

Supplemental Table 1: Median and IQR for cell surface markers in perinatal infections.

	Baseline	Standard (12h PMA/Ionomycin)	Enhanced (18h PHA/PMA/Ionomycin)
CD69	3.62 (1.03 – 4.45)	96.72 (96.62 – 97.95)	98.42 (98.22 – 98.75)
CD25	13.58 (7.05 – 24.11)	70.41 (55.97 – 81.62)	77.88 (58.57 – 84.3)
HLA-DR	4.55 (2.56 – 7.07)	30.86 (21.76 – 37.27)	35.76 (31.27 – 43.97)
PD-1	10.26 (6.17 – 13.31)	20.95 (16.95 – 25.46)	18.35 (15.05 – 26.96)
TIM-3	7.470 (5.08 – 8.85)	13.17 (10.36 – 13.66)	8.96 (5.95 – 9.47)
TIGIT	20.37 (15.6 – 22.7)	17.77 (12.6 – 19.23)	13.67 (11.1 – 17.93)

Supplemental Table 2: Median and IQR for cell surface markers in adult infections.

	Baseline	Standard (12h PMA/Ionomycin)	Enhanced (18h PHA/PMA/Ionomycin)
CD69	5.88 (1.645 – 23.55)	92.95 (64.6 – 98.29)	94.1 (85.05 – 98.37)
CD25	12.59 (4.26 – 16.9)	66.39 (35.95 – 72.28)	68.24 (42.72 – 76.93)
HLA-DR	10.52 (9.068 – 16.99)	35.87 (21.49 – 60.37)	48.52 (22.01 – 75.39)
PD-1	9.43 (2.47 – 15.1)	15.2 (5.4 – 20.83)	14.55 (5.69 – 20.83)
TIM-3	10.61 (7.88 – 14.06)	12.41 (9.31 – 15.01)	8.56 (7.35 – 9.32)
TIGIT	15.96 (11.67 – 20.41)	11.83 (6.34 – 16.04)	9.805 (6.34 – 12.51)

Supplemental Table 3. Primers and Probes used for viral subtypes.

	Tat1.4	Rev	Tat2	HIVFamzen
B¹	TGG CAG GAA GAA GCG GAG A	GGA TCT GTC TCT GTC TCT CTC TCC ACC	ACA GTC AGA CTC ATC AAG TTT CTC TAT CAA AGC A	/56-FAM/TTC CTT CGG /ZEN/GCC TGT CGG GTC CCGTC CC/3IABkFQ/
A/E²	TGG CAG GAA GAA GCG GAA G	TGT CTC TGY CTT GCT CKC CAC C	GCA GTA AGG ATC ATC AAA ATC CTA TAC CAG AGC A	/56-FAM/TTC YTT CGG/ZEN/GCC TGT CGG GTT CC/3IABkFQ
A/D	TGG CAG GAA GAA GCG GAG A	TGG TTC TGY CTT GCT CTC CAC C	GCA GTC AGG ATC ATC AAA ATC CTA TAC CAA AGC A	/56- FAM/TTCYTCCGG/ZEN/GCCTGTC GAGATCC/3IABkFQ/
A/G	TGG CAG GAA GAA GCG GAG A	TGT CTC TGY CTT GCT CKC CAC C	GCC GTC AGG ATC ATC AAA ATC CTG TAC CAA AGC A	/56-FAM/TTC YTT CGG/ZEN/GCC TGT CGG GTT CC/3IABkFQ
A	TGG CAG GAA GAA GCG GAR R	GAT CTG YCT CTG YCT TGC TCT CCA CC	GCA GTA AGG ATC ATC AAA ATC CTR TAC CAA AGC A	/56- FAM/TTCTTCCGG/ZEN/GCCTGTC GGGWYCC/3IABkFQ/
C	TGG CAG GAA GAA GCG GAG A	GAT CTG YCT CTG YCT TGC TCT CCA CC	GCA GTG AGG ATC ATC AAA ATC YTR TAT CAA AGC A	/56- FAM/TTCYTTCGG/ZEN/GCCTGTC GGGTYCC/3IABkFQ/

¹Procopio FA, Fromentin R, Kulpa DA, et al. A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV-infected Individuals. *EBioMedicine* 2015;2:874-83.

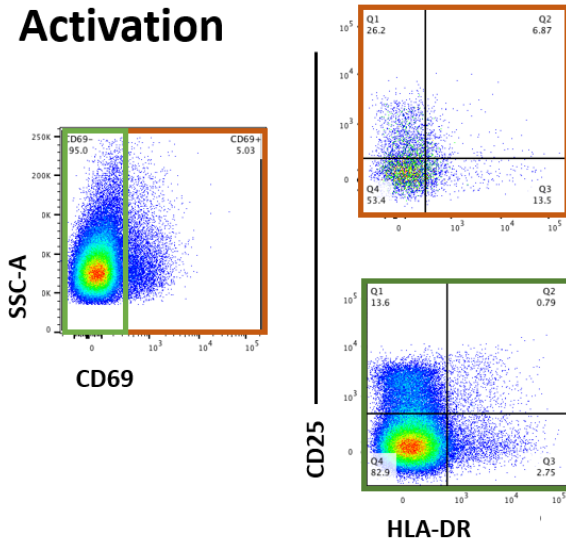
²Colby DJ, Trautmann L, Pinyakorn S, et al. Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. *Nat Med* 2018;24:923-6.

Supplemental Table 4. Antibodies used for flow cytometry.

Antibodies	Clone	Manufacturer
Live/dead FVS780	N/A	BD Biosciences, San Jose, CA
CD8 BV510	SK1	BD Biosciences, San Jose, CA
CD14 BV510	M ϕ P9	BD Biosciences, San Jose, CA
CD19 BV510	SJ25C1	BD Biosciences, San Jose, CA
CD3 APC R700	UCHT1	BD Biosciences, San Jose, CA
CD4 BV421	RPA-T4	BD Biosciences, San Jose, CA
HLA-DR BB515	G46-6	BD Biosciences, San Jose, CA
CD25 PE	MA251	BD Biosciences, San Jose, CA
CD69 FITC	FN50	BD Biosciences, San Jose, CA
TIM-3 PE	7D3	BD Biosciences, San Jose, CA
PD-1 BV786	EH12.1	BD Biosciences, San Jose, CA
TIGIT PerCP eF710	MBSA43	eBioscience, San Diego, CA

A. Activation

Unstimulated



B. Exhaustion

SSC-A

PD-1

SSC-A

PD-1

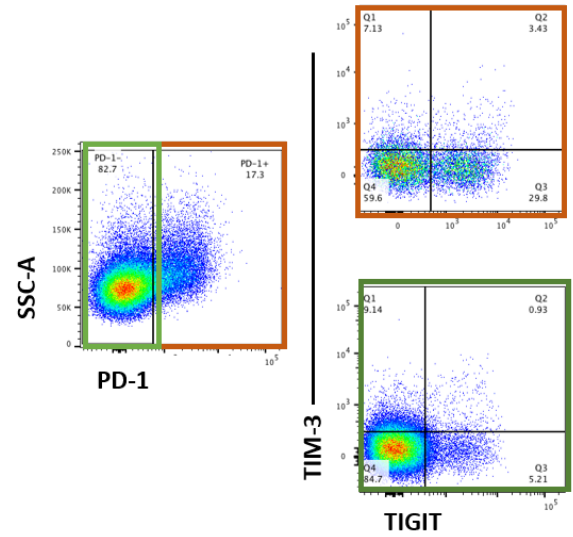
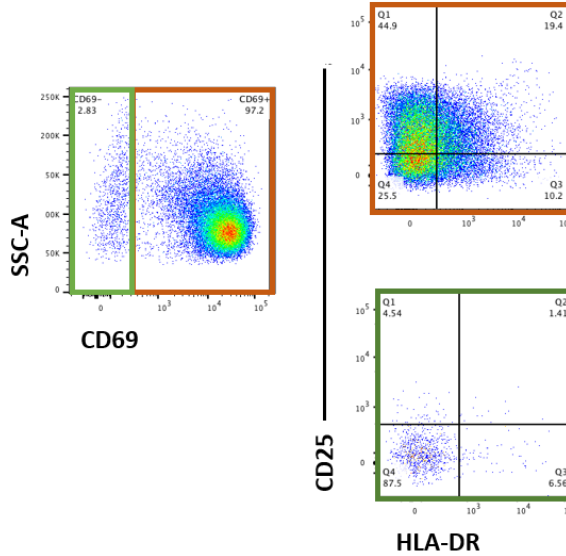
TIM-3

TIGIT

TIM-3

TIGIT

Stimulated



Supplemental Figure 1. Gating strategy for flow cytometry experiments. Cells were gated on lymphocyte, singlet, live CD3+CD8-/CD14-/CD19- and then gated for activation and exhaustion markers as above.