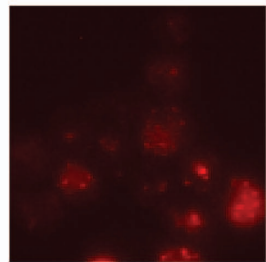
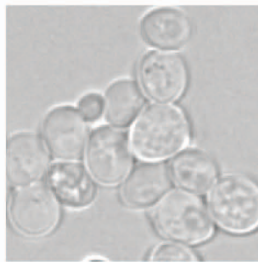
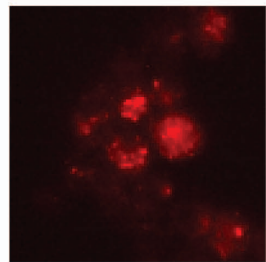
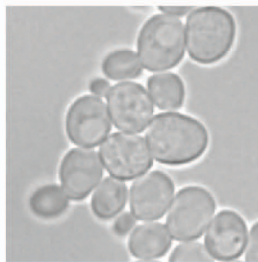


Supplementary Figure 1

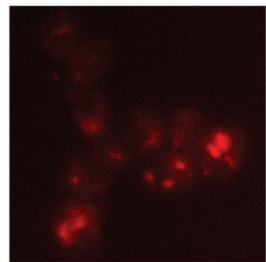
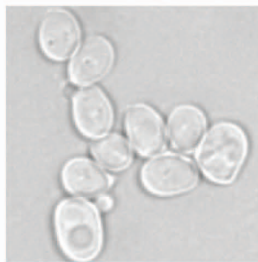
SNF7-mRFP
 $P_{MYO1}::(tetO)_7$
 $P_{CMV}-tTA$
no tetracycline



SNF7-mRFP
 $P_{MYO1}::(tetO)_7$
 $P_{CMV}-tTA$
+ tetracycline



SNF7-mRFP
 $P_{MYO1}::(tetO)_7$
+ tetracycline



DIC

RFP

Supplementary Table 1. Yeast strains used in this study

Strain	Relevant genotype	Source/Reference
SEY6210	<i>MATα leu2-3,112 ura3-52 his3Δ200 trp1-Δ901 lys2-801 suc2Δ9</i>	(Robinson, et al., 1988)
SEY6210.1	<i>MATα leu2-3,112 ura3-52 his3Δ200 trp1-Δ901 lys2-801 suc2Δ9</i>	(Babst, et al., 1997)
SEY6210di ploid ^a	<i>MATα/α leu2-3,112/leu2-3,112 ura3-52/ura3-52 his3Δ200/his3Δ200 trp1-Δ901/trp1-Δ901 lys2-801/lys2-801 suc2Δ9/suc2Δ9</i>	This study
MBY3	SEY6210 <i>vps4Δ::TRP1</i>	(Babst, et al., 1997)
MWY24	SEY6210 <i>snf7Δ::HIS3</i>	(Wemmer, et al., 2011)
GOY65	SEY6210 <i>bro1Δ::HIS3</i>	(Odorizzi, et al., 2003)
EEY2-1	SEY6210 <i>vps20Δ::HIS3</i>	(Babst, et al., 2002)
EEY6-2	SEY6210 <i>vps23Δ::HIS3</i>	(Babst, et al., 2000)
MBY30	SEY6210 <i>vps36Δ::HIS3</i>	(Babst, et al., 2002)
MBY28	SEY6210 <i>vps2Δ::HIS3</i>	(Babst, et al., 2002)
BWY102	SEY6210 <i>vps24Δ::HIS3</i>	(Babst, et al., 1998)
BY4741	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0</i>	(Brachmann, et al., 1998)
BY4742	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0</i>	(Brachmann, et al., 1998)
JTY3562	BY4742 <i>SNF7-mRFP::kanMX</i>	(Huh, et al., 2003)
JTY3986 ^b	BY4741 <i>cdc10-1-GFP::URA3</i>	This study
JTY3992	BY4741 <i>CDC10-mCherry::kanMX</i>	(McMurray and Thorner, 2008)
JTY4000	BY4741 <i>elm1Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY4001	BY4741 <i>gin4Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY4003	BY4741 <i>hsl1Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY4005	BY4741 <i>kcc4Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY4007	BY4741 <i>nap1Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY4008	BY4741 <i>siz1Δ0::kanMX</i>	(Winzeler, et al., 1999)

JTY4457	BY4742 <i>snf7Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY5595	BY4742 <i>vps23Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY5596	BY4742 <i>vps27Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY5597	BY4742 <i>vps36Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY4510 ^c	BY4742 <i>MYO1-GFP::HIS3MX</i>	This study
JTY4953 ^d	BY4742 <i>snf7Δ0::hphMX</i>	This study
JTY4957	BY4741 <i>snf7Δ0::kanMX</i>	(Winzeler, et al., 1999)
JTY4973 ^e	<i>elm1Δ0::kanMX snf7Δ0::hphMX</i>	This study
JTY4974 ^f	<i>cdc10-1-GFP::URA3 snf7Δ0::hphMX</i>	This study
JTY4981 ^g	<i>cla4Δ::LEU2 snf7Δ0::hphMX</i>	This study
YCS639 ^h	JTY3992 <i>snf7Δ::kanMX</i>	This study
YCS640 ⁱ	BY4741 <i>cdc10Δ::kanMX</i>	This study
YMVB12	BY4741 <i>cla4Δ::LEU2</i>	(Versele and Thorner, 2004)
DDY2334	<i>MATα ura3-52 his3 syp1Δ::HIS3 cdc10-1</i>	D. Drubin, Univ. Calif. Berkeley
YEF3923	<i>MATα his3 leu2 lys2 trp1 ura3 CHS2-GFP::kanMX6</i>	E. Bi, Univ. Penn Med. School
JTY5598 ^j	<i>elm1Δ0::kanMX vps23Δ0::kanMX</i>	This study
JTY5599 ^k	<i>elm1Δ0::kanMX vps27Δ0::kanMX</i>	This study
JTY5600 ^l	<i>elm1Δ0::kanMX vps36Δ0::kanMX</i>	This study
TH2808	BY4741 <i>P_{MYO1}::kanR-(tetO)₇-TATA URA3::P_{CMV}-tTA</i>	(Mnaimneh, et al., 2004)
JTY5601 ^m	<i>SNF7-mRFP::kanMX P_{MYO1}::kanR-(tetO)₇-TATA ura3Δ0</i>	This study
JTY5602 ⁿ	<i>SNF7-mRFP::kanMX P_{MYO1}::kanR-(tetO)₇-TATA URA3::P_{CMV}-tTA</i>	This study

^a Cross of SEY6210 with SEY6210.1.

^b BY4741 was transformed with a *cdc10-1-GFP::URA3* cassette made by PCR with template plasmid pCdc10-1-GFP and primers introducing sequences distal to the *URA3* marker that target it for integration at the *CDC10* locus.

^c BY4741 was transformed with a *GFP::HIS3MX* cassette made by PCR with template plasmid pFA6a-GFP(S65T)-HIS3MX using primers that target it for integration at the *MYO1* locus.

^d JTY4457 was transformed with *PvuII*- and *EcoRV*-cut plasmid pAG32.

^e Spore from cross of JTY4000 with JTY4953.

^f Spore from cross of JTY3986 with JTY4953.

^g Spore from cross of YMVB12 with JTY4953.

^h Spore from cross of JTY4457 with YCS640.

ⁱ Spore from JTY4012.

- ^j Spore from cross of JTY4000 with JTY5595.
- ^k Spore from cross of JTY4000 with JTY5596.
- ^l Spore from cross of JTY4000 with JTY5597.
- ^m Spore from cross of TH2808 with JTY3562.

Supplementary Table 2. Plasmids used in this work

Plasmid	Relevant Properties	Reference
FD44	<i>CEN URA3 CLA4</i>	(Cvrckova, et al., 1995)
pAG32	<i>hphMX</i>	(Goldstein and McCusker, 1999)
pCdc10-1-GFP ^a	<i>CEN URA3 cdc10-1-GFP</i>	This work
pFA6a-GFP(S65T)-HIS3MX	<i>GFP(S65T) HIS3MX</i>	(Longtine, et al., 1998b)
pGO216	<i>2 μm URA3 BRO1</i>	(Wemmer, et al., 2011)
pLA10	<i>CEN URA3 CDC10-GFP</i>	(Cid, et al., 1998)
pMWM3	<i>2 μm URA3 BRO1(1-183)</i>	(Wemmer, et al., 2011)
pRS416-SNF7(L121D)-FLAG	<i>CEN URA3 SNF7(L121D)-FLAG</i>	(Saksena, et al., 2009)
pRS416-CUP-ELM1	<i>CEN URA3 P_{CUP1}-ELM1</i>	Amy Trott, Thorner lab
pRS416-SNF7-FLAG	<i>CEN URA3 SNF7-FLAG</i>	(Saksena, et al., 2009)
pRS416-SNF7-GFP	<i>CEN URA3 SNF7-GFP</i>	(Teis, et al., 2008)
YEpl3-ELM1	<i>2 μm LEU2 ELM1</i>	Martin Schmidt, Univ. Pittsburg School of Medicine

^a pLA10 cut with *EcoRI* and *BamHI* was transformed into *cdc10-1* strain DDY2234.

Legend to Supplementary Figure

Supplementary Figure 1.

Single-focal-plane differential interference contrast ('DIC') or epifluorescence ('RFP') micrographs of cells of the indicated genotypes cultured overnight at 30°C in YPD medium containing, where indicated ('+ tetracycline'), 50 $\mu\text{g/ml}$ tetracycline hydrochloride, which allows the product of the *tTA* gene (expressed constitutively from the heterologous *CMV* promoter) to repress transcription from the *MYO1* promoter into which seven tandem copies of the *tetO* sequence are integrated (' $P_{MYO1}::(tetO)_7$ '). Strains were: JTY5602 ('*SNF7-mRFP P_{MYO1}::(tetO)₇ P_{CMV}-tTA*') and JTY5601 ('*SNF7-mRFP P_{MYO1}::(tetO)₇*'). The culture not exposed to tetracycline was instead exposed to the same volume of solvent (100% ethanol).

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