## Supporting Information

# Magnetic domain wall tweezers: a new tool for mechanobiology studies on individual target cells

M. Monticelli, D. V. Conca, E. Albisetti, A. Torti, P. P. Sharma, G. Kidyoor, S. Barozzi, D. Parazzoli, P. Ciarletta, M. Lupi, D. Petti and R. Bertacco

#### 1. Mathematical model of lateral indentation of the cell membrane

An elastic model of the cellular membrane is developed to calculate the mechanical stimulus induced on the cell membrane by the magnetic domain wall tweezers. Let us now consider the mathematical problem of determining the cell deformation under the action of a distributed load imposed by the cluster of beads. Since the observed overall displacement in experiments is small compared to the cell diameter, the external forces are mainly counterbalanced by the bending of the cell membrane, which is considered as a linear elastic shell of thickness *h*, with Young modulus *E* and Poisson ratio *v*. Accordingly, the cell membrane has a bending stiffness  $K_b = \frac{Eh^3}{12(1-v)}$  and a stretching stiffness Ks = Eh. This assumption is valid if we consider a range of applied forces much bigger that the rupture load of a focal adhesion with the substrate, which is in the order of 30 *pN* [M. Sun, J. Graham, B. Heged, F. Marga, Y. Zhang, G. Forgacs, and M. Grandbois. *biophysical journal*, 89(6):4320:4329, 2005.].

Considering a Cartesian coordinate system (x, y) for describing the material position of the doubly-curved cell membrane, its local principal radii of curvature are indicated by  $\rho_{\alpha} = \rho_{\alpha}(x, y)$  with  $\alpha = (x, y)$ . Applying an indentation distributed pressure p = p(x; y) in the *z*-direction (see Figure S1), the linearized equilibrium equations using the theory of thin shallow shells read [E. Ventsel and T. Krauthammer. *CRC press*, 2001.]:

$$K_b \nabla^4 \omega + \nabla_g^2 \phi = p \tag{S1}$$

$$K_S^{-1} \nabla^4 \phi - \nabla_g^2 \omega = 0 \tag{S2}$$

where  $\nabla^2$  is the Laplacian operator,  $\nabla_g^2 = \frac{(.)_{xx}}{\rho_x} + \frac{(.)_{yy}}{\rho_y}$  is the Vlasov operator, comma denotes partial derivative,  $\omega = \omega(x, y)$  is the vertical shell displacement, and  $\phi = \phi(x, y)$  is the Airy stress function, whose derivatives yield the planar stresses. Eqs.(1,2) are valid within the limit  $\varepsilon = \frac{h}{R} \ll 1$ , with  $R = min[\rho_x; \rho_y]$ , and represent a system of partial differential equations whose solution is strongly affected by the shell geometry. Such a geometric dependence can be highlighted by considering the dimensionless variables,  $\overline{\omega} = \frac{\omega K_b}{P R^2}$  and  $\overline{\phi} = \frac{\phi K_b}{P R^3}$  where *P* is the characteristic intensity of the force exerted by the beads. Accordingly, combing the Equation S1 and Equation S2, we get:

$$\varepsilon^2 \nabla^8 \omega + \nabla_g^4 \omega = \varepsilon^2 \nabla^4 p \tag{S3}$$

where we considered dimensionless operators over the coordinates  $\bar{x} = x/R$  and  $\bar{y} = y/R$ , dropping the bars for the sake of simplicity. Eq.(3) is a singularly perturbed linear partial differential equation which is valid far enough from the indenting region. Since  $\epsilon$  is a very small parameter, the leading order solutions for the far-field displacements depends primarily on the Gaussian curvature of the cell surface [A. Vaziri and L. Mahadevan. *Proceedings of the National Academy of Sciences*, 105(23):7913,7918, 2008.].

The complete solution could be derived by imposing the matching between this far-field solution and the one found with asymptotic expansion around the indentation area. Nonetheless, since the cell membrane is a doubly curved surface with positive Gaussian curvature everywhere, Equation S3 is governed by the elliptic operator  $\nabla_g^2$  at the leading order, that is the corresponding solution is concentrated around the indentation area and decays quickly away from it. In such a case, the shell easily buckles, eventually reaching a partly inverted shape around the indentation area, as well known from classical studies on spherical caps [D. Vella, A. Ajdari, A. Vaziri, and A. Boudaoud. *Physical review letters*, 109(14):144302, 2012.]. Therefore, the large deflections (i.e. with respect to *h*, being of the order *R*) of the cell membrane under the indentation pressure exerted by the beads' cluster will be calculated by searching for an elastic solution within the constraint of isometric transformations. The shape of the cell membrane in proximity of the first contact point *C* with the cluster can be approximated by the osculating ellipsoidal paraboloid, having the expression:

$$z = \frac{x^2}{2R_x} + \frac{y^2}{2R_y}$$
(S4)

where the (x,y) plane is tangent to *C* having directions coinciding with the principal direction of the surface, locally characterized by curvature radii *Rx* and *Ry*. If  $2\delta$  is the large indentation provoked by the cluster, we assume that the deformed shape of the cell membrane will be given by reversing the shape of the paraboloid with respect to the plane at  $z = \delta$ , producing a mirror-buckling with a boundary ellipse having semi-axes  $s_x = \sqrt{2\delta R_x}$  and  $s_y = \sqrt{2\delta R_y}$ . Let us now calculate the resulting indentation value using a variational approach. After lengthy manipulations, the total deformation energy *U* of the shell in such a buckled configuration results:

$$U = \frac{c\pi E}{12^{3/4}(1-\nu^2)} h^{5/2} (2\delta)^{3/2} \left(\frac{1}{R_x} + \frac{1}{R_y}\right)$$
(S5)

where c = 1.15 is a constant obtained by minimizing a certain displacement functional under a nonholonomic constraint [A. Pogorelov. *American Mathematical Soc.*, 72, 1988.]. The work *L* of external forces is instead given by:

$$L = 2\delta F_C \tag{S6}$$

for a concentrated force  $F_c$ , or:

$$L = 2\pi \sqrt{R_x R_y} P(\delta_P^2 + 2(h - \delta_P)\delta_P)$$
(S7)

for a pressure *P* distributed over  $z \le h_p$ , with an elliptic boundary having semi-axes  $a = \sqrt{2\delta_P R_x}$  and  $b = \sqrt{2h_p R_y}$ , corresponding to a distributed indentation force  $F_d = 2\delta_P P \sqrt{R_x R_y}$ . Accordingly, the analytic relation between load and indentation can be found by minimizing the elastic functional

Accordingly, the analytic relation between load and indentation can be found by minimizing the elastic functional W = U - L, being:

$$F_{c} = \frac{3c\pi E}{12^{3/4}2(1-\nu^{2})}h^{5/2}(2\delta)^{1/2}\left(\frac{1}{R_{x}} + \frac{1}{R_{y}}\right)$$
(S8)

$$F_{d} = \frac{3c\pi E}{12^{3/4}(1-\nu^{2})}h^{5/2}\frac{(2\delta)^{1/2}}{2(2-\beta)}\left(\frac{1}{R_{x}} + \frac{1}{R_{y}}\right)$$
(S9)

where  $\beta = \delta_p / \delta < 1$  indicates the portion of the free boundary where the pressure is applied.

The latter equation is exploited to estimate the mechanical stimulus exerted on target cells, using the parameters carried out by the experiments and from the referenced works, as reported in the main text.



Figure S1: Sketch of the analytical surface used to model the cell membrane geometry, in proximity to the position where the mechanical load is applied, producing an indentation of  $2\delta$ .

#### 2. Measurement of beads magnetic susceptibility via Vibrating Sample Magnetometer (VSM)

As discussed in the main text, the magnetic susceptibility of the superparamagnetic particles used during the experiments (MyOne-Dynabeads, 1 µm diameter) is modeled according to the Langevin function (see Equation 2), to properly calculate the point-by-point magnetization on the bead volume.

The parameters  $\chi_0$  (linear susceptibility) and  $M_s$  (Magnetization of saturation) in Equation 2 are experimentally determined by a Vibrating Sample Magnetometer (VSM) measurement of the beads magnetic moment, as a function of the applied magnetic field. The measurement is performed on beads in suspension. The total magnetic moment is normalized to the number of beads and divided by the single bead volume, obtaining the magnetization of a single particle, displayed in Figure S2. The experimental data are then fitted with the Langevin function (red line in Figure S2); from this fitting procedure  $\chi_0 = 1.46 \pm 0.02$  and  $M_s = 34.9 \pm 0.2$  KA/m is obtained. The uncertainty is due to the fitting standard error.



Figure S2: Magnetization (M) of a  $1\mu m$  MyOne Dynabead as function of the applied magnetic field (H<sub>A</sub>) measured (black line) by VSM. The experimental data are fitted with a Langevin function (red line, see Equation 2 in the main text).

#### 3. Forces on a magnetic bead in solution

A magnetic bead suspended in a liquid in proximity to magnetic ring-shaped conduits experiments several forces, as shown in Figure S3: the magnetic force ( $\mathbf{F}_{M}$ ), the viscous friction ( $\mathbf{F}_{D}$ ), gravity ( $\mathbf{F}_{G}$ ) and the buoyancy force ( $\mathbf{F}_{B}$ ). The expression for the magnetic force ( $\mathbf{F}_{M}$ ) is reported in Equation 1.

As a matter of fact,  $\mathbf{F}_{\mathbf{M}}$  is the leading contribution and all the other forces can be neglected when one considers the stimulus applied on target cells during the experiments.

In order to confirm this assumption, we calculate the entity of such forces. The viscous friction exerted on a bead by the surrounding fluid is described by the Stokes equation [M. Monticelli, E. Albisetti, D. Petti, D. Conca, M. Falcone, P. Sharma and R. Bertacco, *J. Appl. Phys.*, 117, 17B317, 2015.]:

$$\mathbf{F}_D = 6\pi\eta R_B \mathbf{v} \tag{S12}$$

where v is the bead velocity with respect to the fluid,  $\eta$  the medium viscosity and  $R_B$  the bead radius. Considering a 1µm bead manipulated in PBS ( $\eta \approx 10^{-3} \text{ Pa} \cdot \text{s}$ ) with a typical velocity of 10 µm/s,  $\mathbf{F_D} = 86$  fN, which is negligible compared to  $\mathbf{F_M}$  which is on the order of hundreds of pN (see Figure 2).

Along the vertical direction, the total force due to the gravity and the buoyancy force is given by:

$$\mathbf{F}_{B} + \mathbf{F}_{G} = V_{b}(\rho_{b} - \rho_{f})\mathbf{g} = m_{eff}\mathbf{g}$$
(S13)

where  $V_b$  is the bead volume,  $\rho_b$  is the mass density of the bead,  $\rho_f$  is the mass density of the surrounding medium and  $m_{eff}$  is the effective mass of a body in a fluid. On a 1 µm MyOne dynabead ( $m_{eff} = 9.42 \times 10^{-16}$  kg), the force exerted by gravity and buoyancy force is equal to 9.25 fN, much smaller than **F**<sub>M</sub>. Moreover, a bead in a fluid is affected by the Brownian motion, due to the thermal energy of the surrounding media. Nevertheless, as discussed in the main text, also this contribution is negligible because the thermal energy is much lower than the magnetic potential energy well generated by DWTs



Figure S3: Simplified scheme of the four main forces acting on a magnetic bead suspended in a fluid

### 4. Magnetic beads manipulation to the cellular membrane of living cells

Domain Wall Tweezers (DWTs) technology was intensively tested to prove the effectiveness of beads manipulation in about 70 cells/mm<sup>2</sup> and were let grown in Dulbecco's modified Eagle's medium (DMEM)/F12 (Biowest) supplemented with 10% fetal bovine serum (FSB) (Lonza) and 2% L-glutamine (Biowest). Once attached to the chip, cells were placed under the microscope and magnetic beads were added to the colture medium.different cell culture environments and for different dimensions (radius and width) of the magnetic rings. In addition to the HeLa cells reported in the main text, mouse mammary tumor cells (4T1) were also tested, as illustrated in Figure S4 where 6 frames from a video (see SM1.avi) show the manipulation of a single 1 µm MyOne-Dynabead hitting the cellular membrane.

4T1 cells were plated onto the chip at a density of



Figure S4: Frames from a video showing the manipulation of 1  $\mu$ m bead to the cellular membrane of a target 4T1 cell. The ring-shaped magnetic conduits in Ni<sub>80</sub>Fe<sub>20</sub> are 300 nm wide, 30 nm thick, with a radius of 5  $\mu$ m.

### 5. Algorithm for tracking the beads centre

A particle tracking algorithm was developed to precisely determine the magnetic bead position from the acquired optical images.

In the brightfield images, beads appear as bright spots surrounded by a darker ring as can be clearly evinced by Figure S5a. The image was first inverted and the pixel values rescaled in the interval [0,1]. The contrast between the rings and the background is then enhanced by multiplying by 5 the pixel which value exceeds 0.9. This operation is in principle equivalent to a simple thresholding, but it leaves a faint background as reference. Finally, the image is convolved with an 11 x 11 pixels mask, resembling the beads airy disk. This matrix is generated by the algebraic sum of three Gaussian curves:

$$matrix(i,j) = 2.5exp\left(-\frac{(i-6)^2 + (j-6)^2}{2*5}\right) - 2exp\left(-\frac{(i-6)^2 + (j-6)^2}{2*2}\right) - exp\left(-\frac{(i-6)^2 + (j-6)^2}{2*10}\right)$$
(S14)

where the standard deviation of each Gaussian, as well as the multiplying factor, is manually tuned to reproduce bead airy dish shape. The image intensity is then once again rescaled to the interval [0,1] for clarity.

After the algorithm application, the centre of each bead appears as a bright red spot with a diameter of 1-2 pixels, as illustrated in Figure S5b.

However, the particles centre and, in general, its position can be determined by visual inspection with a precision close to a single pixel.

Note that, the particles centre and, in general, its position can be determined by visual inspection with a precision close to a single pixel. For a pixel size of 120 nm, which is the case of the images reported in Figure 3, the accuracy in the determination of the beads position from our algorithm is in perfect agreement with the one carried out by a direct inspection of the optical images.



Figure S5: **a** Optical microscope image of a ring shaped conduit where some magnetic beads are attracted. **b** Image elaborated by a centroid tracking algorithm which allows to determine the particles centre with a precision of 1-2 pixels.