cells

Supplementary Materials: Activation of Cryptic 3′ Splice-Sites by SRSF2 Contributes to Cassette Exon Skipping

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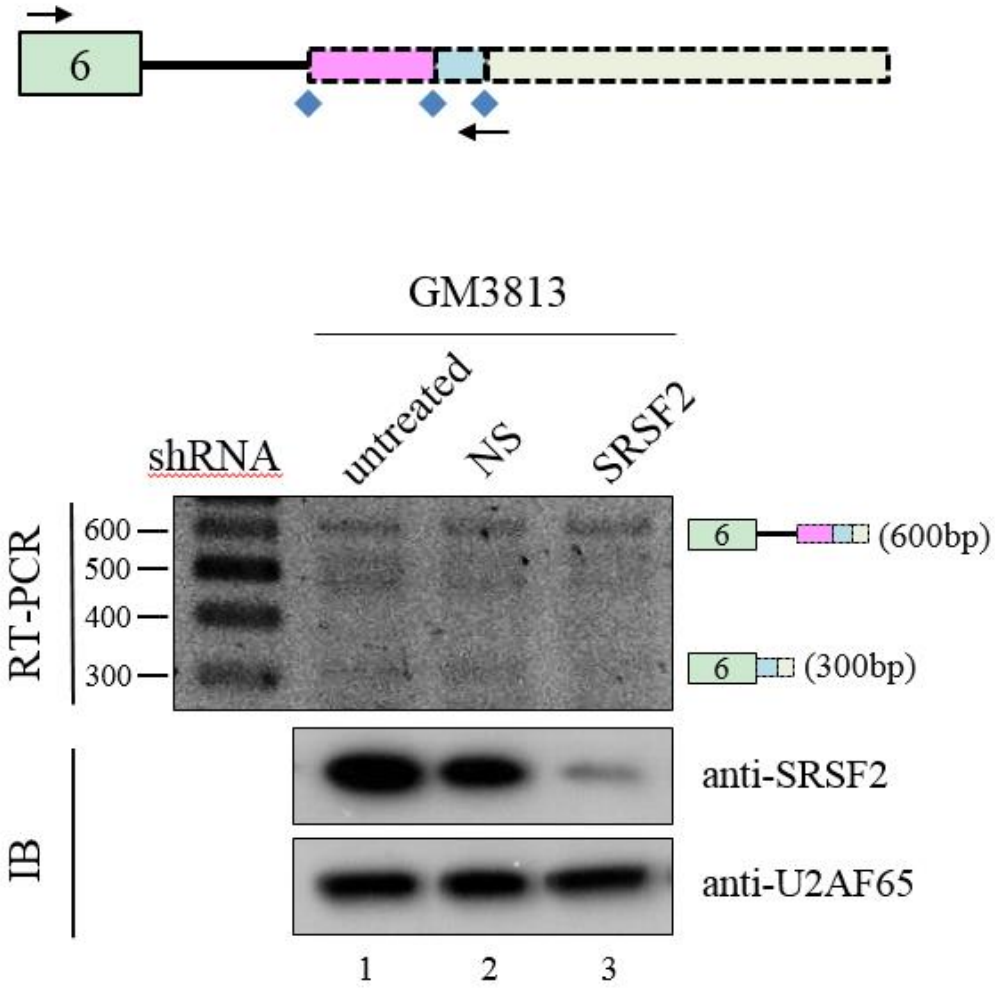

Figure S1. SRSF2 did not affect endogenous 3′AG′ activation. (Upper panel) Primers for 3′AG′ activation are shown with arrows. (Lower panel) RT-PCR analysis to 3′AG′ activation in Spinal Muscular Atrophy patient cells (GM3813) treated with non-silencing and SRSF2-targeting shRNA. Immunoblotting with anti-SRSF2 for SRSF2 expression is shown below, U2AF65 expression is shown as a control.

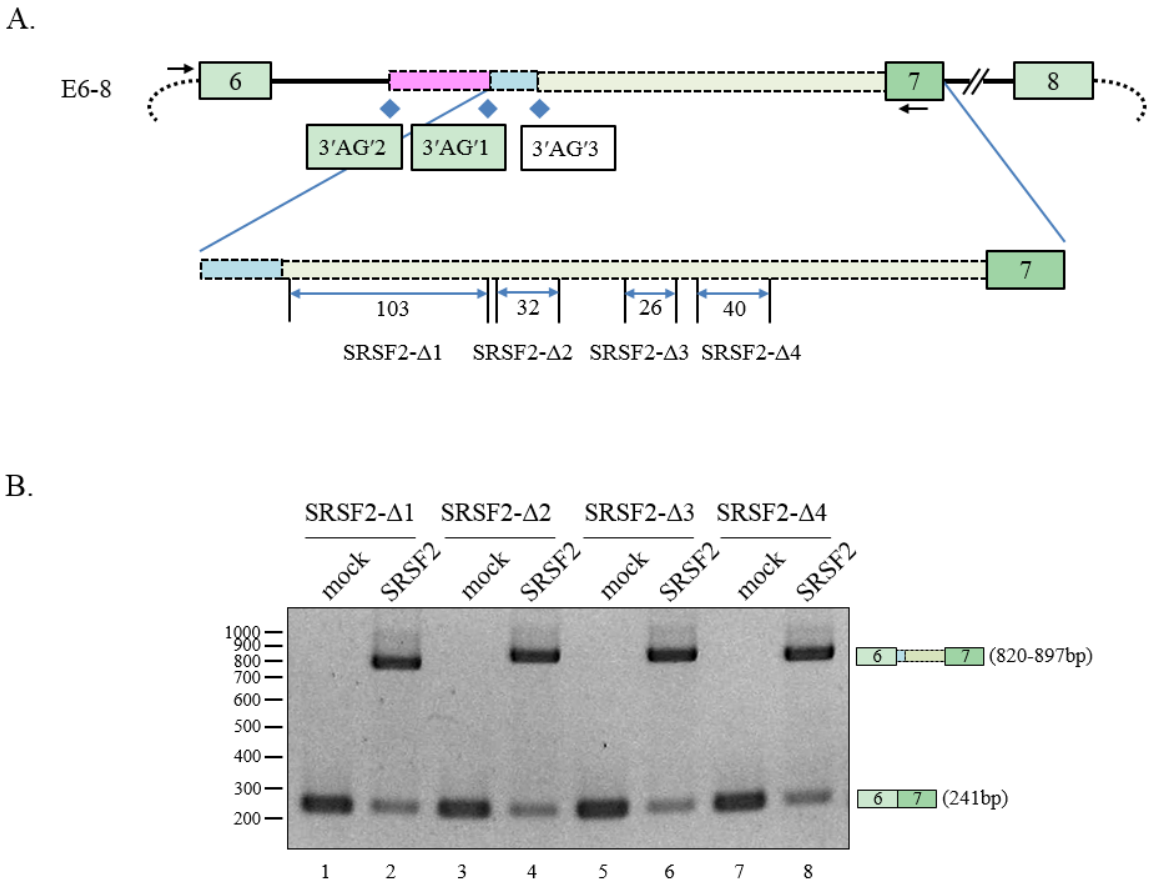

Figure S2. Serial deletions of putative SRSF2 binding sites did not disrupt 3′AG′ activation by SRSF2. (A) Locations of the deleted fragments in the pre-mRNA are indicated. (B) RT-PCR analysis to detect intron6 splicing in cells overexpressing SRSF2 or transfected with pcDNA.

Table 1. Primer list.

PLASMID CONSTRUCTION	
NAME	**SEQUENCE**
SMN.E6(B).F	TACTCGGATCCATAATTCCCCCACCACCTCC
SMN.E8(X).R	CTAACCTCGAGAACAGTACAATGAACAGCCATG
SMN.E7.R	CGATGCTCGAGTCCTTAATTTAAGGAATGTGAG
SMN.E7(I7-20).R	CGGTGCTCGAGCATAATGCTGGCAGACTTACTC
SMN.E7(I7-112).R	CCTAGCTCGAGACCTTTCAACTTTTTAACATCTG
SMN.E7(I7-226).R	CATAGCTCGAGGTTTCTTCCACATAACCAACC
SMN.E7(I7-334).R	AATCGCTCGAGTGCAGTATGCCTAGGTTATCC
SMN.E7(I7-441).R	CCACACTCGAGGCAAATGAGAAATTAGAACCAG
Δ3’AG’1.F	GAAAAGATGGGATAATACGGAGTCTTGCTCTG
Δ3’AG’1.R	CAGAGCAAGACTCCGTATTATCCCATCTTTTC

Δ3’AG’1/2.F	CTTTTGAATTGAAATTATACTTTGGAATGGAAAAGATGGGATAA
Δ3’AG’1/2.R	TTATCCCATCTTTTCCATTCCAAAGTATAATTTCAATTCAAAAG
Δ3’AG’1/2/3.F	GGAATGGAAAAGATGGGATAATGCTGGAGTGCAATGGCGTG
Δ3’AG’1/2/3.R	CACGCCATTGCACTCCAGCATTATCCCATCTTTTCCATTCC
E7-ss-mut1.F	AAATTAAGGAAAAAGTCTGCCA
E7-ss-mut1.R	TGGCAGACTTTTTCCTTAATTT
E7-ss-mut2.F	TTTTCCTTACCCGGTTTTAGAC
E7-ss-mut2.R	GTCTAAAACCGGGTAAGGAAAA
E7/8ex-1.R	CACATAACTACAAAAAAATTG
E7/8ex-2.F	TTTTGTAGTTATGTGGGTTTGTGGAAAACAA
E7/8ex-3.R	GACATGGCTGTTCATTGTACTG
E7/8ex-4.F	CTGTTCATTGTACTGGTAAGTCTGCCAGCATT
E7/8ex-5.R	ATAAAGTTTTACAAAAGTAAG
E7/8ex-6.F	CTTTTGTAAAACTTTATACTTTGTTTTGTAAATT
E6-5’cons.F	ATACTGGCTATTATCAGGTAAGTAATCACTC
E6-5’cons.R	GAGTGATTACTTACCTGATAATAGCCAGTAT
ΔSRSF2.F	ACGGAGTCTTGCTCTGTTGCAAGTTGTGGGATTGTAGGCA
ΔSRSF2.R	TGCCTACAATCCCACAACTTGCAACAGAGCAAGACTCCGT
SRSF2-Δ1.F	CCCAGGCTGGAGTGCAATGTCGTAGCTGGGATTAGAG
SRSF2-Δ1.R	CTCTAATCCCAGCTACGACATTGCACTCCAGCCTGGG
SRSF2-Δ2.F	CCACCACGCCTGTCGTAGCTAATTTTTTGTACTTTCAGTAG
SRSF2-Δ2.R	CTACTGAAAGTACAAAAAATTAGCTACGACAGGCGTGGTGG
SRSF2-Δ3.F	CAGGTGATCCAACTGTCTCGCGTGAGCCACTGTGCCT
SRSF2-Δ3.R	AGGCACAGTGGCTCACGCGAGACAGTTGGATCACCTG
SRSF2-Δ4.F	GTTGCCCAGGGTGGTGTCAAAGTTGTGGGATTGTAGGCA
SRSF2-Δ4.R	TGCCTACAATCCCACAACTTTGACACCACCCTGGGCAAC
E6-A2(1).F	TACTCGCTAGCATAATTCCCCCACCACCTCC
E6-A2(1).R	CTAACGAATTCCCTCTAATCCCAGCTACGAC
E6-A2(2).F	ATCTTGAATTCGCAGTAGTCCAGGGTTTCC
E6-A2(2).R	ATTAGCTCGAGACTGGAAAGACCGCGAAGAG
RT-PCR	
pcDNA.F	CACTGCTTACTGGCTTATCGAA
pcDNA.R	CTAGAAGGCACAGTCGAGGCT
Exon6.F	ATAATTCCCCCACCACCTCC
Exon7.R	AGGTGCTCACATTCCTTAAATT
Exon8.F	GAAATGCTGGCATAGAGCAGC
Exon8.R	ACTACAACACCCTTCTCACAG