Wang et al., http://www.jcb.org/cgi/content/full/jcb.201101109/DC1

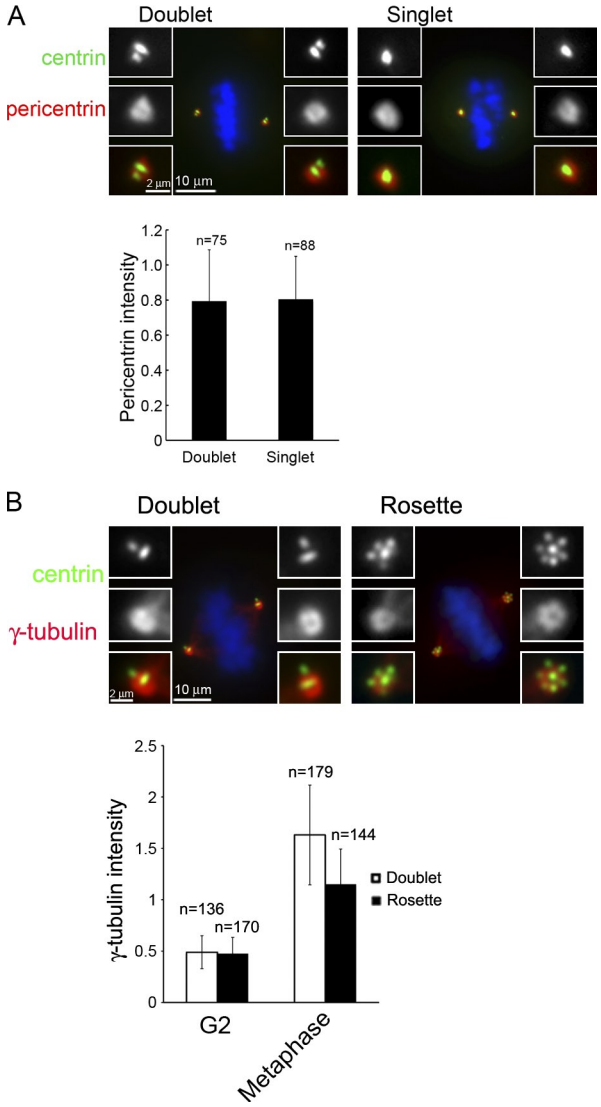

Figure S1. **Daughter centrioles do not contribute to PCM recruitment.** (A) Untreated (doublets) or hSas-6–depleted (singlets) RPE1 cells stably expressing centrin::GFP at metaphase were visualized with centrin::GFP and antibodies against pericentrin. Quantification of pericentrin signals associated with centrosomes is shown. (B) Extra daughter centrioles do not enhance PCM recruitment. Centriole rosettes were conditionally induced by overexpressing wild-type Plk4 in RPE1 cells. Cells were synchronized in G2 or mitosis as described in Fig. 1 A. Wild-type centrioles (doublets) or centriole rosettes containing at least five daughter centrioles in G2 (pictures not depicted) and metaphase were stained with antibodies against γ-tubulin. Centrioles were labeled with centrin::GFP. Quantification is shown for γ-tubulin signals associated with wild-type (doublet) or rosette centrosomes at indicated cell cycle stages. Numbers of centrosomes are indicated. Error bars indicate standard deviations. Insets show a higher magnification of centrosomes.

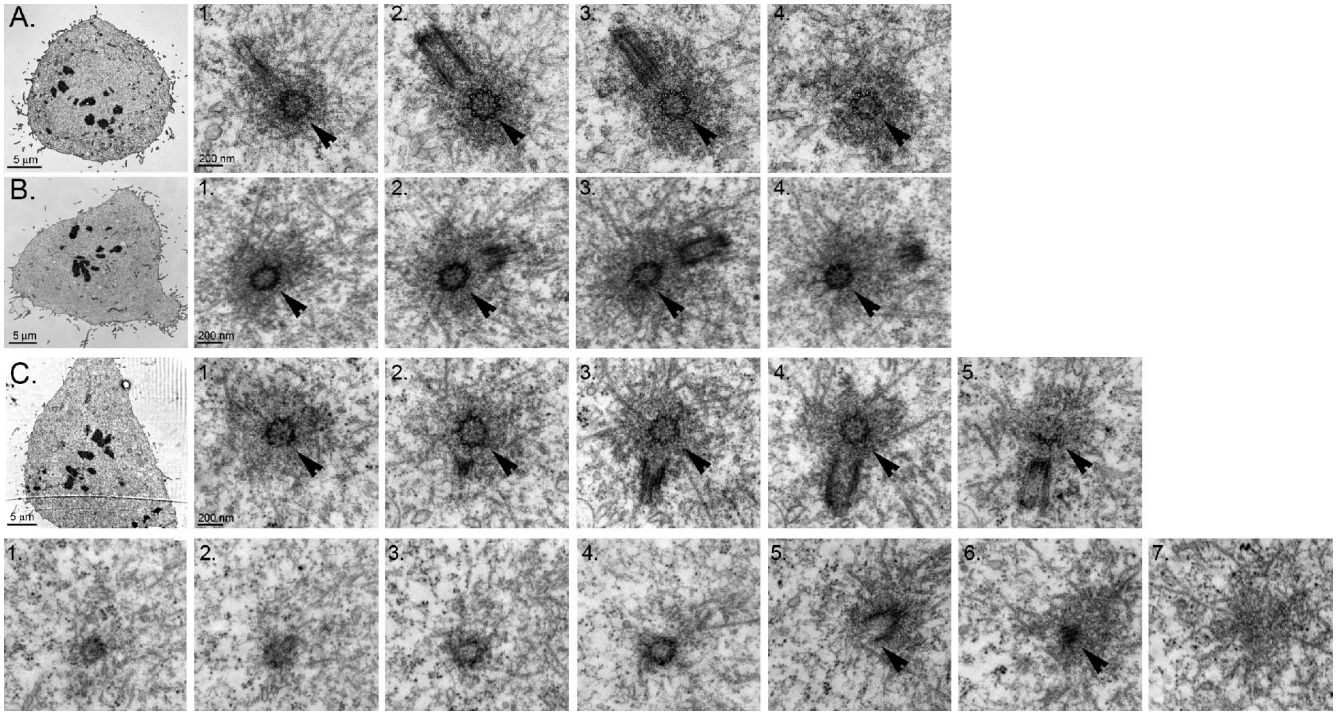

Figure S2. **Serial section transmission EM of mitotic centrosomes.** (A–C) Mitotic RPE1 cells were serially sectioned and examined by EM. Representing images of four centrosomes recovered from three different mitotic cells are shown. Centrioles in three of the centrosomes were positioned in a manner such that mother centrioles were in cross section and the daughter centrioles were longitudinally sectioned. Note that most of the microtubules and electron-dense material are associated with mother centrioles. The fourth centrosome recovered in the cell in C has an opposite orientation, in which the daughter centriole was in cross section; note that PCM (electron-dense material) was clearly associated with the mother, but not daughter, centriole.

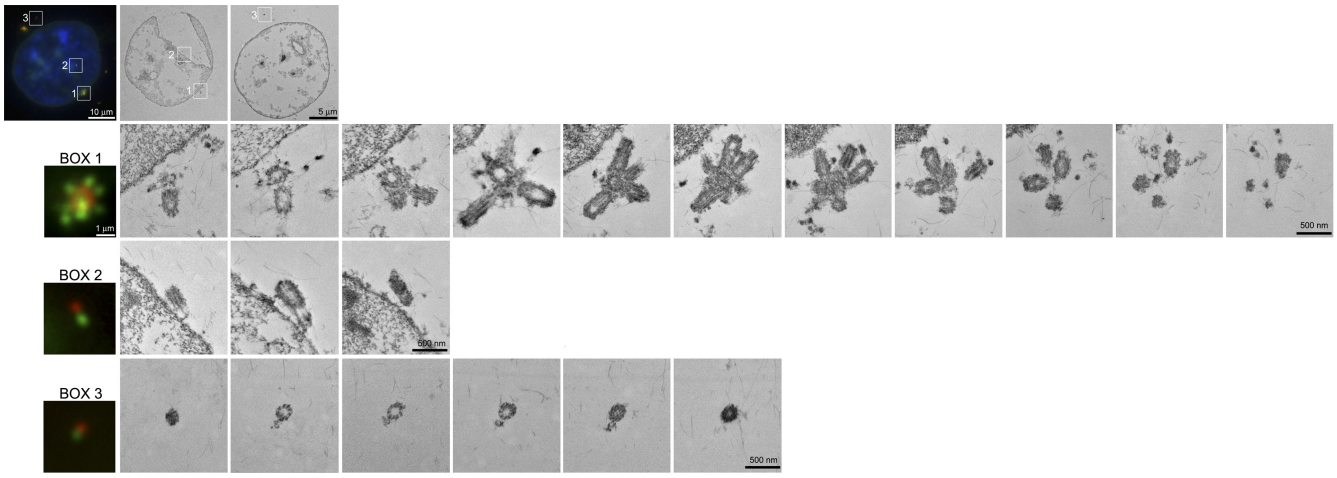

Figure S3. **Analyses of de novo–formed centrioles and centriole rosettes by the correlative light and transmission EM.** Centrioles in RPE1 cells transiently expressing Plk4$^{\Delta SCF}$ as described in Fig. 2 were allowed to enter G2 and labeled with centrin::GFP (green) and anti–hSas-6 antibodies (red). After acquisitions of florescent images, the same cell was processed for serial sectioning and examined by transmission EM. Note that to accommodate the analysis of correlative light EM, cells were permeabilized before centrosome staining and fixation (see Materials and methods). Continuous sections of one centriole rosette (box 1) and two de novo centrioles (box 2 and 3) were shown. Note that the freestanding centrioles in box 2 and 3 were in longitudinal and cross section, respectively.

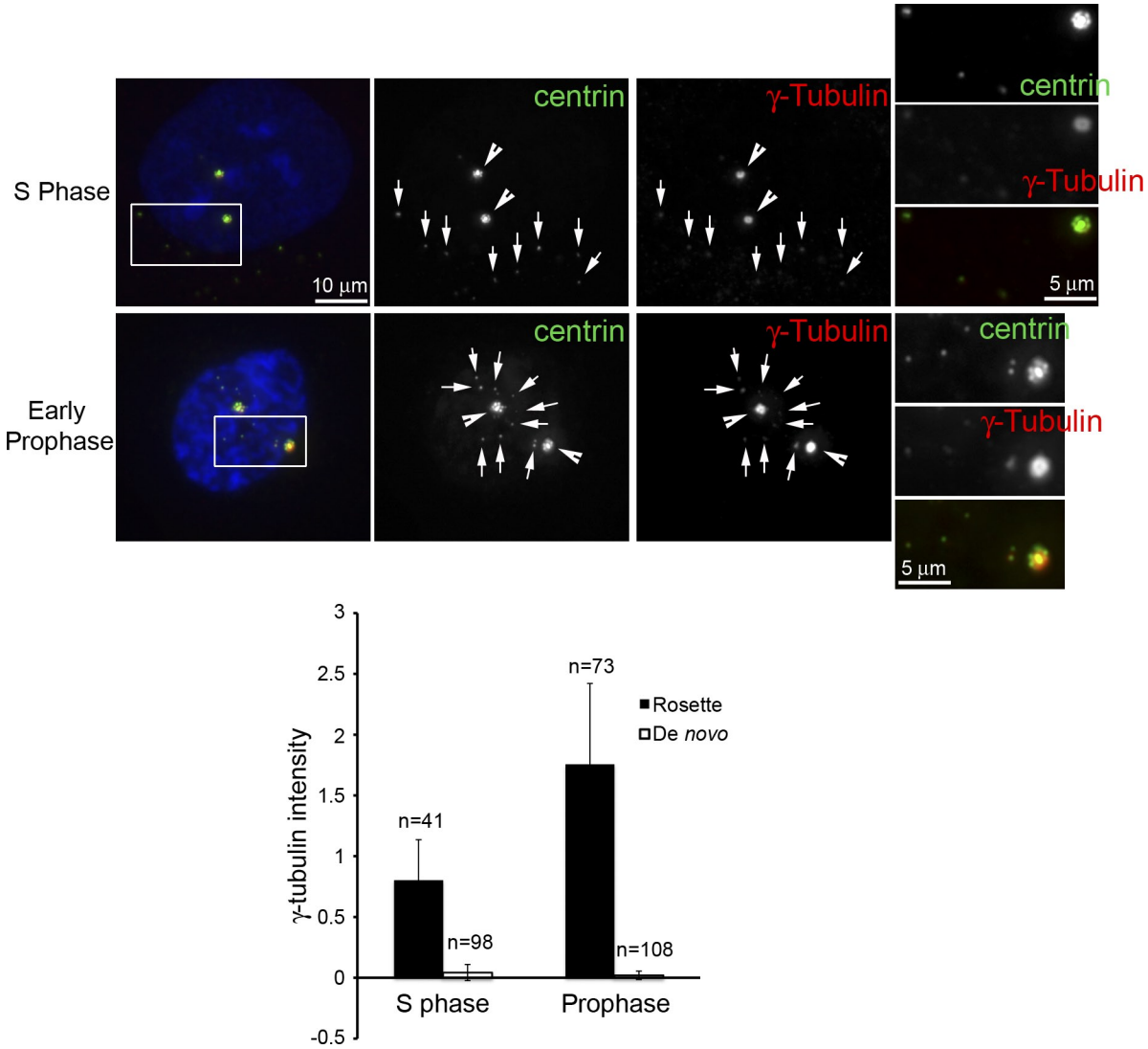

Figure S4. **De novo–formed daughter centrioles contain minimal γ-tubulin signals that are insensitive to cell cycle changes.** De novo centrioles (arrows) and centriole rosettes (arrowheads) were induced in RPE1 cells transiently expressing Plk4$^{\Delta SCF}$ and arrested in S phase as described in Fig. 2. Some cells were then released into early prophase as judged by DAPI staining (blue). Cells were extracted in Pipes buffer before fixation (see Materials and methods). Centrioles were visualized with centrin::GFP and anti–γ-tubulin antibodies. Cropped images containing some of the de novo centrioles and centriole rosettes were magnified for better visualization. γ-tubulin signals associating with centriole rosettes and de novo centrioles were quantified separately at different cell cycle stages. Numbers of centrioles are indicated. Error bars indicate standard deviations. Insets show a higher magnification of centrosomes.

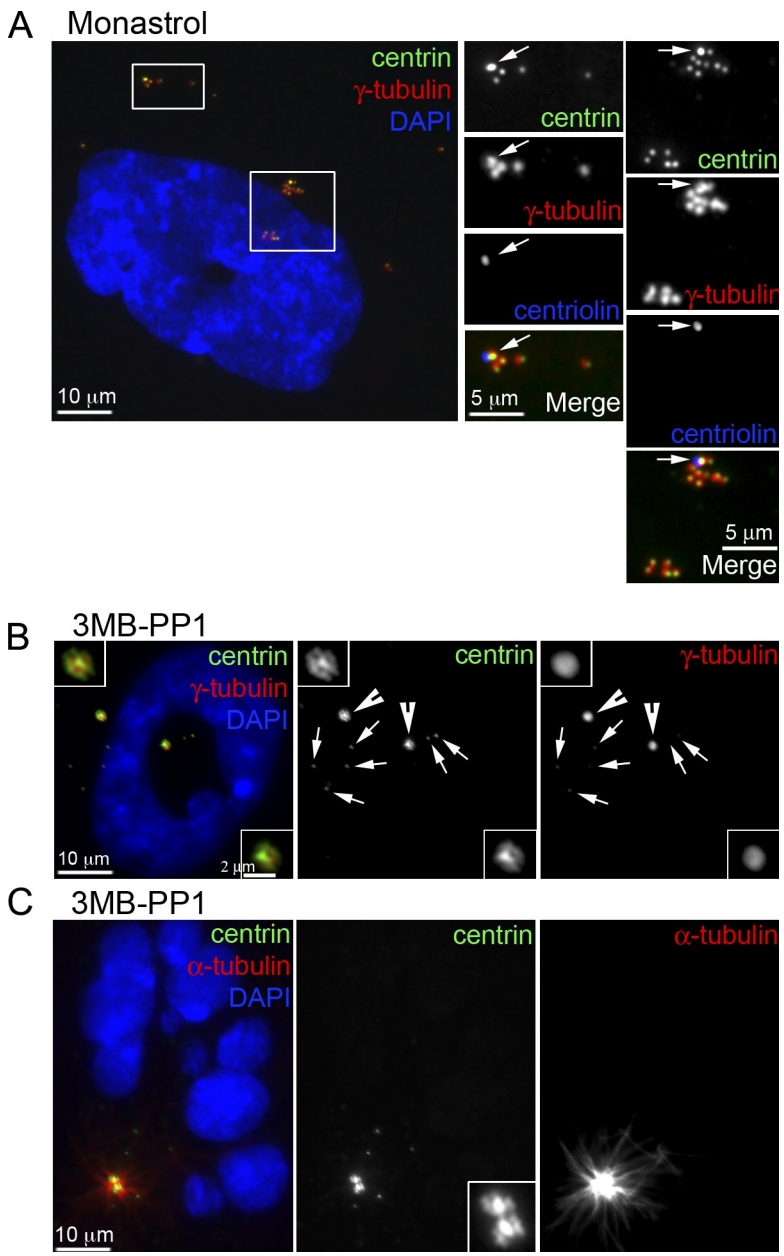

Figure S5. **Plk1 is required for the conversion of de novo–formed daughter centrioles to MTOCs.** (A) The control experiment was performed as follows: de novo centrioles induced in Plk1^{as} cells transiently expressing Plk4$^{\Delta SCF}$ were allowed to pass through mitosis under monastrol inhibition as described in Fig. 4. Cells exiting from mitosis for 5 h were analyzed for centrin, centriolin, and γ-tubulin localizations. Note that all centrioles, including two old/mature mother centrioles marked by centriolin (Gromley et al., 2003), were equally active and recruited similar amounts of γ-tubulin. Arrows indicate old mother centrioles marked by centriolin. (B) De novo centrioles induced as in A passed through mitosis under Plk1 inhibition as described in Fig. 4. Cells exiting from mitosis for more than 16 h were analyzed for centrin and γ-tubulin localizations. In these cells, centriole disengagement failed as the two centriole rosettes remained (insets). The major γ-tubulin foci were formed around the two mother centrioles located at the center of each rosette (arrowheads), whereas de novo centrioles stayed unmodified and contained only minimal centriolar γ-tubulin (arrows). Centrin::GFP is shown. (C) Centrioles in these Plk1-inhibited cells were also analyzed by a microtubule regrowth assay as described in Fig. 2. Microtubule asters formed only around the centriole rosettes, each of which contains a mother centriole actively recruiting PCM. (A and C) Insets show a higher magnification of centrosomes.

Reference

Gromley, A., A. Jurczyk, J. Sillibourne, E. Halilovic, M. Mogensen, I. Groisman, M. Blomberg, and S. Doxsey. 2003. A novel human protein of the maternal centriole is required for the final stages of cytokinesis and entry into S phase. *J. Cell Biol.* 161:535–545. doi:10.1083/jcb.200301105