REFERENCE

E1. Mahapatra S, Mace EM, Minard CG, Forbes LR, Vargas-Hernandez A, Duryea TK, et al. High-resolution phenotyping identifies NK cell subsets that distinguish healthy children from adults. PLoS One 2017;12:e0181134.
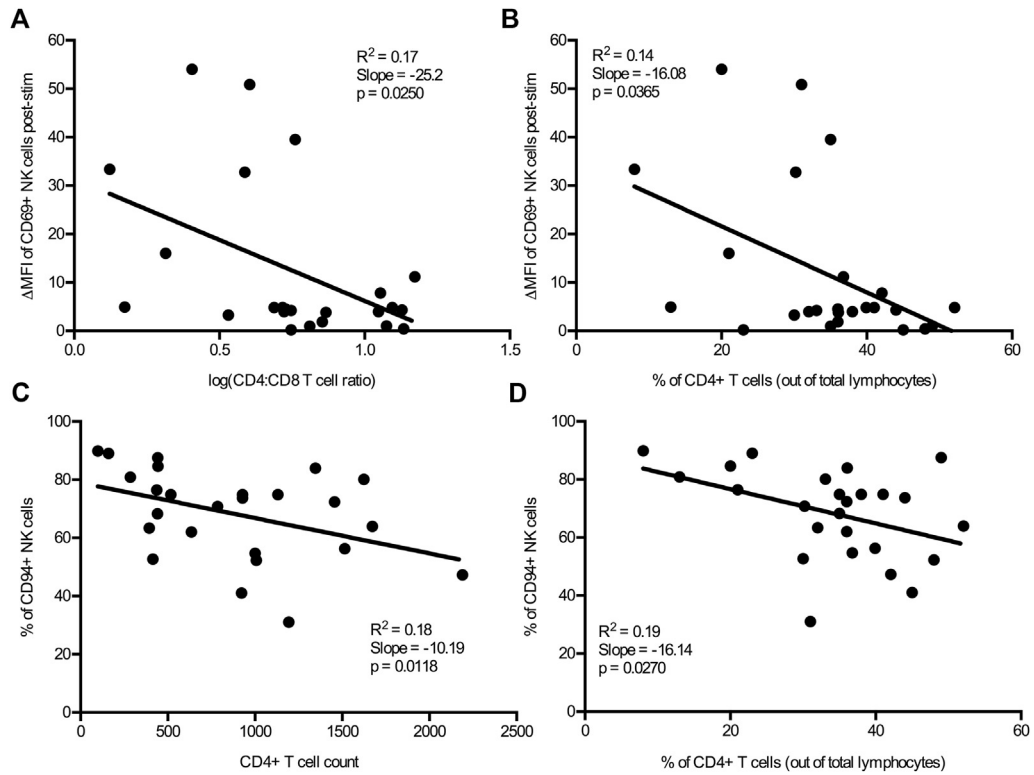
FIG E1. CD69 expression and frequency of CD94 on NK cells negatively associates with CD4/CD8 ratio and CD4^{+} T cells. *stim*, Stimulation.
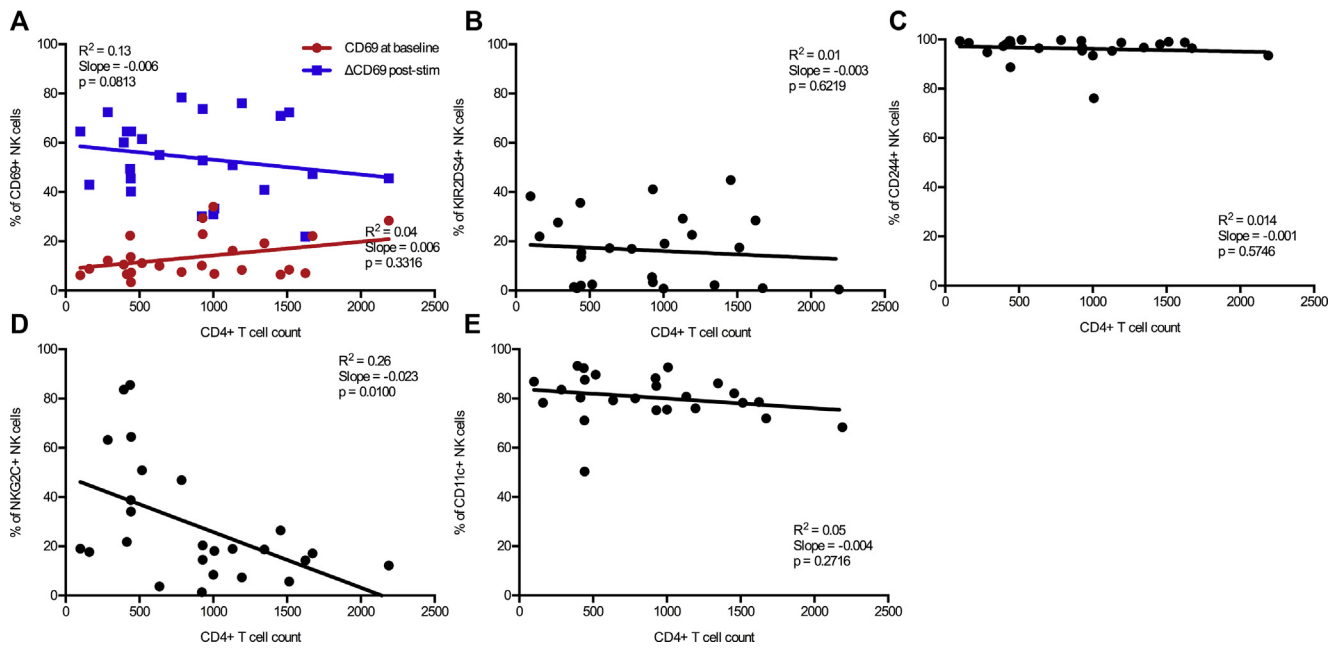
FIG E2. Frequency of NKG2C negatively associates with CD4$^+$ T-cell count. **A,** Relationship between CD4$^+$ T cell count and frequency of NK cells expressing CD69 in the absence of stimulation (red) or increase in CD69 after stimulation with PMA/ionomycin (blue). **B-E,** Relationship between CD4$^+$ T cell count and percentage of NK cells expressing KIR2DS4, CD244, NKG2C, and CD11c. All markers indicate surface expression. Each data point represents a donor; HIV-infected (n = 24). *P* values were identified by linear regression with the coefficient of determination (R^2) and slope defined.

FIG E3. CD57 has an inverse relationship with CD4$^+$ T cells and CD4/CD8 ratio. **A-B**, Relationship between frequency of NK cells expressing CD57 and percentage of CD4$^+$ T cells and CD4$^+$ T cell count, respectively. **C**, Relationship between MFI of CD57 and CD4/CD8 T cell ratio. CD4/CD8 ratios were log transformed after a constant value of 1 was added to each ratio. Each data point represents a donor; HIV-infected (n = 24). *P* values were identified by linear regression with the coefficient of determination (R^2) and slope defined.
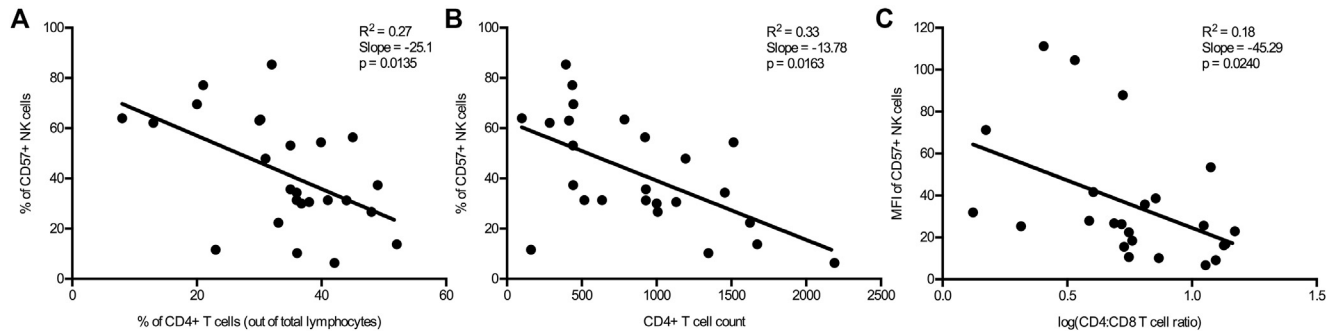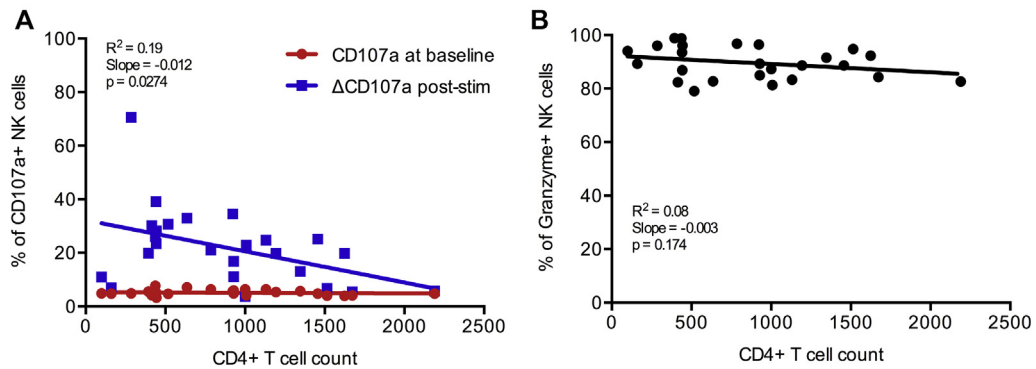
FIG E4. Increase in CD107a frequency on stimulation negatively associates with CD4$^+$ T-cell count. **A,** Relationship between CD4$^+$ T cell count and frequency of NK cells expressing CD107a in the absence of stimulation (red) or increase in CD107a after stimulation (blue). (**B**) Relationship between CD4$^+$ T cell count and percentage of NK cells expressing granzyme in the absence of stimulation. CD107a indicates surface expression while granzyme was detected intracellularly as described in the Materials and Methods section. Each data point represents a donor; HIV-infected (n = 24). *P* values were identified by linear regression with the coefficient of determination (R^2) and slope defined.

FIG E5. Baseline granzyme and increase in CD107a on stimulation reciprocally associate with CD4+ T cells and CD4/CD8 ratio respectively. **A**, Relationship between percentage of CD4+ T cells and percentage of NK cells expressing granzyme in the absence of stimulation. **B**, Relationship between change in CD107a MFI after stimulation and CD4/CD8 T cell ratio. Each data point represents a donor; HIV-infected (n = 24). *P* values were identified by linear regression with the coefficient of determination (R^2) and slope defined.
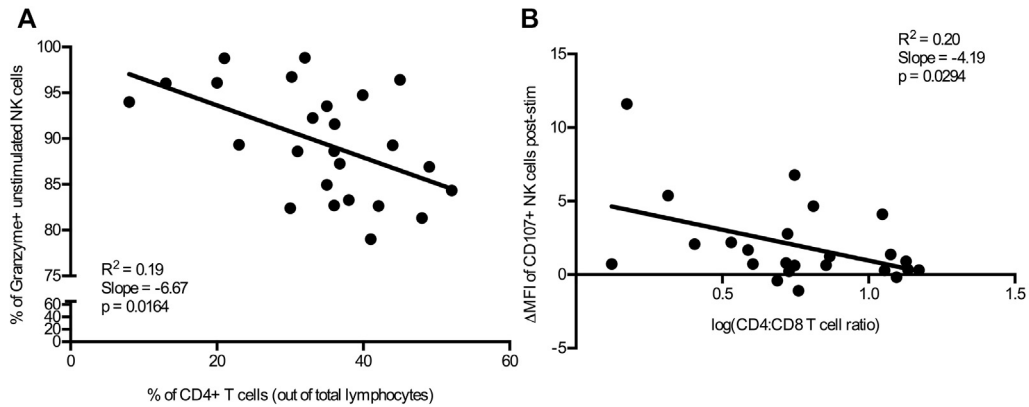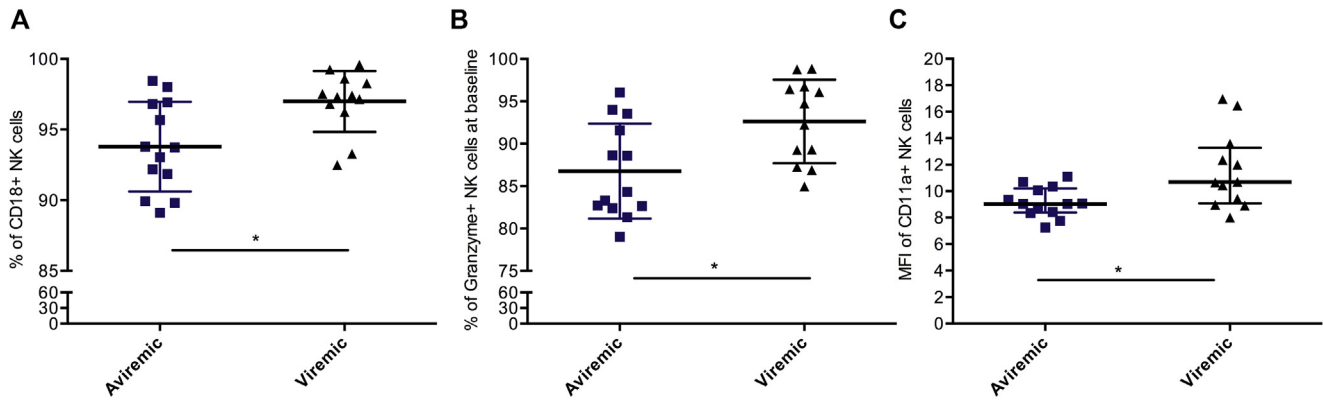
FIG E6. Viremic patients have increased adhesion receptors and baseline granzyme. **A-B,** Frequency of NK cells expressing CD18 and granzyme in the absence of stimulation among patients with detectable (viremic) and undetectable (aviremic) viral loads. **C,** Difference in CD11a MFI between aviremic and viremic patients. Aviremic (n = 13) (blue); Viremic (n = 12) (black). All data shown is mean ± S.D. *P < .05 by Wilcoxon rank-sum test.
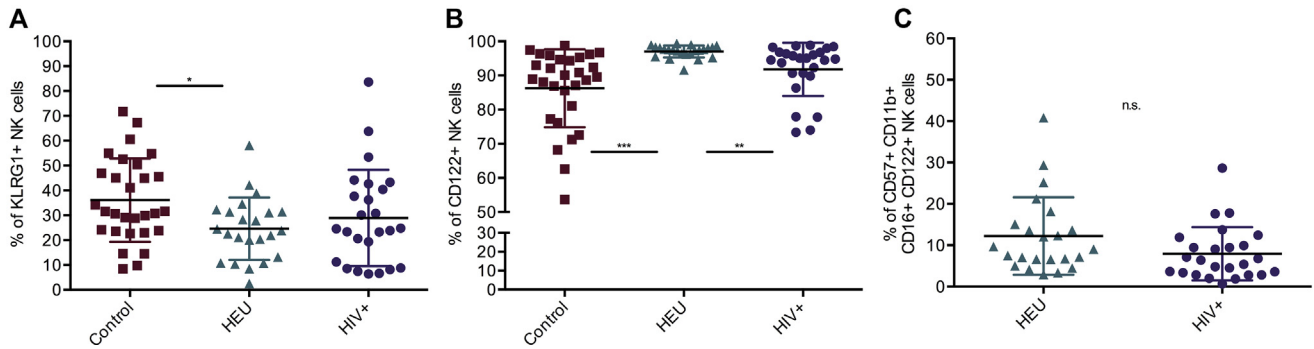
FIG E7. NK cells in HEU children have highest CD122 and increased frequency of mature CD57^{+} subset. **A-C**, Frequency of NK cells individually expressing KLRG1 and CD122 and combinatorially expressing CD57, CD11b, CD16 and CD122. Each data point represents a donor; Control (n = 29) (red); HEU (n = 23) (green); HIV-infected (n = 25) (purple). All data shown is mean ± S.D. *$P <$ .05, **$P <$ .01, ***$P <$ .001 among 3 cohorts by Kruskal-Wallis and *post-hoc* comparison by Wilcoxon rank-sum test using Bonferroni adjustment.

TABLE E1. NK-cell phenotyping panels*

Panel 1: Activating receptors	Panel 2: Adhesion/stimulatory receptors	Panel 3: Inhibitory receptors	Panel 4: Developmental markers	Panel 5: Cytokines and effector molecules
CD45	**CD45**	**CD45**	**CD45**	**CD45**
CD56	**CD56**	**CD56**	**CD56**	**CD56**
CD3	**CD3**	**CD3**	**CD3**	**CD3**
CD8	CD16†	CD158a/KIR2DL1‡	IL-15Rα	Perforin D48
DNAM-1†	CD8	CD158b/KIR2DL2-3‡	CD117/c-kit	Perforin δG9
NKG2D†	CD2†	CD158e/KIR3DL1‡	CD127	Granzyme B
NKp30/NCR3†	CD244/2B4†	KIR2DS4†	CD27	CD107a/LAMP-1
NKp44/NCR2†	CD18	NKG2A‡	CD62L	TNF-α
NKp46/NCR1†	CD11a (LFA-1)	NKG2C†	CD94	IFN-γ
CD69	CD11b (Mac-1, CR3)	CD94	CD122	IL-5
CD25	CD11c (CR4)	KLRG1‡	CD16	IL-13
	CD54/ICAM-1		CD11b	IL-10
	CD28		CD57	

Markers used to distinguish NK cells among PBMCs are highlighted in boldface.

KLRG1, Killer cell lectin-like receptor subfamily G member 1.

*As appears in Mahapatra et al.[E1]

†Activating receptors.

‡Inhibitory receptors.

TABLE E2A. Analysis of experimental variability in controls using Bland-Altman agreement study

NK cell panel	Stimulation	NK-cell marker	Bias (%)	Control vs HIV+	Control vs HEU
Activating receptors	No stimulation	DNAM-1	9.7	No	No
		CD69	−3.02	No	Yes
		NKp30	2.97	NA	Yes
		NKp46	2.35	Yes	Yes
		NKG2C	0.85	Yes	NA
	With stimulation	NKp46	−2.35	No	NA
		CD69	3.02	Yes	NA
Adhesion/stimulatory receptors		CD244	−1.22	No	NA
		CD18	−2.97	No	No
		CD11b	−3.02	No	NA
		CD11c	3.2	NA	NA
		CD2	1.63	Yes	No
Developmental markers		CD16	−0.68	Yes	NA
		CD122	7.25	NA	Yes
Cytokines and effector molecules	No stimulation	IL-5	7.25	No	NA
		CD107a	4.97	Yes	NA
	With stimulation	IL-5	7.25	No	NA
		CD107a	−4.97	NA	Yes

TABLE E2B.

Fluorochrome	Bias 1 (panel 2)	Bias 2 (panel 3)	Bias 3 (panel 4)
BV 650	2.19		8.58
FITC	1.22	9.7	0.23
APC	2.97	2.74	1.56
APC Cy7		7.31	0.18
AF 700		8.9	
PE Cy7	0.02		0.19
PE Texas-Red	3.02		0.68
PerCP Cy5.5	3.2	10.79	
PE Cy5	4.97		1.72
PB	1.63	2.35	2.28
BV 785	0.72		0.86
PE	1.58	0.81	7.25

NA, Not available/applicable.

TABLE E3. HIV-infected cohort medication regimen and asthma history

Age (y)	Viral load	CD4+ T-cell counts		ART regimen		Asthma status
3	UD	1456	NRTI	Protease		None
4	79	1624	NRTI	Integrase		None
5	299	928	NRTI	Integrase		Asthma
5	UD	1347	NRTI	Integrase		None
7	UD	2189	NRTI	NNRTI		None
8	UD	1673	NRTI	Integrase		None
9	UD	1194	NRTI	Protease		None
11	556	1001	NRTI	Protease		None
12	UD	517	NRTI	Integrase		None
12	UD	1131	NRTI	Integrase		Asthma
14	23	1514	NRTI	Protease	Integrase	None
15	UD	1008	NRTI	Protease		Asthma
15	234	929	NRTI	Protease		None
16	4842	437	NRTI	Protease		Asthma
16	UD	635	NRTI	Integrase		Asthma
16	UD	414	NRTI	NNRTI		None
17	UD	286	NRTI	Protease		None
17	UD	441	NRTI	NNRTI		None
18	289	923	NRTI	Protease		None
18	48	442	NRTI	NNRTI		None
18	78	394	NRTI	Integrase		None
19	170	785	NRTI	NNRTI		None
19	UD	99	NRTI			Asthma
19	214	444	NRTI	Integrase		None
19	187	160	NRTI	Integrase		None

NNRTI, Non-nucleoside reverse transcriptase inhibitors; *NRTI*, nucleoside reverse transcriptase inhibitors.

TABLE E4. Top combinatorial subsets in HIV-infected and HEU pediatric patients

Cell type	HEU	Percent	HIV+	Percent
NK cells	DNAM-1−CD8−CD69−NKG2D−NKp30−NKp46−NKp44−CD25−	6.8	DNAM-1−CD8−CD69−NKG2D−NKp30−NKp46−NKp44−CD25−	10.1
CD56^{dim} NK cells	DNAM-1+CD8−CD69−NKG2D−NKp30−NKp46−NKp44−CD25−	12.5	DNAM-1+CD8−CD69−NKG2D−NKp30−NKp46−NKp44−CD25−	13.2
CD56^{bright} NK cells	DNAM-1+CD8−CD69−NKG2D−NKp30+NKp46+NKp44−CD25−	20.3	DNAM-1+CD8−CD69−NKG2D−NKp30+NKp46+NKp44−CD25−	16.7
NK cells	CD16 3G8+CD11a+CD11b+CD11c+CD18+CD54+CD244+CD2+	26.7	CD16 3G8+CD11a+CD11b+CD11c+CD18+CD54+CD244+CD2+	34.9
CD56^{dim} NK cells		26.3		34.9
CD56^{bright} NK cells		29.2	CD16 3G8−CD11a+CD11b+CD11c+CD18+CD54+CD244+CD2+	30.9
NK cells	KIR2DL1−CD158b−CD158e−KIR2DS4−CD94+NKG2A+NKG2C−KLRG1−	23	KIR2DL1−CD158b−CD158e−KIR2DS4−CD94+NKG2A+NKG2C−KLRG1−	20.8
CD56^{dim} NK cells		20.3		17.3
CD56^{bright} NK cells		46.9		49.2
NK cells	CD16 3G8+CD11b+**CD57**+CD94+CD62L−CD27−CD117−CD122+	16.9	CD16 3G8+CD11b+**CD57**−CD94+CD62L−CD27−CD117−CD122+	15.3
CD56^{dim} NK cells		17.8		16.3
CD56^{bright} NK cells	CD16 3G8+CD11b+CD57−CD94+CD62L+CD27+CD117−CD122+	22.9	CD16 3G8+CD11b+CD57−CD94+CD62L+CD27+CD117−CD122+	19.2
NK cells	Perf D48+Perf δG9+Granzyme+CD107a−IFN-γ−TNF-α−IL-5−IL-13−	65.6	Perf D48+Perf δG9+Granzyme+CD107a−IFN-γ−TNF-α−IL-5−IL-13−	62.6
CD56^{dim} NK cells		68.5		65.8
CD56^{bright} NK cells		32.3		32.2

Marker distinguishing top NK cell developmental subset between HIV-infected and HEU is highlighted in boldface.

KLRG1, Killer cell lectin-like receptor subfamily G member 1.