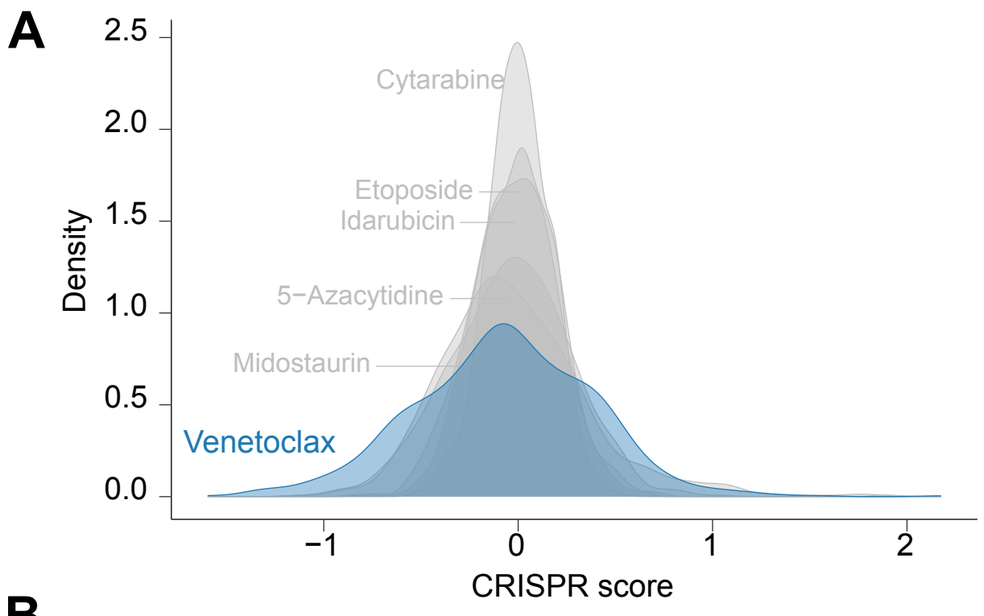
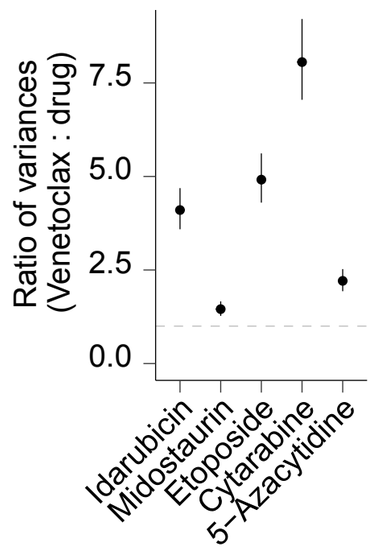
Figure S1, Related to Figure 1. Targeting RNA splicing factors sensitizes AML cells to venetoclax.





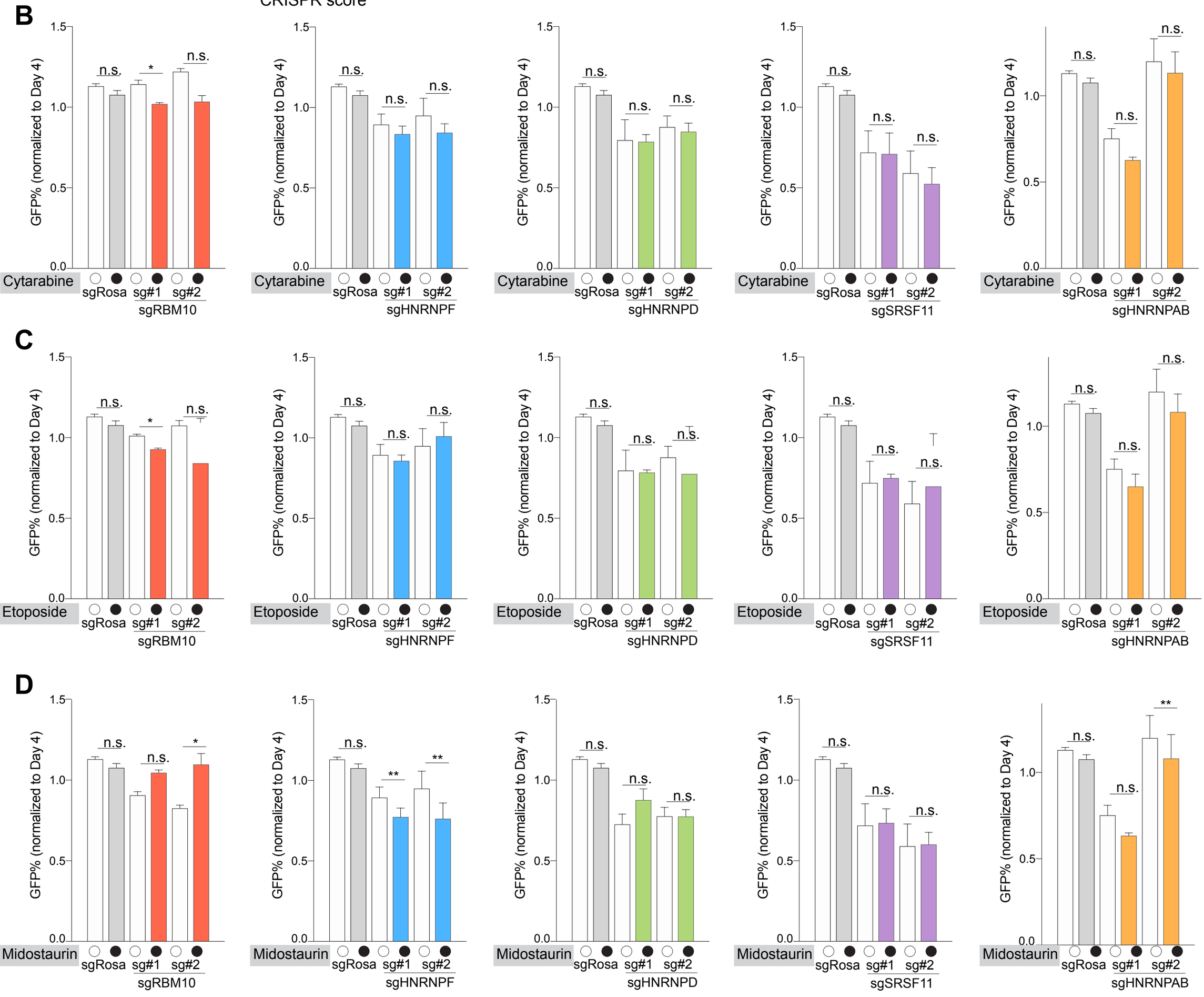


Figure S1, Related to Figure 1. Targeting RNA splicing factors sensitizes AML cells to venetoclax. (A) Distribution of CRISPR scores of sgRNAs targeting RNA processing genes (left) and variance comparisons (against venetoclax; right) from the genome-wide drug screens. The variance ratios and 95% confidence intervals were estimated via an F-test denoted on y-axis. (B) Competition-based assay in MOLM-13 cells 10 days post-transduction with top 2 sgRNAs targeting each splicing factor or non-targeting sgRosa control (n=3, mean+SEM) treated with 50 nM cytarabine, (C) 400 nM etoposide, or (D) 25 nM midostaurin. Statistical analysis was performed using unpaired Student's t-test by Prism GraphPad (*p < 0.05, **p < 0.01, n.s., not significant).

Figure S2, Related to Figure 2. RBM10 ablation sensitizes AML cells to death from venetoclax but RBM10 is not required for normal hematopoiesis.

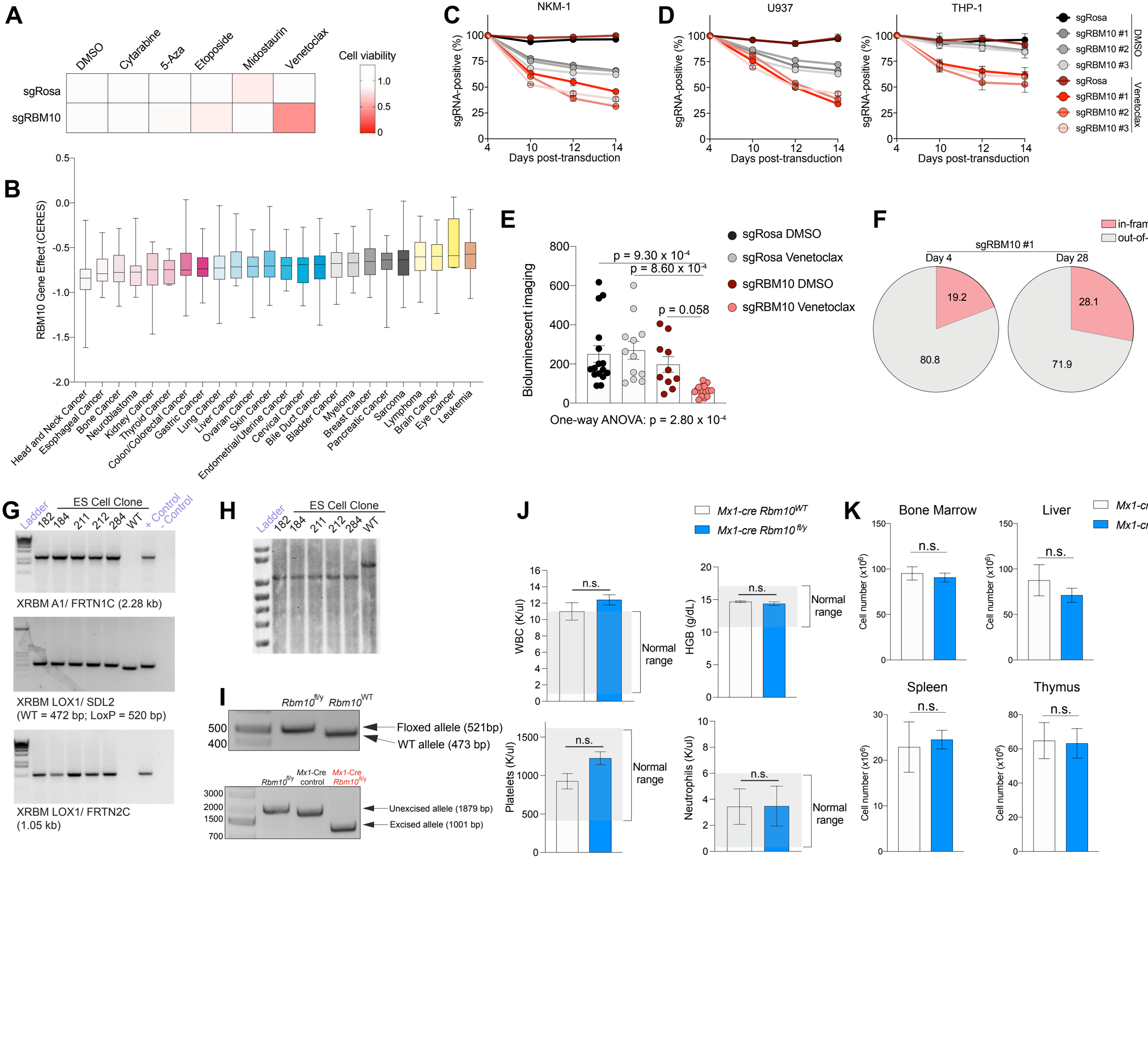
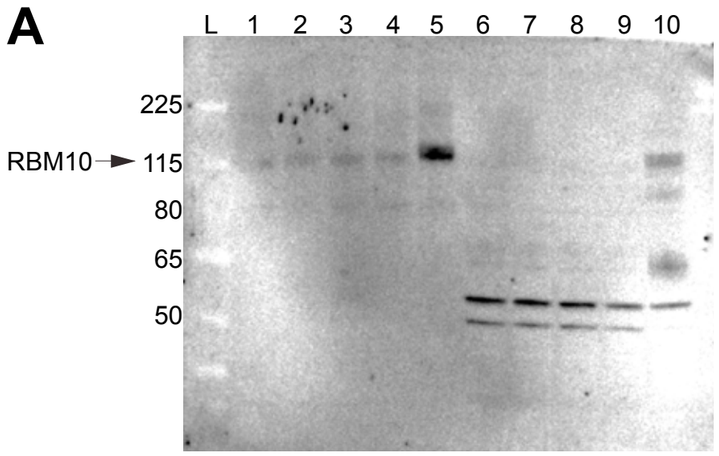
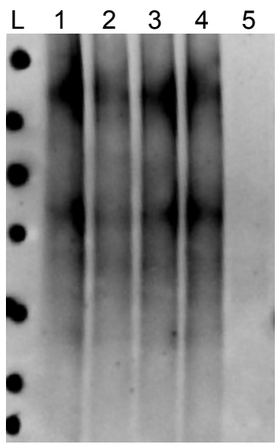


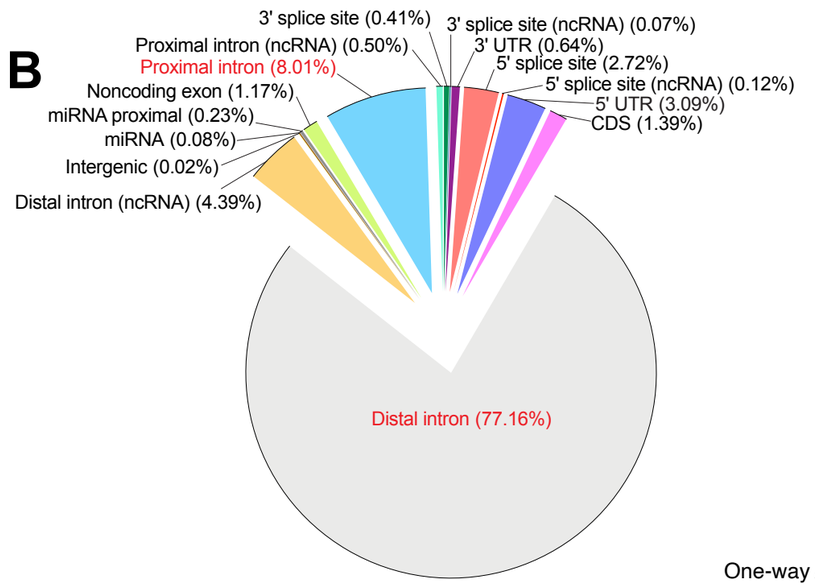
Figure S2, Related to Figure 2. RBM10 ablation sensitizes AML cells to death from venetoclax but RBM10 is not required for normal hematopoiesis. (A) Competition-based assay in MOLM-13 cells transduced with sgRBM10 treated with the indicated drugs (same dose used in CRISPR screens and indicated in the Methods section). Values are normalized to DMSO control. (B) Box-and-whisker plot of RBM10 dependency score (CERES) as indicated on the y-axis across cancer types from DepMap database. Higher negative values are indicative that gene is essential. For the Box-and-whisker plot, the minimum to maximum showing all points, 25th-75th percentiles and median (horizontal line). (C) Competition proliferation assay of sgRBM10 or non-targeting sgRosa in NKM-1 and (D) TP53-mutated cell lines (U937 and THP-1) treated with venetoclax or DMSO (n=3 for each condition, error bars represent SEM). (E) Quantification of bioluminescent images from mice transplanted with RBM10 KO or sgRosa MOLM-13 cells at day 4 post-venetoclax (100 mg/kg) daily treatment (n=3, mean+SEM). (F) CRISPResso indel analysis of RBM10 sgRNAs in MOLM-13 cells at the indicated timepoints. (G) Genotyping of embryonic stem cell clones containing floxed Rbm10 allele using three distinct genotyping primers. (H) Southern blot confirmation of floxed ES cell clones using the Southern probe labeled as “PB3/4.” (I) Validation of Rbm10 floxed alleles and excision of exon 3 of Rbm10 using genomic PCR. (J) Peripheral blood counts and (K) total cell numbers of tissues from primary non-competitive transplantation of Rbm10 cKO (n=4) and Mx1-cre control (n=4) after 1 month of pIpC treatment. Grey represents normal levels of blood counts. Statistical analysis was performed using an unpaired Student’s t-test and error bars represent SEM.

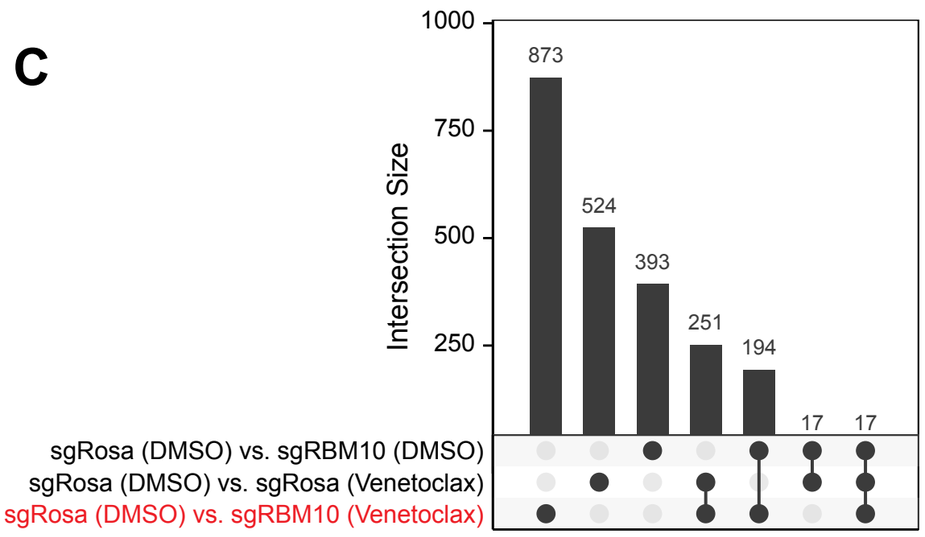
Figure S3, Related to Figure 3. Characterization of RBM10 on RNA splicing and binding in AML cells.

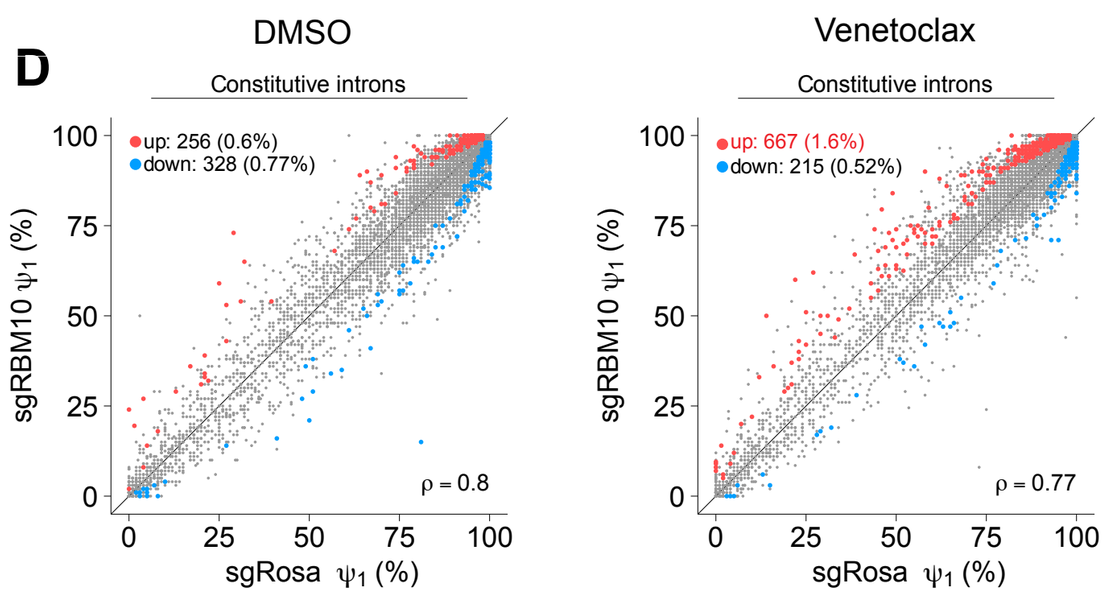


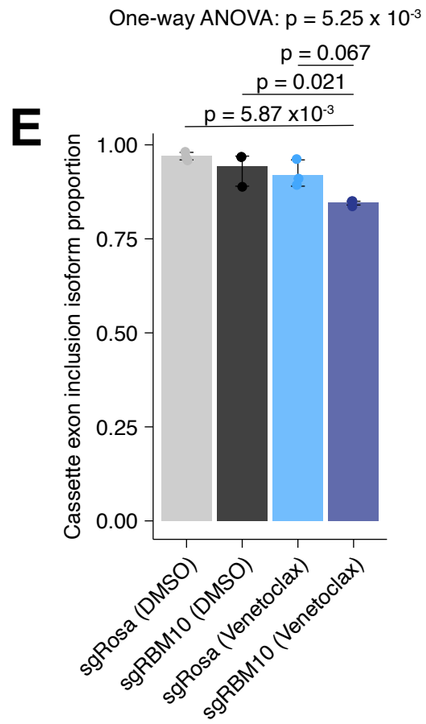


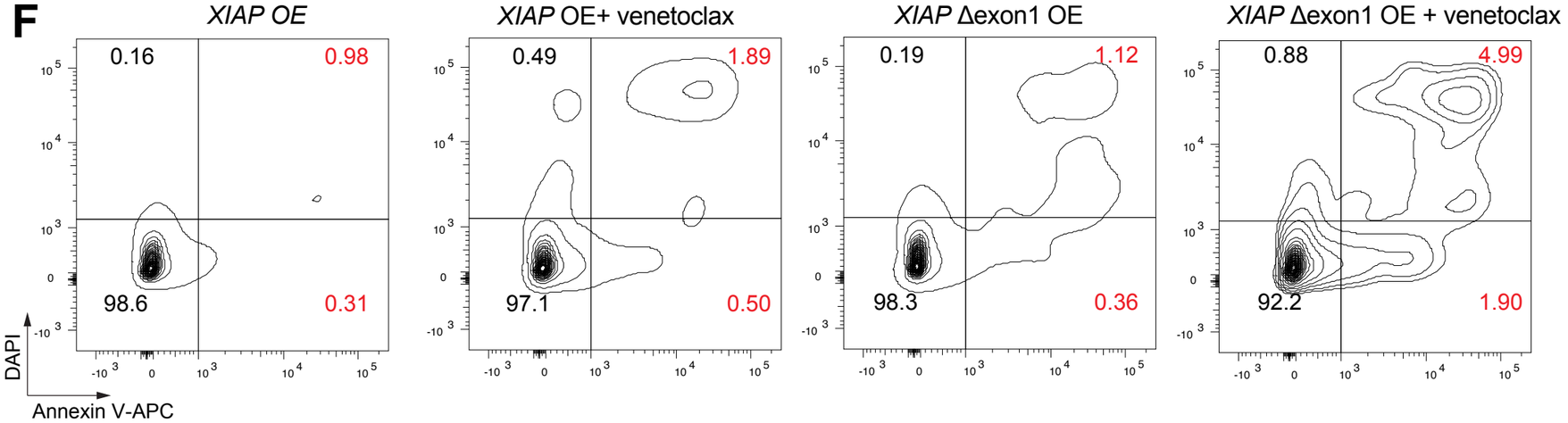
L. Protein Ladder (kDa)
1. RBM10 IP1
2. RBM10 IP2
3. RBM10 IP3
4. RBM10 IP4
5. Positive control IP
6. RBM10 Input 1
7. RBM10 Input 2
8. RBM10 Input 3
9. RBM10 Input 4
10. Negative control Input

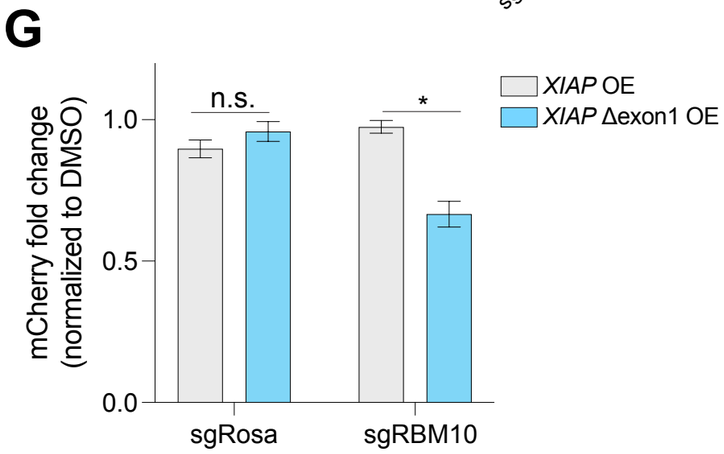


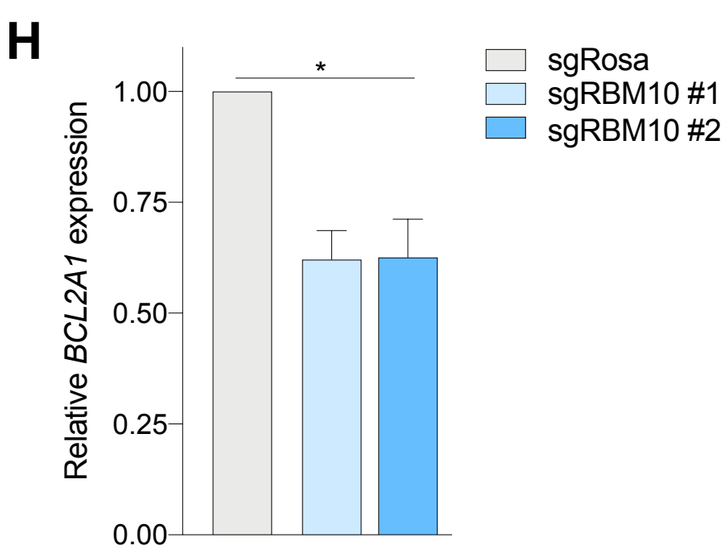












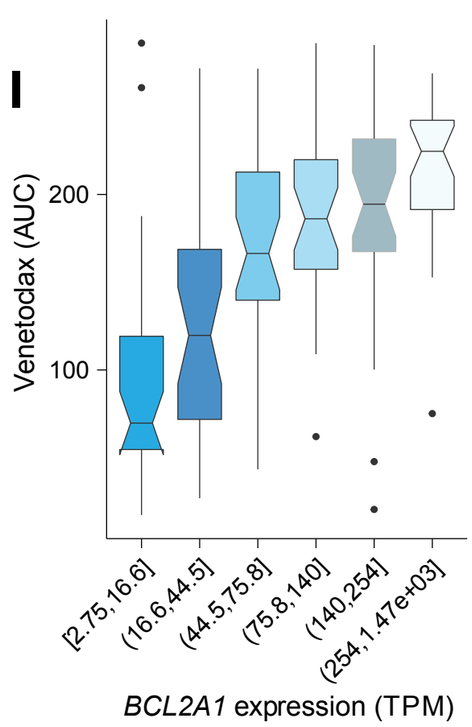


Figure S3, Related to Figure 3. Characterization of RBM10 on RNA splicing and binding in AML cells. (A) Immunoblotting of endogenous RBM10 (left) and RNA visualization (right) of immunoprecipitation in MOLM-13 cells. (B) Genomic distribution of RBM10 eCLIP binding sites in MOLM-13 cells. (C) UpSet plot of overlapping splicing events in RBM10 KO and sgRosa treated with DMSO or venetoclax. (D) Scatter plots of constitutive introns [in MOLM-13 cells transduced with sgRosa (x-axis) or sgRBM10 (y-axis)] in DMSO (left) or venetoclax treatment (right). (E) Percent spliced in (PSI) values of the cassette exon (exon 1) inclusion isoform of XIAP ∆exon1 (n=3, mean+SEM). (F) Annexin V staining of ectopic overexpression of XIAP full-length or XIAP ∆exon1 treated with DMSO or venetoclax for 24 hours and (G) upon RBM10 KO compared to non-targeting sgRosa. (H) RT-qPCR of BCL2A1 mRNA expression in MV4-11 human AML cells with two independent RBM10 sgRNAs and treated with 50 nM venetoclax for 48 hrs (n=3, mean+SEM). Statistical analysis was performed using unpaired Student's t-test by Prism GraphPad (*p < 0.05). (I) Correlation of BCL2A1 expression and venetoclax resistance (AUC) from BeatAML AML patients.

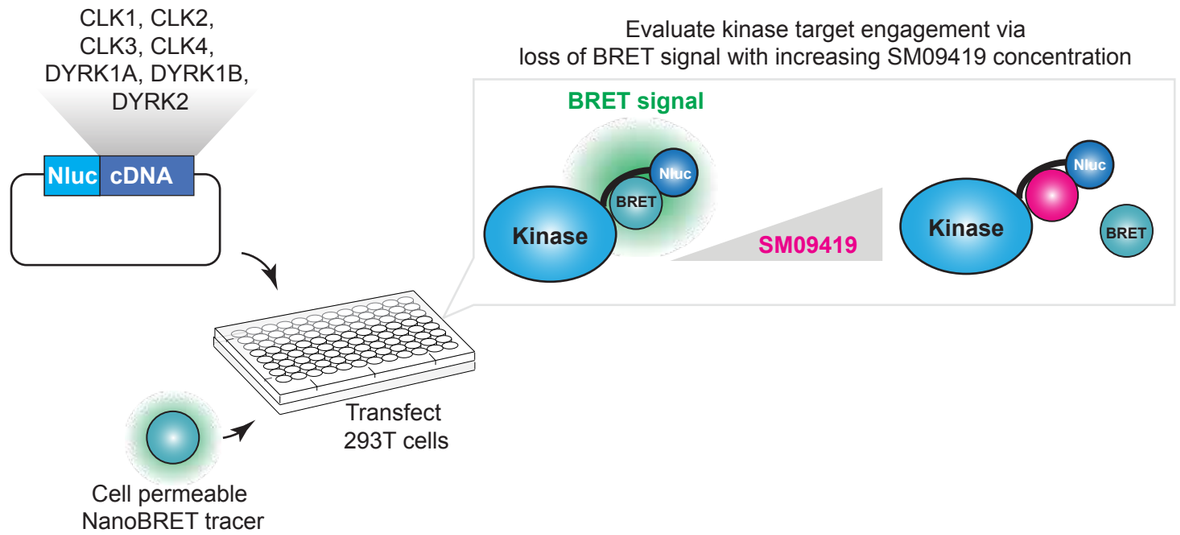
Figure S4, Related to Figure 4. SM09419 is a highly-specific CLK/DYRK inhibitor and synergizes with venetoclax.

A

Top 10 Codependencies for DYRK1A (DepMap)

Gene	Correlations
AMBRA1	0.32
EP300	0.26
MNT	0.26
CLDN14	0.24
CEP350	0.22
BCL2	0.22
PAPOLA	0.21
PDXK	0.21
PCBP3	0.21
CDKN1A	0.21

B



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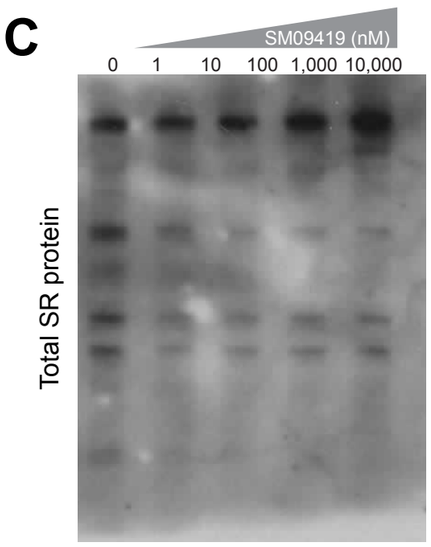

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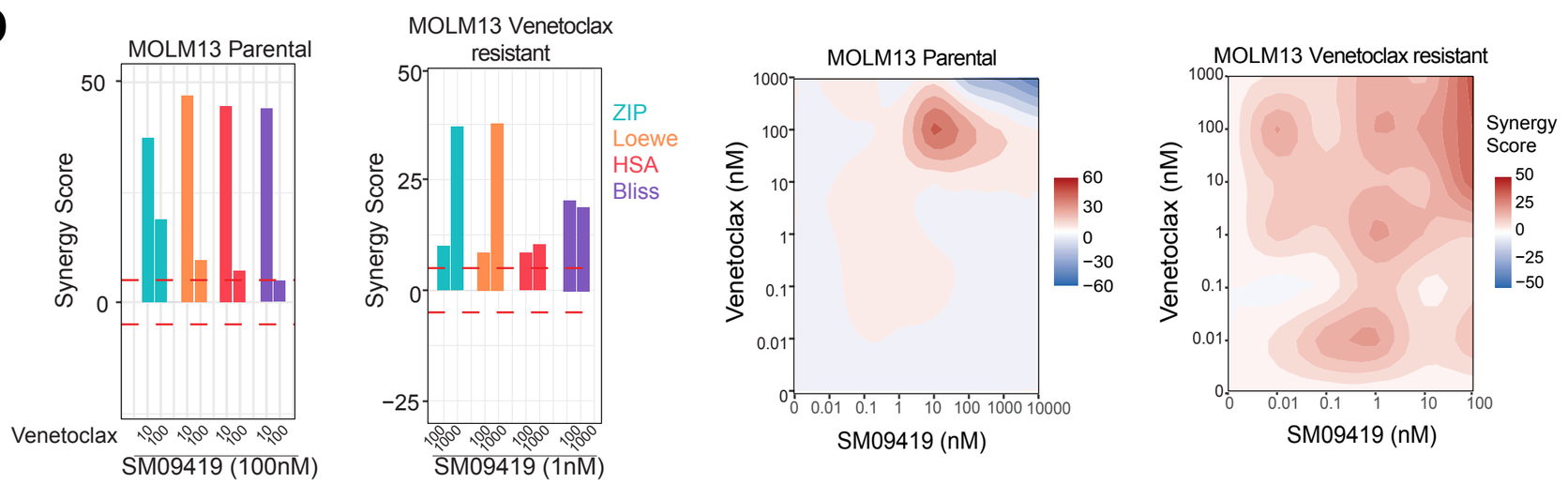

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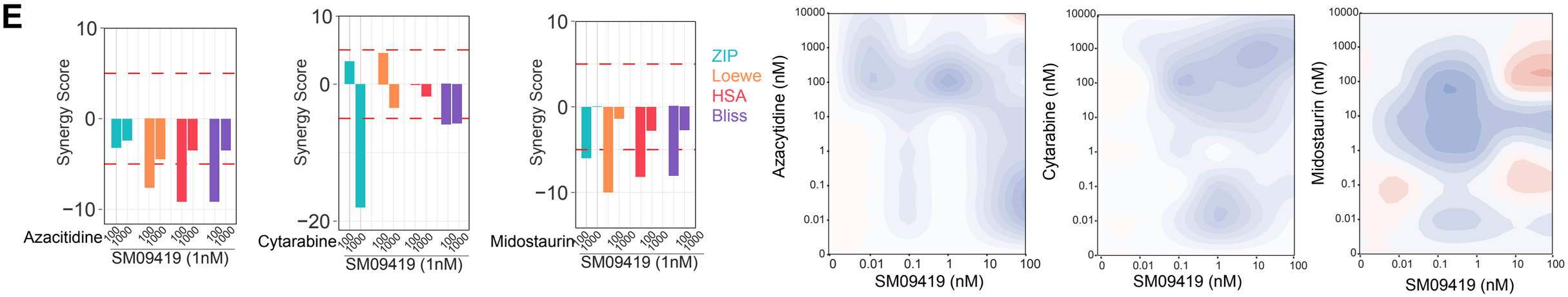
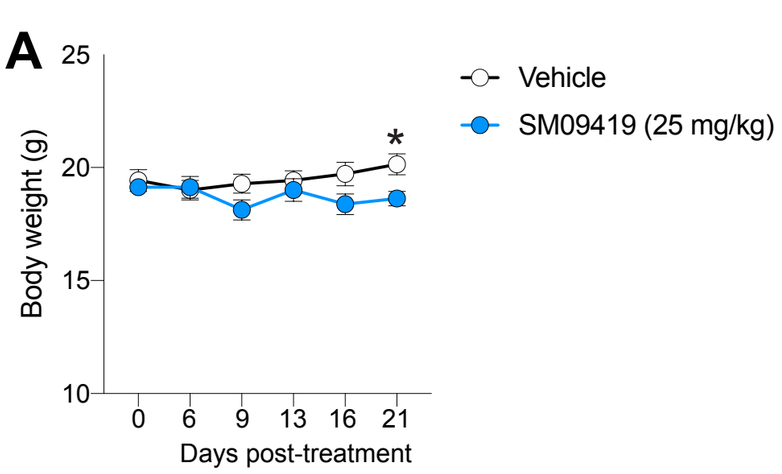
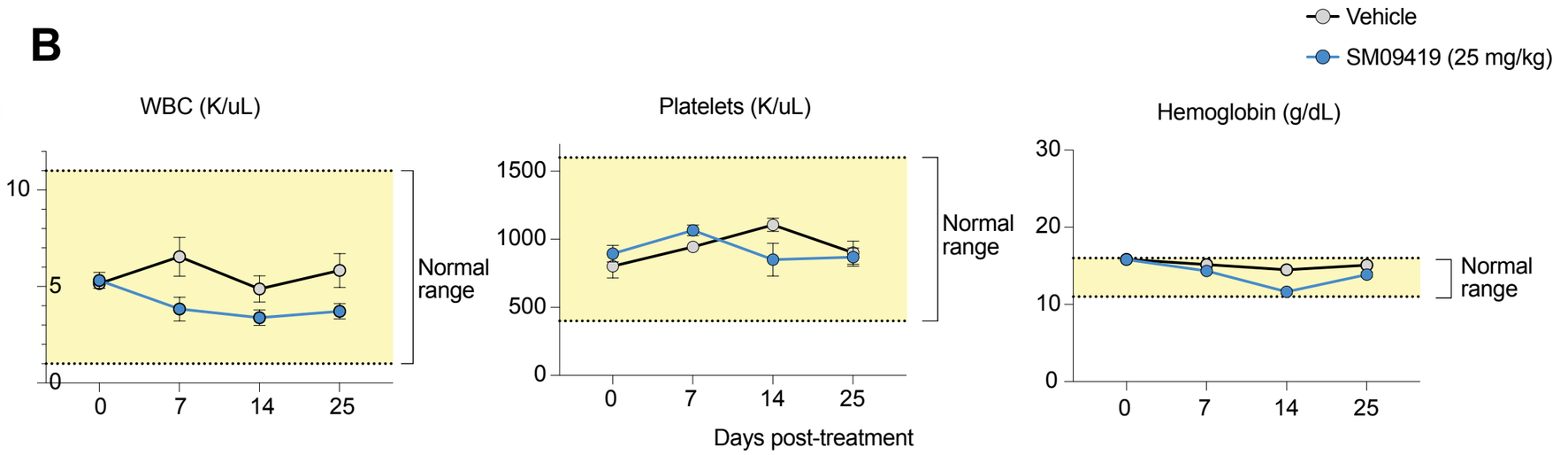
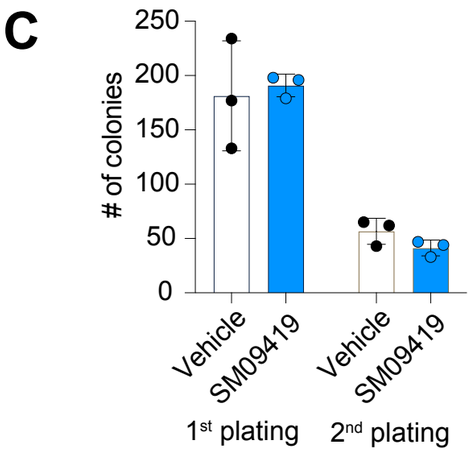


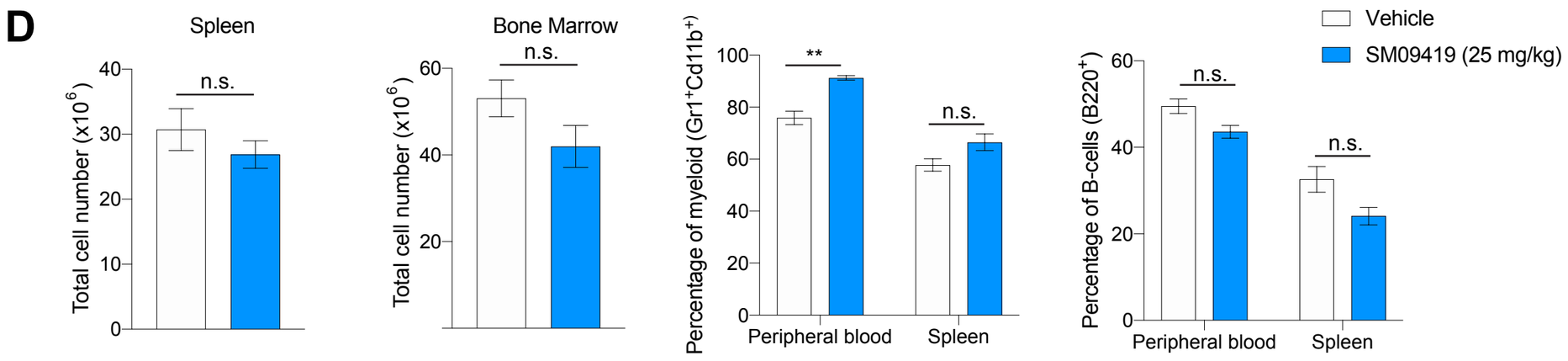
Figure S4, Related to Figure 4. SM09419 is a highly specific CLK/DYRK inhibitor and synergizes with venetoclax. (A) DepMap co-dependency CRISPR screen analysis of DYRK1A. (B) NanoBRET target engagement assay of CLK1-4, DYRK1A/B, and DYRK2 after treatment with varying concentrations of SM09419. (C) Western blot of total SR protein levels in MOLM-13 cells. (D) Four synergy scores (ZIP, Loewe, HSA, and Bliss) (left) and 2D synergy plots (right) of MOLM-13 parental or venetoclax-resistant cells treated with venetoclax, SM09419, or the combination after 48 hours (Values were calculated from technical triplicates per experiment). (E) Same as in (D) except treated with 5-azacytidine, cytarabine, midostaurin, or the combination of each.

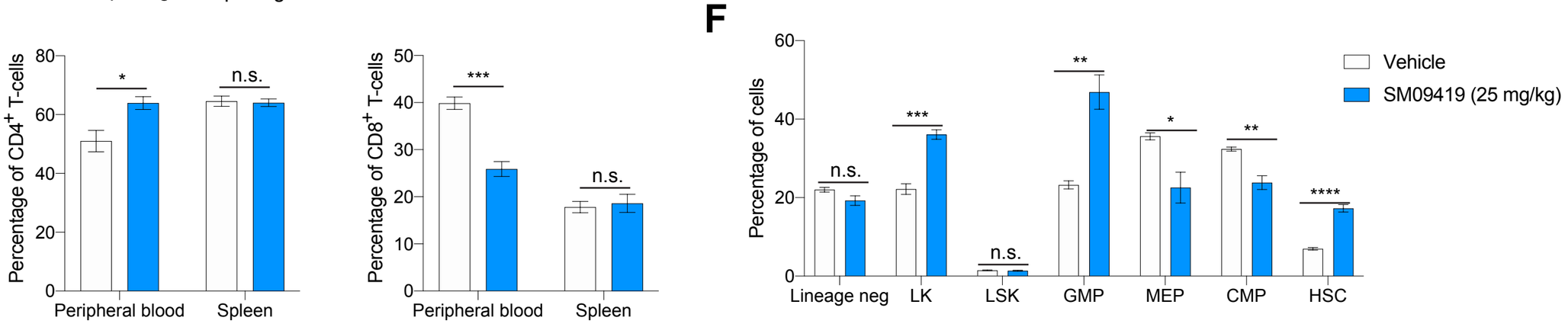
Figure S5, Related to Figure 4. SM09419 is well-tolerable and does not alter normal hematopoiesis.

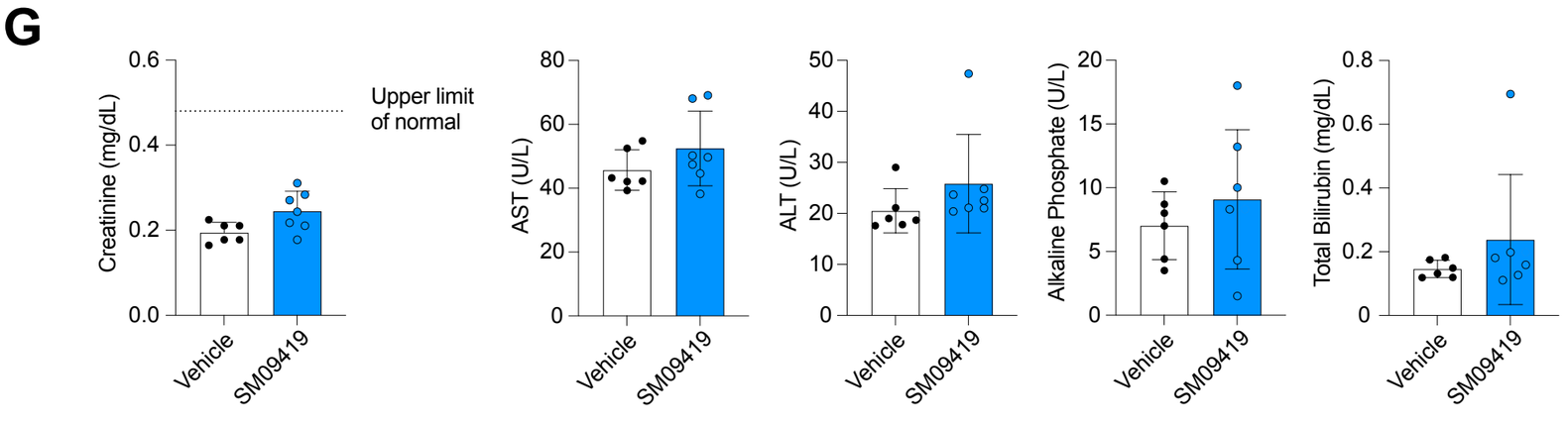












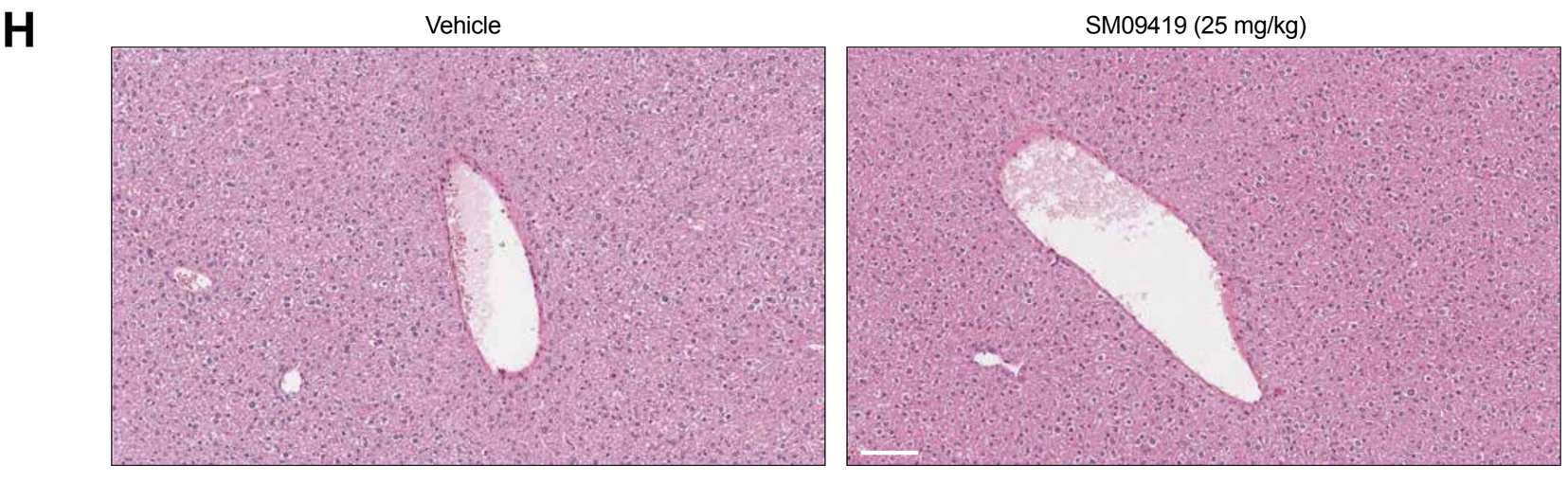
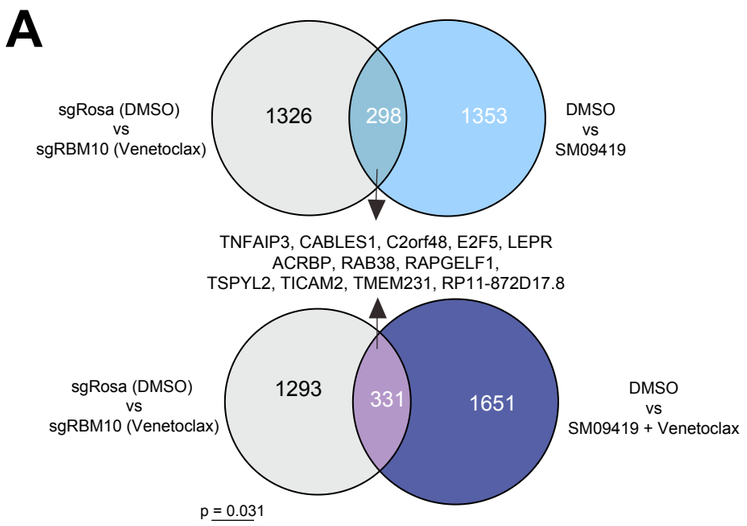
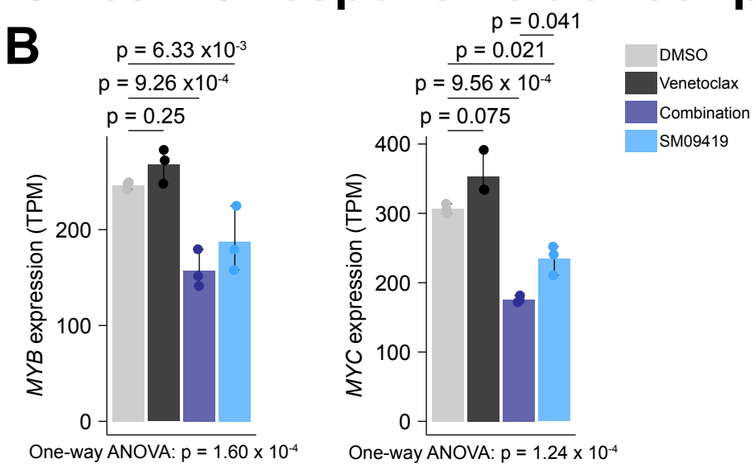
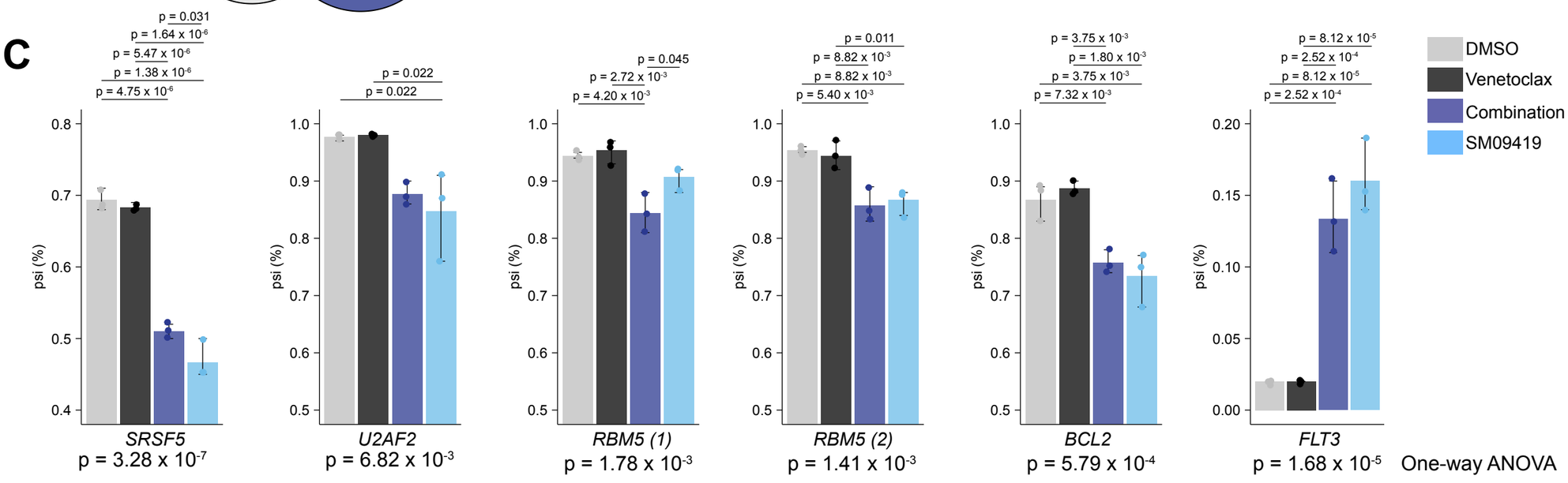


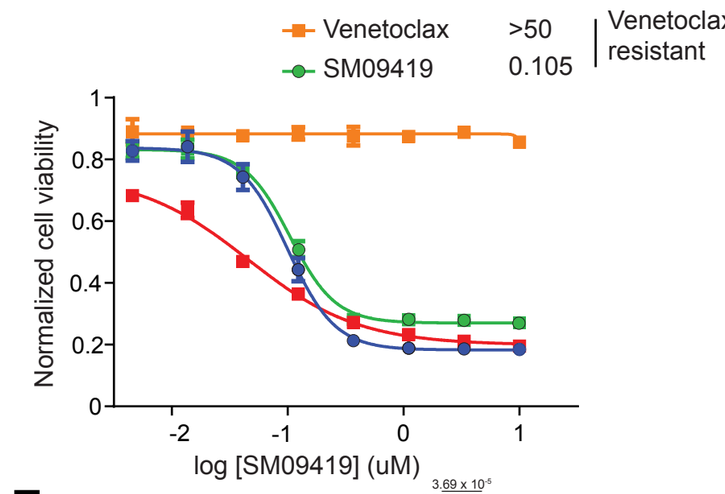
Figure S5, Related to Figure 4. SM09419 is well-tolerable and does not alter normal hematopoiesis. (A) Analysis of normal C57BL/6 mice body weight and (B) complete blood counts (CBCs) at the indicated time-points after daily treatment of SM09419 (25 mg/kg) (n=7) or vehicle (n=6). Yellow area represents the normal ranges for blood counts. (error bars represent mean+SEM). (C) Total number of colony-forming units (CFUs) using methylcelllulose assays with normal C57BL/6 treated with SM09419 daily for 3-weeks (n=3, mean+SEM). Colonies were assessed at day 7 after plating. (D) Total cell numbers of tissues from normal C57BL/6 mice treated daily with SM09419 (25 mg/kg) (n=7) or vehicle (n=6) for 3 weeks (error bars represent SEM). (E) Flow cytometry analysis of T-cells from spleen and peripheral blood after 3 weeks of SM09419 (n=7) or vehicle (n=6) daily treatment (error bars represent SEM). (F) Flow cytometric analysis of hematopoietic stem and progenitor cells (HSPCs) in bone marrow from mice treated daily with SM09419 (n=7) or vehicle (n=6) daily treatment (error bars represent SEM) for 3 weeks. (G) Assessment of kidney function (creatinine test) and liver function (AST, ALT, Alkaline phosphate, total Bilirubin) after treatment with daily SM09419 (n=7) or vehicle (n=6) daily treatment (error bars represent SEM) for 3 weeks. (H) Hematoxylin and eosin (H&E) staining of liver after treatment with daily SM09419 for 3 weeks (bar: 500μM). Statistical analysis was performed using un-paired Student's t-test by Prism GraphPad (*$p < 0.05$, n.s., not significant).

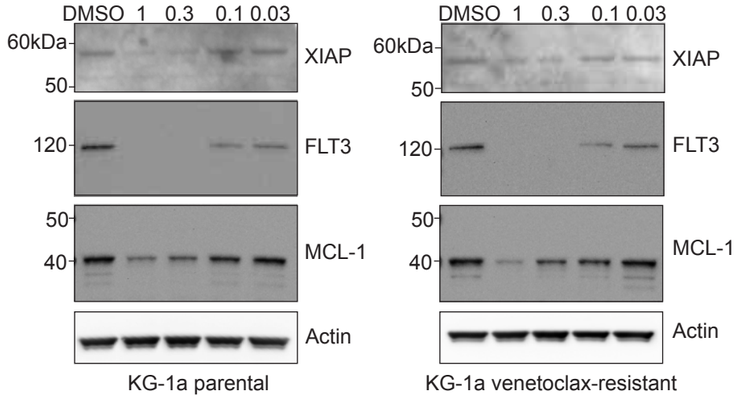
Figure S6, Related to Figure 5. SM09419-responsive transcriptome and splicing changes in AML.











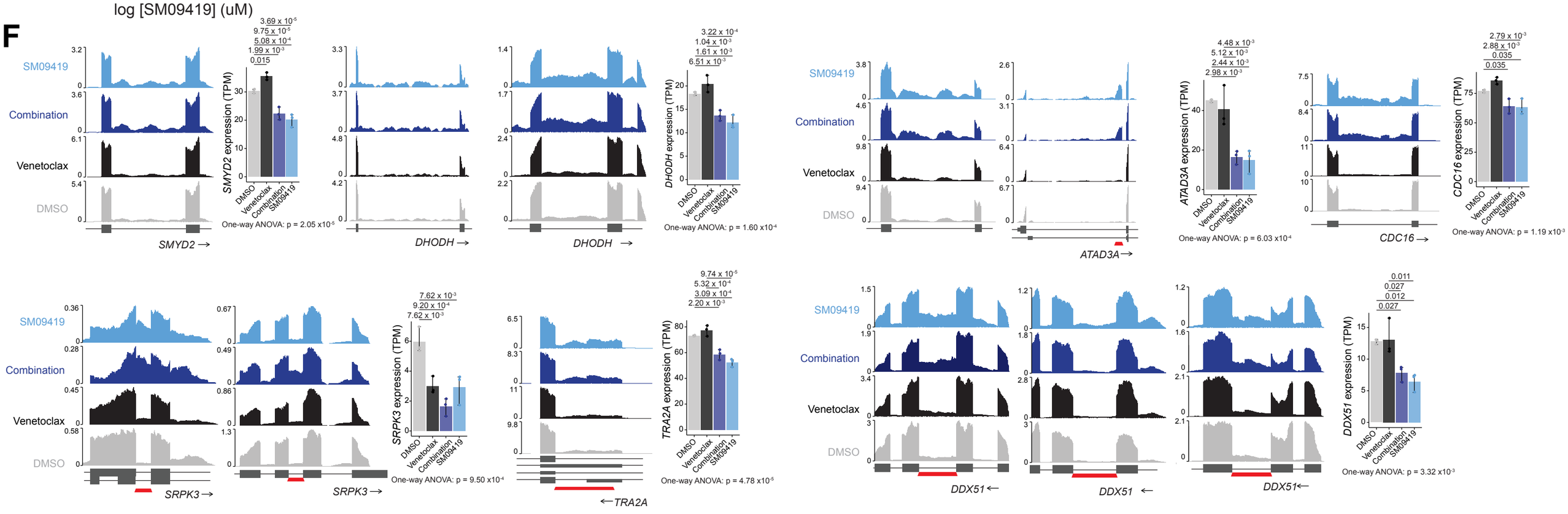


Figure S6, Related to Figure 5. SM09419-responsive transcriptome and splicing changes in AML. (A) Venn diagram of differentially genes expressed in RBM10 KO treated with venetoclax (compared to sgRosa) and SM09419 monotherapy or venetoclax-combined (compared to DMSO) from RNA-seq in MOLM-13 cells. (B) Gene expression for MYC and MYB mRNA from MOLM-13 RNA-seq (n=3, mean+SEM). (C) Mean PSI values of three replicates of each group treated with venetoclax or combination of SM09419 and venetoclax (n=3, mean+SEM). (D) Dose-response curves of KG-1a parental and venetoclax-resistant cell lines treated with venetoclax or SM09419 (normalized to DMSO) after 24 hours (n=3, mean+SEM). (E) Western blotting of XIAP, FLT3, MCL-1, and Actin in KG-1a parental or venetoclax-resistant cells after 24 hours of treatment. (F) Mean PSI values and gene expression of *SMYD2, DHODH, ATAD3A, CDC16, SRPK3, TRA2A*, and *DDX51* of each group treated with venetoclax or combination of SM09419 and venetoclax. p-values were determined by One-way ANOVA with post-hoc testing as indicated (n=3, mean+SEM).