Lambrus et al., http://www.jcb.org/cgi/content/full/jcb.201502089/DC1

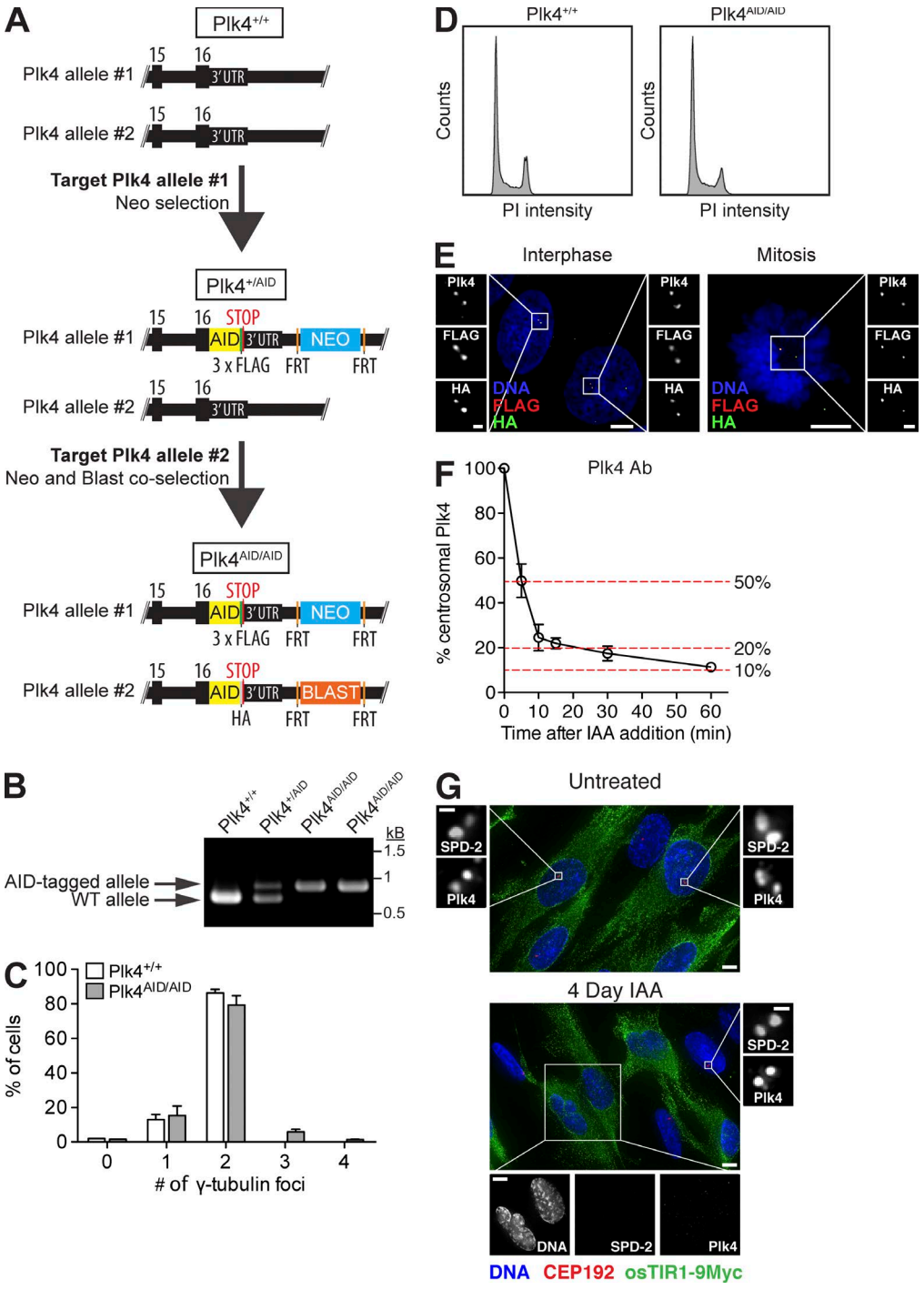


Figure S1. **Generation of Plk4$^{AID/AID}$ cells.** (A) Schematic outlining the strategy for the construction of Plk4$^{AID/AID}$ RPE1 cells. (B) Genotyping of Plk4-AID heterozygous and homozygous cells. Plk4$^{AID/AID}$ cells possess a single band corresponding to the Plk4-AID allele. (C) Quantification of the number of γ-tubulin foci in Plk4$^{+/+}$ and Plk4$^{AID/AID}$ cells. Each bar represents the mean of >100 cells from at least two independent experiments. Error bars represent the SEM. (D) Flow cytometry cell cycle analysis showing normal asynchronous cell cycle profiles for Plk4$^{+/+}$ and Plk4$^{AID/AID}$ cells. (E) Selected images of Plk4$^{AID/AID}$ cells immunostained for Plk4, FLAG, and HA. HA, FLAG, and Plk4 antibody signals colocalize in interphase and mitotic cells. (F) Quantification of the level of Plk4 at the centrosome at the indicated times after IAA addition. Each condition represents the mean of >70 cells from at least two independent experiments. Error bars represent the SEM. (G) Selected images of untreated control or IAA-treated cells immunostained with CEP192, Plk4, and Myc. 4 d after IAA addition, CEP192 and Plk4 are no longer detectable in cells expressing the F-box protein osTIR1-9Myc, but are present in neighboring cells lacking osTIR1-9Myc. Bars: (main) 5 µm; (inset) 1 µm.

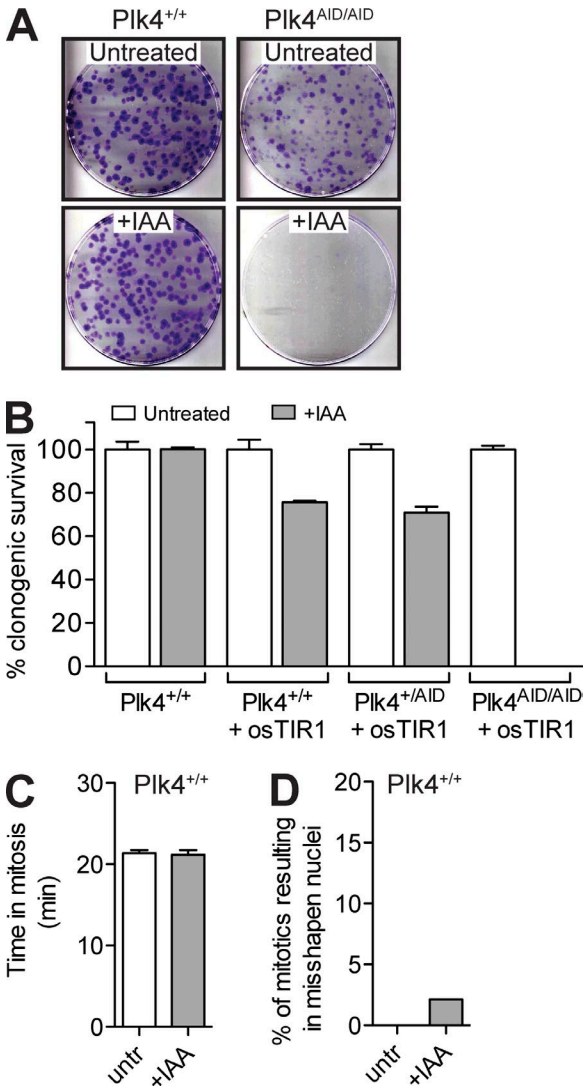


Figure S2. **Destruction of Plk4 leads to a long-term growth arrest.** (A) Representative images of crystal violet–stained colonies formed 2 wk after addition of IAA. (B) Quantification of the percentage of clonogenic survival of the indicated cell lines. Bars represent the mean of at least two independent experiments performed in triplicate. (C and D) Quantification of mitotic duration and the frequency of divisions resulting in the formation of misshapen nuclei in IAA-treated Plk4$^{+/+}$ cells. IAA has a negligible impact on mitotic fidelity. Bars represent the mean of >50 cells from two independent experiments. Error bars represent the SEM.

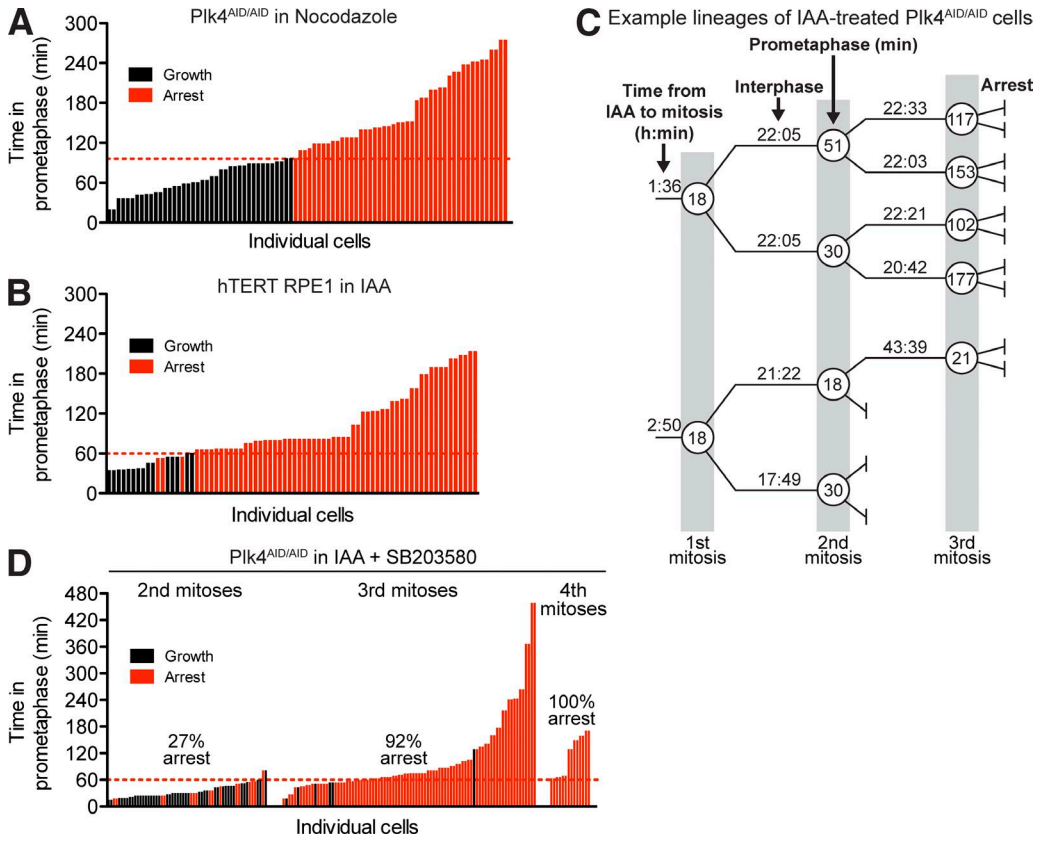


Figure S3. **Inhibiting the activity of p38 MAPK does not prevent the cell cycle arrest caused by centriole duplication failure.** (A–B) Graph showing the prometaphase duration and proliferative capacity of Plk4$^{AID/AID}$ cells or parental RPE1 cells. Each bar represents a daughter cell, its height represents the prometaphase duration of the mother cell, and its color represents the fate of the daughter. The dashed red line indicates the maximum time that cells spend in prometaphase before undergoing a cell cycle arrest. Plk4$^{AID/AID}$ cells were treated with 0.08 µM nocodazole for the first 6 h of imaging, whereas IAA was administered to parental RPE1 cells throughout the experiment. Data were acquired in a single experiment (n = 87 and n = 77 prometaphases, respectively). (C) Example lineages taken from the analysis presented in Fig. 4 D. Note that daughter cells can undergo symmetric or asymmetric fates. (D) Cells were analyzed as in A and B. Only cells that underwent their first mitosis within 6 h of IAA treatment were analyzed. IAA and SB203580 were administered throughout the experiment. Data were taken from two independent experiments (n = 166 prometaphases).

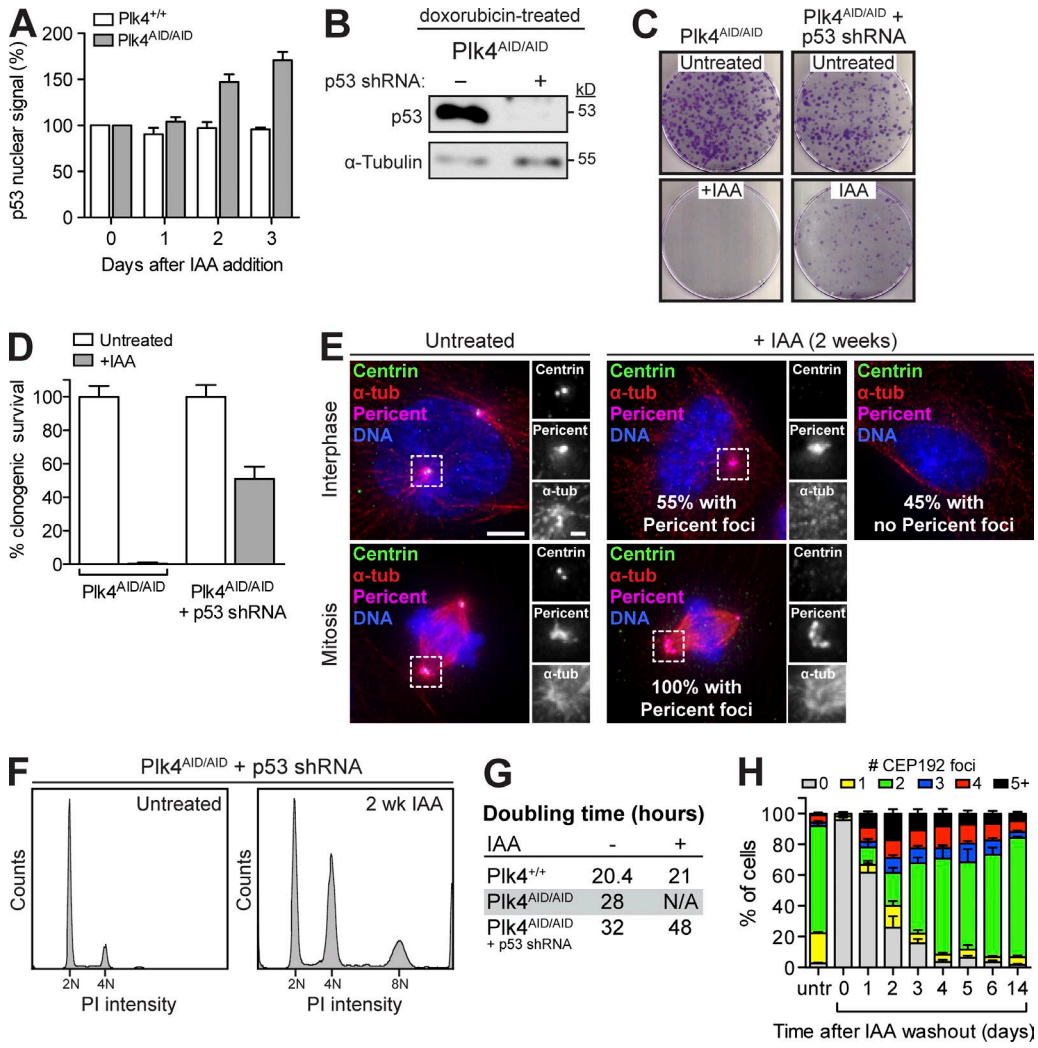


Figure S4. **Cell cycle progression and spindle assembly in the absence of centrioles.** (A) Quantification of the relative level of nuclear p53 in fixed samples at the indicated times after IAA addition. p53 levels increase after Plk4 degradation. Bars represent the mean of >200 cells from at least two independent experiments. (B) Immunoblot showing the level of p53 in doxorubicin-treated cells. (C) Representative images of crystal violet–stained colonies formed 2 wk after addition of IAA. (D) Quantification of the percentage of clonogenic survival of the indicated cell lines. p53 knockdown partly rescues the clonogenic survival of cells lacking Plk4. Bars represent the mean of at least two independent experiments performed in triplicate. All error bars in the figure represent the SEM. (E) Representative images of untreated control or acentriolar cells immunostained with α-tubulin, Pericentrin, and Centrin. Bars: (main) 5 µm; (inset) 1 µm. (F) Flow cytometry cell cycle analysis showing an increase in polyploidy in acentriolar cells. (G) Table displaying the doubling time of the indicated cell lines in the presence and absence of auxin. Doubling times were calculated from the growth curves shown in Figs. 3 A and 5 A. (H) Quantification of the number of CEP192 foci per interphase cell at indicated times after IAA washout in Plk4$^{AID/AID}$;p53 shRNA cells. Bars represent the mean of >200 cells from three independent experiments.

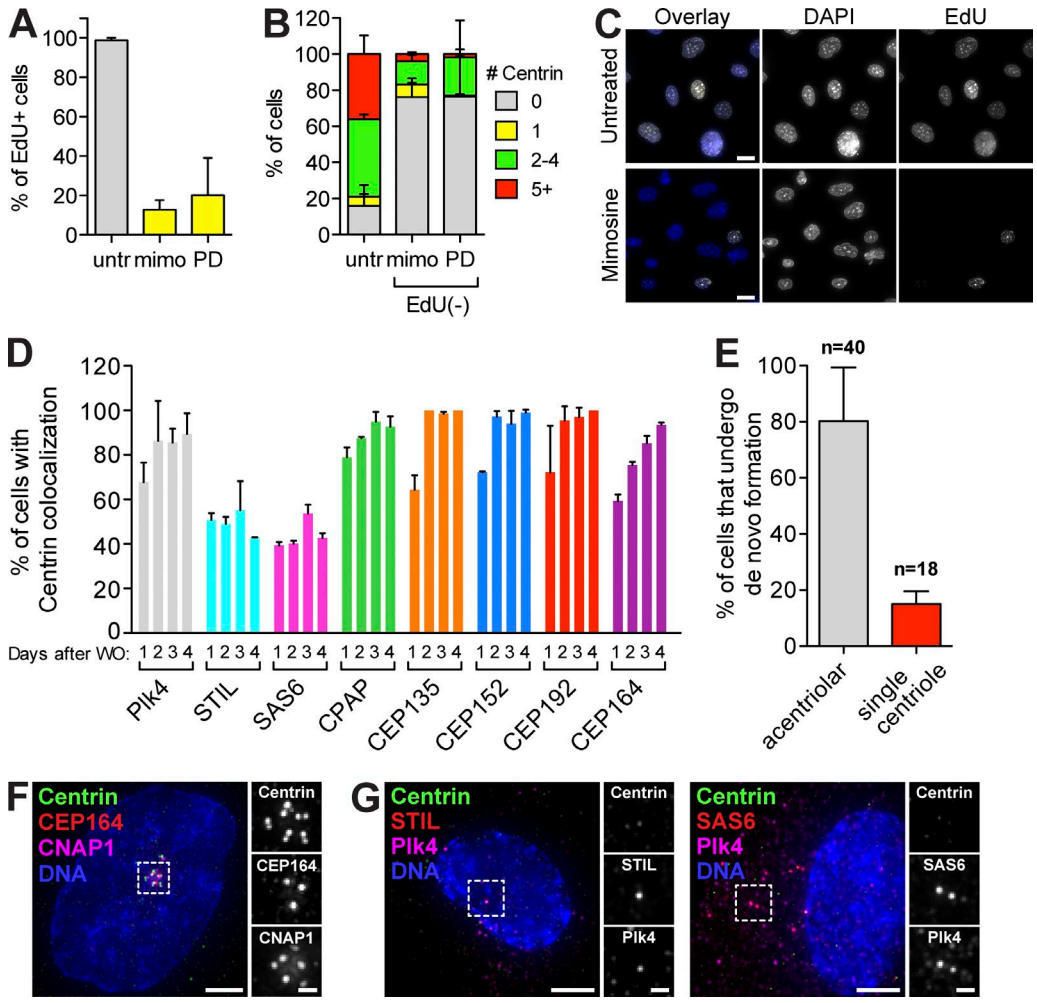
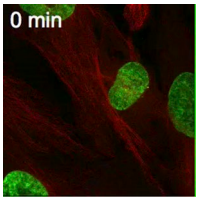
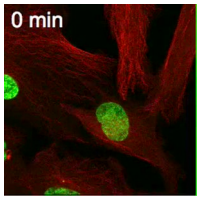


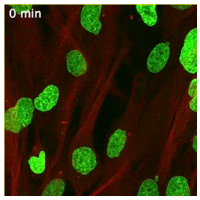
Figure S5. **De novo centriole formation requires cell cycle progression.** (A) Quantification of the fraction of EdU-positive cells. Measurements were made 2 d after release from IAA in the presence or absence of mimosine or PD0332991. Bars represent the mean of >30 cells. (B) Quantification of the fraction of Centrin foci in interphase cells. Measurements were made 2 d after release from IAA in the presence or absence of mimosine or PD0332991. Bars represent the mean of >30 cells. (C) Selected images of control untreated and mimosine-treated cells at 2 d after IAA washout. Cells were costained with DAPI and EdU. Bars, 20 µm. (D) Quantification of the fraction of interphase cells with Centrin-marked de novo centrioles that colocalize with the indicated centriole components. Quantification was derived from fixed images taken 1, 2, 3, and 4 d after IAA washout. Bars represent the mean of >40 cells from two independent experiments. (E) Quantification of the fraction of cells that underwent de novo centriole formation. Acentriolar and single centriole–containing cells expressing EGFP-Centrin were tracked for 60 h by time-lapse microscopy. Bars represent the mean of two independent experiments. All error bars in the figure represent the SEM. (F) Image of de novo centrioles 3 d after IAA washout. Cells were costained with CEP164, CNAP1, and Centrin. (G) Selected images of control de novo centrioles at 1 d after IAA washout. Cells were costained with Centrin, Plk4, and STIL. Plk4 and STIL form foci that do not colocalize with Centrin and likely represent sites of nascent de novo centriole formation. Bars: (main) 5 µm; (inset) 1 µm.



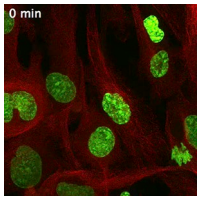
Video 1. **Movie of untreated Plk4$^{AID/AID}$ cell coexpressing TagRFP-tubulin (red), EGFP-Histone H2B (green), and EGFP-Cep63 (green).** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope. Shown in Fig. 3 G (Untreated). One frame captured every 3 min; displayed at 10 frames/s.



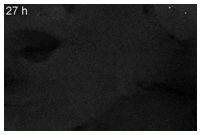
Video 2. **Movie of Plk4$^{AID/AID}$ cell treated for 2 d with IAA and coexpressing TagRFP-tubulin (red), EGFP-Histone H2B (green), and EGFP-Cep63 (green).** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope. Shown in Fig. 3 G (2 days IAA). One frame captured every 3 min; displayed at 10 frames/s.



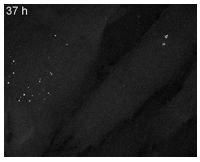
Video 3. **Movie of untreated Plk4$^{AID/AID}$;p53 shRNA cell coexpressing TagRFP-tubulin (red), EGFP-Histone H2B (green), and EGFP-Cep63 (green).** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope. Shown in Fig. 5 E (Untreated). One frame captured every 3 min; displayed at 10 frames/s.



Video 4. **Movie of Plk4$^{AID/AID}$;p53 shRNA cell treated for 2 wk with IAA and coexpressing TagRFP-tubulin (red), EGFP-Histone H2B (green), and EGFP-Cep63 (green).** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope. Shown in Fig. 5 E (Chronic IAA). One frame captured every 3 min; displayed at 10 frames/s.



Video 5. **Movie of an acentriolar Plk4$^{AID/AID}$;p53 shRNA cell expressing EGFP-Centrin.** IAA was washed out at 0 h to allow Plk4 recovery and de novo centriole formation. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope. Shown in Fig. 8 A (Cell 1). One frame captured every 10 min; displayed at 10 frames/s.



Video 6. **Movie of an acentriolar Plk4$^{AID/AID}$;p53 shRNA cell expressing EGFP-Centrin.** IAA was washed out at 0 h to allow Plk4 recovery and de novo centriole formation. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope. Shown in Fig. 8 A (Cell 2). One frame captured every 10 min; displayed at 10 frames/s.