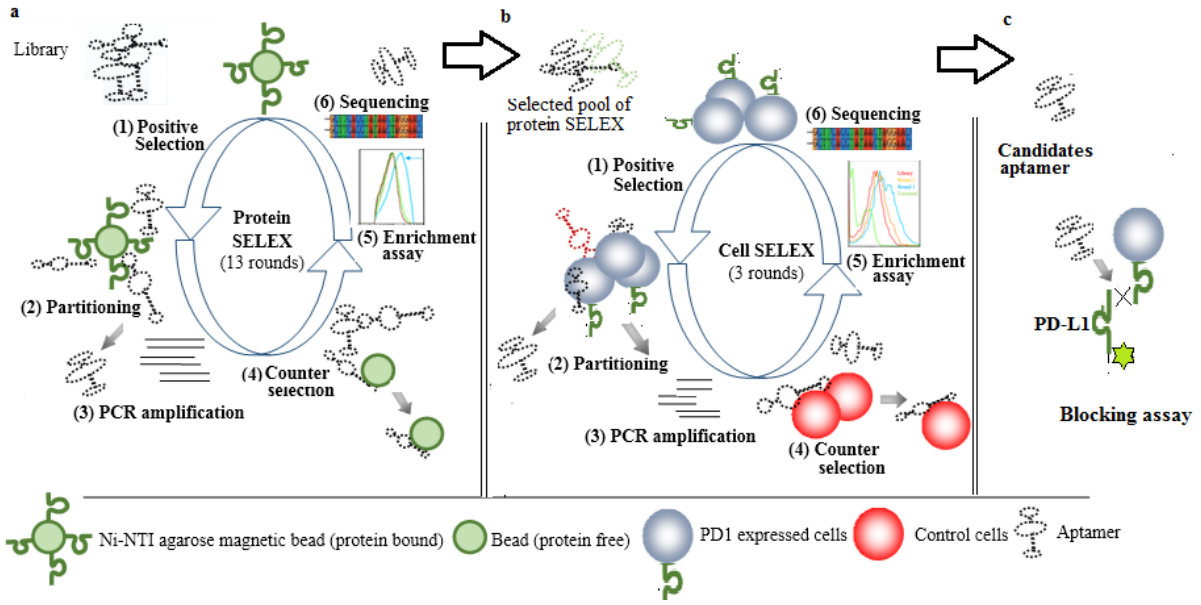
Supplemental Data



Graphical abstract. Using hybrid SELEX for generating ssDNA aptamer against PD1**.** (a) In the initial round of selection, ssDNA library was added to protein-loaded Ni-NTI magnetic beads, unbound aptamer was then eluted and PCR amplification performed. ssDNA was generated from PCR amplified dsDNA and protein-free beads was used for counter selection. After 13 rounds, SELEX process was screened by the flow cytometry of beads. The selected pool of round 11 was transferred to the next phase (cell SELEX) and cloned for sequencing. (**b**) Stimulated Jurkat cells was used as PD1 positive cell and A2780 used as PD1 negative cell in the process of cell SELEX. Selected pool of round 1 and 2 were screened for the enrichment of aptamer by flow cytometry. Pool aptamers from the 1st round of cell SELEX was also selected for cloning and sequencing. **(c)** Aptamers with the ability to bind PD1 were selected for further analysis using blocking assay. PD-L1 with IgG fc-tag was added to PD1-expressing cells then binding of PD-L1 to PD1 was detected using FITC-conjugated antibodies against IgG fc-tag in either the presence or absence of aptamer.

Table S1. Protein SELEX conditions for selecting ssDNA aptamer specific for PD1

No. of round	Protein (μg)	5 % Ni-NTI magnetic beads (μl)	No. of washing	Incubation time (min)
1	5.75	13.2	3	30
2	2.70	6.6	3	30
3	2.70	6.6	3	30
Counter selection	0.00	20.0	0	20
4-6	2.70	66.0	3	30
Counter selection	0.00	20.0	0	20
7-9	2.70	6.6	4	30
Counter selection	0.00	20.0	0	30
10	2.70	6.6	5	30
Counter selection	0.00	20.0	0	30
11	2.70	6.6	6	30
Counter selection	0.00	20.0	0	45
12	1.50	6.6	7	30
Counter selection	0.00	20.0	0	60
13	1.50	6.6	8	30

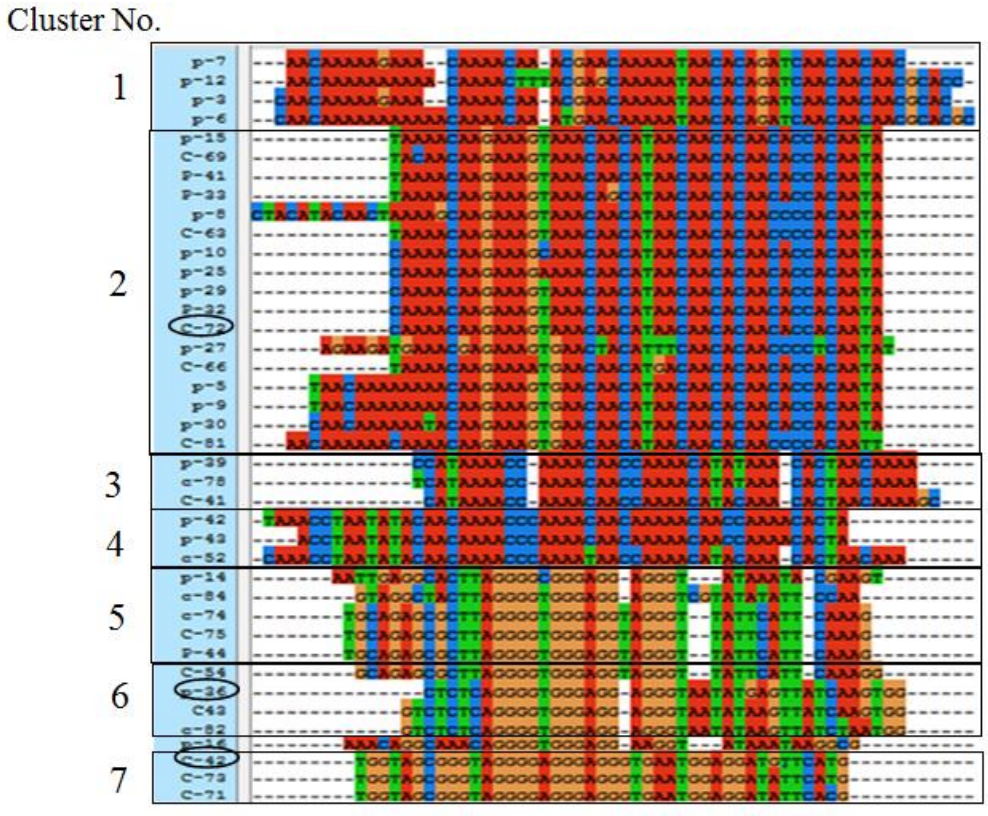


Figure. S1. Alignment of selected aptamers from protein and cell SELEX. Sequences were aligned with ClastalX2 software and clusters were determined based on sequence homology.

Table S2. Proteins targeted with hybrid modeling of SELEX

target	No. of protein SELEX rounds	No. of cell SELEX rounds	K_d (nmol/l)	Crossover direction	reference
CD4	5	10	1.59	Cell to protein	[1]
CD30	3	20	50	Cell to protein	[2]
Dec205	4	1	23	Protein to cell	[3]
Tenascin-C	8	9	5	Cell to protein	[4]
PD1	13	2	16	Protein to cell	This study

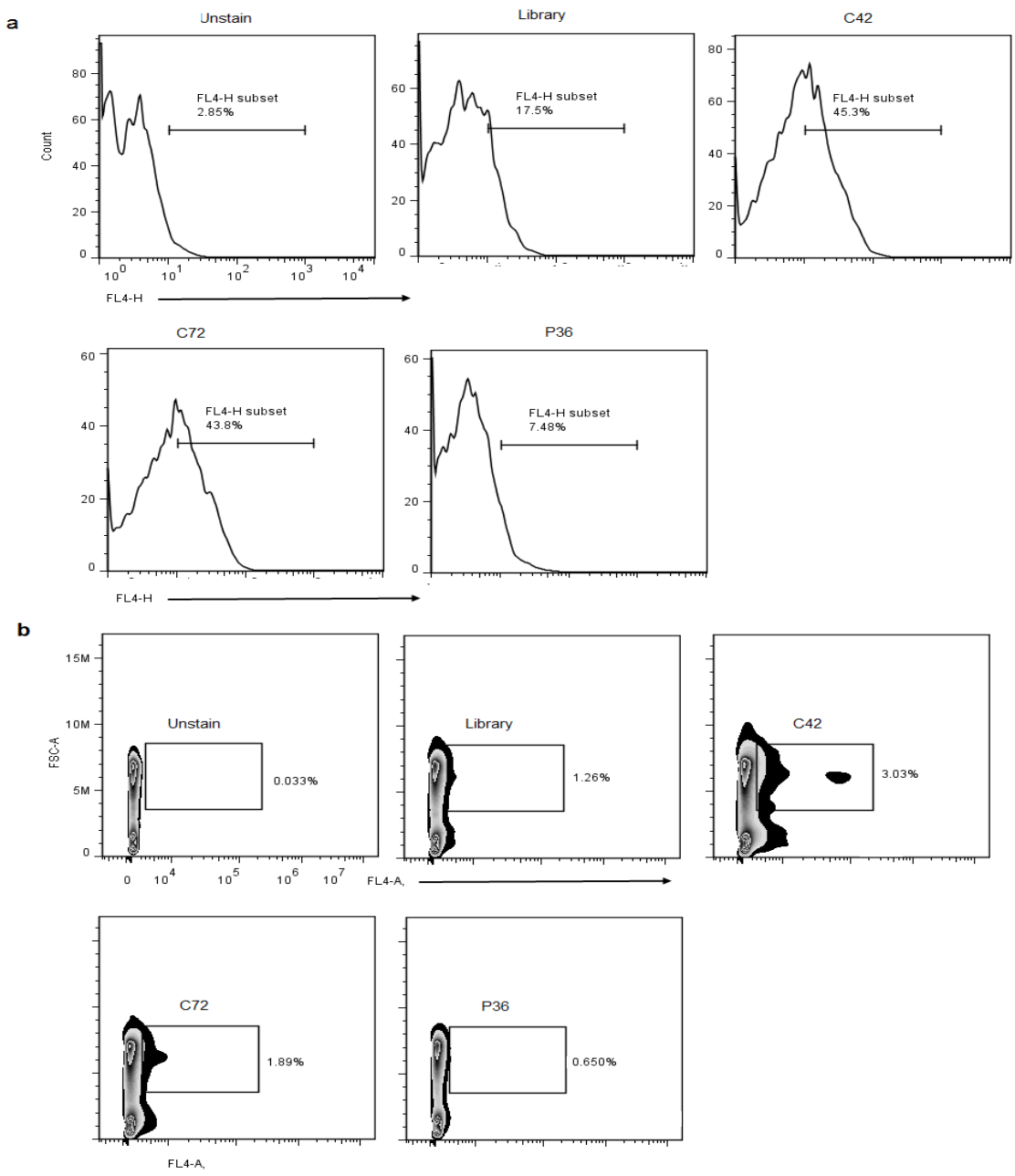


Figure S2. Binding of selected aptamers to PD1 expressing cell line (Jurkat) and peripheral blood mononuclear cell (T CD8+ depleted PBMC). **(a)** Flow cytometry histogram of binding of selected aptamers to PD1 expressing cell line (Jurkat). The percentage of binding was determined as 2.85%, 17.5%, 45.3%, 43.8%, and 7.48% for unstained control, library, C42, C72, and P36, respectively. **(b)** Binding of the selected aptamer to PMBC (T CD8+ depleted PBMC) was evaluated by flow cytometry of Atto-labeled aptamers. Binding of C42 aptamer (3.03%) was more than P36 (0.65%), C72 (1.79%) and library (1.26%).

References

1. Zhao N, Pei S-n, Parekh P, Salazar E, Zu Y (2014) Blocking interaction of viral gp120 and CD4-expressing T cells by single-stranded DNA aptamers. The international journal of biochemistry & cell biology 51:10-18
2. Parekh P, Kamble S, Zhao N, Zeng Z, Portier BP, Zu Y (2013) Immunotherapy of CD30-expressing lymphoma using a highly stable ssDNA aptamer. Biomaterials 34 (35):8909-8917
3. Wengerter BC, Katakowski JA, Rosenberg JM, Park CG, Almo SC, Palliser D, Levy M (2014) Aptamer-targeted antigen delivery. Molecular Therapy 22 (7):1375-1387
4. Hicke BJ, Marion C, Chang Y-F, Gould T, Lynott CK, Parma D, Schmidt PG, Warren S (2001) Tenascin-C aptamers are generated using tumor cells and purified protein. Journal of Biological Chemistry 276 (52):48644-48654