SUPPLEMENTARY MATERIALS AND METHODS

Yeast strains

Yeast strains used were BJ2168 (*MAT*a *prc1 prb1 pep4 leu2 trp1 ura3*), YSCC026 (*MAT*a *prc1 prb1 pep4 leu2 trp1 ura3 PRP5V5*), YSCC024 (*MAT*a *prc1 prb1 pep4 leu2 trp1 ura3 HSH155HA*), YSCC022 (*MAT*a *prc1 prb1 pep4 leu2 trp1 ura3 PRP9V5*), and YSCC027 (*MAT*a *prc1 prb1 pep4 leu2 trp1 ura3 cus2*::*LEU2*).

Oligonucleotides

The following oligonucleotides were used:

A19 CTAAGTCTCATGTACAAACATCGATTGCTT C

A20 GAAGCAATCGATGTTTGTACATGAGACTTAG

A21 CTAAGTCTCATGTACGAACATCGATTGCTTC

A22 GAAGCAATCGATGTTCGTACATGAGACTTAG

A23 CTAAGTCTCATGTACCAACATCGATTGCTTC

P5-1 GGCCGGATCCCTCTGCAAGCAAAAG

P5-2 GGCCAAGCTTTGCAGCCTTTACGACC

P5-3 CCGGACTAGTTAGTTTTTAGAATTTAACTCACTCG

P5-4 CCGGCTCGAGCCAACGTGTGTTTAA

P5-6 GGCCGGATCCGTATGGAAACTATTGATTCG

P5-7 GTATCTGCTTCTGTG

P5-8 CTGGCTCCAACTAGA

P5-9 ATCGCTCCTCTTATC

P5-11 CGTTCGTAGTAATGGATGCGGCAGACAGGCTGTTC

P5-12 GAACAGCCTGTCTGCCGCATCCATTACTACGAACG

P5-13 TTATTGATATATTAACACTAGATGATGGGAAATTACTTAGT

P5-14 ACTAAGTAATTTCCCATCATCTAGTGTTAATATATCAATAA

P5-15 CAGTGTGTTCTATTTACTGCAGGTTTTCCGAACAAACTAC

P5-16 GTAGTTTGTTCGGAAAACCTGCAGTAAATAGAACACACTG

P5-17 GAGATGGAAGTTGAGGCGCTTAGATTTAGTCTG

P5-18 CAGACTAAATCTAAGCGCCTCAACTTCCATCTC

P5-19 CCAGAGCATGATATTGAAAAAGCTGCAGCGGCTGAGTTTATGACGTCATT
G

P5-20 CAATGACGTCATAAACTCAGCCGCTGCAGCTTTTTCAATATCATGCTCTG
G

P5-21 GGCCAAGCTTAAATTTTTCATCTTCTGAATGGC

CU-1 GGCCTCTAGACACTGGTAACTTCTC

CU-2 CAAGGGTCTGCAGCC

CU-3 GGCCGACGTCCCAGACTACGCTTAGAACTGCAGTAAAAAAATG

CU-4 TGAGGATTCCTATATCC

U2-B GCCTCATTGAGGTCATTTCAG

U2-C GAACAGACACTACACTTG

Antibodies and reagents

Anti-V5 antibody was purchased from Serotec, Inc. Anti-HA antibody was produced by immunizing mice with the KLH-conjugated HA-peptide (TY Tsao and SC Cheng, unpublished data). Anti-Prp5, anti-Lea1, anti-Prp8, anti-Ntc20, and anti-Snu114 antibodies were produced by immunizing rabbits with the *E. coli*-expressed recombinant proteins (full-length protein for Lea1 and Ntc20, and amino acid residues 1-115 for Prp8, 654-840 for Prp5, and amino acid residues 1-129 for Snu114). Protein A-Sepharose (PAS), streptavidin

Sepharose and Ni-NTA agarose were obtained from Amersham Bioscience, Sigma-Aldrich and Qiagen, respectively. The site-directed mutagenesis kit was from Stratagene.

Construction of PRP5-V5-tagged and *cus2Δ* strains

For construction of V5-tagged PRP5 yeast strain YSCC026, DNA fragment A was generated by polymerase chain reaction (PCR) using primers P5-1 and P5-2, and digested with *Bam*HI and *Hind*III, and DNA fragment B was generated with primers P5-3 and P5-4, and digested with *Spe*I and *Xho*I. Plasmid pRS406.PRP5C-V5, generated by insertion of fragment A, V5 and fragment B in sequence, into *Bam*HI/*Xho*I-site of pRS406, was linearlized with *Eco*RI and transformed into yeast strain BJ2168 to displace the wild-type allele with the V5-tagged PRP5 allele by the pop-in and pop-out gene displacement method.

For construction of *cus2Δ* strain YSCC027, a *cus2::LEU2* allele was created by replacing the entire Open reading frame of *CUS2* with a 2-kb DNA fragment of the *LEU2* gene. A 0.7-kb DNA fragment C containing the promoter region of *CUS2* gene, and a 0.7-kb DNA fragment D containing the *CUS2* 3'-untranslated region, were generated by PCR using primers CU-1 and CU-2, and CU-3 and CU-4, respectively. Fragments C, LEU2 and D are inserted in sequence into *Xho*I/*Xba*I-site of plasmid vector pBS. The resulted plasmid was digested with *Xho*I and *Xba*I, and transformed into yeast strain BJ2168. Cells were selected for leucine-prototrophy.