Expanded View Figures

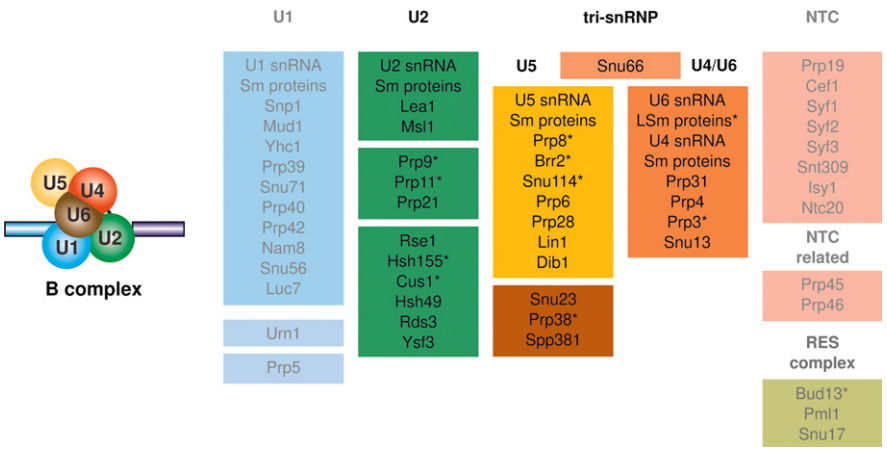

Figure EV1. Protein composition of the spliceosomal B complex from *Saccharomyces cerevisiae*.
The protein composition of the yeast spliceosomal B complex was determined by mass spectrometry, and the proteins (yeast nomenclature) are grouped according to their snRNP association, function or presence in a stable heteromeric subcomplex (Fabrizio *et al*, 2009). Full colours indicate proteins present in stoichiometric amounts, while pale colours and grey letters indicate proteins present in substoichiometric amounts. Asterisks mark the proteins that were located in this study. A schematic drawing of the B complex is given on the left.

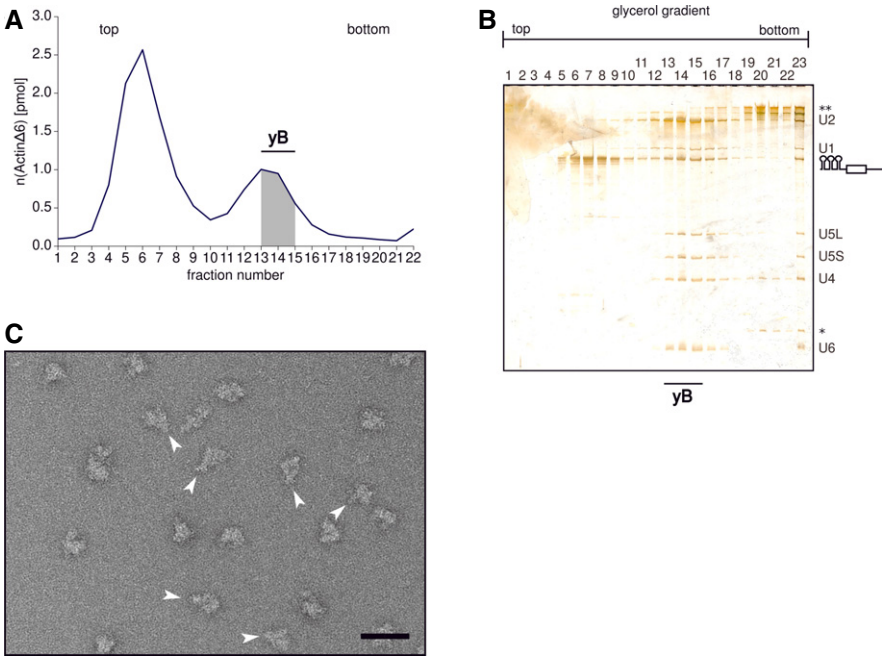

Figure EV2. Purification of yeast spliceosomal B complexes using the modified method.

A Radioactivity profile of the glycerol gradient. The free pre-mRNA migrated in fractions 4–7 and the yeast spliceosomal B complexes (yB) in fractions 13–15.

B RNA composition of the purified yeast spliceosomal complexes. In fractions 13–16 that correspond to the B complex peak (~40S), all five snRNAs (U2, U1, U5L/U5S, U4 and U6) can be found, although the U1 snRNA seems to be present in substoichiometric amounts. The band corresponding to the M3-ActinΔ6 pre-mRNA is labelled with a schematic drawing. * labels the band of 5S ribosomal RNA, and ** marks the bands corresponding to the ribosomal 18S and 28S RNAs that are present as contaminations in the high-density fractions.

C Negative-stain EM overview image (taken at 80,000-fold magnification) of the purified yeast B complexes. The purification yielded a homogenous sample without any aggregates, and a large number of particles display the features characteristic of the main view of the yeast B complex (indicated with white arrows). The scale bar represents 60 nm.

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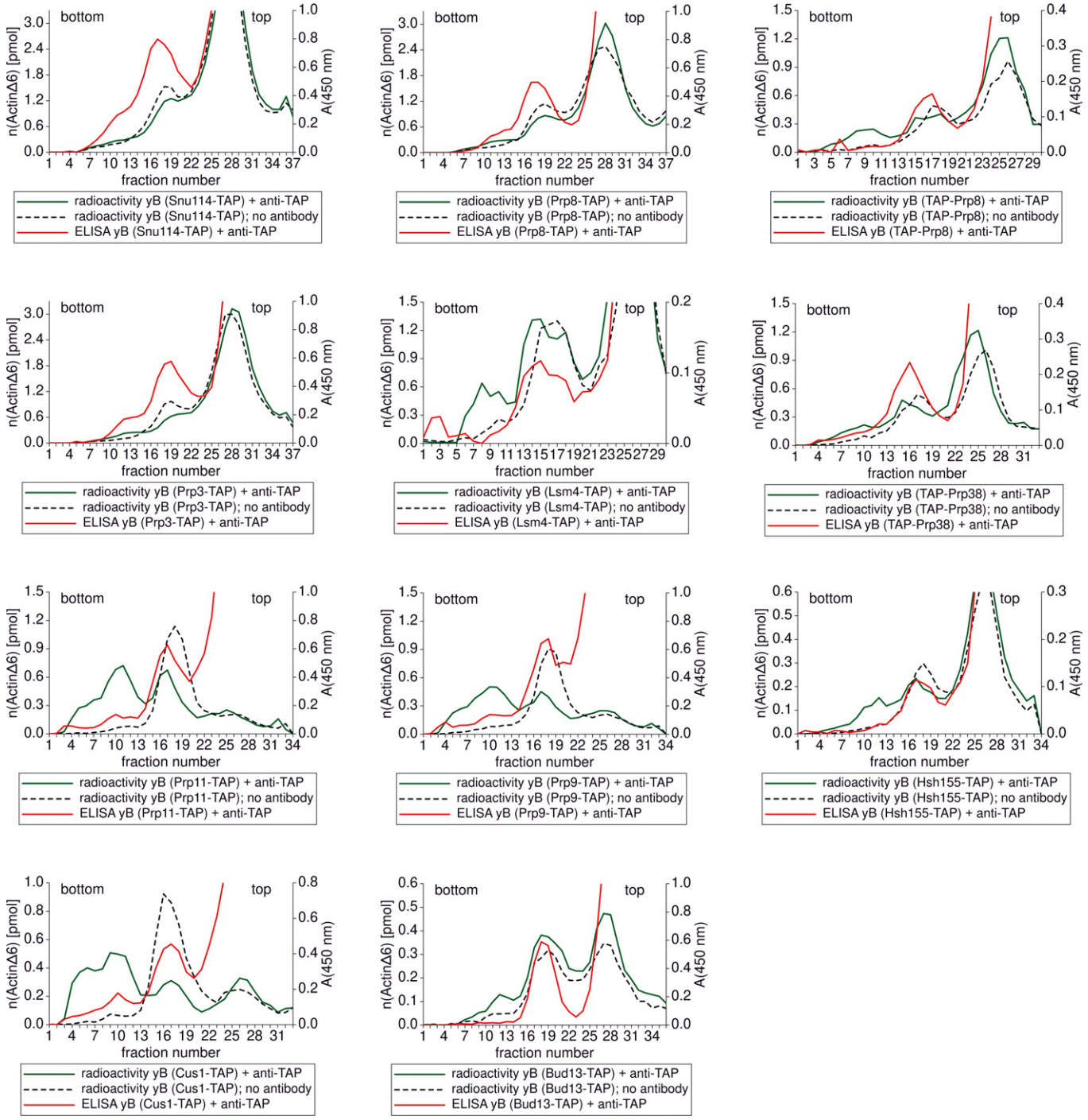

Figure EV3. Gradient profiles of anti-TAP-labelled spliceosomal B complexes harbouring selected TAP-tagged proteins.
The green line represents the radioactivity profile of the gradient on which immuno-labelled B complexes were separated. The dotted line is the radioactivity profile of the control gradient on which B complexes without antibody addition were separated. The signal of the ELISA that was performed to visualise the antibody content of the gradient fractions is shown in red. The protein fused to the TAP tag that was present in spliceosomal B complexes is identified in the legend below each graph. TAP-(protein name) stands for an N-terminal TAP tag and (protein name)-TAP for a C-terminal TAP tag.

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