



**Figure S8. Rescue of TS mutants by TMAO is influenced by restrictive temperature but there is no strict temperature threshold.** (A) Strains from the Boone TS collection were serially diluted and spotted on rich (YPD) medium with or without 0.5 M TMAO and incubated at the indicated temperatures. The top row of images represents an independent experiment from the bottom two rows, which were plated at the same time. Note that

the *scd5-PP1D2* mutant, identified as rescued by TMAO in the initial robotized screen, showed rescue in only one of the experiments shown here. (B) Cells of the indicated genotypes were suspended in 100  $\mu$ L rich medium (YPD) in PCR tubes and incubated in a thermal cycler at the indicated temperatures for 20 hours, then the cultures were transferred to a 96-well plate and the optical density was measured at 600 nm. Two technical replicates were performed for each strain; points represent the mean and error bars are standard deviation. Strains were: BY4741 (“WT”), CBY06147 (“*cdc10-1*”), CBY06420 (“*cdc10-2*”), CBY06421 (“*cdc10-4*”), CBY06424 (“*cdc10-5*”), and CBY05110 (“*cdc12-1*”). (C) Histogram of the first temperature listed (Li *et al.* 2011) (see Table S1) as the restrictive temperature for each TS mutant strain from the Boone collection (“All TS mutants”,  $n = 680$ ) and for the mutants from this collection scored as showing some evidence of rescue by TMAO (“TMAO rescued”,  $n = 139$ ). (D) Venn diagram of overlap between TS mutants from the Boone collection identified as being rescued by the indicated small molecule. Shape sizes are not to scale.

### Literature Cited

Li Z., F. J. Vizeacoumar, S. Bahr, J. Li, J. Warringer, *et al.*, 2011 Systematic exploration of essential yeast gene function with temperature-sensitive mutants. *Nat. Biotechnol.* 29: 361–367.  
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