suggests that the need for Pob3 increases as the temperature increases. Consistent with previous results that showed that Spt16 can be overexpressed independent of Pob3 in yeast cells (11), the level of Spt16 in a *pob3-L78R* strain is close to normal when the cells are growing at 24°, but the excess Spt16 is lost after a shift to 37°. No pools of free Spt16 or Pob3 were detected in normal yeast cells (11), so these results suggest that free Spt16 is unstable at elevated temperatures. Similarly, the level of Pob3 is normal in strains with mutations in *SPT16* that cause temperature sensitivity, and drop after a shift to 37°. Different alleles show somewhat different levels of Spt16 protein under permissive conditions, with Spt16-24 (T434I) consistently displaying the lowest levels at about 20% of the WT. The mutant Spt16 proteins all show some instability at the elevated temperature, but this is difficult to interpret as the magnitude of the decrease is similar to the change observed with WT protein. The instability therefore may contribute to the temperature sensitivity of growth in these strains, but an increased requirement for yFACT may also be responsible.

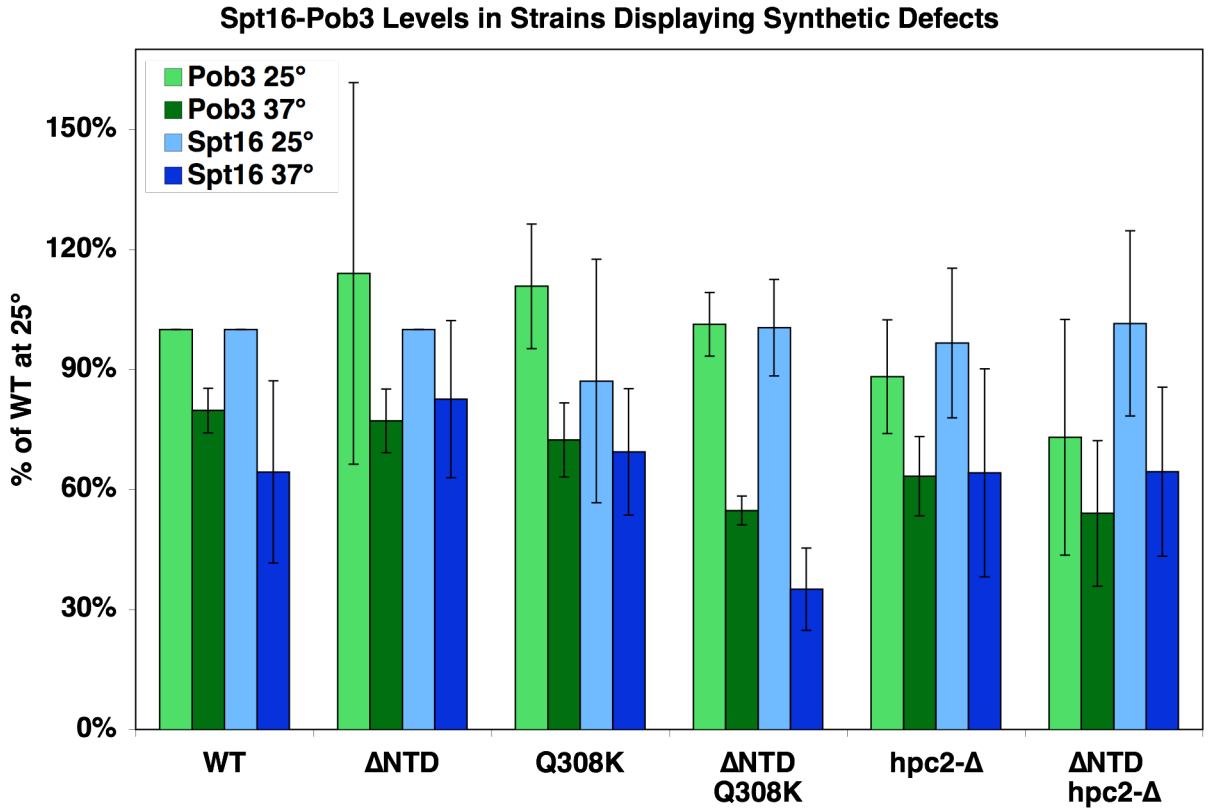

Fig S4B) Pob3 and Spt16 levels were measured in strains lacking the Spt16 NTD, with the *pob3-Q308K* allele, lacking *HPC2*, or with both *spt16-ΔNTD* and the other mutations. The levels of Pob3 and Spt16 decreased 2-3 fold in the *spt16-ΔNTD pob3-Q308K* strain at 37°, but the level of yFACT in these cells remained well above the 10% level shown to be sufficient for growth in panel A. As these cells grew very poorly even at 30° (Fig 3), the 2-3 fold decrease in yFACT level is not likely to be the principle cause of the growth defect. Rather, the cause is likely to be a defect in activity in the doubly mutant complex. Combining a deletion of *HPC2* with *spt16-ΔNTD* also caused a slight drop in yFACT levels at 37°, but comparison with panel A suggests that this is not the cause of the significant growth defect observed with this strain (Fig S5).