## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1. Characterization of the murine MLL::AF9-FKBP12 system, Related to Figure 1**

A. Survival curves of mice injected with $Lin^{-}Sca1^{+}c\text{-}kit^{+}$ (LSK) cells transformed with untagged MLL::AF9 (black), N-terminal HA-FKBP12-MLL::AF9 (blue) or C-terminal MLL::AF9-HA-FKBP12.
B. Markers of leukemogenesis at the time of bone marrow harvesting.
C. Growth curves of untagged MLL::AF9 (black) and MLL::AF9-HA-FKBP12 (red) leukemic cells over 12 days. Data represents the mean and standard deviation of experimental triplicates.
D. Expression of MLL::AF9 target genes in untagged MLL::AF9 (black) and MLL::AF9-HA-FKBP12 (red) leukemic cells from two independently generated leukemias. Data represent the mean and standard deviation of three independent experiments, measured by Taqman.
E. Intracellular FACs: Relative Mean Fluorescent Intensity (MFI) of HA upon treatment with dTAG-13 (500nM) at the indicated timepoints. All MFI signal was normalized to DMSO-treated untagged MLL::AF9 cells. Data represents the mean and standard deviation of three independent experiments.
F. Intracellular FACs: Mean Fluorescent Intensity (MFI) of HA signal upon treatment with DMSO, dNEG-13 (500nM, gray) or dTAG-13 (500nM, red) at the indicated timepoints.
G. Intracellular FACs: Mean Fluorescent Intensity (MFI) of HA signal. Cells were co-treated with DMSO/MLN-4924 (1μM) and DMSO/dNEG-13 (500nM)/dTAG-13 (500nM) for 24 hours. For the proteasomal rescue, cells were co-treated with carfilzomib (500nM) and DMSO/dNEG-13 (500nM)/dTAG-13 (500nM) for 4 hours.
H. (Top) Relative expression of *Meis1* and *HoxA9* after 24 hours of treatment with dTAG-13 in a dose-dependent manner, normalized to DMSO treatment. (Bottom) Relative expression of *Meis1* and *HoxA9* upon treatment with 500nM dTAG-13 in a time-dependent manner, normalized to DMSO treatment. Untagged MLL::AF9 cells are in black and MLL::AF9-HA-FKBP12 transformed cells are in red. Data represents the mean and standard deviation of three independent experiments, measured by Taqman.
I. Relative Expression of *Meis1* and *HoxA9* in MLL::AF9-HA-FKBP12 cells over time with dNEG-13 (500nM, gray) and dTAG-13 (500nM, red) treatment, normalized to DMSO treatment. Data represents the mean and standard deviation of three independent experiments, measured by Taqman.
J. Cell Proliferation of untagged MLL::AF9 transformed cells (black) cells and MLL::AF9-HA-FKBP12 transformed cells (red) assessed as percent DMSO after 6 days with increasing doses of dTAG-13. Data represents the mean and standard deviation of three independent experiments.
K. Cell Proliferation of MLL::AF9-HA-FKBP12 cells assessed as percent DMSO after 6 days with increasing doses of dTAG-13 (red) and dNEG-13 (gray). Data represents the mean and standard deviation of three independent experiments.
L. Cytospins after 5 days of DMSO and dTAG-13 (500nM) treated MLL::AF9-HA-FKBP12 cells. The scale bar marks 90μM.
M. Apoptosis of DMSO and dTAG-13 (500nM) treated MLL::AF9-HA-FKBP12 cells at 3, 5, and 7 days as measured by Annexin V positive, PI negative cells. Data represents the mean and standard deviation of three independent experiments.

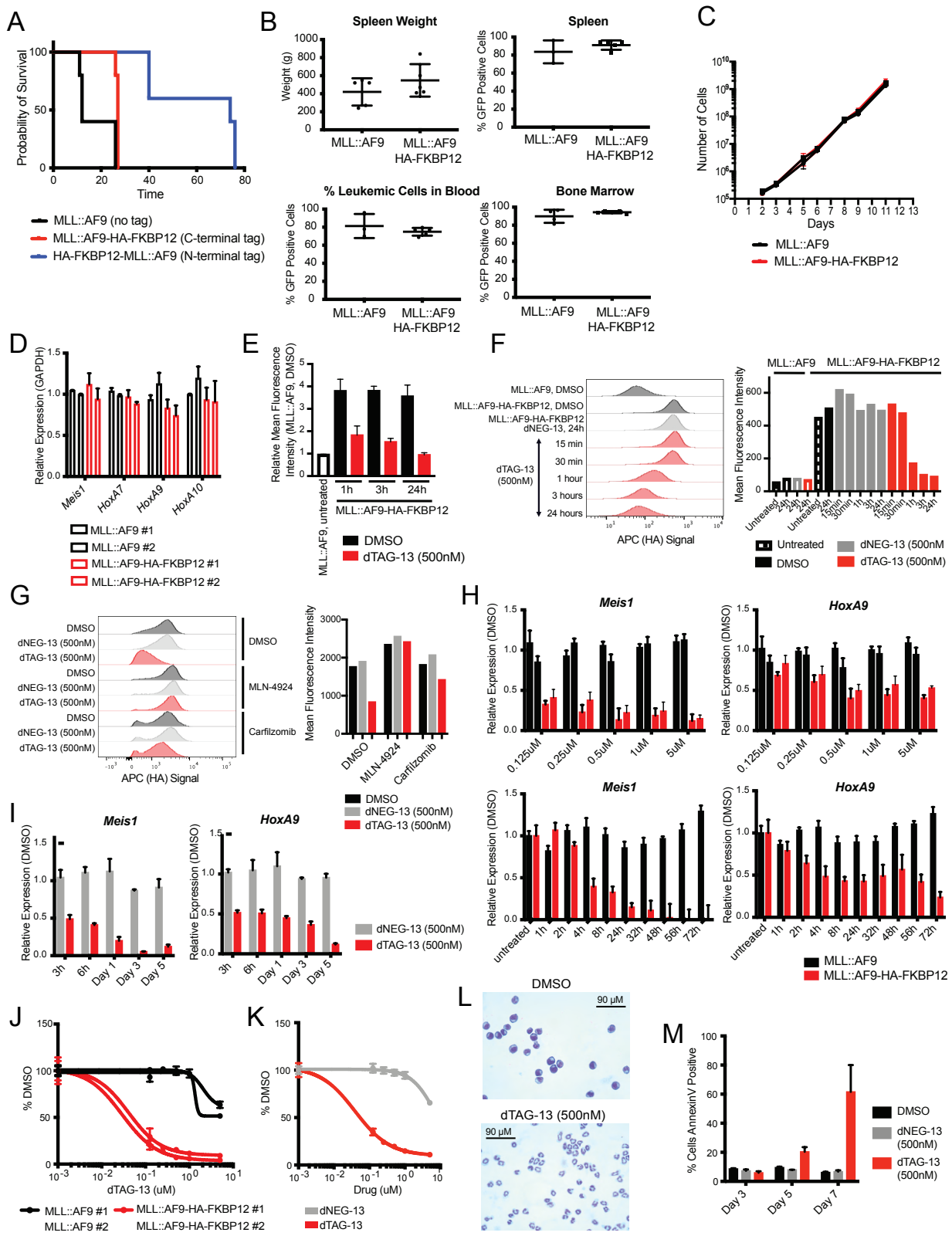
A
Probability of Survival
Time
MLL::AF9 (no tag)
MLL::AF9-HA-FKBP12 (C-terminal tag)
HA-FKBP12-MLL::AF9 (N-terminal tag)
B
Spleen Weight
Weight (g)
Spleen
% GFP Positive Cells
% Leukemic Cells in Blood
Bone Marrow
MLL::AF9
MLL::AF9 HA-FKBP12
C
Number of Cells
Days
MLL::AF9
MLL::AF9-HA-FKBP12
D
Relative Expression (GAPDH)
Meis1
HoxA7
HoxA9
HoxA10
MLL::AF9 #1
MLL::AF9 #2
MLL::AF9-HA-FKBP12 #1
MLL::AF9-HA-FKBP12 #2
E
Relative Mean Fluorescence Intensity (MLL::AF9, DMSO)
1h
3h
24h
MLL::AF9-HA-FKBP12
MLL::AF9, untreated
DMSO
dTAG-13 (500nM)
F
MLL::AF9, DMSO
MLL::AF9-HA-FKBP12, DMSO
MLL::AF9-HA-FKBP12 dNEG-13, 24h
dTAG-13 (500nM)
15 min
30 min
1 hour
3 hours
24 hours
APC (HA) Signal
MLL::AF9
MLL::AF9-HA-FKBP12
Mean Fluorescence Intensity
Untreated
dNEG-13 (500nM
dTAG-13 (500nM)
DMSO
G
DMSO
dNEG-13 (500nM)
dTAG-13 (500nM)
MLN-4924
Carfilzomib
APC (HA) Signal
Mean Fluorescence Intensity
H
Meis1
HoxA9
Relative Expression (DMSO)
0.125uM
0.25uM
0.5uM
1uM
5uM
I
Meis1
HoxA9
Relative Expression (DMSO)
3h
6h
Day 1
Day 3
Day 5
dNEG-13 (500nM)
dTAG-13 (500nM)
J
% DMSO
dTAG-13 (uM)
MLL::AF9 #1
MLL::AF9 #2
MLL::AF9-HA-FKBP12 #1
MLL::AF9-HA-FKBP12 #2
K
% DMSO
Drug (uM)
dNEG-13
dTAG-13
L
DMSO
90 μM
dTAG-13 (500nM)
90 μM
M
% Cells AnnexinV Positive
Day 3
Day 5
Day 7
DMSO
dNEG-13 (500nM)
dTAG-13 (500nM)

**Figure S2. Volcano plots of RNA-Seq and GSEA analyses following MLL::AF9 degradation, Related to Figure 2.**

A. Volcano plots of differentially transcribed genes 3 and 24 hours upon dTAG-VHL (500nM) treatment measured by RNA-Seq in the human MLL::AF9-FKBP12 system (Table S1).
B. Volcano plots of differentially transcribed genes 3 and 24 hours upon dTAG-13 (500nM) treatment measured by RNA-Seq in the murine MLL::AF9-FKBP12 system (Table S1).
C. Gene expression changes in MLL::AF9-HA-FKBP12 transformed murine cells. (Left) Heatmap of downregulated genes (Fold Change > 1.5, $p<0.1$, Table S1) after 3 hours of treatment with either DMSO or dTAG-13 (500nM). (Right) Heatmap of differentially expressed genes (Fold Change > 2, $p<0.1$, Table S1) after 24 hours of DMSO or dTAG-13 (500nM) treatment.
D. GSEA comparing the genes affected by MLL::AF9 degradation in murine cells to the top 100 downregulated genes in the human MLL::AF9-HA-FKBP12 transformed $CD34^+$ system upon MLL::AF9 degradation at 3 hours.
E. GSEA of gene expression changes in murine MLL::AF9-HA-FKBP12 leukemic cells 5 days upon dTAG-13 (500nM) treatment compared with previously published MLL::AF9 (Bernt et al., 2011; Stavropoulou et al., 2016) or MLL-AF4 (Guenther et al., 2008) target gene lists.
F. (Left) GSEA of gene expression changes in murine MLL::AF9-HA-FKBP12 leukemic cells 5 days upon dTAG-13 (500nM) treatment compared with genes upregulated (Fold Change > 2, $p<0.05$, Table S1) in neutrophils vs murine hematopoietic stem and multipotent progenitor cells ($Lin^-Sca1^+c\text{-}kit^+$ (LSK)). (Right) Cytospins after 5 days of DMSO and dTAG-13 (500nM) treated MLL::AF9-HA-FKBP12 cells. The scale bar marks 90μM.

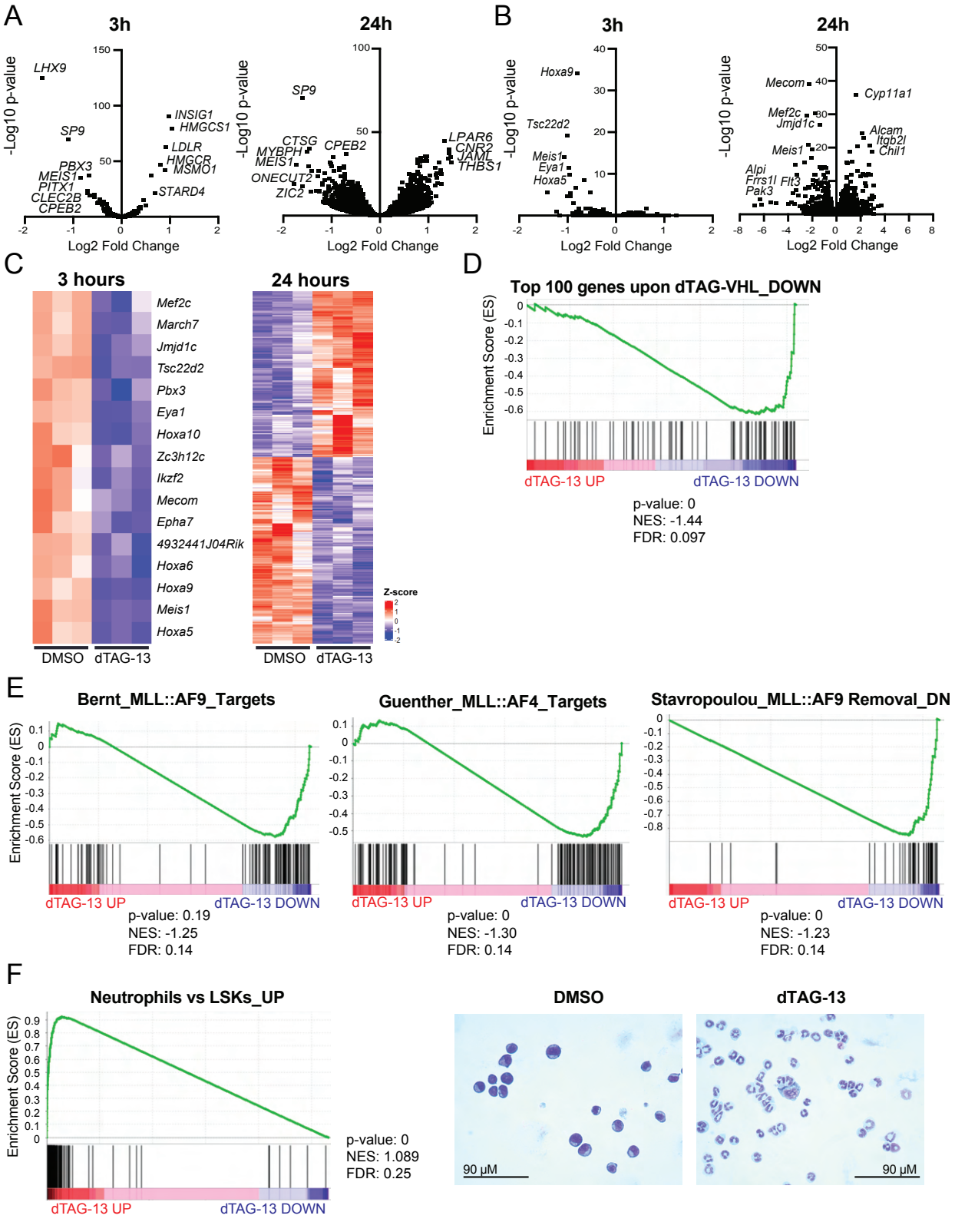
A
3h
24h
-Log10 p-value
Log2 Fold Change
LHX9
SP9
PBX3
MEIS1
PITX1
CLEC2B
CPEB2
INSIG1
HMGCS1
LDLR
HMGCR
MSMO1
STARD4
CTSG
MYBPH
MEIS1
ONECUT2
ZIC2
CPEB2
LPAR6
CNR2
JAML
THBS1
B
Hoxa9
Tsc22d2
Meis1
Eya1
Hoxa5
Mecom
Mef2c
Jmjd1c
Meis1
Alpi
Frrs1l
Pak3
Flt3
Cyp11a1
Alcam
Itgb2l
Chil1
C
3 hours
24 hours
Mef2c
March7
Jmjd1c
Tsc22d2
Pbx3
Eya1
Hoxa10
Zc3h12c
Ikzf2
Mecom
Epha7
4932441J04Rik
Hoxa6
Hoxa9
Meis1
Hoxa5
DMSO
dTAG-13
Z-score
D
Top 100 genes upon dTAG-VHL_DOWN
Enrichment Score (ES)
dTAG-13 UP
dTAG-13 DOWN
p-value: 0
NES: -1.44
FDR: 0.097
E
Bernt_MLL::AF9_Targets
p-value: 0.19
NES: -1.25
FDR: 0.14
Guenther_MLL::AF4_Targets
p-value: 0
NES: -1.30
FDR: 0.14
Stavropoulou_MLL::AF9 Removal_DN
p-value: 0
NES: -1.23
FDR: 0.14
F
Neutrophils vs LSKs_UP
p-value: 0
NES: 1.089
FDR: 0.25
DMSO
dTAG-13
90 μM

**Figure S3. MLL::AF9 degradation in the human MLL::AF9-FKBP12 system induces transcriptional changes within minutes, related to Figure 3.**

A. Heatmap of differentially transcribed genes (Log2 FC>1, p<0.05, Table S2) at 15min/30min/60min/120min following dTAG-VHL (500nM) treatment (Figure 4D) as measured by SLAM-Seq.

B. Venn diagrams depicting the overlap of genes exhibiting decreased transcription (top and middle, Log2 FC>1, p<0.05) upon dTAG-VHL (500nM) treatment and increased transcription (bottom, Log2 FC>1, p<0.05) upon dTAG-VHL (500nM) treatment at the indicated timepoints, as measured by SLAM-seq (Table S2).

C. Heatmap of PRO-Seq signal at MLL::AF9 target genes (Table S1) after 15min/30min/180min following dTAG-VHL (500nM) treatment.

D. Venn diagrams depicting the overlap of genes exhibiting decreased transcription (top, FC>1.5, p<0.0001) upon dTAG-VHL (500nM) treatment and increased transcription (bottom, FC>1.5, p<0.0001) upon dTAG-VHL (500nM) treatment at the indicated timepoints, as measured by PRO-seq (Table S2).

E. Schematic depicting how transcription of a generic gene locus is broken down into promoter proximal (pp, TSS to +150bp) and gene body (gb, +250 to TES) regions. Pausing index (PI) is calculated by dividing the promoter proximal (pp) signal by the gene body (gb) signal.

F. Gene tracks of PRO-seq signal at *MEIS1*, as a representative MLL::AF9 target gene, and *GAPDH*, as a representative housekeeping gene, at the indicated timepoints upon dTAG-VHL (500nM) treatment. The gene tracks were normalized to coverage data (total read counts).

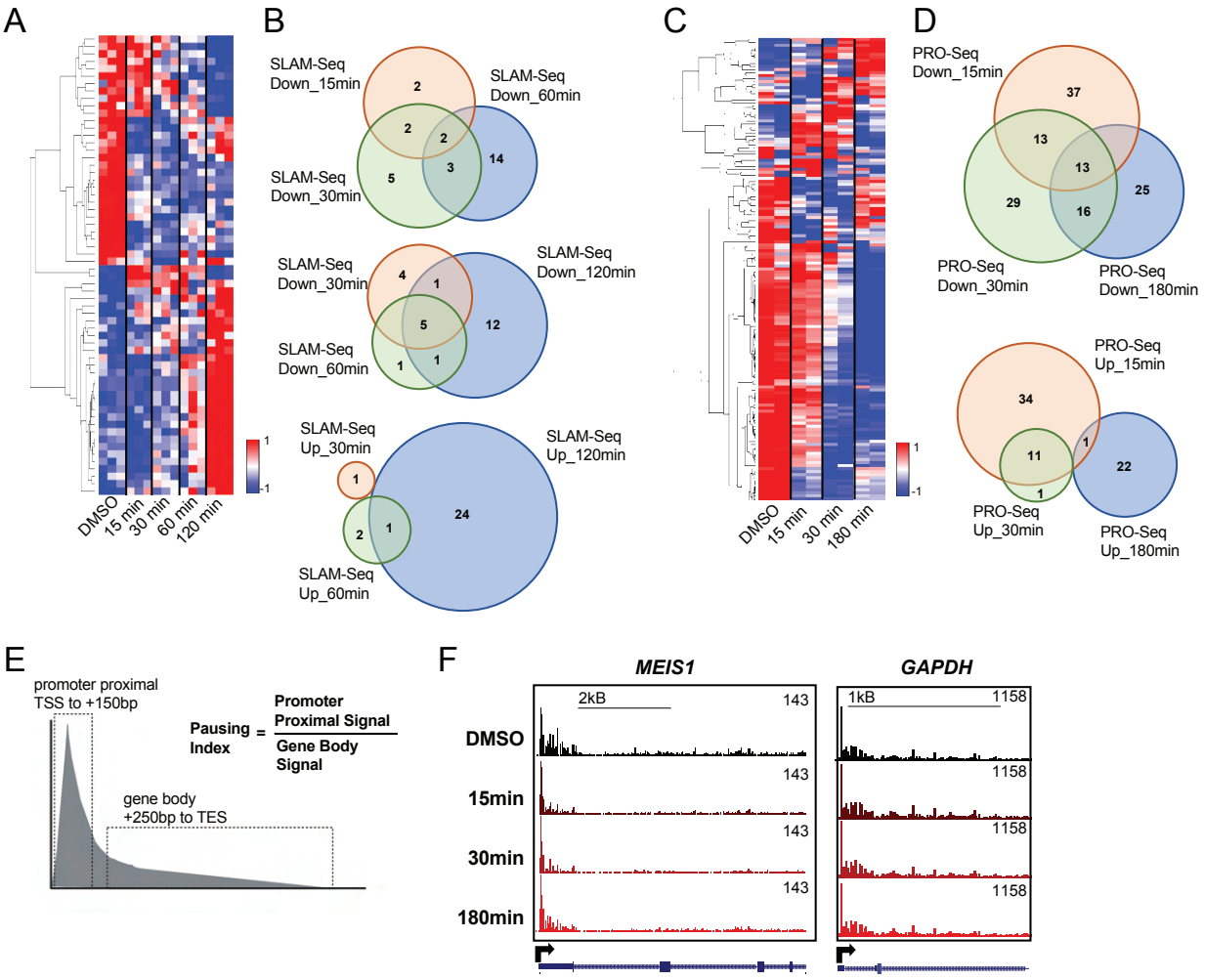

**Figure S4. Additional chromatin occupancy changes upon MLL::AF9 degradation in the human MLL::AF9-FKBP12 system, related to Figure 4.**

A. Boxplot of total reads at the promoter (TSS -1kB/+3kB) following H3K79me2 ChIP-Seq of DMSO-treated cells at 'highly sensitive genes' (red, Table S1), all other MLL::AF9 target genes (black, Table S1) and non MLL::AF9 bound genes (gray).
B. (Left) Boxplots of fold change in ChIP-seq signal for H3K27ac and H3K9ac at promoters (TSS -1kB/+3kB, marked as TSS) 3 or 24 hours upon dTAG-VHL (500nM) treatment, as indicated. (Right) Gene tracks of ChIP-seq signal at MLL::AF9 target genes and *GAPDH*, as a representative housekeeping gene, 3 and 24 hours upon dTAG-VHL (500nM) treatment, as indicated. The gene tracks were normalized to coverage data (total read counts) using IGV.
C. Boxplots of total reads at the promoter (TSS -1kB/+3kB) following H3K27ac and H3K9ac ChIP-Seq of DMSO-treated cells at 'highly sensitive genes' (red, Table S1), all other MLL::AF9 target genes (black, Table S1), and non MLL::AF9 bound genes (gray).
D. Gene tracks of replicate ATAC-seq signal at MLL::AF9 target genes and *GAPDH*, as a representative housekeeping gene, following 3 hours of dTAG-VHL (500nM) treatment. The gene tracks were normalized to coverage data (total read counts) using IGV
E. Boxplot of fold change in ChIP-seq signal for H3K4me3 at promoters (TSS -1kB/+3kB, marked as TSS) 3 hours following dTAG-VHL (500nM) treatment.
F. Boxplots of fold change in ChIP-seq signal for MLL1-N-terminal and MLL1-C-terminal at promoters (TSS -1kB/+3kB, marked as TSS) 3 hours upon dTAG-VHL (500nM) treatment.
G. Gene tracks of ChIP-seq signal at representative genes 3 hours upon dTAG-VHL (500nM) treatment, assessing specificity of initiating (pSer5) versus elongating (pSer2) RNA Pol II signal. The gene tracks were normalized to coverage data (total read counts) using IGV.

ns $p > 0.5$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.001$ (Mann Whitney test)

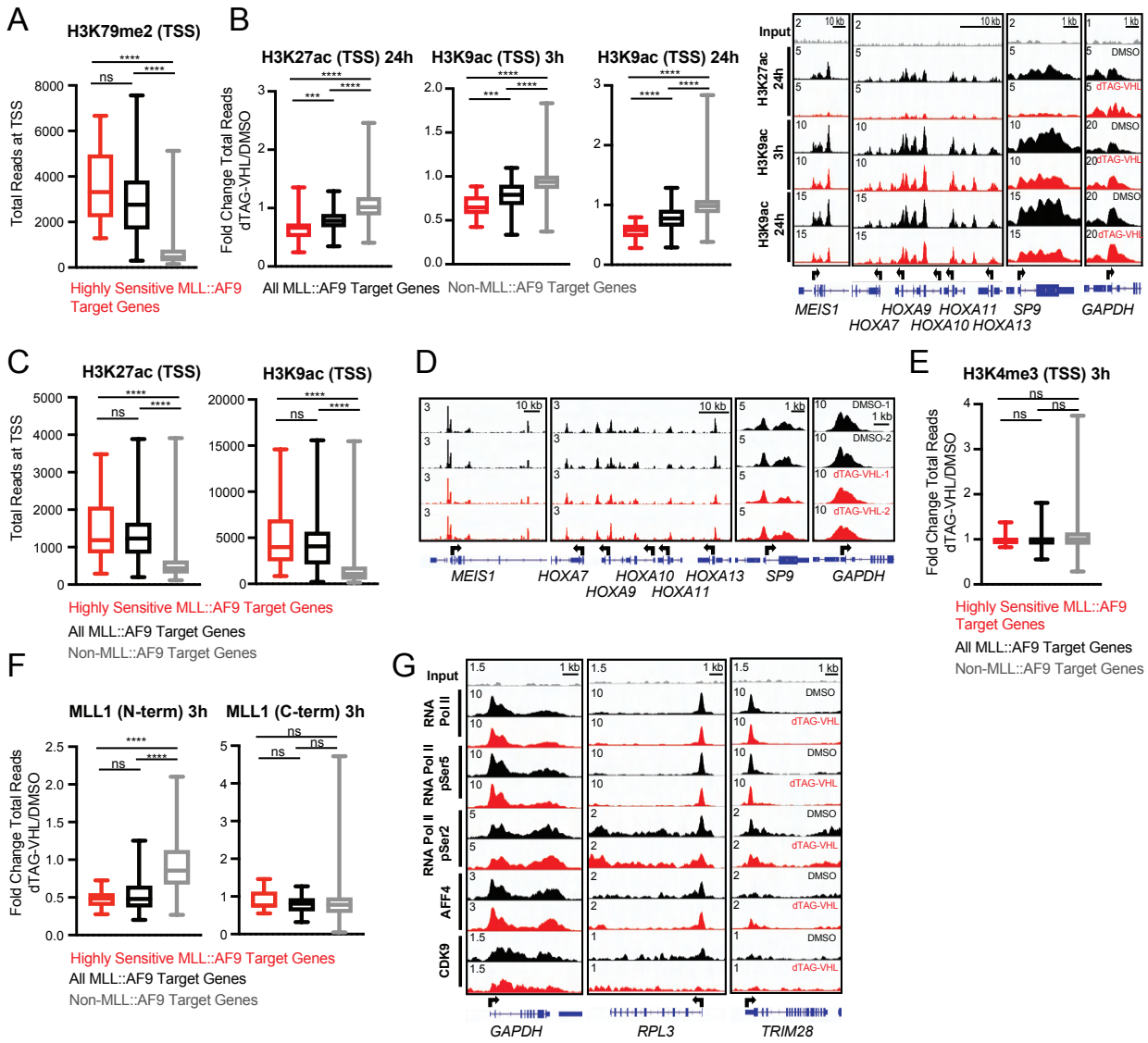
A
H3K79me2 (TSS)
Total Reads at TSS
Highly Sensitive MLL::AF9 Target Genes
B
H3K27ac (TSS) 24h
H3K9ac (TSS) 3h
H3K9ac (TSS) 24h
Fold Change Total Reads dTAG-VHL/DMSO
All MLL::AF9 Target Genes
Non-MLL::AF9 Target Genes
Input
H3K27ac 24h
H3K9ac 3h
H3K9ac 24h
DMSO
dTAG-VHL
MEIS1
HOXA9
HOXA11
SP9
GAPDH
HOXA7
HOXA10
HOXA13
C
H3K27ac (TSS)
H3K9ac (TSS)
Total Reads at TSS
Highly Sensitive MLL::AF9 Target Genes
All MLL::AF9 Target Genes
Non-MLL::AF9 Target Genes
D
DMSO-1
DMSO-2
dTAG-VHL-1
dTAG-VHL-2
MEIS1
HOXA7
HOXA10
HOXA13
SP9
GAPDH
HOXA9
HOXA11
E
H3K4me3 (TSS) 3h
Fold Change Total Reads dTAG-VHL/DMSO
Highly Sensitive MLL::AF9 Target Genes
All MLL::AF9 Target Genes
Non-MLL::AF9 Target Genes
F
MLL1 (N-term) 3h
MLL1 (C-term) 3h
Fold Change Total Reads dTAG-VHL/DMSO
Highly Sensitive MLL::AF9 Target Genes
All MLL::AF9 Target Genes
Non-MLL::AF9 Target Genes
G
Input
RNA Pol II
RNA Pol II pSer5
RNA Pol II pSer2
AFF4
CDK9
DMSO
dTAG-VHL
GAPDH
RPL3
TRIM28

**Figure S5. Cellular consequences are induced more rapidly upon DOT1L/MENIN combination treatment, related to Figure 5.**

A. Cell proliferation of two independently generated human MLL::AF9-HA-FKBP12 leukemic cell lines assessed as percent DMSO after 6 days with increasing doses of either VTP-50469 (left) or EPZ-5676 (right) either as single agent treatment (black) or in combination (red). Data represent the mean and standard deviation of three independent experiments, IC50 values are indicated.

B. Cell proliferation of human *MLL::AF9* rearranged MOLM13 cells assessed as percent DMSO after 6 days with increasing doses of either VTP-50469 (left) or EPZ-5676 (right) either as single agent treatment (black) or in combination (red). Data represent the mean and standard deviation of three independent experiments, IC50 values are indicated.

C. Cell proliferation of human untransformed hematopoietic stem and progenitor cells ($CD34^+$) cells assessed as percent DMSO after 6 days with increasing doses of either VTP-50469 (left) or EPZ-5676 (right) either as single agent treatment (black) or in combination (red). Data represent the mean and standard deviation of three independent experiments.

D. Cell proliferation of human HL60 cells (not *MLL*-rearranged) assessed as percent DMSO after 6 days with increasing doses of either VTP-50469 (left) or EPZ-5676 (right) either as single agent treatment (black) or in combination (red). Data represent the mean and standard deviation of three independent experiments.

E. Cell proliferation of human MLL::AF9-HA-FKBP12 leukemic cells assessed as percent DMSO after 1, 2, 3, 4, 5, and 6 days with increasing doses of dNEG-VHL (black), dTAG-VHL (red), EPZ-5676 (dark green), EPZ-5676 + 100nM VTP-50469 (light green), VTP-50469 (dark blue), and VTP-50469 + 250nM EPZ-5676 (light blue). Data represent the mean and standard deviation of three independent experiments.

F. (Top) Assessment of differentiation via CD14 levels by Mean Fluorescence Intensity after 1, 2, 3 and 4 days of DMSO, dNEG-VHL (500nM, gray), dTAG-VHL (500nM, red), EPZ-5676 (250nM, green), VTP-50469 (100nM, blue), or combination EPZ-5676 (250nM) + VTP-50469 (100nM) (orange) treatment of MLL::AF9-HA-FKBP12 transformed human cells. Data represents the mean and standard deviation of triplicates. (Bottom) Apoptosis of MLL::AF9-HA-FKBP12 human cells measured after 1, 2, 3 and 4 days of DMSO, dNEG-VHL (500nM, gray), dTAG-VHL (500nM, red), EPZ-5676 (250nM, green), VTP-50469 (100nM, blue), or combination EPZ-5676 (250nM) + VTP-50469 (100nM) (orange) treatment of MLL::AF9-HA-FKBP12 transformed human cells. Annexin V positive, PI negative cells were counted and quantified, data represent the mean and standard deviation of three independent experiments.

G. Western blot in the human MLL::AF9-FKBP12 cells treated with DMSO or the irreversible pan-caspase inhibitor Z-VAD-FMK (50μM) and with DMSO, EPZ-5676 (250nM), VTP-50469 (100nM), EPZ-5676 (250nM) + VTP-50469 (100nM), or dTAG-VHL (500nM) as indicated. Cleaved PARP (Cl PARP) and total PARP (T PARP) levels were assessed by immunoblotting at the indicated timepoints.

H. Apoptosis of MLL::AF9-HA-FKBP12 human cells measured after 2, 3 and 4 days of DMSO, dNEG-VHL (500nM), dTAG-VHL (500nM), EPZ-5676 (250nM), VTP-50469 (100nM), or combination EPZ-5676 (250nM) + VTP-50469 (100nM) treatment. Cells were either co-treated with DMSO or Z-VAD-FMK (50μM), as indicated. Annexin V positive, PI negative cells were counted and quantified at the indicated timepoints, data represent the mean and standard deviation of three independent experiments.

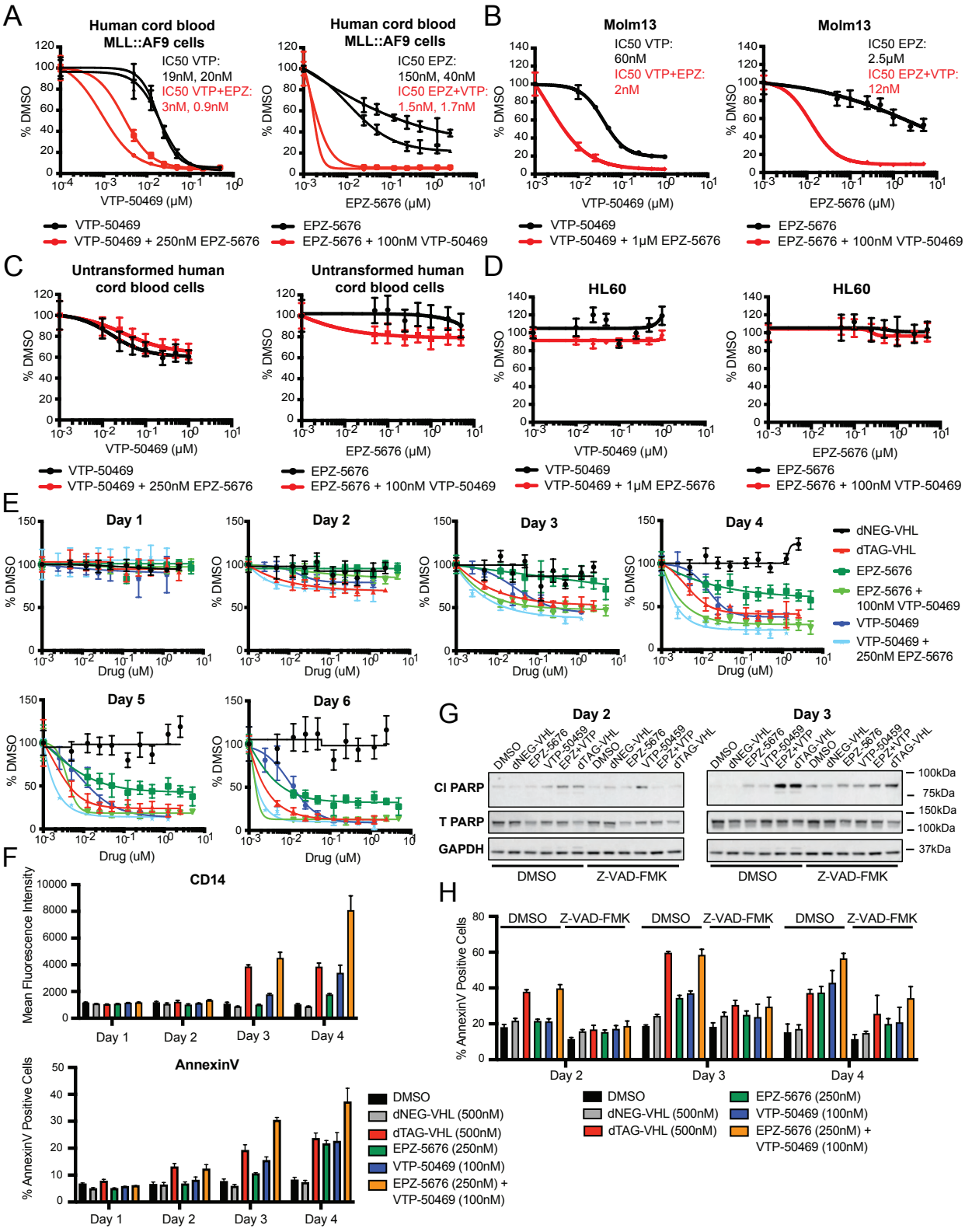
A
Human cord blood MLL::AF9 cells
IC50 VTP: 19nM, 20nM
IC50 VTP+EPZ: 3nM, 0.9nM
% DMSO
VTP-50469 (μM)
VTP-50469
VTP-50469 + 250nM EPZ-5676
Human cord blood MLL::AF9 cells
IC50 EPZ: 150nM, 40nM
IC50 EPZ+VTP: 1.5nM, 1.7nM
EPZ-5676 (μM)
EPZ-5676
EPZ-5676 + 100nM VTP-50469
B
Molm13
IC50 VTP: 60nM
IC50 VTP+EPZ: 2nM
VTP-50469 (μM)
VTP-50469
VTP-50469 + 1μM EPZ-5676
Molm13
IC50 EPZ: 2.5μM
IC50 EPZ+VTP: 12nM
EPZ-5676 (μM)
EPZ-5676
EPZ-5676 + 100nM VTP-50469
C
Untransformed human cord blood cells
VTP-50469 (μM)
VTP-50469
VTP-50469 + 250nM EPZ-5676
Untransformed human cord blood cells
EPZ-5676 (μM)
EPZ-5676
EPZ-5676 + 100nM VTP-50469
D
HL60
VTP-50469 (μM)
VTP-50469
VTP-50469 + 1μM EPZ-5676
HL60
EPZ-5676 (μM)
EPZ-5676
EPZ-5676 + 100nM VTP-50469
E
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
% DMSO
Drug (uM)
dNEG-VHL
dTAG-VHL
EPZ-5676
EPZ-5676 + 100nM VTP-50469
VTP-50469
VTP-50469 + 250nM EPZ-5676
F
CD14
Mean Fluorescence Intensity
Day 1
Day 2
Day 3
Day 4
AnnexinV
% AnnexinV Positive Cells
DMSO
dNEG-VHL (500nM)
dTAG-VHL (500nM)
EPZ-5676 (250nM)
VTP-50469 (100nM)
EPZ-5676 (250nM) + VTP-50469 (100nM)
G
Day 2
Day 3
DMSO
dNEG-VHL
EPZ-5676
VTP-50459
EPZ+VTP
dTAG-VHL
Cl PARP
T PARP
GAPDH
100kDa
75kDa
150kDa
100kDa
37kDa
DMSO
Z-VAD-FMK
H
% AnnexinV Positive Cells
DMSO
Z-VAD-FMK
Day 2
Day 3
Day 4
DMSO
dNEG-VHL (500nM)
dTAG-VHL (500nM)
EPZ-5676 (250nM)
VTP-50469 (100nM)
EPZ-5676 (250nM) + VTP-50469 (100nM)

**Figure S6. Combination of DOT1L and MENIN inhibition mimics the chromatin changes induced by MLL::AF9 degradation, related to Figure 6.**

A. Gene tracks of H3K27ac ChIP-seq signal following 4 days of treatment and RNA-Seq tracks following 5 days of treatment with DMSO (black), EPZ-5676 (250nM, green), VTP-50469 (100nM, blue), EPZ-5676 (250nM) + VTP-50469 (100nM) (orange) or dTAG-VHL (500nM, red) in the human MLL::AF9-FKBP12 system. The gene tracks were normalized to coverage data (total read counts) using IGV.

B. Gene tracks of MLL1 and H3K27ac ChIP-seq signal in MOLM13 cells following 4 days of treatment and representative RNA-Seq tracks following 5 days of treatment with DMSO (black), EPZ-5676 (1μM, green), VTP-50469 (100nM, blue), or EPZ-5676 (1μM) + VTP-50469 (100nM) (orange). The gene tracks were normalized to coverage data (total read counts) using IGV.

C. (Left) Average/meta plot of MLL1 ChIP reads in MOLM13 cells across the promoter (TSS -3kB/+3kB) of MLL::AF9 target genes with detectable MLL1 signal in MOLM13 cells (Table S4), 4 days following DMSO (black), EPZ-5676 (1μM, green), VTP-50469 (100nM, blue), or EPZ-5676 (1μM) + VTP-50469 (100nM) (orange) treatment. (Right) Boxplot of change in ChIP-seq signal for MLL1 at MOLM13-MLL::AF9 target genes (Table S4) around the TSS (TSS -1kB/+3kB) 4 days upon treatment with EPZ-5676 (1μM, green), VTP-50469 (100nM, blue), or EPZ-5676 (1μM) + VTP-50469 (100nM) (orange).
ns p > 0.5; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.001 (Mann Whitney test)