Cell culture

Cell line HL60 (acute myelocytic leukemia) was a generous gift from David Scheinberg lab, Memorial Sloan Kettering Cancer Center. All cells were cultured in RPMI 1640 medium supplemented with 100-units/mL penicillin–streptomycin and 20% fetal bovine serum (heat-inactivated; Invitrogen).

Phosphoramidites

All of the DNA reagents needed for DNA synthesis were purchased from Glen Research or ChemGenes. All the DNA oligo sequences were chemically synthesized with fluorescein-dT at the 3' end using standard solid phase phosphoramidite chemistry on an ABI394 DNA (Biolytics) synthesizer using a 0.2 µmole scale. The completed DNA sequences were deprotected in ammonium hydroxide and purified using HPLC (Waters) equipped with a C-18 reversed phase column (Waters). All in vitro experiments were performed using a binding buffer composed of DPBS and 4.5 g/L glucose (Sigma-Aldrich) and 5 mM $MgCl_2$, 100 mg/L, tRNA (Sigma-Aldrich), 100 mg/L BSA (Sigma-Aldrich). The wash buffer was composed of DPBS with 5 mM $MgCl_2$ and 4.5 g/L glucose (Sigma-Aldrich).

Cell binding assays

Determination of aptamer affinity:

Aptamers were folded for 5 min/100µL at 95.0°C then snap chilled on ice for 45 minutes. The affinities of the aptamer sequences were evaluated by incubating 1.0 × 10^5 HL60 cells with concentrations of 250nM, 125nM, 25nm, 5nM, 1nM and 0nM of fluorescein-dT labeled aptamer in 200µL of binding buffer or random control on ice for 45 min. The cells were then washed once with 2.0mL of wash buffer at 4°C and reconstituted in 250 µL of wash buffer.The binding of the constructs was analyzed using FACS Calibur flow cytometer (Cytek,) by counting 10000 events for each concentration. The equilibrium disassociation constant (Kd) of the aptamer-cell interaction was obtained by plotting the difference in median florescence intensity against concentration. Specific binding was calculated with the equation $Y = \frac{Bmax*X}{Kd+X}$,using GraphPad Prism 5 (La Jolla, CA, USA).

37°C Binding

Aptamers were folded for 5 min/100µL at 95.0°C then snap chilled on ice for 45 minutes. The binding of the aptamer sequences were evaluated by incubating 1.0 × 10^5 HL60 cells with concentrations of 1µM of fluorescein-dT labeled aptamer or random control in 200µL of binding buffer at 37°C for 45min. The cells were then washed once with 2.0mL of 37°C wash buffer at and reconstituted in 250 µL of 37°C wash buffer. The binding of the constructs was analyzed using FACS Calibur flow cytometer (Cytek,) by counting 10000 events for each concentration.

Specificity assays

Aptamers were folded for 5 min/100µL at 95.0°C then snap chilled on ice for 45 minutes. The specificity of the aptamer sequences were evaluated by incubating 1.0 × 10^5 HL60 cells, Jurkat (T cells) and Daudi (B cells) with concentrations of 500nM of fluorescein-dT labeled aptamer separately in 200µL of binding buffer on ice for 45min. The cells were then washed once with 2.0mL of wash buffer and reconstituted in 250 µL of wash buffer. The binding of the constructs was analyzed using FACS Calibur flow cytometer (Cytek) by counting 10000 events for each concentration. The specific binding was determined using the equation $\frac{aptamer-Random\ control}{aptamer} * 100$ and quantified using Graph Pad Prism 5 (La Jolla, CA, USA).

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Nuclease stability

Aliquots of 50 pmol of fluorescently labelled modified aptamers were incubated at 37°C in a final volume of 20µL in human serum and in 20µL of phosphate-buffered saline (PBS) buffer for 0, 0.5 1, 2, 3, 6, and 24 hours. At the end of each time point, the reactions were terminated by adding 20µL 2xTBE Urea loading buffer (Invitrogen-Novex®) 20x SYBR® Green (Lonza) and stored in -80°C. Full-length and digested DNA were analyzed by 10%-TBE urea polyacrylamide gel electrophoresis using Bio-Rad Gel Dock EZ Imager and Image Lab Software version 5.2.1.

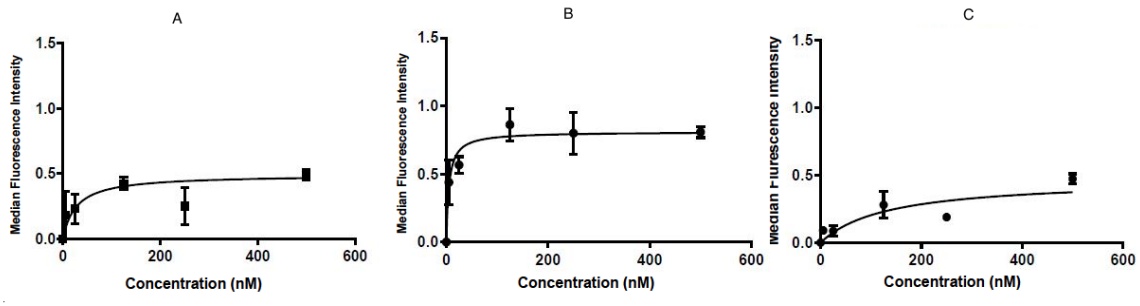

Figure S1. Analysis of affinities of truncated analogues of aptamer KH1C12 at 4°C.

The affinity curve for the analogue KH1C12.O1 (A), KH1C12.O2 (B), KH1C12.O3 (C).
The affinities of the aptamer sequences were evaluated by incubating 1.0 × 10^5 HL60 cells with concentrations of 500nM, 250nM, 125nM, 25nm, 5nM, and 1nM of Fluorescein-dT labeled aptamer in 200µL of binding buffer on ice for 45 min. The cells were then washed once with 2.0mL of wash buffer at 4°C and reconstituted in 250 µL of wash buffer. The binding of the constructs was analyzed using FACS Calibur flow cytometer (Cytek,) by counting 10000 events for each concentration. The equilibrium disassociation constant (Kd) of the aptamer-cell interaction was obtained by plotting the difference in median florescence intensity against concentration. Specific binding was calculated with the equation Y = $Y = \frac{Bmax*X}{Kd+X}$, using GraphPad Prism 5 (La Jolla, CA, USA).

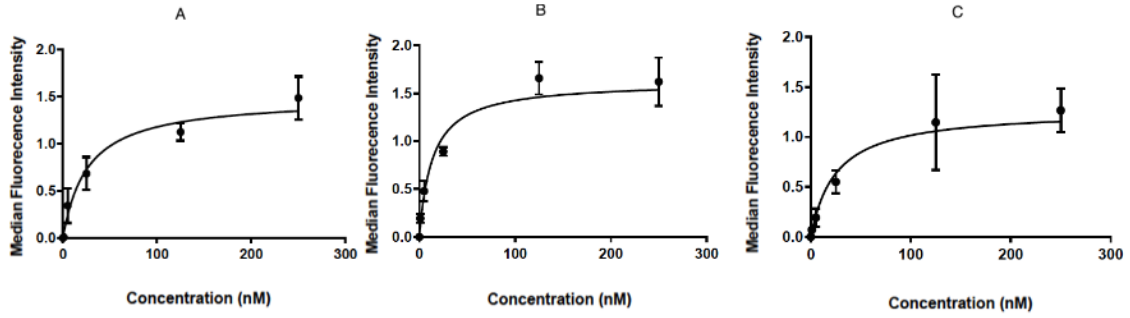

Figure S2. Analysis of affinities of aptamer analogues modified with 2'-O-methyl RNA bases at 4°C.

The affinity curve for the analogue O2.G1 (A), O2.G2 (B), O2.G3 (C).
The affinities of the aptamer sequences were evaluated by incubating 1.0 × 10^5 HL60 cells with concentrations of 500nM, 250nM, 125nM, 25nm, 5nM, and 1nM of Fluorescein-dT labeled aptamer in 200µL of binding buffer on ice for 45 min. The cells were then washed once with 2.0mL of wash buffer at 4°C and reconstituted in 250 µL of wash buffer. The binding of the constructs was analyzed using FACS Calibur flow cytometer (Cytek,) by counting 10000 events for each concentration. The equilibrium disassociation constant (Kd) of the aptamer-cell interaction was obtained by plotting the difference in median florescence intensity against concentration. Specific binding was calculated with the equation $Y = \frac{Bmax*X}{Kd+X}$, using GraphPad Prism 5 (La Jolla, CA, USA).

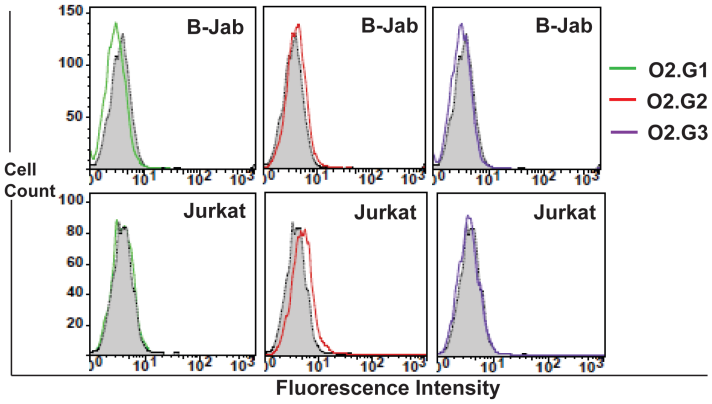

Figure S3. Specificity of aptamer analogues modified with 2'-O-methyl RNA bases at 37°C

Analysis of specificity of 2'-O-methyl RNA-substituted O2.G analogues against three different cell lines. The specificity of the aptamer sequences was evaluated by incubating 1.0×10^5, Jurkat (T cells) and B-Jab (B cells) with concentrations of 1μM of Fluorescein-dT-labeled aptamer separately in 200μL of 37°C binding buffer for 45min. Cells were then washed once with 2.0mL of 37°C wash buffer and reconstituted in 250 μL of 37°C wash buffer.