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Supplemental information

CD3 aptamers promote expansion and persistence of tumor-reactive T cells for adoptive T cell therapy in cancer

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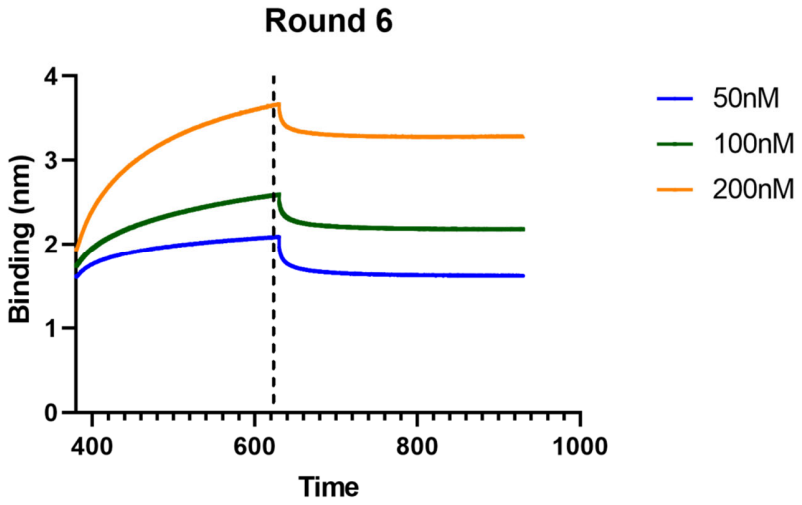


Sample ID	Protein Conc. (nM)	KD (M)	Ka (1/Ms)	ka Error	kd (1/s)	kd Error	R max	R max Error	R equilibrium	X2	R2
ROUND 6 CD3	50	6,66E-07	2,12E+04	1,71E+04	1,41E-02	2,67E-04	8,595	6,806	0,601	1,033	0,946
ROUND 6 CD3	100	3,51E-07	1,23E+04	5,62E+03	4,33E-03	8,36E-05	9,184	4,088	2,036	1,509	0,951
ROUND 6 CD3	200	1,66E-07	1,17E+04	1,88E+03	1,95E-03	2,81E-05	10,550	1,611	5,756	2,114	0,984

Figure S1: Binding of R6 enriched library to rmCD3δε-Fc. The binding affinity KD and dissociation and association constants were determined by OCTEC at three different concentrations of protein.

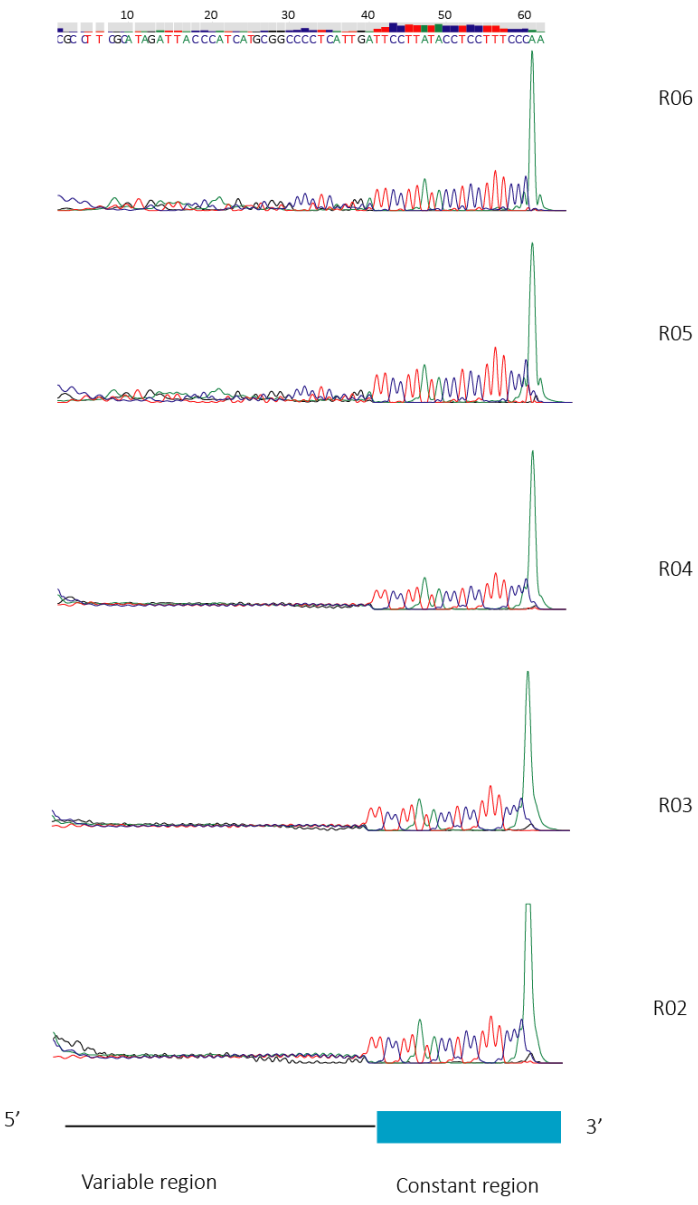


Figure S2: Sanger sequencing chromatograms of the enriched libraries from R02 to R06. Progression of enrichment denoted by the abundance of newly formed peaks in the variable region of the SELEX library after each round of selection.

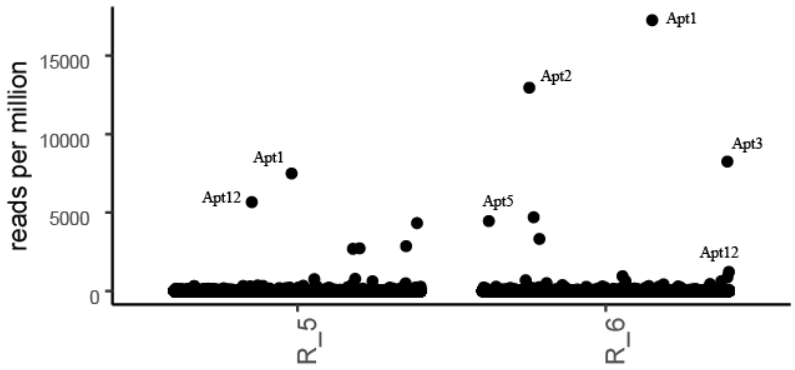


Figure S3: Abundance of top enriched aptamers from R5 and R6 using Ion Torrent high throughput sequencing.

Aptamer	Number of motives	CCTACC	AACTC	CACCGT	CCCGCA	GTCCCCTCGT	CGATTGATTC	CTGTCT	GGCCTT	TCCCTG
Apt 1	2									
Apt 2	3									
Apt 3	3									
Apt 4	1									
Apt 5	1									
Apt 6	1									
Apt 7	2									
Apt 9	2									
Apt 11	3									
Apt 12	2									
Apt 40	2									

Figure S4 : Motive distribution in each candidate aptamer: Graphical representation of motive distribution among CD3 candidate aptamers.

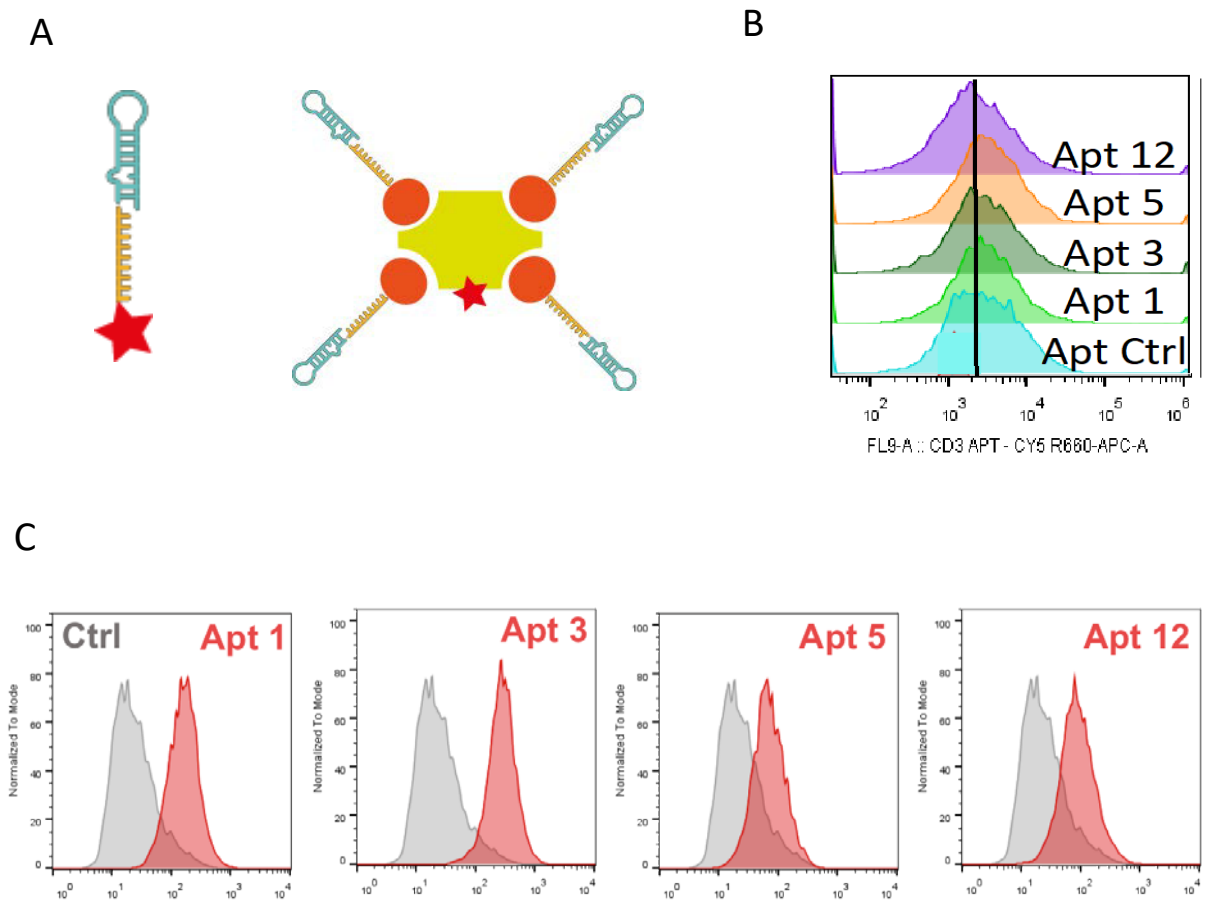
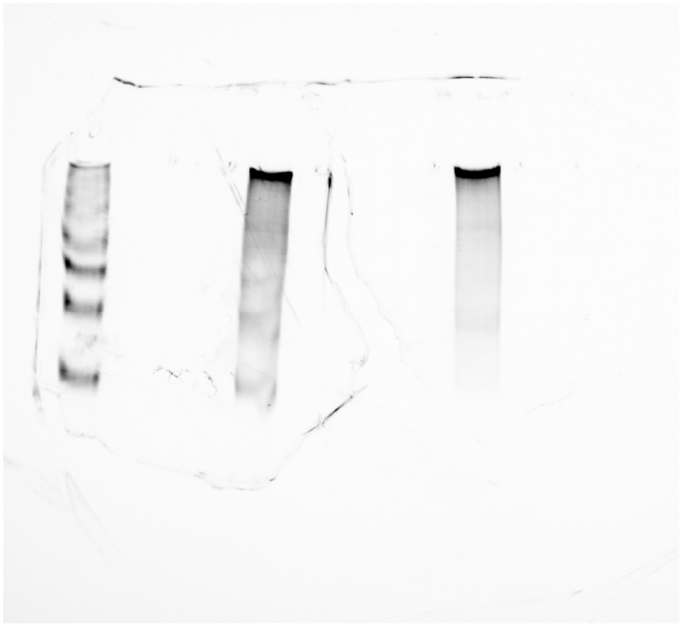


Figure S5: A. Left panel. Monomer aptamer attached with complementary probe labeled with Cy5 fluorophore. Right panel streptavidin protein labeled with fluorophore attached with four biotinylated aptamers. B. Binding of Cy5 labeled monomeric aptamers to EL-4 cells. C. Binding of PE labeled tetrameric CD3 aptamers 1,3, 5 and 12 (red) or Ctrl aptamer (grey) to EL-4 T cells.



1 2 3

1: Streptavidin-PE
2: 1 Streptavidin-PE /2 CD3 aptamer biotin
3: 1 Streptavidin-PE/4 CD3 aptamer biotin

Figure S6: Electrophoretic mobility shift assay (EMSA) of the Streptavidin-PE protein complexed with CD3 biotinylated aptamers. A concentration of 10 µM of Streptavidin-PE protein was incubated with the biotinylated Apt1 at different ratios (1:2 and 1:4) for 15 minutes at room temperature. Subsequently, the samples were loaded onto a non-denaturing 10% acrylamide gel and electrophoresed for 30 minutes at 120 V. The PE fluorescence signal was then determined using an Image ChemiDoc system from Bio-Rad.

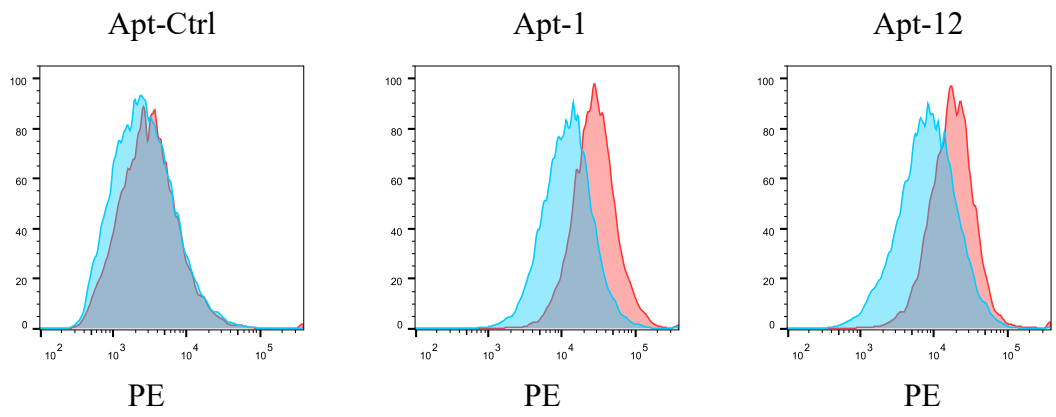


Figure S7. Binding of CD3 aptamers to human Jurkat T cells measured by flow cytometry using streptavidin PE tetrameric aptamers. In blue CD3 epsilon CRISPR knock-out cell line and in red parental Jurkat T cells.

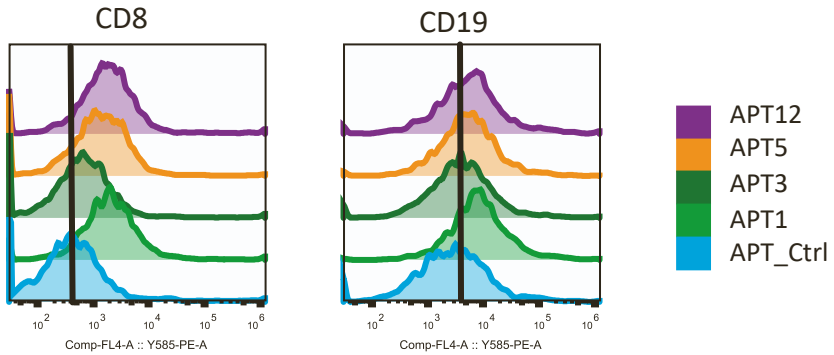
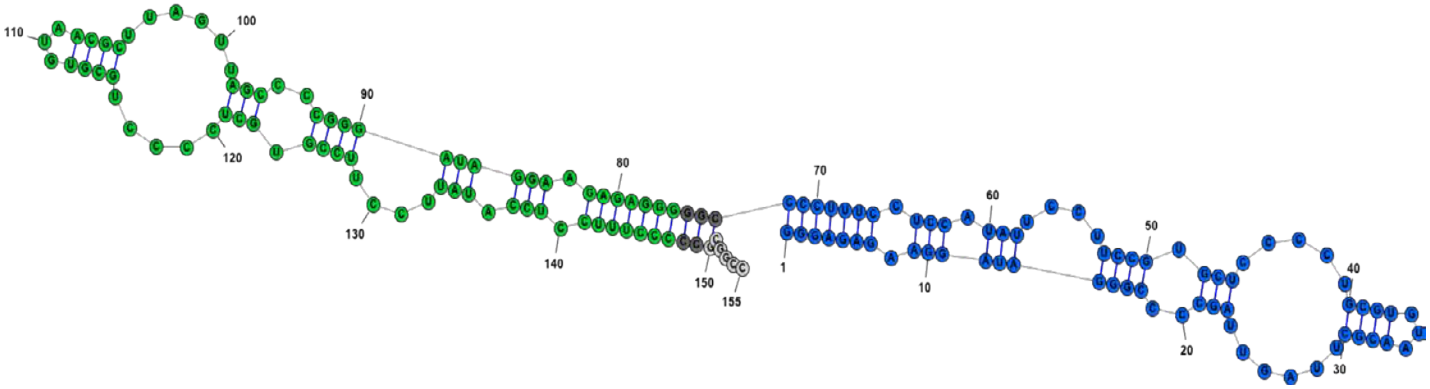


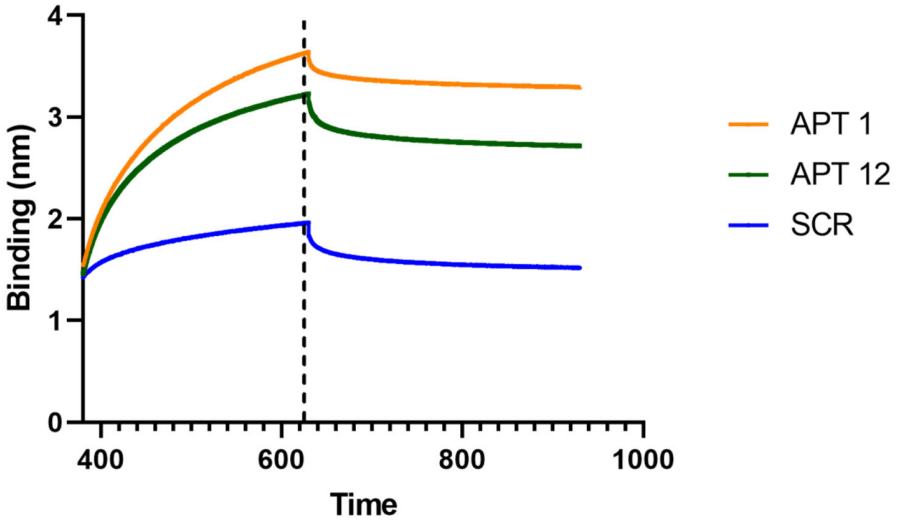
Figure S8: Binding of PE-streptavidin CD3 aptamers to CD8+ (T cells) and CD19+ (B cells) isolated from naïve C57BL6 mouse and detected by flow cytometry.



GGGAGAGAAGGAUAGGGCCCCGAUUGAUUCGCAAUGUGCGUCCCCUCGUGCCUUCCUUAUACCUCCUUUCCCCGGGGGAGA
GAAGGAUAGGGCCCCGAUUGAUUCGCAAUGUGCGUCCCCUCGUGCCUUCCUUAUACCUCCUUUCCCCCGCGGCC

CD3δε monomer - Linker - CD3δε Monomer - Linker - NotI Restriction site overhang

Figure S9: Design and structure of the CD3 dime aptamer: The CD3 dimer aptamer transcribed in vitro as a 155 bp long, single RNA transcript. It consists of two CD3 monomers connected with 3 nucleotide long linker. The sequence and structure of the CD3 dimer of Aptamer 1 is depicted above.



Sample ID	KD (M)	Ka (1/Ms)	ka Error	kd (1/s)	kd Error	R max	R max Error	R equilibrium	X2	R2
DIM APT 1 CD3	2,52E-08	4,37E+04	1,10E+03	1,10E-03	1,87E-05	2,699	0,062	2,156	4,927	0,993
DIM APT 12 CD3	2,18E-07	1,44E+04	1,79E+03	3,13E-03	3,59E-05	11,860	1,482	3,736	4,441	0,990
DIM SCR CD3	1,12E-04	8,06E+03	6,66E+03	1,49E-02	2,56E-04	10,940	8,923	0,560	1,610	0,945

Figure S10: Binding of three different dimers for Apt1, Apt12 and scramble (SCR) used in the functional in vitro and in vivo studies. Binding affinity (KD) and dissociation and association constants were determined by OCTEC.

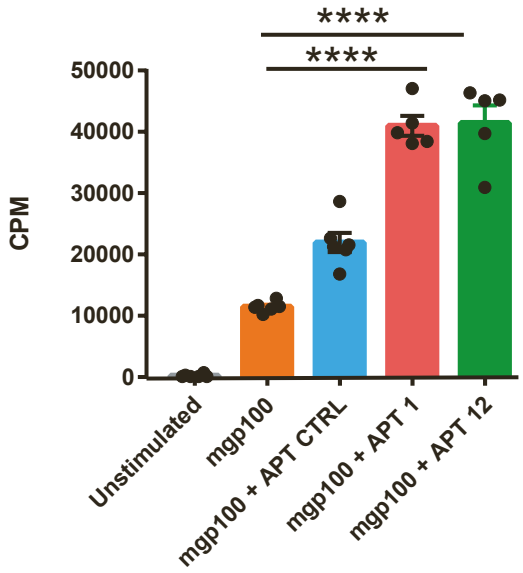


Figure S11: PMEL splenocytes were suboptimally activated with their cognate low affinity melanoma peptide mgp100 and concomitant CD3 aptamer stimulus. Thymidine 3H incorporation was determined by scintillation. Data are represented as mean of n=6 technical replicates of one experiment. Independent experiments with similar results were performed two more times. One-way ANOVA followed by a Bonferroni post hoc test was performed.

Table S1: Primers and DNA oligonucleotide templates used for SELEX and production of CD3 aptamers used in this study.

CD3δε PRIMERS	
CD3δε Sel5 (Forward Primer)	GGGGAATTCTAATACGACTCACTATAGGGAGAGAAGGATAGGG
CD3δε Sel3 (Reverse Primer)	GGGAAAGGAGGTATAAGGAA
CD3δε Sel 3 Extn (Reverse Primer)	GGTTGATGGTATGGATACCCTGGGGGAAAGGAGGTATAA

CD3δε APTAMER DNA TEMPLATES	
CD3δε Apt 1	GGGAAAGGAGGTATAAGGAAGGCACGAGGGGACGCACATTGC GAATCAATCGGGGCCCTATCCTTCTCTCCC
CD3δε Apt 3	GGGAAAGGAGGTATAAGGAATCAGACAGGGCTCATCTTCGAG TTTCAGGTAGGGACCCTATCCTTCTCTCCC
CD3δε Apt 5	GGGAAAGGAGGTATAAGGAAACGGGCAGGGATCACACGTGAA AGCAGGCAAGGCCCTATCCTTCTCTCCC
CD3δε Apt 12	GGGAAAGGAGGTATAAGGAATCAATGCGGGCCAGACGGTGGA TATCGACAGGGGACCCTATCCTTCTCTCCC
CD3δε Apt librery	GGGAAAGGAGGTATAAGGAANNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNCCCTATCCTTCTCTCCCTATAGTGAGTCG TATTAGAATTCCCC
SCR	GGGAAAGGAGGTATAAGGAACAGAGCGATGAGTTACGAACAG GCAGGGGCGCTGCCCCTATCCTTCTCTCCC
Oligo Btn	[Btn]GGTTGATGGTATGGATACCCTGG
Oligo Cy5	[Cy5]GGTTGATGGTATGGATACCCTGG

Table S2: Sequence of the CD3 aptamer dimers used in the in vitro and in vivo studies.

CD3δε DIMER PUC57 DNA Templates cloned into PUC57	
CD3δε Dimer Apt 1	GGGGAATTCTAATACGACTCACTATAGGGAGAGAAGGATAGGGGC AGCGCCCCTGCCTGTTCGTAACTCATCGCTCTGTTCCTTATACCTCCT TTCCCCGGGGGAGAGAAGGATAGGGGCAGCGCCCCTGCCTGTTCG TAACTCATCGCTCTGTTCCTTATACCTCCTTTCCCCCGCGGCCGC
CD3δε Dimer Apt 12	GGGGAATTCTAATACGACTCACTATAGGGAGAGAAGGATAGGGCC CCGATTGATTCGCAATGTGCGTCCCCTCGTGCCTTCCTTATACCTCCT TTCCCCGGGGGAGAGAAGGATAGGGCCCCGATTGATTCGCAATGT GCGTCCCCTCGTGCCTTCCTTATACCTCCTTTCCCCCGCGGCCGC
CD3δε Dimer Apt Ctrl –	GGGGAATTCTAATACGACTCACTATAGGGAGAGAAGGATAGGGTC CCCTGTCGATATCCACCGTCTGGCCCGCATTGATTCCTTATACCTCCT TTCCCCGGGGGAGAGAAGGATAGGGTCCCCTGTCGATATCCACCGT CTGGCCCGCATTGATTCCTTATACCTCCTTTCCCCCGCGGCCGC

T7 RNA Polymerase promoter region Not I Restriction Site Nucleotide Linkers