Supplemental Materials

Molecular Biology of the Cell

Nunes et al.

Supplementary Information for

Centrosome-nuclear axis repositioning drives the assembly of a bipolar spindle scaffold to ensure mitotic fidelity

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This PDF file includes:

Figures S1 to S7
Legends for figures S1 to S7
Captions for Movies S1 to S5

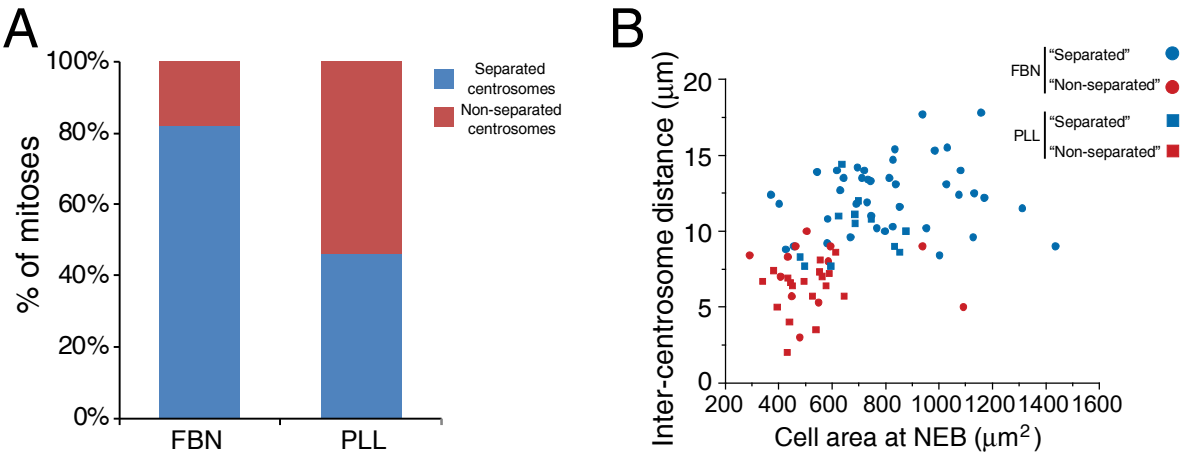

Figure S1 - Effect of substrate coating on centrosome separation efficiency, related to Figure 1

(A) HeLa cells expressing H2B-GFP/mRFP-alpha-tubulin were seeded either on FBN (n=59) or PLL (n=41) and imaged to determine whether they separated ("Separated") or not ("Non-separated") their centrosomes during mitotic entry. (B) Correlation between inter-centrosome distance (µm) and cell area at NEB (µm^2) for cells seeded in FBN or PLL, taking into account their centrosome separation pathway. Cells with decreased area have lower inter-centrosome distances.

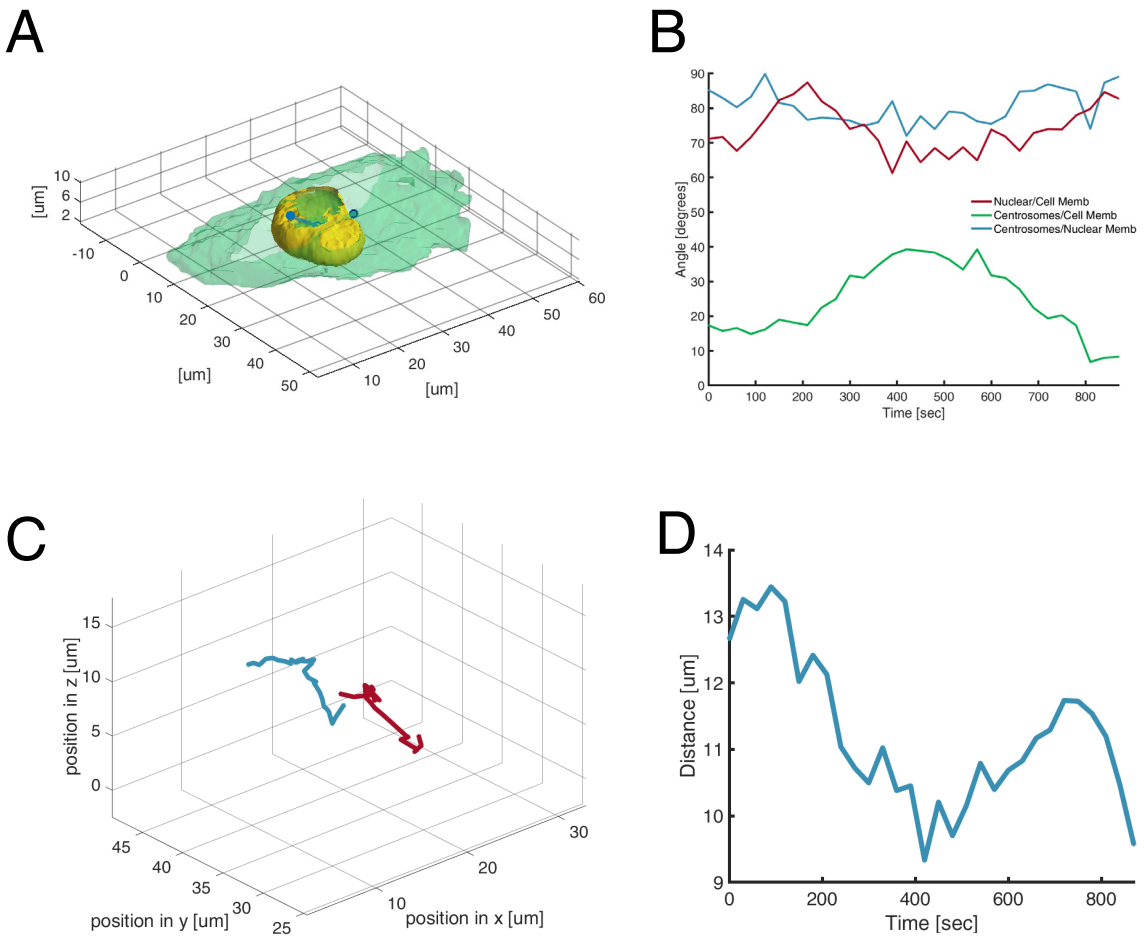

Figure S2 - Computational analysis of centrosomes, cell shape and nuclear shape, related to Figure 1

(A) Representative reconstruction of HeLa cell seeded on a line micropattern, showing centrosomes (blue), nucleus (yellow) and cell membrane (green). Centrosomes are connected by a vector that runs through the nucleus centroid. (B) Plot quantifying angles between centrosomes/nucleus long axis (green), centrosomes/cell membrane (blue) and cell membrane/nucleus (red) over time. (C) Representative 4D reconstruction of centrosomes trajectories. (D) Representative plot showing pole-to-pole distance over time.

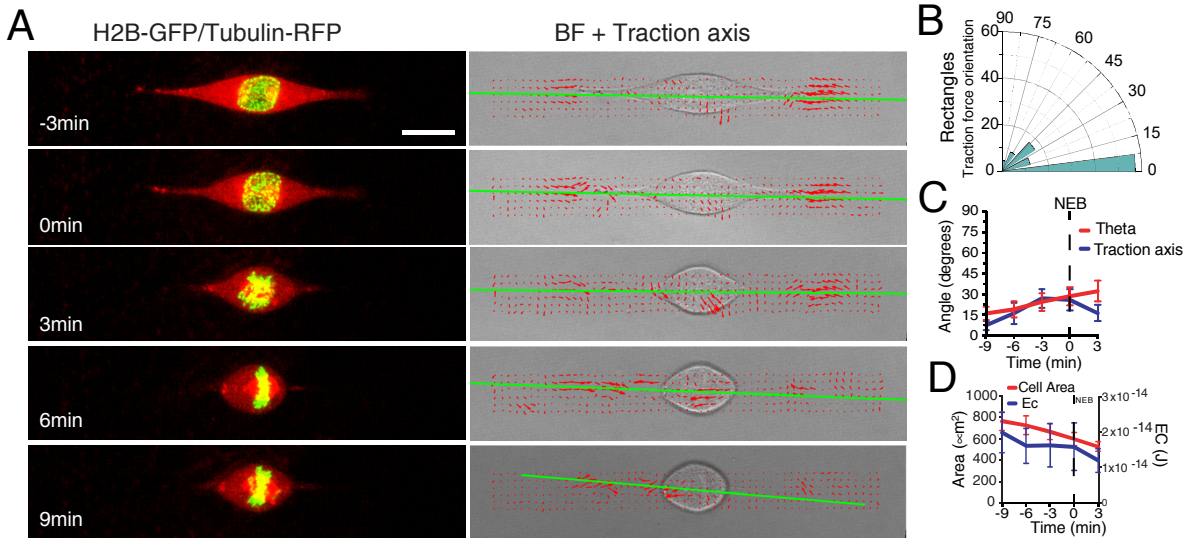

Figure S3 - Cell rounding changes the forces exerted by cells on the substrate , related to Figure 4

(A) Frames from a movie of a HeLa cell expressing H2B-GFP/alpha-tubulin-RFP (left panel) seeded on a PAA hydrogel with a rectangle micropattern. Right panel corresponds to brightfield (BF) image overlaid with the corresponding traction force map (red arrows). Green line corresponds to the main direction of the force dipole. Time is in min. NEB = 0min. (B) Traction force orientation for cells on rectangles (n=14). (C) Correlation between centrosome orientation axis (theta; red) and traction axis (blue). Lines corresponds to average values. Error bars correspond to SEM. (D) Correlation between contractile energy (EC; blue) and cell area (red) for cells seeded on rectangles. Lines corresponds to average values. Error bars correspond to SEM.

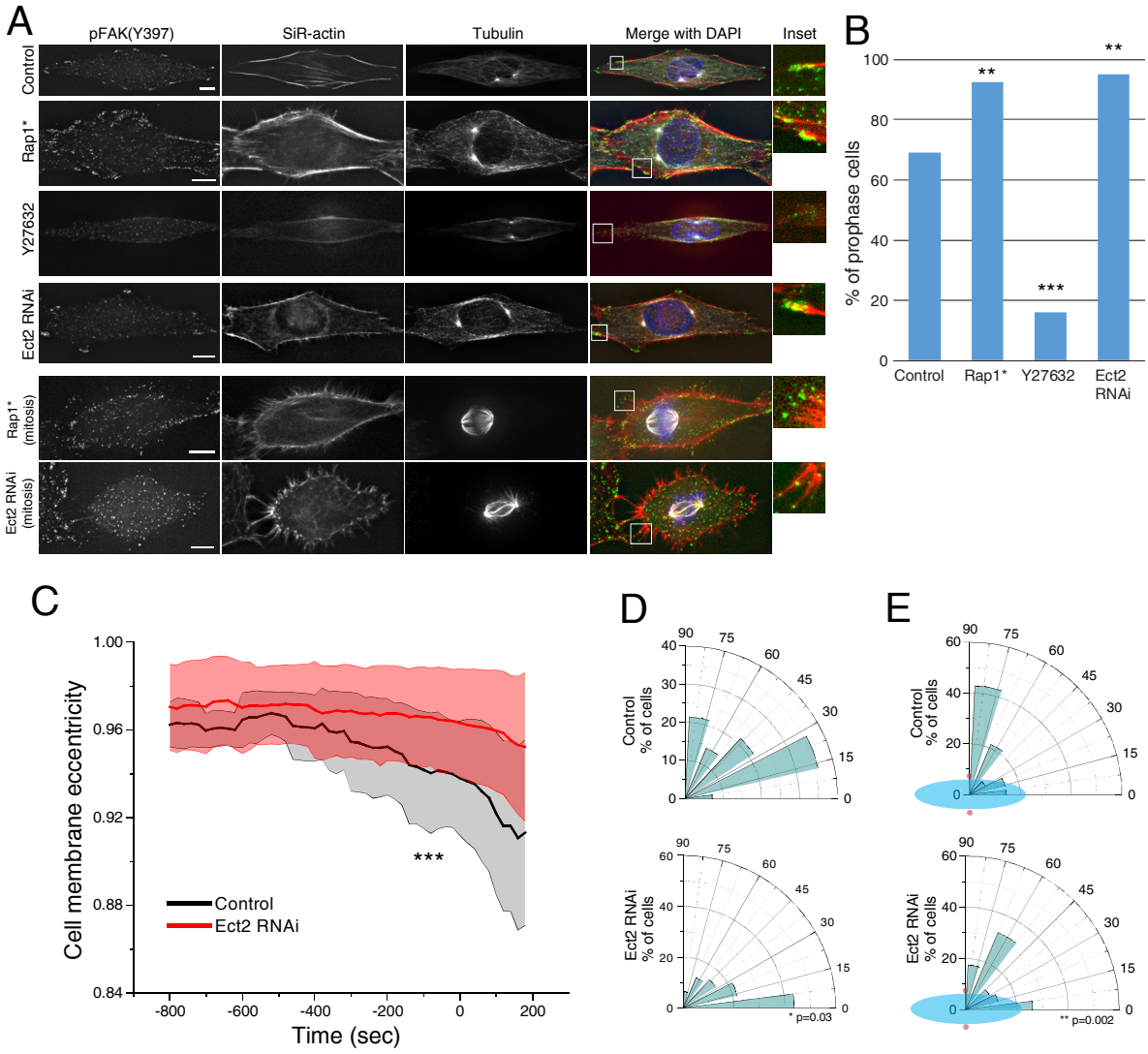

Figure S4 - Adhesion disassembly is required for centrosome positioning on the shortest nuclear axis, related to Figure 4. (A) Immunofluorescence images of pFAK(Y397), SiR-actin, alpha-tubulin and DAPI for controls (n=29), Rap1* (n=53; **p=0.002), Y27632 (n=50; ***p<0.001) and Ect2 RNAi (n=40; **p<0.003). Scale bars, 10μm. Insets show pFAK(Y397) in green and SiR-actin in red. (B) Quantification of the percentage of prophase cells with focal adhesions. (C) Cell membrane eccentricity for controls (n=16) and Ect2 RNAi (n=66; *** p<0.001). (D) Polar plot quantifying alignment of the long nuclear axis with the long cell axis at NEB for Controls and Ect2 RNAi (* p=0.03). (E) Polar plot quantifying centrosome positioning (red circles) relative to the longest nuclear axis (blue ellipse) at NEB for controls and Ect2 RNAi cells (** p=0.002).

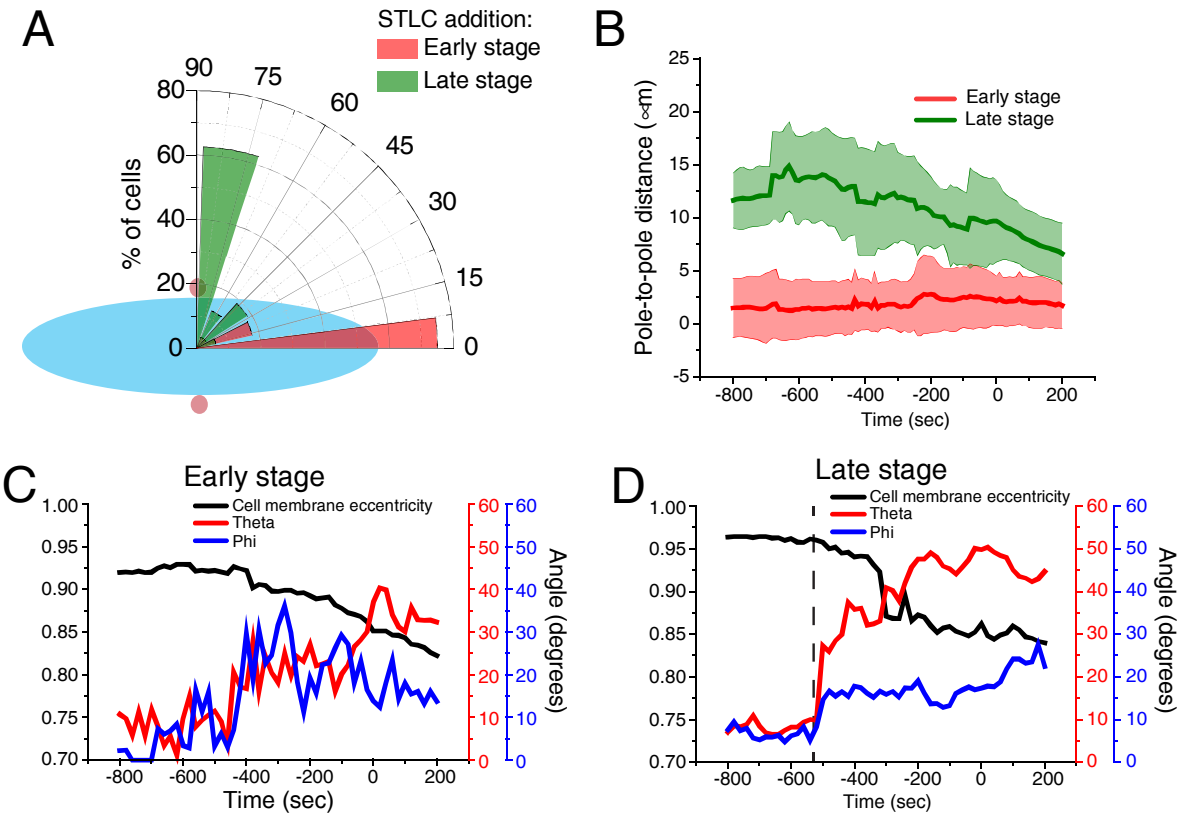

Figure S5 - Eg5 is required for early centrosome separation but not centrosome positioning, related to Figure 5

(A) Polar plot quantifying centrosome positioning (red circles) relative to the longest nuclear axis (blue ellipse) at NEB for cells treated with STLC when centrosomes were already on opposite sides of the nucleus (Late stage; green ; n=12) or when centrosomes were still not completely separated (Early stage; red; n=30). (B) Inter-centrosome distance for cells treated with STLC in either the Early stage (red) or the Late stage (green) of centrosome separation. Note how inter-centrosome distance only decreases in Late stage cells when cell rounding begins (~550sec before NEB). Lines correspond to averages and shaded areas correspond to SD. Correlation between the average theta values (red; corresponding to centrosomes xy orientation), average phi values (blue; corresponding to centrosomes z orientation) and cell membrane eccentricity (black) for cells in early (C) or late (D) stage of centrosome separation. The long axis of the micropattern was oriented horizontally and defined as a reference point, corresponding to a value of zero for theta and phi.

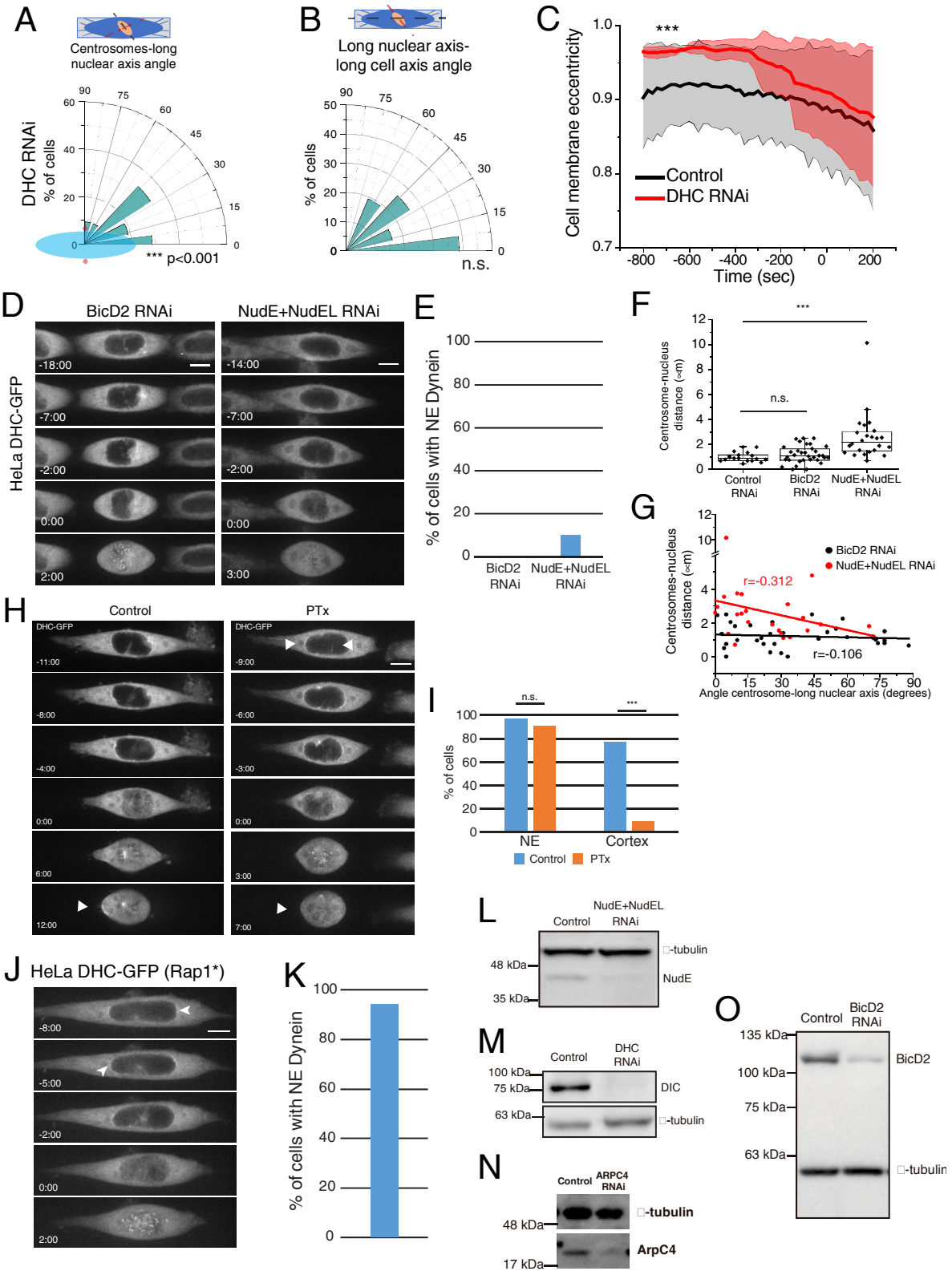
A
Centrosomes-long nuclear axis angle
DHC RNAi
% of cells
*** p<0.001
B
Long nuclear axis-long cell axis angle
% of cells
n.s.
C
Cell membrane eccentricity
***
Control
DHC RNAi
Time (sec)
D
BicD2 RNAi
NudE+NudEL RNAi
HeLa DHC-GFP
E
% of cells with NE Dynein
BicD2 RNAi
NudE+NudEL RNAi
F
Centrosome-nucleus distance (∝m)
***
n.s.
Control RNAi
BicD2 RNAi
NudE+NudEL RNAi
G
Centrosomes-nucleus distance (∝m)
BicD2 RNAi
NudE+NudEL RNAi
r=-0.312
r=-0.106
Angle centrosome-long nuclear axis (degrees)
H
Control
PTx
DHC-GFP
I
% of cells
n.s.
***
NE
Cortex
Control
PTx
J
HeLa DHC-GFP (Rap1*)
K
% of cells with NE Dynein
L
Control
NudE+NudEL RNAi
48 kDa
35 kDa
-tubulin
NudE
M
Control
DHC RNAi
100 kDa
75 kDa
63 kDa
DIC
-tubulin
N
Control
ARPC4 RNAi
48 kDa
17 kDa
-tubulin
ArpC4
O
Control
BicD2 RNAi
135 kDa
100 kDa
75 kDa
63 kDa
BicD2
-tubulin

Figure S6 - Nuclear envelope Dynein is required for centrosome positioning, related to Figure 5

Polar plots for DHC RNAi-treated cells (n=21) quantifying centrosome positioning (red circles) relative to the longest nuclear (A; *** $p<0.001$) or alignment of the long nuclear axis with the long cell axis (B) at NEB. (C) Cell membrane eccentricity for controls and DHC RNAi-treated cells. Line correspond to averages and shaded areas correspond to SD (***$p<0.001$). (D) Representative images of cells expressing DHC-GFP treated with BicD2 (n=11) or NudE+NudEL RNAi (n=10). (E) Quantification of the percentage of cells with NE Dynein. (F) Quantification of centrosome-nucleus distance (μm) for controls (n=18), BicD2 RNAi (n=33) and NudE+NudEL RNAi (n=24; *$p<0.05$). (G) Correlation between the centrosomes-long nuclear axis angle and the centrosome nucleus distance. Note the lack of correlation between the two variables ($r=-0.312$ for NudE+NudEL RNAi and $r=-0.106$ for BicD2 RNAi). (H) Representative images of HeLa DHC-GFP control cells (left panel; n=27) or treated with PTx (right panel; n=11) during mitotic entry. Note how PTx specifically abolishes DHC accumulation on the cell cortex (white arrowhead; *** $p<0.001$). (I) Quantification of the percentage of cells showing DHC accumulation on the NE and cell cortex. (J) Representative images of a cell expressing DHC-GFP and Rap1*. (K) Quantification of the percentage of cells expressing Rap1* with NE Dynein. Scale bars, 10μm. Time-lapse is 20sec. Time is in min:sec. Representative immunoblots to confirm depletion efficiency by RNAi of NudE+NudEL (L), DHC (M), ArpC4 (N) BicD2 (O).

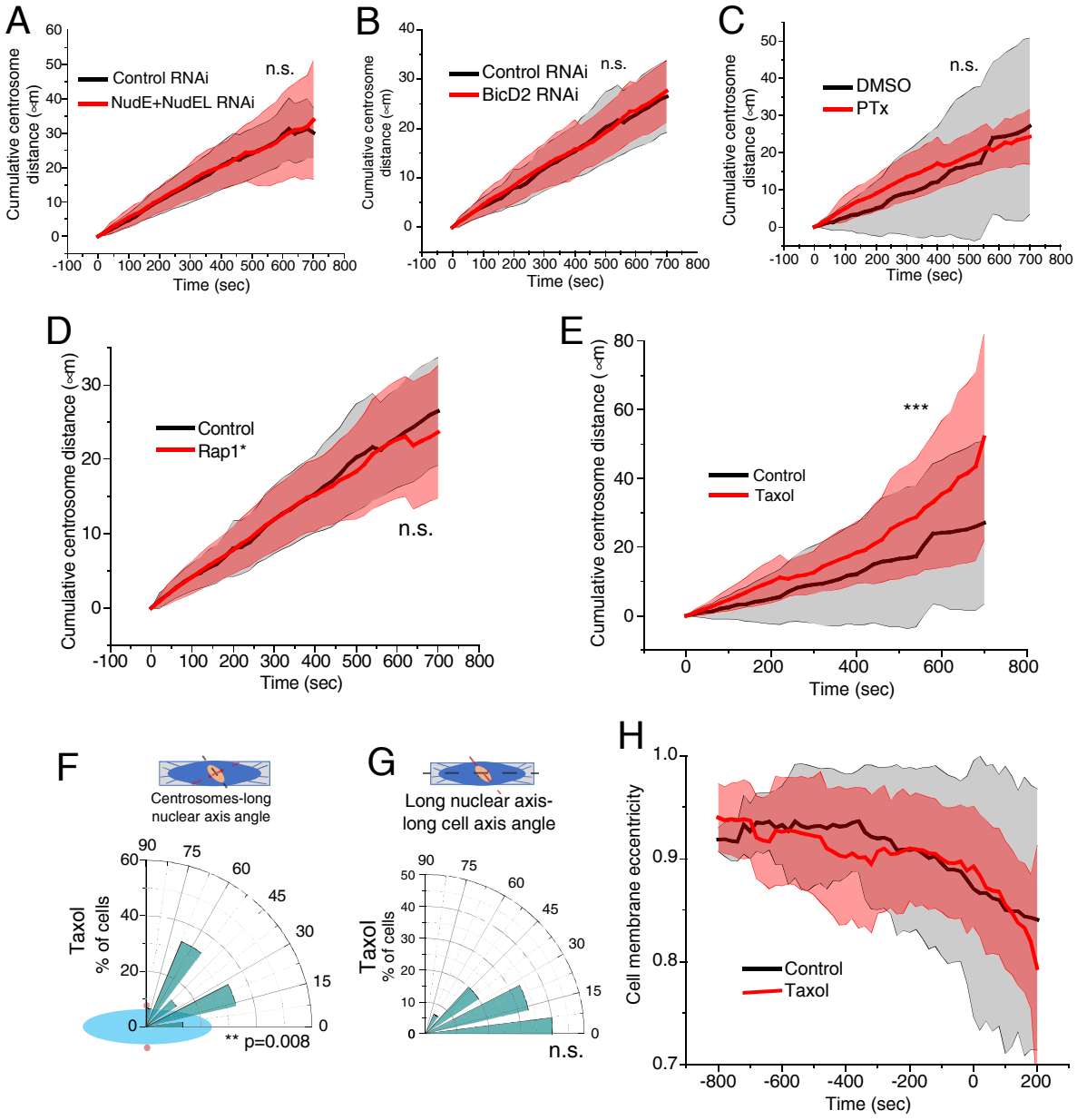

Figure S7 - Microtubule stabilization affects centrosome positioning, related to Figure 6

Quantification of centrosome distance over time for controls and BicD2 (A), NudE+NudEL RNAi (B), PTx (C), Rap1* (D) and Taxol-treated cells (E; ***p<0.001). Polar plots for Taxol-treated cells quantifying centrosome positioning (red circles) relative to the longest nuclear (F; ** p=0.008) or alignment of the long nuclear axis with the long cell axis (G) at NEB. (H) Cell membrane eccentricity for controls and Taxol-treated cells. Line correspond to averages and shaded areas correspond to SD.

Movie S1. HeLa cell expressing EB3-GFP/Lifeact-mCherry seeded on a line micropattern during mitotic entry, related to Figure 1

Movie S2. HeLa cell expressing H2B-GFP/tubulin-RFP treated with NudE+NudEL RNAi, seeded on a line micropattern, related to Figure 5

Movie S3. HeLa cell expressing H2B-GFP/tubulin-RFP treated with ArpC4 RNAi, related to Figure 6

Movie S4. HeLa cell expressing H2B-GFP/tubulin-RFP showing centrosomes on shortest nuclear axis, related to Figure 7

Movie S5. HeLa cell expressing H2B-GFP/tubulin-RFP, showing a missegregation event, related to Figure 7