Supplementary Data

Materials and Methods:

Western immunoblot and Immunoprecipitation (IP) analyses:

Whole cell lyasate were prepared by suspending cell pellets in RIPA buffer for 20min (1% NP-40, 0.1% SDS, 150 mM NaCl, 50 mM Tris, pH 8.0) supplemented with 1X complete EDTA free protease inhibitor (Roche) 1X PhosStop (Roche). Lysates were separated by 4-20% SDS-PAGE and transferred to PVDF membrane (GE Healthcare, Piscataway, NJ). Membranes were blocked with 5% milk or BSA in 1X TBS with 0.1% Tween 20 for 1 hour at room temperature with shaking. Primary antibodies for ACTIN, GAPDH and FLT3 (Santa Cruz Biotechnology, Santa Cruz CA), P65 (Cell signaling), PRMT5 and Sp1 (Millipore), H4R3, H3R8, H3 and H4 (Abcam), were diluted 1:1000 or 1:2000 in 5% milk or BSA and incubated for 1-2hr at room temperature. Membranes were washed using 1X TBS-T, incubated with HRP-conjugated secondary antibodies diluted in 1X TBS-T with 5% milk or BSA, washed, and developed using SuperSignal West Dura Chemiluminescent Substrate (Thermo Scientific). Whole cell lysate (at least 500µg) was used to pull down protein complexes using Catch and Release v2.0 Reversible Immunoprecipitation System (Millipore) and ~4µg antibodies against PRMT5 and Sp1 while 1µg normal IgG was used as negative control (Millipore). Denatured pull down samples were subjected to regular western blotting with exception of using TrueBlot IP secondary antibody (Rockland) for immunodetection.

Flow cytometry and Fluorescence Activated Cell Sorting (FACS):

Cells ($1X10^6$) were washed and passed through a 40µm filter (BD), then resuspended in 200 µl binding buffer with or without 5 µl Annexin V (for apoptosis detection) or CD11b (for maturation detection) antibody (BD Biosciences, Billerica, MA). After 15 min incubation, cells were washed

with PBS, resuspended in 500 µL flow buffer and analyzed on a FACS Calibur cytometer (BD Biosciences). For apoptosis detection 5 µL propidium iodide (PI) (BD Biosciences) was added to celsl before running flow cytometry. Sorting for GFP-positive cells was carried out on BD FACS Aria instrument.

PRMT5 Expression in the TCGA AML Cohort:

PRMT5 expression was assessed in AML cases included in the TCGA AML cohort for which gene expression results are publically available (https://tcga-data.nci.nih.gov/docs/publications/laml_2012/). All level 3 AML version 2 RNA-seq data (i.e. TCGA RNA-seq data for which quantified gene expression values is made publicly available) were downloaded from the TCGA Data Portal (https://tcga-data.nci.nih.gov/docs/publications/laml_2012/). These patients were grouped according to common cytogenetic/genetic classifications. The upper-quantile normalized read counts produced by RSEM program (Deweylab, http://deweylab.biostat.wisc.edu/rsem/) were then used to compare the expression levels among these subgroups of patients.

Chromatin Immunoprecipitation (ChIP):

Crosslinked chromatin was prepared by incubating cells in 1% formaldehyde for 10min at room temperature and quenching with ice cold 1M Glycine. Cells were sonicated in 1% SDS lysis buffer and sheared chromatin was used to pull down protein/DNA complexes using antibodies against Sp1, PRMT5, HDAC2 (ChIP-grade Millipore), H4R3me2, H3R8me2 (Abcam), Anti-Acetyl Lysine (Cell Signaling) and normal IgG (Millipore) as negative control. Reverse crosslinked DNA was purified using QIAquick PCR Purification kit (Qiagen) and quantitative real-time PCR was carried out using SYBER green incorporation and primers designed for either FLT3 promoter region or miR-29b enhancer region. DNA signals were calculated relative to input DNA amount and in comparison to expression values of negative control IgG.

RNA isolation and real-time PCR:

Total RNA was extracted using TriZol reagent (Invitrogen) and subjected to Reverse Transcription using SuperScript III reagents (Invitrogen). Quantitative Real-Time PCR was performed on resulting cDNA using commercially available TaqMan Gene Expression Assay primers and probes, and the 7900HT Fast Real-Time PCR System (Applied Biosystems).

Luciferase reporter assay:

Promoter region of FLT3 (Sequence in supplementary data) was cloned into pGL4.11[*luc2P*] vector. THP-1 cells were transfected with empty vector or FLT3-promoter luciferase plasmid and *Renilla* in presence or absence of HLCL-61. Firefly luciferase and *Renilla* luciferase activity was measured using Dual-Luciferase Reporter Assay System (Promega).

Statistical analysis:

Biological experiments were analyzed with paired or unpaired t-tests using GraphPad Prism and Excel software. Mouse survival was analyzed using the Kaplan-Meier method.

RNA deep sequencing with Illumina Hiseq 2500:

Total RNA samples were isolated (TriZol, Invitrogen) from MV4-11 cells treated with specific shRNA against PRMT5 (shPRMT5) and a scrambled control (scr) using lentivirally stable transduction. Sequencing libraries were prepared with TruSeq RNA Sample Preparation Kit V2 (Illumina) according to the manufacturer's protocol with minor modifications. Briefly, RNA integrity (RIN) was assessed with the Agilent 2100 Bioanalyzer (RNA 6000 Nano LabChip kit, Agilent Technologies). 100 ng of total RNA from each sample with RIN>7 was used for polyadenylated RNA enrichment with oligo dT magnetic beads, and the poly(A) RNA was fragmented with divalent cations under elevated temperature. First-strand cDNA synthesis produced single-stranded cDNA from the fragmented RNA using reverse transcription with

Superscript II (Invitrogen), and followed by the second-strand cDNA synthesis with DNA polymerase I. The double-stranded cDNA underwent end repair, 3′ ends adenylation, and finally the bar-coded adapters (Illumina) were ligated to the cDNA fragments, and a 10 cycles of PCR was performed to produce the final sequencing library. Library templates were prepared for sequencing using cBot cluster generation system with HiSeq SR Cluster Kit V4 (Illumina). Sequencing run was performed in the single read mode of 51 cycles of read1 and 7 cycles of index read using HiSeq 2500 platform with HiSeq SBS Kit V4 (Illumina). Real-time analysis (RTA) 2.2.38 software was used to process the image analysis and base calling.

RNAseq alignment, differential gene expression analysis and DAVID functional annotation: RNAseq reads were aligned to human reference genome assembly 19 (hg19) using TopHat [1] v2.0.8b. Gene level expression of each RefSeq gene was counted by a customized R script developed at City of Hope Integrative Genomics Core. Only genes with RPKM (reads per kilobase of exon model per million mapped reads in exon) greater than 1 in at least one sample were used for differential expression analysis. Gene expression values were normalized with "TMM" method implemented in Bioconductor package "edgeR" [2], and differential expression analysis was also conducted using "edgeR". Genes were identified as being up-regulated in PRMT-5 knockout samples compared to control with 2-fold increase and p-value < 0.05, and as being down-regulated with 2-fold decrease and p-value < 0.05. Functional annotation of these up- and down-regulated genes were performed using DAVID Bioinformatics Resources 6.7 [2] to reveal the enriched biological processes.

1. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, et al. (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biology 14: R36.

2. Huang DW, Sherman BT, Lempicki RA (2008) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protocols 4: 44-57.