Fig S4 Stability of Spt16-Pob3 proteins determined by western blotting.

Strains were grown to logarithmic phase at 24° or 25° C as indicated, then shifted to 37° for 2-4 hours. Cells were harvested and lysed by vortexing with glass beads and SDS sample buffer. Proteins were separated by SDS-polyacrylamide gel electrophoresis, then transferred to nitrocellulose. Spt16 and Pob3 were detected with antisera generated against the purified proteins, then infrared-labeled secondary antibodies were used to detect the primary rabbit antibodies. Blots were scanned using a Li-Cor infrared scanner and quantitated using Odyssey software. This method of detection provides a large window of linear detection, enhancing the reliability of quantitation. In each experiment, the level of intact Spt16 or Pob3 detected in each lane was normalized to the total protein in the lane detected by scanning a parallel gel stained with Coomassie Blue dye, then each signal was normalized to the result for the WT strain at 24°, which was defined to be 100%. This allowed experiments performed on different days to be compared, as the absolute intensities of signals from different blots varied. Comparison with purified standards indicated that WT cells contain about 25,000 copies of Pob3 per cell. Spt16 was more difficult to quantitate as it was more prone to proteolysis during extraction, giving roughly 10,000 copies per cell for the intact form. Given the instability of Spt16 and the heterodimeric nature of yFACT, we assume the number for Pob3 is a more reliable estimate of the number of heterodimers in a living cell. These estimates are consistent with the estimates of 10,000-50,000 copies per cell that have been reported previously (10,11, 12).

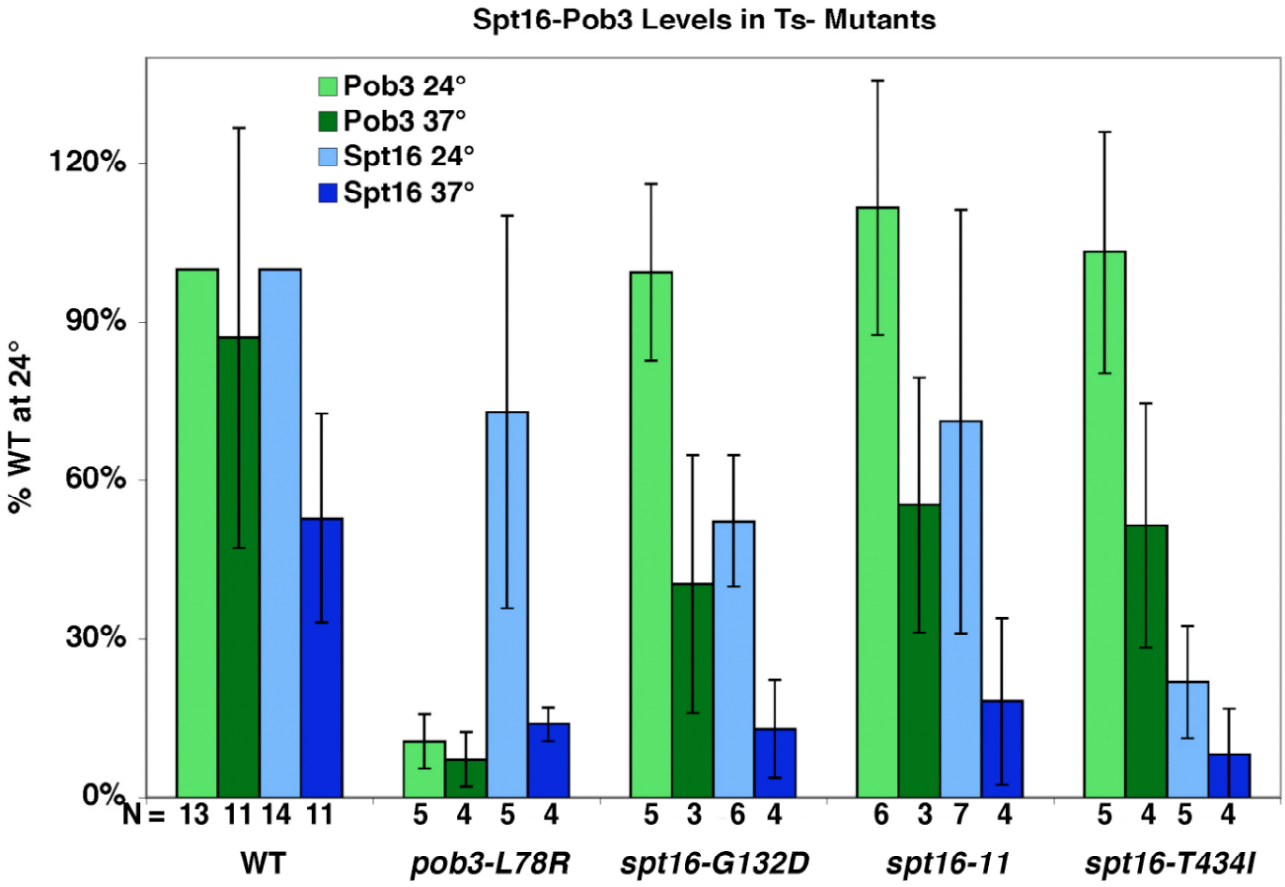

Fig S4A) Several strains with the relevant genotype indicated were tested. Data from A364a, W303, and S288c strain backgrounds were combined; the number of independent experiments for each condition is given (N). Pob3 is stable in a WT strain after a shift to 37°, but the level of Spt16 reproducibly drops about 2-fold. The level of Pob3 detected in a *pob3-L78R* strain growing at the permissive temperature of 24° is decreased about 10-fold relative to a WT strain, and does not decrease dramatically after a shift to 37°. This indicates that cells can grow, although slowly, with about 10% of the normal level of Pob3, and