Electronic Supplementary Material (ESI) for Analyst.

SUPPORTING INFORMATION

Simultaneous imaging of cancer biomarkers in live cells based on DNA-engineered exosomes

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Supporting figures and table

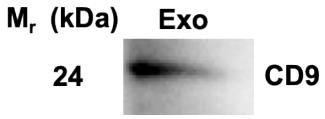

Fig. S1 Western blot image of CD9 protein from purified exosomes using an exoEasy Mid kit. The total protein content of the exosome sample was 20 μg.

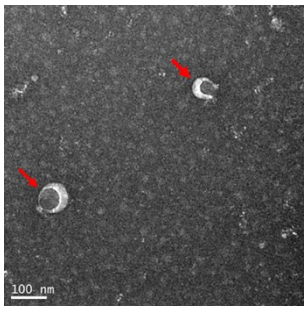

Fig. S2 Transmission electron microscopy image of exosomes purified from HEK 293T cell culture supernatant.

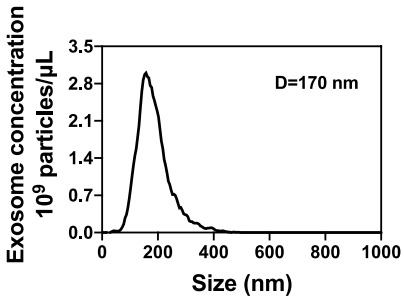

Fig. S3 Characterization of exosome size. The size distribution of purified exosomes was characterized by NTA. The mean diameter of exosome was ca. 170 nm.

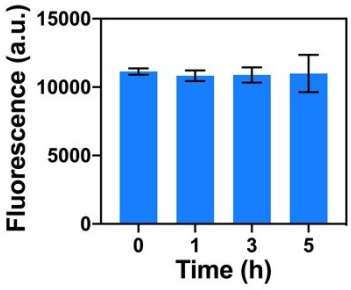

Fig. S4 Stability investigation of FAM-tagged DNA tethers on exosome surface. The FAM fluorescence recovery kinetics of 50 μg/mL FAM-ssDNA-Exos incubated in DMEM medium for different times (0, 1, 3 and 5 h).

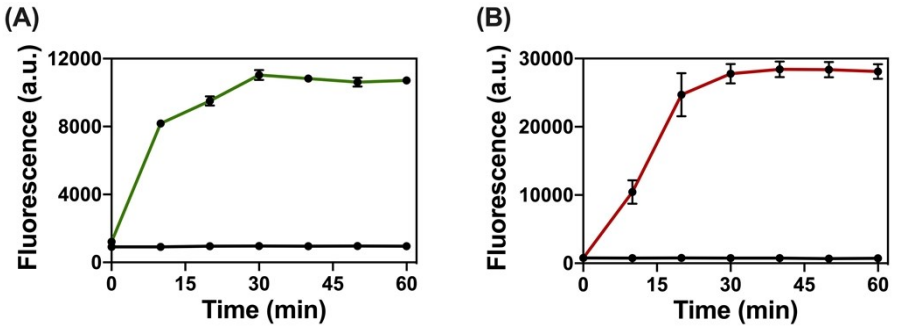

Fig. S5 Feasibility investigation of AP-B and HP towards MUC1 and DNA-21 in vitro, respectively. (A) The FAM fluorescence recovery kinetics of 200 nM AP-B incubated with 200 nM MUC1. (B) The Cy3 fluorescence recovery kinetics of 100 nM HP incubated with 100 nM DNA-21.

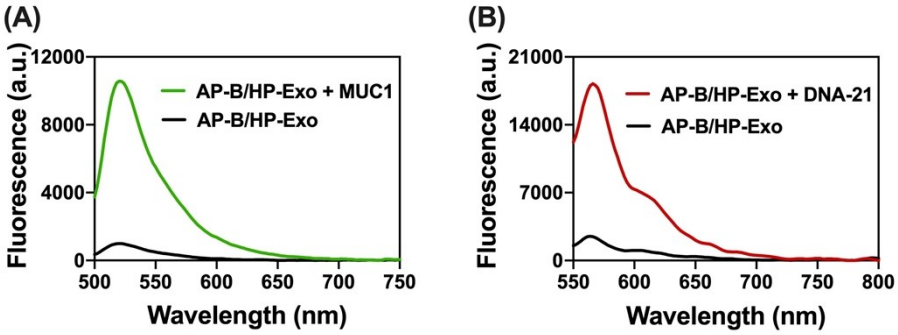

Fig. S6 Feasibility investigation of AP-B/HP-Exo towards detection of MUC1 and DNA-21 in vitro, respectively. (A) The fluorescence spectra of 20 μg/mL AP-B/HP-Exos incubated with 200 nM MUC1 in PBS at 37 °C for 45 min. (B) The fluorescence spectrum changes of 20 μg/mL AP-B/HP-Exos incubated with 100 nM DNA-21 in PBS at 37 °C for 45 min.

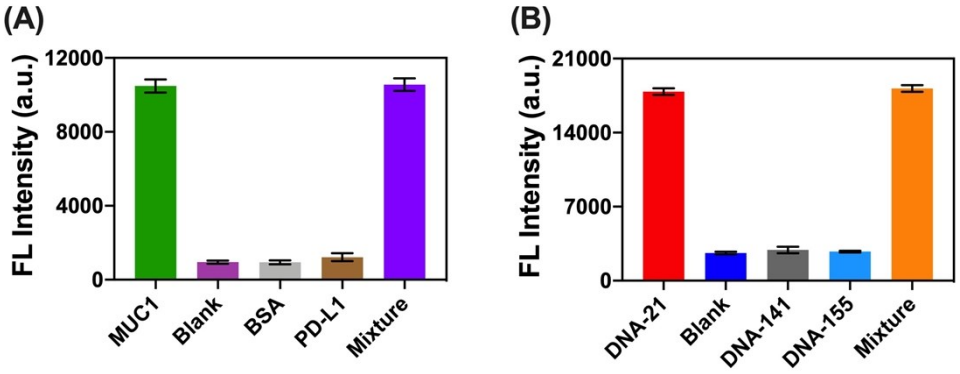

Fig. S7 Selectivity investigation of AP-B/HP-Exo towards detection of MUC1 and DNA-21 in vitro, respectively. (A) The fluorescence intensities of 20 μg/mL AP-B/HP-Exos incubated with 200 nM MUC1 and their analogues and mixture, respectively. (B) The fluorescence intensities of 20 μg/mL AP-B/HP-Exos incubated with 100 nM DNA-21

and their analogues and mixture, respectively.

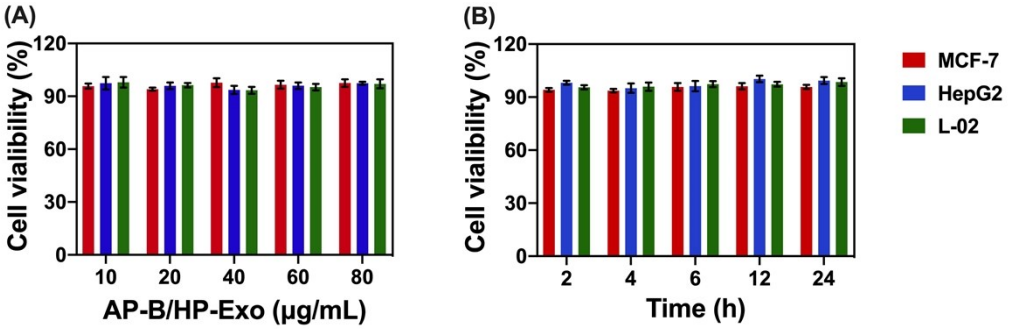

Fig. S8 Cytotoxicity investigation of AP-B/HP-Exo on MCF-7, HepG2 and L-02 cells analyzed with the CCK-8 assay. (**A**) Cell viability of MCF-7, HepG2 and L-02 cells incubated with various concentrations (10, 20, 40, 60 and 80 μg/mL) of AP-B/HP-Exos at 37 °C for 4 h. (**B**) Cell viability of MCF-7, HepG2 and L-02 cells incubated with 40 μg/mL AP-B/HP-Exos for different times (2, 4, 6, 12 and 24 h) at 37 °C.

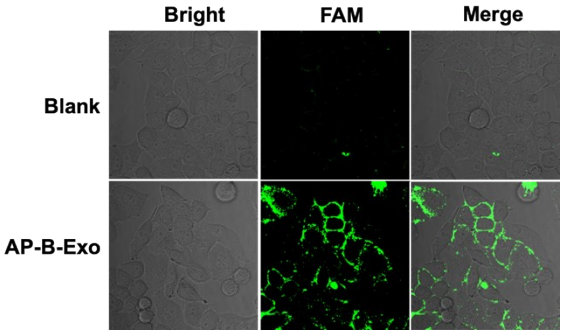

Fig. S9 Confocal microscopy images of MCF-7 cells incubated with 40 μg/mL AP-B-Exos for 90 min. MCF-7 cells in 500 μL DMEM were set as a blank.

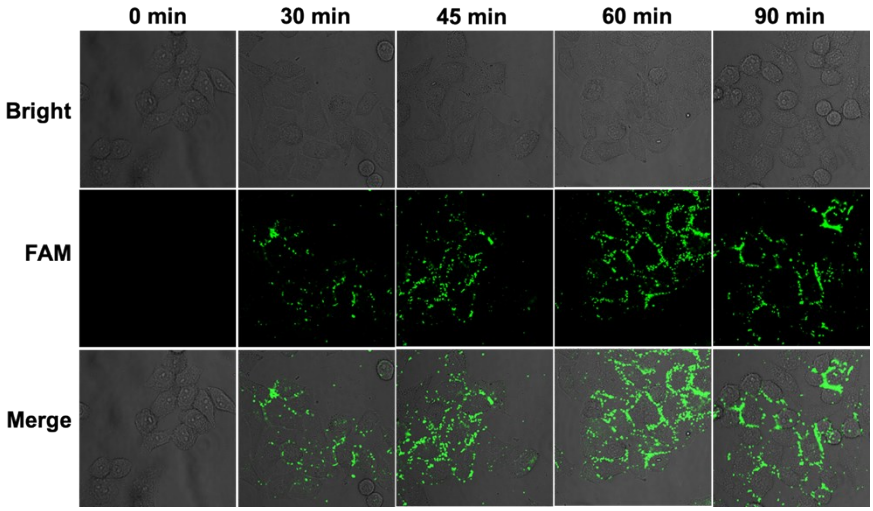

Fig. S10 Investigation of MCF-7 cells incubating with AP-B-Exo at different time points. Confocal microscopy images of MUC1 in MCF-7 cells incubated with 40 μg/mL AP-B-Exos for 0, 30, 45, 60, and 90 min.

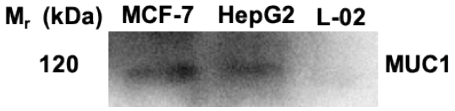

Fig. S11 Western blot analysis of the MUC1 expression in the MCF-7, HepG2, and L-02 cells.

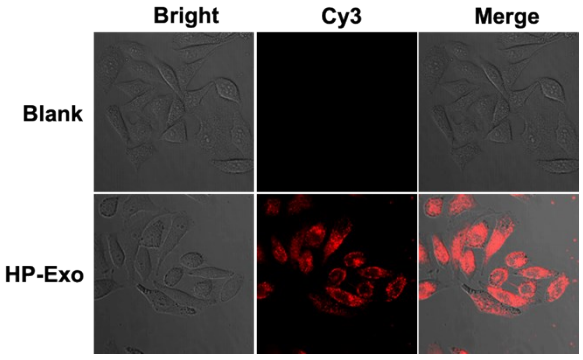

Fig. S12 Confocal microscopy images of MCF-7 cells incubated with 20 μg/mL HP-Exos for 90 min. MCF-7 cells in 500 μL DMEM were set as a blank.

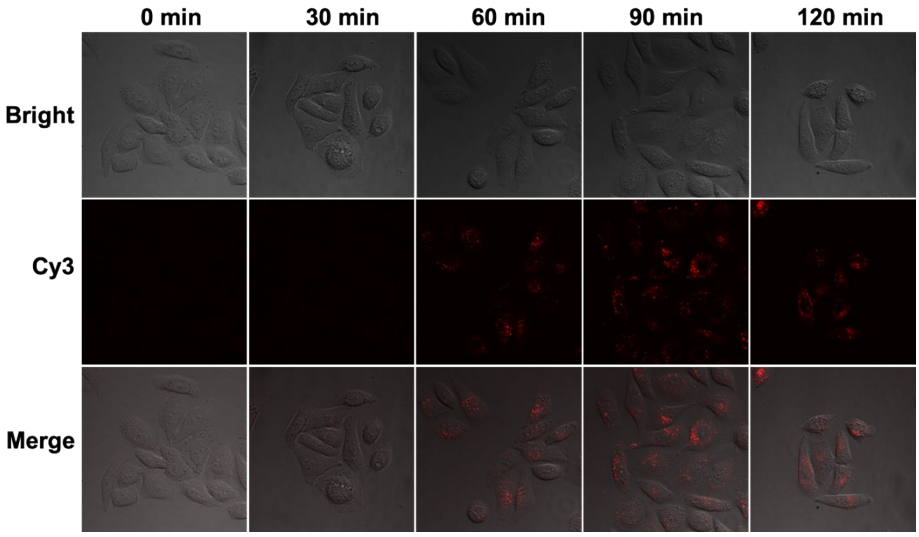

Fig. S13 Investigation of MCF-7 cells incubating with HP-Exo at different times. Confocal microscopy images of miR-21 in MCF-7 cells incubated with 20 μg/mL HP-Exos for 0, 30, 60, 90, and 120 min.

Table S1. Sequence information of oligonucleotides used in this work

Name	Sequence (5′-3′)
AP	FAM-TTTTTTGCAGTTCCTTTGGATACCCTGG
B	BHQ1-CAAACCAACTGCTTTTTTTTT-Cholesterol
HP	BHQ2-TAGCTTATCCAGGGTATCCATCAACATCAGTCTGATAAGCTA-Cy3-TTTTTT-Cholesterol
FAM-ssDNA	FAM-GCAGTTGATCCTTTGGATACCCTGGTTTTTTTTT-Cholesterol
DNA-21	TAGCTTATCAGACTGATGTTGA
DNA-141	CATCTTCCAGTACAGTGTTGGA
DNA-155	TTAATGCTAATCGTGATAGGGGT
miR-21 forward primer	ACACTCCAGCTGGGTAGCTTATCAGACTG
miR-21 reverse primer	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACATC