SUPPLEMENTAL MATERIAL for

New Aspects of Invasive Growth Regulation Identified by Functional Profiling of MAPK Pathway Targets in *Saccharomyces cerevisiae*

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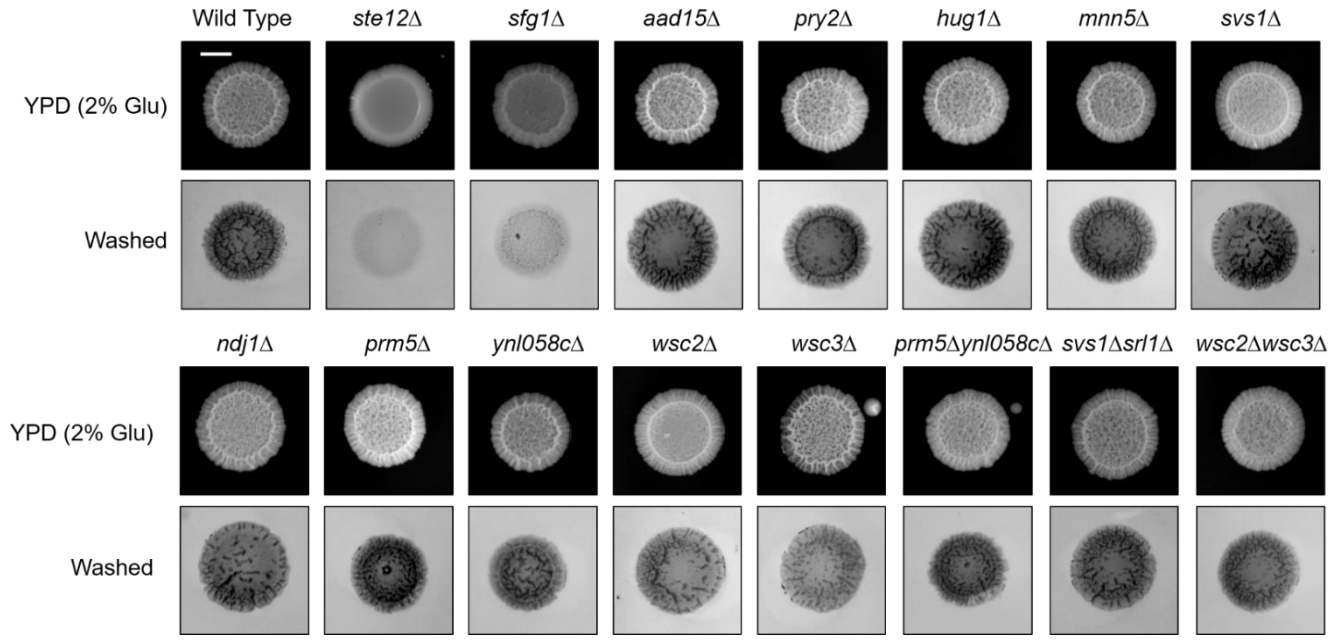


Figure S1. Survey of fMAPK targets by the plate-washing assay. Plate-washing assay for wild type (PC538), *ste12*Δ (PC539), *sfg1*Δ (PC7144), *aad15*Δ (PC7238), *pry2*Δ (PC7240), *hug1*Δ (PC7239), *mnn5*Δ (PC7241), *svs1*Δ (PC7167), *ndj1*Δ (PC7168), *svs1*Δ *srl1*Δ (PC7202), *prm5*Δ (PC7169), *ynl058c*Δ (PC7201), *prm5*Δ *ynl058c*Δ (PC7203), *wsc2*Δ (PC7170), *wsc3*Δ (PC7200), and *wsc2*Δ *wsc3*Δ (PC7243) **s**trains on YPD (2% Glu) for 3 d. Top row, colonies, bottom row, inverted image after wash, bar = 0.5 cm. One target explored was one of the top 5 inhibited targets, *HUG1*, not shown in the volcano plot with no phenotype.

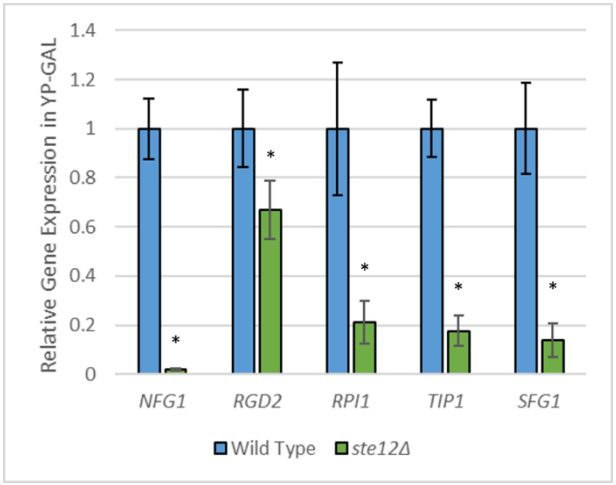


Figure S2. Targets of the fMAPK pathway verified by RT-qPCR. Relative gene expression by RT-qPCR of target gene (*NFG1, RGD2, RPI1, TIP1,* and *SFG1*) mRNA levels between wild-type (PC538+pRS316) and *ste12*Δ (PC539). Normalized to *ACT1* expression, Wild type values set to 1. Cells grown in YP-Gal (2%) liquid medium for 5.5 h. Error represents standard deviation which varied < 20% across 3 trials. Asterisks, p-value < 0.01, by student's t-test compared to wild type**.**

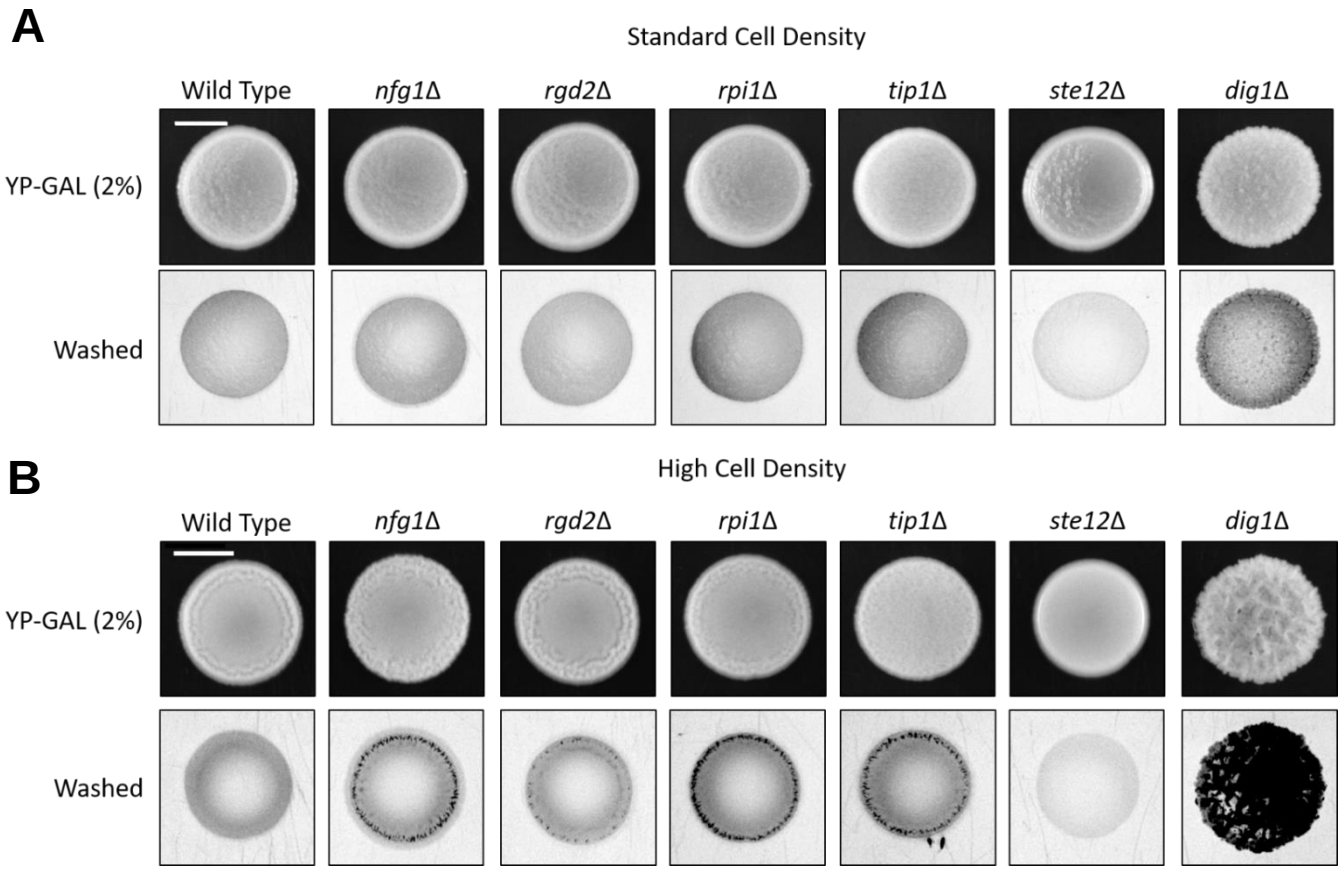


Figure S3. Plate-washing assay of *nfg1*Δ, *rgd2*Δ, *rpi1*Δ, and *tip1*Δ with standard and high cell density on YP-Gal (2%) medium. Plate-washing assay for wild type (PC538+pRS316) and the *nfg1*Δ (PC7147), *rgd2*Δ (PC7146), *rpi1*Δ (PC7145), *tip1*Δ (PC7277), *ste12*Δ (PC539), and *dig1*Δ (PC3039) mutants. Wild type and the *nfg1*Δ mutant data is from Fig. 2B. Top row, colonies, bottom row, inverted images of plates after wash, bar = 0.5 cm. **A)** Cells spotted, OD_{600}= 1.5, and grown on YP-Gal (2%) medium for 3 d. **B)** Cells spotted, OD_{600}= 11, and grown on YP-Gal (2%) medium for 2 d.

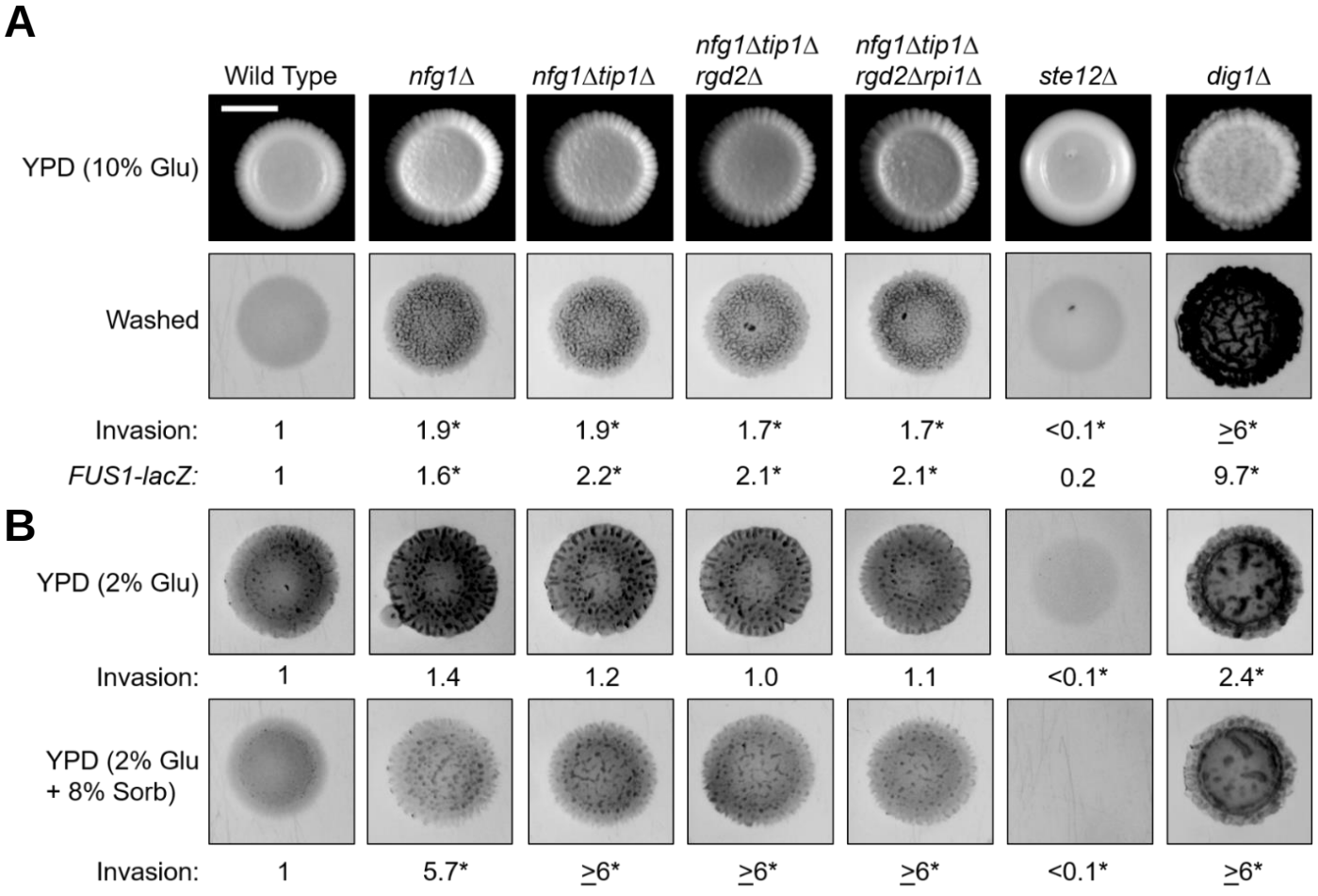
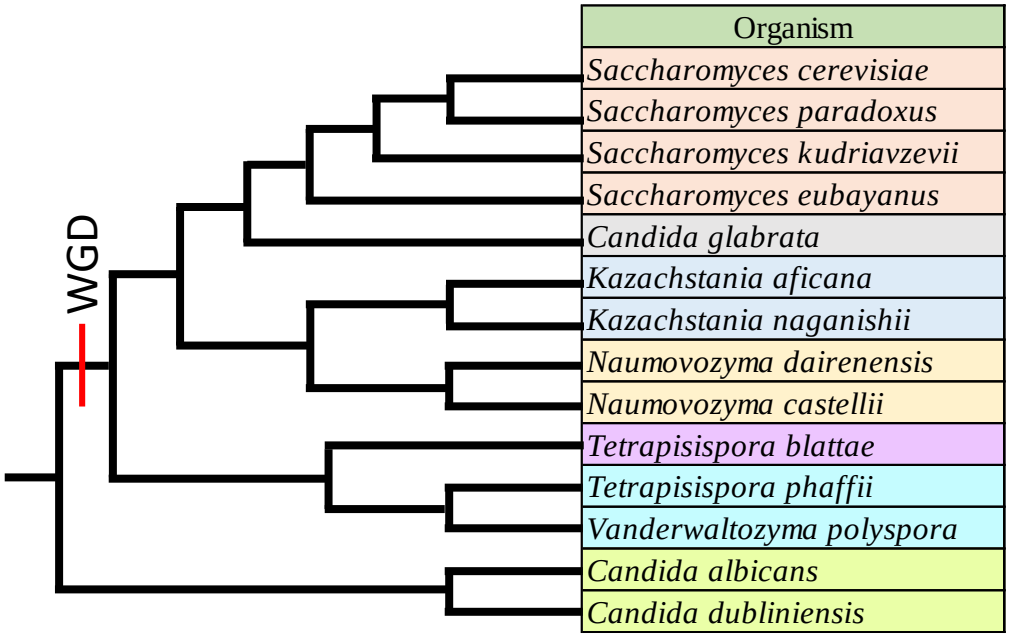


Figure S4. Mutant combinations of negative regulators assessed by invasive growth. A) Plate-washing assay for wild type (PC538+pRS316), and the *nfg1*Δ (PC7147), *nfg1*Δ*tip1*Δ (PC7536), *nfg1*Δ*tip1*Δ*rgd2*Δ (PC7556), *nfg1*Δ*tip1*Δ*rgd2*Δ*rpi1*Δ (PC7557), *ste12*Δ (PC539), and *dig1*Δ (PC3039) mutants spotted onto YPD (10% Glu) for 3 d of growth. Top row, colonies, bottom row, inverted images of plate after wash, bar = 0.5 cm. Invasion, quantification of invasive scars by ImageJ in triplicate, with wild type values set to 1. Error represents the s.e.m., which varied < 20% across trials. Asterisks, p-value ≤ 0.05, by student's t-test compared to wild type. *FUS1-lacZ*, ß-Galactosidase (*lacZ*) assays. Cells grown in SD-URA for 16 h, washed, and resuspended in SD-URA for 5.5 h prior to harvesting cells by centrifugation. **B)** Plate-washing assay on YPD (2% Glu) and high osmolarity medium [YPD (2% Glu + 8% Sorb)] for 3 d. Inverted images of plates after wash for indicated strains. Colonies (not shown) were similar in size and appearance. Invasion, quantification of invasive scars by ImageJ in triplicate, with wild type values set to 1. Error represents the s.e.m., which varied < 30% across trials. Asterisks, p-value < 0.01, by student's t-test compared to wild type.

A



B

	Protein BLAST of Nfg1p		Protein BLAST Rgd2p		Protein BLAST Rpi1p		Protein BLAST Tip1p		Protein BLAST Sfg1p	
Organism	Query Cover	% Identity	Query Cover	% Identity	Query Cover	% Identity	Query Cover	% Identity	Query Cover	% Identity
Saccharomyces cerevisiae	-	-	-	-	-	-	-	-	-	-
Saccharomyces paradoxus	99	83.85	99	95.24	99	75.18	93	79.8	99	87.85
Saccharomyces kudriavzevii	99	67.90	N/A	N/A	99	84.73	N/A	N/A	99	83.82
Saccharomyces eubayanus	99	75.31	99	90.06	99	79.9	93	74.75	99	78.61
Candida glabrata	N/A	N/A	99	55.56	22	59.34	46	53.54	33	45.69
Kazachstania aficana	N/A	N/A	99	54.17	76	33.54	41	45.05	97	36.24
Kazachstania naganishii	N/A	N/A	99	55.6	41	42.35	47	44	96	33.6
Naumovozyma dairenensis	N/A	N/A	99	56.4	75	37.54	56	61.48	99	38.54
Naumovozyma castellii	N/A	N/A	99	56.86	41	49.41	46	70.59	99	40.33
Tetrapisispora blattae	N/A	N/A	N/A	N/A	31	49.62	52	48.18	38	42.25
Tetrapisispora phaffii	N/A	N/A	99	55.75	36	44.97	51	41.07	40	41.03
Vanderwaltozyma polyspora	N/A	N/A	99	61.51	N/A	N/A	N/A	N/A	41	49.06
Candida albicans	N/A	N/A	99	37.33	N/A	N/A	N/A	N/A	45	28.75
Candida dubliniensis	N/A	N/A	99	36.78	N/A	N/A	N/A	N/A	N/A	N/A

Figure S5. *NFG1* is conserved within the *Saccharomyces* clade. A) Cladogram of a subgroup of yeasts (Shen et al., 2018). WGD = whole genome duplication. **B)** Comparative analysis by BLAST for indicated protein sequence. Query cover, percentage of query sequence that overlaps the reference sequence. % identity, percentage of how similar query sequence is to the reference sequence. Significance by E value, all E values were significant being $\leq$ 2E-12, except the protein BLAST for Sfg1p in *Candida albicans* was not significant (E value = 0.67). N/A = not available.

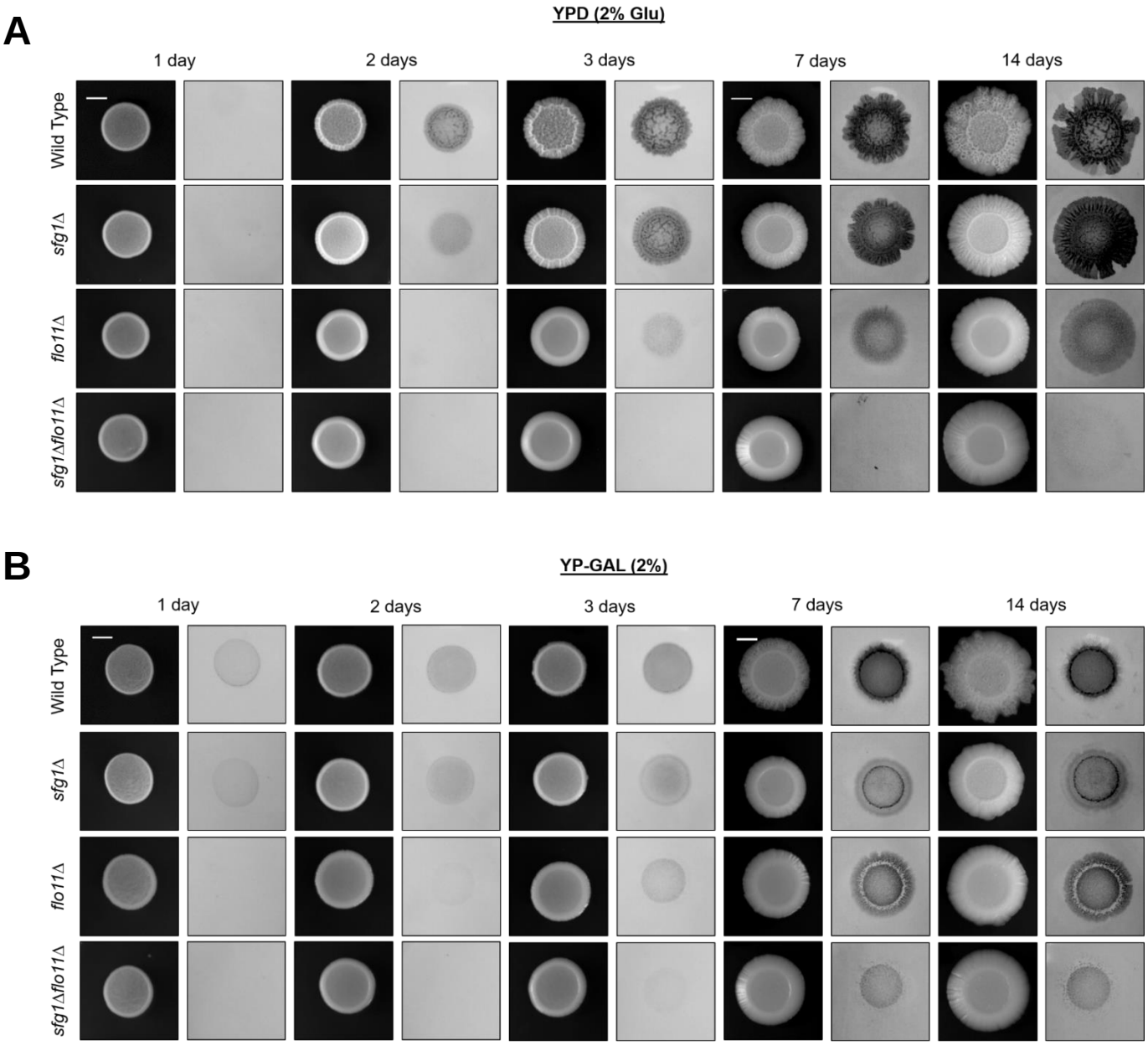


Figure S6. Time course of plate-washing assay over 14 d. Plate-washing assay for wild type (PC538), *sfg1*Δ (PC7144), *flo11*Δ (PC1029), and *sfg1*Δ*flo11*Δ (PC7280) strains grown for 1 d, 2 d, 3 d, 7 d, and 14 d. 7 d data also appears in Figure 4A. Columns with black background, colonies, light background, inverted images of plates after wash, bars = 0.5 cm. **A)** Cells were spotted onto YP-Gal (2%) medium. **B)** Cells were spotted onto YPD (2% Glu) medium.

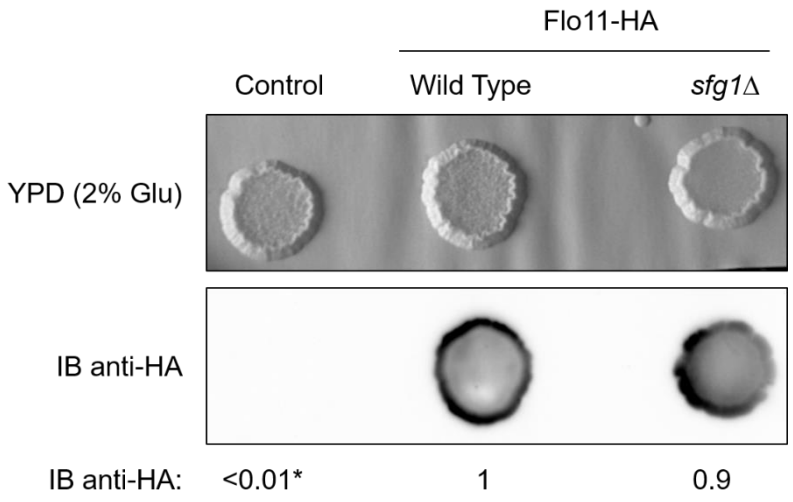


Figure S7. Sfg1p does not regulate Flo11p shedding on YPD (2% Glu). Colony immunoblot analysis to detect HA-tagged Flo11p with anti-HA antibodies was performed for wild type (PC538), *FLO11*-HA (PC2043), and *sfg1*Δ*FLO11*-HA (PC7321) strains grown on nitrocellulose membranes atop 2% glucose YPD semi-solid agar medium for 3 d. Numbers refer to the intensity of anti-HA quantified by image lab. Error is s.e.m. with < 15% variation across trials. Asterisk, p-value < 0.01, by student's t-test compared to wild type.