Supporting Information

"Pre-mRNA splicing is a determinant of histone H3K36 methylation"

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Figure S1

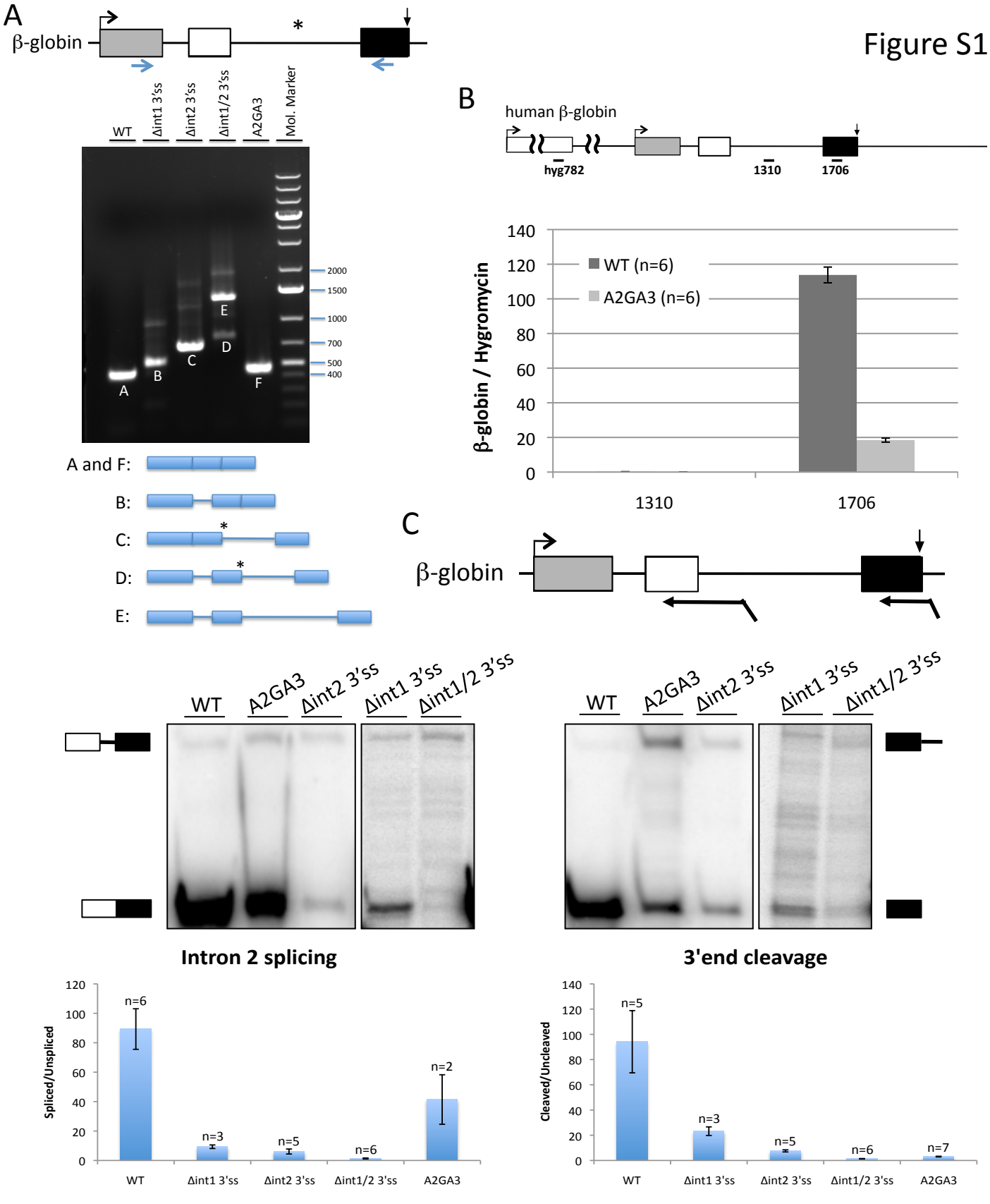

Figure S1. (A) β**-globin transcripts with Δint1 3'ss, Δint2 3'ss, or Δint1/2 3'ss mutation have splicing defects.** Top: Map of β-globin gene with primers used for RT-PCR. Left: RT-PCR products of RNA from doxycycline induced cell lines with integrated WT and mutant globin genes. Bottom: Structure of RT-PCR products as determined by sequencing. * indicates an alternative 3' splice site used when the natural intron 2 3'ss is deleted. (B) Q-RT-PCR analysis of polyadenylated globin transcripts from WT and A2GA3 mutants. cDNA from DNAsed RNA was primed with oligo-dT and quantified with amplicons within exon 3 (1706) and intron 2 (1310) as a negative control. Results were normalized to the amounts of poly(A)+ hygromycin transcripts that was quantified using amplicon hyg 782. (C) RNAse protection assay as in ref 1 of WT and mutant β-globin transcripts. Top: Map with antisense probes (black arrows) indicated. Left: RPA of intron 2 splicing. Right: RPA analysis of poly (A) site cleavage. Bottom: Results were quantified by Molecular Dynamics phosphor-imager and corrected for ^{32}P-U content. Means and SEMs are shown. Note that because of the instability of unprocessed transcripts at least 3X more RNA was analyzed from the mutant cell lines than the WT.

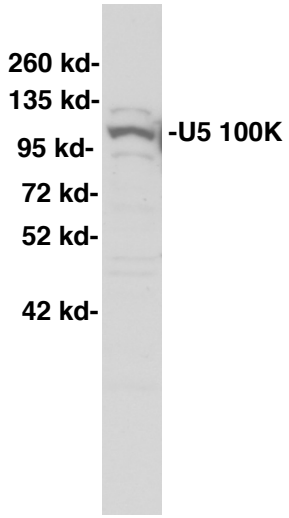

Figure S2

Figure S2.

Western blot of anti-U5 100k antibody (1 μg/ml) using CHO cell whole cell extract.

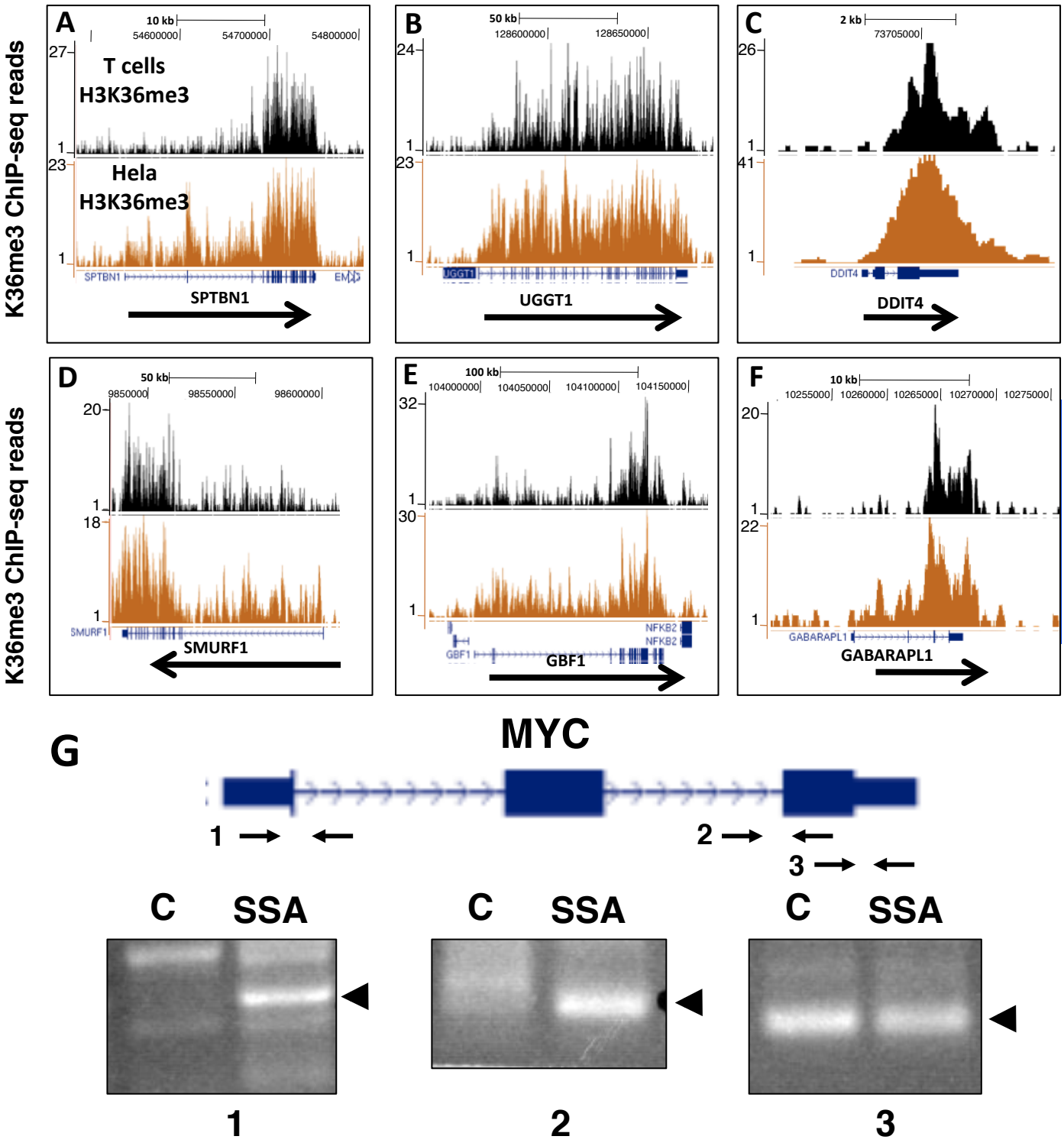

Figure S3.

A-F. Genome browser views of H3K36me3 ChIP-seq reads in control Hela cells obtained using the anti-H3K36me3 antibody described in the Methods section using sheared chromatin (this study) and CD4+ T cells using micrococcal nuclease digested nucleosomes and commercial antibodies (ref 2). Note the close correspondence between results obtained in these two experiments. G. RT-PCR of c-myc transcripts with primer pairs 1-3 in control (C) Hela cells and those treated with SSA (20ng/ml) for 12 hr. Note accumulation of unspliced transcripts (primer pairs 1, 2) in SSA.

Figure S4

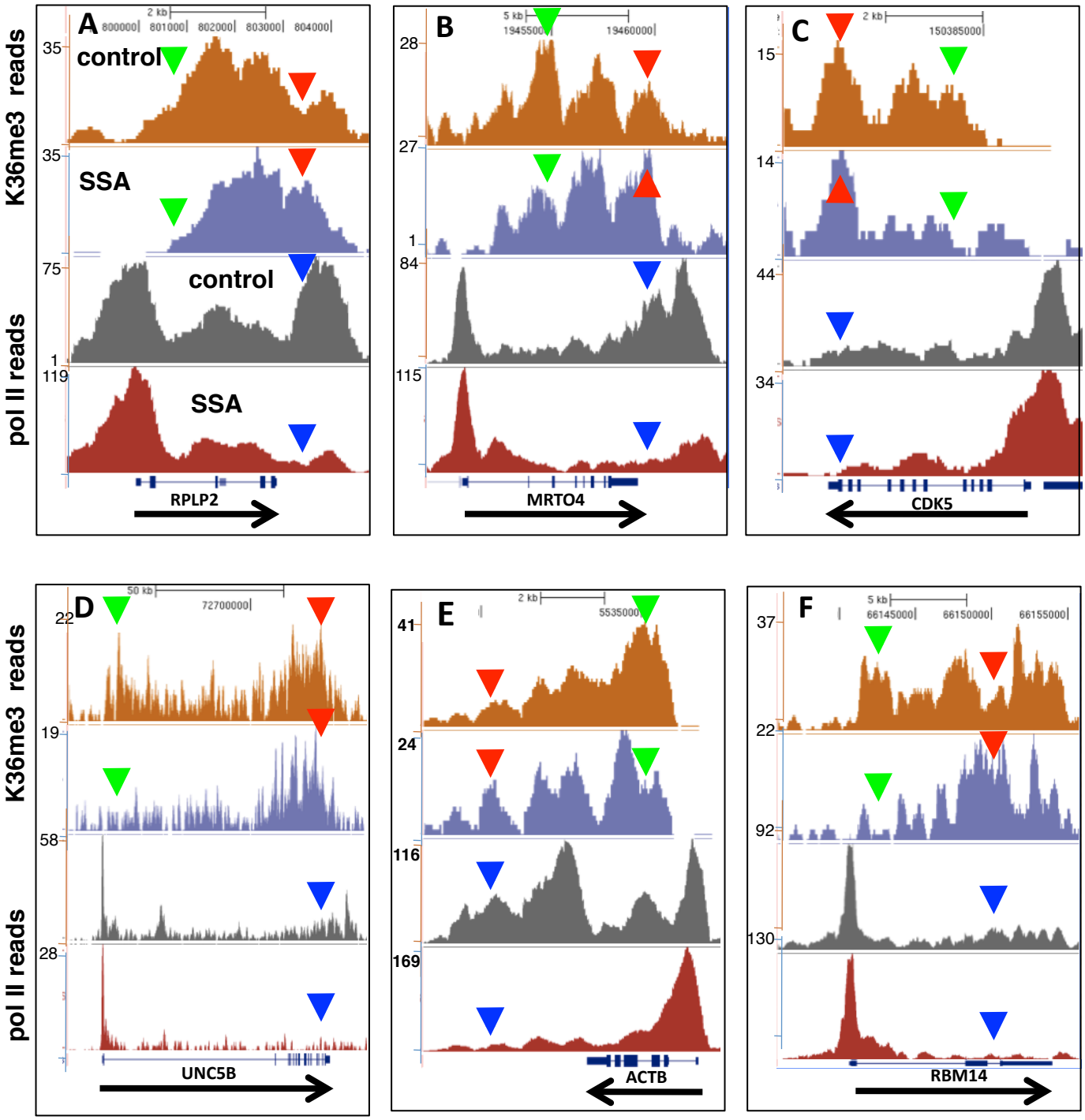

Figure S4. Inhibition of splicing causes widespread re-distribution of histone H3K36 trimethylation toward 3' ends. Genome browser views of H3K36me3 and pol II ChIP-seq reads in control Hela cells and after treatment (20 ng/ml, 12 hr) with SSA to inhibit splicing for several intron-containing genes. Green and red or blue arrowheads mark 5' and 3' positions, respectively, for comparison of signals in WT and SSA. Note that the apparent 5' to 3' shift in H3K36me3 density is not paralleled by a similar shift in pol II density.

References.

1. Fong N, Ohman M, & Bentley DL (2009) Fast ribozyme cleavage releases transcripts from RNA polymerase II and aborts co-transcriptional pre-mRNA processing. *Nature Struct Mol Biol* 16:916-922.
2. Barski A*, et al.* (2007) High-resolution profiling of histone methylations in the human genome. *Cell* 129:823-837.

Table S1.

Oligonucleotide primers used in this study.

Dataset S1.

Column A: List of 1719 genes from the top quartile of genes for H3K36me3 ChIP-seq reads (Figure 4A) with significant 3' shifts ($p < 0.05$, Wilcoxon-Ranksum test) in H3K36me3 distribution when splicing was inhibited with SSA.

Column B: 576 intronless genes analyzed in Figure 4B.