Supplemental material

Ng et al., https://doi.org/10.1083/jcb.201809088

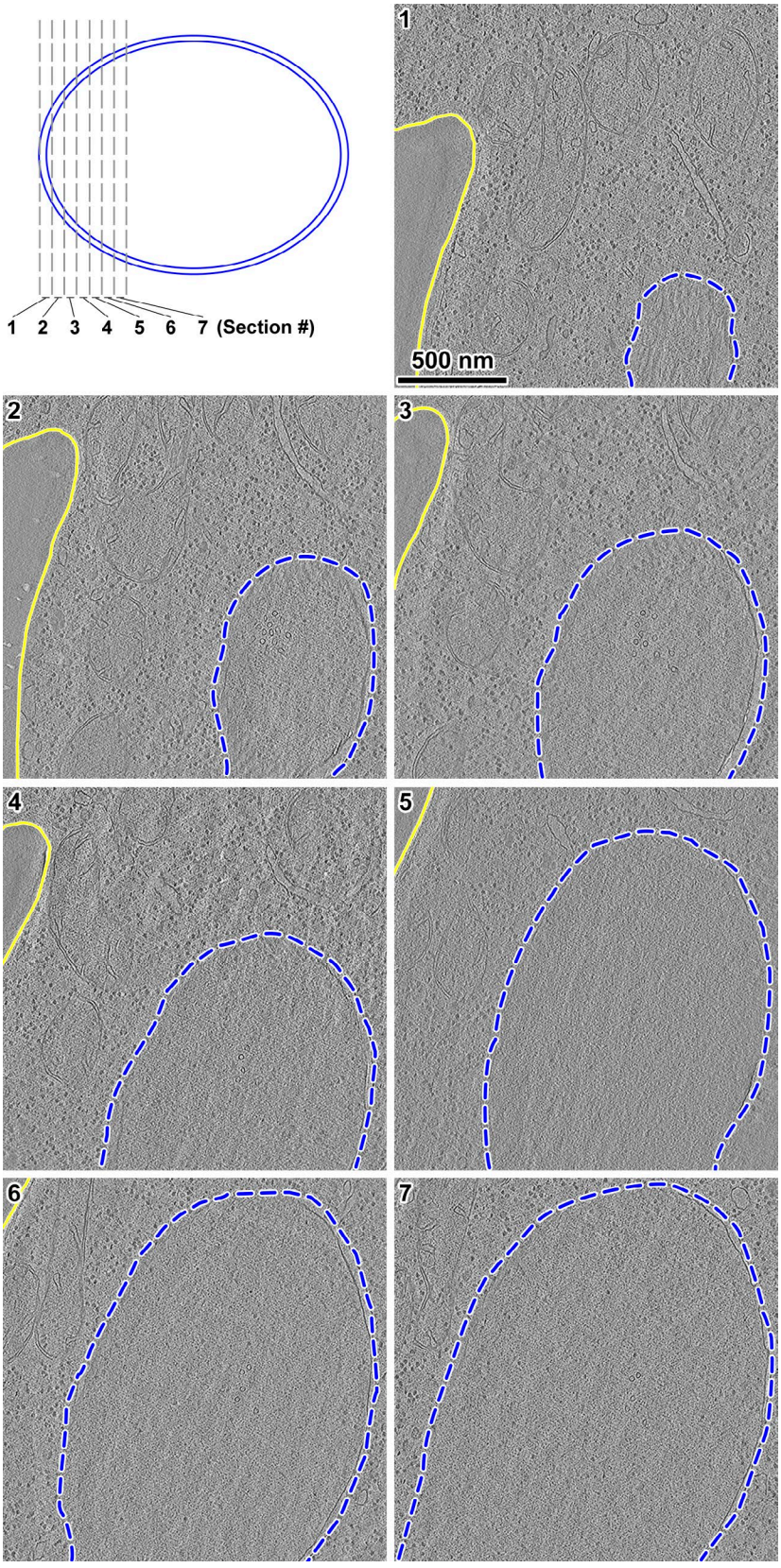


Figure S1. **Serial cryotomograms of a metaphase yeast cell.** Cartoon of a cell nucleus, bounded by a nuclear envelope (top left; double blue lines). Seven sequential sections are shown, bordered by vertical gray dashes. Sections are numbered at the top left of each panel. (1–7) Cryotomographic slices (20 nm) of seven sequential cryosections of a metaphase cell. The outer nuclear membrane is outlined in blue dashes in each panel. The plasma membrane is outlined by a solid yellow line.

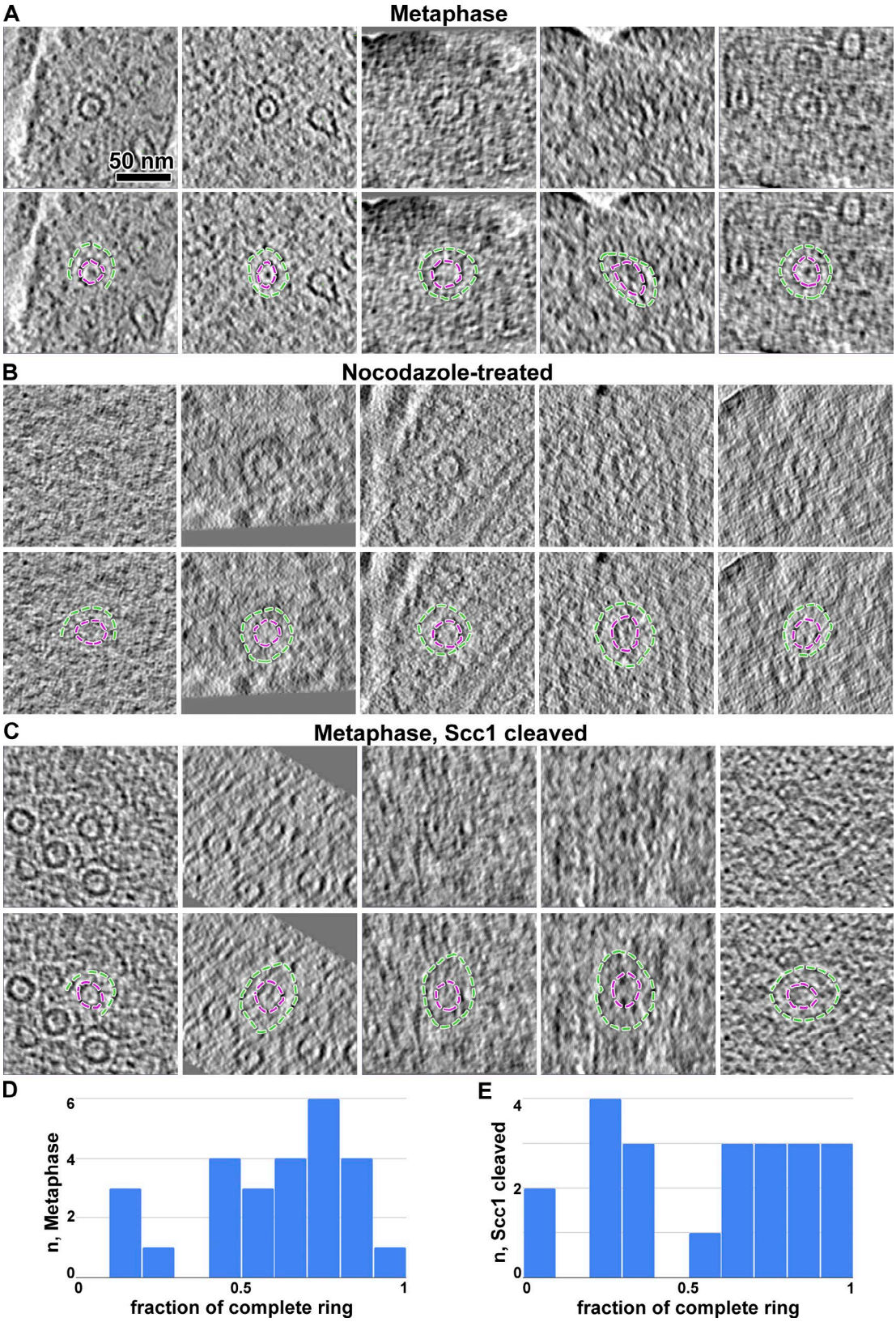


Figure S2. **Gallery of Dam1C/DASH in metaphase and nocodazole-treated cells. (A–C)** Cryotomographic slices (7–9 nm) showing top views of both complete and partial Dam1C/DASH rings attached to kMT walls in metaphase (A), nocodazole-treated cells (B), and metaphase cells with Scc1 cleaved (C). For clarity, the bottom panels show the same densities as the top panels but with annotations. Green dashes indicate Dam1C/DASH complexes. Magenta dashes indicate kMT walls. **(D and E)** Histograms of completeness of partial Dam1C/DASH rings from metaphase (n = 11) and Scc1-cleaved cells (n = 10), respectively.

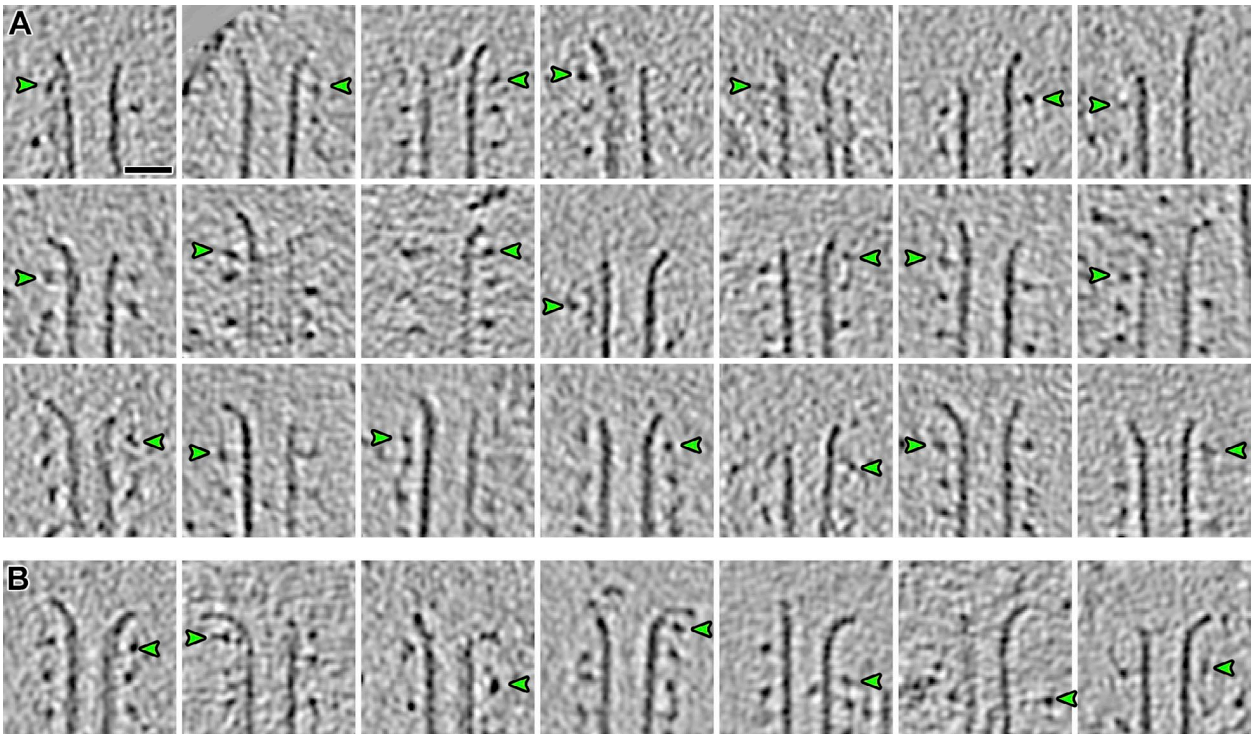


Figure S3. **In vitro examples of Dam1C/DASH near curved tips. (A)** Cryotomographic slices (4.6 nm) of the flared ends of MTs assembled with Dam1C/DASH in vitro. Green arrowheads indicate the Dam1C/DASH density closest to protofilaments' curved tip. Scale bar, 25 nm. **(B)** Same as in A, but for MTs showing the ram's horn tip motifs. Note that some MTs appear narrower than 25 nm in a subset of slices taken closer to the surface of the MT. Another subset of MTs have lower contrast because they were oriented almost perpendicular to the tilt axis; this is a well-known missing wedge effect that changes the appearance of tubular structures.

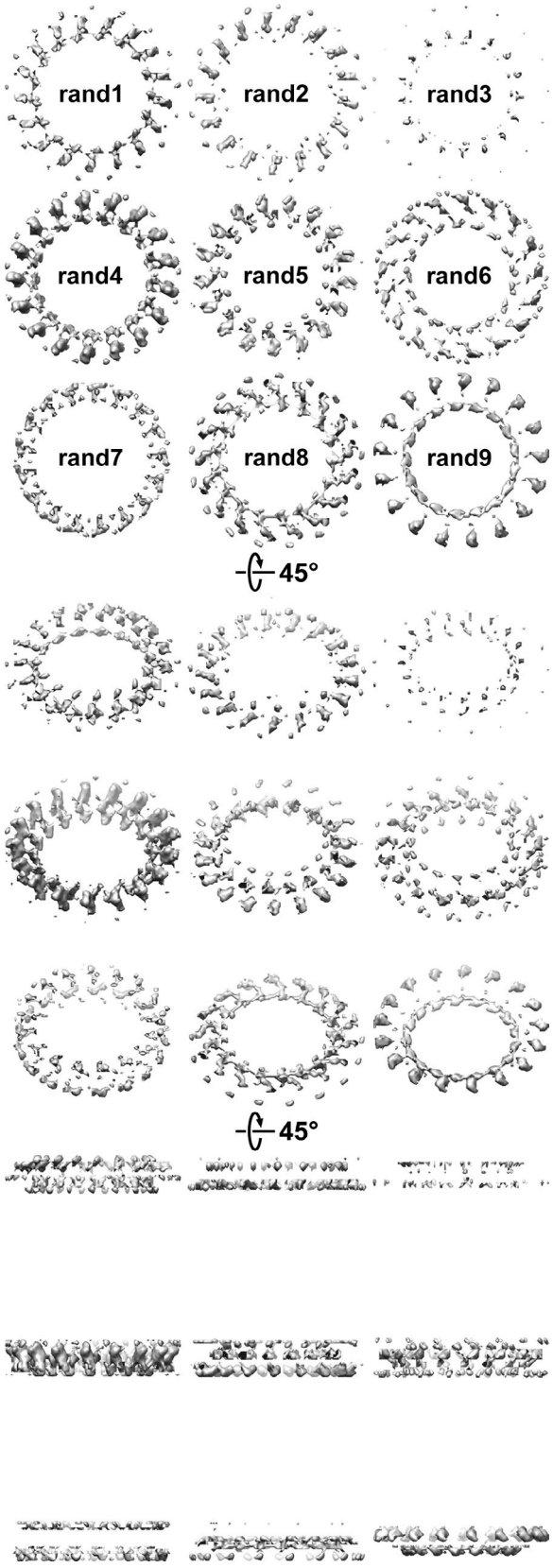
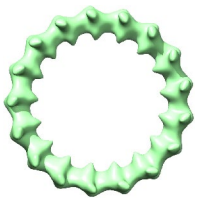


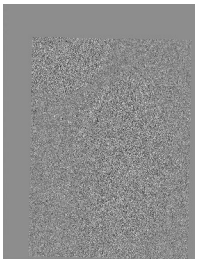
Figure S4. **Control 17-fold symmetrized averages of random nuclear positions.** Nine subtomograms containing random selections of yeast nucleoplasm were symmetrized 17-fold, masked, and contoured just as for the Dam1C/DASH subtomograms in Fig. 3 G. The top 3 × 3 subpanels are viewed along the symmetrization axis, with subtomogram number identified in the middle. The lower two sets of subpanels are sequentially rotated 45° around the horizontal axis.

Table S1. **Imaging parameters**

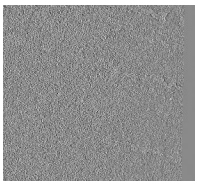
Sample	***S. cerevisiae* cells**	**Dam1C/ DASH + MT, cryosections**	**Dam1C/DASH + MT, plunge frozen**
Grid type	CF-42-2C-T; continuous carbon	CF-22-2C-T (Protochips)	Quantifoil R2/2
Microscope	Titan Krios		
Voltage	300 kV		
Gun type	FEG		
Camera	Falcon II Direct Detector		
Software	TOMO4		
Calibrated magnification	15,678 / 19,167	30,369	30,369
Calibrated pixel	8.93 / 7.3 Å	4.61 Å	4.61 Å
Defocus	−8 to −15 µm Volta: −0.5 µm	−10 µm	−8 to −14 µm
Cumulative dose	100 - 130 e/Å^2		
Dose fractionation	1 / cosine		
Tilt range	±60°	±60°	±66°
Tilt increment	2°		



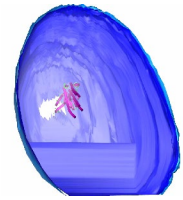
Video 1. **Subtomogram average of Dam1C/DASH in vitro.** A 17-fold symmetrized average of Dam1C/DASH rings assembled around MTs in vitro. This map corresponds to Fig. 1 F and Fig. 2 I. This video is available at https://youtu.be/z38QST8UgjA.



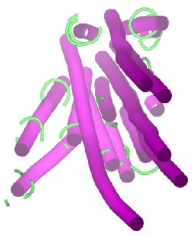
Video 2. **Serial cryotomogram of a metaphase cell.** Tomographic slices through seven serial cryotomograms of a single metaphase cell, which was sectioned perpendicular to the spindle axis. The spindle MTs are visible in the center of the field of view and the nuclear envelope is visible on the right. This video corresponds to the cell presented in Fig. 5 (A–C) and Fig. S1. The full serial reconstruction is available at https://youtu.be/X9-E_3gdmRQ.



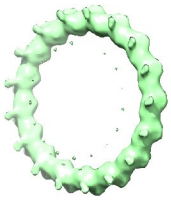
Video 3. **Serial cryotomogram of a metaphase cell, cropped.** This video is cropped to show more details in the nucleus of the cell presented in Video 2 and is available at https://youtu.be/WvJQeBxgJ7s.



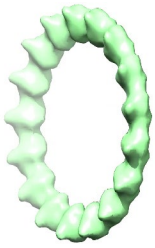
Video 4. **3D model of a budding yeast nucleus and spindle reconstructed by serial cryo-ET.** A model showing the nuclear envelope in dark and light blue, spindle MTs in magenta, and Dam1C/DASH rings in green. This video corresponds to the serial cryotomogram model presented in Fig. 5 B. This video is available at https://youtu.be/BF1UanktHHE.



Video 5. **3D model of a budding yeast spindle reconstructed by serial cryo-ET.** A model of only the spindle MTs (magenta) and Dam1C/DASH (green) in the most complete serial cryotomogram. This video corresponds to the serial cryotomogram model presented in Fig. 5 C. This video is available at https://youtu.be/DiLAchFpdq8.



Video 6. **Dam1C/DASH in vivo, instance 1.** A single Dam1C/DASH ring, symmetrized 17-fold. This video corresponds to the leftmost ring in Fig. 5 G. This video is available at https://youtu.be/sBjVqzmgTvs.



Video 7. **Dam1C/DASH in vivo, instance 2.** Another single Dam1C/DASH ring, symmetrized 17-fold. This video corresponds to the rightmost ring in Fig. 5 G. This video is available at https://youtu.be/oTPv3oNaU-w.