

**Table S1 Strains and plasmids used in this study**

Strain	Genotype	Source	Notes
MS7590	<i>MATa</i> <i>prm3Δ::HIS3 ura3-52 leu2-3,112 trp1Δ1 his3Δ200</i>	Rose Laboratory	
MS7591	<i>MATa</i> <i>prm3Δ::HIS3 ura3-52 leu2-3,112 ade2-101 his3Δ200</i>	Rose Laboratory	
MS7670	<i>MATa</i> <i>kar5Δ::HIS3 ura3-52 leu2-3,112 his3Δ200 trp1Δ1</i>	Rose Laboratory	
MS7673	<i>MATa</i> <i>kar5Δ::HIS3 ura3-52 leu2-3,112 his3Δ200 trp1Δ1</i>	Rose Laboratory	
MS7729	<i>MATa</i> <i>ura3-52 leu2-3,112 his3Δ200 trp1Δ1 ade2-101 SPC42::mRFP-KanMX</i>	Rose Laboratory	
MS7884	<i>MATa</i> <i>prm3Δ::HIS3 ura3-52 leu2-3,112 his3Δ200 trp1Δ1 ade2-101 SPC42::mRFP-KanMX</i>	Rose Laboratory	
MS8020	<i>MATa</i> <i>kar5Δ::HIS3 ura3-52 leu2-3,112 his3Δ200 trp1Δ1 SPC42::mCherry-KanMX</i>	This study	
MS8041	<i>MATa</i> <i>kar2-1 ura3-52 leu2-3,112 trp1Δ1 SPC42::mCherry-KanMX</i>	This study	
MS8087	<i>MATa</i> <i>kar8Δ::NatMX kar5Δ::HIS3 ura3-52 leu2-3,112 his3Δ200 trp1Δ1 SPC42::mCherry-KanMX</i>	This study	
MS8389	<i>MATa</i> <i>kar5Δ::HIS3 ura3-52 leu2-3,112 his3Δ200 trp1Δ1 SPC42::mCherry-KanMX prm3Δ::NatMX [GFP-PRM3 LEU2 CEN4]</i>	This study	
MY7261	<i>MATa</i> <i>his4- HOL1-1 ura3-52</i>	Rose Laboratory	
MY14348	<i>MATa</i> <i>pom152Δ::HygMX ade2-1 his3-11,15 leu2-3,112 trp1Δ1 ura3Δ1 ade3Δ100</i>	This study	
MY14349	<i>MATa</i> <i>pom152Δ::HygMX ade2-1 his3-11,15 leu2-3,112 trp1Δ1 ura3Δ1</i>	This study	
MY14355	<i>MATa</i> <i>pom152Δ::HygMX mps3Δ::NatMX ade2-1 his3-11,15 leu2-3,112 trp1Δ1 ura3Δ1</i>	This study	parent strain for all <i>pom152Δ mps3Δ</i> strains from Sue Jaspersen
MY14357	<i>MATa</i> <i>pom152Δ::HygMX mps3Δ::NatMX ade2-1 his3-11,15 leu2-3,112 trp1Δ1 ura3Δ1</i>	This study	
MY14635	<i>MATa</i> <i>pom152Δ::HygMX ade2-1 his3-11,15 leu2-3,112 trp1Δ1 ura3Δ1 ade3Δ100 SPC42::mCherry-KanMX</i>	This study	
MY14637	<i>MATa</i> <i>pom152Δ::HygMX mps3Δ::NatMX ade2-1 his3-11,15 leu2-3,112 trp1Δ1 ura3Δ1 SPC42::mCherry-KanMX</i>	This study	
MY14667	<i>MATa</i> <i>[GFP11-mCherry-PUS1-LEU2] trp- his- ura-</i>	Jaspersen Laboratory	
MY14668	<i>MATa</i> <i>[GFP11-mCherry-SCS2TM-LEU2] lys- trp- his- ura-</i>	Jaspersen Laboratory	
Plasmid	Relevant markers	Source	
pMR1868	<i>URA3 CEN6 ARS4 amp-r</i>	Rose Laboratory	
pMR4518	<i>KAR5 URA3 CEN6 ARS4 amp-r</i>	Rose Laboratory	
pMR6603	<i>KAR5(C13A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6381	<i>KAR5(C56A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6382	<i>KAR5(C68A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6383	<i>KAR5(C91A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6604	<i>KAR5(C105A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6605	<i>KAR5(C116A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6606	<i>KAR5(C141A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6384	<i>KAR5(C444A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6607	<i>KAR5(C458A) URA3 CEN6 ARS4 amp-r</i>	This study	
pMR6608	<i>KAR5(Δ4-23) URA3 CEN6 ARS4 amp-r (SPΔ)</i>	This study	
pMR6385	<i>KAR5(Δ186-215) URA3 CEN6 ARS4 amp-r (coil1Δ)</i>	This study	
pMR6386	<i>KAR5(Δ401-436) URA3 CEN6 ARS4 amp-r (coil2Δ)</i>	This study	
pMR6609	<i>KAR5(Δ445-465) URA3 CEN6 ARS4 amp-r (TM1Δ)</i>	This study	

pMR6387	<i>KAR5(Δ481-504) URA3 CEN6 ARS4 amp-r (TM2Δ)</i>	This study
pMR6751	<i>KAR5(Δ466-504) URA3 CEN6 ARS4 amp-r (loop-TM2Δ)</i>	This study
pMR6610	<i>KAR5(Δ445-504) URA3 CEN6 ARS4 amp-r (TM1-loop-TM2Δ)</i>	This study
pMR6611	<i>KAR5(Δ116-167) URA3 CEN6 ARS4 amp-r (conserved regionΔ)</i>	This study
pMR6366	<i>KAR5:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6364	<i>KAR5:TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6612	<i>KAR5(C13A):TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6749	<i>KAR5(C56A):TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6750	<i>KAR5(C68A):TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6615	<i>KAR5(C91A):TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6616	<i>KAR5(C105A):TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6617	<i>KAR5(C116A):TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6618	<i>KAR5(C141A):TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6619	<i>KAR5(Δ4-23):TM3:GFP URA3 CEN6 ARS4 amp-r (SPΔ)</i>	This study
pMR6620	<i>KAR5(Δ186-215):TM3:GFP URA3 CEN6 ARS4 amp-r (coil1Δ)</i>	This study
pMR6622	<i>KAR5(Δ116-167):TM3:GFP URA3 CEN6 ARS4 amp-r (conserved regionΔ)</i>	This study
pMR6627	<i>KAR5(Δ445-465):GFP URA3 CEN6 ARS4 amp-r (TM1Δ)</i>	This study
pMR6748	<i>KAR5(Δ481-504):GFP URA3 CEN6 ARS4 amp-r (TM2Δ)</i>	This study
pMR6629	<i>KAR5(Δ466-504):GFP URA3 CEN6 ARS4 amp-r (loop-TM2Δ)</i>	This study
pMR6744	<i>KAR5(Δ445-504):GFP URA3 CEN6 ARS4 amp-r (TM1-loop-TM2Δ)</i>	This study
pMR6082	<i>pADH1-KAR5:HIS4C URA3 2μ amp-r</i>	This study
pMR6081	<i>pADH1-KAR5:TM3:HIS4C URA3 2μ amp-r</i>	This study
pMR6083	<i>pADH1-KAR5(TM2Δ):HIS4C URA3 2μ amp-r</i>	This study
pMR6085	<i>pADH1-SEC63:HIS4C URA3 2μ amp-r</i>	This study
pMR1872	<i>URA3 2μ amp-r</i>	Rose Laboratory
pMR6371	<i>pADH1-KAR5:TM3:GFP URA3 CEN6 ARS4 amp-r</i>	This study
pMR6433	<i>pADH1-mCherry:PRM3 LEU2 CEN6 ARS4 amp-r</i>	This study
pMR6907	<i>pADH1-GFP1-10 URA3 CEN6 ARS4 amp-r</i>	This study
pMR6945	<i>pSEY1-SEY1-GFP1-10 URA3 CEN6 ARS4 amp-r</i>	This study
pMR6932	<i>pKAR5-KAR5:TM3:GFP1-10 URA3 CEN6 ARS4 amp-r</i>	This study

**Table S2 Raw p-values associated with Table 1 (two-sided t-test)**

Available for download as an Excel file at <http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.114.015800/-DC1>