Supplementary Table 1. Raw data of RNA immunoprecipitates for *CLK1* exon 4.

RNA extracted from RNA immunoprecipitation assay (input, HA-IP and IgG-IP) was analyzed for the quantities of *CLK1* exon 4 transcripts using Real-Time quantitative PCR, (described in Material and Methods). Raw Ct data are shown in the table. CT values were used to determine ΔCT, ΔΔCT, and fold enrichment ($2^{-\Delta\Delta Ct}$) of *CLK1* exon 4-containing RNA.

Supplementary Table 2. Sequences of primers and oligonucleotides used for knockout and knockdowns.

The sequences of primers used to monitor production of endogenous *CLK1* splice variants by endpoint RT-PCR and the *CLK1* exon 4 amplicon produced by qRT-PCR are shown. Sequences used for siRNA or shRNA knockdown assays are listed. Targeted sequences and the sequence of oligos used to assemble and clone guide RNAs for the CRISPR/Cas9 SR protein knockouts and the CRISPR/dCas13Rx assays are also shown. The commercial origin of the drugs used to modulate CLK kinase activity is also indicated.

Supplementary Figure 1. Replicates of RT-PCR assays monitoring *CLK1* splicing

RT-PCR reactions to monitor endogenous CLK1 alternative splicing of exon 4 using primers in exons 2 and 5 (see Fig. 1) were fractionated on agarose gel, stained with RedSafe nucleic acid staining soluVon, and imaged using the GelDoc Imaging System. The position of the exon 4-containing (L) and exon 4-lacking (S) products is indicated. A. Samples used for the analysis shown in Figure 1. B. Samples used for the analysis shown in Figure 2. C. Samples used for the analysis shown in Figure 3.

Supplementary Figure 2. Impact of tagged SR proteins on exon 4 splicing in HCT116 cells.

A. HCT116 cells transfected with Myc-, Flag- and His-tagged SR protein expressing plasmids were tested for expression by immunoblotting with the relevant antibody. B. Endpoint RT-PCR was carried out to monitor endogenous *CLK1* exon 4 inclusion. PSI values obtained for each sample were compared with an EMPTY version to obtain ΔPSI values, which are represented in the graph. Gel replicates for each sample displaying the RT-PCR reactions monitoring *CLK1* exon 4 splicing are shown. Statistical significance was evaluated using multiple t-test analysis. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Supplementary Figure 3. Expression of derivatives of HA-SR proteins.

HCT116 cells transfected with the HA-SR plasmids and derivatives were tested for HA-SR expression by immunoblotting with an anti-HA antibody. HA-SRSF3 and HA-TRA2*b* derivatives are shown in panel A while SRSF10 and derivatives are shown in panel B.

Supplementary Figure 4. Validation of the depletion of SR proteins in HCT116 cells.

A. List of SR protein genes targeted by CRISPR/Cas9. The ability (+) or failure (X) to obtain knockout HCT116 cell lines is indicated, as well as the validation method(s) used to confirm the knockout. WB = immunoblots. The source of antibodies used to validate protein expression is also indicated. B. For the siRNAs that were used to target SRSF1, SRSF6 and SRSF7, qRT-PCR was used to quantitate transcript levels. C. *CLK1* exon 4 splicing was monitored by endpoint RT-PCR to assess the impact of depleting SRSF1, SRSF6 and SRSF7. D. Immunoblot showing that the depletion of SRSF3 was achieved by expressing shSRSF3. The antibodies used and their corresponding dilutions are indicated below each immunoblot. A control cell line (HCT1169BB-CTRL) is used for comparison, and actin was used as a loading control.

Supplementary Figure 5. Impact of guide RNAs/dCas13Rx on *CLK1* exon 4 splicing in EcR293 cells.

A. The position on the *CLK1* exon 4 alternative splicing unit of 25 nt-long guide RNAs is indicated. The position of primers used for the RT-PCR is also shown. B. Following transfection of the plasmids expressing guide RNAs and dCas13Rx, RNA was extracted and *CLK1* exon 4 splicing was monitored by RT-PCR. B. ΔPSI values that compare the impact of each guide relative to the gEMPTY plasmid are shown. Duplicates of the RT- PCR products fractionated on agarose gels are shown below the graph. Statistical significance was evaluated using multiple t-test analysis. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Supplementary Figure 6. Triplicates of RT-PCR assays for HCT116 cells treated with drugs.

RT-PCR reactions monitoring *CLK1* exon 4 splicing were fractionated on agarose gels. The position of the exon 4-containing (L) and exon 4-lacking (S) products is indicated. Samples are those used for the analysis shown in Figure 7.

		HA-SRSF10	HA-SRSF10	HA-SRSF10	HA-TRA2β	HA-TRA2β	HA-TRA2β	HA-SRSF12	HA-SRSF12	HA-SRSF12	HA-SRSF3	HA-SRSF3	HA-SRSF3	HA-EMPTY	HA-EMPTY	HA-EMPTY
	Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PLATE1	**INPUT**	24.23	24.69	23.71	23.37	22.30	22.29	23.88	22.17	24.98	24.88	24.99	24.94	22.82	23.50	24.48
	HA-IP	26.47	25.34	26.62	23.75	25.53	24.01	25.69	26.60	26.67	28.07	27.99	28.04	25.87	26.84	27.87
	IgG-IP	29.34	28.13	28.91	26.83	28.97	28.77	29.31	29.80	29.52	29.13	28.84	29.10	26.29	27.18	28.21
PLATE2	**INPUT**	24.31	24.75	23.75	23.17	22.03	22.09	22.48	22.60	23.81	22.88	23.83	23.59	21.79	22.68	23.56
	HA-IP	25.83	25.79	26.44	23.07	23.12	22.71	25.10	26.02	25.92	27.49	27.59	27.55	24.98	26.25	26.22
	IgG-IP	28.81	29.18	28.40	26.25	27.46	27.61	28.78	29.09	28.80	28.49	28.29	28.47	25.25	26.86	27.35
PLATE3	**INPUT**	24.03	23.76	23.66	23.53	22.85	22.53	23.13	23.04	24.62	23.46	22.98	23.05	22.51	22.38	23.42
	HA-IP	26.46	26.28	26.91	22.75	24.38	22.99	25.36	26.30	26.70	27.85	27.46	27.57	25.33	26.52	26.58
	IgG-IP	28.92	29.51	28.85	26.36	27.95	27.83	29.05	29.34	29.56	28.02	28.50	28.47	26.53	26.94	27.52

Supplementary Table 1

SUPPLEMENTARY TABLE 2			
PCR REACTION	**Primer Name**	**Sequence**	
Endogenous CLK1 PCR	CLK1-EX2-FWD	GGA GCA GCA GCA GTC ATA A	
	CLK1-EX5-REV	TGATCGATGCACTCCACAAC	
RNA-IP CLK1-EX4-qRT-PCR	CLK1-EX4-FWD	AAAGAACCAGGAGTGTAGAGG	
	CLK1-EX4-REV	ATCTTGCACTTAGTACGTCTCC	
siRNA knockdown	**Name Catalog #**	**Sequence**	
SRSF1	J-018672-09-0005 ON-TARGETplus Human SRSF1	CGUGGAGUUUGUACGGAAA	
SRSF6	J-016067-09-0005 ON-TARGETplus Human SRSF6	CGCAGUAGAUCUCGAAGUA	
SRSF7	J-015909-05-0005 ON-TARGETplus Human SRSF7	GAGGAGAAACCAAGGUGUA	
shRNA knockdown	**Name**	**Sequence**	
shSRSF3	shScramble forward	GCTAATAATTTCGAAAAATTATTAGCGCTATCGCGCTTTTTTCTAGACCCAGC	
	shScramble reverse	GCTAATAATTTTTCGAAATTATTAGCGCTATCGCGCGGTGTTTCGTCCTTTC	
	shSRSF3 forward	ATCTTGTGGAAAGGACGAAACACCGTAAGAGTGGAACTGTCGAATGCGAACATTCGACAG	
	shSRSF3 reverse	CAAGAAAGCTGGGTCTAGAAAAAATAAGAGTGGAACTGTCGAATGTTCGCATTCGACAG	
CRISPR/Cas9 knockout			
gRNA Name	**gRNA Target Sequence**	**FWD-OLIGO SEQUENCE**	**REV-OLIGO SEQUENCE**
SRSF1-1	CGCGACGGCTATGATTACGA	CACCGCGCGACGGCTATGATTACGA	AAACTCG TAA TCA TAG CCG TCG CGC
SRSF1-2	CGGGTCCTCGAACTCAACGA	CACCGAACGATTGCCGCATCTACGT	AAACACG TAG ATG CGG CAA TCG TTC
SRSF2-1	CCCGCACTCGTTCTCGATCT	CACCGCCCGCACTCGTTCTCGATCT	AAACAGA TCG AGA ACG AGT GCG GGC
SRSF2-2	GAAGACGCGCCTCAGCGTGT	CACCGGAAGACGCGCCTCAGCGTGT	AAACACA CGC TGA GGC GCG TCT TCC
SRSF4-1	AGTCTGTACTCTGTGCGAGT	CACCGAGTCTGTACTCTGTGCGAGT	AAACACT CGC ACA GAG TAC AGA CTC
SRSF4-2	CAATTACTCGCTCACCACAA	CACCGCAATTACTCGCTCACCACAA	AAACTTG TGG TGA GCG AGT AAT TGC
SRSF5-1	ACATACCGTCTATCATTTCG	CACCGACATACCGTCTATCATTTCG	AAACCGA AAT GAT AGA CGG TAT GTC
SRSF5-2	GAGACTAAATCCAGCGGCCA	CACCGGAGACTAAATCCAGCGGCCA	AAACTGG CCG CTG GAT TTA GTC TCC
SRSF6-1	CGCCCCTAGGTACGGCTTCG	CACCGCGCCCCTAGGTACGGCTTCG	AAACCGA AGC CGT ACC TAG GGG CGC
SRSF6-2	CGTTTACGAGCTGAACGGCA	CACCGCGTTTACGAGCTGAACGGCA	AAACTGC CGT TCA GCT CGT AAA CGC
SRSF7-1	AATGATAGATGCTATGAGTG	CACCGAATGATAGATGCTATGAGTG	AAACCAC TCA TAG CAT CTA TCA TTC
SRSF7-2	AGGATCTTCGAATTCCACAA	CACCGAGGATCTTCGAATTCCACAA	AAACTTG TGG AAT TCG AAG ATC CTC
SRSF8-1	AGCTCCGCCCCGTCCATGG	CACCGAGCTCCGCCCCGTCCATGG	AAACCCA TGG ACG GGG CGG AGC TC
SRSF8-2	CGGACGAAAGCGAAGCCCC	CACCGCGGACGAAAGCGAAGCCCC	AAACGGG GCT TCG CTT TCG TCC GC
SRSF9-1	ACCCCGACCTCCATAAGTCC	CACCGACCCCGACCTCCATAAGTCC	AAACGGA CTT ATG GAG GTC GGG GTC
SRSF9-2	GAGTTCCCCAGGACTTATGG	CACCGGAGTTCCCCAGGACTTATGG	AAACCCA TAA GTC CTG GGG AAC TCC
SRSF10-1	CGTGGCCGACGACACCAGGT	CACCGCGTGGCCGACGACACCAGGT	AAAC ACC TGG TGT CGT CGG CCA CGC
SRSF10-2	TTCTACACTCGCCGTCCAAG	CACCGTTCTACACTCGCCGTCCAAG	AAAC CTT GGA CGG CGA GTG TAG AAC
SRSF11-1	GATCAAGATCACGTTCTAGG	CACCGGATCAAGATCACGTTCTAGG	AAACCCT AGA ACG TGA TCT TGA TCC
SRSF11-2	GCAGTGGCAGGTCTTCTGCC	CACCGGCAGTGGCAGGTCTTCTGCC	AAACGGC AGA AGA CCT GCC ACT GCC
SRSF12-1	CCTGTTCATCAGGAACGTCG	CACCGCCTGTTCATCAGGAACGTCG	AAACCGA CGT TCC TGA TGA ACA GGC
SRSF12-2	GAGGACTTGCGCCGTGAGTT	CACCGGAGGACTTGCGCCGTGAGTT	AAACAAC TCA CGG CGC AAG TCC TCC
TRA2A-1	AGGATCTCGTAGTCCATCAA	CACCGAGGATCTCGTAGTCCATCAA	AAACTTG ATG GAC TAC GAG ATC CTC
TRA2A-2	CATATACACCAGAATACCGG	CACCGCATATACACCAGAATACCGG	AAACCCG GTA TTC TGG TGT ATA TGC
TRA2B-1	AGACCACATACGCCAACACC	CACCGAGACCACATACGCCAACACC	AAACGGT GTT GGC GTA TGT GGT CTC
TRA2B-2	CCCATCAAGCTCCATTCCAT	CACCGCCCATCAAGCTCCATTCCAT	AAACATG GAA TGG AGC TTG ATG GGC
CRISPR/dCas13Rx			
Cas13d-gRNA Name	**Target CLK1 sequence**	**FWD-OLIGO-gRNA SEQUENCE**	**REV-OLIGO-gRNA SEQUENCE**
g1	TGAAATTGCTCCTGCCAACCGTTT	AAACAAA CGG TTG GCA GGA GCA ATT TCA	AAAATGA AAT TGC TCC TGC CAA CCG TTT
g2	GTGGCCTGGCGTGTATCTGAATGC	AAACGCA TTC AGA TAC ACG CCA GGC CAC	AAAAGTG GCC TGG CGT GTA TCT GAA TGC
g3	CTGAATATCTCCCCGCTGAATGAAT	AAACATT CAT TCA GCG GGG AGA TAT TCA G	AAAACTG AAT ATC TCC CCG CTG AAT GAA T
g4	TTCGTATTCTGCCCTGAATTCACTC	AAACGAG TGA ATT CAG GGC AGA ATA CGA A	AAAATTC GTA TTC TGC CCT GAA TTC ACT C
g5	GGGTATATTGATTGGCTGGATGATC	AAACGAT CAT CCA GCC AAT CAA TAT ACC C	AAAAGGG TAT ATT GAT TGG CTG GAT GAT C
g6	TTGGTGCCGCCCACTTGACGTTTC	AAACGAA ACG TCA AGT GGG CGG CAC CAA	AAAATTG GTG CCG CCC ACT TGA CGT TTC
g7	TTGACGTTTCCAGAAGAGTCACCGA	AAACTCG GTG ACT CTT CTG GAA ACG TCA A	AAAATTG ACG TTT CCA GAA GAG TCA CCG A
g8	AGTCACCGAAGGAAAAGAACCAGG	AAACCCT GGT TCT TTT CCT TCG GTG ACT	AAAAAGT CAC CGA AGG AAA AGA ACC AGG
g9	GGAAAAGAACCAGGAGTGTAGAGGA	AAACTCC TCT ACA CTC CTG GTT CTT TTC C	AAAAGGA AAA GAA CCA GGA GTG TAG AGG A
g10	AGTGTAGAGGATGATGAGGAGGGTC	AAACGAC CCT CCT CAT CAT CCT CTA CAC T	AAAAAGT GTA GAG GAT GAT GAG GAG GGT C
g11	TGATGAGGAGGGTCACCTGATCTGT	AAACACA GAT CAG GTG ACC CTC CTC ATC A	AAAATGA TGA GGA GGG TCA CCT GAT CTG T
g12	ACCTGATCTGTCAGAGTGGAGACGT	AAACACG TCT CCA CTC TGA CAG ATC AGG T	AAAAACC TGA TCT GTC AGA GTG GAG ACG T
g13	CAGAGTGGAGACGTACTAAGTGCAA	AAACTTG CAC TTA GTA CGT CTC CAC TCT G	AAAACAG AGT GGA GAC GTA CTA AGT GCA A
g14	ACTAAGTGCAAGATGTATAGAATAT	AAACATA TTC TAT ACA TCT TGC ACT TAG T	AAAAACT AAG TGC AAG ATG TAT AGA ATA T
g15	TAGAATATTTTTCAACACTTATTAA	AAACTTA ATA AGT GTT GAA AAA TAT TCT A	AAAATAG AAT ATT TTT CAA CAC TTA TTA A
g16	CTTTTCAGATAACATAATCTATATA	AAACTAT ATA GAT TAT GTT ATC TGA AAA G	AAAACTT TTC AGA TAA CAT AAT CTA TAT A
g17	TAGATTAAGCTTTCAGGGATTTGGAA	AAACTTC CAA ATC CCT GAA AGC TTA ATC TA	AAAATAG ATT AAG CTT TCA GGG ATT TGG AA
g18	ATCTTTTTTTCTTTCTCTTTTTTGTT	AAACAAC AAA AAA GAG AAA GAA AAA AAG AT	AAAAATC TTT TTT TCT TTC TCT TTT TTG TT
DRUG	**Catalog #**	**SUPPLIER**	
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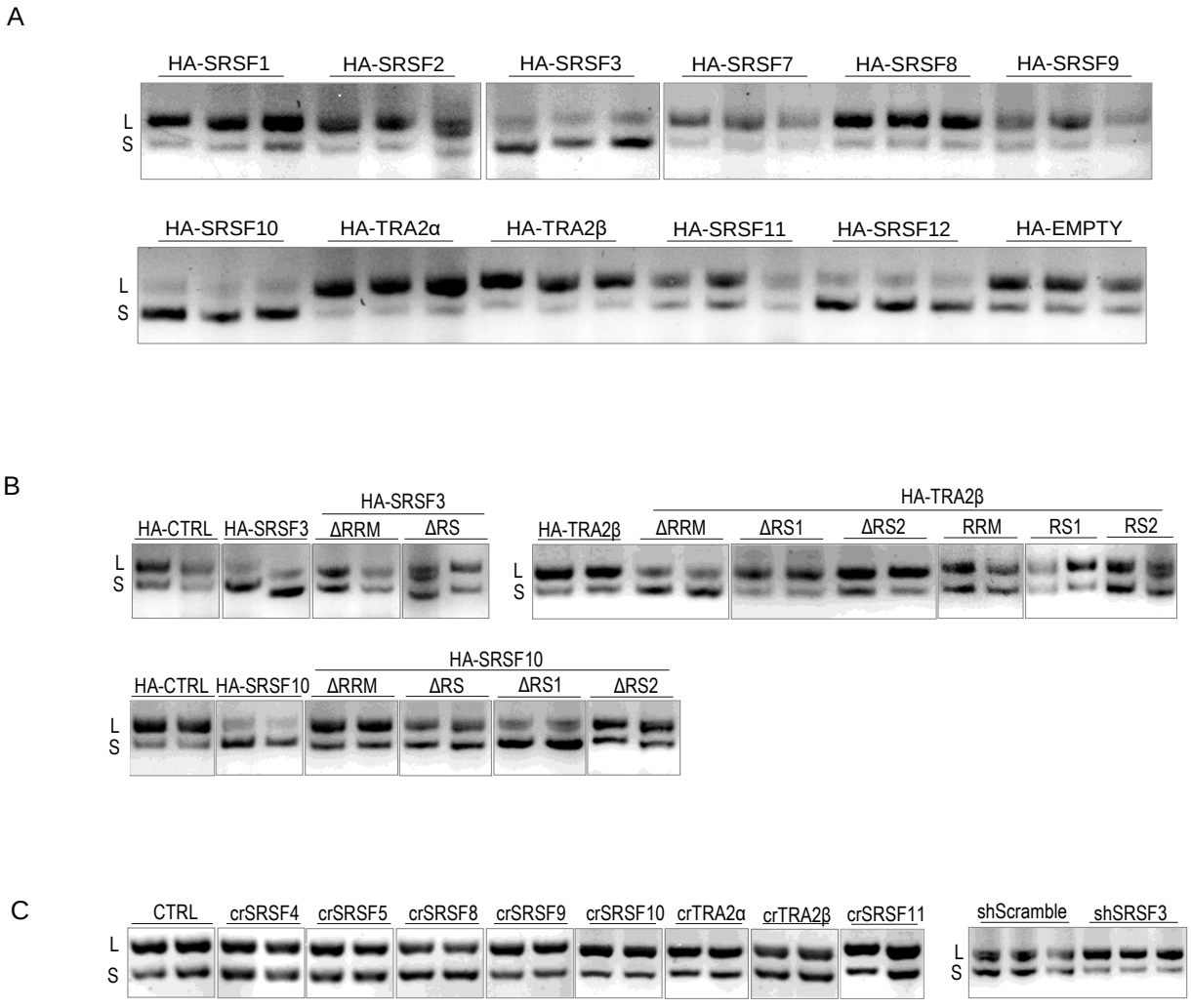
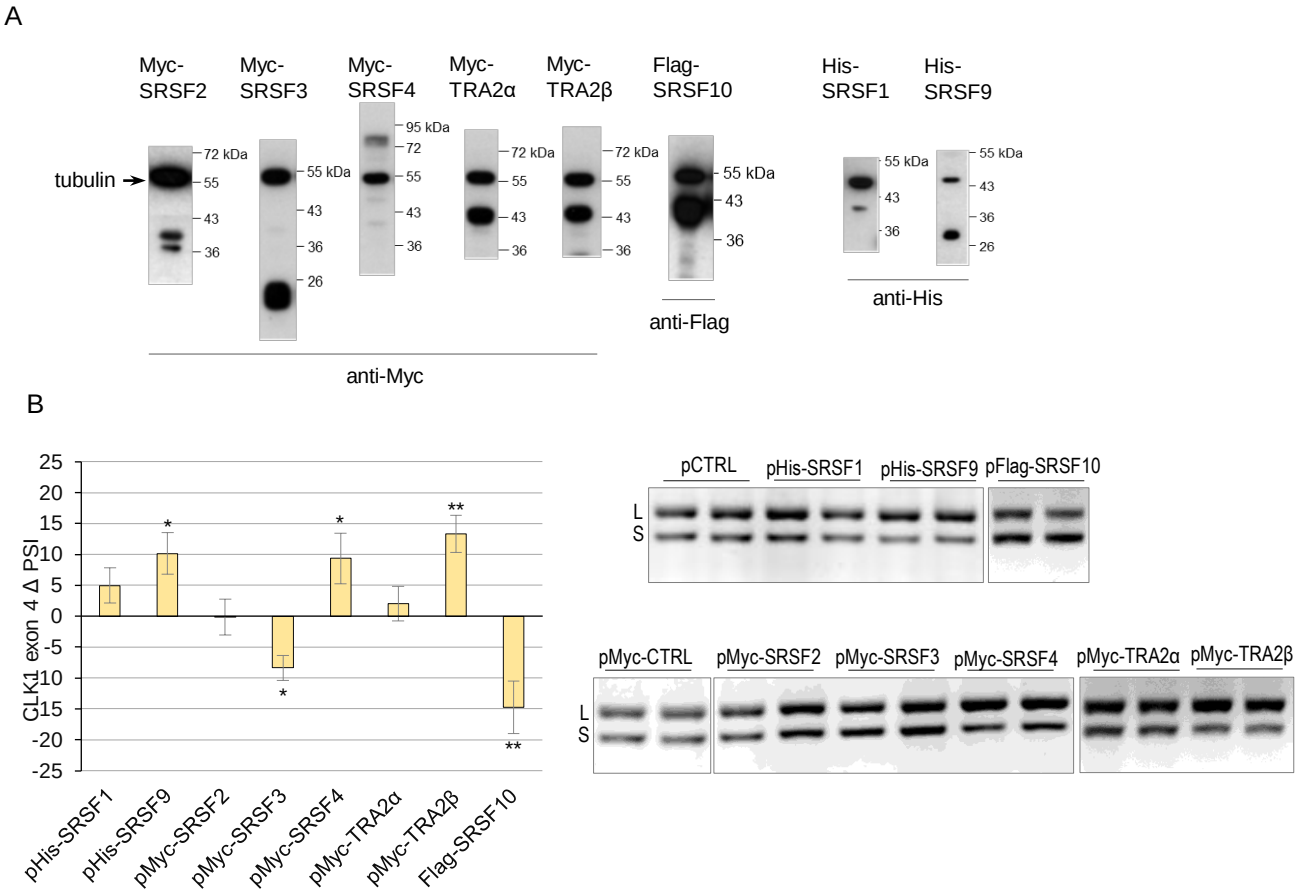
Cantharidin	14589-25	Cayman Chemical Co.-CEDARLANE	



Supplementary Fig. 1



Supplementary Fig. 2

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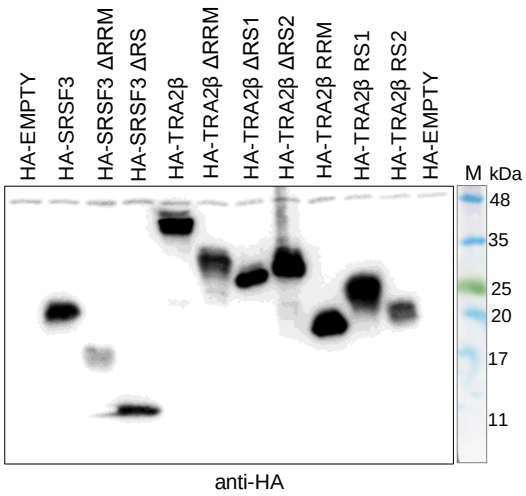

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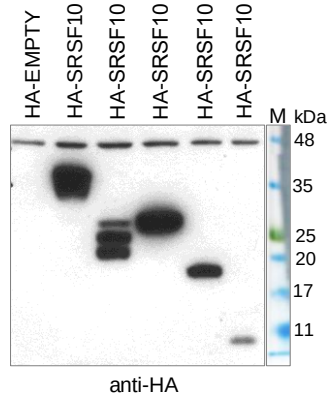
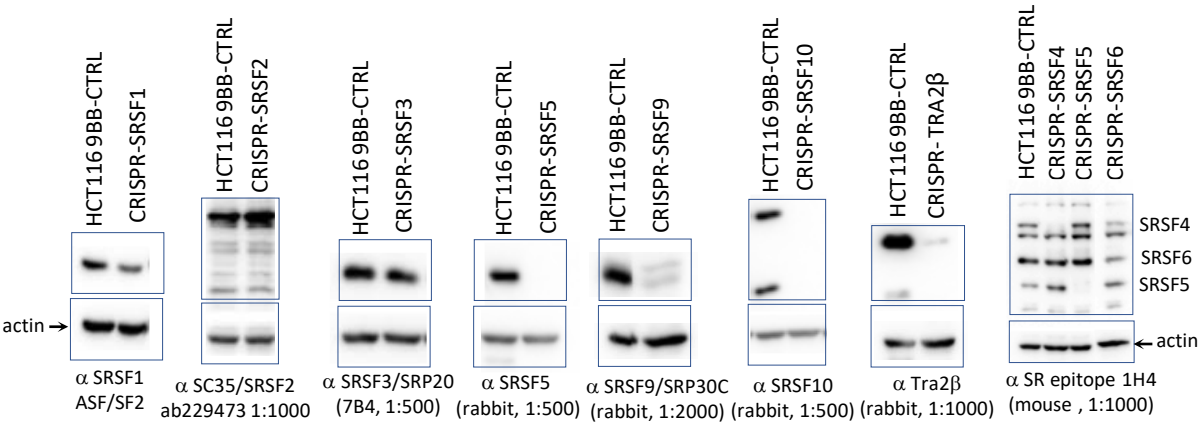


Supplementary Fig.3

A

CRISPR/Cas9			
Target	Impact	Validation Method	Details
SRSF1	X	WB	Invitrogen 32-4600
SRSF2	X	WB & Sequencing	1H4, Abcam ab28428
SRSF3	X	WB	Santa Cruz 7B4 & C8
SRSF4	+	WB	1H4 & Sigma S2570
SRSF5	+	WB	1H4 & Sigma S2695
SRSF6	X	WB	1H4 & Sigma S2820
SRSF7	X	Sequencing	-
SRSF8	+	Sequencing	Insertion of 1 nt, 99%
SRSF9	+	WB	Chemicon 04317 3rd bleed
SRSF10	+	WB	Abcam ab77209
SRSF11	+	Sequencing	Insertion of 1 nt, 76%
SRSF12	X	WB	Novus Biological NBP1-57405
TRA2a	+	Sequencing	Insertion of 1 nt, 99%
TRA2β	+	WB	Abcam ab31353



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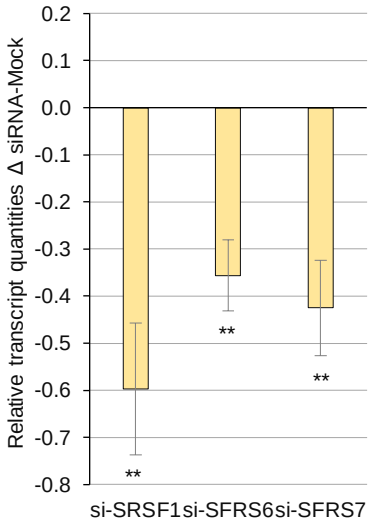

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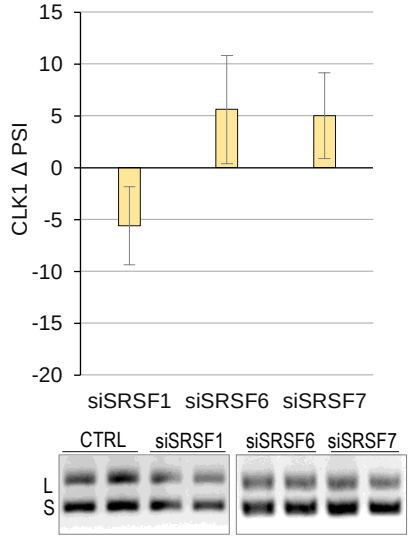

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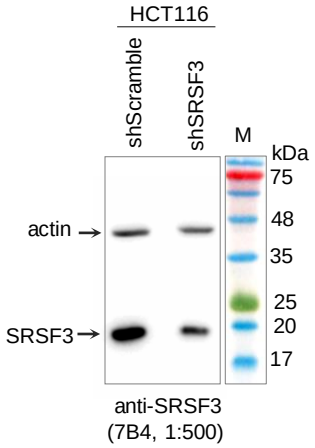

Supplementary Fig. 4

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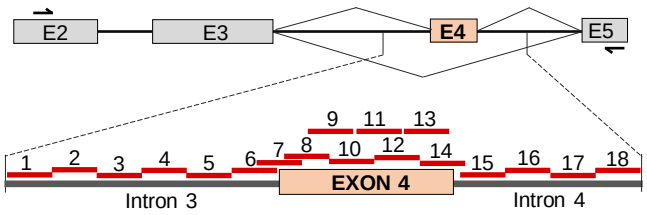

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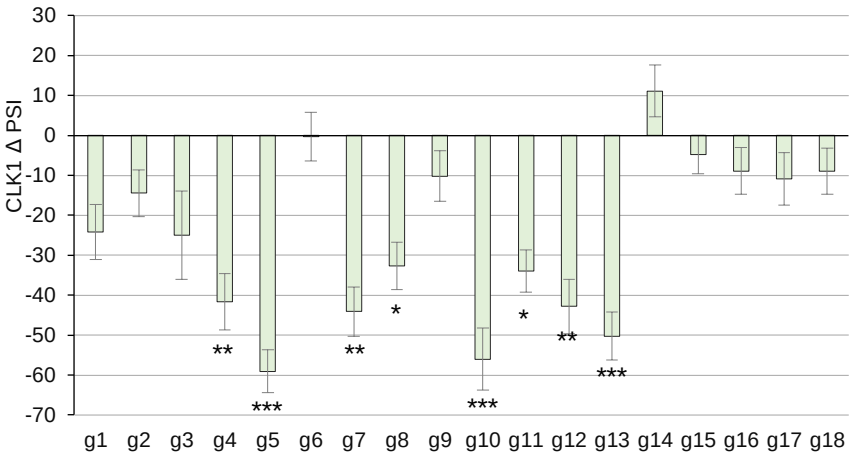

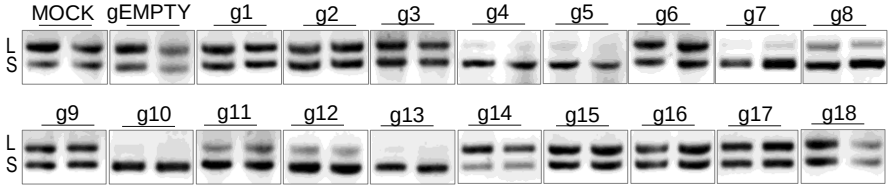

Supplementary Fig.5

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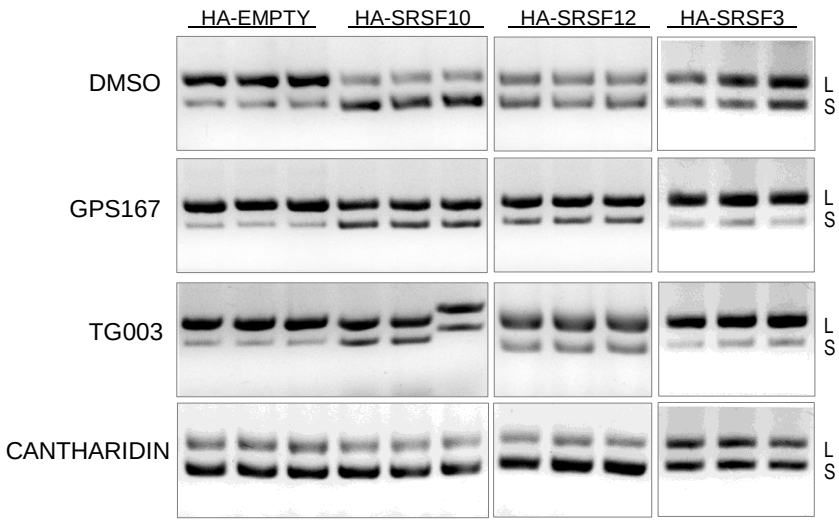

Supplementary Fig.6