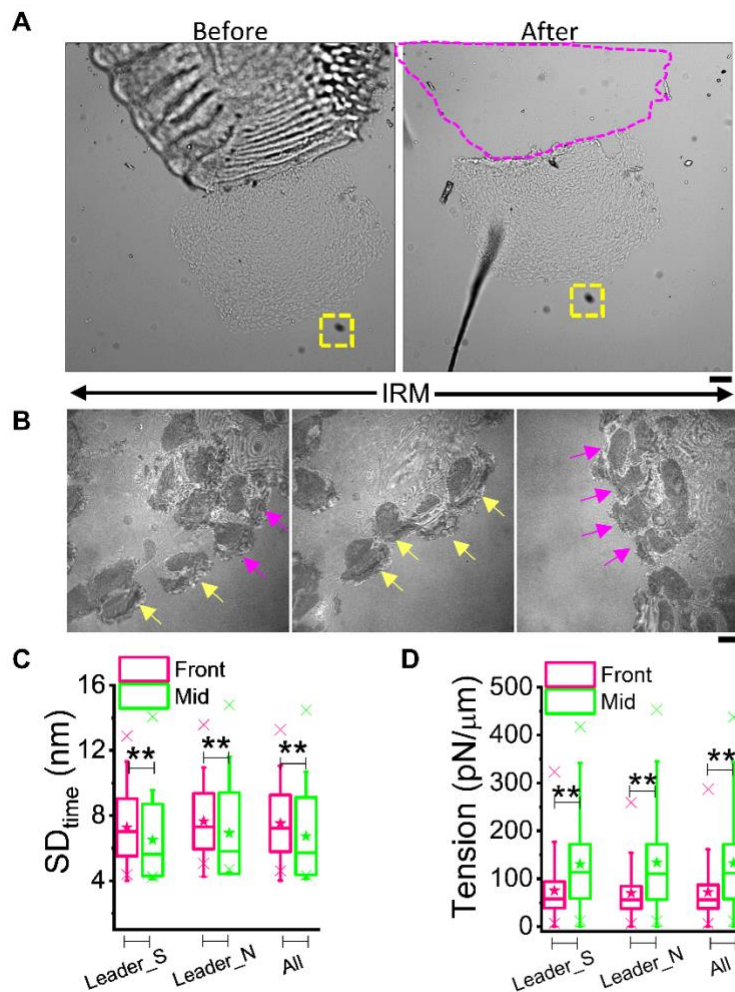
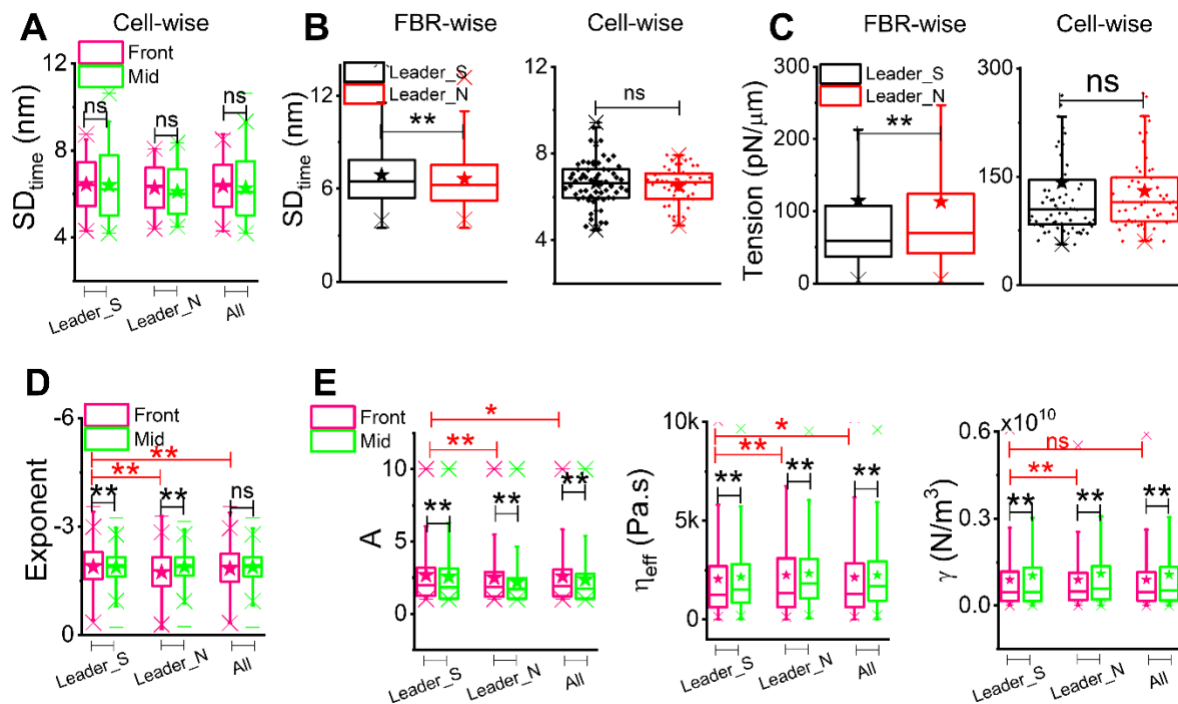


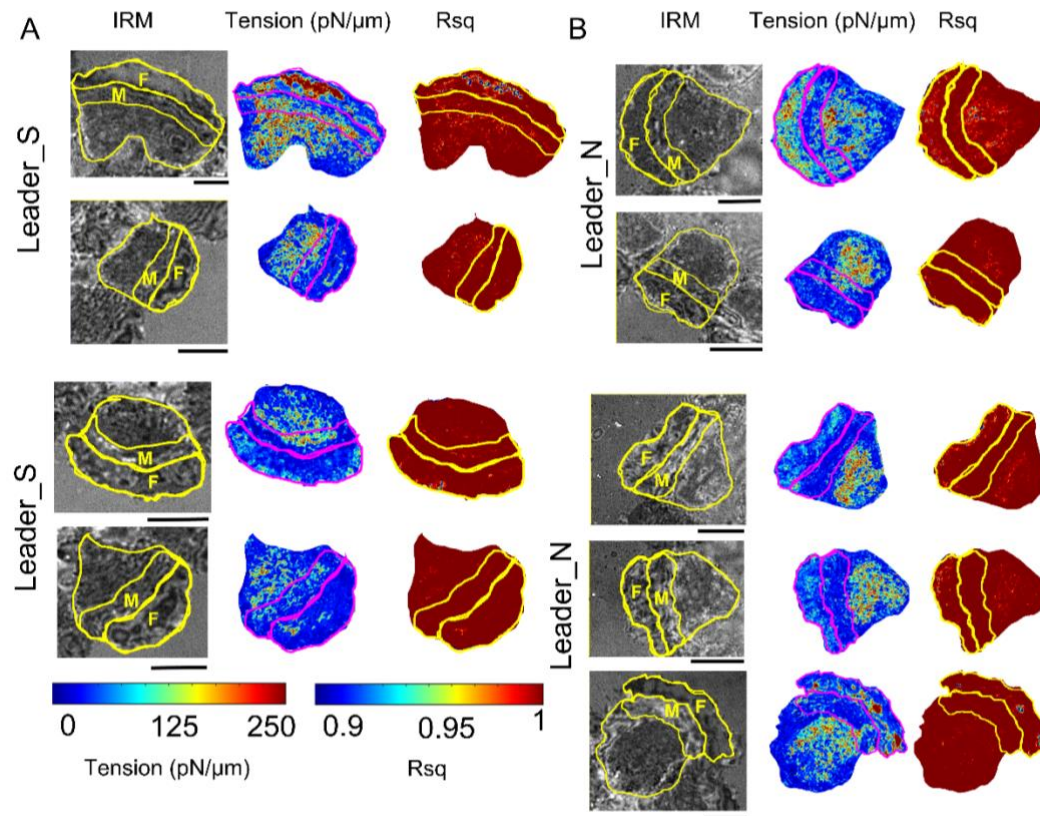
**Fig. S1. Edge velocity calculation** A) Representative DIC image of a typical keratocyte sheet showing all the leaders denoted by yellow arrows and a typical line like pattern at the mid region marked by magenta dotted structure. B) Left: Cells moving out from under a scale, with lines in yellow drawn to analyse from kymographs to measure velocity along those ROIs. Right: A typical kymograph (calculated from pink line ROI displayed in B) showing edge velocity of a cell sheet. Scale bar = 100  $\mu\text{m}$ . Yellow arrow indicates direction of time.



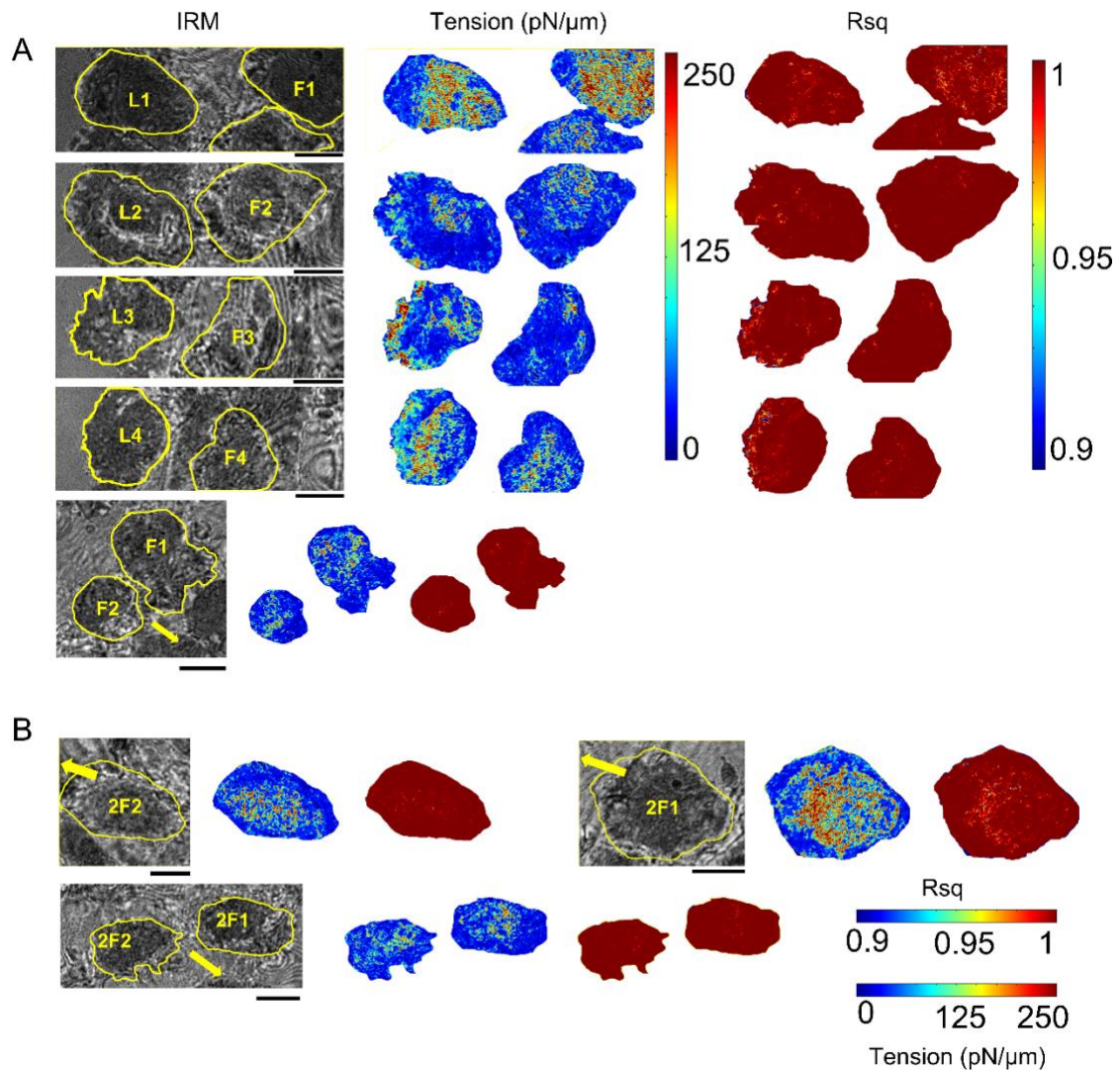
**Fig. S2. Cell sheet after mechanical isolation of scale.** (A) DIC images of cell sheet before (left) and after removal of scale (right), pink dashed outline denotes the old position of scale and yellow boxes are drawn around particular debris on the coverslip to highlight the same area. Scale bar = 100 μm. (B) IRM images of leaders with substrate-adhered followers pointed out by pink arrows (Leader\_S), leaders with substrate-deadhered followers pointed by yellow arrows (Leader\_N), scale bar = 10 μm (C) FBR wise comparison of temporal fluctuations between front and mid regions of Leader\_S and Leader\_N and clubbed together (nFBRs: Leader\_S front: 7565, mid: 5509; Leader\_N front: 14008, mid: 7191) (D) FBR wise comparison of membrane tension between front and mid regions of Leader\_S and Leader\_N and clubbed together (nFBRs: Leader\_S front: 4486, mid: 3510; Leader\_N front: 8610, mid: 4677) Leader\_S: Ncells = 13, Leader\_N: Ncells = 17. Statistical analysis was performed using Mann-Whitney U test. \*\* denotes p value <0.001.



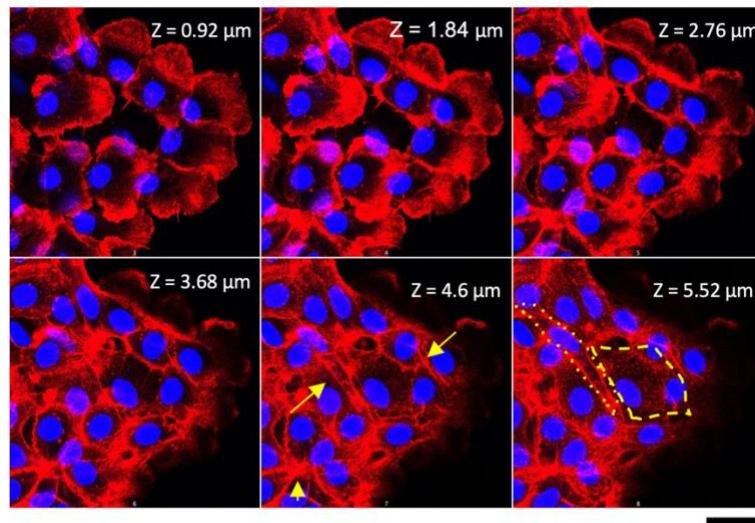
**Fig. S3. Box plot comparison of different mechanical parameters** A) cell wise comparison of  $SD_{time}$  between front and mid parts of leader cells (Leader\_S: Ncells = 66, Leader\_N: Ncells = 53) B) (left) FBR wise comparison of  $SD_{time}$  between Leader\_S and Leader\_N cells with front and mid clubbed together (Leader\_S: nFBRs = 57939; Leader\_N: nFBRs = 47634) (right) cell wise comparison of  $SD_{time}$  with front and mid clubbed together (Leader\_S: Ncells = 66, Leader\_N: Ncells = 53) C) (left) FBR wise comparison of membrane tension between two different pool of leaders with front and mid clubbed together (nFBRs: Leader\_S: 29853, Leader\_N: 26940) (right) cell wise comparison of the same (Leader\_S: Ncells = 66, Leader\_N: Ncells = 53) D) FBR wise comparison of exponent between front and mid among different pool of leaders and all leaders clubbed together (nFBRs : Leader\_S: front: 13373, mid: 981; Leader\_N front: 6580, mid: 5345) E) (left) FBR wise comparison of active temperature between front and mid among different pool of leaders and all leaders clubbed together (centre) FBR wise comparison of effective cytoplasmic viscosity between front and mid among different pool of leaders and all leaders clubbed together (right) FBR wise comparison of confinement between front and mid among different pool of leaders and all leaders clubbed together (nFBRs: Leader\_S front: 15440, mid: 14413, Leader\_N front: 11624, mid: 15316) Statistical analysis was performed using Mann-Whitney U test. ns denotes  $p$  value  $>0.05$ . \* denotes  $p$  value  $<0.05$  \*\* denotes  $p$  value  $<0.001$ .



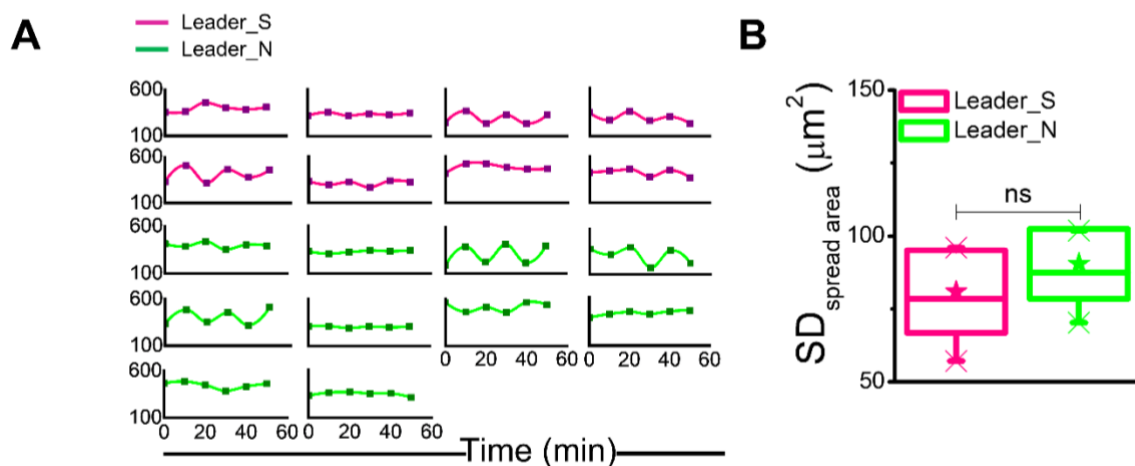
**Fig. S4. Tension and their corresponding  $R^2$  ( $R^2$ ) maps of leaders** A) (left column) Leader\_S with front and mid regions marked as F and M (middle column) respective tension maps, front and mid marked with magenta lines (right column)  $R^2$  maps, front and mid marked with yellow lines B) (left column) Leader\_N with front and mid regions marked as F and M (middle column) respective tension maps, front and mid marked with magenta lines (right column)  $R^2$  maps, front and mid marked with yellow lines. Scale bar = 10  $\mu\text{m}$ .



**Fig. S5. Tension maps and corresponding  $R^2$  (Rsquared) maps of leaders and followers** A) (left panel) (top to bottom) IRM images of leaders with first layer of followers marked as L1-L4 and F1-F4 respectively (middle panel) tension maps of leaders with 1st followers (right panel)  $R^2$  maps of the same B) (top and bottom) IRM images of 2nd layer (2F) of followers with their respective tension and  $R^2$  maps beside them respectively, direction of migration marked with yellow arrows. Scale bar = 10  $\mu$ m.



**Fig. S6. Tight coupling of cells at higher z-planes.** Representative confocal z-slices of a cell sheet with F-actin labelled with Phalloidin Alexa Fluor 568 and DNA with DAPI. Cells are either not attached or less tightly attached with each other at lower planes however are strongly attached at higher z-planes even if sharp-edged hexagonal packing (as in Fig 2A) is not observed. Yellow dashed line at  $z = 5.52 \mu\text{m}$  depicts the presence of a stretched-out cell over a gap at  $z = 0.92 \mu\text{m}$ . The yellow dotted line highlights a highly straightened cell visible only at a high z-planes ( $z > 4.6 \mu\text{m}$ ). Scale bar =  $10 \mu\text{m}$ .



**Fig. S7.** (A) Fluctuations in cell spread area of individual cells during migration. (B) Variability (standard deviation) in cell spread area measured over 50 min.

**Table S1. Descriptive statistics for all parameters plotted in the main figures.** Ncells denote the number of cells and nrois or nFBRs denote the number of ROIs (region of interests) or FBRs respectively used for the corresponding figure.

| Figure 1B                |                   |                    |                   |      |       |       |        |                         |
|--------------------------|-------------------|--------------------|-------------------|------|-------|-------|--------|-------------------------|
| Parameters               | Condition         | N                  | n <sub>rois</sub> | Mean | SD    | SEM   | Median |                         |
| Edge speed (μm/min)      | Growth media      | 4                  | 20                | 1    | 0.4   | 0.1   | 0.9    |                         |
| Figure 3C                |                   |                    |                   |      |       |       |        |                         |
| Parameters               | Condition         | N <sub>cells</sub> | n                 | Mean | SD    | SEM   | Median | p-values (wrt Leader_S) |
| Speed (μm/min)           | Leader_S (41 sec) | 66                 | 66                | 2.19 | 0.92  | 0.11  | 2.21   |                         |
|                          | Leader_N (41 sec) | 53                 | 53                | 1.95 | 0.84  | 0.11  | 1.8    | 0.0935                  |
|                          | Leader_S (10 min) | 67                 | 335               | 2.03 | 0.54  | 0.07  | 1.87   |                         |
|                          | Leader_N (10 min) | 60                 | 300               | 1.89 | 0.48  | 0.06  | 1.76   | 0.14808                 |
|                          | Leader_S (50 min) | 67                 | 46                | 1.99 | 0.56  | 0.07  | 1.83   |                         |
|                          | Leader_N (50 min) | 60                 | 42                | 1.84 | 0.49  | 0.06  | 1.69   | 0.11486                 |
| Figure 4B                |                   |                    |                   |      |       |       |        |                         |
| Parameters               | Condition         | N <sub>cells</sub> | n <sub>FBRs</sub> | Mean | SD    | SEM   | Median | p-values (wrt Front)    |
| SD <sub>time</sub> (nm)  | Front; Leader_S   | 66                 | 33654             | 6.8  | 1.8   | 0.01  | 6.6    |                         |
|                          | Mid; Leader_S     | 66                 | 24285             | 6.9  | 2.5   | 0.02  | 6.3    | 0                       |
|                          | Front; Leader_N   | 53                 | 27381             | 6.7  | 1.8   | 0.01  | 6.4    |                         |
|                          | Mid; Leader_N     | 53                 | 20253             | 6.6  | 2     | 0.01  | 5.9    | 0                       |
|                          | Front; All        | 119                | 61035             | 6.8  | 1.8   | 0.007 | 6.5    |                         |
|                          | Mid; All          | 119                | 44538             | 6.8  | 2.3   | 0.01  | 6.1    | 0                       |
| Figure 4D                |                   |                    |                   |      |       |       |        |                         |
| Parameters               | Condition         | N <sub>cells</sub> | n <sub>FBRs</sub> | Mean | SD    | SEM   | Median | p-values (wrt Front)    |
| Tension (pN/μm) FBR wise | Front; Leader_S   | 66                 | 15440             | 110  | 311.8 | 2.5   | 55.9   |                         |

|                                 |                    |                          |                         |             |           |            |               |                                     |
|---------------------------------|--------------------|--------------------------|-------------------------|-------------|-----------|------------|---------------|-------------------------------------|
|                                 | Mid;<br>Leader_S   | 66                       | 14413                   | 118         | 325.3     | 2.7        | 63.3          | 6E-31                               |
|                                 | Front;<br>Leader_N | 53                       | 11624                   | 110         | 238.3     | 2.2        | 61.8          |                                     |
|                                 | Mid;<br>Leader_N   | 53                       | 15316                   | 115         | 198       | 1.6        | 78.3          | 4E-78                               |
|                                 | Front; All         | 119                      | 27064                   | 110         | 282.6     | 1.7        | 58.5          |                                     |
|                                 | Mid; All           | 119                      | 29729                   | 117         | 267.4     | 1.6        | 70.2          | 3E-118                              |
| <b>Parameters</b>               | <b>Condition</b>   | <b>N<sub>cells</sub></b> | <b>n<sub>FBRs</sub></b> | <b>Mean</b> | <b>SD</b> | <b>SEM</b> | <b>Median</b> | <b>p-values<br/>(wrt<br/>Front)</b> |
| Tension<br>(pN/μm)<br>Cell wise | Front;<br>Leader_S | 66                       |                         | 61.4        | 26.7      | 3.3        | 52.1          |                                     |
|                                 | Mid;<br>Leader_S   | 66                       |                         | 65.5        | 34.5      | 4.3        | 54.5          | 0.94738                             |
|                                 | Front;<br>Leader_N | 53                       |                         | 75.1        | 41.6      | 5.7        | 61            |                                     |
|                                 | Mid;<br>Leader_N   | 53                       |                         | 75.9        | 34.5      | 4.7        | 68.8          | 0.61987                             |
|                                 | Front; All         | 119                      |                         | 67.5        | 34.7      | 3.2        | 56.4          |                                     |
|                                 | Mid; All           | 119                      |                         | 70.1        | 34.8      | 3.2        | 60            | 0.76822                             |

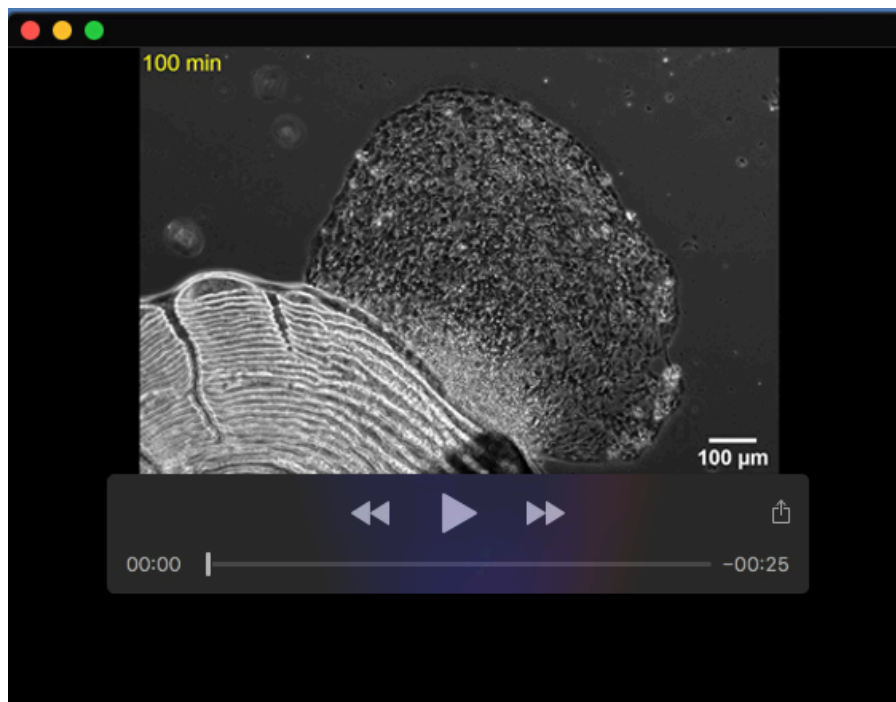


**Table S2. Descriptive statistics for all parameters plotted in supplementary figures.**  $N_{cells}$  denote the number of cells and  $n_{rois}$  or  $n_{FBRs}$  denote the number of ROIs (region of interests) or FBRs respectively used for the corresponding figure.

| Figure S3A              |                 |             |            |       |       |        |        |                      |
|-------------------------|-----------------|-------------|------------|-------|-------|--------|--------|----------------------|
| Parameters              | Condition       | $N_{cells}$ | $n_{FBRs}$ | Mean  | SD    | SEM    | Median | p-value (wrt Front)  |
| SD <sub>time</sub> (nm) | Front; Leader_S | 66          | 66         | 6.44  | 1     | 0.123  | 6.41   |                      |
|                         | Mid; Leader_S   | 66          | 66         | 6.39  | 1.39  | 0.171  | 6.2    | 0.47341              |
|                         | Front; Leader_N | 53          | 53         | 6.29  | 0.91  | 0.126  | 6.32   |                      |
|                         | Mid; Leader_N   | 53          | 53         | 6.10  | 1.03  | 0.142  | 5.99   | 0.258                |
|                         | Front; All      | 119         | 119        | 6.37  | 0.97  | 0.089  | 6.38   |                      |
|                         | Mid; All        | 119         | 119        | 6.27  | 1.25  | 0.114  | 6.07   | 0.18587              |
| Figure S3B              |                 |             |            |       |       |        |        |                      |
| Parameters              | Condition       | $N_{cells}$ | $n_{FBRs}$ | Mean  | SD    | SEM    | Median | p-value (wrt Front)  |
| SD <sub>time</sub> (nm) | Leader_S        | 66          | 5793<br>9  | 6.89  | 2.14  | 0.009  | 6.49   |                      |
|                         | Leader_N        | 53          | 4763<br>4  | 6.63  | 1.92  | 0.009  | 6.26   | 0                    |
|                         | Leader_S        | 66          | 66         | 6.64  | 1.10  | 0.136  | 6.64   |                      |
|                         | Leader_N        | 53          | 53         | 6.49  | 0.84  | 0.116  | 6.68   | 0.60588              |
| Figure S3C              |                 |             |            |       |       |        |        |                      |
| Parameters              | Condition       | $N_{cells}$ | $n_{FBRs}$ | Mean  | SD    | SEM    | Median | p-values (wrt Front) |
| Tension (pN/ $\mu$ m)   | Leader_S        | 66          | 29853      | 114.3 | 318.4 | 1.843  | 59.30  |                      |
|                         | Leader_N        | 53          | 26940      | 112.9 | 216.3 | 1.318  | 70.06  | 8E-99                |
|                         | Leader_S        | 66          | 66         | 141.9 | 102.7 | 12.642 | 105.10 |                      |
|                         | Leader_N        | 53          | 53         | 129.8 | 56.8  | 7.803  | 114.83 | 0.43345              |

| Figure S3D              |                 |                    |                   |        |            |       |        |                     |
|-------------------------|-----------------|--------------------|-------------------|--------|------------|-------|--------|---------------------|
| Parameters              | Condition       | N <sub>cells</sub> | n <sub>FBRs</sub> | Mean   | SD         | SEM   | Median | p-value (wrt Front) |
| Exponent                | Front; Leader_S | 38                 | 1337<br>3         | -1.89  | 0.58       | 0.005 | -1.96  |                     |
|                         | Mid; Leader_S   | 38                 | 9819              | -1.88  | 0.4        | 0.004 | -1.88  | 5E-14               |
|                         | Front; Leader_N | 21                 | 6580              | -1.74  | 0.57       | 0.007 | -1.8   |                     |
|                         | Mid; Leader_N   | 21                 | 5345              | -1.89  | 0.39       | 0.005 | -1.9   | 2E-30               |
|                         | Front; All      | 59                 | 1995<br>3         | -1.84  | 0.58       | 0.004 | -1.91  |                     |
|                         | Mid; All        | 59                 | 1516<br>4         | -1.88  | 0.4        | 0.003 | -1.89  | 0.23452             |
| Figure S3E              |                 |                    |                   |        |            |       |        |                     |
| Parameters              | Condition       | N <sub>cells</sub> | n <sub>FBRs</sub> | Mean   | SD         | SEM   | Median | p-value (wrt Front) |
| A                       | Front; Leader_S | 66                 | 1544<br>0         | 2.6    | 2.8        | 0.01  | 2      |                     |
|                         | Mid; Leader_S   | 66                 | 1441<br>3         | 2.7    | 2.6        | 0.02  | 1.8    | 0                   |
|                         | Front; Leader_N | 53                 | 1162<br>4         | 3.2    | 2.3        | 0.02  | 1.9    |                     |
|                         | Mid; Leader_N   | 53                 | 1531<br>6         | 2.6    | 2.2        | 0.01  | 1.7    | 0                   |
|                         | Front; All      | 119                | 2706<br>4         | 3.2    | 2.6        | 0.01  | 1.9    |                     |
|                         | Mid; All        | 119                | 2972<br>9         | 2.6    | 2.4        | 0.01  | 1.8    | 0                   |
| Parameters              | Condition       | N <sub>cells</sub> | n <sub>FBRs</sub> | Mean   | SD         | SEM   | Median | p-value (wrt Front) |
| η <sub>eff</sub> (Pa.s) | Front; Leader_S | 66                 | 15440             | 2045.5 | 2530.<br>4 | 20.3  | 1254.9 |                     |
|                         | Mid; Leader_S   | 66                 | 14413             | 2158.8 | 2191.<br>7 | 18.2  | 1532.4 | 7E-56               |
|                         | Front; Leader_N | 53                 | 11624             | 2254.3 | 2627.<br>8 | 24.3  | 1348.2 |                     |
|                         | Mid; Leader_N   | 53                 | 15316             | 2341.3 | 2073.<br>3 | 16.7  | 1826.9 | 3E-84               |
|                         | Front; All      | 119                | 27064             | 2135.1 | 2574.<br>7 | 15.6  | 1291.8 |                     |
|                         | Mid; All        | 119                | 2972<br>9         | 2252.8 | 2133.<br>4 | 12.4  | 1680.9 | 7E-153              |

| Parameters                   | Condition          | N <sub>cells</sub> | n <sub>FBRs</sub> | Mean  | SD    | SEM   | Median | p-values (wrt Front) |
|------------------------------|--------------------|--------------------|-------------------|-------|-------|-------|--------|----------------------|
| $\gamma$ (N/m <sup>3</sup> ) | Front;<br>Leader_S | 66                 | 1544<br>0         | 0.9E9 | 1.2E9 | 1E7   | 0.5E9  |                      |
|                              | Mid;<br>Leader_S   | 66                 | 1441<br>3         | 1E9   | 1.4E9 | 1.2E7 | 0.5E9  | 9E-4                 |
|                              | Front;<br>Leader_N | 53                 | 1162<br>4         | 0.9E9 | 1.1E9 | 1E7   | 0.5E9  |                      |
|                              | Mid;<br>Leader_N   | 53                 | 1531<br>6         | 1E9   | 1.4E9 | 1.2E7 | 0.5E9  | 8E-27                |
|                              | Front; All         | 119                | 2706<br>4         | 0.9E9 | 1.2E9 | 0.7E7 | 0.4E9  |                      |
|                              | Mid; All           | 119                | 2972<br>9         | 1E9   | 1.4E9 | 0.8E7 | 0.5E9  | 9E-26                |



**Movie 1.** A representative movie of collective cell migration of cells emerging out of a fish scale. Phase-contrast images were acquired every 10 mins.



**Movie 2.** A representative movie of membrane fluctuations of a leader cell captured by IRM at 50 frames per sec acquisition rate.