Electronic Supplementary Information for

Non-toxic core-shell nanowires for in vitro extracellular vesicle scavenging

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MATERIALS AND METHODS

Cell culture

For culturing MDA-MB-231 (human breast adenocarcinoma cells, American Type Culture Collection), Dulbecco's Modified Eagle Medium (DMEM, Lonza) and 10 % (v/v) exosome-depleted fetal bovine serum (FBS, System Biosciences) were used for the complete growth media. The suspension of 2×10^6 cells/mL was dispersed in a T75 flask with 15 mL of the prepared media and cultured in an incubator (Sanyo) at 37 °C and an atmosphere containing 5 % CO_2. For culturing MCF10A (mammary gland epithelial cells, American Type Culture Collection), the 1×10^6 cells/mL suspension was dispersed in a T25 flask with 5 mL of the Mammary Epithelial Cell Growth Medium (MEGM™, Lonza), and cultured in an incubator (Sanyo) at 37 °C and an atmosphere containing 5 % CO_2. Mammary Epithelial Cell Growth Basal Medium (MEBM™, Lonza) was used for the validation of abnormal cell growth of MCF10A (Fig. S2)

EV collection

After culturing for 48 h, the supernatant of the MDA-MB-231 cells was collected and filtered through a 0.22 μm filter (Merck Millipore) to remove cellular debris. Next, the filtered solution was ultracentrifuged (80 min, 4 °C, 110000 g; CS150FNX, Hitachi) to extract EVs. The supernatant was discarded and 10 mL of 0.22-μm filtered PBS was added to wash the EVs. Then, ultracentrifugation was performed one more time under the same conditions. After discarding the supernatant, 1 mL of 0.22- μm filtered PBS or 1 mL of MEGM™ was added to disperse the EVs, and the EV suspension was stored at 4 °C. The size and concentration of the EVs were measured using a nanoparticle tracking analyzer (NanoSight NTA, Malvern Instruments). The zeta potential of the EVs was measured using a zeta potential analyzer (Zetasizer Nano ZS, Malvern Instruments).

EV-elimination device fabrication and characterization

First, a 15 nm chromium (Cr) layer was deposited onto a silicon substrate (20×20 mm^2) using an electron cyclotron resonance sputtering apparatus (Elionix, EIS-200ERT-YN) for ZnO nanowire seeding. The silicon substrate with the Cr layer was oxidized at 400 °C for 2 h, then immersed in 50 mL growth solution including 15 mM hexamethylenetetramine (HMTA, $C_6H_{12}N_4$, Fujifilm Wako Pure Chemical Corp.) and 15 mM zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, Thermo Fisher Scientific) at 95 °C for 3 h. The metal oxide layers, which included TiO_2 or SiO_2, were deposited on the ZnO nanowires by the atomic layer deposition (ALD) technique. Morphology and composition analyses of ZnO (bare), ZnO/TiO_2 (core/shell), and ZnO/SiO_2 (core/shell) nanowires were made with a field emission scanning electron microscope (FESEM; Jeol), and scanning transmission electron microscopy (STEM) and energy dispersive X-ray spectroscopy (EDS).

EV-elimination method

After setting up the EV-elimination device (Fig. S10), 1 mL of PBS containing dispersed EVs or 1 mL of media containing dispersed EVs was supplied to the device and the EVs were incubated at room temperature for 1, 3, 6, and 12 h while being mechanically shaken. Then, the solutions were recovered for further analyses, including proliferation degree assay. The size and concentration of EVs after incubation were also measured with the nanoparticle tracking analyzer (NanoSight NTA, Malvern Instruments).

Cell proliferation assays

The MCF10A cells were seeded in a 96-well plate (Corning) with a density of 5×10^3 cells/well and incubated at 37 ºC in an atmosphere containing 5 % CO_2. After culturing for 24 h, we replaced the culture media separately with ultracentrifuged EVs or recovered EVs, i.e., the recovered solution from the EV-elimination device. For no EV condition, we continued to culture the cells in the same media. The cell viability was observed using a cell counting kit-8 (Dojindo) on the first and fifth days after

seeding. The absorbance was measured at 450 nm in a microplate reader (POLARstar OPTIMA, BMG Labtech).

In vitro fluorescence imaging of EVs diffused in MCF10A cells

The ultracentrifuged EVs from the MDA-MB-231 cells were labeled using a PKH26 red fluorescent cell linker kit (Sigma-Aldrich). The MCF10A cells were seeded into a 33 mm diameter dish at 5×10^3 cells/200 μL. After culturing for 24 h, we replaced the media with a 20 μL suspension containing labeled EVs in 200 μL of DMEM and 10 % (v/v) exosome-depleted FBS. After that, the cells were cultured for 24 h. Then, the cells were fixed with 10 % (w/v) paraformaldehyde solution (Fujifilm Wako Pure Chemical Corp.), followed by nuclei staining with Hoechst33342 fluorescent dye (Dojindo Molecular Technology) and cytoskeleton Alexa488 fluorescent dye (Thermo Fisher Scientific). Cell morphology and composition were observed using a confocal microscope (Nikon).

EV-derived miRNA sequences

A 1 mL volume of cell lysis buffer (Fujifilm Wako Pure Chemical) was added to the suspended EVs obtained from the MDA-MB-231 cells and this mixture was incubated at room temperature for 5 min. The miRNAs were extracted using a SeraMir Exosome RNA purification column kit (System Biosciences). Finally, miRNA types were analyzed using a next-generation sequencing instrument (HiSeq® 2500/4000, Illumina).

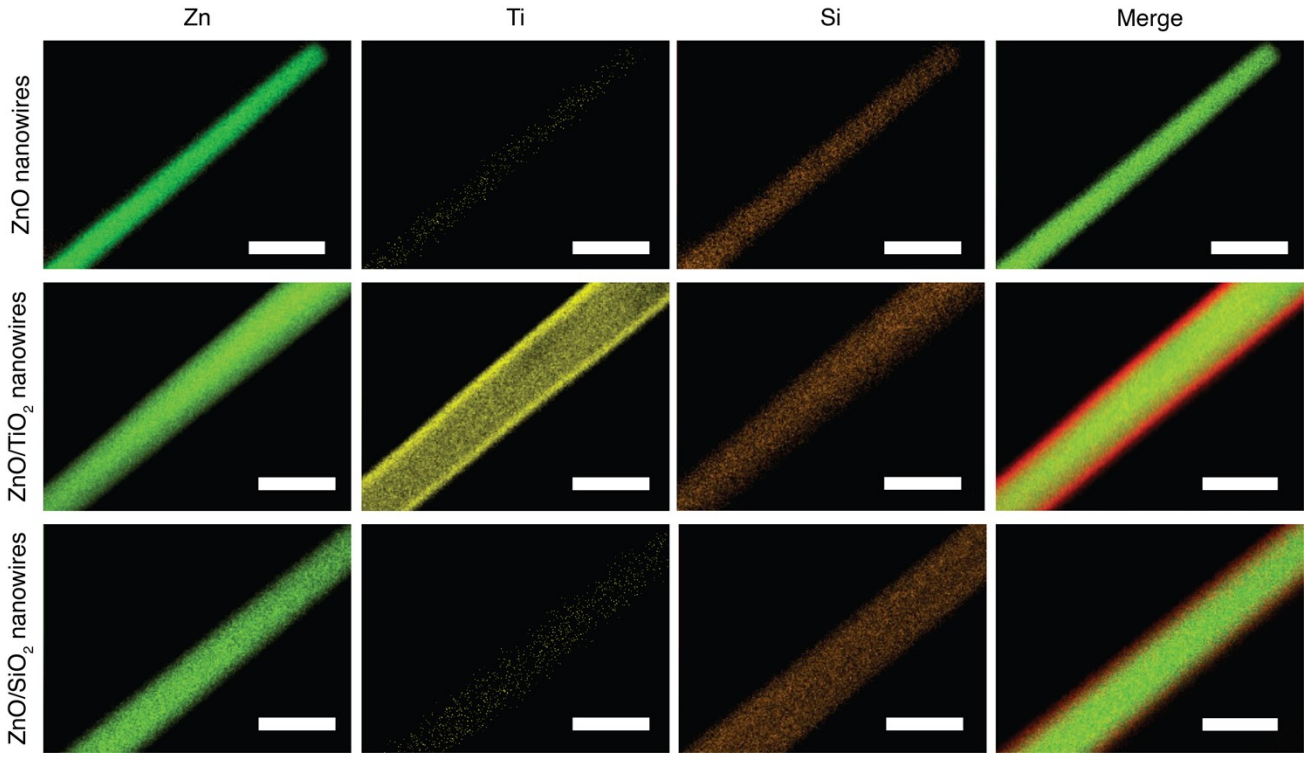

Fig. S1 STEM-EDS elemental mappings of ZnO, ZnO/TiO_2 (core/shell), and ZnO/SiO_2 (core/shell) nanowires. The scale bars are 200 nm, respectively.

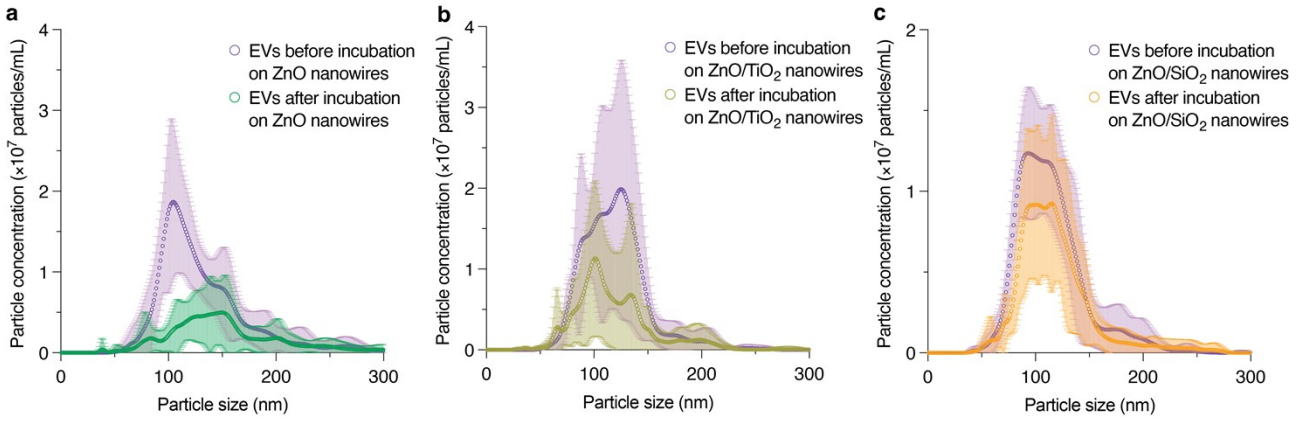

Fig. S2 EV size distribution before and after incubating in our EV-elimination device.

Size distributions of EVs before and after incubating on (a) ZnO nanowires, (b) ZnO/TiO_2 (core/shell) nanowires, and (c) ZnO/SiO_2 (core/shell) nanowires inside the block. Data represent mean ± standard deviation (n = 3).

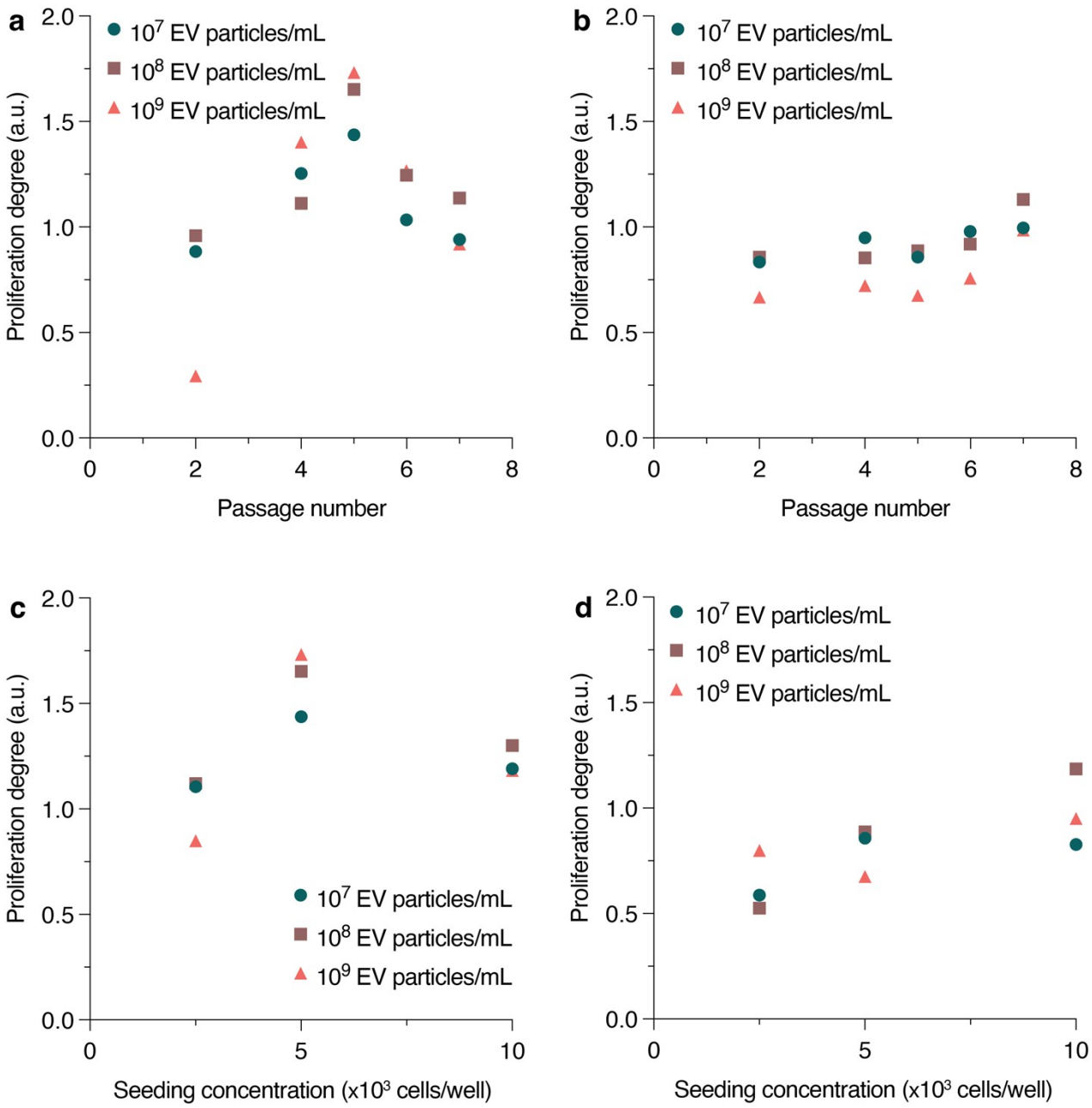

Fig. S3 Cell proliferation evaluation.

The MCF10A cells were cultured for various conditions, including cell culture media, cell passage number, cell seeding condition, and EV concentration. (a) 5000 seeding cells in MEBM. (b) 5000 seeding cells in DMEM. (c) Passage number 5 in MEBM. (d) Passage number 5 in DMEM.

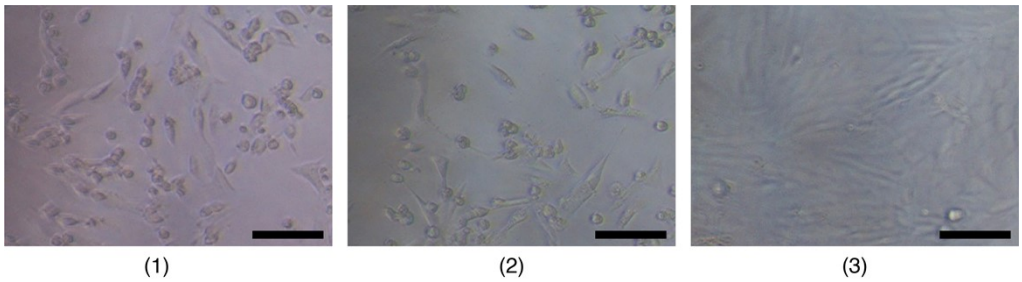

Fig. S4 The microscopic images show MCF10A cells for each culture condition in Fig. 4a. The scale bars are 100 μm.

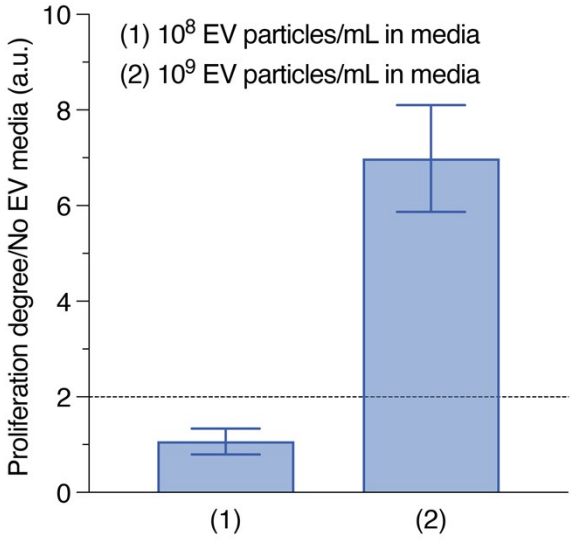

Fig. S5 Cell proliferation ratios of MCF10A cell culturing with different EV concentrations in media.

Data represent mean ± standard deviation (n = 3). The horizontal dash line shows the proliferation ratio of 2.

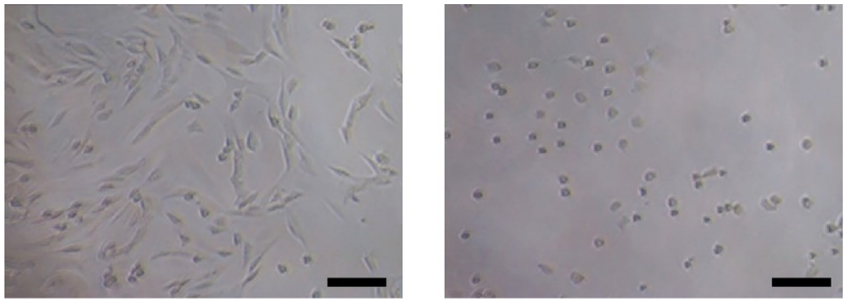

Fig. S6 The microscopic images show MCF10A cells for each culture condition in Fig. 5a. The scale bars are 100 μm.

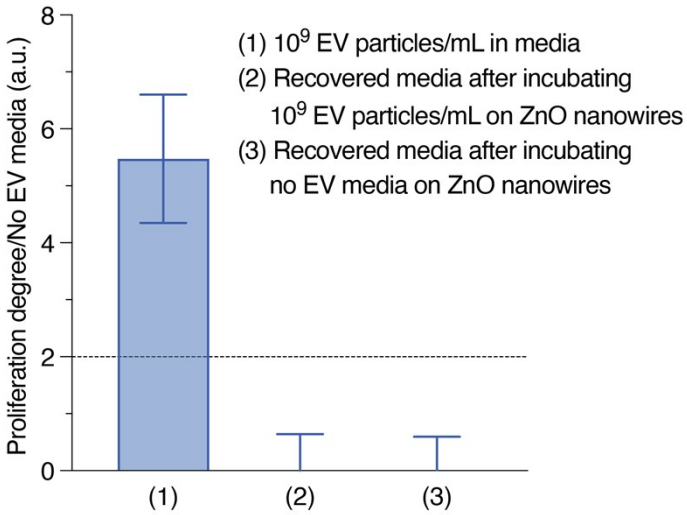

Fig. S7 Cell proliferation ratios of MCF10A cell culturing with no EV media and culturing with recovered media after incubating on ZnO nanowires.

Data represent mean ± standard deviation (n = 3). The horizontal dash line shows the proliferation ratio of 2.

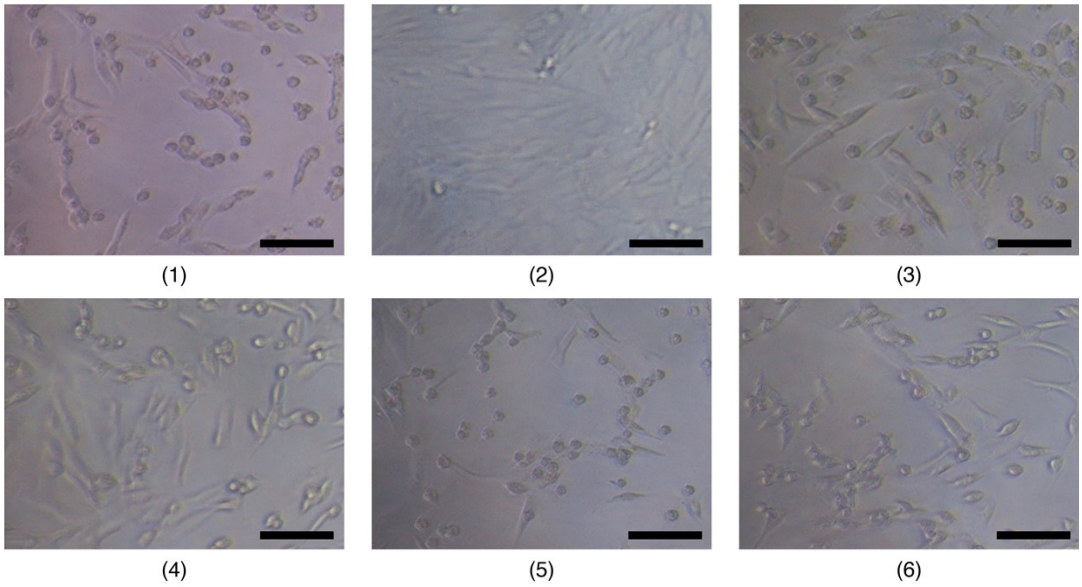

Fig. S8 The microscopic images show MCF10A cells for each culture condition in Fig. 5b. The scale bars are 100 μm.

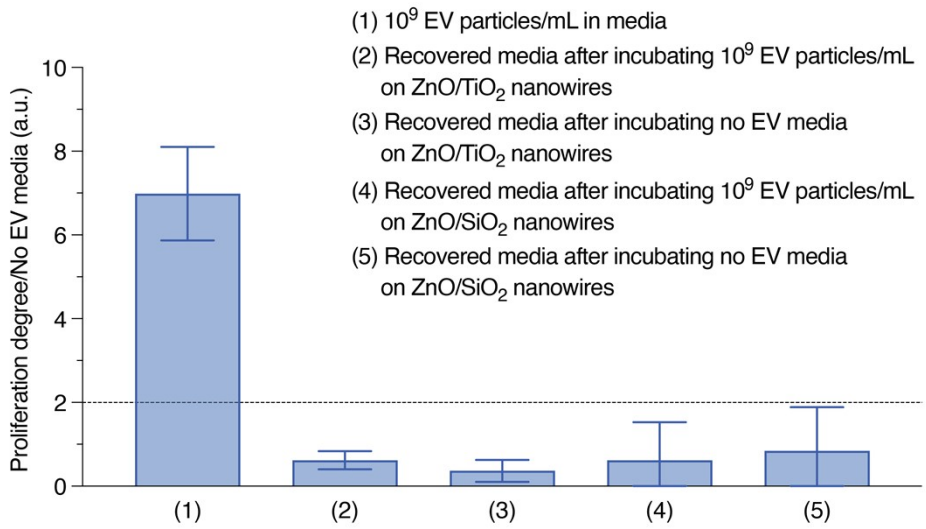

Fig. S9 Cell proliferation ratios of MCF10A cell culturing with no EV media and culturing with the recovered media after incubating on ZnO/TiO_2 (core/shell) or ZnO/SiO_2 (core/shell) nanowires.

Data represent mean ± standard deviation (n = 3). The horizontal dash line shows the proliferation ratio of 2.

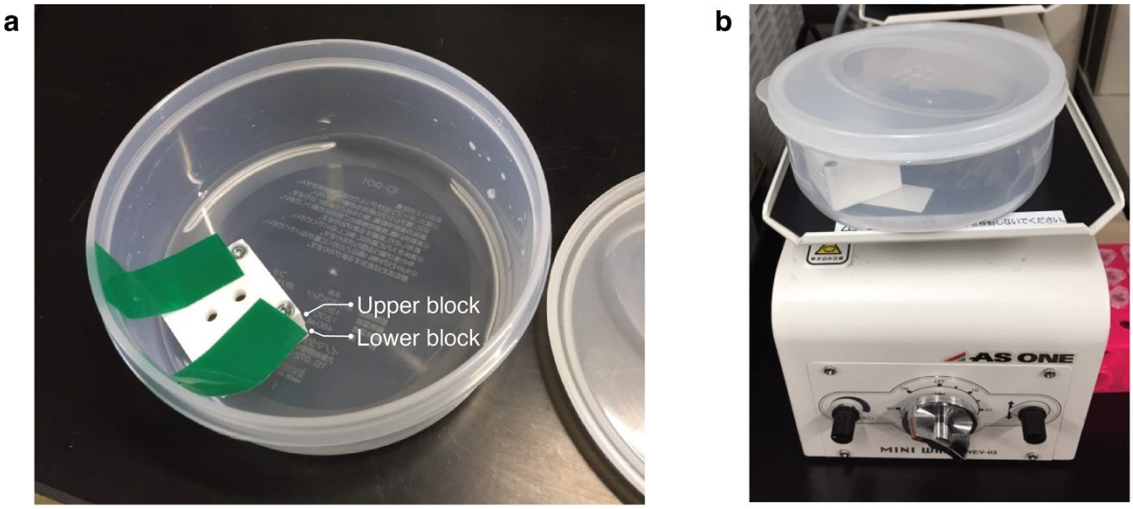

Fig. S10 Experimental setting of EV-elimination device.

(a) The nanowire substrate was set inside the Teflon block that included upper and lower blocks. The assembled Teflon block was set inside a plastic container, and the container was filled with ultrapure water to prevent solvent inside the device from evaporation. (b) The container was set on the mechanical shaker and shaken at room temperature during the incubation for different time periods.

Table S1 Oncogenesis miRNAs. The oncogenesis miRNA expression levels ordered from high to low and their tumor-related functions.

miRNA	Tumor-related function	Reference
miR-122-5p	Oncogenesis	1
miR-151a-3p	Oncogenesis	2
let-7f-5p	Oncogenesis	3
miR-21-5p	Oncogenesis	4
miR-184	Oncogenesis	5
miR-10a-5p	Oncogenesis	6

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