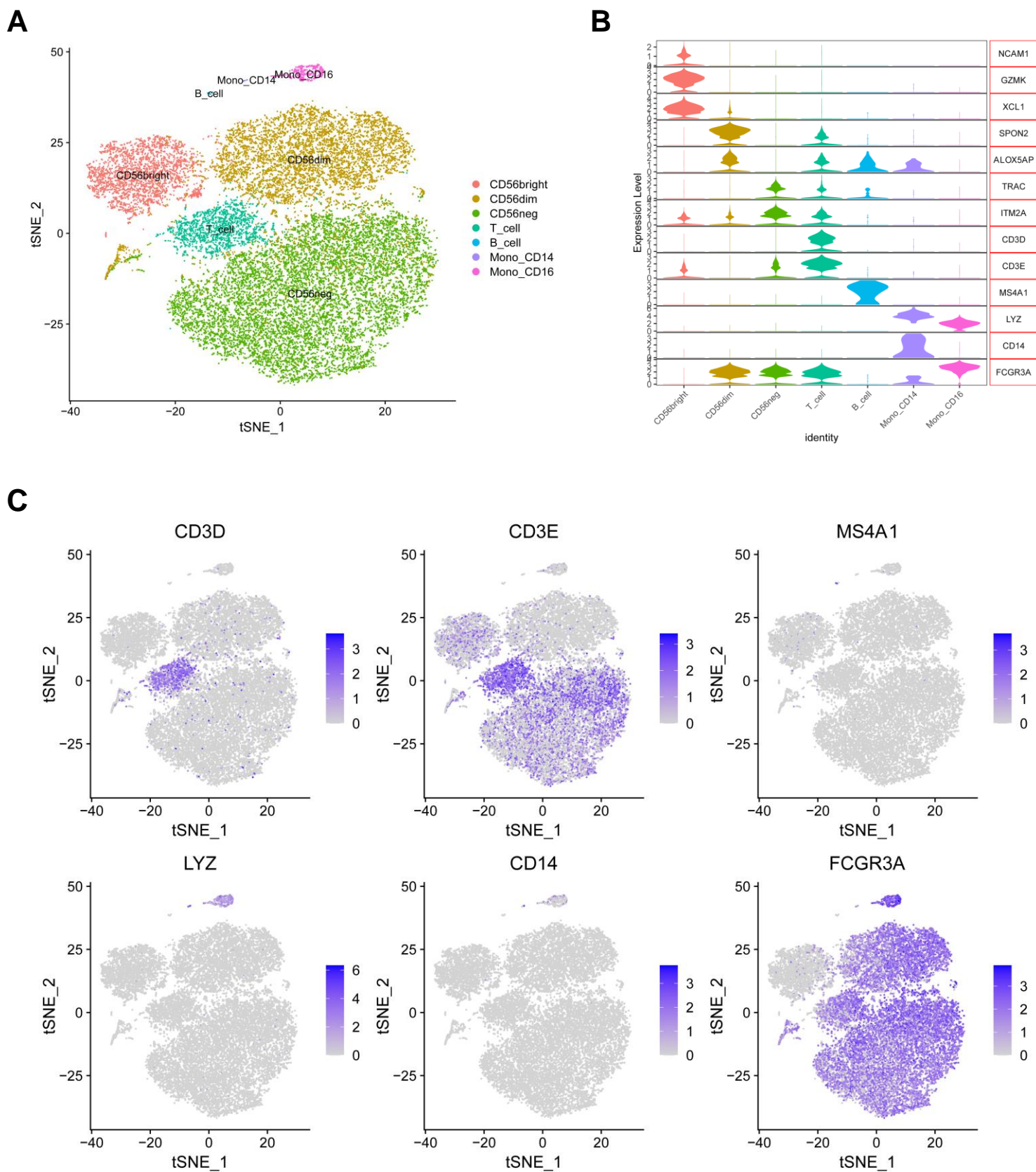# Supplementary Information
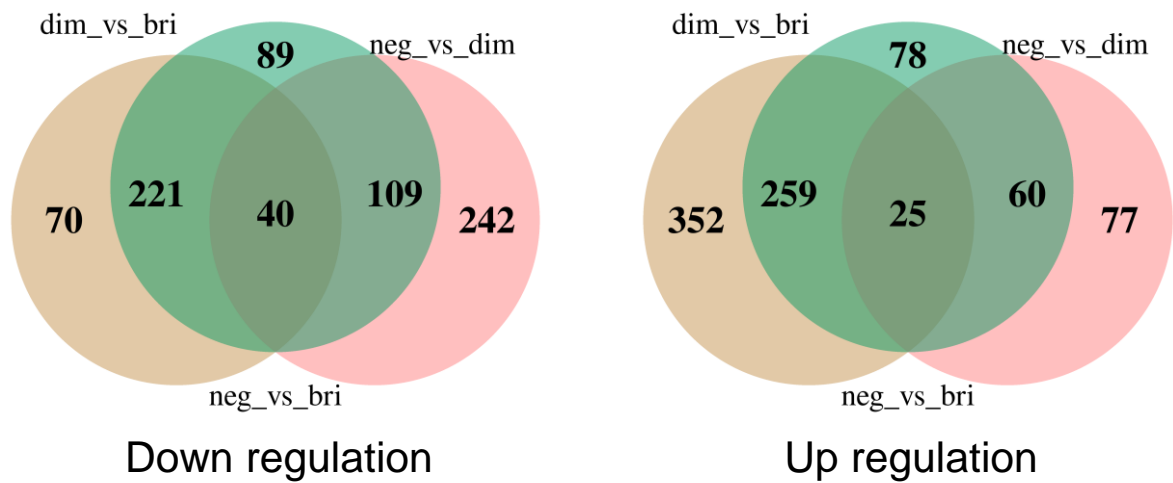
Immune dysfunctions of CD56^{neg} NK cells are associated with HIV-1 disease progression
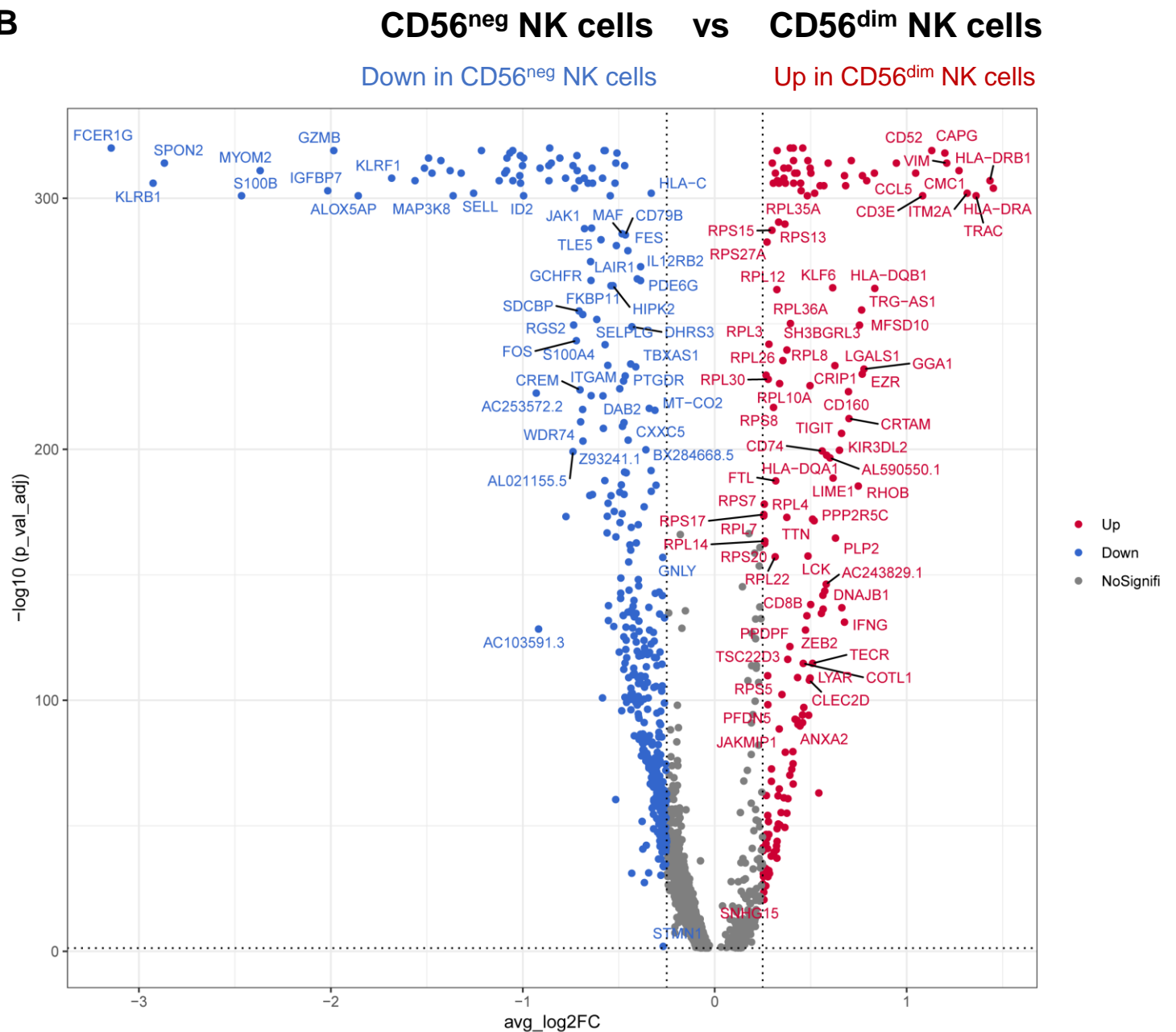
Supplementary Figure 1. Excluding the cells that did not belong to NK cells

(A) TSNE dimplot showed the annotation of all the 7 types of cells in the dataset. (B) Vlnplot showed the expressions of canonical markers of the 7 types of cells. (C) The expression of canonical markers of T cells, B cells, and monocyte (Mono CD14/CD16) were shown.

A

dim_vs_bri | 89 | neg_vs_dim

70 | 221 | 40 | 109 | 242

neg_vs_bri

Down regulation

dim_vs_bri | 78 | neg_vs_dim

352 | 259 | 25 | 60 | 77

neg_vs_bri

Up regulation

B

CD56^{neg} NK cells vs **CD56^{dim} NK cells**

Down in CD56^{neg} NK cells · Up in CD56^{dim} NK cells

$-\log 10\,(p\_val\_adj)$
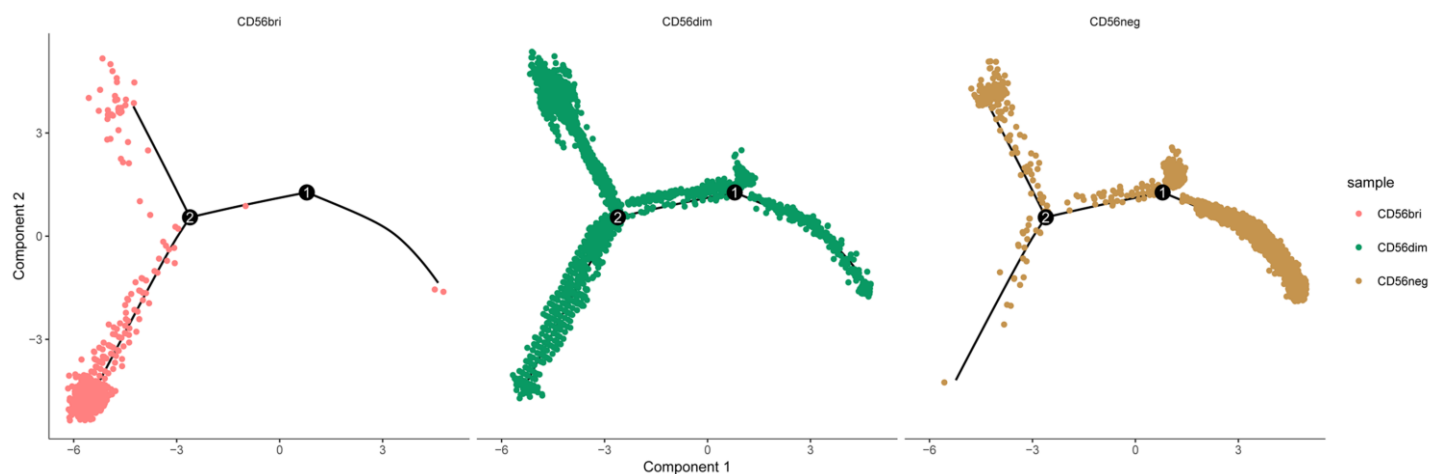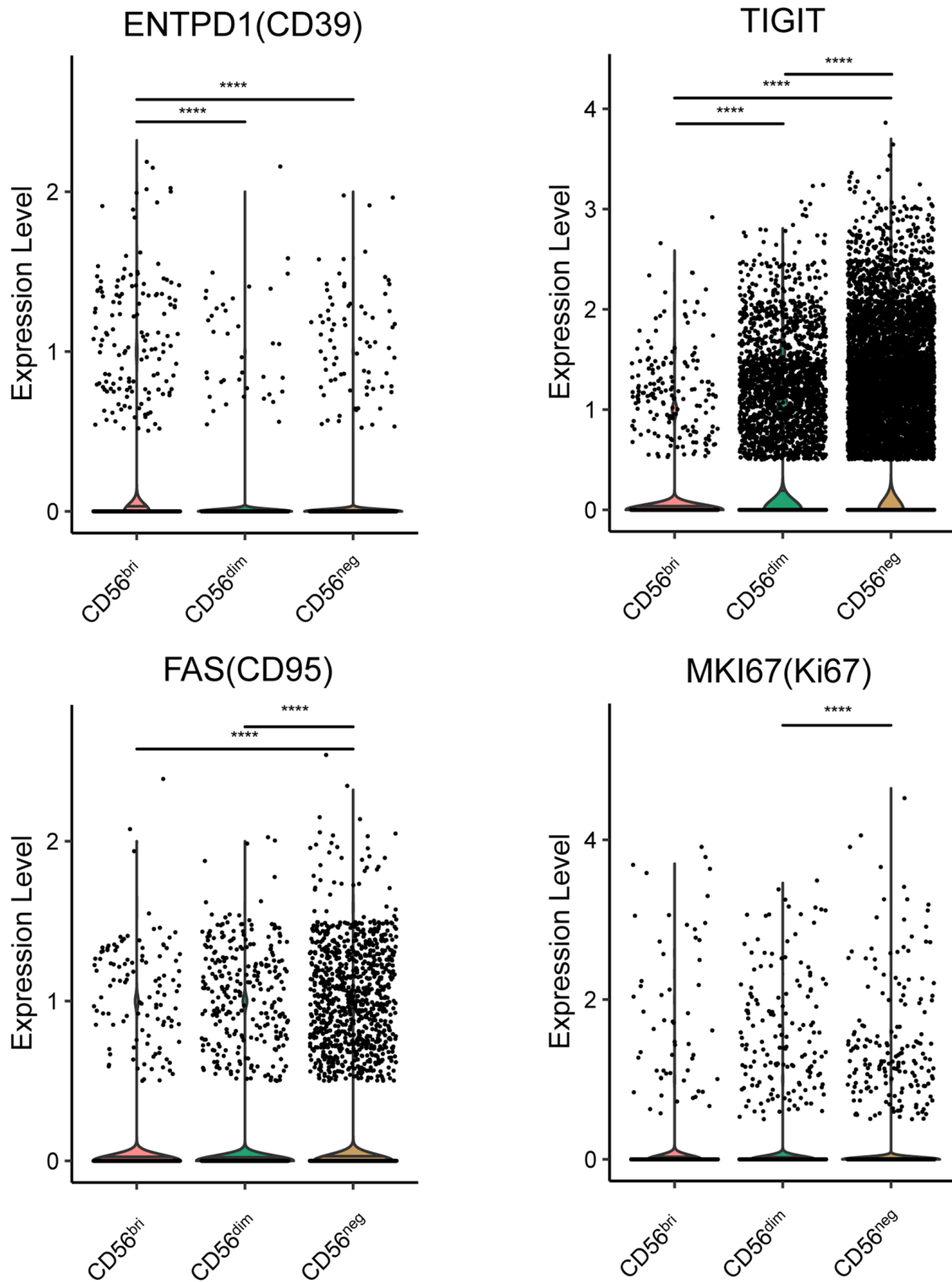
avg_log2FC

- Up
- Down
- NoSignifi

Supplementary Figure 2. Differentially expressed genes (DEGs) in the three NK cell subsets

(**A**) Two Venn diagrams separately showing the relationship of up- and downregulated DEGs between three NK cell subsets. These DEGs were identified using a threshold of "avg_log2FC > 0.25 & p_val_adj < 0.05". (**B**) Volcano plot showing a more detailed analysis of up- and downregulated DEGs between CD56^{neg} and CD56^{dim} NK cells.
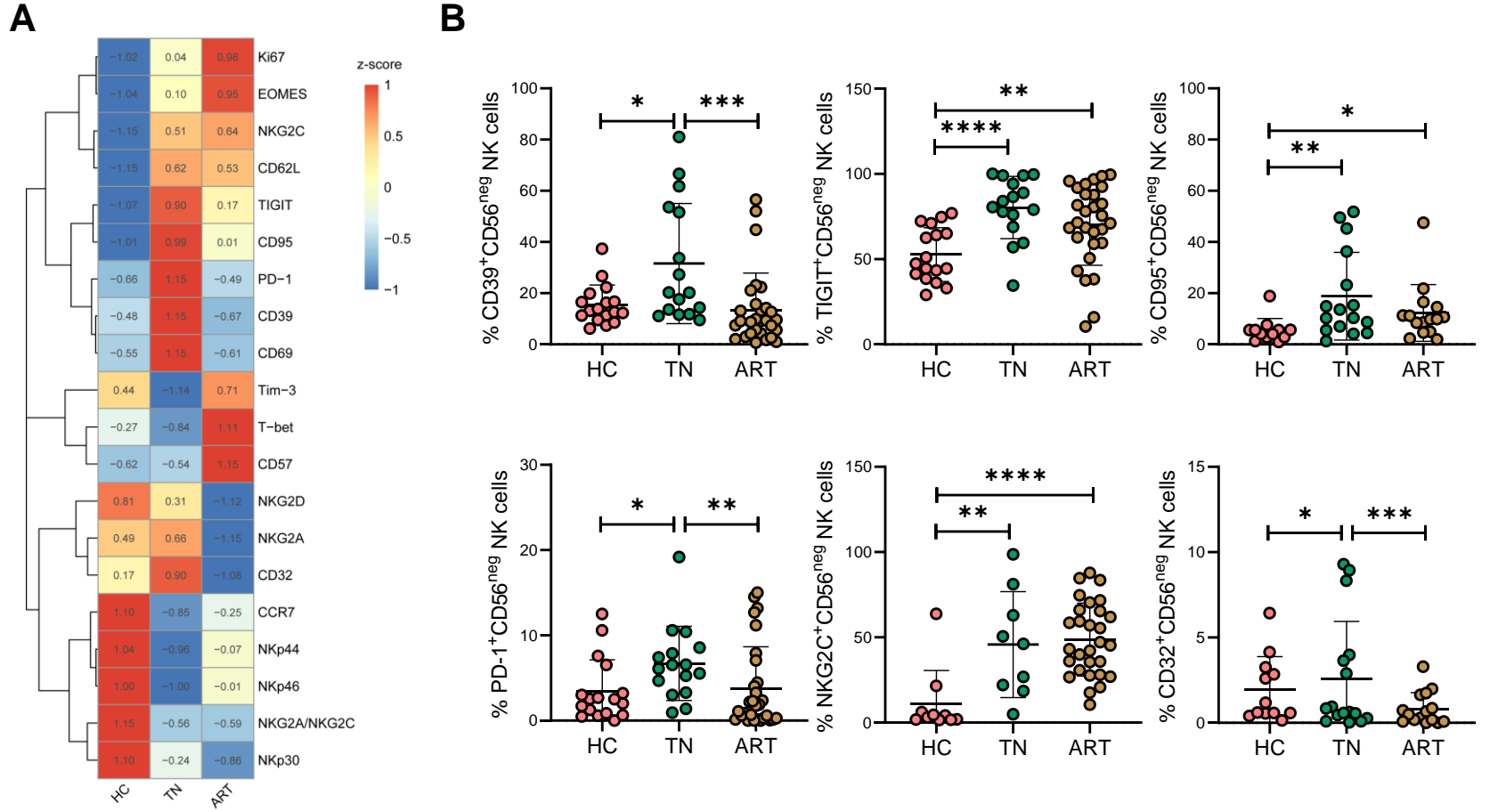
Supplementary Figure 3. The distributions of three NK cell subsets in pseudotime analysis

Three dotplots showing the distributions of three NK cell subsets separately. The DDRTree algorithm was used for dimension reduction.

Supplementary Figure 4. Jitter and violin plots showing single-cell expression levels of selected genes in cells derived from peripheral blood of HIV-1-infected patients

Expression levels were calculated by $log_2(count + 1)$, in which $count$ represents the unique molecular identifier (UMI) count of a particular gene. Statistical significance between two groups was calculated by Mann-Whitney U-test. $*p < 0.05$, $**p < 0.01$, and $****p < 0.0001$

Supplementary Figure 5. Phenotypic of CD56neg NK cells among three groups of participants

(**A**) Heatmap showing the percentage of CD56neg NK cells with specific protein expression across the three groups of participants, which was determined by flow cytometry. Data were scaled using z-score. (**B**) Expression of CD39, TIGIT, CD95, PD-1, NKG2C, and CD32 of CD56neg NK cells in three groups was determined by flow cytometry. Each dot represents one participant. Statistical significance between two groups was determined by Mann-Whitney U-test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$

Supplementary Figure 6. The level of plasma inflammatory markers in healthy controls and HIV-1-infected participants

Comparisons of the plasma levels of sCD14, sCD163, and TGF-β1 in HCs (n = 18), treatment-naïve HIV-1-infected patients (TNs; n = 34), and HIV-1-infected patients with successful antiretroviral therapy (ARTs; n = 15). Each dot represents one participant. Statistical significance between two groups was determined using Mann-Whitney U-test. *p < 0.05, **p < 0.01, and ****p < 0.0001
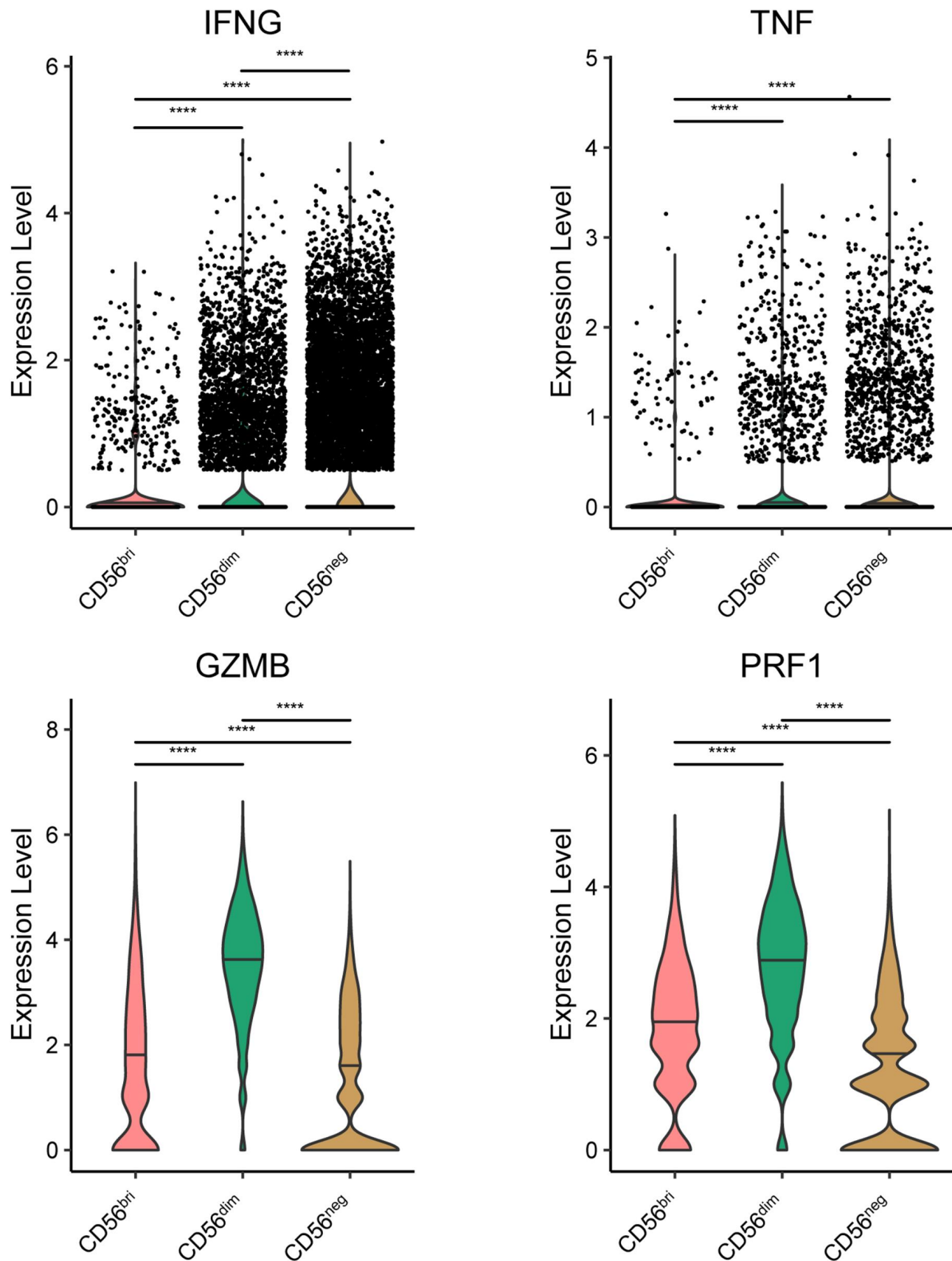
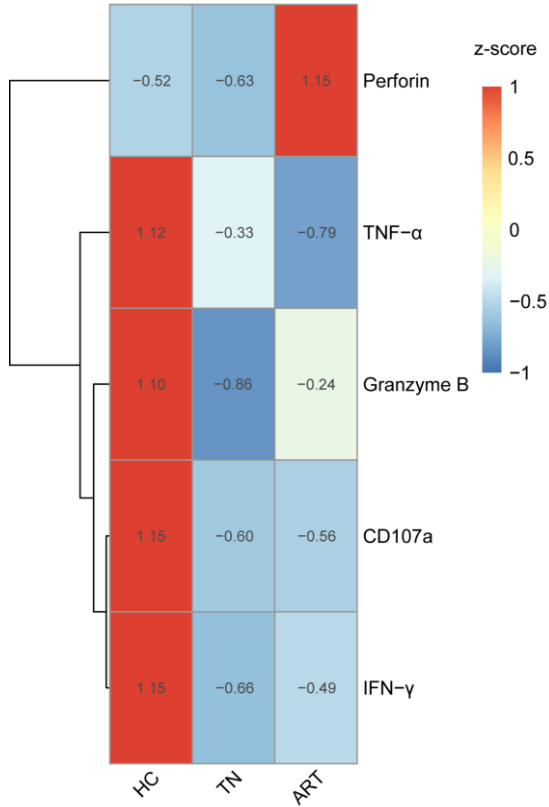**Supplementary Figure 7. Jitter and violin plots showing single-cell expression levels of selected genes in cells derived from peripheral blood of HIV-1-infected patients**

Expression levels were calculated by $log_2(count + 1)$, in which $count$ represents the unique molecular identifier (UMI) count of a particular gene. Statistical significance between two groups was calculated using Mann-Whitney U-test. ****$p < 0.0001$

Supplementary Figure 8. The functions of CD56^{neg} NK cells from three groups of participants

Heatmap (**A**) and scatter plot (**B**) showing the expression of IFN-γ, TNF-α, CD107a, perforin, and granzyme B in CD56^{neg} NK cells from HC, TN, ART. Each dot represents one participant. Statistical significance between two groups was determined by Mann-Whitney U-test. *$p < 0.05$ and **$p < 0.01$
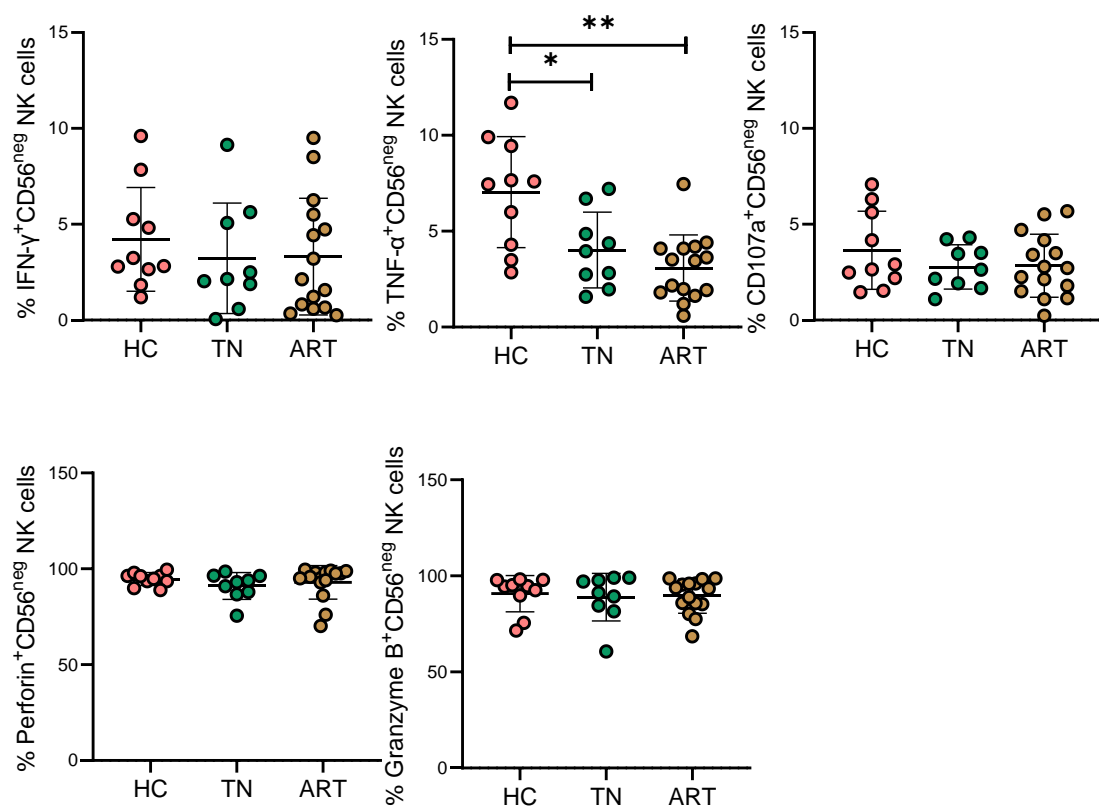
Supplementary Table 1. Characteristics of HIV-1-infected patients with successful antiretroviral therapy (ARTs)

Characteristics	ARTs (n=29)
CD4 Count before ART, cells/μL, Median (IQR)	382 (320~445)
Viral load before ART, Log copies/mL, median (IQR)	4.54 (4.06~4.83)
Duration of ART, months, median (IQR)	38 (26~43)
ART regimen, n (%)	
3TC/TDF/EFV	27 (93)
3TC/AZT/EFV	2 (7)
Transmission route, n (%)	
MSM	26 (90)
Heterosexual	2 (7)
Blood transfusion	1 (3)

All values, except for the ART regimen and transmission route, are expressed as median (interquartile range, IQR). Abbreviations: 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; EFV, efavirenz; AZT, zidovudine; MSM, men who have sex with men

Supplementary Table 2. Reagents used for flow cytometry analyses

Antibodies	Source	Clone	Identifier
APC-Fire 750 anti-human CD3	BioLegend	SK7	344840
APC-Fire 750 anti-human CD14	BioLegend	63D3	367120
APC-Fire 750 anti-human CD20	BioLegend	2H7	302358
PerCP anti-human CD16	BioLegend	3G8	302030
BV421 anti-human CD16	BD	3G8	562874
BV421 anti-human CD56	BioLegend	HCD56	318328
APC anti-human CD56	Tongshengshidai	NA	Z6410017
PE-Cy7 anti-human CD56	BioLegend	HCD56	318318
APC anti-human-CD39	BioLegend	A1	328210
FITC anti-human Ki67	BioLegend	Ki-67	350508
PE-Cy7 anti-human CD62L	BioLegend	DREG-56	304822
AF647 anti-human NKp44	BD	P44-8.1	558563
AF647 anti-human NKp30	BD	P30-15	558408
FITC anti-human NKG2A	MACS	REA110	130-113-565
BV510 anti-human NKp46	BioLegend	9E2	331924
eF660 anti-human EOMES	Thermo Fisher	WD1928	50-4877-42
PE-Cy7 anti-hum Tim-3	Thermo Fisher	F38-2E2	25-3109-42
PE anti-hum Tim-3	BD	7D3	565570
BV421 anti-human T-bet	BioLegend	4B10	644816
BV510 anti-human CD69	BioLegend	FN50	310936
PE-Cy7 anti-human CD69	BioLegend	FN50	557745
PE anti-human NKG2C	BioLegend	S19005E	375004
FITC anti-human CD57	BioLegend	HNK-1	359604
APC anti-human CCR7	BD	G043H7	353214
FITC anti-human CD32	BioLegend	FUN-2	303204
PE anti-human CD95	BD	DX2	555674
BV510 anti-human PD-1	BioLegend	EH12.1	563076
PE anti-human TIGIT	BioLegend	A15153G	372704
PE-Cy7 anti-human NKG2D	BioLegend	1D11	320812
APC anti-human CD7	BioLegend	CD7-6B7	343108
FITC anti-human CD107a	BioLegend	H4A3	328606
FITC anti-human IFN-γ	BioLegend	4SB3	502506
BV421 anti-human TNF-α	BioLegend	MAb11	502932
APC anti-human Granzyme B	BD	GB11	560212
PE-Cy7 anti-human Perforin	BioLegend	dG9	308126

Supplementary Table 3. Selected genes used for calculating five function module scores

Function Modules	Genes Used for Module Score
Cytotoxicity	GZMA, GZMB, GZMH, GZMM, GZMK, GNLY, PRF1, CTSW
Cytokine and Chemokine Receptors	IL7R, IL2RB, IL21R, CXCR3, CXCR4, CCR7, CXCR1, S1PR5
Adhesion Molecules	ITGAL, CD2, CD58, ITGB2, SELL, CD96
Exhaustion	LAG3, TIGIT, PDCD1, HAVCR2, TOX
Apoptosis	FAS, CRIP1, IFI27, CD74, IFNG, HERPUD1, HMGB2, LCK, BCL2A1, RPL11, TYROBP, CD3E, LGALS3, JUN, PRELID1, IFI27L2

Supplementary Table 4. Top 10 upregulated and downregulated biological process

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	Count	Regulation
GO:0006614	SRP-dependent cotranslational protein targeting to membrane	44/147	105/18862	7.93E-67	1.85E-63	1.54E-63	44	Up
GO:0006613	cotranslational protein targeting to membrane	44/147	110/18862	1.06E-65	6.62E-63	5.51E-63	44	Up
GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	45/147	120/18862	1.14E-65	6.62E-63	5.51E-63	45	Up
GO:0045047	protein targeting to ER	45/147	120/18862	1.14E-65	6.62E-63	5.51E-63	45	Up
GO:0072599	establishment of protein localization to endoplasmic reticulum	45/147	124/18862	6.94E-65	3.23E-62	2.69E-62	45	Up
GO:0070972	protein localization to endoplasmic reticulum	45/147	152/18862	3.80E-60	1.47E-57	1.23E-57	45	Up
GO:0019083	viral transcription	46/147	180/18862	3.67E-58	1.22E-55	1.02E-55	46	Up
GO:0006413	translational initiation	46/147	193/18862	1.35E-56	3.93E-54	3.27E-54	46	Up
GO:0019080	viral gene expression	46/147	198/18862	5.01E-56	1.30E-53	1.08E-53	46	Up
GO:0000956	nuclear-transcribed mRNA catabolic process	45/147	208/18862	3.16E-53	7.37E-51	6.14E-51	45	Up
GO:0034341	response to interferon-gamma	27/341	197/18862	2.49E-16	9.64E-13	7.50E-13	27	Down
GO:0071346	cellular response to interferon-gamma	24/341	177/18862	1.53E-14	2.96E-11	2.30E-11	24	Down
GO:0042110	T cell activation	36/341	474/18862	3.61E-13	4.67E-10	3.63E-10	36	Down
GO:0002831	regulation of response to biotic stimulus	32/341	420/18862	7.33E-12	7.10E-09	5.52E-09	32	Down
GO:0007159	leukocyte cell-cell adhesion	29/341	366/18862	2.88E-11	2.23E-08	1.74E-08	29	Down
GO:0042119	neutrophil activation	34/341	500/18862	3.59E-11	2.32E-08	1.80E-08	34	Down
GO:0002683	negative regulation of immune system process	30/341	403/18862	6.08E-11	3.37E-08	2.62E-08	30	Down
GO:0043312	neutrophil degranulation	32/341	485/18862	3.00E-10	1.46E-07	1.13E-07	32	Down
GO:0002283	neutrophil activation involved in immune response	32/341	488/18862	3.51E-10	1.51E-07	1.18E-07	32	Down
GO:0002446	neutrophil mediated immunity	32/341	499/18862	6.13E-10	2.38E-07	1.85E-07	32	Down