

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

Strain	Genotype	Reference or source
YPH499	<i>MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his1-Δ200 leu2-Δ1</i>	(Sikorski and Hieter 1989)
YCF112	YPH499 bar1Δ::NAT rrm3Δ::HIS3	(Chen et al. 2018)
YCF113	YPH499 bar1Δ::NAT pif1-m2	(Chen et al. 2018)
YCF114	YPH499 bar1Δ::NAT pif1Δ::KAN	(Chen et al. 2018)
YCF116	YPH499 bar1Δ::NAT rrm3Δ::HIS3 pif1-m2::HPH	(Chen et al. 2018)
YCF117	YPH499 bar1Δ::NAT rrm3Δ::HIS3 pif1Δ::KAN	(Chen et al. 2018)
YCF118	YPH499 bar1Δ::NAT cbf1Δ::HPH	(Chen et al. 2018)
YCF119	YPH499 bar1Δ::NAT cbf1Δ::HPH pif1-m2	This study
YCF120	YPH499 bar1Δ::NAT cbf1Δ::HPH pif1Δ::KAN	This study
YCF121	YPH499 bar1Δ::NAT cbf1Δ::HPH rrm3Δ::HIS	This study
YCF122	YPH499 bar1Δ::NAT cbf1Δ::HPH rrm3Δ::HIS pif1-m2	This study
YCF123	YPH499 bar1Δ::NAT cbf1Δ::HPH rrm3Δ::HIS pif1Δ::KAN	This study
YCF128	YPH499 tof1Δ::NAT	This study
YCF129	YPH499 tof1Δ::NAT rrm3Δ::HIS	This study
YCF139	YPH499 rnh1Δ::HPH	This study
YCF140	YPH499 rnh1Δ::HPH rnh201 Δ::TRP	This study
YCF141	YPH499 bar1Δ::NAT rnh1Δ::HPH rrm3Δ::HIS pif1-m2	This study
YCF142	YPH499 bar1Δ::NAT rnh1Δ::HPH cbf1Δ::HPH	This study
YCF143	YPH499 bar1Δ::NAT CBF1-MYC9	This study
YCF144	YPH499 bar1Δ::NAT CSE4-MYC9	This study
YCF145	YPH499 bar1Δ::NAT cbf1Δ::HPH CSE4-MYC9	This study
YCF146	YPH499 bar1Δ::NAT rnh1Δ::HPH CSE4-MYC9	This study
YCF147	YPH499 bar1Δ::NAT rrm3Δ::HIS CSE4-MYC9	This study
YCF148	YPH499 bar1Δ::NAT pif1-m2 CSE4-MYC9	This study
YCF149	YPH499 bar1Δ::NAT pif1-m2 rrm3Δ::HIS CSE4-MYC9	This study
YCF396	YPH499 bar1Δ::NAT pif1-m2 rrm3Δ::HIS CBF1-MYC9	This study
YCF399	YPH499 bar1Δ::NAT pif1-m2 CBF1-MYC9	This study
YCF402	YPH499 bar1Δ::NAT rrm3Δ::HIS CBF1-MYC9	This study
YCF407	YPH499 bar1Δ::NAT cbf1Δ::HPH rnh1Δ::HPH CSE4-MYC9	This study
YCF413	YPH499 bar1Δ::NAT rnh1Δ::HPH CBF1-MYC9	This study

- Chen CF, Pohl TJ, Pott S, Zakian VA. 2019. Two Pif1-family DNA Helicases Cooperate in Centromere Replication and Segregation in *Saccharomyces cerevisiae*. *Genetics*.
- Sikorski RS, Hieter P. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**: 19-27.