Cortés et al., http://www.jcb.org/cgi/content/full/jcb.201202015/DC1



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 ags 1 strains containing an integrated ags 1+ GFP copy (see Materials and methods). The ags 1+ 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+ 5' sequence, before the Agel 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. ags 1+ 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 ags 1+-RFP GFP-bgs 1+ 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) Ags 1 is present and localized to all sites of cell wall synthesis during sexual differentiation. Homothallic ags 1+ 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 ags 1+ 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 of 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) $cdc11-119 ags1^+$ -GFP cells were grown and visualized as in B. Two examples representative of Ags1-GFP novement and localization to the septum-like structures (see CW staining) after nuclear mitosis (see histone Hht1-RFP) are shown. (D) $cdc11-119 ags1^+$ -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 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 *cps1-12* and *mok1-664* mutants were grown in YES+S at 37° C for 15 h. The single and double *ags1^+* repression cells were and *mok1-664* mutants 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 as the Ags1-depleted cells, with remnants 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-*ags*1⁺ 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. 81Xags1⁺ 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. 81Xags1⁺ 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	972 h ⁻	P. Munzª
256	cps1-12 leu1-32 ura4-∆18 h	J.C. Ribas
284	leu1-32 ura4-Δ18 his3-Δ1 h ⁻	J.C. Ribas
285	leu1-32 ura4-∆18 his3-∆1 h+	J.C. Ribas
419	<i>leu1-32 ura4-</i> ∆18 h [−]	J.C. Ribas
420	leu1-32 ura4-∆18 h⁺	J.C. Ribas
470	leu1-32 ura4-∆18 his3-∆1 h ⁹⁰	J.C. Ribas
317	leu1-32/leu1-32 ura4-∆18/ura4-∆18 his3-∆1/his3-∆1 ade6-M210/ade6-M216 h ⁻ /h⁺	J.C. Ribas
439	cdc3-6 leu 1-32 h*	I.C. Ribas
572	cdc14-118 leu1-32 ade6-M210 h⁺	I.C. Ribas
574	cdc15-140 leu1-32 h ⁺	I.C. Ribas
577	cdc15-140 leu1-32 h	I.C. Ribas
580	cdc16-116 ura4-0.18 h+	I.C. Ribas
584	teg2-1 leu1-32 gde6-M210 h*	P Nurse ^b
635	mid1-366 leu1-32 h+	P Nurse ^b
899	cdc11-119 leu1-32 his 3-1 h+	LC Ribas
900	cdc11-119 leu1-32 his3-11 h ⁻	J.C. Ribas
953	teg 1-50 lev 1-32 vrg/-0.18 h+	LC Ribas
962	leu 1.32 ura 1.0.18 adeó cro 1+ GEP: KapMY6 h+	F. Chana ^c
071	leu 1-32 ura 4. A 18 adeo cm 1+ GEP: Kan MX6 h	F. Chang ^c
1723	leu 1-32 ura (. A 18 bis 3-A 1 bas 1 A uura (* P GEP. bas 1* ileu 1* b*	I.C. Ribas
1723	een-2 ord-2 or 1.32 ura/. A 18 ht	J.C. Ribas
1005	leu 1.32 ura 1.0.18 ade6 for 30 ··KapMX6 h+	P. Perez ^d
2/92	$e_{0}1-32$ ura/A 18 for 3A ···KanMX6 h	I C Ribas
2933	leu 1-32 ura 4 A 18 cm 1+ GEP·KanMX6 for 3A ··KanMX6 h+	J.C. Ribas
2137	leu 1.32 ura 1.0 18 adeó myo 520 ::ura d* b*	M H. Valdivieso ^d
2156	mok 1-664 Jeu 1-32 ura 4.0.18 h+	P Párez
/781	$m_{0}k_{1}^{-}$ 664 lev 1-32 $v_{1}a_{1}^{-}$ 18 h ⁻	This study
2326	sid2-250 leu 1-32 ura/-18 ade6-M216 h+	LC Ribas
2327	sid2-250 lev 1-32 ura/-0.18 h ⁻	J.C. Ribas
3065	cdc7.24 ura/. 1.8 h ⁺	J.C. Ribas
2523	cdc/2-112 urg/-0.18 h ⁺	P Pérez
2525	$\log 1/32$ ura (A) 18 $\log 1^+$ (GFP ath 2^+ ura (A ⁺ b ⁺)	V Simonis ^e
2025	e_{1-32} ura (1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.	V. Simanis This paper
2100	$ e_0 _{32} u_{a} _{A} B _{B} = u_{a} _{B _{A}} u_{a} _{A} B _{B _{A}} u_{a} _{A} B _{$	I C Ribas
2700	leu 1.32 ura 4.318 P	J.C. Ribas
1127	leu1-32/leu1-32 ura4-\18/ura4-\18 his3-\1/ his3-\1 ade6-M210/ade6-M216	This paper
	$ags1^+/ags1\Delta$::ura4 ⁺ h ⁻ /h ⁺	
1804	leu1-32 ura4-∆18 his3-∆1 ade6-M210 ags1∆::ura4* h [−] p41XH-ags1*	This paper
2881	leu1-32 ura4-∆18 his3-∆1 ade6-M210 ags1∆ h [−] p41XH-ags1 ⁺	This paper
2939	leu1-32 ura4-Δ18 his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1 ₃₇₀₄₋₇₂₃₃ :ura4 ⁺ h ⁻ p41XH-ags1 ⁺	This paper
3166	leu1-32 ura4-∆18 his3-∆1 ade6-M210 ags1∆ 3′UTR _{ags1+} ::ags1+-GFP:leu1+:ura4+ h ⁻	This paper
3265	ura4-Δ18 rlc1+-GFP:KanMX6 h+	V. Simanis ^e
4004	leu1-32 ura4-Δ18 his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+-RFP:leu1+:ura4+ h	This paper
4047	leu1-32 ura4-Δ18 his3-Δ1 ade6 hht1+-RFP:KanMX6 h ⁺	P. Pérez
4090	leu1-32 ura4-Δ18 his3-Δ1 bgs1Δ::ura4 ⁺ P _{bgs1+} ::3XHA-bgs1 ⁺ :leu1 ⁺ h ⁺	J.C. Ribas
4223	leu 1-32? ura4- Δ 18 ade6 ags 1 Δ 3'UTR _{ags1+} ::ags 1+-RFP:leu 1+:ura4+ rlc 1+-GFP:KanMX6 h+	This paper
4083	leu1-32 ura4-Δ18 his3-Δ1 ade6 ags1Δ 3'UTR _{ags1+} ::ags1+-GFP:leu1+:ura4+ hht1+-RFP:KanMX6 h ⁺	This paper
3846	mid1-366 leu1-32 ura4? his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+:GFP:leu1+:ura4+ h+	This paper
3849	$cdc3-6$ leu 1-32 ura4? $ags1\Delta$ 3'UTR _{ags1+} :: $ags1+GFP$:leu 1+: $ura4+h^+$	This paper
3850	cdc15-140 leu1-32 ura4? his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+-GFP:leu1+:ura4 ⁺ h ⁺	This paper
3852	cdc11-119 leu1-32 ura4? his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+-GFP:leu1+:ura4 ⁺ h ⁻	This paper
3853	cdc11-119 leu1-32 ura4? his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+-GFP:leu1+:ura4 ⁺ h ⁺	This paper
3855	cdc14-118 leu1-32 ura4? ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+:GFP:leu1+:ura4 ⁺ h ⁻	This paper
3856	cdc16-116 leu1? ura4-Δ18 his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+-GFP:leu1+:ura4+ h+	This paper
3858	tea 1-50 leu 1-32 ura4-Δ18 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+-GFP:leu 1+:ura4+ h+	This paper
3861	tea2-1 leu1-32 ura4? his3-∆1 ade6-M210 ags1∆ 3'UTR _{ags1+} ::ags1+.GFP:leu1+:ura4+ h ⁻	This paper
4014	sid2-250 leu1-32 ura4- Δ 18 ade6 ags1 Δ 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 ura4-∆18 ade6 ags1∆ 3′UTR _{ags14} ∷ags1+-GFP:leu1+:ura4+ hht1+-mRFP:KanMX6 h+	This paper
4016	cdc12-112 leu1? ura4-Δ18 his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1+-GFP: leu1+:ura4+ h+	This paper
4048	leu1-32 ura4-Δ18 his3-Δ1 ade6-M210 ags1Δ 3'UTR _{ags1+} ::ags1 ⁺ -RFP:leu1+:ura4+ bgs1Δ::ura4+ P _{bgs1+} ::GFP-bgs1+:leu1+ h+	This paper
4067	leu1-32 ura4-Δ18 his3-Δ1 ade6 ags1Δ 3'UTR _{ags1+} ::ags1+.RFP:leu1+:ura4+crn1+.GFP:KanMX6 h ⁻	This paper
4241	cdc3-6 leu1-32 ura4-Δ18? his3-Δ1 ade6 ags1Δ 3′UTR _{ags1+} ::ags1+-RFP:leu1+:ura4+crn1+- GFP:KanMX6 h	This paper
4243	leu1-32 ura4-∆18 his3-∆1 adeó for3∆::KanMX6 ags1∆ 3′UTR _{ags1+} ::ags1+-RFP:leu1+:ura4+crn1+-GFP: KanMX6 h [−]	This paper
4245	sec8-1 leu1-32 ura4-∆18 ade6 ags1∆ 3′UTR _{ags1+} ::ags1⁺-RFP:leu1*:ura4*crn1*-GFP:KanMX6 h [−]	This paper
4247	leu1-32 ura4-Δ18 ade6 myo52Δ::ura4 ⁺ ags1Δ 3'UTR _{ags1+} ::ags1 ⁺ -RFP:leu1 ⁺ :ura4 ⁺ crn1 ⁺ -GFP:KanMX6 h ⁻	This paper
3954	leu1-32 ura4-Δ18 his3-Δ1 ade6-M210 ags1Δ 3'UTR _{aas1+} ::ags1+-GFP:leu1+:ura4+ h ⁹⁰	This paper
4778	cps1-12 mok1-664 leu1-32 ura4-∆18 h [−]	This paper
4786	leu 1-32 ura4-Δ18 ade6 ags1Δ 3'UTR _{ags1+} ::ags1+-GFP:leu1+:ura4+ bgs1Δ::ura4+ P _{bgs1+} ::3XHA-bgs1+:leu1+ h+	This paper
4790	leu1-32 ura4-Δ18 his3-Δ1 ade6 ags1Δ 3'UTR _{ags1+} ::ags1+.RFP:leu1+:ura4+ leu1+::GFP-atb2+:ura4+ h+	This paper
4800	cdc15-140 leu1-32? ura4-Δ18 ags1Δ 3'UTR _{ags1+} ::ags1+-RFP:leu1+:ura4+ rlc1+-GFP:KanMX6 h+	This paper
4802	cdc11-119 leu1-32 ura4-∆18 ade6 ags1∆ 3′UTR _{ags1+} ::ags1+-RFP:leu1+:ura4+ rlc1+-GFP:KanMX6 h	This paper
4825	leu1-32 ura4-Δ18 ade6 P _{nmt1-81X} -ags1+:ura4+ P _{nmt1-81X} -bgs1+ h ⁻	This paper
4844	cdc7-24 mok1-664 leu1-32 ura4-∆18 h [−]	This paper
4846	sid2-250 mok1-664 leu1-32 ura4-Δ18 h ⁺	This paper

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