Electronic Supplementary Material (ESI) for Nanoscale Horizons.


# Supporting Information

## Homotypic and heterotypic adhesion of cancer cells revealed by force-induced remnant magnetization spectroscopy

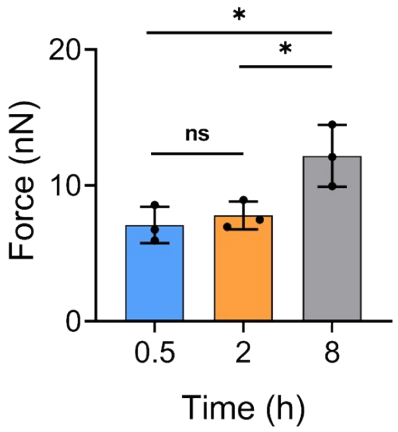


**Fig.S1** Adhesion force measured by FIRMS with various time.

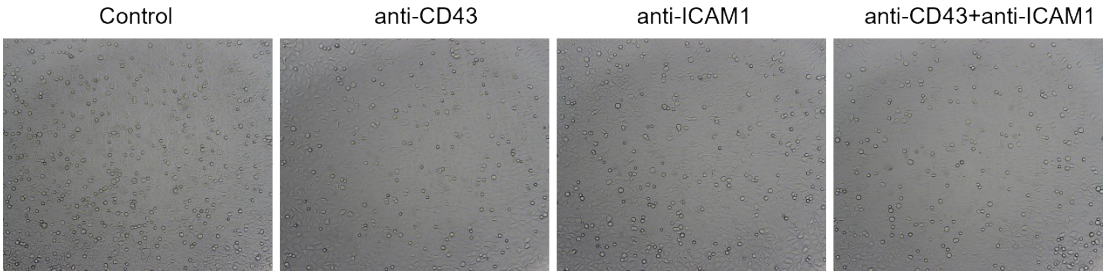


**Fig.S2** Optical microscope images of heterotypic adhesion of MDA-MB-231 cells adhering to ECs while blocking ICAM-1, CD43, and ICAM-1 + CD43.

## Experimental section

### Materials

Carboxyl-magnetic nanoparticles (MNPs) were purchased from Ocean Nano Tech (California, American). Polydimethylsiloxane (PDMS, Sylgard 184) was purchased from Dow Corning Co.Ltd. (3-Aminopropyl) triethoxysilane (APTES), 4',6-diamidino-2-phenylindole (DAPI), 50% glutaraldehyde, fibronectin (Fn), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (USA). Phosphate buffered saline (PBS) was purchased from HyClone/Thermo fisher (Beijing, China). Anti-ICAM-1 antibody was purchased from Beyotime (Beijing, China). Anti-CD43 antibody was purchased from abcam (Beijing, China). MDA-MB-231 cells were purchased from

National Infrastructure of Cell Line Resource (Beijing, China). MCF-7 and MCF-10A cells were purchased from BeNa Culture Collection (Beijing, China).

## Cell culture

MDA-MB-231 and MCF-7 cells were cultured in high glucose containing Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) at 37 °C in a humidified 5% CO2 atmosphere. MCF-10A cells were cultured in Mammary Epithelial Cell Medium (MepiCM), supplemented with 10% FBS, 1% Mammary Epithelial Cell Growth Supplement (MEpiCGS) and 1% P/S. HUVECs were cultured in Endothelial Cell Medium (ECM, **Sciencell**), supplemented with 5% FBS, 1% endothelial cell growth supplement (ECGS) and P/S. Cells were cultured for 24 h to allow cell adhesion prior to the experiments. Carboxyl-MNPs were sterilized prior to the addition to the culture medium by filtration with a sterile membrane filter of 0.22 μm pore diameter.

## Preparation and modification of substrates

Briefly, PDMS mixture was prepared by mixing the elastomer base and curing agent in a 10 : 1 ratio (w/w), followed by a 30 min degassing process under vacuum. Then, the mixture was poured into designed molds and cured at 65°C for 4 h. The solidified PDMS substrates were hydroxylated using a plasma cleaner for 25 s and then incubated with 2% APTES solution of ethanol for 4 h. The resulting substrate was then washed 5 times with ethanol and deionized water, respectively. Subsequently, the aminated PDMS substrates were aldehyde-modified by incubation with 1% glutaraldehyde solution for 2 h. After that, the PDMS substrates were then rinsed with a solvent and dried under a stream of nitrogen for the use of subsequent experiments. The substrates were modified with Fn solution (50 μg • $mL^{-1}$) at 4 °C overnight before seeding cells. Then, the Fn solution was removed and the substrates were incubated with 1% BSA solution (w/v) for 1 h to eliminate nonspecific adsorption.

## Magnetically labelled cell assay

Cells were cultured in 6-well tissue culture plates overnight at 37 °C. The culture medium was then removed and serum-free medium containing 30 nm carboxyl-MNPs (50 μg·$mL^{-1}$) was added. Our previous work shows that 30 nm Carboxyl-MNPs have strong remanence signal and low cytotoxicity internalized in cells at a concentration of 50 μg·$mL^{-1}$,[1] thus 30nm commercial Carboxyl-MNPs were used as nanoprobes in all magnetically labelled cell assays. After 4 hours of incubation, the medium was removed and the cells were rinsed 5 times with PBS to remove free MNPs. Subsequently, cells were digested, collected, and redispersed in complete medium for subsequent adhesion assays. To quantify the mass of intracellular MNPs, we used acid digestion method and inductive coupled plasma emission spectrometer method to assess the mass of $Fe_3O_4$ internalized into cells. In the present work, the average mass of internalized $Fe_3O_4$ was 64.57 ± 3.13 pg per cell for all the experiments.

## FIRMS measurements

Measurements of the magnetic signals of the sample were obtained using an atomic magnetometer. Before measurements, the sample was magnetized for 2 min using a magnet from same distance and direction every time. Mechanical forces of varying amplitudes were produced

by a centrifuge at various speeds. According to previous reports[1, 2], the force amplitude applied on the cells can be given by:

$$F = m \cdot \omega^2 \cdot r$$

where F is the relative centrifugal force. m is the average buoyant mass of magnetically labelled cell, which consists of the average buoyant mass of cell (~0.037 pg) and the average mass of internalized MNPs (10.82 pg per cell). ω is the centrifuge angular velocity, which is speed in rpm multiplied by 2Π/60. And r is the distance of the sample from the rotation center (0.045 m). The remanence signal was subsequently measured after each application of centrifugal force. The relative adhesion force was defined as the force corresponding to the half maximum signal.

## Homotypic adhesion assays

Homotypic adhesion assays were performed using confluent monolayers of MDA-MB-231, MCF-7, or MCF-10A cells. After monolayers were established in PDMS slices, $5 \times 10^4$ MNP-labelled MDA-MB-231, MCF-7, or MCF-10A cells were added to the monolayers of cells. After 30 min adhesion, a 2 min magnetization process was performed using a magnet for subsequent FIRMS measurement assays. Nonspecific adhesions were removed by a 5 min centrifugation at 800 rpm prior to FIRMS measurements.

## Heterotypic adhesion assays

Briefly, $5 \times 10^4$ MNP-labelled cells were seeded on the confluent monolayers of HUVECs cultured in Fn-coated PDMS slices for 30 min, followed by a 2 min magnetization process using a magnet. Nonspecific adhesions were removed by a 5 min centrifugation at 800 rpm prior to FIRMS measurements. For blocking experiments, ICAM-1 on HUVECs and CD43 on breast cancer cells were blocked with specific antibodies (anti-ICAM-1 antibody, 1:200; anti-CD43 antibody, 1:50) for 1 h at 37 °C before the addition of cancer cells.

## Static cell-cell adhesion experiment

HUVECs or cancer cells ($4 \times 10^5$) were seeded in 12-well plates (Nunc, Saint Aubin, France) and grown to confluence for 1 day at 37 °C. Then, $1 \times 10^5$ cancer cells were added per well and incubated for 30 min at 37 °C. Nonadherent cancer cells were removed by gentle washing with complete medium. For blocking experiments, ICAM-1 on HUVECs and CD43 on breast cancer cells were blocked with specific antibodies (anti-ICAM-1 antibody, 1:200; anti-CD43 antibody, 1:50) for 1 h at 37 °C before the addition of cancer cells. Subsequently, we captured 5 different fields of vision from the top, bottom, left, right, and middle using biological microscope at 10x lens, and then counted and calculated the average number of adherent cells for each field. The average number for different groups is in a range of 200-500 per field.

## Immunofluorescence staining

Cancer cells were seeded at $5 \times 10^4$ cells in a 3.5 mm confocal dish and incubated overnight. Then, the medium was removed and the cells were washed three times with PBS. After that, the cells were fixed with 4% glutaraldehyde for 7 min, permeabilized with 0.5% Triton X-100 for 5 min, and then blocked with 2% (w/v) BSA in PBS for 1 h at ambient temperature. After blocking, cells were incubated with anti-CD43 antibody (abcam, 1:50) overnight at 4 °C, followed by 1h Alexa Fluor

488-conjugated goat anti rabbit antibody (1: 200) incubation. Subsequently, cells were washed three times with PBS and stained with DAPI. Samples were then imaged using a confocal microscope (OLYMPUS FV1000-IX81, Zeiss, Germany). The fluorescence intensity was quantified using Image J.

### Western blot analysis

Cancer cells were washed three times with ice-cold PBS. Then, aspirate the PBS and add 1 mL ice-cold RIPA buffer (Solarbio) containing 1 mM phenylmethanesulfonyl fluoride (Solarbio) for 30 min at 4 °C. Scrape cells off the dish using a cold plastic cell scraper, then transfer the cell suspension into a pre-cooled microcentrifuge tube. The soluble proteins were isolated by centrifugation at 12,000 rpm for 5 min and collection of the supernatant. The protein concentration was determined using a BCA protein assay reagent kit (Tiangen). After that, SDS-PAGE of the protein lysates was performed and the proteins were transferred onto a polyvinylidene fluoride membrane activated with methanol for 1 min. Subsequently, the membrane was blocked with 5% non-fat milk for 1 h. After blocking, the membrane was incubated with anti-CD43 antibody (abcam, 1:50) and anti-β-actin antibody (Solarbio, 1:3000) overnight at 4 °C, followed by 1h goat anti-rabbit IgG/HRP (Solarbio, 1: 200) incubation.

## References


1. M. Xu, X. Feng, F. Feng, H. Pei, R. Liu, Q. Li, C. Yu, D. Zhang, X. Wang and L. Yao, *Nanoscale*, 2020, **12**, 14573-14580.
2. C. Yu, D. Zhang, X. Feng, Y. Chai, P. Lu, Q. Li, F. Feng, X. Wang and Y. Li, *Nanoscale*, 2019, **11**, 7648-7655.