Methods

Sample preparation. γTuSC was co-expressed with GST-Spc110p$^{1-220}$ in Sf9 cells and purified as described, in H100 (40 mM Hepes PH 7.6, 100 mM KCl, 1 mM EGTA, 1mM $MgCl_2$)[13,21], except that complexes were eluted from glutathione resin by cleavage of the GST tag with TEV protease as the final purification step. γTuSC rings were formed by a 30 minute incubation on ice after dilution to 0.2 μM in BRB80 (80 mM PIPES pH 6.9, 1mM EGTA, 1mM $MgCl_2$). For cryo-EM γTuSC/Spc110p$^{1-220}$ filaments were at 2 mg/ml total protein in H100. γTuRCs were purified from *Drosophila* embryos as described[2]. Microtubules were grown in the presence of γTuSC rings and prepared for EM as described[3], with the exception that microtubules were crosslinked for 3 minutes in

1% glutaraldehyde before sedimentation. Negative stain samples were prepared as described[26] in 0.75% uranyl formate, and cryo-EM samples were prepared on C-FLAT holey carbon grids[27] using a Vitrobot (FEI Co.).

Electron microscopy and image processing. Negative stain EM was performed on a Tecnai T12 Spirit (FEI Co.) operating at 120 kV, and images acquired on a 4k x 4k CCD camera (Gatan, Inc.). Cryo-EM data were obtained on a Tecnai F20 operating at 200 kV with an 8k x 8k TemCam-F816 camera (TVIPS, GmbH) with a pixel size of 1.19 Å/pixel. Defocus was determined with CTFFIND[28], and each micrograph was corrected by application of a Wiener filter. Particles were boxed out in 505 Å segments, overlapping by 480 Å. After several initial rounds of alignment the particles were centred with respect to the helix axis by integer pixel shifts, then masked to 310 Å along the helical axis and 380 Å perpendicular to the axis with a cosine-edged mask.

Iterative helical real space reconstruction was performed essentially as described by Egelman[17] and Sasche, et al.[25], using SPIDER[29]. Initially, eighteen rounds of projection matching were carried out using 2x binned particles, with the helical symmetry parameters refined and imposed at each step using the programs hsearch_lorentz and himpose[17]. In subsequent rounds, helical symmetry parameters were fixed, real space symmetry was imposed using SPIDER, and refinement was limited to searching within 2° of orientation assignments from the previous round. Seven rounds of refinement were performed, after which particles were re-masked to 160 Å along the helical axis (corresponding to about 1.3 turns of helix) to reduce the effects of bending in the helix. Five rounds of refinement were carried out with these masked particles, then a final fifteen rounds using the unbinned, masked particles.

A 300 $Å^2$ B-factor was applied to the final reconstruction using the program BFACTOR. Volumes were viewed and segmented using Chimera[30]. Automated

segmentation of Spc97p and Spc98p into domains was performed with the Chimera plug-in Segger. The crystal structure of γ-tubulin was fit into the density by initial manual placement and then fitting with the Chimera fitting routine.

Microtubule nucleation assays. The solution nucleation assays were performed essentially as described[21]. Rhodamine labelled tubulin in assembly buffer (BRB80, 25% glycerol, 1 mM GTP) was incubated on ice for five minutes with γTuSC or γTuSC/Spc110p$^{1\text{-}220}$ filaments (150 nM final γTuSC concentration) or H100 as a buffer control. The assembly reactions were transferred to a 37°C waterbath for 4 minutes, diluted tenfold in warm assembly buffer + 1% glutaraldehyde to crosslink microtubules for 3 minutes, then diluted twentyfold in cold BRB80. BRB80 was at pH 6.9 or 7.5, with 1.2 mg/ml tubulin for the pH 6.9 experiments and 2.0 mg/ml for the pH 7.5 experiments.

The crosslinked microtubules were spun through a cushion of BRB80 + 20% glycerol onto poly-lysine coated coverslips. Microtubules were imaged by rhodamine fluorescence on an Axiovert microscope, with images recorded on a CCD camera. Microtubules were counted in fifteen random fields for each experiment, and the values for each experiment were normalized against the buffer control.