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**Supplemental information**

**IL-27 induces IFN/STAT1-dependent  
genes and enhances function  
of TIGIT<sup>+</sup> HIVGag-specific T cells**

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## Supplementary Materials

**Figure S1. T cell expression of IL-27R $\alpha$  and gp130 in PLWH and healthy controls. Related to Figure 1.** PBMCs from PLWH (HIV<sup>+</sup>, frozen n= 10, fresh n= 5) and healthy controls (HC, frozen n= 10) were rested overnight and stained with IL-27R $\alpha$  and gp130 mAbs (Table S2 Panel 1). Representative histogram and expression levels of: **(A)** IL-27R $\alpha$ /WSX-1 and **(B)** gp130 in CD4 and CD8 T cells. The graphs represent expression levels of IL-27R $\alpha$  and gp130 (median fluorescence intensity, MFI) in CD4 and CD8 T cells from PLWH and HC. Graphs represent the box and whisker showing the median value with first and third quartiles in the box, with whiskers extending to the minimum and maximum values. **(C)** Left panel: representative histogram of phosphorylation of STAT1 by increasing concentration of rhIL-27 (5, 10, 25 and 100 ng/ml). Right Panel: PBMC from healthy control (n= 3) were pre-incubated with increasing concentrations of anti-IL-27R $\alpha$  mAb (5, 10, and 20  $\mu$ g/ml) for 20 minutes on ice followed by stimulation with rhIL-27 at 5, 10, 25 and 100 ng/ml for 30 minutes. Phosphorylation of STAT1 was measured by flow cytometry, graph represents median and interquartile range of the frequency of p-STAT1 positive T cells. **(D)** PBMCs from PLWH (HIV<sup>+</sup>, n=10) were rested overnight and incubated with anti-IL-27R $\alpha$  (10  $\mu$ g/mL), anti-gp130 (10  $\mu$ g/ml), alone or in combination. IgG (10  $\mu$ g/mL) was used as control. Cells were stimulated with 10 ng/ml of rhIL-27 for 30 minutes and p-STAT1 and p-STAT3 was measured by flow cytometry. **(E)** Cells were stimulated with 10 ng/mL of IL-6 for 30 minutes and STAT1 and STAT3 phosphorylation was measured by flow cytometry. The graphs are represented by box and whisker showing the median value with first and third quartiles in the box, with whiskers extending to the minimum and maximum values. Comparisons between the culture conditions were performed by Wilcoxon test. *P* value < 0.05 was considered significant.

**Figure S2. DEGs in T cell subsets upon *in vitro* stimulation IL-27. Related to Figure 2.**

RNAseq analysis was performed in sorted naïve (CD45RA<sup>+</sup>CD27<sup>+</sup>) and memory (CD45RA<sup>-</sup>CD27<sup>+</sup>) T cells from PLWH (HIV<sup>+</sup>, n= 5) and healthy controls (HC, n= 3) stimulated *in vitro* with 100 ng/ml of rhIL-27 for 90 minutes. Bar graphs represent Differentially Expressed Genes (DEGs) upregulated (red bars) and downregulated (blue bars) in T cell subsets. The DEGs induced by IL-27 stimulation used were selected based on expression change > 2 -fold ( $|\text{Log}_2 \text{FC}| > 1$ ) compared with unstimulated condition and FDR < 0.05.

**Figure S3. *TBX21* is upregulated by IL-27 stimulation. Related to Figure 3.**

RNAseq analysis was performed in sorted naïve (CD45RA<sup>+</sup>CD27<sup>+</sup>) and memory (CD45RA<sup>-</sup>CD27<sup>+</sup>) T cells from PLWH (HIV<sup>+</sup>, n= 5) and healthy controls (HC, n= 3) stimulated *in vitro* with 100 ng/ml of rhIL-27 for 90 minutes. **(A)** Expression of Th1, Th2, Th17 and Treg core genes in T cell subsets with IL-27 stimulation is represented by heatmap. **(B)** Expression of immune checkpoint receptors in T cell subsets with IL-27 stimulation is represented by heatmap. Gene expression ( $\log_2$  of stimulated /unstimulated ratio) level is indicated by different color. **(C)** PBMCs from healthy control (HC, n= 1) were cultured with rhIL-27 at concentrations: 0.5ng/ml, 5 ng/ml, 25 ng/ml and 100 ng/ml for 6 hours or overnight. Flow cytometry analysis was performed for expression of CD69 in T cells. Line with round symbol for overnight culture and line with triangle symbol for 6 hours culture. **(D)** PBMCs from PLWH (HIV<sup>+</sup>, n= 17) were cultured in the absence (opened symbols) or presence (closed symbols) of rhIL-27 (100 ng/ml) overnight. Flow cytometry analysis was performed for expression of Granzyme A and Granzyme B in CD4 and CD8 T cells. Expression of GZA<sup>+</sup>GZB<sup>+</sup> is represented as frequencies of CD4 and CD8 T cells. The graph is represented by box and whisker showing the median value with first

and third quartiles in the box, with whiskers extending to the minimum and maximum values. Comparisons between culture conditions were performed using non-parametric Wilcoxon test.  $P$  value  $< 0.05$  was considered significant.

**Figure S4. IL-27 promoted cytokine secretion in TIGIT<sup>-</sup> T cells from HIV infected donors.**

**Related to Figure 4.** PBMCs from PLWH (HIV<sup>+</sup>, n=17) were cultured in the absence (red opened symbols) or presence (red closed symbols) of rhIL-27 (100 ng/ml) overnight follow by stimulation with HIV<sub>Gag</sub> peptide pool. DMSO was used as control. PBMCs from and healthy controls (HC, n= 17) were cultured in the absence (black opened symbols) or presence (black closed symbols) of rhIL-27 (100 ng/ml) overnight and stimulated with CEF. DMSO was used as control. Secretion of cytokines by TIGIT<sup>-</sup> T cells was analyzed by flow cytometry. **(A)** Gating strategy for TIGIT<sup>-</sup> CD4 and CD8 T cells. TIGIT<sup>-</sup> CD4 and CD8 T cells were analyzed for expression of: **(B)** CD69<sup>+</sup>CD107a<sup>+</sup>; **(C)** CD69<sup>+</sup>IFN $\gamma$ <sup>+</sup>; and **(D)** CD69<sup>+</sup>TNF $\alpha$ <sup>+</sup>. The graphs are represented by box and whisker showing the median value with first and third quartiles in the box, with whiskers extending to the minimum and maximum values of the frequencies of T cells secreting cytokines. Comparisons between the culture conditions were performed by Wilcoxon test. Comparisons between PLWH and healthy controls were performed by Mann-Whitney test.  $P$  value  $< 0.05$  was considered significant.

**Figure S5. Detection of p24 in the supernatants of PBMCs from PLWH. Related to Figure 4 and 5.** PBMCs from PLWH (n= 4) were cultured in presence or absence of IL-27 100 ng/ml at a cell concentration of 2 to 3x10<sup>6</sup> cells/ml. Supernatants were harvested after overnight and 3 days of culture. PBMCs (n= 4) stimulated with media and CD3/CD28 (TransAct beads) during 4

days were used as controls. P24 was measured in the supernatants by ultrasensitive ELISA (as described by Levinger et al. DOI: 10.21203/rs.3.rs-832339/v1). The graph is represented by box and whisker showing the median value with first and third quartiles in the box, with whiskers extending to the minimum and maximum values. Each color represents one individual donors and viral load and CD4 counts are indicated. The dashed line in the graphs represents the LOD (limit of detection) of p24. Overnight and 3 day is 3.02 fg/mL and 4 day culture LOD for is 7.17 fg/mL.

**Table S1. Patients Characteristics. Related to STAR Methods.**

<b>Cohorts</b>	<b>Cohorts used in the experiments</b>			
	<b>IL-27 Receptor Expression and Blockade</b>	<b>STATs Expression and Activation</b>	<b>RNAseq Analysis</b>	<b>Cytokine Secretion Assay</b>
<b>Gender</b>	F (3), M (12)	F (2), M (10)	F (1), M (4)	F (2), M (15)
<b>Age (median, IQR)</b>	56 (47-58)	52.5 (43.25-58)	60 (48.5-62)	55 (45-60.75)
<b>CD4 count cells/<math>\mu</math>l (median, IQR)</b>	834 (543-1083)	321 (236.3-685.3)	921 (559-1103)	560 (487-757)
<b>CD8 count cells/<math>\mu</math>l (median, IQR)</b>	Not reported	850 (437.5-1258)	Not reported	867 (574.5-1333)
<b>Viral Load copies/ml (median, IQR)</b>	20 (20-20)	50 (20-50)	20 (20-20)	20 (20-20)

\*F: female; M: male, IQR indicated 25-75% range

**Table S2. Antibodies used in Flow Cytometry Experiments. Related to STAR Methods.**

<b>Antibody</b>	<b>Fluorochrome</b>	<b>Clone</b>	<b>Catalog No.</b>	<b>Company</b>
<b>Panel 1</b>				
CD3	BV711	UCHT1	563725	BD Biosciences
CD8	BUV395	RPA-T8	563795	BD Biosciences
CD4	BUV805	SK3	612887	BD Biosciences
IL-27R $\alpha$	PE	191106	FAB14791P	R&D Systems
gp130	PE-Cy7	2E1B02	362008	Biolegend
<b>Panel 2</b>				
CD3	BV711	UCHT1	563725	BD Biosciences
CD4	BUV805	SK3	612887	BD Biosciences
(pY701) STAT1	PE	4a	612564	BD Biosciences
<b>Panel 3</b>				
CD3	BV711	UCHT1	563725	BD Biosciences
CD4	BUV805	SK3	612887	BD Biosciences
CD45RA	BV510	HI100	304141	Biolegend
CD27	BUV737	L128	612829	BD Biosciences
STAT1	A647	1/STAT1	558560	BD Biosciences
STAT3	PerCP-Cy5.5	M59-50	564133	BD Biosciences
(pY701) STAT1	PE	4a	612564	BD Biosciences
(pY705) STAT3	A488	D3A7	4323S	Cell Signaling
<b>Panel 4</b>				
CD3	FITC	OKT3	317306	Biolegend
CD4	PE	L200	550630	BD Biosciences
CD8	APC	RPA-T8	555369	BD Biosciences
CD27	APC-H7	M-T271	560222	BD Biosciences
CD45RA	BV605	HI100	562886	BD Biosciences
<b>Panel 5</b>				
CD3	BV650	SP34-2	563916	BD Biosciences
CD4	BUV805	SK3	612887	BD Biosciences
CD8	BUV395	RPA-T8	563795	BD Biosciences
CD25	BV711	M-A251	356138	Biolegend
CD69	BV785	FN50	563834	BD Biosciences
LAG-3 (CD223)	PE-Dazzle 594	11C3C65	369332	Biolegend
TIGIT	PE-Cy7	A15153G	372714	Biolegend
TIM-3	APC-Cy7	F38-2E2	345026	Biolegend
CD107a	PE-Cy5	H4A3	555802	BD Biosciences
IFN $\gamma$	V570	4S.B3	502534	Biolegend
TNF $\alpha$	A488	MAb11	557722	BD Biosciences
Granzyme A	AF700	CB9	507210	Biolegend
Granzyme B	V510	GB11	563388	BD Biosciences
FOXP3	PE	259D	320208	Biolegend
T-bet	V450	4B10	644816	Biolegend
GATA3	A647	16E10A23	653810	Biolegend



**Table S3. Top 10 canonical pathway analysis in T cells subsets upon IL-27 stimulation. Related to Figure 2.**

Naïve CD4 T cells							
HC				HIV <sup>+</sup>			
Canonical pathway	-log (p-value)	Ratio	Z-score	Canonical pathway	-log (p-value)	Ratio	Z-score
Interferon Signaling	10.5	0.25	2.121	Interferon Signaling	14.1	0.361	2.887
Role of Hypercytokinemia/hyperchemokinaemia in the Pathogenesis of Influenza	9.4	0.128	3.317	Systemic Lupus Erythematosus in B Cell Signaling Pathway	12.2	0.0949	4.315
Prolactin Signaling	7.02	0.105	0.707	Role of Hypercytokinemia/hyperchemokinaemia in the Pathogenesis of Influenza	11.1	0.174	3.873
Systemic Lupus Erythematosus in B Cell Signaling Pathway	6.58	0.0511	3.207	Prolactin Signaling	7.82	0.14	1.897
Coronavirus Pathogenesis Pathway	5.58	0.0547	-2.714	JAK/Stat Signaling	7.04	0.134	1.508
JAK/Stat Signaling	4.98	0.0854	-0.378	Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	6.58	0.0903	2.646
STAT3 Pathway	4.45	0.0593	-0.378	Th1 Pathway	6.2	0.1	0.905
MSP-RON Signaling in Macrophages Pathway	4.05	0.0614	1.134	Activation of IRF by Cytosolic Pattern Recognition Receptors	5.98	0.138	1.667
Necroptosis Signaling Pathway	4.02	0.0516	2.121	Coronavirus Pathogenesis Pathway	5.94	0.0746	-1.604
Tumor Microenvironment Pathway	3.59	0.0447	2.121	Tumor Microenvironment Pathway	5.82	0.0782	2.673
Memory CD4 T cells							
HC				HIV <sup>+</sup>			
Canonical pathway	-log (p-value)	Ratio	Z-score	Canonical pathway	-log (p-value)	Ratio	Z-score
Interferon Signaling	15.8	0.361	2.887	Interferon Signaling	18.3	0.417	3.207
Role of Hypercytokinemia/hyperchemokinaemia in the Pathogenesis of Influenza	11.7	0.163	3.742	Role of Hypercytokinemia/hyperchemokinaemia in the Pathogenesis of Influenza	13.2	0.186	4
Systemic Lupus Erythematosus in B Cell Signaling Pathway	7.39	0.062	3.153	Systemic Lupus Erythematosus in B Cell Signaling Pathway	9.48	0.0766	4.583
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	6.4	0.0774	2.646	Th1 Pathway	6.91	0.1	0.333
Activation of IRF by Cytosolic Pattern Recognition Receptors	5.96	0.123	2.121	Prolactin Signaling	6.47	0.116	0.333
Prolactin Signaling	5.04	0.093	0.378	Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	5.71	0.0774	2.449
Coronavirus Pathogenesis Pathway	4.47	0.0547	-3.317	Activation of IRF by Cytosolic Pattern Recognition Receptors	5.47	0.123	2.121
STAT3 Pathway	4.42	0.0667	-0.816	STAT3 Pathway	4.68	0.0741	-0.378
JAK/Stat Signaling	4.23	0.0854	-0.378	Coronavirus Pathogenesis Pathway	4.56	0.0597	-3.464
Role of RIG1-like Receptors in Antiviral Innate Immunity	3.66	0.109	1.342	Role of PKR in Interferon Induction and Antiviral Response	3.89	0.0662	3
Naïve CD8 T cells							
HC				HIV <sup>+</sup>			
Canonical pathway	-log (p-value)	Ratio	Z-score	Canonical pathway	-log (p-value)	Ratio	Z-score

Interferon Signaling	10.3	0.222	1.89	Interferon Signaling	12.9	0.306	2.53
Systemic Lupus Erythematosus in B Cell Signaling Pathway	7.77	0.0474	3.606	Role of Hypercytokinemia/hyperchemokinaemia in the Pathogenesis of Influenza	10.9	0.151	3.606
Prolactin Signaling	7.2	0.093	1.134	Systemic Lupus Erythematosus in B Cell Signaling Pathway	8.7	0.0657	4.243
Role of Hypercytokinemia/hyperchemokinaemia in the Pathogenesis of Influenza	7.2	0.093	2.828	Th1 Pathway	6.98	0.0917	1
Th1 Pathway	6.08	0.0667	0.378	Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	6.75	0.0774	2.236
Tumor Microenvironment Pathway	5.74	0.0503	2.333	Prolactin Signaling	6.3	0.105	1.414
Coronavirus Pathogenesis Pathway	5.33	0.0448	-3	Coronavirus Pathogenesis Pathway	5.55	0.0597	-2.714
MSP-RON Signaling in Macrophages Pathway	5.14	0.0614	1.134	STAT3 Pathway	5.55	0.0741	0.378
GM-CSF Signaling	4.13	0.0714	1	Tumor Microenvironment Pathway	5.25	0.0615	2.714
Oncostatin M Signaling	3.84	0.093	2	MSP-RON Signaling in Macrophages Pathway	4.38	0.0702	1.414
<b>Memory CD8 T cells</b>							
<b>HC</b>				<b>HIV<sup>+</sup></b>			
Canonical pathway	<b>-log (p-value)</b>	<b>Ratio</b>	<b>Z-score</b>	Canonical pathway	<b>-log (p-value)</b>	<b>Ratio</b>	<b>Z-score</b>
Interferon Signaling	16.5	0.333	2.714	Interferon Signaling	14.7	0.333	2.714
Role of Hypercytokinemia/hyperchemokinaemia in the Pathogenesis of Influenza	11.6	0.14	3.464	Role of Hypercytokinemia/hyperchemokinaemia in the Pathogenesis of Influenza	12.4	0.163	3.207
JAK/Stat Signaling	5.5	0.0854	-0.378	Th1 Pathway	8.17	0.1	-0.302
Coronavirus Pathogenesis Pathway	5.45	0.0498	-2.53	Systemic Lupus Erythematosus in B Cell Signaling Pathway	8.11	0.062	2.183
Th1 Pathway	5.4	0.0667	0.378	JAK/Stat Signaling	7.73	0.122	-0.632
Systemic Lupus Erythematosus in B Cell Signaling Pathway	5.05	0.0401	3.317	IL-23 Signaling Pathway	7.54	0.174	-1.414
STAT3 Pathway	5.02	0.0593	-1.633	Coronavirus Pathogenesis Pathway	6.53	0.0647	-3.051
Activation of IRF by Cytosolic Pattern Recognition Receptors	4.98	0.0923	1.633	GM-CSF Signaling	6.08	0.114	-0.378
MSP-RON Signaling in Macrophages Pathway	3.61	0.0526	0.816	IL-9 Signaling	5.74	0.171	-0.447
Tumor Microenvironment Pathway	3.33	0.0391	1.134	Tumor Microenvironment Pathway	5.4	0.0615	0.302

Lists of differentially expressed genes (DEGs) by IL-27 stimulation selected by criteria:  $|\log_2(\text{FC})| > 1$  compared with unstimulated condition and  $\text{FDR} < 0.05$  were used for the pathway analysis (IPA). The top 10 canonical pathways are presented for the T cell subsets from PLWH (HIV<sup>+</sup>) and healthy controls (HC). Ratio indicated the number of genes predicted by IPA to map the canonical pathway. The canonical pathways in T cell subsets were listed according to their  $p$  value (-log).

**Table S4. Top 20 most-affected categories of diseases and functions predicted by IPA in T cells subsets *in vitro* stimulated with IL-27. Related to Figure 2**

Naïve CD4 T cells									
HC					HIV				
Diseases or Functions Annotation	p-value	Predicted Activation State	Activation z-score	# Mole	Diseases or Functions Annotation	p-value	Predicted Activation State	Activation z-score	# Mole
Organismal death	5.48E-07	Decreased	-4.68	66	Organismal death	5.15E-13	Decreased	-7.21	122
Morbidity or mortality	1.62E-07	Decreased	-4.25	68	Morbidity or mortality	8.37E-14	Decreased	-6.95	125
Quantity of lymphatic system cells	2.03E-19	Increased	4.06	50	Cell viability	2.81E-09	Increased	6.20	73
Quantity of lymphocytes	1.68E-18	Increased	4.01	47	Cell survival	5.04E-09	Increased	5.94	74
Quantity of mononuclear leukocytes	1.70E-18	Increased	3.92	48	Cellular homeostasis	3.30E-19	Increased	5.36	100
Cell viability	1.40E-06	Increased	3.90	43	Cell viability of tumor cell lines	5.32E-07	Increased	4.80	52
Quantity of cells	1.27E-10	Increased	3.78	63	Quantity of cells	5.35E-16	Increased	4.11	108
Quantity of blood cells	2.50E-19	Increased	3.62	58	Hematopoiesis of mononuclear leukocytes	1.40E-24	Increased	4.01	69
Quantity of leukocytes	2.59E-20	Increased	3.44	56	Autophagy	3.62E-07	Increased	3.92	31
Cell death of immune cells	3.62E-17	Increased	3.18	40	Quantity of lymphatic system cells	1.29E-23	Increased	3.87	75
Cellular homeostasis	1.19E-15	Increased	3.18	64	Leukopoiesis	2.52E-25	Increased	3.83	76
Apoptosis of T lymphocytes	8.77E-07	Increased	3.02	15	Cell death of immune cells	3.93E-19	Increased	3.75	57
Viral life cycle	1.01E-06	Decreased	-2.98	10	Quantity of lymphocytes	6.85E-23	Increased	3.72	71
Quantity of T lymphocytes	3.84E-17	Increased	2.94	39	Quantity of mononuclear leukocytes	8.27E-24	Increased	3.58	74
Infection of mammalia	9.89E-16	Decreased	-2.81	28	Migration of cells	5.58E-14	Increased	3.51	108
Replication of viral replicon	7.43E-08	Decreased	-2.80	8	Lymphopoiesis	1.28E-23	Increased	3.49	65
Differentiation of antigen presenting cells	2.86E-09	Increased	2.76	16	T cell development	7.60E-21	Increased	3.46	54
Quantity of B lymphocytes	7.43E-10	Increased	2.71	23	Quantity of leukocytes	5.08E-27	Increased	3.44	88
Autophagy	3.96E-07	Increased	2.71	22	Infection of mammalia	4.24E-16	Decreased	-3.41	37
Cell viability of blood cells	2.80E-10	Increased	2.70	19	Cell movement	3.95E-14	Increased	3.41	117
Memory CD4 T cells									
HC					HIV				
Diseases or Functions Annotation	p-value	Predicted Activation State	Activation z-score	# Mole	Diseases or Functions Annotation	p-value	Predicted Activation State	Activation z-score	# Mole
Organismal death	5.43E-10	Decreased	-5.11	90	Infection of mammalia	9.22E-23	Decreased	-4.13	42
Morbidity or mortality	1.71E-10	Decreased	-4.63	92	Organismal death	1.71E-11	Decreased	-3.92	105
Cell viability	2.80E-07	Increased	4.22	54	Cell viability	3.35E-07	Increased	3.72	60
Cell survival	3.45E-07	Increased	4.01	55	Morbidity or mortality	2.45E-12	Decreased	-3.61	108
Infection of mammalia	1.28E-19	Decreased	-3.92	36	Cell death of immune cells	6.27E-21	Increased	3.50	55
Cell death of immune cells	1.85E-19	Increased	3.88	49	Cell death of blood cells	3.35E-20	Increased	3.47	56

Replication of viral replicon	5.92E-12	Decreased	-3.42	12	Viral life cycle	2.52E-11	Decreased	-3.41	17
Cellular homeostasis	2.55E-17	Increased	3.15	79	Replication of viral replicon	3.28E-15	Decreased	-3.40	15
Viral life cycle	2.73E-09	Decreased	-3.13	14	Cellular homeostasis	2.73E-17	Increased	3.21	87
Autophagy	3.24E-06	Increased	2.93	24	Replication of vesicular stomatitis virus	7.36E-15	Decreased	-3.07	16
Multiple Sclerosis	1.08E-05	Increased	2.63	15	Differentiation of antigen presenting cells	3.43E-08	Increased	3.00	18
Replication of vesicular stomatitis virus	6.31E-12	Decreased	-2.60	13	Multiple Sclerosis	4.01E-06	Increased	2.99	17
Transactivation of RNA	1.35E-11	Increased	2.59	31	Differentiation of phagocytes	4.20E-09	Increased	2.91	22
Hematopoiesis of phagocytes	1.37E-09	Increased	2.59	18	Apoptosis of T lymphocytes	1.19E-07	Increased	2.86	20
Apoptosis of T lymphocytes	2.61E-07	Increased	2.55	18	Cell viability of myeloma cell lines	6.33E-06	Increased	2.77	10
Replication of virus	8.44E-16	Decreased	-2.54	40	Cell death of T lymphocytes	7.33E-11	Increased	2.67	26
Differentiation of phagocytes	2.45E-10	Increased	2.52	22	Replication of Murine herpesvirus 4	9.99E-10	Decreased	-2.65	7
Cell death of fibroblast cell lines	8.01E-06	Increased	2.49	20	Transactivation	3.17E-11	Increased	2.64	34
Dendropoiesis	1.44E-08	Increased	2.49	14	Hematopoiesis of phagocytes	1.47E-08	Increased	2.62	18
Maturation of leukocytes	1.33E-09	Increased	2.48	17	Transactivation of RNA	3.31E-11	Increased	2.58	33

### Naïve CD8 T cells

HC					HIV				
Diseases or Functions Annotation	p-value	Predicted Activation State	Activation z-score	# Mole	Diseases or Functions Annotation	p-value	Predicted Activation State	Activation z-score	# Mole
Cell viability	2.79E-05	Increased	4.49	30	Cellular homeostasis	1.04E-17	Increased	5.22	76
Cellular homeostasis	1.07E-16	Increased	4.22	52	Cell viability	1.34E-07	Increased	4.58	52
Organismal death	3.95E-08	Decreased	-3.88	52	Cell survival	1.56E-07	Increased	4.54	53
Morbidity or mortality	2.03E-08	Decreased	-3.56	53	Hematopoiesis of mononuclear leukocytes	3.90E-23	Increased	4.02	55
Leukopoiesis	3.59E-19	Increased	3.44	40	Infection of mammalia	2.93E-15	Decreased	-3.92	30
Hematopoiesis of mononuclear leukocytes	5.23E-18	Increased	3.31	36	Organismal death	2.83E-13	Decreased	-3.80	93
Differentiation of T lymphocytes	8.61E-14	Increased	3.23	23	Leukopoiesis	2.19E-24	Increased	3.76	61
Autophagy of cells	4.70E-07	Increased	3.23	16	Lymphopoiesis	1.62E-21	Increased	3.60	51
Differentiation of antigen presenting cells	9.43E-11	Increased	3.16	15	T cell development	6.70E-22	Increased	3.59	46
Autophagy	5.25E-08	Increased	3.10	19	Cell death of immune cells	1.45E-18	Increased	3.55	46
Infection of mammalia	4.69E-12	Decreased	-3.09	20	Morbidity or mortality	1.97E-13	Decreased	-3.53	94
Differentiation of phagocytes	5.24E-13	Increased	3.05	19	Homeostasis of leukocytes	4.18E-21	Increased	3.36	47
Lymphopoiesis	1.51E-15	Increased	2.78	32	Viral life cycle	9.21E-08	Decreased	-3.28	12
Apoptosis of cervical cancer cell lines	1.04E-05	Increased	2.74	12	Replication of viral replicon	5.56E-11	Decreased	-3.28	11
Development of phagocytes	7.41E-13	Increased	2.73	18	Cell death of blood cells	4.37E-18	Increased	3.26	47
Hematopoiesis of phagocytes	3.55E-12	Increased	2.70	16	Differentiation of T lymphocytes	4.47E-17	Increased	3.25	34
Homeostasis of leukocytes	1.60E-16	Increased	2.69	31	Differentiation of antigen presenting cells	9.83E-10	Increased	3.04	18
Quantity of lymphatic system cells	1.37E-18	Increased	2.69	40	Maturation of leukocytes	4.17E-10	Increased	3.01	17

Lymphocyte homeostasis	7.17E-16	Increased	2.69	30	Differentiation of phagocytes	5.70E-11	Increased	2.94	22
Cell death of cervical cancer cell lines	5.93E-06	Increased	2.68	14	Neuromuscular disease	2.52E-07	Increased	2.78	38
Memory CD8 T cells									
HC					HIV				
Diseases or Functions Annotation	p-value	Predicted Activation State	Activation z-score	# Mole	Diseases or Functions Annotation	p-value	Predicted Activation State	Activation z-score	# Mole
Organismal death	1.02E-05	Decreased	-4.83	54	Cellular homeostasis	1.12E-13	Increased	3.04	67
Morbidity or mortality	6.48E-06	Decreased	-4.55	55	Replication of RNA virus	3.58E-17	Decreased	-2.97	38
Infection of mammalia	7.42E-16	Decreased	-4.12	26	Infection of mammalia	5.84E-14	Decreased	-2.96	28
Cell viability	2.19E-05	Increased	3.96	35	Cell death of blood cells	5.50E-18	Increased	2.91	46
Cellular homeostasis	6.06E-12	Increased	3.08	51	Cell death of immune cells	1.96E-18	Increased	2.87	45
Cell death of immune cells	1.73E-13	Increased	3.08	32	Viral life cycle	6.23E-08	Decreased	-2.80	12
Differentiation of phagocytes	7.40E-08	Increased	2.84	15	Replication of viral replicon	3.78E-11	Decreased	-2.78	11
Maturation of leukocytes	4.78E-05	Increased	2.82	9	Viral Infection	6.74E-23	Decreased	-2.77	89
Differentiation of antigen presenting cells	8.78E-07	Increased	2.67	12	Activation-induced cell death	1.85E-08	Increased	2.76	10
Cell viability of leukocytes	5.47E-08	Increased	2.65	14	Replication of virus	1.60E-17	Decreased	-2.74	40
Viral life cycle	1.69E-08	Decreased	-2.63	11	Neuromuscular disease	2.21E-06	Increased	2.63	35
Multiple Sclerosis	8.61E-06	Increased	2.63	12	Autophagy of cells	3.47E-06	Increased	2.62	20
Relapsing-remitting multiple sclerosis	2.49E-05	Increased	2.63	7	Replication of vesicular stomatitis virus	5.43E-10	Decreased	-2.58	11
Replication of viral replicon	2.84E-11	Decreased	-2.61	10	Autophagy	5.57E-06	Increased	2.57	22
Hematopoiesis of phagocytes	4.91E-07	Increased	2.60	12	Endotoxicosis	6.23E-08	Increased	2.55	12
Leukopoiesis	2.98E-17	Increased	2.51	42	Cell death of macrophages	2.85E-11	Increased	2.46	15
Quantity of B lymphocytes	6.92E-07	Increased	2.42	17	Apoptosis of cervical cancer cell lines	5.71E-07	Increased	2.45	18
Replication of cells	2.68E-06	Decreased	-2.42	7	Activation-induced cell death of lymphocytes	1.07E-07	Increased	2.39	8
Autophagy	7.92E-05	Increased	2.42	16	Endotoxemia	4.06E-07	Increased	2.36	11
Hematopoiesis of mononuclear leukocytes	1.73E-16	Increased	2.40	38	Endotoxin shock response	2.01E-06	Increased	2.36	9

Lists of differentially expressed gene (DEGs) induced by IL-27 stimulation selected by  $|\log_2(\text{FC})| > 1$  and adjusted  $p$ -value  $< 0.05$  was used as input in the Diseases and Functions (IPA). Top 20 activated functions (Activation Z-score  $> 2$  or  $< -2$ ) predicted by IPA are listed. A positive Z value or negative Z value indicated that a function is predicted to be increased or decreased. \*# Mole: the number of molecules (DEGs) are involved in different functions.

Figure S1

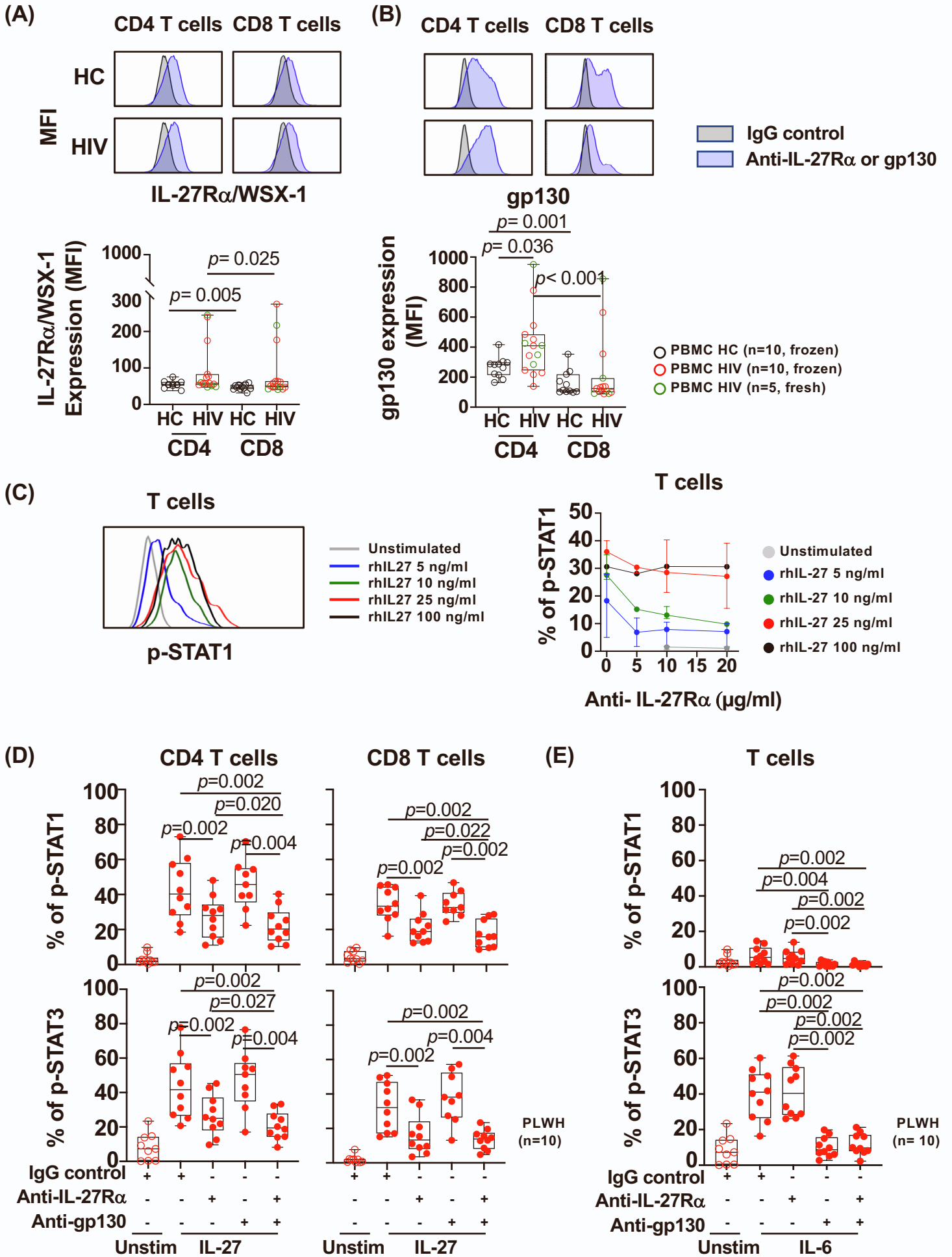


Figure S2

DEGs regulated by IL-27

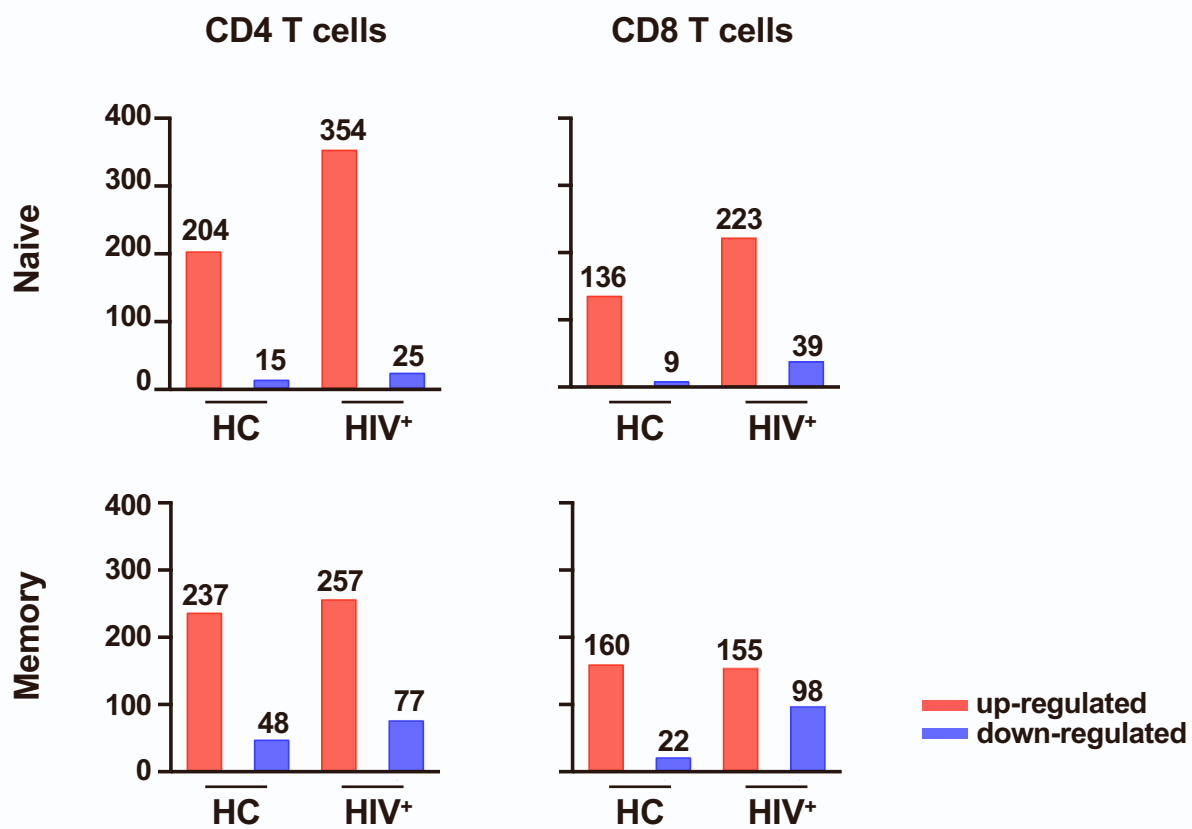


Figure S3

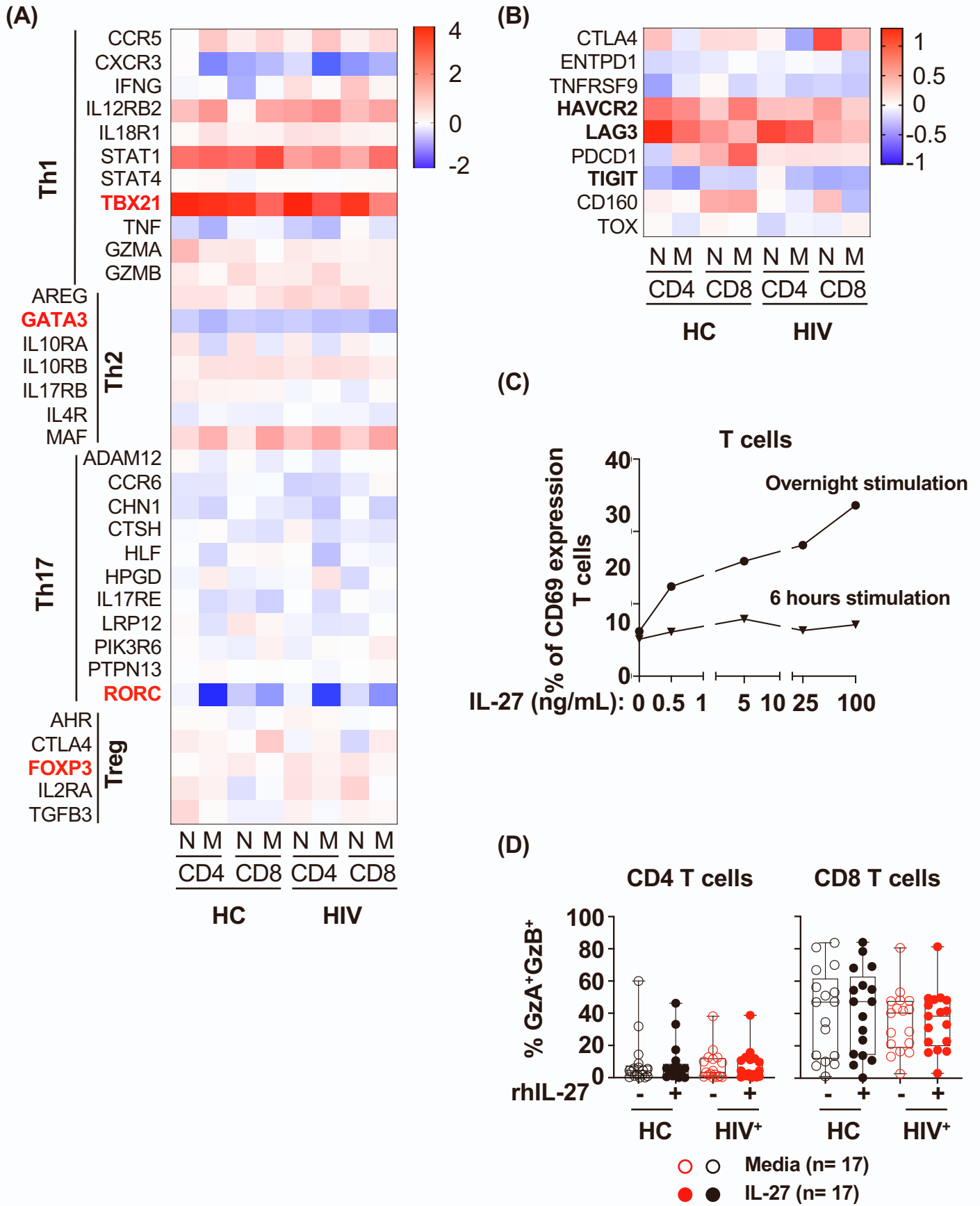




Figure S4

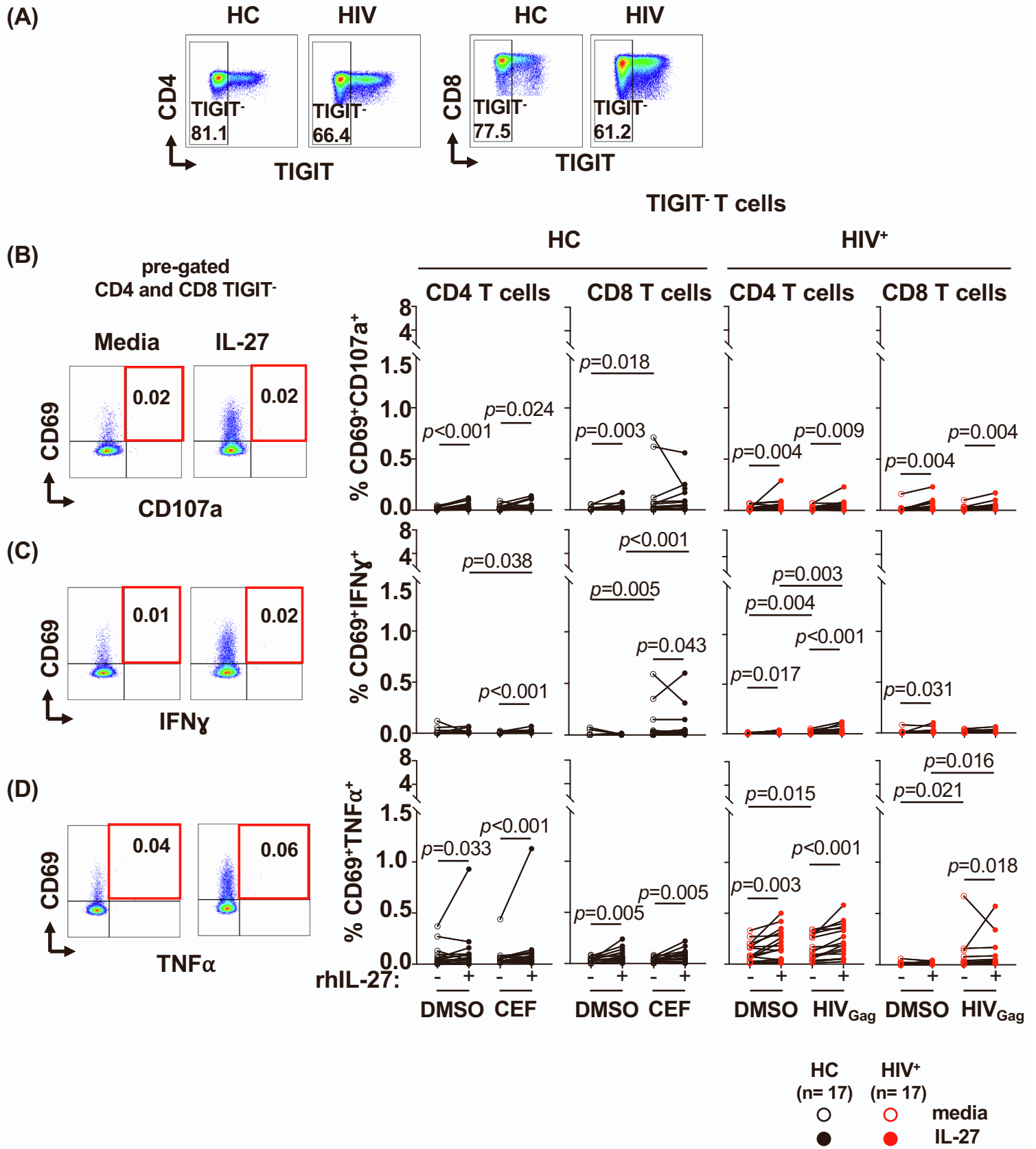
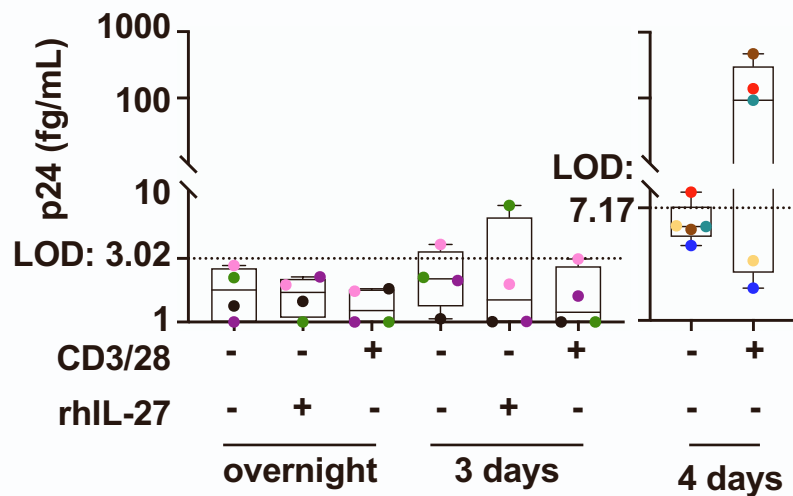


Figure S5



	Viral load (copies/mL)	CD4 (cells/ $\mu$ L)
●	<20	737
●	<20	578
●	<20	835
●	<20	425
●	<20	868
●	<40	1357
●	<40	517
●	<20	854
●	<20	785