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Supplemental Information

Non-muscle Myosin-II Is Required for the Generation of a Constriction Site for Subsequent Abscission

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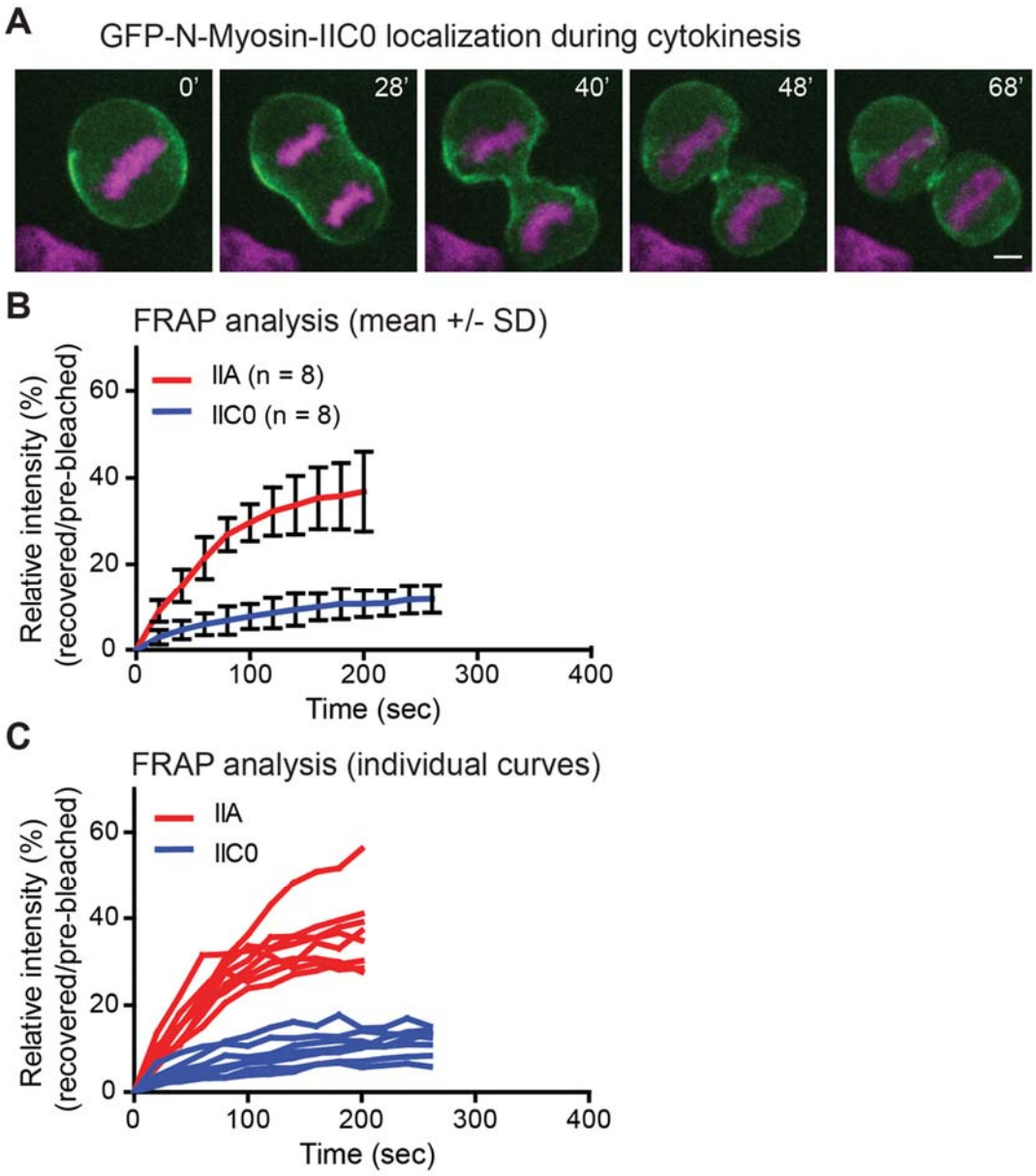

Figure S1. Localization and dynamics of NM-IIC0 during cytokinesis. Related to Figure 1.

(A) Localization pattern of NM-IIC0 during cytokinesis. Scale bar, 5 μm. See also **Movie S2, left**.

(B and C) FRAP analysis of NM-IIC0 during cytokinesis. Data for fluorescence recovery at the bleached region of all cells are presented in the format of mean ± SD (B) or as individual curves (C). See also **Movie S2, right**

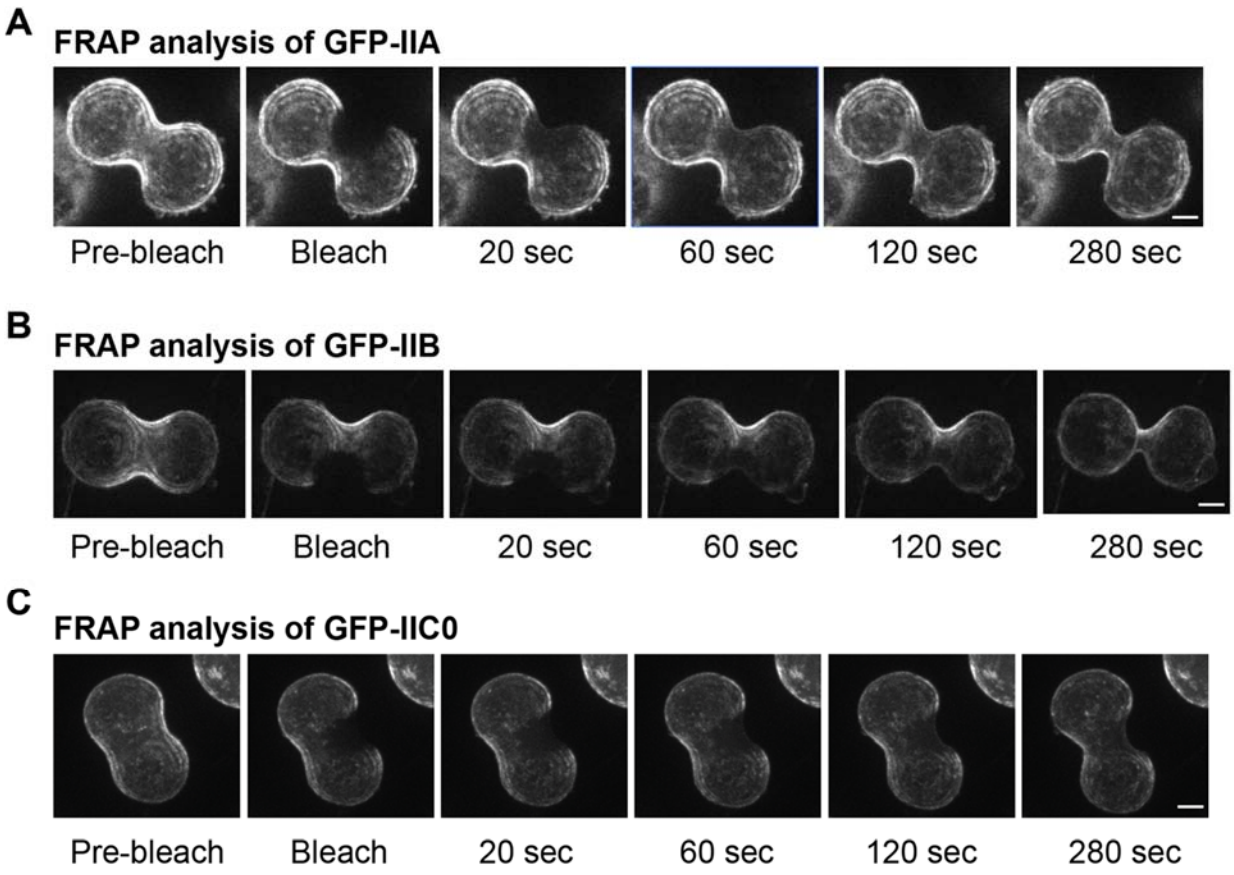

Figure S2. Different NM-II isoforms display distinct dynamics during cytokinesis. Related to Figures 1 and S1.

(A) A representative cell carrying GFP-tagged NM-IIA (A), -IIB (B), or -IIC0 (C) for the FRAP analysis is shown. Scale bar, 5 μm. See also **Movies S2** (right) and **S3**.

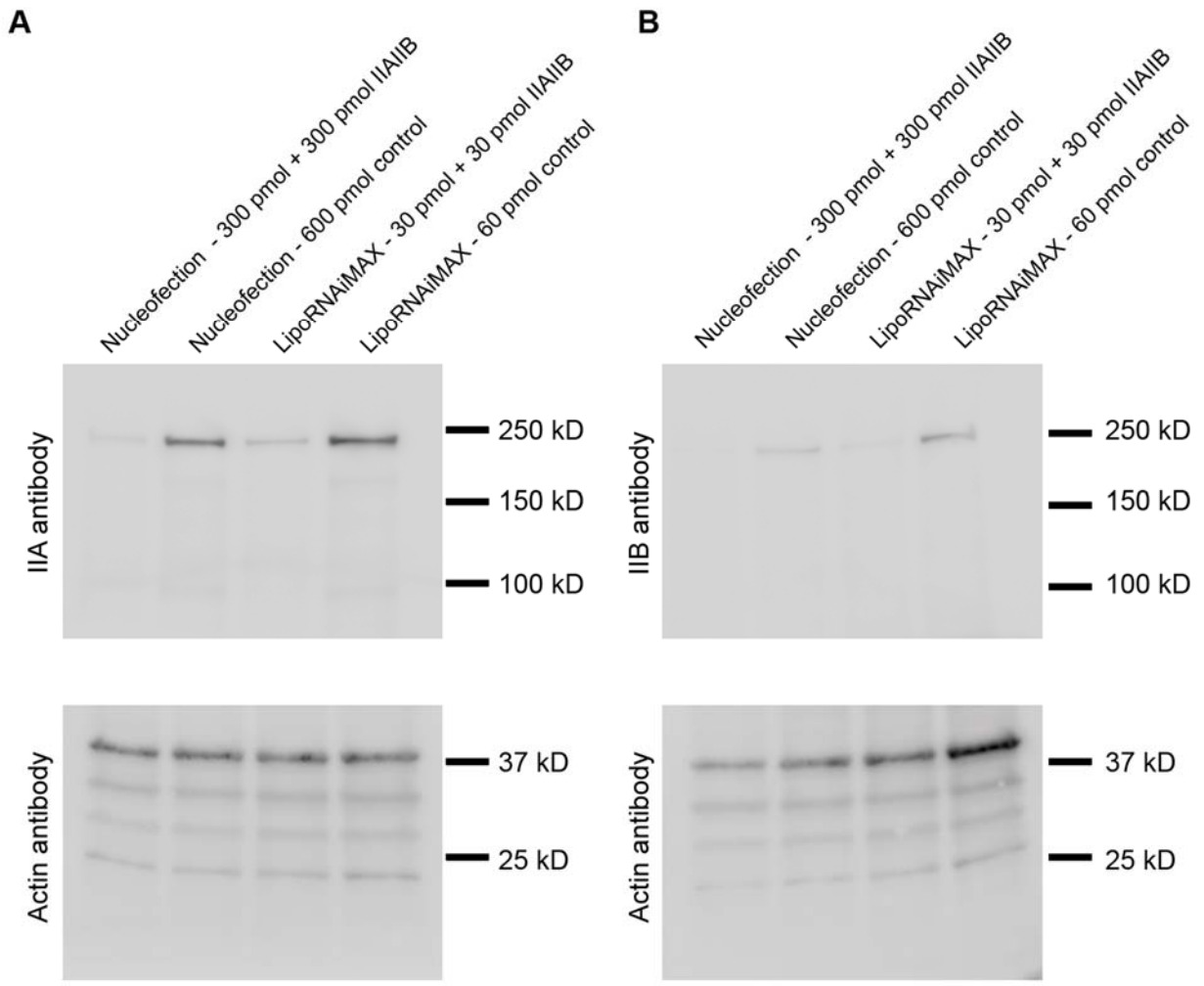

Figure S3. Knockdown of NM-IIA and –IIB expression by siRNAs. Related to Figure 3B.

(A) Efficiency of IIA knockdown by siRNA. Cells were transfected with indicated concentrations of control RNA or siRNAs against IIA and IIB together by lipofectamine or nucleofection. The efficiency of IIA knockdown was determined 72 hours after transfection by Western blotting using an antibody against IIA (top). Sample loading for each lane was determined by Western blotting using an antibody actin (bottom).

(B) Efficiency of IIB knockdown by siRNA. Experiments were performed as described in (A), except that an antibody against IIB was used for Western blotting.

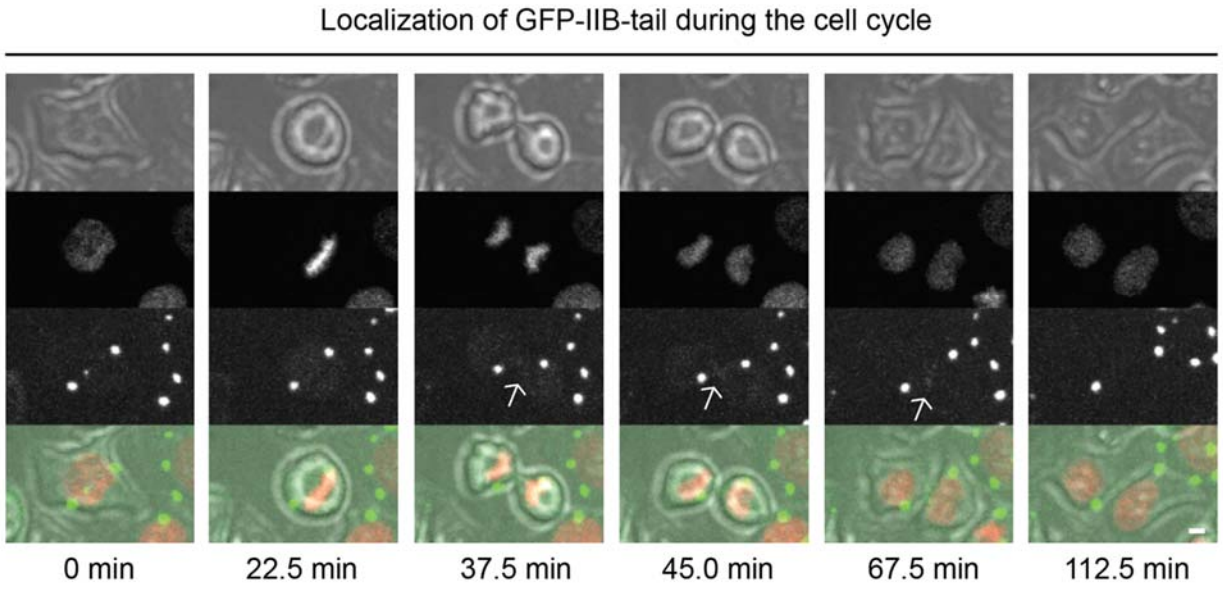

Figure S4. Localization of GFP-IIB-tail during the cell cycle. Related to Figure 2A.
Cells were transfected with the plasmid carrying GFP-IIB-tail, and imaged 24-28 hours after transfection. White arrows indicate the weak localization of GFP-IIB-tail at the division site. Scale bar, 5 μm. See also Movie S4.

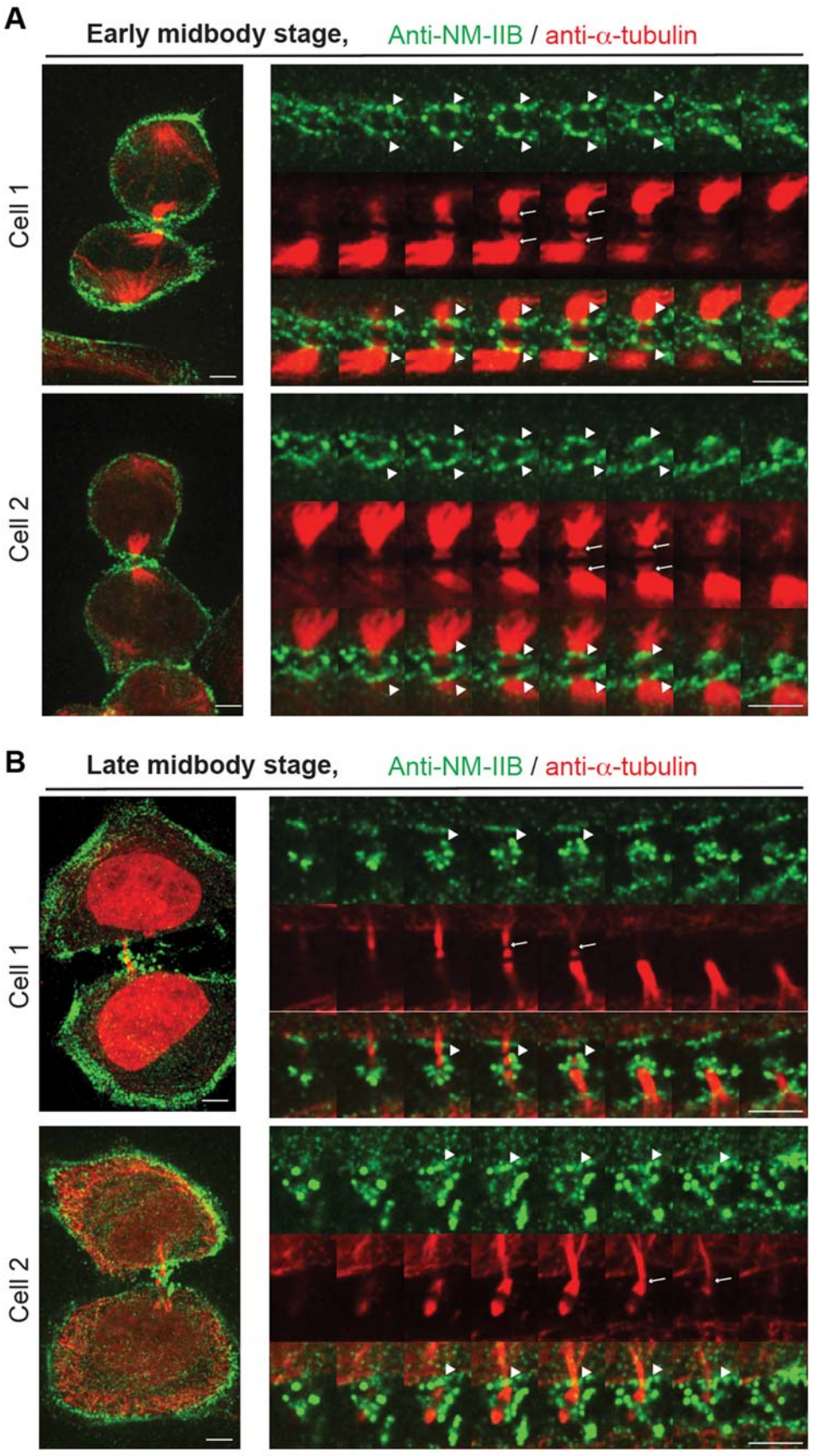
A
Early midbody stage,
Anti-NM-IIB / anti-α-tubulin
Cell 1
Cell 2
B
Late midbody stage,
Anti-NM-IIB / anti-α-tubulin
Cell 1
Cell 2

Figure S5. Localization of IIB at the ICB during different midbody stages. Related to Figure 5B.

(A) Localization of IIB at the ICB during the early midbody stage. Cells were from the culture treated with DMSO for 60 min. IIB (green) and tubulin (red) were immuno-stained with specific antibodies. Left panel, max projection of variable Z sections covering the ICB only; and right panel, montage of Z sections covering the ICB, Z step = 0.6 μm. Arrows, SOCs; and triangles, IIB at the SOCs. Scale bar, 5 μm.

(B) Localization of IIB at the ICB during the late midbody stage. Except that cells were from the culture treated with DMSO for 120 min, all other experimental conditions and denotations are the same as described in panel A.

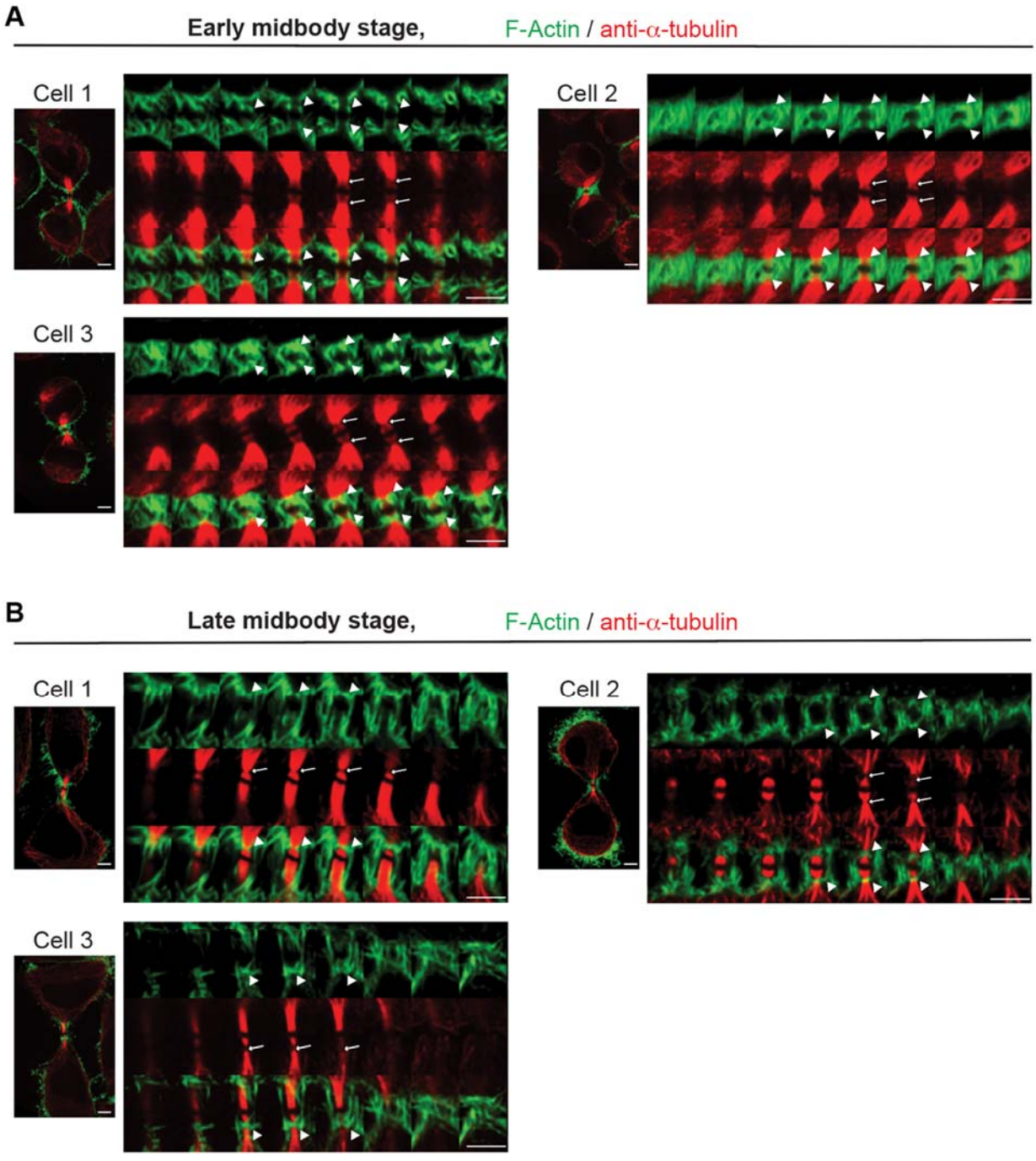
A
Early midbody stage,
F-Actin / anti-α-tubulin
Cell 1
Cell 2
Cell 3
B
Late midbody stage,
F-Actin / anti-α-tubulin
Cell 1
Cell 2
Cell 3

Figure S6. Localization of F-actin at the ICB during different midbody stages. Related to Figure 5C.

(A) Localization of F-actin at the ICB during the early midbody stage. Cells were from the culture treated with DMSO for 60 min. F-actin (green) and tubulin (red) were stained with Alex568 labeled phalloidin and a specific antibody, respectively. Left panel, max projection of variable Z sections covering the ICB only; and right panel, montage of Z sections covering the ICB, Z step = 0.6 μm. Arrows, SOCs; and triangles, F-actin at the SOCs. Scale bar, 5 μm.

(B) Localization of F-actin at the ICB during the late midbody stage. Except that cells were from the culture treated with DMSO for 120 min, all other experimental conditions and denotations are the same as described in panel A.

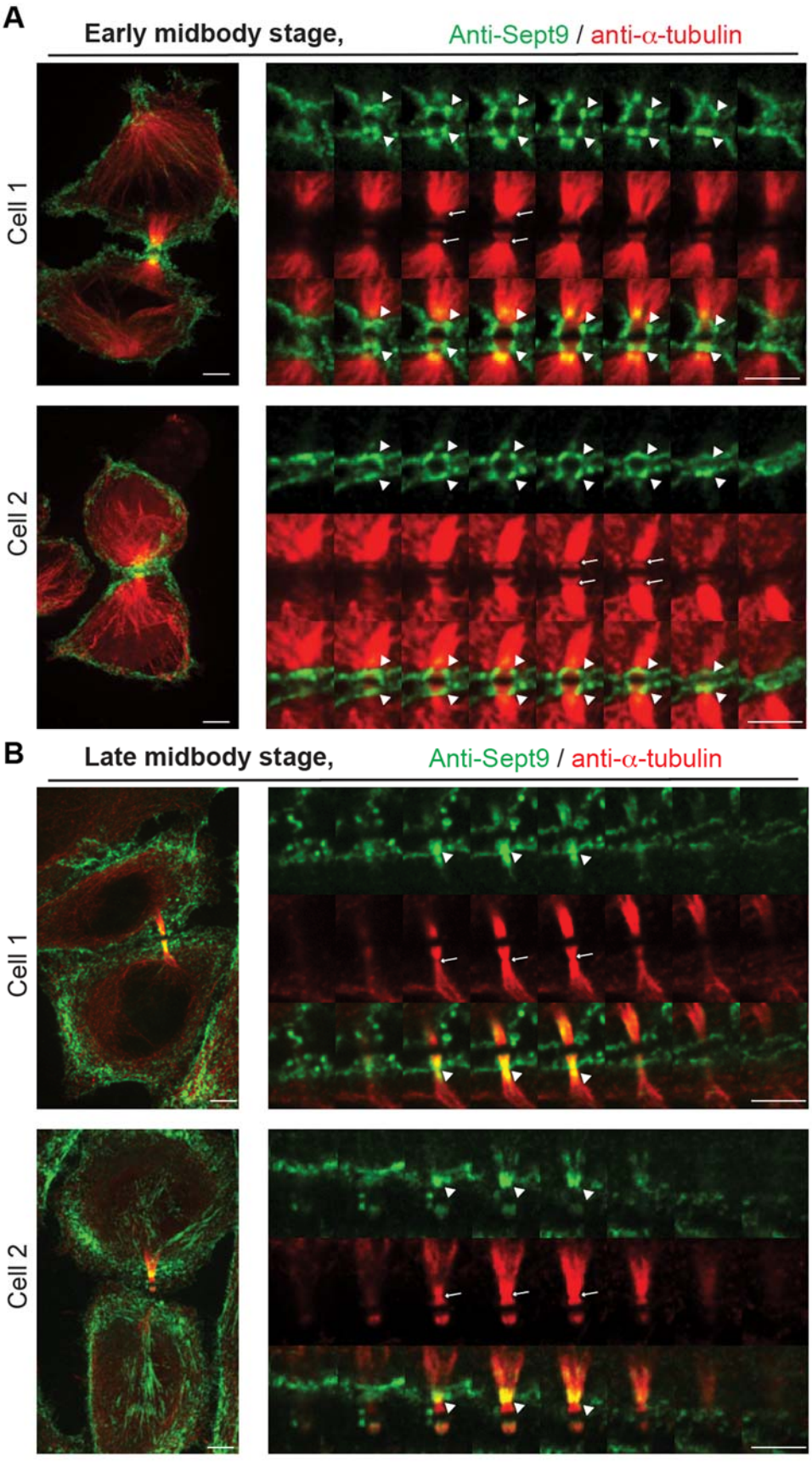
A
Early midbody stage,
Anti-Sept9 / anti-α-tubulin
Cell 1
Cell 2
B
Late midbody stage,
Anti-Sept9 / anti-α-tubulin
Cell 1
Cell 2

Figure S7. Localization of septin 9 at the ICB during different midbody stages. Related to Figure 5D.

(A) Localization of Sept9 at the ICB during the early midbody stage. Cells were from the culture treated with DMSO for 60 min. Sept9 (green) and tubulin (red) were immuno-stained with specific antibodies. Left panel, max projection of variable Z sections covering the ICB only; and right panel, montage of Z sections covering the ICB, Z step = 0.6 μm. Arrows, SOCs; and triangles, Sept9 at the SOCs. Scale bar, 5 μm.

(B) Localization of Sept9 at the ICB during the late midbody stage. Except that cells were from the culture treated with DMSO for 120 min, all other experimental conditions and denotations are the same as described in panel A.

Transparent Methods

EXPERIMENTAL MODEL AND SUBJECT DETAILS

HeLa-Kyoto cells stably expressing histone H2B-mCherry (Schmitz et al., 2010) (kindly supplied by Michael Lampson at the University of Pennsylvania, PA, USA) and HeLa cells stably expressing both EFGFP-α-tubulin and mCherry-H2B (Bastos and Barr, 2010) (kindly provided by Francis A. Barr at the University of Oxford) were used throughout this study.

METHODS DETAILS

Constructs, antibodies, and reagents

Plasmids CMV-GFP-NMHCII-A, CMV-GFP-NMHCII-B, and pEGFP-NMHC II-C0 were purchased from Addgene (Wei and Adelstein, 2000, Golomb et al., 2004). Plasmid carrying CEP55-GFP was provided by Dr. Kerstin Kutsche (Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany) (Martinez-Garay et al., 2006). Adenovirus carrying GFP-tagged LifeAct for live imaging of actin filaments was purchased from IBIDI (Riedl et al., 2008). Small-interference RNAs (siRNAs), including those against IIA (Myh9) and those against IIB (See also KRT) were purchased from Integrated DNA Technologies. The *Silencer*^{TM} Select Negative Control No. 1 was purchased from Invitrogen. The C-terminal constructs of IIA (349 amino acids, residues 1612-1960) and IIB (348 amino acids, residues 1629-1976) were made as follows. The PCR conditions for amplifying the IIA (Myh9) and IIB (Myh10) tail fragments were the same except primers: 1.0 μl PfuUltra II Fusion HS DNA Polymerase (Agilent Technologies), 5 μl 10 X PfuUltra II reaction buffer, 0.1 μl (~~80 ng) Addgene's NMHCII-A or NMHCII-B plasmid as the template DNA, 1.5 μl forward primer (50 μM in stock) (See also KRT for primer sequences), 1.5 μl reverse primer (50 μM in stock), 5 μl 10X dNTPs (2.5 mM each), 2.0 μl $MgCl_2$ (50 mM in stock), 25 cycles with an elongation time of 1 min 10 sec and 4 min post annealing. The PCR-amplified 1050-bp IIA fragment (including stop codon) and the 1047-bp IIB fragment (including stop codon) were subsequently cloned into pcDNA3.1/NT-GFP-TOPO using the NT-GFP Fusion TOPO Expression Kit (ThermoFisher Scientific), resulting in the generation of GFP-IIA-349-tail and GFP-IIB-348-tail under the CMV promoter control. Primary antibodies used in this study include the rabbit polyclonal anti-

NMIIA (Cat#: BT-567) from Biomedical Technologies, rabbit polyclonal anti-Chmp4B (Cat#: 13683-1-AP) and rabbit polyclonal anti-human Cep55 (Cat#: 23891-1-AP) from ProteinTech Group, rabbit polyclonal anti-Sept9 (Cat#: NBP2-3294) from Novus Biological, mouse monoclonal anti-α-Tubulin and rabbit polyclonal anti-actin (1:100 dilution for Western blotting) from Sigma, and rabbit polyclonal anti-mouse NM-IIA (Cat#: 3403) and anti-human NM-IIB antibodies (Cat#: 3404S) (both at 1:1000 dilution for Western blotting) from Cell Signaling Technology. Secondary antibodies used in this study include the peroxidase AffiniPure goat anti-rabbit IgG (1:20,000 dilution for Western blotting) from the Jackson ImmunoResearch Laboratories, Alexa Fluor 488 goat anti-mouse IgG (H+L) (Cat#: A-11001), Alexa Fluor 568 goat anti-mouse IgG (H+L) (Cat#: A-11004), and Alexa Fluor 488 chicken anti-rabbit IgG (H+L) (Cat#: A-21441) from Thermo Fisher Scientific. Alexa Fluor 568 Phalloidin (Cat#: A12380) was purchased from Thermo Fisher Scientific. (-)-Blebbistatin was purchased from Sigma and was dissolved in dimethylsulfoxide (DMSO) (ThermoFisher Scientific) with the stock concentration of 3.4 mM.

Cell culture, transfection, and siRNA knockdown

HeLa-Kyoto cells stably expressing H2B-mCherry were cultured in Dulbecco's Modified Eagle Medium (DMEM) (ThermoFisher Scientific) containing 5% or 10% fetal bovine serum (FBS) (Invitrogen/Gibco) at 37°C in the presence of 5% CO_2. Plasmids were transfected into HeLa-Kyoto cells using Lipofectamine 2000 (ThermoFisher Scientific) following manufacturer's instructions. For the knockdown experiments, siRNAs against IIA (Myh9) and IIB (Myh10) were transfected into HeLa-Kyoto cells using either Lipofectamine RNAiMAX (ThermoFisher Scientific) (30 pmol for siRNAs against IIA or IIB and 60 pmol for the control siRNA) or Nucleofector I (Amaxa) (300 pmol for siRNAs against IIA or IIB and 600 pmol for the control siRNA). The knockdown efficiency was compared 72 hours after transfection by Western blotting. Both methods produced similar results. Since then, only Lipofectamine RNAiMAX was used for our functional studies.

Cell synchronization and immunofluorescence

HeLa-Kyoto cells expressing H2B-mcherry cells were grown on a 15-mm coverslip in a 90-mm dish to 50-60 % confluency. The cells were then synchronized by sequential treatments with

thymidine (a DNA synthesis inhibitor that arrests cells at the G1/S boundary), nocodazole (a microtubule-depolymerization drug that arrests cells in G2 or mitosis), and MG132 (a proteasome inhibitor that arrests cells at metaphase). Specifically, the cells were first treated with 2 mM thymidine (Sigma, Cat#: T9250) (stock concentration: 200 mM in water) for 24 hours. Thymidine was then washed out to let cells progress through the cell cycle for 6 hours. These cells were then treated with 50 ng/μL nocodazole (Sigma, Cat#: SML1665) (stock concentration: 5 mg/mL in DMSO) for 4 hours. Subsequently, nocodazole was washed out, and the cells were treated with 10 μM MG132 (Sigma, Cat#: M7449) (stock concentration: 10 mM in DMSO) for 2 hours. Finally, MG132 was washed out and the cells were allowed to progress to anaphase (~45 minutes after the release). These cells were then treated with DMSO or 7.5 μM Blebbistatin for 60 or 120 minutes to allow them reach the early and late midbody stages, respectively.

The above cells were fixed with 3.7% formaldehyde in PBS for 10 minutes, washed 3 times in PBS, and then treated with 0.2% Triton X-100 (Bio-Rad, Cat#: 1610407) for 10 minutes. After washing 3 times with PBS, cells were blocked in PBS containing 1% BSA for 30 minutes and then incubated with primary antibody (with dilutions for IIA, 1:100; IIB, 1:100; CHM4B, 1:200; Sept9, 1:200; Cep55, 1:200; and α-tubulin, 1:500) in PBS containing 1% BSA at 4°C for overnight. After washing 5 times with PBS, cells were incubated with Alexa Fluor 488 chicken anti-rabbit antibody (1:500) in combination with Alexa Fluor 568 goat anti-mouse antibody (1:500) or Alexa Fluor 488 goat anti mouse antibody (1:500) with Alexa Fluor 568 Phalloidin (1:40) at 25°C for 1.5 hours. After washing 5 times with PBS, cells were mounted with VECTASHIELD Antifade Mounting Medium containing DAPI. Coverslips were sealed with nail polish and imaged with the Nikon microscope described below.

Imaging and analysis

For live imaging, HeLa-Kyoto cells expressing H2B-mCherry were grown in DMEM medium containing 5% or 10% (for **Figures 3D and 3E**) FBS in a 35-mm glass-bottom culture dish (MatTek) that was placed in a Chamlide Incubator System (Live Cell Instrument, Seoul, South Korea) at 37°C in the presence of 5% CO_2. Except where noted, images were acquired on a spinning-disk confocal microscope equipped with a Yokogawa CSU 10 scan head combined

with an Olympus IX 71 microscope and Olympus objectives 20X (0.75 NA, UPlanSAPO, Air/Dry), 40X (0.95 NA, UPlanSAPO, Air/Dry), and 100X (1.4 NA, UPlanSAPO, Oil). Acquisition and hardware were controlled by MetaMorph version 7.7 (Molecular Devices, Downingtown, PA). A Hamamatsu ImagEM EMCCD camera (model C9100-13, Bridgewater, NJ) was used for capture. Diode lasers for excitation (488 nm for GFP and 561 nm for mCherry/RFP) were housed in a launch constructed by Spectral Applied Research (Richmond Hill, Ontario). Bright-field and fluorescence images were taken with 4-10-min intervals as indicated with z-sections of variable step sizes to cover the entire cell. FRAP was performed using a MicroPoint computer-controlled ablation system (Photonic Instruments, St. Charles, IL) consisting of a nitrogen-pumped dye laser (wavelength 435 nm) controlled by MetaMorph. Images were taken with an Olympus 60X objective (1.20 NA UPLSAPO, Water) every 20 seconds (with an appropriate z-stack to cover the entire cell). Quantification was performed with NIH ImageJ, drawing a respective polygon on the region of interest to yield the integrated density for the region (Wloka et al., 2013). This integrated density was used in GraphPad Prism Version 5 (GraphPad Software, La Jolla, CA) to create plots.

For monitoring the impact of Blebbistatin on furrow ingression and abscission (for **Figure 4C**), HeLa cells stably expressing EFGFP-α-tubulin and mCherry-H2B were imaged with 8-min interval in the presence of DMSO or 7.5 μM Blebbistatin with a spinning-disk confocal system that combines the Yokogawa CSU X1 scan head with an Olympus IX 81 microscope equipped with an Olympus objective 100X (1.4NA, UPlanSAPO, Oil) and the Andor iXon X3 EMCCD camera. The same microscope with an Olympus objective 40X (0.6 NA, LUCPlanFLN) was used to image cells with 10-min interval that are presented in **Figures 3D and 3E**.

For imaging the fixed, synchronized, and double-stained cells (**Figures 4A, 5, and 6**), the Nikon microscope (model Eclipse Ti-U, Tokyo, Japan) equipped with a Nikon 100x/1.49NA oil objective (model CFI Apo TIRF 100x), and a Yokogawa spinning-disk confocal scanner unit (model CSU-X1, Tokyo, Japan) was used. Solid-state lasers for excitation (488 nm for GFP and 561 nm for RFP) were housed in a launch constructed by Spectral Applied Research (model ILE-400, Richmond Hill, Ontario, Canada). An Evolve® 512 Delta EMCCD Camera (Tucson, AZ, USA) was used for image capture (15 z-sections with the step size of 0.6 μm). The imaging

system was controlled by MetaMorph version 7.8.10.0 (Molecular Devices, Downingtown, PA, USA).

Supplemental References

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LEGENDS FOR SUPPLEMENTAL MOVIES

Movie S1. Localization of NM-IIA (left) and –IIB (right) during cytokinesis. Related to Figures 1A and 1B.

Movie S2. Localization (left) and FRAP analysis (right) of NM-IIC0 during cytokinesis. Related to Figures 1, B-E; S1; and S2.

Movie S3. FRAP analysis of NM-IIA (left) and –IIB (right) during cytokinesis. Related to Figures 1C and 1D.

Movie S4. Localization of GFP-IIB-tail during the cell cycle. Related to Figure 2A.

Movie S5. Localization of F-actin (GFP-LifeAct) in DMSO (left)- and Blebbistatin (right)-treated cells. Related to Figure 3F.