Electronic Supplementary Information (ESI) for:

DNA Tetrahedron Nanoprobe-Based Fluorescence Resonance Energy Transfer Sensing Platform for Intracellular Tumor-Related miRNA Detection

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1. Supplementary Experimental Section

Cell Viability Assay. The cell viabilities were determined by a standard MTT assay. Briefly, K1 cells were seeded in 96-well microplate with a concentration of 1×10^4 cells per well in a total volume of 100 μL for 24 h. Then the medium was removed and washed with phosphate-buffered saline (PBS) for three times. After that, 100 μL fresh culture medium containing 100 nM DTNP or different concentrations of inhibitors were added to each well and culture for another 24 h. Subsequently, the cells were washed with PBS (3 times), and 100 μL MTT solution (5 mg mL^{-1} in DMEM medium) was added to each well, culturing for another 4 h. After carefully removed the MTT solution, 100 μL of DMSO was added to each well to dissolve the formazan crystals. The cells cultured with media only under same condition were employed as control and the absorbance at 490 nm was measured using PowerWaveXS2 microplate reader (BioTek Co, Inc., USA) after shaking for 10 min.

2. Additional Table S1-S3

Table S1. Sequences of used oligonucleotides in the experiments.

Name	Sequence (5' to 3')	ΔG (kcal mol^{-1})[a]
hsa-miR-146b-5p	**UGAGAACUGAAUUCCAUAGGCU**	Target hybridization -38.92
HP	FAM-GAATTCAGACTTGAGAACTGAATTC-TAMRA	Tail hybridization -32.07
Assistant DNA	*T*A*C*CAGACTTGAGAACTGAA*T*T*C*	Displacement -40.51
T1	GCTTCACCTAAGCATGATTGCCAATAC TAGTGCGTTTGTACCTTCGGAGCTCAT TTTTT**AGCCTATG*GAATTCAGTTCTCA AGTCTGGTA***	
T2	TCTAAGGCAGTTGACATTTACGCACTA GTATTGGCTTATCCATGGTCCAATCGT	
T3	ATGTCAACTGCCTTAGATTGCCAATAC TAGTGCGTTTATGAGCTCCGAAGGTAC	
T4	TCATGCTTAGGTGAAGCTTACGCACTA GTATTGGCTTACGATTGGACCATGGAT	
SNP-Up	TGAG**G**ACTGAATTCCATAGGCT	
SNP-Mid	TGAGAACTGA**G**TTCCATAGGCT	
SNP-Down	TGAGAACTGAATTCCATA**A**GCT	
NC-miRNA	UUGUACUACACAAAAGUACUG	

*Phosphorothioate bonds. The oligonucleotides were purified by HPLC.
[a]ΔG values are calculated using OligoAnalyzer software (https://sg.idtdna.com/calc/analyzer). The tail hybridization ΔG is the energy upon binding T1 of DTNP sequence with the corresponding HP tails. The target hybridization ΔG is the energy upon binding T1 of DTNP sequence with the corresponding target. The Displacement is the energy binding T1 of DTNP sequence with the assistant DNA.

Table S2. Comparison between the proposed assay and other reported methods for miRNA detection.

Analytical method [a]	Dynamic range	Detection limit	References
Fluorescence	1 nM - 16 nM	47 pM	[1]
Electrochemical	1 nM - 50 nM	28.1 nM	[2]
SPR	10 pM – 0.1 nM	5 pM	[3]
Fluorescence	0.1 nM - 10 nM	30 pM	[4]
Colorimetric	0.5 pM -1 nM	0.5 pM	[5]
Fluorescence	0.75 nM - 15 nM	38 pM	[6]
Fluorescence	0.01 nM -10 nM	6 pM	This work

[a] SPR, Surface plasmon resonance.

Table S3. Recoveries for the spiked synthetic hsa-miR-146b-5p in three human serum obtained by DTNP sensor (n=3).

Sample	Spiked (pM)	Measured (pM)	RSD (%)	Recovery (%)
1	10	9.76	5.6	97.6
	100	104.83	3.3	104.8
	1000	964.21	4.8	96.4
2	10	10.74	5.5	107.4
	100	106.32	4.3	106.3
	1000	951.87	4.1	95.2
3	10	9.84	6.8	98.4
	100	102.29	5.1	102.3
	1000	1017.66	3.7	101.8

3. Additional Figure S1-S14

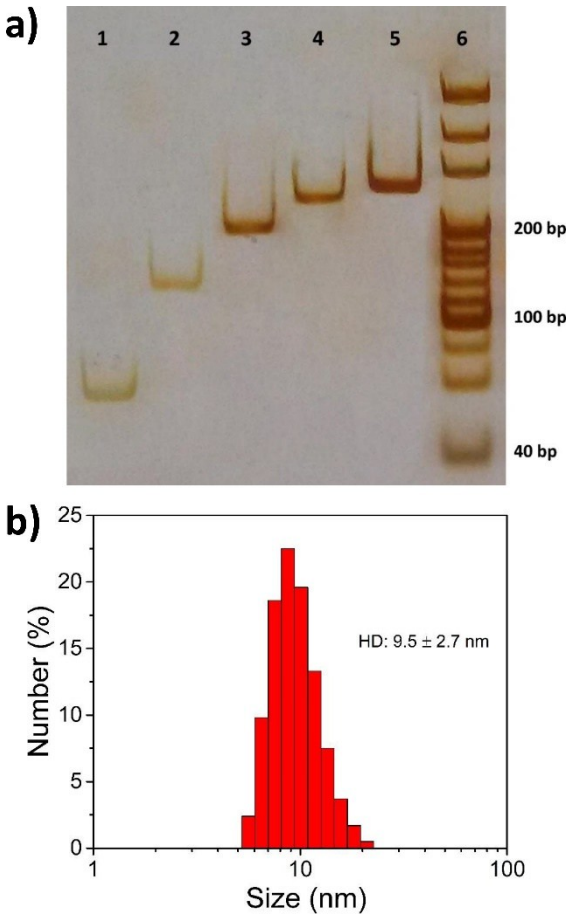

Fig. S1. a) Acrylamide gel electrophoresis analysis of DNA complexes. Lane 1: T2, lane 2: T1+T2, lane 3: T1+T2+T3, lane 4: T1+T2+T3+T4, lane 5: DTNP and lane 6: 20bp marker. b) Hydrodynamic diameter analysis of DTNP.

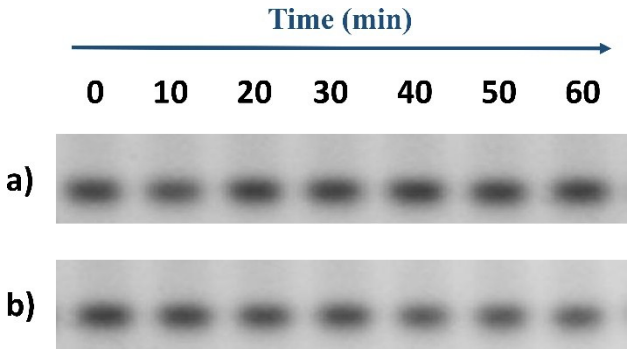

Fig. S2. Agarose gel electrophoresis analysis of DTNP in the presence of 1 U mL^{-1} DNase I (a) and Exo III (b). The DTNPs were incubated with 1 U mL^{-1} nucleases for various time (as indicated in the top of Figure).

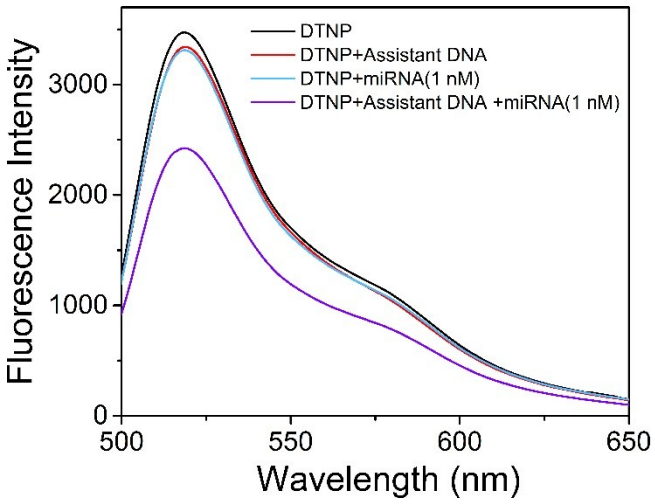

Fig. S3. The enhancement ability of assistant DNA for hsa-miR-146b-5p detection.

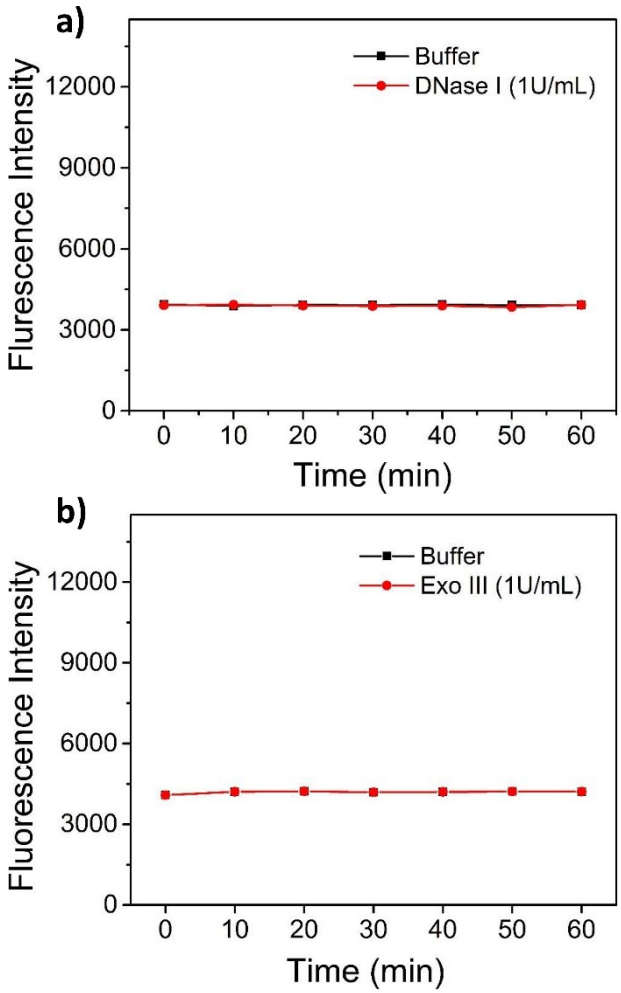

Fig. S4. The maximum fluorescence intensity of DTNP as a function of incubation time. The DTNPs were incubated with 1 U mL^{-1} DNase I (a) and Exo III (b).

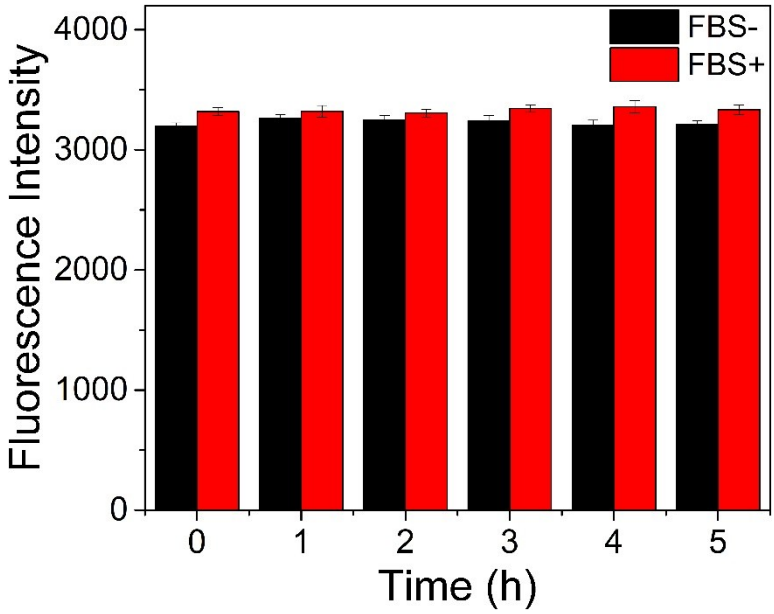

Fig. S5. The maximum fluorescence intensity of DTNP as a function of incubation times. The DTNPs were incubated with medium under FBS+ and FBS-. Error bars mean standard deviations (n=3).

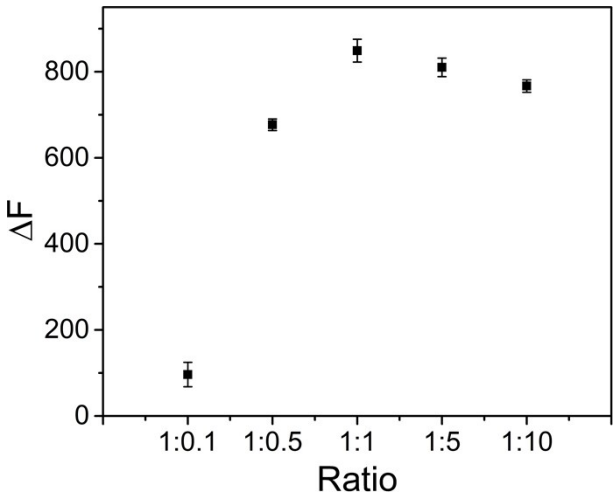

Fig. S6. The maximum fluorescence intensity changes as a function of different ratio of tetrahedron DNA and HP. Error bars mean standard deviations (n=3).

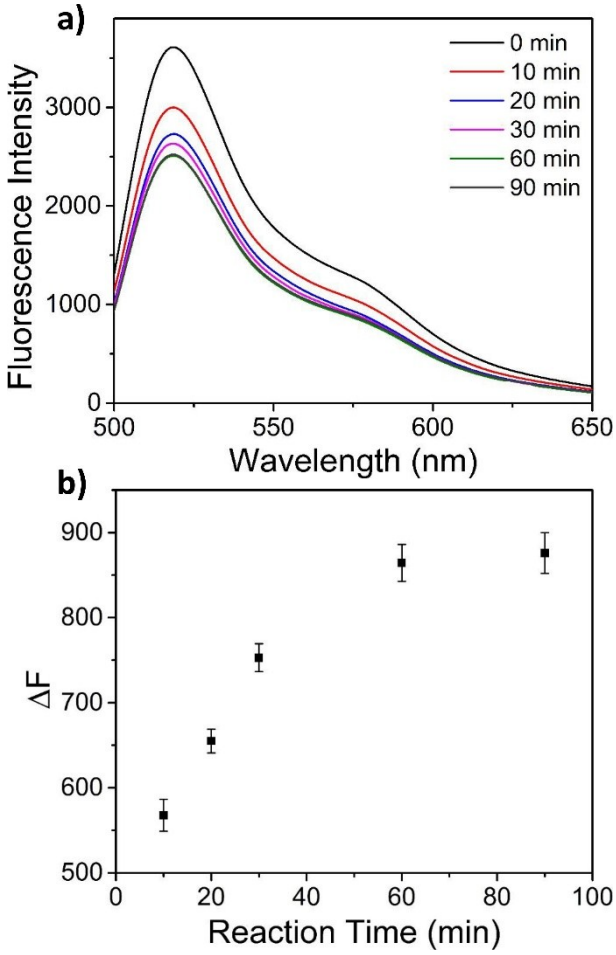

Fig. S7. a) Fluorescence spectra of DTNP sensor for the detection of hsa-miR-146b-5p for different time. b) The corresponding maximum fluorescence intensity changes as a function of reaction time. Error bars mean standard deviations (n=3).

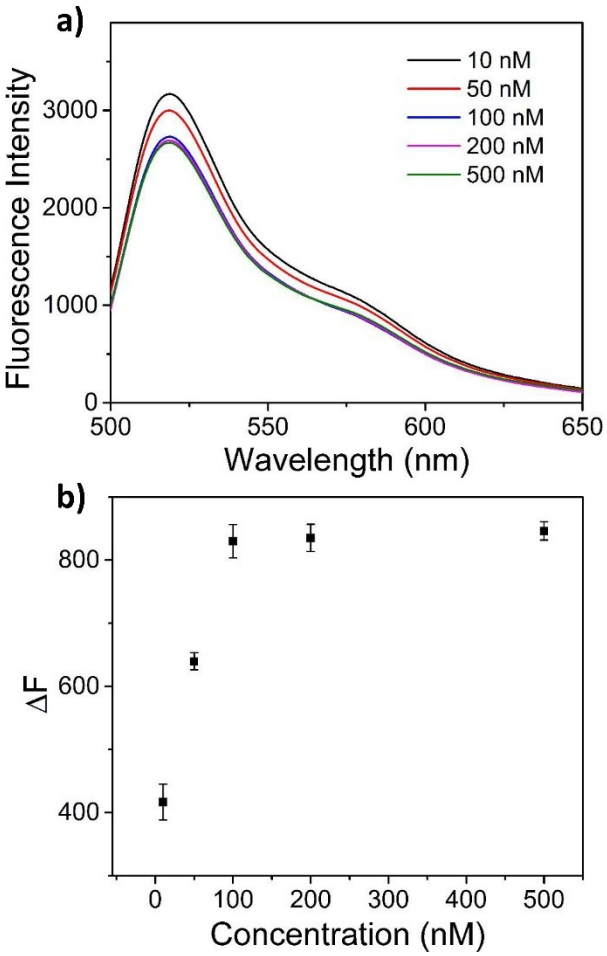

Fig. S8. a) Fluorescence spectra of DTNP sensor for the detection of hsa-miR-146b-5p using different concentrations of assistant DNA. b) The corresponding maximum fluorescence intensity changes as a function of the concentration of assistant DNA. Error bars mean standard deviations (n=3).

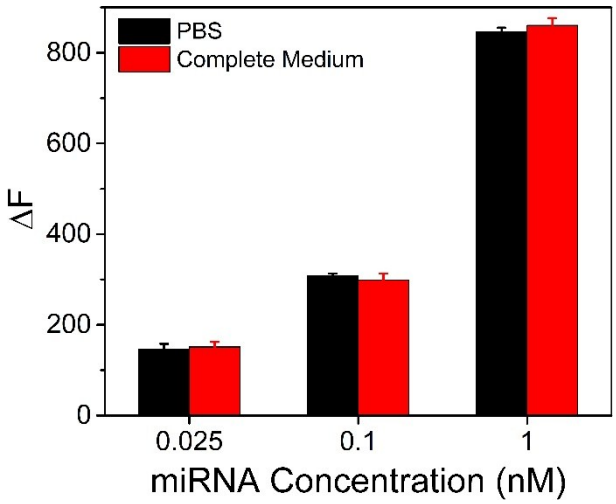

Fig. S9. The maximum fluorescence intensity changes of DTNP sensor in PBS and complete medium as a function of different miRNA concetration. Error bars mean standard deviations (n=3).

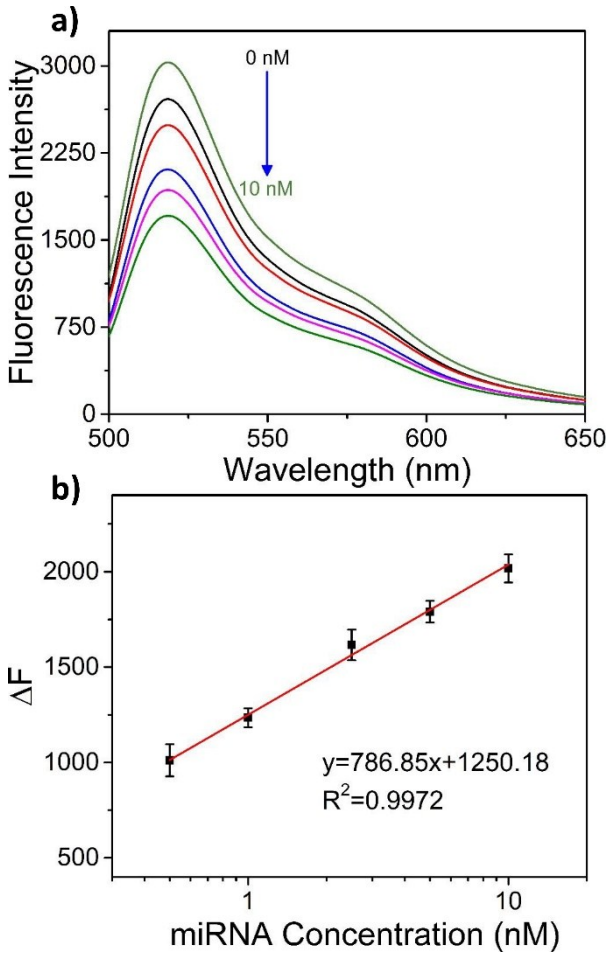

Fig. S10. a) Fluorescence spectra of DTNP incubated with 10% serum spiked with different concentrations of hsa-miR-146b-5p. b) The corresponding maximum fluorescence intensity changes as a function of the concentration of hsa-miR-146b-5p. Error bars mean standard deviations (n=3).

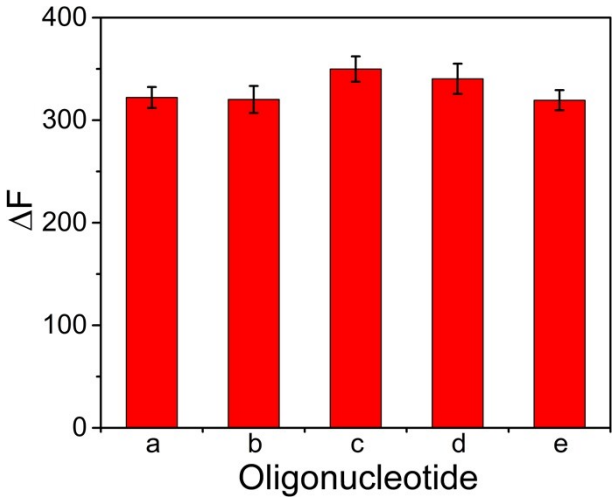

Fig. S11. The maximum fluorescence intensity changes of DTNP sensor tested by interaction with has-miRNA-146b-5p (a) and mixture of has-miRNA-146b-5p with 100-fold NC-miRNA (b), SNP-Up (c), SNP-Mid (d) and SNP-Down (e). The concentration of has-miRNA-146b-5p is 0.1 nM, NC-miRNA, SNP-Up, SNP-Mid and SNP-Down is 10 nM, respectively. Error bars mean standard deviations (n=3).

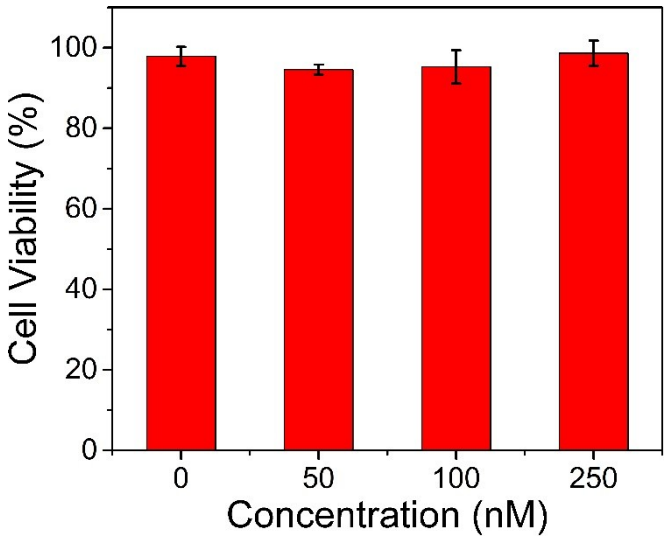

Fig. S12. MTT assays of K1 cells treated with different concentrations of DTNP after incubation for 24 h. Error bars mean standard deviations (n=3).

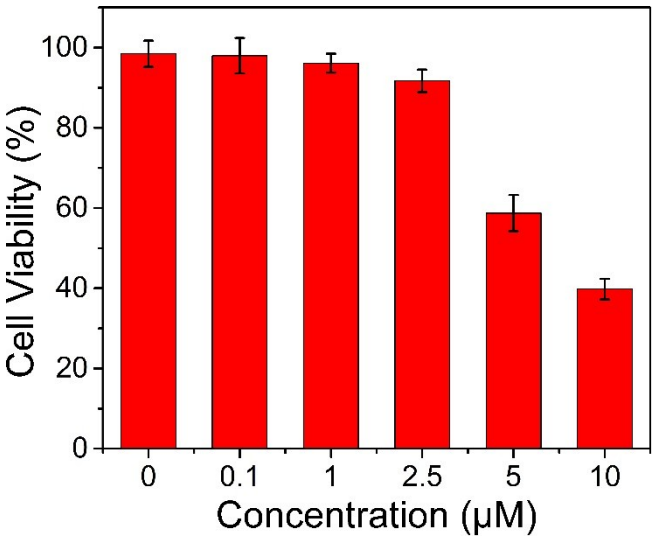

Fig. S13. MTT assays of K1 cells treated with different concentrations of inhibitor (BAY 11-7082) after incubation for 24 h. Error bars mean standard deviations (n=3).

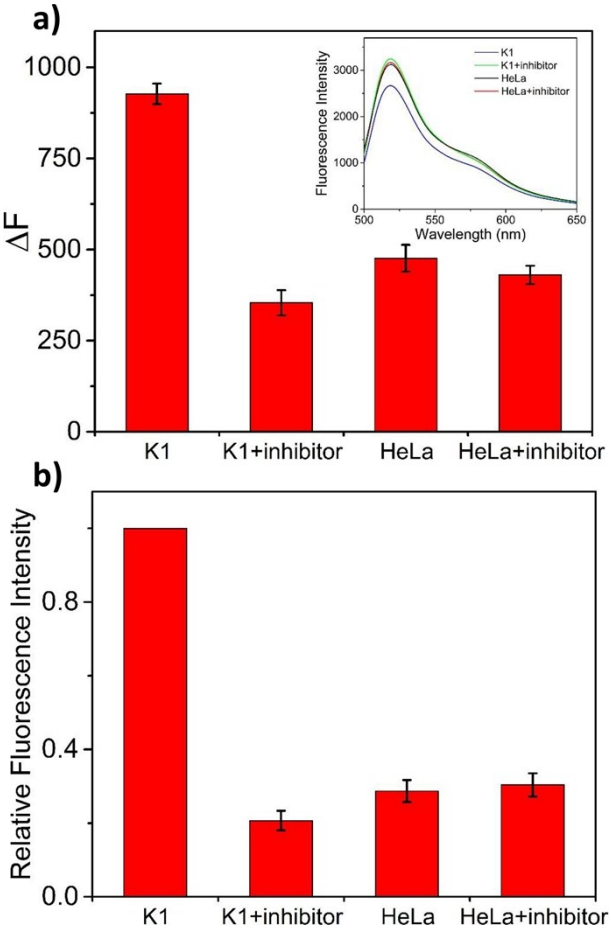

Fig. S14. a) Fluorescence spectra (inset) and corresponding maximum fluorescent intensity changes of the DTNP sensors after incubation with the extracted intracellular miRNA from HeLa and K1 cells pretreated with or without inhibitor BAY 11-7082. b) Relative fluorescence intensity of fluorescence imaging results of DTNP sensors after incubation with the HeLa and K1 cells pretreated with or without inhibitor BAY 11-7082, the fluorescence intensity of K1 cells was set as 1. Error bars mean standard deviations (n=3).

4. Reference

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