Supplemental Materials

Molecular Biology of the Cell

Chang et al.

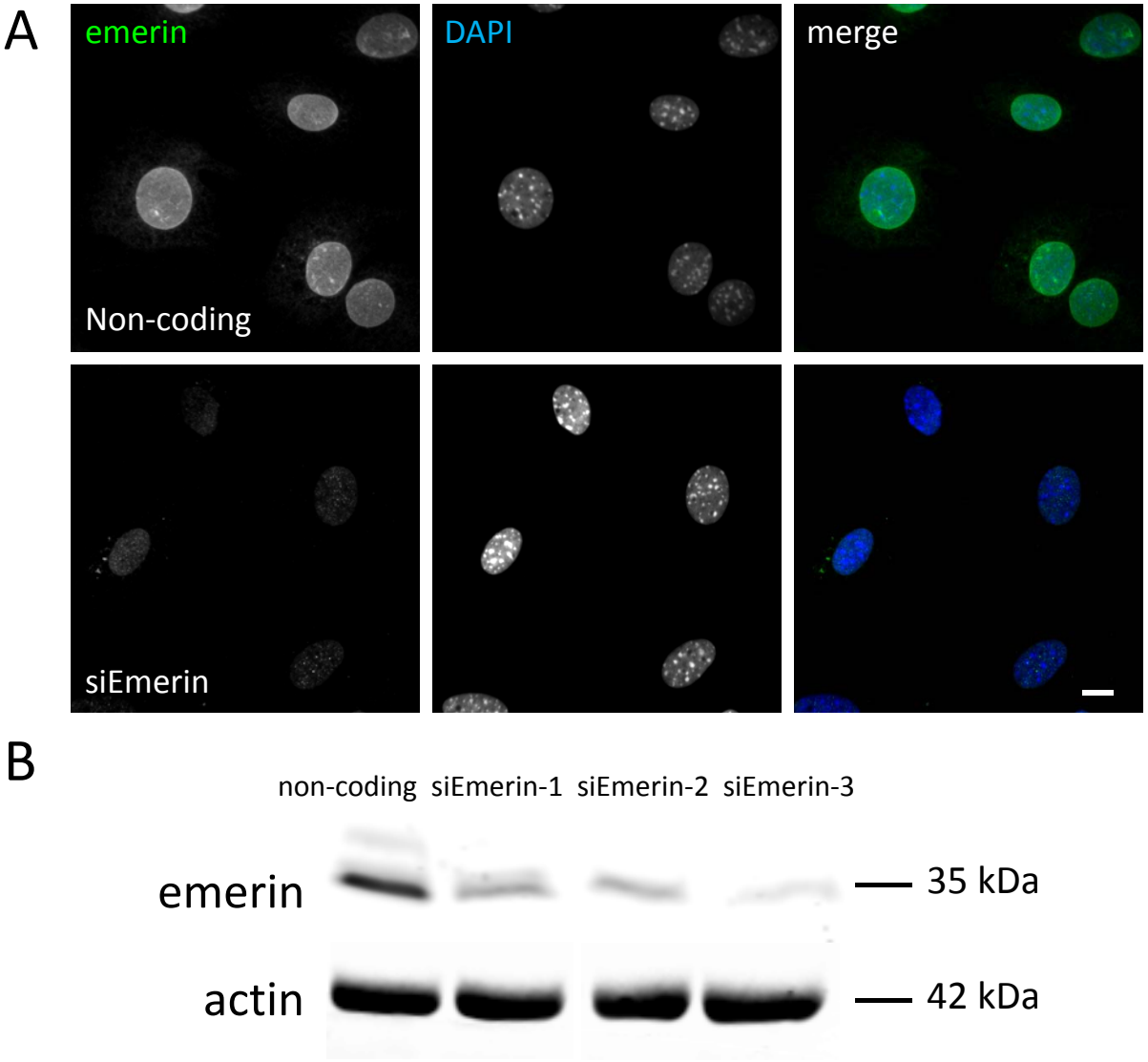

Figure S1. **Analysis of emerin knock down by siRNAs. A)** Emerin immunofluorescence in NIH3T3 cells showing that emerin is depleted from nuclei in emerin siRNA transfected cells compared to non-coding siRNA controls. Bar: 10 µm. **B)** Western blots of emerin levels in lysates prepared from NIH3T3 cells treated with three different emerin siRNAs or non-coding siRNA. Actin is a loading control.

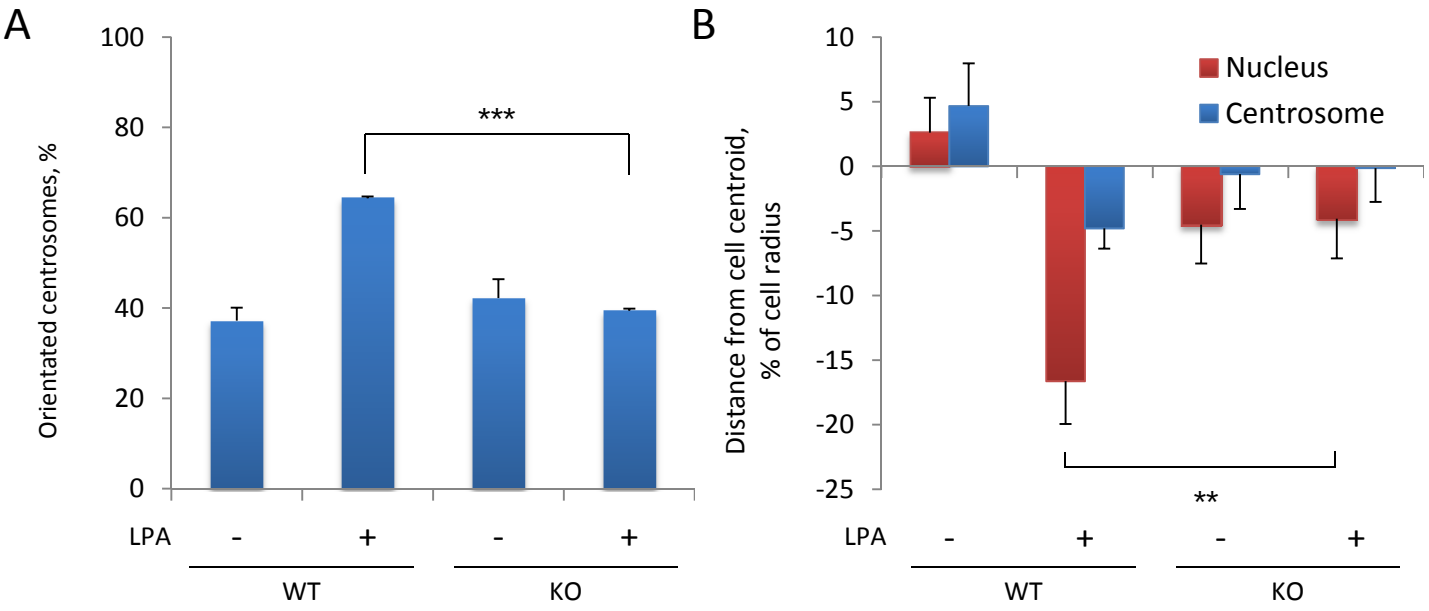

Figure S2. Emerin knockout MEFs are defective in centrosome orientation and nuclear movement. A) Quantification of centrosome orientation in MEFs from and wild type (WT) and emerin-knockout (KO) MEFs after LPA stimulation. Error bars: SD from three experiments (N>120 cells). **B)** Nucleus and centrosome position in cells treated as in A. Error bars: SEM from three experiments (N>100 cells).

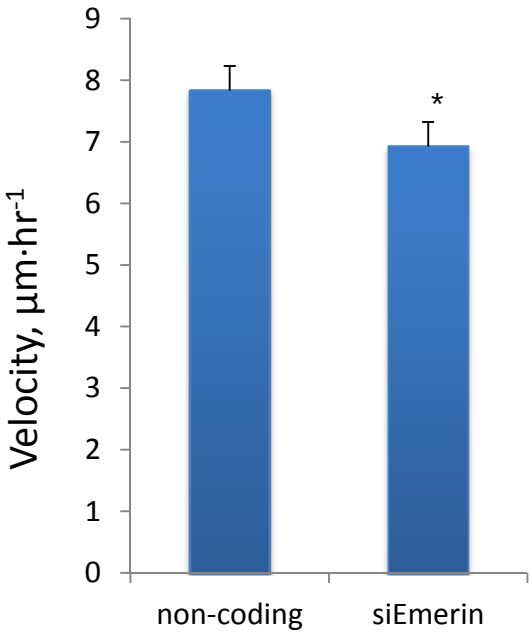

Figure S3. Emerin depletion reduces cell migration. Quantification of cell migration velocity in wound healing assays. Depletion of emerin caused a reproducible reduction in cell migration that was statistically significant. Error bars: SD from three experiments (N>150 cells).

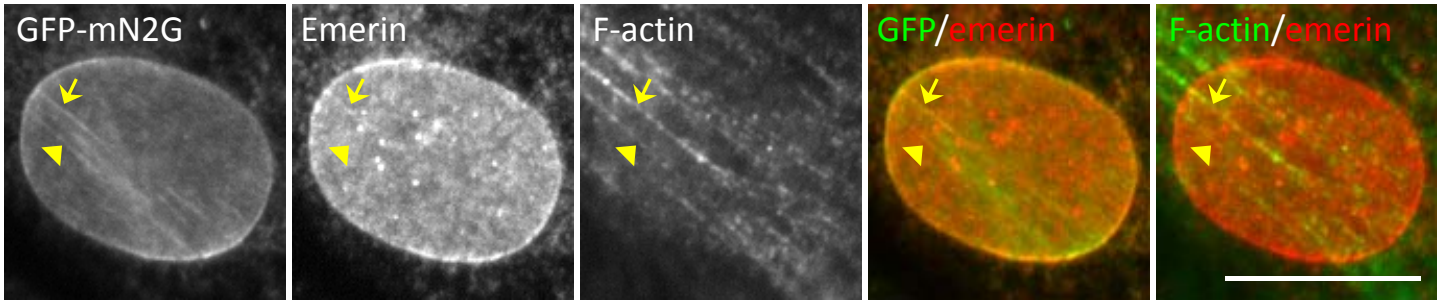

Figure S4. Emerin accumulates in a small subset of TAN lines. Immunofluorescence of GFP-mini-nesprin-2G and emerin and F-actin localization with rhodamine-phallodin in serum-starved NIH3T3 cells expressing GFP-mini-nesprin and stimulated with LPA for 60 minutes. Streaks of emerin above the diffuse level in the nucleus were observed to colocalize with GFP-mini-nesprin-2G TAN lines in ~20% of the cells, although only a subset of the TAN lines appeared to contain emerin. Arrow, example of emerin streak colocalizing with a TAN line; arrowhead, example of a TAN line that does not contain an emerin streak. Bar, 10 µm.

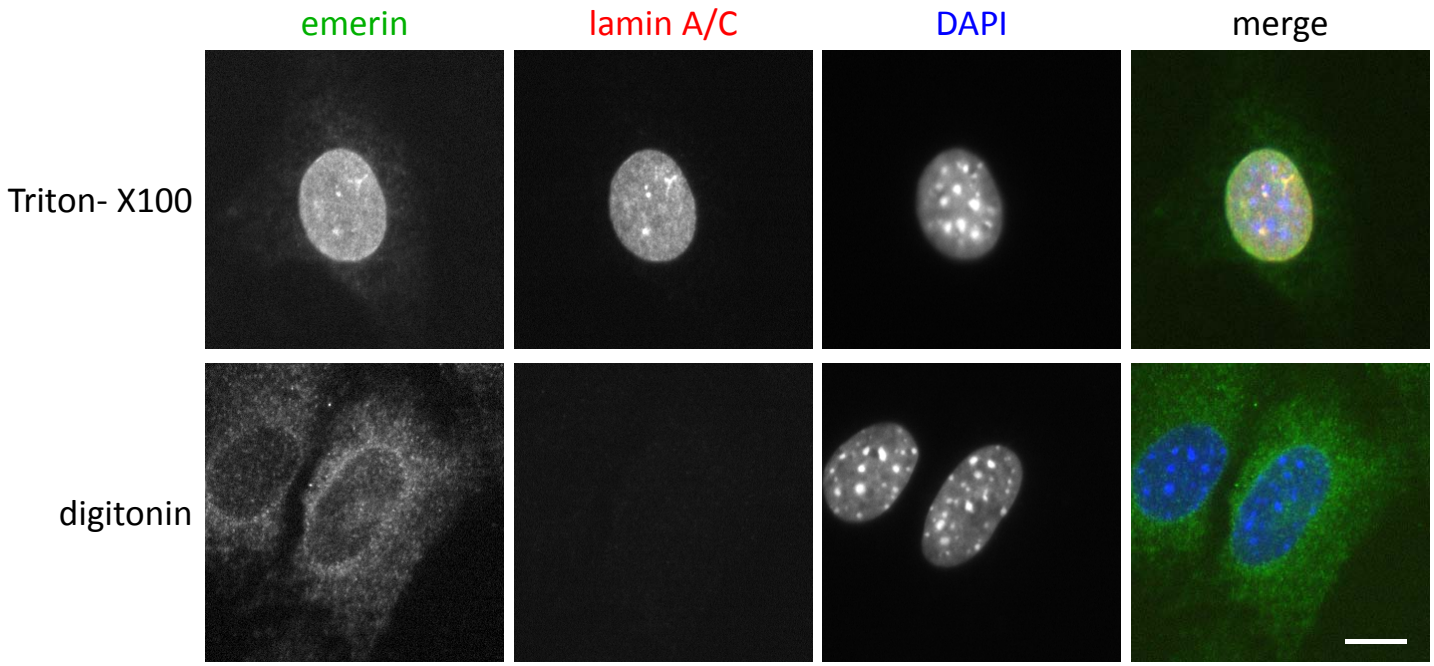

Figure S5. A pool of emerin localizes to the outer nuclear membrane in LPA-stimulated NIH 3T3 cells. Cell were fixed and then permeabilized with either 0.3 % Triton-X 100 or 0.01 % digitonin, which selectively permeabilizes the plasma membrane. Permeabilized cells were immunostained with anti-lamin A/C and anti-emerin antibodies and DAPI to label nuclei. Merge shows emerin (green), lamin A/C (red) and nuclei (blue). The lack of lamin A/C staining in digitonin permeabilized cells confirms that the nuclear envelope is intact. Bar, 10 µm.

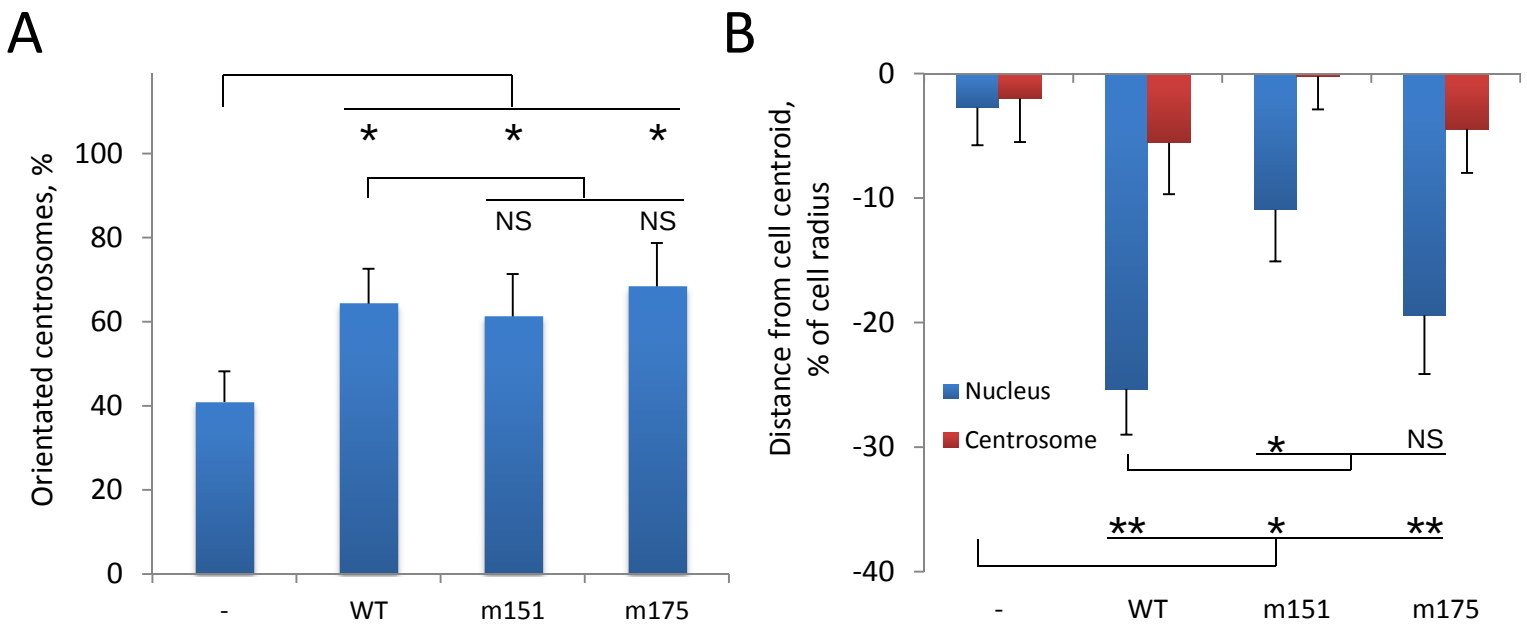

Figure S6. **Emerin-actin interaction is not required for centrosome orientation or nuclear movement. A)** Quantification of LPA stimulated centrosome orientation in emerin-depleted NIH3T3 cells expressing GFP-tagged wild type (WT) emerin or mutants (m151, m175) defective in actin binding. Error bars: SD from three experiments (N>90 cells). **B)** Nucleus and centrosome position in cells treated as in A. Error bars: SEM from three experiments (N>90 cells).

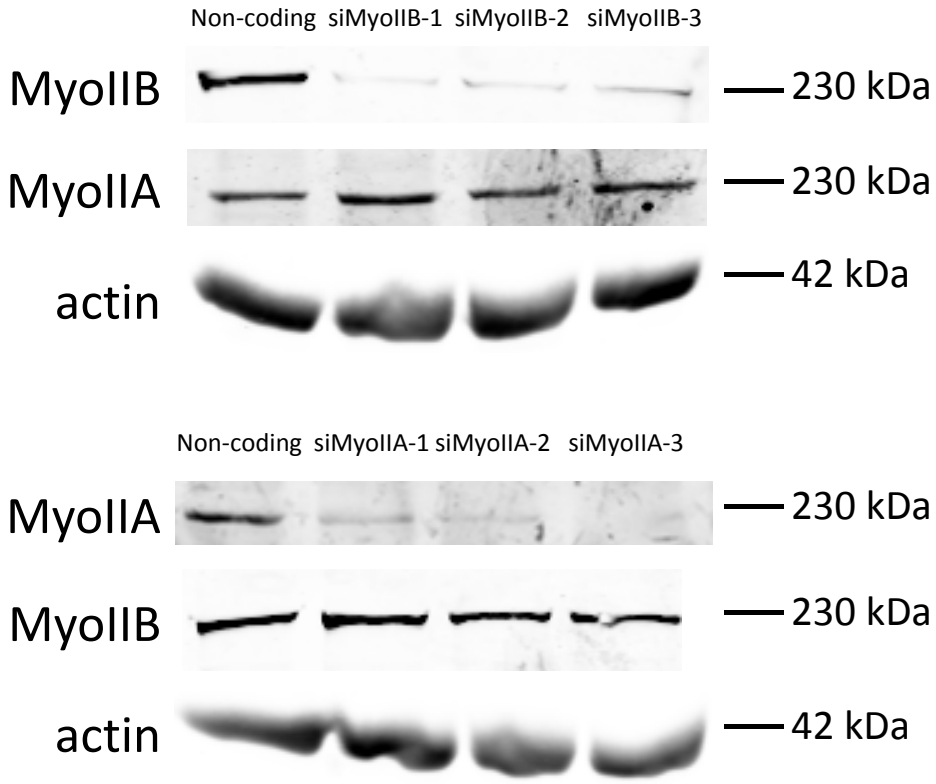

Figure S7. Analysis of myosin II levels in NIH3T3 cells after knock down by siRNAs.
Western blots of myosin IIA and myosin IIB levels in lysates prepared from NIH3T3 cells treated with three different siRNAs to each of the myosin IIs. Not that in each case the level of the targeted myosin II isoform is reduced while the level of the non-targeted isoform is unaffected. Actin is a loading control.

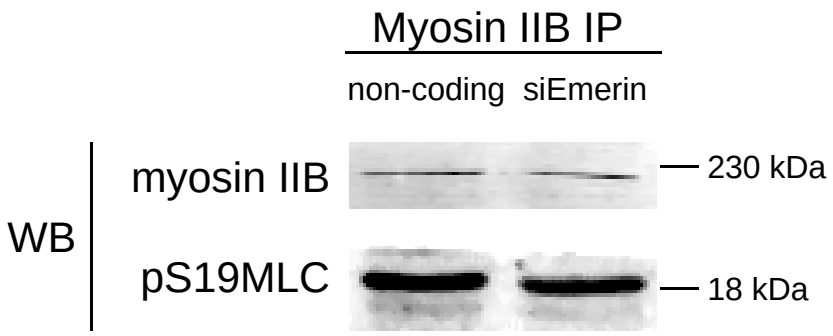

Figure S8. Activation of myosin IIB is not affected by emerin depletion. Myosin activation was measured by the amount of pSer19MLC recognized in the immunoblots shown that co-immunoprecipitated with myosin IIB from lysates of cells treated with non-coding siRNA (non-coding) or siRNA against emerin (siEmerin).

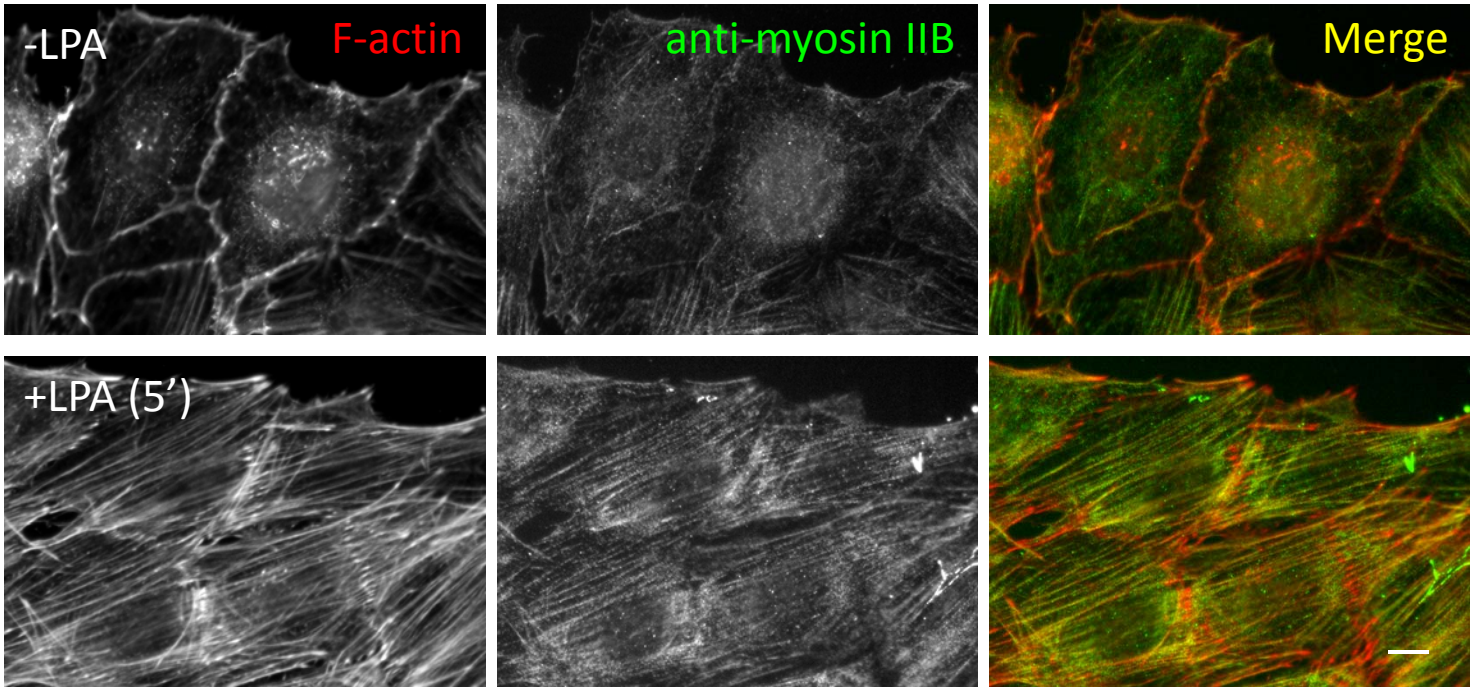

Figure S9. In response to LPA stimulation myosin IIB quickly forms filamentous structures that colocalizes with actin filaments. Starved NIH3T3 cells were fixed before (upper panels) or 5 minutes after LPA treatments and staining for F-actin (red) and myosin IIB (green). Bar, 10 µm.

Supplemental movies:

Movie 1. Retrograde actin flow in control cells revealed by Lifeact-mCherry. Upon LPA stimulation of starved NIH3T3 cells, dorsal actin cables form parallel to the leading edge and undergo retrograde flow and move with the nucleus. In control cells, actin retrograde flow is persistent and unidirectional. Time is in hr:min after LPA stimulation. Frame rate: 3 min/frame. Bar: 10µm.

Movies 2 & 3. Retrograde actin flows in emerin-depleted cells revealed by Lifeact-mCherry. LPA stimulation of emerin-depleted cells results in formation of dorsal actin cables, but their flow is random and frequently changes direction. Time is in hr:min after LPA stimulation. Frame rate: 3 min/frame. Bar: 10µm.

Movie 4. Live cell imaging of emerin-knockdown cells coexpressing mCherry-LifeAct and GFP-mN2G showed slippage of TAN lines. Mini-nesprin-2G and LifeAct form linear structures on nuclear membrane and they move together. Movement of TAN lines over an immobile nucleus indicates slippage of TAN lines over the nucleus. Yellow arrow indicators the wound edge. Bar: 5µm.

Movie 5. Retrograde actin flow in myosin IIB-depleted cells revealed by Lifeact-mCherry. LPA stimulation of myosin IIB-depleted cells results in formation of dorsal actin cables, but their flow is random and frequently changes direction. Time is in hr:min after LPA stimulation. Frame rate: 5 min/frame. Bar: 10µm.