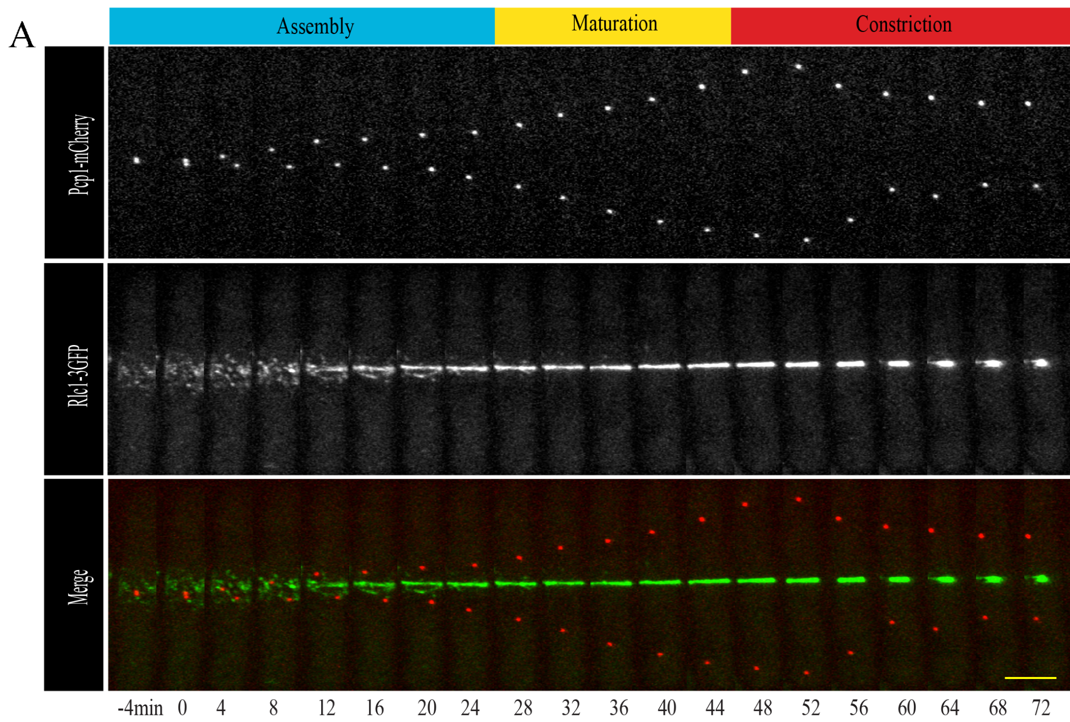
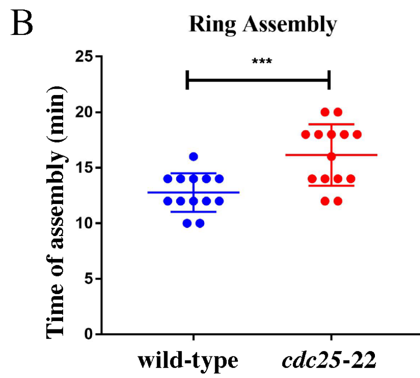
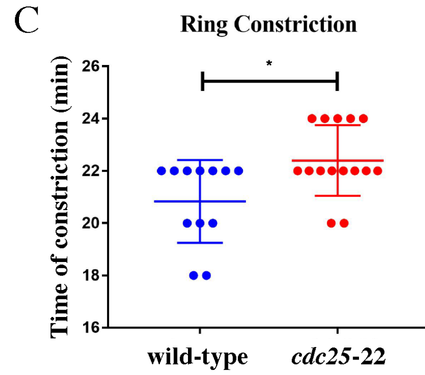
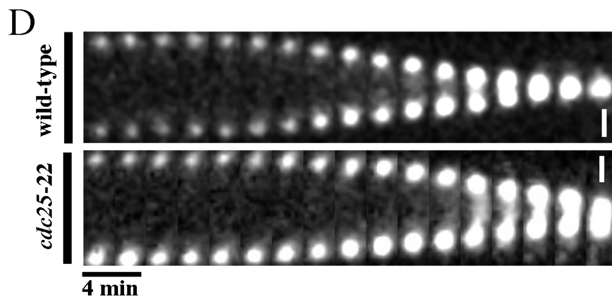
1 # SI Appendix

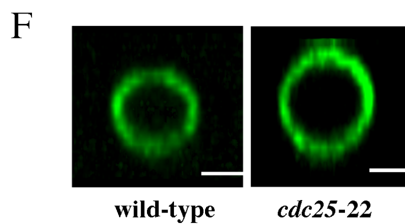
2 ## Supplementary Figures











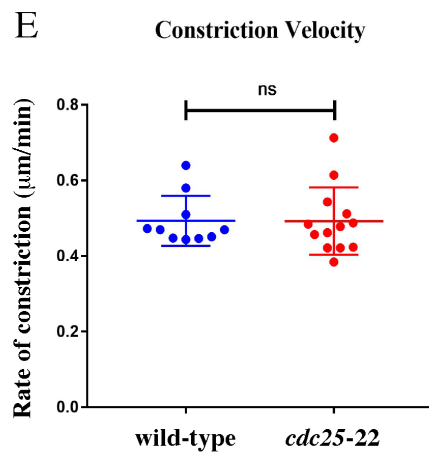


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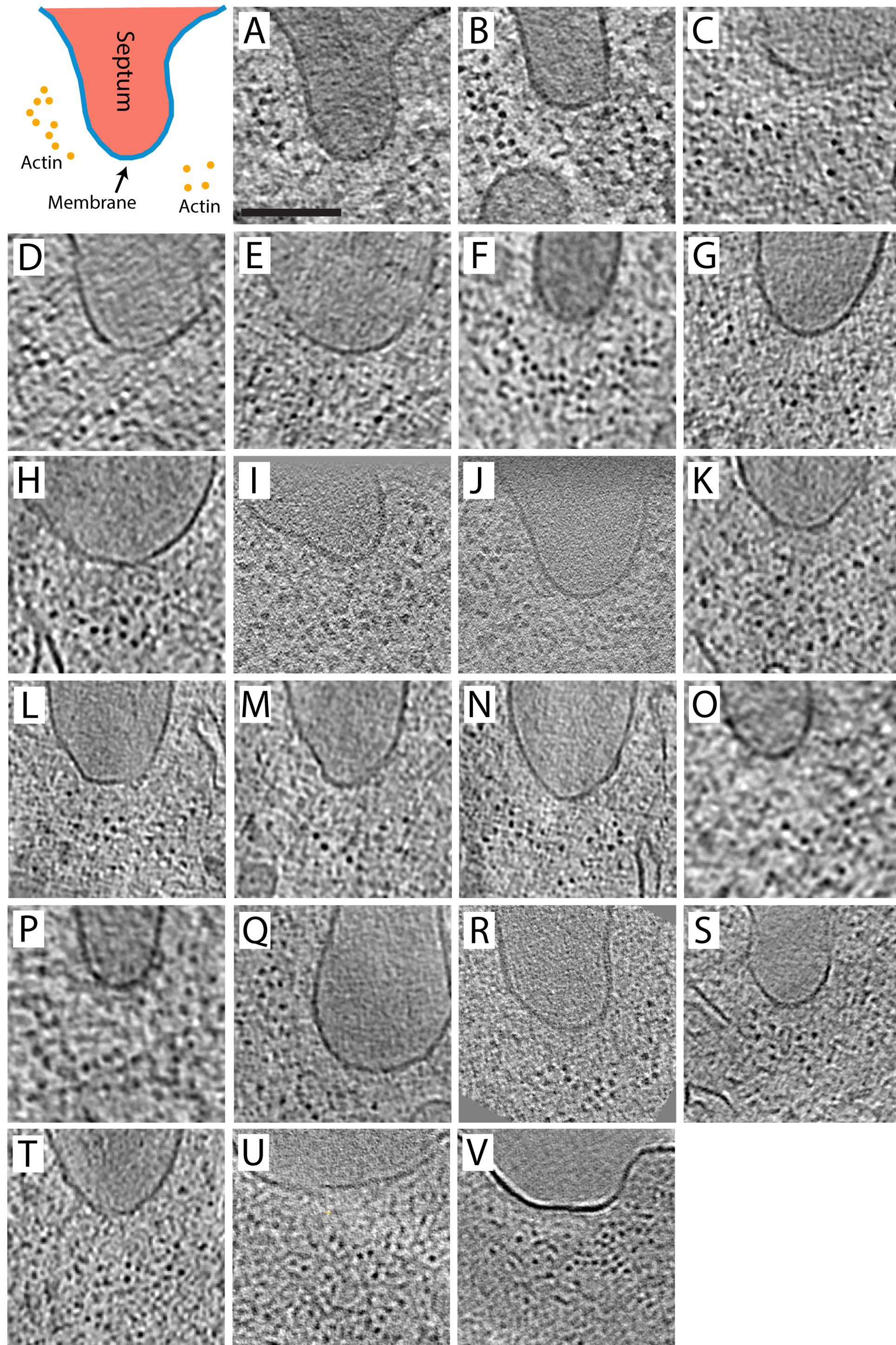
Figure S1: Characterization of cytokinesis in cells synchronized by *cdc25*-22 block and release. (A) Visualization of the mCherry-tagged spindle pole body protein Pcp1 (top), Rlc1-3GFP (middle), and their co-visualization (bottom) in *cdc25*-22 cells released at the permissive temperature of 25°C post 3.5 hours of incubation at the restrictive temperature. Separation of the spindle pole body (mCherry-tagged Pcp1) marks the onset of mitosis. Completion of ring assembly is marked by the appearance of a uniform contractile ring (Rlc1-3GFP) at the equatorial region. Completion of ring constriction is marked by coalescence of Rlc1-3GFP signal to a single bright point. (B) Time taken for ring assembly and (C) for ring constriction compared to wild-type cells ($n > 12$ for all conditions, *** indicates $p < 0.001$, * indicates $p < 0.5$, n.s. indicates non-significant). (D) Visualization of ring constriction of wild-type and *cdc25*-22 cells in a kymograph. (E) Constriction velocity was calculated from the kymograph ($n > 10$ for both wild-type and *cdc25*-22 cells). (F) *cdc25*-22 cells form uniform contractile rings (Rlc1-3GFP) like that of the wild-type. Scale bars represent 5 μm and (A) and 2 μm in (D) and (F).



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18 **Figure S2:** Representative tomographic slices through 22 transverse sections. The top left panel is a
19 cartoon diagram of what is present in the tomographic slices in A, which are the leading edge of the septum
20 (red), the membrane (blue) and visible cross-sections through the long axis of actin filaments (orange dots).
21 (A-V) Cross-sections of filaments are seen to form a bundle near the leading edge of the septum. Each
22 image corresponds to the segmentations in the same position in Fig. 6. Scale bar represents ~100 nm, but
23 some images were cropped to best show the whole septum and filament bundle with slight alteration to the
24 scale of the image.

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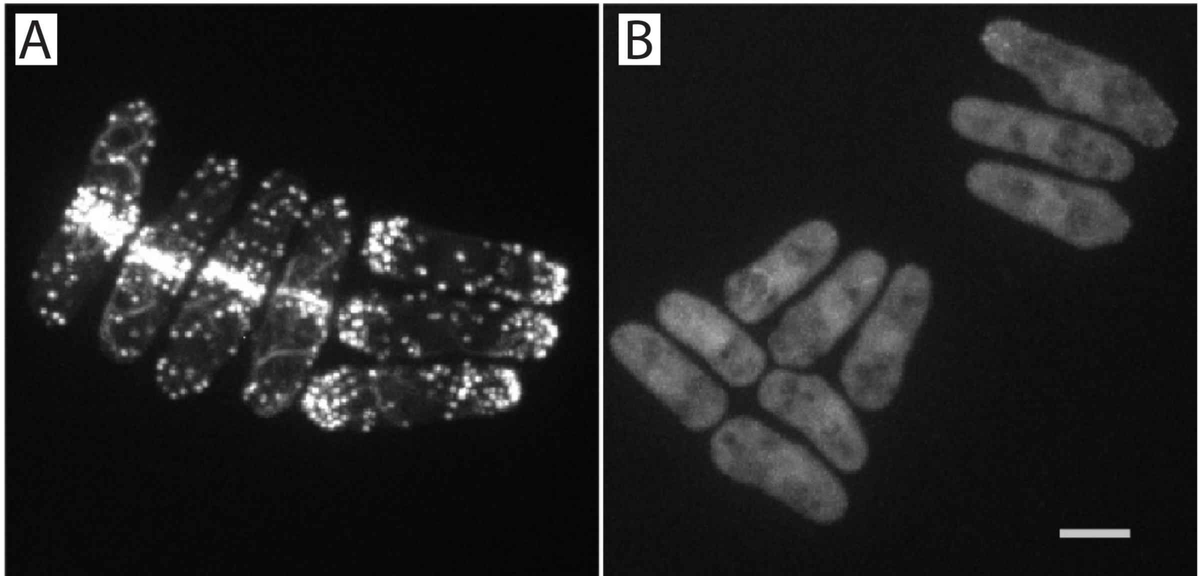

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27 **Figure S3**: Latrunculin A rapidly disassembles actin structures. (A) LifeAct-GFP visualization of F-actin in
28 untreated cells grown in YES media to early log phase. (B) The same LifeAct-GFP visualization in cells
29 after 10 min treatment with 10uM Latrunculin A.

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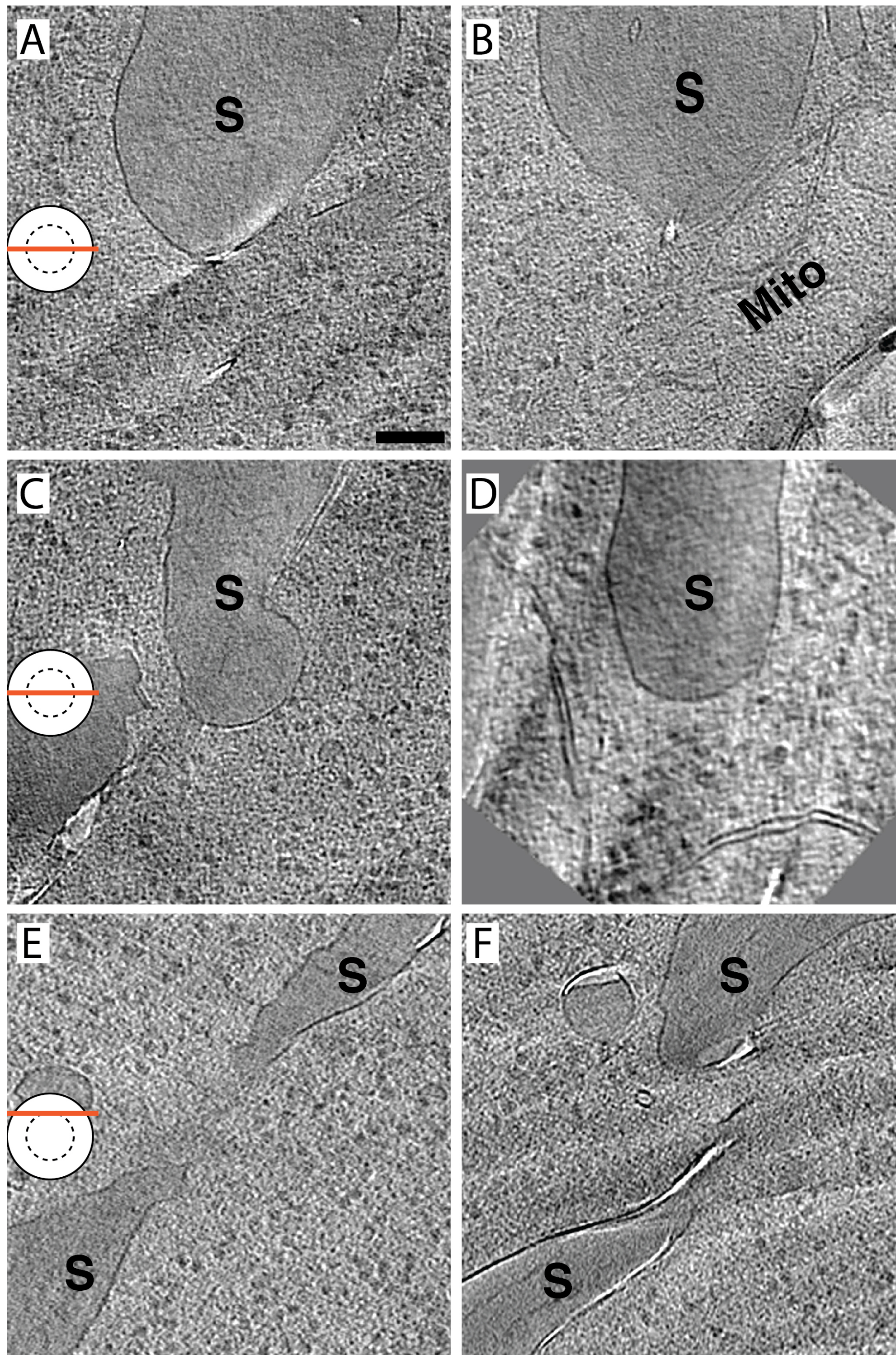

33 **Figure S4:** Tomographic slices of LatA-treated *S. pombe*. (A–D) Tomographic slices of cross-sections
34 through septa, as indicated by the small graphic on the left of panels A and C (where the solid circle
35 represents the cell in cross-section, the dashed circle represents the ingressing septum, and the red line
36 represents the plane of the section through the cell). Note that there are no filaments visible at the leading
37 edge of septa (S) in A–D. The septum in panel B is contacting a mitochondrion, which was never seen in
38 untreated cells. Note that septa in panels A–C are misshapen. (E and F) Tomographic slices from tangential

39 sections through the perimeter of the septum as indicated by the small graphic on the left of panel E.
40 Portions of the septum can be seen coming from both sides of the tomogram. No filaments are seen running
41 laterally between the tips of the sectioned septum. Scale bars represent 100 nm. Tomographic slices are 20-
42 nm thick.
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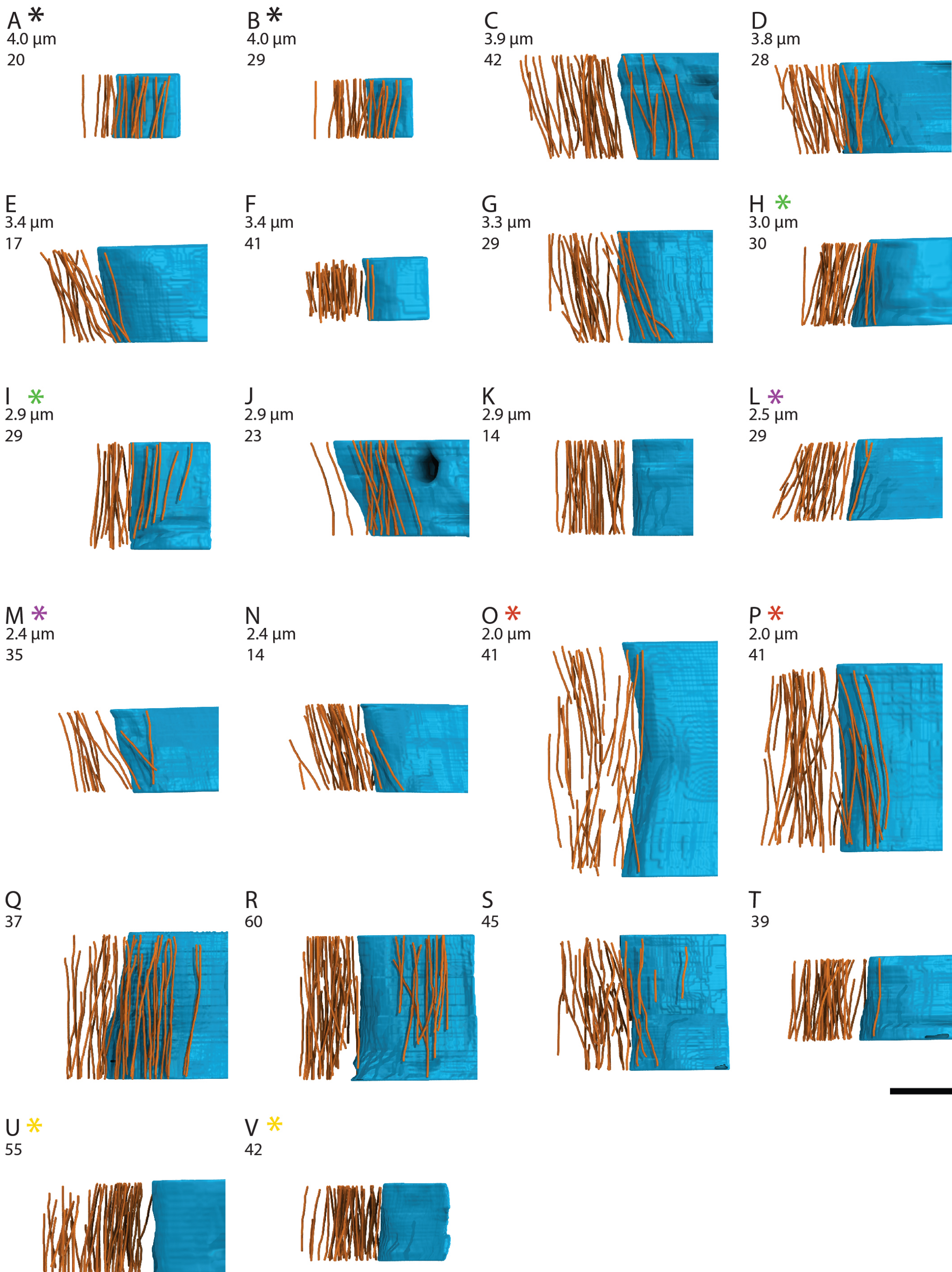

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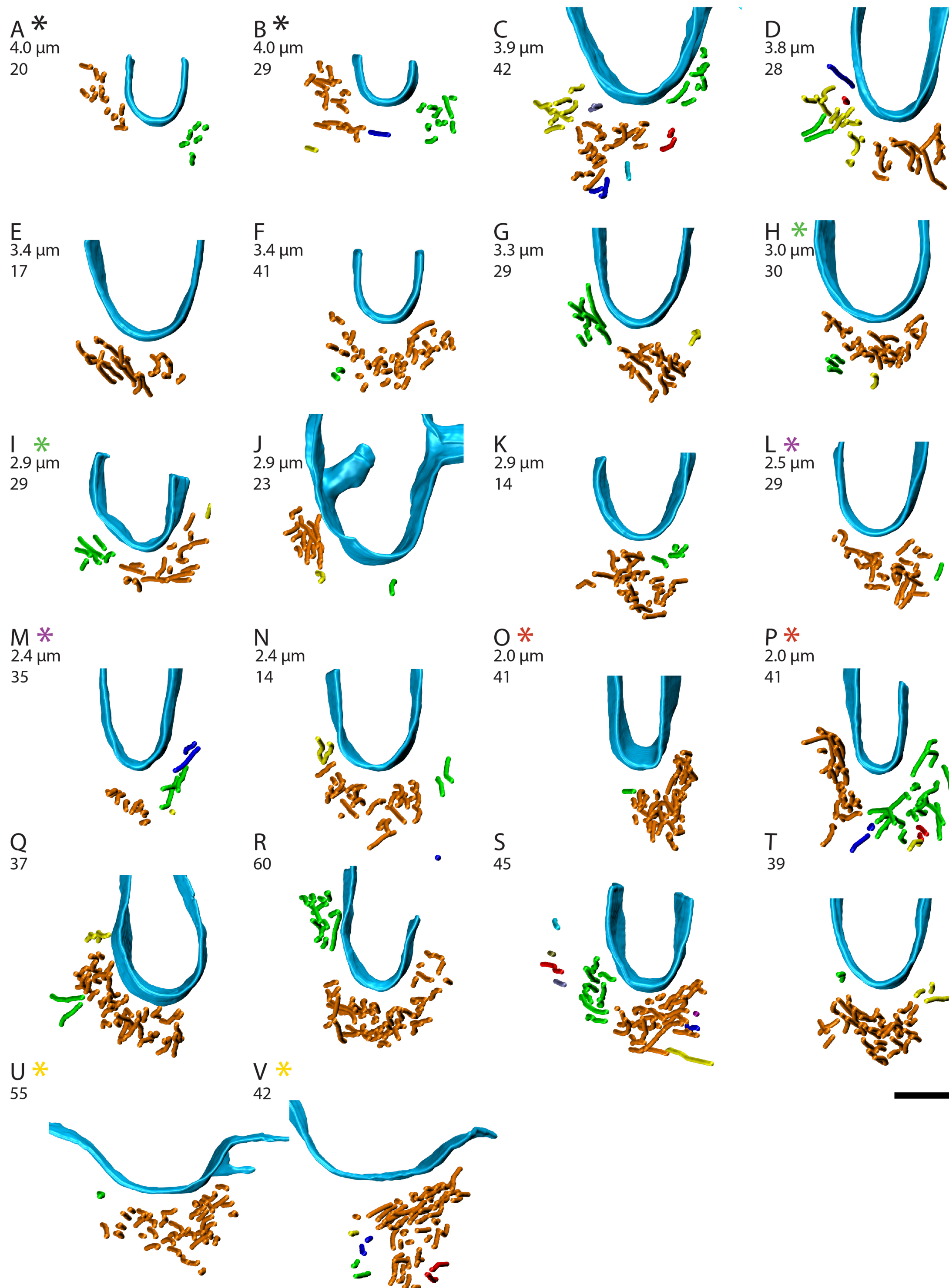
46 **Figure S5:** Side views of 3D segmentations. (A–V) show 22 side views generated from tomographic
47 reconstructions of transverse sections through the actomyosin ring, which illustrate the straightness of
48 filaments (orange) and how they primarily run parallel to the membrane (blue). The calculated diameter of
49 the ring is shown just below the panel letters (panels A–P) and the other number (A–V) indicates the

50 number of filaments. Color-coded asterisks represent pairs of reconstructions from the same cell, but from
51 different tomographic sections. Each image corresponds to the same dataset in Figure 5. Scale bar
52 represents 100 nm.



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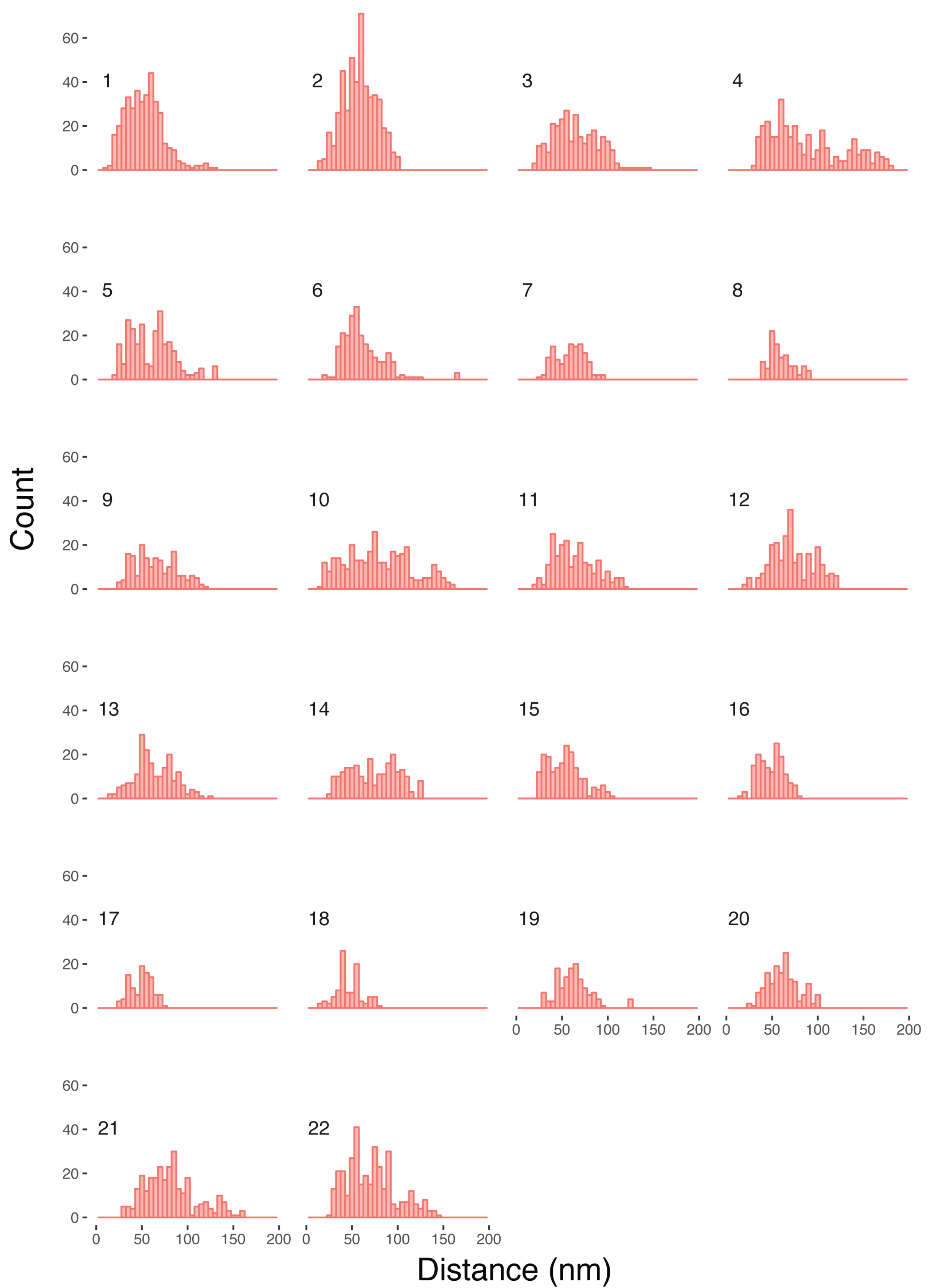
54 **Figure S6:** Visualization of sub-bundles. (A–V) show 22 filament bundles, each divided into sub-bundles

55 that are separated by at least 22 nm, a distance equivalent to the length of the fission yeast α-actinin (See

56 the "General Characteristics" section of the results for how 22 nm was calculated for the length of fission

57 yeast α-actinin. Each image corresponds to the same dataset in Figure 5. Scale bar represents 100 nm.



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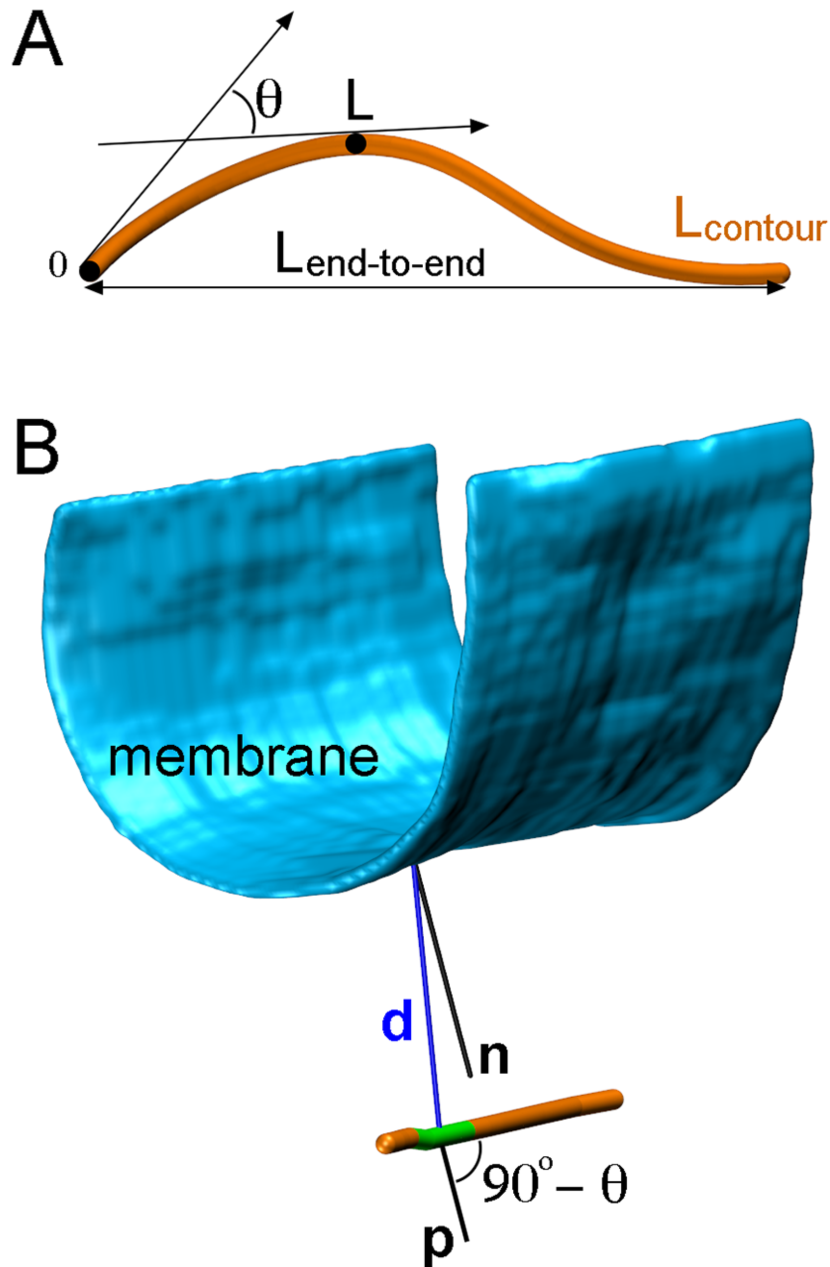
59 **Figure S7:** Histograms of distances measured from the 20-nm segments of the filaments to the membrane

60 in 22 individual tomograms. The bin size equals 5 nm.



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63 **Figure S8:** Schematic of AMR analyses. (A) Straightness of a filament was defined as its end-to-end length
64 divided by its contour length. To calculate the persistence length, the angle θ between the tangent vector at
65 the origin and the tangent vector at a distance L away was calculated for every distance L. (B) To
66 characterize the relationship between the filament bundle and the membrane, the nearest distance d from
67 each 20-nm segment (green) of each filament (orange) to the membrane, and the angle θ between the two
68 were calculated. The normal vector of the membrane at the nearest position from the segment was denoted
69 as n and p was parallel to n.

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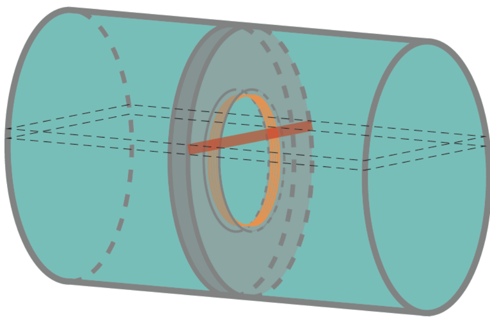

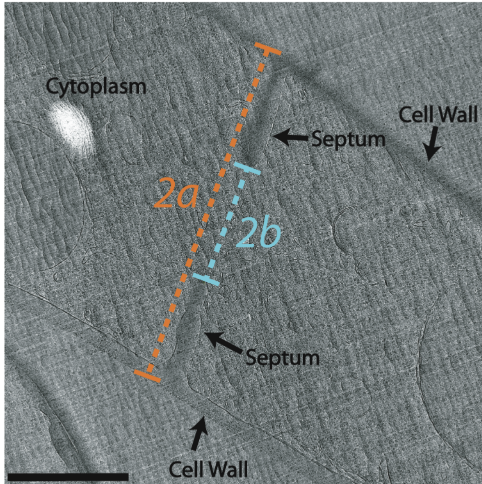

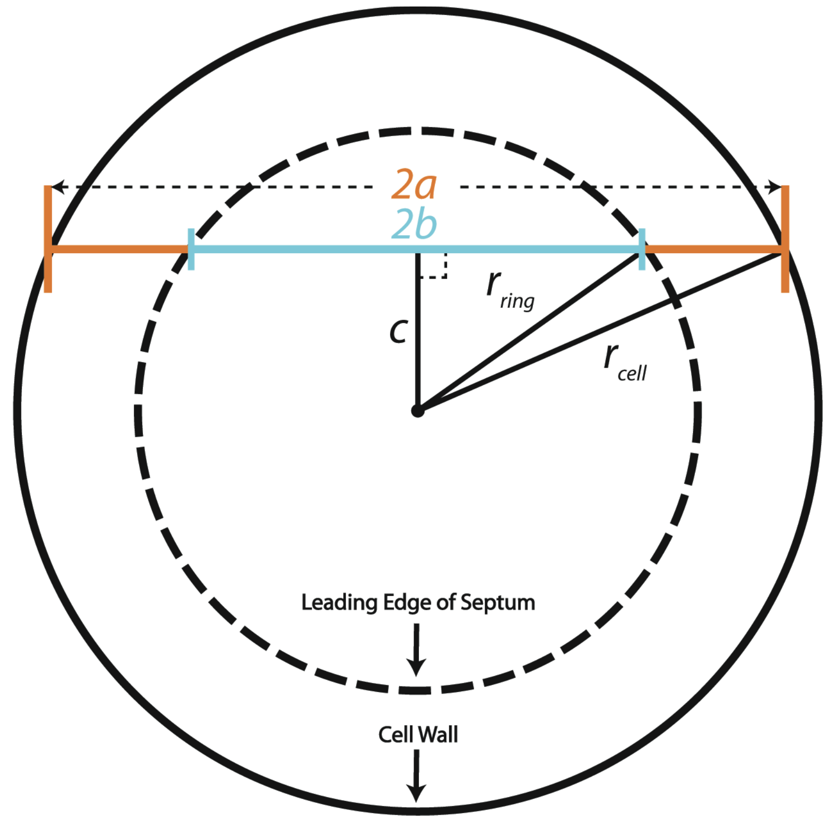

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74 **Figure S9:** Geometry used to calculate the ring diameter in cryosections of *S. pombe* cells. The top left
75 panel is a 3D diagram of a thin section through an *S. pombe* division septum. The bottom left panel
76 illustrates how the values 2a and 2b were measured in lower magnification images of cryosectioned cells.
77 The right panel diagrams the relation between the measurements made on sectioned cells and the diameter
78 of the AMR at the leading edge of the septum. Note $r_{cell} = 2.25$ μm was measured by light microscopy
79 and was assumed to be the same for all cells (see **Methods**).