Choi et al., http://www.jcb.org/cgi/content/full/jcb.201007030/DC1

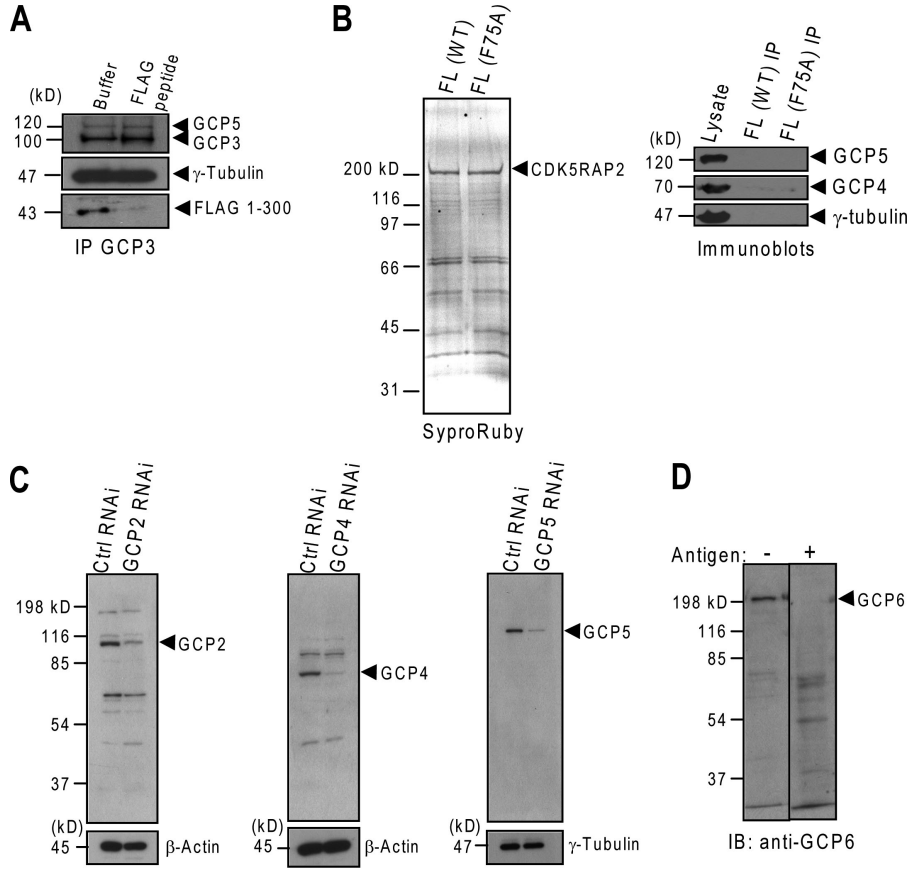

Figure S1. **Characterization of CDK5RAP2 proteins and reagents.** (A) Dissociation of CDK5RAP2 from the γ-TuCs by the Flag peptide. HEK293T ectopically expressing CDK5RAP2 (1-300) was subjected to anti-GCP3 immunoprecipitation (IP). After incubation with or without the Flag peptide, the immunoprecipitates were washed and analyzed by immunoblotting. (B) SDS-PAGE and immunoblotting of Flag-CDK5RAP2 and its F75A mutant isolated from HEK293T. The isolated proteins were resolved by SDS-PAGE for immunoblotting and staining with Sypro ruby. γ-Tubulin and GCPs were not detectable in the purified samples by immunoblotting. (C and D) Characterization of antibodies recognizing GCPs. (C) siRNA-transfected HEK293T extracts were immunoblotted with the respective GCP antibodies and an anti–β-actin or anti–γ-tubulin antibody. (D) The specificity of the anti-GCP6 antibody was tested by immunoblotting (IB) HEK293T extracts with or without preblocking the antibody using the GCP6 antigen. Black line indicates that intervening lanes have been spliced out.

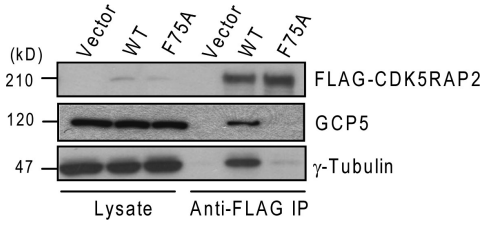

Figure S2. **The mutation of Phe75 abolishes the association of CDK5RAP2 with γ-TuCs.** HEK293T expressing full-length CDK5RAP2 proteins or the vector was subjected to immunoprecipitation of the ectopic tag (anti-Flag IP). The immunoprecipitates were examined on immunoblots. WT, CDK5RAP2 wild type; F75A, CDK5RAP2 (F75A).

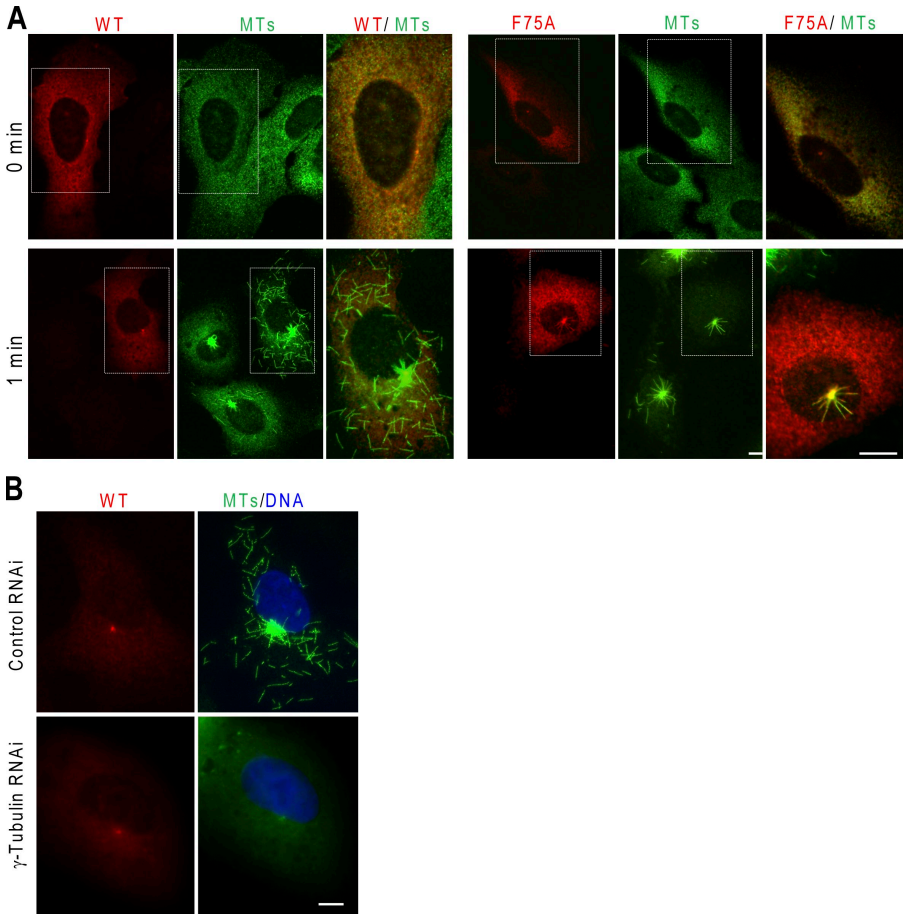

Figure S3. **Overexpression of CDK5RAP2 induces γ-tubulin–dependent nucleation of microtubules.** (A) Microtubule regrowth was performed on U2OS cells expressing the entire CDK5RAP2 protein or its mutant. The cells were immunostained for microtubules (anti–α-tubulin) and the ectopically expressed proteins (anti-Flag). WT, CDK5RAP2 wild type; F75A, CDK5RAP2 (F75A); MTs, microtubules. (right) Enlarged views of boxed areas are shown. (B) CDK5RAP2 was transiently expressed in cells depleted of γ-tubulin by RNAi. Microtubule regrowth was performed for 1 min. Bars, 10 μm.