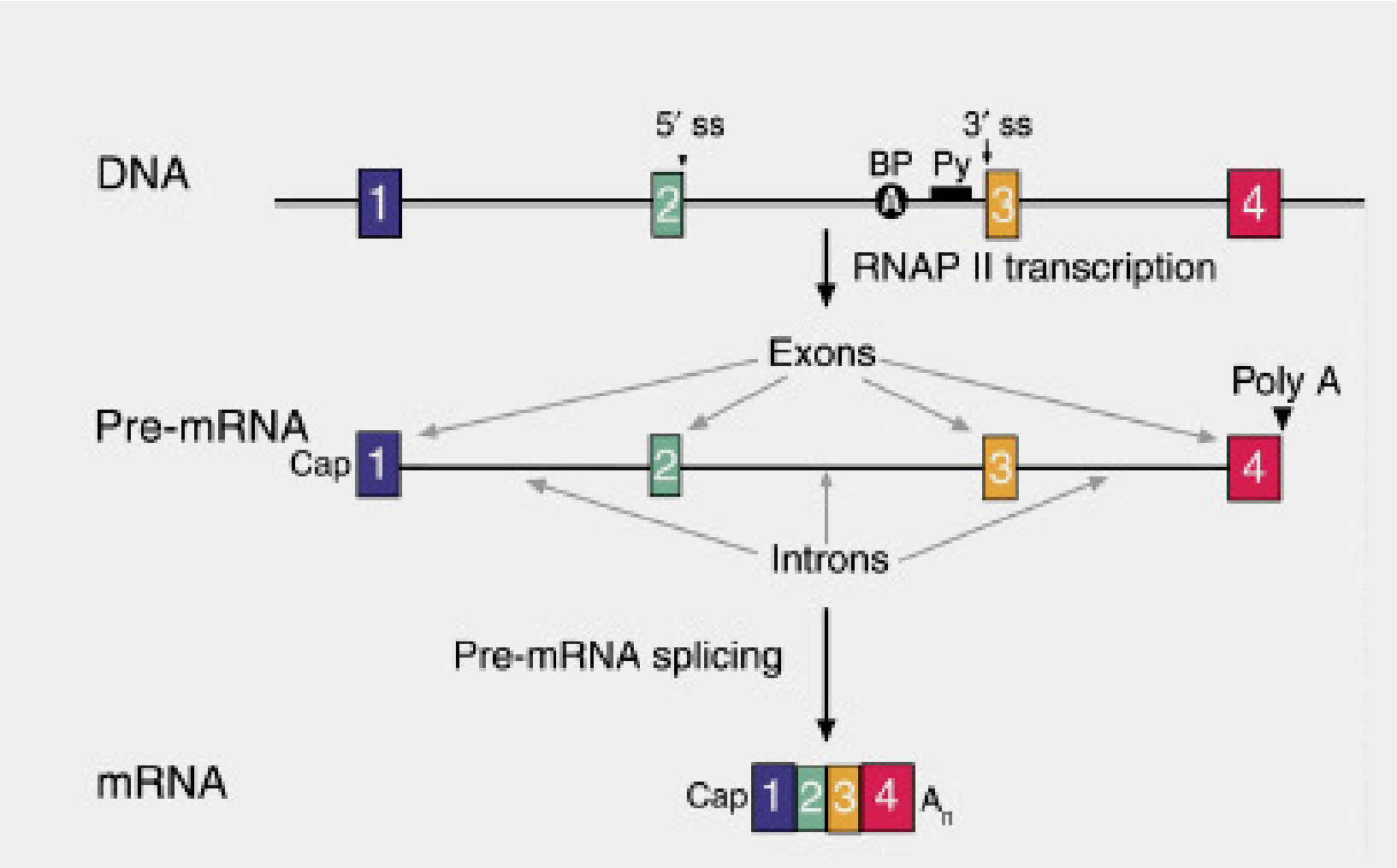
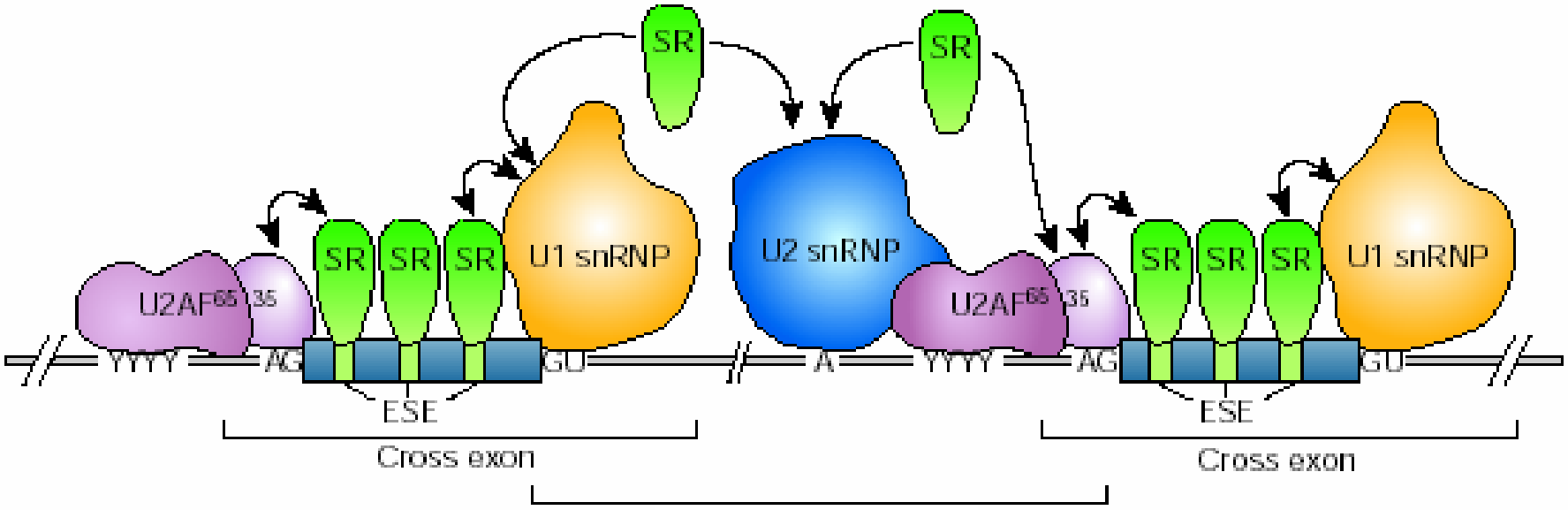
RNA Post-transcriptional processing



ESE: exonic splicing enhancer



Spliceosoma

Negli organismi superiori gli esoni non codificano solamente per le sequenze aminoacidiche ma contengono anche ESE

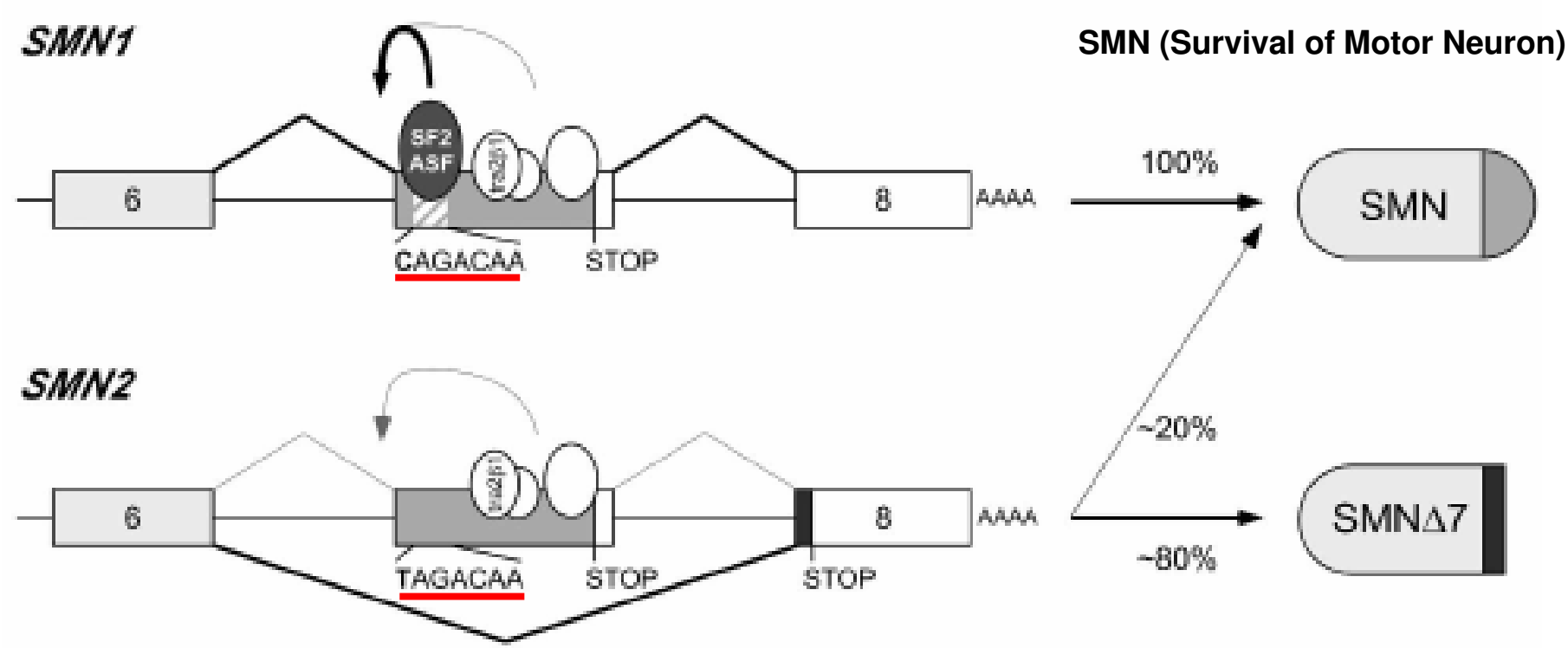
Mutazioni che colpiscono gli ESE possono impedire la formazione dei cross-exon recognition complex.

Come conseguenza si può avere lo "skipping" dell'esone interessato, con produzione di un mRNA maturo mancante di un esone.

Circa il 15% di tutte le mutazioni puntiformi che causano malattie genetiche interferiscono con il processo di definizione degli esoni!

- Serine/arginine-rich proteins are typically involved in positive regulation of splicing, stimulating splicing by interacting with exonic splicing enhancer elements (ESEs) or intronic splicing enhancer elements.
- Negative regulation is promoted most frequently by heterogeneous nuclear ribonucleoproteins (hnRNPs), which function by binding sequences known as exonic splicing silencers (ESSs) or intronic splicing silencers.

Disruption of an SF2/ASF-dependent exonic splicing enhancer in *SMN2* causes spinal muscular atrophy in the absence of *SMN1*



La SMA è un disordine neurodegenerativo pediatrico che deriva da mutazioni nel gene SMN1 (1:10000).

SMN2 è solo parzialmente attivo
ESE?

Luca Cartegni & Adrian R. Krainer nature genetics • volume 30 • april 2002

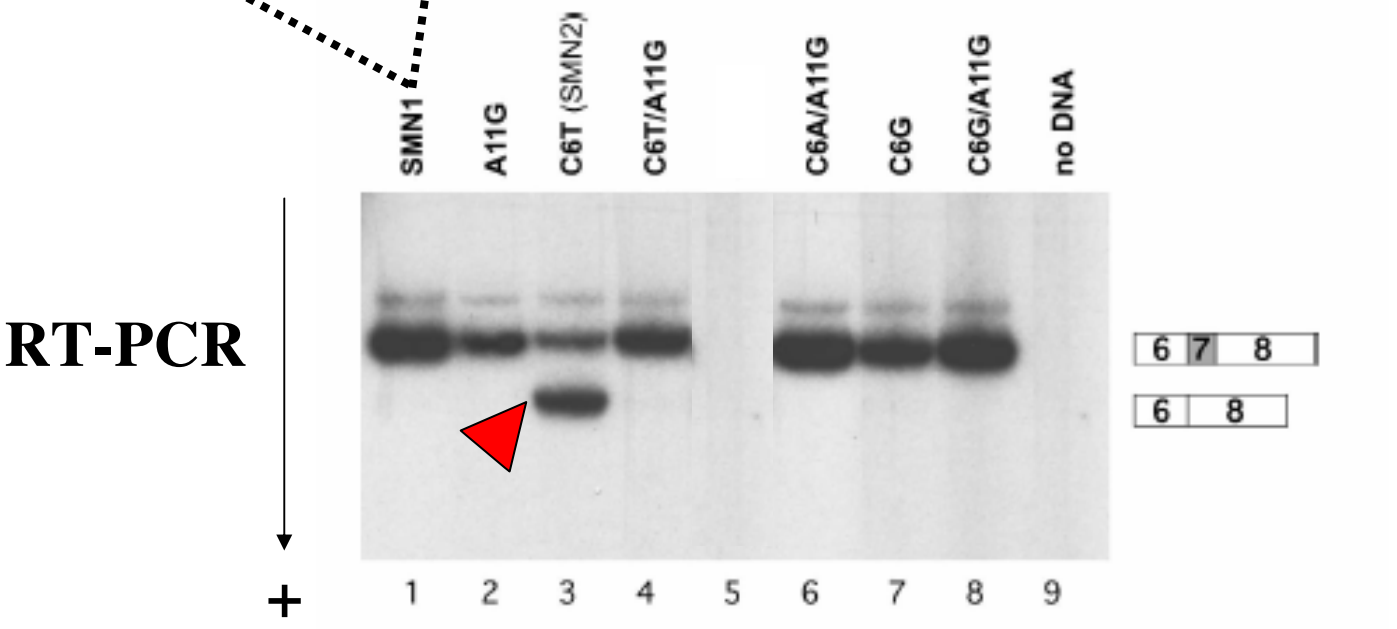
SMN1 (wt)

SF2/ASF

SMN2

SF2/ASF

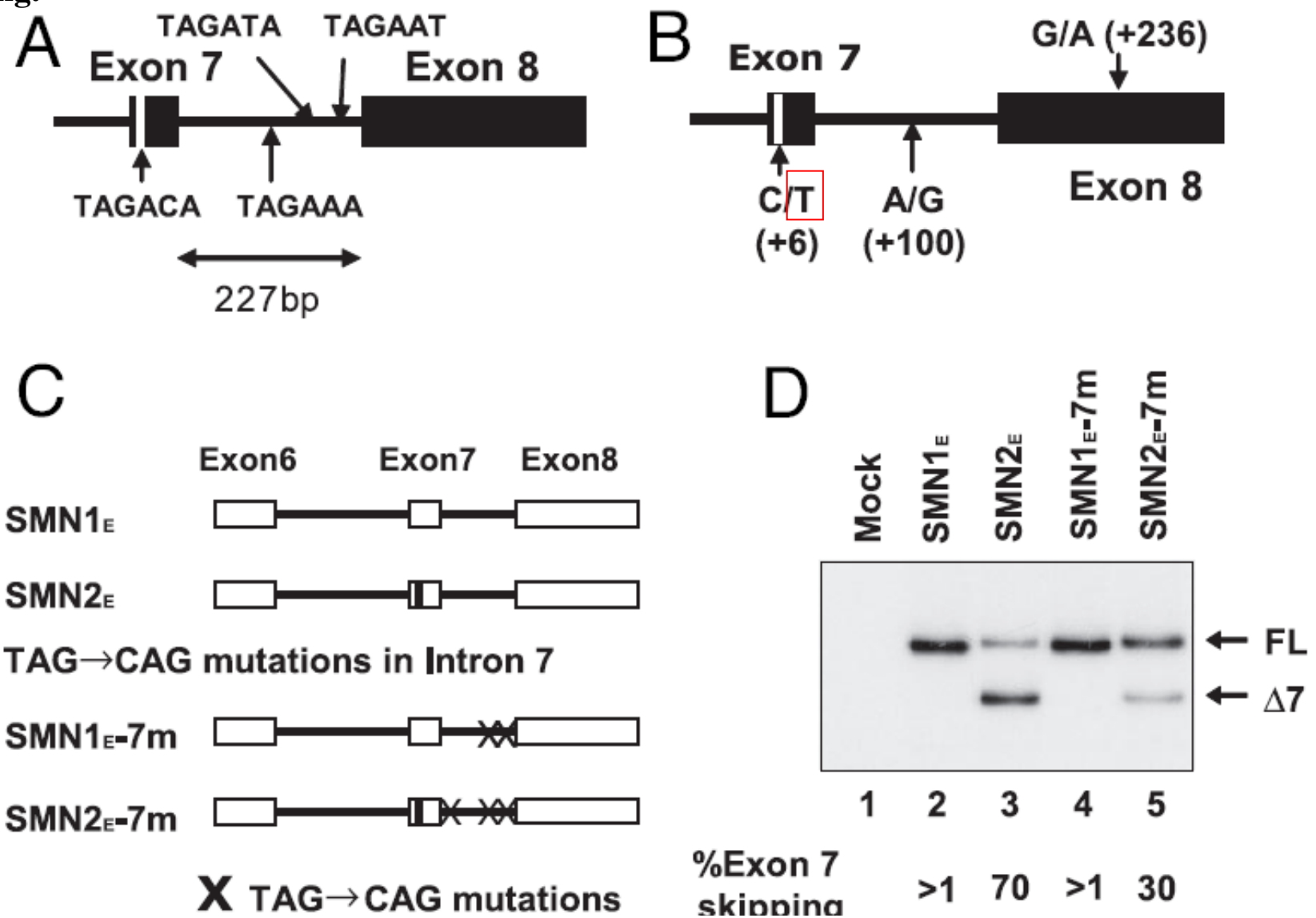
Sequenza consensus ESE



An intronic element contributes to splicing repression in spinal muscular atrophy PNAS07

- **The neurodegenerative disease spinal muscular atrophy is caused by mutation of the *survival motor neuron 1* (*SMN1*) gene.**
- ***SMN2* is a nearly identical copy of *SMN1* that is unable to prevent disease, because most *SMN2* transcripts lack exon 7 and thus produce a nonfunctional protein. A key cause of inefficient *SMN2* exon 7 splicing is a single nucleotide difference between *SMN1* and *SMN2* within exon 7.**
- **We provide evidence that this base change suppresses exon 7 splicing by creating an inhibitory element, a heterogeneous nuclear ribonucleoprotein (hnRNP) A1- dependent exonic splicing silencer. We find that another rare nucleotide difference between *SMN1* and *SMN2*, in intron 7, potentially creates a second *SMN2*-specific hnRNP A1 binding site.**
- **This single base change does indeed create a highaffinity hnRNP A1 binding site, and base substitutions that disrupt it restore exon 7 inclusion *in vivo* and prevent hnRNP A1 binding *in vitro*.**
- **<u>We propose that interactions between hnRNP A1 molecules bound to the exonic and intronic sites cooperate to exclude exon 7</u>**

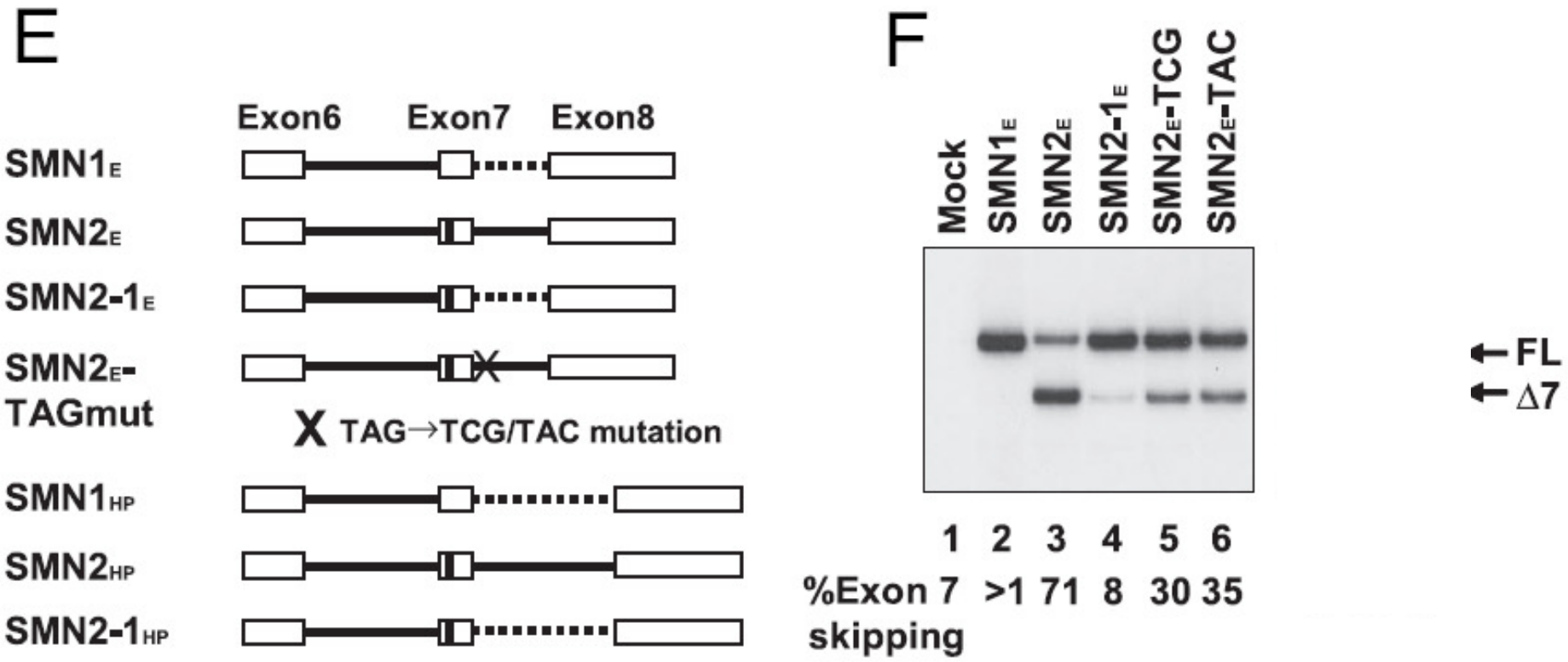
Disruption of consensus hnRNP A1 binding sites (TAGNNA/T) in intron 7 rescues exon 7 splicing.



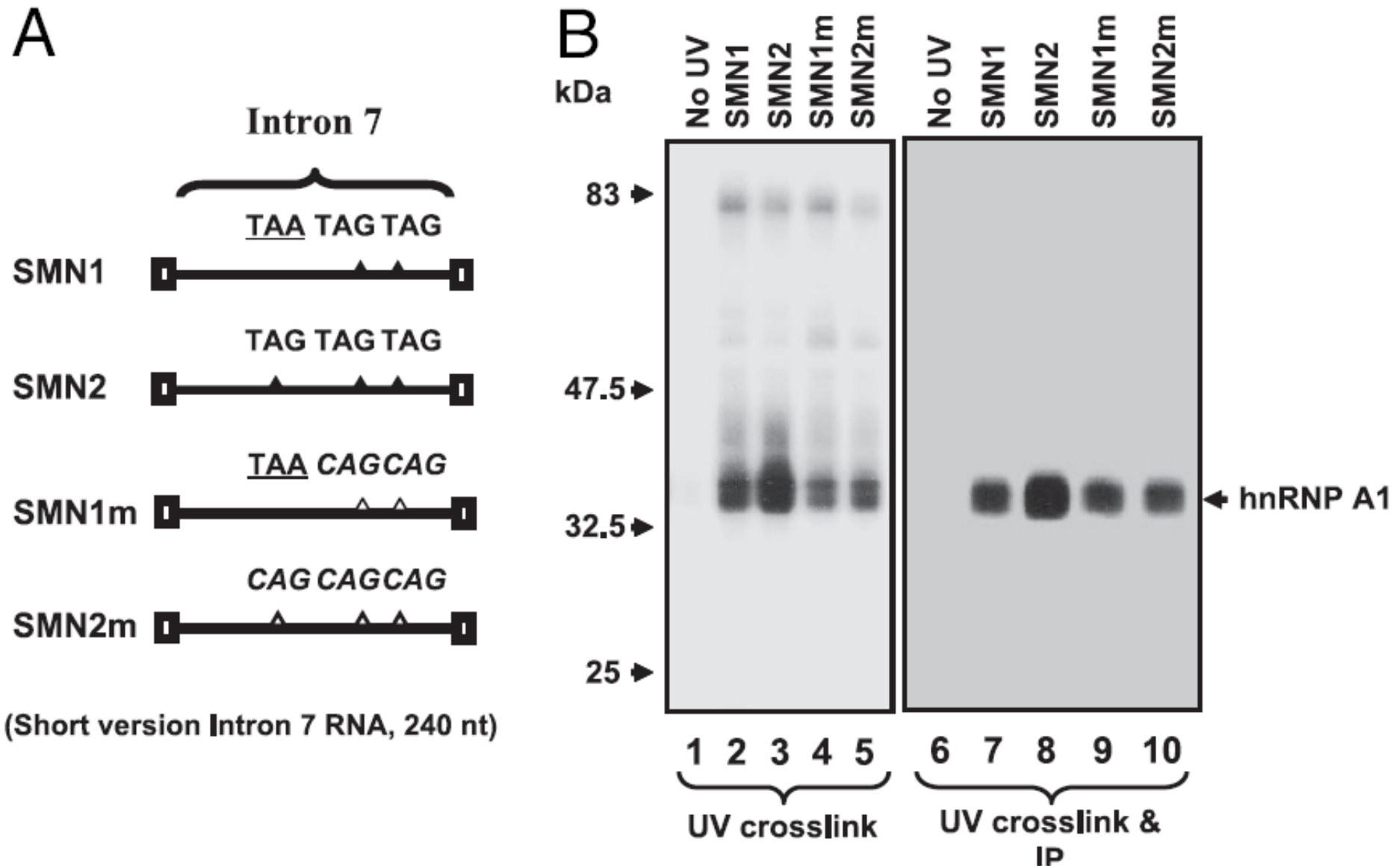
Disruption of consensus hnRNP A1 binding sites in intron 7 rescues exon 7 splicing.

- (*A*) Location and sequence of consensus hnRNP A1 binding sites (TAGNNA/T) in SMN1/2E constructs. *SMN1/2*-common consensus A1 sites are indicated above the diagram, and *SMN2*-specific consensus sites are indicated below the diagram.
- (*B*) Position of single nucleotide differences between *SMN1* and *SMN2* in the SMNE constructs. The exonic 6 C 3 T and intronic 100 A 3 G transitions are indicated below the diagram. Other nucleotide differences are shown above the diagram.
- (*C*) The schematic diagram shows the positions of the T 3 C mutations in the consensus hnRNP A1 sites in intron 7 of the SMN1/2E derivatives. Open boxes indicate exons, solid lines indicate introns, and the vertical thick line in exon 7 denotes the ESS. Crosses indicate positions of mutated consensus A1 sites.
- (*D*) RNA was prepared after 48 h transfection of 293 cells and analyzed by quantitative RT-PCR in the presence of [32P]dCTP. The position of full-length (FL) and exon 7-excluded (7) PCR products are indicated on the right.
- The percentage of exon 7 skipping is indicated below.

Disruption of consensus hnRNP A1 binding sites in intron 7 rescues exon 7 splicing.



- (*E*) Diagram of mutant and intron 7 ‘‘swap’’ constructs. Open boxes indicate exons, dotted lines indicate *SMN1* introns, solid lines indicate *SMN2* introns, and the vertical line in exon 7 denotes the position of ESS. The cross indicates TAG mutations at position 100 in intron 7 in the SMN2E construct.
- (*F*) SMN1/2° and derivative plasmids (4 g) were transfected into 293 cells. RNAs was prepared after 48 h and analyzed by quantitative RT-PCR. Positions of fulllength (FL) and exon 7-excluded (7) PCR products are indicated on the right. The percentage of exon 7 skipping for each sample is indicated below.


A
Intron 7
TAA TAG TAG
SMN1
TAG TAG TAG
SMN2
TAA CAGCAG
SMN1m
CAG CAGCAG
SMN2m
(Short version Intron 7 RNA, 240 nt)
B
kDa
No UV
SMN1
SMN2
SMN1m
SMN2m
No UV
SMN1
SMN2
SMN1m
SMN2m
83
47.5
32.5
25
hnRNP A1
1 2 3 4 5
6 7 8 9 10
UV crosslink
UV crosslink &
IP

hnRNP A1 in HeLa Nuclear Extract (NE) UV-crosslinks preferentially to *SMN2* intron 7RNA.

- (*A*) Diagram of intron 7 RNAs used in UV crosslinking and immunoprecipitation assays. Open boxes indicate exons and solid lines indicate intron 7. Solid triangles indicate consensus A1 sites, and open triangles indicate locations of TAG3CAG mutations.
- (*B*) (*Left*) *SMN1* (lane 1) and *SMN2* (lane 2) wild type and mutant (lanes 3 lane 4, respectively) intron 7 RNAs were incubated with HeLa NE, crosslinked, and analyzed by SDS/PAGE.
- (*Right*) UV crosslinked proteins were immunoprecipitated with anti-hnRNP A1 antibodies (lanes 6–10) and analyzed by SDS/PAGE. The position of hnRNP A1 is indicated on the right, and the position of size marker proteins is indicated on the left.