**Response to reviewers**

Referee #1:

Comments to the Author

The authors have significantly improved their report, but there remain a few minor issues that need to be addressed before publication.

Response: We want to thank the reviewer for providing another very detailed report which has helped us to further improve our paper. Changes have been made throughout the manuscript and the Supplementary Material (highlighted in yellow) according to the constructive comments from the referee.

I agree with Referee 3's earlier concern about the discussion of the theory, and I think it is not completely fixed in this draft. In particular, I think the authors should more clearly state \*in the main paper\* that all the parameters are taken from Ref. 15 (I believe) except the cell length, alpha, and the friction. I believe there are two fitting parameters here: the proportionality between the measured AQP expression and alpha, and the proportionality between the friction and the measured adhesion energy. These need to be stated in more detail. (For instance, how much does the alpha/friction put here compare to those of earlier papers, e.g. Ref. 15?)

Response: Following the reviewer’s suggestion, more detailed discussion on the fitting

parameters have been added in the main text (page 6) as

“*To make a connection to our actual tests, the values of , and L were varied according to different experimental conditions. Specifically, was assumed to be proportional to the measured adhesion strength between the cell and the coated channel surface (Fig. 2C). Furthermore, since the AQP concentration and cell length could be different in each cell line and under different treatment conditions, the values of and L were also varied according to the measured AQP concentration and cell size in each case respectively (see Supplementary Materials – I for more details). Notice that, as a reference, and for A549 cells under BSA coating were taken to be 6.5 Pa. s/m and m Pa-1s-1 , respectively, which are comparable to those ( 8 Pa. s/m and mPa-1 s-1) adopted in [15] for sarcoma cancer S180 cells confined in micro-channels without coating of adhesion proteins.”*

The authors should explain in more detail why, in Fig. 5, the theory is just linearly proportional to AQP concentration, since the expression has a complex dependence on alpha, with alpha in both the numerator and denominator. Can the theory be simplified in the parameter region studied?

Response: We want to point out that, in addition to AQP concentration, the cell length

also varies a lot across different cell types (refer to Table S2). Therefore, *both L and*

*will change* in Eq. (1) eventually leading to an approximate linear relationship between

the AQP concentration and the cell velocity. The following sentences have been

added on page 6 of the revised manuscript to clearly point this out, following the

reviewer’s suggestion

*“In addition,* *the traveling velocities of different cells calculated* *from their measured length and AQP4 concentration (i.e. different L and values as shown in Table S2 and Table S3), are also in agreement with our experiments (Fig. 5).* *Specifically, the shaded region in Fig. 5 represents the range of the predicted cell speed when the cell length is allowed to vary by ±30% from the measured mean value (Table S2). Note that, since all results shown in Fig. 5 were based on collagen I coated channels was fixed as 36 Pa.s/m (Table S4) in this case.”*

Some figures have illegibly small labels (Fig. 2C and Fig S6 in particular); Fig 1C's axes lines are so faint it's hard to understand when printed.

Response: The axis title and label in Fig. 1C, Fig. 2C and Fig. S6 have all been enlarged

to make them clearer.

Eqn. S3 is missing a T, I believe, in a term LRT. The authors should double-check the equations are consistent between main paper, supplement, and the original work.

Response: We want to thank the referee for bringing this to our attention. The error has

been corrected and all equations have been double-checked.

Fig 3B and 2C are not actually beeswarm plots! These are violin plots, which plot a smoothed histogram. A beeswarm plot (or univariate scatter plot) plots all of the experimental data (http://www.cbs.dtu.dk/~eklund/beeswarm/). I would recommend the beeswarm plot here, because for small amounts of data presented here, the violin plots can also be deceptive.

Response: Following the reviewer’s advice, Fig. 2C and 3B have been replaced

with beeswarm plots using the R-script provided in

<http://www.cbs.dtu.dk/~eklund/beeswarm/>. See the revised Fig. 2C appended below

for example

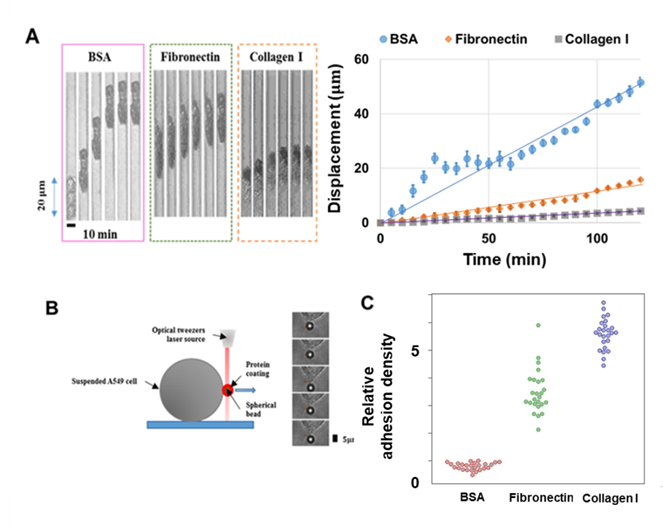


Figure 2C

Fig 5. would be clearer if the data points were plotted in a more highly contrasting color, and if the data were placed "in front of" the theory line and shaded region.

Response: Higher contrasting color has been used in the revised Fig. 5 to make the data

points more clear.

p.4, typo "reduced their sped"

Response: The typo has been corrected.