Recruitment of the mitotic exit network to yeast centrosomes couples septin displacement to actomyosin constriction

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In many eukaryotic organisms cytokinesis is driven by a contractile actomyosin ring (CAR) that guides membrane invagination. What triggers CAR constriction at a precise time of the cell cycle is a fundamental question. In budding yeast CAR is assembled via a septin scaffold at the division site. A Hippo-like kinase cascade, the Mitotic Exit Network (MEN), promotes mitotic exit and cytokinesis, but whether and how these two processes are independently controlled by MEN is poorly understood. Here we show that a critical function of MEN is to promote displacement of the septin ring from the division site, which in turn is essential for CAR constriction. This is independent of MEN control over mitotic exit and involves recruitment of MEN components to the spindle pole body (SPB). Ubiquitination of the SPB scaffold Nud1 inhibits MEN signaling at the end of mitosis and prevents septin ring splitting, thus silencing the cytokinetic machinery.
Cytokinesis is the final stage of mitosis leading to the physical separation of the two daughter cells. In many eukaryotic organisms, such as fungi and animals, cytokinesis is driven by a contractile actomyosin ring (CAR) at the site of cell division. CAR constriction during cytokinesis drives invagination of the overlying plasma membrane inward to cleave the cell in two. Besides generating force, CAR constriction in yeast is also coupled to membrane deposition and formation of a primary septum.

Septins have been implicated, besides CAR, in cytokinesis in many eukaryotes. Septins are cytoskeletal guanosine triphosphate (GTP)-binding proteins that form oligomeric complexes that can in turn self-organize in higher-order structures, such as filaments and rings. Studies in budding yeast and mammalian cells indicate that septins act as scaffolds to recruit cytokinesis factors to the site of cell division and regulate CAR constriction (reviewed in ref. 3). Furthermore, Drosophila and human (but not yeast) septins bundle and bend actin filaments for CAR assembly.

Septins are essential for cytokinesis in the budding yeast Saccharomyces cerevisiae, where they recruit CAR components and other cytokinetic proteins to the division site (reviewed in ref. 3). Budding yeast septins form rod-shaped heteromeric complexes that join end-to-end in nonpolar filaments, which in turn organize in a ring that interacts tightly with the plasma membrane at the bud neck, the constriction between mother and future daughter cell. Septins are first recruited in the G1 phase of the cell cycle to the presumptive bud site as unorganized septin clouds or patches, which are then rapidly transformed into a cortical septin ring. At the time of bud emergence the septin ring expands into an hourglass-shaped septin collar, which spans the whole bud neck. Immediately prior to cytokinesis the septin collar suddenly splits into two distinct rings that sandwich the constricting CAR. This remarkable rearrangement is accompanied by a 90° rotation of septin filaments, which are aligned parallel to the mother-bud axis in the collar while they lie orthogonally to it in the split rings. Furthermore, fluorescence recovery after photobleaching experiments showed that while the septin collar is a rigid structure, split septin rings are dynamic. The relevance of septin ring splitting for cytokinesis is poorly understood, mainly due to the lack of mutants specifically defective in this process. Since both septins and the CAR must contact the plasma membrane, it is plausible that septins impose a physical constraint to CAR assembly or contraction that is overcome by septin splitting. However, this hypothesis could not be experimentally tested so far.

The mitotic exit network (MEN) is an essential Hippo-like kinase cascade that promotes mitotic exit and cytokinesis in budding yeast (reviewed in ref. 12). MEN includes the upstream GTPase Tem1, which activates the Ste20-like Cdc15 kinase that in turn upregulates the NDR kinases Dbf2 and Dbf2O in association with their Mob1 activator. The Tem1 GTPase can be inhibited by the two component GTPase-activating protein (GAP) Bub2-Bfa1, whose activity is antagonized by the polo kinase Cdc5 with their Mob1 activator. The Tem1 GTPase can be inhibited by the two component GTPase-activating protein (GAP) Bub2-Bfa1, whose activity is antagonized by the polo kinase Cdc5.

Results

Septin ring splitting and AMR contraction are spatially and temporally separated. The myosin II Myo1, which is a major CAR component, is first recruited to the septin ring in late G1 and forms the CAR in late mitosis. To determine if the contractile Myo1 ring is still connected to septins after their splitting, we applied super-resolution three-dimensional structured illumination microscopy (3D-SIM) on fixed cells expressing the septin Shs1 tagged with mCherry along with GFP-tagged Myo1. We found that the Myo1 ring has a smaller diameter than the split septin rings (0.6 vs. 1 μm) and it is placed 0.2 μm away from the split septin rings (Fig. 1a). Thus, at the time of cytokinesis CAR and septins are physically separated.

Previous data showed that CAR constriction occurs approximately at the same time as septin ring splitting, however, the exact timing between the two events has not been determined. We therefore carefully quantified the fluorescence associated to Shs1-mCherry and Myo1-GFP at the bud neck during cytokinesis by live cell imaging. Indeed, septin ring splitting is accompanied by loss of septin subunits, which causes a decrease in Shs1 fluorescence. Additionally, the relative density of Myo1 at the CAR remains constant during contraction, decreasing in levels while CAR circumference shrinks. Our measurements indicate that septin ring splitting precedes by 4–5 min CAR contraction (Fig. 1b). We conclude that the two events are spatially and temporally separated.

MEN factors are required for septin ring splitting independently of mitotic exit. To get a comprehensive view of the control of septin ring splitting and CAR constriction by the MEN cascade, we analyzed these events by time lapse imaging in conditional MEN mutants expressing either wild-type CDC14 or the dominant CDC14TA86 allele that partially bypasses MEN requirement for mitotic exit by loosening Cdc14 association with its nucleolar anchor. As expected, the temperature-sensitive ndu1-44, dbf2-2, mob1-77, cdc14-3, as well as the repressible GAL1-UPL-TEM1 and the analogue-sensitive cdc15-as1 mutants, in restrictive conditions arrested in late mitosis with large buds, unsplit septin rings and stable CAR at the bud neck (Supplementary Fig. 1a–f). In agreement with previous data, forcing mitotic exit in GAL1-UPL-TEM1 cells through the
CDC14TAB6-1 allele allowed entry into a new cell cycle without cytokinesis, as assessed by rebudding in the absence of septin ring splitting or CAR constriction (Fig. 2a). Furthermore, fluorescence-activated cell sorting (FACS) analysis on synchronized cell populations showed that while GAL1–UPL–TEM1 cells arrested mainly with 2C DNA content, GAL1–UPL–TEM1–CDC14TAB6–1 cells exhibited mitosis and underwent a second round of DNA replication without cytokinesis, as shown by the accumulation of cells with 4C DNA content (Fig. 2b).

We then asked which MEN components are required for septin ring splitting downstream of Tem1. Similar to Tem1 inactivation, inhibition of the Tem1 effector kinase Cdc15 in cdc15-as1 CDC14TAB6–1 cells prevented both septin ring splitting and CAR constriction (Fig. 2c). This resulted in prominent cytokinesis defects, as shown by FACS analysis of DNA contents on whole cell populations (Fig. 2d).

Cdc15 activates the downstream Dbf2 kinase in association with its activating subunit Mob1, both through Dbf2 phosphorylation and recruitment of the Mob1–Dbf2 complex to SPBs by phosphorylation of the scaffold protein Nud1. Mob1 inactivation through the temperature-sensitive mob1–77 allele in combination with CDC14TAB6–1 led to pronounced cell lysis in most cells in synthetic medium (SD) medium at 32 and 34 °C. However, in a few cells that remained intact during the temperature shift we could observe mitotic exit without concomitant septin ring splitting and CAR constriction (Fig. 2e), consistent with previously reported cytokinesis defects. These were further confirmed by FACS analysis of DNA contents on synchronized cells populations (Fig. 2f). In sharp contrast, inactivation of the Dbf2 kinase through the temperaturesensitive dbf2–2 allele in CDC14TAB6–1 cells did not prevent either septin splitting or CAR constriction (Supplementary Fig. 2a), allowing cytokinesis in virtually all cells at 34 °C (Supplementary Fig. 2b). Similar results were obtained by additionally deleting the Dbf2 paralogue Dbf20 in dbf2–2 CDC14TAB6–1 cells at 35.5 °C, i.e., the maximal temperature at which these cells could still exit mitosis (Supplementary Fig. 2c).

To definitely ascertain if Dbf2 is dispensable for septin ring splitting, we introduced one or three miniAID tags (AID: auxin-inducible degron) at the 3' end of the dbf2–2 open reading frame to allow for the rapid depletion of Dbf2 in the presence of indoleacetic acid (IAA) and upon expression of the E3 ligase OsTir1 from the galactose-inducible GAL1 promoter. Insertion of 1miniAID or 3miniAID at the 3' end of dbf2–2 was lethal in dbf2Δ cells even in the absence of IAA and galactose, suggesting that tagging compromises Dbf2 protein function. In contrast,
**Fig. 2** The MEN factors Tem1, Cdc15, and Mob1 are required for septin ring splitting and CAR contraction independently of mitotic exit. **a**, **c**, **e** Cells with the indicated genotypes were grown in permissive conditions and then shifted to restrictive conditions 60–90 min prior to imaging. Cells were filmed every 2 min (**a**) or 4 min (**c**, **e**) for 4–8 h in restrictive conditions (**a** glucose-containing medium; **c** medium supplemented with 5 µM 1NM-PP1; **e** 32 °C). Arrowheads indicate the appearance of new septin rings (yellow) or CARs (white) before the old structures have been disassembled. DIC differential interference contrast. TL transmitted light. Scale bar: 5 μm. **b**, **d**, **f** Cells with the indicated genotypes were grown in permissive conditions (**b** YEPRG; **d**, **f** YEPD) at 25 °C, arrested in G1 with alpha factor and then released in restrictive conditions (**b** YEPD; **d** YEPD containing 5 µM 1NM-PP1; **f** YEPD at 32 °C). At various time points after release (time 0) cells were collected for FACS analysis of DNA contents. FACS data were plotted after gating out the debris as illustrated in Supplementary Fig. 12.

*dbf20Δ CDC14TAB6-1* cells carrying *dbf2-2-miniAID* constructs were viable and proliferated efficiently in glucose- and galactose-containing medium (*GAL1-OSI1* off and on, respectively; Supplementary Fig. 2f), indicating that entrapment of Cdc14 in the nucleolus is the main cause of the lethality linked to AID-tagging of *dbf2-2*. Furthermore, *dbf2-2-3miniAID dbf20Δ CDC14TAB6-1 GAL1-OSI1* cells stopped proliferating on IAA-containing galactose medium at 30 °C (Supplementary Fig. 2f), indicating that Dbf2 depletion could be efficiently achieved. Imaging of the septin GFP-Cdc12 in these cells dividing in the presence of IAA and galactose at 30 °C confirmed that the Dbf2/Dbf20 kinases are not required for septin ring splitting (Supplementary Fig. 2e), in agreement with previous conclusions. Indeed, all cells that exited mitosis during the movie, as assessed by the appearance of a new bud and a new septin ring, previously split the pre-existing septin ring at the bud neck (*n* = 53).

Thus, the whole MEN cascade is essential for septin ring splitting and CAR constriction through the downstream Cdc14 phosphatase. Additionally, the Tem1 GTPase, its effector kinase Cdc15 and the Mob1 protein, but not its associated kinases Dbf2/Dbf20, are required for these processes also independently of their role in mitotic exit.

**The ubiquitin-ligase Dma2 prevents septin ring splitting and CAR constriction.** We previously showed that overexpression of the E3 ubiquitin ligase Dma2 prevents septin ring splitting and cytokinesis without hampering mitotic exit, thus causing the accumulation of chains of cells with stable septin rings at bud necks and accumulation of ≥4C DNA contents (Fig. 3a). We, therefore, wondered if lack of septin ring splitting was accompanied by a failure to constrict the CAR. Time lapse imaging of cells overexpressing DMA2 from the galactose-inducible GAL1 promoter and expressing Shs1-mCherry along with Myo1-GFP showed indeed that CAR was not contracting. At the end of the cell cycle, cells exited mitosis and rebudded after forming a new septin ring, but kept the old septin collar and unconstricted CAR at the bud neck (Fig. 3b). This prevented formation of a septum between the two dividing cells that in most cases shared a common cytoplasm, as shown by transmission electron microscopy (Fig. 3c). Introducing the *CDC14TAB6-1* allele in *GAL1-DMA2* cells overexpressing DBF2/DBF20 from the galactose-inducible promoter restored septin ring splitting and CAR constriction (Fig. 3d).

**Fig. 3** Overexpression of the E3 ubiquitin ligase Dma2 prevents septin ring splitting and cytokinesis without hampering mitotic exit. **a** Cells with the indicated genotypes and ei3300, a temperature-sensitive allele of the E3 ubiquitin ligase Dma2, were grown in permissive conditions (GAL1-OSI1 or GAL1-OSI1 ei3300, in glucose-containing medium; YEPD containing 5 µM 1NM-PP1 at 32 °C). At 32 °C, or 32 °C + 1% galactose, 0 hr 0 hr 0 hr cells carrying *dbf20Δ CDC14TAB6-1* constructs were viable and proliferated efficiently in glucose- and galactose-containing medium (*GAL1-OSI1* off and on, respectively; Supplementary Fig. 2f), indicating that entrapment of Cdc14 in the nucleolus is the main cause of the lethality linked to AID-tagging of *dbf2-2*. Furthermore, *dbf2-2-3miniAID dbf20Δ CDC14TAB6-1 GAL1-OSI1* cells stopped proliferating on IAA-containing galactose medium at 30 °C (Supplementary Fig. 2f), indicating that Dbf2 depletion could be efficiently achieved. Imaging of the septin GFP-Cdc12 in these cells dividing in the presence of IAA and galactose at 30 °C confirmed that the Dbf2/Dbf20 kinases are not required for septin ring splitting (Supplementary Fig. 2e), in agreement with previous conclusions. Indeed, all cells that exited mitosis during the movie, as assessed by the appearance of a new bud and a new septin ring, previously split the pre-existing septin ring at the bud neck (*n* = 53).

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cells did not improve their ability to split septin rings or to constrict the CAR (Fig. 4c). These data confirm that DMA2 overexpression interferes with, without blocking, some aspects of mitotic exit31. Consistently, the chitin synthase Chs2, which gets overexpression interferes with, without blocking, some aspects of CAR assembly35, we asked if F-actin was timely recruited to the bud neck before septin splitting in all wild-type cells that failed to undergo septin splitting (Supplementary Fig. 3a, b, d).

Since we recently showed that Dma1/2 control the localization of the formins Bni1 and Bnr1 at polarity sites34, which in turn is important for CAR assembly35, we asked if F-actin was timely recruited to the CAR in Dma2-overexpressing cells. To this end, we synchronized wild-type and GAL1-DMA2 cells in G1 and released them in galactose-containing medium. At 120 and 150 min after release (end of the first cell cycle and beginning of the second cycle, respectively) cells were fixed for staining of F-actin with fluorescently labeled phalloidin. An actin ring was clearly visible at the bud neck in a small fraction of wild-type budded cells (Fig. 3d), consistent with the notion that actin is transiently recruited to the CAR shortly before constriction22,23. Similarly, GAL1-DMA2 cells formed actin rings with similar efficiency at the right time. Furthermore, chains of cells appeared often with actin rings, in agreement with lack of CAR constriction and disassembly (Fig. 3d). Consistent with normal F-actin ring assembly, the IQGAP Iggl, which is necessary for this process36, was recruited to the bud neck before septin splitting in all wild-type cells (n = 155; Supplementary Fig. 4a) and DMA2-overexpressing cells (n = 156; Supplementary Fig. 4b).

We, therefore, conclude that the cytokinesis defects of Dma2-overexpressing cells, and in particular the lack of CAR constriction, is not accounted for by inefficient actin recruitment to the division site.

Septin destabilization drives CAR constriction in DMA2-overexpressing cells. On the basis of the above results, we hypothesized that the septin collar might hamper CAR constriction. If this were the case, destabilization of septins could be sufficient to re-establish CAR constriction in mutants affecting septin ring splitting. We, therefore, introduced the cdc12-1 temperature-sensitive mutation in GAL1-DMA2 cells expressing Shs1-mCherry and Myo1-GFP and analyzed their behavior at semipermissive temperature (30 °C). In the majority of the cells analyzed (n = 47/68) Shs1 was cleared from the bud neck at the time of mitotic exit and this was immediately followed by Myo1...
mCherry and Myo1-GFP were grown in SD-raf bar: 5 raf indicated genotypes and expressing Shs1-mCherry and Myo1-GFP were induced with galactose for ~90 min and imaged every 2 min for 2 h at 30 °C in SD-
inference contrast. Arrowheads indicate disassembly of septin rings (yellow) or the onset of CAR constriction (white). DIC differential cell imaging showed that 88% of DMA2 on cytokinesis of cells (during the entire duration of the movie (2 h). Only a minority of 68), and therefore neither septin splitting nor CAR contraction, most of the remaining cells did not undergo mitotic exit (36). We, therefore, asked if deletion of DMA2 had an impact on cytokinesis, as shown by FACS analysis of DNA contents on synchronized cultures (Supplementary Figs. 5c and 6a), suggesting that late cytokinetic processes (e.g., septum formation or cell separation) might also be defective in DMA2-overexpressing cells.

constriction (Fig. 4a, d, e), indicating that septin clearance is sufficient to drive CAR constriction upon DMA2 overexpression. Most of the remaining cells did not undergo mitotic exit (n = 18/68), and therefore neither septin splitting nor CAR constraction, during the entire duration of the movie (2 h). Only a minority of cells (n = 3/68) underwent apparent septin clearance without CAR constriction. Deletion of the SHS1 gene in GAL1-DM2 cells led to similar results, i.e., was sufficient for clearance of the septin collar at mitotic exit and for CAR constriction upon Dma2 overexpression (Fig. 4b).

We, therefore, conclude that septin ring splitting or clearance at the division site is an essential prerequisite for CAR constriction.

The anillin-like protein Bud4 stabilizes septin rings during splitting. We, therefore, asked if deletion of BUD4 had an impact on cytokinesis of DMA2-overexpressing cells. Remarkably, live cell imaging showed that 88% of GAL1-DM2 bud4Δ cells (n = 233) underwent sudden septin disappearance in late mitosis that was shortly followed by CAR constriction (Supplementary Fig. 5a, b), further strengthening the notion that septin destabilization is sufficient to induce CAR constriction upon DMA2 overexpression. However, in the face of an apparently normal CAR constriction, GAL1-DM2 cdc12-1, GAL1-DM2 sha1Δ and GAL1-DM2 bud4Δ remained unable to accomplish full cytokinesis, as shown by FACS analysis of DNA contents on synchronized cultures (Supplementary Figs. 5c and 6a), suggesting that late cytokinetic processes (e.g., septum formation or cell separation) might also be defective in DMA2-overexpressing cells.

Dma2 prevents septin ring splitting through inhibition of MEN signaling. Moderate overexpression of DMA2 to levels that are well tolerated by wild-type cells was toxic for MEN mutants at permissive temperature, with tem1Δ displaying the most dramatic synthetic phenotype (Supplementary Fig. 7 and ref. 31). In light of these genetic interactions and given the remarkable phenotypic similarity between GAL1-DM2 and tem1 or cdc15 mutants forced to exit mitosis, we asked if Tem1 hyperactivation through the GTP-locked TEM1-Q79L allele17 could promote septin ring splitting/disappearance and CAR constriction in DMA2-overexpressing cells. Strikingly, 84% of the GAL1-DM2 TEM1-Q79L cells that we imaged for 2 h (n = 143) underwent septin clearance from the bud neck and CAR constriction shortly afterwards (Fig. 4c–e). Furthermore, TEM1-Q79L restored in most cells bud neck recruitment of Chs2, which then contracted with the CAR (Supplementary Fig. 3c, d).

These results further corroborate the idea that CAR constriction and septum formation are intimately coupled to septin ring
splitting. Furthermore, they suggest that high levels of Dma2 might prevent septin ring splitting along with CAR constriction and septum formation through downregulation of MEN signaling.

In spite of their apparently normal CAR constriction, GAL1-DMA2 TEM1-Q79L cells could not complete cell division, as shown by FACS analysis of DNA contents on synchronized cell cultures (Supplementary Fig. 6a). This result was surprising because we previously showed that deletion of the GTPase-activating protein (GAP) Bub2 or TEM1 overexpression could efficiently rescued the lethality and cytokinesis defects caused by an excess of Dma2. Furthermore, the TEM1-Q79L allele rescued the lethality of GAL1-DMA2 cells on galactose-containing media (Supplementary Fig. 6b), consistent with our previous findings.

We, therefore, wondered if the presence of a wild-type copy of BUD4 that we introduced in our W303 background to follow septin ring splitting (see Methods) could account for the wt kDa.
Dma2 promotes ubiquitination of the MEN scaffold at SPBs

Nud1. The septins Cdc11 and Shs1 were previously shown to be ubiquitinated by Dma1 and Dma237, which could underlie the mechanism by which Dma2 inhibits septin ring splitting. We re-investigated this issue using Ni-NTA pulldowns of ubiquitinated proteins from cells overexpressing untagged or His-tagged ubiquitin, followed by western blot to detect Cdc11-HA or Shs1-HA expressed at endogenous levels from their genomic loci. Unexpectedly, deletion of both DMA1 and DMA2 in our genetic background did not reduce the ubiquitination levels of either Cdc11 or Shs1, but conversely increased them (Supplementary Fig. 8a, b). Additionally, although DMA2 overexpression induced hyper-ubiquitination of both Cdc11 and Shs1 (Supplementary Fig. 8c, d), in agreement with previous data37, this was not suppressed by the TEM1-Q79L allele that allows septin clearance in DMA2-overexpressing cells (Supplementary Fig. 8e), suggesting that other targets might be instrumental for Dma1/2-dependent inhibition of septin ring splitting.

We considered that Tem1 could be a good candidate. Using the same experimental setup that we used for septins, we could clearly detect Tem1 ubiquitination in yeast extracts, consistent with previous data38. However, Tem1 ubiquitination was not affected by either DMA1/2 deletion or DMA2 overexpression (Supplementary Fig. 8f, g), suggesting that Tem1 is not ubiquitinated by Dma1/2.

The constitutive SPB component Nud1 is required for MEN signaling and mitotic exit by recruiting Tem1, Cdc15, and Mob1-Dbf2/20 in a hierarchical manner, thereby leading to Cdc14 release from the nucleolus15,16,18,19. Since Dma1, like its counterpart in Schizosaccharomyces pombe, is present at SPBs39,40, we reasoned that Nud1 could be a likely target of Dma1/2. Furthermore, a small fraction of HA-tagged Dma2 co-immunoprecipitated with 3Flag-tagged Nud1 in anaphase (Supplementary Fig. 9), suggesting that the two proteins physically interact in a cell cycle-regulated fashion. Strikingly, using Ni-NTA pulldown assays as above we found that ubiquitination of Nud1 was markedly affected by deletion of both DMA1 and DMA2 (Fig. 5a), while it was conspicuously upregulated by a short induction of GAL1-DMA2 (Fig. 5b). To get further insights into its physiological significance, we analyzed Nud1 ubiquitination throughout the cell cycle after G1 arrest and release of cells for different times. Interestingly, Nud1 ubiquitination was low in S, G2, and M but markedly induced from mitotic exit to G1 (Fig. 5c), suggesting that Nud1 ubiquitination by Dma2 might silence MEN signaling. Upon DMA2 overexpression Nud1 ubiquitination was enhanced most markedly between late mitosis and G1 (Supplementary Fig. 10a). Furthermore, it could be sterically upon GAL1-DMA2 induction in cells arrested in mitosis by nocodazole treatment, but not in cells arrested in S phase by hydroxyurea (Supplementary Fig. 10b). Altogether, these data suggest that Nud1 might be a direct ubiquitination target of Dma1/2 in late M and G1 phase.

We then investigated the consequences of DMA2 overexpression on the SPB recruitment of MEN factors specifically in anaphase, when the presence of MEN factors at SPBs reaches its peak. To this end, we calculated the ratio between the fluorescence intensity of MEN proteins tagged with GFP and that of the constitutive SPB component Spc42 tagged with mCherry. Additionally, since Tem1 and its GAP Bub2-Bfa1 localize asymmetrically at SPBs and are more concentrated on the bud-directed SPB, Recruitment of Tem1 and the polo kinase Cdc5, which promotes Tem1 activation by inhibiting the Bub2-Bfa1 GAP14, was unaffected by DMA2 overexpression. Conversely, localization of Bub2-Bfa1, Cdc15, and Mob1 at SPBs was inhibited under the same conditions (Fig. 5d and Supplementary Fig. 11a). Furthermore, SPB recruitment of Cdc15 and Mob1 was mildly but significantly stimulated upon deletion of DMA1 and DMA2 (Fig. 5d), suggesting that Nud1 ubiquitination by Dma1/2 antagonizes MEN signaling by attenuating its scaffolding activity toward MEN. Interestingly, localization of Mob1-GFP to the bud neck at cytokinesis19 was also impaired in GAL1-DMA2 cells, perhaps as a consequence of its reduced recruitment to SPBs. Indeed, while wild-type cells transiently showed Mob1-GFP at the bud neck in 43/70 cells during the time frame occurring between its appearance and disappearance at SPBs, only 5/60 GAL1-DMA2 cells did so (Fig. 5e).

As an additional readout of MEN activity at SPBs, we monitored the Cdc15-dependent Nud1 phosphorylation on Ser7816 throughout the cell cycle of wild-type and GAL1-DMA2 cells. Remarkably, while Nud1 Ser78 phosphorylation peaked in late mitosis in wild-type cells, consistent with previous data16, it was largely suppressed upon DMA2 overexpression (Fig. 5f and Supplementary Fig. 11b). Furthermore, total Nud1
Constitutive association between Dma2 and Nud1 prevents mitotic exit and cytokinesis. a Meiotic segregants obtained after sporulation of diploid cells generated by crossing NUD1-GBD with DMA2-eGFP haploid cells. Genotypes were confirmed by PCR. b Serial dilutions of cells with the indicated genotypes were spotted on YEPD and YEPG plates and incubated at 30 °C. c-e NUD1-GALs-DMA2-eGFP cells, either BUD4 or bud4-G2459fs, expressing Shs1-mCherry and grown in SD-raf were induced for 90 min with galactose and then imaged in SD-raf containing medium (i.e., noninduced conditions), causing them to prematurely die and often stop dividing, while NUD1-GBD GALs-DMA2-eGFP cells carrying the truncated bud4-G2459fs allele of W303 (see Methods) were healthy in the same conditions and stopped dividing only after galactose induction, suggesting that the C-terminus of Bud4 might somehow compromise MEN signaling under these sensitized conditions.

Altogether, our data clearly indicate that Dma2 is a powerful inhibitor of MEN signaling at SPBs.
from the nucleolus was also somewhat impaired in the GAL1-DMA2 mutant, with many cells showing only partial or ephemeral release, consistent with the idea that high levels of Dma2 interfere with MEN signaling.

If lack of septin ring splitting in GAL1-DMA2 cells were due to insufficient levels of Cdc14 at SPBs, we might expect to restore efficient splitting and cytokinesis by artificially recruiting Cdc14 to the SPB. To force constitutive anchoring of Cdc14 to SPBs, we expressed in NUD1-GBD cells an extra copy of the CDC14 gene fused to GFP. In fact, given the plethora of functions that Cdc14 plays in several processes, we reasoned that constitutive anchorage of all Cdc14 at SPBs would be lethal. This strategy robustly recruited Cdc14 to mother and daughter SPBs throughout the cell cycle (Fig. 7b and Supplementary Movie 1). Remarkably, this was sufficient to cause septin disappearance from the bud neck and to promote cytokinesis in 97% of DMA2-overexpressing cells (n = 337; Fig. 7b and Supplementary Movie 1). This was confirmed on synchronized cell populations that were released from a G1 arrest in the presence of galactose to induce GAL1-DMA2 overexpression (Fig. 7c): while GAL1-DMA2 NUD1-GBD cells underwent cytokinesis defects that caused the accumulation of cells with DNA contents ≥2C in the second cell cycle, GAL1-DMA2 NUD1-GBD CDC14-GFP cells showed efficient cytokinesis.
Discussion

Although we know since long that in budding yeast CAR constriction takes place between split septin rings, the role and regulation of septin ring splitting has remained mysterious, mainly due to the lack of mutants specifically affecting this process. Previous evidence that Tem1 depletion prevents both septin ring splitting and CAR constriction in cells that are forced to release Cdc14 from the nucleus did not rule out the possibility that some MEN components are involved in both processes independently of their role in mitotic exit. Our data show that during an unperturbed cell cycle septin ring splitting precedes temporally CAR constriction and no physical connections can be detected between septins and CAR by SIM microscopy during cytokinesis.

Furthermore, our results firmly establish that septin displacement from the division site is an absolute requirement for subsequent CAR constriction and cytokinesis. Indeed, mutants affecting septin splitting not only invariably fail to undergo CAR constriction, but septin destabilization (through septin mutant alleles or deletion of the anillin Bud4) also causes disappearance of septins from the bud neck during mitotic exit and is sufficient to promote CAR constriction. Thus, while being necessary for recruitment of CAR components to the bud neck, eviction of the septin collar from the division site is likewise essential for cytokinesis to take place. This provides an intrinsic safe-lock mechanism that ensures the correct temporal order of cytokinetic events.

This mechanism may be conserved in other organisms. In fission yeast, where a septin ring at the medial site is involved in septation but dispensable for CAR assembly, the septin ring also splits in two before cytokinesis, suggesting that septin ring splitting could facilitate CAR constriction. Conversely in Droso- phila, where septins bundle actin filaments for CAR assembly, septins are integral part of the CAR and constrict with it.

How exactly the septin ring restraints CAR constriction in yeast is a crucial question to be addressed in the future. We show here that lack of septin ring splitting also restrains recruitment to the bud neck of the chitin synthase Chs2, whose synthesis of the primary septum is coupled to CAR constriction. However, CAR constriction initiates in the absence of Chs2, suggesting that other factors must be invoked to explain the inhibitory effects of septins on this process. One possibility is that the septin collar acts as a physical constraint by either preventing proper contacts between CAR and plasma membrane or harnessing the CAR in a nonconstrictable arrangement.

The best understood function of MEN is to promote activation of the Cdc14 phosphatase, thereby leading to inactivation of mitotic CDKs (reviewed in ref. 20). Since persistent CDK activity inhibits cytokinesis in many organisms, it is not surprising that MEN mutants are unable to bring about cytokinetic events, including septin ring splitting and CAR constriction. An important issue, however, is whether MEN factors play a direct role in cytokinesis beyond Cdc14-mediated CDK inhibition. Indeed, several MEN factors relocalize to the bud neck in late anaphase. MEN mutants that are allowed to exit mitosis in restrictive conditions through forced nuclear release of Cdc14 (e.g., by NETI deletion or the dominant CDC14<i><sup>TAB6-1</sup></i> allele) or inhibition of mitotic CDKs (e.g., by overexpression of the CDK inhibitor Sic1) have been used to address this issue. This strategy has allowed establishing a key role for the Dbf2 kinase in septum formation through direct phosphorylation of the chitin synthase Chs2 and its regulatory complex Hof1-Inn1-Cyk3. Furthermore, it has implicated the Tem1 GTHase in septin ring splitting and CAR constriction. We have confirmed and extended this result, by showing that the Cdc15 kinase and Mob1 are also required for these processes downstream of Tem1. Surprisingly, the Dbf2/Dbf20 kinase seems to be dispensable for septin ring splitting, as shown by the behavior of mutants where Dbf2 can be either heat-inactivated or depleted through an auxin-inducible degron in cells lacking Dbf20. Although we cannot rule out that residual amounts of Dbf2 are sufficient for septin splitting in our experimental set-up, our results are consistent with published data. Thus, Mob1 could promote septin ring splitting by associating to kinases other than Dbf2/Dbf20, or by directly enforcing Cdc14 recruitment to the SPB.

Our study has revealed a key role for SPB-localized Cdc14 in septin clearance from the bud neck that is independent of its mitotic exit function. The use of Dma2-overexpressing cells that are constitutively defective in septin ring splitting and cytokinesis (see below), while undergoing mitotic exit, has been instrumental to this discovery. Although Cdc14 might be essential for mitotic exit only in budding yeast, its requirement for cytokinesis seems conserved. Potential and established Cdc14 cytokinetic targets have been identified. Although the critical substrates of budding yeast Cdc14 in septin ring splitting remain to be determined, potential candidates include Bud4 and the septin-associated kinase Gin. Additionally, Cdc14 could promote this process also by potentiating MEN signaling through positive feedback controls. The identification of MEN targets in septin ring splitting will be key to further dissect the mechanistic details underlying this process.

Our previous genetic data suggested that Dma1/2 might negatively regulate MEN signaling through an unknown mechanism. Several new observations described here are consistent with this hypothesis: (i) MEN mutants are hypersensitive to moderate Dma2 overexpression; (ii) the cytokinetic defects of Dma2-overexpressing cells are remarkably similar to those of MEN mutants undergoing mitotic exit through the Cdc14<i><sup>TAB6-1</sup></i> allele; (iii) constitutive recruitment of Dma2 to SPBs delays blocks mitotic exit; and (iv) the cytokinetic defects of GAL1-DMA2 cells are bypassed by a GTP-locked variant of Tem1 (Tem1-Q79L). Altogether, these observations indicate that GAL1-DMA2 is a hypomorphic MEN mutant. Consistently, upon DMA2 overexpression Cdc14 nuclear release is somewhat impaired, and degradation of some APC<i>Cdh1</i> targets, such as Cdc5, but not Cbl2, is delayed. We now show that Dma1/2 promote ubiquitination of the SPB component Nud1, thereby weakening its ability to recruit and activate MEN factors, such as Cdc15, Mob1, Bub2-Bfa1, and Cdc14. We further show that Nud1 ubiquitination takes place in late mitosis, presumably after cytokinesis, and is rapidly delayed by Dma2 overexpression preferentially in mitosis and G1 phase, suggesting that Dma2 might directly ubiquitinate Nud1. The finding that Dma1 (and presumably Dma2) localize to SPBs in late mitosis is consistent with this conclusion. We envisage that the physiological role of Nud1 ubiquitination by Dma1/2 during the normal cell cycle is to turn down MEN signaling at SPBs after cytokinesis (see Fig. 8). Indeed, Cdc14 inactivation and re-entrapment in the nucleus is likely an important step for the subsequent cell cycle, as persistent release of Cdc14 from the nucleus interferes with DNA replication. Since deletion of DMA1 and DMA2 is well tolerated by yeast cells, redundant mechanisms obviously participate to timely MEN silencing after mitotic exit and cytokinesis in unperturbed conditions. One of them is degradation of the polo kinase Cdc5.
Together with Sid4, the human protein centrin, which shares homology with S.c. Nud1 and S.p. Cdc11, resides at centrioles and is required for abscission, the latest cytokinetick step. Likely, recruitment of cytokinesis-promoting factors to the SPB/centrosome is an imperative, yet intermediate step in their journey toward the division site, in order to get fully active before proceeding to their final destination and targets. Consistently, Nud1 ubiquitination by Dma1/2 not only lowers the levels of Mob1 at SPBs, but also prevents its translocation to the bud neck in late anaphase. Considering that Nud1 is required for Mob1 localization at the bud neck, we hypothesize that its inability to reach the division site in Dma2-overexpressing cells is a mere consequence of its lousy activation at SPBs.

It is worth noting that the E3 ligase Dma1 in S. pombe negatively controls SIN signaling by ubiquitinating the SPB component Sid4 (related to budding yeast Cnm67), which in turn recruits the Nud1-related protein Cdc11 and downstream SIN factors. This leads to a decrease in polo kinase levels at SPBs, thereby preventing cytokinesis upon spindle depolymerization.

In the future, it will be of great interest to study Dma1 overexpression in budding yeast, which does not interfere with recruitment of the polo kinase Cdc5 to SPBs. However, it is remarkable how the two yeasts, which are evolutionary as distant from one another as each of them is distant from humans, have adopted similar, though distinct, strategies to silence MEN/SIN. Thus, an exciting possibility is that other eukaryotes might have evolved related mechanisms to prevent cytokinesis under adverse conditions in order to preserve genome stability.

Methods

Strains and growth conditions. All yeast strains (Table S1) are congeneric to or at least four times backcrossed to W303 (ade2-1, his3-112, leu2-3,112, lys2-801, met15, trp1-1, ura3-1). W303 bears a single nucleotide deletion in the BUD4 gene (bud4-G2459fs) that results in a premature stop codon. The bud4-G2459fs gene produces a truncated protein of 838 amino acids that lacks 609 amino acids and carries 18 non-natural amino acids at C-terminus (https://www.yeastgenome.org). All strains used for time-lapse video microscopy to look at septin ring splitting/disappearance have been corrected to carry full length BUD4 unless specified. It should be noted that Dma2 overexpression prevents septin ring splitting in both the original bud4-G2459fs and the corrected BUD4 background.

Yeast cultures were grown at 25–30 °C, unless otherwise specified, in either SD supplemented with the appropriate nutrients or YEP (1% yeast extract, 2% bactopeptone, 50 mg/l adenine) medium. Raf (4 µg/ml) in YEP medium containing the appropriate sugar at 23 °C. G1 arrest was monitored under a transmitted light microscope and cells were released in fresh medium (typically after 120–135 min of alpha factor treatment) after being collected by centrifugation at 2000g and washed with YEP containing the appropriate sugar. IAA (3-indoleacetic acid) was dissolved in ethanol as 1000× stock solutions and used at a final concentration of 0.1–0.25 µM.

Generation and integration in the genome of the Gal1-DMA2 construct has been described. The CDC14-GEF plasmid was a generous gift from A. Fatica. One-step tagging techniques were used to generate 3HA-, 3PK-, 3Flag-, GFP-, eGFP-, mCherry-, Yim1AID-, Yim1AID, GBD-tagged proteins at the C-terminus. Yeast cultures were grown at 25–30 °C, unless otherwise specified, in either SD supplemented with the appropriate nutrients or YEP (1% yeast extract, 2% bactopeptone, 50 mg/l adenine) medium. Raf (4 µg/ml) in YEP medium containing the appropriate sugar at 23–25 °C. G1 arrest was monitored under a transmitted light microscope and cells were released in fresh medium (typically after 120–135 min of alpha factor treatment) after being collected by centrifugation at 2000g and washed with YEP containing the appropriate sugar. IAA (3-indoleacetic acid) was dissolved in ethanol as 1000× stock solutions and used at a final concentration of 0.1–0.25 µM.

**Primers used in this study for gene tagging.** Sequences of bold names are used in the table of sequences to tag the tag when tagging Cdc10 and Cdc11 with GFP and tagging Nud1 with 3Flag. MYO1-GFP was a generous gift of J. Pringle; SCP42-mCherry of E. Schwob; GEP-MO1 of F. Luca; GEP-CDC12 of Y. Barral; CDC11-1A and SHS1-1A of E. Johnson; CHS2-GFP of E. Conibear. IQG1-GEF was derived from strain BY25825 of the YGRC that was provided by the NBRP of the MEXT, Japan.

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formaldehyde for 50 min under shaking at R.T. F-actin was visualized with Alexa Fluorescence microscopy.

Fluorescence microscopy. F-actin staining was performed on cells expressing ubiquitin with eGFP or myo1-MBP1 to monitor the dynamics of actin filament formation and elongation. To detect spindle formation and elongation, alpha-tubulin immunostaining was performed on formaldehyde-fixed cells using the YOL3 monoclonal antibody (1:100; MCA78S AbD Serotec, Raleigh, NC), followed by indirect immunofluorescence using Cy2-conjugated anti-rat antibody (1:100; 3164 Pierce Chemical Co.).

Detection of ubiquitin conjugates. Cells growing on coverslips were fixed with 3% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.4 (PB) for 2 h at room temperature, then washed three times with PB buffer containing 2% BSA. Cells were then washed twice with buffer A supplemented with 0.05% Tween 20 and treated with a solution of 0.2 M glycine and 0.05% Tween 20 for 10 min at 4 °C to allow the removal of the BSA and to reduce the background. After washing, cells were resuspended in 50 mM NH4Cl. Cells were subjected to hypotonic shock (0.1 M Tris-Cl, pH 8, 0.5% Triton X-100) for 3 min at 4 °C before fixation with 10% trichloroacetic acid (TCA) at 4 °C. Alternatively, cells were resuspended in 10% TCA and lysed by mechanical shearing with glass beads (Fig. 5c and Supplementary Fig. 2A). TCA precipitates were recovered by centrifugation. Samples were subjected to three cycles of freezing and thawing, followed by centrifugation at 25,000 × g for 20 min at 4 °C. Supernatants were then used for ubiquitin conjugate detection by immunoblotting.

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2. VerPlank, L. & Li, R. Cell cycle-regulated trafficking of Chs2 controls actomyosin ring stability during cytokinesis. *Mol. Biol. Cell* **16**, 2529–2543 (2005).
3. Oh, Y. & Bi, E. Septin structure and function in yeast and beyond. *Trends Cell Biol.* **21**, 141–148 (2010).
4. Mavrikas, M. et al. Septins promote F-actin ring formation by crosslinking actin filaments into curved bundles. *Nat. Cell Biol.* **16**, 322–334 (2014).
5. Barbieri, A. et al. Saccharomyces cerevisiae septins: supramolecular organization of heterooligomers and the mechanism of filament assembly. *Proc. Natl Acad. Sci. USA* **105**, 8274–8279 (2008).
6. Bridges, A. A. et al. Septin assemblies form by diffusion-driven annealing on membranes. *Proc. Natl Acad. Sci. USA* **111**, 2146–2151 (2014).
7. Lippincott, J., Shannon, K. B., Shou, W. & Li, R. The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis. *J. Cell Sci.* **114**, 1379–1386 (2001).
8. Wloka, C. et al. Evidence that a septin diffusion barrier is dispensable for cytokinesis in budding yeast. *Biol. Chem.* **392**, 813–829 (2011).
9. Vrabioiu, A. M. & Mitchison, T. J. Structural insights into yeast septin organization from polarized fluorescence microscopy. *Nature* **443**, 466–469 (2006).
10. Caviston, J. P., Longtine, M., Pringle, J. R. & Bi, E. The role of Cdc42p GTPase-activating proteins in assembly of the septin ring in yeast. *Mol. Biol. Cell* **14**, 4051–4068 (2003).
11. Dobbelere, J., Gentry, M. S., Hallberg, R. L. & Barral, Y. Phosphorylation-dependent regulation of septin dynamics during the cell cycle. *Dev. Cell* **4**, 345–357 (2003).
12. Juanes, M. A. & Pietti, S. The final cut: cell polarity meets cytokinesis at the bud neck in *S. cerevisiae*. *Cell. Mol. Life Sci.* **73**, 3115–3136 (2016).
13. Geymonat, M. et al. Control of mitotic exit in budding yeast. In *vivo regulation of Tem1 GTPase by Bub2 and Bfa1*. *J. Biol. Chem.* **277**, 28439–28445 (2002).
14. Hu, F. et al. Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. *Cell* **107**, 655–665 (2001).
15. Grunenberg, U., Campbell, K., Simpson, C., Grindlay, J. & Schiebel, E. Nud1p links astral microtubule depolymerization and the control of exit from mitosis [in process citation]. *EMBO J.* **19**, 6475–6488 (2000).
16. Rock, J. M. et al. Activation of the yeast Hippo pathway by phosphorylation-dependent assembly of signaling complexes. *Science* **340**, 871–875 (2013).
17. Scarfone, I. et al. Asymmetry of the budding yeast Tem1 GTPase at spindle poles is required for spindle positioning but not for mitotic exit. *PLoS Genet.* **10(e1004938)** (2015).
18. Valero-Santiago, M. & Monje-Casas, F. Tem1 localization to the spindle pole bodies is essential for mitotic exit and impacts spindle checkpoint function. *J. Cell Biol.* **192**, 599–614 (2011).
19. Visintin, R. & Amon, A. Regulation of the mitotic exit protein kinases cdc15 and dbf2. *Mol. Cell Biol.* **12**, 2961–2974 (2002).
20. Stegemeyer, F. & Amon, A. Closing mitosis: the functions of the Cdc4 phosphatase and its regulation. *Annu. Rev. Genet.* **38**, 203–232 (2004).
21. Krapp, A., Gulli, M. P. & Simanis, V. SIN and the art of splitting the fission yeast cell. *Curr. Biol.* **14**, R722–R730 (2004).
22. Bi, E. et al. Involvement of an actomyosin contractile ring in Saccharomyces cerevisiae cytokinesis. *J. Cell Biol.* **142**, 1301–1312 (1998).
23. Lippincott, J. & Li, R. Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis. *J. Cell Biol.* **140**, 355–366 (1998).
24. Wloka, C. et al. Immobile myosin-II plays a scaffolding role during cytokinesis in budding yeast. *J. Cell Biol.* **200**, 271–286 (2013).
25. Shou, W. et al. Net1 stimulates RNA polymerase I transcription and regulates nucleolar structure independently of controlling mitotic exit. *Mol. Cell* **8**, 45–55 (2001).
26. 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 (2001).
27. Luca, F. C. et al. Saccharomyces cerevisiae Moblp1 is required for cytokinesis and mitotic exit. *Mol. Cell Biol.* **21**, 6972–6983 (2001).
28. Kubota, T., Nishimura, K., Kanemaki, M. T. & Donaldson, A. D. The Elg1 replication factor C-like complex functions in PCNA unloading during DNA replication. *Mol. Cell Biol.* **20**, 273–280 (2010).
29. Meitinger, F. et al. Targeted localization of Inn1, Cyk3 and Chs2 by the Cdc42p-activated Mob1 kinase complex. *Mol. Cell Biol.* **20**, 1996–2007 (2000).
30. Smock, J. & Thalhammer, G. M. The spindle position checkpoint by promoting the recruitment of the Elm1 kinase to the bud neck. *Proc. Natl Acad. Sci. USA* **101**, 17458–17463 (2004).
31. Triboulot, S., Bistrocchi, L., Nisse, T., Wenseler, A. & Deshaies, R. J. The role of the formin Chs2 in the bud neck complex. *Nat. Cell Biol.* **14**, 1799–1810 (2012).
32. Bistocchi, L., VerPlank, L. & Li, R. Rho1 directs formin-mediated actin ring assembly during budding yeast cytokinesis. *Curr. Biol.* **12**, 1864–1870 (2002).
36. Epp, J. A. & Chant, J. An IQGAP-related protein controls actin-ring formation and cytokinesis in yeast. *Curr. Biol*. 7, 921–929 (1997).

37. Chahwan, R., Gravel, S., Matsusaka, T. & Jackson, S. P. Dma/RNF8 proteins are evolutionarily conserved E3 ubiquitin ligases that target septins. *Cell Cycle* 12, 1000–1008 (2013).

38. Cassani, C. et al. Saccharomyces cerevisiae Dma proteins participate in cytokinesis by controlling two different pathways. *Cell Cycle* 12, 2794–2808 (2013).

39. Guertin, D. A., Venkatram, S., Gould, K. L. & McCollum, D. Dma1 prevents mitotic exit and cytokinesis by inhibiting the septation initiation network (SIN). *Dev. Cell* 3, 779–790 (2002).

40. Yau, R. G. et al. Release from myosin V via regulated recruitment of an E3 ubiquitin ligase controls organelle localization. *Dev. Cell* 28, 520–533 (2014).

41. Monje-Casas, F. & Amon, A. Cell polarity determinants establish asymmetry in MEN signaling. *Dev. Cell* 16, 132–145 (2009).

42. Pereira, G., Hofken, T., Grindlay, J., Manson, C. & Schiebel, E. The Bub2p ubiquitin ligase controls organelle localization. *J. Cell Biol*. 179, 423–436 (2007).

43. Rothbauer, U. et al. A versatile nanotrap for biochemical and functional studies with fluorescent fusion proteins. *Mol. Cell. Proteom.* 7, 282–289 (2008).

44. Mumborg, D., Müller, R. & Funk, M. Regulatable promoters of *Saccharomyces cerevisiae* can be used for transcriptional activity and their use for heterologous expression. *Nucleic Acids Res.* 22, 5767–5768 (1994).

45. Pereira, G., Manson, C., Grindlay, J. & Schiebel, E. Regulation of the Bfa1p-Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Mol. Cell* 6, 1–10 (2000).

46. Maekawa, H., Priest, C., Lechler, J., Pereira, G. & Schiebel, E. The yeast centrosome translates the positional information of the anaphase spindle into a cell-cycle signal. *J. Cell Biol.* 179, 157–168 (2007).

47. Berlin, A., Paoletti, A. & Chang, F. Mid2p stabilizes septin rings during cytokinesis in budding yeast. *J. Cell Biol.* 160, 1083–1092 (2003).

48. Tasto, J. J., Morrell, J. L. & Gould, K. L. An anillin homologue, Mid2p, acts during fusion yeast cytokinesis to organize the septin ring and promote cell separation. *J. Cell Biol.* 160, 1093–1103 (2003).

49. Fououounou, N., Loyer, N. & Le Borgne, R. Septins regulate the contractility of the actomyosin ring to enable adherens junction remodeling during cytokinesis of epithelial cells. *Dev. Cell* 24, 242–255 (2013).

50. Meitinger, F. et al. Phosphorylation-dependent regulation of the F-BAR protein Ho1 during cytokinesis. *Genes Dev.* 25, 875–888 (2011).

51. Gruneberg, U., Glotzer, M., Gartner, A. & Nigg, E. A. The Cdc14C-14C phosphate is required for cytokinesis in the Caenorhabditis elegans embryo. *J. Cell Biol.* 158, 901–914 (2002).

52. Wang, J., Liu, J., Hu, Y., Ying, S. H. & Feng, M. G. Cytokinesis-required Cdc14 is a signaling hub of axonal development and multi-stress tolerance in *Drosophila* and other organisms. *Cell Discovery* 4, 101–112 (2018).

53. Bloom, J. et al. Global analysis of Cdc14 phosphatase reveals diverse roles in mitotic processes. *J. Biol. Chem.* 286, 5434–5445 (2011).

54. Chen, J. S. et al. Comprehensive proteomics analysis reveals new substrates and regulators of the fission yeast cdc1/cdc14 phosphatase. *Mol. Cell. Proteom.* 12, 1074–1086 (2013).

55. Cremer, C. F., Bennett, A. M., Ma, W. K., Hall, M. C. & Yeong, F. M. Dependence of Chx2 ER export on dephosphorylation by cytoplasmic Cdc14 ensures that septum formation follows mitosis. *Mol. Biol. Cell* 23, 45–58 (2012).

56. Kuijlan, T. et al. Identification of Cdk targets that control cytokinesis. *EMBO J.* 134, 81–96 (2014).

57. Miller, D. P. et al. Dephosphorylation of Iqg1 by Cdc14 regulates cytokinesis in budding yeast. *Mol. Biol. Cell* 26, 2913–2926 (2015).

58. Eluere, R., Varlet, I., Bernadac, A. & Simon, M. N. Cdk and the anillin homolog Bud4 define a new pathway regulating septin organization in yeast. *Cell Cycle* 11, 151–158 (2012).

59. Jasperson, S. L. & Morgan, D. O. Cdc14 activates cdc15 to promote mitotic exit in budding yeast. *Curr. Biol.* 10, 615–618 (2000).

60. Konig, C., Maekawa, H. & Schiebel, E. Mutual regulation of cyclin-dependent kinase and the mitotic exit network. *J. Cell Biol.* 188, 351–368 (2010).

61. Bloom, J. & Cross, F. R. Novel role for cdc14 sequestration: cdc14 inhibits the spindle checkpoint links nuclear migration with mitotic exit. *J. Cell Biol.* 157, 888 (2002).

62. Bloom, J. et al. Global analysis of Cdc14 phosphatase reveals diverse roles in mitotic exit and cytokinesis by inhibiting the septation initiation network (SIN). *Dev. Cell* 3, 779–790 (2002).

63. Visintin, C. et al. APC/C-Cdh1-mediated degradation of the Polo kinase Cdc5 promotes the return of Cdc14 into the nucleolus. *Genes Dev.* 22, 79–90 (2008).

64. Garcia-Rodriguez, L. J. et al. Mitochondrial inheritance is required for MEN-regulated cytokinesis in budding yeast. *Curr. Biol.* 19, 1730–1735 (2009).

65. Magidson, V., Chang, F. & Khodjakov, A. Regulation of cytokinesis by spindle-pole bodies. *Nat. Cell Biol.* 8, 891–893 (2006).

66. Knap, A., Schmidt, S., Cane, E. & Simans, V. S. pombe cdc11p, together with sid1p, provides an anchor for septation initiation network proteins on the spindle pole body. *Curr. Biol.* 11, 1559–1568 (2001).

67. Morrell, J. L. et al. Sid4p-Cdc11p assembles the septation initiation network and its regulators at the S. pombe SPB. *Curr. Biol.* 14, 579–584 (2004).

68. Gromley, A. et al. A novel human protein of the maternal centrosome is required for the final stages of cytokinesis and entry into S phase. *J. Cell Biol.* 161, 535–545 (2003).

69. Johnson, A. E. & Gould, K. L. Dma1 ubiquitinates the SIN scaffold, Sid4, to impede the mitotic localization of Plo1 kinase. *EMBO J.* 30, 341–354 (2011).

70. Murone, M. & Simans, V. F. The fission yeast dma1 gene is a component of the spindle assembly checkpoint, required to prevent septum formation and premature exit from mitosis if spindle function is compromised. *EMBO J.* 15, 6605–6616 (1996).

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**Author contributions**

S.P. conceived the project. D.T., M.A.J., S.I. G.R. and S.P. designed and carried out the experiments and analyzed the relative data. D.T., S.P. and M.A.J. made the figures. S.P. wrote the manuscript with inputs from all the authors.

**Additional information**

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