

# Supplemental Materials

*Molecular Biology of the Cell*

Shukla et al.

## SUPPLEMENTAL DATA

### Supplemental Figures:

**Figure S1. EB1-GFP disperses from comets in biofilms formed under media mixing conditions.** Cells expressing EB1-GFP (strain SO1563) were grown as a static culture at 32°C for 8h followed by growth under mixing by rotation at 100rpm at 23°C. (A) EB1-GFP initially localized to mobile comets. (B) Later EB1-GFP dispersed as the biofilm matured. Scale bar: 10µm.

**Figure S2.** Image of a fungal biofilm after a central vertical region of it had been removed to investigate the effects of partial biofilm removal on MT repolymerization. Cells have been scraped off using a micro-pipette tip.

**Figure S3. ClipA-GFP and NudA-3XGFP localize to immobile foci in benomyl-treated cells.** Image and corresponding kymograph from live cell imaging of (A) ClipA-GFP (strain NS141) and (B) NudA-3XGFP (strain NS176) before, during and after benomyl (2.4 µg/ml) washout. Kymographs for ClipA-GFP represents images at 10s intervals for 2min and at 300ms interval for 30s for NudA-3XGFP. NudA-3XGFP locates to the slow-moving comets (red arrows) at MT plus ends as well as to fast-moving foci that travel along MT cables (blue arrowheads). Scale bar: 10µm.

**Figure S4:** Colonies of the R153 strain grown on minimal media-urea plates for four days at 32°C show growth inhibition. In the absence of growth inhibition, the colony on the top left would have grown uniformly in all directions with a radius of 'b'. However, due to growth inhibition from other colonies, that colony is able to grow only up to a radius of 'a' towards the central region. Hence, the central area despite being equally rich in nutrients as the other areas remains unoccupied.

### Supplemental PowerPoint slides:

**Supplemental PowerPoint 1.** Stitched image showing the dispersed localization of EB1-GFP after lid opening but before removal of a portion of a mature biofilm. By playing the slide show the stitched image can be automatically scanned left to right. Scale bar: 50µm.

**Supplemental PowerPoint 2.** Stitched image showing the different zones of localization of EB1-GFP after removal of a portion of a mature biofilm to the left. The left edge shows that the cells have been scraped out from the biofilm. By playing the slide show the stitched image can be automatically scanned left to right. Scale bar: 50µm.

### Supplemental Videos:

**Video 1.** EB1-CR (strain NS133) localizes to mobile comets that represent localization to the plus-ends of growing TubA-GFP MTs. Images were collected at 1s intervals for 125s. The playback rate is 20fps. Scale bar: 10µm.

**Video 2.** Localization of EB1-CR (strain NS327) to the mitotic spindle and astral MTs during mitosis. GcpC-GFP is a SPB marker. Images were collected at 1m intervals for 13m. The playback rate is 2fps. Scale bar: 10 $\mu$ m.

**Video 3.** Localization of EB1-GFP and DIC imaging (strain SO1563) before and after the halt in cell tip growth that occurs at the bottom of a forming biofilm. Mitotic cells are marked by yellow arrows at the first time point. Imaging was started after growth at 32°C for 6h followed by growth at 23 $\pm$ 2°C for 6h. Imaging was done at 30m intervals for 14h. The images were generated by stitching together multiple fields. The playback rate is 2fps. Scale bar: 50 $\mu$ m.

**Video 4.** EB1-GFP (strain SO1563) transitions from comets to cables that form bars and finally become dispersed by shrinking. Images were collected at 30s intervals for approximately 1h. The playback rate is 20fps. Scale bar: 10 $\mu$ m.

**Video 5.** The distribution of EB1-GFP (strain SO1529) becomes more uniform along the cable followed by breaking and shrinking of the cable to form bars. Images were collected at 10s intervals for approximately 45m. The playback rate is 10fps. Scale bar: 10 $\mu$ m.

**Video 6.** Differential localization of EB1-GFP (strain SO1563) in adjacent hyphal compartments that are separated by a septum (yellow arrow) during early biofilm development. Images were collected at 30s intervals for approximately 53m. The playback rate is 30fps. Scale bar: 10 $\mu$ m.

**Video 7.** EB1-GFP (strain SO1563) returns to mobile comets after lid-removal during early stages of biofilm formation. Imaging was done for the same field as shown in Video 3, at ~2.5m intervals for 25m. The playback rate is 2fps. Scale bar: 50 $\mu$ m.

**Video 8.** EB1-GFP (strain NS326) is dispersed from comets then locates fibers and bars after treatment with 100 $\mu$ M NaHS. Images were collected at 40s intervals for 21.5m. The playback rate is 3fps. Scale bar: 10 $\mu$ m.

**Video 9.** EB1-GFP (strain NS326) rapidly returns to comets after washing out NaHS. Images were collected at 13s interval for 3.5m. The playback rate is 1fps. Scale bar: 10 $\mu$ m.

**Video 10.** After NaHS treatment ClipA-GFP (strain NS141) disperses from comets and localizes to immobile foci, and then to bars. Images were collected at 40s interval for 9.5m. The playback rate is 5fps. Scale bar: 10 $\mu$ m.

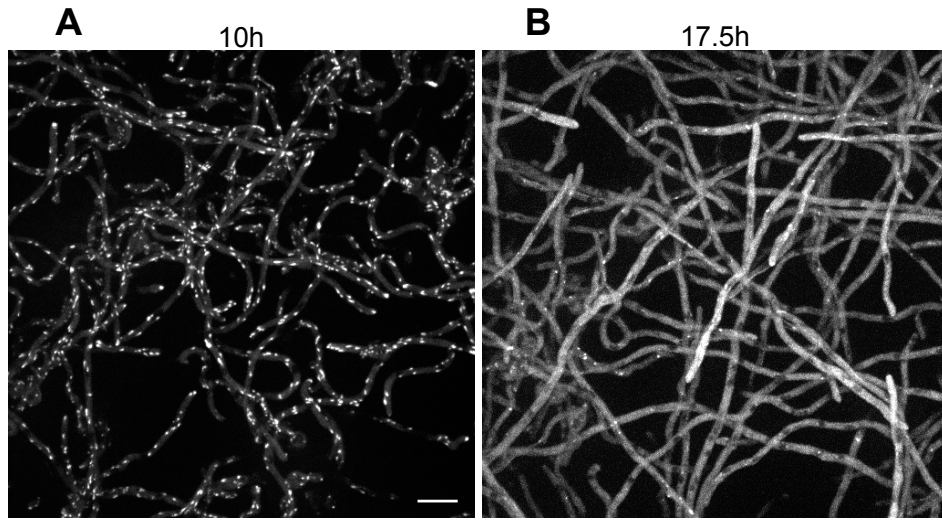
**Video11.** ClipA-GFP (strain NS141) rapidly reappears at mobile comets immediately after washing out NaHS. Images were collected at 10s interval for 2.5m. The playback rate is 5fps. Scale bar: 10 $\mu$ m.

**Video 12.** NudA-3XGFP (strain NS329) localizes to fibers and bars after NaHS treatment. Images were collected with 30s interval for 8m. The playback rate is 5fps. Scale bar: 10 $\mu$ m.

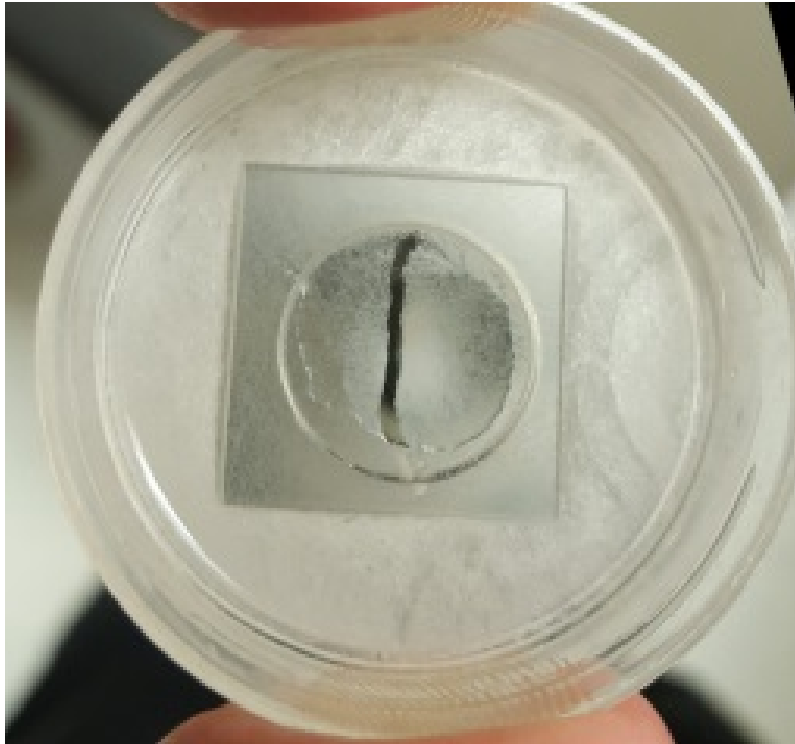
**Video 13.** NudA-3XGFP (strain NS329) rapidly locates back to mobile comets as well as to mobile foci after NaHS washout. Images were collected at 10s intervals for 3.5m. The playback rate is 5fps. Scale bar: 10 $\mu$ m.

**Video 14.** In benomyl-treated cells EB1-GFP (strain NS326) still locates to bars after treatment with NaHS. Images were collected at 40s intervals for 8.5m. The playback rate is 3fps. Scale bar: 10 $\mu$ m.

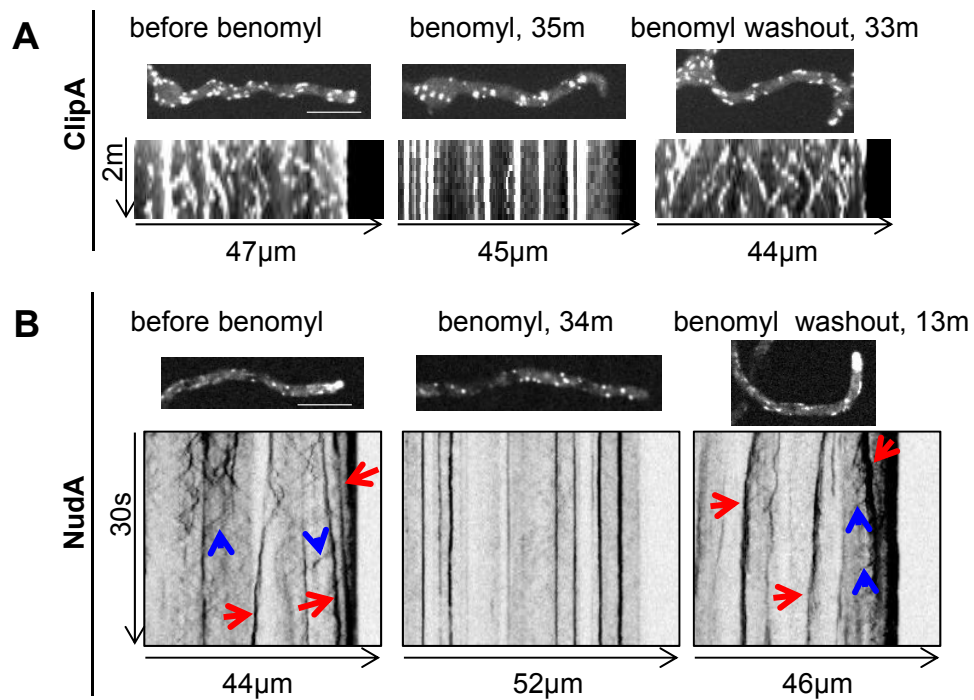
**Video 15.** In benomyl-treated cells ClipA-GFP (strain NS141) still locates to bars after addition of NaHS. Images were collected with 40s interval for 13.5m. The playback rate is 3fps. Scale bar: 10 $\mu$ m.



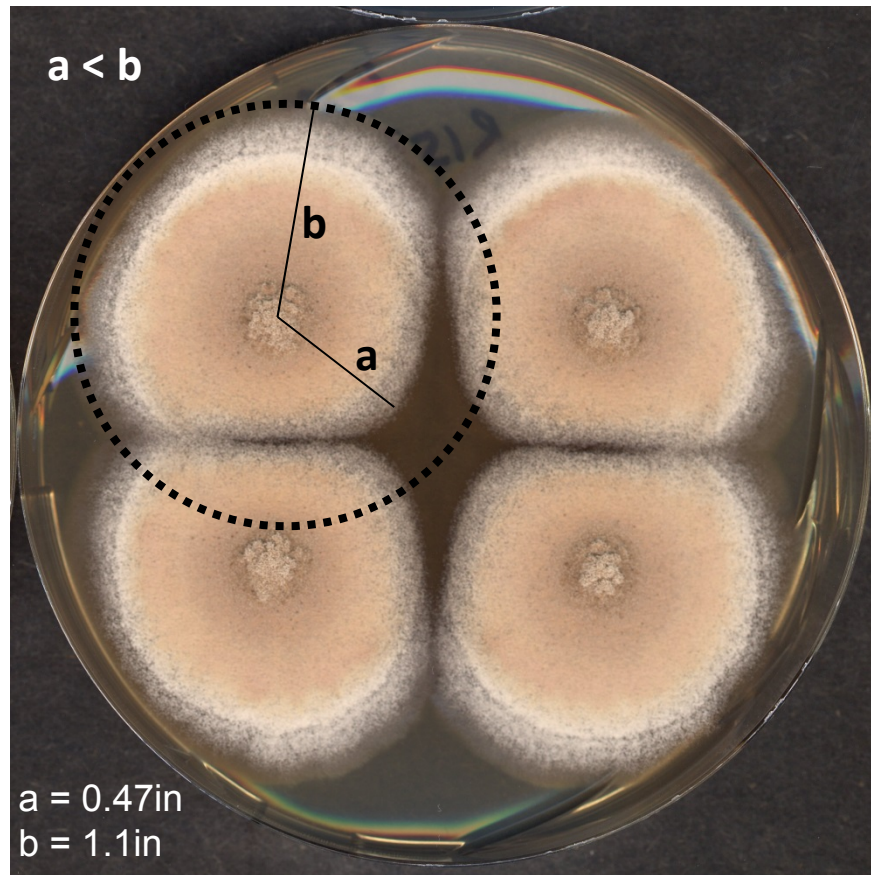
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