

## Supplementary Information

Anillin-related Protein Mid1p Coordinates the Assembly of the Cytokinetic Contractile Ring in Fission Yeast

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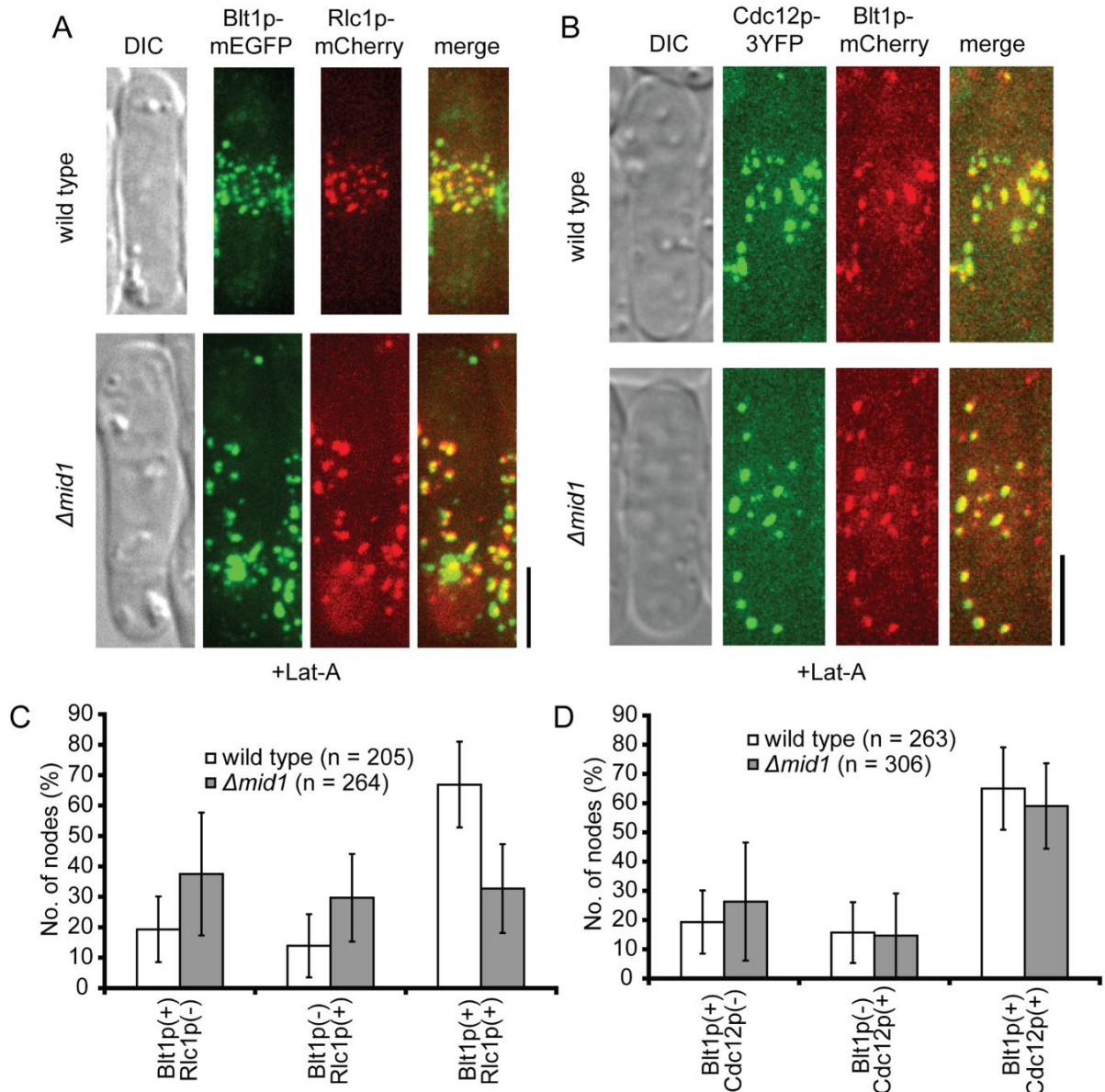
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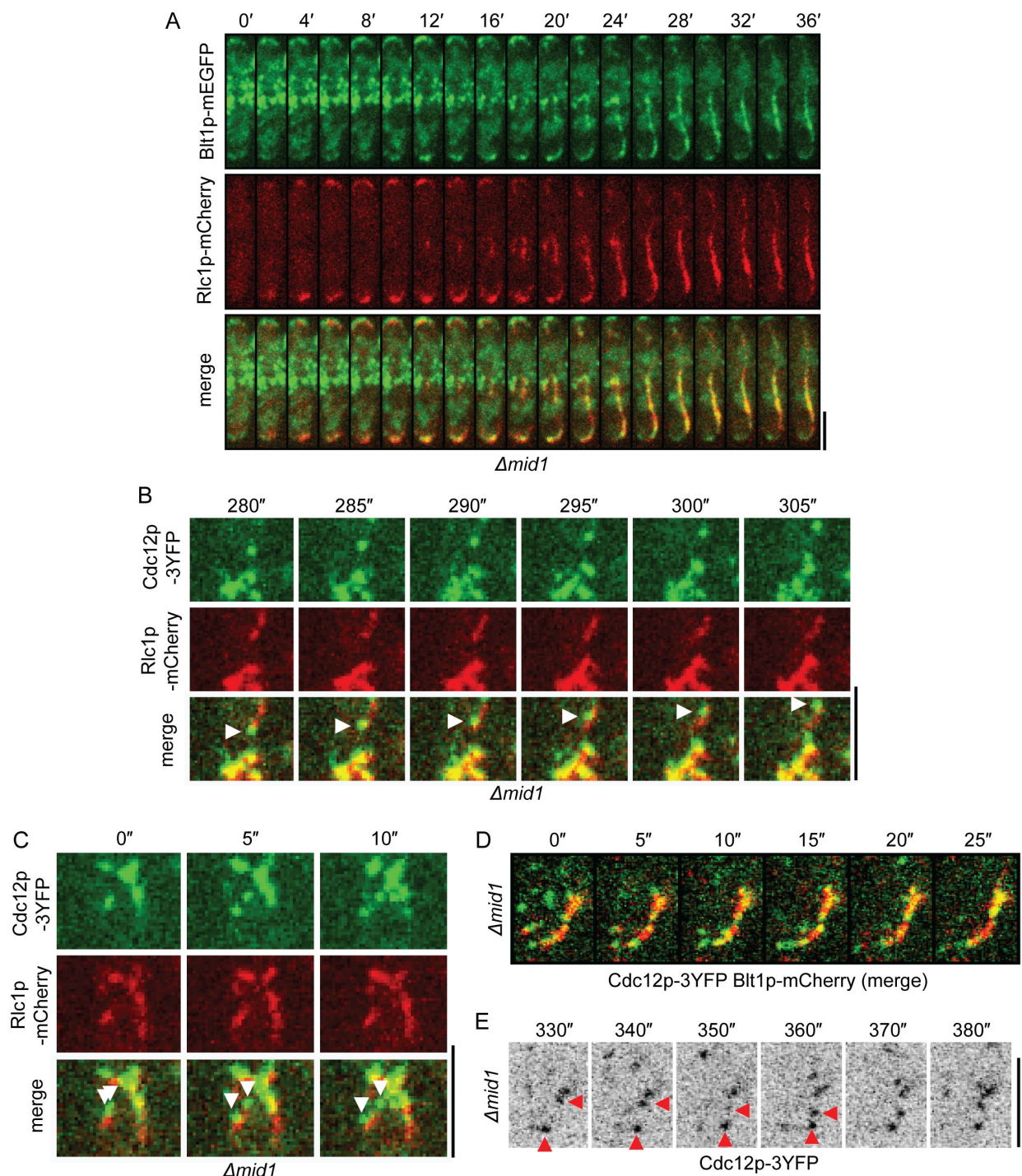
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Running Head: Role of anillin in *S. pombe* cytokinesis



**Figure S1 (related to Figures 1 and 5).** Analysis of colocalization among nodes in wild type and  $\Delta mid1$  cells treated with latrunculin A. (A-D) Analysis of cells treated with 100  $\mu M$  latrunculin A for 30 min prior to imaging, and imaged in presence of latrunculin A. (A-B) DIC and maximum intensity projection fluorescence micrographs of individual wild type and  $\Delta mid1$  cells. (A) Localization of nodes containing Blt1p-mEGFP or Rlc1p-mCherry. (B) Localization of nodes containing Cdc12p-3YFP or Blt1p-mCherry. (C-D) Histograms of colocalization among Rlc1p, Blt1p and Cdc12p in nodes in wild type and  $\Delta mid1$  cells. Micrographs were collected as 32 confocal z slices (slices 0.2  $\mu m$  apart). (C) (□, n = 205) wild type and (■, n = 264)  $\Delta mid1$  cells. We scored the presence of Blt1p-mEGFP and Rlc1p-mCherry in nodes in three-

dimensional reconstructions of 13 wild type and 10 *Amid1* cells containing Rlc1p nodes but lacking septa. (D) (□, n = 263) wild type and (■, n = 306) *Amid1* cells. We scored presence of Blt1p-mCherry and Cdc12p-3YFP in nodes in three-dimensional reconstructions of 10 wild type and 15 *Amid1* cells containing Cdc12p nodes but lacking septa. Scale bars, 5 μm.



**Figure S2 (related to Figure 5).** Contractile ring assembly in  $\Delta mid1$  cells. (A) Time-lapse maximum intensity projection fluorescence micrographs of a  $\Delta mid1$  cell expressing Blt1p-mEGFP and Rlc1p-mCherry. Nodes marked with Blt1p-mEGFP were incorporated into long strands marked with Rlc1p-mCherry, but the cell failed to assemble a contractile ring. (B-E)

Time-lapse single confocal plane fluorescence micrographs acquired every 5 s show the cortical area of part of *Amid1* cells expressing Cdc12p-3YFP and (B-C) Rlc1p-mCherry or (D-E) Blt1p-mCherry. (B-C) Frames from Video 11 showing movements of Cdc12p-3YFP puncta along strands marked with Rlc1p-mCherry. In (B) the arrowhead marks a punctum that moved upward. In (C) arrowheads mark two puncta that traveled in opposite directions. (D) Frames from Video 10 showing puncta marked with Cdc12p-3YFP traveling along a strand marked with Blt1p-mCherry. (E) Frames from Video 10 showing the Cdc12p-3YFP channel in negative contrast. Cdc12p puncta traveled upward (vertical arrowhead) and downward (horizontal arrowhead) along the strand marked with Blt1p-mCherry in (D). Scale bars, 5  $\mu$ m.