A multivalent DNA aptamer specific for the B cell receptor on human lymphoma and leukemia

Supplementary Figures

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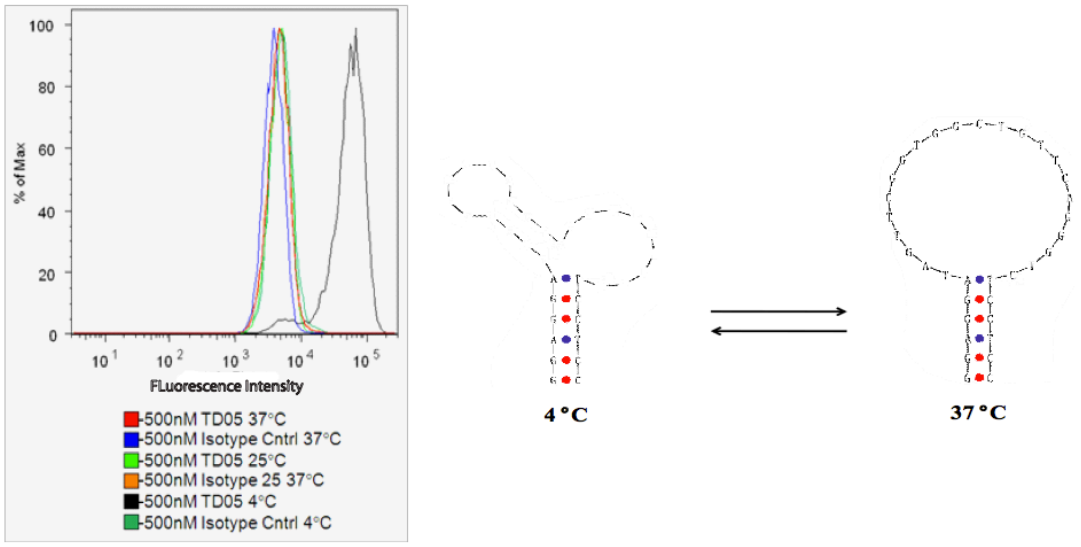

Figure S1. Investigation of binding as a function of temperature. (A) Flow cytometric analysis of TD05 aptamer binding to Ramos cells temperatures above 4°C. The fluorescence shift on the x-axis decreases to its background level at higher temperatures indicating that the K_{off} is higher at temperatures higher than 4°C. (B) Predicted high affinity bulge secondary structure of TD05 changes with temperature to a low affinity stem-loop structure resulting dissociation of the complex.

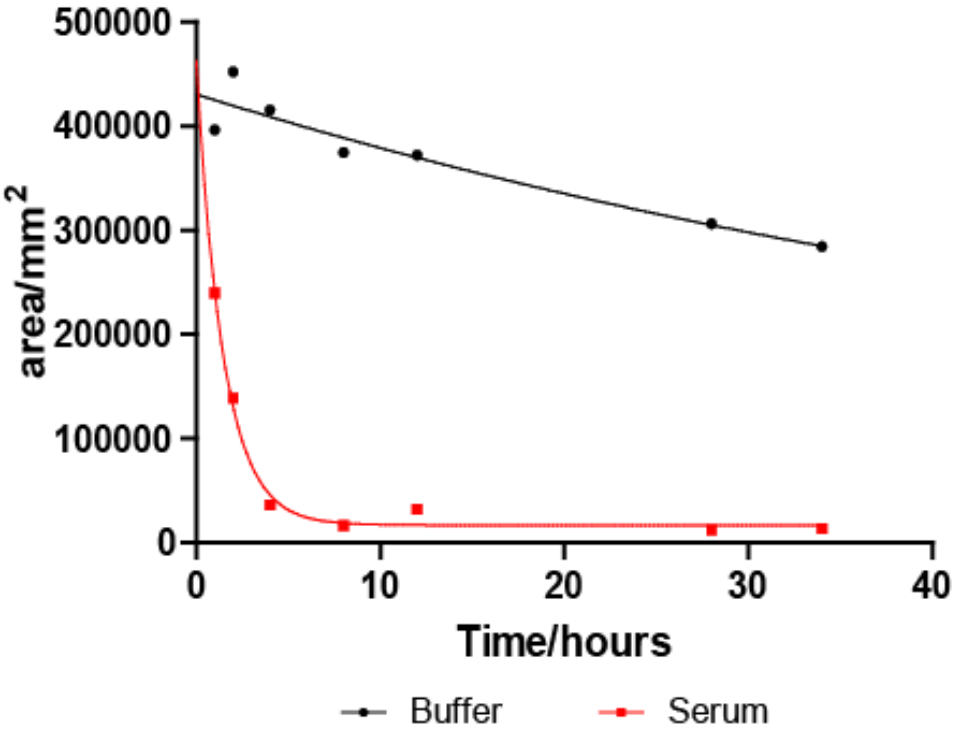

Figure S2. Analysis of nuclease stability of TD05 in serum at physiological temperature using polyacrylamide gel electrophoresis. Fluorescence intensity of full length DNA /area (mm^2) was plotted as a function of time (hours).

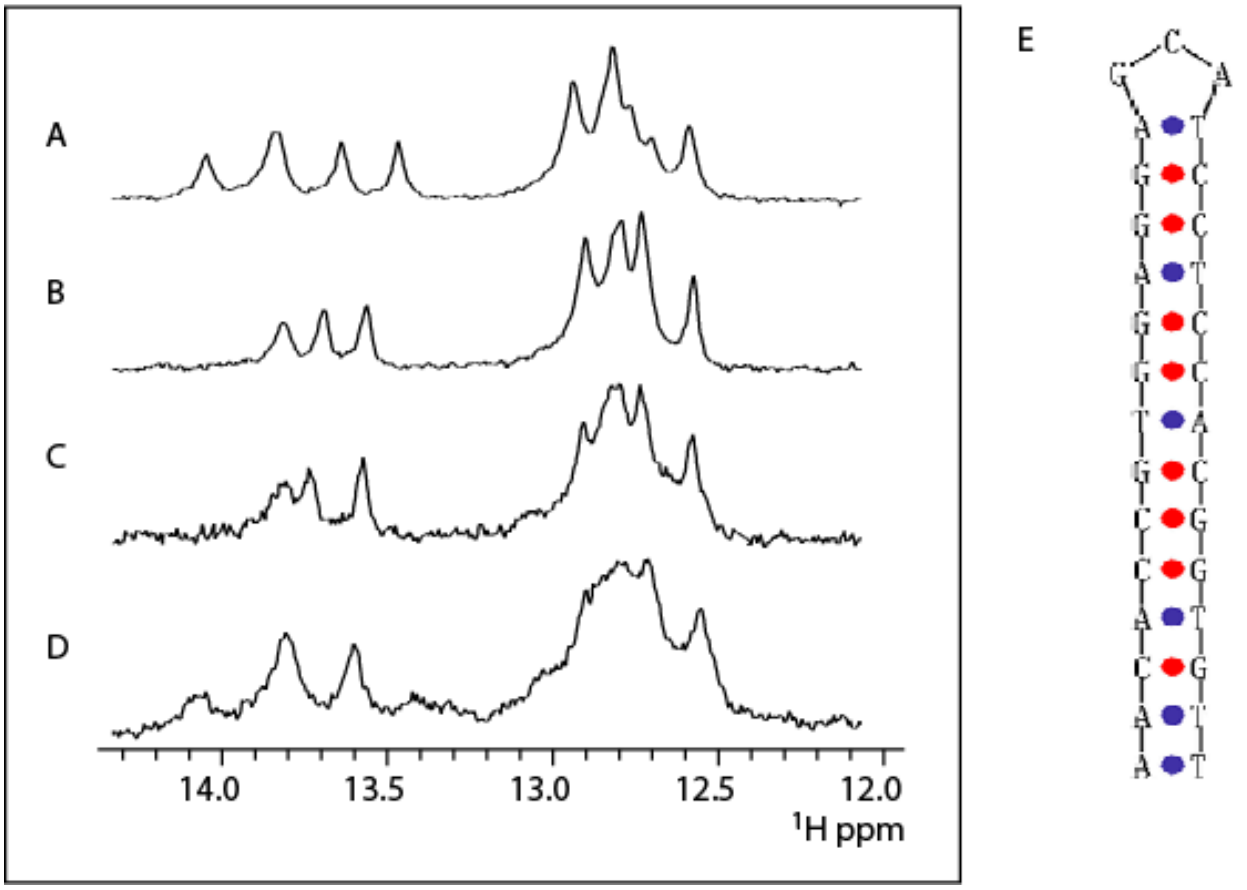

Figure S3. NMR characterization of stem-loop formation. 1D NMR spectra of DNA samples were recorded on 600 MHz Varian Unity-Inova spectrometers with jump-and-return water suppression (512-1024 transients), in 0.5mM $NaHPO_4$ containing 4mM NaCl with 10% of D_2O at temperatures indicated below. (A) NMR spectrum recorded at 37°C for the structure in panel E, which is the palindromic region of TD05 used as the positive control to detect the formation of the stem structure in NMR experiments. Five peaks at 13.5-14ppm (one doubled) for internal AT pairs, and eight GC pairs appear between 12.5-13.0 ppm. (B) NMR spectrum of structure A (in manuscript Figure 1) at 37°C, with three internal AT base pairs and seven GC pairs suggesting that predominant stem formation leads to a more homogeneous structure at 37°C. (C) and (D) NMR spectra of the oligonucleotide A (in manuscript Figure 1) recorded at 25°C and 0°C, indicating contribution of other folds to populations of the molecules at lower temperatures (line broadening and less resolved peaks).

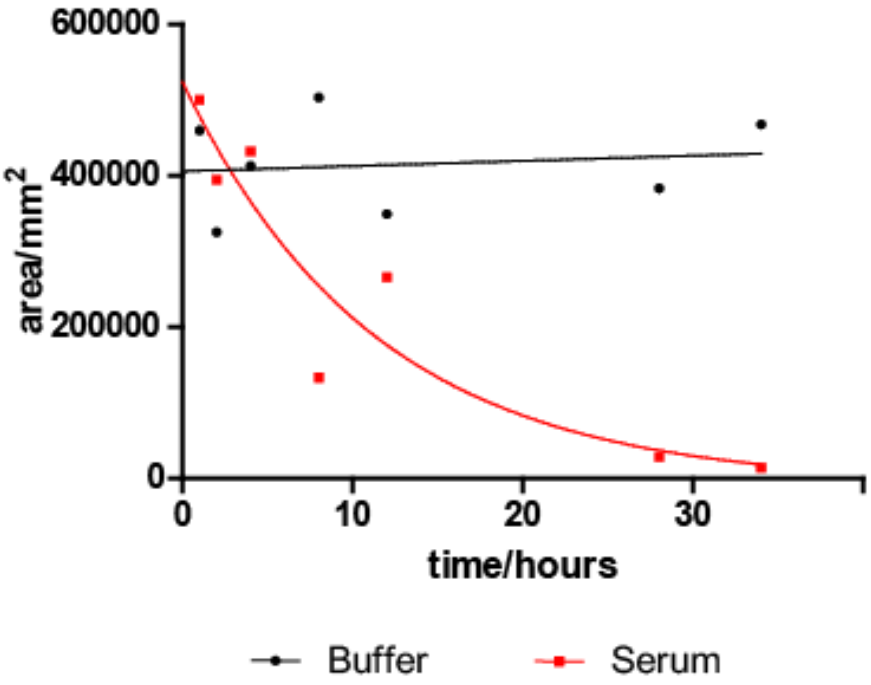

Figure S4. Analysis of nuclease stability of L-BVA.8S in serum at physiological temperature using poly-acrylamide gel electrophoresis. Fluorescence intensity of full length DNA /area (mm^2) was plotted as a function of time (hours).

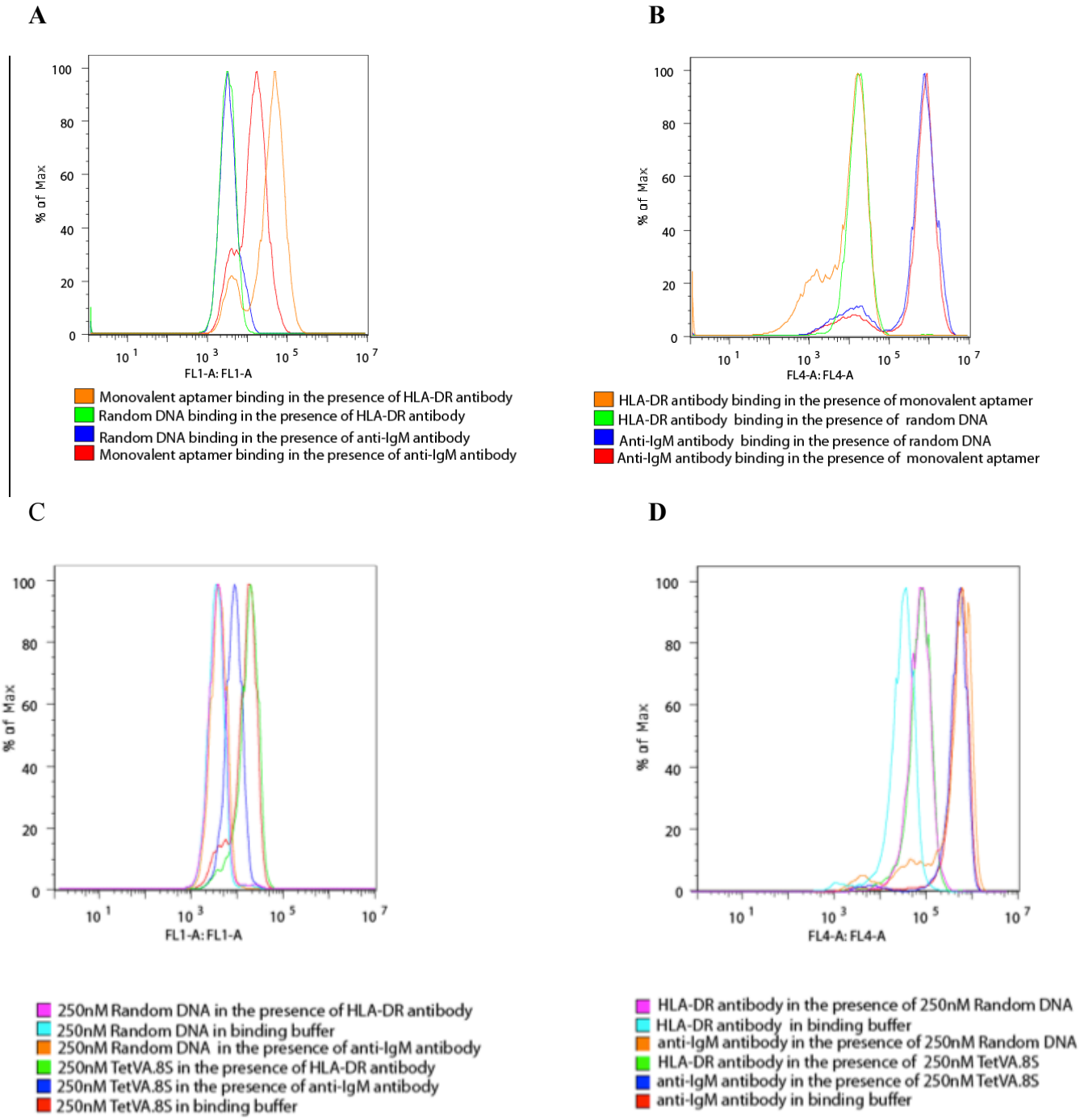

Figure S5. Competition of anti-IgM antibody with monomeric (A-B) and tetrameric aptamer (C-D). In order to investigate the competition between anti-IgM (mu) antibody and tetrameric aptamer, first 0.2ug/mL of cy5-labeled anti-IgM (mu) antibody and isotype control were incubated with 1×10^6 Ramos cells in ice for 30min. Then the free antibody was washed with 1mL of wash buffer, and cells were reconstituted with either 0.5uM of FITC labeled monomeric or tetrameric aptamer, or 0.5uM of random control in 50uL of binding buffer for another 30min in ice. Then the cells were washed with 1mL wash buffer and binding events were monitored in FL1 for the aptamer and FL4 for the antibody counting 10000 events using flowcytometry. Fluorescence intensity on the X axis for FL1 shifts to a lower value in the presence of anti-IgM antibody indicating that the anti-IgM replaces the aptamer.