Supplemental Figure S1. — Cloning of dpb2 alleles and integration of dpb2 alleles into the yeast chromosome (for description see the next page).
Supplemental Figure S1. — Cloning of dpb2 alleles and integration of dpb2 alleles into the yeast chromosome (for illustration see previous page).

The pKF117 integrative plasmid serves as an acceptor vector for introduction of dpb2 alleles into the DPB2 locus. Construction of the pKF117 plasmid is described in Materials and Methods and its features are shown in the upper panel. Filled arrows mark locations and orientations of the ORFs, while other important regions are shown as boxes. Restriction sites, important for a particular step of the procedure, are underlined. Noteworthy is the presence of a truncated DPB2-derivative sequence, i.e. ‘DPB2-T$_{DPB2}$’ (apostrophes indicate the sides of truncation), upstream of the complete P$_{DPB2}$-dpb2::plomba-T$_{DPB2}$ cassette. DPB2 is flanked on the chromosome by two divergently transcribed genes, YPR174C and BET2. The BET2 gene is essential and its ORF is positioned immediately downstream of DPB2 ORF. Therefore, the 3’-terminal region of DPB2 probably serves as transcriptional terminator of BET2. After integration, this feature is provided by the ‘DPB2-T$_{DPB2}$’ fragment. Each dpb2 variant (examples of mutations are shown as pins) was cloned into the pKF117 vector in the place of plomba using XhoI and ClaI sites, naturally occurring in DPB2, or other flanking sites. The resulting plasmid was linearized with BamHI, SpeI and/or XbaI (double BamHI/XbaI digestion preferred) allowing for direct transplacement of the pKF117-derivative plasmid into the DPB2 locus using selection for Ura+. Since the dpb2 allele is not duplicated, a stable single-copy transformant is obtained. The integration into the DPB2 locus was confirmed by PCR with primers 1 and 2, as indicated in the scheme by thin arrows. Although vector sequences were not cured from the strains in the current study, subsequent loss of vector sequences could be selected for in the presence of 5-FOA using CaURA3 as a counterselection marker (GOLDSTEIN et al. 1999) as shown, if desired. The curing of the vector sequence could be subsequently checked by PCR with primers 2 and 3 as indicated on the scheme. In control studies, where we removed vector sequences, we did not observe any difference in the frequency of mutagenesis between DPB2 and DPB2::vector alleles.

More details available on request (mail to kflis@bp.onet.pl).