Supplementary Figure S1

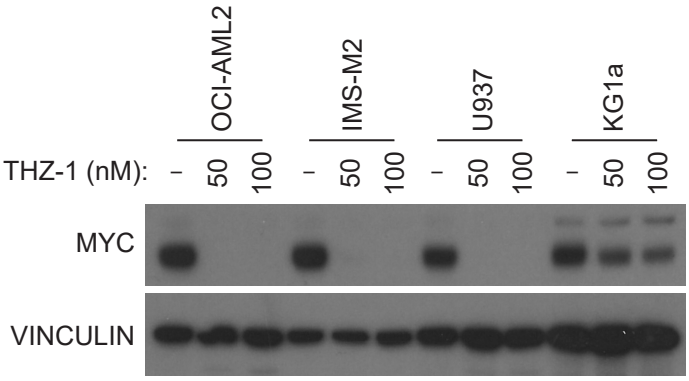


Figure S1: CDK7 inhibitor, THZ-1, decreases MYC expression in AML cells.

Immunoblot for MYC and VINCULIN (loading control) from indicated human AML cell lines treated with indicated THZ-1 concentrations for 24 hours.

Supplementary Figure S2

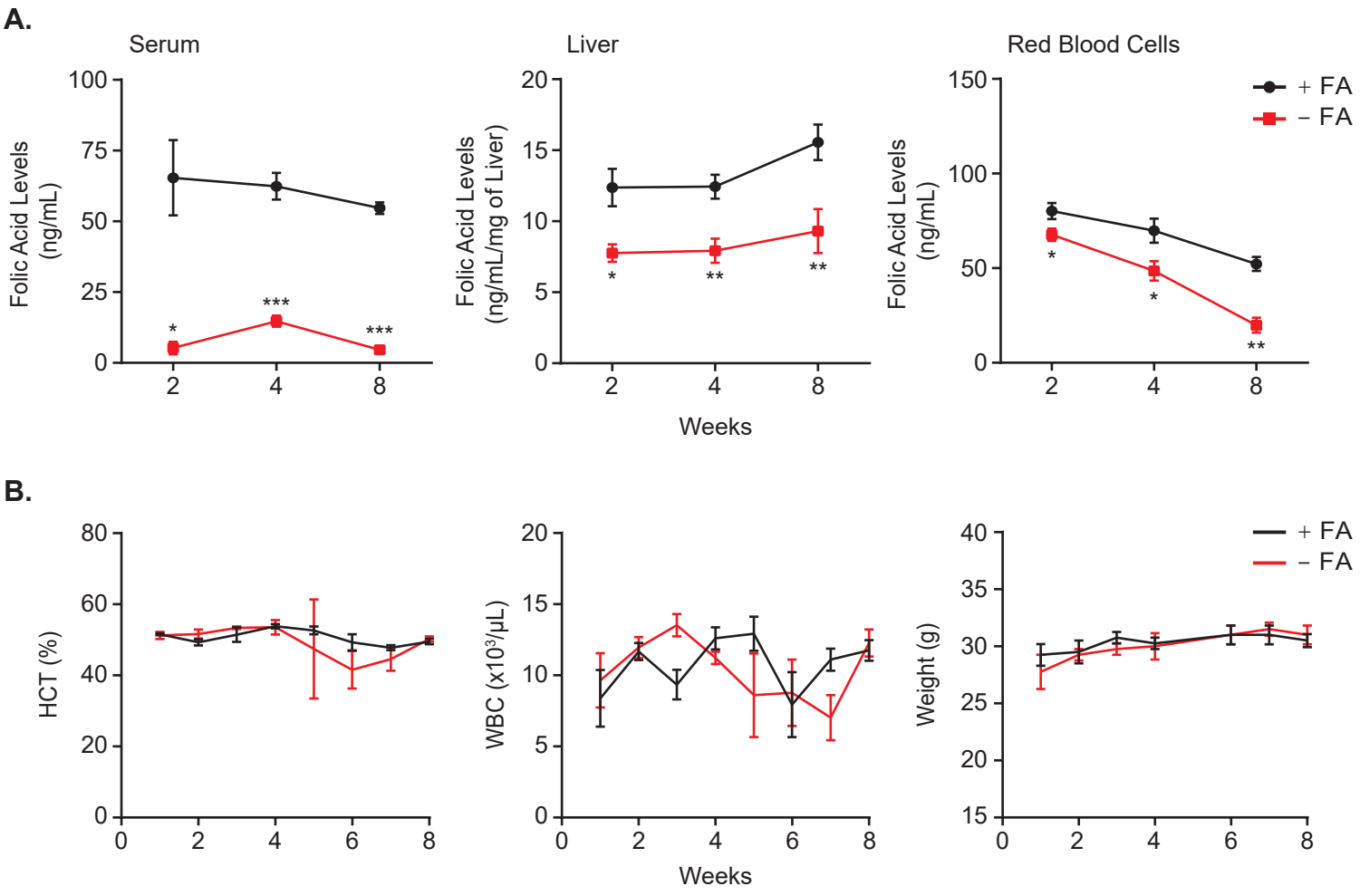


Figure S2: Folic acid withdrawal does not significantly affect mouse hematocrit, white blood count, and weight.

(A) Folic acid levels in serum, liver, and red blood cells (RBC) over a period of 8 weeks from three mice per group fed with either regular or folic acid-restricted diet. *p-value ≤ 0.05, ** ≤ 0.01, and *** ≤ 0.001 by Welch's t-test. Error bars represent mean ± SD.

(B) Effect of regular or folic acid-restricted diet on mouse hematocrit (HCT), white blood cell count (WBC), and weight over a period of 8 weeks from four mice per group. Error bars represent mean ± SD of four technical replicates.

Supplementary Figure S3

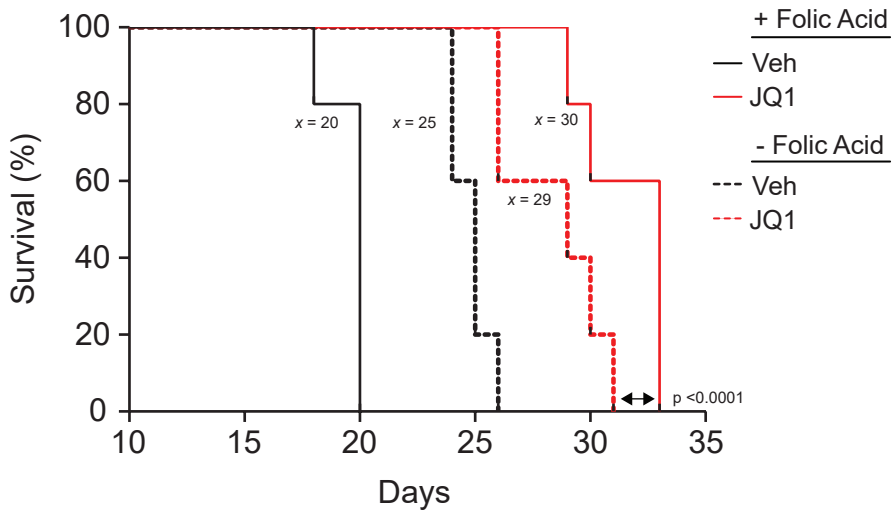


Figure S3: Folic acid withdrawal attenuates leukemic blast response to JQ1.

Kaplan-Meier curves showing overall survival of mice (n = 5 for each group) transplanted with MLL-AF9-positive blasts, fed with either regular or folic acid-restricted diet and treated with either vehicle or 35mg/kg JQ1 for 7 days. *X*, median survival. Statistical significance by log-rank (Mantel-Cox) test.

Supplementary Figure S4

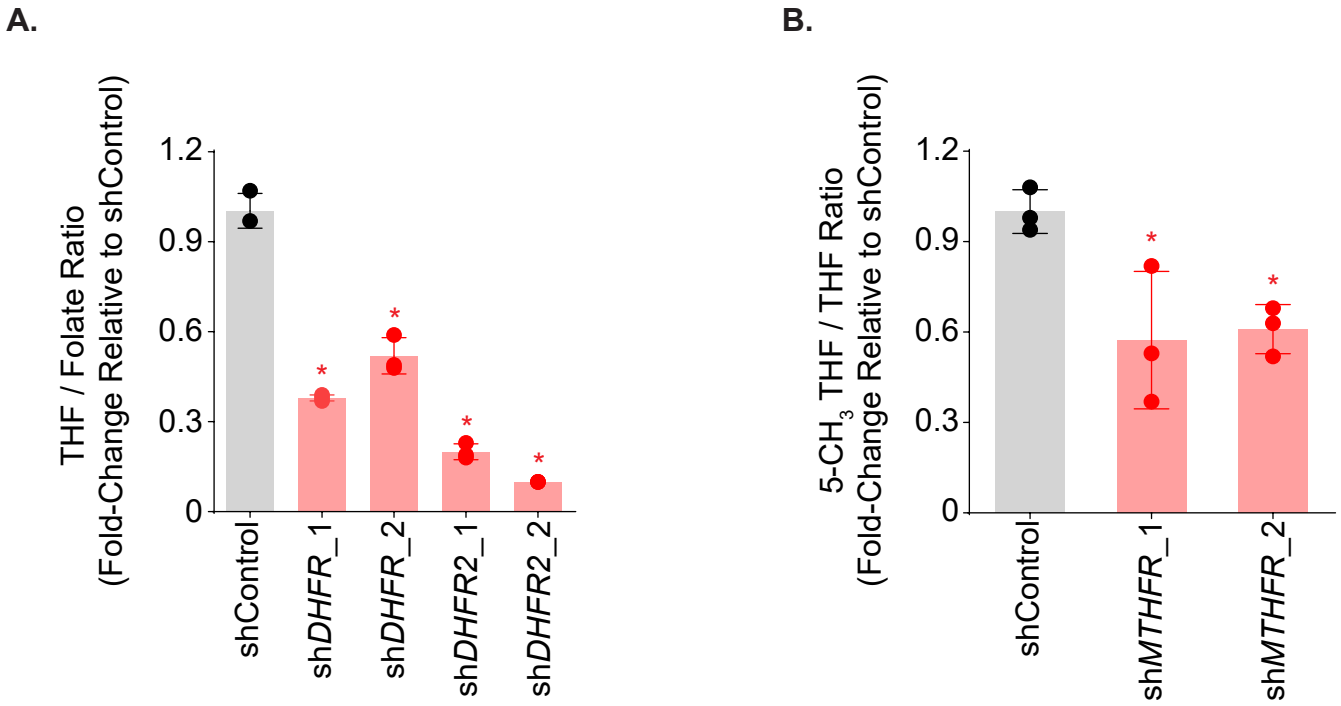


Figure S4: *DHFR*, *DHFR2*, and *MTHFR* knockdown impairs ratios of their respective primary and end-product metabolites.

(A) Fold change in THF/Folate ratio in IMS-M2 cells infected with either a control, two *DHFR*-directed shRNAs (sh*DHFR _1* and sh*DHFR _2*), or *DHFR2*-directed shRNAs (sh*DHFR2 _1* and sh*DHFR2 _2*).

(B) Fold change in 5-CH3 THF/THF ratio in IMS-M2 cells infected with either a control or two *MTHFR*-directed shRNAs (sh*MTHFR _1* and sh*MTHFR _2*).

(A-B) *p-value ≤ 0.05 by t-test. Error bars represent mean ± SD of three biological replicates.

Supplementary Figure S5

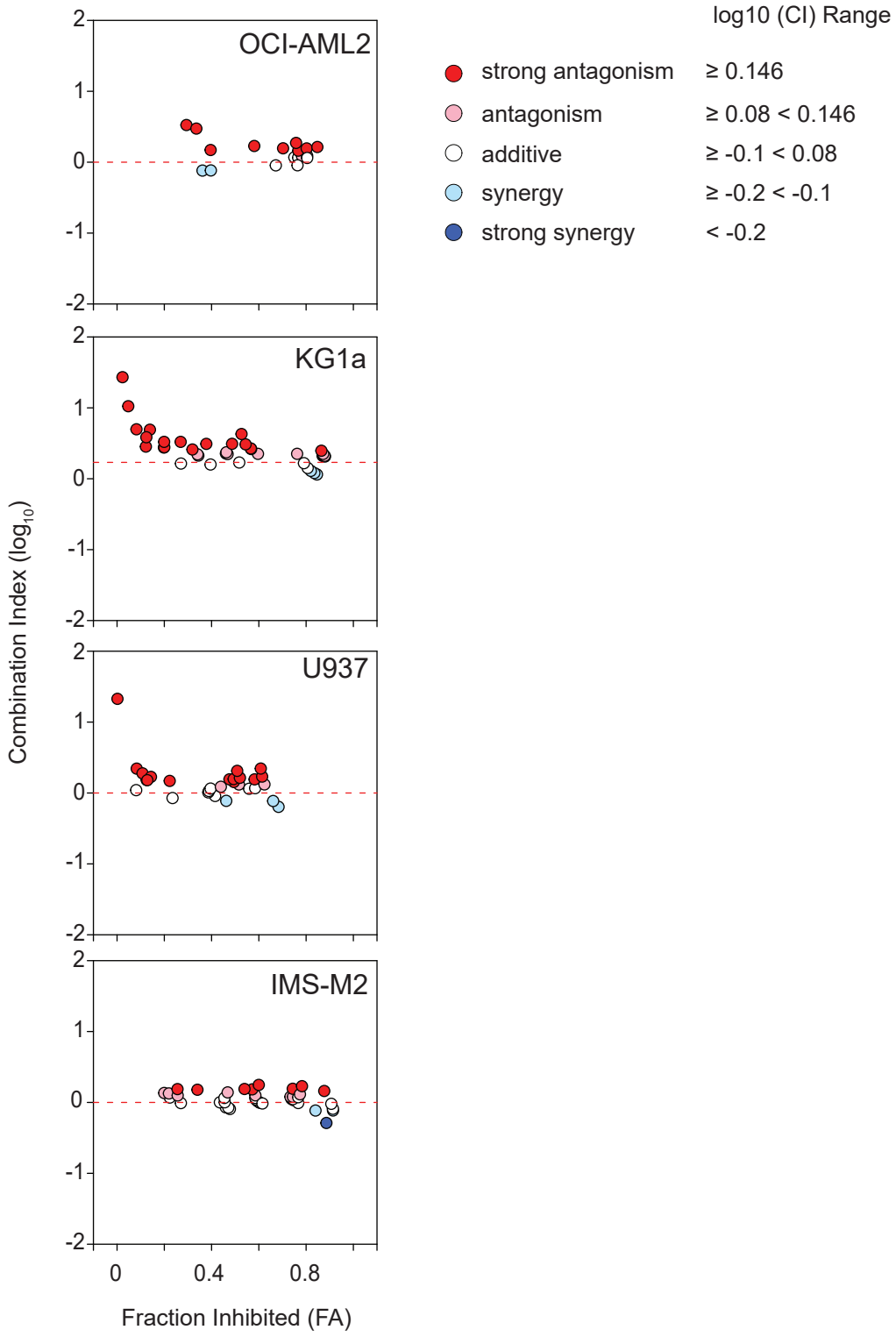


Figure S5: Methotrexate and OTX015 are antagonistic in various AML cell lines.

Combination index analysis for the combinations of OTX015 with Methotrexate in the indicated cell lines treated for 3 days in four technical replicates.

Supplementary Figure S6

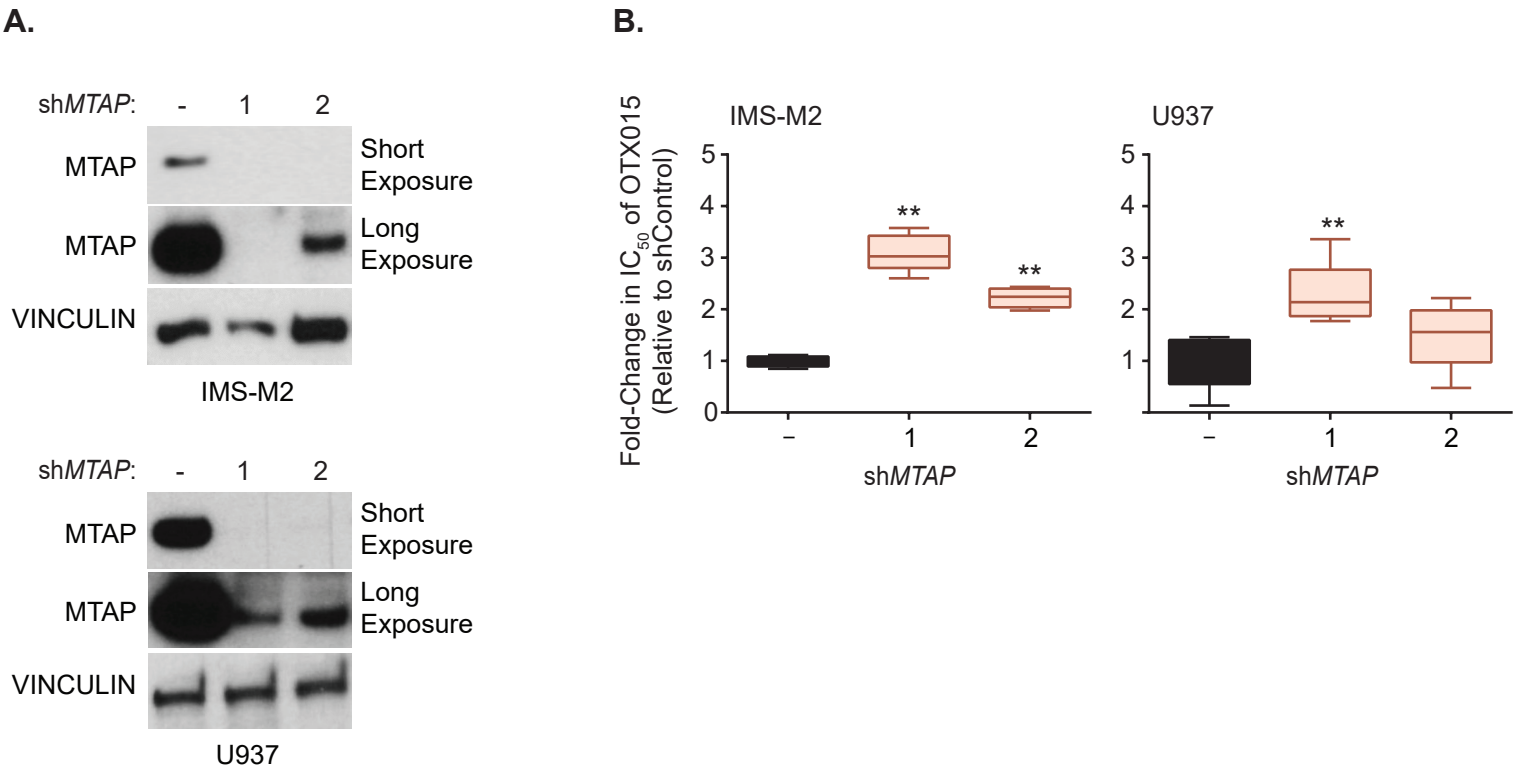


Figure S6: *MTAP* suppression modulates response to BET inhibitors.

(A) Immunoblot for MTAP and VINCULIN (loading control) from human IMS-M2 and U937 cell lines infected with either a control or two *MTAP*-directed sh*RNAs*.

(B) Fold change in IC50 of OTX015 for 5 days in IMS-M2 and U937 cell lines infected with either a control or two *MTAP*-directed shRNAs (sh*MTAP_1* or sh*MTAP_2*). Results shown as fold change of IC50 normalized to average shControl. **p-value ≤ 0.01 by nonparametric Mann-Whitney test. Error bars represent mean ± SD of five technical replicates.

Supplementary Figure S7

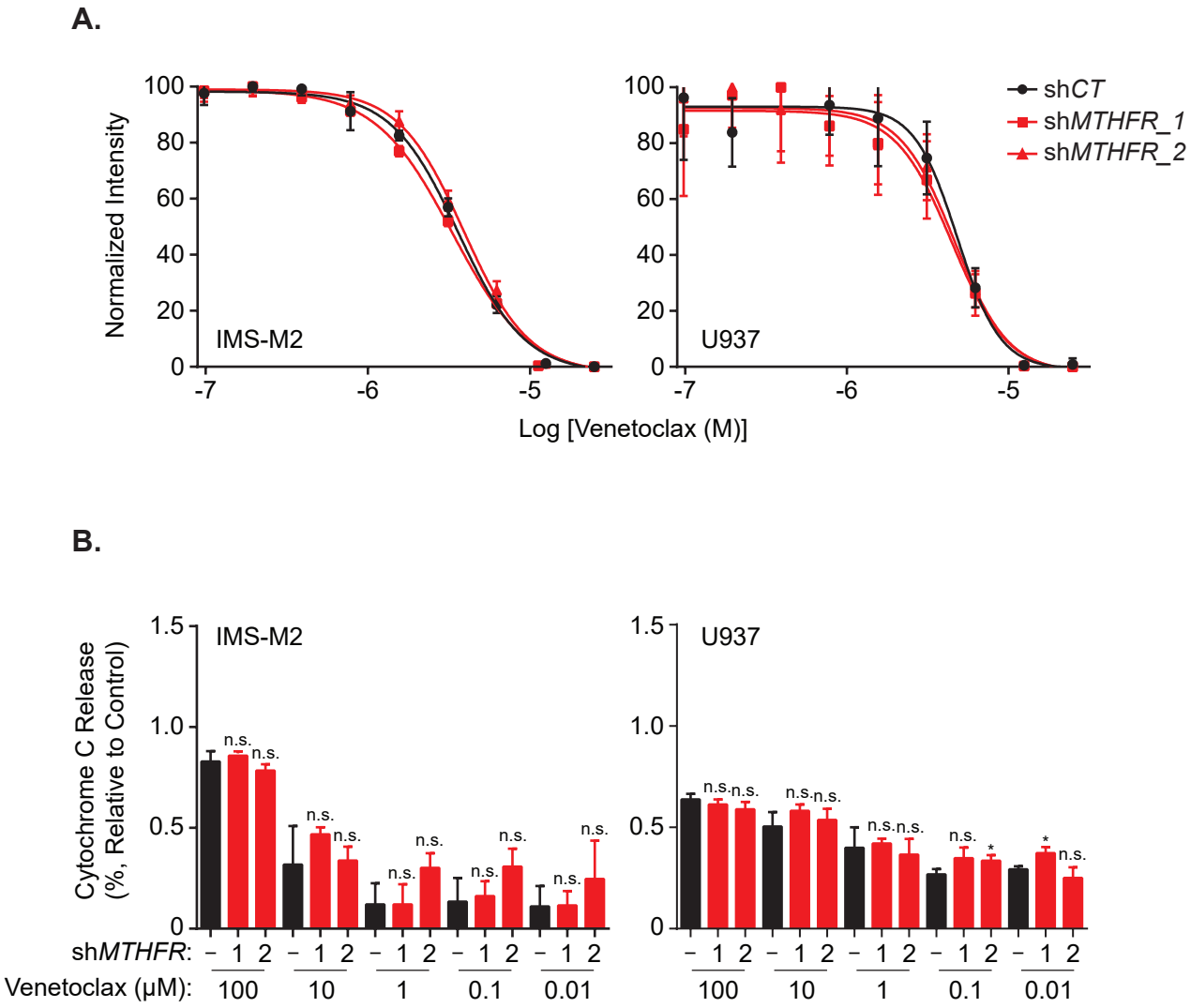


Figure S7: MTHFR suppression does not alter AML cell response to venetoclax.

(A) Growth inhibition of indicated IMS-M2 and U937 cells infected with either a control or two *MTHFR*-directed shRNAs (sh*MTHFR_1* and sh*MTHFR_2*) and treated with increasing concentration of venetoclax for 5 days. Error bars represent mean ± SD of five technical replicates.

(B) BH3 profiling of IMS-M2 and U937 AML cell lines infected with either a control or two *MTHFR*-directed shRNAs (sh*MTHFR_1* and sh*MTHFR_2*) exposed to DMSO or serial concentrations of venetoclax for 1 hour. *p-value ≤ 0.05 by Welch'st-test. n.s, nonsignificant (p > 0.05). Error bars represent mean ± SD.

Supplementary Figure S8

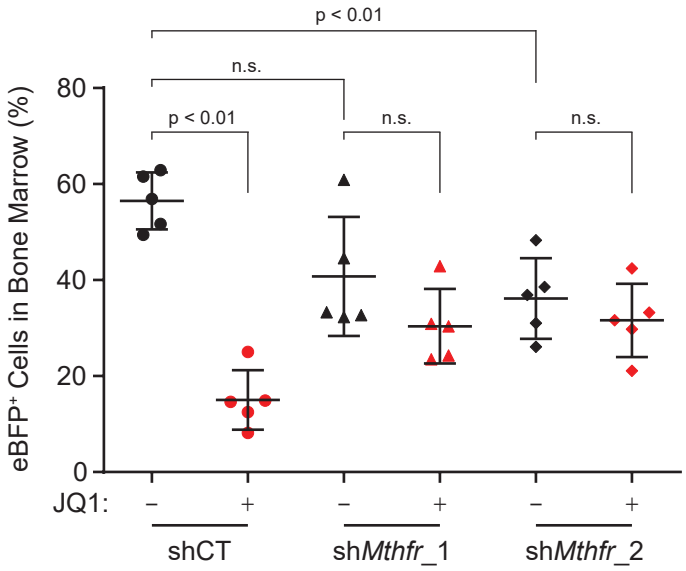


Figure S8: MTHFR suppression attenuates the anti-leukemic effect of JQ1 in an Cbfb-MYH11-driven AML mouse model.

Proportion of eBFP-positive Cbfb-MYH11-driven leukemic cells infected with either a control (shCT) or two *Mthfr*-directed shRNAs (sh*Mthfr_1* and sh*Mthfr_2*) in bone marrow from five mice per group treated with either vehicle or 35mg/kg JQ1 for 7 days. p-value by Mann-Whitney test. n.s, nonsignificant ($p > 0.05$). Error bars represent mean ± SD.

Supplementary Figure S9

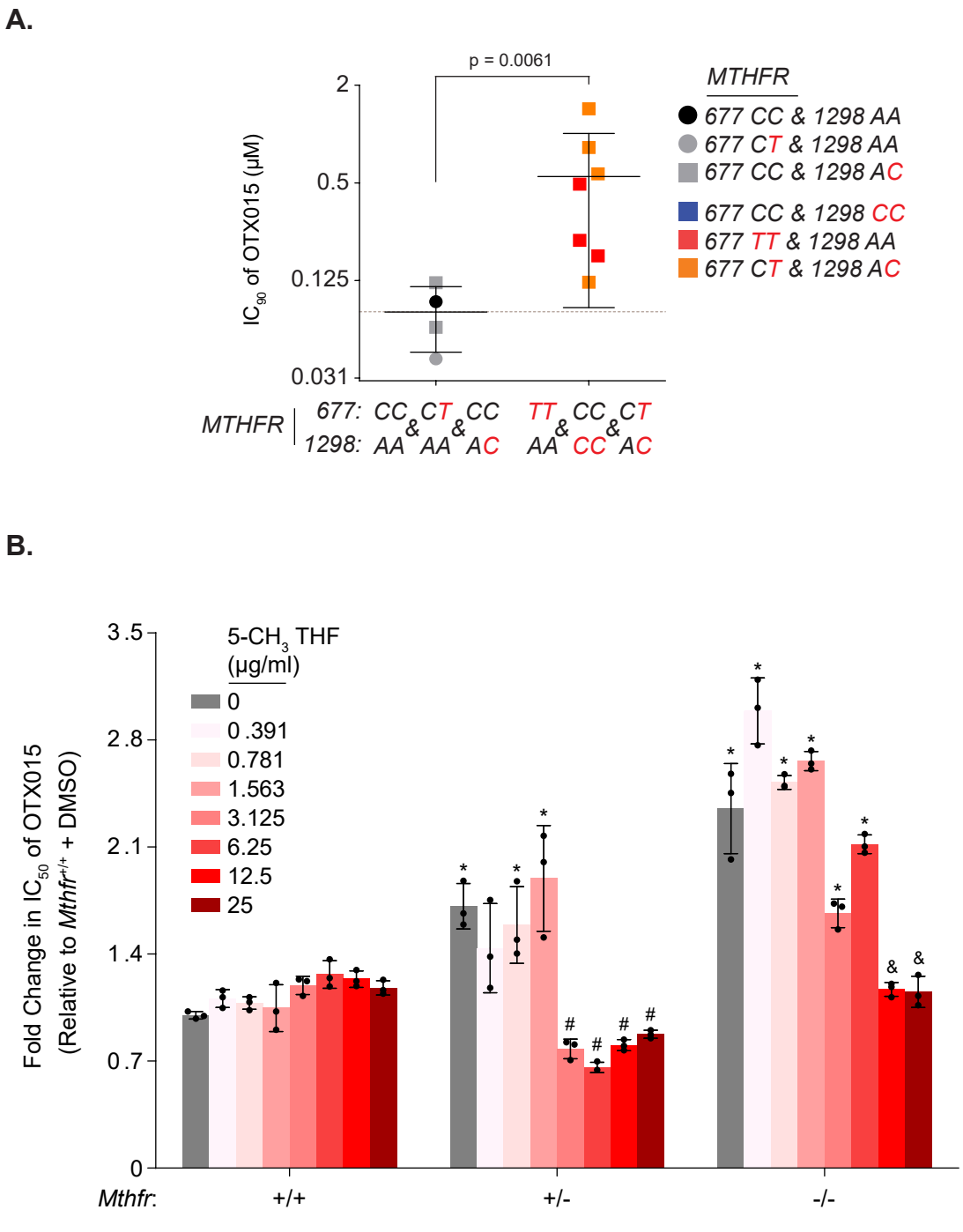


Figure S9: Effect of MTHFR impairment on response to OTX015.

(A) Distribution of IC90 with OTX015 for 5 days of 11 patient samples with Core Binding Factor (CBF) AML divided into 2 subgroups according to *MTHFR* genetic status at C677, A1298. p-value by nonparametric Mann-Whitney test. Error bars represent mean ± SD.

(B) Fold change in IC50 of OTX015 for 5 days in *MLL-AF9*-driven homozygous wild-type (+/+), heterozygous (+/-), and homozygous (-/-) *Mthfr* knockout cells treated with increasing concentration of 5-CH3 THF. *p-value ≤ 0.001 by two-way ANOVA test in comparison with the analogous concentration of 5-CH3 THF in +/+ *Mthfr* cells, # ≤ 0.001 in comparison with 0 µg/ml 5-CH3 THF condition in +/- *Mthfr* cells, & ≤ 0.001 in comparison with 0 µg/ml 5-CH3 THF condition in -/- *Mthfr* cells. Error bars represent mean ± SD.

Supplementary Figure S10

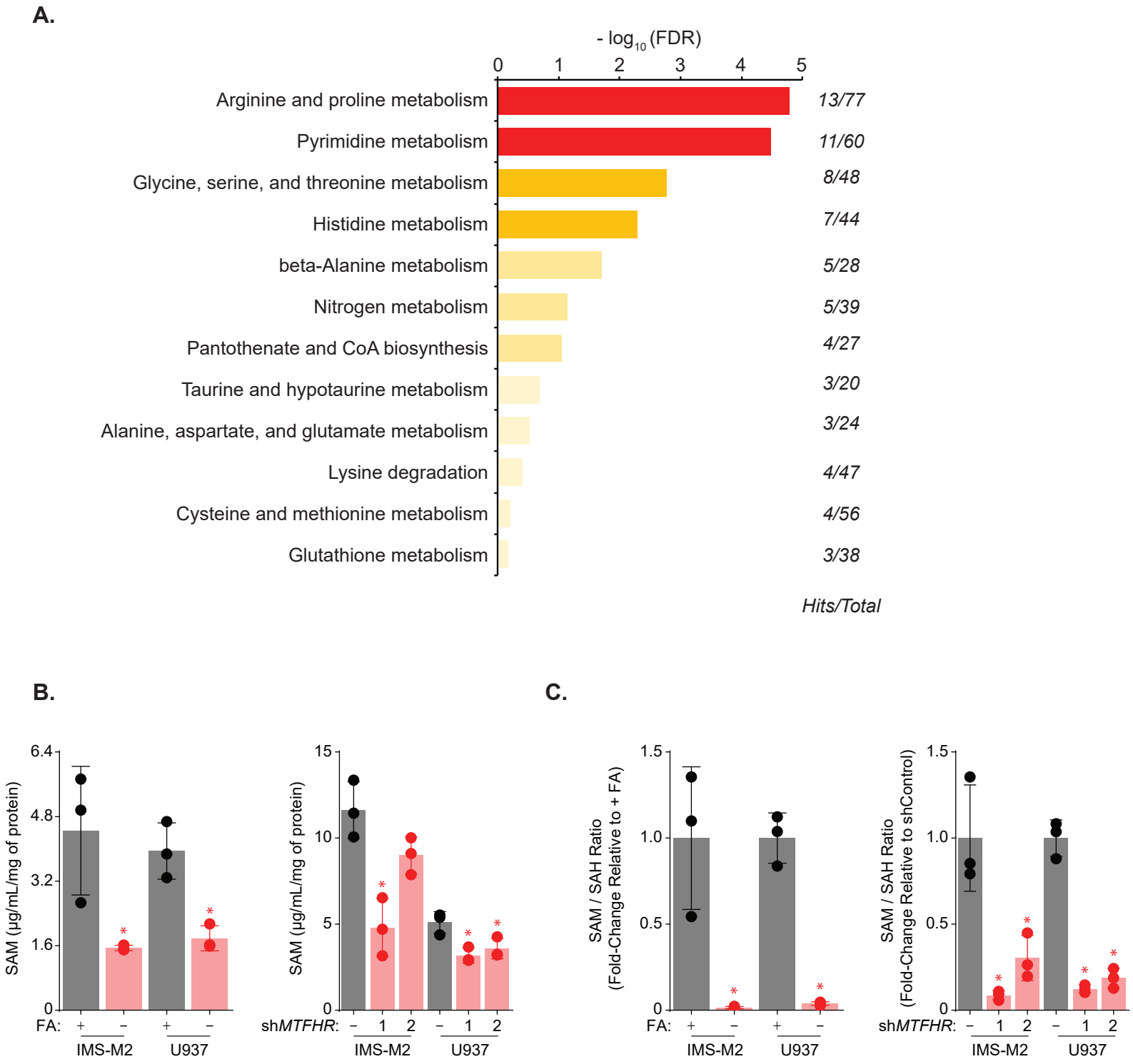


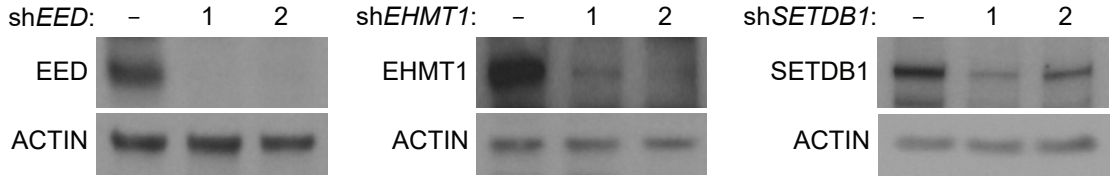
Figure S10: Metabolic perturbations induced by folate starvation in AML cells.

(A) Pathway analysis integrating enrichment and pathway topology analyses (MetaboAnalyst) of top metabolites from panel **(A)** in Figure 4. Pathways ranked by FDR. "Hits" represents number of metabolites that scored in steady-state profile and "Total" represents number of metabolites present in given metabolic pathway.

(B-C) SAM levels **(B)** and **(C)** SAM/SAH ratio in indicated human AML cell lines with folic acid (FA) withdrawal (left panel) or MTHFR suppression using two *MTHFR*-directed shRNAs (sh*MTHFR_1* and sh*MTHFR*_2, right panel). *p-value ≤ 0.05 by Welch's t-test versus respective control. Error bars represent mean ± SD of three technical replicates.
(A-C) + FA = 1000 ng/mL folic acid, - FA = 0 ng/mL folic acid.

Supplementary Figure S11

A.



B.

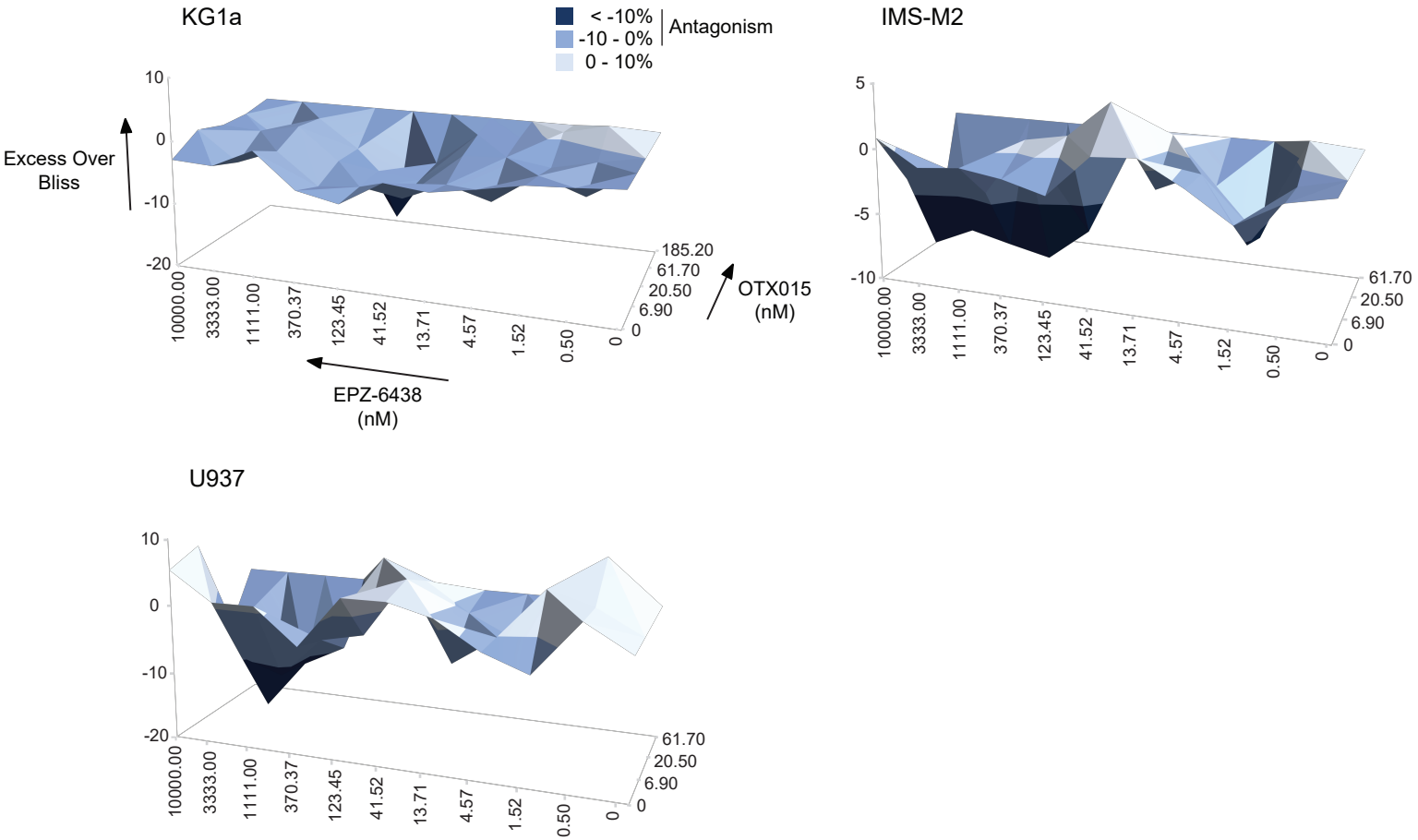


Figure S11: CRISPR screen hit validation.

(A) Immunoblot confirming EED, EHMT1, and SETDB1 knockdown using two hairpins against each target. ACTIN used as loading control.

(B) Excess over bliss for KG1a, IMS-M2 and U937 exposed to a combination of OTX015 and EPZ-3468 for 5 days. Average of four replicates ± SD.

Supplementary Figure S12

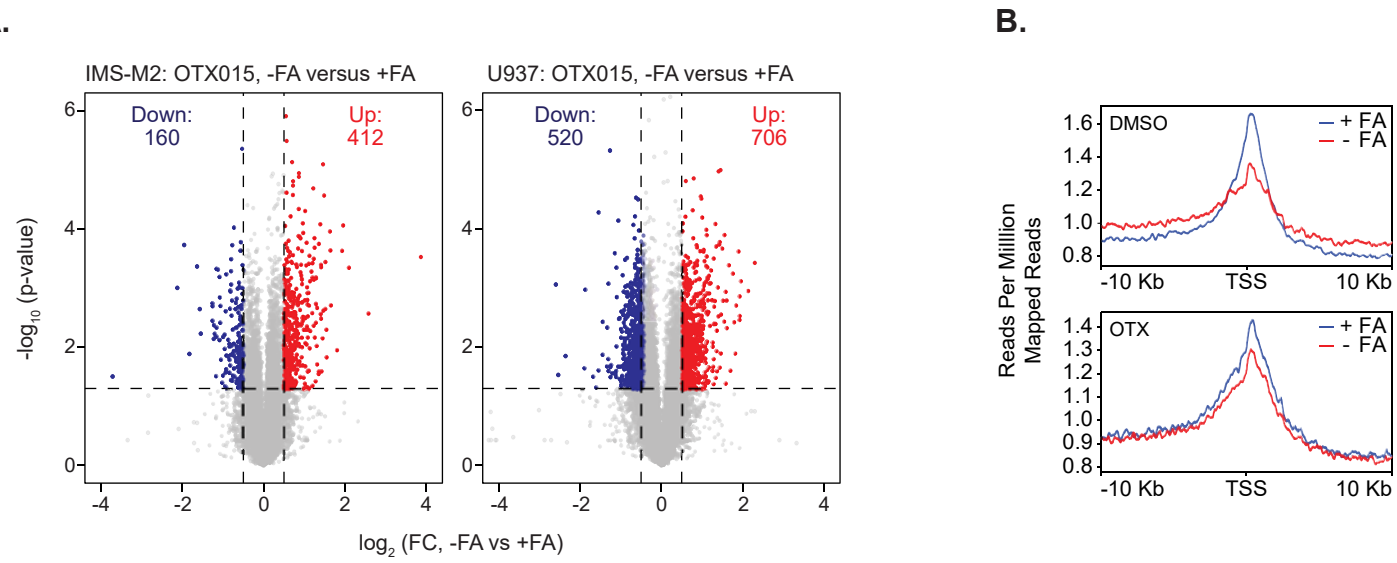


Figure S12: Transcriptional and epigenetic changes induced by folate starvation in AML cells.

(A) Volcano plots highlighting genes differentially expressed in - folic acid (-FA) versus control (+FA) in OTX015-treated IMS-M2 and U937 cells. Number of differentially expressed genes shown in blue and red for down- and up-regulated genes.

(B) Metaplot for average H3K27me3 signal within 10Kb-region flanking gene transcriptional starting sites (TSS) with folic acid starvation (-FA) in DMSO- and OTX015-treated U937 cells.

Supplementary Figure S13

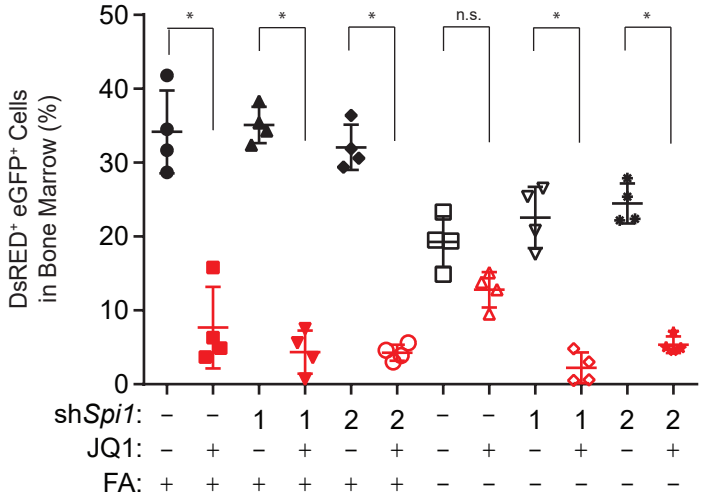


Figure S13: *Spi1* knockdown alleviates the resistance to JQ1 mediated by folic acid withdrawal.

Proportion of DsRed- and eBFP-positive MLL-AF9 leukemic cells infected with either a control (shCT) or two *Spi1*-directed shRNAs (sh*Spi1_1* and sh*Spi1_2*) in bone marrow from four mice per group fed with either regular or folic acid-restricted diet and treated with either vehicle or 35mg/kg JQ1 for 7 days. *p-value ≤ 0.05 by Mann-Whitney test. n.s, nonsignificant ($p > 0.05$). Error bars represent mean ± SD.