Figure S1. AI-10-49 mediated MYC transcriptional changes is specific to inv(16) cells. Related to Figure 1.

(A, B) Gene Set Enrichment Analysis of RNA-seq data depicting pyrimidine metabolism, cell cycle and ribosome biogenesis (A), and pathway signatures (B) positively correlated with AI-10-49 treatment in ME-1 cells. (FDR) false discovery rate; (NES) normalized enrichment score. (C) *MYC* transcript levels in non-inv(16) AML cells (U937, K562, Jurkat, Kasumi-1 and THP-1) treated with DMSO or 1μM AI-10-49 for 6 hrs. (D) Immunoblot depicting MYC and GAPDH protein levels in ME-1 cells treated with 1 μM AI-10-49 for 0 hrs, 2 hrs, 4 hrs, 6 hrs and 8 hrs.

Figure S2. Effect of MYC silencing in inv(16) AML cells. Related to Figure 2.

(A) Time course analysis of cell viability (7AAD- Annexin V-) in ME-1 cells transduced with scramble (Scr) or two *MYC* shRNAs. (B) Flow cytometry analysis of granulocytic differentiation in ME-1 cells transduced with *MYC* shRNAs at day 14. (C, D) Analysis of MYC protein levels assessed by western blot analysis (C) and cell viability (7AAD- Annexin V-; D) of AML cell lines Kasumi-1, NB4, ME-1, THP1, MV4:11 and K562, 14 days after transduction with *MYC* shRNAs; each data point represents the mean of triplicate experiments; error bars represent the SD. (E) Immunoblot analysis of Myc and Gapdh protein levels mouse *Cbfb*$^{+/MYH11}$ leukemic cells transduced with Renila (Ren) or *Myc* shRNAs 1 and 2. (F) Schematic representation of experimental design for *in vivo* evaluation of *Myc* shRNA knockdown experiments. (G) Immunoblot analysis of Myc and Gapdh protein levels in *Cbfb*$^{+/MYH11}$ leukemic cells of leukemic mice (Ren, shMyc1 and shMyc2 groups) from secondary transplant assays shown in Figure 2G. Each band represents Myc total protein levels of leukemic cells isolated from a single mouse. Significance was calculated using Levene's test (D). *P < 0.05, or **P < 0.005.

Figure S3. AI-10-49 cooperates with JQ1 in inv(16) AML. Related to Figure 3.

(A) qRT-PCR analysis of *BRD4* transcript levels in ME-1 cells transduced with scramble (Scr) or two *BRD4* shRNAs (sh1 and sh2). (B) Immunoblot analysis of MYC and GAPDH protein levels in ME-1 cells treated with BET inhibitor JQ1 for 6 hrs. (C) Dose response viability analysis (MTT assay) of ME-1 cells treated with AI-10-49 and/or JQ1 for 72 hrs; the LD_{50} for each compound is: AI-10-49-LD_{50}=0.468 μM, range=0.398-0.537 μM; JQ1-LD_{50}= 0.344μM, range=0.228-0.460 μM; both at 95% confidence intervals. (D) Percentage of c-kit+ (leukemic) cells in peripheral blood 25 days after transplantation in respective groups, assessed by flow cytometry. (E) Viability analysis (MTT assay) of JQ1 and AI-10-49 in human cord blood CD34+ cells 48 hrs after treatment with AI-10-49 and/or JQ1 at the indicated concentrations. (F-J) Toxicology analysis of wild type mice treated with a daily dose of DMSO (D, black) or 200 mg/kg/day AI-10-49 (10 days) and 50 mg/kg/day JQ1 (21 days) (49+JQ1, green). Mice were analyzed 1 day after last treatment dose; body weight (F), spleen weight (G), bone marrow cellularity (H), percentage of stem and early progenitor cells [LSK+: Lin(-) Sca1(+) c-kit(+)] in bone marrow (I), percentage of progenitor cell compartments common myeloid progenitors [CMP: LSK-,CD34(+)CD16/32(-)], megakaryocyte/erythroid progenitors [MEP: LSK-, CD34(-)CD16/32(-)], and granulocyte/monocyte progenitors [GMP: LSK-, CD34(+)CD16/32(+)], in LSK- cells (J). Each symbol represents the mean of values from three animals; error bars represent the S.D. Significance was calculated using unpaired t-test (A) or Levene's test (D). *P < 0.05, or **P < 0.005.

Figure S4. AI-10-49 leads to increased genome wide RUNX1 binding in ME-1 cells. Related to Figure 4.

(A) genomewide (left) and transcription start site (TSS, right) centered RUNX1 aggregated peak signal in ChIP-seq dataset from AI-10-49 or DMSO treated ME-1 cells, and respective heat maps (bottom). (B) Gene distribution of H3K27Ac (top) and RUNX1 (bottom) peaks in ME-1 cells treated with DMSO (left) or AI-10-49 (right).

Figure S5. RUNX1 mediated chromatin changes at *MYC* enhancer elements with AI-10-49. Related to Figure 5.

(A) ATAC-seq and ChIP-seq profiles for K3K27ac and RUNX1 at the +1.7 Mb BDME superenhancer. Five previously reported enhancer regions (E1 to E5) are depicted below the profile. (B) ChIP-seq profiles for K3K27ac and RUNX1 peaks in ME-1 cells treated with DMSO (blue) or AI-10-49 (red) in the 2Mb genomic region upstream of MYC-TSS. (C) 4C-style plots for 15 Kb bins (anchor bins) containing the *MYC* promoter (*Myc-Pr*), *ME1, ME2*, and *E3/E5* enhancers for DMSO and AI-10-49 treated cells. Anchor bins are shown in orange, solid black lines represent the LOWESS mean (the expected interaction frequency as a function of genomic distance) and the dotted black lines are the LOWESS plus and minus 1 standard deviation. Red lines are the observed 5C interaction frequencies. Green dots and vertical dotted lines highlight the positions and interactions between *Myc-Pr, ME1, ME2*, and *E3*. Arrow-heads indicate interactions with CTCF sites around the BDME superenhancer. Arrows indicate peaks of interactions pointing to loci interacting with Myc-Pr, ME1, ME2, and E3. The CTCF binding data were used from ChIP-seq data previously reported in K562 leukemia cells (GSE70764; ref. (Pugacheva et al., 2015)).

Figure S6. Analysis of transcription factor ChIP-seq at *MYC* locus. Related to Figure 5.

Transcription factor ChIP-seq analysis from (GSE46044; ref. (Mandoli et al., 2014) at the 2Mb downstream of the *MYC* TSS. Peak location for MYC promoter (blue) and ME1, ME2 and E3 (black) are shown as dotted line windows.

Figure S7. Related to Figure 6

(A) Immunoblot analysis for BRG1, RING1B and GAPDH in lysates of ME-1 cells treated with 1μM AI-10-49 at 2 to 8 hrs. (B, C) qRT-PCR analysis of *SMARCA4* (B) and *RNF2* (C) transcript levels in ME-1 cells transduced with scramble (Scr) or two gene specific shRNAs. Results from triplicate experiments shown; error bars represent the SD. (D) Evaluation of *MYC* transcript levels in ME-1 cells treated with RING1B inhibitor PRT 4165 for 8 days followed by treatment with DMSO/ AI-10-49 (0.6 μM) for 6 hrs, and *MYC* relative expression levels (REL) were estimated using qRT-PCR. Results from triplicate experiments shown; error bars represent the SD. (E) Co-immunoprecipitation analysis of RUNX1 binding to BRG1 and RING1B in nuclear extracts from ME-1 cells treated with DMSO/ AI-10-49 for 6 hrs. Significance was calculated as unpaired t-test (B-D).

Figure S8, related to Figure 7.

Sequence analysis of deletions (A) by size and (B) by location, in inv(16) AML ME-1 cells treated with CRISPR-Cas9 and sgRNAs for *ME1*, *ME2, N-Me* and *E3*. Analysis performed utilizing CRISPR Genome Analyzer.

Figure S1, related to Figure 1

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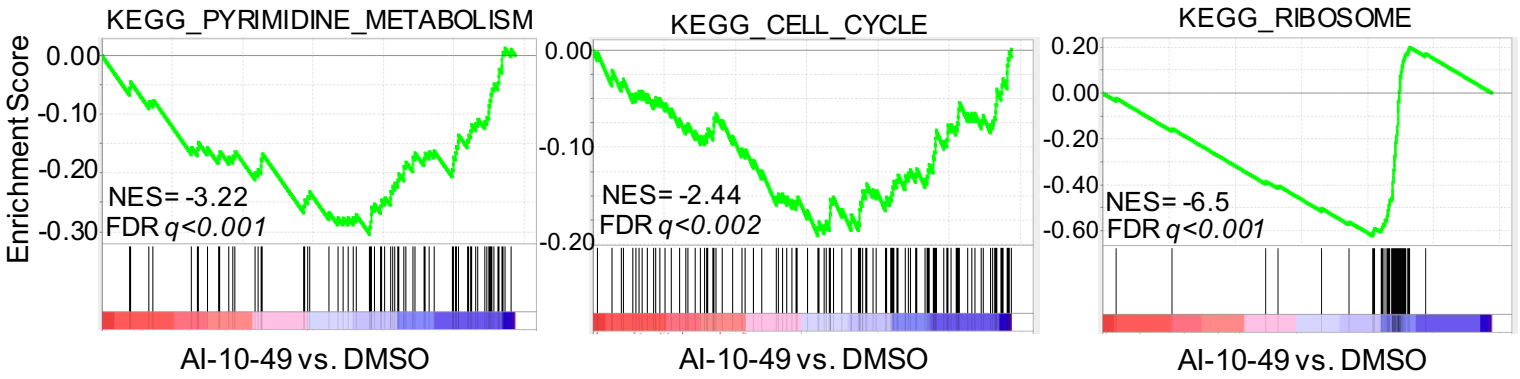

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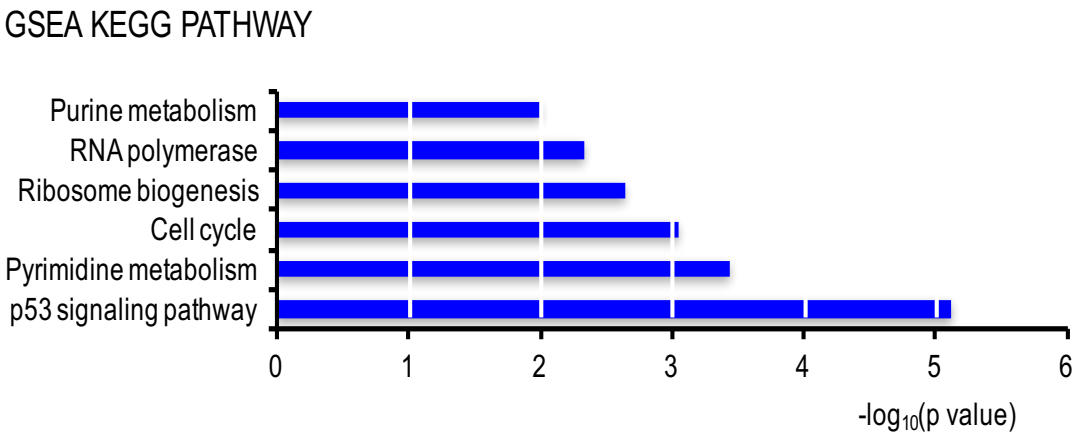

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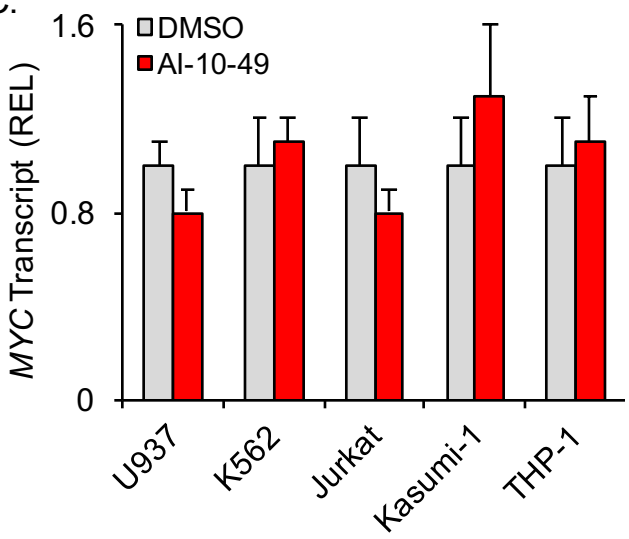

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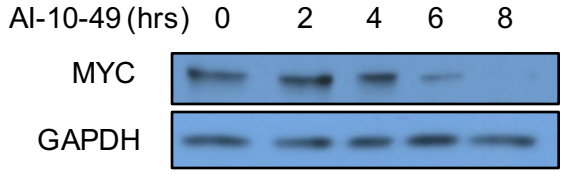


FigureS2, related to Figure 2

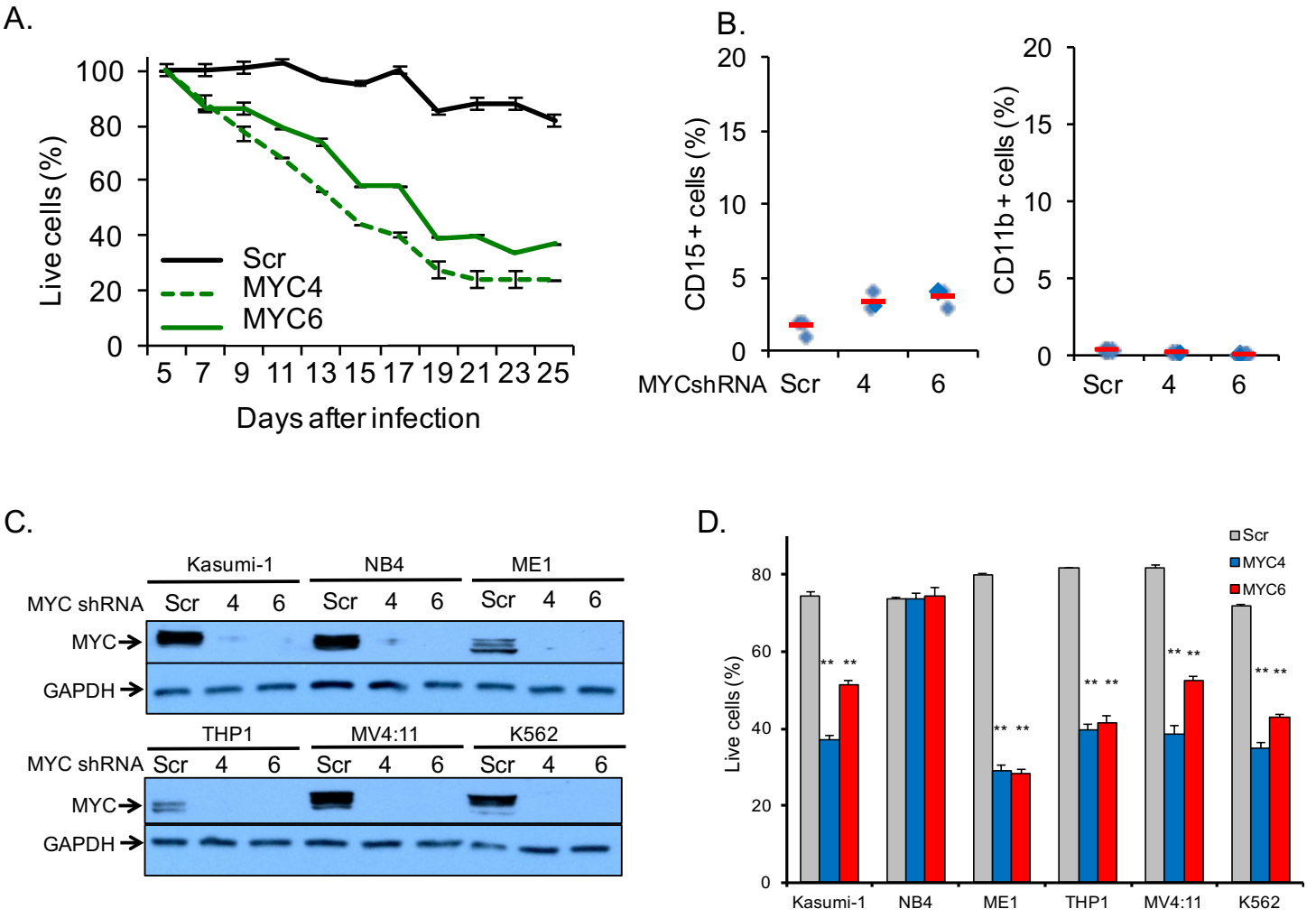


Figure S3, related to Figure 3

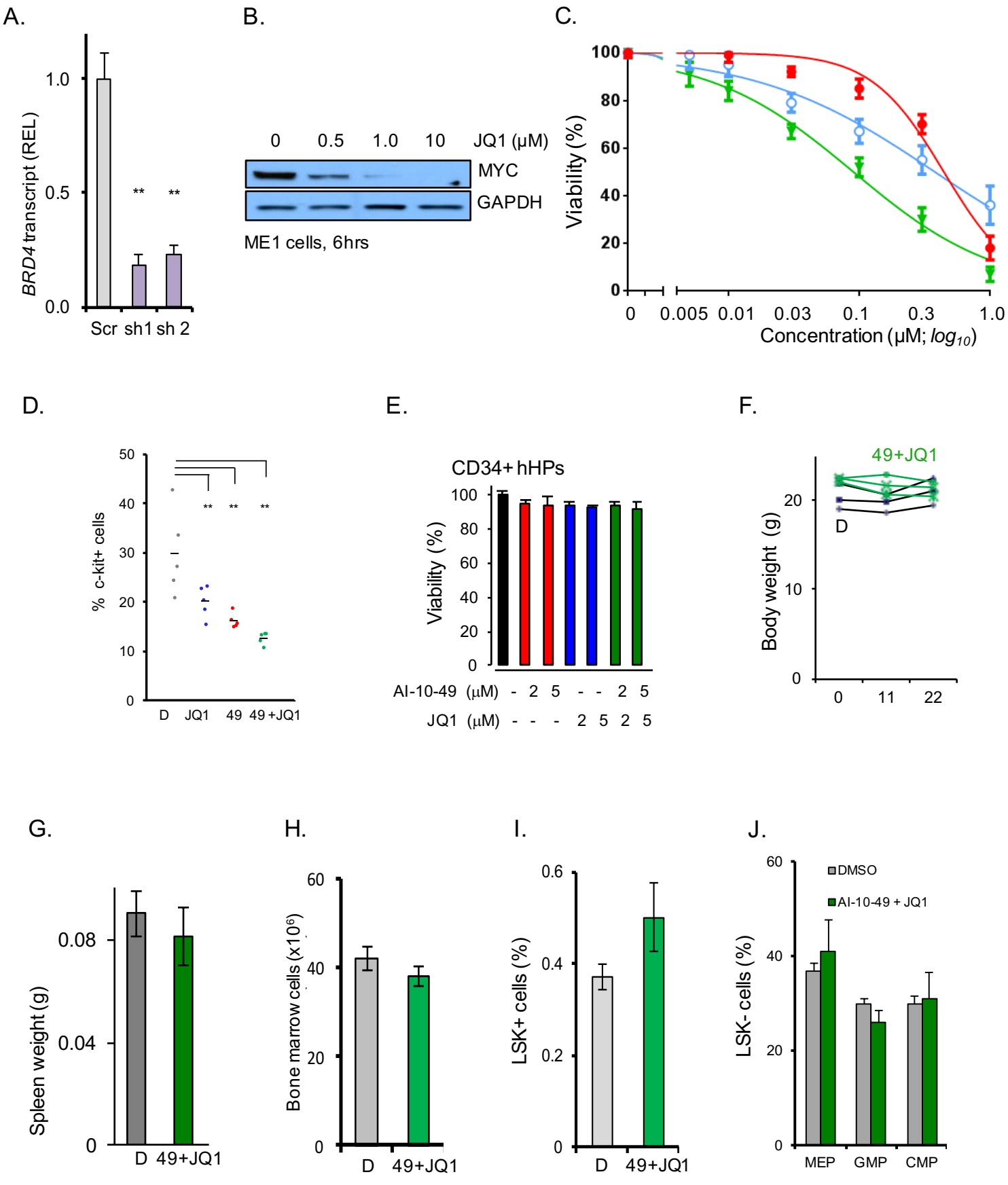
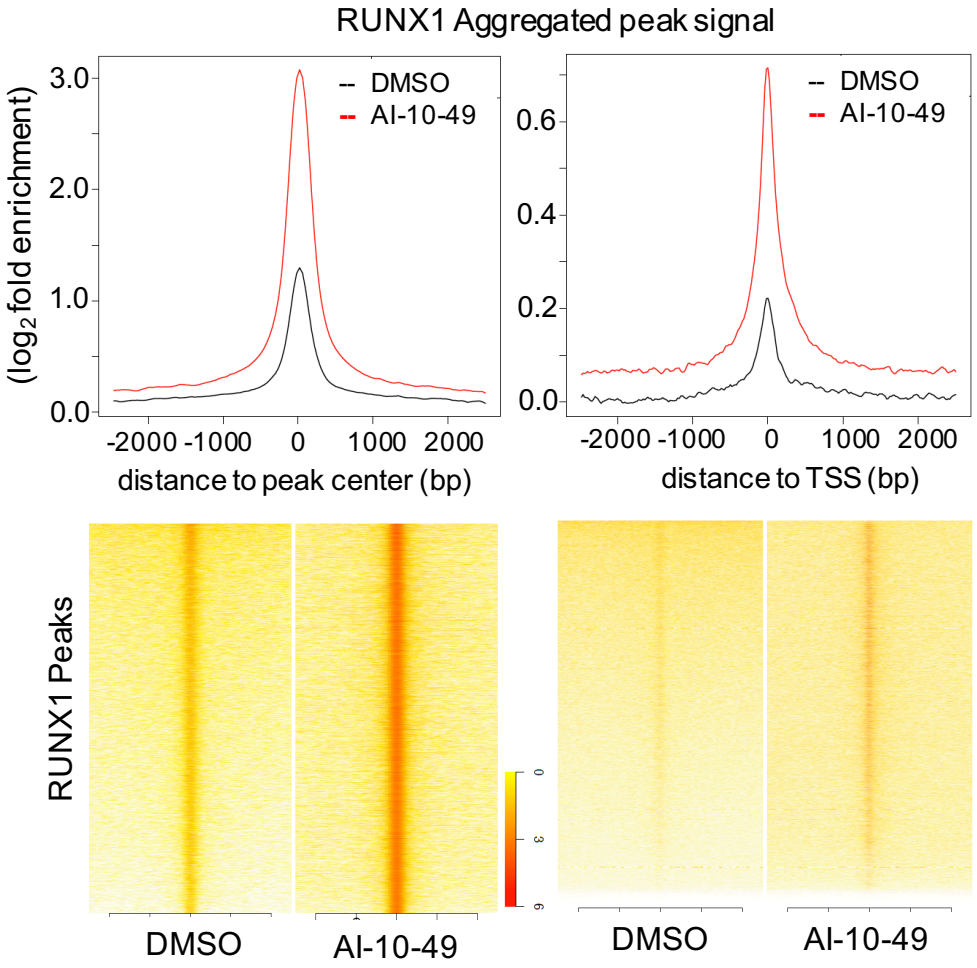


Figure S4, related to Figure 4

A.



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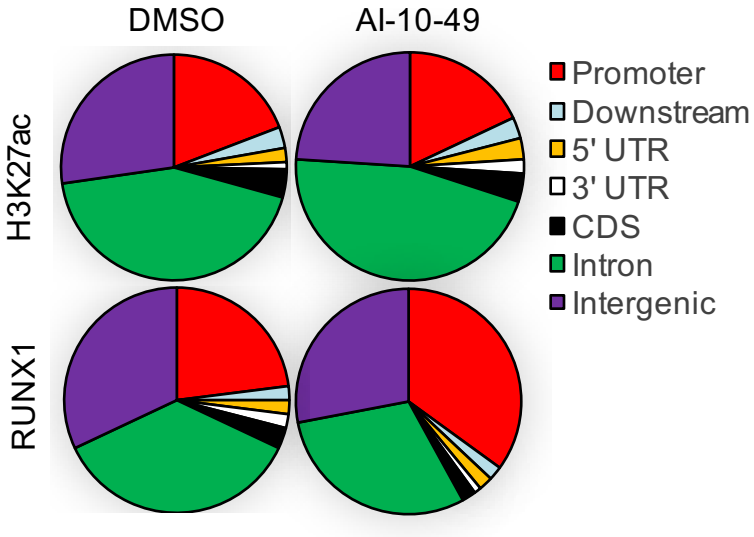
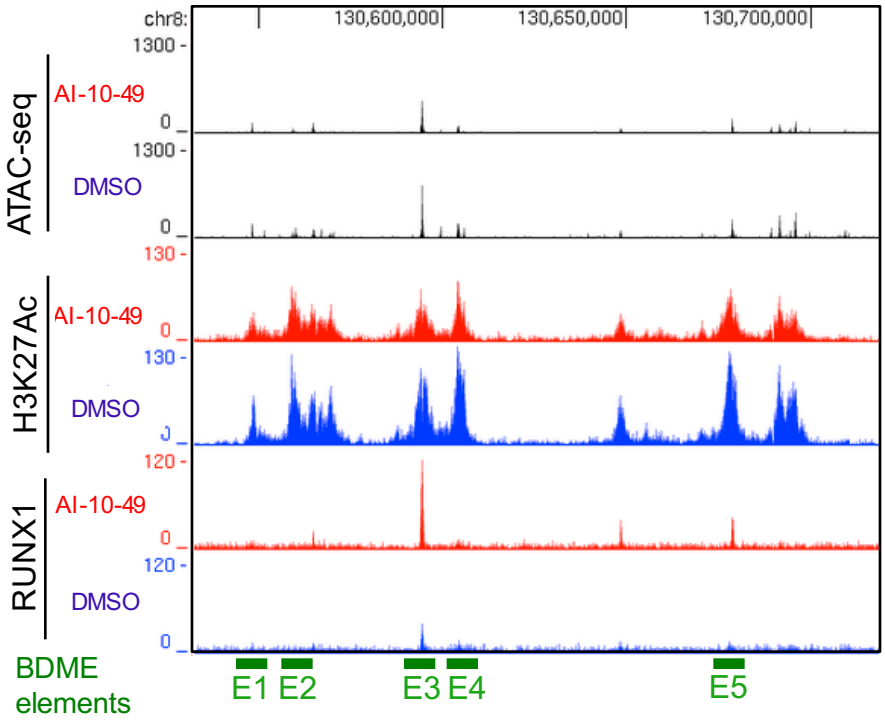


Figure S5, related to Figure 5

A.



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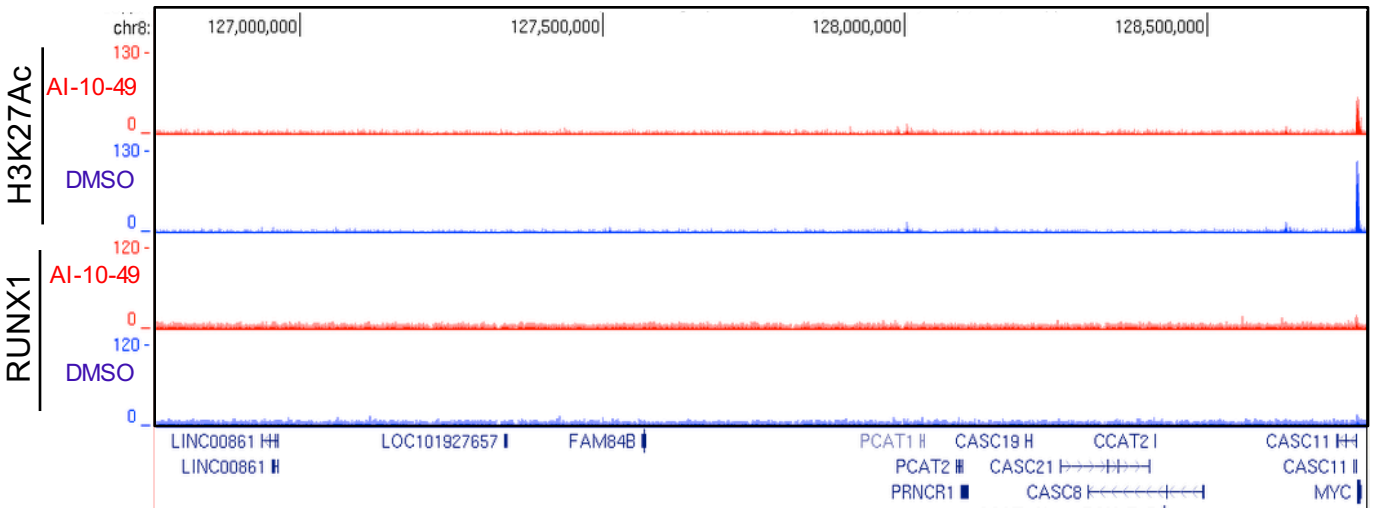


Figure S6
related to Figure 5

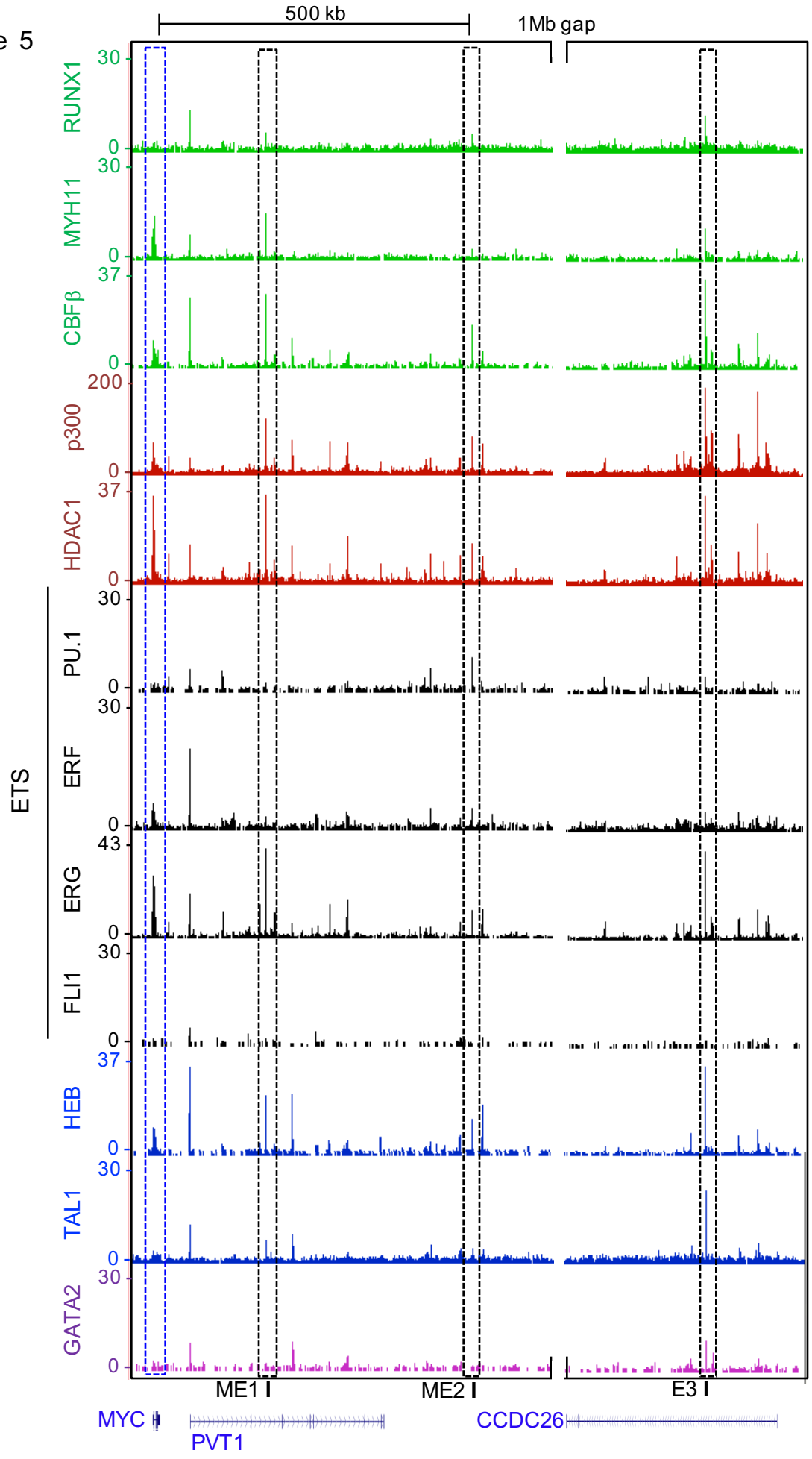


Figure S7
related to Figure 5

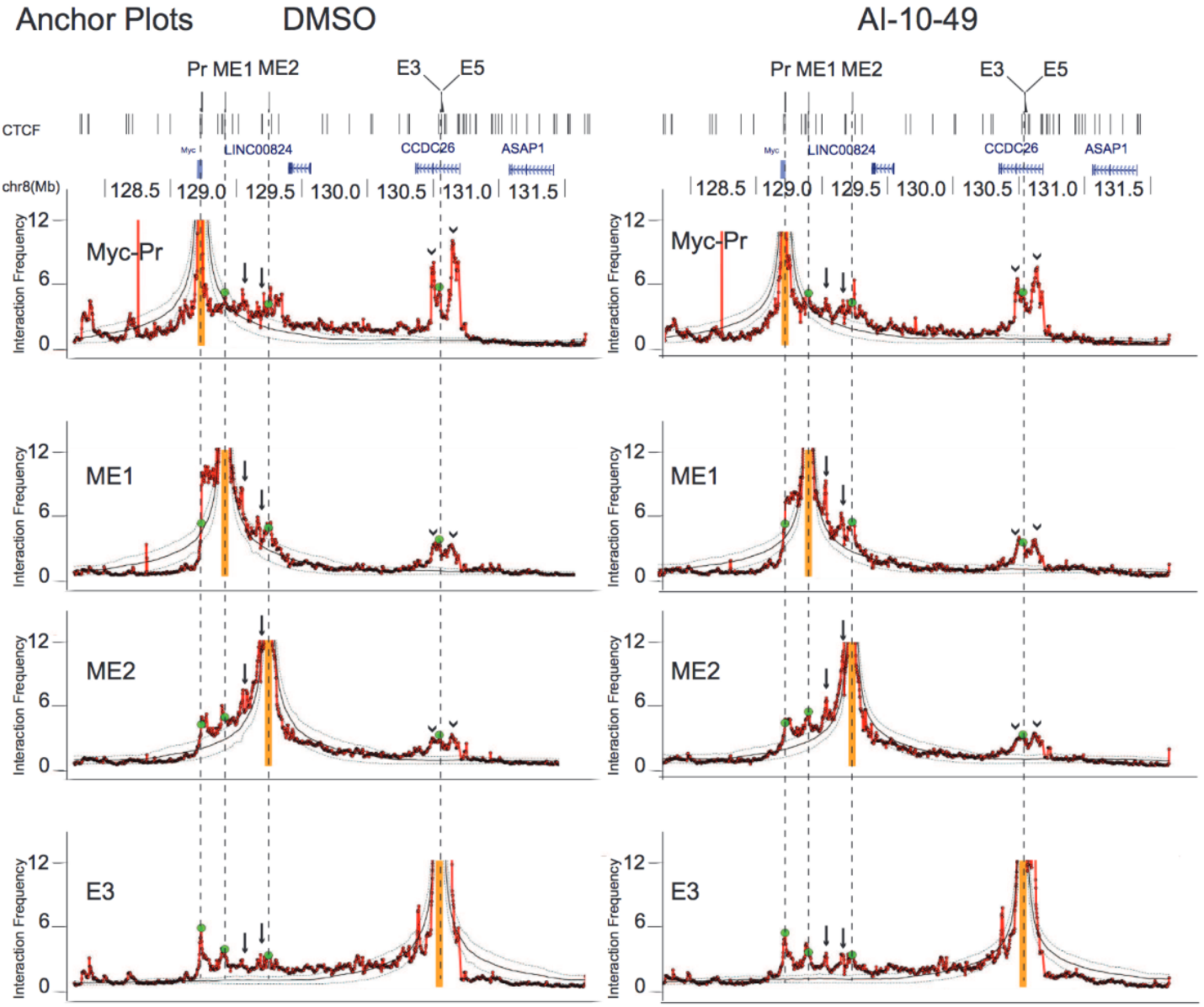
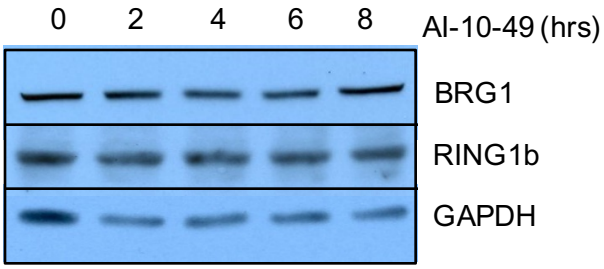


Figure S8, related to Figure 6

A.



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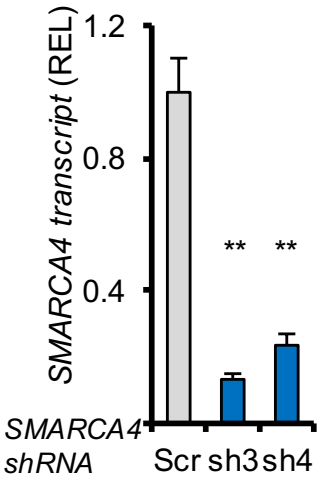

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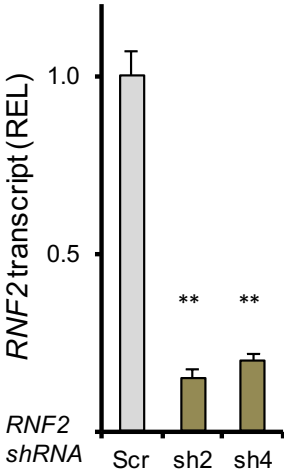

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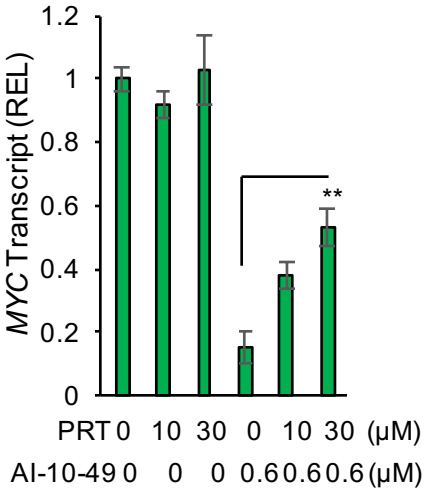

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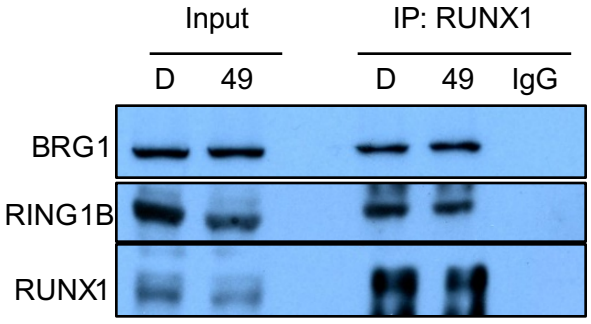
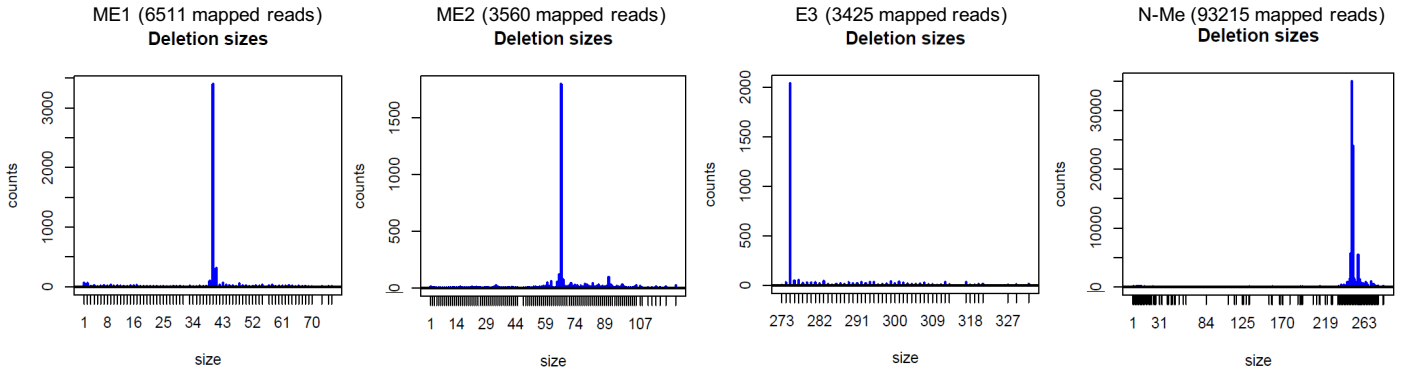


Figure S9, related to Figure 7

A.



B.