Supplemental Materials

Molecular Biology of the Cell

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Supplementary figure legends

Figure S1. Gα-independent membrane association of LGN-N/NuMA-CT complex in HeLa and MDCK cells. Representative fluorescent images of HeLa (A) and MDCK (B) cells are shown. Top panels show cells expressing Venus-LGN1-481 (green). Cells expressing mRFP-NuMA1536- CTδNLS (red) are shown in the second panels from top. The bottom two panels show cells co-expressing Venus-LGN1-481 and mRFP-NuMA1536- CTδNLS.

Figure S2. Lentivirus-mediated stable knocking down of NuMA in MDCK cells. MDCK cells were transduced with lentiviruses expressing control shRNA (top two panels) or NuMA specific shRNA (bottom two panels). Stable, virus-transduced cells were fixed and stained with anti-NuMA antibody (green) and DNA dye (blue). Lentivirus-transduced cells also express H2B-mCherry. Note that knocking down NuMA led to chromosome alignment defect during mitosis (arrows) and the formation of multiple micro-nuclei in post mitotic cells (arrows heads).

Figure S3. Cell cycle-regulated membrane association of NuMA underlies cell cycle-dependent cortical accumulation of DYNC1H1. (A) Membrane association of NuMA is required for anaphase cortical accumulation of dynein. Normal MDCK cells were transduced with lentivirus expressing control shRNA (top panel) or NuMA specific shRNA (second panel from top). In the meantime, MDCK cells expressing Venus-NuMA-FL (third and fourth panels from top) or Venus-NuMA1-2040 (bottom two panels) were also transduced with lentivirus expressing NuMA specific shRNA. Stable, virus-transduced cells were fixed and stained with anti-DYNC1H1 antibody. Note that both lentiviruses also express H2B-mCherry. Bar, 10 μm. (B) Quantification of cortical DYNC1H1 fluorescence intensity from images obtained in (A). Results are from three independent experiments. Error bars represent standard deviation (SD). n=8 for NuMA KD control cells and n=30 for other group of cells. *, $P<0.01$. (C) The spindle pole localization of DYNC1H1 is normal in endogenous NuMA-depleted, Venus-NuMA-FL or Venus-NuMA1-2040-expressing MDCK cells. Data show the quantification of metaphase and anaphase spindle pole DYNC1H1 fluorescence intensity from images obtained in (A). Results are from three independent experiments. Error bars represent standard deviation (SD). n=30 for each group of cells.

Video - Supplementary Movie 1

Time-lapse analysis of the effect of ionomycin treatment on the membrane binding of Venus-NuMA1981-2060. Images were captured at 5 seconds/frame. Movie corresponds to Figure 3F.

4.Video - Supplementary Movie 2

Time-lapse analysis of mitotic Venus-NuMA-FL expressing cells. Merged Venus-NuMA-FL and Hoechst 33342 channels are shown. Images were captured at 10 seconds/frame. Movie corresponds to Figure 5C.

5.Video - Supplementary Movie 3

Time-lapse analysis of mitotic Venus-NuMA1-2040 expressing cells. Merged Venus-NuMA1-2040 and Hoechst 33342 channels are shown. Images were captured at 10 seconds/frame. Movie corresponds to Figure 6A.

6.Video - Supplementary Movie 4

Time-lapse analysis of mitotic Venus-NuMA-T2041E expressing cells. Merged Venus-NuMA-T2041E and Hoechst 33342 channels are shown. Images were captured at 10 seconds/frame. Movie corresponds to Figure 6B.

7.Video - Supplementary Movie 5

Time-lapse analysis of mitotic Venus-NuMA-T2041A expressing cells. Merged Venus-NuMA-T2041A and Hoechst 33342 channels are shown. Images were captured at 10 seconds/frame. Movie corresponds to Figure 6C.

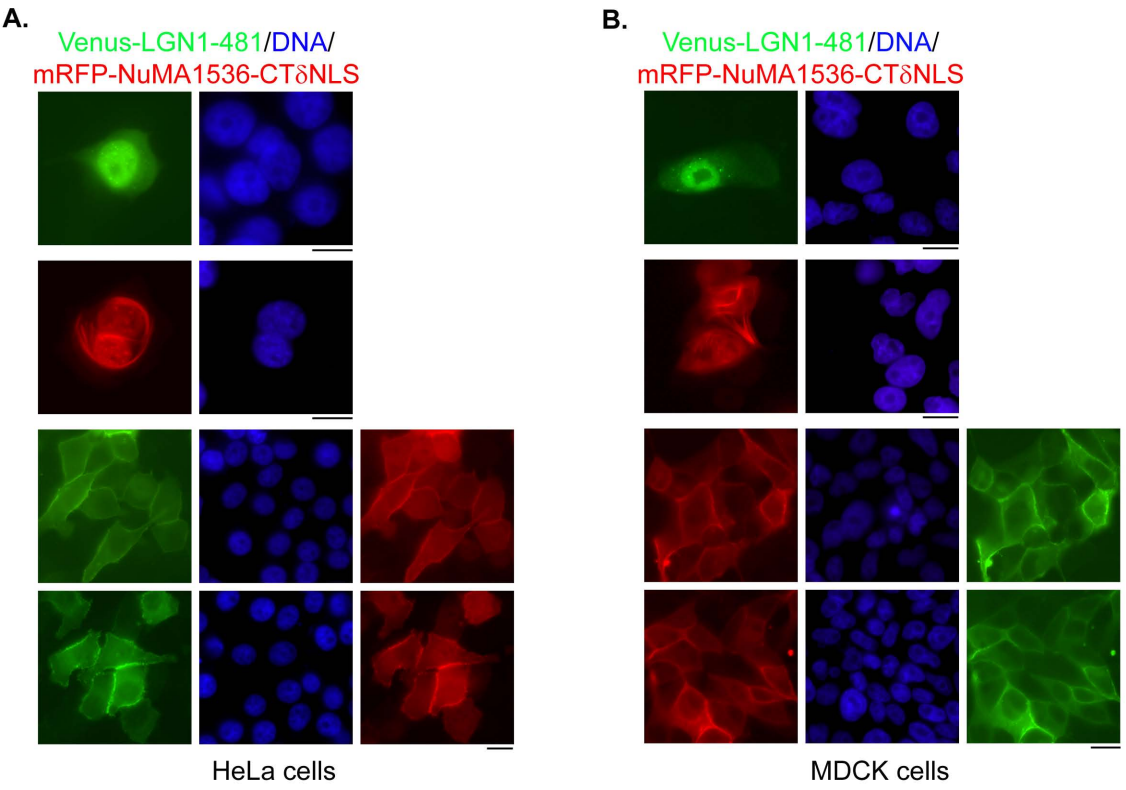
8.Video - Supplementary Movie 6

Time-lapse analysis of mitotic Venus-NuMA-FL expressing cells treated with purvalanol A. Merged Venus-NuMA-FL and Hoechst 33342 channels are shown. Images were captured at 10 seconds/frame. Movie corresponds to Figure 6D.

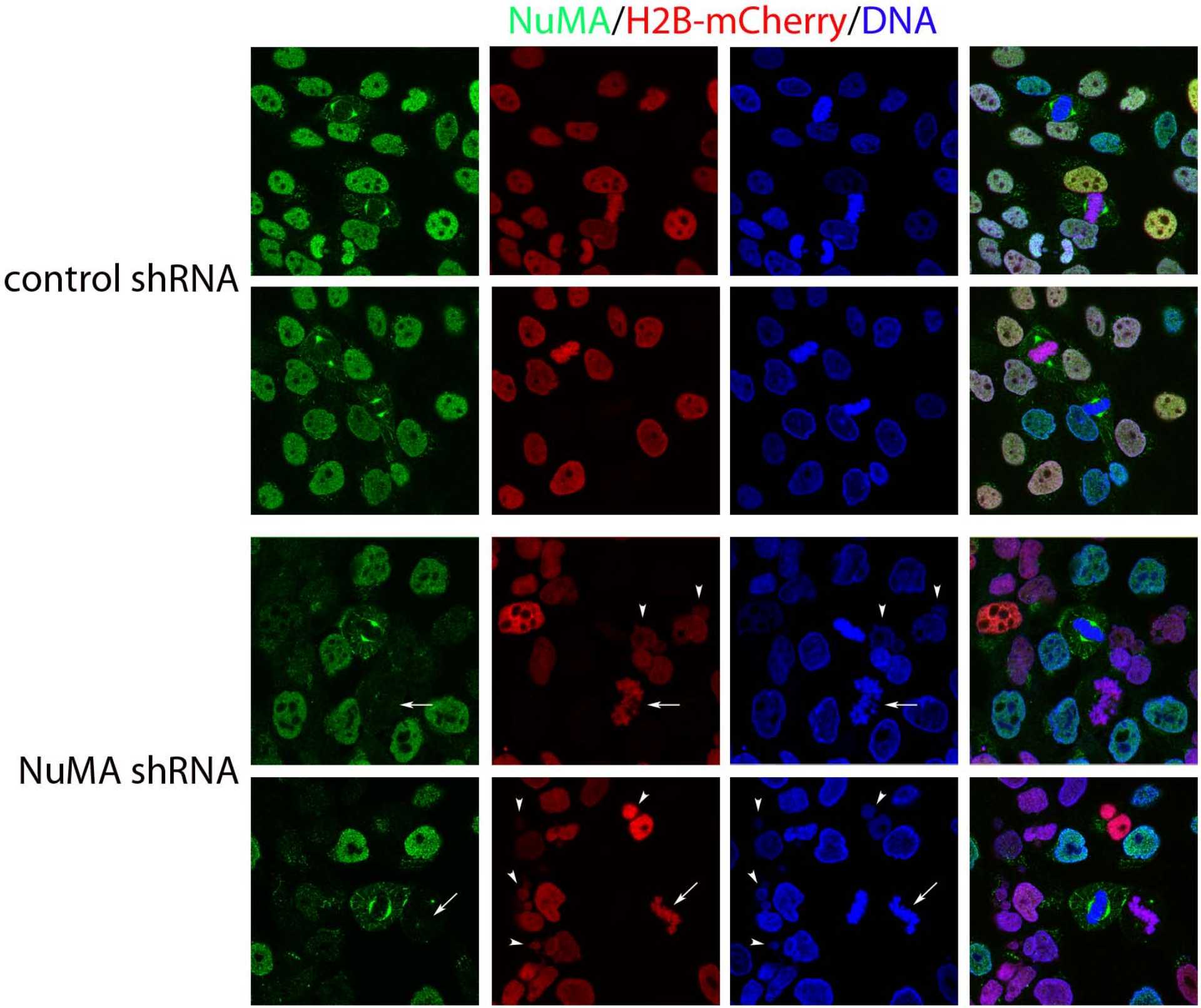
9.Video - Supplementary Movie 7

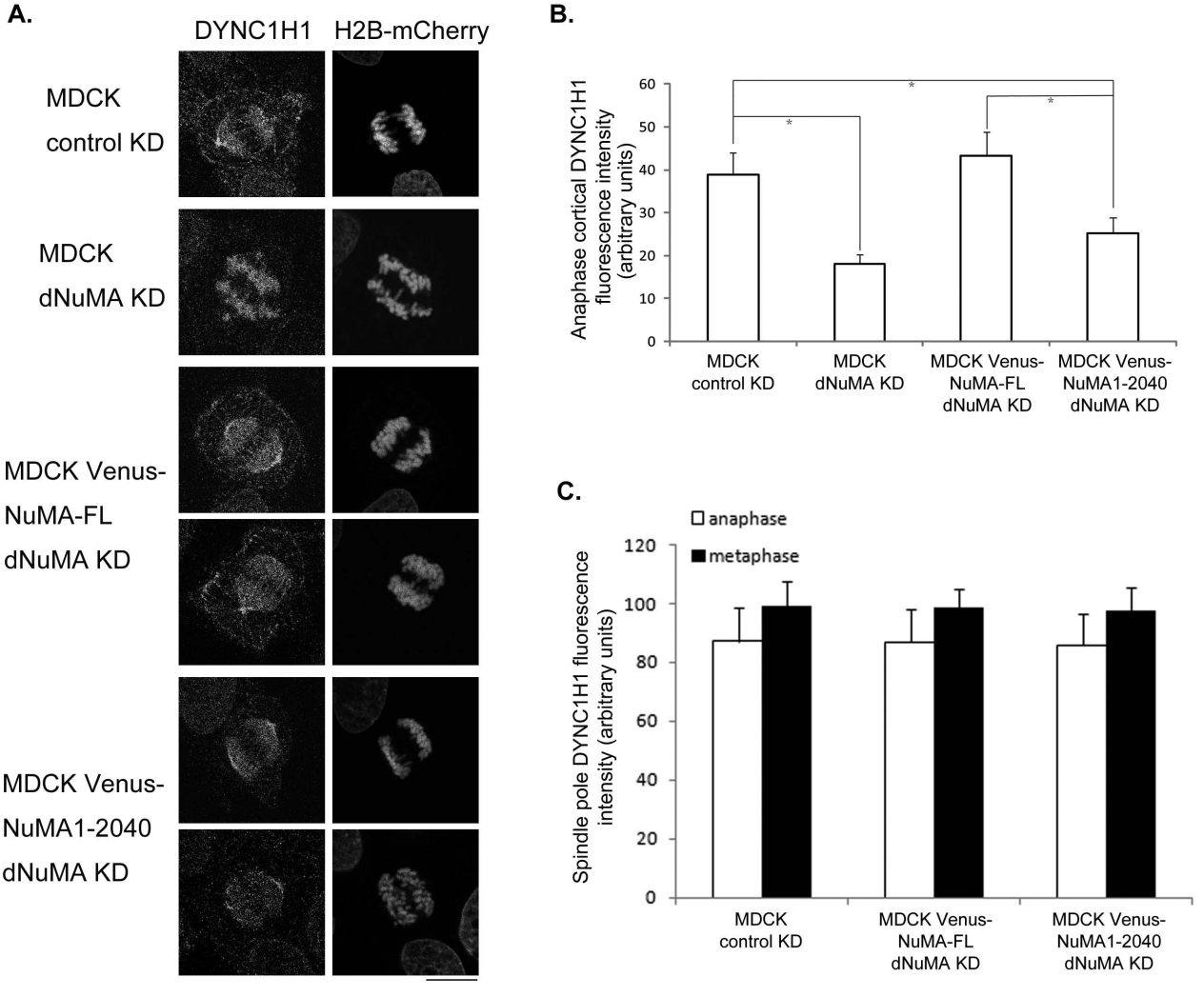
Time-lapse analysis of mitotic Venus-NuMA-FL expressing cells transfected with LGN knockdown plasmid. Venus-NuMA-FL channel is shown. Movie corresponds to Figure 7D.

Supplementary Figure 1



Supplementary Figure 2




A.
DYNC1H1
H2B-mCherry
MDCK control KD
MDCK dNuMA KD
MDCK Venus-NuMA-FL dNuMA KD
MDCK Venus-NuMA1-2040 dNuMA KD
B.
Anaphase cortical DYNC1H1 fluorescence intensity (arbitrary units)
60
50
40
30
20
10
0
*
*
MDCK control KD
MDCK dNuMA KD
MDCK Venus-NuMA-FL dNuMA KD
MDCK Venus-NuMA1-2040 dNuMA KD
C.
anaphase
metaphase
Spindle pole DYNC1H1 fluorescence intensity (arbitrary units)
120
100
80
60
40
20
0
MDCK control KD
MDCK Venus-NuMA-FL dNuMA KD
MDCK Venus-NuMA1-2040 dNuMA KD