Supplementary Material

Table S1. Patient characteristics.

Pt. ID	Age (y.o)	Sex	Race	Duration of Infection (months)	ART Regimen	Time on ART (months)	Time with HIV RNA < 50 copies/ml (months)
1	56	M	AA	291	DRV/r, EFV, RAL	195	25
2	55	M	AA	272	EFV, F/TAF	252	13
3	49	F	AA	312	EVG/c, F/TAF	56	26
4	55	M	AA	315	F/TAF, RAL	113	67
5	38	F	AA	307	ABC, DTG, 3TC	73	70
6	53	M	C	220	ABC, DTG, 3TC	49	35
7	36	F	AA	88	EVG/c, F/TAF	86	85
8	46	F	AA	186	DTG, F/TAF	69	62
9	71	M	C	233	DTG, F/TAF	230	160
10	47	F	AA	271	F/TAF, RAL	65	63
11	51	M	AA	216	F/TAF, RAL	216	>72
12	56	M	C	367	DRV/r,DTG/ABC/3TC	238	120
13	71	M	AA	159	EFV, ABC, 3TC	111	106
14	67	M	C	211	DTG/ABC/3TC	139	137
15	61	M	C	260	DTG, 3TC, DRV/r	258	166
16	59	M	AA	276	DRV/r; DTG; FTC;	229	79
17	55	M	AA	>228	DTG, ABC, 3TC	175	134
18	63	M	AA	200	FTC/ RPV/TAF	195	191
19	67	F	AA	301	DRV/r, DTG, FTC/RPV/TAF	173	165
20	49	M	AA	348	RAL/ETR/MVC	194	65

Abbreviations: African-American (AA), Caucasian (C), Darunavir boosted with Ritonavir (DRV/r), Efavirenz (EFV), Raltegravir (RAL), Emtricitabine-Tenofovir Alafenamide (F/TAF), Elvitegravir boosted with Cobicistat (EVG/c), Abacavir (ABC), Dolutegravir (DTG), Lamivudine (3TC), Emtricitabine (FTC), Rilpivirine (RPV), Maraviroc (MVC), Etravirine (ETR)

Table S2: Quantitative viral outgrowth assay approaches and IUPM values obtained

Separation Method	Pt ID	Subpopulation Tested	IUPM	
			d14	d21
Protocol 1 Negative depletion to isolate CD4+ T cells, then sorting for CD32+ and CD32- cells	1	CD4+CD32+	-	-
		CD4+CD32-	0.08	0.17
	2	CD4+CD32+	-	-
		CD4+CD32-	0.6	1.04
	3	CD4+CD32+	-	-
		CD4+CD32-	5.9	5.9
	4	CD4+CD32+	-	-
		CD4+CD32-	1.4	2.4
	5	CD4+CD32+	-	-
		CD4+CD32-	0.12	0.61
	6	CD4+CD32+	-	-
		CD4+CD32-	0.13	0.36
Protocol 2 Positive vs negative selection to isolate CD4+ T cells	1	positive	0.22	1.42
		negative	0.85	1.09
	7	positive	0.05	0.05
		negative	0.11	0.2
	5	positive	1.15	1.77
		negative	0.9	2.11
	8	positive	1	2.3
		negative	0.8	1.76
	9	positive	2.08	8.2
		negative	4.75	4.75
	10	positive	0.28	0.36
		negative	-	-
Protocol 3 PBMCs for sorted based on CD3, CD4, and CD32	11	CD3+CD4+CD32+	-	-
		CD3+CD4+CD32-	0.14	1.34
		Total CD4+	0.14	0.73
	12	CD3+CD4+CD32+	-	-
		CD3+CD4+CD32-	0.02	0.03
		Total CD4+	0.07	0.14
	13	CD3+CD4+CD32+	-	-
		CD3+CD4+CD32-	0.07	0.1
		Total CD4+	0.07	0.14
	14	CD3+CD4+CD32+	-	-
		CD3+CD4+CD32-	0.18	0.45
		Total CD4+	0.07	0.1
	15	CD3+CD4+CD32+	-	-
		CD3+CD4+CD32-	0.64	1.21
		Total CD4+	0.46	0.8
	16	CD3+CD4+CD32+	-	-
		CD3+CD4+CD32-	40.52	40.52
		Total CD4+	16.25	16.25

Table S3: Frequency and yield of CD32+ Cells from PBMC and CD4+ T cell populations

Pt. ID	%CD32+	CD32+ Count
1	0.2*	37,121
2	0.21*	62,257
3	0.02*	7,487
4	0.02*	2,200
5	0.02*	8,029
6	0.13*	14,108
11	0.03**	16,000
12	0.09**	121,000
13	0.05**	92,500
14	0.13**	230,000
15	0.01**	2,800
16	0.05**	44,680

*Frequency of CD32 as a percent of CD4s
** Frequency of CD32 as a percent of PBMCs

Figure S1

Figure S1. Sort Gating Strategy for Protocol 1
CD4+ T cells were stained with antibodies to CD4 (x-axis) and CD32 (y-axis). Results are shown for subject 1 and are representative of five other subjects analyzed.
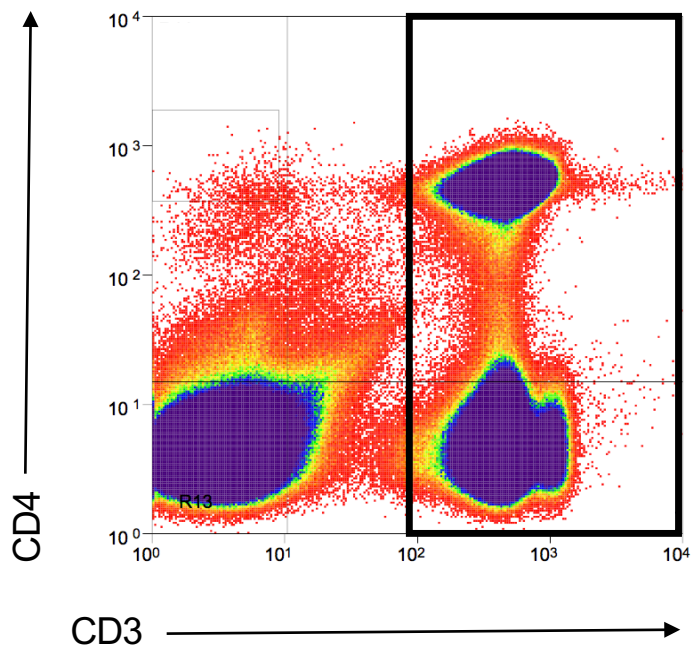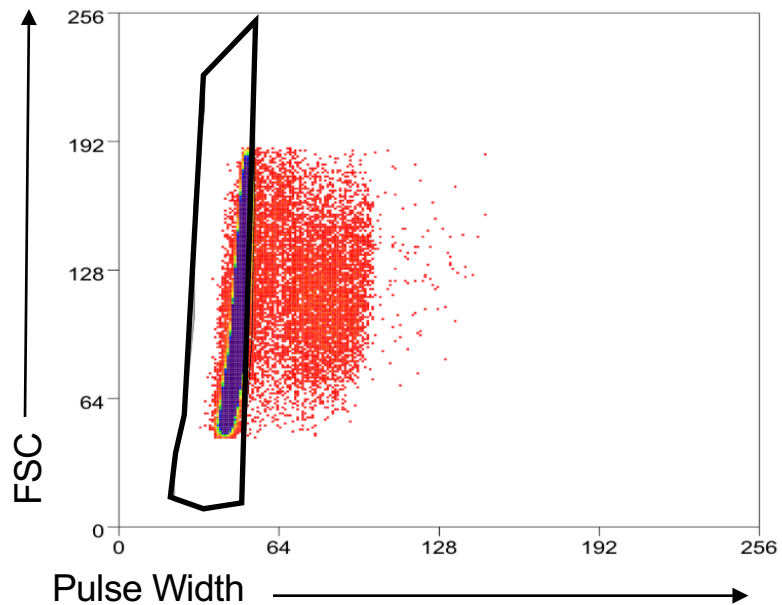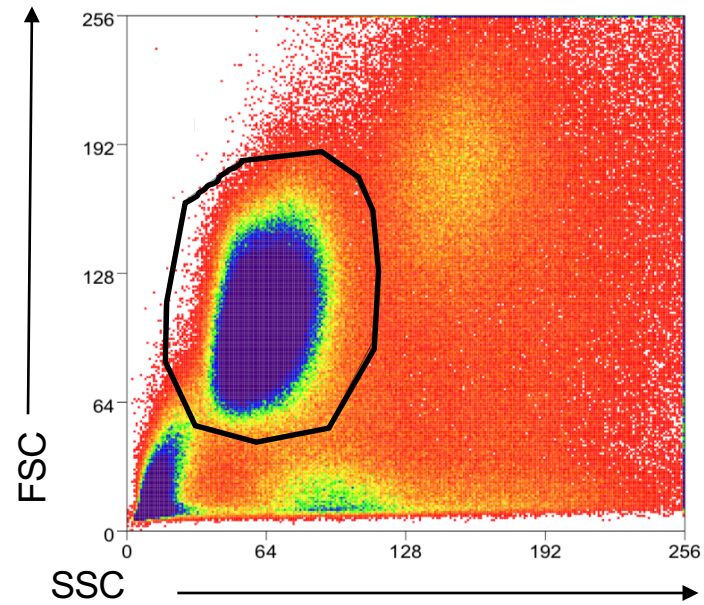
Figure S2

Figure S2. Sort Gating Strategy for Protocol 3

PBMCs were stained with antibodies to CD3 (x-axis; bottom left panel), CD4 (x-axis; bottom right panel), and CD32 (y-axis; bottom right panel). CD3+ cells were selected and used to sort the CD3+CD4+CD32^{hi} and CD3+CD4+CD32^{neg} populations. Results are shown for subject 12 and are representative of five other subjects analyzed.

Supplementary Methods

Study Subjects
This study was approved by the Johns Hopkins Institutional Review Board and written consent clone was obtained from all study participants. Study participants had undetectable plasma HIV-1 RNA (< 50copies/ml) for greater than 6 months. Additional patient data can be found in Table S1.

PBMC Isolation
Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using density centrifugation on a Ficoll-Hypaque gradient.

Healthy Donor Staining
CD4+ T cells were isolated from healthy donors using the EasySep Human CD4+ T Cell Negative Depletion Kit (Stemcell Technologies). Total CD4 T cells were first incubated with FcγR-blocking reagent (BD Pharmingen) for 10 minutes and then stained using FITC-CD32 (Biolegend; FUN-2 clone) and APC-CD4 (BD Pharmingen; RPA-T4 clone). APC-IgG1 and FITC-IgG2b were used as isotype controls. Flow cytometry analysis was performed using the iQue Screener Plus (Intellicyt Corporation).

Negatively Depleted Total CD4s Sorted for CD32
CD4+ T cells were enriched for using negative depletion (CD4+ T Cell Isolation Kit, Miltenyi Biotec). Total CD4+ T cells were first incubated with FcγR-blocking reagent for 10 minutes and then stained with FITC-CD32. Total CD4+ T cells were sorted into CD32+ and CD32- populations using a three-laser Beckman Coulter MoFlo Cell Sorter. FITC-IgG2b was used as an isotype control. Both cell populations were subsequently placed in a quantitative viral outgrowth assay.

Positive Selection vs. Negative Depletion for CD4
Negative depletion of CD4+ T cells was completed using the CD4+ T Cell Isolation Kit (Miltenyi Biotec). Positive Selection of CD4+ T cells was completed using either a Beckman Coulter MoFlo Cell Sorter, a SH800 cell sorter (Sony Biotechnology), or CD4 Microbeads (Miltenyi Biotec). Data on which positive selection method was used for each patient can be found in Table S2. In SH800 and Beckman Coulter MoFlo cell sorted populations, PBMCs cells were stained using PE-CD4 (BD Pharmingen; RPA-T4 clone) and sorted into a CD4+ population. PE-IgG1 was used as an isotype control. Both cell populations, both positively selected and negatively depleted, were subsequently placed in a quantitative viral outgrowth assay.

PBMCs Sorted for CD32
PBMCs obtained by leukapheresis were first incubated with FcγR-blocking reagent for 10 minutes and then stained using PE-CD4, BV421-CD3 (BD Pharmingen; UCHT1 clone), and APC-CD32 (Biolegend; FUN-2 clone). FITC-IgG2b, PE-IgG1, and BV421-IgG1 were used as isotype controls. PBMCs were sorted into CD3+CD4+CD32- and CD3+CD4+CD32+ populations using a three-laser Beckman Coulter MoFlo Cell Sorter. Both cell populations were subsequently placed in a quantitative viral outgrowth assay.

Quantitative Viral Outgrowth Assays (QVOAs)
QVOAs were performed as previously described by Laird *et al.*[7]

P24 Analysis by Quanterix SIMOA Technology

Inactivated culture supernatants were collected on days 5, 9, 14, and 21, frozen at -80⁰C, and sent to measure p24 by SIMOA (Quanterix, MA). The lower limit of quantification (0.01 pg/ml) was used as the cut off to call wells positive. IUPM calculation is based on cell input, fold dilutions and technical replicates.[14]

DNA Extraction and qPCR Analysis
DNA was extracted from 1-3x10^6 sorted T cells using the Gentra Puregene Cell Kit A (Qiagen). When less than 1x10^6 cells were available, C2C12 mouse cells were added to pellet cells. qPCR was performed with primers in the *gag* gene, as previously described.[15] Measurements of the human cellular gene RNaseP in a replicate well by qPCR were used to calculate the frequency of cells. The frequencies of *gag*+ DNA were plotted as a frequency per 10^6 cells.