Supporting Information

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SI Materials and Methods

Gene Cloning. SRSF1 mutants (FL and RRM1/2), premRNA templates β-globin (*Ron*) and β-globin (*Ron*) (ΔAG) were generated by single or sequential PCR mutagenesis or site-directed mutagenesis kit (Stratagene) (primer sequences are listed in Table S1). Template plasmids were generated from the parental pSP64-HβΔ6 plasmid (1). DNA fragments corresponding to U1-70K (FL), N-RRM (1–230), RRM (92–230), RRM (59–215), and RS (230–437) were cloned into pET15b vector using the U1-70K cDNA (open biosystems) as the template. GST-SRSF2 (RRM), (residues 1–93) was cloned in pGEX4T2 using PCR.

In Vitro Transcription/Translation. Different U1-70K constructs (2 μg) were subjected to in vitro transcribed/translation with 2 μl [^{35}S]-Met (1,000 Ci/mmol at 10 mCi/mL) and 40 μl of rabbit reticulocyte lysate mix (TnT system, Promega) in total 50 μl reaction for 2 hours at 30 °C.

Protein Purification. His-SRSF1 (FL, ΔRS2) and SRSF1 (FL) (RARA, RERA, and RERE) mutants were expressed in *Escherichia coli* BL21 (DE3) pLysS and induced with 1 mM IPTG for 3 hours at room temperature. His-tag proteins were purified using the methods as described (2) and purified further by size-exclusion chromatography (Superdex 75, 16/60; GE Healthcare) in 20 mM Tris-HCl (pH 5.0), 500 mM NaCl, 100 mM MOPS, 10% glycerol, and 1 mM DTT. For in vitro splicing and splicing complex formation assay, proteins were dialyzed or purified in 20 mM HEPES (pH 7.9), 300 mM KCl, 20% glycerol, 0.5 mM DTT, and 0.2 mM EDTA) and further separated by size-exclusion chromatography (Superdex 75, 16/60; GE Healthcare). GST-SRSF1 (WT, RSRA, RSRE, RARA, RERA, and RERE), GST-SRSF1 (RRMs) (WT, RRM1, RRM2, and RS), and GST-SRSF2 (RRM) were cloned into pGEX4T2 vector and expressed in *E. coli* BL21 (DE3). Cells were induced at $O.D._{600}$ of 0.4 with 0.5 mM IPTG and cultured for 3 hours at 30 °C. The proteins were purified on DEAE sephacel (Pharmacia) column in tandem with glutathione sepharose (Amersham) column that had been equilibrated with 20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 10% glycerol, 0.2 mM EDTA (pH 8.0), 5 mM DTT, and 1 mM PMSF. Further purification was achieved using size-exclusion chromatography. His-SRSF1 (RRM1/2) WT and mutants were purified in M9-based minimal media and induced with 1 mM IPTG for 3 hour at 37 °C. The protein was purified in 20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 10% glycerol, 10 mM β-mercaptoethanol using Ni-NTA agarose (Qiagen) column and DEAE and further separated using size-exclusion chromatography (Superdex 75, 16/60; GE Healthcare) at room temperature. For the splicing assay, proteins were dialyzed or purified in 20 mM HEPES (pH 7.9), 300 mM KCl, 20% glycerol, 0.5 mM DTT, and 0.2 mM EDTA. His-tag U1-70K (residues 59–215) using a pET15b vector was cultured in M9-based minimal media and induced with 1 mM IPTG for 3 hours at 25 °C. The protein was purified on DEAE sephacel column in tandem with a Ni-NTA agarose (Qiagen) column equilibrated with 20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 10% glycerol, 10 mM β-ME, and 1 mM PMSF. SRPK1 and CLK/STY were purified as described (2).

Protein Phosphorylation. For hypo-phosphorylation, SRSF1 was phosphorylated for 2 hours at 30 °C by His-SRPK1 in kinase buffer (50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM DTT, 5 mM $MgCl_2$, 10% glycerol, 1 mM ATP and 1× phosphatase inhibitor) with a 5:1 molar ratio (SRSF1:SRPK1). For hyper-phosphorylation, SRSF1 was incubated first with SRPK1 for 1 hour and then CLK/STY was added in a 10:1 molar ratio (SRSF1: CLK/STY) for an additional 1 hour at 30 °C.

EMSA. 10 fmol of [γ-^{32}P]-ATP labeled RNA was incubated with SRSF1 for 30 min at 4 °C in buffer A containing 20 mM Tris-HCl (pH 7.5), 75 mM NaCl, 0.01 mg/mL BSA, 0.1% NP40, 1 mM DTT, 5 mM $MgCl_2$, 10% glycerol and 5 U RNase inhibitor with 1 μg polyU as a nonspecific competitor. Binding reaction products were resolved on a 6% or 6.5% polyacrylamide native gel (acrylamide:bisacrylamide = 29:1) containing 5% glycerol in 1X TBE. All gels were ran at 400 V for 1 hour at 4 °C, dried and analyzed by phosphorimaging.

Filter Binding Assay. 10 fmol of [γ-^{32}P]-ATP labeled ESE was incubated with SRSF1 in 100 μl buffer A at 4 °C for 40 min. The reaction mixtures were diluted 1:10 with 900 μl cold buffer A containing 1 μg polyU and immediately filtered through nitrocellulose membranes (Millipore, 0.45 μm) at a flow rate of 0.5 mL/ min and rinsed with 5 mL cold buffer A. Membranes were dried at 60 °C for 1 hour and the amount of bound RNA was measured using liquid scintillation counter. The 0% (baseline) and 100% (maximum binding) was determined by spotting the probe with filtering with and without washing respectively. K_d was calculated as the protein concentration required for 50% RNA binding. Each experiment was repeated at least three times.

GST Pull-Down Assay. GST-fusion proteins (10 ug) were mixed with purified target proteins (10 μg) or in vitro translated U1-70K (5 μl) in buffer B containing 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10% glycerol, 1 mM DTT and 0.5% NP40 at 4 °C for 30 min followed by further incubation with 15 μl glutathione sepharose resin (Amersham) for 30 min at 4 °C. Resins were washed three times with 400 μl buffer B and the bound protein was eluted by boiling with 4× gel loading dye for 5 min at 80 °C and was resolved by SDS/PAGE. Separated proteins were visualized by coomassie staining, western blotting or autoradiography. Quantitation of gel images was performed using GelquantNET (1.7.4).

In Vitro Splicing Assay. β-globin(wt) in pSP64-HβΔ6(1) and β-globin (*Ron*) in pcDNA3 plasmids were linearized and transcribed with SP6 RNA polymerase and T7 RNA polymerase, respectively, in the presence of [α-^{32}P]-UTP. Pre-mRNA was incubated with HeLa nuclear or cytoplasmic S100 extracts in the presence of WT and mutant SRSF1 both full length and RRM as described (3). RNA was extracted and analyzed by denaturing PAGE and phosphorimage analysis on a FUJIFILM FLA-5100 instrument (Fuji Medical Systems USA, Inc.). Quantitation of gel images was performed using GelquantNET (1.7.4).

1. Krainer AR, Maniatis T, Ruskin B, Green MR (1984) Normal and mutant human beta-globin pre-mRNAs are faithfully and efficiently spliced in vitro. *Cell* 36:993–1005.
2. Velazquez-Dones A, et al. (2005) Mass spectrometric and kinetic analysis of ASF/SF2 phosphorylation by SRPK1 and Clk/Sty. *J Biol Chem* 280:41761–41768.
3. Mayeda A, Krainer AR (1999) Preparation of HeLa cell nuclear and cytosolic S100 extracts for in vitro splicing. *Methods Mol Biol* 118:309–314.

Fig. S1. Phosphorylation states of SRSF1 affect ESE binding affinity and specificity. EMSA analysis of unphosphorylated SRSF1, p-SRSF1, and pp-SRSF1 to *Ron* ESE, respectively. The assay was carried out as described in *Materials and Methods*. These results reveal that fully or partially unphosphorylated SRSF1 interacts with *Ron* ESE with high affinity. Affinity is significantly reduced when the RS domain is fully phosphorylated.

Fig. S2. Unphosphorylated RS peptide interacts with the ESE and SRSF1 (RRM1/2). (*A*) EMSA showing the binding of unphosphorylated SRSF1ΔRS2 to *Ron* ESE (left lanes) and p-SRSF1ΔRS2 (right lanes). These results suggest that unphosphorylated RS2 does not play any specific role in binding affinity enhancement. Any unphosphorylated RS segment can enhance binding affinity of the ESE:SRSF1 complex. (*B*) EMSA analysis of interactions between *Ron* ESE (probe) and a 16-mer Arg-Ser peptide (RS_{16}) (left lanes), or phosphorylated form of the same peptide (right lanes). Unphosphorylated RS peptide binds ESE with weak affinity and phosphorylated peptide does not bind ESE. (*C*) EMSA analysis of interactions between *Ron* ESE, SRSF1 (RRM1/2), and RS16. Equimolar amounts of RRM1/2 and unphosphorylated (left lanes) or phosphorylated (right lanes) RS peptide were mixed with ESE (probe) followed by native gel electrophoresis and phosphorimaging. These results clearly show that unphosphorylated RS peptide enhances the affinity of the ESE:SRSF1 (RRMs) complex.

Fig. S3. SRPK1 interacts both with p-SRSF1 and U1-70K. We observed that both p-SRSF1 and pp-SRSF1 bound to U1-70K (FL) and truncated U1-70K proteins (Fig. S3*A*). Direct interaction assay using GST pull-down that SRPK1 interacts with phosphorylated SRSF1 (FL) (Fig. S3*B*). Moreover, we found that SRPK1 interacts with U1-70K independently (Fig. S3*C*) and also contacted in the presence of SRSF1 (Fig. S3*D*). All these proteins (SRSF1, U1-70K, and SRPK1) contain highly charged segments where both acidic and basic residues are present in patches and in some cases phosphorylation augments total negative charge and alters charge distribution. In SRPK1, these patches are present both in the spacer domain, the N-terminal segment and the core kinase domain. We propose that using these charged segments, SRPK1 simultaneously binds to complementary charged segments in p-SRSF1 (or pp-SRSF1) and U1-70K. (*A*) GST pull-down assay between GST-SRSF1 of different phosphorylated states and in vitro transcribed-translated [^{35}S]-met labeled U1-70K constructs in the presence of RNase A (upper) and in presence of its cognate RNAs (*Ron* ESE and U1 snRNA) (lower). Only hypo- and hyper-phosphorylated SRSF1 samples contained kinases with 1 mM ATP. (*B*) Western blot analysis of components of GST pull-down between GST-SRSF1 (FL) and GST-SRSF1 (RS), and His-SRPK1 in the presence of 1 mM ATP was separated by 10% SDS/PAGE. (*C*) Autoradiograph of GST pull-down assay between GST-SRPK1 and in vitro transcribed-translated [^{35}S]-met labeled U1-70K (FL, RS and RRM1/2). (*D*) Autoradiograph of GST pull-down between GST-SRPK1 and in vitro transcribed-translated [^{35}S]-met labeled U1-70K (FL, RS, and RRM1/2) in the presence of SRSF1. (*E*) Bar graph representation of average retention of WT or truncated U1-70K measured based on three independent GST pull-down experiments. One representative pull-down result is shown in Fig. 3*B*. Input indicates 50% of in vitro translated [^{35}S]-met labeled U1-70K proteins used for the pull-down experiments. Bound fraction used 50% input as 1.

Fig. S4. The RRMs of SR proteins directly binds the RRM of U1-70K. (*A*) Coomassie stained SDS/PAGE of the components of GST pull-down experiments under increasing salt concentration between GST-SRSF1 (RRM1/2) and His-U1-70K (RRM) (residues 59–215). 10 μg of each protein was used for this experiment. (*B*) Sequence alignment of SRSF1 (RRM1) and SRSF2 (RRM). The secondary structures of SRSF1 RRM1 are shown. Mutated residues are denoted by the filed circles. (*C*) Autoradiograph of bound radiolabeled proteins after GST pull-down assay between 10 μg of GST-SRSF1 (RRM1/2) or GST-SRSF2 (RRM, 1–93) and 5 μl of in vitro translated [^{35}S]-met labeled U1-70K (N-RRM, 1–230). (*D*) In vitro splicing of β-globin pre-mRNA in S100 complementation assay using WT and mutant SRSF1 (RRM1/2), and relative splicing efficiency is quantified as shown below.

Table S1. Primer list

Name	SEQUENCES	Mutated domain	Template vector
5′SRSF1(1–X)	AATTCATATGATGTAATGTCGGGAGGTGGTGTGATTC		SRSF1(1–X)
3′SRSF1-RA1	GATCGGATCCTTAGGCTCTAGCACGTGCTCTCGCACGGGCACGCGCTCGGGCTCGCGCTC TTCCATAACTTGGACTTCTGGG	RS1 to RA1	SRSF1(1–219)
3′SRSF1-RE1	GATCGGATCCTTACTCTCTTTCACGCTCTCTTTCACGCTCACGTTCTCGCTCTCGTTCTCTTC CATAACTTGGACTTCTGGG	RS1 to RE1	SRSF1(1–219)
3′SRSF1-RSRA	GATCGGATCCTTATGTACGCGCGCGCGCTCTCGCATGACGGGGCGCATAGCGTGGCGCTC CTCTCGCTCTCCTTGGGGAGTAACTGCG	RSRS to RSRA	SRSF1(1–248)
3′SRSF1-RSRE	GATCGGATCCTTATGTACGTTCGCGTTCTCTTTCATGACGGGGTTCATAGCGTGGTTCTCCT CTTTCTCTCCTTGGGGAGTAACTGCG	RSRS to RSRE	SRSF1(1–248)
3′SRSF1-RARA	GATCGGATCCTTATGTACGCGCGCGCGCTCTCGCATGACGGGGCGCATAGCGTGGCGCTC CTCTCGCTCTCCTTGGCGCGTATGCGCGAGCCCTCGCGTTGGCTCTAGCACGTGCTCTCG	RA1 to RARA	SRSF1(1–219, RA1)
3′SRSF1-RERA	GATCGGATCCTTATGTACGCGCGCGCGCTCTCGCATGACGGGGCGCATAGCGTGGCGCTCC TCTCGCTCTCCTTGGTTCGTATTCGCGTTCCCTTTCGTTCTCTCTTTCACGCTCTCTTTCAC	RE1 to RERA	SRSF1(1–219, RE1)
3′SRSF1-RERE	GATCGGATCCTTATGTACGTTCGCGTTCTCTTTCATGACGGGGTTCATAGCGTGGTTCTCCT CTTTCTCTCCTTGGTTCGTATTCGCGTTCCCTTTCGTTCTCTCTTTCACGCTCTCTTTCAC	RE1 to RERE	SRSF1(1–219, RE1)
β-globin (*Ron*)	Forward: CCCTTAGGCTGCTGGTGGGTACCGGCGGAGGAAGCCTTGGACCCAGAGGTTC TTTG Reverse: CAAAGAACCTCTGGGTCCAAGGCTTCCTCCGCCGGTACC CACCAGCAGCCTAAGGG	β-globin (*Ron*) (inserted *Ron* ESE)	β-globin
β-globin (*Ron*) ΔAG	Forward: CTCTCTGCCTATTGGTCTATTTTCCCTCTCTCTCTGGCCA T GTGGTG GGTACCG GCGGAG Reverse: CTCCGCCGGTACCCACCACATGGCCAGAGAGAGAGGGAAAATA GACCAATAGGCAGAGAG	β-globin (*Ron*)ΔAG (3′SS mutation)	β-globin (*Ron*)
SRSF1 (I32A, V35A)	CATCCGAACCAAGGACGCTGAGGACGCGTTCTACAAATACGGC	SRSF1 (FL, RRM1/2) mut1	SRSF1 (FL)
SRSF1 (K38A, Y39A)	GGACATTGAGGACGTGTTCTACGCGGCCGGCGCTATCCGCGAC	SRSF1 (FL, RRM1/2) mut2	SRSF1 (FL)
SRSF1 (D66A, D69A)	GGACCCGCGAGCCGCGGAAGCCGCGGTGTATGG	SRSF1 (FL, RRM1/2) mut3	SRSF1 (FL)
SRSF1 (F56D, F58D)	GCCGCGGGGGACCGCCCGACGCCGACGTTGAGTTCGAGGACCC	SRSF1 (FL, RRM1/2) mut FF-DD	SRSF1 (FL)