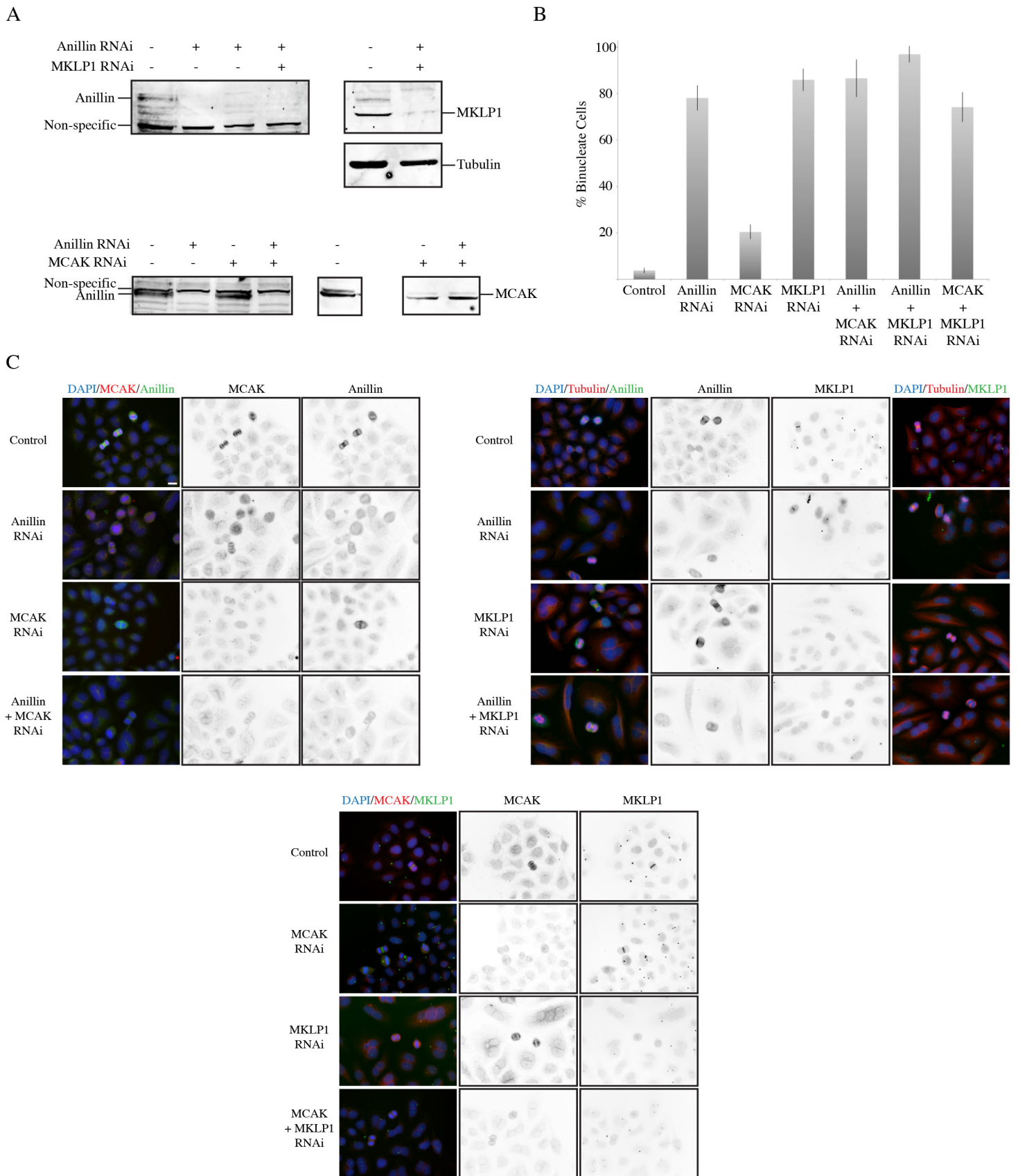
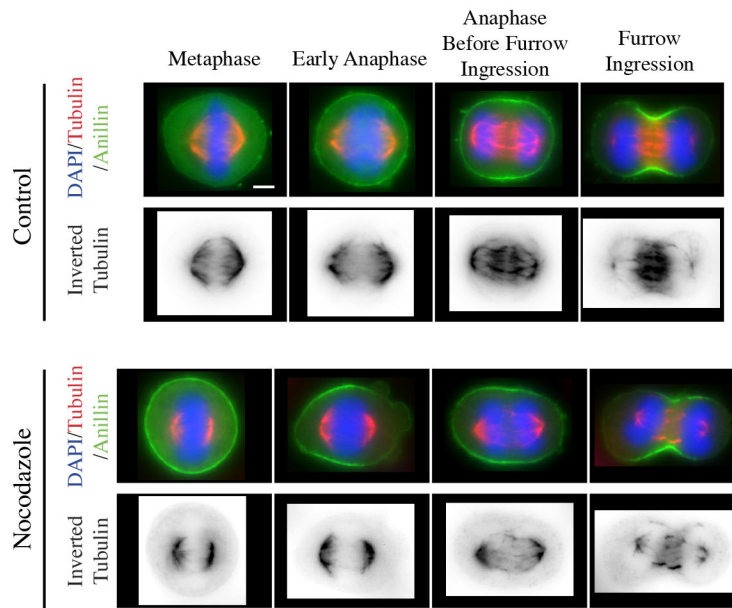


**Figure S1. The effect of various treatments on microtubules and the central spindle.** Fixed HeLa cells treated with MCAK RNAi, MKLPI RNAi, anillin RNAi, or double combinations stained for Tubulin, Incenp (stains central spindle) and DNA (DAPI). Cells at various stages of mitosis are shown as indicated. The scale bar is 10  $\mu$ m.

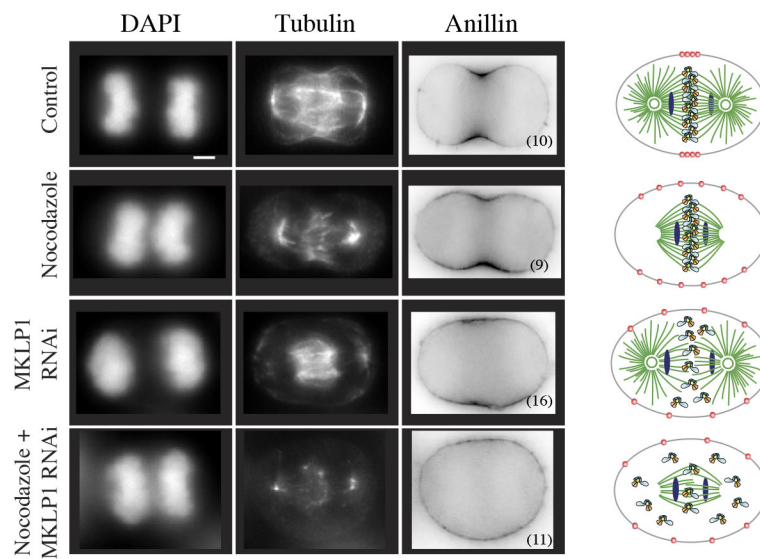


**Figure S2. Characterizing RNAi efficiency.** A) Western blots probed for anillin, tubulin, MCAK or MKLP1 using lysates collected from HeLa cells treated for 30-32 hours with siRNAs for anillin, MCAK, MKLP1 or double combinations. B) A bar graph shows the proportion of binucleate cells after RNAi treatments for 30-32 hours for each single and double RNAi combination used in this study. The bars show standard deviation. C) Fixed HeLa cells after depletion of anillin, MCAK, MKLP1 or double combinations for 30-32 hours, stained for anillin, MCAK, tubulin, and/or MKLP1 are shown. The scale bar is 30  $\mu$ m.

A

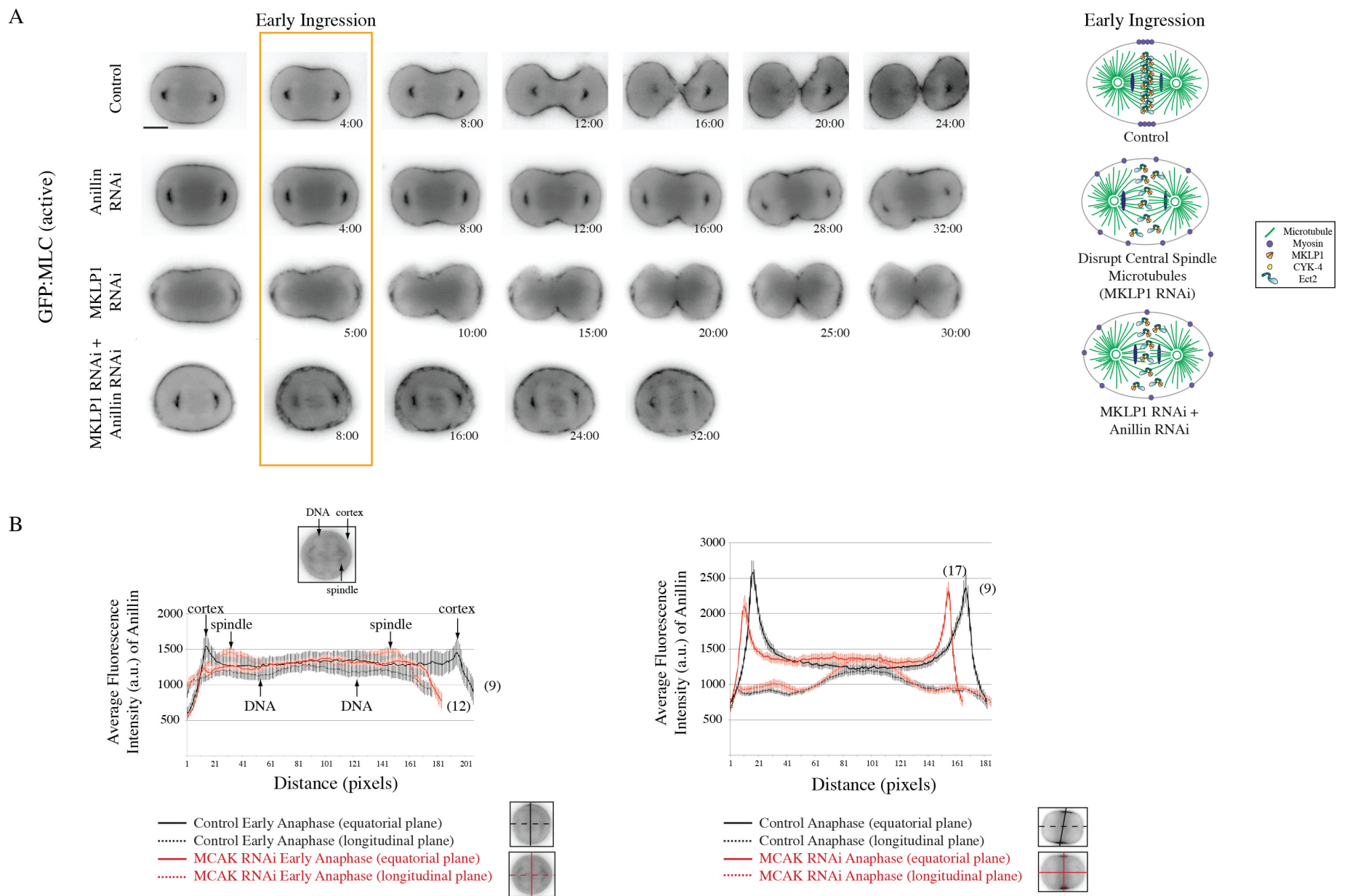


B



**Figure S3. Anillin localization is regulated by astral microtubules.** A) Fixed HeLa cells treated with nocodazole (30 ng/mL) stained for anillin, tubulin and DNA (DAPI). Cells at various mitotic stages are shown. The scale bar is 10  $\mu$ m. B) Fixed HeLa cells treated with nocodazole, MKLP1 RNAi, or both stained for anillin, tubulin and DAPI (n's shown in brackets). Cartoon schematics show the impact of the various treatments on anillin localization.





**Figure S4. Anillin is part of the astral pathway.** A) Images from control live HeLa cells stably expressing GFP:MLC (active) treated with anillin RNAi, MKLP1 RNAi or both are shown. Time points from anaphase onset are indicated. The scale bar is 10  $\mu$ m. Schematics show how myosin localization changes in the different RNAi conditions. B) Graphs show the average fluorescence intensity of anillin (a.u.) across the equatorial plane (solid line; distance in pixels; black for control cells and red for MCAK RNAi cells) and longitudinal plane (dashed line; distance in pixels; black for control cells and red for MCAK RNAi cells). The graph on the left shows early anaphase cells, and regions corresponding to the cortex, spindle and DNA are indicated on the graph and on the picture of the cell above. The graph on the right shows anaphase cells. Bars indicate S.E.M.