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Supplemental Information

BUD13 Promotes a Type I Interferon Response

by Countering Intron Retention in *Irf7*

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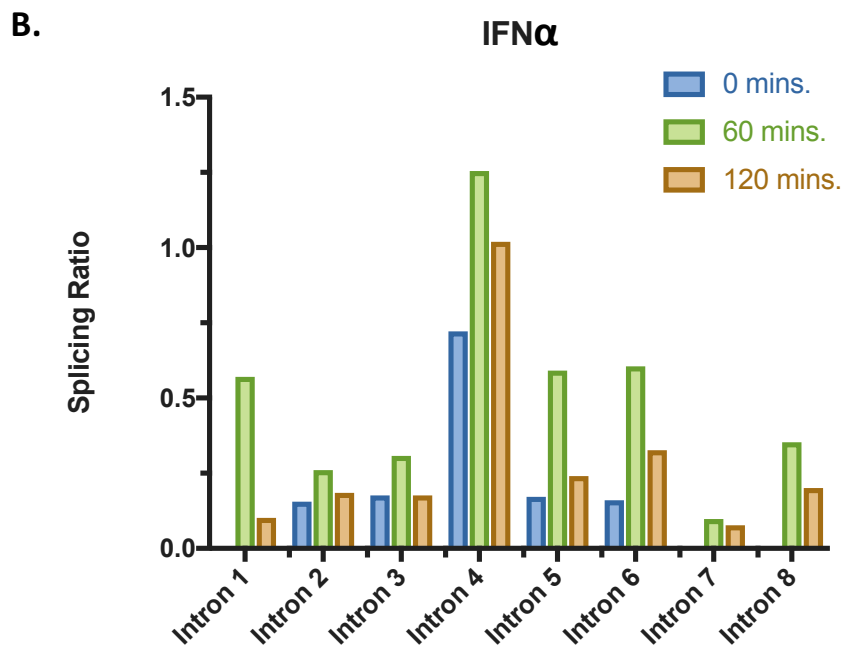
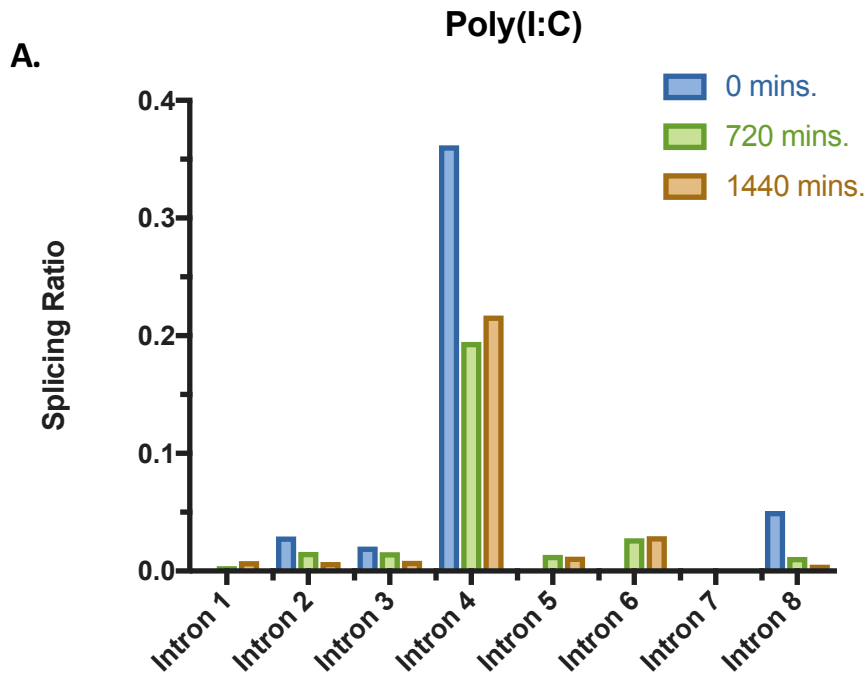


Figure S1: Splicing Ratios across all junctions in *Irf7*. Related to Figure 1.

Splicing ratios calculated for all junctions in the most abundant transcript of *Irf7*. Color represents time-point indicated in legend. **(A)** Poly(I:C) **(B)** IFN α .

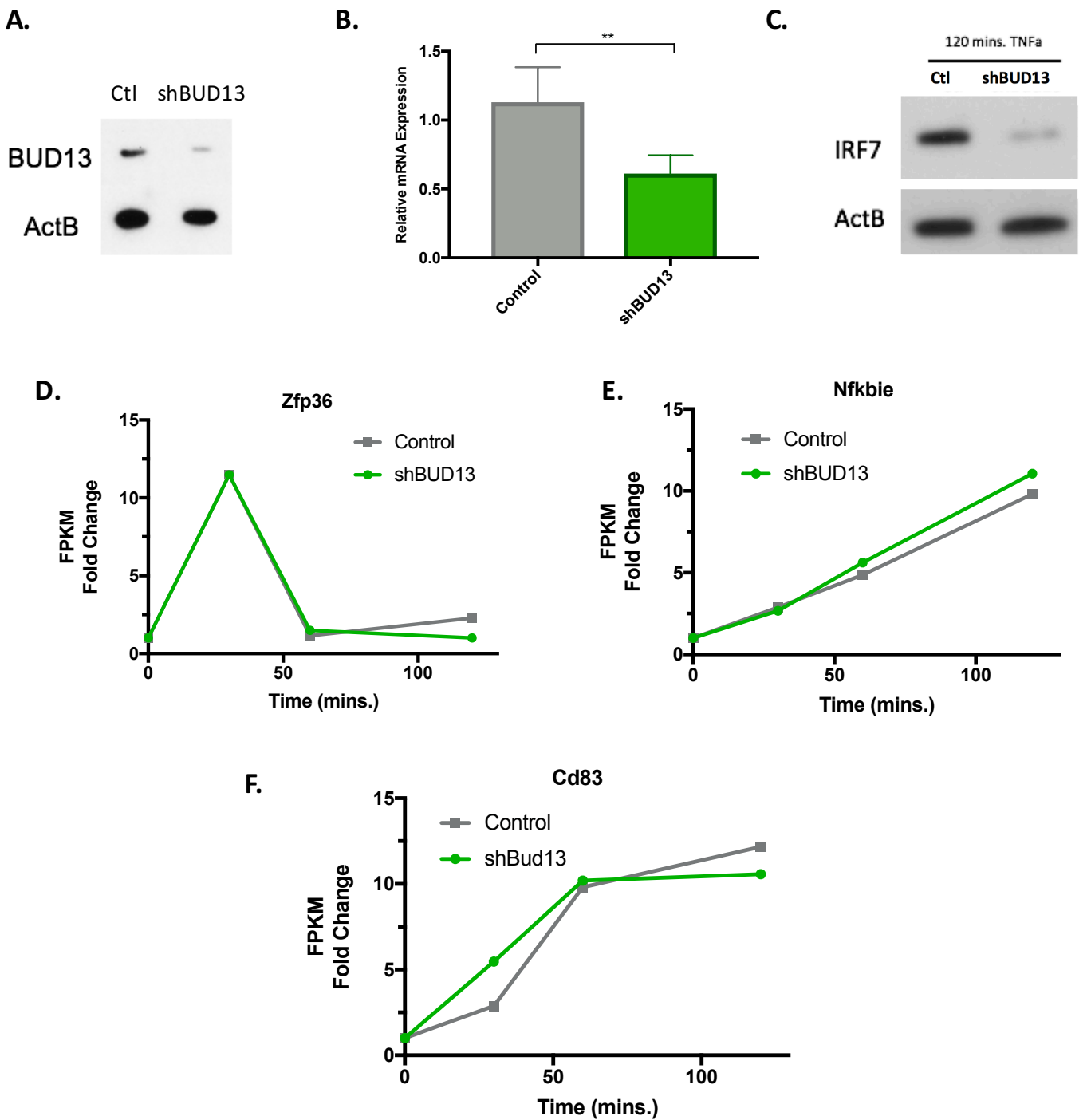


Figure S2: shBUD13 knocks down BUD13 protein and mRNA. Related to Figure 3.

(A) Immunoblot analysis of BUD13 in BMDMs infected with control or shBUD13. ActB serves as loading control. (B) qRT-PCR analysis of *Bud13* mRNA in BMDMs infected with control or shBUD13. (C) Immunoblot analysis of IRF7 protein following 120 mins. TNF α stimulation. (D-F) FPKM fold change with respect to time stimulated (C) Zfp36, (D) I κ B ϵ , and (E) CD83. shBUD13 is shown in green, control is shown in grey. Data is representative of two individual experiments (A, B) and is shown as mean (error bars indicate s.d.) (B). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

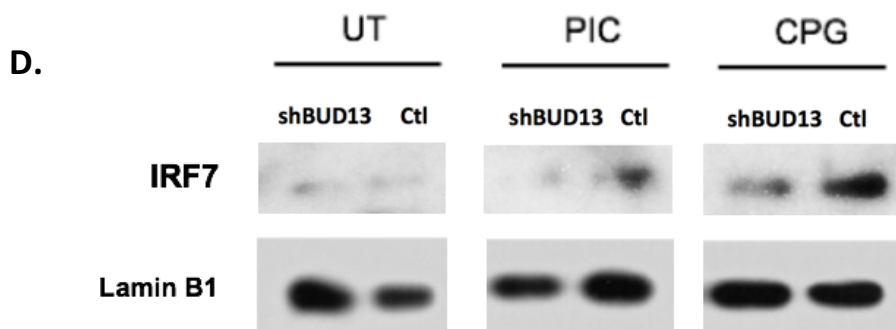
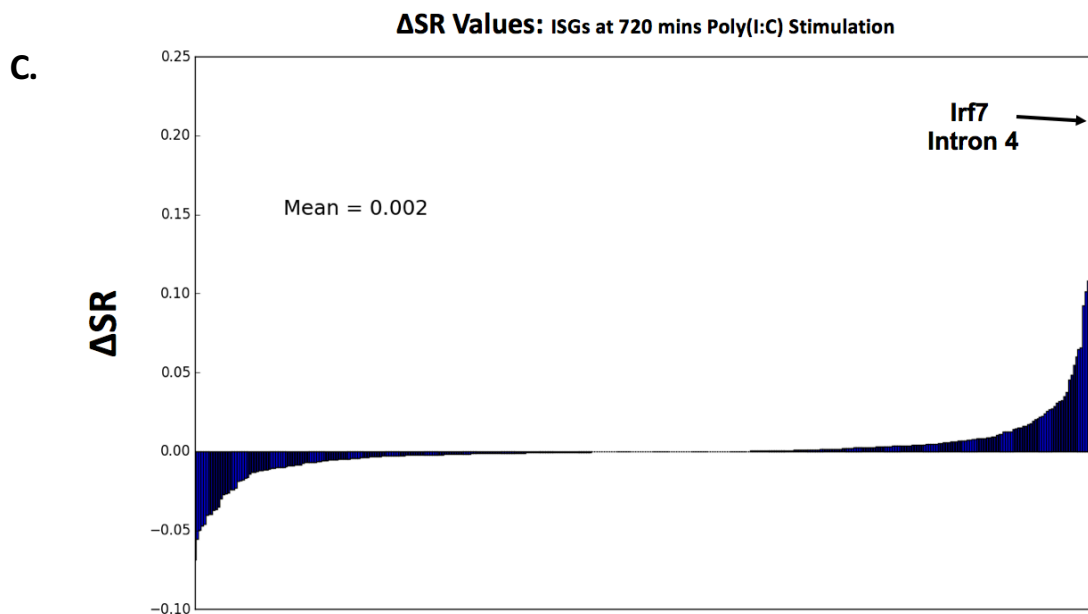
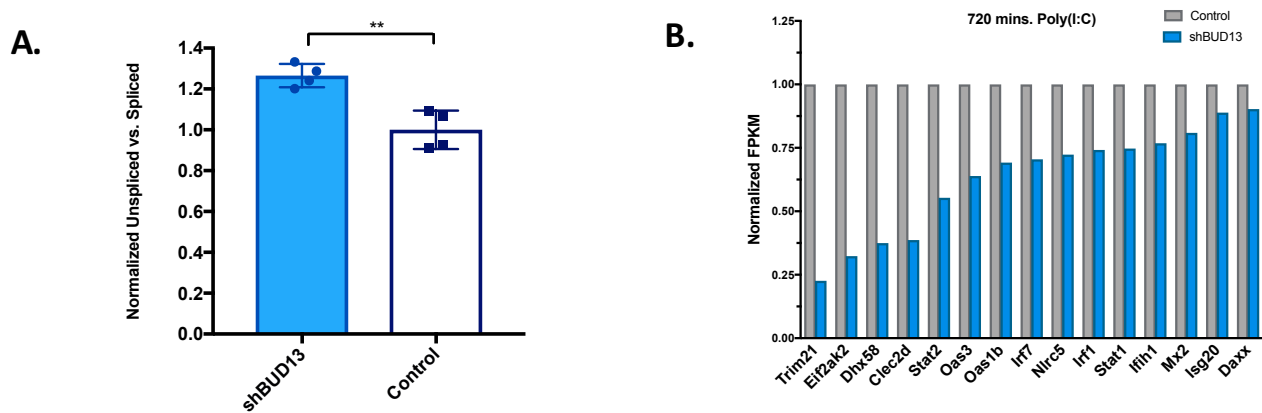


Figure S3: *Irf7* Intron 4 is the most BUD13 knockdown affected junction of all ISGs. Related to Figure 4.

(A) Normalized levels of spliced vs. unspliced intron 4 in control and shBUD13 BMDMs as measure through quantitative RT-qPCR. (B) Normalized FPKM expression levels in shBUD13 and control samples at 720 mins poly(I:C) stimulation for select ISGs). (C) Δ SR was calculated at 720 mins of poly(I:C) stimulation for each ISG junction that passed the transcript and local read count threshold (see methods). Mean Δ SR = 0.002, Median Δ SR = 0. (D) Immunoblot analysis of *Irf7* protein after nuclear fractionation from BMDMs left untreated (UT) or treated with poly(I:C) (PIC) or CpG for 12h. Lamin B1 serves as loading control.

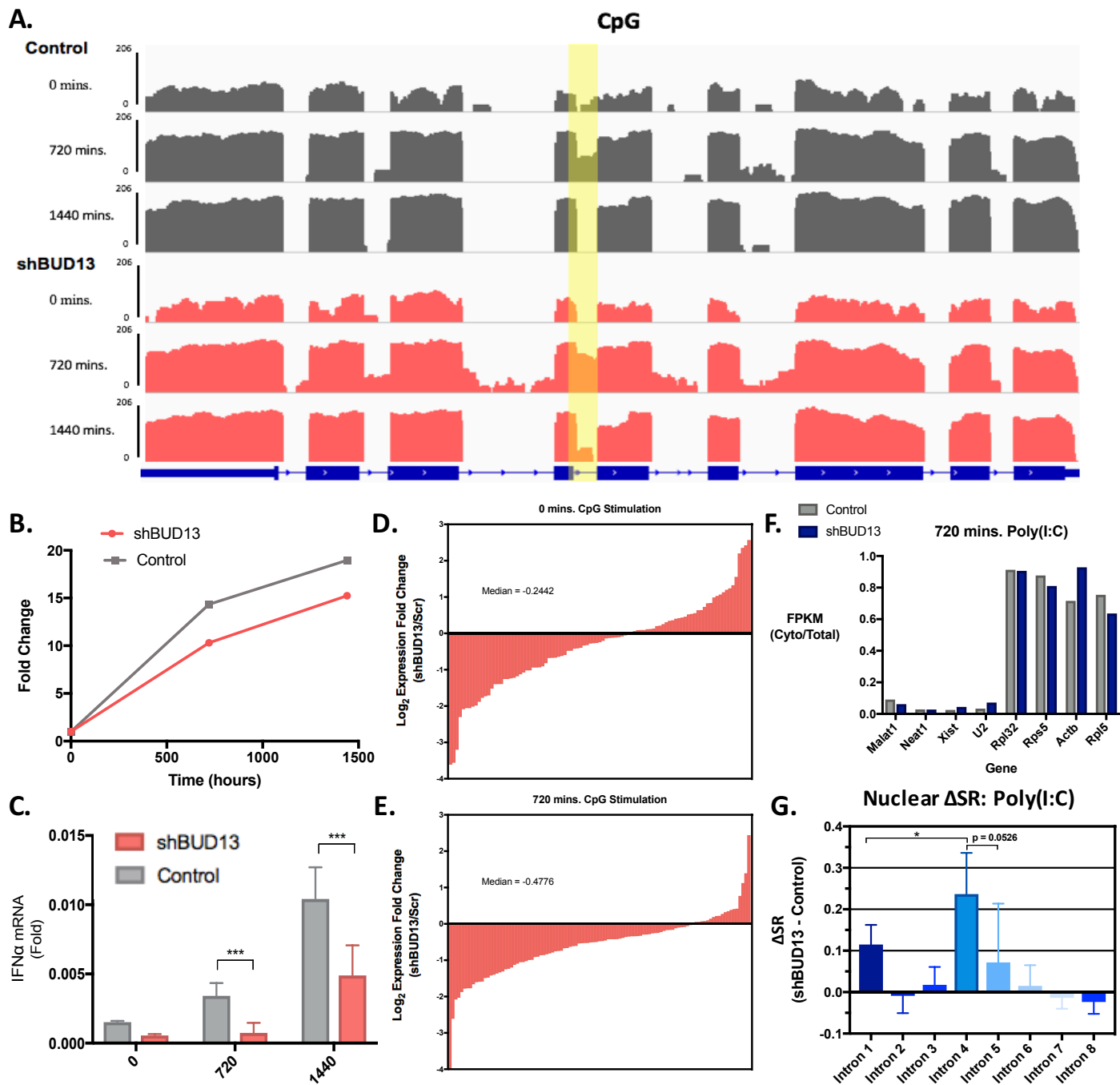


Figure S4: BUD13 knockdown alters the type I interferon response in response to CpG. Related to Figure 4.

(A) Histogram of mapped reads corresponding to the CpG-induced expression of *lrf7*. The poorly spliced fourth intron is highlighted. shBUD13 samples are shown in pink. Control samples are shown in grey. **(B)** *lrf7* FPKM fold change with respect to time stimulated. shBUD13 is shown in pink, control is shown in grey. **(C)** RT-qPCR analysis of IFN α mRNA levels in unstimulated BMDMs and BMDMs stimulated with CpG for 720 and 1440 mins. **(D)** Log₂ expression fold change (shBUD13/Control) for 119 ISGs (selected based on upregulation in response to IFN α) in unstimulated BMDMs (median = -0.2442). **(E)** As in **(D)** for stimulated BMDMs (720 mins CpG (median = -0.4776). Wilcoxon rank-sum between **(D)** and **(E)**, $P < .001$. **(F)** Ratio of cytoplasmic FPKM levels to cytoplasmic and nuclear FPKM levels for transcripts that are primarily nuclear (*Malat1*, *Neat1*, *Xist*, *U2*; left), and primarily cytoplasmic (*Rpl32*, *Rps5*, *Actb*, *Rpl5*; right) (BMDMs – 720 mins poly(I:C) stimulation). **(G)** Nuclear Δ SR calculated for each junction in the *lrf7* transcript. Unless indicated, comparison of intron 4 Δ SR to any other junction is significant (Student's t-test, $p < 0.001$). No other pairwise comparison is significant. Data is represented as mean (error bars indicate s.d.). * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$ using a Student's t test. Results are presented relative to those of *Rpl32*.

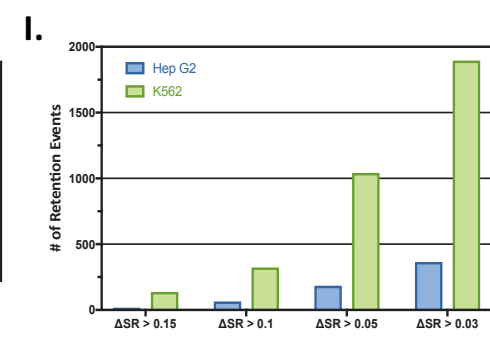
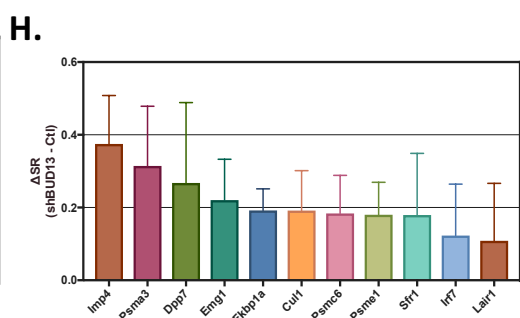
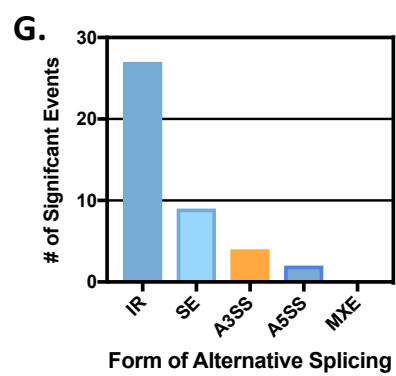
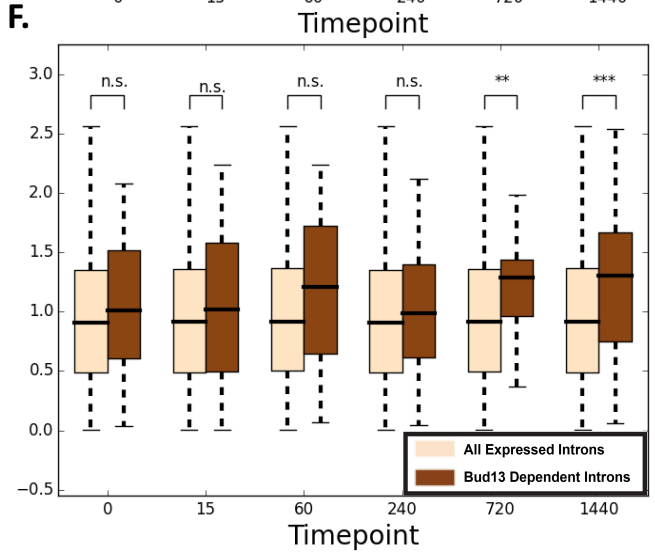
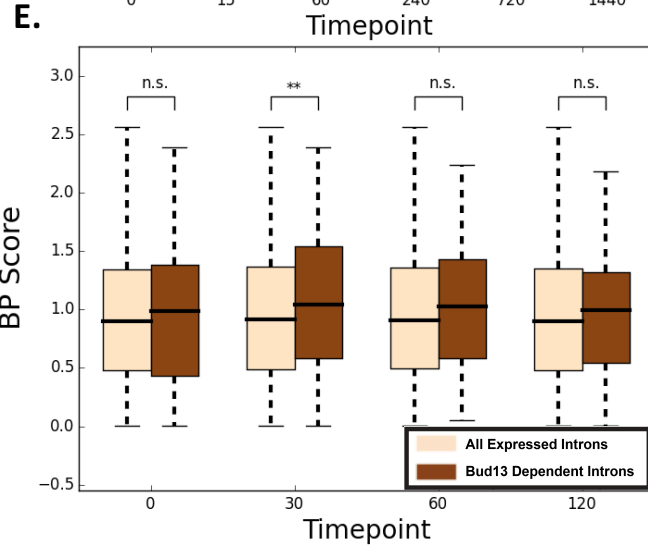
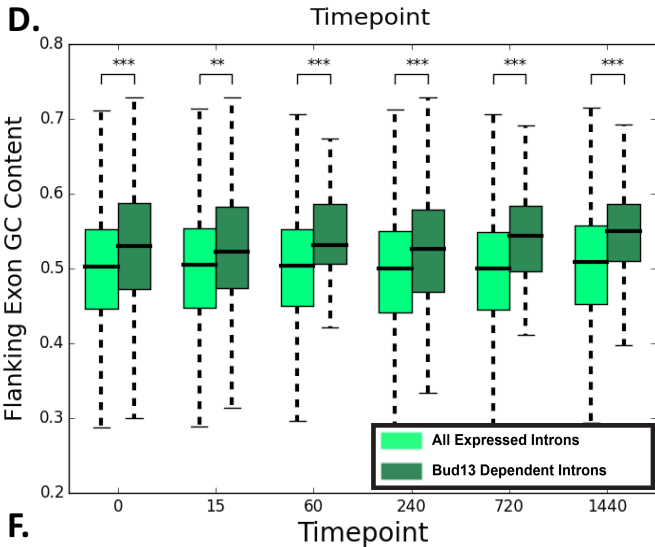
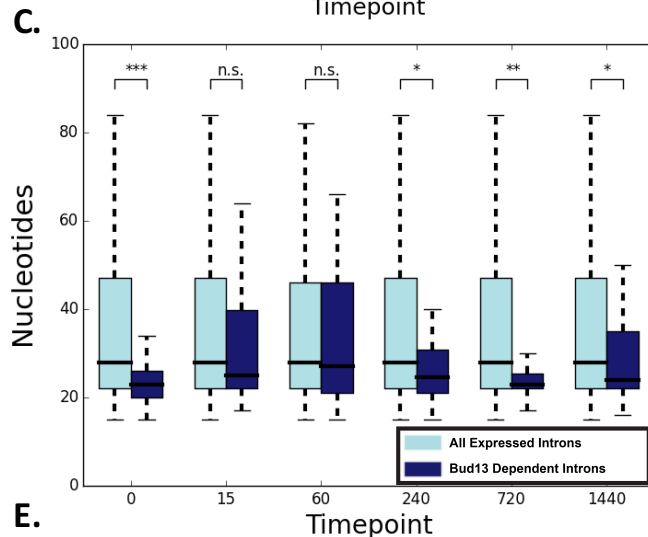
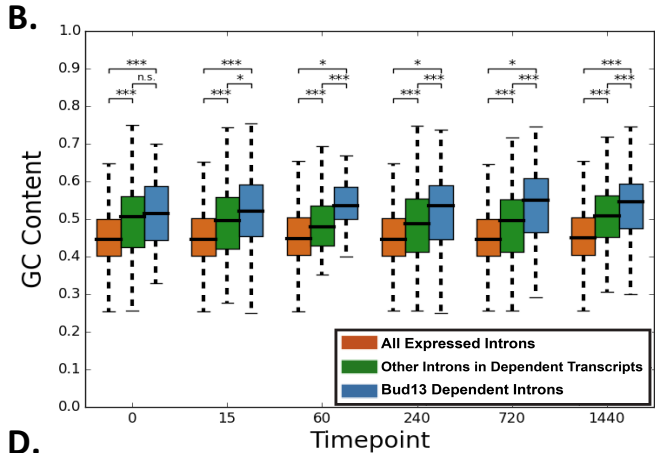
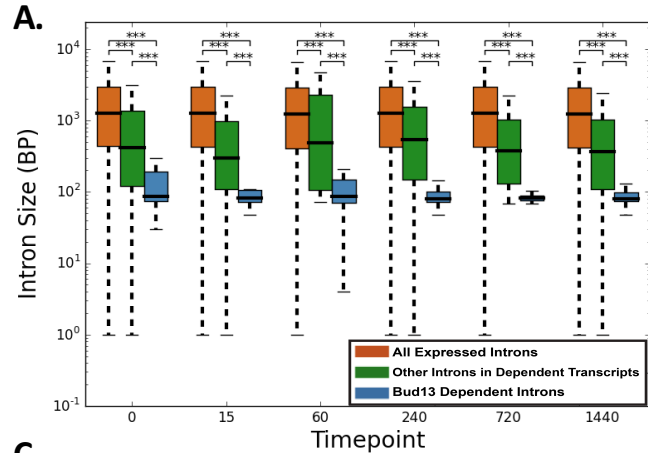


Figure S5: Supplemental global analysis of BUD13. Related to Figure 5 and 6.

(A) Size of intron for introns retained upon BUD13 knockdown (Δ SR. > 0.15) (blue), in introns located in the same transcript as those affected by BUD13 (green), and in introns from all expressed transcripts (orange). **(B)** Same as **(A)** for GC content. **(C)** Flanking exon GC content for exons that flank introns retained upon BUD13 knockdown (Δ SR. > 0.15) (dark green) as compared to exons that flank introns from all expressed transcripts (light green). **(D)** Distance from the branch point to the 3' splice site for introns retained upon BUD13 knockdown (Δ SR. > 0.15) (dark blue) as compared to introns from all expressed transcripts (light blue). **(A-D)** data from BMDM poly(I:C) stimulation. **(E)** Branch point score for introns retained upon Bud13 knockdown (Δ SR. > 0.15) (beige) as compared to introns from all expressed transcripts (dark brown) in TNF α stimulated BMDMs. **(F)** Same as **(E)** but for poly(I:C) stimulated BMDMs. **(G)** Significant number of alternative splicing events across the TNF α time-course as calculated by rMATs. **(H)** Ranked bar chart showing genes with a junction most affected by BUD13 knock-down in all samples during PIC stimulation. **(I)** Box plot showing the number of retention events across replicates at the indicated Δ SR. Box plots show median (center line), interquartile range (box) and tenth and ninetieth percentiles. * P < 0.05, ** P < 0.01 and *** P < 0.001 (Mann-Whitney U -test).

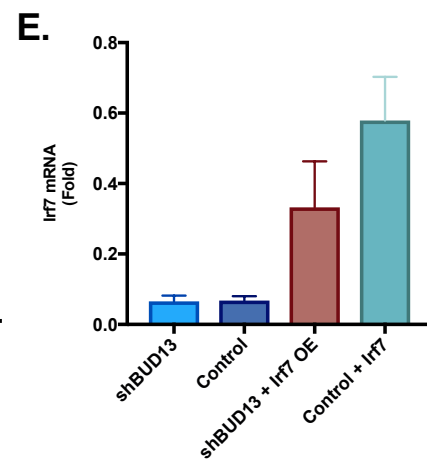
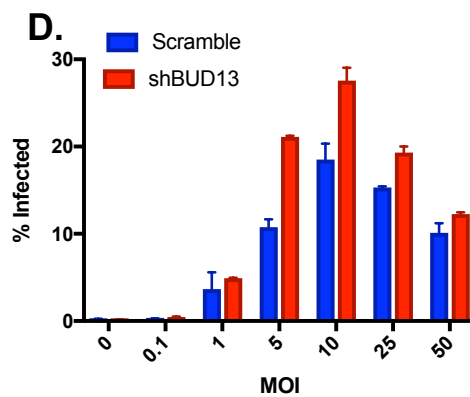
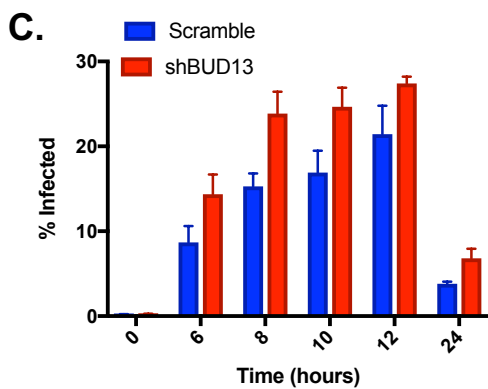
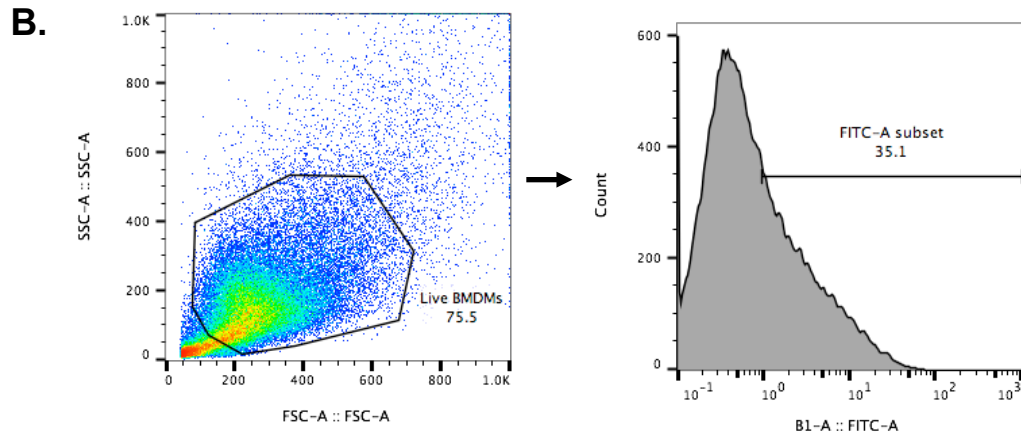
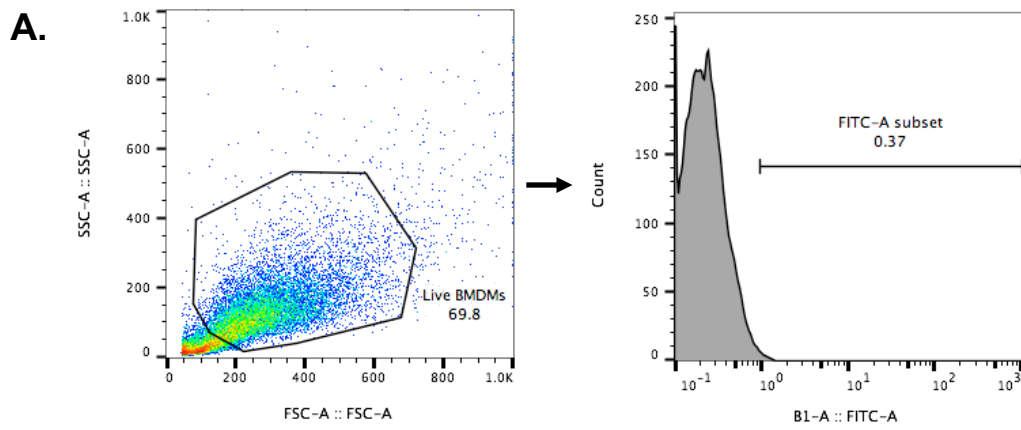


Figure S6: BUD13 knockdown alters the BMDM infection via VSV. Related to Figure 7.

(A) FSC/SSC plot showing the gating of live BMDMs in an uninfected control sample and the subsequent threshold used to calculate infectivity. (B) Same as in (A) but for a control sample infected with VSV-GFP for 12 hours. (C) Percent of live cells infected with VSV-GFP (MOI 10) in both control and shBUD13 BMDMs across a 24-hour time-course. (D) Percent of live cells infected in both control and shBUD13 BMDMs at 12 hours across a range of VSV-GFP MOIs. Data is representative of three (C, D) independent experiments and is represented as mean (error bars indicate s.d.). * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$ using a Student's t test.

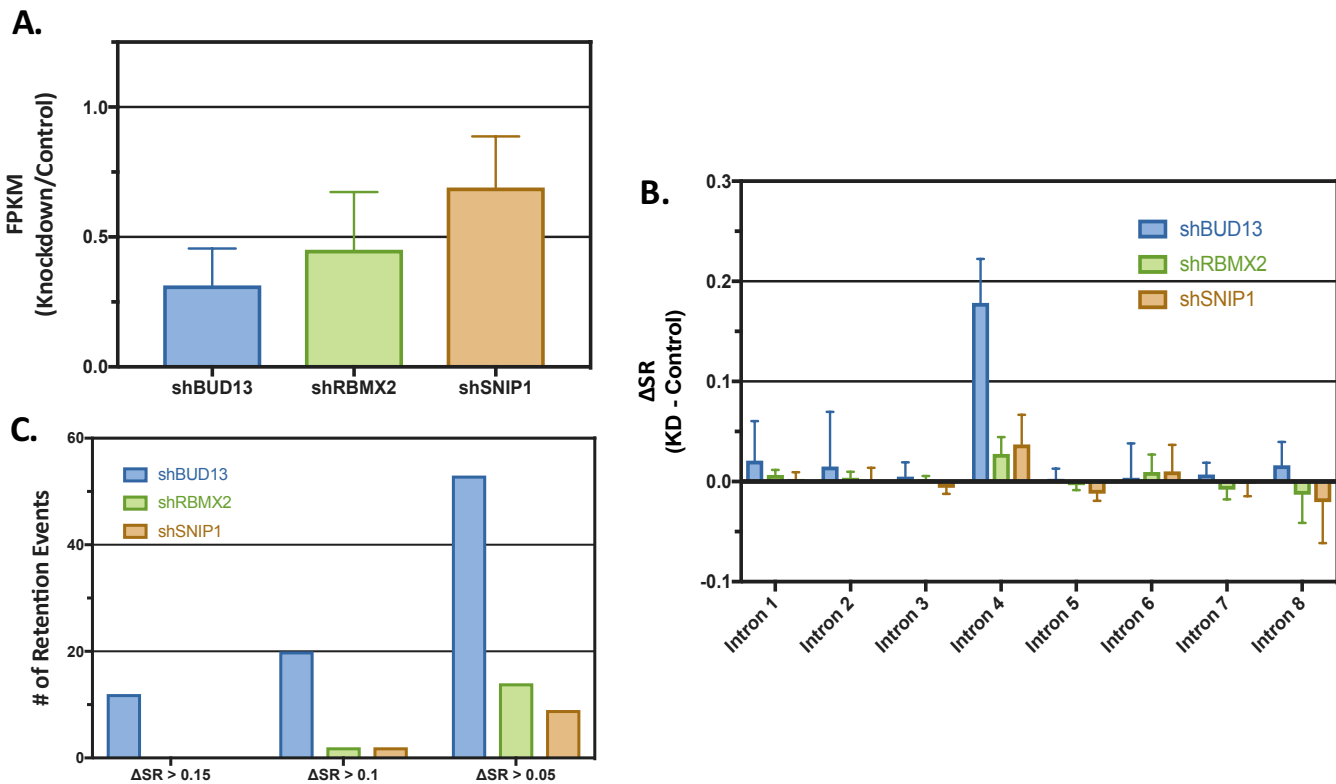


Figure S7: Knockdown of other RES complex proteins. Related to Figure 4.

(A) Bargraph indicating knockdown efficiency for RBMX2 shRNA (green) and SNIP1 shRNA (orange) as compared to BUD13 shRNA (blue). **(B)** Δ SRs calculated for each junction in the *Irf7* transcript for shBUD13 (blue), shRBMX2 (green) and shSNIP1 (orange). The shBUD13 data is from figure 4 and is shown for perspective. Comparison of intron Δ SR at intron 4 to all other junctions is significant (Student's t-test, $p < 0.001$). No other pairwise comparison is significant for shBUD13. No pairwise comparison is significant for other knockdown constructs. **(C)** Box plot showing the number of retention events across replicates at the indicated Δ SR for shBUD13 (blue), shRBMX2 (green), and shSNIP1 (orange).

Table S1: shRNA Sequences

shRNA control (5' - CCTAAGGTTAAGTCGCCCTCGCTCGA GCGAGGGCGACTTAACCTTAGG - 3')	IDT	N/A
shRNA targeting Bud13	IDT	TRCN0000123412
shRNA targeting Rbmx2	IDT	TRCN0000313808
shRNA targeting Snip1	IDT	TRCN0000375040