Electronic Supplementary Material (ESI) for Soft Matter.

Clustering and Jamming in Epithelial-Mesenchymal Co-Cultures

Marielena Gamboa Castro, Susan E. Leggett and Ian Y. Wong

Supporting Information

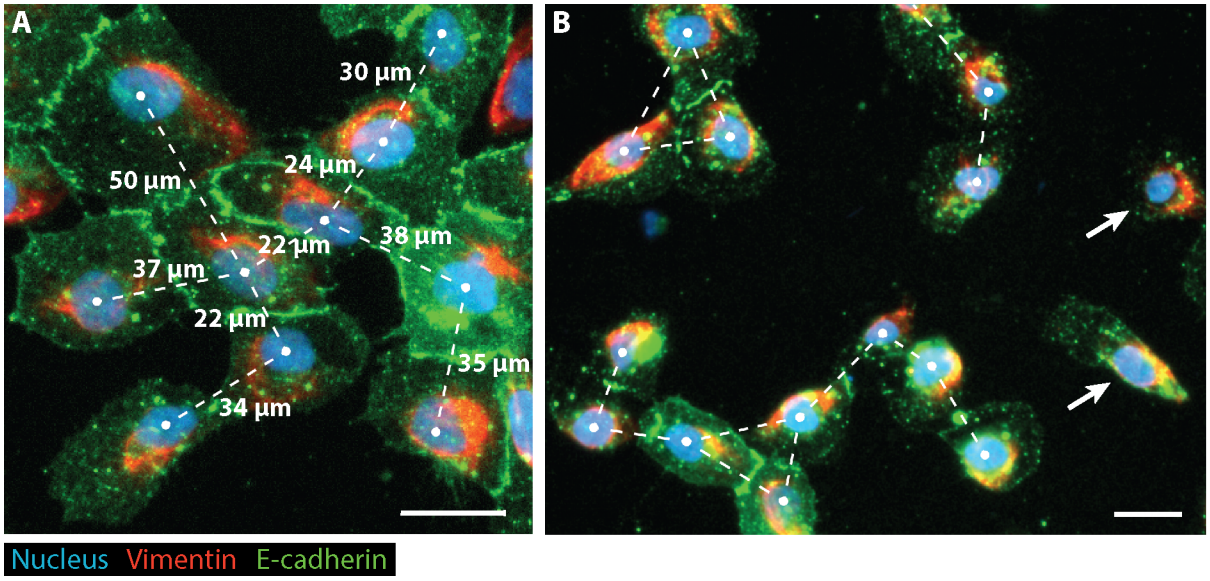

Figure S1. (A) Representative separation distances between nuclei are 50 µm or less in multicellular epithelial clusters with cell-cell junctions (MCF-10A). Immunofluorescent staining corresponds to E-cadherin (green), vimentin (red), and nuclei (blue). (B) Instead, individual cells (arrows) without cell-cell junctions are located 50 µm or more from their nearest neighbors. Scale bar = 25 µm.

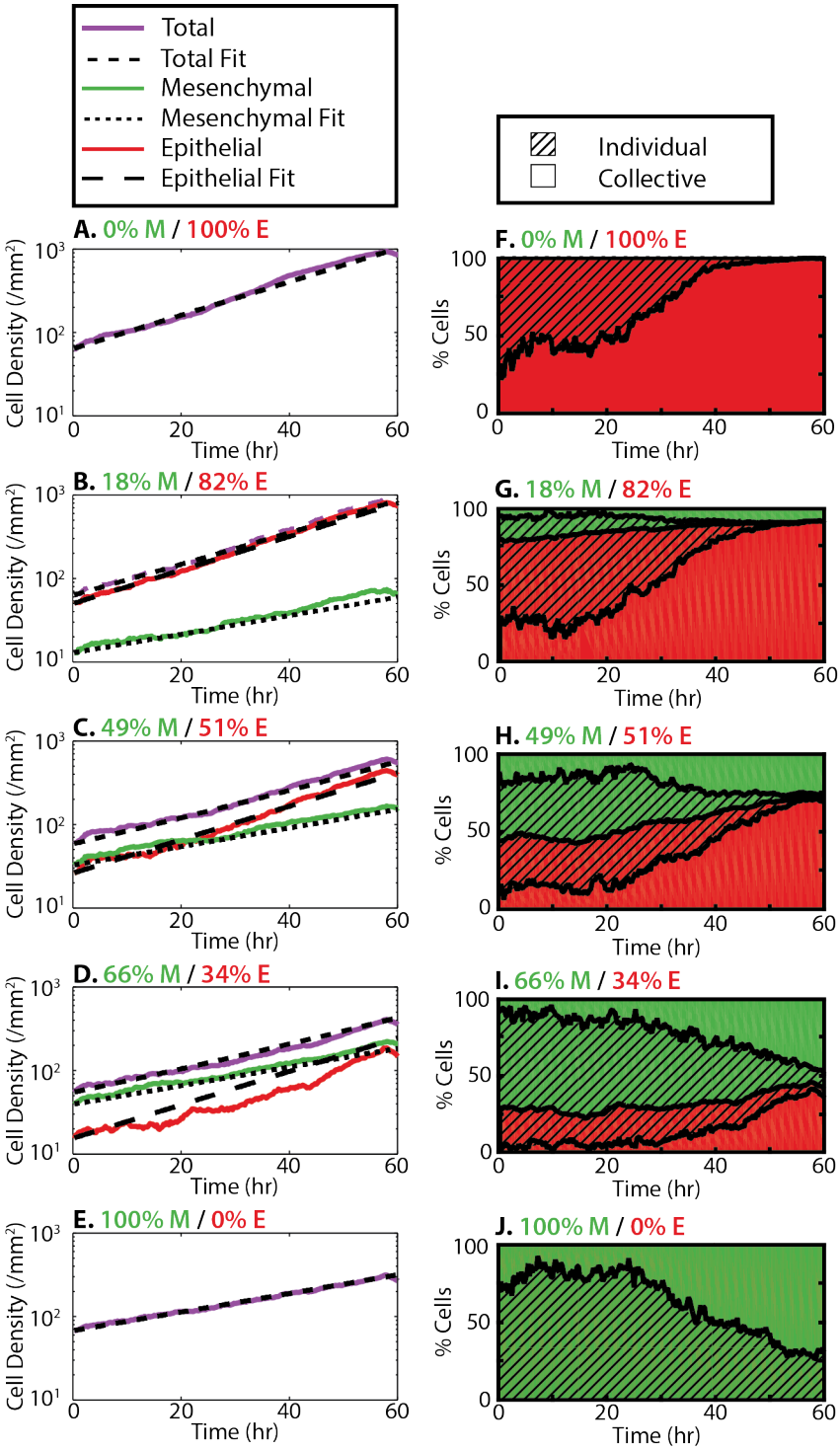

Figure S2. (A-E) Total (purple), mesenchymal (green) and epithelial cell density (red) as a function of time. In monoculture (A, E), the data was fit to an exponential $\sigma = \sigma_0 \exp(t/\tau)$, where σ_0 was the initial cell count and τ was the characteristic timescale. The mesenchymal fit (black dots) showed τ = 39 h, (doubling time of 28 h). The epithelial fit (wide black dashes) showed τ = 21.7 h, (doubling time of 15 h). The total fit (narrow black dashes) was the sum of exponentials for each subpopulation, where $\sigma = \sigma_E \exp(t/\tau_E) + \sigma_M \exp(t/\tau_M)$. (F-J) The percentage of cells in clusters increased over time (open), while the percentage of individual cells (shaded) decreased over time, for both epithelial and mesenchymal subpopulations

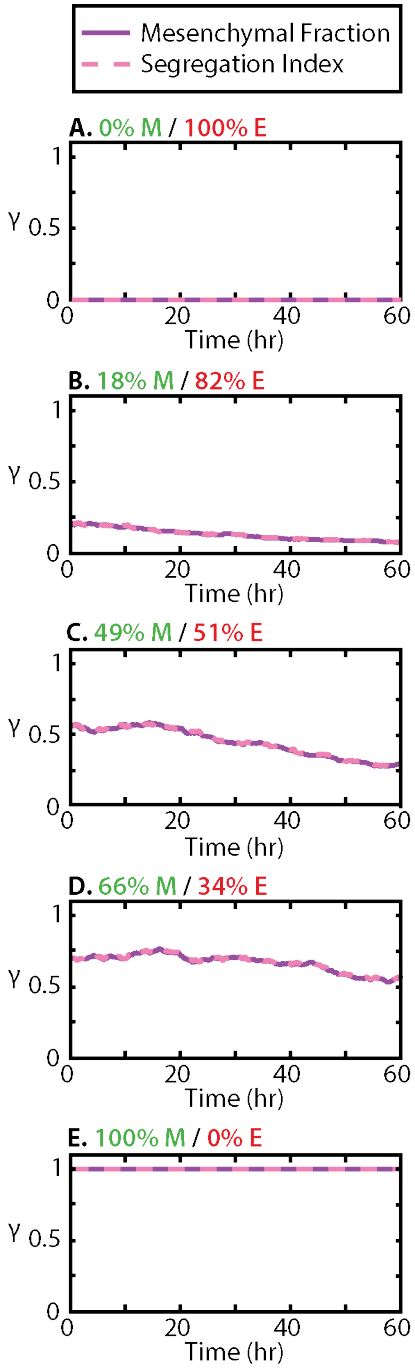

Figure S3. Local cell mixing (segregation index $\gamma = \langle n_M / (n_E + n_M) \rangle$) (pink dashes) and fraction of mesenchymal cells in the whole population (purple line) are roughly consistent and decrease slightly over time, indicative of an increase in nearest neighbors of opposite type. Note that this trend is mirrored by the middle line in Figure 3K-O, which is the percentage of epithelial cells in the total population. Plots are displayed by increasing mesenchymal percentage: 0% (A), 18% (B), 49% (C), 66% (D) and 100% (E).

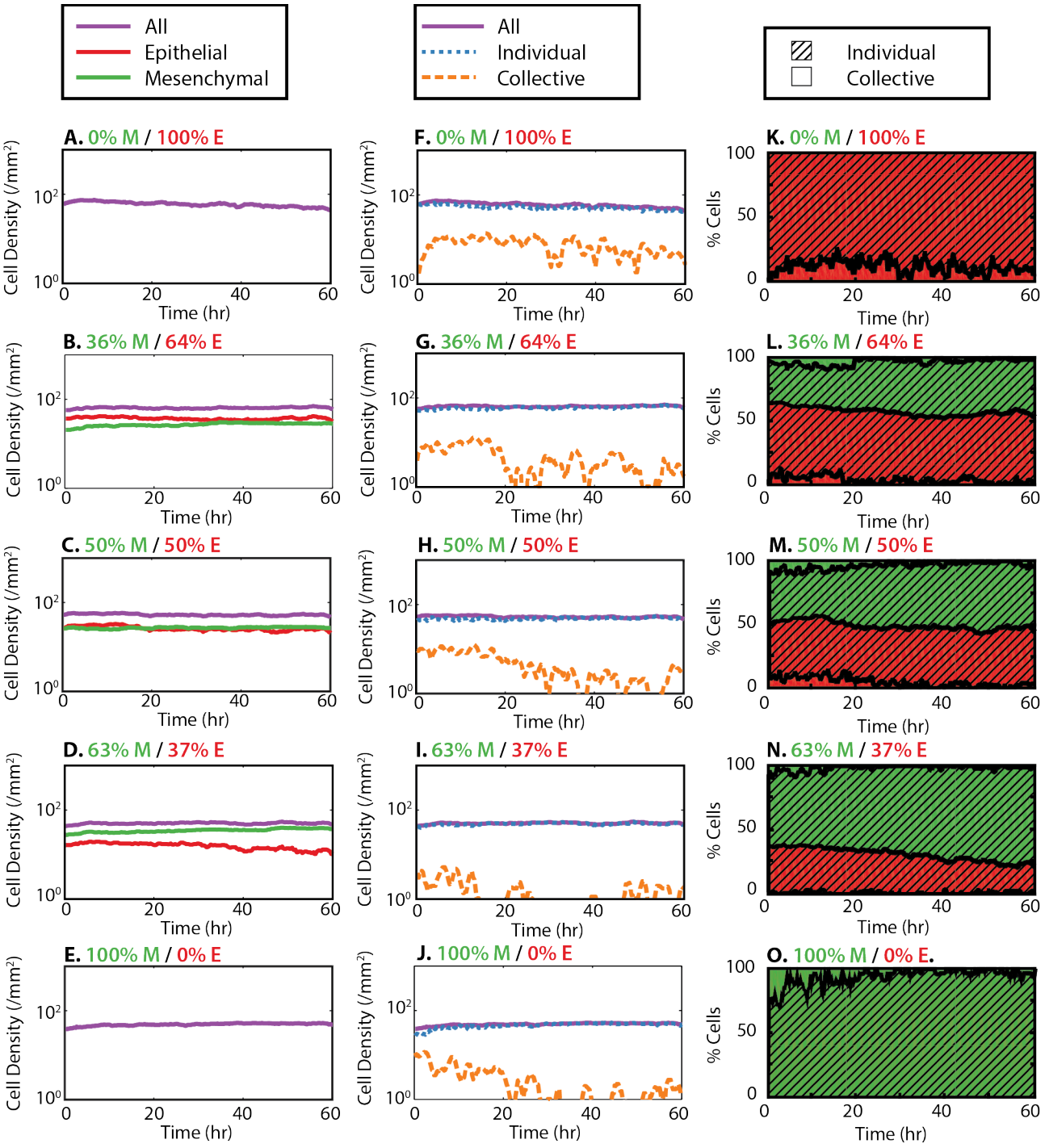

Figure S4. Cell density displayed as a function of time and initial mesenchymal percentage after mitomycin C treatment (top to bottom). (A-E) Total density (purple), mesenchymal density (green) and epithelial density (red line) stay constant since proliferation is blocked. (F-J) Individual density (blue dots) remains constant over time. There are a few cells in multicellular clusters (orange line), which fluctuate dramatically, suggesting these clusters are relatively unstable. (K-O) The relative proportions of epithelial (red), mesenchymal (green), individual (diagonal lines) and collective subpopulations (open) remain roughly consistent over time.

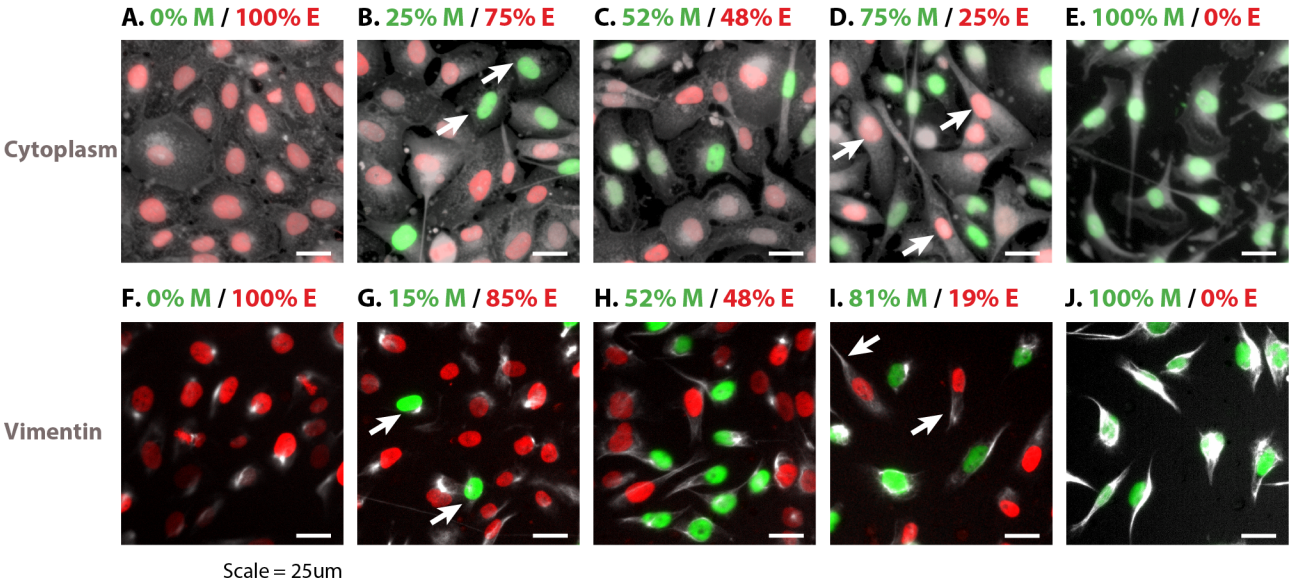

Figure S5. Epithelial cells co-cultured with mesenchymal cells can be individually dispersed, with elongated morphologies and increased vimentin expression, suggestive of EMT (arrows, D & I, respectively). Mesenchymal cells co-cultured with a high percentage of epithelial cells can be compacted with decreased vimentin expression (arrows, B & G, respectively). Immunofluorescent staining of cytoplasm (gray) with epithelial cells (red nuclei) and mesenchymal cells (green) displayed by increasing initial mesenchymal percentage: 0% (A), 25% (B), 52% (C), 75% (D) and 100% (E). Immunofluorescent staining of vimentin (gray) with epithelial cells (red nuclei) and mesenchymal cells (green) displayed by increasing initial mesenchymal percentage: 0% (A), 15% (B), 52% (C), 81% (D) and 100% (E).

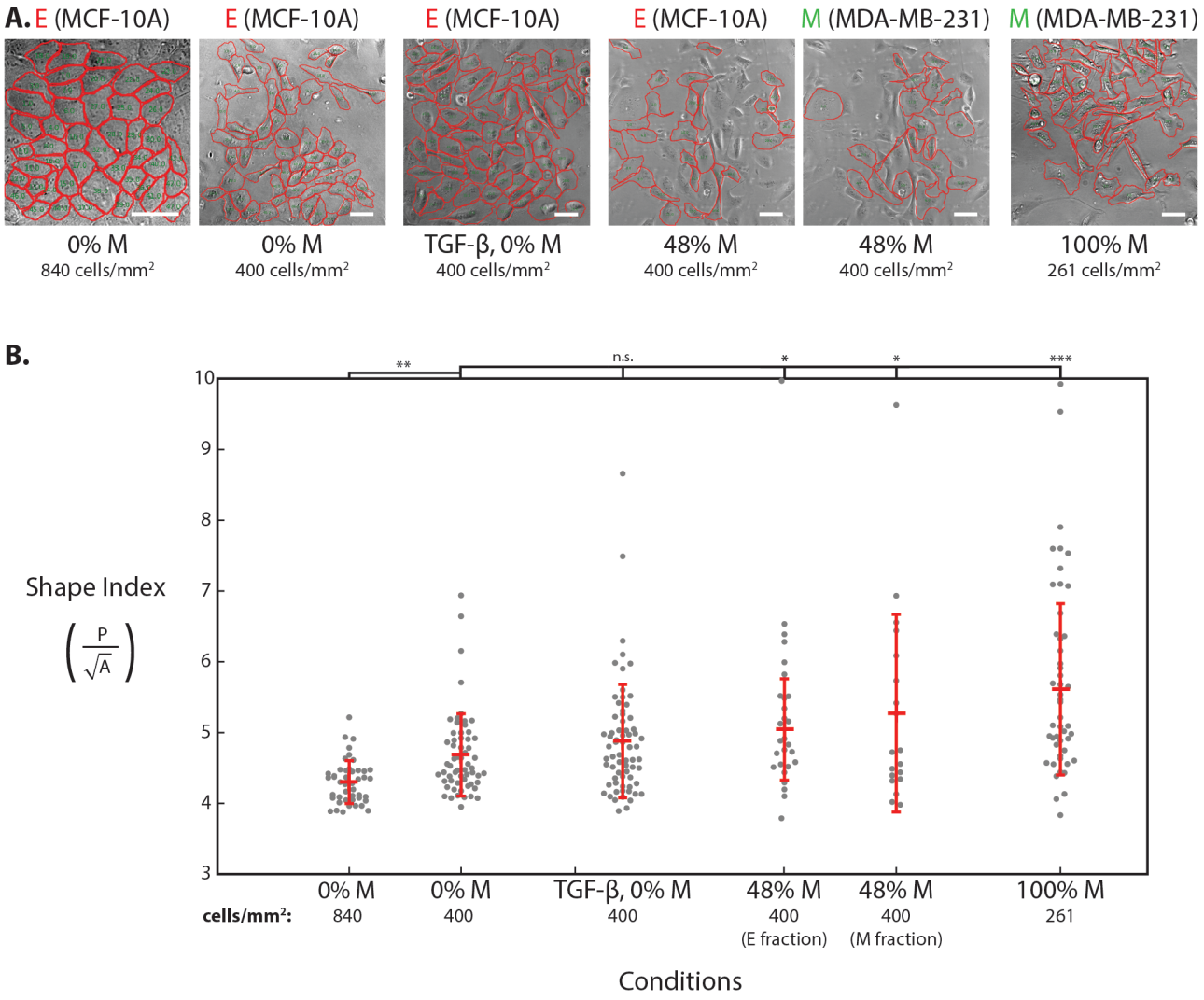

Figure S6. Cell shape index p_i is measured for epithelial (MCF-10A) and mesenchymal (MDA-MB-231) cell lines under various experimental conditions and densities. (A) Phase contrast images overlaid with outlines depicting manual cell segmentation in CellProfiler. From left to right: epithelial cells only (0% M) at high density, epithelial cells only at an intermediate density matching subsequent conditions, epithelial cells treated with TGF-β, epithelial cells co-cultured with mesenchymal cells (48% M), and mesenchymal cells only (100% M). (B) Plot depicting the individual cell values (gray dots), and population median and standard deviation (horizontal red lines) of the cell shape index, which shows an overall increase across the indicated conditions from left to right. Asterisks indicate p-values for statistically significant differences across conditions with respect to the 0% M, intermediate density condition for comparison (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. not significant).

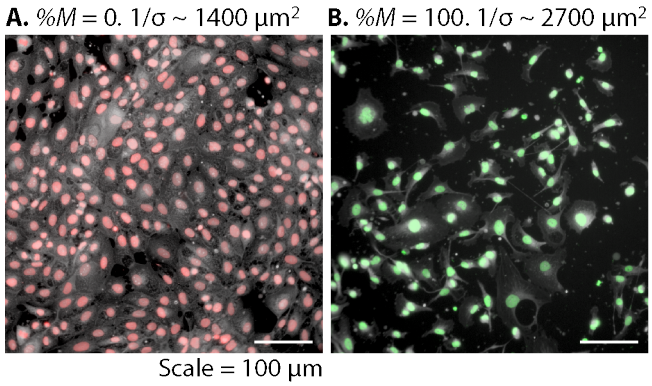

Figure S7. Cytoplasmic staining illustrates relative area coverage of cells. (A) Epithelial cells at 700 cells/mm^2 ($1/\sigma \sim 14 \cdot 10^{-3}$ mm^2) are approaching confluency. (B) Mesenchymal cells at 370 cells/mm^2 ($1/\sigma \sim 270$ mm^2) are subconfluent but still occupy an appreciable part of the field of view due to cell spreading.

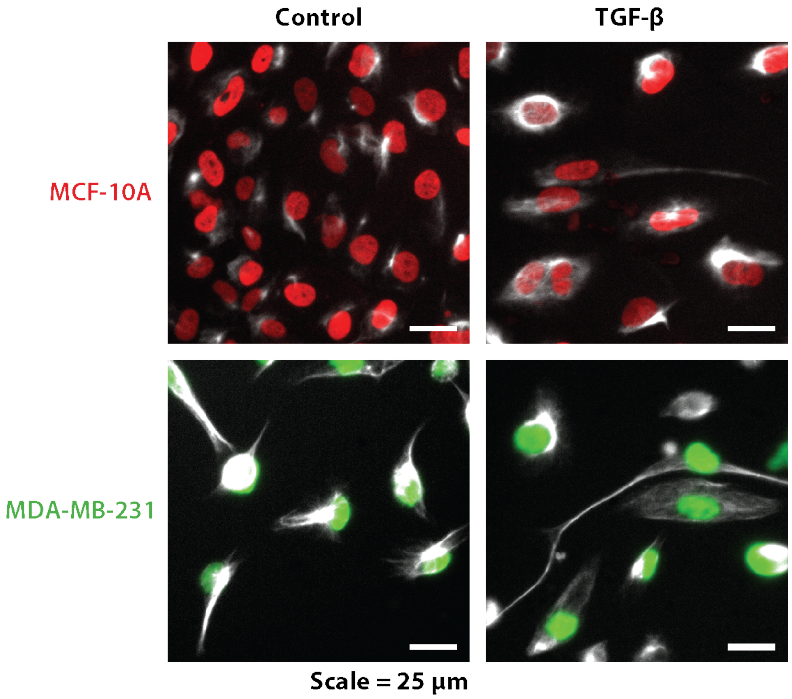

Figure S8. Epithelial and mesenchymal cells treated with TGF-β display enhanced vimentin expression and elongated morphologies relative to controls. These changes are indicative of a more mesenchymal phenotype. Immunofluorescent staining of vimentin (gray) with epithelial cells (red nuclei) and mesenchymal cells (green).

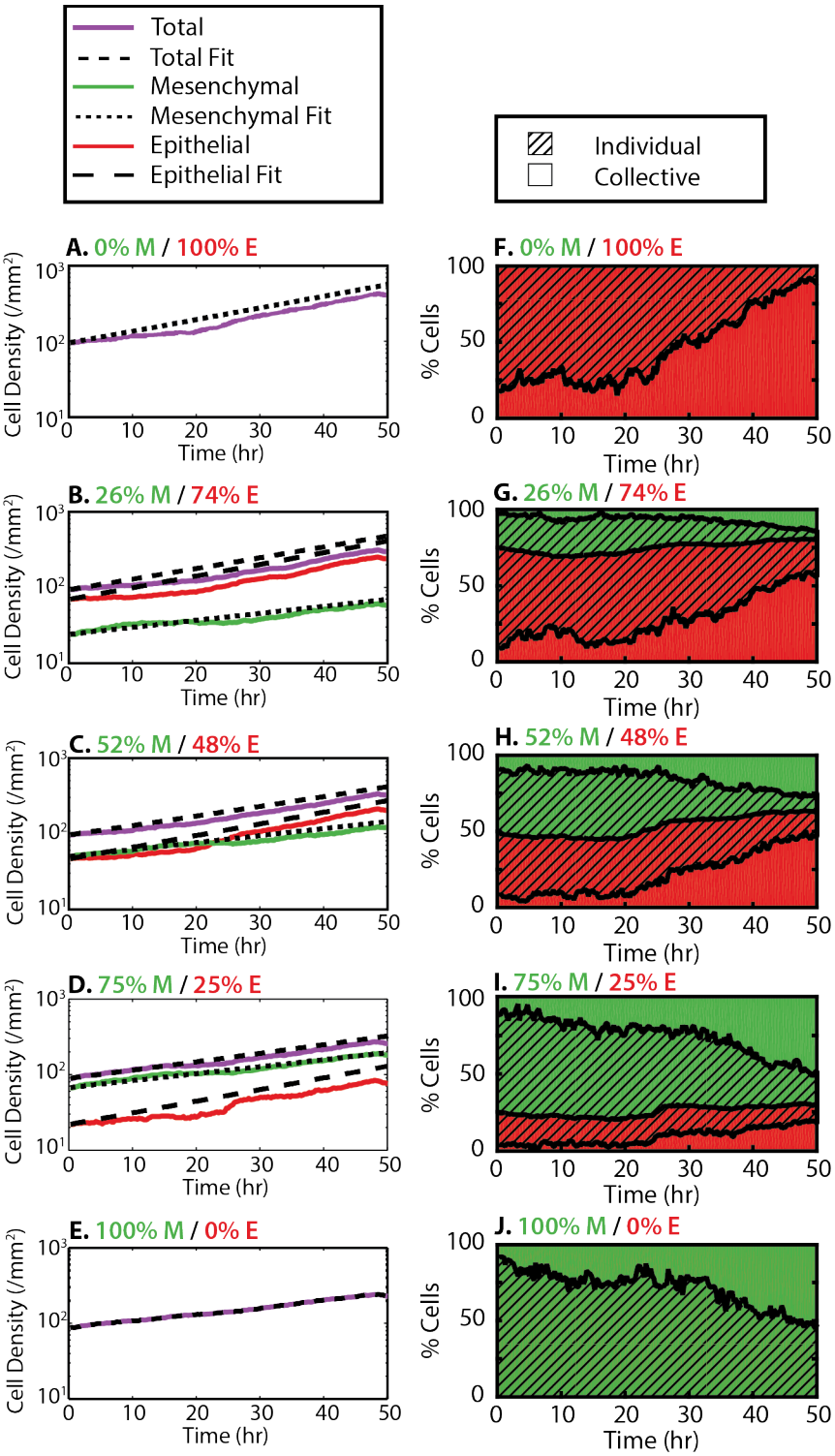

Figure S9. (A-E) Total (purple), mesenchymal (green) and epithelial cell density (red) after TGF-β treatment. In monoculture (A, E), the data was fit to an exponential $\sigma = \sigma_0 \exp(t/\tau)$, where σ_0 was the initial cell density and τ was the characteristic timescale. The mesenchymal fit (black dots) shows τ = 47 h, (doubling time of 32.6 h). The epithelial fit (wide black dashes) shows τ = 28.2 h, (doubling time of 19.5 h). The total fit (narrow black dashes) was the sum of exponentials for each subpopulation, $\sigma = \sigma_E \exp(t/\tau_E) + \sigma_M \exp(t/\tau_M)$. (F-J) The percentage of cells in clusters increased over time (open), while the percentage of individual cells (shaded) decreased over time, for both epithelial and mesenchymal subpopulations

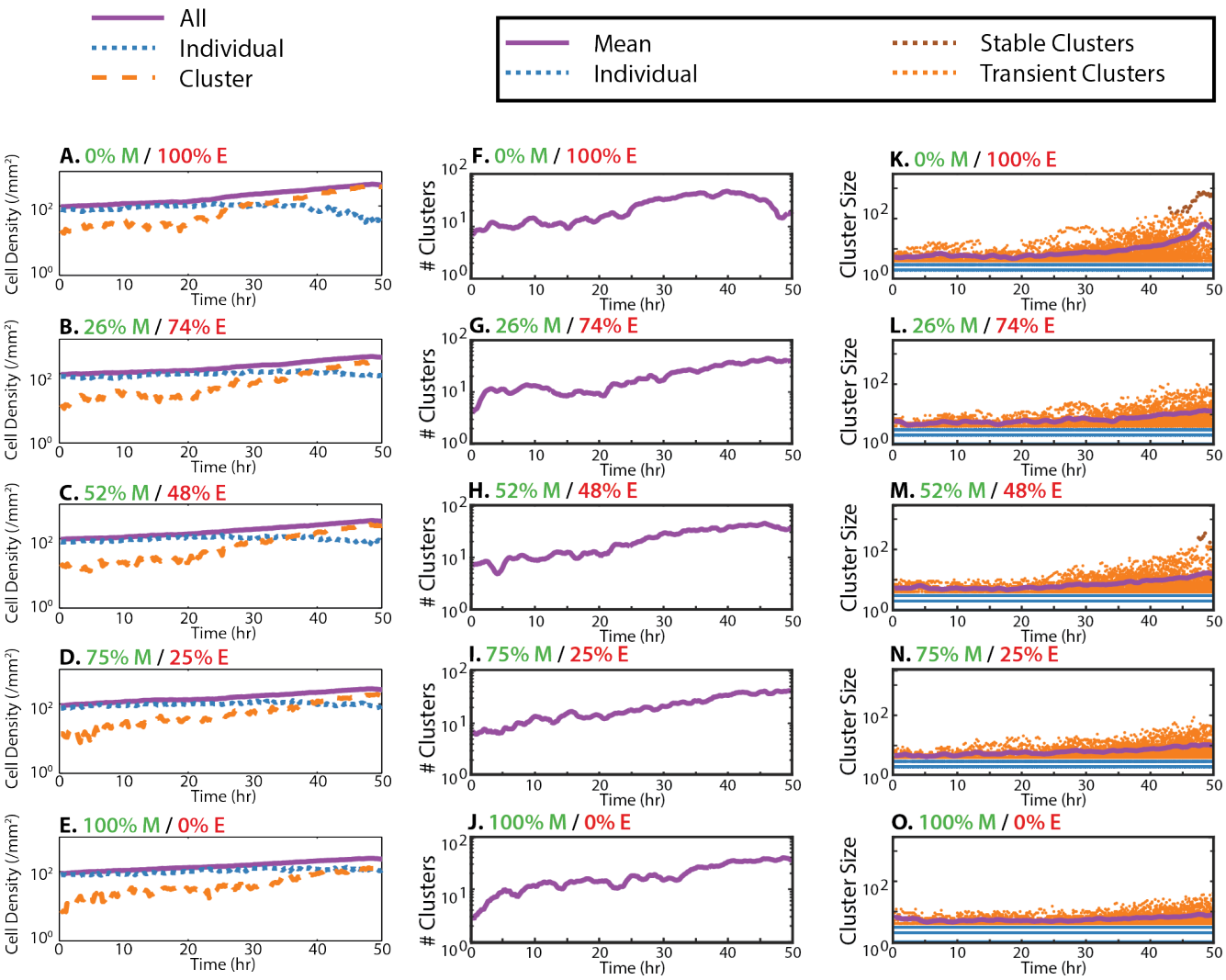

Figure S10. Cell densities, number of clusters and cluster size distribution after TGF-β treatment as a function of time and initial mesenchymal percentage (top to bottom). (A-E) Total (purple) and cluster cell density (orange dashes) increased over time, while individual cell density (blue dots) decreased slightly. (F-J) Number of clusters increased with time (K-M). For low mesenchymal percentage, the cluster size distribution remained roughly constant until ~30 h, when the average cluster size increased rapidly (purple line). Individual cells are colored blue, clustered cells are colored orange and stable clusters are colored brown (N, O). For higher mesenchymal percentage, the average cluster size only increased slightly.

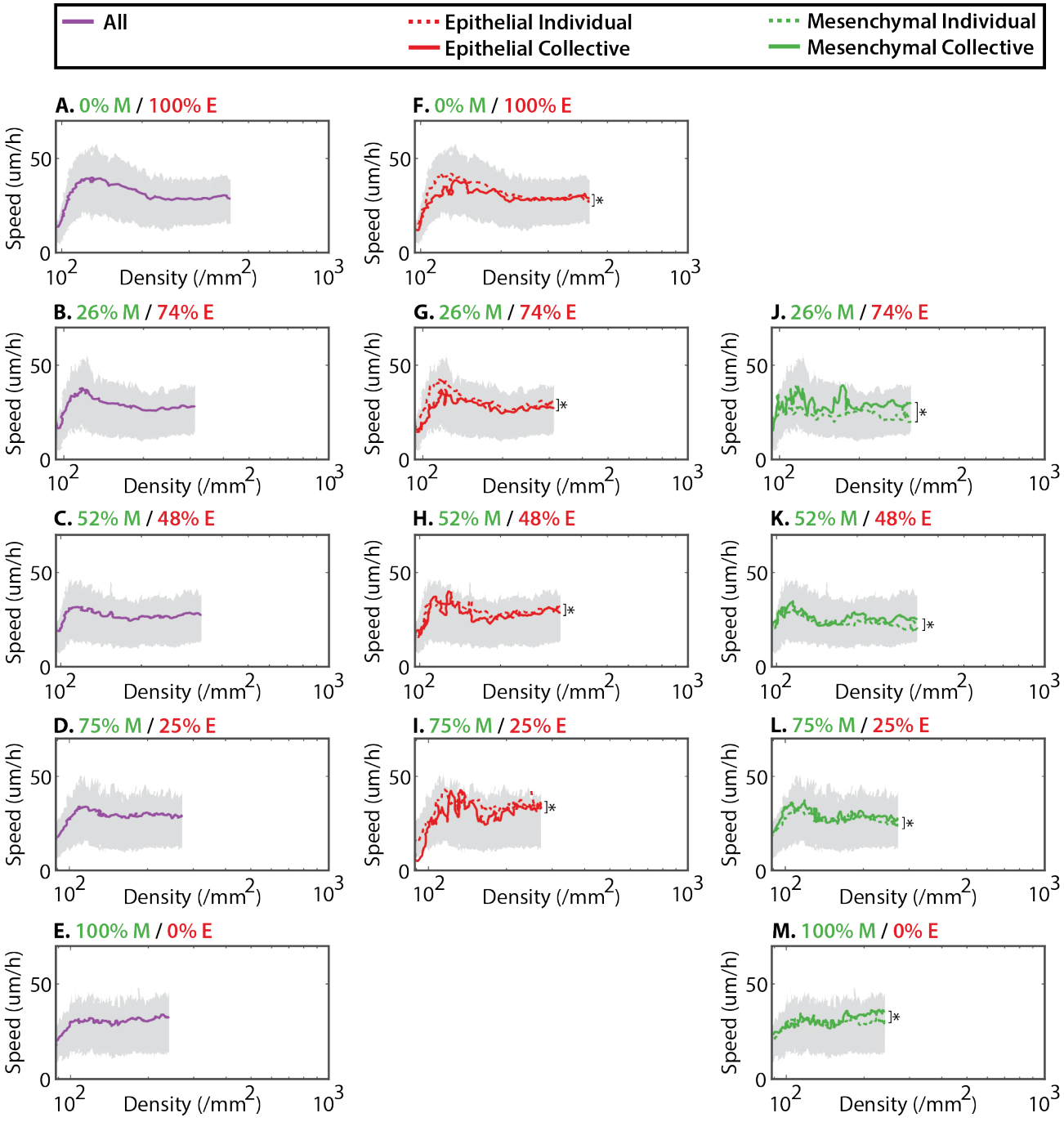

Figure S11. Average root mean squared velocity after TGF-β treatment is displayed as a function of density and initial mesenchymal percentage (top to bottom). Top and bottom quartiles (75%, 25%) are shown in gray for all plots. For low initial mesenchymal percentage, the overall velocity initially increased with density then remained roughly constant (A-C). For high initial mesenchymal percentage, the overall velocity remained roughly constant with density (D-E). For all conditions, epithelial cells were significantly faster as individuals than in clusters (F-I). However, mesenchymal cells displayed similar average velocities as individuals and in clusters (J-M). * denotes statistically significant difference ($p < 0.01$)

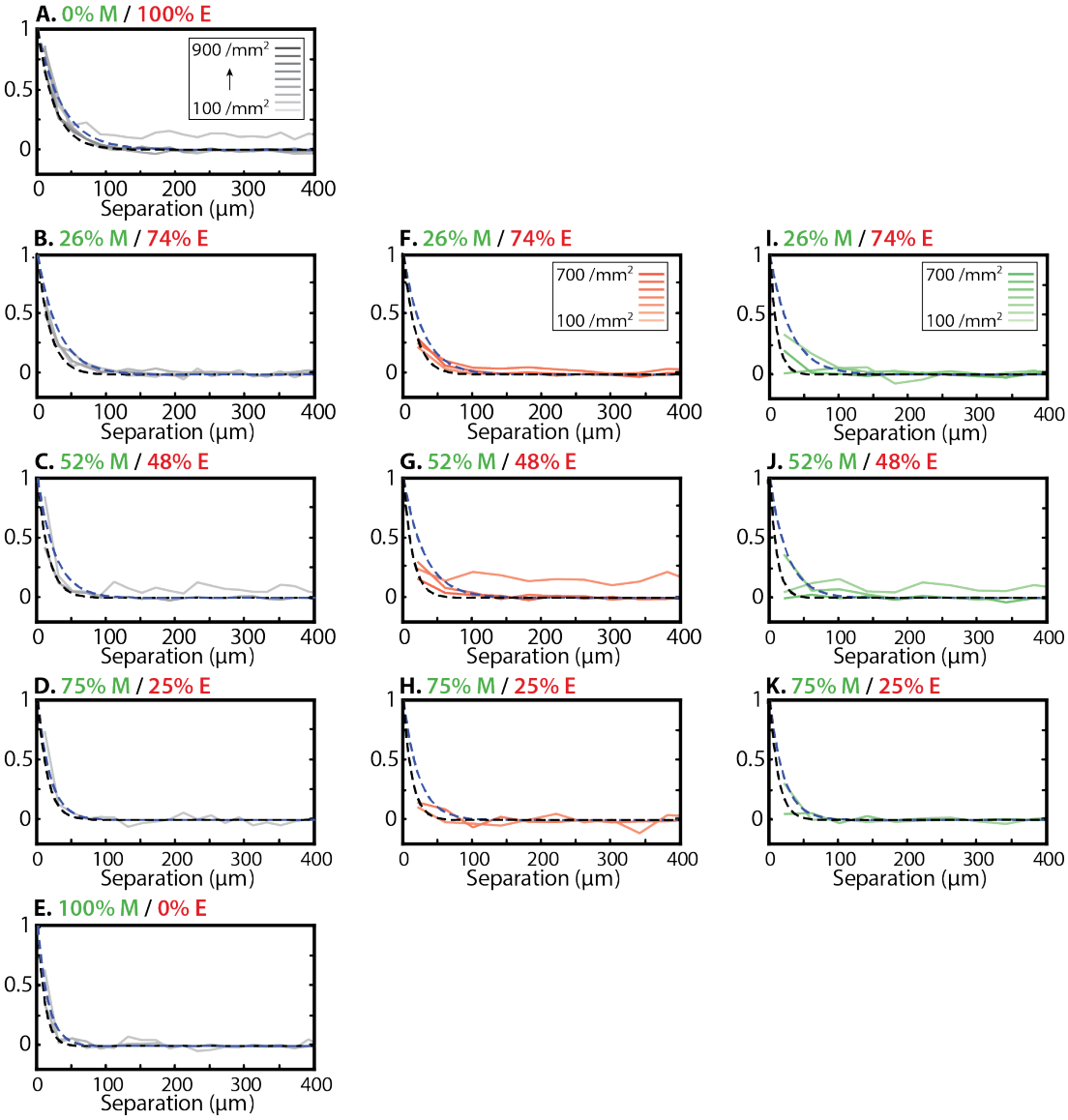

Figure S12. Spatial velocity correlations are displayed as a function of cell-cell separation and initial mesenchymal percentage (top to bottom). Darker lines correspond to increasing times. Black dashed line is the exponential best fit at 9 h and blue dashed line is the exponential best fit at 50 h. In general, spatial correlations became longer-ranged over time at the population level. However, spatial correlations became weaker with increasing initial mesenchymal percentage (A-E). In mixed populations, spatial correlations tended to be longer ranged for epithelial cells (F-H) relative to mesenchymal cells (I-K).

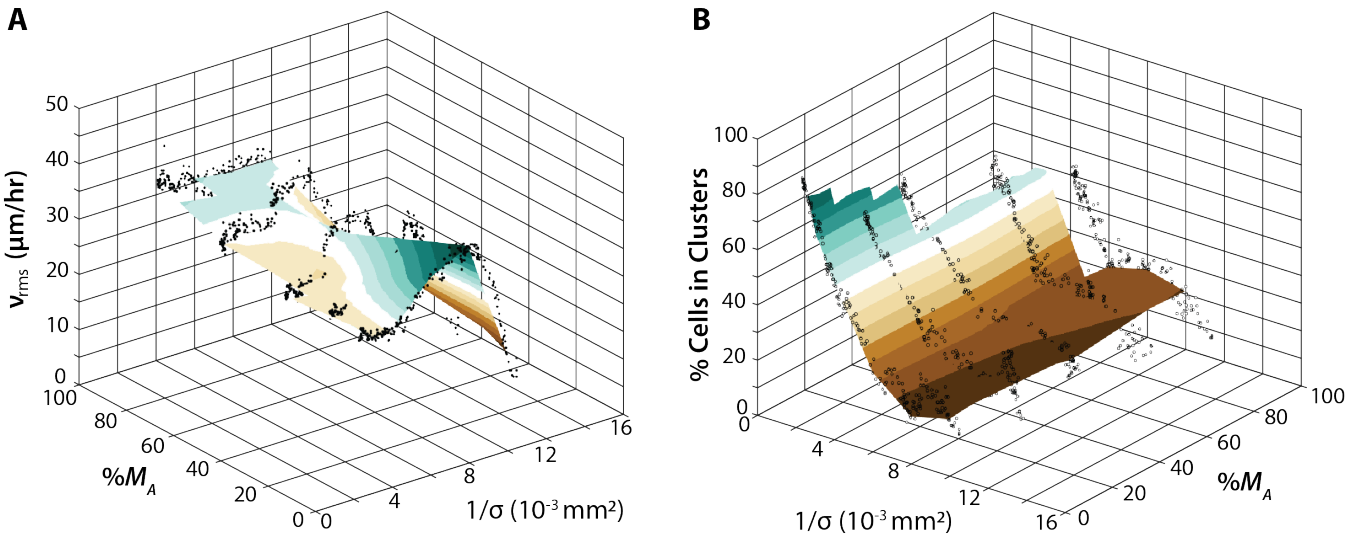

Figure S13. (A) Phase diagram of average cell velocity as a function of reciprocal cell density and actual mesenchymal percentage after TGF-β treatment. Migration was not arrested near the origin for mostly epithelial populations and high cell densities, particularly below $1/\sigma \sim 5 \cdot 10^{-3}$ mm^2, the "critical" cell density observed in untreated conditions. Increasing initial mesenchymal fraction also increased average cell velocity. (B) Phase diagram of percentage of cells in clusters as a function of reciprocal cell density and initial mesenchymal percentage. Incomplete clustering occurred near the origin for mostly epithelial populations and high cell densities. Increased initial mesenchymal fraction also decreases the percentage of cells in clusters.