Electronic Supplementary Material (ESI) for Soft Matter

Supporting Information

Directed persistent motion maintains sheet integrity during multi-cellular spreading and migration

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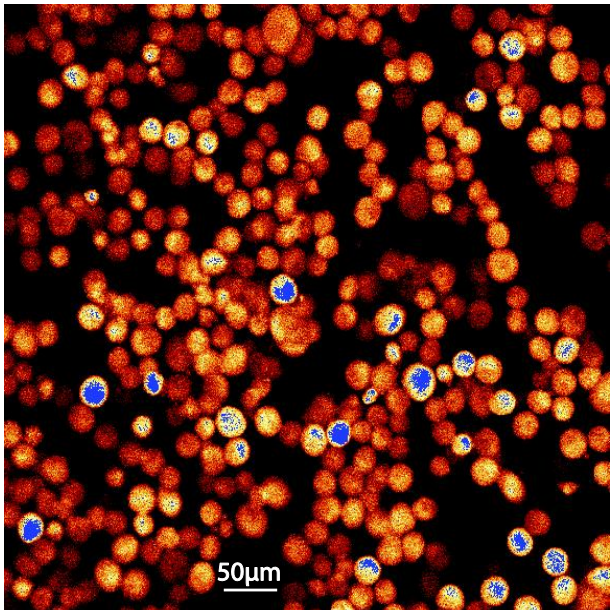

Figure S1: The confocal image that served a reference image from which we determined the height of single cells. We compared the height of the monolayer to that of the single cells to confirm further that we have monolayer. The round shape of the cells shows that not all the cells are completely spread. Therefore, we can estimated the height and diameter of spread cells and cells that are not completely spread.

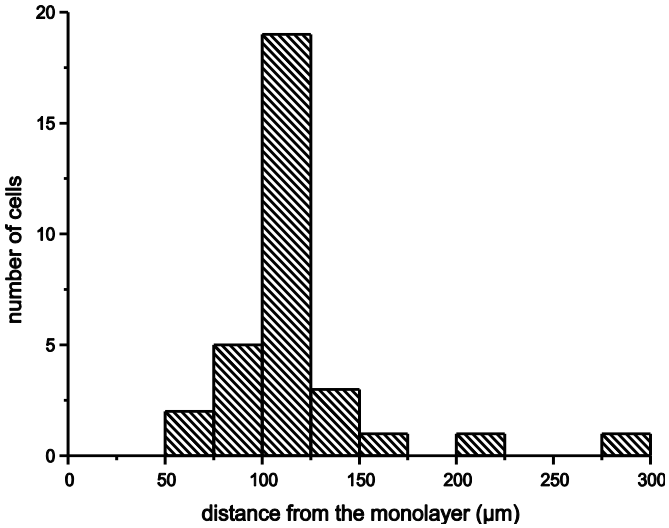

Figure S2: The distance travelled by escaping single cells from the monolayer (n = 32). While some cells could travel very long distance such as 290µm the mean distance travelled by most cells was (115 ± 20) µm. This suggests that there is an area of that slowly evolves in front of

the monolayer containing escaping single cells thus enabling the monolayer to catch up with escaping single cells.

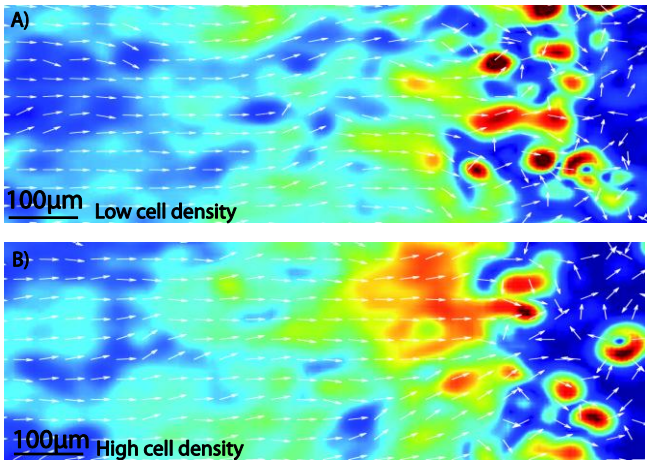

Figure S3: The velocity distribution and displacement fields of MCF-10A cells treated with 4mM EGTA. A) MCF-10A monolayer at a lower cell density (2×10^3 cells/cm^2) at time 1500 min. The displacement fields are uni-directional, thus indicating directed persistent motion while the velocity is heterogeneous. Moreover, single cells escape the monolayer. B) MCF-10A monolayer at a high cell density (1×10^5 cells/cm^2) at time 1500 min. Similar to the low density monolayer, the displacement fields are also uni-directional and the velocity is spatially and temporally heterogeneous. Marginal and escaping single cells have the highest velocity. The magnitude of the velocity is the same as in Fig. 1 and 3 in the paper.

Movie S1
A movie of MCF10A cells seeded at a low density (2×10^3 cells/cm^2) and allowed to move over a very long distance. Some cells escape the sheet as it migrates and the cell density at the front of the monolayer decreased with time. This decrease in cell density results in the formation of gaps within the sheet. However, these gaps are filled up by cell division which creates new cells and by the ballistic motion of the sheet enabling it to catch up with escaping cells.

Movie S2: MCF10A cells seeded at a lower cell density 2×10^3 cells/cm^2 (left hand side) and at a higher cell density 1×10^5 cells/cm^2 (right hand side). At a lower cell density, single cells could escape the monolayer during migration while at the higher cell density; single cells did not escape the monolayer over long migration times.

Movie S3: This movie shows a sheet of NIH 3t3 fibroblast cells (2×10^3 cells/cm^2) seeded on a non-coated glass substrate. When the ibidi insert is removed, the cells escape the sheet and move randomly in front of the cell monolayer.

Movie S4: MCF-10A cells with EGTA (ethylene glycol tetraacetic acid) added in the culture medium to obtain such that the concentration is 4mM. The cells are seeded at a high cell density 1×10^5 cells/cm^2. Single cells detached from the monolayer upon the addition of EGTA. The monolayer is allowed to migrate for 9 h before EGTA is added. However, the monolayer catches up with escaping cells.

Movie S5: MCF-10A cells with EGTA (ethylene glycol tetraacetic acid) added in the culture medium to obtain such that the concentration is 4mM. The cells are seeded at a low cell

density 2×10^3 cells/cm^2. Single cells detached from the monolayer upon the addition of EGTA. The monolayer is allowed to migrate for about 6 h before EGTA is added. However, the monolayer catches up with escaping cells.