Compound Screen

Freshly isolated cbCD34+ cells or cbCD34+ cells transduced with MPC-[nHA-UBTF-TD54], MPC-[nHA-UBTF-TD84], MPC-[RUNX1::RUNX1T1] (transduced cells were at day 40-60 post sorting) were plated at 600cells/30uL/well in 384-well plates using the Multidrop Matrix Wellmate Cell Dispenser (cat# 11002, Thermo Scientific and grown overnight in an incubator (37°C, 5% CO2). Compounds were then added (32 nL at increasing concentrations) using a V&P 10SS pin-tool on a Beckman BioMek FX. Cells were then incubated for 3 days at (37°C, 5% CO2). Plates were then rested at room temperature for 20 min and then added with 25µL/well of Cell TiterGlo (cat# G9242, Promega) with a Multidrop Matrix WellMate and incubated for 10 minutes. Luminescence was then evaluated using a Perkin Elmer Envision. Cytotoxicity data were imported and analyzed using Genedata Screener Analyzer software with the median DMSO signal set as the neutral control and the median signal from 2.75 µM Bortezomib set as the inhibitor control with sixteen replicate wells of each condition per plate. All dose-response curves were then fit using the Genedata Smartfit algorithm with DMSO set to 0 and 2.75 µM Bortezomib set to -100. Data were then exported from Genedata Analyzer, and all curve comparisons across cell lines were performed using the Genedata Screener Hit Profiler software.

Cytopsins of CD34+ cells

For cytospins, 100,000 cells were washed with 1X PBS and spun onto Superfrost Plus Microscope slides (12-660-16, Fisher Scientific) at 800 rpm for 5 min and then subjected to standard Wright-Geimsa staining.

Real-time Quantitative PCR

RNA was isolated using the NucleoSpin RNA purification kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. The RNA concentration and quality were assessed with a NanoDrop (ThermoFisher Scientific). To generate cDNA, 500 ng of RNA underwent reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific) following the manufacturer's protocol. Quantitative real-time polymerase chain reactions (Q-RT-PCR) were conducted in triplicate with 1 µL cDNA using SYBR Green gene expression master mix on a CFX384 Real-Time PCR detection system (Bio-Rad Laboratories, California), 30 seconds at 95°C followed by 35 cycles at 95°C for 15 seconds and 60°C for 30 seconds. Fold change relative to the control condition was calculated by the comparative ΔΔ cycle threshold method using TBP as the housekeeping gene for normalization. Oligo sequences are listed in Supplemental Table 3.

Immunoblotting

Whole-cell protein lysates from 250,000 cells were fractionated by SDS-PAGE on a 10% Protein gel (Bio-Rad, #4561033) and transferred onto nitrocellulose membranes (0.2 µmol/L, Bio-Rad, 1620252). Membranes were incubated overnight at 4°C with rabbit anti-HA-tag (mAb #3724, Cell Signaling Technology, clone: C29F41:1,000 dilution) or Mouse anti-β-actin (mAb #3700, Cell Signaling Technology, clone: 8H10D10, 1:2,000 dilution), or anti-GAPDH (mAb #97166, clone D4C6R, 1:2000). IRDye 800CW Donkey anti-Rabbit IgG (LI-COR Biosciences, #926–32213, 1:15,000) and IRDye 680RD Goat anti-Mouse IgG (LI-COR Biosciences, #926–68070, 1:15,000 dilution) were used as secondary antibody. Imaging was performed on the Odyssey CLx Imaging System (LI-COR Biosciences, 9140).

Histology

For histology, tissue sections from spleens were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were cut at 4 µm then mounted onto glass slides, followed by hematoxylin and eosin (H&E) staining or immunohistochemistry (IHC). For IHC, sections were run on Ventana Discovery ULTRA automated stainer (Ventana Medical Systems, Inc. Tucson, AZ) with cell conditioning media 2, Ventana Medical Systems, Incantigen retrieval and detection by DISCOVERY OmniMap anti-rabbit HRP (760-4311), DISCOVERY ChromoMap DAB kit (760-159); Counterstain Hematoxylin II (Roche, Indianapolis, IN, 790-2208) and Blueing reagent (Roche, 760-2037). The hNuMA1 (Lifespan Biosciences, LS-B11047) primary antibody was used at a dilution of 1:75.

Clonogenic Assay in Methylcellulose

Patient-derived AML cells were subjected to treatment with DMSO and 250 nM SNDX-5613, respectively, and grown for 12 days as previously described. Subsequently, the cells were washed with PBS, mixed at a concentration of 5×10^4 cells/ml with methylcellulose media (Methocult H4435, StemCell Technologies) and plated in triplicates in 24-well plates. Cells were incubated at 37 °C, 5% CO_2 for 14 days before colonies were manually counted. Colonies were imaged using a Leica DMi8 inverted microscope (Leica Microsystems).

RNA-sequencing Analysis

RNA-Seq reads were mapped to GRCh38/hg38 human genome assembly using STAR v2.7.9a [24]. Gene expression count matrix was generated using RSEM (v1.3.0) [25]. The count data were

transformed to log2-counts per million (log2CPM) using Voom [26] available from R package Limma (v3.50.3)[27]. Limma was also used to calculate differential expression between DMSO and dTAG-13 treated samples. P-values were adjusted by the Benjamini-Hochberg method to calculate FDR. Genes with absolute fold change > 2 and FDR < 0.05 were regarded as significantly differentially expressed. GO-Term enrichment and Ingunuity Pathway Analysis (IPA, Qiagen) were performed on significantly differentially expressed genes using default settings.

Differential Peak Analysis

To perform statistical test between experimental groups (DMSO/dTAG or SNDX-5613), we first finalized reproducible peaks for each group as only retained a peak if it called with stringent cutoff (FDR correct p-value < 0.05) in one replicate and at least called with lower cutoff (FDR correct p-value < 0.5) in other replicates. Then reproducible peaks from different groups were merged as reference peak set, fragments for each reference peak were counted with intersect command from pybedtools (v0.9.1) [33,34]. Next, we used limma-voom approach [36,37] to assess the significance of differential peak (DiffPeak) after TMM (trimmed mean of M-values) normalization from edgeR [35], the number of raw reads mapping per peak were also converted to FPKM unit (Fragments Per Kilo base per Million mapped reads). Significant changed peaks were defined by log2FC < -1 or > 1 and FDR < 0.05. Regions were then assigned to genes, with reference gene annotation from Gencode v31 [32] if regions overlapped gene promoters (+/- 2kb from TSS), which was done with bedtools (v2.24.0) [33]. If regions were within rDNA region, they were assigned as "rDNA". One gene could be assigned to multiple peaks. Remaining peaks are assigned to gene with TSS within +/- 50kb, excluding promoter region. Upstream Regulator analysis were generated through the use of QIAGEN IPA (QIAGEN Inc., https://digitalinsights.qiagen.com/IPA) by uploading a gene list of those genes that lost a HA:UBTF-TD peak after dTAG-13 treatment.

CUT&RUN analysis in SNDX-5613 and dTAG-13 treated cells

FKBP12^{F36V}-UBTF-TD54 expressing cbCD34+ cells were treated with 100nM SNDX-5613 or 1μM dTAG-13 for 24 hours. Cells were harvested and then subjected to CUT&RUN protocol and data analysis following our protocol presented here. With the minor difference that after confirmation DiffPeak were not due to IgG, Bigwig files were converted to fold change over IgG samples with pseudo value 1.

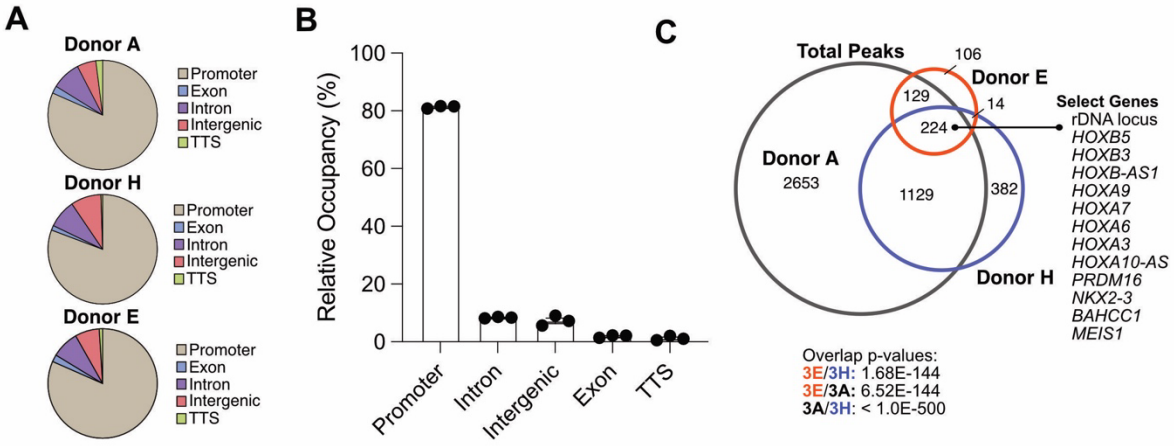
Flow Cytometry

Cells were washed with FACS buffer (1XPBX, 2%FBS) and stained with appropriate antibodies (Supplemental Table 4). For apoptosis assays, cells were stained Annexin V (Biolegend, cat# 640920) after incubation with antibodies per the manufacturer's protocol. Analytical flow cytometry was done using LSR FORTESSAII (BD Biosciences, CA) and analyzed using FlowJo Software (BD, V10.8.1).

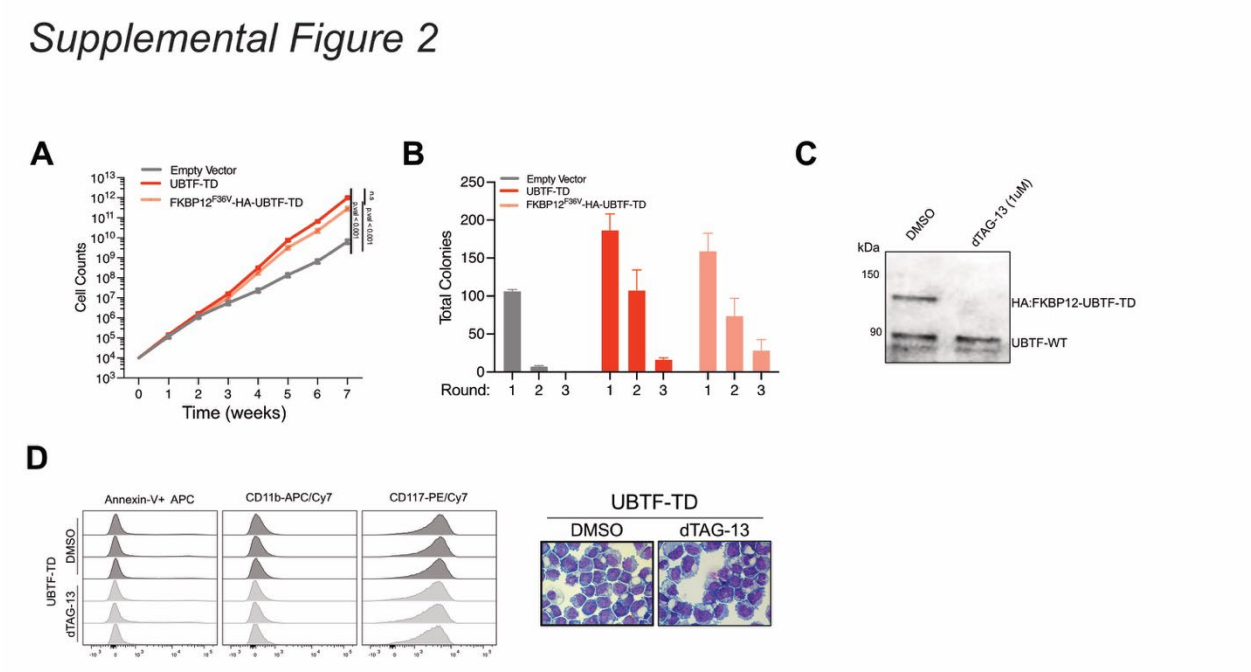
Inhibitor treatment

Following a 4-day incubation period of primary AML cells in co-culture, the cells were reseeded on fresh MSCs and treated with varying concentrations of SNDX-5613 or 0.1% DMSO. Over the course of 14 days, AML cells were reseeded on fresh MSCs every 4 days at a concentration of $5x10^5$ cells/mL while the inhibitor was replenished. Viable cell counts were determined at different time points using 0.4% trypan blue exclusion method (Invitrogen, T10282). IC50 values were determined as 50% of the maximum response to SNDX-5613.

Supplemental Figure 1

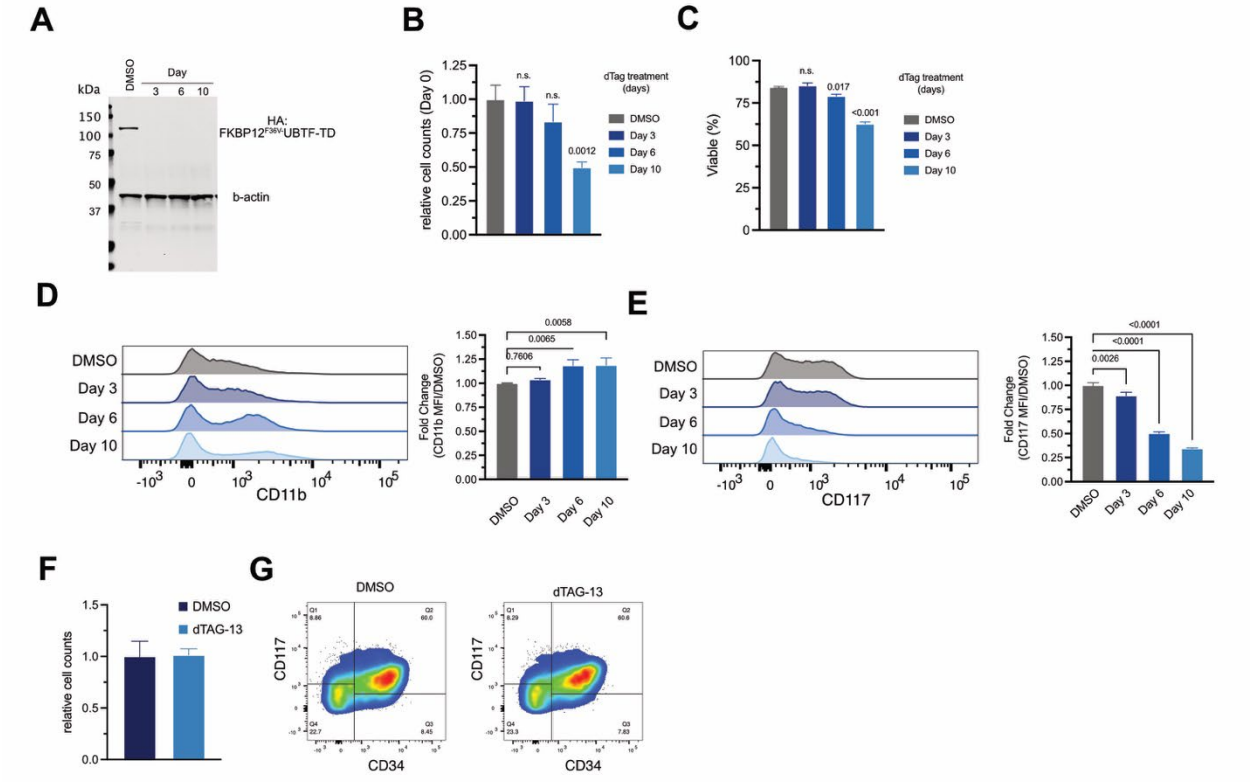


Supplemental Figure 1. Supplemental Figure related to Figure 1. **A.** Genomic annotation of HA:UBTF-TD occupied regions in cbCD34+ nHA-UBTF-TD cells in three biological replicates (cbCD34+ cells from three donors) detected by anti-HA. **B.** Relative genomic distribution of UBTF-TD occupied regions. Relative occupancy was normalized as a percentage of total peaks identified in each biological replicate. **C.** Overlap of genomic regions occupied by UBTF-TD (HA) in all three donors. Closest gene was assigned to each occupied region. Selected genes and regions occupied in all three donors that are dysregulated in *UBTF*-TD leukemias or associated with UBTF function are labeled. Significance of overlap was calculated using hypergeometric distribution.



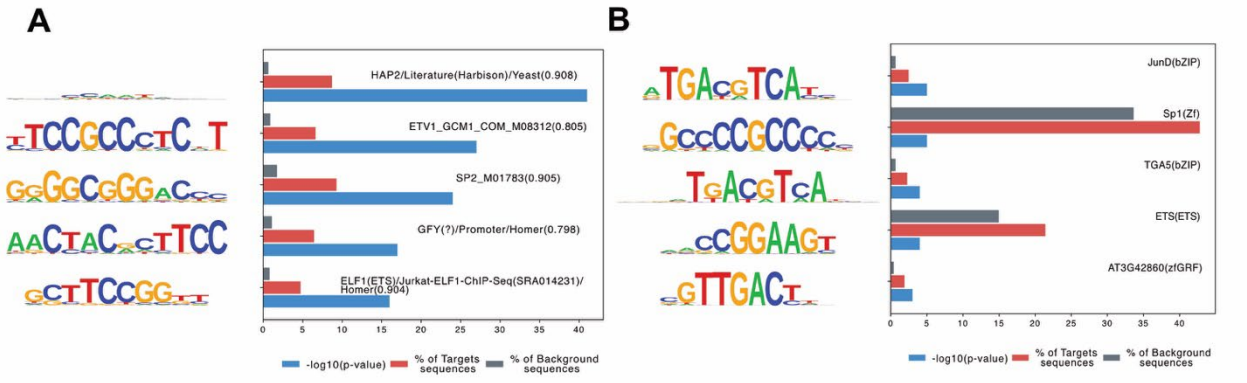
Supplemental Figure 2. Supplemental Figure related to Figure 3. **A.** Growth assay in cbCD34+ cells cultured in StemSpan SFEM II media (#09655, STEMCELL Technologies) supplemented with penicillin–streptomycin, l-glutamine, and recombinant human SCF, FLT-3, TPO, and IL6 (all 50 ng/mL, HHSC6, PeproTech), UM729 (35 nmol/L, 72914, STEMCELL Technologies), and SR-1 (1 µmol/L, 72344, STEMCELL Technologies). **B.** Colony Forming Unit (CFU) in cbCD34+ cells expressing FKBP12^{F36V}-tagged (pink) and un-tagged UBTF-TD (red) lentiviral constructs and compared to empty vector (MND-PGK-mCherry lentiviral vector). Cells were grown in methylcellulose supplemented with human cytokines (SCF, IL-3, EPO, GM-CSF) (#H4434, STEMCELL Technologies). Cells were replated every 10 days. **C.** Immunoblot in FKBP12^{F36V}-HA-UBTF-TD expressing cbCD34+ cells treated with DMSO or dTAG-13 (1 µM). UBTF-specific antibody (cat# ab244287, Abcam) to detect WT-UBTF and FKBP12-tagged UBTF-TD. **D.** Immunophenotyping of UBTF-TD expressing (without the FKBP12^{F36V}-tag) cbCD34+ cells after 10 days of treatment with dTAG-13 with corresponding cytospins.

Supplemental Figure 3



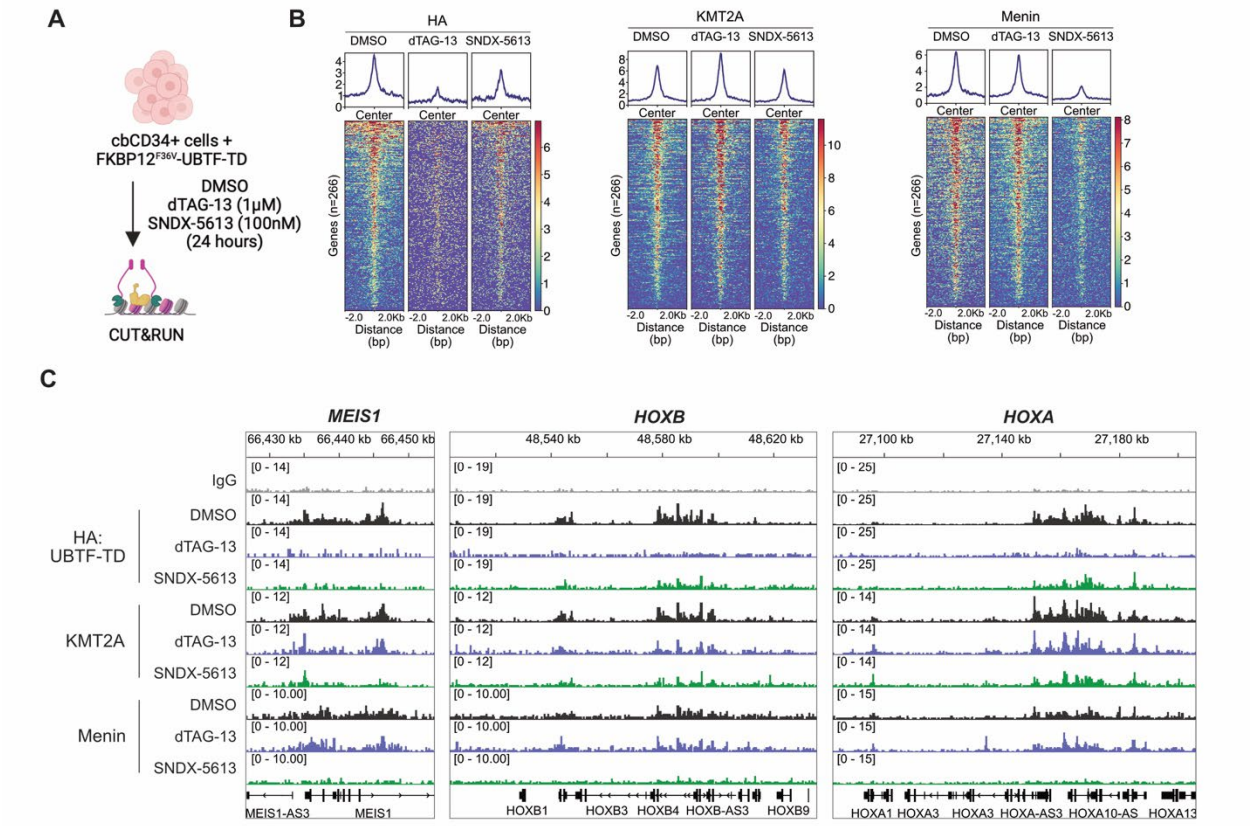
Supplemental Figure 3. Supplemental Figure related to Figure 4. **A.** cbCD34+ cells expressing FKBP12^{F36V}-HA-UBTF-TD were treated in triplicate for 0, 3, 6, and 10 days with dTAG-13. Representative immunoblots using b-actin and HA-specific antibodies from a representative replicate are shown. **B.** Relative cell counts (normalized to DMSO) of cbCD34+ cells expressing FKBP12^{F36V}-HA-UBTF-TD treated with dTAG-13 (1uM) or DMSO after Day 0, 3, 6, 10 of treatment. **C.** Viability of cells from (A) measured by flow cytometry (DAPI positive/Total Cells). **D-E.** Assessment of differentiation of cells from (A) using flow cytometry utilizing CD11b-APC/Cy7 (D) and CD117-PE/Cy7 (E). Mean fluorescent intensity (MFI) is calculated on live singlet cells and normalized to DMSO control. **F.** Relative cell counts (normalized to DMSO) of freshly isolated untransduced cord-blood cells treated with DMSO or 1 μM of dTAG-13 after 10 days of treatment. **G.** Differentiation assessment of cells from (F) using antibodies for CD34-PerCP/Cy5.5 and CD117-PE/Cy7. P-values for all comparisons were calculated using Dunnett's multiple comparison.

Supplemental Figure 4



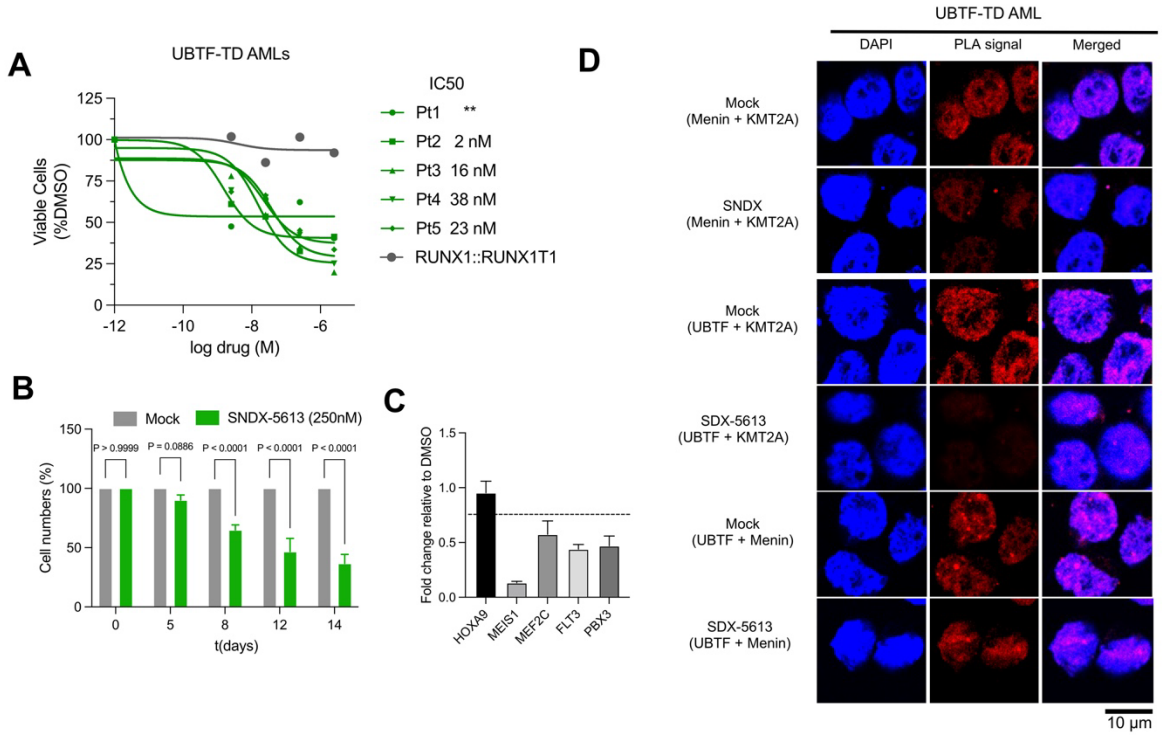
Supplemental Figure 4. Supplemental Figure related to Figure 4. **A.** De novo motif enrichment (HOMER) of regions significantly depleted after dTAG-13 treatment in cbCD34+ cells expressing FKBP12^{F36V}-HA-UBTF-TD. **B**. Known motif enrichment (HOMER) of regions significantly depleted after dTAG-13 treatment in cbCD34+ cells expressing FKBP12^{F36V}-HA-UBTF-TD.

Supplemental Figure 5



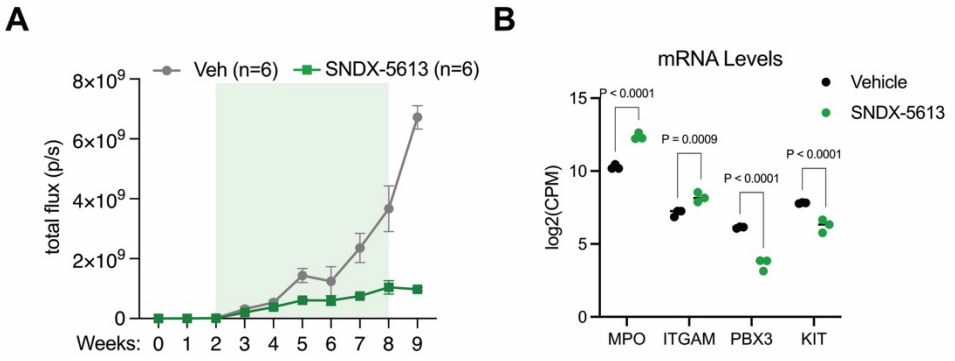
Supplemental Figure 5. Supplemental Figure related to Figure 4. **A.** Experimental schematic of FKBP12^{F36V}-HA-UBTF-TD genomic occupancy experiment. FKBP12^{F36V}-HA-UBTF-TD expressing cbCD34+ cells were treated with either DMSO, dTAG-13 1μM, or SNDX-5613 (100nM) for 24 hours. **B**. Tornado plots depicting the genomic occupancy (representative from n=3 replicates) at significantly depleted HA: FKBP12^{F36V}-HA-UBTF-TD target regions (n=266 regions, FDR <0.5) after treatment with dTAG-13 for 3 days. Occupancy for KMT2A and Menin are also shown (representative of n=2 replicates are shown). **C.** Genomic tracks of representative coverage of HA: FKBP12^{F36V}-HA-UBTF-TD cells treated with DMSO (black), dTAG-13 (blue), or SNDX-5613 (green) for HA, KMT2A, Menin.

Supplemental Figure 6



Supplemental Figure 6. Related to Figure 5. A. SNDX-5613 IC50 calculation for 5 UBTF-TD AMLs and one RUNX1::RUNX1T1 primary patient sample. Average IC50 was calculated using Pt2, Pt3, Pt4, and Pt5. Pt1 was hypersensitive and was excluded from IC50 calculation. **B.** Long-term treatment of 3 primary AML samples (Pt1, Pt2, and Pt3) at 250nM concentration of SNDX-5613. Statistics were assessed using Two-way ANOVA. **C.** Relative mRNA levels of samples from (**B**) at day 14 measured using qPCR. **D.** Proximity labeling assay (PLA) of primary UBTF-TD AML treated with DMSO or SNDX-5613 (250 nM) for 12 days. Proximity was assessed between UBTF and KMT2A or MEN1 in both conditions.

Supplemental Figure 7



Supplemental Figure 7. Related to Figure 6. A. Quantification of total photon flux from panel (Figure 6C). **B.** RNA-seq from human bone marrow cells isolated from NSG-SGM3 UBTF-TD PDX model.