Figure S4. Nhp6 and Spn1 each support functions of Spt6 *in vivo.* Related to Figure 5.

(A) Loss of Nhp6 is detrimental to *spt6* mutants. Strains in the S288C background with the mutations indicated were grown to saturation in rich medium, then 10-fold serial dilutions were spotted to rich medium and incubated for 6 days at 23° C or 3 days at 33° C. Nhp6 is encoded by two very similar genes, *NHP6A* and *NHP6B*, so "*nhp6*-Δ" indicates deletion of both genes. *spt6-1004* is an internal deletion of the helix-hairpin-helix domain within the Tex-like core (Kaplan et al., 2005), *spt6-50* (K1274stop) is a premature termination of the C-terminal domain (deletion of 1274-1451), and spt6-14 is an S952F substitution (F Winston, personal communication); none of these alleles directly affect the Spn1-Spt6 interface. The growth defects caused by these three standard alleles of *SPT6* are all enhanced by loss of Nhp6, especially at elevated temperatures (rows 4, 8, 12), indicating that Nhp6 supports an essential function of Spt6 *in vivo*.

(B, C) As in panel A except the *spn1-F267E* and *spn1-R263D* alleles affecting the Spn1-Spt6 interface are tested in the A364a genetic background. Combining *nhp6*-Δ and *spn1* mutations has more subtle and variable effects, with *spn1-F267E* partially suppressing the growth defect caused by *nhp6*-Δ at 30°, but the double mutant being more impaired than single mutants at 37°. *spn1-R263D* also slightly suppressed *nhp6*-Δ at 30° but did not enhance the Ts- phenotype. These results suggest that Nhp6 does not directly support a function of Spn1, consistent with the model that Spn1 interacts with Spt6 only when Spt6 is not interacting with nucleosomes, and Nhp6 is needed only to support the role of Spt6 when it is interacting with nucleosomes. Panel C also shows the effect of the *spn1-R263A* allele (similar in effect to R263D), and the *spt6-I248K, F249K* double mutation (more severe than F249K alone).