Supplementary Data

Figure Legend

Supplemental Figure 1. Purification of HIS-IN and MBP-IN fusion proteins. **A**. Coomassie blue stained gel showing fractions of HIS-IN eluted from Ni-agarose column at 500mM NaCl. When this purified protein was dialyzed against PBS, a substantial amount of protein was precipitated. **B**. Coomassie blue stained gel showing purified MBP-IN fusion before and after digestion by factor X protease. More than half of the IN protein was degraded after the MBP moiety was separated. MBP alone served as positive control.

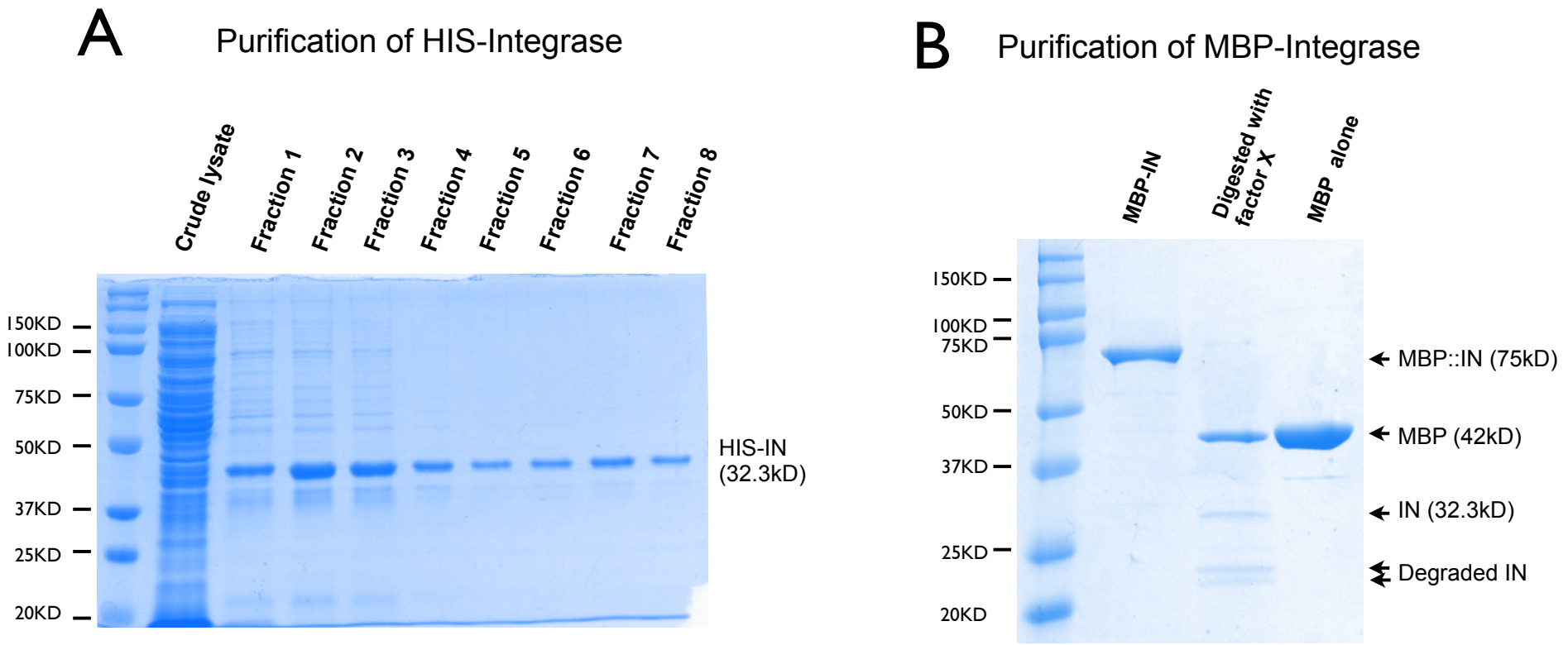
Supplemental Figure 2. Plasmids for functional test of expressed aptamers. In both pJR255 and pJR288, an U6 promoter was used to drive the expression of aptamers, shRNAs or shRNA-aptamer fusions. Oligonucleotide pairs with CACC and AAAA overhangs were cloned to BbsI cut sites. Vectors also contain a Neomycin (G418) resistance cassette and an mCherry visible marker driven by either by CMV (pJR255) or EF1a-HTLV (pJR288) promoters.

Supplemental Figure 3. Aptamers expressed directly from U6 promoter lacked any inhibition on lentivirus HIV7-GFP. **A**. FACS data gated with mCherry and GFP signals in control cells (U6) or cells expressing S1R1, S3R3 or S3R3. A single representative experiment is shown. **B**. Quantification of FACS data shown in panel A. Percentages of double positive (Q2) over total GFP positive (Q2+Q3) in cells expressing S1R1, S3R3 or S3R3 were compared to that of empty vector (U6 only) control (100%). Averages and standard deviations of three independent experiments are shown.

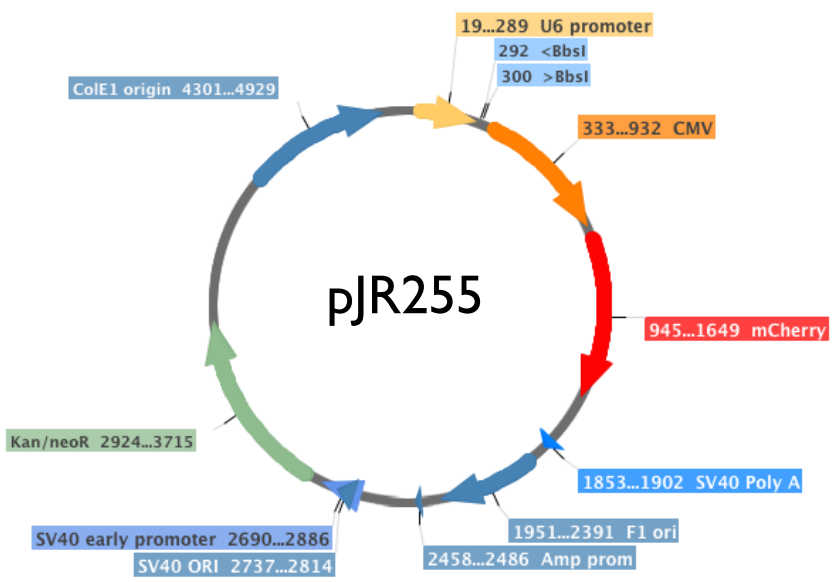
Supplemental Figure 4. Efficacy of shLuc-aptamer fusions in multiple cycle infection. **A**. Change of virus concentration in cultures measured by P24 assay. Ghost3 cells expressing shLuc-aptamer fusions were infected with Ba-L virus. P24 levels were monitored for 9 days. A representative experiment with triplicate samples is shown. Error bar is not shown because of extensive overlapping. **B**. Inhibition of HIV replication at day 9 post-infection. The averages and standard deviations of two independent assays are shown.

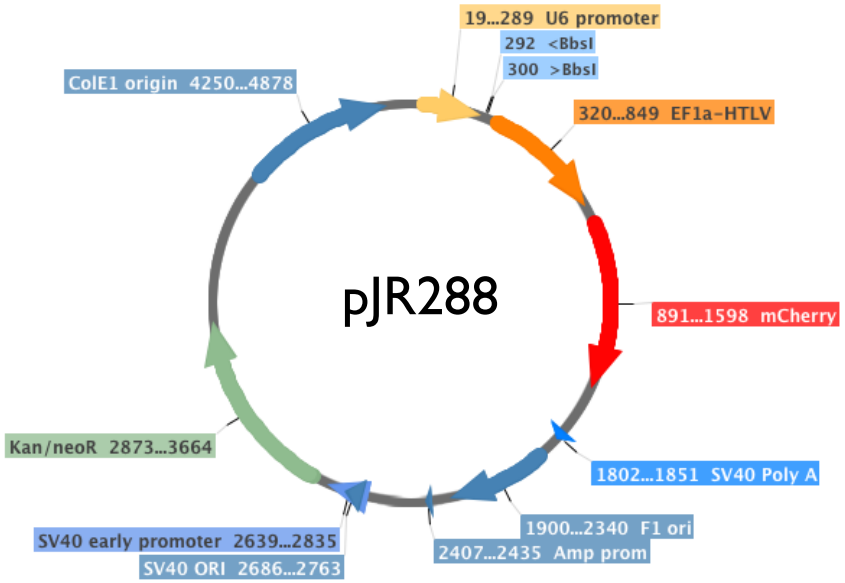
Supplemental Figure 5. A. Aptamer S3R3 had higher binding affinity to HIS-IN than the more abundant S1R1. Single gel shift experiment is shown. Unbound aptamers are indicated by lower arrows (S1R1 and S3R3 are 59-nucleotide long while shLuc-S1R1 is 96-nucleotide long). Bound aptamers appeared to form very large complexes with IN and retained in the wells (upper arrow). Signals from lower bands were used to calculate the value of 50% binding. The average of two independent experiments (47 nM ± 3) was reported in text. **B**. 50% binding of S3R3 to HIS-IN was determined by a single filter binding assay. A large range of binding affinity (10 and 38nM) was observed for two independent experiments, resulting an average of 32 nM ± 20. Therefore, the result from filter retention binding was not shown in the Result section. The assay was performed essential the same as the one with the population of aptamers (See material and method). Purified S3R3 was labeled with ^{32}P and incubated with 10, 20, 40, 80 and 160 nM of HIS-IN for 30 min at 37^{o}C. Aptamer-protein complex were isolated using HAWP filter (0.45μm pore size, 13mm diameter, EMD Millipore, Concord, MA, USA). After washing with 1ml PBS, radioactivity retained on filter was determined by scintillation counter. Binding affinity was determined using Prism 6 software (GraphPad software Inc.).

Supplemental Figure 1



Supplemental Figure 2






```
                                  Bbs-1    Bsb-1
      U6 promoter                   |        |
ATATCTTGTGGAAAGGACGAAA    CACCaaGTCTTCaaGAAGACat    tttttactagtggatcccccggg
TATAGAACACCTTTCCTGCTTTGTGG    ttCAGAAGttCTTCTGtaaaaa    atgatcacctagggggccc
```

cut with BbsI ↓

```
      U6 promoter          Insert annealed oligonucleotide pairs with CACC and AAAA overhangs
ATATCTTGTGGAAAGGACGAAA    CACCGNNNNNNNNNNNNNNNNNN......NNNNNNNNNNNNNNNNN    tttttactagtggatcccccggg
TATAGAACACCTTTCCTGCTTTGTGG    CNNNNNNNNNNNNNNNNNN......NNNNNNNNNNNNNNNNNNAAAA    atgatcacctagggggccc
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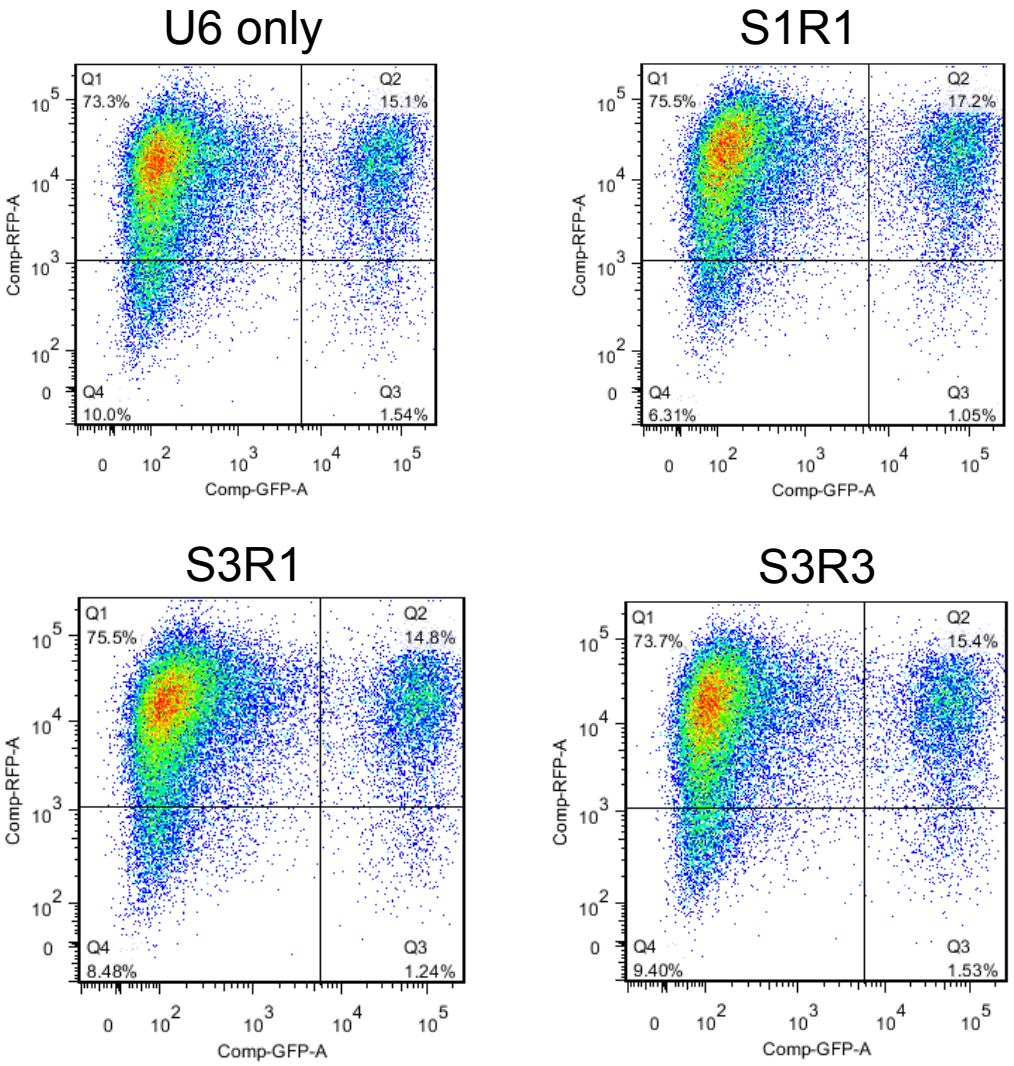
Supplemental Figure 3

A



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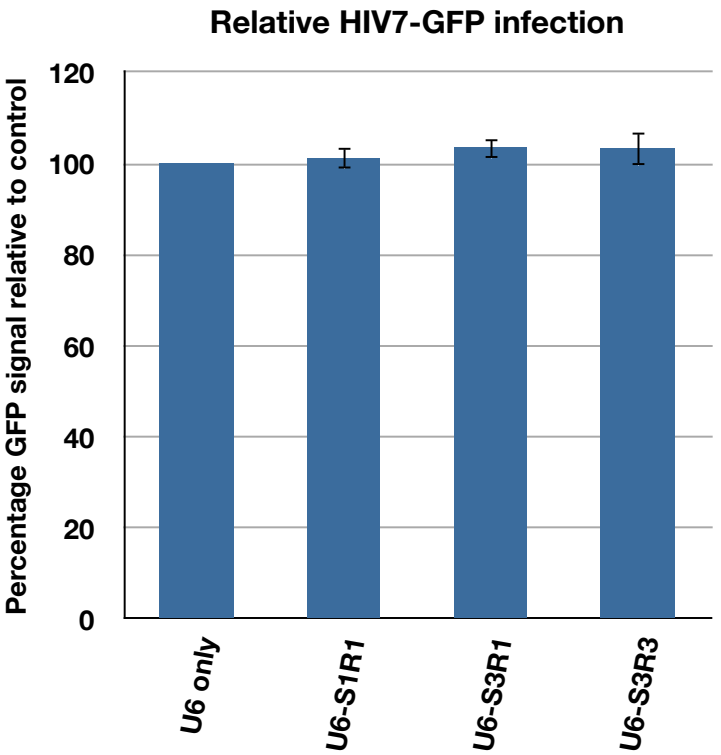
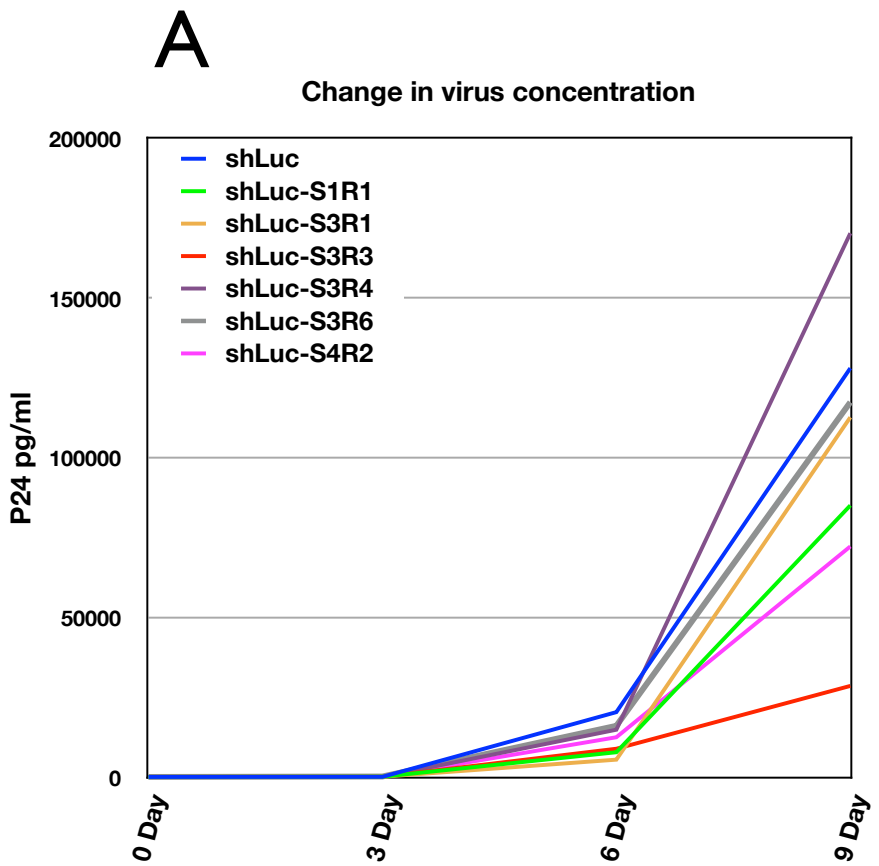
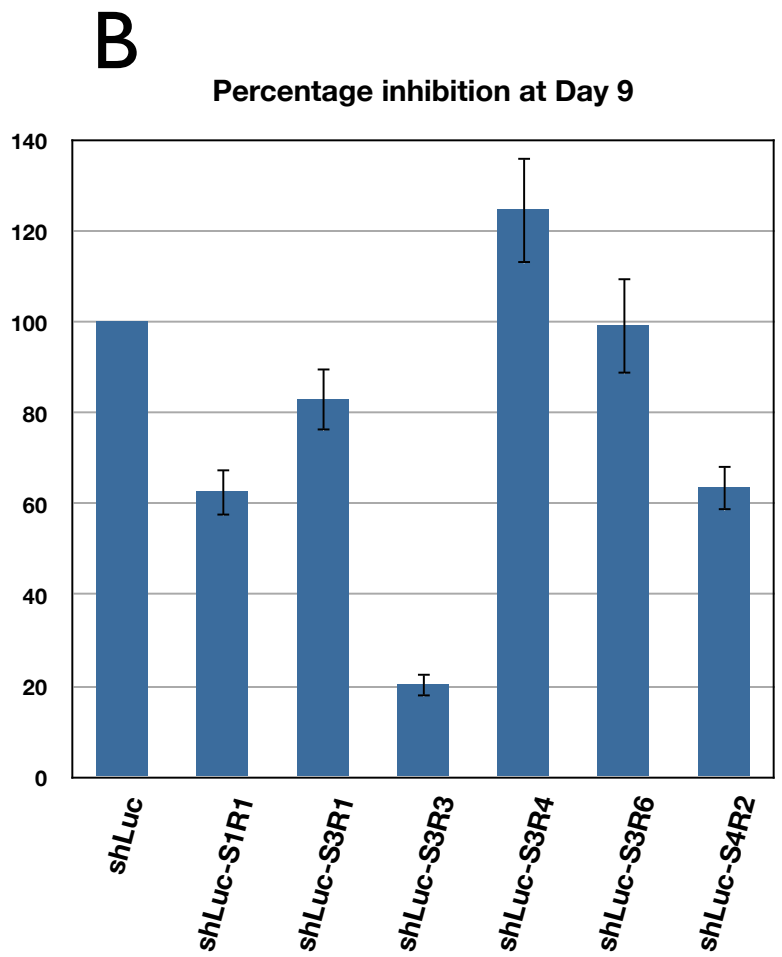
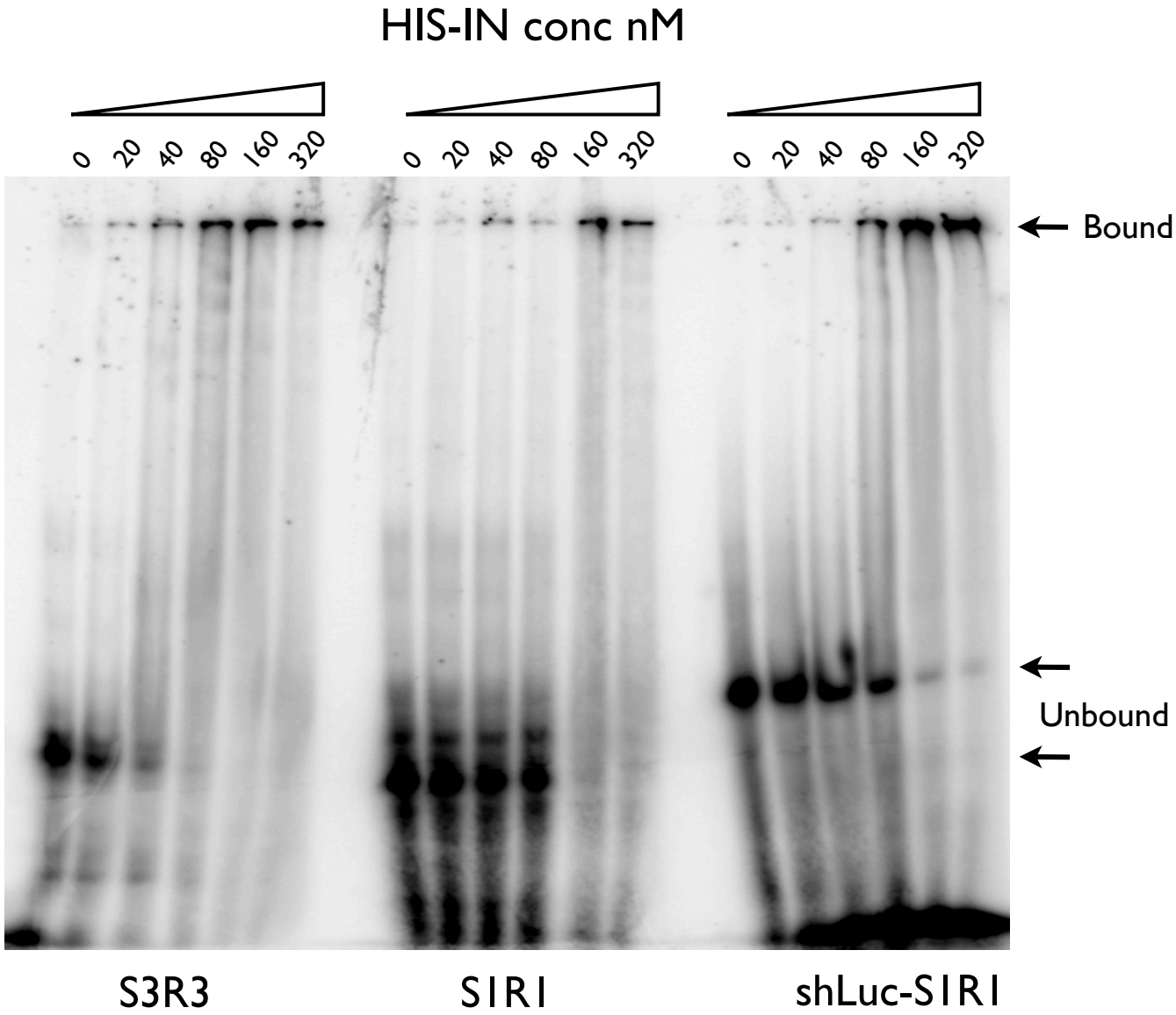


Supplemental Figure 4

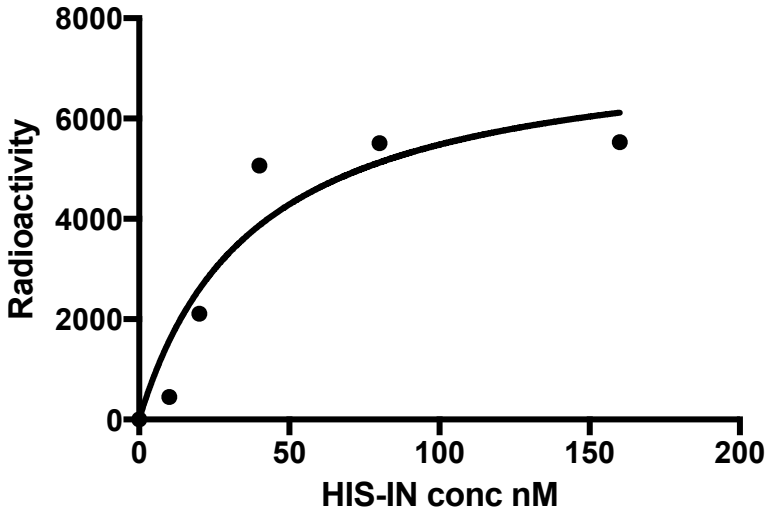




Supplemental Figure 5A



Supplemental Figure 5B



50% binding at 38.5nM

Suppl. Table1 Oligonucleotides used in study

	Oligo name	Oligo sequence
Aptamer library		TAATACGACTCACTATAGGGAGGACGATGCGGGCnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnGGTGGCGCGAGAGGTG
Forward primer T7 promter + library constant region		TAATACGACTCACTATAGGGAGGACGATGCGG
Library reverse primer		CACCTCTCGCGCCACC
For RNA expression		
shLuc	JRO450	CACCGCTTACGCTGAGTACTTCGAAATTGAAGCTTGTTTCGAAGTACTCAGCGTAAG
	JRO451	AAAACTTACGCTGAGTACTTCGAAACAAGCTTCAATTTCGAAGTACTCAGCGTAAGC
shS1	JRO535	caccGCGGAGACAGCGACGAAGAGCattgaagcttgtGCTCTTCGTCGCTGTCTCCGC
	JRO536	aaaaGCGGAGACAGCGACGAAGAGCacaagcttcaatGCTCTTCGTCGCTGTCTCCGC
S1R1	JRO500	caccGATGCGGGCCGTATGGGTGAGCCCGTTAAGATTGCGCGTGGTGGCGCGAG
	JRO501	aaaaCTCGCGCCACCACGCGCAATCTTAACGGGCTCACCCATACGGCCCGCATC
S3R1	JRO502	caccGCGGGCCCTAGACGCGCTGCCGTGGAGGAGGAGGTTGGTGGCGCGAGAGGTG
	JRO503	aaaaCACCTCTCGCGCCACCAACCTCCTCCTCCACGGCAGCGCGTCTAGGGCCCGC
S3R3	JRO504	caccGGGAGGACGATGCGGGCCGTCGTATGCTGCGCCATGGGGTGGACTGGGTGGCGCGA
	JRO505	aaaaTCGCGCCACCCAGTCCACCCCATGGCGCAGCATACGACGGCCCGCATCGTCCTCCC
shLuc-S1R1	JRO452	caccGCTTACGCTGAGTACTTCGAAAtATGCGGGCCGTATGGGTGAGCCCGTTAAGATTGCGCGTGGTGGCGCGAGAGgTTTCGAAGTACTCAGCGTAAG
	JRO453	aaaaCTTACGCTGAGTACTTCGAAAcCTCTCGCGCCACCACGCGCAATCTTAACGGGCTCACCCATACGGCCCGCATaTTTCGAAGTACTCAGCGTAAGC
shLuc-S3R1	JRO478	caccGCTTACGCTGAGTACTTCGAAtGCGGGCCCTAGACGCGCTGCCGTGGAGGAGGAGGTTGGTGGCGCGAGAGGTGgTTCGAAGTACTCAGCGTAAG
	JRO479	aaaaCTTACGCTGAGTACTTCGAAcCACCTCTCGCGCCACCAACCTCCTCCTCCACGGCAGCGCGTCTAGGGCCCGCaTT
shLuc-S3R3	JRO480	caccGCTTACGCTGAGTACTTCGAAacaaGGAGGACGATGCGGGCCGTCGTATGCTGCGCCATGGGGTGGACTGGGTGGCGCGAagTTCGAAGTACTCAGCGTAAG
	JRO481	aaaaCTTACGCTGAGTACTTCGAActTCGCGCCACCCAGTCCACCCCATGGCGCAGCATACGACGGCCCGCATCGTCCTCCttgtTTCGAAGTACTCAGCGTAAGC
shLuc-S3R4	JRO531	caccGCTTACGCTGAGTACTTCGAAGGAGGACGATGCGGGCTATCGCAGCTcTcGCGCCGATGGAGGAGGTGGTGGCGCGAGAGGTGTTCGAAGTACTCAGCGTAAG
	JRO532	aaaaCTTACGCTGAGTACTTCGAACACCTCTCGCGCCACCACCTCCTCCATCGGCGCgAgAGCTGCGATAGCCCGCATCGTCCTCCTTCGAAGTACTCAGCGTAAGC
shLuc-S4R6	JRO482	caccGCTTACGCTGAGTACTTCGAAtCGGGCGCCAATGGGGACCGTCCTATTTGGGATGTCGGTGGCGCGAAATgTTCGAAGTACTCAGCGTAAG

Suppl. Table1 Oligonucleotides used in study

	JRO483	aaaaCTTACGCTGAGTACTTCGAAcATTTCGCGCCACCGACATCCCAAATAGGACGGTCCCC ATTGGCGCCCGaTTCGAAGTACTCAGCGTAAGC
shLuc-S4R2	JRO484	CACCGCTTACGCTGAGTACTTCGAATGGAGGACGATGCGGGCACCTGGCCCCGAAAAATT TCGGGTTGAGCTGGTGGCGCGAGAGGTGGTTCGAAGTACTCAGCGTAAG
	JRO485	AAAACTTACGCTGAGTACTTCGAACCACCTCTCGCGCCACCAGCTCAACCCGAAATTTTTC GGGGCCAGGTGCCCGCATCGTCCTCCATTCGAAGTACTCAGCGTAAGC
shS1-S3R3	JRO529	caccGCGGAGACAGCGACGAAGAGCataaGGAGGACGATGCGGGCCGTCGTATGCTGCGC CATGGGGTGGACTGGGTGGCGCGAgagaGCTCTTCGTCGCTGTCTCCGC
	JRO530	aaaaGCGGAGACAGCGACGAAGAGCtctcTCGCGCCACCCAGTCCACCCCATGGCGCAGCA TACGACGGCCCGCATCGTCCTCCttatGCTCTTCGTCGCTGTCTCCGC
RT 70.15	JRO 566	caccAAAGACGCAGACCTACGAACCCAGGAGATAAGGGGGAAAACTCTGGAAAACCA
	JRO 567	aaaaTGGTTTTCCAGAGTTTTCCCCCTTATCTCCTGGGTTCGTAGGTCTGCGTCTTT
shLuc-70.15	JRO568	caccGCTTACGCTGAGTACTTCGAAAAAGACGCAGACCTACGAACCCAGGAGATAAGGGGG AAAACTCTGGAAAACCATT
	JRO569	CTTACGCTGAGTACTTCGAAtggttttccAGAGttttccCCCttatCTCCtGGGTtcgtaggtctgcgtcttt TTCGAAGTACTCAGCGTAAGC
For mobility assay		
T7-S1R1	JRO469	TAATACGACTCACTATAGGGAGGACGATGCGGGCCGTATGGGTGAGCCCGTTAAGATTGC GCGTGGTGGCGCGAGAGG
	JRO493	CCTCTCGCGCCACCACGCGCAATCTTAACGGGCTCACCCATACGGCccgcatcgtcctccctata gtgagtcgtatta
T7-shLuc-S1R1	JRO512	taatacgactcactataggGCTTACGCTGAGTACTTCGAAAtATGCGGGCCGTATGGGTGAGCCC GTTAAGATTGCGCGTGGTGGCGCGAGAGgTTTCGAAGTACTCAGCGTAAG
	JRO514	CTTACGCTGAGTACTTCGAAACCTCTCGCGCCACCACGCGCAATCTTAACGGGCTCACCCA TACGGCCCGCATATTTCGAAGTACTCAGCGTAAGCCCTATAGTGAGTCGTATTA
T7-S3R3	JRO527	TAATACGACTCACTATAGGgaggacgatgcgggccgtcgtatgctgcgccatggggtggactgggtggcgc gagagag
	JRO528	ctctctcgcgccacccagtccaccccatggcgcagcatacgacggcccgcatcgtcctcCCTATAGTGAGTCGT ATTA
Probes for northern		
Luciferse probe	JRO541	CTTACGCTGAGTACTTCGAAAT
S1R1 probe	JRO456	TCTTAACGGGCTCACCCATA