Supplementary information:

Methods:

Serum stability assay

Five micrograms of refolded aptamer-siRNA conjugates were incubated at 37°C in 100 μL RPMI 1640 Medium containing 50% or 10% (v/v) mouse serum (Sigma), resulting in a concentration of 50 ng/μL RNA. At times 0 min to 72 hours, 10 μL aliquots of the reaction were withdrawn and 10 μL of 2×loading buffer containing 8 M urea, 120 EDTA and 0.1% SDS were added to each sample. The mixtures were heated at 95°C for 5 min and then stored at -80°C until all incubations were completed. 20 μL of each mixture were analyzed by electrophoresis in an 8% denaturing polyacrylamide gel and the RNAs were visualized following ethidium bromide staining usinga a UV-transilluminator.

Cell culture conditions

HEK 293 cells and CEM cells were purchased from ATCC and cultured in DMEM and RPMI 1640 supplemented with 10% FBS. Cells were cultured in a humidified 5% CO_2 incubator at 37 °C.

HIV-1 challenge and p24 antigen assays

CEM cells were infected with HIV strains IIIB for 5 days (MOI 0.001). Prior to RNA treatments the infected cells were gently washed with PBS three times to remove free virus. Next 2×10^4 infected cells and 3×10^4 uninfected cells were incubated with RNAs at 400 nM final concentration in 96-well plates at 37°C. The culture supernatants

were collected at different times post treatment. In this study, the p24 antigen analyses were performed using a Coulter HIV-1 p24 Antigen Assay (Beckman Coulter) according to the manufacturer's instructions.

5'-RACE PCR assay

The experimental RNAs (*Tat/rev* 27-mer siRNA, ***Tat/rev*** **N-1** or **N-2**) and a HIV pNL4-3 luc were co-tranfected into HEK293 cells in presence of Lipofectamine 2000. HIV pNL4-3-luc has the firefly luciferase gene inserted in the *env* gene and is under the control of the Tat responsive HIV LTR. Total RNA was isolated from transfected 293 cells using Trizol as manufacture's protocol. Residual DNA was digested using the DNA-free kit per the manufacturer's instructions (Ambion). Subsequently, total RNAs (5 μg) were ligated to a GeneRacer adaptor (Invitrogen) without prior treatment. Ligated RNA was reverse transcribed using a gene specific primer 1 (GSP-Rev 1: 5'- CCA CTT GCC ACC CAT CTT ATA GCA -3') and SuperScript III reverse transcriptase according to the manufacturer's instructions (Invitrogen). To detect cleavage products, PCR and nested PCRs were performed using primers complementary to the RNA adaptor (5'-cDNA primer: 5'- GGA CAC TGA CAT GGA CTG AAG GAG TA -3') and gene-specific primers (GSP-Rev 2: 5'- CCC AGA AGT TCC ACA ATC CTC GTT -3'; GSP-Rev 3: 5'- TGG TAG CTG AAG AGG CAC AGG CTC -3'; GSP-Rev 4: 5'- CGC AGA TCG TCC CAG ATA AGT GCT AA -3').

Similarly, total RNA (5 μg) from HEK 293 cells transfected with 50 nM experimental RNAs (TNPO3 25/27-mer siRNA, **TNPO3 N-1** or **N-2**) was ligated to a GeneRacer adaptor (Invitrogen, Carlsbad, CA) without prior treatment. Ligated RNA was

reversed transcribed using a gene specific primer 1 (GSP-Rev 1: 5'- TCC CGT AAA GAG GCA TGA GAG TCT GT -3'). To detect cleavage products, PCR was performed using primers complementary to the RNA adaptor (5'-cDNA primer) and gene-specific primer 2 (GSP-Rev 2: 5'- CCG GAT CTG TAA CAA CTG GTC TGA GA -3'). Amplification products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. The specific PCR products were recovered using a QIAquick Gel purification Kit and then were cloned into TOPO TA cloning vector pCR 2.1-TOPO vector (Invitrogen). Individual clones were identified by DNA sequencing.

Mutation assay

PBMCs were obtained from treated mice at three, four and five weeks post-aptamer-cocktailed DsiRNAs conjugates injection and total RNAs were isolated with STAT-60 (TEL-TEST "B") according to the manufacturer's instructions. Residual DNA was removed using the DNA-free kit per the manufacturer's instructions (Ambion). cDNA was synthesized using 1~5 μg of total RNA. Reverse transcription was carried out with SuperScript III reverse transcriptase and an oligo$(dT)_{20}$ primer in a 20 μL reaction according to the manufacturer's instructions (Invitrogen). To detect viral RNAs (intron and exon domains), PCR and nested PCRs were preformed using gene-specific primers (NL4-3 primer S1-F: 5'- TAC AAT GAA TGG ACA CTA GAG -3'; NL4-3 primer S1-R: 5'- TTC TAG GTC TCG AGA TAC TG -3'; NL4-3 primer S2-F: 5'- AGG CGT TAC TCG ACA GAG GA -3'; NL4-3 primer S2-R: 5'- TGG CGA ATA GCT CTA TAA GC -3'; NL4-3 S3-F: 5'- ATG AGA GTG AAG GAG AAG TAT -3'; NL4-3 S3-R: 5'- AAG AGT AAG TCT CTC AAG CG -3'; NL4-3 S4-F: 5'- TCA GCA CTT GTG GAG ATG

GG -3’; NL4-3 S4-R: 5’- TGG TGA ATA TCC CTG CCT AA -3’). Amplification products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. The specific PCR products were recovered using a QIAquick Gel purification Kit. Illumina Deep Sequencing was carried out by the City of Hope DNA sequencing core and data analyses were performed by the City of Hope Bioinformatics Core facility as previously described.(29)

Supplementary information:

Figure Legends:

Figure S1: HIV-1 suppression by chemically synthesized aptamer (A-1-stick) and aptamer-siRNA conjugates. (**A**) Inhibition of HIV-1 replication by the Aptamer A-1, chemically synthesized aptamer (A-1-stick), naked siRNA and different aptamer-siRNA conjugates in CEM cells infected with HIV-IIIB. (**B, C**) Down regulation of *tat/rev* mRNA (B) and TNPO3 mRNA (C) mediated by the aptamer delivered siRNA in HIV-1 IIIB infected CEM cells. Error bars indicate SD (n = 3).

Figure S2: Chemically synthesized aptamer-siRNA conjugates are processed by Dicer and induce mRNA cleavage. (**A, C**) 5'-RACE PCR analysis. The mRNAs from HEK293 cells transfected with the aptamer-*tat/rev* siRNA conjugates (***Tat/rev* N-1** and **N-2**) (**A**) or aptamer- TNPO3 siRNA conjugates (**TNPO3 N-1** and **N-2**) (**C**) were ligated to an RNA adaptor and reverse transcribed using a gene-specific primer. Depicted is an agarose gel electorphoresis of the 5'-RACE-PCR amplification products using a primer specific to the RNA adaptor and a reverse primer to *Tat/rev* or TNPO3. Amplification products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. (**B**, **D**) The specific PCR products were recovered using a QIAquick Gel purification Kit and then were cloned into TOPO TA cloning vector pCR 2.1-TOPO vector (Invitrogen). Individual clones were identified by DNA sequencing and corresponding mRNA cleavage patterns were shown.

Figure S3: 2'-Fluoro modified aptamer-siRNA conjugate in mouse serum. RNA conjugates (Tat/rev N-1) were incubated in mouse serum for the lengths of time indicated and were resolved by 8% denaturing PAGE. Stability of the conjugates was evaluated following incubation in 50% or 10% mouse sera.

Table S1: HIV-1 viral loads in individual RAG-hu mice. Individual and average viral loads are indicated for the experiment described in **Figure 2**. Non-detectable samples were set to a value of 0. Mice from first treatment are depicted in **Figure 2**. **(A)** First-treatment monitoring of HIV-1 viral loads in individual RAG-hu mice. Uninfected mice (n = 2), non-treated mice (n = 8), naked cocktail dsiRNA treated mice (n = 5) and aptamer-cocktailed DsiRNA conjugates treated mice (n = 8) are indicated. **(B)** The re-treatment including twice weekly injections with aptamer-stick-cocktailed DsiRNA conjugates: The viral loads of uninfected mice (n = 2), non-treated mice (n = 4) and aptamer-cocktailed DsiRNA conjugates treated mice (n = 4) are indicated. (**C, D**) *P* values for both experiments were determined.

Table S2: CD4 T-cell levels in individual treated and control RAG-hu mice. CD4:CD3 T-cell ratios were determined by FACS analysis. Cell levels are relative to the baseline established prior to infection. Values are shown for individual mice. Experiment described in **Figure 2A**. **(A)** Uninfected mice (n = 2), non-treated mice (n = 8), naked cocktail dsiRNA treated mice (n = 5) and aptamer-cocktailed DsiRNA conjugates treated mice (n = 8) are indicated. **(B)** *P* values for the experiments were determined.

Figure S3: Sequence analysis of mutant virus from animals treated with aptamer-cocktailed DsiRNA conjugates. Viral RNAs were isolated from plasma of conjugates treated mice and sequence changes in *tat/rev* target region, gp120 and gp41 sequences in the conjugates treated mice were analyzed. Positions of mutations unique to the conjugates treated animals are listed. The gp120 domain is presented in pink whereas the gp41 is in blue. The mutations in the *tat/rev* target domain are marked in yellow.

Supplementary information