Supplementary Figures



Figure S1. Percentage of live cells after exposure to titrated concentrations of ImmTAVs. PHA-stimulated CD4+ T cells and CD8+ T cells (1x10⁵) from 4 healthy donors were cultured with ImmTAVs at the concentrations indicated for 7 days, then analysed by flow cytometry for the proportion of live cells identified by Aqua LiveDead stain among gated lymphocytes (singlets/live cells/CD3+). Individual donor samples were tested in duplicate. Box plots show 10-90th percentile and horizontal lines indicate median values.



Figure S2. Gating strategy for identification of HIV-infected caspase-3-positive CD4+ T cells

that formed doublets with CD8+ T cells.

The left hand plot in the bottom row shows p24+ cell gate, which include both CD4+ and CD4- cells.



Figure S3. Number of conjugates formed between Gag+ CD4+ T cells and autologous or healthy donor CD8+ T cells in the presence of ImmTAVs.

Purified primary CD4+ T cells (1x10⁵) from 5 HIV-positive HLA-A*0201-positive ART-treated subjects (39, 61, 92, 006, 007) were stimulated with PHA to reactivate latent HIV, then cultured in duplicate with either autologous CD8+ T cells or CD8+ T cells from an HIV-negative donor at a CD8+/CD4+ cell ratio of 1:1, alone or with ImmTAVs at the concentrations indicated for 7 days. Conjugates between HIV-infected CD4+ T cells and CD8+ T cells were identified using the gating strategy shown in Figure S2. Data shown indicate mean + SD.



Figure S4. Dose-dependent Inhibition of HIV spread in CD4+ T cells by tenofovir.

PHA-stimulated CD4+ T cells $(1x10^5)$ from 2 healthy donors were infected by spinoculation with HIV IIIB (MOI – 0.01), washed and cultured in duplicate wells for intervals of 4-7 days. Cells were treated with tenofovir $(10\mu M)$ or mock-treated, 3 days after infection. The frequency of Gag p24+ cells was determined by intracellular staining and flow cytometry on the days indicated. Culture supernatants were analysed in duplicate for p24 Ag release during the 24 hours preceding each sampling time point by ELISA.



Figure S5. Gag mean fluorescence intensity (MFI) in HIV-infected CD4+ T cells after culture with tenofovir.

Tenofovir (10μM) was added to PHA-activated CD4+ T cells (1x10⁵) from 3 ART-treated patients (006, 007, 008) immediately after harvesting and 3 healthy donors (HD1, HD2, HD3) immediately after spinoculation with HIV IIIB. Cells were cultured in duplicates for 48h or mock drug-treated, then co-cultured with healthy donor CD8+ T cells and ImmTAVs for a further 48h. Gag expression in residual HIV-infected CD4+ T cells was determined by intracellular staining. Bars indicate 10-90th percentile of MFI values and horizontal lines indicate median values.



Figure S6. Distribution of HIV-uninfected CD4+ T cells among resting and activated subsets. Purified PHA-stimulated CD4+ T cells (1x10⁵) from 5 ART-treated patients were cultured in duplicate with healthy donor CD8+ T cells alone or with ImmTAV m134 (10⁻⁸M). After 7 days, the fraction of HIV-uninfected cells within activated (CD25+/CD69+/HLA-DR+) and resting (CD25-/CD69-/HLA-DR-) CD4+ T cell populations was determined by intracellular and cell surface staining. Data shown indicate mean + SD.