Supporting Information

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SI Materials and Methods

Generation of Strain Cdc24^{pb1}. The ORF encoding Cdc24, the guanine exchange factor for the small GTPase, Cdc42, and 200-bp of its downstream sequence was amplified by PCR from clinical isolate SC5314 using primers CDC24-Amp-ORF-Not1-F2 and CDC24-Amp-ORF-Xba1-R2 (Table S2). The 500-bp region downstream of the Cdc24 stop codon was amplified using primers CDC24-Amp-Term-Sal1-F and CDC24-Amp-term-Kpn1-R. The PCR products were subcloned into TOPO plasmids (Invitrogen) to generate ABp26 and ABp33 (Table S3). The plasmids were digested with NotI/XbaI or SalI/KpnI and the Cdc24 upstream and downstream regions, respectively, were sequentially ligated either side of the *URA3* gene encoding Orotidine-5′-phosphate decarboxylase as a selectable marker, in plasmid pBS-*URA3*, a gift from Carol Munro (University of Aberdeen, Aberdeen, UK), to generate plasmid ABp63, then ABp71.Two rounds of site-directed mutagenesis were carried out to introduce point mutations D802A, D806A, and D813A into the PB1 C-terminal domain of Cdc24 and generate plasmid ABp99. Correct insertion was confirmed by PCR and by sequencing with primers URA3-R2, URA3-R3, URA3-OK-F2, Cdc24-Seq-Up-F, and Cdc24-Seq-Down-F. The CDC24-200t-*URA3*-500t construct was excised from ABp99 by digestion with Not1/Kpn1 and transformed into *Candida albicans* THE1 (1), to replace one *CDC24* allele and generate strain AB299. The Ura-minus strain, AB310, was generated by 5-FOA plating to select for loss of *URA3*. The tetracycline-regulatable promoter (Tet-Off) was PCR-amplified from plasmid CAU1-99 (2) using primers CDC24-Long-Tet-F and CDC24-Long-Tet-R and transformed into strain AB310 to generate strain AB320. PCR analysis, Southern analysis (primers Cdc24-Seq-Up-F and Cdc24-Probe-R), and sequencing were used to select for and confirm insertion of the Tet promoter in front of the wild-type *CDC24* allele and insertion of the mutated allele behind the wild-type promoter in strain Cdc24^{pb1}.

Cell-Wall Composition Analysis. Cell walls were extracted as described previously (3). The hydrolyzed samples were analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection in a carbohydrate analyzer (Dionex) as described previously (4). The total concentration of each cell wall component was expressed as micrograms per milligram of dried cell wall, determined by calibration from the standard curves of glucosamine, glucose, and mannose monomers and converted to a percentage of the total cell wall.

Microscopy. Galvanotropism and thigmotropism bright field images were captured using an Infinity 1 camera fitted to an Olympus BX50 light microscope. Membranes were stained with the lipophilic dye FM4-64 (Molecular Probes) for 40 min, followed by a 40-min chase in the absence of FM4-64 (5). Cell walls were stained with100 μg/mL Calcofluor White. Fluorescence images were captured on a DeltaVision RT microscope (Applied Precision Instruments) equipped with standard filter RD-TR-PE (excitation, 555 nm; emission, 617 nm) and a CoolSNAP HQ camera (Photometrics). Data were recorded using the softWoRx version 3.5.0 software (Applied Precision Instruments). For transmission electron microscopy imaging, samples were subjected to high-pressure freezing with a Leica EM PACT2 (Leica Microsystems) followed by freeze-substitution in substitution reagent (1% OsO_4/0.1% uranyl acetate in acetone) with a Leica EM AFS2. Samples were encapsulated in 3% (wt/vol) low-melting-point agarose before progressing to Spurr resin. Additional infiltration was provided under vacuum at 60 °C before embedding in TAAB capsules and polymerizing at 60 °C for 48 h. Ultrathin sections (60 nm) were prepared with a Diatome diamond knife on a Leica UC6 ultramicrotome and stained with uranyl acetate and lead citrate for examination with a Philips CM10 transmission microscope (FEI U.K. Ltd.) and imaging with a GatanBioscan 792 (Gatan U.K.).

Hyphal Extension in an Electric Field. Yeast cells were adhered to poly-L-lysine–coated microscope slides and incubated in modified Soll's medium (MSM) at pH 7.5 in a Biorad midi-sub cell electrophoresis tank at a temperature of 37 °C ± 1 °C to induce hyphae, as described previously (6). Hyphal length was measured using Improvision Openlab 2.0 software after incubation for 2 h with no electric field, followed by 3 h in an applied electric field of 10 V/cm and a current of 33 ± 2 mA. The mean length ± SD was determined from >100 measurements in a minimum of three independent experiments.

Quantitation of Wild-Type *CDC24* mRNA by Quantitative RT-PCR. PCR primers specific for the wild-type *CDC24* allele, the *CDC24*^{pb1} allele, and the housekeeping gene *ACT1* were designed using the Universal ProbeLibrary Assay Design Center (Roche) (Table S2). Strain Cdc24^{pb1} was grown in yeast-extract peptone dextrose (YPD, 1% wt/vol), 2% (wt/vol) mycological peptone, and 2% (wt/vol) glucose overnight at 30 °C with shaking at 200 rpm, diluted to OD_{600nm} = 0.075 in YPD, and grown in 0, 0.125, or 20 μg/mL doxycycline for 4 h at 30 °C with shaking. Total RNA was isolated using TRIzol reagent (Invitrogen). RNA concentration was quantified using a NanoDrop spectrophotometer (NanoDrop Technologies Inc.). Four micrograms of total RNA treated with DNase I (Invitrogen) was reverse-transcribed using SuperScript II Reverse Transcriptase (Invitrogen) with Oligo(dT) 12–18 primer (Promega). cDNA samples were checked for DNA contamination by PCR using an EFB1 primer pair that spans an intron. Real-time quantitative PCR was performed in 384-well plates using an LC480-II light cycler (F. Hoffmann–La Roche). PCR was carried out in duplicate using a LightCycler 480 Probes Mastermix (Roche): 95 °C for 10 min followed by 45 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 1 min. A melting curve analysis was performed to test for nonspecific PCR products. The efficiencies of qPCR reactions for samples were similar to the standard curve, allowing for calculation of gene expression levels normalized to the geometric mean of the results from *ACT1*.

1. Nakayama H, et al. (2000) Tetracycline-regulatable system to tightly control gene expression in the pathogenic fungus *Candida albicans*. *Infect Immun* 68(12):6712–6719.
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6. Brand A, et al. (2007) Hyphal orientation of *Candida albicans* is regulated by a calcium-dependent mechanism. *Curr Biol* 17(4):347–352.

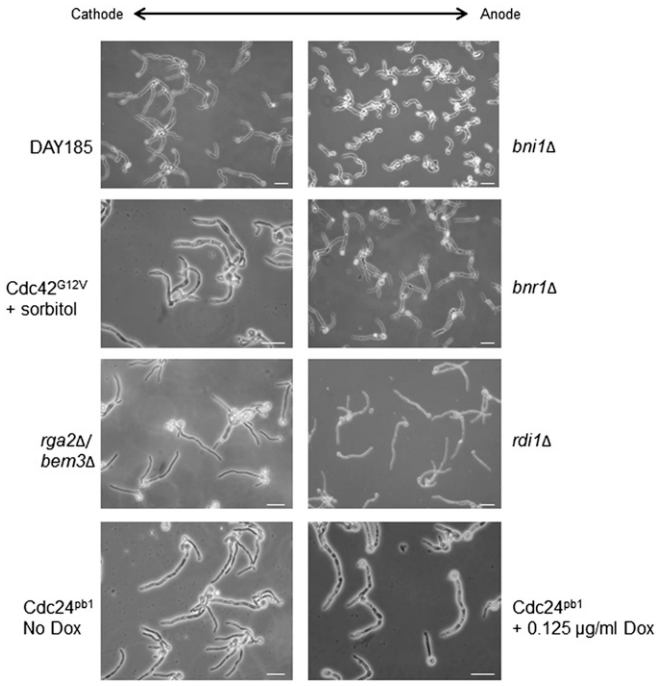

Fig. S1. Final reorientation angles of *C. albicans* hyphae in an applied dc electric field. Yeast cells were adhered to poly-L-lysine slides for 30 min. Slides were placed in a Biorad midi-sub cell electrophoresis tank and hyphal growth was induced by incubation at 37 °C in MSM, as described previously (1). For *CDC42* mutant strains, MSM was supplemented with 2% sorbitol to repress expression of the heterologous copy of *CDC42*. For strain Cdc24^{pb1}, cells were grown in 0.125 μg/mL doxycycline to repress the wild-type copy of *CDC24*. (Scale bars, 20 μm.)

1. Brand A, et al. (2007) Hyphal orientation of *Candida albicans* is regulated by a calcium-dependent mechanism. *Curr Biol* 17(4):347–352.

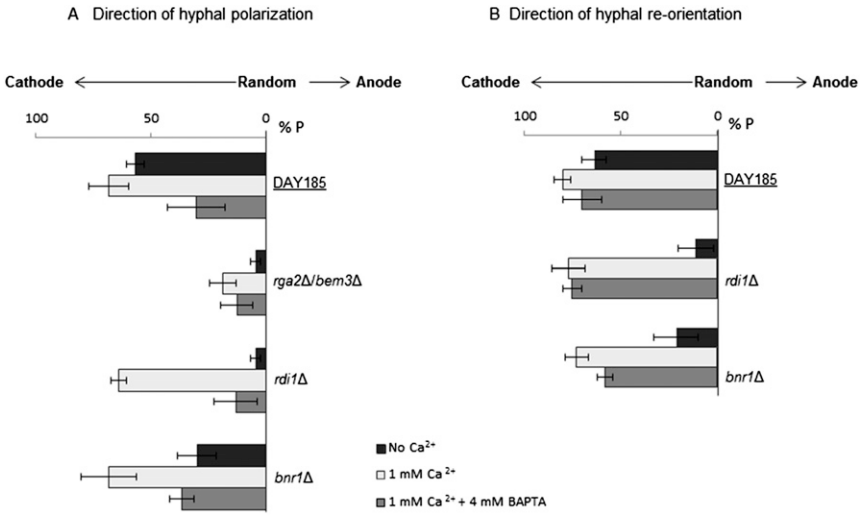

Fig. S2. Addition of 1 mM Ca^{2+} rescues the loss of (*A*) cathodal polarization and (*B*) final hyphal reorientation in a dc electric field in the *rdi1*Δ and *bnr1*Δ mutant. Rescue of cathodal polarization, but not final hyphal reorientation, is reversed by the further addition of the Ca^{2+} chelator, 1,2-bis(2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid (BAPTA) (4 mM), indicating that the two galvanotropic responses are driven by different mechanisms.

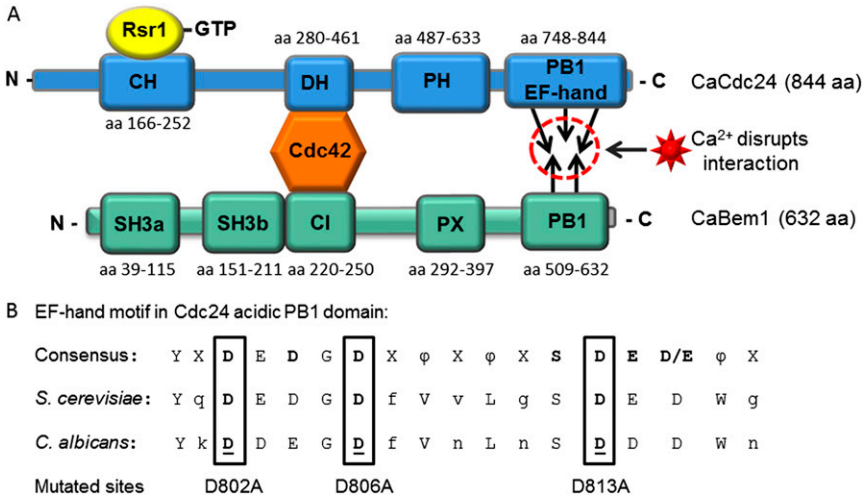

Fig. S3. (*A*) Cdc24-Cdc42-Bem1 structure and interactions. CaCdc24, the guanine exchange factor (GEF) for Cdc42, consists of the following domains: CH [Calponin Homology: ScCdc24 CH domain interacts with Rsr1–GTP and Far1 (1)], DH (Dbl Homology: ScCdc24 Rho–GEF catalytic domain that interacts with Cdc42), PH (Plekstrin Homology: ScCdc24 contains a putative α–lactalbumin-like Ca^{2+}-binding site), and PB1 [Phox& Bem1 Type 1 (acidic): ScCdc24 contains an S110A-like EF-hand (2, 3) and interacts with Bem1-PB1 (4)]. This interaction is disrupted by Ca^{2+} in vitro (5). ScCdc24-PB1 binds an autoinhibitory domain at aa 673–780 (1).CaBem1 domains: SH3a [ScBem1 interacts with Sec15 (6)], SH3b [ScBem1 interacts with Bem2, Boi1, Cla4, Cst20 (7–10)], CI (Cdc42-Interacting (11, 12), PX (PxxP), and PB1 Type 2 (basic) (13). (*B*) Alignment of the consensus acidic PB1 domain in Cdc24 containing a putative EF-hand sequence (14, 15) in *C. albicans* and *Saccharomyces cerevisiae*, where X indicates any amino acid and φ indicates a hydrophobic amino acid. Conserved aspartate residues D802 and D806 (the equivalents of D820 and D824 in *S. cerevisiae*) are critical for Bem1-PB1 binding (13). D802, D806, and D831 (boxed) lie at positions 1, 5, and 12 in the canonical EF-hand structure and contribute Ca^{2+} coordination (16).The amino acid residues mutated in *C. albicans* $Cdc24^{pb1}$ in this study are underlined and numbered.

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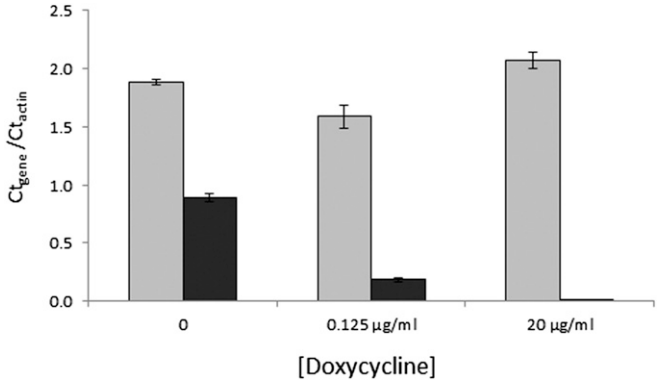

Fig. S4. The wild-type copy of *CDC24* is repressed by doxycycline. Strain Cdc24^{pb1} was grown for 4 h with or without doxycycline at the concentrations indicated. Doxycycline did not significantly affect expression of the mutated copy of *CDC24* from the native *CDC24* promoter. The presence of 0.125 µg/mL doxycycline reduced expression of wild-type *CDC24*, under the control of the Tetp promoter, by fivefold. Expression was reduced 90-fold or was not detectable in the presence of 20 µg/mL doxycycline. In the absence of doxycycline, the expression level of wild-type *CDC24* from the Tet promoter was half that of the native promoter.

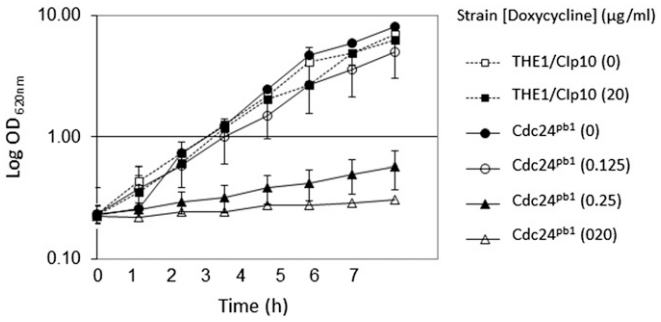

Fig. S5. The Ca^{2+}-binding EF-hand motif is required for growth as yeast in *C. albicans*. The control strain (THE1/Clp10) and Cdc24^{pb1} were grown overnight as yeast without or with doxycycline (0.125 µg/mL, 0.25 µg/mL, or 20 µg/mL) to repress the wild-type copy of *CDC24*. Cells were subcultured at OD_{600} = 0.05 in fresh medium containing the same concentration of doxycycline and the OD_{600} was recorded over 7 h. Error bars indicate SD, n = 3.

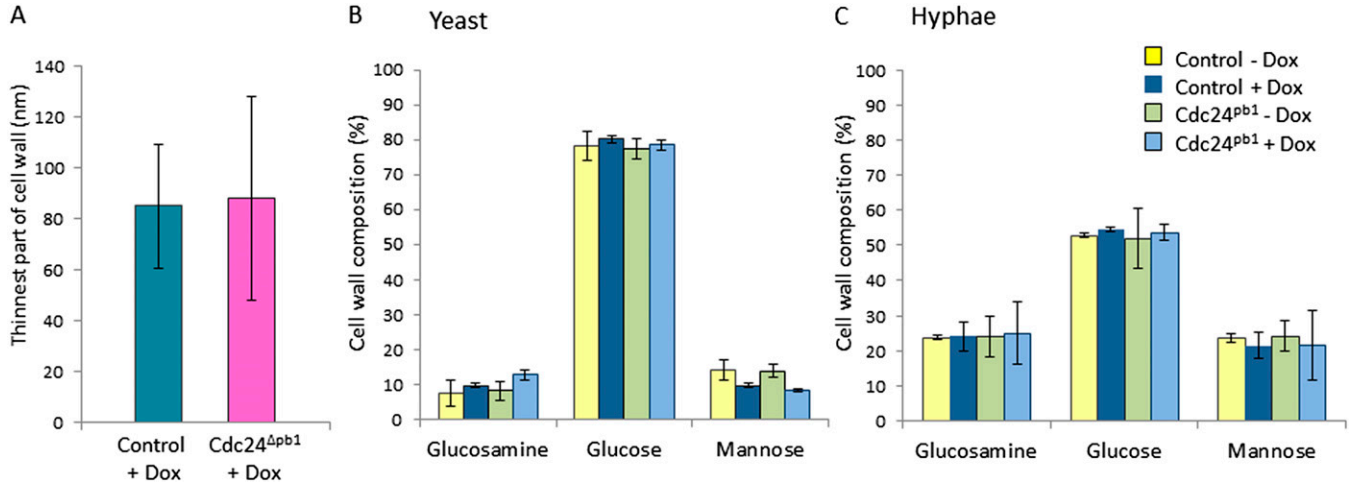

Fig. S6. Mutation of the Cdc24-PB1 domain does not alter cell-wall thickness and composition. (*A*) Measurement of cell-wall thickness from transmission electron microscopy images. (*B* and *C*) HPLC analysis of cell-wall polymers glucosamine (derived from chitin), glucose (β-glucan), and mannose (mannan) in Cdc24^{pb1} yeast and hyphae. Error bars indicate SD, n = 3.

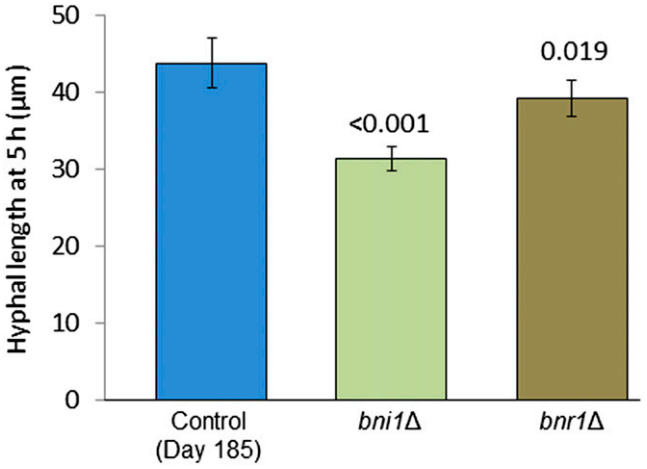

Fig. S7. Hyphae of the formin mutants grew more slowly than wild-type cells in an electric field. Hyphal length was determined after 2 h of hyphal induction without an electric field, followed by 3 h of exposure to10 V/cm. The mean length ± SD was determined from >100 measurements per strain in a minimum of three independent experiments.

Table S1. *C. albicans* strains used in this study

Strain	Alternative name	Genotype	Source
SC5314		Clinical isolate	1
NGY152	CAI4/Clp10	*ura3*Δ-*iro1*Δ::λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434 RPS1*/*rps1*-Clp10-*URA3*	2, 3
SU64	$Cdc42^{G12V}$	*ura3*Δ-*iro1*Δ::λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434, CDC42*/*cdc42*::*hisG*, *PCK1*-*CDC42*^{G12V}::*hisG*-*URA3*-*hisG*	4
SU69	$Cdc42^{D118A}$	*ura3*Δ-*iro1*Δ:: λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434, CDC42*/*cdc42*::*hisG*, *PCK1*-*CaCDC42*^{D118A}::*hisG*-*URA3*-*hisG*	4
SU84	Cdc42 O/E	*ura3*Δ-*iro1*Δ:: λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434, CDC42*/*cdc42*::*hisG*, *PCK1*-*CaCDC42*::*hisG*-*URA3*-*hisG*	4
609	*rga2*Δ	*ura3*Δ-*iro1*Δ::λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434 his1*::*hisG*/*his1*::*hisG* *arg4*::*hisG*/*arg4*::*hisG rga2*Δ::*HIS1*/*rga2*Δ::*ARG4RPS1*/*rps1*-pCAEXP-pMET3-*URA3*	5
611	*bem3*Δ	*ura3*Δ-*iro1*Δ::λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434 his1*::*hisG*/*his1*::*hisG* *arg4*::*hisG*/*arg4*::*hisG bem3*Δ::*HIS1*/*bem3*Δ::*ARG4RPS1*/*rps1*-pCAEXP-pMET3-*URA3*	5
615	*rga2*Δ/*bem3*Δ	*ura3*Δ-*iro1*Δ::λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434 his1*::*hisG*/*his1*::*hisG* *arg4*::*hisG*/*arg4*::*hisG rga2*Δ::*HIS1*/*rga2*Δ::*ARG4bem3*::*ura3*(5′Δ)/ *bem3*::*ura3*(5′Δ) *RPS1*/*rps1*-pCAEXP-pMET3-*URA3*	5
612	*rdi1*Δ	*ura3*Δ-*iro1*Δ::λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434 his1*::*hisG*/*his1*::*hisG* *arg4*::*hisG*/*arg4*::*hisG rdi1*Δ::*HIS1*/*rdi1*Δ::*ARG4 RPS1*/*rps1*-Clp10-*URA3*	5
THE1		*ade2*Δ::*hisG*/*ade2*Δ::*hisG ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434*, *ENO1*/*eno1*Δ::*ENO1*-tetR-ScHAP4AD-3xHA-*ADE2*	6
THE1/Clp10		THE1 *RP10*/*RP10*::*URA3*	7
PY95	*CDC24*/*cdc24*	*ura3*Δ-*iro1*Δ::λ*imm434*/*ura3*Δ-*iro1*Δ:: λ*imm434 his1*::*hisG*/*his1*::*hisG* *arg4*::*hisG*/*arg4*::*hisG CDC24*/*cdc24*Δ::*HIS1*	8
WYL3	*bni1*Δ	*ura3*Δ:: λ*imm434*/*ura3*Δ::λ*imm434 his1*::*hisG*/*his1*::*hisG* *arg4*::*hisG*/*arg4*::*hisG bni1*::*ARG*/ *bni1*::*HIS, URA3*	9
WYL22	*bnr1*Δ	*ura3*Δ:: λ*imm434*/*ura3*Δ::λ*imm434 his1*::*hisG*/*his1*::*hisG* *arg4*::*hisG*/*arg4*::*hisG bn21*::*ARG*/ *bnr1*::*HIS*	9
DAY185		*ura3*Δ:: λ*imm434*/*ura3*Δ::λ*imm434 his1*::*hisG*/*HIS1*::*his1*::*hisG* *arg4*::*hisG*/*ARG4*::*URA3*::*arg4*::*hisG*	10
AB299	THE1-$Cdc24^{pb1}$-*URA3*	THE1 *CDC24*/*cdc24*$^{\Delta pb1}$-*URA3*	This study
AB310	THE1-$Cdc24^{pb1}$	THE1 *CDC24*/*cdc24*$^{\Delta pb1}$	This study
AB320	$Cdc24^{pb1}$	THE1 *URA3*-Tetp- *CDC24*/*cdc24*$^{\Delta pb1}$	This study

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Table S2. Primers used in this study

Primer name	Primer sequence
Cdc24-Amp-ORF-Not1-F2	5′-ctactactaGCGGCCGCatggaacatccaccagcagctctc-3′*
CDC24—Amp-ORF-Xba1I-R2	5′-gatgatTCTAGAtatttcgtcaaaatgtcatttatatatc-3′*
CDC24-Amp-term-SalI-F	5′-cacaGTCGACtttaactacaggaacaaacgc-3′*
CDC24-Amp-term-KpnI-R	5′-aaaaGGTACCgcaatggcttgatgttttgagc-3′*
CDC24-Long-Tet-F	5′cagctttgagttttactagtttgccacctggtttgccacttagttttgccacc aagaagttggactaaagtttatatctgtctcttatataatttaccttatag tcaacttcacttccGTAATACGACTCACTATAGGG – 3′†
CDC24-Long-Tet-R	5′gactgggcccagagaaacaattcttgaagacgaaacagtactt actgaattcaaagatgaagttgattgggttgaaaatgttctgagagct gctggtggatgttccatggttttggaCTAGTTTTCTGAGATAAAGCTG – 3′†
URA3-R2	5′-cttagtgttgactgtcatatc – 3′
URA3-R3	5′-ttataccatccaaatcaattc-3′
URA3-OK-F2	5′-agcactggaactgatattatc-3′
CDC24-Seq-Up-F	5′-ggaagagtttatatatcggag – 3′
CDC24-Seq-Down-F	5′-atcgacagaattgtcagagc-3′
CDC24-WT-Prom-F	5′- ttgctgcatattgaagaaatag – 3′
CDC24-Recom-R2	5 ′-ctgttaattgaatagataatcc – 3′
Tet-Screen-F	5′-ggacttcttcgccagagg – 3′
CDC24-Probe-R	5′-acaattctgtcgatttgtaaa g – 3′
EFB1 forward	5′-aagtcgaatctatcaagtcattgaac -3′
EFB1 reverse	5′-gagtggcagtagtaccatcaatg -3′
Primer set A probe 1 forward	5′-agcggctcattcactttgag-3′
Primer set A probe 1 reverse	5′-cacgtaaacacttttcccattg-3′
Primer set B forward 2	5′-aattatctcccagattatcacttcg-3′
Primer set B reverse 2A	5′-gtctccttcgtcgtctttatat-3′
Primer set C forward 2	5′-aattatctcccagattatcacttcg-3′
Primer set C reverse 2B	5′-caaaagctccttcgtcagctt -3′
Primer set D ACT1 forward	5′-accaccggtattgttttgga -3′
Primer set D ACT1 reverse	5′-agcgtaaattggaacaacgtg-3′

*Uppercase denotes restriction enzyme site.
†Uppercase denotes homology to Tet promoter sequence.

Table S3. Plasmids generated in this study

pBS-*URA3*	pBluescript-*URA3**
ABp26	TOPO-*CDC24*-ORF+200bp
ABp33	TOPO- *CDC24*-t500bp
ABp63	*pBS-URA3*+*CDC24* + t200 bp inserted 3′ to *URA3*
ABp71	ABp63 + t500 bp*CDC24* inserted 5′ to *URA3*
ABp82	ABP71 with *CDC24* point mutations D802A and D806A
ABp99	ABp82 with *CDC24* point mutation D813A

*A gift from Carol Munro (University of Aberdeen, Aberdeen, UK).