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Supplemental Information

Mapping the Lineage Relationship between CXCR5^{+} and CXCR5^{-} CD4^{+} T Cells in HIV-Infected Human Lymph Nodes

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Table S1: Clinical characteristics and demographic information of LN and PBMC samples, related to Figures 1-6.

Donor ID	source	gender	age (years)	CD4 T cell count (cels/ul)	CD4:CD8	pVL (copies/ml)	ART
HIV1	LN, PBMC	M	18	504	0.71	DETECTABLE <40	No
HIV2	LN, PBMC	M	29	367	0.35	DETECTABLE <40	7 months
HIV3	LN	M	22	223	0.08	280700	1 day
HIV4	LN	M	24	6	0.1	714,364	No
HIV5	LN, PBMC	M	26	399	1.03	9610	No
HIV6	LN, PBMC	M	22	538	0.32	9511	No
HIV7	LN, PBMC	M	20	743	0.58	83801	No
HIV8	LN, PBMC	M	22	1136	0.72	45395	No
HIV9	LN	M	19	13	0.27	621,622	No
HIV10	LN	M	33	18	0.32	75,043	No
HIV11	LN	M	28	406	0.46	61586	No
HIV12	LN, PBMC	M	23	473	0.62	208656	No
HIV13	LN	M	28	564	0.4	16647	No
HIV14	LN	M	45	9	0.1	111,403	No
HIV15	LN	M	20	8	0.14	578,155	No
HIV16	LN	M	28	10	0.17	243,150	No
HIV17	LN, PBMC	M	19	157	0.15	63	3 months
HIV18	LN, PBMC	M	19	258	0.41	35847	No
HIV19	LN, PBMC	M	28	25	0.41	31950	No
HIV20	LN, PBMC	M	33	403	0.44	26989	No
HIV21	LN	M	20	8	0.13	5,672,501	No
HIV22	LN	M	26	16	0.29	222428	No
HIV23	LN	M	21	573	0.6	17868	No
HIV24	LN	M	35	676	0.93	25432	No
HIV25	LN	M	29	152	0.21	5915	1 month
HIV26	LN	M	23	16	0.29	3,034	No
HIV27	LN	M	29	241	0.22	447952	No
HIV28	LN, PBMC	M	28	1086	0.77	3964	No
HIV29	LN	M	26	402	0.31	29620	1 month
HIV30	LN	F	20	212	0.28	136190	No
HIV31	LN	M	32	159	0.21	1,387,603	No
HIV32	LN, PBMC	M	40	668	0.57	17247	No
HIV33	LN	M	21	11	0.18	1,227,366.00	No
HIV34	LN	F	41	7	0.06	230948	2 months
HIV35	LN	F	40	18	0.3	111,854	No
HIV36	LN, PBMC	M	29	295	0.42	71	36 months
HIV37	LN	M	23	9	0.11	1,205,036	No
HIV38	PBMC	M	46	666	1.07	9,087.00	No
HIV39	LN, PBMC	M	37	276	0.17	199152	No
HIV40	LN	M	28	22	0.4	18,111	No
HIV41	LN, PBMC	M	38	387	0.4	71004	No
HIV42	PBMC	M	45	212	NA	23065	No
HIV43	PBMC	F	45	7	NA	24448	No
HIV44	PBMC	M	41	336	NA	19700	No
HIV45	LN	M	18	192	0.08	2,613,622.00	No
HIV46	LN	M	21	251	0.19	1,019,989.00	No
HIV47	LN	M	24	716	0.51	322,442.00	No
HIV48	LN	M	27	719	0.39	49,224.00	No
HIV49	LN	M	37	832	0.56	11,026.00	No
HIV50	LN	M	46	622	1.08	848.00	No
HIV51	PBMC	M	25	147	0.13	114,750.00	No
HIV52	LN	M	29	334	0.21	203,393.00	No
HC1	LN	M	1	NA	NA	NA	NA
HC2	LN	F	41	NA	NA	NA	NA
HC3	LN	M	58	NA	NA	NA	NA
HC4	LN	M	18	NA	NA	NA	NA
HC5	LN	M	3	NA	NA	NA	NA
HC6	LN	M	55	NA	NA	NA	NA
HC7	LN	F	37	NA	NA	NA	NA

Table S2: CyTOF antibody panels, related to Figures 1-3.

Panel 1

Antibody	Metal	Clone	Vendor
CD57	113	HCD57	Biolegend
CD3	141	UCHT1	BD
CD5	142	UCHT2	Biolegend
CD8	143	SK1	Biolegend
CD4	144	SK3	Biolegend
CD19	145	HIB19	Biolegend
Granzyme B	146	CLB-GB11	eBioscience
IFN-γ	147	4S.B3	eBioscience
HLA-DR	148	L243	biolegend
CD14	149	M5E2	Bio Legend
CD69	150	FN50	Biolegend
CD38	151	HB-7	Biolegend
TNF-α	152	MAb11	biolegend
CD45RO	153	UCHL1	biolegend
CD27	154	LG.7F9	eBioscience
TCRαβ	155	T10B9.1A-31	BD
CCR5	156	J418F1	Biolegend
	157		
CD71	158	CY1G4	biolegend
CXCR4	159	12G5	biolegend
IL-4	160	8D4-8	BD
CD25	161	M-A251	BD
IL-2	162	MQ1-17H12	biolegend
ICOS	163	c398-4A	biolegend
Ki67	164	B56	BD
Foxp3	165	PCH101	ebiosciences
TCF1	166	C63D9	Cell Signaling
PD-1	167	EH12.2H7	Biolegend
CCR7	168	G043H7	biolegend
CXCR5	169	RF8B2	BD
TCRγδ	170	SA6.E9	invitrogen
CD103	171	B-Ly7	ebioscience
CCR4	172	1G1	BD
CCR6	173	G034E3	biolegend
IL-13	174	JES10-5A2	biolegend
Perforin	175	DG9	biolegend
IL-17A	176	BL168	Biolegend

Panel 2 (Wendel et al., 2018)

antibodies	metal	clone	Vendor
CD57	113	HCD57	Biolegend
CD3	141	UCTH1	BD
CD5	142	UCHT2	Biolegend
CD8	143	SK1	Biolegend
CD4	144	SK3	Biolegend
CD19	145	HIB19	Biolegend
IgD	146	IA6-2	Biolegend
IFN-γ	147	4S.B3	eBioscience
CCR6	148	G034E3	Biolegend
CD14	149	M5E2	Biolegend
CD69	150	FN50	Biolegend
Granzyme A	151	CB9	Biolegend
TNF-α	152	MAb11	Biolegend
CD45RO	153	UCHL1	Biolegend
CD27	154	LG.7F9	eBioscience
TCRab	155	T10B9.1A-31	BD
CCR5	156	J418F1	Biolegend
Ki67	157	B56	BD
BLYS	158	1D6	Biolegend
BCL6	159	K112-91	BD
IL-4	160	8D4-8	BD
CD25	161	M-A251	BD
Eomes	162	wd1928	eBioscience
ICOS	163	c398-4A	Biolegend
IL-5	164	TRFK5	Biolegend
Foxp3	165	PCH101	eBioscience
CD95	166	DX2	Biolegend
CD45RA	167	HI100	eBioscience
CCR7	168	G043H7	Biolegend
CXCR5	169	RF8B2	BD
T-bet	170	4B10	Biolegend
PD-1	171	EH12.2H7	Biolegend
IL-21	172	3A3-N2	Biolegend
IL-2	173	MQ1-17H12	eBioscience
CD24	174	ML5	Biolegend
CXCR3	175	G025H7	Biolegend
CD38	176	HIT2	Biolegend

Table S3: Cell and transcript counts for sequencing experiments, related to Figures 5-7.

Donor ID	Assay Type	T cell Population	Source	Cell count	Mapped reads after QC	Donor ID	Assay Type	T cell Population	Source	Cell count	Mapped reads after QC
HIV15	ATAC-seq	CXCR5-PD-1-ICOS-	LN	20000	135508401	HIV12	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	5992
HIV16	ATAC-seq	CXCR5-PD-1-ICOS-	LN	17544	52354592	HIV13	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	6533
HIV21	ATAC-seq	CXCR5-PD-1-ICOS-	LN	20000	53542641	HIV17	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	8945
HIV4	ATAC-seq	CXCR5-PD-1-ICOS-	LN	10000	49048896	HIV2	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	8777
HIV45	ATAC-seq	CXCR5-PD-1-ICOS-	LN	20000	50769892	HIV23	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	7503
HIV46	ATAC-seq	CXCR5-PD-1-ICOS-	LN	20000	52949511	HIV28	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	2821
HIV15	ATAC-seq	CXCR5-PD-1+ICOS+	LN	20000	76611947	HIV39	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	9033
HIV16	ATAC-seq	CXCR5-PD-1+ICOS+	LN	20000	51161176	HIV41	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	6254
HIV21	ATAC-seq	CXCR5-PD-1+ICOS+	LN	20000	47122262	HIV52	TCR-seq	CXCR5-PD-1-ICOS-	LN	10000	6315
HIV4	ATAC-seq	CXCR5-PD-1+ICOS+	LN	10000	40979805	HIV12	TCR-seq	CXCR5-PD-1+ICOS+	LN	2500	3366
HIV45	ATAC-seq	CXCR5-PD-1+ICOS+	LN	17365	75578156	HIV13	TCR-seq	CXCR5-PD-1+ICOS+	LN	2500	4546
HIV46	ATAC-seq	CXCR5-PD-1+ICOS+	LN	20000	116645531	HIV17	TCR-seq	CXCR5-PD-1+ICOS+	LN	2500	2836
HIV15	ATAC-seq	naïve	LN	20000	50100923	HIV2	TCR-seq	CXCR5-PD-1+ICOS+	LN	5000	3182
HIV16	ATAC-seq	naïve	LN	20000	50541268	HIV23	TCR-seq	CXCR5-PD-1+ICOS+	LN	2500	2552
HIV21	ATAC-seq	naïve	LN	20000	43842949	HIV28	TCR-seq	CXCR5-PD-1+ICOS+	LN	2515	2708
HIV4	ATAC-seq	naïve	LN	10000	41241783	HIV39	TCR-seq	CXCR5-PD-1+ICOS+	LN	4085	3542
HIV45	ATAC-seq	naïve	LN	20000	90993949	HIV41	TCR-seq	CXCR5-PD-1+ICOS+	LN	10000	10290
HIV46	ATAC-seq	naïve	LN	20000	49421306	HIV52	TCR-seq	CXCR5-PD-1+ICOS+	LN	7500	7960
HIV15	ATAC-seq	TFH	LN	20000	85765124	HIV12	TCR-seq	naïve	LN	10000	13225
HIV16	ATAC-seq	TFH	LN	20000	51623107	HIV13	TCR-seq	naïve	LN	10000	18376
HIV21	ATAC-seq	TFH	LN	20000	51243858	HIV17	TCR-seq	naïve	LN	10000	6750
HIV4	ATAC-seq	TFH	LN	3122	44084338	HIV2	TCR-seq	naïve	LN	5000	3502
HIV45	ATAC-seq	TFH	LN	6717	59274617	HIV23	TCR-seq	naïve	LN	10000	10404
HIV46	ATAC-seq	TFH	LN	20000	39057907	HIV28	TCR-seq	naïve	LN	10000	5488
HIV15	RNA-seq	CXCR5-PD-1-ICOS-	LN	5000	118141653	HIV39	TCR-seq	naïve	LN	10000	4314
HIV16	RNA-seq	CXCR5-PD-1-ICOS-	LN	5000	63511176	HIV41	TCR-seq	naïve	LN	10000	5822
HIV21	RNA-seq	CXCR5-PD-1-ICOS-	LN	5000	71005274	HIV52	TCR-seq	naïve	LN	10000	14420
HIV4	RNA-seq	CXCR5-PD-1-ICOS-	LN	5000	61506370	HIV12	TCR-seq	TFH	LN	10000	9804
HIV45	RNA-seq	CXCR5-PD-1-ICOS-	LN	5000	97776218	HIV13	TCR-seq	TFH	LN	10000	17659
HIV46	RNA-seq	CXCR5-PD-1-ICOS-	LN	5000	44654834	HIV17	TCR-seq	TFH	LN	8758	8936
HIV15	RNA-seq	CXCR5-PD-1+ICOS+	LN	5000	93246500	HIV2	TCR-seq	TFH	LN	10000	10900
HIV16	RNA-seq	CXCR5-PD-1+ICOS+	LN	5000	63075958	HIV23	TCR-seq	TFH	LN	10230	8052
HIV21	RNA-seq	CXCR5-PD-1+ICOS+	LN	5000	67697670	HIV28	TCR-seq	TFH	LN	2500	4135
HIV4	RNA-seq	CXCR5-PD-1+ICOS+	LN	5000	51941772	HIV39	TCR-seq	TFH	LN	5000	19342
HIV45	RNA-seq	CXCR5-PD-1+ICOS+	LN	5000	102956361	HIV41	TCR-seq	TFH	LN	10000	10167
HIV46	RNA-seq	CXCR5-PD-1+ICOS+	LN	5000	46350154	HIV52	TCR-seq	TFH	LN	2500	2723
HIV15	RNA-seq	naïve	LN	5000	75327804	HIV13	TCR-seq	CXCR5-PD-1+ICOS+	PBMC	2500	3374
HIV16	RNA-seq	naïve	LN	5000	55014596	HIV17	TCR-seq	CXCR5-PD-1+ICOS+	PBMC	293	496
HIV21	RNA-seq	naïve	LN	5000	62263436	HIV2	TCR-seq	CXCR5-PD-1+ICOS+	PBMC	2500	1711
HIV4	RNA-seq	naïve	LN	5000	53756662	HIV28	TCR-seq	CXCR5-PD-1+ICOS+	PBMC	2267	3016
HIV45	RNA-seq	naïve	LN	5000	86859878	HIV39	TCR-seq	CXCR5-PD-1+ICOS+	PBMC	4487	4536
HIV46	RNA-seq	naïve	LN	5000	39353435	HIV41	TCR-seq	CXCR5-PD-1+ICOS+	PBMC	5000	6640
HIV15	RNA-seq	TFH	LN	5000	126979175						
HIV16	RNA-seq	TFH	LN	5000	60580898						
HIV21	RNA-seq	TFH	LN	5000	63604304						
HIV4	RNA-seq	TFH	LN	5000	50051284						
HIV45	RNA-seq	TFH	LN	5000	89300563						
HIV46	RNA-seq	TFH	LN	5000	64097025						

Table S4: TCRβ sequencing primers, related to Figures 5 and 7.

Reverse Transcription primer:	RT
	ACACTCTTTCCCTACACGACGCTCTTCCGATCT NNNNNNNNNNNN GACCTCGGGTGGGAACAC (N indicates random molecular barcode)
1st PCR Primers:	1st PCR Reverse:
	ACACTCTTTCCCTACACGAC
	1st PCR Forward:
TRBV1	GACGTGTGCTCTTCCGATCTCTGACAGCTCTCGCTTATACCTTCA
TRBV2	GACGTGTGCTCTTCCGATCTGCCTGATGGATCAAATTTCACTCTG
TRBV3	GACGTGTGCTCTTCCGATCTAATGAAACAGTTCCAAATCGMTTCT
TRBV4	GACGTGTGCTCTTCCGATCTCCAAGTCGCTTCTCACCTGAAT
TRBV5-1	GACGTGTGCTCTTCCGATCTCGCCAGTTCTCTAACTCTCGCTCT
TRBV5-2	GACGTGTGCTCTTCCGATCTTTACTGAGTCAAACACGGAGCTAGG
TRBV5-3	GACGTGTGCTCTTCCGATCTCTCTGAGATGAATGTGAGTGCCTTG
TRBV5-4/5/6/7/8	GACGTGTGCTCTTCCGATCTCTGAGCTGAATGTGAACGCCTTG
TRBV6-1	GACGTGTGCTCTTCCGATCTTCTCCAGATTAAACAAACGGGAGTT
TRBV6-2/3	GACGTGTGCTCTTCCGATCTCTGATGGCTACAATGTCTCCAGATT
TRBV6-4	GACGTGTGCTCTTCCGATCTAGTGTCTCCAGAGCAAACACAGATG
TRBV6-5/6/7	GACGTGTGCTCTTCCGATCTGTCTCCAGATCAAMCACAGAGGATT
TRBV6-8/9	GACGTGTGCTCTTCCGATCTAAACACAGAGGATTTCCCRCTCAG
TRBV7-1	GACGTGTGCTCTTCCGATCTGTCTGAGGGATCCATCTCCACTC
TRBV7-2	GACGTGTGCTCTTCCGATCTTCGCTTCTCTGCAGAGAGGACTGG
TRBV7-3	GACGTGTGCTCTTCCGATCTCTGAGGGATCCGTCTCTACTCTGAA
TRBV7-4/8	GACGTGTGCTCTTCCGATCTCTGAGRGATCCGTCTCCACTCTG
TRBV7-5	GACGTGTGCTCTTCCGATCTGGTCTGAGGATCTTTCTCCACCT
TRBV7-6/7	GACGTGTGCTCTTCCGATCTGAGGGATCCATCTCCACTCTGAC
TRBV7-9	GACGTGTGCTCTTCCGATCTCTGCAGAGAGGCCTAAGGGATCT
TRBV8-1	GACGTGTGCTCTTCCGATCTAAGCTCAAGCATTTTCCCTCAAC
TRBV8-2	GACGTGTGCTCTTCCGATCTATGTCACAGAGGGGTACTGTGTTTC
TRBV-9	GACGTGTGCTCTTCCGATCTACAGTTCCCTGACTTGCACTCTG
TRBV10-1/3	GACGTGTGCTCTTCCGATCTACAAAGGAGAAGTCTCAGATGGCTA
TRBV10-2	GACGTGTGCTCTTCCGATCTTGTCTCCAGATCCAAGACAGAGAA
TRBV11	GACGTGTGCTCTTCCGATCTCTGCAGAGAGGCTCAAAGGAGTAG
TRBV12-1/2	GACGTGTGCTCTTCCGATCTATCATTCTCYACTCTGAGGATCCAR
TRBV12-3/4/5	GACGTGTGCTCTTCCGATCTACTCTGARGATCCAGCCCTCAGAAC
TRBV13	GACGTGTGCTCTTCCGATCTCAGCTCAACAGTTCAGTGACTATCAT
TRBV14	GACGTGTGCTCTTCCGATCTGAAAGGACTGGAGGGACGTATTCTA
TRBV15	GACGTGTGCTCTTCCGATCTGCCGAACACTTCTTTCTGCTTTCT
TRBV16	GACGTGTGCTCTTCCGATCTATTTTCAGCTAAGTGCCTCCCAAAT
TRBV17	GACGTGTGCTCTTCCGATCTCACAGCTGAAAGACCTAACGGAAC
TRBV18	GACGTGTGCTCTTCCGATCTATTTTCTGCTGAATTTCCCAAAGAG
TRBV19	GACGTGTGCTCTTCCGATCTGTCTCTCGGGAGAAGAAGGAATC
TRBV20-1	GACGTGTGCTCTTCCGATCTGACAAGTTTCTCATCAACCATGCAA
TRBV21-1	GACGTGTGCTCTTCCGATCTCAATGCTCCAAAAACTCATCCTGT
TRBV22-1	GACGTGTGCTCTTCCGATCTAGGAGAAGGGGCTATTTCTTCTCAG
TRBV23-1	GACGTGTGCTCTTCCGATCTATTCTCATCTCAATGCCCCAAGAAC
TRBV24-1	GACGTGTGCTCTTCCGATCTGACAGGCACAGGCTAAATTCTCC
TRBV25-1	GACGTGTGCTCTTCCGATCTAGTCTCCAGAATAAGGACGGAGCAT
TRBV26	GACGTGTGCTCTTCCGATCTCTCTGAGGGGTATCATGTTTCTTGA
TRBV27	GACGTGTGCTCTTCCGATCTCAAAGTCTCTCGAAAAGAGAAGAGGA
TRBV28	GACGTGTGCTCTTCCGATCTAAGAAGGAGCGCTTCTCCCTGATT
TRBV29-1	GACGTGTGCTCTTCCGATCTCGCCCAAACCTAACATTCTCAA
TRBV30	GACGTGTGCTCTTCCGATCTCCAGAATCTCTCAGCCTCCAGAC
2nd PCR Primers:	2nd PCR Reverse:
	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC
	2nd PCR Forward:
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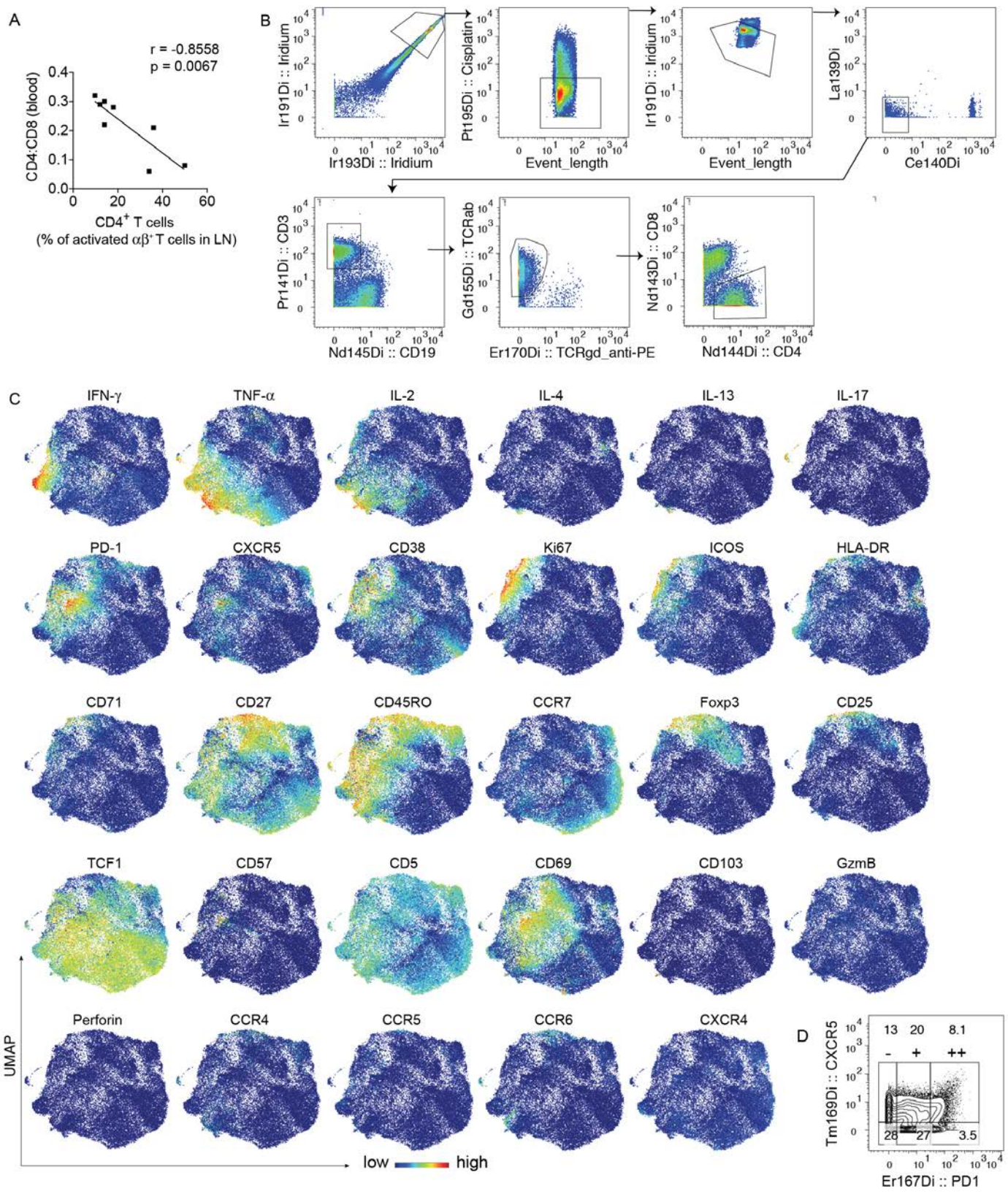


Figure S1: High-dimensional analyses of activated CD4+ T cells in HIV infected LNs by CyTOF, related to Figure 1.

(A) Correlation between peripheral CD4:CD8 ratio and the frequency of $CD38^{+}HLA\text{-}DR^{+}CD4^{+}$ T cells in the LNs. Association was measured by Pearson correlation and the best-fitting line was calculated using least squares fit regression (B) Representative plots showing gating strategy for identifying the input $CD4^{+}$ T cells used for CyTOF analyses. (C) UMAP displays of individual markers as indicated. Markers used to select input cells were excluded. Plots combined $CD4^{+}$ T cells from 8 HIV LN samples. (D) Plots showing CXCR5 and PD-1 expression and the subdivision of $CD4^{+}$ T cells by the intensity of CXCR5 and PD-1 staining in the CyTOF analyses.

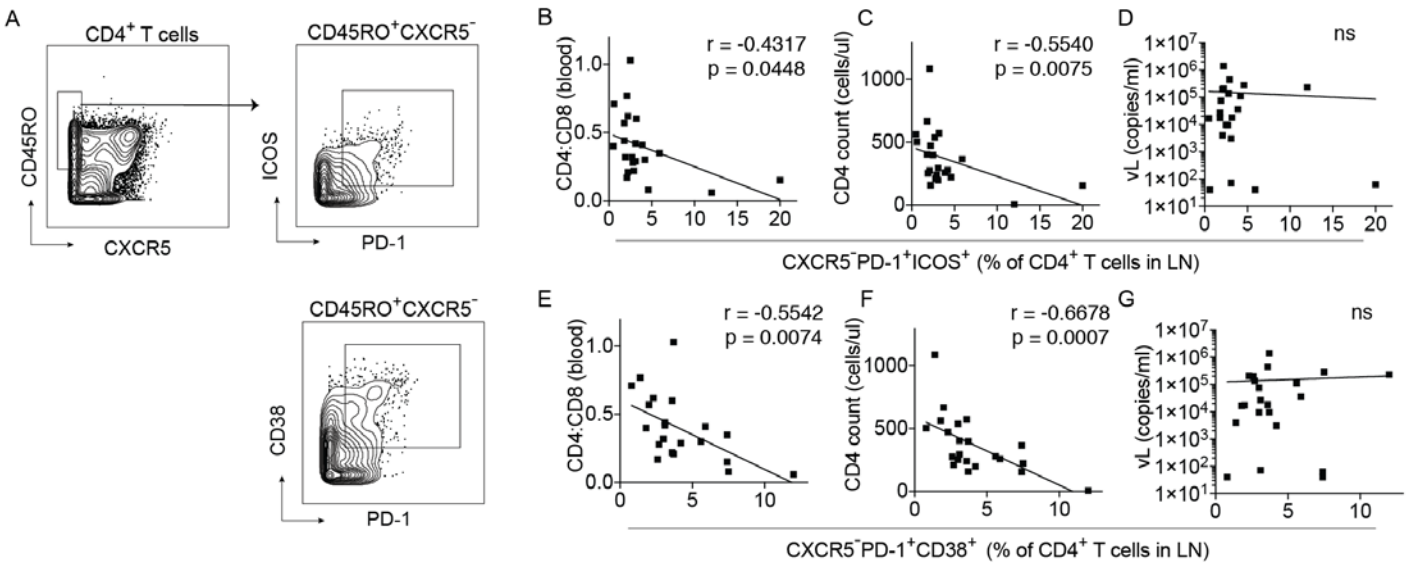


Figure S2: Correlation between subsets of CXCR5$^-$ CD4$^+$ T cells and clinical parameters of HIV infection, related to Figure 1.

(A) Plots showing representative staining and gates for identifying CXCR5$^-$PD-1$^+$ICOS$^+$ T cells (top) or CXCR5$^-$ PD-1$^+$CD38$^+$ T cells (bottom). (B-D) The frequency of CXCR5$^-$PD-1$^+$ICOS$^+$ T cells in the LN is associated with CD4:CD8 ratio (B), CD4$^+$ T cell count (C) but not with viral load (D). (E-G) The frequency of CXCR5$^-$PD-1$^+$CD38$^+$ T cells in the LN is associated with CD4:CD8 ratio (E), CD4$^+$ T cell count (F) but not with viral load (G). Data include 22 HIV LN samples. Association was measured by Spearman or Pearson correlation, depending on normality of the data as determined by D'Agostino & Pearson omnibus normality test. The best-fitting line was calculated using least squares fit regression.

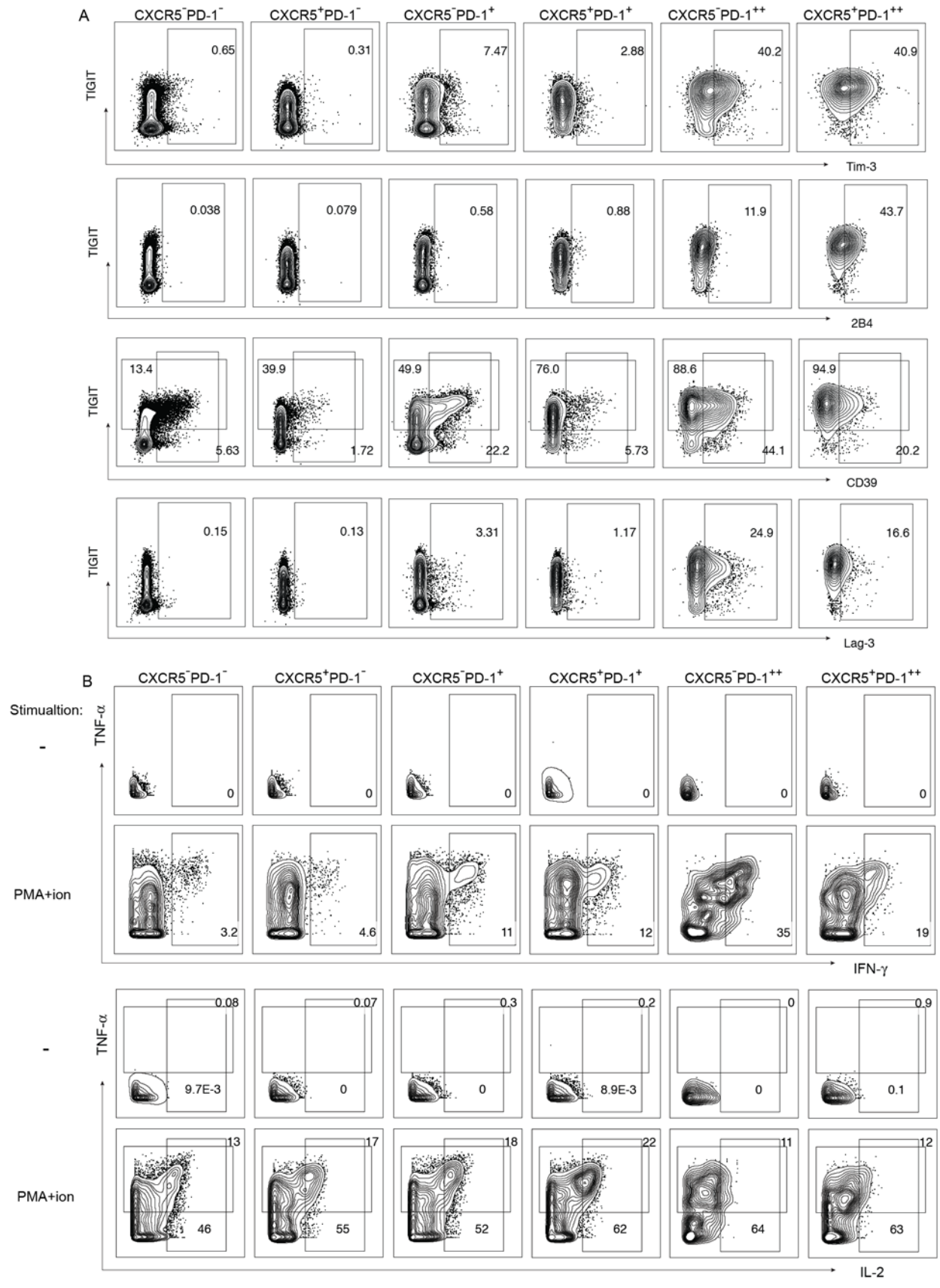


Figure S3: Co-inhibitory receptor expression and cytokine producing function of $CD4^+$ T cell subsets in LNs, related to Figure 2.

(A) Plots showing expression of Tim-3, 2B4, CD39, Lag-3, and TIGIT on CD4$^+$ T cell subsets. (B) Plots showing Boolean gates applied on each CD4$^+$ T cell subset for the indicated cytokines with or without T cell stimulation. Plots are representative of data from 8 HIV LNs acquired by flow cytometry (A) or CyTOF (B).

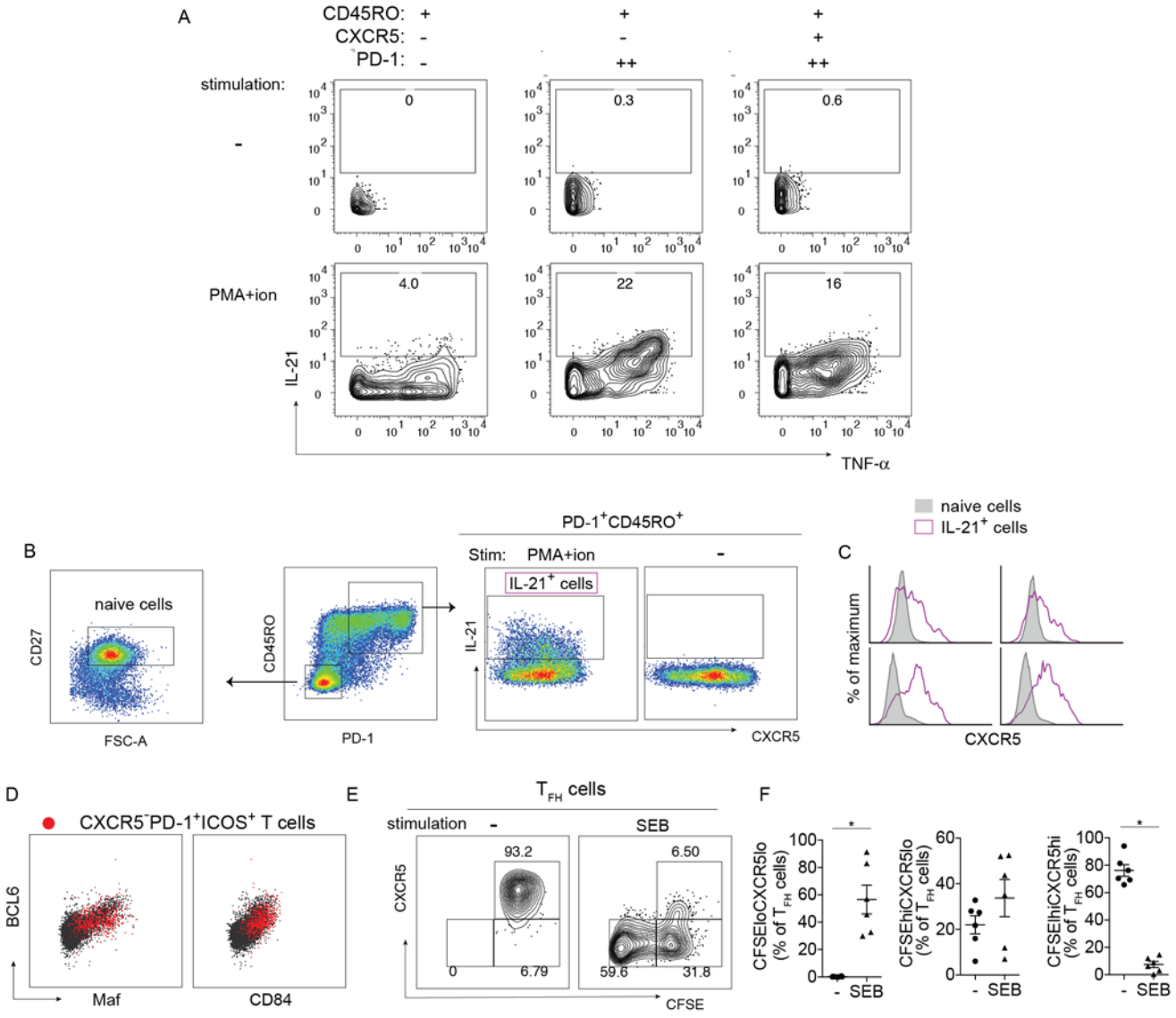


Figure S4: IL-21 production and T_{FH} cell-associated protein expression, related to Figure 2, 3, and 5.

(A) Plots showing IL-21 staining on indicated cell subset with or without T cell stimulation by PMA and ionomycin. Data is representative of 25 LN samples acquired by CyTOF. (B-C) LN cells from 4 HIV$^+$ donors were assayed by flow cytometry for IL-21 expression. Representative plots showing a naïve cell gate and IL-21 expression on PD-1$^+$ memory T cells (B). IL-21$^+$ cells and naïve cells identified in B was used to generate histograms that compares the fluorescent intensity of CXCR5 staining (C). Each histogram represents data from one donor. (D) Plots showing overlay of CXCR5$^-$PD-1$^+$ICOS$^+$ T cells over bulk CD4$^+$ T cells for BCL6, Maf, and CD84 expression. (E) Plots showing CXCR5 staining on CFSE-labeled and sort purified T_{FH} cells cultured in the presence or absence of SEB for 7 days. Cell division was measured by dilution of CFSE staining. (F) Scatter plots summarize the frequency of T_{FH} cells that acquired the indicated staining pattern in stimulated versus unstimulated culture conditions from 6 samples. Wilcoxon matched-pairs signed rank test was used.

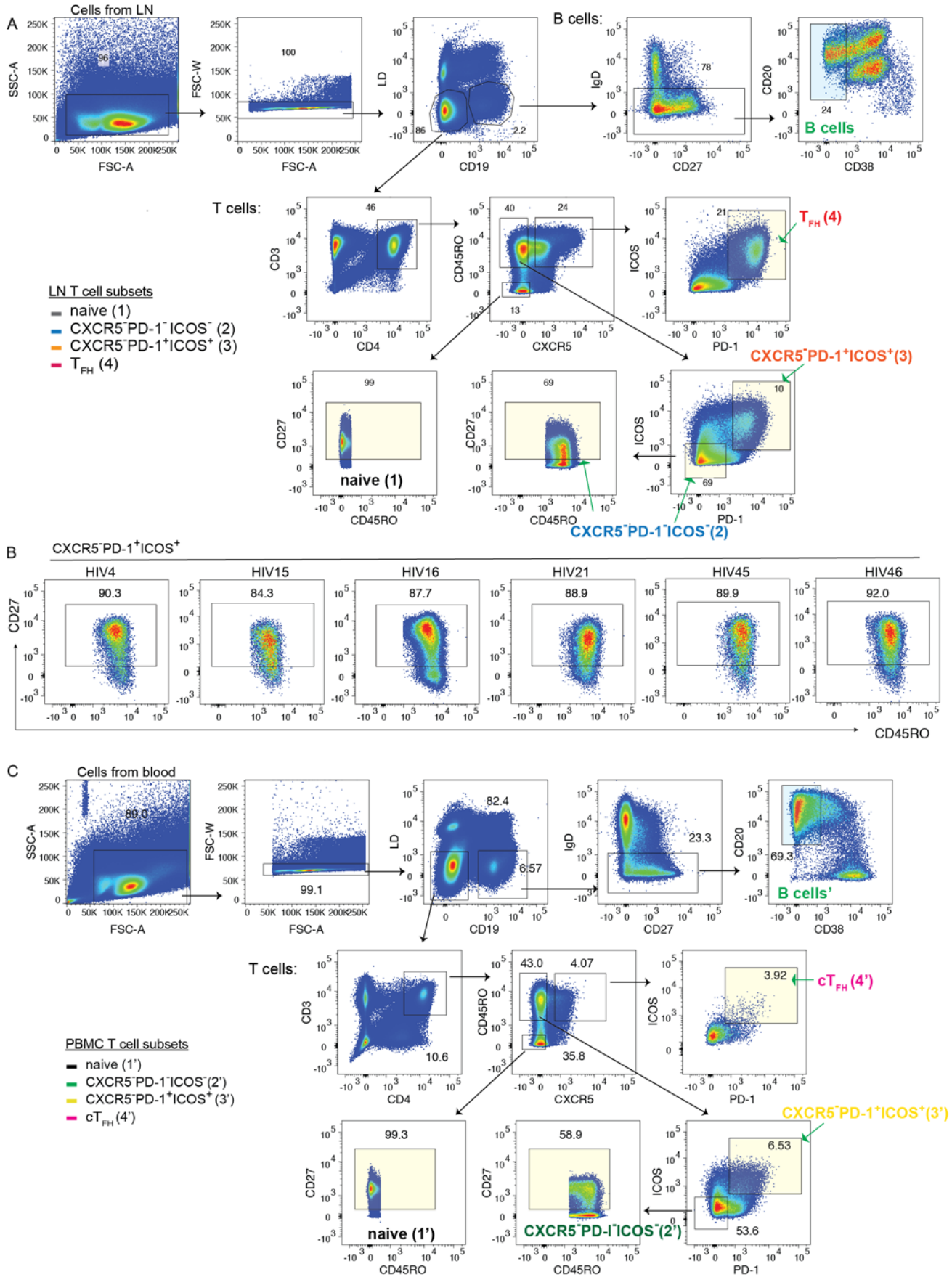

A
Cells from LN
SSC-A
FSC-A
FSC-W
LD
CD19
B cells:
IgD
CD27
CD20
CD38
B cells
T cells:
CD3
CD4
CD45RO
CXCR5
ICOS
PD-1
T_FH (4)
LN T cell subsets
naive (1)
CXCR5⁻PD-1⁻ICOS⁻ (2)
CXCR5⁻PD-1⁺ICOS⁺ (3)
T_FH (4)
CXCR5⁻PD-1⁺ICOS⁺(3)
naive (1)
CXCR5⁻PD-1⁻ICOS⁻(2)
B
CXCR5⁻PD-1⁺ICOS⁺
HIV4
HIV15
HIV16
HIV21
HIV45
HIV46
90.3
84.3
87.7
88.9
89.9
92.0
CD27
CD45RO
C
Cells from blood
B cells'
T cells:
cT_FH (4')
PBMC T cell subsets
naive (1')
CXCR5⁻PD-1⁻ICOS⁻(2')
CXCR5⁻PD-1⁺ICOS⁺ (3')
cT_FH (4')
CXCR5⁻PD-1⁺ICOS⁺(3')
naive (1')
CXCR5⁻PD-1⁻ICOS⁻(2')

Figure S5: Gates used for the analyses of T cell and B cell subsets, related to Figure 3 and 5.

(A) Representative gates used for identification of T cell subsets (FACS analyses, coculture, TCR-seq, RNA-seq, ATAC-seq) and B cells (coculture) from the LN samples. $CXCR5^{-}PD\text{-}1^{-}ICOS^{-}$ T cells were gated on total $ICOS^{-}PD\text{-}1^{-}$ subset except for RNA and ATAC-seq experiments, which was additionally gated on CD27. (B) CD27 antibody stained the majority of $CXCR5^{-}PD\text{-}1^{+}ICOS^{+}$ T cells. Plots show CD27 expression on $CXCR5^{-}PD\text{-}1^{+}ICOS^{+}$ T cells from the six samples used for transcriptomic and epigenetic analyses. (C) Representative gates used for sorting B cells and T cell subsets from the PBMC samples for coculture assays.

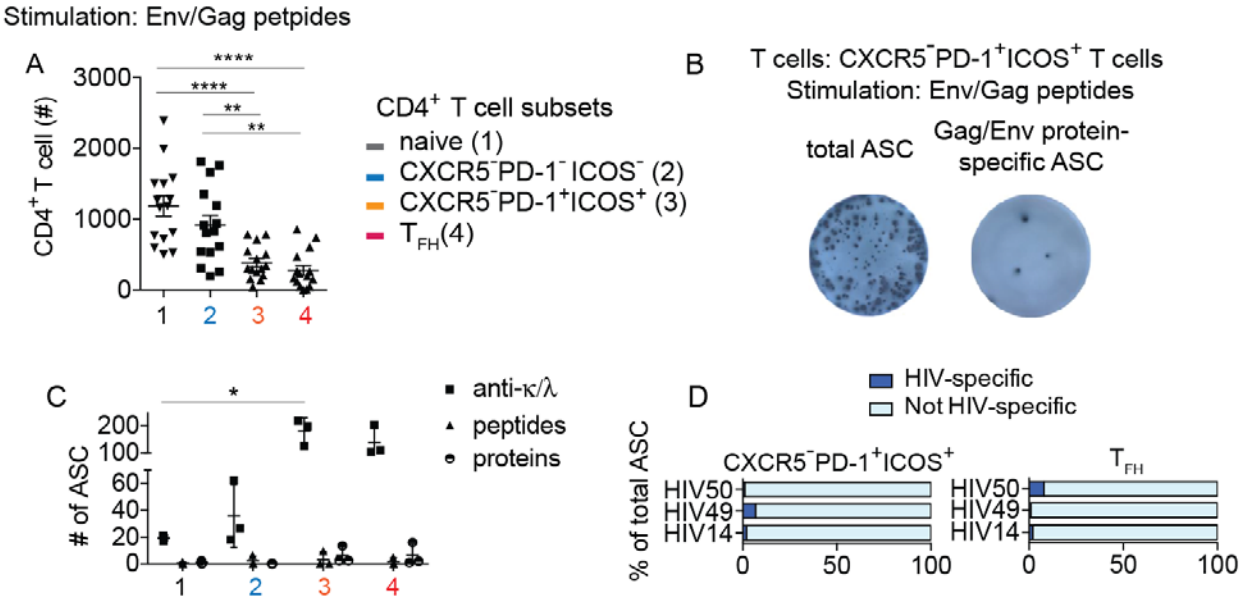
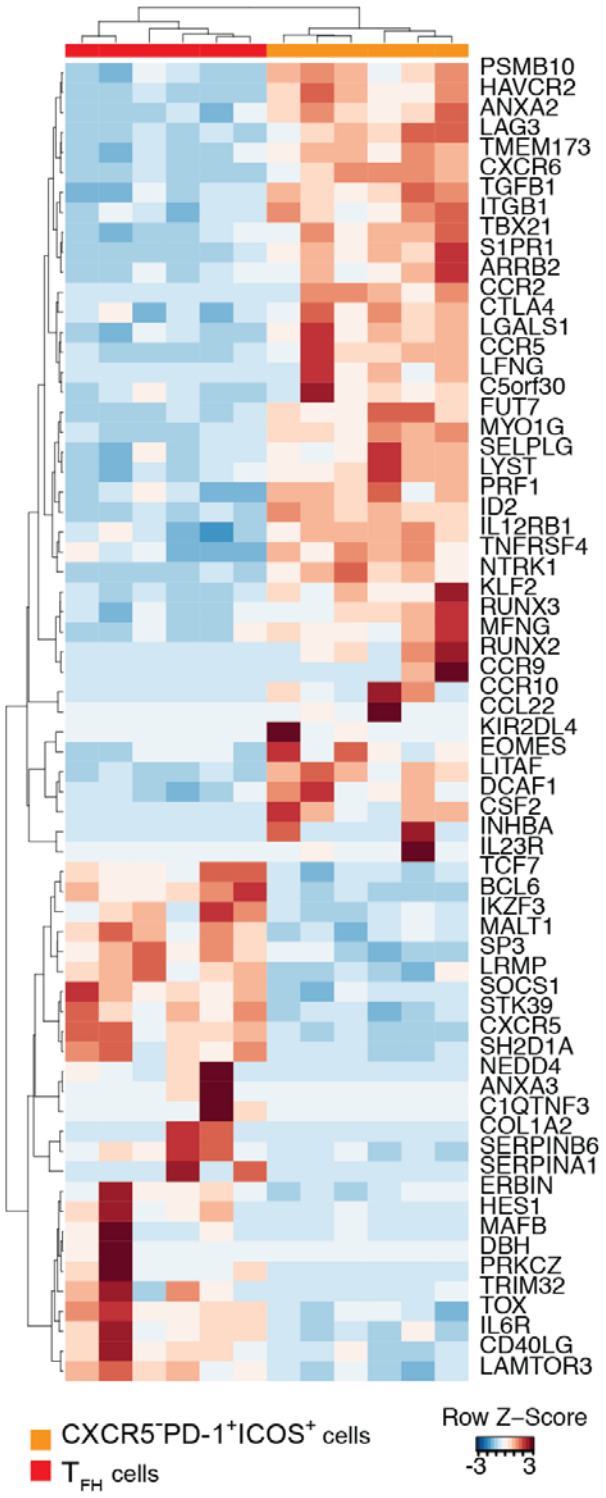


Figure S6: HIV-specific T cell stimulation and induction of B cell responses, related to Figure 4.

(A) Scatter plot quantifies the number of $CD4^{+}$ T cells in B: T cell cocultures after 7 days of Gag/Env peptide stimulation (n = 15 HIV LNs). (B) Representative detection of ASCs induced by HIV peptide stimulated $CXCR5^{-}PD\text{-}1^{+}ICOS^{+}$ T cells. Total ASCs were detected by plates coated with anti-kappa and lambda light chains (left). Plates coated with recombinant HIV proteins were used to detect HIV-specific ASCs (right). (C) Summary plot of total ASCs and antigen-specific ASCs that produced antibodies to HIV peptide or recombinant Gag/Env proteins. PBS coated wells were background subtracted from the data, with the minimum spot number set at 0. (D) Bar-graphs show Gag/Env protein-specific B cells as a percentage of total ASC in the presence of HIV peptide-stimulated $CXCR5^{-}PD\text{-}1^{+}ICOS^{+}$ T cells (left) or T_{FH} cells (right). Each bar indicates one individual. For A and C, Friedman test was performed and corrected using Dunn's multiple comparisons test. C compares differences in total ASCs between subsets.



Genes	log2FC CXCR5$^-$PD-1$^+$ICOS$^+$ vs T_{FH}	p-value	p-adj
CCR2	13.18374732	1.15E-37	1.52E-33
CXCR5	-4.345584277	2.08E-23	1.38E-19
IL23R	23.53263152	4.70E-15	2.96E-12
COL1A2	-23.43132685	6.20E-15	3.28E-12
KIR2DL4	23.34156924	7.81E-15	3.69E-12
SERPINA1	-23.04436844	1.72E-14	6.14E-12
CCR9	22.426547	8.35E-14	1.76E-11
C1QTNF3	-22.34309355	1.05E-13	2.04E-11
ANXA3	-22.30997745	1.14E-13	2.09E-11
S1PR1	2.075975945	6.00E-13	8.02E-11
INHBA	21.54304226	7.58E-13	9.84E-11
MYO1G	2.783981894	3.25E-12	4.13E-10
ID2	2.049002263	4.59E-12	5.78E-10
LITAF	1.868757937	8.99E-12	1.11E-09
BCL6	-2.483762995	1.21E-10	1.46E-08
HAVCR2	3.230451313	8.27E-09	9.12E-07
LAG3	2.799855224	3.36E-08	3.45E-06
PSMB10	0.545990889	4.42E-08	4.47E-06
CSF2	9.289235387	6.49E-08	6.46E-06
ANXA2	1.478376688	1.70E-07	1.63E-05
SH2D1A	-1.349929351	2.89E-07	2.73E-05
LFNG	6.59115525	3.20E-07	3.00E-05
STK39	-1.953466222	3.53E-07	3.24E-05
KLF2	2.057530823	4.42E-07	4.01E-05
NTRK1	6.601930245	8.14E-07	7.08E-05
ITGB1	1.166275232	2.14E-06	0.000177
CCR10	9.279340576	3.74E-06	0.0003
TMEM173	1.299624326	4.24E-06	0.000336
LGALS1	1.814085634	4.57E-06	0.00036
TBX21	1.863239275	5.26E-06	0.000412
SOCS1	-1.127377229	7.16E-06	0.000557
TCF7	-1.148690541	7.72E-06	0.00059
LAMTOR3	-0.805430759	9.29E-06	0.000699
CCL22	10.87534701	2.33E-05	0.001643
RUNX2	4.456308999	2.92E-05	0.002005
PRKCZ	-2.028634309	3.38E-05	0.002247
CCR5	3.126371461	3.69E-05	0.002434
DBH	-9.104564219	3.70E-05	0.002434
LYST	1.558523465	5.05E-05	0.00321
SP3	-0.828300028	5.26E-05	0.003301
MAFB	-9.917340713	5.26E-05	0.003301
LRMP	-1.020543886	8.13E-05	0.004826
TOX	-1.00330743	8.37E-05	0.004925
TNFRSF4	0.744990032	0.000142	0.007891
SELPLG	1.309743626	0.000144	0.007983
TGFB1	1.59554005	0.000171	0.009214
IKZF3	-1.275557009	0.000197	0.010185
CD40LG	-1.061631483	0.000199	0.010185
FUT7	2.992141282	0.000202	0.010266
PRF1	1.240348711	0.000215	0.010722
ARRB2	1.376065016	0.000307	0.014741
CXCR6	3.764771747	0.000382	0.017858
SERPINB6	-1.933736645	0.000407	0.018754
HES1	-2.614818031	0.000444	0.0202
DCAF1	1.276764524	0.000486	0.02174
IL12RB1	0.753241835	0.00056	0.02478
MALT1	-0.5837314	0.000611	0.026777
EOMES	1.199007413	0.000646	0.028108
C5orf30	1.425370663	0.000675	0.029183
RUNX3	1.676585741	0.000699	0.029929
IL6R	-1.281828674	0.000725	0.030745
ERBIN	-1.141940214	0.000796	0.033234
TRIM32	-1.711883014	0.0008	0.03331
CTLA4	0.634138496	0.000839	0.034469
NEDD4	-7.349341402	0.000897	0.036514
MFNG	0.554294779	0.001248	0.048133

Figure S7: Transcriptomic analyses of CXCR5$^-$PD-1$^+$ICOS$^+$ T cells and T_{FH} cells, related to Figure 6. Heatmap and table show the significantly differentially expressed genes between of CXCR5$^-$PD-1$^+$ICOS$^+$ T cells and T_{FH} cells. Gene list included genes in the 8 differential GO terms. Expression values ordered by adjusted p-value were normalized and scaled with DESeq2.