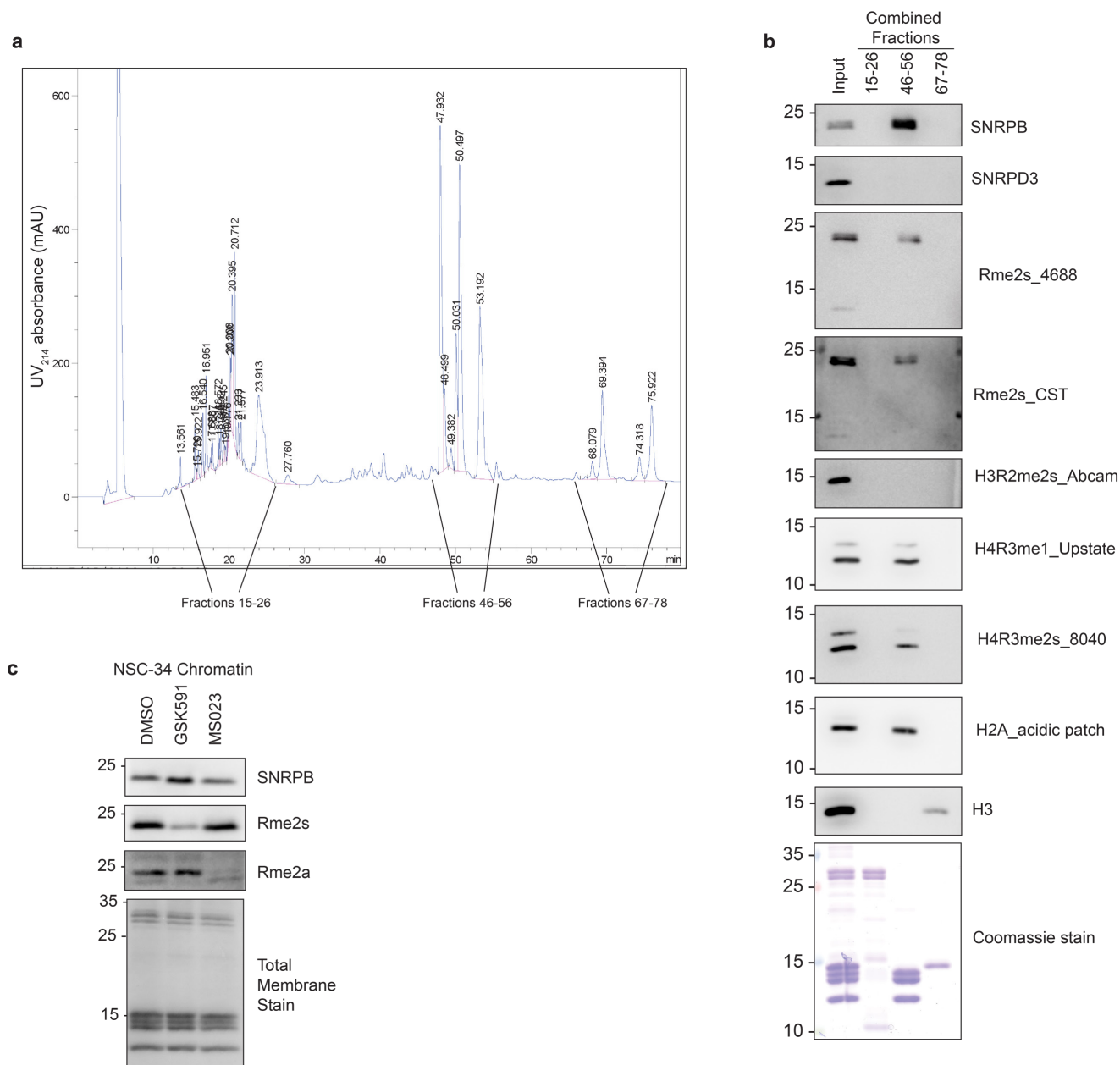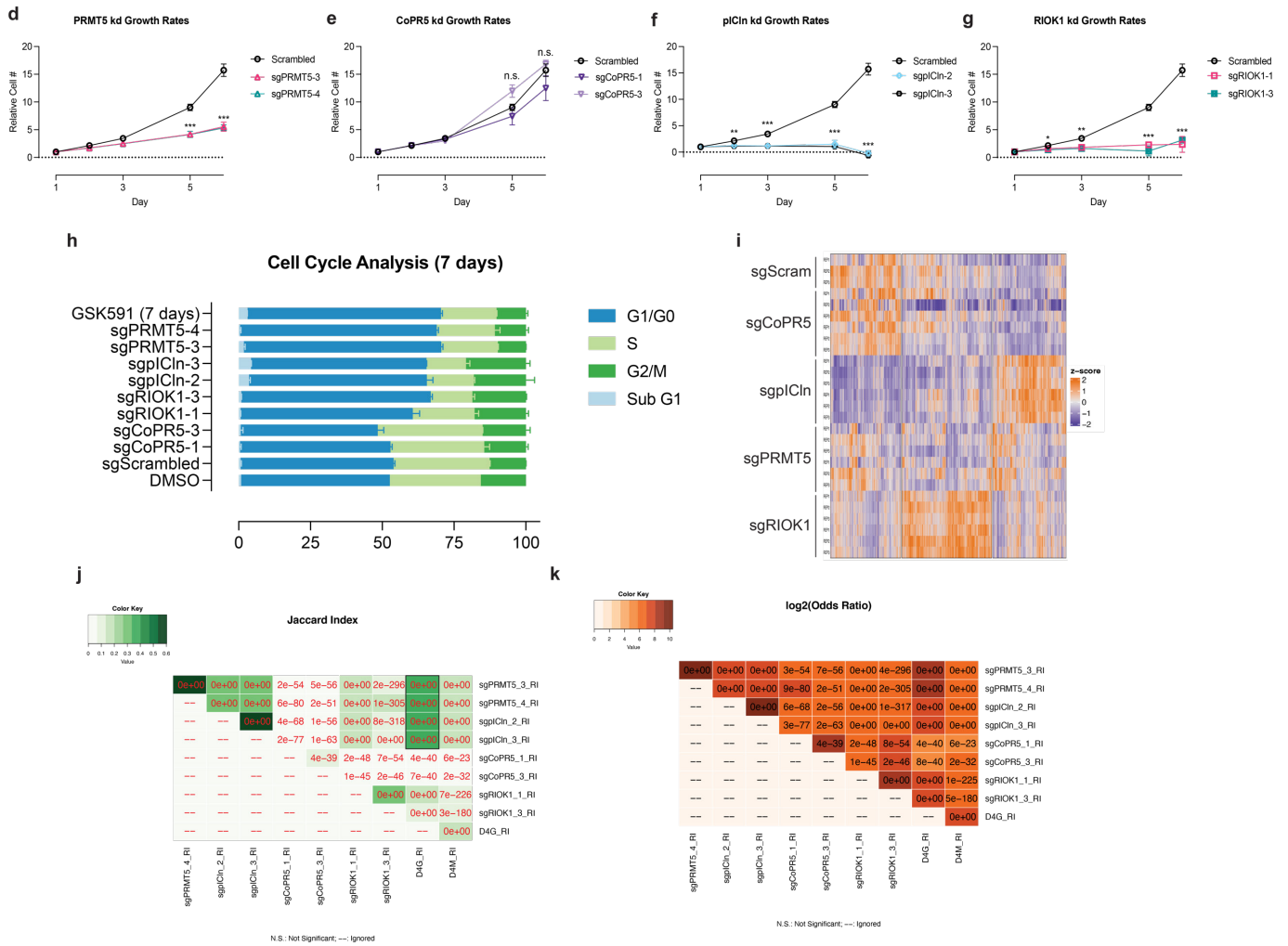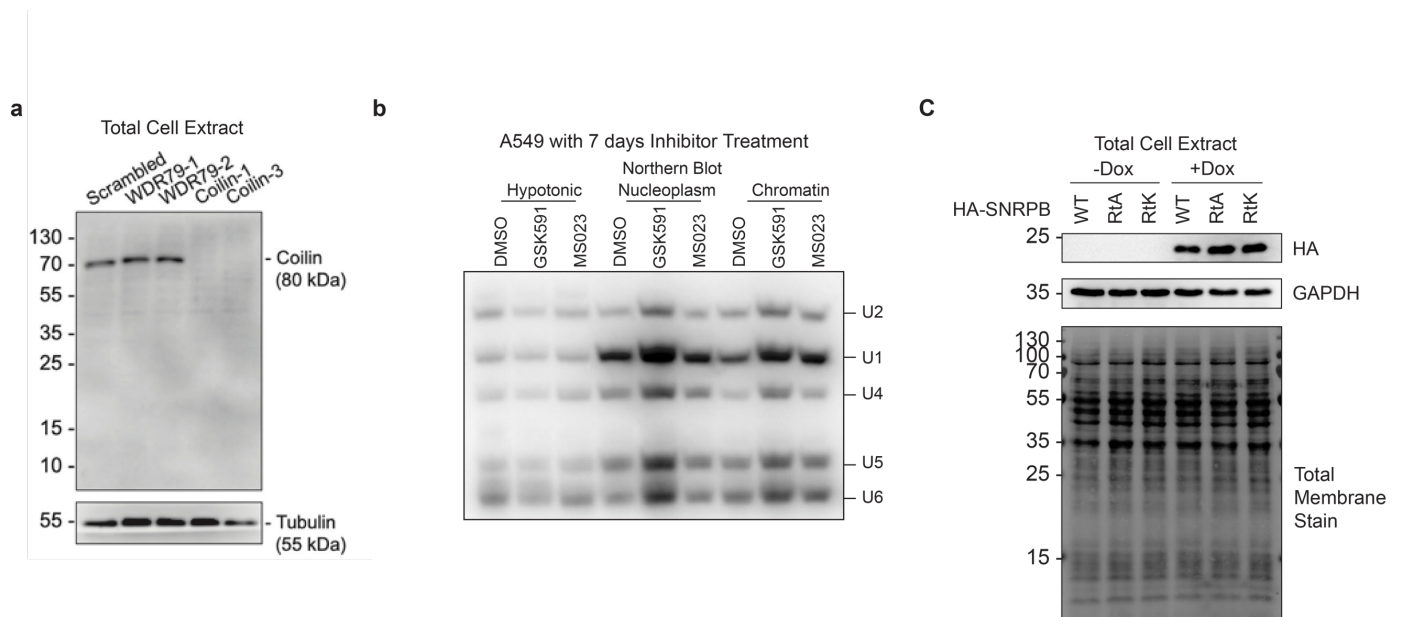**Supplemental Figure S1 (Related to Figure 1). Additional PRMT5 inhibition characterization. a)** Growth curve of GSK591 treatment on A549 cells; **<0.01, ***<0.0001. **b)** Volcano plot of poly(A) RNA-sequencing following two days of GSK591 treatment, compared to DMSO control. **c)** Dot plot of GO Biological Processes (BP) terms for total mRNA sequencing of GSK591 treatment. **d-f)** Dot plots of GO Biological Processes (BP) terms for quadrants of linear correlation plot between GSK591 mRNA-sequencing and PRO-sequencing.

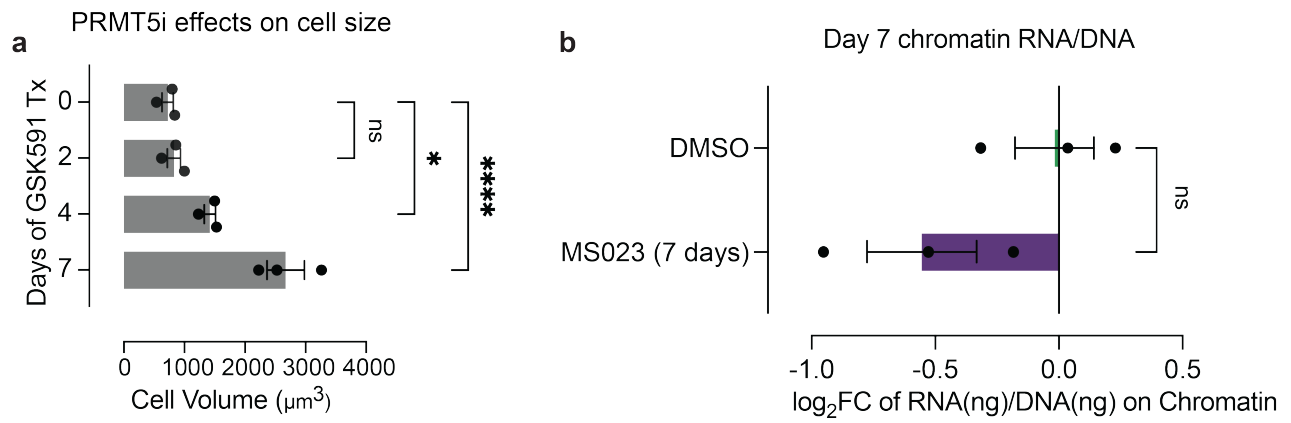**Supplemental Figure S1 (Related to Figure 2). Sm proteins are a contaminant of acid extracted histones. a)** HPLC trace showing UV absorbance at 214nm for a run consisting of acid extracted chromatin. **b)** Western blot of combined fractions, analyzing presence of Sm proteins, histones, and selected histone PTMs. **c)** Immunoblot of chromatin fraction of NSC-34 cells following 2 day treatment of GSK591 or MS023.
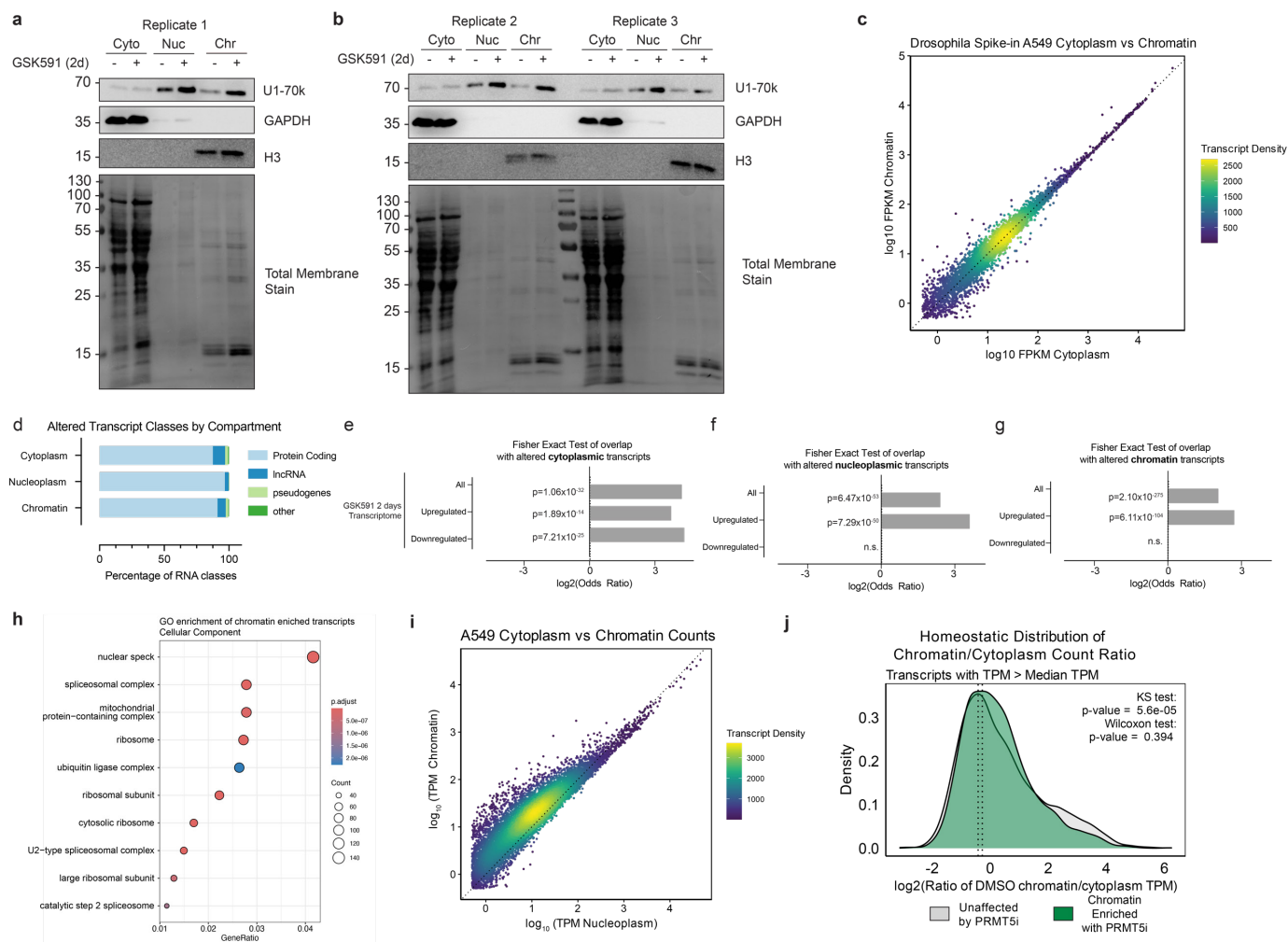
Supplemental Figure S2 continued (Related to Figure 2). Characterization of PRMT5 and adaptor protein knockdown. d-g) Growth curve of knockdown cell lines; *<0.01, **<0.01, ***<0.001, n.s. = not significant **h)** Cell Cycle analysis of PRMT5 and adaptor knockdowns using propidium iodide (PI) staining cell cytometry. **i)** Z-score heatmap TPM counts from RNA sequencing of PRMT5 and adaptor knockdowns. **j)** Heatmap showing the Jaccard Index for the overlap of differentially retained introns for PRMT5 and adaptor protein knockdowns. The p-value of each overlap is shown in the rectangles. Color is proportional to the percentage of intron overlap (Jaccard Index). **k)** Heatmap showing the Odds Ratio for the overlap of the differentially retained introns for PRMT5 and adaptor protein knockdowns. The p-value of the overlap is shows in each rectangle. Rectangle color is proportional to $\log_2$(Odds Ratio).
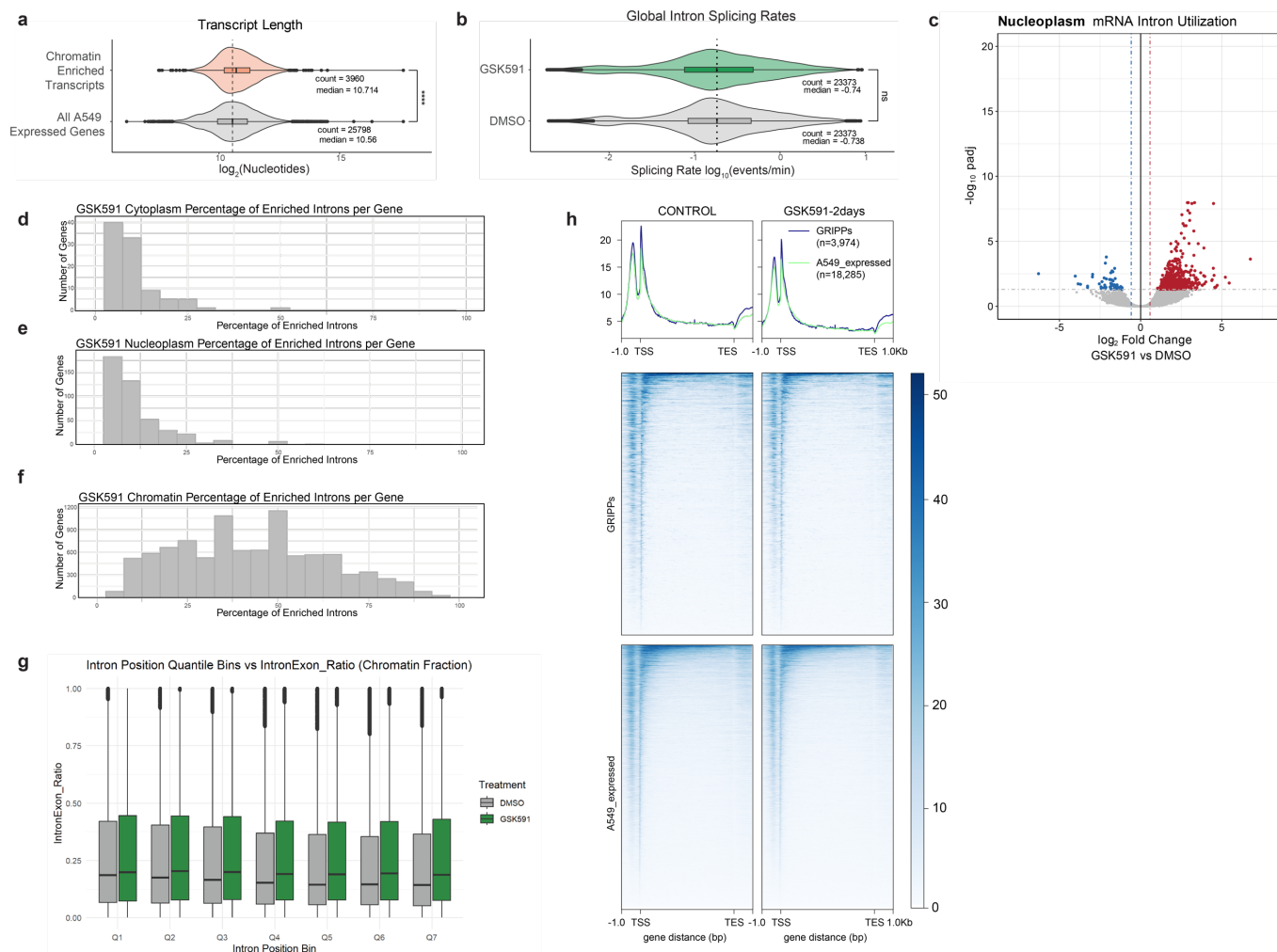
Supplemental Figure S3 (Related to Figure 4). a) Immunoblot controls of Coilin knockdown using CRISPRi in A549 cell. **b)** Northern blot testing snRNP abundance across cellular compartments after PRMT inhibition. **c)** Immunoblot of total cell lysate testing induction levels of mutant SNRPB proteins following four days of DOX (1ug/mL) induction.

a PRMT5i effects on cell size

b Day 7 chromatin RNA/DNA

Supplemental Figure S4 (Related to Figure 5). a) Histogram depicting cell volume as a function of PRMT5 inhibition over time. *p<0.05, ****p<0.0001 **b)** Histogram of the $\log_2$ fold change of total chromatin RNA to DNA following Type I PRMT inhibition with MS023.

Supplemental Figure S5 (Related to Figure 6). a-b) Western blot controls of fractionated samples and replicates used for mRNA-sequencing. **c)** Correlation of the $\log_{10}$ average normalized read counts in FPKM for *D. melanogaster* transcripts in the cytoplasm and chromatin compartments for untreated cells (DMSO). Dotted line represents y=x. Transcript color represents the density of points on the plot. **d)** Relative percentages of RNA classes of significantly altered transcripts (padj<0.05) between DMSO and GSK591 treated cellular compartments. **e-g)** Fisher exact tests of significantly altered transcripts in cellular compartment compared to gene sets from total GSK591 treated mRNA sequencing. **h)** Dot plot of cellular component gene ontology for chromatin-enriched transcripts. Dot size is representative to the number of genes per category and color represents the p-adjusted value. **i)** Correlation of the $\log_{10}$ average normalized read counts in TPM for transcripts in the nucleoplasm and chromatin compartments for untreated cells (DMSO). **j)** Density plots of the ratio of normalized TPM counts per gene (with TPM > median of all expressed genes) between chromatin and cytoplasm compartments, comparing genes found to be enriched on chromatin upon PRMTi. Kolmogorov-Smirnov and Wilcoxon ranked sum tests were used to compare distributions.

Supplemental Figure S6 (Related to Figure 7). a) Violin plots comparing transcript length of chromatin enriched genes upon PRMTi compared to all expressed genes in A549 cells. ****:p<0.0001 using Wilcoxon Ranked Sum test **b)** Splicing rates solved by SKaTER-seq comparing intron splicing rates solved both in GSK591 and DMSO treated conditions. Compared using Wilcoxon Ranked Sum test. **c)** Volcano plot of intron utilization for transcripts in nucleoplasm fraction comparing GSK591 vs DMSO. Red introns have a $\log_2FC>0.58$ and $p_{adj}<0.05$. Blue introns have a $\log_2FC<-0.58$ and a $p_{adj}<0.05$. **d-f)** Histograms depicting the percentage of enriched introns and gene number in GSK591 treated fractions. **g)** Intron:Exon Ratio for DMSO vs GSK591 Chromatin fraction across 7 quantiles throughout the transcripts. **h)** Pro-seq metagene profiles comparing GRIPPs to all genes expressed in A549 cells.

Supplemental Table S5. Antibodies used in this study

Antibody Target	Vendor	Source	Identifiers	Additional Information
SNRPB	Proteintech	Rabbit polyclonal	Cat#: 16807-1-AP	WB: 1:2,000
SNRPD3	Abcam	Rabbit polyclonal	Cat#: ab157118	WB: 1:2,000
H3K27me3	CST	Rabbit monoclonal	Cat#: 9733	WB: 1:100,000
Rme2s	Courtesy of Mark Bedford	Rabbit polyclonal	4688	WB: 1:10,000
Rme2a	Cell Signaling	Rabbit monoclonal	Cat#: 8015S	WB: 1:2,000
Rme1	Cell Signaling	Rabbit monoclonal	Cat# 13522S	WB: 1:2,000
H3	Abcam	Rabbit polyclonal	Cat#: 1791	WB: 1:300,000
PRMT5	Millipore	Rabbit polyclonal	Cat#: 07-405	WB: 1:5,000
MEP50	LPBio	Rabbit polyclonal	Cat#: AR-0145-S	WB: 1:5,000
pICln	Bethyl	Rabbit polyclonal	Cat#: A304-521A	WB: 1:50,000
RIOK1	Proteintech	Rabbit polyclonal	Cat#: 17222-1-AP	WB: 1:10,000
GAPDH	Abcam	Mouse monoclonal	Cat#: ab9484	WB: 1:50,000
U1-snRNP 70 (E-4)	Santa Cruz	Mouse monoclonal	Cat#: SC390988	WB: 1:2,000
SNRPE	Proteintech	Rabbit polyclonal	Cat#: 20407-1-AP	WB:1:10,000
IgG	Abcam	Rabbit polyclonal	Cat#: ab46540	IP: 5 ug
m3G/TMG	MBL Life Science	Mouse monoclonal	Cat#: RN019M	IP: 5 ug
Rme2s	Cell Signaling	Rabbit monoclonal	Cat#: 13222S	WB: 1:2,000
TBP	Cell Signaling	Rabbit polyclonal	Cat#: 8515	WB: 1:10,000
HA	Cell Signaling	Rabbit monoclonal	Cat#: C29F4	WB: 1:100,000
Coilin	BD Biosciences	Mouse monoclonal	Cat#: 612074	WB: 1:250
Tubulin (E7)	DSHB Iowa	Mouse monoclonal	Cat#: AB_2315513	WB: 1:200

Supplemental Table S6. sgRNAs used in this study

Target Gene	Guide #	Sequence
Scrambled Control	1	GTGTAGTTCGACCATTCGTG
PRMT5	3	GGTCCCTCCCGCTGGACACG
PRMT5	4	GAGAAAGATGGCGGCGATGG
COPRS (CoPR5)	1	GCCTGAAGGTCCATGCCCTG
COPRS (CoPR5)	3	CATGGACCTTCAGGCCGCCG
CLNS1A (pICln)	2	GCAGCAGAGTGCGGCAACAC
CLNS1A (pICln)	3	GCTGTGCTCCAACTCCCTCA
RIOK1	1	AACCATTCAGAAGCCAACAG
RIOK1	3	TGGCAGGGTGGTGGATCTGT
Coilin	2	GCCAGCTACCCCGCACTGTA
Coilin	4	CAAGATGGCAGCTTCCGAGA