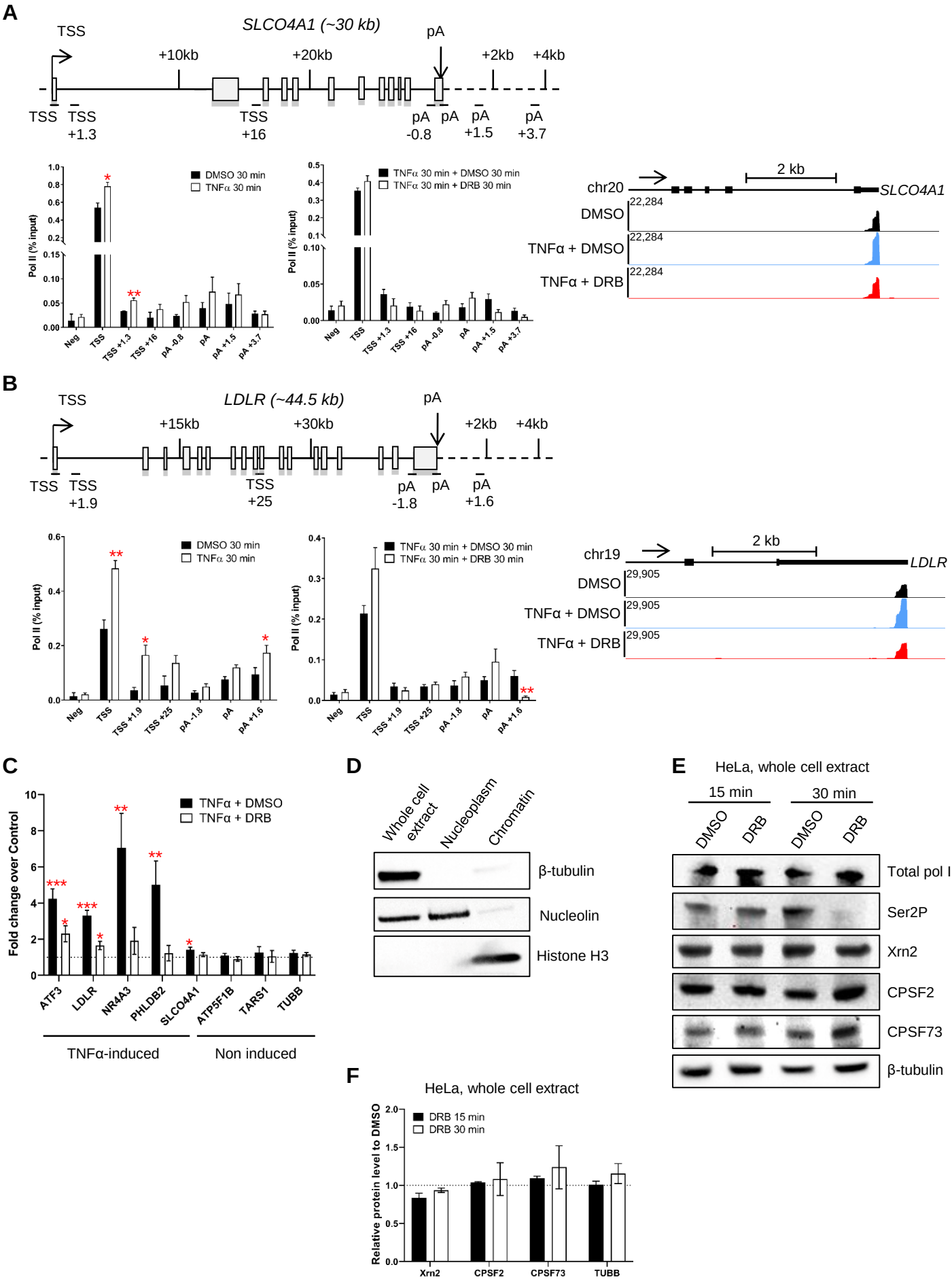
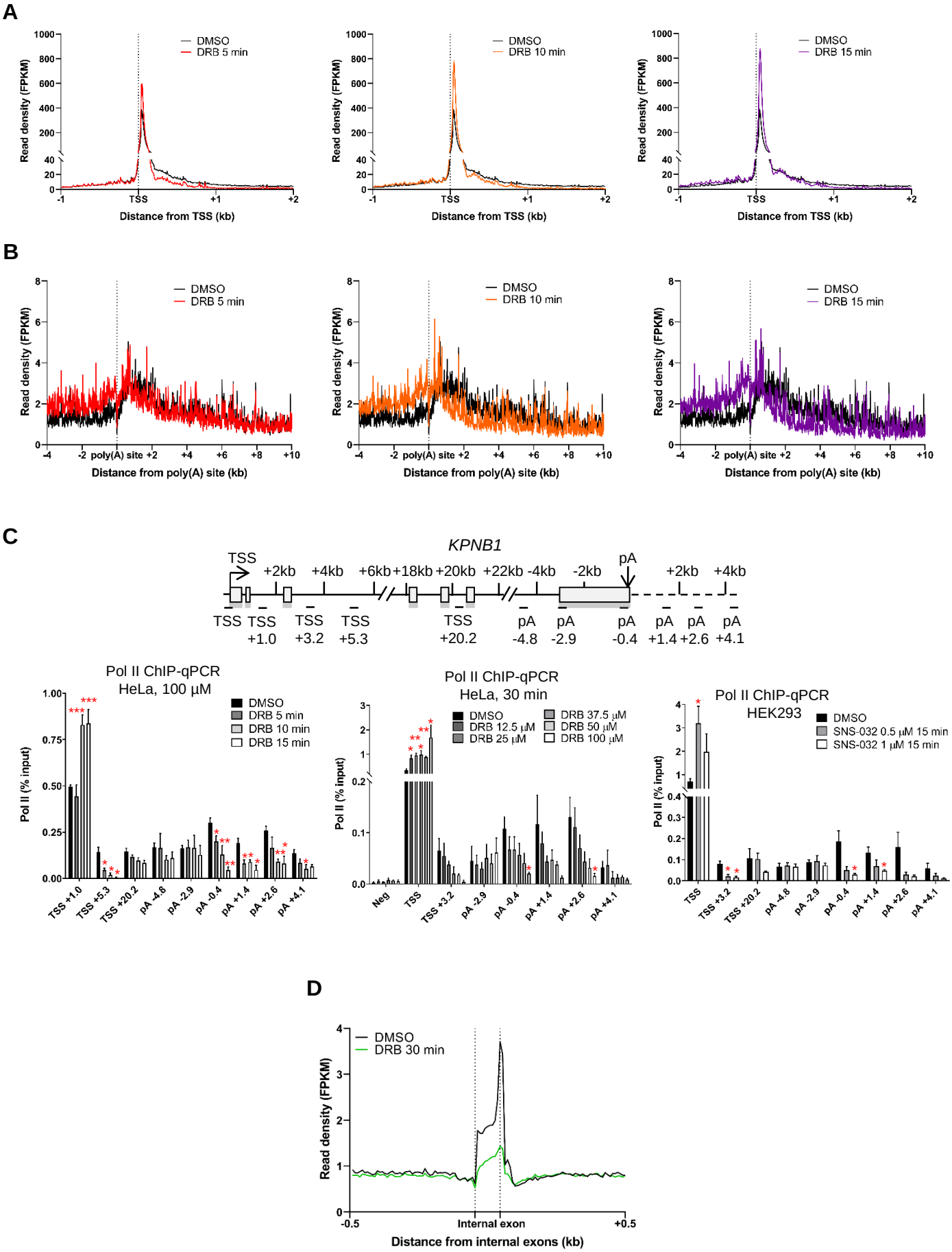


Supplementary Figure 1

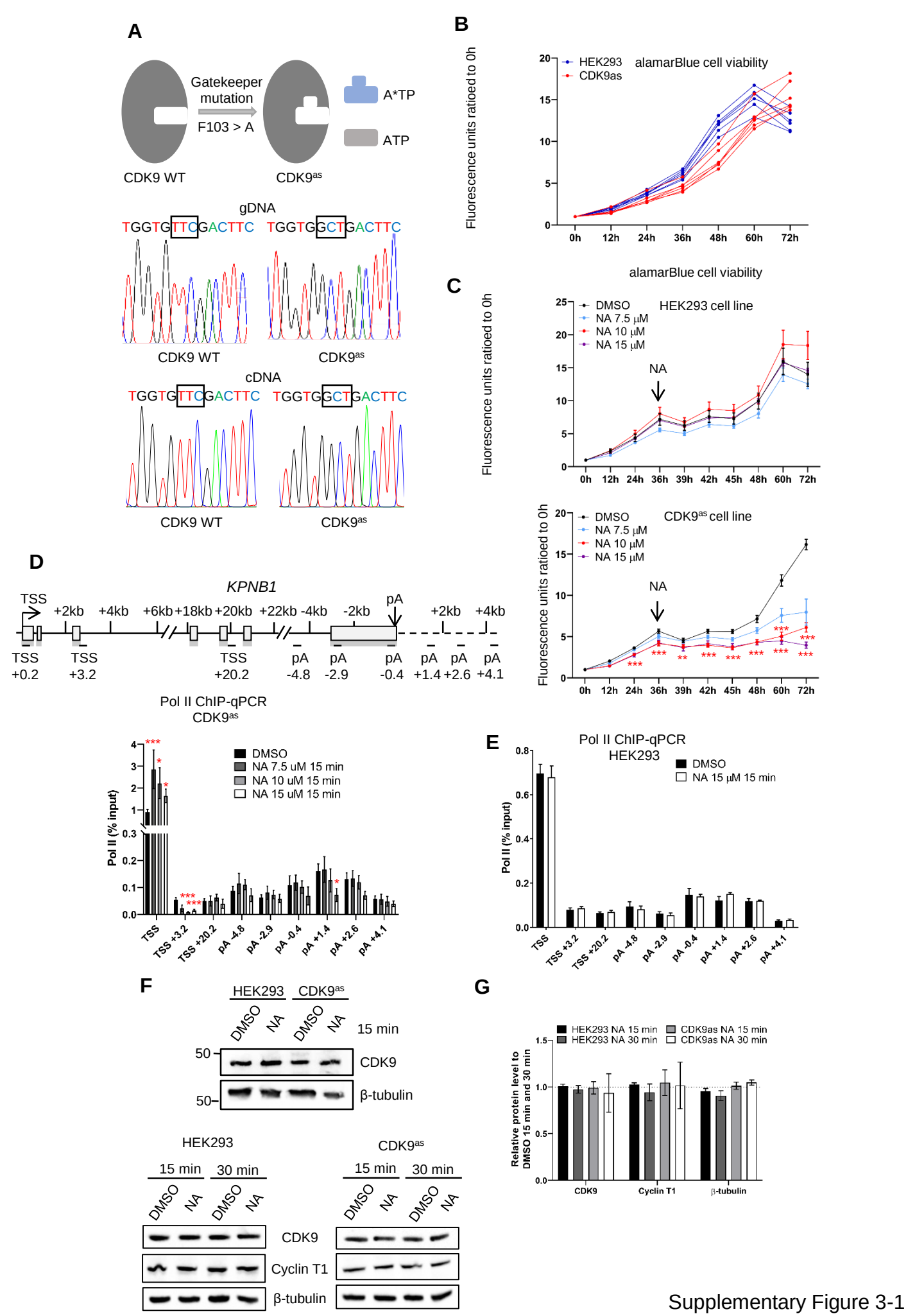
Supplementary Figure 1. CDK9 inhibition causes failure of polyadenylation.
A. Pol II ChIP-qPCR on the TNFα induced gene *SLCO4A1* and screenshot of the genome browser track of the 3'READS experiments for this gene. n=3 biological replicates, p-value: * $p < 0.05$, ** $p < 0.01$. Statistical test: two-tailed unpaired t test. **B.** Pol II ChIP-qPCR on the TNFα-induced gene *LDLR* and screenshot of the genome browser track of the 3'READS experiments for this gene. n=3 biological replicates, p-value: * $p < 0.05$, ** $p < 0.01$. Statistical test: two-tailed unpaired t test. **C.** qRT-PCR of nuclear polyadenylated mRNAs of several TNFα-induced or non-induced genes with a 30 minutes DMSO or a DRB (100 μM here and in all other figures unless stated otherwise) treatment. n=5 biological replicates, p-value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical test: two-tailed unpaired t test. **D.** Western blot of β-tubulin, Nucleolin, and Histone H3 on whole cell extract, nucleoplasm, and chromatin fractions. **E.** Western blot of total pol II, Ser2P, Xrn2, CPSF2, CPSF73, and β-tubulin as a loading control, on whole cell extract. **F.** Quantification of the western blots shown in E. n=2 biological replicates, p-value: n.s. not significant. Statistical test: two-tailed unpaired t test.



Supplementary Figure 2

Supplementary Figure 2. CDK9 inhibition causes an elongation defect starting at the last exon of protein-coding genes.

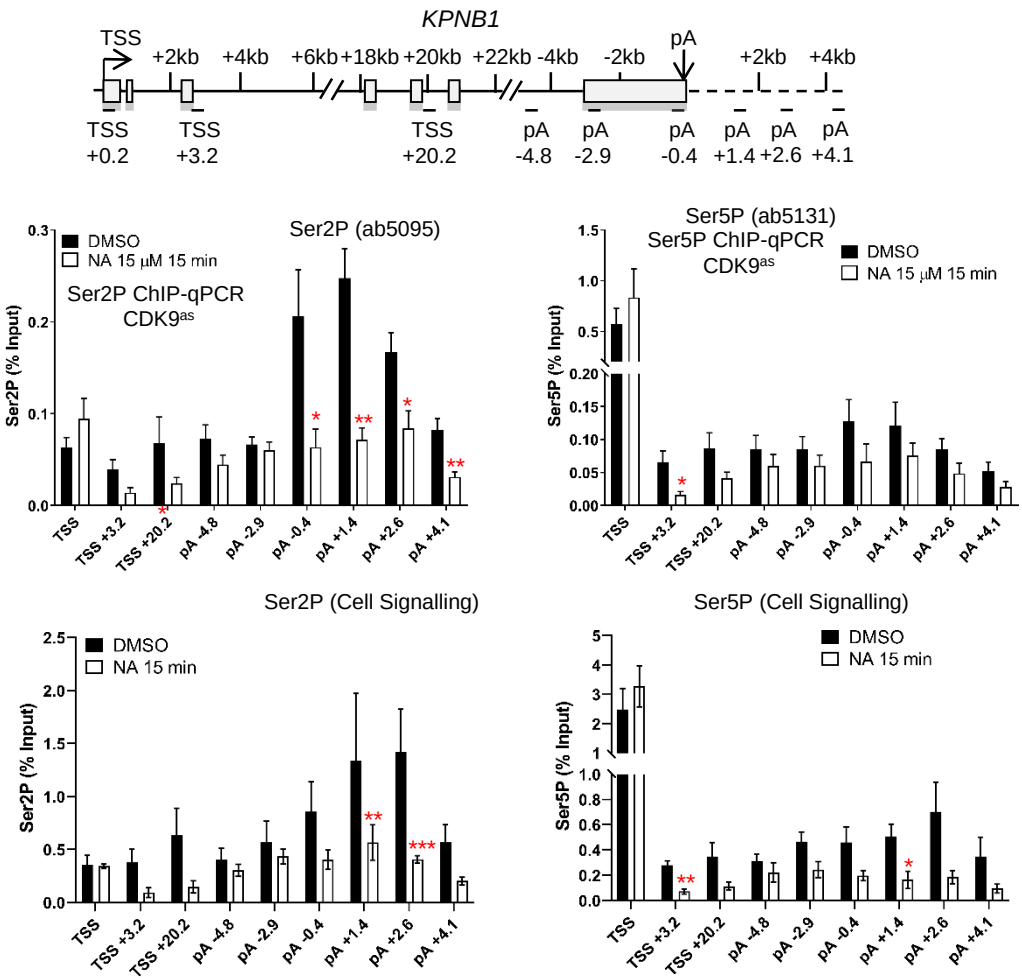
A. Metagene profile of total pol II after treatment with DMSO (black) or 5 (red), 10 (orange), or 15 (purple) minutes with DRB (green) around the TSS of expressed protein-coding genes (n=6,965). **B.** Metagene profile of total pol II after treatment with DMSO (black) or 5 (red), 10 (orange), or 15 (purple) minutes with DRB (green) around the TSS of expressed protein-coding genes longer than 40 kb (n=2,816). **C.** ChIP-qPCR of total pol II with different treatment times or different concentrations of DRB or SNS-032 on the model gene, *KPNB1*. n=3 biological replicates, p-value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical test: two-tailed unpaired t test. **D.** Metagene profile of total pol II after 30 minutes treatment with DMSO (black) or DRB (green) around internal exons, not including first, penultimate or last exons, of expressed protein-coding genes (n=26,094).



Supplementary Figure 3-1

Supplementary Figure 3-1. Inhibition of analog-sensitive (as) CDK9 produces similar results to small molecule CDK9 inhibitors.

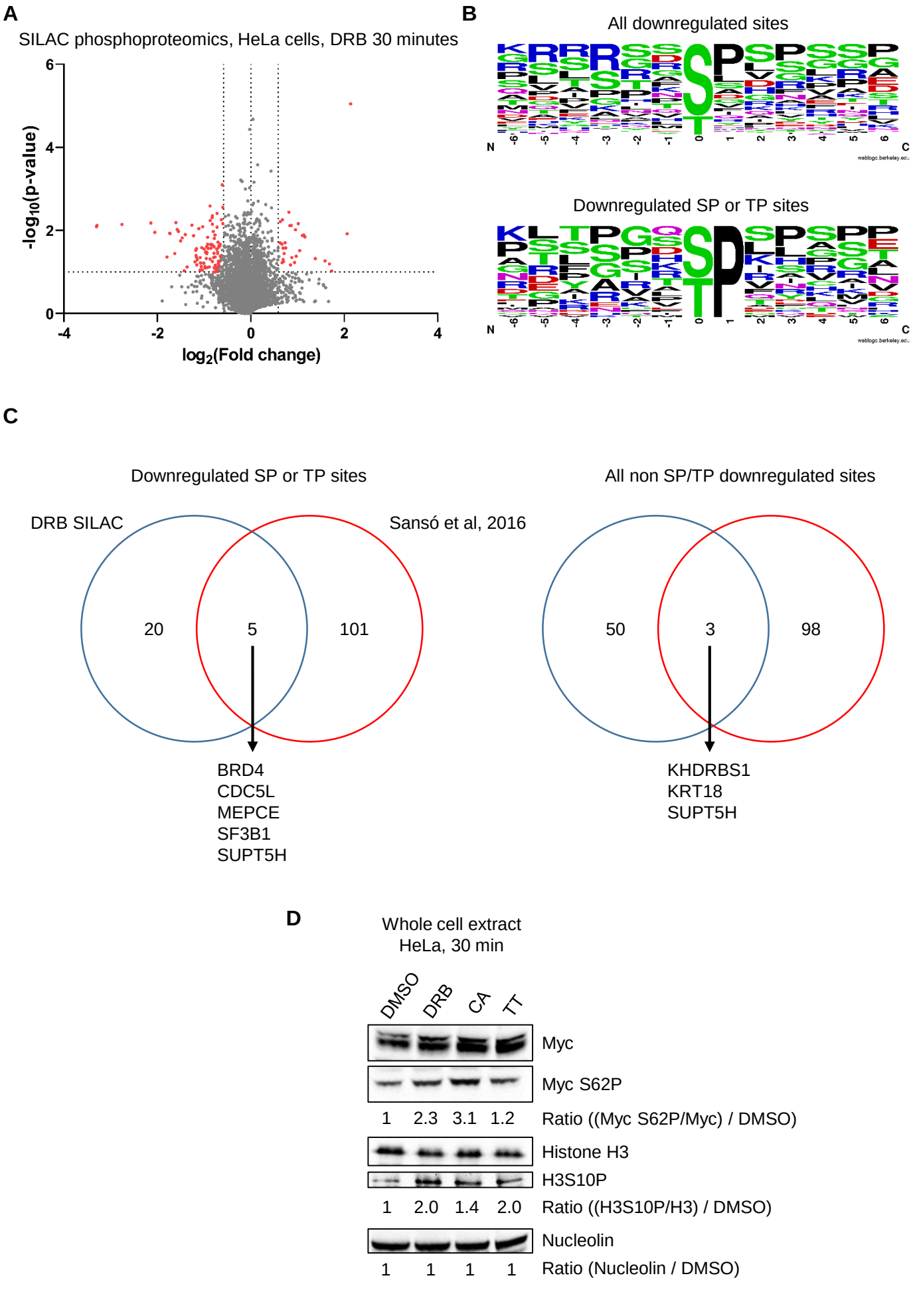
A. Schematic of the genome editing of the CDK9as cell line. **B.** alamarBlue cell viability of wild-type HEK293 and CDK9as cells. Each line represents a biological replicate. **C.** alamarBlueHS cell viability assay of wild-type HEK293 and CDK9as cells with different concentrations of 1-NA-PP1 added at the 36 hour time point. n=3 biological replicates, p-value: ** $p < 0.01$, *** $p < 0.001$. Statistical test: paired t-test with FDR multiple testing correction. **D.** ChIP-qPCR of total pol II with different concentrations of 1-NA-PP1 in CDK9as cells on the model gene, *KPNB1*. n=3 biological replicates, p-value: * $p < 0.05$, *** $p < 0.001$. Statistical test: two-tailed unpaired t test. **E.** ChIP-qPCR of total pol II treated with 1-NA-PP1 in wild-type HEK293 cells on the model gene, *KPNB1*. n=3 biological replicates, p-value: n.s. not significant. Statistical test: two-tailed unpaired t test. **F.** Western blot of CDK9, Cyclin T1, and β-tubulin as a loading control, on whole cell extracts of wild-type HEK293 and the CDK9as cell line treated with DMSO or 1-NA-PP1 for 15 or 30 minutes. **G.** Quantification of the western blots shown in F. n=2 biological replicates, p-value: n.s. not significant. Statistical test: two-tailed unpaired t test.



Supplementary Figure 3-2

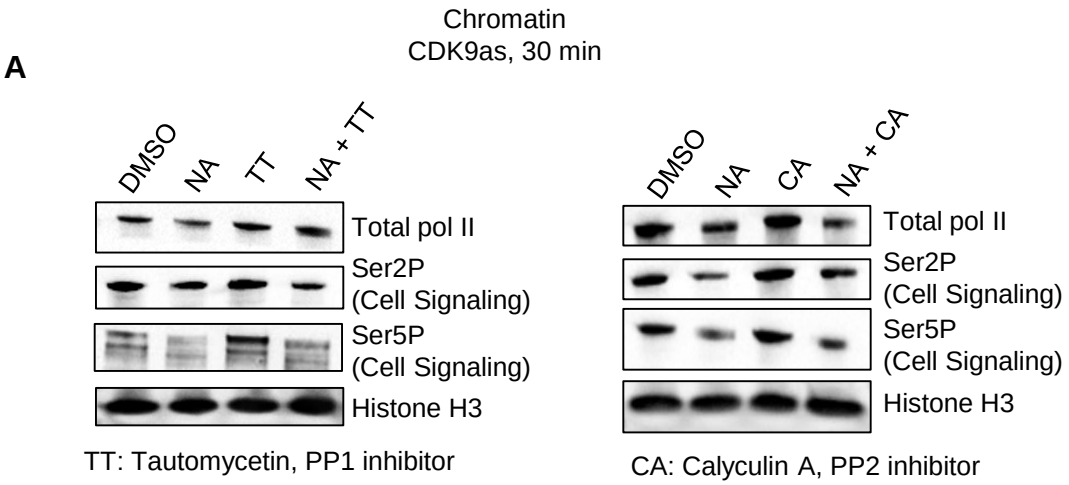
Supplementary Figure 3-2. Inhibition of a CDK9 analog sensitive (as) cell line produces similar results to the current CDK9 inhibitors.

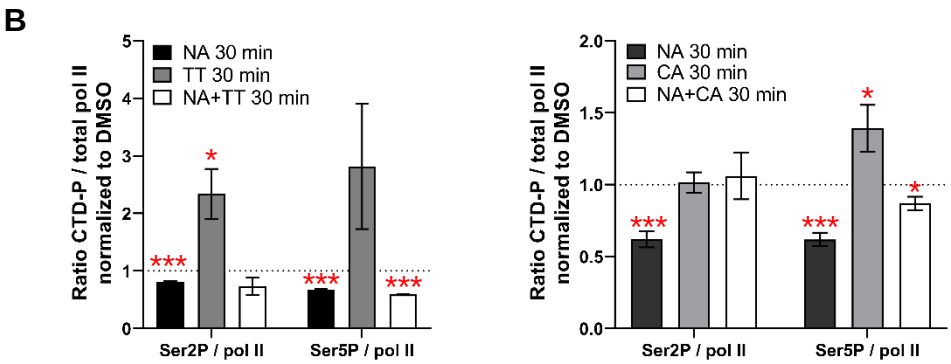
ChIP-qPCR of Ser2P (Abcam or Cell Signaling), and Ser5P (Abcam or Cell Signaling) in CDK9as cells treated with 15μM 1-NA-PP1 for 15 minutes on the model gene, *KPNB1*. n=3 biological replicates, p-value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical test: two-tailed unpaired t test.



Supplementary Figure 4-1

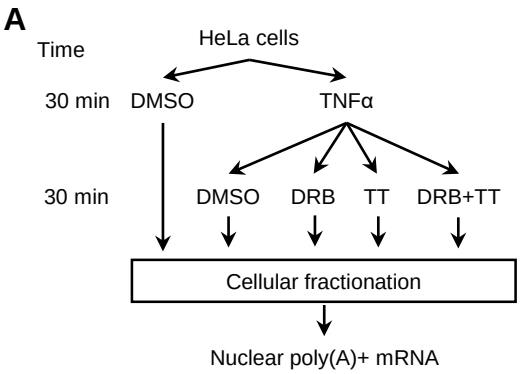
Supplementary Figure 4-1. CDK9 phosphorylates several transcription and splicing factors *in vivo*.
A. Volcano plot of SILAC phosphoproteomics in HeLa cells treated or not with 100 μM DRB for 30 minutes (in red: fold change > 1.5 in both biological duplicates, p-value < 0.1). **B.** Motif found around all the phosphorylation sites decreased following CDK9 inhibition of only the phosphorylation sites containing a ST or TP sites. **C.** Overlap between the proteins found to have at least one phosphopeptides decreased in our study versus an alternative experimental strategy used to identify CDK9 targets in cell extracts (Sanso et al., 2016). **D.** Western blot of Myc, Myc S62P, Histone H3, Histone H3 S10P, and Nucleolin as a loading control, on whole cell extract of HeLa cells treated for 30 minutes with DMSO, DRB, CA, or TT.

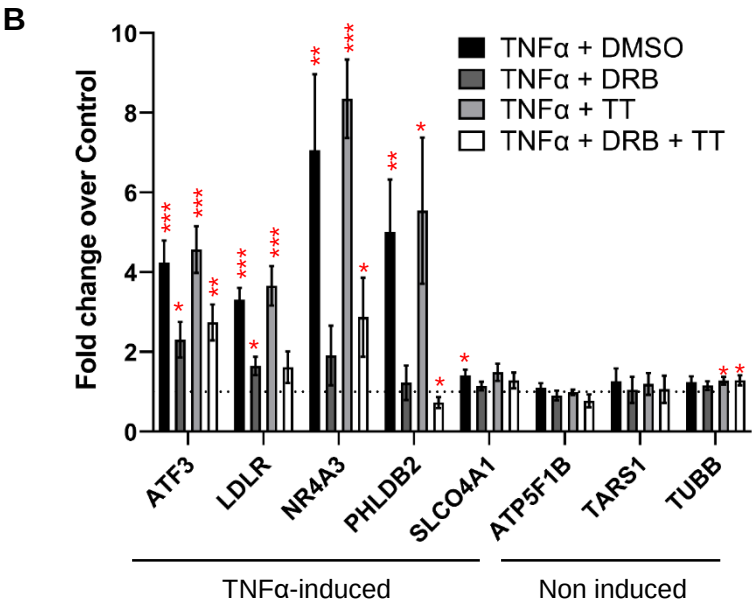


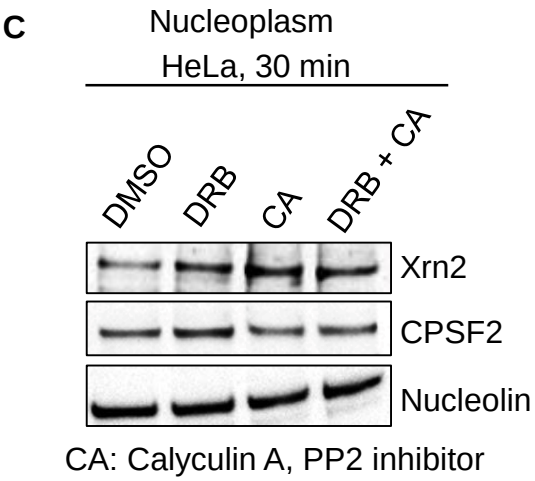


Supplementary Figure 4-2

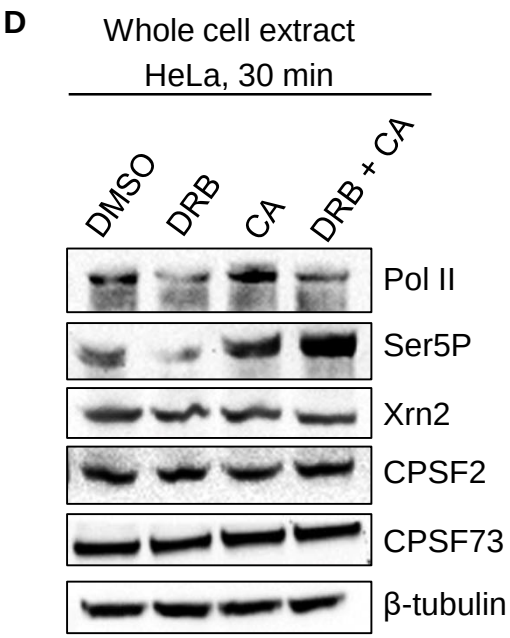
Supplementary Figure 4-2. CDK9 phosphorylates several transcription and splicing factors in vivo.
A. Western blot of total pol II, Ser2P, Ser5P, and histone H3 as a loading control, on the chromatin fraction of CDK9as cells treated for 30 minutes with DMSO, NA, CA, TT, NA+CA, or NA+TT. **B.** Quantification of the western blots shown in C. n=2 biological replicates, p-value: * $p < 0.05$, *** $p < 0.001$. Statistical test: two-tailed unpaired t test.





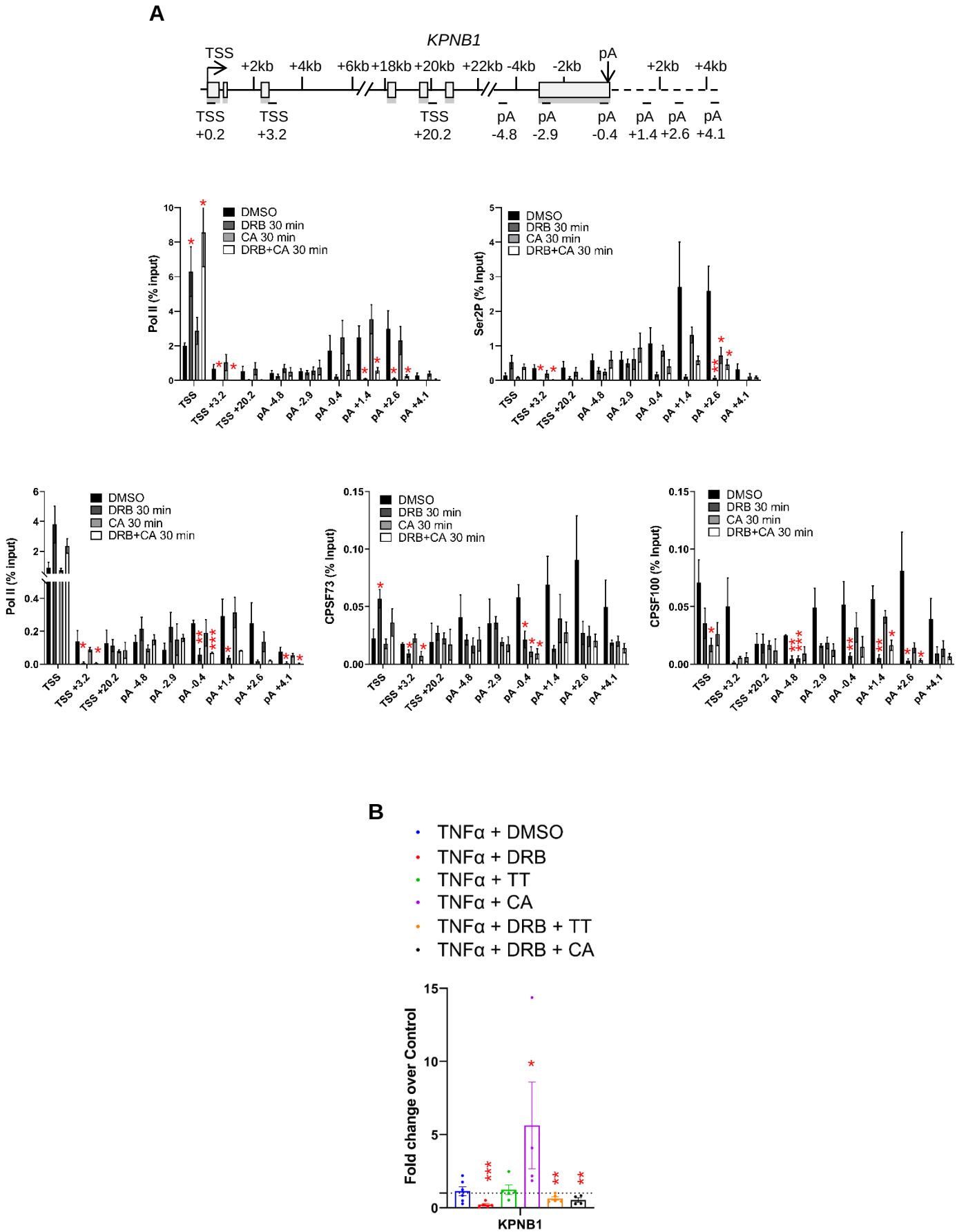


CA: Calyculin A, PP2 inhibitor



Supplementary Figure 6-1

Supplementary Figure 6-1. CDK9 and PP2A regulate mRNA cleavage and polyadenylation.
A. Schematic of the nuclear qRT-PCR experiments. **B.** qRT-PCR of nuclear polyadenylated mRNAs of several TNFα induced or non-induced genes with a 30 minutes DMSO, DRB, TT, or DRB+TT treatment. n=4 biological replicates, p-value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical test: two-tailed unpaired t test. **C.** Western blot of Xrn2, CPSF2, and Nucleolin as a loading control, on the nucleoplasm fraction of HeLa cells after a 30 minutes DMSO, DRB, CA, or DRB+CA treatment. The CPSF73 antibody is not shown at it does not provide reliable results on the nucleoplasm fraction. **D.** Western blot of total pol II, Ser5P, Xrn2, CPSF2, CPSF73, and β-tubulin as a loading control, on whole cell extract of HeLa cells treated for 30 minutes with DMSO, DRB, CA, or DRB+CA.



Supplementary Figure 6-2

Supplementary Figure 6-2. CDK9 and PP2A regulate mRNA cleavage and polyadenylation.
A. ChIP-qPCR of pol II, Ser2P, CPSF73, or CPSF2 after 30 minutes treatment with DMSO, DRB, CA, or DRB+CA on the model gene, *KPNB1*. n=3 biological replicates, p-value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical test: two-tailed unpaired t test. **B.** qRT-PCR of nuclear polyadenylated mRNAs of the *KPNB1* gene with a 30 minutes DMSO, DRB, TT, or DRB+TT treatment. n=4 biological replicates, p-value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical test: two-tailed unpaired t test.

Supplementary Table 1. Results of the DRB vs DMSO SILAC phosphoproteomics in HeLa cells treated for 30 minutes.

Supplementary Table 2. Results of the SF3B1 immunoprecipitation followed by proteomics in CDK9as cells treated for 30 minutes with DMSO or NA.