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Supplementary Materials for

CD32 is expressed on cells with transcriptionally active HIV but does not enrich for HIV DNA in resting T cells

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The PDF file includes:

References (*51*–*53*)

Other Supplementary Material for this manuscript includes the following:
(available at
www.sciencetranslationalmedicine.org/cgi/content/full/10/437/eaar6759/DC1)

Table S4 (Microsoft Excel format). Primary data.

SUPPLEMENTARY MATERIALS:

Materials and Methods

Antibodies used for the phenotyping sections (human). The following antibodies were obtained from Biolegend: CXCR3 Alexa Fluor700 (clone G025H7), CCR7 APC-Cy7 (clone G043H7), CCR6 BV605 (clone G034E3), CD27 BV570 (clone O323), PD-1 BV421 (clone EH12.2H7), CD95 BV711 (clone DX2), CD19 BV510 (clone HIB19), CD14 BV510 (clone M5E2), CD16 BV510 (clone 3G8), CD69 PE-Cy5 (clone FN50), CD57 PE (clone HNK-1), CD3 PE-Cy5 (clone UCHT-1), CD45RO BV605 (clone UCHL1), CD32 Pe-Cy7 (clone FUN-2), HLA-DR PE-Dazzle594 (clone L243), CD3 BV421 (clone UCHT1), CD4 PE-Cy7 (clone RPA-T4), CD32-APC (clone FUN2), CD25 BV605 (clone BC96), and HLA-DR FITC (clone L243). The following antibodies were obtained from BD Biosciences: CD4 AF700 (clone RPA-T4), CD27 FITC (clone M-T271), CCR4 PE-CF594 (clone 1G1), CD32 APC (clone FLI8.26), CD127 BV786 (clone HIL-7R-M21), CD38 BUV661 (clone HIT2), HLA DR BV480 (clone G46-6), CXCR5 BB515 (clone RF8B2), CD8 BUV496 (clone RPA-T8), CD45RA BUV563 (clone HI100), CD25 BUV737 (clone 2A3), Ki67 BUV395 (clone B56), CD3 BUV805 (clone UCHT1), CD69 PE (clone FN50), and CD4 BB790 (clone SK3). FoxP3 PE-Cy7 (clone 236A/E7) was obtained from eBiosciences. The Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen) or Live/Dead fixable Violet Dead Cell Stain kit (Invitrogen) were used for viability exclusion. All antibodies were titrated and tested with Fcγ receptor blocker to optimize staining.

Antibodies used for sorting CD32$^+$ cells (human). The following antibodies were obtained from Biolegend: CCR7 APC-Cy7 (clone G043H7), CD27 BV785 (clone O323), PD-1 BV421 (clone EH12.2H7), CD19 PE-Cy5 (clone HIB19), CD16 PE-Cy5 (clone 3G8), CD4 PE-Cy7 (clone RPA-T4). The following antibodies were obtained from BD Biosciences: CD32 APC (clone FLI8.26), CXCR5 BB515 (clone RF8B2), CD69 PE (clone FN50), CD3 APC-R700 (clone UCHT1), HLA DR BV605 (clone G46-6), CD3 BV421 (clone UCHT1), CD4 PE-Cy7 (clone RPA-T4), CD32-APC (clone FUN2), CD25 BV605 (clone BC96), HLA-DR FITC (clone L243), and CD45RA PE-CF594 (clone HI100). The following antibodies were obtained from BD eBioscience: CD11c PE-Cy5.5 (clone 3.9), CD69 PE (clone FN50), CD8 PE-cy5.5 (clone RPA-T8). CD14 PE-Cy5 (clone 61D3) was obtained from AbCam. The Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen) was used for viability exclusion.

Staining (human). Freshly isolated PBMCs were counted, examined for viability, and were stained for viability exclusion with The Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen) for 10 minutes, and then incubated with Fcγ receptor blocker from Biolegend for 10 minutes. Undiluted extracellular antibody cocktail mix was added and incubated for 20 minutes. Finally, cells were fixed with PBS with 1% paraformaldehyde and stored in the dark at 4°C until acquisition. Cryopreserved PBMC or lymphoid node mononuclear cells (LNMC) were thawed, counted, examined for viability and rested for two hours at 37°C and 5% CO_2 in complete medium (RPMI supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin). Cells were washed in PBS and stained for CXCR3, CCR4, CCR6, and CCR7 at 37°C, 5% CO_2 for 20 minutes. All following incubations were performed in the dark at

room temperature. Cells were stained for viability exclusion with The Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen) for 10 minutes and then incubated with Fcγ receptor blocker from Biolegend for 10 minutes. Undiluted extracellular antibody cocktail mix was added and incubated for 20 minutes. Following staining for cell surface molecules, cells were fixed/permeabilized using the eBiosciences FoxP3 Transcription Factor buffer kit. Intracellular markers were stained with the antibody cocktail for one hour, washed with the eBioscience kit buffer. Finally, cells were fixed with PBS with 1% paraformaldehyde and stored in the dark at 4°C until acquisition. All phenotyping data were collected on a BD FACSymphony flow cytometer, BD LSR II (BD Biosciences), or on an LSR Fortessa (BD Immunocytometry Systems). Data were analyzed using FlowJo software (Treestar).

CD32$^+$ cell sorting (human). We performed sorting on fresh or frozen PBMC and tonsils from ART-treated individuals. All following incubations were performed in the dark at room temperature. Cells were stained for viability exclusion for 10 minutes and then incubated with Fcγ receptor blocker for 10 minutes. Antibody cocktail mix was added and incubated for 20 minutes. Following surface staining, cells were washed and resuspended in complete medium and kept in the dark at 4°C until acquisition. Cells were sorted on a FACSAria II (BD Biosciences) or Sony SH800 cell sorter in purity mode, pelleted and lysed for DNA and RNA extraction using AllPrep DNA/RNA kit (Qiagen) or TRI reagent (Invitrogen).

CD32$^+$ cell immunoprecipitation. Total CD4+ T cells were isolated by negative selection using the total CD4$^+$ T cell isolation kit (STEMCELL; EasySep Human CD4$^+$ T Cell Isolation kit). CD4$^+$ CD32$^+$ T cells were pulled down with antibody beads for CD32. Briefly, mIgG or anti-CD32 antibody (FUN-2 clone) were directly conjugated with Dynabeads according to the Antibody Coupling Kit's instruction (Thermo Fisher). Approximately 4 million each of CD4$^+$ T cells were pelleted down after isolation and resuspended in 1 ml of RPMI (10% serum). 100 µl of mIgG beads were added and mixed for 10 minutes at room temperature on a shaker. Negative selection was performed on a magnet to get CD4$^+$ T cells after pre-clean with mIgG beads. The flow through cells were taken into two tubes that contain FUN-2 beads (100 µl of Ab beads which contains 10 µg antibody) or mock IP as before isolation and incubated on a shaker for 30 minutes. HIV DNA was measured as previously described (*51*). HIV DNA copy number was calculated based on the ratio of HIV DNA over CD3 DNA per CD4$^+$ T cell.

Phenotypical analyses and CD32$^+$ cell sorting from Rhesus macaque. The following antibodies were used to determine the phenotype of LN CD32$^+$ CD4$^+$ T cells in RMs. From Biolegend: CD4 APC-Cy7 (clone OKT4), HLA-DR BV650 (clone L243), PD-1 BV785 (clone EH12.2H7); from BD Biosciences: Ki-67 AL700 (clone B56), CTLA4 TRPE (clone BN13), CD3 BUV395 (clone Sp34-2), CD32 BV421 (clone FLI8.26); from Invitrogen: Aqua LIVE/DEAD amine dye-AmCyan. For the sorting experiments, axillary lymph nodes were collected from SIV$^+$ ART suppressed RMs at the time of necropsy. LN-derived cells were stained for viability and incubated for 5 minutes, then incubated with the antibody cocktail for 30 minutes (incubations were performed in the dark at room temperature). Live, CD3$^+$ CD4$^+$ T cells were sorted according to their expression of CD32 (CD32$^-$ or CD32$^+$) using a FACS Aria II (BD Biosciences).

Purity, checked on $CD3^+$ $CD4^+$ $CD32^-$ cells, was always higher than 98%. Data were analyzed using FlowJo software (Treestar)

qPCR quantification of cellular HIV-1 DNA and RNA. $CD4^+$ T cells were isolated from PBMCS using EasySep Human $CD4^+$ T cell enrichment kit (Stemcell Technologies). Cellular RNA and DNA from total unfractionated PBMCs and isolated $CD4^+$ T cells were purified using the AllPrep DNA/RNA kit (Qiagen, Ventura CA) as specified by the manufacturer, quantified using a Nanodrop (ND-1000) spectrophotometer and normalized to cell equivalents by qPCR using human genomic TERT for DNA and RPLP0 expression for RNA (Life Technologies, Grand Island NY). Total cellular HIV-1 DNA and RNA were quantified with a qPCR TaqMan assay using LTR-specific primers F522-43 (5' GCC TCA ATA AAG CTT GCC TTG A 3'; HXB2522–543) and R626-43 (5' GGG CGC CAC TGC TAG AGA 3'; 626–643) coupled with a FAM-BQ probe (5' CCA GAG TCA CAC AAC AGA CGG GCA CA 3) using the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems). Cell-associated HIV-1 DNA copy number was determined using a reaction volume of 20 µl with 10 µl of 2x TaqMan Universal Master Mix II including UNG (Life technologies), 4 pmol of each primer, 4 pmol of probe, and 5 µl of DNA. Cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes, then 60 cycles of 95°C for 15 seconds and 59°C for 1 minute. Cell-associated HIV-1 RNA copy number was determined in a reaction volume of 20 µl with 10 µl of 2x TaqMan RNA to Ct 1 Step kit (Life Technologies), 4 pmol of each primer, 4 pmol of probe, 0.5 µl reverse transcriptase, and 5 µl of RNA. Cycling conditions were 48°C for 20 minutes, 95°C for 10 minutes, then 60 cycles of 95°C for 15 seconds and 59°C for 1 minute. For HIV-1 DNA measurements, external quantitation standards were prepared from pNL4-3 in a background of HIV-1 negative human cellular DNA, calibrated to the Virology Quality Assurance (VQA, NIH Division of AIDS) cellular DNA quantitation standards. For HIV RNA measurements, external quantitation standards were prepared from full-length NL4-3 virion RNA followed by copy number determination using the Abbott RealTime assay (Abbott Diagnostics, Des Plains Ill) and calibrated to VQA HIV-1 RNA standards. Patient specimens were assayed with up to 800 ng total cellular RNA or DNA in replicate reaction wells and copy number determined by extrapolation against a 7-point standard curve (1–10,000 copies) performed in triplicate.

Quantification of SIV DNA. Quantitative assessment of cell-associated total SIV DNA within LN-derived $CD4^+$ $CD32^+$ and $CD32^-$ T cells was performed using a quantitative nested PCR assay for cell-associated total SIV DNA, as previously described (*52*). Primers targeting the CD3 gene (HCD3OUT 5' and HCD3OUT 3') were added to quantify the exact number of cells in the initial samples. The copy number of total SIV DNA was calculated using a standard curve as a reference. This standard curve consisted in serial dilution of the 3D8 cell lysates (carrying one integrated copy of SIV genome per cell) (*53*).

Supplementary Figure 1

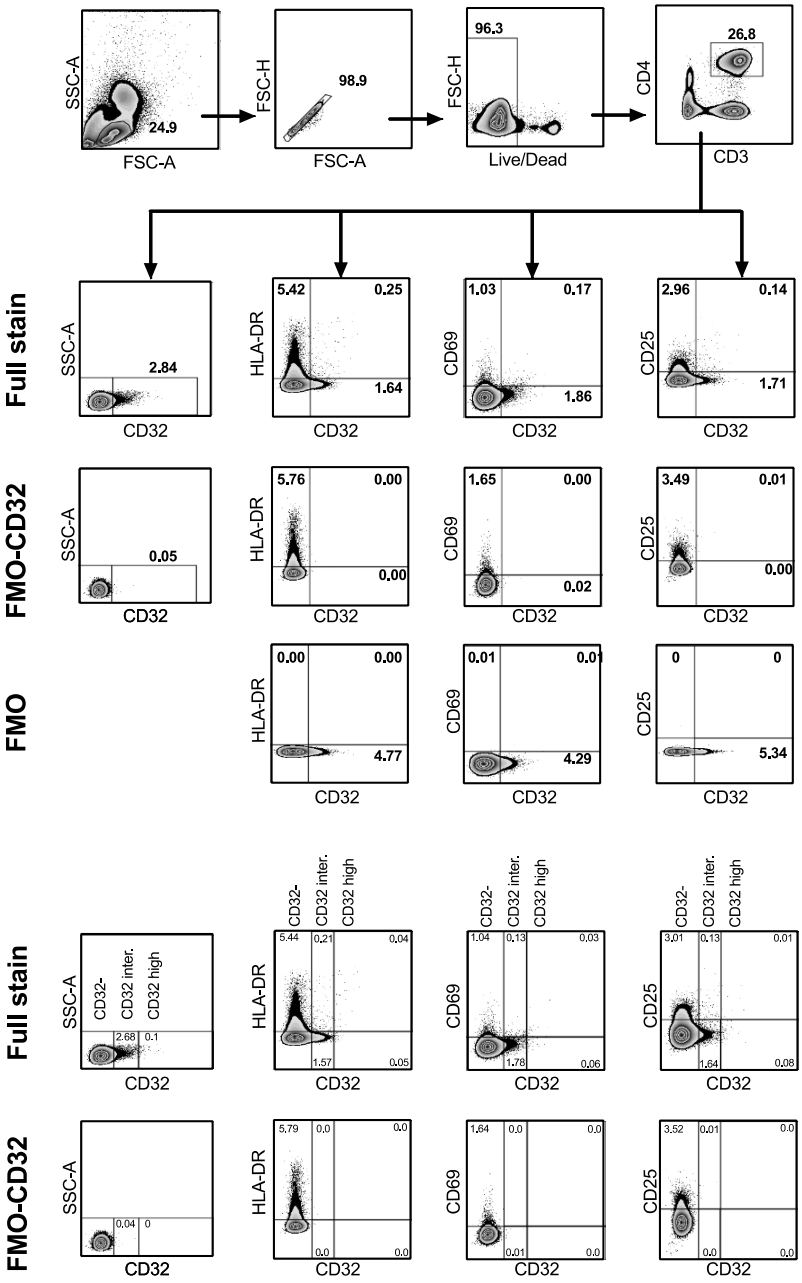

Figure S1. Representative example of the gating strategy used for analyses in Fig. 1. Fluorescence Minus One (FMO) controls were used for all samples. FMOs for CD32, as well as HLA-DR, CD69, and CD25 are presented. FSC = forward scatter, and SSC = side scatter.

Supplementary Figure 2

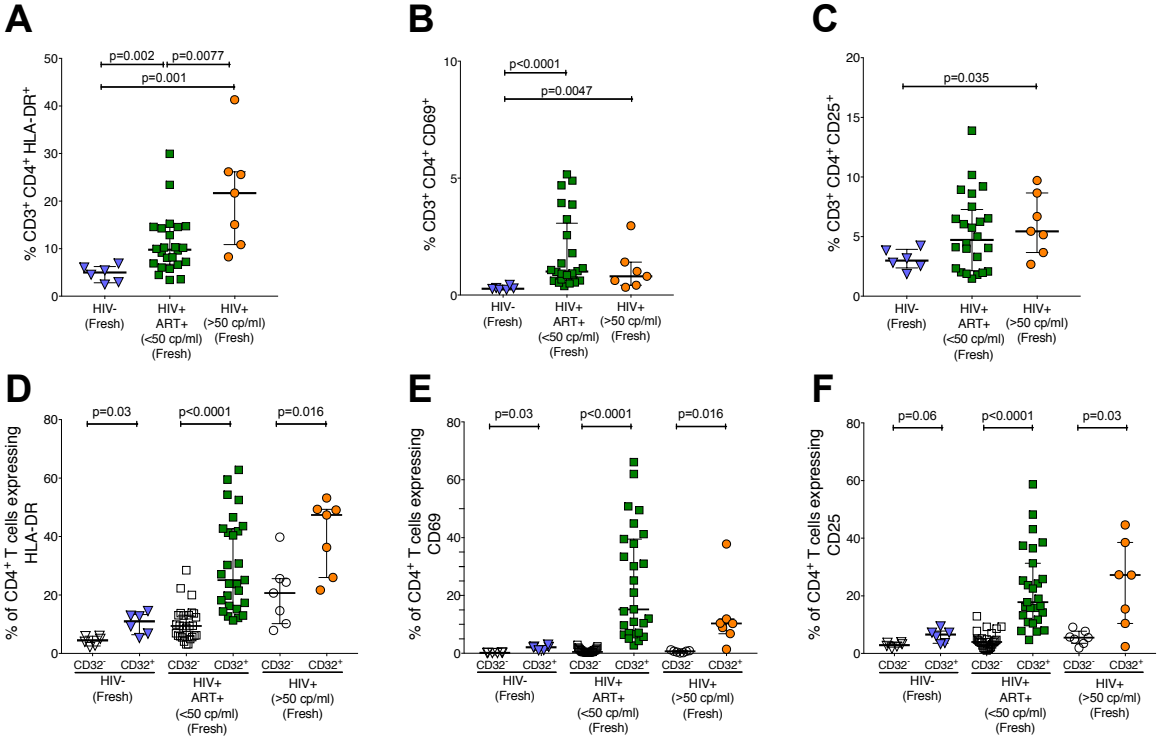

Figure S2. Percentage of cells expressing the activation markers HLA-DR, CD69, or CD25 on total CD4⁺ T cells, CD32⁺ CD4⁺ T cells, and CD32⁻ CD4⁺ T cells. (A-C) Percentage of CD4⁺ T cells expressing the activation markers HLA-DR **(A)**, CD69 **(B)**, or CD25 **(C)** on CD4⁺ T cells. **(D-F)** Percentage of cells expressing the activation markers HLA-DR **(D)**, CD69 **(E)**, or CD25 **(F)** on CD32⁺ CD4⁺ T cells and CD32⁻ CD4⁺ T cells. Lines and error bars represent median and interquartile range. All statistical comparisons were performed using two-tailed Wilcoxson rank tests. N = 6 for HIV-negative controls, 27 for HIV⁺ ART⁺ (<50 copies/ml) individuals, and 7 for HIV⁺ (>50 copies/ml) individuals.

Supplementary Figure 3

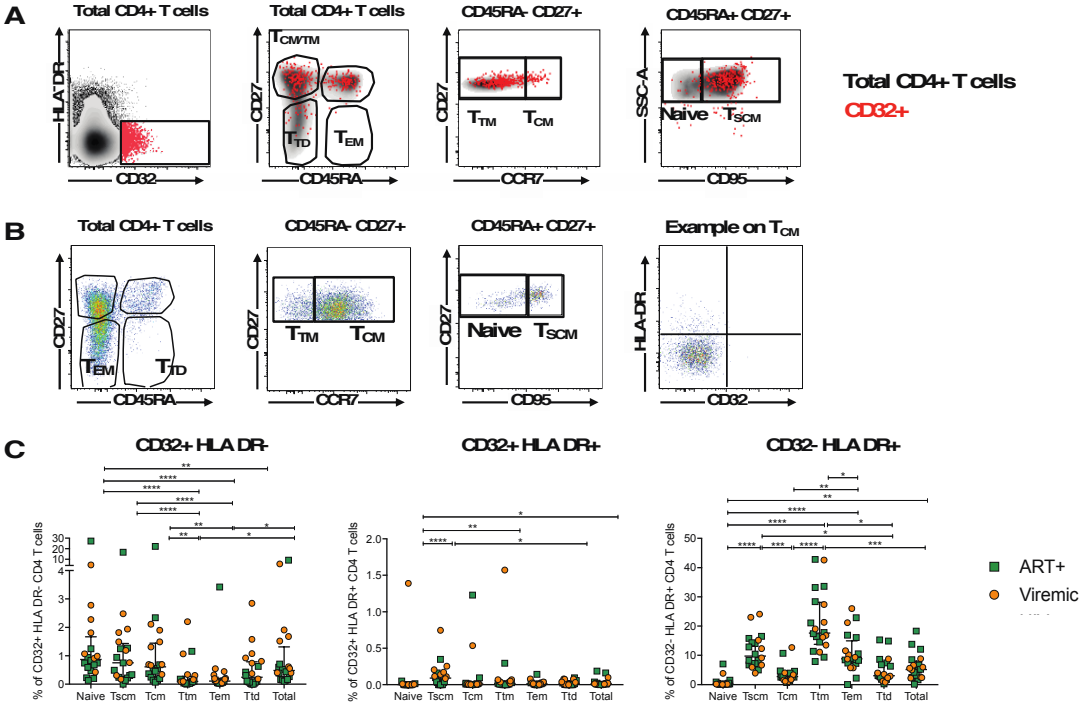

Figure S3. Phenotyping of CD32$^+$ CD4$^+$ cells from peripheral blood. (A) Overlaid plots showing the distribution of CD32$^+$ cells (red) over CD4$^+$ T cell subsets (gray). A representative example of an ART-treated donor from Figure 1. **(B)** Representative example of expression of CD32 and HLA-DR on CD4$^+$ T cell subsets on an ART$^+$ donor. All cells from **(A)** and **(B)** were gated on single viable CD3$^+$ lymphocytes, CD14$^+$, CD16$^+$, CD19$^+$, and CD56$^+$ cells were excluded. CD4$^+$ T cell were then identified, and subsets were gated using CD27, CD45RA/RO, CCR7, and CD95. **(C)** Frequencies of CD32/HLA-DR-expressing cells within each subset of PBMC from viremic and treated HIV$^+$ donors. In all graphs, lines indicate the median of the group. All data were analyzed using a Friedman test (paired, non-parametric) and corrected for multiple comparisons with Dunnett's post-test. **** p<0.0001, *** p<0.001, **p<0.01, *p<0.05. N = 12 for HIV$^+$ ART$^+$ individuals, and 8 for HIV$^+$ viremic individuals.

Supplementary Figure 4

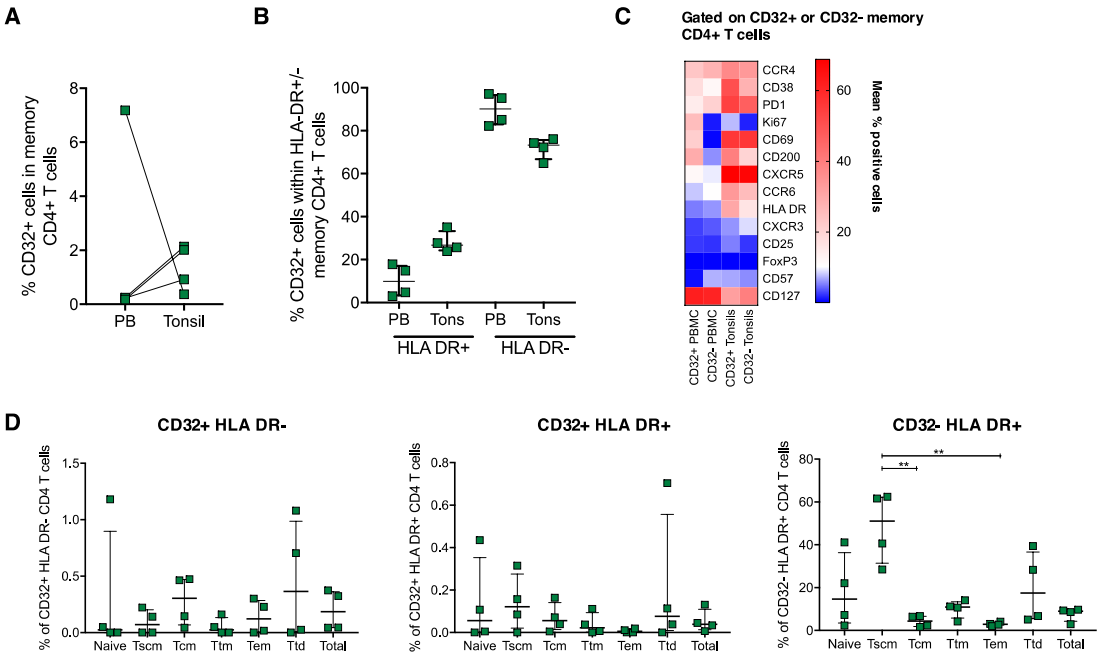

Figure S4. Phenotyping of CD32⁺ CD4⁺ cells from tonsils. (A) Frequency of CD32⁺ cells within memory CD4⁺ T cells from paired PBMC and tonsil samples. **(B)** Frequency of HLA-DR⁺ and HLA-DR⁻ cells within CD32⁺ memory CD4⁺ T cells in paired PBMC and tonsil samples. **(C)** Comparative heatmap showing the mean frequency of the analyzed phenotypic markers in CD32⁺ and CD32⁻ memory CD4⁺ T cells in paired PBMC and tonsil samples. Blue = low mean % positive cells, and red = high mean % positive cells. **(D)** Frequencies of CD32/HLA-DR-expressing cells within each subset in tonsil samples from treated HIV⁺ donors. In all graphs lines indicate the median of the group. All data were analyzed using a Friedman test (paired, non-parametric) and corrected for multiple comparisons with Dunnett's post-test. **p<0.01. N = 4.

Supplementary Figure 5

A

	RJl15	RAk15	RNi15	RWl15	RYj15
Pre-ART VL (copies/ml plasma)	1,960,000	199,000	309,000	442,000	128,000
Started ART (weeks p.i.)	6	6	6	6	6
Length of ART (weeks)	30	30	30	30	30
Weeks with VL <30	18 (with one blip at 111 copies)	26	18	18	26
# CD4+CD32-	380,248	312,661	481,764	373,203	481,764
# CD4+CD32+	24,010	50,203	42,691	11,118	42,987

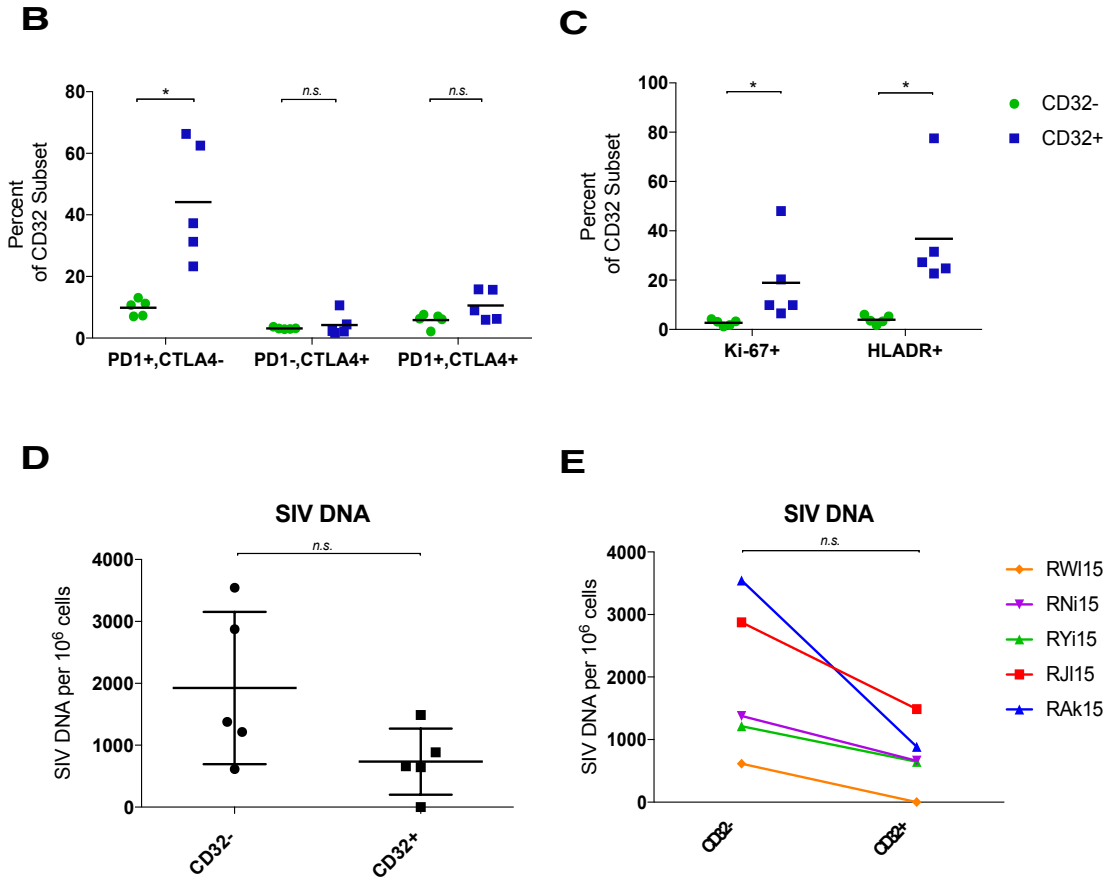

Figure S5. CD4⁺ CD32⁺ T cells in the LN of ART-suppressed, SIV-infected RMs are enriched in activation markers but not in SIV DNA. (A) Analyses were performed in LN-derived cells isolated from five SIV-infected RMs after 30 weeks of ART, initiated at 6 weeks p.i. **(B, C)** The frequency of cells expressing the co-inhibitory receptors PD-1 and/or CTLA-4 **(B)**, or the activation/proliferation markers HLA-DR and Ki67 **(C)**, were compared between $CD32^+$ and $CD32^-$ populations for each animal (lines indicate mean). **(D, E)** SIV DNA content per 10^6 cells

comparison between $CD3^+$ $CD4^+$ T cells based on CD32 expression, indicated as mean and standard deviation **(D)** or as direct comparison between each animal **(E)**. Tests of significance were performed between $CD32^+$ and $CD32^-$ using a two-tailed, Mann-Whitney test; * $p<0.01$; n.s., not significant. N = 5.

Supplementary Figure 6

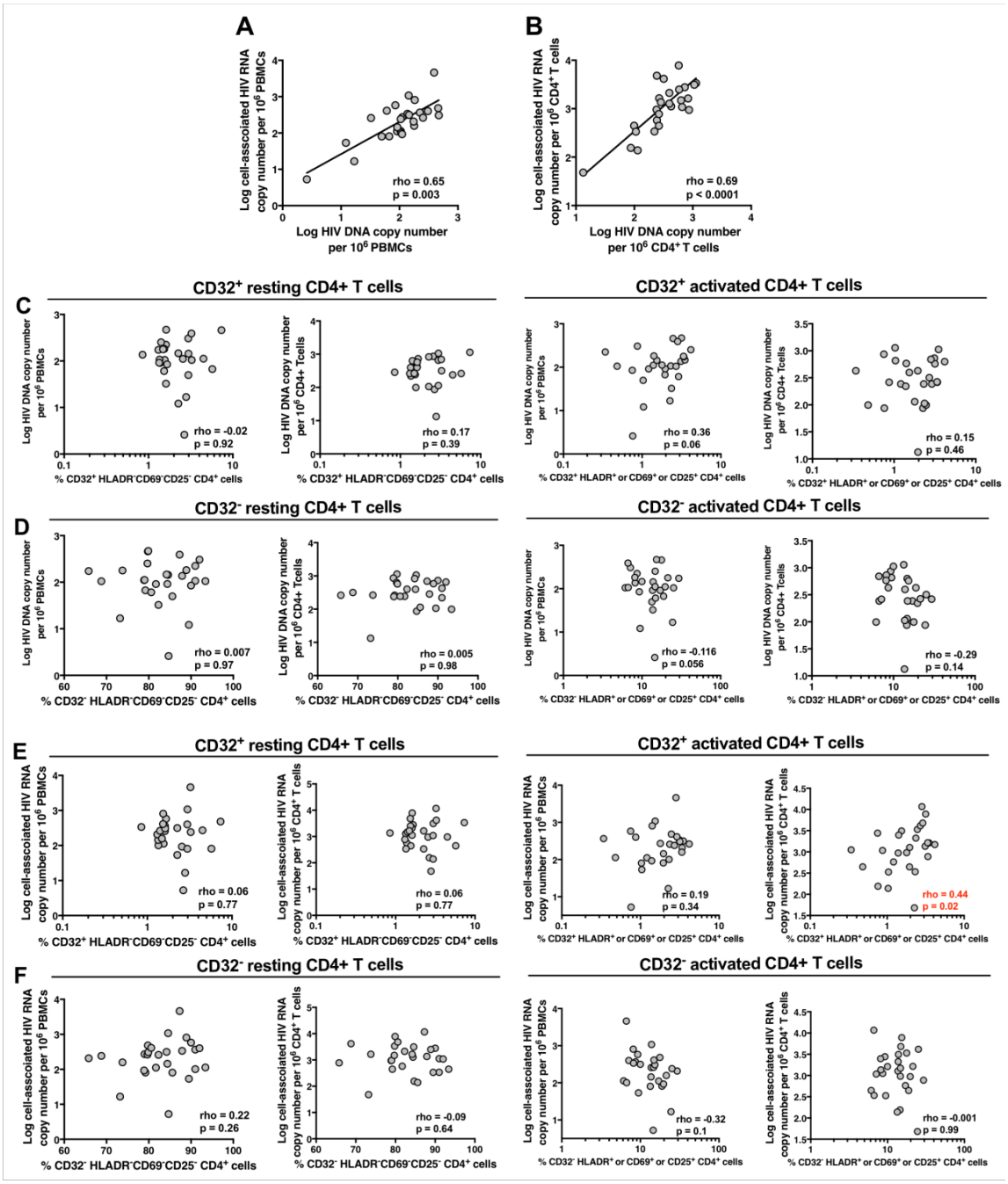

Figure S6. Lack of correlations between frequency of CD32⁺ of resting CD4⁺ T cells and HIV DNA and RNA loads. (A-B) Correlations between HIV DNA and cell-associated HIV RNA measured in **(A)** unfractionated PBMCs, or **(B)** isolated CD4+ T cells. **(C)** Frequency CD32⁺ resting CD4⁺ T cells and frequency of CD32⁺ activated CD4⁺ T cells were examined in relation to total HIV DNA load measured in unfractionated PBMCs and isolated CD4⁺ T cells. **(D)**

Frequency $CD32^-$ resting $CD4^+$ T cells and frequency of $CD32^-$ activated $CD4^+$ T cells were examined in relation to total HIV DNA load measured in unfractionated PBMCs and isolated $CD4^+$ T cells. **(E)** Frequency $CD32^+$ resting $CD4^+$ T cells and frequency of $CD32^+$ activated $CD4^+$ T cells were examined in relation to cell-associated HIV RNA load measured in unfractionated PBMCs and isolated $CD4^+$ T cells. **(F)** Frequency $CD32^-$ resting $CD4^+$ T cells and frequency of $CD32^-$ activated $CD4^+$ T cells were examined in relation to cell-associated HIV RNA load measured in unfractionated PBMCs and isolated $CD4^+$ T cells. Correlations were evaluated using two-tailed Spearman's rank correlation coefficient tests. N = 27 HIV^+ ART^+ individuals.

Supplementary Figure 7

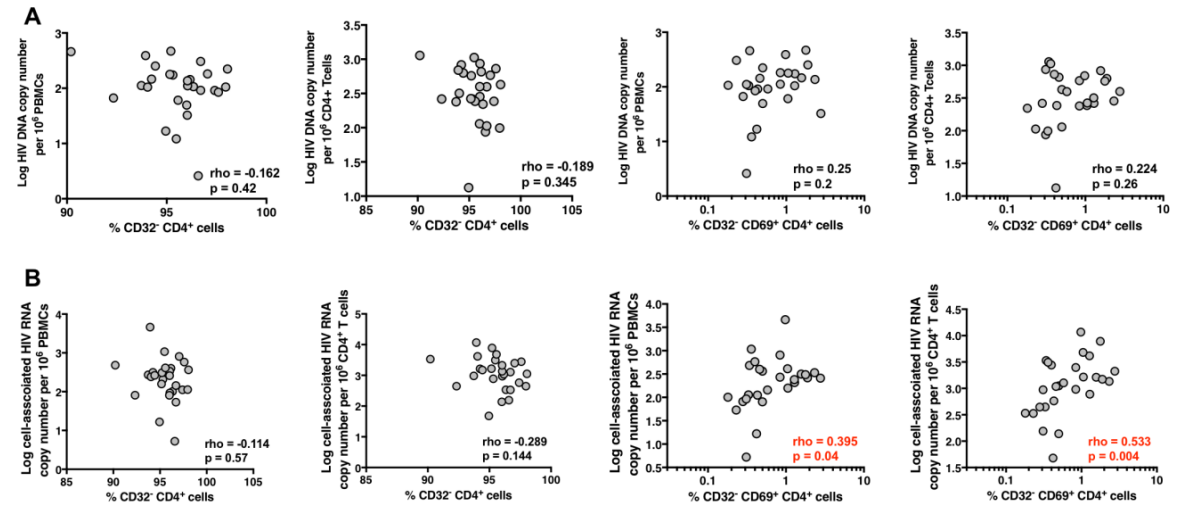

Figure S7. Correlations between frequency of CD32^{-} CD4^{+} T cells and HIV DNA and RNA loads. (A) Frequency CD32^{-} CD4^{+} T cells and frequency of CD32^{-} on CD69^{+} CD4^{+} T cells were examined in relation to total HIV DNA load measured in unfractionated PBMCs and isolated CD4^{+} T cells. **(B)** Frequency CD32^{-} CD4^{+} T cells and frequency of CD32^{-} on CD69^{+} CD4^{+} T cells were examined in relation to cell-associated HIV RNA load measured in unfractionated PBMCs and isolated CD4^{+} T cells. Correlations were evaluated using two-tailed Spearman's rank correlation coefficient tests. N = 27 HIV^{+} ART^{+} individuals.

Supplementary Figure 8

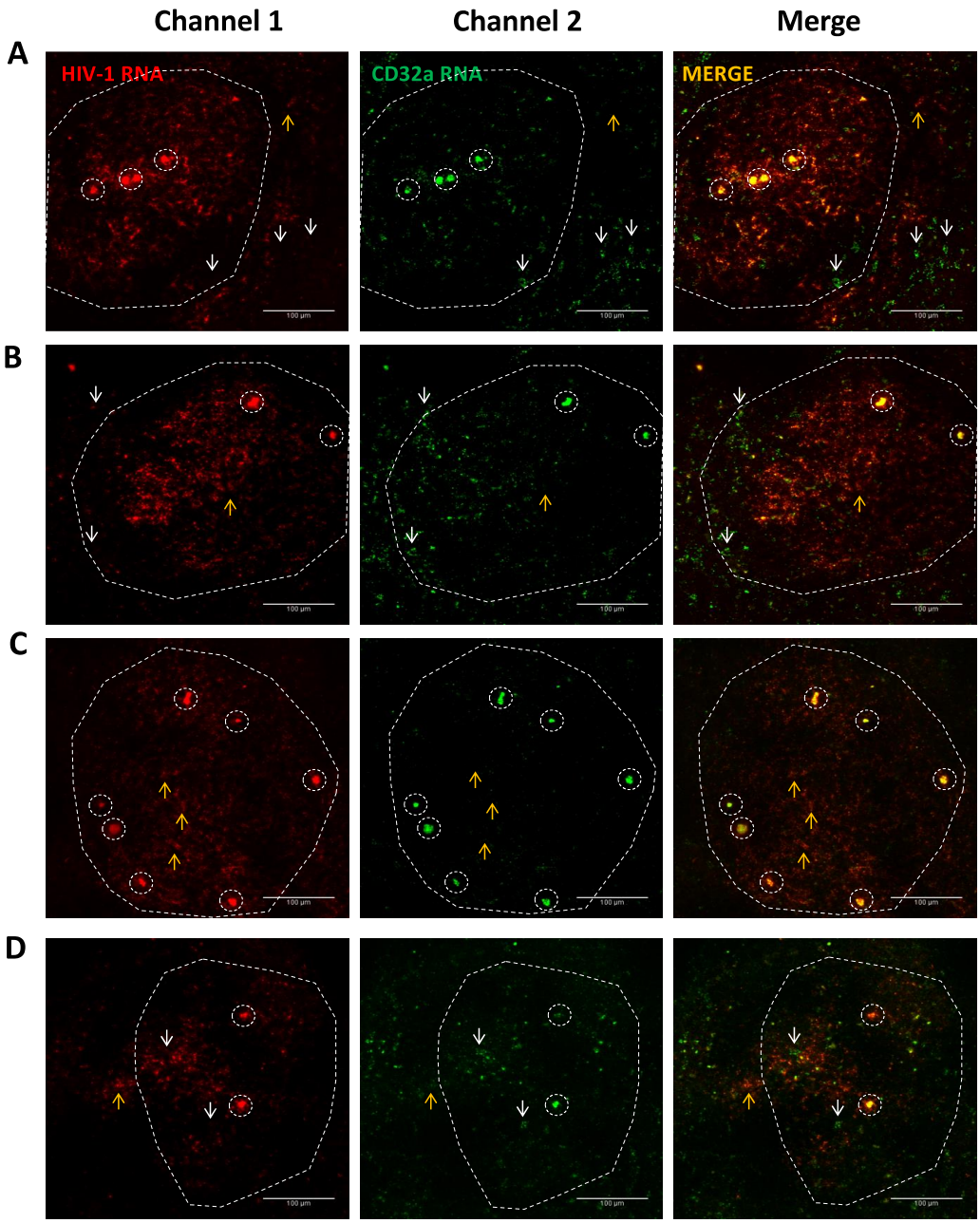

Figure S8. Different HIV RNA and CD32a RNA pattern expression in LN samples from HIV-infected patients. Four different lymph node B cell follicles (**A-D**) of a viremic HIV-infected patient are shown. Channel 1 (red) shows the expression of HIV-RNA, and channel 2 (green) shows the expression of CD32a. White arrows indicate single CD32a RNA positive cells, yellow arrows indicate single HIV-RNA positive cells, and circles show cells co-expressing HIV-RNA and CD32a.

Table S1. Clinical data of patients included in Figs. 1 and 5.

	Donor ID	CD4 count (cells/mm3)	CD4 %	VL (copies/ml)	Gender†	Race¥	Age (years)	Current regime‡	Approx. years on ART	HIV DNA in PBMCs (copies per 10^6 cells)	HIV DNA in CD4 T cells (copies per 10^6 cells	HIV RNA in PBMCs (copies per 10^6 cells)	HIV RNA in CD4 T cells (copies per 10^6 cells
HIV, VL < 50	Donor 1 Visit 1	524	28%	Undetected	M	AA	56	ABC, 3TC, DTG	10	91.1	243.5	112.0	578.2
	Donor 1 Visit 2	460	26%	Undetected	M	AA	56	ABC, 3TC, DTG	10.5	119.3	180.4	223.57	472.89
	Donor 2	739	40%	Undetected	M	AA	37	DTG, RPV	2.5	2.6	86.8	5.3	155.1
	Donor 3 Visit 1	1095	39%	Undetected	M	AA	32	FTC, RPV, TDF	4	223.3	426.4	365.2	1117.4
	Donor 3 Visit 2	1055	38%	Undetected	M	AA	32	FTC, RPV, TDF	4.5	58.6	142.6	738.45	1660.34
	Donor 4	1022	50%	Undetected	M	AA	53	EVG/c, FTC, TDF	5	105.1	99.1	113.0	445.1
	Donor 5	506	38%	Undetected	M	AA	23	EVG/c, FTC, TDF	5	107.0	220.5	101.1	339.3
	Donor 6	452	23%	Undetected	M	AA	55	ABC, 3TC, DTG	7.5	16.8	13.3	16.6	48.0
	Donor 7 Visit 1	482	37%	Undetected	M	AA	27	FTC, RPV, TDF	3	66.8	262.1	81.2	443.7
	Donor 7 Visit 2	516	33%	Undetected	M	AA	27	FTC, RPV, TDF	3.5	151.1	279.5	42.12	1038.93
	Donor 8 Visit 1	542	27%	Undetected	M	AA	37	FTC, TDF, DRV/r	7.5	459.5	1136.0	482.2	3369.9
	Donor 8 Visit 2	575	31%	Undetected	M	AA	37	FTC, TDF, DRV/r	8	116.0	708.6	1319.69	5479.55
	Donor 9	880	27%	Undetected	M	AA	49	ABC, 3TC, DTG	4.5	110.7	862.5	92.7	941.4
	Donor 10 Visit 1	579	25%	Undetected	M	AA	30	ABC, 3TC, DTG	1.75	91.8	396.7	143.9	1272.2
	Donor 10 Visit 2	579	29%	Undetected	M	AA	30	ABC, 3TC, DTG	2	48.7	519.3	99.55	2864.13
	Donor 11 Visit 1	491	25%	Undetected	M	AA	39	ABC, 3TC, DTG	7.5	143.7	1059.7	1077.1	3116.5
	Donor 11 Visit 2	472	24%	Undetected	M	AA	39	ABC, 3TC, DTG	8	48.7	519.3	99.55	2864.13
	Donor 12	695	35%	Undetected	M	AA	25	EVG/c, FTC, TDF	2	49.6	113.9	80.5	137.9
	Donor 13	1035	39%	Undetected	M	C	57	FTC, RPV, TDF	6	84.9	729.9	580.2	2771.8
	Donor 14	938	38%	Undetected	M	AA	29	ABC, 3TC, DTG	6.5	12.1	106.1	53.5	336.0
	Donor 15 Visit 1	542	32%	Undetected	M	C	51	EFV, TDF, FTC	18	305.8	657.2	399.6	1084.6
	Donor 15 Visit 2	573	33%	Undetected	M	C	51	EFV, TDF, FTC	18.5	166.5	319.3	1113.92	4282.48
	Donor 16	960	36%	Undetected	M	AA	52	EVG/c, FTC, TAF	14	60.7	242.6	411.5	4,796.8
	Donor 17	869	40%	Undetected	M	AA	47	FTC, TDF, ATV	7	178.5	265.6	157.8	1,643.8
	Donor 18	1258	46%	Undetected	F	AA	62	3TC, DTG, ABC	15	390.7	694.8	4,606.2	11,689.3
	Donor 19	1309	NA	Undetected	M	C	60	FTC, TDF, DTG	9	111.8	239.3	271.6	957.2

	Donor 20	480	NA	Undetected	F	C	55	FTC, TDF, RAL	10	136.5	284.9	337.2	1,355.9
	Donor 21	425	NA	Undetected	M	C	56	FTC, RPV, TDF	10	145.9	829.2	315.1	1,629.2
	Donor 22	395	NA	Undetected	M	C	37	DRV, FTC, TDF	8	32.5	397.9	258.0	2,119.6
	Donor 23	376	NA	Undetected	M	Hispanic	54	3TC, DTG, ABC	23	173.9	262.1	207.7	774.8
	Donor 24	132	NA	Undetected	F	C	25	EVG/c, FTC, TAF	2	104.9	317.7	241.1	4,140.1
	Donor 25	716	NA	Undetected	M	AA	47	FTC, RPV, TDF	6	182.2	583.7	807.1	2,488.2
	Donor 26	432	NA	Undetected	M	AA	60	DRV/c, FTC, TDF	8	252.4	630.7	262.9	1,488.8
	Donor 27	348		Undetected	F	C	48	FTC, TDF, RTV	8	469.9	574.2	307.4	7,814.2
	Donor 28	829	30	Undetected	F	C	54	TAF / FTC / RAL	5	NA	NA	NA	NA
	Donor 29	781	42	Undetected	M	C	55	TDF / FTC / RAL	6	NA	NA	NA	NA
	Donor 30	623	38	Undetected	M	C	39	TAF / FTC / DRVr	4	NA	NA	NA	NA
	Donor 31	472	24	Undetected	M	C	52	TAF / FTC / DTG	3	NA	NA	NA	NA
	Donor 32	694	32	Undetected	M	C	38	TAF / FTC / RPV	8	NA	NA	NA	NA
	Donor 33	1107	43	Undetected	M	C	32	TAF / FTC / RPV	5	NA	NA	NA	NA
HIV, VL > 50	**Donor 34**	357	25%	15,748	M	AA	29	no ART	-	111.1	703.0	786.5	4142.4
	Donor 35	595	28%	573	F	AA	51	TDF, FTC, RAL	8	208.3	998.8	236.0	381.2
	Donor 36	337	26%	146	M	AA	55	DRV/c, DTG, 3TC	15	329.1	1090.7	120.6	2583.2
	Donor 37	234	7%	27,380	F	AA	50	DRVc, FTC, DTG	7	86.3	923.7	541.3	8231.6
	Donor 38	223	14%	19,220	M	AA	54	no ART	-	643.2	3045.5	3664.7	26714.9
	Donor 39	409	17%	45,333	M	AA	48	no ART	-	1445.2	12406.3	20667.9	107676.6
	Donor 40	137	NA	780	M	AA	52	DRV, RTV, ABC	10	1,453.5	1,984.2	1,806.7	9,178.3
HIV-	**Donor 41**	-	-	-	M	AA	65	-	-				
	Donor 42	-	-	-	M	AA	58	-	-				
	Donor 43	-	-	-	M	AA	48	-	-				
	Donor 44	-	-	-	F	AA	61	-	-				
	Donor 45	-	-	-	F	AA	33	-	-				
	Donor 46	-	-	-	F	C	33	-	-				

† M = male, F = female

¥ AA = African American, C = Caucasian

‡ FTC, emtricitabine; TDF, tenofovir; 3TC, lamivudine; EFV, efavirenz; ABC, abacavir; RAL, raltegravir; EVG/c, elvitegravir boosted with cobicistat; DTG, dolutegravir; DRV/r, darunavir boosted with ritonavir; DRV/c, darunavir boosted with cobicistat; RPV, Rilpivirine, TAF = tenofovir alafenamide fumarate, ATV = atazanavir, RTV = ritonavir.

Table S2. DNA quantity and number of sorted cells examined in Fig. 5C.

	PBMCs		Total $CD4^+$ T Cells		$CD32^-$ $CD4^+$ T Cells		$CD32^+$ $CD4^+$ T Cells		$CD32^+$ resting $CD4^+$ T Cells		$CD32^-$ resting $CD4^+$ T Cells		$CD32^+$ activated $CD4^+$ T Cells		$CD32^-$ activated $CD4^+$ T Cells	
Donor ID	**HIV DNA (copies /10^6 cells)**	**Number of cells examined**	**HIV DNA (copies /10^6 cells)**	**Number of cells examined**	**HIV DNA (copies /10^6 cells)**	**Number of cells examined**	**HIV DNA (copies /10^6 cells)**	**Number of cells examined**	**HIV DNA (copies /10^6 cells)**	**Number of cells examined**	**HIV DNA (copies /10^6 cells)**	**Number of cells examined**	**HIV DNA (copies /10^6 cells)**	**Number of cells examined**	**HIV DNA (copies /10^6 cells)**	**Number of cells examined**
Donor 1 Visit 2	119.3	12260.4	180.4	35971.6	122.1	71678.2	66.5	55964.1	440.0	32996.1	750.9	46544.9	235.0	24359.9	603.2	15197.0
Donor 3 Visit 2	58.6	70329.2	142.6	26261.3	94.7	4902.8	47.0	28132.0	243.5	26037.3	304.6	35523.6	188.1	13882.6	287.1	15372.8
Donor 7 Visit 2	151.1	23469.5	279.5	44526.1	176.3	49616.2	448.9	33233.1	144.3	24720.5	233.5	40246.1	852.8	33458.8	592.8	30790.2
Donor 8 Visit 2	116.0	52867.3	708.6	40353.9	898.9	48103.6	964.4	50883.6	746.9	33272.4	611.4	42226.8	1681.9	35292.9	1368.6	40842.3
Donor 10 Visit 2	48.7	91505.5	519.3	13772.8	84.5	47282.9	475.9	24707.3	150.2	68980.7	248.2	24539.6	77.7	81170.6	330.6	23576.6
Donor 11 Visit 2	286.1	58076.8	1355.1	52412.5	920.4	39857.0	1299.3	49318.4	975.4	36547.0	441.1	41949.8	1890.6	37155.6	995.3	29269.1
Donor 15 Visit 2	166.5	31241.7	319.3	68282.3	241.7	58569.3	247.5	61032.6	601.5	50499.1	222.5	56844.7	894.2	52747.0	948.6	37454.5
Donor 16	60.7	56279.8	242.6	67335.6	156.6	50625.9	144.9	53227.0	180.2	54101.9	143.3	45965.9	534.5	46810.6	452.0	50111.0
Donor 17	178.5	21316.6	265.6	30088.6	348.7	33094.2	171.6	57778.6	373.1	27878.7	196.7	35023.5	394.3	53639.1	249.8	14801.1
Donor 18	390.7	57836.4	694.8	55803.3	1500.6	35554.0	1086.4	48578.7	675.7	68318.7	1314.9	58849.6	633.6	61907.1	677.0	49604.7

Table S3. Clinical data of patients included in Figs. 2, 3, and 6.

Patient ID	CD4 Cell Count (cells/µl)	Viral Load (copies/ml)	Time on ART (months)	ART regimen
1[a]	151	<20	10	TDF, FTC, EVG/c
2[a]	240	272	--	--
3[a]	1150	<20	29	TDF, FTC, RPV
4[a]	660	<20	1	COB, EVG, FTC, TDF
5[a]	700	<20	1	ABC, DTV, 3TC
6[a]	480	178.000	--	--
7[a]	1050	<20	174	COB, DRV
8[a]	1050	<20	84	COB, DRV
9[a]	1150	<20	169	COB, DRV
10[a]	1200	<20	167	COB, DRV
11[b]	820	91	--	--
12[b]	1130	3755	--	--
13[b]	730	1022	--	--
14[b]	800	332257	--	--
15[b]	800	332257	--	--
16[b]	430	170693	--	--
17[b]	280	48425	--	--
18[b]	610	28955	--	--
19[b]	780	13948	--	--
20[b]	840	<50	>100	NA
21[b]	730	<50	13	NA
22[b]	600	<40	6	NA
23[b]	360	<40	7	NA
24[b]	870	<40	14	NA
25[c*]	1136	<40	81	EFV, FTC, TDF
26[c*]	412	<40	52	EFV, FTC, TDF
27[c*]	538	<40	40	FTC, TDF, RAL
28[c*]	412	524[♦]	15	EFV, FTC, TDF
29[b]	450	<50		NA
30[b]	730	152[♦]	12	NA
30[d]	1137	<20	216	TDF, FTC, RAL

[a] Samples obtained at Vall d´Hebron Research Institute, Barcelona. [b] Samples from University of Toronto. [C] Samples from INER-CIENI. [d]Samples from Wistar. *These samples were analyzed for both PBMC and tonsil. [♦]This donor had undetectable viral load for # months, but had a VL blip at the time of sample collection.

FTC, emtricitabine; TDF, tenofovir; 3TC, lamivudine; EFV, efavirenz; ABC, abacavir; RAL, raltegravir; EVG/c, elvitegravir boosted with cobicistat; COB, Cobicistat; RPV, Rilpivirine; NA, not available.