Materials and Methods

Cell lines

The following cell lines were used in our study: THP1 (human acute monocytic leukemia cell line), KBM5 (human transformed chronic myeloid leukemia cell line), HL-60 (human promyelocytic leukemia cell line), HuH-7 (human hepatocellular carcinoma cell line), AN3CA (human endometrial cancer cell line), and SNU-398 (human hepatocellular carcinoma cell line). All cell lines used in the experiments were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured according to instructions provided by ATCC.

Immunoprecipitation and Western blot

For pull-down assays, FITC-labeled peptides and anti-FITC antibody (Catalogue # sc69871, Santa Cruz, CA) were used. 20 μM FITC-labeled peptides were added to the nuclear extracts from $1x10^6$ cells and 5 μg of antibody which was immobilized covalently on the surface of 1 mg Dynabeads (Catalogue # 143.11D, Invitrogen). The magnetic beads were than used in the pull down reaction according to the instructions of the manufacturer with the following minor modifications: 1 mM DTT, 0.1 mM NaF, 1 mM PMSF, 1 mM Na orthovanadate, and 1:100 final dilution of protease inhibitor, (Catalogue # P8340, Sigma Co.) were added to the extraction buffer. The NaCl concentration was optimized to 100 mM. Eluted proteins were submitted to western blotting using standard procedures and the protein bands were visualized using antibodies against HDAC1 (Catalogue # H6287, Sigma Co.) and HDAC2 (Catalogue # H2663,

Sigma Co.). For immunoprecipitation (IP) with SALL4, 20 μM unlabeled peptides were added to nuclear extracts from $1x10^6$ SNU-398 cells and IP was done with SALL4 antibody (Catalogue # sc101147, Santa Cruz), immobilized on magnetic beads as described above. Following IP, eluted proteins were separated and analyzed by western blot using HDAC1 or SALL4 antibody. Other antibodies used in western blots include PTEN (Catalogue # 9559S, Cell Signaling), α-tubulin (Catalogue # T6704, Sigma Co.), pAKT (detecting phosphorylation at residue S473 of AKT) (Catalogue # 9271S, Cell Signaling), and AKT (detecting total AKT) (Catalogue # SAB 4500802, Sigma Co.). For western blot analysis in Figure 3, total cell extracts from $1x10^6$ SNU-398 cells were prepared in 500 μl RIPA buffer (Catalogue # 89901, Pierce) and the protein concentration was determined with protein assay kit (Catalogue # 500-0113, Bio-Rad).

Chromatin-Immunoprecipitation (ChIP) assay

SNU-398 cells ($15x10^6$ cells) were treated with 20 μM peptides and Pep-1 carrier as described earlier. At 12 hr after treatment, the cells ($5x10^6$ cells for each antibody) were cross-linked with 3.7% formaldehyde for 10 min at room temperature and Glycine (125mM) was added to stop crosslinking, and then washed with ice-cold PBS containing proteinase inhibitor (Catalogue # P8340, Sigma). Chromatin was sonicated and immunoprecipitated using anti-acetyl Histone H3 antibody (Catalogue # 06-5990, Millipore) or rabbit IgG as a control, followed by quantitative real-time PCR. For quantitative real-time PCR, PTEN primer specific to the -1706 to -1571bp region of the promoter was used. The following primers were used in qPCR: 5′-GCAAACGAGCCGAGTTACC-3′

(forward-1), 5′-CCCCAAATCTGTGTCCTCA -3′ (reverse-1) for the PTEN promoter. Y axis represent relative fold enrichment obtained with anti-Acetyl Histone H3 antibody compared with IgG, which was used as a negative control. Similar experiments were done using HDAC2 (Catalogue # H2663, Sigma Co.) or SALL4 (Catalogue # sc101147, Santa Cruz) antibodies.

Microscopy

Fluorescent confocal microscopy were performed on cells cultured on Lab-Tek II chamber slides with cover (Catalogue # 154526, Nalge Nunc Inten.) after treated with 20 μM FITC-labeled peptides with or without Pep-1 carrier as described previously. Cells were washed 3 times with PBS (pH 7.4) and mounted in ProLong Gold antifade reagent with DAPI (Catalogue # P36931, Invitrogen). Slides were observed on Zeiss LSM510 Upright Confocal System.

HDAC assay

Assay for histone deacetylase activity was performed with a Fluorescent HDAC Assay Kit (Catalogue # 56200, Active Motif) with the following modifications: following the pull down conditions described under western blot section, proteins bound to the beads were eluted in buffer containing 20 mM HEPES (pH 7.5), 350 mM NaCl, 20% glycerol, 1% Igepal CA-630, 1 mM $MgCl_2$, 5 mM DTT, and Protease inhibitors (Catalogue # P8340, Sigma). The eluates were used as an HDAC enzyme source in an enzymatic reaction for 2 h at 37°C. HDAC activity was detected as fluorescent intensity as instructed by the manufacturer.

Cell Viability assay

CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS), (Catalogue # G5421, Promega) was performed according to the manufacturer's instructions. Briefly, $1x10^4$ cells were seeded in 100 µl culture medium in 96-well plates. After overnight culture, cells were treated with peptides or TSA as indicated in text. 72 h after treatment, 20 µl of 5 mg/ml MTS solution was added to each well. After 3.5 h incubation at 37°C, the medium was removed and the reaction was terminated with a solution of 4 mM HCl, 0.1% Nonidet P-40 (NP-40) in isopropanol in the dark on an orbital shaker for 15 min at room temperature. The absorbance was read at 590 nm with a reference filter of 620 nm in a Spectromax M3 (ABI) spectrophotometer. For PTEN inhibitor experiments, SF1670 (400nM, twice in 24 hours, ref [28]) was added to the cells prior to MTS assay.

Annexin V/ Propidium Iodide (PI) assay

Cells were harvested, washed, and stained with Annexin V and propidium iodide as recommended by the manufacturer (Becton Dickinson, Mansfield, MA). Briefly, phosphate-buffered saline (PBS)-washed cells were incubated with propidium iodide and fluorescein isothiocyanate–conjugated Annexin V antibodies for 15 minutes, washed, processed, and acquired by FACScalibur within 5 minutes of staining.

Lentiviral production

SALL4 shRNA construct (Puri-7412) and scrambled shRNA control vectors have been verified in our previous studies [12,25,33]. The SALL4 shRNA sequence is as follows 5'-GCCTTGAAACAAGCCAAGCTA-3'; Lentiviral

supernatants were obtained in 293T cell cultures by co-transfection of the shRNA plasmids with packaging plasmids containing VSV-G and pHR8.9. For lentiviral infection of primary human AML patient samples, $1x10^5$ cells were seeded in 12-well plates (200 μl per well) with the appropriate culture media. Polybrene (hexadimethrine bromide; Sigma-Aldrich, St Louis, MO) was added at a final concentration of 8 μg/ml. After adding 1ml of lentiviral particles (titer of each lentiviral particles was pre-adjusted to $1x10^6$ transducing unit/ml to achieve a multiplicity of infection of 10), spinoculation was performed at 1800 rpm for 90 minutes at 37°C. Then cells were brought back to 500 μl in volume using the appropriate fresh culture media and incubated at 37°C, 5% CO_2 until use for subsequent experiments.

Supplemental Table 1 AML sample information

	AML diagnosis	Cytogenetic information	Sex	Other information
Sample 1	M4/5	MLL-AF9	F	History of breast cancer
Sample 2	M4/5	MLL-AF9	F	history of breast cancer
Sample 3	M4/5	MLL-AF9	M	History of prostate cancer

Supplemental Table 2 Percentage of peptide uptake on various cells

Cell line	Without Pep-1	With Pep-1
HL-60	50%	>90%
THP-1	<5%	>90%
KBM5	<5%	>90%
SNU-398	<5%	>90%
SNU-387	<5%	>90%
AN3CA	<5%	>90%
Huh-7	<5%	>90%
Primary AML cells	<5%	>90%
MCF7	50%	>90%

Supplemental Table 3 Survival data on NSG recipient transplant after down-regulation of SALL4

Mouse ID	Survival days	Note
Control shRNA recipient 1	15	found dead
control shRNA recipient 2	17	found dead
control shRNA recipient 3	17	found dead, had 27.9% engraftment
Control shRNA recipient 4	33	found dead
Control shRNA recipient 5	35	found dead, had 35.3% engraftment
Control shRNA recipient 6	57	found dead, had 79% engraftment
Control shRNA recipient 7	134	sever ill, killed and had 93.7% engraftment
SALL4 shRNA recipient 1	50	found dead
SALL4 shRNA recipient 2	57	healthy-look, killed as a control for control shRNA recipient 6, less than 1% engraftment found
SALL4 shRNA recipient 3	57	healthy-look, killed as a control for control shRNA recipient 6, less than 1% engraftment found
SALL4 shRNA recipient 4	57	healthy-look, killed as a control for control shRNA recipient 6, less than 1% engraftment found
SALL4 shRNA recipient 5	109	found dead
SALL4 shRNA recipient 6	134	healthy-look, killed as a control for control shRNA recipient 7, engraftment at 0.373%

Supplemental Figure Legends

Supplemental Figure 1 The wild type peptide can bind to the protein complexes in SNU-398 nuclear extracts. Direct binding of labeled wild type or scrambled peptides was measured by fluorescent polarization.1µM of FITC-labeled wild type or scrambled peptides were added to nuclear extracts from $2x10^7$ SNU-398 cells along with increasing concentrations. The fluorescent polarization (y axis) is increased if the FITC-labeled peptide is attached to a larger complex.

Supplemental Figure 2 ChIP–qPCR showed increased H3 acetylation marker but decreased HDAC2 binding on PTEN promoter in THP1 cells upon wild type peptide treatment. THP1 cells treated with SALL4 wild type (wt), scrambled (scr), or mutant (mt) peptide were used for ChIP analysis. ChIP was performed for acetyl-H3 (acH3), HDAC2 or SALL4. Fragments specific for PTEN were amplified and detected using quantitative PCR. Results are expressed as percent input ± SD.

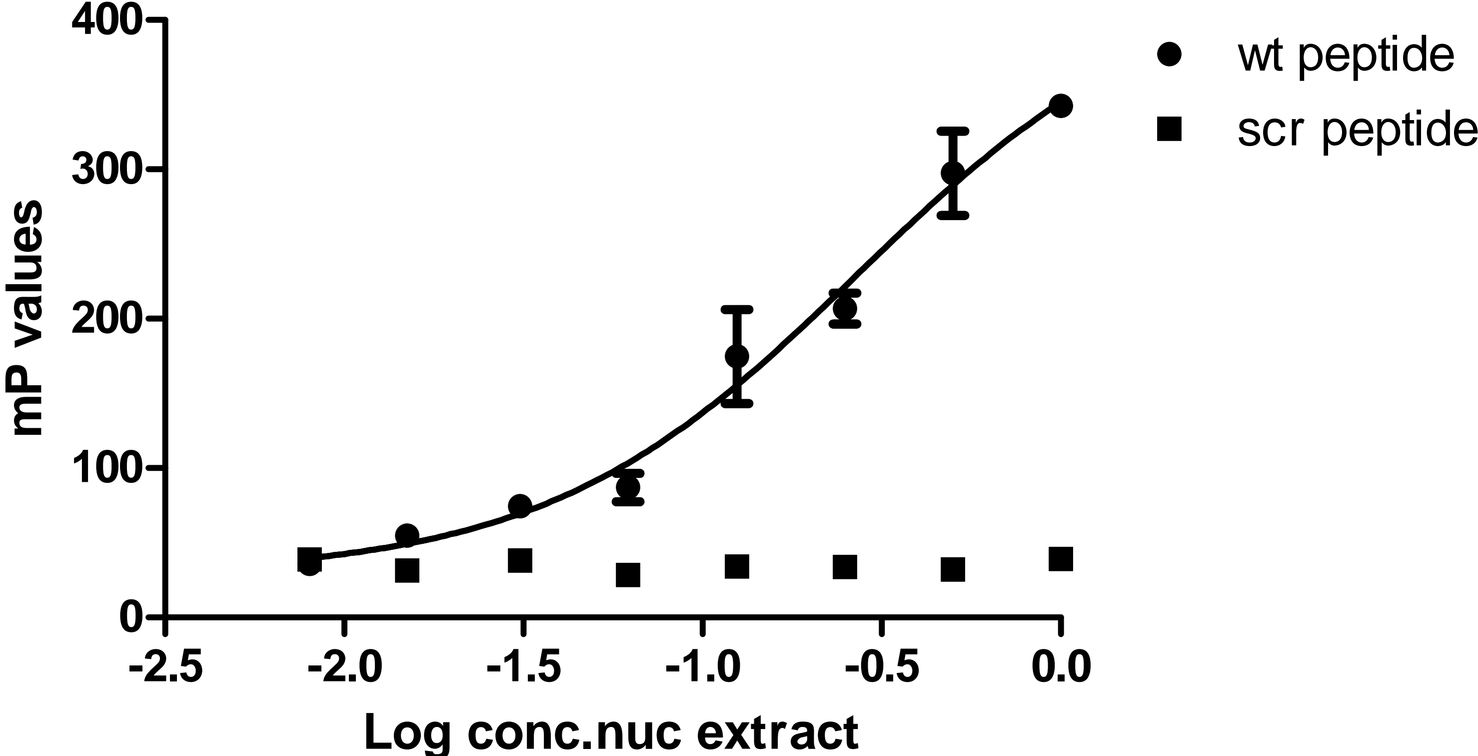
Supplemental Figure 3 No effect on endogenous SALL4 expression after peptide treatment. THP-1 cells were treated with scr or wt peptide similar as in Figure 3D. Western blot showed similar levels of SALL4 expression after scr or wt peptide treatment and α-tubulin was used as a loading control.

Supplemental Figure 4 Early time point engraftment assessment on human leukemic cells after down-regulation of SALL4 in NSG recipients. $1x10^4$ human AML cells were transduced by the methods as described in the methods and similar to that in Fig 4. The cells were cultured for 48 hours prior to transplantation via tail vein injection. The bone marrows of recipient mice were collected at 24 or 48 hours post transplantation. While at 24 hours post transplant, there was no difference in engraftment between the SALL4 shRNA treated

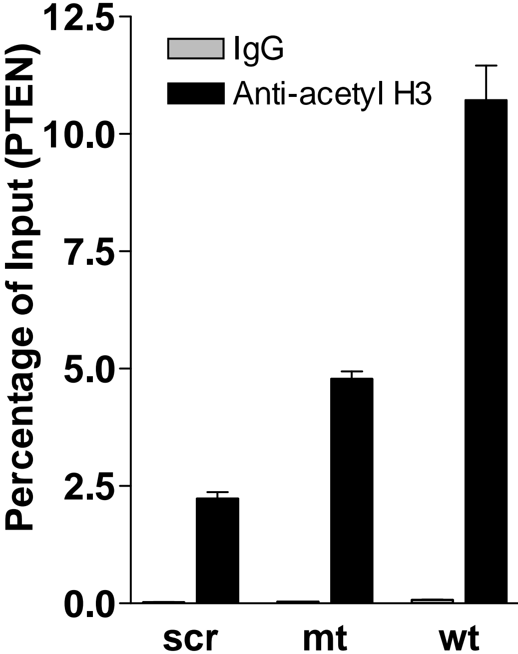
(SALL4 24 hr) and control (scr 24 hr) treated recipients, at 48 hours, SALL4 shRNA treated recipient group (SALL4 48 hr) showed decreased engraftment when compared with that of control treated mice (scr 48 hr). Percentage of human CD45+ cells engrafted in BM was determined by flow cytometry. Statistical summary is shown, N=4 mice per group, $P<0.05$, ANOVA with Tukey's multiple comparison test.

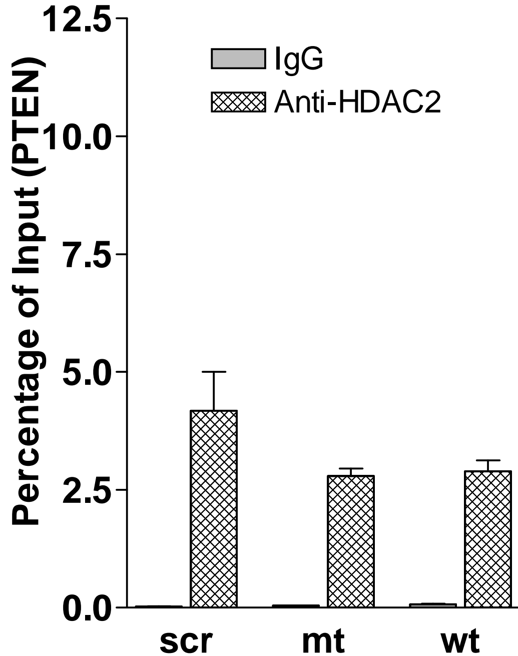
Supplemental Figure 5 Early time point engraftment assessment on human leukemic cells treated with peptide in NSG recipients. Flow cytometry analysis was used to show engraftments of human leukemic cells after peptide treatment in NSG recipients at various early transplantation time points. $1x10^4$ primary human leukemic cells were treated twice with peptides at a 24 h interval as indicated in Figure 5, followed by transplantation into sublethally irradiated NSG mice. Recipient bone marrows were collected at 24 hours, 48 hours or 2 weeks, respectively. SALL4 wild type peptide (wt) treatment significantly impaired human AML cell engraftments in NSG mice analyzed at 2 weeks post-transplantation. Percentage of human CD45+ cells engrafted in BM was determined by flow cytometry. Statistical summary is shown, N>=3 mice per group, $P<0.05$, ANOVA with Tukey's multiple comparison test.

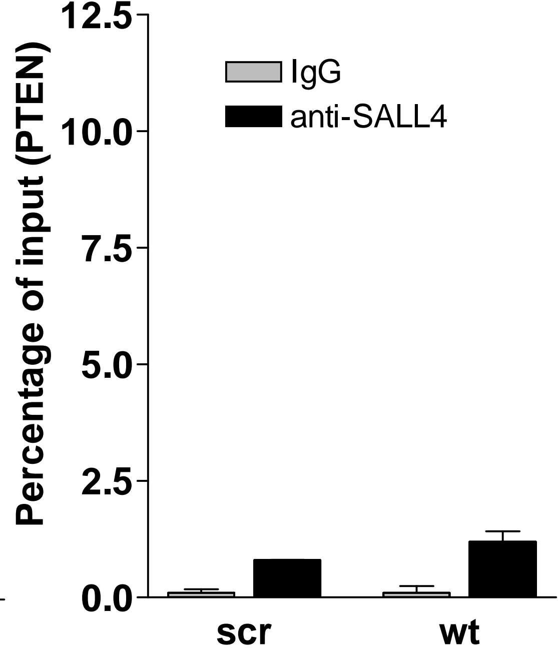
Supplemental Figure 1



Supplemental Figure 2







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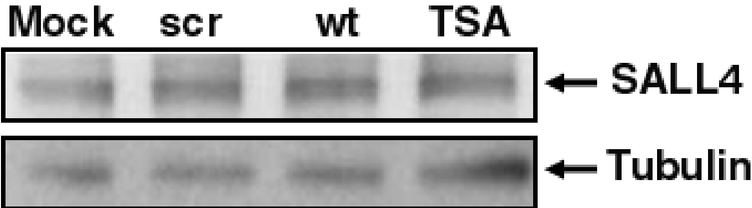
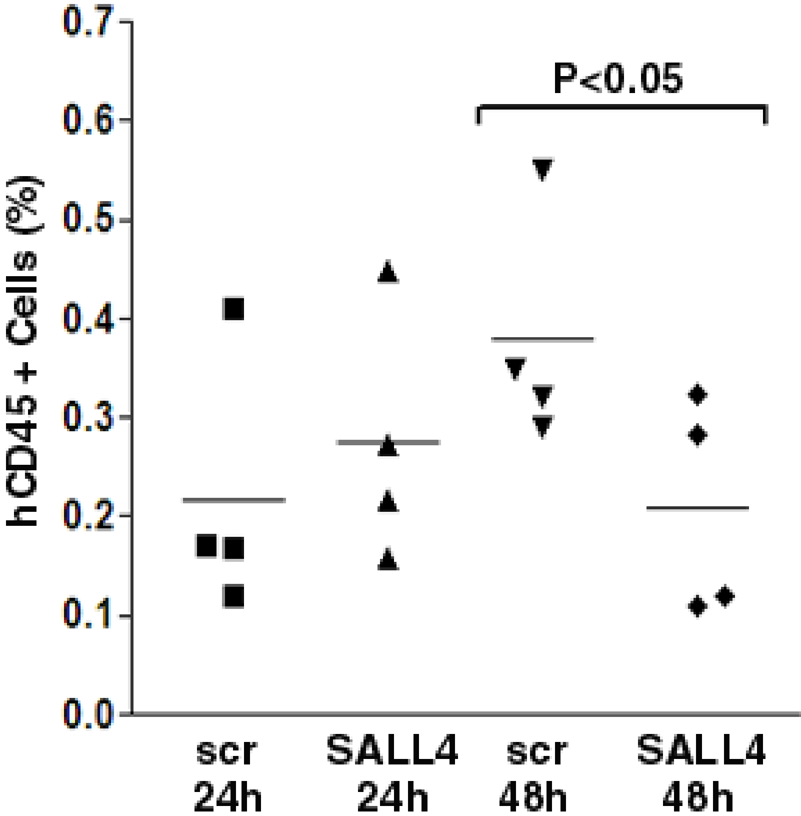


Supplemental Figure 4



Supplemental Figure 5