Supplementary Methods

Confocal microscopy For direct visualization of viral entry, fluorescent HIV-1 virions were generated by co-transfection of a vector containing a full-length R5-tropic HIV-1 provirus (NIH AIDS Research & Reference Reagent Program, Division of AIDS, NIAID, NIH: pWT/BaL (catalog # 11414) [1] and Vpr-GFP into HEK-293T cells as described in [2]. Primary astroglial cells were infected with the HIV-1_{BaL} Vpr-GFP in the absence or presence of bivalent ligand (100 nM) for 18 to 20 h at 37°C, washed in PBS, fixed in 4% paraformaldehyde, and counterstained with DAPI. Cells were imaged using a Zeiss LSM 700 laser scanning confocal microscope equipped with a 63× (1.40 numerical aperture [NA]) objective, using 488-nm laser excitation with a dichroic beam-splitter set at 492 nm to optimize GFP detection. Images are 0.32 µm-thick optical sections from a single Z-plane with the acquisition parameters set to optimize X-, Y-, and especially Z-plane resolution.

Quantitative Real Time PCR Total RNA was isolated using the miRNeasy Mini Kit (Qiagen, Inc.; Valencia, CA, USA) and used to generate cDNA templates by reverse transcription using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Carlsbad, CA, USA) according to the manufacturer's instructions. PCR reactions were performed in a total volume of 20 µL containing SensiMix SYBR qPCR reagents (Bioline USA, Inc.; Tauton, MA, USA) using a Corbett Rotor-Gene 6000 real-time PCR system (Qiagen, Inc.). PCR conditions consisted of an initial hold step at 95°C for 10 min followed by 40 amplification cycles of 95°C for 10 s, 58°C for 30 s, and 72°C for 30 s. Sequences of the primer sets used were forward: 5'-CTTGGAACCCGAAAAGTCTC -3' and reverse: 5'- TGCCATCTAAGTGGGACAAG -3' for MOR; forward: 5'- CTGCTCAACCTGGCCATCTCT -3' and reverse: 5' CTTTTAAAGCAAACACAGCATGGAC -3' for CCR5; forward: 5′-GCG GAT CCA TGG AGC CAG TAG ATC CTA G-3′ and reverse: 5′-T TAT CAT TGC TTT GAT AGA GAA ACT TG-3′ for Tat and forward: 5'- CATGGCACCGTCAAGGCTGAGAA -3' and reverse: 5'- CAGTGGACTCCACGACGTACTCA -3' for GAPDH. The specificity of the amplified products was verified by melting curve analysis and agarose gel electrophoresis.

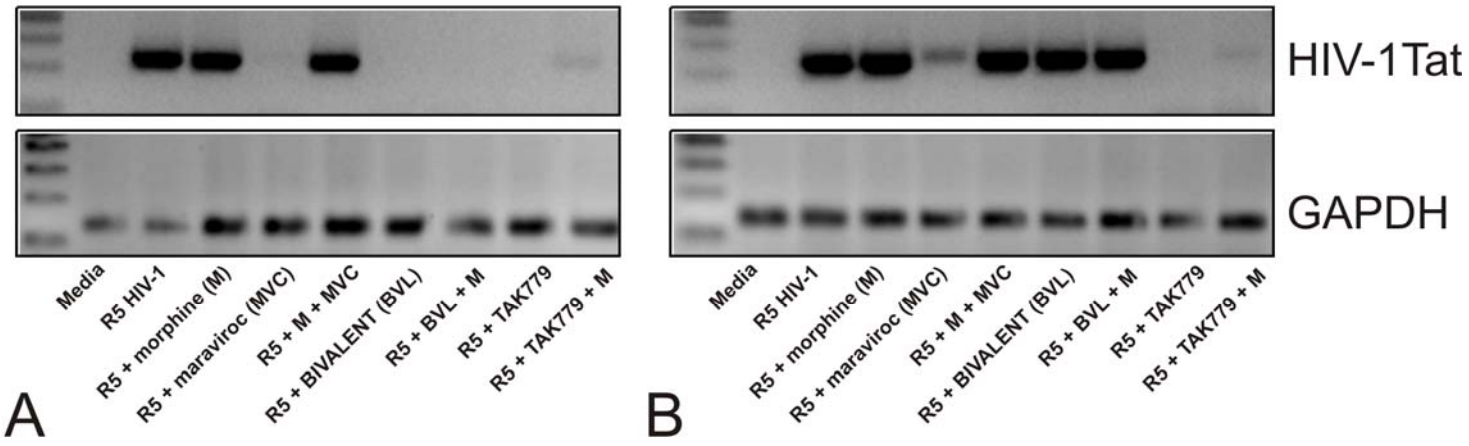
Supplementary Figure 1. RNA expression levels of HIV-1 Tat in astrocytes (A) and microglia (B) with the indicated treatments were examined by qRT-PCR. PCR products were detected using 2 % agarose gels stained with ethidium bromide. Marker indicates 100 base pair (bp) DNA ladder marker where the 100 - 400 bp markers are shown. GAPDH served as a loading control. Bands are representative samples from experiments conducted in Figure 2 and Supplementary Figure 3.

Supplementary Figure 2. Cellular localization of HIV-1 in astrocytes infected with an R5-tropic HIV-1_{Bal}-Vpr-GFP reporter virus (green) was readily detectable (arrows) by confocal microscopy. Cell nuclei are counterstained with DAPI (blue). Incubation with the bivalent ligand (lower panel) inhibits viral entry. Although many cells were not GFP positive, astroglial cells possessing internalized Vpr-GFP were clearly evident and the presence of the bivalent ligand abolished viral entry in all astroglia (lower panel).

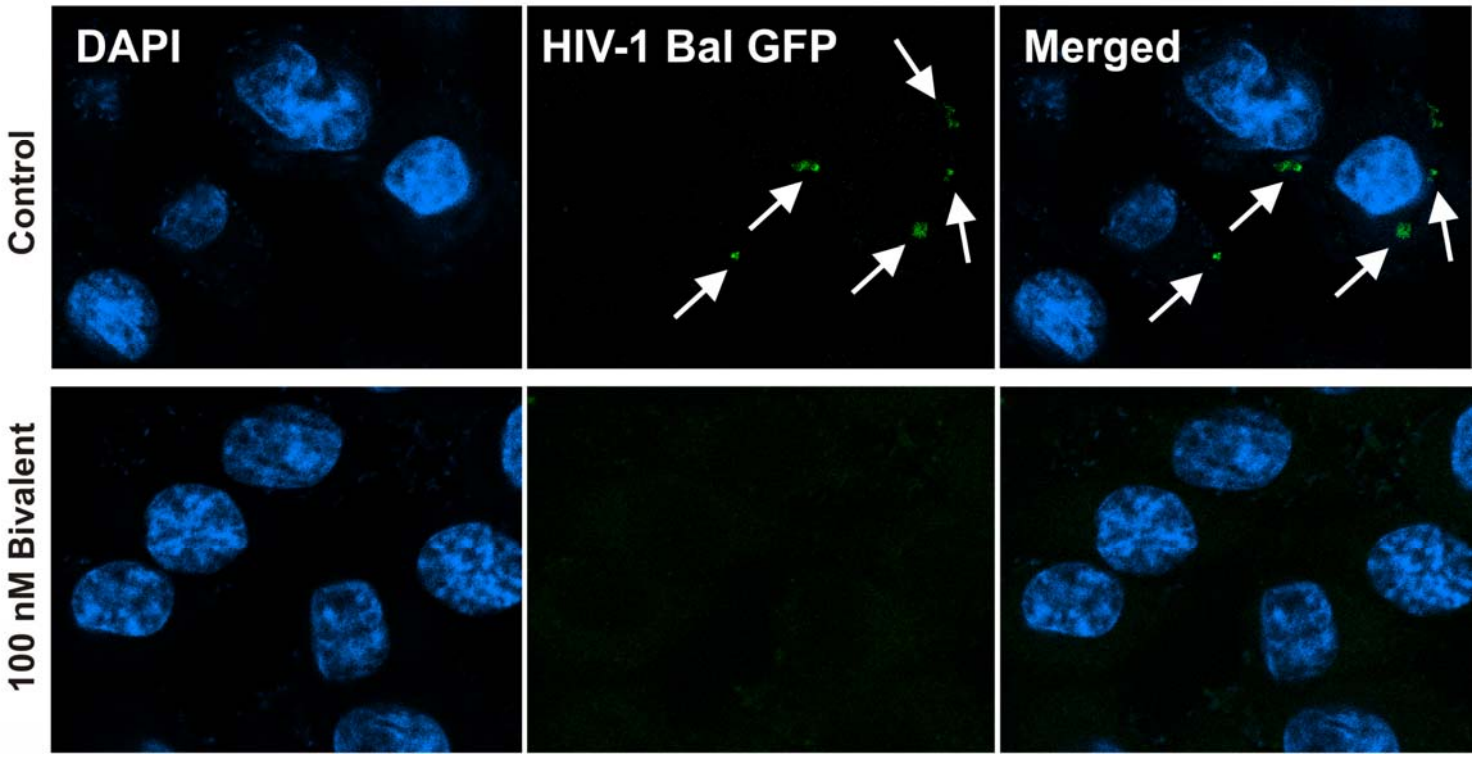
Supplementary Figure 3. Antiviral effect of TAK779 was detected in human astroglia (A) and microglia (B) transfected with the plasmid pBlue3'LTR-luc (NIH AIDS Research & Reference Reagent Program) using Lipofectamine 2000 (Invitrogen). TAK779 at a concentration of 100 nM was used 30-60 min prior to HIV-1 infection to selectively block viral entry. The R5 HIV-1 entry inhibitor TAK779 prevented virus from entering and caused a 1.3- and a 1.5-fold decrease in Tat expression in astrocytes and microglia, respectively, when compared to exposure to virus alone while combination with morphine did not significantly abolish the antiviral effect of TAK779.

Supplementary Figure 4. RNA expression levels of CCR5 and MOR in astrocytes (A) and microglia (B) were detected by qRT-PCR. qRT-PCR data were calculated as relative expression levels by normalization against GAPDH mRNA using the $2^{-\Delta\Delta Ct}$ method [3]**.** The stoichiometric ratio between CCR5 and MOR was 1.6-fold difference in astrocytes and 5-fold difference in microglia. Values are based on SEM of 3 lots of cells from different individuals. (*$p < 0.05$ vs. MOR).

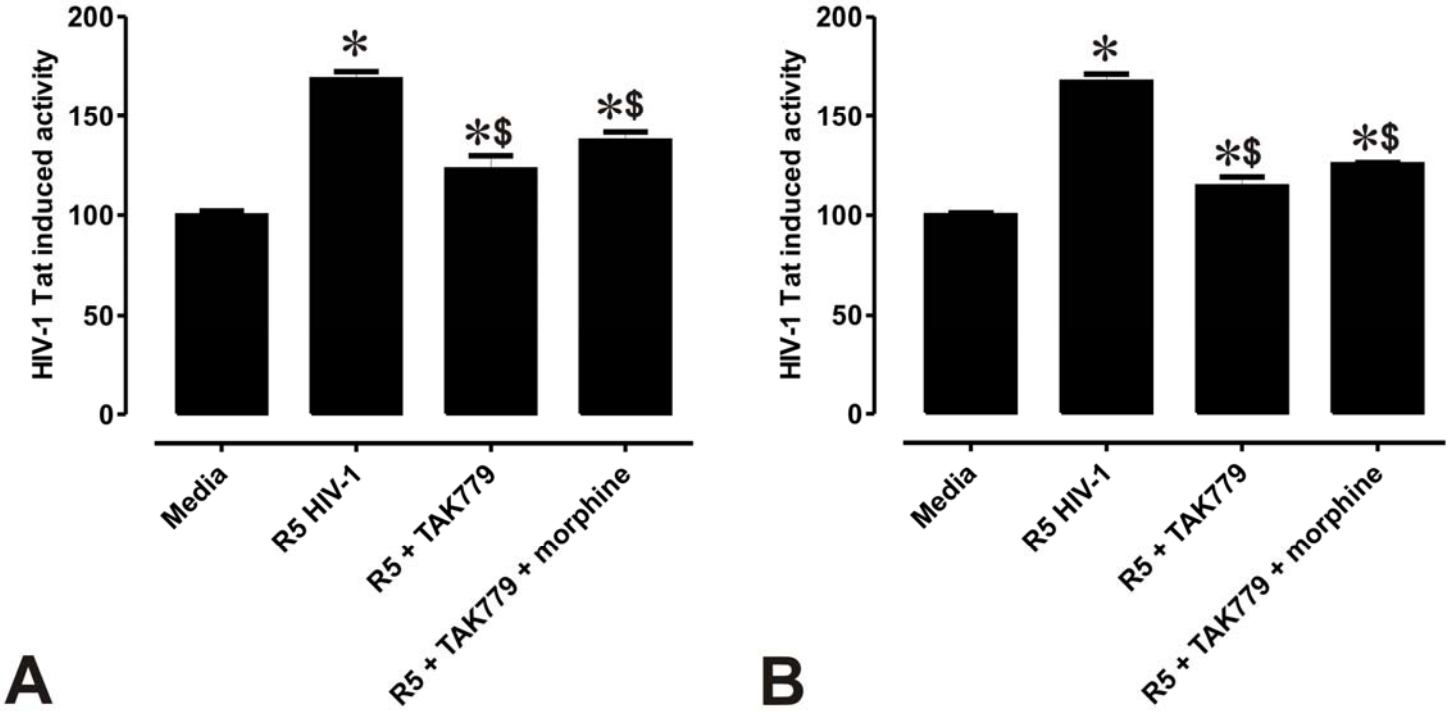
Supplementary Figure 1



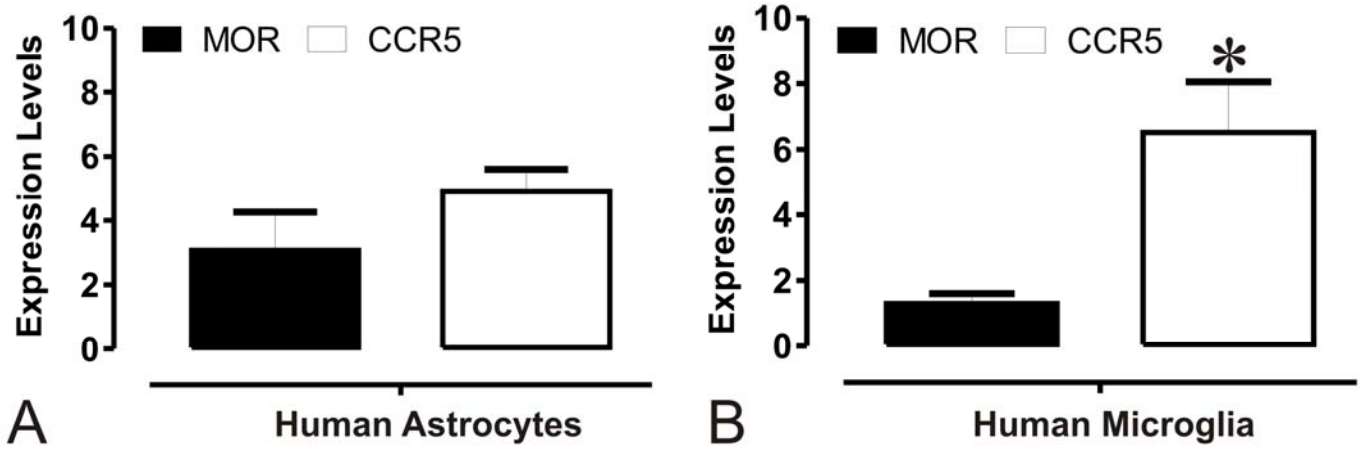
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplemental Reference List

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2. **Suzuki S, Chuang LF, Yau P, Doi RH, Chuang RY. Interactions of opioid and chemokine receptors: oligomerization of mu, kappa, and delta with CCR5 on immune cells. *Exp Cell Res* 2002; 280(2):192-200.**

3. **Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; 25(4):402-408.**