Figure S1

A) DT40 *cdk1as* cells were pre-synchronized in G1 phase by elutriation and progressed through the cell cycle in the presence or absence of 1NMPP1. Samples were taken every 2 hours and analyzed by FACS and immuno-fluorescence to score for centrosome separation. Cells with separated centrosomes were counted. Average of separated centrosomes per cell over time are shown (N>100 for each time point).

B) Transmission electron microscope images of duplicated centrioles in a random section of BI2536 and 1NMPP1 arrested *cdk1as* cells. Quantification of number of centrioles detected in 100 random sections is shown (Scale bar 1μm).

C) Centrosome separation in Plk1WT and *Plk1as* RPE cells after 20 hours 3MBPP1 (20μM) and RO3306 (5μM) treatment. Cells were fixed and analyzed by immuno-fluorescence for γ-tubulin to score centrosomes and CENP-F to identify G2 cells. Images of representative cells (Scale Bar 5μm), quantification of results (N>100) and FACS analysis of PI content are shown.

Figure S2

Representative images of the differentially released cells in Figure 02. Shown are the MIPS of deconvolved 3-D images (Scale bar 5μm) Panels on far right side show close ups the spindle poles as indicated.

Figure S3

A) and B) Tests for phospho-specific Eg5 P-Thr927 antibodies. A) Phosphatase treatment. Chicken Eg5 was immuno-precipitated from mitotic DT40 extracts and treated with alkaline phosphatase. The immuno-precipitates were probed by Eg5 and phospho-specific Eg5 P-Thr 927 antibodies. B) Kinase assays. Bacterially expressed recombinant

GST-Eg5 was subjected to Cdk1 kinase assays and probed with Eg5, and phospho-specific Eg5 P-Thr 927 antibodies. Myc-Cdk1 was stably expressed in DT40 cells and immuno-precipitated after mitotic enrichment by 4 hour nocodazole treatment. Anti Cdk1 antibodies probed the presence of Cdk1 in the immuno-precipitates.

C) Immuno-blots probing extracts of *HeLa* cells at different cell cycle stages with the indicated antibodies. As: asynchronous cells; RO: cells treated for 20 hours with 7.5μM RO3306; RO rel: RO3306 treated cells were released from the G2 arrest by drug removal and blocked in M phase by 20μM MG132 addition for 3 hours; Noc: Cells treated for 20 hours with 0.1μg/ml Nocodazole. D) Immuno-blots of *Hela* cells treated for 22 hours with 10μM RO3306 (RO) and for the final 2 hours with 50μM Roscovitine (RO/Ros). E) FACS profiles of DT40 *cdk1as/cdk2* cells subjected to the indicated treatments. F) Reversal of centrosome separation after inhibition of both Cdk1 and Cdk2. *Cdk1as/cdk2* cells were treated as indicated (see also Figure 5E). Centrosome separation was scored by γ-tubulin immuno-fluorescence using Imaris.

Supplement Movie S1

GFP γ-tubulin expressing *cdk1as* were treated for 8 hours with 100nM BI2536 and 10μM 1NMPP1. The cells were washed 3 times in 50ml medium and then imaged every 30 seconds taking 60x0.4μm stacks for every frame. NEBD occurs between frames 26-28 (at about 13 minutes after the first frame) as judged by the entry of GFP into the nucleus. Centrosomes separate at the onset of the movie and move fast and unidirectional, except for a brief interval at about the time of NEBD.

Supplement Movie S2

As in Movie 1, except that this time 1NMPP1 was kept in the medium and the cells were released only from the Plk1 inhibition. Frames were taken every 2 minutes. Centrosome disjunction first occurs after frame 20 (40 minutes after start) and the movement of the centrosomes is slow and appears to be hindered by opposing forces.

Supplement Movie S3

As in Movie 1, except that this time BI2536 was kept in the medium. The cells were released only from Cdk1 but not Plk1 inhibition. Frames were taken at 30 seconds intervals. NEBD occurs following frame 30 (15 minutes after start). Centrosome initially separate but after NEBD GFP γ-tubulin quickly disperses from the centrosomes, and remains barely visible on what appears to be the mitotic spindle.

Supplement Movie S4

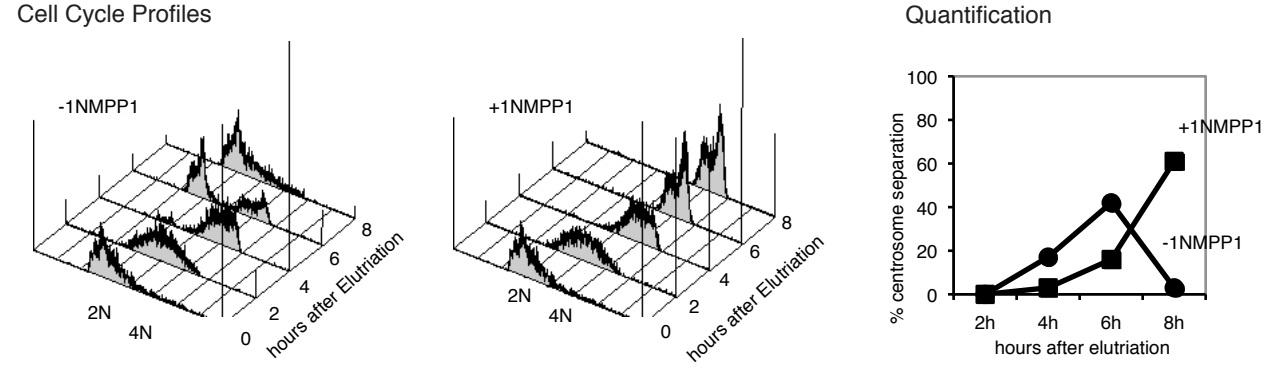
GFP γ-tubulin expressing *cdk1as* cells filmed after 8 hours treatment in 10μM 1NMPP1. Each image is a MIP covering 60 0.4 μM stacks. Frames were taken in 2 minute intervals. Note that the cells also express untagged GFP (see Materials and Methods) that is distributed in the cytoplasm.

Supplement Movie S5

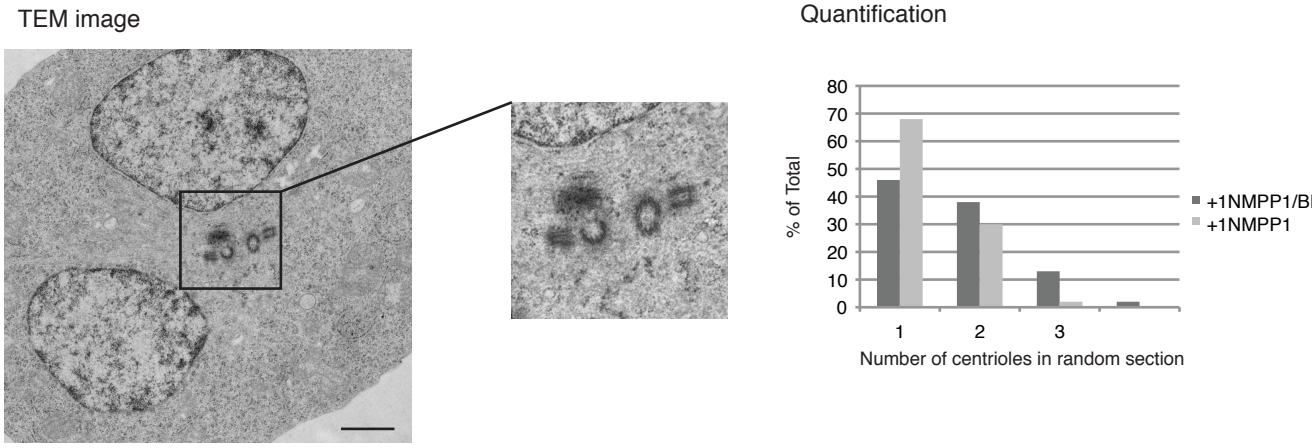
Same as Supplement movie 4 except that 100nM BI2536 was added before imaging.

Figure S1

A) Centrosome separation in pre-synchronized cdk1as cells



B) Random Section of 1NM+BI treated cdk1as cells visualised by TEM



C) Centrosome separation in Plk1as RPE cells

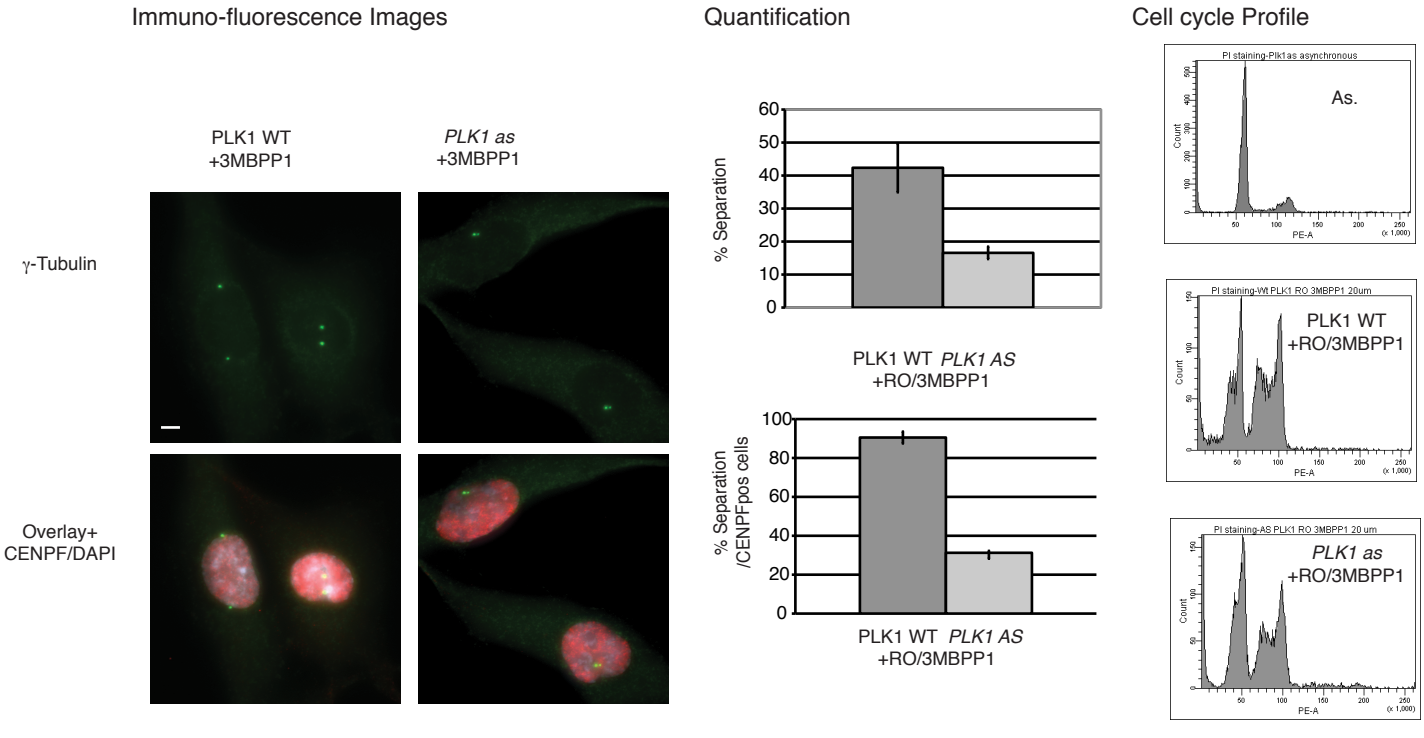


Figure S2 Centrosome separation driven by Cdk1, or Plk1

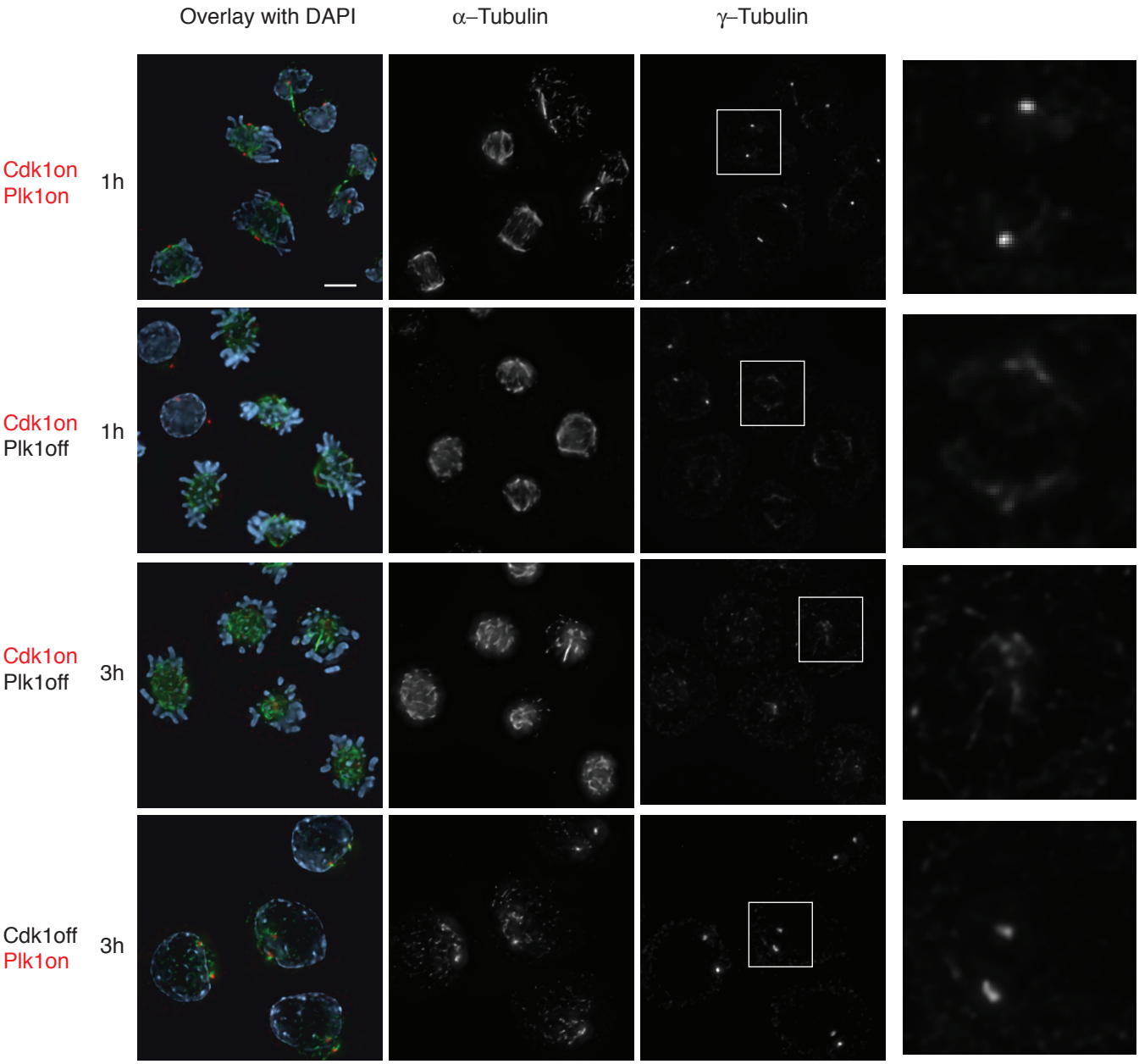
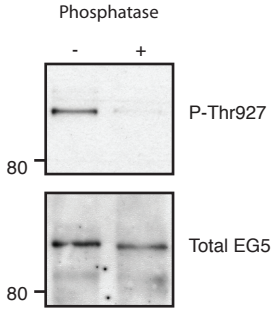
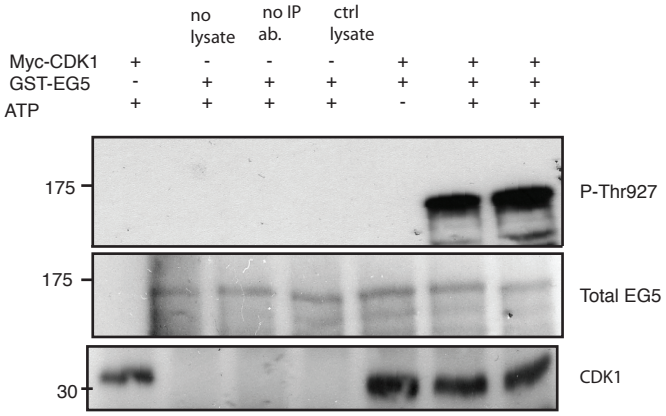


Figure S3

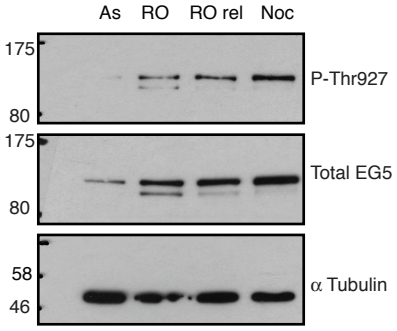
A) Eg5 Thr927P Phospho-specific antibodies Eg5: Phosphatase treatment



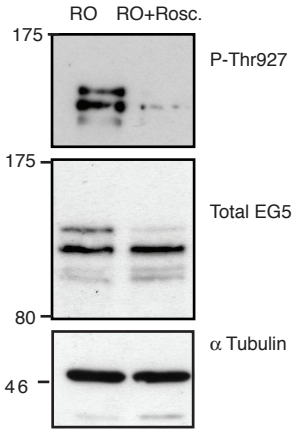
B) Phosphorylation of Eg5 on Thr927 by Cdk1



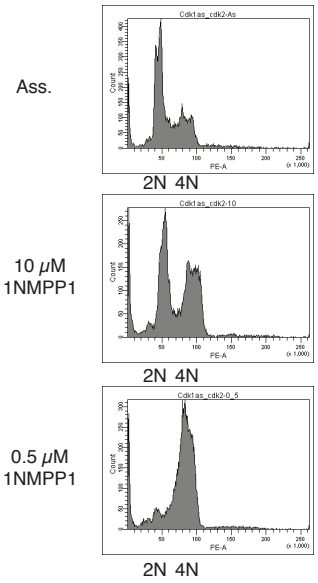
C) Eg5 phosphorylation in HeLa cells



D) Effect of Roscovitine on Eg5 phosphorylation in HeLA cells



E) Effects of 0.5 and 10μM 1NMPP1 on cell cycle progression in *Cdk1as/Cdk2* cells



F) Effects of 0.5 and 10μM 1NMPP1 on centrosome separation in *Cdk1as/Cdk2* cells