

## Supplementary Materials for

### **Sustained IFN-I stimulation impairs MAIT cell responses to bacteria by inducing IL-10 during chronic HIV-1 infection**

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Fig. S1. Frequency and function of MAIT cells in HIV-1–infected patients during ART treatment.

Fig. S2. T cell analysis using Abseq and scRNAseq.

Fig. S3. MAIT cell dysfunction during HIV-1 infection is not ascribed to inhibitory receptors.

Fig. S4. IFN- $\alpha$  does not induce MAIT cell death or apoptosis.

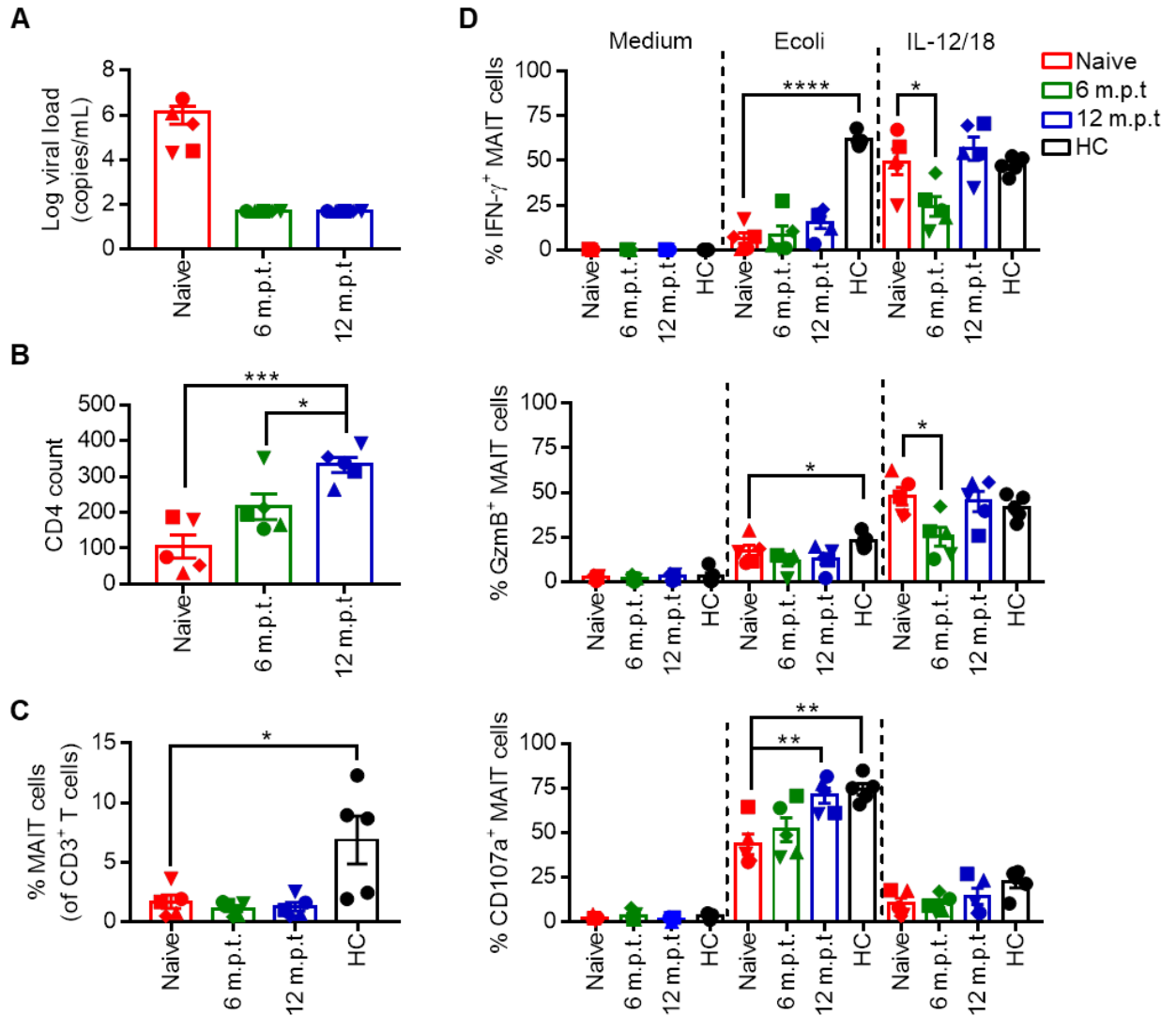
Fig. S5. IFN- $\alpha$ –induced IL-10 inhibits IL-12, thus dampening MAIT cell function.

Table S1. Clinical characteristics of the enrolled participants.

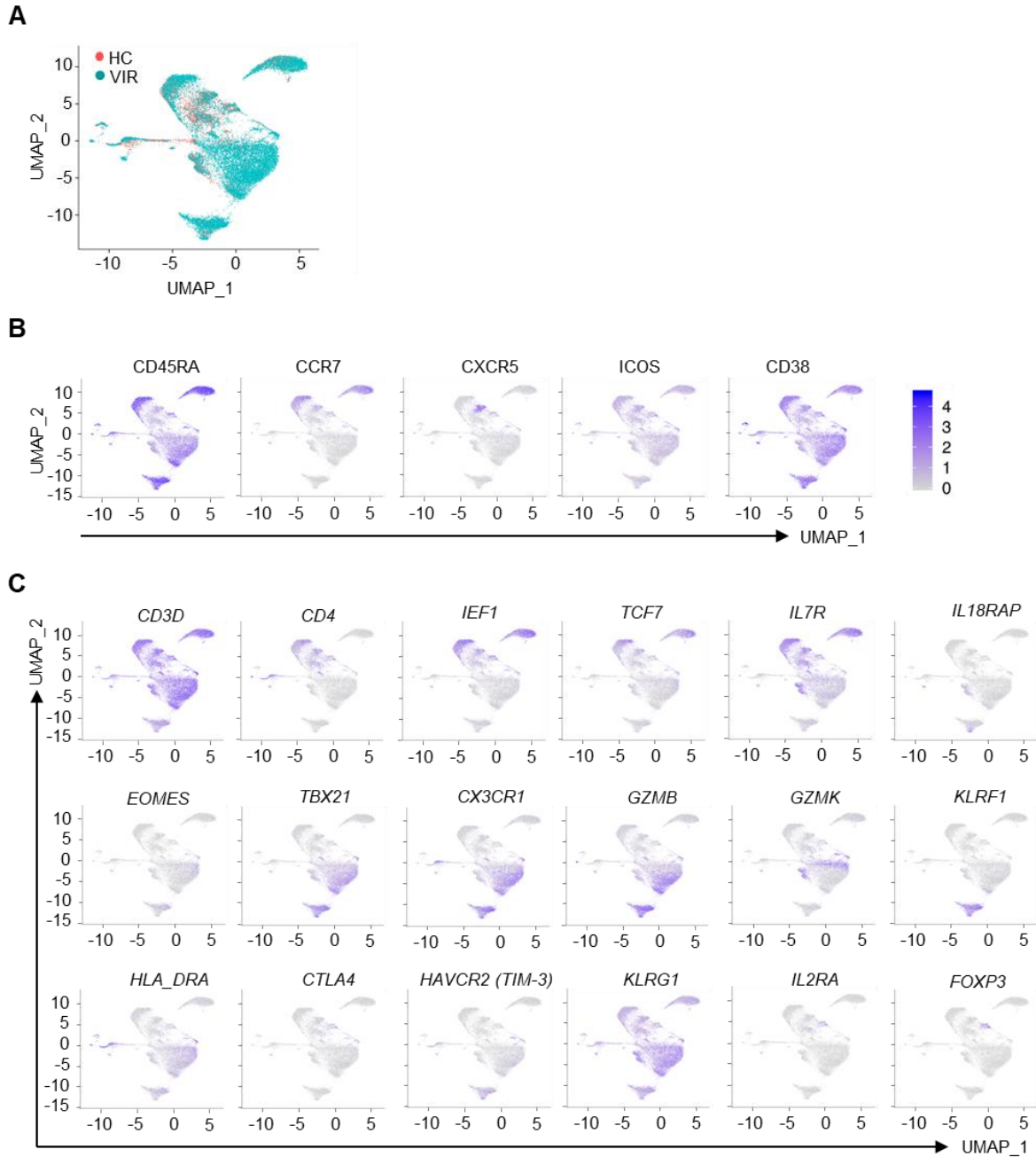
Table S2. BD Rhapsody Abseq antibody panel.

Table S3. BD Rhapsody T cell expression panel.

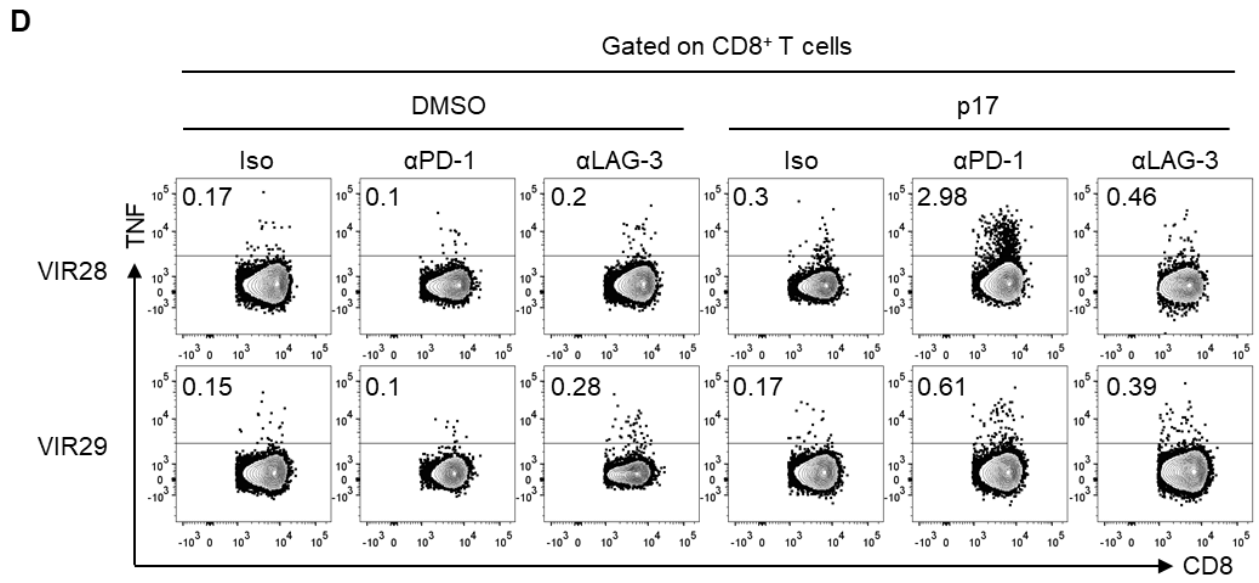
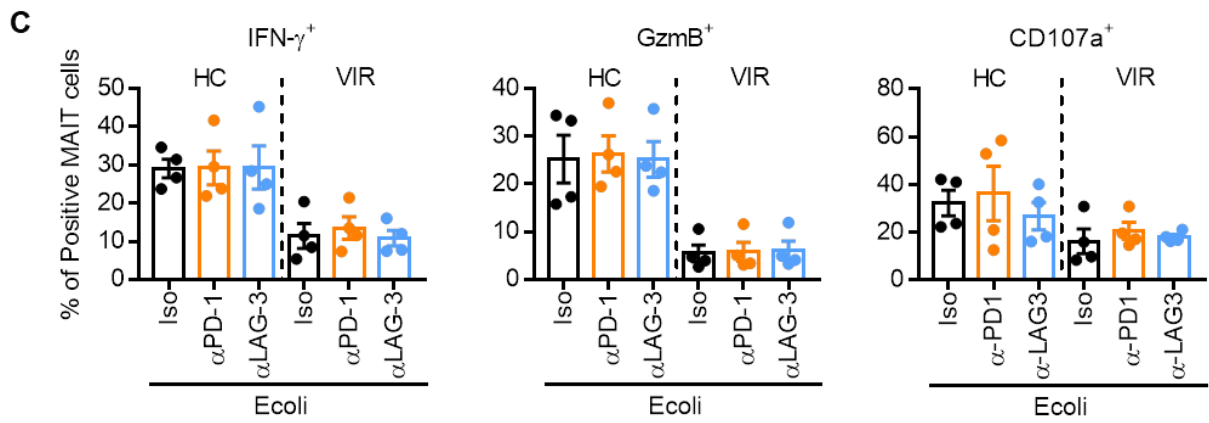
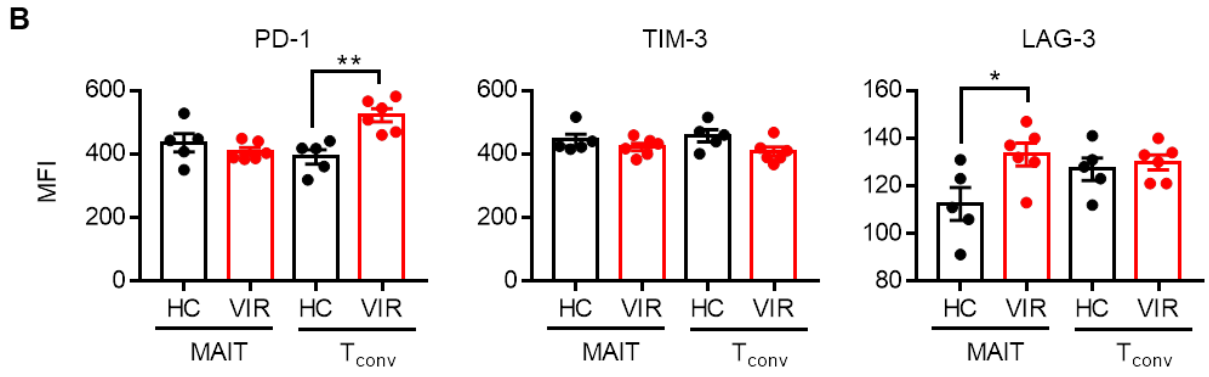
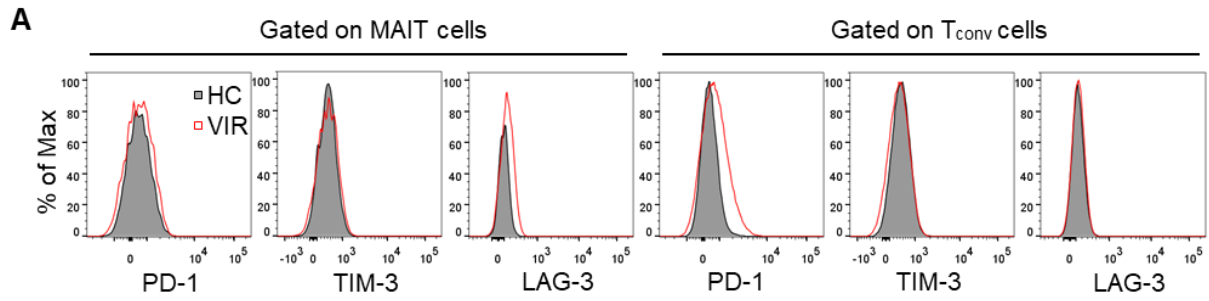
Table S4. List of significantly up-regulated or down-regulated genes in MAIT cells from VIR versus HC subjects.



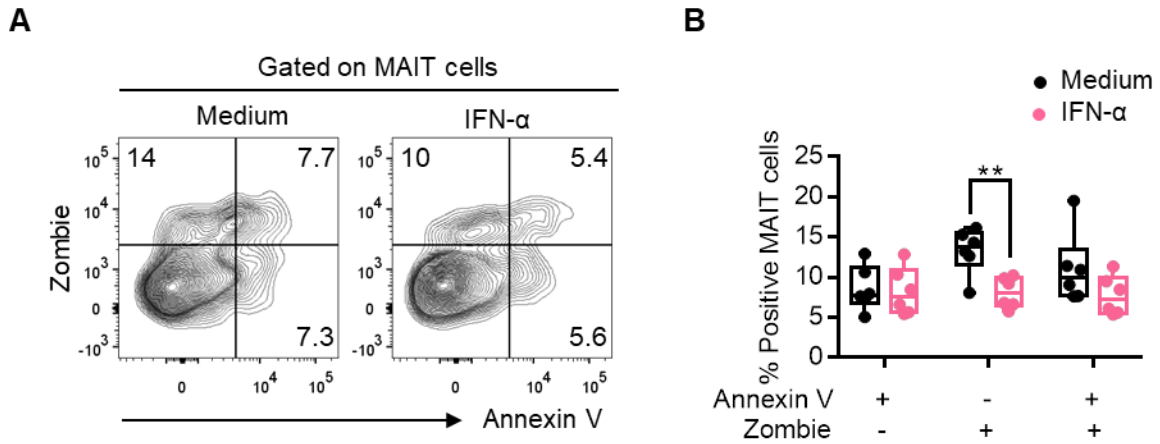
**Fig. S1. Frequency and function of MAIT cells in HIV-1-infected patients during ART treatment.** HIV-1 viral loads (A) and CD4<sup>+</sup> T cell counts (B) in HIV-1-infected patients (n=5) at three time points of treatment: pre-ART, 6 and 12 months post-ART (m.p.t.). (C) The frequency of MAIT cells in frozen PBMCs from those patients and HCs (n=5). (D) The expression level of IFN- $\gamma$ , GzmB, and CD107a in MAIT cells in PBMCs upon stimulation with *E. coli* or IL-12/18.



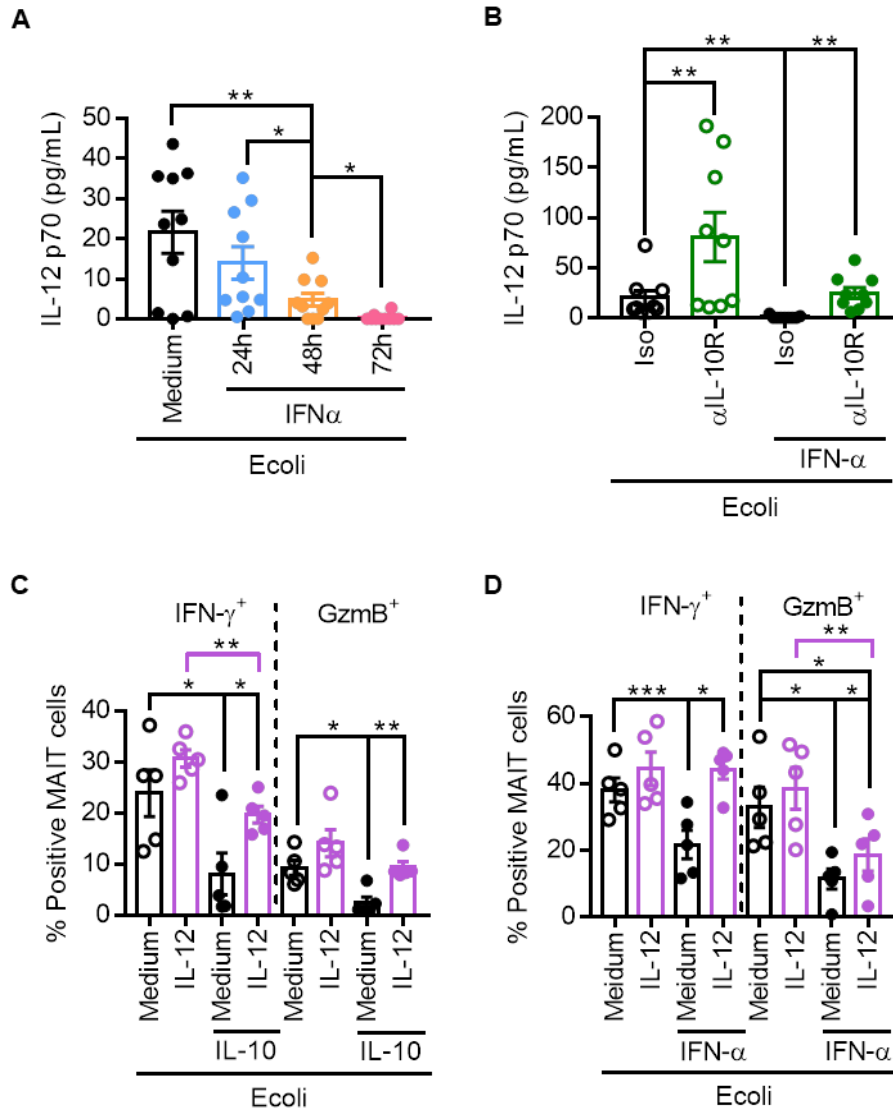
**Fig. S2. T cell analysis using Abseq and scRNAseq.** (A) UMAP plots of cells shown in Figure 2, with color-coding of cells from HC or VIR subjects. (B) UMAP plots highlighting the expression of selected surface proteins. (C) UMAP plots highlighting the expression of selected genes.



**Fig. S3. MAIT cell dysfunction during HIV-1 infection is not ascribed to inhibitory receptors.** (A-B) PD1, TIM3 and LAG3 expression on the cell surface of MAIT cells from HC or VIR subjects as determined by flow cytometry. Representative histograms (A) and statistical analysis (B) are shown. (C) MAIT cells in PBMC freshly isolated from HC (n = 4) or ART-free HIV<sup>+</sup> (n = 4) subjects were stimulated with PFA-fixed *E. coli* for 24 h in the presence of anti-PD-1 ( $\alpha$ PD-1) or anti-LAG-3 ( $\alpha$ LAG-3) blocking antibodies and then assessed for the expression levels of IFN $\gamma$ , GzmB, and CD107a in MAIT cells. (D) PBMCs from VIR subjects (n = 2) were stimulated with an HIV-1 p17 peptide pool or DMSO as a control and then assessed for the production of TNF in CD8<sup>+</sup> T cells.



**Fig. S4. IFN- $\alpha$  does not induce MAIT cell death or apoptosis.** Freshly isolated PBMCs from healthy blood donors ( $n = 6$ ) were left untreated or treated with IFN- $\alpha$  at a concentration of 100 ng/mL for 72 h and assessed for cell death and apoptosis using Zombie Green and Annexin V-APC. Representative FACS plots (**A**) and statistical analysis (**B**) are shown.



**Fig. S5. IFN- $\alpha$ -induced IL-10 inhibits IL-12, thus dampening MAIT cell function.** (A) Levels of IL-12 p70 in the supernatant of PBMCs treated with IFN- $\alpha$  for 24-72 h followed by *E. coli* stimulation. (B) Levels of IL-12 p70 in the supernatant of PBMCs treated with IFN- $\alpha$  for 72 h in the presence of an anti-IL-10R ( $\alpha$ IL-10R, 10  $\mu$ g/mL) or isotype control antibody followed by *E. coli* stimulation. (C-D) Levels of IFN $\gamma$  and GzmB expression in MAIT cells in PBMCs pre-treated with IL-10 (C) or IFN- $\alpha$  (D) at 100 ng/mL for 72 h followed by *E. coli* stimulation in the presence or absence of IL-12 (100 ng/mL).

**Table S1. Clinical characteristics of the enrolled participants.**

Group	HC	VIR	ART	<i>P-value</i>		
				HC vs VIR	HC vs ART	VIR vs ART
n	32	49	35			
Age, yrs	31.59 ± 1.694	34.69 ± 1.652	35.03 ± 1.624	0.2199	0.0787	0.6629
Sex (female/male)	6/21	4/45	4/31	0.182	0.501	0.7137
CD4 counts (cells/ $\mu$ L)	ND	212.2 ± 21.81	512.5 ± 28.45	NA	NA	<0.0001
Viral loads (log copies/mL)	NA	4.702 ± 0.2049	<1.7	NA	NA	<0.0001
Duration of ART (months)	NA	NA	46.54 ± 3.554	NA	NA	NA

ND: not done.

NA: not applicable.



**Table S2. BD Rhapsody Abseq antibody panel.**

Target	Clone	Barcode Sequence
CD3	SK7	AAAGGTAGAGTGTATTGACGTCGGTGTAGGTTGATT
CD4	SK3	TCGGTGTTATGAGTAGGTCGTCGTGCGGTTTGATGT
CD11a	HI111	AAGTAGAGTAGTCGATTGTTTGATGCGCCAGATGTC
CD11b	M1/70	ATCGTTATTCGTTGTAGTTCGCCCCGGTTTGAGTAGT
CD45RA	HI100	AAGCGATTGCGAAGGGTTAGTCAGTACGTTATGTTG
PD1	EH12.1	ATGGTAGTATCACGACGTAGTAGGGTAATTGGCAGT
CD69	FN50	CAATAACGGGTCATAGTAAGTCGCGAGTAAGAGGGC
CD38	HIT2	GTCAACGATGGGTAGCGGTAGAAATAACGGAACTGG
CD62L	DREG-56	ATGGTAAATATGGGCGAATGCGGGTTGTGCTAAAGT
CXCR4	12G5	CAGTGTTTAGAGCGGGTTGCATATGTCGTTTAGAGG
CD127	HIL-7R-M21	AGTTATTAGGCTCGTAGGTATGTTTAGGTTATCGCG
CD134	ACT35	GGTGTGGTAAGACGGACGGAGTAGATATTCGAGGT
CD27	M-T271	TGTCCGGTTTAGCGAATTGGGTTGAGTCACGTAGGT
CD7	M-T701	GTATGTAGGTCTTATGTGTTGGCGTAGTATGCGTTT
CXCR3	1C6/CXCR3	AAAGTGTTGGCGTTATGTGTTTCGTTAGCGGTGTGGG
CD152	BNI3	TAGTATCCGTAGTAGTTATCTGCCCGTTCGTTATGC
CD5	UCHT2	ACGAAGCGAGCGAAGAACCCTATGCGATTGAGTAAGT
CD44	G44-26	CATGATTGTCGGGTGGGTATAGCGTTTCGTGATTTCG
CXCR5	RF8B2	AGGAAGGTCGATTGTATAACGCGGCATTGTAACGGC
ICOS	DX29	ATAGTCCGCCGTAATCGTTGTGTCGCTGAAAGGGTT
CD2	RPA-2.10	AAACGTAGATTAGAGCCGGGTATGTCGCAACTGATT
CD154	TRAP1	TAAGAGGTAAGTGCATTCGGGTATAGGCGTGATTTG
CD137	4B4-1	TGACAAGCAACGAGCGATACGAAAGGCGAAATTAGT
CD335	9E2/NKp46	CAATTTGTTTCGCGTTTAGTAGTCGTCGTCTTATGGG
CD54	HA58	AAGAGAATATATGCGTGCGTTGTTAAGGGAATGCGT
CD9	M-L13	GGGTTGTAAGTCGTCGGAAGTGTGAAGCGTATAGTG
CD94	HP-3D9	GAGGTTAGGATAGGTGTACGGGTCGAGTTGAATTCT
CD126	M5	AATGGTGAATCGCCCTAGCAAGTGGTATCGGAATCG
CD98	UM7F8	GTTGGCGTTAGGTGTTCGATTGTATGGGTTATGCTGC

**Table S3. BD Rhapsody T cell expression panel.**

Gene name	Accession number	Gene name	Accession number
AIM2	NM 004833.1	IL12RB1	NM 005535.2
ANXA5	NM 001154.3	IL12RB2	NM 001559.2
APOBEC3G	NM 021822.3	IL13	NM 002188.2
ARL4C	NM 005737.3	IL15	ENST00000477265.5
AURKB	NM 004217.3	IL15RA	NM 002189.3
B3GAT1	NM 018644.3	IL17A	NM 002190.2
BAX	NM 001291428.1	IL17F	NM 052872.3
BCL11B	NM 022898.2	IL18	NM 001562.3
BCL2	NM 000633.2	IL18R1	NM 003855.3
BCL6	NM 001706.4	IL18RAP	NM 003853.3
BIN2	NM 016293.3	ILIR2	NM 004633.3
BTG1	NM 001731.2	IL2	NM 000586.3
BTLA	NM 181780.3	IL21	NM 021803.3
C10orf54	NM 022153.1	IL22	NM 020525.4
CASP3	NM 004346.3	IL23R	NM 144701.2
CBLB	NM 170662.4	IL25	NM 022789.3
CCL1	NM 002981.2	IL2RA	NM 000417.2
CCL2	NM 002982.3	IL2RB	NM 000878.3
CCL20	NM 004591.2	IL3	NM 000588.3
CCL3	NM 002983.2	IL31	NM 001014336.1
CCL4	NM 002984.3	IL32	NM 004221.4
CCL5	NM 002985.2	IL4	NM 000589.3
CCNB1	NM 031966.3	IL4R	NM 000418.3
CCND2	NM 001759.3	IL5	NM 000879.2
CCR1	NM 001295.2	IL6	NM 000600.4
CCR10	NM 016602.2	IL6R	NM 000565.3
CCR2	NM 001123041.2	IL7R	NM 002185.3
CCR3	NM 001837.3	IL9	NM 000590.1
CCR4	NM 005508.4	IL9R	NM 002186.2
CCR5	NM 000579.3	IRF4	NM 002460.3
CCR6	NM 004367.5	IRF8	NM 002163.2
CCR7	NM 001838.3	ITGA4	NM 000885.5
CCR8	NM 005201.3	ITGAE	NM 002208.4
CCR9	NM 006641.3	ITGAL	NM 002209.2
CD160	NM 007053.3	ITGAM	NM 000632.3
CD2	NM 001767.3	ITGAX	NM 000887.4
CD244	NM 016382.3	ITGB2	NM 000211.4
CD247	NM 000734.3	ITK	NM 005546.3

CD27	NM 001242.4	JUN	NM 002228.3
CD274	NM 014143.3	JUNB	NM 002229.2
CD300A	NM 007261.3	KIT	NM 000222.2
CD3D	NM 000732.4	KLRB1	NM 002258.2
CD3E	NM 000733.3	KLRCI	NM 002259.4
CD3G	NM 000073.2	KLRC3	NM 002261.2
CD4	NM 000616.4	KLRC4	NM 013431.2
CD40LG	NM 000074.2	KLRF1	NM 016523.2
CD44	NM 000610.3	KLRG1	NM 005810.3
CD48	NM 001778.3	KLRKI	NM 007360.3
CD5	NM 014207.3	LAG3	NM 002286.5
CD52	NM 001803.2	LAIR2	NM 002288.5
CD6	NM 006725.4	LAP3	NM 015907.2
CD69	NM 001781.2	LAT	NM 014387.3
CD7	NM 006137.6	LAT2	NM 014146.3
CD70	NM 001252.4	LCK	NM 005356.4
CD8A	NM 001768.6	LEF1	NM 016269.4
CD8B	NM 004931.4	LGALS1	NM 002305.3
CD9	NM 001769.3	LGALS3	NM 002306.3
CHI3L2	NM 004000.2	LIF	NM 002309.4
CLC	NM 001828.5	LILRB4	NM 001278426.3
CLEC2D	NM 013269.5	LRRC32	NM 005512.2
CNOT2	NM 014515.5	LTA	NM 000595.3
CSF2	NM 000758.3	LTB	NM 002341.1
CSF3	NM 000759.3	MKI67	NM 002417.4
CST7	NM 003650.3	MYC	NM 002467.4
CTLA4	NM 005214.4	NAMPT	NM 005746.2
CTSW	NM 001335.3	NCR3	NM 147130.2
CX3CR1	NM 001337.3	NINJ2	NM 016533.5
CXCL10	NM 001565.3	NKG7	NM 005601.3
CXCL13	NM 006419.2	NT5E	NM 002526.3
CXCL8	NM 000584.3	OAS1	NM 016816.3
CXCL9	NM 002416.2	PASK	NM 015148.3
CXCR1	NM 000634.2	PDCDI	NM 005018.2
CXCR3	NM 001504.1	PECAM1	NM 000442.4
CXCR4	NM 003467.2	PIK3PI	NM 052880.4
CXCR5	NM 001716.4	PMCH	NM 002674.3
CXCR6	NM 006564.1	PRDMI	NM 001198.3
DPP4	NM 001935.3	PRF1	NM 005041.4
DUSP1	NM 004417.3	PTGDR2	NM 004778.2
DUSP2	NM 004418.3	PTTG2	NM 006607.2

DUSP4	NM 001394.6	PYCR1	NM 006907.3
EGR1	NM 001964.2	RORA	NM 002943.3
EGR3	NM 004430.2	RORC	NM 005060.3
ENTPD1	NM 001776.5	RUNX3	NM 004350.2
EOMES	NM 005442.3	SIPR1	NM 001400.4
F5	NM 000130.4	SELL	NM 000655.4
FAS	NM 000043.5	SELPLG	NM 003006.4
FASLG	NM 000639.2	SEMA7A	NM 003612.4
FB×022	NM 012170.3	SLAMF1	ENST00000302035.10
FOSB	NM 006732.2	SPOCK2	NM 014767.2
FOSL1	NM 005438.4	SPP1	NM 000582.2
FOXO1	NM 002015.3	STAT1	NM 007315.3
FOXO3	NM 001455.3	STAT3	NM 003150.3
FOXP1	NM 032682.5	STAT4	NM 003151.3
FOXP3	NM 014009.3	STAT5A	NM 003152.3
FYB	NM 001465.4	STAT6	NM 003153.4
FYN	NM 002037.5	TARP_refseq	NM 001003799.1
GAPDH	NM 002046.5	TB×21	NM 013351.1
GATA3	ENST00000346208.3	TCF7	NM 003202.3
GHR	NM 000163.4	TGFB1	NM 000660.5
GIMAP2	NM 015660.2	TGFB3	NM 003239.3
GIMAP5	NM 018384.4	TIAFI	NM 004740.3
GIMAP7	NM 153236.3	TIGIT	ENST00000481065.5
GLG1	NM 012201.5	TK1	NM 003258.4
GNLY	NM 006433.4	TLR2	NM 003264.4
GZMA	NM 006144.3	TLR9	NM 017442.3
GZMB	NM 004131.4	TNF	NM 000594.3
GZMH	NM 033423.4	TNFRSF18	NM 004195.2
GZMK	NM 002104.2	TNFRSF18	NM 001066.2
GZMM	NM 005317.3	TNFRSF25	NM 003790.2
HAVCR2	NM 032782.4	TNFRSF4	NM 003327.3
HLA-A	NM 002116.7	TNFRSF8	NM 001243.4
HLA-C	NM 002117.5	TNFRSF9	ENST00000377507.7
HLA-DMA	NM 006120.3	TNFSF10	NM 003810.3
HLA-DMB	NM 002118.4	TOP2A	NM 001067.3
HLA-DPAI	NM 033554.3	TRAC	ENST00000611116.1
HLA-DPB1	NM 002121.5	TRAT1	NM 016388.3
HLA-DQA1	NM 002122.3	TRBC2	ENST00000466254.1
HLA-DQBI	NM 002123.4	TRDC	ENST00000390477.2
HLA-DRA	NM 019111.4	TRIB2	NM 021643.3
HLA-DRB3	NM 022555.3	TSPAN32	NM 139022.2

HMGB2	NM 002129.3	TXK	NM 003328.2
HMMR	NM 012484.2	TYMS	NM 001071.2
ICAMI	NM 000201.2	UBE2C	NM 007019.3
ICOS	NM 012092.3	VNN2	NM 004665.4
IER3	NM 003897.3	XCL1	NM 002995.2
IER5	NM 016545.4	ZAP70	NM 001079.3
IFNG	NM 000619.2	ZBED2	NM 024508.4
IFNGRI	NM 000416.2	ZBTB16	NM 006006.4
IKZF2	NM 016260.2	ZNF683	NM 173574.3
IL12A	NM 000882.3		

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**Table S4. List of significantly up-regulated or down-regulated genes in MAIT cells from VIR versus HC subjects.**

Gene/protein name	Average log FC	<i>P</i> -value	Adjusted <i>P</i> -value
DUSP1	-1.584601585	8.09E-123	2.33134E-120
CD54-a	-0.913787562	4.18E-90	1.20376E-87
CD7-a	-0.864105545	5.78E-62	1.66503E-59
CD69	-0.844541305	4.29E-60	1.23678E-57
KLRB1	-0.819549243	8.35E-59	2.40494E-56
NKG7	0.737727716	6.78E-49	1.95337E-46
GZMH	1.020772315	1.02E-46	2.95116E-44
CD8B	0.861755354	1.26E-43	3.63106E-41
CD8A	0.835503769	3.25E-43	9.36143E-41
GZMB	1.197902553	1.80E-40	5.19507E-38
CXCR4	-0.64908587	4.23E-37	1.21702E-34
CD38-a	1.558551901	2.29E-36	6.60254E-34
IL32	0.470229004	1.35E-35	3.87822E-33
CX3CR1	0.757646651	1.63E-34	4.70226E-32
CD98-a	-0.467459852	1.05E-33	3.01454E-31
GIMAP5	0.572123556	1.26E-31	3.62880E-29
TRAC	0.602408446	2.35E-31	6.77815E-29
FOSB	-0.58476772	2.82E-31	8.13280E-29
KLRK1	0.584164212	1.40E-29	4.03052E-27
STAT1	0.491179555	2.68E-27	7.72842E-25
CD2	0.529880246	3.56E-26	1.02427E-23
SELPLG	0.497292069	4.22E-26	1.21597E-23
LGALS1	0.851301702	1.60E-22	4.60411E-20
CD300A	0.426056985	2.53E-21	7.28168E-19
CD5	0.42340284	1.16E-19	3.34022E-17
CD94-a	-0.957512967	2.52E-19	7.26533E-17
CD52	0.428199981	4.53E-19	1.30352E-16
TIGIT	0.202308411	5.86E-19	1.68713E-16
CD3E	0.308269713	5.87E-18	1.69131E-15
HLA-DRA	0.561326832	4.49E-17	1.29190E-14
CCR5	0.401857967	4.02E-16	1.15898E-13
GAPDH	0.349232094	7.28E-16	2.09647E-13
CXCL8	0.139620067	7.71E-16	2.22177E-13
HLA-A	0.273311378	1.87E-15	5.39728E-13
TCF7	0.294796703	5.29E-15	1.52228E-12
CD184-a	0.338988499	6.73E-15	1.93800E-12
CD183-a	0.556605511	1.52E-14	4.37494E-12

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CD127-a	-0.444429067	1.54E-14	4.44568E-12
CXCR3	0.212493718	2.65E-14	7.61839E-12
FYB	0.338286776	3.27E-14	9.41287E-12
SELL	0.527898052	1.35E-13	3.89518E-11
NCR3	-0.365416292	1.15E-12	3.30223E-10
CD3D	0.290555439	1.58E-12	4.55273E-10
CD6	0.351351894	2.56E-12	7.38693E-10
CCL5	0.387573042	3.19E-12	9.18402E-10
CTSW	0.32535836	3.67E-12	1.05839E-09
CD11a-a	-0.189026704	4.01E-12	1.15414E-09
CST7	0.317542304	6.09E-12	1.75374E-09
TBX21	0.351706593	6.35E-12	1.82742E-09
GZMK	-0.158421803	7.62E-12	2.19562E-09
LAG3	0.319581747	9.66E-12	2.78115E-09
LCK	0.277566437	1.37E-11	3.95036E-09
CD134-a	0.300989008	2.20E-11	6.34252E-09
ITGB2	0.238001496	4.11E-11	1.18395E-08
BTG1	-0.280904068	4.44E-11	1.27805E-08
CBLB	0.28144266	8.67E-11	2.49809E-08
CHI3L2	0.153630112	2.52E-10	7.25192E-08
GNLY	0.726929694	2.99E-10	8.61008E-08
RUNX3	0.257065603	4.34E-10	1.24904E-07
JUN	-0.272077795	1.34E-09	3.85876E-07
ITGA4	0.299016902	3.37E-09	9.69848E-07
CD27-a	-0.165722802	3.60E-09	1.03685E-06
CD45RA-a	0.754380792	4.32E-09	1.24412E-06
APOBEC3G	0.255887388	1.19E-08	3.42963E-06
CD137-a	-0.110638457	1.87E-08	5.37369E-06
LEF1	0.201835906	4.30E-08	1.23772E-05
CD44-a	0.229608809	4.47E-08	1.28784E-05
FASLG	0.107516061	5.50E-08	1.58432E-05
LTB	0.13603898	1.11E-07	3.19674E-05
NAMPT	-0.080105344	1.32E-07	3.78917E-05
IL7R	0.257292313	2.21E-07	6.36182E-05
EOMES	0.140109742	5.43E-07	0.000156423
ZNF683	0.219688418	7.94E-07	0.000228684
EGR1	-0.227342295	8.76E-07	0.000252394
GZMA	0.269230904	9.33E-07	0.000268565
IL12RB1	0.151541363	1.07E-06	0.000308628
VNN2	0.089362831	1.23E-06	0.000355201
CD44	0.20460463	1.32E-06	0.000381537

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CTLA4	0.105072616	2.15E-06	0.000619001
ITGAX	0.113744118	2.28E-06	0.000657235
B3GAT1	0.094959502	2.41E-06	0.00069388
TRBC2	0.309496005	2.78E-06	0.000801794
IL18R1	0.149758586	3.06E-06	0.000881555
CCL3	0.220579086	6.69E-06	0.001925826
CXCR6	0.26399881	8.47E-06	0.002437977
FAS	0.074408582	9.68E-06	0.002786463
ANXA5	0.17167738	1.12E-05	0.00322552
ITGAM	0.247194143	1.47E-05	0.004227565
KLRC4	0.098583499	1.58E-05	0.004557289
TIAF1	0.130571351	1.85E-05	0.005340459
FOXO1	0.077128225	2.43E-05	0.007012294
CD4-a	-0.401719497	2.88E-05	0.008296524

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