Supporting Information for:

DNA-Templated *in situ* Growth of AgNPs on SWNT: A New Approach for Highly Sensitive SERS Assay of MicroRNA

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EXPERIMENTAL SECTION

Materials. All oligonucleotides were synthesized by TaKaRa Biotechnology Co. Ltd. (Dalian, China). All chemicals were used as received, unless otherwise stated. Cell lines, MCF-7 (human breast cancer cell), HeLa (human cervical carcinoma cancer cell) and MCF-10A (mammary epithelial cell lines) were obtained from the American Type Culture Collection (Manassas, VA). Human serum, breast cancer tissue and normal breast tissue were provided by the Hunan Provincial Tumor Hospital, Central South University (China). SWNTs were purchased from Carbon Nanotechnologies, Inc. (Texas, USA). Streptavidin-coated silica microbeads (SiMBs, 5 μm) were purchased from Ocean Nanotech (USA) and dispersed at 0.1 mg/mL in 20 mM phosphate-buffered saline (PBS), pH 7.4. Ultrapure water obtained from a Millipore water purification system (18 M Ω) was used in all assays. SERS measurements were performed using a confocal microprobe Raman instrument (Ram Lab-3010, Horiba Jobin Yvon, France), and spectra were acquired using an 632.8 nm He-Ne laser and a 50×long working objective lens (8 mm). The AgNPs used in the work were characterized by UV-Vis absorption spectra on a Hitachi U-4100 UV/Vis spectrophotometer (Kyoto, Japan). Atomic Force Microscopy (AFM) images were performed on a SPI3800N-SPA400 (Seiko Instruments, Inc.). The size and shape of SiMBs under different conditions were determined by the transmission electron microscope (TEM) and scanning electron microscope (SEM) images obtained on JEM-100CXII microscope and JSM-6700F microscopes (JEOL, Ltd., Japan), respectively.

Preparation of DNA-Mediated SWNT@AgNPs. Noncovalent functionalization of SWNTs with ssDNA has been well established in earlier reports. The SWNT/ssDNA suspension was then centrifuged at 14000 rpm for 1 h to remove aggregates. The SWNT/ssDNA mixture solution was diluted to 1.0 mL with 20 mM Tris-HNO_3 buffer and aliquot of stock solution of silver nitrate ($AgNO_3$) was transferred into it. The solution was then incubated at 20 ^oC for 15 min to form the SWNTs/ssDNA/Ag^+ complex, following by centrifuged at 14000 rpm for 1 h and then washed with Tri-HNO_3 buffer for three times to remove free $AgNO_3$, and then stored at 4 °C for further usage. To synthesize DNA-mediated SWNT@AgNPs, aliquot of 10 μL of the SWNT/ssDNA/Ag^+ mixture solution was added to a 500 μL volumetric pipe with Tris-HNO_3 buffer. Then, 10 μL sodium borohydride ($NaBH_4$) (5 mM) was added to

the solution with vigorously vortex. After incubation for 100 min at room temperature, the ssDNA-mediated SWNT@AgNPs were formed.

Formation of DNA-Mediated SWNT@AgNPs on the Surface of SiMBs. The biotin-labeled assistant probe (AP) was first incubated with streptavidin-coated SiMBs. The mixtures were vortexed at room temperature for 1 h, followed by washing three times with 10 mM phosphate buffer (PBS containing 5 mM Mg^{2+}, pH 7.4) using centrifugation at 1 000 rpm to remove any DNA that did not conjugate to the surface of SiMBs. The conjugates were dispersed in PBS and stored at 4 ^{o}C at a concentration of 0.1 mg/mL. Then, the capture probe (CP) was added to the solution containing SiMB-conjugated AP (SiMB-AP), and the mixture was vortexed at room temperature for another 0.5 h to form SiMB-AP/CP, followed by washing three times with PBS using centrifugation at 1 000 rpm to remove any CP that did not hybridize with AP. The quantitative analysis of AP and CP on the surface of SiMBs were described in Supporting Information. Next, different concentrations of target were added to the solution containing SiMB-AP/CP, in this case, the specific interaction between target and the CP liberated the AP free following by centrifugation at 1 000 rpm to remove the complex of target and CP, thus SWNT could absorb on the surface of SiMBs efficiently by means of π-π stacking interaction. Finally, SWNT@AgNPs nanocomposite on the surface of SiMB was prepared in the same manner as mentioned above.

Preparation of AgNPs. All glassware was thoroughly cleaned overnight with freshly prepared 3:1 HCl/HNO_3 (aqua regia) and rinsed thoroughly with Mill-Q water prior to use. AgNPs were prepared following the reported protocols published by Lee and Meisel. Briefly, 100 μL of 100 mM $AgNO_3$ were added into 10 mL of water and cooled to ice cold temperature. Then, 30 mL of 2×10^{-3} M $NaBH_4$ solution was prepared. Under vigorous stirring and ice cold conditions, 10 mL of $AgNO_3$ solution were added dropwise to the above $NaBH_4$ solution. The stirring was stopped, and the clear yellow sol showed the characteristic surface plasmon absorption band of nanosized Ag particles at *ca.* 398 nm. The colloid was stored at 4 °C until use.

Quantitative analysis of AP/CP on the surface of SiMBs. The biotin-labeled AP was first incubated with streptavidin-coated SiMBs. The mixtures were vortexed at room temperature for 1 h, followed by washing three times with PBS using centrifugation at 1 000 rpm to remove any AP that did not conjugate to the SiMBs.

The absorption maximums (measured at 260 nm) of the supernatant, containing free AP removed from the SiMBs, were converted to molar concentrations of DNA by UV-Vis absorption using published sequence-dependent absorption coefficients. Finally, the average number of successfully conjugated oligonucleotides on the surface of SiMBs was obtained. The concentration of hybridized CP was also determined using the same method.

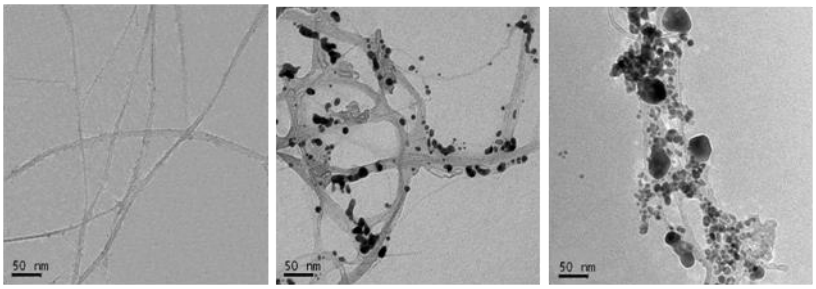
miRNAs extraction from cell lines and tissues. miRNAs were extracted using mirVana™ miRNA Isolation Kit according to the manufacture's protocol. The procedure described briefly as following: firstly, 300-600 μL lysis/binding solution was added to cell pellet and vortex vigorously to completely lyse the cells to obtain a homogenous lysate. Secondly, added 1/10 volume of miRNA homogenate additive and then incubated 10 min on ice. After that, a volume of acid-phenol: chloroform that is equal to the lysate volume was added before addition of the miRNA homogenate additive, following by vortex for 30-60 second to mix, and centrifuged for 5 min at maximum speed (10,000 x g) at room temperature to separate the aqueous and organic phases. Finally, enrich small RNA and the experimental details are shown in Supporting Information and tissues isolation procedures are the same with cells'.

Small RNA enrichment. Firstly, add 1/3 volume 100 % ethanol, and mix thoroughly. Then pass the sample through a filter cartridge, and collect the filtrate. Add 2/3 volume 100 % ethanol and mix thoroughly, pass the mixture through a second filter cartridge, and discard the flow-through. Then, wash the filter with 700 μL miRNA wash solution followed by wash the filter twice with 500 μL wash solution. Finally, elute RNA with 100 μL 95°C elution solution or nuclease-free water. Fresh samples from human breast cancer and paired normal adjacent tissues (NATs, >2 cm from cancer tissue) were obtained from 1 healthy female volunteer and 4 female patients (aged from 45-60 years) in Cancer Hospital (Hunan, China). The fresh specimens were cut to ≤0.5 cm in at least one dimension, stored at 4 ºC for 24 h in RNAlater solution (Ambion Inc.), then at -20 ºC until further use.

Tissue histology. For histology analysis, normal tissues and tumor tissues were fixed in 10 % neutral buffered formalin and frozen sectioned into 5 micron thick slices, stained with hematoxylin & eosin (H&E), and were examined by a digital microscope (Leica QWin).

Table S1. Oligonucleotides Used in This Work*

Entry	Sequence (5′-3′)
ssDNA	ATCGTTATCAGACTGATGGTAT
P_1	TCAACATCAGTCTGATAAGCTA
P_2	biotin-TTTTTATGCTTATCAGACTGATGTTAG
P_3	biotin-TTTTTGTACTTATCAGACTGATGTATG
P_4	biotin-TTTTTATCGTTATCAGACTGATGGTAT
P_5	biotin-TTTTTGCTASATATCAGACTGATTGATG
miR-21	UAGCUUAUCAGACUGAUGUUGA
SM-21	UAGCUA AUCAGACUGAUGUUGA
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-143	UGAGAUGAAGCACUGUAGCUCA



(A) (B) (C)

Figure S1. Representative TEM images of the SWNT upon additions of different concentrations of ssDNA following by $AgNO_3$ and $NaBH_4$ addition. [SWNT]= 0.02 mg/mL, [$AgNO_3$]=100 μM, [$NaBH_4$]= 20 mM. The concentration of ssDNA in A, B and C are 0 nM, 10 nM and 50 nM, respectively. The concentration of SWNT is excessive.

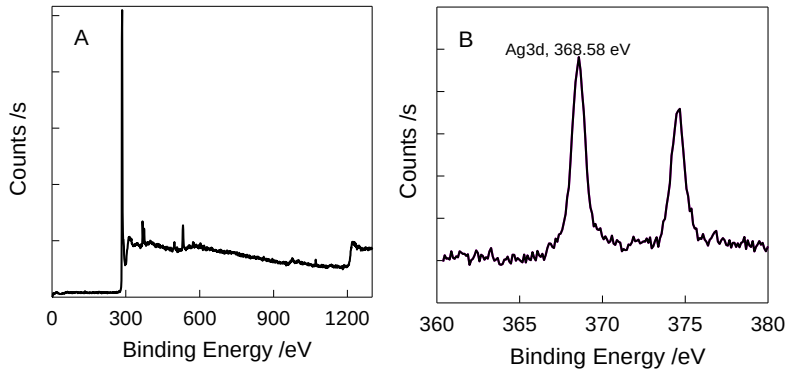


Figure S2. (A) XPS survey spectrum of ssDNA-mediated SWNT@AgNPs nanocomposites. (B) Silver 3d XPS spectra of SWNT@AgNPs nanocomposites.

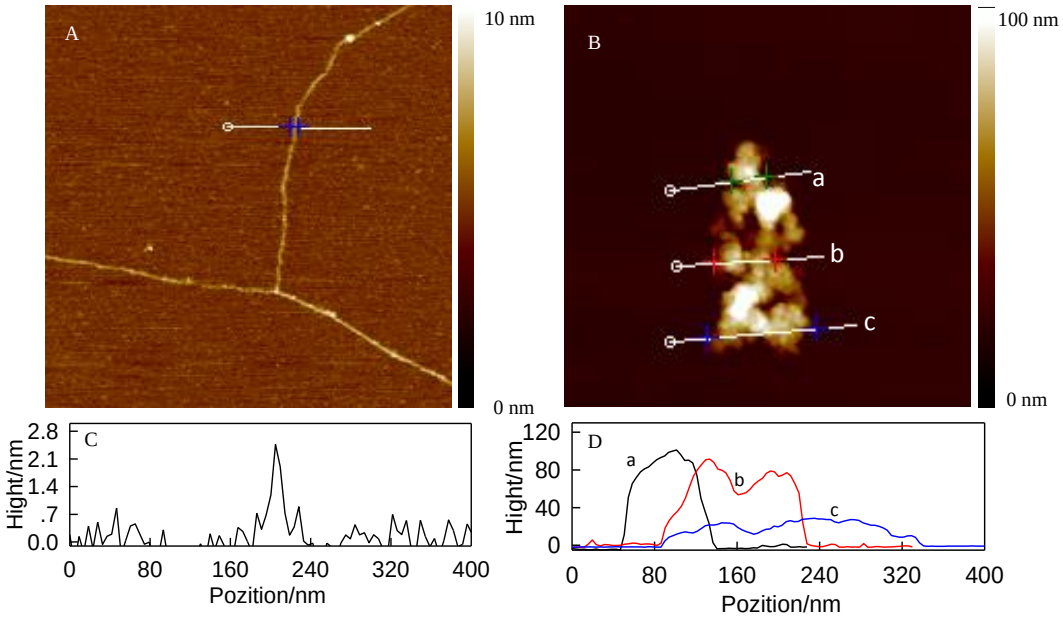


Figure S3. AFM topographic image of SWNT (A) and ssDNA-mediated SWNT@AgNPs nanocomposties (B), and (C) and (D) are the corresponding height profiles of SWNT (A) and the formed SWNT@AgNPs nanocomposites (B), respectively. The curve a, b and c in (D) represent a, b, c in (B), respectively. Scale area: 0.4×0.5 μm.

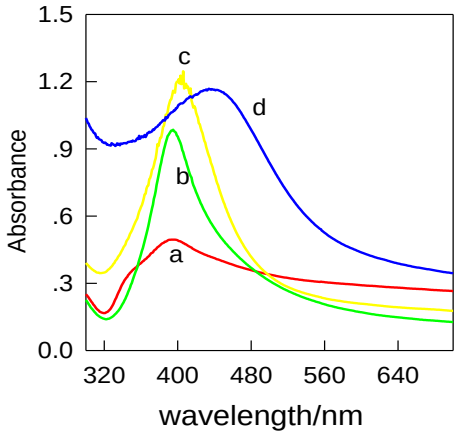


Figure S4. UV-Vis absorption spectra of SWNT@AgNPs nanocomposites mediated by different concentrations of ssDNA. [SWNT]= 0.02 mg/mL, [$AgNO_3$] =100 μM, [$NaBH_4$] = 20 mM. The concentration of ssDNA in a, b, c and d are 20 nM, 50 nM, 80 nM and 200 nM, respectively. The concentration of SWNT is excessive.

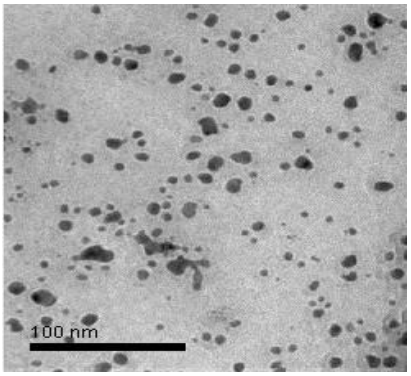


Figure S5. TEM image of free AgNPs. The scale bar is 100 nm.

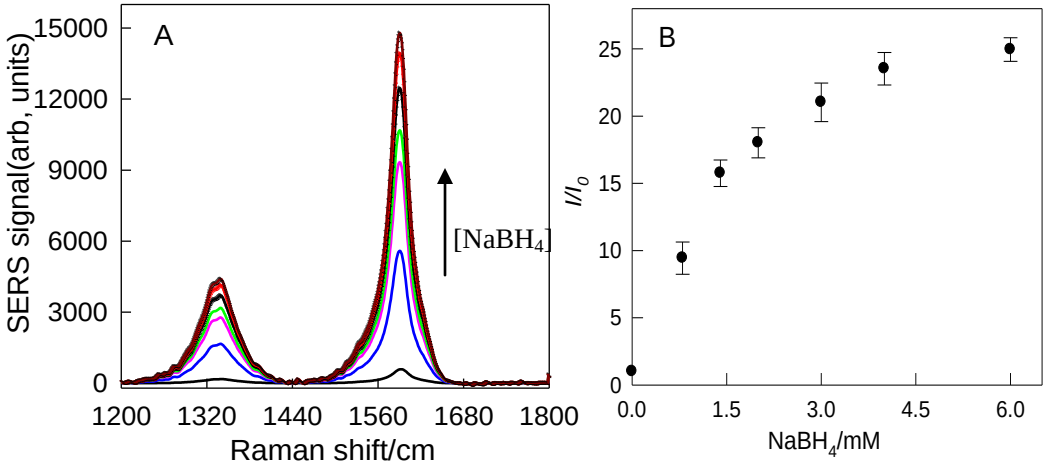


Figure S6. (A) Representative SERS of spectrum of the ssDNA-mediated SWNT@AgNPs nanocomposites obtained by varying the concentration of $NaBH_4$ in 20 mM Tris-HNO_3 buffer. (B) SERS intensity enhancements of the 1605 cm^{-1}-band of SWNT, I/I_0, plotted against the concentration of $NaBH_4$. All error bars were obtained through the detection of six parallel samples. [ssDNA]=300 nM. [$AgNO_3$]=100 μM. The concentration of SWNT is excessive.

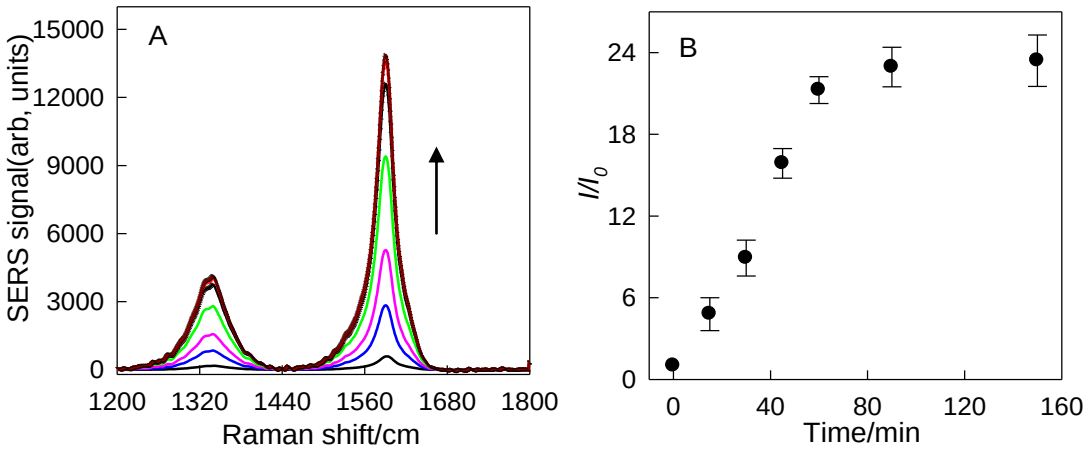


Figure S7. (A) Representative SERS of spectrum of the ssDNA-mediated SWNT@AgNPs nanocomposites obtained by varying the silver growth time. (B) SERS intensity enhancements of the 1605 cm^{-1}-band of SWNT, I/I_0, plotted against the silver growth time. All error bars were obtained through the detection of six parallel samples. [ssDNA]=300 nM. [$AgNO_3$]= 100 μM, [$NaBH_4$]= 6 mM. The concentration of SWNT is excessive.

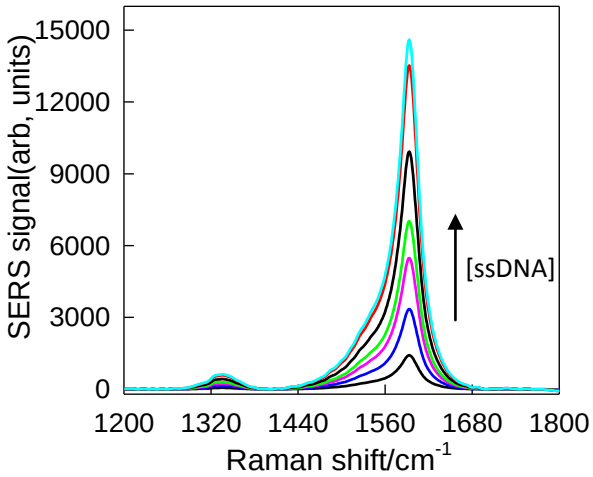


Figure S8. Representative SERS spectrum of the SWNT@AgNPs nanocomposites obtained by varying the concentration of ssDNA in 20 mM Tris-HNO_3 buffer. The arrow indicates the signal changes as ssDNA concentrations increase (0, 0.05, 1.0, 5.0, 10, 50 and 150 nM). [$AgNO_3$] = 100 μM, [$NaBH_4$]= 6 mM. The concentration of SWNT is excessive.

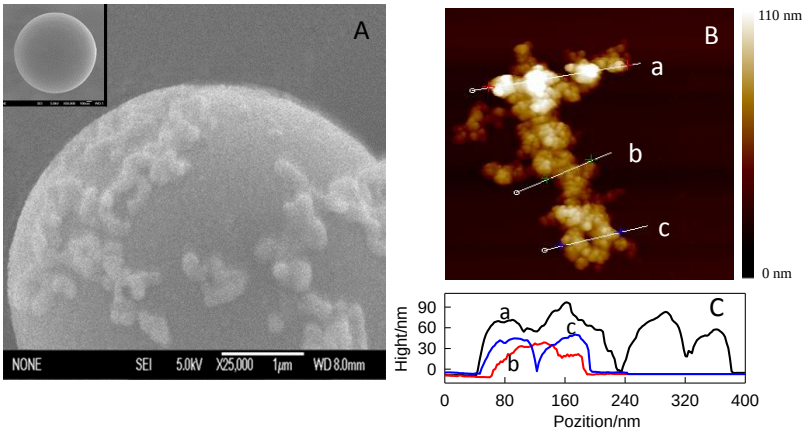


Figure S9. (A) Representative SEM image of the formed SWNT@AgNPs nanocomposites on the surface of SiMBs upon miR-21 addition. Inset: SEM images of the surface of SiMB-P_1/P_4 without miR-21 addition. (B) AFM topographic image of (A), and (C) is the corresponding height profiles of the formed SWNT@AgNPs nanocomposites upon miR-21 addition on the surface of SiMBs. The black, blue and red curve in (C) represents a, b, c in (B), respectively. The measuring conditions as shown in Figure 3. Scale area: 1×1.2 μm.

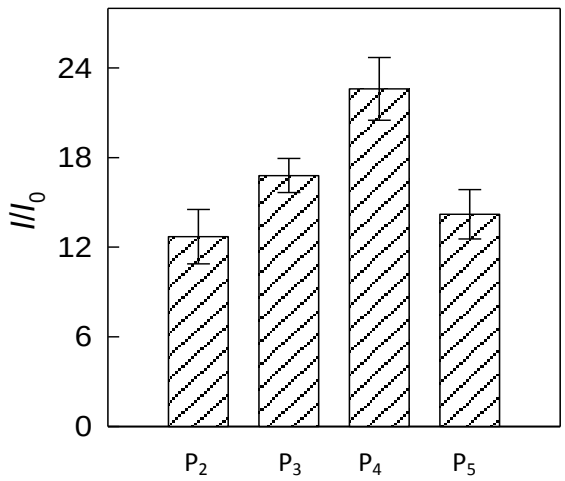
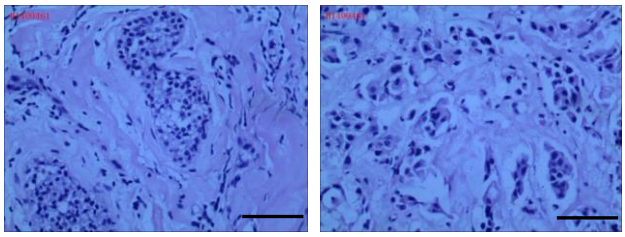


Figure S10. SERS signal enhancement (I/I_0) of the SWNT@AgNPs nanocomposites with different assistant DNA probes in the presence of 200 nM miR-21. The measuring conditions as shown in Figure 3. All error bars were obtained through the detection of six parallel samples.



(A) (B)

Figure S11. H&E staining of residual tissue from the lump in the left normal tissues (A) and invasive ductal carcinoma of the breast (B). Scale bar: 100 μm.

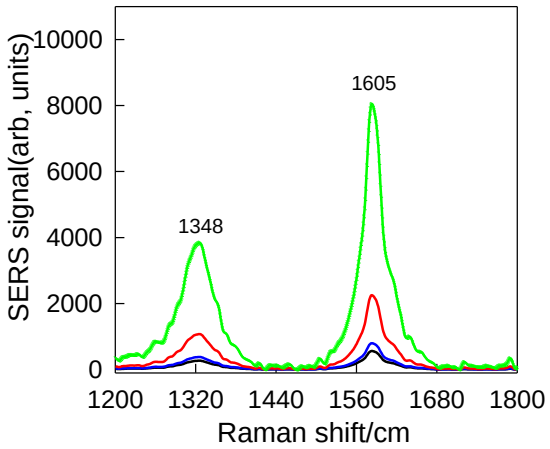


Figure S12. Representative SERS spectrum of the SWNT@AgNPs nanocomposites on the surface of SiMB upon miR-21 extracted from normal breast tissue (blue curve), peficancerous tissue (red curve), breast cancer tissue (green curve) addition. The spectrum of the SWNT@AgNPs nanocomposites without any miR-21 addition is shown as black curve. The measuring conditions as shown in Figure 3.

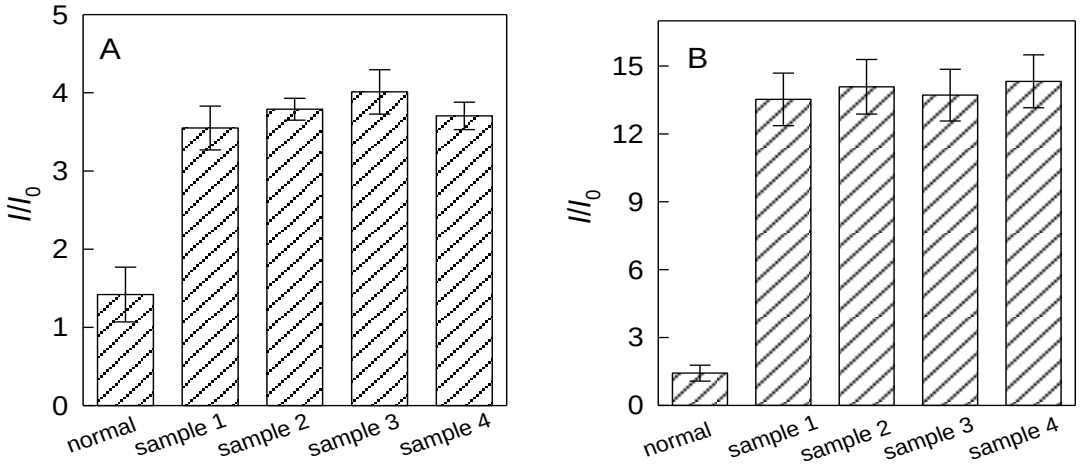


Figure S13. SERS signal enhancement (I/I_0) of the SWNT@AgNPs upon miR-21 extracted from different samples of breast peficancerous tissues (A) and cancer tissues (B) addition. The measuring conditions as shown in Figure 3. All error bars were obtained through the detection of six parallel samples.