Supplemental Figures

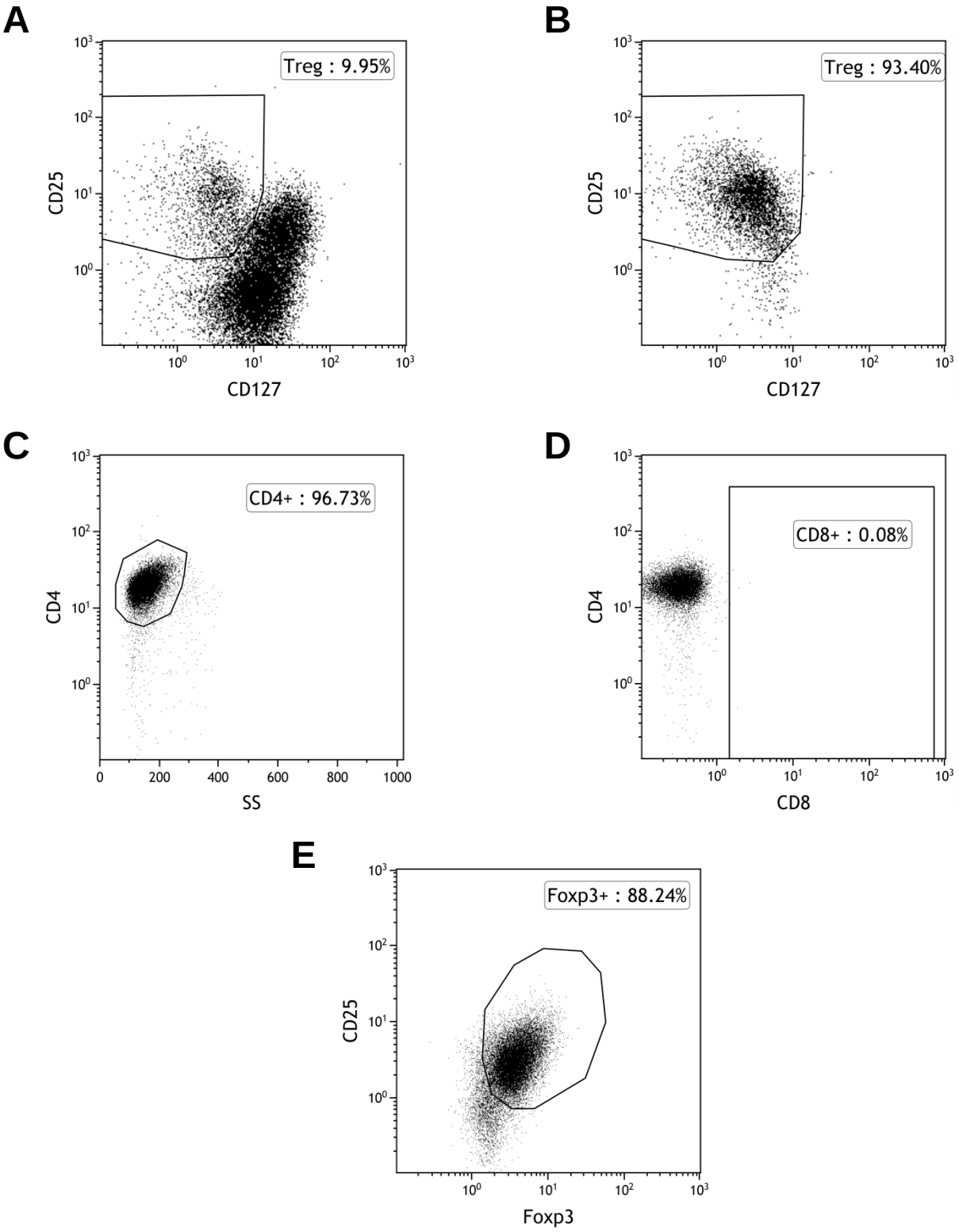

Figure S1.Purity of sorted Treg cells. Dot-plots of sorted Treg cells from a representative donor. Frequency of $CD4^{+}CD25^{+}CD127^{low}$ Treg cells before (A) and after sorter (B). Analysis in ungatedcells showed a purity of more than 95% of $CD4^{+}$ cells (C) and absence of contaminant $CD8^{+}$ T cells (D) in sorted Treg cells. In all experiments purity of sorted Treg was higher than 90% of $CD4^{+}CD25^{+}CD127^{low}$ cells (B) and intracellular staining showed that more than 85% of sorted cells were Foxp3+ (E).

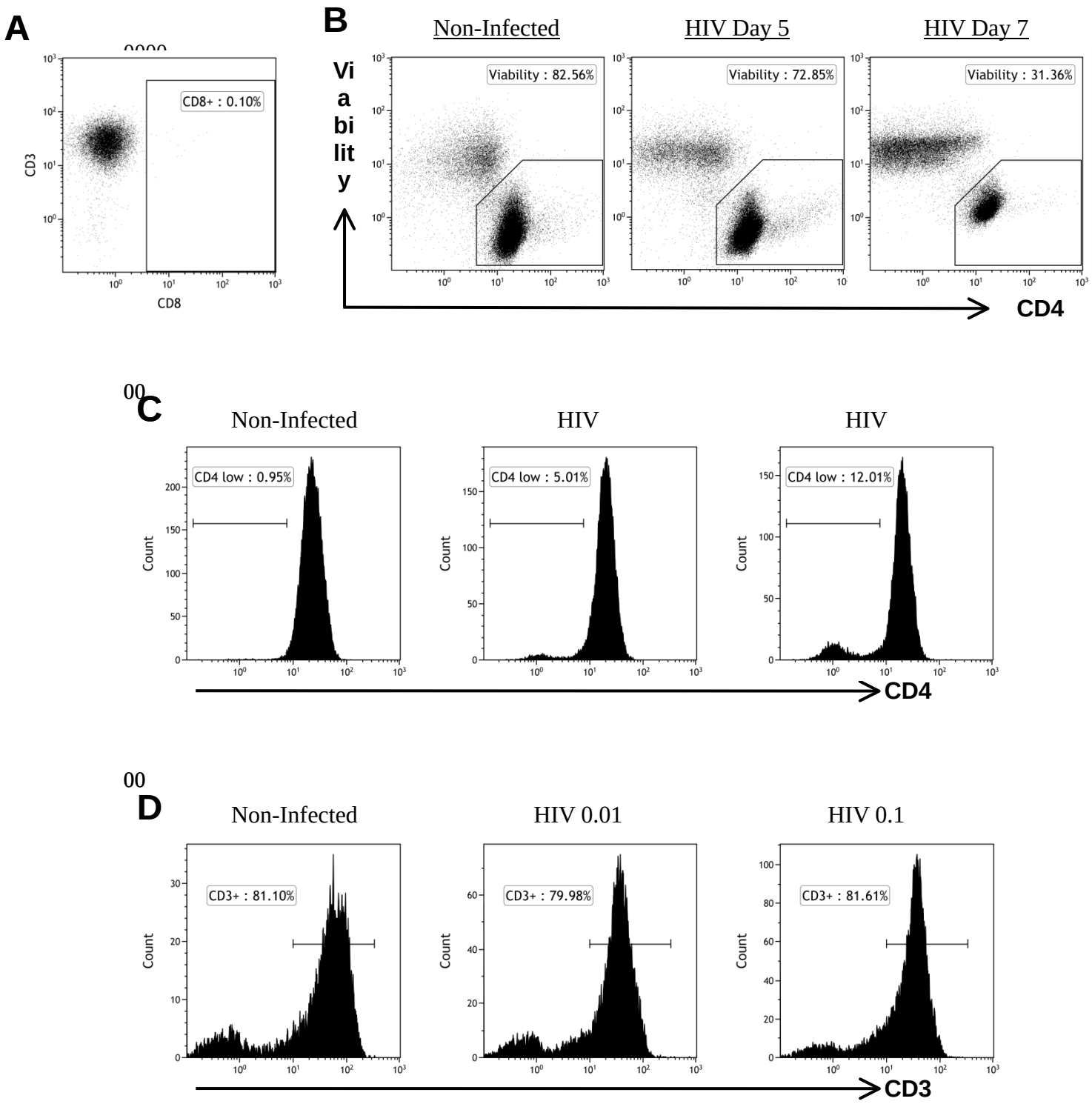

Figure S2. Viability and phenotype of infected Treg cells.(A)Dot-plot and histograms of HIV-infected Treg cells from a representative donor. Analysis in ungated cells showed an absence of contaminant $CD8^+$ T cells at day 5 after infection.(B) Viability of cultured non-infected and HIV-infected Treg cells at day 5 and HIV-infected Treg at day 7 post-infection. (C) Histograms in gated $CD3^+Foxp3^+$ T cells showing a decrease in CD4 frequency in HIV-infected Treg at day 3 postinfection. Numbers represent the percentage of $CD4^{low}$ cells. (D) Histogram from a representative donor showing the percentage of $CD3^+$ T cells in NI-Treg and HIV-infected Treg at day 7 postinfection. Data are representative of 10 different donors.

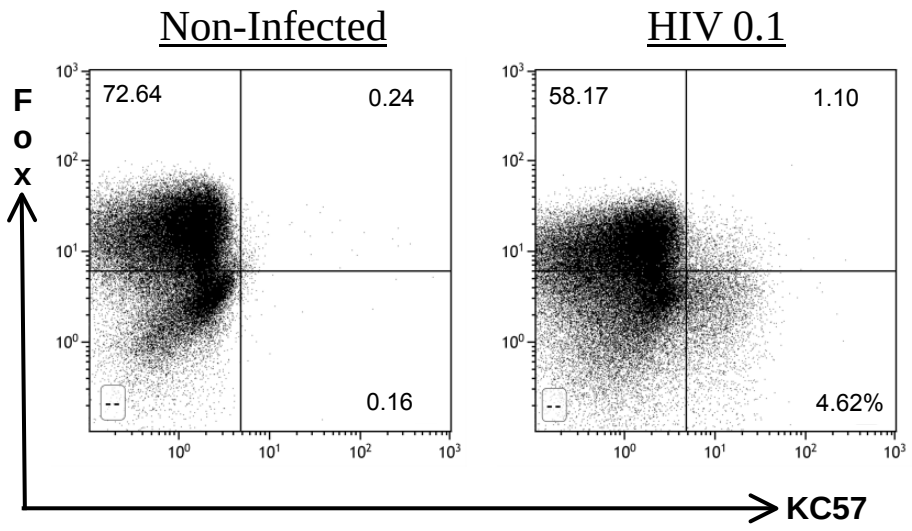

Figure S3.Foxp3 decrease in p24+ HIV-infected Treg. Dot-plotof non-infected and HIV(X4)-infected Treg cells from a representative donor. Analysis showed that Treg cells with higher expression of Foxp3 showed lower expression of intracellular p24.

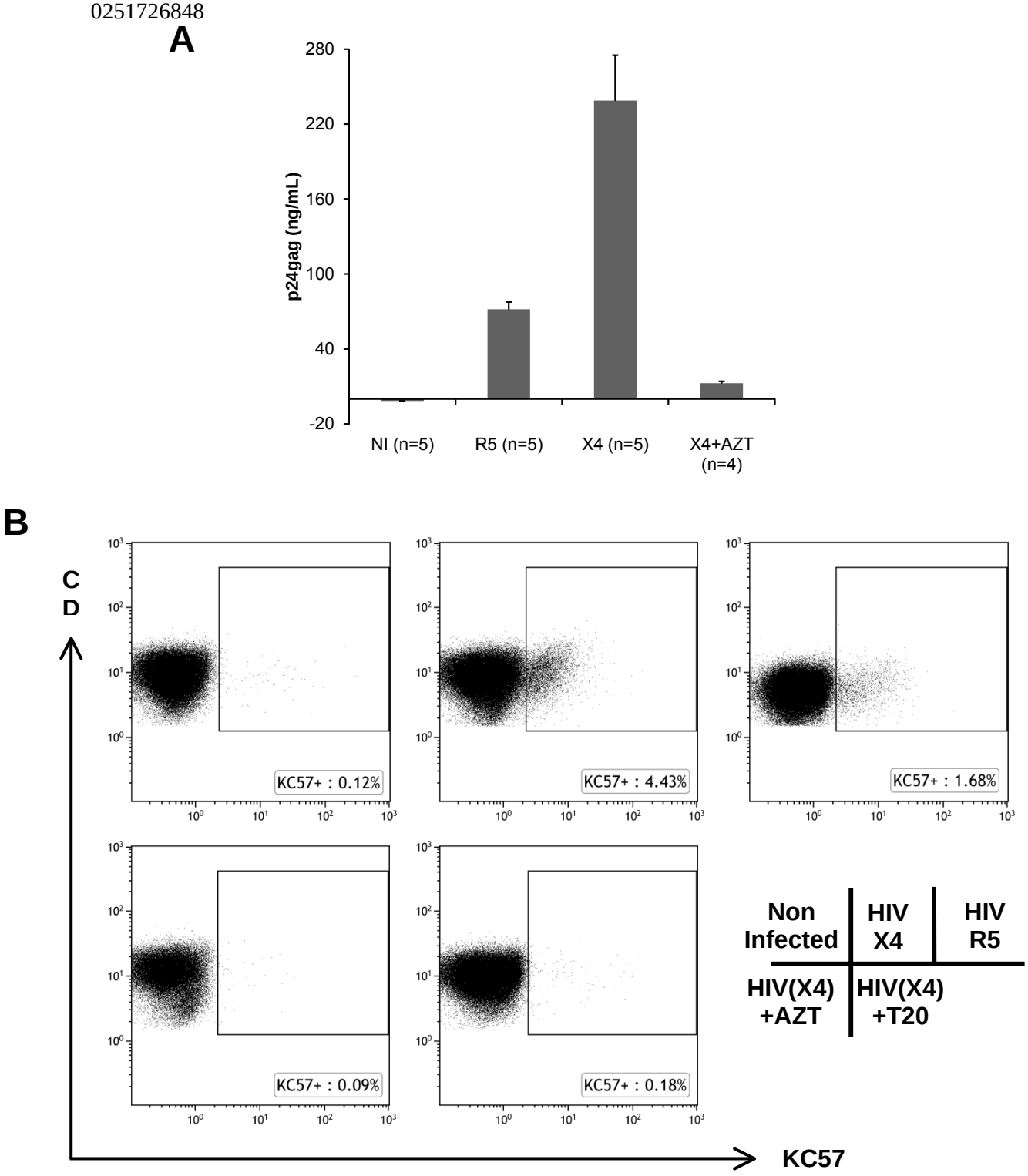

Figure S4.Infection of Treg cells with X4 and R5-tropic HIV. (A) Mean + SEM values of p24gag (ng/ml)production at 5 days postinfection determined by ELISA in supernatants of Treginfected with HIV R5-tropic (MOI 0.1), X4-tropic (MOI 0.1) and X4 + AZT (5 μM).(B) Treg cells infected with R5-tropic virus showed lower frequencies of KC57high (p24+) cells than X4-infected Treg. Presence of AZT (5 μM) or T20 (20 μM) in cultures of Treg infected with X4-tropic virus at MOI=0.1 diminished the percentage of KC57high cells at day 5. Data are representative of four different donors.

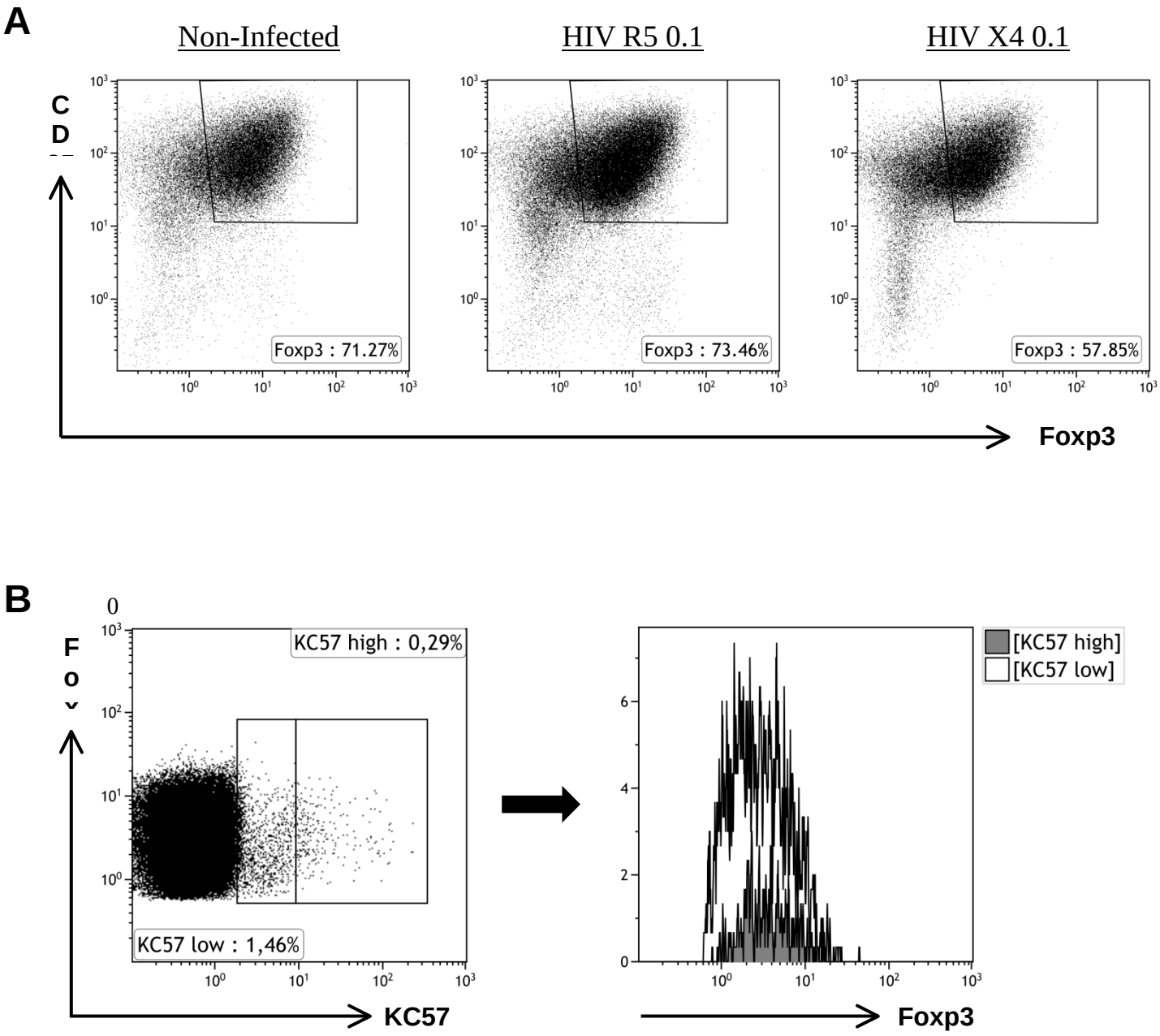

Figure S5.Infectionof Treg cells with X4 and R5-tropic HIV. Dot-plots of cultured Treg cells from a representative donor at day 5 after infection. (A) Analysis in viable cells shown a decrease in Foxp3 expression in X4-infected Treg (MOI=0.1) in comparison to non-infected Treg, but little or no effect in Treg infected with R5-tropic virus (MOI=0.1). (B) Histogram in one representative donor showing the MFI of Foxp3 in gated Treg infected with HIV-R5 (MOI 0.1) at day 5 with high (grey box) or low (white box) intracellular frequency of $p24^{gag}$

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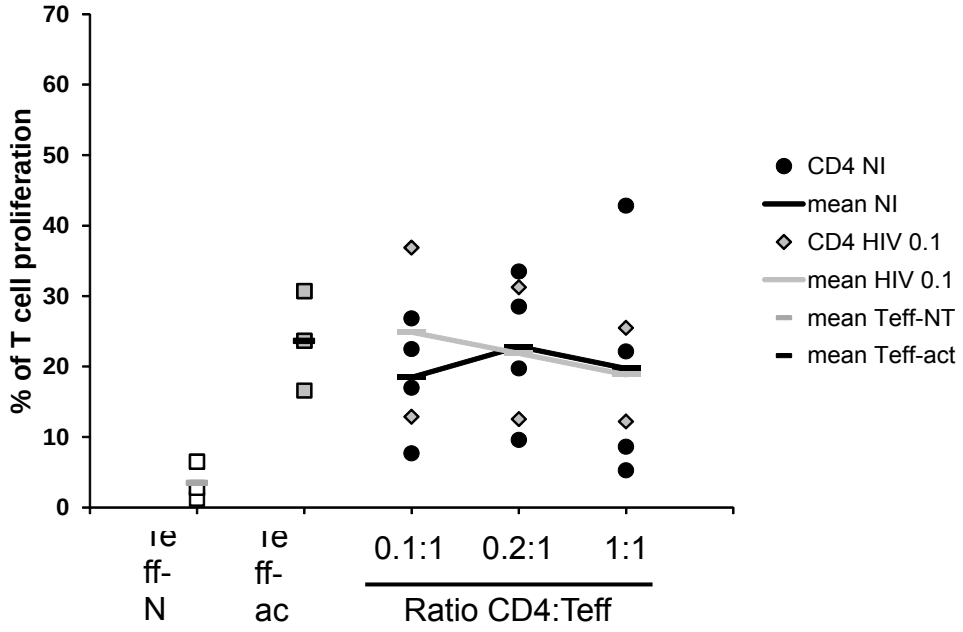

Figure S6. Suppressive capacity of Treg is impaired by HIV-infection. As a control, proliferation of allogenicTeff treated with CFSE was also determined in a co-culture with different amount of infected (n=2) and non-infected (n=4) non-Treg CD4+ T cells at day 4 postinfection.For the 3 CD4:Teff ratios tested, the percentage of Teff proliferation was not significantly different between infected and non-infected CD4+ Tcells ($p>0.05$ [Wilcoxon test]).

Supplemental Table 1

A

Sequence amplification name	Sequence (5'-3')
Foxp3 full length (forward)	CAGCTGCAGCTGCCCACACTG
Foxp3 full length (reverse)	GCCTTGAGGGAGAAGACC
Dnmt1 (forward)	CCGAGTTGGTGATGGTGTGTAC
Dnmt1 (reverse)	AGGTTGATGTCTGCGTGGTAGC
Dnmt3a (forward)	CACAGCGGAGAAGCCCAAGGTCAA
Dnmt3a (reverse)	CGCCGCAGCAGCCCGTAGGTA
Dnmt3b (forward)	GACTTGGTGATTGGCGGAA
Dnmt3b (reverse)	GGCCCTGTGAGCAGCAGA
HPRT (forward)	GCTGAGGATTTGGAAAGGGTG
HPRT (reverse)	TGAGCACACAGAGGGCTACAATG
GAPDH (forward)	ACCACATGCCATGCCATCACT
GAPDH (reverse)	GCCATCACGCCACAGTTTC

B

Sequence amplification name	Bisulfited sequence (5'-3')
Promoter region (forward)	TTTTTGTGGTGAGGGGAAGAAATTATATT
Promoter region (reverse)	TACCATCTCCTCCAATAAAACCCACATC
CNS2 region (forward)	TTTGGGTTAAGTTTGTTGTAGGATAGGGTAGTTAG
CNS2 region (reverse)	AAATCTACATCTAAACCCTATTATCACAACCCC
upstream enhancer region (forward)	AATGTGGGGATTAGGTAAAATTTTT
upstream enhancer region (reverse)	AAACCTTAAAACTACCACTAAC

Supplemental Table S1. List of primers used. (A) List of primers used in RT-PCR for amplification of Foxp3, DNMTs, and housekeeping genes. (B) Specific primers for bisulfited DNA to amplify Promoter, CNS2 and upstream enhancer regions.