Supplementary Material

Functionally Important Structural Elements of U12 snRNA

Kavleen Sikand[1,2] and Girish C. Shukla[1,2,*]

[1]Center for Gene Regulation in Health and Disease, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115, USA

[2]Department of Biological, Geological and Environmental Sciences, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115, USA

*Corresponding author

E-mail: g.shukla@csuohio.edu

Supplementary Figure Legends

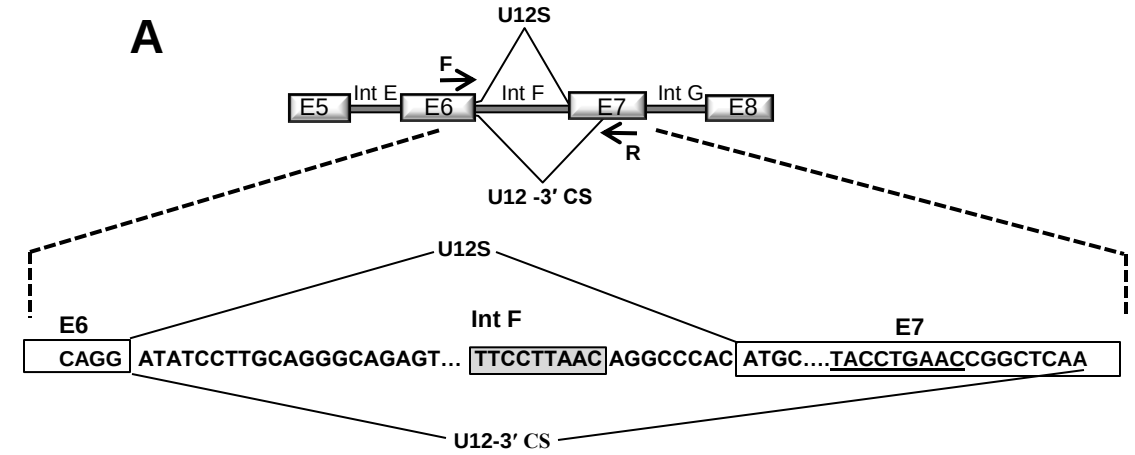
Supplementary Figure S1. Features of the *in vivo* genetic suppression assay. Schematic of the assay system has been modified from Brock *et al*. (1). The *in vivo* suppression system uses a minigene derived from nucleolar proliferating antigen gene P120 or NOLI. The minigene reporter construct contains four exons (E) and three introns (Int). Introns E and G are U2-dependent introns whereas intron F is U12-dependent intron. (A) Diagrams of P120 minigene showing the location of U12-dependent intron F and its splicing patterns. The WT splice sites giving rise to the U12S band shown in (D) are marked. The U12-dependent 3′ cryptic splice site in exon 7 (E7) producing the U12-3′ CS band shown in (D) is also marked. The location of the forward (F: TTGTGCTGCCCCCTGCTGGGGAGATG) and reverse (R: TCAGACAGAGGGAAGAGGTCCATGAG) primers used for PCR is illustrated. The WT U12-dependent branch site sequence in intron F is shaded and the downstream cryptic U12-dependent branch site in exon 7 is underlined. (B) Nucleotide sequences spanning branch site of P120 intron F and branch site binding site of U12 snRNA. Wild type (WT) base pairing between human U12 snRNA and branch site of U12-dependent intron F has been illustrated. The boxed nucleotides were mutated to their complementary nucleotides as shown in (C). (C) The boxed nucleotides correspond to compensatory mutations in human U12 snRNA and branch site of intron F in P120 minigene. GA nucleotides at positions 23/24 in U12 snRNA were mutated to CU and the corresponding nucleotides UC at positions 84/85 in the branch site were mutated to AG. (D) P120 intron F branch site mutation UC84/85AG suppression by first site U12 GA23/24CU mutation in *in vivo* splicing assay. Inverted image of ethidium bromide stained agarose gel shows the *in vivo* spliced phenotypes of P120 intron. Lane M: 100 bp ladder, lane 1: empty vector. Lane 2 shows the spliced phenotypes of P120 WT intron F (labeled US = Unspliced, 331bp; U12S= U12 Spliced, 231 bp). Lane 3: The branch site mutation UC84/85AG

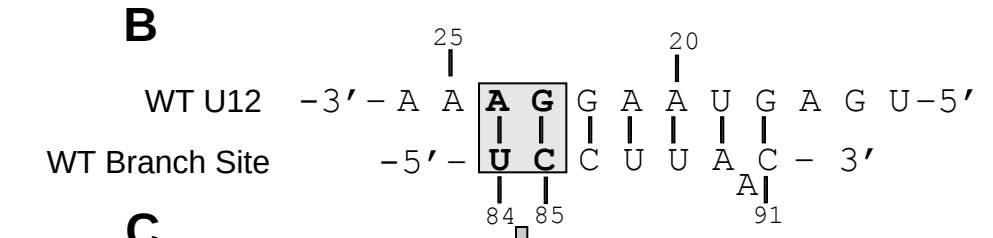
abolishes the splicing of U12-dependent intron from WT splice sites and activates a secondary cryptic branch site in the downstream exon 7. In RT-PCR, this gives rise to a smaller product (labeled U12-3′CS = U12-3′ Cryptic Spliced, 108 bp) compared to WT spliced product. In addition, a large pool of the pre-mRNA remains unspliced (labeled US). Lane 4: Coexpression of U12 suppressor GA23/24CU and P120 UC84/85AG reactivates the splicing of U12-dependent intron from WT branch site region. Hence, the compensatory mutations in U12 snRNA are able to suppress the effects of mutated branch site and restore WT splicing activity. This feature of U12 GA23/24CU suppressor allowed us to test the effects of other sequence and secondary structure alterations in U12 snRNA on its function in *in vivo* splicing of U12-dependent introns.

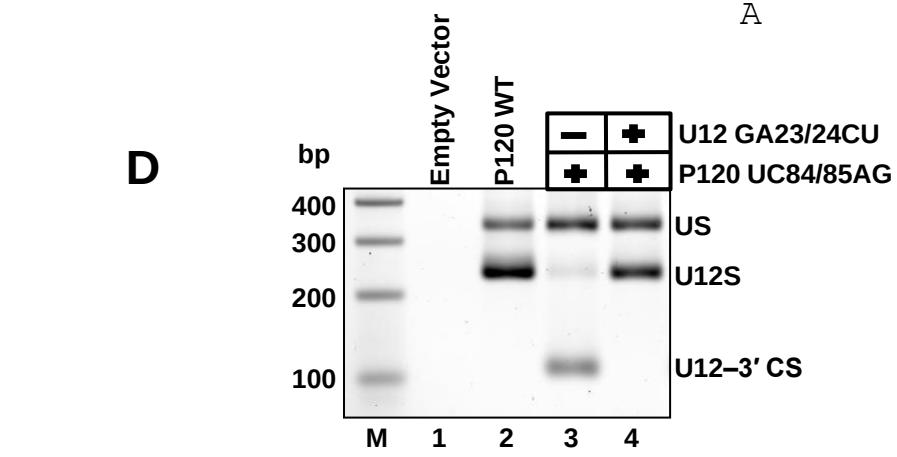
Supplementary Figure S2. Consensus secondary structure model of U12 snRNA. Consensus secondary structure model is constructed from sequence alignment of U12 snRNAs from 17 species. These included *Acanthamoeba castellanii, Ciona intestinalis, Trichinella spiralis, Aplysia californica, Bos taurus, Mus musculus, Rattus norvegicus, Gallus gallus, Xenopus tropicalis, Takifugu rubripes, Tetraodon nigroviridis, Arabidopsis thaliana, Selaginella moellendorffii, Phytophthora infestans, Drosophila melanogaster, Schistosoma mansoni* and *Homo sapiens*. N=A, C, G or U; D=G, U or A. The nucleotide position numbering corresponds to human U12 snRNA. Note that the differences in the lengths of U12 snRNAs are found in the loop of SLIIa and at the 3′ end of consensus U12 snRNA structure.

References:

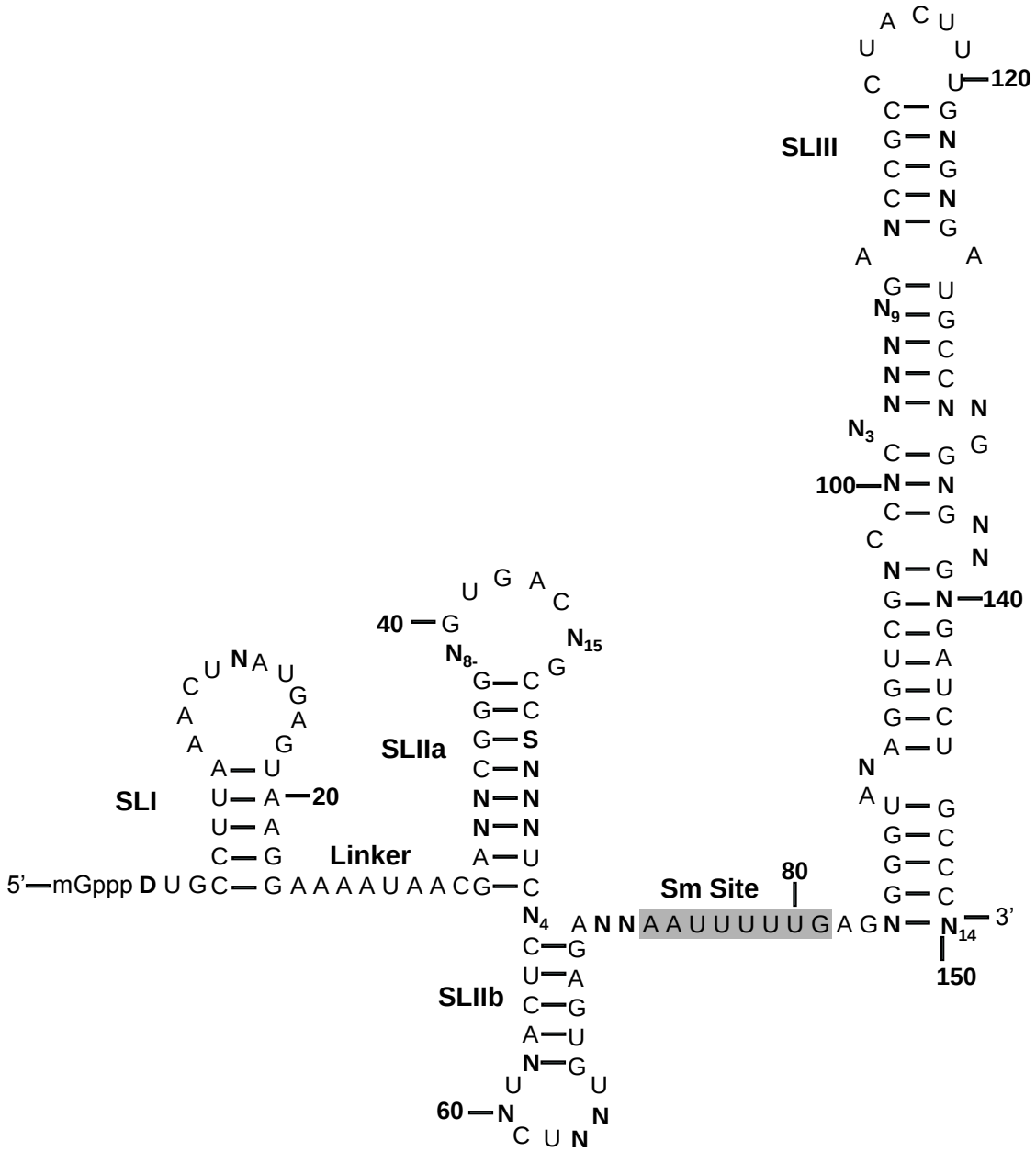
1. Brock,J.E., Dietrich,R.C. and Padgett,R.A. (2008) Mutational analysis of the U12-dependent branch site consensus sequence. *RNA*, **14**, 2430-2439.







Supplementary Figure S1



Supplementary Figure S2