Vernay et al., http://www.jcb.org/cgi/content/full/jcb.201203099/DC1

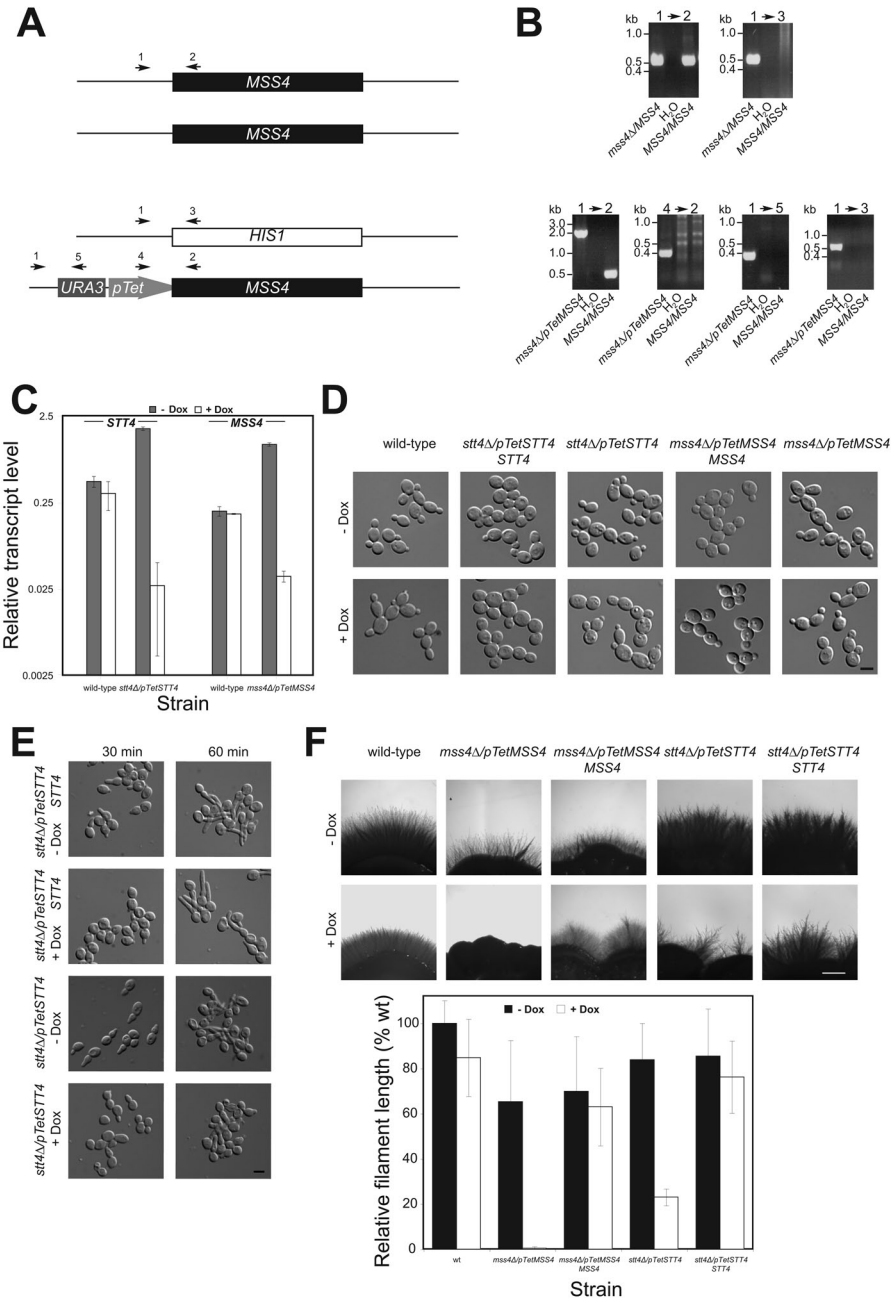


Figure S1. ***stt4* and *mss4* mutants have reduced levels of the respective phosphoinositide phosphate kinases and are defective for invasive filamentous growth.** (A) Schematic representation of *mss4* mutant. Primers used for strain verification are indicated. (B) PCR analyses of *mss4* mutants. Primers indicated in A were used to analyze indicated strains. The *stt4Δ/pTetSTT4* mutant was verified similarly. (C) *STT4* and *MSS4* mRNA transcripts are reduced in *stt4* and *mss4* mutants. qRT-PCR was carried out on indicated strains grown in the presence and absence of Dox. Transcript levels are normalized to the level of *ACT1* transcript and a log scale is shown on the y axis. Values are the average of two independent experiments (2–3 determinations) with bars indicating actual values. (D) *stt4* and *mss4* strains grow normally and do not have substantial morphological defects. Indicated strains were grown in the presence and absence of Dox. Similar results were observed in three independent experiments. Bar, 5 µm. (E) Stt4p is necessary for hyphal growth in response to serum. Indicated strains were incubated in the presence and absence of FCS with and without Dox. Images of cells after indicated incubation times at 37°C. Bar, 5 µm. Similar results were observed in three independent experiments. (F) Mss4p and Stt4p are necessary for invasive filamentous growth. Indicated strains were spotted on FCS containing agar in the presence and absence of Dox and incubated at 30°C for 8 d (top). Bar, 1 mm. Similar experiments were observed in three independent experiments. Quantification of colony filament length of indicated strains from three independent experiments (bottom). Lengths of colony filaments were measured in 4–6 locations and normalized to the wt in each experiment, averages shown with SD indicated.

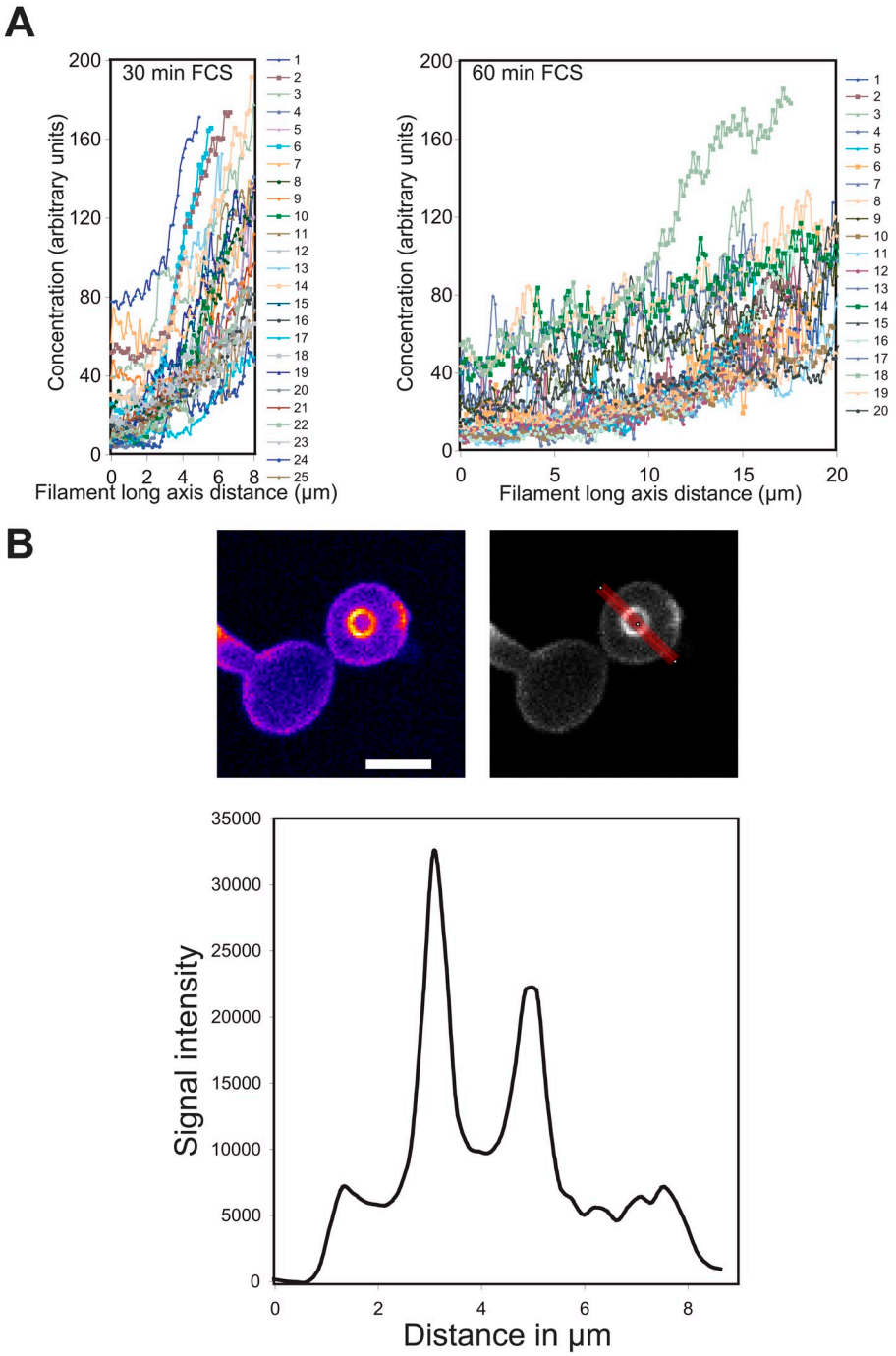


Figure S2. **PI(4,5)P_2 gradients emanating from the extremity of filamentous cells with reduced signal at the tip.** (A) PI(4,5)P_2 gradients from individual cells responding to FCS. Signal concentration (in arbitrary units) was quantified over the long axis of each cell starting from the cell body as described in Fig. 4 C. Profiles are of individual cells used in average shown in Fig. 4 C. Cells incubated with FCS for 30 min at 37°C (left) and 60 min at 37°C (right). (B) A PI(4,5)P_2 gradient ring at the tip of filamentous cells. False-colored sum projection (of 18 deconvoluted spinning-disk z-sections) of germ tube growing in the z-axis. Three z-sections were captured beyond the tip of the germ tube to ensure full sectioning. The line profile region is shown in red and the graph indicates intensity from left to right on this line. The reduction of signal at the tip of the filament was observed in many cells (whose long axis was in the plane of the microscope slide); however, only one cell was observed whose long axis was perpendicular to the plane of the microscope slide. The graph is representative of a single cell in a single experiment.

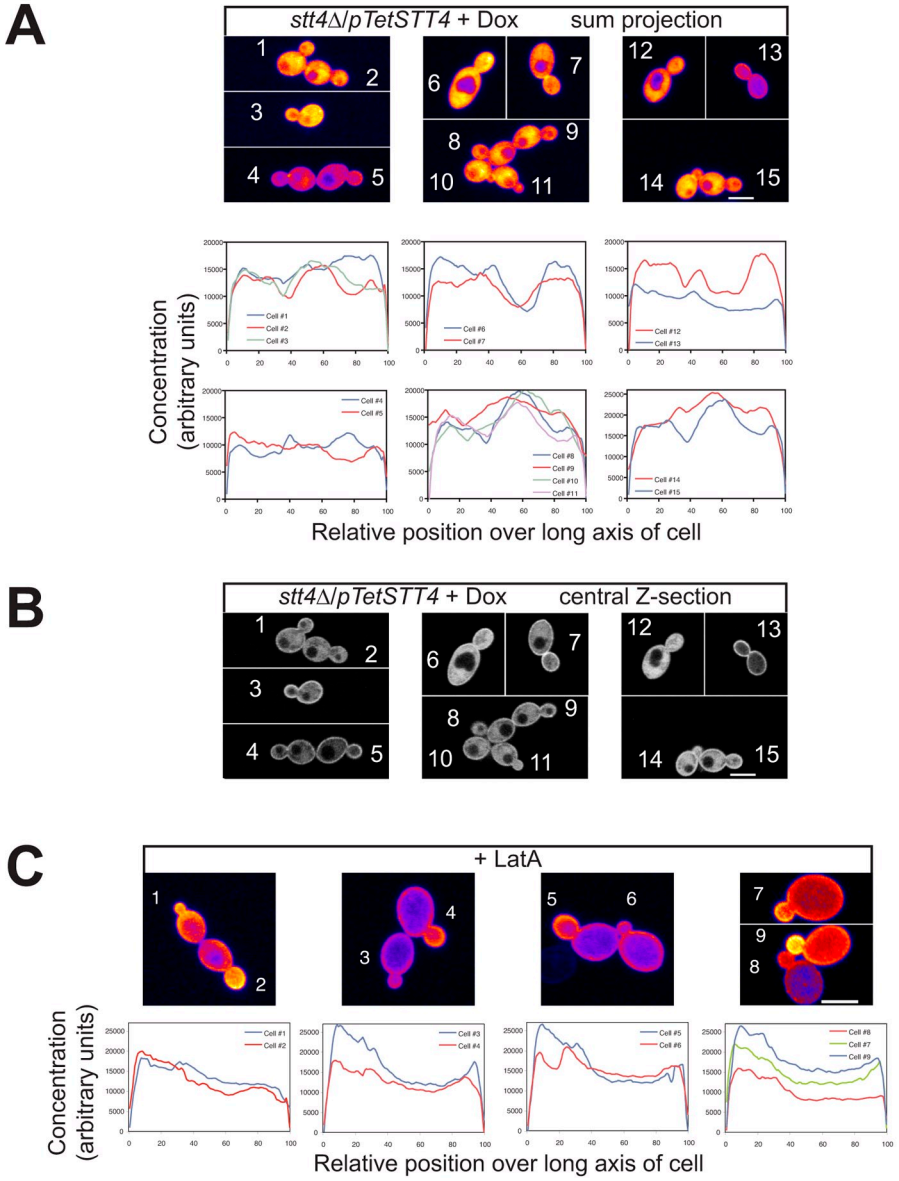
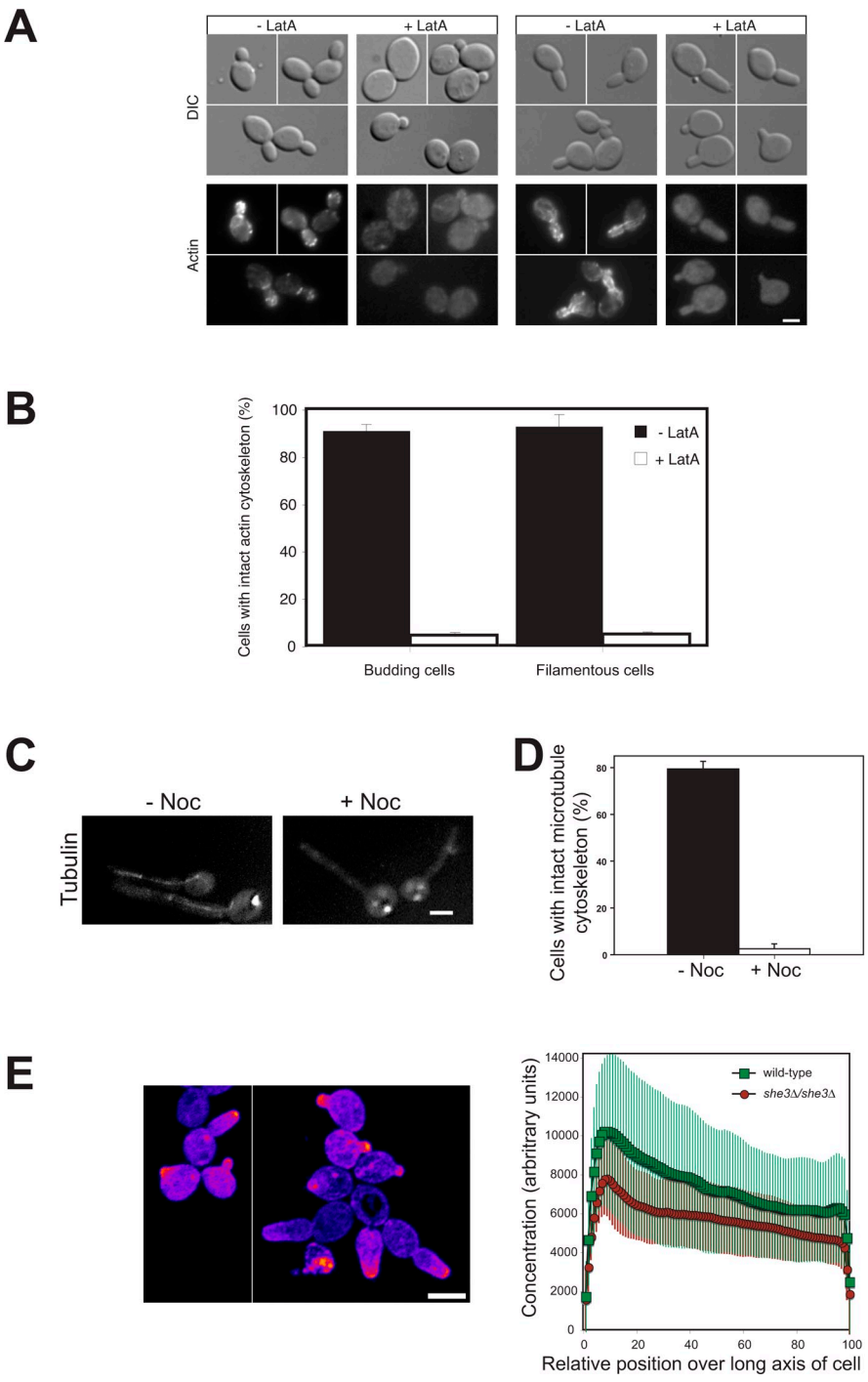


Figure S3. **Effects of depleting Stt4p and disrupting actin on PI(4,5)P_2 distribution in budding cells.** (A) Loss of PI(4,5)P_2 asymmetry upon depletion of PI(4)P. False-colored sum projections from Fig. 5 A of *stt4* cells expressing the GFP-PH$^{Plc\delta}$-PH$^{Plc\delta}$-GFP reporter grown in the presence of Dox with quantification of signal concentration (in arbitrary units) over the long axis of each cell as described in Fig. 4 A. Bar, 5 µm. (B) PI(4,5)P_2 is still observed at the PM in the *stt4* mutant. Central z-section image of representative cells shown in A. The most central z-section is shown; however, this does fall in the center of all cells. Bar, 5 µm. The PM to the cytoplasmic signal ratio was 1.62 ± 0.58 with Dox (with 25% of the cells in Dox having a ratio ≥2.1) compared with 2.98 ± 0.46 in its absence (n = 30 cells). We attribute the difference in loss of plasma membrane PI(4,5)P_2 between these analyses at the cell level (~55 ± 20% remaining in Dox) and at a biochemical level (~80 ± 15% remaining in Dox using an anti-PI(4,5)P_2 mAb; see Fig. 3 D) to the different methods used. In addition, the biochemical method may overestimate PM PI(4,5)P_2 as the P100 fraction does not only contain the PM. (C) F-actin is not required for an asymmetric PI(4,5)P_2 distribution in budding cells. False-colored sum projections (9–12 confocal z-sections) of representative cells from different fields of view (as described in Fig. 4 A) from experiment described in Fig. 6 A. wt cells expressing the PI(4,5)P_2 reporter were treated with 200 µM LatA for 15 min. Signal concentration (in arbitrary units) over the long axis of each cell as described in Fig. 4 A. Bar, 5 µm. Similar results were observed in three independent experiments.

Figure S4. **Disruption of actin and microtubule cytoskeleton and GFP-Mss4 localization in *she3* mutant.** (A) LatA depolymerizes F-actin in budding and hyphal cells. wt cells were treated with 200 μM LatA for 15 min either during budding growth (left) or after 30 min incubation with FCS at 37°C (right) and actin visualized with Alexa-phalloidin; representative cells from different fields of view are shown. Bar, 5 μm. (B) Quantitation of cells with depolymerized actin cytoskeleton. The percentage of cells with an intact actin cytoskeleton was determined from 2–4 independent experiments; *n* = 50–100 cells per experiment. Averages shown with SD indicated. (C) Nocadazole disrupts the MTs. wt cells expressing Tub1-GFP were incubated for 30 min with FCS at 37°C; 40 μM nocodazole was subsequently added (where indicated) and cells incubated for an additional 30 min. Wide-field fluorescence images are shown. Bar, 5 μm. Similar results were observed in three independent experiments. (D) Quantitation of cells with depolymerized MT cytoskeleton by nocodazole. The percentage of cells with intact MTs was determined from three independent experiments; *n* = 50–70 cells per experiment. Averages shown with SD indicated. (E) GFP-Mss4 localizes to the tips of germ tubes in a *she3* mutant. False-colored sum projections of *she3* cells expressing the GFP-Mss4 after 30 min incubation with FCS at 37°C. Representative cells from different fields of view (left). Bar, 5 μm. Similar results were observed in three experiments. Quantification of Mss4 concentration over long axis of filamentous cells using the BP program (right) with average signal concentration over the long axis of cells incubated with FCS shown with SD in gray (*n* = 28 cells).



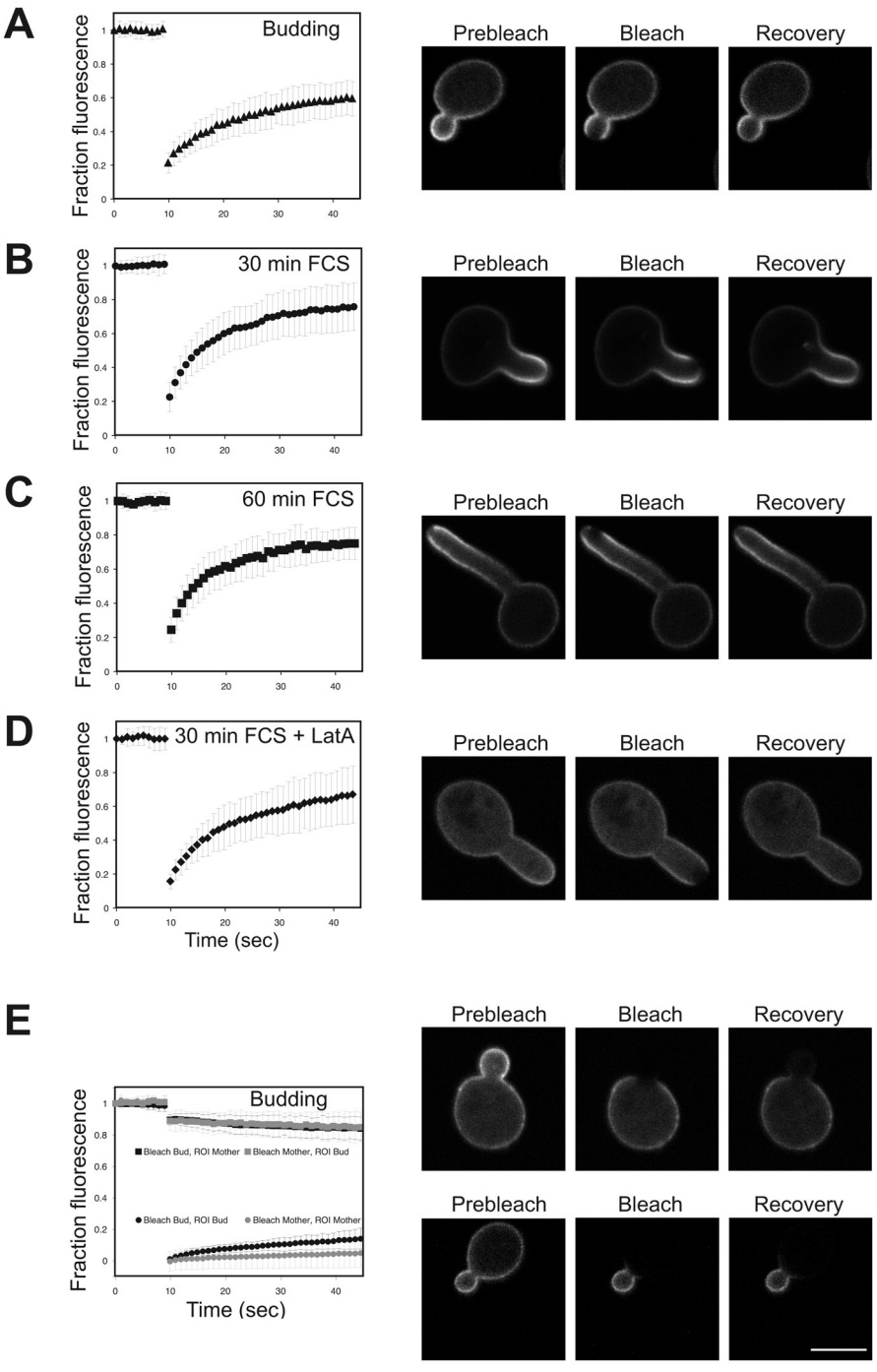
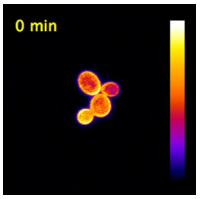


Figure S5. **Dynamics of PI(4,5)P_2 in budding cells and filamentous cells.** (A) Average FRAP curve of budding wt cells expressing the GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP reporter. FRAP was carried out in cells in the indicated condition with averages and SD shown (n = 12 cells). A circular area of 0.6 μm^2 was photobleached. Fluorescence signals were normalized for acquisition-dependent photobleaching and prebleach values set to 1. Note that the mobile fraction in budding cells was substantially less than in filamentous cells; however, this difference is likely to be due to barriers at the bud neck that limit diffusion. Indeed, when we normalized the FRAP data to the bud and not the entire cell this difference in mobile fraction was no longer observable. The average r^2 value for single exponential fits was 0.989 (compared with 0.990 for double exponential fits). Images from a FRAP experiment before, subsequent to photobleaching, and after 20-sec recovery. (B) Average FRAP curve of wt cells expressing the GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP reporter incubated with FCS for 30 min. FRAP was carried out in cells in the indicated condition with averages and SD shown (n = 22 cells). The average r^2 value for single exponential fits was 0.977 (compared with 0.980 for double exponential fits). Images from a FRAP experiment before, subsequent to photobleaching, and after 20-sec recovery. (C) Average FRAP curve of wt cells expressing the GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP reporter incubated with FCS for 60 min. FRAP was carried out in cells in the indicated condition with averages and SD shown (n = 12 cells). The average r^2 value for single exponential fits was 0.954 (compared with 0.960 for double exponential fits). Images from a FRAP experiment before, subsequent to photobleaching, and after 20-sec recovery. (D) Average FRAP curve of wt cells expressing the GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP reporter incubated with FCS for 30 min and then LatA for 15 min. FRAP was carried out in cells in the indicated condition with averages and SD shown (n = 16 cells). The average r^2 value for single exponential fits was 0.979 (compared with 0.982 for double exponential fits). Images from a FRAP experiment before, subsequent to photobleaching, and after 20-sec recovery. (E) Movement of PI(4,5)P_2 between bud and mother cell compartments is impeded. FLIP (top) and FRAP (bottom) curves of wt cells expressing the GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP reporter. Either the entire mother cell or bud was photobleached and loss or recovery of fluorescence signal was followed in the same or remaining compartment (n = 12 cell buds bleached and n = 10 mother cells bleached). Fluorescence signals were normalized for acquisition-dependent photobleaching and prebleach values were set to 1. Images from bud and mother FLIP experiments before, subsequent to photobleaching, and after 20-sec recovery. Bar, 5 μm.



Video 1. **PI(4,5)P_2 asymmetry precedes bud emergence.** Wild-type *C. albicans* cells expressing the GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP PI(4,5)P_2 reporter were incubated at 30°C on a YEPD agar pad. Time-lapse confocal images using a laser-scanning confocal microscope (LSM 510 META; Carl Zeiss) were acquired every 5 min for 65 min. Sum projections of 8 1-µm z-sections are shown.

Table S1. **Strains, plasmids, and oligonucleotides used in this study**

Strains	Genotype	Reference
BWP17	*ura3Δ::λimm434/ura3Δ::λimm434 his1Δ::hisG/his1Δ::hisG arg4::hisG/arg4Δ::hisG*	(Wilson et al., 1999)
PY173	*ade2Δ::hisG/ade2Δ::hisG ura3Δ::λimm434/ura3Δ::λimm434 his1Δ::hisG/his1Δ::hisG arg4Δ::hisG/arg4Δ::hisG ENO1/eno1::ENO1-tetRScHAP4AD-3×HA-ADE2*	This study
PY369	Same as BWP17 with *mss4Δ::URA3/MSS4*	This study
PY399	Same as PY173 with *mss4Δ::HIS1/MSS4*	This study
PY403	Same as PY173 with *stt4Δ::HIS1/STT4*	This study
PY417	Same as PY173 with *stt4Δ::HIS1/stt4::URA3pTet$_{off}$STT4*	This study
PY425	Same as PY173 with *mss4Δ::HIS1/mss4::URA3pTet$_{off}$MSS4*	This study
PY674	Same as PY425 with *RP10::ARG4-pACT1MSS4*	This study
PY702	Same as PY369 with *RP10::ARG4-pACT1mss4[S527P]*	This study
PY739	Same as PY369 with *RP10::ARG4-pACT1MSS4*	This study
PY859	Same as PY417 with *RP10::ARG4-pSTT4STT4*	This study
PY881	Same as PY417 with *URA3::ARG4::arg4::hisG*	This study
PY885	Same as PY425 with *URA3::ARG4::arg4::hisG*	This study
PY977	Same as PY173 with *URA3::ARG4::arg4::hisG*	This study
PY1206	Same BWP17 with *RP10::ARG4-pADH1GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP*	This study
PY1218	Same as PY425 with *RP10::ARG4-pADH1GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP*	This study
PY1480	Same as BWP17 with *mss4Δ::URA3/mss4Δ::HIS1 RP10::ARG4-pACT1mss4[S527P]*	This study
PY1482	Same as BWP17 with *mss4Δ::URA3/mss4Δ::HIS1 RP10::ARG4-pActMSS4*	This study
PY1531	Same as PY425 with *RP10::ARG4-pADH1GFPCDC42*	This study
PY1575	Same as BWP17 with *RP10::pMET3-GFPutr-KAR9*	This study
SE32	*she3Δ::HIS1/she3Δ::LEU2 leu2Δ/leu2Δ his1Δ/his1Δ*	(Elson et al., 2009)
SN152	*arg4Δ/arg4Δ his1Δ/his1Δ URA3/ura3Δ::imm434 IRO1/iro1Δ::imm434*	(Noble and Johnson, 2005)
PY1717	Same as SE32 with *RP10::ARG4-pADH1GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP*	This study
PY1742	Same as PY417 with *RP10::ARG4-pADH1GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP*	This study
PY1813	Same as PY425 with *RP10::ARG4-pMSS4GFPMSS4*	This study
PY1815	Same as BWP17 with *TUB1/TUB1::TUB1GFP-URA3*	This study
PY1825	Same as SN152 with *RP10::ARG4-pADH1GFP-$PH^{Plc\delta}$-$PH^{Plc\delta}$-GFP*	This study
PY1920	Same as PY1480 with *MSS4::SAT1/mss4Δ::HIS1 RP10::ARG4-pACT1mss4[S527P]*	This study
PY1922	Same as PY1482 with *MSS4::SAT1/mss4Δ::HIS1 RP10::ARG4-pACT1MSS4*	This study
PY2145	Same as PY417 with *RP10::ARG4-pSTT4GFPSTT4*	This study
PY2211	Same as SN152 with *RP10::ARG4-pMSS4GFPMSS4*	This study
PY2268	Same as BWP17 with *RP10::ARG4-pADH1yemCh-$PH^{Plc\delta}$-$PH^{Plc\delta}$-yemCh*	This study
PY2270	Same as BWP17 with *RP10::ARG4-pMSS4GFPMSS4*	This study
PY2272	Same as SE32 with *RP10::ARG4-pMSS4GFPMSS4*	This study

Plasmids	Source
pCR2.1 TA	Invitrogen
pExpARG	(Bassilana et al., 2003)
pExpARG-pACT1GFPRac1	This study
pExp-pADH1-GFPRsrIIRac1	(Hope et al., 2008)
pExpArg-pADH-RsrIIRac	(Hope et al., 2008)
pExpArg-pDCK1DCK1	(Hope et al., 2008)
pAW6-X	(Bassilana et al., 2005)
pRS426-PH$^{Plc\delta}$-PH$^{Plc\delta}$-GFP	(Stefan et al., 2002)
pRS426-PH$^{Plc\delta}$-B/P	This study
pRS426-PH$^{Plc\delta}$-PH$^{Plc\delta}$-B/P/S/K	This study
pAW6-PH$^{Plc\delta}$-PH$^{Plc\delta}$-GFP	This study
pAW6-PH$^{Plc\delta}$-GFP	This study
pExpArg-pADH1-PH$^{Plc\delta}$-GFP	This study
pExpARG-pADH1GFP-PH$^{Plc\delta}$-PH$^{Plc\delta}$-GFP	This study
pExpARG-pADH1GFP-PH$^{Plc\delta}$-PH$^{Plc\delta}$-yemCh	This study
pExpARG-pADH1yemCh-PH$^{Plc\delta}$-PH$^{Plc\delta}$-yemCh	This study
pExpARG-pACT1MSS4	This study
pExpARG-pSTT4STT4	This study
pExpARG-pADH1GFPCDC42	(Bassilana et al., 2005)
pExpArg-pMSS4-RsrIIRac1	This study
pExpArg-pMSS4-MSS4	This study
pExpARG-pMSS4GFPMSS4	This study
pExpARG-ACT1MSS4	This study
pExpARG-ACT1mss4-f12	This study
pMSS4-SAT1Flip	This study
Clp-ADH1p-mCherry	(Kepler-Ross et al., 2008)
pExpARG-pSTT4STT4	This study
pExpARG-pSTT4GFPSTT4	This study
pGEMHIS1	(Wilson et al., 1999)
pDDB57	(Wilson et al., 2000)
pRS-ARG-URA-BN	(Davis et al., 2000)
pCAU98	(Nakayama et al., 2000)
pCAITHE5	(Nakayama et al., 2000)
pGFP-URA3	(Gerami-Nejad et al., 2001)
pMet3-GFPutr-Kar9	(Li et al., 2005)
pSFS5	(Sasse et al., 2011)

Oligonucleotides

Primer	Sequence (5′ → 3′)
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MSS4.P2	GGCTTAGTTCCGCTTCTTTTTTAATTGAGTTGTACCTTTCTTTATAAATTCAAGAAATCTATC-ACCATATTTTCCCAGTCACGACGTT
MSS4.P3	AAAGATTCTAATTCTTCTTCTCCTCCACATCCACATCCACATCCACAGTTATTGAAACAT-CCTAAAGATATATTAGACACTTAGGAATTGATTTGGATGG
MSS4.P4	TTTAGATGAATCATTATGGAAATGGTCTTGAGGTGGGTGTGGTGCTGATGATGATGAT-GGACTAATTGTAGATAACATCTAGTTTTCTGAGATAAAGCTG
MSS4.P5	ATGAGCTCGCCTGCTCAATTTCAAAC
MSS4.P6	CTCCGCGGTTAGTTCCGCTTCTTTTTTAATTGAGTTGTACC
MSS4.P7	GTCTCGAGAGGAACAAGGACATACTAG
MSS4.P8	ATGGGCCCATAAACTGGCACTGAAGG
MSS4.P9	TCTGCGGCCGCTTAGGTGTGCGGTGTGTG
MSS4.P10	TGGCATAACTCTCCCTCC
MSS4.P11	CTAGGATCCATGTTATCTACAATTAGTCCATCATC
MSS4.P12	ATGACGCGTTTAGTTCCGCTTCTTTTTTAATTGAGTTGTACC
MSS4.P13	CGTGATATTCATCTTAAATATGATTTAAAAGGGCCCACTTGGGGTAGAAATACCACC
MSS4.P14	GGTGGTATTTCTACCCCAAGTGGGCCCTTTTAAATCATATTTAAGATGAATATCACG
MSS4.P15	ATGCGGCCGCTTCATAATTGTTCTTTTTGTAGAAG
MSS4.P16	CACGGTCCGTGCATTTGATGACAGTGATGATAAAGAAAAATGTTCTTCC
MSS4.P17	GCACGGACCGTGATGTTATCTACAATTAGTCCATCATCATC
MSS4.P18	GTACGCGTCTATTAGTTCCGCTTCTTTTTTAATTGAGTTGTACC
STT4.P1	CAGAAAGAACAGAAAAGCTATGGATTATTCTGGGATTACCCGTGGCTCAATTCGTGCTG-AAGCCCTTAAGTGTGGAATTGTGAGCGGATA
STT4.P2	CTATAGATAAACATACACTATTAACGCCTCCACTAGTAAGGAATACCATTGGTAAGTCTTTGG-AATTCATTTTCCCAGTCACGACGTT
STT4.P3	AATTCCTTCTATATCTACTTTCTTTCTATTTCTTTAATTCTATTTATTTTCATTTGCTGAACTCATTAGTAA-CGGCTTTTATAGGAATTGATTTGGATGG
STT4.P4	ATTTTGAACCGTCAATTCGGCCAATTTCTTAAGGGCTTCAGCACGAATTGAGCCACGGGTAA-TCCCAGAATAATCCATCTAGTTTTCTGAGATAAAGCTG
STT4.P5	GTGGAAGTGTTCCGTCAAAC
STT4.P6	TCTGCGGCCGCCGTGGATCACAACATTGTG
STT4.P7	GTGTTCCGTCAAACTATTGCTCGAGATGTATTAGTTGGTTCATATTGTTCATTGAG
STT4.P8	CTCAATGAACAATATGAACCAACTAATACATCTCGAGCAATAGTTTGACGGAACAC
STT4.P9	AGAAAGAACAGAAAAGCTGAATTCGGACCGTGATGGATTATTCTGGGATT
STT4.P10	AATCCCAGAATAATCCATCACGGTCCGAATTCAGCTTTTCTGTTCTTTCT
PH$^{Plc\delta}$.P1	GGACCTTCAGGCCCTTCTTAAGGGCAGCCAGCTTCTG
PH$^{Plc\delta}$.P2	CAGAAGCTGGCTGCCCTTAAGAAGGGCCTGAAGGTCC
PH$^{Plc\delta}$.P3	CTTCTGAAGGGCAGCCAGCTTTTAAAGGTGAAGTCCAGCTCG
PH$^{Plc\delta}$.P4	GAGCTGGACTTCACCTTTAAAAGCTGGCTGCCCTTCAGAAG
PH$^{Plc\delta}$.P5	CATGAGGTCCCCGGAGTCGCAATTGTTCTCCATCGAGGAC
PH$^{Plc\delta}$.P6	GTCCTCGATGGAGAACAATTGCGACTCCGGGGACCTCATG
PH$^{Plc\delta}$.P7	CACCGCACAGAAGGCCTTGAGAAGTTTGCCCGAGACATC
PH$^{Plc\delta}$.P8	GATGTCTCGGGCAAACTTCTCAAGGCCTTCTGTGCGGTG
PH$^{Plc\delta}$.P9	CTCAGCACTGGGTGCAAGGCCTTCGCAAGATCATCCACCAC
PH$^{Plc\delta}$.P10	GTGGTGGATGATCTTGCGAAGGCCTTGCACCCAGTGCTGAG
PH$^{Plc\delta}$.P11	ATACAAGTCCGGACTCAGATAGATCTATGCACGGGCTCCAGGATGACCC
PH$^{Plc\delta}$.P12	GGGTCATCCTGGAGCCCGTGCATAGATCTATCTGAGTCCGGACTTGTAT
PH$^{Plc\delta}$.P13	CCATGGACCAGCGGCAGAAGTTAATTAATCGACCTCGAGGGGGG
PH$^{Plc\delta}$.P14	CCCCCCTCGAGGTCGATTAATTAACTTCTGCCGCTGGTCCATGG
PH$^{Plc\delta}$.P15	GTCCGTTTAATTAATCACGGGCTCCAGGATGACCC
PH$^{Plc\delta}$.P16	GGATGGTACCGAGCTCTTCTGCCGCTGGTCCATGG
PH$^{Plc\delta}$.P17	CAACAAACGTTATTGTCATACAACAACAACAACAAATACAAAAACAAAGATCTGAATTCGGACC-GTGAGATCTATGCACGGGCTCC
PH$^{Plc\delta}$.P18	GGAGCCCGTGCATAGATCTCACGGTCCGAATTCAGATCTTTGTTTTTGTATTTGTTGTTGTTGTTGTA-TGACAATAACGTTTGTTG
GFP.P1	CAACAAACGTTATTGTCATACAACAACAACAACAAATACAAAAACAAAGATCTGAATTCGGACC-GTGAGATCTATGCACGGGCTCC

Oligonucleotides *(Continued)*

Primer	Sequence (5′ → 3′)
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yemCh.P1	AGGAGCTCGGGTGACGGTGCTGGTTTGATGGTTTCAAAAGGTGAAGAAG
yemCh.P2	TATACGCGTTTATTTATATAATTCATCCATACCACC
yemCh.P3	ATACGGACCGTGATGGTTTCAAAAGGTGAAGAAG
yemCh.P4	CATCACGGTCCGTGCGTAGTCTGGGACATCGTATGGGTAGGATCCCGATCTCTTAATT-AATTTATATAATTCATCCATACCACC
ACT1.p	ATGTTCCCAGGTATTGCTGA
ACT1.m	ACATTTGTGGTGAACAATGG
MSS4.p	AAATCTTTTGATAAACGTGCCCTTA
MSS4.m	AATCCCTCCATCATGACCATAAA
STT4.p	GATCGTGAATGGTCCGCAAT
STT4.m	CGGTACGCAATGGTAATTTATTCAT

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