Supplementary information

Experimental procedure

Materials

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich, all restriction enzymes were obtained from New England BioLabs (NEB) and all cell culture products were purchased from GIBOC (Gibco BRL/Life Technologies, a division of Invitrogen). Sources for the other reagents were: DuraScribe T7 transcription Kit (EPICENTRE Biotechnologies); ThermoScript RT-PCR system (Invitrogen); Silencer siRNA Labeling Kit (Ambion); Hoechst 33342 (nuclear dye for live cells) (Molecular Probes, Invitrogen); M-MLV Reverse transcriptase and Random primers (Invitrogen); Bio-Spin 30 Columns (Bio-Rad); Lipofectamine 2000 (Invitrogen); Trans IT-TKO (Mirus). U373-Magi cells, U373-Magi-CCR5E cells, CEM-NKr cells, CEM-NKr-CCR5 cells, HIV-1_{JR-FL} and HIV-1_{BaL} virus were obtained from the AIDS Research and Reference Reagent Program (Howell, et al., 1985,Lyerly, et al., 1987,Vodicka, et al., 1997).

Primers, DNA library and siRNAs were purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). 51-mer ssDNA oligo library for RNA Library: 5'- GGG AGG ACG ATG CGG - N_{20}- CAG ACG ACT CGC CCG A - 3' (51 nt). Forward primer: 5'- TAA TAC GAC TCA CTA TAG GGA GGA CGA TGC GG -3' (32 mer); Reverse primer: 5'- TCG GGC GAG TCG TCT G -3' (16 mer). CCR5 siRNA sense: 5' P- CUC UGC UUC GGU GUC GAA A dTdT – 3'; Antisense: 5' P-UUU CGA CAC CGA AGC AGA G dTdT - 3'. TNPO3 DsiRNA sense: 5'- CGA CAU UGC AGC UCG UGU ACC AG dGdC -3'; Antisense: 5'- GCC UGG UAC ACG AGC UGC AAU GUC GUU -3'.

Generation of aptamer and aptamer-siRNA chimeras by in vitro transcription

Double-stranded DNA templates were directly generated by PCR, and the resulting PCR products were recovered using a QIAquick Gel purification Kit (Qiagen, Valencia, CA). Chimera sense strands were transcribed from its PCR-generated DNA templates using the DuraScription Kit (EPICENTRE Biotechnologies, Madison, WI) in accordance with the manufacturer's instruction. In the transcription reaction mixture, the canonical cytidine

triphosphate and uridine triphosphate were replaced with 2'-fluoro-cytidine triphosphate and 2'-fluoro-uridine triphosphate to produce RNA that is resistant to RNase A degradation. The reactions were incubated at 37 °C for 6 hours, and subsequently purified using Bio-Spin 30 Columns (Bio-Rad, Hercules, CA) after phenol extraction and ethanol precipitation. Fluorescent dye-labeled RNAs were generated using the Silencer siRNA labeling kit (Ambion, Austin, TX) in accordance with the manufacturer's instructions. The assembly of these chimeric constructs was described previously (Zhou, et al., 2009) and a schematic is presented in **Fig 3A, B**.

G-3 aptamer: 5'- GGG AGG ACG AUG CGG GCC UUC GUU UGU UUC GUC CAC AGA CGA CUC GCC CGA - 3'

G-3-TNPO3 OVH chimera sense strand: 5'- GGG AGG ACG AUG CGG GCC UUC GUU UGU UUC GUC CAC AGA CGA CUC GCC CGA *UU* CAA AGC CGA CAU UGC AGC UCG UGU ACC -3'; **Antisense:** 5'- UAC ACG AGC UGC AAU GUC GGC UUU G -3'

G-3-TNPO3 Blunt chimera sense strand: 5'- GGG AGG ACG AUG CGG GCC UUC GUU UGU UUC GUC CAC AGA CGA CUC GCC CGA *UUUUUUUU* CGA CAU UGC AGC UCG UGU ACC AGG C -3'; **Antisense:** 5'- GCC UGG UAC ACG AGC UGC AAU GUC GGC -3'

G-3-Scrambled siRNA chimera sense strand: 5'- GGG AGG ACG AUG CGG GCC UUC GUU UGU UUC GUC CAC AGA CGA CUC GCC CGA *UU* ACG UGA GAC GUU CGG UGA AUU -3'; **Antisense strand**: 5'- UUC ACC GAA CGU CUC ACG UdTdT -3'

Cell lines and cell culture

All cells were cultured in a humidified 5% CO_2 incubator at 37°C. U373-Magi cells and U373-Magi-CCR5E cells were obtained through the AIDS Research and Reference Reagent Program. Both cell lines were adherent cell lines and split 1:10 or 1:5 once per week upon reaching confluence by washing with DPBS and detaching cells using Cell stripper (Cellgro, Mediatech Inc) in order to minimize the damage to the cellular surface receptors. The cells were stained with Trypan Blue to detect the viability. Above 95% cells were live for seeding. Each cell line was carried for no more than 15 passages. U373-Magi cells were cultured in 90% DMEM supplemented with 10% fetal bovine serum, 0.2 mg/ml G418 and 0.1 mg/ml hygromycin B.

U373-Magi-CCR5E cells were cultured in 90% DMEM supplemented with 10% fetal bovine serum, 0.2 mg/ml G418, 0.1 mg/ml hygromycin B and 1.0 μg/ml puromycin.

CCRF-CEM cells were purchased from ATCC, and CEM-NKr cells and CEM-NKr-CCR5 cells were obtained through the AIDS Research and Reference Reagent Program. CEM cell lines were suspension cell lines and split 1:10 once per week upon reaching confluence. They were cultured in RPMI-1640 supplemented with 10% fetal bovine serum.

PBMCs: Peripheral blood mononuclear samples were obtained from healthy donors from the City of hope National Medical Center. PBMCs were isolated from whole blood by centrifugation through a Ficoll-Hypaque solution (Histopaque-1077, Sigma). CD8 cells (T-cytotoxic / suppressor cells) were depleted from the PBMCs by CD8 Dynabeads (Invitrogen, CA) according to the manufacturer's instructions. CD8+ T cell-depleted PBMCs were washed twice in PBS and resuspended in culture media (RPMI 1640 with 10% FBS, 1×PenStrep and 100 U/ml interleukin-2). Cells were cultured in a humidified 5% CO_2 incubator at 37°C. After cultured for 3 days in the activated T-cell culture medium containing interleukin-2, the cell-surface CCR5 level of PBMCs was detected by flow cytometry as described previously. The cells are ready for HIV-1 protection or HIV-1 challenge assay.

Detect the cell surface target protein (CCR5) expression by flow cytometry analysis

For cell-surface CCR5 protein staining, adherent cell lines (U373-Magi cells and U373-Magi-CCR5E cells) were washed with pre-warm PBS and detached with Cell stripper. Suspension cell lines (CEM-NKr cells, CEM-NKr-CCR5 cells, and PBMCs) were washed with pre-warmed PBS. After counting the cells' number, the desired number of cells was resuspended in 100 μL binding buffer containing DPBS (pH 7.0 ~7.4) and Ca^{2+} and Mg^{2+}, 1 mM $CaCl_2$, 2.7 mM KCl, 1.47 mM KH_2PO_4, 1 mM $MgCl_2$, 136.9 mM NaCl, 2.13 mM Na_2HPO_4, and added APC mouse Anti-human CD195 antibody (BD Pharmingen). For example, 1 μL antibody was enough for 2×10^5 cells in 100 μL reaction system. After incubation for 30 min at room temperature in the dark, cells were washed twice with 500 μL of washing buffer, finally resuspended in 350 μL of DPBS and processed immediately for flow cytometry (BD Fortessa, Flow cytometry core, City of Hope, CA).

Illumina high-throughput sequencing (HTS) and data analysis (please refer the Supplementary information: deep sequencing and data.xlsx).

After 9 rounds of SELEX, the RNA pools for selection rounds 5, 6, 7, 8 and 9 were chosen for Illumina high-throughput sequencing analysis. The sample preparation and sequencing processing were performed by City of Hope DNA sequencing core (City of Hope, CA, USA). Briefly, 1.0 μg of RNA pool was first reverse-transcribed using RT primer (5' -CAG ATT GAT GGT GCC TAC AGT CGG GCG UGT CGT CTG -3') then subjected to PCR amplification for 8 cycles, using the primers JH5 (5' -AAT GAT ACG GCG ACC ACC GAC AGG TTC AGA GTT CGA TCG GGA GGA CGA TGC GG -3') and RT/index primer (5'- CAG ATT GAT GGT GCC TAC AGT CGG GCG UGT CGT CTG -3') then followed by 6% TBE PAGE gel purification with size selection (for targeted smRNAs of 51 nt). The purified library was followed by second round of PCR amplification for 4 cycles with primers PE-mi-index primer (5'- CAA GCA GAA GAC GGC ATA CGA GAT NNNNNN CAG ATT GAT GGT GCC TAC AG -3') and R2 (5'- AAT GAT ACG GCG ACC ACC GA -3') then followed by 6% TBE PAGE gel purification with size selection (for targeted smRNAs of 51 nt). The purified library was followed by quantified using qPCR with a forward primer (5'- CAA GCA GAA GAC GGC ATA CG -3') and a reverse primer (5'- AAT GAT ACG GCG ACC ACC GA -3'). The quantified denatured miRNA library was loaded in 1 mL of hybridization buffer to a final DNA concentration of 10 pM then used for single read flow cell cluster generation and 40 cycle (40 nt) sequencing performed using the Illumina HiSeq2000.

Reads processing and data analysis were conducted by City of Hope Bioinformatics Core facility (Dr. Xiwei Wu, City of Hope, CA). In brief, the processing principles were as follows. Bases after Ns in each read were considered low quality and were removed. The 3'-fixed oligo and 3'-Solexa adapter were identified and trimmed from each reads. The reads with 20-base after processing were considered as usable reads and retained for further analysis. Unique reads in each sample were counted. The most frequent 1,000 unique sequences were identified in each sample. The most frequent 1,000 unique sequence in round 9 were obtained and matched to the other four samples (top 1,000 unique reads) and their frequencies were recorded. The consensus sequence of round 9 was used to compare to the reads in each round. For alignment and grouping

analysis, the top 40 sequences were divided into 6 groups according to their predicted secondary structures by MFold RNA and QuickFold RNA.

Monitor the progress of SELEX by quantitative real-time PCR (qRT-PCR)

After nine selection rounds, we have 10 RNA pools including the initial RNA pool. We therefore applied qRT-PCR methods to monitor SELEX progress. Twenty-four hours before experiment, U373-Magi negative cells and U373-Magi-CCR5 positive cells were seeded at equal density ($3*10^4$ cells per well) on 48-well plate with 250 µL complete culture medium. On the day of experiment, both cells were washed three times with 250 µL per-warmed washing buffer to remove dead cells and then incubated with 250 µL pre-warmed binding buffer supplemented with 100 µg/mL yeast tRNA at 37 °C for 15 min. After incubation, the buffer was removed and the refolded RNA pool (0.1 nmol RNA pool in 250 µL refolding buffer) supplemented with 1 nmol yeast tRNA was added to the U373-Magi negative cells or CCR5 positive cells for 15 min at 37 °C. Following incubation of the RNA pool, the cells were washed six times with 250 µL washing buffer to remove unbound RNA and cell-surface RNAs with weak binding. Cell-surface bound RNA with strong binding affinity and internalized RNA sequences were recovered by TRIzol (Invitrogen) extraction by following the manufacturer's instructions. The recovered RNA pool was reversed transcribed using the ThermoScript RT-PCR system (Invitrogen). The resulting cDNA was further analyzed by quantitative RT-PCR using 2 × iQ SyberGreen Mastermix (Bio-Rad) as described in the manufacturer's instructions and specific primer sets for the RNA pool at final concentrations of 400 nM. Primers were as follows: RNA pool Forward primer: 5'- TAA TAC GAC TCA CTA TAG GGA GGA CGA TGC GG -3' (32 mer); RNA pool Reverse primer: 5'- TCG GGC GAG TCG TCT G -3' (16 mer). GAPDH expression was used for normalization of the qPCR data. GAPDH forward primer: 5'-CAT TGA CCT CAA CTA CAT G-3'; GAPDH reverse primer: 5'-TCT CCA TGG TGG TGA AGA C-3'.

Cell-surface binding of experimental RNAs (Flow cytometry analysis)

Adherent cell lines (U373-Magi cells and U373-Magi-CCR5E cells) were washed with pre-warm PBS and detached with Cell stripper. Suspension cell lines (CEM-NKr cells, CEM-NKr-CCR5 cells, and PBMCs) were washed twice with pre-warmed PBS. After counting the cells' number, the desired number of cells was resuspended in 100 µL binding buffer containing Cy3-labeled

experimental RNA aptamers at different concentrations as shown. After incubation at room temperature for 30 min, cells were washed three times with 500 µL of pre-warmed binding buffer, and finally resuspended in 350 µL of DPBS for flow cytometry analysis (Flow cytometry core, City of Hope, CA). The dissociation constants were calculated using non-liner curve regression with a Graph Pad Prism 6.0.

Similarly, G-3-27-mer-TNPO3 OVH chimera was chosen for binding affinity test with PBMC-CD4+ cells, CEM-NKr-CCR5 cells, and CEM cells. The aptamer-sense strand and antisense strand of the chimera were labeled by Cy3 and Cy5 dye, respectively. And then they (200 nM) were annealed to form aptamer-siRNA chimera for flow cytometry analysis as described above.

Internalization and intercellular localization studies (Live-cell confocal microscopy analyses)

Adherent cell lines (U373-Magi cells and U373-Magi-CCR5E cells) were grown in 35-mm plate pre-treated with poly-lysine (Glass Bottom Dish, MatTek, Ashland, MA, USA) with seeding at 3×10^5 cells in complete cell culture medium to allow about 70-80% confluence in 24 hours. On the day of the experiments, cells were washed with 2 mL of pre-warmed PBS, and incubated with 1.5 mL of pre-warmed, fresh complete growth medium for 30 min at 37 °C. Cy3-labeled, refolded experimental RNAs at a 67 nM final concentration were added to media and incubated for live-cell confocal microscopy in a 5% CO_2 microscopy incubator at 37 °C. The images were collected every 20 min using a Zeiss LSM 510 Meta Inverted 2 photon confocal microscopy system under water immersion at 40× magnification (Confocal microscopy core, City of Hope, CA). After 5~6 h of incubation and imaging, the cells were stained by treatment with 0.15 mg/ml Hoechst 33342 (nuclear dye for live cells, Molecular Probes, Invitrogen, CA, USA) according to the manufacturer's instructions. The images were collected as described previously.

For suspension cell lines (CEM-NKr cells, CEM-NKr-CCR5 cells, and PBMCs), on the day of experiments, cells were washed twice with pre-warmed PBS and seed in the polylysine-coated 35-mm plate as described above with seeding at 1×10^6 cells in the pre-warmed RPMI-1640 medium supplemented with 10% FBS. Cells were incubated for 30-60 min in a humidified 5% CO_2 incubator at 37 °C for attaching on the dish surface. As described above, Cy3-labeled, refolded experimental RNAs at a 67 nM final concentration were added to media and images

were collected using a Zeiss LSM 510 Meta Inverted two proton confocal microscopy system (Confocal microscopy core, City of Hope, CA).

CCR5 knockdown experiment (qRT-PCR and flow cytometry assay)

The CCR5 siRNA (sense: 5' P- CUC UGC UUC GGU GUC GAA A dTdT – 3'; Antisense: 5' P- UUU CGA CAC CGA AGC AGA G dTdT - 3') has been demonstrated previously to knockdown CCR5 expression. The CCR5 siRNA and control non-silencing siRNA NC-1 (IDT, Iowa, USA) were transfected to U373-Magi-CCR5E cells using commercial Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

To assess silencing at the mRNA level, after 48 hours of transfection, total RNA was isolated with STAT-60 (TEL-TEST, Friendswood, TX, USA) according to the manufacturer's instructions. Residual DNA was digested using the DNA-free kit per the manufacturer's instructions (Ambion, CA, USA). cDNA was produced using 2 µg of total RNA Moloney murine leukaemia virus reverse transcriptase (M-MLV RT) and random primers in a 15-µl reaction according to the manufacturer's instructions (Invitrogen, CA, USA). Expression of the CCR5 coding RNAs was analyzed by quantitative RT–PCR using 2× iQ SyberGreen Mastermix (BIO-RAD) and specific primer sets at a final concentration of 400 nM. Primers were as follows: CCR5 forward primer: 5'-AAC ATG CTG GTC ATC CTC AT-3'; CCR5 reverse primer: 5'-AAT AGA GCC CTG TCA AGA GT-3'. GAPDH expression was used for normalization of the qPCR data.

To assess silencing at the protein level, after 48 hours of transfection, cells were washed with 500 µL pre-warmed PBS and detached with Cell stripper. The cell-surface CCR5 protein staining was performed with APC-CD195 antibody as described above. Cells were immediately analyzed by flow cytometry (Flow cytometry core, City of Hope, CA).

IC_{50} value test by HIV-1 protection assay

Human PBMCs were freshly isolated from healthy donors and CD8 cells were depleted as described above. After cultured for 3 days in the activated T-cell culture medium containing interleukin-2, the cell-surface CCR5 level of PBMCs was detected by flow cytometry as described previously. HIV-1 protection assay was performed in 24-well tissue culture plates.

Duplicate nine-point dilution series of experimental RNAs were prepared and refolded in refolding buffer. PBMCs were washed once with pre-warmed PBS, and 4×10^5 PBMCs were seeded to each well of assay plates. Subsequently, experimental RNAs with different concentrations were added. Plates were incubated for 4-6 hours at 37 °C in a humidified 5% CO_2 incubator. Various viruses (R5 strains: JR-FL and Bal, MOI=0.01; or X4 strains: IIIB and NL4-3, MOI=0.001) were added into each well. After 24 hours incubation, the cells were gently washed with pre-warmed PBS to eliminate free viruses and were incubated subsequently at 37 °C in a humidified 5% CO_2 incubator.

The culture supernatants were collected at different time points after infection (3, 5 and 7 days). The HIV-1 p24 antigen analyses were performed using a Coulter HIV-1 p24 antigen assay (Beckman Coulter, Fullerton, CA) in accordance with the manufacturer's instructions. The percentage inhibition of HIV-1 infection for each concentration of experimental RNAs was calculated to determine the anti-HIV-1 IC_{50} value (50% inhibitory concentration) using non-liner curve regression with a Graph Pad Prism 6.0.

Establishment of humanized NSG mice

Establishment of hematopoietic stem/progenitor cell isolation: Human CD34+ HSPCs were isolated from either fetal liver or umbilical cord blood obtained from Novogenix Laboratories following regulatory guidelines approved by the Institutional Review Board at City of Hope, or as waste cord blood material from StemCyte Corp. The fetal liver tissue undergoes collagenase digestion followed by filtration through sterile 70 µm nylon mesh filter. Immunomagnetic enrichment for CD34+ cells was performed using the magnetic-activated cell sorting (MACS) system (Miltenyi Biotec), per the manufacturer's instructions, with the modification that the initial purified CD34+ population was put through a second column and washed three times with 3 ml of the supplied buffer per wash before the final elution. This additional step gave a > 99% pure CD34+ population, as measured by FACS analysis using the anti-CD34 antibody.

NOD.Cg-Prkdc scid Il2rg tm1Wj/SzJ (NOD/ SCID/IL2rγ^{null}, NSG) mice were obtained from Jackson Laboratories. A modified intrahepatic injection technique was used for engraftment of the neonatal pups within 48 hours of birth. We used a custom made Hamilton 80508 syringe/needle set-up for the injections. The needle specifications are 30 gauges, 51 mm long

needle with a beveled edge attached to a 50 µl glass syringe. The maximum volume used for injection with this needle/ syringe was 25 µl. Animals were pre-irradiated with 100 cGy and then transplanted with 0.5-1 × 10^6 $CD34^+/CD90^+$ HSC each. 12-14 weeks after transplantation, blood was collected and the engraftment was verified using multi parameter flow cytometry analysis.

Mouse blood and tissue collection: Peripheral blood samples were collected approximately at 10-12 weeks of age, using retro-orbital sampling. The red blood cells were lysed using Red Cell Lysis buffer solution (Sigma Aldrich) and cells were washed with PBS. This was followed by blocking in FBS and further staining for FACS analyses. Tissue samples were collected at necropsy and processed immediately for cell isolation and FACS analysis. Tissue samples were manually agitated in PBS before filtering through a sterile 70 µm nylon mesh screen (Fisher Scientific) and suspension cell preparations produced as previously described (Holt, et al., 2010). Intestinal samples were processed as per published protocol, with the modification that the mononuclear cell population was isolated after incubation in citrate buffer and collagenase enzyme for 2 hours, followed by nylon mesh filtration and ficoll-hypaque gradient isolation (GE Healthcare).

Analysis of human cells in mouse peripheral blood/tissues: FACS analysis of human cells was performed using a FACS Calibur instrument (BD Biosciences) with either BD Fortessa (BD Biosciences) or FlowJo software version 8.8.6. The gating strategy performed was an initial forward scatter versus side scatter (FSC/SSC) gate to exclude debris, followed by a human CD45 gate. For analysis of lymphocyte populations in peripheral blood, a further lymphoid gate (low side scatter) was also applied to exclude cells of monocytic origin. All antibodies used were fluorochrome conjugated and human specific, and obtained from BD Biosciences: CD45 (clone 2D1), CD19 (clone HIB19), CD14 (clone MϕP9), CD3 (clone SK7), CD4 (clone SK3), CD8 (clone HIT8a) and CCR5 (2D7). Gates were set using fluorescence minus one controls, where cells were stained with all antibodies except the one of interest. Specificity was also confirmed using isotype-matched nonspecific antibodies (BD Biosciences) and with tissues from animals that had not been engrafted with human cells.

***Ex vivo* HIV-1 protection assay**

Human $CD4^+$ T cells from spleens of humanized mice were sorted (≥98% pure) using an ARIA sorter (BD Bioscience, flow cytometry core, City of Hope, CA) according to the $CD45^+CD3^+CD4^+CD8^-$ phenotype. The cell-surface CCR5 expression was detected by flow cytometry as described previously. The human $CD4^+$ T cells from humanized mice were subsequently stimulated for 2-3 days with PHA (4 mg/ml) in RPMI-1640 culture medium supplemented with IL-2 (100 U/ml) and 10% FBS. HIV-1 protection assay was performed in 48-well tissue culture plates as described above. The cells were pre-treated with experimental RNAs with different concentrations for 4-6 hours and then challenged with various virus (R5 strains: Bal or JR-FL, MOI= 0.01 and X4 strain: NL4-3, MOI=0.001). At different time points after infection, the culture supernatants were collected for HIV-1 p24 ELISA assay as described above.

TNPO3 knockdown experiment (qRT-PCR)

Adherent cell lines (U373-Magi cells and U373-Magi-CCR5E cells) were grown in 35-mm plate pre-treated with poly-lysine (Glass Bottom Dish, MatTek, Ashland, MA, USA) with seeding at 3×10^5 cells in complete cell culture medium to allow about 70-80% confluence in 24 hours. On the day of the experiments,

On the day of the experiments (Day 1), a total of 2×10^5 cells (CEM-NKr-CCR5, CEM-NKr negative cells, Primary PBMCs) were seeded in complete cell culture medium in 24-well plate. G-3 aptamer and chimeras at 400 nM of final concentrations were directly incubated with the cells at day 1 and day 2. In parallel, 50 nM of experimental RNAs were transfected at day 1 by a commercial transfection agent (Trans IT-TKO) according to the manufacturer's instructions. As control, irrelative aptamer-siRNA chimera (anti-gp120 A-1 aptamer) and G-3 aptamer-scrambled siRNA chimera were used. After 48 hours of incubation (at day 3), total RNA was isolated with STAT60 (TEL-TEST, Friendswood, TX, USA) according to the manufacturer's instructions. Residual DNA was digested using the DNA-free kit per the manufacturer's instructions (Ambion, CA, USA). cDNA was produced using 2 µg of total RNA. Reverse transcription was carried out using Moloney murine leukaemia virus reverse transcriptase (MMLV-RT) and random primers in a 15 µL reaction according to the manufacturer's instructions (Invitrogen, CA, USA). Expression of the TNPO3 coding RNAs was analyzed by quantitative RT-PCR using 2× iQ SyberGreen Mastermix (BIO-RAD) and specific primer sets at a final concentration of 400 nM. *Gapdh* expression was used for normalization of the qPCR data. Primers were as follows:

TNPO3 Forward primer: 5’ - CCT GGA AGG GAT GTG TGC -3’; TNPO3 Reverse primer: 5’- AAA AAG GCA AAG AAG TCA CAT CA -3’. Experiments were performed in triplicate.

5’-RACE PCR assay to detect *in vivo* RNAi mediated target mRNA cleavage

Total RNA was isolated from CEM-NKr-CCR5 cells treated with different experimental chimeras as described above. Residual DNA was digested using the DNA-free kit per the manufacturer’s instructions (Ambion). Subsequently, total RNAs (10 μg) were ligated to a GeneRacer adaptor (Invitrogen, 5’- CGA CUG GAG CAC GAG GAC ACU GAC AUG GAC UGA AGG AGU AGA AA -3’) without prior treatment. Ligated RNA was reverse transcribed using a gene specific primer 1 (TNPO3 GSP-Rev-1: 5’- CAG GTA ACA CTG TAA GGA TCT CCA GC -3’) To detect cleavage products, nested PCR was 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 primer 2 and 3 (TNPO3 GSP-Rev-2: 5’- TAA AGA GGC ATG AGA GTC TGT GGG GA -3’; and TNPO3 GSP-Rev-3: 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®4-TOPO vector (Invitrogen). Individual clones were identified by DNA sequencing.

Interferon assays by qRT-PCR

As described previously, total RNA was isolated from PBMCs treated with experimental RNAs (400 nM) using STAT-60. Expression of mRNAs encoding p56 (CDKL2) and OAS1 were analyzed by quantitative RT-PCR using 2X iQ SyberGreen Mastermix (BIO-RAD) as described above and specific primer sets for these genes at final concentrations of 400 nM. Primers were as follows: P56 (CDKL2) forward, 5’- TCA AGT ATG GCA AGG CTG TG -3’; P56 (CDKL2) reverse, 5’- GAG GCT CTG CTT CTG CAT CT -3’; OAS1 forward, 5’ - ACC GTC TTG GAA CTG GTC AC -3’; OAS1 reverse, 5’- ATG TTC CTT GTT GGG TCA GC -3’; *gapdh* expression was used for normalization of the qPCR data. INF-α was used as a positive control.

Statistical analysis

Unless otherwise noted, error bars in all the figures, both in the main part of the paper and in the supplementary information, represent standard deviation (SD).

Table S1 is related to Figure 1A and experimental procedures: Live cell-based SELEX.

Table S1: The selection condition. The numbers of cells, plate size, medium volume, the amount of RNA pool and tRNA, washing condition, and incubation time are indicated here.

Figure S1 is related to Figure 1A and experimental procedures: Live cell-based SELEX.

Figure S1: Cell surface expression of CCR5 on U373-Magi-CCR5E cells and U373-Magi cells was measured by Flow cytometry using non-permeabilized cells stained with CCR5 antibody conjugated to APC (APC anti-human CD195).

Figure S2 is related to Table 1 and Figure 1C, D.

Figure S2: Bioinformatics analysis of high throughput sequence data from selection rounds. A) Distribution of frequencies of top 1000 unique sequences at each round. The most frequent 1,000 unique sequences were identified at each selection round. From Round 8, a enrichment saturation was observed. B) Sequence logo for each select round. Bioinformatics analysis of RNA aptamers to identify related sequence and structure families. Through the alignment of primary sequences, the distributions within each round were identified at the 20-nt random domain. From Round 7, highly represented sequences were observed. C) Representative sequences for each group were used to generated predicted secondary structures using Mfold algorithm. RNA mFold (online: http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form). The structures with the lowest energy was indicated here. Group 2, 4 and 5 (G-2, G-4 and G-5 aptames) shared a conserved sequence, which is comprised of 10 nucleotides UUCGUCUG(U/G)G. They also shared a common secondary structural motif (red line).

Figure S3 is related to Figure 2.

Figure S3: Cell surface binding of Cy3-labeled RNAs was assessed by flow cytometry. The 0-RNA pool was used as negative control. Cy3-labeled RNAs were tested for binding to **A)** U373-Magi-CCR5E positive cells and **B)** U373-Magi negative cells. The selected aptamers showed cell-type specific binding affinity. APC-CD195 antibody was used to stain cellular surface CCR5. **C)** One of the best RNA aptamer, G-3, was chosen for further binding affinity test with PBMC-CD4+ cells, CEM-NKr-CCR5 positive cells, CEM negative cells. The G-3 showed cell-type specific binding affinity. **D)** G-3 aptamer was chosen for further binding

affinity test with PBMC-CD4+ cells from different donors. **E, F)** CCR5 siRNA treatment knocked down CCR5 expression. CCR5 siRNA was transfected into U373-Magi-CCR5 positive cells. After 48 hours post-transfection, CCR5 expression was assessed by **E)** qRT-PCR (mRNA level) and **F)** flow cytometry (cell surface CCR5 level). A scrambled siRNA (NC1) was used as negative control. Data represent the average of three replicates. **G)** Knockdown of CCR5 reduced binding affinity of aptamers. CCR5 siRNA was transfected into U373-Magi-CCR5 positive cells. After 48 hours post-transfection, cell surface binding of Cy3-labeled G-3 aptamer or APC-CD195 antibody was assessed by flow cytometry. A scrambled siRNA (NC1) was used as negative control. **H)** Internalization analysis and localization analysis. PBMC-CD4+ cells were grown in 35 mm plates treated with polylysine and incubated with a 67 nM concentration of Cy3-labeled G-3 aptamer in complete culture media for real-time live-cell confocal microscopy analysis. The images were collected using 40×magnification. Z-stack images (from bottom to top of the cells) were shown here.

Figure S4 is related to Table 2.

Figure S4: HIV-1 protection assay. A-D) G-3 aptamers with different concentrations were incubated with primary PBMCs. After 4-6 hours incubation, various viruses (R5 strains: **A)** JR-FL, **B)** Bal; or X4 strains: **C)** IIIB, **D)** NL4-3) were added into each well. The culture supernatants were collected at five days after treatment for HIV-1 p24 antigen ELISA assay. **E-I)** G-3 aptamers with different concentrations were incubated with in vivo generated human $CD4^+$ T-cells. Mature human $CD4^+$ T-cells were isolated from humanized mice. At sacrifice, splenocytes were harvested and human $CD4^+$ T-cells were collected by cell sorting. The CD45 and CCR5 expression of **E**) *in vivo* isolated cells and **F**) human PBMCs was detected by flow cytometry. The sorted $CD4^+$ T-cells were pre-treated with CCR5 aptamer and subsequently were challenged *ex vivo* by R5 stains: **G)** HIV-1 JR-FL, **H)** Bal virus or **I)** NL4-3 virus as described previously. The culture supernatants were collected at five days after treatment for HIV-1 p24 antigen ELISA assay.

Figure S5 is related to Figure 3C, D and E.

Figure S5: Cell surface binding of fluorescent dye –labeled RNAs was assessed by flow cytometry. The Cy3-labeled 0-RNA pool and Cy5-labeld siRNA were used as negative control.

G-3-27-mer-TNPO3 OVH chimera was chosen for binding affinity test with PBMC-CD4+ cells, CEM-NKr-CCR5 positive cells, E) CEM negative cells. The aptamer-sense strand and antisense strand of the chimera were labeled by Cy3 and Cy5 dye, respectively. And then they were annealed to form aptamer-siRNA chimera. G-3 aptamer (non-labeled or Cy3-labeled) could deliver ~70%-80% Cy5 labeled siRNA portion into CCR5 expressing PBMCs and CEM-NKr-CCR5 cells.

Figure S6 is related to Table 2 and Figure 5C.

Figure S6: HIV-1 challenge assay. Primary PBMCs were infected with HIV-1 JR-FL virus (MOI 0.01). After 5 days post-infection, aptamers or aptamer-siRNA chimeras were incubated with HIV-1 infected cells. The culture supernatants were collected at different days after treatment for HIV-1 p24 antigen ELISA assay. The inhibitory activity was calculated by the formula [(p24 value of the cell alone - p24 value of the sample) / p24 value of the cell alone)]. 1 of the inhibitory activity means completed inhibition. A gp120 aptamer (A-1-stick) and an unrelated aptamer (R-1-stick) were used as positive and negative controls, respectively. Data represent the average of triplicate measurements.

References

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Vodicka, M.A., Goh, W.C., Wu, L.I., Rogel, M.E., Bartz, S.R., Schweickart, V.L., Raport, C.J., and Emerman, M. (1997). Indicator cell lines for detection of primary strains of human and simian immunodeficiency viruses. Virology 233, 193-198.

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Table S1

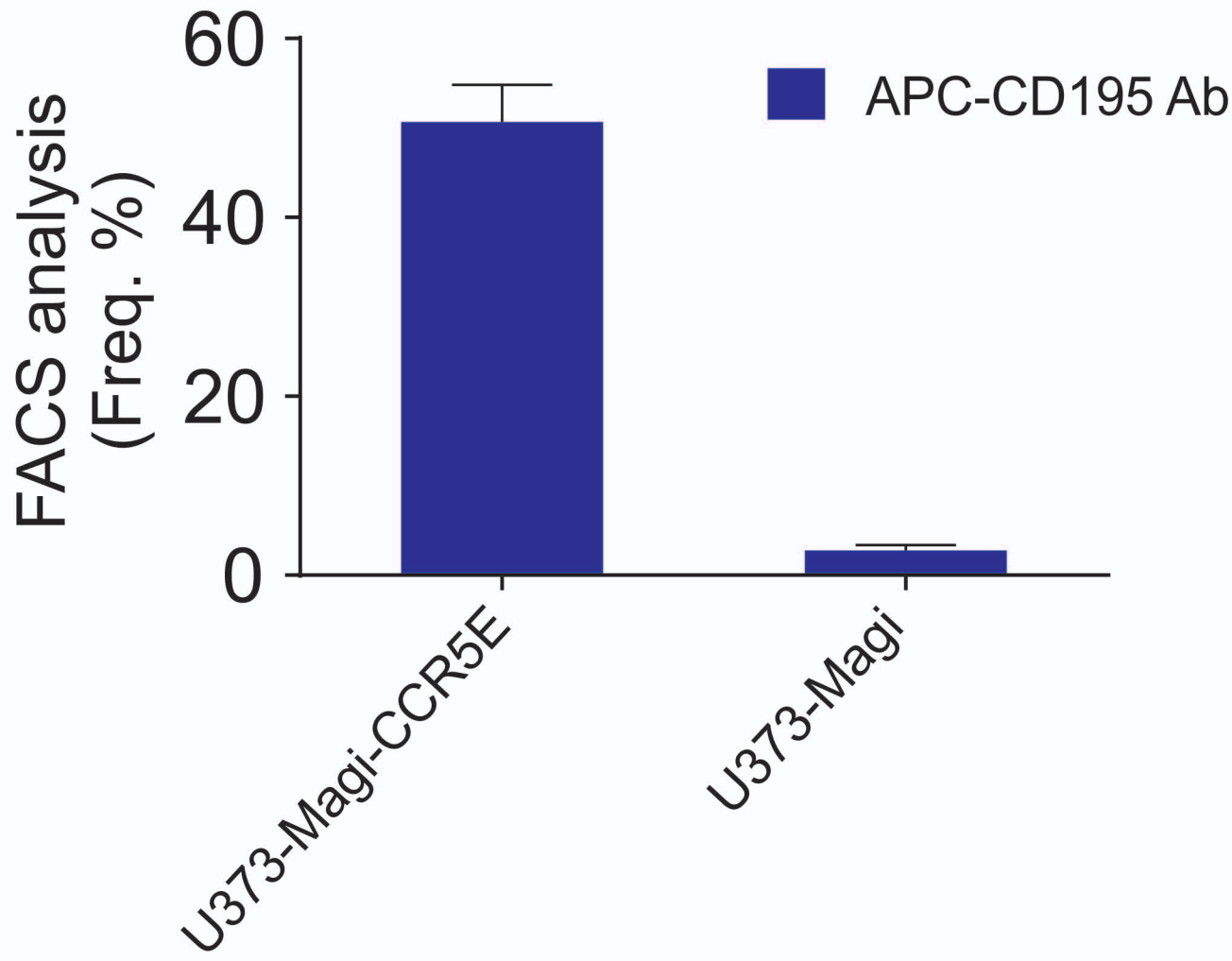
SELEX rounds	Positive cells (plate size and medium volume)	Negative cells (plate size and medium volume)	RNA pool (incubation time)	RNA work Con.	Competitor tRNA	Washing
1	$3*10^6$ cells (15 cm, 15 mL)	$3*10^6$ cells (15 cm, 15 mL)	4 nmol (30 min)	333 nM	0	2 × 12 mL
2	$3*10^6$ cells (15 cm, 15 mL)	$3*10^6$ cells (15 cm, 15 mL)	4 nmol (25 min)	333 nM	2.5 nmol	3 × 12 mL
3	$1.5*10^6$ cells (10 cm, 12 mL)	$3*10^6$ cells (10 cm, 12 mL)	2.5 nmol (25 min)	208 nM	5 nmol	4 × 12 mL
4	$1.5*10^6$ cells (10 cm, 12 mL)	$3*10^6$ cells (10 cm, 12 mL)	2.5 nmol (20 min)	208 nM	15 nmol	5 × 12 mL
5	$7.5*10^5$ cells (6 cm, 8 mL)	$2.25*10^6$ cells (10 cm, 8 mL)	1.5 nmol (20 min)	188 nM	15 nmol	6 × 8 mL
6	$7.5*10^5$ cells (6 cm, 8 mL)	$2.25*10^6$ cells (10 cm, 8 mL)	1.5 nmol (15 min)	188 nM	20 nmol	6 × 8 mL
7	$3*10^5$ cells (3.5 cm, 5 mL)	$1.5*10^6$ cells (6 cm, 5 mL)	0.8 nmol (15 min)	160 nM	20 nmol	7 × 5 mL
8	$3*10^5$ cells (3.5 cm, 5 mL)	$1.5*10^6$ cells (6 cm, 5 mL)	0.8 nmol (10 min)	160 nM	40 nmol	8 × 5 mL
9	$1.5*10^5$ cells (3.5 cm, 5 mL)	$1.2*10^6$ cells (6 cm, 5 mL)	0.5 nmol (10 min)	100 nM	40 nmol	9 × 5 mL

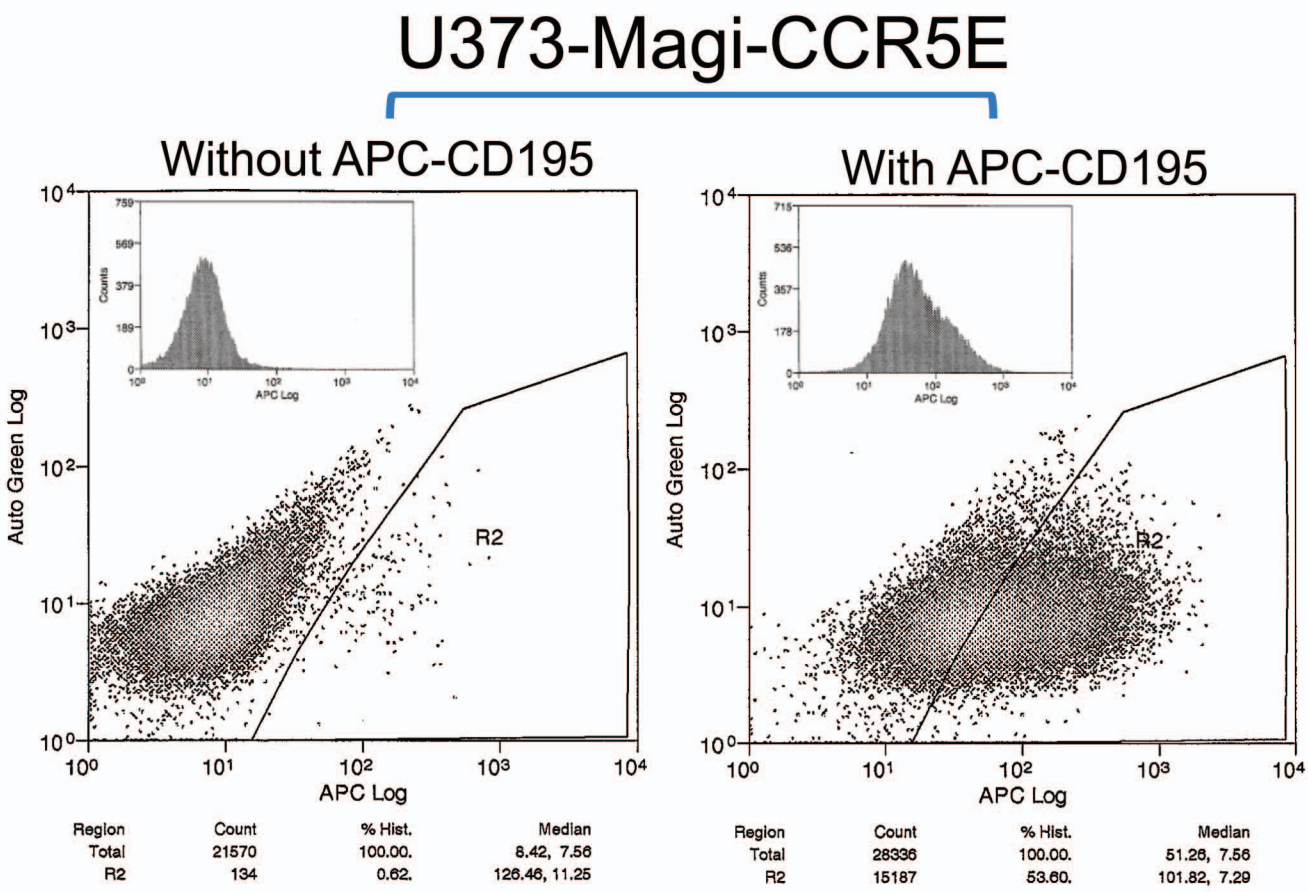
Note:

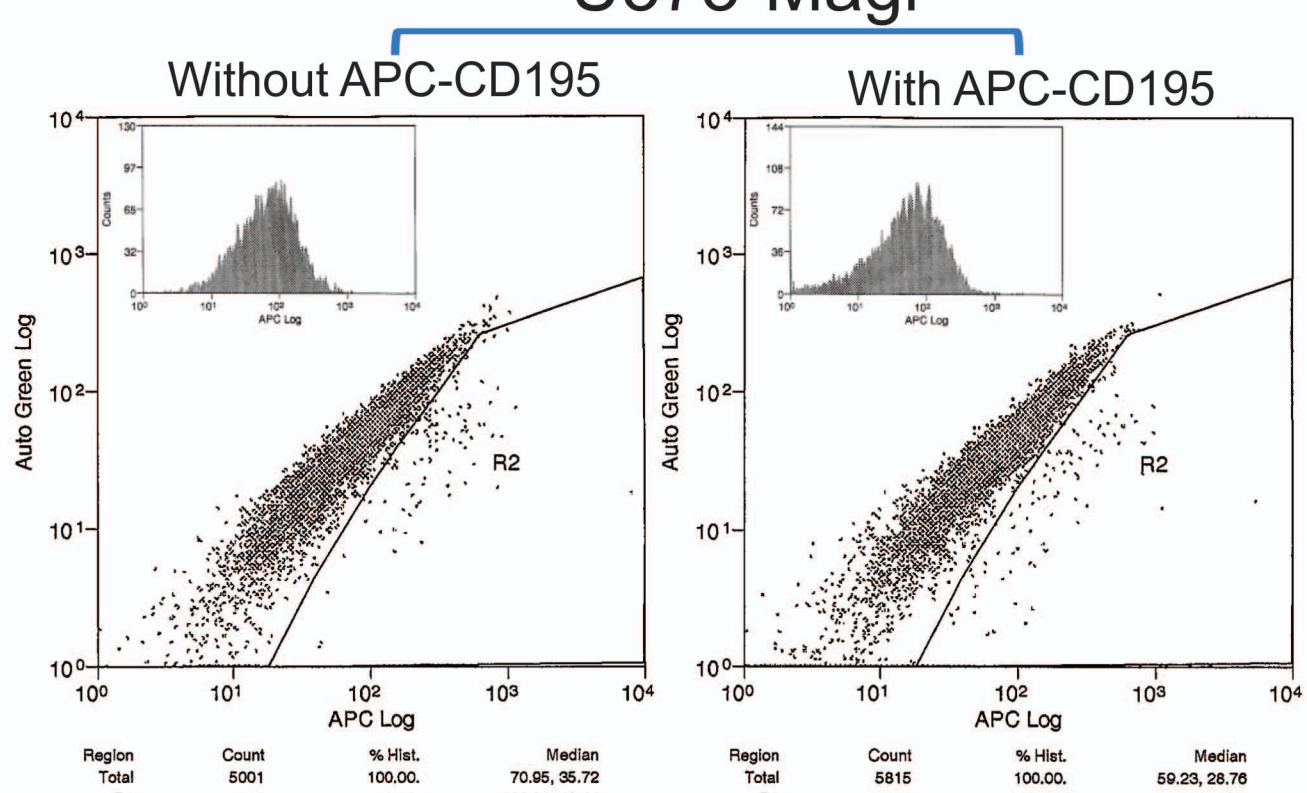
(1) To avoid nonspecifically interaction between nucleic acids and the cell surface, the tRNA (100 μg/mL) as a competitor was first incubated with non-targeted cells or targeted cells at 37 °C for 25 min and then ready for selection step.

(2) Counter-selection: RNA pool was incubated with U373-Magi negative cells at 37 °C for 30 min.

FigS1

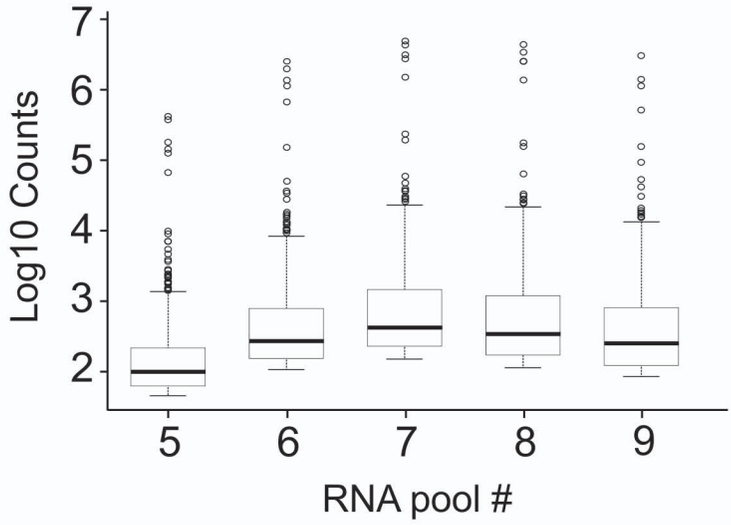

FACS analysis
(Freq. %)
60
40
20
0
APC-CD195 Ab
U373-Magi-CCR5E
U373-Magi


U373-Magi-CCR5E
Without APC-CD195
With APC-CD195
Auto Green Log
APC Log
R2
Counts
Region Count % Hist. Median
Total 21570 100.00. 8.42, 7.56
R2 134 0.62. 126.46, 11.25
Region Count % Hist. Median
Total 28336 100.00. 51.26, 7.56
R2 15187 53.60. 101.82, 7.29

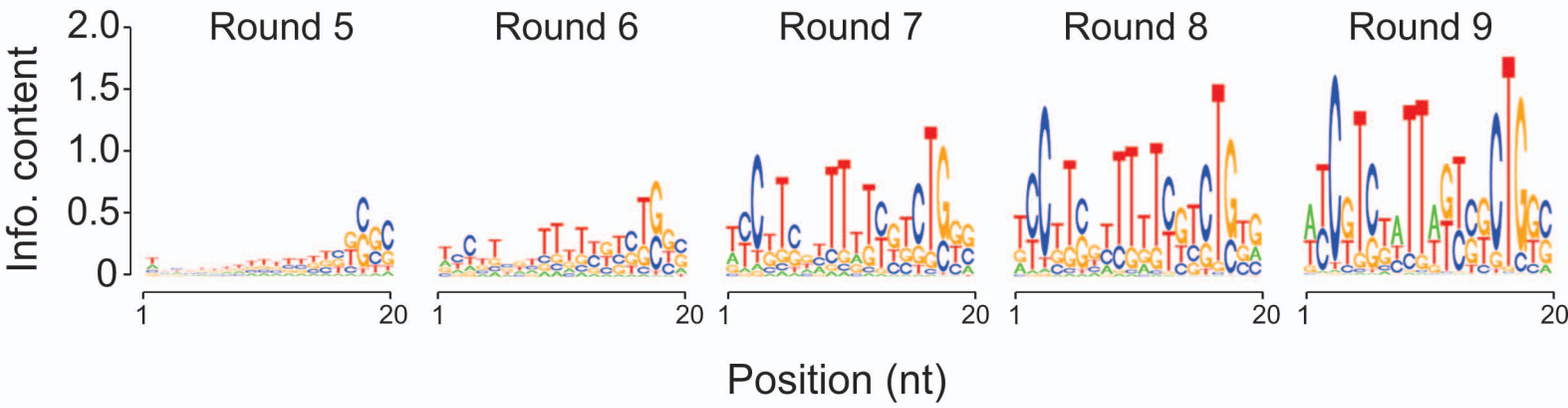

U373-Magi
Without APC-CD195
With APC-CD195
Auto Green Log
APC Log
R2
Counts
Region Count % Hist. Median
Total 5001 100.00. 70.95, 35.72
R2 115 2.30. 300.91, 49.44
Region Count % Hist. Median
Total 5815 100.00. 59.23, 28.76
R2 186 3.20. 85.00, 5.08

FigS2

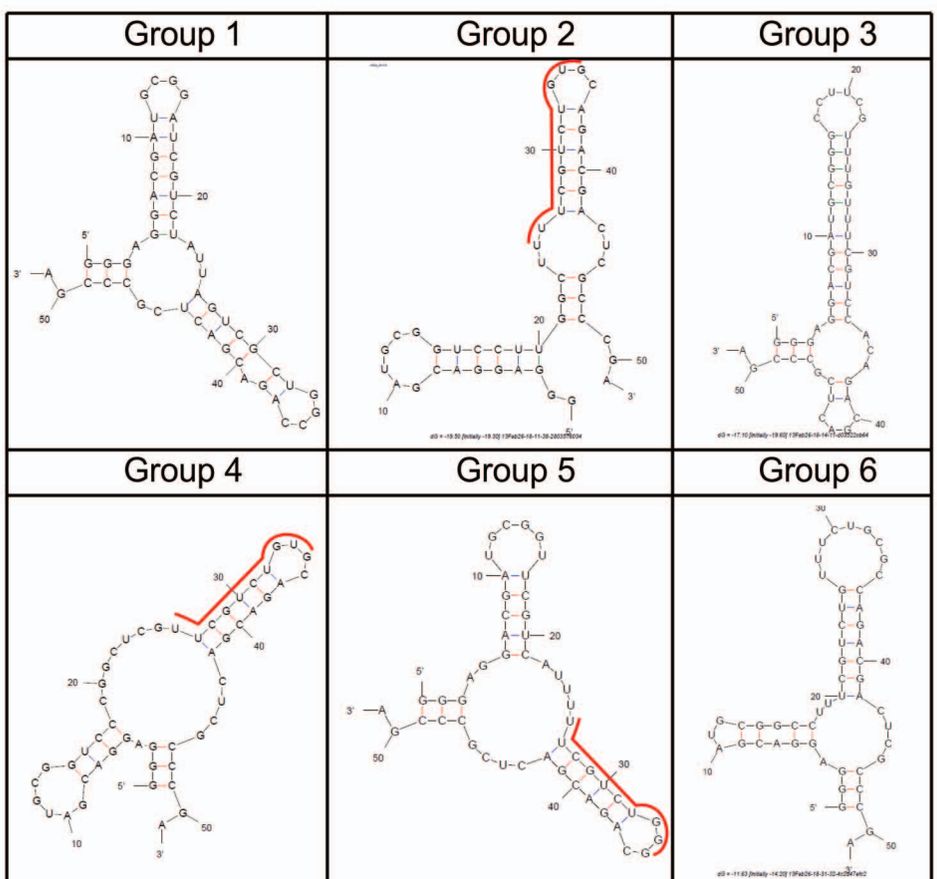
A


Log10 Counts
7
6
5
4
3
2
5
6
7
8
9
RNA pool #

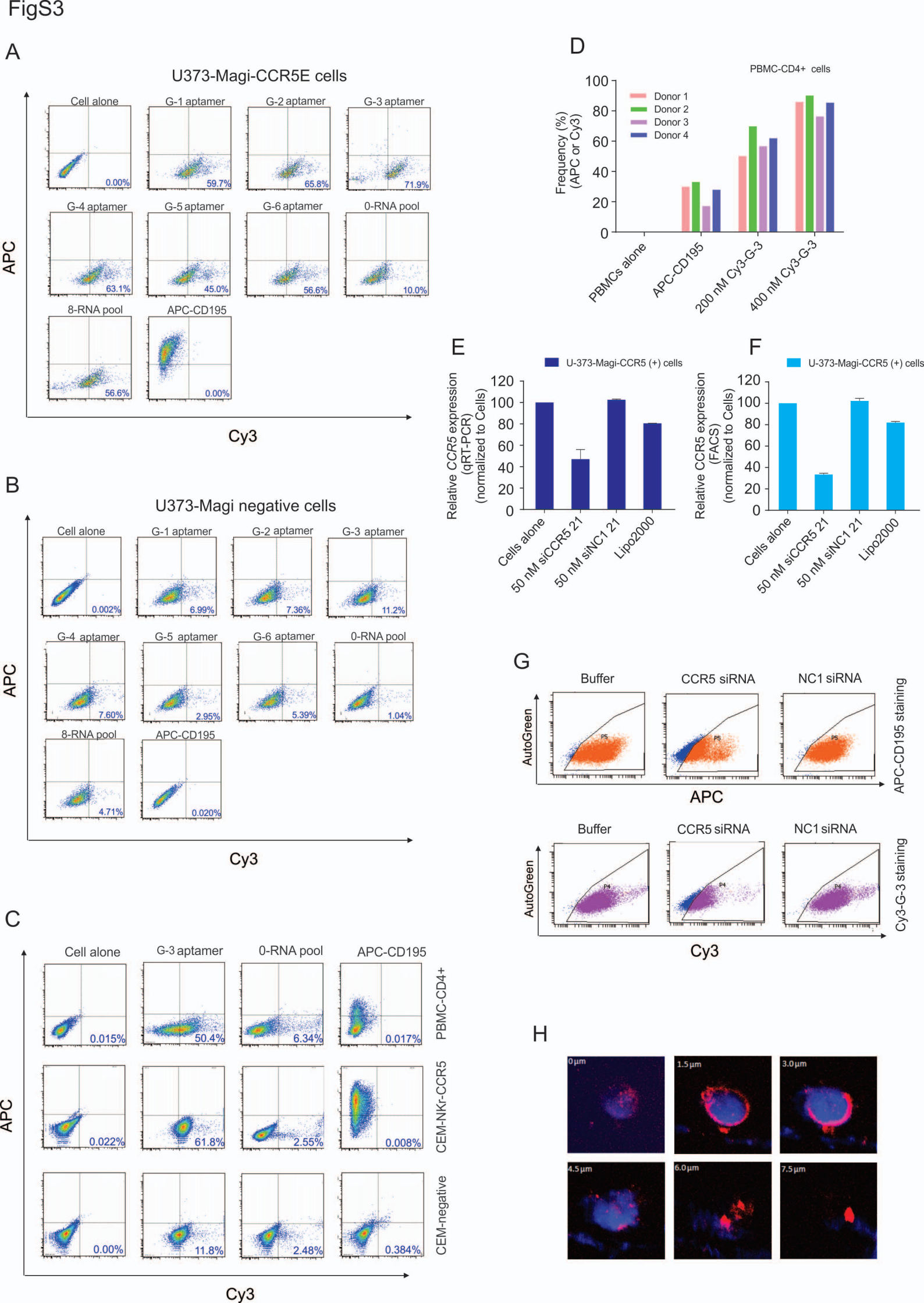
B


Info. content
2.0
1.5
1.0
0.5
0
Round 5
Round 6
Round 7
Round 8
Round 9
1
20
Position (nt)

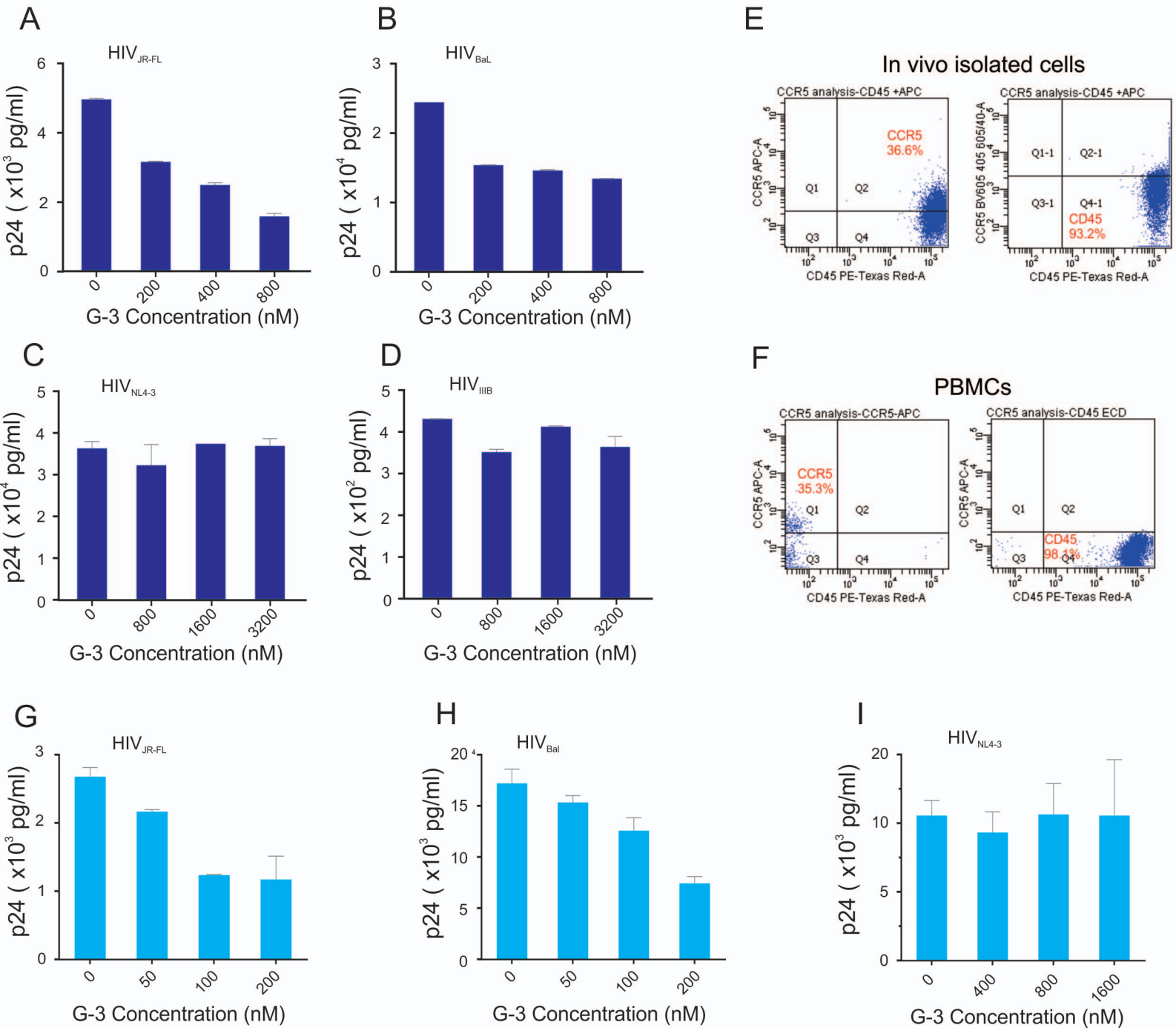
C


Group 1
Group 2
Group 3
Group 4
Group 5
Group 6

FigS3


A
U373-Magi-CCR5E cells
Cell alone
G-1 aptamer
G-2 aptamer
G-3 aptamer
0.00%
59.7%
65.8%
71.9%
G-4 aptamer
G-5 aptamer
G-6 aptamer
0-RNA pool
63.1%
45.0%
56.6%
10.0%
8-RNA pool
APC-CD195
56.6%
0.00%
APC
Cy3
B
U373-Magi negative cells
Cell alone
G-1 aptamer
G-2 aptamer
G-3 aptamer
0.002%
6.99%
7.36%
11.2%
G-4 aptamer
G-5 aptamer
G-6 aptamer
0-RNA pool
7.60%
2.95%
5.39%
1.04%
8-RNA pool
APC-CD195
4.71%
0.020%
APC
Cy3
C
Cell alone
G-3 aptamer
0-RNA pool
APC-CD195
0.015%
50.4%
6.34%
0.017%
PBMC-CD4+
0.022%
61.8%
2.55%
0.008%
CEM-NKr-CCR5
0.00%
11.8%
2.48%
0.384%
CEM-negative
APC
Cy3
D
PBMC-CD4+ cells
Donor 1
Donor 2
Donor 3
Donor 4
Frequency (%) (APC or Cy3)
100
80
60
40
20
0
PBMCs alone
APC-CD195
200 nM Cy3-G-3
400 nM Cy3-G-3
E
U-373-Magi-CCR5 (+) cells
Relative CCR5 expression (qRT-PCR) (normalized to Cells)
120
100
80
60
40
20
0
Cells alone
50 nM siCCR5 21
50 nM siNC1 21
Lipo2000
F
U-373-Magi-CCR5 (+) cells
Relative CCR5 expression (FACS) (normalized to Cells)
120
100
80
60
40
20
0
Cells alone
50 nM siCCR5 21
50 nM siNC1 21
Lipo2000
G
Buffer
CCR5 siRNA
NC1 siRNA
AutoGreen
P5
APC
APC-CD195 staining
Buffer
CCR5 siRNA
NC1 siRNA
AutoGreen
P4
Cy3
Cy3-G-3 staining
H
0 μm
1.5 μm
3.0 μm
4.5 μm
6.0 μm
7.5 μm

FigS4


A
HIV JR-FL
p24 (x10^3 pg/ml)
G-3 Concentration (nM)
B
HIV BaL
p24 (x10^4 pg/ml)
G-3 Concentration (nM)
C
HIV NL4-3
p24 (x10^4 pg/ml)
G-3 Concentration (nM)
D
HIV IIIB
p24 (x10^2 pg/ml)
G-3 Concentration (nM)
E
In vivo isolated cells
CCR5 analysis-CD45 +APC
CCR5 36.6%
CD45 93.2%
CCR5 APC-A
CCR5 BV605 405 605/40-A
CD45 PE-Texas Red-A
F
PBMCs
CCR5 analysis-CCR5-APC
CCR5 analysis-CD45 ECD
CCR5 35.3%
CD45 98.1%
G
HIV JR-FL
p24 (x10^3 pg/ml)
G-3 Concentration (nM)
H
HIV Bal
p24 (x10^3 pg/ml)
G-3 Concentration (nM)
I
HIV NL4-3
p24 (x10^3 pg/ml)
G-3 Concentration (nM)

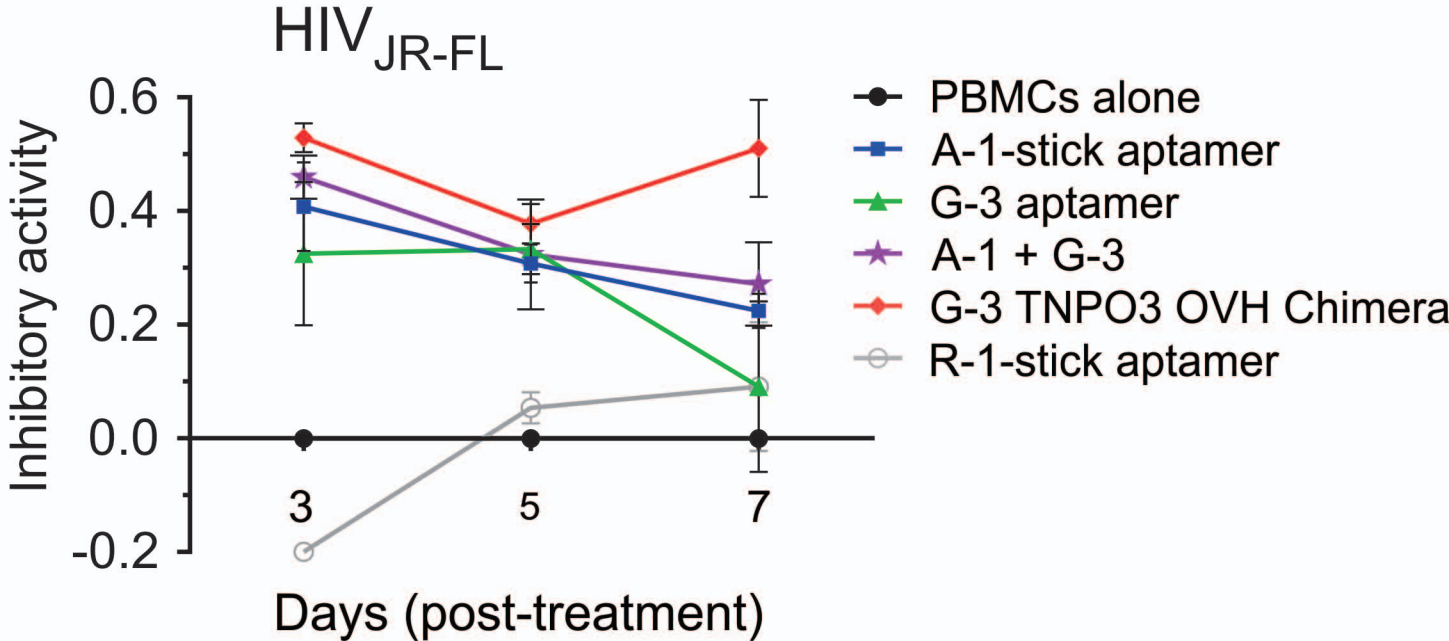
FigS5

PBMCs (donor2)	Cy3 positive (His%)	Cy5positive (His%)	Both positive
Control	0.191	0.279	0.320
Cy3-G-3-siRNA chimeras	78.2	0.00	0.811
G-3-Cy5-siRNA chimeras	0.114	75.1	1.67
Cy3-G-3-Cy5-siRNA chimeras	75.85	76.4	68.8
Cy3-0-RNA pool	7.63	0.082	0.121
Cy5-siRNA	0.246	3.32	1.27

CEM-NKr-CCR5 cells	Cy3 positive (His%)	Cy5 positive (His%)	Both positive
Control	0.076	0.125	0.076
Cy3-G-3-siRNA chimeras	86.0	0.013	0.206
G-3-Cy5-siRNA chimeras	0.132	72.0	0.339
Cy3-G-3-Cy5-siRNA chimeras	79.7	81.1	70.7
Cy3-0-RNA pool	0.808	0.091	0.111
Cy5-siRNA	0.583	0.872	0.472

CEM negative cells	Cy3 positive (His%)	Cy5 positive (His%)	Both positive
Control	0	0.052	0
Cy3-G-3-siRNA chimeras	9.98	0.011	0.066
G-3-Cy5-siRNA chimeras	0.012	5.81	0.147
Cy3-G-3-Cy5-siRNA chimeras	5.4	5.56	3.48
Cy3-0-RNA pool	1.14	0.009	0.052
Cy5-siRNA	0	0.450	0

FigS6


HIV JR-FL
Inhibitory activity
0.6
0.4
0.2
0.0
-0.2
3
5
7
Days (post-treatment)
PBMCs alone
A-1-stick aptamer
G-3 aptamer
A-1 + G-3
G-3 TNPO3 OVH Chimera
R-1-stick aptamer