

Table S1. **Yeast strains used in this study**

Strain	Genotype	Source
YEF473	α/\varnothing <i>his3/his3 leu2/leu2 lys2lys2 trp1/trp1 ura3/ura3</i>	Bi and Pringle, 1996
YEF473A	<i>a his3 leu2 lys2 trp1 ura3</i>	Bi and Pringle, 1996
YEF473B	\varnothing <i>his3 leu2 lys2 trp1 ura3</i>	Bi et al., 2000
YEF1233	As YEF473 except <i>rga1</i> \varnothing :: <i>HIS3/rga1</i> \varnothing :: <i>HIS3</i>	This study ^a
YEF2324	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3</i>	This study ^b
YEF2380	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 rga2</i> \varnothing :: <i>KanMX6 bem3</i> \varnothing :: <i>TRP1</i>	Caviston et al., 2003
YEF2392	As YEF473A except <i>rga2</i> \varnothing :: <i>KanMX6 bem3</i> \varnothing :: <i>TRP1</i>	This study ^b
YEF2921	As YEF473A <i>cdc42</i> ^{V36G}	Caviston et al., 2003
YEF4723	As YEF473A except <i>SHS1-GFP:LEU2 SPC42-GFP:KanMX6 GIC2-PBD-RFP:URA3</i>	This study ^c
YEF4934	<i>a cdc24-4 swe1</i> \varnothing :: <i>TRP1 SHS1-GFP:LEU2 SPC42-GFP:KanMX6 GIC2-PBD-RFP:URA3 his3 leu2 trp1 ura3</i>	This study ^c
LRsY359	As YEF473B except <i>rsr1</i> \varnothing :: <i>TRP1</i>	Pringle, 1991
AM100	As YEF473B except <i>bud3</i> \varnothing :: <i>HIS3</i>	Pringle, 1991
YZT55	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 CDC3-GFP:URA3</i>	This study ^d
YZT82	As YEF473A except <i>CDC3-GFP:URA3</i>	This study ^d
YZT88	As YEF473A <i>rga1</i> \varnothing :: <i>URA3-KanMX6</i>	This study ^e
YZT111	As YEF473A except <i>rga2</i> \varnothing :: <i>KanMX6 bem3</i> \varnothing :: <i>TRP1 CDC3-GFP:URA3</i>	This study ^d
YZT139	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 rsr1</i> \varnothing :: <i>TRP1</i>	This study ^f
YZT146	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 bud3</i> \varnothing :: <i>HIS3</i>	This study ^g
YZT156	As YEF473 except <i>GFP-RGA1/rga1</i> \varnothing :: <i>HIS3</i>	This study ^h
YZT158	As YEF473A except <i>GFP-RGA1</i>	This study ⁱ
YZT166	As YEF473 except <i>CDC3-mCherry-SpHIS5/CDC3</i>	This study ⁱ
YZT194	As YEF473A except <i>HA-RGA1</i>	This study ^k
YZT195	As YEF473A except <i>HA-rga1</i> ^{R829A}	This study ^k
YZT198	As YEF473A except <i>HA-rga1</i> ^{R829A} <i>CDC3-GFP:URA3</i>	This study ^d
YZT211	As YEF473 except <i>GFP-RGA1/GFP-RGA1 CDC3-DsRed:LEU2/CDC3</i>	This study ^l
YZT221	As YEF473 except <i>cdc42</i> \varnothing :: <i>HIS3/cdc42</i> \varnothing :: <i>HIS3 ura3:CDC42-GFP:URA3/CDC42-GFP:URA3 CDC3-DsRed:LEU2/CDC3</i>	This study ^m
YZT232	As YEF473A except <i>GFP-rga1</i> ^{700-1007aa} : <i>URA3 rga1</i> \varnothing :: <i>HIS3</i>	This study ^k
YZT240	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 BUD3-rga1</i> ^{700-1007aa} : <i>GFP:URA3 CDC3-mCherry:LEU2</i>	This study ⁿ
YZT241	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 CYK3-rga1</i> ^{700-1007aa} : <i>GFP:URA3 CDC3-mCherry:LEU2</i>	This study ⁿ
YZT278	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 BUD3-rga2</i> ^{712-1009aa} : <i>GFP:URA3 CDC3-mCherry:LEU2</i>	This study ⁿ
YZT283	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 MLC2-GFP:URA3</i>	This study ^k
YZT284	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 MLC2-rga1</i> ^{700-1007aa} : <i>GFP:URA3</i>	This study ^k
YZT292	As YEF473A except <i>CDC3-GFP:LEU2 GIC2-PBD-RFP::URA3</i>	This study ^o
YZT293	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 CDC3-GFP:LEU2 GIC2-PBD-RFP::URA3</i>	This study ^o
YZT294	As YEF473A except <i>rga2</i> \varnothing :: <i>KanMX6 bem3</i> \varnothing :: <i>TRP1 CDC3-GFP:LEU2 GIC2-PBD-RFP::URA3</i>	This study ^o
YZT295	s YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 rga2</i> \varnothing :: <i>KanMX6 bem3</i> \varnothing :: <i>TRP1 CDC3-GFP:LEU2 GIC2-PBD-RFP::URA3</i>	This study ^o
JGY1621	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 BUD3-GFP:URA3</i>	This study ^k
JGY1622	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 BUD3-rga1</i> ^{700-1007aa} : <i>GFP:URA3</i>	This study ^k
JGY1639	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 BUD3-rga1</i> ^{700-1007aa,R829A} : <i>GFP:URA3</i>	This study ^k
JGY1641	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 BUD3-rga2</i> ^{712-1009aa} : <i>GFP:URA3</i>	This study ^k
JGY1642	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 BUD3-bem3</i> ^{318-1128aa} : <i>GFP:URA3</i>	This study ^k
JGY1644	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 CYK3-GFP:URA3</i>	This study ^k
JGY1645	As YEF473A except <i>rga1</i> \varnothing :: <i>HIS3 CYK3-rga1</i> ^{700-1007aa} : <i>GFP:URA3</i>	This study ^k

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^aYEF1233 is generated by crossing YEF1002 (a *rga1* \varnothing ::*HIS3*) to YEF1000 (\varnothing *rga1* \varnothing ::*HIS3*) (Bi et al., 2000).

^bA segregant of YEF2318 (Caviston et al., 2003).

^cPlasmid Ylp211-GIC2-PBD-RFP was digested with Apal and integrated at the *ura3* locus of Masa1223 (as YEF473A except *SHS1-GFP:LEU2 SPC42-*

GFP::KanMX6; constructed in the Bi laboratory) and Masa1246 (Iwase et al., 2006) to generate YEF4723 and YEF4934.

⁴Plasmid Ylp211-CDC3-GFP (integrative, *URA3*; Caviston et al., 2003) was digested with *SpeI* and integrated at the *CDC3* locus of YEF2324, YEF473A, YEF2392, and YZT195 to generate YZT55, YZT82, YZT111, and YZT198.

⁵*URA3-KanMX6* was amplified from strain Masa1243 (a *myo1 Δ ::URA3-KanMX6*; constructed in Bi laboratory) by a pair of primers containing *RGA1*-flanking sequences and the resulting PCR product was transformed into YEF2324, selecting for Ura⁺Kan^r His⁺ to yield YZT88.

⁶A segregant from the diploid strain generated by crossing YEF2324 to LRSy359.

⁷A segregant from the diploid strain generated by crossing YEF2324 to AM100.

⁸HindIII-digested plasmid pRS315-GFP-RGA1 (Caviston et al., 2003) was transformed into YZT88 to replace *rga1 Δ ::URA3-KanMX6* with *GFP-RGA1* by selecting for Ura⁻ Kan^r GFP⁺ colonies. The resulting strain (a *GFP-RGA1*) was crossed to YEF2325 (\otimes *rga1 Δ ::HIS3*), which is a segregant of YEF2318 (Caviston et al., 2003), to generate YZT156.

⁹A segregant of YZT156.

¹⁰JGY1326 (a *CDC3-mCherry-SpHIS5*; Iwase et al., 2006) was crossed to YEF473B to generate YZT166.

¹¹See description in Materials and methods.

¹²Two segregants of opposite mating types carrying *GFP-RGA1* from YZT156 were crossed to form a diploid strain, which was then transformed with *SpeI*-digested Ylp128-CDC3-DsRed for the integration of the plasmid at the *CDC3* locus to yield YZT211.

¹³YEF1517 (a *cdc42 Δ ::HIS3 ura3::CDC42-GFP:URA3*; Bi et al., 2000) carrying pRS314 (*CEN, TRP1*) was crossed to YEF1518 (\otimes *cdc42 Δ ::HIS3 ura3::CDC42-GFP:URA3*; Bi et al., 2000) carrying pRS315 (*CEN LEU2*) to generate diploids. The resulting strain, after losing both plasmids, was transformed with *SpeI*-digested Ylp128-CDC3-DsRed for the integration of the plasmid at the *CDC3* locus to yield YZT221.

¹⁴Plasmid Ylp128-CDC3-mCherry (integrative, *LEU2*) was digested with *SpeI* and integrated at the *CDC3* locus of JGY1622, JGY1645, and JGY1641 to yield YZT240, YZT241, and YZT278, respectively.

¹⁵Yeast strains YZT292, YZT293, YZT294, and YZT295 were constructed by integrating *BglII*-digested Ylp128-CDC3-GFP (integrative, *LEU2*) and *Apal*-digested Ylp211-GIC2-PBD-RFP (integrative, *URA3*) into strains YEF473A (wild type), YEF2324 (*rga1 Δ ::HIS3*), YEF2392 (*rga2 Δ ::KanMX6 bem3 Δ ::TRP1*), and YEF2380 (*rga1 Δ ::HIS3 rga2 Δ ::KanMX6 bem3 Δ ::TRP1*), respectively.