Supplemental data S1

A, *Schematic representation of the human RBM15B gene organization and its two RNA splice variants*. A partial RBM15B cDNA (clone 33), lacking the 5' end of the full length transcript, was isolated using the CDK11A^{p110} cDNA as bait in a yeast two-hybrid assay. The full length cDNA was obtained by RT-PCR (data not shown). RBM15B is an intronless gene encoding two mRNA splice variants of 3.5 and 6.6 kb with the same 2670 base pair Open Reading Frame (ORF) but distinct poly-adenylation sites. The arrows (►PCR1/2◄) indicate the position of the primers used for the RT-PCR experiments presented in panel B. *B*, *Detection of the large 6.6 kb splice variant by RT-PCR*. cDNAs from human, mouse and rat livers and HFF and HepaRG cells were synthesized using the SuperScript^{TM} II RNase H^{-} Reverse transcriptase kit (Invitrogen) and 5 µg of total RNAs isolated with the RNeasy extraction kit (Qiagen) and treated with RNAse free DNAse I. One µl of each Reverse Transcription (RT) reaction (20 µl reactions) was used to perform Polymerase Chain Reaction (PCR) with various pairs of primers. For PCR1 detecting human, mouse and rat mRNAs of RBM15b primers were: 5'-GAGAACCACTCCAGTGAAGGG (forward) and 5'-GCTGACTGGAGGTACTGCTG (reverse). For PCR2, the primers for mouse and rat RBM15B were: 5'-GCAGCCAGACTTGTTAACTC (forward) and 5'-CTGTGATCTGTGGTGACAAC (reverse). To detect the human 6.6 kb mRNA, the primers were: 5'-CGTGTCCAGTCATACCTAGAG (forward) and 5'-CTCAGGTGTCACTCATGCTAC (reverse). PCR1 detected both transcripts from human (Hs), mouse (m) and rat (r), while PCR2 primers are specific of the long 6.6 kb mRNA from human or rodent species. The use of total RNAs and polyA+ RNAs demonstrated that both transcripts were polyadenylated. mRNAs of human liver and HepaRG cells that were not reverse transcribed were used to demonstrate the absence of contaminating genomic DNA. As positive control for PCR1, the RBM15B cDNA (ORF) was used while negative controls (CTRL minus template and template minus primers) were also performed. The conditions for PCR reactions were: denaturation at 95°C for 3 min, annealing at 60°C for 1.5 min, extension at 72°C for 1 min for 30 cycles. All reactions were carried out using Ex-Taq polymerase (Takara). PCR products were separated on 1% agarose gel, purified, subcloned in Topo TA cloning vector (Invitrogen) and analyzed by sequencing. *C*, RBM15B IVTT products: The full length [S^{35}]-methionine labeled RMB15B (FL) IVTT product was detected at an apparent molecular weight of ~110 kDa comigrating with CDK11^{p110}. The truncated RBM15B protein encoded by the clone 33 migrated at ~75kDa.

Supplemental data S2

Co-immunoprecipitation of endogenous CDK11^{p110} with GFP- and HA-tagged RBM15B. *A*, HEK 293T cells were transiently transfected with expression vectors encoding GFP- or HA-tagged RBM15B proteins or empty plasmid (Vector). Two days after transfection, cell extracts were subjected to immunoprecipitation (IP) of CDK11 using the rabbit polyclonal P1C antibody, the immunoprecipitates were then analyzed by western blotting using anti-GFP and –HA monoclonal antibodies. Efficient IP of CDK11 was verified by immunoblotting using P1C anti-CDK11 antibody. The reverse experiment was also performed by immunoprecipitating GFP- and HA-tagged RBM15B and immunoblotting

with the P1C antibody. Expression of the proteins in the cell lysate was performed using the same antibodies (Input). *B*, Production of the N-Terminal domain of RBM15B. The specific rabbit polyclonal anti-RBM15B antibody was raised against the bacterially produced GST-RBM15B N-terminal protein encoding the first 154 amino acids of RBM15B (RBM15B Δ9 mutant). This antigen was chosen because of the low homology with RBM15/OTT1 and the high yields of the Δ9 protein in bacteria. Specific antibodies against RBM15B were affinity purified first by depleting the rabbit sera against a GST sepharose column then by binding specific antibodies to a GST-RBM15B Δ9 affinity column followed by elution with 100mM glycine (pH 2.5). *C*, Characterization of the specific anti-RBM15B polyclonal antibody. Immunoblotting of endogenous and RBM15B overexpressed in HEK293T cells. Cells were transfected with pCMV-RBM15B expression vector or empty pCMV. The specific antibody detected a single band at ~110 kDa and the signal for RBM15B increased with the amounts of RBM15B expression vector. HSC70 was used as loading control. *D*, The full length [S^{35}]-methionine labeled RMB15B IVTT product (Input) was recognized and immunoprecipitated (IP) by the anti-RBM15B antibody but not by total rabbit IgG used as negative control (CTRL).

Supplemental data S3

A, Alignment of the amino acid sequences of RBM15/OTT1 and RBM15B/OTT3. The most conserved amino acids are located within the RRM and SPOC domains. *B, Schematic representation of the SPOC domain containing proteins.* Spen proteins vary considerably in size (90 to 600 kDa) but are characterized by three RRMs (RNA Recognition Motifs) in their N-terminus and a SPOC domain at the very C-terminal end. Human SHARP and murine Mint also contain nuclear Receptor Interacting Domain (RID) and RBP-Jκ interaction domain (RBPID) thought to be important for repression of transcription.

Supplemental data S4

Partial co-immunolocalization of endogenous RBM15B and CDK11^{p110}.

A, Immunofluorescence of HA-tagged RBM15B in HFFs transiently transfected with the pCMV-HA-RBM15B expression vector. Cells were grown on coverslips and fixed with cold methanol at -20°C for 10 min. Coverslips were rinsed with PBS and incubated with rat HA antibody in PBS containing 0.1% casein for 2h at room temperature. After washing the coverslips were incubated with anti-rat secondary antibody labeled with Alexa Fluor 488 (Molecular probes)a. Overexpressed HA-tagged RBM15B showed strong nuclear staining with diffuse nucleoplasmic signal and intense staining in large speckles but was absent from the nucleoli. *B*, Immunofluorescence of endogenous RBM15B, CDK11^{p110}, SR proteins and PCNA in HFFs using RBM15B, CDK11, 1H4 anti-SR and PCNA specific antibodies and rabbit IgG as negative control. Primary and secondary antibodies [anti-rabbit and anti-mouse fluorescent secondary antibodies respectively labeled with Alexa Fluor 488 (Molecular probes) and Cy3 (Jackson Immunoresearch)] were incubated in the same conditions described for panel *A*. Immunofluorescence experiments demonstrate that endogenous RBM15B proteins localize to both nucleoplasm and nuclear speckles in HFFs and partially co-localize with CDK11^{p110} protein kinase and SR proteins SRp75, SRp55, SRp40, SRp30a/b and SRp20

(merge panels). PCNA and purified rabbit IgG antibodies were used as negative controls for co-localization with CDK11 in nuclear speckles. *Dapi*, 4', 6-diamidino-2-phenylindole. Bar : 5 μm. *C*, Quantitative analysis of the co-immunolocalization of CDK11^{p110} and RBM15B. Axiovision LE Rel 4.3/Inside 4D software (Zeiss) was used to establish the degree of co-localization between CDK11^{p110} and RBM15B in the nuclei of HFF cells. Individual immunofluorescence of RBM15B (FITC channel) and CDK11^{p110} (Texas red channel) were used to set up background fluorescence on Texas red and FITC channels (data not shown), respectively, allowing definition of four quadrants of different fluorescent signal intensities: 1- Texas red (CDK11) negative, FITC (RBM15B) positive (lower right quadrant), 2- Texas red positive and FITC negative (upper left), 3- double positive (upper right) and 4- double negative (lower left) signals. The co-immunofluorescence of RBM15B and CDK11^{p110} in 10 HFF nuclei (large magnification of the nucleus) was then analyzed to define the degree of co-localization in quadrant 3. The degree of co-localization was similar in all nuclei ranging from 45 to 60%.

Supplemental data S5

Expression and localization of endogenous RBM15B and CDK11^{p110} during the cell cycle.

A, Immunoblotting of CDK11^{p110}, RBM15B, p34^{CDK1} and actin in cell cycle synchronized HFF cells. Cells were synchronized by serum starvation for 3 days followed by stimulation with 20% FCS. Percentages of cells in G0/G1, S and G2/M were monitored at different times (0.5 to 48 hours) after serum stimulation by flow cytometry using propidium iodide DNA staining (Cycletest Plus Kit, Becton Dickinson). 0: starved cells arrested in G0/G1, AS: asynchronous log phase proliferating cells. Immunoblotting experiments showed that CDK11^{p110} and RBM15B were expressed throughout the cell cycle, with a slight increase in CDK11 in G2/M phases, while the mitotic CDK1 was only expressed during G2/M. Actin was used as loading control. *B*, immunofluorescence of endogenous RBM15B and CDK11^{p110} in starved cells and in cells progressing through G1 (G1: 8 hours post stimulation), S (24 h) and mitosis (32 h). All cells at 8, 24 and during mitosis showed a similar CDK11^{p110} and RBM15B immunostaining pattern. *Dapi*, 4', 6-diamidino-2-phenylindole. Bar : 5 μm.

Supplemental data S6

RBM15B inhibits intron excision and affects alternative splicing of the E1A preRNA.

A, Human hepatoma HepaRG cells were co-transfected by electroporation with the pCEP4-E1A plasmid (from Dr. Tarn, Institute of Biomedical Sciences, Taipei, Taiwan) and the RBM15B (pCMV-HA-RBM15B) expression vector. The E1A minigene includes 2 exons separated by an intron. This reporter system encodes a pre-RNA and five splice variants (13, 12, 11, 10 and 9S) which are expressed at varying levels depending on the cell types. Alternative splicing takes place in exon 1. *B*, Total plasmid amount was equalized for each transfection using empty pCMV plasmid. Cells were harvested 48 h after transfection, and total RNA was extracted using the RNeasy kit (Qiagen). The RNAs were further treated using the RNase-free DNase I kit (Qiagen). RT was performed using 5 μg of total RNAs, the SuperScript^{TM} II RNase H^{-} reverse transcriptase kit, and the E1A-specific primer p2 (5'-CGGTATTCCACATTTGGACACT-3'). For detection of E1A RNAs, 2 ml of the RT reaction were used as template, and PCR

amplification (Takara ExTaq, 50 ml, 35 cycles) was performed using p1 (5'-CAAGCTTGAGTGCCAGCGAGTAG-3') and p2. PCR products were visualized on 1% agarose gels stained with ethidium bromide using Bio-Vision fluorescence image acquisition system (Vilber-Lourmat, Fisher-Bioblock, France) and quantitated using Bio-1D software (Vilber-Lourmat, Fisher-Bioblock, France) as we previously described (8). As control, RNAs from non transfected cells (-, lane 4) were also used to verify the specific amplification of E1A products. Pre-RNA and 12S cDNAs cloned into TOPO-TA cloning vector (Invitrogen) were also used as templates to generate migration markers (lanes 2 and 3, respectively). In HepaRG cells, the 11S variant is undetectable while the 10S is weakly expressed. Enforced expression of RBM15B was verified by immunoblotting (Supplemental S2C). *C*, The percentage of each transcript signal relative to the amount of the five splice variants was calculated for each sample and expressed relative to the appropriate control splice form percentages, which were set equal to 1. Increasing amounts of RBM15B led to an increased level of unspliced pre-RNA and a decrease in the 12 and 9S splice variants. The results shown represent the mean values and standard deviations of three independent transfection experiments. *, $p<0.05$ for comparison to the control cells (3×10^6 cells in 60 mm dishes) transfected with 7 μg of empty pCMV and 2 μg of pCEP4-E1A plasmids.

Supplemental data S7

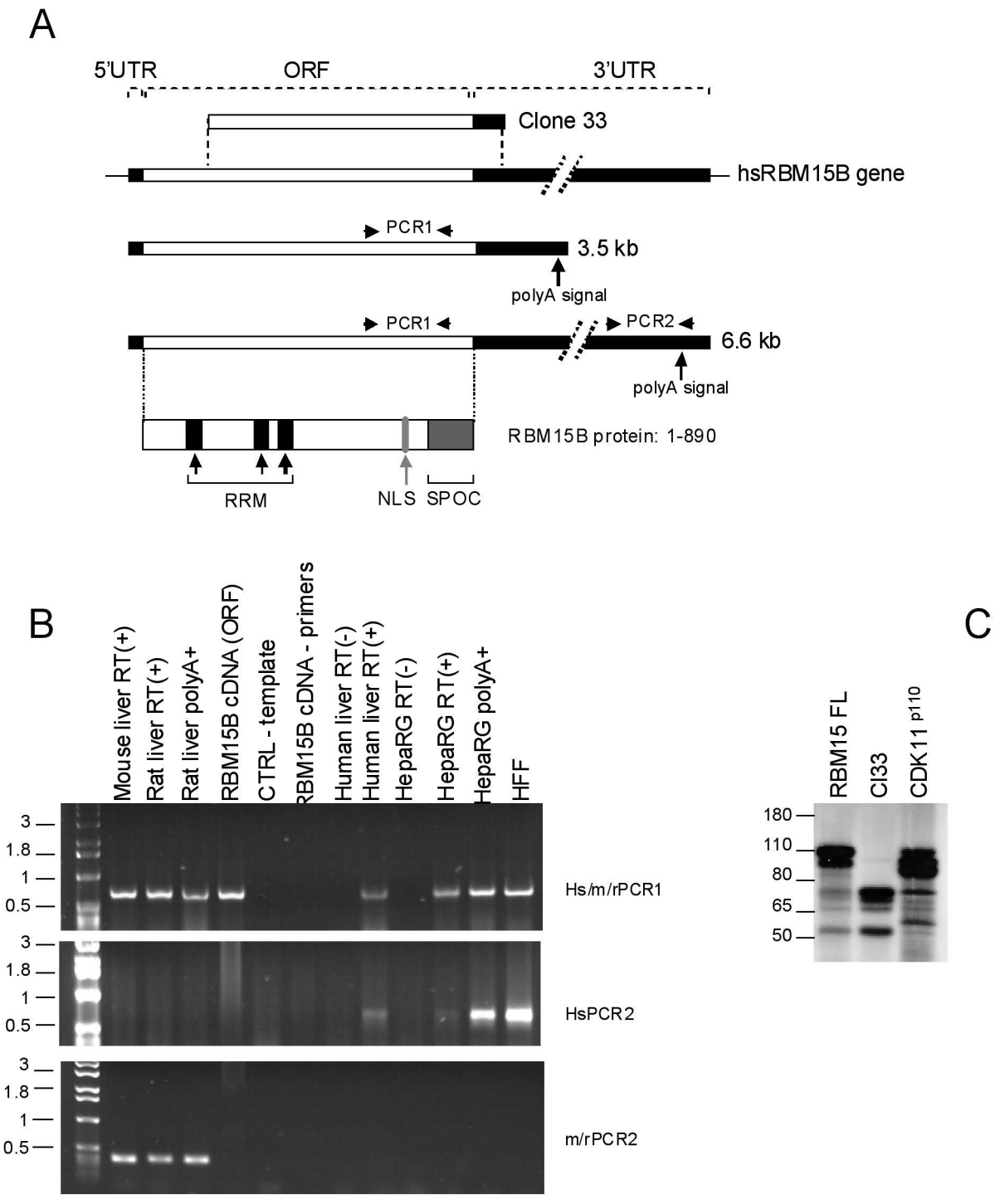
A, Hela nuclear extracts prepared according to Mayeda et *al*. (1999) (48) and the HelaScribe® nuclear extract purchased from Promega exhibit slightly different splicing activities and ratios between β-globin RNA splicing products. Both extracts contained 10 mg/ml of nuclear proteins. HelaScribe® nuclear extract (tested with 2 and 4 μl, 20 and 40 μg of nuclear proteins) always showed a higher splicing activity than the other extract. For experiments using the commercial extract, 20 μg of proteins were used while we used 40 μg of extract prepared according Dr Mayeda's protocol. In addition, with HelaScribe® nuclear extract, the intermediate splice products containing the intron and exon 2 was barely detectable. *B*, The ATP-dependent spliceosomal complexes A, B and C were visualized using native agarose mini-gels as previously described (49). Splicing reactions using the β-globin pre-RNA and HelaScribe® nuclear extract were incubated for various times (0, 5, 30 and 60 minutes at 30°C) and analyzed in native electrophoretic conditions. After 5 minutes, the A complex is formed while the B and C complexes are visible after 30. At 60 minutes the C complex accumulates. In presence of increasing amounts of GST-RBM15B FL (0.5, 2.5 and 5 pmol/splicing reaction, lanes 5, 6 and 7, respectively), none of these complexes were detected as expected from the data in figure 6E showing that the first splicing complex, complex E, did not form in presence of GST-RBM15B. Addition of GST alone (5 pmol/splicing reaction) did not affect formation of complex C (lane 8).

Supplemental data S8

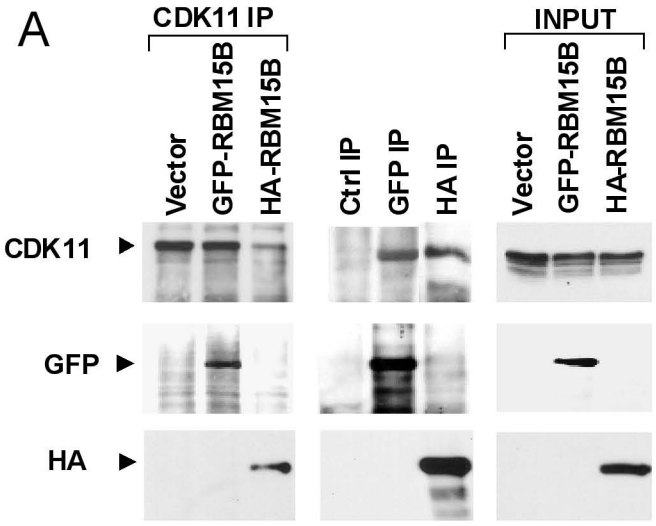
Schematic representation of the RBM15B expression vectors used for binding or splicing assays.

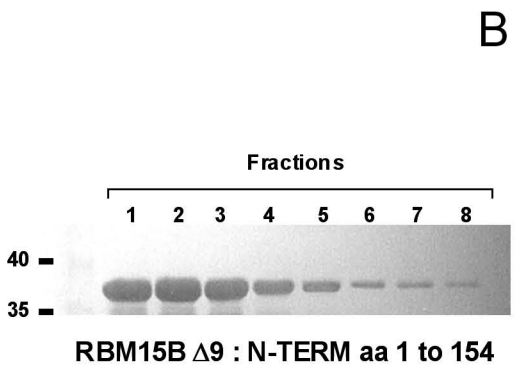
The full length (FL) RBM15B protein was divided in 4 domains based in part on amino acid homology with known functional domains: the N-terminal domain (amino acids 1-154), the RNA Recognition Motifs (RRM) (amino acids 141-489), the C-terminal domain

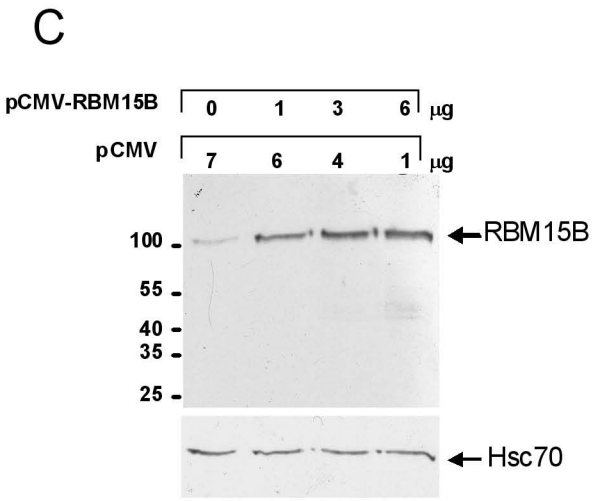
(amino acids 490-720) containing a putative nuclear localization signal (NLS) and the SPOC domain (amino acids 720-890. In the two-hybrid screen, a partial RBM15B cDNA was isolated (clone 33: amino acids 249- 890) lacking the N-Term domain and the first RRM. Seventeen expression vectors of various lengths were generated and used either for GST-pull down binding assays or *in vitro* and *in vivo* splicing assays. These constructs were generated by PCR and amplification products were subcloned in appropriate vectors for subsequent binding or splicing assays.

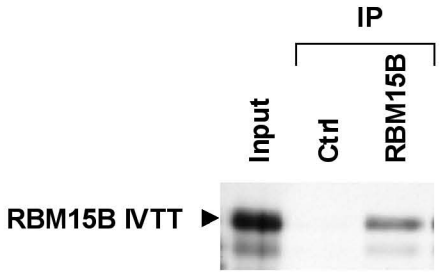


Supplemental S1









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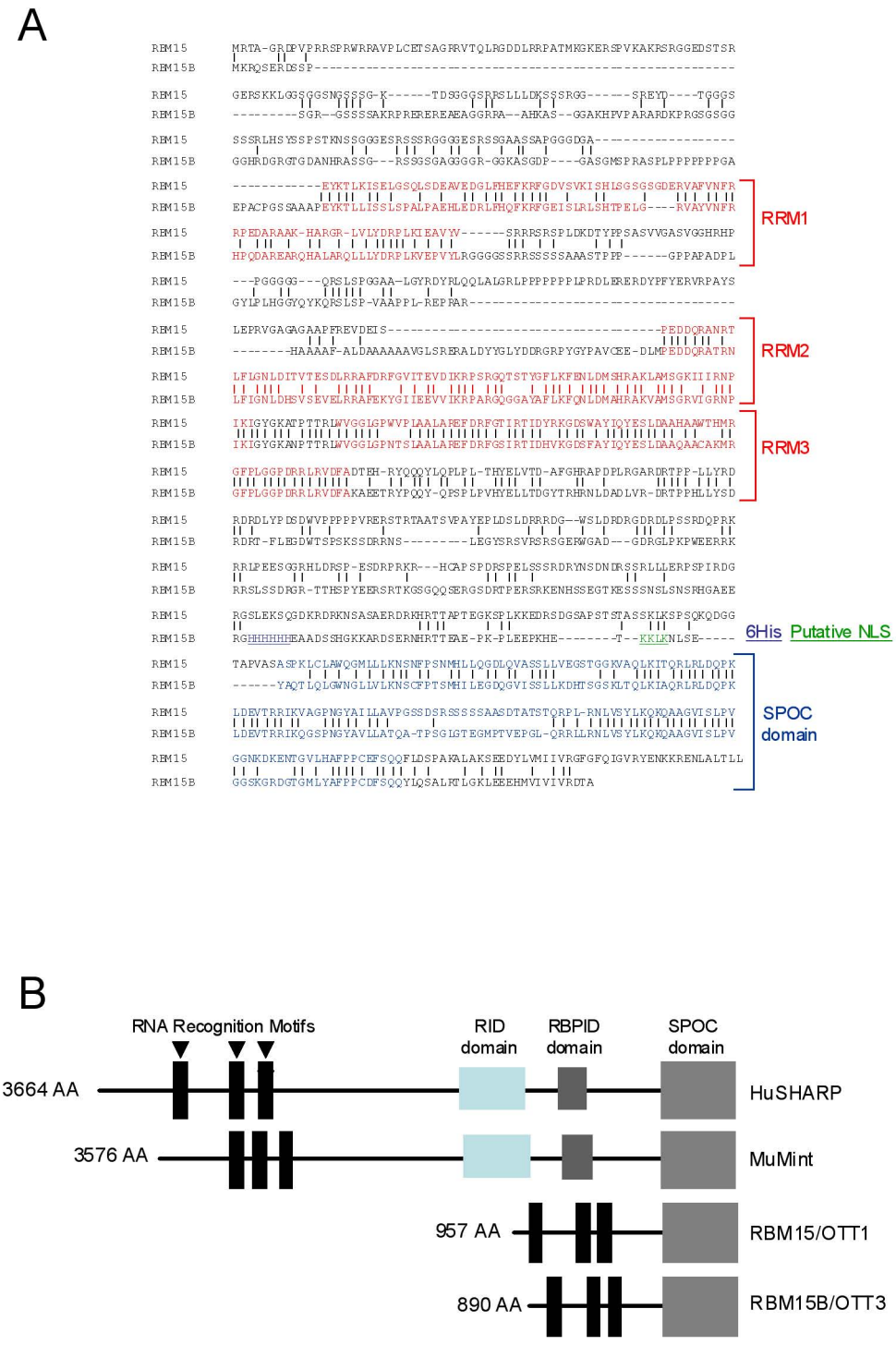
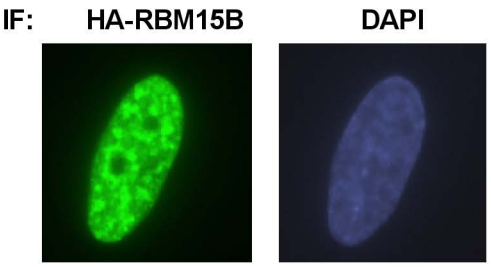


Supplemental S3

A



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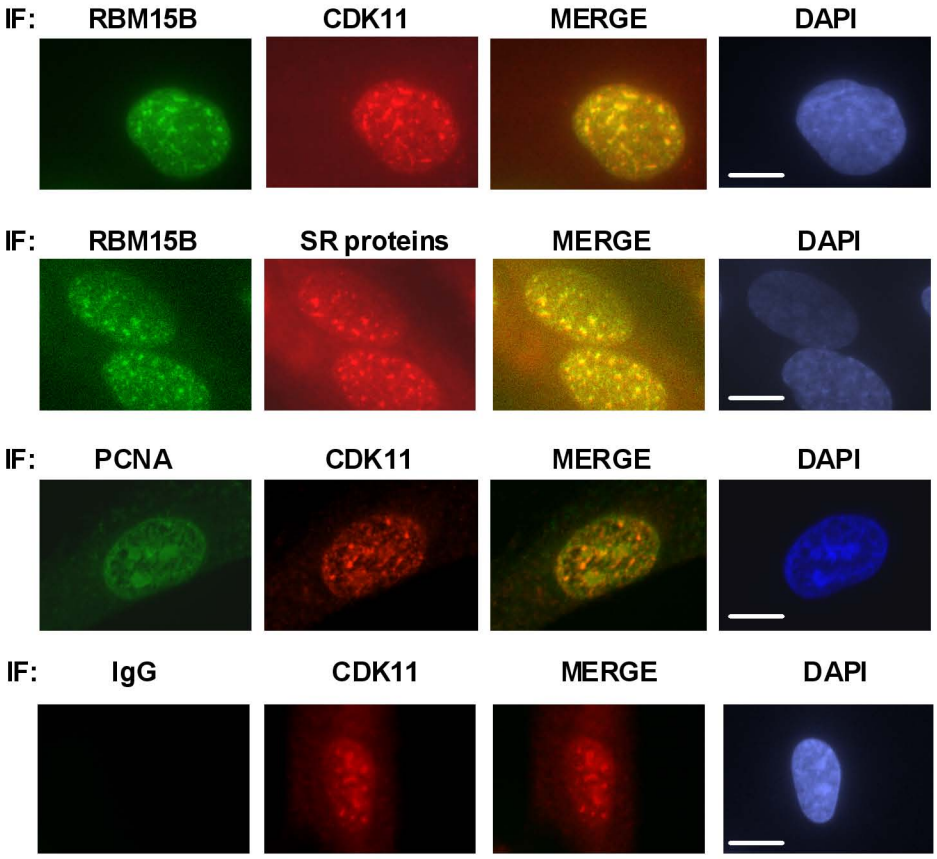

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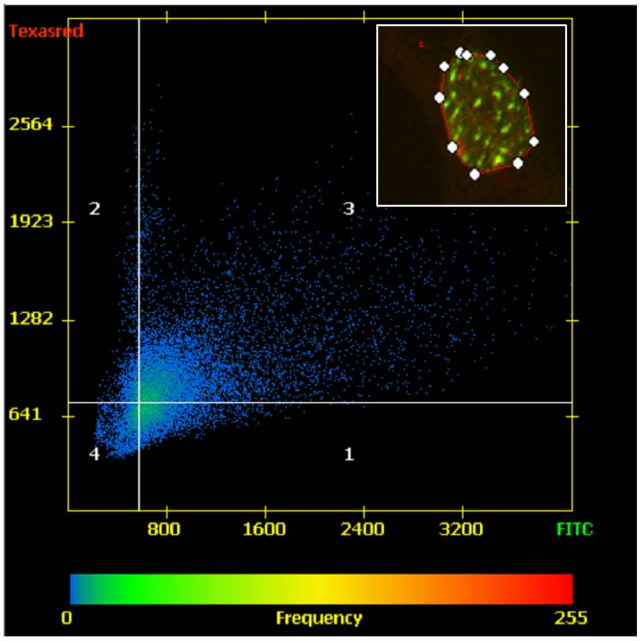
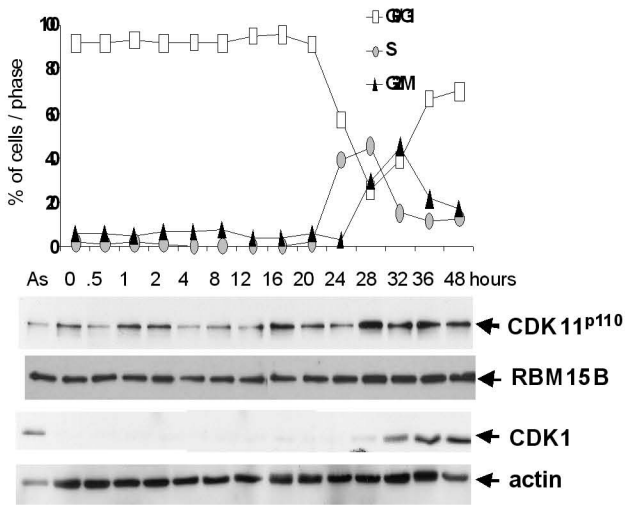


Supplemental S4

A



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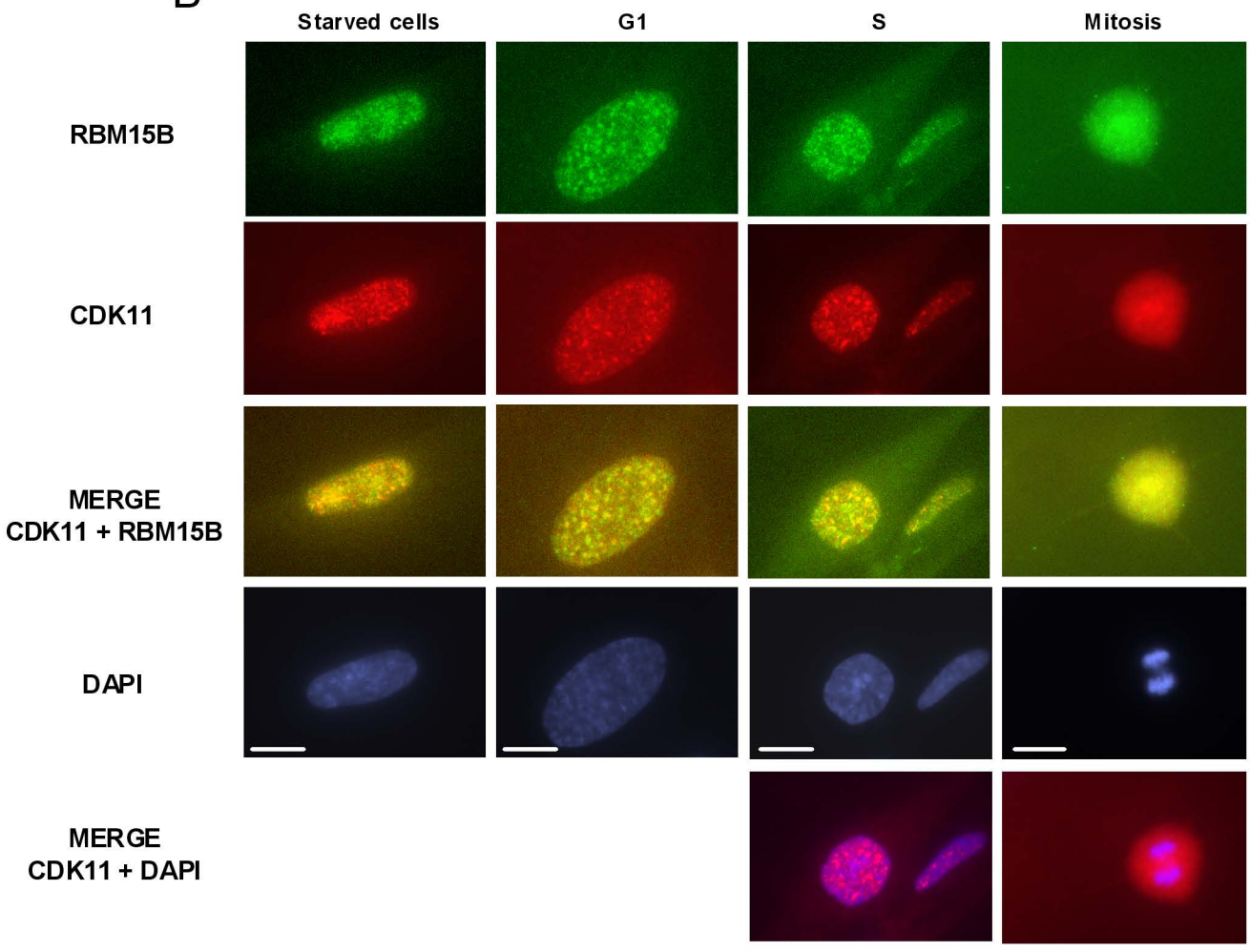
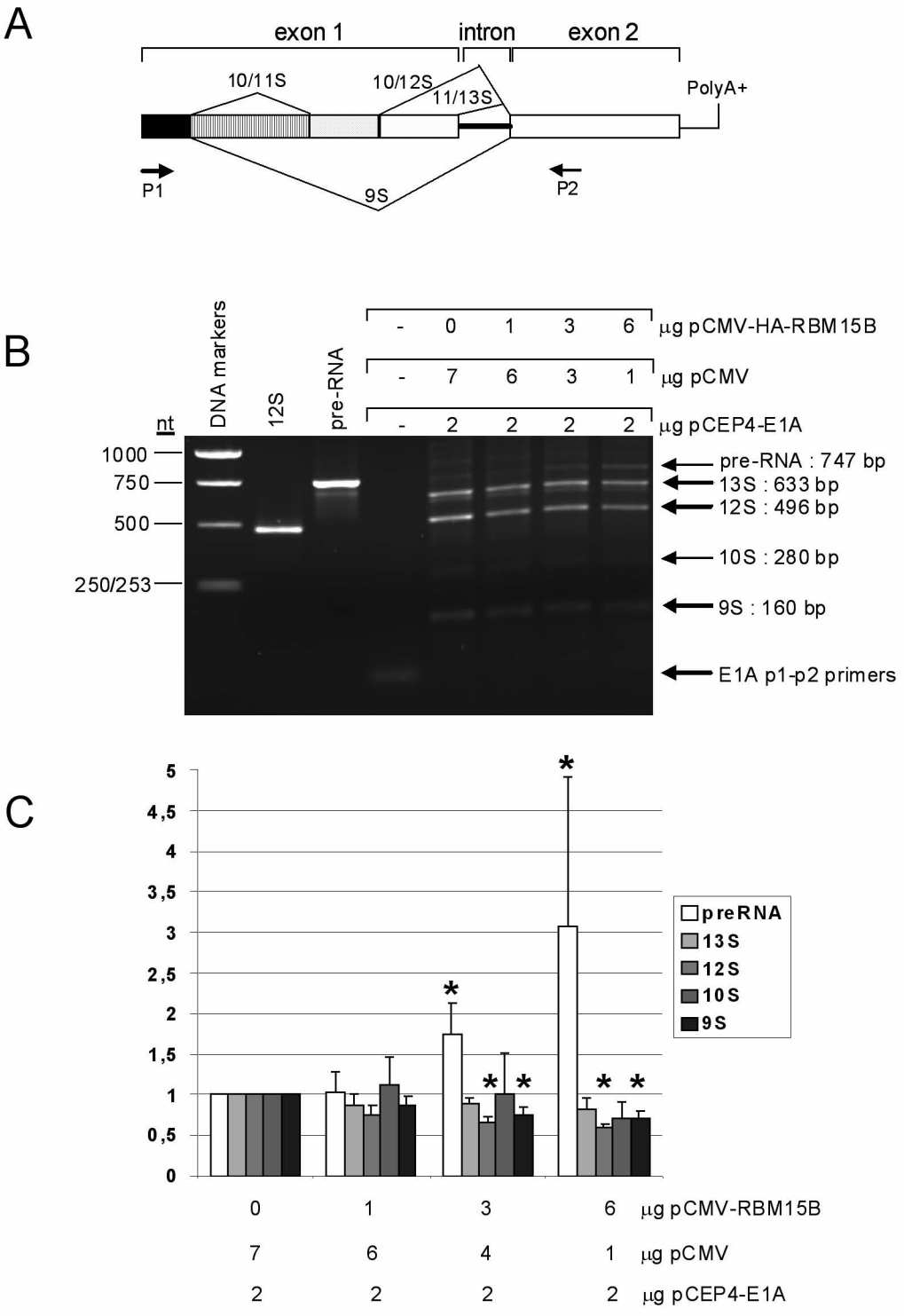
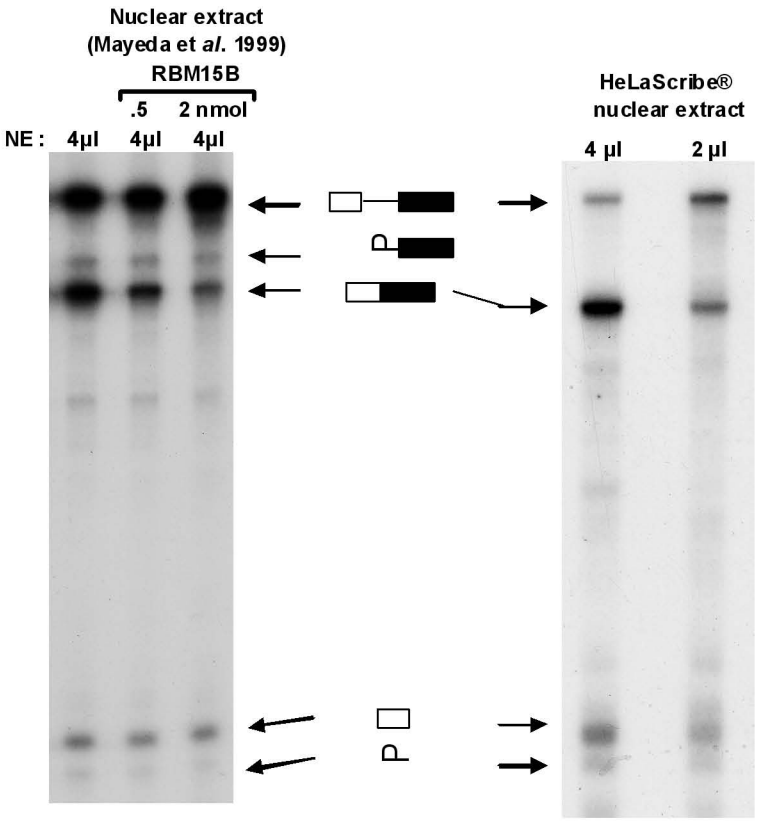


Supplemental S5



Supplemental S6

A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(Mayeda et al. 1999)
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4µl
4µl
HeLaScribe®
nuclear extract
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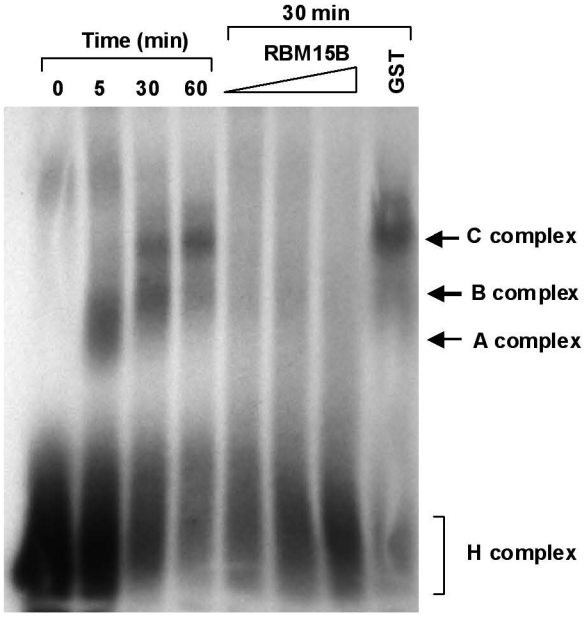
2 µl
P
P

B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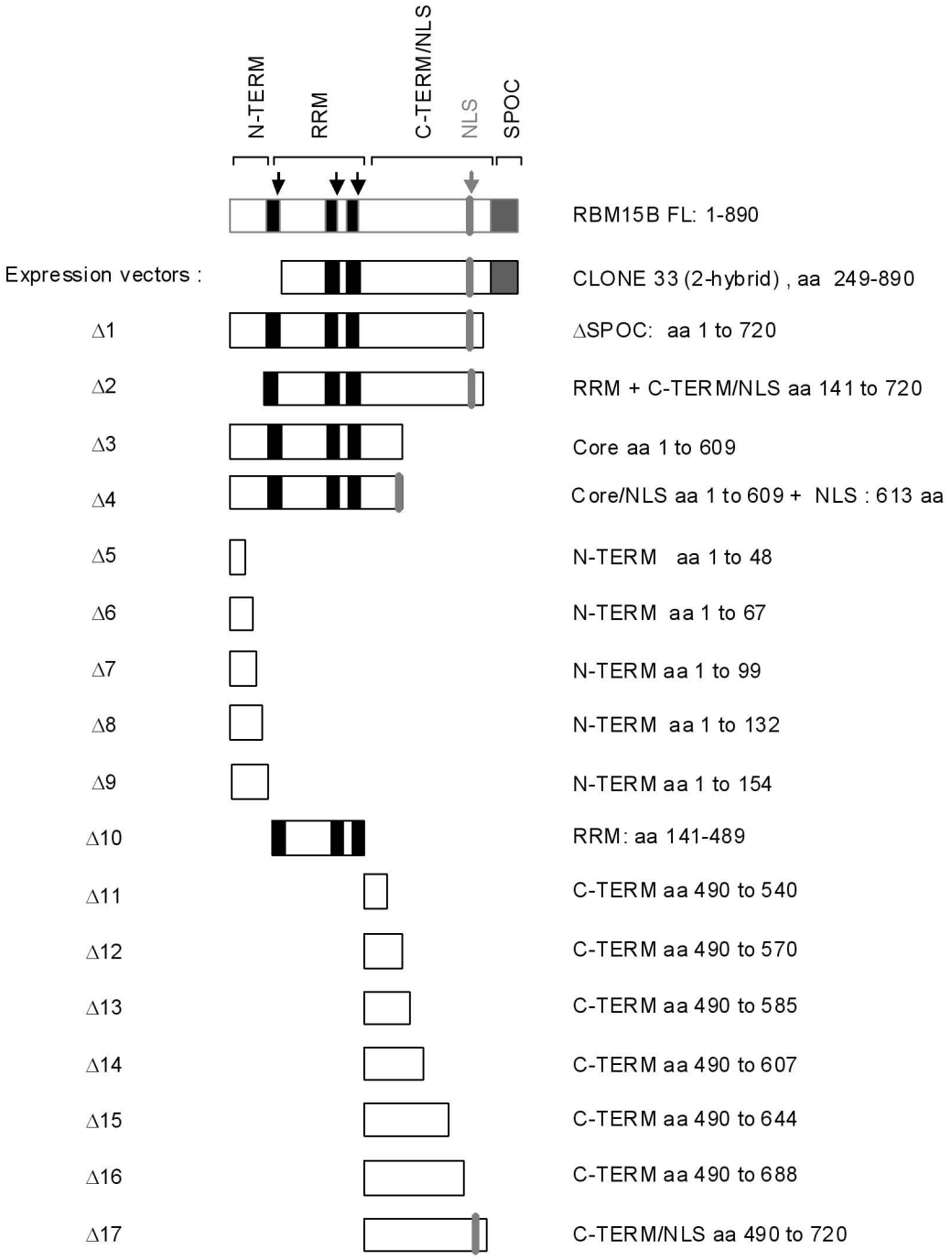

Supplemental S8