Supporting Information

Ultra-Sensitive Dual-Signal Detection of Telomerase and MiR-21 Based on Boolean Logic Operations

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SUPPLEMENTARY EXPERIMENTAL SECTION

Synthesis of MSNs

CTAC (10 g) and TEA (0.4 g) were mixed with 100 mL ultrapure water in a flask and stirred for one hour in a heated magnetic stirrer maintained at 80 °C. Then, TEOS (7.5 mL) was added slowly to the mixture and stored at 80 °C for 1 hour. The CTAC-MSNs suspension was repeatedly washed with ultrapure water and methanol, then mixed with concentrated hydrochloric acid and stirred for 12 hours. Finally, the CTAC was removed by continuous reflux to obtain MSNs. Characterization of MSNs were performed on zeta potential meter and TEM.

Synthesis of AgNPs

We synthesized AgNPs by a simple method. 300 mL of 2 x 10^{-3} M $NaBH_4$ solution was cooled on ice while $AgNO_3$ (2 x 10^{-3} M) was slowly added dropwise and stirred continuously. The solution was centrifuged at 12,000 rpm for 10 min, washed twice and finally dispersed in the ultrapure water. The maximum absorption wavelength of the synthesized AgNPs was at 420 nm.

Agarose gel electrophoresis

Sequences DNA2 (100 mM), DNA3-ROX (100 mM) and miRNA (100 mM) were diluted to 10 mM in PBS (PH=7.4, 0.01 M) and mixed thoroughly in a molar ratio of DNA2: DNA3-ROX: miRNA = 40: 40: 1. The mixture was placed in a constant temperature shaker and kept at 37 °C for 3 h to obtain HCR products.

The HCR products were analyzed by agarose gel electrophoresis. After mixing 242 g Tris, 37.2 g $Na_2EDTA\text{-}2H_2O$ with 800 mL deionized water, 57.1 mL acetic acid was added. Then, deionized water was added to fix the volume to 1 L and we finally obtained 50 x TAE. 50 mL of 1 x TAE was placed in a conical flask, 0.5 g agarose powder was mixed with it and boiled continuously until no bubbles were present. When the temperature of the agarose solution was reduced to 50 °C, 10 μL EB was added. After agarose gel was coagulated, we transferred the DNA samples.

Finally, the DNA samples were kept at 100 V and 80 mA for one hour in the electrophoresis apparatus.

Atomic force characterization (AFM) of HCR products

The HCR products was diluted with deionized water and then transferred onto the mica flakes, which were then blew dry slowly with high-purity nitrogen and the same operation was repeated three times. Finally, the characterization of HCR products was conducted on AFM.

Stability of FOMNs under acidic conditions

2 μg of DNase I endonuclease was added to 1 mL of FOMNs while the pH was adjusted to 6.0 and 7.4, respectively. The fluorescence intensity was recorded by a fluorescence spectrophotometer from 0 to 120 min. After three independent experiments, the data recorded at 608 nm were averaged.

Enhancement of fluorescence signal and Raman signal by AgNPs.

2 μL of DNA3-ROX-BHQ ($1.0x10^{-6}$ M) was placed in a test tube, and sufficient amounts of DNA2 and miR-21 were added to fully react. Under the same conditions, 2 μL of DNA3-ROX-BHQ was placed in a test tube and sufficient amounts of DNA2-Ag and miR-21 were added to fully react. Finally, the fluorescence intensity was measured using a fluorescence spectrophotometer.

Similarly, after taking 1 μL of DNA3-ROX-BHQ ($1.0x10^{-6}$ M) mixed in a solution containing miR-21 and adding DNA2 or DNA2-Ag for a full reaction, the Raman spectra of both solutions were measured using a laser micro Raman spectrometer.

Cell culture

HepG2 (human hepatocellular carcinoma cells), MCF-7 (human breast cancer cells), A549 (human non-small cell lung cancer cells) and L-02 (human normal hepatocytes) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). Petri dishes were placed in a standard humidified incubator containing a 5% CO_2 atmosphere at 37 °C. K562 (Human chronic myeloid leukemia cells) cells were inoculated in RPMI-1640 medium containing 10% FBS and cultured under the same conditions as above.

Cells were grown in DMEM medium or RPMI-1640 medium (1 mL) for 6 hours and then incubated with FOMNs for 10 min, 30 min, 1 h and 2 h, respectively. It was necessary to add Hochest 33342 to stain cell nucleus for 10 min. The medium was discarded and the glass-bottom dish was washed gently three times with PBS before observing the cells by CLSM.

Preparation of miRNAs

MCF-7 cells were used as samples to extract total RNA. The cell suspension was obtained by trypsin digestion and centrifuged at 3,000 rpm for 5 min. Cells were collected and washed three times with PBS at the same centrifugal speed. Cell lysate was added according to the steps provided by mirVana miRNA Isolation Kit (Solarbio, Beijing), and then the total miRNA was further extracted and purified.

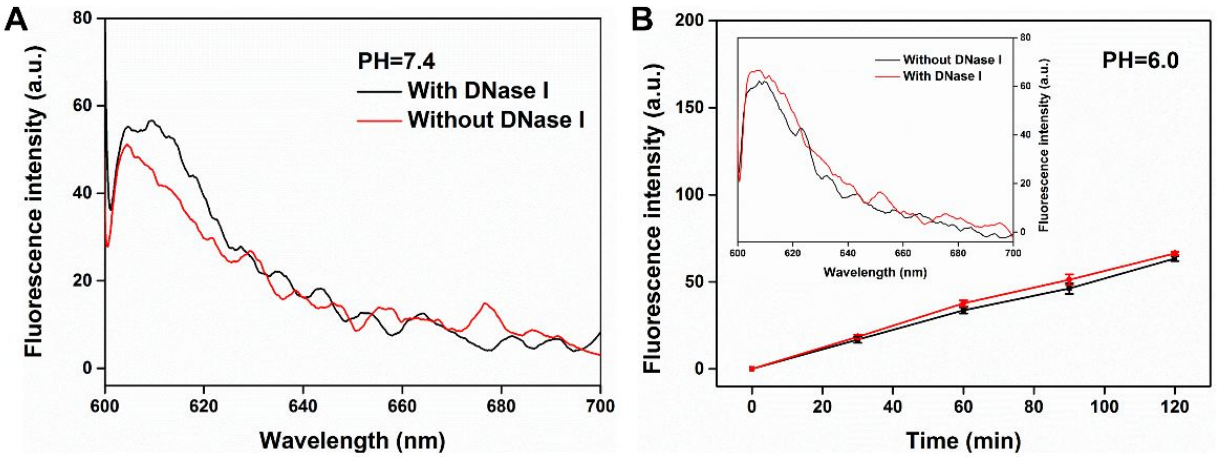

Figure S1. Effect of PH and DNase I on the stability of FOMNs. (A) The stability of FOMNs was investigated in the presence or absence of DNase I treatment for two hours in a pH=7.4 environment. (B) Fluorescence intensity at 608 nm from 0 min to 120 min following the addition or absence of DNase I in a FOMNs suspension at pH=6.0. The inset images represent the fluorescence curves after two hours with or without DNase I.

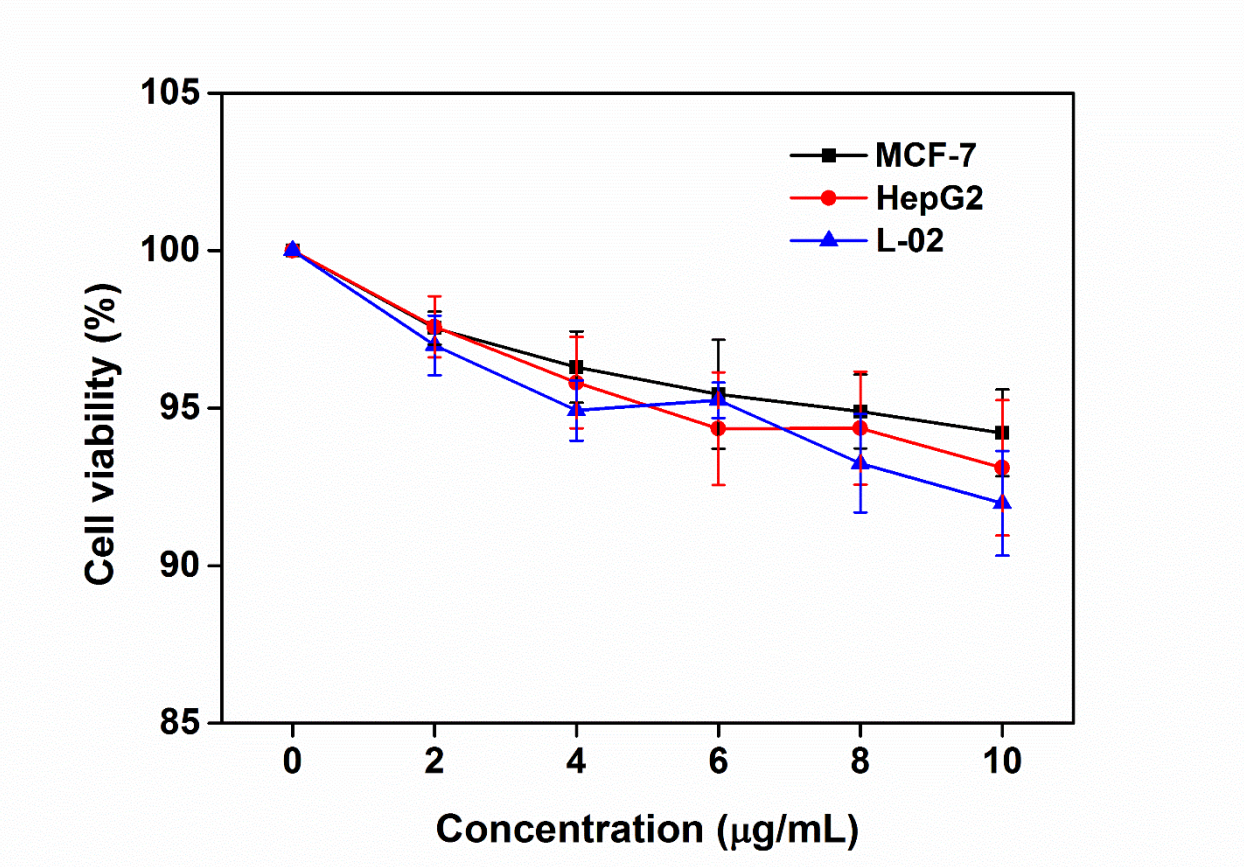

Figure S2. The CCK-8 kit was used to detect the cell viability of MCF-7 cells, HepG2 cells and L-02 cells after incubating with different concentrations of AgNPs for 6 hours.

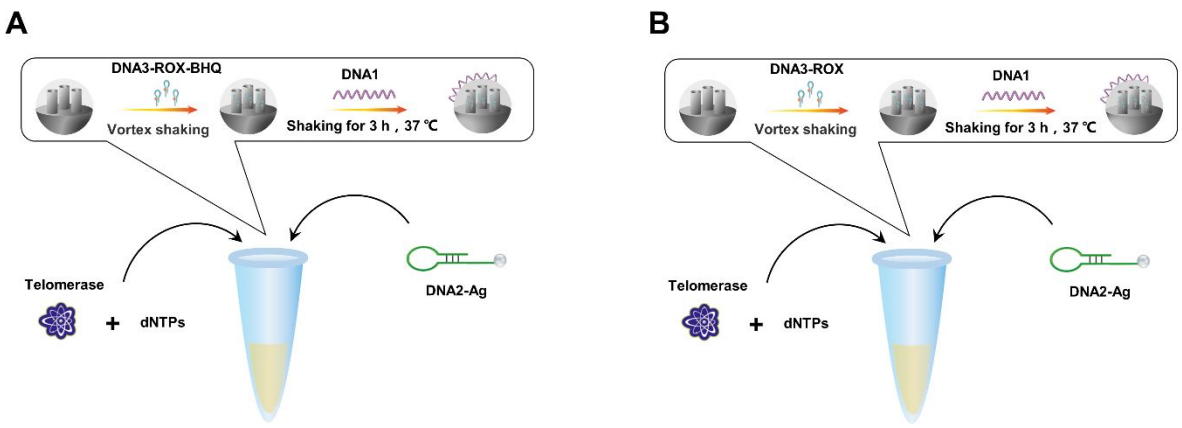

Figure S3. Schematic of the HCR products for (A) fluorescence detection and (B) Raman detection *In Vitro*.

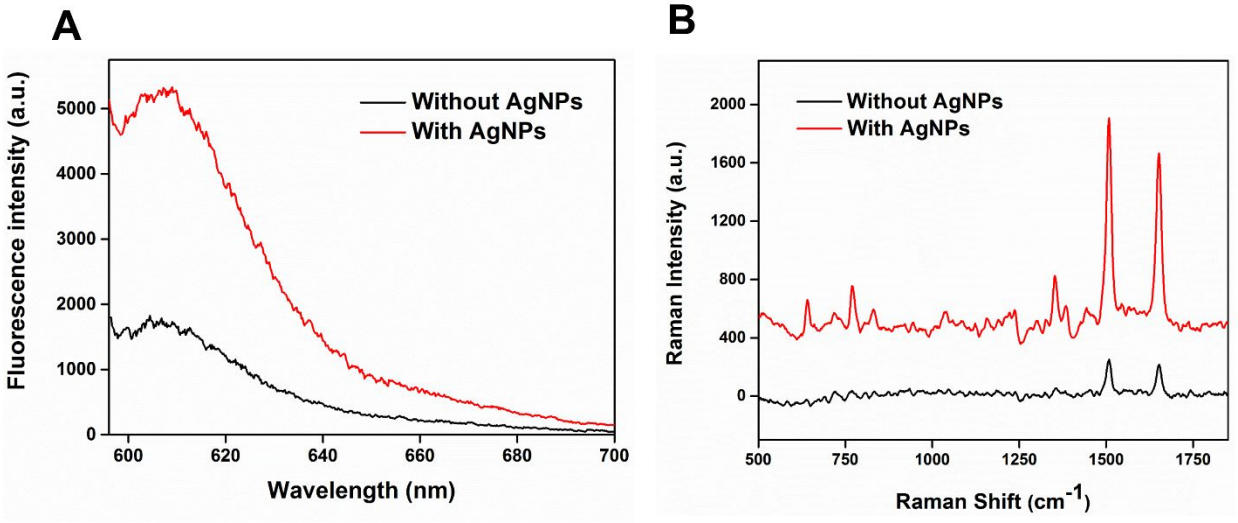

Figure S4. Enhancement of fluorescence signal and Raman signal by AgNPs. (A) Fluorescence intensity in the presence or absence of AgNPs. (B) Raman intensity in the presence or absence of AgNPs.

Telomerase	miR-21	Signal output
0	0	0
0	1	0
1	0	0
1	1	1

Figure S5. The truth table of Boolean logic "AND" strategy. Only in the presence of telomerase and miR-21 can signal amplification be achieved.

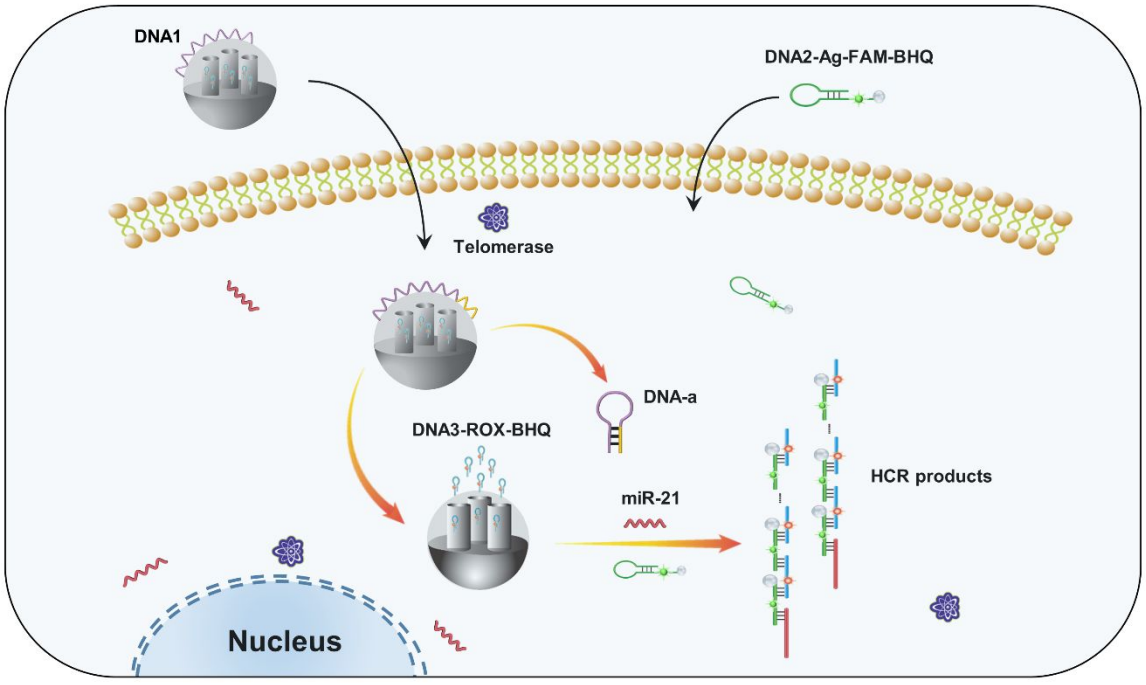

Figure S6. Schematic of HCR in the cells. The CLSM fluorescence channels with laser excitation at 480 nm and 580 nm were used to record the images.

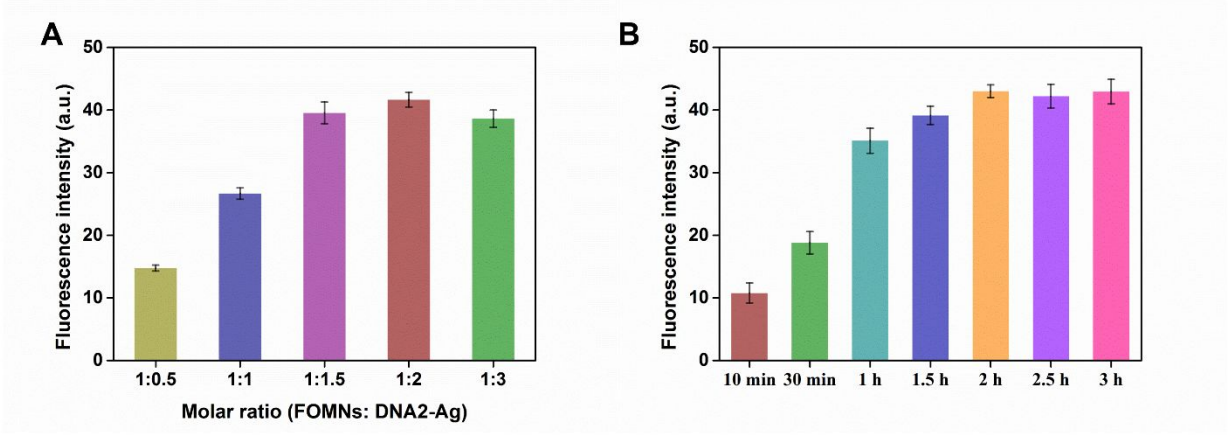

Figure S7. (A) The average fluorescence intensity of FOMNs and DNA2-Ag after 2 hours of incubation with MCF-7 cells in different molar ratios. (B) The average fluorescence intensity of FOMNs and DNA2-Ag incubated with MCF-7 cells for different times in a ratio of 1:2. The mean fluorescence intensity of each group is the average of three independent experiments.

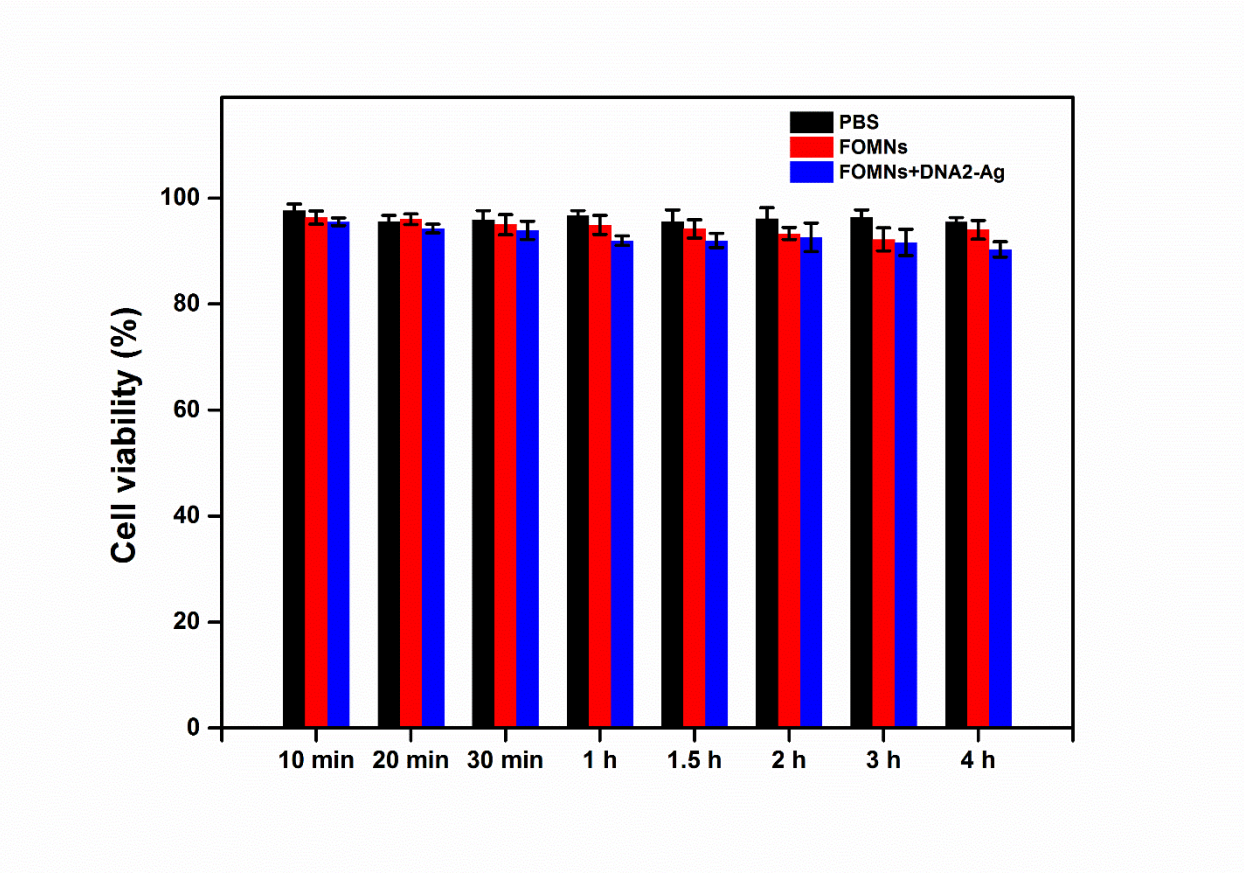

Figure S8. Cell viability of L-02 treated with PBS, FOMNs, FOMNs + DNA2-Ag for 10 min, 20 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, respectively.

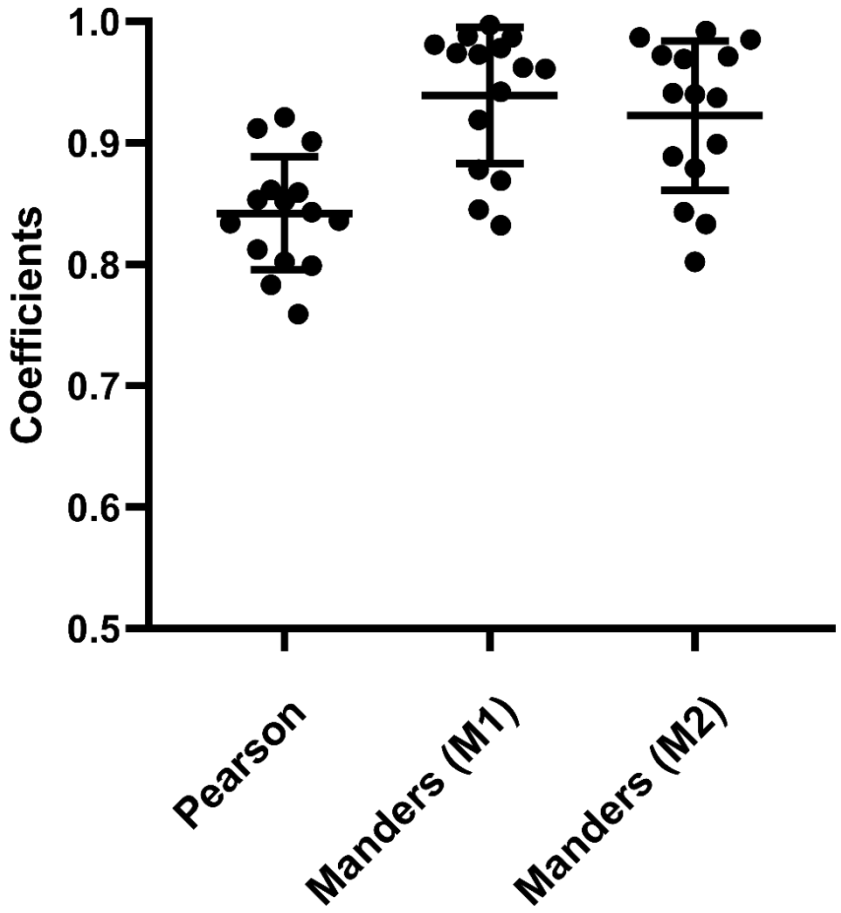

Figure S9. PCC and MCC were calculated by ImageJ. The error bars represent the mean ± SD of three independent experiments. Each group contained 15 cells independently.

Name	Sequence (5'-3')
DNA1	$(CCC\text{-}TAA)_6$ AATCCGTCGAGCAGAGTT
DNA2	ATCAGACTGATGTTGACAAAGTTCAACATCAGTCTGATAAGCTA-biotin
DNA2-FAM-BHQ	ATCAGACT-(FAM)-GATGTTGACAAAGTTCAACATCAGTCTGAT-(BHQ)- AAGCTA-biotin
DNA3-ROX	ACTTTGACATCAGT-(ROX)-CTGATTAGCTTATCAGACT-GATGTTGA

DNA3-ROX-BHQ	ACTTTGACATCAGT-(ROX)-CTGATTAGCTTATCAGACT-(BHQ)-GA TGTTGA
miR-21	UAGCUUAUCAGACUGAUGUUGA

Table S1. Sequences of oligonucleotides