A unique RNA structure formed by a long-distance interaction uncovers the therapeutic potential of a deep intronic sequence

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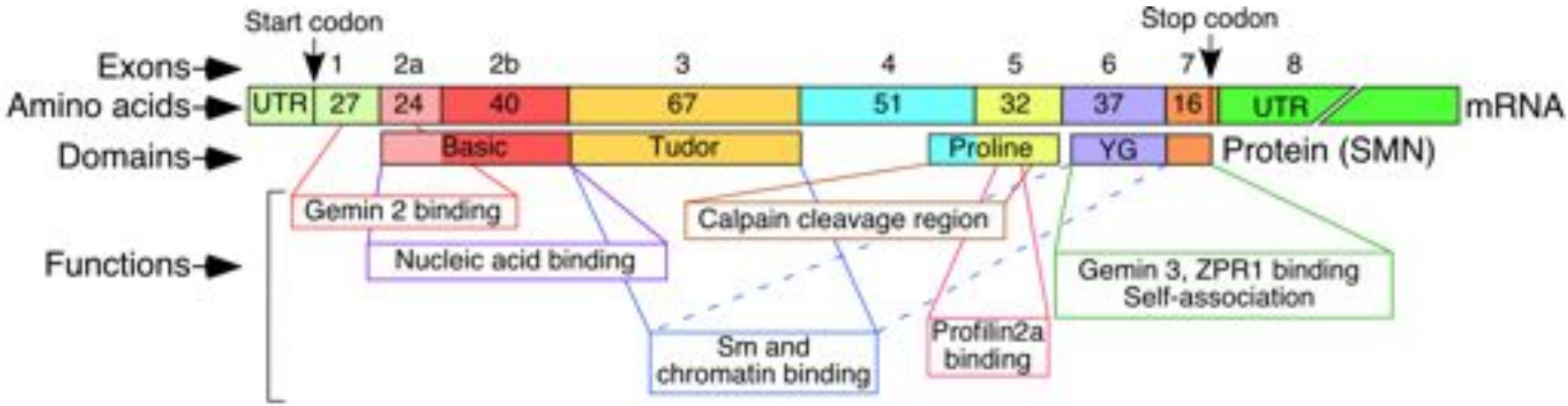
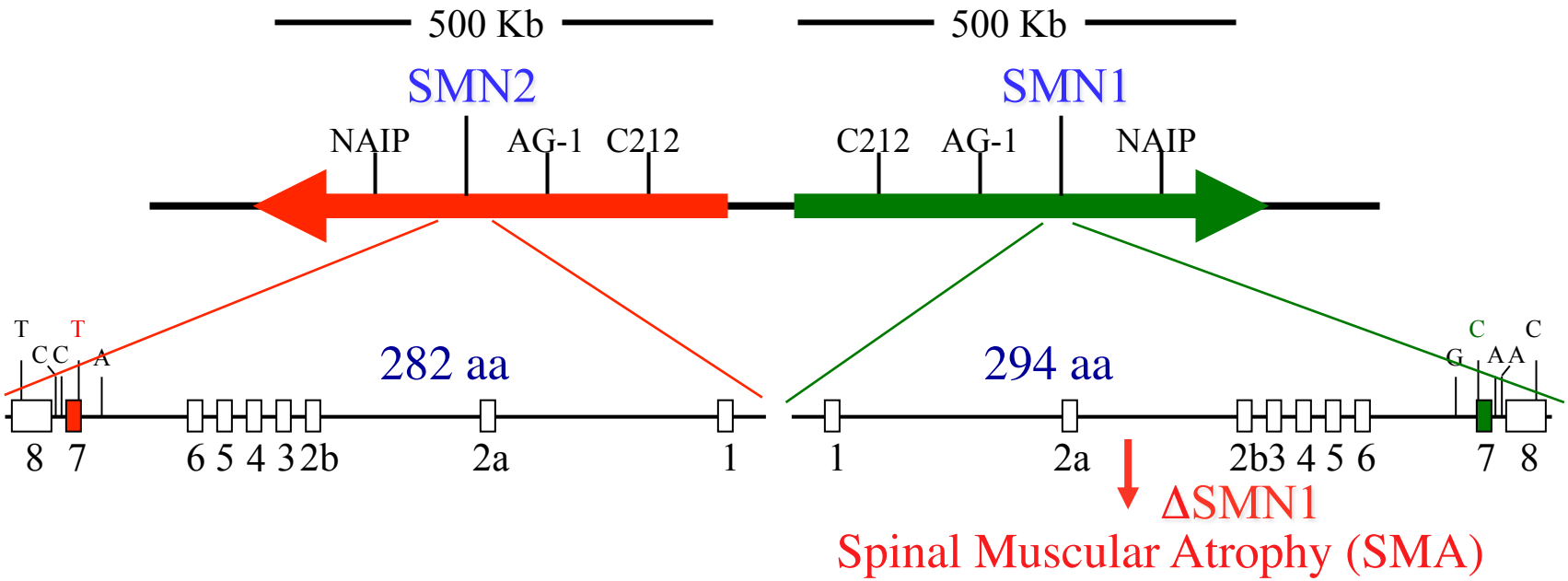
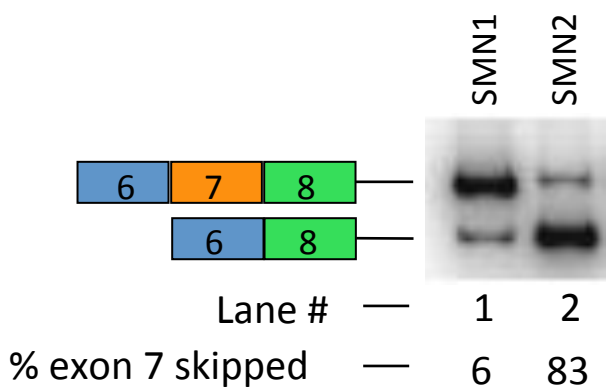
Organization of SMN protein



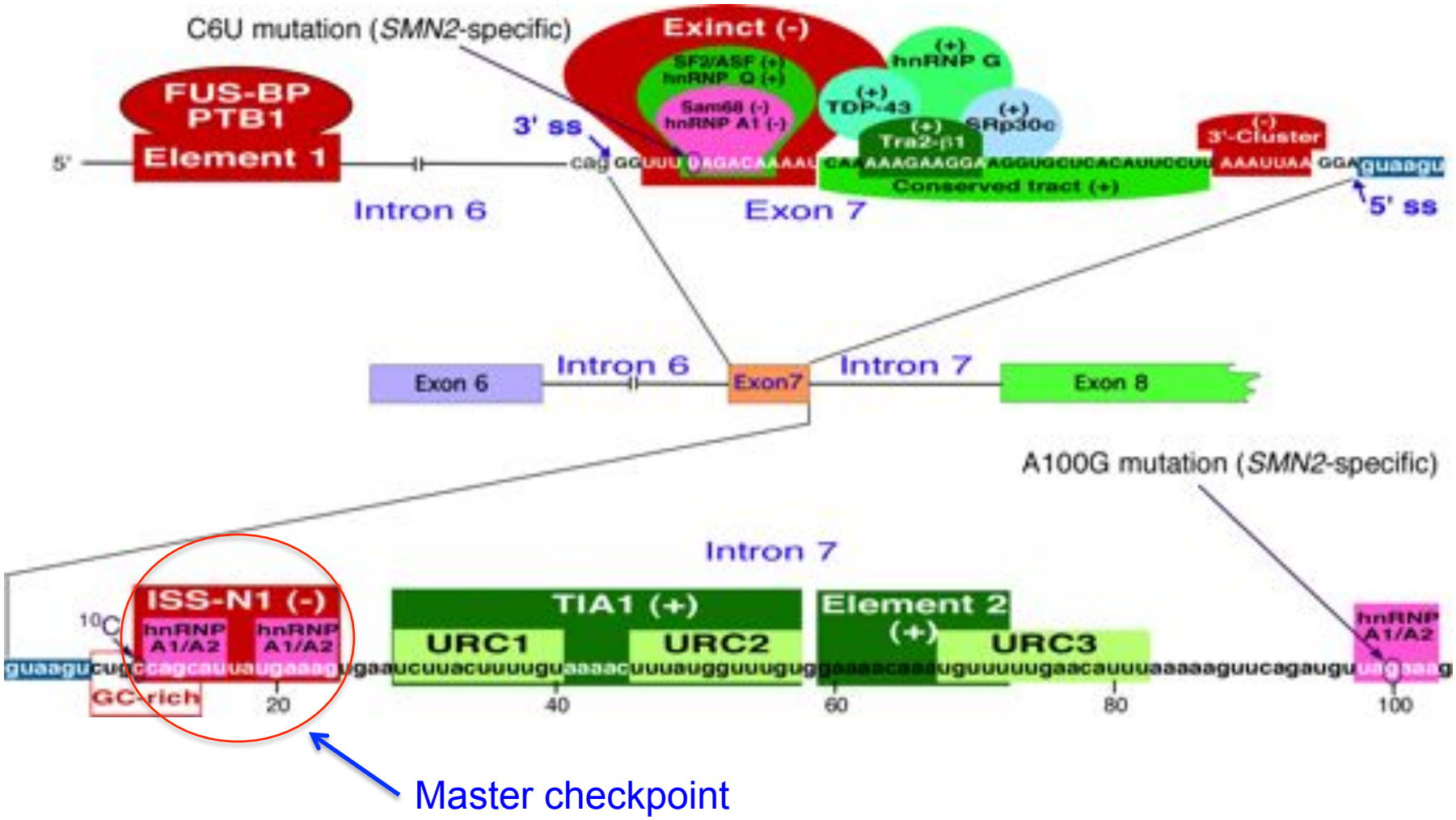
Howell et al., Future Med Chemistry, 6 (9), 1081-1099, 2014

Two *SMN* Genes as inverted repeats on 5q11-q13



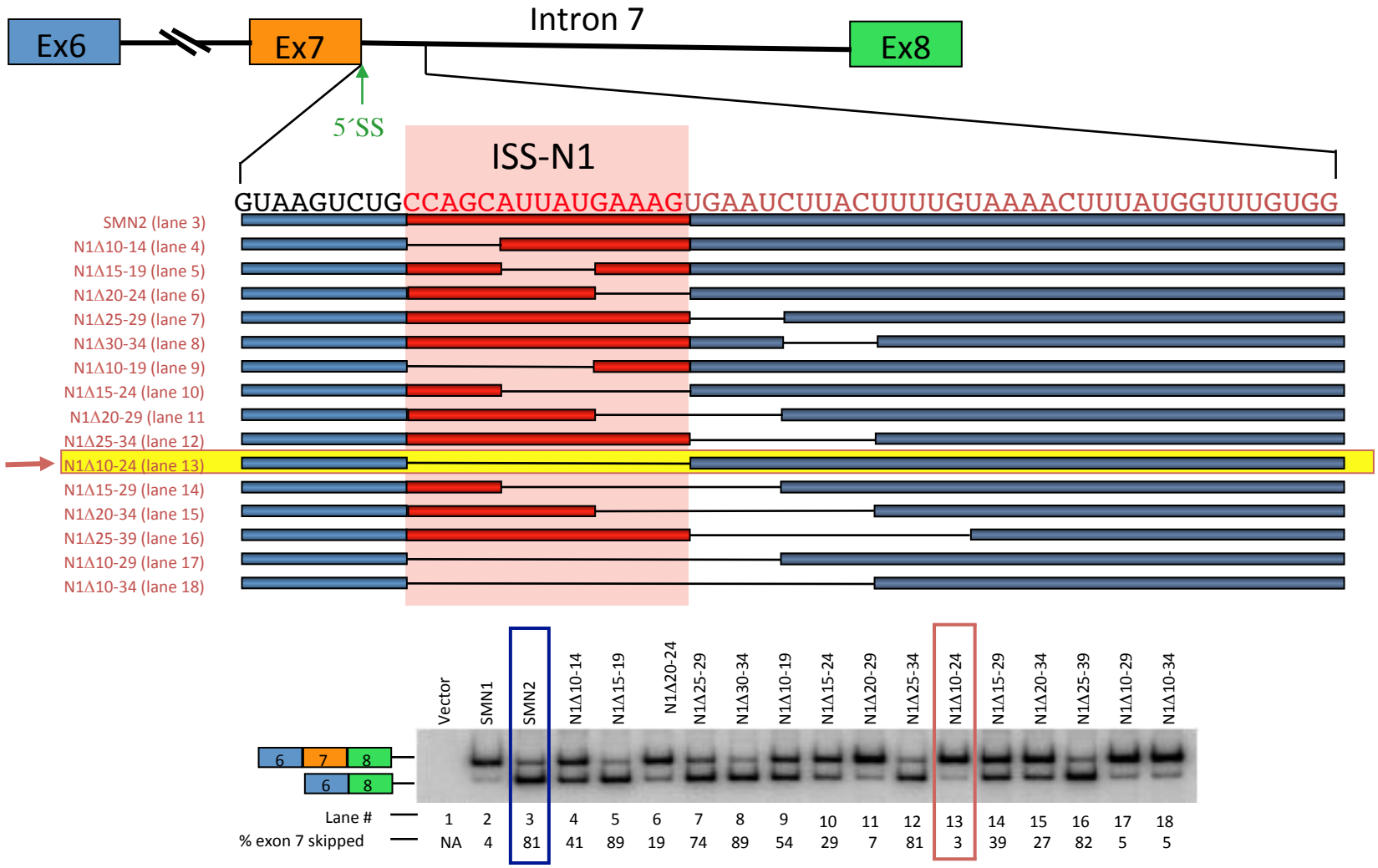


Regulatory elements of *SMN* pre-mRNA



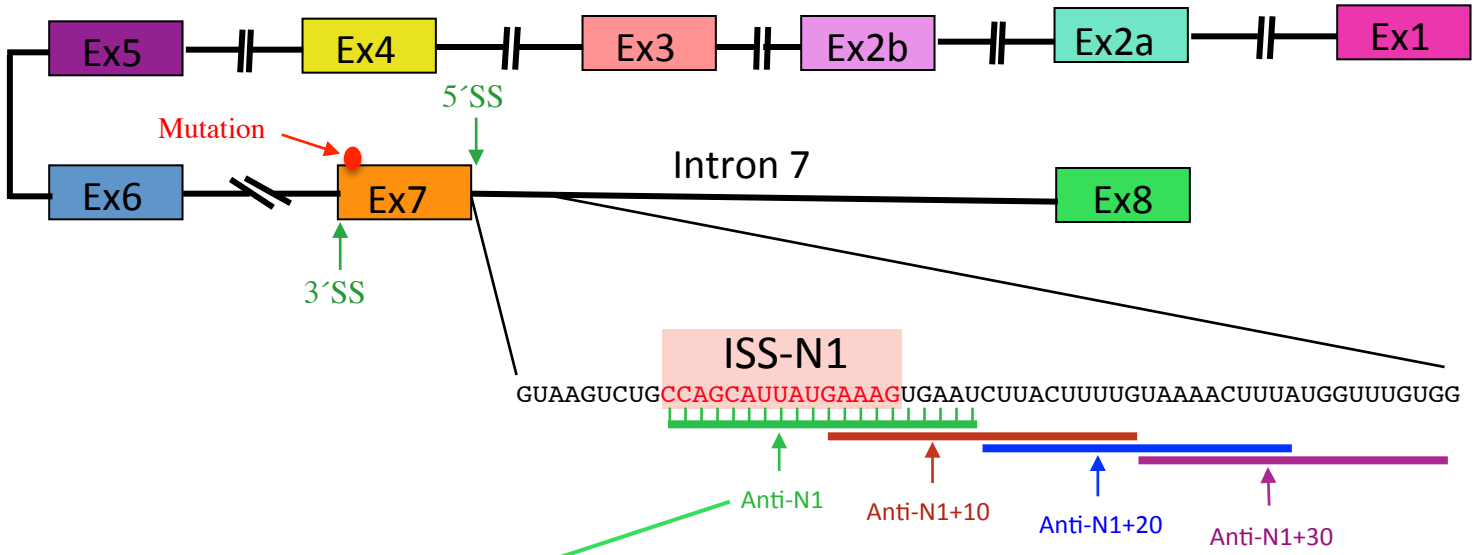
Howell et al., Future Med Chemistry, 6 (9), 1081-1099, 2014

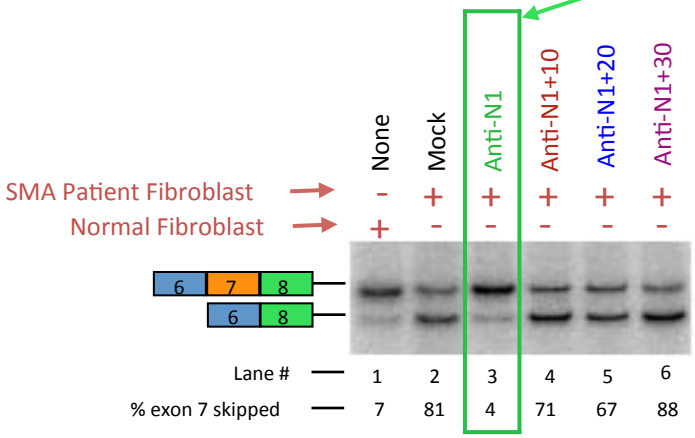
ISS-N1 is a strong intronic splicing silencer



Singh et al. Mol. Cell. Biol. 26, 1333-1346, 2006

ISS-N1 is a target for an antisense oligonucleotide (ASO)-mediated correction of *SMN2* exon 7 splicing





US Patent # US 2007/0292408

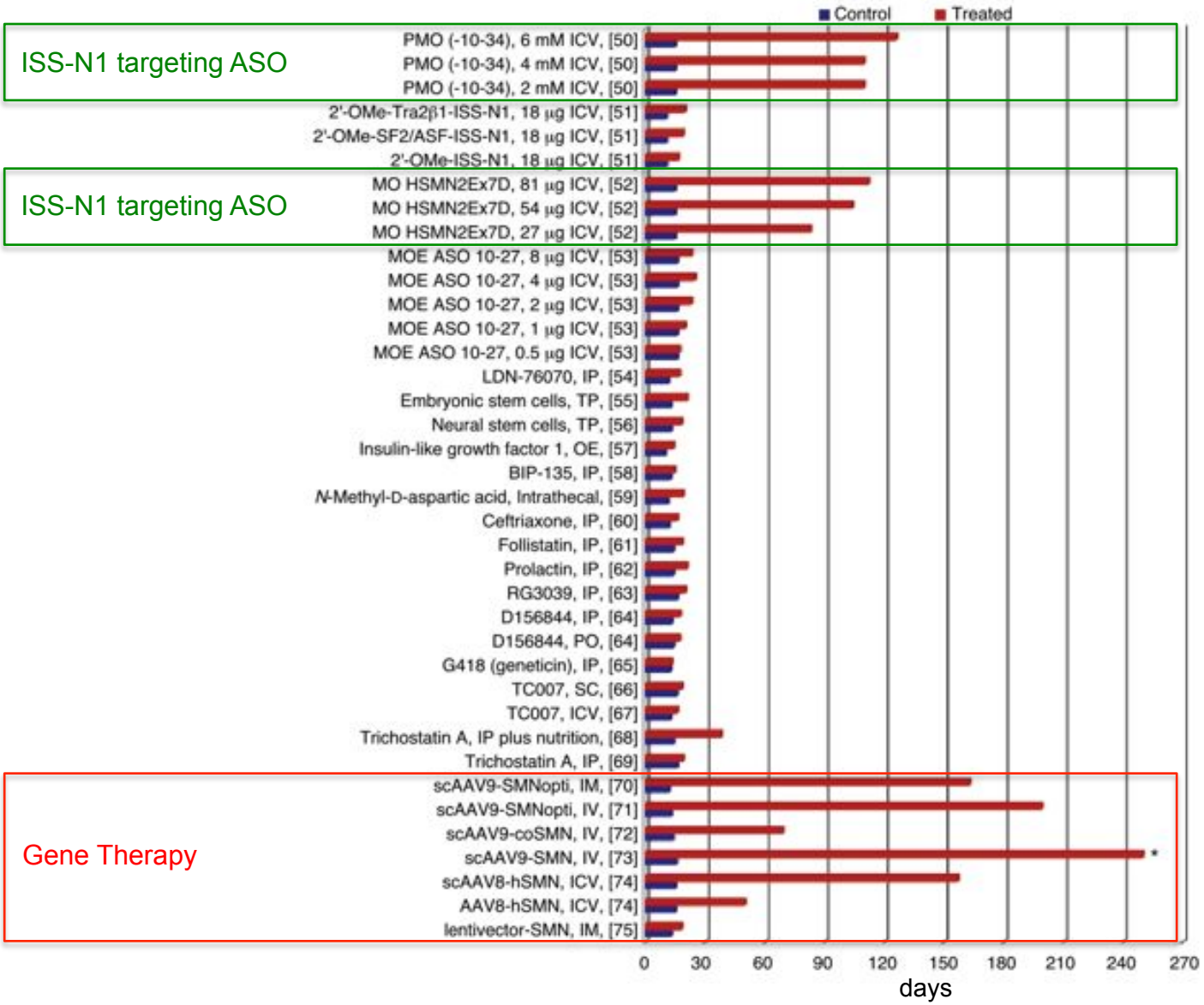
Singh et al. Mol. Cell. Biol. 26, 1333-1346, 2006

Studies with ISS-N1-targeting ASOs

ASO/Chemistry	Annealing positions	Company	Animal Model	References
2'-OMe	ISS-N1 / URC1			
Target	5'-ccagcauuaugaaagugaaucuuacuuuugu-3'			
Anti-N1	3'-GGUCGUAAUACUUUCACUUA-5'	Dharmacon	NA	Singh et al., 2006, Mol. Cell. Biol.
SMA AO	3'-GGUCGUAAUACUUUCACUUA-5'	TriLink	Δ7-SMA	Williams et al., 2009, J. Neurosci.
ISS-N1	3'-GGUCGUAAUACUUUCACUUA-5'	IDT	Δ7-SMA	Osman et al., 2012, Mol. Ther.
MOE	ISS-N1 / URC1			
Target	5'-ccagcauuaugaaagugaaucuuacuuuugu-3'			
ASO 10-27	3'-GGTCGTAATACTTTCACT-5'	ISIS	NA	Hua et al., 2008, Am. J. Hum. Genet.
ASO 10-27	3'-GGTCGTAATACTTTCACT-5'	ISIS	Mild-TW	Hua et al., 2010, Genes Dev.
ASO 10-27	3'-GGTCGTAATACTTTCACT-5'	ISIS	Δ7-SMA	Passini et al., 2011, Sci. Transl. Med.
ASO 10-27	3'-GGTCGTAATACTTTCACT-5'	ISIS	NHP	Passini et al., 2011, Sci. Transl. Med.
ASO 10-27	3'-GGTCGTAATACTTTCACT-5'	ISIS	Severe-TW	Nature et al., 2011, Nature
Morpholino	ISS-N1 / URC1			
Target	5'-ccagcauuaugaaagugaaucuuacuuuugu-3'			
VMO25	3'-GGTCGTAATACTTTCACTTAGAATG-5'	Gene Tools	Severe-TW	Zhou et al., 2013, Hum. Gene Ther.
PMO25	3'-GGTCGTAATACTTTCACTTAGAATG-5'	Gene Tools	Severe-TW	Zhou et al., 2013, Hum. Gene Ther.
PMO20	3'-GGTCGTAATACTTTCACTTA-5'	Gene Tools	Severe-TW	Zhou et al., 2013, Hum. Gene Ther.
ISS-N1 MO	3'-GGTCGTAATACTTTCACTTA-5'	Gene Tools	Zebra fish	Hao et al., 2011, Mol. Neurodegener.
HSMN2Ex7D	3'-GGTCGTAATACTTTCACTTA-5'	Gene Tools	Δ7-SMA	Porensky et al., 2012, Hum. Mol. Genet.
PMO18	3'-GGTCGTAATACTTTCACT-5'	Gene Tools	Severe-TW	Zhou et al., 2013, Hum. Gene Ther.
Bifunctional (2'-OMe)	ISS-N1 / URC1			
Target	5'-ccagcauuaugaaagugaaucuuacuuuugu-3'			
hTra2β1-ISS-N1	3'-GGUCGUAAUACUUUCACUUAGGGAGGAAGGGA 5'-GAAGGAGGGAAGG	IDT	Δ7-SMA	Osman et al., 2012, Mol. Ther.
	ISS-N1 / URC1			
Target	5'-ccagcauuaugaaagugaaucuuacuuuugu-3'			
SF2/ASF-ISS-N1	3'-GGUCGUAAUACUUUCACUUAGGGAGCACACAG 5'-CACACGACACAC	IDT	Δ7-SMA	Osman et al., 2012, Mol. Ther.

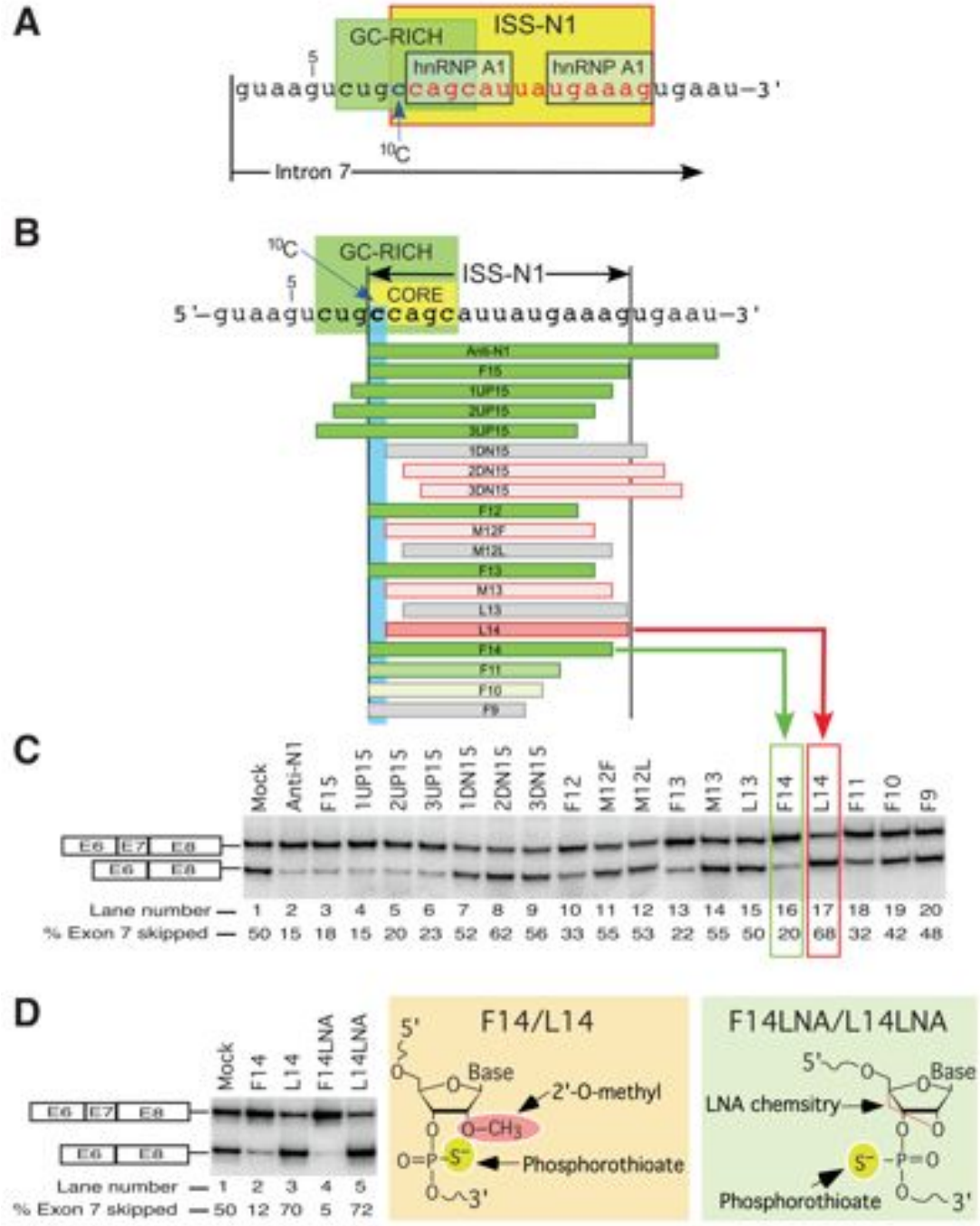
Sivanesan et al., Transl. Neurosci., 4, 1-7, 2013

Relative efficacy of drug candidates in Δ7SMA mice



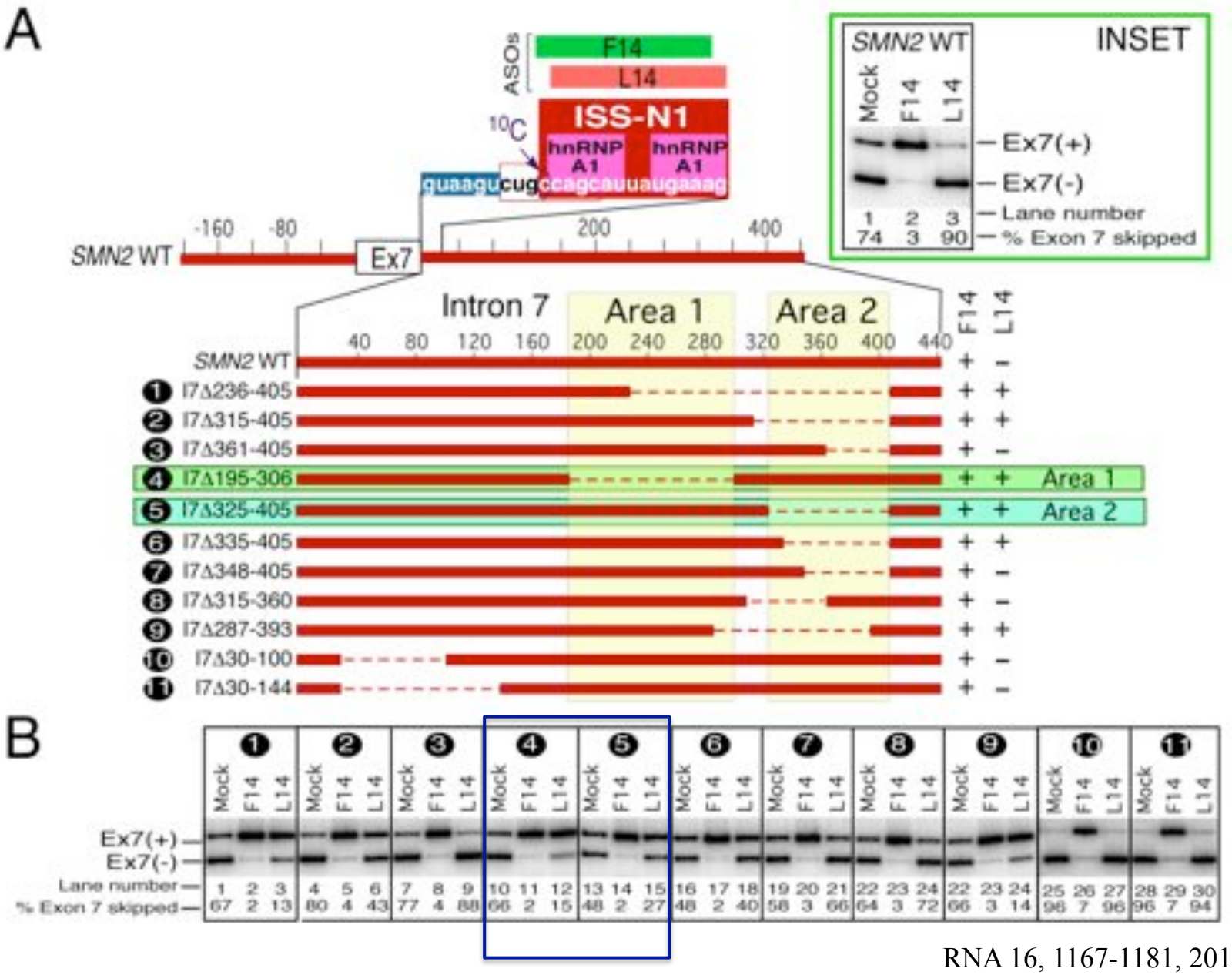
Seo et al., Biochim. Biophys Acta, 1126, 271-83 2013

Antagonistic response of two ASOs that bind to targets differing in a single nucleotide



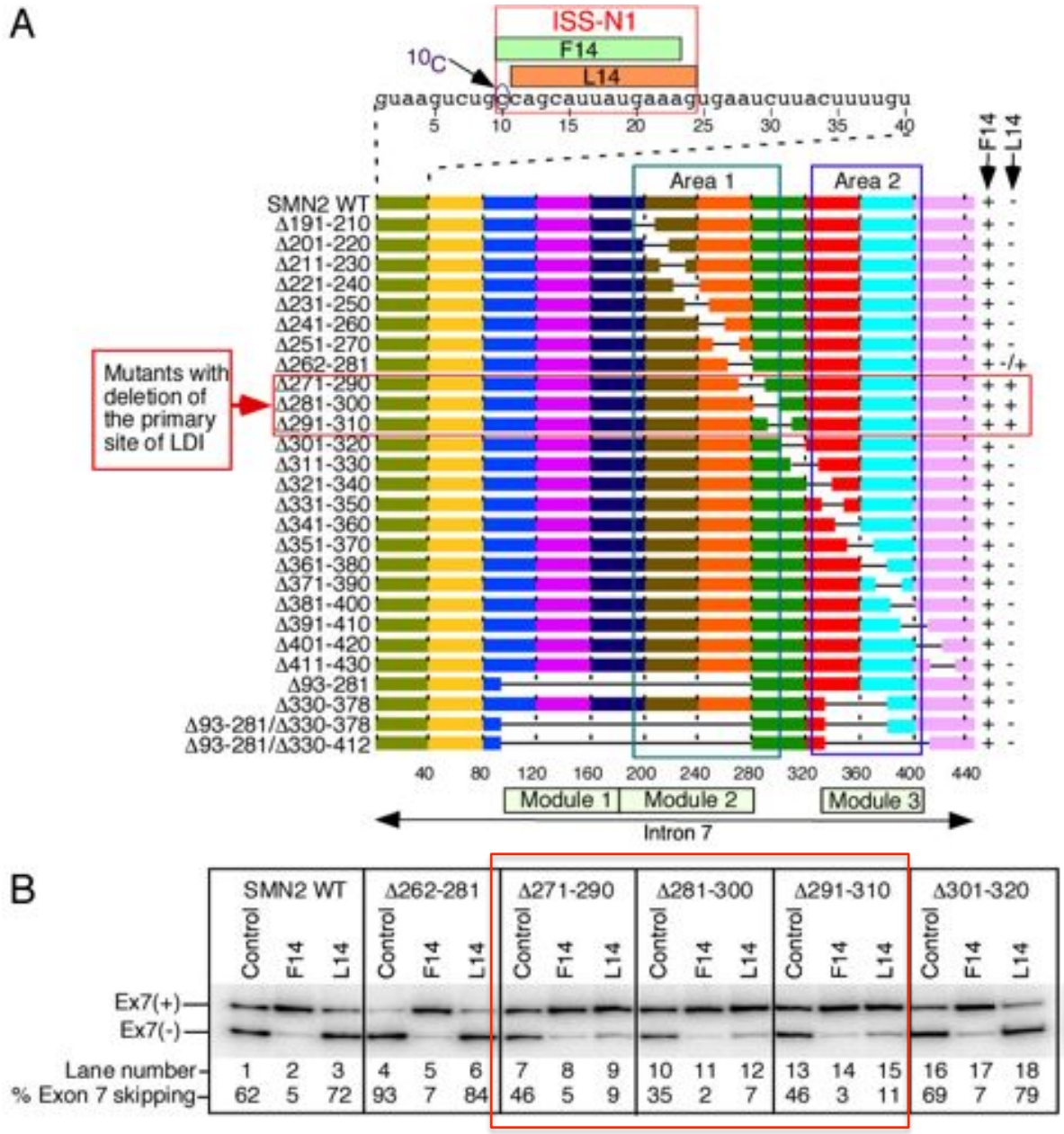
Singh et al., RNA 16, 1167-1181, 2010

ASO-based approach reveals a long-distance interaction (LDI)



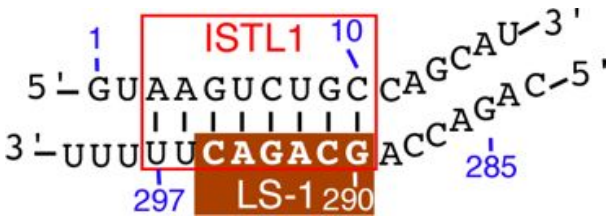
RNA 16, 1167-1181, 2010

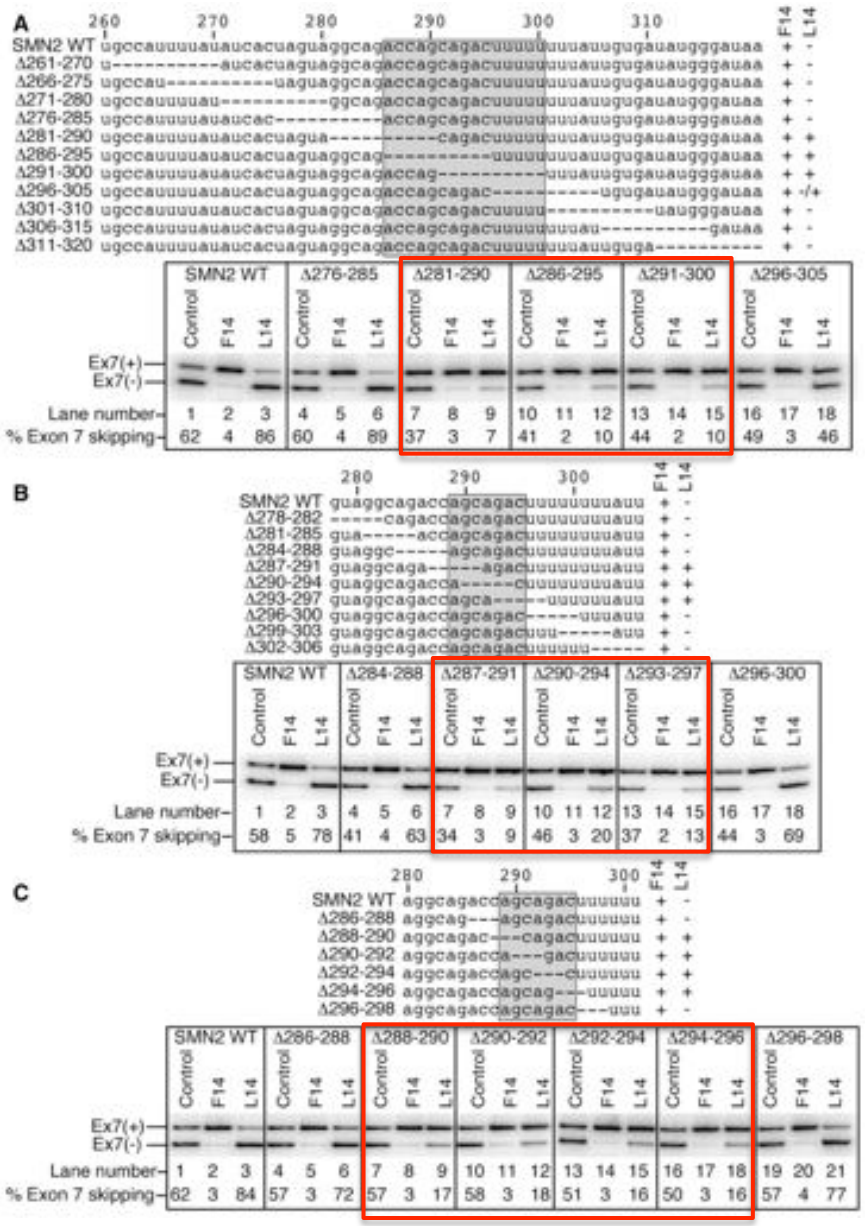
Primary site of LDI



Nucleic Acids Research 41, 8144-8165, 2013

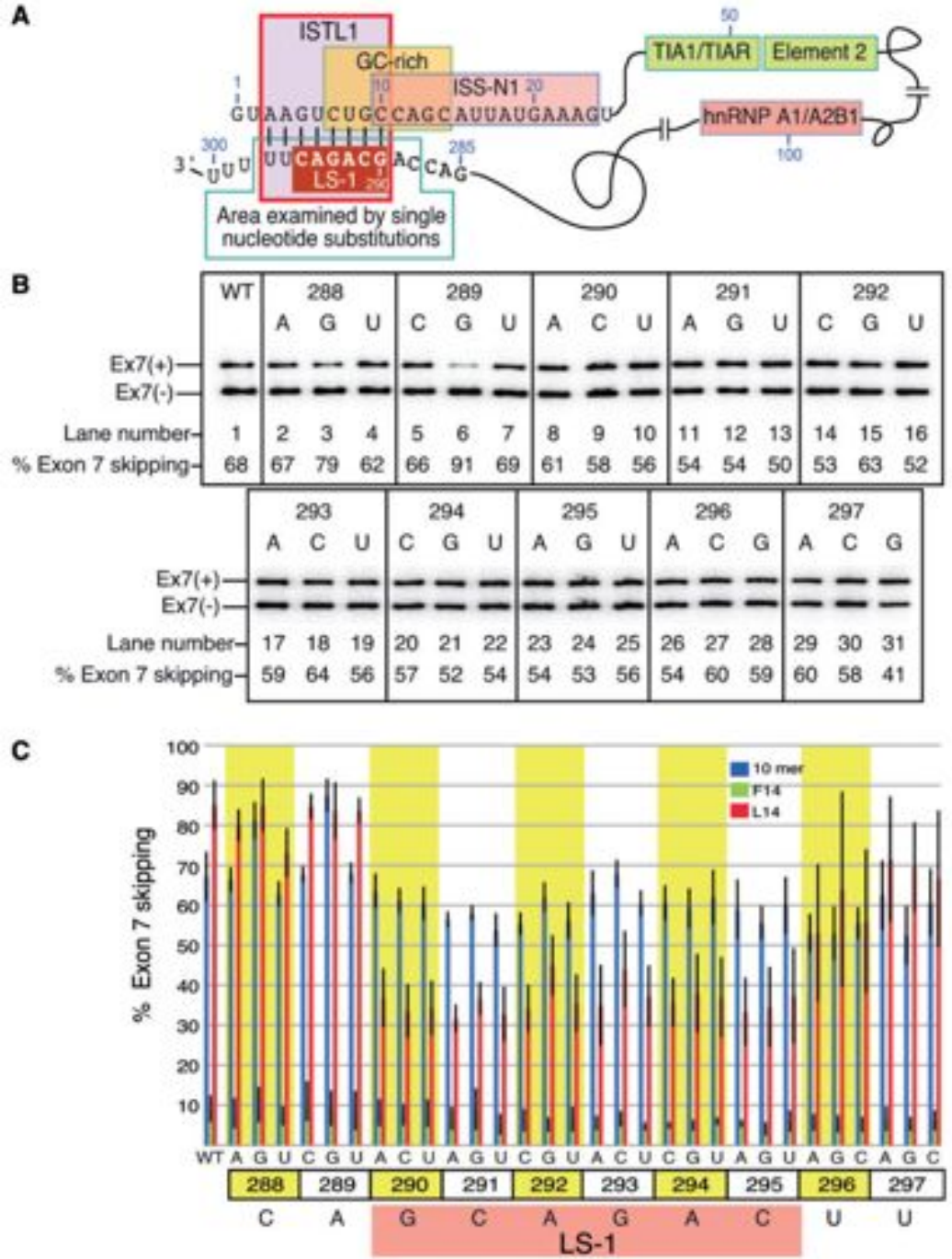
Smallest motif for LDI





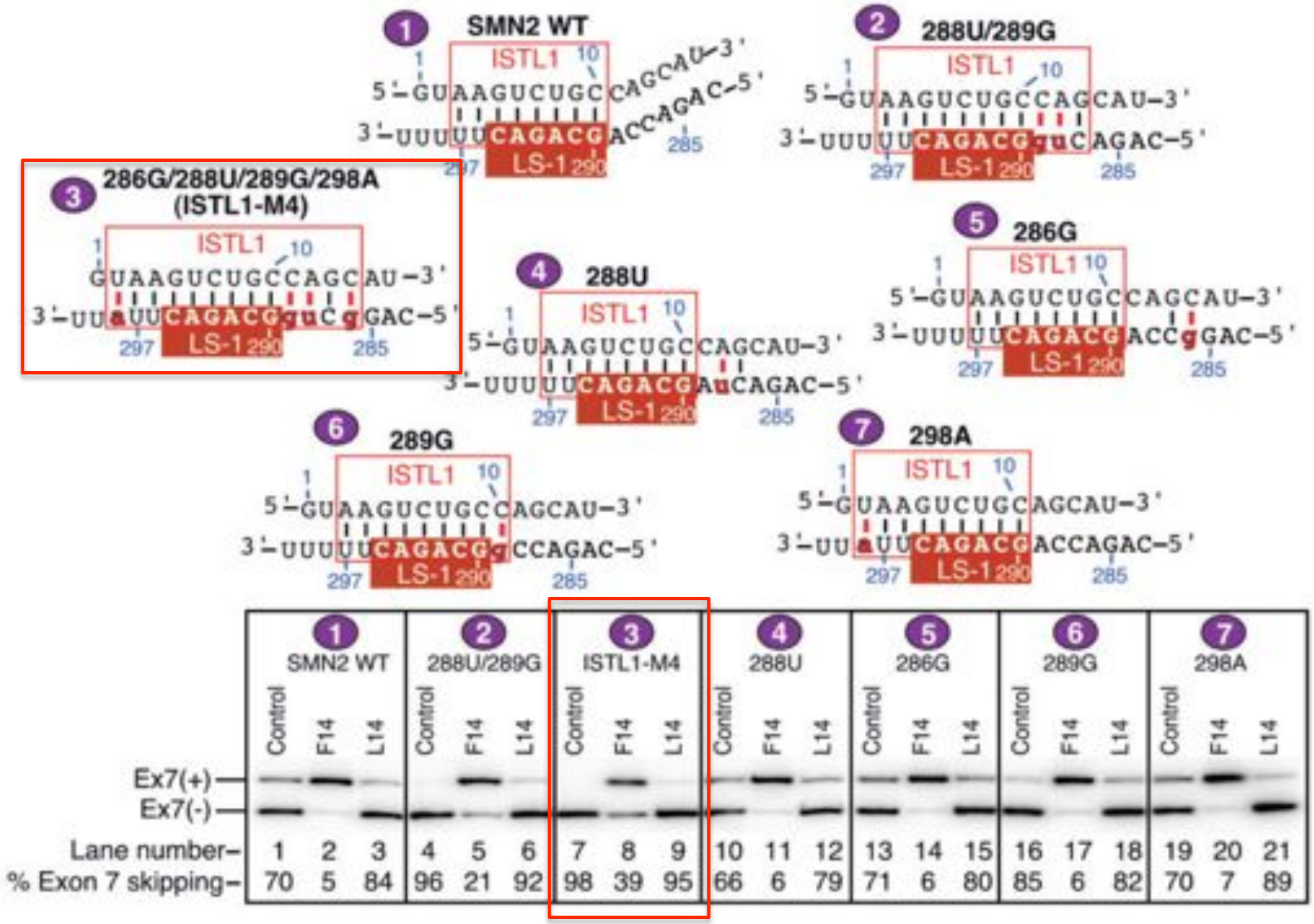
Nucleic Acids Research 41, 8144-8165, 2013

Effect of point mutations within the smallest motif for LDI



Nucleic Acids Research 41, 8144-8165, 2013

Effect of structure-associated mutations on LDI



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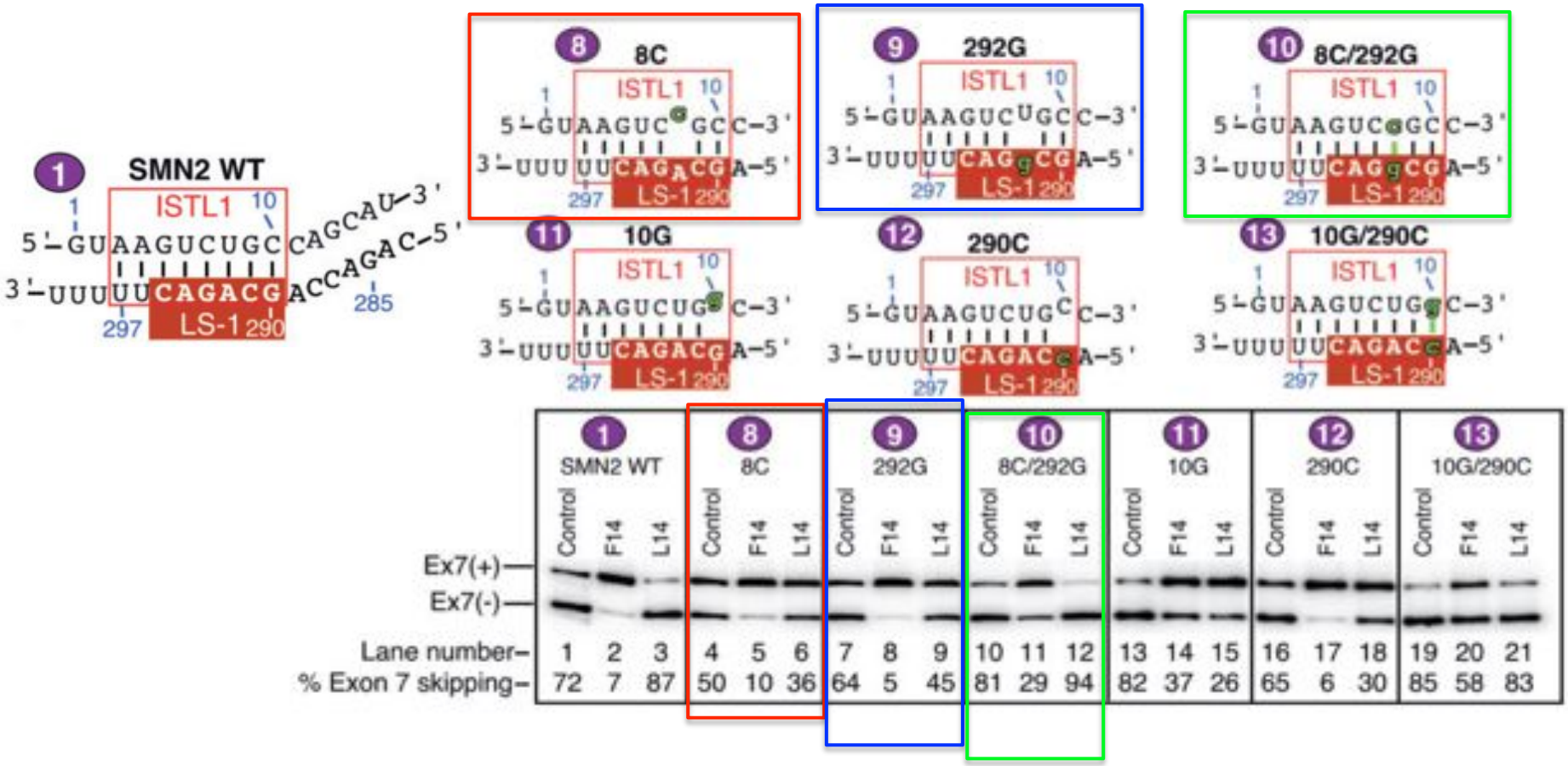
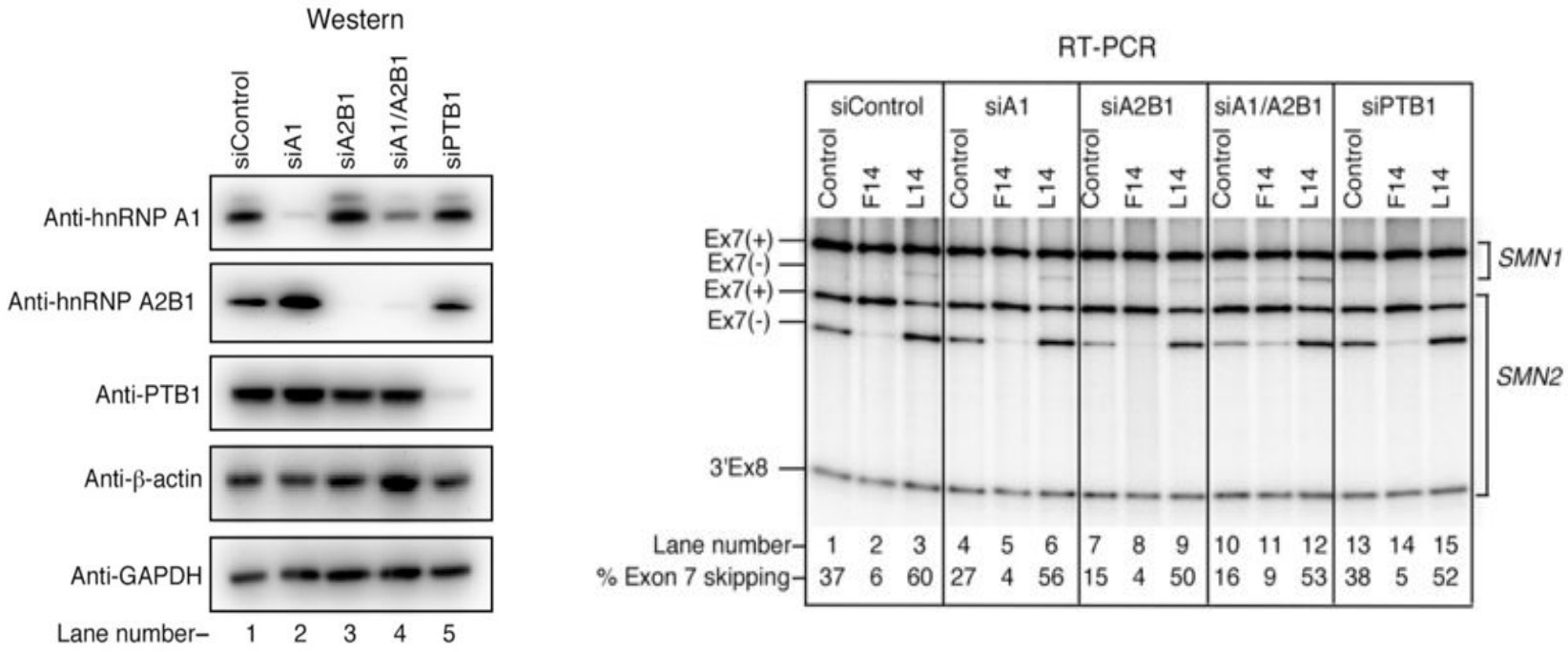
Effect of compensatory mutations on LDI



Nucleic Acids Research 41, 8144-8165, 2013

Effect of negative regulators on LDI



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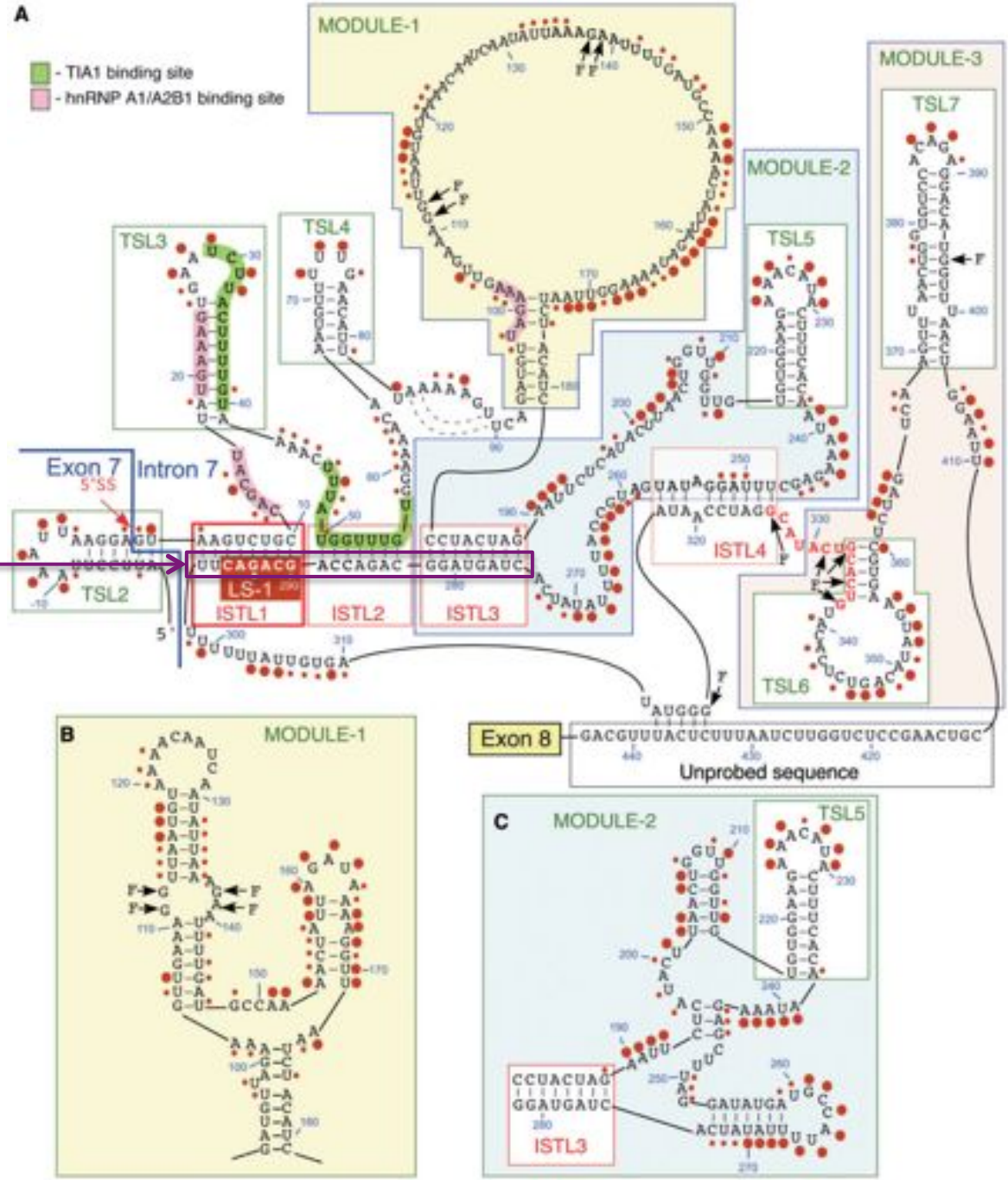
Structure of *SMN2* intron 7 (Probed by SHAPE)

ISS-N2



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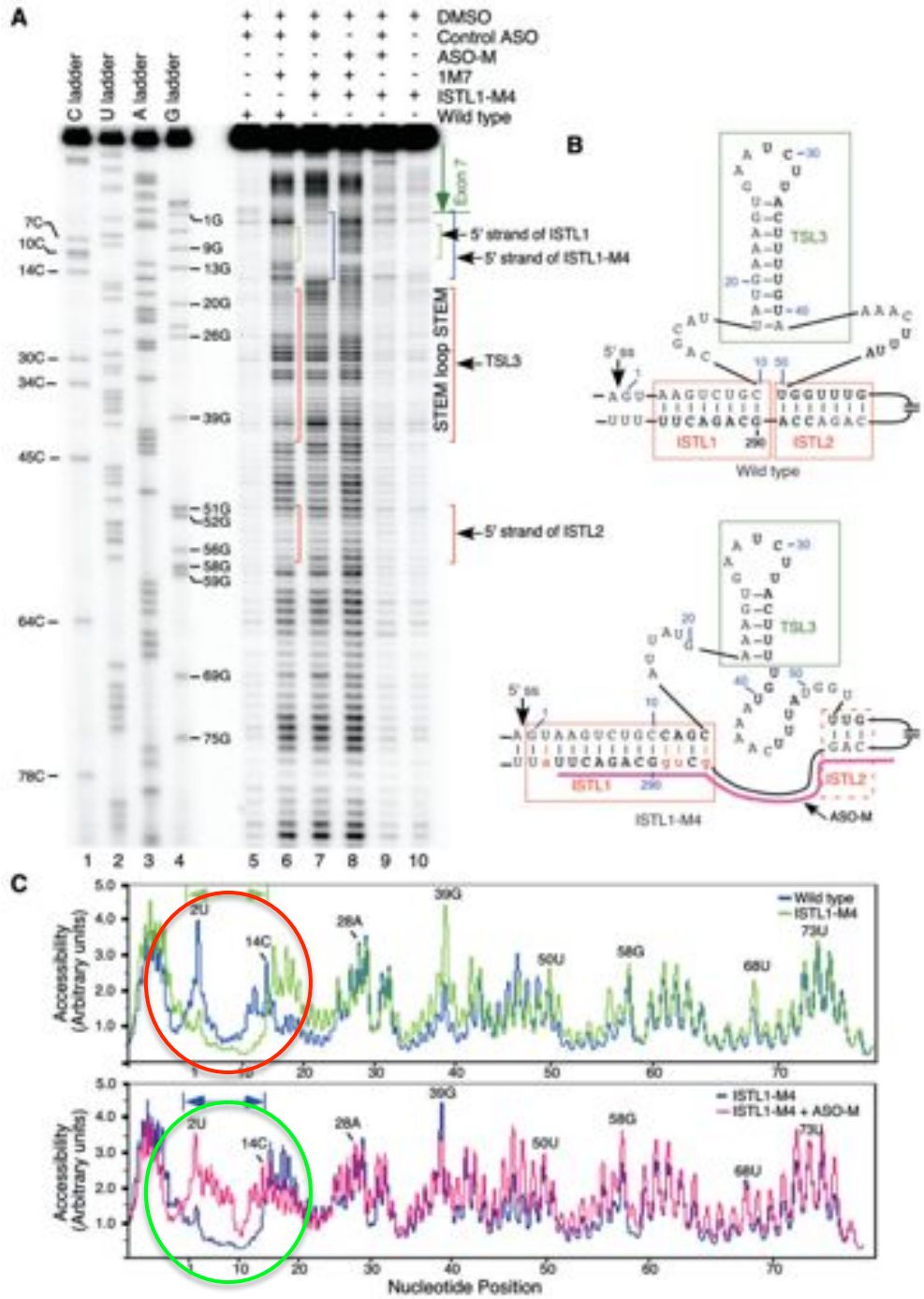
Validation of the 5′ strand of ISTL1



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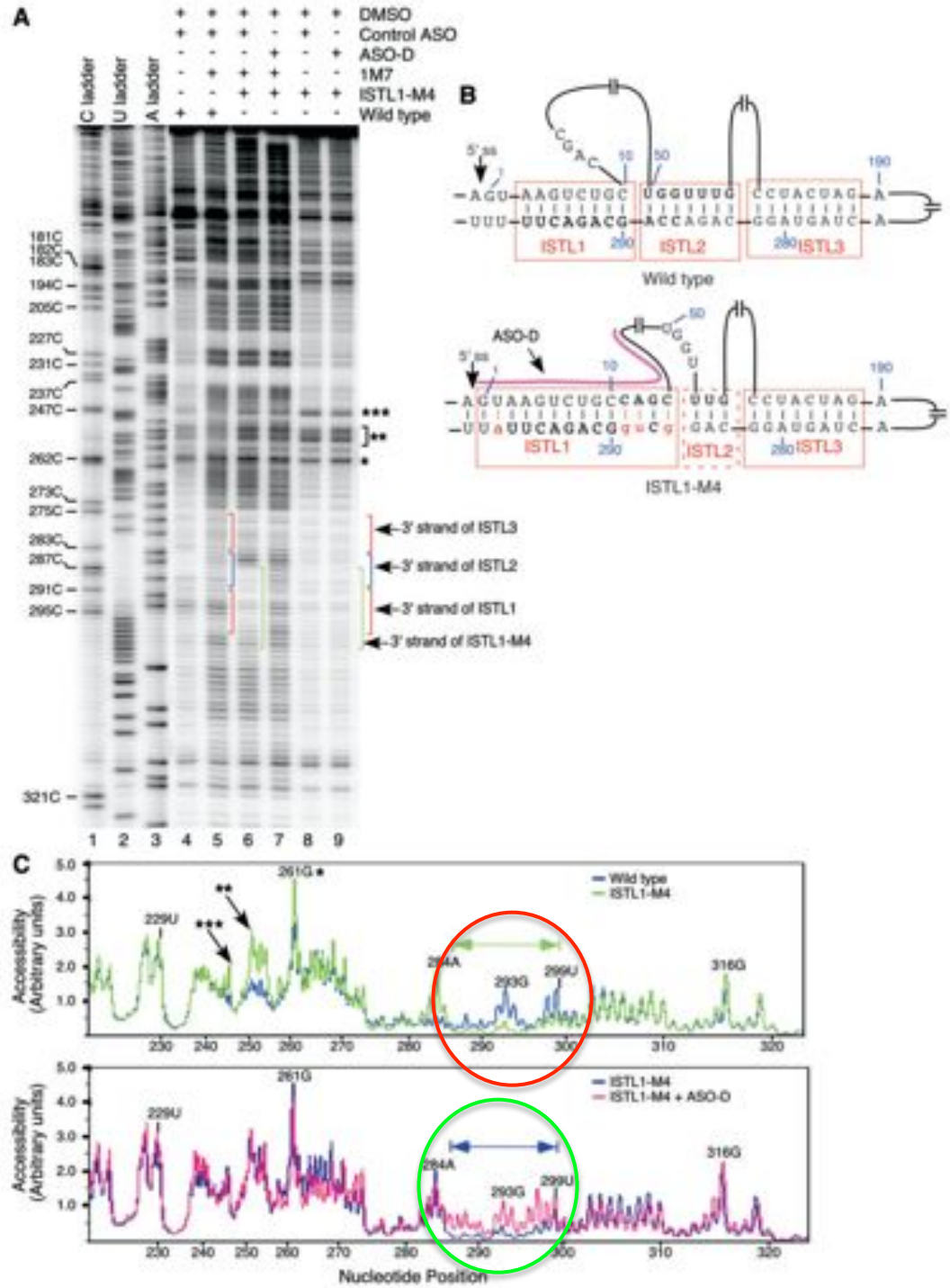
Validation of the 3′ strand of ISTL1 formation



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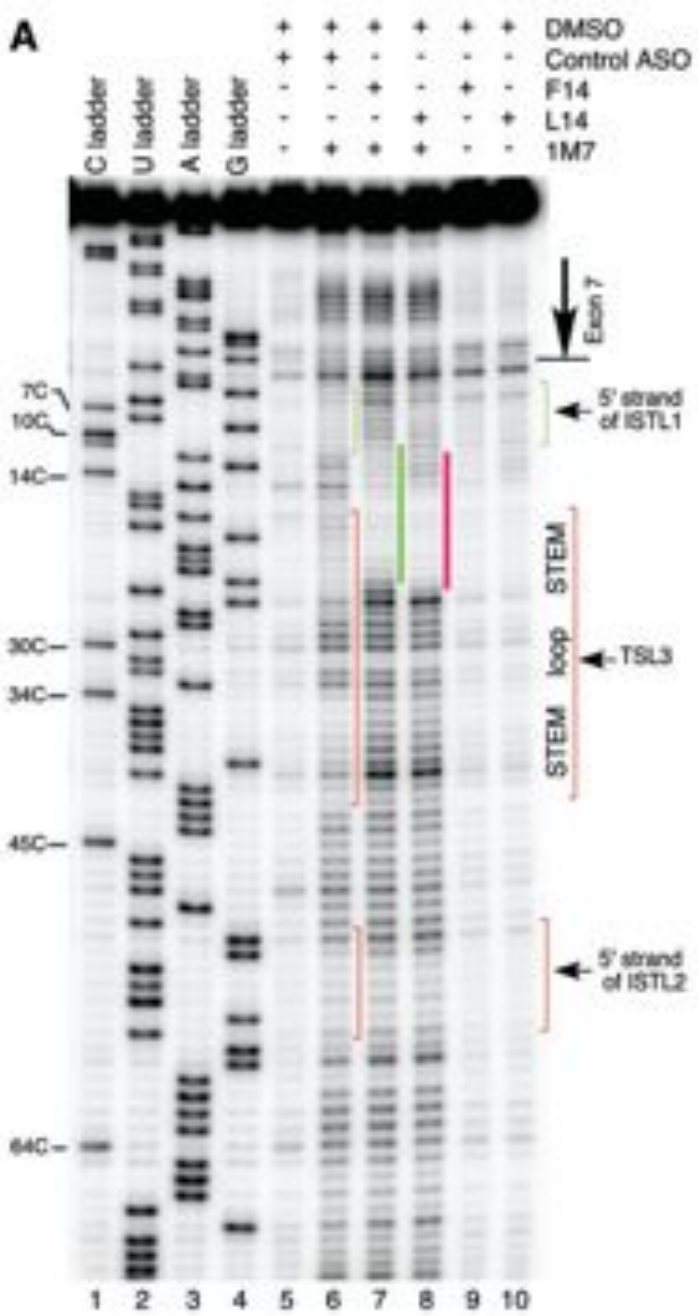
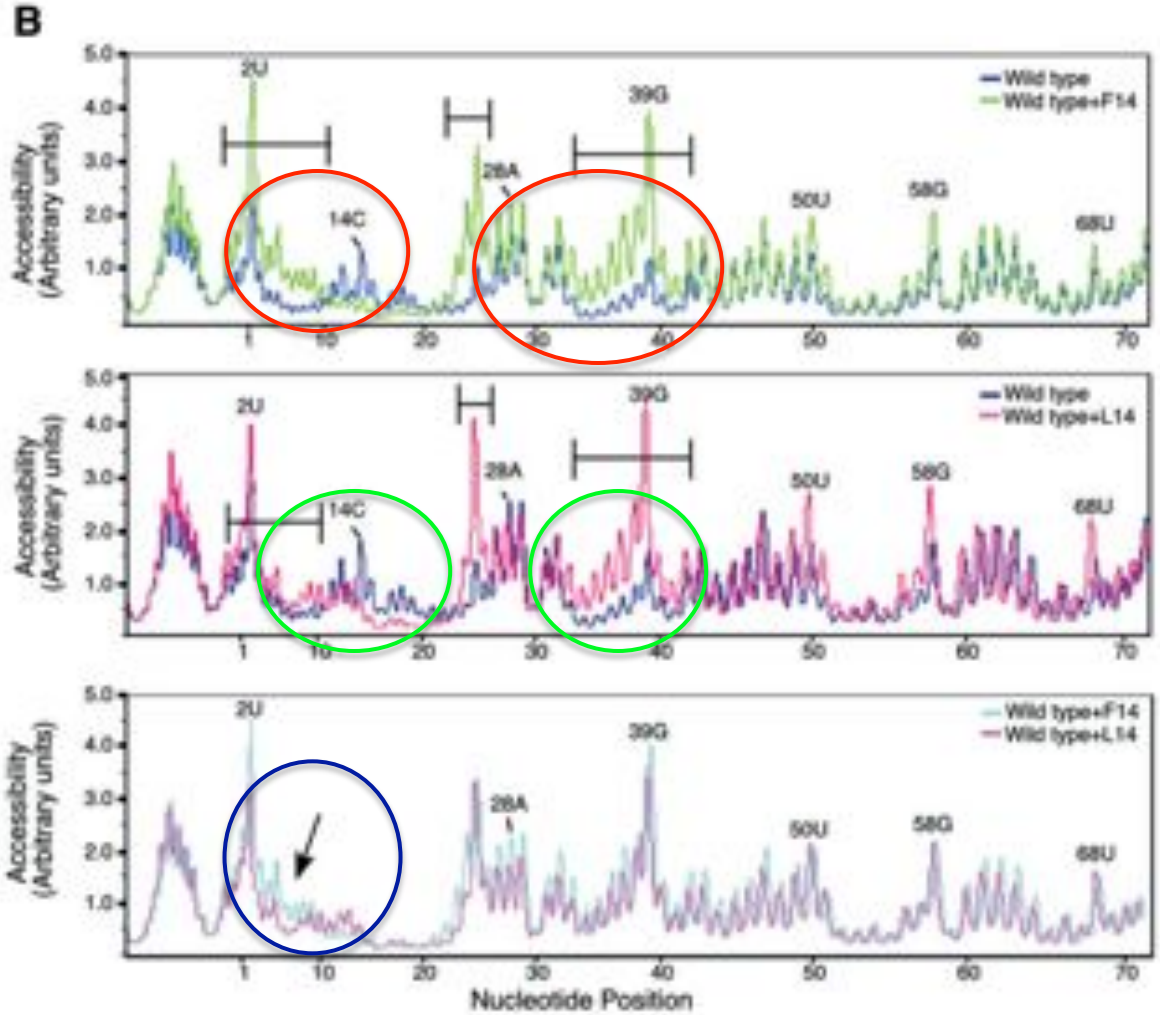
Effect of L14 and F14 on RNA secondary structure of *SMN2* intron 7





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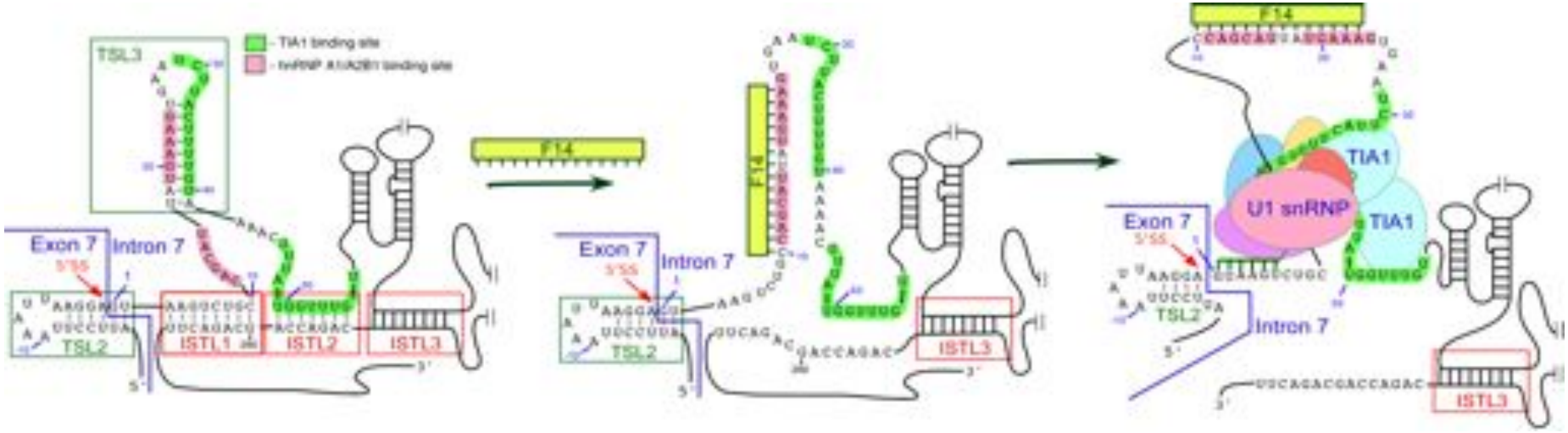
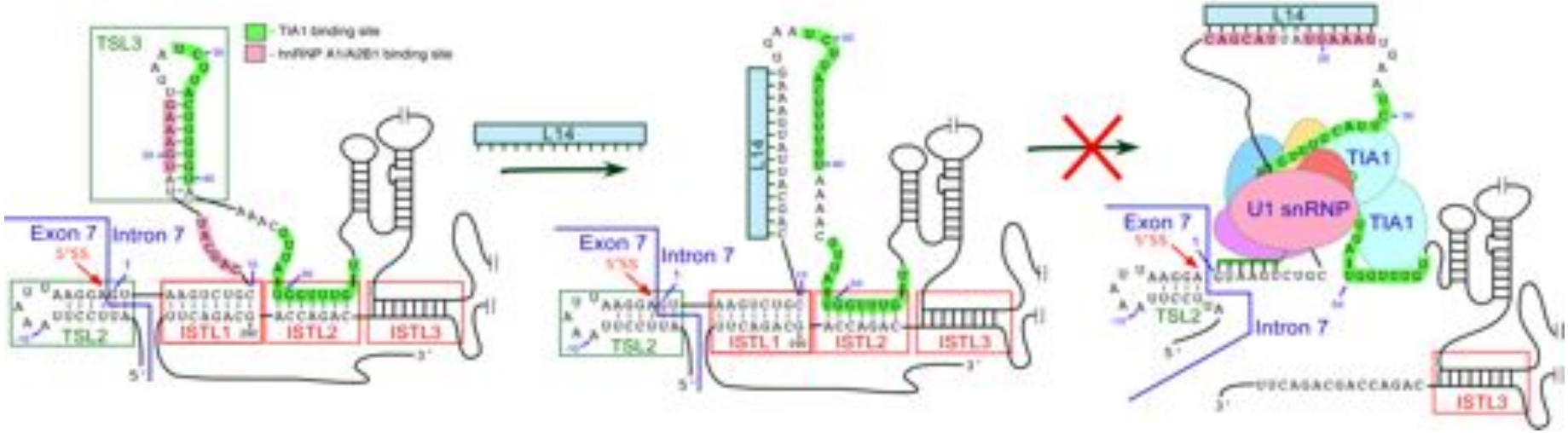
Structural basis of the effect of ASOs

F14 pathway ➔ Exon 7 inclusion



L14 pathway ➔ Exon 7 exclusion



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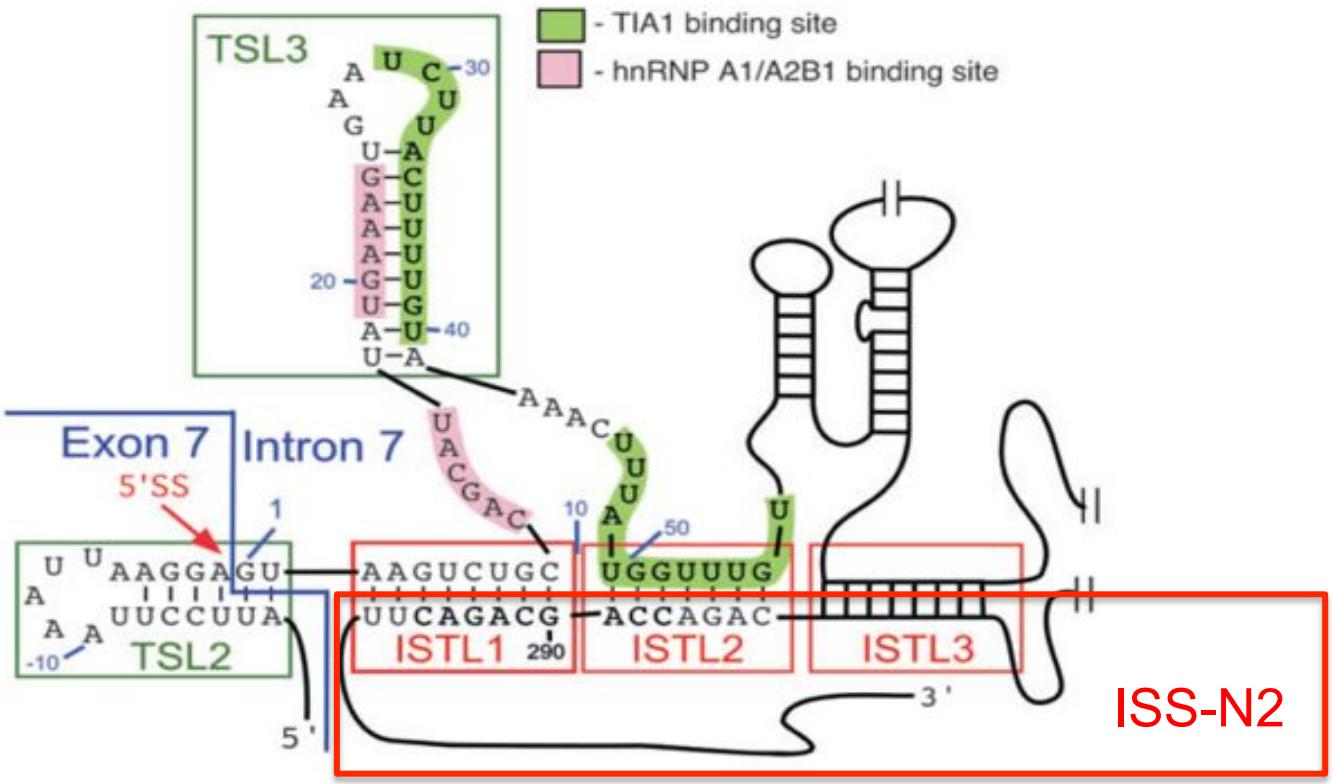
Is ISS-N2 a new antisense target ?



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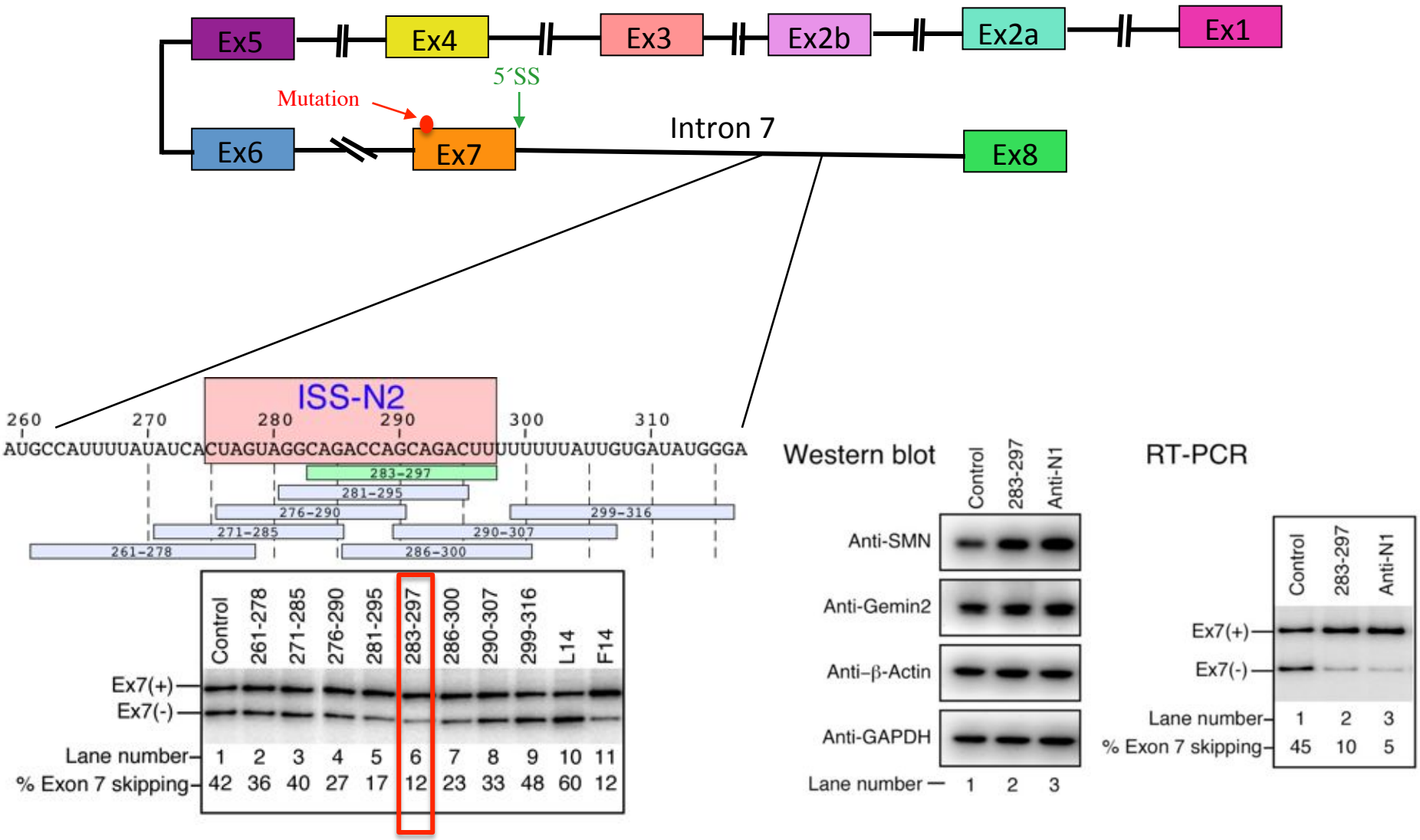
Effect of ISS-N2-targeting ASOs on *SMN2* exon 7 splicing



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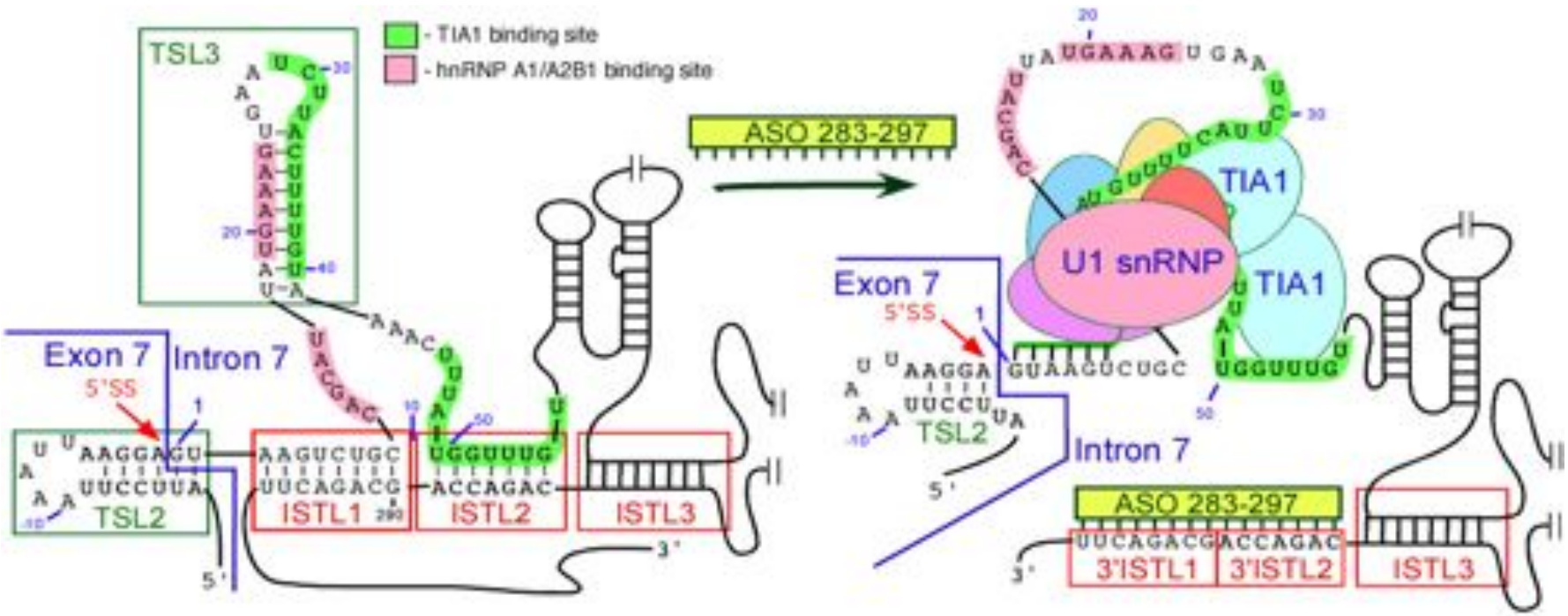
Mechanism of splicing correction by a deep intronic target



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