Supplementary Digital Content
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INFLAMMATORY COLONIC INNATE LYMPHOID CELLS ARE INCREASED DURING UNTREATED HIV-1 INFECTION AND ASSOCIATED WITH MARKERS OF GUT DYSBIOSIS AND MUCOSAL IMMUNE ACTIVATION.

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Table S1. Baseline characteristics of study participants.*

	Uninfected study participants	HIV-1 infected study participants
Number of subjects	10	22
Age (yrs)	29 (23-51)	32 (22-58)
Male/Female Ratio	6/4	17/5
CD4 count (cells/μl)$^{\infty}$	681 (468-1071)	425 (221-1248)
Plasma viral load (HIV-1 RNA copies/ml)	-	51350 (2880-207000)
Years since first HIV-1 seropositive test	-	3.4 (0.2-15)
Ethnicity:		
Non-Hispanic	7 (70%)	17 (77.3%)
Hispanic	3 (30%)	5 (22.7%)
Race:		
White/Caucasian	6 (60%)	16 (72.7%)
Black/African American	2 (20%)	6 (27.3%)
Asian	2 (20%)	0 (0%)
Body Mass Index (kg/m^2) $^{¥}$	25.4 (19.3-32.3)	24.8 (17.4-42.5)
Tobacco use:		
Yes	2 (20%)	12 (54.5%)
No	8 (80%)	10 (45.5%)
Risk Factor:		
MSM^		
Yes	1 (17%)	15 (88%)
No	5 (83%)	2 (12%)
IVDU		
Yes	0 (0%)	5 (22.7%)
No	10 (100%)	17 (77.3%)

* Measurement of cytokine-expressing colonic NKp44^{+} ILCs was not performed for two (N=1 uninfected; N=1 HIV-1 infected) study participants due to the acquisition of an insufficient number of cells for subsequent analysis. Values are shown as median (range) or the number (percentage) of each cohort. $^{¥}$One subject had no weight or height values recorded at time of study therefore N=9. MSM: Men who have sex with men; IVDU: IV drug use. Statistical analysis performed using Mann-Whitney test for comparisons between uninfected and HIV-1 infected study participants and the Fisher Exact test or Chi-square test for comparison of categorical data. $^{\infty}$p=0.006; ^p=0.003.

Table S2: Correlations between numbers of IFNγ-expressing NKp44$^+$ ILCs and clinical, virological, immunological and microbial parameters.

	IFNγ$^+$ NKp44$^+$ CD56$^-$ ILCs	IFNγ$^+$ NKp44$^+$ CD56$^+$ ILCS
Clinical Parameters		
Blood CD4 T cell count	R= 0.28, P=0.22	R=-0.23, P=0.30
Plasma HIV-1 viral load	R=-0.07, P=0.76	R= 0.17, P=0.44
Systemic inflammation, immune activation, microbial translocation and epithelial barrier disruption parameters		
Plasma IL-6	R=-0.12, P=0.60	R=-0.16, P=0.49
Plasma C-reactive protein	R=-0.23, P=0.33	R=-0.03, P=0.88
Plasma TNFα	R=-0.05, P=0.84	R= 0.02, P=0.95
Plasma IFNγ	R= 0.18, P=0.52	R= 0.19, P=0.47
Plasma IL-10	R= 0.03, P=0.93	R= 0.13, P=0.63
Activated blood CD4 T cells[#]	R=-0.17, P=0.47	R= 0.27, P=0.24
Activated blood CD8 T cells[#]	R=-0.17, P=0.45	R= 0.25, P=0.27
Plasma soluble CD14 (sCD14)	R= 0.11, P=0.66	R= 0.17, P=0.50
Plasma lipopolysaccharide (LPS)	R=-0.37, P=0.12	R= 0.20, P=0.41
Plasma lipoteichoic acid (LTA)*	R=-0.49, P=0.04	R=-0.51, P=0.03
Plasma intestinal fatty acid binding protein (iFABP)	R=-0.04, P=0.86	R=-0.04, P=0.86
Colonic immunity parameters		
HIV-1 RNA levels	R=-0.13, P=0.58	R= 0.07, P=0.78
Number of CD1c$^+$ mDC[‡]	R= 0.47, P=0.06	R= 0.14, P=0.58
Number of pDC	R= 0.44, P=0.06	R= 0.39, P=0.09
CD40 expression levels on CD1c$^+$ mDC	R= 0.14, P=0.59	**R= 0.56, P=0.02**
CD40 expression levels on pDC	R=-0.19, P=0.45	R= 0.04, P=0.98
Percent of CD83-expressing CD1c$^+$ mDC	**R=-0.51, P=0.03**	R=-0.01, P=0.98
Percent of CD83-expressing pDC	R=-0.15, P=0.53	R= 0.23, R=0.32
Number of CD4 T cells	R= 0.17, P=0.47	R=-0.13, P=0.57
Number of activated CD4 T cells	R= 0.17, P=0.46	**R= 0.43, P<0.05**
Number of activated CD8 T cells	R= 0.38, P=0.09	**R=0.64, P=0.002**
Number of IFNγ-expressing CD4 T cells	**R= 0.53, P=0.01**	R= 0.39, P=0.07
Number of IL-17-expressing CD4 T cells	R= 0.39, P=0.08	R= 0.22, P=0.34
Number of IL-22-expressing CD4 T cells	R= 0.30, P=0.18	R= 0.18, P=0.41
Number of IFNγ-expressing CD8 T cells	R= 0.18, P=0.44	R= 0.15, P=0.62
Mucosa-associated microbiome[†]		
Phylum:		
Proteobacteria	R=-0.10, P=0.74	R=-0.05, P=0.84
Firmicutes	R=-0.04, P=0.88	R= 0.04, P=0.88
Families:		
Xanthomonadaceae	**R= 0.55, P<0.05**	R= 0.37, P=0.18
Lachnospiraceae	R= 0.18, P=0.55	R= 0.14, P=0.63
Ruminococcaceae	R=-0.03, P=0.90	R= 0.02, P=0.95
Prevotellaceae	**R= 0.68, P=0.009**	R= 0.50, P=0.06
Bacteroidaceae	R=-0.37, P=0.20	R=-0.31, P=0.26
Genera:		

Acinetobacter	R= 0.09, P=0.76	R= 0.01, P=0.98
Blautia	R=-0.28, P=0.33	R=-0.18, P=0.53
Coprococcus	R= 0.007, P=0.98	R= 0.08, P=0.79
Prevotella	**R= 0.69, P=0.008**	R= 0.50, P=0.06
Bacteroides	R=-0.37, P=0.20	R=-0.31, P=0.26
Alistipes	R= 0.13, P=0.65	R= 0.20, P=0.46
Species:		
Prevotella copri	**R= 0.68, P=0.01**	R= 0.50, P=0.06
Prevotella stercorea	**R= 0.63, P=0.02**	R= 0.41, P=0.13
Prevotella oris	R= 0.40, P=0.15	R= 0.23, P=0.40
Bacteroides stercoris	R=-0.53, P=0.05	R=-0.48, P=0.07
Bacteroides dorei	R=-0.40, P=0.16	R=-0.36, P=0.17
Blautia luti	R= 0.20, P=0.48	R= 0.24, P=0.39
Blautia glucerasei	R= 0.19, P=0.51	R= 0.26, P=0.34
Rumminococcus bromii	R=-0.27, P=0.35	R=-0.38, P=0.16
Acidaminococcus intestini	R= 0.08, P=0.78	R=-0.06, P=0.64
Acinetobacter junii	R= 0.21, P=0.48	R= 0.20, P=0.47
Schlegelella thermodepolymerans	R= 0.05, P=0.85	R=-0.13, P=0.59

Statistical analysis performed using the Spearman test. #Assessed as percent of CD4 or CD8 T cells that co-express HLA-DR and CD38. *When potential outlier value removed: IFNγ^+NKp44$^+$CD56$^-$ ILCs: R=-0.43, P=0.08; IFNγ^+NKp44$^+$CD56$^+$ ILCs: R= -0.42, P=0.08. ‡Number refers to the number of cells of interest per gram of tissue. †Associations between phylum, families, genera and species that were significantly different between HIV-1 infected versus uninfected study participants. Only those taxa which were detectable in >60% of HIV-1 infected study participants are detailed.

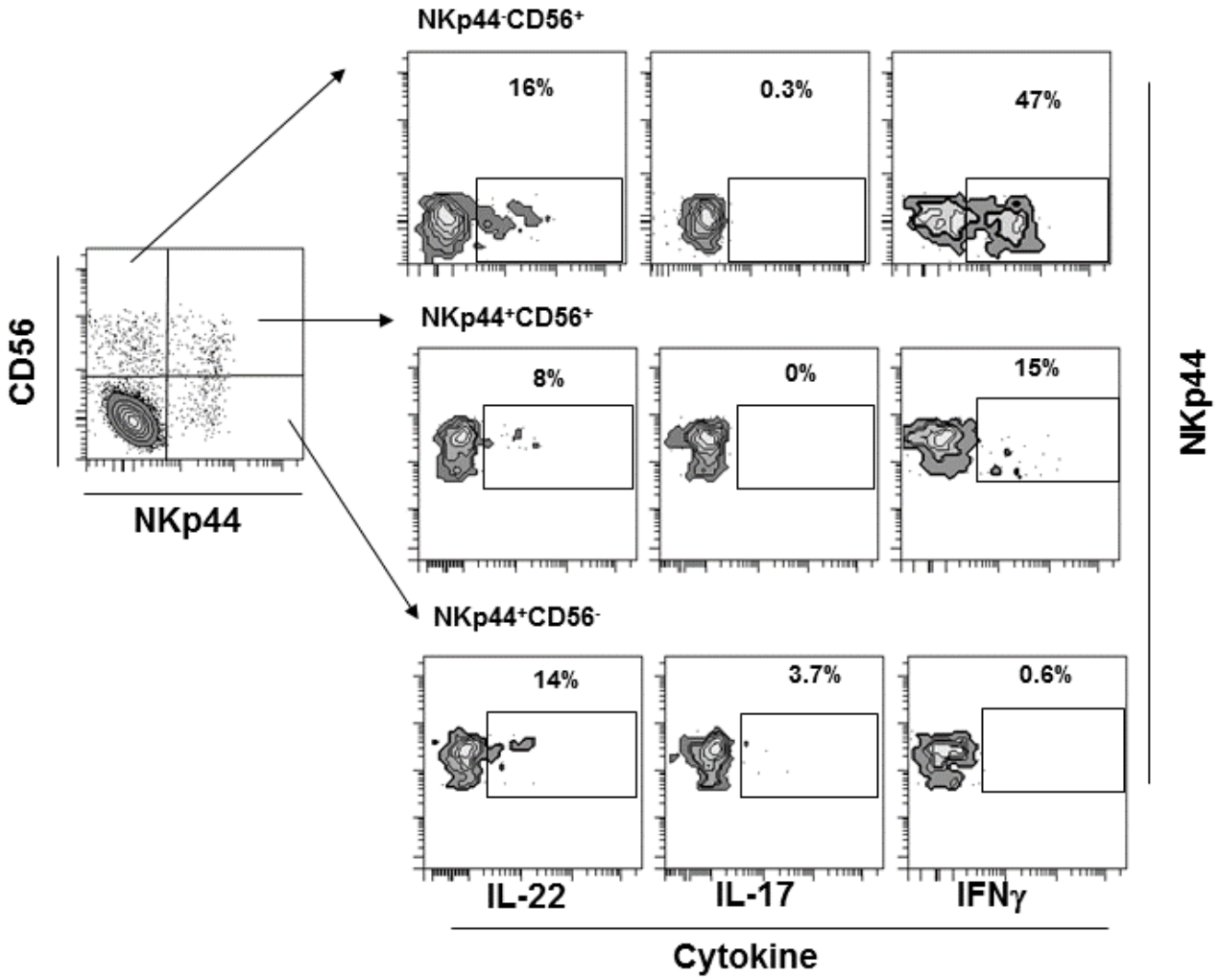

Figure S1. Representative gating strategy of $CD3^-$ colonic $NKp44^-CD56^+$ (top panel), $NKp44^+CD56^+$ (middle panel) and $NKp44^+CD56^-$ (bottom panel) ILCs in an HIV-1 uninfected individual to assess percentages of cytokine-expressing (IL-22, IL-17, IFNγ) cells in each ILC subset after *in vitro* mitogenic stimulation. Live $CD3^-$ ILCs were identified based on forward/side scatter properties in viable, $CD45^+$ colonic cells. Appropriate cytokine isotype controls were used to establish antibody specific staining.

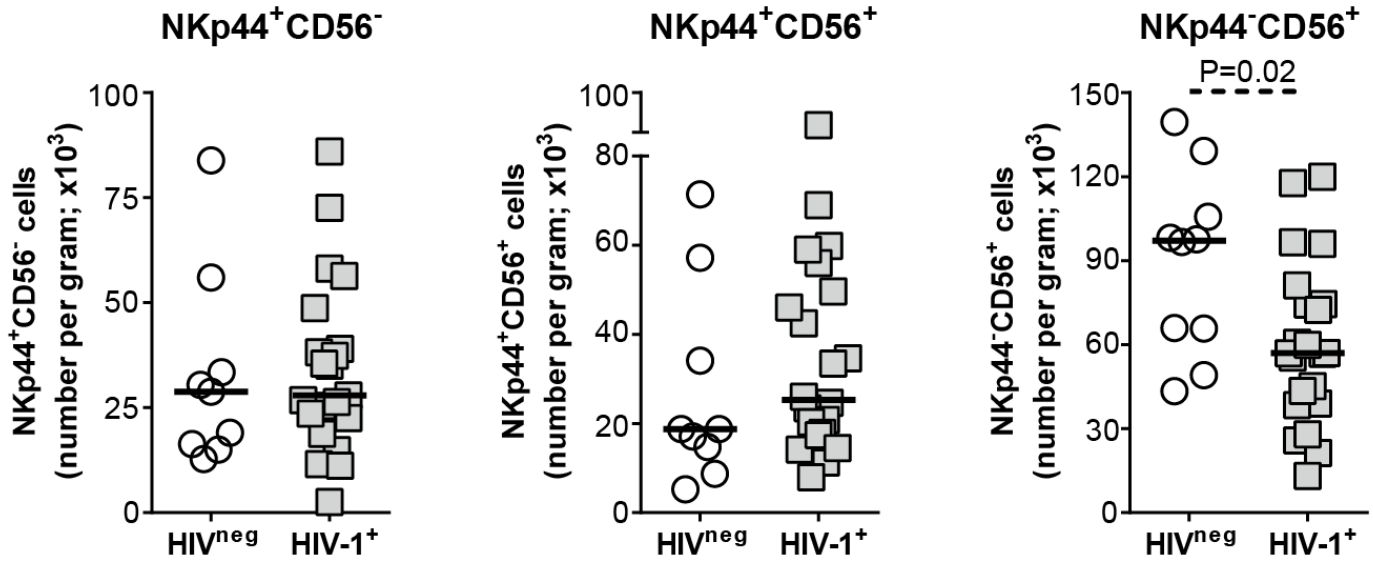

Figure S2. Absolute numbers of colonic ILC subsets. Absolute numbers of colonic NKp44$^+$CD56$^-$, NKp44$^+$ CD56$^+$ and NKp44$^-$CD56$^+$ ILCs measured after mitogenic stimmulation in uninfected (HIV^{neg}: NKp44$^+$CD56$^-$ N=9; NKp44$^+$CD56$^+$ N=9; NKp44$^-$CD56$^+$ N=10) and HIV-1 infected (HIV-1$^+$: NKp44$^+$CD56$^-$ N=21; NKp44$^+$ CD56$^+$ N=22; NKp44$^-$CD56$^+$ N=22) study participants and are expressed as total number of ILC subsets per gram of tissue. Lines represent median values. Statistical analysis to compare between independent groups were made using the Mann-Whitney test.