ELUCIDATION OF THE ASSEMBLY EVENTS REQUIRED FOR THE RECRUITMENT OF Utp20, Imp4 AND Bms1 ONTO NASCENT PRE-RIBOSOMES

By

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SUPPLEMENTARY MATERIAL

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1. Legends to Supplementary Figures

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1. LEGENDS TO SUPPLEMENTARY FIGURES

FIGURE S1. Schematic representation of the pre-rRNA processing pathways. The 35S pre-rRNA precursor contains the nucleotide sequences for the 18S, 5.8S, and 25S rRNAs. These sequences are flanked by 5' and 3' external transcribed spacers (ETS) and separated by two internal transcribed spacers (ITS). In the main processing pathway, the 35S pre-rRNA is cleaved at site A_0, producing the 33S pre-rRNA species. This molecule is then cleaved at sites A_1 and A_2 to produce the 20S and $27SA_2$ pre-rRNAs. The 20S pre-rRNA is exported to the cytoplasm where undergoes dimethylation by Dim1 and further cleavage at site D to generate the mature 18S rRNA. $27SA_2$ follows a series of complex processing steps to produce the 5.8S and 25S rRNAs (not shown for simplicity). Cells can also use an alternative pathway, in which cleavage at A_3 occurs prior to cleavages at A_0-A_2 sites. This yields a 23S species that comprises the 5' ETS, the 18S rRNA and the ITS1 fragment. It has been suggested that this abnormal 23S species cannot be further processed to generate the 18S rRNA (1). In rapidly growing cells, ~ 70% of nascent pre-rRNAs are cleaved at the A_0-A_2 sites co-transcriptionally, and the rest (~ 30%) are processed after complete transcription of the 35S pre-rRNA (2,3). More detailed information about the pre-rRNAs processing pathways can be found elsewhere (4,5).

FIGURE S2. Sedimentation behavior of Utp20-MYC, Imp4-MYC and Bms1-MYC in wild type cells. Cellular extracts prepared from the indicated yeast strains (top) grown in medium containing glucose were resolved in 7-50% linear sucrose gradients. After ultracentrifugation, 0.5 ml fractions were collected from the top of the tube. The content of Utp20-MYC (A), Imp4-MYC (B) and Bms1-MYC (C) in each fraction was analyzed by anti-MYC immunoblotting (first panels from top). In parallel, total RNAs were prepared from each fraction and analyzed by Northern blot with either an

oligonucleotide probe to the 35S A_2-A_3 region (second panels from top) or to the U3 snoRNA (bottom panels). The number of each fraction and the sedimentation positions of 40S and 80S particles are indicated at the bottom. Glu, glucose; WB, Western blot; NB, Northern blot.

FIGURE S3. Protein composition analysis of the complexes formed by Utp20, Imp4 and Bms1 in wild type and Rrp5-, Nan1- or Pwp2-depleted cells. (A-L) The MYC-tagged proteins used as baits, and the proteins depleted before complex purifications are indicated on the top. The proteins co-purifying with the bait were purified using an anti-MYC affinity matrix, fractionated electrophoretically, silver-stained and identified by mass spectrometry. The identified proteins are indicated on the left, and the migration of molecular weight markers is indicated on the right.

FIGURE S4. Protein composition analysis of pre-ribosomal complexes in cells depleted of Nan1, Rrp5, Utp20, Imp4 or Bms1. (A-F) The MYC-tagged proteins used as baits, and the proteins depleted before complex purifications are indicated on the top. The proteins co-purifying with the baits were purified using an anti-MYC affinity matrix, fractionated electrophoretically, silver-stained and identified by mass spectrometry. The identified proteins are indicated on the left, and the migration of molecular weight markers is indicated on the right.

2. REFERENCES TO SUPPLEMENTARY INFORMATION

1. Lamanna, A.C. and Karbstein, K. (2011) An RNA Conformational Switch Regulates Pre-18S rRNA Cleveage. *J. Mol. Biol.* **405**, 3-17.
2. Kos, M. and Tollervey, D. (2010) Yeast pre-rRNA processing and modification occur cotranscriptionally. *Molecular cell*, **37**, 809-820.
3. Osheim, Y.N., French, S.L., Keck, K.M., Champion, E.A., Spasov, K., Dragon, F., Baserga, S.J. and Beyer, A.L. (2004) Pre-18S Ribosomal RNA Is Structurally Compacted into the SSU Processome Prior to Being Cleaved from Nascent Transcripts in Saccharomyces cerevisiae. *Molecular cell*, **16**, 943-954.
4. Venema, J. and Tollervey, D. (1999) Ribosome synthesis in Saccharomyces cerevisiae. *Annu Rev Genet*, **33**, 261-311.
5. Fatica, A. and Tollervey, D. (2002) Making ribosomes. *Current opinion in cell biology*, **14**, 313-318.

3. SUPPLEMENTARY FIGURES S1-S4

Figure S1
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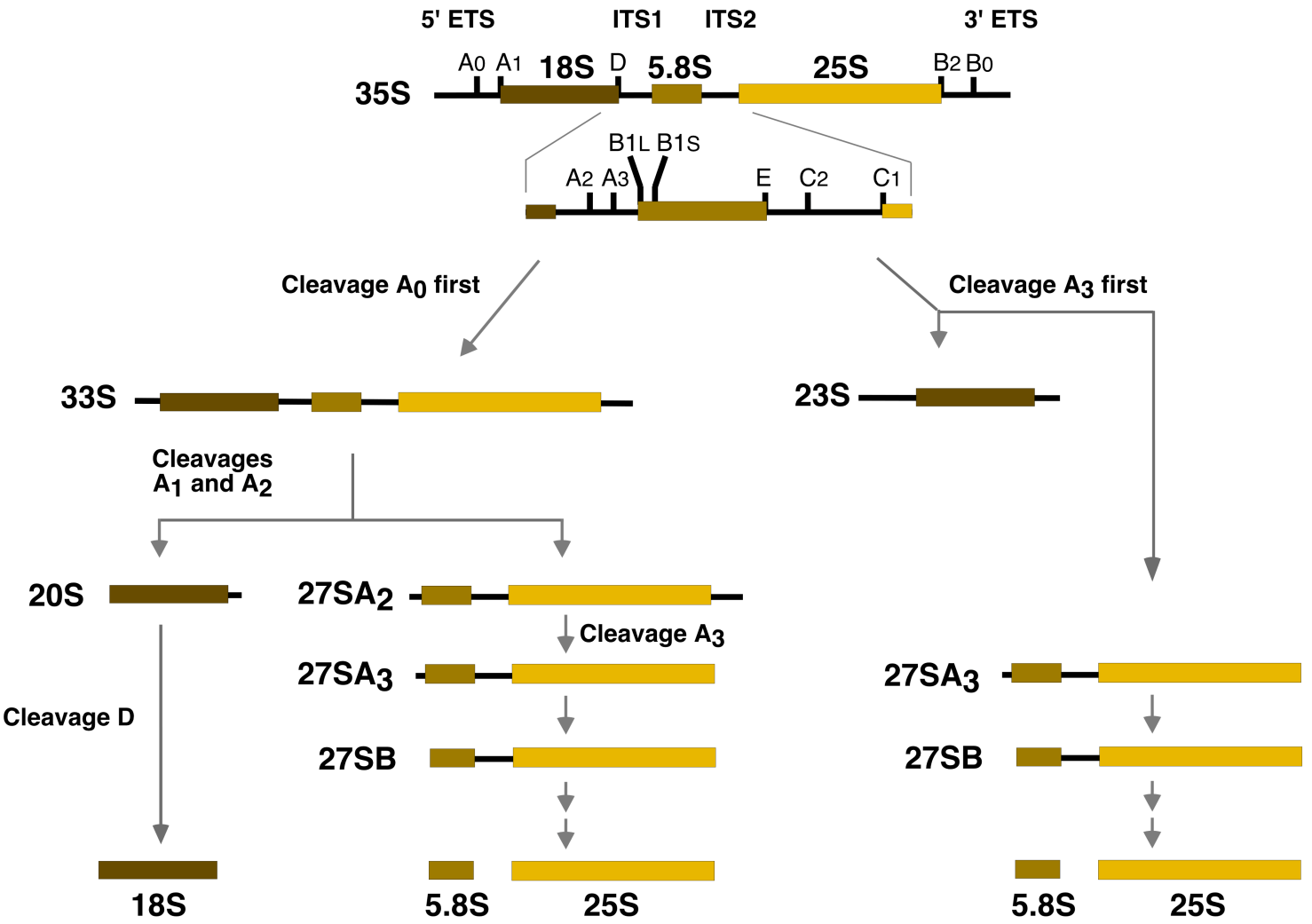

Figure S2
Perez-Fernandez et al.

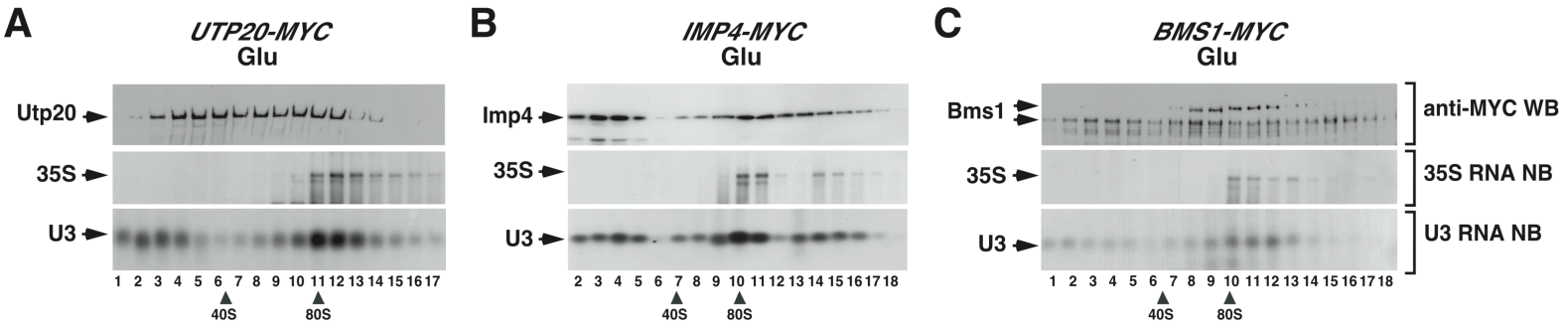

Figure S3
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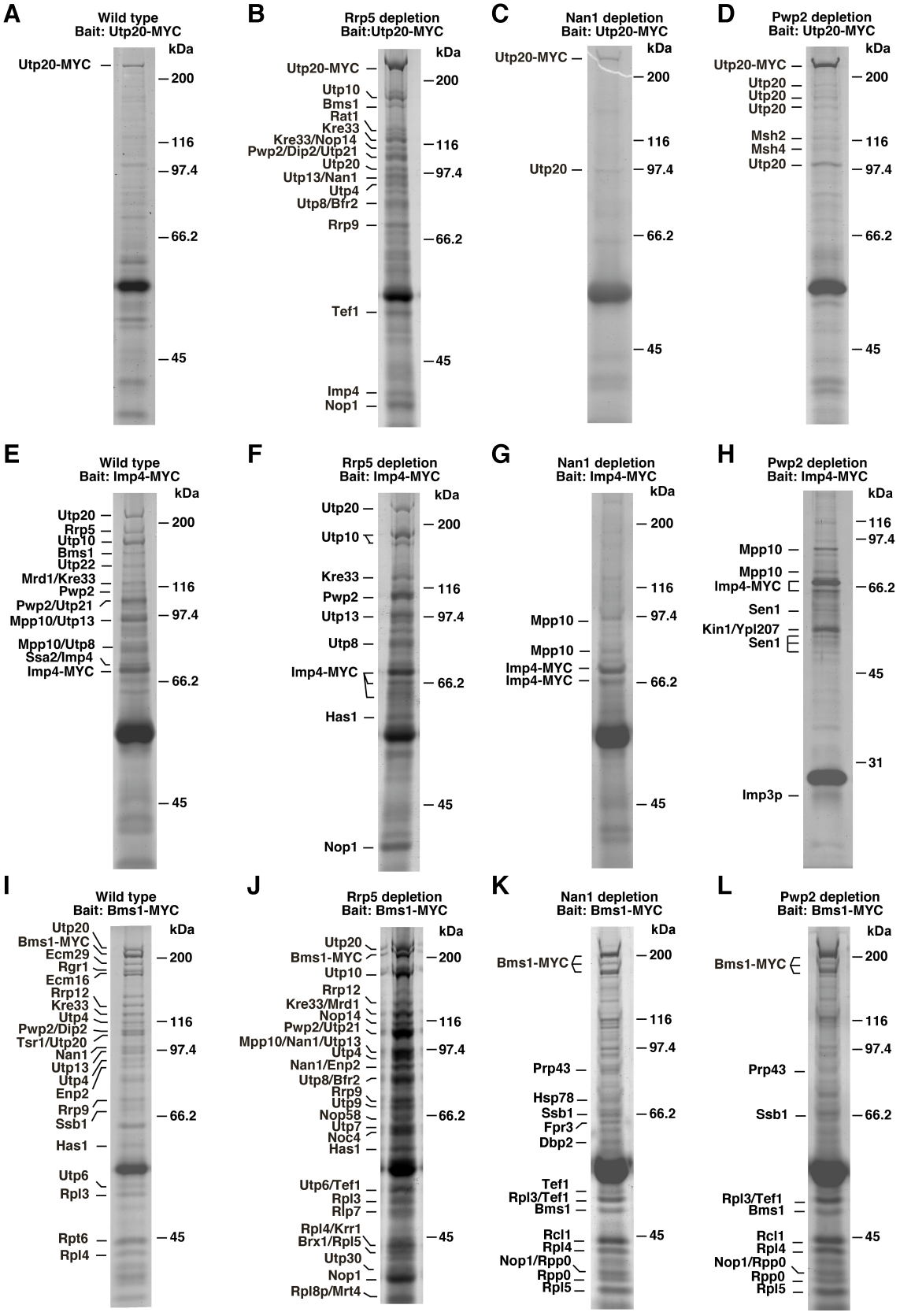

Figure S4
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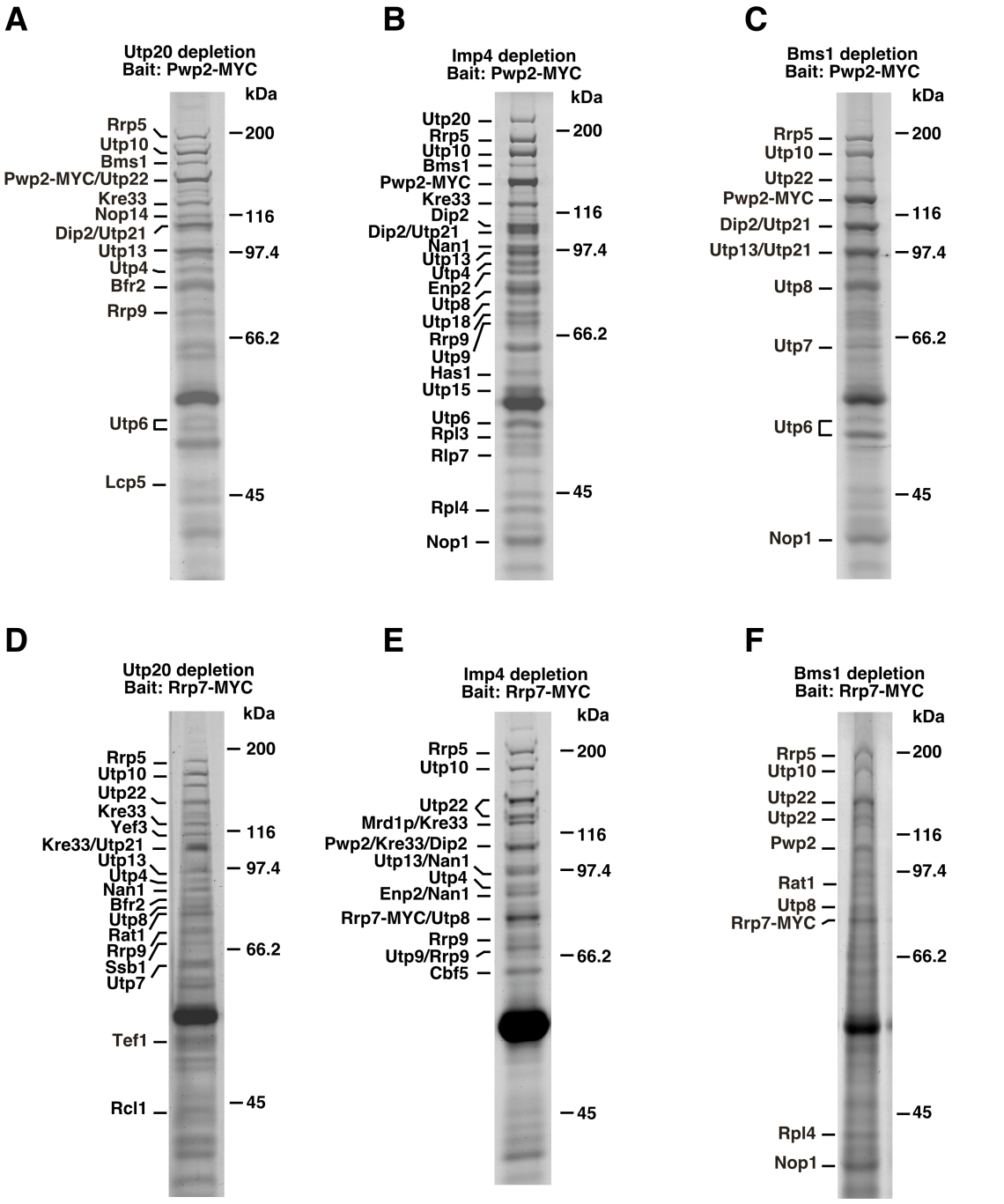

TABLE S1. *S. cerevisiae* strains used in this study

Name	Genotype	Source
W303a	*MATa, ade2, his3, leu2, trp1, ura3*	Euroscarf 20000D
YJP28	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-NAN1, PWP2-MYC::HIS3*	Pérez-Fernández et al, 2007
YJP30	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-NAN1, IMP4-MYC::HIS3*	This study
YJP36	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-NAN1, RRP7-MYC::HIS3*	Pérez-Fernández et al, 2007
YJP93	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-RRP5, RRP7-MYC::HIS3*	Pérez-Fernández et al, 2007
YJP95	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-RRP5, PWP2-MYC::HIS3*	Pérez-Fernández et al, 2007
YJP139	*MATa, ade2, his3, leu2, trp1, ura3, UTP20-MYC::HIS3*	This study
YJP149	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-NAN1, UTP20-MYC::HIS3*	This study
YJP151	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-PWP2, UTP20-MYC::HIS3*	This study
YJP153	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-RRP5, UTP20-MYC::HIS3*	This study
YJP157	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-UTP20, PWP2-MYC::HIS3*	This study
YJP159	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-UTP20, RRP7-MYC::HIS3*	This study
YJP169	*MATa, ade2, his3, leu2, trp1, ura3, BMS1-MYC::HIS3*	This study
YJP173	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-BMS1, PWP2-MYC::HIS3*	This study
YJP177	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-IMP4, PWP2-MYC::HIS3*	This study
YJP181	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-RRP5, IMP4-MYC::HIS3*	This study
YJP183	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-PWP2, BMS1-MYC::HIS3*	This study
YJP187	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-RRP5, BMS1-MYC::HIS3*	This study
YJP191	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-BMS1, RRP7-MYC::HIS3*	This study
YJP195	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-NAN1, BMS1-MYC::HIS3*	This study
YMD68	*MATa, ade2, his3, leu2, trp1, ura3, IMP4-MYC::HIS3*	Dosil et al, 2004
YMD73	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-PWP2, IMP4-MYC::HIS3*	Dosil et at, 2004
YPM16	*MATa, ade2, his3, leu2, trp1, ura3, KANMX6-GAL::HA-IMP4, RRP7-MYC::HIS3*	This study