Extended Materials and Methods

Reproducibility and Double-Blinded acquisition of data.

In vitro and *in vivo* functional assays in this study were performed in a double-blinded manner to remove investigator bias whenever possible as described in more detail below. Analysis of MTF2 and H3K27me3 expression in diagnostic AML patient bone marrow samples was performed without knowledge of the clinical outcome. Likewise, the Kaplan-Meier analysis of our AML cohort was also performed without knowledge of the molecular analysis of patient samples. Furthermore, to ensure reproducibility, the *in vitro* functional assays were performed by 2 different individuals (HBM, HJ). Data analysis was performed in an unbiased manner by using preset algorithms to analyze data with the FlowJo Dean Fox Jett, Amnis IDEAS and ImageJ OpenComet software.

Isolation of UCB mononuclear cells

Hespan (B.Braun Medical Inc) was used for mononuclear cell isolation from UCB. Cord blood was mixed with Hespan at a final ration of 5:1 and centrifuged at 50 RCF for 10 minutes at room temperature. Post-centrifugation the serum supernatant containing the mononuclear cells was collected and centrifuged at 400 RCF for 10 minutes. Red blood cells were then lysed with Red Blood Cell lysis buffer (1g/L $KHCO_3$, 8.2g/L NH_4Cl, 0.37g/L EDTA) to eliminate residual red blood cells before being frozen down in 10% DMSO using an ethanol-based control rate freezer (Kinetic), allowing high viability after thawing.

Enrichment of stem and progenitor populations from UCB

To reduce heterogeneity for *in vitro* experiments and next generation sequencing experiments, an effort was made to enrich for human stem and progenitor cells (HSPCs).

Lineage-negative (Lin^-) UCB or BM cells were obtained using the EasySep™ Human Progenitor Cell Enrichment Kit with Platelet Depletion to enrich for stem and progenitor cells (HSPCs). $CD34^+$ positive selection was performed on Lin^- cells, using the EasySep™ Human $CD34^+$ selection kit (Stem Cell Technologies).

Lentiviral-mediated shRNA knockdown of primary progenitors from UCB or AML patient BM

The following shRNAs encoded by pGIPZ GFP-tagged lentivirus vectors (ThermoScientific) were used in the knockdown experiments:

Target shRNA	**shRNA Sequence**
Scrambled shRNA	GTTACACGATATGTTATC
MTF2 shRNA Clone 3	TAATGTATGTCATAAGC
MTF2 shRNA Clone 7	TTGGCTTTATGTCCATCC
EZH2 shRNA	TGTGCTATCACACAAGG
EZH1 shRNA	TTCTCTTTCTTGTTACTG
EED shRNA	TGCATTTCTTTGACTTCC
SUZ12 shRNA	AGCATTAAGAGCATAAC

Lentivirus production and transduction of primary cells were as described in the Methods section. During the lentivirus transduction of UCB Lin^-CD34^+, cells were maintained in IMDM media containing BIT9500 (Stem Cell Technologies), 1% PenStrep (ThermoFisher), SCF (100ng/mL), TPO (50ng/mL), FLT3 (100ng/mL), 1% Glutamax (Invitrogen), and LDL (1μg/ml) (Calbiochem). Growth factors were purchased from Peprotech.

To maintain consistency with the UCB-derived Lin^-CD34^+ cells, Lin^-CD34^+ patient derived xenograft (PDX)-expanded primary AML BM cells were maintained in IMDM media containing SCF (100ng/mL), FLT3 (50ng/mL), IL-3 (20ng/mL), G-CSF

(20ng/mL), 10^{-4} M β-mercaptoethanol and 15% BIT and cultured at a density of 200,000 cells during the course of the transduction experiments (1).

Lentiviral-mediated rescue of MTF2 expression within patient leukemic cells

Sorted $CD34^+$ (Clone 4H11) $CD38^-$ (HIT2) primary AML BM cells were maintained in IMDM media containing SCF (100ng/mL), FLT3 (50ng/mL), IL-3 (20ng/mL), G-CSF (20ng/mL), 10^{-4}M β-mercaptoethanol and 15% BIT and cultured at a density of 200,000 cells during the course of the transduction experiments. The cells were cultured in a 96-well plate (Falcon, 353072). Growth factors were purchased from Peprotech.

In vitro EZH1 and EZH2 inhibition

Lin^-CD34^+ UCB cells and Lin^-CD34^+ primary AML BM cells were maintained in IMDM media containing SCF (100ng/mL), FLT3 (50ng/mL), IL-3 (20ng/mL), G-CSF (20ng/mL), 10^{-4}M β-mercaptoethanol and 15% BIT. Growth factors were purchased from Peprotech. Cells were treated with UNC1999 (2uM) which inhibits EZH1 and EZH2 or EPZ005687 (2uM) which inhibits EZH2 alone for 3 days.

During the course of drug treatment, the cells were maintained using the fed-batch system at a density of 200,000 cells in 100uL within a 96-well plate (Falcon, 353072) at 37°C, 5%CO_2. Both the inhibitors were purchased through Cedarlane.

Fed Batch System

Cells were cultured using a fed batch system as described by Csaszar et al. (2). On day 0, UCB Lin^-CD34^+ cells or Lin^-CD34^+ leukemic cells were plated at a density of 200,000 cells in 100uL of in IMDM media containing BIT9500, 1% PenStrep, SCF (100ng/mL),

TPO (50ng/mL), FLT3 (100ng/mL), 1% Glutamax, and LDL (1μg/ml), within a 96-well plate. On day 1, 50 uL of fresh media was added to each well. If the cells needed to be transduced (shRNA or expression vector) or treated with inhibitors (such as UNC1999 or EPZ005687), the fresh media contained the virus or the inhibitors. Each well on day 1 contained a total volume of 150uL. On day 2, 50 uL fresh media was added to each well. The wells were than split into 2 wells (each containing 100uL). This fed batch process of half media exchange every day and splitting the cells every 48 hours was followed during the virus transduction and inhibitor treatment (UNC1999 or EPZ005687) experiments mentioned above. The cells were maintained in a humidified incubator set at 37°C, 5%CO_2.

Screening H3K27me3 levels within Immunophenotypic AML subpopulations

Mononuclear cells were isolated from 32 AML BM aspirates and 7 healthy BM aspirates as described above. The cells were stained with CD34 (Clone 4H11) and CD38 (HIT2) antibodies and the various immunophenotypic subpopulations ($CD34^+CD38^-$, $CD34^+CD38^+$, $CD34^-$) were sorted using Beckman Coulter Astrios sorter. The sorted cell populations were subjected to intracellular labeling (see next section) with the H3K27me3 antibody (Cell Signaling, clone c36B11) and appropriate secondary antibody and ran on the LSRFortessa™ Cell Analyzer (BD Biosciences). The H3K27me3 levels within different immunophenotypic subpopulations sorted from each AML BM aspirate were compared to the average of H3K27me3 levels found within the respective subpopulations isolated from 7 healthy BM aspirates. Data analysis was performed using FlowJo v10.2 to compare mean fluorescent intensity values.

Intracellular staining

Cells were sorted using cell surface markers, then fixed in 4% PFA, permeabilized with 0.3% Triton and incubated with antibodies against MTF2 (Genway, clone M96), EZH2 (Millipore, clone AC22), SUZ12 (Millipore, clone 2AO9), H3K27me3 (Cell Signaling, clone c36B11), EZH1 (ThermoFisher, PA5-40850), PCNA (Santa Cruz, clone C10), p53 (Cell Signaling, clone 1C12), or MDM2 (Santa Cruz, clone C18) and appropriate secondary antibodies. Protein expression was determined by flow cytometry compared with an isotype only control and ran on the LSRFortessa™ Cell Analyzer (BD Biosciences). Data analysis was performed using FlowJo v10.2 to compare mean fluorescent intensity values.

BrdU Assay

Cells were cultured over 48 hours until collected for their respective time points. 24 hours prior to collection, they were pulsed with 1μM of BrdU, fixed in ice-cold ethanol, treated with 1.5M HCl for 30 min and then stained overnight with an antibody against BrdU (Millipore) and a fluorescence-conjugated secondary antibody the following day for one hour before flow cytometric analysis. Cells were stained with Hoechst (Life Tech) to assess cell cycle state.

Imaging Flow Cytometry – Amnis

For cell cycle analysis, cells were fixed and stained with DRAQ5 (BD Biosciences). To assess p53 expression in MTF2 rescue experiments, cells were incubated with antibodies against p53, MDM2 or H3K27me3 as described above and with DRAQ5 for nucleic acid detection. Cells were analyzed using the ImageStream imaging flow cytometer (Amnis).

The nuclear contents of each protein were determined using the preset wizard tool "Nuclear Localization", within the Amnis IDEAS analysis software.

Apoptosis assay

Apoptosis was assessed by the dual staining of AnnexinV and 7-AAD (eBiosciences). Flow cytometric analyses was done using BD LSRFortessa™ and the analysis of the treated samples were performed consistently, using the same gating strategy throughout. Viable cells were categorized as being AnnexinV and 7-AAD negative, late apoptotic/dead cells were categorized as being AnnexinV and 7-AAD positive.

Comet assay

Alkaline comet assays were performed using GelBond Films and buffers were prepared as follows (3). In preparation for the comet assay, the Lysis Solution (2.5 M NaCl, 100 mM EDTA, 10 mM Trizma base, 1% sodium lauryl sarcosinate, 1% Triton X-100 added fresh, pH 10) was chilled on ice for at least 20 minutes prior to use. 1% low melting-point agarose (LMA) was made and melted by submerging in a beaker of boiling water for 5 minutes with the cap loosened. The 1% LMA was maintained in a liquid state by transferring to a 37°C water bath. The LMA was allowed to cool at 37°C for at least 20 minutes prior to use. Cell samples, at a concentration of $1x10^5$ cells/mL, were combined with LMA at a ratio of 1:10 cells:LMA and immediately pipetted onto labeled Gelbond film strip (agarose gel support medium by Lonza) in 75uL. Cells were applied to the hydrophilic side of the film to ensure that the sample spread evenly into a circle of approximately 25mm in diameter. Films were placed flat at 4°C in the dark for 10 minutes or until the gel solidified. Films were then immersed in pre-chilled Lysis Solution and incubated at 4°C for 45 minutes. After incubation, the lysis solution was removed and the slides were immersed in freshly prepared Alkaline Unwinding Solution pH13 (200 mM NaOH, 1 mM EDTA) for 45 minutes in the dark. Films were removed

from the alkaline solution and transferred to a horizontal electrophoresis apparatus where they were placed equidistant from each electrode in alkaline electrophoresis solution (300 mM NaOH, 1 mM EDTA, pH13). Temperature fluctuations were minimized in the non-buffered system by running the electrophoresis at 4°C in walk-in refrigerator. The voltage was set to 30V for 30 minutes at constant amperage of 300mA. Following electrophoresis, the slides were rinsed several times in ddH_2O then immersed in 70% ethanol for 5 minutes for fixation. Films were dried in an air-tight container containing desiccant overnight at room temperature. The following day, films were loaded onto microscope slides with the hydrophobic side facing upward. They were stained by submerging in SYBR Gold stain at 1:10000 in TE buffer pH 7.5 for 30 minutes. Slides were then removed, washed with H_2O to remove excess staining solution and coverslips were mounted on each slide (3, 4). Slides were imaged using Zeiss Axio2 Imaging inverted microscope equipped with a 5× Plan-NEOFLUAR 0.3NA objective and an AxioCam MRm camera through a FITC-compatible filter. At least 10 random fields containing a minimum of 20 non-overlapping comets in each group total were photographed. Blinded imaging acquisition and analysis was performed using ImageJ software (NIH) and the OpenComet comet assay plugin calculating Olive moment (arbitrary units) on the basis of comet head and tail sizes (measured in pixels) and their integral intensity. The magnitude of these parameters depends on time of electrophoresis, staining brightness and image magnification, which were constant within each assay and between experiments. Comet assays were independently repeated using HSPCs isolated from 3 different UCB samples. Statistical significance was determined by two-way ANOVA using GraphPad Prism software version 6.

H3K27me3 antibody specificity validation

Internal standard calibrated chromatin immunoprecipitation (ICeChIP) was performed as described by Grzybowski et. al., (5) with following changes: 0.6 μg of each antibody

(CST c36B11 AM61017, AM61435, AM61015) was immobilized on 5 μl of Protein G Dynabeads (Invitrogen) and presented to 0.8 μg ($1A_{260}$=50ng/μl) of chromatin isolated from mESC E14 cell line spiked with semi-synthetic nucleosome standards; for 304M3B we used 2 μg of the Fab immobilized on 40 μl of Streptavidin M-280 Dynabeads (Invitrogen) and presented to 5 μg of spiked chromatin. Streptavidin beads after antibody immobilization were washed two additional times with 200 μl ChIP buffer 1 supplemented with 5 μM D-biotin and 100 μg/ml BSA (NEB) with 10 minute, 4°C incubations, on a rotator, to block free streptavidin. Briefly, Dynabeads were washed twice with 200 μl of ChIP buffer 1 (25 mM Tris pH 7.5, 5 mM $MgCl_2$, 100 mM KCl, 10% (v/v) glycerol, 0.1% (v/v) NP-40 substitute), antibodies were diluted in 100 μl of ChIP buffer 1 with 100 μg/ml BSA (NEB) and exposed to Dynabeads for at least an hour in 4°C, on a rotator. Beads-antibody conjugates were washed twice with 200 μl ChIP buffer 1 with 100 μg/ml BSA (NEB). Spiked mononucleosomal chromatin, diluted to 20 ng/μl with ChIP buffer 1 with 100 μg/ml BSA (NEB), was incubated with antibody-beads conjugate for 10 minutes at 4°C, on a rotator. Immediately after, beads were washed twice with 200 μl ChIP buffer 2 (25 mM Tris pH 7.5, 5 mM $MgCl_2$, 300 mM KCl, 10% (v/v) glycerol, 0.1% (v/v) NP-40 substitute, 100 μg/ml BSA(NEB)), and once with 200 μl ChIP buffer 3 (10 mM Tris pH 7.5, 250 mM LiCl, 1mM EDTA, 0.5% Na•Deoxycholate, 0.5%(v/v) NP-40 substitute, 100 μg/ml BSA(NEB)) for 10 minutes per wash at 4°C, followed by tube changes between each wash. Finally, beads were briefly washed with 200 μl ChIP buffer 1 and 200 μl TE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA). DNA was eluted by incubating beads 5 minutes at 55°C with 50 μl ChIP elution buffer (50 mM Tris pH 7.5, 1 mM EDTA, 1% w/v SDS). Proteins were digested by 2 hours, 55°C incubation with 200 ng/μl Proteinase K (NEB). DNA was isolated using 3 volumes of Serapure SPRI magnetic beads (1mg/ml of 1μm, hydrophobic, carboxylated, Sera-Mag SpeedBeads (GE 65152105050250), 20% PEG-8000, 2.5 M NaCl, 10 mM Tris pH 7.5, 1 mM EDTA, 0.05% Tween-20). DNA from 300 ng of

chromatin input was isolated with the same procedure. Specificity of antibodies was evaluated by qPCR (Biorad CFX384) using TaqMan Gene Expression Master Mix (Applied Biosystems). Specificity was calculated using ΔΔCt method.

RNA-seq bioinformatic analysis pipeline

Replicate data was analyzed using TopHat v1.4.1 and Cuffdiff v1.3.0 (6) to map reads to a reference human genome assembly (hg19) and determine expression differences against the Ensembl release 67 gene model. For each sample, the average ERCC spike-in reads were used to calculate the normalization factor, while track hubs were normalized by read depth. Significant fold changes were determined using Benjamini-Hochberg corrected *p* value of 0.05. Raw RNA-seq data is available in GEO (GSE98343). Data was analyzed using DAVID bioinformatics tool for functional annotation (7, 8) and Cytoscape with Enrichment Map plugin for visualization (9, 10). RNA-seq targets were validated by RT-qPCR after RNA was converted to cDNA using Superscript II (LifeTech). The qPCR experiments were performed on LightCycler 480 (Roche).

Principal component analysis (PCA)

A BED file of non-overlapping 20kb windows was generated to cover the human (hg19) genome using the BEDTools 'makewindow' command (11). The BEDTools 'multicov' command was used to count the number of reads overlapping each window (calculated separately for H3 and H3K27me3 ChIP-seq data), and the data were loaded into DESeq2 (12). For each sample, the ratio of *Drosophila* spike-in reads to the number of spike-in reads in the 'Basal-1' sample was used in lieu of '*estimateSizeFactors'*, and the DESeq2 '*estimateDispersions'* and '*nbinomWaldTest'* functions were applied to the data set. The normalized count matrix was then transformed using the '*rlog*' regularized log transformation, and PCA was performed on the rlog-transformed normalized count

matrix using the DESeq2 '*plotPCA*' function on the most variable 5000 20kb windows (*ntop=5000* parameter for *plotPCA*).

Differential H3K27me3 coverage

Regions of differential H3K27me3 coverage were detected in the ChIP-seq data using diffReps (13) to compare coverage in refractory MTF2 patient $CD34^+CD38^-$ cells against coverage in induction therapy responsive patient $CD34^+CD38^-$ BM cells with normal (basal) MTF2 levels, and in MTF2 deficient $CD34^+CD38^-$ cells (transduced with MTF2 shRNA SH3 or SH7) against healthy $CD34^+CD38^-$ BM cells. For each comparison, H3K27me3 ChIP-seq reads were first filtered to remove reads mapping to ENCODE ChIP-seq blacklist regions, and diffReps was run using normalization factors calculated as described above for PCA analysis. Association between regions of reduced H3K27me3 and genes was taken directly from the diffReps output files.

Hierarchical clustering

The matrix of rlog-transformed count values was used to generate a matrix of pairwise Euclidian distances between samples. These distances were hierarchically clustered using complete linkage clustering, and a heatmap plotted to illustrate the distance relationships between samples.

Genome coverage calculations

BEDTools was used to convert BAM files of mapped reads into BED files, extend reads to 200bp length, and to calculate coverage depth across the human genome. The coverage depth was scaled by the ratio of *Drosophila* spike-in reads in the 'Basal-1' sample to the number of spike-in reads in the sample in question (*i.e.* the inverse of the size factor estimates used for DESeq2 analysis), and converted to bigWig format using the UCSC 'bedGraphToBigWig' tool (14).

Validation of ChIP-seq targets was performed using both the formaldehyde cross-linked (X-ChIP) and Native-ChIP strategies followed by qPCR. All qPCR analysis was completed on a Roche Light Cycler 480 using Sybr Green MasterMix (Roche) and 0.1mM primers. Primer sequences are listed in Extended Data Table 6.

ChIP-seq validation by N-ChIP-qPCR

Native chromatin IP was performed as described previously by Brand et al. (15). Briefly, 250,000 GFP^+ scramble or MTF2 (SH3 or SH7) knockdown $CD34^+CD38^-$ cells were used per biological replicate and the experiment was performed using 3 biological replicates. Buffer volumes were adjusted according to the low cell number. For each chromatin IP experiment, 2.5ug of MNase-digested and hydroxyapatite-purified chromatin was used with 1ul H3K27me3 antibody (c36B11, Cell Signaling Technology) or 1ul H3 antibody (Abcam, ab1791). All qPCR analysis was completed on a Roche Light Cycler 480 using Sybr Green MasterMix (Roche) and 0.1mM primers. Primer sequences are listed in Extended Data Table 6.

Transcription Factor ChIP-qPCR

UCB Lin^-CD34^+ cells were transduced with different knockdown lentivirus (MTF2 shRNA, EZH2 shRNA, EED shRNA, SUZ12 shRNA) as discussed above while maintaining the cells in the fed batch culture. 72 hours post-transduction, lentivirus transduced GFP^+ Lin^-CD34^+ cells were sorted using BDAstrios. 500,000 GFP^+ sorted Lin^-CD34^+ cells were crosslinked with 1% formaldehyde and ChIP was performed (as described above) using the following antibodies EZH2 (Millipore, clone AC22), EZH1 (ThermoFisher, PA5-40850) and H3K27me3 (Cell Signaling, clone c36B11).

Methyl-ChIP-qPCR

Methyl ChIP-qPCR analyses were performed using the EpiMark Methylated DNA Enrichment Kit (New England Biosciences), according to manufacturer's instructions. Briefly, DNA was isolated from Lin$^-$CD34$^+$ cells isolated from the BM aspirates of healthy individuals and patients with AML. DNA was fragmented and combined with methyl-CpG-binding domain protein 2 (MBD2) bound to magnetic beads to capture methylated DNA. Methylated CpG DNA was eluted from beads. Enriched DNA was used for RT-qPCR using primers listed in Extended Data Table 6.

Isolation of mononuclear cells from bone marrow aspirates

Ficoll (GE Healthcare Life Sciences) was used for mononuclear cell isolation from bone marrow aspirates. Density centrifugation to isolate mononuclear cells using Ficoll was done at 400 RCF for 20 minutes. All density centrifugations were done at room temperature, without brakes. Red blood cells were lysed and frozen down in 10% DMSO using an ethanol-based control rate freezer (Kinetic), allowing high viability after thawing.

AML patient-derived xenograft (PDX) mouse model

NOD-*scid* IL2Rγ^{null} (NSG) (Jackson Laboratories) were housed in sterile ventilated cages of up to 5 mice per cage. Mice were kept on a 12:12 light:dark cycle and given irradiated chow and autoclaved acidified water ad libitum. Animal protocols were approved by the University of Ottawa Animal Care Committee, in accordance with the Canadian Council on Animal Care Standards and the Province of Ontario's Animals for Research Act. Anonymized, coded primary diagnostic AML BM samples were obtained from consented patients as described above. Researchers were blinded to the clinical diagnosis and outcome of the AML patients until the MTF2 and H3K27me3 analysis was complete. Frozen diagnostic AML patient bone marrow samples, were thawed viable and the AML

patient BM cells were expanded in NSG mice to obtain large numbers of PDX cells for the animal studies. Briefly, NSG mice were sub-lethally irradiated with 300 Rads (Gammacell 3000) and transplanted via tail vein with 1 million BM cells. After 6-8 weeks, 40-50 million BM cells from the tibiae and femurs of the NSG mice were harvested and frozen at a density of 20 million per mL in 10% DMSO using a controlled rate freezer (Kinetic) and stored at -150°C for future studies. The marrows consisted of ~ 50% human $CD45^+$ cells.

In vivo Treatment

Female NSG mice (>7 week old) were sub-lethally irradiated with 300 Rads and transplanted via tail vein with 1 million bulk PDX cells; n=8 mice were transplanted per AML PDX sample. (Note that these cells were not manipulated in any way prior to their injection into the irradiated NSG mice.) Pilot experiments were done using PDX expanded cells to determine the time required to achieve >20% $CD45^+CD33^+$ cell engraftment in the peripheral blood for each patient sample that turned out to be between 50-57 days. The peripheral blood of the transplanted mice was collected from the saphenous vein 3-4 weeks to verify engraftment of human $CD45^+CD33^+$ cells, followed by a collection approximately 4 weeks later to ensure >20% $CD45^+CD33^+$ cells in the peripheral blood before treatment.

Upon the presence of >20% $CD45^+CD33^+$ cells in the peripheral blood (~50-57 days post transplantation), mice were randomized into 4 treatment groups (n=6 PDX samples (4 from refractory AML and 2 from basal AML); n=2 mice per group; n=8 mice total per group) and treated with i) DMSO vehicle control, ii) nutlin3A (12mg/kg), iii) induction therapy or iv) combination therapy (see below for details). To conceal the identity of the treatment, syringes containing the vehicle control or drugs were prepared and coded by

one person, while another person administered the treatment intravenously via tail vein. Coded treatments were assigned to the corresponding mouse.

For mice receiving induction or combination therapy, mice were treated for the first three days with both Cytarabine (50 mg/kg) and Daunorubicin (1.5mg/kg), while during the last 2 days the mice were treated with Cytarabine (50 mg/kg) alone. Mice belonging to the combination therapy cohort, were given the 5+3 treatment regimen in combination with Nutlin3A (12mg/kg). Nutlin3a was given for the entire duration of the 5 days. Weights were taken daily during treatment by the animal care staff, who were blinded to assigned treatment cohort, and doses were recalculated by the researcher preparing the syringes to ensure that the mice received a consistent dose.

Post-treatment, the weights of the mice were monitored every 5 days. Humane moribund endpoint was determined based on >20% loss in body weight, loss of mobility, loss of appetite and hunched posture. Otherwise if mice survived, endpoint was at 16 weeks post-treatment, when the experiment was terminated. Upon reaching endpoint, the BM of the mice was harvested and human cell engraftment was analyzed by flow cytometry (described below).

Lineage determination of PDX in vivo samples

To analyze PDX cells post-treatment, cells were harvested from the peripheral blood or BM of NSG mice. To assess human cell engraftment in the mice, red blood cells were lysed and mononuclear cells were incubated with antibodies directed against human specific lineage markers CD34 (Clone 4H11), CD38 (HIT2), CD45 (HI130), CD33 (P67.6), CD19 (HIB19), CD15 (HI98), CD3 (OKT30), CD4 (OKT4), CD8 (OKT8), CD14 (61D3) (eBioscience). Cells were then analyzed by flow cytometry using the BD LSR Fortessa II.

Immunohistochemistry

Blinded analysis of BM cytospins of PDX mice was performed using a total of 500,000 sorted human $CD45^+$ cells per slide at end point. The slides were stained with Wright-Giemsa stain for 5 minutes and de-stained in water (PH 7.2) for 2 minutes. This process was repeated twice for each slide and photomicrographs were obtained using a Zeiss Inverted LSM510 microscope. The pictures were taken at a magnification of 40X.

Cell viability assays

Lin^-CD34^+ cells isolated from either UCB or primary AML patient BM aspirates were transduced as above, sorted for GFP^+ and cultured in 96-well plates at 100,000 cells/100uL/well in duplicate. Daunorubicin, Cytarabine, Nutlin3a and MI773 were dissolved in DMSO. Cytarabine and Daunorubicin were added at concentrations of 1μM and 0.5μM, respectively. After 1 hour of incubation with Daunorubicin, cells were collected and transferred into fresh media to remove Daunorubicin from the media. The MDM2 pathway inhibitors, Nutlin3a and MI773 were added at concentrations of 1 μM. The apoptosis assay was performed over a duration of 48 hours and to maintain appropriate drug concentrations, no media exchange was performed during this period. Apoptosis and cell death was measured using AnnexinV and 7AAD at 24hrs and 48hrs. The percentages of dividing cells, were measured by analyzing the percentage of PCNA positive cells.

TCGA AML cohort analysis

The clinical data (including mutations data) for this cohort was obtained through cBioPortal, as well as the original publication (16). The gene expression data was obtained through the Gene Expression Omnibus (GSE68833). From the original 200 patients, only treated patients with available gene expression and cytogenetics data

(n=165) were used for the Kaplan Meier curves, the clinical characteristics table and the multivariate analysis (see Supplemental Tables S4, S5 and S9-11). In addition, the multivariate analyses were performed again using only patients bearing no p53 mutations (n=153). Since this cohort contains no healthy controls, it was impossible to determine a normal expression range for any marker. Therefore, for each marker, the fraction above the median was compared to the one below.

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