Manuscript title: Aptamer-RT interactions govern antiviral specificity and viral encapsidation of broad spectrum anti-HIV RT aptamers.

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Supplemental Materials & Methods

Primer Extension Assay. Primer extension was carried out essentially as described (11). Briefly, a Cy3-labeled, 18-mer DNA oligonucleotide corresponding to the 3′ end of $tRNA^{Lys3}$ was mixed with a 31-mer template in a 1:3 ratio to ensure that all primer was pre-bound to template. This mixture was heated to 90 °C in a heat block for 2 minutes and then annealed by cooling to room temperature. A reaction master mix was assembled to contain (final concentrations) 30 μmol/l dNTPs, 0.5 mmol/l ethylenediaminetetraacetic acid (EDTA), 50 mmol/l Tris–HCl pH 7.8, 50 mmol/l $NaCl_2$, 10 mmol/l dithiothreitol and 20 nmol/l RT, with the RT and dithiothreitol added last. The reaction master mix of 14 μl was aliquoted to each tube, along with either 2 μl of aptamer solution (final concentration 100 nmol/l unless otherwise noted) or water. Reactions were initiated by adding 4 μl of a solution containing annealed primer/template and $MgCl_2$ (final concentration 20 nmol/l and 6 mmol/l, respectively). After incubating at 37 °C for 10 min, reactions were stopped by adding 20 μl of 90% formamide, 50 mmol/l EDTA, and a trace amount of bromophenol blue. Samples were heated to 90 °C for 2 min immediately before loading onto a 15% polyacrylamide, 8 mol/l urea denaturing gel. Gels were scanned for Cy3 fluorescence with a FLA9000 phosphorimager (Fujifilm, Valhalla, NY). The fraction of primer converted to full-length product was determined by quantifying band intensities using ImageQuant software (Pharmacia, Piscataway, NJ).

Supplemental Table

Table S1.

Aptamer	Sequence including 5’ and 3’ constant regions
Arbitrary	GGGAAAAGCGAATCATACACAAGAAATTTGGACTTTCCGCCCTTCTTGGC CTTTATGAGGATCTCTCTGATTTTTCTTGCGTCGAGTTTTCCGGGGGCATA AGGTATTTAATTCCATA
70.05 (F1pk)	GGGAAAAGCGAATCATACACAAGATCCGAGGCAGAACGGGAAAATCTGC GAAGTAACTGTGGAATCCGTGACCTTTGACGTGAAAACCGCGAGGGCAT AAGGTATTTAATTCCATA
70.08 (F2Pk)	GGGAAAAGCGAATCATACACAAGACGAGACAAGTACCGAAAAAGAGATCT GGCAGTGTCACAACCAGGAAAAAGACACGACGAACACGCCGCACGGGCA TAAGGTATTTAATTCCATA
88.1 (6/5AL)	GGGAAAAGCGAATCATACACAAGACATTGACCAAAAGGGTCGATAGCGTC GTGTAGATTGCACCCATGACTGAGCTACTGCCAAATCCACCCACGGGCAT AAGGTATTTAATTCCATA
80.103 (UCAA)	GGGCATAAGGTATTTAATTCCATAGCAACCGGTTGTCTACACGCGGCGAA TAGAGCCCGGTTCAAGGACACCGCCACTGCTGTCGACATTTCCTTAGTGC TAGATTGATTCGGATGCTCCGGTAGCTCAACCTG

Table S1. Sequences of aptamers used in this study. Sequences are represented in the 5’→3’ orientation and include constant regions utilized for aptamer amplification by PCR. All aptamers with the exception of aptamer 80.103 (UCAA) contain a 70N random region and utilize the same constant regions. Aptamer 80.103 contains a 80N random region and has different constant regions.

75 **Supplemental Figure Legends**

76 **Supplemental Fig. S1**. (A) Silent mutations near 5' and 3' edges of RT-coding segment of HIV-
77 1. Protease cleavage sites that define boundaries of mature p66 subunit of RT are shaded.
78 Point mutations that introduce *XmaI*, *NheI* and *AgeI* sites are shown in blue. (B) Replace RT
79 DNA with cloning sites. In step 1, amplicons were generated from plasmid v550 extending from
80 upstream of the *SpeI* site in *gag* to the 5' edge of the RT-encoding segment (green), or
81 extending from the 3' edge of the RT-encoding segment to downstream of the *EcoRI* site in *tat*
82 (gold). Primers near the edges of RT included synonymous substitutions that introduced *XmaI*
83 and *NheI* restriction sites, along with a *NotI* site. In Step 2, those amplicons were inserted into
84 pTA-Topo and their orientations relative to the vector were confirmed by sequencing. In Step 3,
85 the plasmid carrying the upstream fragment (pTA-DeltaL(Xma)) was digested either with
86 *SpeI/XmaI* or with *SpeI/EcoRI*; the plasmid carrying the downstream fragment (pTA-
87 DeltaR(Nhe)) was digested either with *XmaI*/*EcoRI*. The three indicated fragments were gel
88 purified and ligated to generate the 5.84kb plasmid pTA-DeltaLR(Xma/Nde) in which the *SpeI*-
89 *EcoRI* fragment of the original plasmid has been replaced by three restriction sites. (C) Insert
90 RT deletion into proviral vector. Proviral vector pNL4-3-CMV-EGFP carries an *NheI* site just
91 upstream of the EGFP reporter that must be removed prior to preparing the vector. A common
92 approach is to digest the DNA, fill in with a DNA polymerase, and then re-ligate. However, for
93 *Nhe*I, this duplicates the original site. Therefore, synthetic oligos were annealed to generate
94 *Nhe*I overhangs. Upon re-ligation with vector, the recognition site is lost (five nucleotides are left
95 on each side, in green), and an *Xba*I site is inserted. Orientation was confirmed by DNA
96 sequencing to generate plasmid v550_DeltaNhe. Finally, the 3.7kb *SpeI-EcoRI* fragment of
97 v550_DeltaNhe was removed and replace with the corresponding 2.0 kb *SpeI-EcoRI* fragment
98 from plasmid pTA-DeltaLR(Xma/Nde).

99 **Supplemental Fig. S2. Building recombinant reporter viruses.** (A) RT expression plasmids
100 (8) served as templates for generating amplicons encoding RT using the primers shown for the
101 various phylogenetically diverse sources. Where the full pol gene is available (NL4-3, HXB2,
102 primary isolates, clinical samples, *gag-pol* clones), only the outer 3' primer is needed.
103 Sequential amplification with overlapping primers appended the protease-encoding segment
104 between the 3' end of RT and the *NheI* cloning site. All PR-derived sequence is based on HIV-1
105 strain NL4-3. The build-out process is shown explicitly for the first primer. (B) Sequence of the
106 cloning region of plasmid pNL4-3-CMV-GFPDeltaRT is shown. Amplicons from (A) were
107 digested with *XmaI/NheI* and inserted into the corresponding sites of plasmid pNL4-3-CMV-
108 GFPDeltaRT. The ligation products were back-digested with *NotI* to reduce background. All
109 plasmids were confirmed by DNA sequencing. Yellow arrows indicate primers used in
110 generating initial amplicons from Fig. S1B.

111 **Supplemental Fig. S3. A single point mutation can abolish aptamer binding to RT and**
112 **aptamer-mediated inhibition of HIV-1 replication, but does not abolish aptamer**
113 **encapsidation within viral particles.** (A) VSV-pseudotyped virus was generated in 293FT
114 cells co-transfected with aptamer-expressing constructs (1000 ng), and their infectivity on fresh
115 293FT cells shown as % EGFP-positive cells normalized to p24 was determined using flow
116 cytometry. The arbitrary control for each proviral construct was set to 1. Values are shown as
117 the mean ± SD for three experiments. (B) Transfected cells from (A) were harvested in Trizol
118 reagent for RNA analysis of aptamer expression. cDNA was synthesized from 500 ng of RNA
119 and subjected to quantitative real time PCR using primers to amplify the aptamer cassette and
120 primers for the housekeeping genes, 18s rRNA. "No RT" controls were included for each
121 sample, and each sample was assayed in triplicate. Each quantity was normalized to the
122 housekeeping gene, then to the pcDNA3.1 control, and averaged. Values are shown as the
123 mean ± SD. Representative data for a single aptamer are shown. Experiments were repeated

124 three times. (C) Viral RNA was harvested from virus produced by transfection in the presence of
125 aptamer. cDNA was synthesized from 500 ng of RNA and subjected to RT-qPCR using primers
126 specific for the aptamer constant region and HIV-1 *gag*. “No RT” controls were included for each
127 sample, and each sample was assayed in triplicate. Each quantity was normalized to the HIV-1
128 *gag* reference gene, then to the empty control, and averaged. Values are shown as the average
129 of three technical repilcates ±SD. Experiments were performed three times, and a
130 representative experiment is shown.
131 **Supplemental Fig. S4**. **Encapsidation within the viral particle is required for aptamer-**
132 **mediated inhibition.** Virus expressing subtype B RT was produced in 293FT cells in the
133 absence of aptamer by co-transfection of the indicated proviral plasmid with pMD-G. Separately,
134 293FT cells were transfected in triplicate with aptamer-expressing constructs at the indicated
135 doses. Aptamer-expressing cells were infected with subtype B viruses at 6 (A), 24 (B), or 48 (B
136 and C) hours post-transfection. Cells were harvested 24 hours post-infection and fixed with 2%
137 paraformaldehyde. Infectivity was determined using flow cytometry (% EGFP positive cells).
138 Arbitrary controls were set to 1. Experiments were performed (A) once, (B and C) twice. Values
139 are shown as the mean ± SD for two experiments in (B) and (C).

140 **Supplemental Fig. S5**. **Cellular aptamer expression increases with increasing aptamer**
141 **dose**. (A) Aptamer-transfected cells (from samples in Fig. 3) were harvested in TRIzol reagent
142 for RNA analysis of intracellular aptamer expression. cDNA was synthesized from 500 ng of
143 RNA and subjected to RT-qPCR using primers specific to the aptamer constant region and for
144 the cellular reference gene, 18s rRNA. “No RT” controls were included for each sample, and
145 each sample was assayed in triplicate. Each quantity was normalized to the reference gene,
146 then to the 0 ng control, and averaged. Values are shown as the average of three technical
147 replicates ±SD. Representative data for a single proviral construct (subtype B) are shown.
148 Experiments were repeated three times. (B and C) Viral RNA was harvested from cell-free virus

149 produced by co-transfection of aptamer expressing plasmids (0-1000 ng), DNA filler plasmid (1-
150 1000 ng), proviruses expressing either HXB2 (B) or the HXB2 point mutant R277k (C) (150 ng),
151 and VSV-G (50 ng). cDNA was synthesized from 500 ng of viral RNA and subjected to qRT-
152 PCR using primers to amplify aptamers and HIV-1 *gag*. "No RT" controls were included for each
153 sample, and each sample was assayed in triplicate to determine technical variability. Samples
154 were normalized to HIV-1 *gag* reference gene and the 0 ng control, and then averaged (relative
155 quantity method). Values shown are the mean relative quantity of three technical replicates
156 ±SD. A representative experiment of three total experiments is shown. An additional experiment
157 is represented in Figure 4.

158 **Supplemental Fig. S6. Aptamers inhibit the p66/p66 homodimer for Subtype B RT.** Primer
159 extension assays were performed using a Cy3-labeled, 18-mer DNA oligonucleotide
160 corresponding to the 3′ end of $tRNA^{Lys3}$. The 18-mer was incubated with RT with or without
161 aptamer in primer extension buffer and samples were analyzed on polyacrylamide gels. Gels
162 were scanned for Cy3 fluorescence with a FLA9000 phosphorimager and the fraction of primer
163 converted to full-length product was determined by quantifying band intensities using
164 ImageQuant software and is shown as percent extended. Data are shown as the average of
165 three experiments ±SD.

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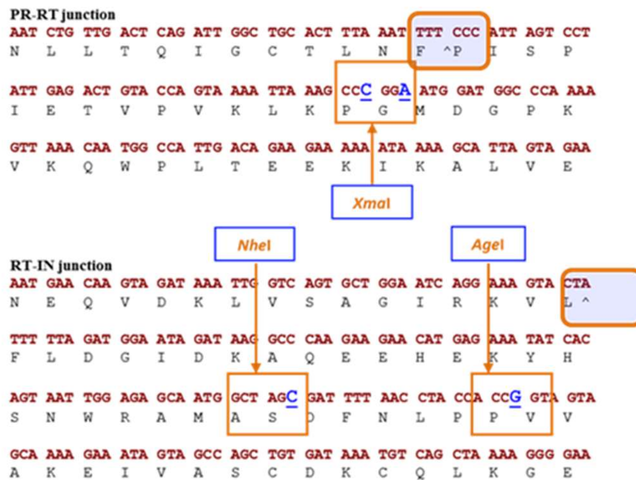
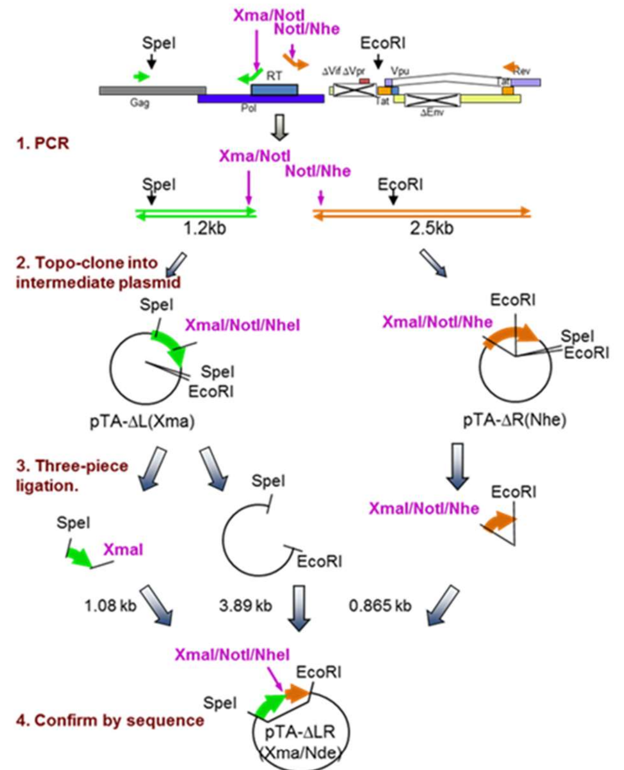
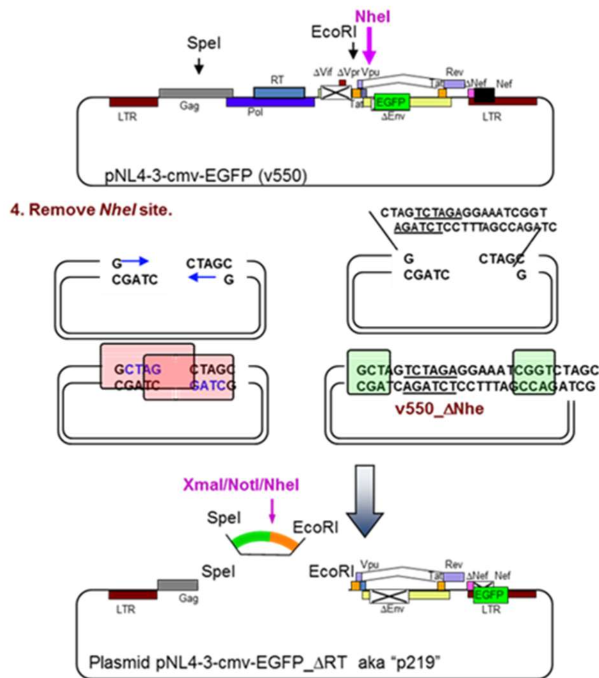
182 Figure S1.







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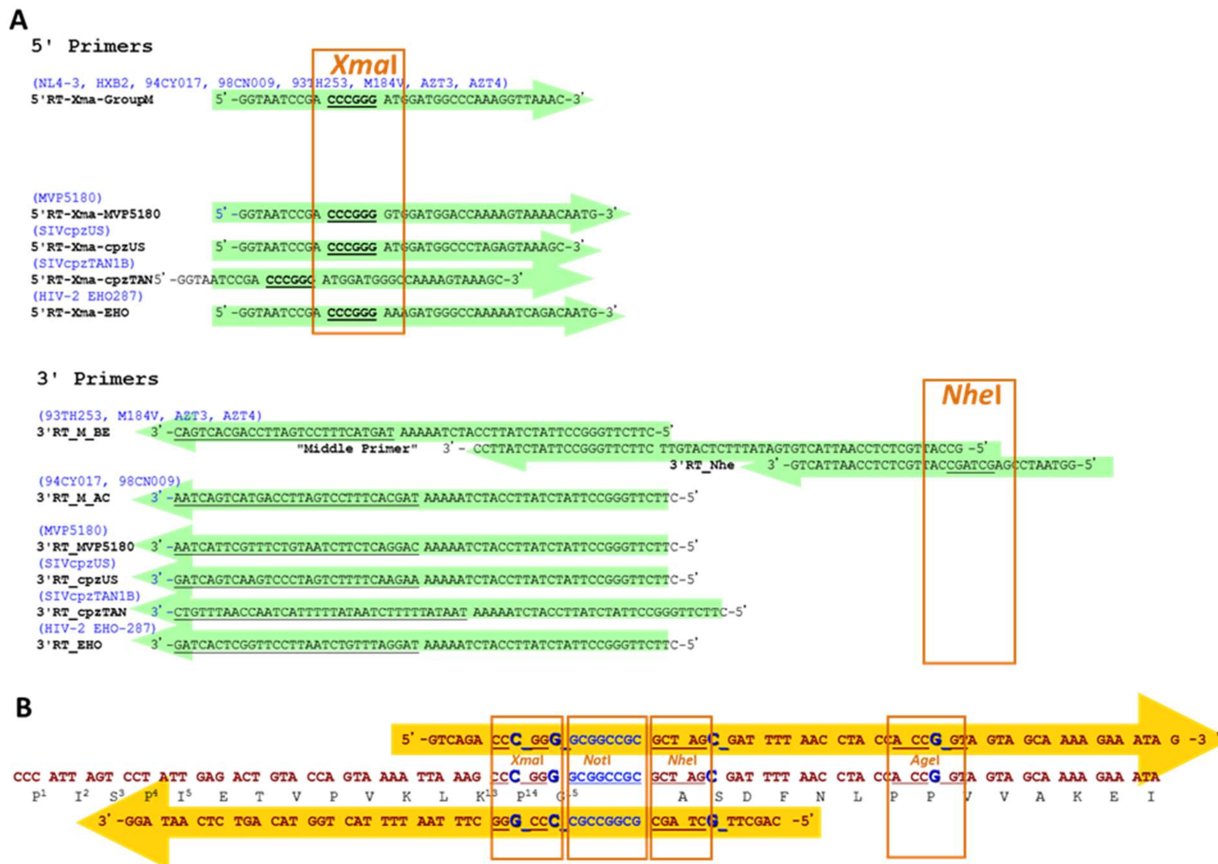
187 Figure S2.



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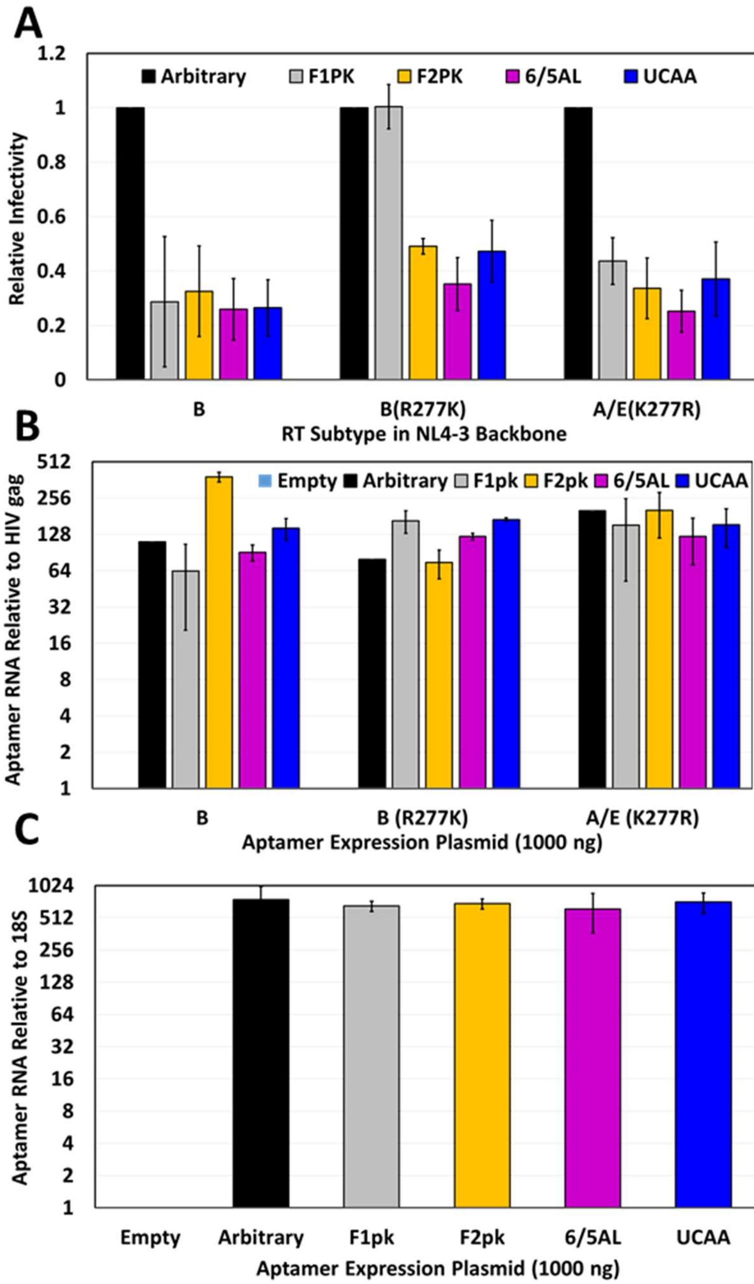
197 Figure S3.



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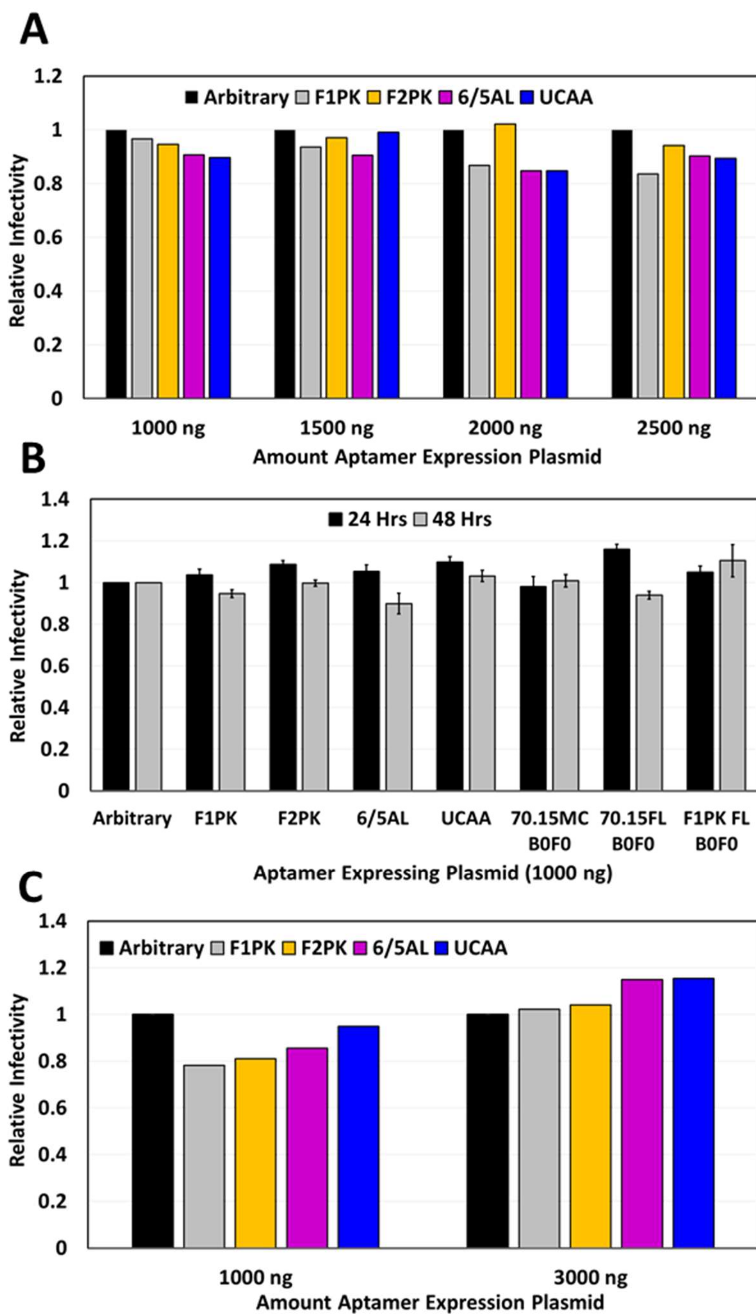
202 Figure S4.



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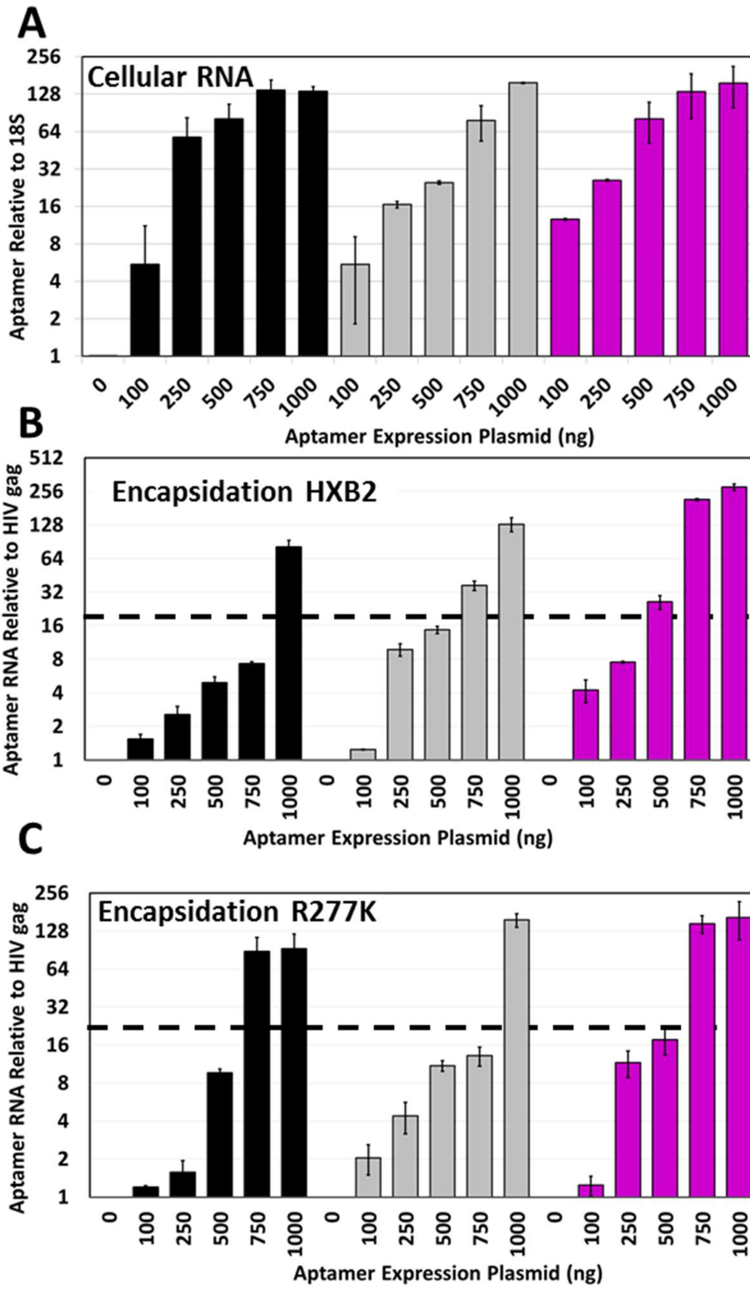
207 Figure S5.



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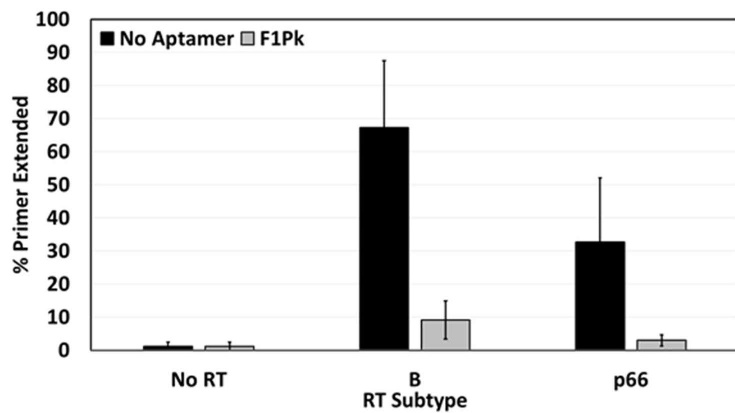

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