Supplementary Material

A label-free activatable biosensor for in situ detection of exosomal microRNAs based on DNA-AgNCs and hairpin type nucleic acid probes

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1. Materials and reagents

All nucleic acid fragments (**Table S1**) were purchased from Beijing Hippo Biotechnology Co., Ltd (Beijing, China) and purified by high performance liquid chromatography. Ovarian cancer cells (SKOV3) and human normal ovarian epithelial cells (IOSE-80) were purchased from the American type of culture collection (Shanghai, China). Silver nitrate ($AgNO_3$), sodium borohydride ($NaHB_4$), sodium dihydrogen phosphate dehydrates ($NaH_2PO_4 \cdot 2H_2O$) and disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Magnesium chloride was purchased from Shanghai Shanpu Chemical Co., Ltd (Shanghai, China). Exosome depleted fetal bovine serum (FBS) was purchased from Shanghai XP Biomed Co., Ltd (Shanghai, China). Tris (hydroxymethyl) methyl aminomethane and sodium dodecyl sulfate were purchased from Biosharp (Wuhan, China). Exosome Extraction Kit was purchased from Shanghai Bebop Biotechnology Co., Ltd (Shanghai, China). Clarity Max™ Western ECL Substrate was purchased from Bio-Rad Laboratories Hercules (Hercules, USA). Phosphotungstic acid negative staining solution (3%) was purchased from Beijing Solaibio Science&Technology Co., Ltd (Beijing, China). All other chemicals used were of analytical grade.

2 Agarose Gel Electrophoresis

3% agarose gel was prepared to detect the hybridization of each sequence, including the groups in which they were alone, in pairs, in three hybridizations and in all hybridizations. The reaction was carried out at 25° C in the dark for 1 h, followed by agarose gel electrophoresis for about 30 min, and the hybridization results of each sequence were observed using a gel imager (Syngene, English).

Table S1. Summary of nucleic acid sequences.

Name	Sequences (5'-3')
HC	**CCCTTAATCCCC**TATAATAAATTTAAATATTATTTATTAAT
CC-G	ATTAATAAATAATATTTAAAATTTATTATA**GGGTGGGGTGGGGTGGGG**
HG	ATTTAAATTTTAATCAGTCTGAATATTTAAAATTTATTATA**GGGTGGGGTGGGGTGGGG**
HT	AATTCAGACTGATTAAAATTTTTCAACATCAGTCTGATAAGCTA
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
let-7a	UGAGGUAGUAGGUUGUAUAGUU
miRNA-155	UUAAUGCUAAUUGUGAUAGGGGU
single-base mismatch	UAGCUUAUCA**C**ACUGAUGUUGA
double-base mismatch	UAGCUUAU**A**AGACU**A**AUGUUGA

The bold segment in HC is the DNA template sequence for the synthesis of AgNCs. CC-G and HG in bold are G-rich sequences. Mismatched bases are highlighted in bold in single base mismatch and double base mismatch.

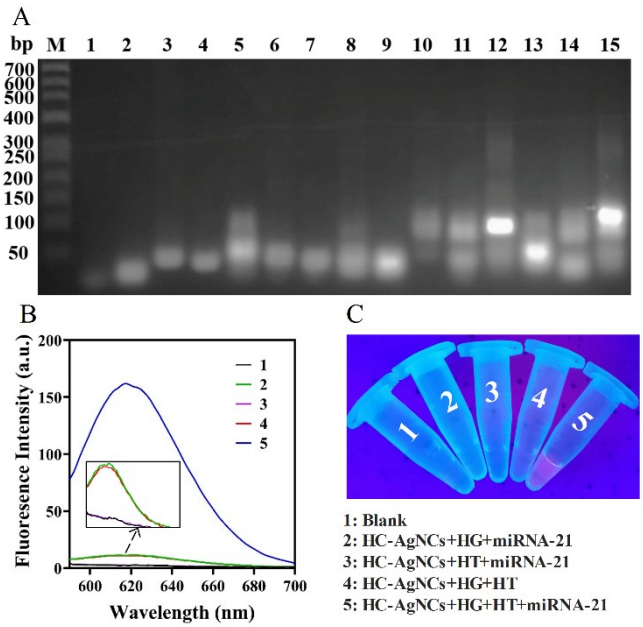

Fig. S1. Feasibility verification for miRNA-21 in solution. (A) Agarose gel electrophoresis analysis. Lane M: Marker, 1: miRNA-21, 2: HT, 3: HG, 4: HC-AgNCs, 5: miRNA-21+ HT, 6: miRNA-21+ HG, 7: miRNA-21+ HC-AgNCs, 8: HT+ HG, 9: HT+ HC-AgNCs, 10: HG+ HC-AgNCs, 11: miRNA-21+ HG+ HC-AgNCs, 12: miRNA-21+ HT+ HG, 13: miRNA-21 + HT+ HC-AgNCs, 14: HT+ HG+ HC-AgNCs, 15: miRNA-21+ HT+ HG+ HC-AgNCs. (B) Fluorescence spectrum analysis. (C) UV transmittance images analysis; The final concentrations of miRNA-21, probes HT, HG and HC-AgNCs were 1.2 μmol/L, respectively.

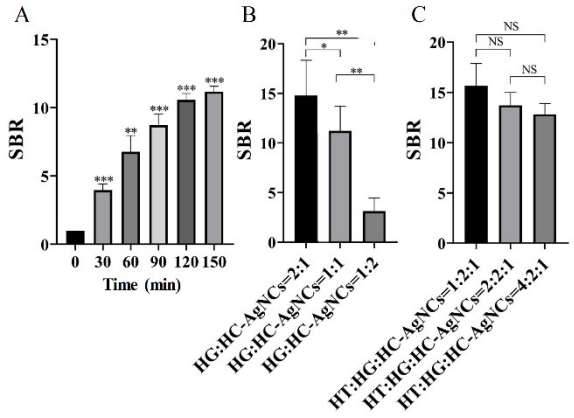

Fig. S2. Optimization of experimental conditions. Optimizations of (A) incubation time, (B), concentration ratio of the probe HG and HC-AgNCs, (C) concentration ratio of the probes HT, HG and HC-AgNCs. Statistic significant differences were indicated: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, n=3.

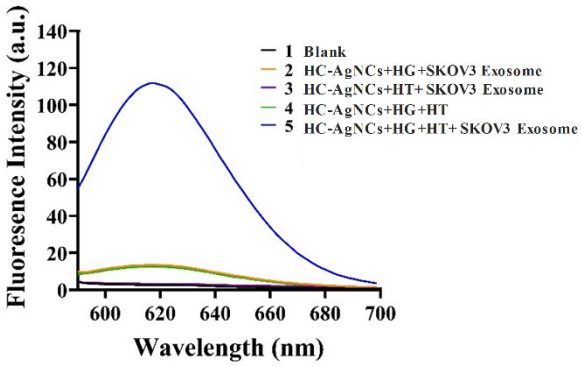

Fig. S3. Feasibility verification for in situ detection of exosomal miRNA-21 in SKOV3 cells.