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Supplemental Information

A CTLA-4 Antagonizing DNA Aptamer with Antitumor Effect

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MTNA-17-519-R2: "A CTLA-4 antagonizing DNA aptamer with antitumor effect." Supporting Information

Table.S1

Table.1A

Cluster reads	Number of cluster	%	Accumulated %	Sum of reads	%	Accumulated %	Next step
>=7000	28	1.73	1.73	529780	55.94	55.94	Yes
1000-6999	105	6.47	8.19	276740	29.22	85.15	No
100-999	345	21.26	29.45	111815	11.81	96.96	No
10-99	794	48.92	78.37	26299	2.78	99.74	No
6-9	351	21.63	100.00	2491	0.26	100.00	No
Total	1623	100.00		947125	100.00		

Table.1B

	Representative sequences from cluster (reads>=7000)	QGRS	Sum of reads in this cluster	%	ID
1.	TCCCTACGGCGCTAACGATGGTGAAAATG**GG**CCTA**GGG**T**GG**AC**GG**TGCCACCGTGCTACAAC	Yes	46235	4.88	aptCTLA-4
2.	TCCCTACGGCGCTAACCCGAAGTGGGATGAGTTGTCGATTGACGTAGCCACCGTGCTACAAC	No	61237	6.46	
3.	TCCCTACGGCGCTAACTGCAAAGTATGTTCATCAATCGATTCGGTAGCCACCGTGCTACAAC	No	51783	5.46	
4.	TCCCTACGGCGCTAACACGAACACAAAATATAAGGGATGCACTGGCGCCACCGTGCTACAAC	No	32783	3.46	
5.	TCCCTACGGCGCTAACTCAGTGCCAAACTCTGTCGGGTGACTAGTAGCCACCGTGCTACAAC	No	17716	1.87	
6.	TCCCTACGGCGCTAACTCCGACGAGGGCACTCTAGTATAGGTCTGTGCCACCGTGCTACAAC	No	16800	1.77	
7.	TCCCTACGGCGCTAACCCGCTGGAGTTGCGAATCACATTCACTGTAGCCACCGTGCTACAAC	No	15360	1.62	
8.	TCCCTACGGCGCTAACTCCGATGTGGGTAAATTGTAGACGGCTAGCGCCACCGTGCTACAAC	No	11567	1.22	
9.	TCCCTACGGCGCTAACCAGATACAACGCAGATGCCAATCGCACGGAGCCACCGTGCTACAAC	No	10416	1.09	
10.	TCCCTACGGCGCTAACATTAGGGCTCTCTTTGCGTATGTTATGTATGCCACCGTGCTACAAC	No	15519	1.63	
11.	TCCCTACGGCGCTAACTCCGAGGTAGGAAGATTAATAATCATTGTAGCCACCGTGCTACAAC	No	22729	2.39	
12.	TCCCTACGGCGCTAACACAATCAATGACAAATTTAAAGGGACTGTTGCCACCGTGCTACAAC	No	32746	3.45	
13.	TCCCTACGGCGCTAACATCGAATGATTTAATACTGGGATCCGGTTAGCCACCGTGCTACAAC	No	15213	1.61	
14.	TCCCTACGGCGCTAACAGATAGGGCAAGTAGCGTTCTGTTTATATTGCCACCGTGCTACAAC	No	20968	2.21	
15.	TCCCTACGGCGCTAACTACTCACATGCAAAAATCAAGAGGGCTGAAGCCACCGTGCTACAAC	No	9215	0.97	
16.	TCCCTACGGCGCTAACAGGTTACAGAATACAAAGGGAATTGGCGATGCCACCGTGCTACAAC	No	8545	0.90	
17.	TCCCTACGGCGCTAACAATTGGGTGTCGTGCGTTTGTTAATTTGAAGCCACCGTGCTACAAC	No	7823	0.82	
18.	TCCCTACGGCGCTAACCGATCACAAATGACAAAGGACTGTATGCATGCCACCGTGCTACAAC	No	8114	0.85	
19.	TCCCTACGGCGCTAACCTCTCGCAAAGATTCAAAGGGATTGGTGTTGCCACCGTGCTACAAC	No	16906	1.78	
20.	TCCCTACGGCGCTAACCAAACGTGAATAATAAGAGGGAATGTATGTGCCACCGTGCTACAAC	No	19009	2.00	
21.	TCCCTACGGCGCTAACGTCCACACTCAGAAAACAGAATAGGGGGTAGCCACCGTGCTACAAC	No	7640	0.80	
22.	TCCCTACGGCGCTAACCACAAAGATACAAAATACAAAAGGGAATGTGCCACCGTGCTACAAC	No	20023	2.11	
23.	TCCCTACGGCGCTAACCAGCATGTTGAAAATAAAGGGATATGGAGTGCCACCGTGCTACAAC	No	9842	1.03	
24.	TCCCTACGGCGCTAACTCAACACCACACAAAATGAATAAGGGATCAGCCACCGTGCTACAAC	No	16541	1.74	
25.	TCCCTACGGCGCTAACAGACACAATGATAAACTGTATAAAGGGACAGCCACCGTGCTACAAC	No	9522	1.00	
26.	TCCCTACGGCGCTAACCAAATGGCAAAACATAATGGGATCCTGATAGCCACCGTGCTACAAC	No	9361	0.98	
27.	TCCCTACGGCGCTAACCAGACTGCACTGAATTCAAAAGGGATCATAGCCACCGTGCTACAAC	No	7958	0.84	
28.	TCCCTACGGCGCTAACCACAGCTATCGAAAATAAAAGGGAGAGTTGGCCACCGTGCTACAAC	No	8209	0.86	

Fig.S1

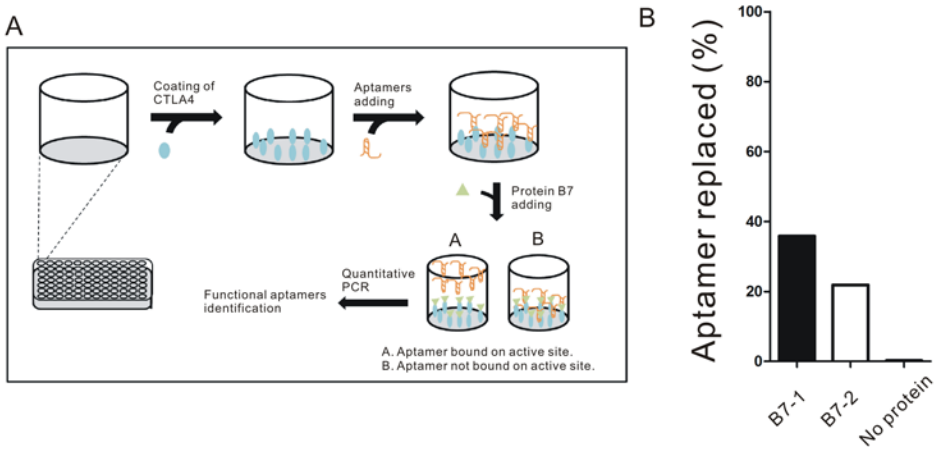


Figure S1. B7 proteins and aptCTLA-4 compete for the shared binding sites A. 2 μg/ml human CTLA-4 proteins were first coated onto ELISA plates. The coated wells were then washed and blocked with 1% BSA. After blocking, 50nM aptamers were added into wells and incubated at 37°C for 1hr. Unbound aptamers were removed by washing with 1X SELEX buffer. B7-1 or B7-2 (250 μg/ml) proteins were then added into wells and incubated at 37°C for 10 mins. Aptamers that shared common binding sites with B7 proteins were replaced and collected in the portion A. Aptamers that do not share common binding sites with B7 proteins were eluted by heating (95°C for 2 min) and collected in the portion B. The aptamers in the portion A and B were then quantified by quantitative PCR. The ratio of replaced aptamer were calculated with equation of Ratio = A/ (A+B). **B.** The replaced ratio for atpCTLA-4 was 35.8% with B7-1, 21.9% with B7-2 and 0.29% when no protein was added for competition.

Fig.S2

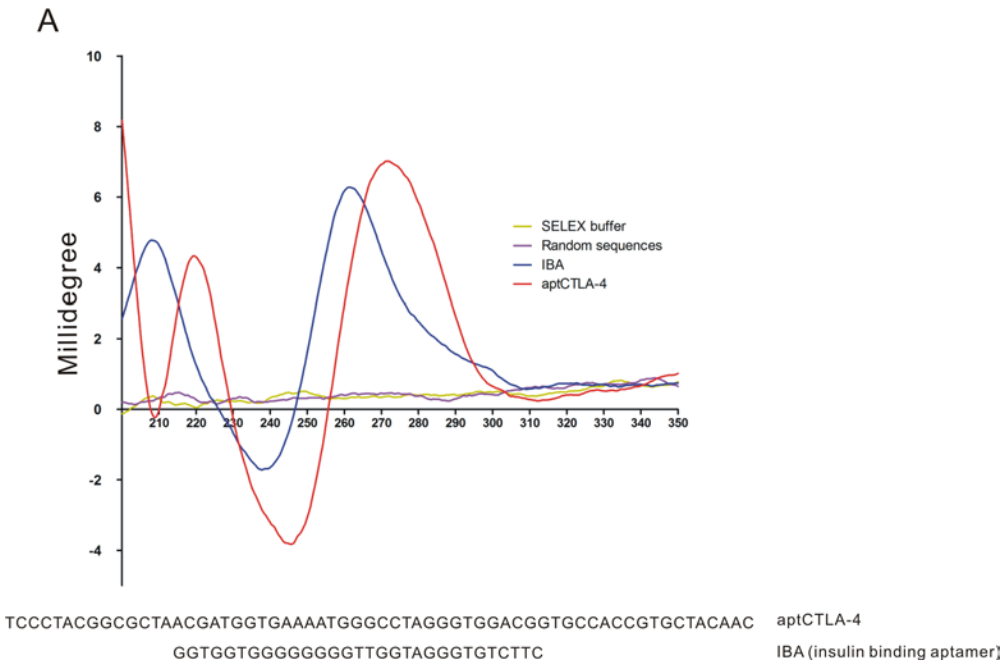


Figure S2. CD spectra of aptCTLA-4 in SELEX buffer. A. CD spectra collected on a Circular Dichroism spectropolarimeter J815 at 200-350 nm. IBA: insulin-binding aptamer.

Fig.S3

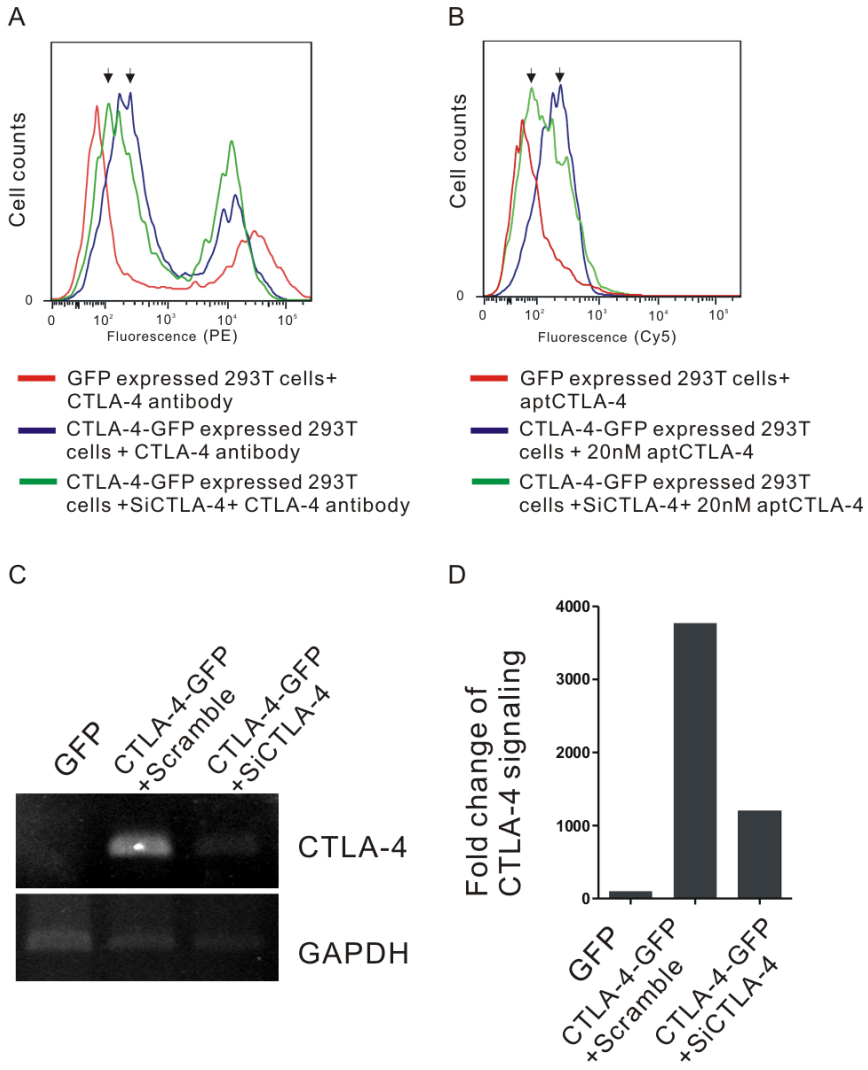


Figure S3. AptCTLA-4 specifically binds to CTLA-4. A-B. The PE-labeled CTLA-4 antibody (10μg/ml) and the Alexa Fluor 647-labeled aptCTLA-4 (20 nM) were incubated with GFP, CTLA-4-GFP overexpressing HEK293T cells or CTLA-4-GFP overexpressing HEK293T cells that were cotransfected with CTLA-4 siRNA. Fluorescence intensities were analyzed by flow cytometry. **C-D**. CTLA-4-GFP expression was efficiently knocked down as measured by semi-quantitative RT-PCR and normalized with GAPDH.

Fig.S4

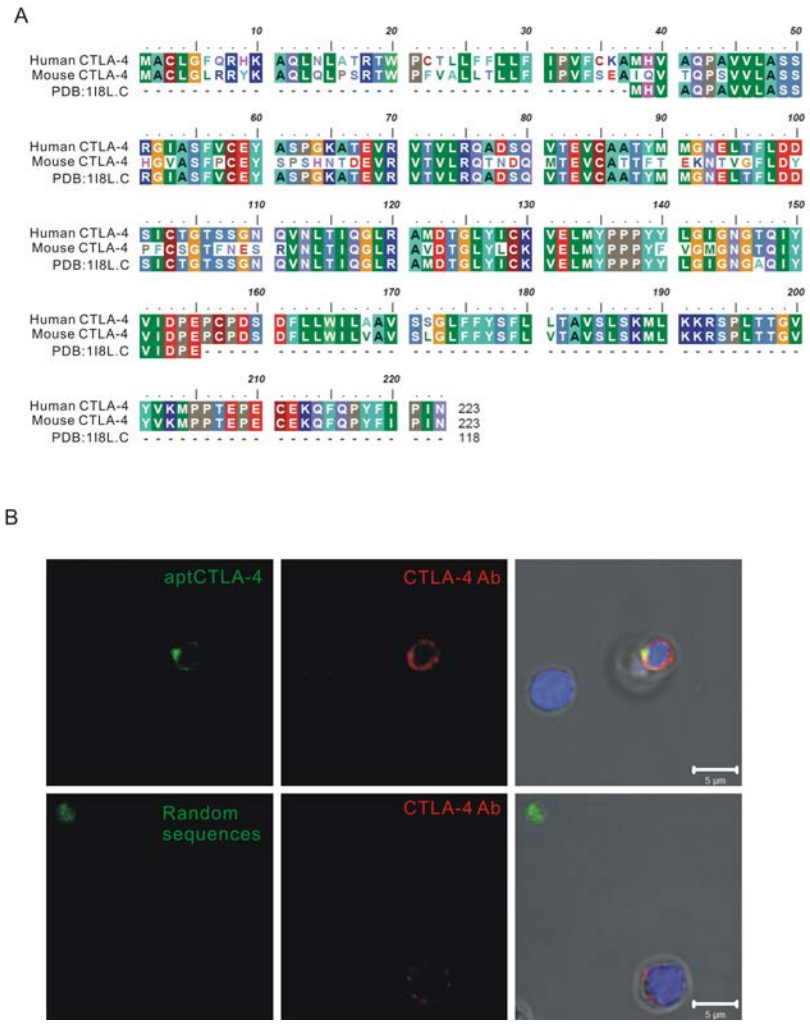
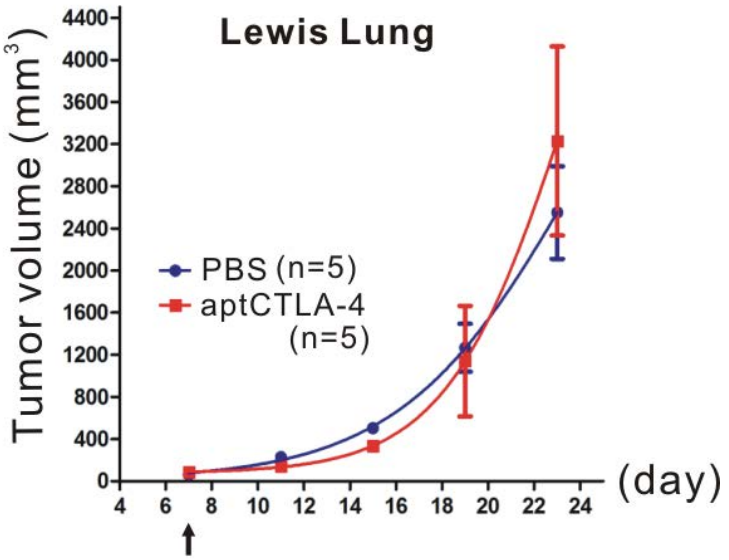


Figure S4. AptCTLA-4 recognizes mouse CTLA-4 proteins. A. Alignment of human and mouse CTLA-4 proteins revealed an amino acid sequence homology of 76%. PDB:1I8L.C is the published CTLA-4 protein sequence used in the 3D crystal structure. **B**. Confocal microscopy revealed the co-localization of FITC-conjugated aptCTLA-4 (green) and PE-conjugated anti-mouse CTLA-4 antibody (red) on mouse T cells.

Fig.S5

A



B

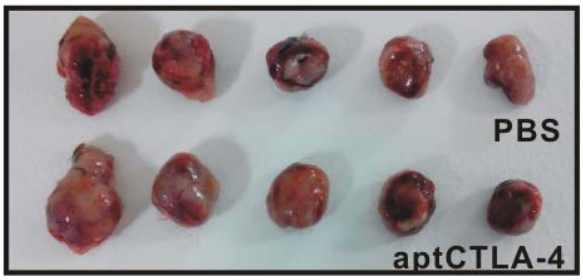


Figure S5. **Single-dose intraperitoneal administration of aptCTLA-4 at the dosage of 0.2 mg/kg failed to suppress tumor growth in a murine Lewis Lung syngeneic tumor model. A**. No significant tumor inhibition effect in aptCTLA-4 single dose treatment in Lewis lung tumor model. **B**. Selected tumor pictures from sacrificed mice on day 23.

Fig.S6

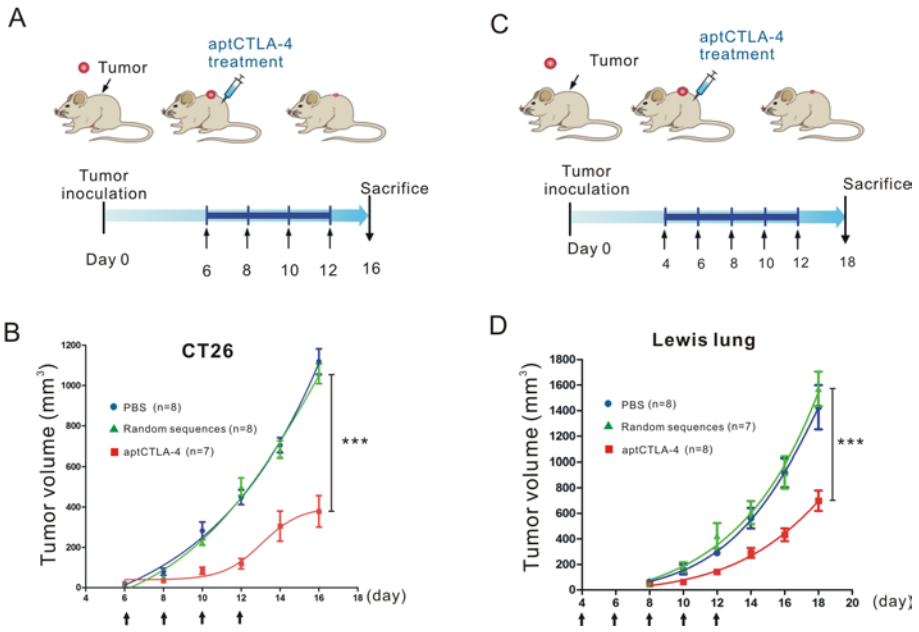


Figure S6. A. CT26 cells (2 x 10^5) were subcutaneously inoculated onto BALB/c mice and aptCTLA-4 (2 mg/kg) treatment was begun four days after tumor cell inoculation. **B.** aptCTLA-4 treatment effectively inhibited tumor growth. **C.** Lewis lung cells (1 x 10^5) were subcutaneously inoculated onto C57BL/6 mice and aptCTLA-4 (2 mg/kg) treatment was begun four days after inoculation. **D.** aptCTLA-4 treatment inhibited tumor growth. These data are presented as mean ± standard error of the mean and were analyzed by Student's t-test. Asterisks denote statistical significant differences ($P < 0.001$).

Fig.S7

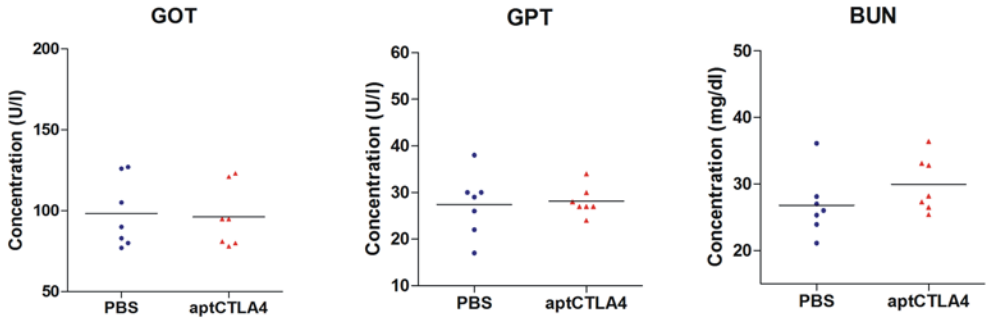


Figure S7. AptCTLA-4 treatment does not cause changes in GOT, GPT and BUN.