**Supporting information**

# Simultaneous detection of exosomal membrane protein and RNA by highly sensitive aptamer assisted multiplex-PCR

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**Table S1**. The sequences of PLA, primers, TaqMan probes and scramble DNA sequence (Rc) used in this study.

| Sequence | 5'-3' |
|---|---|
| CD63 PLA | ACTTCAGTCCATCTCTCGCTCACCCCACCTCGCTCCCGTGACACTA ATGCTAAGTTCCTATCAGATCAACCT |
| CD63-F | ACTTCAGTCCATCTCTCGCT |
| CD63-R | AGGTTGATCTGATAGGAACT |
| CD63-T | FAM-ACCCCACCTCGCTCC-MGB |
| PDL1 PLA | ATTATAGAGGACACCTAGTACGGGCCACATCAACTCATTGATAGA CAATGCGTCCACTGCCCGTGTTATTACGACATTTTGGAA |
| PDL1-F | ATTATAGAGGACACCTAGT |
| PDL1-R | TTCCAAAATGTCGTAATAAC |
| PDL1-T | FAM-CGGGCCACATCAACT-MGB |
| Rc | ATTATAGAGGACACCTAGTCATATTATTTTTTAATTATTTATATTAT ATTGTTATTACGACATTTTGGAA |
| IDO1-F | TGGAGAAAGCCCTTCAAGTG |
| IDO1-R | CCAGAACCCTTCATACACCAG |
| IDO1-T | VIC-ACCAAATCCACGATCATGTGAACCCA-BHQ1 |
| SLC25A6-F | GGCCTACTTCGGCGTGTAC |
| SLC25A6-R | GAAGGGGTAGGACACCACG |
| SLC25A6-T | Cy5-TCACGGTCTGCGCGATCATCCA-BHQ3 |

The underlined sequences were original aptamers. F-Forward Primer, R-Reverse Primer, T-TaqMan Probe.

**Table S2**. The information of cancer patients.

| | Tumor type | Stage | Treatment |
|---|---|---|---|
| P1 | Lung adenocarcinoma | IV | Pemetrexed, Cisplatin, Nivolumab |
| P2 | Lung adenocarcinoma | IV | Pemetrexed, Cisplatin, Endostar, Nivolumab |
| P3 | Lung squamous cell carcinoma | IV | Gemcitabine, Cisplatin, Endostar, Nivolumab |
| P4 | Lung squamous cell carcinoma | IV | Paclitaxel, Carboplatin, Cisplatin, Gemcitabine, Nivolumab |
| P5 | Lung adenocarcinoma | IIIB | Pemetrexed, Lobaplatin, Endostar, Nivolumab |

**Table S3**. The zeta potentials of COOH-MBs before and after modification by $NH_2$-S-S-$NH_2$ ($NH_2$-S-S-MBs) and DSPE-PEG-NHS (DSPE-S-S-MBs).

| | COOH-MBs | $NH_2$-S-S-MBs | DSPE-S-S-MBs |
|---|---|---|---|
| Zeta potential/mV | -33.5±4.82 | 22.3±3.90 | -34.9±5.11 |

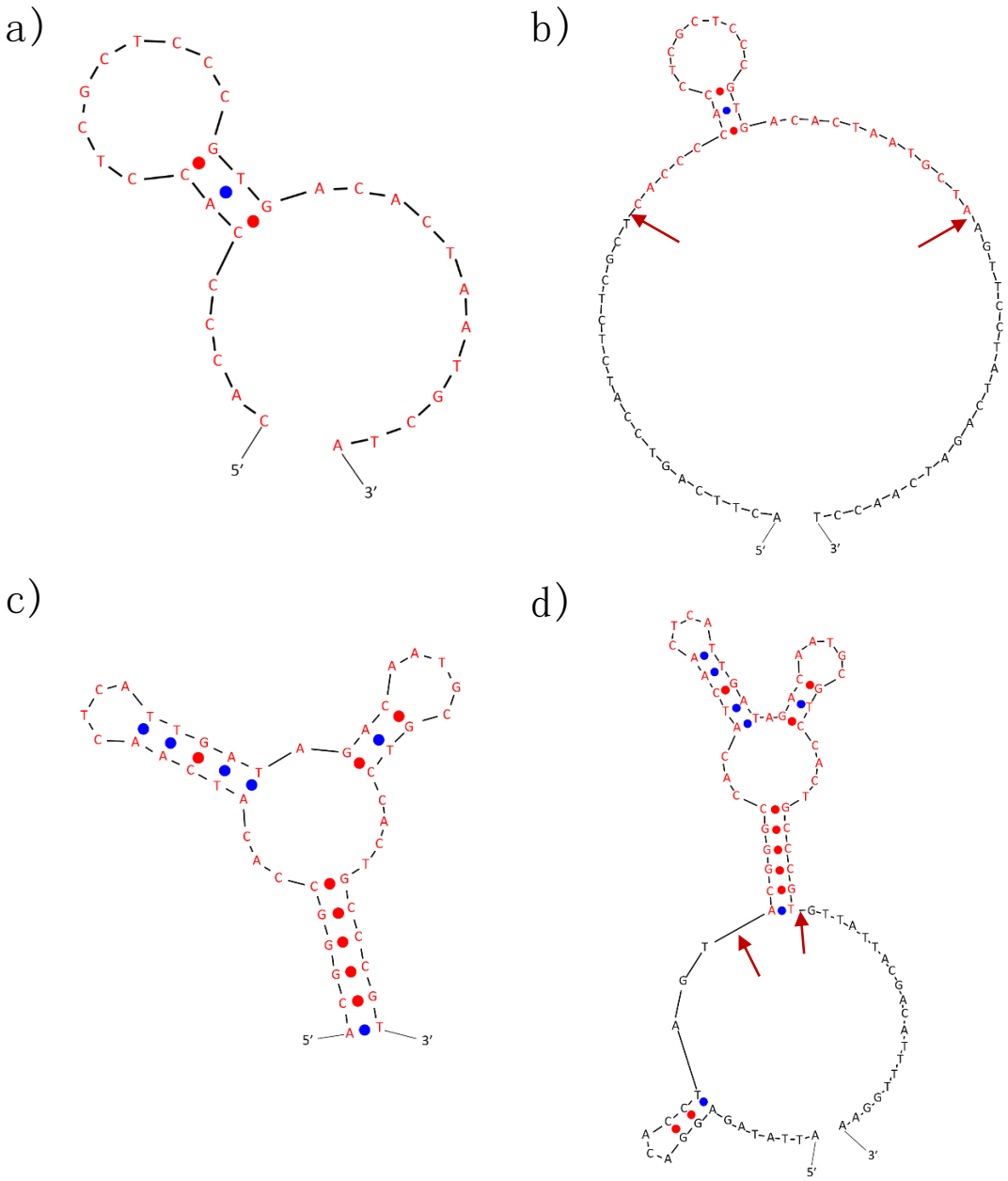


**Figure S1**. Conformational simulation of a) CD63 aptamer, b) CD63 PLA, c) PD-L1 aptamer and d) PD-L1 PLA by OligoAnalyzer. The two primers were attached to the 5' and the 3' end of aptamer, respectively (pointed by red arrows).

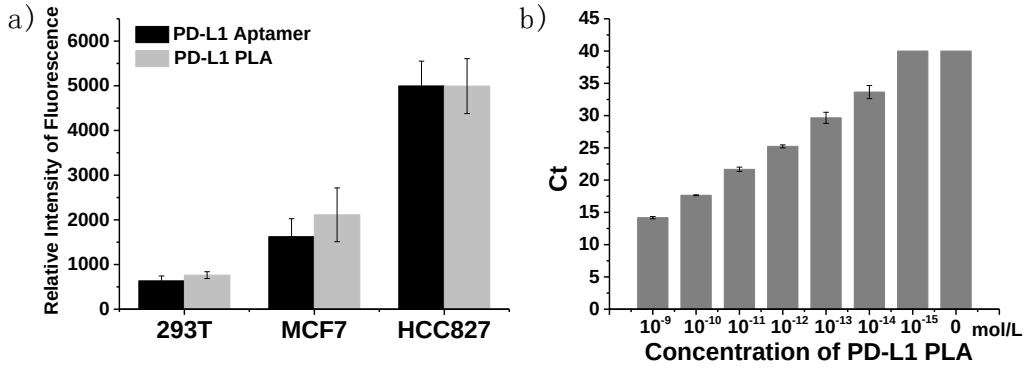


**Figure S2**. a) Flow cytometry results of different cells coupled with fluorescein-labeled PD-L1 aptamer and PLA, respectively. b) The PCR results from different concentrations of PD-L1 PLA. The error bars represent the standard deviations of three repetitive measurements.

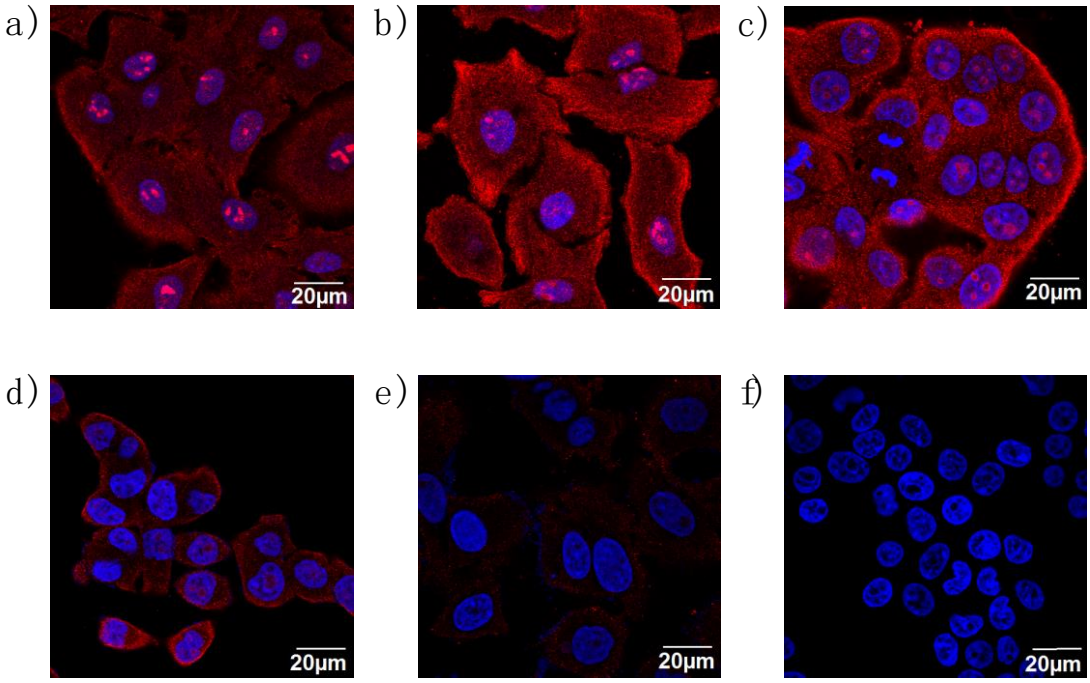


**Figure S3**. Immunofluorescence staining images of different cells: a) A549, b) A549+IFN-γ, c) HCC827, d) A375, e) MCF7 and f) 293T cells. The cells were treated with Alexa 555-secondary antibody for the fluorescence labeling of PD-L1 protein (red). The cell nucleuses were labeled with Hoechst 33258 (blue).

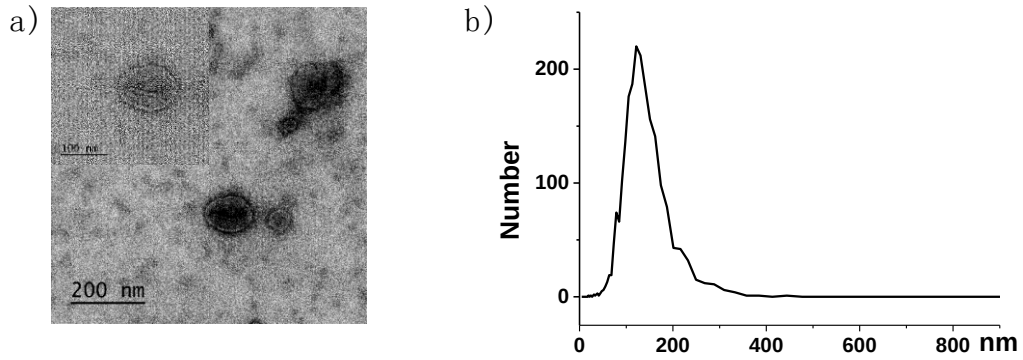


**Figure S4.** a) Typical TEM image of the A375 cell-derived exosomes. b) The size distribution of A375 cell-derived exosomes measured by NTA.

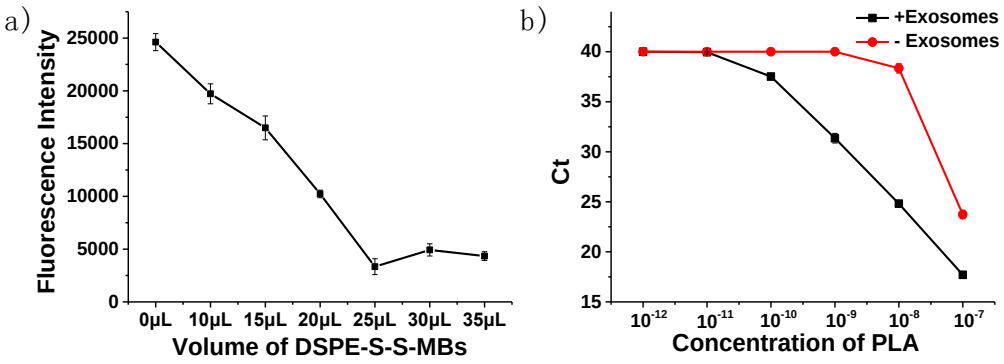


**Figure S5**. a) The fluorescence intensity of the supernatant after removing DSPE-S-S-MBs captured fluorescein-labeled exosomes with different amount of DSPE-S-S-MBs. b) Ct values corresponding to different concentrations of PLA in the presence (+Exosome) or absence (-Exosome) of exosomes. The concentrations of exosomes were $1\times10^5$ particles/μL. The error bars represent the standard deviations of three repetitive measurements.

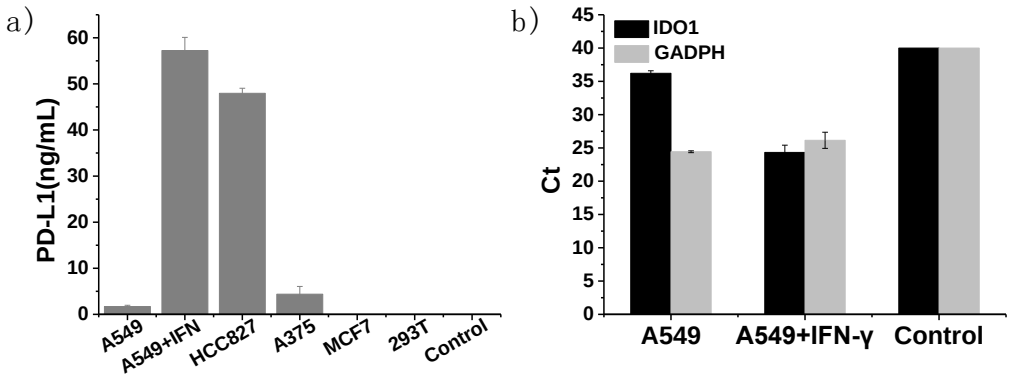


**Figure S6**. a) Detection of PD-L1 from exosomes ($1\times10^7$ particles/μL) of different cell lines by PD-L1 protein ELISA kit. Control was the sample diluent provided by the ELISA kit and used as a negative sample. b) PCR results of cellular IDO1 RNA and GADPH (glyceraldehyde-3-phosphate dehydrogenase) RNA from A549 cells with or without IFN-γ. GADPH was the reference RNA of cells. Control was the negative sample ($H_2O$) to ensure that the PCR was free of contamination. The error bars represent the standard deviations of three repetitive assays.

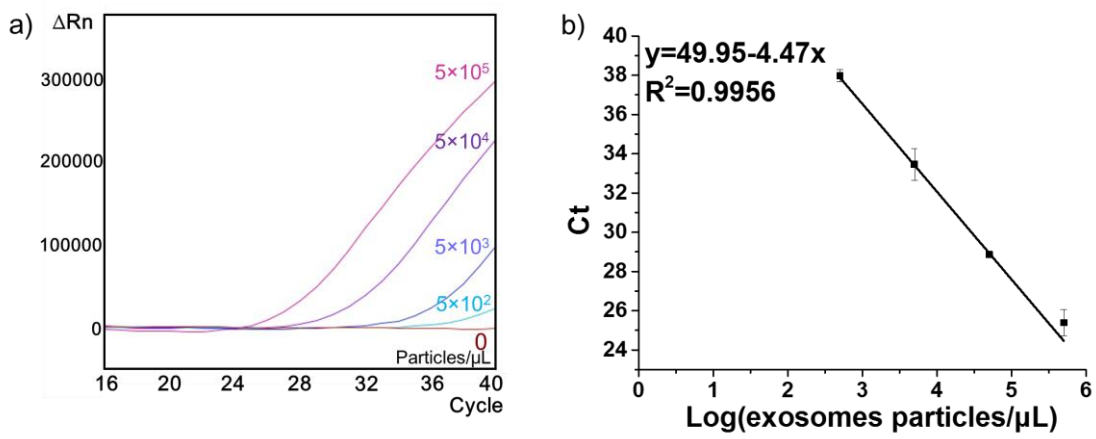


**Figure S7**. a) Results of different concentrations of HCC827 cell-derived exosomes detected by PD-L1 aptamer assisted multiplex-PCR. b) The linear relationship results from Figure S7a.

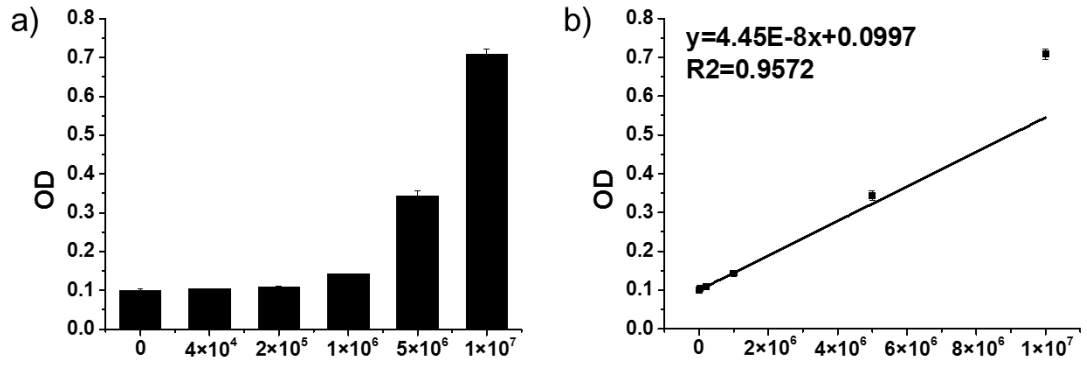


**Figure S8**. a) Results of different concentrations of HCC827 cell-derived exosomes detected by PD-L1 protein ELISA kit. b) The linear relationship of results from Figure S8a.