Figure S1  Sequence of the molecule to integrate Flag\(^{\text{5}}\)-tag TRA1. The ATG translational start preceding the tag is in bold. A genomic Hind\(\text{III}\) fragment encoding URA3 (~1.1 kbp) was inserted at the underlined Hind\(\text{III}\) site. TRA1 sequences in frame with the NotI site (Saleh et al., 1998) were cloned 3' to the NotI.
**Figure S2**  Suppression of tra1-F3744A phenotypes by tti2-F328S and tti2-I336F. Yeast strains CY4353 (TRA1 TTI2), CY4350 (tra1-F3744A TTI2), CY5667 (tra1-F3744A tti2-F328S), CY5843 (tra1-F3744A tti2-I336F), CY5665 (TRA1 tti2-F328S), or a mec2-1 strain (Weinert et al., 1994) were grown to stationary phase diluted 1/10⁴ and serial dilutions spotted onto selection plates as follows: YPD at 30°, YPD at 37°; YPD at 30° containing 0.03% methyl methanesulfonate 0.03% (MMS), 1.0 μg/mL phleomycin, YPD depleted of phosphate, 7.5 μg/mL Calcofluor white, 6% ethanol, or 1.0 μg/mL tunicamycin; YP containing 2% galactose, and YPD at pH 8.0. Note that some images are composites from two otherwise identical plates.
Figure S3  Localization of eGFP-Tra1-F3744A with RFP-tagged Cop1, Snf7 and pex3 in SC media containing ethanol. Diploid strains containing a single copy of each tagged allele were grown to stationary phase in SC media, diluted 1:4 in SC containing 8% ethanol, grown a further 18 hr, and visualized by fluorescence microscopy. A 10 μm scale bar is shown in the bottom right.
Figure S4  tti2-F328S does not suppress the temperature sensitivity of mec1-W2368A. A. pCB2317 for converting tryptophan 2368 of Mec1 to alanine. The altered codon is underlined. HIS3 was inserted at the BamHI site. A BclI site was placed downstream of the stop codon to identify the allele. B. mec1-W2368A-HIS3 was integrated into a diploid strain heterozygous for tti2-F328S (CY6045). The TTI2 allele of spore colonies growing on media depleted of histidine were sequenced. CY6071 and CY6072 were TTI2, CY6078 was tti2-F328S. The strains were streaked onto a YPD plate and grown at 37° for 3 days.