Supporting Information

Force Spectroscopy on a Cell Drum: AFM Measurements on the Basolateral side of Cells via Inverted Cell Cultures

Joo Hyoung Kim,^a Kristina Riehemann^{a, *} and Harald Fuchs,^a

^{a.} Physikalisches Institut, WWU Münster, D-48149 Münster, Germany and Center for Nanotechnology (CeNTech)

Corresponding Author

*K. Riehemann@uni-muenster.de



Figure S1. Scheme of the individual steps of sample preparation for basolateral measurement. 1. Bonding of substrate on stainless steel plate; 2. Covering the net with poly-L-lysine; 3. Growth of cells; 4. Prepared sample; illustration bottom left: clarification of the $0^{\circ}/180^{\circ}$ net rotation (top: visualization of sample during the cell growth; below: orientation of the sample during the measurement).



Figure S2. Light microscopy images of cancer cells growing on the bottom side of the grid marked in red (left: PaTu8988S; right: PaTu8988T). The black shadow shows the cantilever above the grid which is out of focus.



Figure S3. Force-displacement curves recorded within one cycle on a cell attached under a mesh (left curve, positions 2-3) and on the bar of the grid (right curve, position 1). The sketch below indicates the corresponding positions 1-3 of the tip in the F-D curves (in green: cell; in black: mesh):

1. F–D curve recorded on a bar. As expected, the curve shows the characteristics of a hard sample (deflection d=z).

2. This position correlates the second recorded F–D curve on a mesh. After an additional tip movement of approximately 5 μ m, the tip touches the cell surface.

3. A further approach leads to a bent shape in the curve (deflection $d=z-\delta$).

Elastic Modulus 1.61 kPa 1.0nt Elastic Modulus 2.03 kPa 400pN 0.8 300 0.6 200 Force e 0.4 · 0.2 0.0 -0.2 0.4 0.5 1.0µm 0.5 1.0 -0.5 -0.5 -1.0 -1.0µm 0.0 Ind Elastic Modulus 1.26 kPa Elastic Modulus 1.35 kPa 600p 400 Force 800 200

-1.0

-0.5

0.0

0.5

1.0µm

(a) Apical measurements with PaTu8988S on coverslips



600

-200

400

200

Ind

400



(c) Basolateral measurements with PaTu8988S beneath the grids



S-5



(d) Basolateral measurements with PaTu8988T beneath the grids



(e) Apical measurements with PaTu8988S on the grids





(f) Apical measurements with PaTu8988T on the grids









S-7



(i) Apical measurements with PaTu8988S after cytochalasin D treatment on coverslips



(j) Apical measurements with PaTu8988T after cytochalasin D treatment on coverslips



Figure S4. Representative raw AFM data traces as well as their fits. Apical measurements with PaTu8988S and PaTu8988T cells on coverslips ((a) and (b) respectively), basolateral

measurements with PaTu8988S and PaTu8988T cells beneath grids ((c) and (d)), apical measurements with PaTu8988S and PaTu8988T cells on grids ((e) and (f)), apical measurements after treatment of cytochalasin D with PaTu8988S and PaTu8988T cells on grids ((g) and (h)), finally apical measurements after treatment of cytochalasin D with PaTu8988S and PaTu8988T cells on coverslips ((i) and (j)) as comparison.

Table S1. Average of the E-moduli of apical measurements on various substrates with and

 without treatment of cytochalasin D.

Substrate type	Cell type	Value [Pa]	Error [Pa]	relative Error [%]
On grid	PaTu8988S	1170	335	29
(w/o cytochalasin D)	PaTu8988T	731	150	21
On coverslip	PaTu8988S	634	211	33
(w/ cytochalasin D)	PaTu8988T	558	180	32
On grid	PaTu8988S	422	204	48
(w/ cytochalasin D)	PaTu8988T	370	182	49