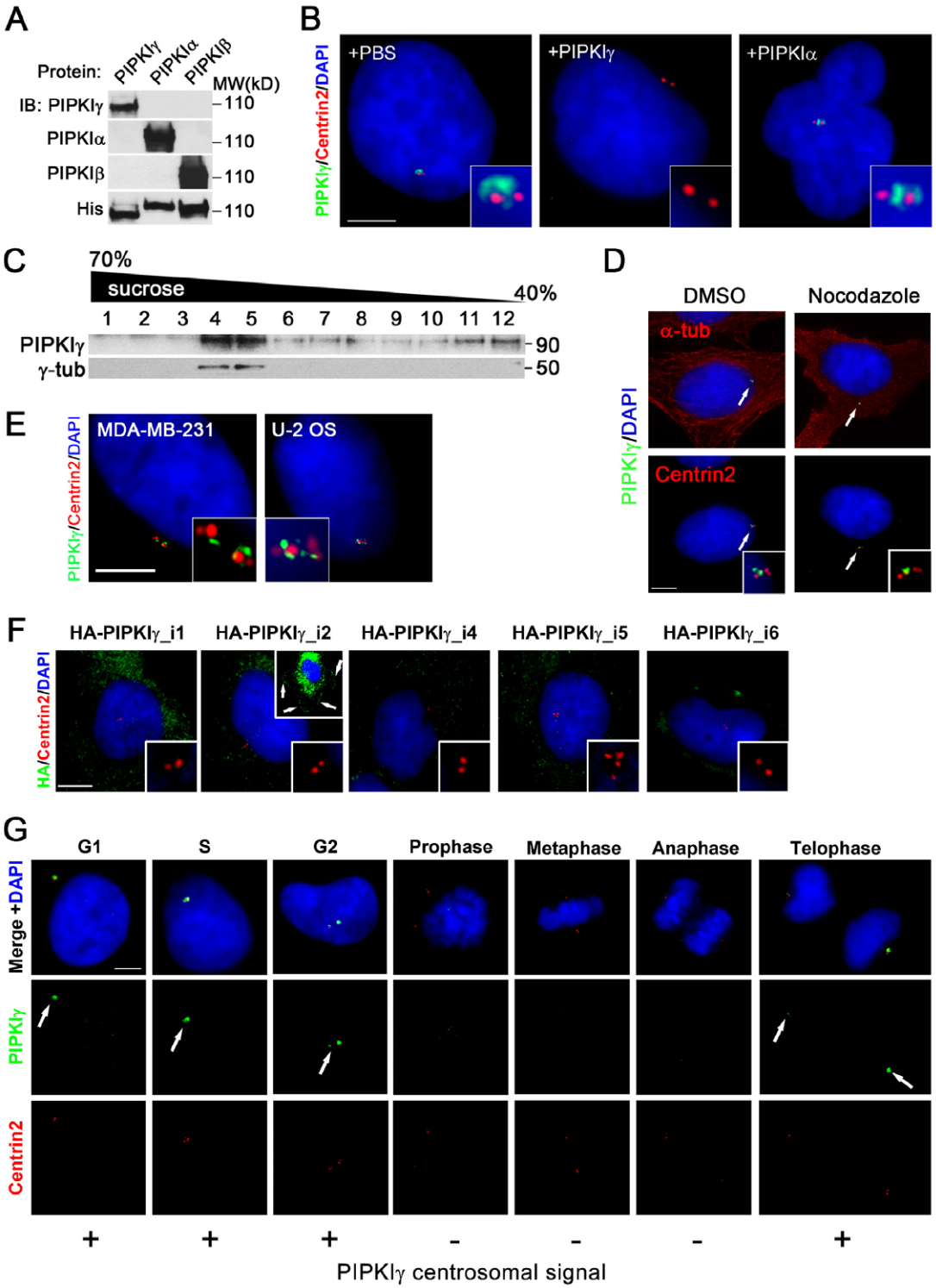

Fig. S1. PIPKIγ associates with the centrosome and it is regulated within the cell cycle. (A) Purified anti-PIPKIγ, PIPKIα, and PIPKIβ antibodies are specific. Same amount of recombinant GST-His-PIPKIα, GST-His-PIPKIβ and His-PIPKIγ proteins were blotted with indicated antibodies. (B) Staining of PIPKIγ at centrosome was abolished by excessive purified recombinant PIPKIγ, but not PIPKIα (20-fold higher than PIPKIγ antibody). (C) PIPKIγ co-sediments with centrosomes isolated from mouse kidneys using 40-70% sucrose gradients. (D) Association of PIPKIγ with centrioles is independent of the integrity of microtubule network. HeLa cells were treated with 10 μM nocodazole for 1 hour, and stained with indicated antibodies. Arrows show centrioles. Cells treated with DMSO were used as a negative control. (E) PIPKIγ localizes at the centrosome in MDA-MB-231 and U-2 OS cells. (F) HeLa cells were transfected with HA-tagged HA-PIPKIγ_i1, i2, i4, i5, and i6, then fixed and stained with anti-HA and Centrin2 antibodies. Insert at top right of the HA-PIPKIγ_i2 panel shows the focal adhesion (FA) targeting of HA-PIPKIγ_i2 (indicated by arrows) at the bottom of the same cell below the centriole focus plane. (G) The association of PIPKIγ with centrosome is regulated within the cell cycle. PIPKIγ targets to the centrosome in G1, S, and G2 phases, but is absent from the centrosome in M phase until telophase. HeLa cells were stained with anti-PIPKIγ and Centrin2 antibodies, as well as DAPI. Arrows indicate PIPKIγ signal on centrosomes. (B, D, E, F and G) Scale bars, 5 **μm**. Inserts at the bottom show magnified centrosome images.

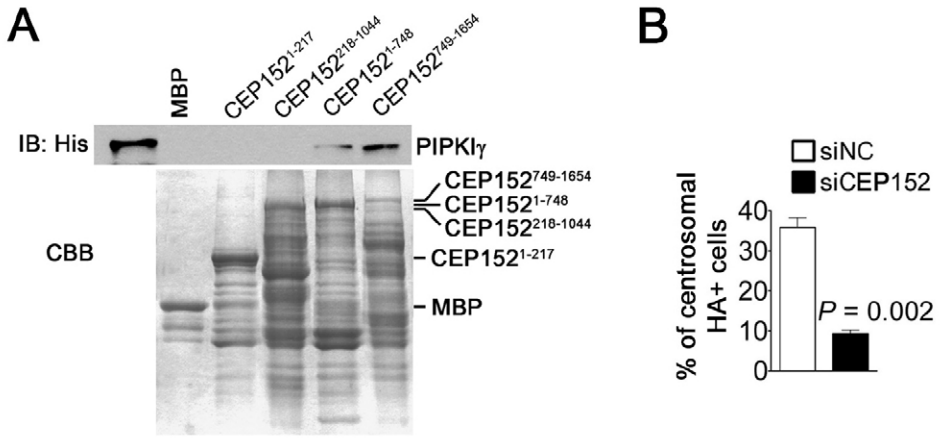

Fig. S2. CEP152 regulates PIPKIγ targeting to the centrosome. (A) PIPKIγ interaction with CEP152 fragments. His-PIPKIγ was incubated with purified recombinant MBP or indicated MBP-tagged CEP152 fragments in MBP pull-down assay. The precipitates were analyzed by immunoblotting using anti-His antibody. Loading of MBP and MBP-fused CEP152 was shown by Coomassie Brilliant Blue (CBB) staining. (B) Loss of CEP152 abolished the centrosomal targeting of exogenous PIPKIγ. HeLa cells treated with control or CEP152 specific siRNAs were transfected with HA-PIPKIγ$^{1-445}$, and then stained with anti-HA and Centrin2 antibodies and analyzed by fluorescence microscopy. Cells with centrosomal HA signal were quantified and plotted from three independent experiments (n>200).

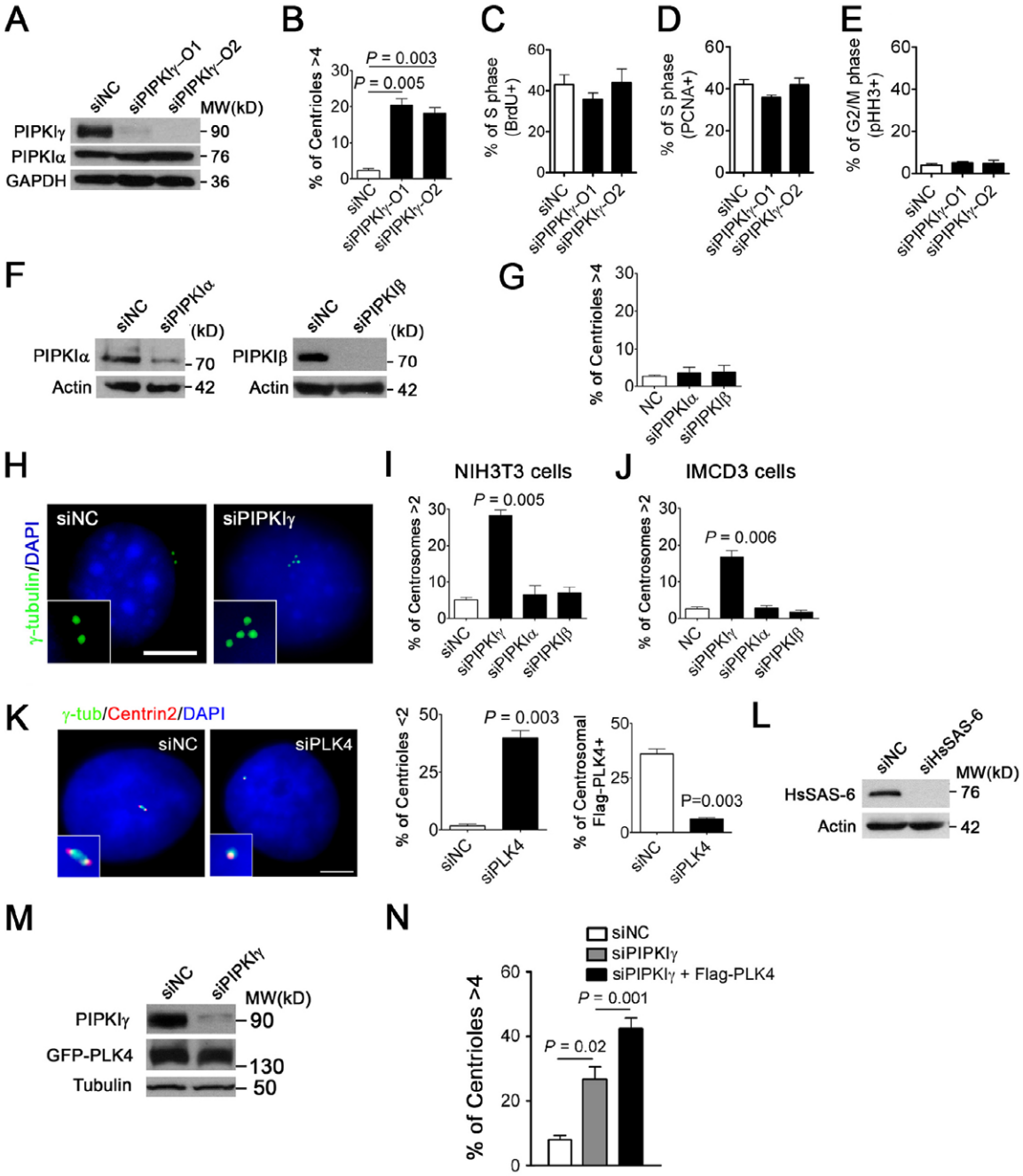

Fig. S3. Depletion of PIPKIγ induces excessive Centrin2 foci in cells, but does not arrest cells in S- or G2/M- phases. (A, B) Depletion of PIPKIγ by two independent siRNAs (siPIPKIγ-O1, siPIPKIγ-O2) results in centriole amplification. HeLa cells treated with control (siNC) or PIPKIγ-specific (siPIPKIγ-O1, siPIPKIγ-O2) siRNA were stained with anti-Centrin2 antibody and DAPI. Percentage of cells with >4 centrioles was quantified and plotted. (C-E) Depleting PIPKIγ does not cause S- or G2/M-phase arrest. HeLa cells treated with indicated siRNAs were subjected to BrdU incorporation assay (C), PCNA staining (D), and phosphor-Histon H3 (pHH3) staining (E). Percentage of cells with positive signal in each group was quantified. No significant difference was observed between the control and PIPKIγ depleted cells. (F) Specific depletion of endogenous PIPKIα (siPIPKIα), PIPKIβ (siPIPKIβ). (G) No amplification of Centrin2 foci was observed in PIPKIα or PIPKIβ depleted cells. Percentage of cells with >4 centrioles was quantified in each group. (H-J) Depletion of PIPKIγ induces centrosome amplification in NIH3T3 and IMCD3 cells. NIH3T3 cells treated for 5 days (H, I) and IMCD3 cells were treated for 3.5 days (J) with indicated siRNAs. The percentage of cells with >2 centrosomes was quantified in each group. (K) PLK4-specific siRNA strongly suppresses the expression and function of PLK4. Left panels, HeLa cells were treated with control (siNC) or PLK4-specific (siPLK4) siRNA and stained with indicated antibodies and DAPI. Middle panel, percentage of cells with less than two centrioles was quantified. Right panel, expression of presence of Flag-PLK4 at the centrosome was abolished by treated cells with PLK4 siRNA. (L) HsSAS-6 specific siRNA efficiently knocked down endogenous HsSAS-6. (M) Depletion of PIPKIγ had no effect on GFP-PLK4 level. (N) Depletion of PIPKIγ enhances centriole amplification resulted from PLK4 overexpression. HeLa cells were treated with siNC or PIPKIγ siRNA (siPIPKIγ) for 48 hours, and then were transfected with Flag-PLK4 for additional 12 h before fixation. Percentage of cells with >4 centrioles in control cells (siNC), PIPKIγ-depleted cells (siPIPKIγ), or PIPKIγ-depleted cells transfected with Flag-PLK4 (siPIPKIγ + Flag-PLK4) was quantified. (B-E, G, I-J, K-N) n > 200, at least three independent experiments. (H, K) Scale bars, 5 **μm**.

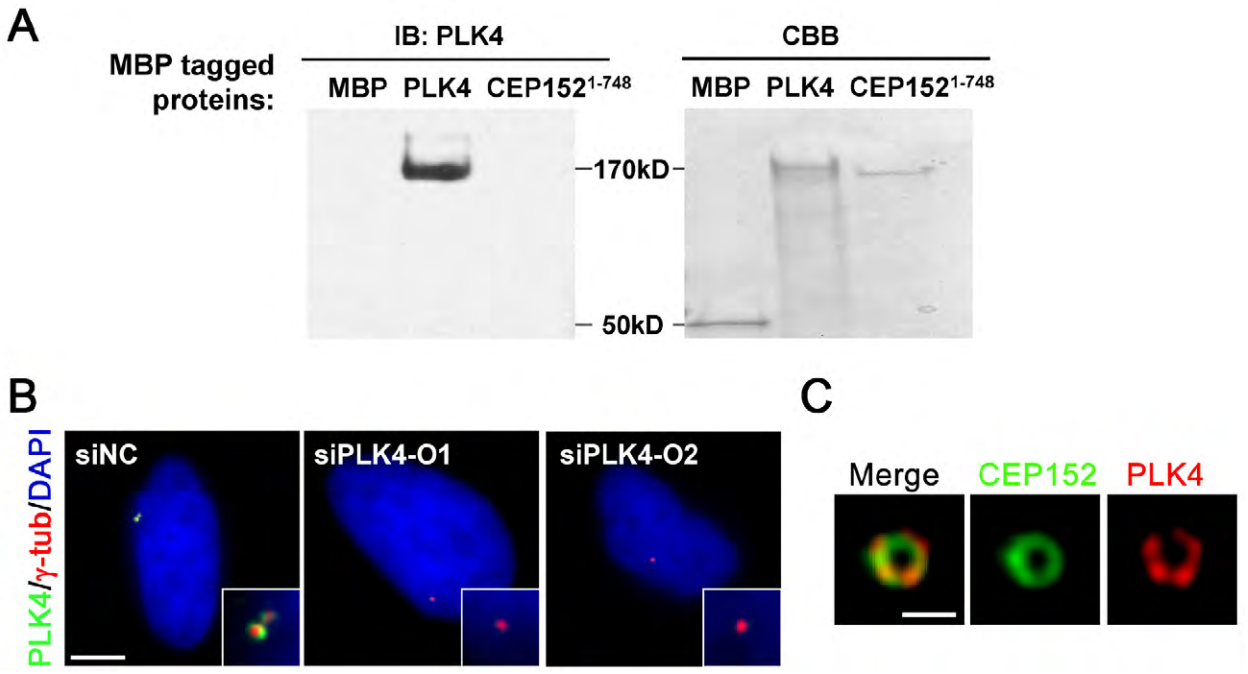

Fig. S4. Characterization of a new monoclonal PLK4 antibody. (A) The in-house monoclonal PLK4 antibody specifically recognized PLK4 by immunoblotting. Same amount of recombinant MBP, MBP-PLK4, and MBP-CEP152$^{1-748}$ proteins were blotted with indicated antibodies. Loading of each protein was shown by Coomassie Brilliant Blue (CBB) staining. (B) The in-house PLK4 antibody specifically recognized endogenous PLK4 at the centrosome by immunofluorescence. Depletion of PLK4 using two independent PLK4 specific siRNAs (siPLK4-O1, siPLK4-O2) abolished the centrosome signal recognized by anti-PLK4 antibody. Anti-γ-tubulin was used to label centrosomes. Enlarged centrosome images were shown as inserts. DNA was stained with DAPI. Scale bar, 5 μm. (C) PLK4 and CEP152 colocalize at the centrosome. 3D-SIM images showed that PLK4 colocalizes with CEP152. HeLa cells were processed through indirect immunofluorescence with antibodies against CEP152 and PLK4, and analyzed by 3D-SIM. Scale bar, 0.5 μm.