Supplemental Materials

Molecular Biology of the Cell

Gayek et al.

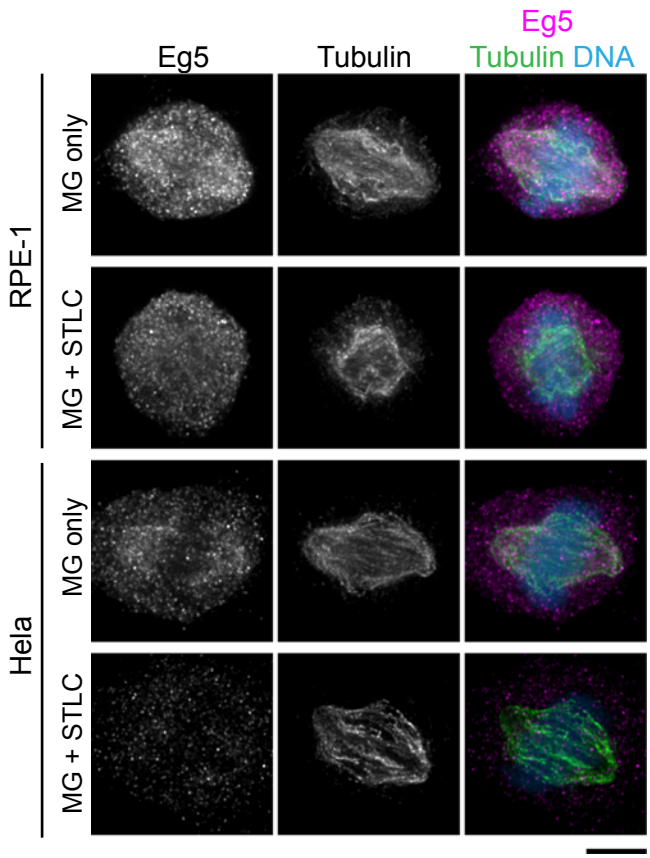

Figure S1. STLC displaces Eg5 from the spindle in RPE-1 and HeLa cells. Cells were arrested in mitosis with 5 μM MG-132 for 90 min, then treated with MG-132 with or without 10 uM STLC for 10 min. Eg5 (magenta) and tubulin (green) were detected by immunostaining. DNA (blue) was counterstained with Hoechst 33342. LUTs were scaled identically. Scale bar, 5 μm.

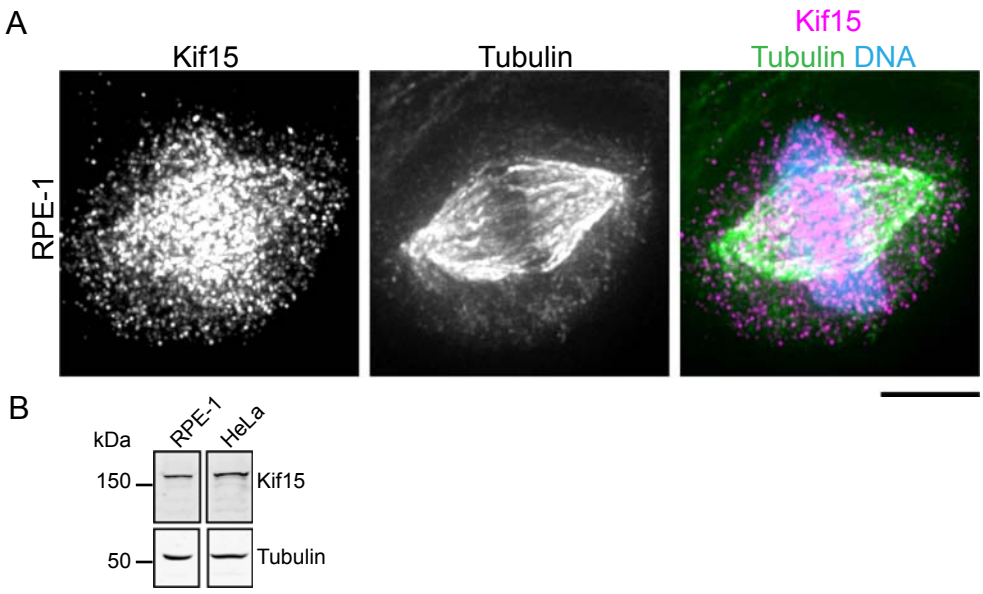

Figure S2. Kif15 expression and localization in RPE-1 cells. A) Kif15 binds the spindle in RPE-1 cells. Cells were treated with 5 μM MG-132 for 90 min. Kif15 (magenta) and tubulin (green) were detected by immunostaining. DNA (blue) was counterstained with Hoechst 33342. Scale bar, 5 μm. B) Immunoblots of Kif15 against mitotic lysates prepared from RPE-1 and HeLa cells. Tubulin is shown as a loading control.

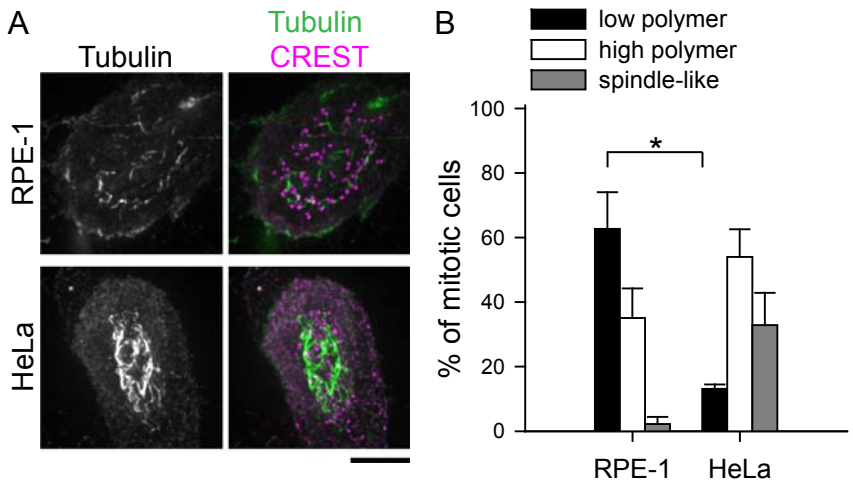

Figure S3. Cold treatment reveals differential K-MT stability in RPE-1 and HeLa cells. A) Images of RPE-1 or HeLa cells arrested in mitosis with 5 μM MG-132 for 90 min, then placed on ice with 5 uM MG-132 for 29 min, extracted on ice for 1 min, and fixed. Tubulin (green) and kinetochores (magenta; CREST) were detected by immunostaining. LUTs were scaled identically. Scale bar, 5 μm. B) Quantification of residual MT polymer levels following cold treatment. Puncta or short streaks were scored as "low polymer;" long streaks joined at nodes were scored as "high polymer;" long streaks joined in two poles were scored as "spindle-like." Data represent the mean ± SEM; n≥79 cells from 3 experiments. *p<0.05.

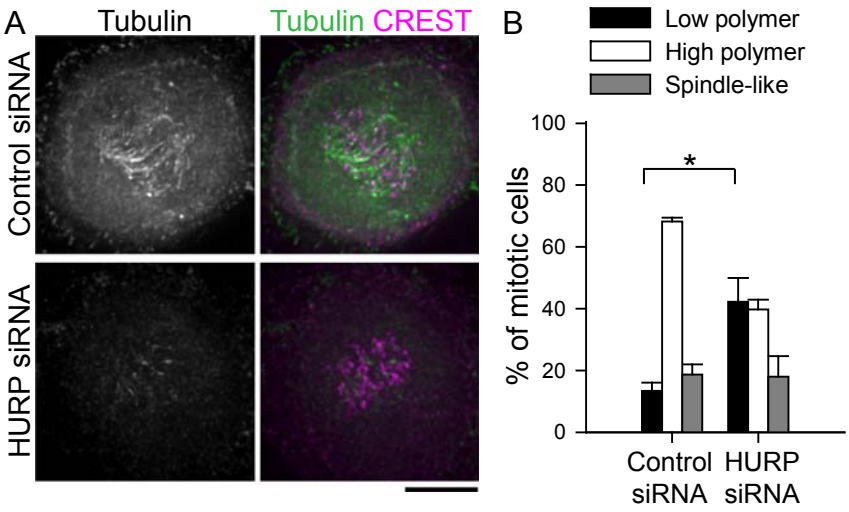

Figure S4. HURP depletion destabilizes K-MTs. A) HeLa cells transfected with control or HURP siRNAs were arrested in mitosis with 5 μM MG-132 for 90 min, then treated with 5 μM nocodazole for 6 min, extracted for 20 sec, and fixed. Tubulin (green) and kinetochores (magenta; CREST) were detected by immunostaining. LUTs were scaled identically. Scale bar, 5 μm. B) Quantification of residual MT polymer levels following nocodazole treatment. Puncta or short streaks were scored as "low polymer;" long streaks joined at nodes were scored as "high polymer;" long streaks joined in two poles were scored as "spindle-like." Data represent the mean ± SEM; n≥100 cells from at least 3 experiments. *p=0.052.

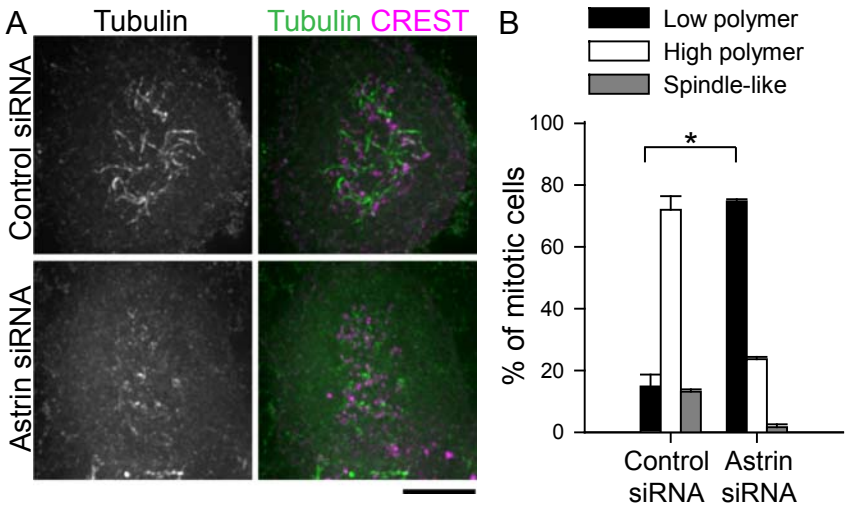

Figure S5. Astrin depletion destabilizes K-MTs. A) HeLa cells transfected with control or Astrin siRNA were arrested in mitosis with 5 μM MG-132 for 90 min, then treated with 5 μM nocodazole for 6 min, extracted for 20 sec, and fixed. Tubulin (green) and kinetochores (magenta; CREST) were detected by immunostaining. LUTs were scaled identically. Scale bar, 5 μm. B) Quantification of residual MT polymer levels following nocodazole treatment. Puncta or short streaks were scored as "low polymer;" long streaks joined at nodes were scored as "high polymer;" long streaks joined in two poles were scored as "spindle-like." Data represent the mean ± SEM; n≥100 cells from at least 3 experiments. *p<0.005.