Atkins et al., http://www.jcb.org/cgi/content/full/jcb.201301090/DC1

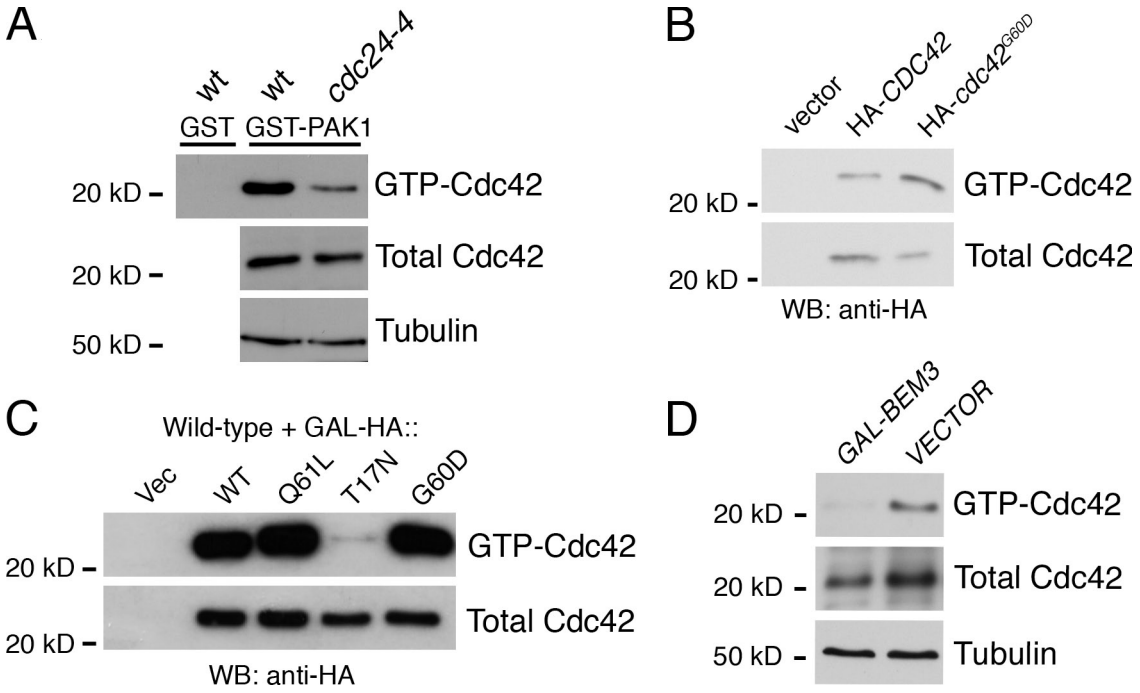

Figure S1. **Additional control experiments for CRIB pull-down assays.** (A) Similar ability of GST-PAK1-CRIB to detect active Cdc42 as for GST-Ste20-CRIB (Fig. 1). Wild-type (wt) and *cdc24-4* cells were grown to early log phase, shifted to 37C° for 3 h, harvested, and subjected to the GST-CRIB pull-down assay using purified GST-PAK1-CRIB. (B) GST-Ste20-CRIB pulls down more *cdc42*^{G60D} than wild-type Cdc42. Wild-type cells were transformed with the indicated plasmids, grown to early log phase, and subjected to the GST-CRIB pull-down assay. (C) GST-Ste20-CRIB binds to dominant-active, but not dominant-negative, versions of Cdc42. Cells were transformed with the indicated plasmids, grown to early log phase, and subjected to the GST-CRIB pull-down assay. (D) Bem3 overexpression reduces global Cdc42 activity. Cells were transformed with the indicated plasmid, grown to early log phase, shifted to media containing galactose for 3 h, and subjected to the GST-CRIB pull-down assay. Vec, vector; WB, Western blot.

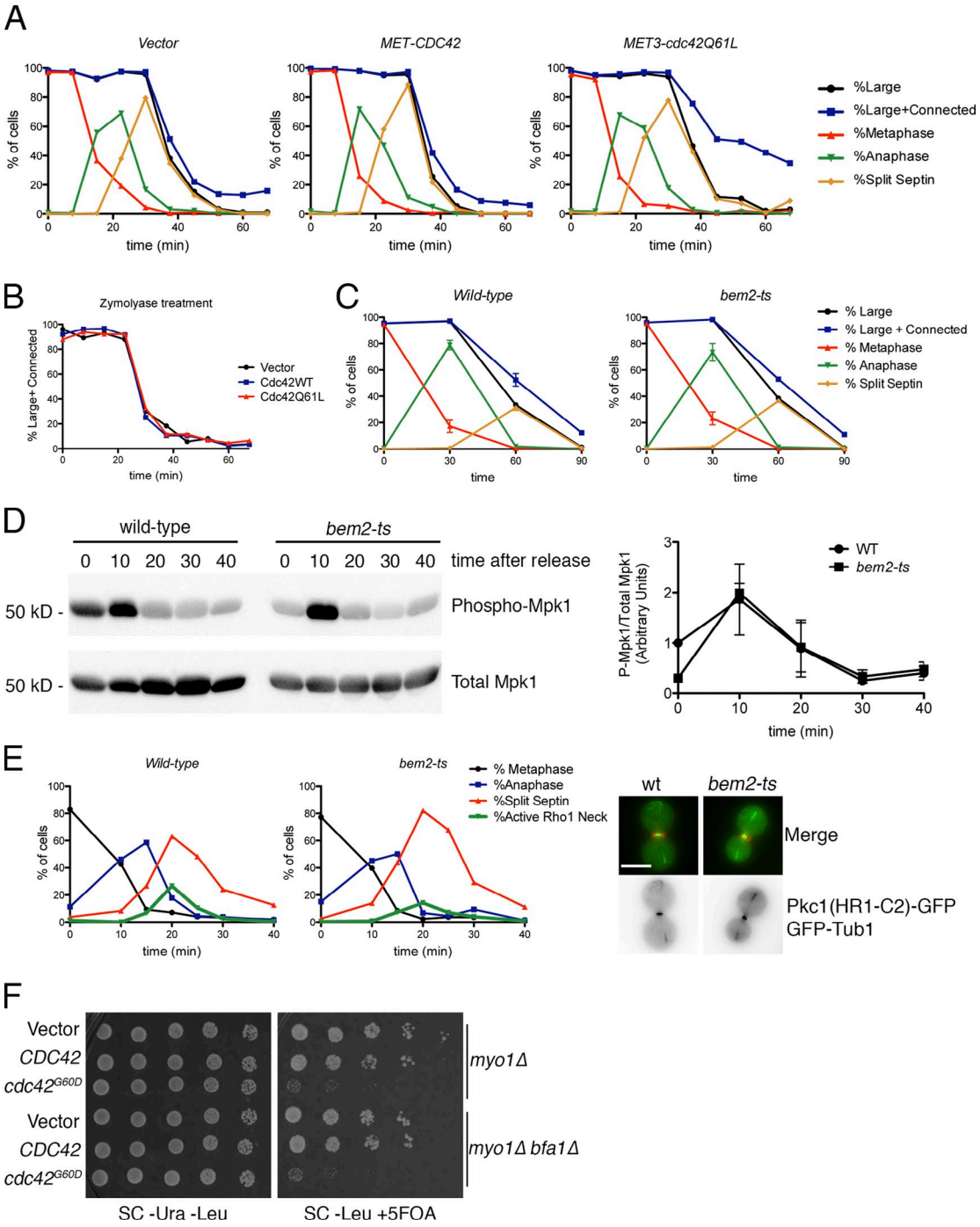

Figure S2. **Cdc42 activation during mitosis causes a cell separation defect.** (A) Expression of GTP-locked Cdc42 at metaphase causes a cell separation defect. *GALL-CDC20 GFP-TUB1 SHS1-GFP* cells were transformed with the indicated plasmids, arrested in metaphase for ∼3 h by depletion of Cdc20, shifted to media lacking methionine for 1 h to express Cdc42 or the indicated variants, and then released into the cell cycle by addition of galactose. Cells were sampled at the indicated time points, fixed, and scored by fluorescence microscopy. The percentage of cells displaying the indicated bud, spindle, or septin morphology is plotted. Percent large indicates the percentage of cells that were large budded. (B) Cells expressing GTP-locked Cdc42 have a cell separation defect. Samples from an experiment in A were treated with zymolyase. The percentage of cells that were large budded or connected was scored at each time point. (C) *bem2-ts* cells undergo normal cell separation at the permissive temperature. Cells were arrested in metaphase for ∼3 h by depletion of Cdc20 and then released into the cell cycle at 25°C and processed and scored as in Fig. 3 E. (D) Mpk1 phosphorylation is unaffected in synchronized *bem2-ts* cells. Cells were synchronized as in Fig. 3 E, and samples taken from the indicated time points (minutes) were processed for Western blotting. Graph shows means ± SEM from two independent experiments. (E) The percentage of cells displaying bud neck localization of Pkc1(HR1-C2) is moderately reduced in *bem2-ts* cells. Cells expressing Pkc1(HR1-C2)-GFP were synchronized as in Fig. 3 E. Percent active Rho1 neck indicates the percentage of cells showing bud neck localization of Pkc1(HR1-C2)-GFP. The data are representative of two independent experiments. (right) Example *GFP-TUB1 SHS1-mCherry* ± *bem2-ts* cells expressing Pkc1(HR1-C2)-GFP are shown. Bar, 5 µm. (F) *BFA1* deletion (to hyperactivate mitotic exit network signaling) fails to suppress the sensitivity of *myo1Δ* cells to *cdc42*^{G60D} expression. Results are representative of two independent transformants; cells were grown at 25°C for 3 d after fivefold serial dilution. The strains are complemented with a *MYO1 CEN URA3* plasmid that is counterselected by 5-fluoroorotic acid (5FOA). SC, synthetic complete; WT, wild type.

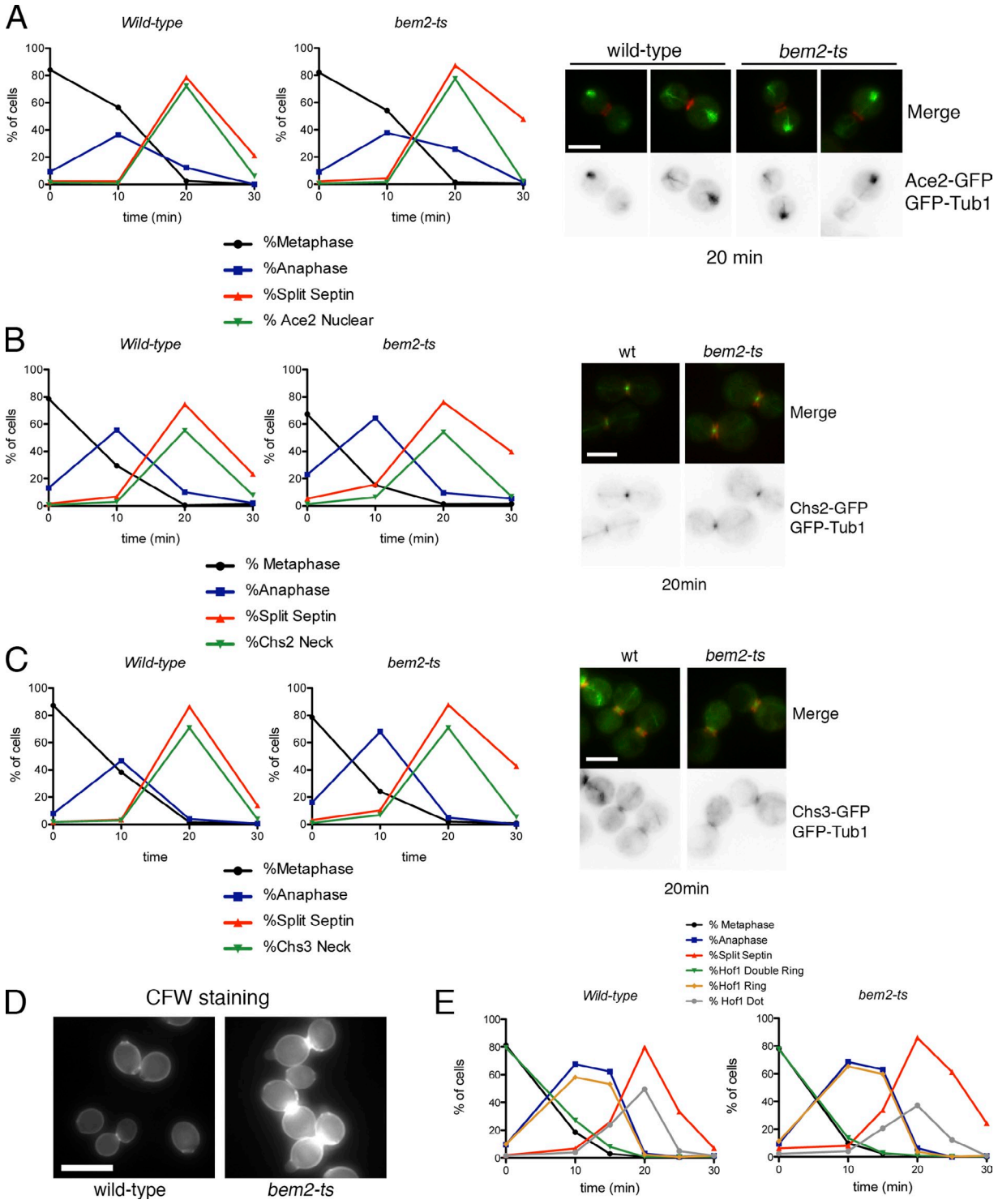

Figure S3. **Localization of cytokinesis and cell separation proteins in *bem2-ts* cells.** (A) Ace2-GFP localizes normally in *bem2-ts* cells undergoing mitotic exit. *MET3-CDC20 GFP-TUB1 SHS1-mCherry ACE2-GFP* ± *bem2-ts* cells were synchronized as in Fig. 3 E. Percent Ace2 nuclear indicates the percentage of cells displaying asymmetric nuclear localization of Ace2. (right) Montage of representative cells (a single focal plane is shown for clarity). Example is representative of two independent experiments. (B) Chs2-GFP localizes normally in *bem2-ts* cells undergoing mitotic exit. *MET3-CDC20 GFP-TUB1 SHS1-mCherry CHS2-GFP* ± *bem2-ts* cells were synchronized as in Fig. 3 E. Graph shows percentage of cells with the indicated spindle or septin morphology. Percent Chs2 neck indicates the percentage of cells showing Chs2-GFP localized to the bud neck. (right) Representative image of cells from 20 min after release (single focal plane for clarity). Example is representative of two independent experiments. (C) Chs3-GFP localizes normally in *bem2-ts* cells undergoing mitotic exit. *MET3-CDC20 GFP-TUB1 SHS1-mCherry CHS3-GFP* ± *bem2-ts* cells were synchronized as in Fig. 3 E. Graph shows percentage of cells with the indicated spindle or septin morphology. Percent Chs3 neck indicates the percentage of cells showing Chs3-GFP localized to the bud neck. (right) Single focal plane of representative cells 20 min after release. Example is representative of two independent experiments. (D) Abnormal chitin deposition at the bud neck in *bem2-ts* cells. Cells sampled 45 min after release from a *CDC20* arrest were stained with calcofluor white (CFW) to visualize chitin. (E) Hof1 localizes normally in *bem2-ts* cells undergoing mitotic exit. *MET3-CDC20 GFP-TUB1 SHS1-mCherry HOF1-GFP* ± *bem2-ts* cells were synchronized as in Fig. 3 E. Graph shows percentage of cells with the indicated spindle or septin morphology. Hof1 localization to the bud neck was scored as a double ring, a ring, a contracted dot, or absent. wt, wild type. Bars: (A–C) 5 µm; (D) 10 µm.

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

Strain	Relevant genotype	Source
PY3295	BY4741 *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATa*	Pellman permanent collections
PY7205	YEF473a S288c *his3 leu2 trp1 ura3 MATa*	E. Bi[a]
PY7206	YEF473a S288c *his3 leu2 trp1 ura3 cdc24-4 MATa*	E. Bi
PY7207	BY4741 *cln1::natMX cln2::hygR GAL1-CLN3-kanR MATa*	This study
PY3665	BY4741 *bar1::kanR MATa*	Pellman permanent collections
PY7208	BY4741 *GALL-CDC20-kanR GFP-TUB1-URA3 MYO1-GFP-HIS3 MATa*	This study
PY6573	BY4741 *GALL-CDC20-kanR GFP-TUB1-URA3 SHS1-GFP-HIS3 MATa*	This study
DLY13157	YEF473a *BEM1-GFP-LEU2 Gic2(CRIB)-tdTomato-URA3 MATa/α*	Howell et al., 2012
PY7209	*cdc5-2::URA3* 5× backcross to BY4741 *MATa*	Yoshida et al., 2006
PY5220	*cdc15-2* 5× backcross to BY4741 *MATa*	Yoshida et al., 2006
PY6200	BY4741 *cdc20-3::kanR MATa*	C. Boone[b]
SY1108	BY4741 *Bem3(1–500)-13myc-HIS3 cdc15-2 MATa*	This study
SY940	BY4741 *Bem3(1–500)-3HA-HIS3 cdc5-2::URA3 MATα*	This study
SY944	BY4741 *Bem3(1–500)-3HA-HIS3 cdc15-2 MATa*	This study
SY938	BY4741 *Bem3(1–500)-3HA MATa*	This study
PY6545	BY4741 *hof1::his3MX6 [HOF1 CEN URA3] MATa*	This study
PY5453	BY4741 *myo1::his3MX6 [MYO1 CEN URA3] MATa*	This study
PY5032	BY4741 *cyk3::kanR MATa*	Pellman permanent collections
PY7210	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR MATa*	This study
PY7211	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR bem2-ts(#84)-HIS3 MATa*	This study
PY7212	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR MYO1-GFP-kanR MATa*	This study
PY7213	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR MYO1-GFP-kanR bem2-ts(#84)-HIS3 MATa*	This study
PY7214	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR IQG1-GFP-kanR MATa*	This study
PY7215	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR IQG1-GFP-kanR bem2-ts(#84)-HIS3 MATa*	This study
PY7216	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR INN1-GFP-kanR MATa*	This study
PY7217	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR INN1-GFP-kanR bem2-ts(#84)-HIS3 MATa*	This study
PY7218	BY4741 *MET3-CDC20-TRP1 SHS1-mCherry-hygR IQG1-GFP-kanR MATa*	This study
PY7219	BY4741 *MET3-CDC20-TRP1 SHS1-mCherry-hygR IQG1-GFP-kanR bem2-ts(#84)-LEU2 MATa*	This study
PY7220	BY4741 *MET3-CDC20-TRP1 SHS1-mCherry-hygR INN1-GFP-kanR MATa*	This study
PY7221	BY4741 *MET3-CDC20-TRP1 SHS1-mCherry-hygR INN1-GFP-kanR bem2-ts(#84)-LEU2 MATa*	This study
PY7222	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR ste20::kanR MATa*	This study
PY7223	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR bem2-ts(#84)-HIS3 ste20::kanR MATa*	This study
PY7224	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR IQG1-kanR ste20::URA3 MATa*	This study
PY7225	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR IQG1-kanR bem2-ts(#84)-HIS3 ste20::URA3 MATa*	This study
PY7226	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR CHS2-GFP-kanR MATa*	This study
PY7227	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR CHS2-GFP-kanR bem2-ts(#84)-HIS3 MATa*	This study
PY7228	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR CHS3-GFP-kanR MATa*	This study
PY7229	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR CHS3-GFP-kanR bem2-ts(#84)-HIS3 MATa*	This study
PY7230	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR ACE2-GFP-kanR MATa*	This study
PY7231	BY4741 *MET3-CDC20-TRP1 GFP-TUB1-LEU2 SHS1-mCherry-hygR ACE2-GFP-kanR bem2-ts(#84)-HIS3 MATa*	This study

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[b]University of Toronto, Toronto, Ontario, Canada.

Table S2. **Plasmids used in this study**

Plasmid	Relevant features	Source
PB70	pRS316 *URA3 CEN AmpR*	Pellman perms
PB72	pRS415 *LEU2 CEN AmpR*	Pellman perms
PB158	*2μ URA3*	Pellman perms
PB3050	pRS415 *GAL1-HA-CDC42 LEU2 CEN AmpR*	This study
PB3051	pRS415 *GAL1-HA-CDC42^{Q61L} LEU2 CEN AmpR*	This study
PB3052	pRS415 *GAL1-HA-CDC42^{T17N} LEU2 CEN AmpR*	This study
PB3053	pRS415 *GAL1-HA-CDC42^{G60D} LEU2 CEN AmpR*	This study
PB3054	pRS415 *HA-CDC42 LEU2 CEN AmpR*	This study
PB3055	pRS415 *HA-cdc42^{G60D} AmpR*	This study
PB1622	pGEX-5X-1 *GST AmpR*	Pellman perms
PB3056	pGEX-5X-1 *GST-STE20(332–411) AmpR*	This study
PB3057	pRS415 *MET3-HA-CDC42 CEN LEU2 AmpR*	This study
PB3058	pRS415 *MET3-HA-CDC42^{Q61L} CEN LEU2 AmpR*	This study
YEp351-*CDC24*	*CDC24 LEU2 2μ AmpR*	D. Johnson[a]; Richman et al., 1999
pAJ044	*IQG1 2μ URA3*	J. Heinisch[b]; Jendretzki et al., 2009
PB2680	GST-PAK1 *AmpR*	Y. Zheng[c]
E735	pRS316-HOF1 *HOF1 CEN URA3 AmpR*	E. Bi[d]; Vallen et al.,2000
E1361-1	yIP211-GIC2-PBD-tdTomato-*URA3 AmpR*	E. Bi; Tong et al., 2007
pVD63	*PKC1*(HR1-C2)-GFP *URA3 CEN AmpR*	M. Cyert[e]; Denis and Cyert, 2005
pMK187	*GALS-BEM3-GFP CEN URA3 AmpR*	M. Peter[f]; Knaus et al., 2007
PB3063	pRS316 MYO1 CEN URA3 *AmpR*	This study
PB3064	*pMET3-CDC20-TRP1 AmpR*	F. Uhlmann[g]; Uhlmann et al., 2000
PB3065	pDLB3138 *bem2-ts(#84)::LEU2* integrative *AmpR*	This study
PB3066	*bem2-ts(#84)::HIS3* integrative *AmpR*	This study
pEL45	*ste20::URA3* integrative	M. Whiteway[h]; Leberer et al., 1992

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[b]University of Osnabrück, Osnabrück, Germany.
[c]Cincinnati Children's Hospital, Cincinnati, OH.
[d]University of Pennsylvania, Philadelphia, PA.
[e]Stanford University, Stanford, CA.
[f]ETH Zurich, Zurich, Switzerland.
[g]London Research Institute, London, England, UK.
[h]Concordia University, Montreal, Quebec, Canada.

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