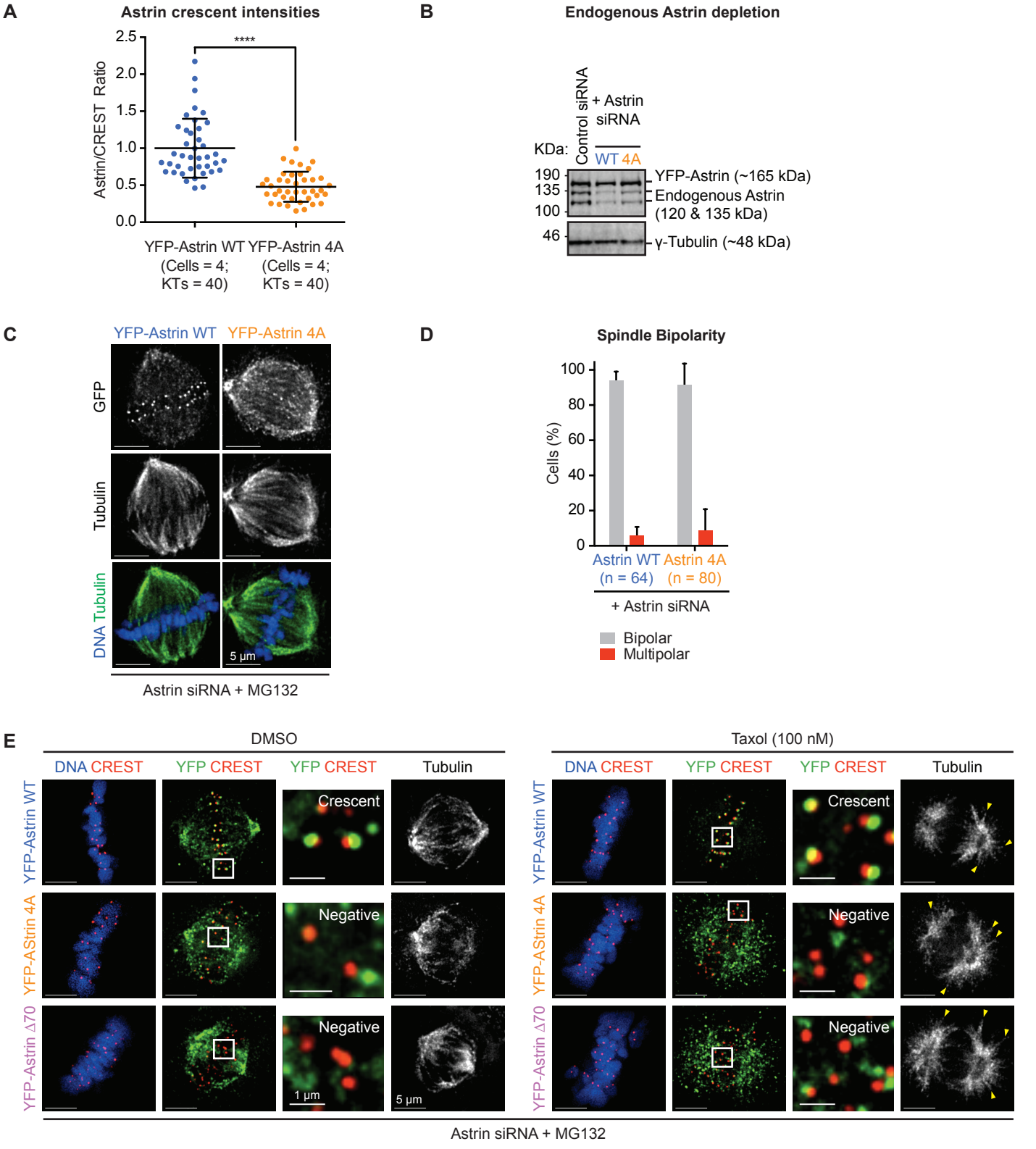

Figure 3 supplement 3. AAAA mutation in Astrin-Tail disrupts Astrin levels at kinetochores but not spindle bipolarity
(A) Graph shows normalised Astrin/CREST signal intensities in HeLa cells expressing YFP-Astrin WT or 4A mutant treated as in **Figure 3A**. Each dot represents value from one kinetochore. Black bars and whiskers mark average value and standard deviation, respectively, of kinetochore intensities across cells in a single experiment. "*" indicates statistically significant differences. **(B)** Immunoblots show the extent of endogenous Astrin protein depletion in cells treated with control or Astrin siRNA (as in **Figure 3C**) and harvested 14h after starting the live-cell imaging. For control siRNA condition, HeLa FRT-TO YFP-Astrin-4A cell line was used. Immunoblots were probed using antibodies against Astrin and γ-Tubulin (loading control). **(C)** Representative images show the rescue of spindle bipolarity defects in Astrin siRNA treated HeLa cells transiently expressing an siRNA resistant form of YFP-Astrin WT or 4A mutant, as indicated, following an hour of MG132 treatment. Cells were immunostained with antibodies against GFP and Tubulin and co-stained with DAPI for DNA. **(D)** Graph of percentage of mitotic cells treated as in **C** displaying either a bipolar or multipolar spindle. Bars and whiskers mark average value and standard deviation, respectively, across at least three experimental repeats. WT values are the same from **Figure 2E**. **(E)** Representative deconvolved images showing no changes in the kinetochore levels of YFP-Astrin (WT, 4A or Δ70 as indicated) in Astrin siRNA treated HeLa cells expressing exposed to Taxol or DMSO, as indicated. Taxol treatment was maintained for 15 minutes following an hour of MG132 treatment. Cells were fixed and immunostained with antibodies against YFP and CREST antisera and co-stained with DAPI for DNA. Yellow arrowheads highlight stabilised astral microtubule fibers.