

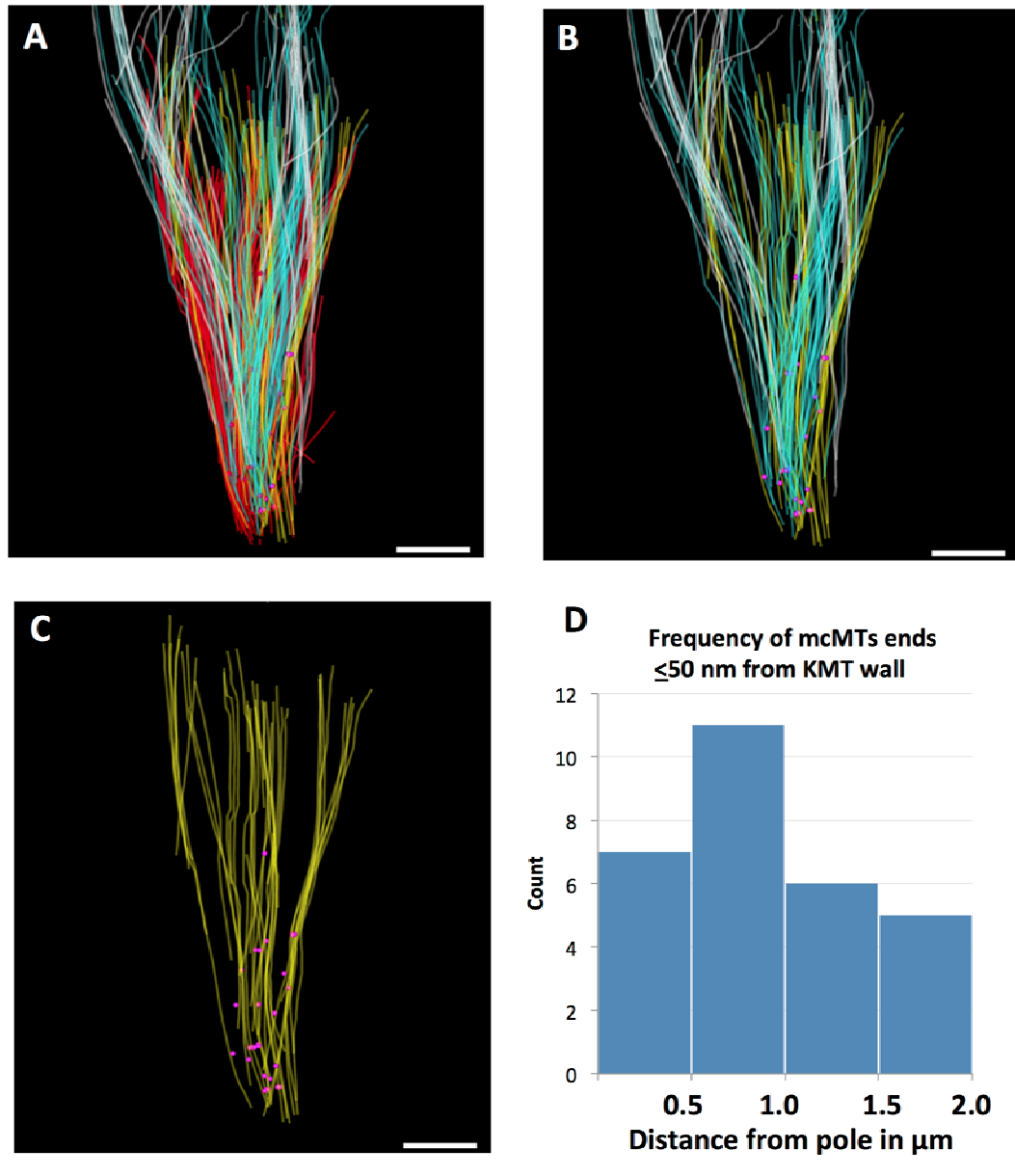
# Supplemental Materials

*Molecular Biology of the Cell*

O'Toole et al.

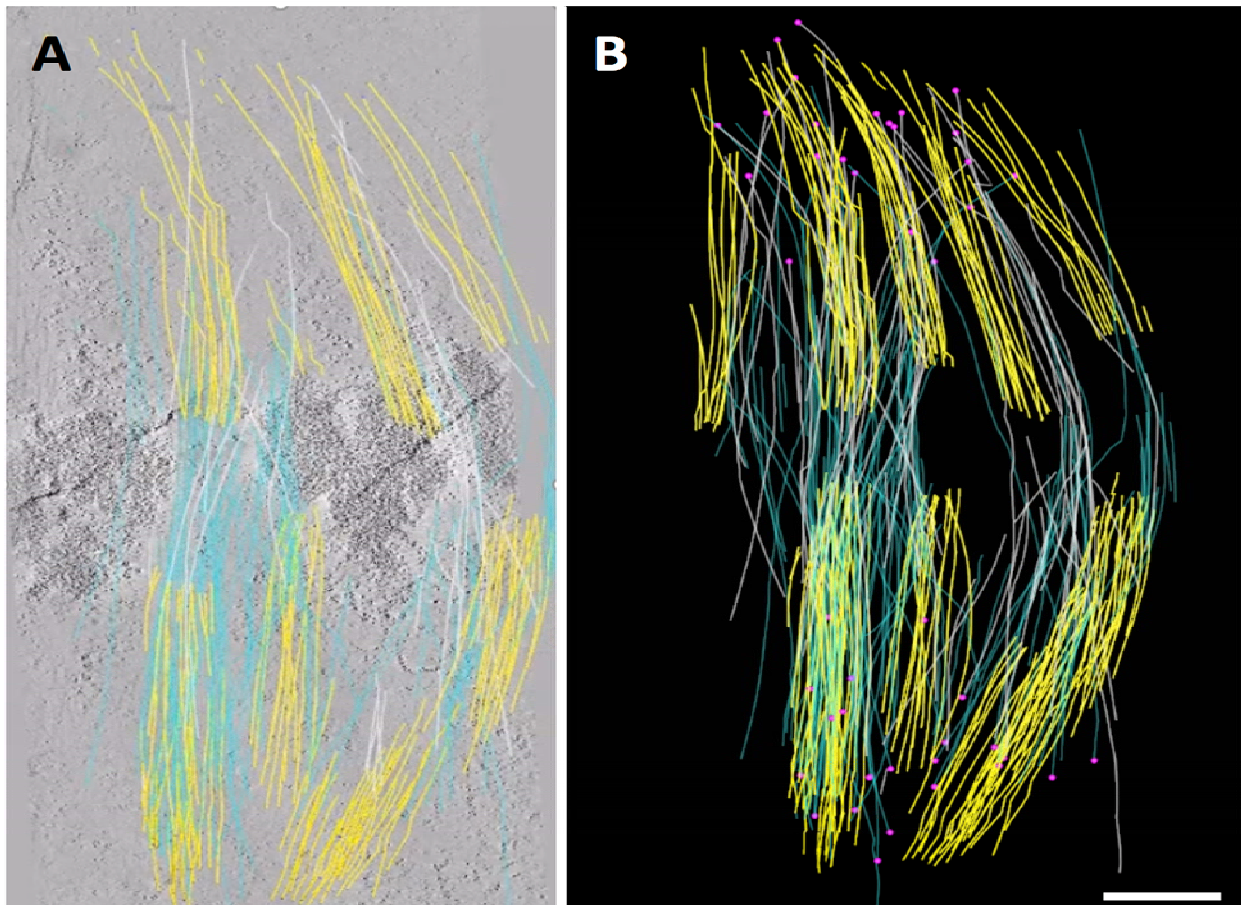
## Supplementary Materials

### Supplementary Figures.



**Figure S1.** Part of another *Chlamydomonas* metaphase spindle. This model was built from 6 serial 250nm thick sections. (A) The model shows KMTs (yellow; n=47) and two groups of mcMTs: white (n=42) for association with the upper spindle pole, which lies outside the

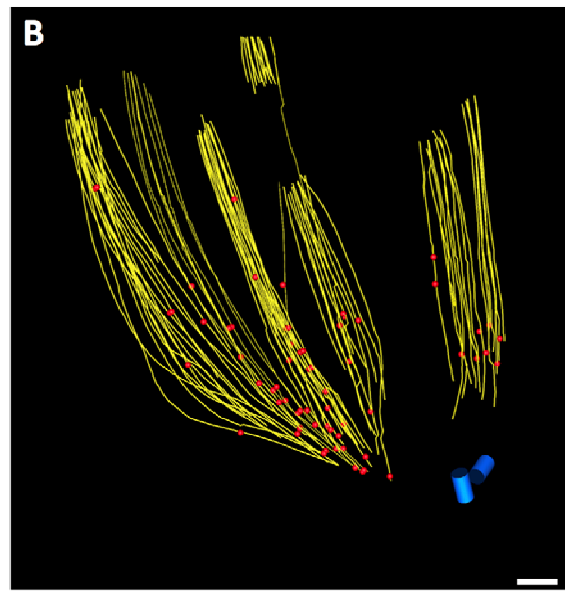
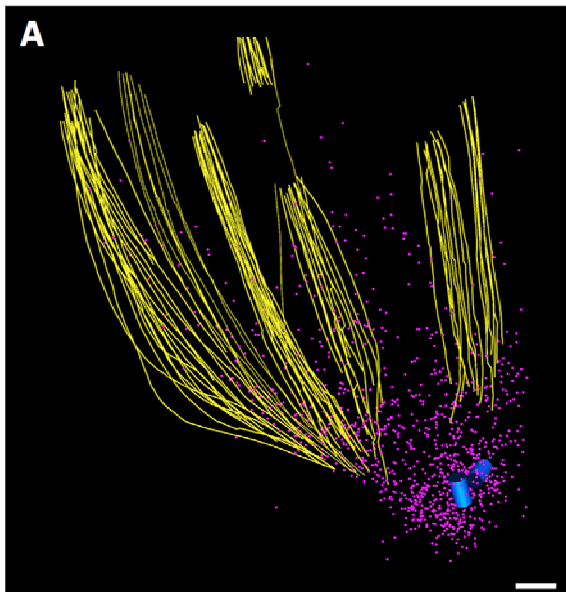
reconstructed volume, and light blue (n=58) for association with the lower pole. MTs that either ended before reaching the chromosomes or went out of the volume of the reconstruction are red (n=193). (B) Model showing KMTs (yellow) and mcMTs (white, blue). (C) Model showing KMTs (yellow) and positions of close approach of mcMT ends (pink circles; n=29). (D) Distribution of the frequency of places where mcMTs end  $\leq 50$  nm from a KMT as a function of distance from the pole.



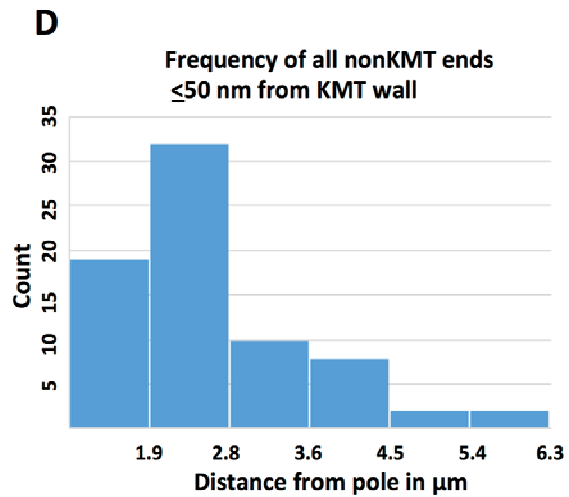
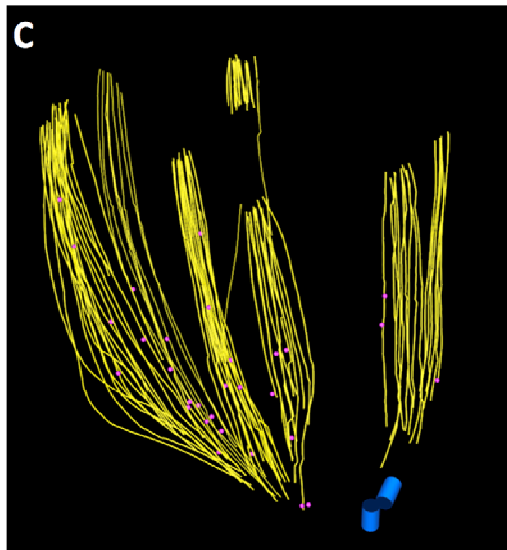
**Figure S2.** Longitudinal view of an RPE1 metaphase spindle. (A) Projection of a 3D model showing KMTs (yellow), ipMTs (white, blue) and their pole proximal ends (pink circles; n=45). This model was built from 4 serial, 250 nm sections. Bar = 1  $\mu$ m.

All pole-proximal nonKMT ends

All nonKMT ends within 50nm of a KMT



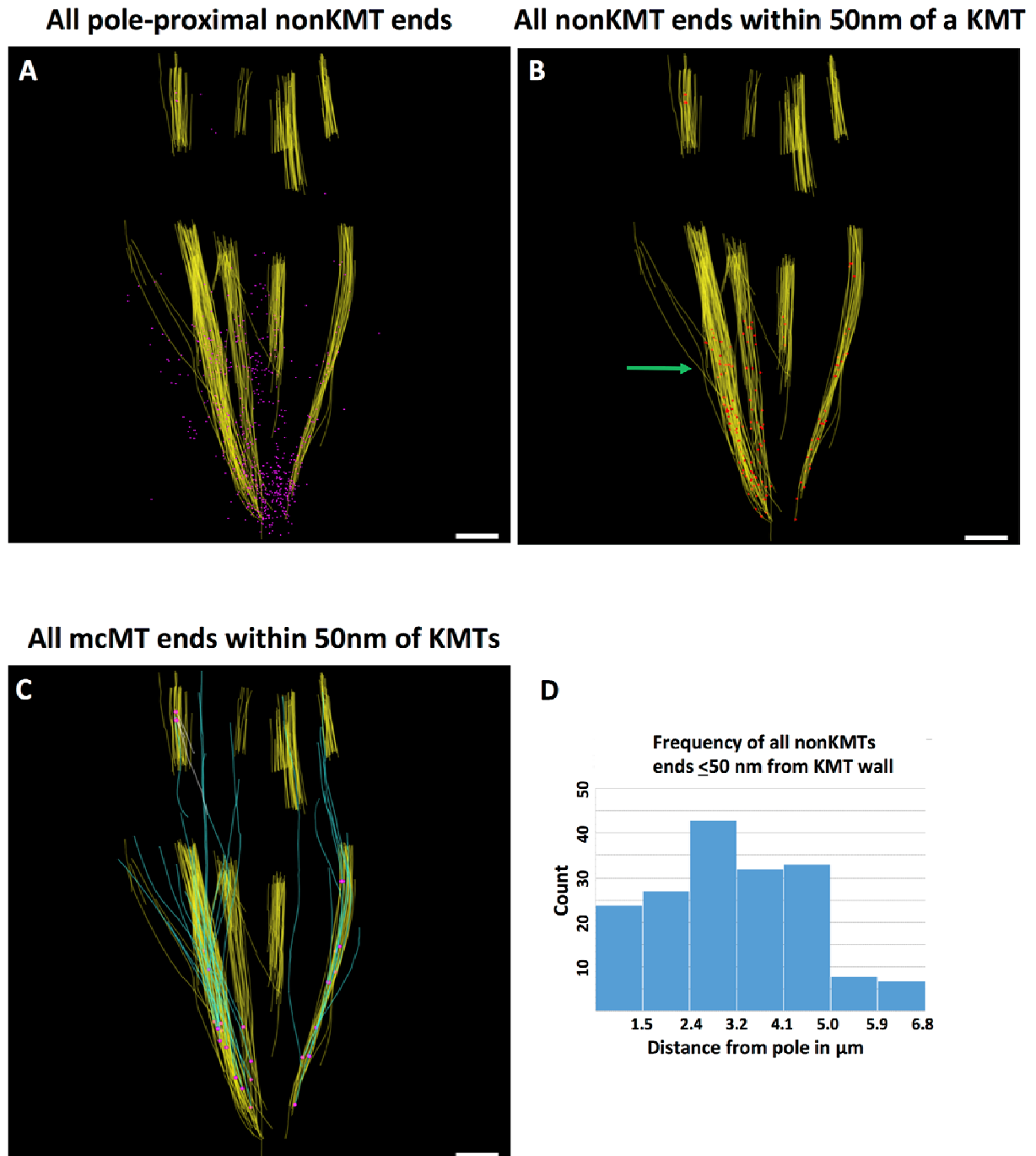
mcMT ends within 50nm of KMTs



**Figure S3.** Distributions of subsets of MT ends from the RPE1 model shown in Fig 5 (A) All the KMTs (yellow) and the pole-proximal (minus) ends of all nonKMTs (purple dots;  $n=1061$ ). (B) All KMTs and the minus ends of nonKMTs that lie within 50 nm of the wall of a KMT (red dots;



n=73). Note that these dots can be seen in K-fibers, starting about 2  $\mu\text{m}$  from each kinetochore. (C) All KMTs and the minus ends of all mcMTs (pink dots; n=32). Bars = 500 nm. (D) Histogram of the frequency of all nonKMT minus ends that are within 50 nm of KMTs as a function of distance from the pole.



**Figure S4.** Distributions of subsets of MT ends from the PTK model shown in Fig 7 (A) All the KMTs (yellow) and the pole-proximal (minus) ends of all nonKMTs (purple dots; n=657). Note that these dots can be seen in k-fibers, starting about 2  $\mu\text{m}$  from each kinetochore. (B) All KMTs and the minus ends of nonKMTs that lie within 50 nm of the wall of a KMT (red dots; n=181). Blue arrow indicates a distance of about 2  $\mu\text{m}$  from the kinetochores. (C) All KMTs and the minus ends of all mcMT ends that are within 50 nm of KMTs (pink dots; n=41). Bars = 500 nm. (D) Histogram of the frequency of all nonKMT minus ends that are within 50 nm of KMTs as a function of distance from the pole.

## **Movies**

**Movie 1** Serial tomographic slices through a volume containing part of a *Chlamydomonas* metaphase spindle. The tomographic volume was built from four, 250 nm sections. The nuclear envelope, chromosomes, basal bodies and numerous spindle MTs can be seen. A 3D projection of the model shows KMTs (yellow) and mcMTs (light blue). Red indicates MTs that either end before reaching the chromosomes or end outside the reconstructed volume. The duplicated basal bodies are at the plasma membrane, not at the spindle pole. Scale bar = 500nm.

**Movie 2** The model shown in Figure 2C and Movie 1 was resampled along the spindle axis to display the model points in cross section. The movie begins at the spindle pole, goes to the metaphase plate then back to the pole. Yellow circles indicate positions of KMTs and blue circles indicate mcMTs. A comingling of these two MT classes can be seen throughout the spindle.

**Movie 3** Serial tomographic slices through a volume containing the midzone of a *Chlamydomonas* metaphase spindle. The tomographic volume as built from four, serial 250 thick sections. The model shows KMTs (yellow), and red indicates MTs that either end before reaching the chromosomes or end outside the reconstructed volume. mcMTs from either pole are colored in white and light blue, respectively. Bar = 500nm

**Movie 4** The model in Figure 3A and Movie 3 was resampled along the spindle axis to display the model points in cross section. Yellow circles indicate positions of KMTs, blue and white

circles indicate mcMTs from either pole, respectively. Bundles of mcMTs are well organized in the spindle midzone and these MTs comingle with KMTs.

**Movie 5** Serial, tomographic slices and projected 3D model of a spindle from the human cell line, RPE1. The tomographic volume was built from five, serial 300nm thick sections and comprises ~1/5 the total spindle volume. KMTs are yellow (n=76), nonKMTs are red (n=1252). Pole distal and pole proximal ends are marked by blue and green circles, respectively. A centriole pair (blue cylinders) marks the centrosome of the spindle pole. Bar = 1µm.

**Movie 6** The model in Figure 6A was resampled along the spindle axis to display the model points in cross section. The movie begins at the pole and ends at the metaphase plate. Yellow circles indicate positions of KMTs, blue and pink circles indicate two bundles of mcMTs associated with the same pole. White circles represent mcMTs from the opposite pole. Bundles of mcMTs are well organized in the spindle midzone and these MTs initially associate laterally with specific KMT bundles. The mcMTs comingle with KMTs, but nearer the pole, they diverge to mingle with other KMT bundles.

**Movie 7** Serial, tomographic slices and projected 3D model of a spindle from the mammalian tissue culture cell line, PtK<sub>2</sub>. The tomographic volume was built from six, serial 300nm thick sections. KMTs are shown in yellow (n=255), nonKMTs are in red (n=1384).