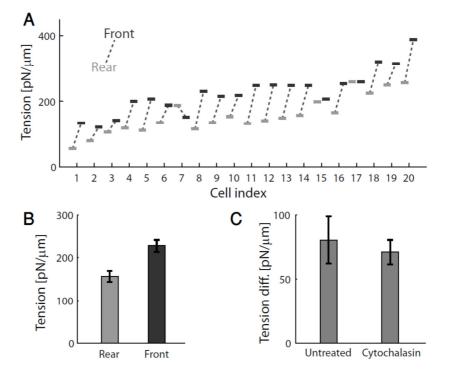
# **Supplementary Materials**

## Front-to-rear membrane tension gradient in rapidly moving cells

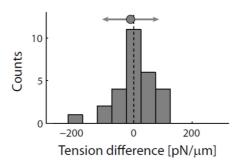
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### **Supplementary Figures**



**Figure S1.** *Membrane tension difference in Cytochalasin-treated cells.* (A) Front (dark) and rear (light) tension measurements for different Cytochalasin-treated cells. (B) Bar plot showing the population averaged membrane tension values at the front and rear for Cytochalasin-treated cells (mean±SEM). The tension values in Cytochalasin-treated cells are significantly lower than in untreated cells (Fig. 1). (C) Bar plot showing the population average front-to-rear tension difference in untreated cells and Cytochalasin-treated cells. The average front-to-rear tension difference of 71±10 pN/ $\mu$ m (mean±SEM) in Cytochalasin-treated cells, is not significantly different from the average in untreated cells 85±19 pN/ $\mu$ m. Cytochalasin treatment slows down cell movement and influences the lamellipodial actin network structure (3) and the density of pushing filaments at the leading edge (20). Cytochalasin treatment may also change the density of cytoskeletal-attached adhesions and anchors in the membrane; however detailed information

regarding the direction and magnitude of these changes is not available. According to our theoretical model (9), the front-to-rear tension difference will depend on model parameters such as the anchor density, the adhesion density and the density of pushing filaments in a non-trivial manner (9). However, since we cannot characterize how Cytochalasin treatment influences these model parameters, notably the anchor density and the adhesion density, we are unable to use this perturbation to test our model predictions, even in a qualitative way.



**Figure S2. Sequential membrane tension measurements at the same position**. A histogram of the tension difference between the first and second measurement in control experiments in which sequential membrane tension measurements were performed at the same position are shown. The mean and standard deviation are indicated above the histogram. The average tension difference between sequential measurements is not significantly different from zero in the control experiments.

## **Supplementary Movies**

#### Movie 1. Tether pulling at the leading edge of a cell.

This movie shows bright-field images of a keratocyte during a tether pulling experiment from the leading edge. An image of the whole cell (top) is shown together with a zoomed view of the bead (inset). The location of the center of the laser trap is indicated (red cross). The presence of a tether, which is invisible by bright-field, is evident from the displacement of the bead from the trap center. The field of view is 51  $\mu$ m wide, and the movie is played at 4× real time.

### Movie 2. Tether pulling from the cell body at the rear.

This movie shows bright-field images of the same keratocyte shown in Movie 1 during a tether pulling experiment from the rear of the cell body. An image of the whole cell (top) is shown together with a zoomed view of the bead (inset). The location of the center of the laser trap is indicated (red cross). The tether force displaces the bead from the trap center. The field of view is 51  $\mu$ m wide, and the movie is played at 4× real time.

### Movie 3. Measurements of front-to-rear membrane tension difference in fragments.

This movie shows bright-field images of a keratocyte fragment during a tether pulling experiment (top), together with a zoomed view of the bead (inset). The location of the center of the laser trap is indicated (red cross). A membrane tether is first pulled from the leading edge of the fragment. By moving the stage, the same bead is then brought to contact with the fragment rear. A tether is formed at the rear on the third attempt. The field of view is 45  $\mu$ m wide, and the movie is played at 4× real time.