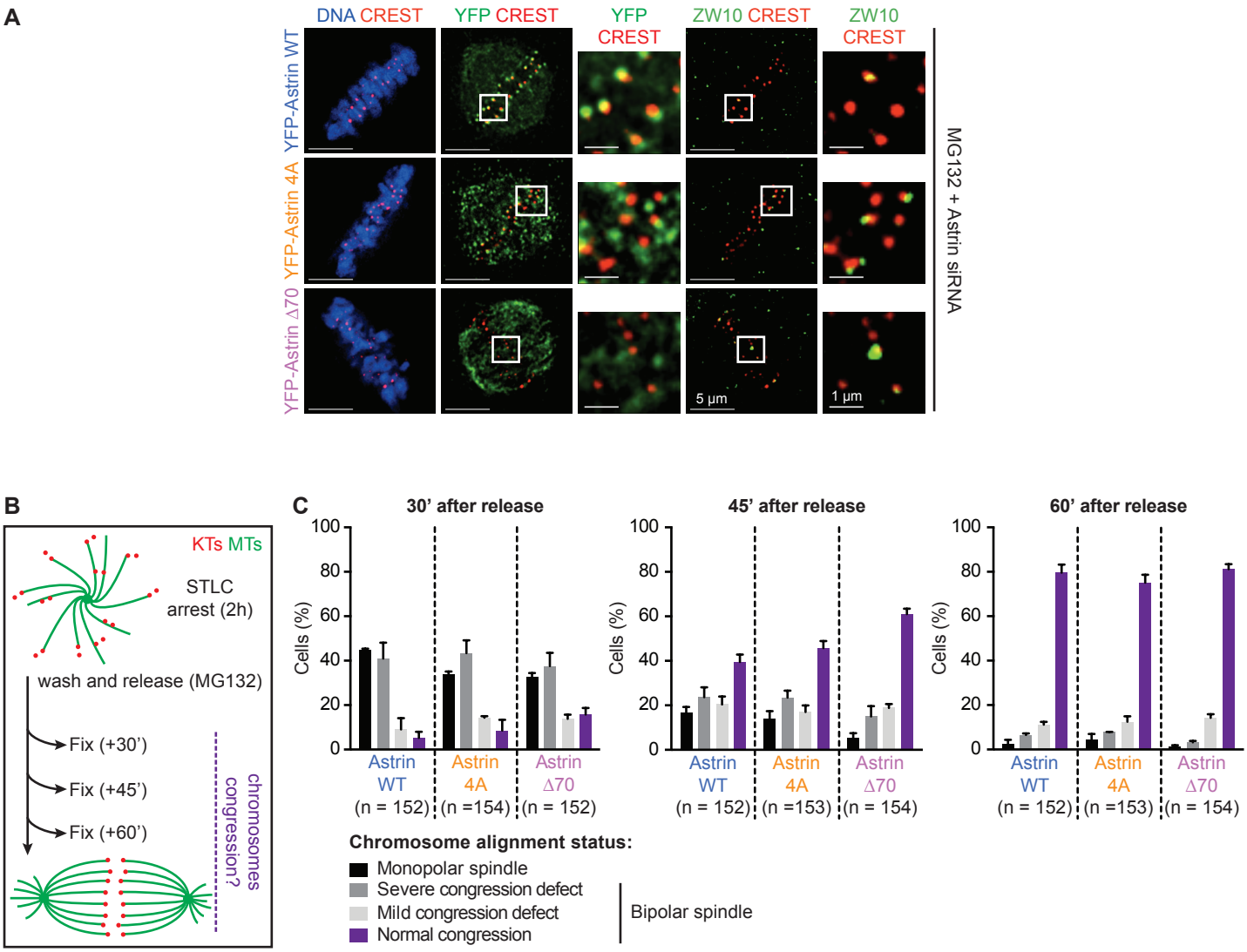

Figure 5 supplement 1. Checkpoint signalling and chromosome congression efficiency in Astrin:PP1 docking mutant expressing cells
(A) Representative images show ZW10 levels at kinetochores in HeLa cells depleted of endogenous Astrin and transiently expressing YFP-Astrin (WT, 4A or Δ70 mutant). Cells were arrested in metaphase with MG132 for 1h before fixation and were immunostained with antibodies against GFP, ZW10, CREST antiserum and stained with DAPI for DNA. Cropped images highlight ZW10 or YFP-Astrin levels at kinetochores identified using CREST antisera (a centromere marker). Scale as indicated. **(B)** Cartoon of experimental methodology to test chromosome congression efficiency in HeLa cells by first inducing monopolar spindles using STLC (Eg5 inhibitor) and then releasing them into MG132 (proteasome inhibitor) for measuring chromosome congression efficiency in bipolar spindles 30, 45 or 60 minutes after release from STLC treatment. Cells were fixed at different timepoints and processed for immunostaining using anti-tubulin antibody and CREST antisera (a centromere marker) and co-stained with DAPI for DNA. **(C)** Bar graphs show percentage of cells with fully aligned or congressed chromosomes (normal metaphase), 1-5 unaligned chromosomes (mild congression defect), more than 6 unaligned chromosomes (severe congression defect) or no obvious alignment despite release from monopolar spindles.