SUPPLEMENTARY INFORMATION

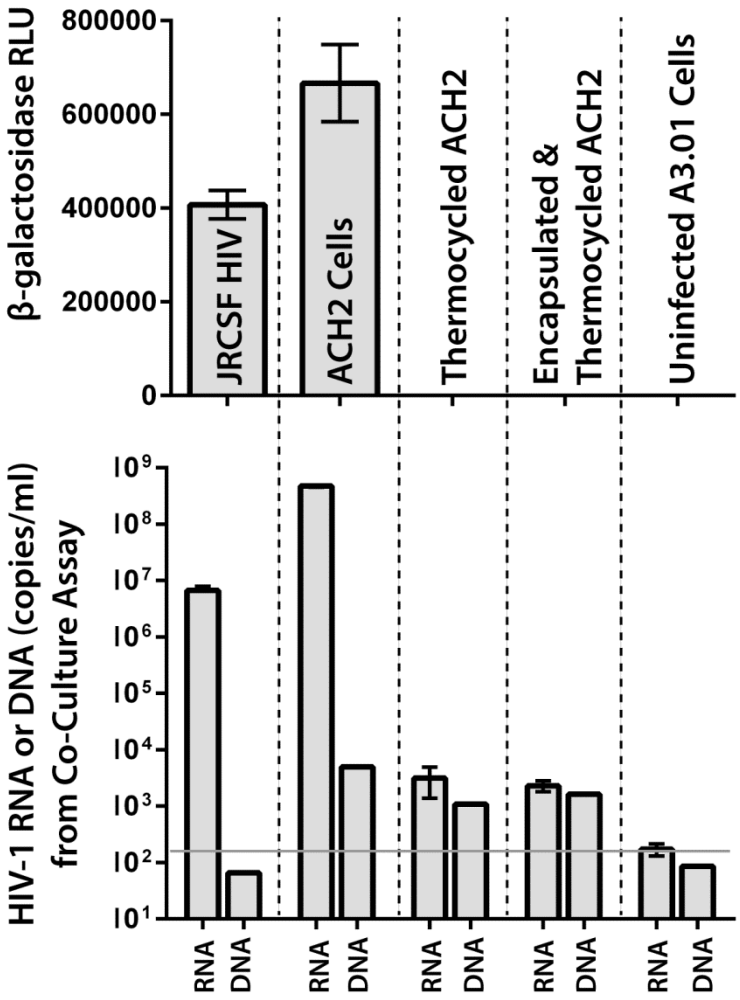
Supplementary Table 1. scdPCR mastermix and thermocycling conditions.

	msRNA			usRNA		
	Conc.		µL / Reaction	Conc.		µL / Reaction
Enzyme	2x	One-Step RT ddPCR Supermix (Biorad)[a]	10	2x	One-Step RT ddPCR Supermix (Biorad)[a]	10
Mn Acetate	25 mM		0.8	25 mM		0.8
Forward Primer	10 µM	ks1: 5'-CTTAGGCATCTC CTATGGCAGGAA[c]	0.12	100 µM	5'- TACTGACGCTCTCGCACC[d]	0.12
Reverse Primer	10 µM	mf-83: 5'-GGATCTGTCTCTGT CTCTCTCTCCACC	0.12	100 µM	5'-TCTCGACGCAGGACTCG	0.12
Probe	10 µM	ks2: 5'-FAM-TTCCTTCGGGCC TGTCGGGTCCC	0.5	5 µM	FAM-CTCTCTCCTTCTAGCCTC	1
Cell suspension	10^6 cells/mL		10	10^6 cells/mL		10
Lysis buffer			2[b]			2[b]
Cycling conditions	60°C, 30 min / 95°C, 5 min / 94°C, 30 sec; 60°C, 1 min 40x / 98°C, 10 min / 4°C hold					

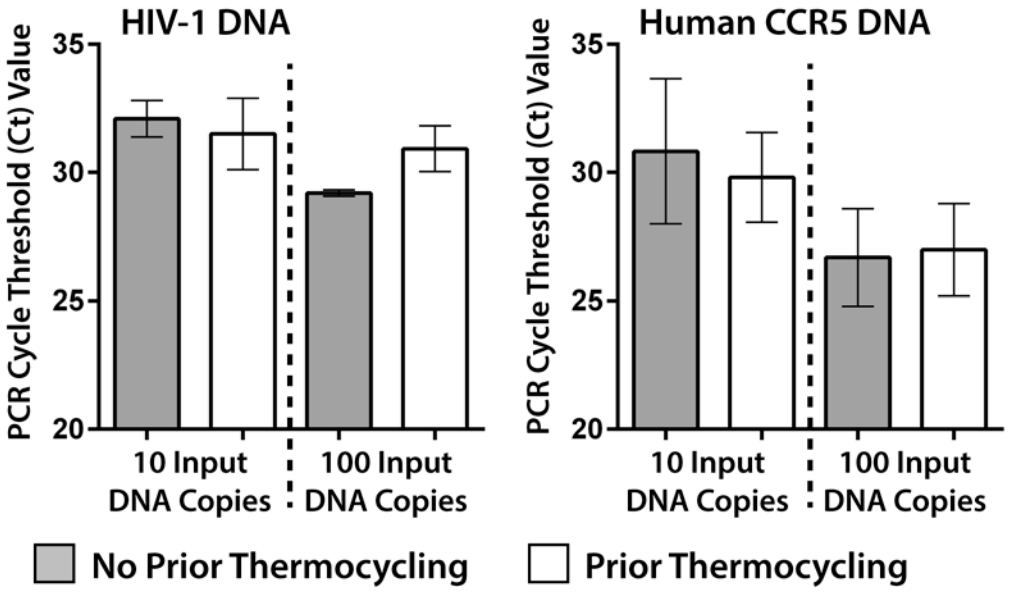
[a] Samples from Participant B and C were used using the updated BioRad One-Step RT-ddPCR Advanced Kit which eliminates that need for addtional Mn Acetate and uses additional DTT. Assay volumes and concentrations of primer and probes were kept the same.
[b] lysis buffer added immediately prior to cell encapsulation
[c] {Kiselinova, 2014 #1131}
[d] {Malnati, 2008 #449}

Supplementary Table 2. Percent decrease in cell viability 24 hours following T-cell receptor or romidepsin pulse stimulation compared with unstimulated cells

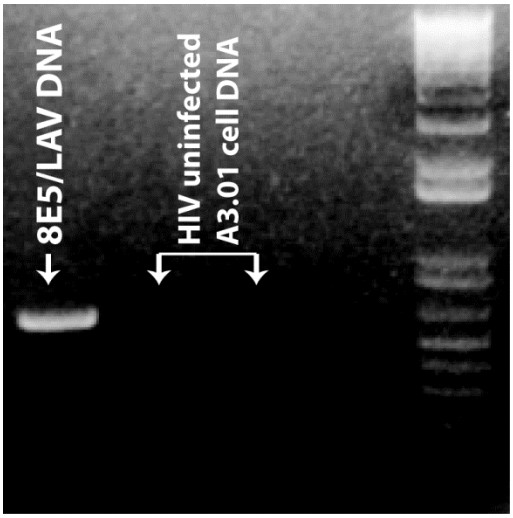
Participant	% Decrease in Live Cells	
	T-cell receptor (αCD3/ αCD28) stimulation	Romidepsin pulse
A	1.3	0
B	1.9	4.6
C	4.4	9.8
D	2.7	13.3
E	4.1	16.7
F	5.2	16.5



Supplementary Figure 1. Infectivity and replication capacity of infected ACH2 cells following scdPCR. High levels of β-galactosidase, a surrogate for HIV-1 entry into TZM-bl cells, were observed in cell infectivity experiments incorporating unmanipulated ACH2 cells or infectious HIV-1 JRCSF; no β-galactosidase activity was detected in experiments including either encapsulated or non-encapsulated, thermocycled ACH2 cells (top). Encapsulated, thermocycled ACH2 cells did not lead to increasing HIV-1 RNA in supernatants over 14 days of co-culture, with RNA levels being similar to DNA in supernatants derived from dead or lysed cells (bottom).



Supplementary Figure 2. Real-time PCR cycle threshold values for the detection of genomic HIV-1 integrase or human CCR5 DNA do not decrease following thermocycling using scdPCR conditions. Error bars represent standard error from duplicate experiments. These results suggest that secondary genomic DNA targets can be quantified following scdPCR screening for cells harboring HIV-1 RNA.



Supplementary Figure 3. Agarose gel electrophoretic band of HIV-1 envelope DNA amplified from genomic DNA extracted from isolated droplets containing 8E5/LAV or A3.01 cells following scdPCR. DNA was only able to be obtained from fluorescent droplets containing infected 8E5/LAV cells.