Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information

Duplex-specific nuclease mediated target recycling amplification for fluorescence detection of microRNA

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Table S1 Details of the sequence of miRNA and DNA probes used in this study

Name	Sequence (5′-3′)
DNA probe	GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTT CAA CAT CAG TCT GAT AAG CTA-FAM
Control DNA probe	GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT GTA CCT TAC ATT TCT TCA TAC CTC-FAM
miR-21	5’-UAG CUU AUC AGA CUG AUG UUG A-3′
miR-122	5’-UGG AGU GUG ACA AUG GUG UUU G-3’
miR-328	5’-ACG GAA GGG CAG AGA GGG CCA G-3’
miR-197	5’-UUC ACC ACC UUC UCC ACC CAG C-3′
miR-141	5′-UAA CAC UGU CUG GUA AAG AUG G-3′
Forward primer for U6	5′-CTC GCT TCG GCA GCA CA-3′
Reverse Primer for U6	5′-AAC GCT TCA CGA ATT TGC GT-3′
Forward primer for miR-21	5′-ACACTCCAGCTGGGTAGCTTATCAGACTG-3′
Reverse Primer for miR-21	5′-TGG TGT CGT GGA GTC G-3′

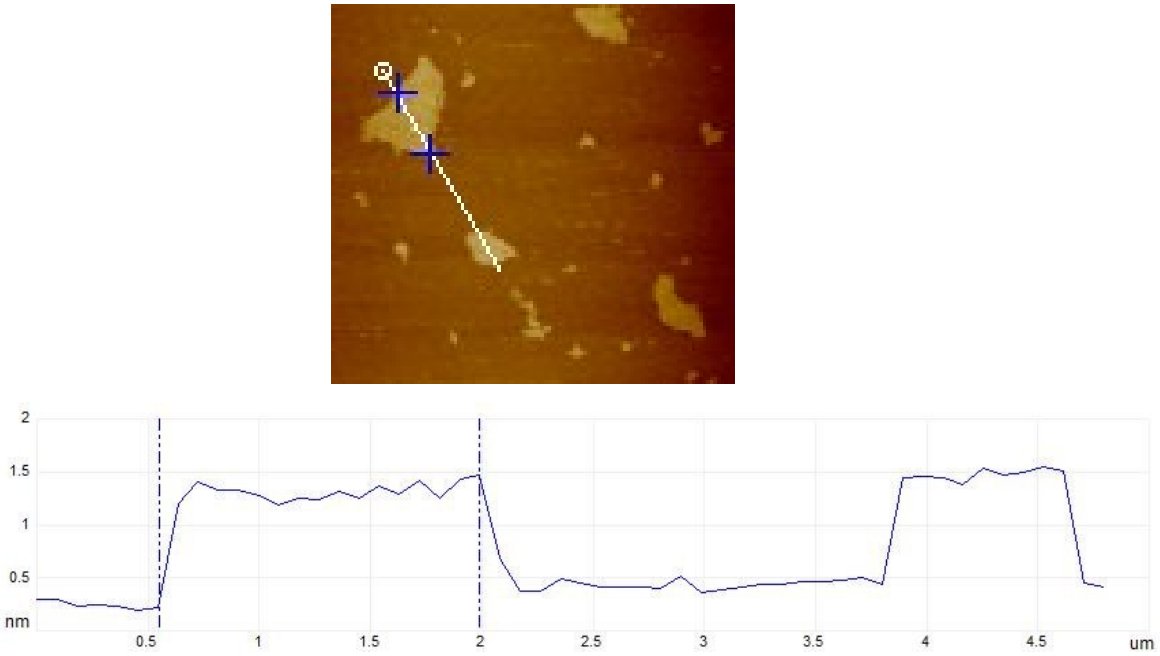

Figure S1 AFM height image of GO nanosheets deposited on mica substrates

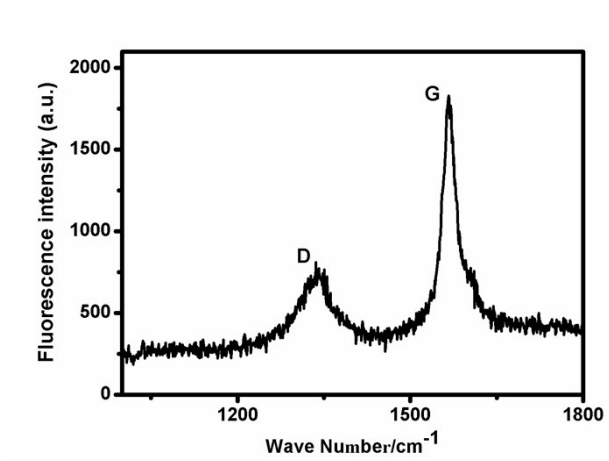

Figure S2 Raman spectrum of GO nanosheet

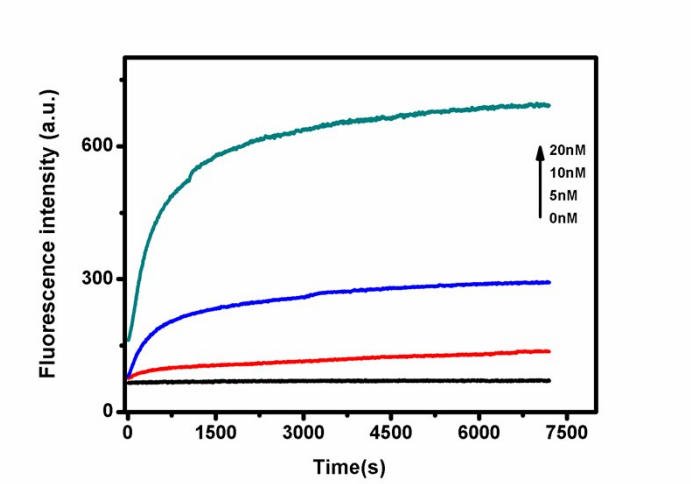

Figure S3 The time course of fluorescence recovery of GO-absorbed DNA probe at different target concentrations (0, 5, 10, 20 nM).

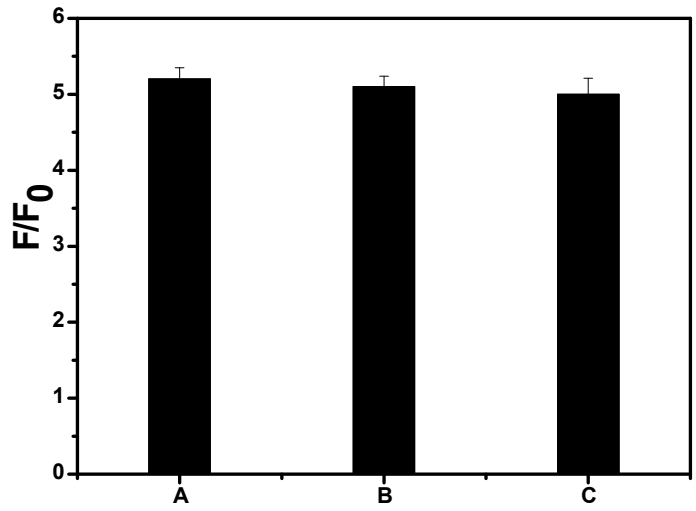

Figure S4. Fluorescence responses upon different conditions of the assay, where F_0 and F are the fluorescence signals in the absence and the presence of 1 nM miR-21, respectively. A: normal condition, B: in the presence of 5 U DNase I, C: in the presence of 10% FBS). Error bars are standard deviation of three repetitive experiments.

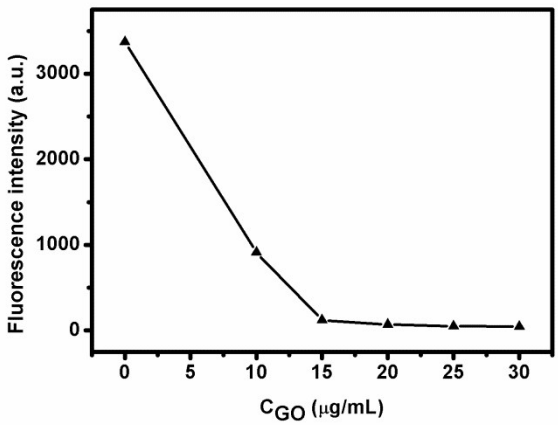

Figure S5 Influence of the concentration of GO on fluorescence recovery.

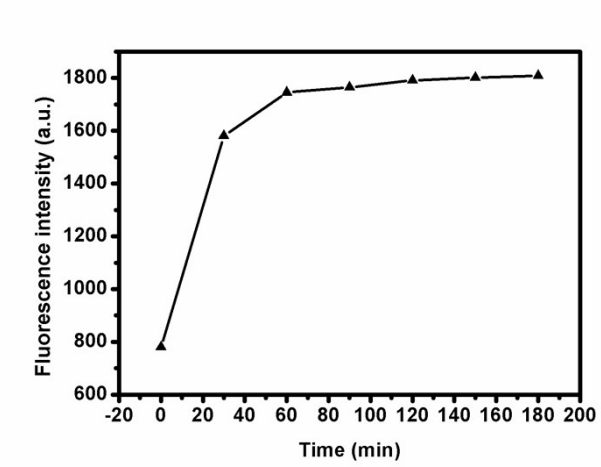

Figure S6 Influence of incubation time of DSN on fluorescence recovery.

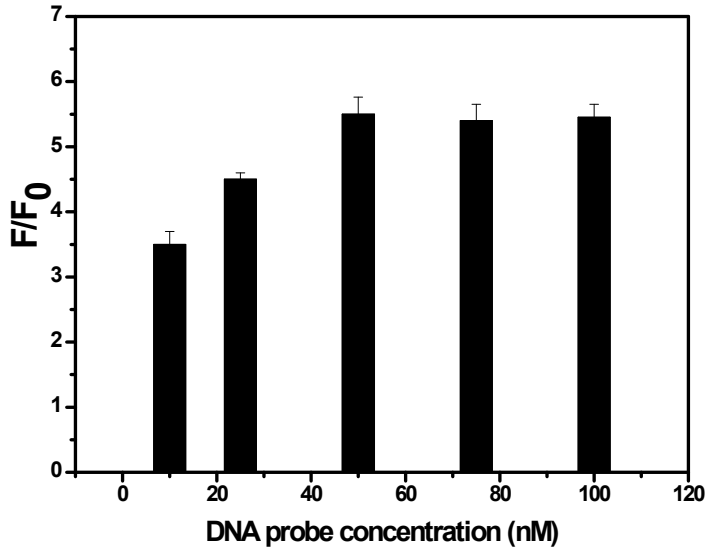

Figure S7. Fluorescence responses upon addition of different DNA probe concentrations (10 nM, 25 nM, 50 nM, 75 nM, and 100 nM) in the assay, where F_0 and F are the fluorescence signals in the absence and the presence of 1 nM miR-21, respectively. Error bars are standard deviation of three repetitive experiments.

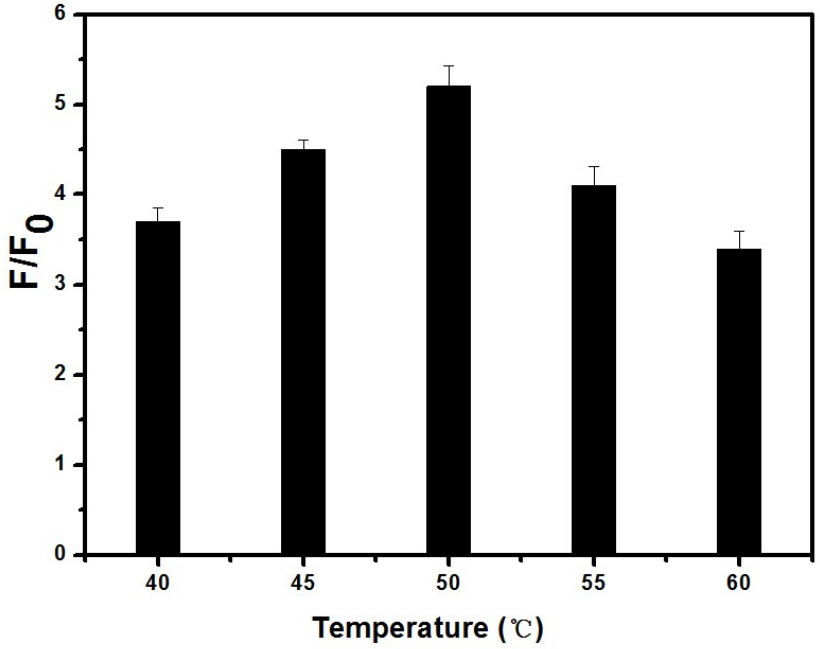

Figure S8. Fluorescence responses under different reaction temperatures (40°C , 45°C, 50°C, 55°C and 60°C) in the assay, where F0 and F are the fluorescence signals in the absence and the presence of miR-21, respectively. Error bars are standard deviation of three repetitive experiments.

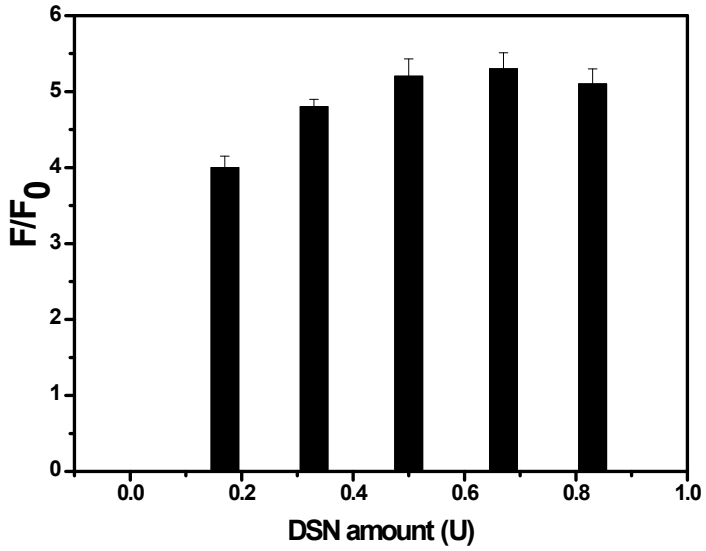

Figure S9. Fluorescence responses upon addition of different DSN amount (017 U, 0.33 U, 0.50 U, 0.67 U and 0.83 U) in the assay, where F_0 and F are the fluorescence signals in the absence and the presence of 1nM miR-21, respectively. Error bars are standard deviation of three repetitive experiments.

Table S2 Recovery experiments of miR-21 spiked in cell lysates of Hela.

Cell lines	Detected (pM)	Added (pM)	Found (pM)	Recovery (%)	CV (%)
		100	175.6	98.46	1.196
Hela	78.34	150	219.8	96.26	1.319
		300	387.1	102.3	1.369

Table S3 Recovery experiments of miR-21 spiked in cell lysates of Hela. The assay was used BSA for blocking after DNA probe absorption

Cell lines	Detected (pM)	Added (pM)	Found (pM)	Recovery (%)	CV (%)
		100	173.6	98.38	1.192
Hela	76.45	150	216.4	95.56	1.324
		300	384.2	102.0	1.257

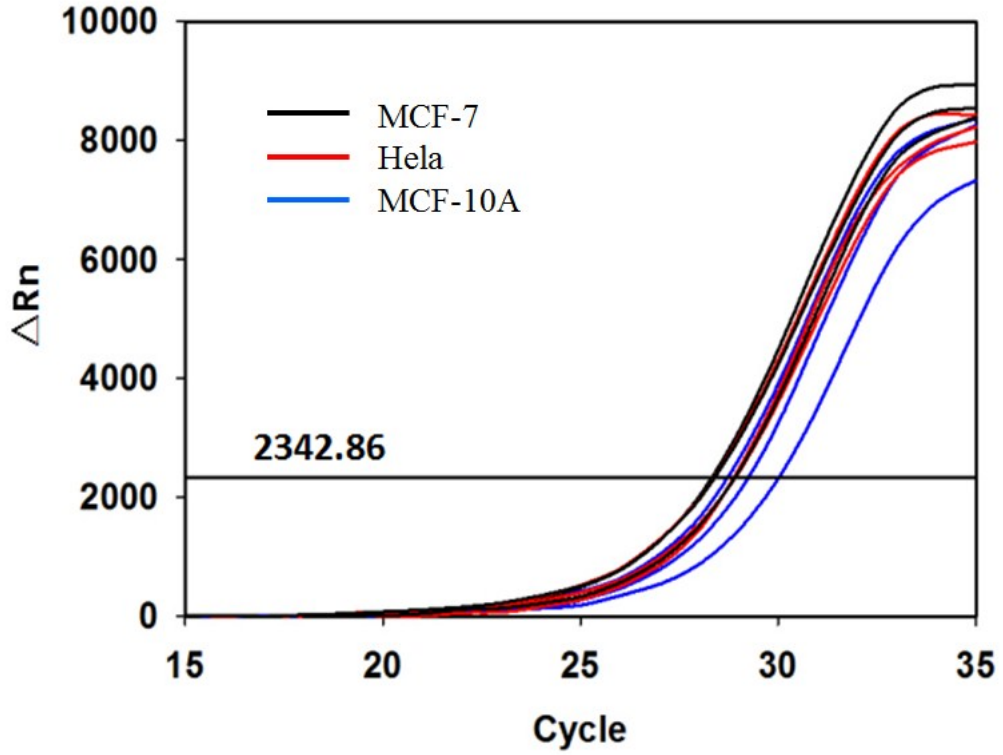

Figure S10. Expression analysis of miR-21 for different cell lines by RT-qPCR experiments.

Table S3. Average Ct values in q-PCR assay of miR-21

Cell line	Ct(miR-21)	Ct(U6)	ΔCt	ΔΔCt	$2^{-(\Delta\Delta Ct)}$
MCF-7	29.12	20.91	8.21	0	1
HeLa	28.03	18.95	9.08	0.87	0.55
MCF-10A	28.62	18.64	9.98	1.77	0.29

The relative expression level was estimated by the values of $2^{-(\Delta\Delta Ct)}$ and U6 gene was used as reference. From the data, expression of miR-21in MCF-10A and Hela were estimate to be 0.29 and 0.55 fold of that in MCF-7 cell line, respectively.