Supporting information:

A Highly Sensitive and Selective Strategy for MicroRNA Detection Based on WS_2 Nanosheet Mediated Fluorescence Quenching and Duplex-Specific Nuclease Signal Amplification

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Experimental Section

Reagents and Apparatus. HPLC-purified miRNAs, RNase inhibitor and DEPC treated water were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The FAM-labeled probe was synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China), 5× TBE buffer (225 mM Tris-Boric Acid, 50 mM EDTA, pH 8.0) was obtained from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). The sequences of these miRNAs and FAM-labeled probe are given in Table S1. Duplex-specific nuclease (DSN) was purchased from Evrogen Joint Stock Company (Moscow, Russia). The monolayer Tungsten Disulfide (WS_2) nanosheet was purchased from Nanjing XFNano Material Tech Co., Ltd. (Nanjing, China). GeneRuler Ultra Low Range DNA Ladder was obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Other chemicals used in this work were of analytical grade and directly used without further purification. The solutions used in all experiments were prepared using DEPC treated water.

The fluorescence spectra were recorded at room temperature in a quartz cuvette on an F-7000 fluorescence spectrophotometer (Hitachi, Japan). The excitation wavelength was fixed at 495 nm with a recording emission range from 505 to 600 nm and the excitation and emission slits were set at 5 nm.

MiRNAs detection. The amplified detection of miRNA was performed in a 30 μL reaction mixture containing 1× DSN master buffer (50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$ and 1mM DTT), 0.2 U DSN, 20 U RNase inhibitor, 100 nM probe and different

concentrations of target miRNA at 50 °C for 30 min. Then 30 μL 20 μg/mL WS_2 nanosheets solution and DEPC treated water were added into the reaction with final reaction volume of 100 μL. The mixture was incubated at room temperature for 10 min before allowing fluorescence detection.

Gel electrophoresis analysis. Gel electrophoresis analysis was carried out on 4% (w/w) agarose gels containing 0.5 μg/mL GoldView and the same concentration of ethidium bromide running in 0.5× TBE buffer at room temperature. The electrophoresis was performed at a constant potential of 101 V for 2 h with loading of 10 μL of each sample into the lanes. After electrophoresis, the gel was visualized via a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company, China).

Cell culture and sample preparation. MCF-7 (human breast cancer cell lines) and Hela (cervical cancer cell lines) were cultured in RPMI 1640 medium (Thermo Scientific Hyclone) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. MCF-10A (mammary epithelial cell lines) was cultured in DMEM/F-12 medium supplemented with 5% horse serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.5 mg/mL hydrocortisone, 100 ng/mL cholera toxin, 10 mg/mL insulin, 10 ng/mL epidermal growth factor, and 1% $_L$-glutamine. These cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Cells (1×10^6) were dispensed in an RNase-free 1.5 mL centrifuge tube, washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4) at 2000 rpm for 3min, and then

suspended in 100 μL lysis buffer (10 mM Tris-HCl with pH 8.0, 150 mM NaCl, 1%(w/v) NP-40, 0.25 mM sodium deoxycholate, 1% glycerol and 0.1 mM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride). The lysates were incubated for 30 min on ice, and then centrifuged at 12000 rpm for 20 min at 4 °C. The extract was used immediately for miRNA assay or stored at -80 °C.

Table S1. Sequences of miRNAs and DNA probes used in this work

Name	Sequence (5′-3′)
5-F	FAM-TCAAC
9-F	FAM-TCAACATCA
13-F	FAM-TCAACATCAGTCT
17-F	FAM-TCAACATCAGTCTGATA
22-F	FAM-TCAACATCAGTCTGATAAGCTA
P-21	FAM-TCAACATCAGTCTGATAAGCTA
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-143	UGAGAUGAAGCACUGUAGCUCA
SM miR-21	UAGCUUAUCAGACUGAUGAUGA

SM: single-base mismatched RNA, the mismatched position is marked in red.

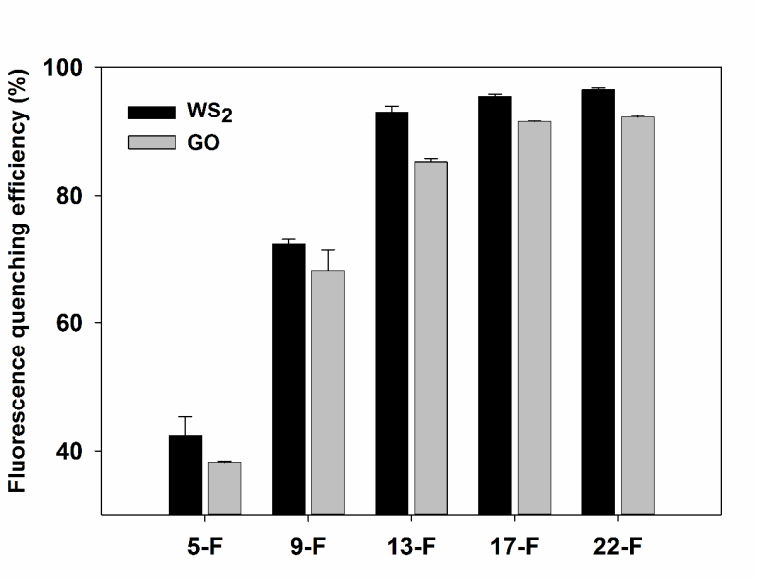

Figure S1. Fluorescence quenching efficiencies (F_0-F/F_0) of WS_2 nanosheet versus GO for single-stranded DNA probes of different lengths. The concentrations of WS_2 nanosheet and GO were fixed at 6 μg/mL. 5-F, 9-F, 13-F, 17-F, and 22-F denote the probes given in Table S1. Error bars are standard deviation of three repetitive experiments.

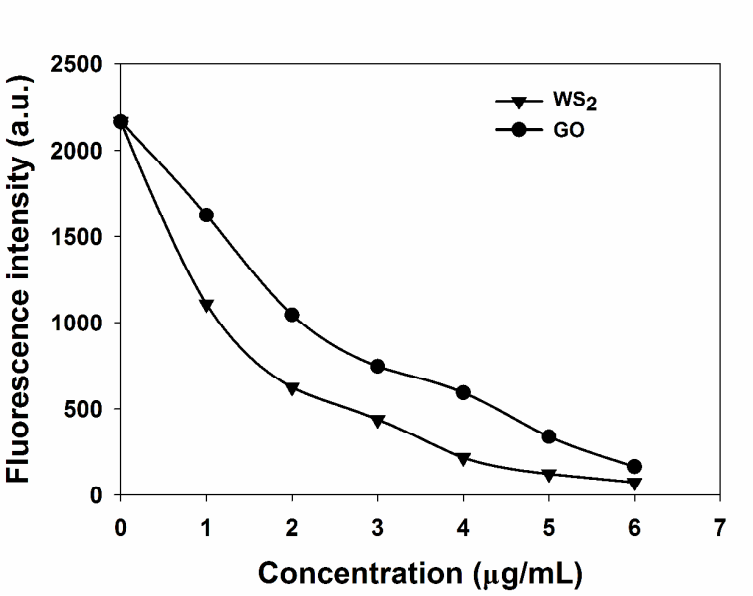

Figure S2. Fluorescence quenching assay of WS_2 nanosheet versus GO. The fluorescence intensities recorded at 520 nm were obtained by gradually adding WS_2 nanosheet or GO at equal concentrations in a solution of 100 nM FAM-labeled DNA probe P-21.

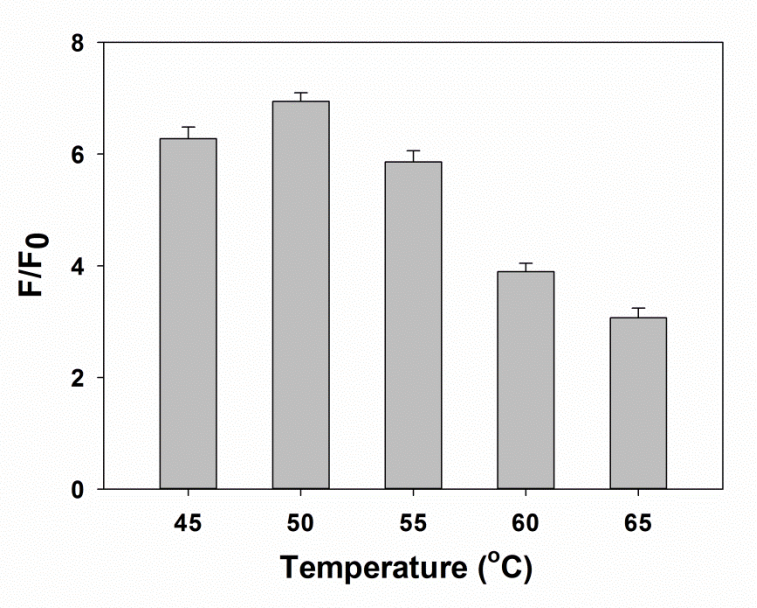

Figure S3. The performance of the assay was evaluated by the F/ F_0 at different temperatures, where F_0 and F are the fluorescence signals in the absence and the presence of miR-21, respectively. Error bars are standard deviation of three repetitive experiments.

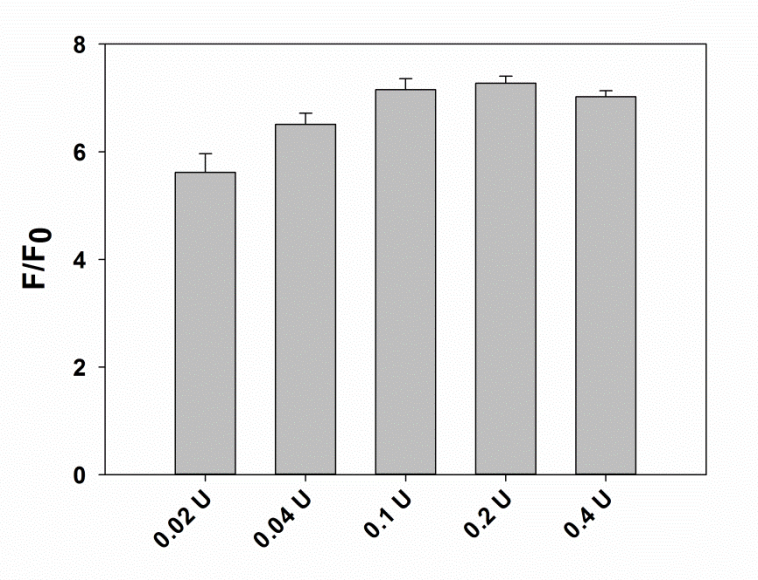

Figure S4. The variation in fluorescence signal ratio upon addition of different amounts of DSN enzyme, where F_0 and F are the fluorescence signals in the absence and the presence of miR-21, respectively. Error bars are standard deviation of three repetitive experiments.

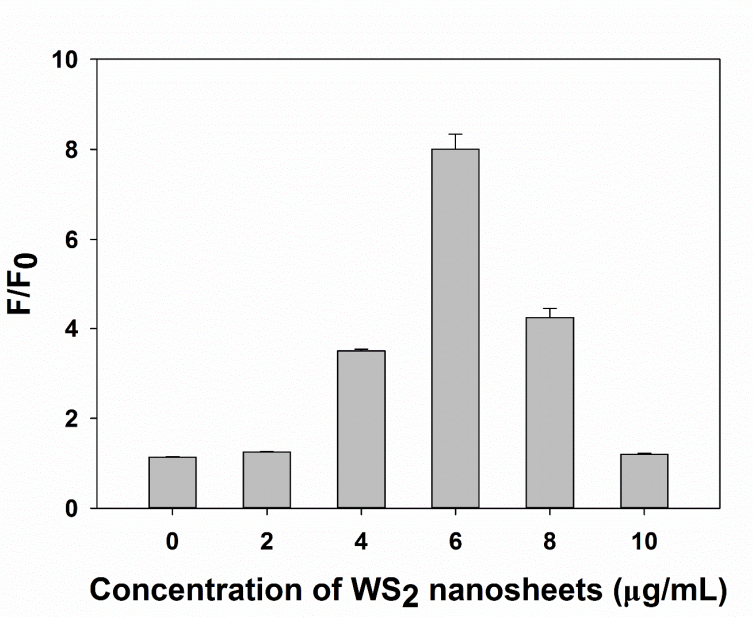

Figure S5. The effect of a change in the quantity of WS_2 nanosheets in this assay, where F_0 and F are the fluorescence signals in the absence and the presence of miR-21, respectively. Error bars are standard deviation of three repetitive experiments.

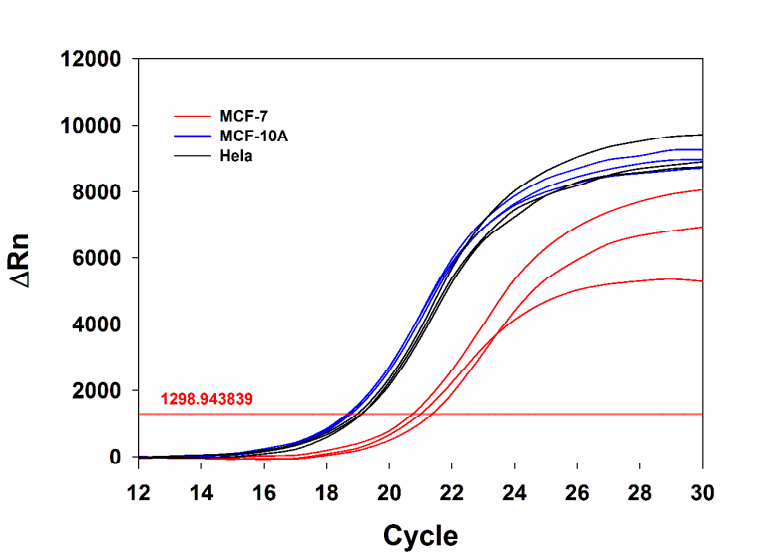

Figure S6. qPCR curves for U6 small RNA. Each sample was detected in three repetitive assays. The pink horizontal line represents the threshold line, and the number on the line indicates the threshold value.

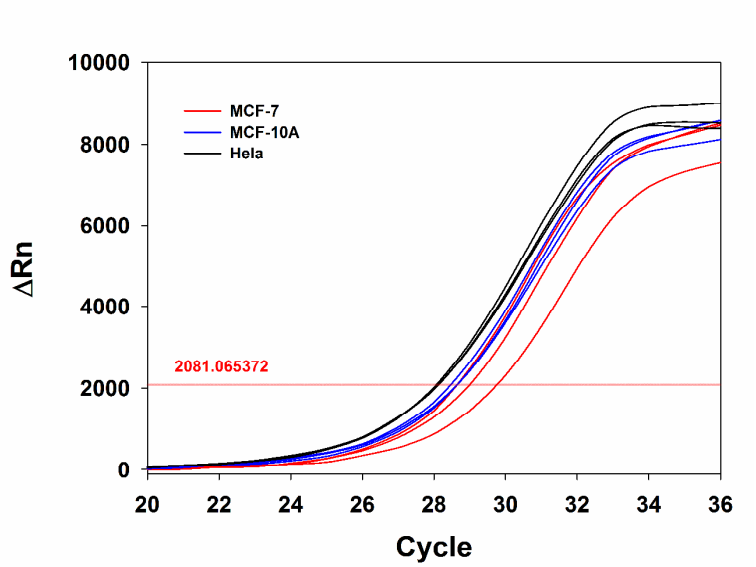

Figure S7. qPCR curves for miR-21. Each sample was detected in three repetitive assays. The pink horizontal line represents the threshold line, and the number on the line indicates the threshold value.

Table S2. Average C_t values in qPCR assay of miR-21.[a]

Cell line	miR-21	U6	ΔCt	ΔΔCt	$2^{-(\Delta\Delta Ct)}$
MCF-7	29.10634	20.98045	8.125891	0	1
MCF-10A	28.57015	18.71297	9.857185	1.731295	0.301182
Hela	28.06944	19.02447	9.04497	0.919079	0.528847

a. The relative expression level can be estimated by the values of $2^{-(\Delta\Delta Ct)}$. From the data, the expression level of miR-21 in MCF-10A and Hela cell line were estimated to be 0.301182 and 0.528847 fold of that in MCF-7 cell line, indicating the up-regulation of miR-21 in MCF-7 cell lines as compared with MCF-10A and Hela cell lines.

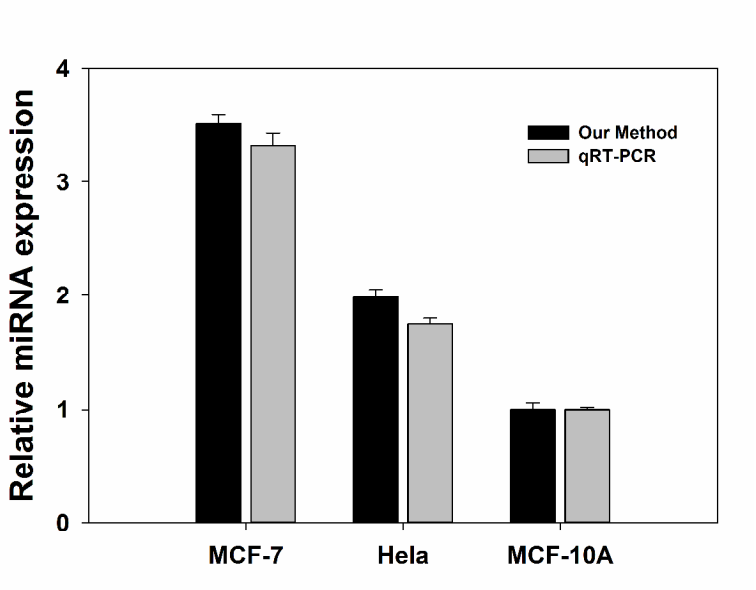

Figure S8. Detection of miR-21 expression levels in different cell lysates, as measured by using our proposed method and qRT-PCR method. Error bars are standard deviation of three repetitive experiments.

Table S3. Recovery experiments of miR-21 spiked in cell lysates of MCF-10A.

Cell lines	Detected (pM)	Added (pM)	Found (pM)a	Recovery (%)	CV (%)
MCF-10A	416	50	474 ± 7	102	1.5
		100	499 ± 16	97	3.2
		500	951 ± 57	104	6.0

[a] Average of three determinations ± standard deviation

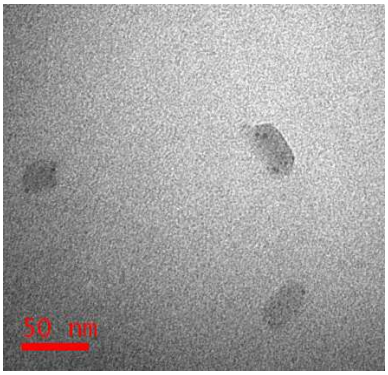

Figure S9. TEM image of WS_2 nanosheets.

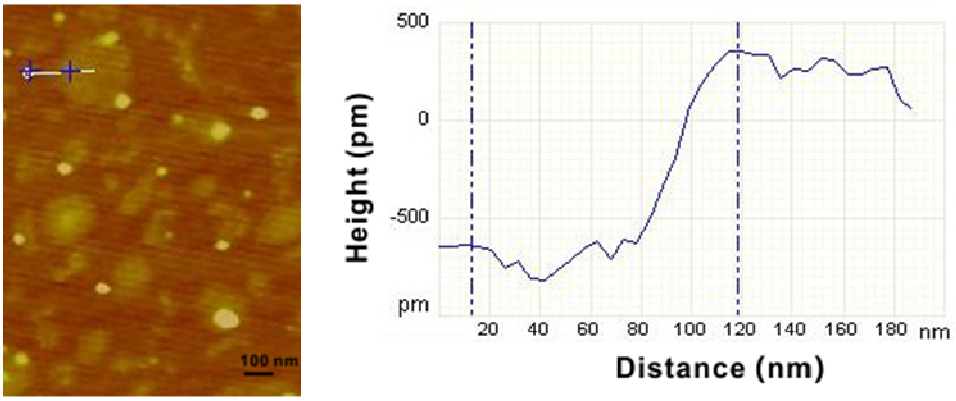

Figure S10. AFM image and height profile of WS_2 nanosheets on mica substrate.