Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Double-stranded probe modified AuNPs for sensitive and selective detection of microRNA 30a in solution and live cell

Juan Yao [a], Zhang Zhang [a], Yingze Zhao [b], Wanli Jing [c], Guowei Zuo [a,*]

[a] Key Laboratory of Laboratory Medical Diagnostics of Education, Department of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, P. R. China

[b] State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, P. R. China

[c] Department of Orthopaedics, Tianjin First Center Hospital, Tianjin 300192, P. R. China.

* Correspondence author：Fax：+86 23 68485240,

E-mail address：**gwzuo@qq.com** or **17305105@qq.com**

Text S1. RNA extraction and Quantitative reverse transcription PCR

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Complementary DNA was synthesized by the Reverse Transcriptase M-MLV kit with random hexamer primers. Gene expression levels in each group were measured using Real-Time RT-PCR of CFX Connect™ Real-Time System (Bio-Rad) and the SYBR Premix Ex Taq (TaKaRa). U6 was used as an internal control. MiRNA expression was quantified by the $2^{-\Delta\Delta Ct}$ method. All primer efficiencies were confirmed to be high (>90%) and comparable (Table S1).

Text S2. CCK8 assay

Cell proliferation and toxicity detection was analyzed with the CCK8 assay. Briefly, cells infected with gold nanoparticles by liposome package were incubated in 96-well plate at a density of 1×10^4 cells per well with DMEM supplemented with 10 % FBS. At the indicated time (0 h, 0.5 h, 2 h, 4 h, 8 h), 10 μl of the CCK8 reagent (5 mg/ml) was added to each well and the mixture was then incubated for 4 h. The color reaction was measured at 490 nm with enzyme immunoassay analyzer (Bio-Rad, Hercules, CA, USA).

Table S1. DNA sequence and design principle

Oligonucleotides	Sequences(5' to 3')
Thiolated complement	CTTCCAGTCGAGGATGTTTACAGATGCT$_{10}$(CH$_2$)$_6$-SH
protector	FAM-GCATCTGTAAACATCCTCGA
Target	UGUAAACAUCCUCGACUGGAAG
One mismatch	UGUAAACAUCCGCGACUGGAAG
One deletion	UGUAAACAUCCCGACUGGAAG
One insertion	UGUAAACAUCCGUCGACUGGAAG
Non-complement	UGUAGUAUUAAUCAUUAAUCGA
Hsa-miR-30a	RT primer: GTCGTATCCAGTGCAGGGTCCGAGGTATTCG CACTGGATACGACCTTCCA
	Forward: GGCTTGTAAACATCCTCGAC
	Reverse: GTGCAGGGTCCGAGGT
U6	RT primer: AAAATATGGAACGCTTCACGAATTTG
	Forward: CTCGCTTCGGCAGCACATATACT
	Reverse: ACGCTTCACGAATTTGCGTGTC

Table S2 Thermodynamic analysis of strand displacement reaction

	A + **B/C** ⇌ **A/B** + **C**				**ΔG^0(Kcal/mol)**	K_{eq}	Reaction yield	DF
Target	-1.46	-28.9	-31.8	+0.38	-1.06	6.03	7.1 E-02	-
Mismatch	-1.68	-28.9	-29.0	+0.38	1.96	3.6 E-02	1.6 E-02	4.5
Deletion	-1.83	-28.9	-26.9	+0.38	4.21	8.0 E-04	2.7E-03	25.9
Insertion	-1.87	-28.9	-27.9	+0.38	3.25	4.0 E-03	6.0E-03	11.9
Random	-2.26	-28.9	-9.5	+0.38	22.04	-	-	-

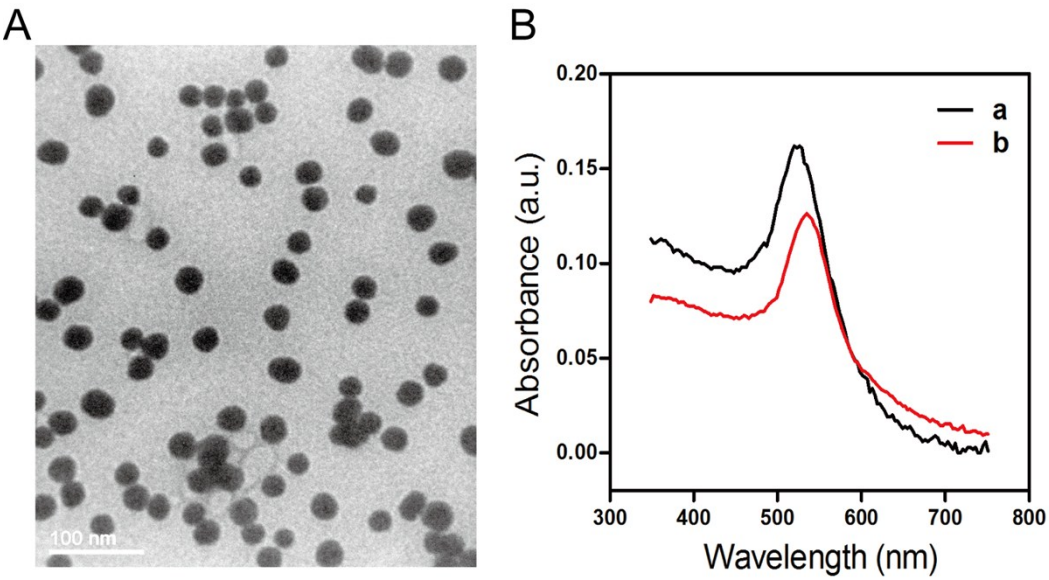

Figure S1 (A) Typical TEM image of the prepared AuNPs. (B) UV–vis spectra of AuNPs before (a) and after (b) assembling of double strand probe.

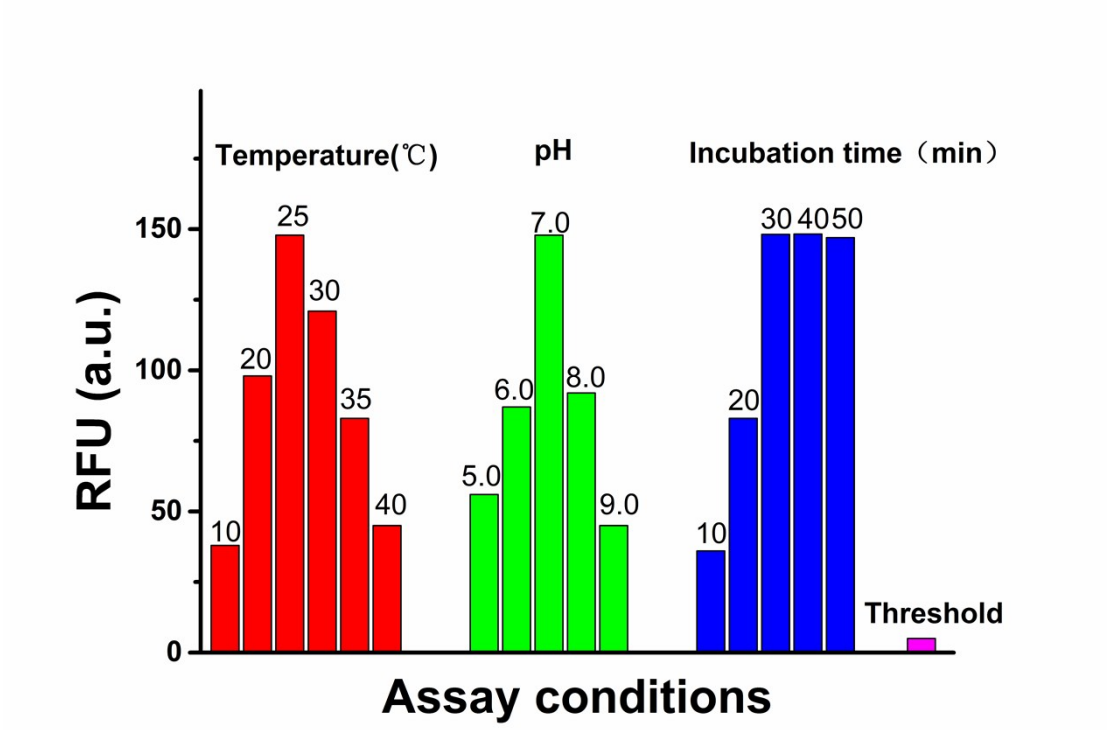

Figure S2 Optimization of experimental conditions

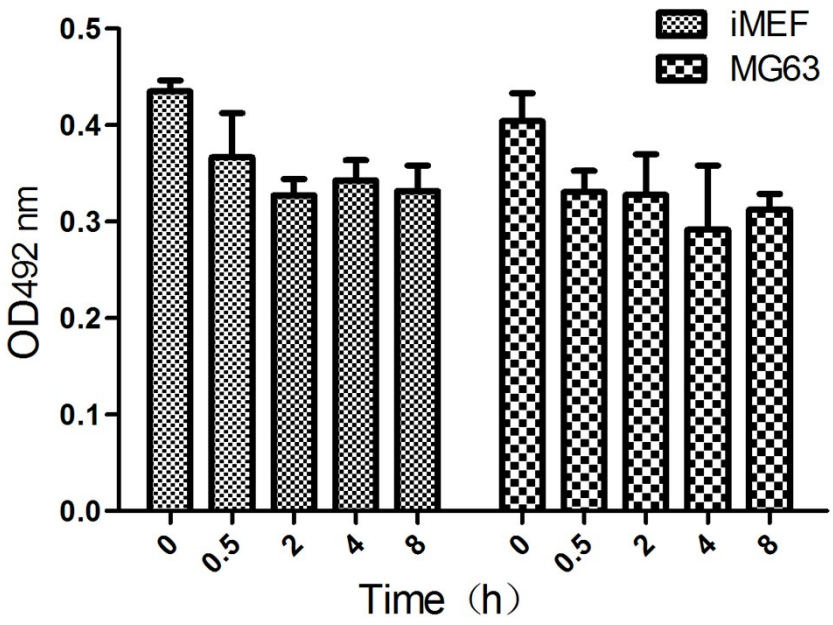

Figure S3 CCK8 assay was used for investigating the cytotoxicity of AuNPs and liposome to iMEF and MG63 cell lines.

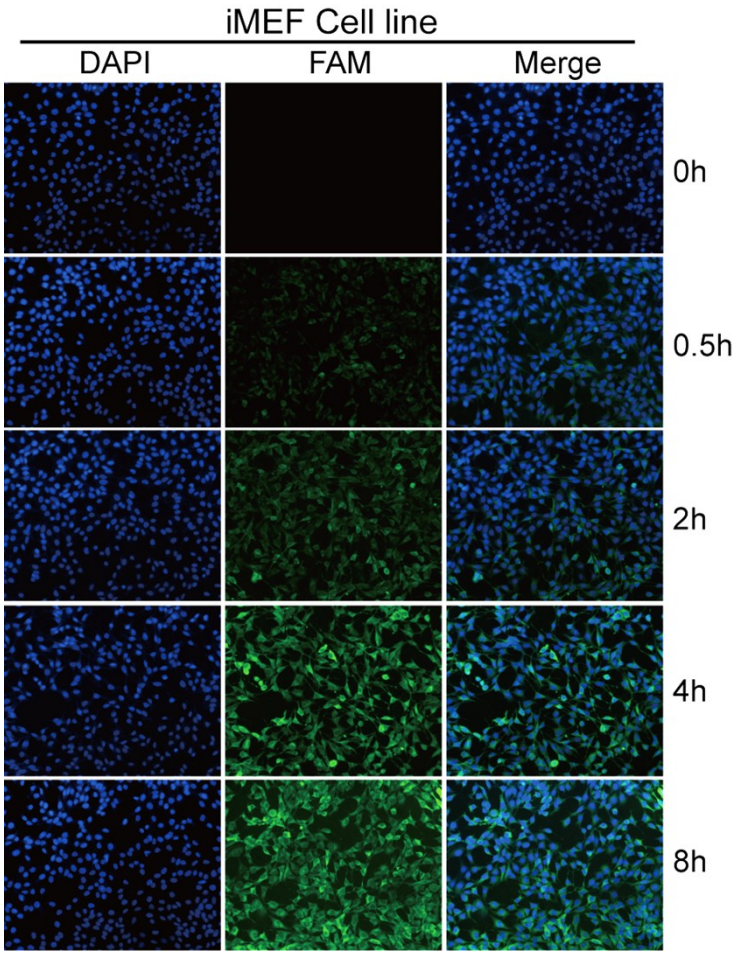

Figure S4 Fluorescence signals were visualized for iMEF cell line as incubation time increased from 0 to 8 h.