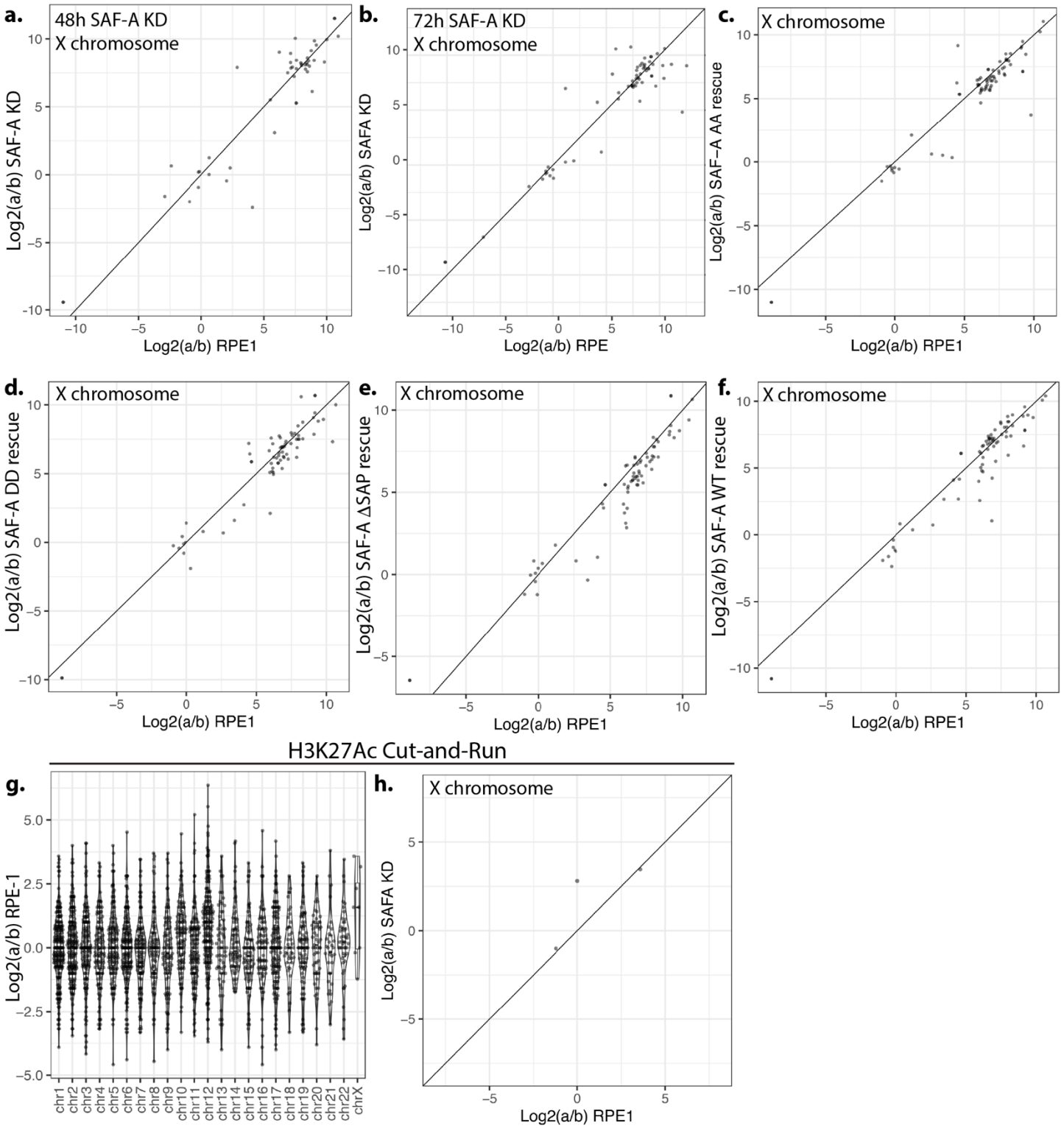
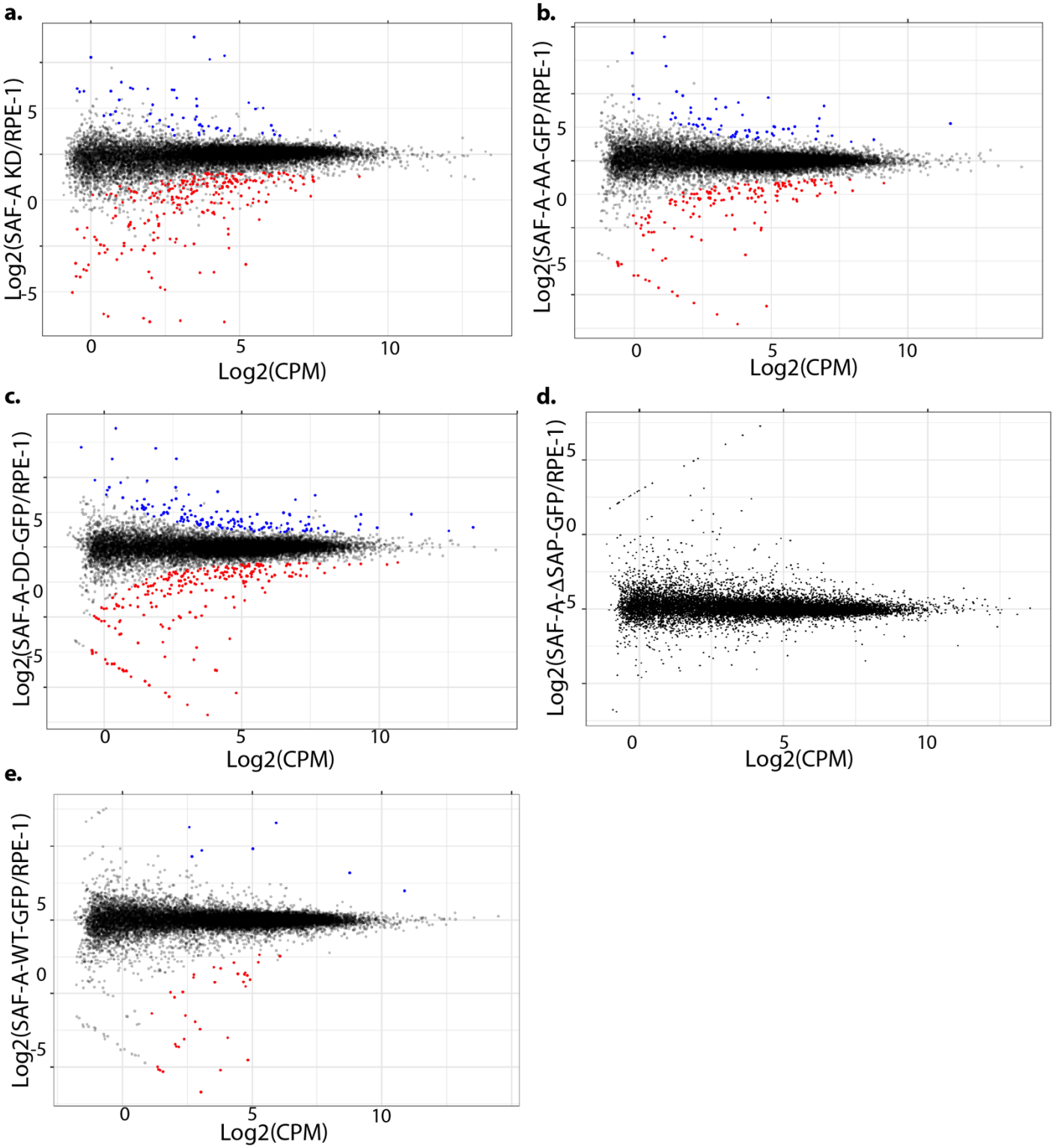
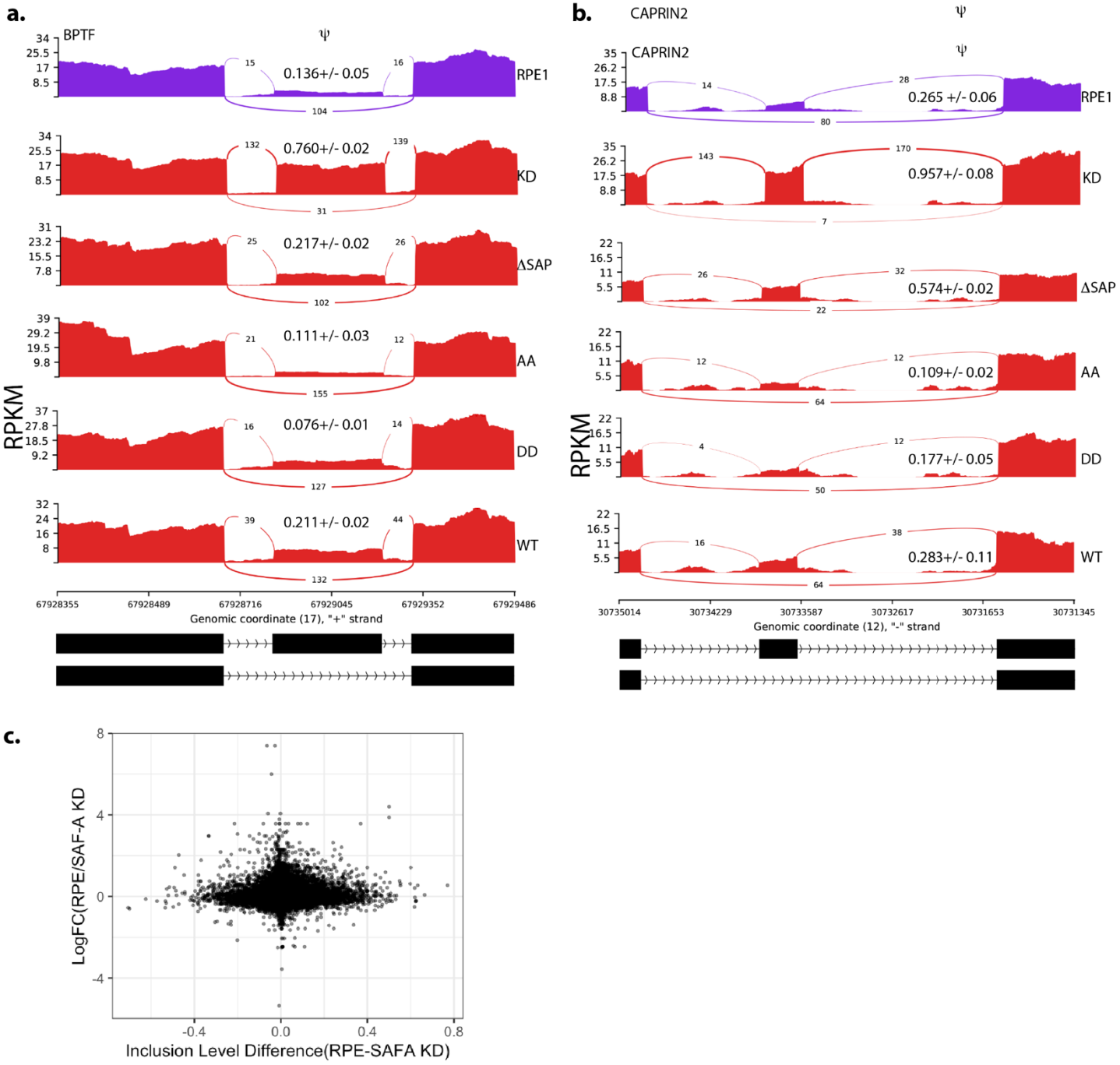


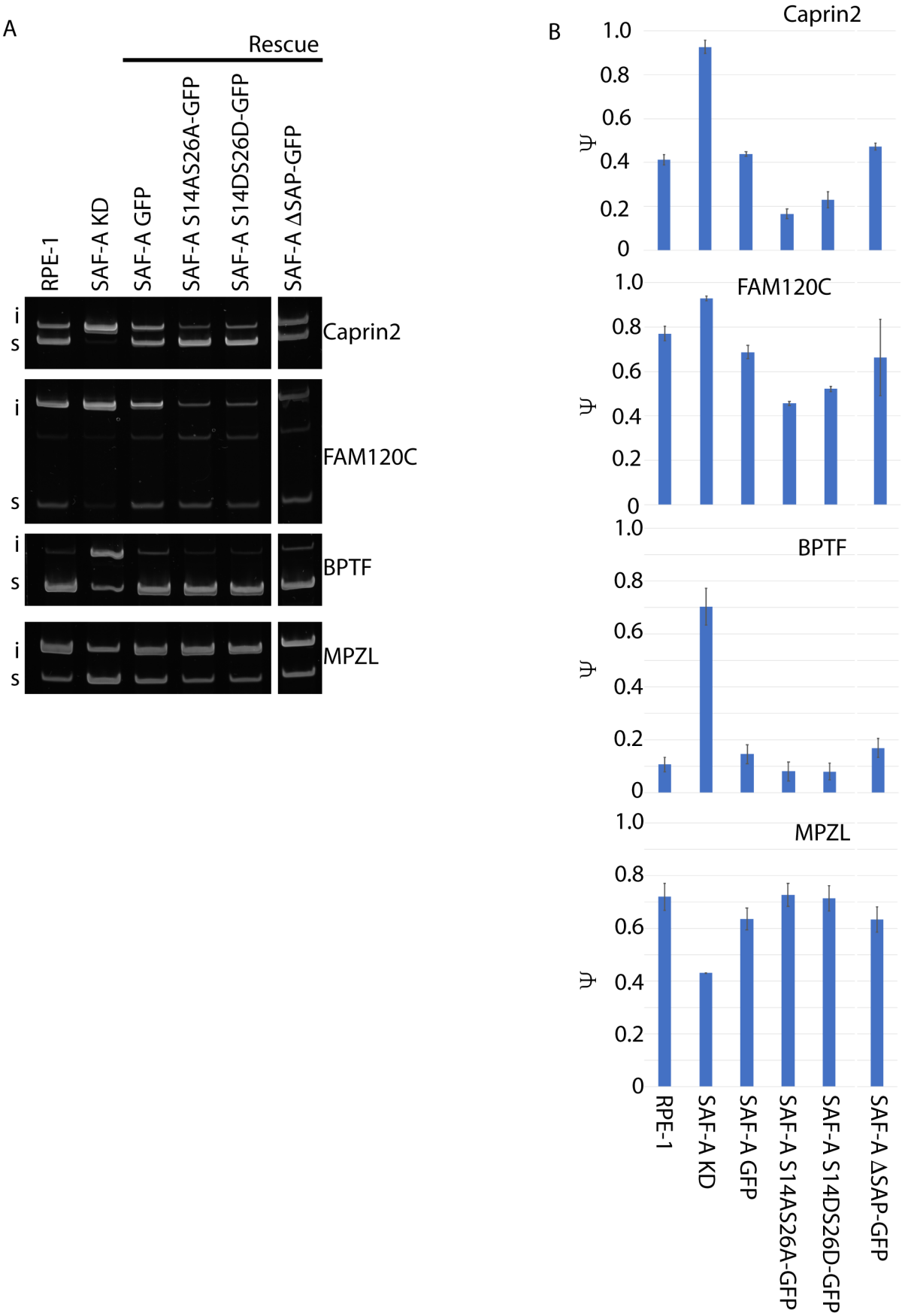
Supplemental Figure 1. Analysis of X-linked chromatin and gene expression in SAF-A mutants. A. Comparison of 'a/b' ratios for all X-linked genes after 48 hours of SAF-A KD. B. Comparison of 'a/b' ratios for all X-linked genes after 72 hours of SAF-A depletion. C.F. Comparison of 'a/b' ratios for all X-linked genes after in all SAF-A SAP domain mutants. G. Allele-specific Cut-and-Run was performed with H3K27Ac antibodies and calculated using PAC. 'a/b' ratios are plotted for each gene by chromosome. H. Comparison of 'a/b' ratios for X linked genes in SAF-A depleted cells.



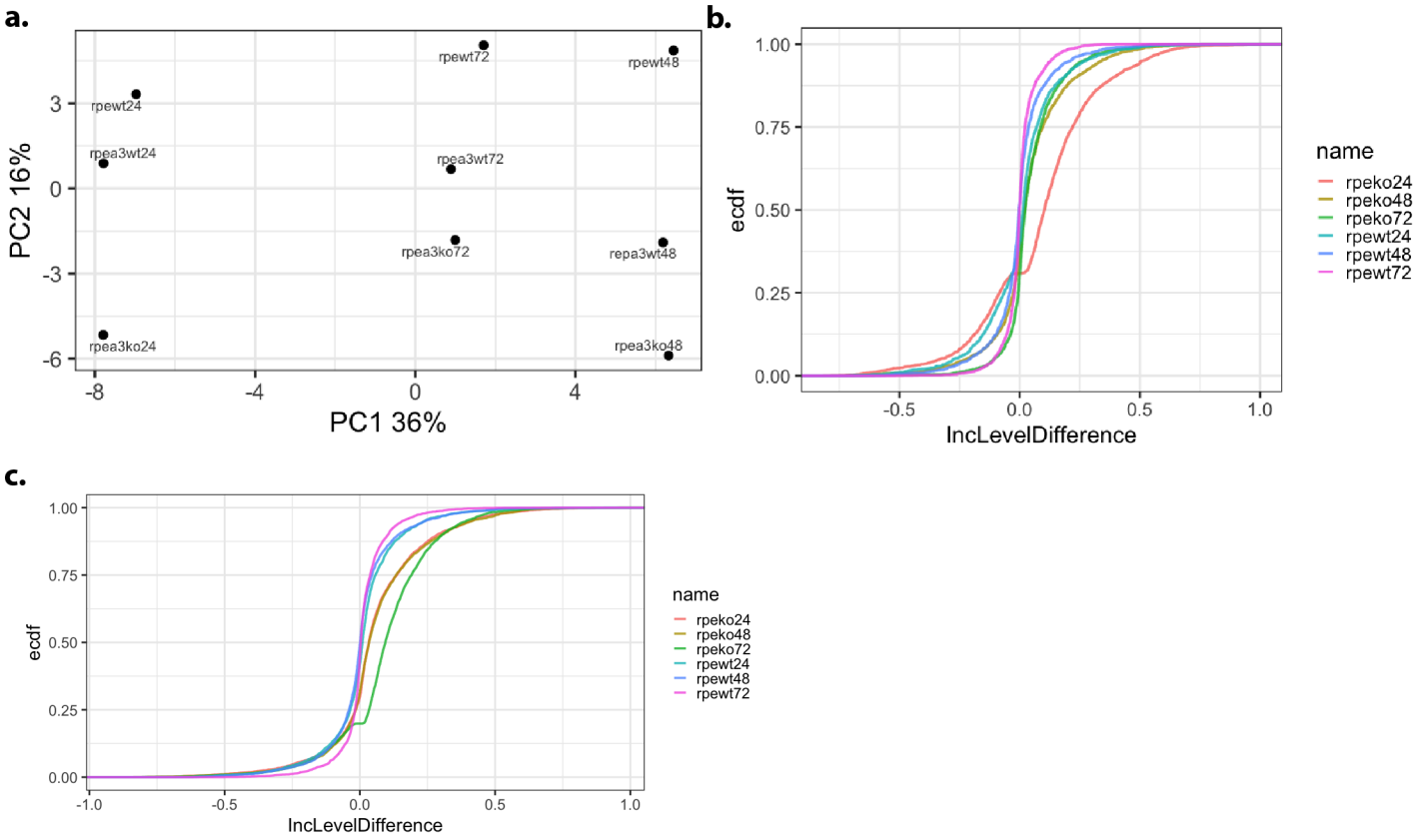
Supplemental Figure 2. SAF-A mutants do not alter gene expression at 24 hours. A-E. SAF-A was depleted by auxin addition and various mutants were expressed by the addition of doxycycline. Gene expression was evaluated at 24 hours using RNA-seq and EdgeR. MD plots depict significantly differentially expressed genes for each mutant (FDR < 0.01).



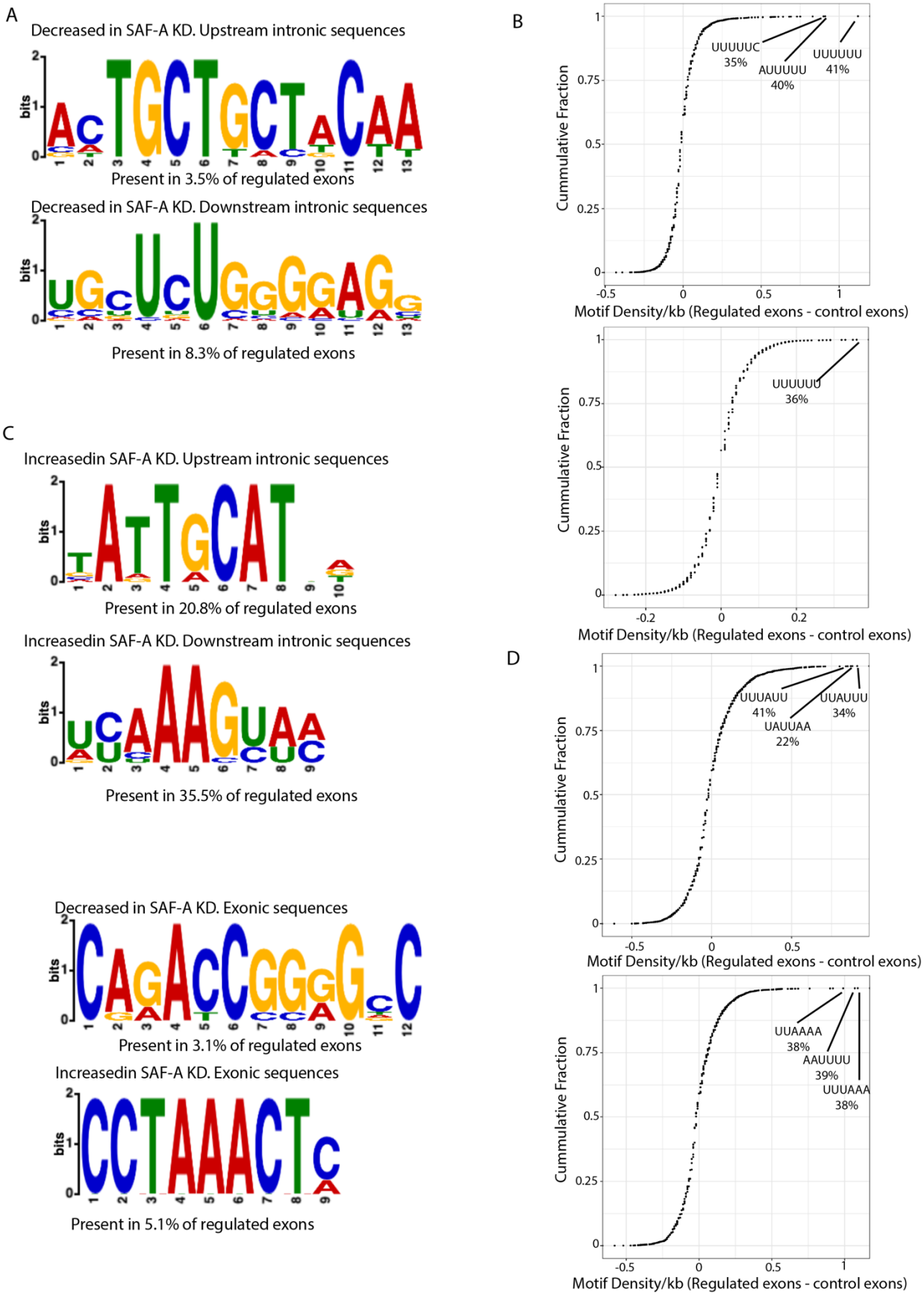
Supplemental Figure 3. MISO plots of additional regulated exons in each SAF-A mutant. A-B. MISO plots of SE events in WT RPE-1 cells, SAF-A depleted cells, and SAP domain mutants. C. Scatterplot comparison of changes in gene expression at 24h to changes in mRNA splicing.



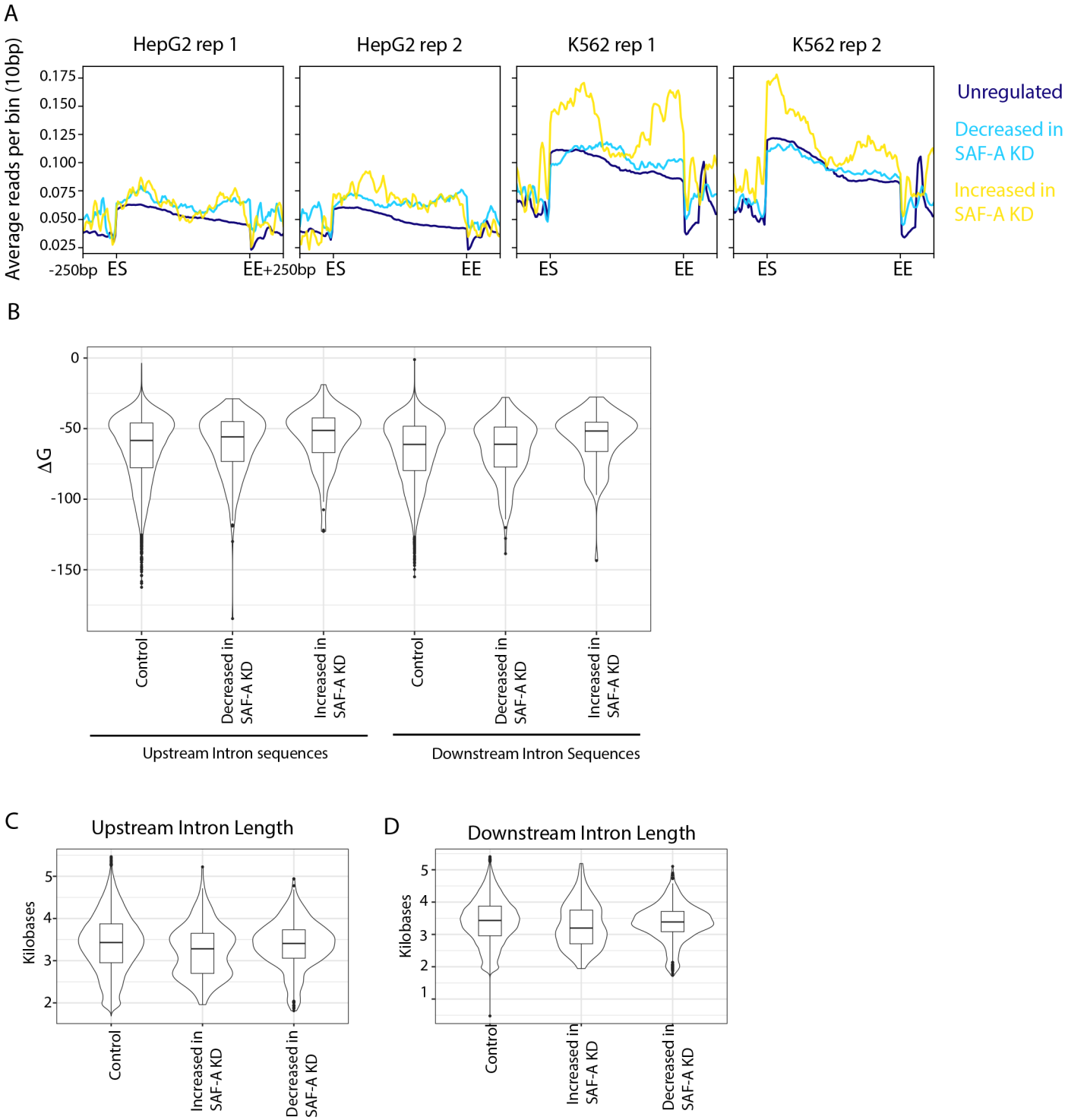
Supplemental Figure 4. Validation of predicted splicing changes using RT-PCR. A. PAGE gel analysis of exon inclusion for 4 different genes with predicted changes in exon inclusion in SAF-A depleted cells. B. Quantitation of percentage spliced in in each mutant from three biological replicates.



Supplemental Figure 5. Splicing defects do not increase with time of SAF-A depletion. A. PCA analysis of skipped exons in SAF-A KD, RPE-1, and SAF-A KD + SAF-A-GFP WT at each time point. B. CDF analysis of SE altered in SAF-A KD cells at 24 hours in each cell type at each time point. C. Same analysis as in B but with SE significantly altered in SAF-A KD at 72 hours.



Supplemental Figure 6. Motif analysis of SAF-A regulated exons. A. STREME analysis of upstream and downstream intronic sequences surrounding SAF-A regulated exons. B. Hexamer enrichment in the same sequences as in A. C. C-D STREME and hexamer analysis of sequences in and surrounding SAF-A regulated exons. Sequence sets tested are indicated on the plots.



Supplemental Figure 7. Analysis of SAF-A eCLIP data surrounding regulated exons. A. ENCODE project eCLIP data was analyzed using Deeptools in both HepG2 and K562 cells at SAF-A regulated exons (increased or decreased) and at unregulated genes. Average coverage and heatmap are depicted for all datasets. B. mFold calculation of ΔG for indicated sequenes. C-D. Calculated lengths of indicated sequence groups.