Supplementary Material

Figure Legends:

Figure S1: Schematic of the matched CRE-containing and completely defined synthetic promoters with luciferase and ED I minigene reporter constructs tested.

Figure S2: (A) Location of previously described primers (pSV5´J, pSV3´J) (Caputi et al, 1994) used for radiolabeled RT-PCR and Taqman real-time PCR Primer/Probe sets utilized for the detection and quantitation respectively of alternatively spliced products originating from the minigene reporters are indicated by arrows. Location of the FAM-labeled probes are indicated by a red bar. (B) Sequences of the Taqman Primer/Probe sets designed and used throughout this study.

Figure S3: (A) Representative western blot of extracts from HEK293T cells overexpressing FLAG-tagged CRTCs. Top panel probed with α-FLAG-HRP. Bottom panel probed with α-Tubulin. (B) CRTC coactivators regulate alternative exon splicing and transcriptional activation in multiple cell lines that lack SV40 large T-antigen or adenovirus E1A. Effect of CRTCs on alternative exon splicing. Transient transfection assays of *EVX1* ED I minigene reporter cotransfected into PC12, Huh7, or C2C12 cells with CRTC1 and/or A-CREB. Top: Taqman real-time qPCR analysis of CRTCs affects on *EVX1* ED I minigene exon skipping. The relative exon skipping levels are expressed as the ratio of skipped versus included transcripts. Bottom: Taqman real-time qPCR analysis of CRTCs affects on *EVX1* ED I minigene reporter gene expression. Relative

amounts of ED I reporter transcripts obtained by each transfection were plotted as folds relative to basal transcription (*n*=3 experiments; mean ± SEM; asterisk denotes *P*-value ≤ 0.05).

Figure S4: CRTC-dependent ED I exon skipping is not due to saturation of splicing factors. (A) Transient transfection assays of increasing amounts (0.25, 2.5, and 25 ng) of the *EVX1* luciferase reporter with CRTCs 1, 2, or 3. Relative light units for CRTC 1, 2 or 3 activation of the *EVX1* luciferase reporter are shown (n=6 wells; mean ± SEM).
(B) Increasing amounts of the EVX1 ED I minigene reporter plasmid (1, 10, 100 ng) was transiently transfected into HEK293T cells with CRTCs 1, 2, or 3. RT-PCR products were separated by PAGE and splice variants analyzed to determine the effect of CRTCs on splice site selection. The ratio (ED I-/ED I+) represents the relative amount of spliced transcripts that have skipped the ED I exon for each transfection experiment are graphed (n=3 experiments; mean ± SEM).

Figure S5: Real-time Taqman qPCR analysis of luciferase transcription (n=3 experiments; mean ± SEM).

Figure S6: Schematic of the HIV-1 minigene splicing substrate relative to the HIV-1 genome. Components of the HIV-1 genome used to generate the minigene are indicated by dashed lines. The various splice products generated as a result of alternative 3' splice site selection are highlighted and illustrated (Bottom).

Figure S7: CRTC coactivator regulation of alternative 3´ splice site selection is not due to promoter strength or saturation of splicing factors. (A) qPCR quantification of the transcripts expressed by the HIV-1 derived reporter constructs. Relative amounts of HIV-1 reporter transcripts obtained by each reaction were normalized and plotted as the percentage of total gene products expressed (unspliced + spliced) versus the amount expressed by each respective S1X-R construct (n=3 experiments; mean ± SEM). (B) Increasing amounts of the indicated reporter plasmids were transiently transfected in HEK293T cells and the relative amounts of HIV-1 reporter transcripts (spliced + unspliced) was calculated as a percentage of transcript expressed versus the amount expressed from 100 ng of each respective plasmid. For each reporter plasmid the amount expressed with 100 ng of input DNA was set to 1 (n=3 experiments; mean ± SEM). (C) RT-PCR products were separated by PAGE and splice variants quantified. The ratio represents the sum of the total amount of spliced transcripts versus unspliced transcripts for each transfection experiment (n=3 experiments; mean ± SEM).

Figure S8: CRTC promotes alternative splicing of endogenous *NR4A2.* (A) Representative western blot analysis of endogenous CRTC2 protein in non-stimulated and stimulated cells transduced with either Adeno-NS shRNA or Adeno-CRTC2 shRNA. Bottom panel probed with α-Tubulin as loading control. (B) Original data scans of the panels shown in Figure 4C were from the same gel and had the same exposure. Cells were infected with two different MOI; lanes 1 and 4 represent MOI=20 while lanes 2 and 5 represent MOI=40. Lanes 3 and 6 are replicates of MOI=40. (C) Analysis of ND-PC12 cells co-transduced with adenovirus expressing either GFP or CRTC2 and either Non-

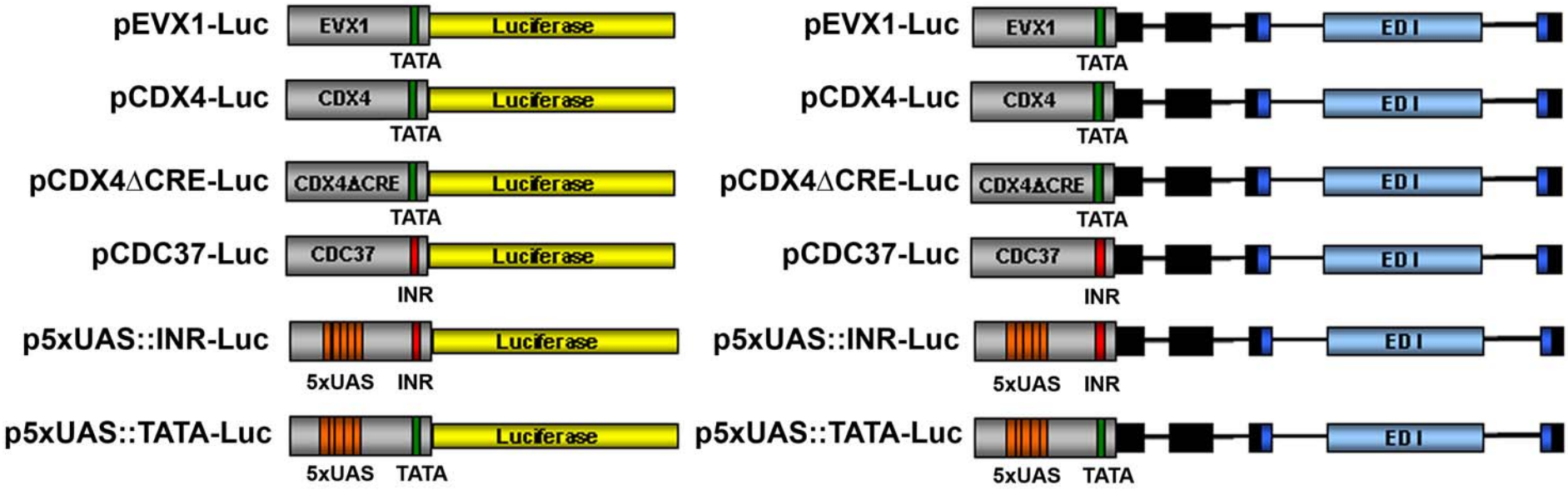
Specific (NS) or CRTC2-specific shRNAs. Ratio of alternatively spliced exons are indicated and graphically displayed (*n*=3 experiments; mean ± SEM; single asterisk denotes *P*-value ≤ 0.05; double asterisk for shRNA-mediated inhibition denotes *P*-value ≤ 0.05). (D) Taqman real-time qPCR analysis of relative CRTC RNA levels. The RNA levels are expressed relative to non-transduced (Mock) cells (n=3 experiments; mean ± SEM). (E) Levels of Adeno-CRTC overexpression relative to endogenous CRTC. Representative western blot analysis showing amounts of overexpressed Adeno-CRTC2 (HA-CRTC2) and endogenous CRTC2 protein levels in non-transduced and transduced cells. Top panel probed with α-CRTC2 (#3364). Bottom panel probed with α-Tubulin.

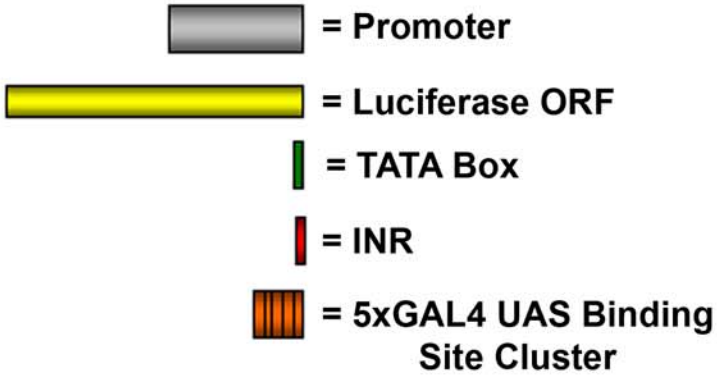
Figure S9: Nr4a2 splice variants act as dominant negative proteins by inhibiting full-length Nr4a2 transcription factor activity. (A) Transient cotransfection assays of PC12 cells with a luciferase reporter containing tandem Nr4a2 canonical transcription factor binding sites (3xNBRE-Luc) with full-length Nr4a2 (Nurr1) in the presence or absence of Nurr1 splice variants. Transfection of PC12 cells was achieved via NeuroMag™ magnetic transfection (n=6 wells; mean ± SEM). (B) Transient cotransfection assays of HEK293T cells with a luciferase reporter containing tandem Nr4a2 canonical transcription factor binding sites (3xNBRE-Luc) with full-length Nr4a2 (Nurr1) in the presence or absence of Nurr1 splice variants in (n=6 wells; mean ± SEM).

Figure S10: CRTCs elicit alternative ED I splicing independent of core promoter context. (A) Effect of CRTC1 or CBP on transcriptional activity with TATA-less and

TATA-containing core promoters. Transient transfection assays of *GAL4 UAS* luciferase reporter cotransfected into HEK293T cells with CRTC1 or CBP along with full-length GAL4-CREB or the phospho-serine mutant GAL4-CREB-S133A were analyzed for luciferase activity. (B) Effect of CRTC1 or CBP on alternative exon splicing with TATA-less and TATA-containing core promoters. Transient transfection assays of *GAL4 UAS* EDI reporter cotransfected into HEK293T cells with CRTC1 or CBP along with full-length GAL4-CREB or the phospho-serine mutant GAL4-CREB-S133A. RT-PCR products were separated by PAGE and splice variants analyzed to determine the effect of CRTC expression on splice site selection. Identities of spliced and unspliced amplicons are indicated on the right side of the panel. (C) Taqman real-time qPCR analysis of CRTC1 or CBP on alternative exon splicing with TATA-less and TATA-containing ED I minigenes. The relative exon skipping levels are expressed as the ratio of skipped versus included transcripts (n=3 experiments; mean ± SEM). (D) Effect of CRTC1 on transcriptional activity with TATA-less and TATA-containing core promoters. Transient transfection assays of *GAL4 UAS* luciferase reporter cotransfected into HEK293T cells with CRTC1 along with the CRTC-interacting domain of CREB (GAL4-bZIP) or the phospho-serine mutant GAL4-bZIP R314A were analyzed for luciferase activity. (E) Effect of CRTC1 on alternative exon splicing with TATA-less and TATA-containing core promoters. Transient transfection assays of *GAL4 UAS* EDI reporter cotransfected into HEK293T cells with CRTC1 along with the CRTC-interacting domain of CREB (GAL4-bZIP) or the phospho-serine mutant GAL4-bZIP R314A. RT-PCR products were separated by PAGE and splice variants analyzed to determine the effect of CRTC expression on splice site selection. Identities of spliced and unspliced

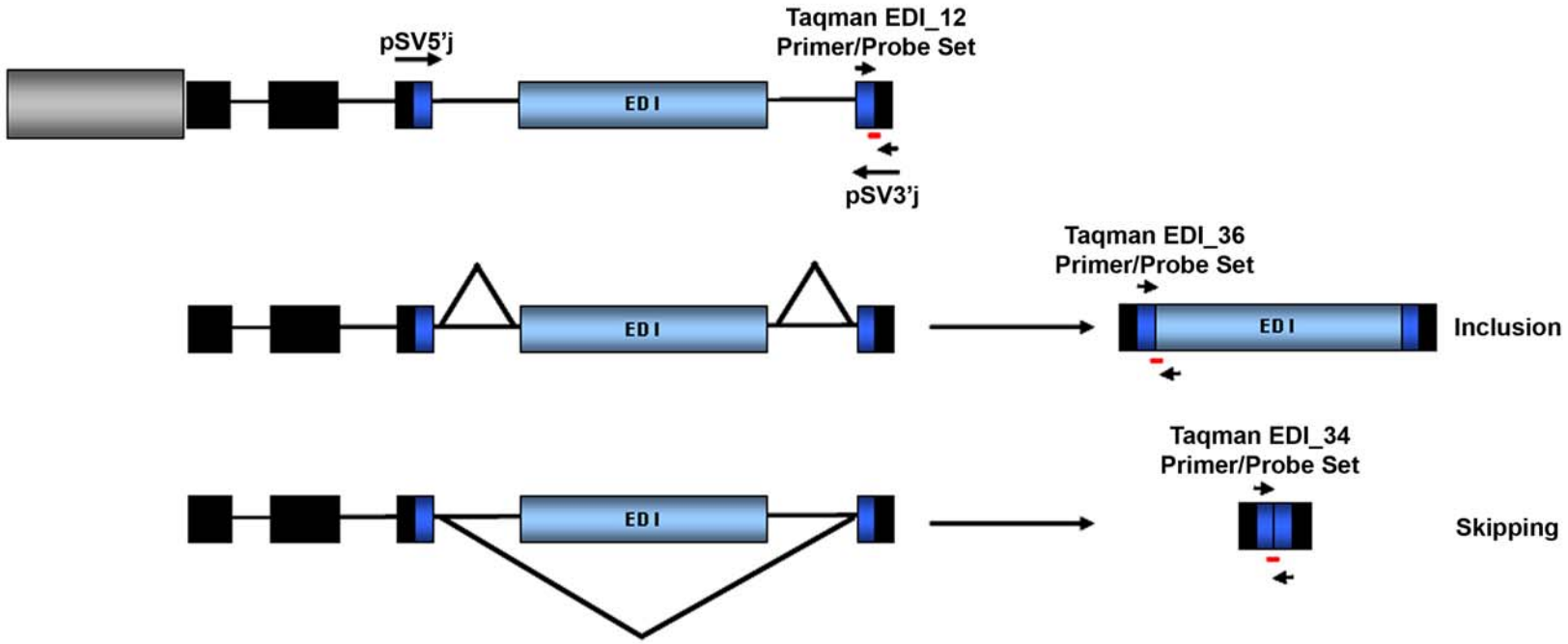
amplicons are indicated on the right side of the panel. (F) Taqman real-time qPCR analysis of CRTC1 on alternative exon splicing with TATA-less and TATA-containing ED I minigenes. The relative exon skipping levels are expressed as the ratio of skipped versus included transcripts (n=3 experiments; mean ± SEM). (G) Effect of direct GAL4-CRTC1 recruitment to the *5xUAS* TATA-less and TATA-containing luciferase reporters. Transient transfection assays of each *GAL4 UAS* luciferase reporter cotransfected into HEK293T cells with GAL4-CRTC1 were analyzed for luciferase activity. (H) Effect of direct GAL4-CRTC1 recruitment to the *5xUAS* TATA-less and TATA-containing ED I minigene. RT-PCR products were separated by PAGE and splice variants analyzed to determine the effect of CRTC expression on splice site selection. Identities of spliced and unspliced amplicons are indicated on the right side of the panel. (I) Taqman real-time qPCR analysis of direct GAL4-CRTC1 recruitment on alternative exon splicing with TATA-less and TATA-containing ED I minigenes. The relative exon skipping levels are expressed as the ratio of skipped versus included transcripts (n=3 experiments; mean ± SEM).





Supplemental Figure S1

A

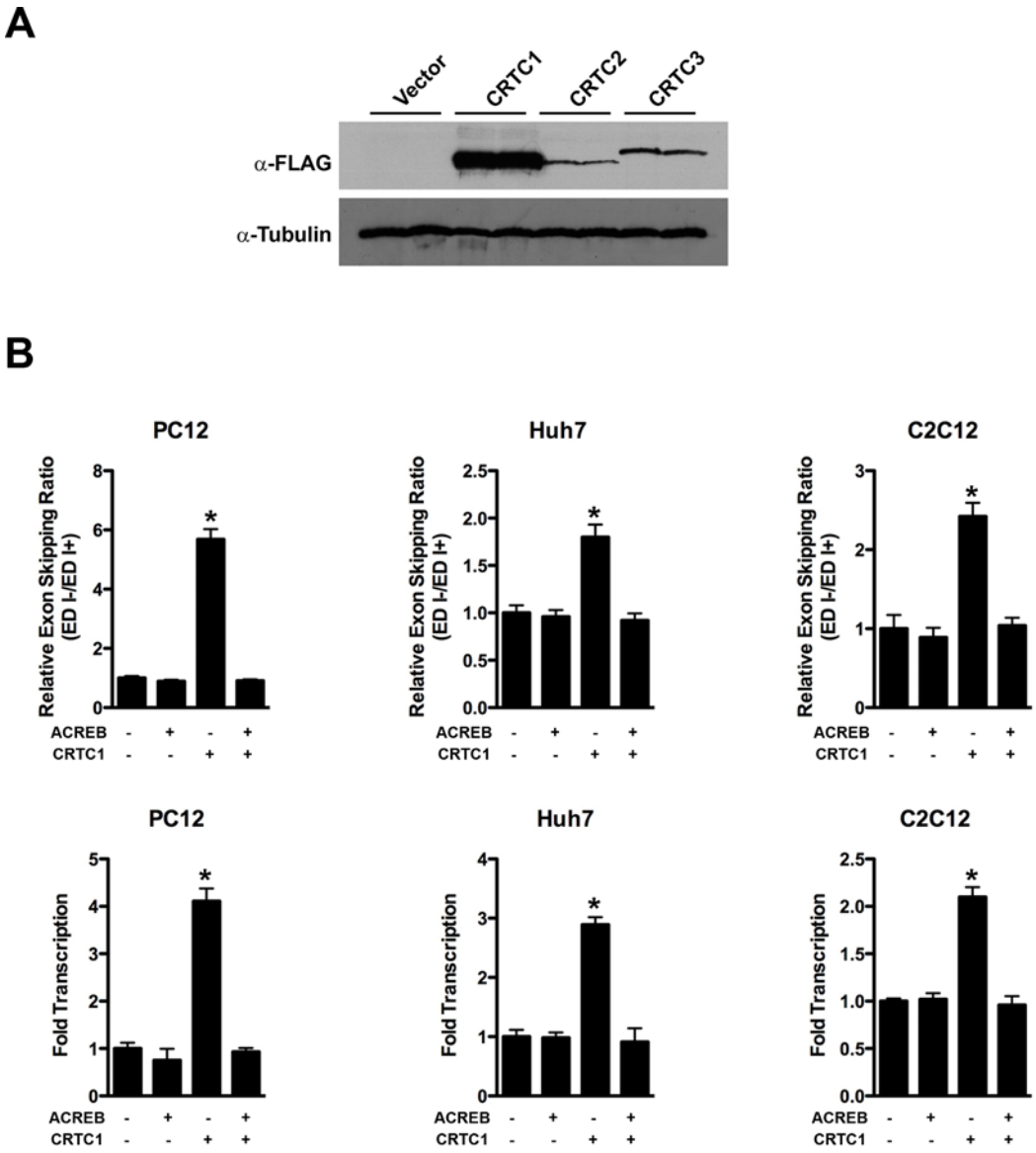


B

Taqman Real-time PCR primer and probe sequences

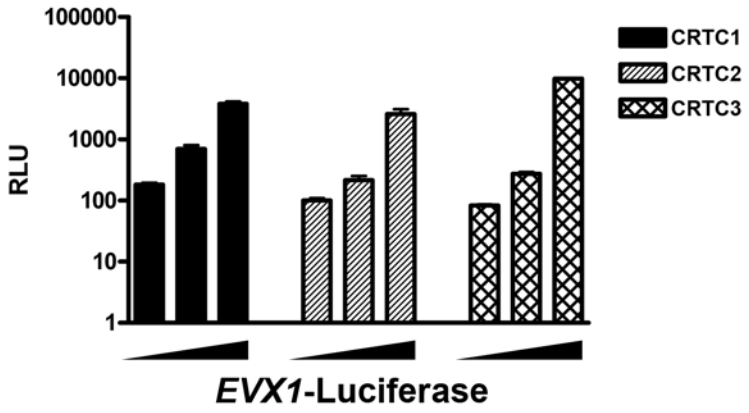
Target	Sequence (5' to 3')
XP2_Luc	GTACGCGGAATACTTCGAAATGTC (Forward) TGCATACGACGATTCTGTGATTTGT (Reverse) TCGGTTGGCAGAAGCTAT (Probe)
EDI_12	CCCAATGTTCAGCTCACTGGATA (Forward) CCAGGGAGGCGTGCA (Reverse) CCAGGGTCACGATCCAG (Probe)
EDI_36	AGAATCCAAGCGGAGAGAGTCA (Forward) ATCAGTGAATGCCAGTCCTTTAGG (Reverse) CTGCAGTAACCAACATTG (Probe)
EDI_34	AGAATCCAAGCGGAGAGAGTCA (Forward) GGTGTGACCTGAGTGAACTTCA (Reverse) ACTGCAGTAACCACTATTC (Probe)

Supplemental Figure S2

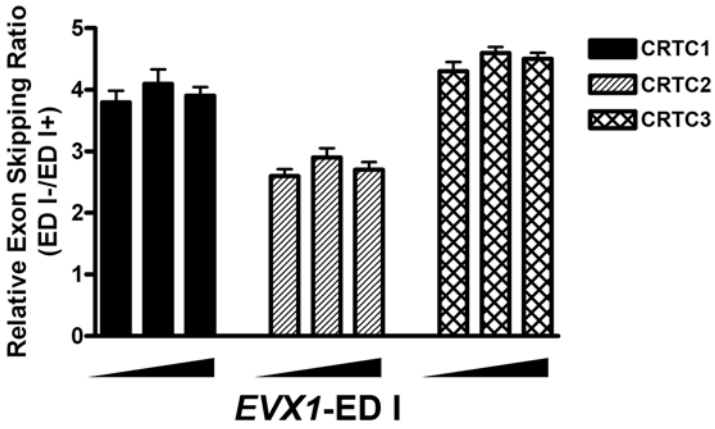


Supplemental Figure S3

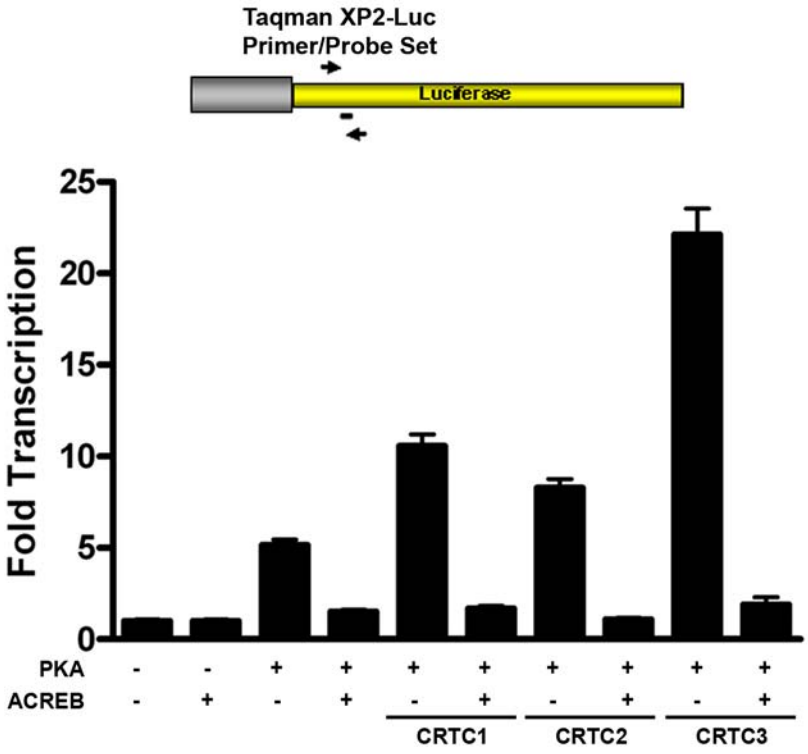
A



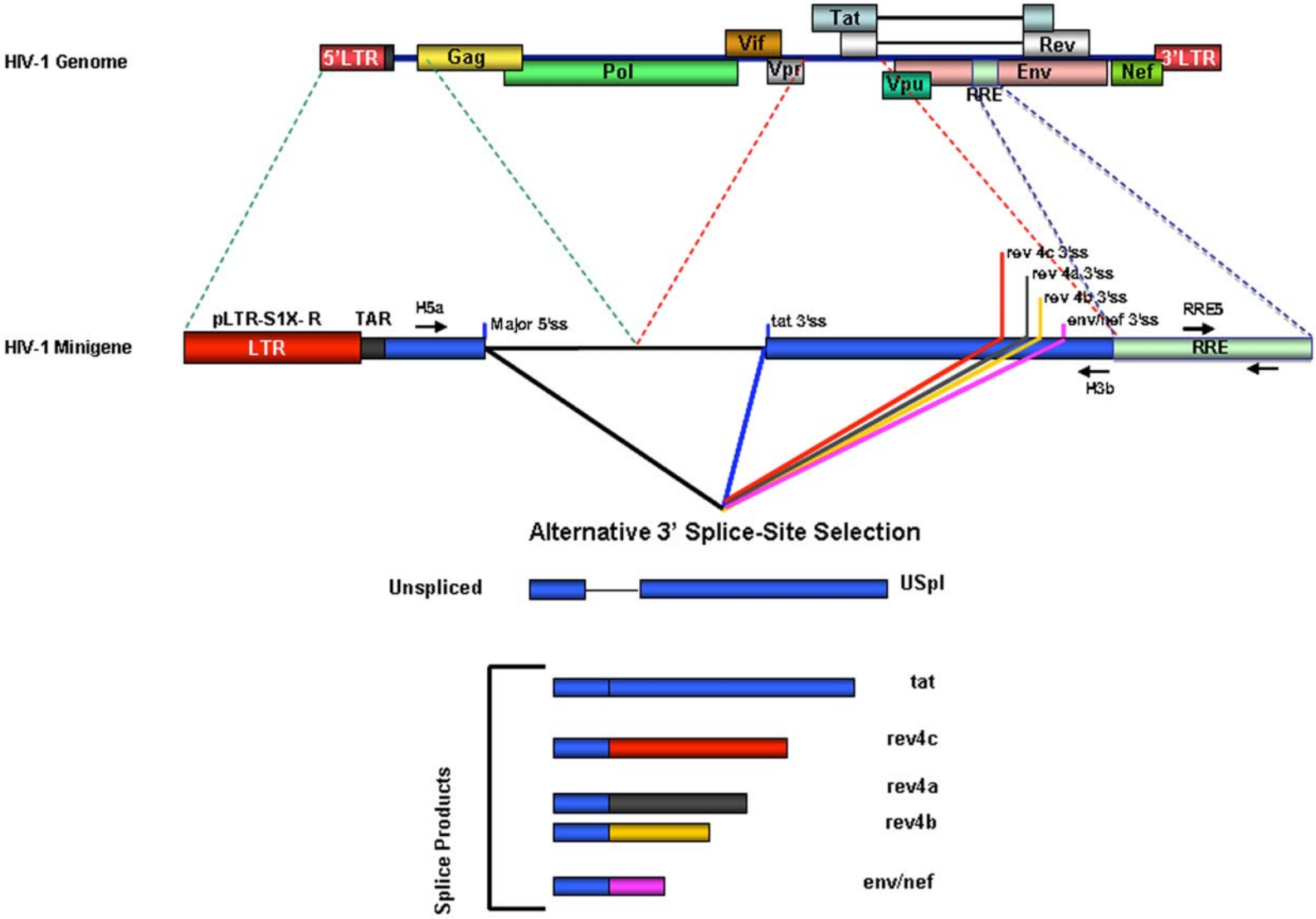
B



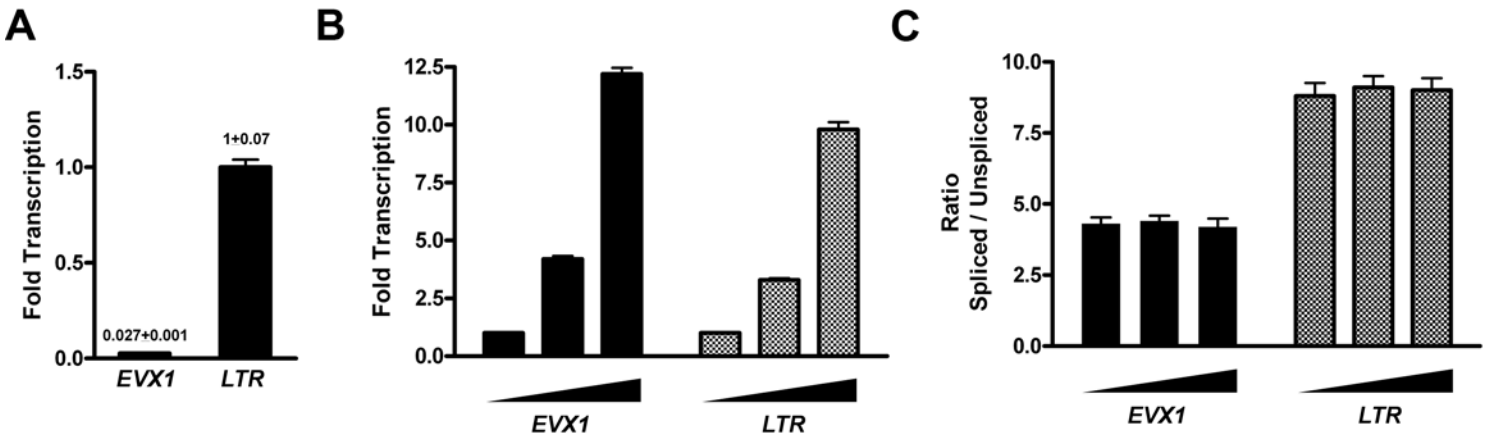
Supplemental Figure S4



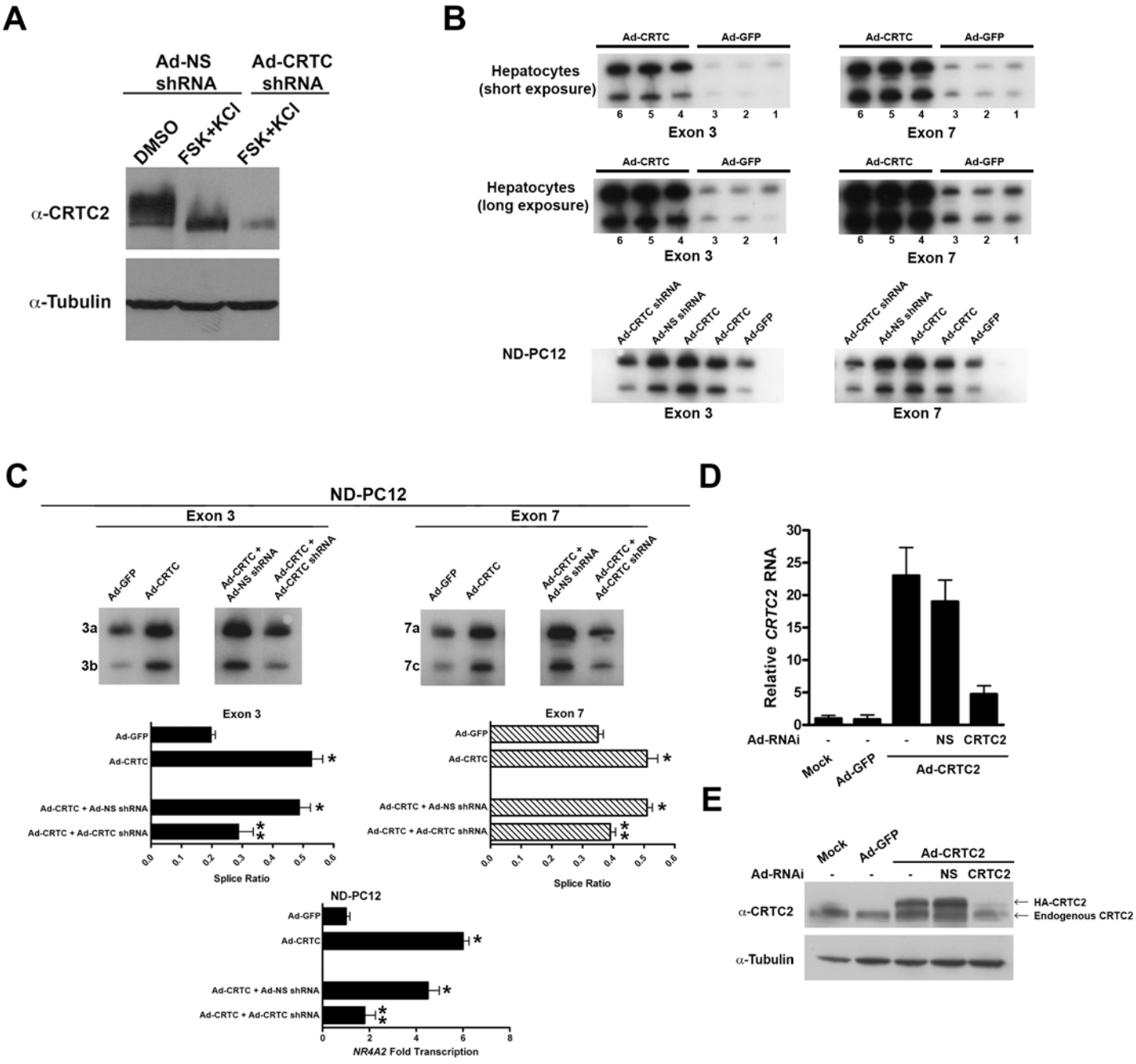
Supplemental Figure S5



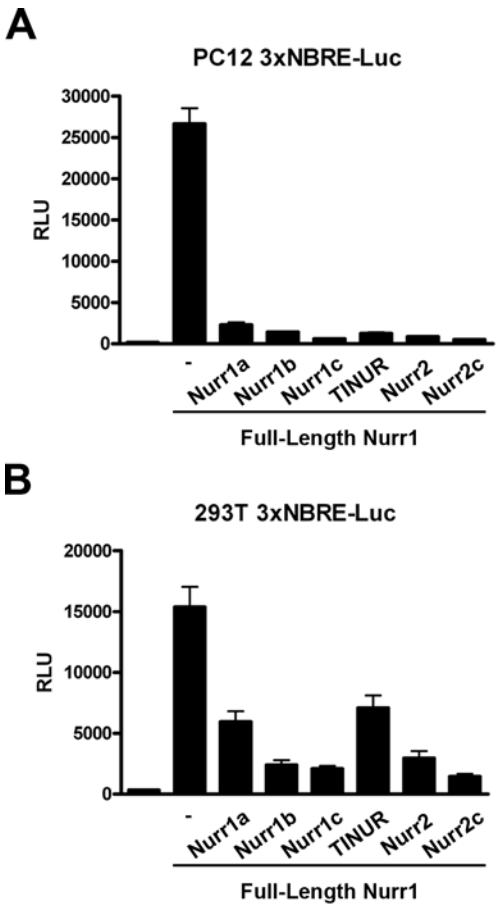
Supplemental Figure S6



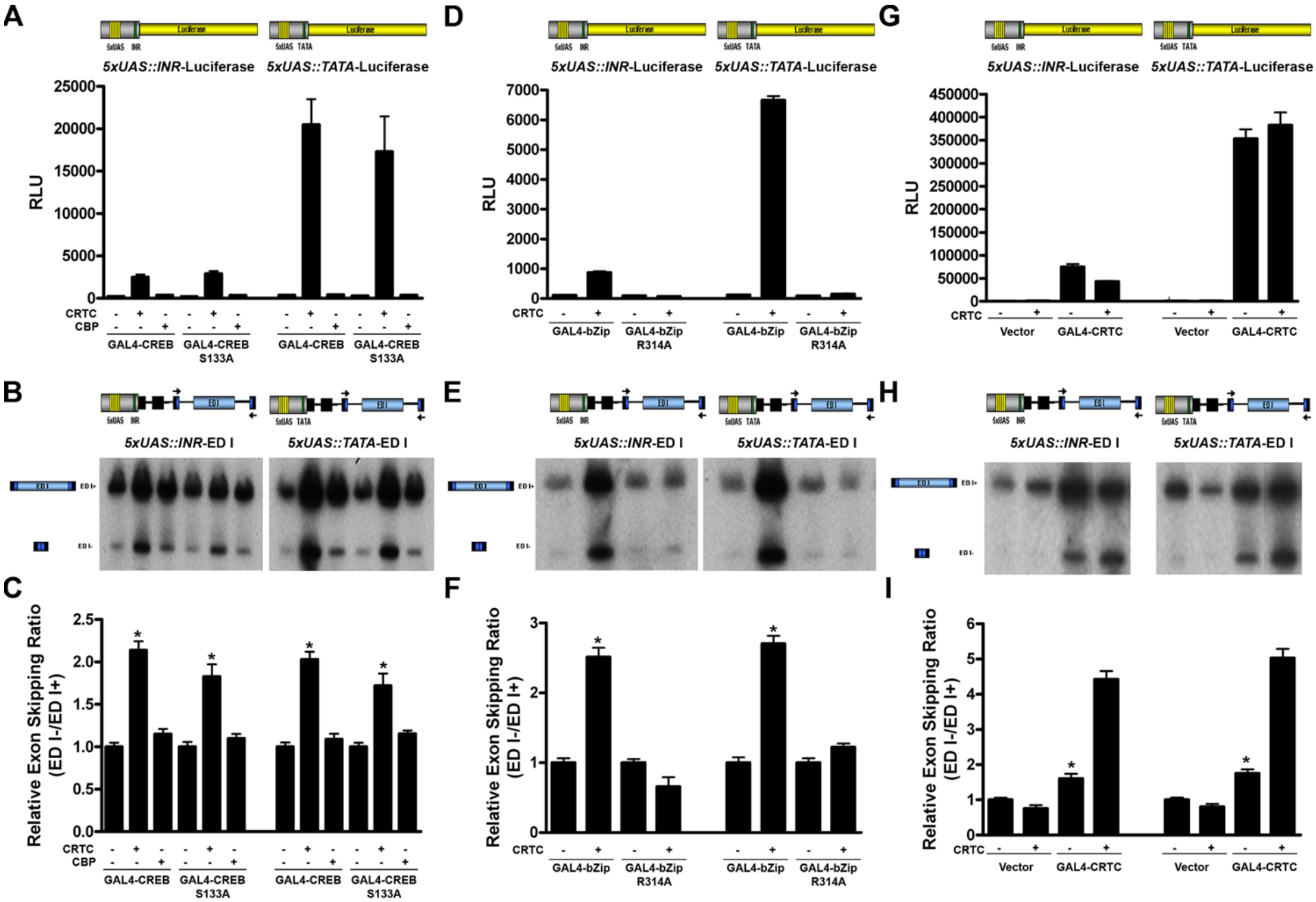
Supplemental Figure S7



Supplemental Figure S8



Supplemental Figure S9



Supplemental Figure S10