Expanded View Figures

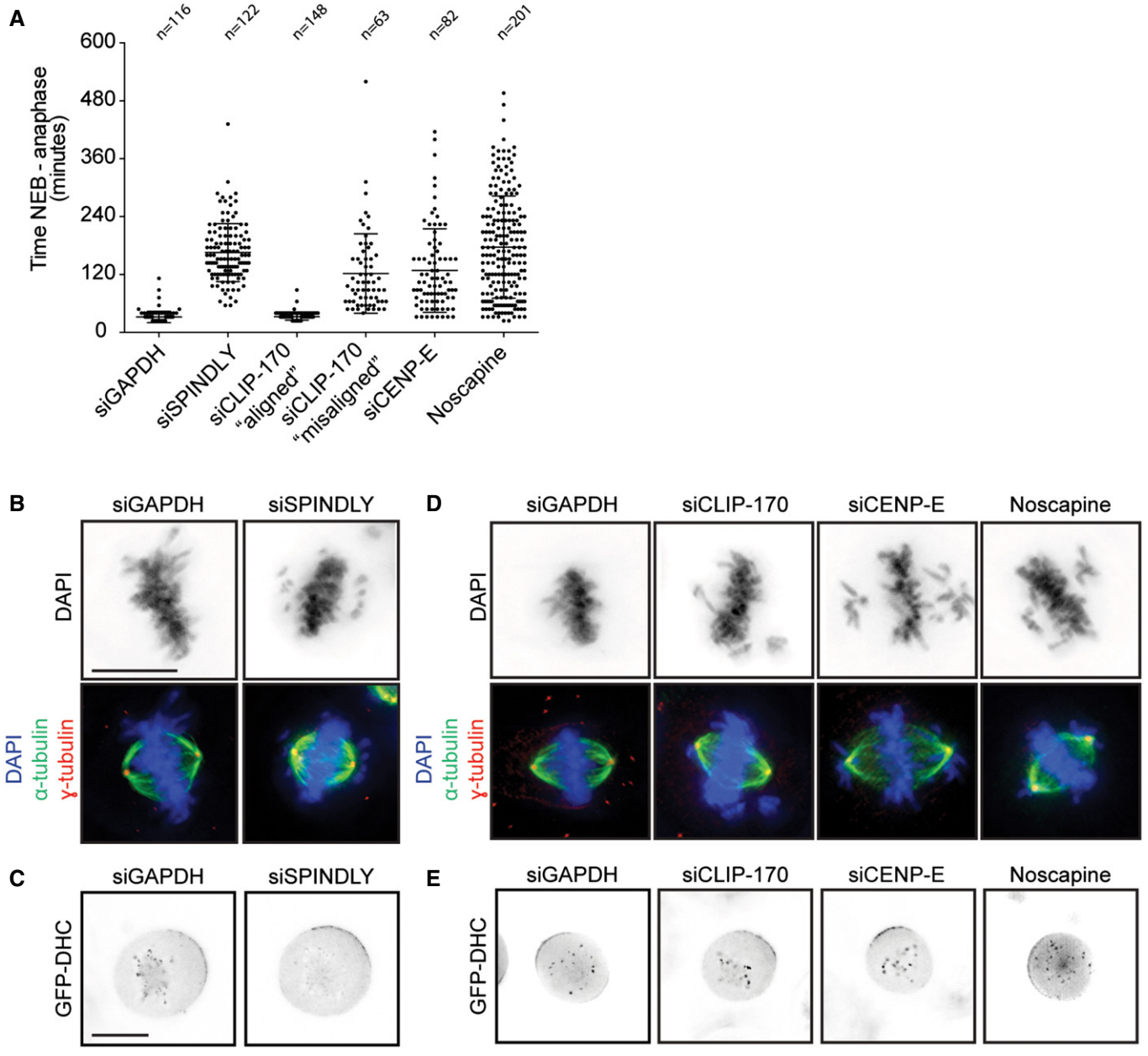

Figure EV1. Spindly, CLIP-170, and CENP-E are required for chromosome alignment, but not for cortical dynein recruitment.

A Average time in mitosis (nuclear envelope breakdown till anaphase) of U2OS cells transfected with GAPDH, Spindly, CLIP-170, and CENP-E siRNA or treated with noscapine. Data adapted from experiments shown in Fig 1. Graph shows mean ± SD. (B, C) Scale bars represent 10 μm.

B Representative image of the mitotic phenotype observed after Spindly depletion. Cells were stained with α-tubulin, γ-tubulin, and DAPI.

C Representative live-cell microscopic images of HeLa cells stably expressing GFP-DHC from a bacterial artificial chromosome [49]. Cells were transfected with the indicated siRNAs for 48 h. One hour before images were taken, cells were treated with the Eg5 inhibitor STLC to arrest them in mitosis with a monopolar spindle and to allow the visualization of KT- and cortical dynein. Scale bar represents 10 μm.

D Cells were transfected with CLIP-170 or CENP-E siRNA for 48 h or treated for 30 min with noscapine and fixed and stained as in (B).

E Same experiment as described in (C) to examine GFP-DHC localization after CLIP-170 and CENP-E depletion and noscapine treatment.

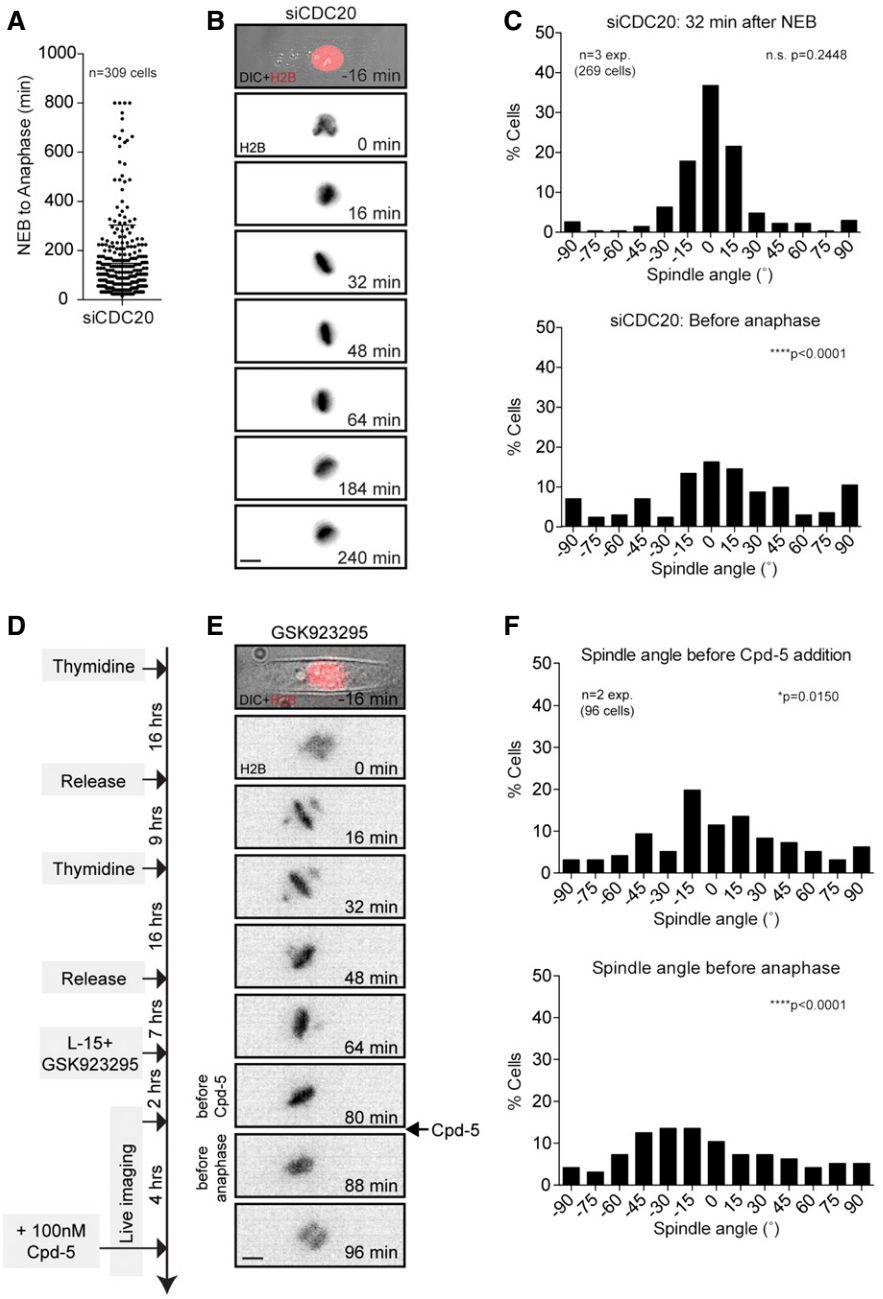

Figure EV2. Chromosome misalignments induce spindle misorientation independent of SAC activity.

A Average time in mitosis of U2OS cells transfected with CDC20 siRNA for 24 h. Error bar represents mean ± SD.

B, C Live-cell images and histograms of GFP-H2B U2OS cells filmed on rectangular micropatterns after CDC20 depletion. The spindle angles were scored at 32 min after NEB (top) and one frame before cells eventually exit mitosis (bottom).

D Schematic showing the experimental setup for the synchronization and live-cell imaging of micropatterned U2OS cells that enter mitosis in the presence of a CENP-E inhibitor to induce chromosome misalignments and subsequently are forced out of mitosis by the addition of the Mps1 inhibitor Cpd-5.

E, F Example cell and spindle angle histograms of cells one frame before the addition of Cpd-5 (top) and one frame before mitotic exit (bottom).

Data information: (B, E) Scale bars represent 10 μm. Distributions in (C, F) were compared against the distributions of Fig 1B (control siGAPDH) using non-parametric Kolmogorov-Smirnov test. Statistical difference between the distributions is indicated by: ****$P < 0.0001$ and *$P < 0.05$.

Figure EV3. Ran depletion causes a global reduction in cortical LGN recruitment.

A Representative immunofluorescence image of a U2OS cell after Ran depletion. Cells were either mock-transfected or with Ran siRNA for 24 h. After fixation, the cells were stained with α-tubulin, HURP, and DAPI. For the quantification of the HURP staining, the spindle area was sectioned into two parts, one half in the vicinity of the chromosomes and the other half in the vicinity of the spindle pole. The mean HURP signal intensity within the section of the spindle close to the chromosomes was normalized to its mean intensity in the region close to the poles. $n = 40$ cells per condition from two experiments. ****$P < 0.0001$ and unpaired t-test. Graph shows mean ± SD.

B Representative live-time microscopy images of mitotic HeLa cells stably expressing GFP-LGN and transiently expressing RFP-H2B after mock and Ran depletion. Time is relative to NEB.

C HeLa cells stably expressing GFP-LGN and transiently expressing RFP-H2B were treated for 30 min with 5 μM MG-132. Subsequently, DMSO or 40 μM importazole was added to the medium while filming. Images were taken every 8 min. The graph on the right shows the quantification of cortical and cytoplasmic GFP-LGN levels over time. Intensity values are normalized over the background intensities measured around the cell. ANOVA Dunnett's multiple comparisons test. Graph shows mean ± SEM. (****$P < 0.0001$, **$P < 0.003$, *$P < 0.05$).

D HeLa cells stably expressing GFP-LGN were transfected for 48 h with mCherry-RanT24N or mCherry-RanQ69L constructs and subsequently filmed at 8-min intervals. The presence or absence of cortical LGN was scored at 32 min after NEB. $n = 3$ experiments, *$P = 0.0014$, multiple t-test. Graph shows mean ± SEM.

E HeLa cells stably expressing GFP-LGN and transiently expressing RFP-H2B were mock- or siRNA-transfected for 24 h. Cells were treated with 12.5 μg/ml noscapine to induce chromosome misalignments. After 30 min, Hoechst 33342 was added to the medium to visualize the chromosomes. Another 30 min later, live images of mitotic cells with misaligned chromosomes were taken within 1 h. Images were later used to measure the distance of misaligned chromosomes to the closest cortical region and to score for cortical LGN presence or absence in the same region.

F Representative images of mock- and Ran-depleted cells from the experiment described in (D). The histograms show the effect of misaligned chromosome position on cortical LGN enrichment. The frequency of incidents was plotted where cortical LGN was excluded (black bars) or enriched (red bars) from the nearest misaligned chromosome.

Data information: (A–D and F) Scale bars represent 10 μm.

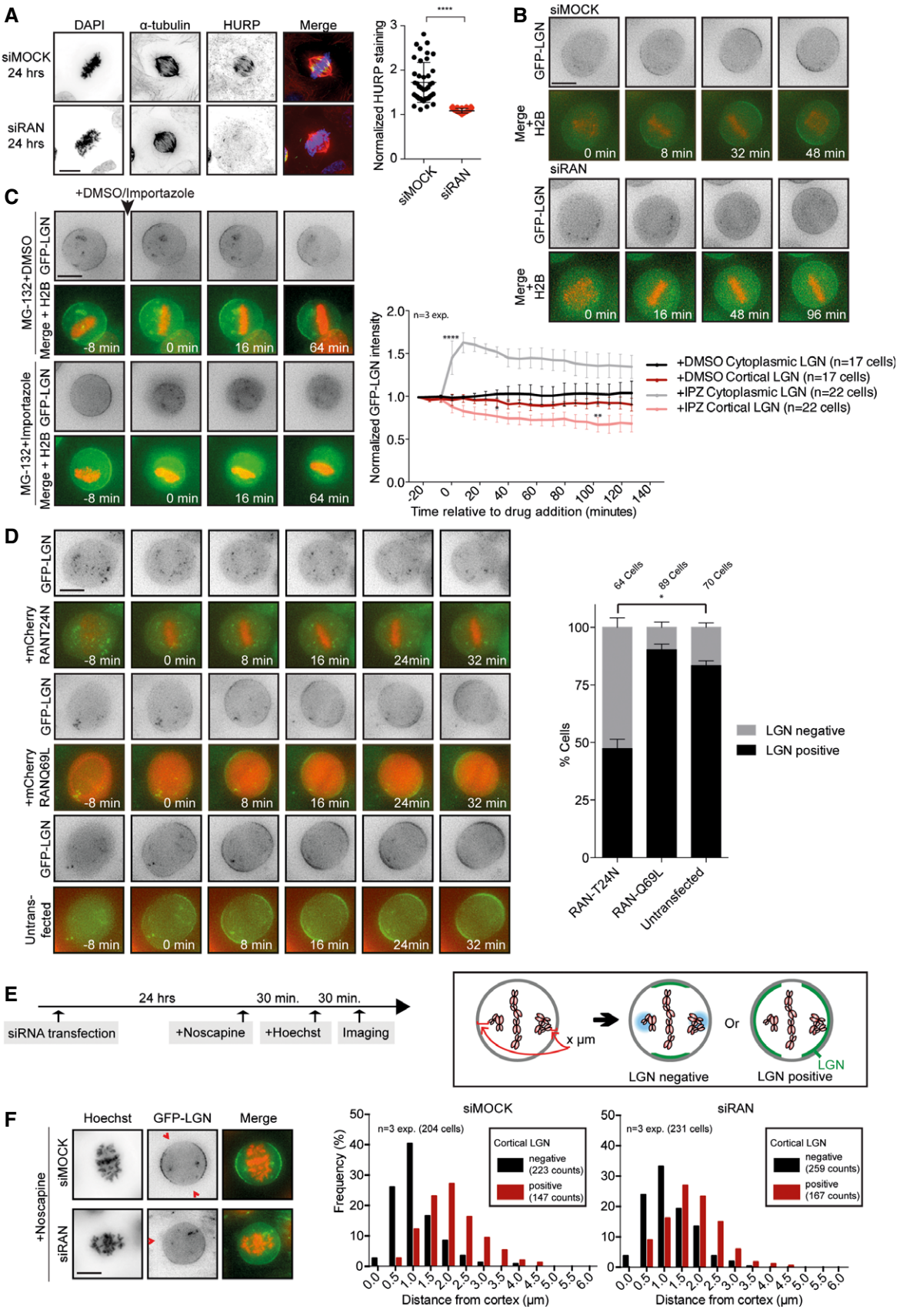
A
DAPI
α-tubulin
HURP
Merge
siMOCK 24 hrs
siRAN 24 hrs
Normalized HURP staining
siMOCK
siRAN
B
siMOCK
GFP-LGN
Merge + H2B
0 min
8 min
32 min
48 min
siRAN
16 min
96 min
C
+DMSO/Importazole
MG-132+DMSO
MG-132+Importazole
-8 min
64 min
n=3 exp.
Normalized GFP-LGN intensity
+DMSO Cytoplasmic LGN (n=17 cells)
+DMSO Cortical LGN (n=17 cells)
+IPZ Cytoplasmic LGN (n=22 cells)
+IPZ Cortical LGN (n=22 cells)
Time relative to drug addition (minutes)
D
+mCherry RANT24N
+mCherry RANQ69L
Untrans-fected
24min
64 Cells
89 Cells
70 Cells
% Cells
LGN negative
LGN positive
RAN-T24N
RAN-Q69L
Untransfected
E
24 hrs
30 min.
siRNA transfection
+Noscapine
+Hoechst
Imaging
x µm
Or
LGN
LGN negative
LGN positive
F
Hoechst
GFP-LGN
Merge
+Noscapine
siMOCK
siRAN
n=3 exp. (204 cells)
n=3 exp. (231 cells)
Cortical LGN
negative (223 counts)
positive (147 counts)
negative (259 counts)
positive (167 counts)
Frequency (%)
Distance from cortex (µm)

Figure EV3.

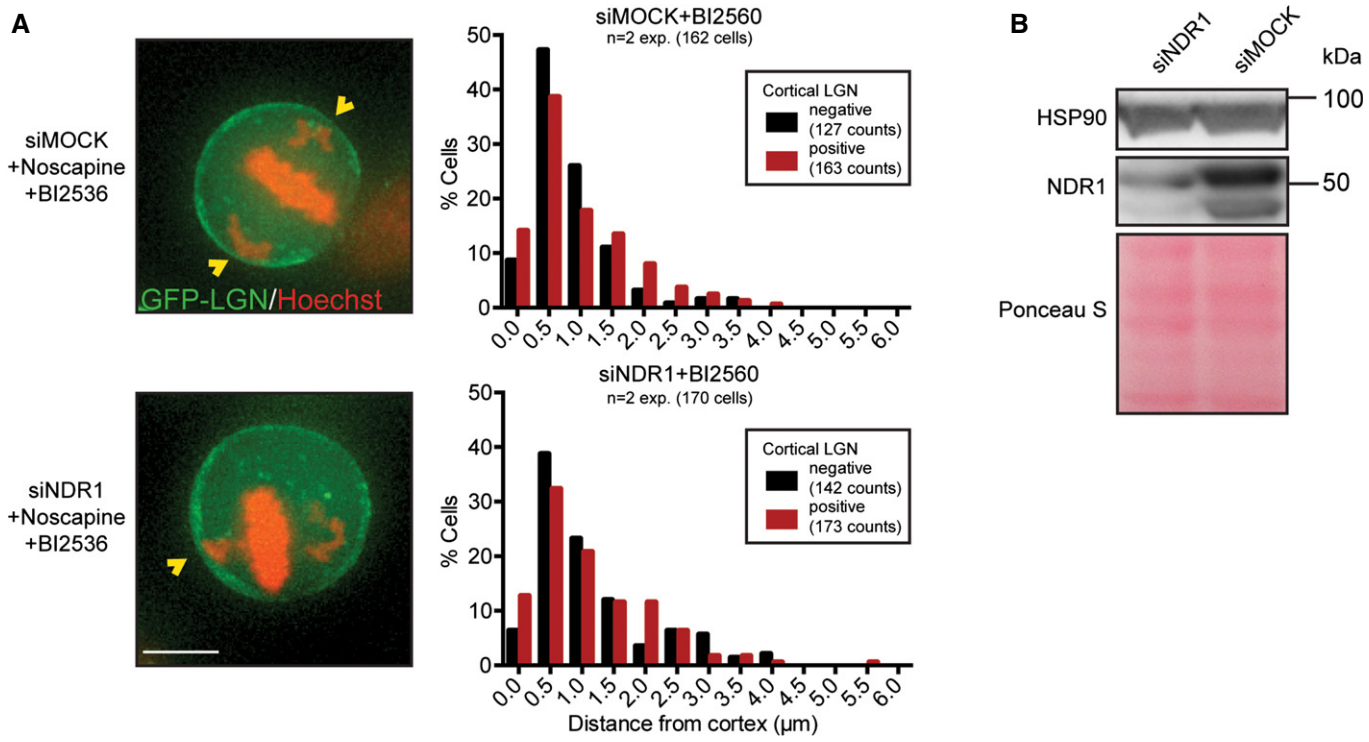

Figure EV4. Plk1 does not function through NDR1 to displace cortical LGN.

A Representative images and histograms showing the effect of chromosome position on cortical LGN enrichment after 48 h of mock and NDR1 depletion and co-inhibition of Plk1 with BI2560. Scale bar represents 10 µm.

B Western blot showing the depletion level of NDR1 after transfection of cells with indicated siRNAs for 48 h.

Figure EV5. Spindle pole-localized Plk1 restricts cortical LGN localization.

A, B Kymographs showing the cortical enrichment sites of GFP-LGN and mCherry-Arp1 switching in a single HeLa cell. Images were taken every 5 min. As an example, a cell was selected that took almost 200 min to complete mitosis to be able to show multiple switching events. Line scans were made from the kymographs of both the "upper" and the "lower" cortical regions. Fluorescence intensity values are corrected for background fluorescence.

C, D GFP-LGN- and mCherry-Arp1-expressing HeLa cells were treated with STLC to induce monopolar spindles and filmed for 160 min. Subsequently, DMSO or BI2536 was added (at t = 0 min) and filmed for an additional 160 min. The number of switching events observed for cortical GFP-LGN before and after the addition of the secondary drug is plotted on the right. Graphs show mean + SD.

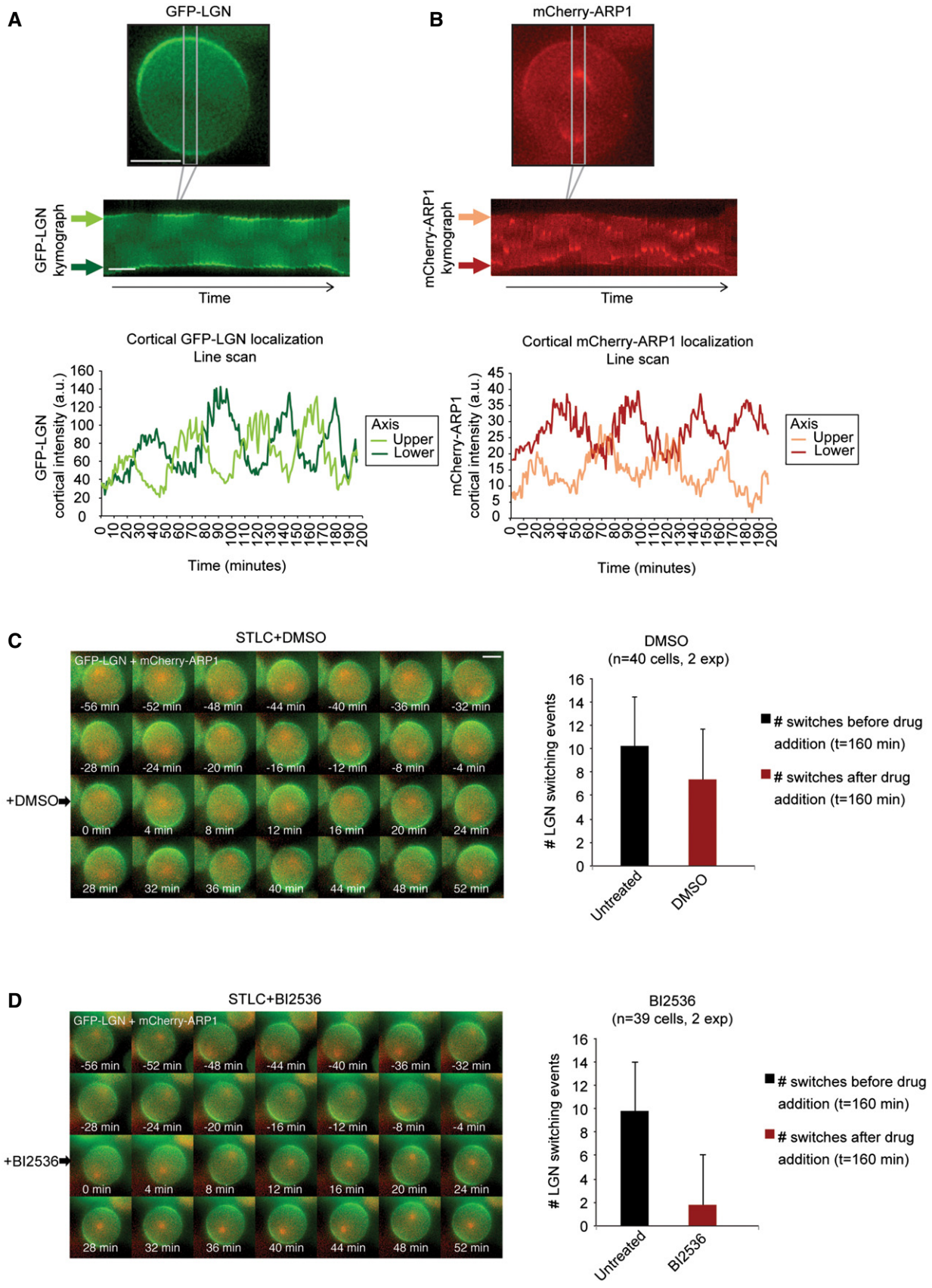

Figure EV5.