

## SUPPLEMENTAL INFORMATION

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### Supplemental Figure legends

#### **FIGURE S1. Analysis of U6 snRNA structure in the B<sup>act</sup> and B\* complexes.**

(A) DMS modifications and SHAPE (1M7) analysis of U6 snRNA.

(B), (C) CMCT and KE modifications of U6 snRNA.

(D), (E) Analysis of N7 positions in U6 snRNA with DMS and DEPC plus aniline.

Chemicals employed and complexes analysed are shown above the panels. For orientation, important features of the RNAs are shown on the right.

#### **FIGURE S2. Analysis of U2 snRNA structure in the B<sup>act</sup> and B\* complexes.**

(A) DMS and DMS/aniline modifications, and SHAPE analysis of U2 snRNA.

(B), (C), and (D) CMCT (B), KE (C) modifications and DEPC (D) analysis of U2 snRNA.

Labelling is as in Supplemental Fig. S1, important features of the RNAs are shown on the right.

#### **FIGURE S3. Analysis of pre-mRNA structure in the B<sup>act</sup> and B\* complexes.**

(A), (B), (C), and (D) RNA structure probing of nucleotides around the 5' splice site. DMS and CMCT (A) and KE (B) modifications and analysis of N7 positions ((C) and (D)).

(E), (F), and (G) RNA structure probing of nucleotides around the branch site. DMS and CMCT modifications (E) and analysis of N7 positions ((F) and (G)).

Labelling is as in Supplemental Fig. S1. The 5' splice site (5' ss) and the branch site (BS) are indicated.

**FIGURE S4. Structural analysis of the 3' region of the intron RNA in the B<sup>act</sup> and B\* complexes.**

Dots above the RNA sequence indicate the Watson-Crick reactivity of nucleotides against the chemical compounds, while dots below the sequence refer to the SHAPE reactivity. The accessibility of N7 positions is indicated by arrows: dashed ones, poorly protected; closed ones, fully protected. The degree of accessibility and the complexes analysed are coded as shown in the inset.

**FIGURE S5. The exon binding channel in the vicinity of the exon nucleotides with diminished flexibility after Prp2 activation.**

(A) Overview of the cryo-EM structure of the yeast B<sup>act</sup> spliceosome [5LQW, Rauhut et al. (2016)] illustrating the 5' exon binding channel. The major landmarks are labelled according to Rauhut et al. (2016).

(B) Close up of the path of the 5' exon in the yeast B<sup>act</sup> spliceosome from (A). Red: nucleotides with reduced flexibility in the B\* complex. All involved components are labelled and the catalytic centre is behind the plane of the image.

(C) Close up of the electrostatic surface composed of sections of Prp8 and Cwc22 in the cryo-EM structure of the yeast B<sup>act</sup> [5GM6, Yan et al. (2016)]. The particular architecture creates a channel for entry of the 5' exon that funnels it towards the catalytic centre. In this respect, the exon is held in position independently of its interaction with U5 snRNA loop 1. The heat map coloration is as described in Fig. 4.

**FIGURE S6. U2-A30 is the pivot point for the rotational movement of the complete 3' domain of the U2 snRNP.**

(A), (B) and (C) Overlay of the RNA-networks of the yeast B<sup>act</sup>, C, and C\* complexes, all aligned on U5 snRNA. PDB accession codes are given in brackets. Blue nucleotides: branch

point (BP) and 5' ss. The pivotal U2-A30 is in red and is part of the flexible linker 1, AAGU (nts 30-33 of yeast U2 snRNA; Fig. 2B).