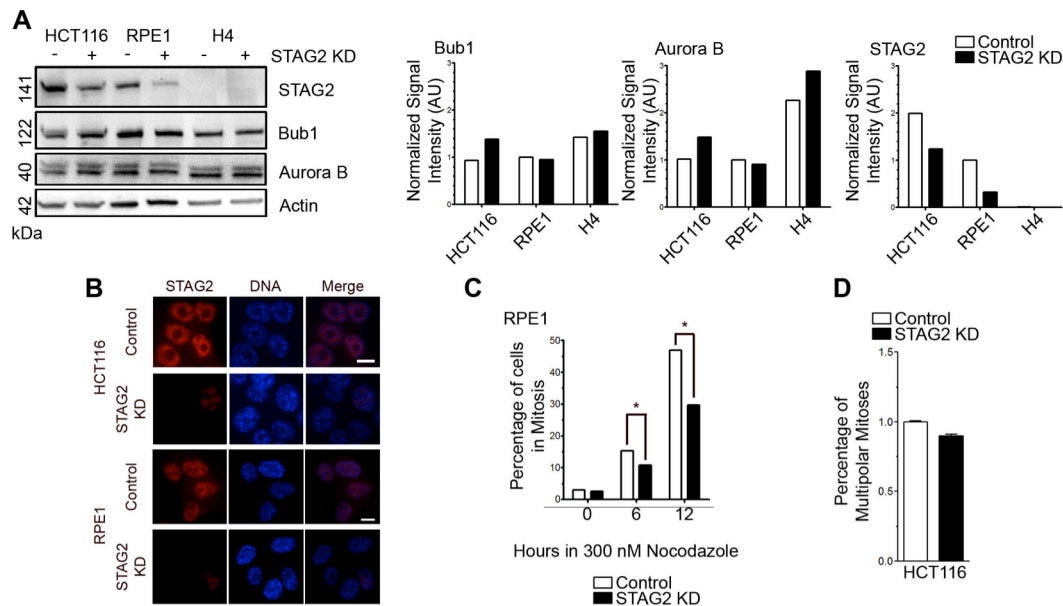


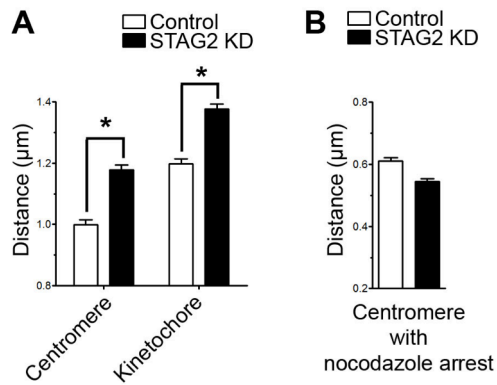
## Supplemental Information

### Supplementary Figure 1.



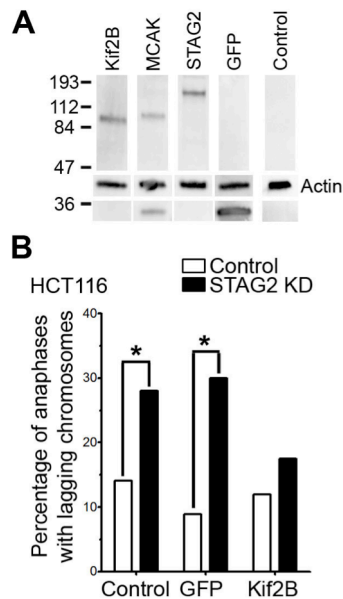
**Figure S1.** (A) Immunoblot of asynchronous cells 48 hours after STAG2 KD in the four cell lines analyzed. Graphs are normalized to actin loading control levels. (B) STAG2 staining of cells 48h post-transfection with STAG2-specific siRNA. Scale bar = 10  $\mu$ m. (C) Mitotic index of RPE1 cells with STAG2 KD in 300 nM nocodazole after 0, 6 or 12 hours of arrest measured using HOECHST and centromere co-staining. \* $p \leq 0.0001$  using Chi-squared test. (D) Percentage of multipolar spindles in HCT116 cells that were untreated (Control) or transfected with siRNA specific to STAG2 (STAG2 KD) and measured by HOECHST, centromere, and tubulin co-staining. N = 200 cells.

## Supplementary Figure 2.



**Figure S2.** (A) Sister centromere (via human anti-centromere serum) and kinetochores (via Hec1 staining) distances in RPE1 cells that were untreated (Control) or transfected with siRNA specific to STAG2 (STAG2 KD). N = 200 centromeres, \* $p \leq 0.001$ . (B) Sister centromere (via human anti-centromere serum) and kinetochores (via Hec1 staining) in RPE1 cells with STAG2 depleted cells treated with 300 nM nocodazole for 2 hours. N = 100 centromeres.

### Supplementary Figure 3.



**Figure S3.** (A) Immunoblot using antibodies specific for GFP or actin of total cell lysates prepared from asynchronous H4 clones overexpressing their respective GFP constructs. The small GFP-positive protein observed in the GFP-MCAK clone is likely from the breakdown of the GFP-MCAK fusion protein. (B) Lagging chromosomes in anaphase of HCT116 cells that were untreated (Control) or transfected with siRNA specific to STAG2 (STAG2 KD) and then un-transfected (Control) or transfected with plasmids to overexpress either GFP alone (GFP) or GFP-Kif2B (Kif2b). N ≥ 50 cells, \*p ≤ 0.05.