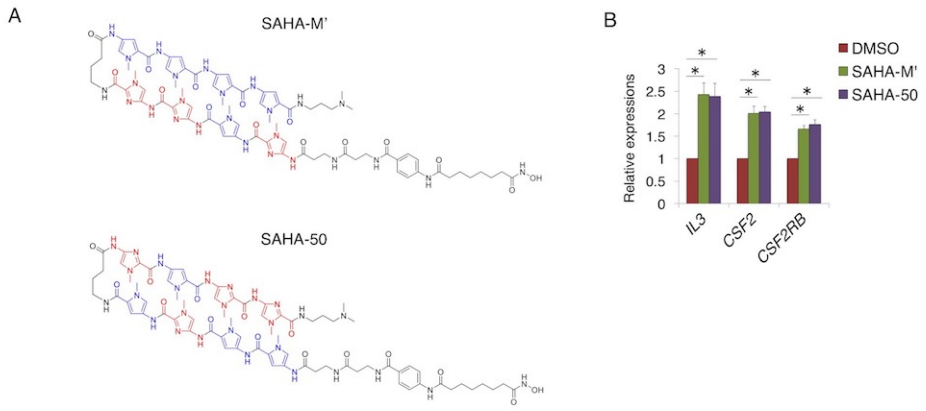
(B) Immunoblotting of p53, phosphor-p53 (Ser-15), TRIM24, BCL11A, RUNX1, RUNX2, RUNX3 and GAPDH in AML-derived MV4-11 cells, gastric cancer-derived MKN45 cells and lung cancer-derived LU99A cells transduced with control or shRNAs targeting all *RUNX* family members (*RUNX1*, *RUNX2* and *RUNX3*). Cells were treated with 3μM doxycycline for 48 hours.

(C) Growth curves of MV4-11, MKN45 and LU99A cells transduced with control or shRNAs targeting all *RUNX* family members. Cells were cultured with 3μM doxycycline (n = 3).

Data are mean±SEM values. * $P < 0.05$, by two-tailed Student's *t* test.

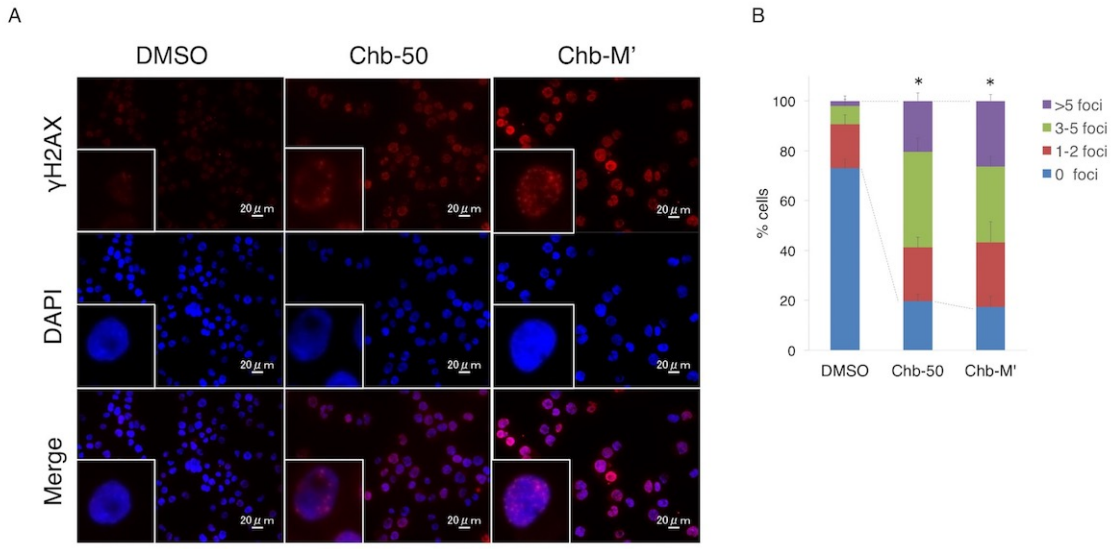


Supplemental Figure 10
Alteration frequency of *RUNX1*, *RUNX2* and *RUNX3* in 681 human cancer samples out of 18,754 patients from 126 genomics studies on various cancer types. Data are analyzed and visualized by the cBioPortal for Cancer Genomics software (1).



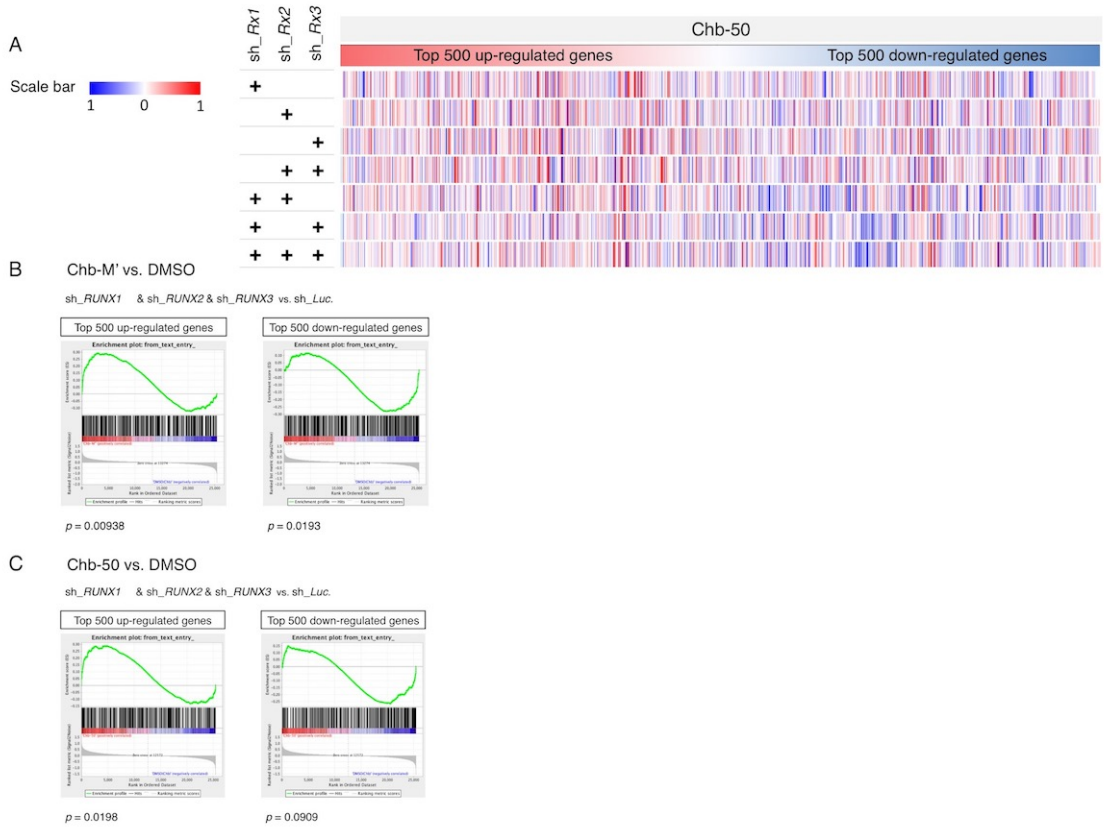
Supplemental Figure 11

(A) Chemical structures of the synthetic SAHA - PI polyamide conjugates targeting 5'-TGTGGT-3' (SAHA-M') and 5'-TGCGGT-3' (SAHA-50).
(B) SAHA-M' and SAHA-50 stimulate the expression levels of RUNX1-target genes. MV4-11 cells were treated with 20 μM SAHA - PI polyamide conjugates for 12 hours, then total RNA was prepared and analyzed by real-time RT-PCR. Values are normalized to that of DMSO treated cells (n = 3).
Data are mean±SEM values. * $P < 0.05$, by two-tailed Student's *t* test.



Supplemental Figure 12

(A and B) γ-H2AX assay detecting DNA repair dynamics following Chb-50 or Chb-M' treatment. MV4-11 cells were cultured in the presence of 1 μM Chb-50 or Chb-M' for 24 hours. (A) Immunofluorescence image of γ-H2AX foci formation. (B) Formation of foci was counted in 100 randomly sampled cells from each slide (n = 3).
Data are mean±SEM values. * $P < 0.05$, by two-tailed Student's *t* test.

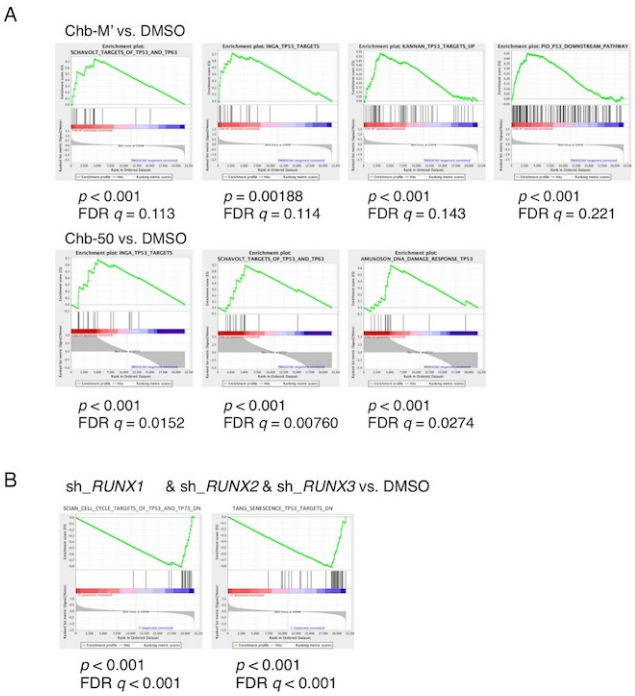


Supplemental Figure 13

(A) Heatmap showing top 500 up-regulated and down-regulated genes in MV4-11 cells treated with 1 µM Chb-50 for 6 hours in comparison to transcripts following *RUNX* family knockdown.

(B) GSEA pathway analysis conducted in MV4-11 cells treated with 1 µM Chb-M' for 6 hours to compare the variance of top 500 transcripts following *RUNX* family knockdown.

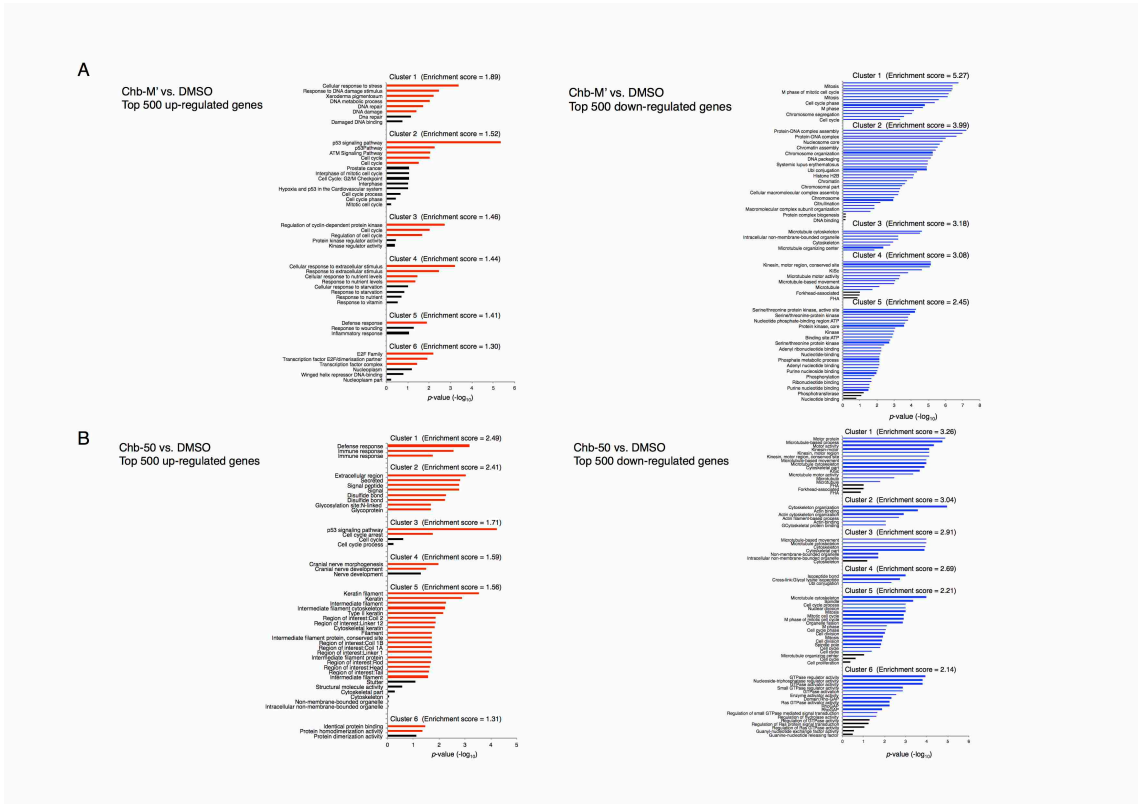
(C) GSEA pathway analysis conducted in MV4-11 cells treated with 1 µM Chb-50 for 6 hours to compare the variance of top 500 transcripts following *RUNX* family knockdown.



Supplemental Figure 14

(A) GSEA pathway analysis conducted in MV4-11 cells treated with 1 μM Chb-M' or Chb-50 for 6 hours. Results of p53 pathways are shown.

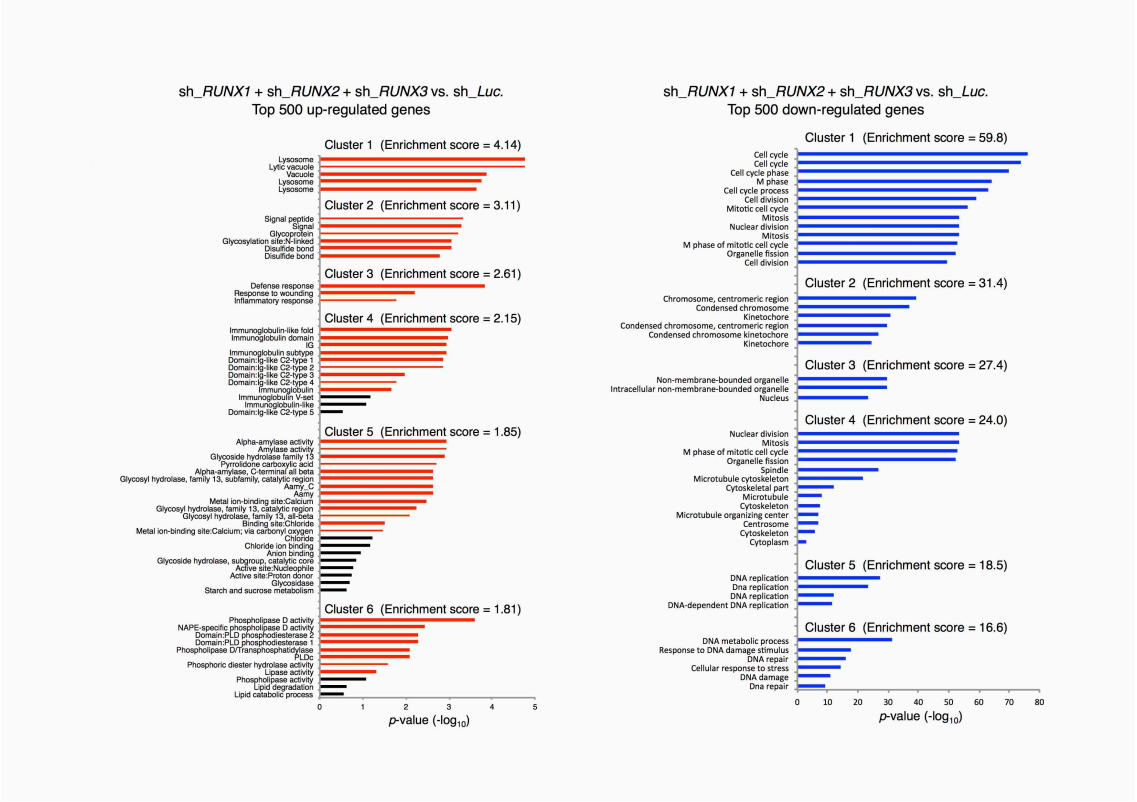
(B) GSEA pathway analysis conducted in MV4-11 cells transduced with control or shRNAs targeting *RUNX1*, *RUNX2* and *RUNX3*. Cells were treated with 3μM doxycycline for 48 hours. Results of p53 pathways are shown.



Supplemental Figure 15

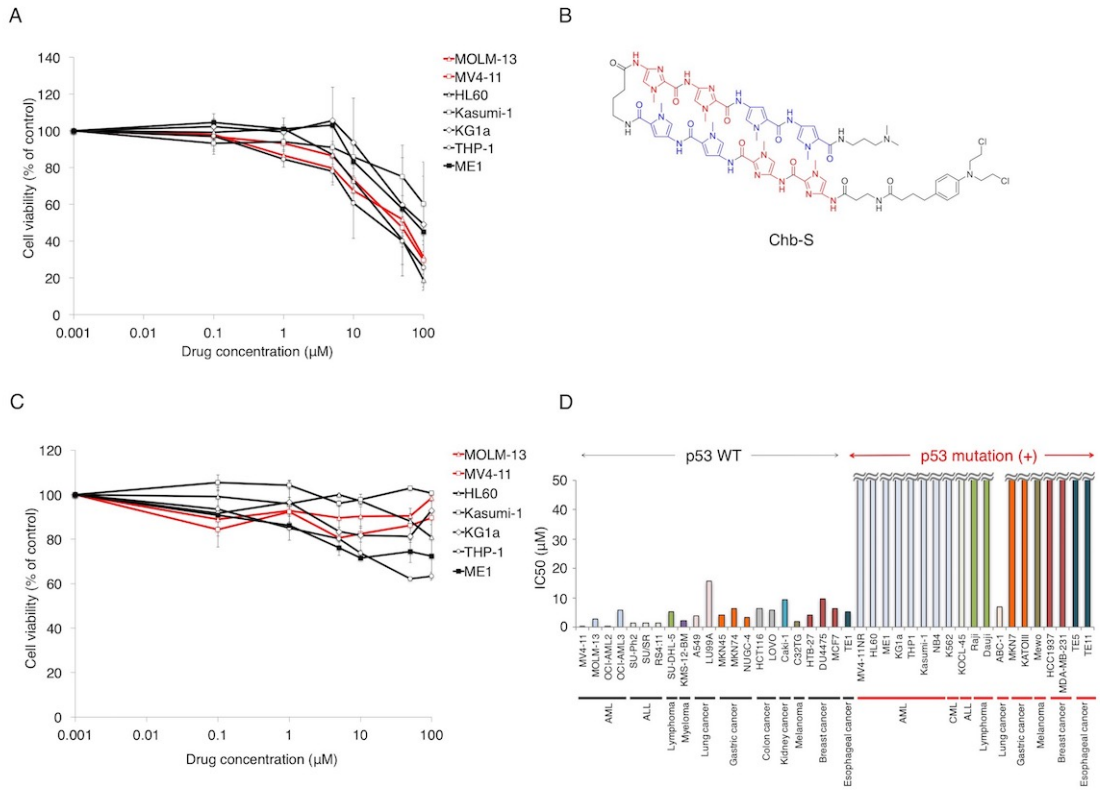
(A) Gene ontology enrichment analysis by DAVID software conducted in top 500 up-regulated and down-regulated genes in MV4-11 cells treated with 1 μM Chb-M' for 6 hours.

(B) Gene ontology enrichment analysis by DAVID software conducted in top 500 up-regulated and down-regulated genes in MV4-11 cells treated with 1 μM Chb-50 for 6 hours.



Supplemental Figure 16

Gene ontology enrichment analysis by DAVID software conducted in top 500 up-regulated and down-regulated genes in MV4-11 cells transduced with control or shRNAs targeting *RUNX1*, *RUNX2* and *RUNX3*. Cells were treated with 3μM doxycycline for 48 hours.



Supplemental Figure 17

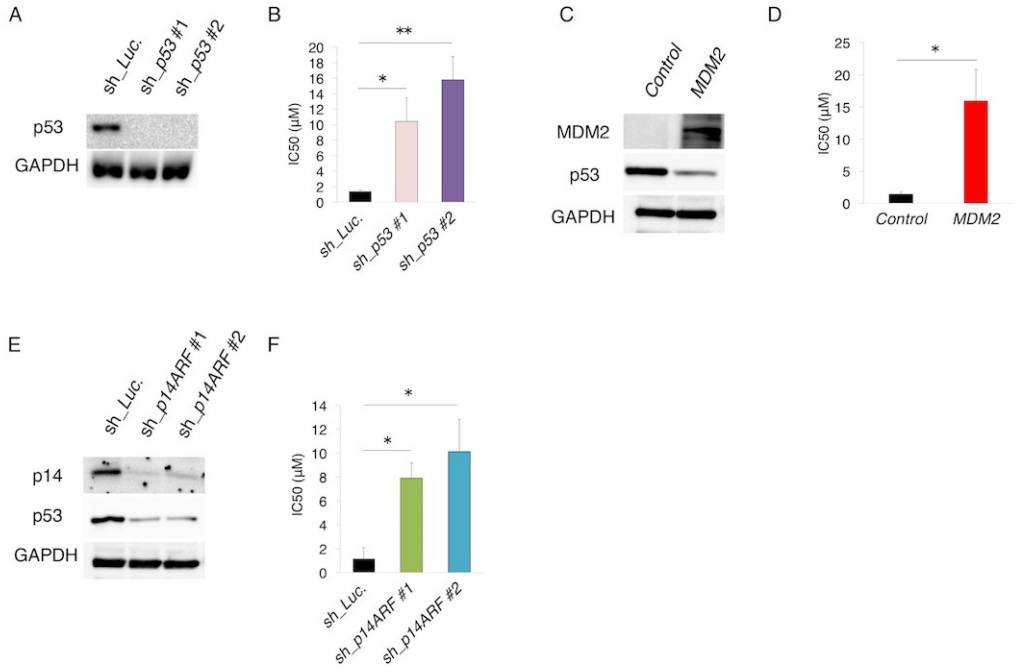
(A) Dose-response curves of Chlorambucil in AML cells (MV4-11, MOLM-13, HL60, Kasumi-1, KG1a, THP-1 and ME1 cells). Cells were treated with Chlorambucil at various concentrations for 48 hours (n = 3).

(B) Chemical structure of the synthetic chlorambucil - PI polyamide conjugates targeting 5'-TGGCCT-3' (Chb-S).

(C) Dose-response curves of Chb-S in AML cells (MV4-11, MOLM-13, HL60, Kasumi-1, KG1a, THP-1 and ME1 cells). Cells were treated with Chb-S at various concentrations for 48 hours (n = 3).

(D) IC50 values of Chb-50 are calculated in human cancer cell lines of various origins (n = 3).

Data are mean±SEM values.



Supplemental Figure 18

(A) Immunoblotting of p53 and GAPDH in MV4-11 cells transduced with control or shRNAs targeting *p53* (sh*_p53* #1 and sh*_p53* #2). Cells were treated with 3μM doxycycline for 48 hours.

(B) IC50 values of Chb-M' are calculated in cells utilized in (A) (n = 3).

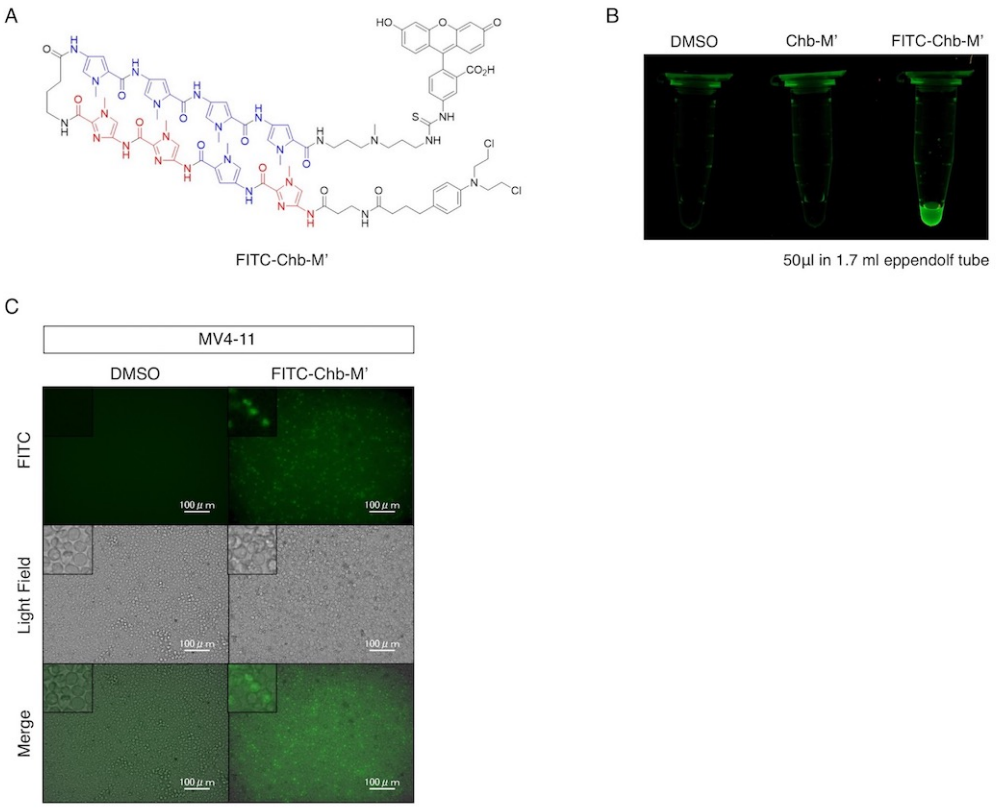
(C) Immunoblotting of MDM2, p53 and GAPDH in MV4-11 cells transduced with control or MDM2 expression vectors. Cells were treated with 3μM doxycycline for 48 hours.

(D) IC50 values of Chb-M' are calculated in cells utilized in (C) (n = 3).

(E) Immunoblotting of p14, p53 and GAPDH in MV4-11 cells transduced with control or shRNAs targeting *p14ARF* (sh*_p14ARF* #1 and sh*_p14ARF* #2). Cells were treated with 3μM doxycycline for 48 hours.

(F) IC50 values of Chb-M' are calculated in cells utilized in (E) (n = 3).

Data are mean±SEM values. * $P < 0.05$ ** $P < 0.01$, by two-tailed Student's *t* test.



Supplemental Figure 19

(A) Chemical structure of the synthetic Chb-M' conjugated with FITC immunofluorescence protein (FITC-Chb-M').

(B) Immunofluorescence images of DMSO, Chb-M' and FITC-Chb-M'. Immunofluorescence activity of each agent was detected with ChemiDoc™ XRS+ System (BioRad).

(C) Incorporation of Chb-M' was visualized in MV4-11 cells. Cells were treated with 1μM FITC-Chb-M' for 24 hours and immunofluorescence was detected with BZ-X700 Fluorescence Microscope (KEYENCE).

A

		Control	Chb-M' 1 mg/kg	Chb-M' 3.2 mg/kg	Chb-M' 10 mg/kg
RBC	(10^6 / μL)	8.272±0.568	8.108±0.216	8.156±0.363	8.104±0.548
Hb	(g / dL)	13.50±0.69	13.34±0.36	13.16±0.76	13.08±0.54
Ht	(%)	42.16±2.29	41.66±1.28	41.72±1.77	41.56±1.27
MCV	(fL)	50.98±1.08	51.38±0.79	51.20±1.00	51.42±1.93
MCH	(pg)	16.36±0.50	16.44±0.43	16.16±0.67	16.18±0.50
MCHC	(g / dL)	32.08±0.36	32.04±0.90	31.54±0.70	31.44±0.44
Ret.	(%)	2.36±0.45	2.66±0.24	3.38±0.36	3.06±0.67
Plat.	(10^3 / μL)	1008.0±97.2	963.6±66.2	850.0±61.1	733.0±89.2
WBC	(10^3 / μL)	3.066±1.077	2.428±0.613	3.124±0.651	1.892±0.860
Neutro.	(10^3 / μL)	0.526±0.236	0.380±0.182	0.558±0.287	0.320±0.131
Lymph.	(10^3 / μL)	2.364±0.936	1.878±0.536	2.365±0.412	1.440±0.715
Mono.	(10^3 / μL)	0.058±0.022	0.044±0.018	0.050±0.029	0.036±0.023
Eosino.	(10^3 / μL)	0.112±0.027	0.118±0.053	0.144±0.049	0.092±0.028
Baso.	(10^3 / μL)	0.002±0.004	0.000±0.000	0.004±0.005	0.000±0.000
LUC	(10^3 / μL)	0.006±0.005	0.004±0.005	0.014±0.005	0.004±0.005
Neutro.	(%)	17.84±5.45	15.58±5.98	17.16±5.35	17.46±3.81
Lymph.	(%)	75.22±8.68	77.30±7.26	75.76±4.01	75.10±4.27
Mono.	(%)	2.04±0.97	1.94±0.69	1.76±1.17	1.76±0.74
Eosino.	(%)	4.62±3.37	4.96±2.18	4.76±1.77	5.38±1.85
Baso.	(%)	0.10±0.00	0.10±0.00	0.12±0.08	0.10±0.12
LUC	(%)	0.22±0.04	0.18±0.08	0.42±0.22	0.22±0.08

B

		Control	Chb-M' 1 mg/kg	Chb-M' 3.2 mg/kg	Chb-M' 10 mg/kg
AST	(IU / L)	45.6±5.9	54.4±11.1	46.2±4.4	48.2±4.0
ALT	(IU / L)	22.6±4.3	35.2±17.8	22.4±2.1	31.2±10.1
ALP	(IU / L)	245.4±43.9	224.2±53.7	222.0±39.3	194.6±41.8
CK	(IU / L)	48.6±11.4	53.0±24.2	45.4±31.5	48.0±14.0
T-Bil	(mg / dL)	0.072±0.008	0.062±0.028	0.068±0.008	0.064±0.005
TP	(g / dL)	4.48±0.22	4.48±0.13	4.62±0.15	4.56±0.09
TG	(mg / dL)	78.0±36.6	81.4±31.9	79.0±22.7	85.0±39.9
T-cho	(mg / dL)	92.2±8.2	84.6±20.2	83.8±22.2	81.4±13.5
Glucose	(mg / dL)	231.8±27.8	218.2±15.3	212.2±15.0	216.4±12.5
UN	(mg / dL)	19.00±3.92	19.02±5.39	18.16±3.04	19.00±1.19
Cre	(mg / dL)	0.104±0.009	0.112±0.020	0.108±0.008	0.112±0.004
IP	(mg / dL)	3.294±0.707	3.904±0.985	3.954±1.224	3.032±0.826
Ca	(mg / dL)	8.60±0.19	8.74±0.21	8.70±0.31	8.46±0.41
Na	(mEq / L)	150.8±0.8	151.8±1.3	152.8±1.8	152.2±0.8
K	(mEq / L)	3.58±0.28	3.32±0.43	3.44±0.23	3.34±0.43
Cl	(mEq / L)	120.4±1.7	120.2±1.6	121.4±1.7	121.0±1.9
Albumin	(g / dL)	3.12±0.15	3.08±0.16	3.12±0.08	3.16±0.11
Globulin	(g / dL)	1.36±0.11	1.40±0.07	1.50±0.12	1.40±0.07

C

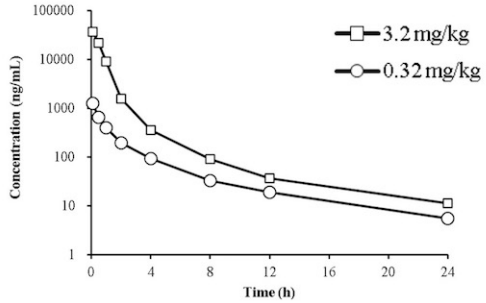
Day	Control	Chb-M' 1 mg/kg	Chb-M' 3.2 mg/kg	Chb-M' 10 mg/kg
-1	31.56±0.89	31.38±1.38	31.6±0.68	31.52±0.84
0	31.60±1.34	31.24±1.61	31.62±0.87	31.72±1.08
1	31.40±1.48	31.00±1.51	31.16±1.26	31.06±0.93
2	31.34±1.48	31.22±1.56	31.34±0.84	31.58±0.90
3	31.60±1.83	31.18±1.56	31.94±0.94	31.64±0.88
4	31.92±1.89	31.06±1.40	32.24±1.00	31.66±0.65
5	32.10±2.00	31.26±1.59	31.58±0.97	31.62±0.46
6	31.72±2.27	31.36±1.56	31.34±0.94	31.50±0.80
7	31.32±2.07	30.94±1.44	31.60±0.73	31.54±1.00
8	31.24±2.14	31.24±1.64	31.70±0.86	31.32±0.83
9	31.16±1.99	31.48±1.14	32.08±0.79	31.48±0.45

Body weight (g)

Supplemental Figure 20

(A-C) Acute toxicological testing of Chb-M' at various concentrations in NOG mice. Results of complete blood cell counts (A), blood biochemistry (B) and body weight (C) are shown (n = 5).

Data are mean±SEM values.



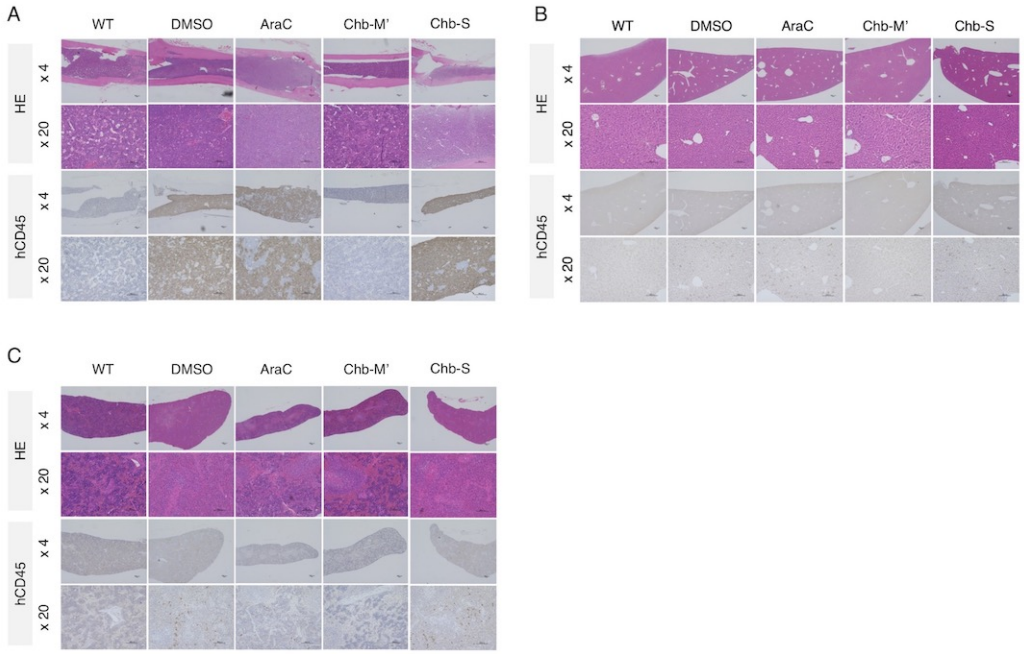
Pharmacokinetic Parameters of Chb-M' after single intravenous administration of Chb-M'

Dose (mg/kg)	C_0 (ng/mL)	λz (1/h)	$t_{1/2}$ (h)	$AUC_{0\text{-}24h}$ (ng*h/mL)	$AUC_{0\text{-}inf}$ (ng*h/mL)	Vd (mL/kg)	CL (mL/h/kg)	$MRT_{0\text{-}inf}$ (h)
0.32 mg/kg	1410	0.108	6.41	1840	1890	1560	169	3.96
3.2 mg/kg	40900	0.123	5.64	31700	31800	819	101	1.01

C_0; Concentration, λz; Terminal elimination rate constant, $t_{1/2}$; Elimination half-life, AUC; Area under the curve, Vd; Volume of distribution, CL; Clearance, MRT; Mean residence time.

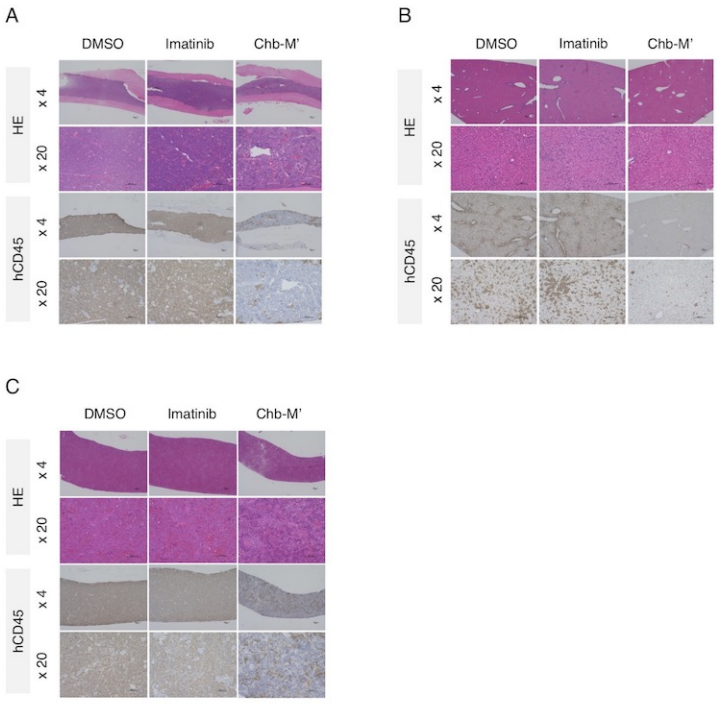
Supplemental Figure 21

In vivo pharmacokinetic profiles of Chb-M' was determined. Crl:CD1(ICR) mice were given single dose of 320 μg/kg body weight or 3.2 mg/ kg body weight Chb-M' i.v. and its concentrations in the peripheral blood plasma were sequentially examined up to 24 hours after initial injection. Concentration-Time curve and mean values of pharmacokinetic parameters were shown (n = 12).

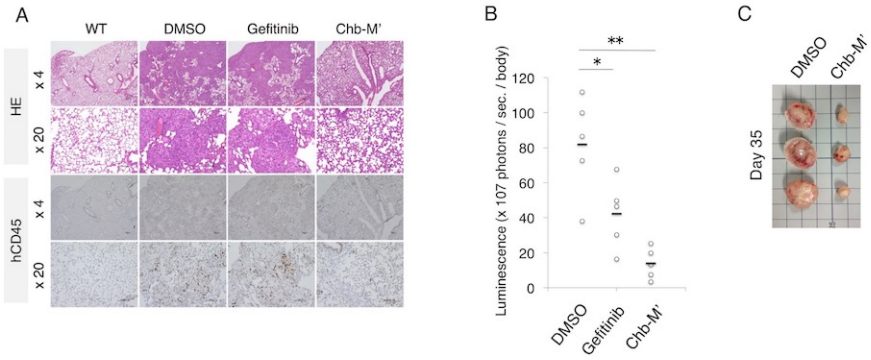


Supplemental Figure 22

(A-C) Representative microscopic images of the organs from an AML xenograft mouse with MV4-11 cells (14 days post-transplantation). Hematoxylin and eosin (H&E) staining and immunohistochemical staining with anti-human CD45 antibody were done for each slide (original magnification 4× and 20×, Scale bars, 100 μm). (A) Bone marrow, (B) liver, (C) spleen.



Supplemental Figure 23
(A-C) Representative microscopic images of the organs from an ALL xenograft mouse with SU/SR cells (14 days post-transplantation). Hematoxylin and eosin (H&E) staining and immunohistochemical staining with anti-human CD45 antibody were done for each slide (original magnification 4× and 20×, Scale bars, 100 μm). (A) Bone marrow, (B) liver, (C) spleen.



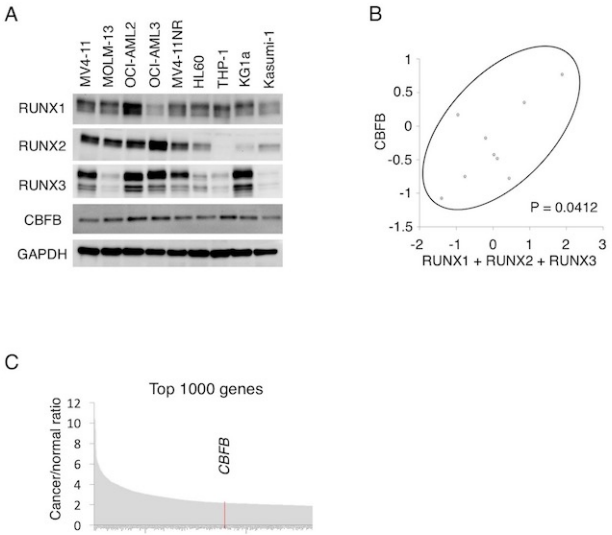
Supplemental Figure 24

(A) Representative microscopic images of the lungs from a lung cancer xenograft mouse with A549 cells (14 days post-transplantation). Hematoxylin and eosin (H&E) staining and immunohistochemical staining with anti-human Ki-67 antibody were done for each slide (original magnification 4× and 20×, Scale bars, 100 μm).

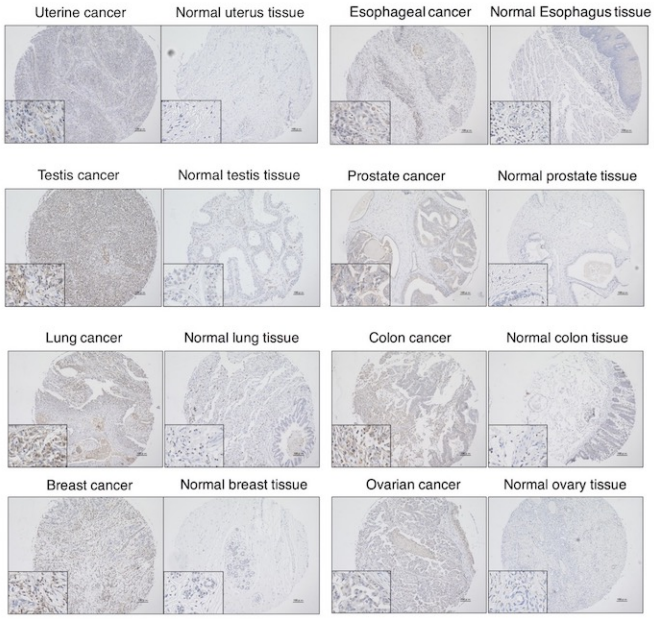
(B) Human lung cancer xenotransplant model in NOG mice transplanted with A549 cells followed by treatment with DMSO, gefitinib or with Chb-M'. Quantification of the IVIS bioluminescent signal intensity at 21 days post-transplantation (n = 5).

(C) Human stomach cancer xenotransplant model in NOG mice transplanted with MKN45 cells followed by treatment with DMSO or with Chb-M'. Representative macroscopic images of tumors at 35 days post-transplantation.

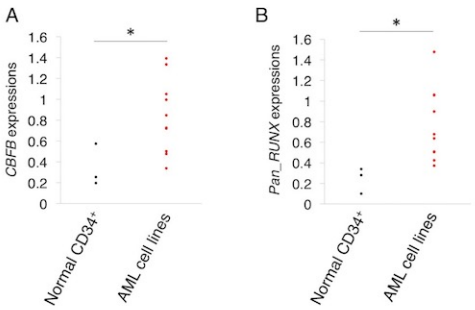
Data are mean±SEM values. * $P < 0.05$ ** $P < 0.01$, by two-tailed Student's *t* test.



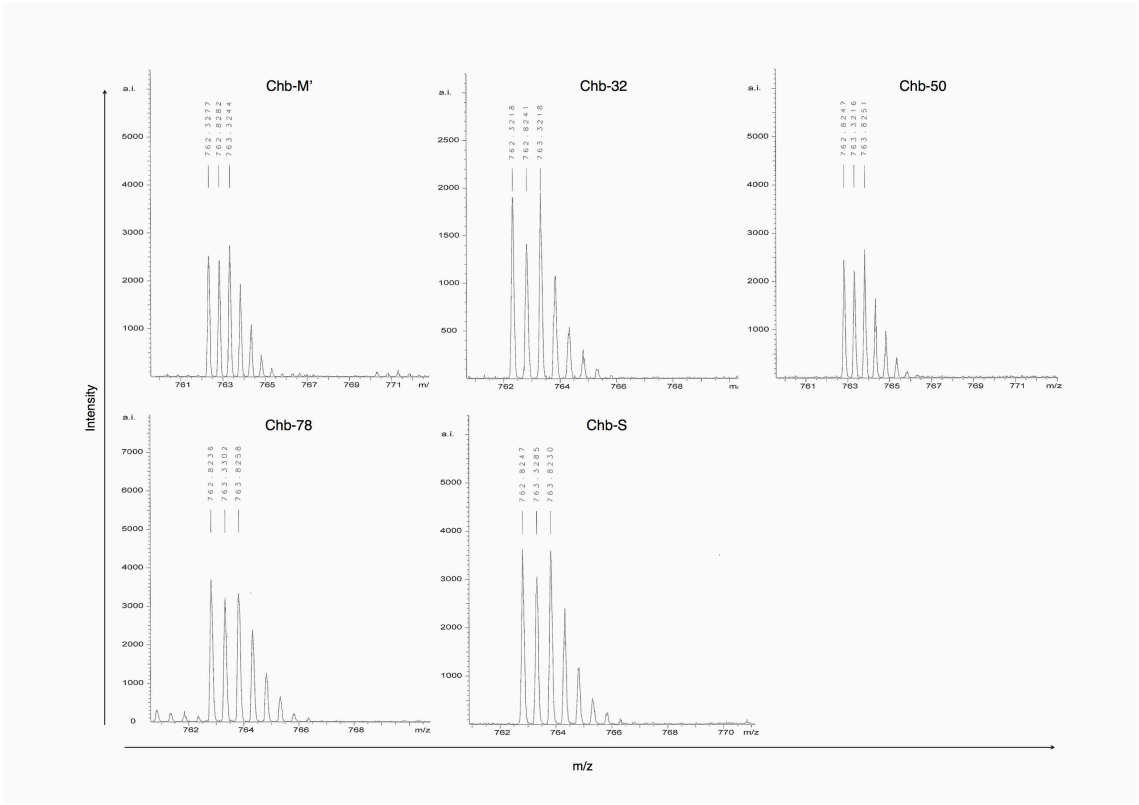
Supplemental Figure 25
(A) Immunoblot of RUNX1, RUNX2, RUNX3,CBFB and GAPDH in various AML cell lines.
(B) Correlation between the expression levels of CBFB and RUNX1 + RUNX2 + RUNX3 in AML cell lines (n = 9). $P < 0.05$, by Spearman's correlation.
(C) Top 1000 genes differentially expressed in cancer cell lines compared to normal tissues. Genes are ordered according to the ratio of expressions in cancer cell lines to those in normal tissues.



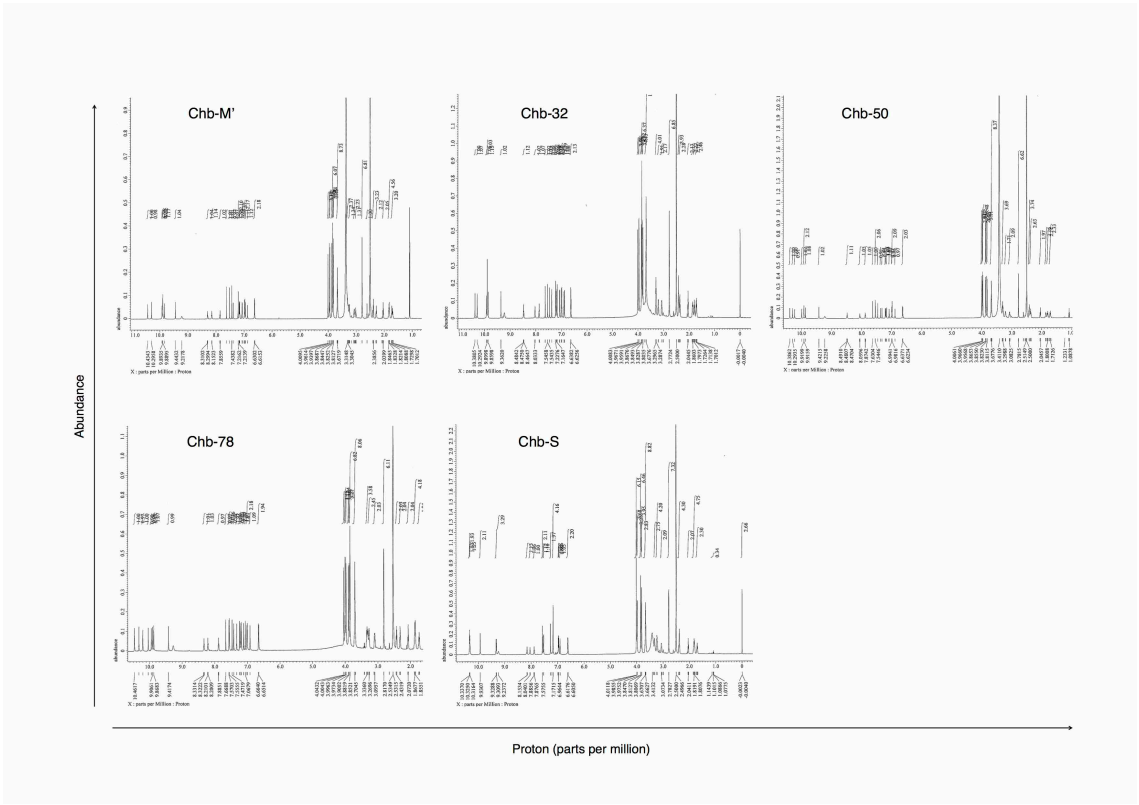
Supplemental Figure 26
Immunohistochemistry of CBFB in patient-derived cancer tissues and their adjacent normal counterparts obtained from the identical patients.



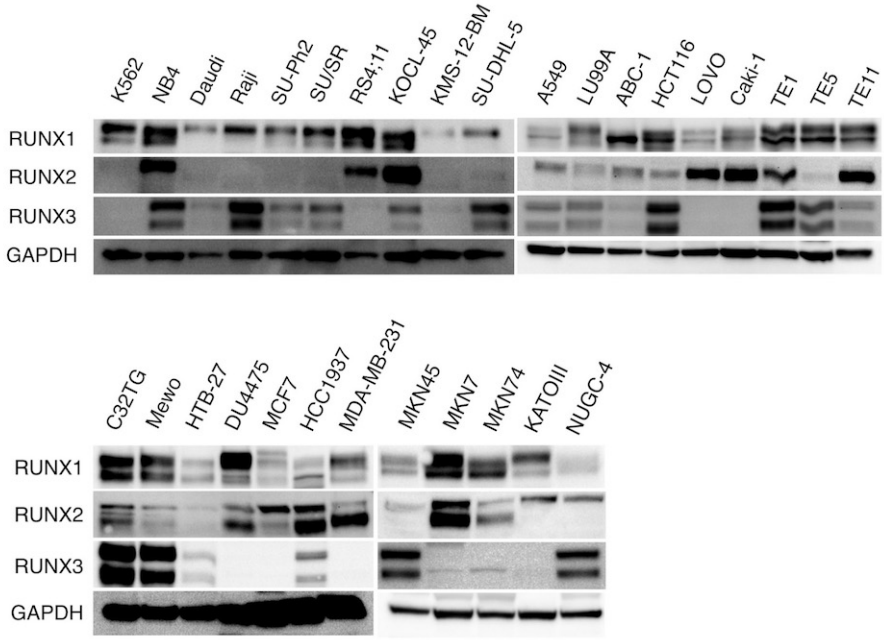
Supplemental Figure 27
(A and B) Expression levels of (A) *CBFB* and (B) *pan_RUNX* in human CD34+ normal hematopoietic cells (n = 3) and in AML cell lines (n = 9).
Data are mean±SEM values. * $P < 0.05$, by two-tailed Student's *t* test.



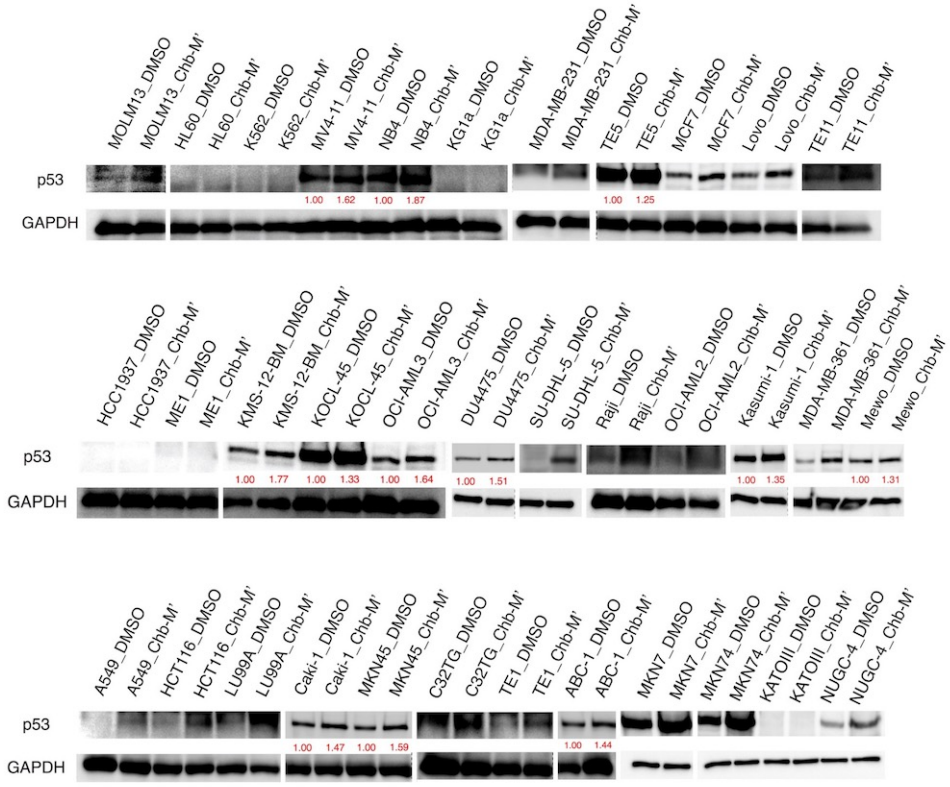
Supplemental Figure 28
Chemical analysis of PI polyamides used in this study by ESI-TOF MS performed on a mass spectrometer.



Supplemental Figure 29
Chemical analysis of PI polyamides with ^{1}H NMR.



Supplemental Figure 30
Immunoblot of RUNX1, RUNX2, RUNX3 and GAPDH in various cell lines utilized in this study.



Supplemental Figure 31
Immunoblot of p53 and GAPDH with or without Chb-M' treamtent in various cell lines utilized in this study. Cells were treated with DMSO or 1μM Chb-M' for 24 hours.

Supplemental Tables

Supplemetal Table 1

Commonly up-regulated genes in RUNX1 high-expressing AML patients. GSE21261, GSE19577, GSE22845, GSE67936		
GUCY1A3	EXTL2	MDM1
CEP70	MGC3032	SRBD1
KIAA0125	VAV3	SOCS2
ANGPT1	SLC24A1	ZMYM4
MLLT11	TCEAL1	FLT3
CPA3	CXORF45	USP11
WT1	CRNKL1	POT1
ZNF573	GRB10	PLAGL1
RUNX1	ZNF271	LAPTM4B
B4GALT6	CCDC41	DHTKD1
KIT	TARBP1	COL4A5
ITPR2	C12ORF24	RPP40
ANKRD28	RNF170	TRIM5
ARHGAP22	TCF12	SENP6
ACSM3	PRKACB	ITPR1
OSBPL3	SSBP2	GLMN
AGPAT5	BBS10	PHF16
ARMCX1	SMC6	NCBP2
SEPP1	MAGED1	PCCA
TCF4	MEF2C	EXOC1
ATP1B1	EHBP1	FAIM
MAP7	CBFA2T3	IFT88
ZNF84	SPATA7	CSTF3
TXK	AKAP11	C14ORF104
LIMA1	ZNF83	ZMYND11
BCL11A	CNOT6	PUS7
DPY19L4	CASP6	SLC15A2
ABCC1	RASGRP3	MRE11A
FHL1	ZNF195	KIF2A
MLC1	SOS1	PHACTR2
PKD2	VPS13C	CRYZ
TNFSF4	CEP110	TOP2B
FANCL	KIAA1797	PRKX
DAPK1	MEIS1	C5ORF28
GPR125	TRIM24	BMI1
IL12A	CEP170	ALDH18A1
WBP5	NME7	BAZ2B
LRBA	TMEM106B	SS18L1
DEPDC6	UPF3B	CEP350
UTP14C	FAM38B	TRIM68
DNMT3B	ABCE1	ACYP1
TEX10	RALA	P15RS
FBXO41	ZNF148	FASTKD1
ZNF189	METTL3	PTPLAD1

Supplemetal Table 2

Commonly up-regulated genes in RUNX1 high-expressing AML patients are collapsed to ChIP-seq data.	
ABCC1	ITPR2
ACYP1	KIT
AGPAT5	LRBA
AKAP11	MDM1
ALDH18A1	MEF2C
ANGPT1	MEIS1
ANKRD28	METTL3
ARMCX1	MLLT11
ATP1B1	MRE11A
BAZ2B	NCBP2
BBS10	NME7
BCL11A	OSBPL3
CASP6	PCCA
CBFA2T3	PHACTR2
CCDC41	PRKACB
CEP110	PRKX
CEP350	RALA
CEP70	RASGRP3
CNOT6	RPP40
CPA3	RUNX1
CRNKL1	SENP6
DAPK1	SEPP1
DEPDC6	SLC24A1
EHBP1	SRBD1
EXOC1	SSBP2
FAIM	TCF12
FASTKD1	TCF4
FHL1	TEX10
FLT3	TOP2B
GRB10	TRIM24
IFT88	USP11
IL12A	VAV3
ITPR1	ZMYM4

Supplemental Table 3

PCR primers used for RT-qPCR	Forward (5' → 3')	Reverse (5' → 3')
RUNX1	CTGCTCCGTGCTGCCTAC	AGCCATCACAGTGACCAGAGT
RUNX2	GGTTAATCTCCGCAGGTCACT	CACTGTGCTGAAGAGGCTGTT
RUNX3	CAGAAGCTGGAGGACCAGAC	GTCGGAGAATGGGTTCAGTT
CBFB	TGTGAGATTAAGTACACGG	TAATGCATCCTCCTGCTGGGCT
GAPDH	CATGTTCGTCATGGGGTGAACCA	AGTGATGGCATGGACTGTGGTCAT
BCL11A	AACCCCAGCACTTAAGCAAA	GGAGGTCATGATCCCCTTCT
TRIM24	GCGCCTACTTTTATTTCTTTACTG	AATGCTTTTGAGGCGTTTCTT
IL3	AATCTCCTGCCATGTCTGCC	AGATCGCGAGGCTCAAAGTC
CSF2RB	AGCCCAGATGCAGGGGA	CCCAGGATGTCAGGTAGGGA
p53	CCCCTCCTGGCCCCTGTCATCTTC	GCAGCGCCTCACAACCTCCGTCAT
CSF2	GGCCAGCCACTACAAGCAGCACT	CAAAGGGGATGACAAGCAGAAAG
p21	TGTGGACCTGTCACTGTCTTG	AATCTGTCATGCTGGTCTGC
BAX	CATGTTTTCTGACGGCAACTTC	AGGGCCTTGAGCACCAGTTT
PUMA	GCAGGCACCTAATTGGGCT	ATCATGGGACTCCTGCCCTTA
Pan_*RUNX* (*RUNX1 + RUNX2 + RUNX3*)	GCACCGACAGCCCCAACTT	GTCTTGTTGCAGCGCCAGTG

Supplemental Table 4

PCR primers used for ChIP	Forward (5' → 3')	Reverse (5' → 3')
TRIM24 R1	GAGACGGCGAAGAGAAATAA	CCGCCAAGTGTTCTACCC
TRIM24 R2	ACGACTGGAAAGTTCGAACAG	ATGCCTGTAATCCCAGCTACTC
TRIM24 R3	CAAAGTGCTGGGATTACAAGTG	AATCGCTTGAACCAGGGAG
BCL11A R1	ATTTCACCCTGGGCATATTATG	GCACTTAGTTACAAGTAGGTCTGCT
BCL11A R2	ATTGGAAATAGCCACAGATAGCA	AATGAGCGAGTGAGTGTTGTG
BCL11A R3	AGACATTCTTTTGGTGACTCCAA	TTGTTTCCCTCCAGTTTGTG
BCL11A R4	ACAGAATTTGAGGTCTCCCTCC	GCAAGCGGCTGTTTATTCCT
RUNX1 P1	TAAAGTTGTCCATTTAGGGGGAA	GGTAACGTCTATCATGGCATAAGTG
RUNX1 P2	AAGGACTTAACTCTCCCGGAG	CTCAATGGTCTTTGCTGATTTAGT
RUNX1 P3	AATTGAGATGGGCTGTGGAA	TTGGTGATGCTCACCACG
RUNX2 P1	CCAAATCCTCATGAGTCACAAA	TGCAAGCACTATTACTGGAGAGG
RUNX3 P1	TTCTCGCATCCTGTGAGCT	AATGAATGAATGAGGCTTACCC

Supplemental Table 5

Target sequences for shRNA knockdown experiments.	5' → 3'
sh *RUNX1* #1	AGCTTCACTCTGACCATCA
sh *RUNX1* #2	AACCTCGAAGACATCGGCA
sh *RUNX2*	AAGGTTCAACGATCTGAGATTT
sh *RUNX3*	AAGCAGCTATGAATCCATTGT
sh *pan-RUNX* #1	ACCGACAGCCCCAACTTCCT
sh *pan-RUNX* #2	GCACTGGCGCTGCAACAAGA
sh *Luc.*	CGTACGCGGAATACTTCGA
sh *p53* #1	ACCATCCACTACAACTACA
sh *p53* #2	GTCCAGATGAAGCTCCCAA
sh *p14ARF* #1	GTGCTGATGCTACTGAGGA
sh *p14ARF* #2	AGAACATGGTGCGCAGGTT

Supplemental Methods

Cell lines

AML derived THP-1 and KG-1a cells, CML derived K562 cells, lung cancer derived A549 cells, and esophageal cancer derived TE-1, TE-5 and TE-11 cells were purchased from RIKEN biological resource center (BRC), Japan. AML derived Kasumi-1 and HL60 cells, lung cancer derived LU99A and ABC-1 cells, gastric cancer derived MKN7, MKN45, MKN74, NUGC-4 and KATOIII cells, melanoma derived C32TG and Mewo cells, kidney cancer derived Caki-1 cells, colon cancer derived HCT116 and LOVO cells, and embryonic kidney derived HEK293T cells were from Japanese Collection of Research Bioresources (JCRB), Japan. AML derived OCI-AML2, OCI-AML3 and MOLM13 cells were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany. AML derived MV4-11 and KG-1a cells, ALL derived RS4;11 cells, lymphoma derived SU-DHL-5, Raji and Dauji cells, nyeloma derived KMS-12-BM cells, lung cancer derived NCI-H2228 cells, and breast cancer derived DU4475, MCF7, HCC1937, MDA-MB-231 and HTB-27 cells were from American Type Culture Collection (ATCC), USA. ALL derived SU-Ph2 and SU/SR cells were kindly provided by Dr. A. Kanamaru (Department of Internal Medicine, Kinki University School of Medicine, Osaka, Japan). ALL derived KOCL-45 cells were a gift from Dr. K. Sugita (Department of Pediatrics, Yamanashi University, Yamanashi, Japan). AML derived MV4-11NR cells harboring *TP53* R248W mutation were kindly provided by Dr. T. Ikezoe (Department of Hematology and Respiratory Medicine, Kochi University, Kochi, Japan). Caki-1 and HEK293T cells were maintained in Dulbecco's modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (PS) at 37 °C, 5 % CO_2. The other

cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% FBS and 1% PS at 37 °C, 5 % CO_2. Protein Expressions of RUNX1, RUNX2 and RUNX3 in cell lines utilized in this article are shown in Supplemental Figure 25A and 30. Protein expressions of p53 in response to Chb-M' treatment in these cell lines were also determined by immunoblotting and shown in Supplemental Figure 31.

Synthesis of PI polyamides

Chb-M'

Py-Im polyamide supported by oxime resin was prepared in a stepwise reaction by Fmoc solid-phase protocol. The product with oxime resin was cleaved with *N*,*N*-dimethyl-1,3-propane diamine (1.0 mL) at 45 ℃ for 3 h. The residue was dissolved in the minimum amount of dichloromethane and washed with diethyl ether to yield a 59.6 mg. To the crude compound (59.6mg, 48.1 μmol), a solution of chlorambucil (32.6 mg, 107 μmol), PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate) (101 mg, 195 μmol), and *N*,*N*-diisopropylethylamine (100 μL, 581 μmol) in *N*,*N*-dimethylformamide (DMF) (300 μL) was added. The reaction mixture was incubated for 1.5 h at room temperature, washed with diethyl ether and DMF for three times, and dried *in vacuo*. The crude product was purified by reversed-phase flash column chromatography (water with 0.1% trifluoroacetic acid/MeCN). After lyophilization, product was obtained (30.2 mg, 19.8 μmol). The other conjugates were prepared by the same procedure.

^{1}H NMR (600 MHz, DMSO (dimethyl sulfoxide)-d_6): d = 10.43 (s, 1H), 10.30 (s, 1H),

9.92 (s, 1H), 9.90 (s, 1H), 9.894 (s, 1H), 9.890 (s, 1H), 9.83 (s, 1H), 9.44 (s, 1H), 8.30 (t, J = 6.2 Hz, 1H), 8.15 (t, J = 6.2 Hz, 1H), 7.86 (t, J = 5.9 Hz, 1H), 7.63 (s, 1H), 7.52 (s, 1H), 7.44 (s, 1H), 7.39 (d, J = 2.0 Hz, 1H), 7.22 (d, J = 1.4 Hz, 2H), 7.18 (d, J = 1.3 Hz, 1H), 7.17 (d, J = 1.3 Hz, 1H), 7.15 (d, J = 1.3 Hz, 1H), 7.073 (d, J = 2.1 Hz, 1H), 7.066 (d, J = 2.0 Hz, 1H), 6.98 (d, J = 8.9 Hz, 2H), 6.95 (d, J = 2.0 Hz, 1H), 6.88 (d, J = 1.4 Hz, 1H), 6.62 (d, J = 8.9 Hz, 2H), 4.01 (s, 3H), 3.96 (s, 3H), 3.94 (s, 3H), 3.87 (s, 3H), 3.84 (s, 6H), 3.83 (s, 3H), 3.81 (s, 3H), 3.67 (m, 8H), 3.32-3.23 (m, 6H), 3.07 (m, 2H), 2.79 (d, J = 4.8 Hz, 6H), 2.52 (m, 2H), 2.40 (apparent t, J = 7.6 Hz, 2H), 2.28 (apparent t, J = 7.2 Hz, 2H), 2.04 (apparent t, J = 7.4 Hz, 2H), 1.82 (m, 4H), 1.70 (m, 2H).

ESI-TOF-MS m/z calcd for $C_{71}H_{90}Cl_2N_{24}O_{11}^{2+}$ $[M + 2H]^{2+}$ 762.3293, 763.3279, found 762.3277, 763.3244.

Chb-32

^{1}H NMR (600 MHz, DMSO-d_6): d = 10.38 (s, 1H), 10.29 (s, 1H), 9.93 (s, 1H), 9.90 (s, 3H), 9.86 (s, 1H), 9.36 (s, 1H), 8.48 (t, J = 5.9 Hz, 1H), 8.03 (t, J = 5.5 Hz, 1H), 7.87 (t, J = 5.5 Hz, 1H), 7.64 (d, J = 1.4 Hz, 1H), 7.54 (d, J = 1.4 Hz, 1H), 7.47 (d, J = 1.4 Hz, 1H), 7.38 (s, 1H), 7.23 (s, 2H), 7.20 (s, 1H), 7.18 (s, 1H), 7.16 (s, 1H), 7.07 (s, 1H), 7.06 (s, 1H), 6.99 (d, J = 7.6 Hz, 2H), 6.90 (s, 1H), 6.88 (s, 1H), 6.63 (d, J = 7.6 Hz, 2H), 4.01 (s, 3H), 3.97 (s, 3H), 3.96 (s, 3H), 3.87 (s, 3H), 3.85 (s, 6H), 3.83 (s, 3H), 3.80 (s, 3H), 3.68 (m, 8H), 3.30 (apparent quint, J = 5.8 Hz, 4H), 3.21 (apparent q, J = 6.2 Hz, 2H), 3.07 (m, 2H), 2.78 (d, J = 3.4 Hz, 6H), 2.41 (m, 4H), 2.36 (apparent t, J = 7.6 Hz, 2H), 2.04 (apparent t, J = 7.2 Hz, 2H), 1.86 (m, 2H), 1.80 (m, 2H), 1.71 (m, 2H).

ESI-TOF-MS *m/z* calcd for $C_{71}H_{90}Cl_2N_{24}O_{11}^{2+}$ $[M + 2H]^{2+}$ 762.3293, 763.3279, found 762.3218, 763.3218.

Chb-50

^{1}H NMR (600 MHz, DMSO-d_6): *d* = 10.38 (s, 1H), 10.29 (s, 1H), 10.22 (s, 1H), 9.99 (s, 1H), 9.920 (s, 1H), 9.916 (s, 1H), 9.86 (s, 1H), 9.42 (s, 1H), 8.48 (t, *J* = 6.2 Hz, 1H), 8.06 (t, *J* = 5.5 Hz, 1H), 7.87 (t, *J* = 5.8 Hz, 1H), 7.63 (s, 1H), 7.545 (s, 1H), 7.538 (s, 1H), 7.46 (s, 1H), 7.37 (s, 1H), 7.32 (s, 1H), 7.21 (s, 1H), 7.19 (s, 1H), 7.17 (s, 1H), 7.08 (s, 1H), 6.99 (d, *J* = 7.6 Hz, 2H), 6.98 (s, 1H), 6.89 (s, 1H), 6.63 (d, *J* = 8.2 Hz, 2H), 4.01 (s, 3H), 3.98 (s, 3H), 3.97 (s, 3H), 3.96 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.68 (m, 8H), 3.30 (apparent quint, *J* = 6.2 Hz, 4H), 3.21 (apparent q, *J* = 6.2 Hz, 2H), 3.07 (m, 2H), 2.78 (d, *J* = 4.8 Hz, 6H), 2.43-2.34 (m, 6H), 2.05 (t, *J* = 7.6 Hz, 2H), 1.86 (quint, *J* = 7.6 Hz, 2H), 1.80 (quint, *J* = 7.6 Hz, 2H), 1.71 (quint, *J* = 7.6 Hz, 2H).

ESI-TOF-MS *m/z* calcd for $C_{70}H_{89}Cl_2N_{25}O_{11}^{2+}$ $[M + 2H]^{2+}$ 762.8270, 763.8255, found 762.8247, 763.8251.

Chb-78

^{1}H NMR (600 MHz, DMSO-d_6): *d* = 10.46 (s, 1H), 10.33 (s, 1H), 10.20 (s, 1H), 10.04 (s, 1H), 9.94 (s, 1H), 9.91 (s, 1H), 9.87 (s, 1H), 9.42 (s, 1H), 8.33 (t, *J* = 5.5 Hz, 1H), 8.21 (t, *J* = 5.5 Hz, 1H), 7.89 (t, *J* = 5.5 Hz, 1H), 7.67 (s, 1H), 7.57 (s, 1H), 7.56 (s, 1H), 7.47 (s, 1H), 7.42 (s, 1H), 7.33 (s, 1H), 7.25 (s, 1H), 7.22 (s, 1H), 7.19 (s, 1H), 7.12 (s, 1H), 7.07 (s, 1H), 7.01 (d, *J* = 7.6 Hz, 2H), 6.92 (s, 1H), 6.66 (d, *J* = 7.6 Hz, 2H), 4.04

(s, 3H), 4.004 (s, 3H), 3.996 (s, 3H), 3.97 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 3.85 (s, 6H), 3.70 (m, 8H), 3.36-3.25 (m, 6H), 3.10 (m, 2H), 2.82 (d, J = 3.1 Hz, 6H), 2.51 (m, 2H), 2.43 (m, 2H), 2.32 (t, J = 7.5 Hz, 2H), 2.07 (t, J = 7.2 Hz, 2H), 1.86 (m, 4H), 1.73 (quint, J = 7.5 Hz, 2H).

ESI-TOF-MS m/z calcd for $C_{70}H_{89}Cl_2N_{25}O_{11}^{2+}$ $[M + 2H]^{2+}$ 762.8270, 763.8255, found 762.8236, 763.8258.

Chb-S

^{1}H NMR (600 MHz, DMSO-d_6): d = 10.34 (s, 2H), 10.33 (s, 1H), 10.32 (s, 1H), 9.93 (s, 2H), 9.33 (s, 1H), 9.31 (s, 1H), 8.15 (t, J = 5.5 Hz, 1H), 8.04 (t, J = 5.2 Hz, 1H), 7.89 (t, J = 5.5 Hz, 1H), 7.58 (s, 2H), 7.55 (s, 1H), 7.52 (s, 1H), 7.26 (s, 2H), 7.17 (s, 4H), 6.97 (d, J = 7.6 Hz, 2H), 6.95 (s, 1H), 6.91 (s, 1H), 6.61 (d, J = 7.6 Hz, 2H), 4.01 (s, 6H), 3.99 (s, 3H), 3.98 (s, 3H), 3.85 (s, 6H), 3.813 (s, 3H), 3.807 (s, 3H), 3.66 (m, 8H), 3.32 (q, J = 6.2 Hz, 2H), 3.23 (m, 4H), 3.06 (m, 2H), 2.79 (d, J = 3.4 Hz, 6H), 2.52 (m, 2H), 2.38 (m, 4H), 2.04 (t, J = 7.5 Hz, 2H), 1.82 (m, 4H), 1.70 (m, 2H).

ESI-TOF-MS m/z calcd for $C_{70}H_{89}Cl_2N_{25}O_{11}^{2+}$ $[M + 2H]^{2+}$ 762.8270, 763.8255, found 762.8247, 763.8230.

Poly-Acrylamide Gel Electrophoresis (PAGE)

The 5′ -Texas Red-labeled DNA fragments (6.0 nM) were alkylated by Chb-M' in 10 μL of 5.0 mM sodium phosphate buffer (pH 7.0) containing 10% DMF at 23 ℃ for 18 h. The reaction was quenched by adding calf thymus DNA (10 mg/mL, 1 μL) and heated at 95 °C for 10 min. The DNA was recovered by vacuum centrifugation. The pellet was

dissolved in 7 μL of loading dye (formamide with New fuchsin) and heated at 95 °C for 25 min, and then immediately cooled to 0 °C. A 1.8 μL aliquot was loaded on a 6% denaturing polyacrylamide gel containing 6.0 M of urea and electrophoresed using SQ5500-E Sequencer (HITACHI). For preparation of 500 mL of this gel, 183 g of urea and 60 mL of 50% Long Ranger gel solution (Lonza Rockland, Inc.) were added to *ca*. 200 mL of water and stirred for 30 min with 6 g of anion and cation exchange resin (AG 501-X8 Resin, Bio-Rad Laboratories, Inc.). After filtration, the resin was rinsed with 60 mL of 10x TBE, and water was added to the filtrate to a 500 mL. Electrophoresis was conducted under 1.5 kV, *ca.* 16 mA, and 40 °C.

Preparation of 209 bp DNA fragments used in PAGE

All DNA fragments and primers were purchased from Sigma-Aldrich. Two DNA fragments (34 bp) were annealed in a final volume of 50 μL containing 10 μM of each strand (5′ - ACCACATTAACCACAATTACCACATATAGGCCAA -3′

and 5′ - TGGCCTATATGTGGTAATTGTGGTTAATGTGGTA -3′) and ligated into pGEM-T Easy vector (Promega). *E. coli* JM109 competent cells were transformed and cultured on an LB plate with 100 μg/mL ampicillin overnight at 37 °C. White colonies were identified by colony direct polymerase chain reaction (PCR) in 15 μL of the reaction mixtures containing 300 nM of each primer

(SP6 primer: 5′ -ATTTAGGTGACACTATAGAATAC-3′ , T7 primer: 5′ -TAATACGACTCACTATAGG-3′), 2 x Go Taq Green Master Mix (Promega). Amplification cycles were carried out with an T100 Thermal Cycler (Bio-Rad). The reaction mix was incubated at 95 °C for 2 min, followed by 30 cycles of 95 °C for 20 s,

55 °C for 30 s, 72 °C for 20 s, with a final extension step of 72 °C for 7 min. The insertion-confirmed colony was selected for transfer to 5 mL of LB medium with 5 μL of ampicillin (100 μg/mL) and cultured at 37 °C for 15 h. The plasmid was extracted using GenElute Plasmid Miniprep Kit (Sigma-Aldrich). PCR condition from this inserted pGEM-T Easy vector: 95 °C for 2 min, followed by 34 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, with a final extension step of 72 °C for 7 min. The fragment was purified by QIAquick PCR purification kit (QIAGEN).

The sequence of this PCR product (209 bp) is 5′-TAATACGACTCACTATAGGGCGA ATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCGGGAATTCG ATTACCACATTAACCACAATTACCACATATAGGCCAAATCACTAGTGAATTCG CGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCAT AGCTTGAGTATTCTATAGTGTCACCTAAAT-3′. The complementary fragment (209 bp) was obtained by PCR using 5′-Texas Red-labeled SP6 primer and T7 primer.

Cell cycle and apoptosis assay

For cell cycle analysis, cells were fixed in fixation buffer and permeabilized with permeabilization wash buffer (BioLegend) followed by an incubation with PBS containing 3 % heat-inactivated FBS, DAPI and 100 μg/mL of RNase A. Cells were then subjected to flow cytometric analysis. For apoptosis assay, apoptotic cells were detected by Annexin V Apoptosis Detection Kit APC (eBioscience Inc.). In brief, approximately 2×10^5 cells of the indicated control and experimental groups were washed in PBS, suspended in annexin-binding buffer, and then mixed with 5 uL of annexin V. The reaction mixtures were incubated for 30 min. After incubation, cells

were diluted, stained with DAPI and processed for flow cytometric analysis.

Flow cytometry

Detection and isolation of c-kit^{+} or Lin^{-} (CD3^{-} Ly-6G/Ly-6C^{-} CD11b^{-} CD45R/B220^{-} TER-119^{-}) Sca-1^{+} c-kit^{+} (LSK) fraction from mice normal or leukemic bone cells and isolation of leukemia cell lines transduced with immunofluorescent color markers of GFP, Kusabira-Orange and Venus were performed using FACSAria III (BD) cell sorter. MLL-ENL fusion gene was retrovirally-transduced to c-kit^{+} primary mice bone marrow cells to obtain immortalized mice AML cells. FITC anti-mouse Lineage Cocktail (#133301), APC anti-mouse CD117 (c-Kit) Antibody (#105812) and PE anti-mouse Ly-6A/E (Sca-1) Antibody (#108107) (BioLegend) were used in this study.

Colony forming cell assay

Mice cells were plated into MethoCult™ M3434 and human AML cells were to MethoCult™ H4434 Classic media (StemCell Technologies). Colonies of each dish were counted on day 7.

γ-H2AX assay

The cells were grown and cytospun onto glass slides, washed in PBS, fixed in 4% paraformaldehyde/PBS for 30 min, then permeabilized in 0.5% Triton X for 15 min, followed by blocking with 1% bovine serum albumin in PBS for 30 min. Immunostaining was performed using an Alexa Fluor® 647 anti-H2A.X Phospho (Ser139) Antibody (#613407, BioLegend) for 12 hours at 4 ℃ in a dark humidified

chamber. After three 10 min washes, DNA was stained using ProLong Gold Antifade Mountant with DAPI (P36931, Thermo Fisher Scientific). Confocal immunofluorescence microscopy imaging was performed using an BZ-X700 Fluorescence Microscope (KEYENCE). The γ-H2AX foci were counted in 100 randomly sampled cells from each slide. Three independent experiments were performed.

IC50 evaluation

For cell survival assay, cells were seeded at a density of 1×10^5 cells/mL. The indicated concentrations of PI polyamides or drugs were added to the culture medium and cells were incubated for 48 hours. Cell viability was then assessed by Cell Count Reagent SF (nacalai tesque, Inc.) and Infinite® 200 PRO multimode reader (TECAN) according to the manufacturer's instructions. Percent inhibition curves were drawn and IC50 of the indicated compounds was calculated on the basis of the median-effect method (2).

Immunohistochemistry

IHC was performed on formalin-fixed paraffin-embedded tissue sections using antibodies directed against human CD45 antigen (IR751, DAKO) for xenograft experiments or human CBFB (sc-56751, Santa Cruz Biotechnology, Inc.) for patient-derived clinical samples (Multiple organ tumor and adjacent normal tissue array, BCN963a, US Biomax, Inc.) respectively. The antigen–antibody complexes were visualized with Histofine Simple Stain MAX PO (414151F, Nichirei Bioscience). The

tissue section images were captured using BZ-X700 All-in-One Fluorescence Microscope (Keyence, Japan).

Human samples

Normal human $CD34^+$ bone marrow or peripheral blood cells were purchased from STEMCELL Technologies (ST-70002), PromoCell GmbH (C-12921) and American Type Culture Collection (ATCC) (PCS-800-012). Total protein lysate of human normal kidney tissue (P1234142), human normal skin tissue (P1234218), human normal breast tissue (P1234086) and human normal lung tissue (P1234152) were purchased from BioChain Institute Inc.

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2. Chou TC, and Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul.* 1984;22(27-55.