Supplementary Material

Case reports:

Patient EXID#1

49 years-old African woman (Zambia) originally diagnosed with HIV/AIDS after an infectious work-up for prolonged history of headache, sore throat and cervical lymphadenopathy. On presentation, HIV-1 plasma viral load was >100,000 copies/mL, CD4 count was 102 cells/μL (7%), and she had infection with a circulating recombinant form (CRF) BC. She had no AIDS defining illnesses nor clinical or laboratory evidence of malignancy, lymphoproliferative or hematologic disorders but reported a past medical history of malaria and herpes zoster, 1 year prior the HIV/AIDS diagnosis. ARV treatment with TFV/FTC/EFV was started and resulted in consistent suppression of viral load below the limit of detection but CD4 remained as low as 36 cells/μL, 5%, after 192 weeks of ART**.**

Patient EXID#2

18 years-old African man (Congo) originally diagnosed with HIV-1 infection during medical screening performed for immigration purposes. On presentation, HIV-1 plasma viral load was 82,000 copies/mL and CD4 count was 211 cells/μL, 12%, had HIV-1 subtype C infection. He did not have any opportunistic infection nor clinical, radiological or laboratory evidence of malignancy, lymphoproliferative or hematologic disorders but developed a diffuse monomorphic papular eruption consistent with lichen spinolosus which required topical steroid treatment. ARV treatment with AZT/3TC/NLF was started with CD4 count of 315 cells/μL, 15%, at time of ART initiation. ART resulted in prompt and consistent suppression of viral load below the limit of detection but CD4 progressively declined with a nadir of 22 cells/μL, 5%, after 339 weeks of ART with 4 different regimens and remained 52 cells/μL, 7%, after 357 weeks of ART.

Patient EXID#3

13 years-old African male (Burundi) with history of perinatal infection and originally diagnosed with HIV-1 infection during medical screening performed for immigration purposes. On presentation, HIV-1 plasma viral load was 13,163 copies/mL, CD4 count was 179 cells/μL, 8%, and had HIV-1 subtype C infection. He did not have any opportunistic infection, clinical,

radiological or laboratory evidence of malignancy nor lymphoproliferative or hematologic disorders but developed recurrent tinea capitis, molluscum contagiosum and skin warts. ARV treatment with AZT/3TC/rLPV was started with CD4 count of 179 cells/μL, 8%, at time of ART initiation. ART resulted in prompt and consistent suppression of viral load below the limit of detection but CD4 progressively declined with a nadir of 9 cells/μL, 2%, after 112 weeks of ART with 4 different regimens and remained 26 cells/μL, 6%, after 357 weeks of ART.

Patient EXID#4

25 years-old male (Israel) originally diagnosed with HIV-1 infection during routine screening test prompted by high risk male-who-have-sex-with-male (MSM) activity. Previous negative HIV test was obtained six months prior. At time of HIV-1 infection diagnosis, HIV-1 plasma viral load was 110 copies/mL, CD4 count 764 cells/μL, 39%, HIV subtype was a CRF AG. He did not have any opportunistic infection or any significant medical history. ARV treatment with TFV/FTC/Rilpivirine was started with CD4 count of 561 cells/μL, 32%, and HIV-1 plasma viral load of 660 copies/mL. ART resulted in prompt and consistent suppression of viral load below the limit of detection but CD4 progressively declined and remained as low as 68 cells/μL, 11%, after 144 weeks of ART with 3 different regimens. During his clinical course he developed recurrent episodes of lichenoid dermatitis requiring intermittent courses of topical steroids, but did not develop any opportunistic infection.

Patient EXID#5

40 years-old African female (Zambia), diagnosed with HIV/AIDS after an infectious work-up for oral and esophageal candidiasis. At time of HIV/AIDS diagnosis, HIV-1 plasma viral load was >100,000 copies/mL, CD4 count was 33 cells/μL, 4%, and she had HIV-1 subtype D infection. ARV treatment was started and resulted in prompt and consistent suppression of viral load below the limit of detection but CD4 progressively declined and remained as low as 9 cells/μL, 1%, after 192 weeks of ART with 3 different regimens. After approximately 226 weeks of ART, CD4-T-cell count started to progressively increase (highest on week 249 post-ART: 119 cell/μL, 12%). Alongside such CD4-T-cell reconstitution, EXID5 developed mental status decline with radiological evidence of basilar arteritis and cerebral ischemic lesions. A clinical diagnosis of CNS tuberculosis was formulated but did not result in significant clinical improvement in her mental

status. The patient was lost to follow-up after family requested her discharge from an skilled nursing facility.

Supplementary Figures Legend

Figure 1. Proportion of CD4 and CD8-T cells in HC, INR, IR and EXID. The median (red bar), interquartile range (error bar) and each subject (symbols) is presented for HC (n=13), IR (n=8), INR (n=15), EXID (n=5). Each EXID subject is identified by a different grey filled shape.

Figure 2. Distribution of CD4-T-cell maturation subsets in HC, INR, IR and EXID based on expression of CD27 and CD45RO. The median (red bar), interquartile range (error bar) and each subject (symbols) is presented for HC (n=5), IR (n=8), INR (n=15), EXID (n=5). Each EXID subject is identified by a different grey filled shape. *$p \leq 0.05$ in the comparison indicated by the black horizonal line as determined by Kruskal-Wallis test followed by Dunn`s post-hoc test.

Figure 3. Distribution of CD8-T-cell maturation subsets in HC, INR, IR and EXID based on expression of CD27 and CD45RO. The median (red bar), interquartile range (error bar) and each subject (symbols) is presented for HC (n=5), IR (n=8), INR (n=15), EXID (n=5). Each EXID subject is identified by a different grey filled shape. *$p \leq 0.05$ in the comparison indicated by the black horizonal line as determined by by Kruskal-Wallis test followed by Dunn`s post-hoc test.

Figure 4. Correlation between the serum levels of IL-7 and CD4-T-cell counts in IR (n=8), INR (n=13), EXID (n=5). Each EXID subject is identified by a different grey filled shape. Spearman **ρ**: -0.53 (CI 95%: -0.76, -0.17), $p<0.004$.

Figure 5. Ingenuity pathway analysis (IPA) of genes differentially expressed in PBMC from patients with EXID compared to PBMC of ARV-treated immunological responders. Canonical pathways significantly differentially regulated are listed according to their p value (left Y axis, -Log, height of bars), the regulation z-score algorithm to identify pathways that are upregulated (positive z-score, orange) or downregulated (negative z-score, blue) and the ratio of list genes found to be differentially regulated in each pathway compared to the total number of genes in that pathway (right Y axis, orange squares).

Figure 6. Telomeres length in different peripheral blood mononuclear cell types in EXID2.

Figure 7. CD4-T-cell trends in EXID1 (**A**) and EXID5 (**B**). The dotted line indicates the pre-ART CD4-T-cell count level.

Figure 8. Clinical, radiological and histopathological findings in EXID4. **A.** Right palpebral edema, ptosis and hypotropia, subsequently resolved (**B**) after initiation of infliximab. **C.** Hypermetabolic right orbital mass infiltrating the right superior extraocular muscle and modeling of the superior right orbital bone as seen on PET-CT. **D.** Right oribital mass biopsy with granulomatous inflammation and dense fibrosis. The asterisk denotes the area showed at higher magnification in the left panel. **E.** Immunostaining for Myxovirus resistance gene A (MxA), an interferon alpha induced gene, Collagen-1 (**F**), CD68/CD163 and CD4 in the germinal center (GC) and T-cell zone (TCZ) (**G**) in lymph nodes of ICL, HIV-1 infected individuals (pre-ART and 24 weeks post-ART) and EXID4 (52 weeks post-ART).

Figure 9. CD4-T-cell trends and quantitative Imaging in lymph node biopsy for EXID4**. A**. CD4-T-cells and HIV-1 viral load trends in relation to different ART treatment and infliximab. **B**. The percentage area positive for MxA in lymph node biopsies from a subject with ICL, HIV/AIDS immunological responder (IR) pre- and post-ART and in EXID4 as was quantified using Cell-profiler-version 3.1.5 and compared to health subjects (red dotted line). **C**. The percentage area positive for Collagen-1 in lymph node biopsies from a subject with ICL, HIV/AIDS immunological responder (IR) pre- and post-ART and in EXID4 as was quantified using Cell-profiler-version 3.1.5 and compared to health subjects (red dotted line). **D** The percentage area positive for CD4 in lymph node biopsies from a subject with ICL (yellow), HIV/AIDS immunological responder (IR) pre- and post-ART (green) and in EXID4 (grey hatched) as was quantified using Cell-profiler-version 3.1.5 and compared to healthy subjects (red dotted line) in germinal center (GC) and paracortical T-cell zone (TCZ).

Figure 10. Quantification of active caspase-1 in T lymphocytes**.** PBMCs from patients and a healthy donor were incubated with the fluorochrome inhibitor of caspase-1 (FAM-FLICA), stained for lymphocyte identification and acquired by Imaging flow cytometry. The number of CD4-T cells **(A)** or CD8-T cells **(B)** showing spontaneous caspase-1 activation was assessed as the percentage of FLICA positive cells, by using IDEAS software.

Supplementary Tables

Supplementary Table 1. T cell subpopulations in healthy subjects (HC, n=13), immunological-responders (IR, n=8), immunological non-responders (INR, n=15) and extreme immune-decline (EXID, n=5). Data expressed as median percentage and interquartile range (25^{th}-75^{th} percentile).

T cell subpopulations	HC	IR	INR	EXID
CD4*	68 (62-73)	31 (27-39)	19 (14-25)	9 (4-12)
CD8	24 (19-29)	58 (53-63)	66 (59-75)	63 (48-83)
^CD4 Naïve (CD27+CD45RO-)	43 (25-51)	43 (30-62)	32 (17-47)	4 (1-26)
CD4 Central Memory (CD27+CD45RO+)	50 (43-59)	46 (33-60)	45 (40-60)	15 (12-37)
CD4 Effector Memory (CD27-CD45RO+)	5 (3-10)	8 (5-12)	14 (9-26)	66 (9-16)
CD4 Effector (CD27-CD45RO-)	0 (0-3)	1 (0-1)	0 (0-1)	2 (0-14)
°CD8 Naïve (CD27+CD45RO-)	55 (47-62)	30 (13-65)	21 (12-35)	8 (4-21)
CD8 Central Memory (CD27+CD45RO+)	33 (20-38)	15 (13-18)	19 (16-27)	11 (9-19)
CD8 Effector Memory (CD27-CD45RO+)	6 (4-10)	19 (8-43)	30 (16-41)	32 (15-60)
CD8 Effector (CD27-CD45RO-)	6 (3-11)	20 (14-29)	20 (16-25)	36 (24-54)
^CD4 CD45RO+Ki67+	2 (2-8)	6 (4-18)	21 (9-28)	9 (6-16)
°CD8 CD45RO+Ki67+	1 (0.7-1.3)	2 (2-3)	3 (2-18)	4 (1-13)
^CD4 HLA+CD38+	0.6 (0.4-1.2)	2 (1-2)	3 (2-3)	7 (1-9)
°CD8 HLA+CD38+	1 (0.7-3.3)	3 (2-5)	6 (4-10)	3 (2-18)
^CD4 CD45RO+PD1+	8 (3-12)	13 (3-18)	10 (6-30)	59 (34-90)
°CD8 CD45RO+PD1+	14 (9-23)	8 (5-16)	9 (5-20)	21 (9-25)
^Regulatory T cells (CD4+CD25+FoxP3+)	2 (1-3.7)	3 (3-6)	6 (4-8)	1 (0.8-3)
^CD4 CD27+CD45RO- CD127+	89 (83-95)	79 (69-84)	76 (72-80)	79 (62-87)†
CD4 CD27+CD45RO+ CD127+	84 (79-87)	72 (69-81)	67 (60-70)	46 (38-70)†
CD4 CD27-CD45RO+ CD127+	94 (83-96)	82 (66-90)	71 (60-84)	55 (27-65)†
°CD8 CD27+CD45RO- CD127+	89 (86-92)	93 (79-95)	87 (71-91)	32 (32-55)†
CD8 CD27+CD45RO+ CD127+	83 (76-88)	72 (67-77)	65 (57-74)	39 (17-39)†
CD8 CD27-CD45RO+ CD127+	46 (36-83)	31 (21-57)	28 (23-38)	20 (5-32)†
CD8 CD27-CD45RO- CD127+	28 (15-40)	21 (11-39)	14 (13-18)	11 (4-12)†

*% of total CD3+ lymphocytes; ^ % of total CD3+CD4+; ° % of total CD3+CD8+.

†Sufficient material for the evaluation of the expression of CD127 was available from 3 patients with EXID.

Supplemetary Table 2. Plasma concentration of cytokines/chemokines in healthy subjects (HC, n=10), idiopathic CD4-lymphopenia (ICL, n=11) immunological-responders (IR, n=8), immunological non-responders (INR, n=15) and extreme immune-decline (EXID, n=5). Data expressed as median picogram per milliliter (pg/mL) and interquartile range (25^{th}-75^{th} percentile).

Cytokine/Chemokine	HC	ICL	IR	INR	EXID
IL-1β	ND	ND	ND	ND	ND
IL-2	0.2 (0.07-0.25)	0.3 (0.2-0.35)	0.17 (0.09-0.22)	0.19 (0.14-0.3)	0.34 (0.18-0.4)
IL-4	ND	ND	ND	ND	ND
IL-6	0.4 (0.3-1.17)	0.5 (0.35-1.32)	1.22 (0.27-2.5)	0.5 (0.3-0.78)	0.16 (0.13-0.48)
IL-7*	20.2 (13.5-25.7)	30 (21-41)	21 (18-22)	23.6 (18-32)	44.8 (38-58)
IL-8	1.6 (0.76-3.3)	1.8 (1.6-3.7)	1.3 (0.9-1.8)	3.4 (2.2-4)	1.6 (1.2-4.3)
IL-10	0.3 (0.26-0.49)	0.3 (0.36-0.45)	0.3 (0.2-0.4)	0.24 (0.2-0.4)	0.07 (0.04-1.5)
IL-12p70	0.33 (0.2-0.4)	0.1 (0.1-0.38)	0.2 (0.1-0.4)	0.1 (0.1-0.3)	0.1 (0.06-0.1)
IL-13	0.67 (0.1-1.3)	0.15 (0.1-0.15)	0.15 (0.1-1)	0.15 (0.1-0.5)	0.1 (0.1-10.15)
IL-18	1357 (1007-1586)	2150 (1760-2707)	1273 (790-1373)	1490 (1159-1821)	1611 (882-2554)
TNFα	2.5 (2.2-2.8)	3.3 (2.2-4)	3 (2.6-4.6)	3.3 (2.9-4.6)	2 (1.7-2.25)
Eotaxin	95 (83-177)	94 (71-114)	108 (84-137)	139 (86-182)	76 (47-105)
Eotaxin-3	33 (22-38)	24 (19-68)	17.7 (15-86)	26 (20-31)	16 (11-92)
IFNγ	2.5 (1.8-3.1)	2.8 (2.3-4.5)	2.7 (1.7-5.9)	3.9 (2.9-7.5)	1.6 (0.8-10.5)
IP-10	207 (164-264)	371 (248-626)	361 (260-576)	509 (400-687)	267 (223-490)
MCP-1	54 (45-69)	55 (41-71)	46 (41-64)	61 (49-80)	38 (25-88)
MCP-4	70 (36-83)	47 (39-85)	58 (40-99)	64 (37-81)	58 (50-113)
MDC	1170 (838-1488)	1004 (746-1223)	841 (674-1056)	868 (710-969)	1065 (668-1511)
MIP-1α	13.6 (9.6-16)	15.4 (14-27.4)	14 (10.4-16.2)	15.3 (14-27)	9.1 (6.7-15.9)
MIP-1β	46.5 (35-89)	50 (42-117)	50 (36-60)	59 (39-93.7)	65.7 (45-96)
TARC	38 (21-95.7)	59 (23-213)	43 (28-79.7)	41 (30-52)	67 (48-264)

ND: not detected; Interleukin- (IL-); Tumor necrosis factorα (TNFα); Interferonγ induced-protein-10 (IP-10; Monocyte chemoattractant protein- (MCP-); Macrophage-derived chemokine (MDC); Monocyte inflammatory protein- (MIP-); Thymus and activation regulated chemokine (TARC).
*IL-7 concentrations were measured in serum and in a different number of HC, ICL, IR and INR subjects compared to other cytokines/chemokines (HC, n=39; ICL: n=60; INR: n=13; IR: n=8).

Supplementary Table 3. Number of differentially expressed genes (DEGs) in transcriptome analysis of PBMC from EXID, ICL, HC, HIV/AIDS subjects before and after 96 weeks of ART.

Comparison	DEGs
EXID vs untreated HIV/AIDS	269
EXID vs ART-treated HIV/AIDS IR	313
EXID vs HC	138
EXID vs ICL	24
Untreated HIV/AIDS vs HC	426
ART-treated HIV/AIDS vs untreated HIV/AIDS	857
ART-treated HIV/AIDS vs HC	0

Supplementary Table 4. Legend of the differentially regulated genes in PBMC of EXID, HC, and paired HIV/AIDS-untreated subjects pre-ART and after 96 weeks of ART as shown in the dendrogram in **Figure 5A**. Red and Green and Blue genes clusters distinguish EXID from HC, HIV/AIDS-untreated subjects pre-ART and HIV/AIDS-untreated subjects post-ART.

LEPROT	ISCA1	PAIP2B
LGALSL	PLVAP	AKT3
TAGLN2	TSPAN5	CDK20
PTGS1	STAU1	ZC4H2
CALM3	EIF2AK1	PACS2
PRKAR2B	RIOK3	TIAM1
PPBP	OAZ2	ULK2
IFNL1	BCL2L13	ZFYVE9
XCL1	XK	PWAR5
HES1	MPP1	FBLN7
IFRD2	MXI1	ACVR1C
ULK4	MKRN9P	PARK2
PGLS	IFI27	PFN2
TNF	TOP2A	PIK3R1
IL1B	GBP1P1	MORC4
NR4A1	TLN2	IFNG
SPATA6	UBALD1	CD69
NR4A2	MAP2K3	ICOSLG
TSPAN4	OAZ1	PASK
DRAM2	NPRL3	IL11RA
IFI27L2	WBP2	AXIN2
IFI30	FOXO3	PEX7
CD68	WNK1	FBLN5
TYROBP	TAL1	TNFRSF25
IFITM10	TNIP1	TCF7
OASL	UBALD2	OXNAD1
CD63	MXD4	DOCK9
RETN	FOXO4	TRABD2A
THEMIS2	TCF3	NR3C2
RASGRP4	NPLOC4	TCEA3
DOK3	MAP2K7	NOG
MKNK1	CCR5	PEX3
P2RX1	AXIN1	TESPA1
TNFAIP2	ESCO2	LEPROTL1
MAP3K11	NEDD4L	CAMK4
PPCDC	OLFM4	CD28
MBD6	BPI	CD40LG
NOTCH1	LCN2	IL7R
MBOAT7	CD24	ITGA6

DOCK5	DEFA4	SERINC5
SCARF1	CEACAM6	TRAT1
LPPR2	MPO	CCR7
RARA	DEFA3	PRKCQ-AS1
ATG16L2	ELANE	WNT7A
TREM1	TYMS	EPHX2
SBNO2	KIFC1	N6AMT2
ITGAX	MKI67	IL7
TYK2	NCAPG	TNFRSF17
REC8	KIF2C	IFNLR1
ULK1	CDK1	IFI27L1
NOD2	MXD1	IFITM4P
RELT	IFRD1	THEMIS
ATG2A	IFITM2	CD3G
NCF1B	IL1R1	CD3D
HLX	LILRA6	CD8A
TYMP	IFNAR2	CD8B
IRF9	IFNAR1	ITK
HSPA6	ATG3	CTLA4
HELZ2	DDX60L	CD2
IL17RA	IFIT2	MICA
MXD3	MX2	DPP4
MRVI1	IL18RAP	CD3E
LILRB2	ITGAM	S1PR1
IGF2R	DYSF	INPP4B
P2RY14	FCGR1B	NOSIP
WARS	IFNGR2	LCK
P2RY13	IFNGR1	NPM1
TLN1	DRAM1	CD27
SYK	SERPINB1	GCNT4
CD4	FCGR1A	PWARSN
MSR1	TNFAIP6	LDHB
CD300E	S100A9	PIK3IP1
	S100A8	LEF1
	BST1	CD5
	MAPK14	FLT3LG
	NCOA7	ICOS
	DDX58	IL32
	IFI16	SNORD116-1
	IFIT3	SNORD116-2
	IFIT1	SNORD116-24
	MX1	SNORD116-8
	IFIT5	SNORD116-16

	XAF1	SNORD116-23
	PLSCR1	RTKN2
	F5	IFNG-AS1
	IFIH1	PAIP2B
	NCAPH	AKT3
	IRF7	CDK20
	OTOF	ZC4H2
	PARP11	PACS2
	AIM2	TIAM1
	C3AR1	ULK2
	CD177	ZFYVE9
	IL18R1	PWAR5
	TNFSF13B	FBLN7
	PADI2	ACVR1C
	IFITM3	PARK2
	IFITM1	PFN2
	PARP12	PIK3R1
	IFI35	MORC4
	OAS3	IFNG
	USP18	CD69
	ISG15	ICOSLG
	IFI6	PASK
	OAS1	IL11RA
	OAS2	AXIN2
	IFI44	PEX7
	IFI44L	FBLN5
	SPATS2L	TNFRSF25
	SCARB2	TCF7
	NEDD4	OXNAD1
	S100P	DOCK9
	ARG1	TRABD2A
	CEACAM1	NR3C2
	PTX3	TCEA3
	SERPINB10	NOG
	MMP8	PEX3
	KIF4A	TESPA1
	PDCD1LG2	LEPROTL1
	UBAP1	CAMK4
	JAK2	CD28
	NLRC4	CD40LG
	VNN1	IL7R
	SAMD9L	ITGA6
	SAMD9	SERINC5
	EIF2AK2	TRAT1

	DDX60	CCR7
	S100A12	PRKCQ-AS1
	MMP9	WNT7A
	GBP3	EPHX2
	PIK3CB	N6AMT2
	ITSN1	IL7
	NPL	TNFRSF17
	BRCA1	IFNLR1
	MBOAT2	IFI27L1
	STAT2	IFITM4P
	PARP14	THEMIS
	TNFSF10	CD3G
	GBP1	CD3D
	STAT1	CD8A
	PARP9	CD8B
	OLR1	ITK
	NCAPG2	CTLA4
	BCL2L15	CD2
	CAMP	MICA
	TCN1	DPP4
	CRISP3	CD3E
	RMI1	S1PR1
	CASP3	INPP4B
	CASP10	NOSIP
	KMO	LCK
	INPP1	NPM1
	CXCL10	CD27
	PDCD10	GCNT4
	ATG2B	PWARSN
	C3	LDHB
	GBP4	PIK3IP1
	PLD1	LEF1
	HP	CD5
	PGLYRP1	FLT3LG
	ORM1	ICOS
	PADI4	IL32
	IFIT1B	SNORD116-1

Supplementary Table 5. Top differentially regulated genes in EXID compared to ART-treated HIV/AIDS subjects (in blu top downregulated genes: log_2 fold change ≤ 1.5, 20 genes; in red upregulated genes: log_2 fold change ≥ 1.5, 18 genes), as shown in Figure 5C.

Gene ID (name)	Pathway / Function	Log_2 FC	Adjusted p Value
FBLN5 (Fibulin 5)	Integrin-binding extracellular matrix protein	-2.69	0.00075
GCNT4 (Glucosaminyl transferase 4)	Glycans synthesis: high thymic expression, role in lymphocytes trafficking	-2.61	0.00075
TCEA3 (transcription elongation factor 3)	Regulator of efficiency of transcription	-2.61	0.00061
LEF1 (lymphoid enhancer binding factor 1)	T and B cell specific transcriptional factor	-2.50	0.00061
CAMK4 (calcium/calmodulin protein kinase 4)	Cellular kinase implicated in transcriptional regulation in lymphocytes	-2.48	0.00087
CD5	Regulator of TCR mediated signal transduction.	-2.17	0.00075
SNORD116-1 (small nucleolar RNA116)	Transcription regulator	-2.13	0.00733
TRAT1 (TCR transmembrane adaptor1)	Associated with TCR, stabilized and regulate its signaling	-2.11	0.00075
CD28	Costimulation of TCR for T cells activation, survival and proliferation	-2.02	0.00485
DOCK9 (Dedicator of cytokinesis 9)	Guanine nucleotide exchange factor: cell cycle regulation/actin remodeling	-1.98	0.00087
IL7R (IL-7 receptor, CD127)	T cell development and survival	-1.87	0.00218
FLT3LG (Fms-related tyrosine kinase 3 ligand)	Growth and differentiation factor for several hematopoietic lineages	-1.83	0.00545
CD3D (CD3 delta chain)	Component of TCR-CD3 complex	-1.76	0.00640
THEMIS (Thymocyte Selection Associated)	Regulator of late thymocyte development	-1.76	0.00640
CD27	Costimulatory molecule for B and T cells activation	-1.74	0.00927
TESPA1 (Thymocyte Expressed Positive Selection Associated 1)	Regulator TCR signaling in T cell development and activation	-1.58	0.00522
NOSIP (Nitric Oxide Synthase Interacting Protein)	Modulate activity of nitric oxide (NO) synthase and thus NO production	-1.58	0.00061
ITK (IL2 Inducible T Cell Kinase)	Regulator T cell activation and differentiation	-1.54	0.00218
RUVBL1 (RuvB Like AAA ATPase 1)	ATP-dependent chromatin remodeling and histone modification	-1.50	0.00203
CD3G (CD3 gamma chain)	Component of TCR-CD3 complex	-1.50	0.00548
LILRA6 (Leukocyte Immunoglobulin Like Receptor A6)	Orphan surface receptor with presume immunomodulant function	2.76	0.00075
ATG16L2 (Autophagy Related 16 Like 2)	Role in autophagy	2.50	0.00075
DYSF (Dysferlin)	Adhesion of monocytes and expression in muscle skeletal cells	2.32	0.00073
TNFAIP2 (TNF Alpha Induced Protein 2)	Mediator of Inflammation and angiogenesis	2.21	0.00069
CACNA1D (Calcium Voltage- Gated Channel Subunit Alpha1 D)	Calcium signaling in lymphocytes and multiple other cell types	2.18	0.00069
LOC146880 (Pseudogene)	Pseudogene of Rho GTPase Activating Protein 27	2.17	0.00075
ULK1 (Unc-51 Like Autophagy Activating Kinase 1)	Role in autophagy	2.07	0.00044
NCF1B (Pseudogene)	Pseudogene of neutrophil cytosolic factor 1B	2.03	0.00073
ST3GAL6 (Sialyltransferase 6)	Production of trisaccharide (CD22 ligand): lymphocytes adhesion/activation	1.98	0.00043
NBEAL2 (Neurobeachin Like 2)	Platelets development and function	1.96	0.00500
TCIRG1 (T Cell Immune Regulator 1)	T cell activation, regulation of intracellular pH	1.94	0.0070
P2RX1 (Purinergic Receptor P2X 1)	Intracellular signaling in response to extracellular ATP	1.94	0.0052
PLEKHO2 (Pleckstrin Homology Domain Containing O2)	M-CSF-mediated macrophages survival	1.93	0.0052
NOD2 (Nucleotide-binding oligomerization domain- protein 2)	Pattern recognition receptor involved in autophagy and innate responses	1.86	0.0037
FAM63A (Family with sequence similarity 63)	Ubiquitous expression, unknown function	1.71	0.0019
MAML3 (Mastermind Like Transcriptional Coactivator 3)	Transcriptional coactivator NOTCH: cell development and T cell function	1.70	0.0055
CMIP (C-Maf Inducing Protein)	T cell function and cytoskeletal organization	1.57	0.0021
NACC2 (NACC Family Member 2)	Transcriptional repressor and regulator p53 pathway	1.52	0.0073

Supplementary Methods

Transcriptome bioinformatic analysis

25 libraries were run as 2 x 100 bp paired end reads on 4 lanes of an Illumina Hiseq 2500 sequencer, which produced ~19.7 million reads per sample. Reads were trimmed for the Clontech Template Switching Oligo, adapter sequence and trimmed and filtered for low quality sequence using the FASTX-Toolkit. Remaining reads were mapped to the human genome assembly NCBI hg38 using Hisat2. Reads mapping to genes were counted using htseq-count. Differential expression analysis was performed using the Bioconductor package DESeq2. Further analysis was performed using Partek Genomic Suite.

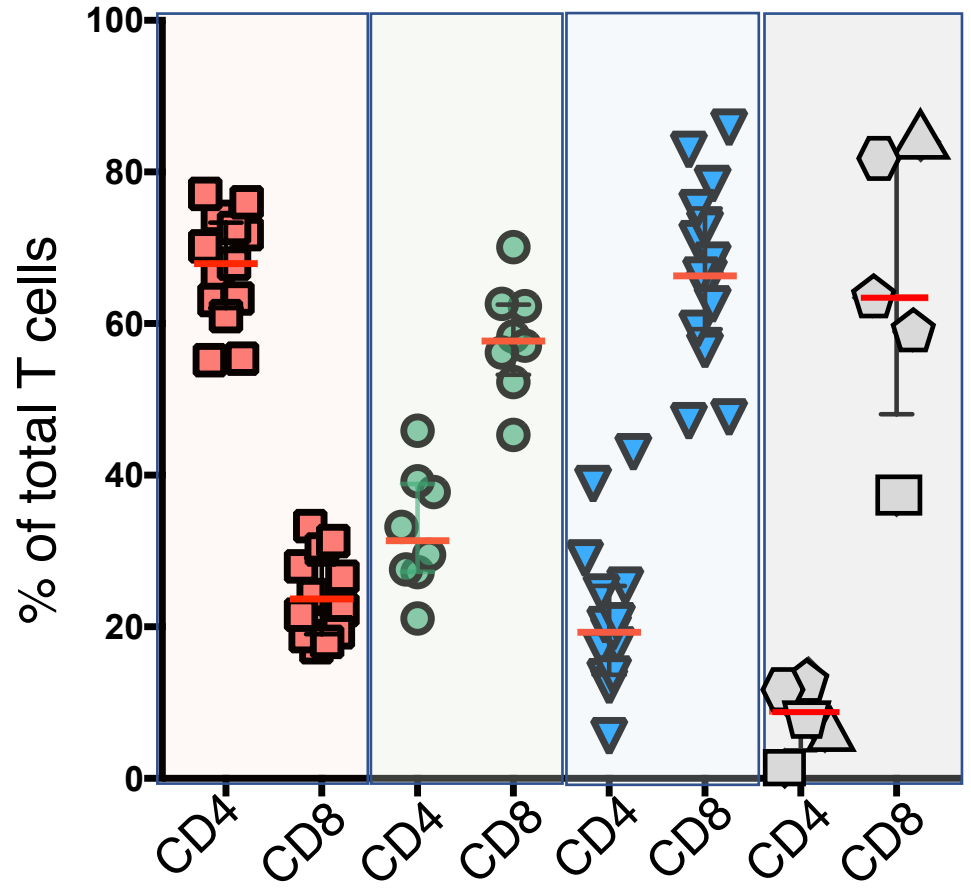
Immunohistochemistry

Immunohistochemical staining of lymph node and orbital mass biopsies was performed on 5-µm tissue sections. Heat-induced epitope retrieval was performed by heating sections in 0.01% citraconic anhydride containing 0.05% Tween-20 then incubated with antibody to MxA (M143, 1:2000) or Collagen 1 (C2456, Sigma, 1:800) or CD4 (ab133616, Abcam, 1:100) in combo with CD163 (NCL-CD163, Novo Castra, 1:500) and CD68 (CM033C, Biocare, 1:500) diluted in blocking buffer overnight at 4°C. Slides were washed in 1× TBS with 0.05% Tween-20, endogenous peroxidases blocked using 1.5% (v/v) H_2O_2 in TBS, pH 7.4, for 5 min, incubated with rabbit or mouse Polink- 1 or 2 horseradish peroxidase (HRP) and developed with Immpact^{TM} DAB (3,3′-diaminobenzidine; Vector Laboratories) and wrap red according to manufacturer's recommendations. All slides were washed in H_2O, counterstained with haematoxylin, mounted in Permount (Fisher Scientific), and scanned at high magnification (x200) using the ScanScope CS System (Aperio Technologies), yielding high-resolution data from the entire tissue section. Representative regions of interest (0.4mm^2) were identified and high-resolution images extracted from these whole-tissue scans. The percentage area positive for MxA, Collagen1 and CD4+T cells was quantified using Cell profiler version 3.1.5.

Inflammasome and caspase-1 analysis

Cells were acquired using an Amnis ImageStreamX Mark II (Millipore Sigma) imaging flow cytometer and the integrated software INSPIRE (Millipore Sigma) was used for data collection.

Images were analyzed by using image-based algorithms in the ImageStream Data Exploration and Analysis Software (IDEAS 6.2.64.0, Millipore Sigma).

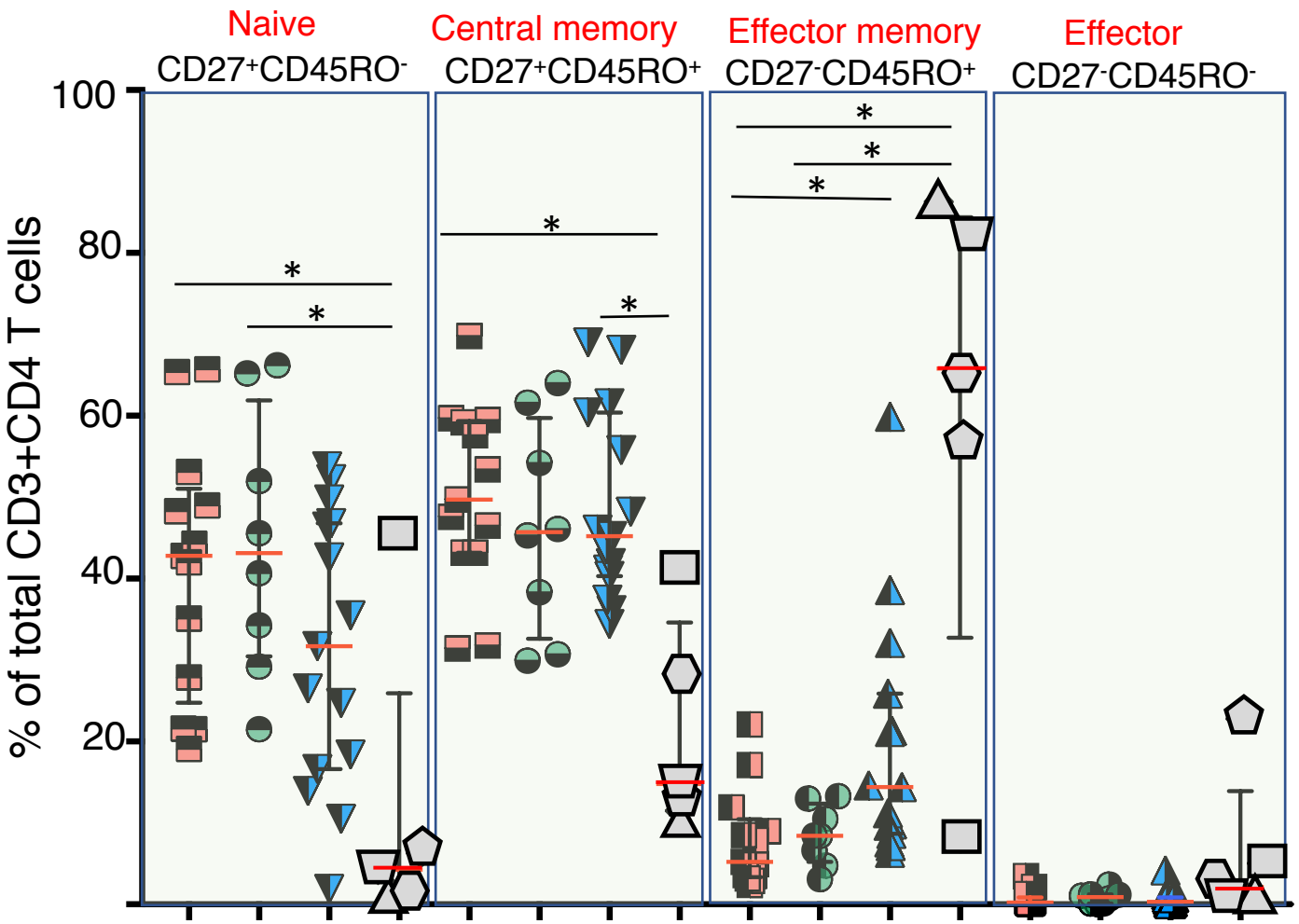


Healthy subjects (HC)
Immunological Responders (IR)
Immunological non-responders (INR)

EXID1
EXID2
EXID3
EXID4
EXID5

Extreme immunological decline (EXID)

Supplementary Figure 1



Healthy subjects (HC)

Immunological Responders (IR)

Immunological non-responders (INR)

EXID1

EXID2

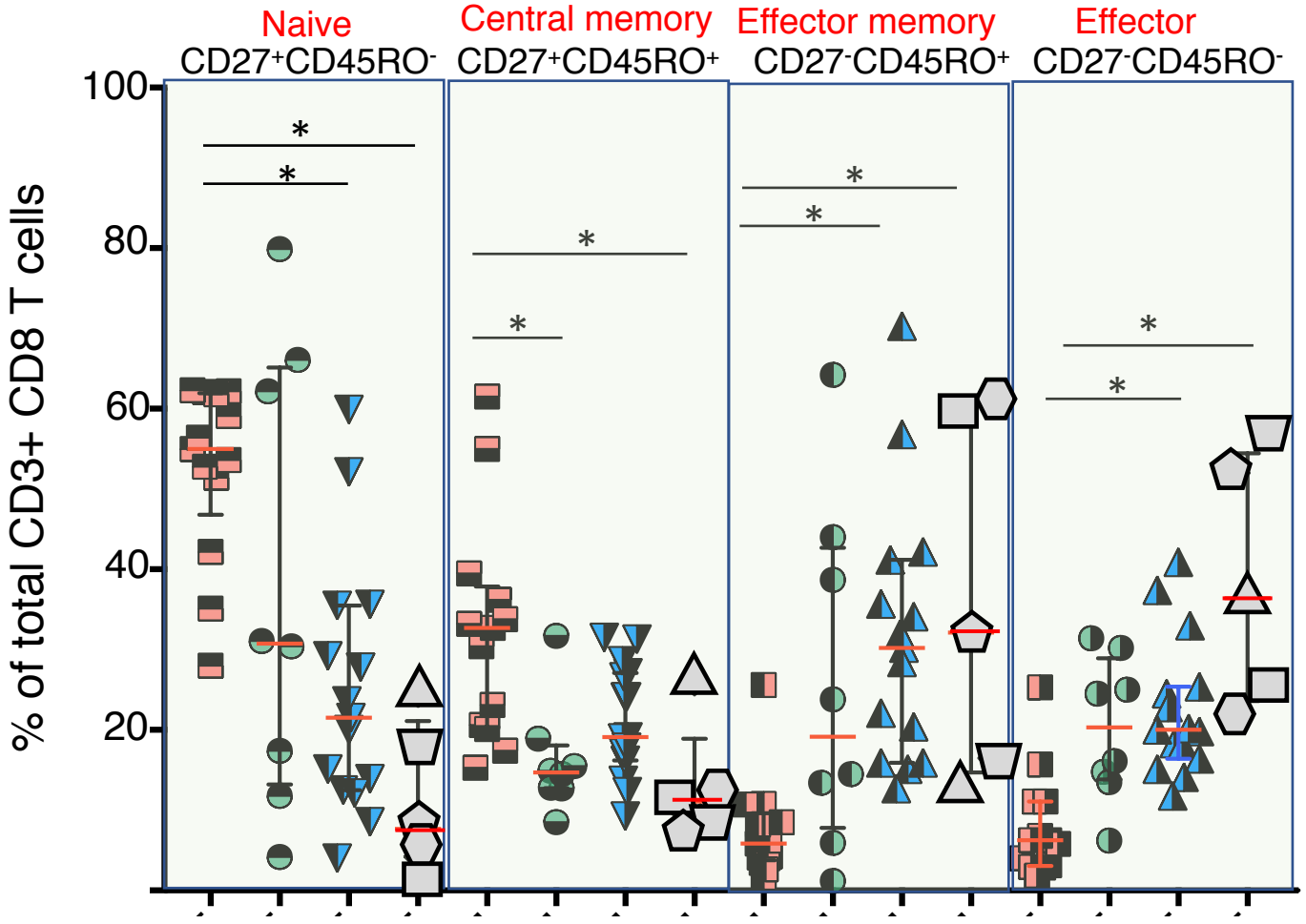
EXID3

EXID4

EXID5

Extreme immunological decline (EXID)

Supplementary Figure 2

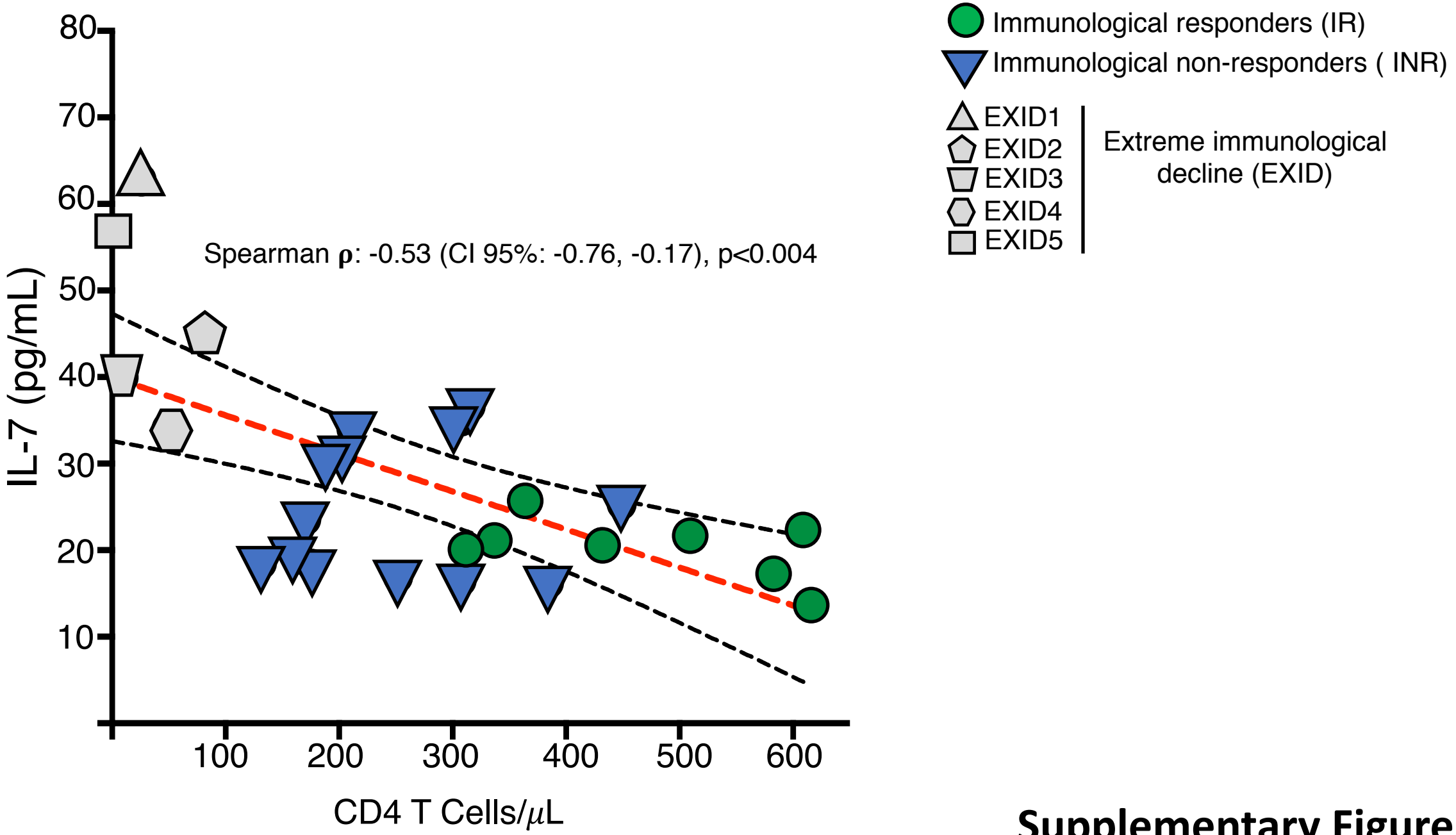


Healthy subjects (HC)
Immunological Responders (IR)
Immunological non-responders (INR)

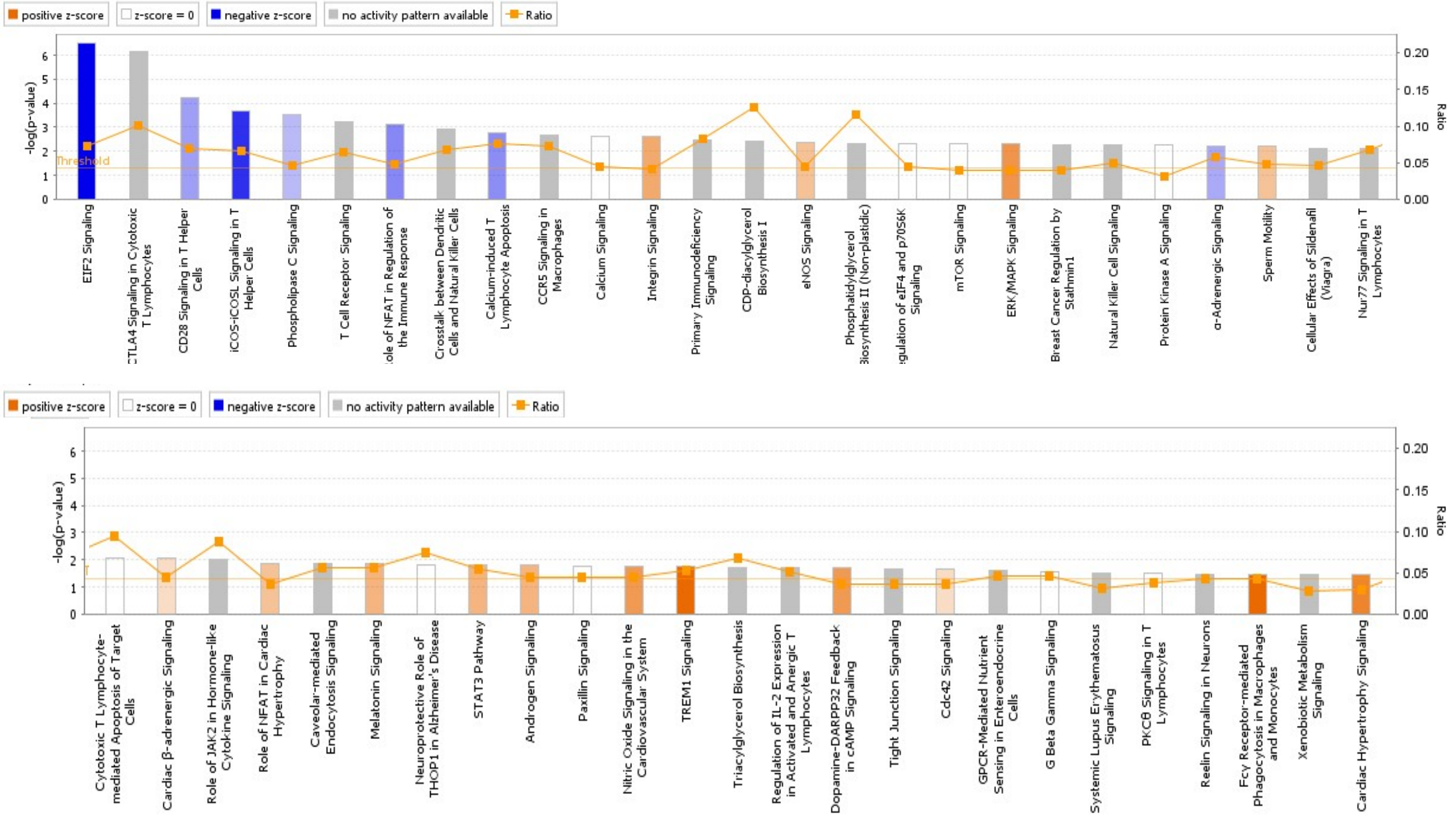
EXID1
EXID2
EXID3
EXID4
EXID5

Extreme immunological decline (EXID)

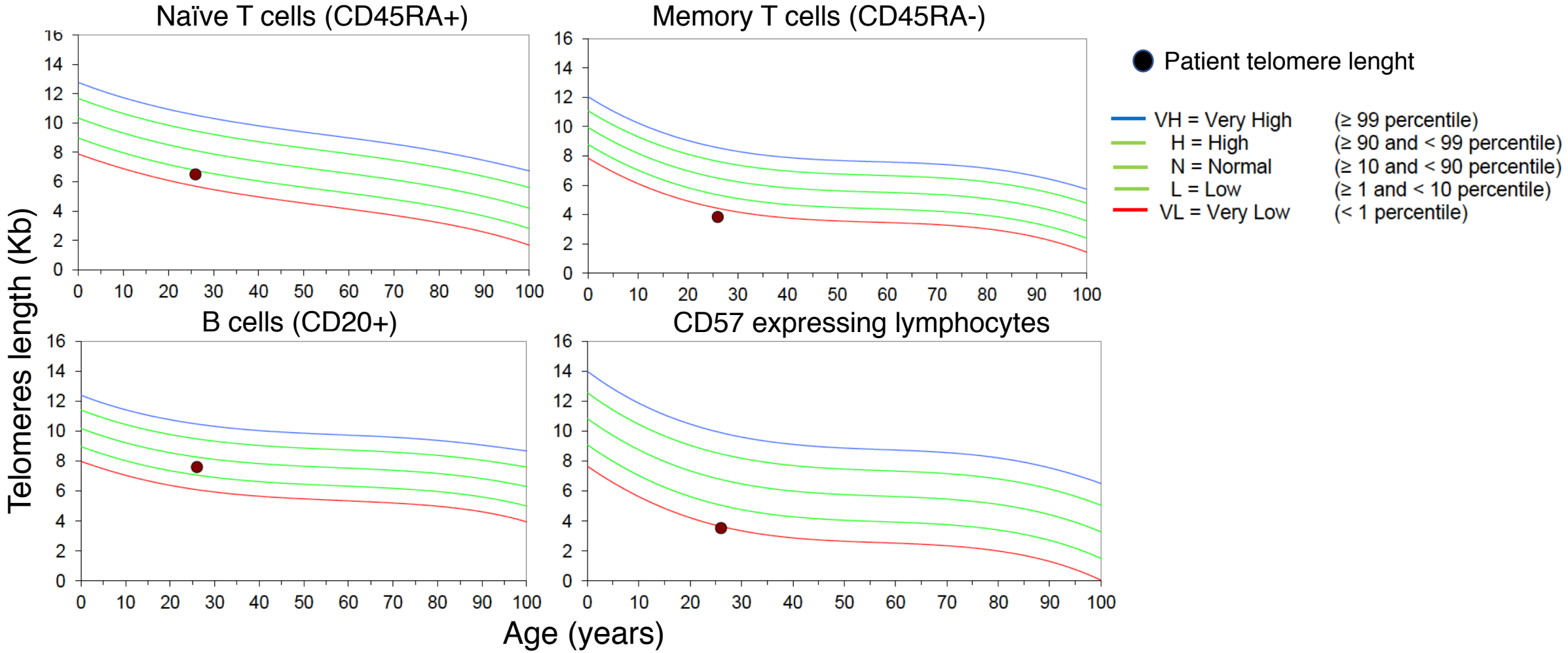
Supplementary Figure 3



Supplementary Figure 4



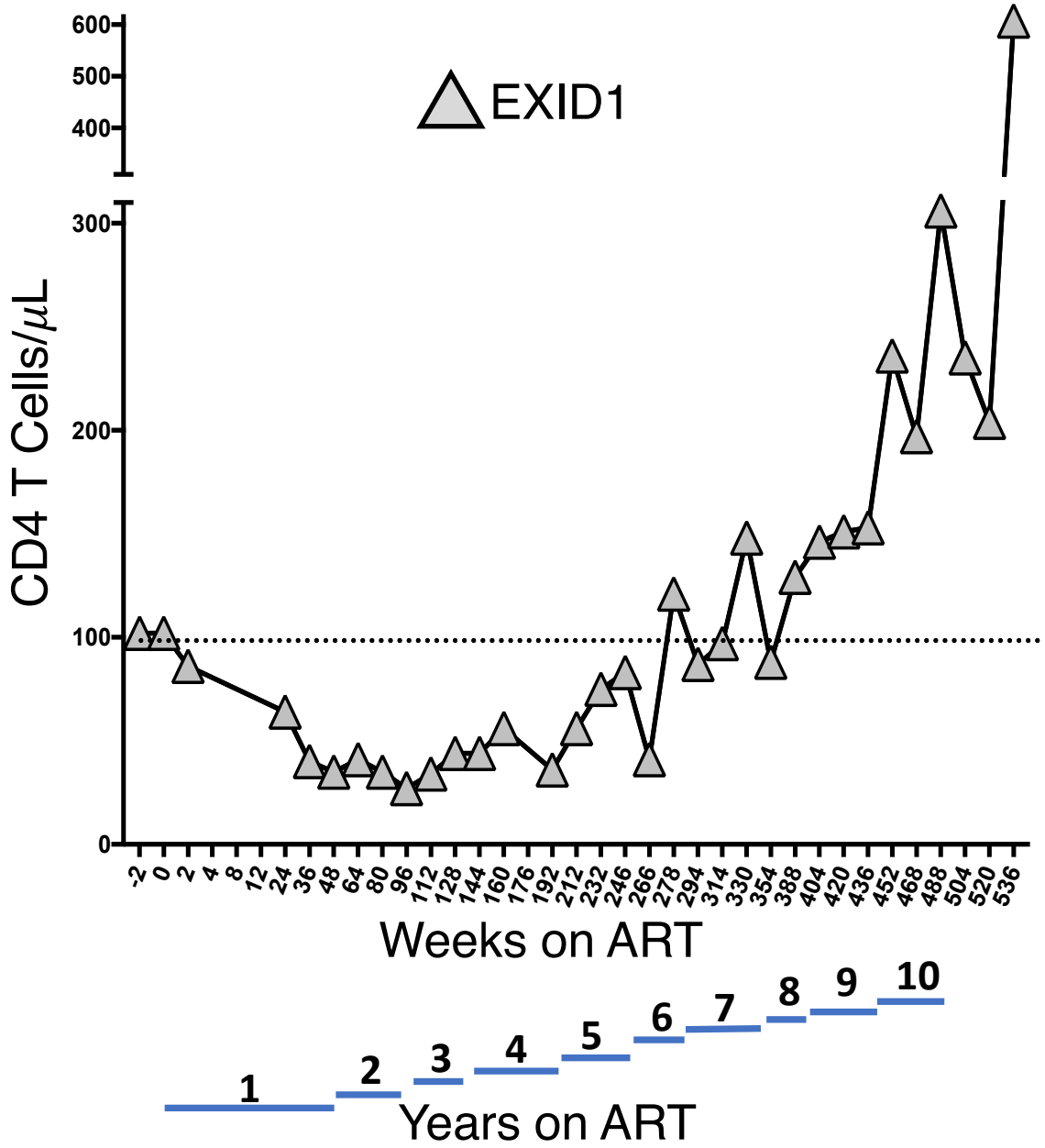
Supplementary Figure 5

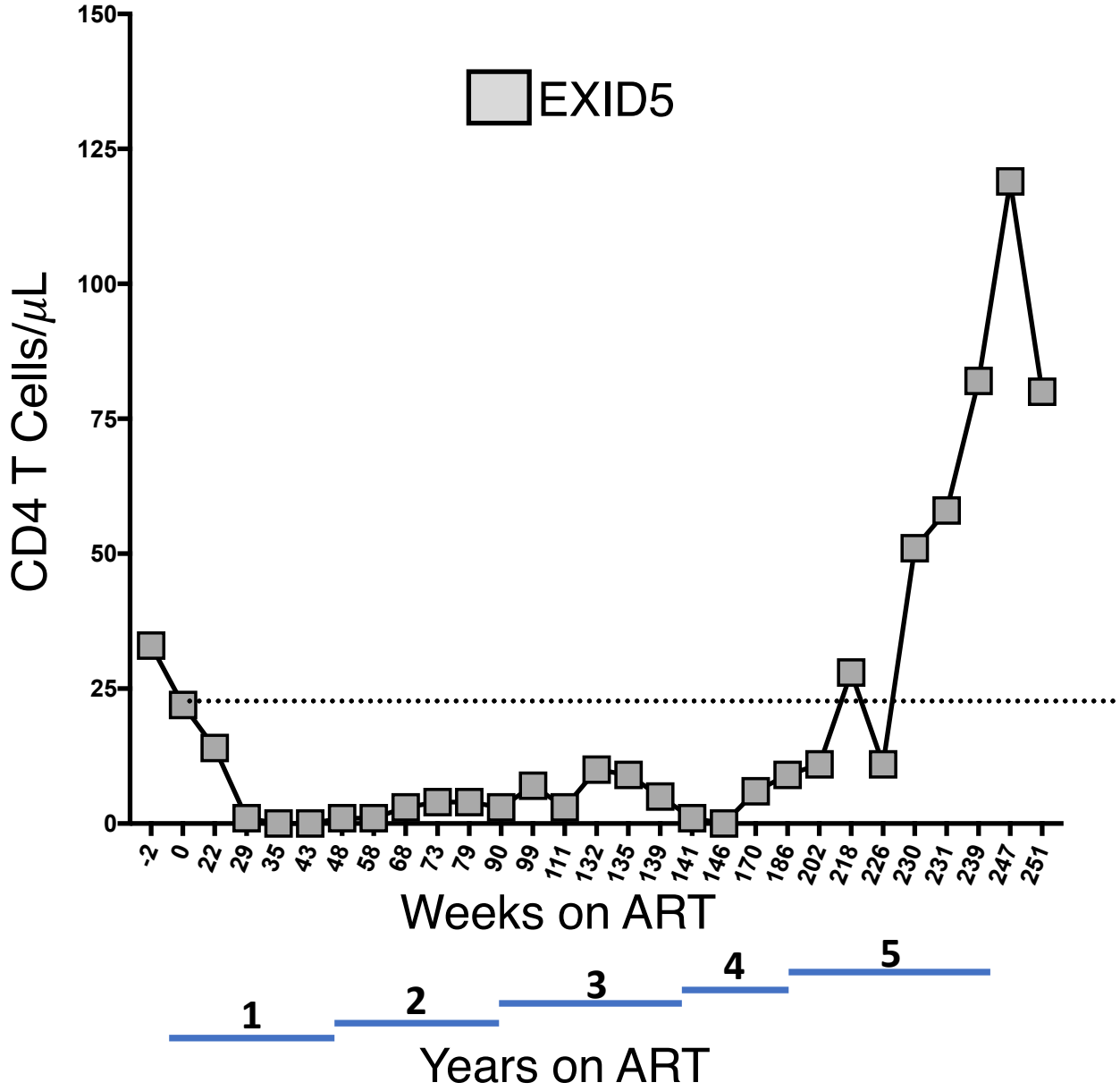


Lymphocytes			Granulocytes			CD45RA+ (Naïve T)			CD45RA- (Memory T)			CD20+ (B Cells)			CD57+ (NK Cells)		
MTL	MTLN	INT	MTL	MTLN	INT	MTL	MTLN	INT	MTL	MTLN	INT	MTL	MTLN	INT	MTL	MTLN	INT
(kb)	(kb)		(kb)	(kb)		(kb)	(kb)		(kb)	(kb)		(kb)	(kb)		(kb)	(kb)	
4.7	7.5	VL	**6.7**	8.6	L	**6.5**	8.1	L	**3.8**	6.5	VL	**7.6**	8.3	N	**3.5**	6.8	VL

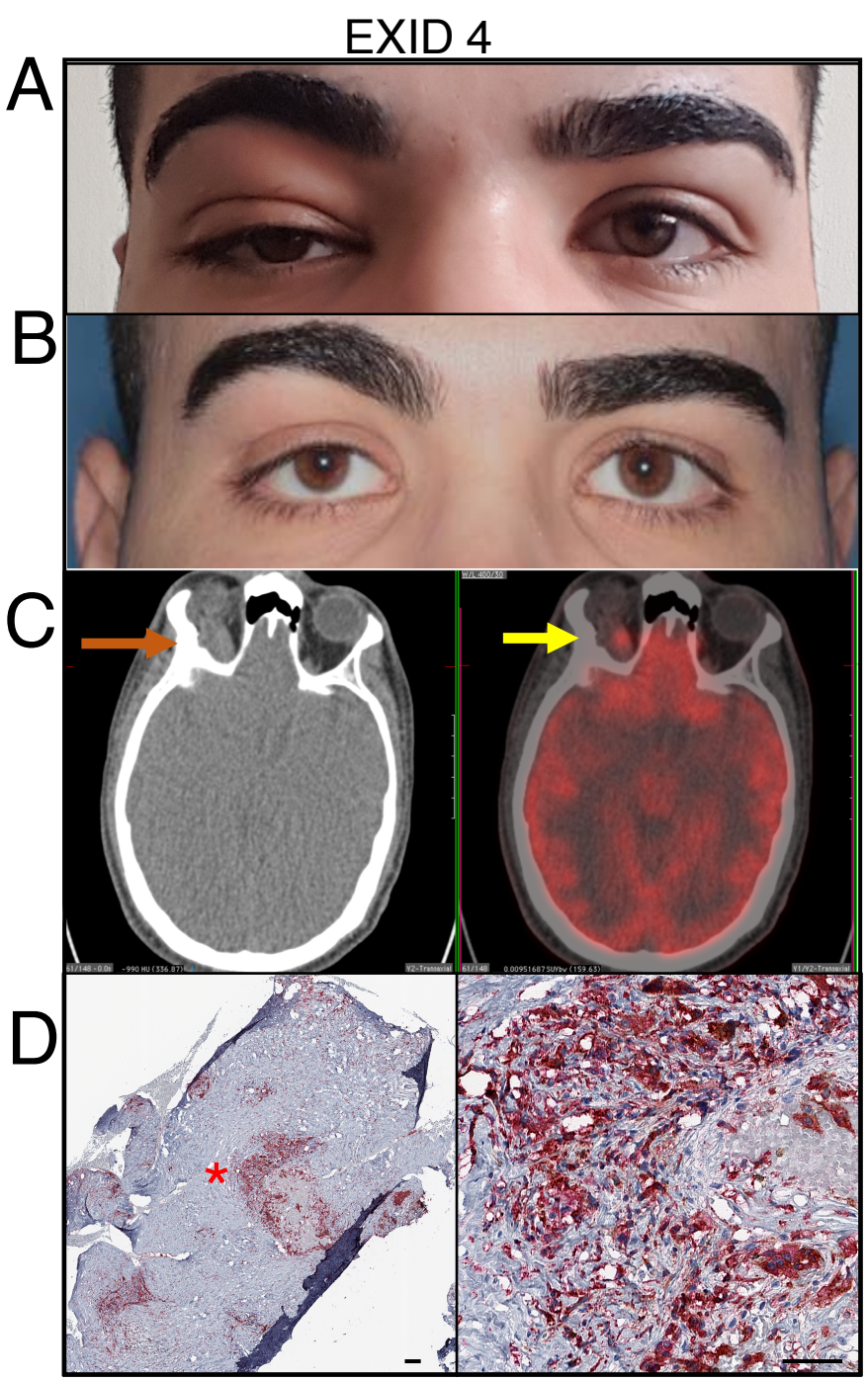
MTL = Patient Median Telomere Length
MTLN = Normal MTL at age (50th percentile)
INT = Telomere length interpretation

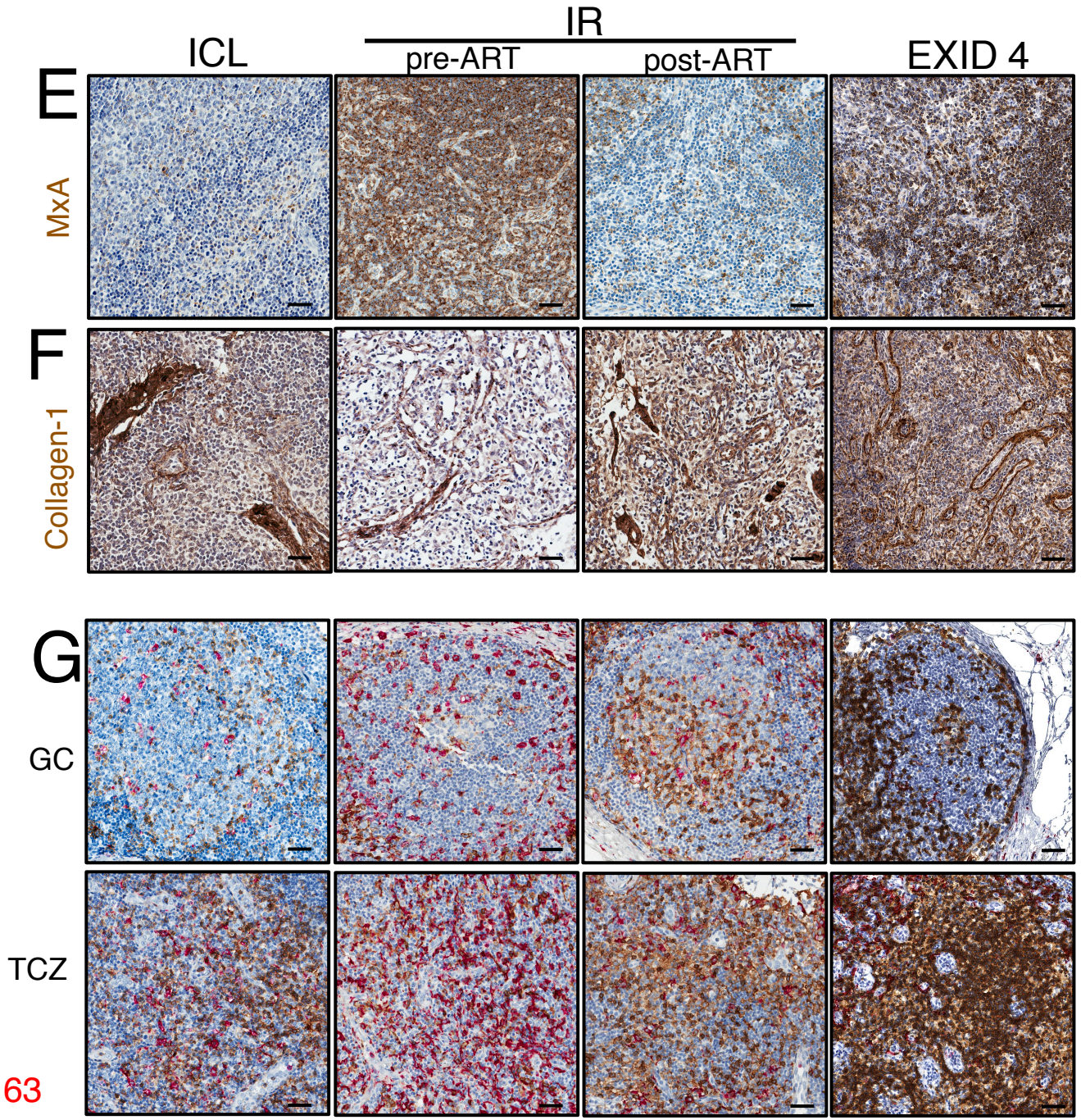
Supplementary Figure 6



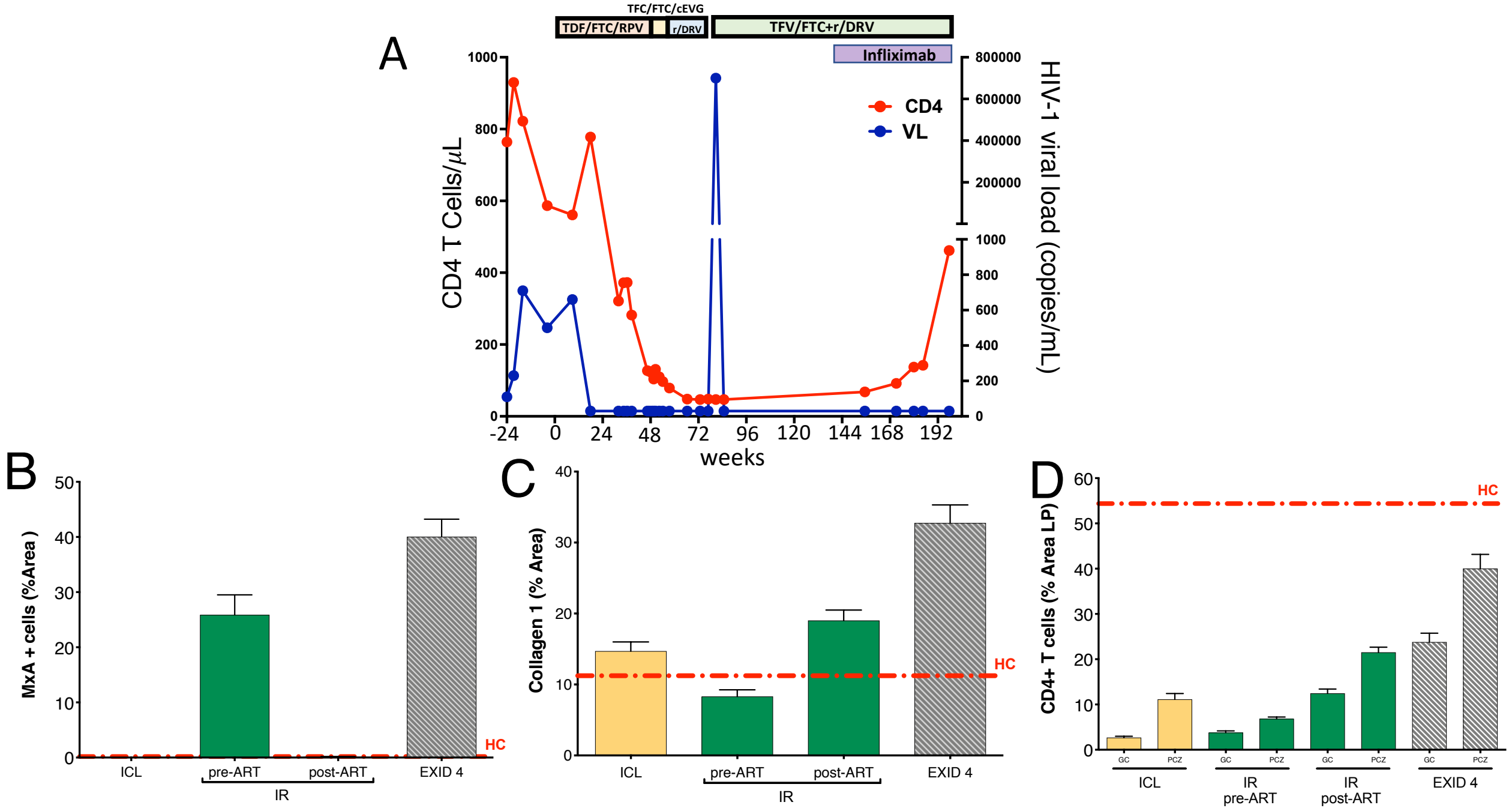


Supplementary Figure 7



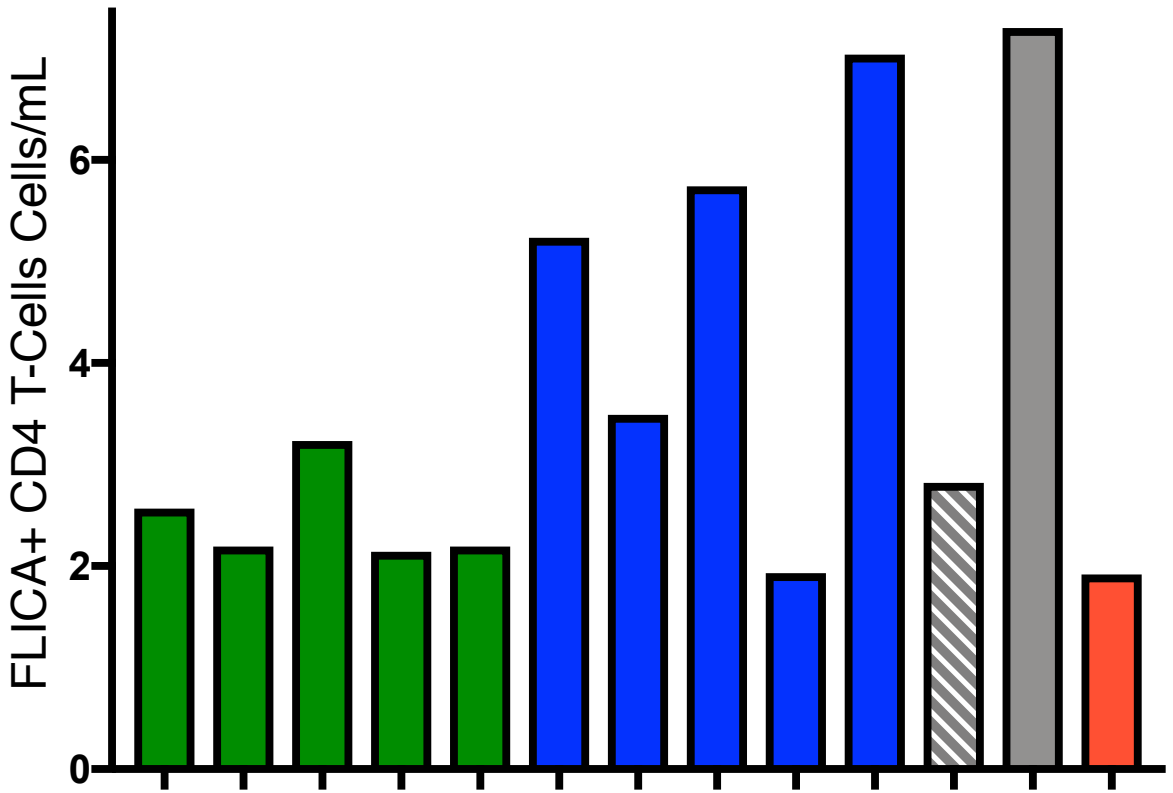


Supplementary Figure 8

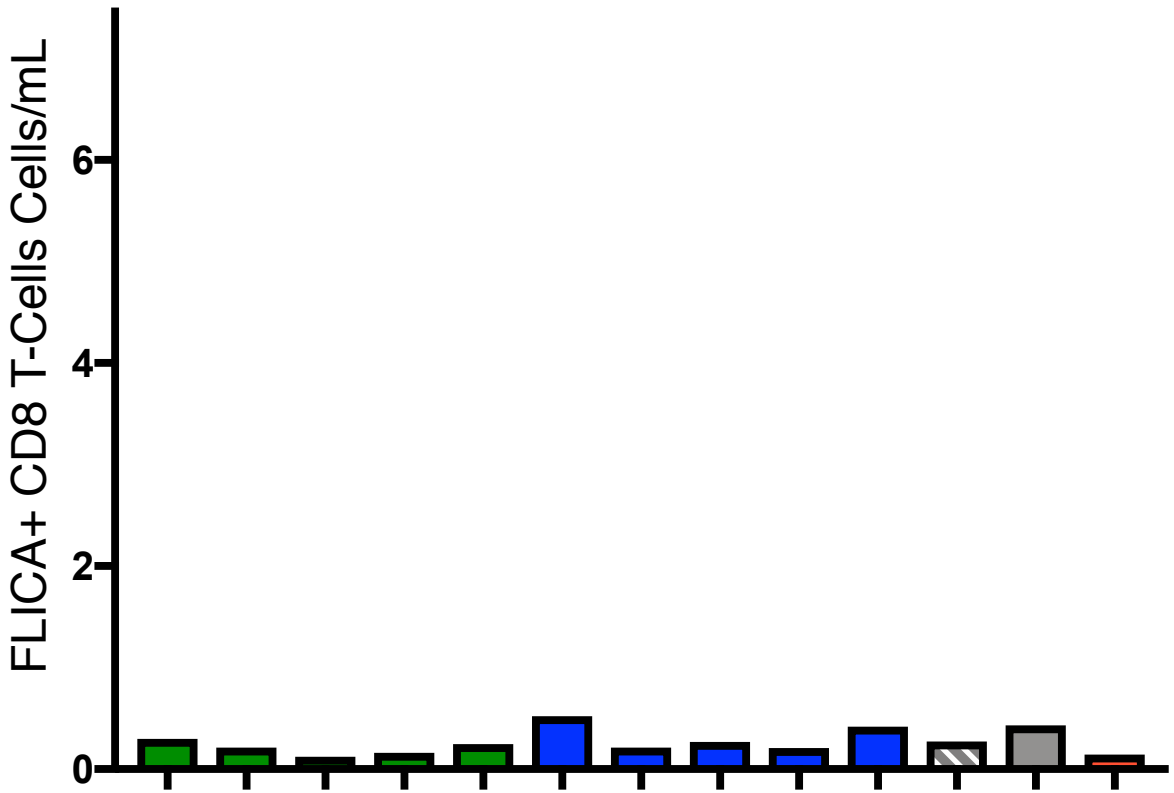


Supplementary Figure 9

A



B



IR
INR
EXID 4
EXID 2
HC

Supplementary Figure 10