create a duplication of the *SPN1* locus. Recombinants that popped out the plasmid were obtained by selection on medium containing 5-FOA (Boeke et al., 1987), then these were screened for temperature sensitivity. Successful mutation of the *SPN1* gene without other changes was confirmed by DNA sequencing.

Other strains were derived by standard crosses within isogenic backgrounds.

Plasmid Construction

The integrating vector YIplac211 (Gietz and Sugino, 1988) was digested with BamHI and SacI, then a PCR product containing the *SPN1* ORF along with 332 bp of sequence upstream and 50 bp downstream was inserted. The WT sequence was verified by sequencing, and variants with the K192N and R263D mutations were derived using the Quikchange strategy (Stratagene). The mutated plasmids were digested with KpnI to target integration of the K192N mutation to the *SPN1* locus. The inserts from these plasmids were transferred to the high copy vector YEplac112 (Gietz and Sugino, 1988) for the experiment shown in Figure 5B.

pLK04 containing the *SPT6* gene was constructed by inserting DNA fragments flanking the *SPT6* locus into a low copy vector, then using gap repair from a WT strain to fill in the remainder of the gene. This resulted in a plasmid with 495 bp upstream (including the final 11 bp of the adjacent *DAM1* locus to insure inclusion of the entire intergenic region) and 161 bp downstream of the *SPT6* ORF. The sequence was confirmed by DNA sequencing; pLK04 complemented the lethality of an *spt6*-Δ deletion and the temperature sensitivity of all *spt6* alleles tested, but either *spt6* mutant or *SPT6* WT strains with this low copy version of *SPT6* displayed the Spt^- phenotype. Expression of *SPT6* from the plasmid context is therefore aberrant and is sufficient to cause a defect in regulation of transcription.