SUPPLEMENTARY INFORMATION

Inducible nuclear import by TetR aptamer-controlled 3' splice site selection

Adam A. Mol[1], Marc Vogel[1] and Beatrix Suess[1,2]

[1] Department of Biology, Technical University of Darmstadt, Schnittspahnstrasse 10, D-64287 Darmstadt, Germany, Tel.: +49 6151 16 22000, Tel.: +49 6151 16 22003

[2] Centre for Synthetic Biology, Technical University of Darmstadt

Correspondence should be addressed to BS (bsuess@bio.tu-darmstadt.de).

Running title: TetR aptamer controlled splicing

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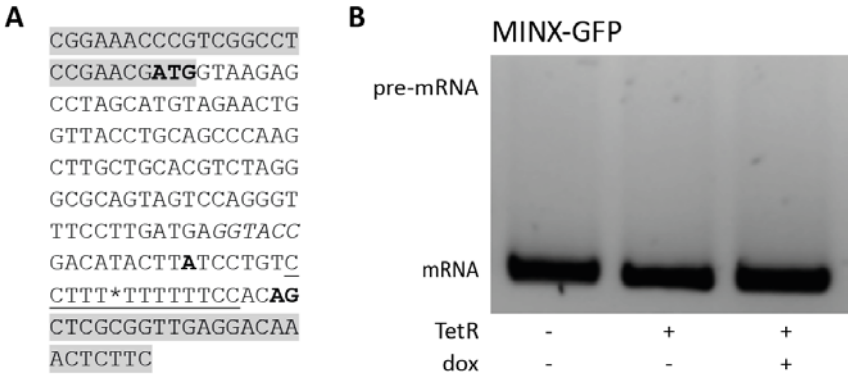
Adam Mol

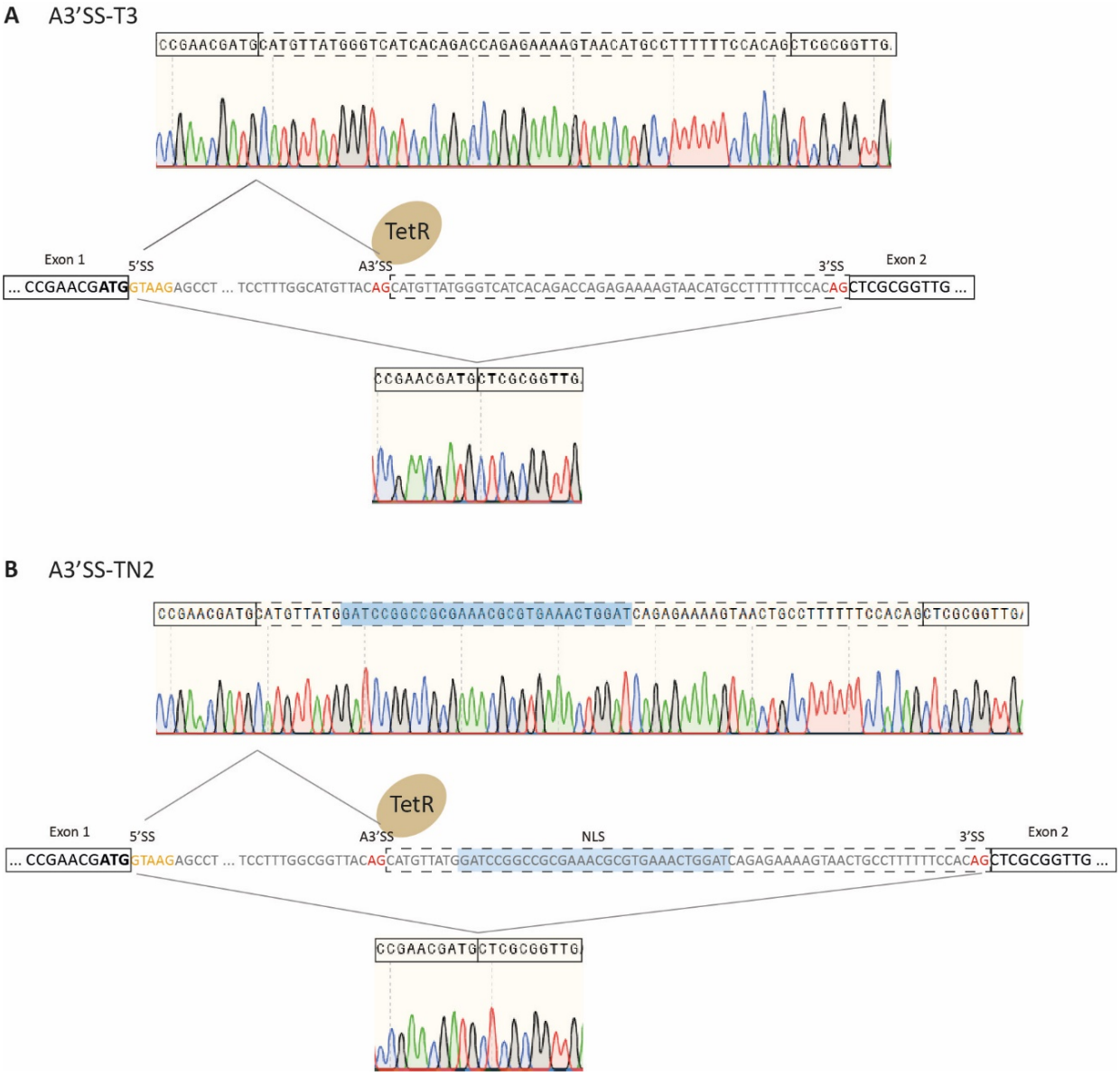
CONTENT

Supplementary Figure S1



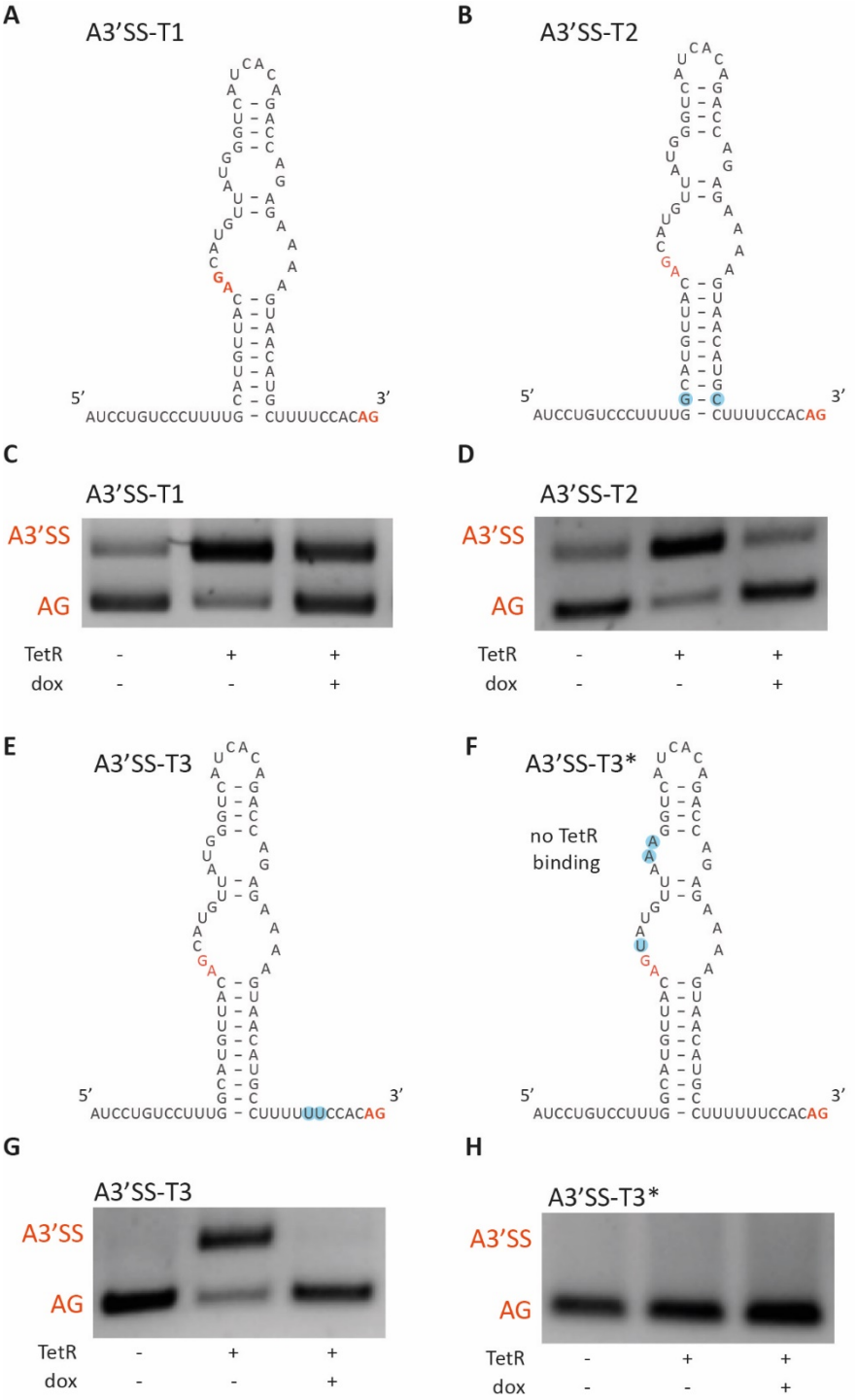
MINX intron (A) Sequence of the MINX intron used. Flanking exons are highlighted in gray. The start codon, the branch point adenosine and the AG are indicated in bold, the pyrimidine-tract is underlined. An introduced *KpnI* restriction site is shown in italics. The position of the aptamer insertion is indicated by *. **(B)** The splicing pattern of the control construct MINX-GFP is visualized by RT-PCR. HeLa cells were transiently transfected with the control construct MINX-GFP, transfected with a plasmid expressing TetR and treated with (+) or without (-) 50 µM doxycycline (dox) for 24 h. Total RNA was prepared and used for RT-PCR with primer pairs binding to both exons. The band corresponds to the spliced mRNA. The experiment was repeated three times with similar results.

Supplementary Figure S2



Electropherogram demonstrating alternative 3'SS usage. (A) Construct A3'SS-T3, (B) construct A3'SS-TN2. Exon sequences are presented as boxes. The sequence additionally spliced in after TetR binding is shown as box with a dashed line. The 5'SS is highlighted in orange, the 3'SS in red, the start codon is given in bold, the nuclear localization sequence (NLS) is displayed in blue. Colors of the electropherogram: A: green, C: blue, G: black, T: red. The corresponding sections of the electropherogram of the (alternatively) spliced RNA is shown below and above the sequence, respectively.

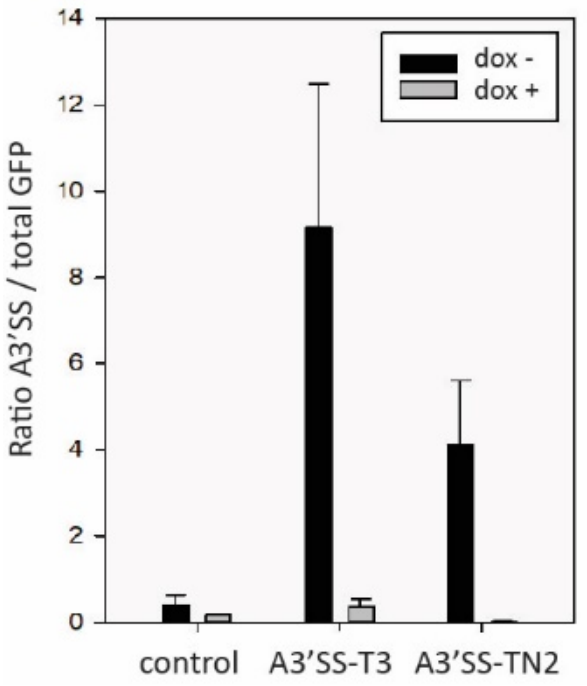
Supplementary Figure S3



TetR aptamer in the context of the MINX intron (A,B,E,F) Secondary structure of the TetR aptamer located at the 3' end of the intron. Changes in the sequence are highlighted in blue. The A3'SS and the canonical AG are highlighted in red. The mutations introduced into T3* prevent TetR binding. **(C,D,G,H)** Splicing patterns were visualized by RT-PCR. HeLa cells were transiently transfected with the constructs A3'SS-T1, -T2, -T3 and -T3*, co-transfected with a plasmid expressing TetR and treated with (+) or without (-) 50 µM dox for 24 h. Total RNA was prepared and used for RT-PCR with primer pairs binding to both exons. The spliced products were cloned and sequenced for verification. The upper band corresponds to usage of A3'SS and the lower to distal AG. All experiments were repeated three times with similar results.

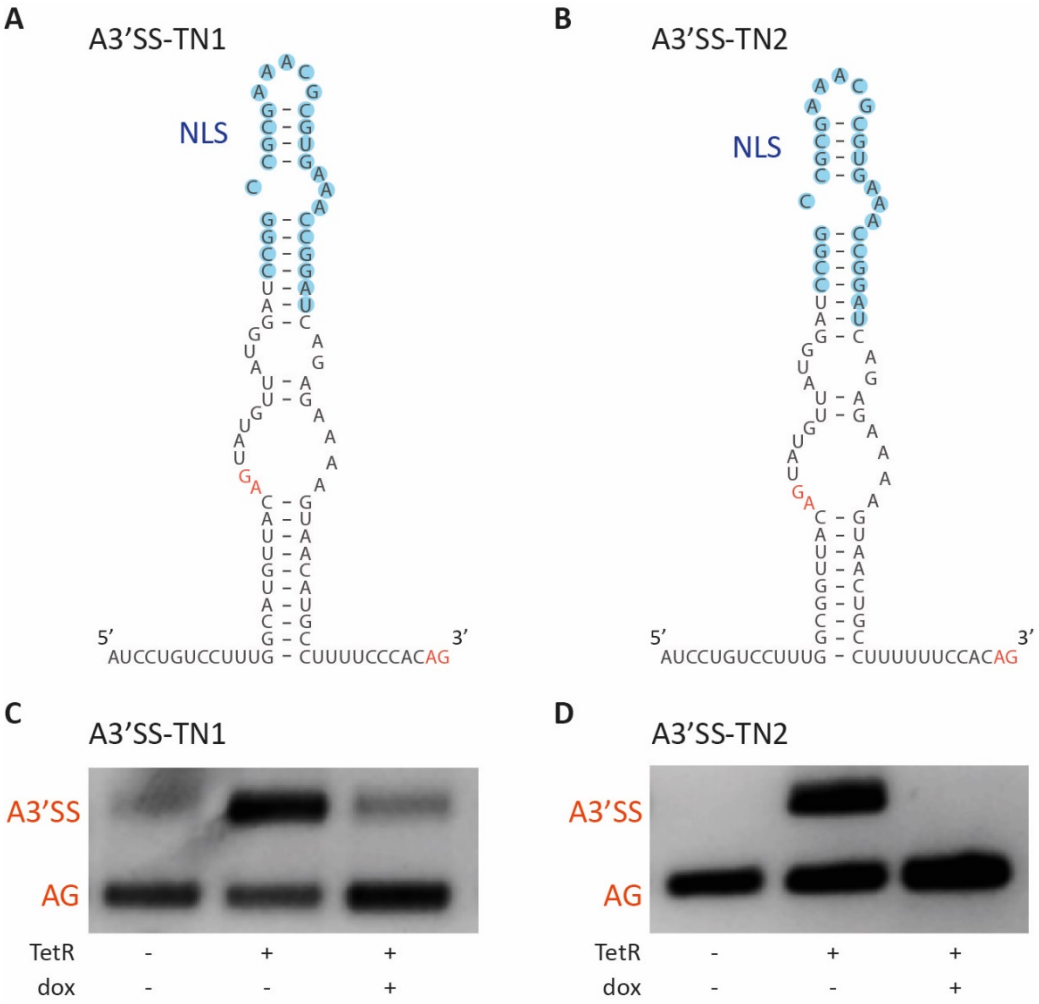
Adam Mol

Supplementary Figure S4



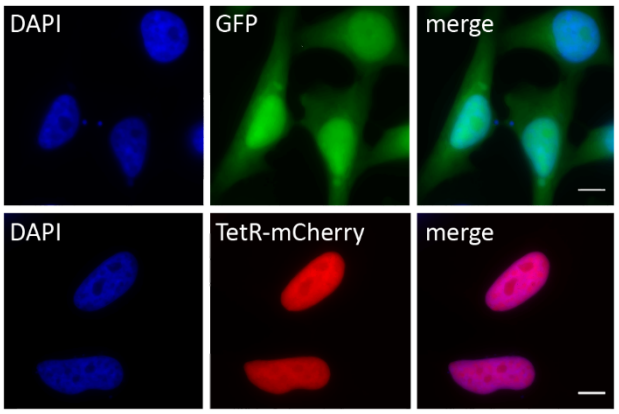
qRT-PCR analysis of A3'SS activation Cells were transiently transfected with a plasmid expressing TetR (control) and the constructs A3'SS-T3 or A3'SS-TN2 and treated with (+) or without (-) 50 µM dox for 24 h. Total RNA was prepared and used for qRT-PCR for quantification. Results were normalized as $2^{\Delta\Delta-Ct}$. Error bars represent the standard deviation from the means from three independent experiments.

Supplementary Figure S5



Insertion of a NLS sequence within the TetR aptamer (A,B) Sketches of the A3'SS-TN1 and A3'SS-TN2 constructs with inserted NLS sequence (in blue) replacing the terminal stem-loop structure of TetR aptamer. Alternative A3'SS and distal AG are displayed in red. **(C,D)** Splicing pattern visualized by RT-PCR. HeLa cells were transiently transfected with the constructs A3'SS-T1 and A3'SS-T2 and co-transfected with plasmid expressing TetR (+) and treated with (+) or without (-) 50 µM dox for 24 h. Total RNA was prepared and used for RT-PCR with primer pairs binding to both exons. The upper band corresponds to usage of A3'SS and the lower to distal AG. The spliced products were cloned and sequenced for verification. Experiments were repeated three times with similar results.

Supplementary Figure S6



Microscopic visualization of GFP and TetR-mCherry of the control construct without the aptamer (A) GFP is stably integrated into a HeLa HF1-3 cell line using the Flp-In system. The cells were transiently transfected with mCherry-tagged TetR. Cells were fixed and stained with DAPI. Scale bar 10 µm.