



**Figure 4 supplement 1. Astrin purifies with GST-PP1<sub>γ</sub>**  
(A) Methodology: Control or Astrin siRNA treated cells were exposed to STLC. STLC treated HeLa cell lysates (WCL) were cleared by centrifugation (Input lysate) and incubated with either GST-PP1<sub>γ</sub> or GST immobilised on Glutathione beads or Glutathione beads alone. All beads were washed four times. Precipitates bound to beads (Pull-down samples) were assessed using immunoblotting. Unbound supernatant from all wash steps were stored. Only supernatants exposed to GST-PP1<sub>γ</sub> immobilised beads are shown (right). (B) Blot includes area presented in **Figure 4D**. Immunoblot is representative of two independent repeats and was probed using an anti-Astrin antibody. Yellow boxes mark area used for measuring Astrin intensities. (C) Immunoblot shows the specificity of anti-Astrin antibody in recognising mitotic forms of Astrin. STLC treated and mitotically enriched lysates of cells treated with Astrin or Control siRNA were used. Lysates of unsynchronised cells were used as interphase control. In mitotic cell lysates, three forms of Astrin are clearly separated (filled green triangles): in addition to the two forms (~120 kDa and ~135 kDa) found in interphase lysates, higher molecular weight forms (>135 kDa) are present, which are all lost following Astrin siRNA treatment. Red asterisk refers to a nonspecific band found in Astrin siRNA treated and untreated mitotic and interphase lysates. PP1 was used as a loading control.