Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figure Legends

SUPPLEMENTAL FIGURE 1: (A) Quantification of mitotic defects in HeLa cells arrested in metaphase with MG132 prior to importazole treatment. (B) Representative micrograph of a mitotic HeLa cell displaying the double spindle phenotype. Dashed white line indicates the cell cortex. N=3, 100 mitotic cells counted per condition in (A). Bars represent standard error. Asterisks indicate statistical significance ($p < 0.005$). Scale bar = 10 μm.

SUPPLEMENTAL FIGURE 2: Representative micrograph of a metaphase HeLa cell treated with 40 μM importazole displaying a defect in spindle centering and cortical LGN staining in line with the axis of the metaphase plate. Scale bars = 10 μm.

SUPPLEMENTAL FIGURE 3: Relative abundance of LGN and NuMA in mitotic HeLa cells treated with DMSO (Control) or 40 μM importazole as measured by quantitative Western blot. Protein levels are normalized to DMSO treatment. N=4, bars represent standard error.

SUPPLEMENTAL FIGURE 4: (A) Mean spindle movement speed derived from 60-minute movies of mitotic HeLa cells stably expressing GFP-tubulin and mCherry-H2B treated with DMSO, 40 μM importazole, 20 nM nocodazole, or 20 nM nocodazole and 40 μM importazole. (B) The percentage of asynchronously growing mitotic HeLa cells displaying spindle and chromosomal defects after treatment for one hour with DMSO, 40 μM importazole, 20 nM nocodazole, or 20 nM nocodazole and 40 μM importazole. Spindles from six cells were analyzed for each condition in (A). N=3, 100 cells counted per condition in (B). Bars represent standard error.

SUPPLEMENTAL FIGURE 5: (A) Quantification of spindle assembly and chromosome congression defects as a percentage of total mitotic cells. Asynchronously growing HeLa cells expressing GFP-CLASP1 or YFP alone (control) were treated with DMSO, 20 μM, or 40 μM importazole for 1 hour before fixation. CLASP1 overexpression resulted in a small but significant reduction in the incidence of cells displaying spindle assembly defects at 20 μM importazole. Neither spindle assembly nor chromosome congression was affected by CLASP1 overexpression under any other conditions. (B) Mean astral MT number and length in HeLa cells treated with DMSO or 40 μM importazole for 1 hour before fixation. (C) Representative micrographs of astral microtubules in HeLa cells treated with DMSO or 40 μM importazole. N=3, 100 metaphase cells counted per condition in (A). N=3, 20 cells counted or 100 astral

microtubules measured per condition in (B). Bars represent standard error. Asterisks denote statistical significance ($p < 0.05$). Scale bars = 10 μm.

SUPPLEMENTAL MOVIE 1: 60 minute movie of HeLa cell stably expressing GFP-tubulin treated with DMSO. The spindle progresses to the middle of the cell before entering anaphase. Scale bar = 10 μm.

SUPPLEMENTAL MOVIE 2: 60 minute movie of HeLa cell stably expressing GFP-tubulin treated with 40 μM importazole. The spindle moves throughout the cytoplasm. Scale bar = 10 μm.

SUPPLEMENTAL MOVIE 3: 60 minute movie of HeLa cell stably expressing GFP-tubulin treated with 40 μM importazole. The spindle moves throughout the cytoplasm before progressing through anaphase. Scale bar = 10 μm.

SUPPLEMENTAL MOVIE 4: 2 minute movie of HeLa cell stably expressing GFP-EB3 treated with 20 nM nocodazole. Astral MTs are severely disrupted. Scale bar = 10 μm.

SUPPLEMENTAL MOVIE 5: 60 minute movie of HeLa cell stably expressing GFP-tubulin treated with 20 nM nocodazole. The spindle remains close to the center of the cell and moves little. Scale bar = 10 μm.

SUPPLEMENTAL MOVIE 6: 60 minute movie of HeLa cell stably expressing GFP-tubulin treated with 20 nM nocodazole and 40 μM importazole. The spindle remains close to the center of the cell and moves little. Scale bar = 10 μm.

SUPPLEMENTAL MOVIE 7: 2 minute movie of HeLa cell stably expressing GFP-EB3 treated with DMSO. Astral MTs are clearly visible. Scale bar = 10 μm.

SUPPLEMENTAL MOVIE 8: 2 minute movie of HeLa cell stably expressing GFP-EB3 treated with 40 μM importazole. Astral MTs are clearly visible. Scale bar = 10 μm.

SUPPLEMENTAL METHODS

Mitotic Defects with MG132 Arrest

HeLa cells were treated for three hours with 10 μM MG132 to induce arrest at metaphase. During the final 30 minutes of MG132 treatment, cells were treated with either DMSO or 40 μM importazole. Cells were then washed two times with fresh media, and placed into fresh media with DMSO or 40 μM importazole for an additional 30 minutes before fixation and immunostaining for tubulin as described in the methods section. Cells were imaged via widefield fluorescence microscopy.

LGN and NuMA stability

Cells were first synchronized via double thymidine block as described in the materials and methods section. Cells were treated for one hour prior to collection with

DMSO or 40 μM importazole. Metaphase mitotic cells were collected via shake off, and lysed in lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1% Triton X-100, 10 μM Chymostatin, 1 μM Pepstatin A, 10 μM Leupeptin) at 4° C. Samples were then analyzed via Western blot using anti-LGN or anti-NuMA antibodies at 1:10000 in 4% milk, and protein levels were quantified using a LI-COR® Odyssey® CLx imaging system. Protein levels were normalized to the DMSO condition.

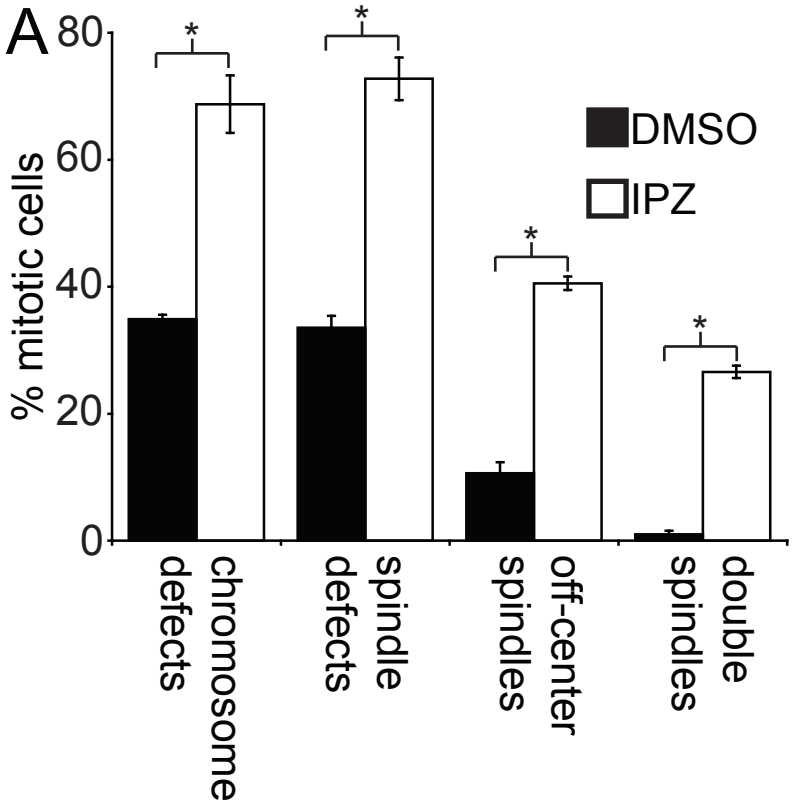
Spindle movement speed

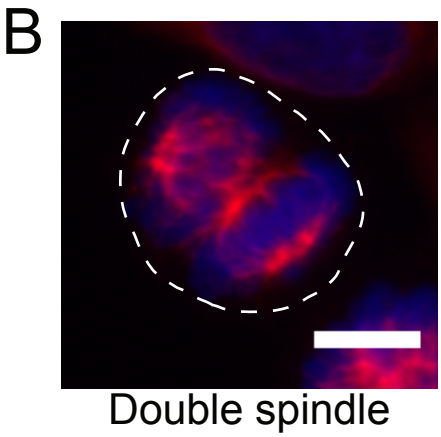
Movement of mitotic spindles was quantified as discussed in the materials and methods section. For each cell, the speed of spindle movement was calculated at each frame of a sixty-minute movie with frames taken every two minutes. The results were then averaged to derive the mean spindle movement speed for each cell. Spindles in a total of six cells were measured for each condition.

Astral microtubule imaging

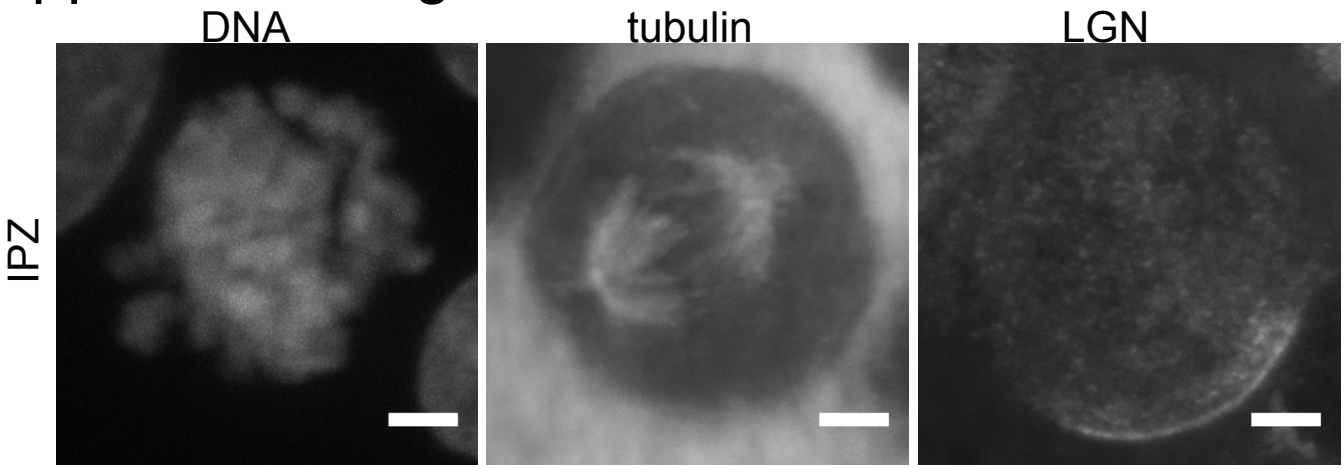
Cells were washed once with PBS prior to fixation with MeOH plus 1 mM EGTA for five minutes at -20° C. Cells were then washed three times with PBS, blocked with PBS plus 4 % BSA and 0.1 % triton X-100, and stained for tubulin as described in the methods section. Astral microtubules were counted and measured in a single z plane bisecting the spindle poles.

Supplemental Figure 1

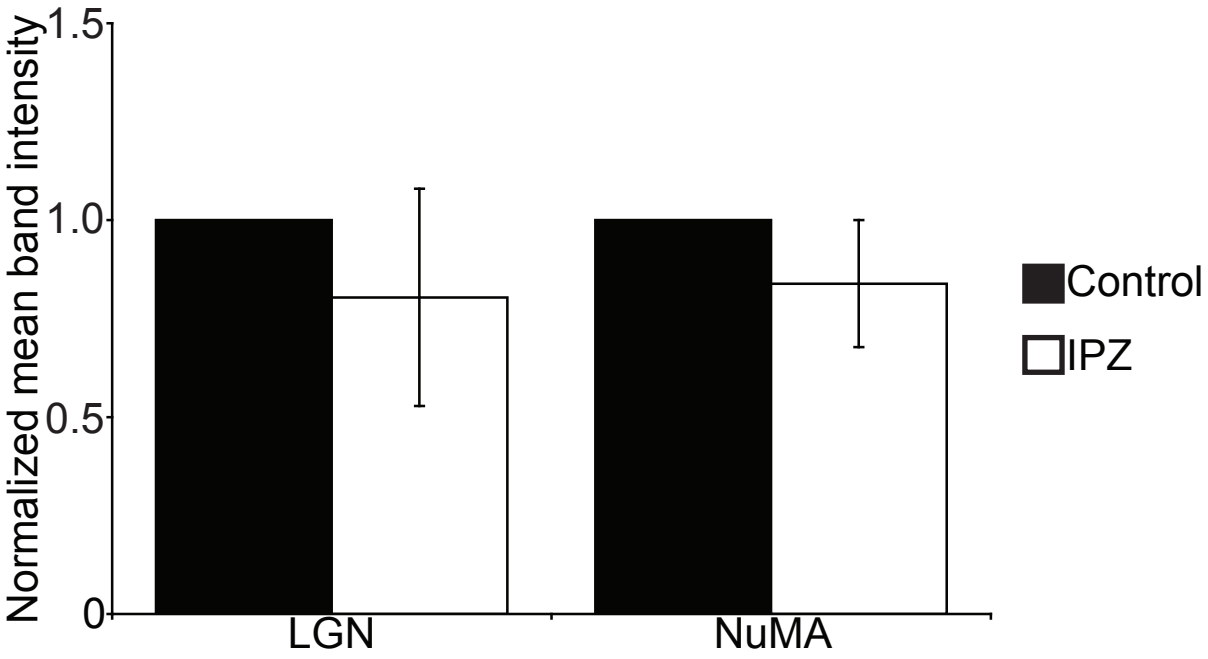




Supplemental Figure 2

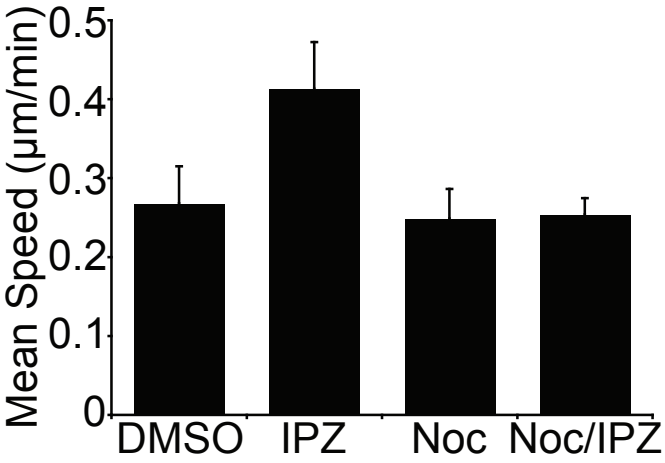


Supplemental Figure 3



Supplemental Figure 4

A



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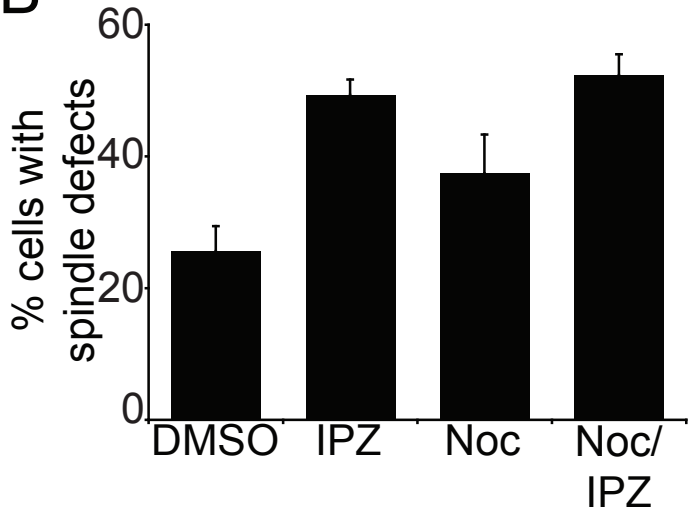

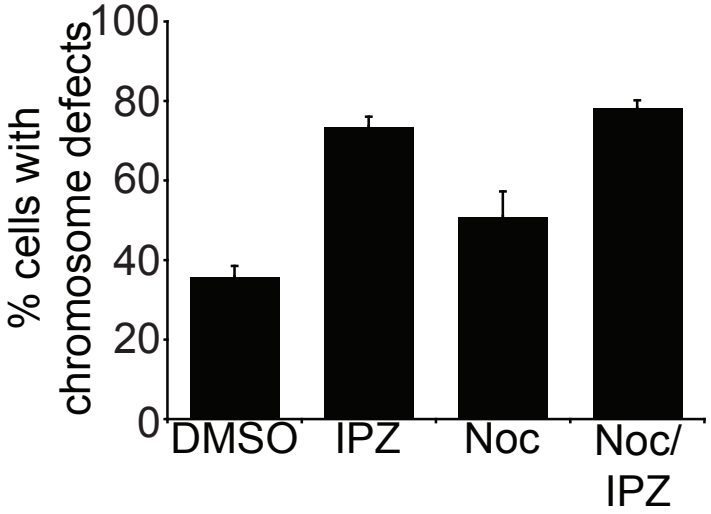

Supplemental Figure 5