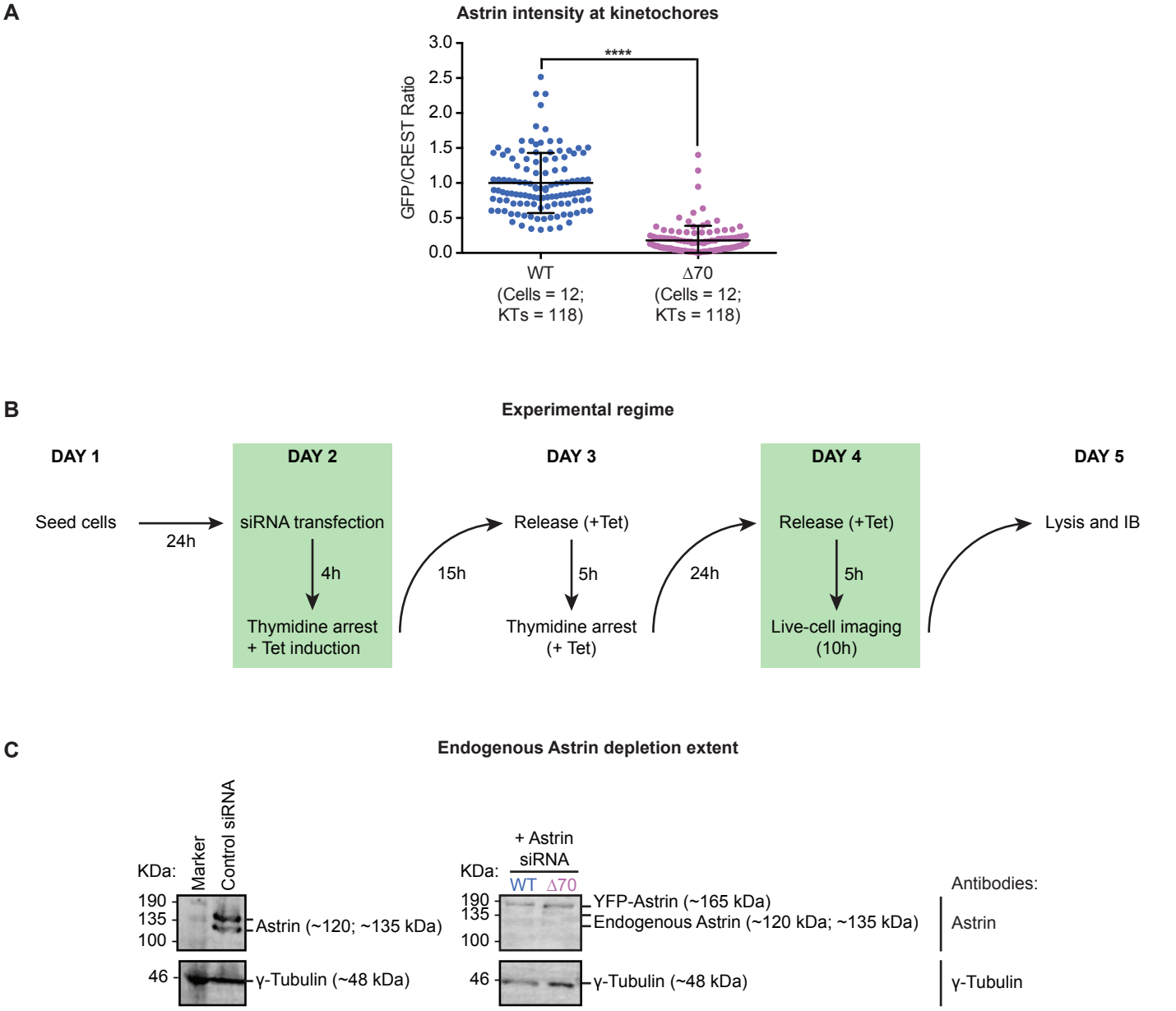

Figure 2 supplement 1. Depletion of endogenous Astrin and conditional expression of Astrin mutants
(A) Graph shows normalised Astrin/CREST signal intensities in HeLa cells expressing YFP-Astrin WT or Δ70 mutant. HeLa cells depleted of endogenous Astrin, expressing siRNA resistant YFP-Astrin (WT or Δ70) were arrested with MG132, immunostained with antibodies against GFP and HEC1 and CREST antisera and stained with DAPI for DNA. Each dot represents value from one kinetochore. Black bars and whiskers mark average value and standard deviation, respectively, of kinetochore intensities across cells in three independent repeats. "*" indicates statistically significant differences. **(B)** Experimental regime: HeLa FRT/TO cells expressing siRNA resistant YFP-Astrin (WT or Δ70 mutant) were treated with Control or Astrin siRNA and induced with Tetracycline containing media for 48 hours prior to imaging overnight for 10 hours (images in **Figure 2F**) and collecting lysates for immunoblots to assess the extent of endogenous Astrin depletion. Cells were treated with a double thymidine block to enrich for the mitotic population of cells at the time of imaging. **(C)** Immunoblots show the extent of endogenous Astrin protein depletion in cells as in **B**. For control siRNA condition, HeLa FRT/TO cell line was used. Immunoblots were probed using antibodies against Astrin and γ-Tubulin (loading control).