David Myszka, personal communication). These experiments show that yFACT can interact with the N-terminal tails of histones, but that the Spt16 NTD has no more than a minor role in this binding.

Genetic effects caused by mutation of the Spt16 NTD are not due to instability of yFACT proteins.

The structure of the Spt16 NTD explains the behavior of some previously reported mutant proteins. Partial deletion of the Spt16 NTD and some point mutations in this region cause a Ts- phenotype (failure to grow at elevated temperatures) even though deletion of the entire domain does not (7). This suggested that improper folding of the NTD could destabilize the entire Spt16 protein (7). Consistent with this, mutations such as G132D that cause the Ts- phenotype are found to disturb residues in the hydrophobic core of the structure of the Spt16 NTD, which is expected to make the resulting protein more difficult to fold.

In contrast, substitution of surface residues did not cause the Ts- phenotype or instability of either Spt16 or Pob3 proteins (Fig S4). It was therefore a surprise that combining some of these *spt16* mutations with the *pob3-Q308K* allele caused temperature sensitivity. This result either indicates that the combination of mutations caused yFACT proteins to become unstable or that yeast cells require higher levels of yFACT activity at elevated temperatures. We therefore used quantitative western blots to determine the level of Spt16 and Pob3 proteins in WT, single mutant, and double mutant cells both in cultures grown under permissive conditions (25°) and after shifting cultures to 37° for several hours. Fig S4 shows that Spt16 and Pob3 proteins were stable even in mutant combinations that fail to support growth at elevated temperatures. Levels of yFACT did drop about 2-fold in some cases, but mutations that cause temperature sensitivity on their own, such as *pob3-L78R* and *spt16-T434I*, displayed reductions of 5-10 fold even in cells grown under permissive conditions. We conclude that the 2-fold changes observed with some double mutants may be real but cannot account for the phenotypes observed, because even lower levels of yFACT can be tolerated. Instead, we propose that the Ts- phenotype results from the loss of a function whose role increases in importance as the temperature increases. For example, coordination of events during replication or reassembly of nucleosomes may become more difficult at elevated temperatures. Consistent with the idea that elevated temperatures alone cause replication stress, we note that the toxicity of HU increases at elevated temperatures even with WT strains (Fig S2). Misfolding of the Spt16 NTD therefore can cause defects in yFACT, but the point mutations in surface residues described here appear to disturb a function that overlaps with Pob3-M without causing destabilization of the yFACT complex.

The Spt16 NTD does not appear to have peptidase activity

Due to the low level of sequence similarity between the Spt16 NTD and several types of peptidases, we considered the possibility that yFACT and the Spt16 NTD have protease activity using a standard assay *in vitro*. 100 µl samples containing 110 nM Spt16-Pob3 or 380 nM Spt16 NTD and 100 µM peptide were incubated at RT for 2 hours. Peptidase activity was measured as fluorescence due to liberation of the MCA moiety by hydrolysis of the peptide bond. Consistent with the lack of active site residues in the Spt16 NTD known to participate in proteolysis (the metal-coordinating residues D209, D220, H284, E313 and E327 in the prolidase structure 1PV9 align with S289, N300, S366, S397, and A417 in Spt16, and the conserved active site histidine in creatinase aligns with V271 in Spt16), no significant activity was detected in any reaction, including substrates listed below that should reveal dipeptidase or prolidase activity. Any of the substrates with an unblocked N-terminus should detect peptidase activity. We therefore conclude that yFACT does not have peptidase activity. We thank Greg Pratt and Marty Rechsteiner for performing these assays.

Substrates tested:
N-terminus unblocked:
Gly-Pro-MCA
Lys-Ala-MCA
Leu-MCA
Pro-Phe-Arg-MCA