Zyss et al., http://www.jcb.org/cgi/content/full/jcb.201106025/DC1

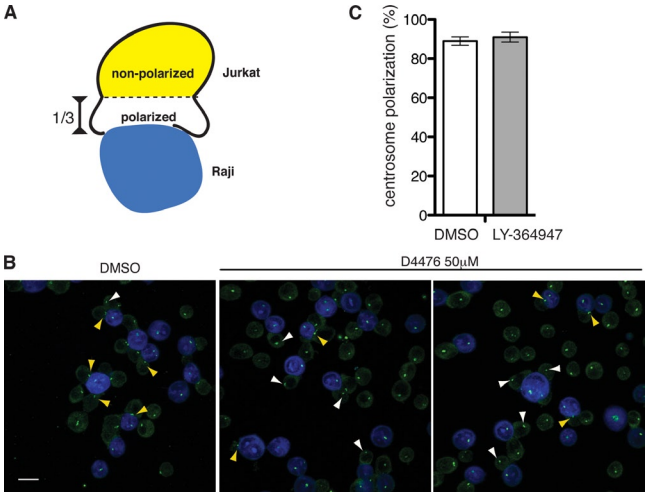


Figure S1. **CKI inhibition blocks centrosome polarization.** (A) The schematic shows the criterion used to quantify centrosome polarization to the IS. (B) Fields of Jurkat cells treated with DMSO or 50 μM D4476 (for 1 h) and conjugated with SEE-pulsed Raji cells represent examples of images used to generate quantitative data in Fig. 1 A. Centrosomes were stained with anti–γ-tubulin antibodies. Raji cells are shown in blue. White and yellow arrowheads mark arbitrary examples for nonpolarized and polarized centrosomes, respectively. Bar, 10 μm. (C) In addition to CKI, D4476 also inhibits activin receptor–like kinase ALK5 (also known as TGF-β type I receptor). Jurkat cells were treated with the ALK5 inhibitor LY-364947 (at 50 μM for 2 h) before conjugate formation with SEE-pulsed Raji cells and scored for centrosome polarization to the IS (n = 3 independent experiments; 200 conjugates were scored per experiment). Error bars are mean ± SD.

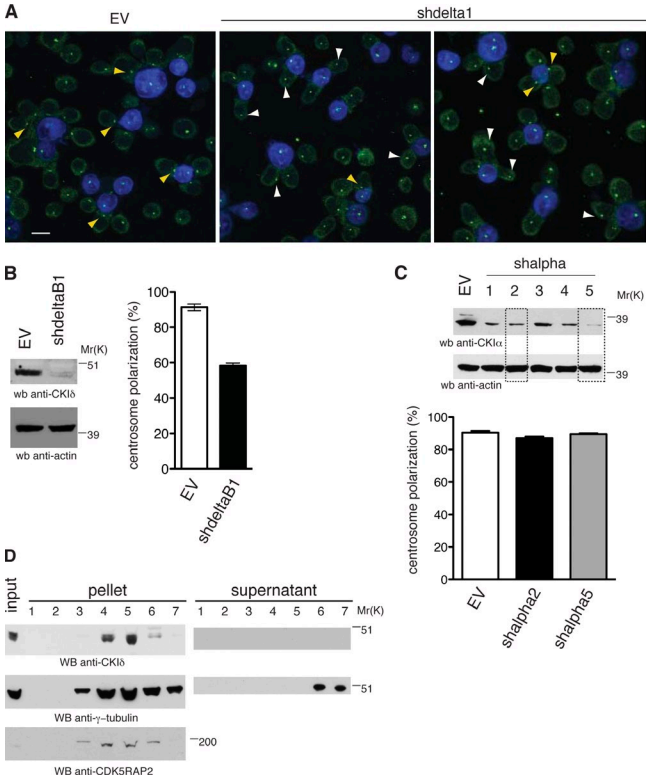


Figure S2. **CKIδ, but not CKIα, is required for centrosome polarization to the IS.** (A) Fields of conjugates between control (EV) or CKIδ-depleted (shdelta1) Jurkat and SEE-pulsed Raji cells represent examples of images used to generate quantitative data in Fig. 1 D. Centrosomes were stained with anti–γ-tubulin antibodies. Raji cells are shown in blue. White and yellow arrowheads mark arbitrary examples of nonpolarized and polarized centrosomes, respectively. Bar, 10 µm. (B) Cytoplasmic cell extracts of Jurkat cells containing stably integrated EV (control) or vector encoding the *CSNK1D*-specific shRNA shdeltaB were immunoblotted with the indicated antibodies. shdeltaB shRNA achieved an 80% depletion in clone shdeltaB1. Actin serves as a loading control. Control (EV) or shdeltaB1 Jurkat cells were conjugated to SEE-pulsed Raji cells and scored for centrosome polarization to the IS (three independent experiments; 200 conjugates were scored per experiment). Error bars are mean ± SD. wb, Western blotting. (C) Cytoplasmic cell extracts of Jurkat cells containing stably integrated EV (control) or vector encoding *CSNK1A*-specific shRNA were immunoblotted with the indicated antibodies. shalpha shRNA achieved a 73 and 81% depletion in clones shalpha2 and shalpha5, respectively, as detected by anti-CKIα antibody. Actin serves as a loading control. Control (EV) or CKIα-depleted (clones shalpha2 and shalpha5) Jurkat cells were conjugated to SEE-pulsed Raji cells and scored for centrosome polarization to the IS ($n = 3$ independent experiments; 200 conjugates were scored per experiment). Dotted rectangles on Western blots correspond to the clones scored for centrosome polarization. (D) CKIδ cosediments with centrosomal proteins γ-tubulin and CDK5RAP2 during centrosome purification, indicating that it is an integral centrosomal protein in Jurkat cells.

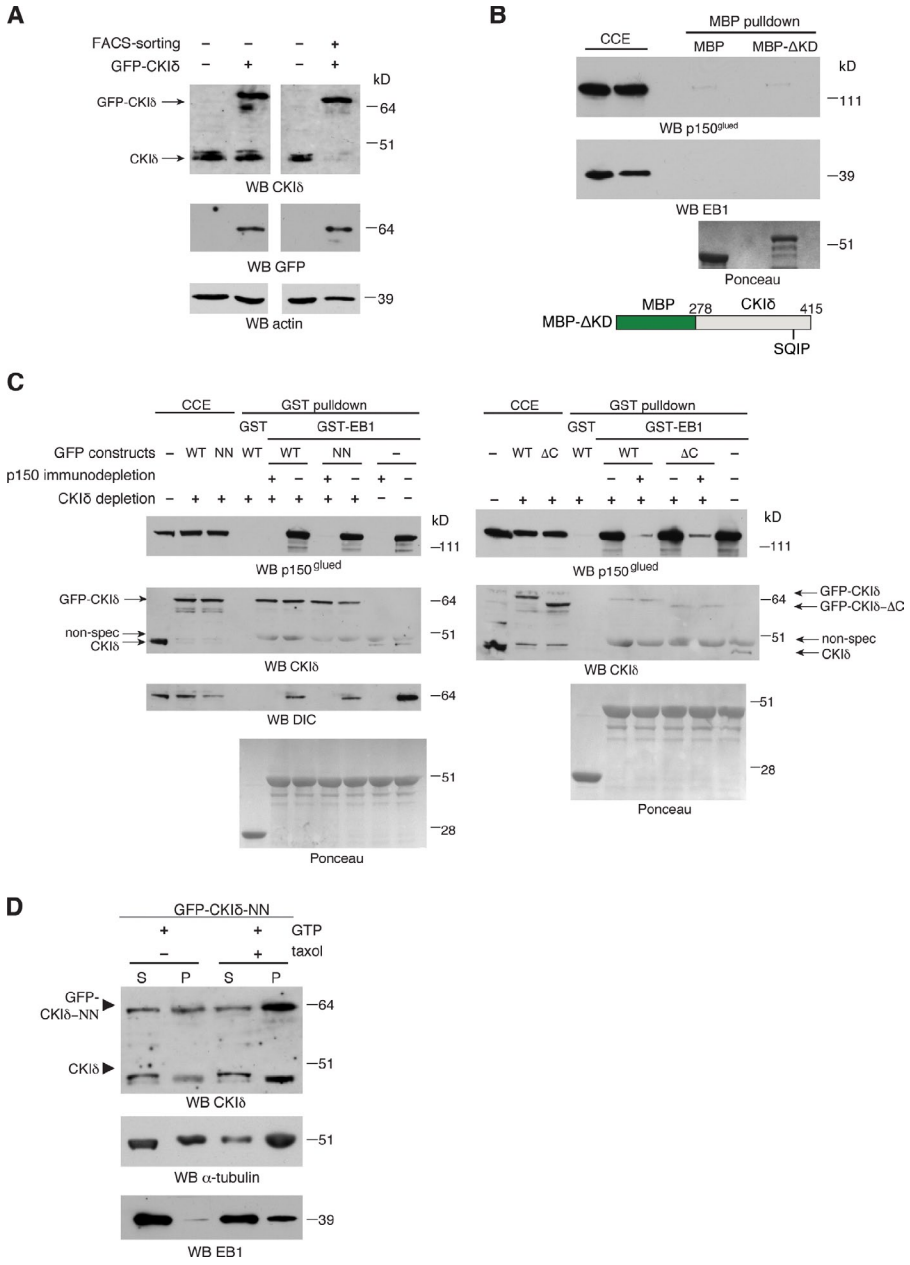


Figure S3. **CKIδ lacking the SQIP domain still interacts with EB1.** (A) Cytoplasmic cell extracts from Jurkat cells transiently transfected with GFP-CKIδ are shown. GFP-positive cells were selected by fluorescence-activated cell sorting (right) and analyzed by immunoblotting with CKIδ antibodies. Note the marked decrease in endogenous CKIδ levels in sorted GFP-positive populations. WB, Western blotting. (B) Cytoplasmic cell extracts (CCE) of Jurkat cells were subjected to pull-down assays with MBP or MBP-ΔKD and immunoblotted with antibodies as indicated. Recombinant MBP products are visible in Ponceau staining below. (C) Cytoplasmic cell extracts of EV or CKIδ-depleted Jurkat cells transiently transfected with GFP-CKIδ (wild type [WT]) and GFP-CKIδ–NN (NN; left) or with GFP-CKIδ (wild type) and GFP-CKIδ–ΔC (ΔC;right) were mock depleted with random IgG (−) or immunodepleted of $p150^{glued}$ (+). Depleted extracts were subjected to pull-down assays with GST or GST-EB1 (EB1) and immunoblotted with antibodies as indicated. Recombinant GST products are visible in Ponceau staining below. Note that a band the size of GST-EB1 appears as a nonspecific (non-spec) band in all GST pull-downs, probably as a result of the high concentrations. (D) In vitro microtubule-pelleting assay. Pure tubulin was incubated in the presence of GTP without (−) or with (+) taxol before being added to cytoplasmic extracts of Jurkat cells transiently transfected with GFP-CKIδ–NN. P, pellet; S, supernatant.

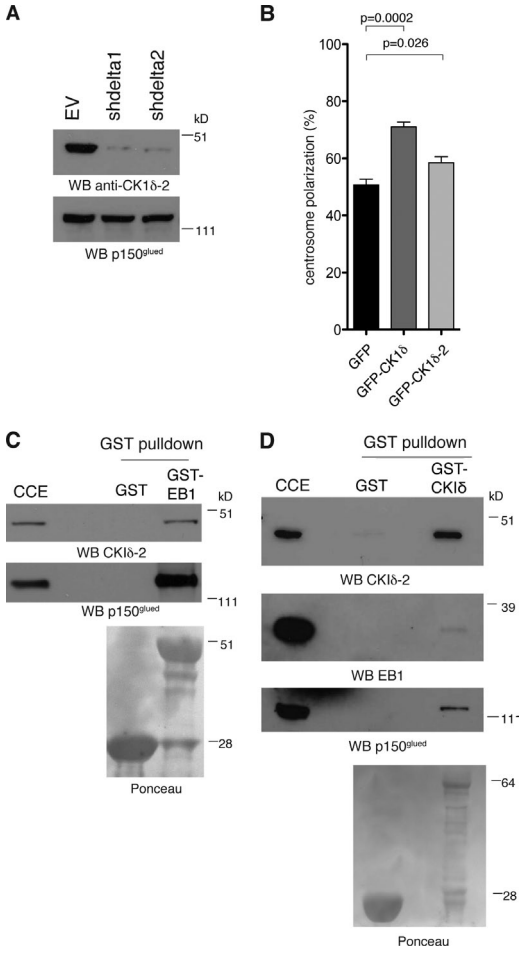


Figure S4. **CKIδ isoform-2 (CKIδ-2) promotes centrosome polarization to the IS and forms a complex with EB1 and the canonical CKIδ isoform.** (A) Cytoplasmic cell extracts of Jurkat cells containing stably integrated EV (control) or vector encoding *CSNK1D*-specific shRNA (clones shdelta1 and shdelta2) were immunoblotted with a CKIδ-2–specific antibody. This antibody does not cross-react with canonical CKIδ.WB, Western blotting. (B) Control (EV) or CKIδ-depleted (shdelta) Jurkat cells were transiently transfected with the indicated GFP fusion constructs and conjugated to SEE-pulsed Raji cells, and GFP-positive cells were scored for centrosome polarization to the IS (three independent experiments; 200 conjugates were scored per experiment). Error bars are mean ± SD. (C) Cytoplasmic cell extracts (CCE) of Jurkat cells were subjected to GST pull-down assays with GST alone (GST) or GST-EB1 (EB1) and immunoblotted with antibodies as indicated. GST and GST fusion products are shown on Ponceau staining below. (D) Cytoplasmic cell extracts of Jurkat cells were subjected to GST pull-down assays with GST alone (GST) or GST-CKIδ (CKIδ; i.e., canonical isoform) and immunoblotted with antibodies as indicated. GST and GST fusion products are shown in Ponceau staining below.

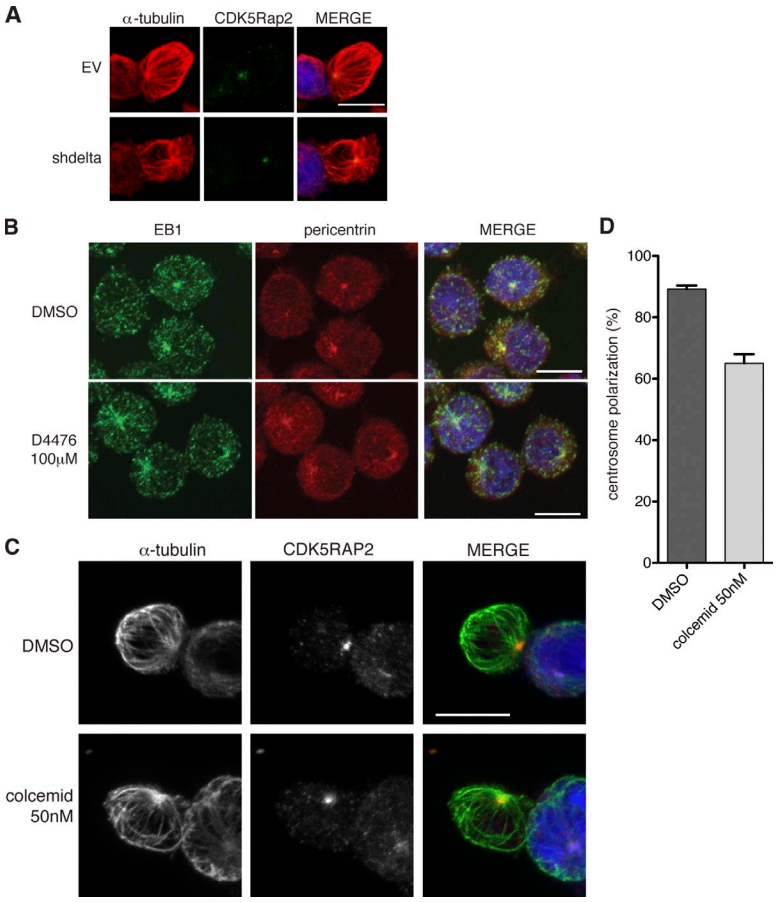
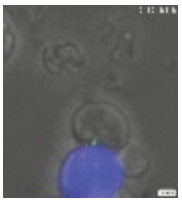
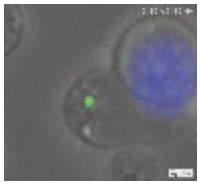


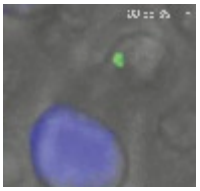
Figure S5. **CKIδ depletion or inhibition does not obliterate microtubule assembly in Jurkat cells.** (A) Conjugates between control (EV) or CKIδ-depleted (shdelta) Jurkat and SEE-pulsed Raji cells were stained with anti–α-tubulin and anti-CDK5RAP2 antibodies. (B) Jurkat cells were treated for 16 h with DMSO or 100 μM D4476 and stained with anti-EB1 and antipericentrin antibodies. (C) Jurkat cells treated with DMSO or 50 nM colcemid were stained with anti–α-tubulin (green in merge) and anti-CDK5RAP2 (red in merge) antibodies. (A–C) Bars, 10 μm. (D) DMSO- or colcemid-treated Jurkat cells were conjugated to SEE-pulsed Raji cells and scored for centrosome polarization to the IS (two independent experiments; 200 conjugates were scored per experiment). Error bars are mean ± SD.



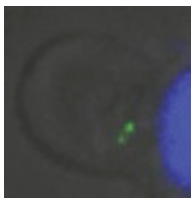
Video 1. **Conjugate formation between an SEE-pulsed Raji cell (CMAC; blue) and a control (EV) Jurkat cell transfected with centrin-GFP (green).** The fluorescence is superimposed on grayscale brightfield images to illustrate conjugate formation. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Note that time 0 in Fig. 2 A refers to first frame after conjugate formation and not to the start of the video. Frames were taken every 3 min for 102 min. The display rate is two frames per second.



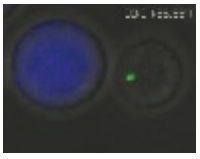
Video 2. **Conjugate formation between an SEE-pulsed Raji cell (CMAC; blue) and a control (EV) Jurkat cell transfected with centrin-GFP (green).** The fluorescence is superimposed on grayscale brightfield images to illustrate conjugate formation. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Note that time 0 in Fig. 2 A refers to first frame after conjugate formation and not to the start of the video. Images were acquired every 3 min for 87 min. The display rate is two frames per second.



Video 3. **Conjugate formation between an SEE-pulsed Raji cell (CMAC; blue) and a CKIδ-depleted (shdelta) Jurkat cell transfected with centrin-GFP (green).** The fluorescence is superimposed on grayscale brightfield images to illustrate conjugate formation. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Note that time 0 in Fig. 2 A refers to first frame after conjugate formation and not to the start of the video. Images were acquired every 4 min for 155 min. Thr display rate is two frames per second.

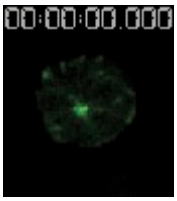


Video 4. **Conjugate formation between an SEE-pulsed Raji cell (CMAC; blue) and a CKIδ-depleted (shdelta) Jurkat cell transfected with centrin-GFP (green).** The fluorescence is superimposed on grayscale brightfield images to illustrate conjugate formation. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Images were acquired every 4 min for 80 min. The display rate is three frames per second.



Video 5. **Conjugate formation between an SEE-pulsed Raji cell (CMAC; blue) and a GFP-CKIδ–transfected Jurkat cell (green).** Note the diffuse centrosomal signal of GFP-CKIδ throughout centrosome polarization. The fluorescence is superimposed on grayscale brightfield images to illustrate conjugate formation. Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Images were acquired every 90 s for 18 min.

Video 6. **Detection of EB3-GFP comets in a control (EV) Jurkat cell.** Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Images were acquired every 0.5 s (displayed at 10 frames per second).



Video 7. **Detection of EB3-GFP comets in a CKIδ-depleted Jurkat cell.** Images were analyzed by time-lapse confocal microscopy using a spinning-disk confocal microscope (PerkinElmer). Images were acquired every 0.5 s (displayed at 10 frames per second).