METHODS

Inclusion criteria for participant enrollment. Twenty nine subjects were enrolled during the acute/early primary HIV infection (PHI) by the *Grupo Argentino de Seroconversión* Study Group [1] under the following inclusion criteria: (1) detection of HIV RNA or p24 antigen with a simultaneous negative or indeterminate Western Blot assay; or (2) positive Western Blot with a negative test within the previous six months.

Phenotypic and functional analysis of the immune response. Functionality and phenotype of T-cells were assayed using thawed and overnight rested PBMCs cultured in RPMI-1640 medium (Gibco BRL, USA) plus 10% fetal bovine serum (SFB, Gibco BRL), 2 mM L-glutamine (Gibco BRL), 100 U/ml penicillin (Gibco BRL), 100 µg/ml streptomycin (Gibco BRL) and 10 mM HEPES (Gibco BRL). Polyfunctionality of HIV-specific $CD8^+$ T-cells were assayed by intracellular cytokine staining (ICS) as reported previously by our group [2]. Potential T-cell epitope (PTE) peptide panels (NIH AIDS Reagent Program) spanning the proteins Nef, Gag and Env were used as stimuli [3, 4]. Briefly, cells were stimulated with the designated peptide pool (2µg/ml) plus costimulatory antibodies (anti-CD28 and anti-CD49d; 1 µg/ml; BD Biosciences), monensin (Golgistop, 0.7 µl/ml; BD Biosciences) and brefeldin A (10 µg/ml; BD Biosciences) for 5 hours at 37°C. Anti-CD107a/b-FITC antibodies (BD Biosciences) were also added to identify degranulating cells. After stimulation, cells were stained with Zombie NIR™ Fixable Viability Kit (Biolegend, USA), in order to exclude dead cells, and with surface antibodies anti-CD3-BV786 and anti-CD8-APC (BD Biosciences). Then, cells were permeabilized and fixed using the Cytofix/Cytoperm kit (BD Biosciences) and subsequently stained using anti-IL-2–PerCP Cy5.5, anti-TNF-α-PECy7 and anti-IFN-γ–BV711 (polyfunctionality panel). Samples were analysed by flow cytometry as described below.

T-cell phenotype markers (CCR7, CD45RO and PD-1) were studied in bulk and HIV-specific $CD8^+$ T-cells as described previously by our group [5]. Additionally, PD-1 expression was analysed in bulk and HIV-specific $CD4^+$ T-cells. Concisely, cells were stimulated as described above and afterwards stained with Zombie NIR™ Fixable Viability Kit plus the following surface antibodies: anti-CCR7-Alexa700, anti-PD-1-PE, anti-CD3-BV786, anti-CD8-APC, anti-CD4-BV650 and anti-CD45RO-PerCPCy5.5 (BD Biosciences, USA). Then, cells were permeabilized and stained intracellularly with anti-IL-2, anti-TNF-α and anti-IFN-γ antibodies, all of them conjugated to FITC (BD Biosciences) to identify specific cells regardless of its function (phenotype panel).

Flow cytometry data acquisition was performed on a 3-laser 14-color BD FACSAria using the BD FACSDiva v 8.0.1 software (BD Biosciences). Instrument setting and fluorescence compensation were performed on each testing day using unstained samples and compensation beads (BD

Biosciences). Isotype controls consisting of stimulated cells stained with conjugated antibodies to surface molecules –CD3 and CD8- (or CD4-) and isotype controls corresponding to phenotype (CCR7, CD45RO and PD-1) and intracellular markers were performed for each individual in order to accurately set negative populations. Gating strategy was performed as in [2, 5, 6] (Figure S1). Briefly, after removing doublets in a forward scatter (FSC) area (FSC-A) versus FSC-Height (FSC-H) plot; and excluding dead cells (on the bases of Zombie NIR™ fluorescence), the small lymphocyte population was selected in a FSC-A versus side scatter (SSC) plot. At least 80,000 events were acquired in the lymphocyte gate. Subsequently, $CD3^+$ $CD8^+$ (or $CD4^+$) cells were gated in dot plots. In the phenotype panel, HIV-specific $CD8^+$ (or $CD4^+$) T-cells were identified in a CD8 (or CD4) vs. cytokines and CD107A/B (FITC) density plots. A positive cytokine response was defined as at least twice the background value, >0.05% after subtraction of background and at least 50 events. This criterion was established to minimize the possibility of error due to a low number of events when further subdividing these cells into the memory subsets. To analyse the distribution of the different phenotype subsets, CD45RO vs. CCR7 density plots were constructed on both bulk and HIV-specific $CD8^+$ T-cells. Moreover, PD-1 density plots were performed on bulk and HIV-specific $CD8^+$ (or $CD4^+$) cells. In cells stained with the polyfunctional panel, 1-function, 2-function and 3-function positive populations were studied by applying a boolean gating strategy (FlowJo software).

Immune Activation. $CD4^+$ and $CD8^+$ T lymphocytes activation was analysed by determining the co-expression of CD38 and HLA-DR on thawed and overnight rested PBMCs by flow cytometry, as in [2, 5, 6].

Quantitative real-time PCR for Cell Associated (CA) HIV RNA and DNA. CA HIV DNA and unspliced (US) RNA were quantitated by real-time PCR on samples obtained once study subjects were on cART. $CD4^+$ T-cells were isolated from frozen PBMCs using an immunomagnetic selection kit (STEMCELL technologies, Canada) and only 90% purity samples (determined by flow cytometry) were assayed. DNA and RNA were extracted using Qiagen mini kit (AllPrep DNA/RNA Mini Kit, Qiagen, Germany), quantitated and stored at -80°C until use. All samples from each participant were extracted at the same time, run on the same PCR plate and subsequently analysed together. Total HIV DNA was quantitated as described previously in [7]. Briefly, a single-step real-time PCR was performed using 5µl of extracted DNA as input per 50µl of reaction, in triplicate. HIV DNA copy numbers were standardized to cellular equivalents using a CCR5 SYBR green real time PCR. The low limit of detection (LLOD) was 1 copy per well. For quantitation of US-RNA, Pasternak's protocol was used [8]. Briefly, a hemi-nested PCR was performed with 16 cycles of amplification followed by a second amplification round of quantitative real time PCR. US-RNA copy numbers were standardized to cellular equivalents using a 18s RNA real time PCR

(Invitrogen). The LLOD was 1 copy per well. Each sample was assayed in quadruplicate and a no reverse transcriptase control was used to evidence DNA contamination. Quantitative real time PCR assays were run for 40 cycles.

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Figure S1: Gating strategy used for the identification of the studied cellular populations, by flow cytometry. (A) To study $CD8^+$ T-cell polyfunctionality, initial gating was performed on a forward scatter area (FSC-A) versus FSC-height (FSC-H) plot to remove doublets. Dead cells were then excluded on the bases of Zombie NIR fluorescence. Then, small lymphocytes were selected in FSC versus side scatter (SSC) plot. Subsequently, $CD3^+$ cells were gated in a CD3 versus SSC-H dot plot, and then a CD8 versus CD4 dot-plot was constructed to identify $CD8^+$ events. Plots were derived from the $CD8^+$ gate to study each particular function: degranulation (evidenced as CD107A/B mobilization) and production of IL-2, IFN-γ and TNF-α. Cells capable of exerting multiple functions simultaneously (degranulating and/or secreting multiple cytokines) were identified using the Boolean gating strategy available at FlowJo v10 software. (B) For the phenotype panel, the initial gating strategy was identical to the polyfunctionality panel up-to-the point of CD8 versus CD4 plot. There, a CD8 versus FITC plot was derived to identify HIV-specific $CD8^+$ T-cells (defined as the ones degranulating and/or expressing cytokines, all stained in FITC). Subsequent analyses were performed on both populations (Bulk and HIV-specific cells) as shown by overlaid dot-plots and overlaid histograms. To analyze the distribution of the different phenotype subsets, CD45RO vs. CCR7 density plots were constructed to identify: naïve or naïve-like (NL) T-cells ($CCR7^+/CD45RO^-$) central memory T-cells (CM, $CCR7^+/CD45RO^+$), effector memory T-cells (EM, $CCR7^-/CD45RO^+$) and terminal effector T-cells (TE, $CCR7^-/CD45RO^-$). Additionally, percentage of PD-1 expression was evaluated. For certain analysis, $PD\text{-}1^+$ events were subdivided into $PD\text{-}1^{Low}$ and $PD\text{-}1^{High}$ according to the intensity (on the basis of mean fluorescence intensity) of PD-1 expression. In A and B illustration data represent cells derived from one representative subject.

Supplementary 1

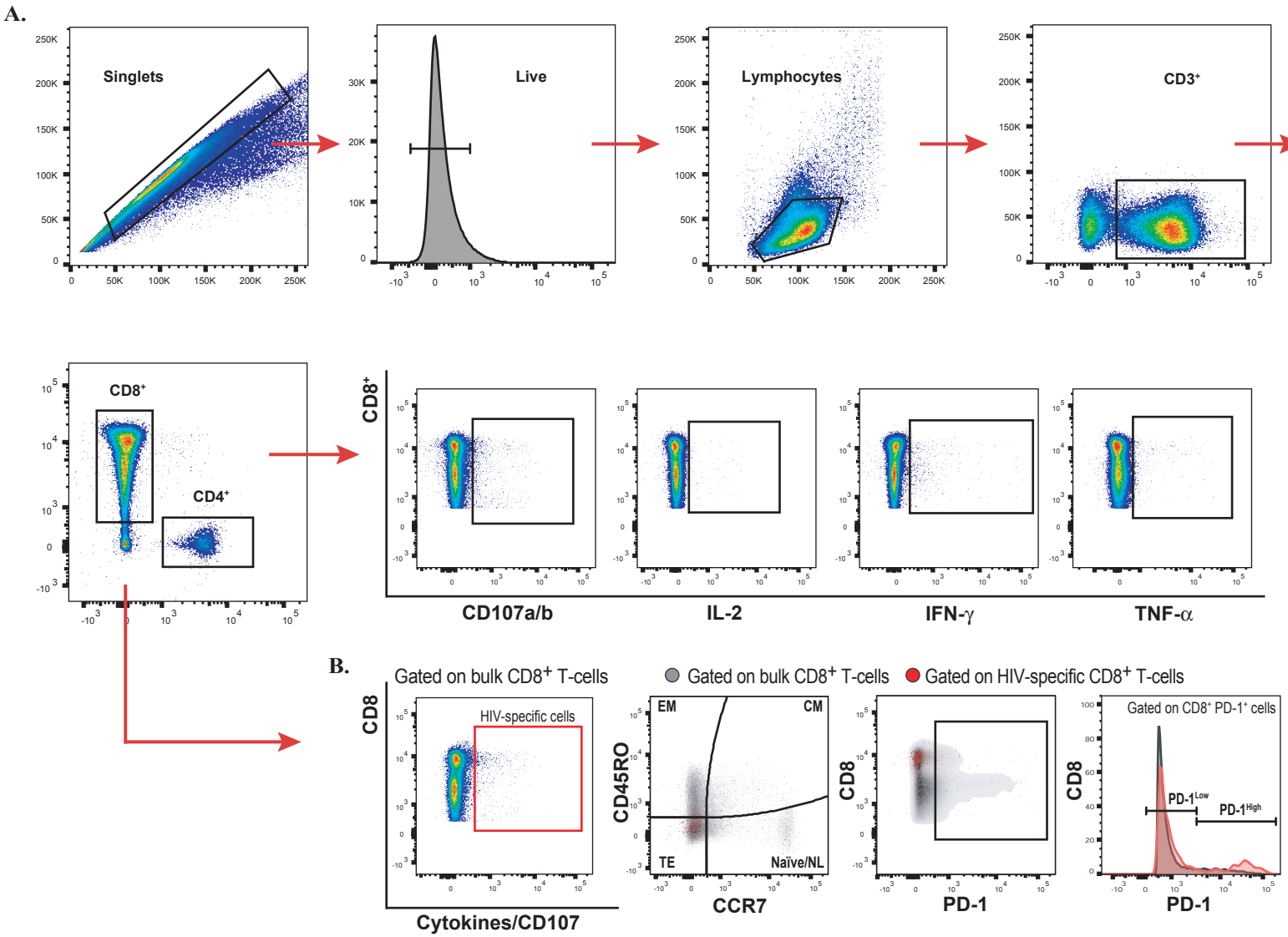

Table S1: Characteristics of HIV^{+} subjects at enrollment

Subject ID	Arm group	Gender	Age (Years)	Fiebig stage	AIDS defining illnesses	ART regimen used	Status		Viral Load (VL)[a]		CD4[b]		CD8[b]		CD4/CD8 (Ratio)
							HBV (anti-HBc/ HBsAg)	HCV (anti-HCV Ab)	RNA (copies/ ml)	Log_{10}	count (cells/ µl)	% (cells/µl)	count (Cells/µl)	% (cells/µl)	
PHI 1	Early	F	27	V	No	TDF/FTC/EFV	-/-	-	19434	4,3	327	19	690	40	0,47
PHI 2	Early	F	41	VI	No	TDF/FTC/ATV/rtv	-/-	-	102297	5,0	222	7	2471	78	0,09
PHI 3	Early	M	28	NA	No	3TC/AZT/NVP	-/-	-	44828	4,7	593	24	1327	53	0,32
PHI 4	Early	M	30	VI	No	TDF/FTC/EFV	NA	NA	17292	4,2	612	21	1828	62	0,33
PHI 5	Early	M	29	V	No	TDF/FTC/EFV	-/-	-	7643	3,9	328	21	803	52	0,41
PHI 6	Early	M	50	IV	No	3TC/AZT/EFV	-/-	-	283441	5,5	187	14	928	69	0,20
PHI 7	Early	M	29	V	No	TDF/FTC/EFV	+/-	-	>500000	>5,7	365	26	744	54	0,49
PHI 8	Early	M	24	NA	No	TDF/FTC/ATV/rtv	-/-	-	>500000	>5,7	238	28	424	50	0,56
PHI 9	Early	M	55	NA	No	TDF/FTC/EFV	-/-	-	20106	4,3	787	36	743	34	1,06
PHI 10	Early	M	46	NA	No	TDF/3TC/ATV/rtv	NA	NA	36338	4,6	768	40	649	33	1,18
PHI 11	Early	M	31	NA	No	ABC/3TC/ATV/COBI	-/-	-	>500000	>5,7	206	15	791	57	0,26
PHI 12	Early	M	74	NA	No	TDF/3TC/ATV/rtv	-/-	-	>500000	>5,7	421	18	1379	59	0,31
PHI 13	Early	M	43	NA	No	TDF/FTC/ATV/rtv	-/-	-	>500000	>5,7	627	32	943	48	0,66
PHI 14	Early	M	49	V	No	TDF/FTC/RAL	+/-	-	>500000	>5,7	341	19	951	53	0,36
PHI 15	Early	M	45	NA	No	TDF/FTC/ATV/rtv	-/-	-	>500000	>5,7	213	9	1126	50	0,19
PHI 16	Delayed	F	27	VI	No	ABC/3TC/EFV	-/-	-	5030	3,7	399	28	670	47	0,60
PHI 17	Delayed	F	30	VI	No	TDF/3TC/DOR	-/-	-	17420	4,2	685	22	1776	57	0,39
PHI 18	Delayed	F	44	VI	No	TDF/3TC/ATV	-/-	-	3662	3,6	302	16	1324	70	0,23
PHI 19	Delayed	F	40	V	No	TDF/FTC/ DRV/rtv	+/-	-	198770	5,3	250	9	2259	84	0,11
PHI 20	Delayed	F	40	V	No	3TC/AZT/ LPV/rtv	-/-	-	9532	4,0	590	34	694	40	0,44
PHI 21	Delayed	M	50	VI	No	3TC/LPV	-/-	-	363614	5,6	496	18	1721	61	0,29
PHI 22	Delayed	M	42	VI	No	3TC/AZT/EFV	+/-	-	473037	5,7	282	11	1771	66	0,16
PHI 23	Delayed	M	47	V	No	ABC/3TC/EFV	+/-	-	110105	5,0	436	22	753	38	0,58
PHI 24	Delayed	M	32	VI	No	3TC/AZT/EFV	-/-	-	37169	4,6	505	21	1419	59	0,36
PHI 25	Delayed	M	30	V	No	NA	-/-	-	1093	3,0	1012	37	1157	42	0,87
PHI 26	Delayed	M	54	VI	No	TDF/3TC/EFV	-/+	-	8613	3,9	778	35	1021	42	0,76
PHI 27	Delayed	M	31	VI	No	TDF/FTC/EFV	-/-	NA	>500000	>5,7	333	7	3564	75	0,09
PHI 28	Delayed	M	44	V	TB	3TC/AZT/EFV	+/-	+	1315	3,1	504	26	930	48	0,54
PHI 29	Delayed	M	40	VI	No	TDF/3TC/ATV/rtv	-/-	-	53303	4,7	393	28	545	39	0,72

[a] Branched-DNA (bDNA), VERSANT® HIV-1 RNA 3.0 Assay. Lower-upper detection limits: 40-500000 RNA copies/ml (1.6-5.7 log_{10}).

[b] TruCount absolute-count, flow cytometry FACSCalibur (BD Biosciences, USA).

Fiebig stages (Fiebig et al. 2003 AIDS, 17:1871-9)

F: Female. M: Male. NA: not available. TB: Tuberculosis.

Drugs= EFV: Efavirenz; TDF: Tenofovir; FTC: Emtricitabine; AZT: Zidovudine; NVP: Nevirapine; 3TC: Lamivudine; rtv: Ritonavir; ABC: Abacavir; COBI: Cobicistat; ATV: Atazanavir; DORA: Doravirine; DRV: Darunavir; LPV: Lopinavir; RAL: Raltegravir.