Movie 1 and Movie 2. Sep7-GFP assembles into cortical rings at growing tips and branch points

Cells expressing Sep7-GFP (AG127) were grown on media-supplemented agarose gel. Fluorescence z-stacks of 14 slices with a spacing of 1μm (green) and phase contrast z-stacks of 5 slices with a spacing of 1μm (red) were acquired every 20 minutes. Movies 1 and 2 are 10 hours 40 minutes and 14 hours 40 minutes, respectively. The maximum projections of both channels are shown here at 3 frames per second. Both movies were used for analysis, shown in Figures 2 and 3. Bar is 8μm.

Movie 3. Stress induces septin ring splitting

A cell expressing Sep7-GFP (AG127) was grown on media-supplemented agarose gel. The cell undergoes rapid, cell-wide septation (asterisks). Additionally, a hypha (arrow) stops growing (at 02:00:00.000), as septins proceed to disappear from the hyphal tip. ~1μm septin circles form (as in Figure 1Q) at the cortex and are maintained until the cell re-establishes polarity and growth begins again. Septin rings less than 2 hours old did not septate. Maximum projection of fluorescence z-stacks (14 slices with a spacing of 1μm) were acquired every 20 minutes and are shown here at a rate of 1 frame per second. Bar is 8μm.

Movie 4. ~1μm septin circle forms after branch formation fails

A cell expressing Sep7-GFP (AG127) recruits septin protein to a new branch site. This branch formation fails, Sep7-GFP is released from the area and a ~1μm septin circle (arrow) forms in the vicinity and persists for hours. This is a clip from Movie 1. Bar is 8μm.

Movie 5. ~1μm circle forms and disassembles coincident with hyphal growth

A cell expressing Sep7-GFP (AG127) forms a new branch hypha but growth stalls when the tip hits a neighboring branch (1st arrow). Upon stalling, a ~1μm septin circle (2nd arrow) is formed between the tip and the branch base. A new branch is eventually formed across from the ring and then the ectopic ring dissolves. This is a clip from Movie 1. Bar is 8μm.

Movie 6. *elm*Δ, *SEP7-GFP* cells do not form IR rings

A single *elm*Δ *SEP7-GFP* cell (AG120) was imaged for 4 hours. Acquisition settings were identical to Movie 1, acquiring z-stacks every 40 minutes and are shown at 1 frame per second. Examples of branch rings are noted with a "B". Bar is 8μm.

Movie 7. *gin4*Δ *SEP7-GFP* cells do not form IR rings

A single *gin4*Δ *SEP7-GFP* (AG121) cell was imaged for 3 hours. Acquisition settings were identical to Movie 1 and are shown at 1 frame per second. Examples of branch rings are noted with a "B". Bar is 8μm.

Supplemental Figure 1. Sep7 mutants lack all higher-order septin structures

Cells were grown on agar pads and imaged live. A) *CDC11a-mCherry* (AG230) B) *sep7*Δ *CDC11a-mCherry* (AG311). mCherry fluorescence (red) and phase contrast cell outlines (blue) are shown. Bar is 5μm.

Supplemental Figure 2. All septins localize to all class of rings

Cells were grown on agar pads and imaged live. Maximum projections of fluorescence image stacks of *SEP7-GFP* (AG127), *CDC3-CFP(*AG245*)*, *CDC10-YFP* (AG306), *CDC11a-mCherry* (AG230), and *CDC12-Venus* (AG308) are shown. Bar is 5μm.

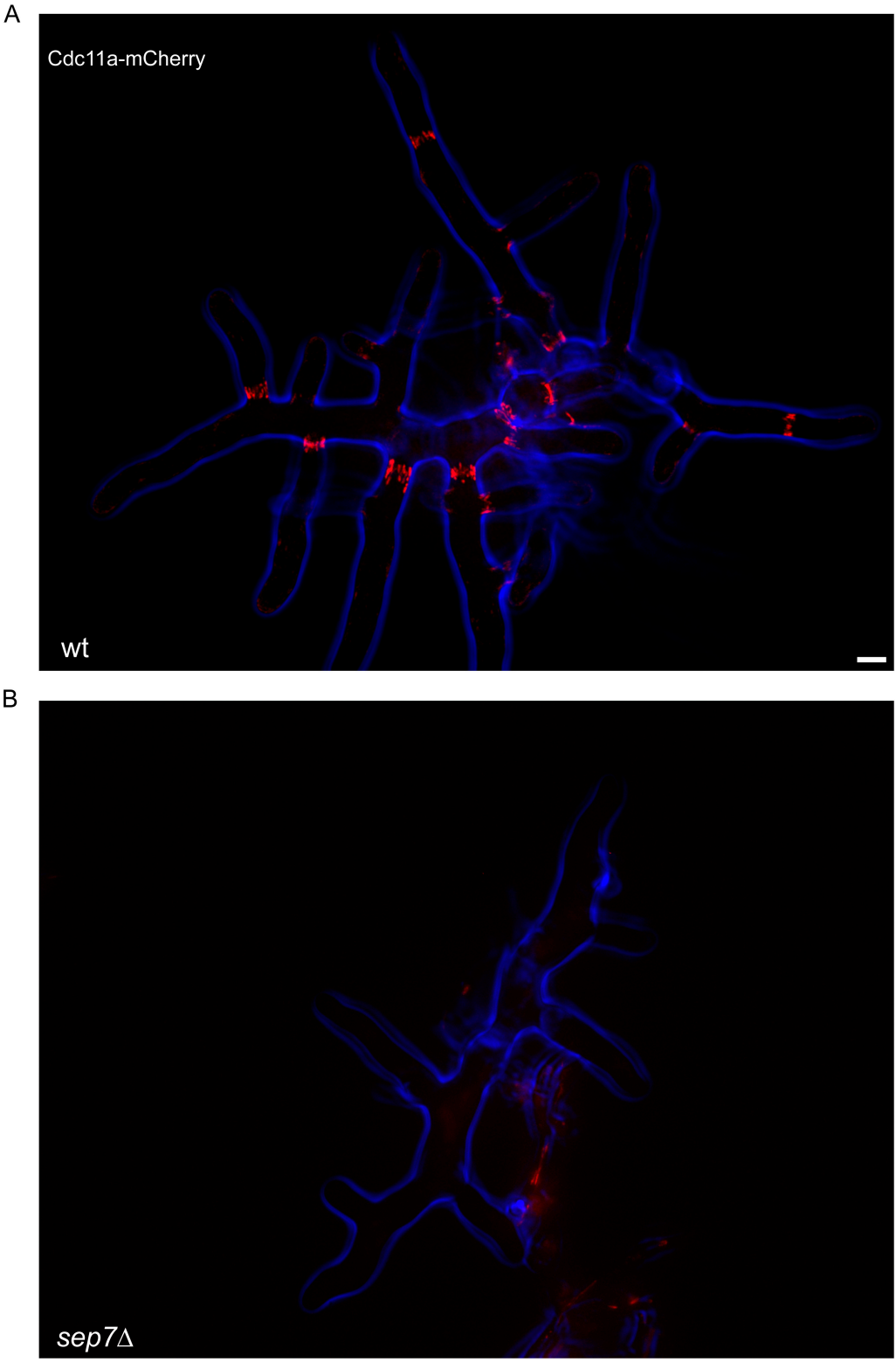
Supplemental Figure 3. Cdc11a-mCherry localizes similarly to Sep7-GFP in septin regulator mutants

Cells were grown on agar pads and imaged live. Maximum projections of fluorescence image stacks of *CDC11a-mCherry* (AG230), *gin4*Δ *CDC11a-mCherry* (AG312), *elm1*Δ *CDC11a-mCherry* (AG313), and *nap1*Δ *CDC11a-mCherry* (AG314) are shown with phase contrast cell outlines (blue). Bars are 5μm.

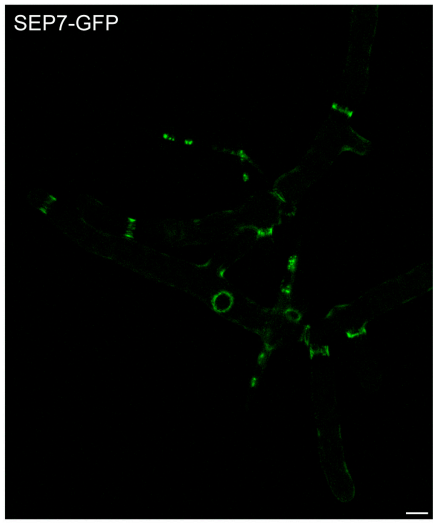
Supplemental Figure 4. Deletion of septin regulators disrupts formation of septa. Wild-type *SEP7-GFP* (AG127), *gin4*Δ *SEP7-GFP* (AG121), *elm1*Δ *SEP7-GFP* (AG120), and *nap1*Δ *SEP7-GFP* (AG209) cells grown in liquid media, then stained with calcofluor (white). Bar is 10μm.

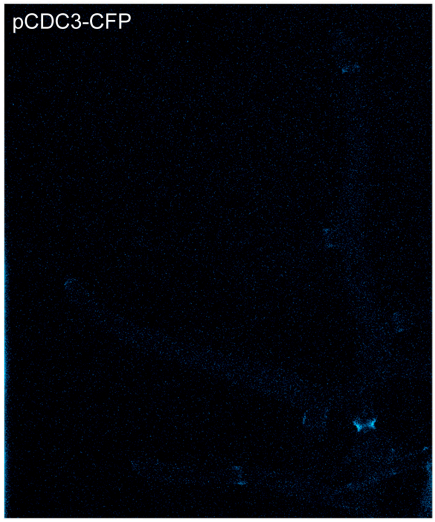
Supplemental Figure 5. Strains lacking IR rings are susceptible to whole cell death. Live cell fluorescence time-lapse images of single wild-type *SEP7-GFP* (AG127), *gin4*Δ *SEP7-GFP* (AG121), and *elm1*Δ *SEP7-GFP* cells grown on agarose pads. Arrows in top-right box highlight portions of the wild-type cell which continue to grow while many other sections die (autofluorescence) and asterisks show positions of septa that form which insulate these regions from the dying areas. GFP fluorescence (green) and phase contrast cell outlines (red) are shown. Bar is 10μm.

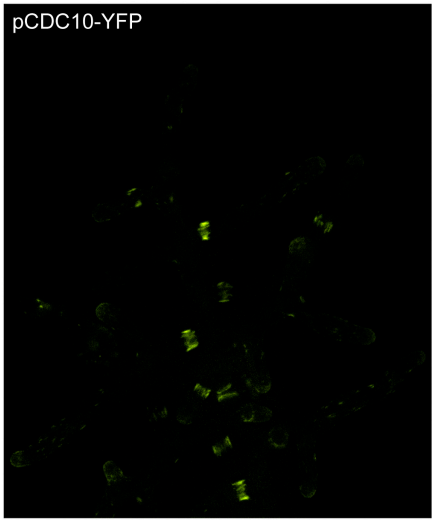
Supplemental Figure 1, DeMay et al.

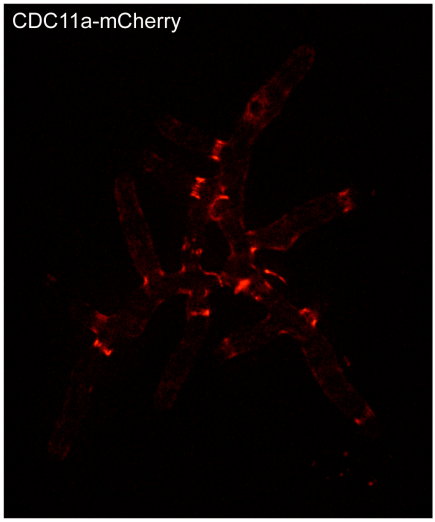


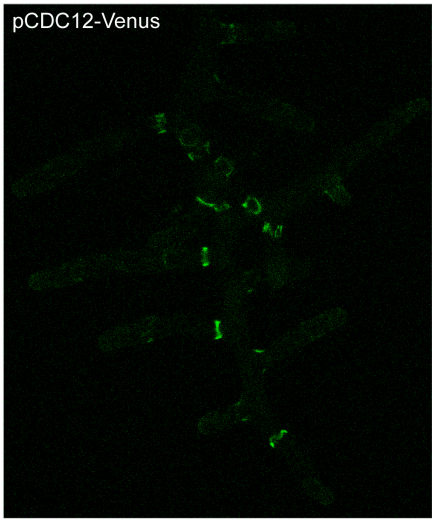
Supplemental Figure 2, DeMay et al.





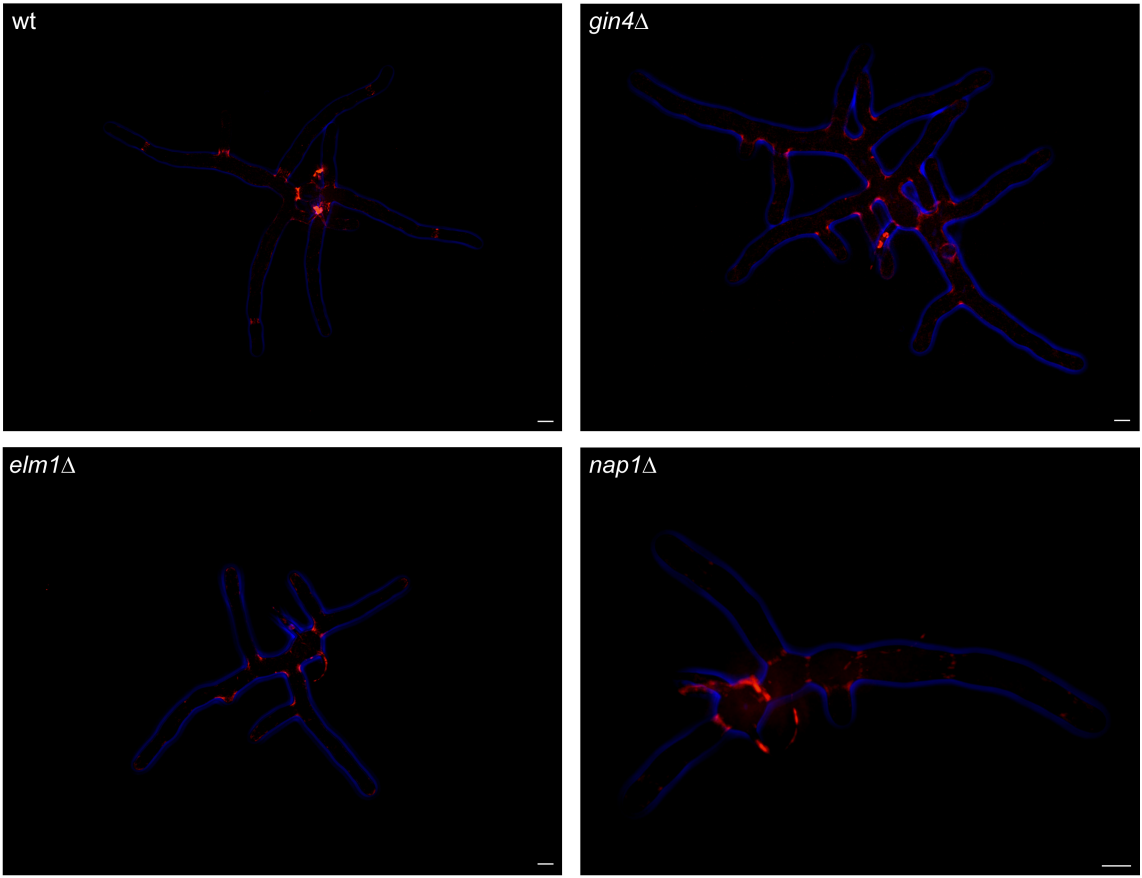




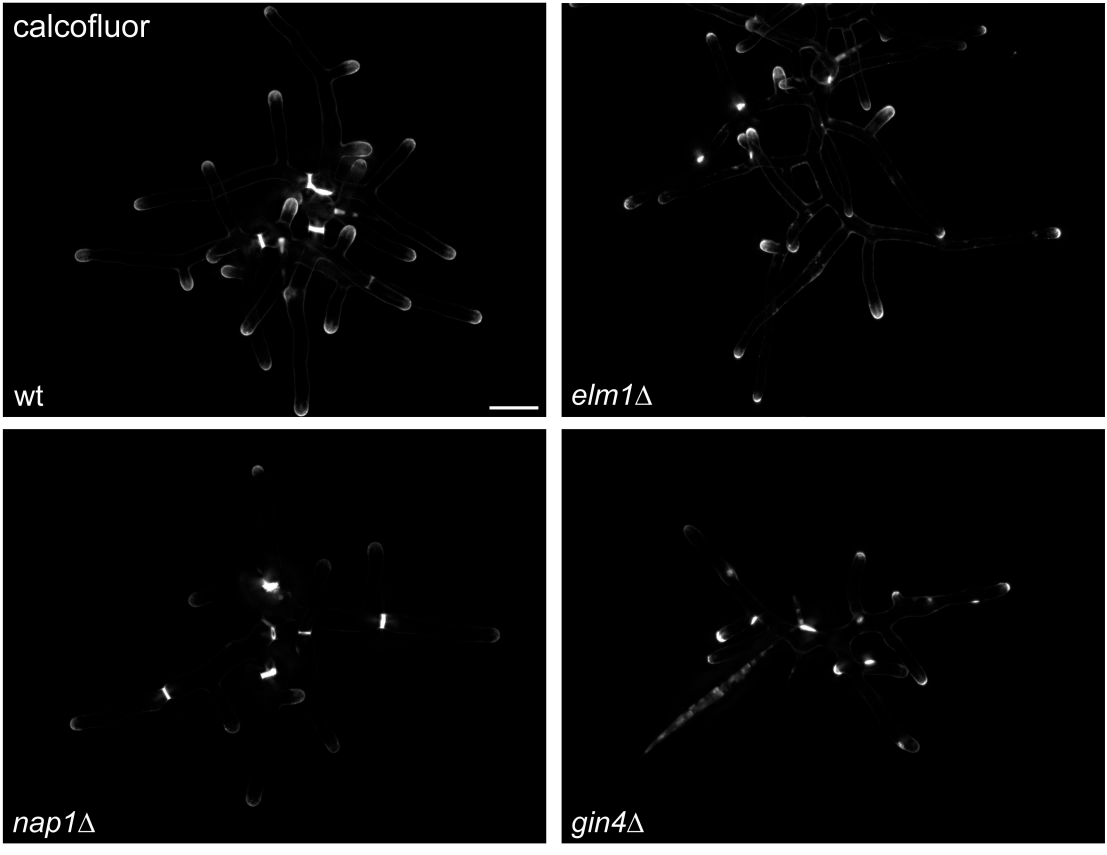


Supplemental Figure 3, DeMay et al.

Cdc11a-mCherry



Supplemental Figure 4, DeMay et al.



Supplemental Figure 5, DeMay et al.