Supporting Information

Shen *et al.* 10.1073/pnas.0809147105

SI Text

Plasmid Construction. Previously published plasmids used in this study are shown in Table S1. Primers used are listed in Table S2. All cloned PCR products were sequence verified.

pJK890, used to delete the *PES1* ORF from the putative start to putative stop codons, was made from pJK863 by amplifying regions of homology 587 nt upstream and 403 nt downstream of the *PES1* ORF from SC5314 genomic DNA, using primers 676 and 677 (upstream), and 678 and 679 (downstream), and cloning the products into pJK863 using the KpnI/ApaI sites (upstream) and NotI/SacI sites (downstream).

pJK896 was used to replace 200 nt of the *PES1* promoter with *pMAL2* in the chromosomal *PES1* locus. The *MAL2* promoter was amplified with primers 741 and 742 from SC5314 genomic DNA and cloned into the NotI and SacII sites of pJK863. Upstream of the *PES1* locus 397 nt homology was amplified with primers 732 and 733 from SC5314 genomic DNA and cloned into the KpnI/ApaI sites of the resulting construct. The first 404 nt of the *PES1* ORF were then amplified with primers 734 and 735 from SC5314 genomic DNA and cloned into the SacII and SacI sites.

pJK945 contains the *PES1* ORF with 280 nt upstream and 245 nt downstream sequence in pBluescript SK+, amplified from SC5314 genomic DNA with primers 882 and 883, and TA cloned into the EcoRV site of the vector.

pJK954 contains the *PES1* ORF with its promoter and terminator in pRS316. A BamHI/XhoI fragment of pJK945, comprising the insert, was cloned into the same sites of pRS316.

pJK955 contains the *PES1* ORF with its promoter and terminator in pRS315. A BamHI/XhoI fragment of pJK945, comprising the insert, was cloned into the same sites of pRS315.

pJK957 contains the *PES1* ORF with its promoter and terminator in pRS425. A BamHI/XhoI fragment of pJK945, comprising the insert, was cloned into the same sites of pRS425.

pJK959 contains the *PES1* ORF with its promoter and terminator in pRS426. A BamHI/XhoI fragment of pJK945, comprising the insert, was cloned into the same sites of pRS426.

pJK1000 was used to replace 200 bp of the *PES1* promoter with *tetO* in the *PES1* locus. *tetO* was amplified from pNIM1 using primers 950 and 890. *pMAL2* was excised from pJK896 with NotI/SacII and replaced with the *tetO*-containing PCR product, cut with the same enzymes, generating pJK980. The KpnI/NcoI fragment of the *PES1* ORF, from pJK945, was cloned into the same sites of Litmus28 (New England Biolabs), generating pJK998. The KpnI fragment of pJK980 was then cloned into the KpnI site of pJK998.

pJK1026 contains the *PES1* ORF, amplified with primers 943 and 944 from pJK945 plasmid DNA, blunt cloned in the EcoRV site of pBluescript SK+.

pJK1047 contains the *PES1* ORF with a W416R amino acid substitution, and with elimination of the BsrGI site at nt 1233 of the *PES1* ORF. It was generated from pJK1026 by site-directed mutagenesis using mutagenic primers 973 and 974 from pJK1026 plasmid DNA template.

pJK1061 was used to introduce mutations encoding a W416R amino acid substitution, and loss of a native BsrGI site, into the *PES1* chromosomal locus. Downstream of the *PES1* ORF 403 nt homology were amplified from SC5314 genomic DNA using primers 901 and 937 and cloned into the KpnI/ApaI sites of pJK863 to make pJK986. The *PES1* promoter and ORF (2026 nt total) were amplified from SC5314 genomic DNA, using primers 895 and 900. This fragment was cloned into the SacII/SacI sites of pJK986. The BglII/SacII fragment of pJK1047 was then cloned into the resulting construct.

pLC52 contains a *C. albicans* codon optimized tetracycline-repressible transactivator, CaTAR, in the *FLP-CaNAT1* cassette vector. To convert a *C. albicans* codon optimized tetracycline-inducible transactivator (7) into a tetracycline-repressible transactivator we introduced five amino acid mutations into pNIM1 through site directed mutagenesis: G12S, G19E, P56A, E148D, and R179H. Four sequential rounds of site-directed mutagenesis were performed following standard protocols with the following oligos: G12S, G19E: oLC207 and oLC208; P56A: oLC209 and oLC210; E148D: oLC229 and oLC230; R179H: oLC213 and oLC214. The tetracycline-repressible transactivator under the control of the *ADH1* promoter and followed by the *ACT1* terminator (as described for pNIM1) was amplified with oLC221 and oLC222 and cloned into the ApaI site of pJK863.

pLC53 targets CaTAR of pLC52 to the *C. albicans HIS1* locus. Upstream of *HIS1* (up to and including the start codon) 416 nt of homology was amplified from SC5314 genomic DNA with oLC231 and oLC232 and was cloned into pLC52 at KpnI. Homology downstream of *HIS1* (295 nt starting at the stop codon) was amplified with oLC233 and oLC234 and cloned at SacII into pLC52 containing the upstream *HIS1* homology.

1. Fonzi WA, Irwin MY (1993) Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* 134(3):717–728.
2. Winzeler EA, *et al.* (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285(5429):901–906.
3. Kinoshita Y, *et al.* (2001) Pescadillo, a novel cell cycle regulatory protein abnormally expressed in malignant cells. *J Biol Chem* 276(9):6656–6665.
4. Adams CC, Jakovljevic J, Roman J, Harnpicharnchai P, Woolford JL, Jr. (2002) *Saccharomyces cerevisiae* nucleolar protein Nop7p is necessary for biogenesis of 60S ribosomal subunits. *RNA* 8(2):150–165.
5. Mumberg D, Muller R, Funk M (1994) Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. *Nucleic Acids Res* 22(25):5767–5768.
6. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122(1):19–27.
7. Park YN, Morschhäuser J (2005) Tetracycline-inducible gene expression and gene deletion in *Candida albicans*. *Eukaryot Cell* 4(8):1328–1342.
8. Shen J, Guo W, Köhler JR (2005) *CaNAT1*, a heterologous dominant selectable marker for transformation of *Candida albicans* and other pathogenic *Candida* species. *Infect Immun* 73(2):1239–1242.

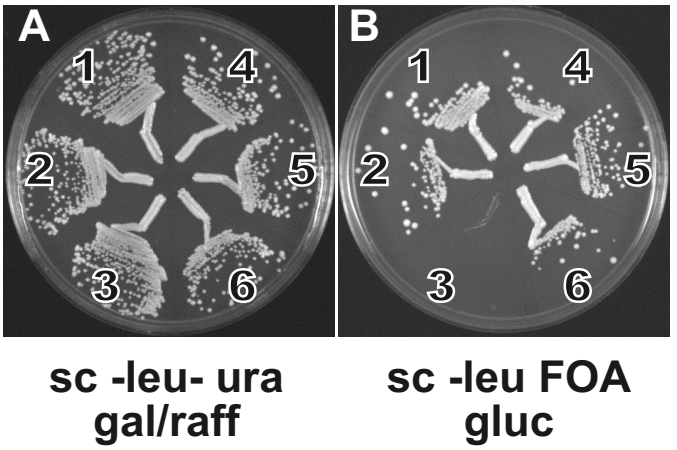


Fig. S1. *PES1* in low-copy number permits growth of *S. cerevisiae* mutants in *YPH1*. *S. cerevisiae* strains were streaked on synthetic complete (sc) medium without leucine and uracil, containing 1% galactose and 1% raffinose (*A*) and sc medium without leucine, containing uracil, 5-fluoroorotic acid and 2% glucose (*B*) and incubated at 30 °C for 4 days. (*1*) JKY1314 (MY1400, *MATa ura3*Δ *leu2*Δ *his3*Δ transformed with pRS315 and with pRS416-*pGAL1-L-CEN URA3*). (*2*) JKY1315 (MY1400 transformed with pRS315 and with pJW6004, *pGAL1-ScYPH1-CEN-URA3*). (*3*) JKY1316 (JKY1297, *MATa ura3*Δ *leu2*Δ *his3*Δ *yph1*::*kanR* transformed with pRS315 and with pJW6004). (*4*) JKY1317 (MY1400 transformed with pJK955, *PES1* in pRS315 *CEN LEU2* and pRS416-*pGAL1-L*). (*5*) JKY1318 (MY1400 transformed with pJK955 and with pJW6004). (*6*) JKY1319 (JKY1297 transformed with pJK955 and with pJW6004).

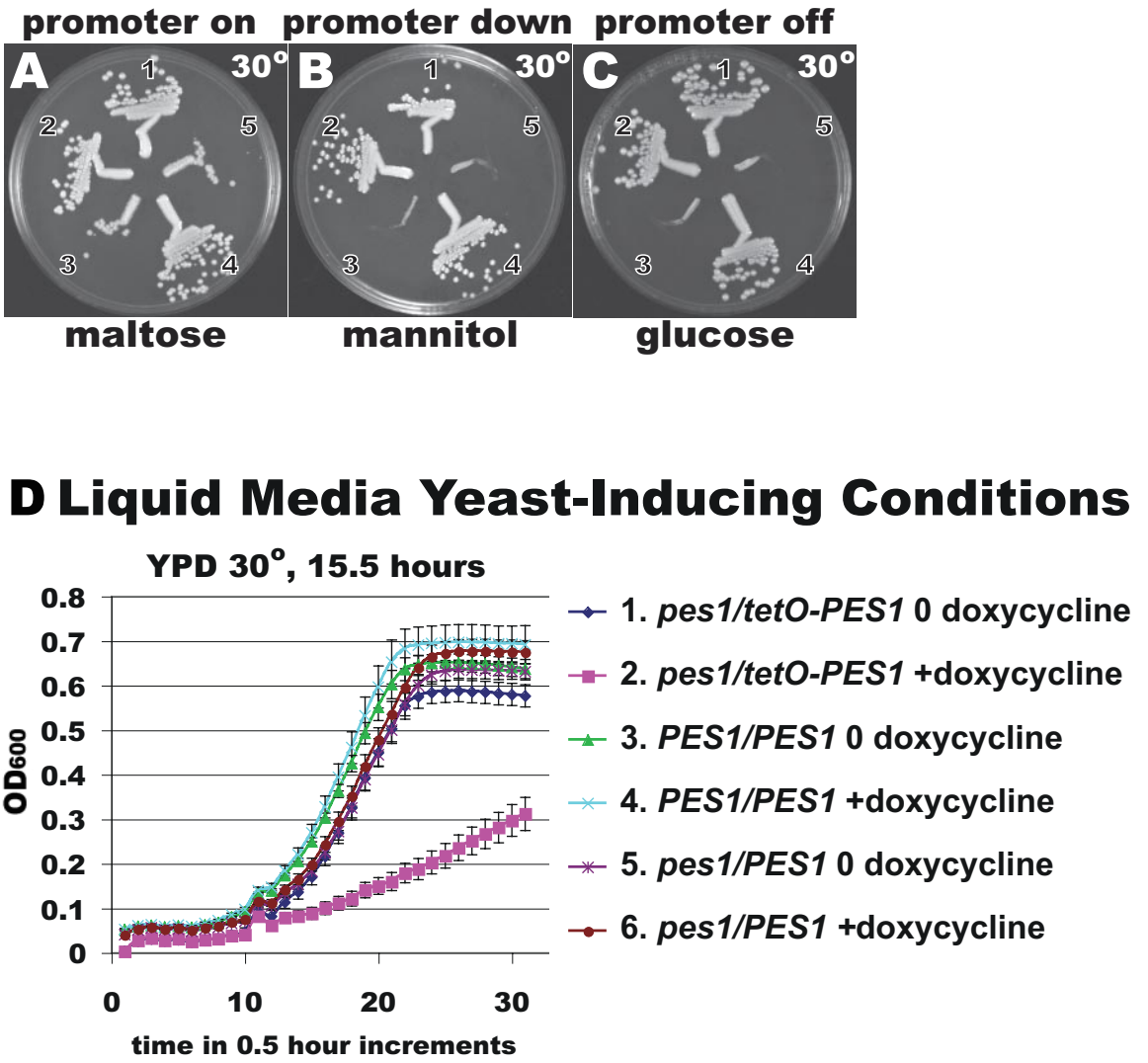
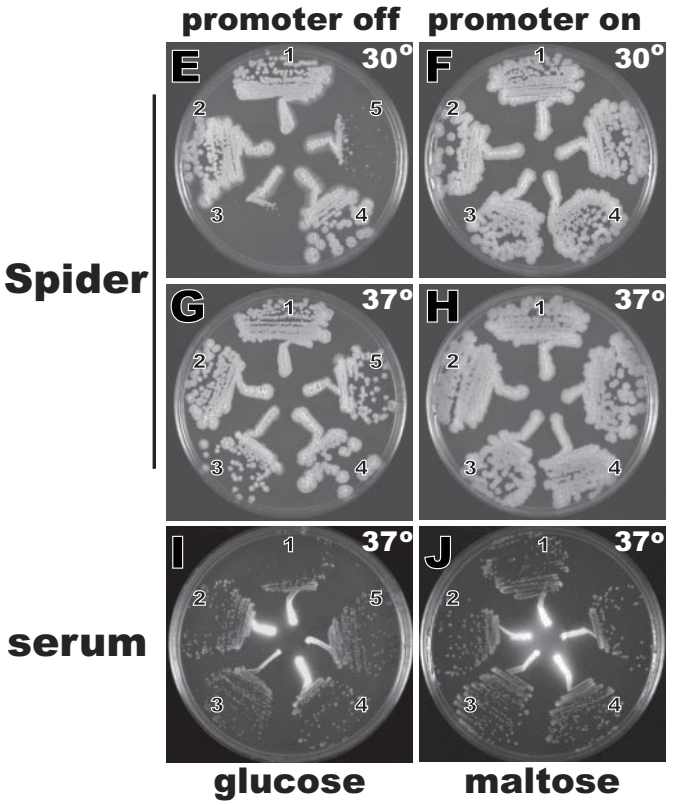


Fig. S2. *PES1*-depleted cells have severe growth defects in yeast-inducing, but not in hyphae-inducing conditions. (*A–C*) *C. albicans* strains were streaked on YP with 2% maltose (*A*), YP with 2% mannitol (*B*), and YPD (*C*) and incubated at 30 °C for 3 days. (*1*) SC5314 (*PES1/PES1*). (*2*) JKC621 (*PES1/pes1*::*FRT*). (*3*) JKC681 (*pes1*::*FRT/FRT-pMAL2-PES1*). (*4*) JKC622 (*PES1/pes1*::*FRT*). (*5*) JKC713 (*pes1*::*FRT/FRT-pMAL2-PES1*). (*D*) *C. albicans* strains in stationary phase were diluted into liquid YPD or YPD with 20 μg/ml of doxycycline to an OD_{600} of 0.05 and incubated at 30 °C for 15.5 h. OD_{600} readings were obtained every 30 min. (*1* and *2*) JKC1143 (*pes1*::*FRT/FRT-tetO-PES1 HIS3/his3*::*FRT-tetR*). (*3* and *4*) JKC915 (*PES1/PES1 HIS3/his3*::*FRT-tetR*). (*5* and *6*) JKC962 (*PES1/pes1*::*FRT HIS3/his3*::*FRT-tetR*). (*E–J*) *C. albicans* strains were streaked on Spider plates containing 2% glucose (*E* and *G*) or 2% maltose (*F* and *H*) instead of mannitol as the carbon source, and on 10% serum plates containing 2% glucose (*I*) or 2% maltose (*J*). They were incubated for 3 days at 30 °C (*E* and *F*) or at 37 °C (*G–J*). (*1*) SC5314 (*PES1/PES1*). (*2*) JKC621 (*PES1/pes1*::*FRT*). (*3*) JKC681 (*pes1*::*FRT/FRT-pMAL2-PES1*). (*4*) JKC622 (*PES1/pes1*::*FRT*). (*5*) JKC713 (*pes1*::*FRT/FRT-pMAL2-PES1*). (*K* and *L*) *C. albicans* strains in stationary phase were diluted into liquid media without or with 20 μg/ml of doxycycline to an OD_{600} of 0.05 and incubated at 37 °C for 24 h. OD_{600} readings were obtained every 30 min. (*1* and *2*) JKC1143 (*pes1*::*FRT/FRT-tetO-PES1 HIS1/his1*::*FRT-tetR*). (*3* and *4*) JKC915 (*PES1/PES1 HIS1/his1*::*FRT-tetR*). (*5* and *6*) JKC962 (*PES1/pes1*::*FRT HIS1/his1*::*FRT-tetR*). (*K*) Liquid Spider medium. (*L*) Liquid 20% serum in 2% glucose and water.

Solid Media Hyphae-Inducing Conditions



Liquid Media Hyphae-Inducing Conditions

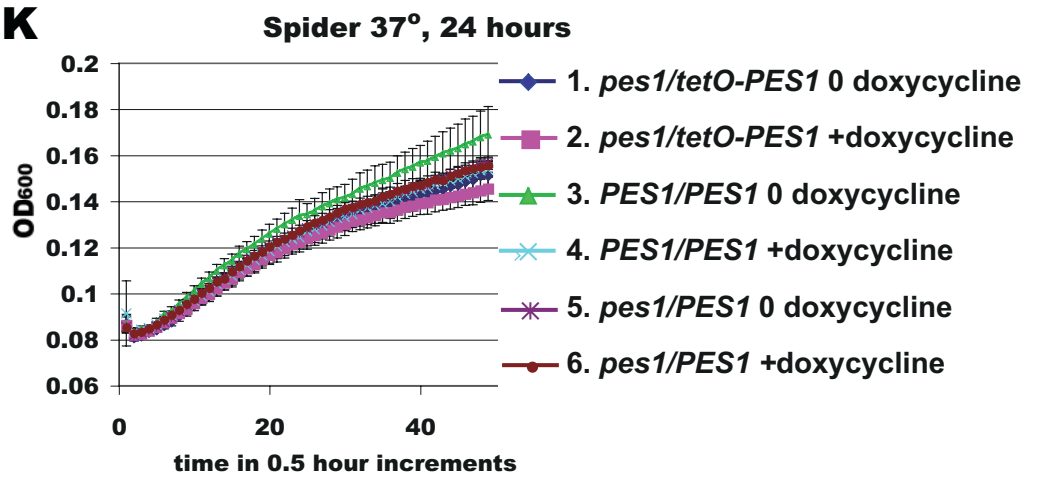


Fig. S2 continued

Liquid Media Hyphae-Inducing Conditions

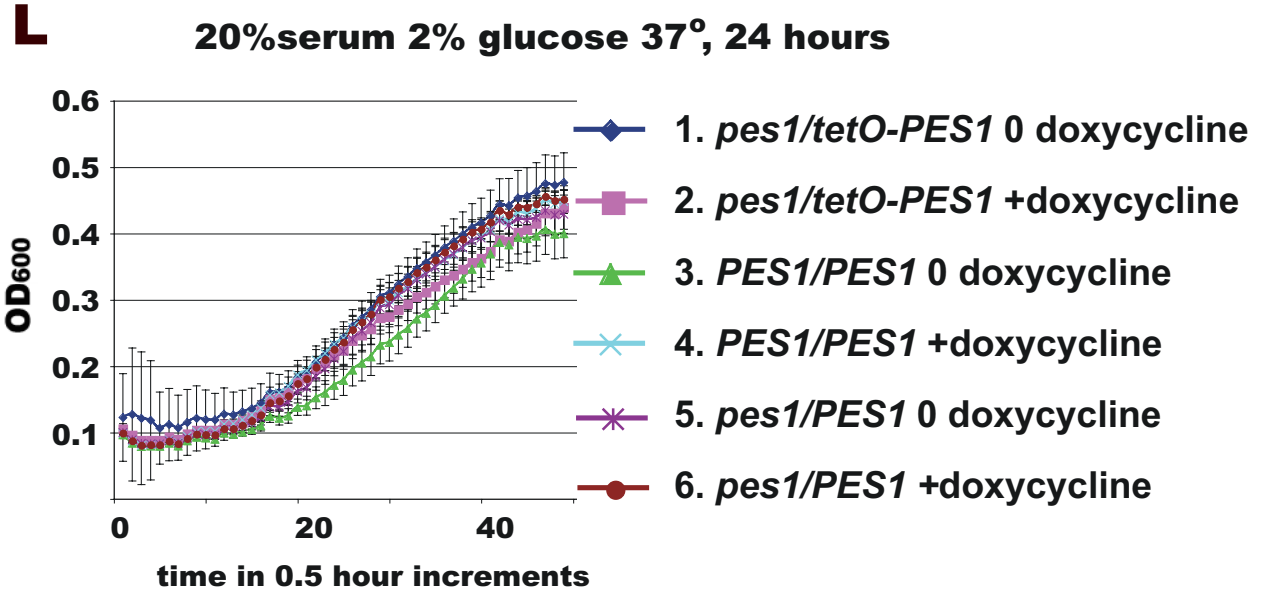


Fig. S2 continued

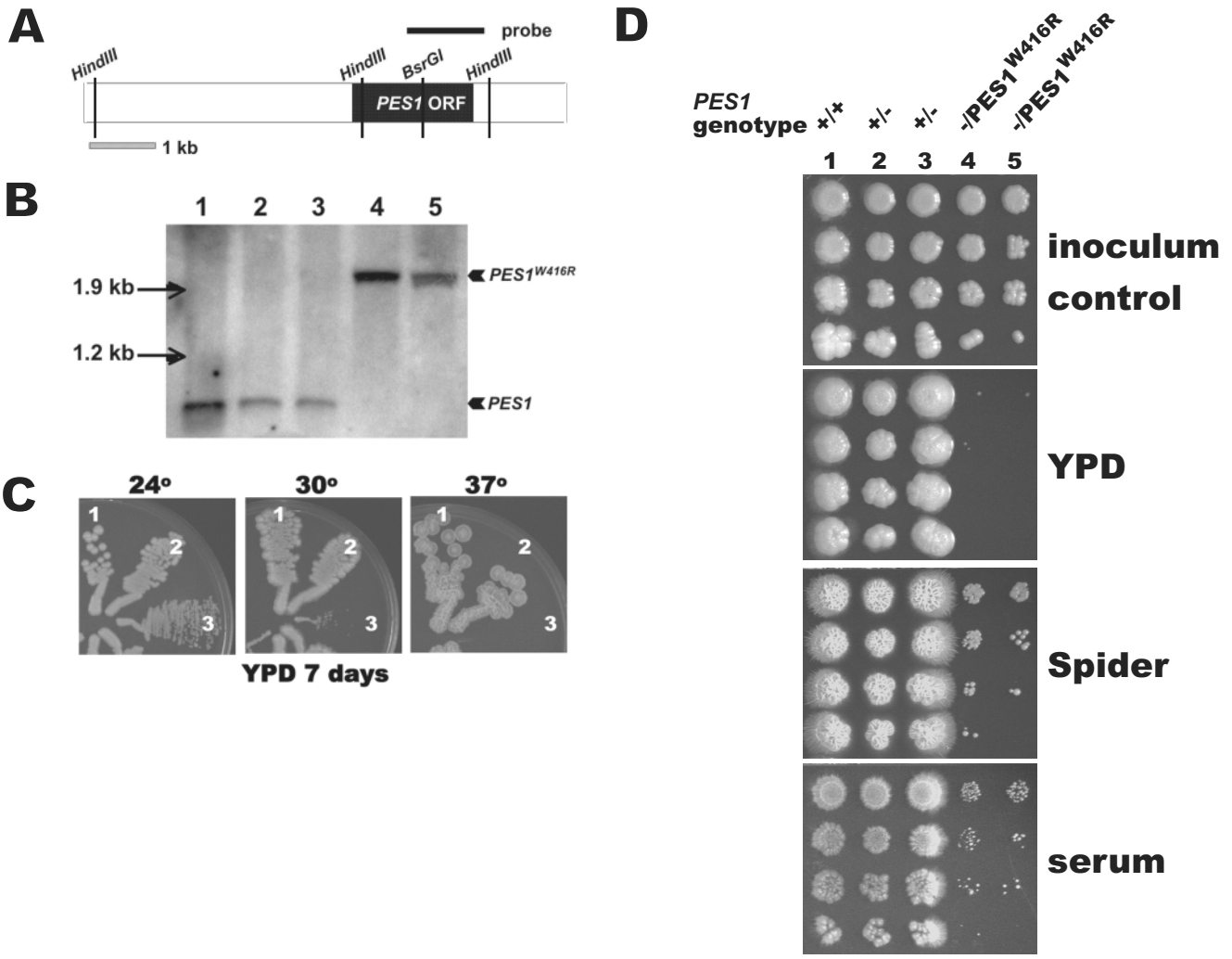


Fig. S3. Temperature-sensitive mutants in *PES1*, derived from two independent heterozygotes, die at the nonpermissive temperature in yeast-inducing, but not in hyphae-inducing conditions. (*A*) Schematic of *PES1* locus and Southern blot probe. (*B*) Genomic DNA was digested with HindIII and BsrGI, blotted, and probed with a PCR product comprising part of the *PES1* ORF and 3′-UTR. A 6-kb *pes1*::*FRT* band was cut off. The wild-type *PES1* allele is digested to a 992- and 945-bp fragment; these appear as one band. Strains are (*1*) SC5314 (*PES1*/*PES1*), (*2*) JKC621 (*PES1*/*pes1*::*FRT*), (*3*) JKC622 (*PES1*/*pes1*::*FRT*), (*4*) JKC1155 (*pes1*::*FRT*/*FRT-PES1*^{W416R}), and (*5*) JKC1160 (*pes1*::*FRT*/*FRT-PES1*^{W416R}). (*C*) Strains were streaked on YPD and plates were incubated at the indicated temperatures for 7 days: (*1*) SC5314, (*2*) JKC621, and (*3*) JKC1155. (*D*) Strains were grown overnight in YPD at 24 °C and diluted to an initial density of OD_{600} 0.1. Fivefold dilutions were spotted onto YPD (two plates), Spider, and 10% serum in water. One YPD plate was incubated at 24 °C for 8 days as an inoculum control. The others were incubated at the restrictive temperature of 37 °C for 3 days and then at the semipermissive temperature of 24 °C for 5 days. Strains are: (*1*) SC5314 (*PES1*/*PES1*), (*2*) JKC621 (*PES1*/*pes1*::*FRT*), (*3*) JKC622 (*PES1*/*pes1*::*FRT*), (*4*) JKC1155 (*pes1*::*FRT*/*FRT-PES1*^{W416R}), and (*5*) JKC1160 (*pes1*::*FRT*/*FRT-PES1*^{W416R}).

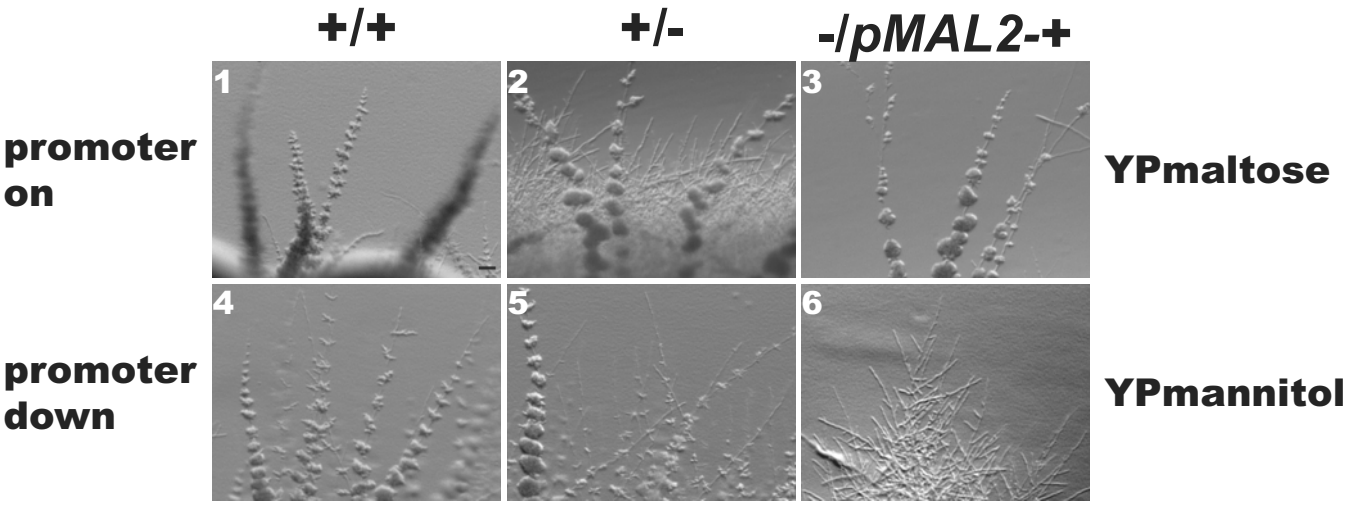


Fig. S4. Lateral yeast growth is defective when *PES1* expression is repressed from the *MAL2* promoter. Strains were streaked on YP with 2% maltose (*1*, *2*, and *3*) to induce *PES1* expression, or with 2% mannitol (*4*, *5*, and *6*) to repress *PES1*, and incubated at 30 °C for 7 days. (*1* and *4*) SC5314 (*PES1*/*PES1*). (*2* and *5*) JKC621 (*PES1*/*pes1*::*FRT*). (*3* and *6*) JKC681 (*pes1*::*FRT*/*FRT-pMAL2-PES1*). Arrowhead, cluster of yeast cells on filament. (Scale bar, 100 μm.)

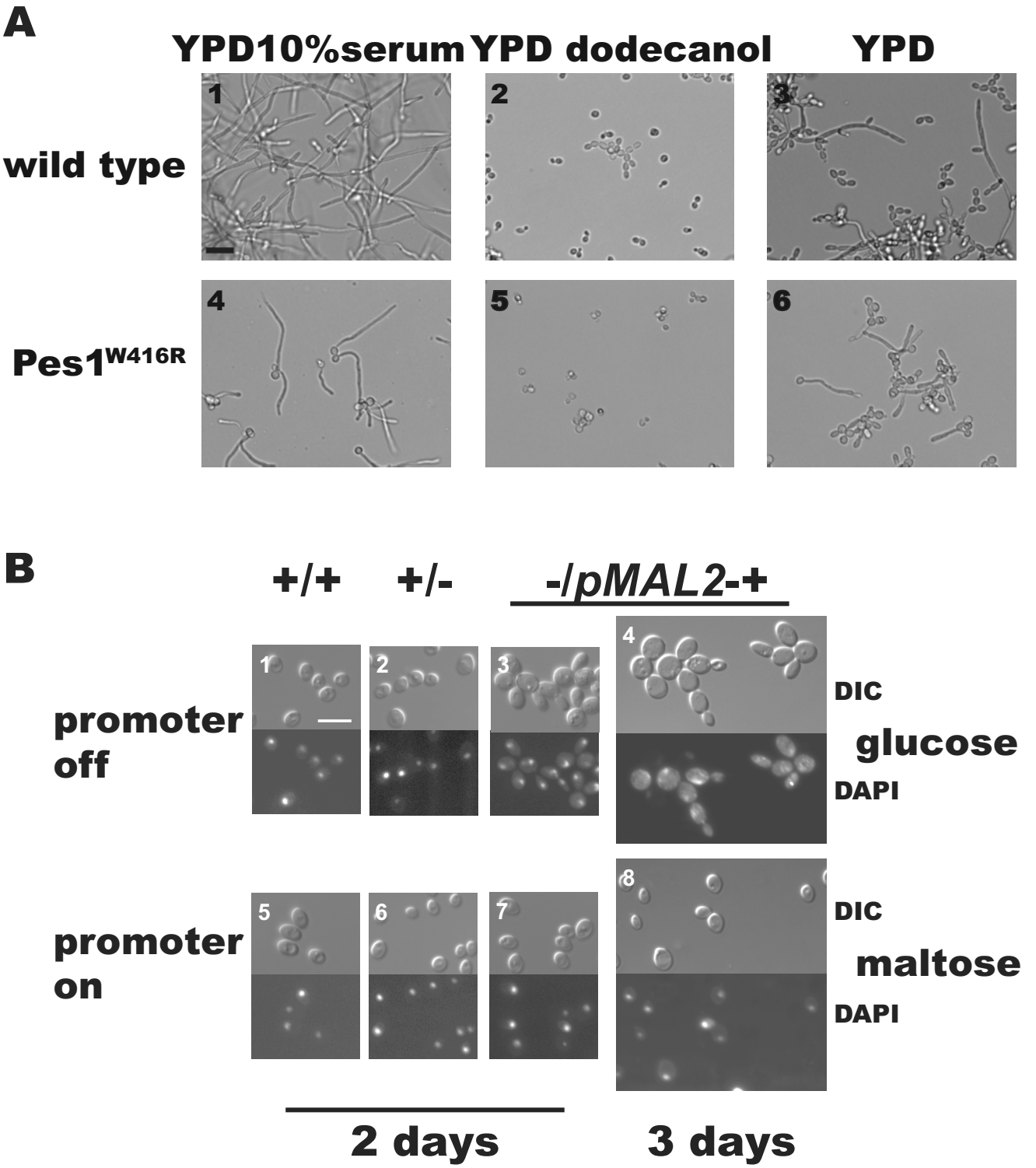


Fig. S5. Mutants in *PES1* are not trapped in a polarized growth mode and can respond to quorum-sensing molecules. (*A*) Wild type and a *pes1*Δ/*PES1*^{W416R} strain were grown overnight at 24 °C and diluted to an OD_{600} of 0.025 into 250 μl of three media: YPD with 10% serum (*1* and *4*), YPD with 200 μM dodecanol in DMSO for a DMSO concentration of 0.5% (*2* and *5*), and YPD with 0.5% DMSO (*3* and *6*). Inocula were incubated for 7 h at 37 °C in a 24-well dish and photographed. (*1*, *2*, and *3*) Wild-type SC5314. (*4*–*6*) JKC1155 (*pes1*::*FRT*/*FRT-PES1*^{W416R}). (Scale bar, 20 μm.) (*B*) Cells were grown in liquid YP with 2% glucose (*1*–*4*) or YP with 2% maltose (*5*–*8*) at 30 °C for 2 (*1*, *2*, *3*, *5*, *6*, and *7*) and 3 (*4* and *8*) days, fixed, and stained with DAPI. (*1* and *5*) SC5314 (*PES1*/*PES1*). (*2* and *6*) JKC621 (*PES1*/*pes1*::*FRT*). (*3*, *4*, *7*, and *8*) JKC681 (*pes1*::*FRT*/*FRT-pMAL2-PES1*). (Scale bar, 10 μm.)

Table S1. Strains used in this study

C. albicans strain name	Genotype	Construction	Reference
SC5314	*C. albicans* wild type		(1)
JKC619	*PES1/pes1*::*FLP-CaNAT1*	SC5314 transformed with pJK890, cut KpnI/SacI	This work
JKC620	*PES1/pes1*::*FLP-CaNAT1*	SC5314 transformed with pJK890	This work
JKC 621	*PES1/pes1*::*FRT*	Derived from C619 by inducing FLP	This work
JKC622	*PES1/pes1*::*FRT*	Derived from C620 by inducing FLP	This work
JKC673	*pes1*::*FRT/FLP-CaNAT1-pMAL2-PES1*	Derived from C621 transformed with pJK896, cut PvuII/SacI	This work
JKC665	*pes1*::*FRT/FLP-CaNAT1-pMAL2-PES1*	Derived from C622 transformed with pJK896, cut PvuII/SacI	This work
JKC681	*pes1*::*FRT/FRT-pMAL2-PES1*	Derived from C673 by inducing FLP	This work
JKC713	*pes1*::*FRT/FRT-pMAL2-PES1*	Derived from C665 by inducing FLP	This work
JKC915	*HIS1/his1*::*FRT-tetR*	SC5314 transformed with pLC53	This work
JKC935	*HIS1/his1*::*FRT-tetR PES1/pes1*::*FLP-CaNAT1*	C915 transformed with pJK890, cut KpnI/SacI	This work
JKC937	*HIS1/his1*::*FRT-tetR PES1/pes1*::*FLP-CaNAT1*	C915 transformed with pJK890, cut KpnI/SacI	This work
JKC956	*HIS1/his1*::*FRT-tetR PES1/pes1*::*FRT*	Derived from C935 by inducing FLP	This work
JKC962	*HIS1/his1*::*FRT-tetR PES1/pes1*::*FRT*	Derived from C937 by inducing FLP	This work
JKC1129	*HIS1/his1*::*FRT-tetR pes1*::*FRT/FLP-CaNAT1-tetO-PES1*	Derived from C956 by transforming with pJK1000, cut PvuII/BglII	This work
JKC1132	*HIS1/his1*::*FRT-tetR pes1*::*FRT/FLP-CaNAT1-tetO-PES1*	Derived from C962 by transforming with pJK1000, cut PvuII/BglII	This work
JKC1137	*HIS1/his1*::*FRT-tetR pes1*::*FRT/FRT-tetO-PES1*	Derived from C1129 by inducing FLP	This work
JKC1143	*HIS1/his1*::*FRT-tetR pes1*::*FRT/FRT-tetO-PES1*	Derived from C1132 by inducing FLP	This work
JKC1155	*pes1*::*FRT/FRT-pes1*^{W416R}	Derived from C681 transformed with pJK1061, cut AscI/SacI	This work
JKC1160	*pes1*::*FRT/FRT-pes1*^{W416R}	Derived from C713 transformed with pJK1061, cut AscI/SacI	This work
S. cerevisiae strain name	Genotype	Strain background/construction	Reference
Clone 24733 from Open Biosystems	*MATa/a his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 MET15/met15Δ0 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 YPH1/yph1*::*kanR*	BY4743, derived from S288C	(2)
MY1386	*MATa ura3Δ0*	Σ1278b, isogenic series ″Sigma 2000″	Gift of Todd Milne, Microbia
MY1387	*MATa ura3Δ0*	Σ1278b, isogenic series ″Sigma 2000″	Gift of Todd Milne, Microbia
MY1394	*MATa ura3Δ0 leu2Δ0*	Σ1278b, isogenic series ″Sigma 2000″	Gift of Todd Milne, Microbia
MY1397	*MATa ura3Δ0 his3Δ0*	Σ1278b, isogenic series ″Sigma 2000″	Gift of Todd Milne, Microbia
MY1400	*MATa ura3Δ0 leu2Δ0 his3Δ0*	Σ1278b, isogenic series ″Sigma 2000″	Gift of Todd Milne, Microbia
YJMF29	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1*::*HIS3 yph1–24*::*LEU2*		(3)
YJMF30	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1*::*HIS3 yph1–45*::*LEU2*		(3)
JKY573	*MATa/a ura3Δ/ura3Δ*	Σ1278b, cross of MY1386 and MY1387	This work
JKY1287	*MATa/a ura3Δ0/ura3Δ0 LEU2/leu2Δ0 HIS3/his3Δ0*	Σ1278b, cross of MY1394 and MY1397	This work
JKY1293	*MATa/a ura3Δ0/ura3Δ0 LEU2/leu2Δ0 HIS3/his3Δ0 YPH1/yph1*::*kanR*	Σ1278b, transformation of JKY1287 with a product of primers 886 and 887, using genomic DNA of clone 24733 (Open Biosystems) as template	This work
JKY1297	*MATa ura3Δ0 leu2Δ0 his3Δ0 yph1*::*kanR* < *pGAL1,10 - YPH1 URA3 CEN*>	Σ1278b, spore of JKY1295	This work
JKY1299	*MATa ura3Δ0 yph1*::*kanR* < *pGAL1,10 - YPH1 URA3 CEN*>	Σ1278b, spore of JKY1295	This work
JKY1302	*MATa ura3Δ0 leu2Δ0 his3Δ0 yph1*::*kanR* < *pGAL1,10 - YPH1 URA3 CEN*>	Σ1278b, spore of JKY1295	This work
JKY1314	*MATa ura3Δ0 leu2Δ0 his3Δ0* <*pGAL1,10L URA3 CEN*> <*LEU2 CEN*>	Σ1278b, MY1400 transformed with p416GALL and pRS315	This work
JKY1315	*MATa ura3Δ0 leu2Δ0 his3Δ0* <*pGAL1,10 - YPH1 URA3 CEN*><*LEU2 CEN*>	Σ1278b, MY1400 transformed with pJW6004 and pRS315	This work
JKY1316	*MATa ura3Δ0 leu2Δ0 his3Δ0 yph1*::*kanR* < *pGAL1,10 - YPH1 URA3 CEN*> <*LEU2 CEN*>	Σ1278b, JKY1297 transformed with pJW6004 and pRS315	This work

C. albicans strain name	Genotype	Construction	Reference
JKY1317	*MATa ura3Δ0 leu2Δ0 his3Δ0 < pGAL1,10 - YPH1 URA3 CEN> <PES1 LEU2 CEN>*	Σ1278b, MY1400 transformed with p416GALL and pJK955	This work
JKY1318	*MATa ura3Δ0 leu2Δ0 his3Δ0 < pGAL1,10 - YPH1 URA3 CEN> <PES1 LEU2 CEN>*	Σ1278b, MY1400 transformed with pJW6004 and pJK955	This work
JKY1319	*MATa ura3Δ0 leu2Δ0 his3Δ0 yph1::kanR < pGAL1,10 - YPH1 URA3 CEN> <PES1 LEU2 CEN>*	Σ1278b, JKY1297 transformed with pJK955	This work
JKY1320	*MATa ura3Δ0 leu2Δ0 his3Δ0 < pGAL1,10L URA3 CEN> <LEU2 2 μ>*	Σ1278b, MY1400 transformed with p416GALL and pRS425	This work
JKY1321	*MATa ura3Δ0 leu2Δ0 his3Δ0 < pGAL1,10 - YPH1 URA3 CEN> <LEU2 2 μ>*	Σ1278b, MY1400 transformed with pJW6004 and pRS425	This work
JKY1322	*MATa ura3Δ0 leu2Δ0 his3Δ0 yph1::kanR < pGAL1,10 - YPH1 URA3 CEN> <LEU2 2 μ>*	Σ1278b, JKY1297 transformed with pRS425	This work
JKY1323	*MATa ura3Δ0 leu2Δ0 his3Δ0 < pGAL1,10L URA3 CEN> <PES1 LEU2 2 μ>*	Σ1278b, MY1400 transformed with p416GALL and pJK957	This work
JKY1324	*MATa ura3Δ0 leu2Δ0 his3Δ0 < pGAL1,10 - YPH1 URA3 CEN> <PES1 LEU2 2 μ>*	Σ1278b, MY1400 transformed with pJW6004 and pJK957	This work
JKY1325	*MATa ura3Δ0 leu2Δ0 his3Δ0 yph1::kanR < pGAL1,10 - YPH1 URA3 CEN> <PES1 LEU2 2 μ>*	Σ1278b, JKY1297 transformed with pJW6004 and pJK957	This work
JKY1340	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1::HIS3 yph1–24::LEU2 < URA3 CEN>*	YJMF29 transformed with pRS316	This work
JKY1342	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1::HIS3 yph1–24::LEU2 < PES1 URA3 CEN>*	YJMF29 transformed with pJK954	This work
JKY1344	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1::HIS3 yph1–24::LEU2 < URA3 2 μ>*	YJMF29 transformed with pRS426	This work
JKY1346	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1::HIS3 yph1–24::LEU2 < PES1 URA3 2 μ>*	YJMF29 transformed with pJK959	This work
JKY1348	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1::HIS3 yph1–45::LEU2 < URA3 CEN>*	YJMF30 transformed with pRS316	This work
JKY1350	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1::HIS3 yph1–45::LEU2 < PES1 URA3 CEN>*	YJMF30 transformed with pJK954	This work
JKY1352	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1::HIS3 yph1–45::LEU2 < URA3 2 μ>*	YJMF30 transformed with pRS426	This work
JKY1354	*MATa ura3–52 lys2–801 ade2–101 his3-Δ200 trp1-Δ63 leu2-Δ1 yph1::HIS3 yph1–45::LEU2 < PES1 URA3 2 μ>*	YJMF30 transformed with pJK959	This work

Plasmid name	Insert, markers	Reference
pJW6004	*YPH1* controlled by *pGAL1,10, CYC1* terminator, *CEN, URA3, ampR*	(4)
p416GAL-L	*pGAL1,10* with 2/3 UAS, *CYC1* terminator, *CEN, URA3,ampR*	(5)
pRS315	*LEU2, CEN, ampR*	(6)
pRS425	*LEU2,2 μ, ampR*	(6)
pRS316	*URA3, CEN, ampR*	(6)
pRS426	*URA3, 2 μ, ampR*	(6)
pNIM1	*tetR, tetO, GFP, SAT1,* ampR	(7)
pJK863	*FLP-CaNAT1,* ampR	(8)

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Table S2. Primers used in this study

Primer name	Purpose	Sequence 5′ to 3′	Comment
676	Reverse to amplify *PES1* deletion upstream homology	gcgcat**gggccc**gatggagtctgaacaaagtg	Restriction site in boldface
677	Forward to amplify *PES1* deletion upstream homology	gcatgc**ggtacc**gctgctcatccaattaatca	Restriction site in boldface
678	Forward to amplify *PES1* deletion downstream homology	cgtgatta**gcggccgc**atcggaaatgtgaatagcag	Restriction site in boldface
679	Reverse to amplify *PES1* deletion downstream homology	cagtc**gagctc**tcacaatctctggcgacacc	Restriction site in boldface
732	Forward to amplify *PES1* promoter replacement upstream homology	cgtata**ggtacc**ggcgcgccgctcatccaattaatcaactc	Restriction site in boldface
733	Reverse to amplify *PES1* promoter replacement upstream homology	cgagatgggccc**ggcgcgcc**gaatcaactgtctatccagc	Restriction site in boldface
734	Forward to amplify *PES1* promoter replacement downstream homology	ctagat**ccgcggttaattaa**cataaaatggccagaataaag	Restriction site in boldface
735	Reverse to amplify *PES1* promoter replacement downstream homology	gacgat**gagctcttaattaa**caagtcacgcaatgcgtcagc	Restriction site in boldface
741	Forward to amplify *pMAL2*	gtcgca**gcggccgc**gattgatatttttgtctagtacc	Restriction site in boldface
742	Reverse to amplify *pMAL2*	gtcgca**gcggccgc**gattgatatttttgtctagtacc	Restriction site in boldface
847	Reverse to amplify *PES1* Southern probe	ggttgagatgatccagtaggc	
871	Reverse to amplify *GSP1* Northern probe	gcatcatcttcatcaggcaatgg	
882	Forward to amplify the genomic locus of *PES1*	cgtgca**gcggccgc**taagcagcaagaacccaagtg	Restriction site in boldface
883	Reverse to amplify the genomic locus of *PES1*	gtcacg**ccgcgg**gaccttagtatggatttcatg	Restriction site in boldface
886	Forward to amplify *yph1*::*kanR* allele from *S. cerevisiae* deletion collection heterozygote	cgttctagcactatgggtaag	
887	Reverse to amplify *yph1*::*kanR* allele from *S. cerevisiae* deletion collection heterozygote	ggactcgagggtagcacatgg	
890	Reverse to amplify *tetO*	gcactg**gcggccgc**ttggttcagcaccttgtcgag	Restriction site in boldface
895	Forward to amplify *PES1* promoter and *ORF*	gtcgct**gagctc**caagtgaccaattataatga	Restriction site in boldface
900	Reverse to amplify PES1 ORF and His-FLAG tag	gctgca**ccgcgg**ttatttatcatcatcatctttataatcaccaccat gatgatgatgatgatgcacgtgattcaatttttttcaattgttc	Restriction sites in boldface
901	Forward to amplify PES1 downstream homology	cgtcga**gggccc**atcggaaatgtgaatagcag	Restriction site in boldface
909	Forward to amplify *PES1* Southern probe	gtagaagatattgaattggac	
937	Reverse to amplify *PES1* downstream homology	gc**ggtaccggcgcgcc**tcacaatctctggcgacacc	Restriction site in boldface
943	Reverse to amplify *PES1* ORF	caattgaaaaaattgaattgag**gtcgac**gc	Restriction site in boldface
944	Forward to amplify *PES1* ORF	gcg**ggatcc**ctatggccagaataaagaagaggg	Restriction site in boldface
950	Forward to amplify *tetO*	gtg**ccgcgg**gtcgactatttatatttgtatgtgtgtagg	Restriction site in boldface
973	Forward to introduce a W416R aa substitution, and to abolish the BsrGI site at nt 1233 of the *PES1* ORF	cccggaagaacttatgtt**c**aaccacag**aga**gtttttgact ccatcaac	Introduced mutations in boldface and underlined
974	Reverse to introduce a W416R aa substitution, and to abolish the BsrGI site at nt 1233 of the *PES1* ORF	gttgatggagtcaaaaac**tct**ctgtggttg**a**acataagttct tccggg	Introduced mutations in boldface and underlined
998	Forward to amplify *GSP1*Northern probe	ggctcaagaagttcctacattc	
oLC207	Forward to introduce G12S, G19E aa substitutions in CaTAR	gataaaagtaaagtgattaac**a**gcgcattagagttgcttaatg**a** ggtcggaatcgaaggtttaac	Introduced mutations in boldface and underlined
oLC208	Reverse to introduce G12S, G19E aa substitutions in CaTAR	gttaaaccttcgattccgacc**t**cattaagcaactctaatgcgc**t** gttaatcactttacttttatc	Introduced mutations in boldface and underlined
oLC209	Forward to introduce P56A aa substitution in CaTAR	cgggctttgctcgacgcctta**g**cc**a**ttgagatgttagataggcac	Introduced mutations in boldface and underlined
oLC210	Reverse to introduce P56A aa substitution in CaTAR	gtgcctatctaacatctcaat**gg**c**t**aaggcgtcgagcaaagcccg	Introduced mutations in boldface and underlined
oLC213	Forward to introduce R179H aa substitution in CaTAR	caagctatcgaattatttgat**cac**caaggtgcagagccagccttc	Introduced mutations in boldface and underlined
oLC214	Reverse to introduce R179H aa substitution in CaTAR	gaaggctggctctgcaccttg**gtg**atcaaataattcgatagcttg	Introduced mutations in boldface and underlined

Primer name	Purpose	Sequence 5′ to 3′	Comment
oLC221	Forward to amplify mutagenized CaTAR from pNIM1	ttgc**gggccc**ttgagatggagccgtcaaatatcc	Restriction site in boldface
oLC222	Reverse to amplify mutagenized CaTAR from pNIM1	accg**gggccc**gacattttatgatggaatgaatgg	Restriction site in boldface
oLC229	Forward to introduce E148D aa substitution in CaTAR	ggttgcgtattggaaga**t**caagagcatcaagtcgctaaagaa gaaaggg	Introduced mutations in boldface and underlined
oLC230	Reverse to introduce E148D aa substitution in CaTAR	ccctttcttctttagcgacttgatgctcttg**a**tcttccaatacgcaacc	Introduced mutations in boldface and underlined
oLC231	Forward to amplify *HIS1* upstream homology	cgg**ggtacc**caatttggggacagaagagg	Restriction site in boldface
oLC232	Reverse to amplify *HIS1* upstream homology	cgg**ggtacc**cattatcggtagttggtgg	Restriction site in boldface
oLC233	Forward to amplify *HIS1* downstream homology	tcc**ccgcgg**taaaagaagtgatagtttctc	Restriction site in boldface
oLC234	Reverse to amplify *HIS1* downstream homology	tcc**ccgcgg**gaattggatagattatttggg	Restriction site in boldface