

Supplemental Materials

Molecular Biology of the Cell

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Figure S1: Supporting data for Figure 1.

(A) Representative SIM images of fixed and phalloidin stained wildtype, *hof1* Δ , *bnr1* Δ , and *hof1* Δ *bnr1* Δ strains after treatment with 100 μ M CK666. Scale bar, 5 μ m. (B) CoV analysis on actin cable fluorescence distribution in mother cells. Cells were treated with CK666 to remove actin patches, then stained with AlexaFluor-488 phalloidin, and imaged by confocal microscopy. Cells were binned by bud area. Error bars, SD. Statistical significance calculated by one-way ANOVA (n.s. no significance $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

Figure S2: Supporting data for Figure 3.

(A) Representative TIRF images of 2 μ M actin (10% oregon green labeled) in the presence of indicated concentration of Hof1 polypeptide. Corresponding control image (no Hof1) is in Figure 3B. (B) Distribution of distances ($n = 115$ measurements) between Hof1-FL particles decorating actin filaments, from EM images as in Figure 3E.

Figure S3: Supporting data for Figure 4.

(A) Representative cell images showing Hof1-GFP and Hof1 Δ linker-GFP localization acquired by confocal microscopy. In both images, yellow arrowheads indicate GFP signal at the bud neck. In the Hof1 Δ linker-GFP image, the red arrowhead indicates additional GFP signal that is observed at the bud tip in a fraction of small budded cells. (B) Quantification of total cellular levels of Hof1-GFP and Hof1 Δ linker-GFP fluorescence in cells grown asynchronously. Images acquired on an Airyscan microscope. Each data point corresponds to one cell. Statistical significance calculated by unpaired t-test. (n.s. no significance $p > 0.05$). (C) Comparison of Hof1-GFP and Hof1 Δ linker-GFP signals at the bud neck, binned by bud size. Images were acquired on an Airyscan microscope. Each data point corresponds to one cell. Statistical significance in each bin calculated by unpaired t-test. (**** $p \leq 0.0001$).

Figure S4: Supporting EM data for Figure 5.

EM images of negatively stained yeast septin filaments (Cdc3, SNAP-Cdc10, Cdc11, Cdc12) assembled in the presence and absence of 500 nM Hof1-FL, showing that Hof1 bundles septin filaments.

Figure S5: Septin axial pillars imaged by Airyscan microscopy.

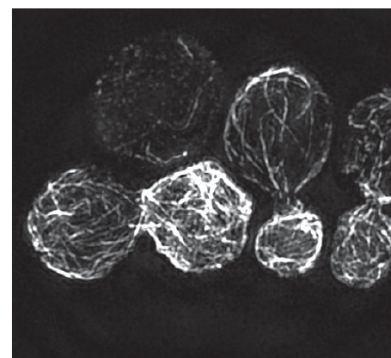
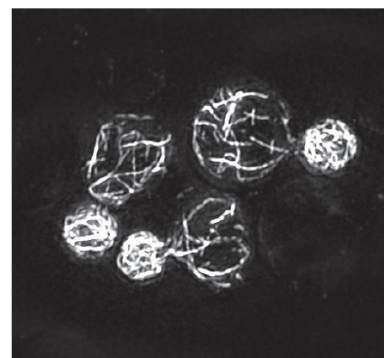
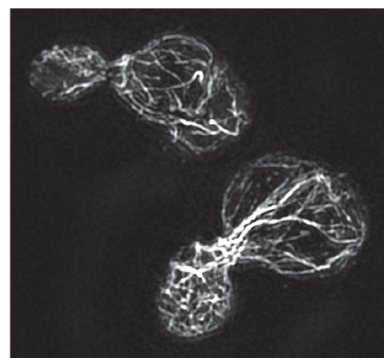
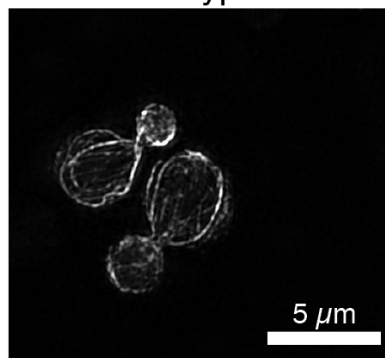
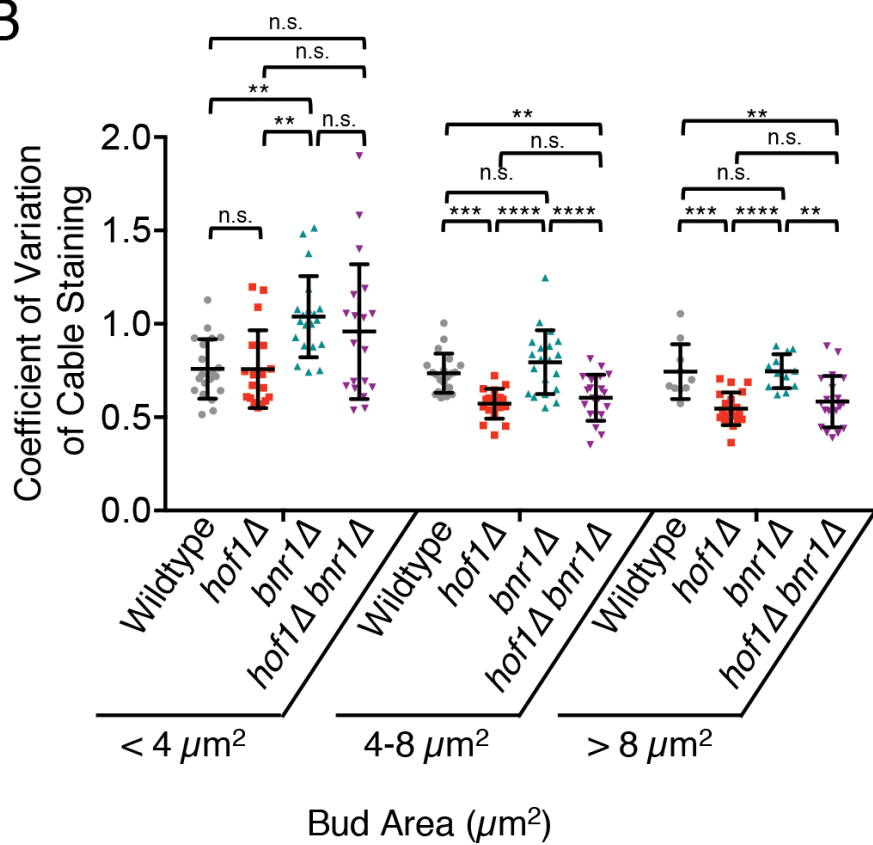
(A) Representative image of Cdc3-yomApple localization at the bud neck imaged by Airyscan microscopy. Scale bar, 1 μ m. (B) Representative image of Cdc10-GFP at the yeast bud neck imaged by Airyscan microscopy. Scale bar, 1 μ m.

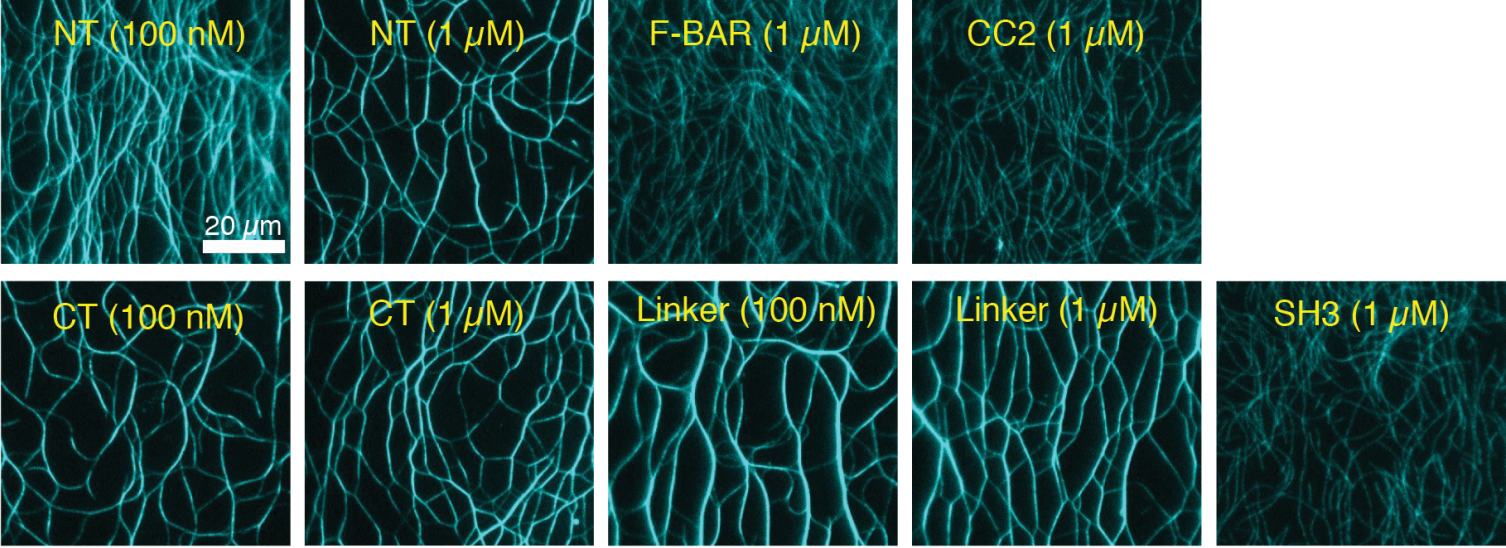
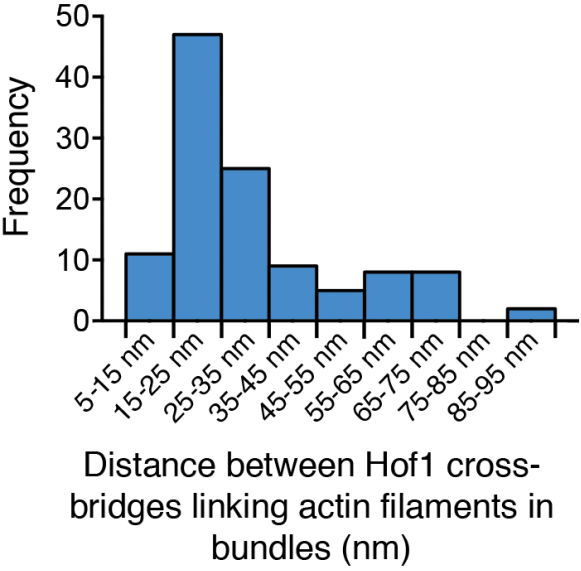
A

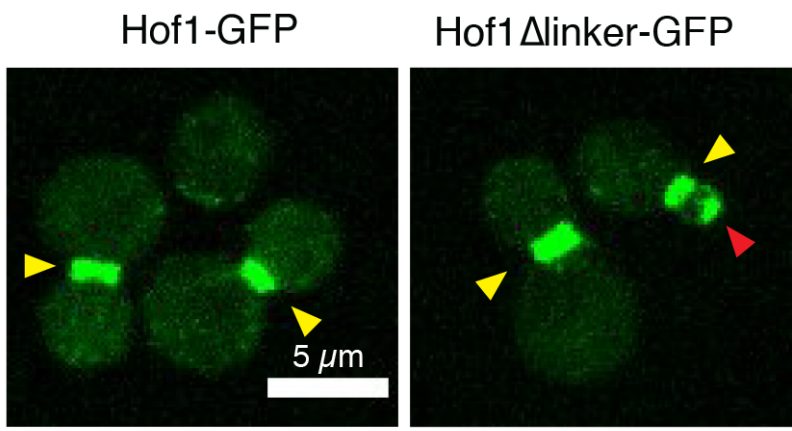
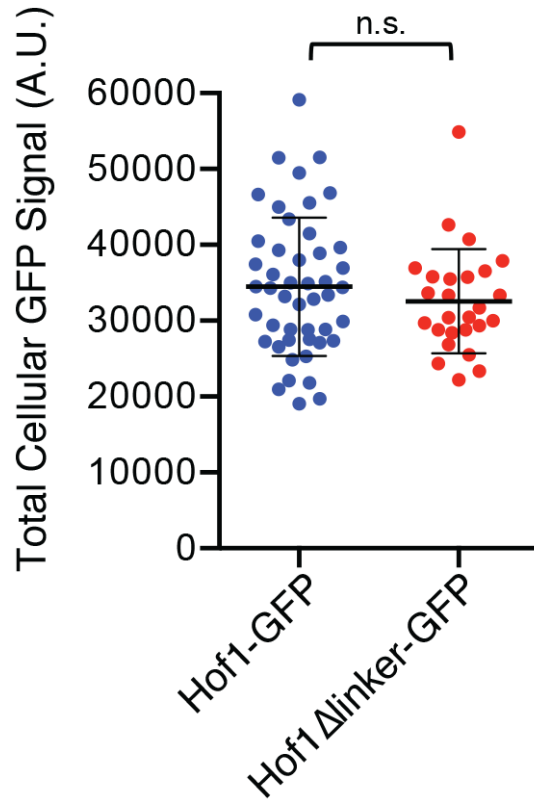
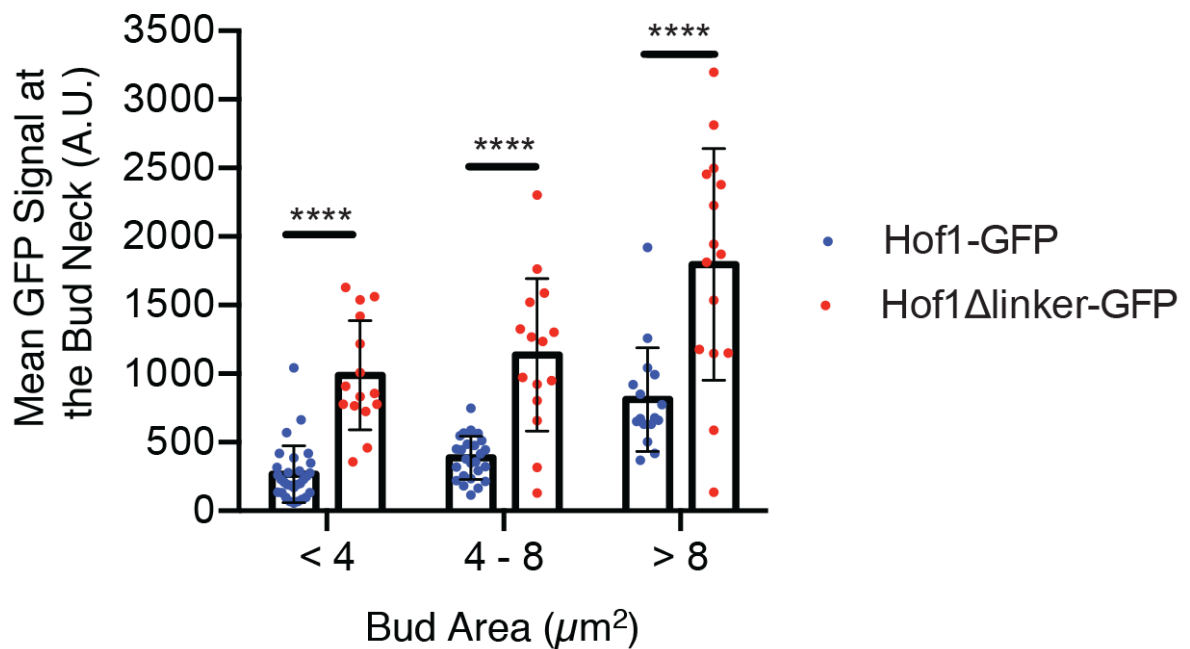
Wildtype

hof1 Δ *bnr1* Δ *hof1* Δ *bnr1* Δ

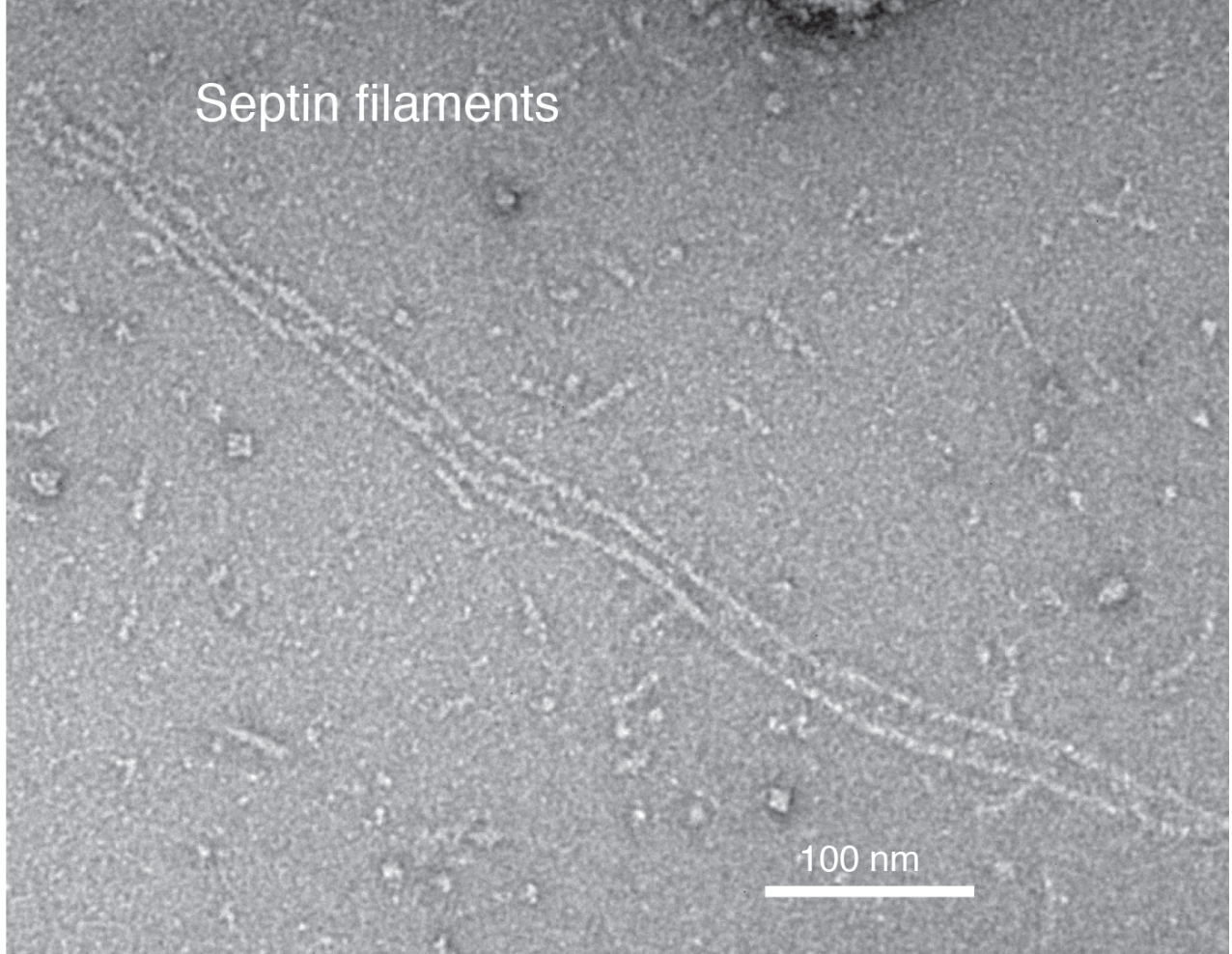
+ CK666

**B**

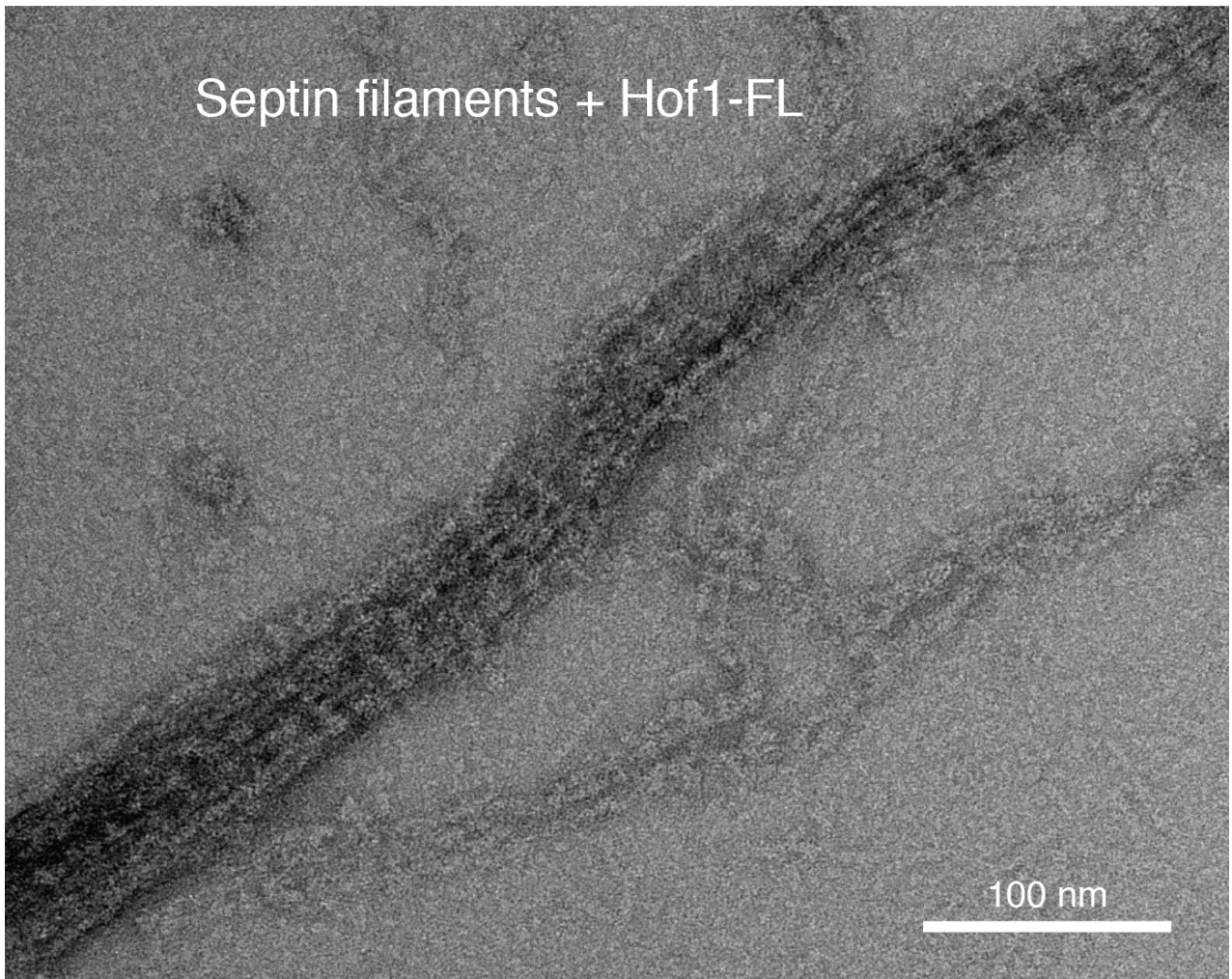
A**B**

A**B****C**

Septin filaments

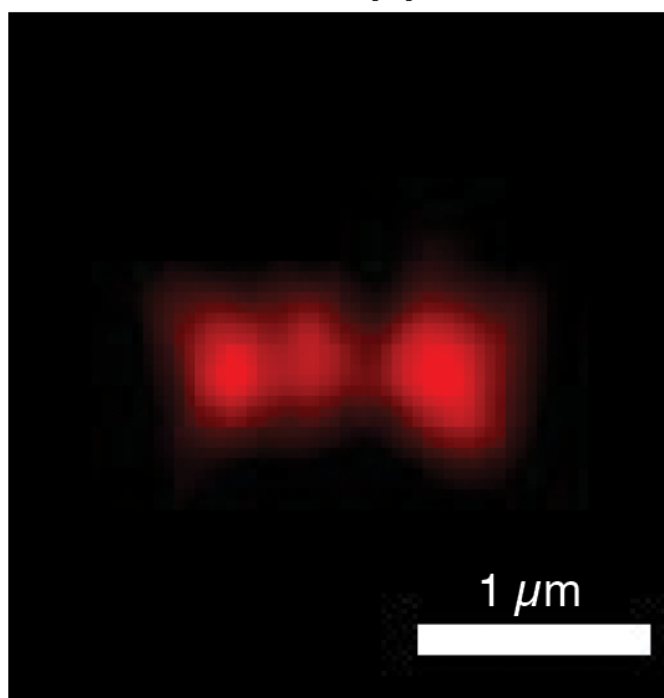


Septin filaments + Hof1-FL



A

Cdc3-Apple



B

Cdc10-GFP

