Supplemental Materials

Molecular Biology of the Cell

Fenix et al.

Supplemental Figure Legends

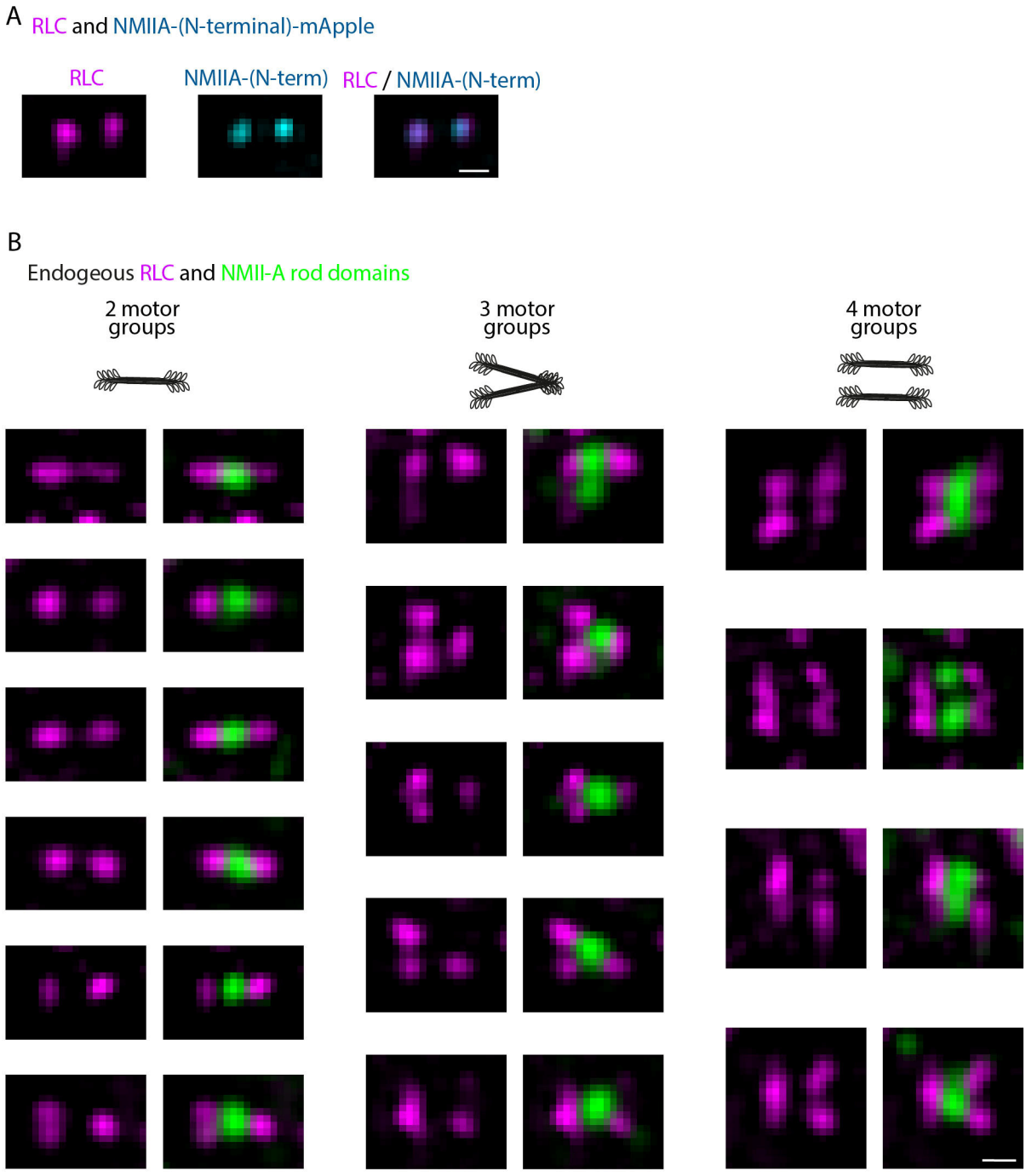
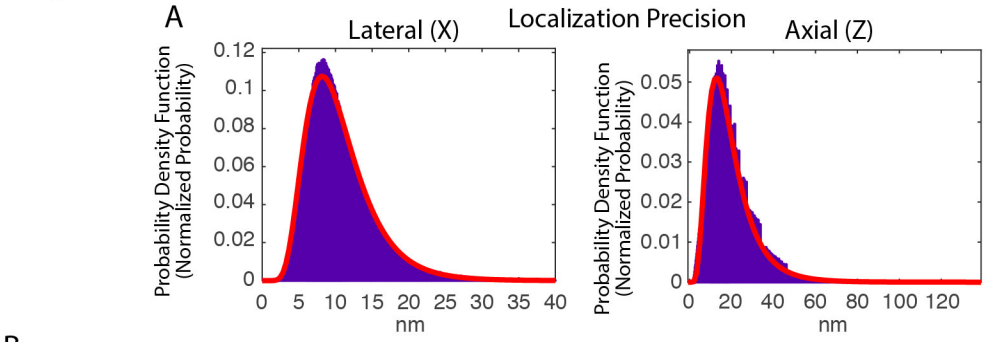
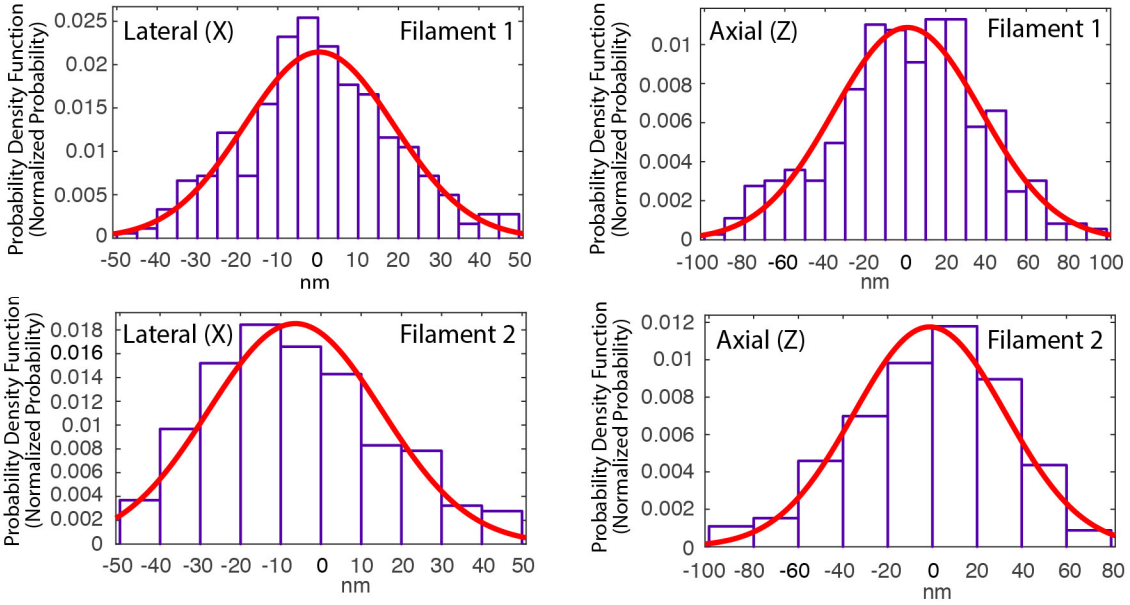


Figure S1-1: Endogenous NMIIA-F organization
A) SIM of RLC (magenta) and NMIIA-(N-terminal)-mApple motor domains (green). Signal from RLC channel aligns with NMIIA-(N-terminal)-mApple motor domains, and thus can be used to quantify motor domains. B) Examples of 2-motor-group, 3-motor-group, and 4-motor-group NMIIA-Fs at the leading edge of crawling U2-OS cells. Scale bars, 200 nm.



B 2-motor-group filaments from Figure 1I



C 3-motor-group filaments from Figure 1J

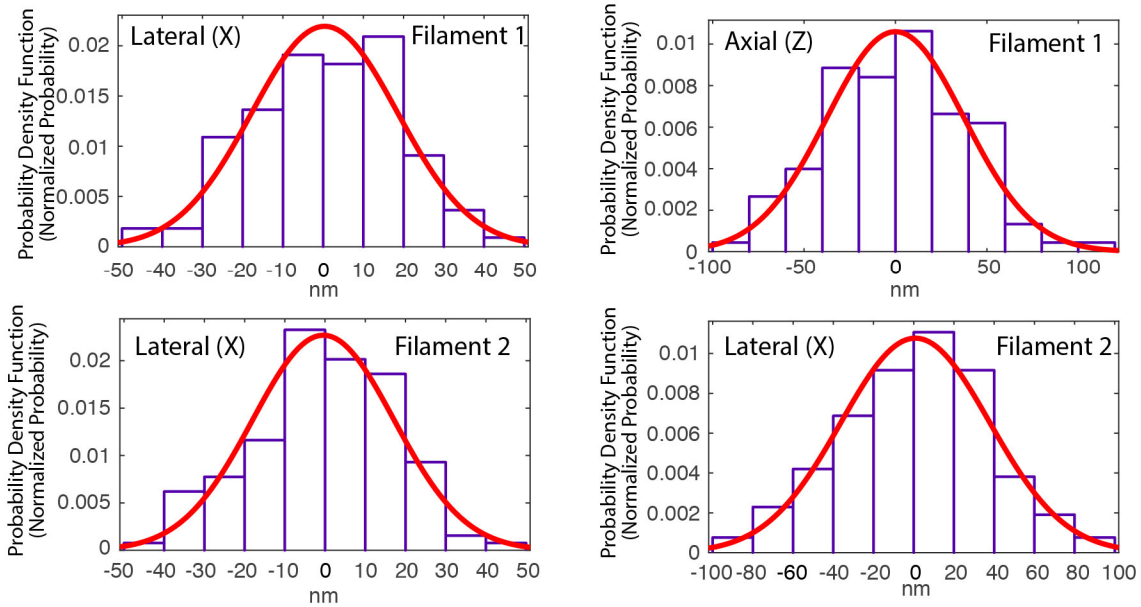
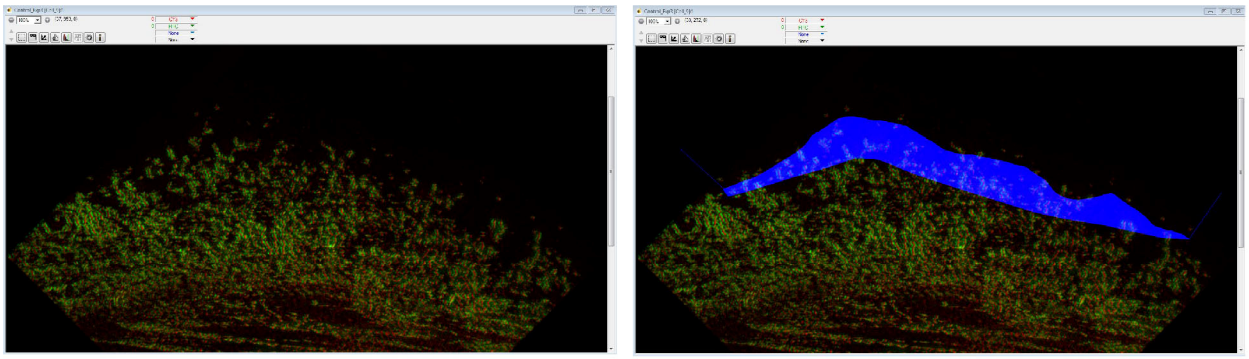


Figure S1-2: Spatial distributions and localization precision of NMIIA-mEOS 3D-PALM measurements.
A) Localization precision for all NMIIA-mEOS2 molecules detected within a cell. The mean of the localization precision was extracted from a lognormal fit to the histogram distribution (μ_z = 20 +/- 11 nm and μ_x = 11+/- 5 nm; error represents S.D.). B) Representative histogram distributions of individual NMIIA-mEOS2 localized within clusters from 2-motor-group filament. Isolated clusters of NMIIA-mEOS2, interpreted to represent NMIIA motor groups, were segmented, normalized to their respective means, and quantified along X and Z spatial dimensions. Histograms were fit to a normal distribution model and the standard deviation was extracted (σ). Cluster dimensions for Filament 1 were σ_x = 19 and σ_z = 37 nm and for Filament 2 were σ_x = 22 and σ_z = 34 nm. C) Representative histogram distributions of individual NMIIA-mEOS2 localization clusters from 3-motor-group filament. Isolated clusters of NMIIA-mEOS2, interpreted to represent NMIIA motor groups, were segmented, normalized to their respective means, and quantified along X and Z spatial dimensions. Histograms were fit to a normal distribution model and the standard deviation was extracted (σ). Cluster dimensions for Filament 1 were σ_x = 18 and σ_z = 38 nm and for Filament 2 were σ_x = 18 and σ_z = 37 nm.

A
Control



B
5 μm Blebb

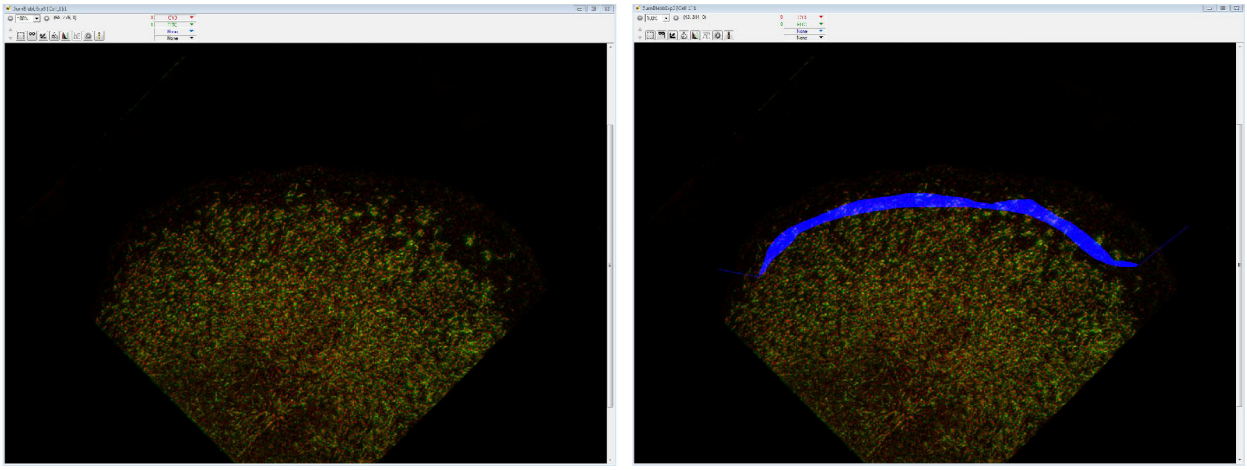
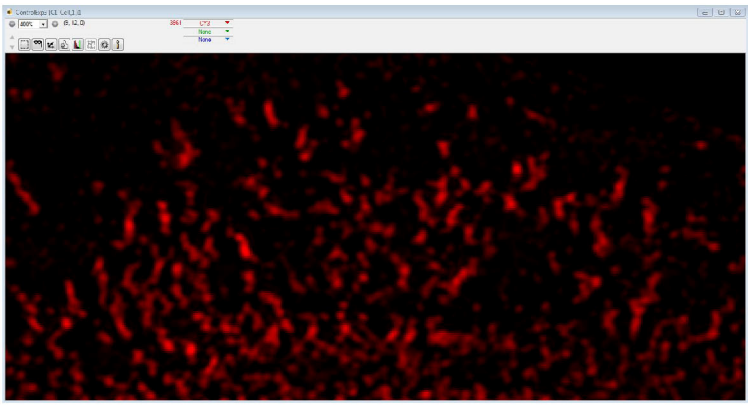
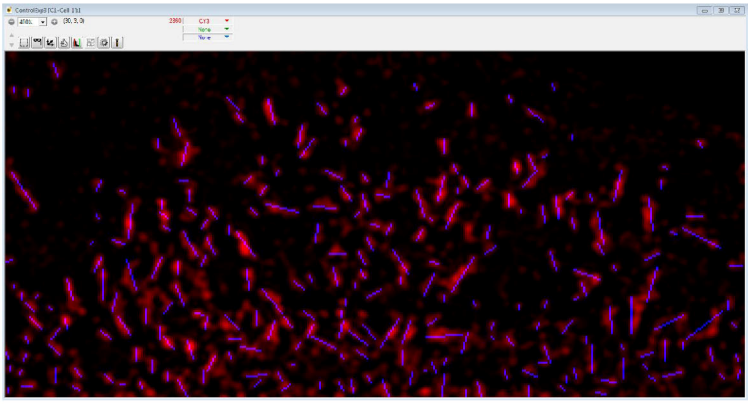


Figure S3-1: Defining region for 2, 3, 4, and >4 motor-group NMIIA-Fs
Actual screen shots of data analyzed in Slidebook from cells visualized by endogenous RLC (green) and NMIIA (red). A) Left screen shot shows a control cell without the region in which 2, 3, 4, >4 motor-group NMIIA-Fs were quantified. A) Right screen shot shows the same control cell with the region in which 2, 3, 4, >4 motor-group NMIIA-Fs were quantified. NMIIA-Fs were quantified above the blue shaded area. Region was made by measuring 1.5 μm towards cell body from the first visualized NMIIA-Fs along the edge of a migrating cell. Width of blue shaded area did not factor into quantification. B) Left screen shot shows a cell treated with 5 μm blebbistatin before quantification, and the right screen shot shows the same cell including the region (blue shaded area), above which 2, 3, 4, >4 motor-group NMIIA-Fs were quantified.

A

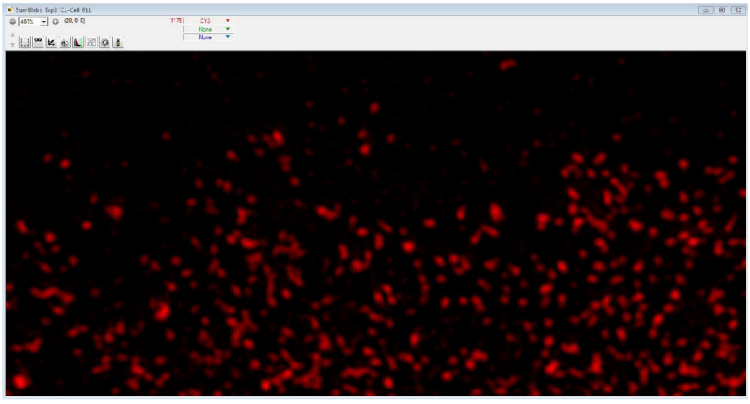
Control





B

5 μm Blebb



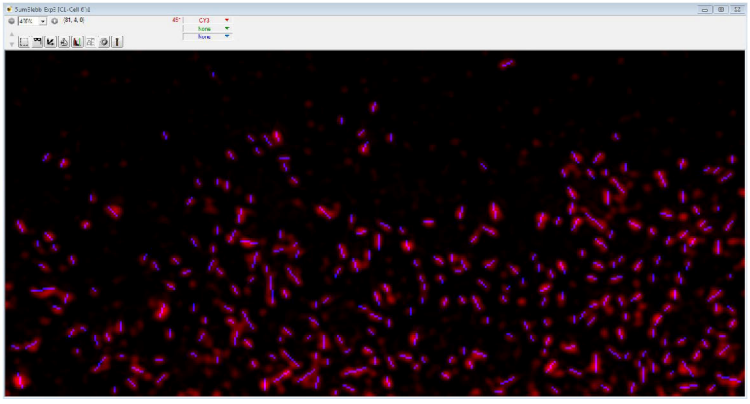


Figure S3-2: Measuring NMIIA-F stack length

A 15x7 μm region was used to crop cells visualized by endogenous NMIIA (red), starting at the first visualized NMIIA-F. Shown are screen shots from Slidebook, as in Fig. S3-1. a) Top screen shot shows the cropped region of a control cell. Bottom screen shot shows the same cell, including a mask of all NMIIA-Fs (blue lines), which give the number of overall NMIIA-Fs, and length of each filament. b) Top screen shot shows the cropped region of a cell treated with 5 μM blebbistatin. Bottom screen shot shows the same cell including a mask of all NMIIA-Fs (blue lines), which give the length and number of overall NMIIA-Fs.

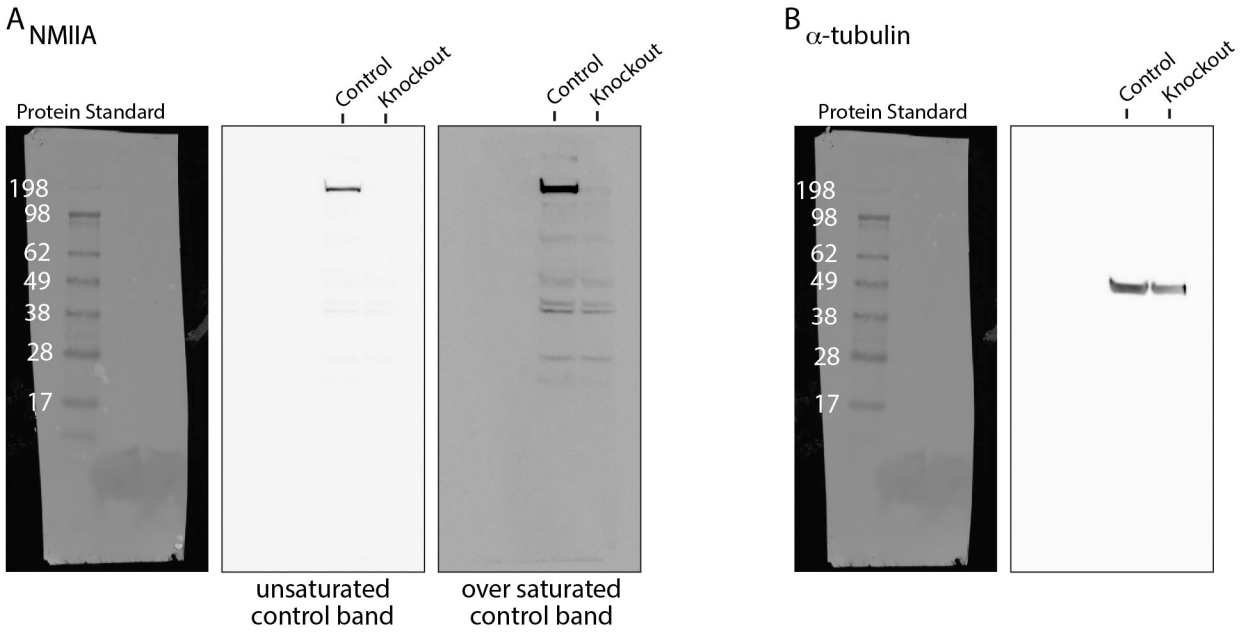


Figure S3-3: Western blot analysis of HAP1 NMIIA knockout cells

A) Cell lysates from control and myosin IIA knockout cells probed for NMIIA. Control cells show a prominent band at ~200kD corresponding to NMIIA heavy chain, while knockout cells lack this band. B) α-tubulin loading control from the same gel as (A).

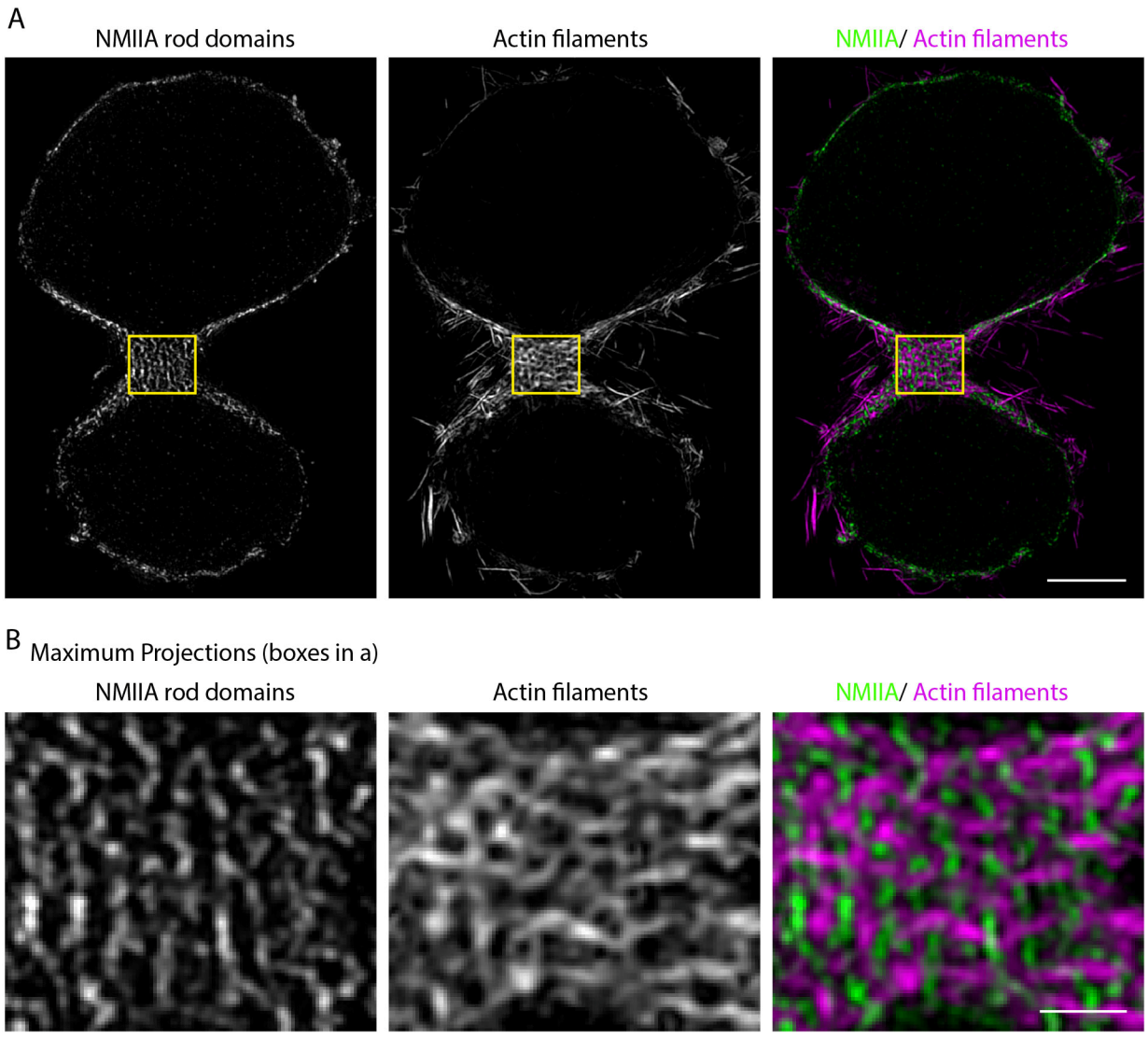


Figure S5-1: Relative NMIIA-Fs and actin filament bundles in the cleavage furrow A) Whole cell view of endogenous NMIIA rod domains, actin filaments (visualized with phalloidin), and overlay of NMIIA rod domains (green) and actin filaments (magenta) at the bottom of a cleavage furrow in a HeLa cell. B) High mag views of the boxes in (A). Note the positions of actin filament bundles are consistent with published electron microscopy data(Sanger and Sanger, 1980). In addition, the NMIIA filaments and actin bundles resemble the sarcomere-like organization of the stress fibers at the leading edge of U2-OS cells (Burnette et al., 2014). This cell was fixed with 4% paraformaldehyde without live-cell extraction (i.e., the same protocol as used for U2-OS cells) as the unlabeled phalloidin in the extraction buffer interfered with labeling with fluorescent Alexa-488 phalloidin. Scale bars: (a), 5 μm; (b), 1 μm.

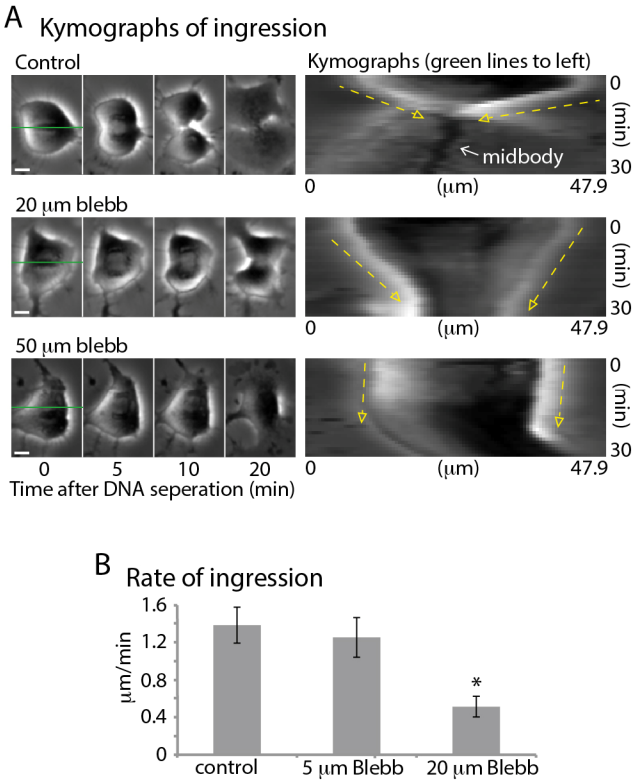


Figure S5-2: Rates of cleavage furrow ingression during cell division

A) Phase contrast time-montages of control, 20 μM blebbistatin, 50 μM blebbistatin treated cells during. First frame is the time-point before chromosome separation. Kymographs showing ingression rates were created from the lines indicated in the first frame. Arrows in kymographs show ingression. Kymographs were created with Nikon Elements. B) Rates of ingression for control cells (N = 43 cells, 3 experiments), cells treated with 5 μM blebbistatin (N = 37 cells, 3 experiments), and cells treated with 20 μM blebbistatin (N = 36 cells, 3 experiments). * denotes $P < 0.001$. Scale bars, 10 μm.

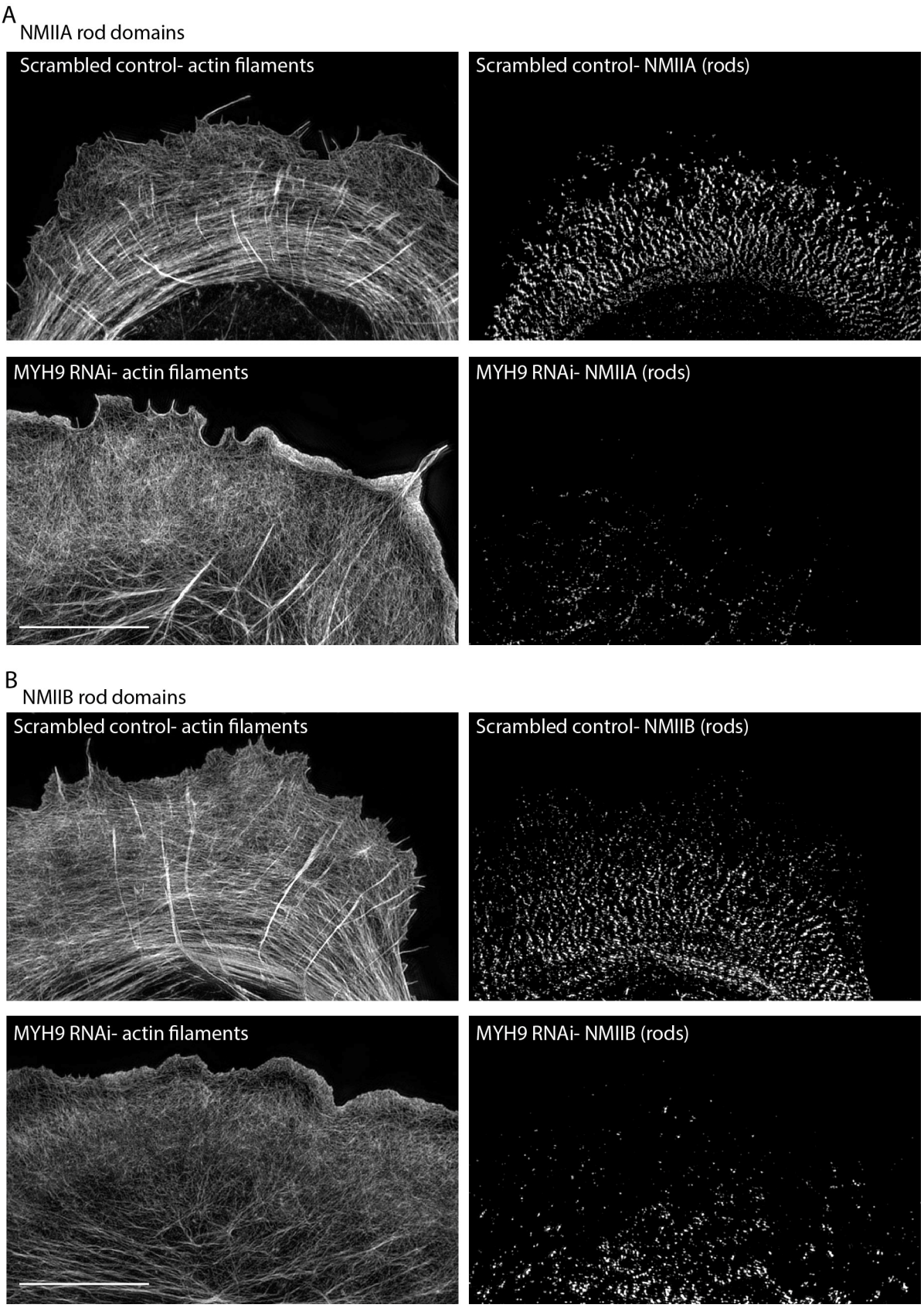


Figure S6: NMIIB-F stacks are absent after knockdown of NMIIA in U2-OS cells

A) Actin filaments localized with phalloidin and NMIIA-F rod domains localized with an antibody in a cell treated with an RNA molecule of a random sequence (Scrambled control) or a pool of RNA molecules design to silence the expression of MYH9 (NMIIA). Images were acquired and are displayed using the same parameters. B) Actin filaments localized with phalloidin and NMIIB rod domains localized with an antibody in a cell treated with an RNA molecule of a random sequence (Scrambled control) or a pool of RNA molecules designed to silence the expression of MYH9 (NMIIA). Images were acquired and are displayed using the same parameters. Note the absence of stack-like arrays of NMIIA-F when RNAi reduces the amount of NMIIA. Scale bars, 10 μm.