Supplemental Material

Figure S1. Differences in pressure and TEER occur independent of ZO-1 integrity and p-MLC expression. (A) Immunofluorescent images of MDCK I and II cells stained for the tight junction protein, Z0-1 show no differences in intensity between MDCK I and II cell lines. Bar = 20μm. (B) Quantification of ZO-1 at the cell-cell junctions. ($n \geq 30$, N =3). Not significant (ns), P = 0.8808 (C) A representative western blot of MDCKI and MDCKII cells where p-MLC levels were compared. While there was some variation in p-MLC between the two cell lines, the differences were not statistically significant. Quantified in (D) (N=3). p-MLC, ns, P=0.8346.

Figure S2. **The reduction of cytoplasmic pressure in response to HGF treatment precedes changes in E-cadherin and vimentin expression.** (A) A representative western blot of parental MDCK cells after 1 hour of HGF treatment. There was no significant change in E-cadherin or vimentin expression compared to control cells. Vimentin expression did show greater variability in cells treated with a combination of HGF and hypo-osmotic media or hyper-osmotic media alone, but the differences were not statistically significant compared to control cells. Quantified in (B) (N = 3). E-cadherin, ns, P > 0.1 versus control. Vimentin, ns, P > 0.07 versus control.

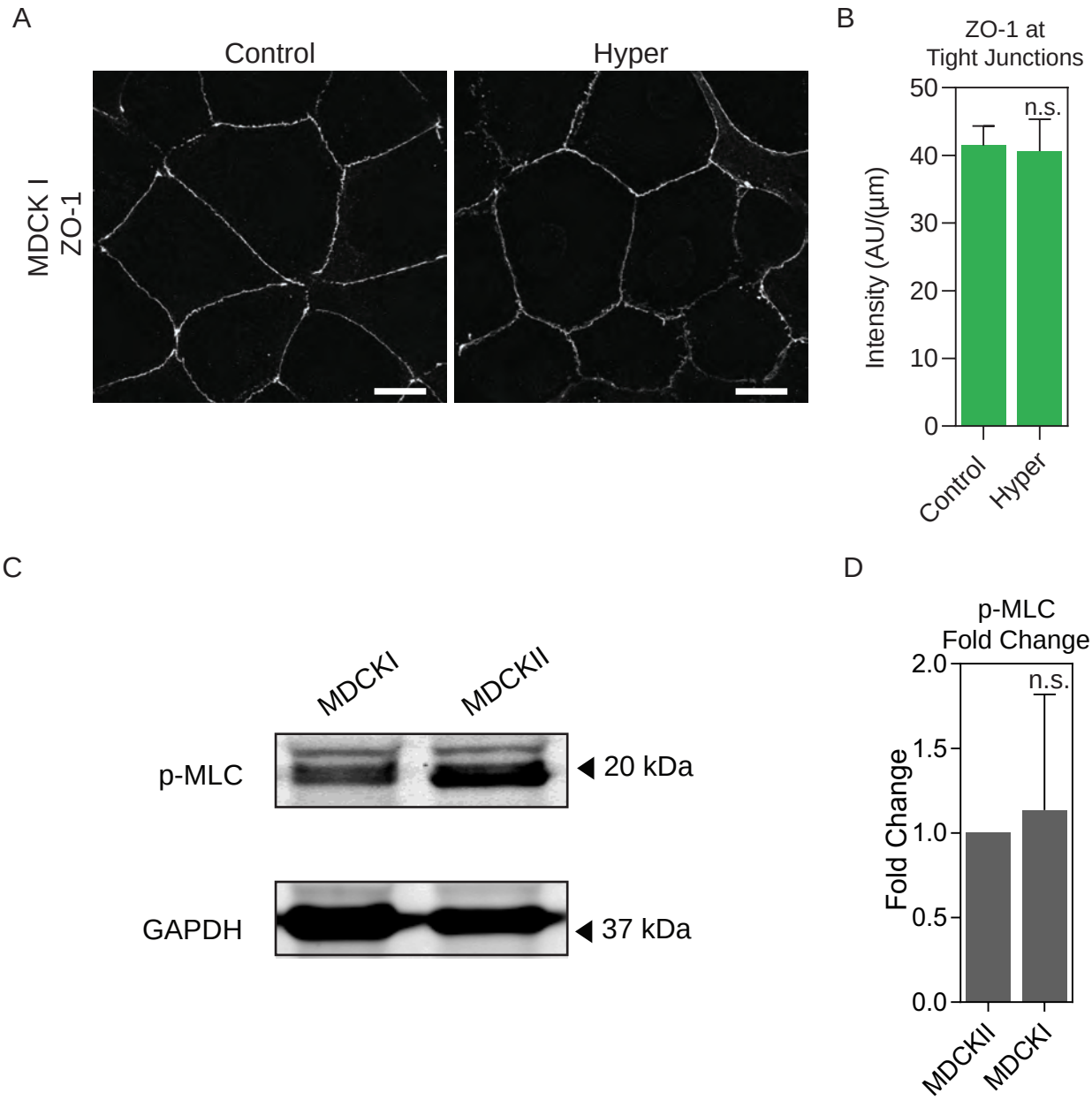
Figure S3. **NMII inhibition re-localizes Arp2 to the cell periphery.** (A) NMII inhibited, low-pressure MDCK cells re-localize Arp2 to the cell periphery (white arrowheads) compared to control cells which have higher cytoplasmic Arp2 localization. MDCK cells were treated with NMII inhibitor blebbistatin and stained for Arp2. Bars = 10 μm. Quantified in (B) (n ≥ 30, N=3). *P=0.0162.

Movie S1. **HGF treatment triggers epithelial cell scattering.** Image sequence of untreated control (left panel) and HGF treated parental MDCK cells (right panel). The increase in movement and the dissociation of cells from each other following exposure to HGF corresponds with the significant drop in pressure in Fig. 2 D.

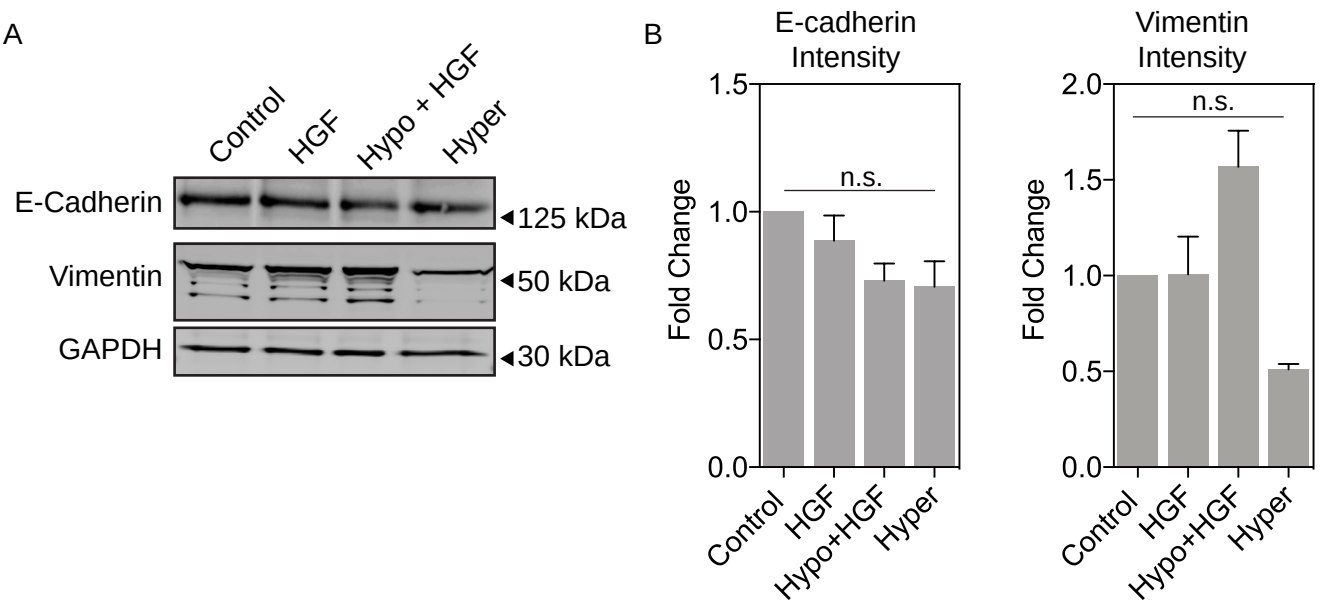
Movie S2. **Inhibition of NMII is sufficient to trigger epithelial cell scattering.** Image sequence of untreated control (left panel) and blebbistatin treated parental MDCK cells (right panel). Similar to HGF treatment, inhibition of NMII activity triggers loss of cell-cell contacts and

increases cell motility, as reported previously (1). This increase in cell migration following NMII inhibition corresponds with the significant drop in cytoplasmic pressure in Fig. 2 E.

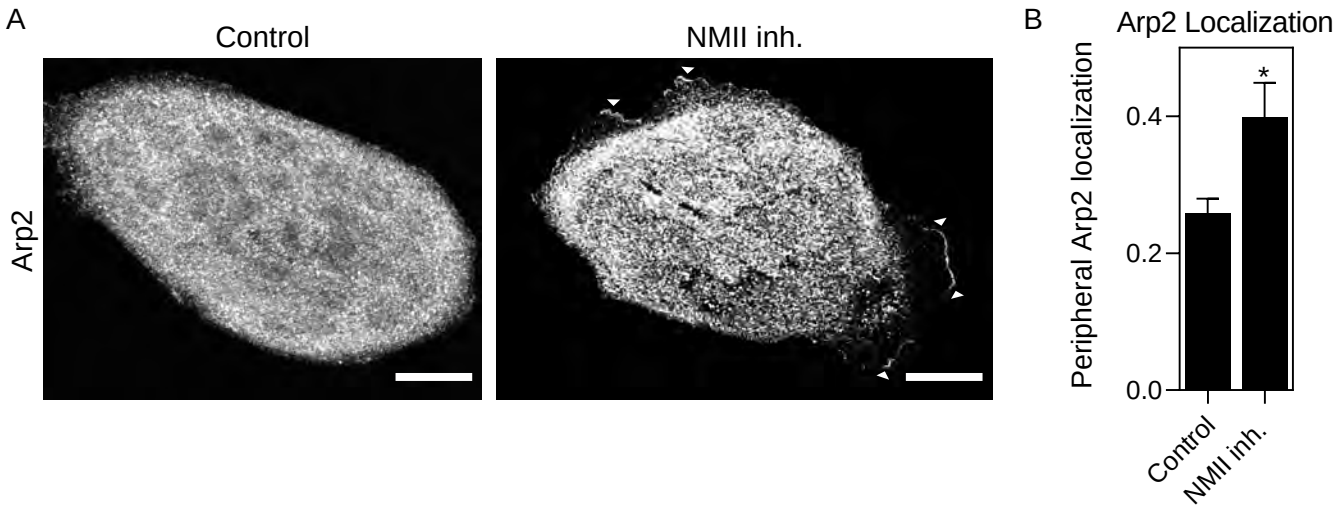
Movie S3. **Arp2/3 activity is required for the increase in epithelial cell motility following HGF treatment.** Image sequence of untreated control cells (upper left panel), HGF treated cells (upper right panel), CK666 and CK869 treated cells (lower left panel), and cells treated with a combination of HGF and the Arp2/3 inhibitors (lower right panel). Inhibiting Arp2/3 when treating with HGF significantly reduces cell velocity compared to cells treated with HGF alone. This reduced velocity corresponds with elevated intracellular pressure (Fig. 5 C).



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3