Supplementary Figure S1 PLK2-dependent CPAP phosphorylation site mapping. In vitro kinase assays of PLK2 were performed with CPAP truncated mutant fusion proteins. The PLK2 enzymes were prepared from 293T cells by immunoprecipitation (Myc-PLK2-WT and Myc-PLK2-KD, **A**) or from bacteria by purification (GST-PLK2-kido-WT and GST-PLK2-kido-KD, **B-D**; GST-PLK2-FL-WT and GST-PLK2-FL-KD, **E**). Bacterially expressed GST-CPAP truncated proteins were used as substrates (**A-D**). A point mutant of CPAP (GST-CPAP$^{563\text{-}613,SSAA}$) was also used as a substrate (**E**). The phosphorylation activity was determined by autoradiogram. The amounts of the proteins were determined by Coomassie blue staining and immunoblotting. Asterisks indicate degraded forms of GST-PLK2-kido or FL.

Supplementary Figure S2 Alanine substitutions at S589 and S595 of CPAP do not interfere with the binding of CPAP with microtubules. (**A**) Truncated GST-CPAP proteins were incubated with purified tubulins as described in Materials and Methods. After polymerization and centrifugation, the equal ratio of supernatant (S) and pellet (P) were subjected to SDS-PAGE and the proteins were stained by Coomassie brilliant blue. (**B**) Forty-eight hours after transfection of *pMyc-CPAP* or *pMyc-CPAP^{SSAA}*, the U2OS cells were treated with 2 μg/ml nocodazole (NZ) or 15 μM taxol (TX) to depolymerize or stabilize microtubules, respectively. Four hours later, the cells were fixed and coimmunostained with antibodies specific to Myc (green) and α-tubulin (red). The scale bar represents 10 μm.

Supplementary Figure S3 CPAP is required for centriole duplication. (**A**) HeLa cells were transfected with *siCTL* or *siCPAP* and cultured for 48 h. The cell lysates were then subjected to immunoblot analysis with antibodies specific to CPAP and α-tubulin. (**B**) Six hours after *siRNA* transfection, the U2OS cells were treated with hydroxyurea (HU) to arrest the cell cycle at S phase. Seventy-two hours later, the cells were fixed and coimmunostained with antibodies specific to γ-tubulin (red) and PCNA (green). The cells with more than two centrosomes were counted by γ-tubulin dots. (**C**) The *siCTL*- or *siCPAP*-transfected HeLa cells were coimmunostained with antibodies specific to CP110 (red) and β-tubulin (green). Centriole duplication was determined by counting CP110 dots at spindle poles. The scale bar represents 10 μm. For statistical analysis, over 50 cells were counted and the experiments were repeated three times. The results are presented as means and standard errors. **, $P < 0.01$, analyzed with the paired *t*-test. Insets are magnified views of the centrosomes.

Supplementary Figure S4 Phosphorylation of CPAP S589 and S595 is critical for centriole duplication. HeLa cells were initially transfected with *siCTL* or *siCPAP-U* and then with *pMyc-CPAP* or *pMyc-CPAP^{SSAA}*. The cells were coimmunostained with antibodies specific to Myc and centrobin to determine centriole duplication. The scale bar represents 10 μm. For statistical analysis, over 50 cells were counted and the experiments were repeated three times. The results are presented as means and standard errors. *, $P < 0.05$, analyzed with the paired *t*-test. Insets are magnified views of the centrosomes.

Supplementary Figure S5 Depletion of CPAP or PLK2 does not induce cell cycle arrest at G1 phase. HeLa cells were transfected with *siCTL*, *siCPAP*, *siPLK2* or *siCPAP-U* and cultured for indicated time periods. The cells were collected and analysed by FACS.

Supplementary Figure S6 Ectopic expression of CPAP phospho-mimetic mutants does not impair centriole duplication. HeLa cells were transfected with *pMyc-CPAP*, *pMyc-CPAP^{S589E}*, *pMyc-CPAP^{S595E}*, *pMyc-CPAP^{SSEE}* or *pMyc-CPAP^{SSAA}*. Forty-eight hours later, the cells were coimmunostained with antibodies specific to Myc (red) and centrobin (green), and centriole duplication was determined by counting centrobin dots. The scale bar represents 10 μm. For statistical analysis, over 50 cells were counted and the experiments were repeated three times. The results are presented as means and standard errors. ***, $P < 0.001$, analyzed with the paired *t*-test. Insets are magnified views of the centrosomes.

Supplementary Figure S7 Specificity of the pS589CPAP and pS595CPAP antibodies. (**A**) HeLa cells were transfected with *siCTL* or *siCPAP*. The cells were then synchronized at G1/S phase by a double thymidine block and immunostained with the CPAP or phospho-CPAP (αpS589CPAP and αpS595CPAP) antibodies (green) along with the γ-tubulin antibody (red). (**B**) HeLa cells were transfected with *siCTL*, *siPLK2* or *siPLK4* and the cells were immunostained with the CPAP or phospho-CPAP (αpS589CPAP and αpS595CPAP) antibodies (green). Immunostaining intensities of the centrosomal CPAP and phospho-CPAP proteins were determined densitometrically relative to the intensity of *siCTL*-transfected cells. The scale bar represents 10 μm. For statistical analysis, immunofluorescent intensities of at least 20 cells were determined and the results are presented as means and standard errors. **, $P < 0.01$; ***, $P < 0.001$, analyzed with the *t*-test. Insets are magnified views of the centrosomes.

Supplementary Figure S8 Specificity of the CPAP antibody (**A**) 293T cell lysates were subjected to immunoblot analysis with the CPAP antibody or with pre-immune serum. (**B**) HeLa cells were coimmunostained with antibodies specific to CPAP (red) and γ-tubulin (green). The scale bar represents 10 μm. Insets are magnified views of the centrosomes, which are indicated by arrows. (**C**) 293T cells were lysed and centrosomes were enriched by sucrose density gradient centrifugation. The collected fractions were analyzed by immunoblotting with antibodies specific to CPAP and γ-tubulin. Whole cell lysates (WCL) were used as a control. (**D**) Centriolar localization of CPAP. HeLa cells were coimmunostained with antibodies specific to CPAP (red) and CP110 (green). The upper and lower panels show unduplicated and duplicated centrioles, respectively. The scale bar represents 2 μm.

Supplementary Figure S9 HeLa cells were accumulated at G2 phase by thymidine block and release for 7 h to easily detect the cells containing separated centrosomes. The cells were coimmunostained with indicated antibodies. Each magnified picture shows one separated centrosome which contains a pair of centrioles. The scale bar represents 1 μm.

Supplementary Materials and Methods

Flow cytometry

HeLa cells were trypsinized and suspended in 100 µl PBS. We added 1.4 ml 75% cold ethanol and this was then discarded following centrifugation. The cell pellets were resuspended in PBS containing 100 µg/ml RNase A and 10 µg/ml propidium iodide, and then incubated at 37°C for 30 minutes. The stained cells were loaded onto a FACSCalibur flow cytometer (Becton Dickinson) and analyzed with CellQuest Pro software.

Centrosome fractionation

To disrupt cytoskeletons, cells were treated with 10 µg/ml nocodazole and 1 µg/ml cytochalasin D for 1 h at 37°C. The cells were sequentially washed with PBS, 1 mM HEPES (pH 7.2) with 8% sucrose, and 1 mM HEPES, and then lysed in lysis buffer (1 mM HEPES, 0.5% NP-40, 0.5 mM $MgCl_2$, 0.1% β-mercaptoethanol, 50 mM NaF, 1 mM Na_3VO_4, protease inhibitor cocktail) for 10 min on ice. To remove nuclei, the lysates were centrifuged at 2,500 × g for 10 min and the supernatant was filtered through a 50-µm nylon mesh. The concentration of HEPES was adjusted to 10 mM and DNase I was added (2 units/ml). The lysates were incubated for 30 min on ice, layered onto 0.5 ml 60% sucrose solution (60% wt/wt sucrose in 10 mM PIPES (pH 7.2), 0.1% Triton X-100, 0.1% β-mercaptoethanol), and centrifuged at 10,000 × g for 30 min. The interface was then loaded onto a discontinuous sucrose gradient (0.5 ml of 70%, 0.3 ml of 50%, and 0.3 ml of 40% sucrose) and centrifuged at 120,000 × g for 1 h. Fractions were collected from the top, individually diluted in 10 mM PIPES, and centrifuged at 13,000 rpm for 15 min; the pellets were dissolved in SDS sample buffer.

Microtubule sedimentation assay

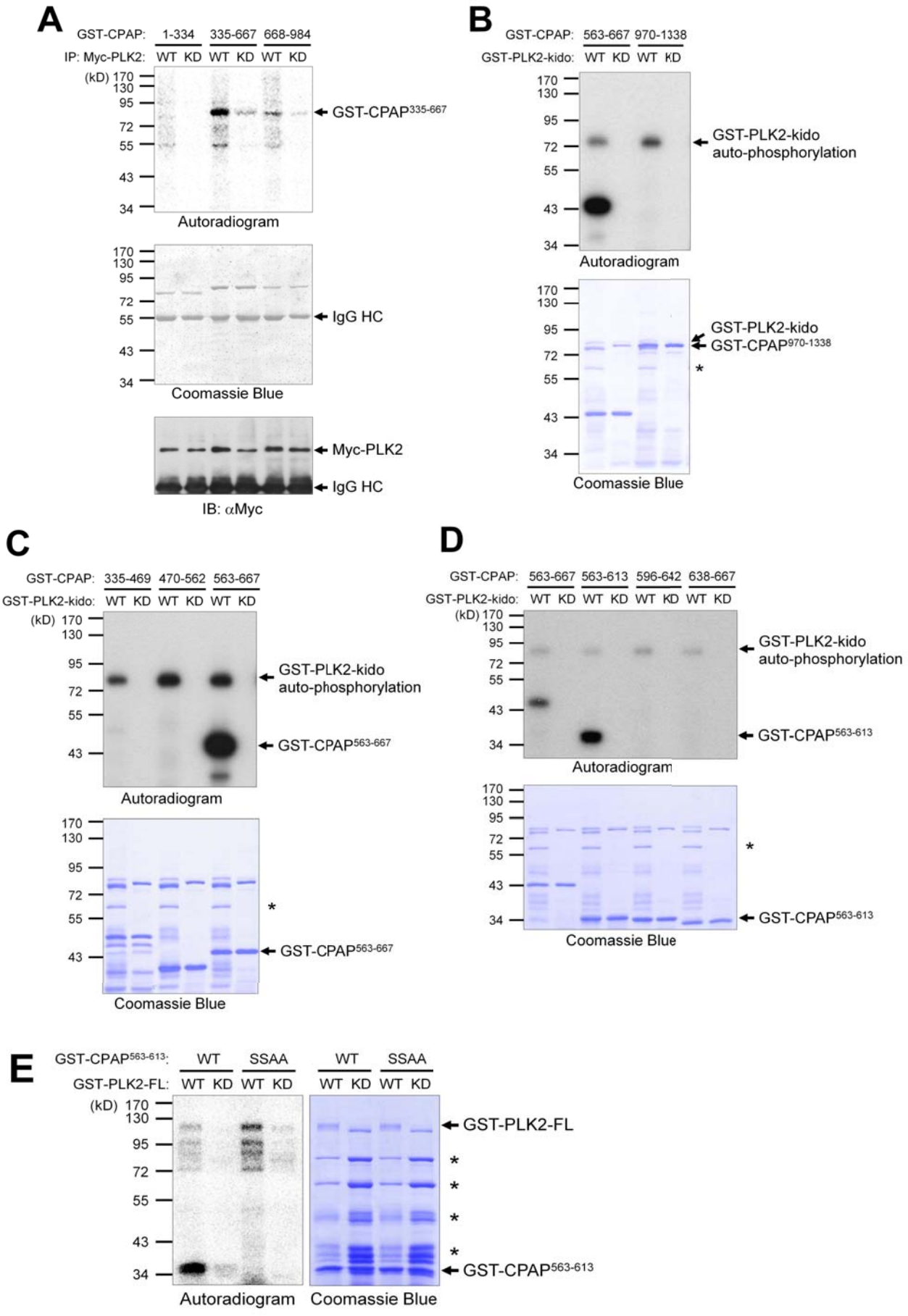
The assay was performed as previously described (Hung *et al*., 2004; Hsu *et al*., 2008). In brief, 40 µg of purified α/β-tubulins (Cytoskeleton) were incubated with 5 µg of GST or GST-CPAP in RG1 buffer (80 mM PIPES, pH 6.8, 1 mM EGTA, 1 mM $MgCl_2$ and 1 mM GTP) for 30 min at 37°C. For microtubule polymerization, the samples were mixed and incubated with GMRG buffer (RG1 buffer containing 12 mM $MgCl_2$ and 50% glycerol) for 2.5 hours at 37°C, and centrifuged at 300,000 × g for 30 min at 37°C using a TLA-100.2 ultracentrifuge (Beckmann Coulter). Total amount of supernatants and pellets were subjected to SDS-PAGE and the proteins were stained by Coomassie blue.

Supplementary References

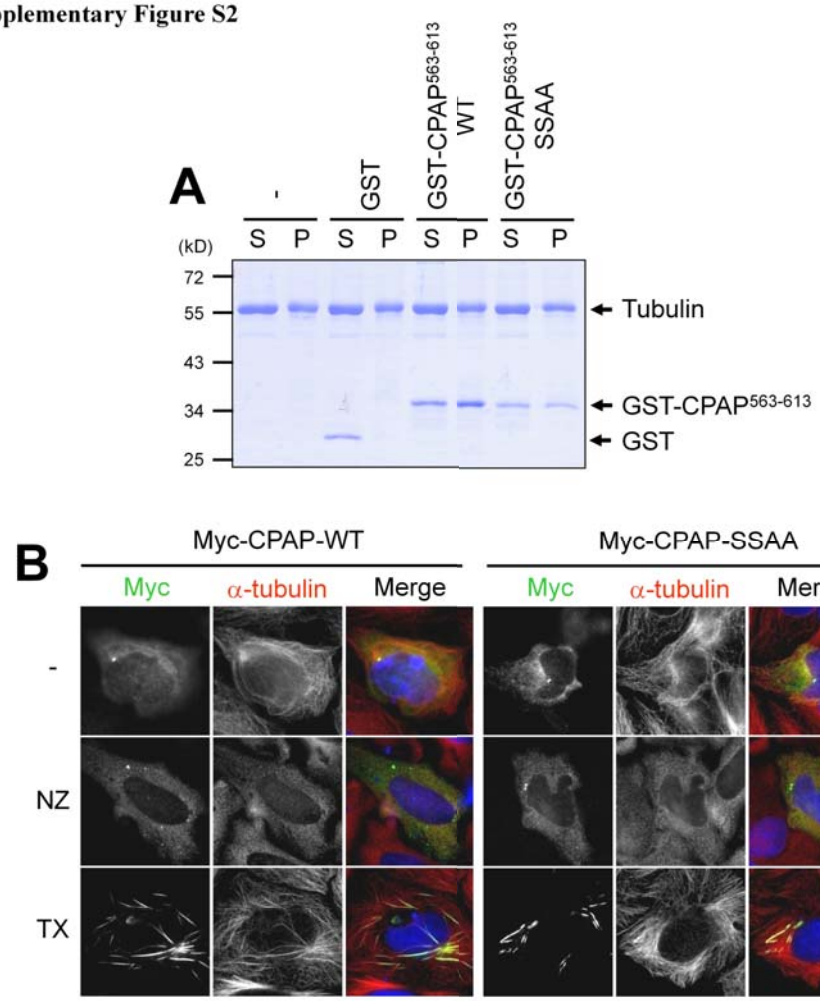
Hsu WB, Hung LY, Tang CJ, Su CL, Chang Y, Tang TK (2008) Functional characterization of the microtubule-binding and -destabilizing domains of CPAP and d-SAS-4. *Exp Cell Res* **314**(14)**:** 2591-2602

Hung LY, Chen HL, Chang CW, Li BR, Tang TK (2004) Identification of a novel microtubule-destabilizing motif in CPAP that binds to tubulin heterodimers and inhibits microtubule assembly. *Mol Biol Cell* **15**(6)**:** 2697-2706

Supplementary Figure S1



Supplementary Figure S2



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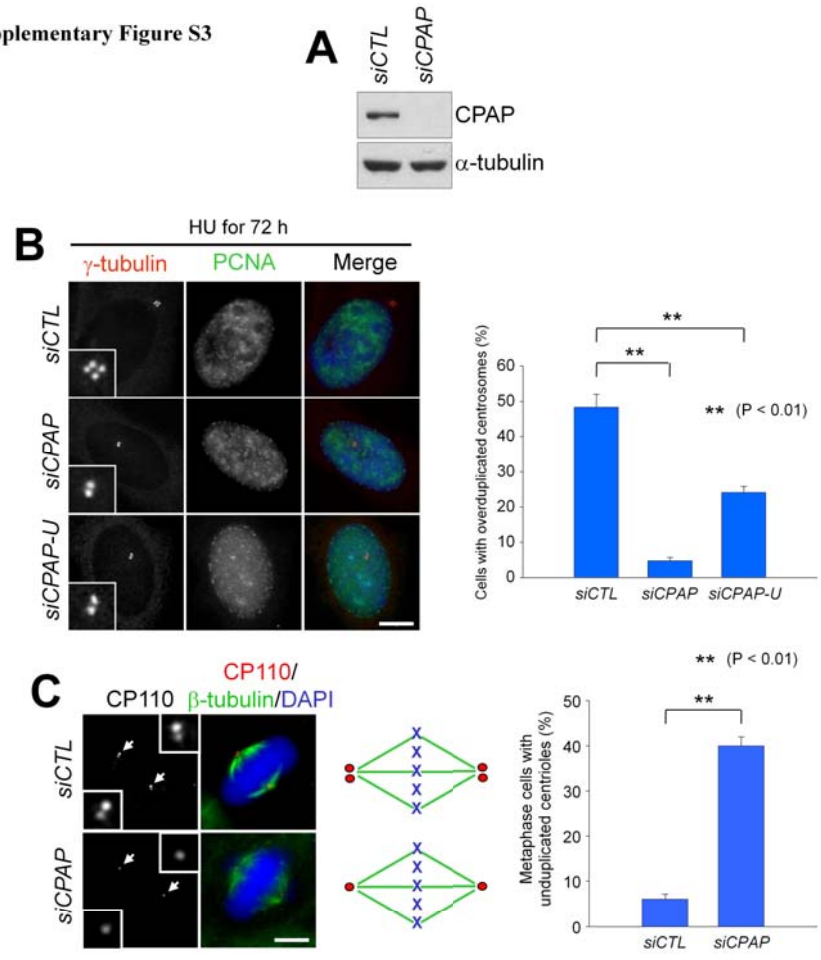

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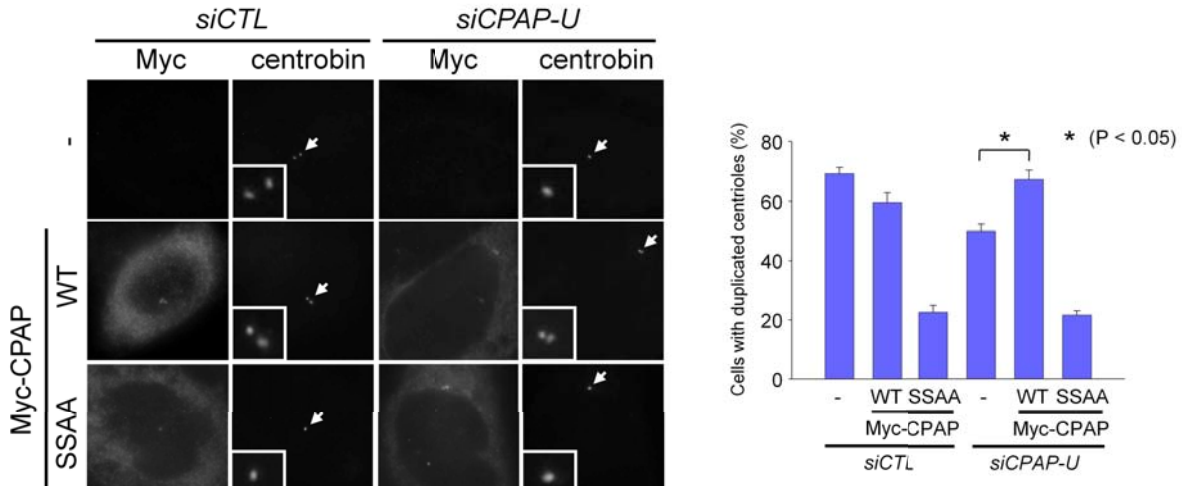

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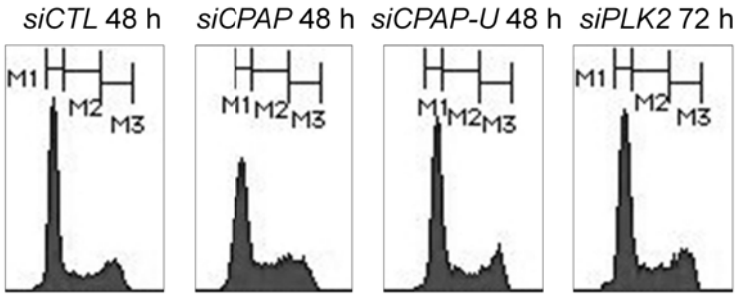
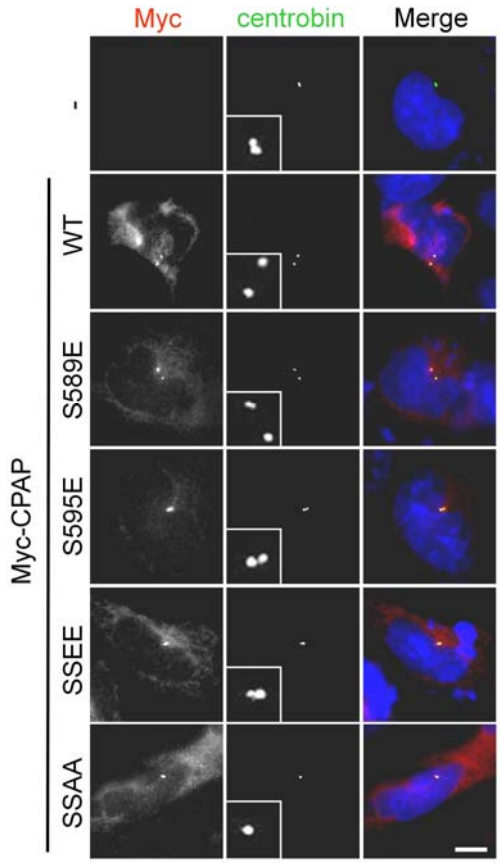
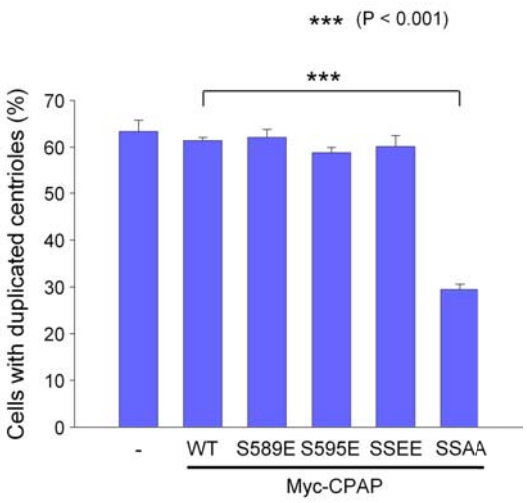


	siCTL 48 h	siCPAP 48 h	siCPAP-U 48 h	siPLK2 72 h
M1 (G1)	64.95%	57.36%	50.30%	58.78%
M2 (S)	17.35%	23.19%	28.73%	20.76%
M3 (G2/M)	17.22%	19.50%	20.75%	20.18%

Supplementary Figure S6





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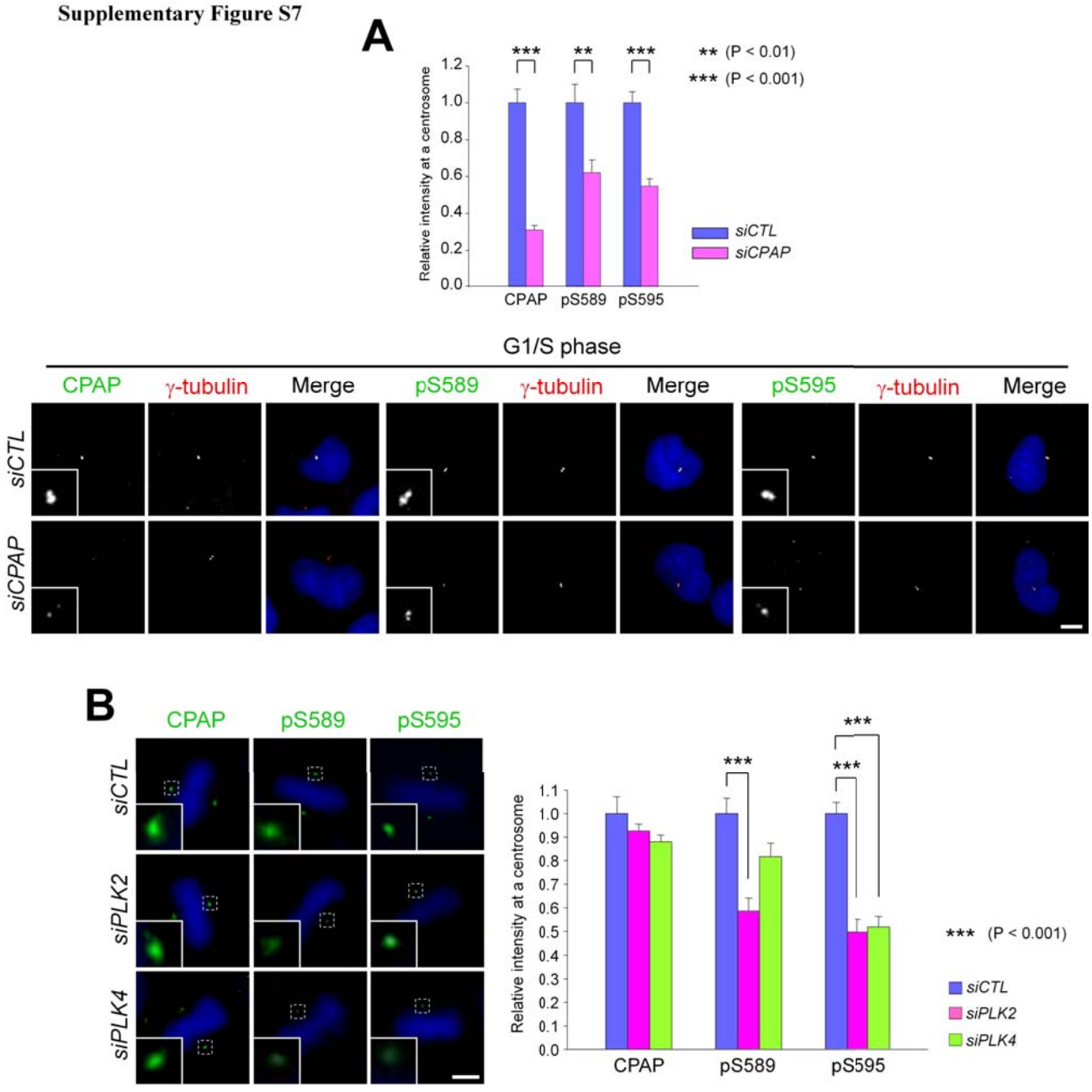
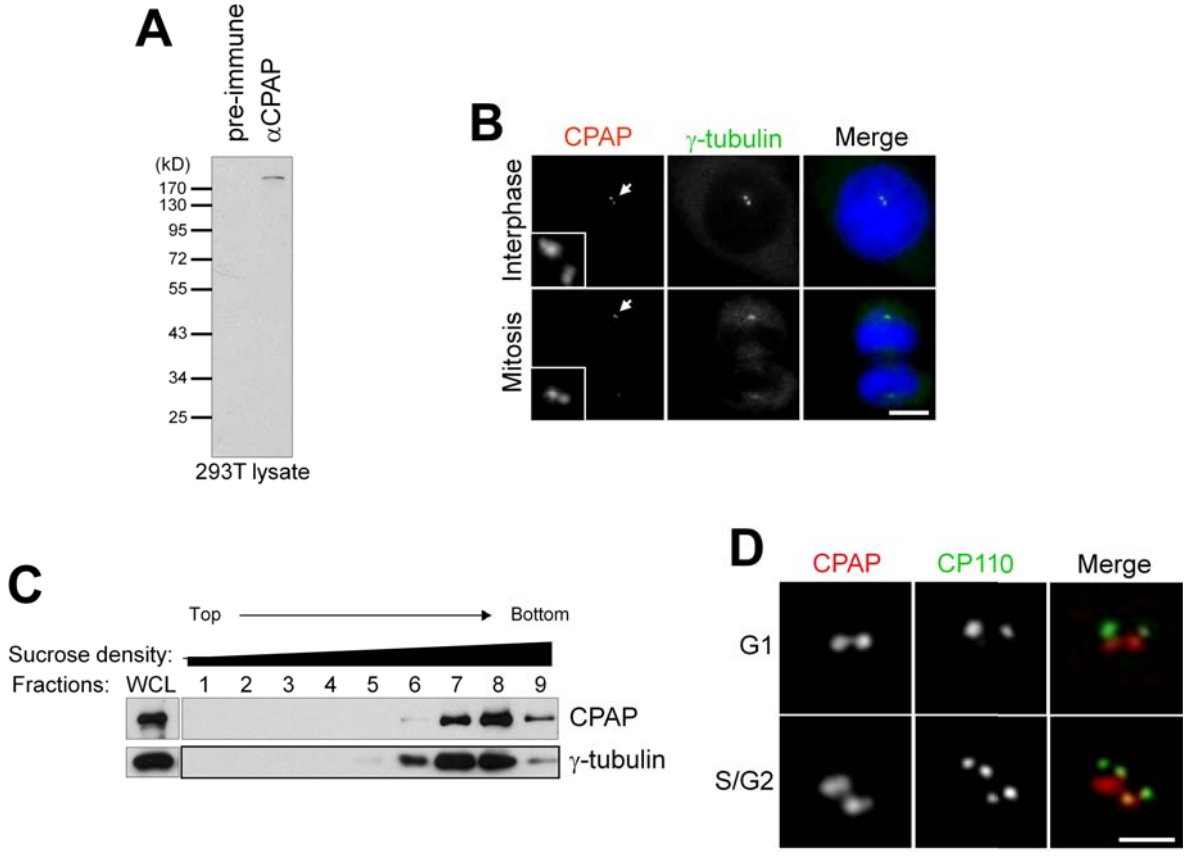


Supplementary Figure S8



Supplementary Figure S9