The mechanical force of minority cells modulates the tumor taxol-resistance and progression

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Materials

Fetal Bovine Serum (FBS) was purchased from Gibco (Thermo Fisher Scientific inc.), Penicillin and streptomycin, Phosphate-Buffered Saline (PBS), DMEM medium and Trypsin obtained from HyClone/Thermofisher. (3-aminopropyl) triethoxysilane (APTES), Fibronectin (FN), 50% glutaraldehyde, Gelatinase, Diphenyl Oxide, Iron (III) acetylacetonate ($Fe(acac)_3$), DSPE-PEG-NHS, L-α-Phosphatidylcholine, cholesterol were purchased from Sigma-Aldrich. Oleylamine, and 3, 4-dihydroxyhydrocinnamic acid (DHCA) were all purchased form Sigma-Aldrich (USA). A549 cell lines were purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Taxol-resistance A549 (chronically exposed to Taxol (230 nM)) was purchased from Shanghai Gefan Biothechnology. CO, LTD. 4',6-diamidino-2-phenylindole (DAPI) was purchased from Beyotime, China. Cell culture plates were purchased from Coning Company.

METHODS

Synthesis of magnetic nanoparticles.

(4 nm): 4 nm-nanoparticles were synthesized according to the previous report[1].

(10 nm): $Fe(acac)_3$ (2 mmol), oleic acid (4 mmol), 1-octadecene (20 mL) were mixed and magnetically stirred (1600 rpm) under a flow of nitrogen and heated to 300 ℃ (1 h). The black-brown mixture was cooled to room temperature by removing the heat source. Under ambient conditions, ethanol (50 mL) was added to the mixture, and a black material was precipitated and separated via centrifugation (6000 rpm) for 10 min. The black product was dissolved in hexane and centrifuged (6000 rpm) for 10 min. The supernatant was re-precipitated with ethanol for 3 times and finally the products were dispersed into hexane.

(20 nm): $Fe(acac)_3$ (2 mmol), oleic acid (6 mmol), oleylamine (6 mmol), and 1-octadecene (20 mL) were mixed and magnetically stirred (1600 rpm) under a flow of nitrogen and heated to 200 ℃ (30 min), and then heated to 300 ℃ (30 min). The same precipitated method was carried out as that of treatment of 10 nm NPs.

(40 nm): $Fe(acac)_3$ (2 mmol), oleic acid (6 mmol), oleylamine (6 mmol), and 1-octadecene (10 mL were mixed and magnetically stirred (1600 rpm) under a flow of nitrogen and heated to 200 ℃ (30 min), and then heated to 300 ℃ (30 min). A black material was precipitated and separated via centrifugation (1700 rpm) for 10 min. The black product was dissolved in chloroform and centrifuged (3000 rpm) for 5 min for removing the precipitation and the supernatant was stored at 4 ℃.

(60 and 80 nm): 60 and 80 nm-nanoparticles were synthesized according to the previous report[2]. Briefly, $Fe(acac)_3$ (2 mmol) was added to a mixture of oleic acid (1.13 g) and benzyl ether (10.4 g). The mixture solution was degassed at room temperature for 1 h and then heated to 290 ℃ under vigorous magnetic stirring. The reaction mixture was maintained at this temperature for 30 min. The precipitated process was the same as that of 40 nm NPs.

Synthesis of VS-4718⊂L and taxol⊂L nanoparticles.

VS-4718⊂L and taxol was added into the ethanol solution of mixutre (L-α-Phosphatidylcholine (PC, 3.04 mg), cholesterol (CH, 0.72 mg) and DSPE-PEG-NHS (0.3 mg). VS-4718⊂L and taxol⊂L were formed by rapidly injecting the ethanol solution into PBS (1 mL), followed by adding 0.02 mg peptide RGD which increase the targeted ability of VS-4718⊂L and taxol⊂L to tumor cells, stirring for 2 h. The ethanol in the liposome solution was evaporated with rotatory evaporator. Store the dialyzed nanopaticles at 4^oC.

Characteristics of magnetic nanoparticles. The synthesized magnetic nanoparticles were characterized with transmission electron microscope (TEM) and dynamic light scattering (DLS). The morphology and size of NPs were examined on a Tecnai G2 20 S-TWIN TEM at an acceleration voltage of 200 kV. DLS experiments were performed with a Zetasizer Nano instrument (Zetasizer Nano ZS) equipped with a 10-mW helium-neon laser (λ = 632.8 nm) and thermoelectric temperature controller. And the sample was dispersed in the PBS buffers (pH = 7.4) or chloroform. Aqueous phase transfer of hydrophobic magnetic nanoparticles was based on the previous report[3].

Examination of the remanence signal of optimal nanoparticles (20 nm) by FIRMS. Nanoparticles with an average diameter of 20 nm were used as optimal NPs. 10-μg NPs were dissolved in PBS and dropped on the FIRMS slice, finally immobilized on the slice surface by solvent evaporation. The remanence signal was examined through FIRMS after magnetization in the vertical direction for approximately 2 min by using a permanent magnet. Control experiments were carried out without solvent evaporation or magnetization. The signal stability of NPs was examined by FIRMS from 1 h to 48 h after magnetization. The sensitivity of NPs was investigated in the mass range: 0, 0.5, 1, 2, 4, 8, 10 μg.

FIRMS slice modification. The FIRMS slice was designed by utilizing polydimethylsiloxane (PDMS) slice with a hole (4 mm of dimeter, 1 mm of depth). After hydroxylation by plasma cleaner for 25 s, the APTES solution of ethanol was quickly added on the activity surface of PDMS and incubated for 4 h. The slices were subsequently rinsed with solvent and dried under a stream of nitrogen. The silanized surface was activated by incubation in a solution of glutaraldehyde (1% v/v). Then the solution was replaced by FN solution (20 μg/mL). The slices were kept at 4 ℃

overnight, and subsequently rinsed. Before FIRMS measurement, the BSA solutions (1% w/v) were added on the slice for the elimination of nonspecific adsorption.

Cell culture. Taxol-resistant human lung cancer cells (RCs, taxol-resistant A549 cell lines) and taxol-sensitive cancer cells (SCs) were incubated with DMEM mediums supplemented with 10% FBS, 1% penicillin and streptomycin at 37 ^{o}C in humidity and 5% CO_2.

Cytotoxicity.

NPs: Cells (SMRCs and SCs) were seeded in 96-well plates at a density of ~1×10^5 cells per well and cultured further for 17 h. The different concentrations of NPs (0, 5, 10, 20, 50 μg/mL) was added into the medium, respectively. Then the cell viabilities were investigated through cells counting kits (CCK-8) assay after 36 h incubation. The effect of NPs on cell mechanical profiles: The cells was incubated with Taxol (1 nM) and/or PBS for 36 h and then incubated with low and high concentrations of NPs (25 and 50 μg/mL) in 6-well plates for 4 h. The cells was digested by trypsin (0.25%, 3 min) and re-seeded on the FIRMS slices to incubate for 30 min. After magnetization, the slice incubated with cells was examined by using FIRMS. The curve of force-dependent relative magnetic signal was obtained to test cell mechanical profiles.

VS-4718⊂L and taxol: The cells (SMRCs and SCs) were seeded in 96-well plates at a density of ~1×10^5 cells per well and cultured further for 17 h. The different concentration of taxol, VS-4718 ⊂L or VS-4718⊂L and taxol⊂L was added into the medium, respectively. Then the cell viabilities were investigated through cells counting kits (CCK-8) assay after 36 h incubation.

Feasibility of FIRMS for investigation of the deformability and adhesion of magnetically labelled cells. The cells were incubated in the FIRMS slice for 17 h. The nanoprobes (NPs, 20 μg/mL) were added into the cell medium for cell uptaking. The free NPs in medium were removed and washed with PBS for three times. After sample magnetization, 40-μL trypsin (0.25%) as

mimetic force driver was added into FIRMS slice. The time-dependent signal changes induced by cellular deformation and detachment were recorded by FIRMS. Meanwhile, in control experiments, cells were treated with the same conditions in the absence of trypsin, where the cellular signal kept stable in 2 h. When the NPs-labeled cells were fixed by glutaraldehyde (4%) for 20 min and the measured the signal.

The working profile of FIRMS

The working profile of FIRMS is that an external force with varying amplitudes is applied on the magnetically labeled samples which interact with target molecules immobilized on the substrates and show strong remanence signal after magnetization[4]. When the exerted force is equal to the interaction force between two molecules, the magnetically labeled samples will detach from their target molecules, resulting in the decrease of overall remanence signal. This process is recorded by an atomic magnetometer.

Determination of adhesion force, adhesion stability and the cell-cell force by FIRMS. The cells (SMRCs and SCs) were seeded in 6-well plates at a density of ~1×10^6 cells per well and cultured further for 17 h. After 4 h-incubation with NPs (20 μg/mL), the Taxol (1 nM) dispersed in BSA solution (2%) was added into the medium and cultured for 10 h or 36 h. Then the cells were digested with trypsin (0.25%) and reseeded on the special FIRMS slice coated with FN (26 μg/mL). Then measure the mechanical properties using FIRMS.

Adhesion force: According to short-term adhesion assay[5, 6], the cells were incubated on the slice for 30 min. Initial magnetic signal of cells were detected after magnetization for 2 min. Then the centrifugal force was applied to the adhered cells, reducing cell detachment. FIRMS slice with cultured cells kept perpendicular to the centrifugal force and faced outward. The force-dependent cells detachment was recorded through the decreased magnetic signal.

Cell-cell force: Culture the treated cells for another 17 h, and then added the same number cells in the medium, letting these fresh cells interacted with the pre-seeded cells for 30 min. The cells were magnetized for 2 min and then applied the centrifugal force to record the cell detachment through decreased magnetic signal.

The centrifugal force exerted on the cells was calculated according to previous report[6]:

$$F = (\rho_{cell} - \rho_{medium}) \cdot V_{cell} \cdot \omega^2 \cdot r$$

where F is the relative centrifugal force, ρ_{cell} and ρ_{medium} is the specific density of the cells ($\sim 1.07 \times 10^3$ kg/m^3) and the medium ($\sim 1.00 \times 10^3$ kg/m^3), respectively. V_{cell} is the cell volume (~525 μm^3). r is the radius of the rotation (0.045 m). The relative adhesion force was defined as the force corresponding to the half maximum signal. In control experiment of integrin blocking, after the cells were treated with taxol for 36 h, the cell medium was changed to the complete medium. The peptide (PLGVRGRGD, 5 mg/mL) which synthesized according our previous report[7] was added into the medium and cultured for 15 min at 37 °C. Then the cells were digested to examine the mechanical behavior. In control experiment of FN abrogating, the FIRMS slice was not modified with FN, but just cultured with BSA (1%) solution before utilization.

Adhesion stability: The cells were incubated on the slice for 30 min. A constant force (25 pN) was imposed on the cells with 6 repeats. The adhered cells were counted by microscopy. Measurement of contraction force via elastic micropillar system according to the manufacturer's instructions from Microduits Company (Switzerland).

Investigation of cell promigratory and migration via FIRMS.

Promigratory: Cells ($\sim 1.5 \times 10^5$) were incubated on the FIRMS slices coated with FN. After the cells (SCs and SMRCs) were incubated on the slice for 17 h, the medium was changed with the complete DMEM medium containing taxol and incubated for 36 h. NPs (20 μg/mL) were added

into the cells medium without FBS and incubated for 4 h, and then the medium was washed with PBS for 3 times. The trypsin (0.25%) was added into the solution of medium. The time-dependent signal decrease induced by cellular deformation and promigratory was monitored by FIRMS.

Migration: Cells were incubated on the FIRMS slice for 8 h with DMEM mediums supplemented with 10% FBS, 1% penicillin and streptomycin at 37 ^{o}C in humidity and 5% CO_2 atmosphere. The fresh cells medium without FBS was changed to incubate cells with NPs (20 μg/mL). After incubation of 4 h, the slice was washed 3 times with the medium. The cells were further incubated with taxol (1 nM) for 10 h and 36 h, then digested with trypsin (0.25%) and re-seeded on the FIRMS slice for 1 h. After magnetization, the migration-induced signal decrease was recorded by FIRMS.

Wound healing assay. Cells were seeded into 6-well plates. The migration velocity of treated cells was examined through a thin "wound" introduced by scratching with a pipette tip. Cells at the wound edge polarized and migrated into the wound space, and the migration rates were analyzed by wound space distance at 36 h[8].

Investigation of cell polarization via microscopy. Cells (SMRCs and SCs) were seeded in PDMS slice at a density of ~1×10^6 cells and cultured further for 17 h. The Taxol (1 nM) dispersed in BSA solution (2%) was added into the medium and cultured for 36 h. The cells polarization was observed by F-actin polymerization through Actin-stain 555 phalloidin staining. The polarization ratio of the cells was calculated from the ratio of the significant polarized cells (length: width > 2) to total cells in microscopy images.

Immunofluorescence staining. Cells were fixed and processed for immunofluorescence as described in previous reports[9]. Briefly, the treated cells were fixed in 4% paraformaldehyde for incubation 15 min after washing 5 times with warmed-PBS. All these processes were performed

at 37 ^{o}C. After the fixation step, the cells were treated with 0.5% Triton X-100 (5 min) for permeabilization in DPBS at room temperature, and then the cells were washed three times with DBS to remove the detergent. To enhance the specific interaction of antibody to targeted proteins, non-specific antibody binding was blocked by cell incubation with 1% (w/v) BSA in PBST (0.1% v/v Tween 20) at room temperature for at least 1 h. The cells were then washed briefly with DPBS for 3 times. F-actin staining: the working stock (100 nM) of Actin-stain 555 phalloidin (Sigma-Aldrich) diluted with DPBS (0.1% BSA) was added into the cells for incubation of 1 h. P-MLC II, vinculin, merlin, tubulin, P-, E-cadherin, and phosphorylated (pSer518) merlin staining: the primary antibody (Abcam, P-cadherin, ab242060; E-cadherin, ab1416; merlin, ab88957, P-MLC II, ab2480, vinculin ab129002, tubulin, ab6046) diluted in 1:300 ratio in DPBS with 0.1% BSA were added into the cells and incubation overnight at 4 ^{o}C. After incubation and washing 3 times with DPBS, cells were incubated with secondary antibody tagged with the fluorescent dye of Alexa fluor 677 (Sigma-Aldrich) diluted in 1:300 (v/v) ratios with DBPS (0.1% BSA) for 1 h at room temperature. The nuclei were stained with 4',6-diamidino 2-phenylindole (DAPI) for 10 min at room temperature with washing for 5 min per times (5 times). The image series were acquired using a 100X phase objective lens of confocal microscope (OLYMPUS FV1000-IX81) at the 405 nm, 559 nm and 633 nm of laser excitation. All the images analysis was dependent on the Image J software. All experiments performed at least three times.

Young's modulus measurement using AFM

AFM measurements were performed as previously described. Briefly, the cells were cultured in the cell culture dishes with complete medium and placed on the stage for AFM measurements. AFM studies were conducted using a Dimension FastScan Bio Atomic Force Microscope (AFM, Bruker) with a combined inverted optical microscope. The measurements were collected at 37^{o}C

using silicon nitride cantilevers with spring constants of 0.07 N/m and a tip radius of 20 nm (MLCT-BIO-DC, Bioscope catalyst AFM from Bruker). The scan size for all measurements was set to 0 nm to maintain a constant position over the cell. By using the AFM software, the tip was brought into contact with the central region of the cell (Wu HW, et al., *Scanning*, 20, 389–397 (1998); Sarah E. Cross, et al., *Nature Nanotechnology*, 2, 780-783 (2007)). The Young's modulus was identified using a modified Hertz model and the data were analyzed using Nanoscope software.

Micro-fabricated elastic micropillar assay

The micropillar devices were purchased from MicroDuits (www.microduits.com). The assay was run according manufacture's illustration and the image was analyzed with their Open Source Software "MechProfiler". Briefly, the glass substrate with micropost array was put in a 12-well plate and wet it by adding 1 mL ethanol (99%) and incubated at room temperature for 20-30 s. Then 1-mL DI water was added to dilute the ethanol and then 1 mL was aspirated away and repeat this step at least 3-times. Replace the DI-water with PBS in the same manner and then 1mL was also aspirated away. Repeat this step for 3-times. After these steps, the PBS was replaced by adding 1 mL cell culture medium and then take out 1 mL of medium. This step was also repeated at least 3-times. Then the 1-mL cell solution (250000 cells) was added on the top of the micropost array and cultured in incubator (CO_2, 5%; 37 ^oC) for 6-7 hours. The cells were fixed with 3.7% formaldehyde solution for 5 minutes and then the cells were stained with dye (0.05% Coomassie Brilliant Blue in 50% water, 40% ethanol and 10% acetic acid) for 90 s. The micropost arrays was observed using 20X objective of Confocal with transferring micropost array to imaging dish with the microposts facing up. After obtaining the images with micropost tips are in focus, the MechProfiler software was used to analyzed images under the illustration of manufacture.

Western blot.

Western blot procedure was performed according the general protocol for western blotting from Bio-rad. Briefly, the cells were washed three times after transfection and the cells were collected in a 1.5 mL tube. Then the RIPA lysis, extraction buffer and 1 × proteinase inhibitor were added to the samples and maintained constant agitation for 30 min at 4 ℃. During this time, the samples were sonicated 3 time for 15 sec each. Samples were spun down at 16,000 g for 20 min in a 4 ℃ precooled centrifuge. The supernatant was transferred to a fresh tube, kept on ice, and the pellet was discarded. 20 μL of lysate was removed to perform a protein assay (BSA assay, Thermo Fisher Scientific) for quantifying total protein concentration. The other samples were mixed with 5× Laemmli sample buffer and boiled each cell lysate in sample buffer at 95 ℃ for 5 min. The cell debris was removed by centrifugation. The extracted protein samples were separated by 10% SDS-PAGE and transferred onto a membrane. The transferred membrane was blocked with dry milk (5%, in 1X Tris-Buffered Saline and 0.1% Tween® 20, TBST) for 1 h and then incubated overnight at 4 ℃ with P-cadherin primary antibody (1:1000), merlin antibody (1:500) and GAPDH antibody (1:1000). Then the blotted membrane was washed three times for 5 min each and then cultured with Goat anti-Rabbit IgG (H+L)-HRP conjugate (Jackson, 111035003) and Goat anti-mouse IgG(H+L)-HRP conjugate (Jackson, 115035003) for 1 h. The protein was finally detected using the chemiluminescent method.

Flow cytometry.

The cells were collected after 48 h incubation and then were centrifuged to remove supernatant. During the experiments, the cells always were put on the ice. The ice-cold methanol (100%) was slowly added to pre-chilled cells, while gentle overtaxing, to a final concentration of 90% methanol. After 15 min, the cells were centrifuged and washed with PBS twice. The pellet cells were

suspended using 100 μL diluted primary antibody (P-cadherin 1:200, CST, #14029) and incubated for 1 h at room temperature. Before adding the secondary antibody, the cells were washed with PBS twice and cultured the cells in the diluted secondary antibody for 30 min at room temperature with protecting from light. The cells were finally suspended into 500 μL PBS and analyzed on flowcytometer after twice washing with PBS.

Transfection.

Sensitive cells were transfected with RNAi-Lipofectamine® 2000 complexes according to the transfection procedure suggested by Thermo Fisher Scientific company. (P-cadherin siRNA, Thermo Fisher, s2774, #4392420; Merlin siRNA, Sigma, #NM_000268). Briefly, siRNA (30 pmol) and Lipofectamine® 2000 (3 μL) was diluted in 150 μL of Opti-MEM® I Reduced Serum Medium, respectively. Then the solution was mixed together gently and incubated for 15 minutes at room temperature. The RNAi-Lipofectamine® 2000 complexes were added into well containing cells and medium (1mL) and cultured for 48 h.

Cell labeling assay. MRCs and SCs were seeded in 6-well plates at a density of ~2×10^6 cells per well and cultured further for 17 h, respectively. DiI and DiO (6 μM) were added into the medium of MRCs and SCs, respectively, and cultured for 5 min. The cells were digested using trypsin (0.25%) for 3 min at 37 ^{o}C. The labeled cells (MRCs, 0.5%, and SCs, 99.5%) were mixed to form SMRCs, then reseeded in confocal plates for 17 h and treated with Taxol (1 nM) for 10 h or 36 h. The cells behavior was observed by using confocal microscope (OLYMPUS FV1000-IX81) at the 488 nm and 555 nm of laser excitation.

Conditioned medium culture. Taxol-resistance cells (~1×10^5) were seeded in the 96-well plates, and the media were conditioned for 36 h by culturing Taxol-resistance cells with Taxol (1 nM). And then the supernatant (200 μL) collected as conditioned medium were added into the SCs

(~1×10^5) and incubated in 96-well plates. The adhesion force and migration rate of SCs incubated with conditioned medium were examined as above methods.

3D tumor spheroid culture. 5.0×10^4 cells (SCs or SMRCs) were seeded in 400 μL assay medium (DMEM, 10% FBS, 1% penicillin and streptomycin, and 2% Matrigel) in 6-well chamber slides with 60 μL of matrigel matrix (BD)[10]. Tumor spheroid formed and cultured for 15 days. The medium (DMEM, 10% FBS, 1% penicillin and streptomycin, and 2% matrigel) was exchanged per 4 days.

Cell invasion in 3D culture system: At the 15 days, the two groups of tumor spheroids (SCs and SMRCs) were treated with taxol (1 nM). The other two groups were treated with the same volume of PBS. Finally, the invasion cells were imaged by microscope with 10× lens.

Cell viability in 3D culture system: after 15 days, the tumor spheroid was treated with PBS, taxol⊂L (10 nM), VS-4718 ⊂L (10 μM), taxol⊂L (10 nM)+VS-4718 ⊂L (10 μM), respectively, for 24 h. The cell viability was measure using the CCK-8 kits. The tumor spheroids were observed using live/dead cell staining kits.

Transwell migration and invasion assays. 12-well cell migration assay kits with 8-μm pores were obtained from BD Company. Serum-starved cells were seeded in the upper chamber and allowed to migrate for 36 h into DMEM/10% FBS in the lower chamber. Invasiveness of all cells lines was assayed by migration through a commercially available 12-well invasion assay (BD BioCoat Matrigel Invasion Chamber). As the manufacturer's instructions, matrigel inserts on an 8 μm pore-size filter were rehydrated with warm DMEM for 2 h, then serum-starved cells were added to the upper chamber and allowed to invasion for 36 h into DMEM containing 10% FBS. Cells that passed through were detached from the membrane and quantified using the CCK-8 kits supplied in a Microplate reader with 450 filter set.

Microfluidic assay. Microfluidic device was prepared by PDMS with micro-chamber (0.5x4x4.5 mm^3). The micro-chamber surface was modified with FN. The PDMS microfluidic channel was incubated with DMEM/10% FBS for at least 1 h. Then the pre-treated cells were injected into microfluidic channel under different shear stress by adjusting the fluid velocity (50, 100, 150, 250 μL/min) during 30 min. The adhered cells number was recorded through microscopy. And the sheer force of fluid was calculated through the method reported in previous study[5].

***In vivo* invasion study.** All animal experiments were performed under the NIH guidelines for the care and use of laboratory animals and according to the protocol approved by the Institutional Animal Care. Cells (SMRCs and SCs) xenografted tumors were inoculated into the right flank of the 6 ~ 8 week-old female BALB/c nude mice. When the tumors had developed to about 4 mm in diameter, the mice were randomly divided into four groups (n=6 mice per group) in a way to minimize weight and tumor size differences among the groups and administered with PBS and taxol in BSA (0.2 mg/mL) (200 μL, pH 7.4). The drug was intravascularly injected once a time per five days (d = 0, 5, 10, 15, 20). On 25 days, the tumor cells were resected and isolated according to the previous reports. The tumor cells were digested by 1% trypsin in 10 mL HBSS solution containing $CaCl_2$ (1 M) for 1 h at 37 ºC. After getting the cells suspension, they were filtered through a sterile filter (70 μm, BD) and centrifuged (1000 rpm) for 5 min. The tumor cells were re-suspended into DMEM complete medium further cultured for 4 generation. And then the metastasis contribution factors (mechanical force and migration) investigated with FIRMS and microscopy. All experiments were performed three times.

***In vivo* metastasis experiments.** Six-week old female BALB/c nude mice were injected with 2.0 $\times 10^5$ cells in 100 μL of PBS+10% OptiMem into the tail vein on day 0. Mice were injected with each cell line, for 6 mice per group. On day 25, all animals were euthanized with CO_2. Organs

(Lungs, hearts, kidneys, livers and spleens) were then removed and fixed in 15 mL 10% neutral-buffered formalin overnight. After fixation for 16 h, metastases were counted and tissue was imaged. The metastasis ratio was calculated from the metastatic mice divided by total mice per group.

Tumor therapy.

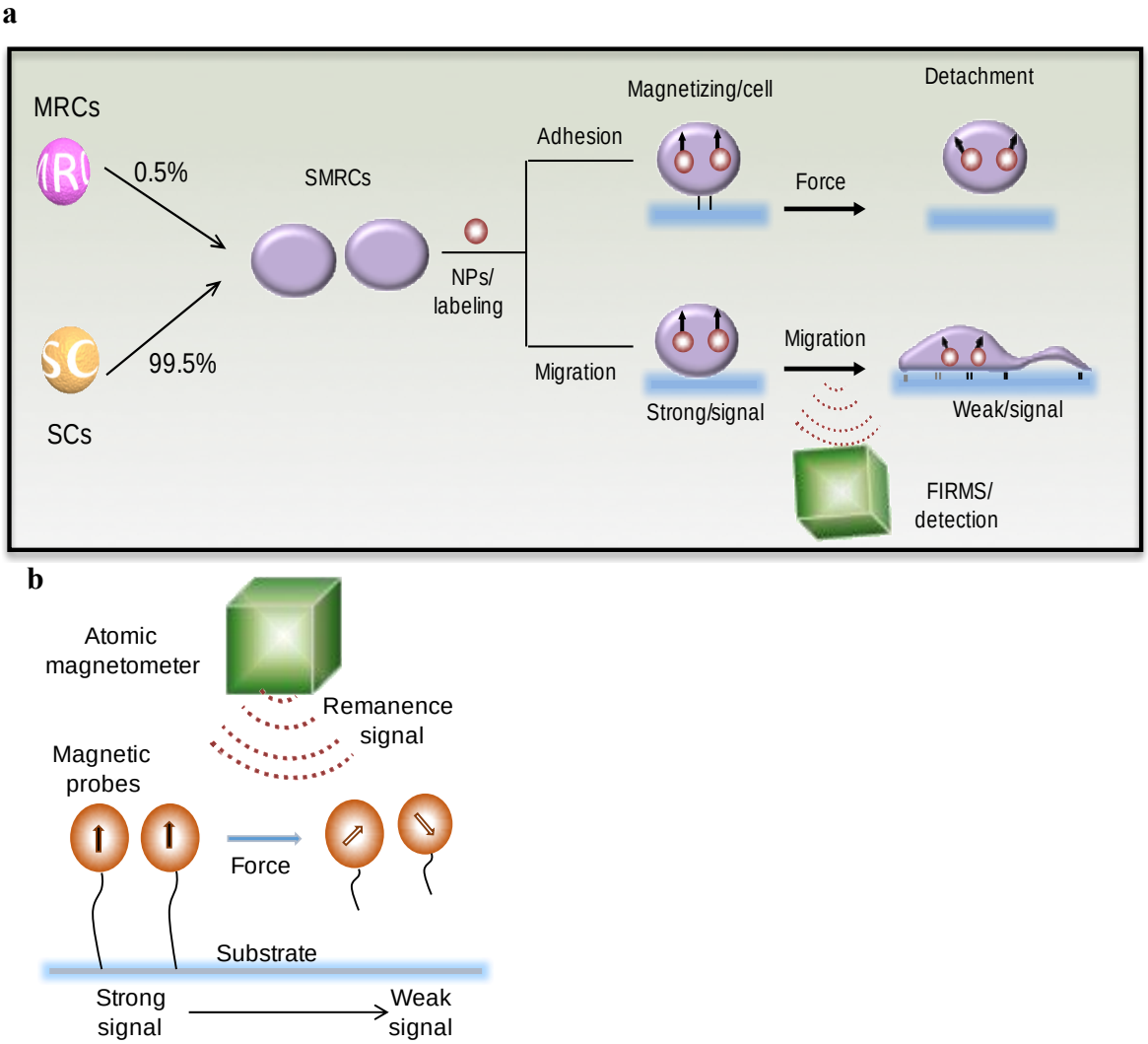
SMRCs were inoculated into the right flank of the 6 ~ 8 week-old female BALB/c nude mice. When the tumors had developed to about 4 mm in diameter, the mice were randomly divided into four groups (n=5 mice per group) in a way to minimize weight and tumor size differences among the groups. The mice were administered with PBS, taxol⊂L (0.1 mg/mL, 200 μL), VS-4718 ⊂L (1 mg/mL), taxol⊂L+VS-4718 ⊂L, respectively, through tail vein injection. The drug was injected once a time per there days at day 11, 13, 15, 17, 19.

Tissue histology and immunohistochemistry. Formalin-fixed and paraffin embedded mouse xenografts primary tumors, organ tissues (lung, heart, liver, spleen, and kidney) were sectioned and stained with H&E and Picrosirius Red. And for immunohistochemistry, the slice was stained overnight at 4 ^{o}C with antibodies against collagen I (Abcam) followed by incubation with Alexa fluor 674 tagged secondary antibody and Actin-stain 555 phalloidin (Beyotime Biotechanology, China) for 2 h at room temperature.

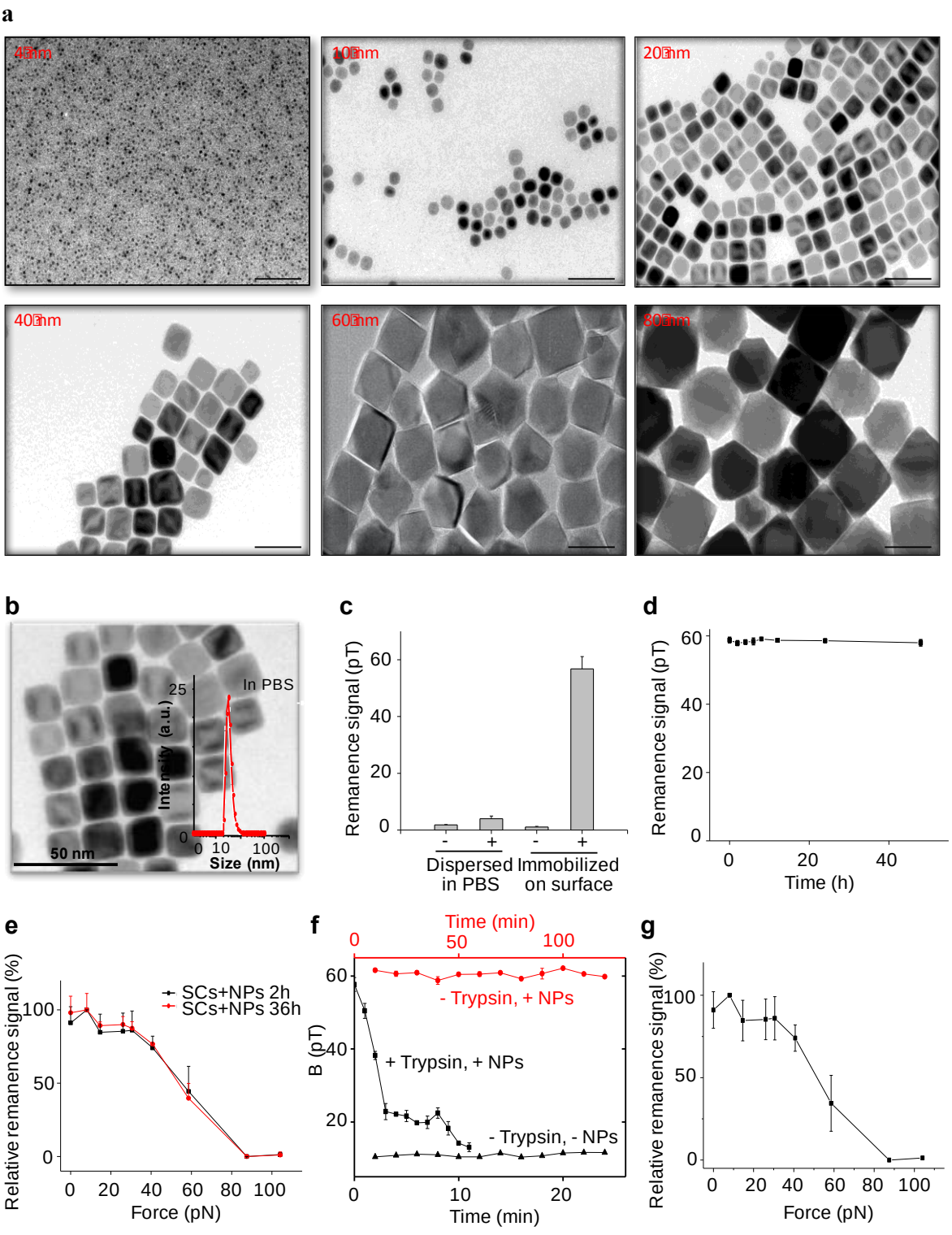
RNA sequence experiments.

The SMRCs and SCs were cultured for 48 h, and then collected the cells into 1.5 mL RNAase-free tubes, and then stored them in liquid nitrogen. And the RNA extraction and sequence analysis ware performed by company Novogene.

Supplementary Figures

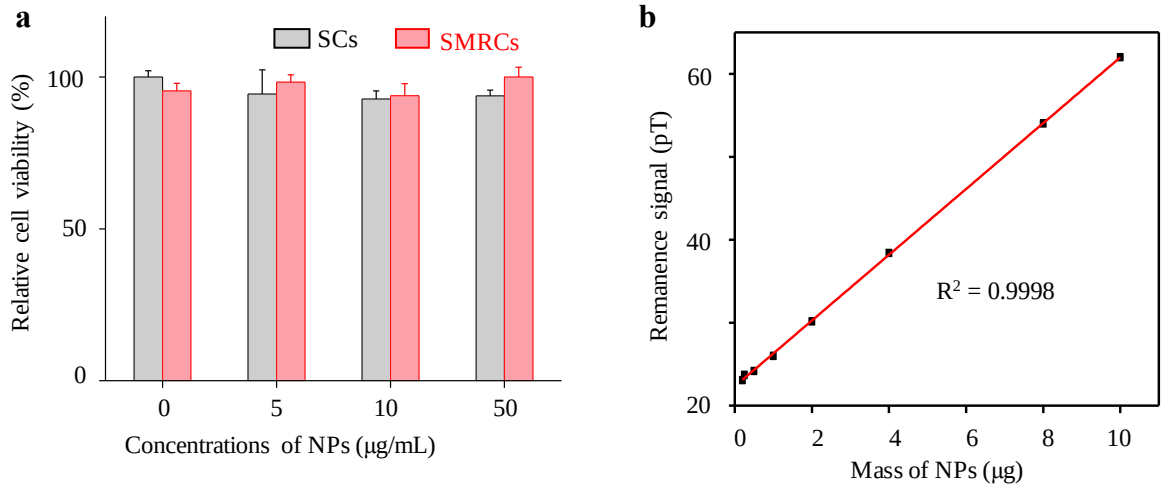


Supplementary Figure 1 | Schematic of measuring cell adhesion and migration through FIRMS and FIRMS working profiles.

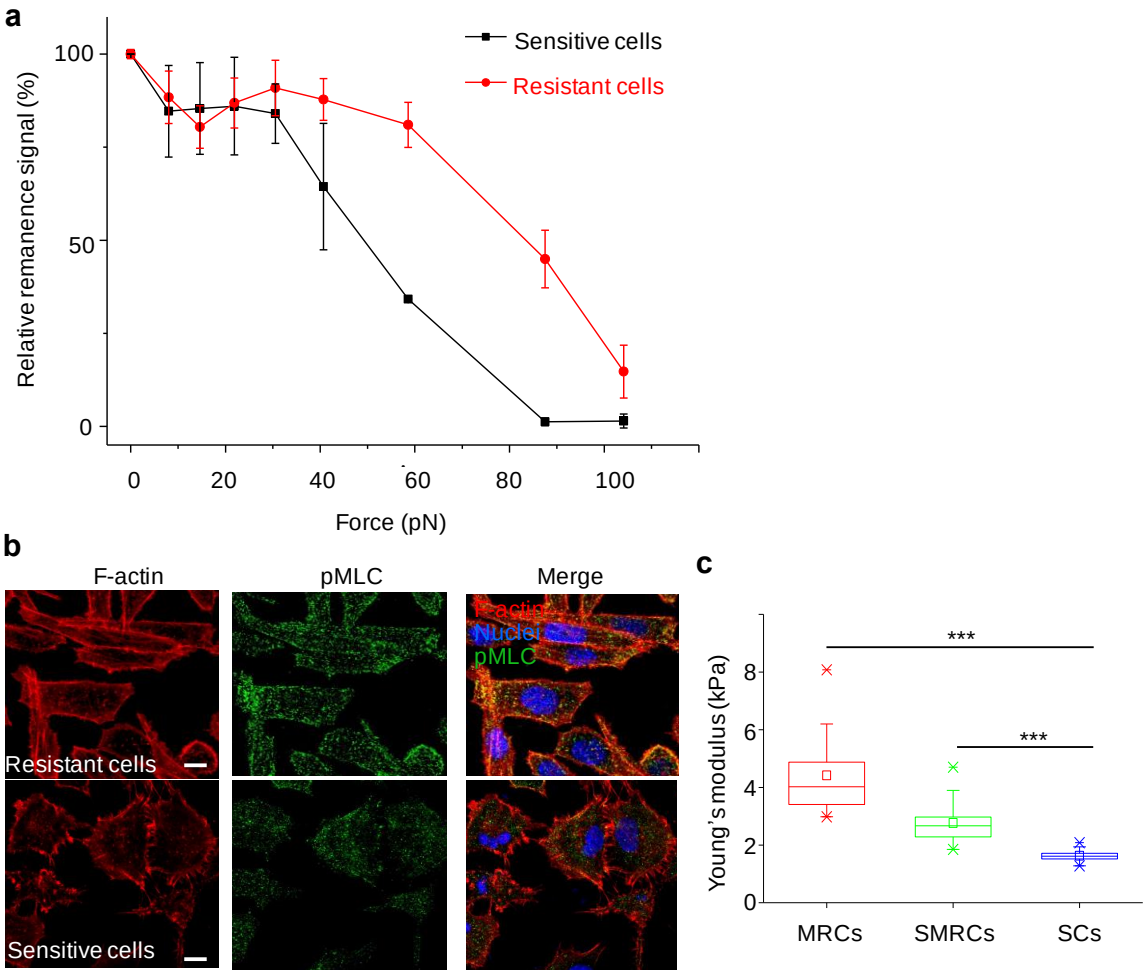


Supplementary Figure 2 | The characterization of NPs for accurately measuring cell adhesion force using FIRMS. a, Morphology and size of magnetic nanoparticles with different size from 4 nm to 80 nm. **b**, Optimized NPs (20 nm), characterized by TEM and DLS. **c**, Magnetic

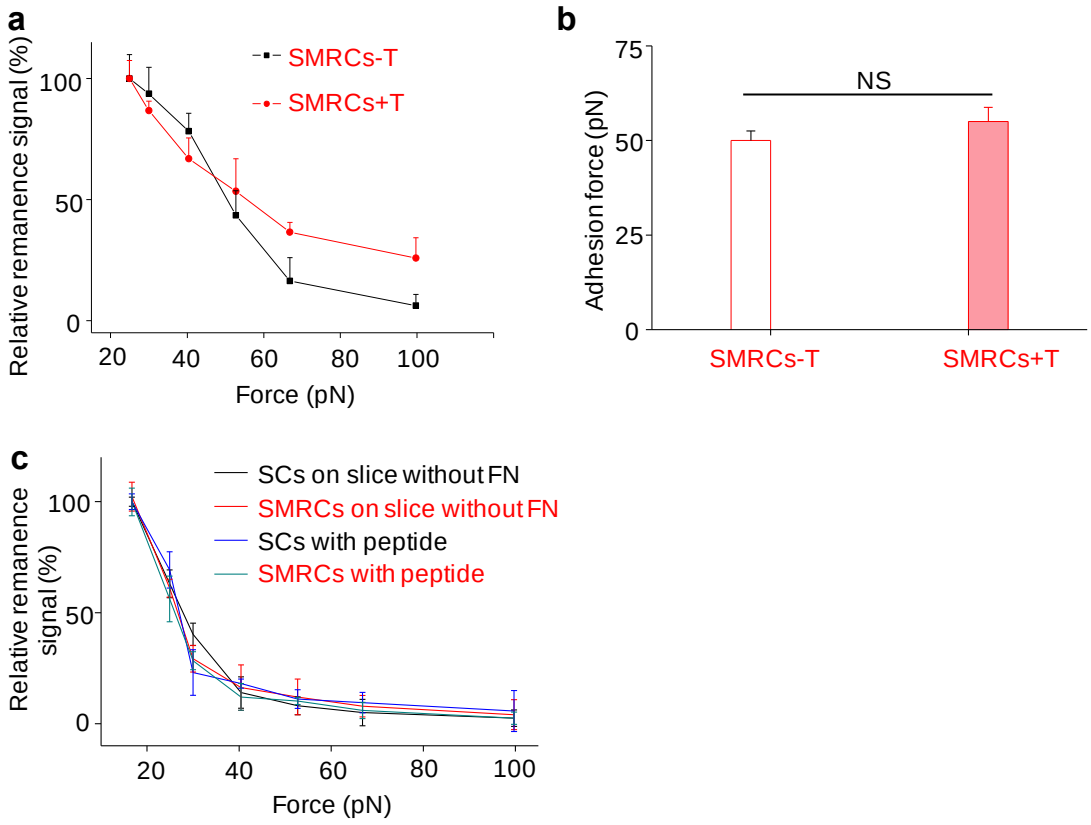
signals of the magnetized NPs (10 μg). + and –represented NPs with and without magnetization, respectively. Results are presented as the mean ± SD. **d**, Time-dependent signal stability of NPs (10 μg) immobilized on the slice surface. Remanence signal was monitored by FIRMS from 1 h to 48 h after magnetization. Data are mean ± sd.. **e**, Curves of force-induced remanence signal decrease for evaluating the effect of NPs on adhesion force. **f**, Curves of remanence signal changes imposed by the external mimic force (0.25%, trypsin) for evaluating the sensitivity of FIRMS on the measurement of deformation, migration, and detachment of NP-labeled cells. **g**, Curves of force-dependent relative signal decrease of A549 cells recorded by FIRMS to measure the cell adhesion force. Data are mean ± s.d., n=3 independent experiments.



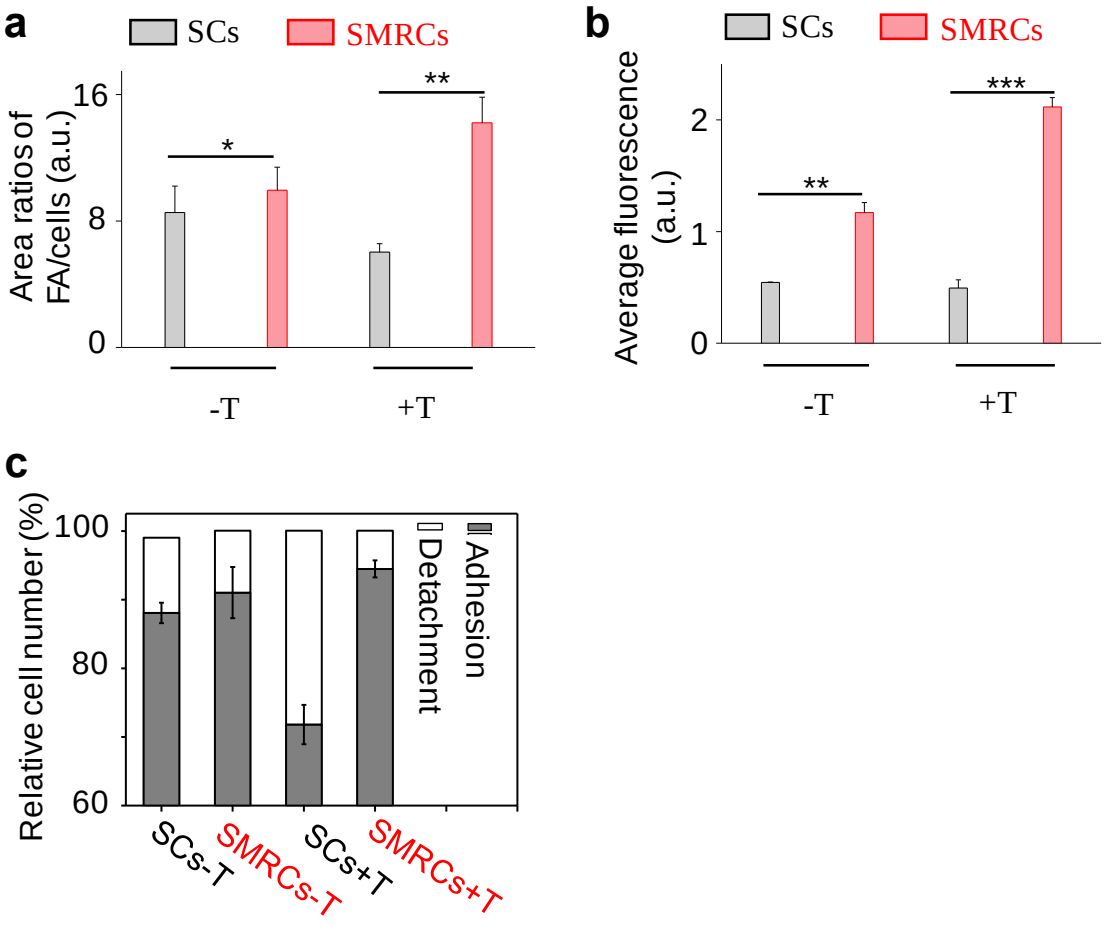
Supplementary Figure 3 | Characteristics of NPs. a, Effect of NPs on cells viability. Cells were treated with different concentrations of NPs (0, 5, 10 and 50 μg/mL) for 36 h, respectively. **b**, Linear relationship between magnetic signal and the mass of NPs.



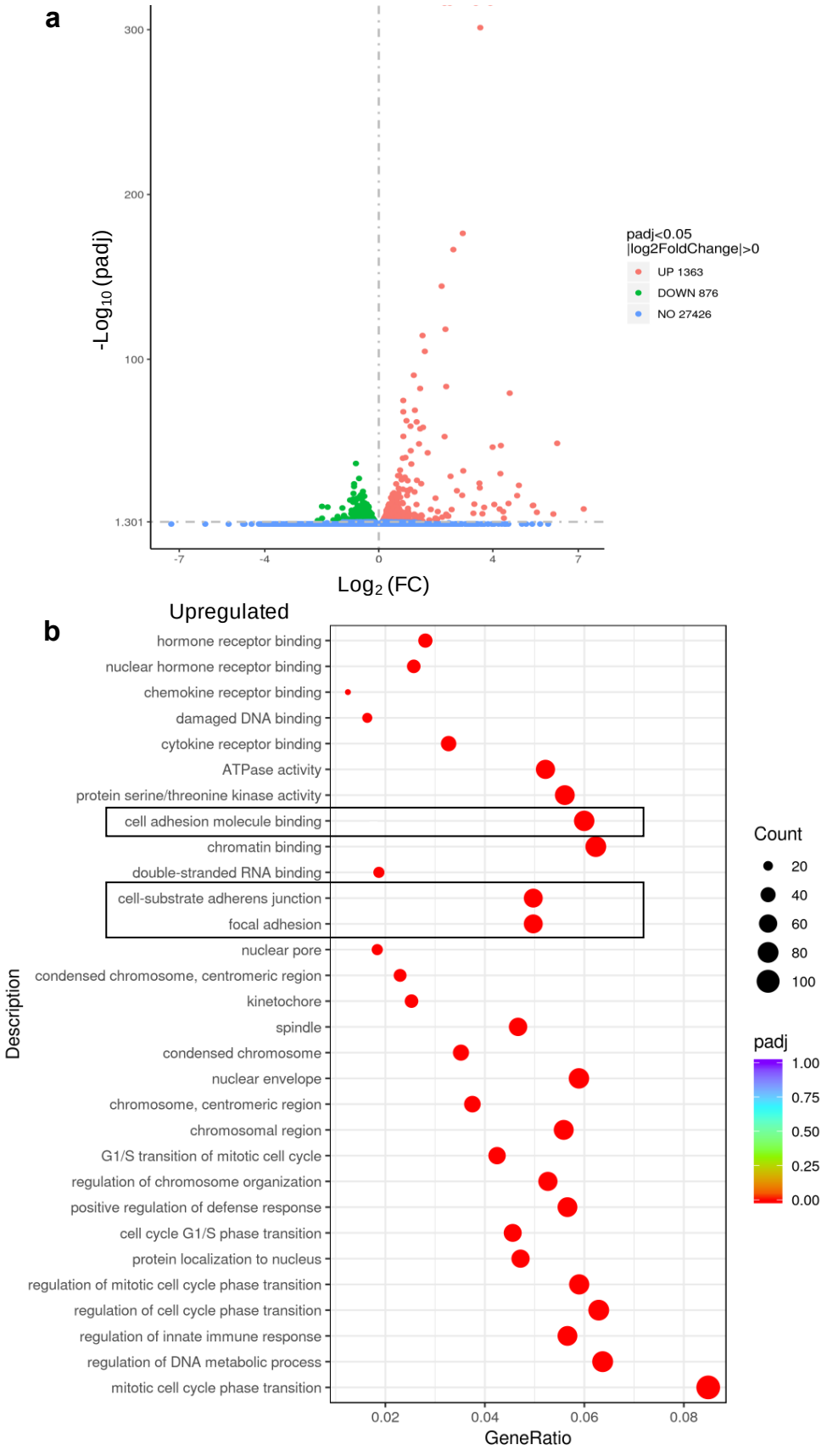
Supplementary Figure 4 | Mechanical properties of MRCs. a, Curves of force-dependent relative signal decrease of resistant and sensitive cells. **b**, Actomyosin contractility of resistant and sensitive cells. F-actin (red) and pMLC (green) and nuclei (Blue). Scale bar, 10 µm. **c**, Young's modulus of SCs, SMRCs and MRCs investigated with AFM. n ≥ 300 cells.



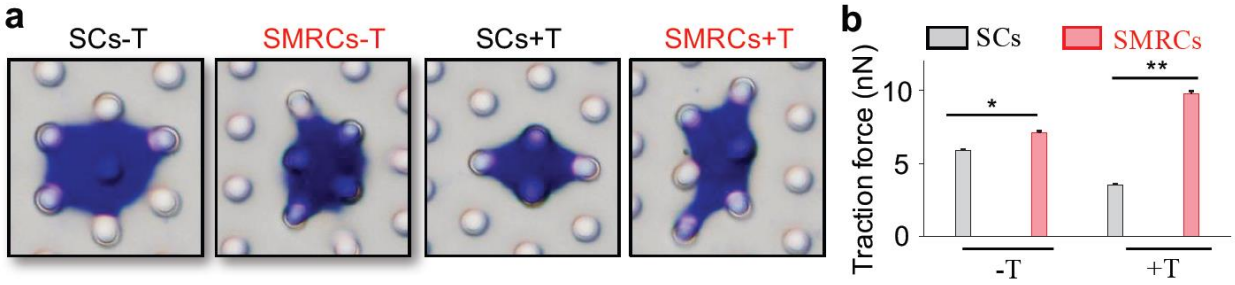
Supplementary Figure 5 | The contribution of MRCs to the integrin-based adhesion force of SMRCs. a and **b**, Curves of force-dependent relative signal decrease (**a**) and quantification of cell adhesion forces (**b**) of SMRCs when the mechanical properties of MRCs were inhibited by blebbistatin for 2 h (+B, 50 μM). Data are mean ± s.d. **c**, Adhesion force of the cells cultured on the substrates without FN modification and the cells treated with RGD-peptide.



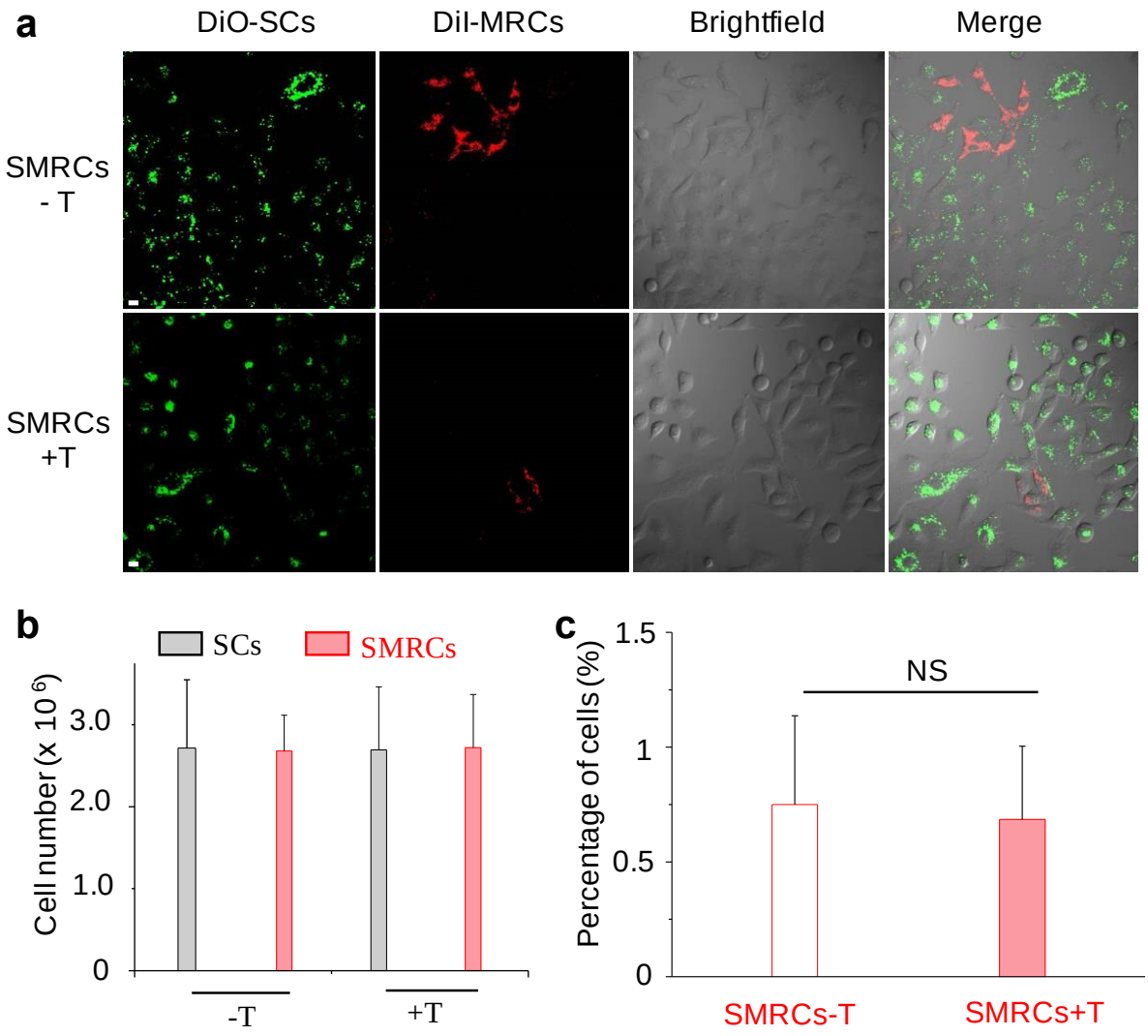
Supplementary Figure 6 | Quantification of FAs and the adhesion stability of cells with different treatments. a and **b**, Quantification of area ratios of FAs to cells (a) and average fluorescence intensity of FAs in cells (b) under different treatments. The fluorescence was quantified using Image J. **c**, The percentage of adhered to detached cells after applying force the sixth times. Force = 25 pN. Data are mean ± s.d., * $P \leq 0.05$, ** $P \leq 0.01$,*** $P \leq 0.001$, Student's t-test.


a
300
200
100
1.301
-Log10 (padj)
-7
-4
0
4
7
Log2 (FC)
padj<0.05
|log2FoldChange|>0
UP 1363
DOWN 876
NO 27426
b
Upregulated
hormone receptor binding
nuclear hormone receptor binding
chemokine receptor binding
damaged DNA binding
cytokine receptor binding
ATPase activity
protein serine/threonine kinase activity
cell adhesion molecule binding
chromatin binding
double-stranded RNA binding
cell-substrate adherens junction
focal adhesion
nuclear pore
condensed chromosome, centromeric region
kinetochore
spindle
condensed chromosome
nuclear envelope
chromosome, centromeric region
chromosomal region
G1/S transition of mitotic cell cycle
regulation of chromosome organization
positive regulation of defense response
cell cycle G1/S phase transition
protein localization to nucleus
regulation of mitotic cell cycle phase transition
regulation of cell cycle phase transition
regulation of innate immune response
regulation of DNA metabolic process
mitotic cell cycle phase transition
Description
0.02
0.04
0.06
0.08
GeneRatio
Count
20
40
60
80
100
padj
1.00
0.75
0.50
0.25
0.00

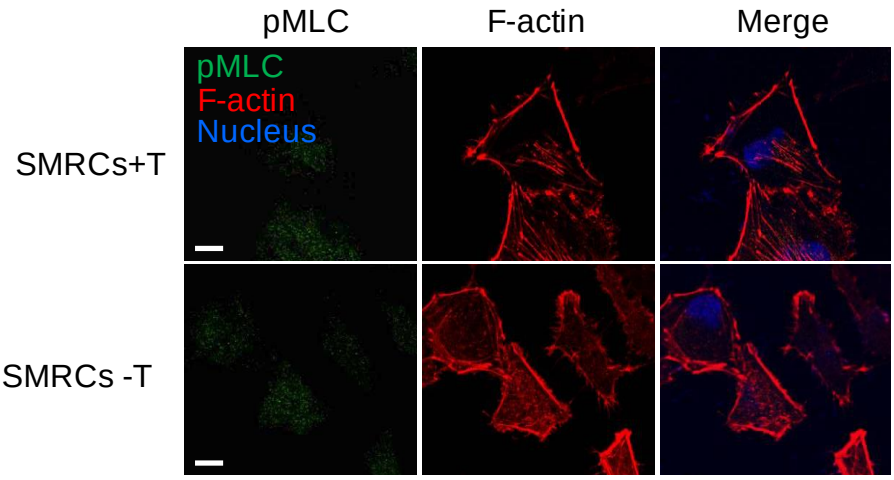
Supplementary Figure 7 | RNA sequence analysis. a, Volcano plot showing log10 padj versus log2 fold change showed summary of significant up- and down-regulated genes between SMRCs and SCs. **b**, Go term enrichment analysis based on the RNA-sequence data of the up-regulated genes in SMRCs versus SCs.



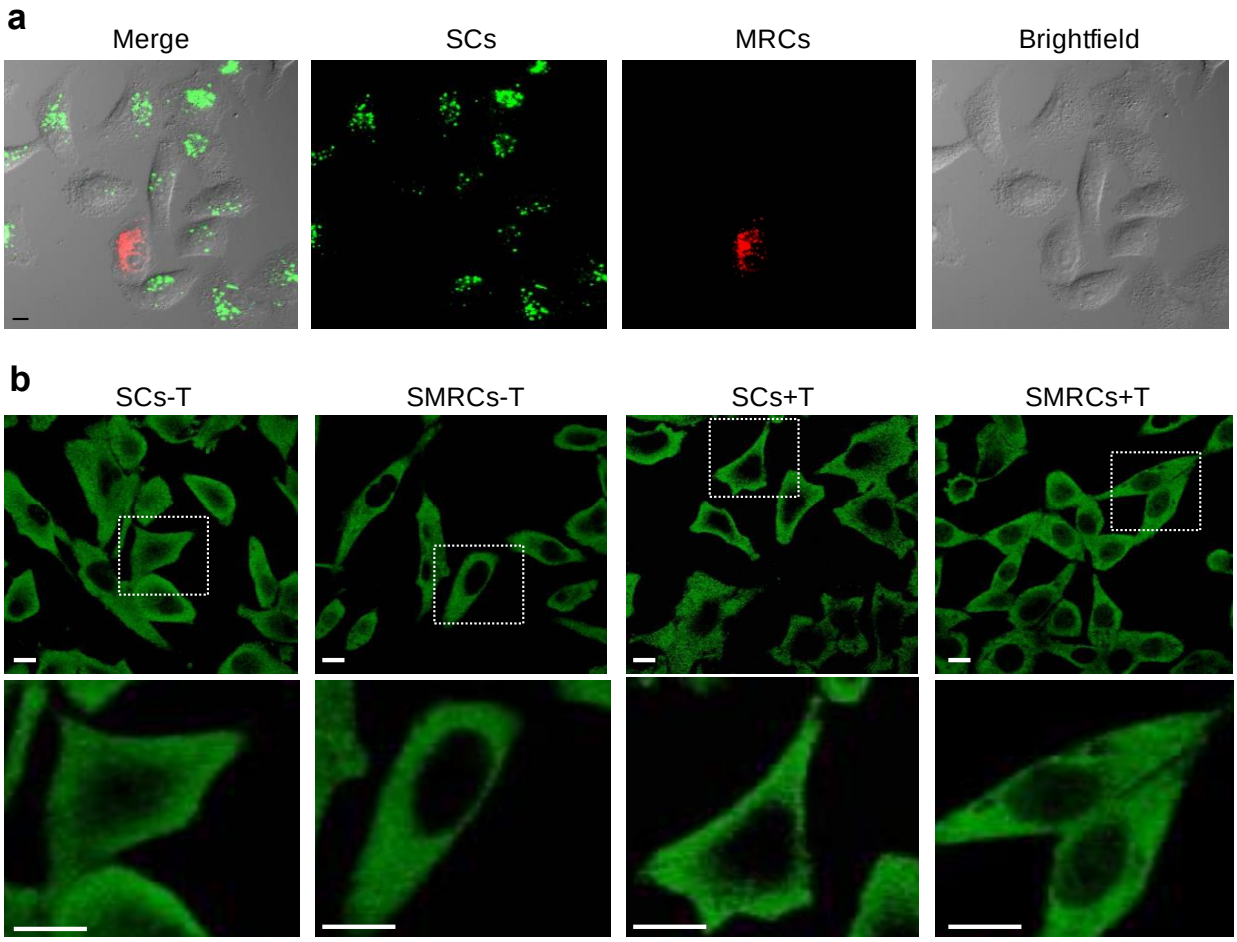
Supplementary Figure 8 | The traction force of cells measured by micropillar assay. a and **b**, Representative Trypan blue-dye-stained cells on FN-fabricated elastic micropillar (**a**) and quantifications of average adhesion forces exerted by cells under different treatments (**b**). Measured through ~200 cells per replicate.



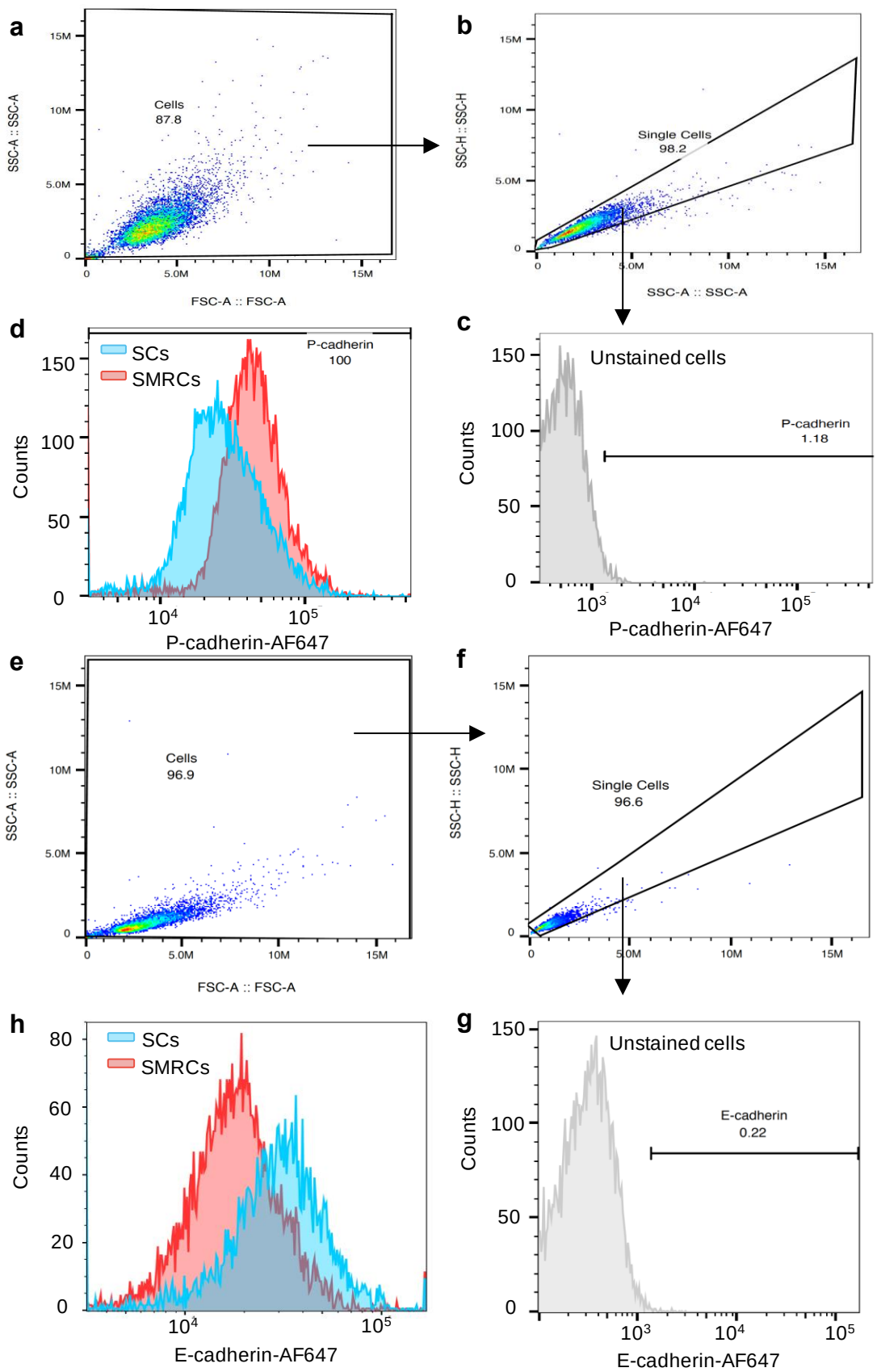
Supplementary Figure 9 | The total cell number and the ratio of the subpopulation in SMRCs. **a**, Representative subpopulation in SMRCs after 36 h incubation. Scale bar, 10 μm. Before co-culturing, MRCs and SCs were stained with DiI and DiO for 5 min at 37 ºC, respectively. n = 6 replicates. **b**, Total cell number of SCs and SMRCs after 36 h incubation under different treatments. Data are mean ± s.d. n = 6 replicates. **c**, Percentage of MRCs in SMRCs with or without Taxol treatment after 36 h incubation. Data are mean ± s.d., NS, no significant difference, n = 6 replicates.



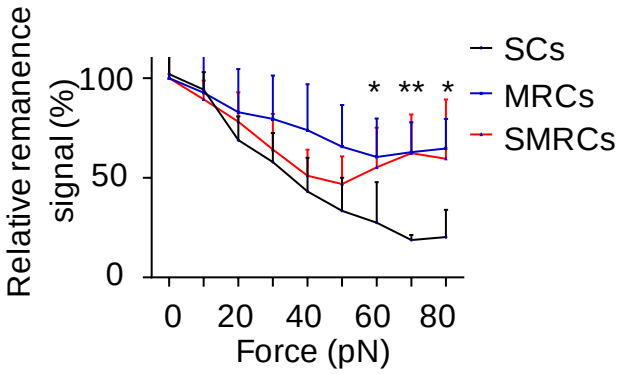
Supplementary Figure 10 | The mechanical properties of SMRCs in which the mechanical force of MRCs was inhibited before co-culturing. Representative confocal images of F-actin and pMLC in SMRCs with and without taxol by inhibiting the contractility of MRCs by adding the myosin inhibitor, blebbistatin (+B, 50 μM), F-actin (Red), pMLC (Green) and nuclei (Blue). Scale bar, 10 μm.



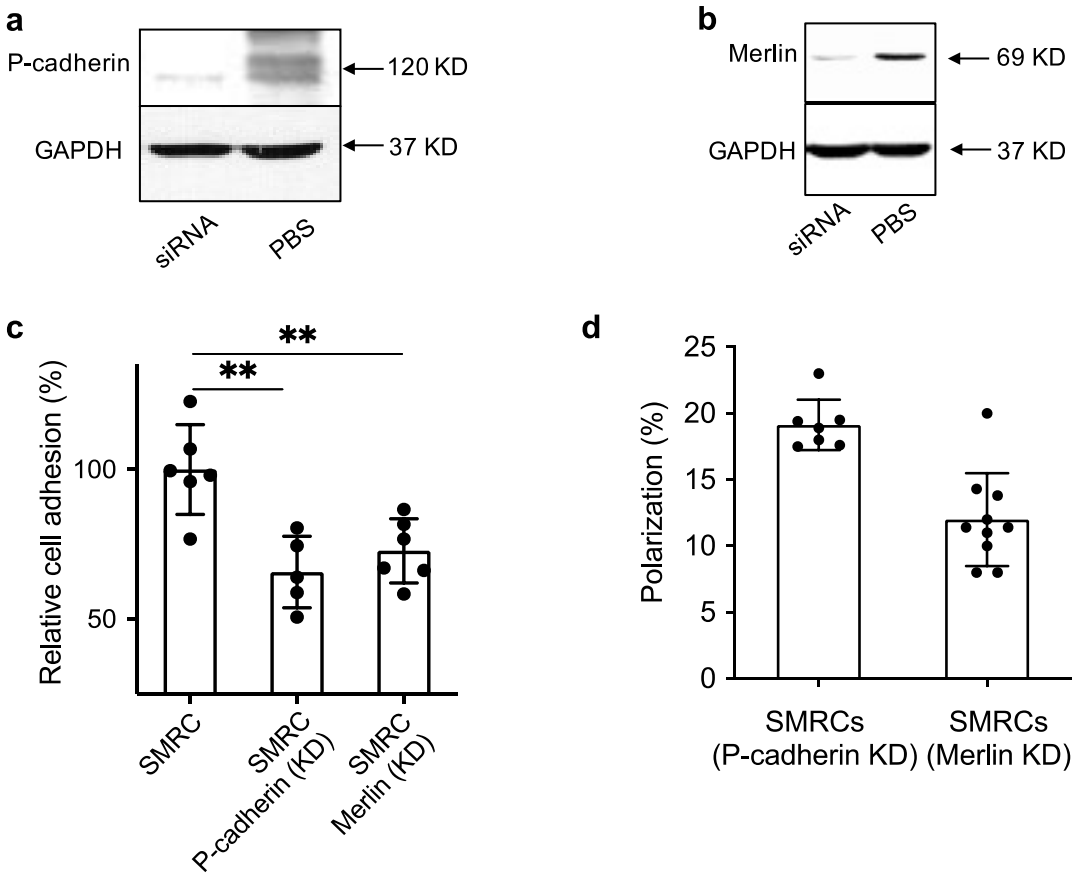
Supplementary Figure 11 | The mechanism of the enhanced mechanical force of SMRCs. a, The polarization of MRCs as leader cells was disturbed by inhibiting the mechanical properties of MRCs before co-culturing. Before co-incubation, MRCs and SCs were stained with DiI and DiO, respectively for 5 min at 37 ºC. Scale bar, 10 μm. **b**, Representative immunofluorescence images of phosphorylated (pSer 518) merlin. The images showed in bottom were the region of dashed square in corresponding top images. Scale bar, 10 μm.

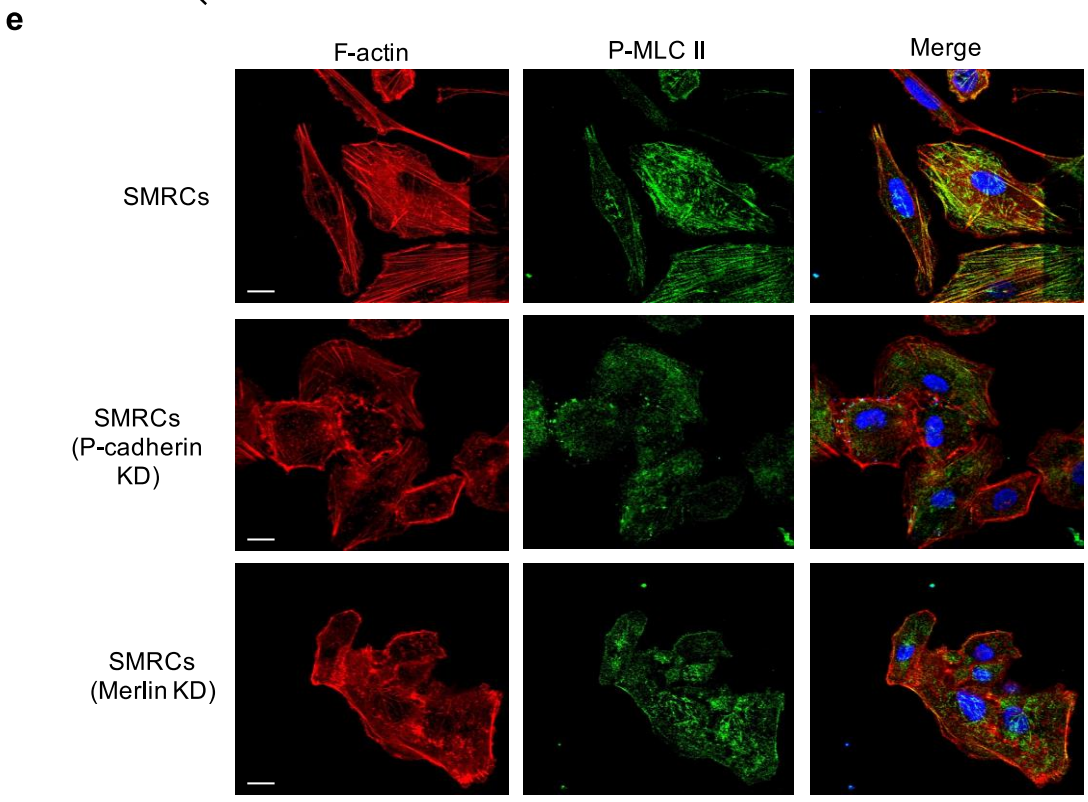

a
Cells
87.8
SSC-A :: SSC-A
FSC-A :: FSC-A
b
Single Cells
98.2
SSC-H :: SSC-H
SSC-A :: SSC-A
c
Unstained cells
P-cadherin
1.18
Counts
P-cadherin-AF647
d
SCs
SMRCs
P-cadherin
100
Counts
P-cadherin-AF647
e
Cells
96.9
SSC-A :: SSC-A
FSC-A :: FSC-A
f
Single Cells
96.6
SSC-H :: SSC-H
g
Unstained cells
E-cadherin
0.22
Counts
E-cadherin-AF647
h
SCs
SMRCs
Counts
E-cadherin-AF647

Supplementary Figure 12 | Representative flow cytometry gating strategies (a-c, P-cadherin, e-g, E-cadherin) and analysis of P-cadherin (d) and E-cadherin (h) expression in SCs and SMRCs.

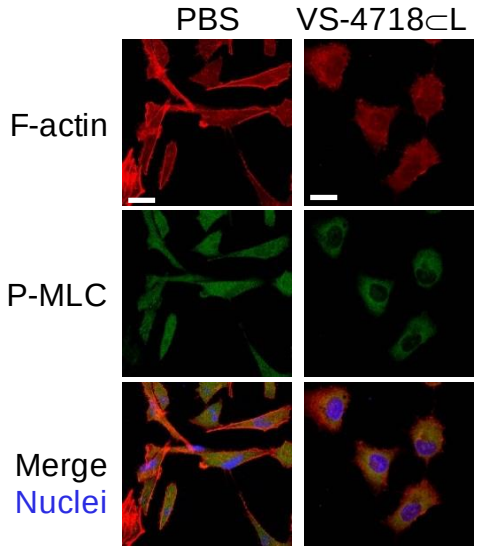


Supplementary Figure 13 | The cell-cell interaction force among SCs, MRCs and SMRCs measured by FIRMS.

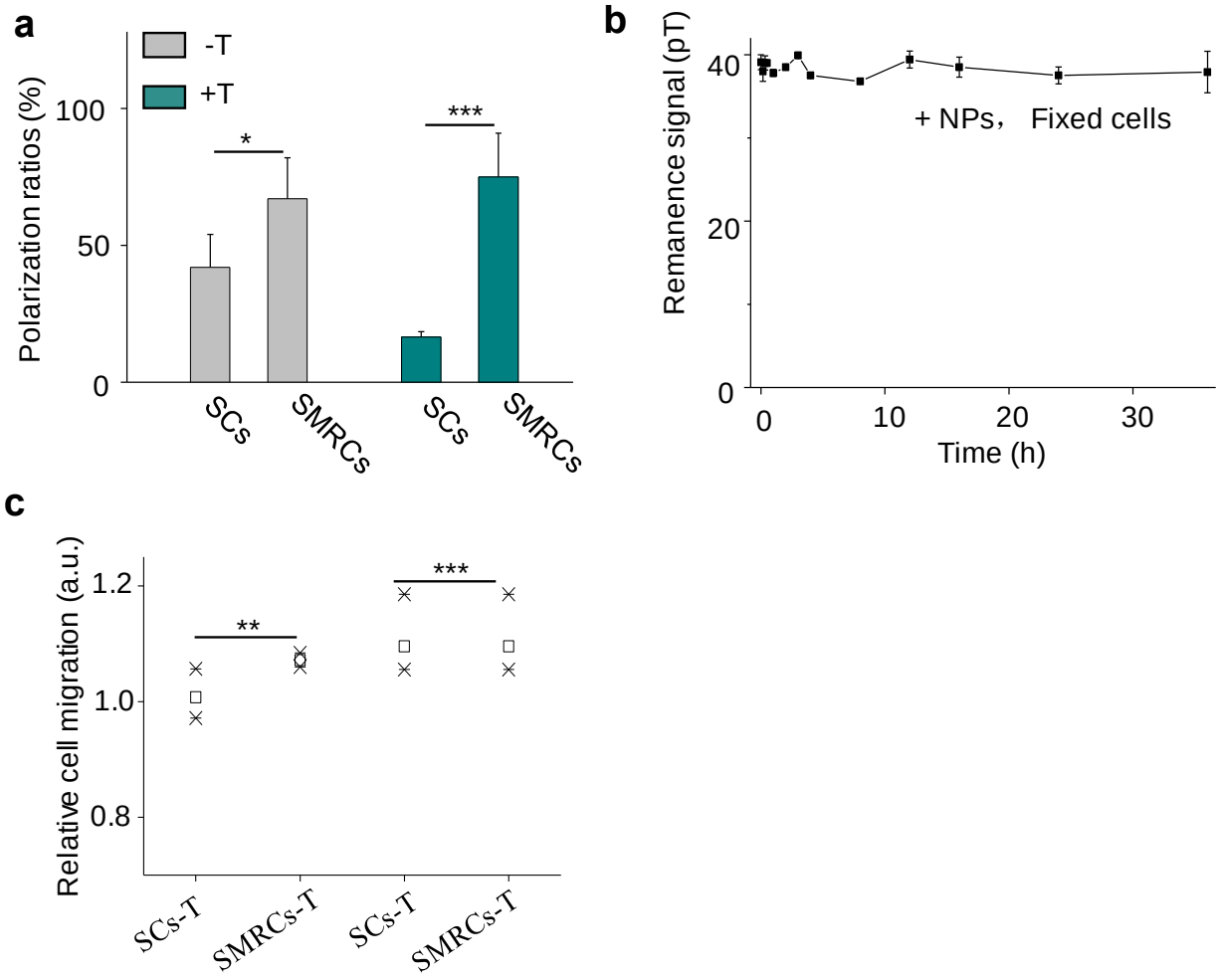

a
P-cadherin
120 KD
GAPDH
37 KD
siRNA
PBS
b
Merlin
69 KD
GAPDH
37 KD
siRNA
PBS
c
**
**
Relative cell adhesion (%)
100
50
SMRC
SMRC P-cadherin (KD)
SMRC Merlin (KD)
d
Polarization (%)
25
20
15
10
5
0
SMRCs (P-cadherin KD)
SMRCs (Merlin KD)


e
F-actin
P-MLC II
Merge
SMRCs
SMRCs (P-cadherin KD)
SMRCs (Merlin KD)

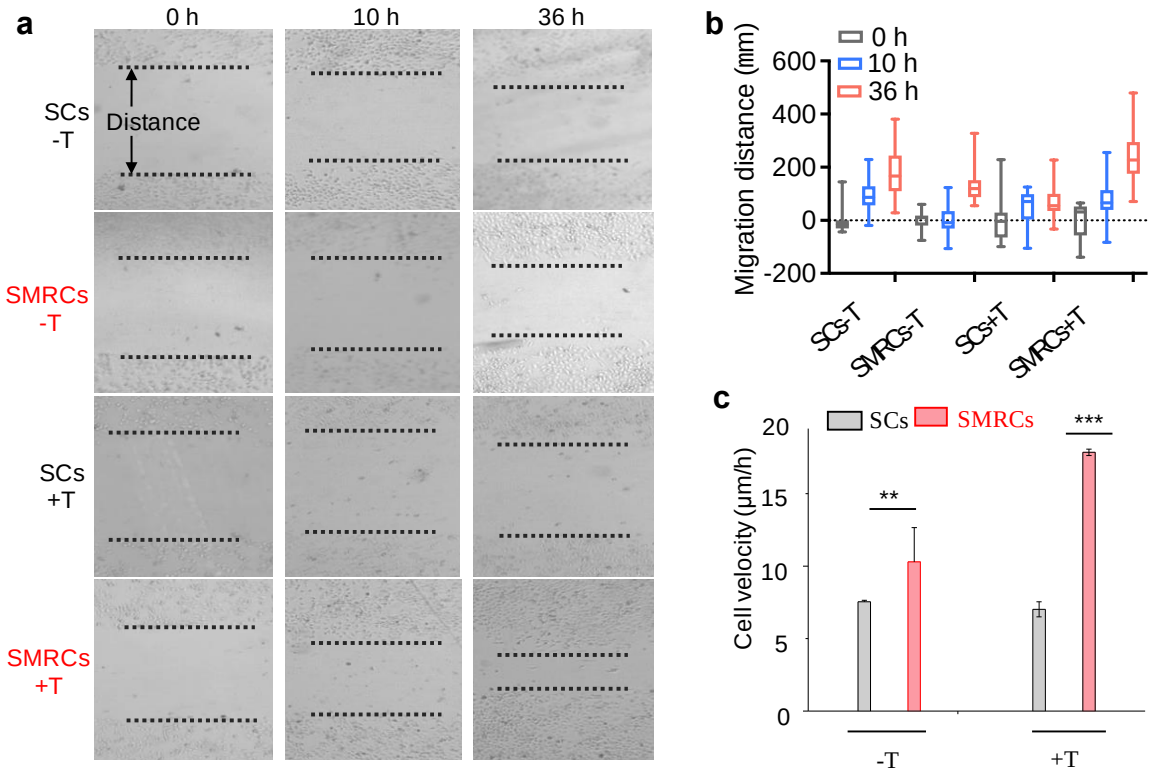
Supplementary Figure 14|. Knockdown P-cadherin and merlin decrease the mechanics of SMRCs. a and **b**, Western blot analysis of protein expression in A549 sensitive cells transfected with P-cadherin-siRNA (**a**) and merlin-siRNA (**b**) after 48 h transfection compared with cells treated with PBS. c, The relative adhesion of SMRCs to SMRCs with P-cadherin and merlin knockdown (KD). Data are shown as the mean ± s.d., * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, Student's t-test. d, The cell polarization of SMRCs compared with SMRCs with P-cadherin and merlin knockdown. Analysis cell number >300. e, Representative cell contraction (F-actin and P-MLC II) of SMRCs to SMRCs with P-cadherin and merlin knockdown (KD). F-actin (Red), pMLC (Green) and nuclei (Blue). Scale bar, 10 μm.



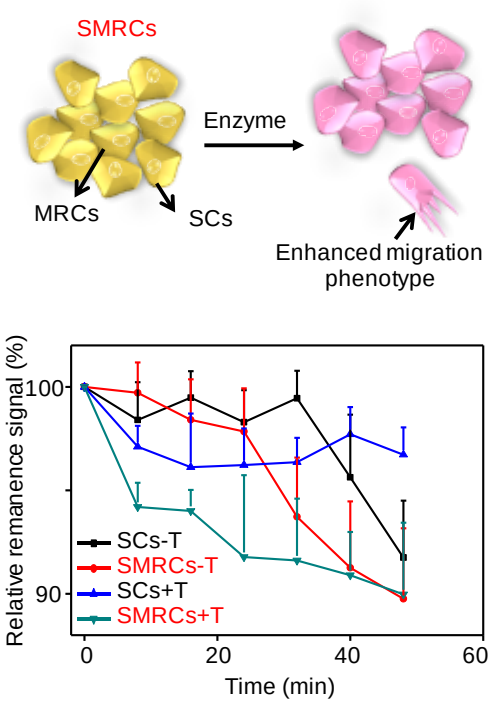
Supplementary Figure 15 | The contraction (F-actin and P-MLC II) of SMRCs was decreased by VS-4718⊂L.



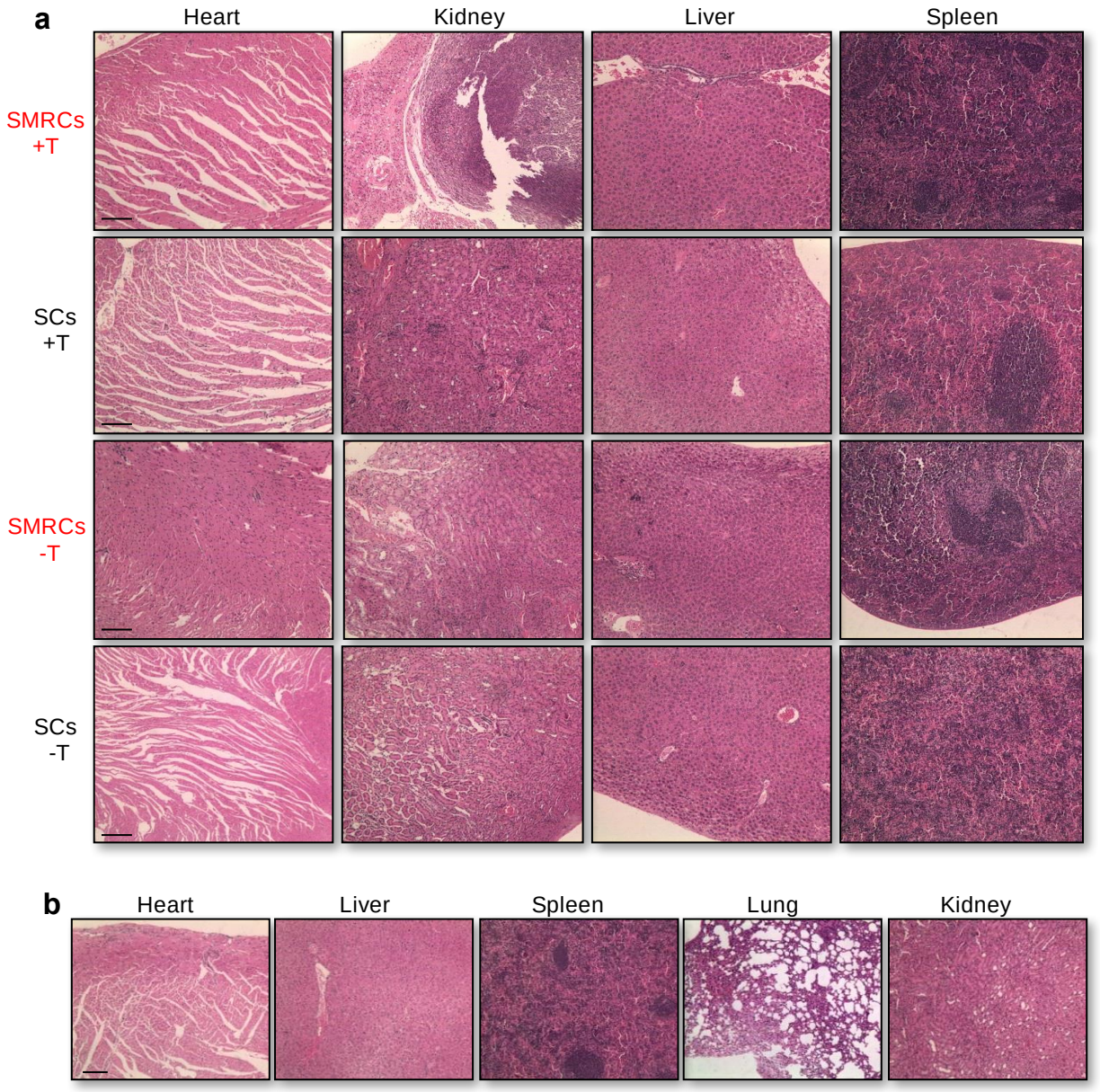
Supplementary Figure 16 | The enhancement of migration in SMRCs. a, Polarization ratios of cells through statistical analysis of the protrusions spreading out distinctly in one or two directions. n ≥ 300 cells. **b**, The stability of remanence signal in fixed cells. **c**, Cell migration rates obtained from FIRMS after 36 h treatment. Data are shown as the mean ± s.d., * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, Student's t-test.



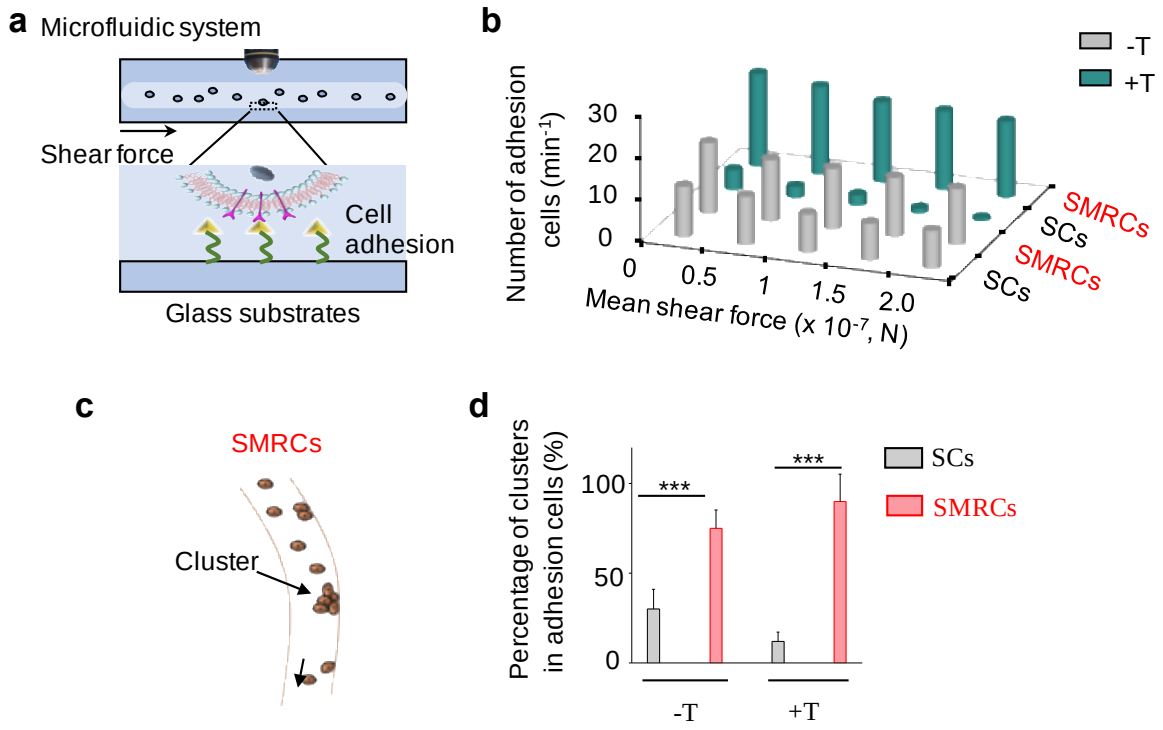
Supplementary Figure 17 | The SMRC migration was enhanced by MRCs measured through wound healing and transwell migration assays. a, Representative images of cells migration across of wound space recorded by microscopy. Cells were incubated on the 6-well plates and treated with Taxol or PBS for 10 h and 36 h, respectively. Then, a thin wound was introduced by scratching with a pipette tip in the middle of the plates. Cells at the wound edge migrated into the wound space. **b**, Statistical results of cell migration distance based on microscopy records. n = 6 replicates. **c**, Statistical results of cell migration rates based on microscopy records from the results of wound healing assay. Data are shown as the mean ± s.d., * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, Student's t-test.



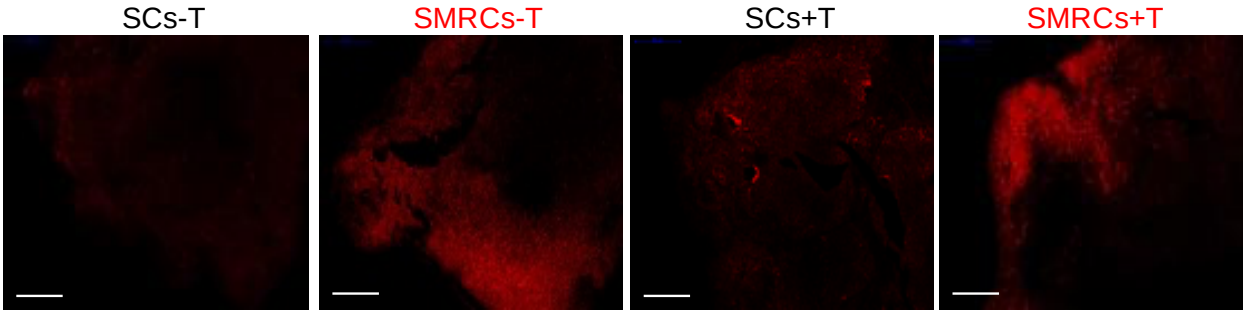
Supplementary Figure 18 | SMRCs easily obtained the pre-migratory phenotype. (Up) Schematic illustration of the individual cell in SMRCs obtaining pre-migratory phenotype upon exposure to the digestive enzyme. (Down), Curves of time-dependent signal decrease recorded by FIRMS after cells were exposed to trypsin (37 ^{o}C, 0.25%). Enzyme-induced cell deformation or migration resulted in the remanence signal decrease. n = 6 replicates.



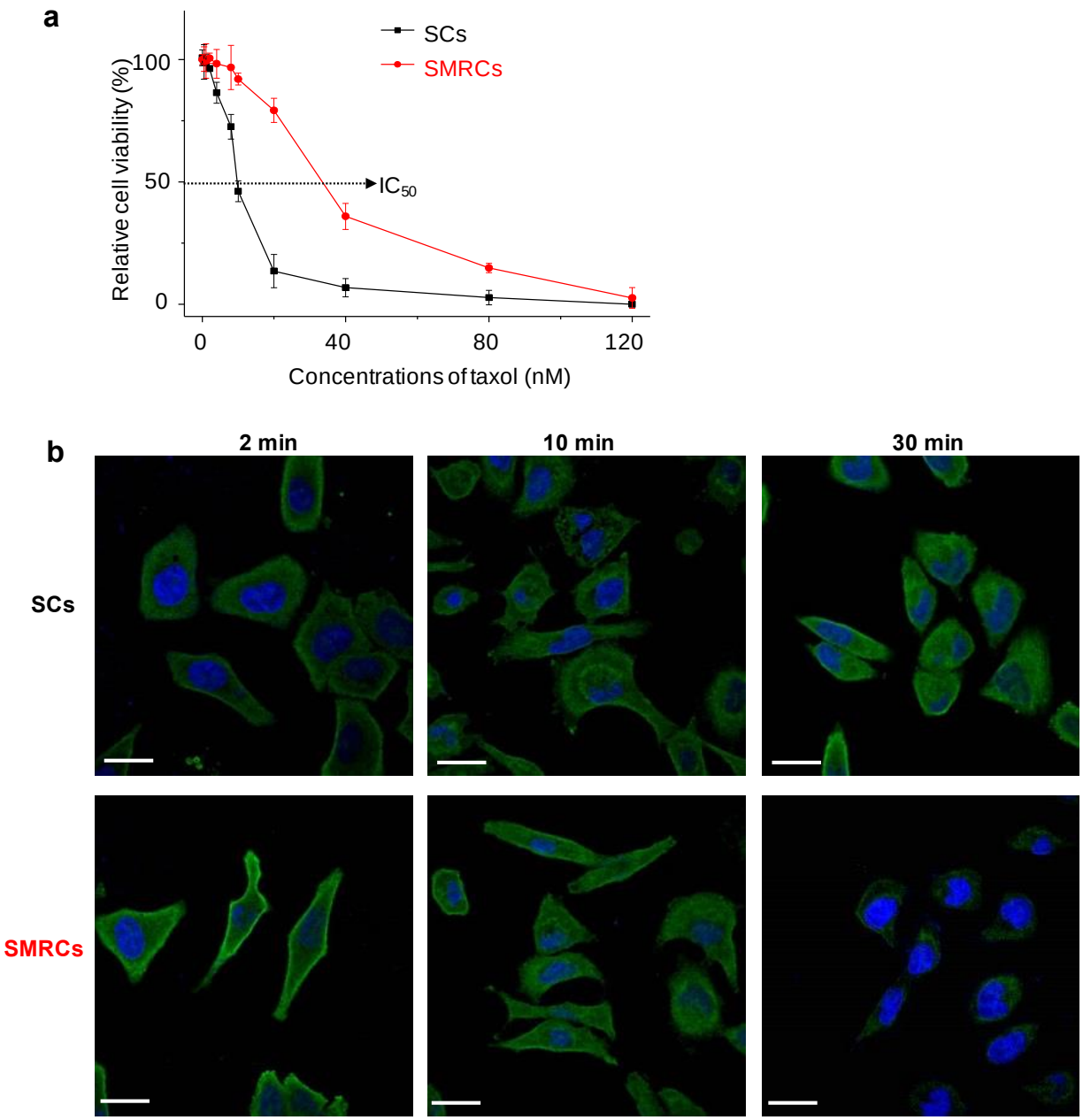
Supplementary Figure 19 | MRCs promote metastasis *in vivo*. a, Representative images of H&E staining of tissues in different organs from mice subjected to tail vein injection with SCs and SMRCs, respectively. **b**, Representative images of H&E staining of tissues in different organs from mice subjected to tail vein injection with SMRCs in which MRC mechanical properties were inhibited by Blebbistatin (50 μM) before co-incubation. Scale bar, 500 μm.



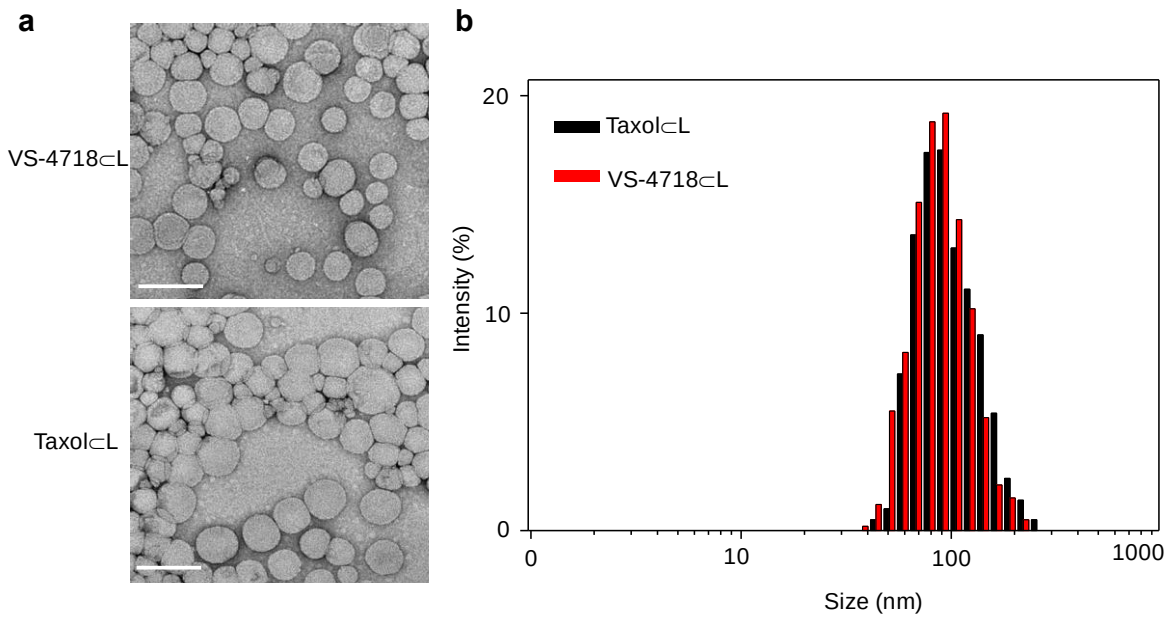
Supplementary Figure 20 | The microfluidic system demonstrated the enhanced adhesion force of SMRCs in microfluidic channels. a, Schematic illustration of microfluidic device combined with microscopy which was utilized to mimic the vascular system. The microfluidic substrate modified with FN for arresting cells by integrin-based adhesion. **b**, The number of adhered cells under different shear force in microfluidic substrates. **c**, Schematic illustration of SMRC-cluster formation in microfluidic channel. **d**, Percentage of clusters in the adhered cells in microfluidic substrates recorded by microscopy.



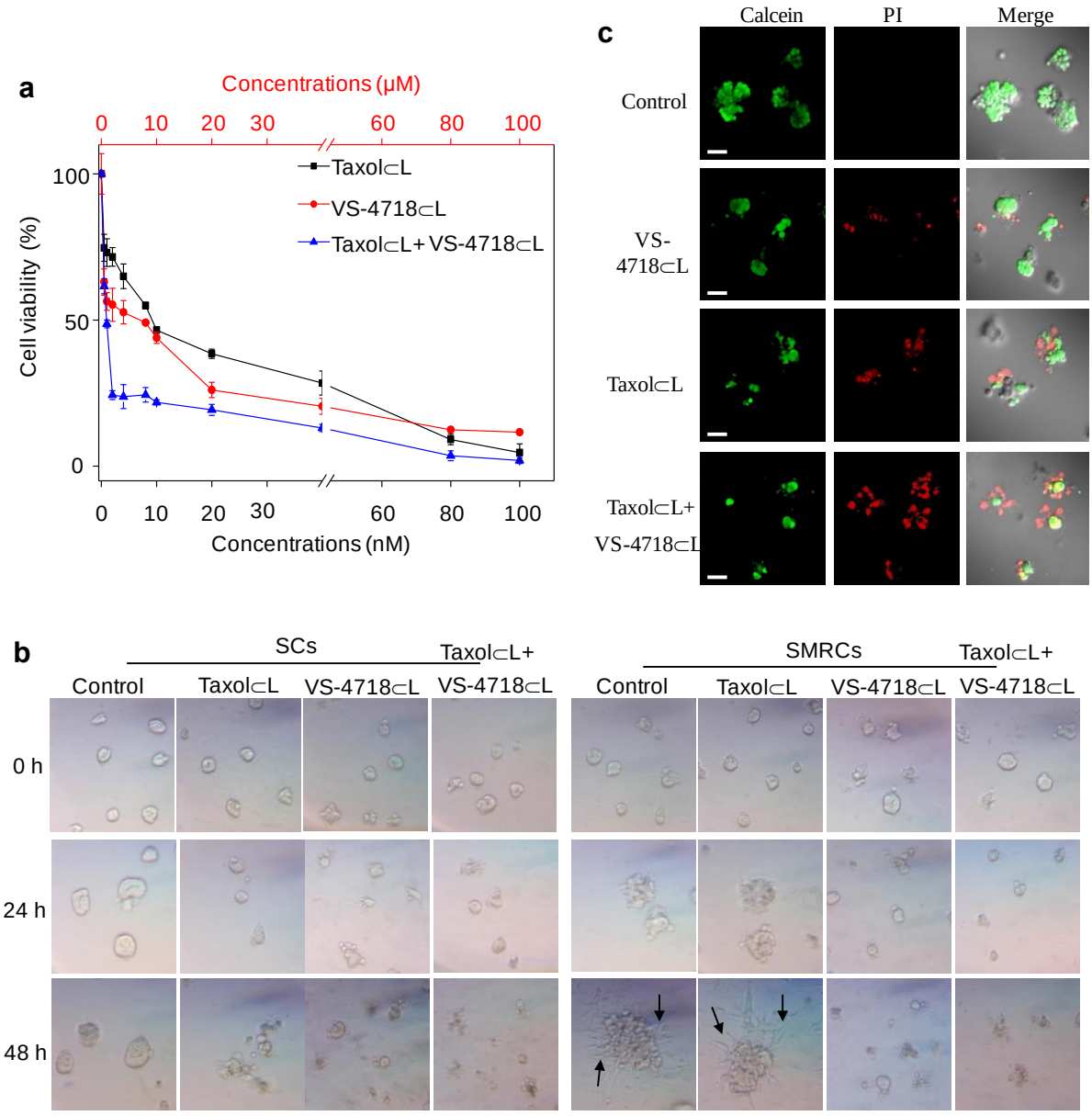
Supplementary Figure 21 | The enhanced mechanical properties of SMRCs in metastatic foci. The tumor tissues of mice were stained with phalloidin. Scale bar, 500 μm.



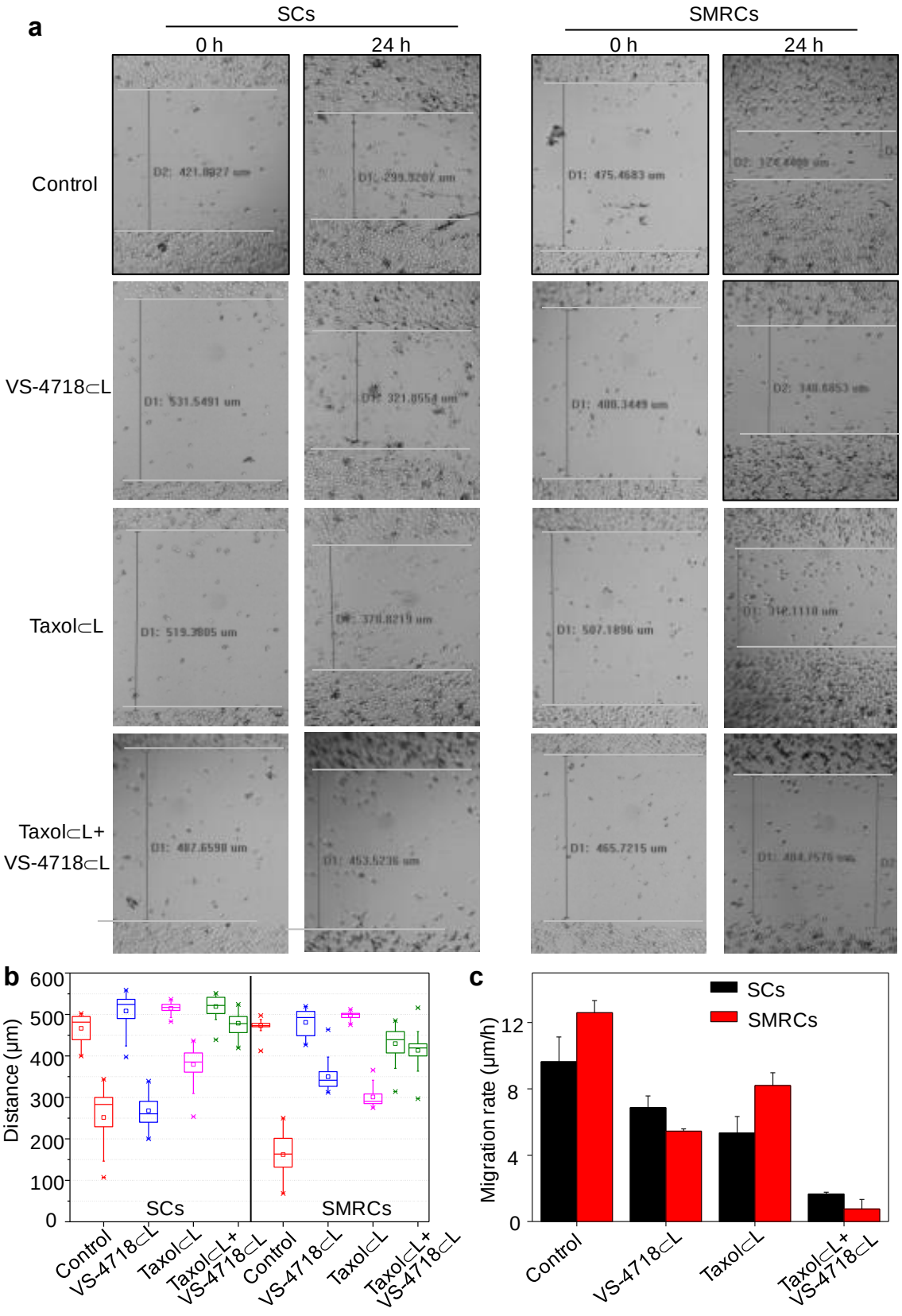
Supplementary Figure 22 | SMRCs obtained the drug resistance through alteration of growth and shrinking of microtubules. a, Relative cell viability of cells (SCs and SMRCs) cultured with different concentrations of Taxol for 24 h. n = 3 replicates. Data are mean ± s.d. **b**, Representative the dynamics of microtubules in the SCs and SMRCs. Scale bar, 20 μm. Tubulin (green) and nuclei (blue).



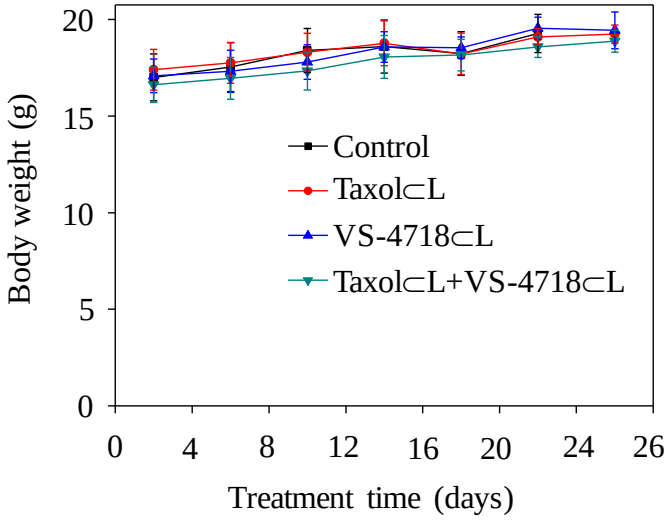
Supplementary Figure 23 |Characteristics of the synthesized nanoparticles. a and **b,** TEM images (**a**) and the hydrodynamic size distribution (**b**) of taxol⊂L and VS-4718⊂L nanovesicles. Scale bar = 200 nm.



Supplementary Figure 24 |Cell viability and proliferation in 2D and 3D culture system. a, Relative cell viability of SC cells after treatments with taxol⊂L, VS-4718⊂L, together with taxol⊂L+VS-4718⊂L, compared with PBS. **b**, Microscopy images of time-dependent 3D tumor sphere (SCs and SMRCS) growth under different treatments. Arrow in SMRC tumor sphere treated with PBS or taxol⊂L represented the invading cells from tumor sphere to surrounding environment. **c**, Confocal images of the SC-tumor sphere growth and activity, detected through the live/dead cell staining. Scar bar = 200 nm.


a
SCs
SMRCs
0 h
24 h
0 h
24 h
Control
VS-4718⊂L
Taxol⊂L
Taxol⊂L+
VS-4718⊂L
b
Distance (μm)
600
500
400
300
200
100
0
SCs
SMRCs
Control
VS-4718⊂L
Taxol⊂L
Taxol⊂L+
VS-4718⊂L
Control
VS-4718⊂L
Taxol⊂L
Taxol⊂L+
VS-4718⊂L
c
Migration rate (μm/h)
12
8
4
0
SCs
SMRCs
Control
VS-4718⊂L
Taxol⊂L
Taxol⊂L+
VS-4718⊂L

Supplementary Figure 25 | The cell migration was inhibited after together treated with taxol⊂L and VS-4718⊂L. a, Representative images of cells migration across of wound space recorded by microscopy. Cells were incubated on the 6-well plates and a thin wound was introduced by scratching with a pipette tip in the middle of the plates, then the cells treated with PBS, taxol⊂L, VS-4718⊂L or together with taxol⊂L+VS-4718⊂L for 24 h. Cells at the wound edge migrated into the wound space. **b**, Statistical results of cell migration distance based on microscopy records. The two statistical boxes under each group were the wound width before (first) and after (second) migration. **c**, Statistical results of cell migration rates based on microscopy records from the results of wound healing assay. Data are shown as the mean ± s.d., * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, Student's t-test.



Supplementary Figure 26 | Body weight of mice with different treatments.

Reference

1. Sun, S. et al. Monodisperse mfe2o4 (m= fe, co, mn) nanoparticles. *J. Am. Chem. Soc.* **126**, 273-279 (2004).
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Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials **67**, 328-333 (2003).
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