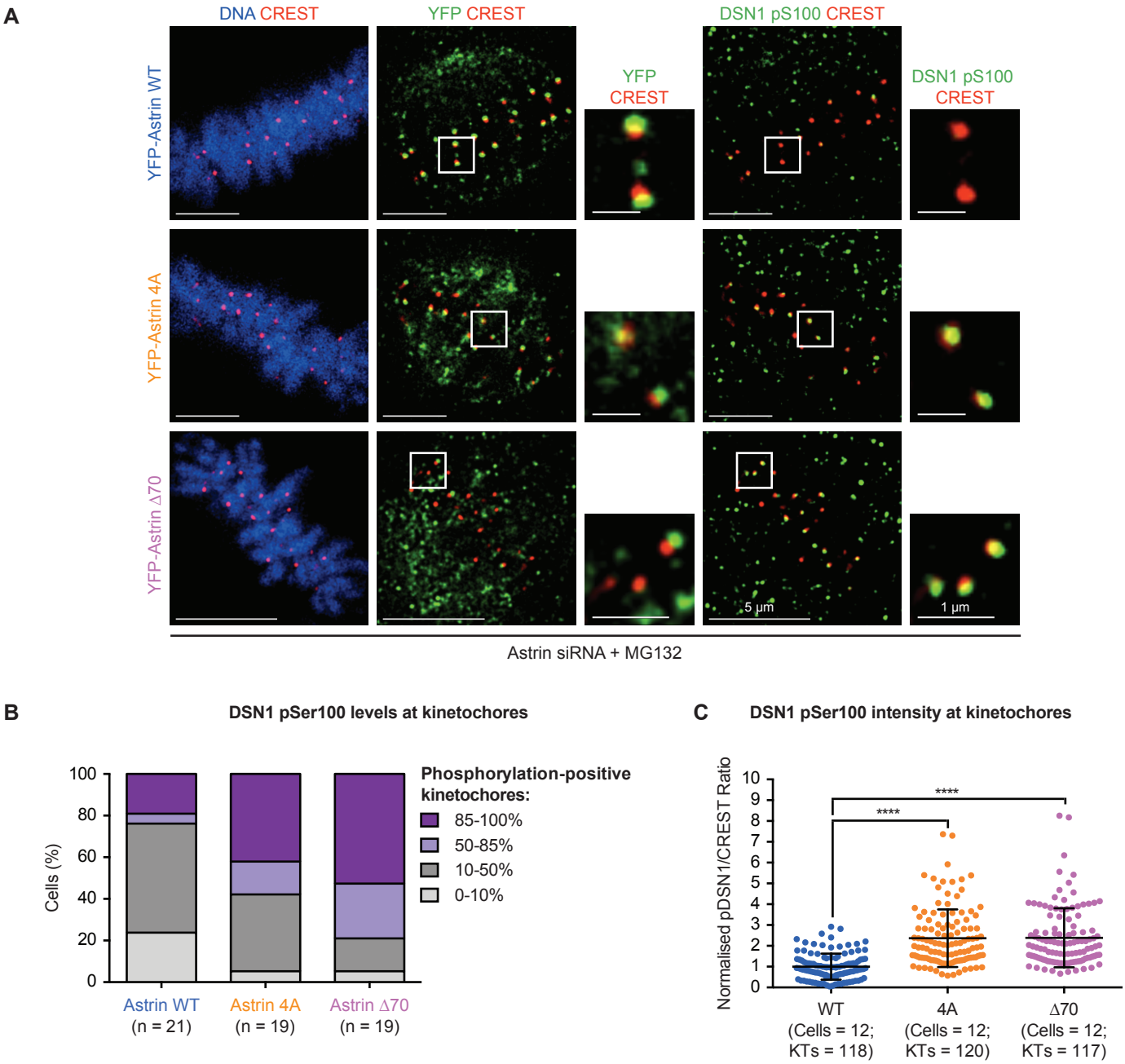

Figure 5 supplement 2. Status of DSN1 Ser100 phosphorylation in Astrin:PP1 docking mutant expressing cells
(A) Representative images show levels of phosphorylated DSN1 Ser100 levels at kinetochores in HeLa cells depleted of endogenous Astrin and transiently expressing YFP-Astrin (WT, 4A or Δ70 mutant). Cells were arrested in metaphase with MG132 for 1h before fixation and were immunostained with antibodies against GFP, DSN1 pSer100, CREST antiserum and stained with DAPI for DNA. Cropped images highlight DSN1 pSer100 or YFP-Astrin levels at kinetochores identified using CREST antisera (a centromere marker). Scale as indicated. **(B)** Bar graphs show percentage of cells with phospho-DSN1 positive kinetochores in 85-100%, 50-85%, 10-50% or 0-10% of metaphase kinetochores in cells treated with MG132 and expressing Astrin WT or mutants as indicated. Number of cells indicated. Data represents values from at least three experimental repeats. **(C)** Scatter plot of the integrated fluorescence intensity ratio of DSN1 pSer100 and CREST signals at each kinetochore. 9-10 KTs/cell were randomly picked for intensity measurements. Black bars and whiskers mark average value and standard deviation, respectively, of kinetochore intensities across cells in three independent repeats. "*" indicates statistically significant differences.