Online supplementary material:

Five supplementary figures and three supplementary movies are included in the manuscript.

Supplementary figure 1 (Figure S1): shRNA-mediated knockdown of DYNC1H1.

Supplementary figure 2 (Figure S2): Effects of 50 nM nocodazole treatment on astral microtubules in MDCK cells.

Supplementary figure 3 (Figure S3): Lat-A-induced cortical dissociation and spindle pole accumulation of LGN in MDCK cells.

Supplementary figure 4 (Figure S4): Effects of Lat-A treatment on LGN and NuMA localization in MDA-231 cells.

Supplementary figure 5 (Figure S5): Live cell analysis of Lat-A-induced cortical dissociation and spindle pole accumulation of LGN and the rescue process of nocodazole.

Supplementary movie 1: Time-lapse FRAP analysis of cortical Venus-LGN in an untreated cell.

Supplementary movie 2: Time-lapse FRAP analysis of cortical Venus-LGN in a cell treated with 50 nM of nocodazole.

Supplementary movie 3: Time-lapse FRAP analysis of cortical Venus-LGN in a cell transfected with DYNC1H1 shRNA.

Supplementary movies correspond to Figure 4A.

Supplementary figure legends

FIGURE S1. shRNA-mediated knockdown of DYNC1H1. (A). Western blot analysis of MDCK II cells transfected with plasmids expressing control shRNA or two different shRNAs against canine DYNC1H1. Transfected cells were maintained in media containing G418 for 5 days. Cell lysates were harvested and subjected to western blot using anti-DYNC1H1 and anti-α-tubulin antibodies. (B) Fluorescent images of MDCK II cells transfected with plasmids expressing control shRNA or two different shRNAs against canine DYNC1H1. 48 h after transfection, cells were fixed and stained with anti-DYNC1H1, anti-α-tubulin antibodies and DNA dye.

FIGURE S2. Effects of 50 nM nocodazole treatment on astral microtubules in MDCK cells. MDCK II cells were either untreated or treated with 50 nM nocodazole for 20 min. Cells were pre-extracted with microtubule stabilization buffer containing 0.2% Triton X-100, then fixed with 4% PFA. Fixed cells were stained with anti-α-tubulin (red) antibody and DNA dye (blue). Representative images were shown. Magnified images from selected area (white square circled) were presented on the right. Bar, 10 μm.

FIGURE S3. Lat-A-induced cortical dissociation and spindle pole accumulation of LGN in MDCK cells. (A) Effects of Lat-A treatment on LGN and actin filaments in MDCK cells. MDCK II cells were treated as in Fig. 5A. Cells were fixed after treatments and stained with anti-LGN (green), rhodamine phalloidin (red) and DNA dye (blue). (B) Lat-A-induced spindle pole accumulation of LGN staining is not observed in LGN knockdown cells. MDCK II cells stably transduced with control lentivirus or lentiviruses expressing two different shRNA against LGN were treated with 1 μM of Lat-A for 45 min. Cells were fixed and stained with anti-LGN antibody (red) and DNA dye. Transduced cells express GFP.

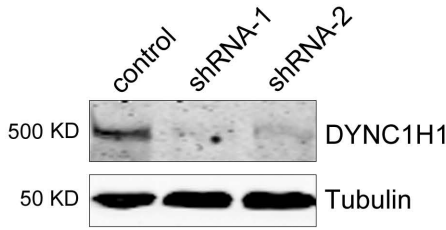
FIGURE S4. Effects of Lat-A treatment on LGN and NuMA localization in MDA-231 cells. (A). Effects of Lat-A treatment on LGN localization in MDA-231 cells. MDA-231 cells were either untreated (control), treated with 1 μM of Lat-A for 45 min (Lat-A) or treated with 50 nM of nocodazole plus 1 μM of Lat-A for 45 min (Nocodazole + Lat-A). 5 μM of MG132 was added 1 h before treatments and maintained during treatments. Cells were fixed after treatments and stained with anti-LGN (green), anti-α-tubulin (red) antibodies and DNA dye (blue). (B). Effects of Lat-A treatment on NuMA localization in MDA-231 cells. Cells were treated as in A and stained with anti-NuMA (green), anti-α-tubulin (red) antibodies and DNA dye (blue).

FIGURE S5. Live cell analysis of Lat-A-induced cortical dissociation and spindle pole accumulation of LGN and the rescue process of nocodazole. Representative images from live cell time-lapse series were shown. MDCK cells expressing low level of Venus-LGN were treated with 1 μM of Lat-A and

recorded for 30 min. Then 50 nM nocodazole was added in the medium and the cells were monitored for another 30 min. Treatments and time points were indicated for each image.

Figure S1

A.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shRNA-2
500 KD
DYNC1H1
50 KD
Tubulin

B.

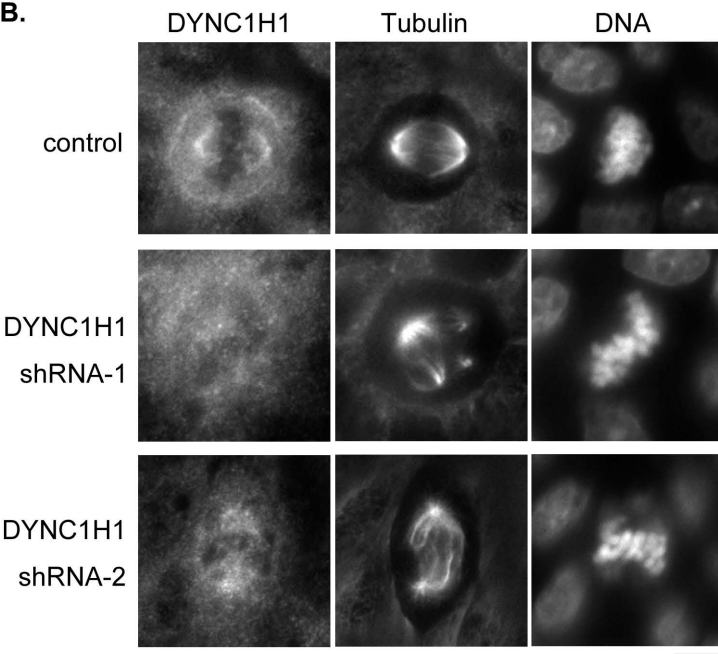

DYNC1H1
Tubulin
DNA
control
DYNC1H1
shRNA-1
DYNC1H1
shRNA-2

Figure S2

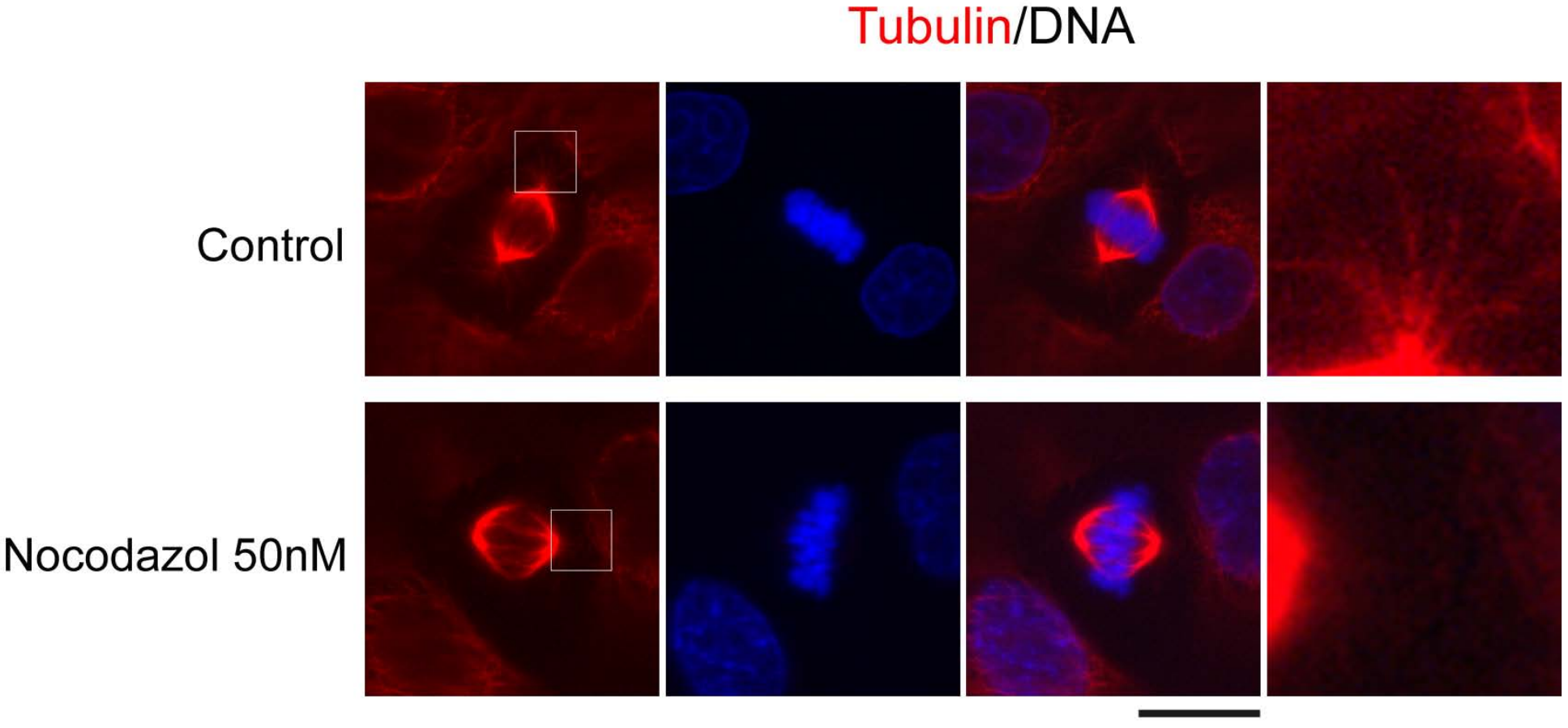


Figure S3

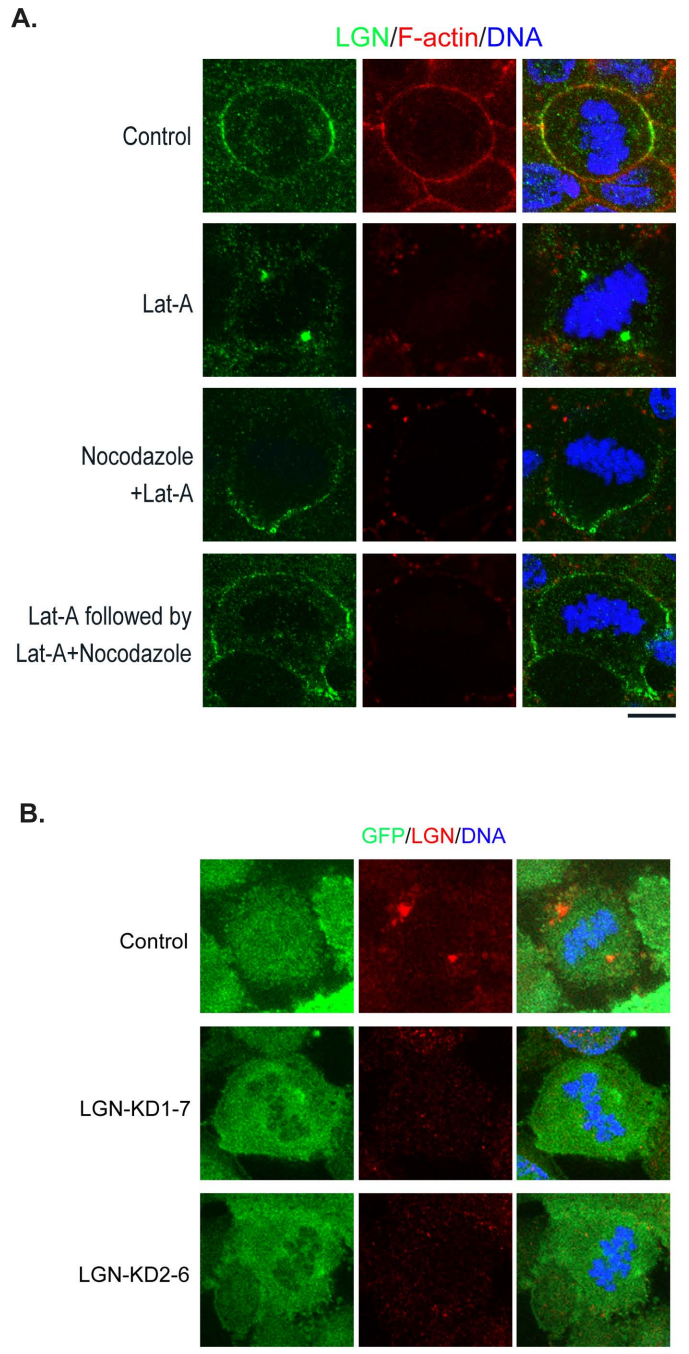

A.
LGN/F-actin/DNA
Control
Lat-A
Nocodazole
+Lat-A
Lat-A followed by
Lat-A+Nocodazole
B.
GFP/LGN/DNA
Control
LGN-KD1-7
LGN-KD2-6
Lat-A-treated MDCK cells

Figure S4

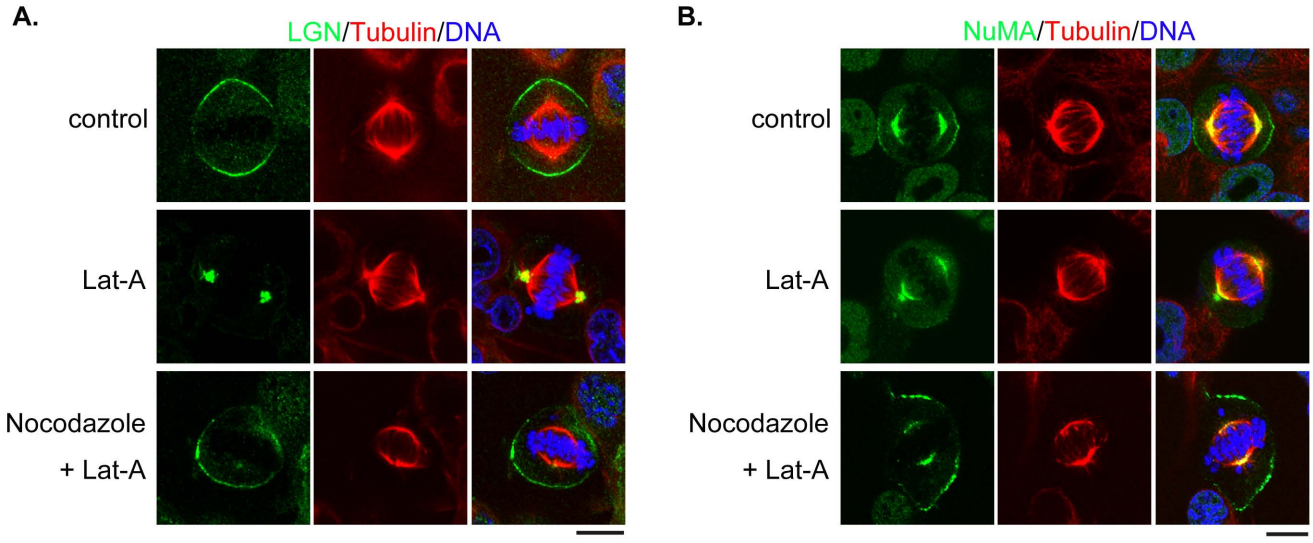
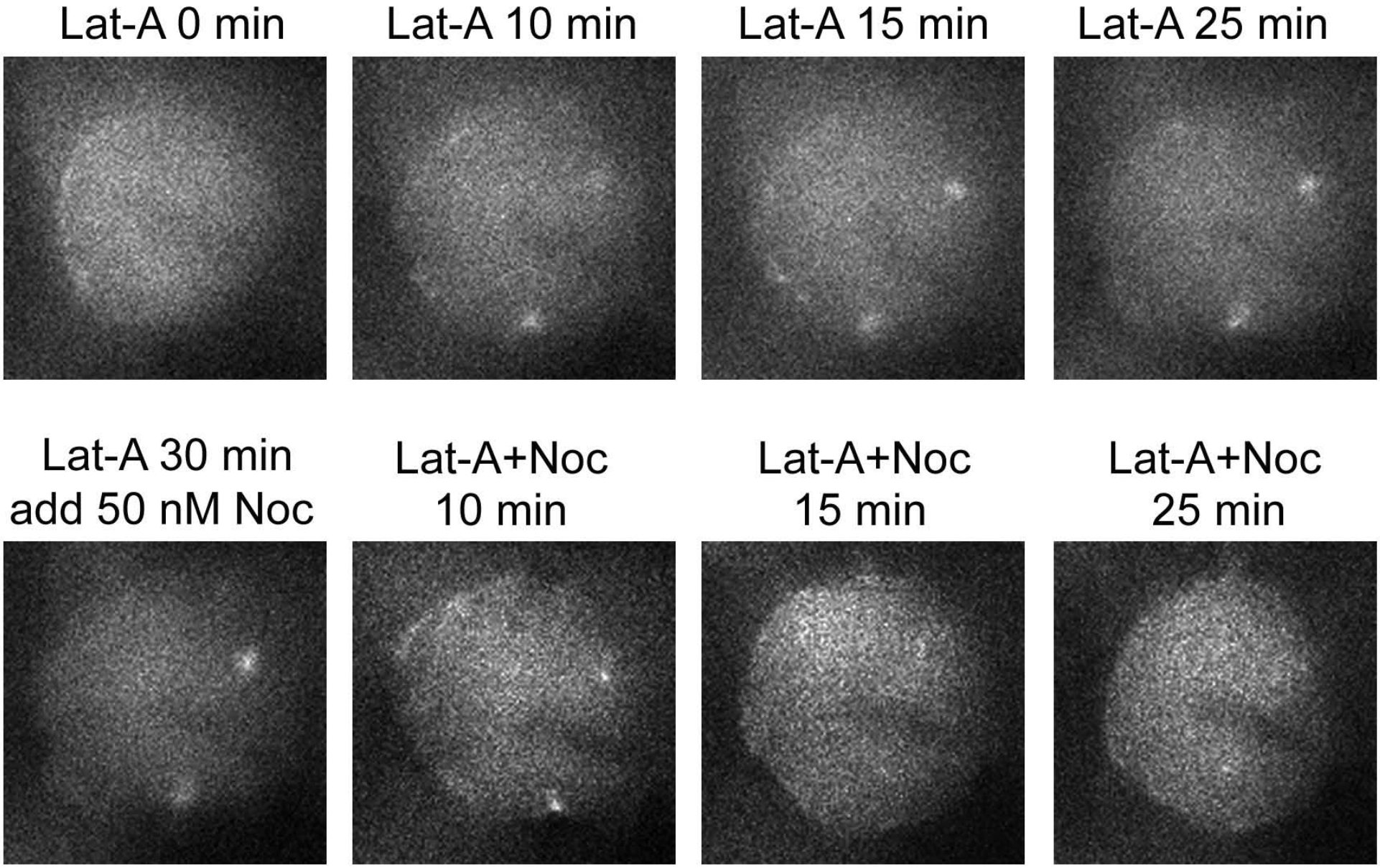

A.
LGN/Tubulin/DNA
control
Lat-A
Nocodazole
+ Lat-A
B.
NuMA/Tubulin/DNA
control
Lat-A
Nocodazole
+ Lat-A

Figure S5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Lat-A 10 min
Lat-A 15 min
Lat-A 25 min
Lat-A 30 min
add 50 nM Noc
Lat-A+Noc
10 min
Lat-A+Noc
15 min
Lat-A+Noc
25 min