SUPPLEMENTAL MATERIALS AND METHODS

for

Cdc42-Interacting Protein Bem4 Regulates the Filamentous Growth MAP Kinase Pathway

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Role of Sbe2p/Sbe22p and septins in regulating Bem4p function in the filamentous growth pathway. A) Sbe2p/Sbe22p do not regulate the filamentous growth pathway. Equal numbers of wild type (PC538), *bem4Δ* (PC3016), *sbe2Δ* (PC4056), *sbe22Δ* (PC3874), *sbe2Δ sbe22Δ* (PC4060), and *ste12Δ* (PC2382) cells were spotted onto SD+AA, SD-HIS, or YEPD plates and incubated for 3d. The *FUS1-HIS3* growth reporter (typically a mating pathway reporter but in a *ste4* strain shows dependence on filamentous growth pathway regulators) was used to evaluate the activity of the pathway of strains spotted onto SD+AA and SD-HIS (left panels). The plate-washing assay was used to evaluate the filamentation pathway by spotting cells onto YEPD media and performing the plate-washing assay (right panels). **B)** Overexpression of *SBE2* or *SBE22* does not rescue the signaling defect of a *bem4* mutant. Equal numbers of wild type (PC538) and *bem4Δ* (PC3016) cells containing either a control plasmid pRS316 (PC4204) or overexpression plasmids *pGAL-Sbe2* or *pGAL-Sbe22* (Open Biosystems) were spotted onto SGAL-URA (left) or SGAL-URA-HIS (right) plates and photographed after being grown for 2d. **C)** The septin defect seen in the *bem4* mutant does not account for its role in the filamentation pathway. Wild type (PC538), *bem4Δ* (PC3016), and *ste12Δ* (PC2382) strains expressing pCdc12p-GFP (PC1364)(from J. Pringle) were grown to mid-log phase in YEPD, and protein localization was examined at 100X magnification. Cdc12p localization was observed in *bem4* mutants with normal (84%) and abnormal (15%) morphology. DIC, top panels; FITC bottom panels; Bar, 10 microns. Approximately15% of *bem4* cells showed abnormal septin staining, whereas a >4-fold defect in MAPK activity was observed.

Supplemental Figure 2. Bem4p regulates filamentous growth and the filamentous growth pathway. A) Examples of pseudohyphal growth of wild type (PC344) and *bem4Δ* (PC3908) homozygous diploid strains. Bar, 50 microns. **B)** Examples of diploid colonies producing pseudohyphae on nitrogen-limited medium. Boxed colonies were used for **Fig. 1D**. **C)** Activity of the cross talk reporter (*FUS1-HIS3*) in *ste4* mutants that contain the conditional protein glycosylation mutant *pmi40-101*. Equal numbers of *pmi40-101* (PC2148), *pmi40-101msb2Δ* (PC979), *pmi40-101sho1Δ* (PC448), *pmi40-101 bem4Δ* (PC3525), and *pmi40-101 ste12Δ* (PC389) cells were spotted onto YEPD or SD-HIS media supplemented with 200 mM mannose (High Man) or no mannose (Limiting Man). Plates were incubated for 48h and photographed. In high mannose, the glycosylation deficiency is suppressed, and cells are His-. In limiting mannose, the filamentous growth pathway is activated (His+), which is dependent on Msb2p, Sho1p, Bem4p, and Ste12p. Activation of the pathway contributes to viability of *pmi40-101*, as seen by the growth defect of the *msb2Δ, sho1Δ, bem4Δ,* and *ste12Δ* mutants on YEPD limiting mannose. **D)** P~ Kss1p in the *pmi40-101* mutant +/-50 mM mannose (+). Asterisk refers to a

Kss1p-dependent band seen in some conditions. **E)** Pectinase assay showing secretion of pectinase in the indicated mutants. **F)** Flo11p-HA shedding in wild-type cells and the *bem4Δ* and *ste12Δ* mutants. Colonies were grown on nitrocellulose filters on YEPD media for 2d (top panel). After washing, the filter was probed by anti-HA antibodies to detect shed Flo11p-HA. The level of Flo11p-HA shedding was determined by densitometry by ImageJ of immunoblots following background subtraction. Values were normalized to colony area and set to a value of 1 for wild type. Other values were determined accordingly. Flo11p-HA shedding was measured at 1d and 2d time periods; the 2d time period is shown.

Supplemental Figure 3. Role of Bem4p in regulating the mating pathway. A) Shmoo formation. Strains were grown to mid-log phase in YEPD and treated with 1mM of α-factor for the times indicated. Shmoos were evaluated by microscopy. Time 0h, shmoos were counted prior to the addition of α-factor. The average of two independent trials is shown; error bars show the standard deviation between trials. More than 300 cells were counted for each time point. **B)** Mating efficiency. Wild type, *bem4Δ*, and *ste12Δ* strains were grown to saturation and mixed for 8h at 30˚C in YEPD. Diploid formation was assessed by the presence of antibiotic resistance markers. **C)** Quantitation of halo assay. Two concentrations (10^5 and 10^6) of wild-type (PC313), *bem4Δ* (PC3343), *far1Δ* (PC623), and *ste12Δ* (PC350) cells were spread onto YEPD media. 1μM and 5μM of α-factor were applied to plates, which were incubated for 24h at 30˚C. The values represent the size of halos determined by quantifying the area using the circle tool in ImageJ. **D)** Clustering of GFP-Cdc42p in cells exposed to alpha factor. Wild type (PC313) and *bem4Δ* (PC3343) strains harboring pGFP-Cdc42p were grown to mid-log phase in YEPD. Cells were washed three times and treated with 5 μM α-factor. Values represent the percentage of cells displaying polarization of GFP-Cdc42p at shmoo tips during the indicated time points. At least 200 cells were counted, and the experiment was performed in duplicate. **E)** Genetic suppression analysis for Fig. 3A.

Supplemental Figure 4. Clustal W2 alignment of Bem4p with other proteins. Homology between Bem4p and members of the Smg GDS family of proteins. Proteins that bear homology to Smg GDS proteins from *Homo sapiens* (Rap1, GTP-GDP dissociation stimulator 1, isoform CRA_c, accession number EAX06070.1)*, Mus musculus* (EDL12082.1)*, Xenopus laevis* (NP_001088766.1)*, Drosohila melanogaster* (NP_477305.1)*,* and *S. cerevisiae* Bem4p (NP_015164.1). Red, identical residues between Bem4p and at least 4 other proteins. Pink shows similarity for aliphatic (L, I, V) or charged (D and E) amino acids. The GEF domain is marked by a black box. The blue lines mark conserved regions of the GEF domain, which are not present in Bem4p. Manual alignments were made at positions 474 for Bem4p. Lower panel, Arm-like repeats in the Bem4p protein. Asterisk, fully conserved residue; colon, residues with strongly similar properties (> 0.5 in Gonnet PAM 250 matrix); period; residues with weakly similar properties (=< 0.5 in the Gonnet PAM 250 matrix).

Supplemental Figure 5. Yeast MAPK pathways that share components incorporating the discovery of Bem4p. The mating, filamentous growth and HOG pathways shown as different pathways (A), or a core pathway with different pathway-specific regulators (B) in their respective colors. The discovery of Bem4p suggests that a core pathway (composed of Cdc42p, Ste20p, Ste11p, and Ste50p) diversified by the evolution of pathway-specific factors.

Supplemental Figure 6. Role of Bem4p in the localization of GFP-Cdc42p. A) Additional examples of cells expressing GFP-Cdc42p. Bar, 5 microns. **B)** Quantitation of the localization of GFP-Cdc42p from the cell interior to the cell exterior. The average fluorescence intensity for five cells is shown using the line feature of ImageJ. A line was drawn perpendicular to the plane of the PM. The first interior value was set to 100% for all samples; other values were adjusted accordingly. Samples were corrected against background levels. Raw data was averaged before background subtraction and adjustment to 100%. **C)** Example of fluorescence intensity from a single wild-type cell (top line) and a single *bem4*Δ mutant cell (bottom line).

SUPPLEMENTAL TABLES

Table S1. Yeast strains.

Strain	Genotype	Reference
BY4741[c]	*MAT***a** *his3Δ0 leu2Δ0 ura3Δ0 met15Δ0*	BRACHMANN *et al.*, 1998
PC284	*PJ69-4***a** *MAT***a** *trpl-901 leu2-3,112 ura3-52 his3-200 ga14A ga18OA LYSZ::GALl-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	JAMES *et al.*, 1996
PC312	*MATα ura3-52*	LIU *et al.*, 1993
PC313	*MAT***a** *ura3-52*	LIU *et al.*, 1993
PC344	*MAT***a** *ura3-52 / MATα ura3-52*	CULLEN *et al.*, 2004
PC350	*MAT***a** *ura3-52 ste12::KlURA3*	This study
PC389[d]	MATα *ste4 FUS1-HIS3 ura3-52 pmi40-101 ste12::KlURA3*	CULLEN *et al.*, 2000
PC446[d]	MATα *ste4 FUS1-HIS3 ura3-52 pmi40-101 ste50::KlURA3*	CULLEN *et al.*, 2000
PC448[d]	MATα *ste4 FUS1-HIS3 ura3-52 pmi40-101 sho1::KlURA3*	CULLEN *et al.*, 2000
PC479	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 rga2::KlURA3*	SMITH et al. 2002
PC480	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bem3::KlURA3*	SMITH et al. 2002
PC522[d]	MATα *ste4 FUS1-HIS3 ura3-52 pmi40-101 ste20::KANMX6*	CULLEN *et al.*, 2000
PC538[a]	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52*	CULLEN *et al.*, 2004
PC539	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ste12::KlURA3*	PITONIAK *et al.*, 2009
PC544	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52*	CULLEN and SPRAGUE, 2002
PC549	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ste20::URA3*	CULLEN and SPRAGUE, 2002
PC551	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pea2::KlURA3*	CULLEN and SPRAGUE, 2002
PC552	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 hsl1::KlURA3*	CULLEN and SPRAGUE, 2000
PC554	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 spa2::KlURA3*	CULLEN and SPRAGUE, 2002
PC555	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 hsl7::KlURA3*	CULLEN and SPRAGUE, 2000
PC559	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 gal4::KlURA3*	CULLEN and SPRAGUE, 2000
PC560	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 snf1::URA3*	CULLEN and SPRAGUE, 2000
PC563	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bud8::KlURA3*	CULLEN and SPRAGUE, 2002
PC568	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 sip4::KlURA3*	CULLEN and SPRAGUE, 2000
PC587	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 snf4::KlURA3*	CULLEN and SPRAGUE, 2000
PC622	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-SHO1::KanMX6*	CULLEN *et al.*, 2004
PC623	*MAT***a** *ura3-52 far1::KlURA3*	This study
PC624	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-SHO1::KanMX6 ste12::KlURA3*	PITONIAK *et al.*, 2009
PC637	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bud4::KlURA3*	CULLEN and SPRAGUE, 2002
PC642	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pbs2 sho1::KlURA3*	This study
PC644	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pbs2 ste12::KlURA3*	This study
PC648	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 STE20*$^{\Delta CRIB}$	This study
PC673	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ste20::KlURA3*	PITONIAK *et al.*, 2009
PC717	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 rsr1::KlURA3*	CULLEN and SPRAGUE, 2002
PC720	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ste5::KlURA3*	This study
PC842	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pGAL-GFP-BUD14::KlURA3*	CULLEN and SPRAGUE 2002
PC916	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bud6::KlURA3*	CULLEN and SPRAGUE 2002
PC948	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 msb2::KanMX6*	CULLEN *et al.*, 2004
PC955	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 gip1::KanMX6*	This study
PC961	*MAT***a** *ura3-52 msb2::KanMX6*	This study
PC967	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 mad2::KanMX6*	This study
PC968	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 mad3::KanMX6*	This study
PC969	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cla4::KanMX6*	This study
PC986[b]	*MAT***a** *ura3-52 leu2 his3 trp1*	ROBERTS *et al.*, 2000
PC979[d]	MATα *ste4 FUS1-HIS3 ura3-52 pmi40-101 msb2::KANMX6*	CULLEN *et al.*, 2000
PC999	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA*	CULLEN *et al.*, 2004

PC1029	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 flo11::KanMX6*	PITONIAK *et al.*, 2009
PC1063	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 rdi1::KlURA3*	This study
PC1241	*MAT***a** *ura3-52 msb2::HYG / MATα ura3-52 msb2::NAT*	CULLEN *et al.*, 2004
PC1242	*MAT***a** *ura3-52 sho1::HYG / MATα ura3-52 sho1::NAT*	CULLEN *et al.*, 2004
PC1436	*MAT***a** *ura3-52 leu2 his3 cdc24-4*	A. Bender
PC1437	*MAT***a** *ura3-52 leu2 his3 trp1 cdc42-1*	D. Johnson
PC1509	*MAT***a** *ura3-52 far1::NAT*	This study
PC1516	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52* $MSB2^{\Delta 100\text{-}818}$	VADAIE *et al.*, 2008
PC1519	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pgu1::KanMX6*	PITONIAK *et al.*, 2009
PC1523	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ssk1::NAT*	PITONIAK *et al.*, 2009
PC1531	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 sho1::HYG*	CULLEN *et al.*, 2004
PC1558	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 sho1::NAT ssk1::HYG*	This study
PC1620	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52* $MSB2^{\Delta 100\text{-}818}$ *sho1::HYG*	VADAIE *et al.*, 2008
PC1811	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52* $MSB2^{\Delta 100\text{-}818}$ *ste12::KlURA3*	VADAIE *et al.*, 2008
PC1894	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 leu2::HYG*	CULLEN *et al.*, 2004
PC1994	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 rga1::URA3*	SMITH et al. 2002
PC2043	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA*	KARUNANITHI *et al.*, 2010
PC2053	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pbs2::KanMX6*	PITONIAK *et al.*, 2009
PC2061	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ssk1::NAT ste11::KlURA3*	PITONIAK *et al.*, 2009
PC2110	*MAT***a** *ste4 fus1-lacZ::NAT FUS1-HIS3 ura3-52 pbs2::KanMX6*	PITONIAK *et al.*, 2009
PC2148[d]	MATα *ste4 FUS1-HIS3 ura3-52 pmi40-101*	CULLEN *et al.*, 2000
PC2184	*MATα leu2::ura3 ste12::KlURA3*	PITONIAK *et al.*, 2009
PC2382	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ste12::KanMX6*	This study
PC2519	*MAT***a** *ste4 fus1-lacZ::NAT FUS1-HIS3 ura3-52*	PITONIAK *et al.*, 2009
PC2522	*MAT***a** *ste4 fus1-lacZ::NAT FUS1-HIS3 ura3-52 sho1::KanMX6*	PITONIAK *et al.*, 2009
PC2544	*MAT***a** *ste4 fus1-lacZ::NAT FUS1-HIS3 ura3-52 ste11::KanMX6*	This study
PC2545	*MAT***a** *ste4 fus1-lacZ::NAT FUS1-HIS3 ura3-52 ste12::KanMX6*	PITONIAK *et al.*, 2009
PC2687	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA ste12::KlURA3*	PITONIAK *et al.*, 2009
PC2691	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA ste12::KlURA3*	KARUNANITHI *et al.*, 2010
PC2738	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bem4::KlURA3*	This study
PC2754	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4-GFP::KanMx6*	This study
PC2964	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ssk1::NAT bem4::KlURA3*	This study
PC3014	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ssk1::NAT ste11::URA3 bem4::HYG*	This study
PC3016	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bem4::HYG*	This study
PC3098	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 mig1::HYG*	KARUNANITHI and CULLEN 2013
PC3166	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 rdi1::KlURA3*	This study
PC3206	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cap2::KlURA3*	This study
PC3214	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 aip1::KlURA3*	This study
PC3226	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bbc1::KlURA3*	This study
PC3286	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-BEM4::KanMx6*	This study
PC3341	*MAT***a** *ura3-52 bem4::KlURA3*	This study
PC3343	*MATα ura3-52 bem4::HYG*	This study
PC3387	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-SHO1::KanMX6 bem4::KlURA3*	This study
PC3390	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52* $MSB2^{\Delta 100\text{-}818}$ *bem4::KlURA3*	This study
PC3391	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 rga1::NAT*	This study
PC3392	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ste12::KanMX6*	This study

	rga1::NAT	
PC3393	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bem4::HYG rga1::NAT*	This study
PC3398	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4-HA::KanMx6*	This study
PC3448	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4-HA::KanMx6 pGFP-CDC42 URA3*	This study
PC3495	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ent2::KlURA3*	This study
PC3496	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 far1::KlURA3*	This study
PC3497	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 msb1::KlURA3*	This study
PC3498	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 msb4::KlURA3*	This study
PC3499	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 tos2::KlURA3*	This study
PC3500	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 sla2::KlURA3*	This study
PC3501	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bem2::KlURA3*	This study
PC3502	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 mdg1::KlURA3*	This study
PC3503	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bzz1::KlURA3*	This study
PC3525[d]	MATα *ste4 FUS1-HIS3 ura3-52 pmi40-101 bem4::KlURA3*	This study
PC3526	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 leu2::HYG BEM4-HA::GENT*	This study
PC3541	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pGFP-CDC42 URA3*	This study
PC3542	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bem4::HYG pGFP-CDC42 URA3*	This study
PC3544	*MATα ura3-52 bem4::NAT*	This study
PC3551	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 leu2::HYG bem4::NAT*	This study
PC3722	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4*$^{\Delta 600\text{-}633}$*-HA::KanMx6*	This study
PC3750	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 tmn2::KlURA3*	This study
PC3752	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 opy2::KlURA3*	KARUNANITHI and CULLEN 2013
PC3754	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 skm1::KlURA3*	This study
PC3756	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 boi1::KlURA3*	This study
PC3758	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 boi2::KlURA3*	This study
PC3760	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 rho2::KlURA3*	This study
PC3762	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 rho4::KlURA3*	This study
PC3766	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 gic1::KlURA3*	This study
PC3771	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 gic2::KlURA3*	This study
PC3862	*MAT**a** ura3-52 ste4 FUS1-lacZ FUS1-HIS3 ste11::NAT*	This study
PC3863	*MAT**a** ura3-52 ste4 FUS1-lacZ FUS1-HIS3 ste50::NAT*	This study
PC3865	*MAT**a** ura3-52 ste4 FUS1-lacZ FUS1-HIS3 get3::KlURA3*	This study
PC3867	*MAT**a** ura3-52 ste4 FUS1-lacZ FUS1-HIS3 gin4::KlURA3*	This study
PC3870	*MAT**a** ura3-52 ste4 FUS1-lacZ FUS1-HIS3 opy1::KlURA3*	This study
PC3874	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 sbe22::NAT*	This study
PC3876	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pbs2 bem4::KlURA3*	This study
PC3884	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 leu2::HYG bem4::NAT pCDC24-GFP LEU2*	This study
PC3908	*MAT**a** ura3-52 bem4::HYG / MATα ura3-52 bem4::NAT*	This study
PC3924	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bag7::KlURA3*	This study
PC3998	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-BEM4*$^{\Delta 1\text{-}300}$*::KanMx6*	This study
PC4056	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 sbe2::KlURA3*	This study
PC4060	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 sbe22::NAT sbe2::KlURA3*	This study
PC4094	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-BEM4*$^{\Delta 1\text{-}200}$*::KanMx6*	This study
PC4182	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA bem4::KlURA3*	This study
PC4256	*MAT**a** ura3-52 ste4 FUS1-lacZ FUS1-HIS3 rsr1::NAT*	This study
PC4382	*MAT**a** ura3-52 ste4 FUS1-lacZ FUS1-HIS3 gic1::KlURA3 gic2::NAT*	This study
PC4729	*MAT**a** ura3-52 ste12::NAT*	This study
PC4805	*MAT**a** ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4*$^{\Delta 16\text{-}99}$*-GFP::KanMx6*	This study

PC4807	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4*$^{\Delta 99\text{-}200}$*-GFP::KanMx6*	This study
PC4809	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4*$^{\Delta 200\text{-}300}$*-GFP::KanMx6*	This study
PC4811	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4*$^{\Delta 300\text{-}400}$*-GFP::KanMx6*	This study
PC4882	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4*^{T619A}*-GFP::KanMx6*	This study
PC4950	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 STE11-GFP::KanMX6*	This study
PC4952	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 bem4::HYG STE11-GFP::KanMX6*	This study
PC4954	*MAT***a** *ura3-52 ste4 FUS1-lacZ FUS1-HIS3 BEM-HA::NAT STE11-GFP::KanMX6*	This study
PC4970	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT pRS316-CDC24*	This study
PC4982	*MAT***a** *ura3-52 ste50::NAT*	This study
PC5124	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT pYEp351-CDC24-GFP*	This study
PC5125	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT pYEp351-MYR-CDC24-GFP*	This study
PC5376	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 BEM4-GFP::KanMX6 sho1::NAT*	This study
PC5387	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 leu2::HYG pCDC24-GFP LEU2*	This study
PC5388	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 leu2::HYG BEM4-HA::GENT pCDC24-GFP LEU2*	This study
PC5965	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT sho1::KanMX6 YEp351-CDC24-GFP*	This study
PC5970	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT bem4::KanMX6 YEp351-CDC24-GFP*	This study
PC5971	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT bem4::KanMX6 YEp351-MYR-CDC24-GFP*	This study
PC6013	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT sho1::KanMX6 YEp351-CDC24-GFP*	This study
PC6080	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT pRS315-cdc24-4*	This study
PC6081	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 cdc24::NAT pRS425-cdc24-4*	This study

a. All strains are Σ1278b background unless otherwise indicated.

b. S288c background ordered deletion collection control strain.

c. Deletion strains from the *MAT***a** ordered deletion collection in the S288c background were also used in this study.

d. 246-1-1 strain background.

Table S2. Plasmids used in this study.

Strain	Description	Reference
PC1027	*pMSB2*^{CG}*-lacZ URA3*	PITONIAK *et al*., 2009
PC1041	*pKSS1-lacZ LEU2*	ROBERTS *et al*., 2000
PC1042	*pPGU1-lacZ LEU2*	ROBERTS *et al*., 2000
PC1043	*pSVS1-lacZ LEU2*	ROBERTS *et al*., 2000
PC1044	*pYLR042c-lacZ LEU2*	ROBERTS *et al*., 2000
PC1236	*pλYES-GAL-MID2 URA3*	BIRKAYA *et al.,* 2009
PC1364	*pCDC12-GFP URA3*	LIPPINCOTT and LI, 1998
PC1368	*pFgTY-lacZ URA3*	LALOUX *et al*., 1994
PC1405	*pFRE-lacZ URA3*	MADHANI *et al*., 1997
PC1422	*pRS315*	SIKORSKI and HEITER, 1989
PC1441	p*STE11-4 YCp50 URA3*	STEVENSON *et al*., 1992
PC1442	p*STE11-1 YCp50 URA3*	STEVENSON *et al*., 1992
PC1601	*pRS316-SHO1-GFP URA3*	MARLES *et al.*, 2004
PC1614	*pRS316-SHO1-GFP (D16H) URA3*	MARLES *et al.*, 2004
PC1696	*pMSB2-GFP URA3 (pMSB2*)*	VADAIE *et al*., 2008
PC1883	*pGFP-BUD8 LEU2*	HARKINS *et al.*, 2001
PC1885	*pMPY-3xHA*	SCHNEIDER *et al*. 1995
PC2205	*pNAT*	GOLDSTEIN *et al*., 1999
PC2206	*pHYG*	GOLDSTEIN *et al*., 1999
PC2242	*pGEX-2T-PBD*	OTSUKI *et al*., 2001
PC2432	*pRS316 SHO1-HA::KANMX6 (D16H) URA3*	VADAIE *et al*., 2008
PC2433	*pRS316 SHO1 (P120L)-HA::KANMX6 (D16H) URA3*	VADAIE *et al*., 2008
PC2435	*pRS316 SHO1 (S220F)-HA::KANMX6 (D16H) URA3*	VADAIE *et al*., 2008
PC2445	*pRS316-SHO1-ΔSH3-GFP URA3*	ZARRINPAR *et al.,* 2004
PC2560	*pRS426 GFP-2xPH URA3*	GARRENTON *et al*., 2010
PC3132	*pMSB2-lacZ URA3*	PITONIAK *et al*., 2009
PC3133	*pHKR1-lacZ URA3*	PITONIAK *et al*., 2009
PC3483	*pOAD LEU2*	MCCRAITH *et al*., 2000
PC3484	*pOAD-CDC42 LEU2*	DREES *et al*., 2001
PC3485	*pOAD-CDC24 LEU2*	DREES *et al*., 2001
PC3486	*pOBD-2-CDC11 TRP*	DREES *et al*., 2001
PC3488	*pOBD-2-CDC42 TRP*	DREES *et al*., 2001
PC3489	*pOBD-2-CDC42*^{D57Y} *TRP*	DREES *et al*., 2001
PC3621	*pGFP-CDC24 LEU2*	WEDLICH-SOLDNER *et al*., 2004
PC4190	*pOAD-BEM4 LEU2*	DREES *et al*., 2001
PC4204	*pRS316*	SIKORSKI and HEITER, 1989
PC4206	*pOBD-2 TRP*	MCCRAITH *et al*., 2000
PC4214	*pBEM4-lacZ URA3*	This study
PC4222	*pGBDU-C1-RAS2 URA3*	This study
PC4223	*pGBDU-C1-RAS2(V19) URA3*	This study
PC4224	*pGBDU-C1-PBS2 URA3*	This study
PC4225	*pGBDU-C1-CDC24 URA3*	This study
PC4226	*pGBDU-C1-CDC24*$^{\Delta PB1}$ *URA3*	This study
PC4369	*pGBDU-C1-STE11 URA3*	This study
PC4370	*pGBDU-C1-STE50 URA3*	This study
PC4394	*pRL116 pGFP-STE20 URA3*	LAMSON *et al*., 2002
PC4460	*pOBD-2-BEM4*$^{\Delta 1-200}$ *TRP*	This study
PC4462	*pOBD-2-BEM4*$^{\Delta 1-300}$ *TRP*	This study
PC4464	*pOBD-2-BEM4*$^{\Delta 1-400}$ *TRP*	This study
PC4466	*pOBD-2-BEM4*$^{\Delta 1-500}$ *TRP*	This study
PC4506	*pOAD-BEM4*$^{\Delta 1-100}$ *LEU2*	This study
PC4507	*pOAD-BEM4*$^{\Delta 1-200}$ *LEU2*	This study
PC4509	*pOAD-BEM4*$^{\Delta 1-300}$ *LEU2*	This study

PC4511	*pOAD-BEM4*$^{\Delta 1-400}$ *LEU2*	This study
PC4513	*pOAD-BEM4*$^{\Delta 1-500}$ *LEU2*	This study
PC4590	*pOBD-2-RSR1 TRP*	DREES *et al*., 2001
PC4683	*pGBDU-C1-KSS1 URA3*	This study
PC4684	*pGBDU-C1-STE7 URA3*	This study
PC4686	*pGBDU-C1-STE11-1 URA3*	This study
PC4687	*pGBDU-C1-CDC42*$^{Q61L/C188S}$ *URA3*	This study
PC4688	*pGBDU-C1-STE11-4 URA3*	This study
PC4691	*pGBDU-C1-CDC42*^{D118A} *URA3*	This study
PC4697	*pGBDU-C1-STE11-Nterm*	This study
PC4399	*pRS316-CDC24*	This study
PC4700	*pGBDU-C1-STE11-kinase*	This study
PC4731	*pCTT1-lacZ URA3*	This study
PC4878	*pFUS1-lacZ URA3*	This study
PC5155	*pRS315-CDC24-GFP*	This study
PC5157	*pYEp351-CDC24-GFP*	This study
PC5161	*pYEp-351-MYR-CDC24-GFP*	This study
PC5806	*pGEX4T1-CDC24*	This study
PC5807	*pMAL-C2-BEM4*	This study
PC5960	*pET28b-CDC42*	This study
PC6045	*pGEX4T1-STE11*	This study
PC6077	*pRS315-CDC24-4*	This study
PC6078	*pRS425-CDC24-4*	This study
PC6235	*pMAL-C2*-Bem4 1-100	This study
PC6236	*pMAL-C2*-Bem4 1-200	This study
PC6237	*pMAL-C2*-Bem4 1-300	This study
PC6238	*pMAL-C2*-Bem4 1-400	This study
PC6239	*pMAL-C2*-Bem4 1-500	This study
PC6240	*pMAL-C2*-Bem4 1-600	This study
PC6272	*pYEp351-pTEF2-BEM4-GFP*	This study
PC6273	*pYEp352-pTEF2-BEM4-GFP*	This study
PC6274	*pGEX4T1-CDC24-PB1Δ*	This study
PC6275	*pGEX4T1-CDC24-CHΔ*	This study
PC6276	*pGEX4T1-CDC24-PHΔPB1Δ*	This study
PC6277	*pGEX4T1-CDC24-CHΔPB1Δ*	This study
PC6278	*pGEX4T1-CDC24-PHΔ*	This study
PC6279	*pGEX4T1-CDC24-PH* domain only	This study
PC6280	*pRS425-CDC24-PHΔ*	This study

Table S3. Deletion analysis of Cdc42p netowrk components for phenotypes in the filamentous growth response.

Gene Deleted	Strain	Plate-washing (a)	*ste4 FUS1-HIS3* (b)	Kss1~P	Score	Final
Wild Type Control	PC999	+++	HIS +	+++	0	
MSB2	PC948	+/-	HIS -	+	-2	
SHO1	PC1531	+/-	HIS -	+	-2	
OPY2	PC3752	-	HIS -	-	-3	
STE20	PC549	-	HIS -	-	-3	
STE50	PC3863	-	HIS -	-	-3	
STE11	PC2544	-	HIS -	-	-3	
STE12	PC539	-	HIS -	-	-3	
STE4	PC538	+++	HIS +	++++	-1	
MSB1	PC3497	+++	HIS +	N/D	0	
GIP1	PC955	+++	HIS +	N/D	0	
MSB4	PC3498	+++	HIS +	N/D	0	
MAD2	PC967	+++	HIS +	N/D	0	
MAD3	PC968	+++	HIS +	N/D	0	
BEM4	PC2738	-	HIS -	-	-3	
GAL4	PC559	+++	HIS +	N/D	0	
SIP4	PC568	+++	HIS +	N/D	0	
SNF4	PC587	+++	HIS +	N/D	0	
MIG1	PC3098	+++	HIS +	+++	0	
SNF1	PC560	+	HIS +	+	-1	
RHO2	PC3760	+++	HIS +	N/D	0	
RHO4	PC3762	+++	HIS +	N/D	0	
BOI2	PC3758	+++	HIS +	N/D	0	
BOI1	PC3756	+++	HIS +	N/D	0	
RSR1	PC717	+++	HIS -	N/D	-1	
SBE2	PC4056	+++	HIS +	N/D	0	

SBE22 (c)	PC3875	+++	HIS +	N/D	0
RDI1	PC3166	+++	HIS +	N/D	0
GIC1	PC3766	+++	HIS +	N/D	0
GIC2 (d)	PC3771	+++	HIS +	N/D	0
FAR1	PC3496	+++	HIS +	N/D	0
STE5	PC720	+++	HIS +	N/D	0
ENT2	PC3495	+++	HIS +	N/D	0
TOS2	PC3499	+++	HIS +	N/D	0
RGA1	PC1994	+++	HIS +++	N/D	3
RGA2	PC479	+++	N/D	N/D	0
BEM3	PC480	+++	N/D	N/D	0
BZZ1	PC3503	+++	HIS +	N/D	0
BBC1	PC3226	+++	HIS +	N/D	0
CAP2	PC3206	+++	HIS +	N/D	0
SLA2	PC3500	+++	HIS +	N/D	0
AIP1	PC3214	+++	HIS +	N/D	0
BNI1	PC544	+++	HIS +	N/D	0
PEA2	PC551	+++	HIS +	N/D	0
SPA2	PC554	+++	HIS +	N/D	0
BUD6	PC916	+++	HIS +	N/D	0
CLA4	PC969	+++	HIS +++	N/D	2
BEM2	PC3501	+++	HIS +	N/D	0
BUD14	PC842 (e)	+++	HIS ++	N/D	1
TMN2	PC3750	+++	HIS +	N/D	0
MDG1	PC3502	+++	HIS +	N/D	0
HSL1	PC552	+++	HIS +	N/D	0
HSL7	PC555	+++	HIS +	N/D	0
SKM1	PC3754	+++	HIS +	N/D	0
GET3	PC3865	+++	HIS +	N/D	0

GIN4	PC3867	+++	HIS +	N/D	0
OPY1	PC3870	+++	HIS +	N/D	0
BAG7	PC3924	+++	HIS +	N/D	0
BEM1 (f)	N/A	N/A	N/A	++	0

a. The plate-washing assay was performed on mutants grown for 72h at 30C on YEPD semi-solid agar media.

b. Genes were disrupted in PC538 *(MAT* **a** *ste4 FUS1-HIS3 FUS1-lacZ ura3-52)* using the *KlURA3* cassette or GENT cassette. Reporter activity was assessed by growth on SD-HIS media compared to SD+AA media.

c. The *sbe2 sbe22* double mutant (PC4060) was also examined.

d. The *gic1 gic2* double mutant (PC4382) was also examined.

e. *GAL-GFP-BUD14* allele was examined.

f. Bem1p is essential in the □1278b background and was tested in S288c background by Kss1p phosphoblot analysis.

Fig._S1

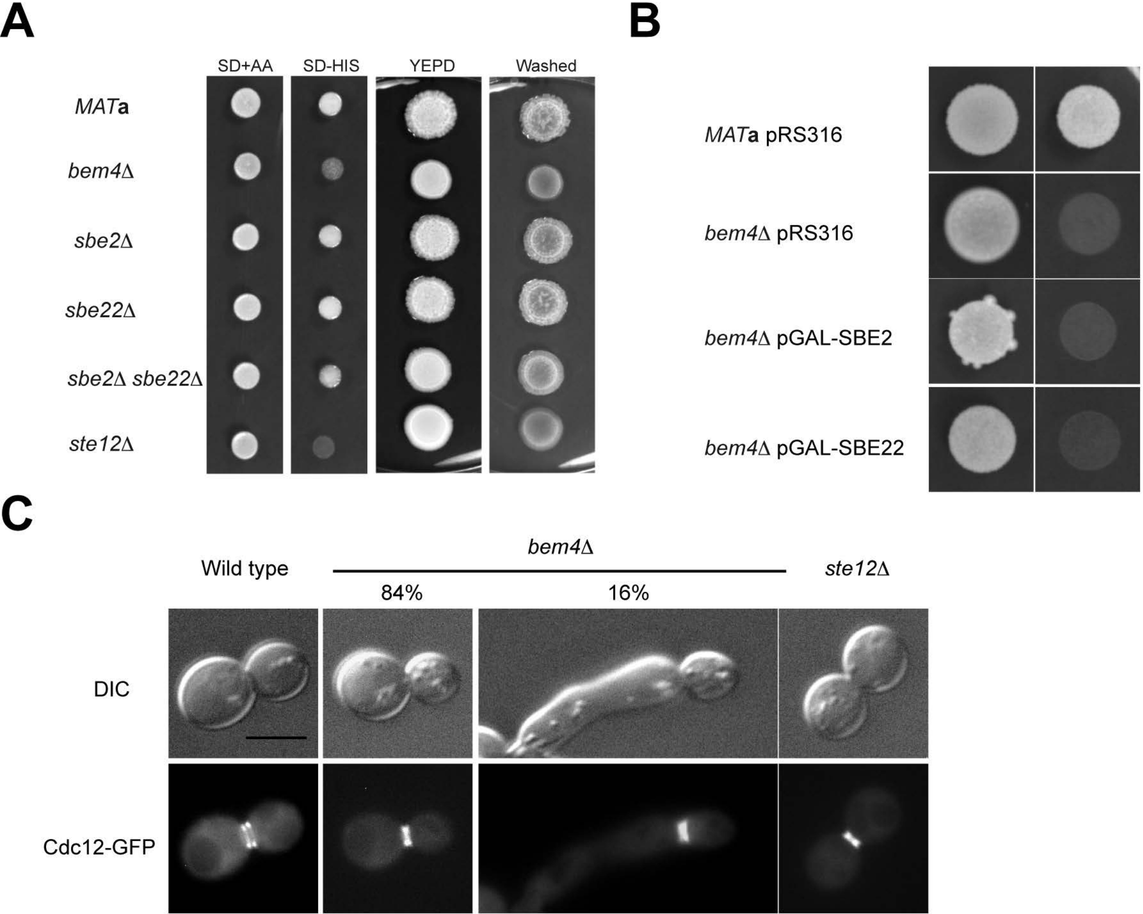
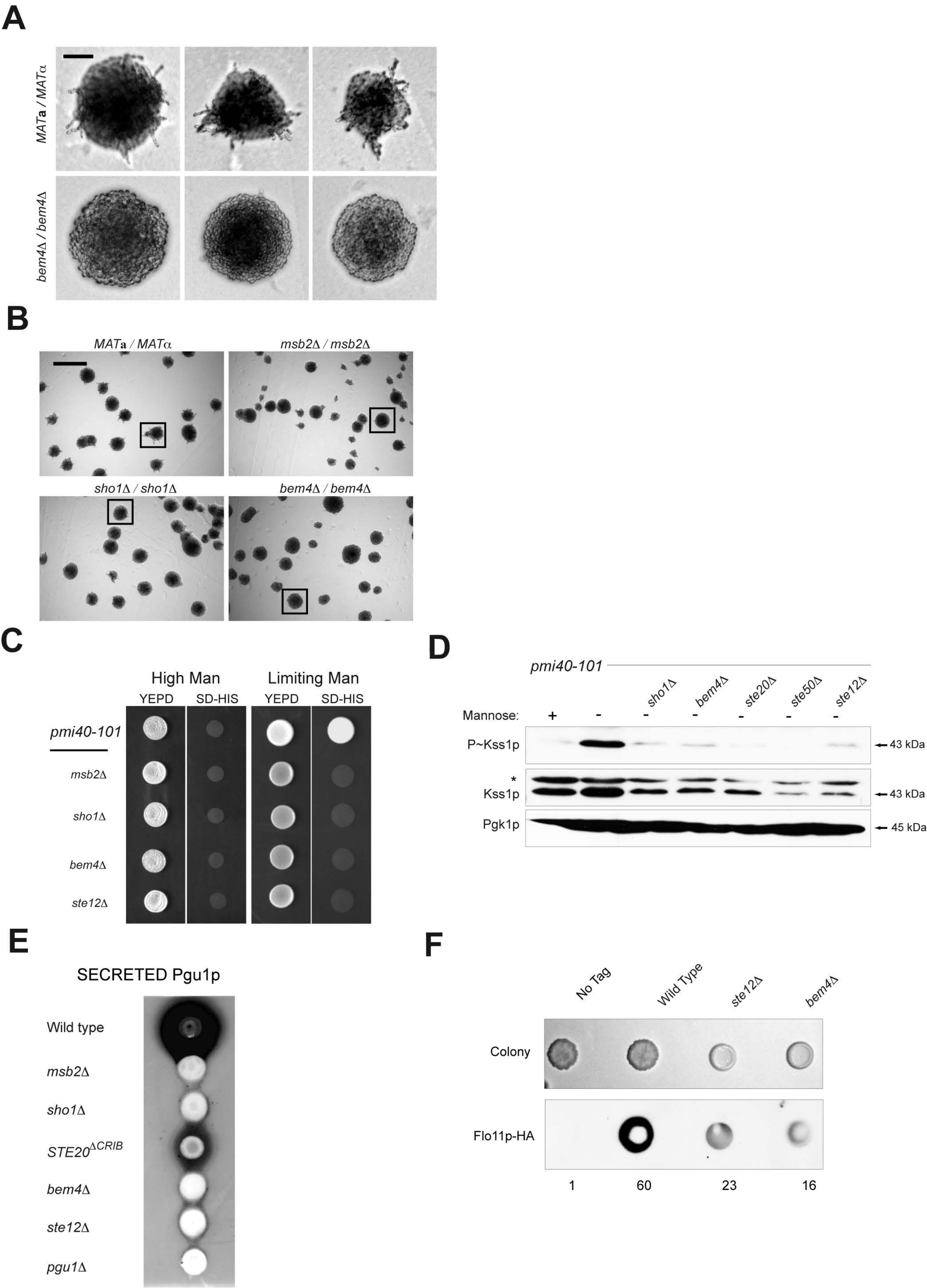

A
SD+AA
SD-HIS
YEPD
Washed
MATa
bem4Δ
sbe2Δ
sbe22Δ
sbe2Δ sbe22Δ
ste12Δ
B
MATa pRS316
bem4Δ pRS316
bem4Δ pGAL-SBE2
bem4Δ pGAL-SBE22
C
Wild type
bem4Δ
ste12Δ
84%
16%
DIC
Cdc12-GFP

Fig._S2



A
MATa / MATα
bem4Δ / bem4Δ
B
MATa / MATα
msb2Δ / msb2Δ
sho1Δ / sho1Δ
bem4Δ / bem4Δ
C
High Man
Limiting Man
YEPD
SD-HIS
YEPD
SD-HIS
pmi40-101
msb2Δ
sho1Δ
bem4Δ
ste12Δ
D
pmi40-101
sho1Δ
bem4Δ
ste20Δ
ste50Δ
ste12Δ
Mannose:
+
-
-
-
-
-
-
P~Kss1p
43 kDa
*
Kss1p
43 kDa
Pgk1p
45 kDa
E
SECRETED Pgu1p
Wild type
msb2Δ
sho1Δ
STE20^ΔCRIB
bem4Δ
ste12Δ
pgu1Δ
F
No Tag
Wild Type
ste12Δ
bem4Δ
Colony
Flo11p-HA
1
60
23
16

Fig._S3

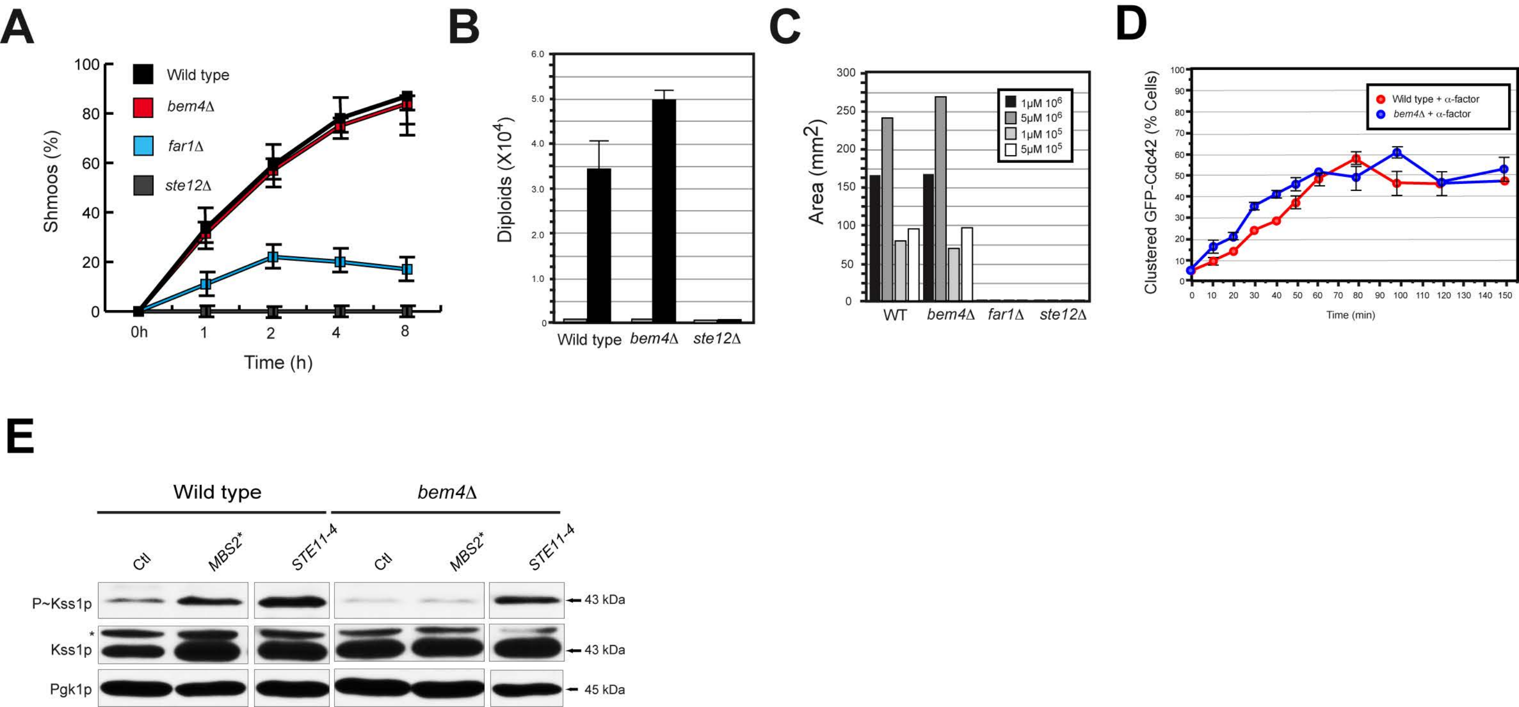

A
Shmoos (%)
Time (h)
0h
1
2
4
8
0
20
40
60
80
100
Wild type
bem4Δ
far1Δ
ste12Δ
B
Diploids (X10^4)
0
1.0
2.0
3.0
4.0
5.0
6.0
Wild type
bem4Δ
ste12Δ
C
Area (mm^2)
0
50
100
150
200
250
300
1μM 10^6
5μM 10^6
1μM 10^5
5μM 10^5
WT
bem4Δ
far1Δ
ste12Δ
D
Clustered GFP-Cdc42 (% Cells)
Time (min)
Wild type + α-factor
bem4Δ + α-factor
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
E
Wild type
bem4Δ
Ctl
MBS2*
STE11-4
Ctl
MBS2*
STE11-4
P~Kss1p
*
Kss1p
Pgk1p
43 kDa
43 kDa
45 kDa

Fig._S4

```
H. sapiens      ------------------------------------------------------------
X. laevis       -MDNLNDALEKLKLTGTECTSDKLDGCLDCLLQALGHNNTESSEKIQQSGILQLFASILN 59
M. musculus     -TDNLSDTLKKLKITAADRTEGSLEGCLDCLLQALAQNNAETSEKIQGSGILQLFANLLT 59
D. melanogaster MATEIDDLIEKLKTTSVSPAN---TTNLLCEISATKDPKLFDKHELAECFLGLTKCDDTN 57
S. cerevisiae   --MDYEEILFGLQP----------------ILNASSIKDVPMNDVYLGSYLAVMDQLAVS 42

H. sapiens      ---------------------MRIPCVDAGLISPLVQLLNSK-DQEVLLQTGRALGNICY 38
X. laevis       SQSSCASKVAHIVAEIAKNELMRIPCVEADLIPPLVQLLHSK-DQEVLLQTGRALGNICY 118
M. musculus     PQASCTAKVADIIAEVAKNEFMRIPCVDAGLISPLVQLLNSK-DQEVLLQTGRALGNICY 118
D. melanogaster VRKEAAKCIAEITKSEVQRKKFTKRNIIAAFLECLRQVPTSDGSMELPIQICRALGNICY 117
S. cerevisiae   LREPSNRDIVGKTGLLLNLVRVLEQALDICFHDTSISINDKIAFYEISSEVIRCIANAII 102

H. sapiens      DSQ--------------------------------------------------------- 41
X. lavies       DNHEGRRTVDQEGGAQIVVDHLRSRCTLTDPSSEKLMTVFCGMLMNYSSENDSLQTQLIQ 178
M. musculus     DSHS-------------------------------------------------LQAQLIS 129
D. melanogaster LNDEARDLILELEGDAVLLRLLDITTIEDVANAAQFIKVRGGLLSNYLLGGEGLAKRAME 177
S. cerevisiae   DNDDNREILLDSGGKKLLNYYIGGVLQLDEISSDK----------SEDSLVDKLQMRSVV 152

H. sapiens      -------------------------------------SSKEQFASTNIAEELVKLFKKQI 64
X. lavies       MGVIPILVDLLAVHSQNTALTEMCLVAFGNLA--ELESSKEQFAATNVAEVIVKLFKKQT 236
M. musculus     MGVIPTLVKLLGIHCHNAALTEMCLVAFGNLA--ELESSKEQFASTNIAEELVKLFKKQI 187
D. melanogaster LGVMKKLQGIIDIGASNVEQHEDLLLNTLPLLSILTENVSDLNFDSSLNIQLSRILAAST 237
S. cerevisiae   LLRNFCIGNLKYTENLAPFIRGPLFVLLKTTQYSYLSPEKVVLGSDLLNDILKVNYSNV 212

H. sapiens      EHDKREMIFEVLAPLAENDA-IKLQLVEAGLVECLLEIVQQKVDSD-------KEDDITE 116
X. lavies       EHEKREVIFEVLAPLAENDA-IKMQLVEAGLVECLLDIVQQTVNSE-------KDDDVAE 288
M. musculus     EHDKREMIFEVLAPLAENDA-IKLQLVEAGLVECLLEIVQQKVNSN-------KEDDVAE 239
D. melanogaster NPDLAEMCLELLHYQAESDE-VKLILAKDGLCETIYNLLEKYKTLAS------TSEARAL 290
S. cerevisiae   QISDLFFLSQYIKKISSNVQNKELQAMEDGAVEAYSNTETQKFAGQGNQEYIEKEEEDDE 272

H. sapiens      LKTGSDLMV-------LLLGDESMQKLFEGGKGSVFQRVLSWIPSNNHQLQLAGALAIA 169
X. lavies       LKTSSDLMV-------LLLGDESMQKLFEGGKGSVFQRVLSWLPSNNHQLQLAGALAIA 341
M. musculus     LKTASDLMV-------LLLGDESMQKLFEGGKGSVFQRVLSWIPSNNHQLQLAGALAIA 292
D. melanogaster MKLACELIV-------LILTGDDSMHYLYTTP---LLKNMVDWLDSTDIDLLTTGVLALG 340
S. cerevisiae   EDVNCELLLNLSTCLETIVAKDETINFTNEEQLVLSMQKNLILSLVCLESKTFNNKLIVM 332

H. sapiens      NFARNDANCIHMVDNGIVEKLMDLLDR-HVEDGNVTVQHAALSALRNLAIPVINKAKMLS 228
X. lavies       NFARNDGNCIHMVDSEIVQKLLDLLDR-HVEDGNVTVQHAALSALRNLAIPVVNKAKMLS 400
M. musculus     NFARNDGNCIHMVDNGIVEKLMDLLDR-HVEDGNVTVQHAALSALRNLAIPVVNKAKMLS 351
D. melanogaster NFARTDSHCIYFVEQQTMNKLLEVLAKNNGVKDDVRLQHALLSALRNLVIPKPNKNAVIQ 400
S. cerevisiae   RRLISCAGNISANLTNSNKREQSLCIETIKSSASSYALAAALMILCNSVASKSDAVALLK 392

H. sapiens      AGVTEAVLKFLKSEMPPVQFK-LLGTLRMLIDAQAEAAEQLGKNVKLVERLVEWCEAKDH 287
X. lavies       AGVTEEVLKFLPSEMPPVQFK-LLGTLRMLIDAQAEAAEQLGKNEKLVERLVEWCEAKDH 459
M. musculus     AGVTETVLKFLKSEMPPVQFK-LLGTLRMLIDAQAEAAEQLGKNAKLVERLVEWCEAKDH 410
D. melanogaster AGLVQTILPMLEIHQPPVVFK-LLGTLRMTVDGQEKLALELLKNKTLIEQLVHWSKSSDY 459
S. cerevisiae   LISLSELIQVGSLLQDPLQYQGFLDLLRKLLNLENTMWLDIKDLFTLFQIMRRCHEQTKY 452

H. sapiens      AG-VMGESNRLLSALIRHSK-SKDVIKTIVQ----------------------------- 316
X. lavies       AG-VMGESNRLLSALIRHSK-SKDVIRTTVQ----------------------------- 488
M. musculus     AG-VMGESNRLLSALIRHSK-SKDVIKTIVQ----------------------------- 439
D. melanogaster AG-VTGESLRLMAWLIKHAYLSKIAYALPRKGDAPAEQIADKIPLTQDYDRSSLSEFLAN 518
S. cerevisiae   YNNLRSLLTNLLNKTLTVLPSSKIHNSISSD-----------------------PTIISF 489

H. sapiens      SGGIKHLVTMATSEHVIMQNEALVALALIAALELGTAEKDLESAKLVQILHRLLADERSA 376
X. lavies       SGGIKHLVTMATSEHVIMQNEALVALGLIAALELQAAERDLESAKLVEVLHRLLSDERSA 548
M. musculus     SGGIKHLVTMATSEHVIMQNEALVALALIAALELGPAEKDLASAQLVQILHRLLADERSA 499
D. melanogaster EGTVEAMVSMLTAQHLVMQNEALIALCILSVVYLSQPSEAAQAQLLQDELVKCEVGKKLA 578
S. cerevisiae   IAEHGTLTSCIAMDKLLVSKKALPKEAITSLWDSIFKFQNLGQAEQLSISDLFHITKTVG 549

H. sapiens      PEIKYNSMVLICALMGS-ECLHKEVQDLAFLDVVSKLRSHENKS-------------VAQ 422
X. lavies       PEIKYNSMVLICAVMGS-EPLHKEVQKLAFLDVVSKLRSHENKT-------------VAQ 594
M. musculus     PEIKYNSMVLICALMGSGKASSCRSVSMWLSSDVKRWIINDNKG-------------LKR 546
D. melanogaster ELISKSSDTMTKEIVEN--LQNCVNLLKSSEQLVAHLEQHNINE-------------LLK 623
S. cerevisiae   IYLKDSSVTADVNPIENILFKDYIQKLTLILETILSFKENKDKGSESCFNNGKFIAGIIL 609

H. sapiens      QASLTEQRLTVES------------- 435
X. lavies       QASLTEQKFTVQS------------- 607
M. musculus     SSKSFERGYRGQNGVSDNCSHNHRQF 572
D. melanogaster SIPILTEYCTL--------------- 634
S. cerevisiae   NIVKNTKCLTPEEQNLEALAKSFF-- 633
```

Arm-like repeats of Bem4p

```
280 LLNLSTCLETIVAKDETINFTNEEQLVLSMQKNLIL--SLV--CLES--KT
325 FNNKLIVMRRLISCAGNISAN-LTNSNKREQS-LCIETIKΔSYALAAALMI
377 LCNSVASKSDAVALL--K-LISLSELIQVG-S-L-LQDPL---QYQGFLDL
419 LRKLLN-LENTMW-LDIKDLFTLFQ-IMRR---CHEQTKYYNΔLLNKTLTV
471 LPSSKIHNS-ISSDPTIIS-FIA-EHGTLT-S-CIAMDKLLVS-----KKA
512 LPKEAIT-SLWDSIFKF--QN-LGQAEQLSISDLFHITKTVGIYL-KDSSV
```

Fig._S5

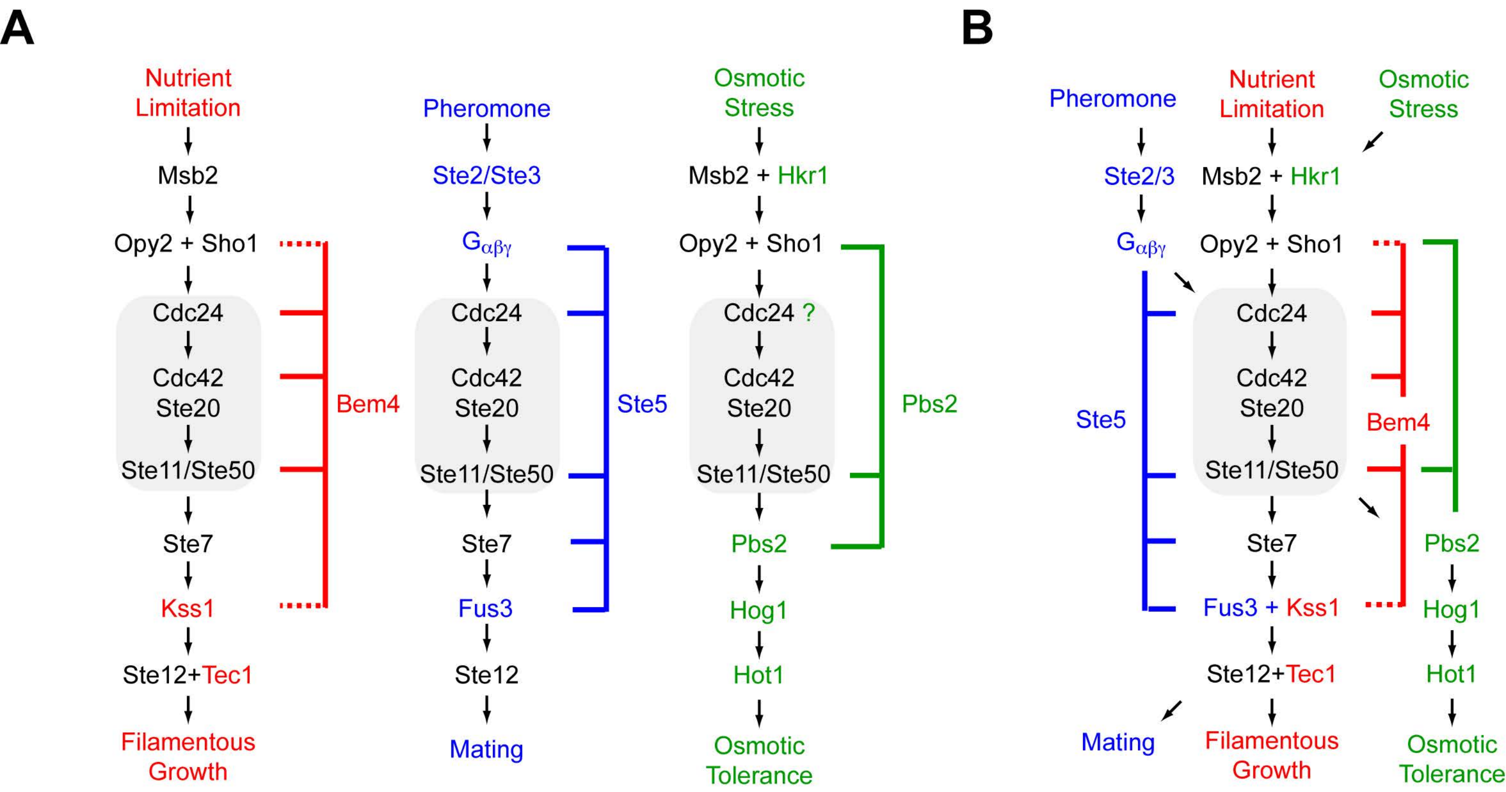
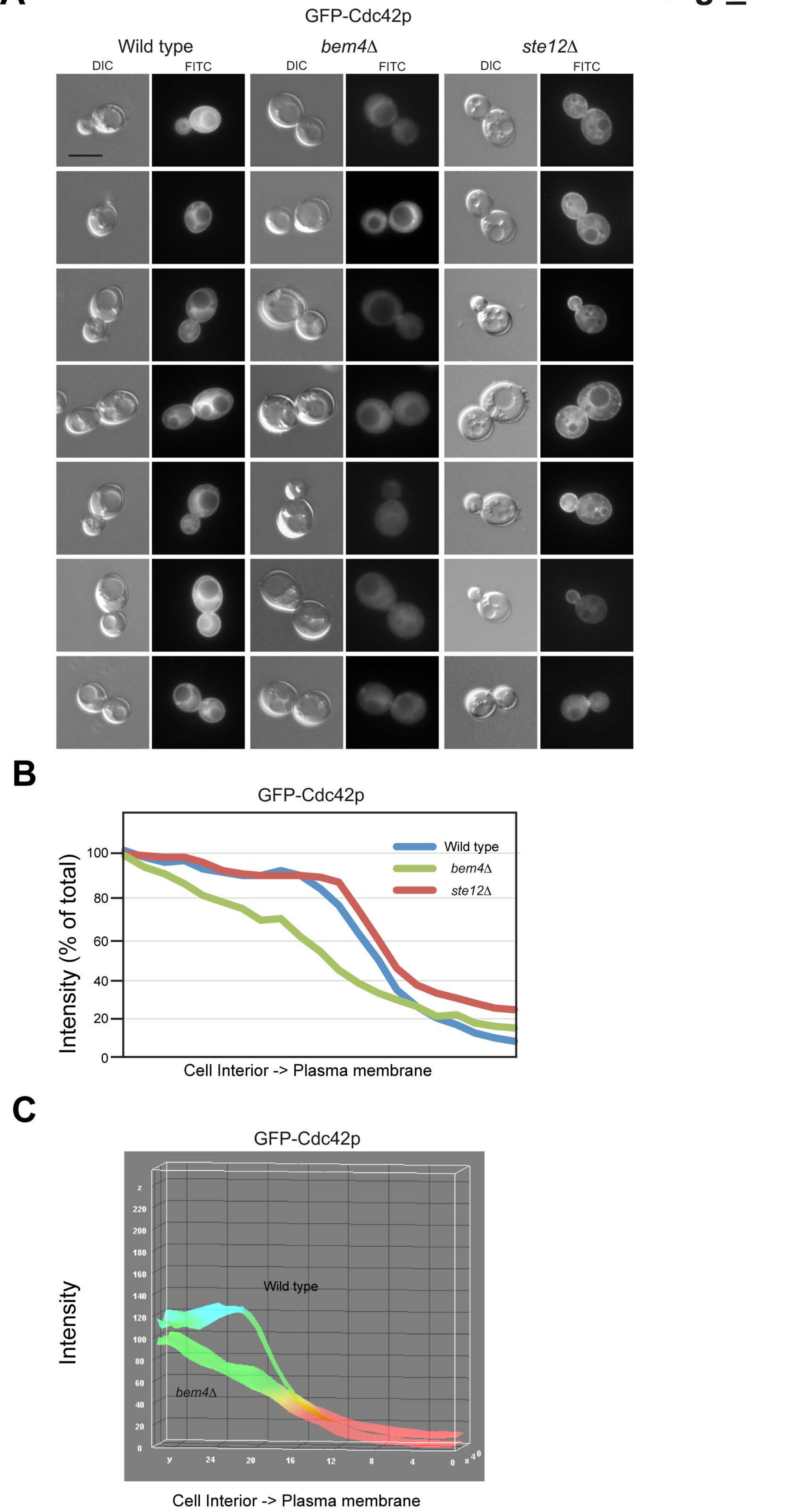

A
Nutrient Limitation
Msb2
Opy2 + Sho1
Cdc24
Cdc42
Ste20
Ste11/Ste50
Ste7
Kss1
Ste12+Tec1
Filamentous Growth
Bem4
Pheromone
Ste2/Ste3
Gαβγ
Cdc24
Cdc42
Ste20
Ste11/Ste50
Ste7
Fus3
Ste12
Mating
Ste5
Osmotic Stress
Msb2 + Hkr1
Opy2 + Sho1
Cdc24 ?
Cdc42
Ste20
Ste11/Ste50
Pbs2
Hog1
Hot1
Osmotic Tolerance
Pbs2
B
Pheromone
Nutrient Limitation
Osmotic Stress
Ste2/3
Msb2 + Hkr1
Gαβγ
Opy2 + Sho1
Cdc24
Cdc42
Ste20
Ste11/Ste50
Ste7
Fus3 + Kss1
Ste12+Tec1
Ste5
Bem4
Pbs2
Hog1
Hot1
Mating
Filamentous Growth
Osmotic Tolerance

Fig._S6


A
GFP-Cdc42p
Wild type
bem4Δ
ste12Δ
DIC
FITC
B
GFP-Cdc42p
Intensity (% of total)
Wild type
bem4Δ
ste12Δ
100
80
60
40
20
0
Cell Interior -> Plasma membrane
C
GFP-Cdc42p
Intensity
Wild type
bem4Δ
z
220
200
180
160
140
120
100
80
60
40
20
0
y
24
20
16
12
8
4
0
x
Cell Interior -> Plasma membrane