SUPPLEMENTAL FIGURES



Figure S1, Related to Figure 1. pMRLC- and ppMRLC-labeled NMII species revealed by immunogold PREM in detergent-extracted and gelsolin-treated REF52 fibroblasts. Gold particles are highlighted in yellow.

- (A) Bipolar filaments in untreated cells.
- (B) Unfolded NMII monomers in untreated cells.
- (C, F) Bipolar filaments in blebbistatin-treated cells.
- (D, G) Unfolded NMII monomers in blebbistatin-treated cells.
- (E) "Half-filaments" in blebbistatin-treated cells labeled at one end.

Bar, 100 nm.



Figure S2, Related to Figure 3. Heteropolymers of NMIIA and NMIIB in cell lamellae.

(A) Heteropolymers in HUVECs. Double immunogold PREM with antibodies to NMIIA (12 nm gold, yellow) and NMIIB (18 nm gold, blue).

(B) Heteropolymers in REF52 cells labeled with an alternative set of antibodies, NMIIA from BTI (Cat. # BT-567) and NMIIB from Iowa Hybridoma Bank (Cat. #CMII 23). Bar, 100 nm.



Figure S3, Related to Figure 5. Phalloidin staining of the cells shown in Figure 5A.

Yellow outlines are examples of regions used for determination of correlation coefficients in lamellipodia (Lp), lamellae (La) and cell bodies (Body). Bar, 10 µm.

SUPPLEMENTAL TABLE	

	NMIIA		NMIIB		NMIIA/NMIIB		
	N	%	Ν	%	Ν	%	I Otal N
Untreated	76	75%	2	2%	26	25%	104
Blebbistatin	20	59%	3	9%	11	32%	34
1 min washout	21	54%	2	5%	16	41%	39
5 min washout	59	73%	1	1%	21	26%	81
15 min washout	61	68%	0	0%	29	32%	90

Table S1, Related to Figure 3. Frequency of different types of bipolar filaments in different experimental conditions.