Supplementary Information

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Fig. S1

A. Different fragments of hSAS-6 as preys with various portions of hSAS-6 (prey) in a yeast two-hybrid assay. The positive interaction between hSAS-6 and CEP135 was evidenced by the growth of mating colonies on DQO plates (SD minimal medium, -Trp, -Leu, -Ade, and -His) and the activation of beta-galactosidase activity. P53 (bait) and SV40-large T-Ag (prey) were used as a positive control.

B. Fine mapping the CEP135-interacting domain in hSAS-6. HEK293T cells were transfected with various myc-tagged hSAS-6 constructs, and IP was performed using an anti-CEP135 antibody. Here, a different batch of secondary antibody was used to avoid the detection of a non-specific band shown in Figure 2D.
Figure S2. The recruitment of hSAS-6 to the centriole appears to be weakly or not affected by CEP135 depletion. Because hSAS-6 is absent from G1 cells, we arrested cells at early S phase with aphidicolin to ensure the detection of hSAS-6 after siCEP135 treatment. U2OS-based EGFP-centrin-expressing cells (A, C) or U2OS cells (B, D) were transfected with siCEP135 (A, B) or sihSAS-6 (C, D), and 72 hr after transfection, the cells were synchronized at early S phase by aphidicolin treatment for another day. The cells were then analyzed by confocal microscopy using the indicated antibodies (A, C). Quantification of centriolar signals of hSAS-6 (B) or CEP135 (D) in the siRNA-treated U2OS cells. Bar values are the means +/- s.d (n=100 cells) of three independent experiments.
Figure S3. CEP135 depletion inhibits PLK4-induced centriole amplification. U2OS cells were transfected with siControl or siCEP135 for 2 days, and then co-transfected with mCherry-PLK4 with or without siRNA-resistant CEP135-R-myc for another 2 days. The cells were then analyzed by immunofluorescent confocal microscopy (A) and immunoblotting (B) using the indicated antibodies. (C) Histogram illustrating the percentages of cells showing the various centriole numbers (n=100 cells in triplicate). (D) A protocol showing the timing and procedures of siRNA treatment and rescue experiments.
Figure S4. Mapping the interacting domains of CEP135 and CPAP. (A) CEP135, CPAP, and hSAS-6 may form a complex in cells. Full-length recombinant GST-hSAS-6 or GST-CPAP (895-1338) were used to pulldown the indicated proteins from HEK 293T cell lysates, and the precipitates were analyzed by immunoblotting. (B) GST-CEP135 (1-460) directly interacts with CPAP. Full-length 35S-methionine-labeled CPAP proteins were incubated with bead-bound GST or recombinant GST-CEP135 and analyzed by SDS-PAGE and autoradiography. (C) GST-CPAP (895-1338) directly binds to CEP135-His (1-460), but not to CEP135-His (650-1140). (D) GST-CPAP (895-1338) directly interacts with full-length 35S-methionine-labelled CEP135 proteins. Asterisks indicate the major purified proteins used for GST pulldown assays.