Supplementary information for:

Counteractive control of polarized morphogenesis during mating by MAPK Fus3 and G1 cyclin dependent kinase

Lu Yu, Maosong Qi, Mark A. Sheff and Elaine A. Elion*
Department of Biological Chemistry and Molecular Pharmacology
Harvard Medical School
240 Longwood Avenue
Boston, MA 02115- 5730

***Corresponding author:** **Elaine A. Elion**
elaine_elion@hms.harvard.edu
tel: 617-432-3815
fax: 617-738-0516

Supplemental materials and methods.

Strain construction

CUP1p-GFP-STE5 gene has SuperGlo™ green fluorescent protein with F64L, S65C and I167T mutations (Mahanty *et al*., 1999). EYL4653 and EYL4654 were created by C-terminally tagging *STE5* in S288C *MAT**a*** (BY4741) cells with *3xGFP-KAN* and *3xYFP-KAN* respectively, using pFA6a-3xGFP-KAN and pFA6a-3xYFP-KAN as templates (gift from Jian-Qiu Wu and Thomas Pollard, Yale University; Wu and Pollard, 2005). The pFA6a 3xXFP linker sequence in Ste5-3xYFP3 and Ste5-3xGFP3 is GRRIPGLINGTKAGGS. The GFP sequence in the 3xGFP plasmid was made from codon optimized yEGFP1 (Cormack *et al.*. 1997). The YFP sequence in the 3xYFP plasmid is not codon optimized and matches YFP in pDH5 (Hailey *et al.*. 2002). The 3xYFPs were introduced into the genome by PCR amplification using oligo Ste5_3xGFP-f (which contains 40bp homologous DNA upstream of the *STE5* stop codon followed by18 bp homology to the pFA6a plasmids and anneals at the linker nucleotides encoding GRRIIPG; Wach *et al.*. 1997) and oligo Ste5_xFP-r (containing 40bp homologous DNA downstream of Ste5 stop codon followed by 18 homologous DNA to the pFA6a plasmids). The PCRs were performed with the Expand Long Template System (Roche) adhering to the manufacturers parameters, with an annealing temperature of 54°C and an elongation time of 210 seconds. The resulting PCR products were then purified on PCR purification columns (Qiagen), eluted in 50 µl buffer and transformed into BY4741 following the high efficiency transformation method (Amberg *et al*., 2005; Gietz *et al*., 1995) using 100 µg of carrier DNA, heat shock for 50 min., and incubating overnight at 30°C on YPD plates before replica plating the next day to YPD plates containing 250 µg/ml G418 (Calbiochem). G418 resistant colonies were purified on YPD G418+ plates and verified using colony PCR to confirm the presence of the tag and absence of the untagged gene (Amberg *et al*., 2005; Petracek and Longtine, 2002). Ste5-3xGFP could promote approximately

80% wild type diploid formation in quantitative matings. The level of diploid formation conferred by Ste5-3xYFP was approximately 30% wild type levels, most likely because of lower expression levels due to noncodon-optimized YFP. EYL4640 was made by deleting *GPA1* from EY1775 using the hphMX4 hygromycin phopsphotransferase disruption cassette (Goldstein and McCusker, 1999) using oligos Gpa1_delf and Gpa1_delr, consisting, respectively, of 40 nucleotides upstream of the *GPA1* start codon followed by 18 bp homology to pFA6a plasmid (pAG32, Goldstein and McCusker, 1999) and 40 nucleotides downstream of the *GPA1* stop codon followed by 18 bp homology to pFA6a (pAG32, Goldstein and McCusker, 1999) and the hygromycin gene as marker (Sheff and Thorn, 2004). This PCR was done with *Pfu Ultra* (Stratagene), an annealing temperature of 53.8°C and elongation time of 2 minutes. Hygromycin resistant transformants were purified and verified by colony PCR. Strains EYL4649, EYL4684, and EYL4685 are *ste5Δ::hphMX4* derivatives of strains EY1093, EY1094, and EYL1664 respectively. These strains were constructed following the same methodology as for EYL4640, using oligos Ste5_delf and Ste5_delr (18bp pFA6a homology, 40bp up/downstream of *STE5*) and PCR conditions described in (Wach *et al.*, 1994). All restriction enzymes were purchased from New England Biolabs. The *cdc28-4* strains were made by crossing strain AFY367 (EY2504 + pRS314; EY2504=PY1237, gift of D. Pellman) *MATα cdc28-4 ura3-1 leu2-3,112 ade2-1 trp1-1 his3-11,15 can1-100 [CEN TRP1] Gal+* to strain AFY368 (EY1110 + pYEE81) *MATa bar1Δ fus3-6::LEU2 kss1::ADE2 FUS1p-HIS3::lys2 his3Δ200 ura3-1 leu2-3, 112 trp1-1 ade2-1 can1-100 [CEN URA3 FUS3] Gal1*$^{+}$ by selecting for Ura+ and Trp+ prototrophy to isolate diploids. Diploids were then sporulated and dissected and ascospores were scored after transfer over SC plates containing 5-fluoroorotic acid to identify cells that had lost pYEE81. Ascospores were scored for temperature sensitivity by a double lift-off replica plating as well as for other markers, sensitivity to α factor and ability to mate in qualitative plate assays. The relevant *cdc28-4* strains were then subjected to

introduction of *STE5-3xGFP* in place of chromosomal *STE5* as previously described. Comparison with a *cdc28-4* strain at room temperature showed that the expression of Ste5-3xGFP did not interfere with Cdc28 function.

Tagged derivates of Ste5 not bright enough to detect cortical pool Fluorescent tags that were not bright enough when inserted in single copy at the C-terminus of Ste5 as previously described (Sheff and Thorn, 2004) included: yEGFP, yEmCit, yEVenus (gifts of Kurt Thorn, Bauer Center, Harvard University). Although cytoplasmic and nuclear pools and tip staining could be detected with these tags, it was weak, albeit slightly brighter than Ste5-GFPS65T (Huh *et al.*, 2003). None of the aforementioned fluorescent protein fusions permitted detection of the basal pool at the plasma membrane prior to or immediately after α factor addition with our microscope setups.

Supplemental References

Amberg, D.C., Burke, D., and Strathern, J.N. (2005). Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, Cold Spring Harbor, N.Y.

Andersson, J., Simpson, D.M., Qi, M., Wang, Y. and Elion, E.A. (2004). Differential input by Ste5 scaffold and Msg5 phosphatase route a MAPK cascade to multiple outcomes. EMBO J. *23*, 2564-2576.

Cormack, B.P., Bertram, G., Egerton, M., Gow, N.A., Falkow, S., Brown, A.J. (1997). Yeast-enhanced green fluorescent protein (yEGFP)a reporter of gene expression in Candida albicans. Microbiology *143*, 303-311.

Elion, E.A., Satterberg, B., Kranz, J.E. (1993). *FUS3* phosphorylates multiple components of the mating signal transduction cascade: evidence for *STE12* and *FAR1*. Mol Biol Cell *4*, 495–510.

Gietz, R.D., Schiestl, R.H., Willems, A.R., Woods, R.A. (1995). Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast *11*, 355-360.

Goldstein, A.L., McCusker, J.H. (1999). Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast *15*, 1541-1553.

Hailey, D.W., Davis, T.N., Muller, E.G. (2002). Fluorescence resonance energy transfer using color variants of green fluorescent protein. Methods Enzymol. *351*, 34-49.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S. and O'Shea, E.K. (2003). Global analysis of protein localization in budding yeast. Nature *425*, 686-691.

Yu, L., Qi, M., Sheff, M.A., Elion, E.A. E07-08-0757 in press

Lee, L., Klee, S.K., Evangelista, M., Boone, C. and Pellman, D. (1999).. Control of mitotic spindle position by the *Saccharomyces cerevisiae* formin Bni1p. J. Cell Biol. *144*, 947-961.

Mahanty SK, Wang Y, Farley FW, Elion EA. (1999). Nuclear shuttling of yeast scaffold Ste5 is required for its recruitment to the plasma membrane and activation of the mating MAPK cascade. Cell *98*, 501-512.

Petracek, M.E., Longtine, M.S. (2002). PCR-based engineering of yeast genome. Methods Enzymol. *350*, 445-469.

Pruyne, D., Legesse-Miller, A., Gao, L., Dong, Y. and Bretscher, A. (2004). Mechanisms of polarized growth and organelle segregation in yeast. Annu Rev Cell Dev Biol. *20*, 559-591.

Sheff, M.A., Thorn, K.S. (2004). Optimized cassettes for fluorescent protein tagging in *Saccharomyces cerevisiae*. Yeast *21*, 661-670.

Toenjes, K. A., Sawyer, M. M., and Johnson, D. I. (1999). Curr. Biol. *9*, 1183-1186

Wach, A., Brachat, A., Alberti-Segui, C., Rebischung, C., Philippsen, P. (1997). Heterologous *HIS3* marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. Yeast *13*, 1065-1075.

Wu, J.Q., Pollard, T.D. (2005). Counting cytokinesis proteins globally and locally in fission yeast. Science *310*, 310-314.

Supplemental figure legends and Supplemental tables.

Supplemental Figure 1. Description of pattern of GFP-Ste5 localization in *fus3Δ* mutants. Cells from the experiment described in Figure 2B were photographed and labeled as shown.

Supplemental Figure 2. Localization of Ste5-3XGFP in *FUS3* and *fus3Δ* strains of S288c background. Strains EYL4653 and EYL4697 (which are *BAR1*) were grown at 30^{o}C to logarithmic phase in liquid YPD medium, pelleted and resuspended in fresh medium prewarmed to 30^{o}C and treated with 5μM α factor for the indicated time periods and photographed and tallied as shown.

Supplemental Figure 3. Ste5-3xGFP localization in *cdc28-4* cells. (A-B) Pattern of cortical recruitment of Ste5-3xGFP in *cdc28-4*, *cdc28-4 fus3Δ* and *cdc28-4 bni1Δ* cells. Strains *cdc28-4* (EYL4705), *cdc28-4 fus3Δ* (EYL4710) and *cdc28-4 bni1Δ* (EYL4711) with integrated Ste5-3xGFP were pregrown at room temperature overnight, then shifted to pre-warmed 37^{o}C medium for 3 hours followed by incubation at same temperature with or without addition of 50nM α factor for 90 minutes.

Supplemental Table 1. Yeast strains used in this study.

Strains	Genotype	Source
Isogenic to W303a		
EY699	*MATa trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)*	E. Elion
EY701	*MATa trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+) fus3-7::HIS3*	E. Elion
EY725	*MATa trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+) kss1Δ::URA3*	E. Elion
EY957	*MATa bar1⊗ trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)*	Elion *et al.,* 1993
EY1093	*MATa bar1⊗ trp1-1 his3-11,15 ura3-1 can1-100 fus3-8::ADE2 cln2Δ::LEU2 (Gal+)*	E. Elion
EY1094	*MATa bar1⊗ trp1-1 his3-11,15 ura3-1 can1-100 fus3-8::ADE2 cln1Δ::TRP1 Gal+*	E. Elion
EY1095	*MATa bar1⊗ trp1-1 his3-11,15 ura3-1 can1-100 fus3-8::ADE2 (Gal+)*	E. Elion
EY1262	*MATa bar1Δ far1Δ trp1-1 his3Δ200 leu2-3,112 ade2-1 ura3-1 can1-100 FUS1p-HIS3::lys2 (Gal+)*	E. Elion
EY1774	*MATa bar1⊗ ste5⊗::TRP1 fus3-8::ADE2 trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)*	E. Elion
EY1775	*MATa bar1⊗ ste5⊗::TRP1 trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)*	E. Elion
EY2019	*MATa bar1Δ ste5⊗::TRP1 far1Δ trp1-1 his3Δ200 leu2-3,112 ade2-1 ura3-1 can1-100 FUS1p-HIS3::lys2 (Gal+)*	E. Elion
EYL917	*MATa bar1⊗ bni1⊗::KAN ura3-1 leu2-3 trp1-1 his3-11,15 ade2-1 can1-100 (Gal+)*	Qi and Elion, 2005
EYL2184	*MATa bar1⊗ cdc28-4 fus3-6::LEU2 kss1Δ::ADE2 ura3-1 trp1-1 his3-11,15 (Gal+)*	A. Flotho**

EYL2190 *MATa bar1⊗cdc28-4 ura3-1 leu2-3 trp1-1 his3-11,15 ade2-1 can1-100 (Gal+)* A. Flotho**

EYL4640 *MATa bar1⊗ste5⊗::TRP1 gpa1⊗::Hygro trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)* M. Sheff*

EYL4649 *MATa bar1⊗ste5⊗::HYGRO fus3⊗::ADE2 cln2⊗::LEU2 trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)* M. Sheff*

EYL4684 *MATa bar1⊗cln1⊗::TRP1 fus3-8::ADE2 ste5⊗::HYGRO trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)* M. Sheff*

EYL4685 *MATa bar1⊗ste5⊗::HYGRO fus3-6::LEU2 tec1⊗::HIS3 trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)* M. Sheff*

EYL4692 *MATa bar1⊗ ste5⊗::TRP1 fus3K42R::HIS3 trp1-1 his3-11,15 leu2-3,112 ade2-1 ura3-1 can1-100 (Gal+)* M. Sheff*

EYL4704 *MATa bar1Δ cdc28-4 fus3-6::LEU2 ura3-1 leu2-3,112, his3-11,15 ade2-1 trp1-1 can1-100 (Gal+)* M. Sheff*

EYL4705 *MATa bar1⊗cdc28-4^{ts} STE5-3xGFP::KAN ura3-1 leu2-3 trp1-1 his3-11,15 ade2-1 (Gal+)* M. Sheff*

EYL4710 *MATa bar1⊗cdc28-4^{ts} fus3Δ::HYGRO STE5-3xGFP::KAN ura3-1 leu2-3 trp1-1 his3-11,15 ade2-1 (Gal+)* M. Sheff*

EYL4711 *MATa bar1⊗cdc28-4^{ts} bni1Δ::HYGRO STE5-3xGFP::KAN ura3-1 leu2-3 trp1-1 his3-11,15 ade2-1 (Gal+)* M. Sheff*

Isogenic to S288c

EYL4653 *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 STE5-3xGFP::KAN* M. Sheff*

EYL4654 *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 STE5-3xYFP::KAN* M. Sheff*

EYL4697 *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 STE5-3xGFP::KAN fus3Δ::HYGRO* M. Sheff*

*This study

**Strain may be *his3Δ200* instead of *his3-11,15*

[ts]: temperature sensitive

Supplemental Table 2. Plasmids used in this study.

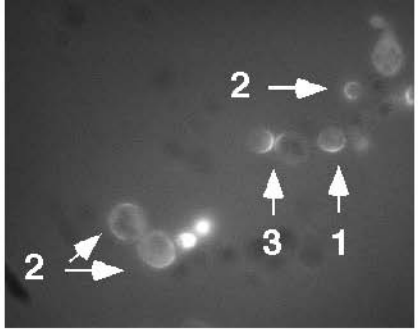
Plasmids	Description			Source
EB562/pYBS351	*fus3K42R-HA*	*CEN*	*HIS3*	Elion collection
EBL206	*GAL1p-STE5-CTM*	*CEN*	*HIS3*	P. Pryciak
EBL334/PB1229	*ADH1p-BNI1-GFP*	*CEN*	*URA3*	D. Pellman
pSKM21	*CUP1p-GFP-STE5*	*CEN*	*URA3*	Mahanty *et al.,* 1999
pRL116 (EBL511)	*GFP-STE20*	*CEN*	*URA3*	E. Leberer
EBL510	*GFP-STE20Δ334-369*	*CEN*	*URA3*	P. Pryciak
EBL664	*p415MET25GFP-8A-Cdc24*	*CEN*	*LEU2*	Toenjes *et al.* 1999
p11-4	*STE11-4*	*CEN*	*HIS3*	H. Madhani
pSKM12	*STE5*	*CEN*	*URA3*	Mahanty *et al.,* 1999
pSKM32	*ste5Δ49-66*	*CEN*	*URA3*	Mahanty *et al.,* 1999
B1820		*CEN*	*URA3*	Elion collection
pSKM17	*CUP1p-STE5-MYC9*	*CEN*	*URA3*	Mahanty *et al.,* 1999

Supplemental Table 3. Oligos used in this study.

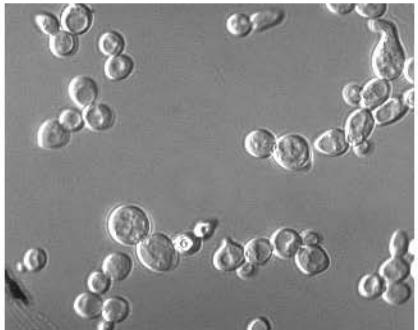
Oligos	Sequence (5'--> 3')
Sst2_delf	*GTTATAGGTTCAATTTGGTAATTAAAGATAGAGTTGTAAG*GCCTCGTCCCCGCCGG
Sst2_delr	*GTGCAATTGTACCTGAAGATGAGTAAGACTCTCAATGAAA*TCGATGAATTCGAGCTCG
Ste5_delf	*AAAAAAGGAAGATACAGGATACAGCGGAAACAACTTTTAA*GCCTCGTCCCCGCCGG
Ste5_delr	*ATGCTTTCTTTTTATTATTGCATAAAATTTAGTGTATACT*TCGATGAATTCGAGCTCG
Gpa1_delf	*ATCCAGAGGTGTATAAATTGATATATTAAGGTAGGAAATA*GCCTCGTCCCCGCCGG
Gpa1_delr	*AATTTACGTATCTAAACACTACTTTAATTATACAGTTCCT*TCGATGAATTCGAGCTCG
STE5_xFPr	*ATGCTTTCTTTTTATTATTGCATAAAATTTAGTGTATACT*TCGATGAATTCGAGCTCG
Ste5_3GFPf	*GAGCGGTAATAACAACTGTCCCCTCCATA-*
	*TGGATTATATA*GGTCGACGGATCCCCGGG

underline=template homology italics=40bp of gene specific homology

GFP-Ste5



Nomarski



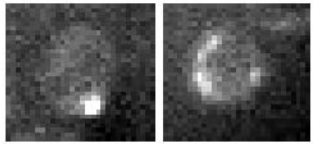
GFP-Ste5 in *fus3Δ* after 90 min α factor induction

1. Emerging shmoo with mislocalized recruitment not in line with axis of polarization

2. Greater and more depolarized recruitment

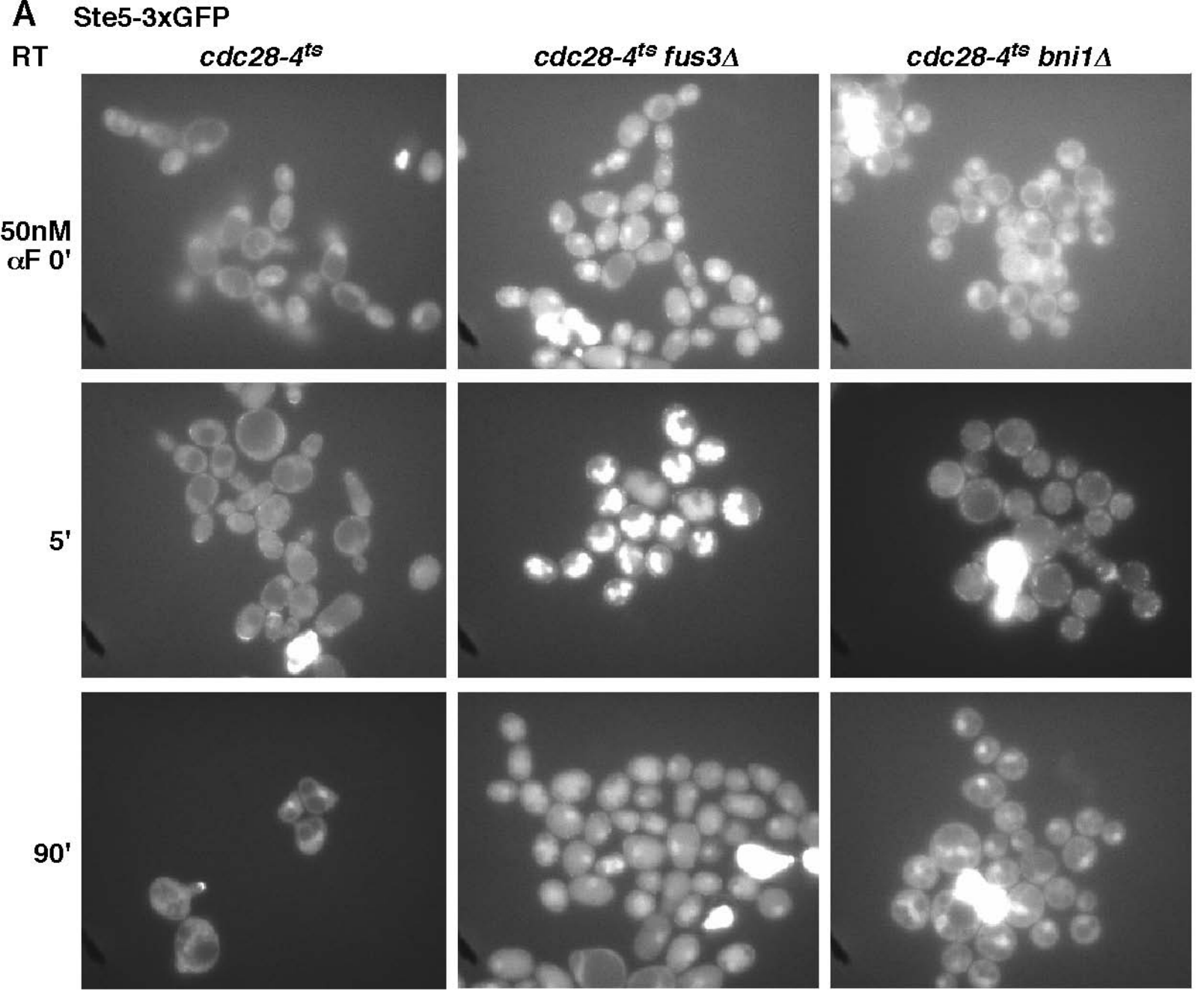
3. Stable recruitment in budding cells

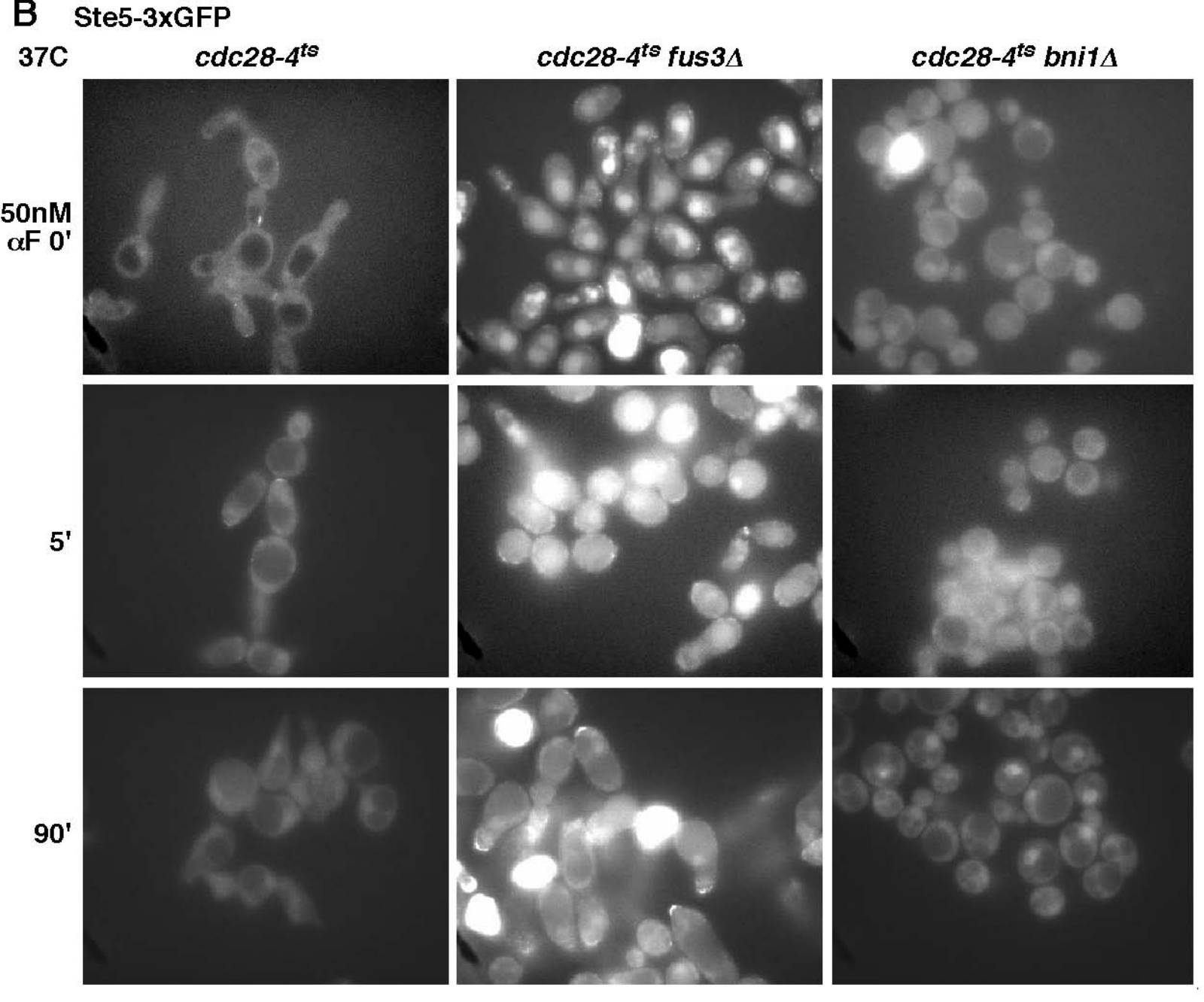
S288c-Ste5-3xGFP



YPD

	% total within each category									% total
										
FUS3-αF5min	8	4	8	0	60	5	0	0	0	1
fus3Δ-αF5min	5	0	89	0	19	81	0	0	0	2
FUS3-αF60min	33	33	0	0	63	0	67	0	0	53
fus3Δ-αF60min	10	0	55	30	26	74	33	11	56	17
FUS3-αF90min	0	0	0	0	100	0	79	0	0	82
fus3Δ-αF90min	0	0	0	100	9	91	27	33	40	32


A
Ste5-3xGFP
RT
cdc28-4ts
cdc28-4ts fus3Δ
cdc28-4ts bni1Δ
50nM αF 0'
5'
90'


B
Ste5-3xGFP
37C
cdc28-4ts
cdc28-4ts fus3Δ
cdc28-4ts bni1Δ
50nM αF 0'
5'
90'