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Supporting Information

Construction of fluorescence logic gates responding to telomerase and miRNA based on DNA-templated silver nanoclusters and hybridization chain reaction

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EXPERIMENTAL SECTION

Materials and instruments. All DNA and RNA oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). Their sequences were listed in Table S1. 20 bp DNA Ladder was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Graphene oxide (GO) was from Nanjing XFNANO Materials Tech Co., Ltd (Nanjing, China). Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), diethypyrocarbonate (DEPC), Tween 20, glycerol, 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonic acid (CHAPS), ethylene glycol bis(aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), phenylmethylsulfonyl fluoride (PMSF), and deoxynucleotide triphosphates (dNTPs) were purchased from Sigma (USA). Bull serum albumin (BSA), human serum albumin (HSA), and 3'-Azido-3'-deoxythymidine (AZT) were purchased from Aladdin Biological Technology Co., Ltd (Shanghai, China). HeLa cells were from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), trypsin and fetal bovine serum (FBS) were purchased from Gibco (Gaithersburg, USA). Enhanced Cell Counting Kit-8 was purchased from Shanghai Beyotime Biotechnology Co. Ltd. (Shanghai, China). Other reagents were of analytical grade and used without further purification. Double-distilled water was used throughout the experiments, which was firstly purified with a Millipore system under 18 MΩ cm resistivity and then treated with DEPC (0.1%). Telomerase-related experiments were performed using RNase-free water.

Fluorescence spectra were acquired with a Hitachi F-4600 Fluorescence Spectrophotometer (Hitachi, Japan). UV-vis absorption spectrum was measured by Synergy HT multifunction microplate reader (BioTek Instruments, Inc., USA). Transmission electron microscopic (TEM) images were taken

by using a FEI Tecnai G20 transmission electron microscopy (FEI, USA). The cell images were taken with a Leica TCS SP5 laser scanning confocal microscope (Leica, Germany).

Synthesis of DNA-templated AgNCs and GO based quenching. H2 was applied as the template, which was firstly mixed with $AgNO_3$ in PBS (pH7.4) and heated at 95 °C for 5 min. After cooling to room temperature, freshly prepared $NaBH_4$ solution was added with vigorous shaking for 1 min. The final volume of the mixture was 600 μL and the ratio of H2, $AgNO_3$, $NaBH_4$ was 1:7:7. The concentration of $NaBH_4$ was 7 μM. Subsequently, the resulted solution was incubated at 4 °C in dark for 3 h. For fluorescence quenching, GO with a series of concentrations (0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 45, 50 μg mL^{-1}) were added to the synthesized AgNCs for 10 min before fluorescence measurements.

Cell culture, telomerase extraction and cytotoxicity assessment. HeLa cells were cultured in DMEM supplemented with 10% FBS at 37 °C in an atmosphere of 5% CO_2. During the exponential phase of growth, cells were collected and washed twice with PBS buffer. Telomerase was extracted by the CHAPS method. 10^6 cells were firstly resuspended in 200 μL of ice-cold CHAPS lysis buffer (10 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 0.5% (w/v) CHAPS, 10% (v/v) glycerol, pH 7.5) by pipetting several times. After completely lysing of the cells, an ice bath for 30 min was performed. Next, the solution was centrifuged at 12000 rpm for 30 min at 4 ℃. The supernatant containing telomerase was carefully collected and diluted using CHAPS lysis buffer, which was stored frozen at −80 °C before use. To assess the cytotoxicities of AgNCs and GO, various concentrations of the nanomaterials were added to the HeLa cells in the 96 well plate. After 24 h, 10 μL of CCK-8 standard solution was added into each well and further cultivation for 1 h was performed.

Finally, the absorbance value of each well at 450 nm was read. Relative cell viabilities were then calculated.

Gel electrophoresis analysis. Different combinations of nucleic acids were firstly mixed and incubated. Afterward, 15 μL of the samples were blended with 3 μL of 6×loading buffer, which were then injected into a 12% non-denaturing polyacrylamide hydrogel in tris-borate-EDTA (TBE) buffer. Electrophoresis was carried out at 100 V for about 80 minutes. After staining with ethidium bromide, the gel was photographed under UV light by a Gel Doc™XR+ system (Bio-Rad, USA).

Logic gates operations. miRNA-21 and telomerase extracts from HeLa cells were applied as two inputs. For (1,0) and (0,1) inputting cases of OR gate, the two inputs with various concentrations were separately blended with the mixture of DNA probes ($H0_{OR}$, TP_{OR}, and H1) and GO@H2-tempalted AgNCs. Briefly, 10 μL of the inputs were added into the 600 μL of the DNA solutions. After incubation at room temperature for 2 h, 20 μL of telomerase extension solution (20 mM Tris-HCl, 1.5 mM $MgCl_2$, 63 mM KCl, 10 mM dNTPs, 0.05% Tween 20, 1 mM EGTA, pH 8.3) was added and reacted at 37 °C for 2 h. Afterward, fluorescence emission spectra were recorded using a Hitachi F-4600 fluorescence spectrophotometer at the optimal excitation wavelength of 500 nm. For (0,0) and (1,1) cases of OR gate, the two inputs were removed or added simultaneously while the other operations were the same. For AND gate, the DNA solutions were changed to be the mixture of TP_{AND}, $H0_{AND}$, HP and H1.

Intracellular imaging. 500 μL of HeLa cells (10^6 mL^{-1}) were seeded in a confocal dish and incubated for 24 h. Next, GO and various DNA sequences according to OR and AND gates were added to the cell-adhered dish for further incubation at 37 ℃ for 4 h. Afterward, the cells were observed by a TCS SP5 laser scanning confocal microscope.

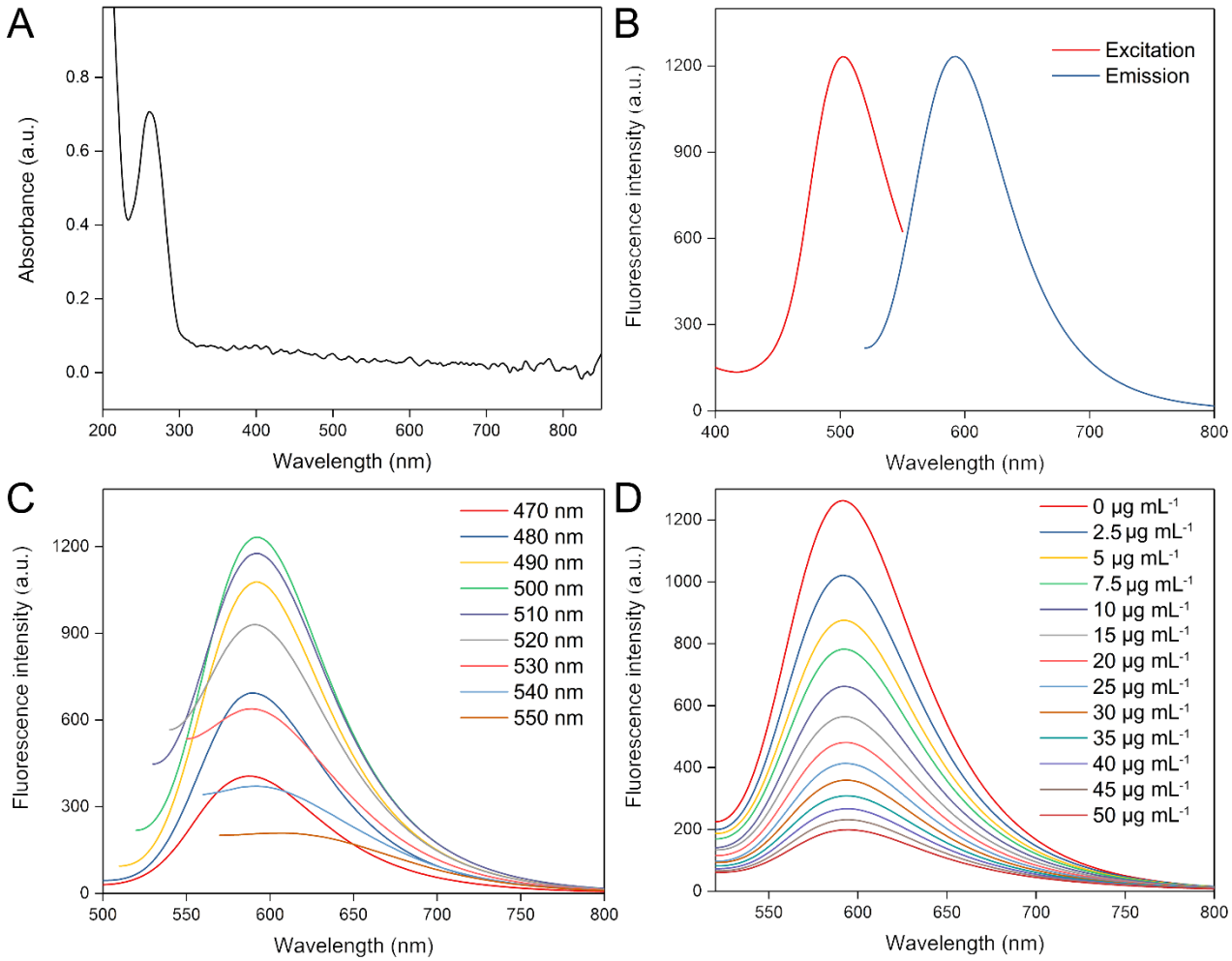

Figure S1. (A) UV−vis absorbance spectrum of DNA-templated AgNCs. (B) Fluorescence excitation and emission spectra of the prepared AgNCs. (C) Fluorescence emission spectra of AgNCs under excitation wavelengths from 470 to 550 nm. (D) Fluorescence emission spectra of AgNCs treated with various concentrations of GO.

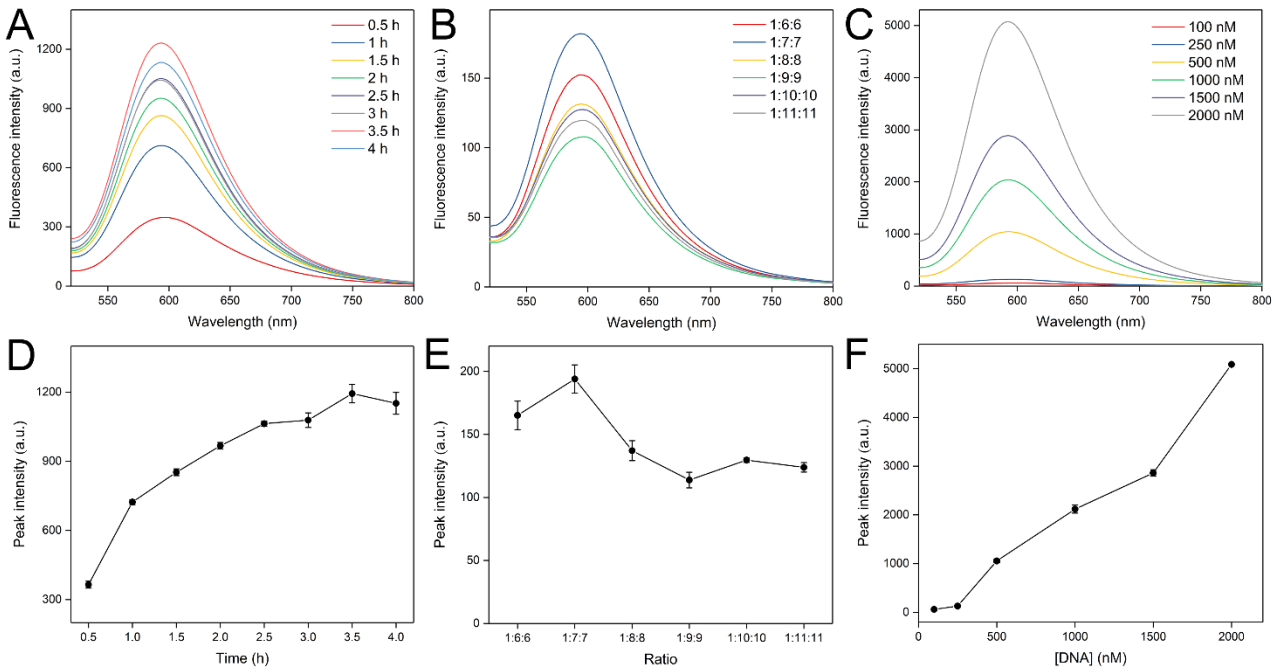

Figure S2. Optimization of the synthesis conditions of AgNCs: (A, D) reaction time of $NaBH_4$; (B, E) the ratio of H2, Ag^+ and $NaBH_4$; (C, F) the concentration of DNA template.

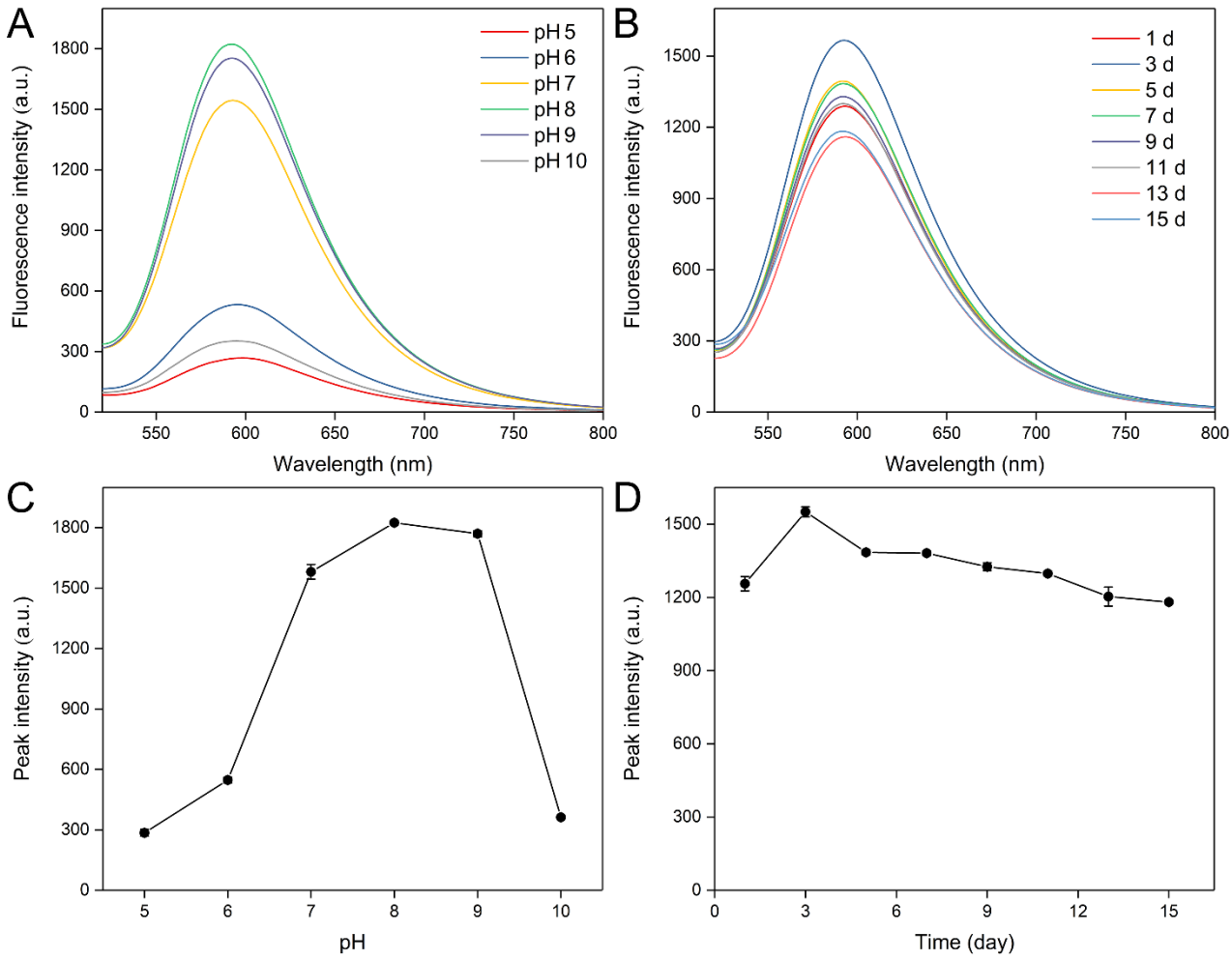

Figure S3. (A, C) The fluorescence response at various pH conditions. (B, D) Fluorescence emission response of AgNCs within 15 days.

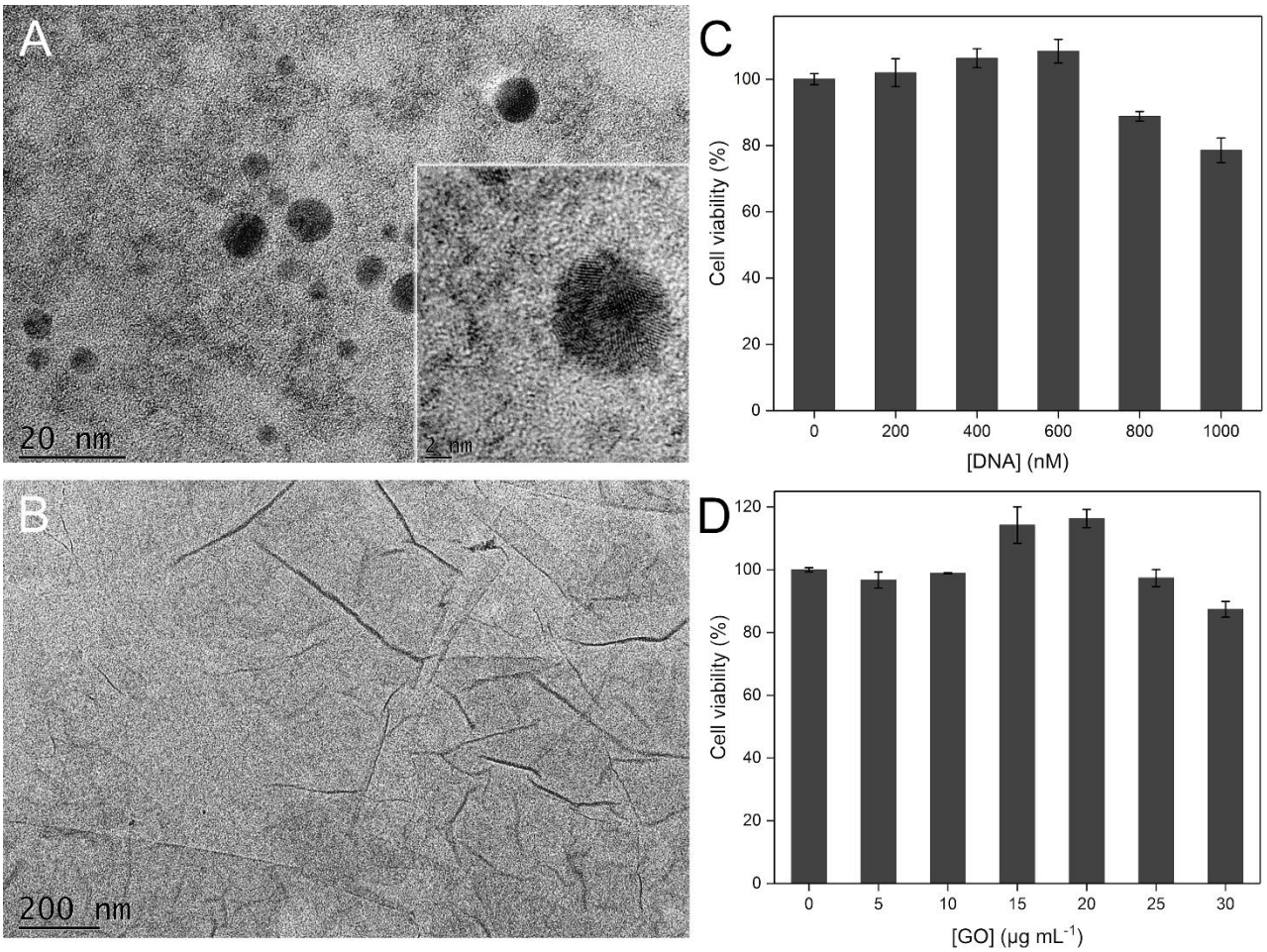

Figure S4. TEM images of (A) DNA-templated AgNCs and (B) GO. Cell viabilities after treated with (C) DNA-templated AgNCs and (D) GO with different concentrations for 24 h.

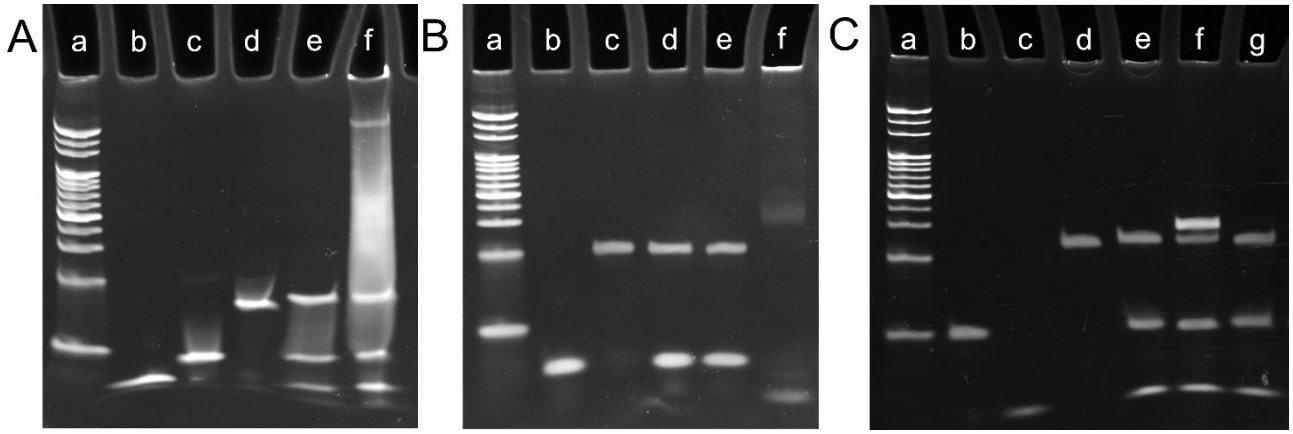

Figure S5. (A) Gel electrophoresis analysis of HCR: lane a, 20 bp DNA Ladder; lane b, $H0_{OR}$; lane c, H1; lane d, H2; lane e: $H0_{OR}$ and H1; lane f: $H0_{OR}$, H1 and H2. (B) Gel electrophoresis analysis of telomerase elongation reaction for OR gate: lane a, 20 bp DNA Ladder; lane b, $H0_{OR}$; lane c, TP_{OR}; lane d, $H0_{OR}$ and TP_{OR}; lane e, $H0_{OR}$, TP_{OR} and dNTPs; lane f, $H0_{OR}$, TP_{OR}, dNTPs and cell extracts. (C) Gel electrophoresis analysis of telomerase elongation reaction for AND gate: lane a, 20 bp DNA Ladder; lane b, $H0_{AND}$; lane c, HP; lane d, TP_{AND}; lane e, $H0_{AND}$, HP and TP_{AND}; lane f, $H0_{AND}$, HP, TP_{AND}, miR-21, dNTPs and cell extracts; lane g, $H0_{AND}$, HP, TP_{AND}, dNTPs and cell extracts.

Table S1. DNA and RNA sequences.

Name	Sequence (5'-3')
miR-21	UAGCUUAUCAGACUGAUGUUGA
Mismatch 1	UAGAUUAUCAGACUGAUGUUGA
Mismatch2	UAGACUAUCAGACUGAUGUUGA
Mismatch 3	UAGACGAUCAGACUGAUGUUGA
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-183	UAUGGCACUGGUAGAAUUCACU
miR-155	UUAAUGCUAAUCGUGAUAGGGGUU
TP_{OR}	CAGTCTGATAAGCTAACCCTAACCCTAACCCTAAAATCCGTCGAGCAG AGTT
$H0_{OR}$	**CTCGAGATAAGGTT**AGCTTA
H1	ACGTTA**AACCTTATCTCGAG**TTCGACGAATAAGGTT
H2	ATAAGGTTTAACGTAACCTTATTCGTCTTAAAAACCCCCTAATTCCCCC
TP_{AND}	TCAACATCAGTCTGATAAGCCCTAACCCTAACCCTAAAATCCGTCGAGC AGAGTT
$H0_{AND}$	**CTCGAGATAAGGTT**AGACTGATGTTGA
HP	TTAGGGCTTATC**CCT**