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Electronic Supplementary Information

Establishing biodegradable single-layer MnO_2 nanosheet as a platform for live cell microRNA sensing

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EXPERIMENTAL SECTION

Reagents and solutions. The manganese chloride tetrahydrate ($MnCl_2$•$4H_2O$), hydrogen peroxide (H_2O_2, 30 wt%), tetramethylammonium hydroxide pentahydrate (TMA•OH), 3-(4,5-dimethylthialzol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and tris(hydroxymethyl)aminomethane were purchased from Alfa Aesar (Tianjin, China)., DMSO, KCl, NaCl, and $MgCl_2$ were obtained from Aladdin (Shanghai, China). N-ethylmaleimide was purchased from Sigma-Aldrich. All chemical reagents were of analytical grade and were used as received without further purification. All aqueous solutions were prepared using ultrapure water, which was prepared through a Millipore Milli-Q water purification system, with an electrical resistance >18.2 MΩ. The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co., Ltd (Shanghai, China) and purified using high-performance liquid chromatography. Their sequences were listed in Table S1. HeLa cancer cell line (human cervical carcinoma cell) was obtained from the cell center of Xiangya medical school. L02 normal cell line was obtained from the Shanghai cell bank, Chinese Academy of Medical Sciences.

Apparatus and characterization. Ultraviolet-visible light (UV-vis) absorption spectra were recorded on a UV-800 UV-vis spectrometer. Fluorescence spectra were collected using a Hitachi Model F-7000 Fluorometer. Transmission electron microscope image (TEM) was obtained on a F20 field-emission transmission electron microscope and an accelerating voltage of 200 KV. Zeta potential and DLS measurements were performed at 25 °C using a Nano ZS90 laser particle analyzer. Fourier transform infrared (FT-IR) spectra were obtained from a TENSOR 27 spectrometer. The MTT assay was obtained in a Benchmark Plus, Biorad Instruments Inc. The confocal laser scanning microscopy (CLSM) images were obtained on a Fluoview FV500, Olympus.

Synthesis of single-layer MnO_2 nanosheets (SLMONs). SLMONs were synthesized according to a literature procedure reported previously. In a typical reaction, 20 mL of mixture containing 0.6 M TMA•OH and 3.0 wt% H_2O_2 were added to 10 mL aqueous solution of 0.3 M $MnCl_2$•$4H_2O$ within 15 s. The resulting mixture a dark brown

suspension was formed, then the mixture solution was stirred vigorously overnight in the open air at room temperature, which was accompanied by the generation of oxygen. Subsequently, the crude product was collected by centrifugation at 10000 rpm for 10 min, washed with ethanol and water several times. Finally, the purified solid (30 mg) was dispersed in 30 mL ultrapure water and was degraded by ultrasonic cleaning machine and ultrasonic cell crasher to form SLMONs with small size.

Immobilization of MB probe on the surface of SLMONs. An amount of 100 μL of the obtained SLMONs (0.1 mg mL^{-1}) was mixed with 10 μL of MB probe (100 μM) and sonicated for 30 min at room temperature in shaker to prepare SLMONs/MB conjugates. The adsorption ability of SLMONs toward MB probes was investigated by the hairpin DNA labelled with FAM dye. The immobilizing amount of hairpin DNA was calculated from the difference in the concentration of the initial and left hairpin DNA by UV-vis spectroscopy. In order to show the protective properties of SLMONs, the SLMONs/MB conjugates were treated with DNase I (1 unit) for 15 min. Samples were then analyzed by the fluorescence spectrophotometer. As a comparison, the free MB probe was treated with DNase I with the same procedure.

In vitro DNA detection. To investigate the detection of target miRNA-21 (miR-21) in vitro, its DNA analog was employed to replace the target miR-21. The in vitro DNA detection protocol involved the duplex formation between the MB probe released from the SLMONs surface and the DNA analog of miR-21 added to the mixed solution. To perform these experiments, SLMONs/MB solution was prepared as 2 μg mL^{-1} (5 nM loaded MB) in Tris-HCl buffer (pH 7.5, 100 mM NaCl, 5 mM KCl and 5 mM $MgCl_2$) at room temperature. Various concentrations (0-2000 pM) of the synthetic target DNA was added to the hybrid nanocomplex solution. After that, the sample was collected and the fluorescence signal was measured using a fluorometer. In addition, 100 pM of the mismatched DNA sequences were added to the hybrid nanocomplex solution to evaluate the selectivity of probe.

Cytotoxicity assay of SLMONs. HeLa cells (1.0×10^4) were cultured at 37 °C for 12 h in a 96-well microplate. Subsequently, the culture medium was removed, and the RPMI 1640 medium (100 μL) or medium containing various concentrations of

SLMONs was added in each well. After being incubated for 24 h, MTT solution (0.5 mg mL^{-1}) was added to each well and then the cells were incubated for another 4 h. After that, the MTT medium was removed, and DMSO (100 μL) was added to dissolve the formazan crystal. Absorbance values of of each well were detected using a Bio-Rad model-680 microplate reader at 570 nm. The cytotoxicity of SLMONs was estimated by the cell viability calculated with the formula. Viability = $(A_{treated}/A_{control}) \times 100\%$, where $A_{treated}$ was obtained from the cells treated by nanocomposites and $A_{control}$ was obtained from the cells without any treatments.

MiR-21 sensing in living cancer cells. HeLa cancer cells (1.0×10^4) were incubated for 12 h in 24-well plates containing RPMI 1640 medium (1.0 mL) with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere with 5% CO_2. To determine the expression level of target miR-21 in living cancer cells, the fresh RPMI 1640 medium (1.0 mL) containing SLMONs/MB (10 μg mL^{-1}, 25 nM loaded MB) was added to the each well. After incubating for 3 h, the cells were washed with PBS buffer (0.1 M, pH = 7.4). The fluorescence signal of each well was collected by the flow cytometer. The fluorescence activation caused by the hybridization interaction between the MB probe and the target miR-21 was investigated by CLSM. HeLa cells were also treated with SLMONs only (10 μg mL^{-1}, unloaded MB) and then investigated by CLSM. In order to reduce the concentration of free miR-21 in living cells, the inhibitor probe was used and loaded onto the SLMONs to yield SLMONs/IP. Then, SLMONs/IP (10 μg mL^{-1}, 25 nM loaded IP) was added to the medium containing HeLa cells and incubated for 4 h prior to the addition of SLMONs/MB. As a control, L02 normal cells were incubated with different nanoprobes under the same conditions. To correlate fluorescence intensity from our probe with actual amount of induced miR-21 in the HeLa cells, inhibitor probe was treated to miR-21expressing HeLa cells. The SLMONs/MB complex was treated to cells following inhibitor treatment at indicated time point and intervals. Fluorescence signals of FAM were monitored with a flow cytometer.

Prior to imaging experiments, HeLa cells were treated with N-ethylmaleimide (GSH scavenger, 500 μM) for 20 min. Furthermore, the cells were incubated with 1, 2-bis-

(2-pyren-1-ylmethylamino-ethoxy) ethane (NPEY) and SLMONs/MB probes for 3h, washed using cell culture media and subsequently imaged at ambient temperature.

Table S1. Oligonucleotides used in this study

Oligonucleotide	**Sequence (5'→3')**
Target miR-21	UAG CUU AUC AGA CUG AUG UUG A
DNA analog of miR-21 (DNA1)	TAG CTT ATC AGA CTG ATG TTG A
1-Base mismatched DNA (DNA2)	TAG CTT AT**A** AGA CTG ATG TTG A
2-Base mismatched DNA (DNA3)	TAG CTT AT**A** AGA CT**A** ATG TTG A
Random DNA (DNA4)	CAG TGG TTT TAC CCT ATC ATA G
Molecular beacon probe for miR-21 (MB)	FAM- CCG GGT TCA ACA TCA GTC TGA TAA GCT A ACC CGG -Dabcyl
Control MB probe (cMB)	FAM- CCG GGT CTA TGA TAG GGT AAA ACC ACT G ACC CGG -Dabcyl
FAM-labelled hairpin DNA (hDNA)	FAM- CCG GGT TCA ACA TCA GTC TGA TAA GCT A ACC CGG
Inhibitor probe (IP)	TCA ACA TCA GTC TGA TAA GCT A

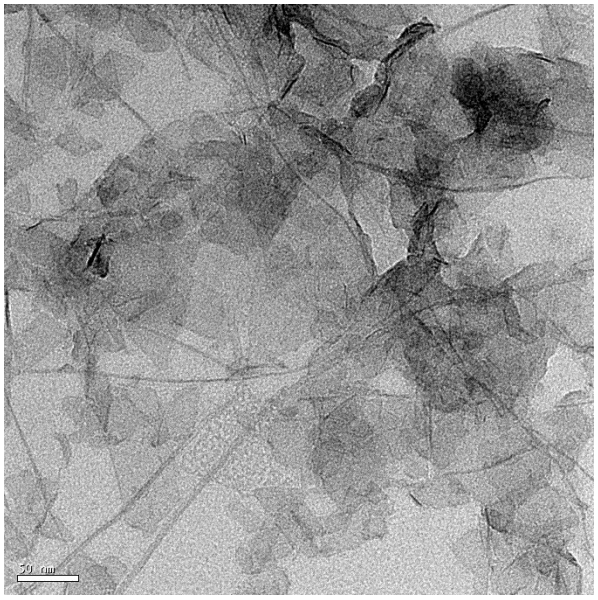

Fig. S1 TEM image of SLMONs.

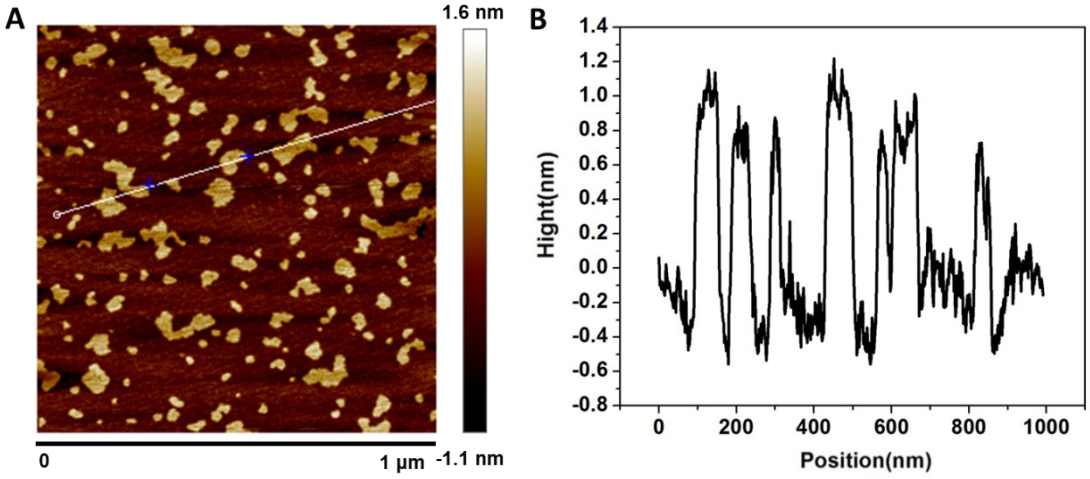

Fig. S2 AFM image of SLMONs deposited on mica substrate.

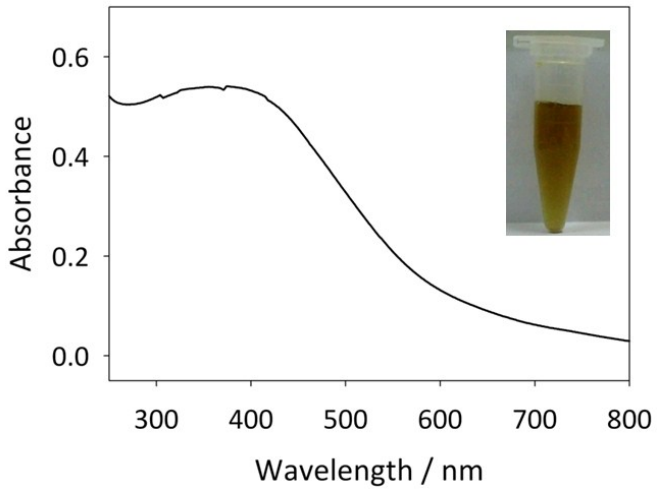

Fig. S3 UV-vis absorption spectra of the SLMONs in water. Inset: Photograph of the SLMONs solution under visible light.

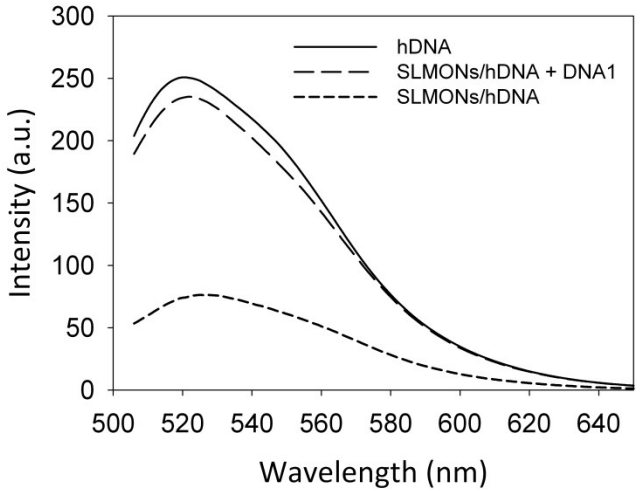

Fig. S4 Fluorescence emission spectra of dye-labelled hDNA probe (5 nM) before and after immobilization onto the surface of SLMONs.

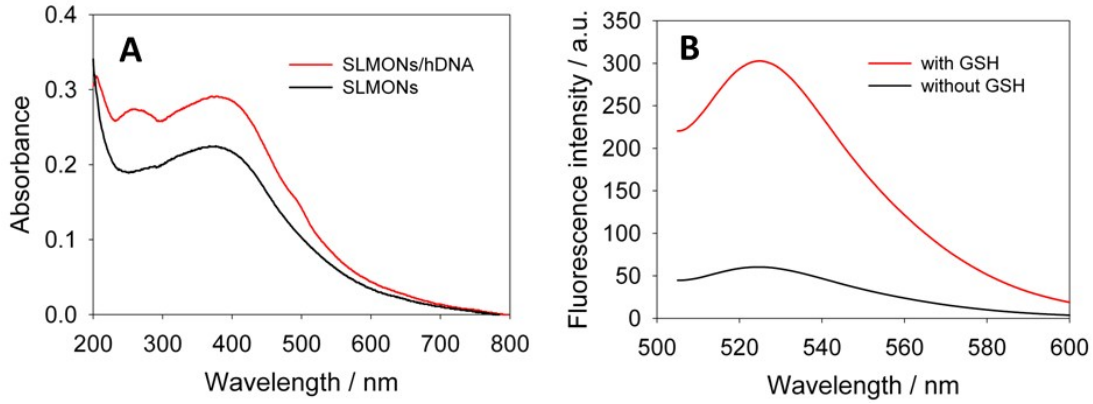

Fig. S5 (A) UV-vis spectra of SLMONs before and after the adsorbtion of FAM-labelled hDNA. (B) Fluorescence intensity the supernatant solution of SLMONs/hDNA in the absence and presence of GSH.

The adsorption of SLMONs towards FAM-labelled hDNA was investigated by UV-vis absorption spectra. UV-vis spectra demonstrated a characteristic absorbance peak at 260 nm from DNA and the wide band in the range of 310-500 nm with a peak located at 380 nm from MnO_2 (Fig. S5A). This result proved the formation of SLMONs/hDNA nanocomplex. Fig. S5B showed that the fluorescence of FAM-labelled hDNA adsorbed on the surface of SLMONs was significantly quenched after immobilization, further confirming the strong interactions between the SLMONs and DNA. In the presence of GSH, SLMONs can be degraded and the adsorbed hDNA was released to the solution, thus resulting in the fluorescence recovery.

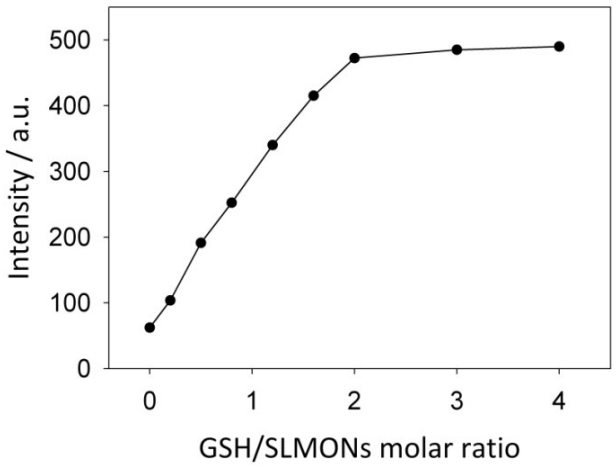

Fig. S6 Plot of fluorescence intensity at 521 nm against the GSH/SLMONs molar ratio in water.

During this redox reaction, GSH was oxidized to generate glutathione disulfide (GSSG) through thiol-disulfide exchange as shown in following equation.

$$MnO_2 + 2GSH + 2H^+ \rightarrow Mn^{2+} + GSSG + 2H_2O$$

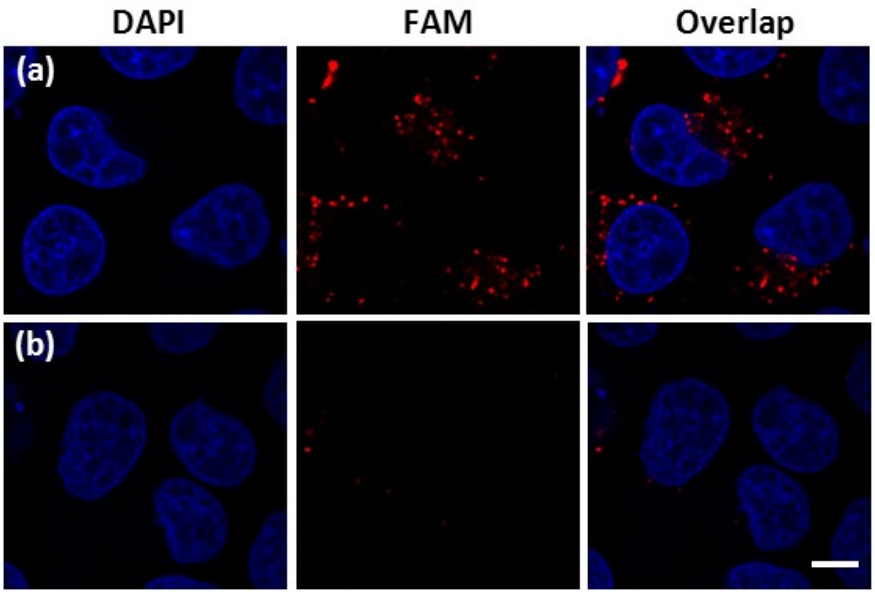

Fig. S7 Specificity of the SLMONs/MB nanoprobes in living cells. CLSM images of HeLa cells treated with (a) non-recognition SLMONs/cMB nanoprobes and (b) miR-21-recognition SLMONs/MB nanoprobes (λ_{ex} = 488 nm). In each image, left panel is DAPI fluorescence, middle panel is FAM fluorescence and right panel is the overlay of DAPI fluorescence and FAM fluorescence image. Scale bar is 5 μm.

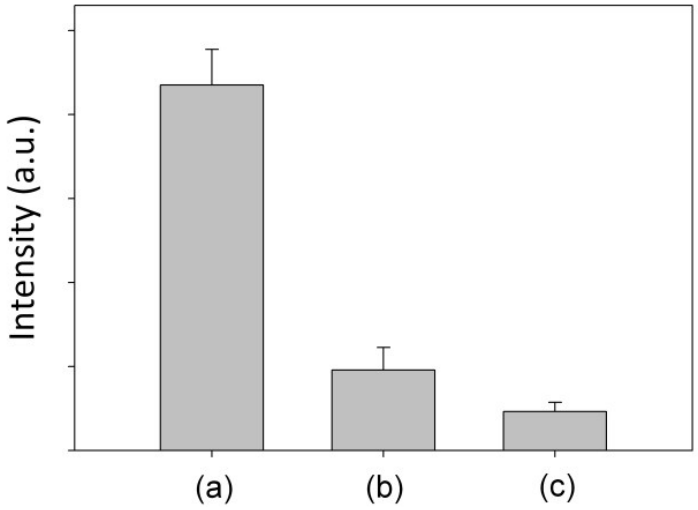

Fig. S8 Histogram of mean fluorescence intensities of HeLa cells treated without (a) and with (b) SLMONs/IP, and L02 cells (c) by SLMONs/MB nanoprobes from flow cytometric analysis.

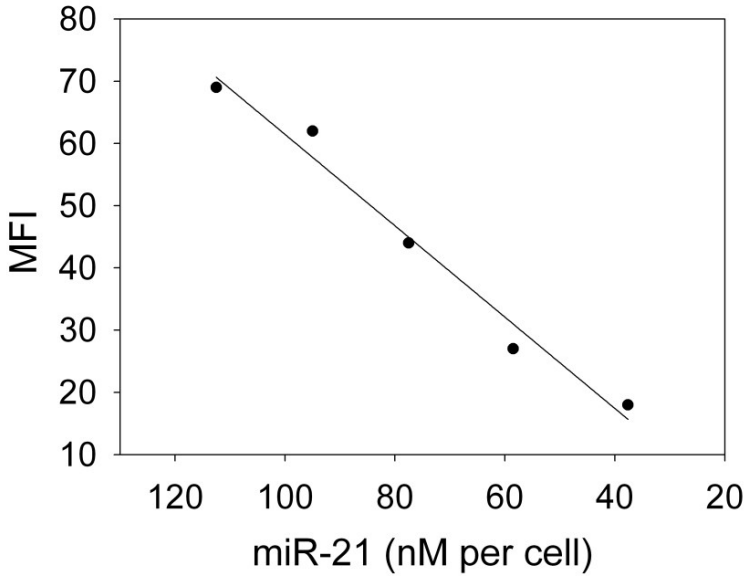

Fig. S9 A plot of mean fluorescence intensities from flow cytometric analysis versus the intracellular levels of miR-21 estimated from quantitative real-time PCR indicates that the present SLMONs/MB nanoprobes can quantitatively monitor miR-21 expression levels inside living cells (coefficient of correlation, R = 0.9812). MFI represents the mean fluorescent intensity of probes.

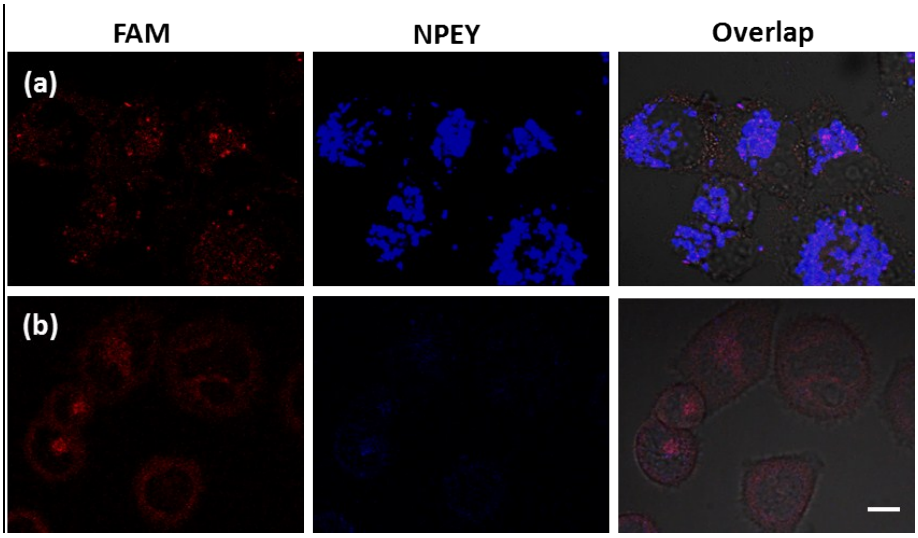

Fig. S10 CLSM images of HeLa cells in the presence of SLMONs/MB nanoprobes after being treated without (a) and with (b) N-ethylmaleimide (GSH scavenger). In each image, left panel is FAM fluorescence, middle panel is NPEY (Mn^{2+} probe) fluorescence and right panel is the overlay of FAM fluorescence and NPEY fluorescence image. Scale bar is 5 μm.