Supplemental Materials

Molecular Biology of the Cell

Li et al.

Figure S1. The growth phenotypes of polarisome component mutations in wt or *ptc1*Δ cells. (**A**) Serial dilutions of the overnight cultures from indicated strains were plated on YPD plates and grown for 2 days at different temperatures as shown. (**B**) Synthetic lethality between *msb3*Δ *msb4*Δ and *ptc1*Δ. Dissection plates are of the following diploid strains: *msb3*Δ *msb4*Δ crossed to *ptc1*Δ. The *msb3*Δ*msb4*Δ double mutant (representative spores are squared) grows slower than WT. Inferred triple mutant spores (*msb3*Δ*msb4*Δ*ptc1*Δ*)* are circled.

Figure S2. Deletion of all individual polarisome component genes (except *msb3* and *msb4*) significantly suppresses the ER inheritance defects in *ptc1*Δ mutant cells. Shown are the representative GFP fluorescence of the ER marker Hmg1-GFP and DIC images of the following strains: (top) wild type, *sph1*Δ*, pea2*Δ*, spa2*Δ, *msb3*Δ*, msb4*Δ*,* (bottom) *ptc1*Δ, *sph1*Δ*ptc1*Δ*, pea2*Δ*ptc1*Δ*, spa2*Δ*ptc1, msb3*Δ*ptc1, and msb4*Δ*ptc1*. Arrowheads point to small buds. Bars, 5μm. The percentage of small buds that contained normal cortical ER in these strains is shown in Figure 1B.

Figure S3. Deletion of *PEA2* or *SPA2* affects Slt2-GFP localization at the bud tip or bud neck, while deletion of other polarisome component genes does not. Shown are Slt2-GFP fluorescence and DIC images of representative cells from more strains: *ptc1*Δ*, sph1*Δ*, spa2*Δ*, msb3*Δ, *msb4*Δ *and msb3*Δ*msb4*Δ, which are not shown in Figure 2. Arrowheads and arrows point to bud tips in small buds and bud necks in large buds, repectively. Bars, 5μm. The quantification results of the Slt2-GFP localization in small buds from these

strains are shown in Figure 2B.

Figure S4. Deletion of *PEA2* or *SPA2* affects Mkk1-GFP localization at the bud tip or bud neck. A plasmid expressing an Mkk1-GFP fusion from the *ADH1* promoter was transformed into the indicated strains. Cells were grown to early log phase in SC medium at 25°C and examined by fluorescence microscopy. Mkk1-GFP localization at polarized sites was quantified. The error bars in the bar graph represent the SEM from three independent experiments.

Figure S5. The effects of Latrunculin A (Lat-A) treatment on the time course of cER inheritance in *ptc1*Δ or *sec3*Δ mutant cells. Arrowheads point to small buds. *ptc1*Δ and *sec3*Δ cells expressing the ER marker Hmg1p-GFP were treated with 200μM Lat-A on the slide. Time lapse images were acquired by fluorescence microscopy immediately after Lat-A addition. Selected frames from the time series of representive cells are shown. Bar, 5μm.

Figure S6. The effects of Latrunculin A (Lat-A) treatment on Mitochondrial inheritance or cER inheritance in *ptc1*Δ mutant cells. Wild-type and *ptc1*Δ cells expressing the ER marker Hmg1p-GFP and containing a plasmid expressing the F_0ATP synthase mitochondrial targeting sequence fused to RFP (Mito-RFP) were treated with control DMSO or 200μM Lat-A for 10 min. Cells were immediately fixed, washed and then imaged by fluorescence microscopy. **(A)** Representative cell images. Arrowheads point to the cER staining in small buds. Arrows point to the efficient or deficient mitochondria

staining. **(B)** Percentage of small buds containing normal cER staining or normal mitochondrial formation with or without Lat-A treatment. The error bars in the bar graph are the SEM from three independent experiments.

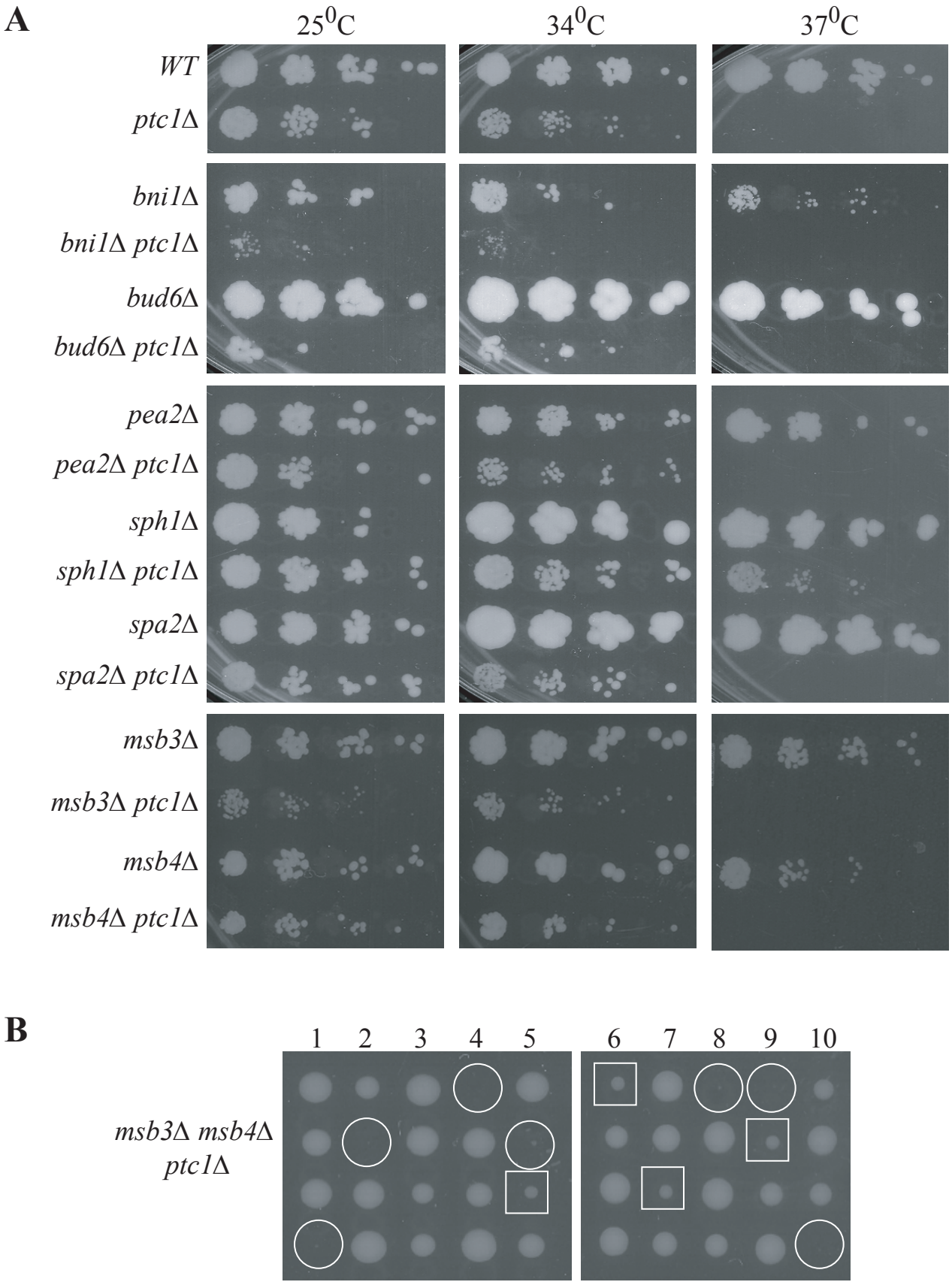
A
25⁰C
34⁰C
37⁰C
WT
ptc1Δ
bni1Δ
bni1Δ ptc1Δ
bud6Δ
bud6Δ ptc1Δ
pea2Δ
pea2Δ ptc1Δ
sph1Δ
sph1Δ ptc1Δ
spa2Δ
spa2Δ ptc1Δ
msb3Δ
msb3Δ ptc1Δ
msb4Δ
msb4Δ ptc1Δ
B
1 2 3 4 5 6 7 8 9 10
msb3Δ msb4Δ
ptc1Δ

Figure S1

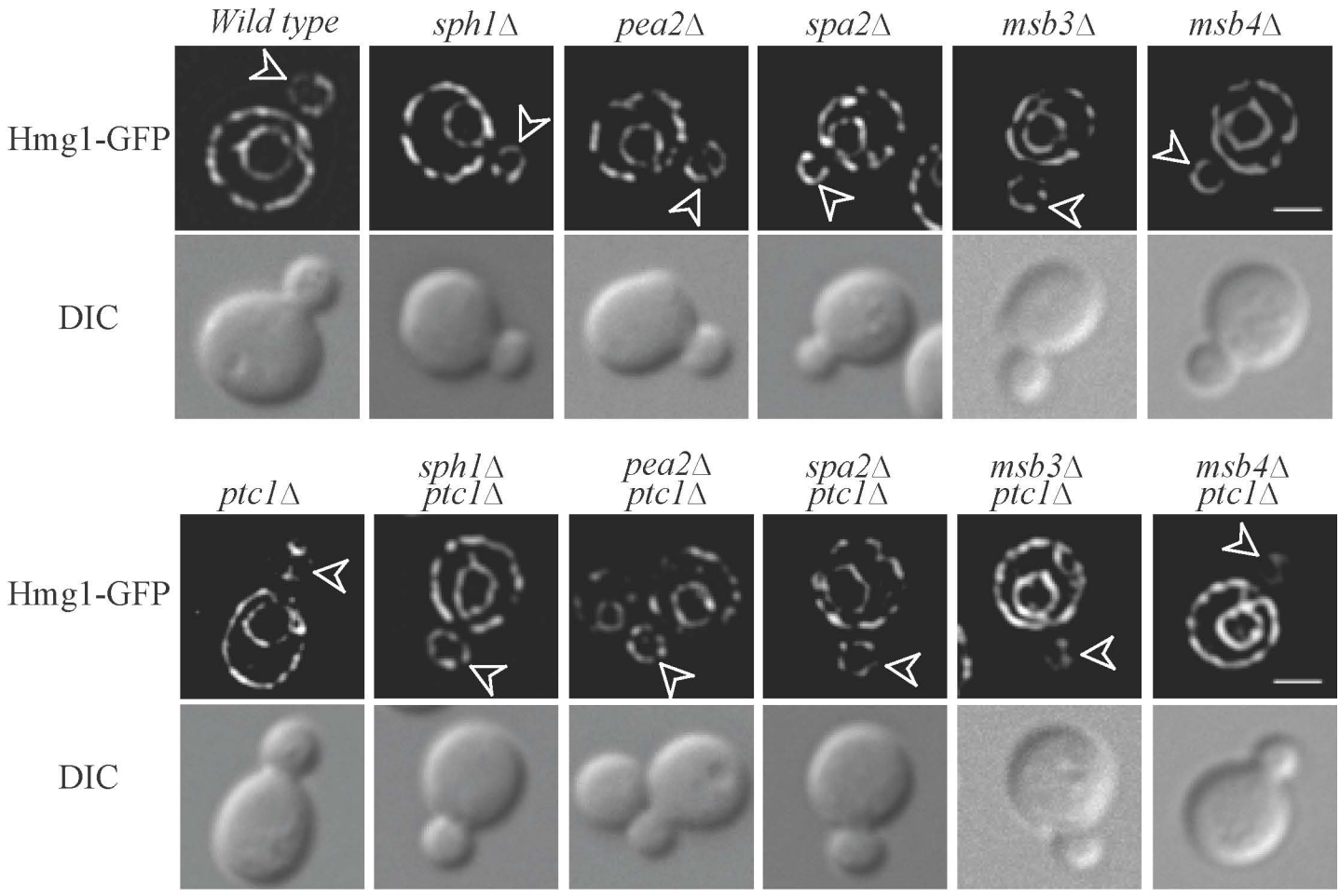
Wild type
sph1Δ
pea2Δ
spa2Δ
msb3Δ
msb4Δ
Hmg1-GFP
DIC
ptc1Δ
sph1Δ ptc1Δ
pea2Δ ptc1Δ
spa2Δ ptc1Δ
msb3Δ ptc1Δ
msb4Δ ptc1Δ
Hmg1-GFP
DIC

Figure S2

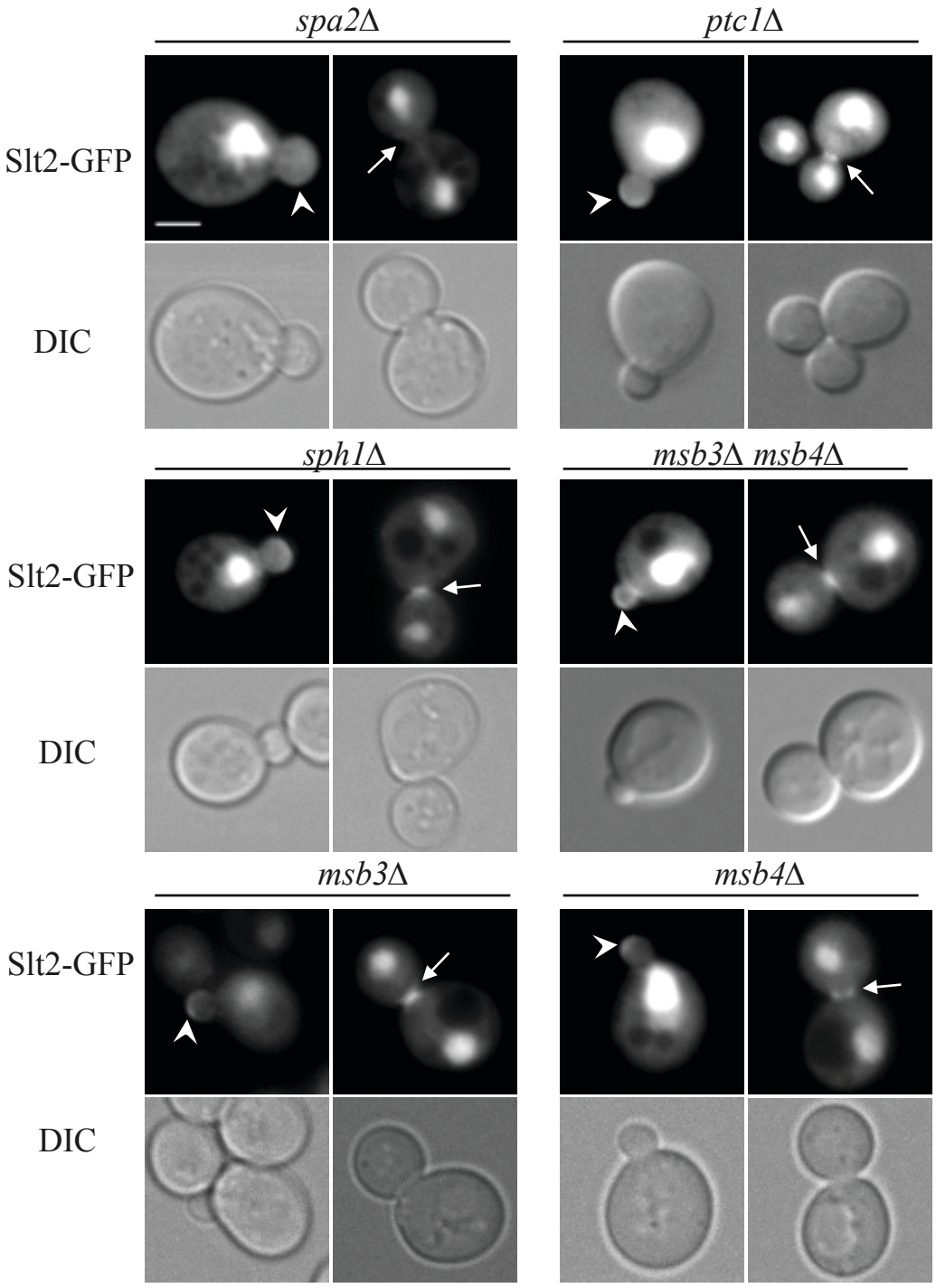
spa2Δ
ptc1Δ
Slt2-GFP
DIC
sph1Δ
msb3Δ msb4Δ
Slt2-GFP
DIC
msb3Δ
msb4Δ
Slt2-GFP
DIC

Figure S3

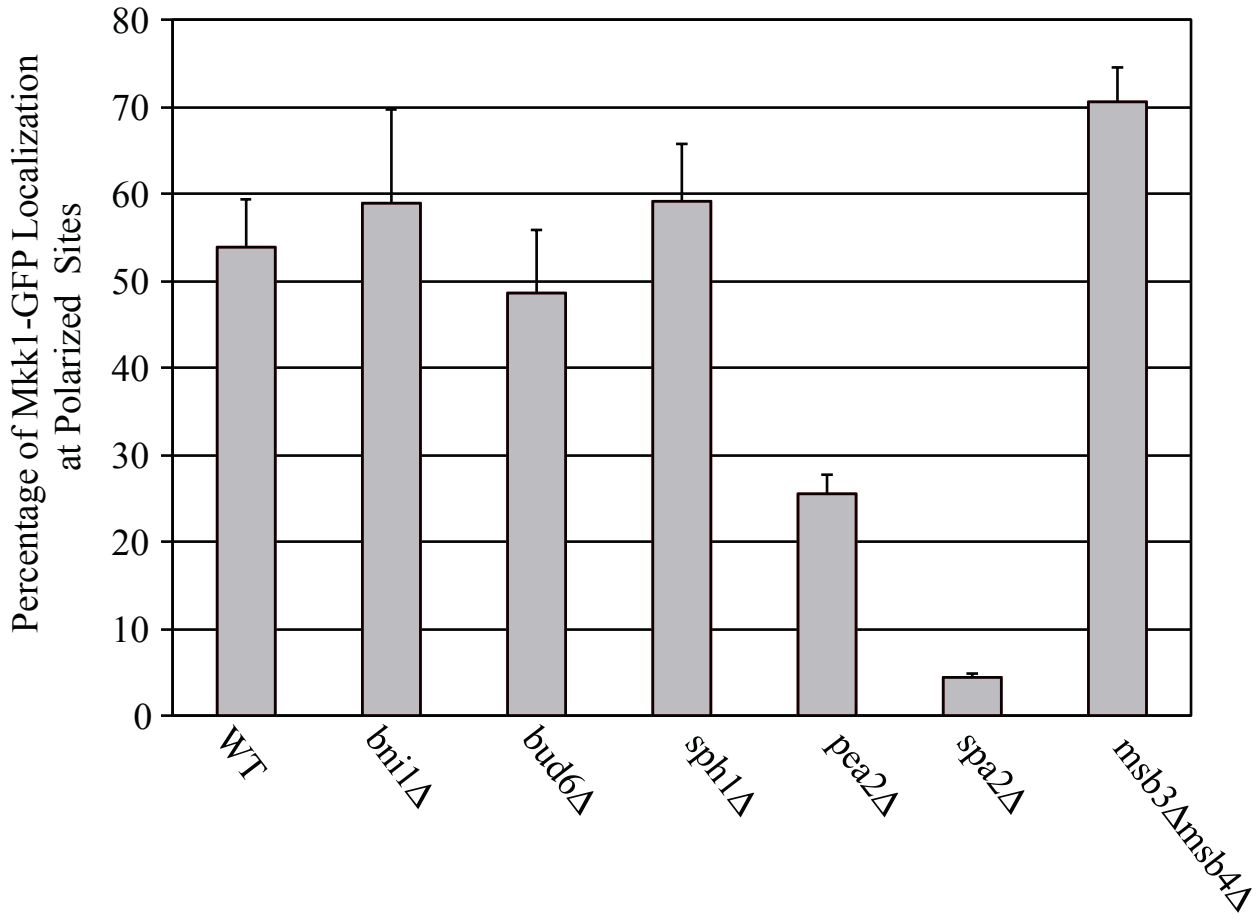
Percentage of Mkk1-GFP Localization at Polarized Sites
80
70
60
50
40
30
20
10
0
WT
bni1Δ
bud6Δ
sph1Δ
pea2Δ
spa2Δ
msb3Δmsb4Δ

Figure S4

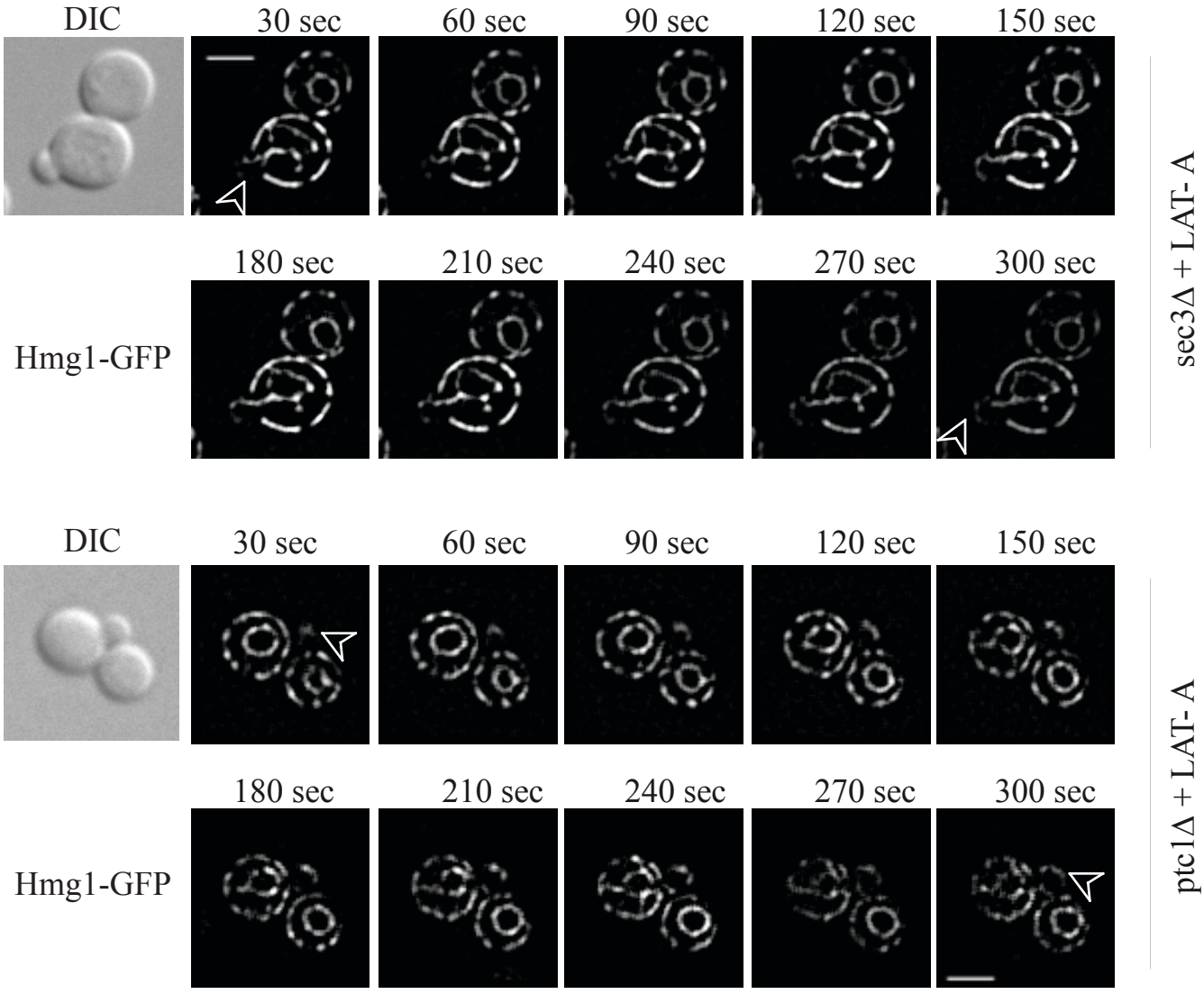
DIC
30 sec
60 sec
90 sec
120 sec
150 sec
180 sec
210 sec
240 sec
270 sec
300 sec
Hmg1-GFP
sec3Δ + LAT- A
DIC
30 sec
60 sec
90 sec
120 sec
150 sec
180 sec
210 sec
240 sec
270 sec
300 sec
Hmg1-GFP
ptc1Δ + LAT- A

Figure S5

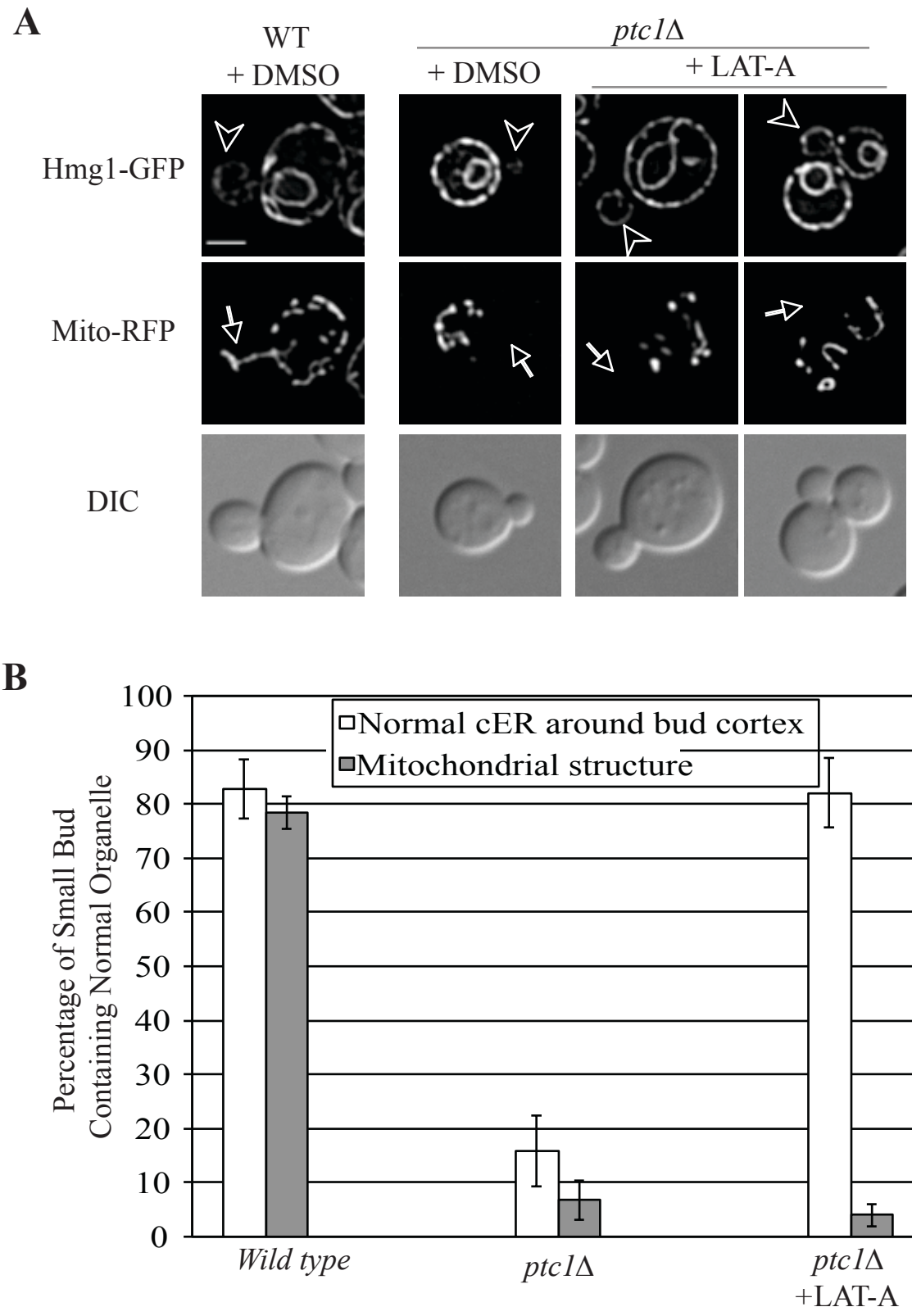
A
WT
+ DMSO
ptc1Δ
+ DMSO
+ LAT-A
Hmg1-GFP
Mito-RFP
DIC
B
Normal cER around bud cortex
Mitochondrial structure
Percentage of Small Bud Containing Normal Organelle
100
90
80
70
60
50
40
30
20
10
0
Wild type
ptc1Δ
ptc1Δ +LAT-A

Figure S6