



Figure 6 supplement 3. SKA3 localisation is not abrogated due to the lack of Astrin

(A) Representative deconvolved images show SKA3 enrichment at kinetochores of HeLa cells treated with Control or Astrin siRNA. Cells were arrested in metaphase using MG132 for 1h prior to fixation and were immunostained with antibodies against Astrin and SKA3 and CREST antiserum (to mark centromeres) and stained with DAPI for DNA. Cropped images show Astrin or SKA3 enrichment at kinetochores. **(B)** Representative images show SKA3 enrichment at kinetochores of HeLa cells treated with Astrin siRNA before transfection with eukaryotic protein expression vectors encoding YFP-Astrin (WT, 4A or $\Delta 70$, as indicated). Cells were arrested in metaphase using MG132 for 1h prior to fixation and were immunostained with antibodies against GFP (for detecting YFP-Astrin) and SKA3 and CREST antiserum and co-stained with DAPI for DNA. Cropped images show YFP-Astrin or SKA3 enrichment at kinetochores. Scale as indicated.