Supplementary materials

Supplementary figures

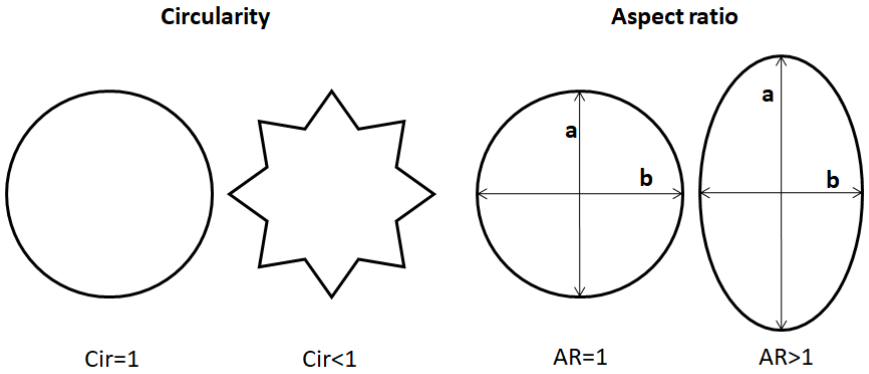


Fig S1. Shape descriptors to characterize morphological changes. Circularity (4π (Area/Perimeter^2)) with a value of 1 represent a perfect circle and lower values indicate formation of protrusions and cell polarization. Aspect ratio is the ratio of the length of the major axis (a) to the minor axis (b) of the cell and a high aspect ratio indicate an elongated morphology.

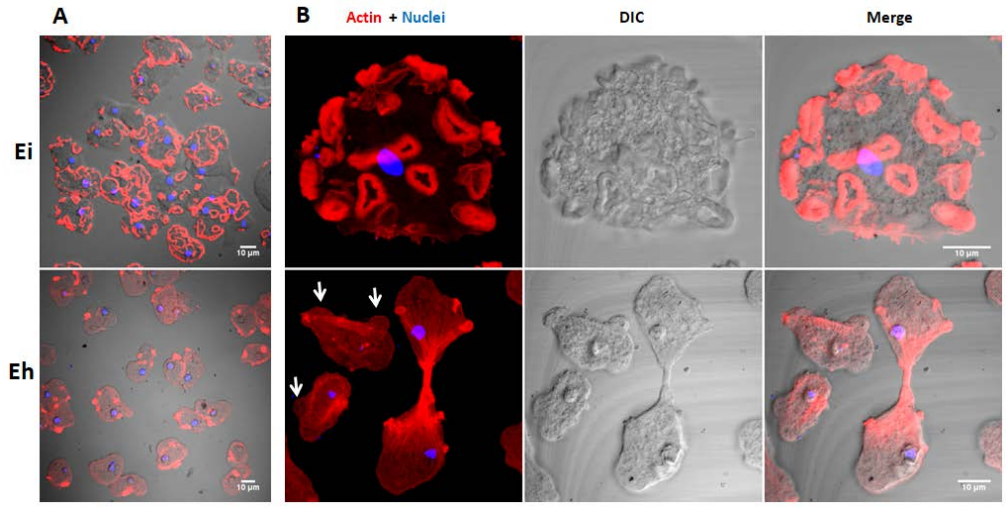


Fig S2. Cellular localization of F-actin in unpolarized trophozoites of *E. invadens* and *E. histolytica*. **(A)** In unpolarized trophozoites, F-actin is found in phagocytic and pinocytic invaginations on cell surface and in stress fibers at the ventral side of the cells. **(B)** *E. invadens* trophozoites contained more phagocytic and pinocytic invaginations and *E. histolytica* showed more blebs (arrow) on the cell surface. Scale bar: 10 µm.

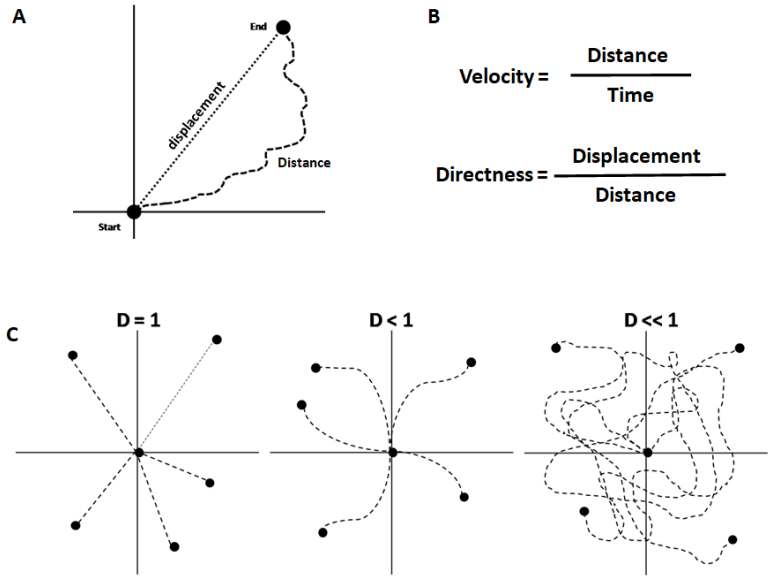


Fig S3. Parameters for analyzing 2D motility. (A) For each cell, 2D trajectory plot was generated by tracking its position using time-lapse microscopy. The displacement of the cell was calculated from the start point and end point, and the distance was calculated from the position of cell in each image. **(B)** Velocity of the cell is calculated as the distance divided by time. The directness (D) of cell motility is the ratio displacement to the distance. **(C)** The directness is a measurement of the persistence of migration. For a cell moving in straight line, the directness is near to one. As the cells deviate from straight line, the directness decreases, and for random motility it is much less than one.

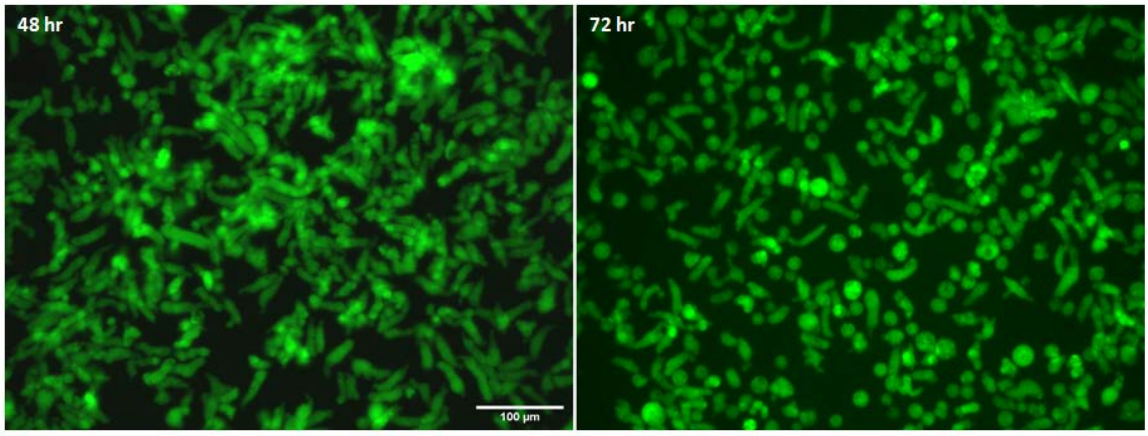


Fig S4. Ptx and cell viability. Fluorescein diacetate (FDA) hydrolysis assay showed that the *Entamoeba* cells treated with 10 mM Ptx for 48 and 72 hours produced and retained Fluorescein indicating Ptx does not cause cell death. Scale bars: 100 µm.

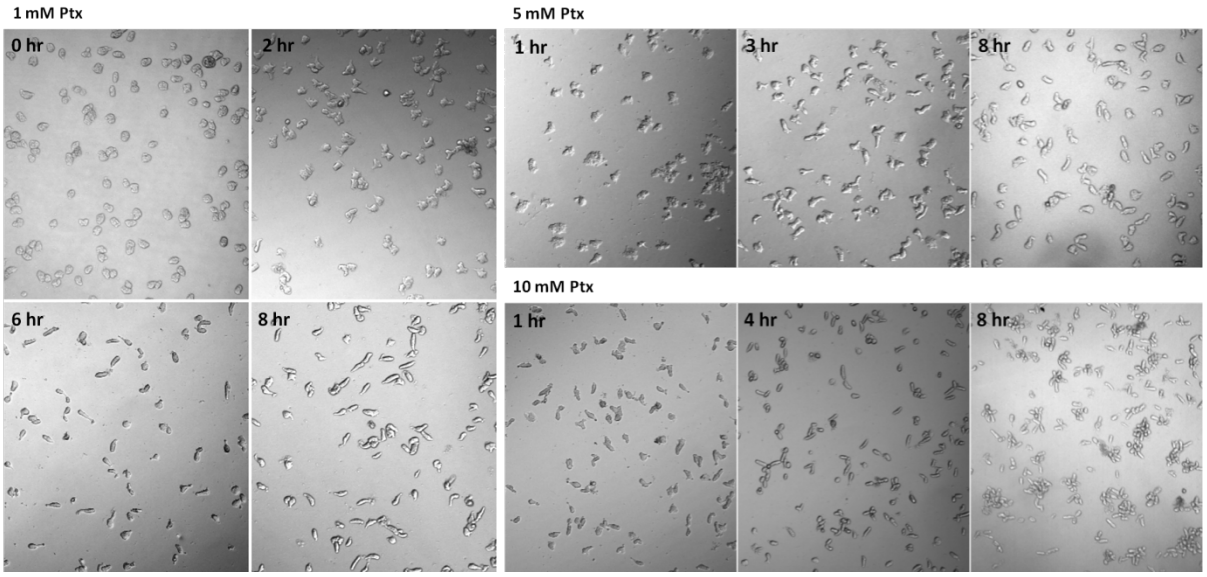


Fig S5. Morphological changes in *E. invadens* after treatment with different Ptx concentration (1, 5, 10 mM). The cell polarization in *Ei* depended on the Ptx concentration. At lower Ptx concentrations (0.5-1 mM), the cell polarization took nearly 6-8 hours, but with higher Ptx concentrations (5-10 mM), the polarization was faster.

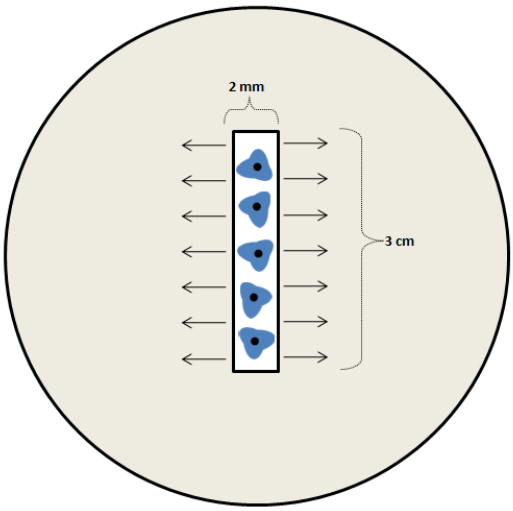


Fig S6. Schematics of the under-agar assay to study *Entamoeba* motility in confinement. 0.7% agarose was dissolved in TYI without glucose (LG100) and poured into Petri dishes and allowed to solidify. A single trough was made in the middle of the agarose plate. Cells were deposited in the trough and it was covered with a coverslip. The cells spontaneously exit from both side of the trough and move under the agarose. To induce cell polarization, Ptx was added to the agarose prior to pouring into the plate. The cell movement under the agarose was then recorded using video microscopy.

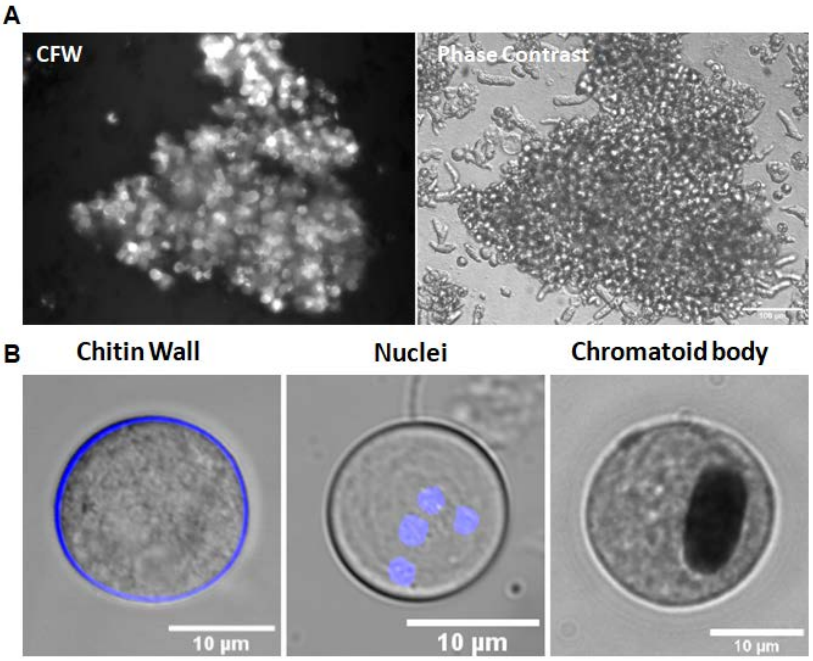


Fig S7. Ptx-induced cell aggregates contained mature cysts. (A) Cell aggregates formed in response to 1 mM Ptx in encystation media. The cysts formed in these aggregates are shown by calcofluor white staining. Scale bar: 100 µm. **(B)** These cysts showed the three features of a mature *Entamoeba* cyst; chitin wall, four nuclei, and chromatoid body shown by staining with calcofluor white, DAPI and toluidine blue respectively. Scale bar: 10 µm.

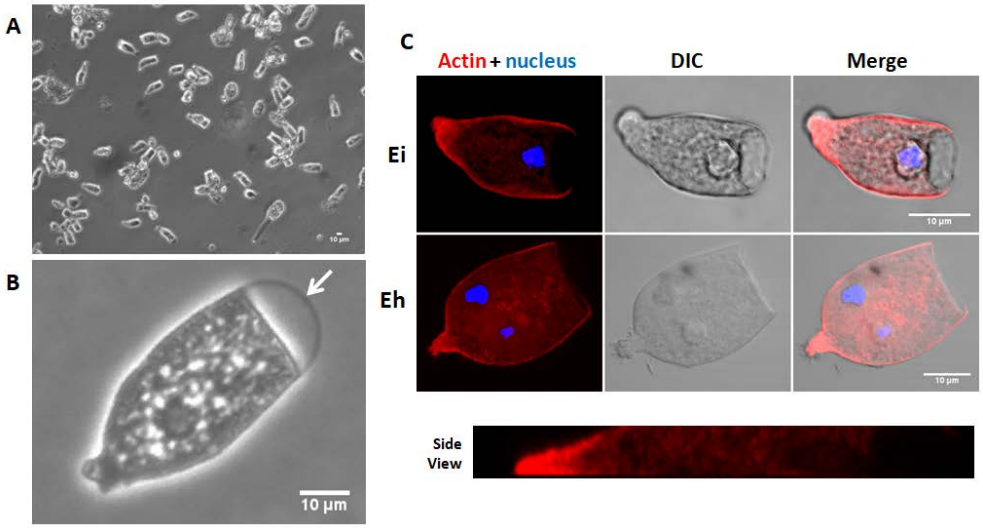
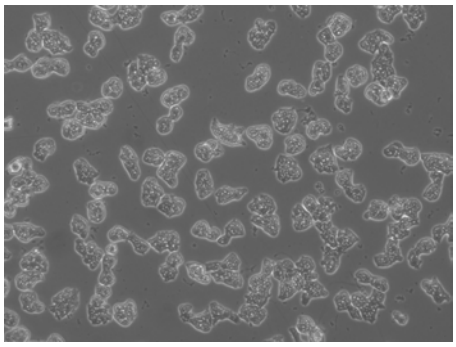
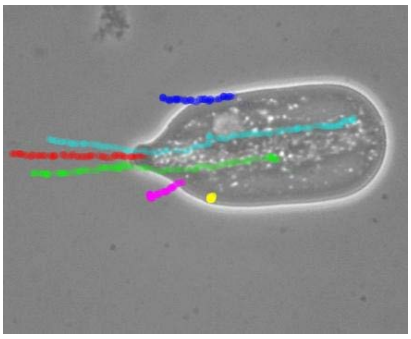


Fig S8. Polarized morphology with hyaline cap. (A, B) When exposed to continuous stress, *Entamoeba* formed an amoeboid form with a fluid filled hyaline cap at the leading edge. **(C)** Cell shape and F-actin distribution of these cells were similar to that of stable bleb cells. Scale bar: 10 µm.

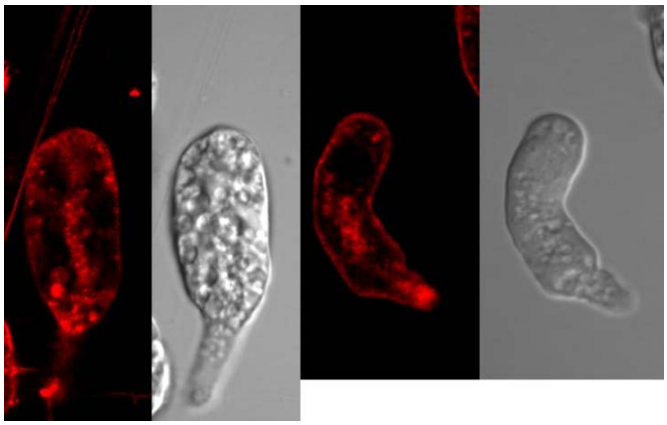
Supplementary Movies



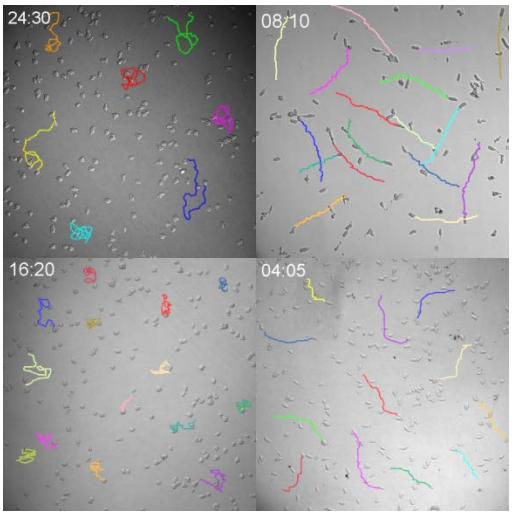
Supplementary Movie 1. Morphological and motility changes in *E. invadens* after Pentoxifylline treatment. Pentoxifylline was added at time 0 and the cell culture was immediately placed under a microscope and imaging was started. Time is shown in hours.



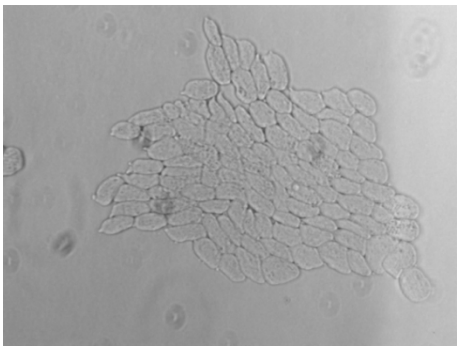
Supplementary Movie 2. The cytoplasm of polarized *Entamoeba* was divided into a fluid endoplasm and a gelled ectoplasm as shown by the speed of cytoplasmic particles (coloured tracks) compared to the cell track (red).



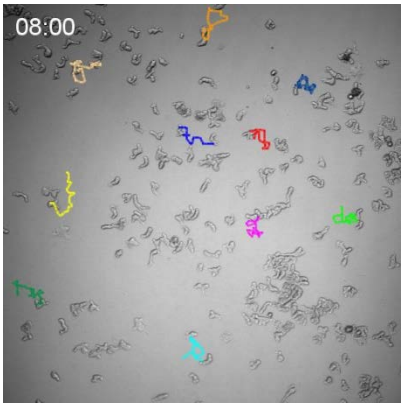
Supplementary Movie 3. Staining the polarized *Entamoeba* with membrane stain CellMask Orange, showed the transport of membrane from the uroid to the leading edge through the endoplasm as a continuous posterior to anterior flow or clumps of fluorescing vesicles.



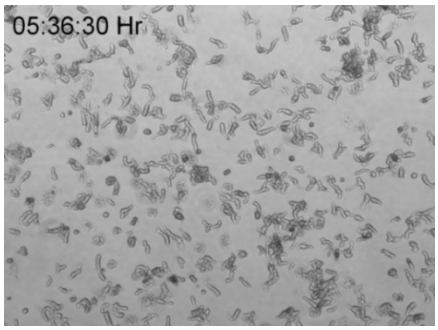
Supplementary Movie 4. Motility pattern of unpolarized and polarized cells of *Entamoeba* shown by cell motility tracks. Time is shown in minutes.



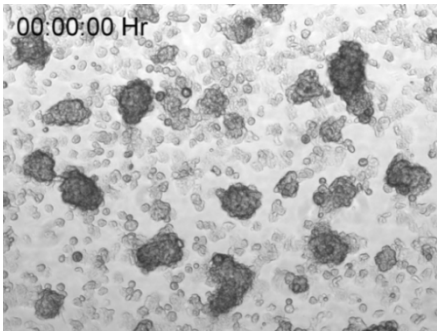
Supplementary Movie 5. The Ptx induced polarized cells of *E. invadens* moved collectively in a head to tail alignment under the agarose.



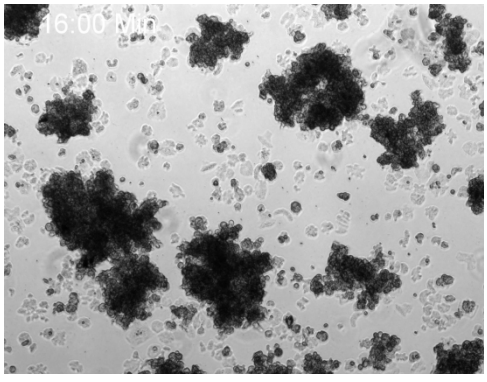
Supplementary Movie 6. Motility pattern of adenosine treated cells of *E. invadens* shown by cell tracks. After adding 0.1mM adenosine, the cell culture was immediately placed under a microscope and imaging was started. Time is shown in minutes.



Supplementary Movie 7. Cell polarization in response to encystation media. Growth medium was replaced with encystation medium and the culture was immediately placed under a microscope and the recording of the cell response was started at time 0. Time is shown in hours.



Supplementary Movie 8. Migration of the polarized morphology towards smaller aggregates in the encystation media. Growth medium was replaced with encystation medium and the culture was placed immediately under a microscope and the recording of the cell response was started at time 0. Time is shown in hours.



Supplementary Movie 9. ATP addition caused dispersion of cell aggregates in confluent growth medium and in encystation medium. ATP (0.5 mM) was added at time 0 and the cell culture was immediately placed under a microscope and imaging was started. Time is shown in minutes.