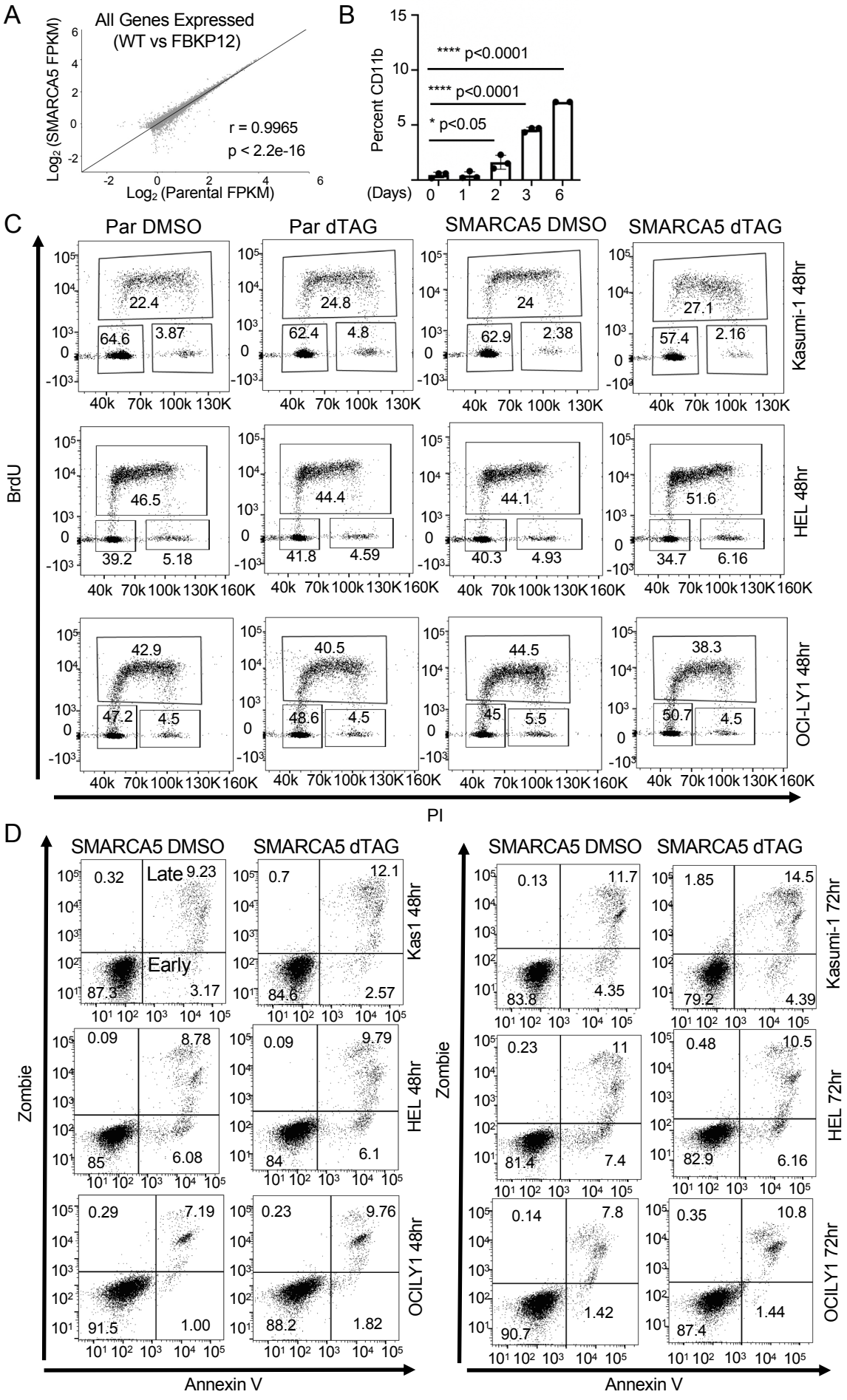
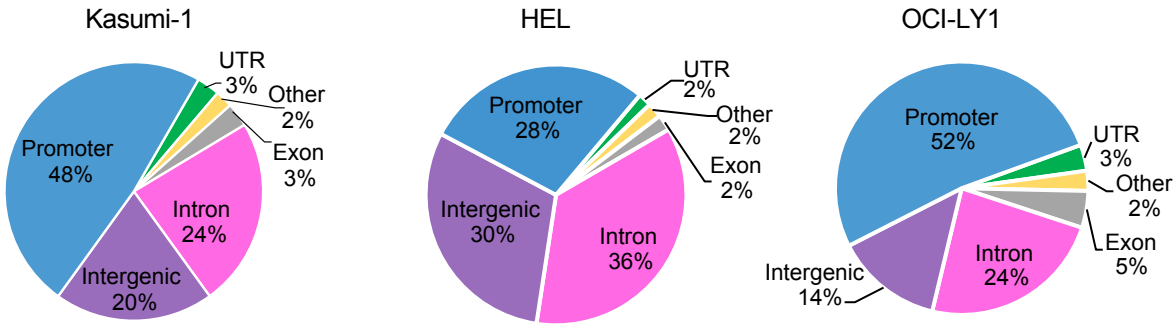
Supplemental Figure 1



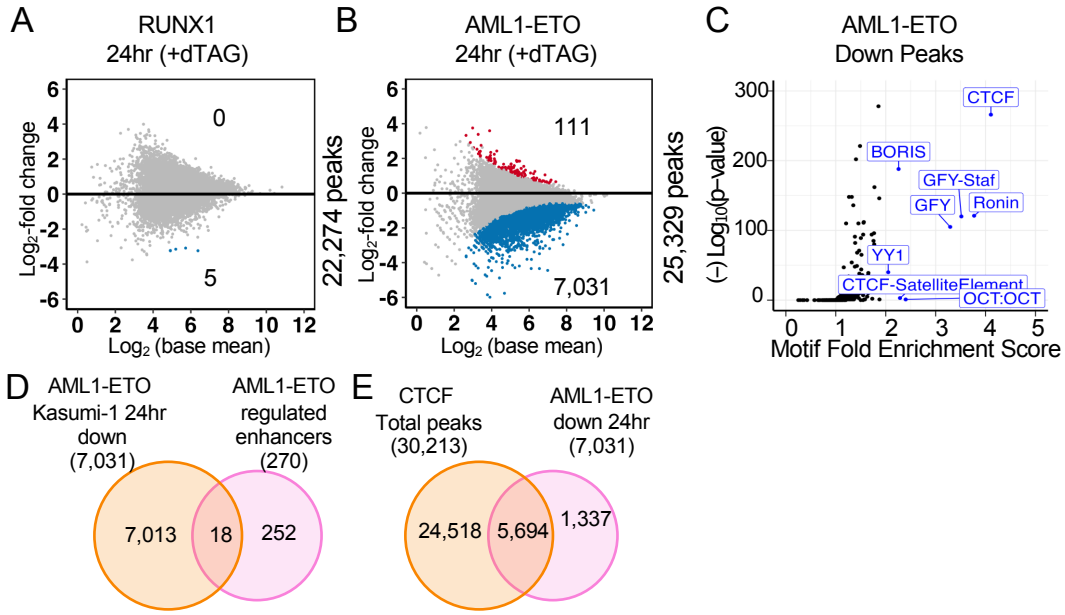
Supplemental Figure 1, Related to Figure 1. Characterization of SMARCA5-FKBP12$^{F36V}$–Flag tagged cell lines. (A) Scatter plot of $\log_2$ normalized counts from RNA-seq comparing the $\log_2$ transformation of FPKM of all genes expressed in Kasumi-1 Parental versus Kasumi-1 SMARCA5-FKBP12$^{F36V}$-FLAG-expressing cells. (B) Quantification of CD11b expression by flow cytometry as a marker of myeloid differentiation of parental Kasumi-1 or SMARCA5-FKBP12$^{F36V}$-FLAG–expressing cells at 1, 2, 3, and 6 days after SMARCA5 degradation (n=3). (C) Flow cytometry analysis of BrdU incorporation versus propidium iodide (PI) staining of Kasumi-1, HEL, and OCI-LY1 Parental versus SMARCA5-FKBP12$^{F36V}$-FLAG-expressing cells at 48hr following SMARCA5 degradation. (D) Flow cytometry analysis of Annexin V-FITC staining and Zombie-NIR staining of Kasumi-1, HEL, and OCI-LY1 SMARCA5-FKBP12$^{F36V}$-FLAG cells 48hr and 72hr after degradation of SMARCA5 compared to DMSO control samples.

Supplemental Figure 2



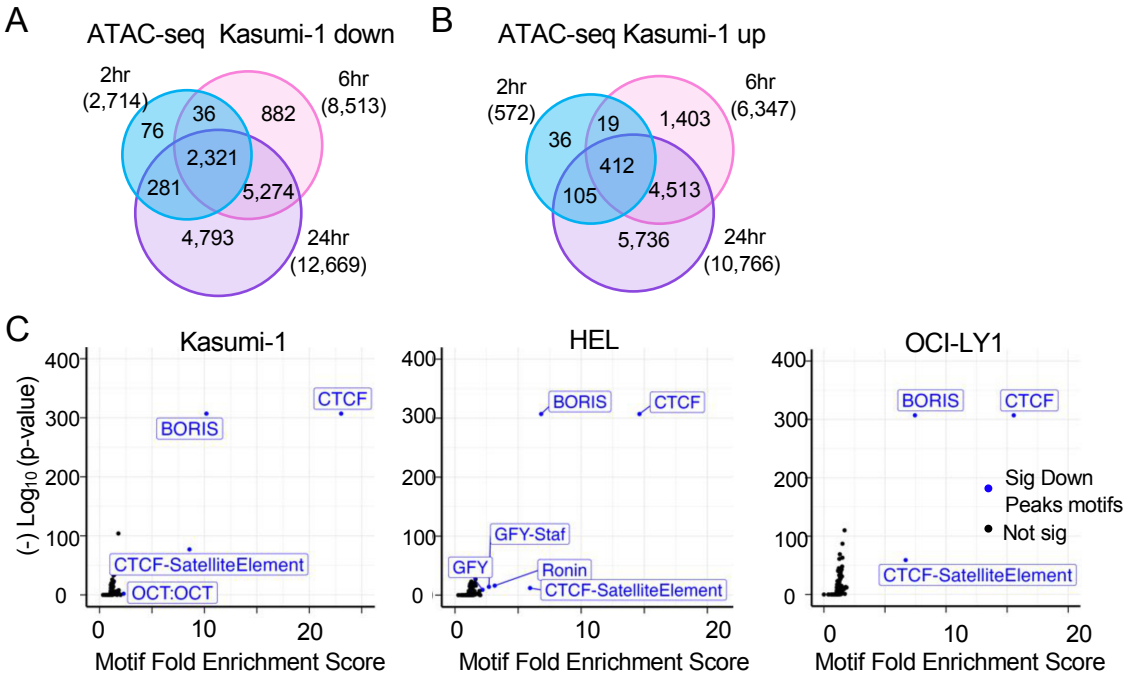

Supplemental Figure 2, Related to Figure 2. Genomic annotations of the SMARCA5 peaks using nearest neighbor analysis.

Supplemental Figure 3



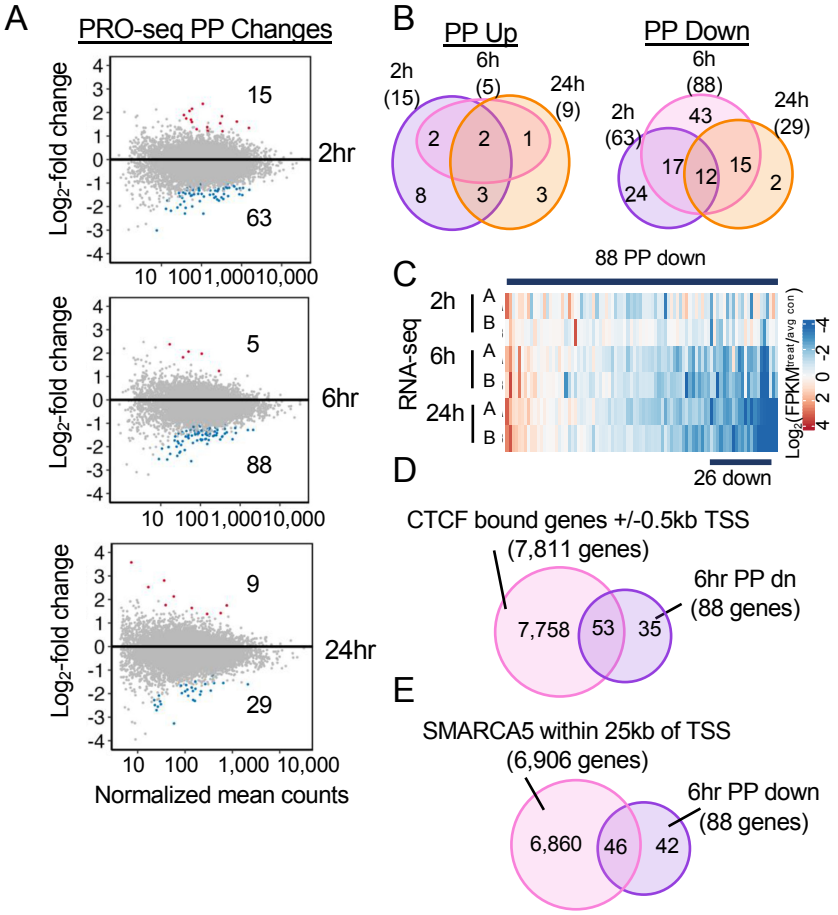

Supplemental Figure 3, Related to Figure 3. SMARCA5 is not required for RUNX1 localization. (A and B) MA plots of differential analysis of (A) RUNX1 CUT&RUN or (B) AML1-ETO CUT&RUN peaks 24hr after degrading SMARCA5-FKBP12$^{F36V}$-FLAG in Kasumi-1 cells (n=2). (C) Volcano plots of the negative $Log_{10}$ (p-value) versus motif enrichment scores from the down-regulated AML1-ETO peaks in B. (D) Venn diagram showing the over lap between the AML1-ETO peaks down-regulated upon degradation of SMARCA5-FKBP12$^{F36V}$-FLAG with the previously identified AML1-ETO regulated enhancer peaks (Stengel et al., 2021). (E) Venn diagrams comparing the AML1-ETO down-regulated peaks with total CTCF CUT&RUN peaks.

Supplemental Figure 4



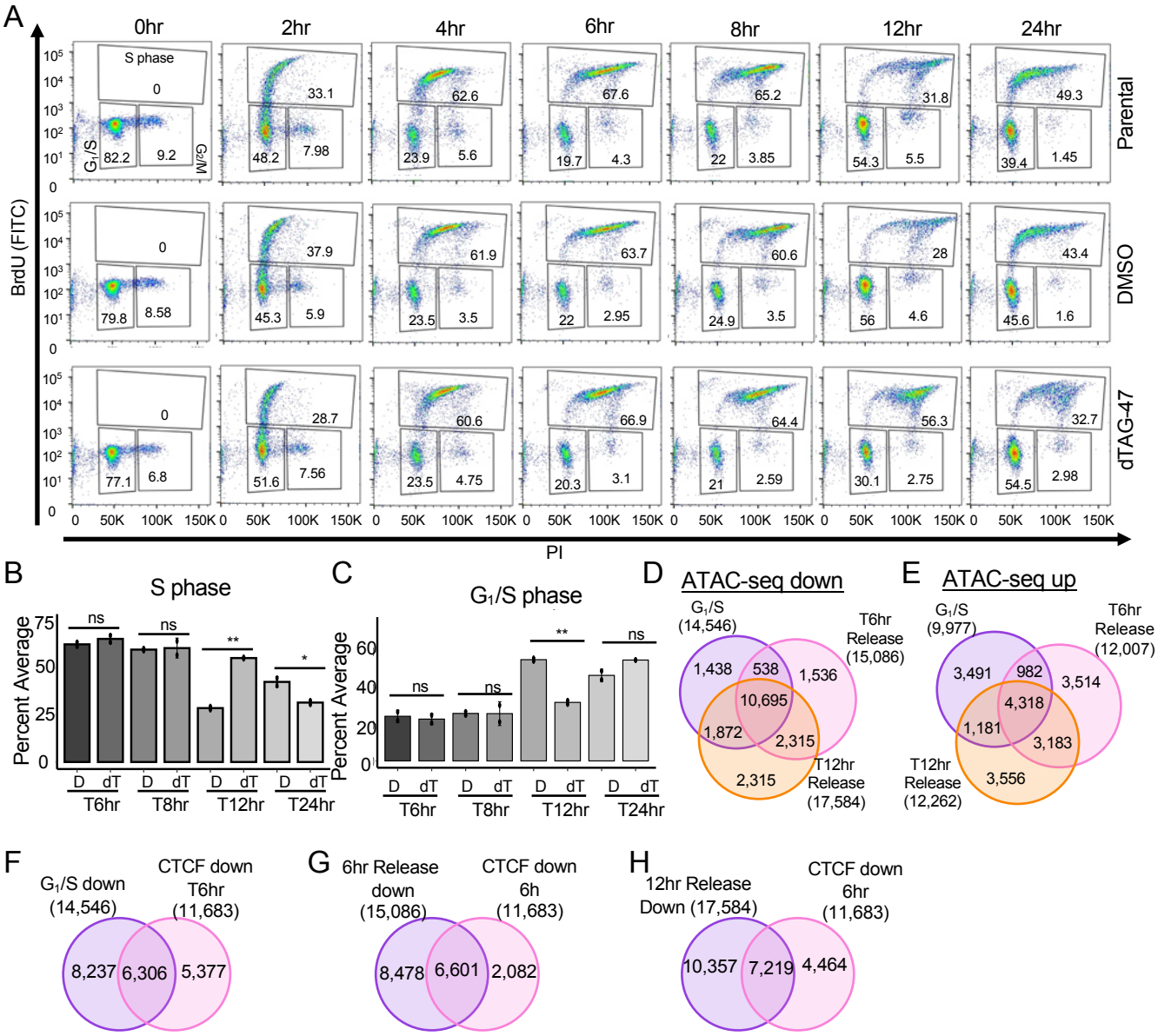

Supplemental Figure 4, Related to Figure 4. SMARCA5 degradation affects chromatin accessibility. (A and B) Venn diagrams showing the loss of in ATAC-seq peaks (A) or gain of peaks (B) over the time course of degradation of SMARCA5-FKBP12$^{F36V}$-FLAG in Kasumi-1 cells. (C) Volcano plots of the negative $Log_{10}$ of the p-value versus the motif enrichment score of ATAC-seq peaks 6hr after degradation of SMARCA5-FKBP12$^{F36V}$-FLAG in Kasumi-1, HEL, and OCI-LY1 cells. The blue dots represent motifs enriched by at least 2-fold.

Supplemental Figure 5



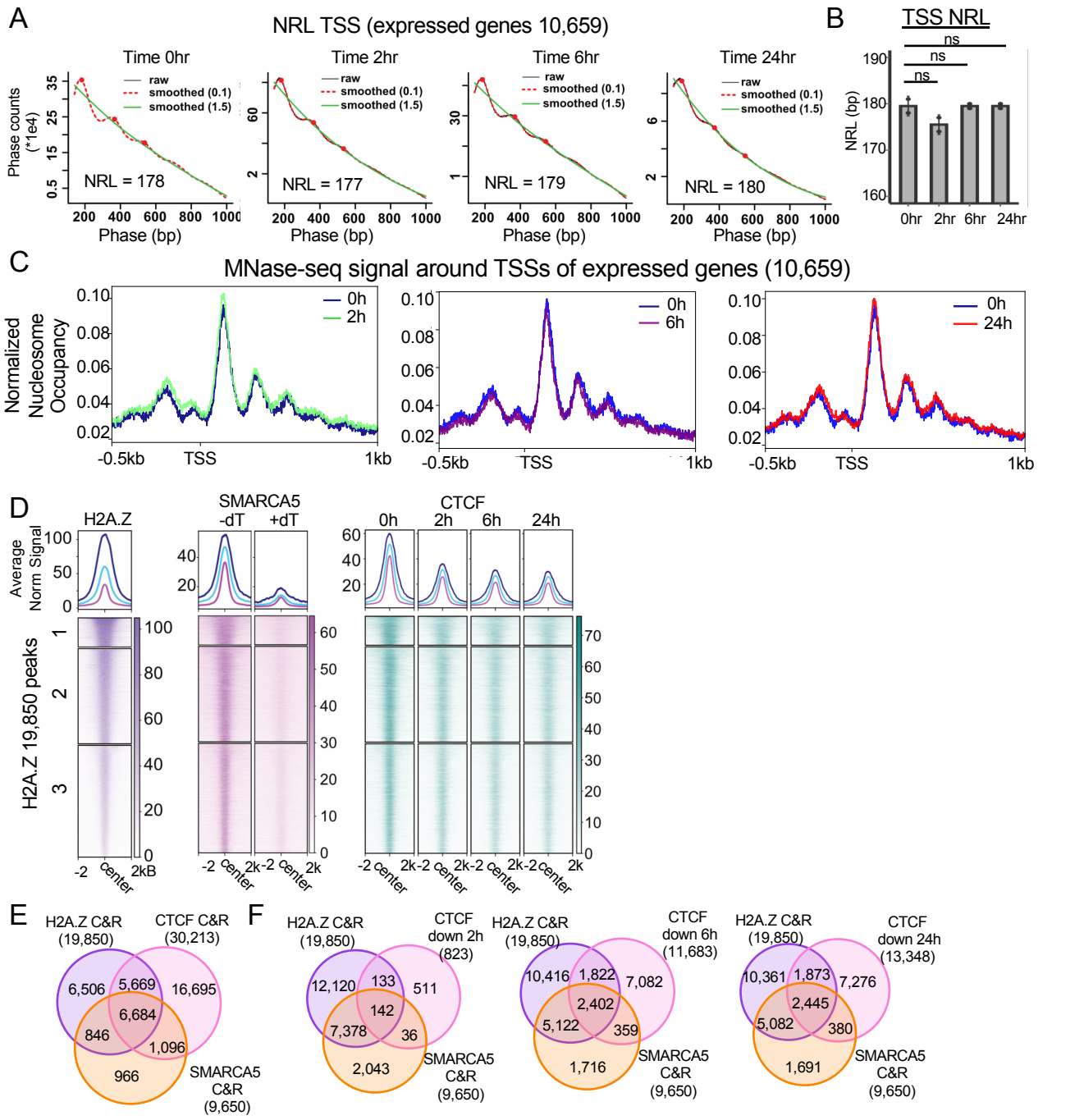

Supplemental Figure 5, Related to Figure 5. Degradation of SMARCA5 causes changes in RNA polymerase pausing. (A) MA plots of the log2-fold change vs the normalized mean counts of the promoter proximal regions of all expressed genes. The colored dots are the significant promoter proximal gene changes (adjusted p-value < 0.05 and fold change > 2; red = Up, blue = Down). (B) Venn diagrams showing the overlap between promoter proximal changes of up- or down-regulated genes at 2, 6, 24hr. (C) Heatmap of $Log_2$ (FPKM/avgFPKM) of each RNA-seq replicate of the time course of SMARCA5 degradation. Shown are the relative changes of gene expression of the 88 genes that displayed a loss of paused polymerase at 6h after degradation of SMARCA5. (D) Venn diagram showing the overlap between promoter proximal genes up- or down-regulated 6hr after SMARCA5 degradation intersected with CTCF CUT&RUN peaks annotated to +/-500bp of the TSS of the nearest neighbor gene. (E) Venn diagram showing the overlap between promoter proximal changes of up- or down-regulated genes at 6hrs after SMARCA5 degradation intersected with SMARCA5 CUT&RUN peaks annotated to +/-25kb of the TSS of the nearest neighbor gene.

Supplemental Figure 6




Supplemental Figure 6, Related to Figure 6. SMARCA5 loss affects chromatin accessibility during the cell cycle. (A) Flow cytometry analysis of BrdU incorporation versus propidium iodide staining of Kasumi-1-SMARCA5-FKBP12$^{F36V}$-FLAG cells after a double thymidine block, followed by treatment with dTAG-47 for 2hr to ensure SMARCA5 degradation, before release into the S phase for the times indicated above each plot. Parental cells are unmodified Kasumi-1 cells and DMSO are the *SMARCA5-FKBP12$^{F36V}$*-expressing cells that were mock treated with DMSO. (B) Bar graph of quantification of flow cytometry analysis of BrdU incorporation from cells released at 6h, 8h, 12h, and 24h (n=2). (C) Bar graph of quantification of flow cytometry analysis of $G_1$ phase cells at 6h, 8h, 12h, and 24h after release (n=2). (D) Venn diagram of the down-regulated ATAC-seq peaks at $G_1$S, 6h after release (mid S), and 12h after release (late S). (E) Venn diagram of the up-regulated ATAC-seq peaks at $G_1$S, 6h after release (mid S), and 12h after release (late S). (F-H) Venn diagrams of the down-regulated CTCF peaks from Figure 3 intersected with the down-regulated ATAC-seq peaks at $G_1$/S (F), 6h after release (G), and 12hr after release (H).

# Supplemental Figure 7




Supplemental Figure 7, Related to Figure 7. SMARCA5 and CTCF localize around H2A.Z sites (A) "Phasogram" of the nucleosome repeat length using the MNase-seq signal of a representative replicate after degradation of SMARCA5 at the indicated times +/-1kb from the TSSs of all expressed genes, as determined from the PRO-seq data in Figure 5. (B) Bar graph quantification of the nucleosome repeat lengths of biological replicates calculated using the around the TSS of the expressed genes at each of the indicated time points. (C) Histograms of nucleosome occupancy using MNase-seq data plotted from 500bp upstream to 1000bp downstream of all expressed TSSs from the PRO-seq data in Figure 5. (D) Heatmaps of H2A.Z, SMARCA5, and CTCF CUT&RUN at the indicated timepoints around H2A.Z – containing peaks +/- 2kb. H2A.Z sites were clustered by k-means using deepTools. (E) Venn diagram showing the overlap between H2A.Z and SMARCA5 with all CTCF binding sites. (F) Venn diagrams showing the overlap between H2A.Z and SMARCA5 with CTCF binding sites lost at 2, 6, and 24hrs in Kasumi-1 cells.