Supplementary Figure 1

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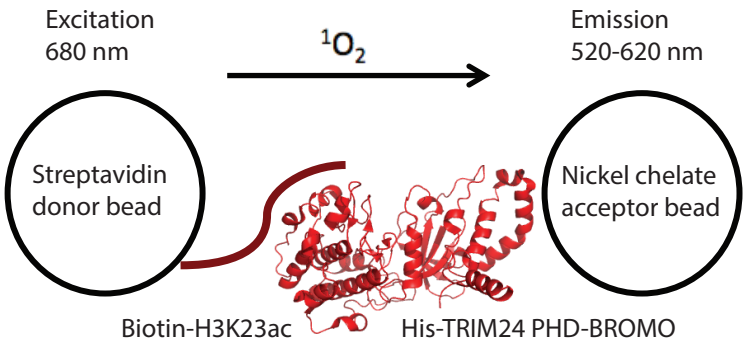
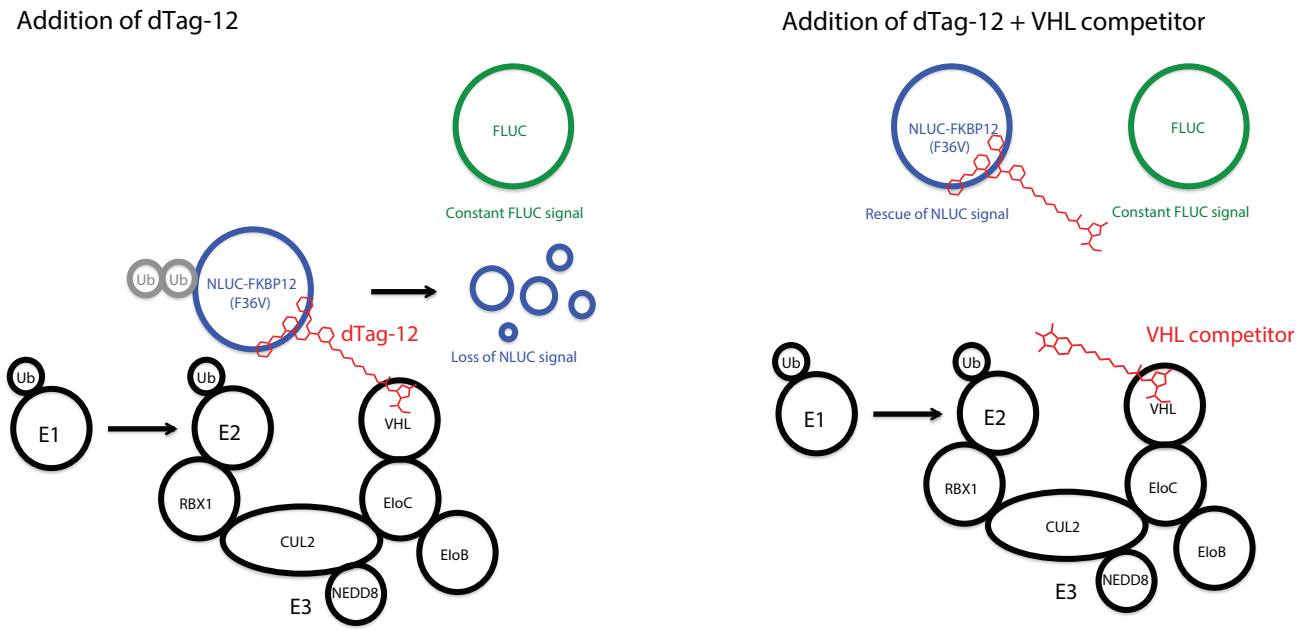


b

Compound Name	Gene Symbol	Kd (nM)
IACS-7e	TRIM24(PHD,Bromo.)	11
IACS-9571	TRIM24(PHD,Bromo.)	2.7
dTRIM24	TRIM24(PHD,Bromo.)	42
dTRIM24	BRD1	130
dTRIM24	BRD4	10000
dTRIM24	BRPF1	52
dTRIM24	TAF1	2900

c



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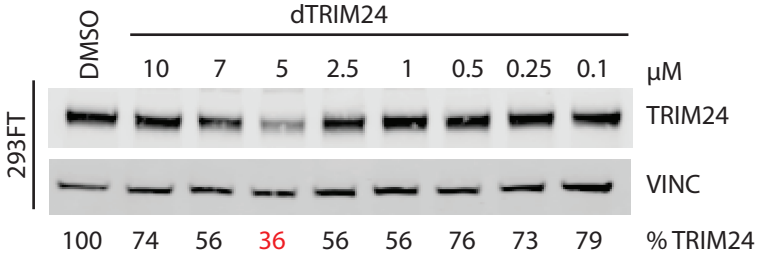
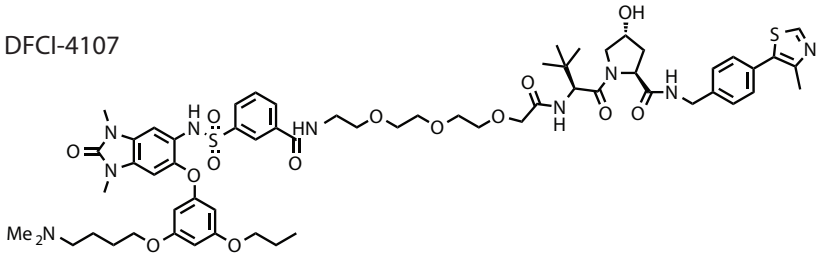


Supplementary Figure 1. Biochemical characterization of dTRIM24

(a) Schematic of TRIM24 ligand displacement assay. **(b)** KD binding constant values for compounds dTRIM24, IACS-7e, and IACS-9571 to several bromodomains in singlicate (BromoScan). **(c)** Schematic of intracellular VHL degron displacement assay with the addition of either dTag-12 alone or dTag-12 plus a VHL competitor. The VHL competitor competes for binding of VHL with dTag-12 and displaces its binding from VHL, therefore preventing its degradation, resulting in an increase in luciferase signal. **(d)** Immunoblot of TRIM24 and Vinculin following 24 hours of incubation with the indicated concentrations of dTRIM24 in 293FT cells. Percentages were calculated by normalization of the band intensity to the loading control and relative to DMSO. This immunoblot was repeated three times, with one representative blot shown. Full immunoblot shown in Supplementary Fig. 11.

Supplementary Figure 2

a



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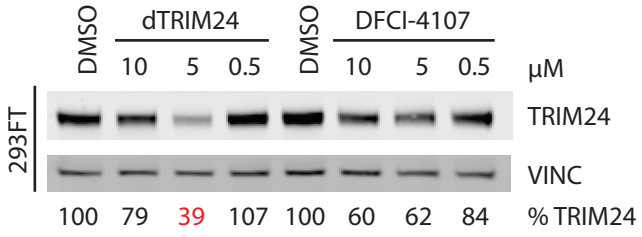

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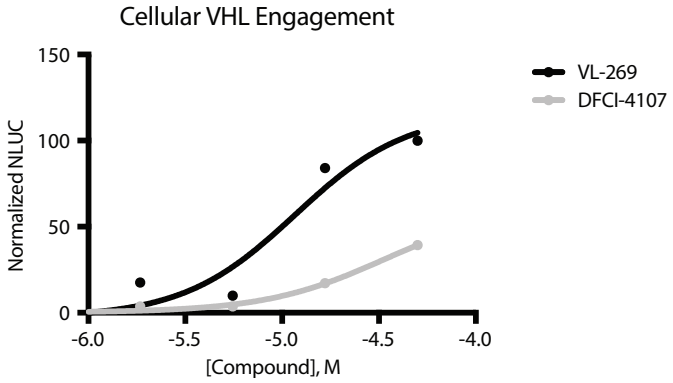
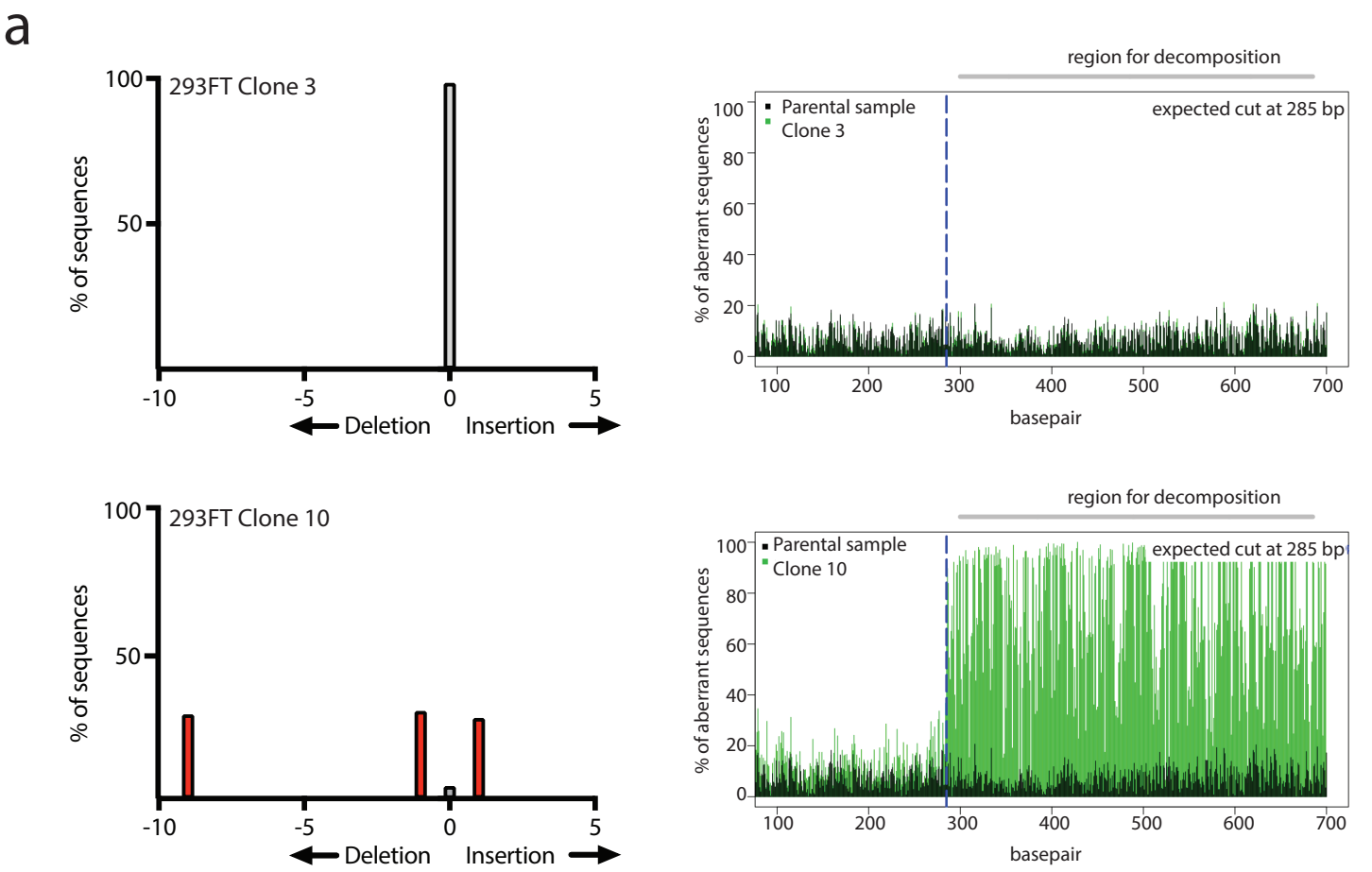


Supplementary Figure 2. Assessment of DFCI-4107 as a degrader of TRIM24

(a) Chemical structure of DFCI-4107. **(b)** Immunoblot of TRIM24 and Vinculin following 24 hours of treatment with indicated concentrations of DFCI-4107. Percentages were calculated by normalization of the band intensity to the loading control and relative to DMSO. This immunoblot was repeated three times, with one representative blot shown. Full immunoblot shown in Supplementary Fig. 11. **(c)** Intracellular VHL degron displacement assay with DFCI-4107 and control VL-269 (values represent means normalized to 50 μM VL-269 calculated from duplicate technical replicates). n=2 independent experiments with one representative experiment shown.

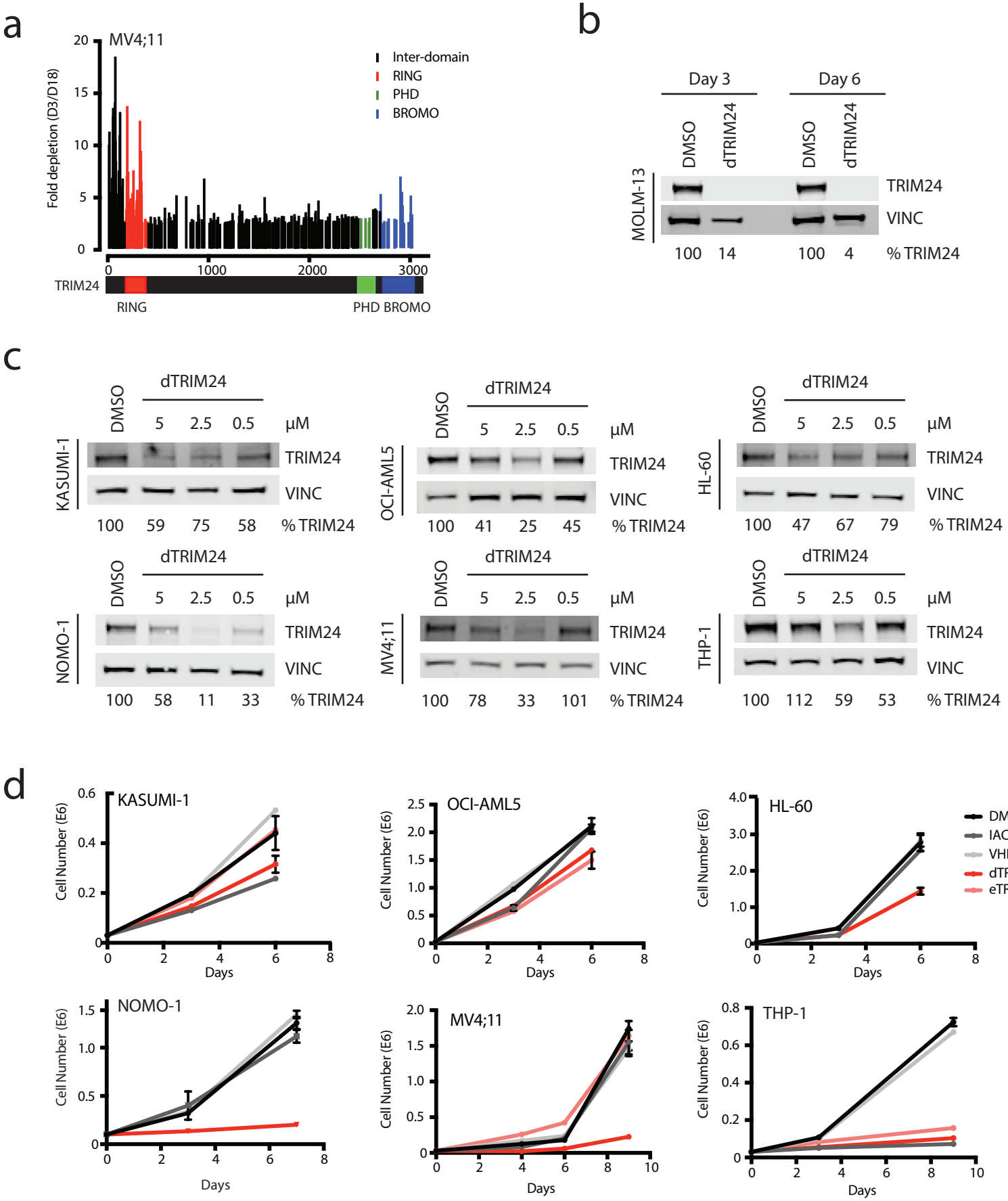
Supplementary Figure 3



Supplementary Figure 3. Expression status and perturbation of endogenous VHL

(a) Genetic validation of modification at the VHL locus in 293FT Clone 10, as compared to a sister clone, Clone 3, by Sanger sequencing of the VHL locus followed by TIDE analysis in singlicate. **(b)** Immunoblot of VHL (antibody: SantaCruz SC-17780) and Actin to validate the absence of VHL in the 786-O renal cell adenocarcinoma line by comparison to 293FT cells treated with NTC or ON-TARGETplus Human VHL siRNA (GE Dharmacon, INC # L-003936-00-0005) for 72 hours. **(c)** Immunoblot of TRIM24 and Actin following 24 hours of treatment with 5 μM IACS-7e or dTRIM24 in the VHL-null 786-O cell line. **(d)** VHL expression levels as detected by immunoblot in the cell lines employed in our studies (antibody: Cell Signaling 68547). For c-d, each immunoblot was repeated twice, with one representative blot shown. Full immunoblots shown in Supplementary Fig. 11. **(e)** mRNA expression level of VHL across the panel of cells used in the Cancer Cell Line Encyclopedia (CCLE) project[32], cell lines used in our study are highlighted in red and labeled.

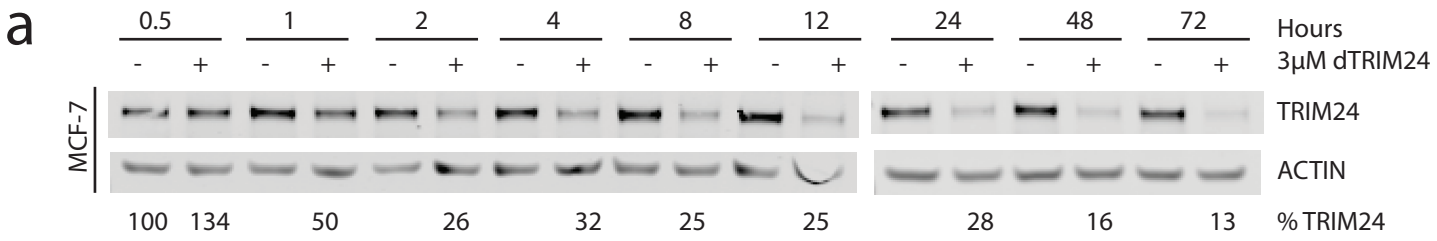
Supplementary Figure 4

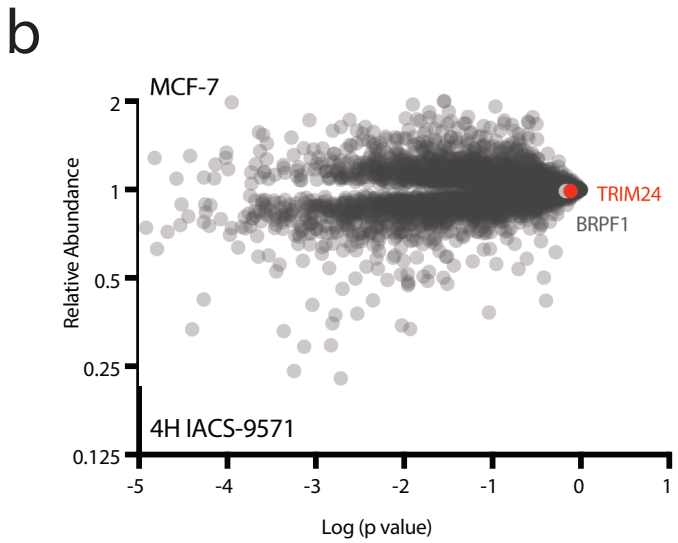


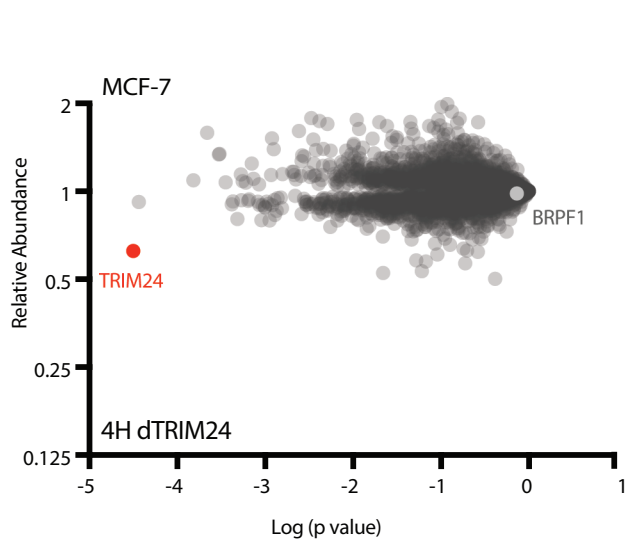
Supplementary Figure 4. Genetic and chemical modulation of TRIM24 in AML lines

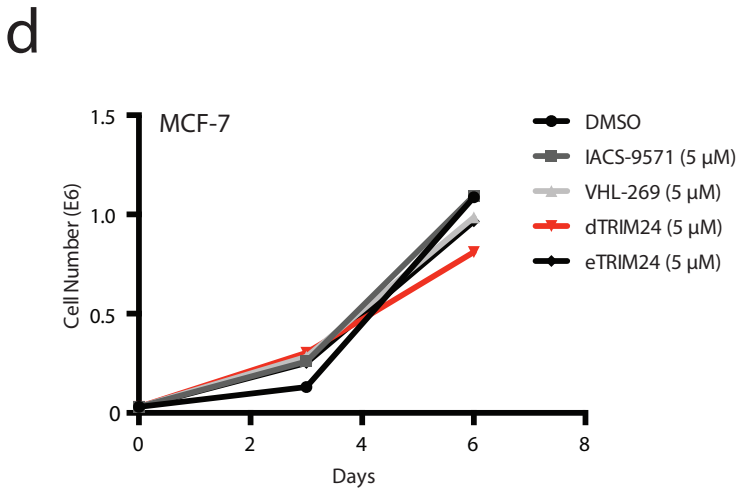
(a) CRISPR/Cas9-mediated mutagenesis tiling TRIM24 in MV4;11 cells stably expressing Cas9. After transduction with a pool of all sgRNAs, fold change from Day 3 to Day 18 was calculated for each guide by next generation sequencing at each timepoint, in singlicate. **(b)** Immunoblot of TRIM24 and Vinculin following 3 and 6-day incubation with 5 μM of dTRIM24 in MOLM-13 cells. **(c)** Immunoblots of TRIM24 and Vinculin following 24 hours of incubation with the indicated concentrations of dTRIM24 in AML cell lines. For b-c, percentages were calculated by normalization of the band intensity to the loading control and relative to DMSO. n=3 independent experiments with one representative experiment shown. Full immunoblots shown in Supplementary Fig. 11 and Supplementary Fig. 12. **(d)** Growth over time of KASUMI-1, OCI-AML5, HL-60, NOMO-1, MV4;11 and THP-1 cells treated with 5 μM of indicated compounds (values represent means normalized to DMSO +/- standard deviation, n=3 independently conducted experiments).

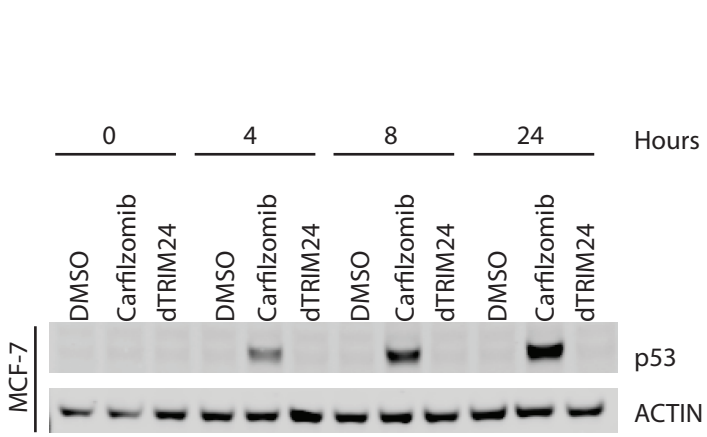
Supplementary Figure 5

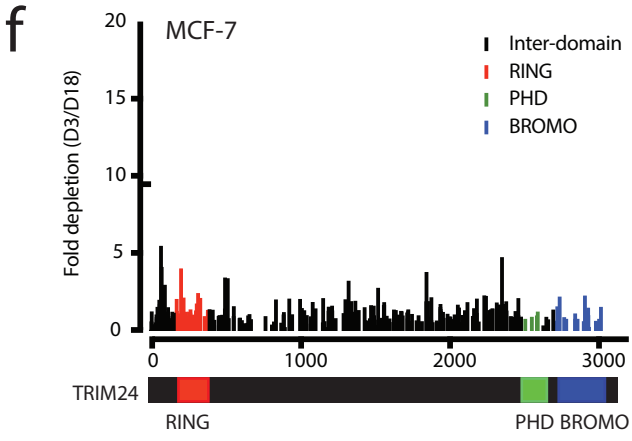








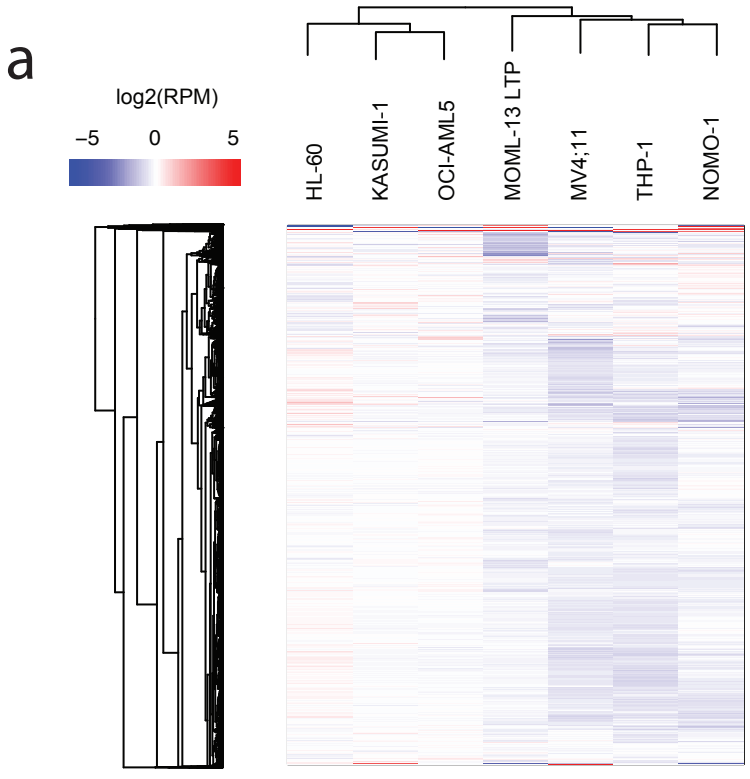


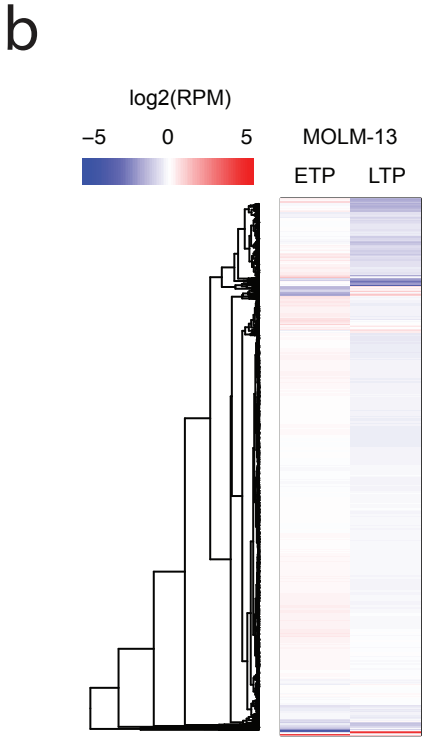


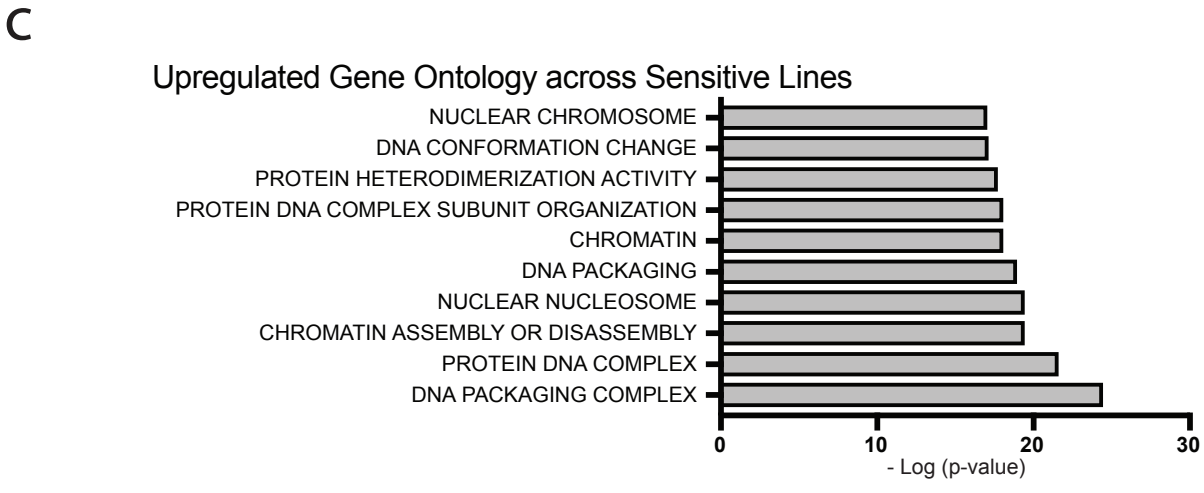
Supplementary Figure 5. Genetic and chemical modulation of TRIM24 in MCF-7

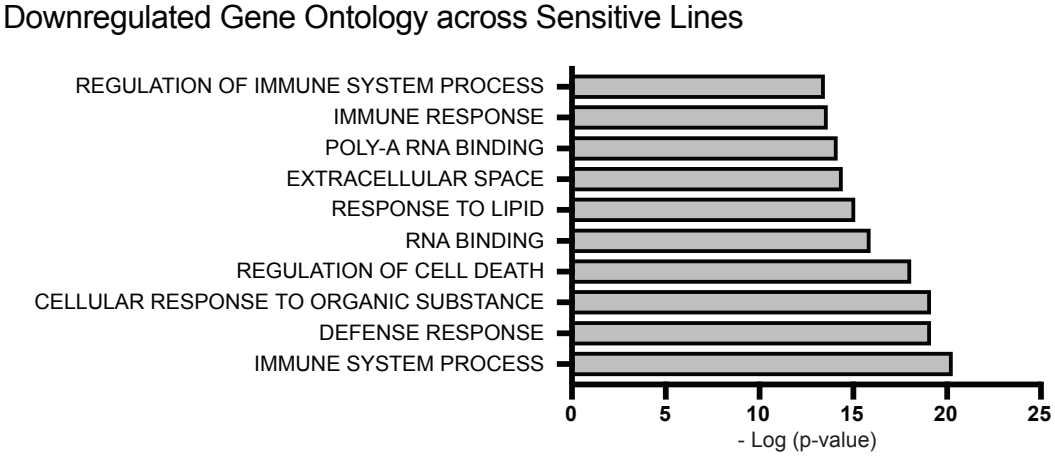
(a) Immunoblot of TRIM24 and Actin following treatment of MCF-7 cells with 3 μM dTRIM24 for the indicated incubation times. Percentages were calculated by normalization of the band intensity to the loading control and relative to DMSO at each timepoint. **(b)** Proteomic analysis in MCF-7 cells treated for 4 hours with DMSO, 3 μM of dTRIM24, or 3 μM of IACS-9571. Fold-change in the abundance of 6622 proteins from IACS-9571 to DMSO treatment versus p-value (t-test; triplicate analysis). **(c)** As described in (b), but comparing treatment of 3 μM dTRIM24 to DMSO. **(d)** Growth over time of MCF-7 cells treated with 5 μM of indicated compounds over 6 days (values represent means normalized to DMSO, calculated from n=3 technical replicates). **(e)** Immunoblot of p53 and Actin with MCF-7 cells treated with dTRIM24 or positive control carfilzomib for p53 stabilization at indicated durations of treatment. For a,e, n=2 independent experiments with one representative experiment shown. Full immunoblots shown in Supplementary Fig. 13. **(f)** CRISPR/Cas9-mediated mutagenesis tiling TRIM24 in MCF-7 cells stably expressing Cas9. After transduction with a pool of all sgRNAs, fold change from Day 3 to Day 18 was calculated for each guide by next generation sequencing at each timepoint in singlicate.

Supplementary Figure 6





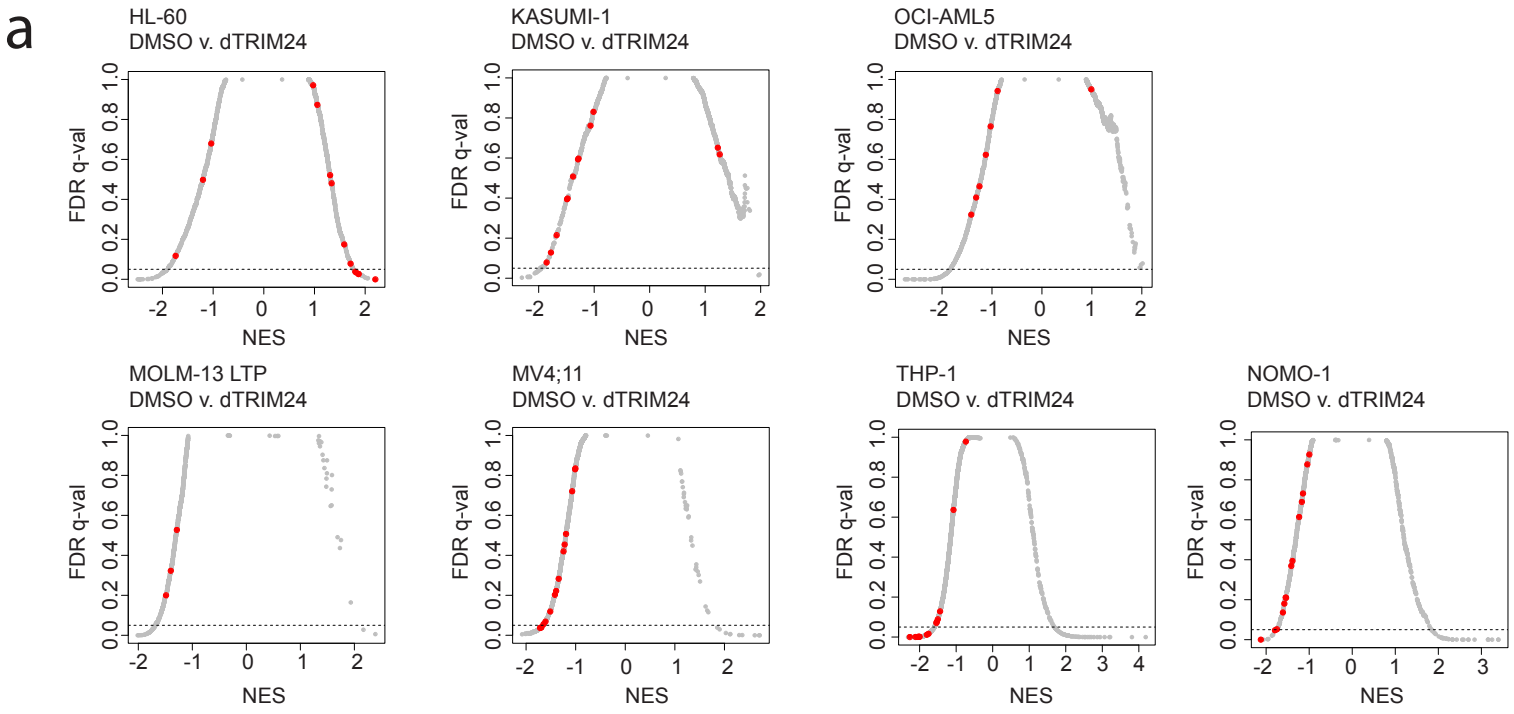


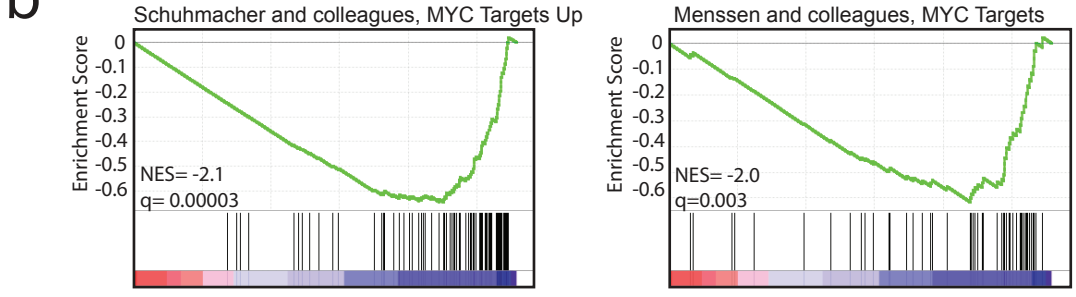


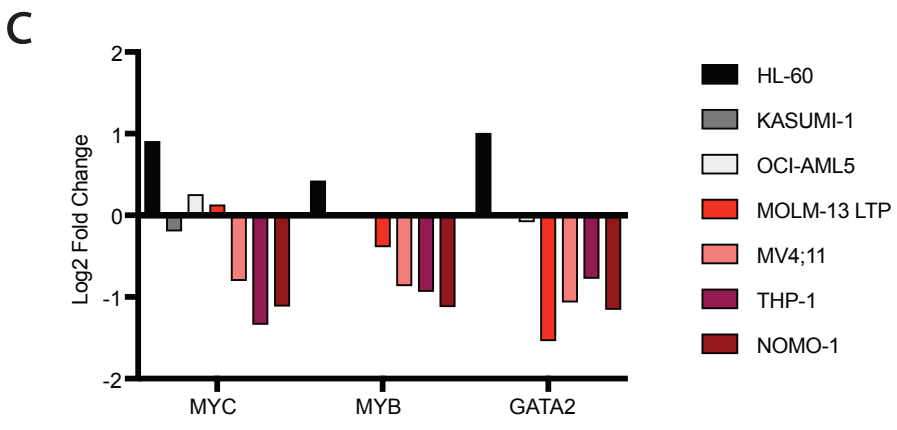
Supplementary Figure 6. Transcriptional profiling of AML lines with TRIM24 degradation

(a) Unbiased hierarchical clustering of the transcriptional profile of sensitive (MOLM-13 LTP, MV4;11, THP-1, NOMO-1) and insensitive (HL-60, KASUMI-1, OCI-AML5) cell lines to TRIM24 degradation after dTRIM24 treatment compared to DMSO. **(b)** Transcriptional profile of MOLM-13 cells at an Early Time Point (ETP) and Late Time Point (LTP) after dTRIM24 treatment. **(c)** Gene Ontology in sensitive lines for transcripts upregulated or downregulated at least 1.5 fold in the sensitive cell lines. For a-c, data represents biological triplicates.

Supplementary Figure 7



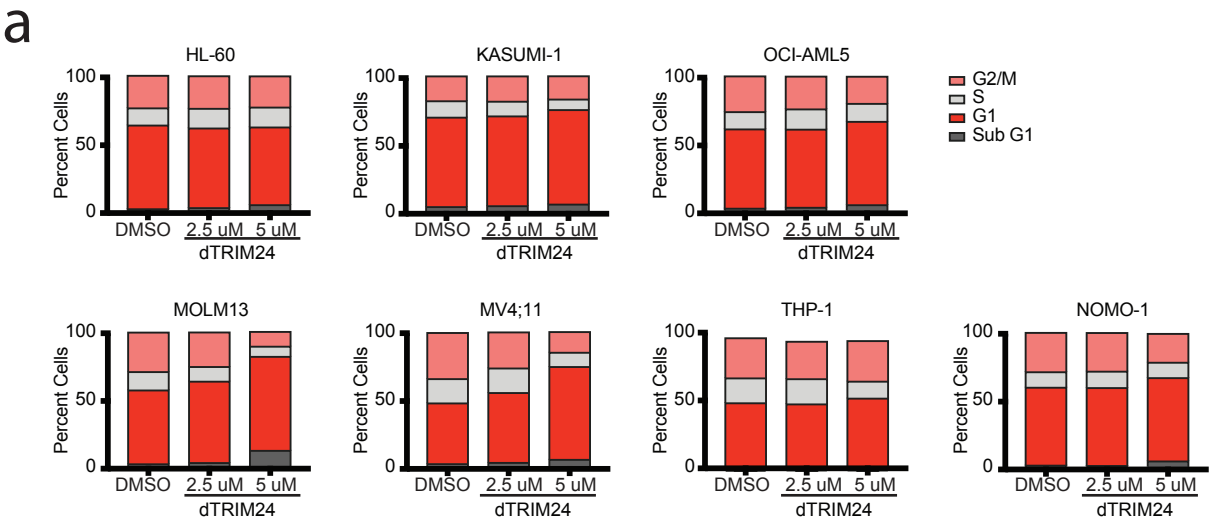


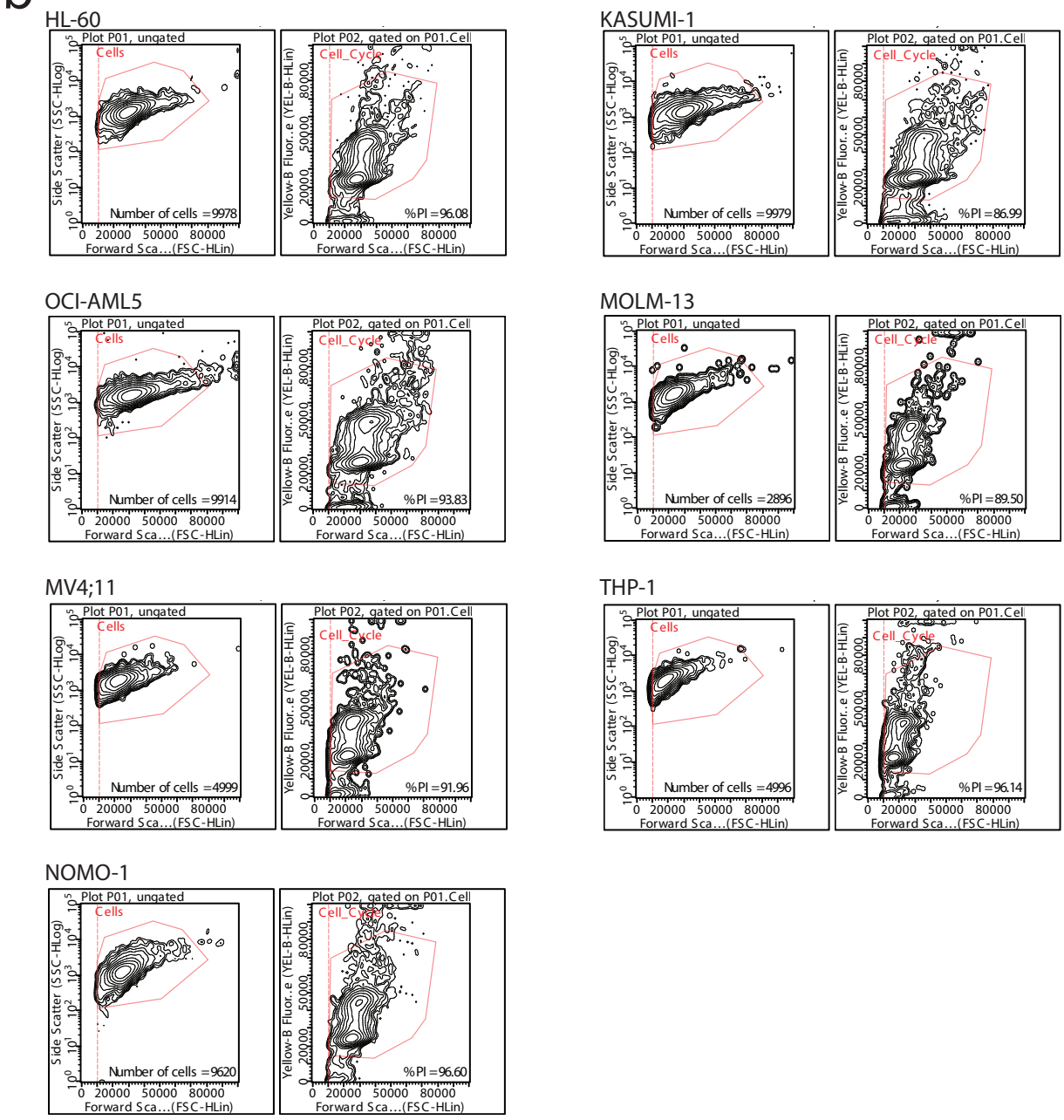


Supplementary Figure 7. Deregulation of master transcription factors in AML lines sensitive to dTRIM24

(a) Differential enrichment of the MYC upregulated signature across the AML lines tested, highlighted in red. **(b)** GSEA of two of the most enriched MYC gene sets in transcriptional profiles of the NOMO-1 cell line after treatment with dTRIM24. **(c)** Log2 fold change in master transcription factors, MYC, MYB, and GATA2 across sensitive and insensitive cell lines treated with dTRIM24. For a-c, data was derived from transcriptional fold change from biological triplicates in each cell line.

Supplementary Figure 8





Supplementary Figure 8. Cell cycle arrest induced by treatment with dTRIM24

(a) Cell cycle analysis of indicated cell lines treated with DMSO or dTRIM24 at 2.5 µM and 5 µM by flow cytometry (means calculated from n=3 technical replicates). **(b)** Gating strategy for cell cycle analysis with the DMSO replicate 1 as an example per cell line. The first gate indicates FCS/SSC to select the single cell population and the second gate indicates FCS/Yellow-B-Fluorescence to select the positive propidium iodide stained population.

Supplementary Figure 9. Full blots for indicated figure panels.

Figure 1F: 293FT

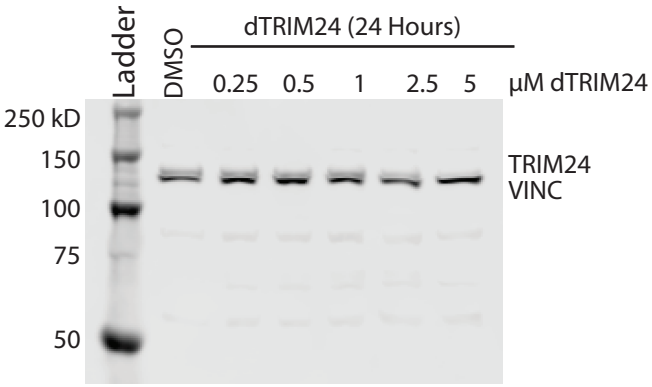


Figure 1G: 293FT

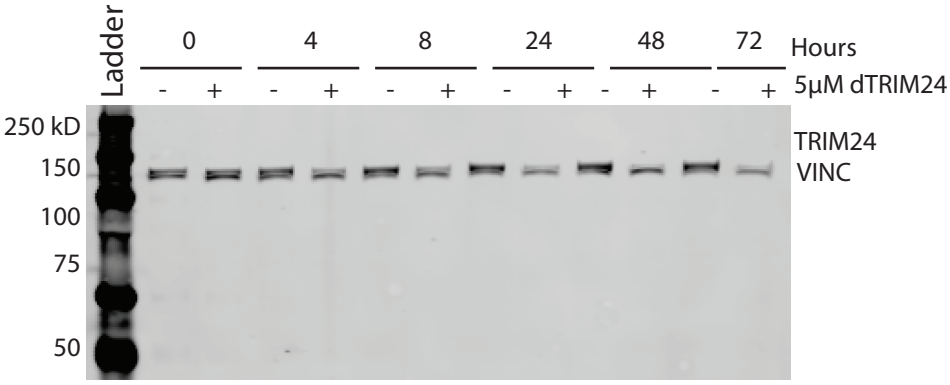


Figure 2A: 293FT

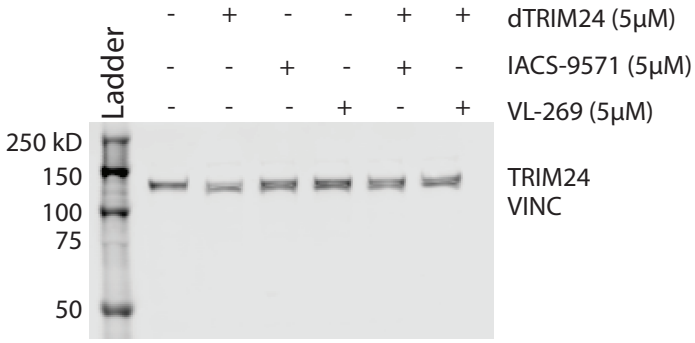


Figure 2B: 293FT

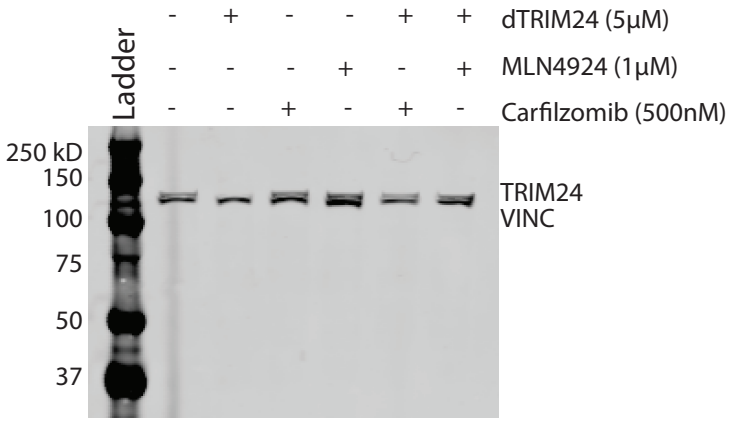


Figure 2C: 293FT

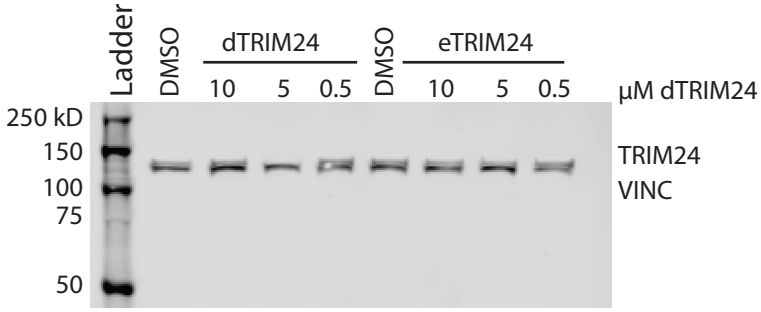


Figure 2D: 293FT

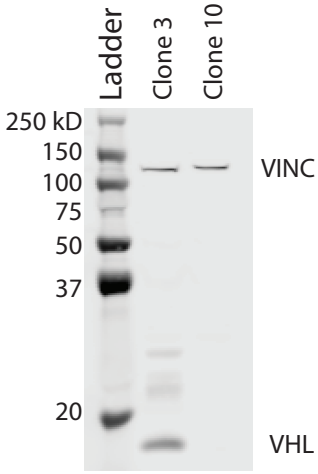
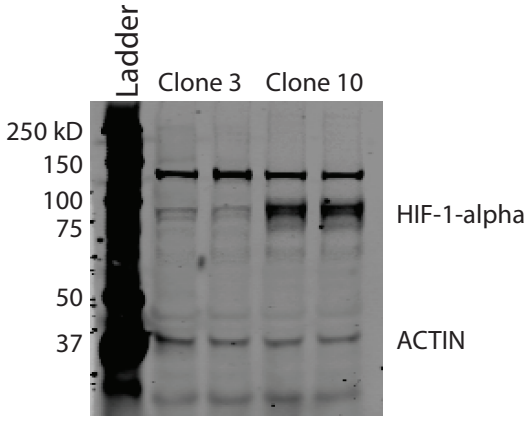


Figure 2E: 293FT



Supplementary Figure 10. Full blots for indicated figure panels.

Figure 2F: 293FT

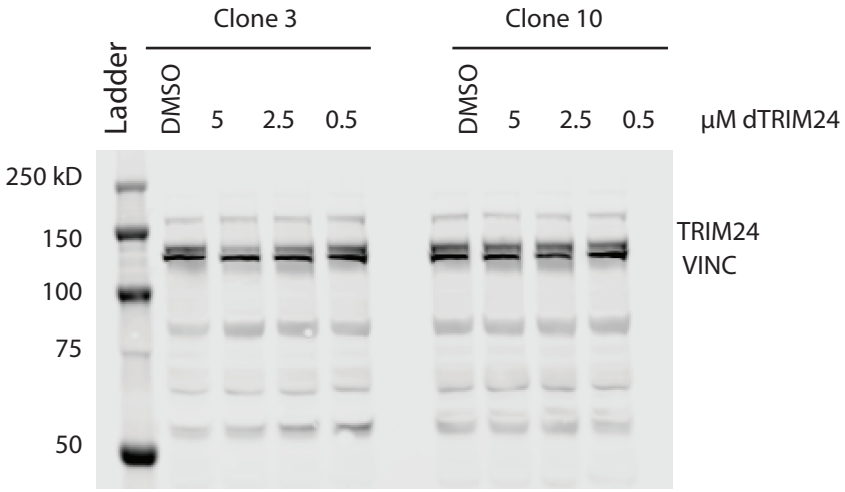


Figure 4A: MOLM-13

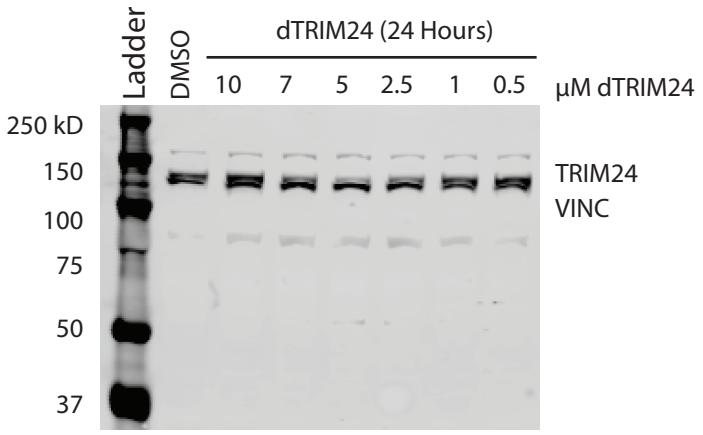


Figure 4B: MOLM-13

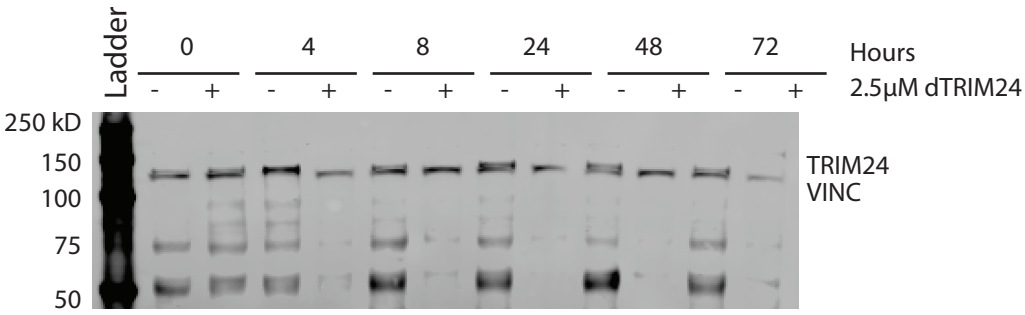
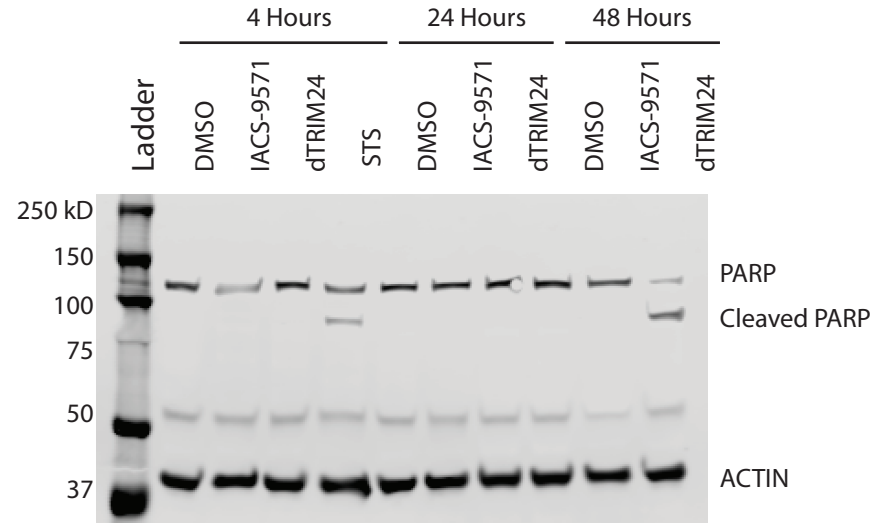
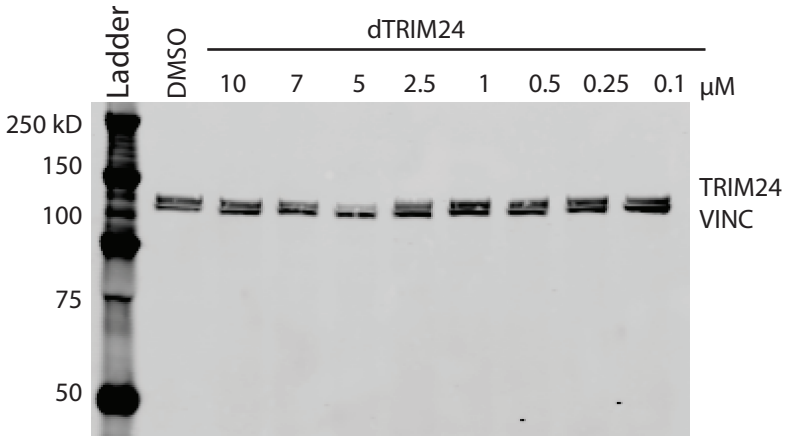


Figure 4F: MOLM-13

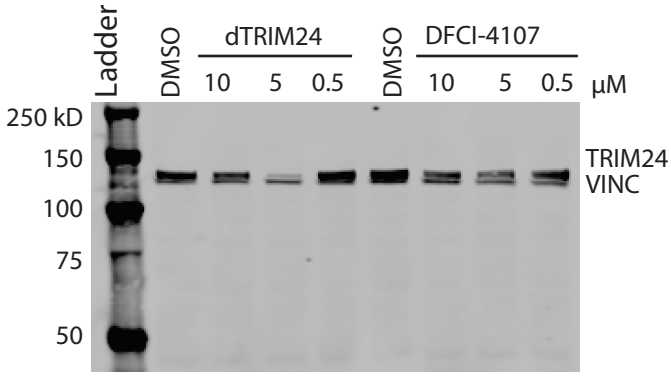


Supplementary Figure 11. Full blots for indicated figure panels.

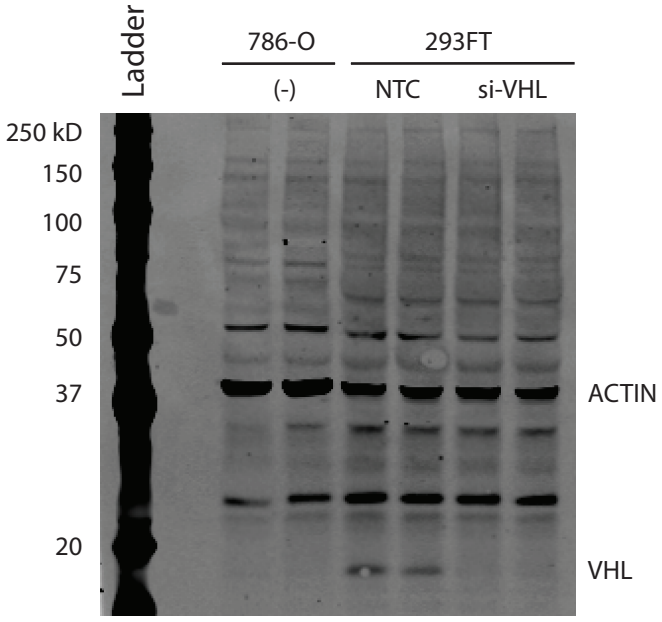
Supplementary Figure 1D: 293FT



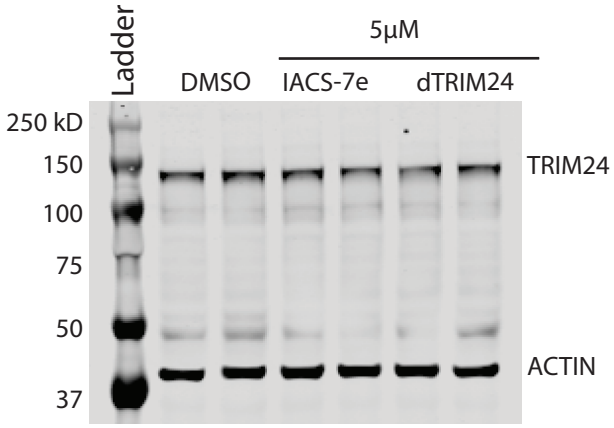
Supplementary Figure 2B: 293FT



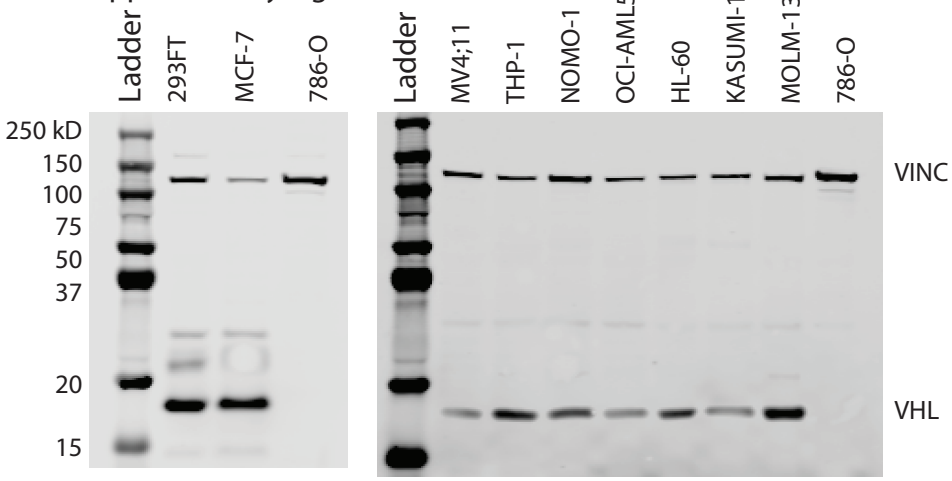
Supplementary Figure 3B



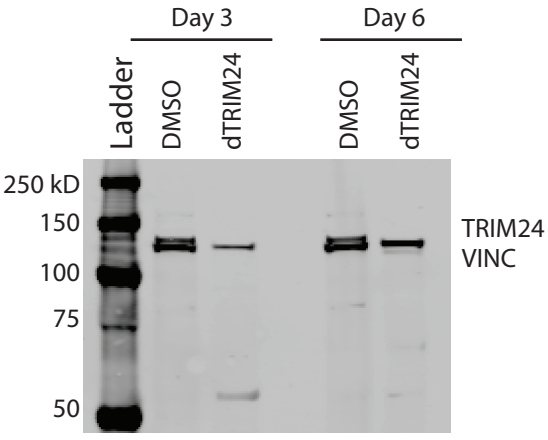
Supplementary Figure 3C: 786-O



Supplementary Figure 3D



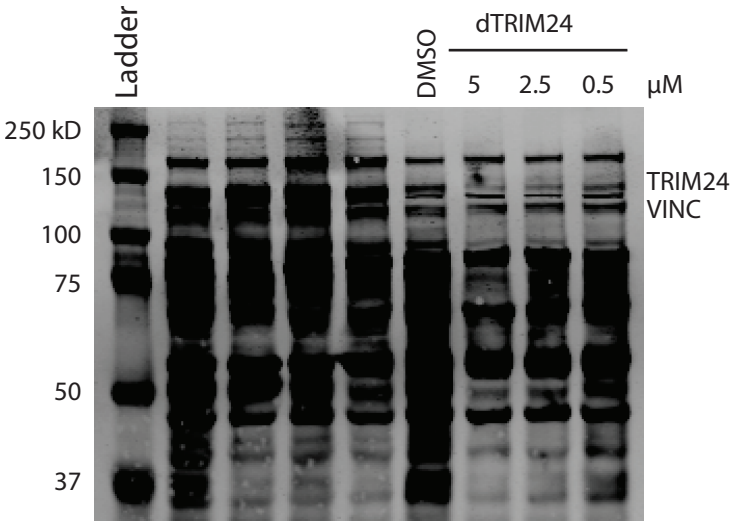
Supplementary Figure 4B: MOLM-13



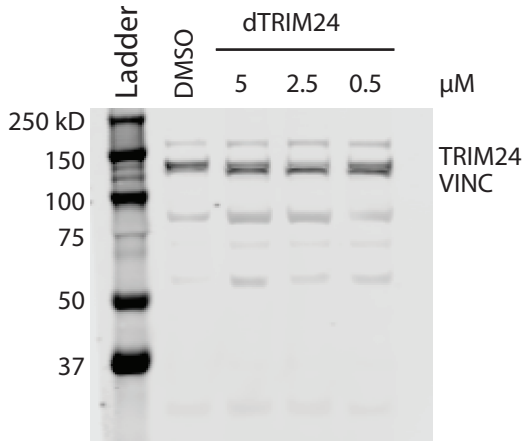
Supplementary Figure 12. Full blots for indicated figure panels.

Supplementary Figure 4C

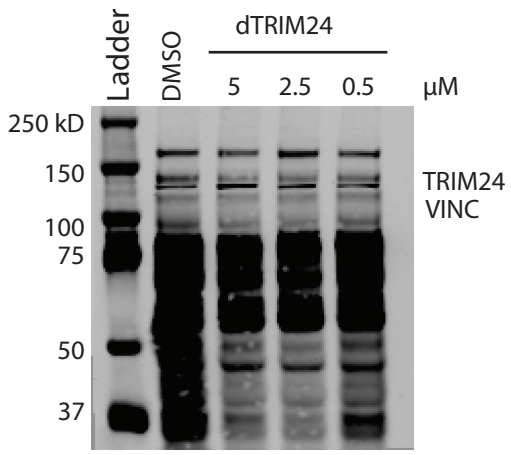
KASUMI-1



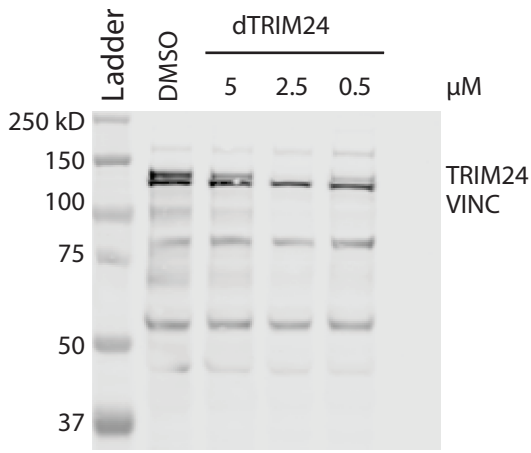
OCI-AML5



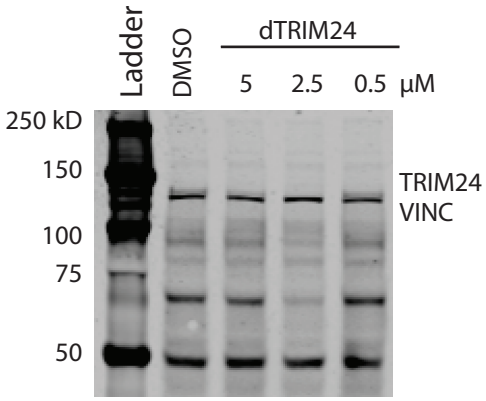
HL-60



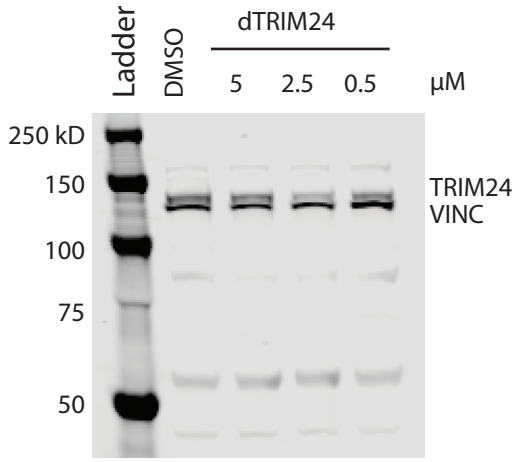
NOMO-1



MV4;11

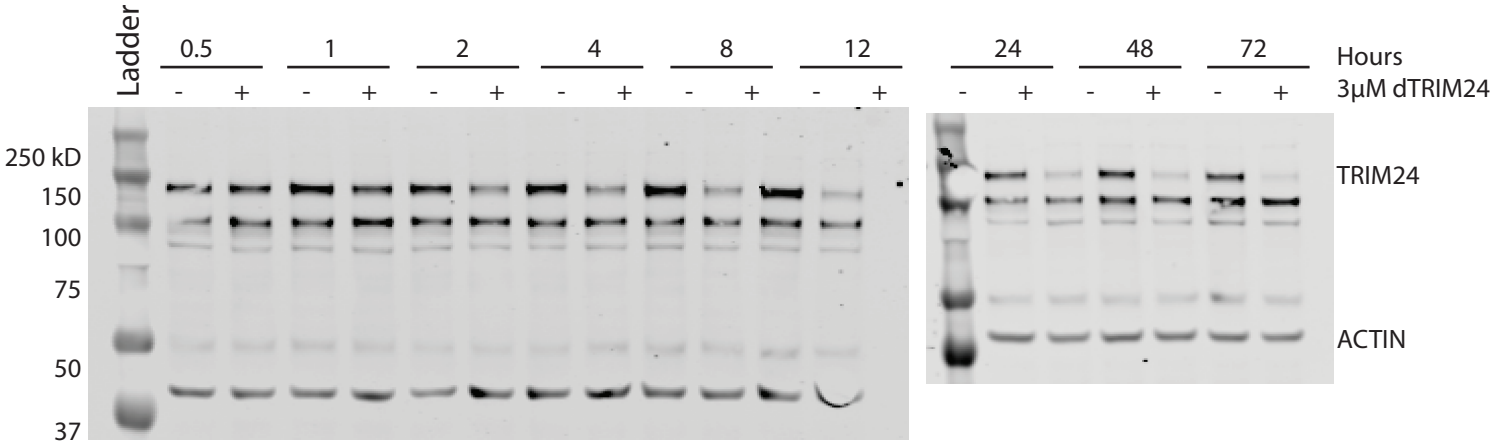


THP-1

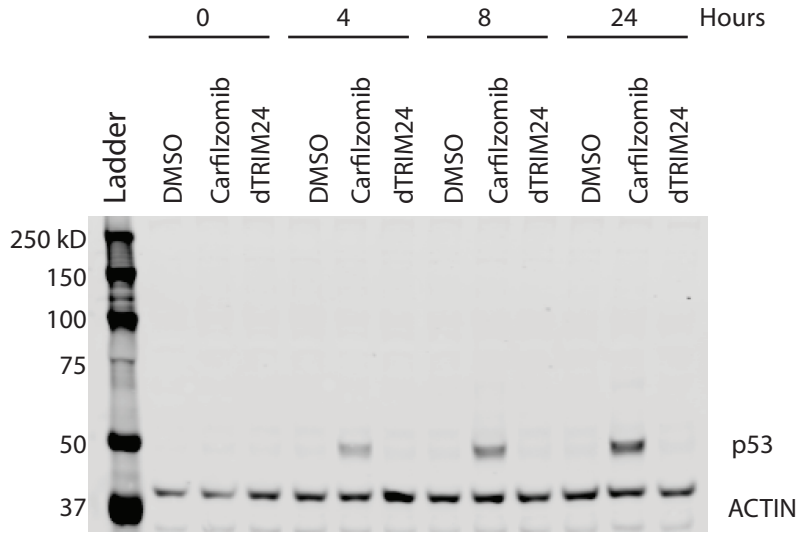


Supplementary Figure 13. Full blots for indicated figure panels.

Supplementary Figure 5A: MCF-7



Supplementary Figure 5e: MCF-7



SUPPLEMENTARY DATASETS

Supplementary Dataset 1: dTRIM24 Proteomics MCF-7

Supplementary Dataset 2: dTRIM24 Proteomics MOLM-13