Supplemental figure 1. Inter-centrosomal distance in prophase in EICs.

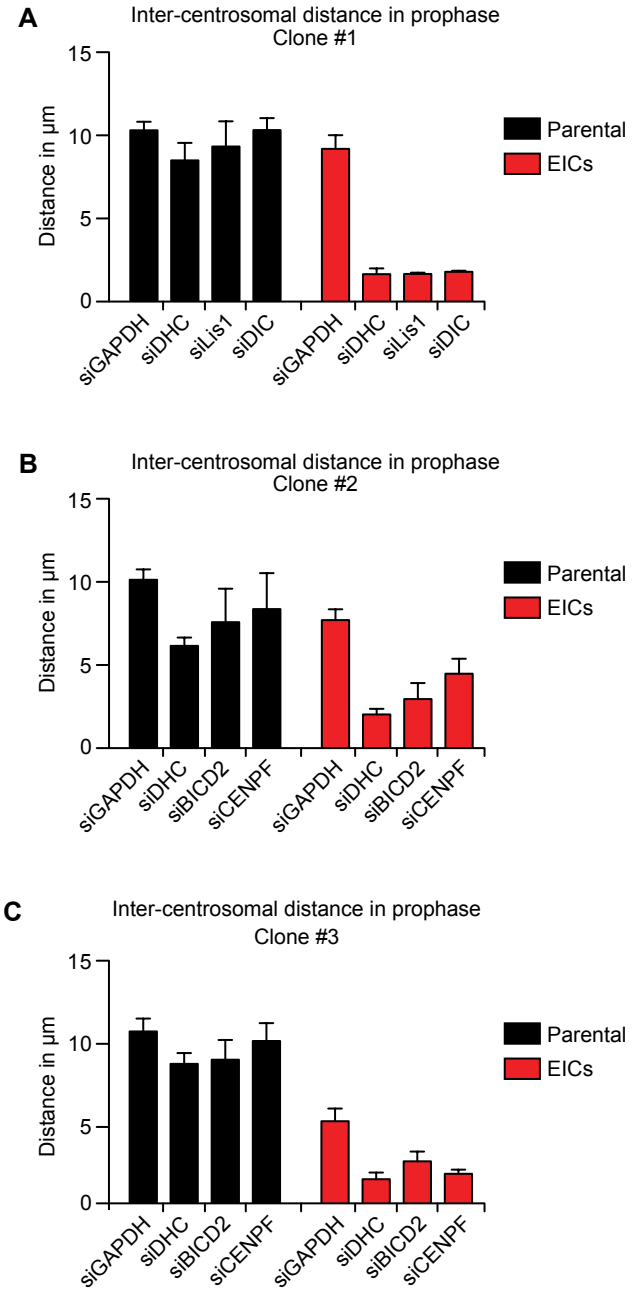

Figure S1. Dynein is required for prophase centrosome separation in EICs.
(A) Parental and EICs (clone #1) were treated for 72 hours with either control (GAPDH), DHC, Lis1 or DIC siRNA, and fixed with formaldehyde. Centrosomes (γ-tubulin) and DNA (DAPI) were stained. Inter-centrosomal distance in prophase was calculated using Zeiss LSM 510 confocal software (n = 45 per condition). (B, C) Parental and EICs (clone #2 and #3) were treated for 72 hours with either control (GAPDH), DHC, BICD2 or CENPF siRNA, and fixed with formaldehyde and stained as in (A). Inter-centrosomal distance in prophase was calculated as in (A) (n = 45 per condition). Results in (A), (B) and (C) are averages of at least three independent experiments. Error bars represent SD.

Supplemental figure 2. NE dynein activity is lost after depletion of BICD2 or CENPF

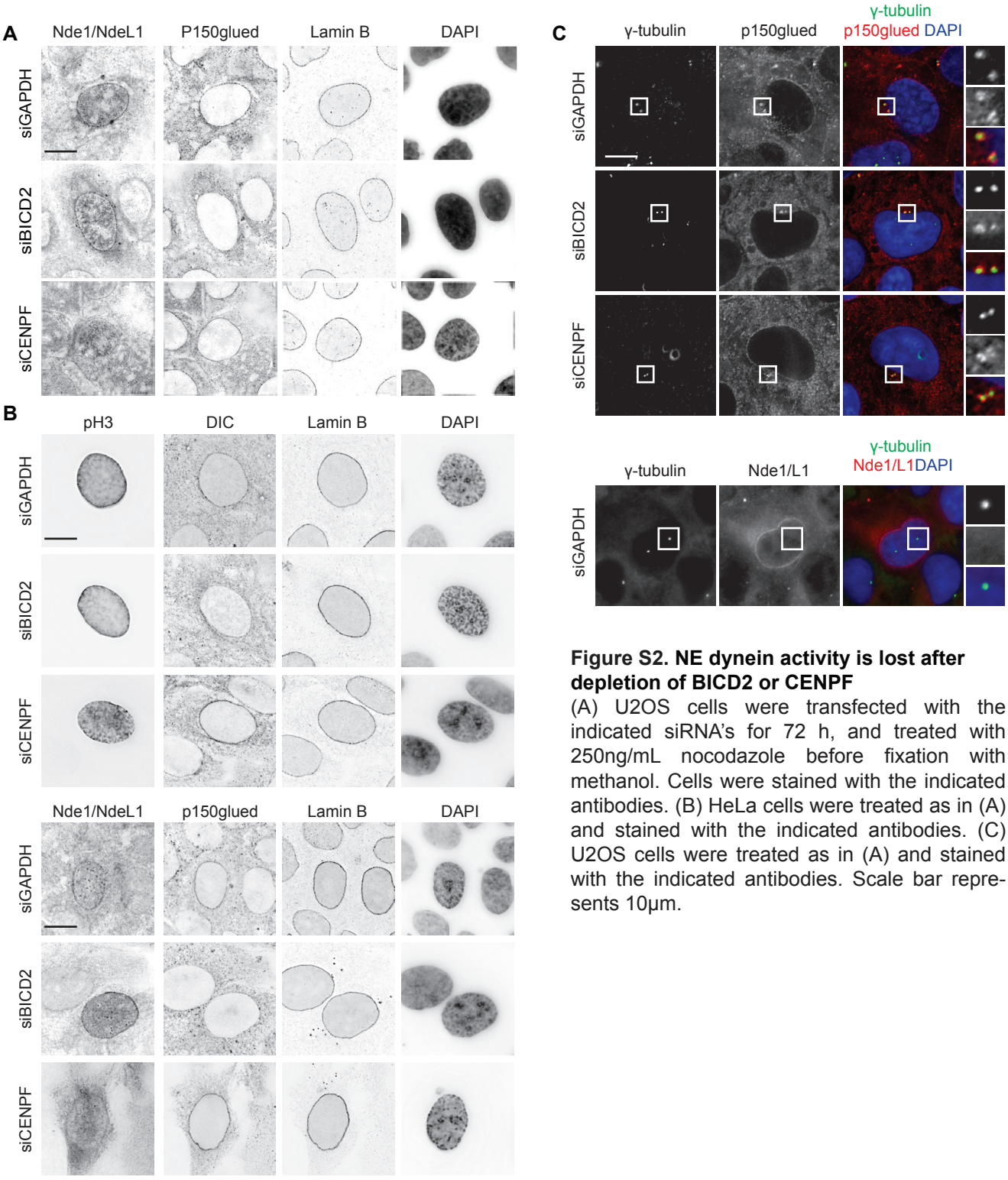

Figure S2. NE dynein activity is lost after depletion of BICD2 or CENPF

(A) U2OS cells were transfected with the indicated siRNA's for 72 h, and treated with 250ng/mL nocodazole before fixation with methanol. Cells were stained with the indicated antibodies. (B) HeLa cells were treated as in (A) and stained with the indicated antibodies. (C) U2OS cells were treated as in (A) and stained with the indicated antibodies. Scale bar represents 10μm.

Supplemental figure 3. Characterization of BICD2 and CENPF siRNA's

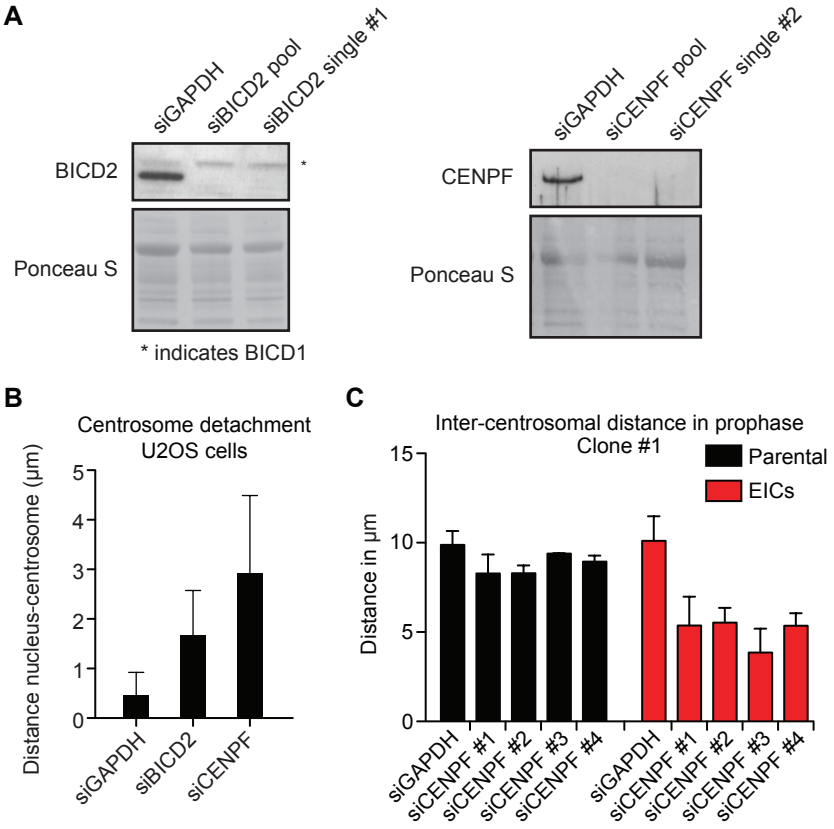

Figure S3. Characterization of BICD2 and CENPF siRNA's
(A) U2OS cells were transfected with indicated siRNA's and harvested 72h after transfection. Total protein levels of BICD2 and CENPF were analyzed by western blot. (B) U2OS cells were treated with the indicated siRNA's and fixed with formaldehyde 72h post-transfection. Cells were stained for centrosomes (γ-tubulin) and DNA (DAPI). (C) Parental and EICs (clone #1) were treated for 72 hours with the indicated siRNA's and fixed with formaldehyde. Centrosomes (γ-tubulin) and DNA (DAPI) were stained. Centrosome detachment and separation in prophase was calculated using Zeiss LSM 510 confocal software (n = 45 per condition). Results in (B) and (C) are averages of at least three independent experiments. Error bars represent SD.

Supplemental figure 4. Centrosome detachment is independent of Eg5 activity

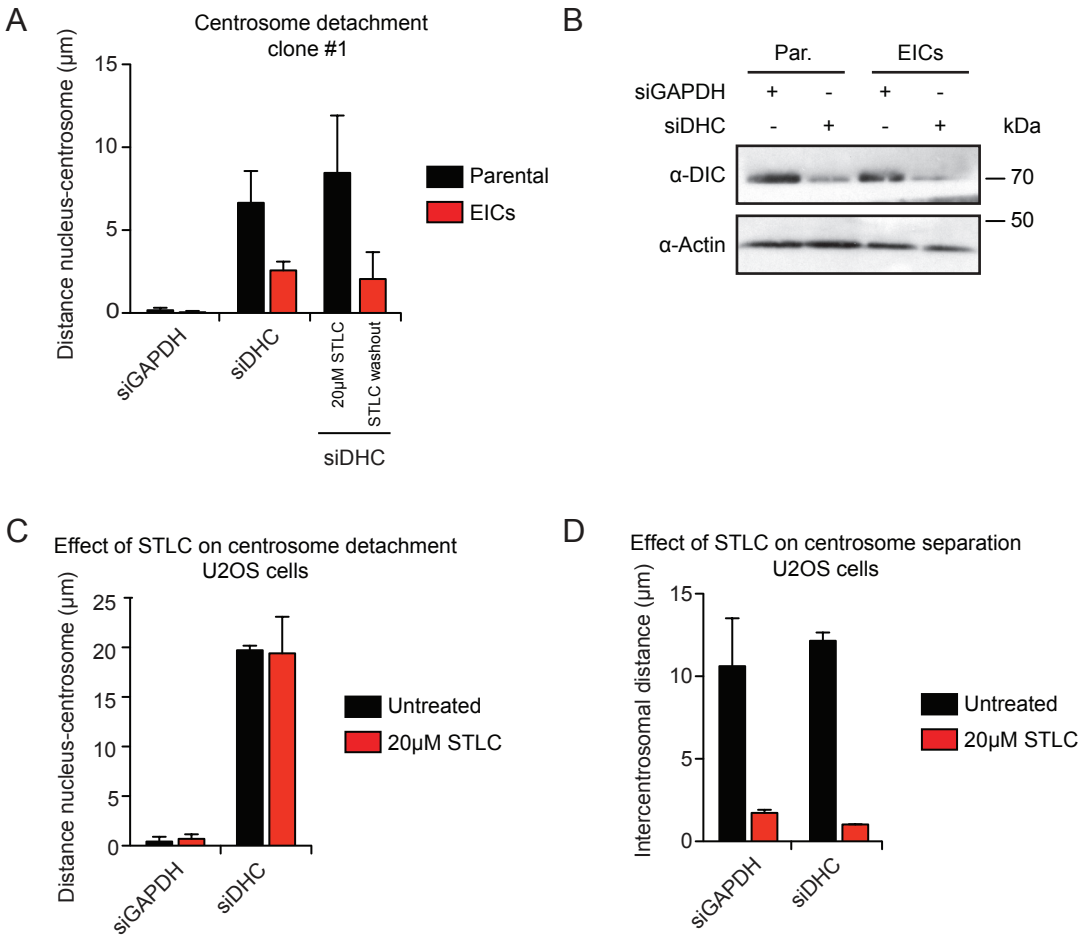

Figure S4. Centrosome detachment is independent of Eg5 activity.
(A) Parental and EICs (clone #1) were treated for 72 hours with either control (GAPDH) or DHC siRNA, and fixed with formaldehyde and stained for γ-tubulin to visualize the centrosomes, DAPI to visualize the nucleus and pH3 as a mitotis marker. The distance between centrosomes and nucleus was determined in prophase cells (n = 45 cells per condition). (B) Parental and EICs (clone #1) were transfected with either GAPDH or DHC siRNA and protein levels were analyzed by western blot 72 hours post-transfection. (C) U2OS cells were transfected with either control (GAPDH) or DHC siRNA for 72 hours and treated with a high dose of STLC (20µM) 16 hours before fixation or left untreated. Cells were stained and stained as in (A) Inter-centrosomal distance in prophase was determined (n = 45 per condition). (D) Inter-centrosomal distance in cells from (C). Results in (A), (C) and (D) are averages of at least three independent experiments. Error bars represent SD.

Supplemental figure 5. Depletion of kinesin-1 does not affect centrosome separation

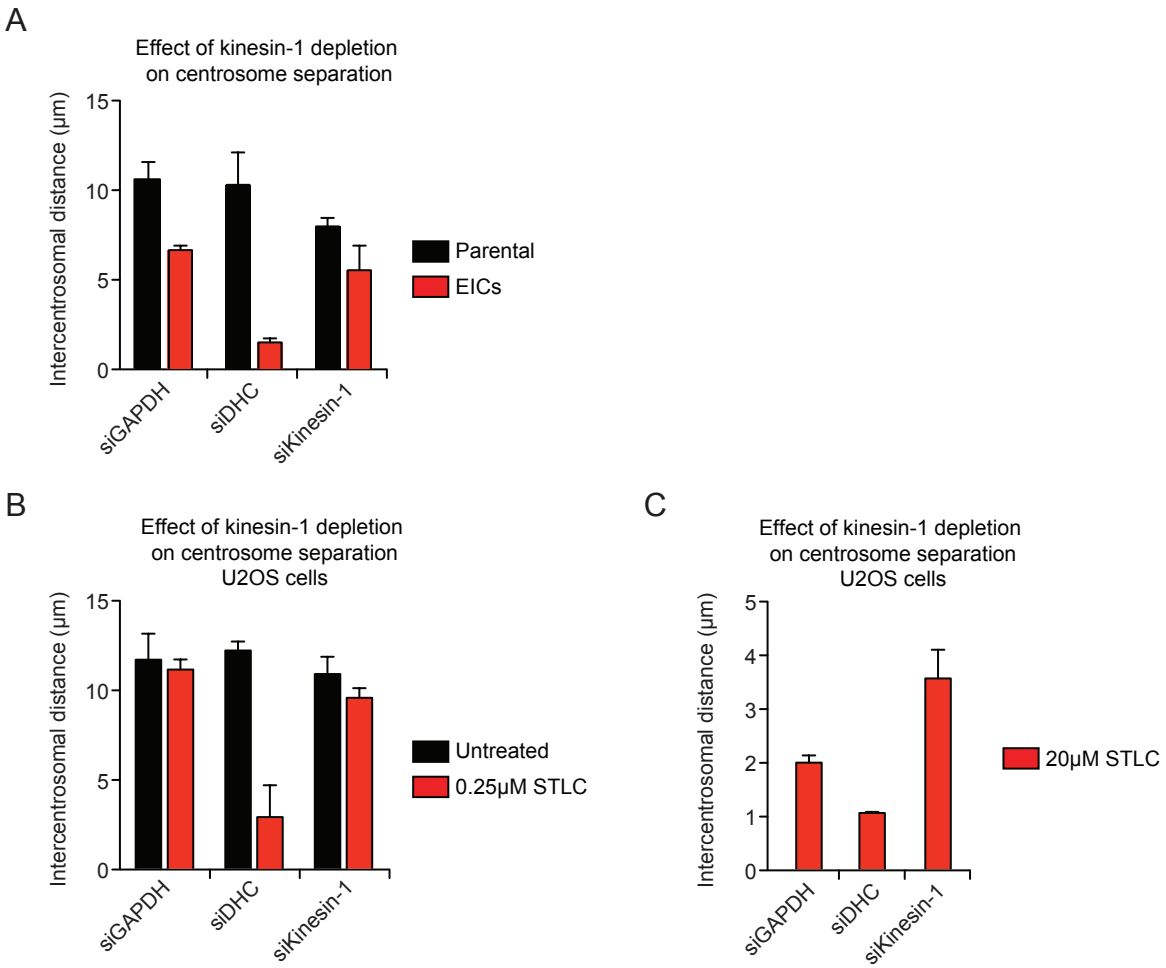

Figure S5. Depletion of kinesin-1 does not affect centrosome separation.
(A) Parental and EICs (clone #1) were treated for 72 hours with either control (GAPDH), DHC, or Kinesin-1 siRNA. Cells were stained for centrosomes (γ-tubulin), DNA (DAPI) and pH3 as a mitosis marker. Intercentrosomal distance was determined in prophase cells (n = 45 per condition). (B) U2OS cells were treated for 72 hours with either control (GAPDH), DHC, or Kinesin-1 siRNA. 0.25 µM STLC was added to the cells 16 hours before fixation or cells were left untreated. Cells were stained as in (A) and intercentrosomal distance was determined (n = 45 per condition). (C) U2OS cells were transfected as in (B). 20 µM STLC was added 16 hours before fixations. Cells were stained as in (A) and intercentrosomal distance was determined (n = 45 per condition). Results in (A), (B) and (C) are averages of at least three independent experiments. Error bars represent SD.

Supplemental figure 6. DIC, P150glued, CENPF and BICD2 levels are not increased at the nuclear envelope in the EICs

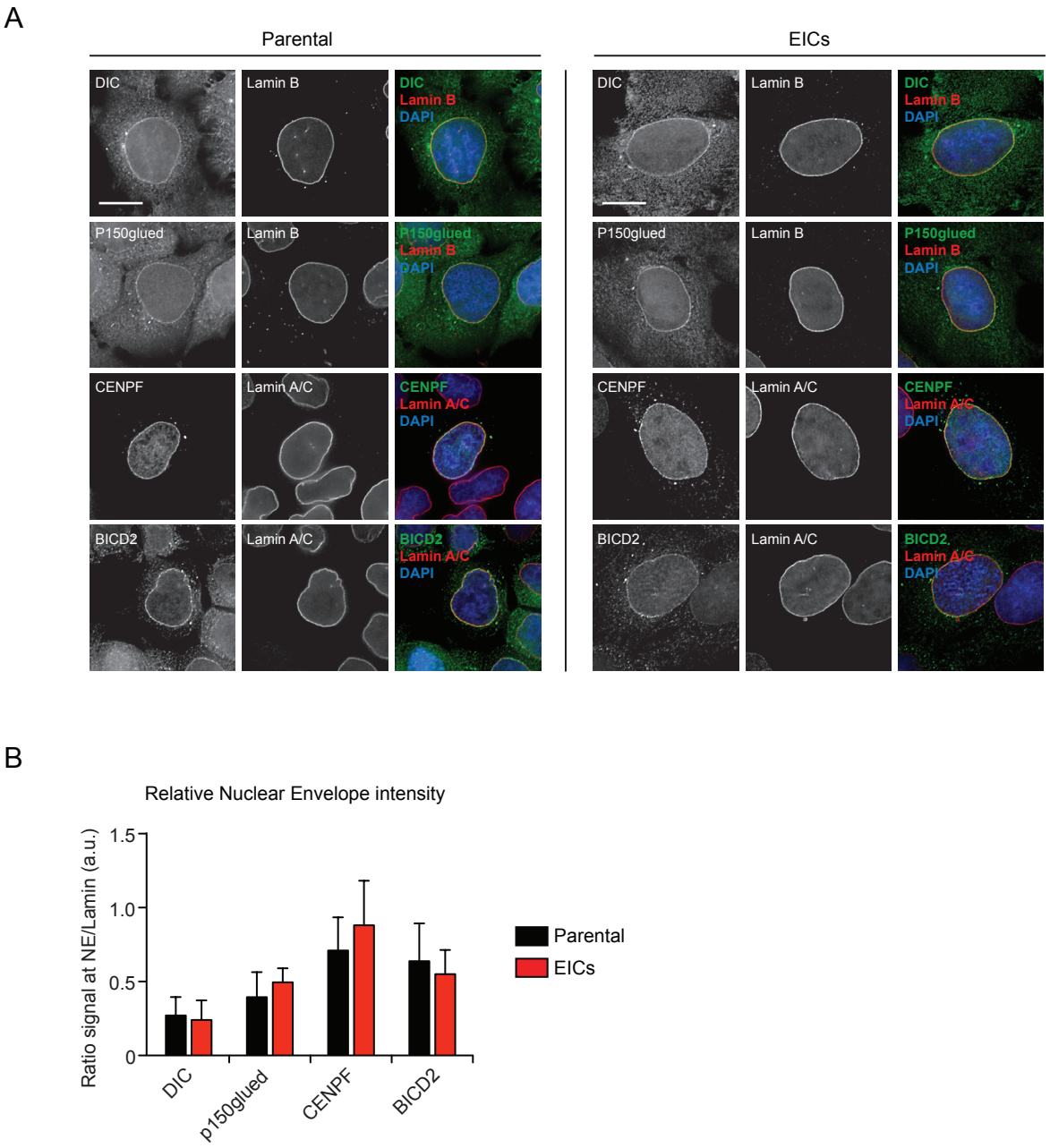

Figure S6. DIC, P150glued, CENPF and BICD2 levels are not increased at the nuclear envelope in the EICs
(A) Parental and EICs (clone #1) were treated with 250ng/mL nocodazole for five hours and fixed in MeOH. Cells were stained with indicated antibodies. Representative images for each staining are showed. Scale bars represent 10 µm (B) Quantification of NE-stainings from (A). Ratio between indicated NE factor and either Lamin B or Lamin A/C was determined for at least 10 cells per condition. No statistical significant differences were found between parental and EICs. Error bars indicate SD.

Supplemental figure 7. Inter-centrosomal distance in prophase in HeLa cells.

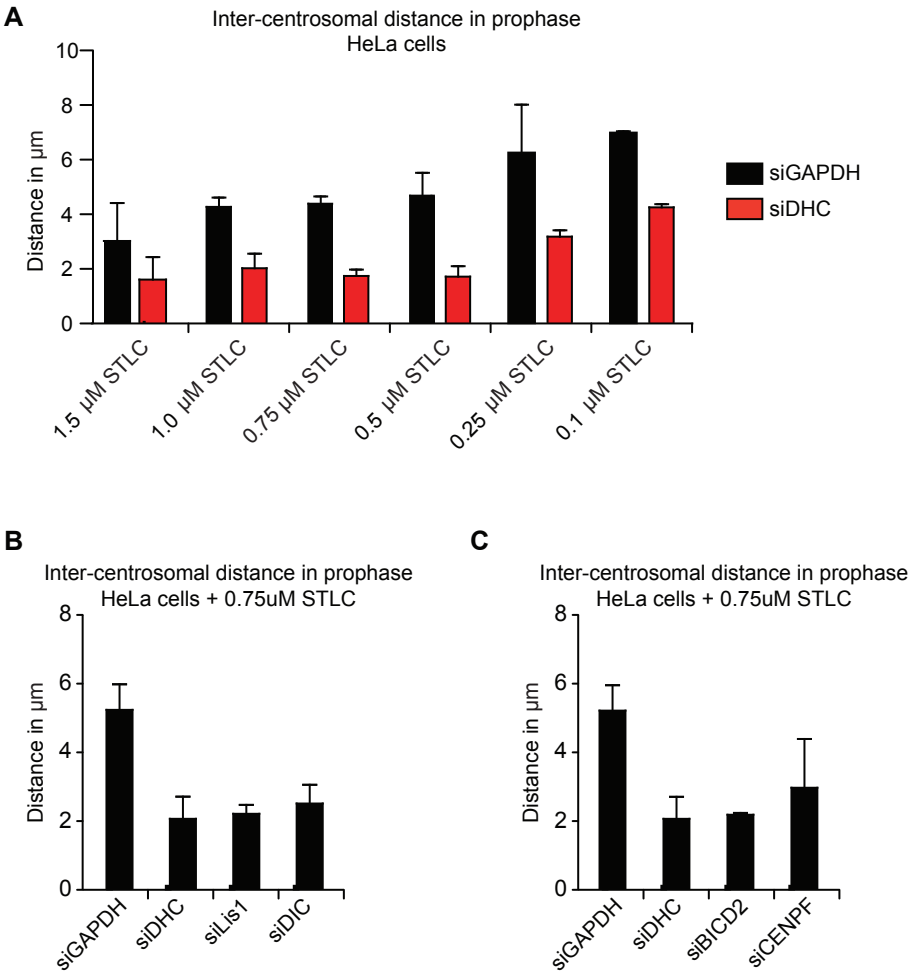

Figure S7. Inter-centrosomal distance in prophase in HeLa cells.
(A) STLC titration curve. HeLa cells were transfected with siRNAs targeting either control (GAPDH) or DHC. The indicated concentrations of STLC were added to the cells 16 hours before fixation (n = 30 per condition). Cells were fixed with formaldehyde and centrosomes (γ-tubulin) and DNA (DAPI) were stained. (B) HeLa cells were treated with siRNAs targeting the indicated dynein components. 0.25 µM STLC was added to the cells 16 hours before fixation. Cells were fixed and stained as in (A). Inter-centrosomal distance in prophase was calculated as in Fig. S1A (n = 30 per condition). (C) HeLa cells were treated for 72 hours with either control (GAPDH), DHC, BICD2, or CENPF siRNA. Cells were treated with 0.25 µM STLC 16 hours before fixation. Cells were fixed and stained as in (A). Inter-centrosomal distance in prophase was calculated as in Fig. S1A (n = 30 per condition). Results in (A), (B) and (C) are averages of at least three independent experiments. Error bars represent SD.

Supplemental Figure 8. The dynein complex cooperates with Eg5 to drive prophase centrosome separation.

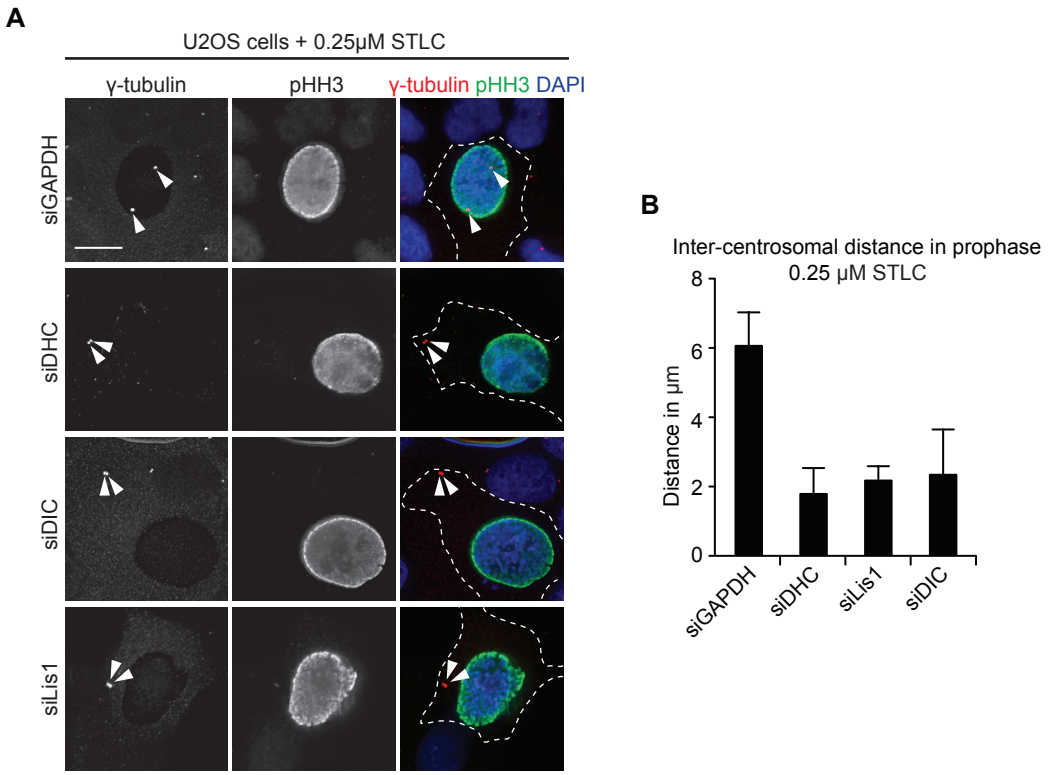

Figure S8. The dynein complex cooperates with Eg5 to drive prophase centrosome separation.
(A) B) U2OS cells were treated for 72 hours with either control (GAPDH), DHC, DIC, or LIS1 siRNA. 16 hours before fixation, cells were treated with 0.25 μM STLC. Centrosomes (γ-tubulin), prophase cells (pH3) and DNA (DAPI) were stained. (B) Quantification of inter-centrosomal distance in prophase from (A) (n = 30 per condition).

Supplemental figure 9. Plk4 depletion inhibits centriole duplication.

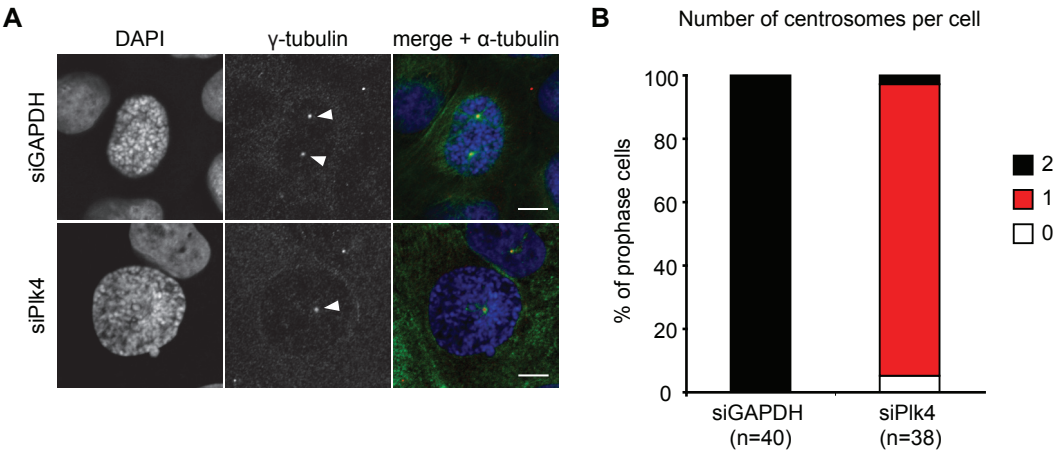

Figure S9. Plk4 depletion inhibits centriole duplication.
(A) U2OS cells were treated for 48 hours with either control (GAPDH) or Plk4 siRNA, and fixed with formaldehyde. Centrosomes (γ-tubulin), microtubules (α-tubulin) and DNA (DAPI) were stained. The number of centrosomes was counted in prophase cells. Arrows mark the centrosomes. (B) Quantification of the number of centrosomes from (A). Scale bars represent 5 μm.

Supplemental Movie 1.
Tracking of individual centrosome in U2OS cell stably expressing mCherry-α-tubulin depleted for Plk4. Images were acquired every 60 seconds.

Supplemental Movie 2.
Tracking of individual centrosome in U2OS cell stably expressing mCherry-α-tubulin depleted for Plk4 and DHC. Images were acquired every 60 seconds.

Supplemental Movie 3.
Tracking of individual centrosome in U2OS cell stably expressing mCherry-α-tubulin depleted for Plk4 and BICD2. Images were acquired every 60 seconds.