Figure S1 (related Figure 1)

Qi et al

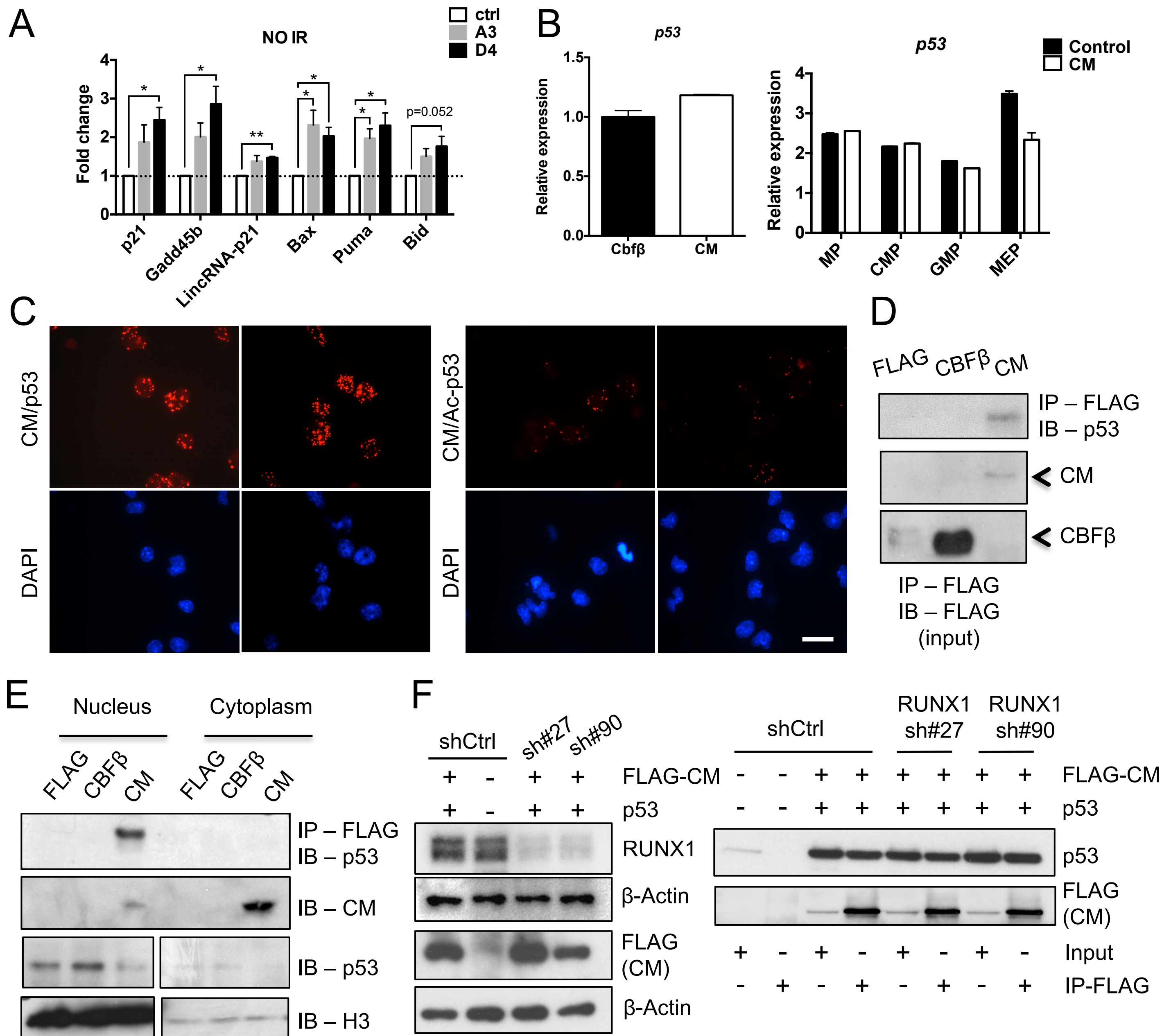

Figure S1. CM fusion protein binds to p53 and impairs p53 acetylation.

A. Relative expression of p53 target genes in sorted AML cells transduced with ctrl-shRNA or CM-shRNA (A3, D4) without IR. Shown are fold change (mean ± SD) relative to ctrl-shRNA-expressing cells, performed in triplicate. **B.** Relative expression of p53 mRNA in 32D-Cbfβ, 32D-CM cells (left) or primary CM pre-leukemic progenitors as determined by qRT-PCR. Phenotypic progenitor subsets are defined as myeloid progenitors (MPs) ($Lin^-/ckit^+/Sca1^-$), common myeloid progenitors (CMPs) ($Lin^-/ckit^+/Sca1^-/CD34^+/FcgR^{lo}$), granulocyte-macrophage progenitors (GMPs) ($Lin^-/ckit^+/Sca1^-/CD34^+/FcgR^{hi}$), and megakaryocyte-erythroid progenitors (MEPs) ($Lin^-/ckit^+/Sca1^-/CD34^-/FcgR^{lo}$). Shown are mean $\pm$ SD, performed in triplicate. **C.** Duolink *in situ* PLA in 32D-CM cells using mouse anti-CBFβ, rabbit anti-p53 or anti-Ac-p53 (K379) and PLA probes. Red fluorescent spots indicate CM-p53 or CM-Ac-p53 protein interactions (top), DAPI-stained nuclei are in blue (bottom); scale bar, 10 μm. **D.** Co-IP (anti-FLAG) and IB (anti-p53) analysis in 32D FLAG, CBFβ or CM cells. Input of CBFβ or CM is shown using anti-FLAG (IP, IB). **E.** Co-IP (anti-FLAG) and IB (anti-p53) analysis in nuclear and cytoplasmic fractions. Western blots of CM, p53, and Histone H3 for each fraction is shown. **F.** Western blot of RUNX1, FLAG-CM, and β-actin showing RUNX1 knock-down in 293T cells co-transfected with RUNX1 or control (Ctrl) shRNA vector (pLKO), (left). Co-IP (anti-FLAG) followed by IB (anti-p53 or anti-FLAG) in Ctrl- or RUNX1-shRNA transduced cells (right).

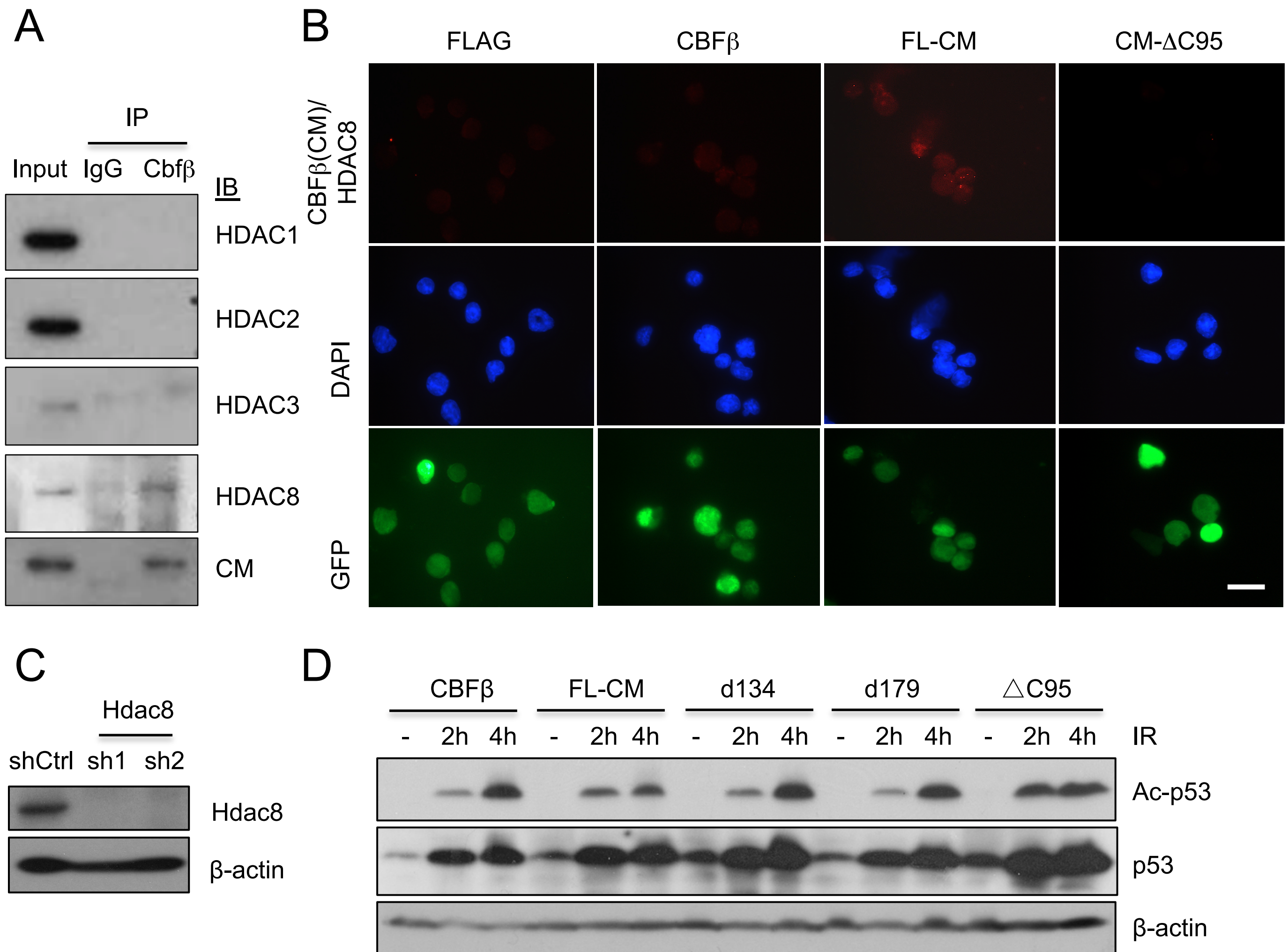

Figure S2. CM fusion protein recruits HDAC8 and p53 in a protein complex and promotes the deacetylation of p53 by HDAC8 .

A. Co-IP (IgG or anti-Cbfβ) and IB (anti- HDAC1, HDAC2, HDAC3, HDAC8, CBFβ) in 32D-CM cells. **B.** Duolink *in situ* PLA in 32D cells expressing FL-CM, deletion mutant ΔC95 using mouse anti-CBFβ, rabbit anti-HDAC8 and PLA probes. Red fluorescent spots indicate CM-HDAC8 protein interactions (top), DAPI-stained nuclei are blue (center) and green indicates transduced cells expressing GFP reporter (bottom); scale bar, 10 μm. **C.** Western blot of Hdac8 and β-actin in 32D-CM cells expressing pLKO.1 Ctrl- or Hdac8-shRNA (sh1 or sh2) vectors. **D.** Western blot of Ac-p53 (K379), total p53 levels in 32D cells expressing CBFβ, CM, CM-d134, CM-d179 or CM-ΔC95 deletions. Cell lysate were isolated before, 2h or 4h after IR (3Gy). Levels of β-actin were detected as loading control.

Figure S3 (related to Figure 3) Qi et al

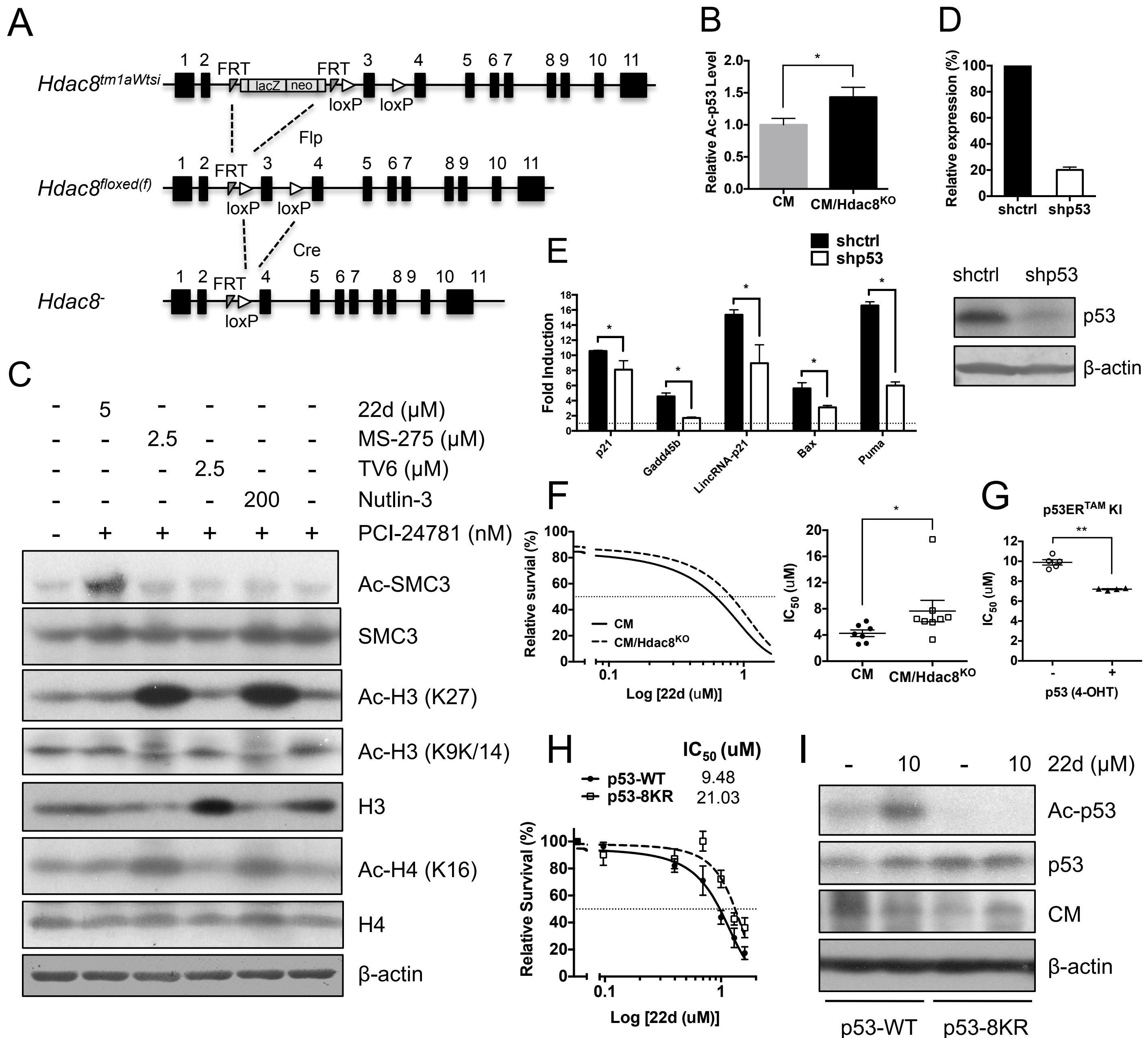

Figure S3. Selective inhibition of HDAC8 enhances p53 activity and inhibits cell survival.
A. *Hdac8*^{tm1aWtsi} knockout first allele was converted by Flp-mediated deletion into the floxed allele in which exon 3 is flanked by loxP sites. *Hdac8*^{floxed} mice were crossed with *Mx1-Cre* and induced with pIpC to generate *Hdac8*$^{-(KO)}$. **B.** Relative quantitation of Ac-p53 levels in CM and *CM/Hdac8*^{KO} pre-leukemic BM progenitor cells (n=3; P=0.0199). **C.** Western blot analysis of Ac-SMC3, SMC3, acetylated histones H3K27, H3-K9/14, H4K16 and total H3 and H4 in 32D-CM cells treated with indicated inhibitors. Levels of β-actin serve as loading control. **D.** Relative expression levels of p53 in 32D-CM cells transduced with pLKO.1 lentivectors expressing shp53 or non-silencing control (shctrl) normalized to levels of β-actin (top). Western blots of p53 and β-actin in 32D-CM cells expressing shp53 or shctrl (bottom). Shown are mean ± SEM. **E.** Fold activation of p53 target genes in 32D-CM cells expressing shctrl or shp53 and treated with 22d (10 μM) for 16 h, determined by qRT-PCR. Relative expression of each target gene was normalized to levels of *ACTB*. * $P < 0.05$. **F.** Survival inhibition dose-response curve (left) and IC_{50} (right) of HDAC8i 22d for primary CM (n=7) or CM/Hdac8^{KO} (n=8) BM cells (P=0.0159). **G.** Survival inhibition IC_{50} of HDAC8i 22d for p53 deleted (p53ER^{TAM} KI) or p53 restored (+4-OHT, 100 nM) BM cells (n=5; P=0.0079). **H.** Survival inhibition IC_{50} of HDAC8i 22d for K562 cells co-expressing CM and p53-WT or the p53-8KR mutant. **I.** Western blot of Ac-p53, p53, CM and β-actin in vehicle or 22d (10 μM) treated K562 cells co-expressing CM and p53 WT or the p53-8KR mutant.

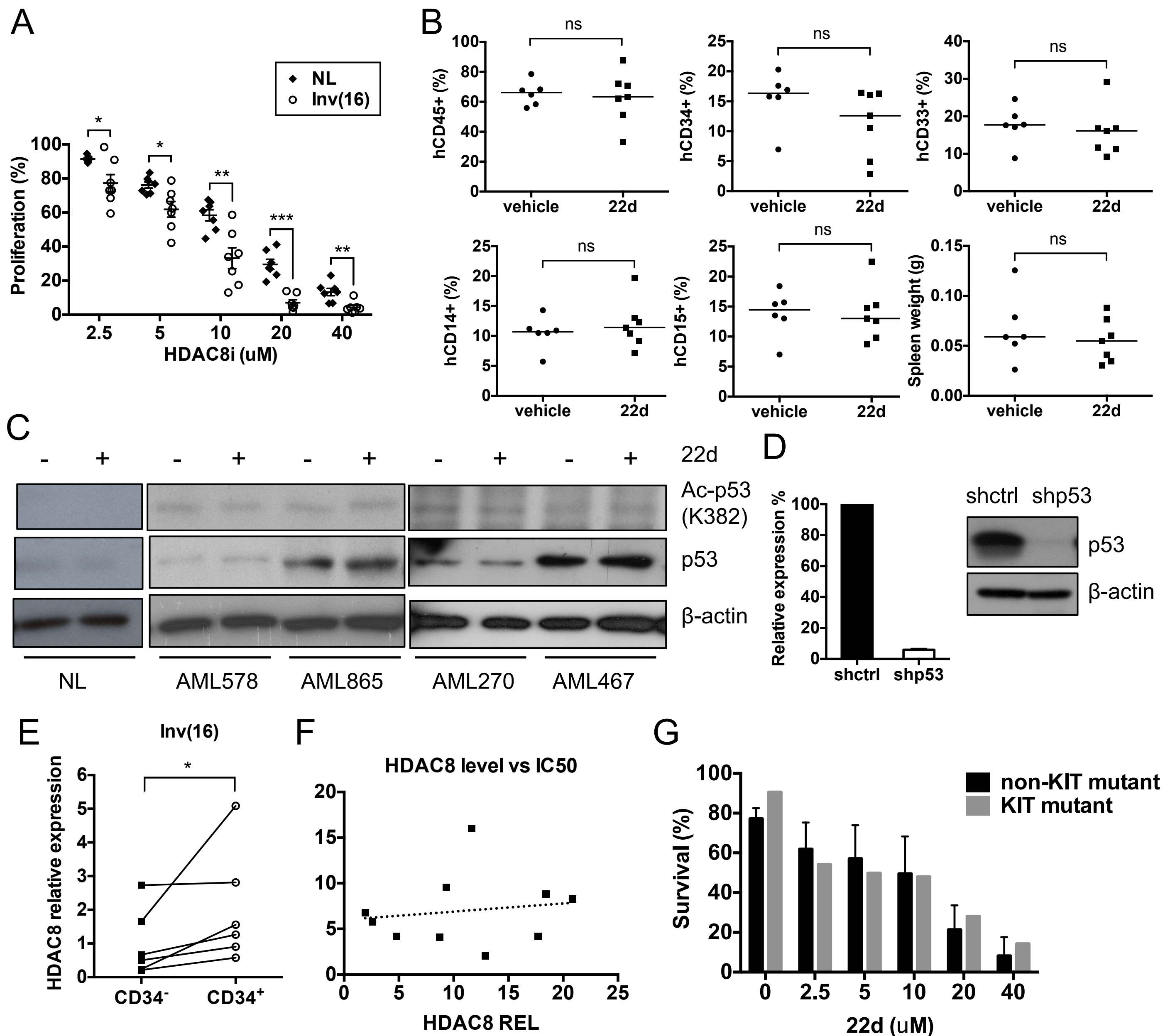

Figure S4. HDAC8i treatment selectively reduce survival of inv(16)+ AML CD34+ cells.

A. Relative proliferation of normal (NL; n=7), inv(16)+ AML (n=12) CD34+ cells treated with HDAC8i 22d for 48 h compared to vehicle treated controls. Each dot represents an individual patient and lines indicate the mean ± SEM (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). **B.** Engraftment of normal CB CD34+ cells treated with 22d (10 μM) or vehicle for 48 h into sub-lethally irradiated NOD/SCID/interleukin-2 receptor-g chain deficient (NSG) mice. Shown are engraftment levels of each population (CD45+, CD34+, CD33+, CD14+, CD15+) in the BM and spleen weight at 16 weeks after transplantation. Each dot represents result from an individual mouse and line indicates medium. ns, not significant. **C.** Western blotting of Ac-p53 (K382), and p53 levels in non-inv(16) AML or NL CD34+ cells treated with 22d (10 μM) for 6 h. Levels of β-actin were detected as loading control. **D.** Relative p53 mRNA level in GFP sorted MV4-11 cells transduced with pLKO.1-GFP lentivirus expressing sh-p53 or non-silencing control (sh-ctrl) normalized to levels of b-actin (left). Shown are mean ± SEM. Western blot analysis of p53 in GFP sorted MV4-11 cells expressing sh-p53 or sh-ctrl (right). Levels of β-actin serve as loading control. **E**. Relative HDAC8 expression levels in CD34+ and CD34- cells from inv(16)+ AML patients (n=6), assessed by qRT-PCR. Dots connected by line represent an individual patient; mean of triplicated assays for each patient is shown (P=0.0183). **F.** Correlation analysis of IC_{50} and HDAC8 relative expression level (REL) for inv(16)+ AML. Dashed line indicates correlation analysis (r = 0.2). **G.** Percent survival of inv(16)+ AML CD34+ cells with or without KIT mutation treated with various concentrations of HDAC8i 22d.

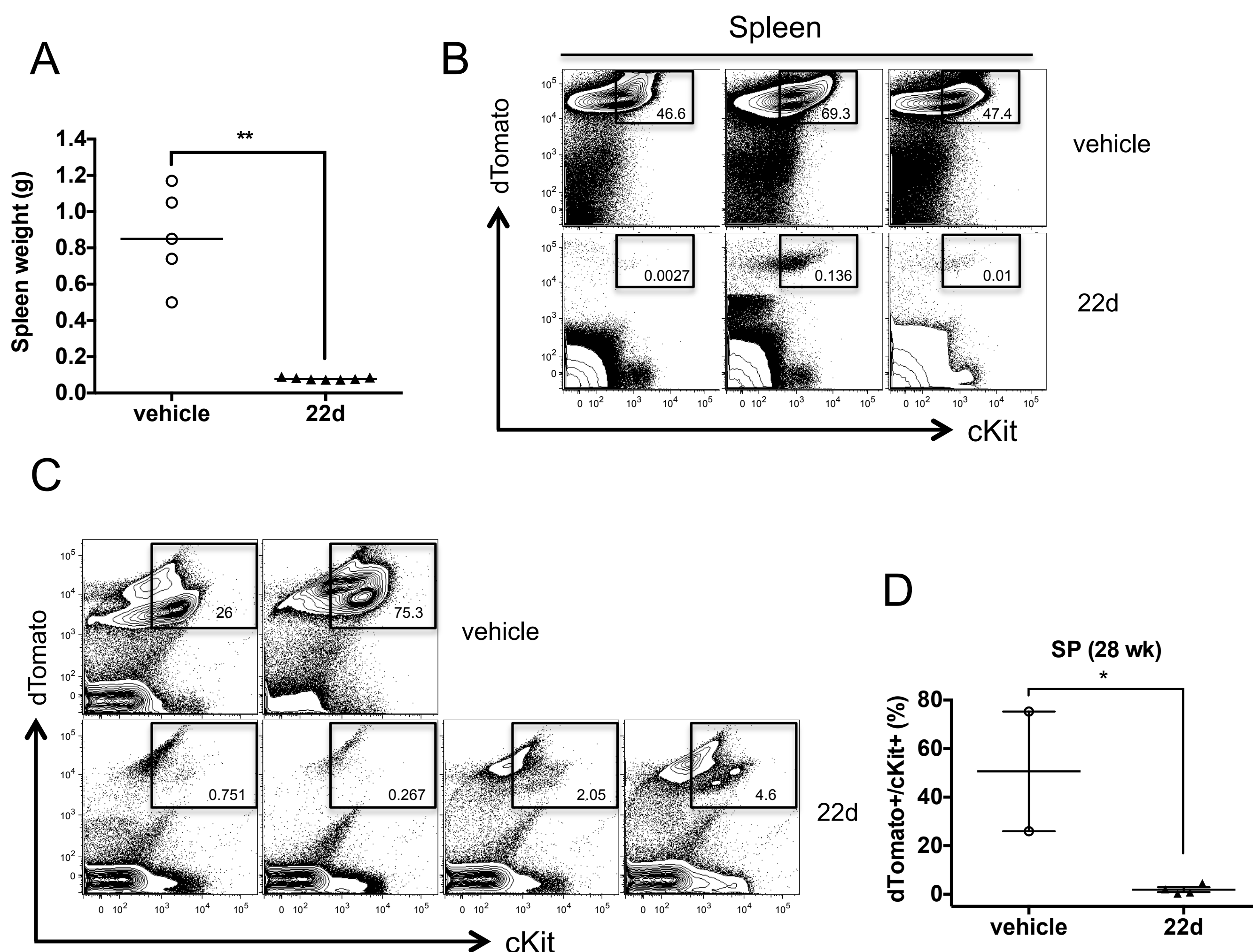

Figure S5. Pharmacological inhibition of HDAC8 eliminates LSC survival and engraftment.
A. Weight of spleens isolated mice transplanted with vehicle treated (n=5) or 22d treated cells (n=7). Each dot represents results from individual mice and line indicate median (P=0.0025). **B.** Representative FACS plots and frequencies of engrafted $dTomato^{+}/cKit^{+}$ AML cells in the spleen at 8 weeks after transplantation. **C.** Representative FACS plots and frequencies of $dTomato^{+}/cKit^{+}$ AML cells in recipients transplanted with equal numbers of live 22d- or vehicle-treated cells ($2x10^{6}$). **D.** The frequency of dTomato+/ckit+ cells in the SP 28 weeks after transplantation of $2x10^{6}$ live cells treated with 22d (n=4) or vehicle (n=2; another 2 had succumbed to AML prior to analysis) *ex vivo* for 48 h. Each dot represents results from an individual mice and line indicate mean $\pm$ SEM (*P=0.0325).

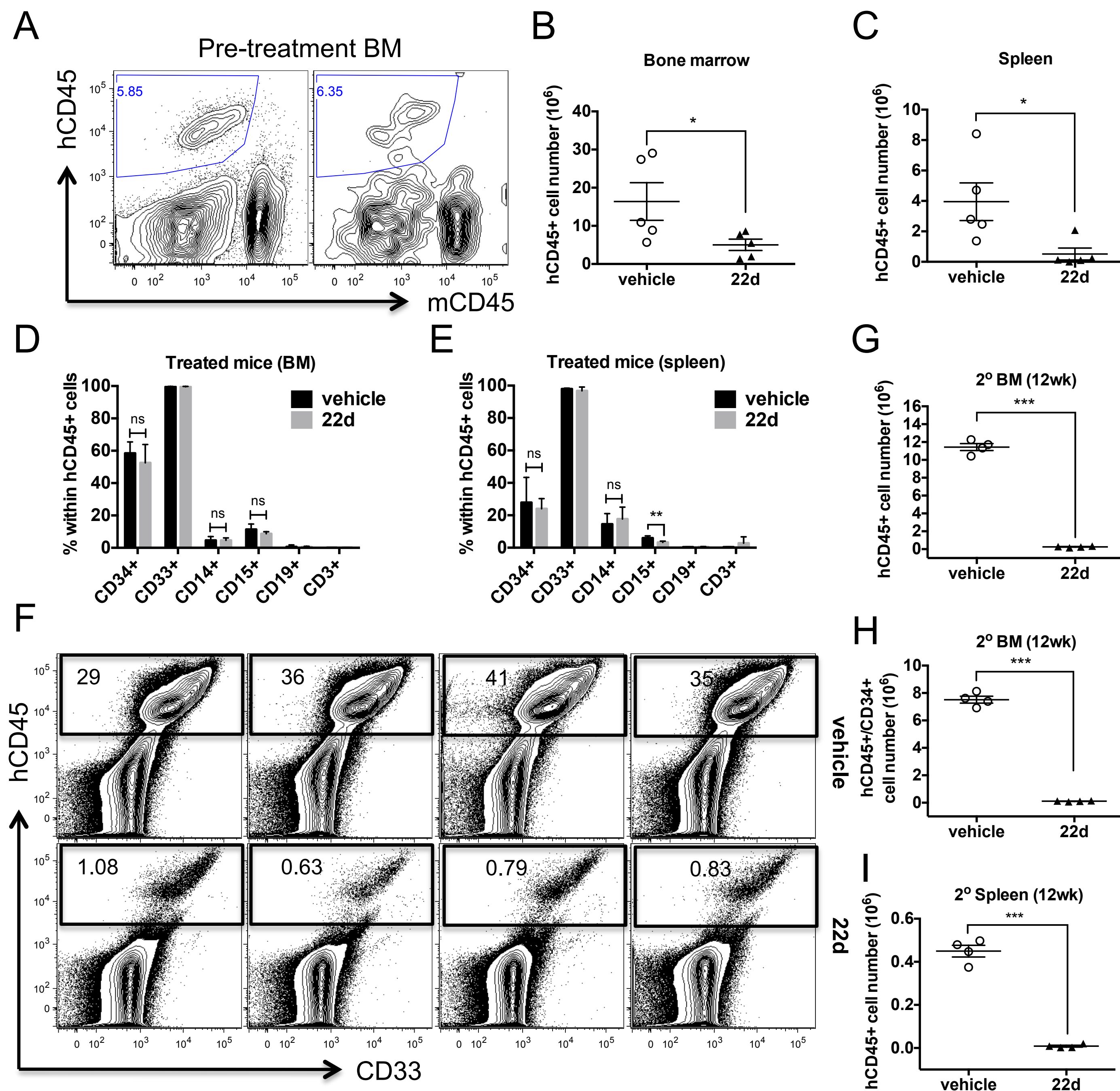

Figure S6. *In vivo* administration of HDAC8i 22d diminished AML propagation.
A. Representative FACS plots showing pre-treatment engraftment levels of $hCD45^+$ AML cells in the bone marrow of NSGS mice. **B.** Total number of $hCD45^+$ AML cells in the BM of vehicle (n=5) or 22d (n=5) treated mice (*P=0.0317). **C.** Total number of $hCD45^+$ AML cells in the spleen of vehicle (n=5) or 22d (n=5) treated mice (*P=0.0159). **D.** Frequency of immunophenotypic populations within remaining hCD45+ AML cells in the bone marrow after 2-week treatment with HDAC8i 22d or vehicle (n=5). Bars show mean ± SD; ns, not significant. **E.** Frequency of immunophenotypic populations within remaining hCD45+ AML cells in the spleen after 2-week treatment with HDAC8i 22d or vehicle (n=5). Bars show mean ± SD (** P=0.0058). **F.** Representative FACS plots showing $hCD45^+$ AML cells in secondary recipient BM 12 weeks after transplantation. **G.** Total number of $hCD45^+$ AML cells in the BM of secondary recipients transplanted with vehicle (n=4) or 22d (n=4) treated BM at 12 weeks (***P < 0.0001). **H.** Total number of $hCD45^{+/}CD34^+$ AML cells in the BM of secondary recipients transplanted with vehicle (n=4) or 22d (n=4) treated BM BM at 12 weeks (***P < 0.0001). **I.** Total number of $hCD45^+$ AML cells in the spleen of secondary recipients transplanted with vehicle (n=4) or 22d (n=4) treated BM BM at 12 weeks (***P=0.0004).

Table S1 (related to Figure 4 & Experimental Procedures)

Qi et al

Table S1. Characteristics of patient samples used in this study

Sample ID	Sex	Age	Diagnosis	FAB Classification	Sample Type	Disease Status At Sample Collect	Risk Status	Cytogenetic	Other Mutation	WBC (K/uL)	Blasts In PB (%)	Blasts In BM (%)
AML020	F	47	AML	M4eo	PB	Untreated	Better-risk	inv(16)	FLT-3 ITD Neg	50.8	67	90
AML021	F	42	AML	M4	BM	Untreated	Better-risk	inv(16)		39.2	60	40
AML033	M	69	AML	M4	PB	Untreated	Intermediate-risk	inv(16), trisomy 22		6.3	30	98
AML041	F	56	AML	M2	PB	Untreated	Better-risk	inv(16), trisomy 8		38.8	63	44
AML111	M	51	AML	M2	BM	Untreated	Intermediate-risk	Inv(16), Trisomy 8, trisomy 21		13.4	70	75
AML163	M	57	AML	M4	PB	Relapsed	Intermediate-risk	t(16;16), trisomy 21, trisomy 22		82.8	94	67
AML319	F	46	AML	M4	PB	Untreated	Better-risk	CBFB/16q22 rearrangement		1.4	0	
AML987	M	50	AML	M4	PB	Relapsed	Intermediate-risk	t(16;16), trisomy22, CBFB rearrangenent	FLT-3 ITD Neg, FLT-3 D835 Pos., NPM1 Neg, C-KIT Neg	64.1	90	
AML1052	F	23	AML	M4	BM	Relapsed	Intermediate-risk	inv(16), trisomy 8, trisomy 22	FLT-3 ITD Neg, FLT-3 D835 Neg	9.9	64	87
AML1070	F	37	AML	M4Eo	BM	Relapsed	Better-risk	der(16) inv(16) (p13.1q22) del(16) (q22.1q22.?2) [16]/[22] 44% CBFB rearrangement by FISH	NPM1 Neg	2.7	3	30
AML1241	M	64	AML	M4Eo	BM	Relapsed	Better-risk	inv(16),	C-KIT Neg,		2	
AML1702	F	53	AML	M4	BM	Untreated	Poor-risk	t(16;16)(p13;q22)[8]/[20]; sl,del(7)(q31.2q34)[12]/[20]	FLT3 ITD Neg., FLT3 TKD Pos., NPM1 Neg., CEBPA Neg., C-Kit Pos., CBFB Pos.		61	84
AML270	M	61	AML		PB	Untreated	Poor-risk	Complex abnormalities, including 11q23, MLL gain, loss of TP53/17p13.1, add(2), add(6), add(22), ider(11), del(11)	FLT-3 ITD Neg., NPM1 Neg.	4.4	5	50
AML467	F	61	AML		PB	Untreated	Poor-risk	Complex abnormalities with trisomy 8, trisomy 9 and trisomy 22.	FLT-3 ITD Neg, FLT-3 D835 Neg	44.7	51	80
AML578	M	38	AML	M5b	PB	Refractory/Induction failure	Poor-risk	Trisomy 8, del(9q), t(2;18), trisomy 13	FLT-3 ITD Pos, FLT-3 D835 Neg, NPM1 Neg	1.3	41	8
AML865	F	60	AML		PB	Untreated	Poor-risk	Normal cytogenetics	FLT-3 ITD Pos, FLT-3 D835 Neg, JAK2 Neg	86.3	90	90
AML098	F	40	AML	M2	PB	Relapsed	Better-risk	Variant t(8;21); RUNX 1/RUNX1T1		8.6	80	
AML107	F	40	AML	M2	BM	Untreated	Better-risk	t(8;21), RUNX1/RUNX1T1		6.5	32	40
AML113	M	50	AML	M2	BM	Untreated	Better-risk	t(8;21); RUNX1/RUNX1T1	C-KIT Pos	9.4	50	95

Table S2. Primers used in the study

Gene	Cells	Primer type	Sequence
p53	Mouse	Forward	AAGATCCGCGGGCGTAA
	Mouse	Reverse	CATCCTTTAACTCTAAGGCCTCATTC
p21	Mouse	Forward	GCGGCTGTTTTTCTTGGTAG
	Mouse	Reverse	AGACGAGGAAAGCAGTTCCA
Mdm2	Mouse	Forward	TTAGTGGCTGTAAGTCAGCAAGA
	Mouse	Reverse	CCTTCAGATCACTCCCACCT
Gadd45b	Mouse	Forward	CTGCCTCCTGGTCACGAA
	Mouse	Reverse	TTGCCTCTGCTCTCTTCACA
LincRNA-p21	Mouse	Forward	CCTGTCCACTCGCTTTC
	Mouse	Reverse	GGAACTGGAGACGGAATGTC
Bax	Mouse	Forward	GTGAGCGGCTGCTTGTCT
	Mouse	Reverse	GGTCCCGAAGTAGGAGAGGA
Bid	Mouse	Forward	GACAGCTAGCCGCACAGTT
	Mouse	Reverse	GGCCAGGCAGTTCCTTTT
Puma	Mouse	Forward	TTCTCCGGAGTGTTCATGC
	Mouse	Reverse	TACAGCGGAGGGCATCAG
Stag1	Mouse	Forward	GCACTTCTGGATTTAATCAACTTTTT
	Mouse	Reverse	CGAAACATCTCTATTCTGACCGTA
Hprt	Mouse	Forward	TCCTCCTCAGACCGCTTTT
	Mouse	Reverse	CCTGGTTCATCATCGCTAATC
HDAC8	Human	Forward	GGTGACGTGTCTGATGTTGG
	Human	Reverse	GACACTTGCCAATTCCCACT
p53	Human	Forward	CCGCAGTCAGATCCTAGCG
	Human	Reverse	AATCATCCATTGCTTGGGACG
p21	Human	Forward	TACCCTTGTGCCTCGCTCAG
	Human	Reverse	CGGCGTTTGGAGTGGTAGA
MDM2	Human	Forward	CCTTCGTGAGAATTGGCTTC
	Human	Reverse	CAACACATGACTCTCTGGAATCA
PUMA	Human	Forward	GACCTCAACGCACAGTACGAG
	Human	Reverse	AGGAGTCCCATGATGAGATTGT
ACTB	Human	Forward	GTGGATCAGCAAGCAGGAG
	Human	Reverse	TTTGTCAAGAAAGGGTGTAACG

Supplementary Experimental Procedures

***In situ* proximity ligation assay (PLA)**

The Duolink kit (Sigma, St. Louis, MO) was used to perform *in situ* PLA according to the manufacturer's instructions. Antibodies used included mouse anti-CBFβ (Santa Cruz), rabbit anti-Ac-p53 (K379) (Cell Signaling) and rabbit anti-p53 (Cell Signaling). Slides were mounted using in an anti-fade medium containing DAPI (Santa Cruz) and imaged using a Zeiss upright LSM 510 2-photon confocal microscope.

Cell culture and cell transduction

Bones were crushed with mortar and pestle in 1X PBS/3% FBS/1mM EDTA, filtered using 0.45 μm nylon filter and cells were preceded for RBC lysis followed by two washes with the same buffer. Single cell suspension was obtained and used for protein lysis or cell culture. Isolated BM cells were maintained in RPMI-1640 media supplemented with 10% FBS, antibiotics, 6 ng/mL interleukin-3 (IL-3), 10 ng/mL interleukin-6 (IL-6) and 10 ng/mL stem cell factor (SCF)(PeproTech, Rocky Hill, NJ). $CD34^+$ cells were cultured in medium supplemented with growth factors including IL-3 (25 ng/mL), IL-6, (10 ng/mL), Flt-3 ligand (100 ng/mL), SCF, (50 ng/mL), and thrombopoietin (100 ng/mL). AML cells were cultured in IMDM media supplemented with 20% FBS, antibiotics, 20 ng/mL Tpo, 20 ng/mL SCF and 10 ng/mL IL-6 for 48 h or 72 h with HDAC8i 22d (10 μM) or vehicle (control). For 32D cell lines, cells were cultured in RPMI-1640 supplemented with 10% FBS, 20% WEHI-3 conditioned media and antibiotics at 37°C with 5% CO_2 and humidified conditions. $CD34^+$ cells were cultured in Stemspan serum-free medium (StemCell Technologies), supplemented with low concentrations of growth factors (200 pg/ml granulocyte-macrophage colony-stimulating factor [GM-CSF], 1ng/mL granulocyte colony-stimulating factor [G-CSF], 200 pg/mL SCF, 1 ng/mL IL-6, 200 pg/mL

macrophage inflammatory protein-1α [MIP-1α], and 50 pg/ml leukemia inhibitor factor [LIF]), at 37 °C with 5% CO_2. BM cells or 32D cells were transduced with MIG based retroviruses or lentiviruses (pLKO.1 or HIV-7) (Lo et al., 2007) by spinoculation in the presence of 5 ug/mL polybrene (American Bioanalytical, Natick, MA). Human $CD34^+$ cells were transduced with pLKO.1 lentivirus by two rounds of spinoculation.

Nuclear/ cytoplasmic fractionation

Cells were lysed in ice-cold NP-40 lysis buffer [10mM Tris (pH 7.4), 3mM MgCl2, 10mM NaCl, 0.5% NP-40, containing protease inhibitor] and incubated for 10 minutes followed by centrifugation at 1500rpm at 4°C. The pellet was resuspend gently in ice-cold hypotonic buffer [10mM HEPES (pH 7.9), 1.5mM MgCl2, 10mM KCl, containing protease inhibitor] followed by centrifugation at 7000rpm for 1 minute at 4°C. The supernatant is removed as cytoplasmic fraction while the pellet were resuspended in ice-cold extraction buffer [20mM HEPES (pH 7.9), 1.5mM MgCl2, 420mM KCl, 0.2mM EDTA, 20% glycerol, containing protease inhibitor] and incubated for 45 minutes at 4°C with vigorous shaking. After centrifugation at 10,000 rpm for 5 minutes at 4°C, the supernatant was collected as nuclear fraction. Protein concentration was estimated using the BCA protein assay (Pierce, Rockford, IL).

Quantitative RT-PCR

RNA was isolated using Trizol reagent (Invitrogen) or by RNeasy micro Kit (Qiagen) following standard manufacturer's protocol. First-strand cDNA was generated using SuperScript III reverse transcriptase (Invitrogen). Quantitative (q)RT-PCR was performed using TaqMan® assays (Life technologies) or LightCycler 480 SYBR Green I master mix (Roche Applied Science) containing 0.2μM gene-specific primers and detected with a LightCycler 480 real-time PCR system (Roche Applied Science). Gene

Specific primers are listed in Table S2. Relative expression level was determined by normalizing to levels of *Hprt* or *ACTB*.

Annexin-V staining

Annexin-V staining was performed following the manufacturer's protocol (BD Biosciences). Cells were washed twice with ice cold 1X PBS and resuspended at a concentration of 10^6 cells/ml in 1X binding buffer, then incubated with APC-Annexin-V and 7-AAD (vital dye) at room temperature for 15 minutes in dark. Flow cytometry was performed on a 4-laser, 14-detector FACS-LSRII (BD Biosciences).

Cell proliferation assay

Cell proliferation was measured utilizing the Cell Titer-Glo Luminescent Cell Viability Assay Kit (Promega Corp., Madison, WI). Addition of the CellTiter-Glo solution to cells in culture will result in cell lysis and the generation of a luminescent signal proportional to the amount of ATP present, which signals the number of metabolically active cells present in culture. Briefly, cells were cultured under different treatment conditions for 48 h and transferred to a 96-well plate to equilibrate for 30 min before assay. The substrate and buffer reagents were equilibrated to room temperature and mixed to obtain a homogeneous solution. After that, 100 ul assay solution was added into each well and mixed for 2 min on an orbital shaker to obtain cell lysis. The cell lysis was then incubated at room temperature for 10 min to stabilize the luminescence signal, and results were read on a Microplate reader (Beckman culture DTX880). All plates had control wells containing medium without cells to obtain a value for background luminescence. Data are expressed as percentage of untreated cells, mean ± SE, for three replications.

Flow cytometry

Cells were resuspended in 5 mL PBS/0.5% BSA and filtered through a 70-μm filter (BD Biosciences). The following antibodies were used for fluorescence activated cell-sorting (FACS) analyses: CD117 (c-kit, clone 2B8, eBioscience), Ly-6A/E (Sca-1, clone E13-161.7, BioLegend), CD150 (SLAM, clone TC15-12F12.2, BioLegend), CD48 (clone HM48.1, BioLegend), CD34 (clone RAM34, eBioscience), CD16/CD32 (clone 93, eBioscience), CD45.1 (clone A20, BioLegend), CD45.2 (clone 104, BioLegend), CD11b (clone M1/70, eBioscience), Ter119 (clone TER-119, BioLegend), CD45R (clone RA3-6B2, BD Biosciences), CD3 (clone 17A2, eBioscience), Ly-6G/Ly-6C (Gr1, clone RB6-8C5, BioLegend), human CD45 (2D1, eBioscience), human CD34 (4H11, eBioscience), human CD33 (HIM3-4, eBioscience), human CD14 (61D3, eBioscience), human CD15 (HI98, eBioscience), human CD19 (HIB19, eBioscience), human CD3 (SK7, eBioscience). Flow cytometry analysis was performed on a 5-laser, 15-detector LSRII. Cell sorting was performed on a 4-laser, 15-detector FACSAria-III or a 6-laser, 18-detector FACSAria II SORP (BD Bioscience, San Jose, CA).

Immunoprecipitation (IP) and Western blotting

Additional antibodies used for IP and Western blots include anti-HDAC1, anti-HDAC2, anti-HDAC3, anti-Ac-H3K27, anti-Ac-H3K9/K14, anti-Ac-H4K16, anti-RUNX1 (Cell Signaling), anti-RUNX2 (MBL international).

Luciferase reporter assays

K562 cells (purchased from ATCC) were co-transfected with pMIC-CM, p53 responsive reporter (BP100) and internal control (Renilla-TK) plasmids, together with p53-WT or an acetylation-defective p53 mutant (p53-8KR) using 4D-nucleofector system (Lonza). HDAC8i 22d (10 uM) or vehicle (DMSO) was added to the cells at 24 h post-transfection.

Sixteen-hours after HDAC8i treatment, cells were lysed by 1×PLB buffer (Promega) for 15 min and Luciferase assays were performed using the dual-luciferase reporter assay system (Promega). The data are represented as the fold induction of luciferase activity in HDAC8i treated cells normalized to levels in vehicle-treated cells.

Compounds and DNA constructs

Compound 22d was synthesized as previously described (Huang et al., 2012). For animal use, 22d was prepared in water containing 20% (m/v) 2-hydroxypropyl–betacyclodextrin (Cyclodextrin Technologies Development Inc. Alachua, FL) and 1% DMSO. PCI-34051 and PCI-24781 were provided by Dr. Sriram Balasubramanian (Pharmacyclics). Nutlin-3, TV-6, MS-275 were purchased from Cayman Chemical (Ann Arbor MI) and cytarabine was purchased from Selleck Chemicals (Houston TX). MIG-CM d134 (residues 134-236 deleted) and d179 (residues 179-221 deleted) vectors were provided by Dr. Pual Liu (NIH). MIG-CM ΔC95 were generated by PCR directed mutagenesis. Anti-p53 shRNA sequence from pLKO-p53 shRNA vector (Addgene) was cloned into a pLKO-Puro-IRES-GFP vector. The pcDNA3-p53 and MDM2-luc (BP100) plasmids were provided by Dr. Mu-Shui Dai (Oregon Health & Sciences University). Plasmids pTRE2-hyg-p53 and pTRE2-hyg-p53-8KR were provided by Dr. Wei Gu (Columbia University). Hdac8 shRNA vectors used were TRCN0000087998 and TRCN0000087998 (Sigma-Aldrich). Human HDAC8 cDNA were cloned into pMICII vector.