Supporting Information for

Isolation of DNA Aptamer Targeting PD-1 with Antitumor Immunotherapy Effect

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MATERIALS

Cell lines and buffers

CHO-K1 (Chinese hamster ovary cell), Hep G2 (human liver hepatoma cell) and Jurkat (human acute T leukemia cell) were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). PD-1 (cells overexpressed PD-1 protein) and CHO-K1 cells were maintained in F12K medium. Jurkat cells were cultured in RPMI 1640 medium. Hep G2 cells were cultured in Eagle's Minimum Essential Medium, and all medium was supplemented in 10% FBS and 100 U/mL penicillin-streptomycin. Cells were rinsed before and after incubation with washing buffer (4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS without calcium and magnesium). Binding buffer used for selection was prepared by adding yeast tRNA (0.1 mg/mL, Sigma) and BSA (1 mg/mL, Sigma) into washing buffer.

ssDNA library and primers

The ssDNA library (m-Lib) composed of a 40 random-nucleotide (40-mer) region and two conservative 18-nt primers for PCR amplification flanking the center (5′-ATACCAGCTTATTCAATT-40N-AGATAGTAAGTGCAATCT-3′). The forward primer P1 (5′-ATACCAGCTTATTCAATT-3′), the FAM-labeled forward primer FAM-P1 (5′-(FAM)-ATACCAGCTTATTCAATT-3′), the biotinylated reverse primer biotin-P2 (5′-Bio-AGATTGCACTTACTATCT-3′) and the ssDNA library were all synthesized by Sangon Biotechnology Co., Ltd. (China).

Table S1 The details of cell-SELEX procedure.

Round	(ssDNA pool)/nmol	Positive selection (dimension of culture dish/mm)	Incubation time (min) for positive selection	Counter selection (dimension of culture dish/mm)	Incubation time (min) for counter selection
1	10	100	60	-	-
2	8	100	60	-	-
3	6	100	60	-	-
4	4	100	60	60	30
5	2	100	50	60	40
6	1.2	100	50	60	40
7	0.9	60	40	60	50
8	0.7	60	40	60	50
9	0.6	60	40	100	50
10	0.5	60	30	100	60
11	0.3	60	30	100	60
12	0.2	60	30	100	60

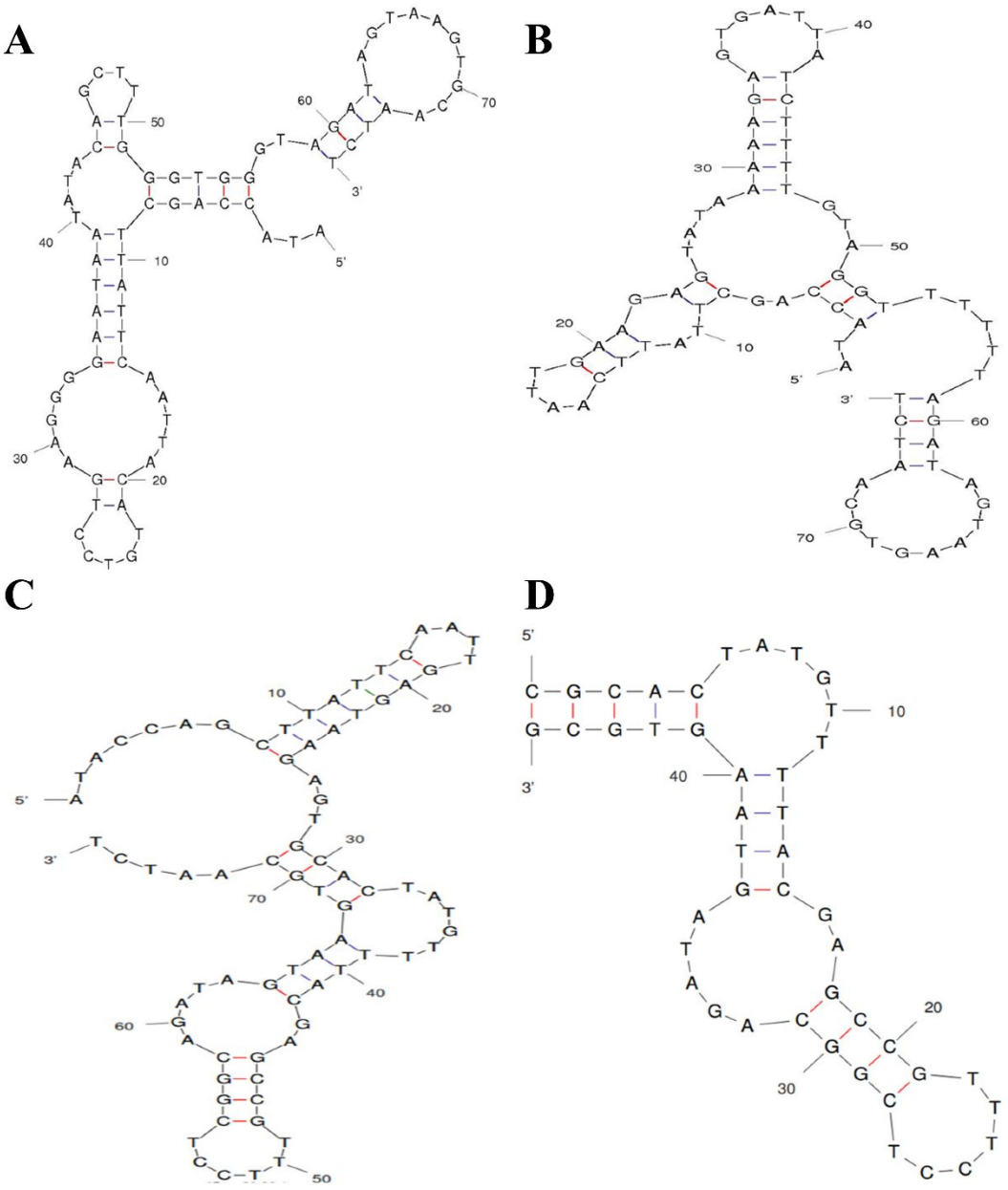

Figure S1. The predicted secondary structure of aptamer candidates PD2 (A), PD27(B), PD4 (C), and PD4S (D) by M-fold (http: //mfold.rna.albany.edu/) under 4 ^{o}C, in which the concentration of sodium and magnesium were 140 mM and 5 mM, respectively.

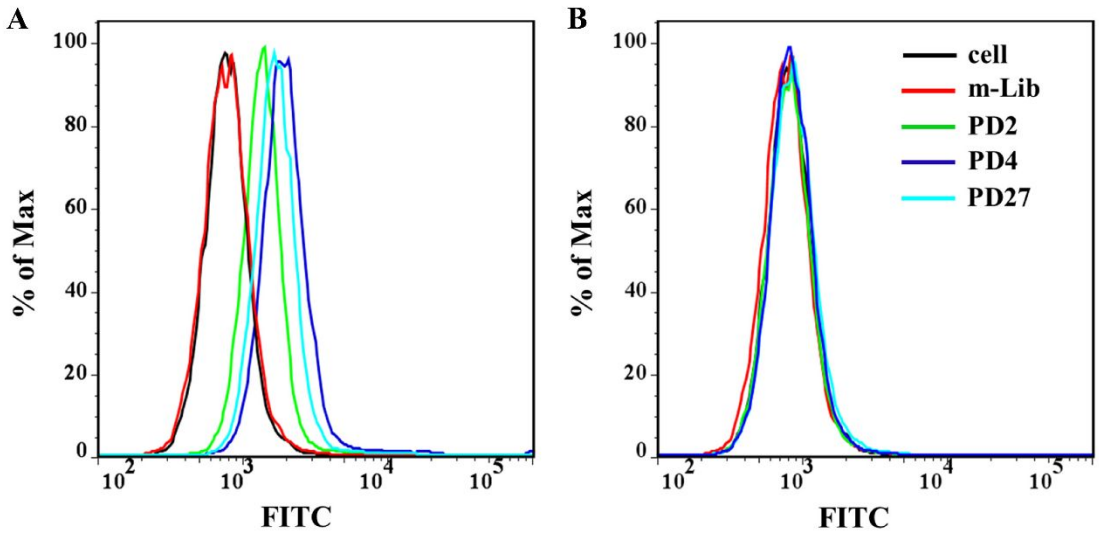

Figure S2. Binding assays of selected aptamer candidates with PD-1 and CHO-K1 cells. Flow cytometry assays for the binding of FAM-labelled PD2, PD4, and PD27 with PD-1 (A) and CHO-K1 cells (B); The initial pool (m-Lib) was used as the negative control; The final concentration of FAM-labeled sequences is 250 nM.

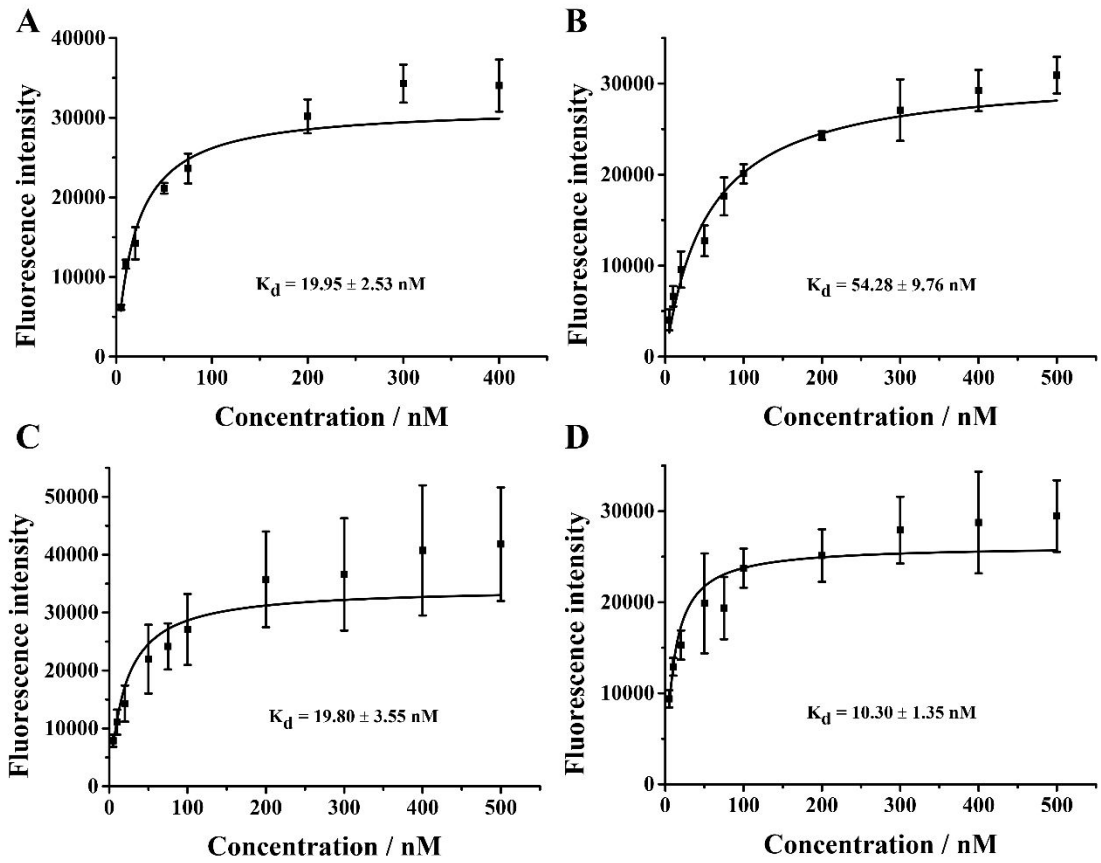

Figure S3 Equilibrium dissociation constant (K_d) curve of aptamer candidates PD2 (A), PD27(B), PD4 (C) and PD4S (D) targeting PD-1 cells (n=3).

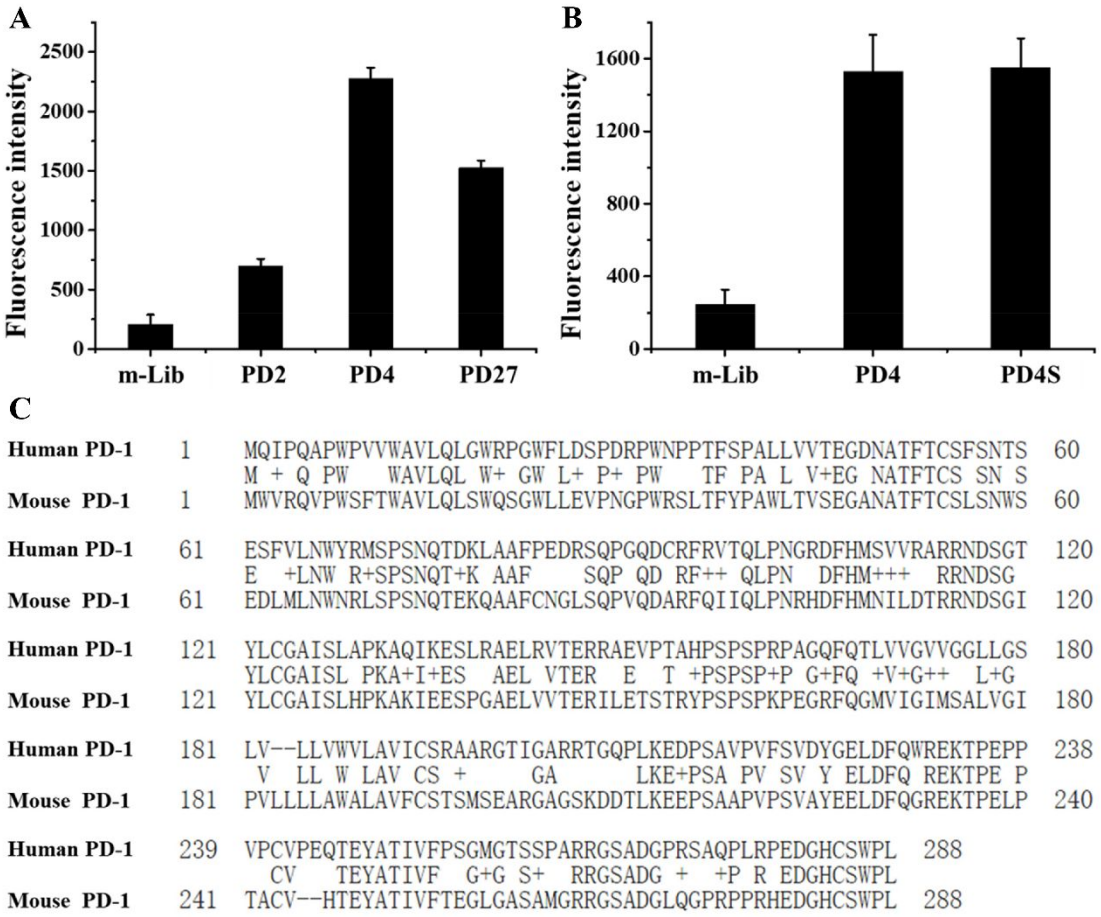

Figure S4 Anti-PD-1 aptamer candidates bind to the PD-1 proteins. The binding of the selected aptamer to the human recombinant PD-1 protein (A)
and the mouse recombinant PD-1 protein (B) (n=3); Alignment of human and mouse PD-1 proteins revealed an amino acid sequence homology of 59% (C); UniProtKB-Q15116 (human) and UniProtKB-Q02242 (mouse) were used in the alignment.

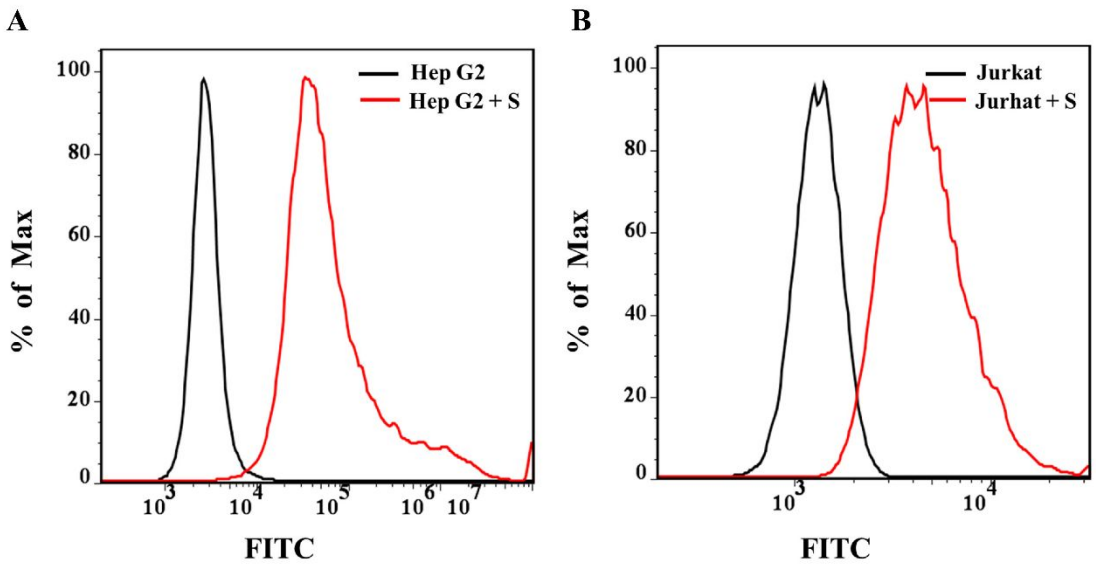

Figure S5 The Flow cytometry assays for investigating the PD-L1 expression on the Hep G2 cells after the IFN-γ stimulation (A) and PD-1 expression on the Jurkat cells activated by the anti-CD3 and CD28 antibody (B).

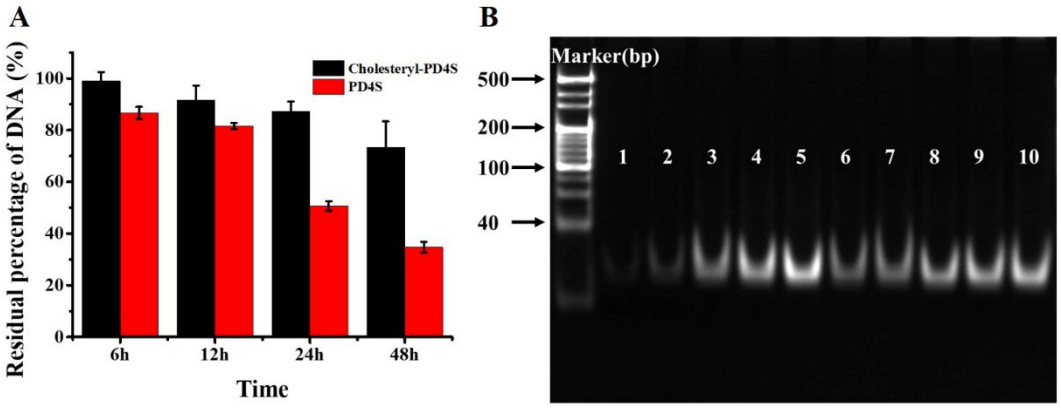

Figure S6. The serum stability of free aptamer PD4S (PD4S) and cholesteryl-modified aptamer PD4S (Cholesteryl- PD4S) in 10% FBS at 37 ^{o}C for 6, 12, 24 and 48 h was evaluated by ultrafiltration centrifuge (A) and native PAGE using 12% polyacrylamide gel (B) The 1-5 and 6-10 bands represent the free PD4S and Cholesteryl- PD4S respectively at 48, 24, 12, 6 and 0 h.

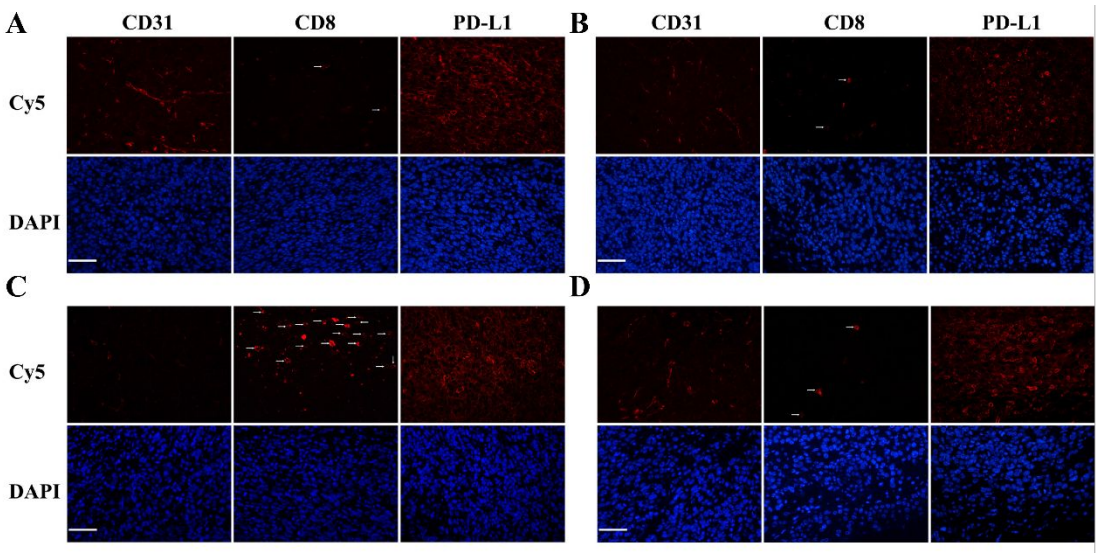

Figure S7 The immunofluorescence images of the tumor from the PBS- (A), random sequence- (B), anti-PD-1 antibody- (C) and isotype antibody-treated group (D); The scale bar in the images is 50 μm.