

Supplementary Methods:

Patients:

34 patients with HIV infection were enrolled in this study. 10 of these patients had developed PML in the past or suffered from acute PML at the time of blood withdrawal. More detailed patient information is given in Supplementary table 1. The study was approved by the local ethics committee and informed written consent was obtained from all participants. This study was performed according to the Declaration of Helsinki.

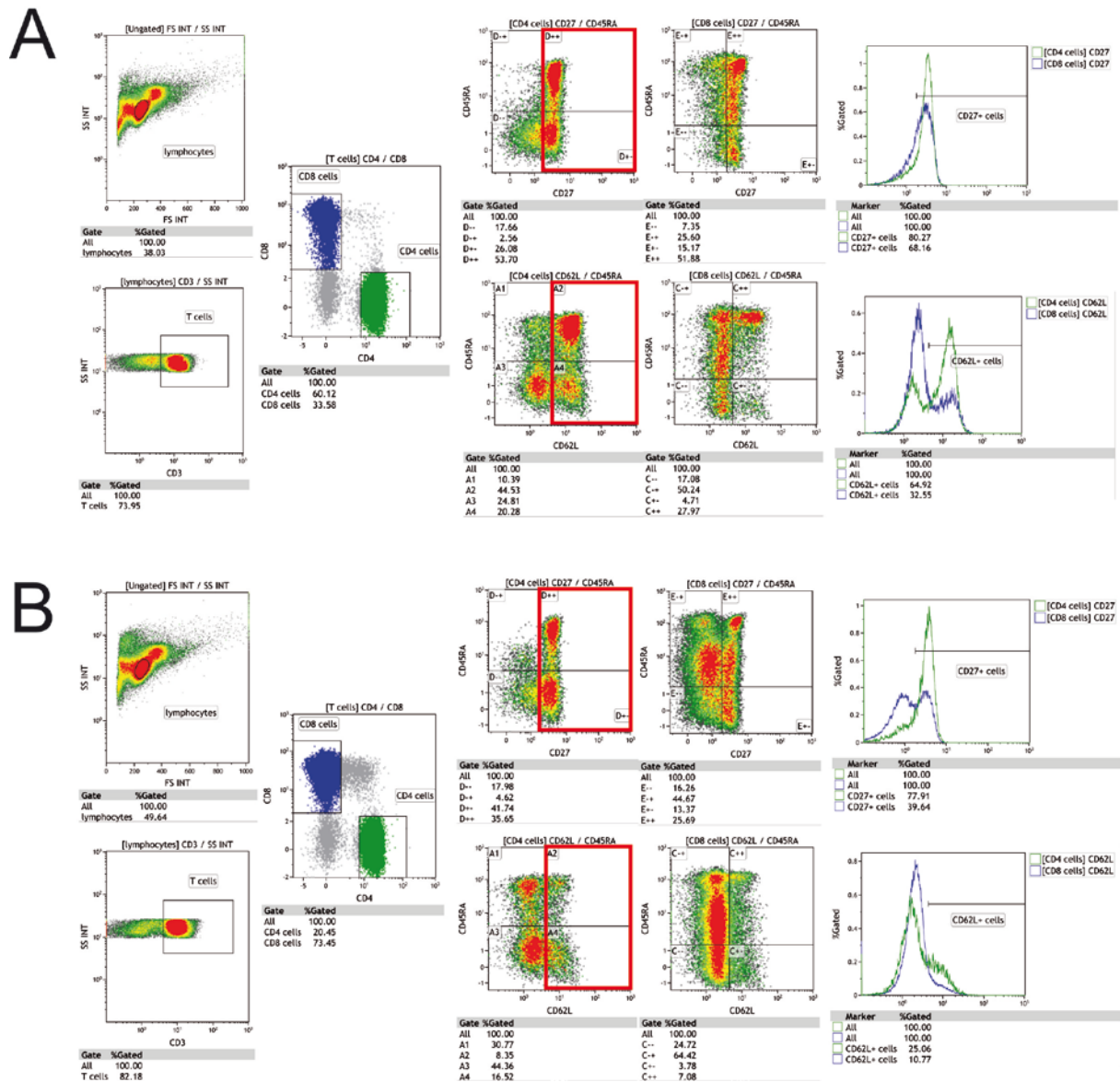
Statistics:

For statistics between two groups, an unpaired, two-tailed, non-parametric Mann-Whitney *U* test was performed. Correlation between two parameters was performed using linear regression algorithms in GraphPad Prism 5.0 (GraphPad Software, San Diego California, USA).

Biomaterials and flow cytometry:

Biomaterials were isolated and processed as published previously[21,22]. In short, peripheral blood mononuclear cells (PBMC) were isolated from EDTA blood by ficoll density gradient. Cells were then cryopreserved in liquid nitrogen using freezing medium (50% RPMI 40% FCS 10% DMSO) in concentrations of 1×10^7 PBMC/ml. All assessments were made on cells that had been frozen and thawed. After gentle thawing at 37° C, cells were immediately added to 10ml RPMI (Invitrogen) and centrifuged to remove DMSO. Cells were washed with staining buffer (PBS supplemented with 0.1% BSA and 200 mM EDTA) and subsequently stained with fluorescence-labeled monoclonal antibodies (see Supplementary Table 2) at room temperature for 15 min. After washing once with staining buffer, cells were immediately measured on a Gallios™ (Beckman Coulter, Krefeld, Germany) flow cytometer and analyzed using Kaluza V1.2 (Beckman Coulter) software. The stainings, measurements, and data analysis were performed blinded.

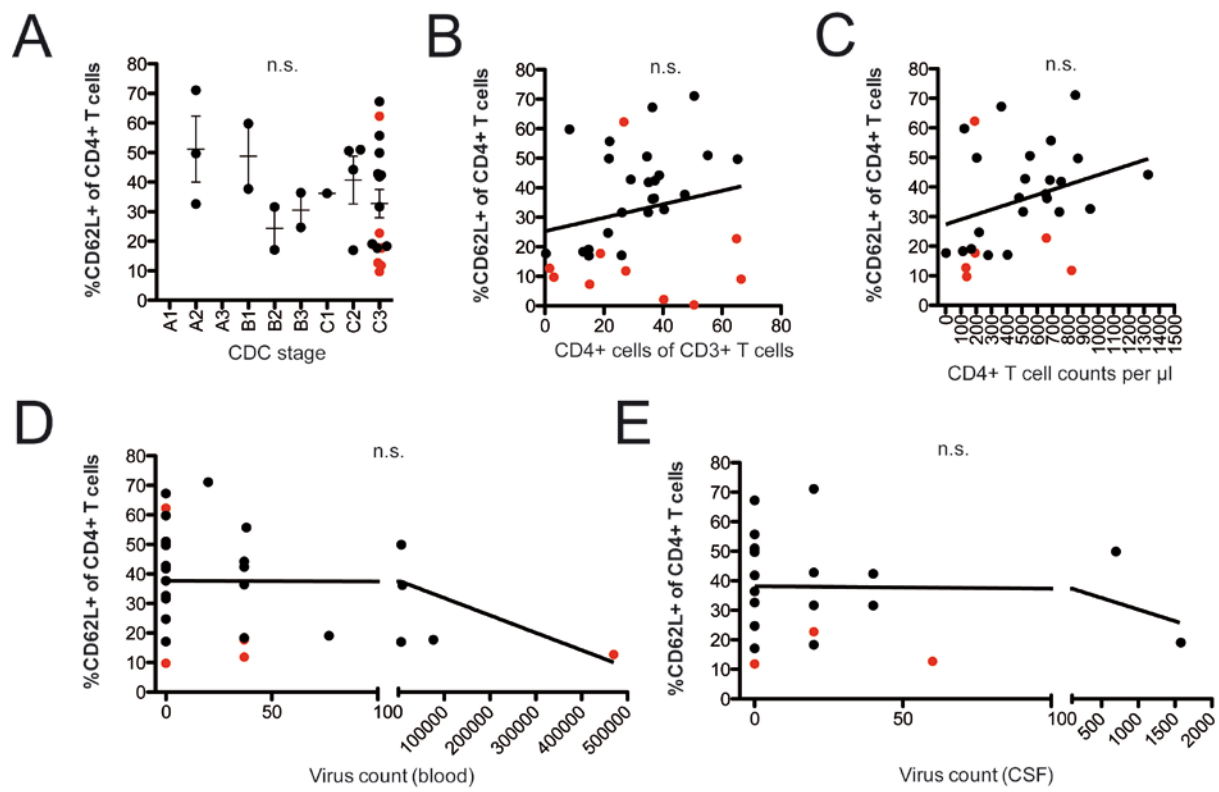
Supplementary Figure 1:



Supplementary Figure 1: Flow cytometry gating strategy and examples of selective loss of CD62L

Shown are one representative healthy control (A), one HIV patient (B), and the flow cytometry gating strategy: gating on live lymphocytes (FSC/SSC blot), then CD3⁺ T cells (CD3/SSC blot), then CD4⁺ or CD8⁺ T cells (CD4/CD8 blot) and their respective distribution of CD45RA/CD27 or CD45RA/CD62L. Red frames emphasize the fact that while healthy CD4⁺CD27⁺ T cells (=naïve and central-memory cells) usually co-express CD62L (A), CD4⁺ T cells from HIV patients show selective loss of CD62L with intact expression of CD27 (B).

Supplementary Figure 2:



Supplementary Figure 2: Correlations of CD62L expression with clinical parameters

Shown is the correlation of CD62L expression on CD4⁺ T cells with: (A) CDC stage, (B) percentage of CD4⁺ T cells of total CD3⁺ T cells, (C) CD4⁺ T cell count, (D) HI virus count in peripheral blood, and (E) HI virus count in the CSF. Red dots indicate individual PML patients.

Supplementary Table 1: patients and parameters

Patient #	Date of birth	Sample date	Year of HIV diagnosis	CDC stage	Polyneuropathy	Severity or neurocognitive dysfunction	Other opportunistic infections	Lymphocytes (per µl)	T-Lymphocytes (per µl)	CD4 cell count (per µl)	CD4 (% of CD3)	CD4/CD8 ratio	HIV count (CSF) (per µl)	HIV count (blood) (per µl)	Opportunistic CNS infection	Brain atrophy (severity)	HIV encephalopathy	Acute encephalitis	Severity of signal intensity abnormalities (T2)	PML
1	11/71	11/2010	2002	C3	-	3	-	1821	1131	661	36,3	1,6	<20	n.a.	+	+	+	-	2	+
2	02/68	11/2010	2005	C3	-	1	-	1296	916	193	14,9	0,3	<20	<37	+	-	-	-	0	+
3	02/69	8/2011	2007	C3	+	2	-	1361	1089	191	14	0,2	n.a.	0	+	-	-	-	0	+
4	04/62	9/2011	2001	C2	-	1	-	2266	2076	279	12,3	0,2	n.a.	6033	-	+	+	-	2	-
5	11/68	9/2011	n.a.	C3	+	3	+(CMV)	340	178	89	26,3	1,3	n.a.	0	-	+	+	-	4	-
6	02/58	9/2011	n.a.	C3	+	2	+(HSV)	476	405	3	0,7	0	n.a.	76500	-	+	+	-	2	-
7	12/65	9/2011	2003	C2	+	1	-	3586	2725	1327	37	0,9	n.a.	<37	-	+	+	-	2	-
8	11/74	10/2011	n.a.	C3	-	n.a.	+	1053	858	113	10,7	0,2	<20	<37	+	-	+	-	n.a.	-
9	09/60	10/2011	2001	B1	-	1	-	1909	1460	657	34,3	0,9	n.a.	0	-	-	-	-	n.a.	-
10	12/70	11/2011	2005	C3	-	3	-	2160	1950	138	6,4	0,1	n.a.	0	+	-	+	-	2	+
11	02/70	11/2011	2009	C3	+	n.a.	+	1671	1417	169	10,1	0,1	1580	77	+	-	+	-	n.a.	-
12	05/58	11/2011	1993	C2	+	2	-	n.a.	n.a.	n.a.	n.a.	n.a.	0	0	-	+	+	-	3	-
13	10/61	11/2011	1997	C3	+	1	+	2210	1525	508	23	0,5	<20	0	-	+	-	-	2	-
14	03/57	11/2011	1988	B3	+	1	+	1714	1047	482	28,1	0,9	0	<37	-	+	+	-	2	-
15	05/82	11/2011	2003	C3	-	1	+	1871	1521	522	27,9	0,5	<20	0	-	-	-	-	0	-
16	06/76	11/2011	2002	C3	-	1	-	2114	1163	366	17,3	0,5	0	0	+	-	-	-	0	-
17	06/70	12/2011	2002	C3	-	n.a.	-	3637	2411	826	22,7	0,6	0	<37	+	+	-	-	2	+
18	01/72	12/2011	1998	C3	+	2	+	722	582	204	28,3	0,6	697	6377	-	-	-	-	0	-
19	11/70	3/2012	2003	A2	-	2	-	1992	1430	851	42,7	1,6	<20	<20	-	+	+	-	2	-
20	05/73	5/2012	2002	B2	+	2	-	2377	2047	746	31,4	0,7	40	0	-	-	-	-	2	-
21	03/63	5/2012	1990	C3	+	3	-	2504	2046	691	27,6	0,5	0	38	-	+	-	-	2	-
22	05/63	7/2012	2002	A2	+	1	-	2576	1819	948	36,8	1,1	0	0	-	+	-	-	1	-
23	01/96	8/2012	n.a.	B1	-	1	+	2111	1883	122	5,8	0,1	n.a.	0	-	-	-	-	0	-
24	08/78	8/2012	n.a.	C3	-	n.a.	+	2367	1969	684	28,9	0,5	<40	<37	-	-	-	-	n.a.	-
25	01/45	10/2012	1993	C3	+	3	-	2765	2262	758	27,4	0,5	0	0	-	+	-	-	3	-
26	06/39	11/2012	2012	B2	+	n.a.	-	1563	1485	406	26	0,4	0	0	-	+	-	-	3	-
27	06/50	11/2012	2005	B3	-	3	-	1191	807	220	18,5	0,4	0	0	-	+	+	-	4	-
28	05/68	11/2012	1999	C2	+	2	-	1976	1522	553	28	0,6	0	0	-	+	-	-	1	-
29	01/72	1/2013	2012	C3	+	2	-	1953	1703	133	6,8	0,1	60	470000	-	-	-	-	n.a.	+
30	01/63	3/2013	2000	A2	-	3	-	1847	1450	868	46	1,4	0	0	-	+	-	-	n.a.	-
31	1956	7/2009	n.a.	C3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.	n.a.	+
32	1971	7/2009	n.a.	C3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.	n.a.	+
33	1976	5/2009	n.a.	C3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.	n.a.	+
34	1969	6/2009	n.a.	C3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	n.a.	n.a.	n.a.	+

Supplementary Table 2: monoclonal antibodies for flow cytometry

<i>antibody against</i>	<i>clone</i>	<i>manufacturer</i>
CD3	UCHT1	Beckman Coulter
CD4	Okt4	BioLegend
CD8	B9.11	Beckman Coulter
CD27	O323	BioLegend
CD45RA	HI100	BioLegend
CD62L	DREG-56	BioLegend