

**S2 Fig.** Flow cytometric assessments of ploidy.

**A,** Parental *K. phaffii* strains and diploids formed by mating. AMY429 and BY4742 are *S. cerevisiae* diploid and haploid strains respectively, included as controls. Dashed red lines indicate haploid *K. phaffii* cell populations in the G1 and G2 phases of the cell cycle.

**B, C,** Ploidy screening of **B**, superior segregants and **C**, inferior segregants, from Cross 1. The top two histograms in each group show the DNA content of the diploid (CBS\_BGL9/Pp2\_BGL5, light red) and one haploid parent (Pp2\_BGL5, blue) for comparison as diploid and haploid controls respectively. Segregants that were not included in the final pools of 30 segregants are named in parentheses (segregants were excluded if they had diploid profiles, inconsistent re-phenotyping results, or were simply supernumerary).

**D, E,** Ploidy screening of **D**, superior segregants, and **E**, inferior segregants, from Cross 2. The top two histograms in each group show the DNA content of the diploid (CBS\_BGL9/Pp4\_BGL3, blue) and one haploid parent (Pp4\_BGL3, red) for comparison as diploid and haploid controls respectively. Segregants that were not included in the final pools of 30 segregants are named in parentheses.

Raw data (FCS files) have been deposited at FlowRepository (https://flowrepository.org/).

