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Supplemental information

Lymph node dendritic cells harbor inducible replication-competent HIV despite years of suppressive ART

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Supplemental Fig. 1

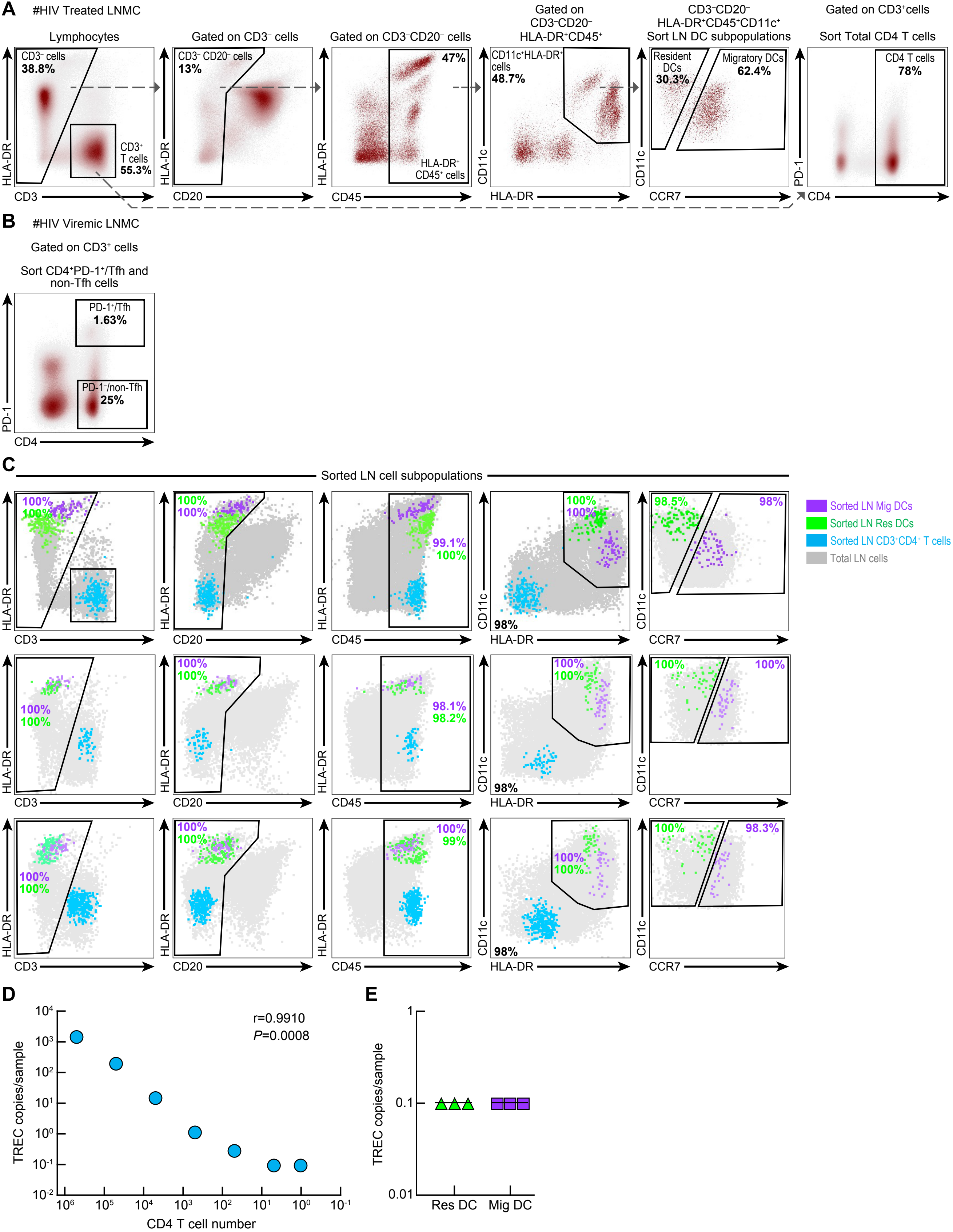

Figure. S1 (Related to Figure 1 and Figure 4): Gating strategy for sorting LN resident and migratory DCs and purity analyses. (**A**) Representative flow cytometry profiles of the gating strategy for sorting LN resident and migratory DCs from a representative ART treated HIV-infected individual (#136). Upon exclusion of T and B cells, myeloid DCs were identified within the $CD11c^+$ fraction and distinguished into LN resident DCs ($CD3^-CD20^-HLA\text{-}DR^+CD45^+CD11c^+CCR7^-$) and LN migratory DCs ($CD3^-CD20^-HLA\text{-}DR^+CD45^+CD11c^+CCR7^+$). (**B**) Representative flow cytometry profile of the gating strategy for sorting LN $PD\text{-}1^+$/Tfh and $PD\text{-}1^-$/non Tfh cells from a representative viremic HIV-infected individual (#1024). (**C**) Purity analysis of sorted LN cell populations *i.e.* LN resident and migratory DCs and CD4 T cells overlaid on unsorted total LN cells from three representative ART treated HIV-infected individuals. Purity for all sorting experiments was ≥ 98%. **(D)** Detection of TREC copies in sorted CD4 T cells at various cell concentrations (5.10^5-1 cell). (**E**) Detection of TREC copies in 40,000 sorted LN resident and migratory DCs from HIV uninfected individuals (N=3). Total LN cells are represented in grey (**C**), LN migratory DCs are depicted in violet (**C and E**), LN resident DCs are depicted in green (**C and E**) and LN CD4 T cells are depicted in blue (**C and D**). Red bars correspond to mean ± SEM **(E)**. Statistical significance ($*=P<0.05$) was obtained using Spearman rank test (**D**).

Supplemental Fig. 2

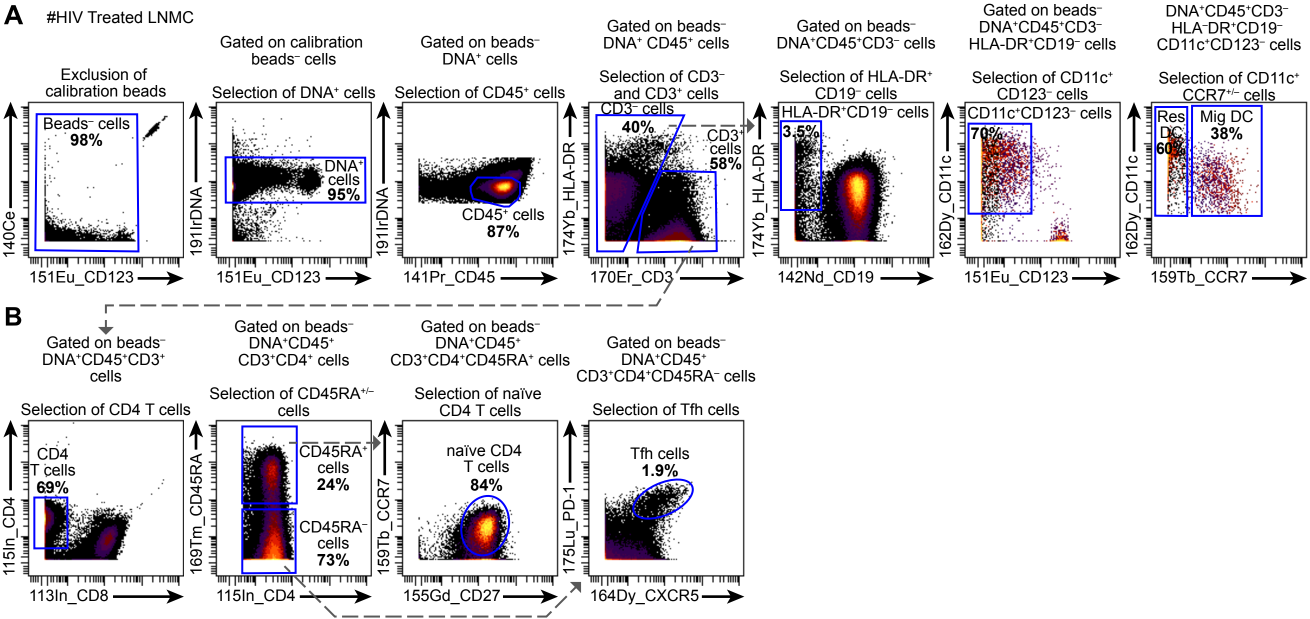

Figure. S2 (Related to Figure 2): Mass cytometry gating strategy for LN mononuclear cell populations. (**A**) Representative example of gating strategy for LN resident and migratory DCs of an aviremic ART treated HIV-infected individual (#155). (**B**) Representative example of gating strategy for naïve CD4 T cells and LN Tfh cells of an aviremic ART treated HIV-infected individual (#155).

Supplemental Fig. 3

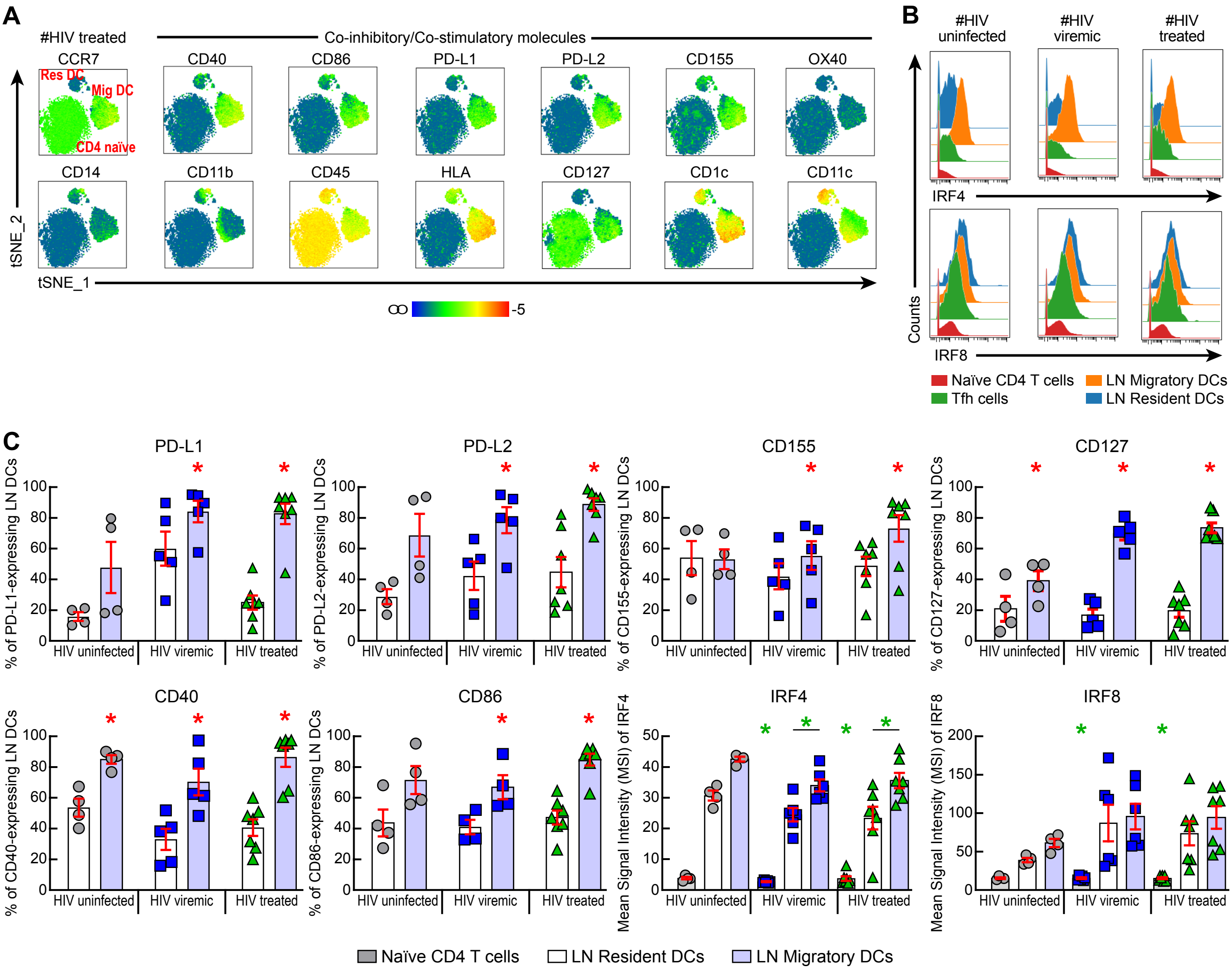

Figure. S3 (Related to Figure 2): **Phenotypic characterization of LN resident and migratory DCs by mass cytometry.** (**A**) Representative t-distributed stochastic neighbor embedding (t-SNE) plots representing the surface expression patterns of co-stimulatory molecules (CD40, OX-40 and CD86), immunoregulatory molecules (PD-L1, PD-L2 and CD155) and other molecules (CD14, CD11b, CD45, HL-DR, CD127, CD1c and CD11c) on naïve CD4 T cells, LN migratory and LN resident DCs from a representative ART treated HIV-infected individual (#161). (**B**) Representative mass cytometry profile of expression levels of transcription factors IRF4 and IRF8 in naïve CD4 T cells, LN Tfh cells, LN resident and LN migratory DCs isolated directly *ex vivo* from a representative HIV uninfected, viremic (#1009) and aviremic ART treated HIV-infected individual (#163).(**C**) Cumulative data representing the frequencies of LN migratory and resident DCs expressing PD-L1, PDL2, CD155, CD127, CD40 and CD86 in HIV-uninfected (N=4), viremic (N=5) and aviremic ART treated HIV-infected individuals (N=7) or the Mean signal intensity (MSI) values of IRF4 and IRF8 in naïve CD4 T cells, LN migratory and resident DCs isolated directly *ex vivo* from HIV uninfected (N=4), viremic (N=6) and ART treated HIV-infected individuals (N=7). Red histograms correspond to naïve CD4 T cells, green histograms correspond to LN Tfh cells, orange histograms correspond to LN migratory DCs and blue histograms correspond to LN resident DCs **(B)**. Grey circles correspond to HIV-uninfected individuals, blue squares corresponds to viremic HIV-infected individuals and green triangles correspond to aviremic ART treated HIV-infected individuals **(C)**. Red bars correspond to mean ± SEM **(C)**. Red stars indicate statistical significance (*=P<0.05) for intra-group comparisons. Green stars indicate statistical significance (*=P<0.05) for intra-group comparisons of LN migratory DCs, LN resident DCs and naïve CD4 T cells. Statistical significance (P values) was obtained using one-way ANOVA (Kruskal-Wallis test) followed by a two-tailed paired t-test.

Supplemental Fig. 4

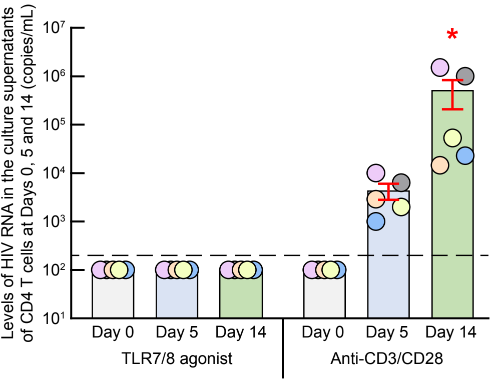

Figure. S4 (Related to Figure 6): **Levels of HIV RNA in the culture supernatants of LN CD4 T cells exposed to TLR7/8 agonist treatment.** Levels of HIV production in purified LN CD4 T cell culture supernatants of ART treated HIV-infected individuals (N=5) at days 0, 5 and 14 post-exposure to TLR7/8 agonist as assessed by HIV RNA levels (copies/mL). HIV-infected individuals are color coded. Red bars correspond to mean ± SEM **(C)**. Red stars indicate statistical significance (*=$P<0.05$). Statistical significance (*P* values) was obtained using one-way ANOVA (Kruskal-Wallis test) followed by Wilcoxon Matched-pairs one-tailed Signed Rank test.

Supplemental Table 3 (Related to Figure 1). HIV-specific CD8 T-cell responses in ART treated HIV-infected individuals

HIV treated individuals	Peptide sequences	SFU/million
#136	RDYVDRFFKTL	227
	TPQVPLRPM	192
	SPAIFQSSM	162
	WHLGHGVSI	140
	total SFU/million	**721**
#142	YPLTFGWCY	**233**
#169	VPLDEDFRKY	150
	IEELRQHLL	140
	total SFU/million	**290**

Supplemental Table 4 (Related to Figure 4). All Flip-seq sequences obtained for viremic HIV-infected individuals

								Defective					
Patient	LN cell population	Cell count before extraction	LTRgag Count after extraction	% of cells with LTR*gag*	Total cells assayed	Total HIV Sequences	Intact	Defects in PSI	LD	LD/ Hypermutation	LD/ Internal Inversion	PSC	Hypermutation
1024	Mig DC	3.00E+05	1.76E+03	0.6	5,684	1	1	-	-	-	-	-	-
	Res DC	2.80E+05	1.40E+03	0.5	7,146	2	-	-	1	1	-	-	-
	PD-1$^+$/Tfh	3.00E+05	2.08E+04	6.9	5,292	9	2	0	5	2	-	-	-
	PD-1$^-$	3.00E+05	2.64E+03	0.9	41,967	5	-	-	4	-	-	1	-
1019	Mig DC	1.96E+05	4.32E+02*	0.2	3,476	1	-	-	1	-	-	-	-
	Res DC	1.80E+05	1.80E+02*	0.1	8,322	1	1	-	-	-	-	-	-
	PD-1$^+$/Tfh	2.70E+05	3.29E+04	12.2	1,826	4	2	-	1	1	-	-	-
	PD-1$^-$	3.00E+05	7.81E+03	2.6	3,743	-	-	-	-	-	-	-	-
1025	Mig DC	3.00E+05	1.10E+03	0.4	6,600	1	1	-	-	-	-	-	-
	Res DC	3.00E+05	7.77E+01*	0.03	19,305	2	2	-	-	-	-	-	-
	PD-1$^+$/Tfh	3.00E+05	4.33E+04	14.4	3,663	2	-	-	1	-	1	-	-
	PD-1$^-$	3.00E+05	2.31E+03*	0.8	1,601	-	-	-	-	-	-	-	-

Asterix (*) refers to quantifications at limit of detection. When no intact HIV-1 sequences were detected, the limit of detection was calculated as two times the number of cells analyzed without target identification. Percentage of HIV-infected cells harboring at least 1 copy of HIV was calculated as follows: LTR HIV*gag* count (copies) / cell count before extraction*100.

Supplemental Table 5 (Related to Figure 4). All MIP-seq sequences obtained for viremic HIV-infected individuals

					Defective					
Patient	LN cell population	Total cells assayed	Total HIV Sequences	Intact	Defects in PSI	LD	LD/ Hypermutation	LD/ Internal Inversion	PSC	Hypermutation
1024	Mig DC	23,012	6	2	-	2	-	-	2	-
	Res DC	36,286	9	4	-	5	-	-	-	-
	PD-1$^+$/Tfh	619	4	1	-	3	-	-	-	-
	PD-1$^-$	0	-	-	-	-	-	-	-	-
1019	Mig DC	3,476	1	1	-	-	-	-	-	-
	Res DC	11,927	1	0	-	1	-	-	-	-
	PD-1$^+$/Tfh	348	2	0*	-	-	-	-	-	-
	PD-1$^-$	0	-	-	-	-	-	-	-	-
1025	Mig DC	5,984	1	1	-	-	-	-	-	-
	Res DC	26,255	0	0	-	-	-	-	-	-
	PD-1$^+$/Tfh	228	3	0*	-	-	-	-	-	-
	PD-1$^-$	NA	NA	NA	NA	-NA	NA	NA	NA	NA

Abbreviations: LD: Large deletions; Pre-mature stop codon. *Short sequences (<~1200 bp) were not sequenced. NA; Not applicable

Supplemental Table 6 (Related to Figure 6). Percentage of homology between HIV sequences obtained from VOA culture supernatants of LN CD4 T cells and LN DC subpopulations of treated HIV-infected individuals.

Volunteer #104			
Reverse Transcriptase (1158 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		99 (6 mismatch)	100
Resident DCs	99 (6 mismatch)		99 (6 mismatch)
Migratory DCs	100	99 (6 mismatch)	
Protease (297 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		99 (3 mismatch)	100
Resident DCs	99 (3 mismatch)		99 (3 mismatch)
Migratory DCs	100	99 (3 mismatch)	
Integrase (867 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		100 (2 mismatch)	100
Resident DCs	100 (2 mismatch)		100 (2 mismatch)
Migratory DCs	100	100 (2 mismatch)	
Volunteer #109			
Reverse Transcriptase (1158 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		NA	NA
Resident DCs	NA		99 (8 mismatch)
Migratory DCs	NA	99	
Protease (297 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		NA	NA
Resident DCs	NA		98 (8 mismatch)
Migratory DCs	NA	98	
Integrase (867 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		NA	NA
Resident DCs	NA		100 (1 mismatch)
Migratory DCs	NA	100	
Volunteer #133			
Reverse Transcriptase (1158 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		100	100
Resident DCs	100		100
Migratory DCs	100	100	
Protease (297 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		100	100
Resident DCs	100		100
Migratory DCs	100	100	
Integrase (867 nucleotides)			
Cell populations	CD4 T cells	Resident DCs	Migratory DCs
CD4 T cells		100	100
Resident DCs	100		100
Migratory DCs	100	100	

*NA. Not applicable