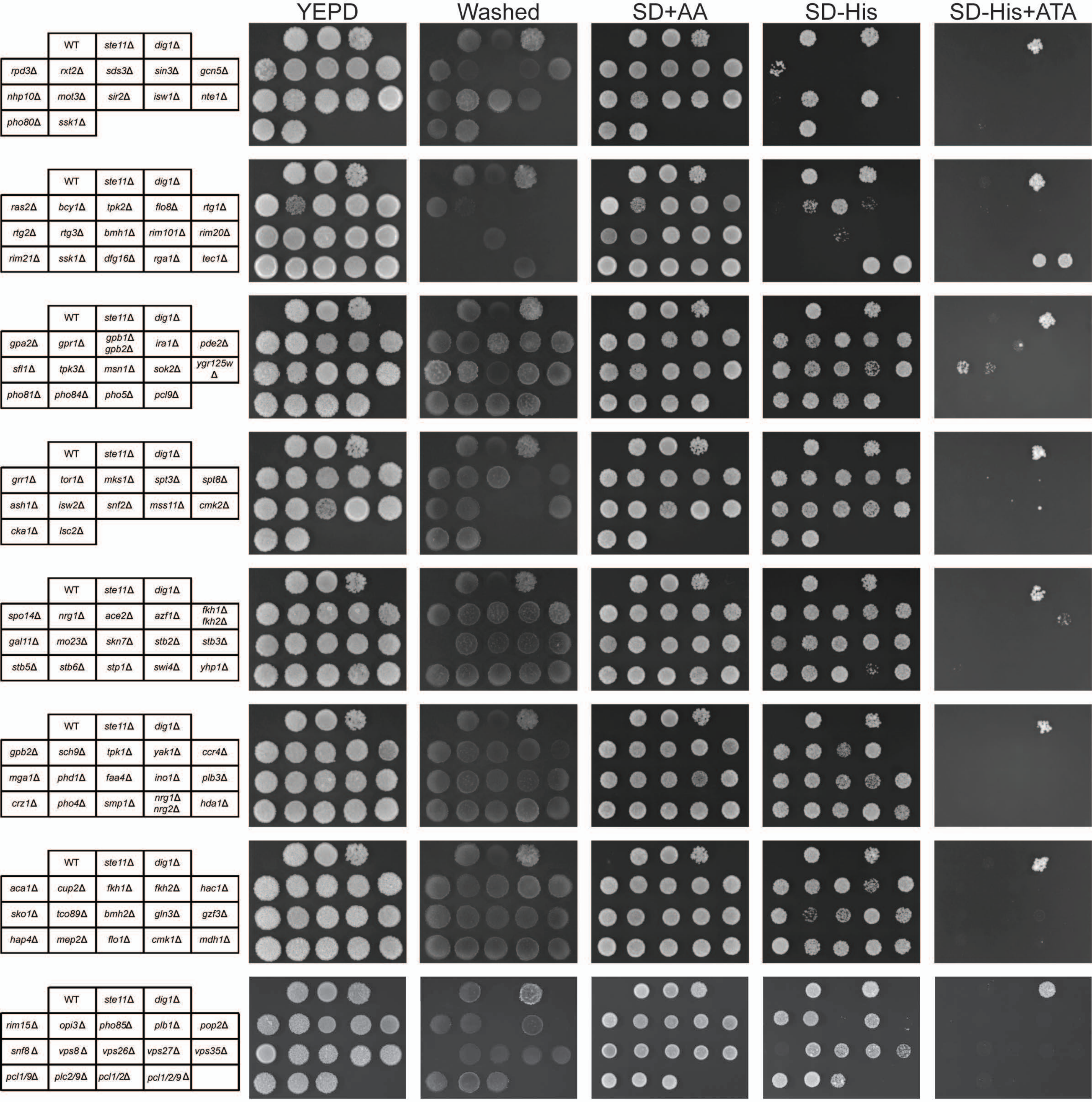


Figure S1. Evaluation of candidate regulators of the filamentous growth MAPK pathway by the plate-washing assay and cross-talk reporter (*FUS1-HIS3*). Wild type and control strains and the indicated mutants were spotted on to YEPD, SD-HIS, and SD-HIS + 2.5 mM ATA and incubated for 2d. No growth on SD-HIS indicates a defect in filamentous growth MAPK pathway activity. Growth on SD-HIS + 2.5 mM ATA indicates elevated filamentous growth MAPK pathway activity. YEPD plates were photographed, washed in a stream of water to reveal invaded cells, and photographed again (Washed).

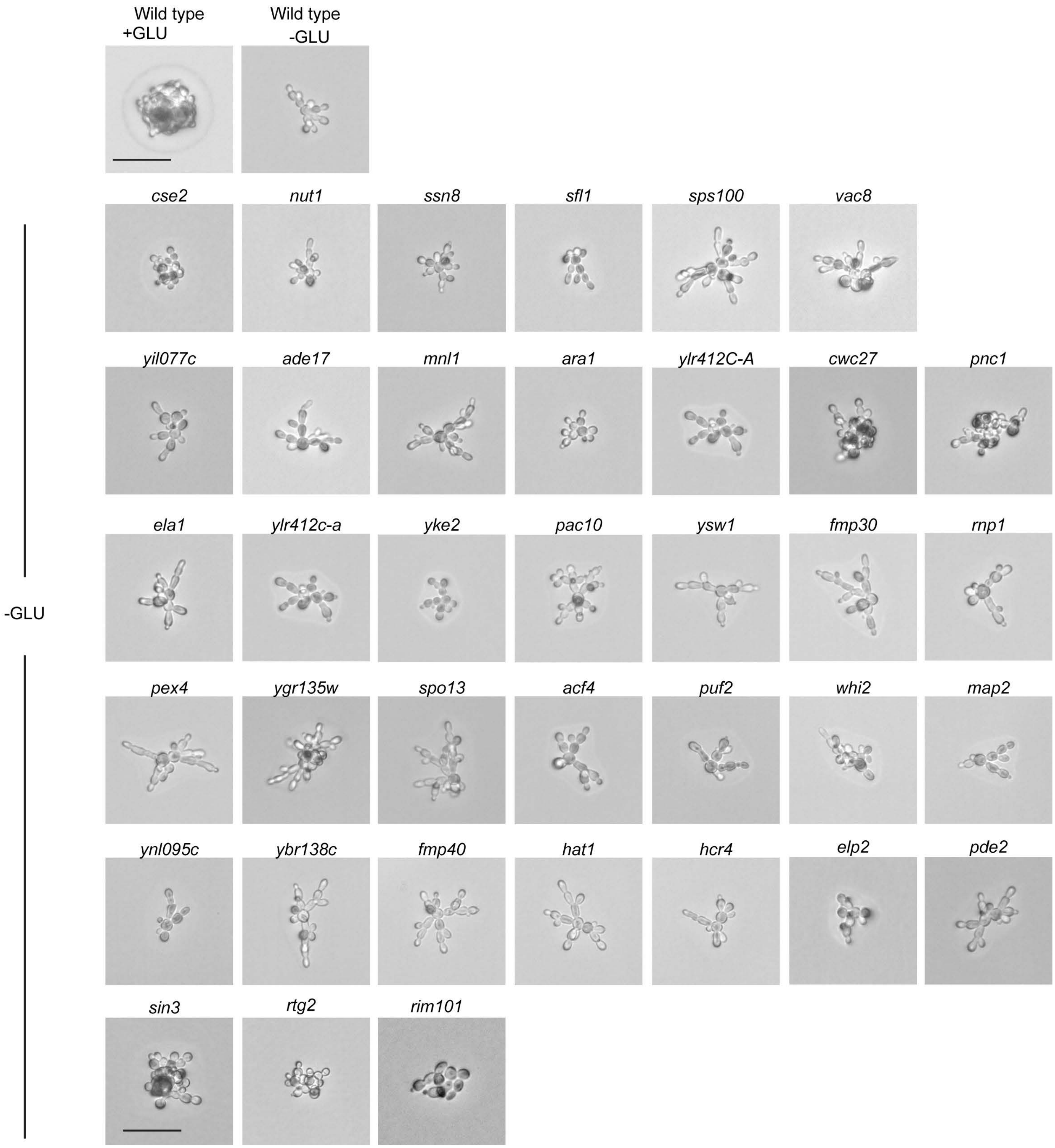
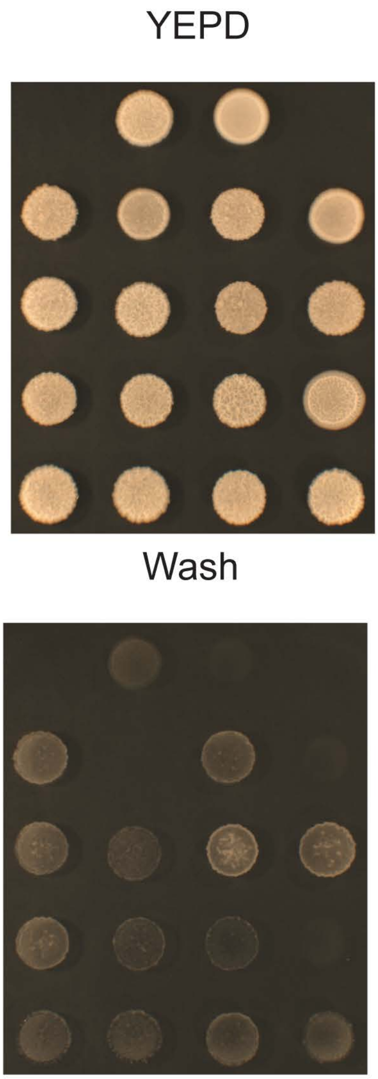


Figure S2. The role of filamentous growth MAPK pathway regulators in filament formation by the single cell invasive growth assay. Wild-type strain and the indicated mutants were grown on S-GLU medium for 16 hr and photographed at 100X. Bar, 20 microns.

A

Plate Guide

	WT	*ste12Δ*	
mcy1Δ	*mcy1Δ ste12Δ*	*pnc1Δ*	*pnc1Δ ste12Δ*
mnl1Δ	*mnl1Δ ste12Δ*	*rxt3Δ*	*rxt3Δ ste12Δ*
cwc27Δ	*cwc27Δ ste12Δ*	*ssn8Δ*	*ssn8Δ ste12Δ*
nut1Δ	*nut1Δ ste12Δ*	*ela1Δ*	*ela1Δ ste12Δ*



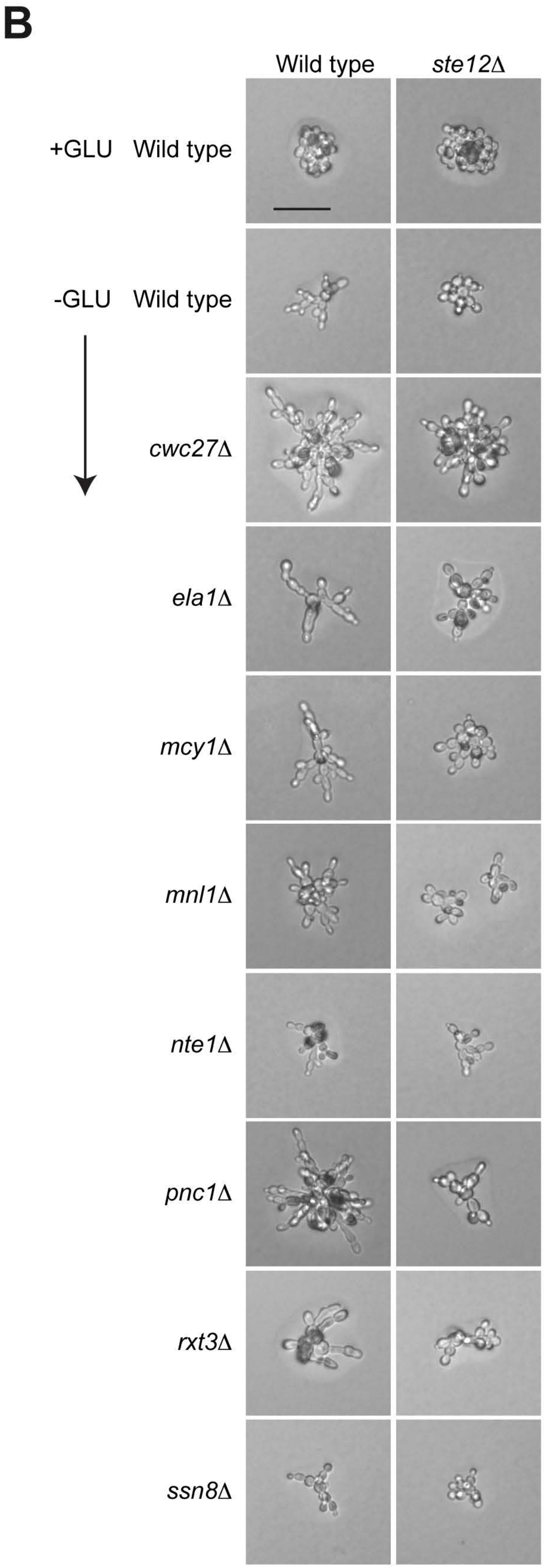


Fig. S3. Suppression of hyper-invasive growth phenotypes of mutants identified in the screen by deletion of *STE12*. A) Wild-type and *ste12*Δ mutant combinations as indicated were examined by the plate-washing assay, or in **B)** by the single cell assay. Bar, 30 microns. The *mcy1* mutant may contain a second mutation based on retesting.

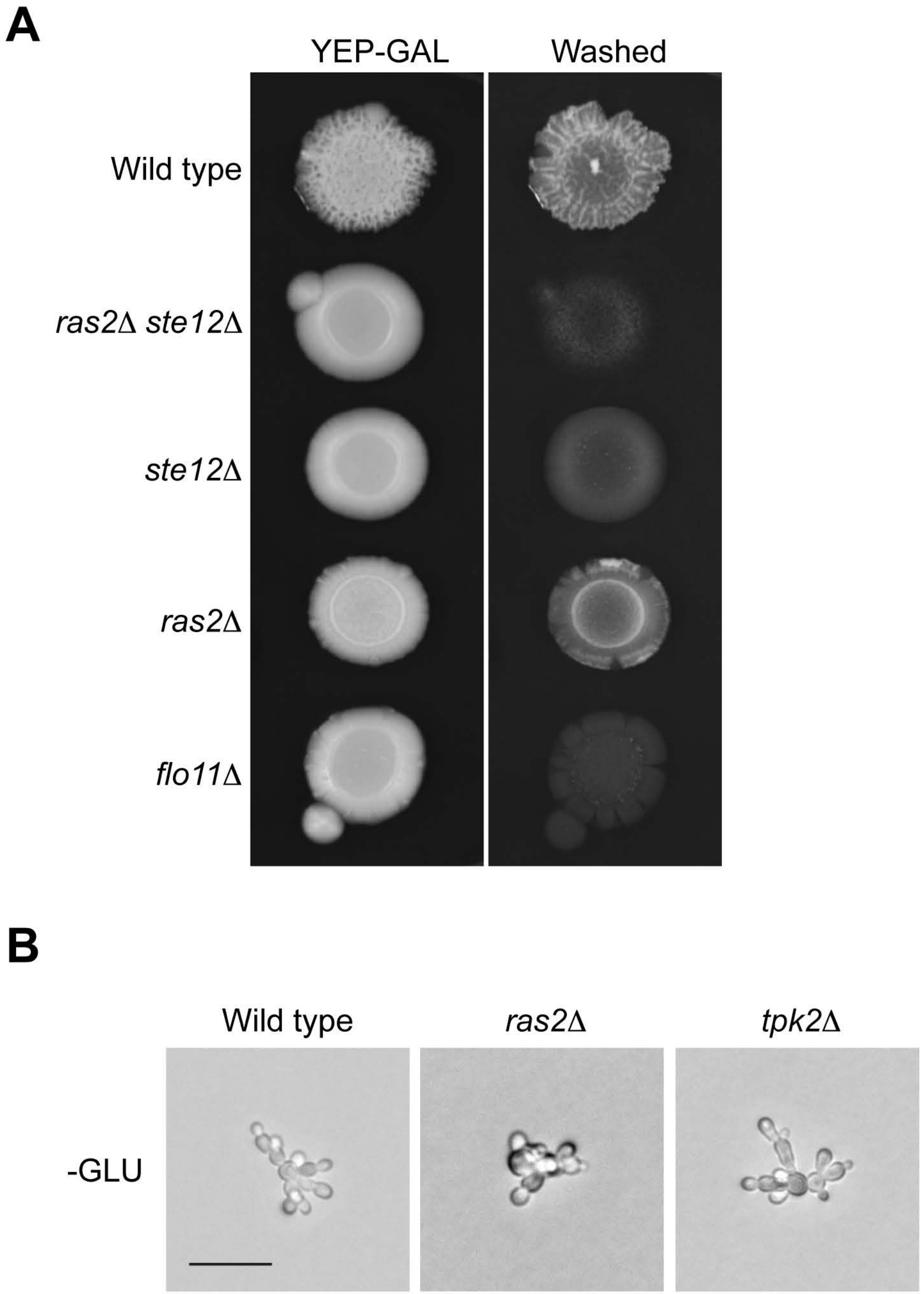


Figure S4. Role of Ras2p and Tpk2p in conditional regulation of the filamentous growth MAPK pathway. A) The plate washing assay on YEP-GAL medium of wild-type cells, and the *ste12*Δ, *ras2*Δ, and *ste12*Δ *ras2*Δ double mutants. **B)** Single cell assay of the *ras2*Δ and *tpk2*Δ mutant. Bar, 20 microns.

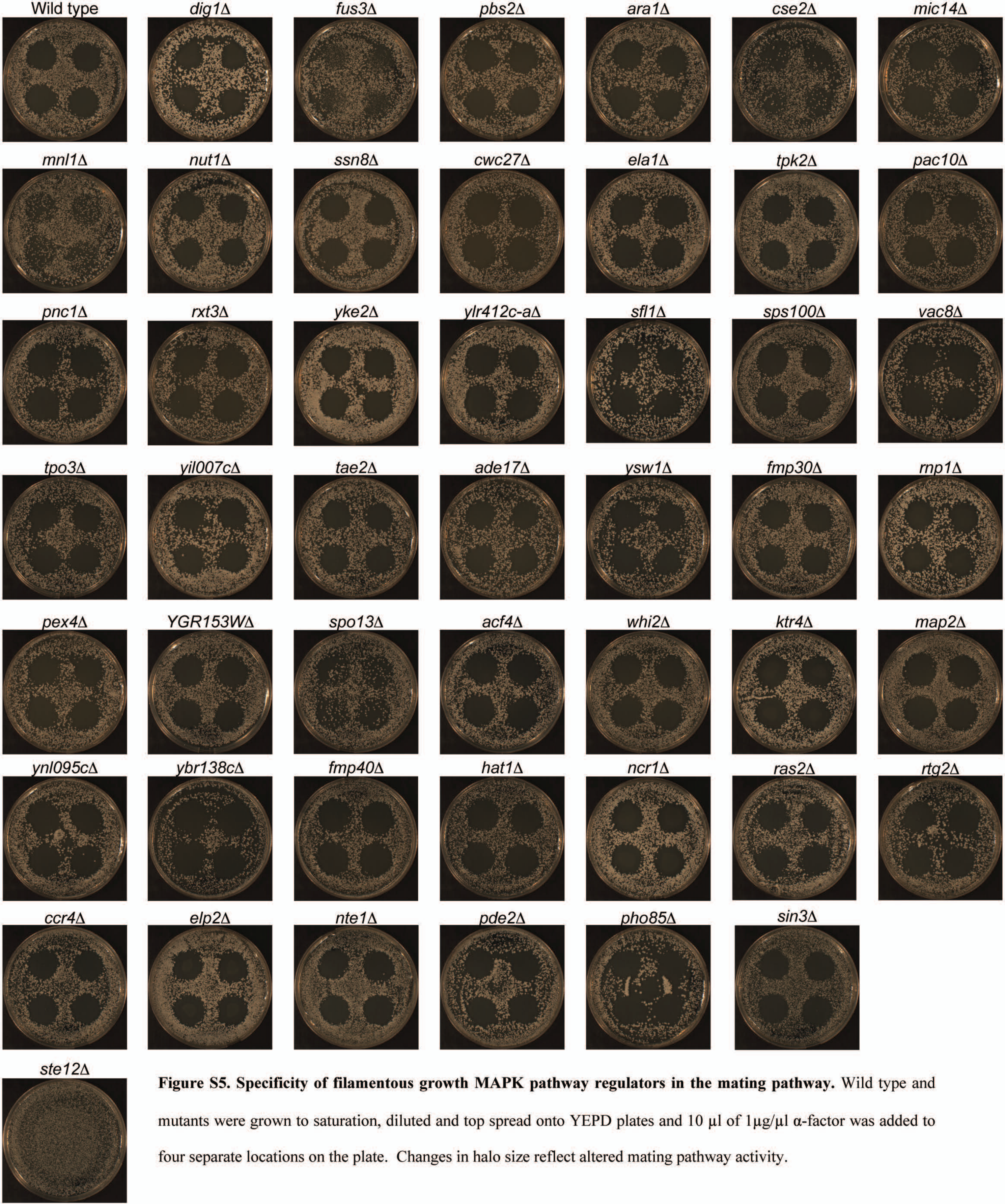


Figure S5. Specificity of filamentous growth MAPK pathway regulators in the mating pathway. Wild type and mutants were grown to saturation, diluted and top spread onto YEPD plates and 10 µl of 1µg/µl α-factor was added to four separate locations on the plate. Changes in halo size reflect altered mating pathway activity.

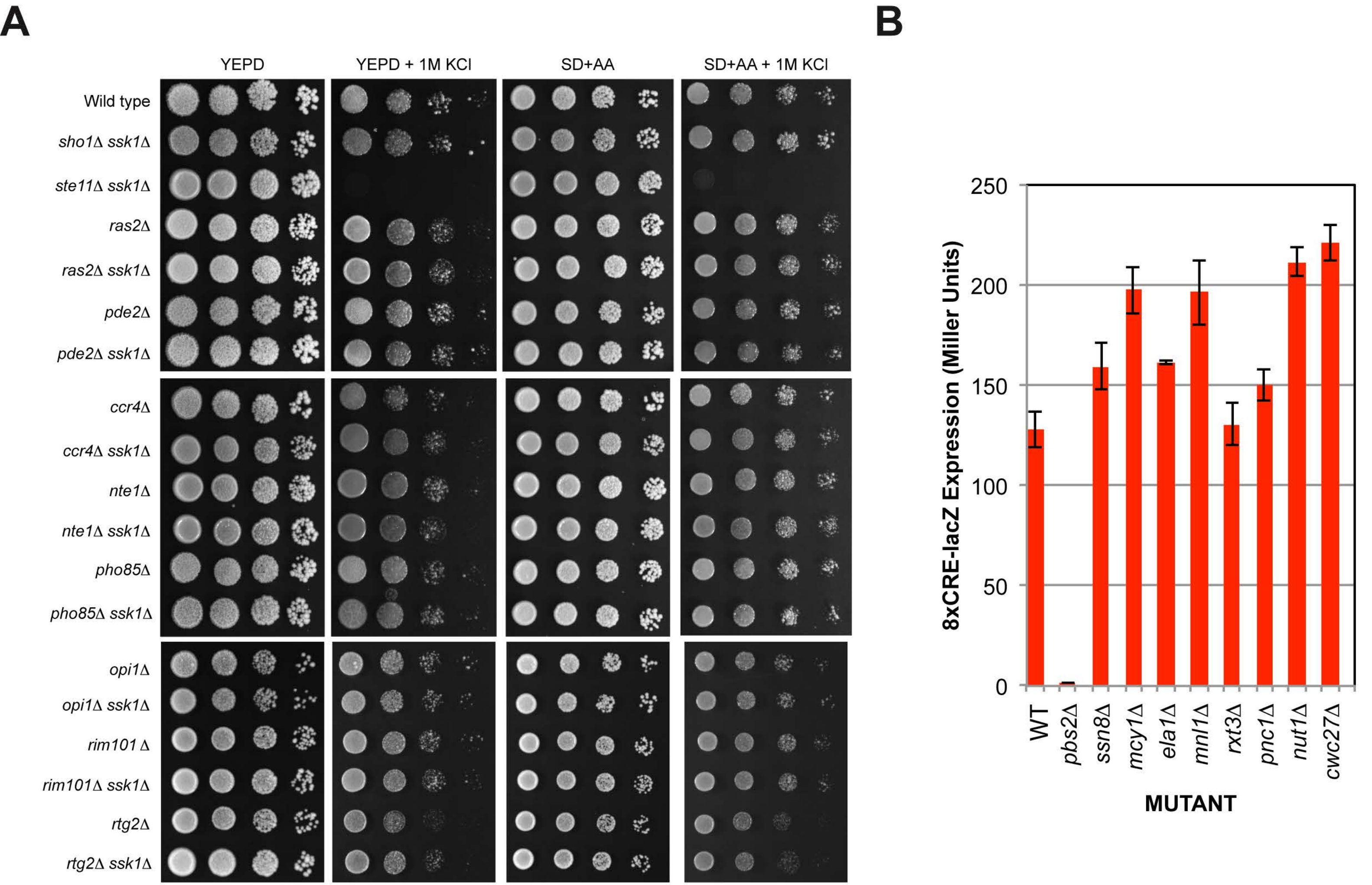
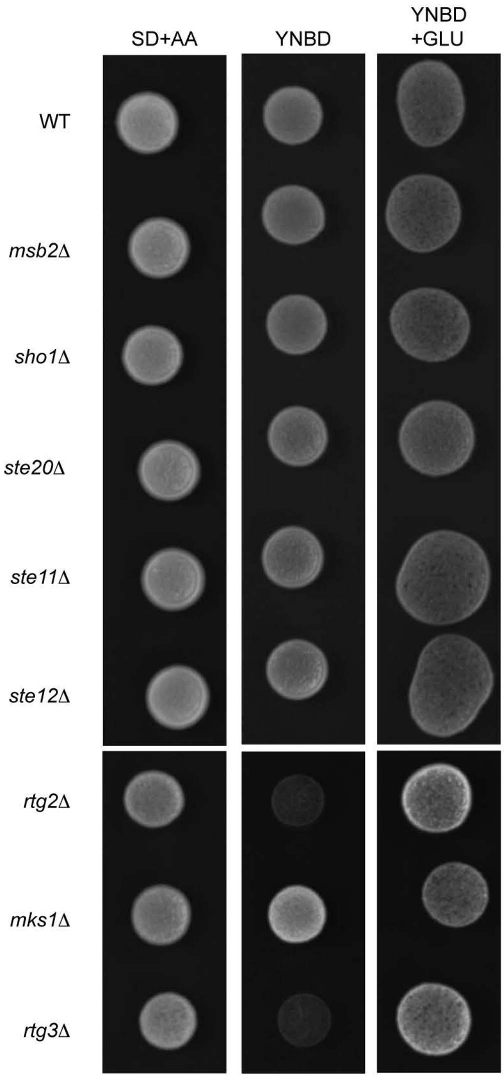


Figure S6. Specificity of filamentous growth MAPK pathway regulators in the HOG pathway. A) Wild type and indicated mutants were spotted on YEPD, YEPD + 1M KCl, SD + AA, and SD + AA + 1M KCl and grown for 2d. **B)** Level of p*8X-CRE-lacZ* activity in selected mutants that show filamentous growth MAPK pathway hyper-activation. Strains were grown in YEPD for 6 h then shifted to YEPD + .4M KCl for 30 min.

A



B

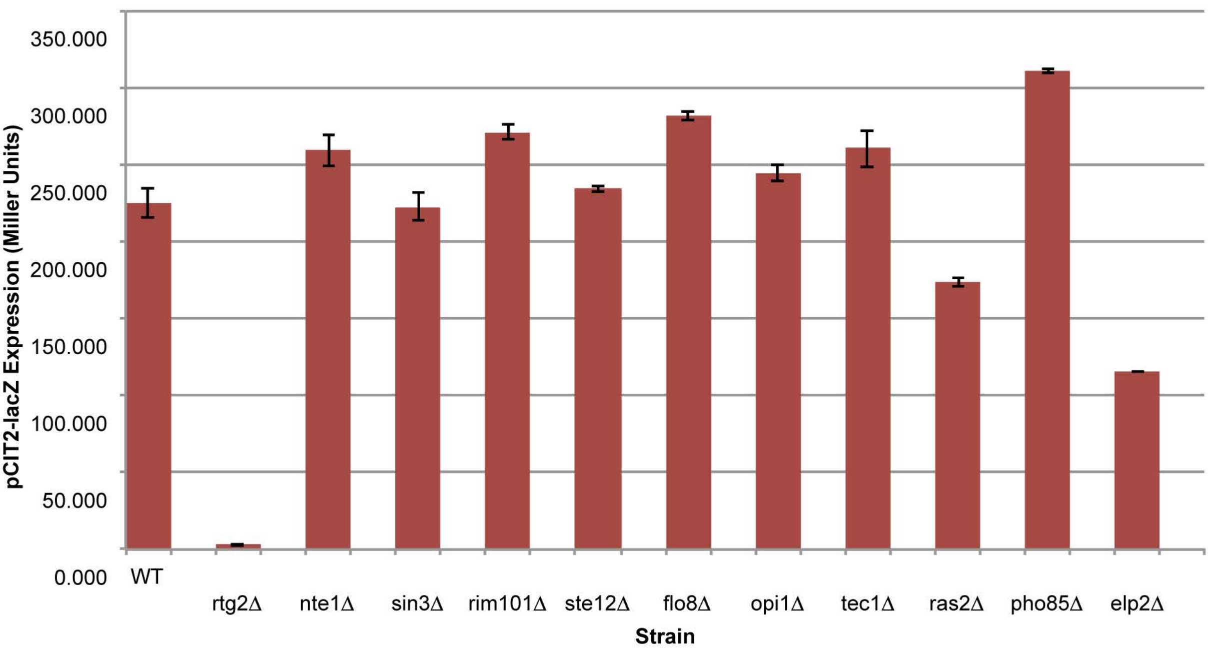


Figure S7. The filamentous growth MAPK pathway does not regulate the RTG response. A) Strains were spotted onto plates containing 6.7% Yeast Nitrogen Base without amino acids and supplemented with uracil with or without glutamate. Glutamate auxotrophy reflects a defective RTG pathway. **B)** *CIT2-lacZ* analysis of selected mutants. ß-galactosidase assays were performed in duplicate; error bars represent standard deviation between samples.

Files S1-S2

Available for download as .mov files at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.168252/-/DC1

File S1 Serial Z-stack images of rhodamine phalloidin stained wild-type cells grown for 16h in S-GLU.

File S2 Serial Z-stack images of rhodamine phalloidin stained *MSB2 cells grown for 16h in S-GLU.**

Tables S1-S3

Available for download as Excel files at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.168252/-/DC1

Table S1 **Analysis of invasive growth mutants for a role in filamentous growth MAPK pathway regulation. References for previously identified regulators of filamentous growth not identified in the text are as follows: (Liu *et al.* 1993; Gimeno and Fink 1994; Stevenson *et al.* 1995; Ward *et al.* 1995; Gavrias *et al.* 1996; Lorenz and Heitman 1997; Mosch and Fink 1997; Tedford *et al.* 1997; Ramezani Rad *et al.* 1998; Entian *et al.* 1999; Gagiano *et al.* 1999; Johnson 1999; Kobayashi *et al.* 1999; Conte and Curcio 2000; Pan and Heitman 2000; Harashima and Heitman 2002; Kohler *et al.* 2002; Laprade *et al.* 2002; Smith *et al.* 2002; Breitkreutz *et al.* 2003; Bao *et al.* 2004; Wu and Jiang 2005; Bester *et al.* 2006; Bhattacharyya *et al.* 2006; Ishigami *et al.* 2006; Frydlova *et al.* 2007; Tiedje *et al.* 2007; Valerius *et al.* 2007; Fidalgo *et al.* 2008; Kim and Siede 2011; Laxman and Tu 2011; Lo *et al.* 2012; Vandenbosch *et al.* 2013).**

Table S2 **Analysis of invasive growth and colony morphology.**

Table S3A **Activity of the *FRE-lacZ* reporter in mutants that show hyper-filamentous growth. (See sheet 2 for *FRE-lacZ* analysis of hypo- filamentous growth mutants).**

Table S3B **Activity of the *FRE-lacZ* reporter in mutants that show hypo-invasvie growth. (See sheet 1 for *FRE-lacZ* analysis of hyper-filamentous growth mutants).**

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