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Supplementary Information

DNA-templated silver nanoclusters locate microRNAs in nuclei of gastric cancer cells

Jingpu Zhang [a,b], Yanlei Liu [a], Xiao Zhi [a],Chunlei Zhang [a], Tiefu Liu [b*], Daxiang Cui [a,c*]

[a]Institute of Nano Biomedicine and Engineering, Key Laboratory for Thin Film and Microfabrication of the Ministry of Education, Department of Instrument Science and Engineering, School of Electronic Information and Electrical Engineering, Shanghai Jiao Tong University, P. R. China; [b]Shanghai Public Health Clinical Centre, Fudan University, Shanghai, P. R. China; [c]National Center for Translational Medicine, Shanghai Jiao Tong University, P. R. China

**To whom correspondence should be addressed.*

Tel: 0086-21-37990333; Fax: 0086-21-37990333; Email: liutiefu@shaphc.org

Tel: 0086-21-34206886; Fax: 0086-21-34206886; Email: dxcui@sjtu.edu.cn

SUPPLEMENTARY METHODS

Cell Culture

Cells were maintained at 37°C in a humidified air atmosphere containing 5% carbon dioxide in Dulbecco's Modified Eagle's Media supplemented with 10% FBS.

Establishment of MGC803 cells with over-expressed miR-101-3p

The miR-101-3p sequence in a length of 490bp was amplified from HEK293T cells and constructed into the lentivirus expression vector pCDH1-CMV-MCS-EF1a-GFP-T2A-Puroto. The primers used were miR-101-1F: CCTGAATTCATTCTAATTTAATTCAACTGG and miR-101-1R: TATGG ATCCTCAGCACAACATGGCTGCAC. The acquired vectors were transfected into HEK293T cells using GM easy™ lentivirus packaging kit (Genomeditech) to generate lentivirus. MGC803 cells were infected with the recombinant lentivirus-transducing units plus 8 μg/mL Polybrene (Sigma). The stable colonies (MGC803(101))were obtained after selection on puromycin [1].

RNA extraction and qRT-PCR

10^6 cells were collected for RNA extraction according to the method described earlier[2]. The extracted RNA was detected on NanoDrop 1000 (NanoDrop Technologies, Delaware, USA) and stored at -80°C for further use. After that, miR-101-3p, miR-16-5p and miR-19b-3p were detected by TaqMan qRT-PCR as described in our previous methods[2, 3], in which the spiked-in cel-miR-39 was used as an external control for normalized quantification.

SUPPLEMETARY TABLES AND FIGURES

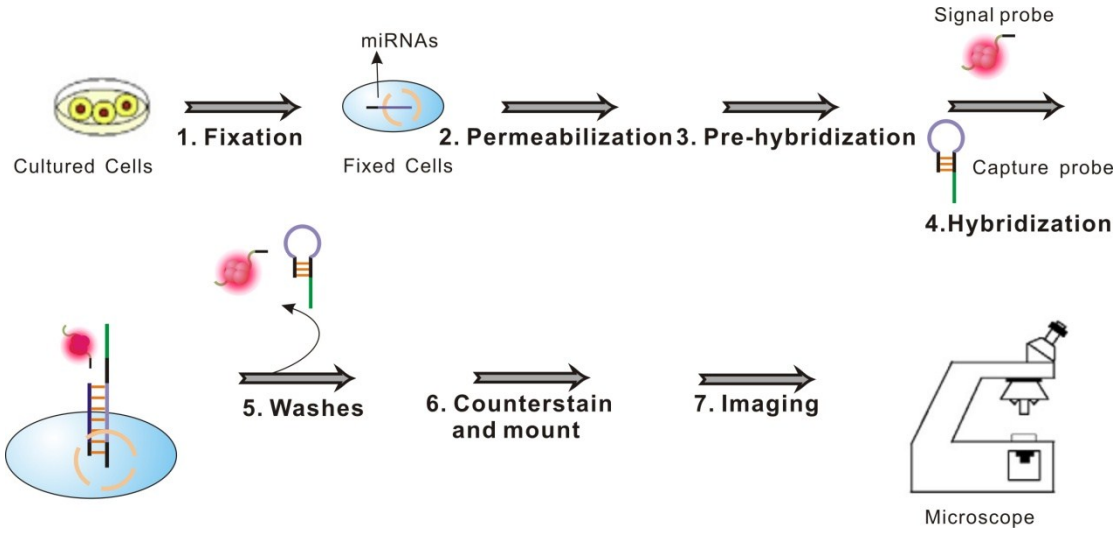

Fig. S1 The protocol flowchart of the main steps for fluorescence *in situ* hybridization detection of miRNAs based on AgNCs/DNAs.

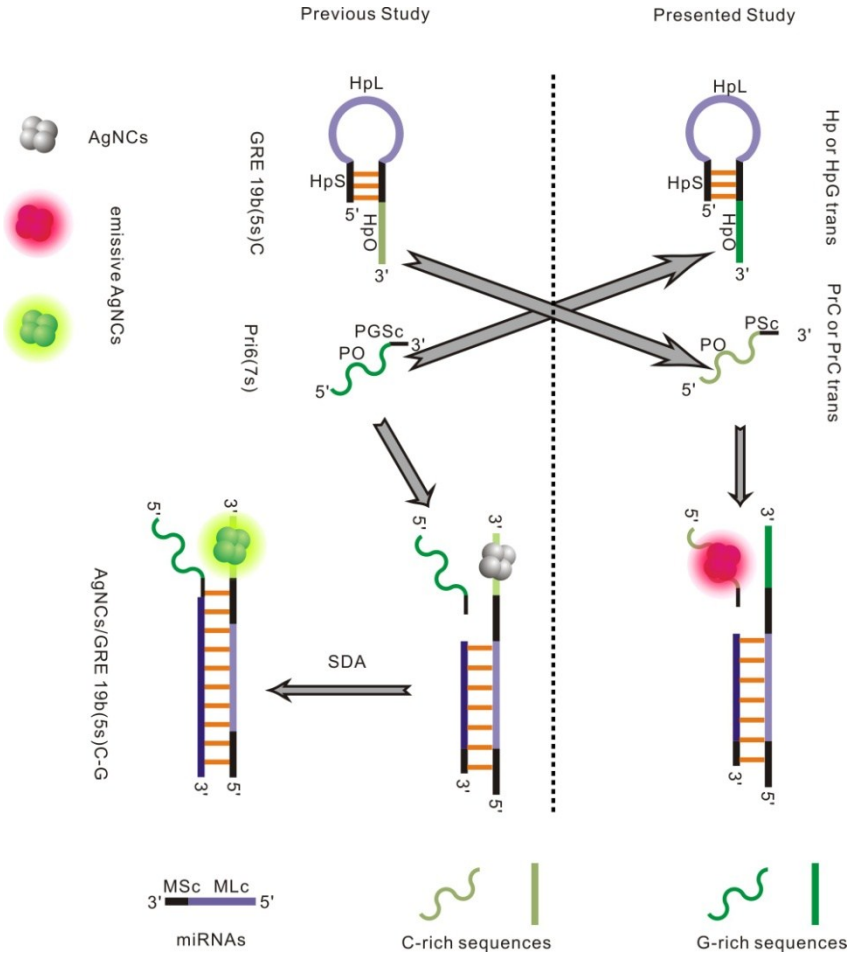

Fig. S2 Schematic illustration of the relationship of our previous study[2] with the presented study.

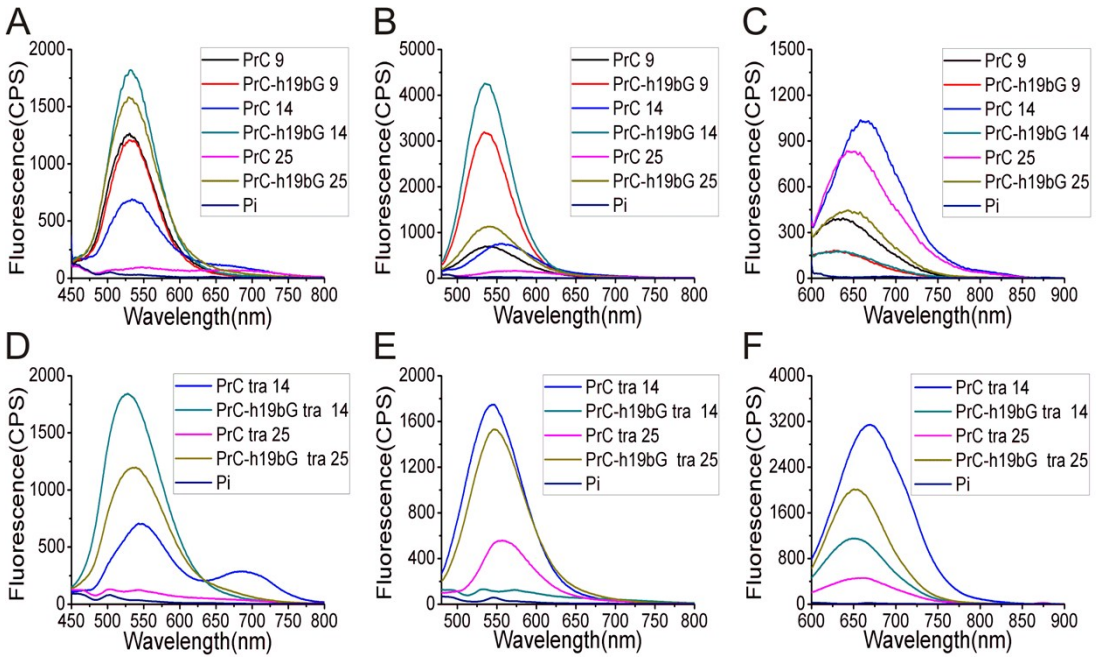

Fig. S3 Fluorescence spectra of the AgNCs/DNAs and their hybrids prepared in different molar ratios of DNAs : $AgNO_3$: $NaBH_4$. **(A-C)** AgNCs/PrC (PrC) and its hybrids (PrC-h19bG) via hybridization with Hph19bG in molar ratios of DNAs:$AgNO_3$:$NaBH_4$ as 1:9:9, 1:14:14 or 1:25:25 (pH8.0) (A, λ_{ex} = 430 nm; B, λ_{ex} = 460 nm; C, λ_{ex} = 580 nm). **(D-F)** AgNCs/PrC trans (PrC tra) and its duplex (PrC-h19bG tra) via hybridization with Hph19bG trans in molar ratios of DNAs:$AgNO_3$:$NaBH_4$ as 1:14:14 or 1:25:25 (pH 6.6) (D, λ_{ex} = 430 nm; E, λ_{ex} = 460 nm; F, λ_{ex} = 580 nm). Sodium phosphate buffer (Pi) at the same pH was used as blank control.

Table S1 Integral areas in the fluorescence spectra for AgNCs/DNAs and their hybrids prepared in different molar ratios of DNAs : $AgNO_3$: $NaBH_4$

DNAs: $AgNO_3$: $NaBH_4$	**AgNCs/DNAs 或 AgNCs/DNA-HpDNAs**	**430nm (450-800nm)**		**460nm (480-800nm)**		**580nm (600-900nm)**	
		***A*[a]**	***R*[b]**	***A*[a]**	***R*[b]**	***A*[a]**	***R*[b]**
1:9:9	PrC	316.744	-	199.757	-	117.189	-
	PrC-h19bG	296.150	0.93	723.702	3.62	50.935	0.43
1:14:14	PrC	225.079	-	241.491	-	351.189	-
	PrC-h19bG	434.725	1.93	958.817	3.97	54.087	0.15
1:25:25	PrC	61.761	-	71.672	-	282.769	-
	PrC-h19bG	411.551	6.66	317.953	4.44	140.185	0.50
1:14:14	PrC tra	282.915	-	915.671	-	1081.408	-
	PrC-h19bG tra	539.396	1.91	497.329	0.54	352.105	0.33
1:25:25	PrC tra	63.369	-	161.540	-	154.751	-
	PrC-h19bG tra	384.306	6.06	443.783	2.75	581.519	3.76

[a,b] elucidated in Table 2.

Table S2 Integral areas in the fluorescence spectra for AgNCs/DNAs and their hybrids prepared at different pH

pH	AgNCs/DNAs 或 AgNCs/DNA-HpDNAs	430nm (450-800nm)		460nm (480-800nm)		580nm (600-900nm)	
		A^a	R^b	A^a	R^b	A^a	R^b
pH6.6	PrC	961.628	-	563.627	-	145.474	-
	PrC-h101G	466.972	0.49	354.567	0.63	23.651	0.16
	PrC-h16G	532.758	0.55	401.970	0.71	34.925	0.24
	PrC-h19bG	462.761	0.48	275.550	0.49	18.767	0.13
	PrC tra	191.670	-	213.175	-	809.598	-
	PrC-h101G tra	1541.494	8.04	946.905	4.44	1458.880	1.80
	PrC-h16G tra	1098.513	5.73	786.435	3.69	764.732	0.94
	PrC-h19bG tra	1190.606	6.21	827.569	3.88	1778.794	2.20
pH7.2	PrC	560.763	-	399.668	-	266.014	-
	PrC-h101G	601.154	1.07	1163.578	2.91	71.696	0.27
	PrC-h16G	552.638	0.99	625.675	1.57	128.778	0.48
	PrC-h19bG	490.693	0.88	1008.772	2.52	48.061	0.18
	PrC tra	144.613	-	308.033	-	972.755	-
	PrC-h101G tra	869.546	6.01	725.160	2.35	671.586	0.69
	PrC-h16G tra	710.756	4.91	692.310	2.25	846.998	0.87
	PrC-h19bG tra	873.168	6.04	737.936	2.40	775.566	0.80
pH8.0	PrC	432.245	-	309.824	-	145.396	-
	PrC-h101G	463.907	1.07	1190.910	3.84	20.114	0.14
	PrC-h16G	495.800	1.15	1369.513	4.42	36.534	0.25
	PrC-h19bG	474.091	1.10	1063.243	3.43	21.037	0.14
	PrC tra	149.375	-	470.589	-	598.073	-
	PrC-h101G tra	791.429	5.30	689.830	1.47	216.737	0.36
	PrC-h16G tra	760.788	5.09	716.006	1.52	211.523	0.35
	PrC-h19bG tra	984.388	6.59	829.842	1.76	290.965	0.49

[a,b] elucidated in Table 2.

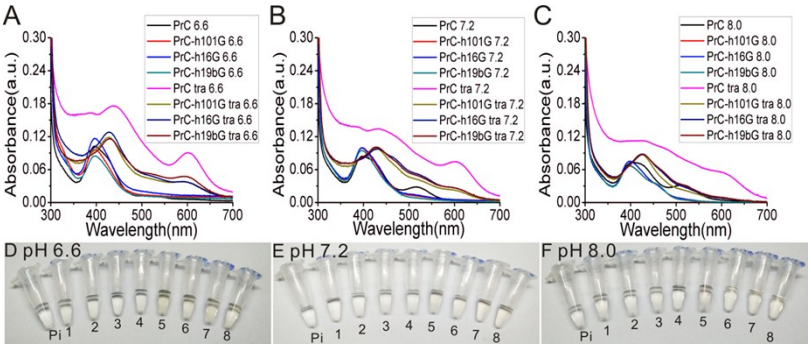

Fig. S4 (A-C) The absorbance spectra of the AgNCs/DNAs and their hybrids prepared at different pH(A, pH 6.6; B, pH 7.2; C, pH 8.0). **(D-F)** Photos of the solutions taken under white light in (A-C) (D, A; E, B; F, C). Subjects and No. labels were illustrated in Fig. 2. The AgNCs/DNAs probes (PrC) without hybridization at the same pH were used as controls.

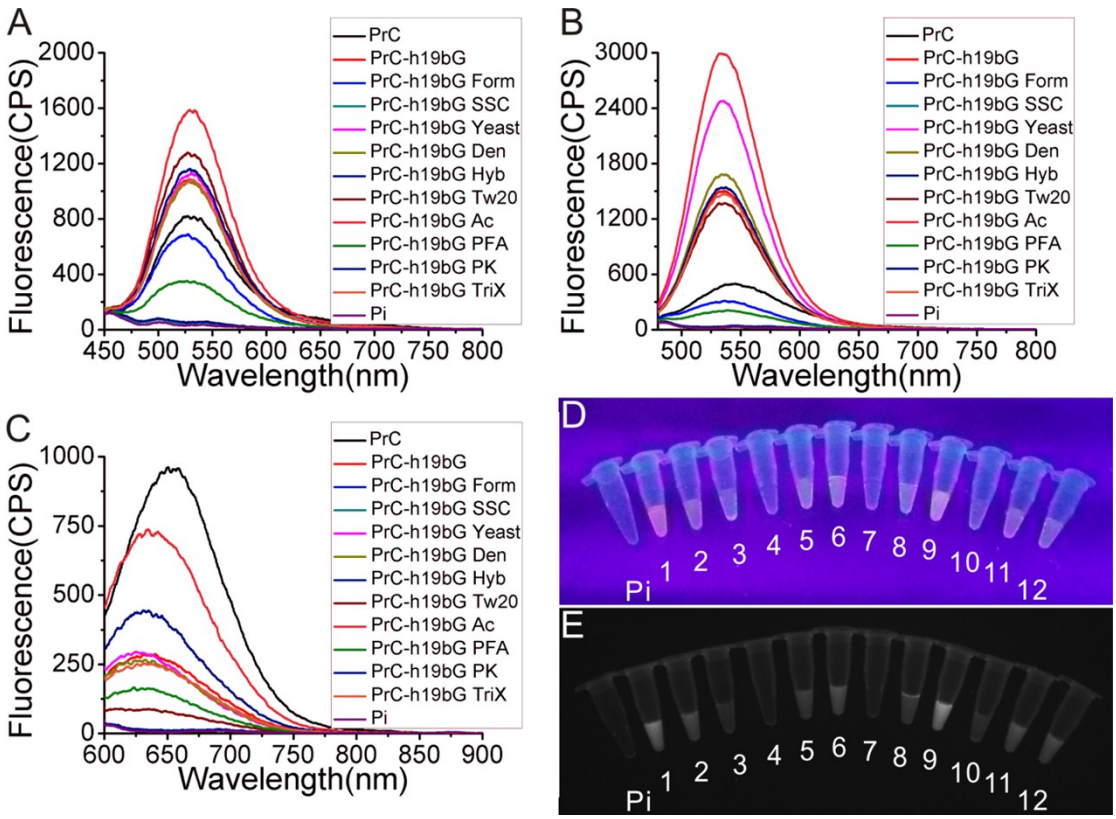

Fig. S5 Effect of components in FISH on the AgNCs/DNA probe and its fluorescence enhancement. **(A-C)** Fluorescence spectra of AgNCs/PrC (PrC) and its hybrid AgNCs/PrC-h19bG (PrC-h19bG) with the following components: formamide(Form), SSC (SSC), yeast tRNA(Yeast), Denhardt' s solution (Den), Hybridization solution (Hyb), Tween 20 (Tw20), acetic anhydride (Ac), PFA (PFA), proteinase K (PK), Triton X-100 (Trix) (A, λ_{ex} = 430 nm; B, λ_{ex} = 460 nm; C, λ_{ex} = 580 nm). **(D, E)** (D) Photo and (E) image taken under UV light by Gel Image System for the solutions in (A-C). 1-12 in (D-E) corresponded to the labels from top to bottom in (A-C). Sodium phosphate buffer (Pi) at the same pH was used as blank control.

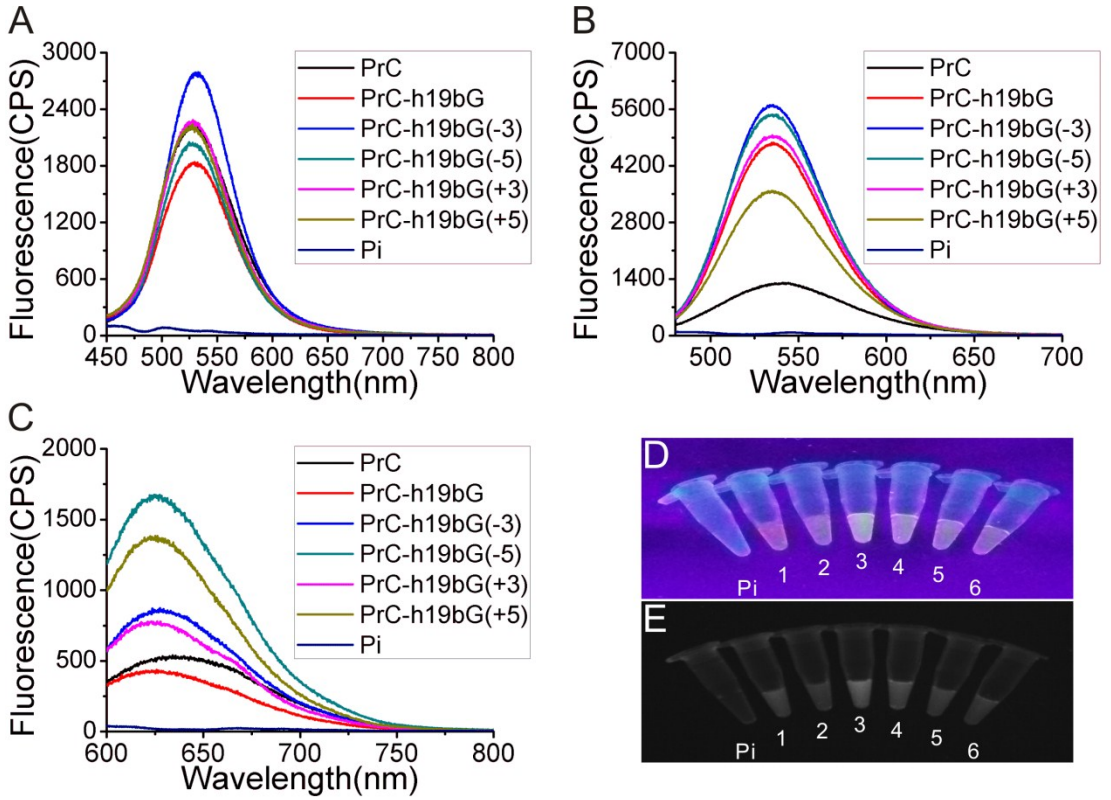

Fig. S6 Effect of the self-prepared hybridization solution in FISH on the AgNCs/DNA probe and its fluorescence enhancement. **(A-C)** Fluorescence spectra of AgNCs/PrC (PrC) and its hybrid AgNCs/PrC-h19bG (PrC-h19bG) with the hybridization solutions (A, λ_{ex} = 430 nm; B, λ_{ex} = 460 nm; C, λ_{ex} = 580 nm). **(D, E)** (D) Photo and (E) image taken under UV light by Gel Image System for the solutions in (A-C). 1-6 corresponded to the labels from top to bottom in (A-C). (-3), (-5), (+3) and (+5) were illustrated in Experimental Section. Sodium phosphate buffer (Pi) at the same pH was used as blank control.

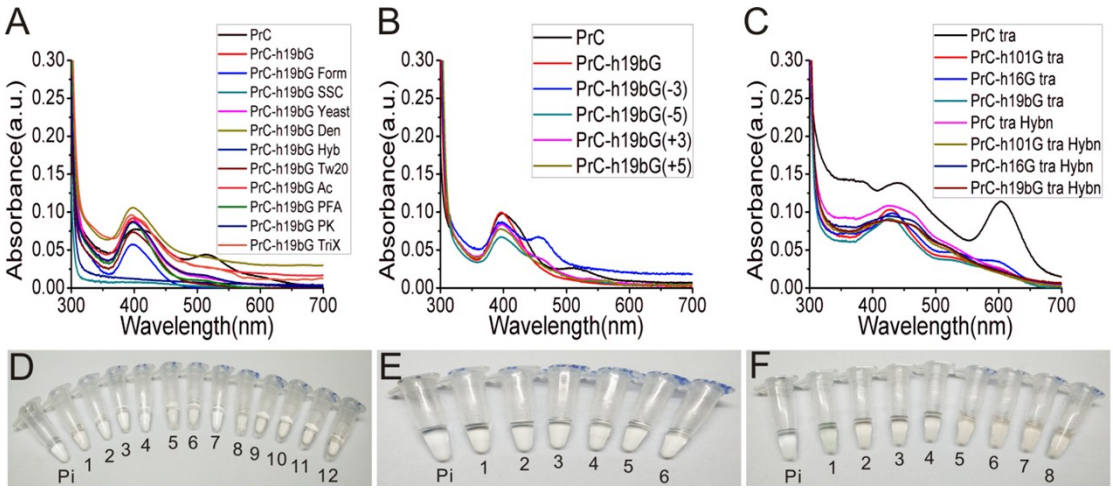

Fig. S7 (A-C) Absorbance spectra of the solutions respectively in (A) Fig.S3, (B) Fig. S4 and (C) Fig. 3. **(D-F)** Photos taken under white light of the solutions in (A-C) (D, A; E, B; F,C). Subjects and No. labels were illustrated respectively in Fig.S4, Fig.S5 and Fig. 3. The AgNCs/DNAs probes (PrC or PrC tra) without hybridization at the same pH were used as controls.

Table S3 Integral areas in the fluorescence spectra for the AgNCs/PrC probe and its hybrids

AgNCs/DNA or AgNCs/DNA-HpDNAs [c]	430 nm (450-800 nm)		460 nm (480-800 nm)		580 nm (600-900 nm)	
	A [a]	***R*** [b]	***A*** [a]	***R*** [b]	***A*** [a]	***R*** [b]
PrC	555.595	-	368.371	-	160.561	-
PrC-h19bG	451.265	0.81	1077.577	2.93	116.439	0.73
PrC-h19bG(-3) [c]	634.116	1.14	1245.243	3.38	224.364	1.40
PrC-h19bG(-5) [c]	489.979	0.88	1242.099	3.37	421.378	2.62
PrC-h19bG(+3) [c]	543.431	0.98	1137.579	3.09	198.858	1.24
PrC-h19bG(+5) [c]	535.404	0.96	852.855	2.32	334.993	2.09

[a,b] elucidated in Table 2. [c] the AgNCs/PrC probe and its hybrids as illustrated in Fig. S6.

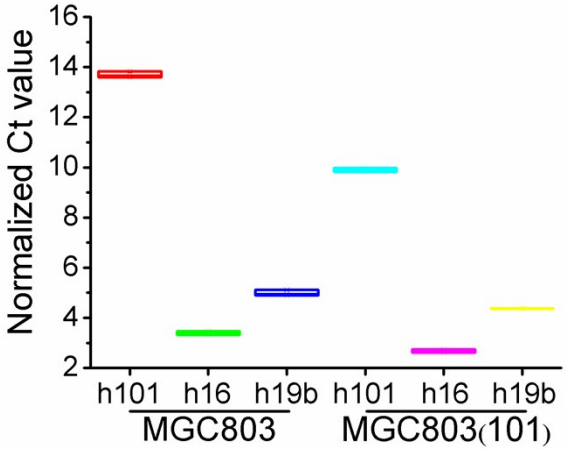

Fig. S8 Quantification of miR-101-3p, miR-16-5p and miR-19b-3p in MGC803 cells and miR-101-3p over-expressed MGC803 cells (MCC803(101)) based on qRT-PCR.

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