Supplementary Information

Measurement of tissue viscosity to relate force and motion in collective cell migration

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Supplemental Figures

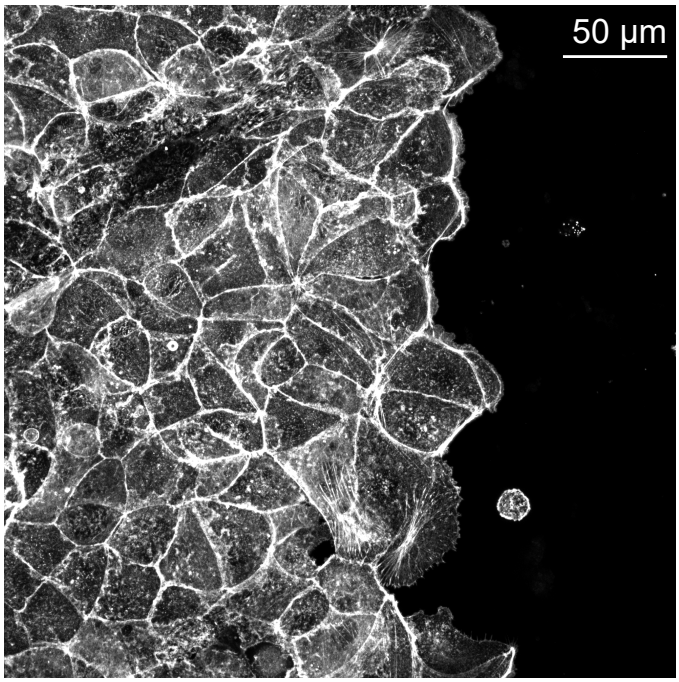

Figure S1: Fluorescent labeling of the actin cytoskeleton at the edge of a cell monolayer at the time of barrier removal. The fluorescent intensity is similar at the edge of the monolayer and at cell-cell interfaces, indicating the absence of a multicellular actin cable.

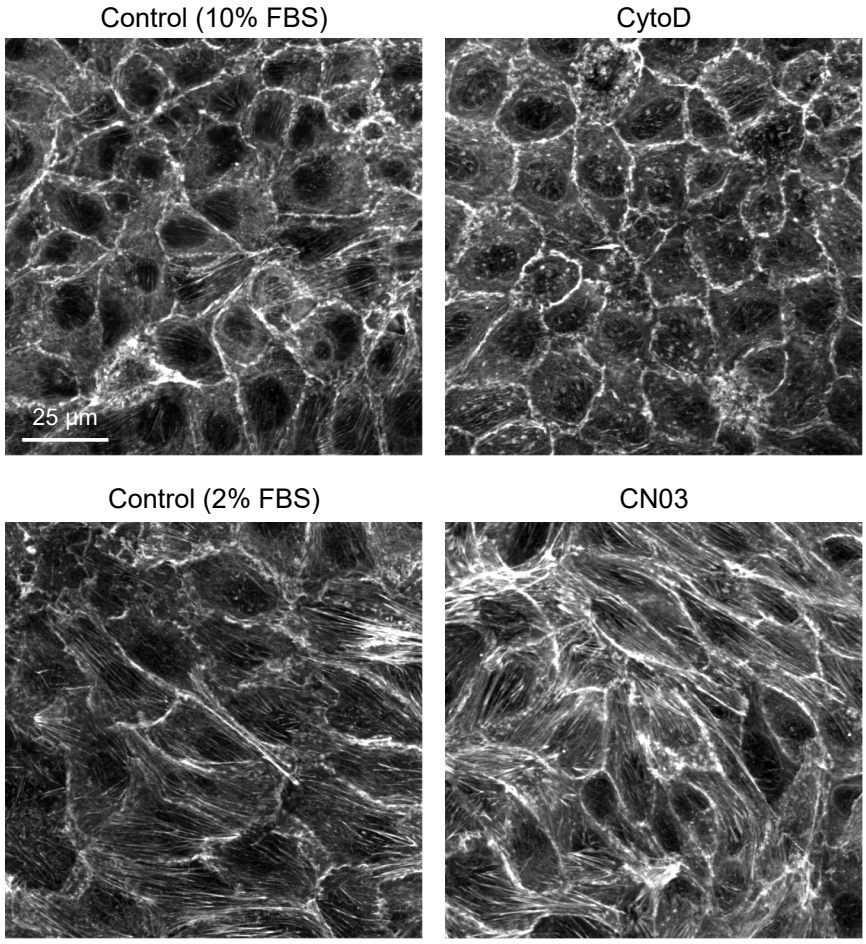

Figure S2: Enlarged images of fluorescently labeled actin in response to cytochalasin D and CN03. Compared to their respective controls, treatment with cytochalasin D reduced the amount of stress fibers and treatment with CN03 increased the amount of stress fibers.

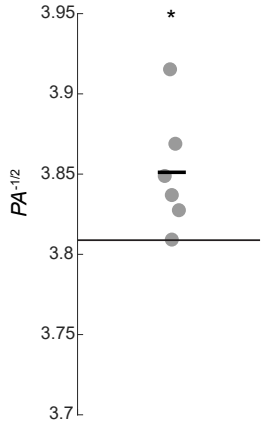

Figure S3: Cell monolayers are within the viscous regime. Cells were segmented, and each cell's perimeter P and area A were measured. The quantity $P/\sqrt{A}$ was measured for each cell. Dots show averages of $P/\sqrt{A}$ over independent cell monolayers. Black bar indicates the mean. The data are statistically different from 3.81 ($p = 0.04$ one-sample t-test in comparison to 3.81), indicating the cells are in the fluid regime.

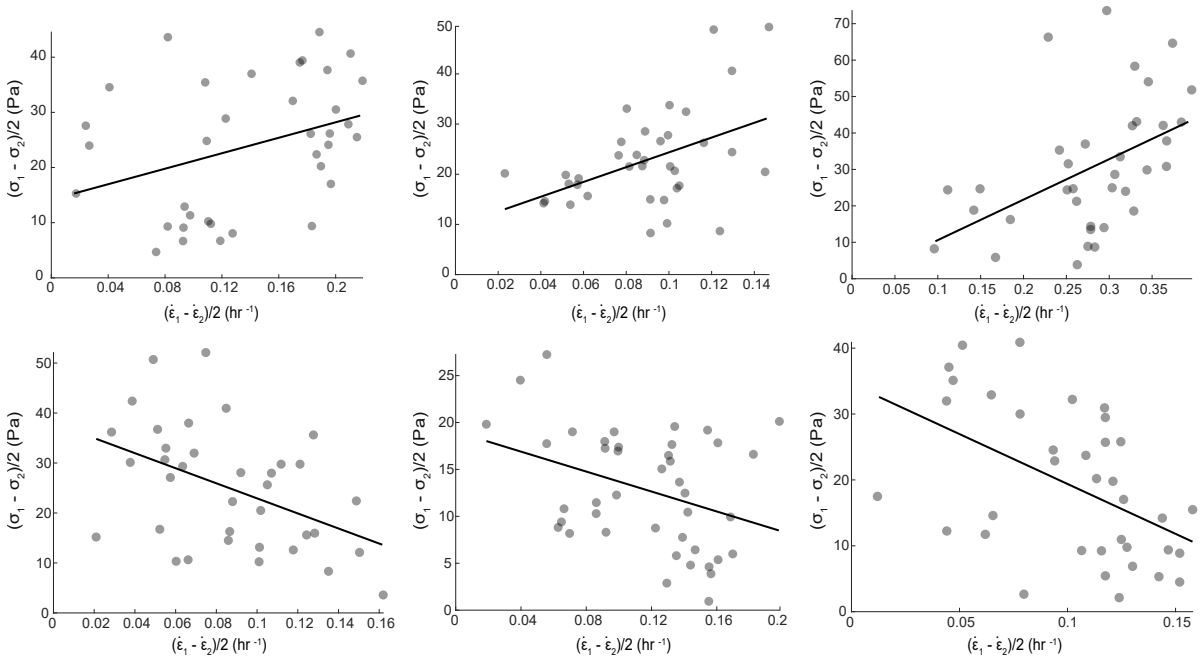

Figure S4: Representative scatter plots of the relationship between shear stress and shear strain rate within different 62×62 μm^2 windows chosen at random locations within one monolayer. Gray dots indicate points within each window, and the black lines indicate the linear fits.

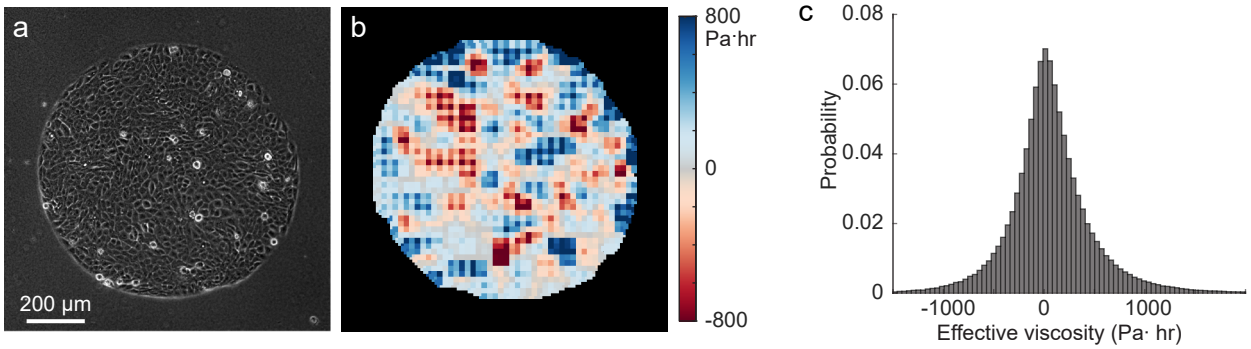

Figure S5: Measurement of effective viscosity within HaCaT cell monolayers. (a) Representative phase contrast image of a HaCaT cell island. (b) Color map of effective viscosity. (c) Histogram of effective viscosity, showing a broad width and slightly positive mean.

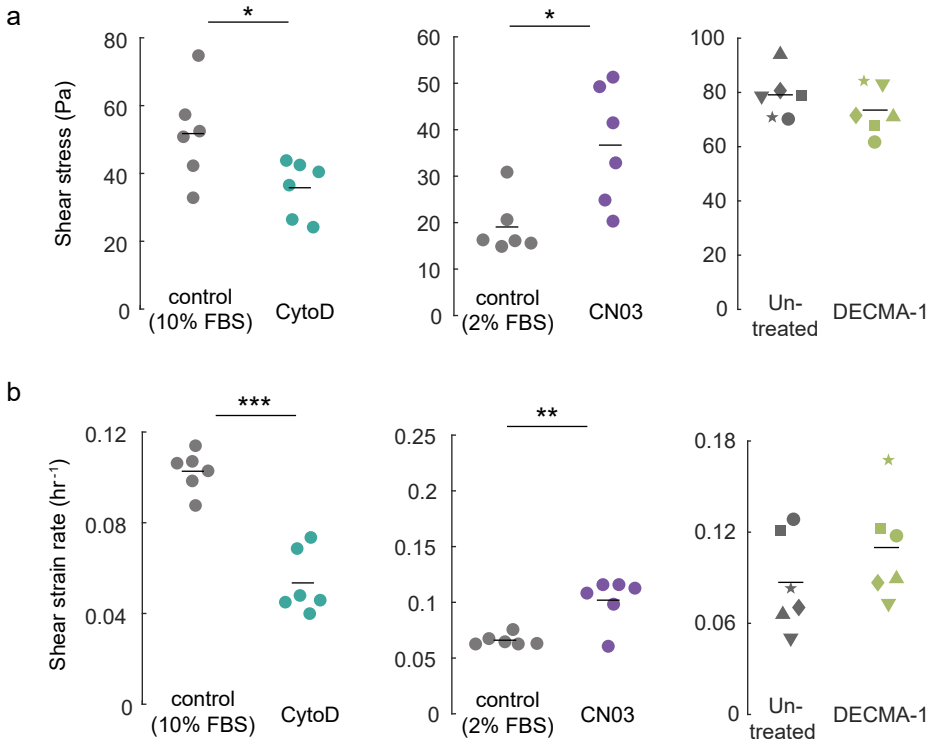

Figure S6: Shear stress and shear strain rate for various treatments. (a) Shear stress, $(\sigma_1 - \sigma_2)/2$, for treatments and their respective controls (CytoD $p = 0.039$, CN03 $p = 0.012$, two-sample t-tests; DECMA-1 $p = 0.33$, one-sample t-test) Each dot represents a different cell island. For DECMA-1 treatment, the marker shape is the same cell island before and after treatment. (b) Shear strain rate, $(\dot{\varepsilon}_1 - \dot{\varepsilon}_2)/2$, for treatments and their respective controls (CytoD $p < 0.0001$, CN03 $p = 0.002$, two-sample t-tests; DECMA-1 $p = 0.15$, one-sample t-test). Each dot represents a different cell island. For DECMA-1 treatment, the marker shape is the same cell island before and after treatment.

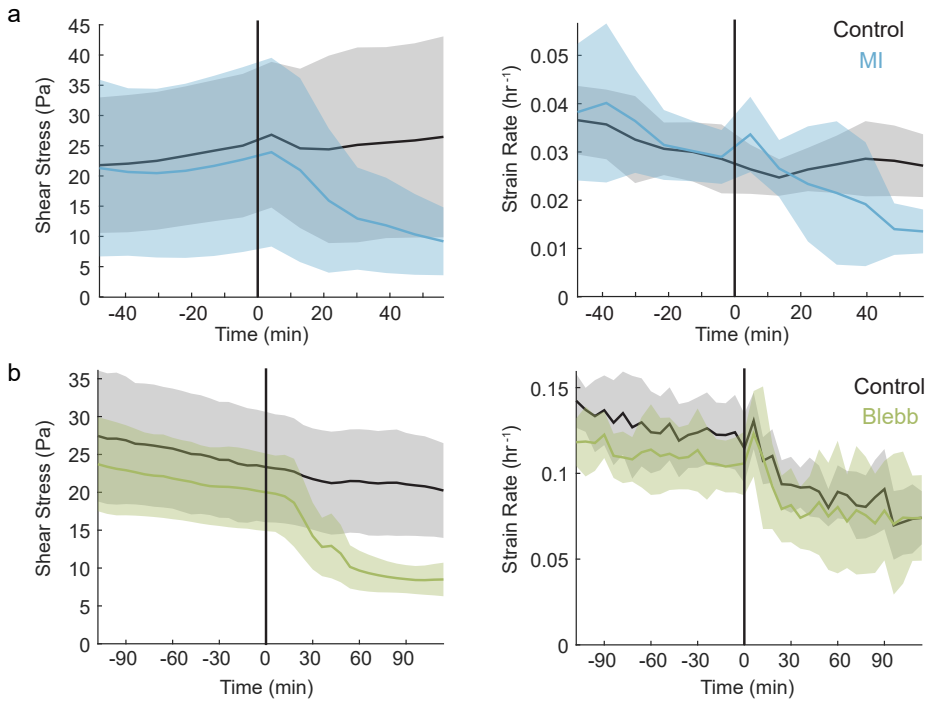

Figure S7: Shear stress and shear strain rate before and after metabolic inhibition and treatment with blebbistatin. (a) Shear stress and shear strain rate over time in response to metabolic inhibition. Metabolic inhibition occurred at $t = 0$. Solid lines indicate the mean and shading indicates the standard deviations over 6 (control) or 7 (treatment) cell islands. (b) Shear stress and shear strain rate over time in response to blebbistatin. Cells were treated with blebbistatin at $t = 0$. Solid lines indicate the mean and shading indicates the standard deviations over 6 (control) or 7 (treatment) cell islands. Both treatments reduced the shear stress and strain rate, but both shear stress and strain rate remained above zero for the duration of the experiment.

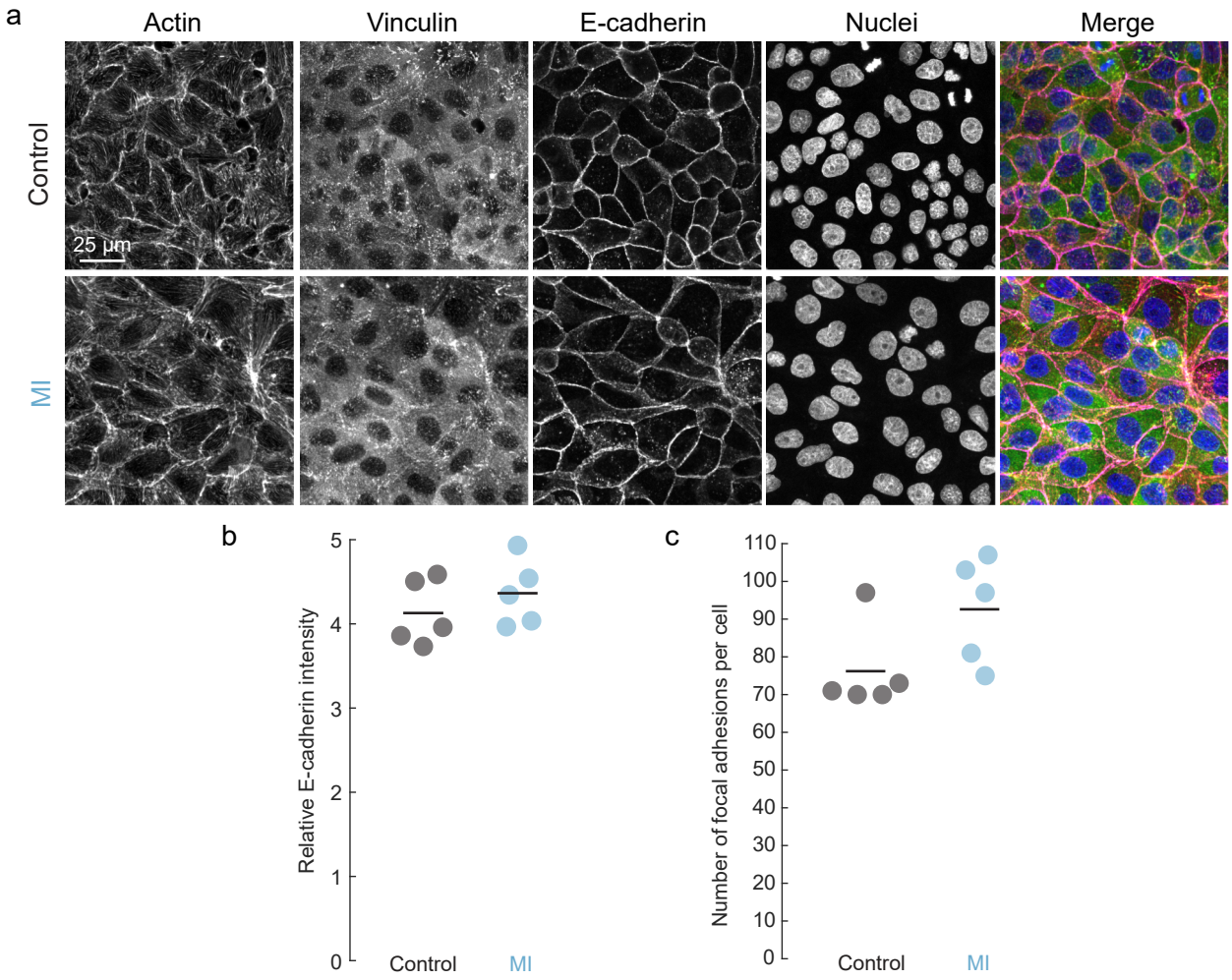

Figure S8: Fluorescent labeling of the cytoskeleton, cell-substrate adhesions, and cell-cell adhesions for metabolic inhibition experiment. (a) Fluorescent images of actin, vinculin, E-cadherin, nuclei, and merged channels for control and metabolic inhibition groups. (b) Relative E-cadherin intensity and (c) average number of fofcal adhesions per cell for control and metabolic inhibition. Each dot represents the average over a field of view, and black bars indicate means.