as an indication that yFACT has a function in a pathway that overlaps nucleosome deposition (6). Combining a deletion of the Spt16 NTD with H4-K5R, K12R also caused an additive defect for HU sensitivity (Fig S5A). This pattern of interactions suggests that the Spt16 NTD is not responsible for recognizing the modified H4 N-terminal tail directly. Instead, it appears to have an indirect role in a process that involves nucleosome deposition, and this role becomes more important when cells are subjected to a replication stress. However, it is also possible that the H3 tail, the H4 tail, or the Spt16 NTD contribute to HU resistance in multiple independent and non-overlapping ways.

The Spt16 NTD is not required for nucleosome binding or reorganization by yFACT (additional description of Fig 6)

If the Spt16 NTD is involved in recognizing the N-terminal tails of histones then yFACT complexes lacking this domain should have lower binding affinity for nucleosomes. To test this, we constructed expression plasmids lacking residues 2-468 of the Spt16 NTD, coexpressed this Spt16(469-1035) (Spt16-ΔNTD) in yeast cells along with a His_{12}-tagged Pob3 protein, then purified the Pob3 protein by nickel affinity and size exclusion chromatography. Spt16-ΔNTD copurified as a 1:1 complex with Pob3, consistent with previous results showing that the Spt16 NTD is not required for heterodimer formation (6,7). We titrated this complex and WT complexes with nucleosomes in an EMSA experiment to determine the relative binding affinities. As shown in Fig 6B, the two complexes were able to bind nucleosomes at similar concentrations, indicating that deletion of the Spt16 NTD did not cause decreased affinity for intact nucleosomes. We also asked whether mutation of the potential peptide binding surface on Pob3-M contributes to the interaction between yFACT and nucleosomes using the EMSA assay. Complexes with the Pob3-Q308K mutation and intact Spt16 or Spt16-ΔNTD were readily purified, and both were found to bind nucleosomes with the same affinity as WT complexes in an EMSA test (Fig S4B). This mutation of Pob3 therefore also does not significantly alter the affinity of yFACT for nucleosomes.

yFACT-mediated reorganization of nucleosomes is detected *in vitro* as increased accessibility of nucleosomal DNA to nucleases (4,8). We therefore asked whether the normal binding to nucleosomes observed above with mutant yFACT complexes resulted in normal reorganization. yFACT complexes lacking the Spt16 NTD, containing the Pob3-Q308K mutation, or with both alterations were tested for their ability to alter accessibility of nucleosomal DNA to DNAse I or to the restriction endonuclease *Dra*I. All four versions of yFACT were essentially identical in the DNase I assay (not shown) and in a DraI accessibility assay (Fig 6C). yFACT can therefore bind to and alter the properties of nucleosomes without the Spt16 NTD, with a mutation in Pob3-M, or with both changes. The defects caused by these mutations individually and when combined *in vivo* are therefore not related to a gross inability to effect nucleosome reorganization as detected *in vitro*.

yFACT displayed reduced affinity for nucleosomes whose N-terminal histone tails had been removed with trypsin (Fig 6A), suggesting that yFACT can bind to histone tails. We tested this directly using a surface plasmon resonance assay (SPR). Various biotinylated peptides representing the N-terminal tails of histones were synthesized and immobilized on a streptavidin surface, then different fragments of Spt16-Pob3 were tested for binding to this surface. WT Spt16-Pob3 bound to histone H3 and H4 peptides with high affinity, displaying a Kd of about 2-6 nM. However, this number is a rough approximation because the kinetics of binding and dissociation were complex and did not fit a simple 2-component binding model (Fig 6D and not shown). The interaction appeared to be at least somewhat specific, as no binding was detected with the similarly charged H2A tail peptide assayed in parallel. Spt16-Pob3 therefore is able to bind to isolated histone tail peptides with high affinity but this interaction is complex. The same assay was performed with Spt16-ΔNTD-Pob3 and with the purified Spt16 NTD. Spt16-ΔNTD-Pob3 still bound to histone tail peptides, but the kinetics of binding were somewhat altered compared with WT Spt16-Pob3, suggesting that the Spt16 NTD contributed in some way to the complex interaction with peptides but was not responsible for the interaction itself (Fig S5D). Consistent with this, the isolated Spt16 NTD did not display robust binding to any histone peptides in this assay (not shown;