Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figure Legends

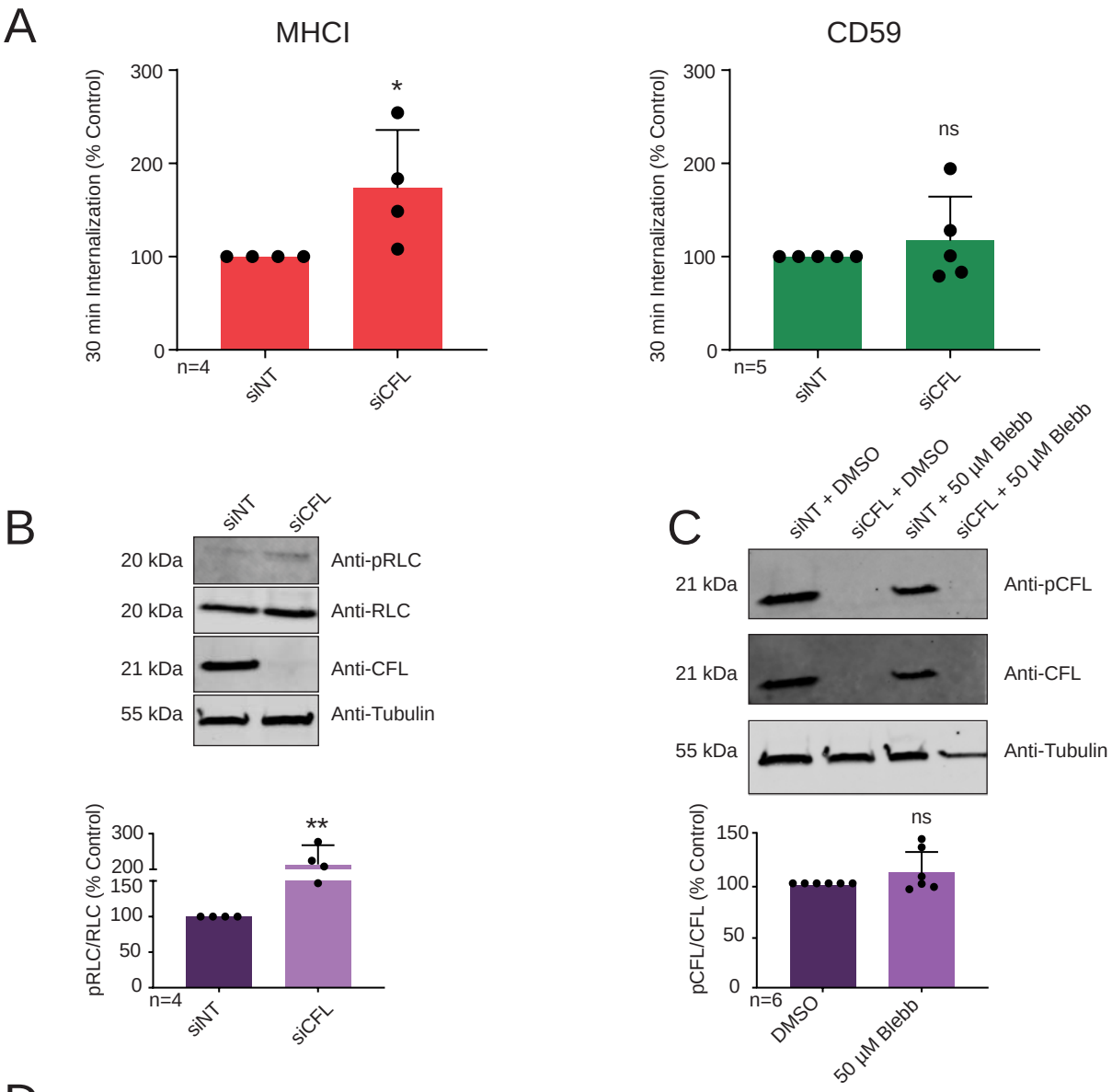
Supplemental Figure 1. (A) 30 min antibody internalization of MHCI or CD59 on siRNA transfected HeLa cells (B,C) Western blot and quantification of phosphorylated (pCFL) or total cofilin (CFL), phosphorylated (pRLC) or total myosin light chain (RLC), or tubulin (B,C) from siRNA transfected HeLa lysates with (C) or without (B) 60 min inhibitor pre-treatment or DMSO. (D) Maximum intensity projections of confocal z-series of fluorescent phalloidin in siRNA transfected HeLa cells. Bars, 5 μm. Yellow boxes in enlarged in insets, Bar, 1 μm. N = number of replicates (B,C), or replicates of 6 fields and approximately 25 cells per field (A) from at least three independent experiments, data expressed as mean ± S.D. (error bars). ns: P> 0.05; *: P≤ 0.05; **: P≤ 0.01; ***: P≤ 0.001; ****: P ≤ 0.0001.

Supplemental Figure 2 (A-G) X-Z projections of confocal Z-series (above, XZ), and either a single X-Y confocal image ((A-C) below, Apical, XY), with the position of the Z-plane indicated as a yellow line and arrow and the distance from the dorsal surface labeled, or a maximum intensity projection of a confocal Z-series of the apical-most 1 μm ((E-G), below, Apical Max, XY)). (A) Immunostaining of myosin IIA (Myo2A, magenta) and myosin IIB (Myo2B, green) or (B) CD59 (green) and MHCI (magenta). (C,E) 60 min internalization (T60int) of antibodies to CD59 that were loaded apically (C (bottom), E (top)) or to MHCI that were loaded basally (green, C (top), E (bottom)) on polarized Caco-2 monolayers with (E) or without (C) siRNA transfection. (D) Quantification of western blot from Fig 4G. (F,G) phalloidin (Actin, red) and immunostaining of ZO-1 and E-cadherin (as noted) on polarized Caco2 cells which were preincubated with either vehicle (DMSO, top) or blebbistatin (50 μM Blebb, bottom) prior to immunostaining (F) or transfected with indicated siRNA (G). Bars, 5 μm. N= number of replicates from at least three independent experiments, data expressed as mean ± S.D. (error bars). ns: P> 0.05; *: P≤ 0.05; **: P≤ 0.01; ***: P≤ 0.001; ****: P ≤ 0.0001.

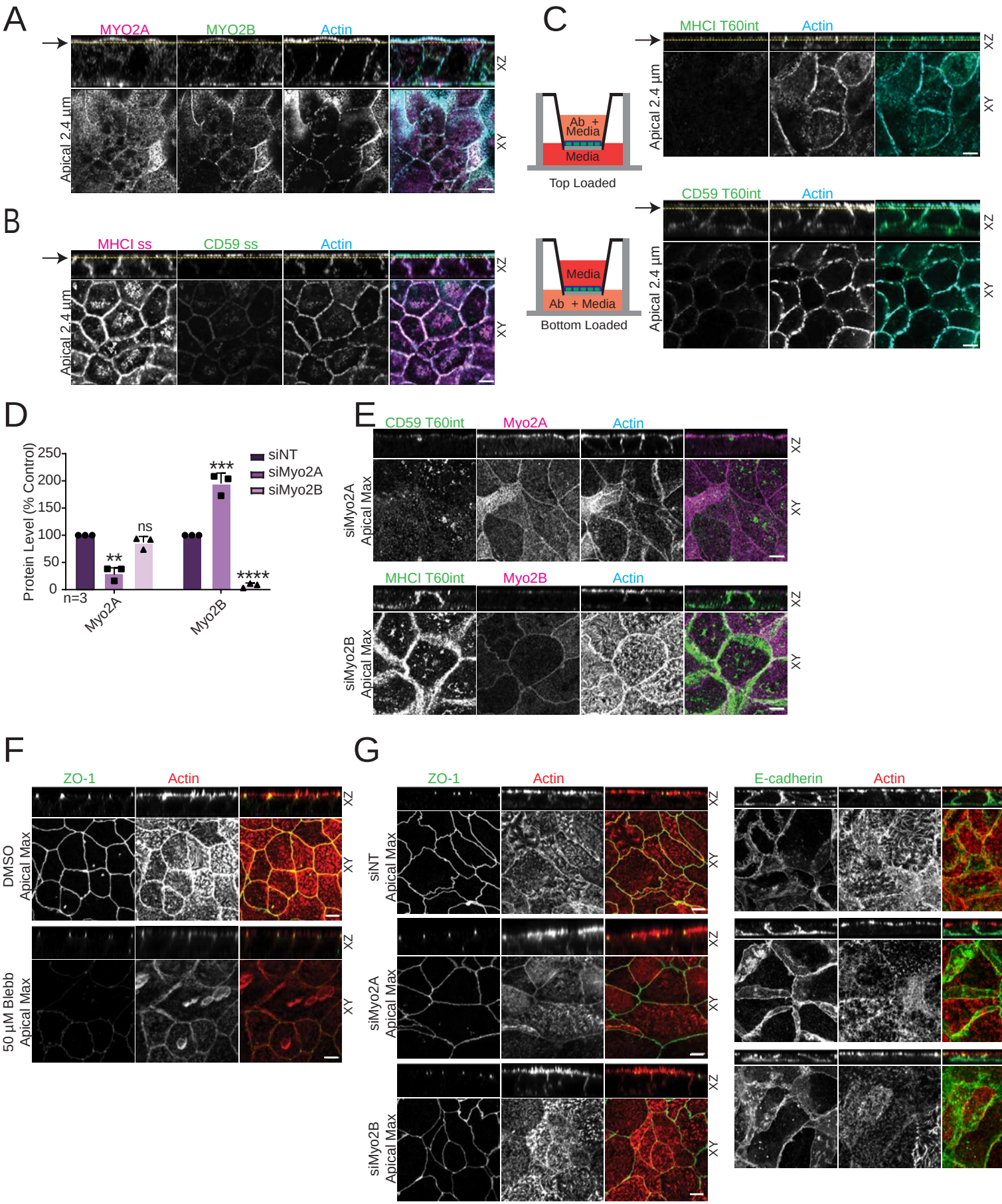
Supplemental Figure 3 (A,B) X-Z projections of confocal Z-series (above, XZ) and a maximum intensity projection of confocal Z-series of the apical-most 1 μm (below, Apical Max, XY) of polarized Caco-2 cells following siRNA transfection. Immunostaining of (A) CD59 (green) and MHCI (red) or (B) Ezrin (green) and actin (red). (C) Epithelial height (μm) of siRNA-treated caco-2 monolayers. Scale bars = 5 μm. N= number of replicates from at least three independent experiments, data expressed as mean ± S.D. (error bars). ns: P> 0.05; *: P≤ 0.05; **: P≤ 0.01; ***: P≤ 0.001; ****: P ≤ 0.0001.

Supplemental Figure 4. Model. (A) In polarized Caco2 intestinal epithelial cells, CD59 is localized and internalized at the apical plasma membrane, while MHCI is localized and internalized from the basolateral domain. ROCK2 mediated myosin II contractility is required for the efficient internalization of both cargo proteins. Myosin IIA localizes at the basal cortex and apical brush border and mediates basolateral internalization of MHCI, while myosin IIB localizes to the basal cortex and apical cell-cell junctions and promotes the internalization of apical CD59, perhaps through modulation of apical epithelial tension. (B). In myosin IIA-silenced Caco2 cells, there is less basolaterally localized myosin IIA to assist in the efficient internalization of MHCI. (C) In myosin IIB silenced cells, there is less apical membrane tension being generated from the interaction between myosin IIB at the apical junctions, the actin cytoskeleton and the plasma membrane and thus the overall apical epithelial tension is decreased. Insufficient membrane tension leads to less CIE of CD59, perhaps through the inability to form an endocytic pit.

Supplemental Figure 1

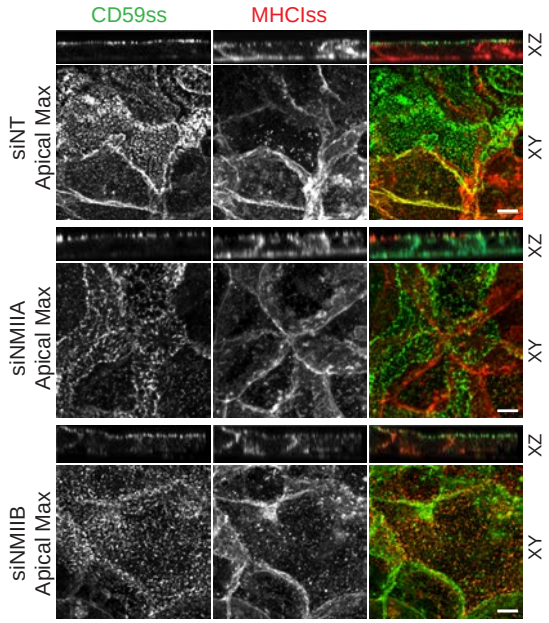


Supplemental Figure 2



Supplemental Figure 3

A



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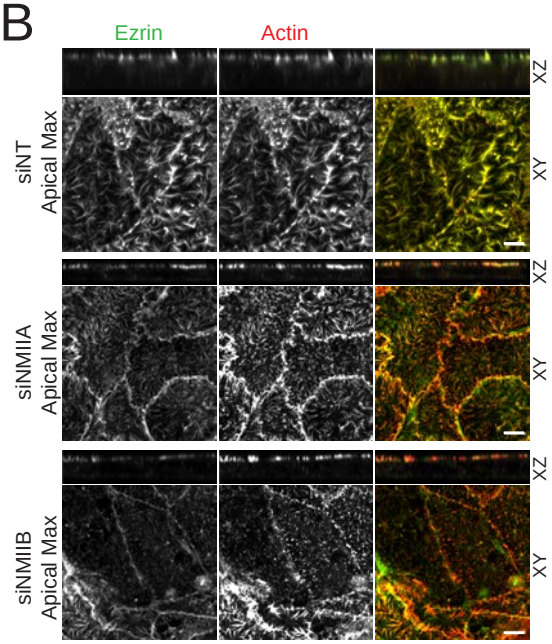

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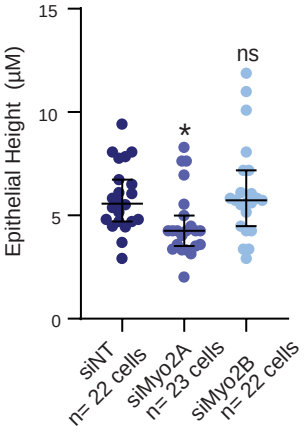
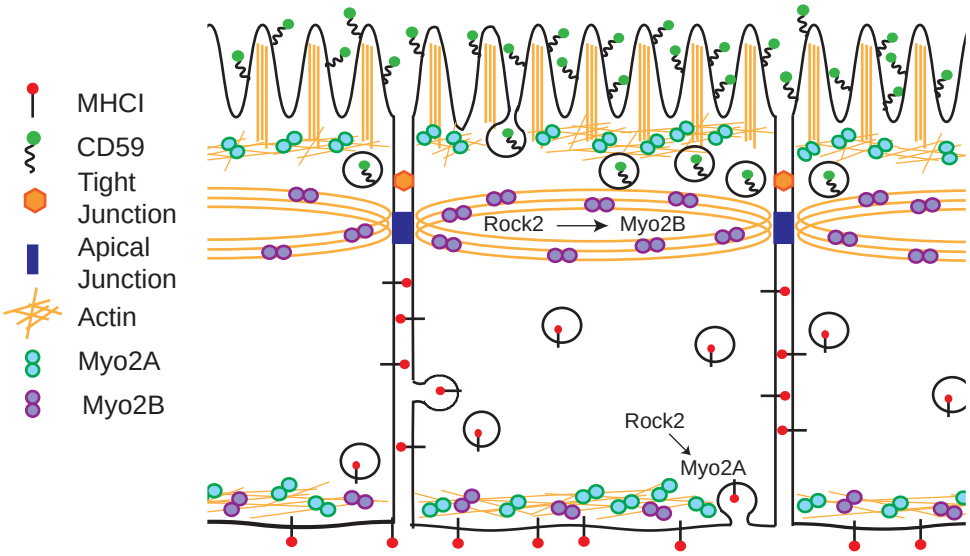
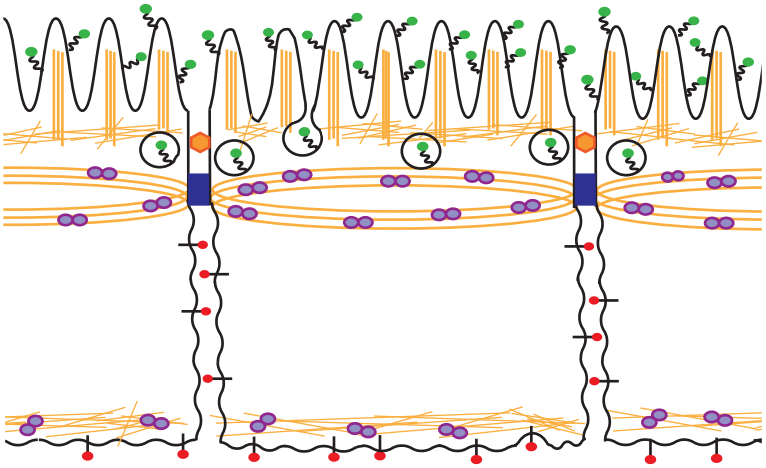


Supplemental Figure 4

A. Caco-2 cells



B. Caco-2 cells, NMIIA depleted



C. Caco-2 cells, NMIIB depleted