Supplemental Materials

Molecular Biology of the Cell

Alessi Wolken et al.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Aim44p-GFP is a functional fusion protein. The fusion of a C-terminal GFP tag to Aim44p does not produce a multibudded phenotype in cells in late-log phase liquid culture (SC), indicating that the tag does not interrupt its normal function in promoting cell division. The percentage of multibudded cells in the Aim44p-GFP expressing cells is not statistically different from wild-type cells ($p = 0.9$, Student's t-test). *aim44Δ* cells are multibudded compared to wild-type and Aim44p-GFP expressing cells ($p = .0085$ and $p = 0.01$, respectively, Student's t-test). Error bars represent standard deviation of 3 independent experiments. $n \geq 100$ cells for each strain in each independent experiment.

Supplemental Figure 2: Localization of Cdc3p-mCherry and Myo1p-GFP during cell division in wild-type cells. Cells expressing *MYO1* tagged with GFP at its chromosomal locus and plasmid-borne Cdc3p-mCherry were grown to mid-log phase and imaged by fluorescence microscopy. Deconvolved projections of representative images of Cdc3p-mCherry (red) and Myo1p-GFP (green) at the bud neck of cells with small, medium, and large buds (**a-c**, respectively) and separated mother and daughter cells **(d)**. **(a)** Myo1p co-localizes with a single septin ring in a small-budded cell. **(b-c)** In cells bearing medium to large buds, the septin ring thickens and forms 2 rings that surround the single Myo1p ring. **(d)** After contractile ring closure and cell separation, mother and daughter cell each have a single septin ring. Scale bar = 0.3 μm.

Supplemental Figure 3: (A) *aim44*Δ yeast expressing a plasmid-borne septin, Cdc3p-mCherry (red), were grown to mid-log phase and imaged by fluorescence microscopy. Cdc3p-mCherry forms single and double rings in *aim44*Δ cells. (B) Myo1p is not required for localization of Aim44p to a ring at the bud neck. *myo1Δ* cells in which *AIM44* was tagged with GFP at its chromosomal locus were grown in SC to mid-log phase and imaged by fluorescence microscopy. Deletion of *MYO1* did not prevent localization of Aim44p-GFP to the bud neck. Scale bar = 1 μm.

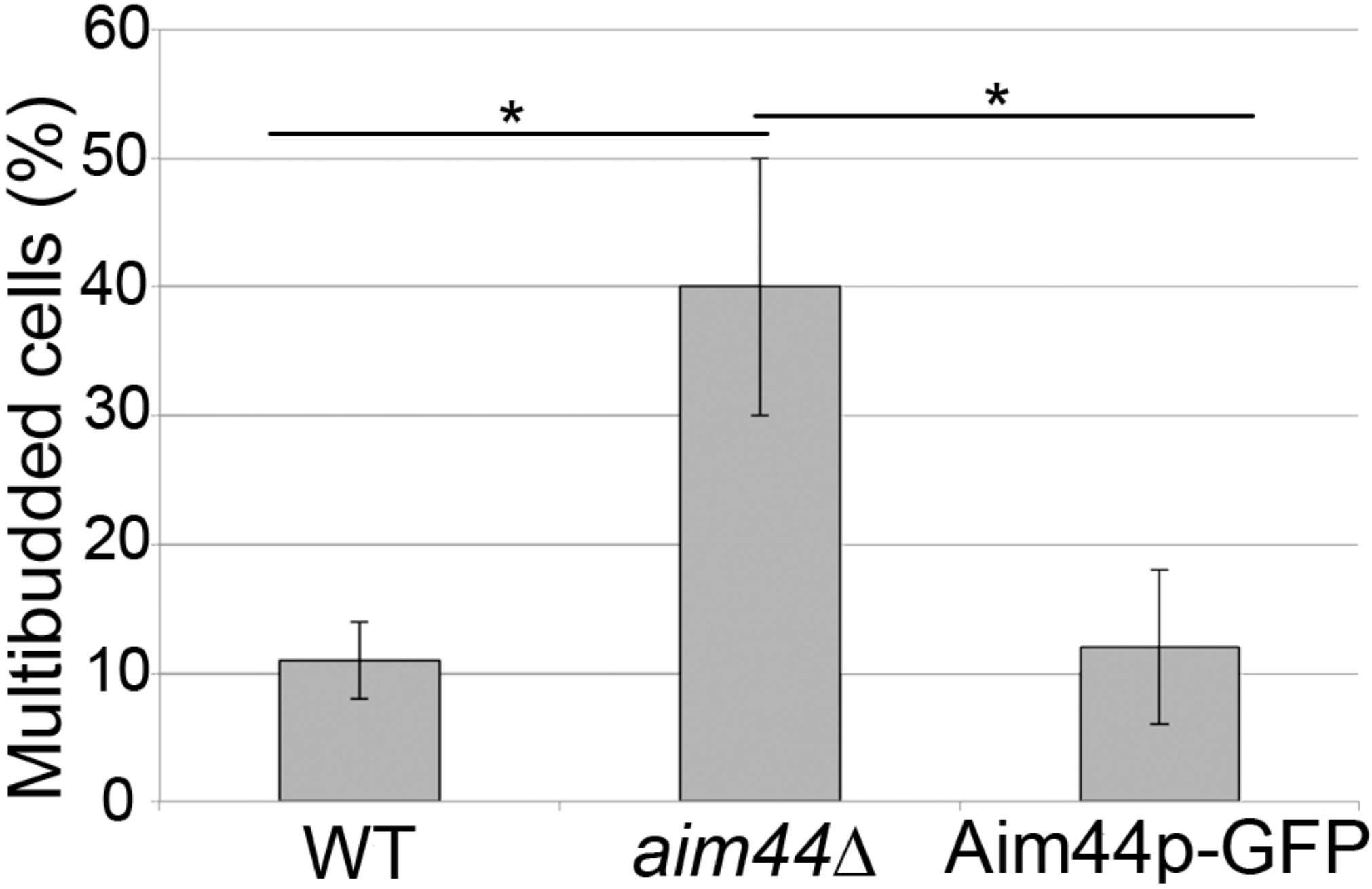
Supplemental Figure 4: The lower-mobility band containing Hof1p-13Myc is due to phosphorylation of the protein. Wild-type and *aim44Δ* cells expressing Hof1p tagged with 13Myc at its chromosomal locus were synchronized as for Figure 1. An aliquot was removed 90 min after release from G_1 arrest for extraction of protein. Half of the protein sample was left untreated and the other half was treated with 10 units of calf intestinal alkaline phosphatase (CIP) for 1 hr at 37°C. Western blots are shown in which Hof1p was detected using anti-Myc antibody and the load control, hexokinase, was detected using a polyclonal anti-hexokinase antibody. *, lower mobility band(s) of Hof1p-13Myc. The lower mobility Hof1p-13Myc band(s) is phosphorylated, as measured by sensitivity to CIP treatment.

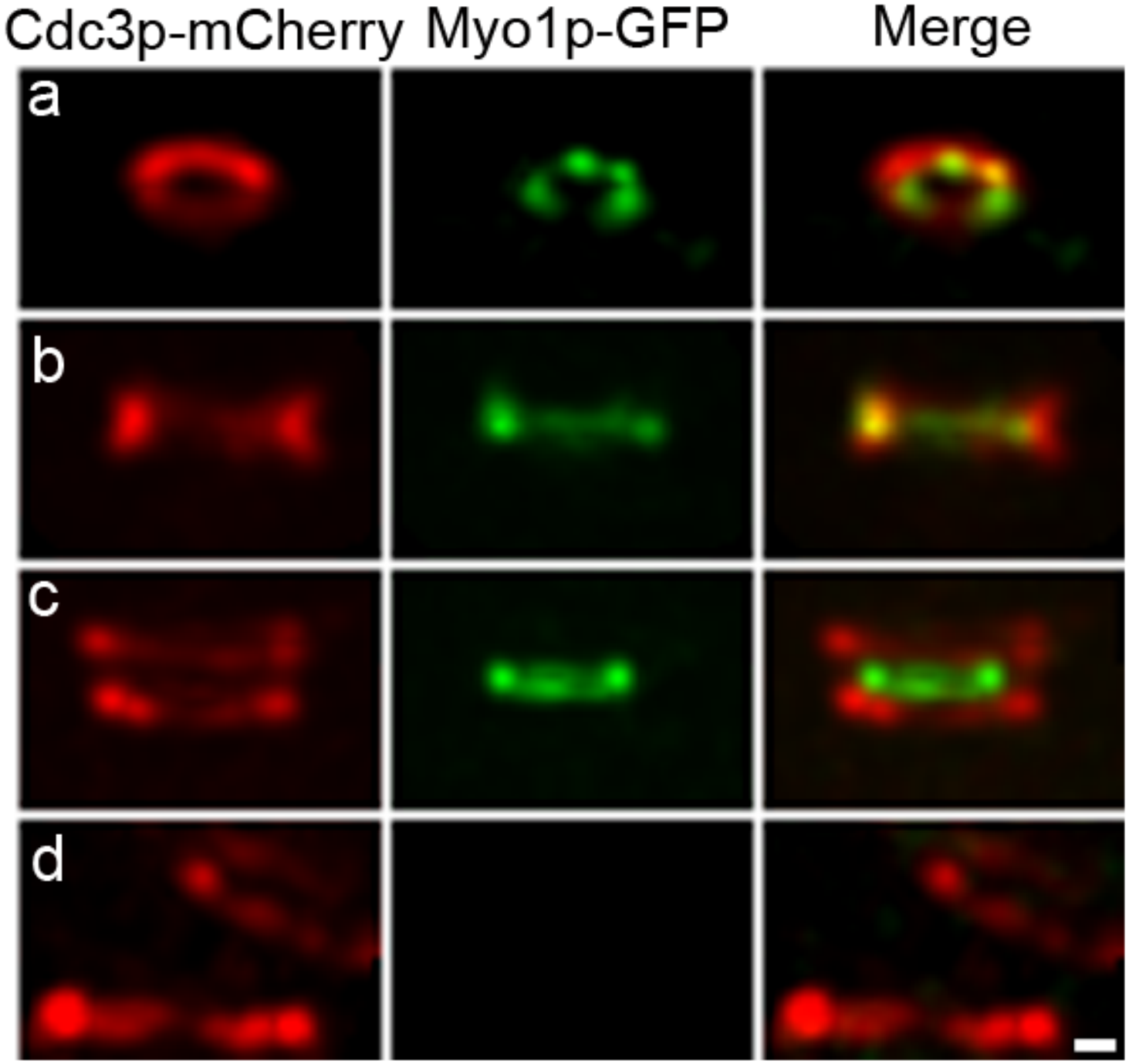
Supplemental Figure 5: Deletion of *AIM44* inhibits cytokinesis but does not affect rates of cell cycle progression. Cell cycle progression was analyzed by quantifying spindle length every 15 min for 90 min after release from pheromone-induced arrest in G_1 phase in wild-type and *aim44Δ* cells expressing tubulin tagged at its C-terminus with GFP. Cells were fixed using paraformaldehyde and stained with the DNA-binding dye DAPI and imaged. These data are pooled from two independent experiments and measurements average $n \geq 100$ cells per strain. Error bars represent standard error of the mean.

Supplemental Table 1: Yeast strains used in this study were constructed as described in the Methods section. All yeast strains were created in the *BY4741* wild-type background except for the temperature-sensitive septin strain (*YEF743 cdc12-6*) and the wild-type control (*YEF6866 CDC12*). The two *YEF* strains were kindly provided by Dr. Erfei Bi.

Strain	Genotype	Source
BY4741	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems (Huntsville, AL)
7394	*MAT**a** aim44Δ::kanMX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
ISY007	*MAT**a** cbk1Δ:HIS3 leu2Δ0 met15Δ0 ura3Δ0*	(García-Rodríguez *et al.*, 2009)
THY158	*MAT**a** MYO1-GFP::HIS3 leu2Δ0 met15Δ0 ura3Δ0*	(Huckaba *et al.*, 2006)
RHY045	*MAT**a** his3Δ1 myo1Δ::LEU2 met15Δ0 ura3Δ0*	(Higuchi *et al*., 2013)
DAY001	*MAT**a** aim44Δ::kanMX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* [pYIP128-CDC3-mCherry::LEU2]	This study
DAY002	*MAT**a** MYO1-GFP:HIS3 aim44Δ::kanMX6 leu2Δ0 met15Δ0 ura3Δ0*	This study
DAY003	*MAT**a** AIM44-GFP::HIS3 leu2Δ0 met15Δ0 ura3Δ0*	This study
DAY004	*MAT**a** AIM44-GFP::HIS3 leu2Δ0 met15Δ0 ura3Δ0* [pYIP128-CDC3-mCherry::LEU2]	This study
DAY005	*MAT**a** AIM44-GFP::HIS3 myo1Δ::LEU2 met15Δ0 ura3Δ0*	This study
DAY006	*MAT**a** AIM44-GFP::HIS3 MYO1-mCherry::hphMX4 leu2Δ0 met15Δ0 ura3Δ0*	This study
DAY007	*MAT**a** MYO1-GFP::HIS3 CDC3-mCherry::hphMX4 leu2Δ0 met15Δ0 ura3Δ0*	This study
DAY008	*MAT**a** HOF1-13Myc::HIS3 leu2Δ0 met15Δ0 ura3Δ0*	This study
DAY009	*MAT**a** HOF1-13Myc::HIS3 aim44Δ::LEU2 met15Δ0 ura3Δ0*	This study
LPY057-1	*MAT**a** HOF1-GFP::HIS3 leu2Δ0 met15Δ0 ura3Δ0*	This study
DAY010	*MAT**a** aim44Δ::KANMX6 HOF1-GFP::HIS3 met15Δ0 ura3Δ0*	This study
DAY011	*CDC12+ AIM44-GFP::KanMX6 ura3 leu2Δ0 HIS3+ TRP1+* [pYIP128-CDC3-mCherry::LEU2]	This study

Strain	Genotype	Source
DAY012	*cdc12-6 AIM44-GFP::KanMX6 ura3 leu2Δ0 HIS3+ TRP1+* [pYIP128-CDC3-mCherry::LEU2]	This study
DAY013	*MAT**a** his3Δ1 TUB1-GFP::LEU2 met15Δ0 ura3Δ0*	This study
DAY014	*MAT**a** aim44Δ::kanMX6 his3Δ1 TUB1-GFP::LEU2 met15Δ0 ura3Δ0*	This study
DAY015	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [*pGB1805-*DBF2::URA3]*	This study
DAY016	*MAT**a** aim44Δ::kanMX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [*pGB1805-*DBF2::URA3]*	This study
DAY017	*MAT**a** AIM44-3HA::kanMX6 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
DAY018	*MAT**a** AIM44-3HA::kanMX6 HOF1-13Myc::HIS3 leu2Δ0 met15Δ0 ura3Δ0*	This study


Multibudded cells (%)
60
50
40
30
20
10
0
*
*
WT
aim44Δ
Aim44p-GFP

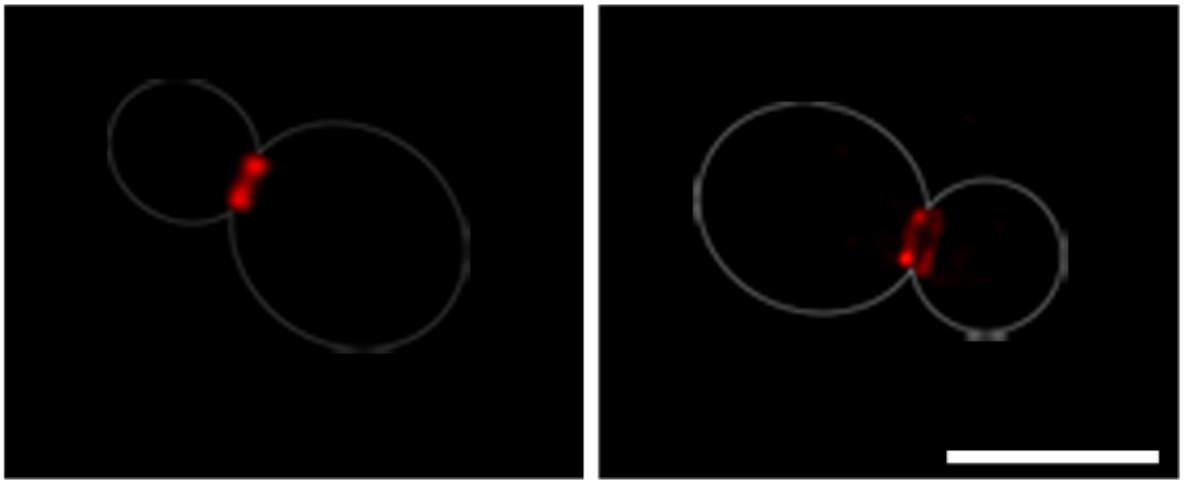

Cdc3p-mCherry
Myo1p-GFP
Merge
a
b
c
d

A

aim44Δ Cdc3p-mCherry

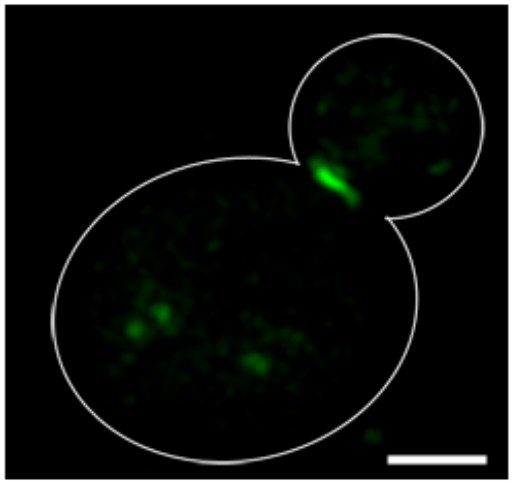
Single Ring

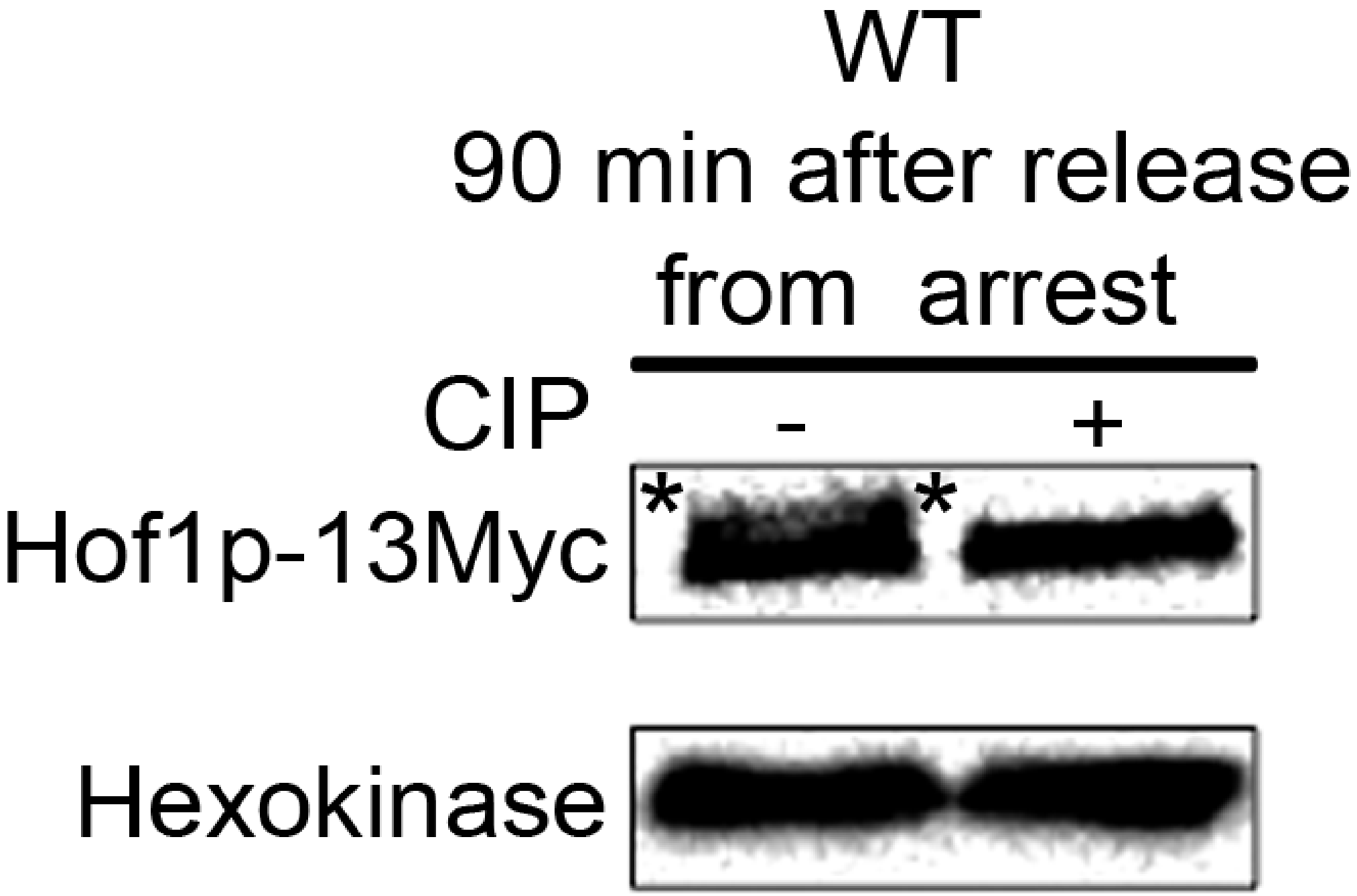
Double Ring

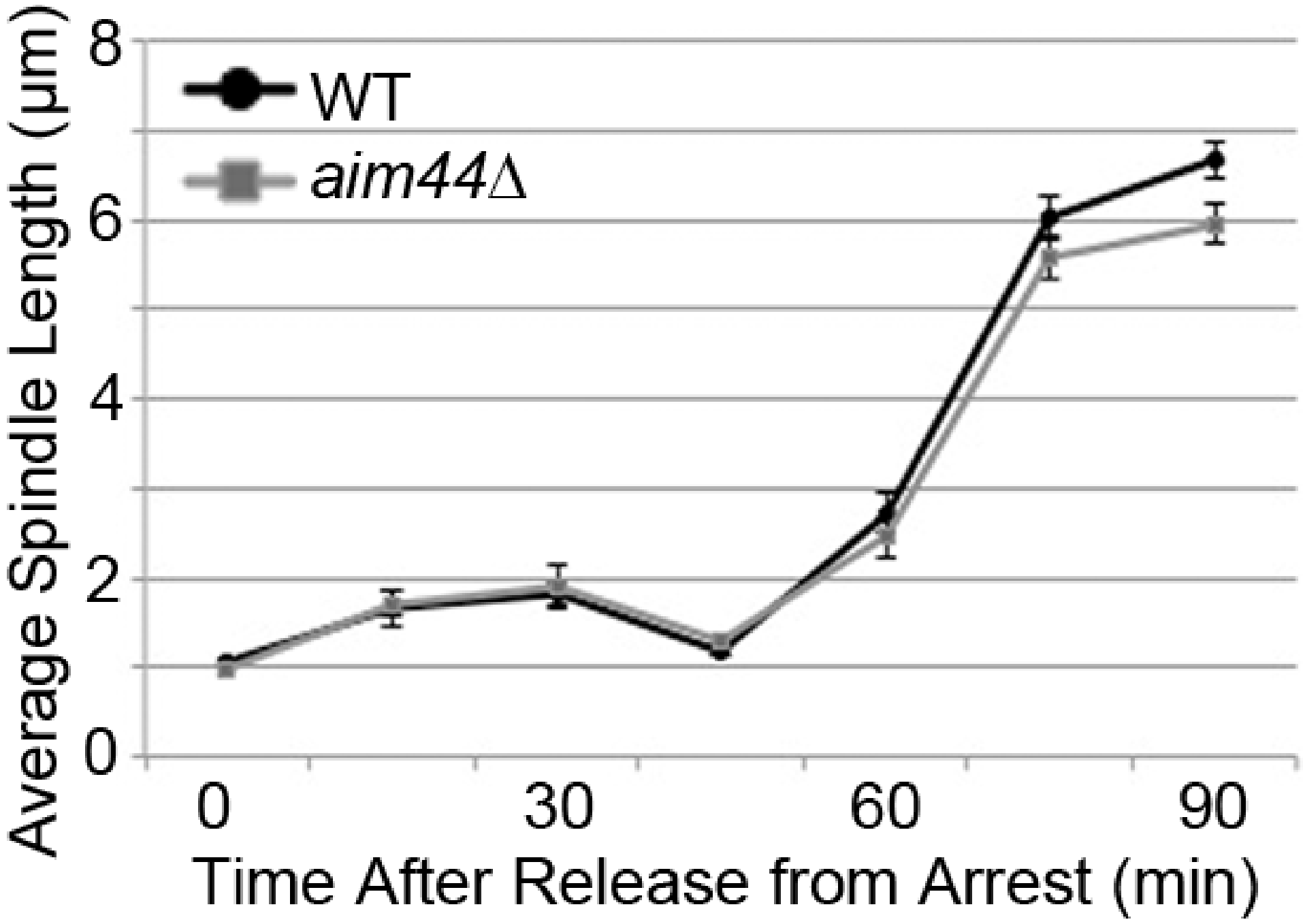


B

myo1Δ
Aim44p-GFP




WT
90 min after release
from arrest
CIP
-
+
Hof1p-13Myc
*
*
Hexokinase


Average Spindle Length (µm)
0
2
4
6
8
WT
aim44Δ
0
30
60
90
Time After Release from Arrest (min)