Cappell and Dohlman Supplemental Material

Table S1
Strains used in this study

Strain Name	Genotype	Source
BY4741*	*MAT**a** leu2Δ met15Δ his3-1 ura3Δ*	(1)
$TetO_7$-STT4	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-STT4*	(2)
$TetO_7$-PIK1	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1*	(2)
$TetO_7$-WT	*MAT**a** URA3::CMV-tTA*	(2)
$TetO_7$-PIK1 *kss1Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 kss1::LEU2*	This study
$TetO_7$-PIK1 *ste4Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 ste4::LEU2*	This study
$TetO_7$-PIK1 *ste5Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 ste5::LEU2*	This study
$TetO_7$-PIK1 *ste7Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 ste7::LEU2*	This study
$TetO_7$-PIK1 *ste11Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 ste11::LEU2*	This study
$TetO_7$-PIK1 *ste20Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 ste20::LEU2*	This study
$TetO_7$-PIK1 *ste50Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 ste50::LEU2*	This study
$TetO_7$-PIK1 *cla4Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 cla4::LEU2*	This study
$TetO_7$-PIK1 *ssk1Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 ssk1::LEU2*	This study
$TetO_7$-PIK1 *opy2Δ*	*MAT**a** URA3::CMV-tTA* kan^R*-*$TetO_7$*-*$TATA_{CYC1}$*-PIK1 opy2::LEU2*	This study
vps34Δ	*MAT**a** vps34*::kan^R	Invitrogen

*All strains derived from BY4741

Table S2
Plasmids used in this study

Fig	Plasmid Name	Alias	Description	Source
1	pRS315	SC-2-013	CEN LEU2 vector	(3)
5	pRS425	SC-2-006	2μM LEU2 vector	(4)
1	pRS423 FUS1-LacZ	SC-2-001	2μM HIS3 P_{FUS1}-lacZ	(5)
1	pRS315-STT4	SC-2-075	CEN LEU2 STT4	(6)
1	pRS315-PIK1	SC-2-076	CEN LEU2 PIK1	(6)
5	pRS425-STE11-4	SC-2-025	2μM LEU2 STE11-4	(6)
5	pRS315 GAL1-STE4	SC-2-024	CEN LEU2 P_{GAL1}-STE4	(7)
3	pRS313 GAL1-STE5-CTM	SC-2-035	CEN HIS3 P_{GAL1}-STE5-CTM	This Study
	pGS5-CTM	SC-2-027	CEN TRP1 P_{GAL1}-STE5-CTM	(8)
2	pRS316 STE5-GFPx3	SC-1-055	CEN URA3 GFP-STE5x3-T_{CYC1}	(9)
S2	pRS426 GFP-2xPH-PLCδ	SC-3-008	2μM URA3 GFP-2xPH-PLCδ	(10)
S2	pRS426 GFP-2xPH-FAPP	SC-3-009	2μM URA3 GFP-2xPH-FAPP	(10)
S2	pRS416 GFP-Lact-C2	SC-3-010	CEN URA3 GFP-Lact-C2	(11)

Figure S1

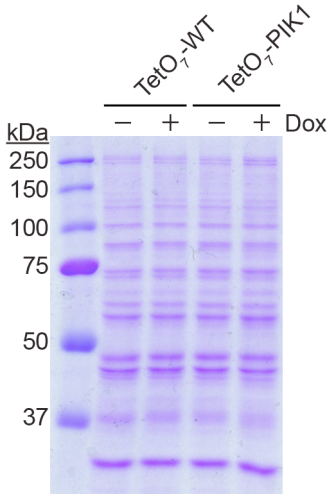

Figure S1. **Knockdown of *PIK1* does not affect global protein expression.** Whole cell lysates stained with Coomassie Brilliant Blue. Cells were treated with 10 μg/ml doxycycline (Dox) for 15 h.

Figure S2

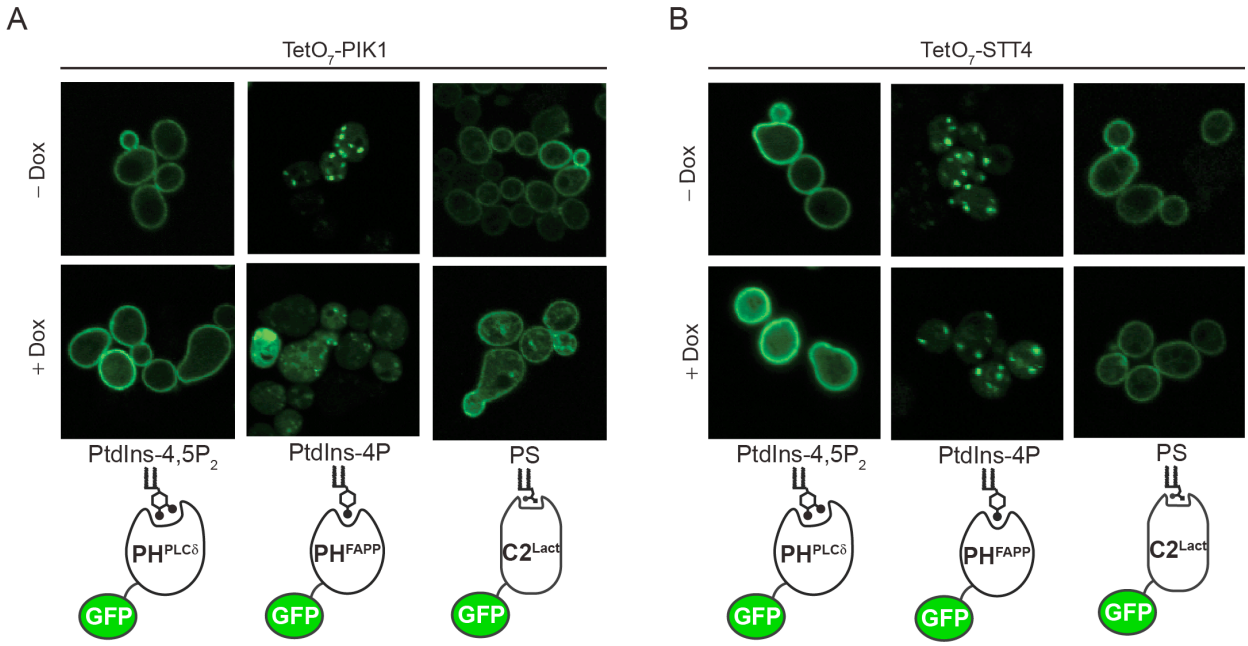

Figure S2. **Knockdown of *PIK1* or *STT4* results in loss of PtdIns 4-P *in vivo*.** GFP fluorescence of cells expressing a single copy plasmid pRS316 containing GFP fusion proteins of PH^{PLCd}, PH^{FAPP1}, or $C2^{Lact}$ and treated with 10 μg/ml doxycycline (Dox) for 15 h, as indicated. *A*, $TetO_7$-PIK1. *B*, $TetO_7$-STT4.

Figure S3

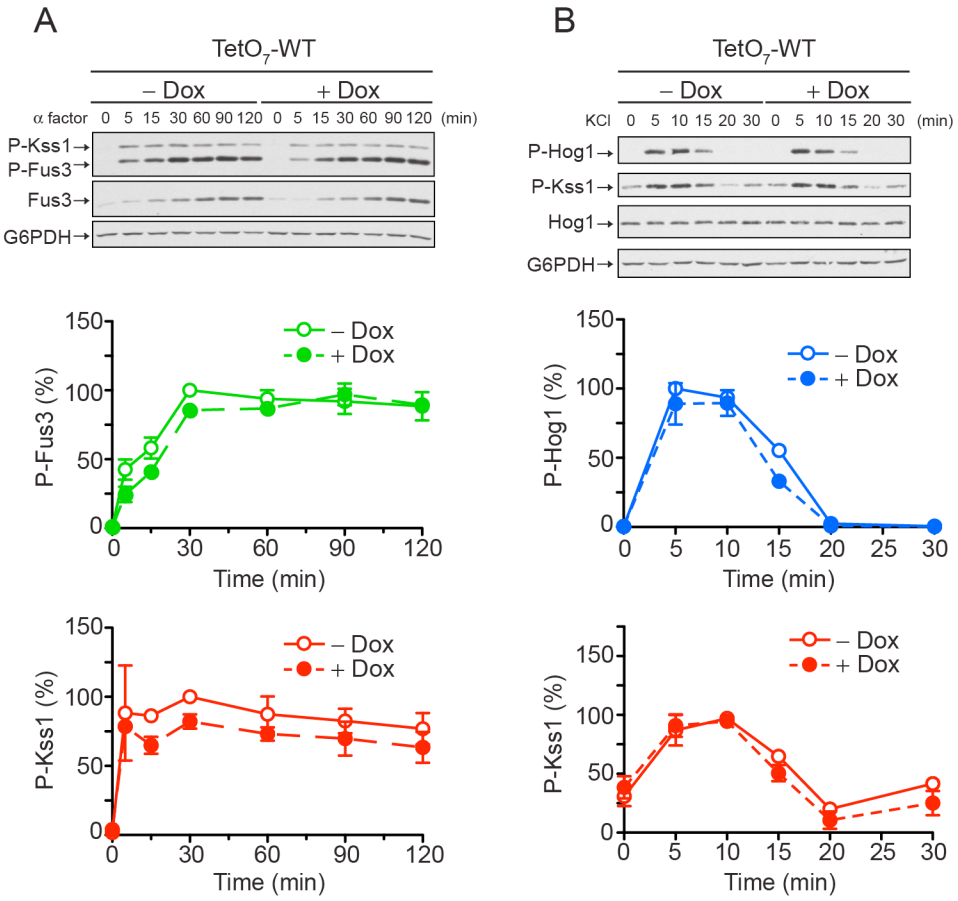

Figure S3. **Doxycycline treatment does not affect Fus3, Kss1, or Hog1 activation.** *A*, Cells harboring a doxycycline-repressible promoter attached to a non-expressible genetic element ($TetO_7$-WT) were treated with 10 μg/ml doxycycline (Dox) for 15 h and 3 μM α factor pheromone for the times indicated. Cell lysates were resolved by 12.5% SDS-PAGE and immunoblotting with phospho-p44/42 antibodies (P-Fus3, P-Kss1), Fus3 antibodies, Hog1 antibodies, or G6PDH antibodies as a loading control. Note that pheromone stimulation induces *FUS3* but not *KSS1* expression. Bands were quantified by scanning densitometry and analyzed with ImageJ software. Results are the mean ± S.E. for three individual experiments. *B*, as in *A*, but cells were treated with 0.5 M KCl instead of pheromone.

Figure S4

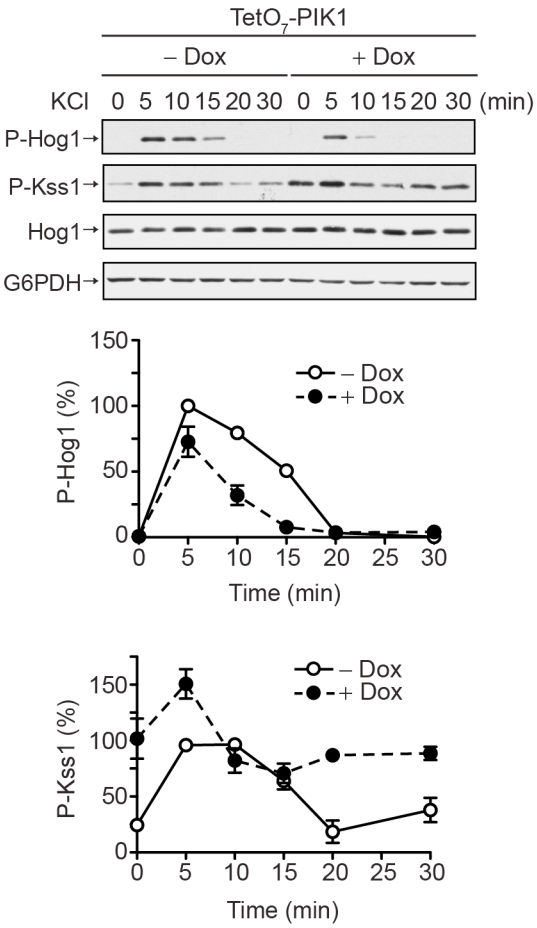

Figure S4. **Knockdown of Pik1 inhibits Hog1 activation.** $TetO_7$-PIK1 cells were treated with doxycycline for 15 h and 0.5 M KCl for the times indicated and analyzed by immunoblotting with phospho-p38 (P-Hog1) antibodies, phospho-p44/42 (P-Kss1) antibodies, Hog1 antibodies, or G6PDH antibodies as a loading control. All bands were quantified by scanning densitometry and analyzed with ImageJ software. Results are the mean ± S.E. for three individual experiments.

Figure S5

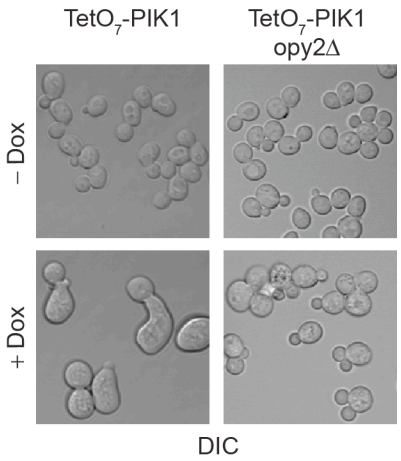

Figure S5. **Deletion of *OPY2* restores normal cell morphology upon *PIK1* knockdown.** DIC image of $TetO_7$-PIK1 and $TetO_7$-PIK1 *opy2Δ* cells treated with doxycycline for 15 h where indicated (+ Dox).

SUPPLEMENTAL REFERENCES

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