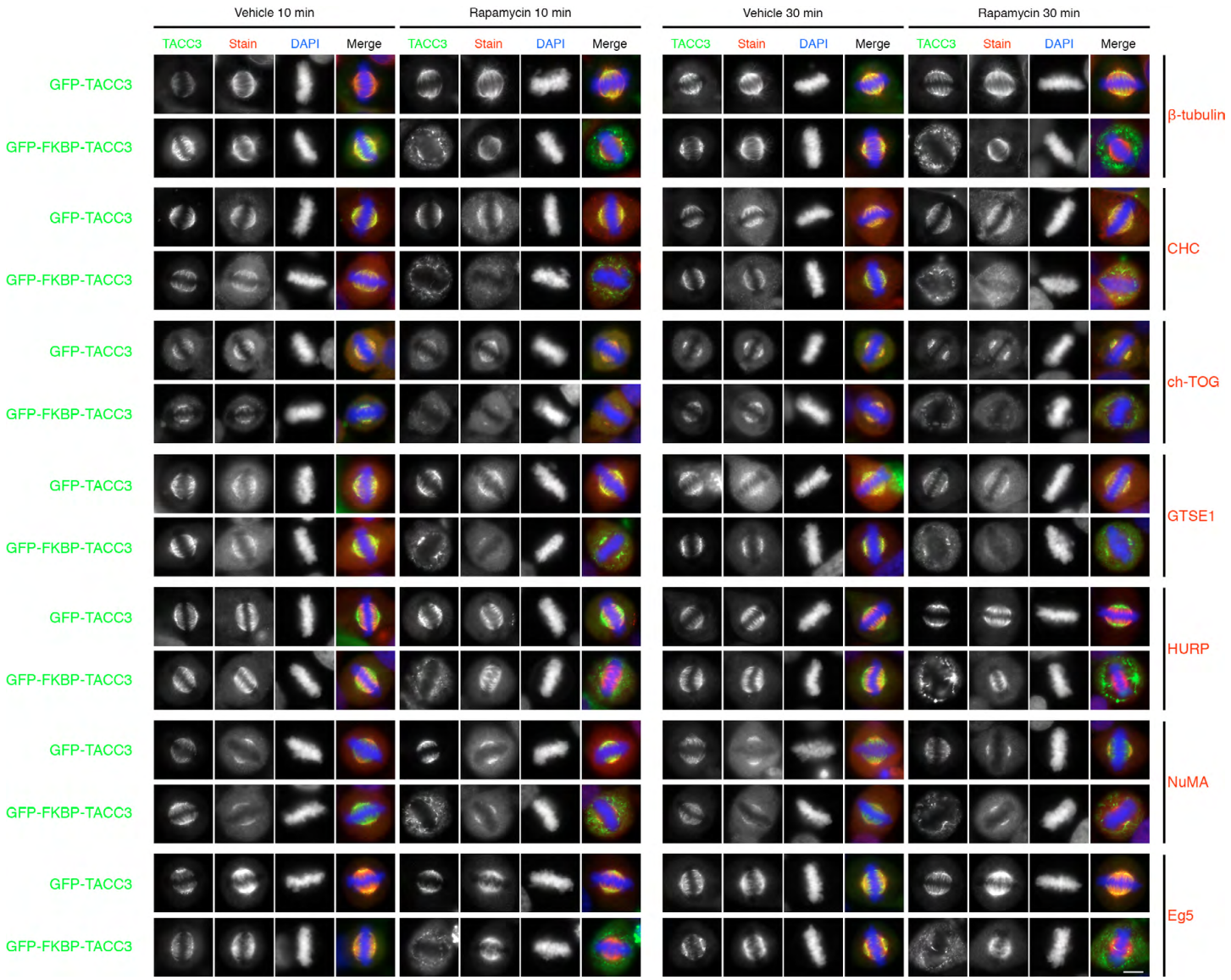


Fig. S1. Effect of TACC3 KS on other spindle proteins. Representative micrographs of TACC3-depleted HeLa cells expressing PAGFP-MitoTrap and either GFP-TACC3 or GFP-FKBP-TACC3 and treated with vehicle or rapamycin (200 nM) for 10 min or 30 min. Cells were fixed and stained for other spindle proteins (red). TACC3 KS caused removal of its partners (ch-TOG, clathrin and GTSE1). The gross morphology of the spindle was not affected (tubulin). Other spindle proteins (HURP, NuMA, Eg5) were unaltered. There were no differences between the two timepoints, indicating that removal of TACC3/ch-TOG/clathrin by TACC3 KS did not alter the localization of other spindle proteins over time. An abbreviated version of this figure showing only 1 control and the 10 min timepoint is shown as Fig. 2. Scale bar: 10 μm.

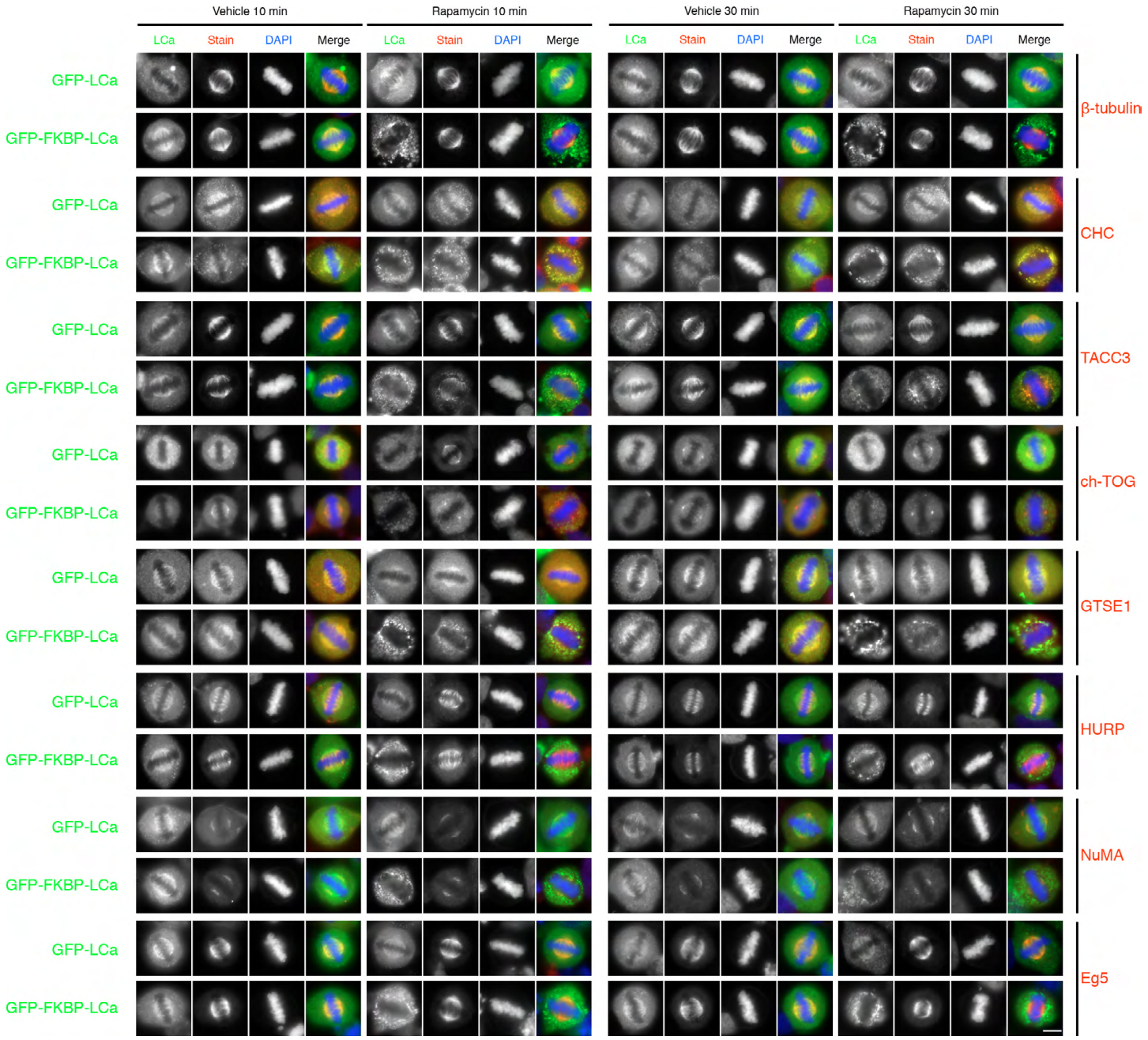


Fig. S2. Effect of clathrin rerouting on other spindle proteins. Representative micrographs of HeLa cells expressing PAGFP-MitoTrap and either GFP-LCa or GFP-FKBP-LCa and treated with vehicle or rapamycin (200 nM) for 10 min or 30 min. Cells were fixed and stained for other spindle proteins (red). LCa rerouting also removed clathrin heavy chain, ch-TOG, TACC3 and GTSE1 from the spindle. Again, tubulin, HURP, NuMA and Eg5 were unaltered after 10 and 30 min rapamycin application, indicating that removal of TACC3/ch-TOG/clathrin complexes by LCa rerouting did not alter the localization or function of other spindle proteins. Scale bar: 10 μm.

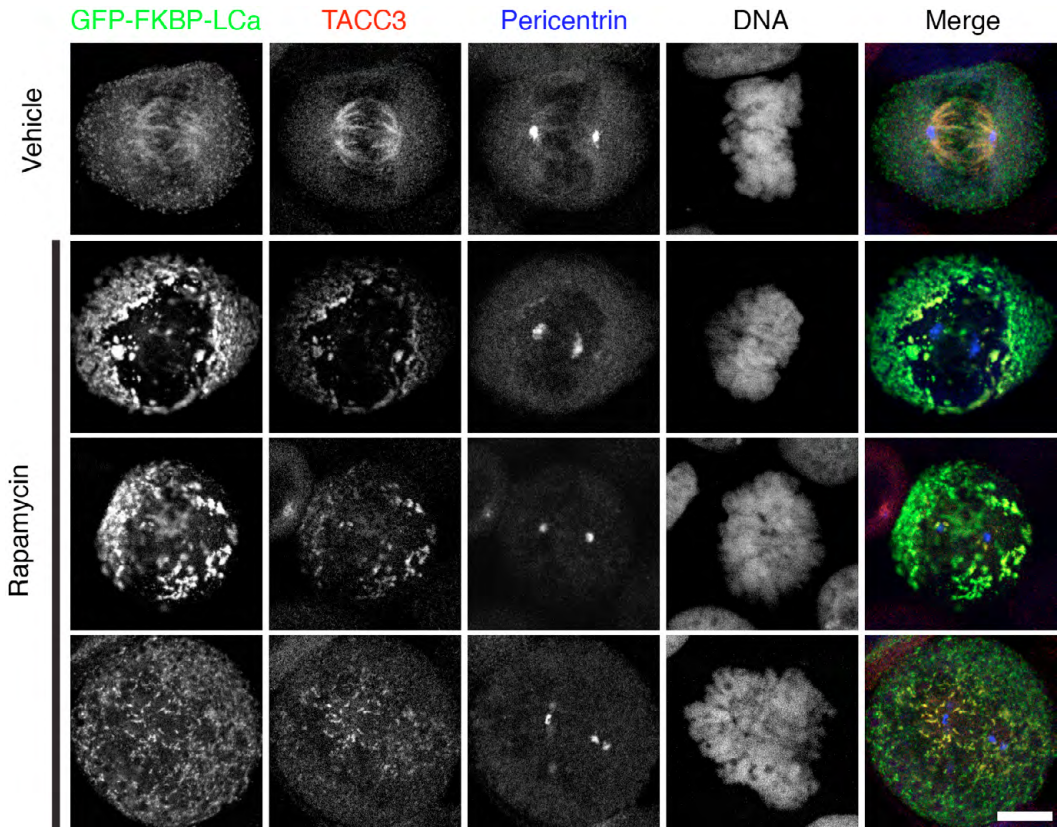


Fig. S3. No evidence for clathrin-independent TACC3 on spindle MTs or centrosomes. Representative confocal micrographs of a clathrin rerouting experiment (as shown in supplementary material Fig. S2). Treated cells were fixed and stained for TACC3 (red) and pericentrin (blue). Note the complete co-localization of clathrin and TACC3 and the lack of TACC3 on spindle MTs and centrosomes. Scale bar: 10 μm.

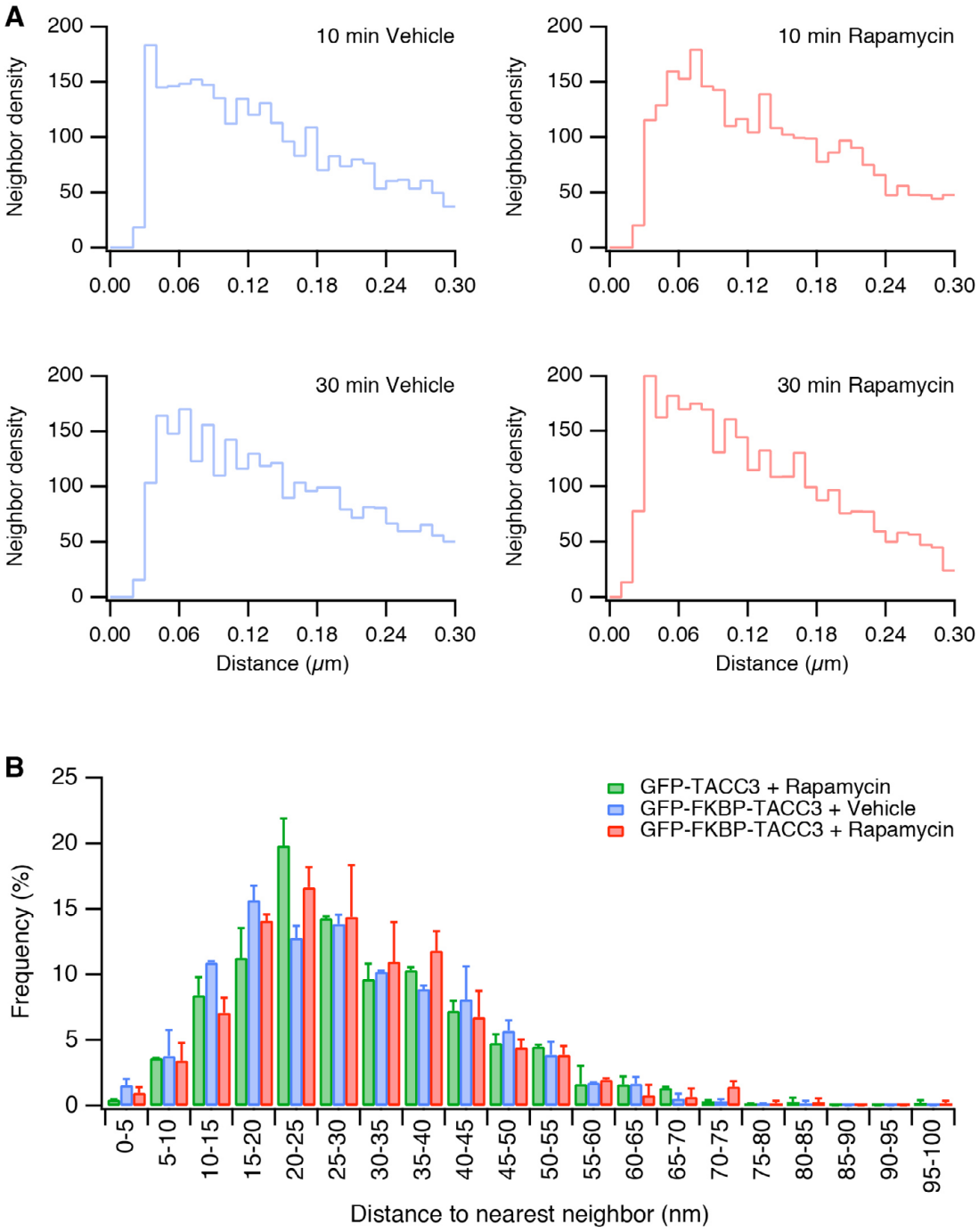
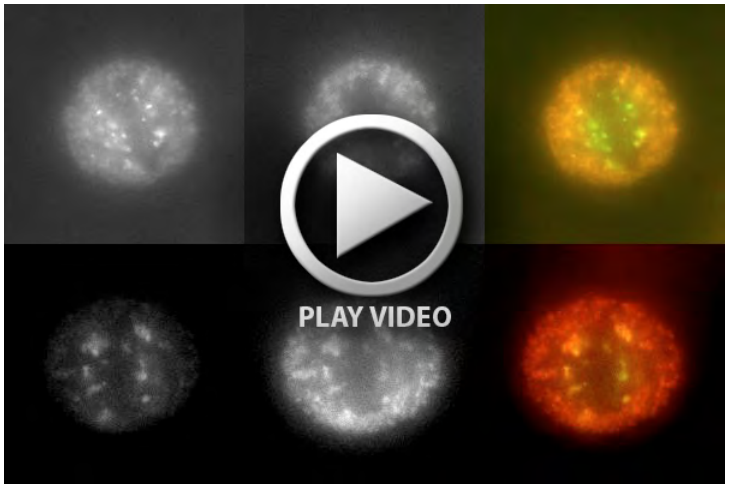
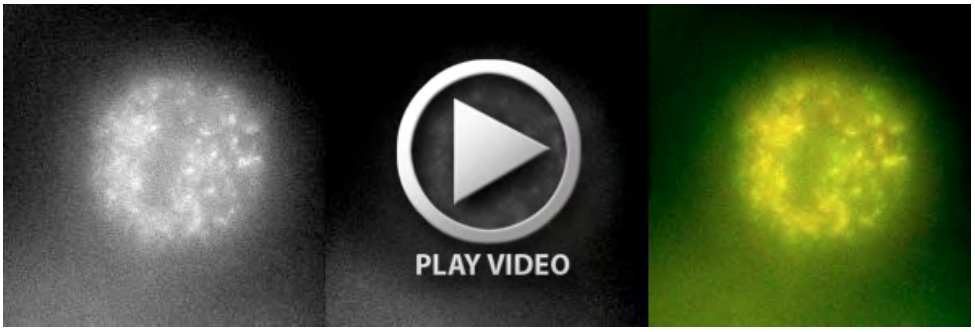


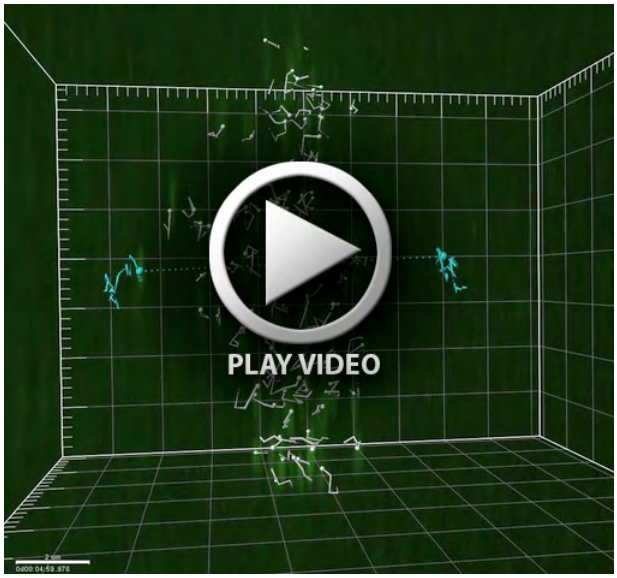
Fig. S4. TACC3 KS causes no change in the spatial organization of K-fibers. (**A**) Typical neighbor density analysis (nda) plots (McDonald et al., 1992) are shown for GFP-FKBP-TACC3 expressing K-fibers treated with vehicle or rapamycin for 10 or 30 min. These plots show the preferred spacing within a K-fiber and no obvious qualitative difference was seen following TACC3 KS. (**B**) Bar chart to show the mean±s.e.m. of the distance of each MT to its nearest neighboring microtubule.



Movie 1. Live-cell imaging of rapamycin-induced rerouting of TACC3 to mitochondria. Two mitotic TACC3-depleted HeLa cells expressing GFP-FKBP-TACC3 (left, green) and mCherry-MitoTrap (middle, red). Rapamycin (200 nM) is added part way through the movie. Movie plays at 30× real time. See Fig. 1B for still images of upper cell.



Movie 2. Live-cell imaging of rapamycin-induced rerouting of clathrin to mitochondria. Mitotic HeLa cell expressing GFP-FKBP-clathrin light chain a (LCa) and mCherry-MitoTrap. Rapamycin (200 nM) is added part way through the movie. Movie plays at 30× real time. Relates to supplementary material Fig. S2.



Movie 3. 3D kinetochore and spindle pole tracking. An example of a tracking experiment. Rapamycin-treated mCherry-TACC3 (control) TACC3-depleted HeLa cell stably expressing CENP-A-GFP and Centrin-GFP. Automated tracking of kinetochores (white) and spindle poles (cyan), outlier kinetochores that were rejected are shown in red. Relates to Fig. 8.