Supplemental Materials and Methods

Quantitative Real-Time PCR (Q-RT-PCR) for hTERT expression. T cells were isolated and stimulated as described above. On day 12, when the activation-induced telomerase activity had returned to baseline, $CD8^+$ T cells were isolated by negative selection and re-plated in 24-well plates with complete RPMI. TAT2 (1 μM) or DMSO (0.1%) was added to cells, which were harvested at various times (3, 6, 12, 24 hours) after treatment. RNA was extracted using the RNeasy Mini Kit (Qiagen), and RNA concentrations were determined using the Quant-iT Ribogreen RNA Assay Kit (Molecular Probes). 2 μg of RNA were converted to cDNA with the iScript cDNA synthesis kit (BioRad). Q-RT-PCR was performed by using the iQ SYBR Green SuperMix and IQCycler (Bio-Rad). As an internal control, Q-RT-PCR for the housekeeping gene GAPDH was performed. The sequences of the primers for amplification were GAPDH-F-429 (5′-CCT CAA GAT CAT CAG CAA TGC CTC CT-3′) and GAPDH-R-528 (5′-GGT CAT GAG TCC TTC CAC GAT ACC AA -3′). For the hTERT gene expression measurement, we used primers hTERT-F-1627 (5′-AAG TTC CTG CAC TGG CTG ATG AGT -3′), and hTERT-R-1758 (5′-GCT TTG CAA CTT GCT CCA GAC ACT -3′). Samples were run in triplicate in a 96-well plate in the I-CycleriQ Multicolor Real-Time Detection System (BioRad). Each well contained 10 μL of cDNA (1:10 dilution of cDNA reaction), 12.5 μL of iQ SYBR Green Super Mix, 0.75 μL of each primer (from 10 μM stock), and 1 μL of H_20, for a total of 25 μL. The IQcycler program consisted of initial denaturation at 95°C for 3 minutes, followed by 40 PCR cycles at 95°C for 15 seconds, 61°C for 30 seconds, and 72ºC for 30 seconds (single fluorescence measurement). Standard curves were created for both GAPDH and hTERT. The relative GAPDH and hTERT copy numbers and were calculated according to the standard curve method.

p24 production by $CD4^+$ T lymphocytes treated with TAT2. T cells from HIV+ donors were isolated as described and $CD4^+$ T cells were purified from this population by negative selection using the $CD8^+$ microbead purification kit (Miltenyi). The isolated $CD4^+$ T cells were cultured in complete RPMI supplemented with IL-2 (50 U/mL). Every 48 hours, IL-2 (20 U/mL) was added to wells. The amount of HIV-1 released by the $CD4^+$ T lymphocytes was evaluated by a commercial p24 ELISA kit (Perkin Elmer).

At least three dilutions of each culture supernatant were tested in triplicate. At least one of these dilutions must fall within the linear range of the standard curve for the ELISA. The assay is performed according to the manufacturer's instructions, and the plates are read at 490 or 492 nm, with a reference filter > 600 nm.

Treatment of Jurkat cells with TAT2. Jurkat cells were cultured in complete RPMI in a 24 well plate at a concentration of $5x10^5$/mL and treated with TAT2 (10 μM, 1 μM, and 0.01 μM) or DMSO (0.01%). Every 48 hours, half the media from each well was removed and replaced with fresh media supplemented with corresponding concentration of TAT2 or DMSO. 7 days after culture was started, cells were harvested and assayed for telomerase activity using TRAPeze detection kit.

Monitoring of EBV-induced transformation. PBMC were isolated from HIV+/EBV+ donors and cultured in complete RPMI media at a concentration of $1\text{-}2x10^6$. EBV supernatant was added to cells at a ratio of 1:1 with cell suspension and cells were plated in 60 wells of a 96 well plate. To 30 of the wells, TAT2 (1 μM) was added and to the other 30 wells, DMSO (0.01%) was added. After 7 days, half of media was removed and replaced with fresh media supplemented with either TAT2 or DMSO. This process was repeated on day 14. After 21 days in culture each well was scored for positive or negative growth.

Supplemental Figure Legends

Supplemental Figure 1. **TAT2-treatment of resting T cells leads to an increase in hTERT transcript.** Fold-increase in amount of hTERT transcript in $CD8^+$ T cells treated with TAT2 versus DMSO, as detected by real-time quantitative PCR (n = 6). Cells were stimulated with antibody-coated beads and cultured for 12 days to allow telomerase levels to return to baseline. Cells were then treated for 3 hrs, 6 hrs, 12 hrs, and 24 hrs before harvest and RNA extraction.

Supplemental Figure 2. TAT2 treatment of HIV-infected $CD4^+$ T lymphocytes does not increase viral production. $CD4^+$ T lymphocytes from HIV-infected individuals were isolated and cultured in the presence of IL-2 (20 U/mL). Every 48 hours, TAT2 or DMSO was added to cells. After 7 days in culture,

media was collected and tested for p24 concentration. Data shows p24 concentrations for triplicate wells for 3 separate donors of varying viral loads. VL = viral load. Error bars indicate s.d.

Supplemental Figure 3. TAT2 has minimal effect on telomerase activity in a transformed T lymphocyte line. Jurkat cells were treated with TAT2 at 3 different concentrations or DMSO for 24 hours in culture. Cells were then harvested and assayed for telomerase activity. DMSO-treated cells were given a telomerase activity value of 1 for calculation of relative telomerase activity of TAT2-treated cells. Results were averaged from 3 separate wells for each treatment. Error bars indicate s.d.

Supplemental Figure 4. TAT2 treatment of B lymphocytes does not increase rate of EBV-induced transformation. PBMC were isolated from HIV+/EBV+ donors (n=6). EBV-containing supernatant from B95-8 cells along with either TAT2 or DMSO was added to cells. TAT2 or DMSO was added to cells again on days 7 and 14 and cell growth was monitored on day 21. The number of wells (out of 30 total wells for each condition) showing abnormal growth for each donor was scored by microscopic evaluation. Error bars indicate s.d.

Supplemental Figure 5. Telomerase up-regulation effects of TAT2 are short-term and reversible. PBMC were isolated from HIV+ donors and treated with TAT2 or DMSO for 24 hours, at which point cells were washed, stimulated with CD2/CD3/CD28 Ab-coated beads, and re-plated in complete media without TAT2 or DMSO. Cells were harvested every 24 hours and assayed for telomerase activity. N = 5. Error bars indicate s.d. * indicates P value < 0.05.