Strain Construction

The *URA3* gene was inserted 424 bp upstream of the *SPT6* ORF, which is 60 bp downstream of *DAM1*. This site was chosen to minimize potential interference with either of these essential genes, and the insertion itself caused no detectable phenotype in any of our tests. Genomic DNA from this strain was then used as the template in a PCR amplification using one wild type primer 343 bp upstream of the insertion and one mutant primer downstream of the insertion containing the F249K mutation followed by 31 nucleotides of WT sequence. This PCR product was used to transform a WT strain. Ura+ transformants were screened for integration of the F249K mutation, indicating recombination between the PCR product and the genomic DNA in the 31 bp interval between the mutation and the end of the product. The transformation was performed in a diploid strain, which was then sporulated to derive haploids. All Ura+ derivatives displayed a severe growth defect initially in the A364a genetic background (but not in the S288C background), but this became more subtle during subsequent vegetative growth. This does not appear to be due to acquisition of a suppressor mutation, as only a mild growth defect was observed segregating 2:2 with the *URA3* marker in subsequent crosses.

Similarly, the *TRP1* gene was integrated 49 bp downstream of the *SPN1* ORF (which is also 161 bp downstream of the convergently transcribed gene *RPS23B*) without any detected phenotype, and genomic DNA from this strain was used as the template to transfer the *TRP1* gene along with the R263D and F267E mutations to a fresh diploid strain. After confirming the integration of the mutation by DNA sequencing, the diploids were sporulated to produce haploid strains with the desired mutation linked to the *TRP1* marker.

The *spn1-K192N* mutation was introduced into an integrating plasmid carrying the *SPN1* and *URA3* genes (described below), and this plasmid was integrated into a haploid yeast strain to