Supplemental Materials

Molecular Biology of the Cell

Shen et al.

Supplemental Table 1: Primers Used in This Study

Name	Purpose	Sequence
HP15	*nimA* GFP-tagging	GAACACCATTCTACATGTCTCCAG
HP16	*nimA* GFP-tagging	CCTCAATTGCGAATCACCTTTC
HP17	*nimA* GFP-tagging	TGAAAACGCAAGATCCTAACC
HP18	*nimA* GFP-tagging	TGGCGTACGAGCGCTTCAAAACTG
HP19	*nimA* GFP-tagging	GGGGAAGAAAGGTGATTCGCAACTTGAGGGGAGCTGGTGCAGGCGCTGGAGCCAAAG
HP20	*nimA* GFP-tagging	ACCGCCGGTTAGGACGATCTTGCGTTTTCACTGTCTGAGAGGAGGCACTGATGCGTG
KP44	HexA GFP-tagging and HexA deletion	ACTTGACCTTGCCTG
KP45	HexA deletion	CCTACCTCACCTCACCTTGC
KP46	HexA deletion	GAAGAGCATTGTTTGAGGCGCTTGCGAGCCTTGGGGTGAT
KP47	HexA GFP-tagging	GTTACCGTGACGGCGTCAAG
KP48	HexA GFP-tagging	GGCTCCAGCGCCTGCACCAGCTCCTAGACGGGAAGAGTGGATGA
KP49	HexA GFP-tagging and HexA deletion	GCATCAGTGCCTCCTCTCAGTAAGCTGTCTACAGGGGTTC
KP50	HexA-GFP-tagging and HexA deletion	GATAGCCTACCTGTGG
KP51	HexA GFP-tagging and HexA deletion	CTGTGCTCCATTACATGCCA
KP52	HexA-GFP-PTS1-tagging	CCTGTAGACAGCTTAGAGGCGAGATTTGTATAGTTCATCCATGC
KP53	HexA-GFP-PTS1-tagging	GATGAACTATACAAATCTCGCCTCTAAGCTGTCTACAGGGGTTC
KP54	HexA-GFP-PTS1-tagging	CAAGGAGCCGAATCAGATCA
KP55	HexA-GFP-PTS1-tagging	AGGCGAAGGTGAGATTCAGG
KP56	SepA GFP-tagging	GAATGTGCAGGCGAGCTTGG
KP57	SepA GFP-tagging	CCGTGTCAGCCAGATTACTC
KP58	SepA GFP-tagging	GGCTCCAGCGCCTGCACCAGCTCCGCTGGTCGAGGGGCGATGTTC
KP59	SepA GFP-tagging	GCATCAGTGCCTCCTCTCAGTAGCTGTTTCTCAAGCAAAG
KP60	SepA GFP-tagging	GTAGATGGTGCGAACGGCTG
KP61	SepA GFP-tagging	GCTTGGCCACAAGGTTCGTC
KP137	MyoB chRFP-tagging	CGCCTCCGAGAAGAACTTGA
KP138	MyoB chRFP-tagging	GCCAAGGAGAAGTCACGTAT
KP139	MyoB chRFP-tagging	GGCTCCAGCGCCTGCACCAGCTCCAAGAAAACCTTTGGTGTTGCTA
KP140	MyoB chRFP-tagging	GCATCAGTGCCTCCTCTCAGTGAGCGAAAGCCGCTTGTTAAG
KP141	MyoB chRFP-tagging	CCACTTCTCGCGCATGAGTT
KP142	MyoB chRFP-tagging	CACCACCAGGTATCTCACGA
KP156	SO deletion	GGTCAAGATGGCGGCATTGG
KP157	SO deletion	CAAGCGGCGACGACAAGTTC
KP158	SO deletion	GAAGAGCATTGTTTGAGGCGCGTGACGGCAGTGTTTCCTC
KP162	SO deletion	GCATCAGTGCCTCCTCTCAGTGATTGATGCCGAGTCTTTTTAATG
KP163	SO deletion	TAGACACCGTGTTCCGCCTG
KP164	SO deletion	CCGCTCTCGCAGGAACAGAA

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KP165	Express GFP-S-Tag under the promoter of AN1553	GCACCGGATCGCAGCAATTC
KP166	Express GFP-S-Tag under the promoter of AN1553	GCGGACACAAGCGACATAGT
KP167	Express GFP-S-Tag under the promoter of AN1553	GAAAAGTTCTTCTCCTTTACTCCCCGGTTCCATCTTGAATGAATTG
KP168	Express GFP-S-Tag under the promoter of AN1553	GCATCAGTGCCTCCTCTCAGGCAGACCCCCTTGACGGCCAAT
KP169	Express GFP-S-Tag under the promoter of AN1553	GGTGGATCGAACGGTATATG
KP170	Express GFP-S-Tag under the promoter of AN1553	CGTGGTTGGCTTGCAGTAGG
SO445	SPA3 GFP-tagging	CCTTCATCAGGATCACGACG
SO446	SPA3 GFP-tagging	CACGATAGACAGCCATGGAG
SO447	SPA3 GFP-tagging	ATCAGTGCCTCCTCTCAGACAGTAACTGATGGAAGCCGAGA
SO448	SPA3 GFP-tagging	CTCCAGCGCCTGCACCAGCTCCACTCTTCTGCGACGATCTTGGC
SO449	SPA3 GFP-tagging	CTGCGCCTAATTCTCGTCTC
SO450	SPA3 GFP-tagging	CGTCATGGCCACATCAGATC
SO457	SPA10 GFP-tagging	GGACGACATGGAAGCTCTGC
SO458	SPA10 GFP-tagging	CTGTTCCAGAAGCCGATGAG
SO459	SPA10 GFP-tagging	ATCAGTGCCTCCTCTCAGACAGTAATAAGTGCGCGAGTTTGAC
SO460	SPA10 GFP-tagging	CTCCAGCGCCTGCACCAGCTCCGTAGTCATGCTCCTCGTCAAC
SO461	SPA10 GFP-tagging	CCCGAGTTCATTGATGCGTG
SO462	SPA10 GFP-tagging	GAAAAACACCTGCTTGTCCC
SO469	SPA13 GFP-tagging	CGGCTAGAGGATCATAGACG
SO470	SPA13 GFP-tagging	CGCGAGCTCTCCGATTACAG
SO471	SPA13 GFP-tagging	ATCAGTGCCTCCTCTCAGACAGTGAACATGAACATGACCATCAC
SO472	SPA13 GFP-tagging	CTCCAGCGCCTGCACCAGCTCCTTTCATTGTATGGCTTTTTCTGAC
SO473	SPA13 GFP-tagging	GAACGGACTATCCAGCTAGC
SO474	SPA13 GFP-tagging	GAAGCTGGTTTTGAGAGCGG
SO505	KfsA GFP-tagging	CAGAGCGACAGATCCAAGAC
SO506	KfsA GFP-tagging	GAAACCTACCGGCCAAGAAC
SO507	KfsA GFP-tagging	ATCAGTGCCTCCTCTCAGACAGTAGAGGGGCCAGCTGCTCTA
SO508	KfsA GFP-tagging	CTCCAGCGCCTGCACCAGCTCCGCGGTCGTCCCCAACTCTGGATTT
SO509	KfsA GFP-tagging	CACACAGTACGCGAACGAAG
SO510	KfsA GFP-tagging	CCACGCAACTTCTGTGCTCG

Supplemental Figures:

A.

B.
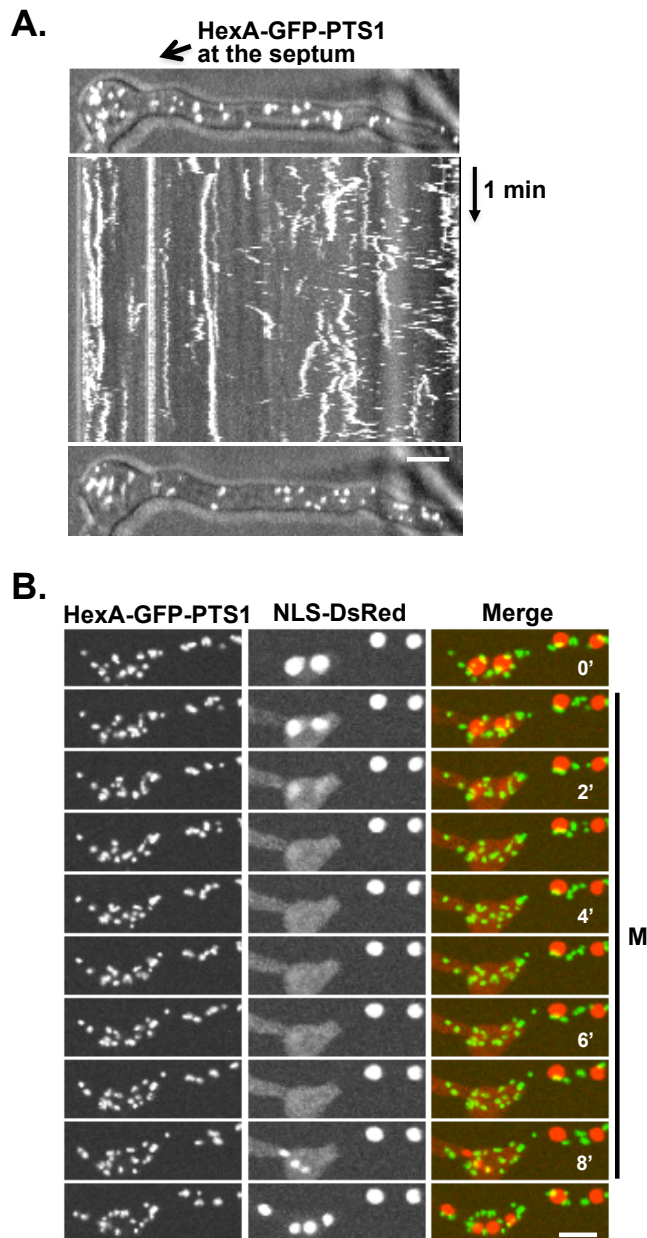
Figure S1. Immobile Woronin bodies locate adjacent to septa during interphase and mitosis. (A) A kymograph of HexA-GFP-PTS1 and the corresponding DIC image of strain KF095 showing HexA-GFP-PTS1 labelled Woronin bodies located adjacent to septum do not move while all other Woronin bodies are not as stationary. Images were captured at 2 sec intervals. (B) Time-lapse images of strain KF95 showing HexA-GFP-PTS1 labeled Woronin bodies and NLS-DsRed during mitosis. The Woronin bodies located near a septum stay adjacent to the septum during mitosis. Images were collected with 10 sec delays with images displayed at one minute intervals. Bars, 5 µm.
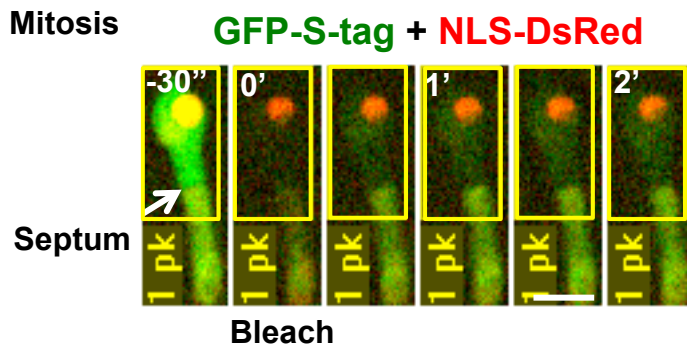
Figure S2. A mitotic septal pore is closed. A septum (arrowed) separates one cell in mitosis at the bottom (NLS-DsRed dispersed) from one in interphase (NLS-DsRed nuclear). As depicted by the rectangle, the top cell was photobleached and the recovery of GFP-S-tag monitored by live cell imaging. This revealed a barrier preventing rapid movement of GFP-S-tag through the septal pore when one cell is in mitosis. One pre-bleach and five post-bleach images were captured with 30 sec intervals using strain KF491.
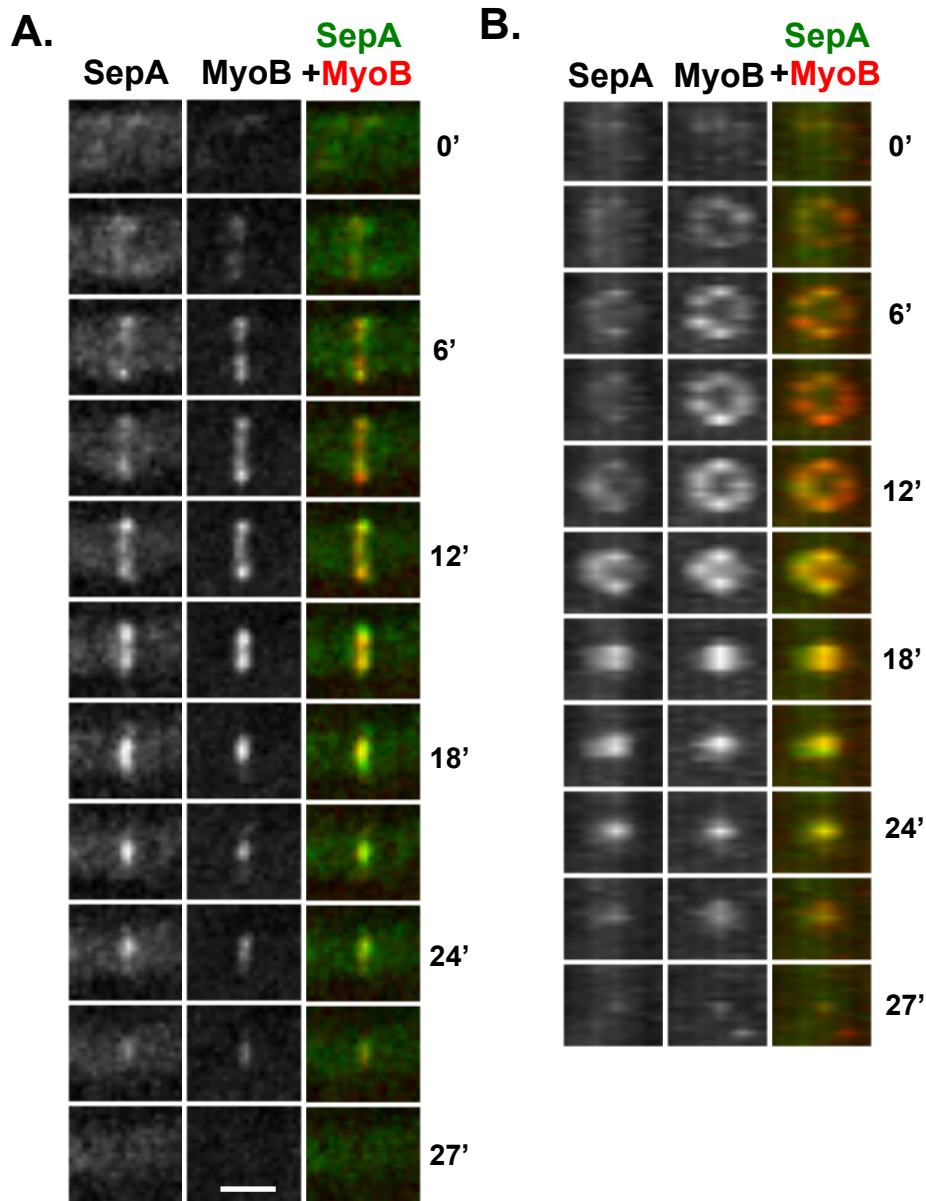
Figure S3. SepA co-localizes with MyoB at forming septa. (A) Time-lapse images showing SepA-GFP co-localizes with MyoB-chRFP at forming septa. Through-Z images were captured at 30 sec intervals. (B) Images of (A) were rotated 90° (using ImageJ software) for an end-on view of septum formation. Both SepA-GFP and MyoB-chRFP appear at forming septa as transient rings and then the rings fill and contract. Both SepA-GFP and MyoB-chRFP are removed as the septum is formed. Strain KF298 was used. Bars, 5 µm.
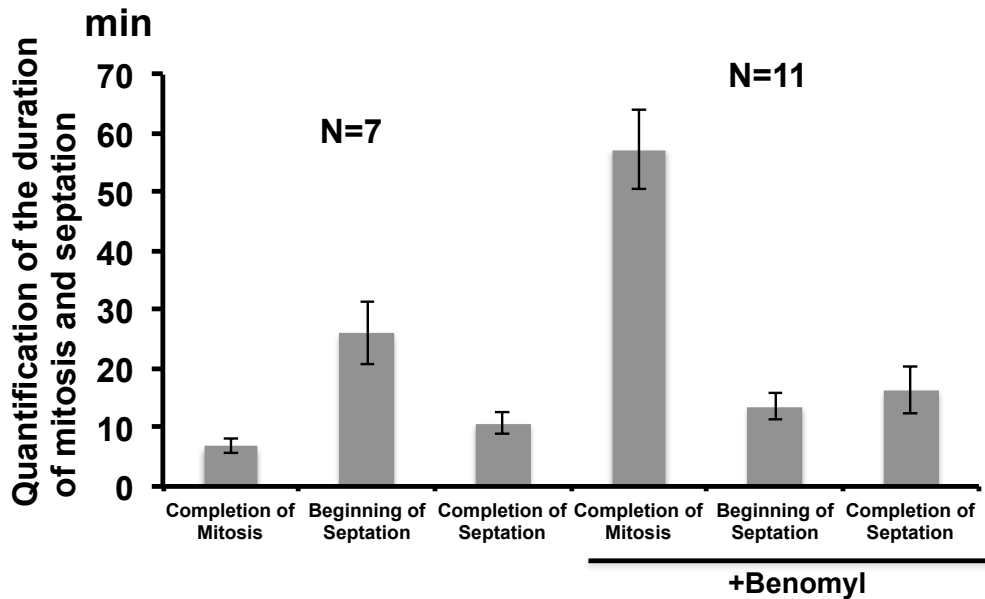
Figure S4. Quantification of mitosis and septation times with and without microtubule functions. In the absence or presence of benomyl, strain KF033 containing NIMA-GFP and NLS-DsRed was used for imaging and the duration of mitosis and septation was determined using NLS-DsRed as a mitotic marker and NIMA-GFP as a septation marker. The duration of mitosis was measured as the time from the dispersal of NLS-DsRed into the cytoplasm to the re-import of NLS-DsRed into the nucleus. The time between completion of mitosis and the beginning of septation was measured as the time from when the nuclear re-import of NLS-DsRed occurred to the appearance of NIMA-GFP at septation sites. The time to complete septation was measured as the time from the appearance of NIMA-GFP at septation sites to the time when NIMA-GFP first became concentrated at the middle of septa.

Supplementary Movie Information:
Movie 1: 3D reconstruction imaging of NIMA-GFP at the site of forming septum.
Frame rate = 30 fps. Rotation angle increment: 10°. Length of movie = 20 minutes.