Electronic supplementary information: RSC Advances

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Supplementary figures

Figure S1

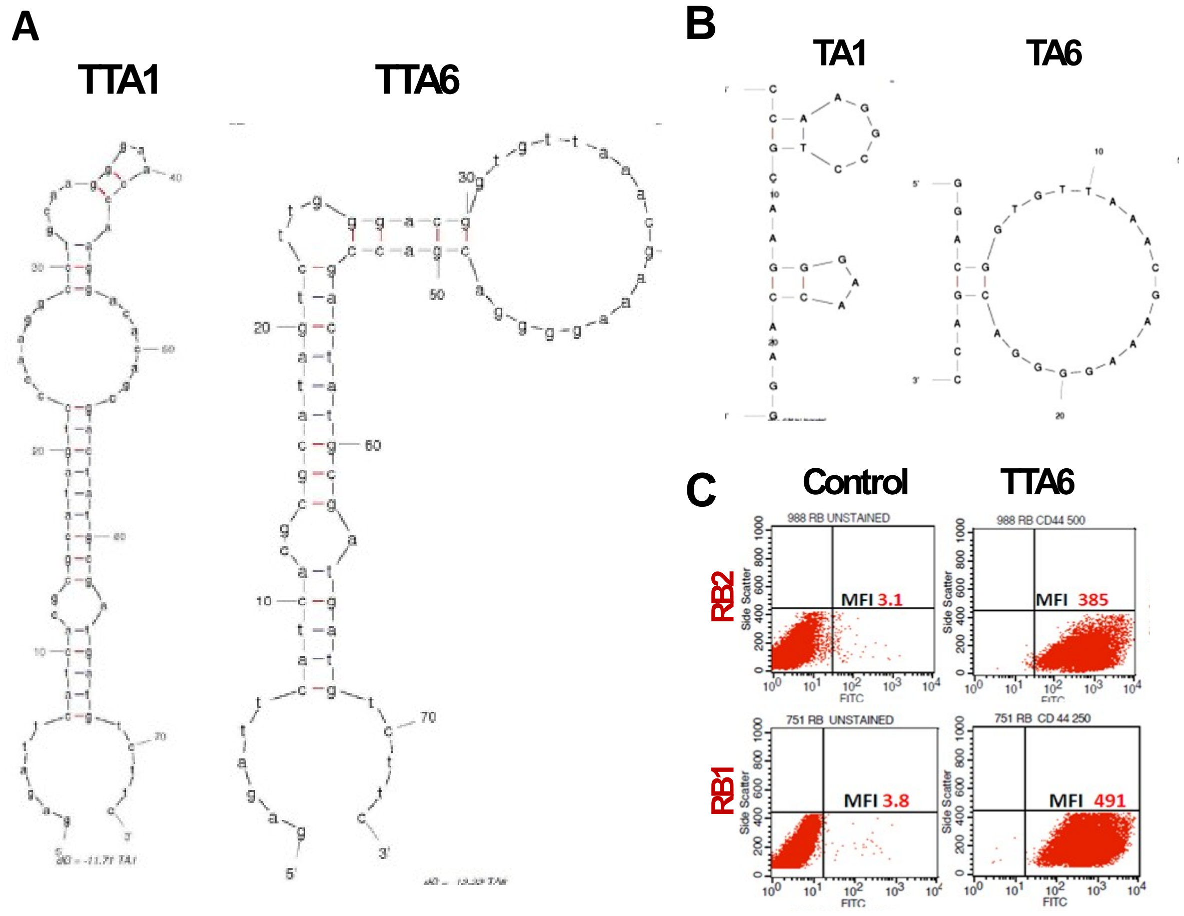

Figure S1 Truncation of CD44 aptamer and binding of full length aptamer. A. Mfold structure of CD44 aptamer TTA1 (left) and TTA6 (right) clone obtained from combinatorial screening of DNA with thio-modification for stability. The aptamer is selected against the HA binding domain. **B.** Mfold structure of the truncated aptamersTA1, derived from the TTA1 and TA6, derived from the TTA6. **C.** Scatter plot of RB tumor cells bound to CD44 full length aptamer TTA6. The mean fluorescence intensity (MFI) is shown within each panel.

Figure S2

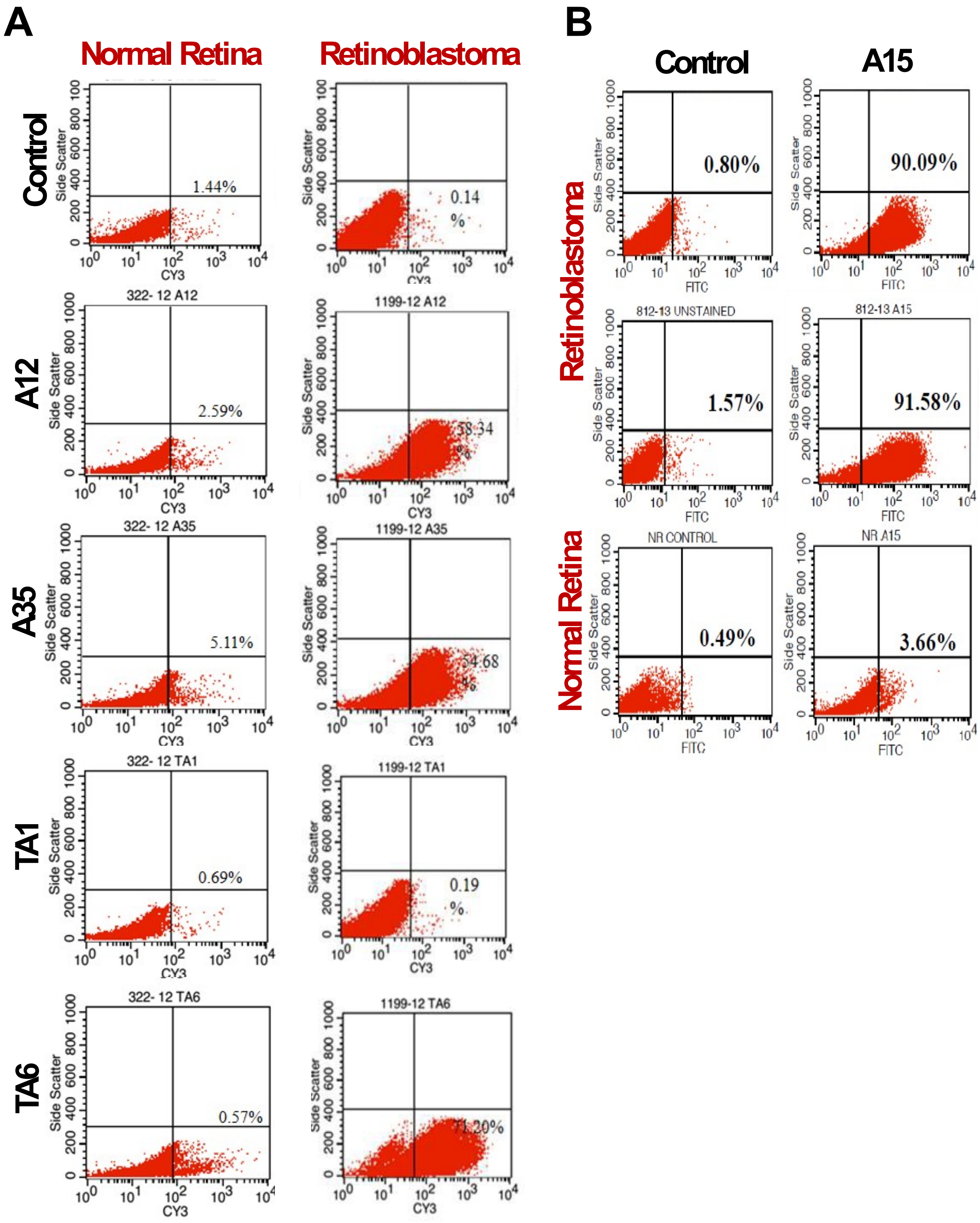

Figure S2 A. Scatterplot of CD44 and ABCG2 aptamers binding to the RB primary tumors and the non malignant cadaveric retina. **B.** Scatter plot of CD133-A15 aptamer binding to the RB primary tumors and the non malignant cadaveric retina.

Figure S3

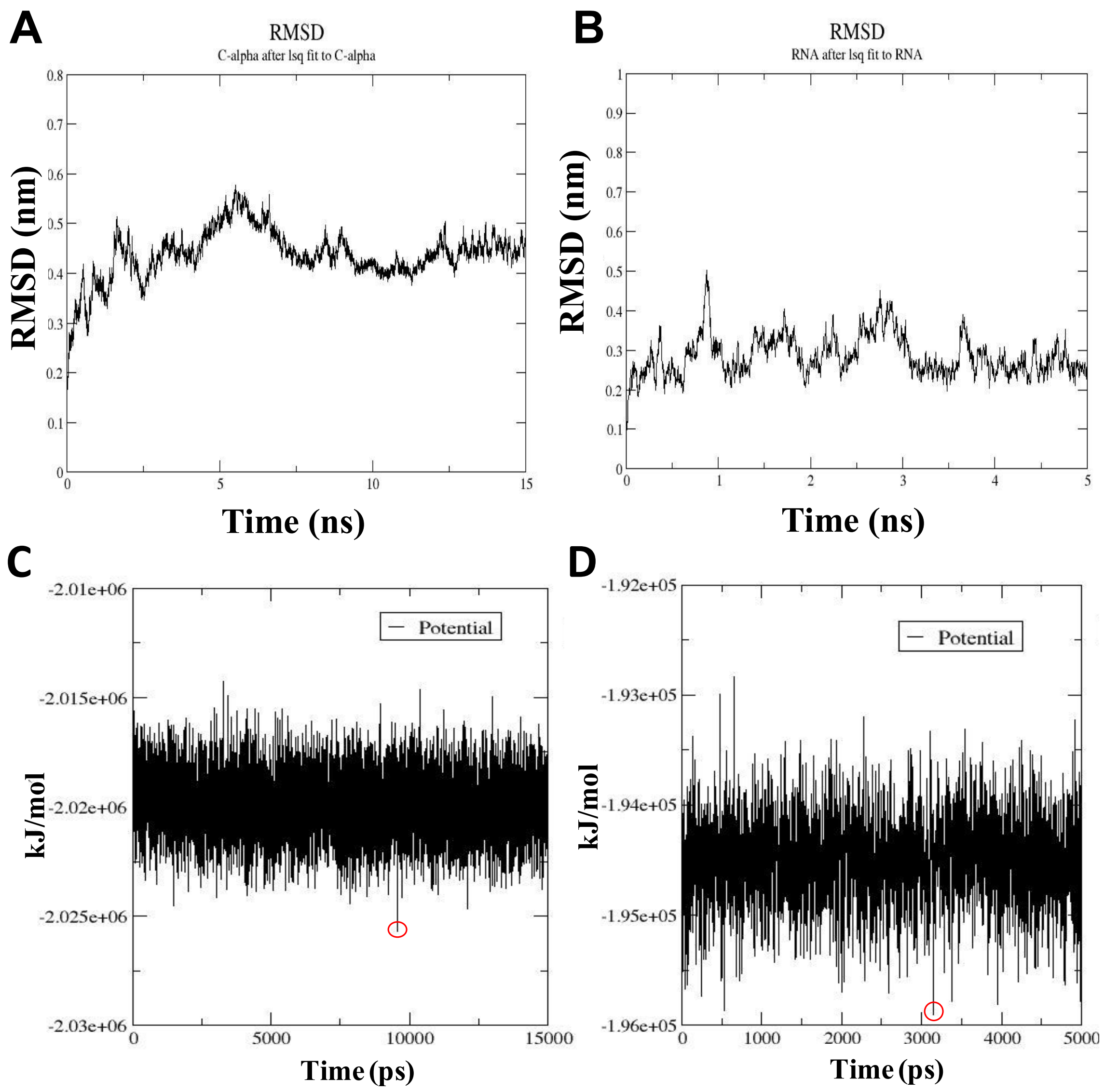

Figure S3. Molecular simulation of CD133 ExD2 and A15 aptamer. A. C alpha RMSD trajectory of CD133 extracellular region during Molecular dynamics Simulation. B. RMSD Trajectory of A15 CD133 RNA aptamer during Molecular dynamics Simulation. Graph obtained from molecular dynamics simulation showing the lowest energy conformation as observed at 9580th frame for the modeled extracellular domain 2 of CD133 (C) and A15 CD133 aptamer (D).

Table S1. List of primers used to study the expression status of CD44 variants (CD44v), CD44 standard (CD44s) and CD44 total (CD44T) forms by qRT PCR.

Name	5’-Primer sequences -3’
hCD44s FP	AAAGGAGCAGCACTTCAGGA
hCD44s RP	TGTGTCTTGGTCTCTGGTAGC
hCD44v6 FP	AGGAACAGTGGTTTGGCAAC
hCD44v6 RP	CGAATGGGAGTCTTCTCTTGG
hCD44v8 FP	TCAGCCTACTGCAAATCCAA
hCD44v8 RP	GAGGTCCTGTCCTGTCCAAA
hCD44v9 FP	GGCTTGGAAGAAGATAAAGACC
hCD44v9 RP	TGCTTGATGTCAGAGTAGAAGTTG
hCD44v10 FP	GGAATGATGTCACAGGTGGA
hCD44v10 RP	AGGTCACTGGGATGAAGGTC
hCD44T FP	CCGCTATGTCCAGAAAGGA
hCD44T RP	CTGTCTGTGCTGTCGGTGAT

Table S2.The percentage uptake of aptamers by primary RB tumors and normal retina.

Aptamers (DNA/RNA)	sequence (5’-3’)
A15 (RNA)	CCCUCCUACAUAGGG
TA1 (DNA)	CCAAGGCCTGCAAGGGAACCAAGG
TA6 (DNA)	GGACGGTGTTAAACGAAAGGGGACGACC
A12 (DNA)	ACGCTCGGATGCCACTACAGGCCCACCCTCATGGACGTGCTGGTGAC
A35 (DNA)	ACGCTCGGATGCCACTACAGATCGCCCCTCACCTCATGGACGTGCTGGTGAC

Table S3. Clinicopathological features of primary retinoblastoma tumors and gene expression profile by qRT-PCR.

S.NO	AGE/ SEX	Clinicopathological descriptions	CD44-S	CD44v6	CD44v8	CD44v9	CD44v10	CD44-T
1	1/F	RB, WD, Focal invasion of RPE, CI<3mm	NS	↑	↑	NS	↑	↑
2	2/F	RB, WD, Focal Invasion of RPE, CI<3mm, No invasion of ON.	NS	↓	↓	↓	↓	NS
3	2/M	RB, PD, Focal RPE invasion, Focal CI<3mm, Pre laminar& Post laminar invasion of ON.	NS	↓	NS	↓	↓	↑
4	2/M	RB, UD, CI>3mm, Tumor cells invading in anterior border of sclera.	↓	↓	NS	NS	NS	NS
5	2/F	RB, PD, CI>3mm, Pre laminar & Post laminar invasion of tumor.	NS	↓	↓	↓	↓	↓
6	6/M	RB, CI>3mm, Tumor cells touching the anterior border of the sclera.	NS	NS	↓	↓	↓	NS
7	3/F	RB, MD, Focal CI> 3mm, Pre laminar& Post laminar invasion of ON.	NS	↑	↑	↑	↑	↑
8	4/M	RB, PD, CI>3mm, Pre laminar & Post laminar invasion, Tumor invasion upto posterior border of sclera.	↓	↑	↑	↑	NS	↓
9	3/F	RB, MD, Focal CI> 3mm, Pre laminar& Post laminar invasion of ON.	NS	NS	↑	↑	NS	↑
10	4/M	RB, PD, CI>3mm, Pre laminar & Post laminar invasion, Tumor invasion upto posterior border of sclera.	↓	↓	↑	↑	↑	NS

F: Female; M: Male; RB: Retinoblastoma; PD: Poorly Differentiated; MD: Moderately Differentiated; WD: Well Differentiated; UD: Un-Differentiated; CI: Choroid Invasion; ON: Optic Nerve; RPE: Retinal pigmented epithelium ↑: Up-regulation above 0.8 fold change (Log 2 ratio); ↓ : Down-regulation above 0.8 fold change (Log2 ratio);NS: Not Significant fold change.

Table S4.The percentage uptake of aptamers by primary RB tumors and normal retina.

Sample	Control cy3	A12 cy3	A35 cy3	TA1 cy3	TA6 cy3	Control FITC	A15 FITC
RB1	0.27	67.50	77.11	19.31	73.04	0.80	90.09
RB2	1.02	57.30	58.81	0.90	71.20	0.57	95.81
RB3	0.14	58.34	54.68	0.19	70.89	1.57	91.58
RB4	0.49	41.97	44.52	0.61	62.40	0.74	84.82
RB5	0.42	55.94	55.24	0.47	64.95	0.49	90.43
NR1	1.44	2.59	5.11	0.69	0.57	1.19	8.42