Expanded View Figures

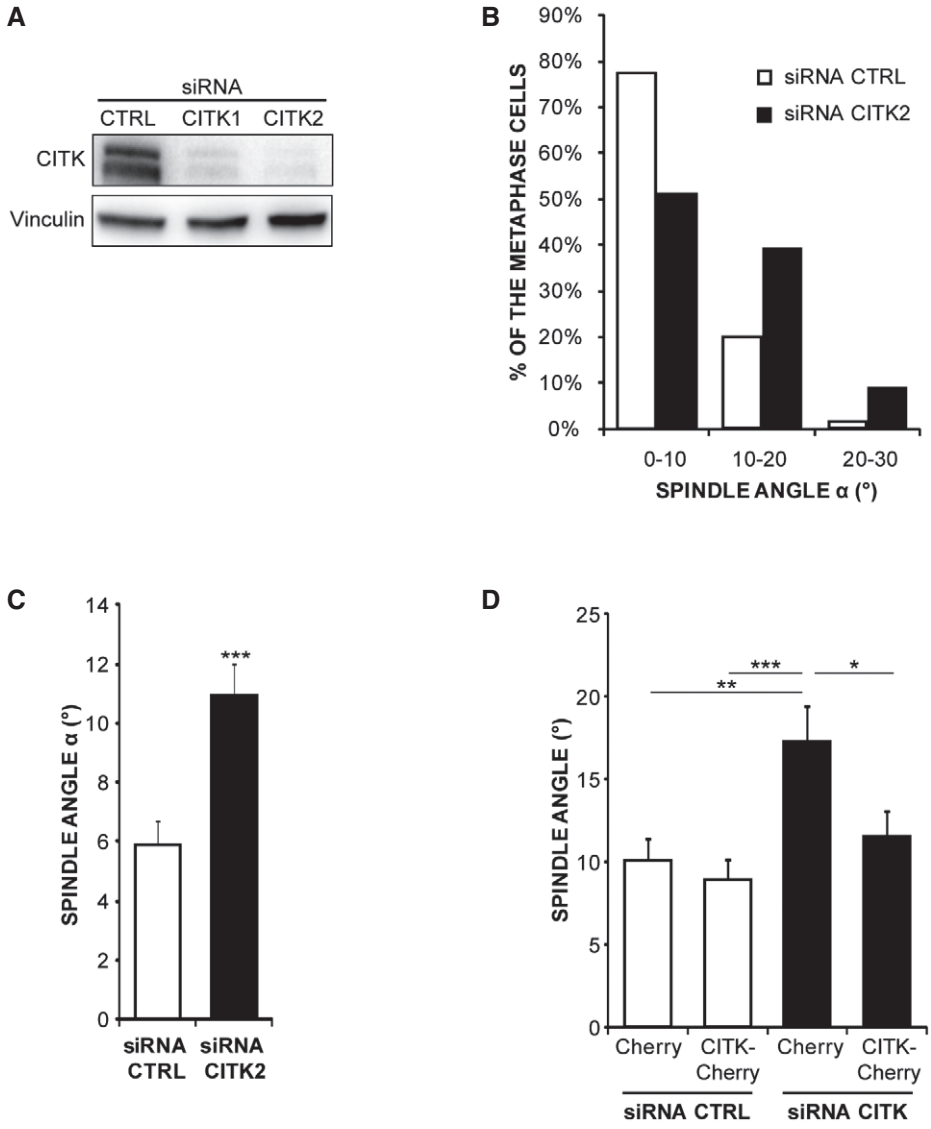

Figure EV1. Specificity of the CITK-RNAi experiments.

A Western blot analysis performed with the indicated antibodies on lysates of HeLa cells transfected with control or with two different CITK-specific siRNAs (CITK1 and CITK2). The results of experiments shown in the main figures were obtained with the CITK1 sequence.

B Distribution of spindle angles (°) in HeLa cells depleted of CITK using the CITK2 sequence or transfected with control sequence. The values represent the angles between the axis crossing the two poles of metaphase spindles and the coverslip ($n \geq 150$ cells in three independent experiments).

C Quantification of average and distribution of spindle angles (°) in HeLa cells depleted of CITK using the CITK2 ($n \geq 50$ cells in three independent experiments).

D Quantification of angles average in HeLa cells transfected with either control or CITK1 siRNAs and cotransfected with expression plasmids expressing mCherry alone or fused to RNAi-resistant (mouse) CITK wild-type sequence ($n \geq 50$ cells in three independent experiments).

Data information: Data presented are means ± SEM. Statistical significance was assessed using a two-tailed Student's *t*-test. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

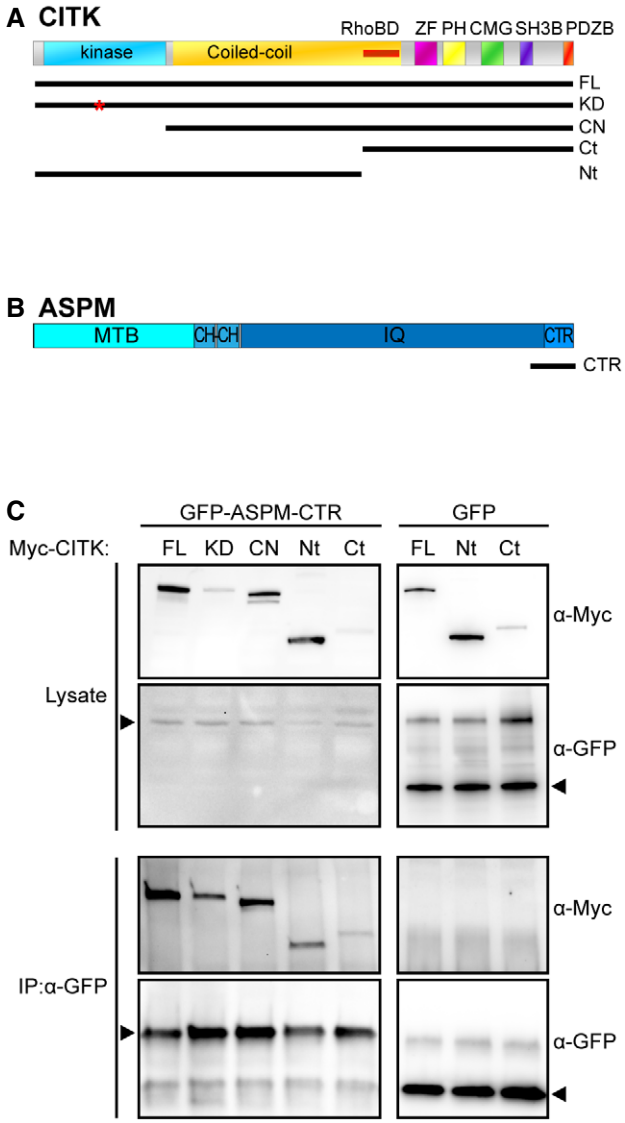

Figure EV2. Analysis of CITK regions involved in the interaction with ASPM.

A Representation of domain structure of CITK and its fragments: Ser/Thr kinase domain (kinase), coiled-coil region (Coiled-coil), Rho-binding domain (RhoBD), type-2 zinc finger (ZF), pleckstrin homology domain (PH), CMG domain (CMG), proline-rich, putative SH3 binding domain (SH3B), PDZ binding domain (PDZB); full-length CITK (FL), kinase-dead full-length CITK (KD), citron-N (CN), and the N-terminal (Nt) and the C-terminal halves of CITK (Ct).

B Representation of domain structure of ASPM and CTR fragment: putative microtubule binding domain (MTB), calponin homology domain (CH), calmodulin binding domains (IQ), and the C-terminal region (CTR).

C The indicated Myc-CITK fusion constructs were cotransfected with ASPM-CTR-GFP in HEK293T. After 48 h, immunoprecipitations were performed from total cell lysates using anti-GFP antibody and the immunoprecipitated proteins were revealed by Western blotting for the GFP and Myc epitopes. The results shown are representative examples of at least three different experiments.

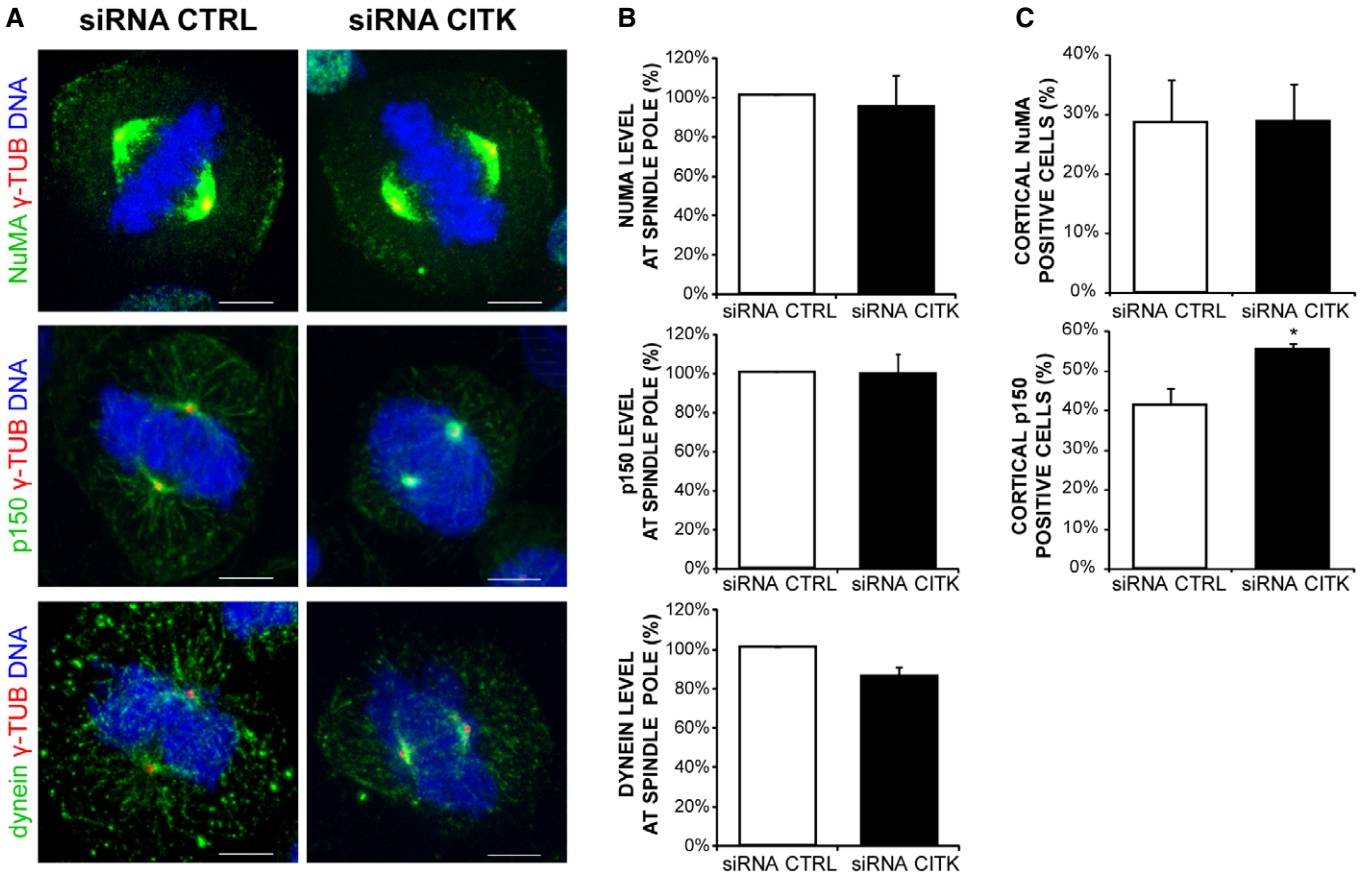

Figure EV3. Loss of CITK does not impair the localization of the dynein/dynactin motor complex and NuMA.

A Control and CITK-depleted cells were immunostained for γ-tubulin (red), DNA (blue), and NuMA, dynactin (p150), or dynein (green). Images represent maximum-intensity projections of confocal z-stacks. Scale bars, 5 μm.

B Quantification of fluorescence intensity at spindle poles of NuMA, dynactin (p150), or dynein in HeLa cells transfected with either CTRL or CITK siRNA ($n > 150$ cells, five independent experiments).

C Percentage of mitosis with cortical localization of NuMA or dynactin (p150) in HeLa cells transfected with either CTRL or CITK siRNA. The results shown are representative examples of at least three different experiments.

Data information: Data are presented as mean ± SEM. Statistical significance was assessed using a two-tailed Student's *t*-test. *$P < 0.05$.

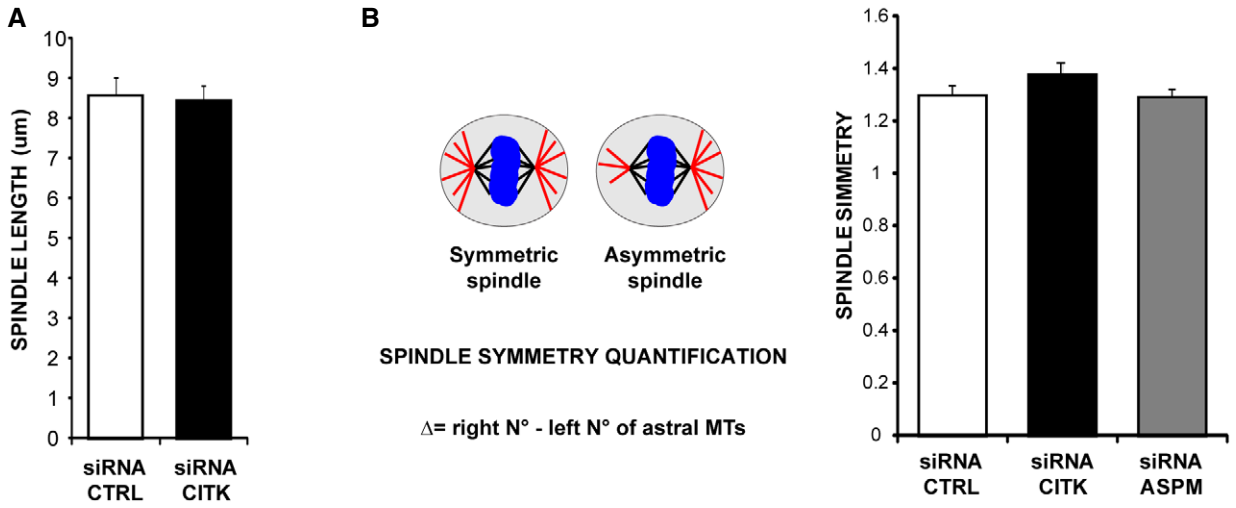

Figure EV4. Loss of CITK does not alter spindle length or symmetry.

A Quantification of spindle length in HeLa cells transfected with control sequence or with CITK-specific siRNA.
B Quantification of spindle symmetry in HeLa cells transfected with control sequence or with CITK-specific siRNA.

Data information: Data are presented as mean ± SEM. Differences in both panels were not statistically significant ($n \geq 150$ cells in three independent experiments). Statistical significance was assessed using a two-tailed Student's *t*-test.