## **Supplementary Information**

**Text S1.** The reversibility of the Stokes flow means that it has the same shear stress profile for either forward or reverse flow. The well-known general governing equation of fluid flow is the Navier-Stokes equation as follows

$$(\rho (\partial v / \partial t) + v \cdot \nabla v) = -\nabla P + \eta \nabla 2v + f$$
(1)

where  $\rho$  is density, v is velocity, t is time, P is pressure, and f is body force). When the Reynolds number is Re  $\ll$  1 (creeping flow rate), the inertia term of equation (1) should be ignored and the Navier-Stokes equation is modified to the following Stokes equation.

$$\rho(\partial \mathbf{v}/\partial \mathbf{t}) = -\nabla \mathbf{P} + \eta \nabla 2\mathbf{v} + \mathbf{f}$$
<sup>(2)</sup>

Note that Re represents the ratio of the inertial forces to the viscous forces. Re is approximately  $1 \times 10^{-3}$  in our system and thus corresponds to creeping flow. The same shear stress trend for both forward and reverse flow is shown in Fig. S4.



**Figure S1.** Osmosis-driven pump performance test. Osmosis-driven flow rate by osmotic pressure between DI water and PEG solution was tested by controlling the molar concentration of the PEG solution. This flow rate test was performed using our shear stress gradient chip. A 0.5 M PEG solution was selected to achieve the appropriate flow rate (30  $\mu$ L / h) (n = 5).



**Figure S2**. Single-cell simulation grid test. Grid test was performed to verify the grid independence and accuracy of the simulation, using two mesh numbers (2,000,000 and 1,460,000 grid elements). Shear stress levels were plotted to compare the shear stress magnitudes of the two meshes, and the difference was less than 0.05%. Note that the flow was in the x direction. Inset shows the line at which the shear stress is measured.



**Figure S3**. Single-cell simulation. a) 3D view of shear stress distribution on the single cell surface. b) Flow velocity of the microchannel (flow in +x direction). Scale bars are 50  $\mu$ m.



**Figure S4**. Shear stress distribution according to flow direction; a) x-direction and b) reverse x-direction. Since the flow regime is in extremely low Re (<<1), the reversible Stokes equation governs the flow characteristics, which implies that opposite flow directions give the same shear stress results. Different shear stress on each pseudopodium was calculated when the cell was exposed to flow in the reverse x-direction (see the arrow); the shear stress on the vertical pseudopodium (①) was the highest, and the shear stress on the horizontal pseudopodium (②) was the lowest.



**Figure S5**. Pseudopodia angle and length distribution in three regions; a) high, b) medium, and c) low shear stress regions. The plot radius is the pseudopodia length, and the plot angle is the pseudopodia angle from the reference line (parallel to the flow direction, see Fig. 5d).



**Figure S6**. Red color intensity regression curves of 18 samples (each of 6 plots includes 3 sample data). Red color intensity data of each pixel (point) were dotted in the graph with regression lines. Average slope of regression curves is  $0.0046 \pm 0.0034$ .

Movie 1. Channel flow visualization. Fresh nutrient (DMEM) was changed every 30 min with 30  $\mu$ L/h flow rate by osmotic pump.