Fungal cytokinesis requires the assembly of a dividing septum wall. In yeast, the septum has to be selectively digested during the critical cell separation process. Fission yeast cell wall α(1-3)glucan is essential, but nothing is known about its localization and function in the cell wall or about cooperation between the α- and β(1-3)glucan synthases Ags1 and Bgs for cell wall and septum assembly. Here, we generate a physiological Ags1-GFP variant and demonstrate a tight colocalization with Bgs1, suggesting a cooperation in the important early steps of septum construction. Moreover, we define the essential functions of α(1-3)glucan in septation and cell separation. We show that α(1-3)glucan is essential for both secondary septum formation and the primary septum structural strength needed to support the physical forces of the cell turgor pressure during cell separation. Consequently, the absence of Ags1 and therefore α(1-3)glucan generates a special and unique side-explosive cell separation due to an instantaneous primary septum tearing caused by the turgor pressure.

Introduction

Fungal cytokinesis requires concomitant contractile actomyosin ring (CAR) closure and the synthesis of a special cell wall structure termed septum (Pollard and Wu, 2010). Schizosaccharomyces pombe medial fission displays several stages: CAR positioning and assembly, activation of CAR contraction and septum formation by the septation initiation network (SIN), septum synthesis, and cell separation (Sipiczki, 2007; Krapp and Simanis, 2008). The septum is a three-layered structure composed of a middle disk named primary septum, flanked at both sides by the secondary septum (Johnson et al., 1973). The last step of cytokinesis is cell separation that requires degradation of the primary septum and the adjacent cell wall (septum edging) by glucanases. Therefore, correct assembly and structural integrity of the septum are vital for cell survival.

The fission yeast cell wall consists of an outer layer rich in galactomannanproteins and an inner layer comprised of β(1-3), β(1-6), and α(1-3)glucans (Pérez and Ribas, 2004; Grün et al., 2005). Immunoelectron microscopy (IEM) studies delimited the branched β(1-6)glucan in the cell wall and secondary septum, the branched β(1-3)glucan in the cell wall and both primary and secondary septum, and a linear β(1-3)glucan (L-BG) mainly present in the primary septum and a small amount in the cell wall (Humbel et al., 2001; Cortés et al., 2007). L-BG is necessary but not sufficient for primary septum formation and is the polysaccharide that specifically interacts with the fluorochrome Calcofluor white (CW) in S. pombe (Cortés et al., 2007). In addition to β-glucans, other polysaccharides, like the α(1-3)glucan, may be present in the primary septum as well. α(1-3) and branched β(1-3)glucans are essential for cell shape maintenance. However, their importance for cell wall and septum structure and function is still unknown (Ribas et al., 1991; Hochstenbach et al., 1998; Katayama et al., 1999).

S. pombe contains three essential β(1-3)glucan synthases (BGSSs) that localize in CAR, septum, growing poles, and sites of wall synthesis during sexual differentiation. Bgs1 appears earlier in the division site and is responsible for the L-BG and primary septum synthesis. Bgs4 is essential for cell integrity and is the only subunit shown to form part of the BGSS enzyme. Bgs3 function remains unknown (Cortés et al., 2002, 2005, 2007; Liu et al., 2002; Martín et al., 2003; Martins et al., 2011).
Ags1 (Mok1) is a putative α-glucan synthase (αGS) essential for cell integrity. Indirect immunofluorescence detected Ags1 in dividing and growing areas (Hochstenbach et al., 1998; Katayama et al., 1999). *S. pombe* contains four additional Ags1 homologues (Mok11–14), which are only detected during sporulation (García et al., 2006). αGS orthologues are not found in budding yeasts but are widely extended in pathogenic fungi (Edwards et al., 2011; Henry et al., 2012).

In this work, we have investigated the localization and requirements of Ags1 and found a tight colocalization with Bgs1, although they differ in their SIN dependence for medial positioning. We show for the first time that α(1-3)glucan is essential for both secondary septum formation and the primary septum robustness needed to support the turgor pressure during cell separation. Our findings bring to light convergent similarities between the primary septum α(1-3)glucan and the lamellapectin of plants, as both are essential for the adhesion and separation functions within similar structures.

**Results**

**Ags1 localizes in the growing sites during vegetative and sexual phases**

We examined the physiological localization of Ags1 by generating functional Ags1-GFP and -RFP fusions (Fig. 1 A, Fig. 51 A, and Materials and methods). During polar growth Ags1 was localized to the growing ends. Before the primary septum was detected, Ags1 simultaneously appeared in the growing ends and as a medial ring. After the primary septum was detected, Ags1 spread flanking the emerging septum and accumulating in the CAR. An obvious signal remained along the invaginated membrane, appearing as two separated bands after septum completion (Fig. 1, B and C; and Fig. 51 B).

Like the Bgs subunits, Ags1 was also present in all of the sites of wall synthesis during sexual differentiation: mating, spor formation, and spore germination (Fig. 1, D and E; and Fig. 51, D and E). These data suggest that Ags1 cooperates with the Bgs1 homologues and Bgs proteins in cell fusion, spor wall formation, and spore germination.

**Ags1 coincides spatially and temporally with Bgs1 in the growing sites: poles, CAR, and septum**

As Ags1 and Bgs1 are the only GSs found in the medial region before the septum is detected, their localization and displacement from the tips to the CAR during mitosis was simultaneously analyzed. Time-lapse images showed that before the primary septum was detected (Fig. 2 A, arrow) Ags1 and Bgs1 colocalized as medial faint dots (Fig. 2 A, arrowhead). Furthermore, both Ags1 and Bgs1 concentrated in a ring that moved with the edge of the growing septum. However, the Ags1 signal remaining along the invaginated membrane was more intense than that of Bgs1 (Fig. 2 B and Fig. 51 C). During cell separation, Ags1 and Bgs1 remained at both sides of the degrading septum and moved simultaneously to the old end of each cell (Fig. 2 C).

We tested for a physical interaction finding that physiological Bgs1 and Ags1 coimmunoprecipitate (Fig. 2 E). This suggests either a physical interaction or the close presence of both proteins in the same plasma membrane domains.

**Ags1 localization depends on the polarity establishment proteins, the polarized actin patches, and the polarized exocytosis**

Ags1 localized to the poles during polar growth. *tea1-1* and *tea2-1* mutants fail to reinstate polarized growth along the long axis of the cell (Verde et al., 1995). In these mutants, Ags1 localized to the incorrect new growing tips (Fig. 3 A, left). On the contrary, the microtubule cytoskeleton was dispensable for the correct localization and displacement of Ags1 to poles and septum (Fig. 3 A, right and bottom).

Actin localization coincides with that of Ags1 throughout the cell cycle (Katayama et al., 1999). Therefore, we analyzed whether the actin cytoskeleton is involved in the correct Ags1 localization. In the actin mutant *cps8-188* the actin patches appeared depolarized (Ishiguro and Kobayashi, 1996), and Ags1 extended from the septum and poles along the plasma membrane all around the cell (Fig. 3 B, left). In contrast, polymerized F-actin was unnecessary for stable Ags1 localization in growing sites (Fig. 3 B, middle).

Formin For3 and type V myosin Myo52 are responsible for polarized actin cable assembly and transport of cargoes along the actin cables, respectively (Feierbach and Chang, 2001; Win et al., 2001). In the absence of For3 or Myo52, Ags1 localized correctly to the growing poles and septum (Fig. 3 B, right). These findings are in agreement with the fact that loss of function of profilin Cdc3, essential for actin cable formation (Balasubramanian et al., 1994), still permitted polarized growth and localization of Ags1 in cell tips (Fig. 3 B, bottom).

The exocyst is essential for polarized fusion of secretory vesicles with the plasma membrane (Wang et al., 2002). In the exocyst mutant *sec8-1*, Ags1 accumulated in the surrounding areas of tips and septa but no signal was detected in the plasma membrane (Fig. 3 C). Thus, Ags1 localization to regions of active cell wall synthesis depends on the correct actin patch localization and the polarized exocytosis.

**Ags1 localization in the division site depends on the CAR formation and positioning, but not on the SIN pathway**

To study the Ags1 requirements during septation, Ags1 localization in mutants affected in CAR positioning and formation was analyzed. In *mid1* mutants (Chang et al., 1996), CAR and...
Figure 2. *Ags1* coincides spatially and temporally with *Bgs1* in the growing sites: CAR, septum, and poles. (A) *Ags1* and *Bgs1* colocalize in the medial zone before septum synthesis. *ags1-1 RFP GFP-bgs1* cells were imaged by time-lapse microscopy. Arrowhead: appearance of *Ags1* and *Bgs1* in the cell middle; arrow: appearance of septum (CW staining). (B) *Ags1* and *Bgs1* colocalize in the CAR and septum membrane. (C) *Ags1* and *Bgs1* colocalize in the old end before cytokinesis completion. Elapsed time is shown in minutes. (D) *Ags1* and *Bgs1* colocalize in the aberrant septa of the medial ring mutants. *mid1-366 ags1-1 RFP GFP-bgs1* cells were shifted to 32°C for 6 h. (E) *Bgs1* coimmunoprecipitates with *Ags1*. Solubilized membrane proteins (MP; see Materials and methods) from the indicated strains were immunoprecipitated (IP) with anti-GFP serum. Solubilized MP (left) and IP (right) were transferred to the same membrane and blotted with monoclonal anti-GFP or anti-HA antibodies. Bars, 2.5 µm.
primary septum are positioned at arbitrary sites, always coincident with Bgs1, but not with Bgs4 (Cortés et al., 2005). In the mid1-366 mutant, Ags1 was always coincident with septum alterations and Bgs1 localization (Fig. 2 D).

In the CAR formation mutants (Balasubramanian et al., 1994; Fankhauser et al., 1995) Ags1 was detected at cell poles and/or coincident with the incorrect septum structures (Fig. 4 A and unpublished data). The F-Bar protein Cdc15 is essential for

Figure 3. **Ags1 localization depends on the polarity establishment proteins, polarized actin patches, and polarized exocytosis.** (A) Ags1 localization depends on the polarity proteins but not on the microtubule cytoskeleton. Left: ags1<sup>1</sup>-GFP mutant cells were shifted to 36°C for 6 h. Right and bottom: ags1<sup>1</sup>-RFP, GFP-atb2<sup>2</sup> cells were transferred to medium containing 25 µg/ml MBC for 30 min and imaged. Arrow: Ags1 displacement to the middle of the cell. Elapsed time is shown in minutes. (B) Ags1 localization depends on the correct actin patch polarization, but not on the polymerized actin, actin cables, and type V myosins. Left: cps8-188 ags1<sup>1</sup>-GFP cells were shifted to 32°C for 4 h. Middle: ags1<sup>1</sup>-RFP, crn1<sup>1</sup>-GFP cells were transferred to medium containing 100 µM Lat A for 30 min. Right and bottom: ags1<sup>1</sup>-RFP crn1<sup>1</sup>-GFP mutant cells were grown at 28°C (for3<sup>Δ</sup> and myo52<sup>Δ</sup>) or at 25°C and shifted to 37°C for 2 h (cdc3-6). (C) Ags1 localization depends on the exocytosis. sec8-1 ags1<sup>1</sup>-GFP cells were shifted to 37°C for 2 h. Bars, 5 µm.
presence, time-lapses of SIN mutants expressing both Ags1-GFP and Hht1-RFP (nucleus labeling) or Ags1-RFP and Rlc1-GFP were performed. During mitosis and after CAR assembly (Fig. 4 D, arrowhead) Ags1 disappeared from the tips and concentrated in the cell cortex surrounding the CAR and the mitotic nuclei. However, Ags1 never became concentrated as a ring structure; instead, it spread as a broad band as CAR collapsed (Fig. 4, C and D, arrow; and Fig. S2 B). When the nuclei were closely paired and the cell reinitiated bipolar growth, Ags1 moved to both cell tips. Similar Ags1 medial localization and dynamics were observed during the second round of mitosis (Fig. 4 C, arrow).

Occasionally some SIN mutant cells maintained the CAR longer, and in this case Ags1 concentrated as a ring overlapping the CAR that was able to constrict, giving rise to CW-stained septum structures (Fig. S2, C and D [Ags1, arrow; CW, arrowhead]). Interestingly, the emergence of these septum structures frequently coincided with cell death before growth reinitiation, but never with septum completion.

In agreement with the SIN dependence of Cdc15 recruitment to the CAR, the cdc15-140 mutant shows a similar cytokinesis CAR maturation. The cdc15-140 mutant does not form aberrant septum structures but generates an unstable CAR that rapidly disappears (Hachet and Simanis, 2008). To know whether this defective CAR is sufficient for a transient Ags1 localization to the cell middle, time-lapse of cdc15-140 cells at restrictive temperature and expressing both Ags1-RFP and Rlc1-GFP (CAR labeling) was performed (Fig. 4 B). In these cells Ags1 was never detected in the medial cortex, indicating that a stable CAR is essential for Ags1 localization in the division site.

The SIN is a signaling network required for CAR contraction and septum formation in S. pombe (Barral and Liakopoulos, 2009). Thus, we examined whether Ags1 medial localization depends on the SIN. The SIN mutants suffer a cytokinesis blockage after mitosis, building a CAR that disassembles prematurely rather than undergoing constriction and septum synthesis (Sparks et al., 1999; Krapp et al., 2001; Rosenberg et al., 2006). In these mutants, Ags1 localized to the poles and as a broad band in the cell middle (Fig. S2 A, arrow; and unpublished data), indicating that the SIN is not necessary for Ags1 displacement to the middle during cytokinesis.

To know if the Ags1 medial localization in SIN mutants occurs specifically during mitosis and during or after CAR presence, time-lapses of SIN mutants expressing both Ags1-GFP and Hht1-RFP (nucleus labeling) or Ags1-RFP and Rlc1-GFP were performed. During mitosis and after CAR assembly (Fig. 4 D, arrowhead) Ags1 disappeared from the tips and concentrated in the cell cortex surrounding the CAR and the mitotic nuclei. However, Ags1 never became concentrated as a ring structure; instead, it spread as a broad band as CAR collapsed (Fig. 4, C and D, arrow; and Fig. S2 B). When the nuclei were closely paired and the cell reinitiated bipolar growth, Ags1 moved to both cell tips. Similar Ags1 medial localization and dynamics were observed during the second round of mitosis (Fig. 4 C, arrow).

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Ags1 is essential for the septum integrity at the start of cell separation

To study the lethal effect of the absence of Ags1, a strain expressing a single integrated ags1+ copy under the control of the thiamine-repressible 81X-nmt1+ promoter was analyzed (see Materials and methods). Cell growth arrested after 4 h of ags1+ repression and osmotic stabilization protected the cells, delaying the growth arrest to at least 12 h (Fig. 5 A, left). Morphological observations revealed that Ags1 depletion promotes cell lysis and cytokinesis release from the lateral region of the poles (Fig. 5 B, arrowhead) and mostly from the septum (Fig. 5 B, arrow). Total lysis reached 70% of the cells (n = 420) after 7 h of repression, and sorbitol reduced it to 7% (n = 986; Fig. 5 A, right). A similar lysis phenotype was observed in the mok1-664 mutant at a restrictive temperature (Fig. 5 C). In agreement with previous data from ags1+ point mutants (Hochstenbach et al., 1998; Katayama et al., 1999), the Ags1-depleted cells showed a considerable reduction (53%) in the cell wall α-glucan content (Table 1).

The septum lysis process of ags1+ -repressed cells was examined in detail by time-lapse (Fig. 5 D and Video 1). The lysis always appeared after septum completion, coincident with the start of cell separation. After 3 h of ags1+ repression, lysis mostly occurred at the septum–cell wall border in only one cell (15% cells, n = 238; Fig. 5 D, top graph), generating one dead cell (arrowhead, top) attached to the living cell. After 7 h, lysis mostly occurred in both cells (41% cells, n = 420; Fig. 5 D, bottom graph) at both sides of the septum–cell wall border (arrowhead, bottom). Sorbitol delayed the cell lysis to 7 h. At this time most of the cells were still normal in growth and morphology (see graphs in Fig. 5, A and D).

Because Ags1 and Bgs1 might collaborate in the initial steps of septum formation, a possible genetic interaction was tested. Bgs1 and Ags1 interact genetically because the cps1-12 mok1-664 mutant was more sensitive to temperature than the single mutants (Fig. S3 A). The SIN mutants do not form septa, and therefore, the lysis of mok1-664 mutant at the start of cell separation was abolished. Instead, a new and abundant lysis at the pole tip appeared (Fig. S3 C), indicating that Ags1 is also essential for cell wall integrity during polarized growth. In some cases, the SIN mutants lysed at the septum. This was due to the initiation of defective septa that cannot be completed. As expected, the mok1-664 mutation increased the septum lysis defect of the SIN mutants (Fig. S3 C).

The absence of Ags1 alters the cell wall ultrastructure and leads to cell lysis at the lateral region of the poles

To further investigate the cell integrity defect caused by the absence of Ags1, the cell wall ultrastructure was examined (Fig. 6, A and C). The wild-type (WT) cell wall presented a three-layered structure (Fig. 6 A), whereas in the absence of Ags1 the cell wall became amorphous, thicker, and looser, with no signs of the outer dense layer. In the presence of sorbitol the cell wall appeared stratified with remedial internal dense and transparent layers (Fig. 6 A, ICW). This is in agreement with the different cell wall amounts detected with and without sorbitol. The cell wall increase detected with sorbitol was only due to β-glucan, suggesting a compensatory mechanism (Table 1).

The galactomannan has been detected in the outer and inner electron-dense layers (Horisberger and Rouvet-Vauthey, 1985). Because the outer layer was absent in Ags1-depleted cell walls, WT cells expressing Hht1-RFP (nuclear RFP) and ags1+ -repressed cells (no nuclear RFP) were mixed, stained with FITC-concanavalin A, and visualized for FITC and RFP (Fig. 6 B). Concanavalin A specifically binds to mannose residues, which can be used for indirect measurement of the mannan content and localization in the cell surface (Goldstein, 2002). Quantitative analysis of the FITC signal showed that in Ags1-depleted cells the outer galactomannan content was significantly reduced (Fig. 6 B), in agreement with the 36% decrease observed by cell wall fractionation (Table 1).

WT cells are surrounded by a uniform cell wall (Figs. 6 C and 7 A). In contrast, the cell wall of Ags1-depleted cells was irregular and displayed erosions in the pole lateral region (Fig. 6 C, open arrowhead; see also Fig. 9 C) and attached wall material from the sister cell (Fig. 6 C, closed arrowhead; see also Fig. 9 C).

The Ags1-depleted cells demonstrated cell lysis and cytoplasm release during polar growth. To study more precisely cell wall breakage, time-lapse of ags1+ -repressed cells was performed. Lysis always occurred not at the cell tip, but at the pole side region (Fig. 6 D, arrowhead). Lysis was detected at early repression times and increased during repression, reaching 8% of the cells at 7 h (n = 420; Fig. 6 D, graph). In the presence of sorbitol, lysis was delayed and reduced to 2% after 7 h of repression (n = 986; Fig. 6 D, graph). Interestingly, when the cells grown with sorbitol were imaged in the absence of sorbitol, the pole lateral lysis increased to 28% while the septum lysis remained in 6% (n = 453, not depicted).

A possible genetic interaction between ags1+ and SIN genes was also tested, finding that the mok1-664 SIN double mutants are more sensitive to temperature than the single mutants (Fig. S3 A). The SIN mutants do not form septa, and therefore, the lysis of mok1-664 mutant at the start of cell separation was abolished. Instead, a new and abundant lysis at the pole tip appeared (Fig. S3 C), indicating that Ags1 is also essential for cell wall integrity during polarized growth. In some cases, the SIN mutants lysed at the septum. This was due to the initiation of defective septa that cannot be completed. As expected, the mok1-664 mutation increased the septum lysis defect of the SIN mutants (Fig. S3 C).
Figure 5. Absence of Ags1 promotes cell lysis and release of cytoplasmic material from either the septum region or the lateral region of the pole. (A) Sorbitol partially suppresses both cell growth arrest and cell lysis promoted by ags1+ repression. 81X-agst+ cells were grown either without or with thiamine (−T, induced; +T, repressed) and 1.2 M sorbitol (S). Cell growth (left) and lysis (right) were monitored at the indicated times. (B) Absence of Ags1 promotes cell lysis at the septum and poles. Cells were grown as in A and visualized at the indicated times. (C) Loss of Ags1 function promotes similar cell lysis to that of Ags1 absence. mok1-664 cells were grown in YES+S and shifted to 37°C for 5 h. Arrowhead: lysis in the pole side region; arrow: lysis in the septum region. (D) ags1+ repression promotes cell lysis and release of cytoplasm from the division site, at the start of cell separation, resulting in the death of either one (top panels) or both sister cells (bottom panels). 81X-agst+ cells were grown in EMM+T for 3 h and visualized by time lapse. Arrowhead: lysed cell. Elapsed time is shown in seconds. The percentage of each cell lysis was quantified. Bars, 5 µm.
These data indicate that Ags1 and the corresponding α(1-3)glucan are essential to maintain cell wall structure and integrity during cell growth.

**Ags1 is responsible for both secondary septum synthesis and correct primary septum formation**

To study in more detail the role of Ags1 in septum synthesis, the septum ultrastructure of Ags1-depleted cells was analyzed. WT cells presented a three-layered septum structure (Fig. 7 A). As the primary septum grew, the secondary septum was laid down at both sides. After septum closure, the septum thickness increased in a maturation process involving additional secondary septum synthesis. The final septum displayed a primary septum invading the cell wall (arrow) and flanked by two triangular electron-dense structures termed “materiel triangulaire dense” (MTD; Johnson et al., 1973; Fig. 7 A, bottom).

The Ags1-depleted cells presented strong septum defects of abruptly twisted (Fig. 7, B–D) and not anchored primary septa (arrow) and disorganized MTD structures (Fig. 7, C and D). This indicates that Ags1 is essential for a straight primary septum synthesis, and suggests an important Ags1 cooperation with Bgs1 and the CAR. In addition, Ags1 is also essential for primary septum anchorage and MTD assembly. Sorbitol delayed the lytic phenotype, revealing a primary septum that was slowly degraded (Fig. 8 E). Interestingly, cell separation in Ags1-depleted cells was instantaneous and asymmetrical, with a ripped primary septum, usually giving rise to one sister cell remaining attached to the well surface, and the other cell quickly jumping but staying connected by its side to the sister cell (Videos 3 and 4). This side-explosive phenotype was caused by asymmetrical degradation and/or breakage of the septum edging followed by an abrupt tear of a weak primary septum and an instantaneous curvature of the secondary septum to reach the most stable conformation in the new end.

Interestingly, cell separation in Ags1-depleted cells was instantaneous and asymmetrical, with a ripped primary septum, usually giving rise to one sister cell remaining attached to the well surface, and the other cell quickly jumping but staying connected by its side to the sister cell (Videos 3 and 4). This side-explosive phenotype was caused by asymmetrical degradation and/or breakage of the septum edging followed by an abrupt tear of a weak primary septum and an instantaneous curvature of the secondary septum to reach the most stable conformation in the new end.

After side-explosive separation, the sister cells remained attached by one side of the septum edging through remnants of a primary septum that was slowly degraded (Fig. 8 E). Importantly, the new end of both sister cells displayed considerable amounts of primary septum remnants, indicating that the immediate cell separation is caused by a fast internal tear of a weak primary septum that cannot withstand the cell pressure that curves the secondary septum (Fig. 8 E). This shows that the α(1-3)glucan forms part of the primary septum and is essential to confer it the strength needed to support the cell internal pressure during cell separation.

We examined if the primary septum weakness also affected its linkage to the secondary septum, resulting in cell separation through not only internal tearing but also in the primary–secondary septum boundary. As expected, all the *ags1*-repressed (*n* = 612) and *mok1-664* cells (*n* = 358) separated asymmetrically.

### Table 1. Incorporation of [14C]glucose into cell wall polysaccharides during *Ags1* depletion either in the absence or presence of 1.2 M sorbitol

| Growth | Strain | Thiamine* | Cell Wall | α-Glucan | β-Glucan | Galactomannan |
|--------|--------|-----------|-----------|----------|----------|--------------|
| EMM    | Control| – (on, wt)| 28.1 ± 2.8 (100)* | 5.5 ± 0.3 (19.8) | 19.2 ± 1.6 (68.5) | 3.3 ± 0.8 (11.7) |
| EMM    | 81X-ags1* | + (off) | 21.2 ± 0.7 (100) | 2.6 ± 0.1 (12.1) | 16.5 ± 1.2 (77.8) | 2.1 ± 0.6 (10.1) |
| EMM+S  | Control| – (on, wt)| 26.8 ± 3.0 (100) | 5.3 ± 1.0 (19.9) | 17.9 ± 1.3 (66.9) | 3.6 ± 0.8 (13.2) |
| EMM+S  | 81X-ags1* | + (off) | 32.0 ± 2.7 (100) | 3.4 ± 0.6 (10.9) | 25.3 ± 3.2 (77.7) | 3.6 ± 0.5 (11.4) |

*a* = *ags1*-repressed cell cultures were grown for 3 (EMM) or 7 h (EMM+S) in the presence of thiamine. [14C]glucose was added 1.5 or 4 h before harvesting, respectively, 5, 1.2 M sorbitol.

bPercent incorporation of [14C]glucose = cpm incorporated per fraction × 100/total cpm incorporated. Values are the means and SDs calculated from three independent experiments.

cValues are percentages of the corresponding polysaccharide in the cell. Values in parentheses are percentages of the polysaccharide in the cell wall.
The absence of Ags1 causes a final cell separation defect. Only primary septum CW staining ensures that two cells closely observed by microscopy are physically attached. In Ags1-depleted cells, the CW staining of the side-contact region eventually disappeared. However, many cells appeared in close lateral contact (Figs. 8 A and 9A), suggesting that they remain attached by their side. To find out how and when the side-contact region is degraded to complete cell separation, time-lapse of paired Ags1-depleted cells connected by their side region was carried out. Depending on the localization of primary septum remnants (Fig. S4, D and E, arrow), three types of paired cells were detected: (1) both new ends exhibiting CW-stained material, indicating an internal primary septum tearing; (2) only one new end presenting CW-stained material, suggesting a tearing through the contact region between primary and secondary septum; and (3) in later separation stages, the new ends did not display detectable CW-stained material, but the cells were still firmly connected through a small cell wall area with little or no CW staining (Fig. 9 A and Fig. S4 E, circle).
Figure 8. Ags1 is responsible for the strength of the primary septum structure needed during cell separation. (A) Ags1-depleted cells exclusively display asymmetrical cell separation and remain attached by their side. Arrow: normal straight separation; arrowhead: asymmetrical separation. Cells were grown in EMM+T+S for 8 h. The percentage of each cell separation was quantified. (B) WT cells separate gradually (top panels), whereas the Ags1-depleted cells suffer an immediate side-explosive separation. Cells were grown in EMM+T for 3 h and imaged. Asterisk, time spent for maximal new end curvature.
performed (Fig. 9 B, arrowhead). After 240 min the cells still remained connected (arrowhead), each already containing a new septum ready to be ripped. In this example, the side-explosive separation of the new septum (Fig. 9 B, a1–a2) released the connected cells from the cell wall surface (path shown by broken arrow). In other cases, the side-explosive separation did not necessarily cause detachment from the surface (Video 1). During movement, the cells still stayed connected through the initial side-contact region (Fig. 9 B, arrowhead, a1–b1, t = 237:40 to 255:00; and Video 10). Under our time-lapse conditions, the cells stayed connected for more than one cell cycle, whereas in shaking cultures only paired cells were detected. This result suggests that final cell separation of Ags1-depleted cells is a mechanical rather than enzymatic process.

Ultrastructure analysis of ags1Δ-repressed cells confirmed the absence of degradation of the side-contact area maintaining both cells connected after separation (Fig. 9 C, arrow). This non-degraded area was located close to the fission scar, corresponding to the septum edging of the previous septum. The separated cells presented in the pole lateral region cavities and attached wall fragments (Fig. 9 C, open and closed arrowheads), suggesting a physical tear of the cell wall that connected both cells.

Discussion

In fungal cytokinesis, CAR contraction is intimately accompanied by centripetal assembly of a septum wall that physically separates the sister cells (Bathe and Chang, 2010). However, little is yet known about how the different essential GSs cooperate to assemble the septum structures. Once septation is accomplished, subsequent cell separation requires the selective degradation of the primary septum (Sipiczki, 2007). Because of the high turgor pressure, even a minor rupture of the cell wall structure might conduct to lysis and cell death. Therefore, correct assembly and structural integrity of the cell wall and specialized septum are vital for cell survival (Cabié et al., 2001).

S. pombe is an attractive model for morphogenesis whose growth pattern likely diverged from filamentous fungi in response to the loss of hyphal growth (Harris, 2011). Like fission yeast, the cell wall of filamentous fungi also contains β- and α-glucans (Latgé, 2007). S. pombe IEM studies allowed the localization of the β-glucans in specific sites of cell wall and septum (Humbel et al., 2001; Cortés et al., 2007). To date, the cell wall α(1-3)glucan distribution and function is unknown (Sugawara et al., 2003; Grün et al., 2005). Ags1 was described as a putative αGS essential for cell integrity, but a specific role for Ags1 in the cell wall and septum architecture has not yet been described (Hochstenbach et al., 1998; Katayama et al., 1999).

We succeeded in generating a fully functional Ags1-GFP, and interestingly, Ags1 localizes very early in division sites and poles, coinciding exactly with Bgs1. Both Ags1 and Bgs1 localize before and ahead of the growing primary septum. Ags1 and Bgs1, but not Bgs4, overlap with the aberrant CW-stained septum material of the CAR positioning mutant mid1-366. Importantly, Bgs1 communoprecipitates with Ags1, and ags1Δ and bgs1Δ interact genetically, suggesting a tight cooperation between both GSs in primary septum assembly. In fact, Bgs1 depletion produces septa with no primary septum but still containing diffuse L-BG, suggesting that L-BG association with branched β(1-3) and or α(1-3)glucan is critical for primary septum assembly (Cortés et al., 2007).

Independently of the close Ags1–Bgs1 cooperation and although both are GSs, Ags1 is structurally very distinct and presents a relevant difference with the Bgs family with respect to their dependence on the SIN for medial localization (Cortés et al., 2005), indicating different requirements for medial localization and/or activation. In the absence of a functional SIN, only Ags1 is able to localize in the division site during mitosis and in the presence of the CAR. In cdc15-140 cells, Ags1 does not localize to the division site in the absence of the CAR and spreads along the medial cortex in the presence of a nonfunctional CAR, indicating that CAR serves as a landmark for Ags1 incorporation to the division site, whereas the SIN is exclusively involved in its ring localization and activation.

Ags1 α-glucan is essential for the primary septum strength needed during cell separation

S. pombe cell separation is the most critical step of the cell cycle, where the degrading primary septum must withstand the internal pressure and gradual secondary septum curvature to reach the most stable conformation in the new end. The normal septum structure is able to support the mechanical force of the internal pressure, allowing a symmetrical and progressive primary septum degradation and secondary septum curvature (Fig. 10 A). Our results show that Ags1 absence or loss of function generates a primary septum that is highly vulnerable to the separation internal pressure. After asymmetrical septum edging degradation, the primary septum is instantly torn by a side-explosive cell separation (Fig. 10 B), creating two sister cells with primary septum remnants in the new ends. These observations indicate that Ags1 synthesizes an α-glucan of the primary septum that is essential to confer it the mechanical strength needed to face the separation internal pressure.

In contrast to the chitin-rich primary septum of budding yeast, our data show that in addition to β-glucans, the primary septum of S. pombe also contains α-glucan. The primary septum is essential for S. pombe viability, whereas in S. cerevisiae it is dispensable. These differences might account for distinct division patterns and/or cell wall compositions: (i) S. pombe does not contain chitin, whereas the budding yeast primary septum is made of chitin. It seems reasonable to think that linkages between α- and β-glucans are required for septum strength in S. pombe, whereas in budding yeast a different mechanism is engaged.
Absence of Ags1 results in a defective final cell separation step. (A) Ags1-depleted cells stay attached through the septum edging for a long time, even after disappearance of the remaining CW-stained primary septum. Cells were grown as in Fig. 8 A. Circle: side-contact region with different amounts of CW-stained primary septum. Bar, 5 µm. (B) The Ags1-depleted sister cells remain attached by their septum edging (arrowhead) for at least two cell cycles. Cells were grown in EMM+T+S for 4 h. Arrowhead: side-contact region. Broken arrow: movement of the attached cells. Square: magnified area. Elapsed time is shown in minutes. Bars, 5 µm. (C) Ultrastructure of Ags1-depleted cells attached by the septum edging (arrow). The cells display irregular walls with cavities (open arrowhead) and attached wall fragments (closed arrowhead) at the pole lateral region. Cells grown as in Fig. 6 A were analyzed by TEM (top) or scanning electron microscopy (SEM, bottom). Bars, 1 µm.
β-glucans would contribute to the mechanical strength of the primary septum. (ii) *S. pombe* primary and secondary septum assembly is simultaneous and closely coordinated, whereas the *S. cerevisiae* septum is synthesized sequentially. (iii) *S. pombe* septum covers the entire cell diameter, whereas in budding yeast it just closes the narrow mother-bud neck. (iv) *S. pombe* septum degradation gives rise to full-diameter new growth ends, whereas in *S. cerevisiae* it results in a small non-growing area. (v) *S. pombe* full-diameter septum degradation supports a considerable internal pressure, whereas in *S. cerevisiae* it must only support the pressure of the small disk area (Johnson et al., 1973; Schmidt et al., 2002; Cortés et al., 2007).

As we advance in our knowledge of fission yeast septation, we find new and surprising evolutionary convergent structural and functional similarities between fission yeast and plant septa: (i) as in *S. pombe*, the plant cell plate is made of callose (L-BG) and the protein involved is the Bgs homologue CalS1 (Verma and Hong, 2001; Cortés et al., 2007); (ii) *S. pombe* α-glucan and plant pectin (an α-linked polysaccharide) assume the same function in a similar structure. Both are essential for wall adhesion and safe cell separation, maintaining the linkages between polysaccharides in equivalent structures, the fission yeast primary septum and the plant mature cell plate (middle lamella; Iwai et al., 2002; Roberts and Gonzalez-Carranza, 2007).

**Ags1 is responsible for secondary septum formation and cell wall integrity**

Ags1 depletion generates fragile and twisted primary septa with no secondary septa, indicating that (i) Ags1 is responsible for the secondary septum assembly; and (ii) the perpendicular and straight primary septum deposition needs to be closely accompanied by a flanking secondary septum. Alternatively, the twisted...
primary septum might be due to an essential Ags1 cooperation with Bgs1 and the CAR. Analysis of revertant protoplasts suggested that the primary cell wall formation step is the assembly of β-glucan microfibrils, whereas α-glucan might be involved in glucan bundle formation (Osumi et al., 1989; Konomi et al., 2003). Our results indicate that in the absence of α-glucan, the β-glucan microfibrils are unable to evolve into bundles, and therefore, the glucan network is not generated and the secondary septum is not assembled.

*S. cerevisiae* association between chitin and β-(1-3)glucan grants to the wall the mechanical strength needed to withstand the internal pressure (Hartland et al., 1994). As *S. pombe* does not contain chitin, α- and β-glucans and their corresponding GSs have probably assumed multiple essential functions during cell wall synthesis, as is the case for the three chitin synthases in *S. cerevisiae* (Shaw et al., 1991). Our results show that Ags1 is also essential for cell wall integrity: (i) Ags1 localizes to the growing poles, suggesting that Ags1 synthesizes an α-glucan of the cell wall. (ii) Ags1 is essential for the correct cell wall architecture. In its absence the cell wall is amorphous, thicker, and looser, with no signs of the outer dense layer, reduced concanavalin A binding to the wall surface, and reduced cell wall galactomannan content. It is unknown how galactomannan is integrated into the cell wall, although our results suggest that part of the mannose residues might associate with the α-glucan fibrils. (iii) Ags1 depletion generates a predominant septum lysis at the start of cell separation, suggesting that Ags1 is essential to compensate an excess of cell wall degradation during separation. In addition to α-glucan, the secondary septum contains branched β-(1-3)glucan. Bgs4*-repressed cells also display septum lysis at the beginning of cell separation (Cortés et al., 2005). Thus, it will be necessary to evaluate whether Bgs4 is responsible for the secondary septum β-(1-3)glucan and how Ags1 and Bgs4 cooperate to assemble this structure.

Ags1 and Bgs4 are also essential for cell integrity during apical growth, although by different mechanisms (Fig. 10 C). Bgs4 absence generates a thin-tip cell wall leading to lysis at the pole tip (unpublished data). However, Ags1 absence produces lysis at the pole lateral region. Interestingly, the pole lateral region presents cavities from a defective cell separation, suggesting that lysis may be indirectly caused by the separation defect (Fig. 10 C).

Sorbitol protects the cells, allowing the activation of a compensatory mechanism that generates a remedial cell wall that extends as a secondary septum at both sides of the twisted primary septum, in a sequential process similar to that of *S. cerevisiae* (Cabib et al., 2001). In some cases, only one sister cell displays primary septum remnants in the new end after side-explosive separation, suggesting a defective primary–secondary septum association and a linking role for α-glucan. Additionally, primary and secondary septum synthesis might need to be coupled in order to generate the required linkages for a compact septum. Similar compensatory mechanisms have been described in Bgs1- and Bgs4-depleted cells and other α-(1-3)glucan-containing fungi (Cortés et al., 2005, 2007; Maubon et al., 2006; Reese et al., 2007; Farkas et al., 2009). *S. pombe* contains four additional Ags proteins, which are detected during sporulation (García et al., 2006).

Evaluation of the activities induced by this compensatory mechanism will require further genetic and molecular analysis of Ags and Bgs subunits.

**Materials and methods**

**Strains and culture conditions**

The *S. pombe* strains used in this study are enumerated in Table S1. *ags1*Δ deletion was performed in a diploid strain by removing the entire coding sequence of an *ags1* copy, as described for *bgs1*Δ, *bgs3*Δ, and *bgs4*Δ gene deletions (Cortés et al., 2002, 2005, 2007). Tetrad analysis of sporulated ags1Δ/ags1Δ diploids showed Ags1 to be essential, as described by previous work (Katayama et al., 1999). Haploid *ags1*Δ strains were maintained viable by transforming the heterozygous *ags1*Δ/ags1Δ diploid with *ags1*-expressing plasmids and selecting haploid spore clones containing the *ags1*Δ deletion, which contained the corresponding *ags1*Δ plasmid needed to maintain the cell viable.

*ags1*Δ::ura4+ *p41XH-ags1* strain 1804 (his3* selection) contains *ags1* expressed under the control of the 41X version (medium expression) of the thiamine-repressible *nmt1* promoter (Moreno et al., 2000). Other strains with different levels of *ags1* repression were made by genetic cross with strain 285 (Leu+, Ura+, His+) transformed with the corresponding version of p81X-ags1 and p41XH-ags1 (S. cerevisiae LEU2 selection) and selection of Leu+, Ura+, His*^-^ clones.

81X-agS1 strain 2086 contains the selection marker ura4+ adjacent to the *nmt1*-81X promoter, followed by the *agS1* ORF. This strain was made from a diploid strain by homologous recombination of an ApA–ApA fragment of pSK-81X-agS1-ura4+81X-agS1 1,2003 (see below) and sporulation. The resulting 81X-agS1Δ haploid strain contained one single integrated *ags1* copy under the control of the 81X promoter and exhibited a strong lytic phenotype in the presence of thiamine (repressed conditions) and a wild-type phenotype in its absence (induced conditions).

81X-agS1Δ Bgs1Δ-81X-bgs3Δ strain 4825 was made by a genetic cross between strains 2086 (81X-agS1Δ, Ura- and 2234 (B1X-bgs3Δ, Ura- 5-fluoroorotic acid [FOA] selection) and random spore selection of Ura* clones (81X-agS1ΔΔ+), followed by the analysis of the possible differential phenotypes in the presence of thiamine between single 81X-agS1ΔΔ+ and double 81X-agS1ΔΔ+bgs3Δ- strains. PCR analysis of positive clones confirmed the presence of the 81X promoter and the absence of the original promoter in both genes.

The 11.3 kb *ags1*Δ containing fragment seemed to be toxic for cloning. Therefore, the ags1Δ strain containing an integrated *ags1*Δ-GFP copy was made sequentially with two overlapping fragments that restore the entire *ags1*Δ sequence (Fig. S1 A). First, strain 1804 (ags1Δ::ura4+ p41XH-agS1Δ, his3* selection) was made Ura+ by transformation with a ura4*-empty ags1Δ deletion cassette and colony selection in minimal medium containing FOA to make strain 2881 (ags1Δ p41XH-agS1Δ). Then, the ags1Δ p41XH-agS1Δ strain was transformed with Spel-cut pSK-ura4+81X-agS1Δ::ura4+ (ura4+ selection, 20% bgS1Δ ORF, and 1.3 kb 3'UTR), which directed its integration at the Spel site at position +431 of aags1Δ 3'UTR. Correct integration was confirmed by PCR analysis using pairs of oligonucleotides external and internal to the integrated fragment. The resulting strain 2939 (ags1Δ 3'UTRagS1Δ::ags1Δ 3'UTRagS1Δ::ura4+ p41XH-agS1Δ) was transformed with AgeI-cut pJK-ags1Δ:GFP (Leu* selection, 2.9 kb promoter, 6.2 kb ags1Δ 5'ORF, and 0.7 kb 12A-GFP, 12A inserted in frame at base 5866 of aags1Δ ORF, see below), which directed its integration at the Ags1 site at base 6025 in the aags1Δ 3'ORF, and restored the complete aags1Δ coding sequence (Fig. S1 A). To eliminate p41XH-agS1Δ, to confirm ags1Δ restoration, and to analyze Ags1-GFP functionality, this strain was crossed with strain 285 (Leu+, Ura+, His+) and tetrad analysis was performed. Leu and Ura phenotypes always co-segregated 2+2, as expected after ags1Δ restoration. p41XH-agS1Δ was lost in most of the clones as a result of the sporulation process. The obtained ags1Δ aags1Δ-GFP strain displayed a wild-type phenotype under all tested conditions and expressed Ags1-GFP at physiological levels from a single integrated aags1Δ-GFP gene under the control of its own promoter. Similarly, an aags1Δ aags1Δ-RFP strain expressing a fully functional Ags1-RFP (variant strain, Shaner et al., 2005) from pJK-agS1Δ:GFP-Cherry was also made.

Standard complete yeast growth (YES), selective (EMM), and sporulation (SPA) media (Egel, 1984; Alfa et al., 1993) have already been described. Cell growth was monitored by measuring the A600 of early log-phase cell cultures in a Coleman Junior II spectrophotometer (OD600 0.15 = 107 cells/ml). For serial dilution drop tests of growth, early log-phase cells growing at 25°C were adjusted to 105 cells/ml and then serially diluted.

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1.1.0. The different dilutions were spotted onto YES and YES + 1.2 M sorbitol plates, incubated for 2–3 d at the indicated temperatures, and photographed. Latrunculin A (Lot A; Enzo Life Sciences) was used at a 100-[mu]M final concentration (from a stock of 10 mM in DMSO). Methyl benzimidazol-2-yl-carbamate (MBC; Sigma-Aldrich) was used at a 25-[mu]g/ml final concentration (from a stock of 5 mg/ml in DMSO). General procedures for yeast and bacterial culture and genetic manipulations were performed as described previously (Moreno et al., 1991; Sambrook and Russell, 2001).

**Plasmid and DNA techniques**

pSK-ags1-1 is an 11.3-kb EcoRI-Apal DNA fragment containing the ogs1-1 gene sequence cloned in two steps into the Bluescript SK+ vector. First, a 7.9-kb EcoRI-Sall-5'-ogs1 fragment (5.1 kb of ogs1 1-ORF) from pB248X-ags1-1 (a gift from Takashi Toda, London Research Institute, Cancer Research UK, London, UK), and then a 3.4-kb Sall-Apal 3'-ogs1 fragment (2.1 kb of ogs1 1-ORF) PCR-amplified from genomic DNA, were cloned.

The 7.2-kb ags1 1-ORF sequence from pSK-ags1-1 containing the sites NotI before the start codon and BamHI after the termination codon, was cloned into NotI–BamHI fragment of pBR2-81XL, pBR2-41XL, and pBR2-41XH (S. cerevisiae LEU2 and S. pombe his3) selection, and 81X and 41X versions of the thiamine-repressible nmt1 promoter, respectively; Moreno et al., 2000). The levels of ags1 1 repression from the resulting 81X and 41X plasmids were assessed in an ags11 bak mutant background. Although the expression displayed a heterotic phenotype in induced conditions (presence of thiamine) even with the 41X promoter, and the wild-type phenotype in induced conditions (absence of thiamine). As expected, lysis and cell growth arrest appeared earlier in 81X-ags1 1 strains due to their reduced ags1 1 expression. To find the optimal ags1 1 repression level, three deleting deletions (Asc1–NotI, Sac1–NotI and Nrd1–NotI) in the multiple cloning site (MCS) were made to approach ags1 1 ORF and 81X promoter, thus gradually increasing the ags1 1 expression level as described for ags1 1 (Cortes et al., 2007). The optimal 81X-ags1 1 repression conditions were achieved with the MCS NruI–NotI deletion between 81X promoter and ags1 1 ORF. The lysis was only slightly slower but the appearance of revertant survivors observed with the other constructs decreased.

Due to the subunit phenotype of strains containing multicopy pB18X-ags1 1 plasmids was homogeneous and the appearance of revertant or attenuated clones was detected. To obtain a strain with a more uniform and stable ags1 1 shut-off phenotype that could be useful to study the Ags1 absence effect, a strain with an integrated 81X-ags1 1 single copy was made (see above). To ensure that the low expression level of a single 81X-ags1 1 copy would still be able to maintain wild-type cells under induced conditions, the 81X-ags1 1 MCS-Nrd1-NotI sequence was selected. pSK-Pags1 1-ura4-81X-ags1 1-2626 contains an ags1 1 promoter fragment (−1,123 to −1,099), the ura4 sequence, and an 81X-ags1 1 5′ ORF fragment (+1 to +2,626) from pB18X-ags1 1 MCS-Nrd1-NotI. An Apal–Apal Pags1 1-ura4-81X-ags1 1-2626 fragment was used as a substitution cassette to make the integrated 81X-ags1 1 strains.

Similarly, a 41X-ags1 1 MCS-Nrd1-NotI strains was made and analyzed as well.

pSK-ura4-ags1 1-2670-2725 (ura4 selection) is a 4.8-kb BstBI-Apal fragment containing 3.5 kb of ags1 1 3′ ORF and 1.3 kb of ags1 1 3′ UTR cloned into Cia1–Apal sites of pSK-ura4 and pSK-ura4-1-1226 is the integrated pSK-ura4-1-1226 sequence. pSK-ura4-1-1226 selection was with 9.1-kb Apal–Nhel fragment, containing 2.9 kb of ags1 1 promoter and 6.2 kb of ags1 1 5′ ORF, overlapping 2.5 kb of the above ags1 1 3′ ORF fragment (Fig. S1 A). Coudirected integration and recombination between both plasmids restored the complete ags1 1 ORF sequence (see above strains).

To provide a flexible linker between GFP and the target proteins, a 12-alanine coding sequence was fused to the GFP 5′ and/or 3′-end of pKS-GFP (Cortes et al., 2002), making pKS-GFP-12A, pKS-12A-GFP, and pKs-12A-GFP. The pKS-GFP-12A is essentially described elsewhere (Shiba et al., 1997). Extracted from pJK148 (provided by R.Y. Tsien, University of California, San Diego, La Jolla, CA). pKS-Bsa-Cherry-12A is pKS-Cherry with 8- and 12-alanine coding sequences fused to Cherry 5′ and 3′-end, respectively. pJK-ag-1226-Cherry is pJK-ag-1226 inserted at base 5866 (amino acid 1956). The ags1 1 ags1 1-GFP strain (Table S1) expressed a functional Ags1 1-GFP and therefore, it was selected for Ags1 1 localization studies at the physiological level. (Fig. 1 A).

**Immunoprecipitation and immunoblot analysis**

10° early log-phase cells expressing the different tagged proteins were harvested, washed with stop solution (154 mM NaCl, 10 mM EDTA, 10 mM NaNO3, and 10 mM NaF), then with buffer (50 mM Tris-Cl, pH 7.5, 5 mM EDTA) suspended in lysis buffer (50 mM Tris-Cl pH 7.5, 5 mM EDTA, 200 mM NaCl containing 100 mM phenylmethylsulphonylfluoride and 2 µg/ml leupeptin and aprotonin) and broken with glass beads (Fastprep FP120, BioSpec, 5 s, speed of 15,101). Samples were centrifuged (4,500 g, 1 min, 4°C). The supernatants were collected and cell membranes were pelleted by centrifugation (16,000 g, 1 h, 4°C), suspended in 200 µl of immunoprecipitation buffer (IPB, 50 mM Tris-Cl, pH 7.5, 5 mM EDTA, 200 mM NaCl, 0.5% Tween 20, 100 µM phenylmethylsulphonylfluoride, and 2 µg/ml leupeptin and aprotonin), and agitated (1,300 rpm, 15 min, 1°C, Thermomixer Comfort, Eppendorf). Next, the membrane suspension was centrifuged (20,000 g, 30 min, 4°C), the supernatant was collected, diluted with IPB, and the solubilized membrane proteins were incubated with protein G-Sepharose 4 Fast Flow beads (GE Healthcare) coated with anti-GFP serum (Molecular Probes, Invitrogen) for 4 h at 4°C. The beads were washed three times with IPB and suspended in sample buffer.

The proteins were separated by 3–8% Tris-Acetate SDS–PAGE (NuPAGE, Invitrogen), transferred to Immobilon-P membranes (EMD Millipore), and blotted to detect the GFP- or HA-fused epitopes with the corresponding antibodies (monoclonal JL-8 anti-GFP 1:500 [Living colors; Takara Bio Inc.]; and monoclonal anti-HA 1:10,000 [Roche]), and the enhanced chemiluminescence (ECL) detection kit (GE Healthcare). Western blot analysis of solubilized membrane proteins (40 µg of total protein) was performed to determine the total amount of tagged protein.

**Labeling and fractionation of cell wall polysaccharides**

[14C]glucose labeling and fractionation of cell wall polysaccharides were performed as previously described (Shiba et al., 1997). Early log-phase cells were harvested at early logarithmic phase, supplemented with unbleached as the carrier, washed twice with 1 mM EDTA, and broken with glass beads in a Fastprep FP120 apparatus (3 × 20 s, speed of 6.0 [BIO 101; Savant]). Cell walls were purified by repeated washing and differential centrifugation (once with 1 mM EDTA, twice with 5 mM NaCl, and twice with 1 mM EDTA) at 1,000 g for 5 min. Finally, purified cell walls were heated at 100°C for 5 min. Cell wall samples were extracted with 6% NaOH for 60 min at 80°C and neutralized with acetic acid.
Precipitation of the galactomannan fraction from the neutralized supernatant was performed with FeHLing reagent by adding unlabeled yeast mannan as the carrier, as described previously (Algranati et al., 1966). Other samples of cell wall suspension were incubated with Zymolyase 100T (250 ng of enzyme and 1/10 volume of cell wall suspension; AMS Biotechnology) in 50 mM citrate-phosphate buffer (pH 5.6) for 24 h at 30°C. Samples without enzyme were included as a control. After incubation, samples were centrifuged and washed with the same buffer. 1 ml of 10% trichloroacetic acid was added to the pellets, filtered in Whatman GF/C glass fiber filters (3 x 1 ml of 10% trichloroacetic acid and 2 x 1 ml of ethanol), and their radioactivity levels were measured in a scintillation counter (Beckman Coulter). These pellets were considered the α-glucan fraction, and supernatants were the β-glucan-plus-galactomannan fraction. β-Glucan was calculated as radioactivity remaining after subtraction of galactomannan and α-glucan from total cell wall incorporation. All determinations were performed in duplicate, and data for each strain were calculated from three independent cultures.

**Microscopy techniques**

For Calcofluor white fluorescence, early logarithmic-phase cells were visualized directly by adding a solution of Calcofluor white [CW; 50 µg/ml final concentration] to the sample and using the appropriate UV filter. Images were obtained with a fluorescence microscope (model DM RXA; Leica), a PL APO 63x/1.32 oil PH3 objective, a digital camera (model DFC350FX; Leica) and CW4000 cytoSHIFt software (Leica). Images were processed with Adobe Photoshop software. For time-lapse imaging, 0.3-0.6 ml of log-phase cells were collected by low speed centrifugation (3,000 g for 1 min), suspended in 0.3 ml of EMM or EMM + 1.2 M sorbitol (with 20 µg/ml thiamine for cells repressing of bgs1+ cells) containing CW (5 µg/ml final concentration) when necessary, and placed in a well in a μ-Slide 8 well (80821-Uncoated; Ibidi) previously coated with 10 µl of 1% mg/ml soybean lectin (L1395; Sigma-Aldrich). Time-lapse experiments were performed at 37°C or the specified temperature by acquiring epifluorescence and/or phase-contrast cell images in single planes and 1 x 1 binning on an inverted microscope (model IX71; Olympus) equipped with a PlanApo 100x/1.401X70 objective and a Personal DeltaVision system (Applied Precision). Images were captured using a CoolSnap HQ2 monochrome camera (Photometrics) and softWoRx 5.5.0 release 6 imaging software (Applied Precision). Subsequently, GFP and RFP time-lapse images were restored and corrected by 3D Deconvolution (conservative ratio, 10 iterations and medium noise filtering) through softWoRx imaging software. Next, images were processed with Image (National Institutes of Health, Bethesda, MD) and Adobe Photoshop software.

For FITC-conjugated concanavalin A staining, equal amounts of log-phase HT1-RFP [control wild type, nucleus labeling] and 81D-ags1- cells were mixed, washed with PBS (3,000 g for 1 min), suspended in 0.5 ml PBS containing 100 µg/ml FITC-conjugated concanavalin A (C7642; Sigma-Aldrich), and incubated at 37°C for 20 min in the dark. After incubation, the cells were washed three times with PBS (3,000 g for 1 min), re-suspended in 10 µl PBS, and visualized for FITC and RFP fluorescence. Fluorescence intensity analysis of FITC-conjugated concanavalin A staining was performed with ImageJ software by placing a fixed square on the cell poles and measuring maximal fluorescence.

**Transmission electron microscopy**

Early log-phase cells were fixed with 2% glutaraldehyde EM [GA; Electron Microscopy Sciences] in 50 mM phosphate buffer, pH 7.2, and 150 mM NaCl (PBS) for 2 h at 4°C, post-fixed with 1.2% potassium permanganate overnight at 4°C, and embedded in Quetol 812 as described previously (Konami et al., 2003). Ultrathin sections were stained in 4% uranyl acetate and 0.4% lead citrate, and viewed with a transmission electron microscope (model DSM 940; Carl Zeiss) operating at 30 kV.

**Scanning electron microscopy**

Early log-phase cells were placed in a slide coated with 5 mg/ml soybean lectin (L1395; Sigma-Aldrich), prefixed overnight with 2.5% glutaraldehyde at 4°C, washed three times with 100 mM phosphate buffer, pH 7.4, and postfixed with 1% osmium tetroxide (OsO4) at 4°C for 1 h. Next, the cells were washed three times with water, dehydrated in graded acetone series (30, 50, and 70% for 10 min; 90% for 20 min; and 100% twice for 20 min and 30 min), critical-point dried, coated with gold, and visualized with a scanning electron microscope (model DSM 940; Carl Zeiss) operating at 30 kV.

**Online supplemental material**

Fig. 1E shows a scheme of the steps followed to obtain a fully functional ags1+–GFP strain and images of Ags1 localization during the cell cycle and sexual differentiation and of Ags1–Bgs1 colocalization. Fig. 2 shows time-lapse images of Ags1 localization in different SIN mutants, showing that Ags1 does not depend on the SIN pathway for its movement from the poles to the medial zone during cytokinesis. Fig. S3 shows genetic interactions of ags1+ with bgs1+ and SIN genes. The loss of Bgs1 or SIN function suppresses the lytic phenotype of Ags1 absence or mok1-664 mutant showing the CW-stained primary septum remnant in the new pole of both or just one sister cell. Video 1 shows time-lapse of a representative field of lysis in either one or both sister cells at the beginning of cell separation of Ags1-depleted cells. Videos 2 and 5 show time-lapses of a representative field (2) or a single cell (5) of the progressive and symmetrical cell separation of wild-type cells. Videos 3, 4, and 6 show time-lapses of representative fields (3, 4) or single cells (6–9) of the instantaneous and asymmetrical cell separation of Ags1-depleted cells, either without [3, 6, and 7] and or with sorbitol [4, 8, and 9]. Video 10 shows time-lapse of Ags1-depleted sister cells remaining attached by their side-contact region for at least two cell cycles. Table S1 lists the fission yeast strains used in this study. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201202015/DC1.

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Figure S1. **Like Bgs1, Ags1 localizes to all sites of wall synthesis during both the vegetative and sexual phases of the life cycle.** (A) Scheme showing the sequential steps followed to obtain *ags1*Δ strains containing an integrated *ags1*Δ-GFP copy (see Materials and methods). The *ags1*Δ 5' and 3' fragments are shown as gray boxes, in which the 2.5-kb overlapping region is shown as a light gray box. The 0.7-kb 12A-GFP-12A fragment, shown as a black box, can be inserted into any site of *ags1*Δ 5' sequence, before the AgeI linearization site. (B) *Ags1* localizes like *Bgs1* in the contractile ring very early in cytokinesis, before the septum is detected by Calcofluor white (CW) staining. However, contrary to *Bgs1*, *Ags1* does not disappear from the poles during cytokinesis until late septation. *ags1*Δ-GFP cells growing in YES medium at 28°C were transferred to fresh YES medium containing 5 µg/ml CW and visualized by time-lapse CW staining and GFP fluorescence microscopy at 28°C. Arrowhead: localization of *Ags1* in the medial ring before septum synthesis; Arrow: appearance of the septum structure. Elapsed time is shown in minutes. (C) *Ags1* and *Bgs1* colocalize at the division site (contractile ring and septum) and growing poles. Early log-phase *ags1*Δ-RFP *GFP-bgs1*Δ cells growing in YES medium at 28°C were visualized for CW staining (50 µg/ml) and GFP and RFP fluorescence. Bar, 5 µm. (D) *Ags1* is present and localized to all sites of cell wall synthesis during sexual differentiation. Homothallic *ags1*Δ-GFP-H90 cells grown in EMM to early stationary phase were transferred onto SPA plates and incubated at 28°C. Samples were collected after 3, 5, 8, 24, and 48 h and visualized by phase contrast (not depicted), CW staining (50 µg/ml), and GFP fluorescence. Cells and zygotes representative of each mating and sporulation step were selected and ordered to show a sexual phase progression. (E) *Ags1* is present and localized to all sites of cell wall synthesis during spore germination. Homothallic *ags1*Δ-GFP-H90 strain was grown and sporulated for 10 d as in D. The spores were collected and incubated in YES liquid medium at 28°C. Samples were taken after 2, 3, 7, 8, 9, 10, and 11 h, and observed as in D. Spores illustrative of each germination step were selected and ordered to show a germination progression. Bar, 5 µm.
Figure S2. Ags1 does not depend on the SIN pathway for its movement from the poles to the medial zone during cytokinesis. (A) SIN mutant cells expressing ags1-GFP were grown in YES medium at 25°C and shifted to 36°C for 4 h. (B) sid2-250 ags1-GFP hht1-RFP cells growing in YES medium at 25°C were shifted to 36°C for 2 h, transferred to fresh YES medium containing 5 µg/ml CW, and visualized by time-lapse CW staining (5 µg/ml) and GFP and RFP fluorescence at 36°C. Arrow: Ags1-GFP localization in the medial region after nuclear mitosis in the SIN mutant cell. Two examples representative of Ags1-GFP movement from the poles to the medial region after nuclear mitosis (see histone Hht1-RFP) are shown. (C) Ags1 localizes to the medial septum-like structures formed in the absence of a functional SIN. cdc11-119 ags1-GFP hht1-RFP cells were grown and visualized as in B. Two examples representative of Ags1-GFP movement and localization to the septum-like structures (see CW staining) after nuclear mitosis (see histone Hht1-RFP) are shown. (D) cdc11-119 ags1-RFP rlc1-GFP cells were grown and visualized as in B. Arrow: Ags1-GFP localization in the septum-like structure after mitosis and CAR assembly in the SIN mutant cell; arrowhead: septum-like structure detected by CW staining. Elapsed time is shown in minutes. Bar, 5 µm.
Figure S3. **ags1**+ presents genetic interactions with **bgs1**+ and SIN genes. The loss of Bgs1 function suppresses the lytic phenotype of Ags1 absence or mok1-664 mutation and promotes strong cytokinesis defects. The absence of septa of the SIN mutants suppresses the septum lysis phenotype of mok1-664 but promotes a new lysis at the poles. (A) The double mutants of mok1-664 with the Bgs1 mutant cps1-12 or with the SIN mutants cdc7-24 or sid2-250 are more thermosensitive than the single mutants. Early log-phase cells were adjusted to 10^7 cells/ml, 1:10 serial diluted, spotted onto YES and YES+S plates, and incubated at 25, 28, 32, 35, and 37°C for 3–4 d. The plates that displayed differences in growth between single and double mutant are shown. (B) The Bgs1 absence or cps1-12 mutation suppresses the lytic phenotype of Ags1 absence or mok1-664 mutation. In both cases the double mutants present strong septation defects. The cps1-12 mok1-664 double mutation originates arrested cells with no septa and the combined Bgs1 Ags1 absence promotes stronger septation defects. The single and double cps1-12 and mok1-664 mutants were grown in YES+S at 37°C for 15 h. The single and double ags1+ and bgs1+ repression cells were grown in EMM+S+T for 15 h. The cells were visualized by phase-contrast and CW-staining microscopy. Arrow: increased septation defects of double ags1+ bgs1+ repression cells. (C) The absence of septa of SIN mutants (high restrictive temperature) suppresses the septum lysis phenotype of mok1-664 mutant but promotes a new lysis at the poles, whereas when the SIN mutants are able to form septa (low restrictive temperature) the septum lysis phenotype of mok1-664 mutant is greatly increased. The single and double cdc7-24 or sid2-250 and mok1-664 mutants were grown in YES for 6 h at 37°C (top panels) or for 2 h at 28°C (sid2-250) or 32°C (cdc7-24) (bottom panels). Cells were imaged as in B. Bar, 5 μm.
Figure S4. Ags1-depleted or defective sister cells display a side-explosive cell separation due to tearing of a weak primary septum that remains attached to the new pole of one or both sister cells. After the side-explosive cell separation the cells remain attached through the septum edging area. (A) Steady cell separation in wild-type cells. Cells were grown in EMM+S+T for 7 h and visualized by time-lapse phase-contrast and CW (5 µg/ml)-staining microscopy. Elapsed time is shown in seconds. (B) The absence of Ags1 originates cells with an immediate side-explosive cell separation. Cells were grown and imaged by time-lapse phase-contrast microscopy as in A. Asterisk: time needed after cell separation start for maximal new end curvature. (C) Wild-type cells separate symmetrically and gradually. Phase-contrast and CW-staining micrographs of log-phase wild-type cells grown in EMM+S+T at 28°C. (D) Ags1-depleted sister cells show the remains of primary septum in the pole of either both (top panels) or only one cell after asymmetrical side-explosive cell separation and tearing of primary septum. Ags1-depleted cells were grown in EMM+S+T for 8 h and visualized as in C. (E) The Ags1-defective mok1-664 mutant shows the same side-explosive cell separation as the Ags1-depleted cells, with remnants of primary septum in one or both poles or just the septum edging. The cell separation occurs instantly (curved arrow) during the time spent for two image captures of the same cell. Cells were grown in YES+S at 37°C for 7 h and visualized as in C. Arrow: CW-stained remains of primary septum in the new end of both or just one sister cell. Circle: residual CW-stained primary septum in the septum edging. Bar, 5 µm.
Video 1. **Representative field showing the lysis of either one or both sister cells at the beginning of cell separation of *S. pombe Ags1-depleted cells.*** 81X-ags1+ cells were grown in the presence of thiamine for 3 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 120 min. To decrease the movie size, the interval of frames around each cell lysis is exclusively shown.

Video 2. **Representative field showing the progressive and symmetrical cell separation of *S. pombe* wild-type cells.*** Wild-type cells were grown in the presence of thiamine for 3 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 89 min. To decrease the movie size, the frames exclusively show the cell separation stage.

Video 3. **Representative field showing the instantaneous and asymmetrical cell separation of *S. pombe* Ags1-depleted cells.*** 81X-ags1+ cells were grown in the presence of thiamine for 3 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 89 min. To decrease the movie size, the frames exclusively show the cell separation stage.

Video 4. **Representative field showing the instantaneous and asymmetrical cell separation of *S. pombe* Ags1-depleted cells growing in the presence of sorbitol.*** 81X-ags1+ cells were grown in the presence of thiamine and 1.2 M sorbitol for 4 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 69 min. To decrease the movie size, the frames exclusively show the cell separation stage.

Video 5. **Progressive and symmetrical cell separation of a *S. pombe* wild-type cell.*** Wild-type cells were grown in the presence of thiamine for 3 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 6 min.

Video 6. **Instantaneous and asymmetrical cell separation of an Ags1-depleted cell.*** 81X-ags1+ cells were grown in the presence of thiamine for 3 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 6 min.
Video 7. Instantaneous and asymmetrical cell separation of an Ags1-depleted cell. 81X-ags1+ cells were grown in the presence of thiamine for 3 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 6 min.

Video 8. Instantaneous and asymmetrical cell separation of an Ags1-depleted cell growing in the presence of sorbitol. 81X-ags1+ cells were grown in the presence of thiamine and 1.2 M sorbitol for 4 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 6 min.

Video 9. Instantaneous and asymmetrical cell separation of an Ags1-depleted cell growing in the presence of sorbitol. 81X-ags1+ cells were grown in the presence of thiamine and 1.2 M sorbitol for 4 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 6 min.

Video 10. After instantaneous and asymmetrical cell separation, the Ags1-depleted sister cells remain attached by their lateral septum cell wall region for at least two cell cycles. 81X-ags1+ cells were grown in the presence of thiamine and 1.2 M sorbitol for 4 h and imaged by time-lapse phase-contrast microscopy, using an inverted microscope (model IX71; Olympus) equipped with a Personal DeltaVision system (Applied Precision). Frames were taken every 20 s for 20 min.
Table S1. Fission yeast strains used in this study

| Strain | Genotype | Source |
|--------|----------|--------|
| 33     | leu1-32 ura4Δ18 | P. Munera^a |
| 256    | leu1-32 ura4Δ18 | J.C. Ribas |
| 284    | leu1-32 ura4Δ18 | J.C. Ribas |
| 289    | leu1-32 ura4Δ18 | J.C. Ribas |
| 419    | leu1-32 ura4Δ18 | J.C. Ribas |
| 420    | leu1-32 ura4Δ18 | J.C. Ribas |
| 470    | leu1-32 ura4Δ18 | J.C. Ribas |
| 317    | leu1-32 ura4Δ18 | J.C. Ribas |
| 439    | cdc3-6 leu1-32 | J.C. Ribas |
| 572    | cdc14-118 leu1-32 | J.C. Ribas |
| 574    | cdc5-140 leu1-32 | J.C. Ribas |
| 577    | cdc5-140 leu1-32 | J.C. Ribas |
| 580    | mid1-366 leu1-32 | J.C. Ribas |
| 899    | cdc11-119 his3Δ1 | J.C. Ribas |
| 900    | cdc11-119 leu1-32 | J.C. Ribas |
| 953    | tea1-50 leu1-32 | J.C. Ribas |
| 962    | leu1-32 ura4Δ18 ade6 cen1::GFP:KanMX6 h^+ | F. Chang^e |
| 971    | leu1-32 ura4Δ18 ade6 cen1::GFP:KanMX6 h^+ | F. Chang^e |
| 1723   | leu1-32 ura4Δ18 his3Δ1 bgs1::ura4^+ P_bgs1::GFP-bgs1::leu1^+ h | J.C. Ribas |
| 1771   | sec8-1 leu1-32 ura4Δ18 h | J.C. Ribas |
| 1905   | leu1-32 ura4Δ18 ade6 for3::KanMX6 h | J.C. Ribas |
| 2492   | leu1-32 ura4Δ18 for3::KanMX6 h | J.C. Ribas |
| 2933   | leu1-32 ura4Δ18 cen1::GFP:KanMX6 for3::KanMX6 h | J.C. Ribas |
| 3137   | leu1-32 ura4Δ18 ade6 myo52::ura4^+ | M.H. Valdivieso^d |
| 2156   | mok1-666 leu1-32 ura4Δ18 h | P. Pérez |
| 2326   | sid2-250 leu1-32 ura4Δ18 ade6-M216 h | J.C. Ribas |
| 2327   | sid2-250 leu1-32 ura4Δ18 h | J.C. Ribas |
| 3065   | cdc7-24 ura4Δ18 h | J.C. Ribas |
| 2523   | cdc12-112 ura4Δ18 h | J.C. Ribas |
| 2525   | leu1-32 ura4Δ18 ade6 cen1::GFP:alb2::ura4^+ h | V. Simanis^e |
| 2086   | leu1-32 ura4Δ18 ade6 P_mel1::ags1::ura4^+ h | This paper |
| 2100   | leu1-32 ura4Δ18 P_mel1::ags1::ura4^+ h | J.C. Ribas |
| 2234   | leu1-32 ura4Δ18 P_mel1::ags1::ura4^+ h | J.C. Ribas |
| 1127   | leu1-32 ura4Δ18 ura4Δ18 his3Δ1/his3Δ1 ade6-M210/ade6-M216 ags1::ags1::ura4^+ h | This paper |
| 1804   | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1::ura4^+ h p41XH-ags1^+ | This paper |
| 2881   | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1::ura4^+ h p41XH-ags1^+ | This paper |
| 2939   | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1::3'UTR_ags1::ags1::ura4^+ h p41XH-ags1^+ | This paper |
| 3166   | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 3265   | ura4Δ18 rlc1::GFP::KanMX6 h | V. Simanis^e |
| 4004   | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:RFP:leu1::ura4^+ h | This paper |
| 4047   | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1::3'UTR_ags1::ags1::RFP:KanMX6 h | V. Simanis^e |
| 4090   | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1::3'UTR_ags1::ags1::3XHA-bgs1::leu1^+ h | J.C. Ribas |
| 4223   | leu1-32 ura4Δ18 ade6 P_mel1::ags1::ura4^+ h rlc1::GFP::KanMX6 h | This paper |
| 4083   | leu1-32 ura4Δ18 ade6 P_mel1::ags1::ura4^+ h rlc1::GFP::KanMX6 h | This paper |
| 3846   | mid1-366 leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 3849   | cdc6-3 leu1-32 ura4Δ18 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 3850   | cdc15-140 leu1-32 ura4Δ18 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 3852   | cdc11-119 leu1-32 ura4Δ18 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 3853   | cdc11-119 leu1-32 ura4Δ18 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 3855   | cdc14-118 leu1-32 ura4Δ18 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 3856   | cdc16-116 leu1-32 ura4Δ18 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 3861   | tea1-1 leu1-32 ura4Δ18 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
| 4014   | mid1-366 leu1-32 ura4Δ18 ade6-M210 ags1::3'UTR_ags1::ags1::GFP:leu1::ura4^+ h | This paper |
Table S1.  **Fission yeast strains used in this study** (Continued)

| Strain  | Genotype                                                                 | Source          |
|---------|---------------------------------------------------------------------------|-----------------|
| 4179    | sid2-250 leu1-32 ura4Δ18 ade6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-GFP:leu1<sup>+</sup>:ura4<sup>+</sup> hkl1<sup>+</sup>-mRFP:KanMX6 h<sup>+</sup> | This paper      |
| 4016    | cdc12-112 leu1<sup>+</sup> ura4Δ18 his3Δ1 ade6-M210 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-GFP:leu1<sup>+</sup>:ura4<sup>+</sup> h<sup>+</sup> | This paper      |
| 4048    | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup> bgs1Δ<sup>+</sup> | This paper      |
| 4067    | leu1-32 ura4Δ18 his3Δ1 ade6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup>crn1<sup>+</sup>-GFP:KanMX6 h<sup>+</sup> | This paper      |
| 4241    | cdc3-6 leu1-32 ura4Δ18? his3Δ1 ade6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup>crn1<sup>+</sup>-GFP:KanMX6 h<sup>+</sup> | This paper      |
| 4243    | leu1-32 ura4Δ18 his3Δ1 ade6 for3::KanMX6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup>crn1<sup>+</sup>-GFP:KanMX6 h<sup>+</sup> | This paper      |
| 4245    | sec8-1 leu1-32 ura4Δ18 ade6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup>crn1<sup>+</sup>-GFP:KanMX6 h<sup>+</sup> | This paper      |
| 4247    | leu1-32 ura4Δ18 his3Δ1 ade6 myo52Δ::ura4<sup>+</sup> ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup>crn1<sup>+</sup>-GFP:KanMX6 h<sup>+</sup> | This paper      |
| 3954    | leu1-32 ura4Δ18 his3Δ1 ade6-M210 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-GFP:leu1<sup>+</sup>:ura4<sup>+</sup> h<sup>+</sup>   | This paper      |
| 4778    | cps1-12 mok1-664 leu1-32 ura4Δ18 h<sup>+</sup>                           | This paper      |
| 4786    | leu1-32 ura4Δ18 ade6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-GFP:leu1<sup>+</sup>:ura4<sup>+</sup> bgs1Δ::ura4<sup>+</sup> P<sub>bgs1</sub>::3XHA-bgs1<sup>+</sup>:leu1<sup>+</sup> h<sup>+</sup> | This paper      |
| 4790    | leu1-32 ura4Δ18 his3Δ1 ade6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup> leu1<sup>+</sup>::GFP-atb2<sup>+</sup>-ura4<sup>+</sup> h<sup>+</sup>   | This paper      |
| 4800    | cdc15-140 leu1-32 ura4Δ18 ade6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup> leu1<sup>+</sup>::GFP-atb2<sup>+</sup>-ura4<sup>+</sup> h<sup>+</sup> | This paper      |
| 4802    | cdc11-119 leu1-32 ura4Δ18 ade6 ags1Δ 3′UTR<sub>agpl1</sub>...:ags1<sup>+</sup>-RFP:leu1<sup>+</sup>:ura4<sup>+</sup> rlcl1<sup>+</sup>-GFP:KanMX6 h<sup>+</sup> | This paper      |
| 4825    | leu1-32 ura4Δ18 ade6 P<sub>ad1</sub>::ags1<sup>+</sup>-ura4<sup>+</sup> P<sub>ad1</sub>::3XHA-bgs1<sup>+</sup>:bgs1<sup>+</sup> h<sup>+</sup> | This paper      |
| 4844    | cdc7-24 mok1-664 leu1-32 ura4Δ18 h<sup>+</sup>                           | This paper      |
| 4846    | sid2-250 mok1-664 leu1-32 ura4Δ18 h<sup>+</sup>                           | This paper      |

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