Supplementary Materials

Supplementary Tables

Supplementary Table 1: Characteristics of HIV-1-infected individuals.

Characteristics	Values
Number of subjects	42
Age (years)	33 (25–48)
Plasma HIV RNA load (copies/mL)	<40
Median duration of HIV infection (years), medians (IQRs)	9.00 (7.50–11.25)
Median duration of ART (years), medians (IQRs)	7.50 (5.38–9.00)
HIV-1 subtype, *n* (%)	
CRF01_AE	24 (57.1)
CRF07_BC/CRF08_BC	11 (26.2)
B/B′	2 (4.8)
CRF55_01B	2 (4.8)
URF	3 (7.1)
$CD4^+$ T-cell counts (cells/μL), medians (IQRs)	552 (457–765)
$CD8^+$ T-cell counts (cells/μL), medians (IQRs)	746 (559–914)
CD4/CD8 T-cell ratio, medians (IQRs)	0.80 (0.59–1.05)

ART: Antiretroviral therapy; HIV: Human immunodeficiency virus; IQRs: Interquartile ranges; URF: Unique recombinant subtypes.

Supplementary Table 2: Sequences of primers and probes used in total HIV DNA and integrated HIV DNA assays.

Target	Step	Name	Sequence (5′ to 3′)	Position
Total DNA	ddPCR	HIV-FWD-1	CCYgggAgCTCTCTggCT*	nt482–nt489 (LTR 5')
		HIV-RES-1	CTGAGGGATCTCTAGTTACC	nt582–nt682 (LTR 5′)
		HIV-Probe-FAM	5′-FAM-CACTCAAggCAAGCTTTATTgAgg C-3′-BHQ3	nt526–nt550 (LTR 5′)
Integrated DNA	Pre-amplification	ULF1	ATGCCACGTAAGCGAAAC TCTGGGTCTCTCTDGTTAGAC†	nt452–471 (LTR 5′)
		Alu1	TCCCAGCTACTGGGGAGG CTGAGG	NA
		Alu2	GCCTCCCAAAGTGCTGGG ATTACAG	NA
	ddPCR	Lambda T	ATGCCACGTAAGCGAAACT	NA

		HIV-RES-1	CTGAGGGATCTCTAGTTACC	nt582–nt682 (LTR 5′)
		HIV-Probe-FAM	5′-FAM-CACTCAAggCAAGCTTTATTgAggC-3′-BHQ3	nt526–nt550 (LTR 5′)
***CD3* gene**	ddPCR	CD3-FWD	gTATCCCTAACCTTCAgCC	NA
		CD3-RES	CTTCTCCTCCATgggACAC	NA
		CD3-probe-VIC	5′-VIC-AGCAGAGAACAGTTA AGAGCCAT-3′-BHQ1	NA

*Y=C/T, †D=G/A/T. ddPCR: Droplet digital polymerase chain reaction; HIV: Human immunodeficiency virus; NA: Not applicable.

Supplementary Table 3. Reproducibility of the total HIV ddPCR assay

	Measured HIV copies/10^6		
HIV copies/10^6 PBMCs	**PBMCs**	**SD**	**CV%**
1000	546.00 (528.00–643.00)	72.61	13.29
500	306.16 (232.00–364.00)	54.82	17.91
100	54.33 (39.00–66.00)	11.26	20.71

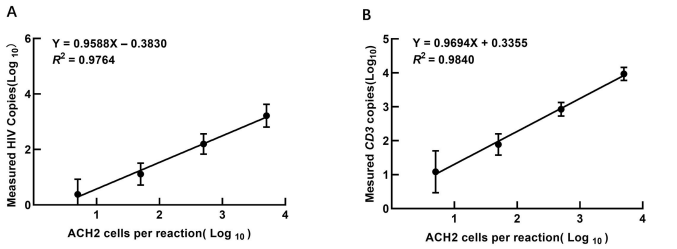
Data are presented as medians (IQRs). CV%: Coefficient of variation; ddPCR: Droplet digital polymerase chain reaction; HIV: Human immunodeficiency virus; IQRs: Interquartile ranges; PBMCs: Peripheral blood mononuclear cells. SD: Standard deviation.

Supplementary Table 4. Reproducibility of the integrated HIV ddPCR assay

	Measured HIV copies/10^6		
HIV copies/10^6 PBMCs	**PBMCs**	**SD**	**CV%**
1000	314 (250–365)	40.15	12.76
500	208 (156–240)	30.16	14.39
100	19 (14–25)	4.34	22.82

Data are presented as medians (IQRs). CV%: Coefficient of variation; ddPCR: Droplet digital polymerase chain reaction; HIV: Human immunodeficiency virus; IQRs: Interquartile ranges; PBMCs: Peripheral blood mononuclear cells. SD: Standard deviation.

Supplementary Figures



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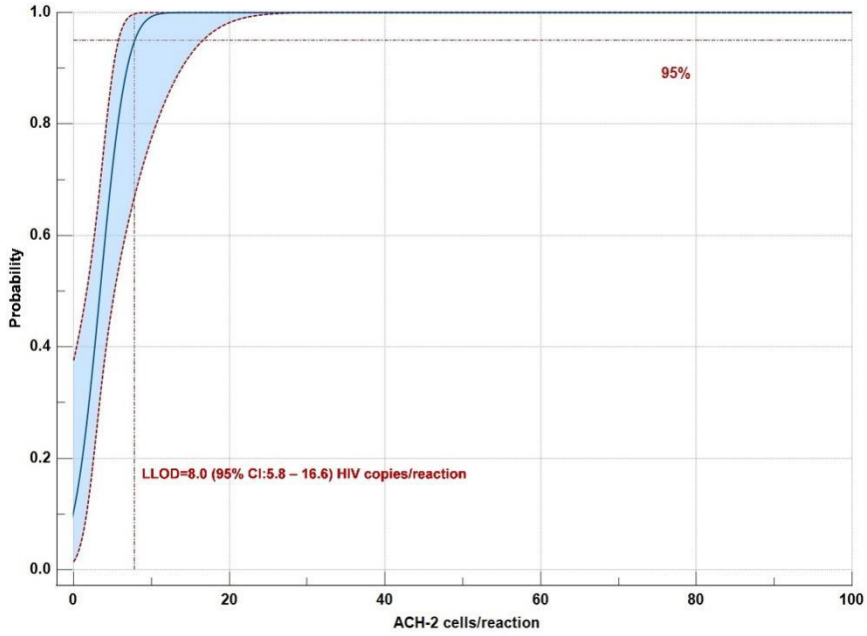
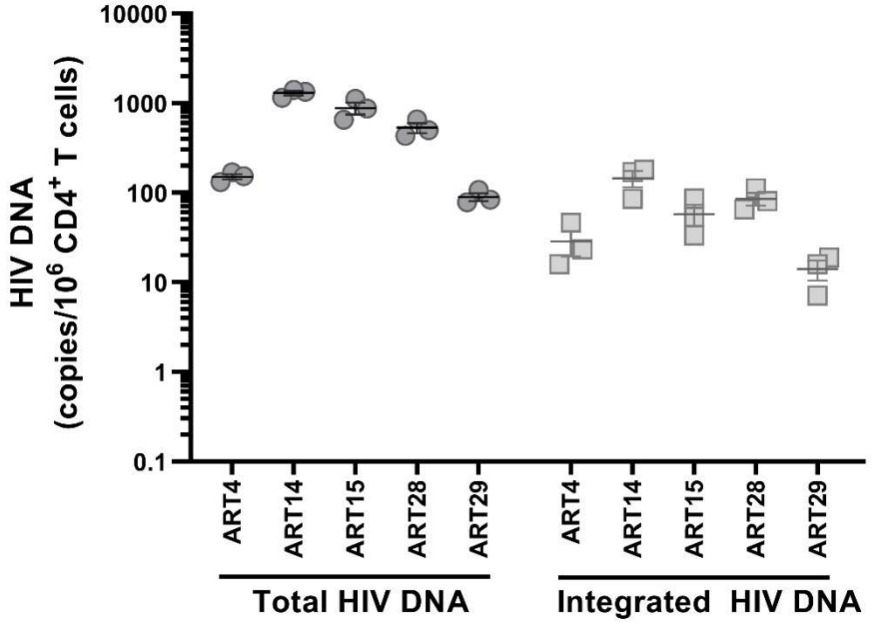
ACH-2 cell Input	HIV copies measured	*CD3* copies measured
5000	1727.54 ± 680.80	9418.85 ± 1659.70
500	162.88 ± 54.81	858.47 ± 160.63
50	13.56 ± 4.66	79.82 ± 24.19
5	2.62 ± 1.36	13.61 ± 8.19

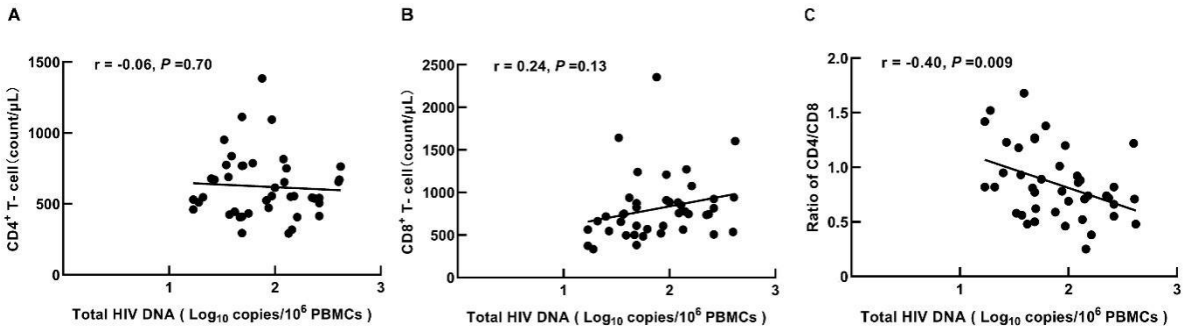
Supplementary Figure S1. The linear dynamic range of integrated HIV droplet digital PCR (ddPCR) assays. Panel (A) and Panel (B) show linearity experiments where the data was fit with a linear model, the concentrations ranged from 5000 to 5 ACH-2 cells/reaction. Panel (C) provides quantification data for panel (A) and panel (B). Panel Error bars indicate 95% total Poisson CIs for three replicates, and in some cases, the error bars are too small to visualize.



Supplementary Figure S2. Lower limit of detection (LLOD) of integrated HIV DNA droplet digital PCR assay. The probability of detecting HIV (%) in 1:2 serial dilutions of ACH-2 cells from 100 to 1.6 input copies/reaction was analyzed by probit regression. The red dotted line depicts the 95% probability of detection.



Supplementary Figure S3. **Reproducibility of total and integrated DNA from 5 clinical samples.** Total HIV DNA and integrated HIV DNA were measured in $CD4^+$ T cells isolated from 5 virally suppressed individuals receiving ART in 3 independent experiments. Values are presented as the mean ± standard deviation.



Supplementary Figure S4. Relationship between immunologic parameters and total HIV DNA in PBMCs. (A). Correlation between total HIV DNA in PBMCs and $CD4^+$ T-cell counts. (B). Correlation between total HIV DNA in PBMCs and $CD8^+$ T-cell counts. (C). Correlation between total HIV DNA in PBMCs and the CD4/CD8 T-cell ratio. Linear regression analysis was performed with a significance level of 0.05.