**Supplemental data**

**Characterization of *cis*-acting elements that control oscillating alternative splicing**

Gesine Goldammera, Alexander Neumanna, Miriam Straucha, Michaela Müller-McNicollb, Florian Heyda\*, Marco Preußnera\*

*aFreie Universität Berlin, Institute of Chemistry and Biochemistry, Laboratory of RNA Biochemistry, Takustrasse 6, 14195 Berlin, Germany.*

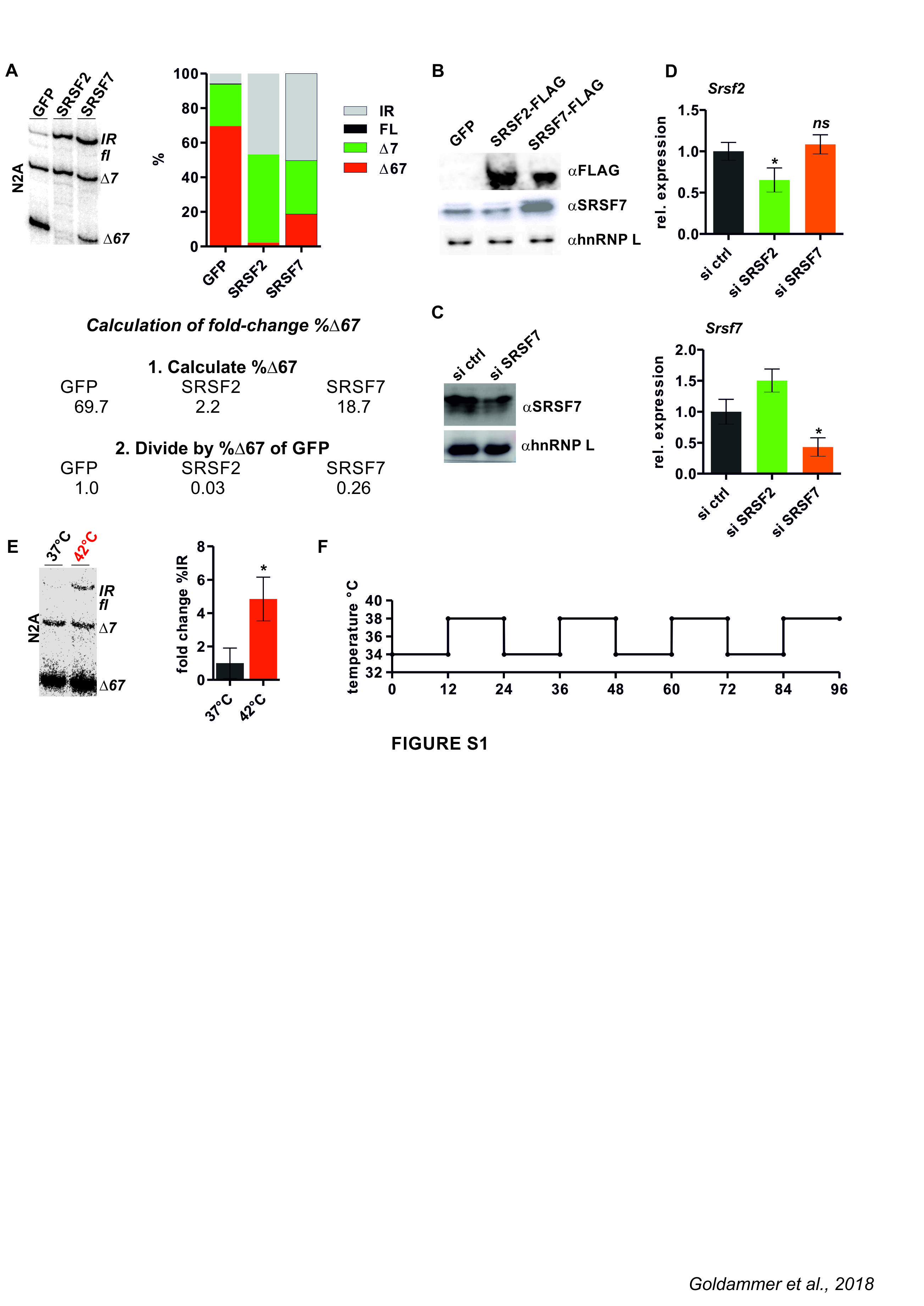
*bGoethe University Frankfurt, Institute for Cell Biology and Neuroscience, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany.*

\*Co-corresponding Authors: mpreussner@zedat.fu-berlin.de, florian.heyd@fu-berlin.de

Phone: +49 30 83862938

FAX: +49 30 838-4-62938

Supplementary Figures



**Figure S1. Overexpression and knockdown of Srsf2 and Srsf7 regulated U2af26 *wt* minigene splicing**

(A) Calculation of fold change %67 after overexpression of Srsf2 or Srsf7. N2A cells were co-transfected with the U2af26 *wt*-minigene and SRSF2 or 7. AS was analyzed using minigene-specific primers (left, representative gel as in Figure 1B). The percentage of each splicing isoform was quantified and is depicted on the right (in this example for the particular gel shown on the left). Bottom: To determine the fold change %67, the %67 for each condition was divided by the %67 of the control condition (here GFP).

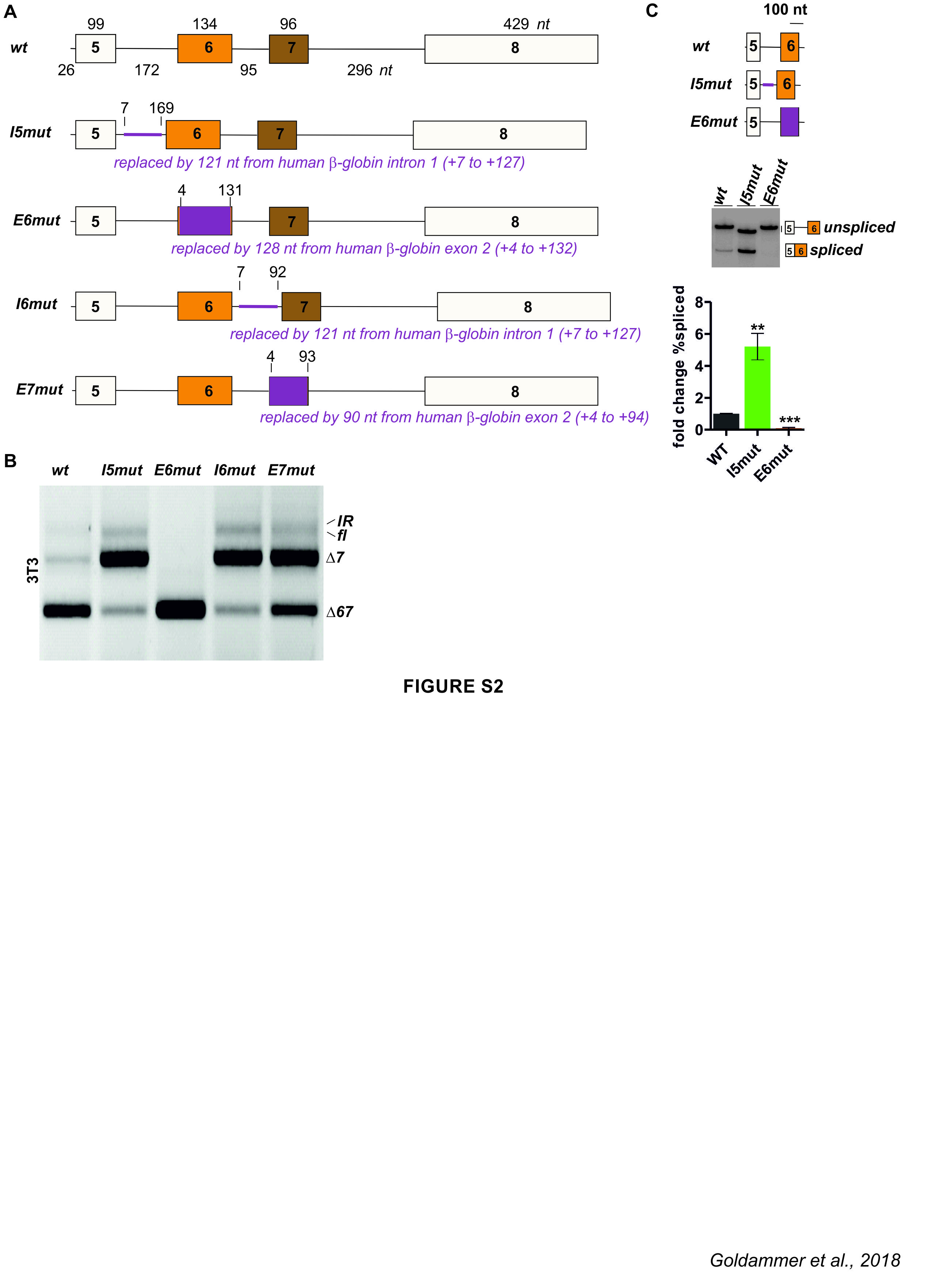
(B) Western blot confirming overexpression of SRSF2 and 7 (anti-FLAG or specific antibody against endogenous SRSF7) with HNRNPL as loading control.

(C) Western blot confirming knockdown of SRSF7 with HNRNPL as loading control.

(D) Knock down validation. N2A cells as in Figure 1C were analyzed for *Srsf2* and *Srsf7* expression using qPCR. Expression is depicted relative to the housekeeping gene *hypoxanthine guanine phosphoribosyl transferase (Hprt)* and ctrl siRNA for *Srsf2* (left) and *Srsf7* (right, n=3, mean +/- SD, \*Students t-test derived p value < 0.05).

(E) The *U2af26 wt-*minigene recapitulates splicing regulation by heat shock in N2A cells. N2A cells were transfected with the *wt*-minigene and heat shocked (42°C, red) for 2 hours. AS was analyzed using minigene-specific primers (left, representative gel). %IR-isoform was quantified relative to 37°C (n=3, mean +/- SD, \*p<0.05). In support of Figure 1D.

(F) Temperature profile of square-wave temperature rhythms (related to Figure 1F).

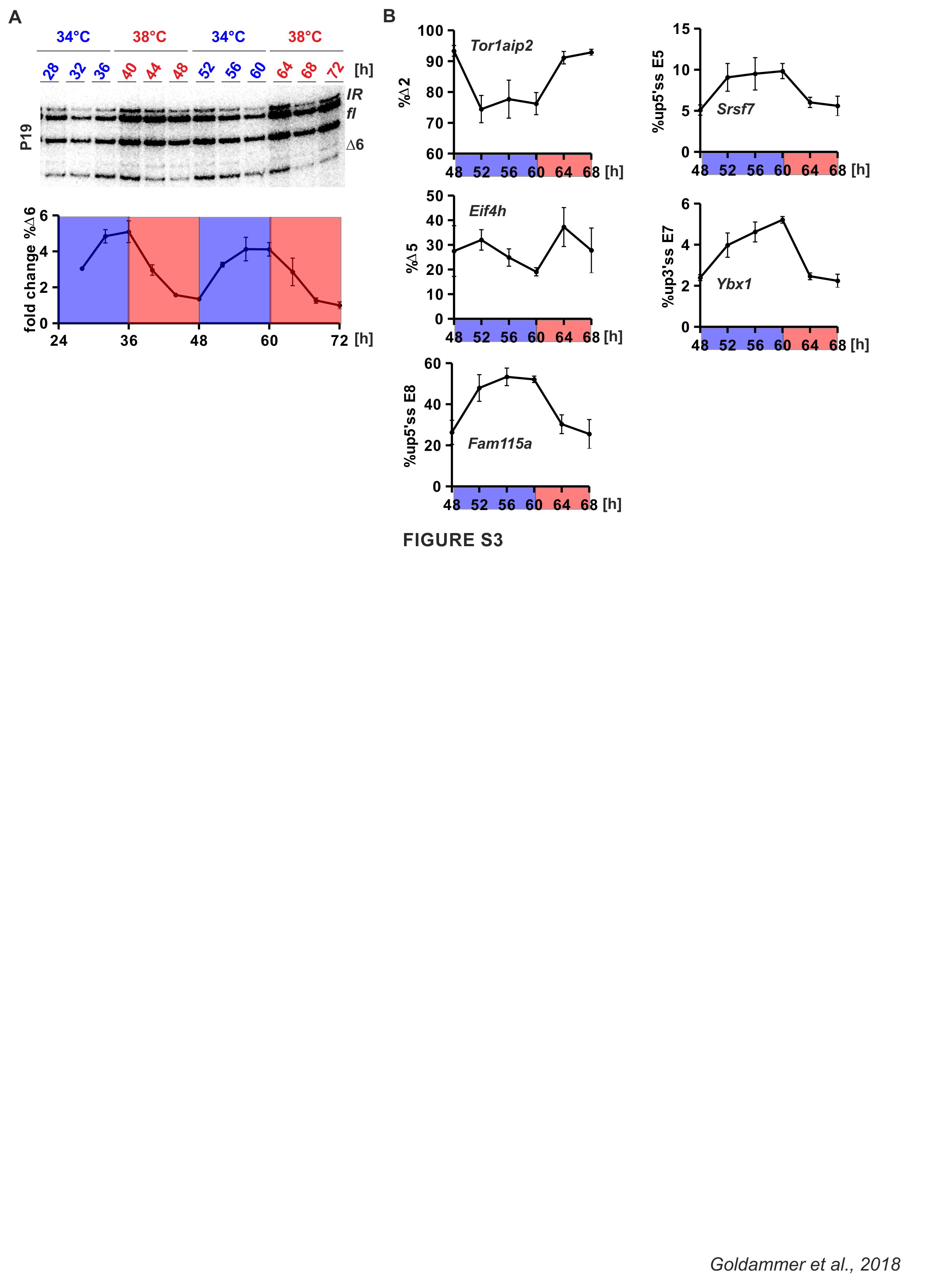
**Figure S2. 4-exon and 2-exon minigene reporters**

(A) Detailed exon/intron structure of minigene reporters used in Figure 2. From the *wt*-minigene (containing genomic exon 5 to 8 sequence) exons and introns were individually replaced by -globin sequence (marked in violet). Fur each mutant the start and end position in *U2af26* of replaced sequence is indicated on top. Sequences from human -globin are indicated below. Exons 6 and 7 were replaced by -globin exon 2 sequence of the same length, introns 5 and 6 were replaced by complete -globin intron 1 including the branch point adenosine, resulting in constructs with a slightly different length.

(B) 3T3 cells were transfected with the indicated minigenes and AS was analyzed using minigene-specific primers by conventional PCR and run on a 2% agarose gel (representative result).

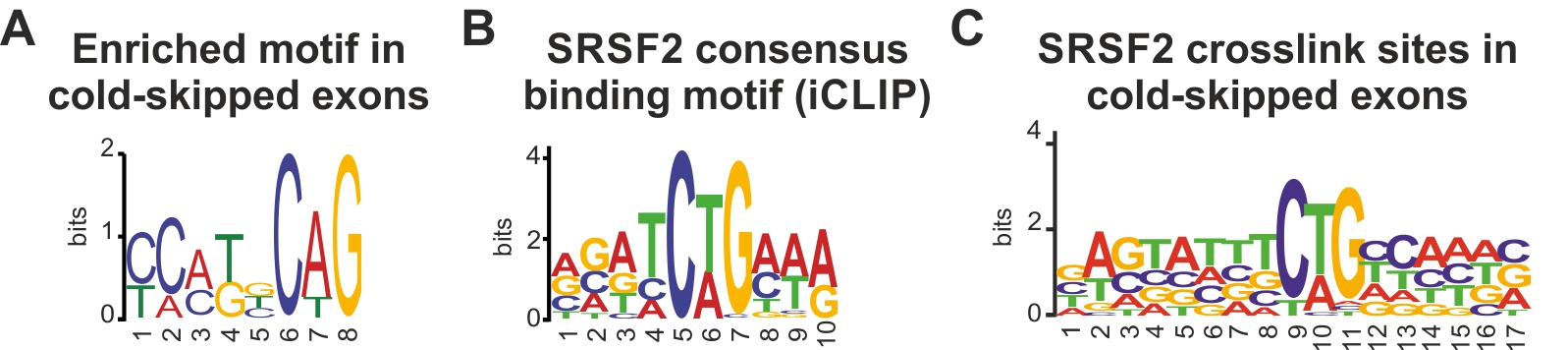
(C) Structure of 2-exon constructs (top), cloned using a reverse primer in intron 6 and the respective 4-exon constructs as a template. N2A cells were transfected with the indicated minigenes and splicing efficiency of intron 5 was analyzed using minigene-specific primers (middle, representative gel). Splicing efficiency (% spliced) was quantified relative to the *wt* (n=3 (for *wt* n=2), mean +/- SD, \*\*p<0.01, \*\*\*p<0.001).

Figure S3. Temperature-dependent rhythmic alternative splicing



(A) P19 cells were transfected with the *E7-8mut*-minigene and treated with square-wave temperature cycles (12h 34°C (blue)/12h 38°C (red), see Figure S1D for a temperature scheme). AS was analyzed at the indicated time points using minigene-specific primers (top, representative gel). %6-isoform was quantified relative to 72 hours (n=2, mean +/- SD).

(B) Square-wave temperature rhythms. In N2A cells 5 exemplary targets were analyzed by splicing sensitive radioactive RT-PCR and splicing isoforms were quantified. For each example, the percentage of one isoform (for SE events always the skipping isoform) was calculated (n=3, mean +/-SD).



**Figure S4. SRSF2 binding motifs in cold-skipped exons**

(A) Enriched sequence motifs in 20 cold-skipped exons searched with MEME using default settings and a 0-order model of sequences as background. The E-value estimates the expected number of motifs with the given log likelihood ratio, and with the same width and site count, that one would find in a similarly sized set of random sequences.

(B) Consensus *in vivo* binding motif of SRSF2 derived from enriched 8-mers (Z-score > 50) located around significant binding sites (FDR < 0.05) using all transcribed regions as input.

(C) CAG/CTG binding motif in cold-responsive exons exhibiting significant binding sites (FDR < 0.05; 11 out of 20 exons).

Supplementary Materials and Methods

|  |  |
| --- | --- |
| T7.fwd (for minigene analysis):  BGH.rev (for minigene analysis): | GACTCACTATAGGGAGACCC  TAGAAGGCACAGTCGAGG |
| U2af26.E4.fwd:  U2af26.E8.rev: | CGTGGAGGTGCAAGAACAC  GCTGGGACTGGAGAGTGAAG |
| Mettl3.E3.fwd:  Mettl3.E5.rev: | TGGCCTCTTCAGCATCAGAA  ATTTGCAGGTGTCCATGTGG |
| BAT2D.E14.fwd:  BAT2D.E18.rev: | CACCACGCCAACATCTAGTC  TCACTCAGCGTTTCAGGGTT |
| Atp13a3.E32.fwd:  Atp13a3.E34.rev: | TCAATGCCTTCGTGTCTATCA  GCTCCTGTGCTAAGTACATGT |
| PPP3cb.E12.fwd:  PPP3cb.E14.rev: | TAGTGGAGTGTTGGCTGGAG  TTCTCAGTGGTATGTGCGGT |
| Mff.E4.fwd:  Mff.E8rev: | CTGGCACTGAAAACACCACC  GCTCCTTCAATGGCTGCATC |
| EIF4H.E4.fwd:  EIF4H.E6.rev: | AGGTGGCTTTGGATTCAGGA  TGTGGGTTCTCTGAAGTCCA |
| TOR1AIP2.E1.fwd:  TOR1AIP2.E3.rev: | ATCCTGACTGTCCCGTGTTT  GATCCGGTGAGTCTTCTGCT |
| FAM115A.E8.fwd:  FAM115A.E9.rev: | AAGCTTAGCCTATCTGCCCG  CGCCATGTTTCCCGATCATT |
| YBX1.E6.fwd:  YBX1.E7.rev: | GCAGGAGAGCAAGGTAGACC  TGTCCTCTTCATTGCCATCCT |
| SRSF7.E5.fwd:  SRSF7.E6.rev: | GCCGATCAAGGTCTGTGTCT  ACCTGGATCTTGATCTCGACC |

Table S2: Primer Sequences

Minigene Sequences

*wt (exons 5-8 uppercase; introns 4-7 lowercase; restriction sites used: yellow):*ggatccgctgagcttctccccatcttctgcagGAGGTATTCACAGAACTGCAGGAGAAGTATGGAGAGATTGAAGAGATGAATGTGTGTGACAACCTCGGGGACCACCTCGTGGGCAATGTCTACGTTAAGgtgcctgttgccccctgcttagaacccagaagtggaaacatttcagtgcctatggccaccactgaaacctcccaacctgaggactatcgataagttgagtccagcctcagagctgactagtcccctgaagatcttgaattccacagcccatggtcacccactttcccctcagTTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGCCGAGCTGTCTCCTGTCACTGACTTCCGAGAGTCCTGCTGCCGGCAGTATGAGATGGGgtaaggtagtgtaaaagtaggttgggcatcagagtcccagaagcttgagtgtttgatagggagttcttagtgatggtggccttctctgtctttagGGAATGCACCCGAGGTGGCTTCTGCAACTTTATGCACCTACGGCCCATATCTCGGAACTTGCGCCGGCAGCTCTATGGGCGAGGACCCAGGCATAGgtacctcagacccagggtgctgtgttctcctgtaccctaggatgaacctgatgctaacttgtgggctcttgtgctttaaatagagttttgagccttgcctgggtagaagtaccttagtcttagtgtcccaaacagtgtaaaccccatatccacccccagcttgggtacctgtccctgagtaccaatcctcagatgacattaccagccacctgtccctaagagacaggcctggaaaaatcttgaccactcttaactATTCCTACCCATCTCTGTCCACTCCTCTTCCTAATTCTTAGGTCACCTCCAAGGTCCCACACAGGTCACCGTCCCCGAGAAAGGAACCGACGTCGTTCCCCAGACCACCGGCATGGTCGCTTCTGAGACGGGTGCCCTTGCTCTCCACCCAAGGGCATAGATGTTCCTGTCCAGCGTCCCTTTTCAAAGTGCCCTTCACTCTCCAGTCCCAGCATCCCCAGGCTTCGGAGCTTCATAATATAATCTGTTCGACACAGGAACCTCCTCCTCCTGTCCCTCCTCCAATAAAGGTTAAGAAGTTTGTCAATCAAGGTCTGTGGTTTCCTTCCACCCCCACACCTAAGAAAGGGTGTAAGCCAACCACAGTCTCAGCAGAGGAACTTGGAGCCAGCTTGGGGTCTGGGTCTGCACTGGTAAGTCGGGGCTGCTTTAATACCTGTAACCTCAGATTATCCATTCTAGGTGCCTTTctcgag

*I5mut (human -globin intron 1 sequence marked in red):*ggatccgctgagcttctccccatcttctgcagGAGGTATTCACAGAACTGCAGGAGAAGTATGGAGAGATTGAAGAGATGAATGTGTGTGACAACCTCGGGGACCACCTCGTGGGCAATGTCTACGTTAAGgtgcctatcaaggttacaagacaggtttaaggagaccaatagaaactgggcatgtggagacagagaagactcttgggtttctgataggcactgactctctctgcctattggtctattttcccaccctcagTTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGCCGAGCTGTCTCCTGTCACTGACTTCCGAGAGTCCTGCTGCCGGCAGTATGAGATGGGgtaaggtagtgtaaaagtaggttgggcatcagagtcccagaagcttgagtgtttgatagggagttcttagtgatggtggccttctctgtctttagGGAATGCACCCGAGGTGGCTTCTGCAACTTTATGCACCTACGGCCCATATCTCGGAACTTGCGCCGGCAGCTCTATGGGCGAGGACCCAGGCATAGgtacctcagacccagggtgctgtgttctcctgtaccctaggatgaacctgatgctaacttgtgggctcttgtgctttaaatagagttttgagccttgcctgggtagaagtaccttagtcttagtgtcccaaacagtgtaaaccccatatccacccccagcttgggtacctgtccctgagtaccaatcctcagatgacattaccagccacctgtccctaagagacaggcctggaaaaatcttgaccactcttaactATTCCTACCCATCTCTGTCCACTCCTCTTCCTAATTCTTAGGTCACCTCCAAGGTCCCACACAGGTCACCGTCCCCGAGAAAGGAACCGACGTCGTTCCCCAGACCACCGGCATGGTCGCTTCTGAGACGGGTGCCCTTGCTCTCCACCCAAGGGCATAGATGTTCCTGTCCAGCGTCCCTTTTCAAAGTGCCCTTCACTCTCCAGTCCCAGCATCCCCAGGCTTCGGAGCTTCATAATATAATCTGTTCGACACAGGAACCTCCTCCTCCTGTCCCTCCTCCAATAAAGGTTAAGAAGTTTGTCAATCAAGGTCTGTGGTTTCCTTCCACCCCCACACCTAAGAAAGGGTGTAAGCCAACCACAGTCTCAGCAGAGGAACTTGGAGCCAGCTTGGGGTCTGGGTCTGCACTGGTAAGTCGGGGCTGCTTTAATACCTGTAACCTCAGATTATCCATTCTAGGTGCCTTTctcgag

*E6mut (human -globin exon 2 sequence marked in red):*ggatccgctgagcttctccccatcttctgcagGAGGTATTCACAGAACTGCAGGAGAAGTATGGAGAGATTGAAGAGATGAATGTGTGTGACAACCTCGGGGACCACCTCGTGGGCAATGTCTACGTTAAGgtgcctgttgccccctgcttagaacccagaagtggaaacatttcagtgcctatggccaccactgaaacctcccaacctgaggactatcgataagttgagtccagcctcagagctgactagtcccctgaagatcttgaattccacagcccatggtcacccactttcccctcagTTCGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGGGgtaaggtagtgtaaaagtaggttgggcatcagagtcccagaagcttgagtgtttgatagggagttcttagtgatggtggccttctctgtctttagGGAATGCACCCGAGGTGGCTTCTGCAACTTTATGCACCTACGGCCCATATCTCGGAACTTGCGCCGGCAGCTCTATGGGCGAGGACCCAGGCATAGgtacctcagacccagggtgctgtgttctcctgtaccctaggatgaacctgatgctaacttgtgggctcttgtgctttaaatagagttttgagccttgcctgggtagaagtaccttagtcttagtgtcccaaacagtgtaaaccccatatccacccccagcttgggtacctgtccctgagtaccaatcctcagatgacattaccagccacctgtccctaagagacaggcctggaaaaatcttgaccactcttaactATTCCTACCCATCTCTGTCCACTCCTCTTCCTAATTCTTAGGTCACCTCCAAGGTCCCACACAGGTCACCGTCCCCGAGAAAGGAACCGACGTCGTTCCCCAGACCACCGGCATGGTCGCTTCTGAGACGGGTGCCCTTGCTCTCCACCCAAGGGCATAGATGTTCCTGTCCAGCGTCCCTTTTCAAAGTGCCCTTCACTCTCCAGTCCCAGCATCCCCAGGCTTCGGAGCTTCATAATATAATCTGTTCGACACAGGAACCTCCTCCTCCTGTCCCTCCTCCAATAAAGGTTAAGAAGTTTGTCAATCAAGGTCTGTGGTTTCCTTCCACCCCCACACCTAAGAAAGGGTGTAAGCCAACCACAGTCTCAGCAGAGGAACTTGGAGCCAGCTTGGGGTCTGGGTCTGCACTGGTAAGTCGGGGCTGCTTTAATACCTGTAACCTCAGATTATCCATTCTAGGTGCCTTTctcgag

*I6mut (human -globin intron 1 sequence marked in red):*ggatccgctgagcttctccccatcttctgcagGAGGTATTCACAGAACTGCAGGAGAAGTATGGAGAGATTGAAGAGATGAATGTGTGTGACAACCTCGGGGACCACCTCGTGGGCAATGTCTACGTTAAGgtgcctgttgccccctgcttagaacccagaagtggaaacatttcagtgcctatggccaccactgaaacctcccaacctgaggactatcgataagttgagtccagcctcagagctgactagtcccctgaagatcttgaattccacagcccatggtcacccactttcccctcagTTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGCCGAGCTGTCTCCTGTCACTGACTTCCGAGAGTCCTGCTGCCGGCAGTATGAGATGGGgtaaggatcaaggttacaagacaggtttaaggagaccaatagaaactgggcatgtggagacagagaagactcttgggtttctgataggcactgactctctctgcctattggtctattttcccacccttagGGAATGCACCCGAGGTGGCTTCTGCAACTTTATGCACCTACGGCCCATATCTCGGAACTTGCGCCGGCAGCTCTATGGGCGAGGACCCAGGCATAGgtacctcagacccagggtgctgtgttctcctgtaccctaggatgaacctgatgctaacttgtgggctcttgtgctttaaatagagttttgagccttgcctgggtagaagtaccttagtcttagtgtcccaaacagtgtaaaccccatatccacccccagcttgggtacctgtccctgagtaccaatcctcagatgacattaccagccacctgtccctaagagacaggcctggaaaaatcttgaccactcttaactATTCCTACCCATCTCTGTCCACTCCTCTTCCTAATTCTTAGGTCACCTCCAAGGTCCCACACAGGTCACCGTCCCCGAGAAAGGAACCGACGTCGTTCCCCAGACCACCGGCATGGTCGCTTCTGAGACGGGTGCCCTTGCTCTCCACCCAAGGGCATAGATGTTCCTGTCCAGCGTCCCTTTTCAAAGTGCCCTTCACTCTCCAGTCCCAGCATCCCCAGGCTTCGGAGCTTCATAATATAATCTGTTCGACACAGGAACCTCCTCCTCCTGTCCCTCCTCCAATAAAGGTTAAGAAGTTTGTCAATCAAGGTCTGTGGTTTCCTTCCACCCCCACACCTAAGAAAGGGTGTAAGCCAACCACAGTCTCAGCAGAGGAACTTGGAGCCAGCTTGGGGTCTGGGTCTGCACTGGTAAGTCGGGGCTGCTTTAATACCTGTAACCTCAGATTATCCATTCTAGGTGCCTTTctcgag

*E7mut (human -globin exon 2 sequence marked in red):*ggatccgctgagcttctccccatcttctgcagGAGGTATTCACAGAACTGCAGGAGAAGTATGGAGAGATTGAAGAGATGAATGTGTGTGACAACCTCGGGGACCACCTCGTGGGCAATGTCTACGTTAAGgtgcctgttgccccctgcttagaacccagaagtggaaacatttcagtgcctatggccaccactgaaacctcccaacctgaggactatcgataagttgagtccagcctcagagctgactagtcccctgaagatcttgaattccacagcccatggtcacccactttcccctcagTTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGCCGAGCTGTCTCCTGTCACTGACTTCCGAGAGTCCTGCTGCCGGCAGTATGAGATGGGgtaaggtagtgtaaaagtaggttgggcatcagagtcccagaagcttgagtgtttgatagggagttcttagtgatggtggccttctctgtctttagGGAGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAATAGgtacctcagacccagggtgctgtgttctcctgtaccctaggatgaacctgatgctaacttgtgggctcttgtgctttaaatagagttttgagccttgcctgggtagaagtaccttagtcttagtgtcccaaacagtgtaaaccccatatccacccccagcttgggtacctgtccctgagtaccaatcctcagatgacattaccagccacctgtccctaagagacaggcctggaaaaatcttgaccactcttaactATTCCTACCCATCTCTGTCCACTCCTCTTCCTAATTCTTAGGTCACCTCCAAGGTCCCACACAGGTCACCGTCCCCGAGAAAGGAACCGACGTCGTTCCCCAGACCACCGGCATGGTCGCTTCTGAGACGGGTGCCCTTGCTCTCCACCCAAGGGCATAGATGTTCCTGTCCAGCGTCCCTTTTCAAAGTGCCCTTCACTCTCCAGTCCCAGCATCCCCAGGCTTCGGAGCTTCATAATATAATCTGTTCGACACAGGAACCTCCTCCTCCTGTCCCTCCTCCAATAAAGGTTAAGAAGTTTGTCAATCAAGGTCTGTGGTTTCCTTCCACCCCCACACCTAAGAAAGGGTGTAAGCCAACCACAGTCTCAGCAGAGGAACTTGGAGCCAGCTTGGGGTCTGGGTCTGCACTGGTAAGTCGGGGCTGCTTTAATACCTGTAACCTCAGATTATCCATTCTAGGTGCCTTTctcgag

*wt (2-exon reporter, exons 5-6 uppercase; introns 4-6 lowercase; restriction sites used: yellow; mutations in the 2-exon context are identical to the mutations in I5mut and E6mut in the 4-exon context):*ggatccgctgagcttctccccatcttctgcagGAGGTATTCACAGAACTGCAGGAGAAGTATGGAGAGATTGAAGAGATGAATGTGTGTGACAACCTCGGGGACCACCTCGTGGGCAATGTCTACGTTAAGgtgcctgttgccccctgcttagaacccagaagtggaaacatttcagtgcctatggccaccactgaaacctcccaacctgaggactatcgataagttgagtccagcctcagagctgactagtcccctgaagatcttgaattccacagcccatggtcacccactttcccctcagTTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGCCGAGCTGTCTCCTGTCACTGACTTCCGAGAGTCCTGCTGCCGGCAGTATGAGATGGGgtaaggtagtgtaaaagtaggtctcgag

*E6het (heterologous CamKII sequence (constitutive exons 10 and 14 and adjacent introns) underlined, U2af26 exon 6 was cloned using NotI):*ggatcctttctagAGTAAAAGTATGATTACGAAAAAAAGTGAAGGATCGCAAGTTAAAGAAAAAGAATCTACTGACTCAAGTACAACCATTGAAGATGATGATGTGAAAGgttagtgcaaattgatttttaaattaagataatgaaaatggctaagtattttcgaagagaacctatatatgttgatatttttctacaaatttaaatttcataccaaatcagcggccgcctgactagtcccctgaagatcttgaattccacagcccatggtcacccactttcccctcagTTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGCCGAGCTGTCTCCTGTCACTGACTTCCGAGAGTCCTGCTGCCGGCAGTATGAGATGGGgtaaggtagtgtaaaaggcggccgctatagctagcctcatgagacgatattagttttagaattttcgacttataagaaatgattaatatttaagctttaaggatggaacacactattaaaacaatgaaccagaaaaaaccaatttaaagaaaataacaaagcaatattacacttaaatattaaatgaaatgttatgtttttttatgttggatgttaatctacttaaagaactgattgtgtaaaatagttttgtgtaacaaaatttaaataatttcaaagttatagtttaatcttgaaaagatatttttgaaaaatttagctgctctttactatttatataataaaacatatatctgagaacctttgtactaaaggttaaaatacaataaaatttaagtatctgaattattatttgatttttggattatcatttcactgagaaatgattccatattagtcttttgtatttcatttgtctaagaacaaaccagtttgaaaaactagtaattgtaaaatatgtttatattataatattgcttaaaatataaaattgtgttttccataaaaaatgcaaaccactaaaatcaacattttatagtaaaacttgagccctttgatgttttatttttgttaactccttagctaattaacatttataactgcattttttataataacatttgtttgaacttcctgagacataatattttttaacatgaaaaacatgtagatttatttgtatgtacattcaaaaaaattacctaaatgtgtacacaatataagaattctcctgaaaatcacatctagtaaaacgtaatttttaatttattttatttcgatcttctttcggattagCTCGGCGTCAGGAAATCATCAAAATGACTGAACAACTTATCGAAGCCATCAATAATGGAGATTTTGAAACATATACgtgagttgaacctctagattctcgag

*mutCC (point mutations marked in red, shown is only exon 6):*TTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGAAGAGCTGTCTAATGTCACTGACTTAAGAGAGTAATGCTGAAGGCAGTATGAGATGGG

*E5mut (human -globin exon 1 and intron 1 sequence marked in red):*

ggatccACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGgttggtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcatgtggagacagagaagactcttgggtttctgataggcactgactctctgaattccacagcccatggtcacccactttcccctcagTTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGCCGAGCTGTCTCCTGTCACTGACTTCCGAGAGTCCTGCTGCCGGCAGTATGAGATGGGgtaaggtagtgtaaaagtaggttgggcatcagagtcccagaagcttgagtgtttgatagggagttcttagtgatggtggccttctctgtctttagGGAATGCACCCGAGGTGGCTTCTGCAACTTTATGCACCTACGGCCCATATCTCGGAACTTGCGCCGGCAGCTCTATGGGCGAGGACCCAGGCATAGgtacctcagacccagggtgctgtgttctcctgtaccctaggatgaacctgatgctaacttgtgggctcttgtgctttaaatagagttttgagccttgcctgggtagaagtaccttagtcttagtgtcccaaacagtgtaaaccccatatccacccccagcttgggtacctgtccctgagtaccaatcctcagatgacattaccagccacctgtccctaagagacaggcctggaaaaatcttgaccactcttaactATTCCTACCCATCTCTGTCCACTCCTCTTCCTAATTCTTAGGTCACCTCCAAGGTCCCACACAGGTCACCGTCCCCGAGAAAGGAACCGACGTCGTTCCCCAGACCACCGGCATGGTCGCTTCTGAGACGGGTGCCCTTGCTCTCCACCCAAGGGCATAGATGTTCCTGTCCAGCGTCCCTTTTCAAAGTGCCCTTCACTCTCCAGTCCCAGCATCCCCAGGCTTCGGAGCTTCATAATATAATCTGTTCGACACAGGAACCTCCTCCTCCTGTCCCTCCTCCAATAAAGGTTAAGAAGTTTGTCAATCAAGGTCTGTGGTTTCCTTCCACCCCCACACCTAAGAAAGGGTGTAAGCCAACCACAGTCTCAGCAGAGGAACTTGGAGCCAGCTTGGGGTCTGGGTCTGCACTGGTAAGTCGGGGCTGCTTTAATACCTGTAACCTCAGATTATCCATTCTAGGTGCCTTTctcgag

*E7-8mut (human -globin exon 2-3 sequence marked in red):*ggatccgctgagcttctccccatcttctgcagGAGGTATTCACAGAACTGCAGGAGAAGTATGGAGAGATTGAAGAGATGAATGTGTGTGACAACCTCGGGGACCACCTCGTGGGCAATGTCTACGTTAAGgtgcctgttgccccctgcttagaacccagaagtggaaacatttcagtgcctatggccaccactgaaacctcccaacctgaggactatcgataagttgagtccagcctcagagctgactagtcccctgaagatcttgaattccacagcccatggtcacccactttcccctcagTTCCGGCGGGAGGAGGATGCAGAGCGGGCTGTAGCGGAACTCAATAACCGCTGGTTCAACGGGCAGGCTGTGCATGCCGAGCTGTCTCCTGTCACTGACTTCCGAGAGTCCTGCTGCCGGCAGTATGAGATGGGgtaaggtagtgtaaaagtaggttgggcatcagagtcccagaagcttgagtgtttgatagggagttcttagtgatggtggccttctctgtctttagGGAGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACTTCAGGgtgagtctatgggacgcttgatgttttctttccccttcttttctatggttaagttcatgtcataggaaggggataagtaacagggtacagtttagaatgggaaacagacgaatgattgcatcagtgtggaagtctcaggatcgttttagtttcttttatttgctgttcataacaattgttttcttttgtttaattcttgctttctttttttttcttctccgcaatttttactattatacttaatgccttaacattgtgtataacaaaaggaaatatctctgagatacattaagtaacttaaaaaaaaactttacacagtctgcctagtacattactatttggaatatatgtgtgcttatttgcatattcataatctccctactttattttcttttatttttaattgatacataatcattatacatatttatgggttaaagtgtaatgttttaatatgtgtacacatattgaccaaatcagggtaattttgcatttgtaattttaaaaaatgctttcttcttttaatatacttttttgtttatcttatttctaatactttccctaatctctttctttcagggcaataatgatacaatgtatcatgcctctttgcaccattctaaagaataacagtgataatttctgggttaaggcaatagcaatatctctgcatataaatatttctgcatataaattgtaactgatgtaagaggtttcatattgctaatagcagctacaatccagctaccattctgcttttattttatggttgggataaggctggattattctgagtccaagctaggcccttttgctaatcatgttcatacctcttatcttcctcccacagCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAActcgag