

Figure S1. **Activity and localization of Rho1 was not significantly affected in PP2A mutants, associated with Fig. 3.** (A) Rho1 activity was does not appear to be significantly affected by loss of *CDC55*. The total cellular amount of active Rho1 (Rho1-GTP) was analyzed in the indicated strains by a previously established pull-down assay for active RhoA/Rho1 using GST-rhotekin RhoA binding domain (RBD) beads (Kimura et al., 2000; Kono et al., 2008; Yoshida et al., 2009). Rho1 was detected by Western blot analysis using a custom-made rabbit anti-Rho1 antibody (Y1486). Ponceau staining is shown as a loading control for GST-rhotekin RBD. (B and C) Rho1 is localized to the bud cortex in the absence of PP2A subunits. GFP-Rho1 (B) and GFP-Rho1 Q68L (C) were expressed under the *RHO1* promoter from the centromeric plasmids, and images were taken in living cells at RT. Note that these experiments were performed in a *swe1Δ* background. Bars, 5 μ m. (D) Bud tip and bud neck localization of Lrg1 was not detectably affected by deletion of *CDC55*. In *swe1Δ* strains, Lrg1-GFP was expressed at the endogenous locus, and live cells were imaged at RT. WT, wild type.

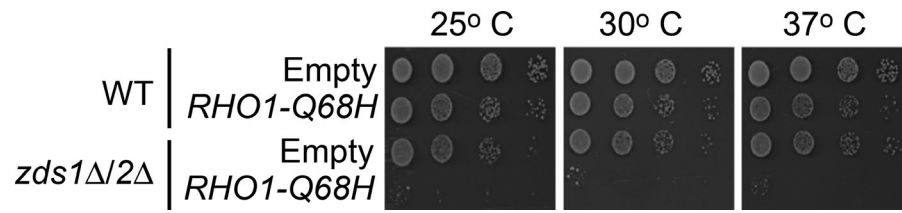


Figure S2. *zds1Δ zds2Δ* cells are sensitive to expression of active Rho1, associated with Fig. 4. Serial dilutions of yeast strains expressing *RHO1-Q68H* (active Rho1) under the control of the *RHO1* promoter were spotted on SC-URA plates and grown at different temperatures for 3 d before the plates were imaged. WT, wild type.

Table S1. Yeast strains used in this study

Strain	Genotype	Source
PY3295	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0</i>	Open Biosystems
PY3296	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0</i>	Open Biosystems
SY1327	<i>MATa rom1Δ::KanMX6 rom2Δ::KanMX6 tus1Δ::KanMX6 + <CEN-ROM2-URA3></i>	Yoshida et al., 2009
SY1854	<i>MATa zds1::HIS3MX6::pGAL₁-3xHA-ZDS1-GFP::KanMX6</i>	This study
VR334	<i>MATa cdc55::CDC55-13xMyc::KanMX6</i>	This study
VR371	<i>MATa cdc55::CDC55-13xMyc::KanMX6 zds1::KanMX6 zds2::HIS3MX6</i>	This study
YSY4	<i>MATα ade2 his3 leu2 lys2 trp1 ura3 rho1Δ::HIS3 ade3::RHO1::LEU2 (YOC764)</i>	Saka et al., 2001
YSY6	<i>MATα ade2 his3 leu2 lys2 trp1 ura3 rho1::LYS2 ade3::rho1-2::HIS3 (YOC729)</i>	Saka et al., 2001
YSY74	<i>MATa cdc55Δ::KanMX6</i>	Rossio and Yoshida, 2011
SY1763	<i>MATa zds1Δ::KanMX6 zds2Δ::HIS3MX6</i>	Rossio and Yoshida, 2011
VR368	<i>MATα pph21Δ::KanMX6</i>	Open Biosystems
VR406	<i>MATα pph22Δ::KanMX6</i>	Open Biosystems
VR408	<i>MATα pph21Δ::KanMX6 pph22Δ::KanMX6</i>	This study
VR403	<i>MATα sit4Δ::KanMX6</i>	Open Biosystems
SY1529	<i>MATa lrg1Δ::KanMX6</i>	Yoshida et al., 2009
SY1390	<i>MATa swe1Δ::LEU2</i>	Rossio and Yoshida, 2011
YSY71	<i>MATa swe1Δ::LEU2 cdc55Δ::KanMX6</i>	Rossio and Yoshida, 2011
YSY80	<i>MATa swe1Δ::LEU2 zds1Δ::KanMX6 zds2Δ::HIS3MX6</i>	Rossio and Yoshida, 2011
SY1630	<i>MATa zds1::ZDS1-GFP::HIS3MX6</i>	Rossio and Yoshida, 2011
VR63	<i>MATa zds1::ZDS1-GFP::HIS3MX6 zds2Δ::HIS3MX6</i>	Rossio and Yoshida, 2011
SY1856	<i>MATa zds1::Zds1ΔC400-GFP::HIS3MX6</i>	Rossio and Yoshida, 2011
VR58	<i>MATa zds1::Zds1ΔC400-GFP::HIS3MX6 zds2Δ::HIS3MX6</i>	Rossio and Yoshida, 2011
SY1857	<i>MATa zds1::Zds1ΔC800-GFP::HIS3MX6</i>	Rossio and Yoshida, 2011
VR60	<i>MATa zds1::Zds1ΔC800-GFP::HIS3MX6 zds2Δ::HIS3MX6</i>	Rossio and Yoshida, 2011
SY1779	<i>MATa cdc55::CDC55-GFP::HIS3MX6</i>	Rossio and Yoshida, 2011
SY1811	<i>MATa cdc55::Cdc55-GFP-NES::KanMX6</i>	Rossio and Yoshida, 2011
SY1808	<i>MATa cdc55::Cdc55-GFP-NLS::KanMX6</i>	Rossio and Yoshida, 2011
SY1793	<i>MATa cdc55::CDC55-GFP::HIS3MX6 zds1::KanMX6 zds2Δ::HIS3MX6</i>	Rossio and Yoshida, 2011
VR21	<i>MATa cdc55::Cdc55-GFP-NES::KanMX6 zds1::KanMX6 zds2Δ::HIS3MX6</i>	Rossio and Yoshida, 2011
YPH499	<i>MATa ade2 his3 leu2 lys2 trp1 ura3</i>	Sikorski and Hieter, 1989
YOC2581	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 zds1Δ::cgHIS3</i>	Sekiya-Kawasaki et al., 2002
SY1762	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 zds1Δ::cgHIS3 zds2Δ::KanMX6</i>	This study
YSY123	<i>MATa mpk1Δ::KanMX6</i>	This study
VR160	<i>MATa mpk1Δ::KanMX6 cdc55Δ::KanMX6</i>	This study
VR194	<i>MATa mpk1Δ::KanMX6 zds1Δ::KanMX6 zds2Δ::HIS3MX6</i>	This study
YSY83	<i>MATa swe1Δ::LEU2 lrg1::LRG1-3xHA::HIS3MX6</i>	This study
YSY85	<i>MATa swe1Δ::LEU2 cdc55Δ::KanMX6 lrg1::LRG1-3xHA::HIS3MX6</i>	This study
YEJ56	<i>MATa lrg1::pGAL₁-3xHA-LRG1::HIS3MX6</i>	This study
YEJ96	<i>MATa cdc55Δ::KanMX6 lrg1::pGAL₁-3xHA-LRG1::HIS3MX6</i>	This study
YEJ968	<i>MATa swe1Δ::LEU2 cdc55Δ::HIS3MX6</i>	This study
YEJ972	<i>MATa swe1Δ::LEU2 cdc55Δ::HIS3MX6 lrg1Δ::KanMX6</i>	This study
YEJ900	<i>MATa swe1Δ::LEU2 sac7::SAC7-3xHA::HIS3MX6</i>	This study
YEJ904	<i>MATa swe1Δ::LEU2 cdc55Δ::KanMX6 sac7::SAC7-3xHA::HIS3MX6</i>	This study
YEJ163	<i>MATα his3Δ200 leu2Δ1 ura3-52 cim3-1,ts (CMY763)</i>	Ghislain et al., 1993
YEJ815	<i>MATa CIM3 sac7::SAC7-3xHA::HIS3MX6</i>	This study
YEJ803	<i>MATa CIM3 cdc55Δ::KanMX6 sac7::SAC7-3xHA::HIS3MX6</i>	This study
YEJ811	<i>MATa cim3-1,ts sac7::SAC7-3xHA::HIS3MX6</i>	This study
YEJ807	<i>MATa cim3-1,ts cdc55Δ::KanMX6 sac7::SAC7-3xHA::HIS3MX6</i>	This study
YSY186	<i>MATa ade2 his3 leu2 lys2 trp1 ura3 fks1Δ::HIS3 fks2Δ::LYS2 ade3::fks1-1154::TRP (YOC1087)</i>	Sekiya-Kawasaki et al., 2002
YSY124	<i>MATα leu2-3,112 ura3-52 trp1-1 his4 can1' pkc1ΔLEU2 <YCP50-pkc1-2,ts></i>	Levin and Bartlett-Heubusch, 1992
SY1895	<i>MATa swe1Δ::LEU2 lrg1::LRG1-GFP::HIS3MX6</i>	This study
SY1897	<i>MATa swe1Δ::LEU2 cdc55Δ::KanMX6 lrg1::LRG1-GFP::HIS3MX6</i>	This study
SY1806	<i>MATa sac7Δ::HIS3MX6</i>	Yoshida et al., 2009

Unless otherwise indicated, all yeast strains used in this study were isogenic or congenic to BY4741 named PY 3295 or BY4742 named PY 3296.

Table S2. Plasmids used in this study

Plasmid	Gene	Marker and vector type	Source
PB2308	<i>ROM2</i>	URA3, CEN	Yoshida et al., 2009
pGEX4T-1	GST	Amp ^R	NA
pSY371	GST-Rho1-Q68L	Amp ^R	This study
pSY372	<i>ZDS1-GFP</i>	NA	This study
pSY373	<i>CDC55-GFP</i>	NA	This study
pSY374	<i>ZDS1</i>	URA3, <i>leu2Δ</i> , 2μ (gTOW6000)	Makanae et al., 2013
pSY65	<i>GFP-RHO1</i>	URA3, CEN	Yoshida et al., 2009
pSY63	<i>GFP-Rho1-Q68L</i>	URA3, CEN	Yoshida et al., 2009
PB1561	<i>RHO1-Q68H</i>	URA3, CEN	Yoshida et al., 2009
PB1544	<i>PKC1-R398P</i>	URA3, CEN	BYP5216 (YGRC)
VR11	<i>CDC55</i>	URA3, 2μ	This study
pSY375	<i>pGAL₁-6xHis-SAC7</i>	URA3, 2μ	This study
PB1354	<i>pGAL₁-PKC1-R398P</i>	NA	Kono et al., 2012
pNV7-MKK1P386	<i>pGAL₁-MKK1-S386P</i>	URA3, Yep (pAT597)	Watanabe et al., 1995
PB1543	<i>PKC1</i>	URA3, 2μ	This study

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