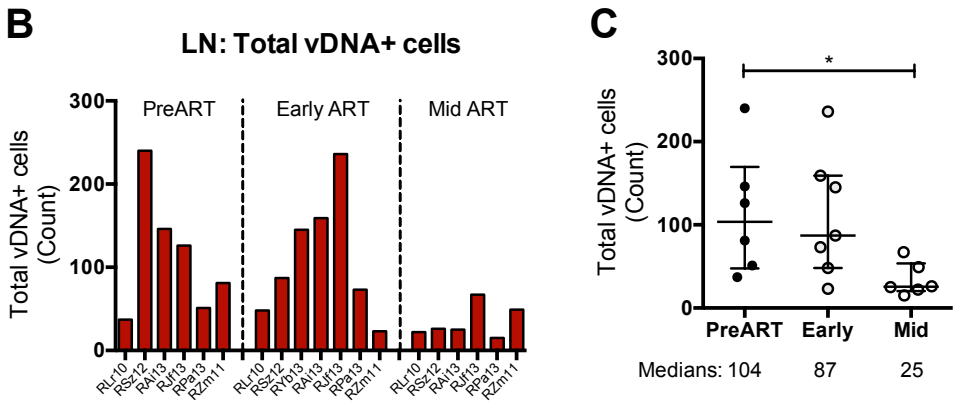
Supplemental Data Items

Figure S1. ART significantly reduces plasma viremia and SIV DNA levels in lymphoid tissues of SIV-infected RMs. Related to Figure 1

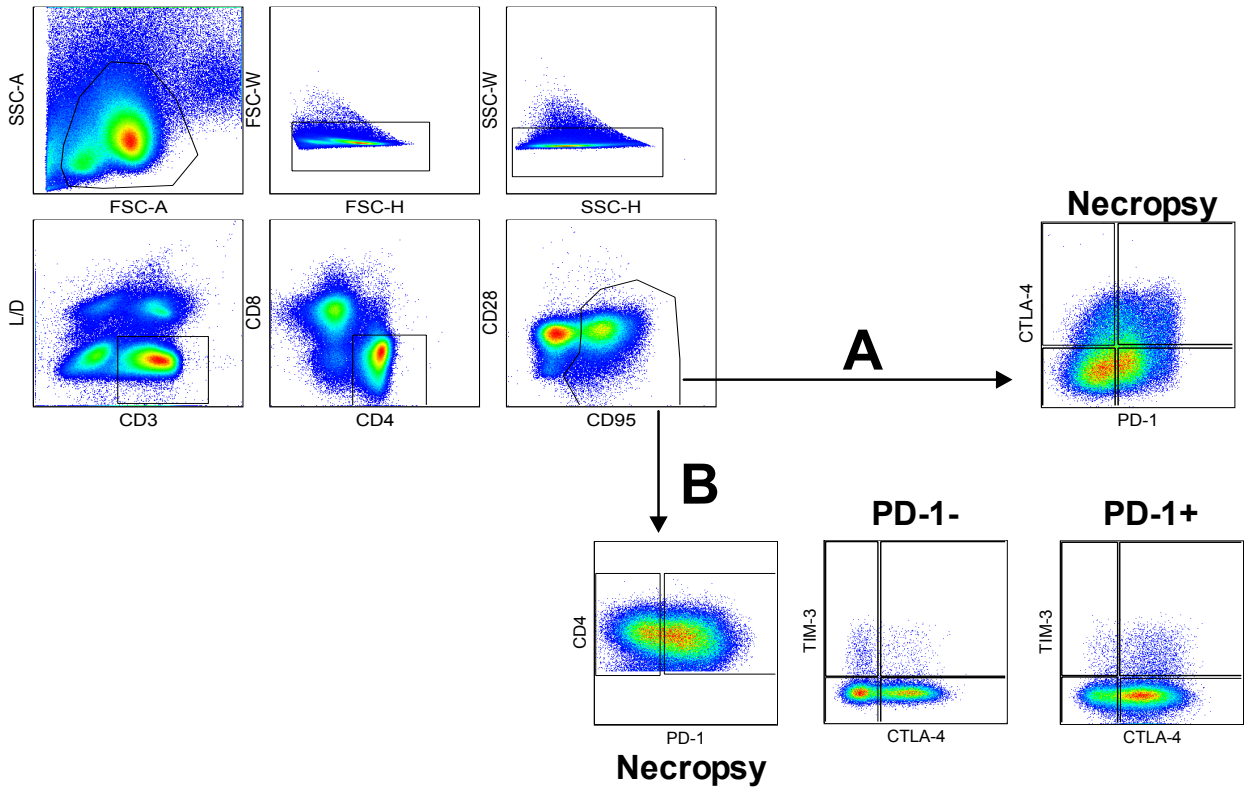
A

Macaque	Days on ART *(years)*	% VL Reduction *(Nx/PreART VL)*
RKa13	n/a*	n/a*
RLr10	220	100.0%
RSz12	218	99.96%
RYb13	421	99.94%
RAi13	425	100.0%
RJf13	327	100.0%
RPa13	331	100.0%
RZm11	404	100.0%
RPa10	242	99.99%
RWo10	236	100.0%



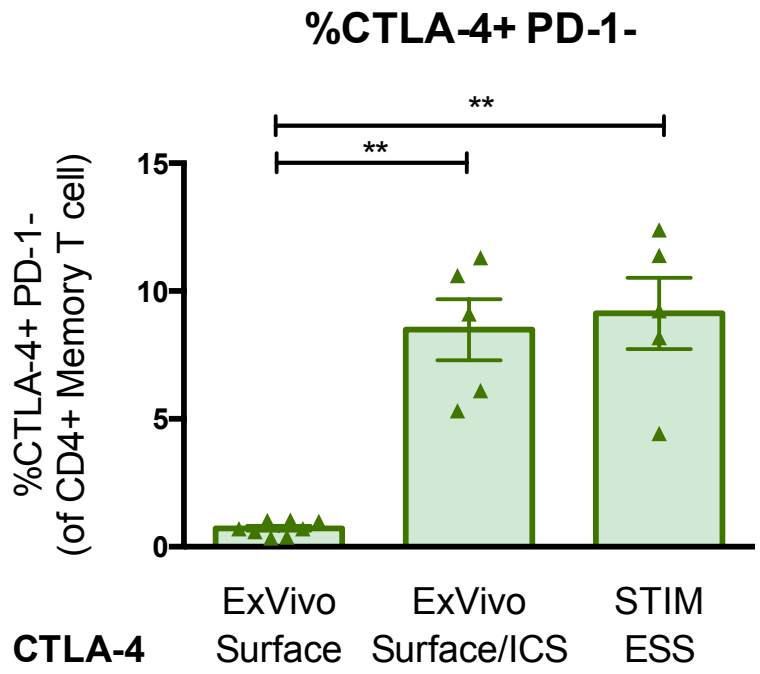
(**A**) The above table illustrates the duration of uninterrupted ART administration and the reduction in plasma viremia as a result of this ART regimen. The percent reduction was calculated between plasma viral load measurements at animal necropsy and the final time point prior to ART initiation (PreART), where undetectable plasma viral load measurements are represented as one-half of the LOD (30 copies/mL). (**B**) Quantitative image analysis following DNAscope hybridization for the LN represents the number of vDNA+ cells present in SIV-infected RMs (n=7) at 3 separate time points: PreART, Early ART (90 days post-SIV infection, 38-41 days post-ART initiation), and MidART (average time since last undetectable plasma viral load being approximately 80 ± 40 d). (**C**) The aggregate data of vDNA+ cells in the LN are shown for 6 SIV-infected RMs (7 at Early ART). Medians are indicated by the horizontal bars on the graph (±IQR) and numerically below. *, $p<0.05$.

Figure S2. Gating strategy for sorted CTLA-4 and PD-1 memory CD4+ T-cell subsets. Related to Figure 1



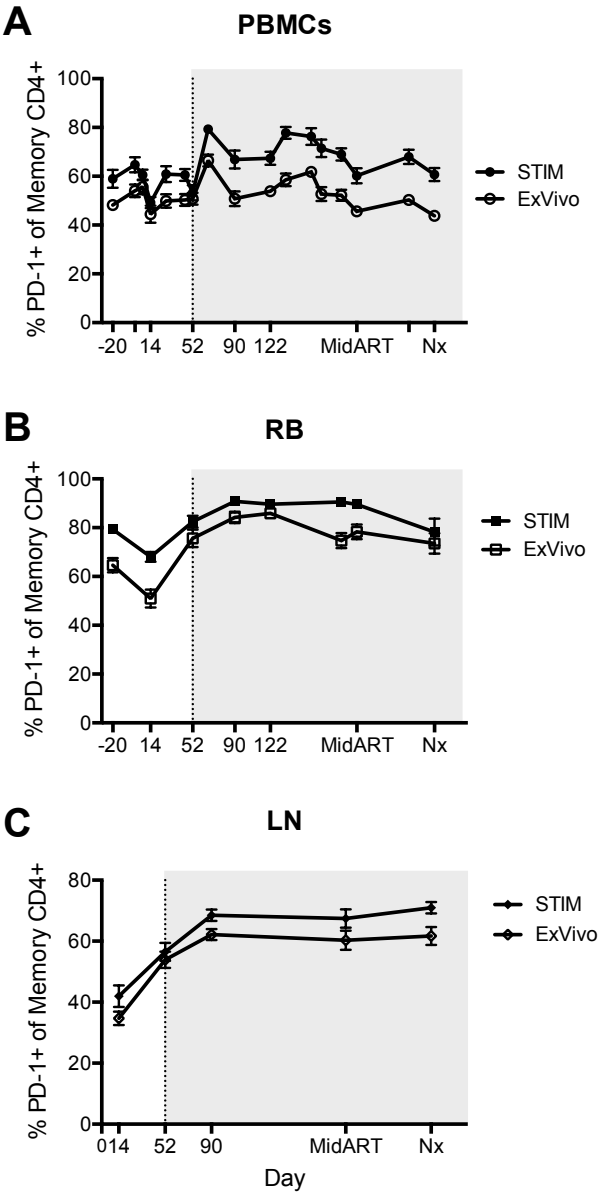
(**A**) Memory CD4+ T-cell subsets were sorted on the basis of Co-IR expression using a FACS AriaII (BD Biosciences). Isolated lymphocytes were first stimulated for 3 hours with PMA and Ionomycin (See Methods), during which anti-CTLA-4 antibody was added with the stimulation media. Memory CD4+ T-cells (CD95+) were then sorted from the PBMCs and LN at MidART and Necropsy (shown) according to their expression of CTLA-4 and PD-1. (**B**) At necropsy, memory CD4+ T-cells were first sorted from the PBMCs, LN, and spleen based on PD-1 expression, and then each PD-1 subset was further sorted according to TIM-3 and CTLA-4 expression. Pooled gut tissue samples at necropsy were sorted solely by their CTLA-4 and PD-1 expression, given their low starting material. LN staining from a representative ART-treated, SIV-infected RM is shown.

Figure S3. Enhanced surface staining captured rapidly upregulated surface CTLA-4 expression and reflect intracellular CTLA-4 levels prior to stimulation. Related to Figure 1



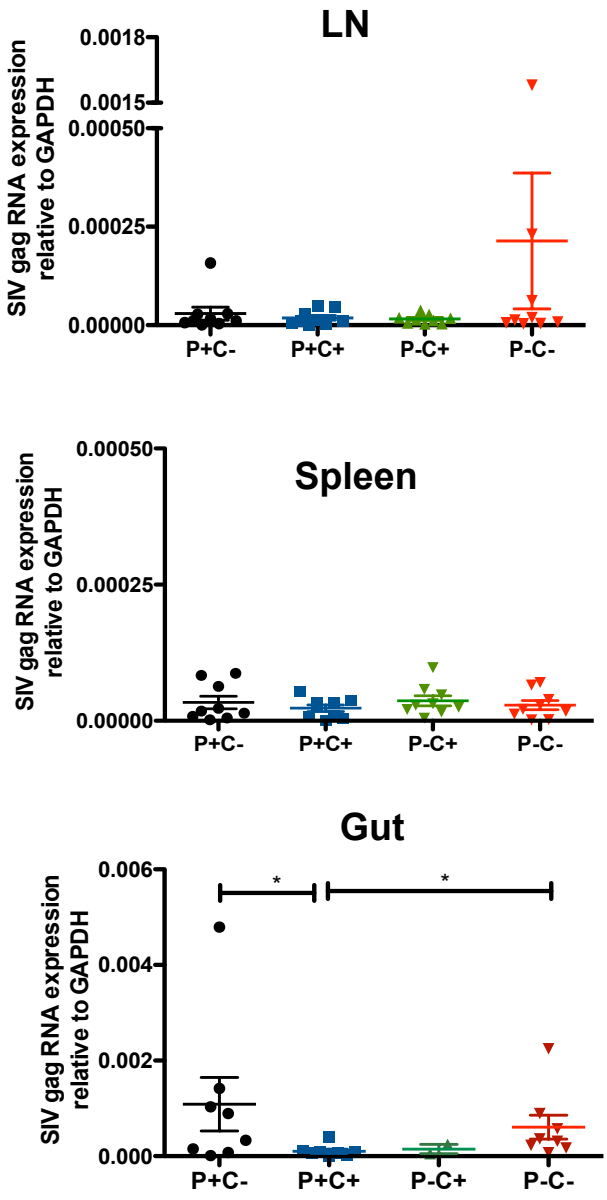
Frequencies of CTLA-4+PD-1- (green) memory CD4+ T-cells, measured as a percentage of the memory CD4+ T-cells were quantified in superficial LN from SIV-infected, ART-treated RMs. The results shown are a comparison of CTLA-4 capture conditions, *ex vivo* versus 3hr PMA/Ionomycin stimulation, and the route of CTLA-4 staining (surface versus surface with ICS versus ESS). Nine RMs were used for the *ex vivo* surface-only condition; however, due to a lack of cells, five RMs were used for the latter conditions, of which three came from a different study. All animals possessed undetectable viral loads for a minimum of three months. Averaged data are presented as the mean ± SEM, and unpaired, nonparametric Wilcoxon matched-pairs signed rank tests used to compare differences between subsets. ****, $p<0.0001$.

Figure S4. Enhance surface staining does not affect the kinetics of PD-1+ memory CD4+ T-cells during SIV infection. Related to Figure 1



Frequencies of PD-1+ memory CD4+ T-cells, expressed as percentages of memory CD4+ T-cells, were measured longitudinally in the blood (PBMCs; A), gut (RB; B), and LN (C) during SIV infection and ART suppression (gray box) for 9 RMs. ExVivo represents lymphocytes stained immediately following isolation, while STIM represents lymphocytes stained following 3 hour PMA/Ionomycin stimulation (see Methods). Averaged data are presented as the mean ± SEM. The MidART time point represents values measured at the time of sorting for each animal, with the average time since first undetectable plasma viral load being approximately 80±40 d; similarly, necropsy (Nx) refers to the animal's final access point, with average time of ART-mediated viral suppression being 125±76 days.

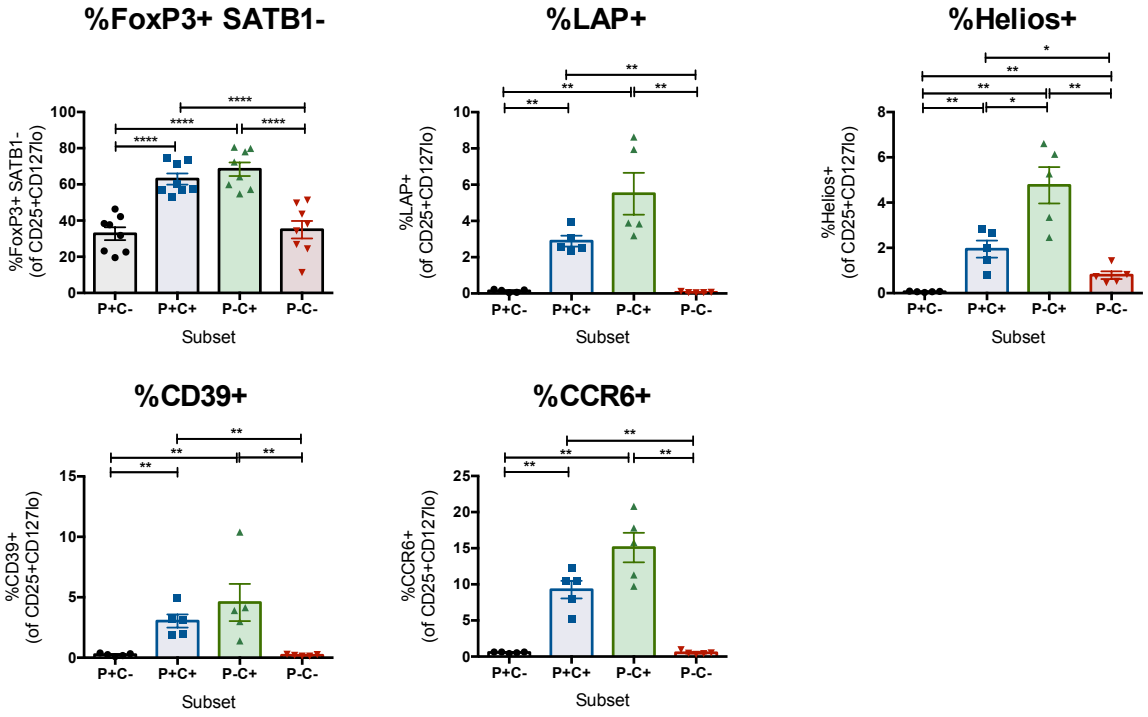
Figure S5. SIV-RNA levels in CTLA-4+PD-1- memory CD4+ T-cells are comparable to those found in CTLA-4- cells within the LN, spleen, and gut. Related to Figure 1



Levels of cell-associated SIV*GAG* RNA were quantified from the CTLA-4 and PD-1 sorted subsets in LN, spleen, and gut of 9 ART-treated, SIV-infected RMs at least 3 months following the first undetectable viral load measurements (163 ± 42 d). Data from subsets with less than 10,000 sorted cells were excluded when undetectable. Sample averages are indicated by the horizontal bar on each graph (± SEM), and t-tests were used to compare DNA levels between subsets (P, PD-1; C, CTLA-4). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

Figure S6. CTLA-4-expressing memory CD4+ T-cells contain higher frequencies of Treg functional markers. Related to Figure 2

A

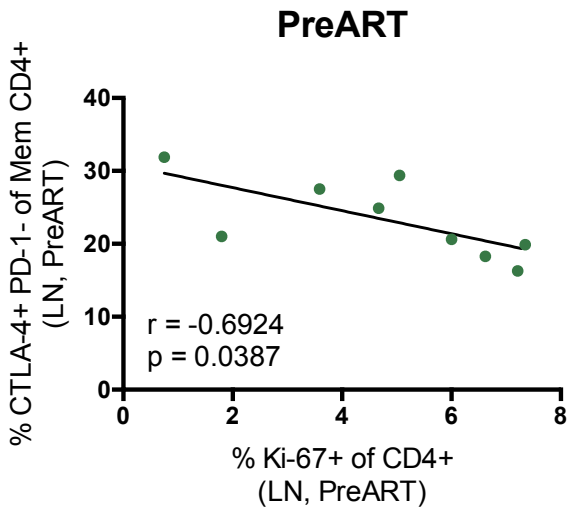


B

Marker	Relative Fold Change of P-C+ Against P+C-	Relative Fold Change of P-C+ Against DN
%FoxP3+SATB1-	2.237 ± 0.6530	2.281 ± 1.093
%LAP+	60.41 ± 62.02	91.86 ± 21.28
%Helios+	75.89 ± 20.99	7.238 ± 4.816
% CD39+	18.68 ± 9.909	21.47 ± 11.41
%CCR6+	27.53 ± 10.79	30.29 ± 5.950

(**A**) Within CD25+CD127low memory CD4+ T-cells, frequencies of FoxP3+ SATB1- cells were quantified between each CTLA-4 and PD-1-expressing subset in the LN of 9 ART-treated, SIV-infected RMs at day 90 post-SIV infection (38-41 days post-ART initiation). The frequencies of LAP+, Helios+, CD39+, and CCR6+ cells were quantified between each CTLA-4 and PD-1-expressing subset in the superficial LN of 5 ART-treated, SIV-infected RMs with a minimum of three months of virological suppression. The results shown are from unstimulated lymphocytes, where CTLA-4 expression was measured intracellularly. Averaged data are presented as the mean ± SEM, and ANOVAs using Tukey's adjustment for multiple comparisons were used to compare differences between subsets. ****, $p<0.0001$. (**B**) Inside the CD25+CD127low memory CD4+ T-cells, the frequency of each marker (FoxP3+ SATB1-, LAP+, Helios+, CD39+, and CCR6+) for the CTLA-4+PD-1- subset is given as a relative fold change against the CTLA-4-PD-1+ and CTLA-4-PD-1- (DN) subsets.

Figure S7. CTLA-4+PD-1- memory CD4+ T-cell frequencies are inversely related to CD4+ T-cell proliferation. Related to Figure 5



The correlation between CTLA-4+PD-1- cell frequencies, as a percentage of memory CD4+ T-cells, in the LN and the fraction of Ki-67+ CD4+ T-cells in the LN, both prior to ART initiation (D43 or D52 p.i.) is shown for 9 SIV-infected RMs. Statistical analysis was performed using the Pearson product-moment correlation test.

Table S1. Characteristics of the 10 RMs infected with SIVmac251. Related to Figure 1 and Experimental Procedures: Animals, SIV-infection, and antiretroviral therapy

Macaque	Age *(years)*	VL at ART initiation[1] *(copies RNA/mL)*	ART regimen[2]	Time to first suppression[3] *(days)*	**Mid-ART time point**		**Necropsy time point**	
					Time since first supp. *(days)*	VL *(copies RNA/mL)*	Time since first supp. *(days)*	VL *(copies RNA/mL)*
RKa13	4.39	1.09×10^8	PMPA, FTC, RAL, DRV/r	n/a*	n/a*	n/a*	n/a*	n/a*
RLr10	9.20	7.46×10^4	PMPA, FTC, RAL, DRV/r, MRV	14	134	<60	206	<60
RSz12	4.43	5.30×10^4	PMPA, FTC, RAL, DRV/r, MRV	14	134	<60	204	<60
RYb13	4.35	2.80×10^8	PMPA, FTC, RAL, DRV/r, MRV	288	83	151	133	<60
RAi13	4.33	8.99×10^6	PMPA, FTC, RAL, DRV/r, MRV	317	64	233	108	166
RJf13	4.35	3.20×10^6	PMPA, FTC, RAL, DRV/r, MRV	116	147	<60	211	<60
RPa13	4.44	3.65×10^6	PMPA, FTC, RAL, DRV/r, MRV	195	68	85.6	136	<60
RZm11	7.34	1.98×10^6	PMPA, FTC, RAL, DRV/r, MRV	290	62	<60	114	<60
RPa10	10.44	2.81×10^5	PMPA, FTC, RAL, DRV/r	41	111	<60	201	<60
RWo10	9.50	2.61×10^6	PMPA, FTC, RAL, DRV/r	83	69	<60	153	<60

[1]Viral load was measured by quantitative RT-PCR with a limit of detection of 60 copies/mL of plasma.
[2]Antiretroviral therapy (ART) abbreviations: PMPA, tenofovir; FTC, emtricitabine; RAL, raltegravir; DRV/r, darunavir boosted by ritonavir; MRV, maraviroc
[3]Time to first suppression represents the number of days RMs were on ART before their first measurement of undetectable plasma viremia. This time point was then used to establish MidART and necropsy time points, which reflect at least one and three months after this time, respectively.

Table S2. Profiles of 6 virally suppressed HIV-infected individuals. Related to Figure 7 and Experimental Procedures: Patient Population

Patient Number	Sex	Age *(years)*	Plasma HIV RNA[1] *(copies/mL)*	CD4+ cell count *(cells/mm^3)*	Treatment[2]	Duration of HIV infection[3] *(months)*	Duration of aviremia[4] *(months)*
25	F	59	<50	383	FTC/TDF/EFV	53	35
26	M	40	<50	313	FTC/TDF/ATV/r	103	22
27	M	23	<50	527	FTC/TDF/DRV/r	33	28
28	M	44	<50	664	FTC/TDF/ATV/r; FTC/TDF/DRV/c	43	15
29	M	48	<50	301	FTC/TDF/ATV/r; FTC/TDF/RAL/ ATV/r	43	36
30	M	64	<50	308	FTC/TDF/EFV	122	50

[1]Viral load was measured by quantitative RT-PCR with a limit of detection of 50 copies/mL of plasma.
[2]Antiretroviral therapy (ART) abbreviations: FTC, emtricitabine; TDF, tenofovir; EFV, efavirenz; ATV/r, atazanavir boosted by ritonavir; DRV/r, darunavir boosted by ritonavir; /c, cobicistat; RAL, raltegravir. Patient 28 switched to a DRV-containing ART regimen following 19.3 months of therapy. Patient 29 switched to a RAL-containing ART regimen following 2.3 months of therapy.
[3]Duration of HIV infection was calculated as the time between the first time point with detectable plasma viremia and the date of lymph node biopsy.
[3]Duration of aviremia was calculated as the time between the first time point with undetectable plasma viremia and the date of lymph node biopsy. Patients 27, 28, and 29 each experienced two viral blips in this time period, at the following levels: Patient 27 (630, 130 copies/mL); Patient 28 (80, 90 copies/mL); Patient 29 (60, 140 copies/mL).