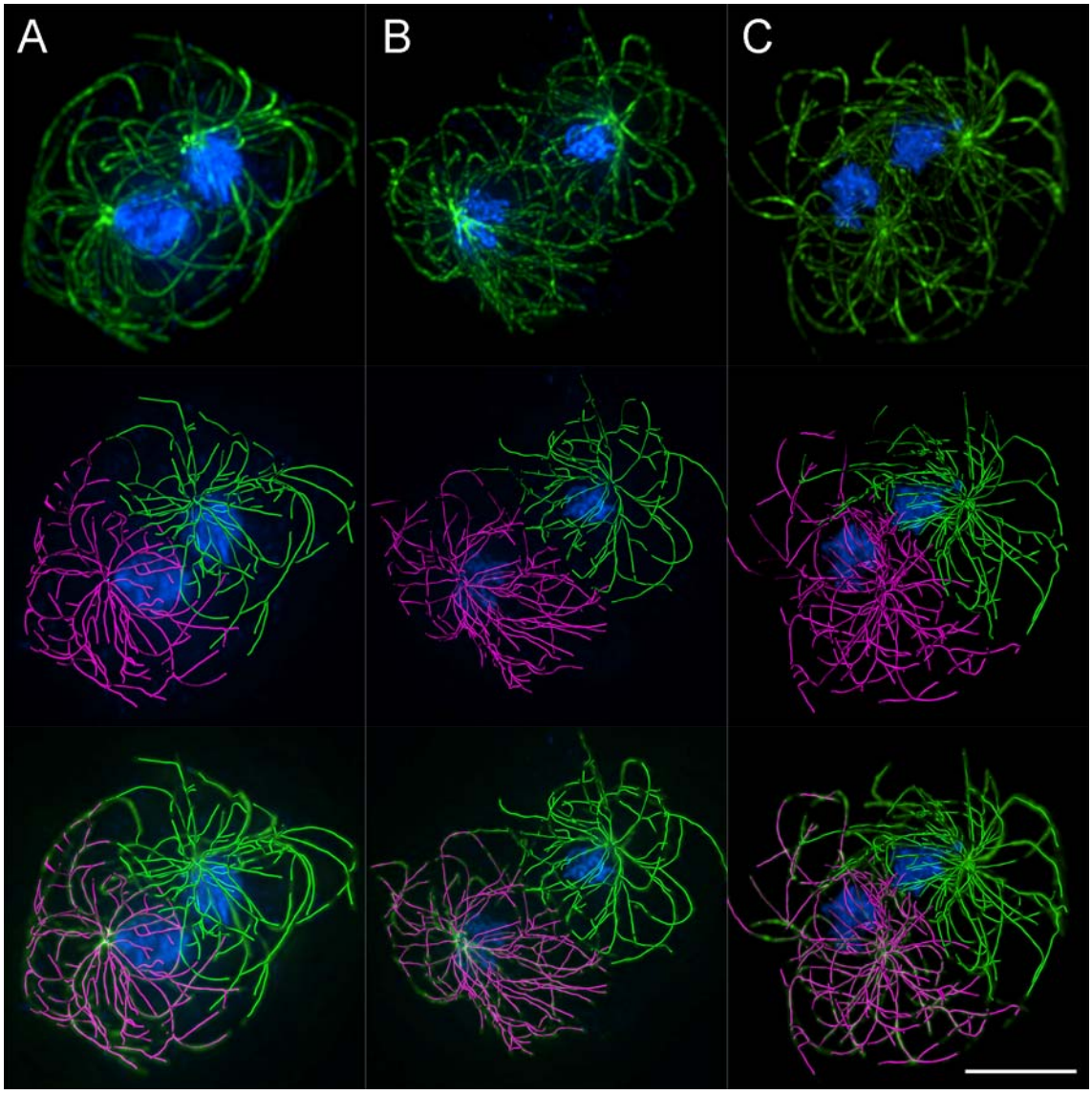
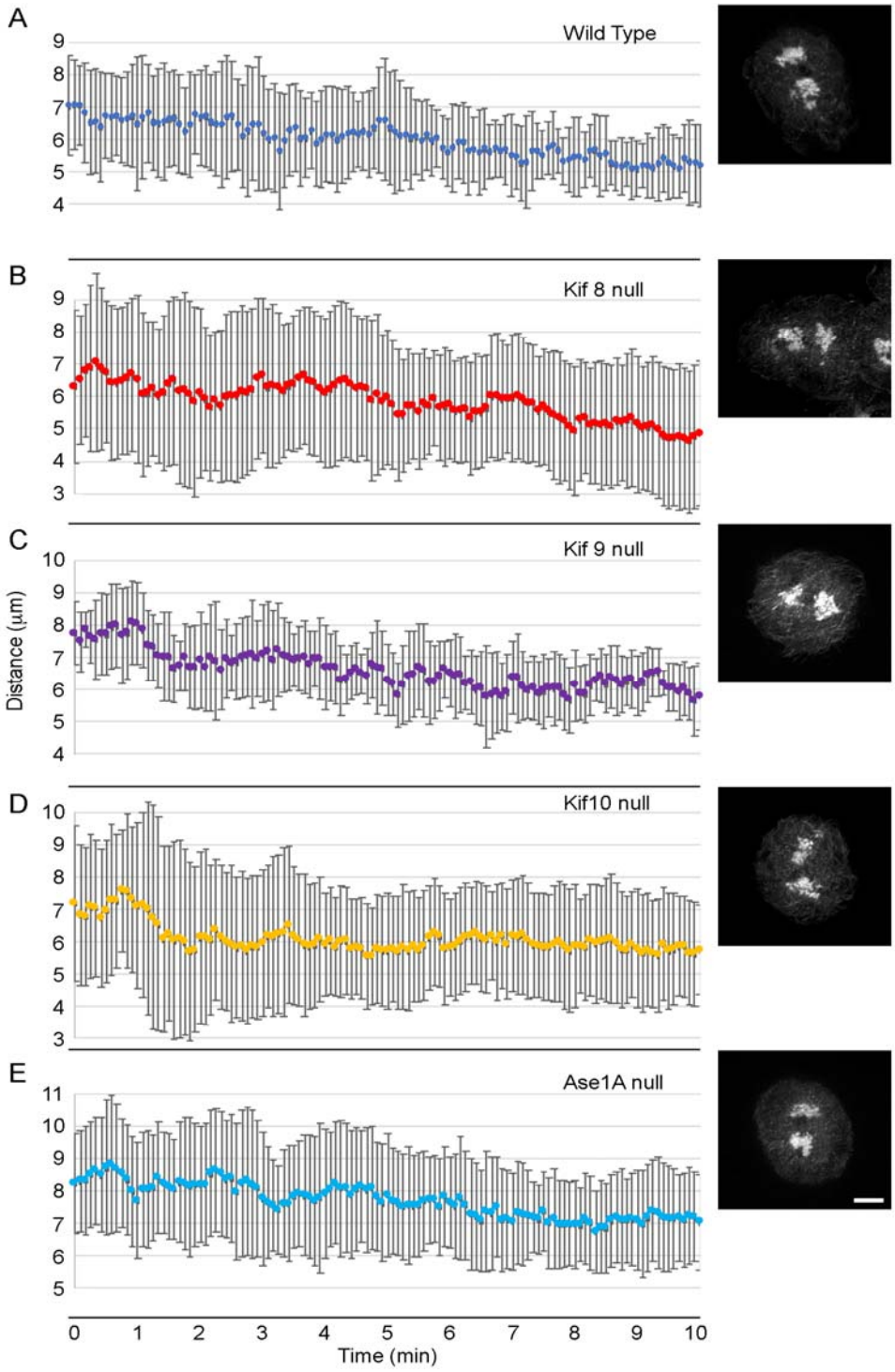
Supplemental Materials

Molecular Biology of the Cell

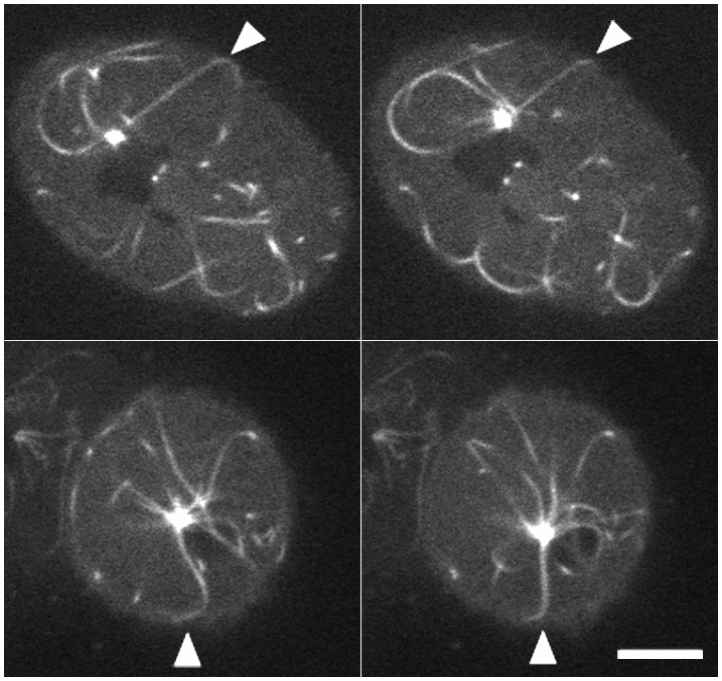
Odell et al.



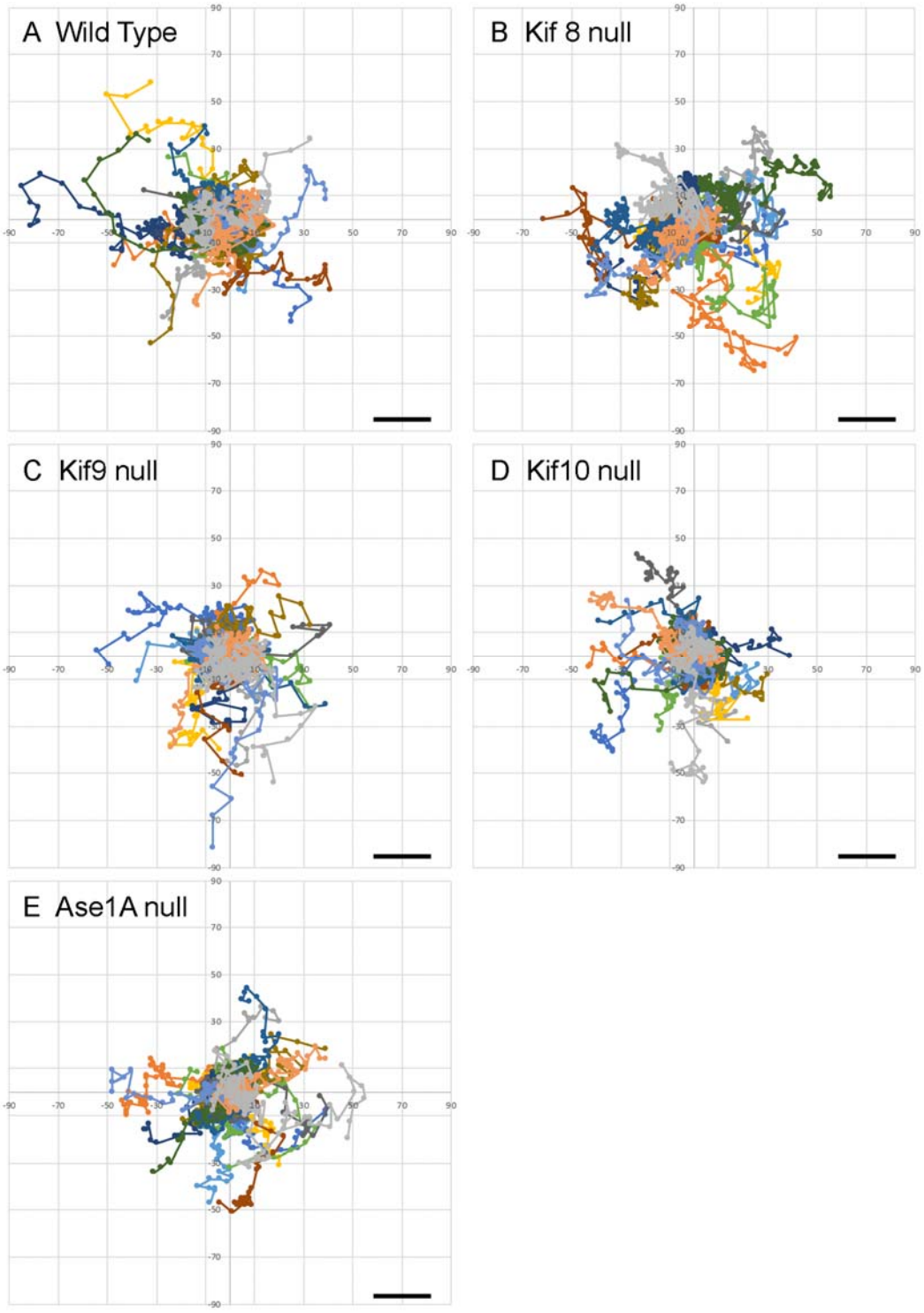
Supplemental Figure 1. MT tracking in binucleate cells. Automated tracking that shows the extent of coverage for each MT arrangement in three representative cells. A-C. The top row shows 2D maximum intensity projections of image stacks collected from cells stained with a tubulin antibody (green) and Hoechst label for DNA (blue). The middle row shows same view projections of the MT arrays tracked using the FilamentTracer module in Imaris software. MTs are color coded either magenta or green depending on their centrosomal origin. The bottom panel shows a merged view of the light microscopy and computational panels. No attempt was made to manually fill in obvious missing segments of the MTs. Even though the correspondence is not perfect, the tracing algorithm demonstrates there is minimal overlap between the MT arrays shown here. Rotation of the 3D volumes further show minimal obvious close contact between MT ends of opposite polarity. Scale bar = 5 μm.



Supplemental Figure 2. Cortical Force Transients. Two examples of MT-cortex engagements that generate a pulling force sufficient to move the centrosome toward the point marked by the arrowhead. Both sequences (frames are 10-s apart) are from DdKif8 null cells, the top row is during the initial period of movement to the cell center, the bottom row is from a different cell, during the oscillatory period after centering. Also note here that the MT ends extend beyond the point of force generation. Scale bar = 5 μm.



Supplemental Figure 3. Inter-centrosome dynamics in binucleate mutant cells. Average distance plus error bars (s.d.) for each of the mutant cell lines. Panel A duplicates the wild type data presented in Figure 1 and is shown here for reference (n=8 cells). Panels B - E show average distance over a 10 min time window in each of the four mutant cell lines (n=10 cells for each tracing). As in Figure 1, the light micrographs show representative 2D maximum intensity projections of centrosome positions within a single binucleate. Scale bar = 5 μm.



Supplemental Figure 4. Compilation of post ablation centrosome movements. In each panel, the 15 individual tracks for wild type and the four mutant cell lines summarized in figure 4 are color coded and superimposed. The start positions for each track are located on the periphery and the tracks converge toward the cell center. Each dot is separated by 5-s intervals. Scale bars = 2 μm.

Movie 1. Non-irradiated control binucleate WT cell demonstrating dynamics of centrosomes and interphase MTs. Frames recorded at 5-s intervals.

Movies 2, 3, 4. Examples of centrosome/MT array movement following ablation of one of two centrosomes in binucleate cells. Frames recorded at 5-s intervals.