**Candida albicans** Cdc15 is essential for mitotic exit and cytokinesis

Steven Bates

_Candida albicans_ displays a variety of morphological forms, and the ability to switch forms must be linked with cell cycle control. In budding yeast the Mitotic Exit Network (MEN) acts to drive mitotic exit and signal for cytokinesis and cell separation. However, previous reports on the MEN in _C. albicans_ have raised questions on its role in this organism, with the components analysed to date demonstrating differing levels of importance in the processes of mitotic exit, cytokinesis and cell separation. This work focuses on the role of the Cdc15 kinase in _C. albicans_ and demonstrates that, similar to _Saccharomyces cerevisiae_, it plays an essential role in signalling for mitotic exit and cytokinesis. Cells depleted of Cdc15 developed into elongated filaments, a common response to cell cycle arrest in _C. albicans_. These filaments emerged exclusively from large budded cells, contained two nuclear bodies and exhibited a hyper-extended spindle, all characteristic of these cells failing to exit mitosis. Furthermore these filaments displayed a clear cytokinesis defect, and _CDC15_ over-expression led to aberrant cell separation following hyphal morphogenesis. Together, these results are consistent with Cdc15 playing an essential role in signalling for mitotic exit, cytokinesis and cell separation in _C. albicans_.

Exit from mitosis following the successful segregation of chromosomes requires the inactivation of the cyclin-dependent kinase (Cdk), and is followed by the activation of cytokinesis and preparation for the onset of the next cell cycle. In budding yeast _Saccharomyces cerevisiae_ exit from mitosis and the activation of cytokinesis is regulated by the Mitotic Exit Network (MEN), a GTPase regulated kinase cascade that displays similarity to the fission yeast Septation Initiation Network (SIN) and Metazoan Hippo pathway1–3. The core elements of this pathway in budding yeast include the GTPase Tem1, a two-component GTPase activating protein Bub2-Bfa1 and potential GTPase exchange factor Lte1, and the downstream kinases Cdc15 and Dbf2-Mob1 which ultimately regulate the activity of the Cdc14 phosphatase.

In budding yeast Cdc14 is held inactive for the majority of the cell cycle through its interaction with Net1 which sequesters it to the nucleolus. The key output of the MEN is the sustained release of Cdc14 from the nucleolus, which then drives mitotic exit through terminating Cdk activity and activating expression of the Cdk inhibitor Sic14–7. During early anaphase Cdc14 is initially transiently released from the nucleolus to the nucleus through the action of the Cdc14 early anaphase release (FEAR) network8. Subsequently, in late anaphase, the MEN then drives the sustained release of Cdc14 into the cytoplasm where it associates with the spindle pole bodies (SPB) and bud neck and drives mitotic exit1–3,10. The regulation of the MEN is mainly through the spatial and temporal regulation of the GTPase Tem1, which localises to SPB alongside the GAP complex Bub2-Bfa1 which holds it inactive11–13. The activity of the GAP complex is maintained by the protein kinase Kin4, which localises to the mother cell cortex and mother cell localised SPB14–16. Conversely, Lte1 localises to the daughter cell cortex and is thought to activate Tem1 through preventing the low levels of Kin4 present in the bud from associating with the SPB17–20. Therefore the MEN is controlled through spindle position, with Tem1 being activated following the migration of the SPB into the bud. Following its activation Tem1 then signals through the Ste20-like kinase Cdc15 and LATS/NDR kinase Dbf2. GTP-bound Tem1 first activates Cdc15 through its recruitment to the SPB12,13, which in turn recruits the Dbf2-Mob1 complex through the phosphorylation of the SPB scaffold protein Nud121. Ultimately, the Dbf2-Mob1 complex then drives the sustained release of Cdc14 to the cytoplasm through its phosphorylation22,23.

In addition to signalling mitotic exit more recent work has also suggested the MEN plays a direct role in signalling for cytokinesis and cell separation26,27. Firstly, following the drop in Cdk activity, a number of the MEN components have been seen to relocalse to the bud neck. Furthermore, MEN mutants that bypass their mitotic exit defect subsequently exhibit defects associated with actomyosin ring dynamics24–31. There is also evidence that activation of Cdc14 results in the repolarisation of the cytoskeleton to the bud neck through impacting on the...
phosphorylation status of the formins32, and that MEN components may also regulate other cytokinesis factors such as Chs2, Hof1 and Inn132. Finally, Cdc14 may also play a role in promoting cell separation through its interaction with the RAM (regulation of Ace2 and morphogenesis) signalling cascade which regulates the enzymes required for septum degradation and cell separation34,35. The finding that the MEN is involved with cytokinesis in budding yeast is also in keeping with the role of analogous pathways in other fungi, such as the fission yeast SIN, where their primary role is in coordinating cytokinesis. Furthermore, a recent re-evaluation of the role of Cdc14 in budding yeast suggests that coordinating cytokinesis is its most conserved function, and that its role in signalling mitotic exit may be through acting on a small set of substrates with additional phosphatases required for widespread Cdk substrate dephosphorylation7.

_Candida albicans_ is an important opportunistic human fungal pathogen, capable of causing both superficial and life-threatening systemic infections. It can grow in a wide variety of morphological forms, and the ability to switch forms has been linked with virulence. As the process of morphogenesis impacts on the extent of polarised growth, nuclear dynamics, and septation and cell separation its progression must be tightly linked with cell cycle switching forms has been linked with virulence. As the process of morphogenesis impacts on the extent of polarised growth, nuclear dynamics, and septation and cell separation...
cassette\textsuperscript{39}, was also employed. Screening of sixty arginine and uridine prototrophic recombinants derived from a $CDC15/cdc15::UAU1$ strain demonstrated all had undergone a triplication event and carried a wild type copy of $CDC15$, consistent with $CDC15$ being essential. Furthermore, cells depleted of Cdc15 were ultimately seen to lose viability. In liquid culture this was first seen following 20 h growth of the TET-V5-CDC15 strain under repressing conditions with a significant drop in viability to $75.77 \pm 5.99\%$ ($p < 0.001$), and subsequently to $24.91 \pm 2.06\%$ ($p < 0.0001$) at 24 h, as opposed to under non-repressing conditions where viability was maintained at over 95% (Fig. 2B). These results are in contrast to a previous large scale screen of kinases in \textit{C. albicans} where insertional mutants in $CDC15$ were reported\textsuperscript{40}. However, the two mutants previously reported would have only affected the extreme C-terminus of the protein, resulting in a slight truncation (deletion of either the C terminal 86 or 125 Aa of the 1126 Aa protein) and would not have impacted on the kinase domain. The results presented here therefore clearly demonstrate that $CDC15$ is essential in \textit{C. albicans}.

Cells depleted of Cdc15 form elongated filaments. To characterise the terminal phenotype associated with loss of $CDC15$ cellular morphology was monitored following the repression of $CDC15$ expression in the TET-V5-CDC15 strain. Following overnight growth in non-repressing conditions the TET-V5-CDC15 strain was inoculated into repressing conditions (SC + Dox) at $2.5 \times 10^3$ cells/ml, and cells collected at various time points. Under these conditions the cells grew normally for the first 12 h before starting to arrest as large budded cells (Fig. 3A,B), such that by 16 h $71.01 \pm 5.37\%$ of the population was present as large budded cells compared to $42.38 \pm 1.9\%$ in an exponential culture under non-repressing conditions (SC). Western blot analysis of the TET-V5-CDC15 strain grown under repressing conditions also demonstrated that V5-Cdc15 levels were almost undetectable following 12 h growth (Fig. 2C), therefore the arrest seen broadly correlated with the depletion of Cdc15. Following their arrest as large budded cells the Cdc15 depleted cells subsequently became enlarged and initiated filamentous growth. In terms of cell size, under the conditions tested, non-repressed cells demonstrated an average width of $4.37 \pm 0.32\mu m$ ($n = 53$), however, between 16 and 20 h the cells were seen to swell from a width of $4.14 \pm 0.55\mu m$ ($n = 50$) at 16 h to $6.57 \pm 1.25\mu m$ ($n = 66$) at 20 h. From 16 h these cells also started to demonstrate filamentous growth, with filaments emerging exclusively from large budded cells, such that by 24 h the majority of cells in the population ($91.13 \pm 1.40\%$) displayed filamentous growth. Growth of the TET-V5-CDC15 strain, under non-repressing conditions for yeast like growth, displayed no obvious phenotype, indicating that the arrest followed by cell swelling and initiation of filamentous growth was due to the loss of $CDC15$.

Cdc15 is required for mitotic exit and cytokinesis. Our previous work has shown that Tem1 is required for mitotic exit and cytokinesis, and suggested that its essential function is signalled through Cdc15\textsuperscript{37}. To confirm that depletion of Cdc15 also results in nuclear division defects the TET-V5-CDC15 cells were collected following

Figure 1. Cdc15-GFP localises to spindle pole bodies in \textit{C. albicans}. (A) Cdc15-GFP, under control of the $CDC15$ native promoter, was visualised in \textit{C. albicans} grown as yeast (YEPD, 30 °C), pseudohyphae (YEPD, 37 °C) or true hyphae (YEPD + 20% Serum, 37 °C). (B) Cdc15-GFP was demonstrated to co-localize with RFP tagged gamma-tubulin (Tub4-RFP). Differential interference contrast (DIC) and fluorescent images were taken of unfixed cells, and arrows indicate the position of Cdc15-GFP and Tub4-RFP. Bars, 5µm.
Figure 2. CDC15 is essential in C. albicans. (A) Growth of C. albicans TET-V5-CDC15 on SC medium (CDC15 expression on) and SC medium plus 20 µg/ml doxycycline (SC + Dox, CDC15 expression off). (B) Viability of Cdc15 depleted cells. The TET-V5-CDC15 strain was grown from 2.5 × 10⁶ cells/ml under non-repressing (SC) or repressing conditions (SC + Dox) and viability determined at the time points indicated. (C) V5-Cdc15 protein levels following promoter shut off. TET-V5-CDC15 was grown under repressing conditions for the times indicated and V5-Cdc15 protein levels determined by western blotting.

repression and nuclear material visualised through staining with DAPI. Following 16 h repression, when the majority of cells were arrested, over 90% of these cells were binucleate, with a small proportion of multinucleate cells. Furthermore, at all subsequent time points this was maintained, with over 90% of cells remaining binucleate (Fig. 3). Following the formation of filaments by the repressed cells nuclear migration was also seen. Initially one nucleus from the original budded cell body was seen to migrate into the filament, and it was subsequently followed by the second. As a result, following 24 h repression, both nuclei were present in the filaments of 50% of the arrested cells (Fig. 3A,C).

The nuclear dynamics seen following Cdc15 depletion are in keeping with these cells displaying a defect in mitotic exit and failing to re-enter the cell cycle. To confirm this mitotic exit defect spindles were visualised through GFP tagging Tub1 in the CDC15 tetracycline repressible strains. Under repressing conditions these strains were also seen to arrest as large budded cells which subsequently formed filaments. However, the GFP tagging of Tub1, in both the TET-CDC15 and TET-V5-CDC15 strains, resulted in the cells showing much less synchrony in their response to Cdc15 depletion. The cells did all arrest, generally earlier than the untagged strain, but a greater level of variation was seen in the timing of this. As such at time points following CDC15 repression the cell population would demonstrate a range of morphologies associated with Cdc15 depletion. Importantly however, the vast majority of these arrested cells displayed an extended spindle (Fig. 4), characteristic of the cells arresting in late anaphase or early telophase and failing to exit from mitosis. The mitotic spindle in these cells was also seen to become highly elongated, displaying a fish hook or lasso morphology, with the spindle wrapped along the cell periphery. A similar phenotype has been reported in a subset of S. cerevisiae mutants, including a number in the MEN 41–44. Thus Cdc15 depleted cells display both a defect in mitotic exit and the regulation of spindle length.

The presence of a hyper-extended spindle and nuclear migration into the filaments, following Cdc15 depletion, is suggestive of cytokinesis being blocked in these cells. To confirm this the Cdc15 depleted cells were first stained with calcofluor white (CFW), and the arrested cells were seen to display only a single chitin band at the original mother-bud neck with no bands present in the filaments (Fig. 5). In order to follow this further the septin Cdc3 was GFP tagged in the TET-V5-CDC15 strain. In wild type cells a septin ring is laid down early in the cell cycle at the site of bud emergence, and this then switches to an hourglass-shaped collar across the mother-bud neck before finally splitting into two rings for cytokinesis⁴⁵,⁴⁶. Following Cdc15 depletion Cdc3-GFP was only seen at the original mother-bud neck of the arrested cells, with no structures present in the filaments (Fig. 5).
Figure 3. Cdc15 depleted cells form filaments and fail to exit mitosis. (A) The TET-V5-CDC15 strain was grown, from 2.5 × 10^3 cells/ml, under non-repressing (SC) or repressing conditions (SC + Dox) for the times indicated, fixed and stained with DAPI. Representative cells are shown. Bars 5 µm. The TET-V5-CDC15 cultures were also scored for cellular morphology (B) and nuclear position (C), with at least 100 cells counted for each time point. (B) For cellular morphology cells were classed as either unbudded, small budded, large budded or large budded with a filament emerging. (C) The binucleate large budded cells and large budded cells with filaments emerging were scored according to nuclear position with: one nucleus in original mother and daughter cell, one nucleus in mother and one in filament, one nucleus in daughter and one in filament, and both nuclei in the filament.

Figure 4. Cdc15 depleted cells display a hyper-extended mitotic spindle. Tub1-GFP was visualised in the TET-V5-CDC15 strain background after 16 h growth in non-repressing (SC) or repressing (SC + Dox) conditions. Cells representative of the various morphologies displayed following Cdc15 depletion are shown. Bars, 5 µm.
Furthermore, the septins in these cells all displayed the hourglass collar structure, consistent with the cells having arrested before the signal for the septin rings to separate and the actomyosin ring to assemble. Cdc15 depleted cells are therefore one continuous compartment and have a clear cytokinesis defect.

Finally, evidence that Cdc15 also plays a role in signalling for cell separation was seen through following hyphal growth in the TET-CDC15 strain, which under non-repressing conditions overexpresses CDC15. This strain grew normally under non-repressing conditions that promote yeast growth, and it also switched to hyphal growth as expected in response to serum and temperature. However, in contrast to wild type cells, where cell separation is blocked following filamentation, the filaments formed under non-repressing conditions in response to serum by the TET-CDC15 strain displayed partial or total separation (Fig. 6A). The Ace2 transcription factor is known to drive expression of genes involved with cell separation, such as DSE1 and SCW11\textsuperscript{35,47}. Expression analysis clearly demonstrated that these genes were repressed as expected in wild type hyphae, however the TET-CDC15 strain still displayed their expression when grown in hyphal form (Fig. 6B). A similar phenotype has been reported in a sep7 mutant which lacks hyphal specific modifications to the septin ring, and mutants in mob2 which is part of the RAM network involved in regulating Ace2\textsuperscript{48,49}. This would suggest that the MEN is involved with activating Ace2 and signalling for cell separation, however this signal must normally be overridden in hyphal cells. Overall the phenotypes associated with CDC15 are therefore consistent with it playing a key role in the MEN and signalling for mitotic exit, cytokinesis and cell separation.

Discussion

This report focuses on the role of Cdc15 in the processes of mitotic exit, cytokinesis and cell separation in C. albicans. Cdc15 was seen to be essential in C. albicans and cells depleted of it displayed phenotypes highly similar to cells depleted of Tem1. This, together with the previous finding that overexpression of CDC15 rescues the essential phenotype associated with the loss of Tem1\textsuperscript{37}, is in keeping with Cdc15 signalling downstream of Tem1 and playing an essential role in mitotic exit. The essentiality of CDC15 was demonstrated through two approaches, plus further supported by the loss of viability seen following Cdc15 depletion. It was however noticeable that when using the tetracycline repressible promoter system the resulting strain completed multiple rounds...
of cell division before arrest was seen. This would suggest that Cdc15 is a stable protein and only low levels are required for its essential function. A similar result was seen following Tem1 depletion, plus the essentiality of Dbf2 following its depletion was only observed when coupled with epitope tagging. This observation, that only low levels of MEN components are required for viability, is also supported by a recent re-evaluation of the role of Cdc14 in *S. cerevisiae* which suggested that only trace levels of the protein are required for signalling mitotic exit.

When cells were depleted of Cdc15 they formed wide filamentous structures, typically lacking constrictions. The activation of polarised growth by *C. albicans* is a common response to cell cycle arrest, and is seen to occur independent of the stage of the cell cycle the cells have arrested in. However, the morphology seen in the progenitor cell giving rise to the filament will vary depending on the stage of arrest. In the case of the Cdc15 depleted cells, similar to what was previously reported with cells depleted of Tem1 or the G2/M cyclin Clb2, the filaments appeared exclusively from large budded cells. This phenotype is therefore consistent with Cdc15 depleted cells displaying a defect late in the cell cycle. Furthermore, nuclear dynamics and the presence of a hyper extended spindle are indicative of Cdc15 depleted cells failing to exit mitosis. The filamentous cells formed predominantly contained two nuclear bodies, indicating that Cdc15 depleted cells were arrested after nuclear division. These cells also displayed an extended spindle, indicative of the cells arresting in either late anaphase or early telophase and failing to exit mitosis. The filamentous cells were arrested late and were wrapped around the cell periphery, which would suggest that the inactivation of mitotic cyclins is required to promote spindle disassembly. A similar phenotype has been seen in specific groups of *S. cerevisiae* mutants, in particular members of the CFT19 complex which associates with the kinetochore, the FEAR network, and importantly the MEN.

In addition to the mitotic exit defect there was also evidence for a cytokinesis defect in Cdc15 depleted cells, as demonstrated by the lack of septa being laid down along the filaments. There was some evidence of a chitin band at the original mother-bud neck, however, the migration of nuclei across the neck and ultimately into the filaments is supportive of the view that these cells consist of one continuous compartment. Furthermore, septins were also present at the original mother-bud neck in the arrested cells, and were limited to their hourglass collar structure failing to separate into two rings. A similar phenotype was seen in *S. cerevisiae*, and following Tem1 depletion in *C. albicans*, and is supportive of the view that the MEN also acts in signalling for cytokinesis. Evidence was also seen supporting the view that the MEN promotes cell separation. When *C. albicans* forms hyphae cell separation is normally blocked, however, the overexpression of *CDC15* overcame this and resulted in cell separation. Previous work has shown that Cdc14 plays a role in promoting cell separation, and that this is blocked in hyphae through the action of the hyphal specific cyclin Hgc1 which prevents Cdc14 localising to the

**Figure 6.** Hyphae overexpressing *CDC15* activate cell separation. (A) Wild type (NGY152) and the TET-CDC15 strain, which overexpresses *CDC15*, were grown for 3 h in hyphal inducing conditions, fixed, and stained with CFW. Bar, 5 µm. (B) Expression analysis of Ace2-regulated genes *DSE1* and *SCW11* in yeast form (Y) or hyphal form (H), *EFB1* was used as a loading control.
![S. cerevisiae and C. albicans](image)

**Figure 7.** Key roles of MEN components in *S. cerevisiae* and *C. albicans.*

...site of septation. The overexpression of Cdc15 may potentially be outcompeting the signal from the Hgc1-Cdk complex and driving localisation of Cdc14 to the septum region. This could be achieved either though Cdc15 directly driving its activation and localisation or potentially through impacting on the hyphal specific septin (Sep7) which has been seen to block Cdc14 recruitment. Either way this overexpression ultimately led to the aberrant activation of the RAM signalling cascade in hyphae which then drove expression of genes required for cell separation. Cross talk between the MEN and RAM signalling cascade has been reported in *S. cerevisiae* and this work alongside previous reports would suggest that this also occurs in *C. albicans* yeast cells but this signal is overridden in hyphal cells to block cell separation.

Cdc15 was seen to localise to the SPB, and this localisation was cell cycle regulated. Similar to the findings in *S. cerevisiae*, and for Tem1 in *C. albicans*, Cdc15 localised to the SPB commensurate with the G1/S phase transition. In *S. cerevisiae* Cdc15 has also been reported to relocalse to the bud neck at the end of mitosis, as have Dbf2 and Cdc14 in *C. albicans*. However, no evidence was seen of Cdc15 relocalse to the bud neck in *C. albicans*. It instead remained exclusively localised to the SPB, similar to the localisation pattern previously reported for Tem1. A similar pattern of localisation exclusive to the SPB has also been seen for the Cdc15 homolog (Sid1) in *S. pombe*, although here localisation was asymmetrical and only seen on one SPB. Overall the localization of Cdc15 in *C. albicans* would suggest that its role in cytokinesis and cell separation must therefore be signalled through other factors, such as Dbf2 and Cdc14.

This report, alongside the previous analysis of *C. albicans* MEN components, is supportive of the network playing a key role in driving mitotic exit, cytokinesis and cell separation similar to the situation in *S. cerevisiae*. However, although the overall role of the network is conserved, there are key differences in the role of the individual components of the network in these two yeasts. In *S. cerevisiae* the key signalling components of the network, Tem1, Cdc15, Dbf2/Dbf20 and Cdc14, are all essential and signal for mitotic exit through the release and activation of Cdc14, following which they act to drive cytokinesis and cell separation. As such mutants in these components all share a common phenotype, arresting as large budded cells with an extended neck. In contrast cells depleted of either Tem1 or Cdc15 arrest after nuclear division with an elongated spindle, consistent with these factors signalling for mitotic exit. In contrast previous reports suggest the primary role of Dbf2 is in signalling cytokinesis, whereas the role of the non-essential Cdc14 is in driving cell separation. Therefore, although the MEN is conserved between *C. albicans* and *S. cerevisiae*, and Tem1 and Cdc14 signal for mitotic exit in both species, the final output from the network that drives mitotic exit must differ. Whereas the network signals for mitotic exit through Cdc14 in *S. cerevisiae*, in *C. albicans* this must be achieved through other factors downstream of Cdc15, perhaps through the PP1 and PP2A phosphatases as seen in other systems. Indeed the initial analysis of PP1 and PP2A components in *C. albicans* has provided some evidence of them playing an essential role in late cell cycle progression.

**Methods**

**Strains, media and growth conditions.** All strains used and constructed in this study are listed in Table 1. *C. albicans* strains were grown at 30°C in YEPD (1% yeast extract, 2% peptone, 2% glucose) or in SC (0.67% yeast nitrogen base with ammonium sulphate without amino acids, 2% glucose, 0.077% complete supplement mixture minus uracil), supplemented with 50µg/ml uridine as required. Nourseothricin resistance was selected for through growth on Sabouraud dextrose agar (1% mycological peptone, 4% glucose, 1.5% agar) containing 200µg/ml nourseothricin (Werner BioAgents). Hyphal growth was induced by growing the cells at 37°C in YEPD medium plus 20% fetal calf serum. To repress expression from the tetracycline-regulated promoter doxycycline was added to the media at a final concentration of 20µg/ml. To score for viability the cells were first washed in PBS and the total cell number established through a haemocytometer count, the proportion of viable cells was then determined following the plating of serial dilutions onto YEPD-agar medium.

**Strain construction.** The strains constructed in this study (Table 1) were generated through a targeted recombination approach using PCR-generated disruption or tagging cassettes, and the oligonucleotides used are listed in Table S1. To regulate expression of *CDC15* a conditional mutant was generated that carries a single
copy of CDC15 under the control of a tetracycline-regulated promoter\(^{38}\). For this the first copy of CDC15 was disrupted using the URA3 recyclable PCR-directed disruption system\(^{38}\) in the THE1 strain which expresses the tetracycline transactivator\(^{38}\). The disruption cassette was first amplified from pDBB57 using the primer pair CDC15-KO-F and CDC15-KO-R and transformed into the THE1 strain. The URA3 marker was then recycled through selection for ura3 auxotrophs on SC medium plus 5-fluoroorotic acid (1 mg/ml) and uridine (50 µg/ml). The promoter replacement cassette was then amplified from p99CAU1\(^{57}\) using primer pair CDC15-TET-F and CDC15-TET-R, and transformed into the heterozygote mutant to generate the TET-CDC15 strain (SBC189). In addition, to allow the levels of Cdc15 to be monitored following promoter switch off, the tetracycline-repressible system (Promega) to generate pURA3-TET-V5. The promoter replacement cassette was then amplified from this vector using primer pair CDC15-TET-F and CDC15-TET-R and cloned into the pGEM-T Easy vector system (Promega) to generate pURA3-TET-V5. The promoter replacement cassette was then amplified from this vector using primer pair CDC15-TET-F and CDC15-TET-V5-R and transformed into the heterozygote mutant to generate the TET-V5-CDC15 strain (SBC190). CDC15 was also disrupted using the UAU1 cassette\(^{58}\), for this the cassette was amplified from the plasmid pBME101 using the primer pair CDC15-UAU3-F and CDC15-UAU3-R and transformed into strain SN148.

For the C- terminal tagging of Tub1 and Cdc3 GFP-NAT1 tagging cassettes were amplified from pGFP-NAT1\(^{59}\) using primer pairs TUB1-GFP-F and TUB1-GFP-R and TUB4-GFP-NAT1 and TUB4-GFP-NAT1-R. The resulting cassettes were then transformed into the TET-V5-CDC15 strain with selection for nourseothricin resistance. For localisation studies Cdc15 was C-terminally tagged with GFP, for this a GFP-NAT1 tagging cassette\(^{60}\) was amplified using primer pair CDC15-GFP-F and CDC15-NAT1-R and transformed into the NGY152 strain. In addition a strain was also constructed where CDC15-GFP was overexpressed through replacing the native promoter with the tetracycline-regulated promoter. For this the promoter replacement cassette was first amplified from p99CAU1 using primer pair CDC15-TET-F and CDC15-TET-R and transformed into the heterozygote mutant to generate the TET-V5-CDC15 strain (SBC190). CDC15 was also disrupted using the UAU1 cassette\(^{58}\), for this the cassette was amplified from the plasmid pBME101 using the primer pair CDC15-UAU3-F and CDC15-UAU3-R and transformed into strain SN148.

### Cell staining and microscopy

For microscopy cell samples were washed in PBS and viewed live for visualising the fluorescent protein fusions and calcocollor white (CFW) staining, or following a 10 min fixation in 70% ethanol for 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) staining. To visualise septa and nuclei cells were stained for 5 min with CFW (5µg/ml) or DAPI (1µg/ml) respectively. Epifluorescence and differential interference microscopy was carried out using a Zeiss Axioskop microscope equipped with a Plan Neofluor 100x/1.30 oil objective. Images were captured using a Spot Pursuit™ 1.4 MP Monochrome CCD camera and VisiView software (Visitron Systems). Images were subsequently handled and measurements taken using the Fiji software\(^{62}\). Statistical differences were determined using a one way ANOVA with Turkey’s post hoc test.

### References

1. Bardin, A. J. & Amon, A. Men and Sin: what's the difference? Nat Rev Mol Cell Biol 2, 815–826, https://doi.org/10.1038/35099020 (2001).
2. Hergovich, A. & Hemmings, B. A. Hippo signalling in the G2/M cell cycle phase: lessons learned from the yeast MEN and SIN pathways. Semin Cell Dev Biol 23, 794–802, https://doi.org/10.1016/j.semcdb.2012.04.001 (2012).
3. Krapp, A., Gulli, M. P. & Simanis, V. SIN and the art of splitting the fission yeast cell. Curr Biol 14, R722–730, https://doi.org/10.1016/j.cub.2004.08.049 (2004).

| Strain | Genotype | Reference |
|-------|----------|-----------|
| CAI-4 | ura3Δ::mma434/ura3Δ::mma434 |  |
| THE1 | ura3Δ::mma434/ura3Δ::mma434 ade2Δ::hisG/ade2Δ::hisG ENO1::ENO1::tetR-S hap4AD-3LexA-ADA2 |  |
| SN148 | urg1Δ::dlp200/urg1Δ::dlp200, leu2Δ::dlp200/leu2Δ::dlp200, his3Δ::hisG/his3Δ::hisG, ura3Δ::mma434/ura3Δ::mma434 |  |
| NGY152 | As CAI-4 but RPS1/rps11Δ::Cbp10 | This study |
| SBC187 | As THE1 but CDC15/cdc15Δ::dlp200-U3A-dlp200 | This study |
| SBC188 | As THE1 but CDC15/cdc15Δ::dlp200 | This study |
| SBC189 | As THE1 but URA3-TETp-CDC15/cdc15Δ::dlp200 | This study |
| SBC190 | As THE1 but URA3-V5-TETp-CDC15/cdc15Δ::dlp200 | This study |
| SBC191 | As THE1 but URA3-V5-TETp-CDC15/cdc15Δ::dlp200, TUB1/TUB1-GFP-NAT1 | This study |
| SBC192 | As NGY152 but CDC15/CDC15-GFP-NAT1 | This study |
| SBC193 | As THE1 but CDC15/URA3-TETp-CDC15 | This study |
| SBC194 | As THE1 but CDC15/URA3-TETp-CDC15-GFP-NAT1 | This study |
| SBC195 | As THE1 but CDC15/ura3-TETp-CDC15-GFP-NAT1 | This study |
| SBC196 | As THE1 but CDC15/ura3-TETp-CDC15-GFP-NAT1, TUB4/TUB4-RFP-U3A | This study |
| SBC197 | As SN148 but CDC15/cla15::UAU1 | This study |
| SBC198 | As THE1 but URA3-V5-TETp-CDC15/cla15Δ::dlp200, CDC3/CDC3-GFP-NAT1 | This study |

Table 1. Strains used in this study.
4. Shou, W. et al. Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. Cell 97, 233–244 (1999).
5. Stegmeier, F. & Amon, A. Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. Annu Rev Genet 38, 203–232, https://doi.org/10.1146/annurev.genet.38.072902.093051 (2004).
6. Visintin, R. et al. The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. Mol Cell 2, 709–718 (1998).
7. Powers, B. L. & Hall, M. C. Re-examining the role of Cdc14 phosphatase in reversal of Cdk phosphorylation during mitotic exit. J Cell Sci 130, 2673–2681, https://doi.org/10.1242/jcs.201102 (2017).
8. Rock, J. M. & Amon, A. The FEAR network. Curr Biol 19, R1063–1068, https://doi.org/10.1016/j.cub.2009.10.002 (2009).
9. Jaspersen, S. L., Charles, J. F., Tinker-Kulberg, R. L. & Morgan, D. O. A late mitotic regulatory network controlling cyclin destruction in Saccharomyces cerevisiae. Mol Biol Cell 9, 2803–2817 (1998).
10. Visintin, R., Hwang, E. S. & Amon, A. Cbl1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. Nature 398, 818–823, https://doi.org/10.1038/19775 (1999).
11. Bardin, A. J., Visintin, R. & Amon, A. A mechanism for coupling exit from mitosis to partitioning of the nucleus. Cell 102, 21–31 (2000).
12. Lee, S. E., Frenz, L. M., Wells, N. J., Johnson, A. L. & Johnston, L. H. Order of function of the budding yeast mitotic exit-network proteins Tem1, Cdc15, Mob1, Dbf2, and Cdc5. Curr Biol 11, 784–788 (2001).
13. Pereira, G., Hofken, T., Grindlay, J., Manson, C. & Schiebel, E. The Bub2p spindle checkpoint links nuclear migration with mitotic exit. Mol Cell 6, 1–10 (2000).
14. Pereira, G. & Schiebel, E. Kin4 kinase delays mitotic exit in response to spindle alignment defects. J Cell Biol 157, 1364–1378, https://doi.org/10.1083/jcb.200905114 (2009).
15. Mah, A. S., Jang, J. & Deshaies, R. J. Protein kinase Cdc15 activates the Dbf2-Mob1 kinase complex. Proc Natl Acad Sci USA 98, 7325–7330, https://doi.org/10.1073/pnas.141098998 (2001).
16. Rock, J. M. & Amon, A. Regulation of the mitotic exit protein kinases Cdc15 and Dbf2. Mol Biol Cell 12, 2961–2974 (2001).
17. Bertazzi, D. T., Kurtulmus, B. & Pereira, G. The cortical protein Lte1 promotes mitotic exit by inhibiting the spindle position checkpoint kinase Kin4. J Cell Biol 193, 1033–1048, https://doi.org/10.1083/jcb.201101056 (2011).
18. Chan, L. Y. & Amon, A. Spindle position is coordinated with cell-cycle progression through establishment of mitotic exit-activating and -inhibitory zones. Mol Cell 39, 444–454, https://doi.org/10.1016/j.molcel.2010.07.032 (2010).
19. Falk, J. E., Chan, L. Y. & Amon, A. Lte1 promotes mitotic exit by controlling the localization of the spindle position checkpoint kinase Kin4. Proc Natl Acad Sci USA 108, 12584–12590, https://doi.org/10.1073/pnas.1107784108 (2011).
20. Geymonat, M., Spanos, A., de Bettignies, G. & Sedgwick, S. G. Lte1 contributes to Bfa1 localization rather than stimulating nucleotide exchange by Tem1. J Cell Biol 187, 497–511, https://doi.org/10.1083/jcb.200905114 (2009).
21. Visintin, R. & Amon, A. Regulation of the mitotic exit protein kinases Cdc15 and Dbf2. Mol Biol Cell 12, 2961–2974 (2001).
22. Visintin, R. & Amon, A. Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. Mol Cell 2, 709–718 (1998).
23. Jaspersen, S. L., Charles, J. F., Tinker-Kulberg, R. L. & Morgan, D. O. A late mitotic regulatory network controlling cyclin destruction in Saccharomyces cerevisiae. Mol Biol Cell 9, 2803–2817 (1998).
24. Bardin, A. J., Boselli, M. G. & Amon, A. Mitotic exit regulation through distinct domains within the protein kinase Cdc15. Mol Cell 46, 5730–5736 (2012).
25. Meitinger, F. & Amon, A. Integrating high-throughput genetic interaction mapping and high-content screening to explore yeast spindle morphogenesis. J Cell Biol 188, 69–81, https://doi.org/10.1083/jcb.200909013 (2010).
44. Jensen, S., Johnson, A. L., Johnston, L. H. & Segal, M. Temporal coupling of spindle disassembly and cytokinesis is disrupted by deletion of LTE1 in budding yeast. Cell Cycle 3, 817–822 (2004).
45. Sudbery, P. E. The germ tubes of Candida albicans hyphae and pseudohyphae show different patterns of septin ring localization. Mol Microbiol 41, 19–31 (2001).
46. Warenda, A. J. & Konopka, J. B. Septin function in Candida albicans morphogenesis. Mol Biol Cell 13, 2732–2746 (2002).
47. Kelly, M. T. et al. The Candida albicans CaNKC2 gene affects morphogenesis, adherence and virulence. Mol Microbiol 53, 969–983, https://doi.org/10.1111/j.1365-2958.2004.04185.x (2004).
48. Gonzalez-Noxo, A. et al. Sep7 is essential to modify septin ring dynamics and inhibit cell separation during Candida albicans hyphal growth. Mol Biol Cell 19, 1509–1518, https://doi.org/10.1091/mbc.E07-09-0876 (2008).
49. Gutierrez-Escobano, P. et al. CDK-dependent phosphorylation of Mob2 is essential for hyphal development in Candida albicans. Mol Biol Cell 22, 2458–2469, https://doi.org/10.1091/mbc.E11-03-0265 (2011).
50. Bensen, E. S., Clemente-Blanco, A., Finley, K. R., Correa-Bordes, J. & Berman, J. The mitotic cyclins Clb2p and Clb4p affect morphogenesis in Candida albicans. Mol Biol Cell 16, 3387–3400, https://doi.org/10.1090/mc04-12-1081 (2005).
51. Xu, S., Huang, H. K., Kaiser, P., Latterich, M. & Hunter, T. Phosphorylation and spindle pole body localization of the Cdc15p mitotic regulatory protein kinase in budding yeast. Curr Biol 10, 329–332 (2000).
52. Guettin, D. A., Chang, L., Ireshad, F., Gould, K. L. & McColum, D. The role of the sid1p kinase and cdc14p in regulating the onset of cytokinesis in fission yeast. EMBO J 19, 1803–1815, https://doi.org/10.1093/emboj/19.8.1803 (2000).
53. Grollert, A. et al. A PP1-PP2A phosphatase relay controls mitotic progression. Nature 517, 94–98, https://doi.org/10.1038/nature14019 (2015).
54. Wurzenberger, C. & Gerlich, D. W. Phosphatases: providing safe passage through mitotic exit. Nat Rev Mol Cell Biol 12, 469–482, https://doi.org/10.1038/nrm3149 (2011).
55. Hu, K. et al. Shp1, a regulator of protein phosphatase 1 Glc7, has important roles in cell morphogenesis, cell cycle progression and DNA damage response in Candida albicans. Fungal Genet Biol 49, 433–442, https://doi.org/10.1016/j.fgb.2012.04.004 (2012).
56. Liu, Q. et al. Tpd3-Pph21 phosphatase plays a direct role in Sep7 dephosphorylation in Candida albicans. Mol Microbiol 101, 109–121, https://doi.org/10.1111/mmi.13576 (2016).
57. Nakayama, H. et al. Tetracycline-regulatable system to tightly control gene expression in the pathogenic fungus Candida albicans. Infect Immun 68, 6712–6719 (2000).
58. Wilson, R. B., Davis, D., Enloe, B. M. & Mitchell, A. P. A recyclable Candida albicans URA3 cassette for PCR product-directed gene disruptions. Yeast 16, 65–70 (2000).
59. Milne, S. W., Cheetham, J., Lloyd, D., Aves, S. & Bates, S. Cassettes for PCR-mediated gene tagging in Candida albicans utilizing nourseothricin resistance. Yeast 28, 833–841, https://doi.org/10.1002/yea.1910 (2011).
60. Gerami Nejad, M., Berman, J. & Gale, C. A. Cassettes for PCR-mediated construction of green, yellow, and cyan fluorescent protein fusions in Candida albicans. Yeast 18, 859–864, https://doi.org/10.1002/yea.738 (2001).
61. Gerami-Nejad, M., Dalmage, K. & Berman, J. Additional cassettes for epitope and fluorescent fusion proteins in Candida albicans. Yeast 26, 399–406, https://doi.org/10.1002/yea.1674 (2009).
62. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676–682, https://doi.org/10.1038/nmeth.2019 (2012).
63. Fonzi, W. A. & Irwin, M. Y. Isogenic strain construction and gene mapping in Candida albicans. Genetics 134, 717–728 (1993).
64. Noble, S. M. & Johnson, A. D. Strains and strategies for large-scale gene deletion studies of the diploid human fugal pathogen Candida albicans. Eukaryot Cell 4, 298–309, https://doi.org/10.1128/EC.4.2.298-309.2005 (2005).
65. Brand, A., MacCallum, D. M., Brown, A. J., Gow, N. A. & Odds, F. C. Ectopic expression of URA3 can influence the virulence phenotypes and proteome of Candida albicans but can be overcome by targeted reintroduction of URA3 at the RPS10 locus. Eukaryot Cell 3, 900–909, https://doi.org/10.1128/EC.3.4.900-909.2004 (2004).

Acknowledgements
Thanks to Jill Cheetham and Stephen Milne for technical support, and Isabelle Jourdain for critical reading of the manuscript. This work was supported by a UK Biotechnology and Biological Sciences Research Council (BBSRC) project grant (No. BB/F009232/1) to S.B.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-27157-y.

Competing Interests: The author declares no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.