Supplemental material

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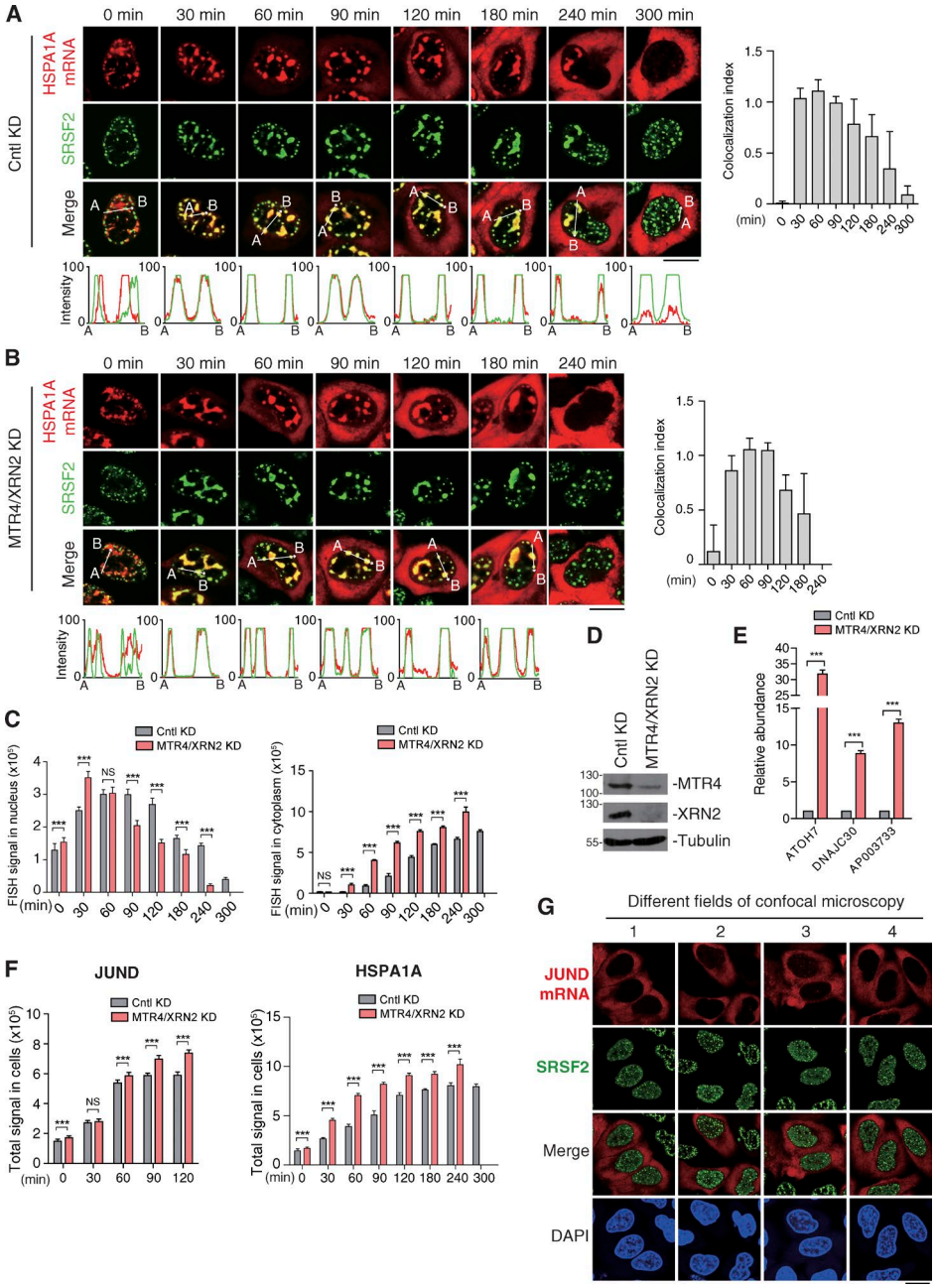


Figure S1. **Evidence that speckle-localized HSPA1A mRNAs were released to the cytoplasm rather than being degraded. (A)** HeLa cells were treated with Cntl siRNA for 72 h. The HSPA1A mRNA transcribed from the microinjected reporter construct was detected by FISH with the 3′ vector probe at the indicated time after addition of α-amanitin (4 µg/ml). NSs were detected by the SRSF2 antibody. Confocal microscopy was used to visualize the cells. The red and green lines in the line scan graphs demonstrate the intensities of the mRNA and the SRSF2 IF signals, respectively. Colocalization indexes of the HSPA1A FISH foci and SRSF2 dots at each time point are shown on the right. Data represent the mean ± SD from three independent experiments; $n = 10$. **(B)** Same as in A, except that the Mtr4 and XRN2 siRNA were used. **(C)** Quantification of HSPA1A mRNA FISH signals in nucleus or cytoplasm from cells in A and B. Data represent the mean ± SD from three independent experiments; $n = 10$. **(D)** Western blotting analysis of XRN2 and Mtr4 shown the knockdown (KD) efficiency. Molecular masses are given in kilodaltons. **(E)** RT-qPCRs examine the relative abundance of three exosome targets (ATOH7, DNAJC30, and AP003733 mRNAs) in HeLa cells treated with Cntl or MTR4/XRN2 siRNAs. Data represent the mean ± SEM; $n = 3$. **(F)** Quantification of JUND mRNA and HSPA1A mRNA FISH total signals in cells in Fig. 1 C and panels A and B. Data represent the mean ± SD from three independent experiments; $n = 10$. **(G)** Multiple fields of cells at the 120-min time point shown in Fig. 1 C. FISH with the 3′ vector probe and IF with the SRSF2 antibody were performed to indicate the mRNAs and NSs. Confocal microscopy was used to visualize the cells. Bars, 20 µm. Statistical analysis was performed using an unpaired *t* test. ***, $P < 0.01$.

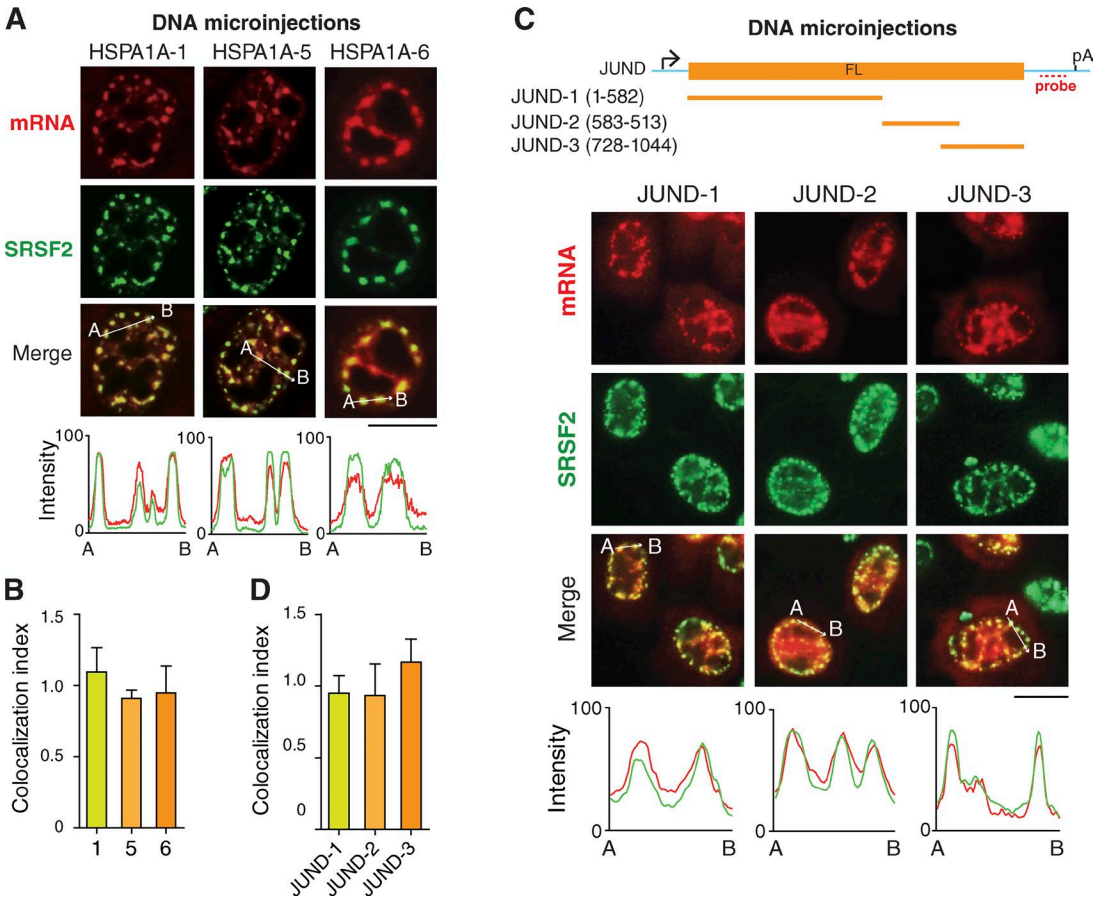


Figure S2. **Multiple fragments of HSPA1A and JUND mRNAs associate with NSs. (A)** Constructs expressing the no. 1, no. 5, and no. 6 HSPA1A fragments shown in Fig. 5 A were microinjected into HeLa nuclei, and α-amanitin (4 µg/ml) was added 20 min after injection. 30 min after the addition of α-amanitin, FISH with the 3′ vector probe and IF with the SRSF2 antibody were performed. Confocal microscopy was used to visualize the cells. The red and green lines in the line scan graphs demonstrate the intensities of the mRNA and the SRSF2 IF signals, respectively. **(B)** The colocalization index of RNA foci with SRSF2 speckles in each group shown in A. **(C)** Top: Schematic of JUND fragment constructs. The promoter and polyA (pA) site are shown. Sequences from the vector and JUND gene fragments are indicated as a cyan line and an orange bar respectively. The position of the FISH probe is marked with a red broken line. Bottom: Equal amounts of JUND fragment constructs shown in the top were microinjected into HeLa nuclei. α-Amanitin (4 µg/ml) was added 20 min after microinjection. 40 min after the addition of α-amanitin, FISH with the 3′ vector probe and IF with the SRSF2 antibody was performed. Confocal microscopy was used to visualize the cells. The red and green lines in the line scan graphs demonstrate the intensities of the mRNA and the SRSF2 IF signals, respectively. FL, full length. **(D)** The colocalization indexes of mRNA foci and SRSF2 speckles in each group are shown in C. Bars, 20 µm. Data represent the mean ± SD from three independent experiments; *n* = 10.

Figure S3. **The presence of ESEs in naturally intronless genes. (A)** The graph shows the frequency distribution of ESEs in naturally intronless genes. **(B)** The graph shows the normalized distribution of ESEs in naturally intronless genes. $\mu = 30.08$; $\sigma = 17.21$. **(C)** ESEs that are highly enriched in intronless genes are highlighted in STE1 and STE2. **(D)** The distribution of the 5′-GACGTC-3′ and 5′-GAGGAG-3′ ESE motifs in the HSPA1A coding region are highlighted with red and cyan, respectively. **(E)** Introducing ESEs did not cause cryptic splicing events of the cG mRNA. Left: Schematic of β-globin constructs. The position of the primers (red arrows) and the size of the PCR products are indicated. Right: The RT-PCR product from each mRNA run on an agarose gel, and a size standard was loaded on the left.

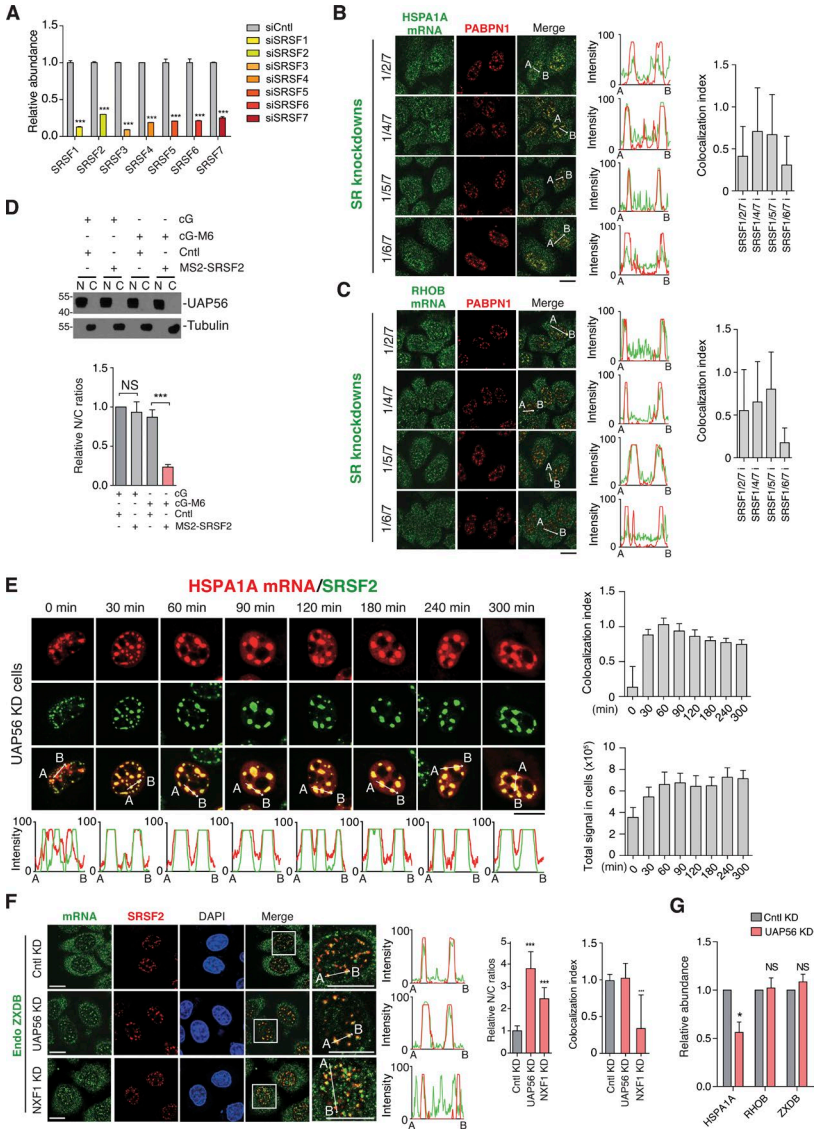


Figure S4. **The roles of SR and TREX proteins in speckle localization and nuclear export of intronless mRNAs. (A)** Cntl siRNA or siRNAs targeting different SRSF proteins as indicated were transfected to HeLa cells. 72 h later, RT-qPCRs were performed to examine the knockdown (KD) efficiencies. Data represent the mean ± SEM; *n* = 3. **(B)** Cntl siRNA or combination of siRNAs targeting different SRSF proteins as indicated was transfected to HeLa cells. 72 h later, distribution of the endogenous HSPA1A mRNA was detected with its specific probe. PABPN1 IF shows the location of NSs. Confocal microscopy was used to visualize the cells. The green and red lines in the line scan graphs demonstrate the intensities of the mRNA and the PNBPN1 IF signals, respectively. Colocalization indexes of the HSPA1A mRNA foci and PABPN1 dots at each time point are shown on the right. Data represent the mean ± SD from three independent experiments; *n* = 10. **(C)** Same as in B, except the endogenous RHOB mRNA was detected. **(D)** The cG or cG-M6 (100 ng/µl) construct was cotransfected with the GFP-MS2 (Cntl) or MS2-SRSF2 (10 ng/µl) construct into HeLa nuclei. N/C separations were performed 24 h after transfection followed by RNA extraction and RT-qPCRs. Western blotting was used to examine the purities of nuclear and cytoplasmic fractions by using UAP56 and tubulin as nuclear and cytoplasmic markers, respectively. Ratios of nuclear to cytoplasmic β-globin mRNAs after normalization to NEAT1 (nucleus) and GAPDH (cytoplasm) in each fraction are presented in the bar graph. Data represent the mean ± SEM; *n* = 3. Molecular masses are given in kilodaltons. **(E)** HeLa cells were treated with UAP56/URH49 siRNA for 48 h to achieve efficient mRNA nuclear retention and to avoid a secondary effect. The HSPA1A mRNA transcribed from the microinjected reporter construct was detected by FISH with the 3′ vector probe at the indicated times after addition of α-amanitin (4 µg/ml). NSs were detected by the SRSF2 antibody. Confocal microscopy was used to visualize the cells. The red and green lines in the line scan graphs demonstrate the intensities of the mRNA and the SRSF2 IF signals, respectively. The colocalization indexes of the HSPA1A FISH foci and SRSF2 foci at each time point are shown in the right top graph. The total FISH signal at each time point is shown in the right bottom graph. Data represent the mean ± SD from three independent experiments; *n* = 10. **(F)** Distribution of the endogenous (Endo) ZXDB mRNA was detected with its specific probe in cells treated with indicated siRNAs (note that to achieve efficient mRNA nuclear retention and to avoid a secondary effect, the cells were treated with UAP56/URH49 or NXF1 siRNAs for 48 h). SRSF2 IF shows the location of NSs. Higher magnification of the boxed regions is shown. Confocal microscopy was used to visualize the cells. The green and red in the line scan graphs demonstrate the intensities of the mRNA and the SRSF2 IF signals, respectively. The bar graphs show the average N/C ratios and colocalization indexes in each treatment group. Data represent the mean ± SD from three independent experiments; *n* = 10. **(G)** HeLa cells were treated with UAP56/URH49 siRNA for 48 h to achieve efficient mRNA nuclear retention and avoid a secondary effect. Total RNA was extracted, followed by RT-qPCRs. The bar graphs show the relative abundance of endogenous HSPA1A, RHOB, or ZXDB mRNAs. Data represent the mean ± SEM; *n* = 3. Bars, 20 µm. Statistical analysis was performed using an unpaired *t* test. *, P < 0.05; ***, P < 0.01.

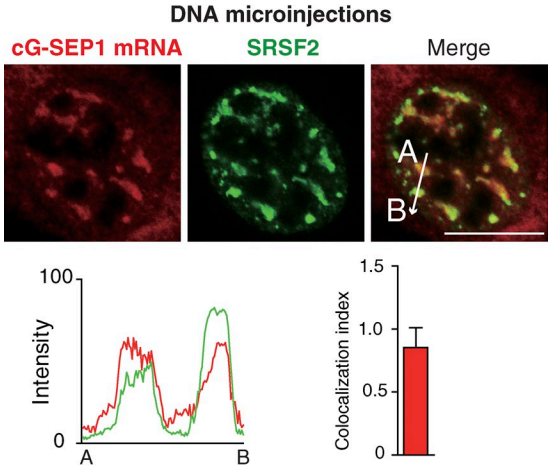
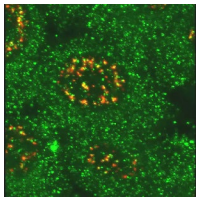
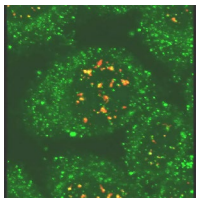


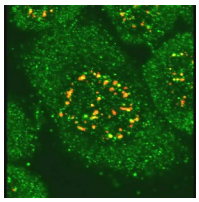
Figure S5. **SEP1 facilitates speckles association of cDNA transcripts.** The cG-SEP1 construct was microinjected into HeLa nuclei. α-Amanitin (4 µg/ml) was added 20 min after microinjection. To examine speckle localization, 2 h after the addition of α-amanitin, FISH with the 3′ vector probe and IF with the SRSF2 antibody were performed. Bar, 20 µm. Confocal microscopy was used to visualize the cells. The red and green lines in the line scan graphs demonstrate the intensities of the mRNA and the SRSF2 IF signals, respectively. The colocalization indexes of mRNA foci and SRSF2 speckles are shown. Data represent the mean ± SD from three independent experiments; *n* = 10.



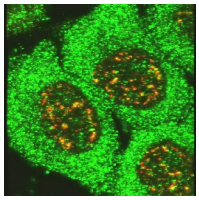
Video 1. **Colocalization of JUND mRNA with SRSF2.** 3D reconstitution of JUND mRNA (green) and SRSF2 (red) in HeLa cells was performed with FV10-ASW software based on z stack images captured with a FLUOVIEW FV1000 microscope. The frame is 70 × 70 µm.



Video 2. **Colocalization of HSPA1A mRNA with SRSF2.** Same as Video 1, except that the HSPA1A mRNA was detected.



Video 3. **Colocalization of RHOB mRNA with SRSF2.** Same as Video 1, except that the RHOB mRNA was detected.



Video 4. **Colocalization of ZXDB mRNA with SRSF2.** Same as Video 1, except that the ZXDB mRNA was detected.

Table S1. **siRNA and ASO targeting sequences**

Name	Target sequence (5′–3′)
si Cntl	CGTACGCGGAATACTTCGA
si SRSF1	CGACGGCTATGATTACGAT
si SRSF2	AATCCAGGTCGCGATCGAA
si SRSF3	CTATGTGGCTGCCGTGTAA
si SRSF4	GAATCACGCTCCAGATCAA
si SRSF5	CAAGGGATATGGACGGATA
si SRSF6	GCAGATCTAAGGATGAGTA
si SRSF7	CCTCGACGATCAAGATCTA
si UAP56	AAGGGCTTGGCTATCACAT
si URH49	AAAGGCCTAGCCATCACTT
si NXF1	GCGCCATTCGCGAACGATT
si MTR4	GCACAGATTGCAATAGATA
si XRN2	ACACTGTAGTCAGTATTAA
Cntl ASO	GGAGGAACCTTCAGGGAAGG
HSPA1A ASO	GCGTCCGGAAGGACCGAGCT
JUND ASO	ACGCGCTGACGCTGAGCCTG
RHOB ASO	CCATCCCAGTGTCTGTGTGC
ZXDB ASO	CCGAGTCCACCGAGGCCCTG

Table S2. **Probe sequences**

Name	Gene ID	Sequences (5′–3′)
3′ vector probe	—	AAGGCACGGGGGAGGGGCAAACAACAGATGGCTGG
		CAACTAGAAGGCACAGTCGAGGCTGATCAGCGGGT
5′ vector probe	—	AAGTTTAAACGCTAGCCAGCTTGGGTCTCCCTATAGT
		GAGTCGTATTAATTTCGATAAGCCAGTAAGCAG
β-globin probe	—	CACGTTGCCCAGGAGCCTGAAGTTCTCAGG
Cntl probe	NR_045321.1	Sequence of mouse Gm14635 lncRNA region (682–1,222 nt)
HSPA1A probe	NM_005345.5	Antisense sequence of human HSPA1A mRNA region (51–1,206 nt)
JUND probe	NM_005354.5	Antisense sequence of human JUND mRNA region (173–1,216 nt)
RHOB probe	NM_004040.3	Antisense sequence of human RHOB mRNA region (408–1,409 nt)
ZXDB probe	NM_007157.3	Antisense sequence of human ZXDB mRNA region (214–1,206 nt)
NEAT1 probe	NR_028272.1	Antisense sequence of human NEAT1 lncRNA region (913–1,967 nt)

Table S3. **Primer sequences**

Name	Sequence (5′–3′)
β-globin F	AGAACTTCAGGCTCCTGGGC
β-globin R	TCTGGGACGTCGTATGGGTAG
Smad F	ACACCAGCAGTTTTACCACCTG
Smad R	ATGGAATGCGTGTAGTCATCAAG
HSPA1A F	TGCTCCGACCTGTTCCG
HSPA1A R	CTCGTCGGGGTTGATGC
JUND F	CGCCTCCTACCCCCCTG
JUND R	AGCCCGTTGGACTGGATGAT
RHOB F	AAGGGTGGTGATGGGTG
RHOB R	CTGGTGGAGGGTTCGCA
ZXDB F	GATAATGGAGCAGTGGAGG
ZXDB R	TAGTTGGAATGGGAGTTGG
GAPDH F	AGGGCTGCTTTTAACTCTGGT
GAPDH R	CCCCACTTGATTTTGGAGGGA
NEAT1 F	GCGAGGTGCCTTTACTACAT
NEAT1 R	TGGAACCCAGAAGACAGAA
SRSF1 F	CAGAGTGGTTGTCTCTG
SRSF1 R	CTCCACGACACCAGTGCC
SRSF2 F	GGACGCCGGAGCCGCAG
SRSF2 R	GAGATCGAGAACGAGTGC
SRSF3 F	AATGGCAACAAGACGGAAT
SRSF3 R	AGTTCCACTCTTACACGGC
SRSF4 F	CGAGGAGAGAGTGAGAATG
SRSF4 R	GCTACGGCTACCAAACAT
SRSF5 F	GATCCAAGGGATGCAGATG
SRSF5 R	CTATCATTTCGAGGTCTGCG
SRSF6 F	GTGGATACAGCAGTCGG
SRSF6 R	CTGGATCTGCTTCCAGAG
SRSF7 F	GGTCTAGATCACATTCTCG
SRSF7 R	CCAGACCTAGATCTTCTG
pre-COL1A1 F	GCTGGGGAGCAAAGTTCTA
pre-COL1A1 R	GCTGGGGAGCAAAGTTCTA
pre-PTB1 F	GGCAGGCAGCAGGAGAC
pre-PTB1 R	TCTGCACTAGGGCGTTCTC
pre-DDX39B F	AACCCTGGGGGGATTGG
pre-DDX39B R	CGACGATATGCGGGCAGT
T7 F	AATACGACTCACTATAGGG
SP6 R	ATTTAGGTGACACTATAG