

Supplementary Material

Methods

Soluble biomarkers

The following inflammatory-related biomarkers were measured: high-sensitivity C-reactive protein (hsCRP) and β 2-microglobulin levels were determined by an immunoturbidimetric assay and homocysteine and lactate dehydrogenase (LDH) were determined by photometry, all of them using Cobas 701 (Roche Diagnostics, Mannheim, Germany). The soluble biomarker D-dimer was considered an inflammatory biomarker in the absence of venous thrombosis, and levels were determined with an automated latex-enhanced immunoassay (HemosIL D-Dimer HS 500, Instrumentation Laboratory, Bedford, Massachusetts). Ferritin was measured by particle-enhanced immunoturbidimetric assay in a Hitachi Cobas C702 modular analyzer (Roche Diagnostics, Rotkreuz, Switzerland). Soluble cytokines related to inflammation measured by ProcartaPlex™ Human Inflammation Panel 20plex included cytokines, chemokines and cell adhesion molecules: GM-CSF, E-selectin, P-selectin, ICAM-1, IFN- α , IFN- γ , IL-1 α , IL-1 β , IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IP-10, MCP-1, MIP-1 α , MIP-1 β and TNF α . Analysis was performed in a Luminex xMAP 200 instrument (Austin, TX. USA) using manufacturer's settings.

Immunophenotyping by mass cytometry

After thawing, 5 million PBMCs were incubated with 20 U/ μ L of DNase in RPMI media for 1 hour at 37°C and 5% CO₂. Samples were used only when cell viability was >85%. PBMCs were then washed and incubated with FcR-blocking reagent (Human TruStain FcX, Fluidigm) for 10 minutes at RT. Blocked cells were transferred to a 5 mL tube containing a dry pellet of MaxPar Assay antibodies. Additional antibodies (pre-conjugated and in-house labeled) were then added to the tube and incubated for 30 minutes at RT. After washing, stained PBMCs were fixed incubating with 1.6% formaldehyde for 10 min. Cells were then incubated overnight with 125 nM of Cell-ID Intercalator-Ir solution for

cell viability at 4°C. Finally, cells were washed and stored at -80°C until acquisition. All antibodies used for mass cytometry were obtained from the indicated companies in **Supplementary Table 1** as pre-conjugated or purified antibodies with in-house labeling that was performed using Maxpar X8 and MCP9 antibody labeling kits (Fluidigm). This panel allowed us to identify populations of innate (monocytic and polymorphonuclear myeloid-derived suppressor cells, classical, intermediate and non-classical monocytes, early and late NK cells, plasmacytoid and myeloid dendritic cells) and adaptive (recent thymic emigrants, naïve, effector memory, central memory and TemRA CD4 and CD8 T-cells, CD4+CD8+ and CD4-CD8- T-cells, Th1, Th2, Th17, Treg, $\gamma\delta$ T-cells, naïve and memory B-cells, plasmablasts) immunity, as well as additional function-related markers (CD57, CD38, HLA-DR, CD31, CD95, PD1, CD98, Glut1, CD36, OX40, $\alpha4\beta7$ integrin and ICOS), which were assessed in all the mentioned cellular subsets. Anti-CD31/PECAM-1, CD33, CD36 and CD98 antibodies were labeled using the Maxpar MCP9 protocol. Briefly, MCP9 polymer was loaded with Cd metal solution by incubating them at 37° C for an hour. Then, the complex was retrieved by filtering through a 3 kDa filter (Amicon Ultra-0.5 Centrifugal Filter Unit, MilliporeSigma). Simultaneously, purified antibodies were reduced with 4 mM Tris(2-carboxyethyl) phosphine hydrochloride (TCEP, MilliporeSigma) for 30 minutes at 37° C and the product was retrieved by filtering through a 50 kDa filter. Both products were mixed, transferred to a 100 kDa filter for 90 minutes at 37° C, retrieved and quantified. Finally, the conjugated antibody was diluted in HRP-protectorTM peroxidase stabilizer solution (Boca Scientific). Anti-Glut1 and $\alpha4\beta7$ integrin antibodies were labeled using the Maxpar X8 protocol. First, lanthanides (Tb and Ho) were loaded to the X8 polymer by incubating for 40 minutes at 37°C, then the metal-loaded polymer was retrieved by filtering in a 3 kDa filter. Purified antibodies were reduced with 4 mM TCEP for 30 minutes at 37° C and retrieved in a 50 kDa filter. Then, both products were mixed in the 50 kDa filter by incubating for 90 minutes at 37° C and quantified. Finally, the conjugated antibody was diluted in Antibody Stabilizer PBS (Boca Scientific).

ELISpot assay

After thawing PBMCs were incubated with 20 U/ μ L of DNase for 1 hour. Then, 250,000 PBMCs (100,000 PBMCs for positive control) were seeded in an anti-IFN γ (1-D1K) or anti-IL-2 (MT2A91/2C95) antibody pre-coated 96 well plates (previously conditioned with RPMI +10% of FBS during at least 30 minutes) and stimulated by CEFTA (2 μ g/mL; peptide pool for CD4; ref. 3617-1, Mabtech), CEF (2 μ g/mL; peptide pool for CD8 supplied by NIH AIDS Research and Reference Reagent Program, catalog #9808), or HIV-1 consensus B Gag (2 μ g/mL; HIV-specific pool also supplied by NIH, catalog #12425) peptide pools. As positive control, anti-CD3-2 antibody stimulation was used and, as negative control, unstimulated PBMCs. After a 48-hour stimulation at 37°C and 5% CO₂, cells were removed by washing 4 times with PBS. ALP-conjugated IFN γ antibody (7-B6-1-ALP conjugate) and biotinylated IL-2 antibody (MT8G10) were added to the plates and incubated for 2 hours. IL-2 plates were additionally washed 5 times and incubated with Streptavidin-ALP for 1 hour. Both IFN γ and IL-2 plates were washed 5 times and incubated with a substrate solution (BCIP/NBT-plus) for 30 minutes. Color development was stopped by removing substrate solution and washing with water. Plates were left overnight to dry and analyzed in an Astor ELISpot Reader (Mabtech). Positive response was considered when Spots Forming Units (SFUs) $>$ 50 after subtracting SFUs from the negative control.

Supplementary Tables

Supplementary Table 1. Antibodies used for immunophenotyping by mass cytometry.

Antibody (Clone)	Isotope Tag	Conjugation	Source
CD45 (HI30)	89Y	MaxPar Assay	Fluidigm
CD36 (5-271)	106Cd	In-house	Biolegend
CD31/PECAM-1 (WM59)	112Cd	In-house	Biolegend
CD33 (WM53)	114Cd	In-house	BD
CD98 (UM7F8)	116Cd	In-house	BD
CD196/CCR6 (G034E3)	141Pr	MaxPar Assay	Fluidigm
OX40/CD134 (ACT35)	142Nd	Pre-conjugated	Fluidigm
CD123 (6H6)	143Nd	MaxPar Assay	Fluidigm
CD19 (HIB19)	144Nd	MaxPar Assay	Fluidigm
CD4 (RPA-T4)	145Nd	MaxPar Assay	Fluidigm
CD8a (RPA-T8)	146Nd	MaxPar Assay	Fluidigm
CD11c (Bu15)	147Sm	MaxPar Assay	Fluidigm
CD16 (3G8)	148Nd	MaxPar Assay	Fluidigm
CD45RO (UCHL1)	149Sm	MaxPar Assay	Fluidigm
CD45RA (HI100)	150Nd	MaxPar Assay	Fluidigm
CD161 (HP-3G10)	151Eu	MaxPar Assay	Fluidigm
CD194/CCR4 (L291H4)	152Sm	MaxPar Assay	Fluidigm
CD25 (BC96)	153Eu	MaxPar Assay	Fluidigm
CD27 (O323)	154Sm	MaxPar Assay	Fluidigm
CD57 (HCD57)	155Gd	MaxPar Assay	Fluidigm
CD183/CXCR3 /G025H7)	156Gd	MaxPar Assay	Fluidigm
CD185/CXCR5 (J252D4)	158Gd	MaxPar Assay	Fluidigm
Glut1 (202915)	159Tb	In-house	R&D
CD28 (CD28.2)	160Gd	MaxPar Assay	Fluidigm
CD38 (HB-7)	161Dy	MaxPar Assay	Fluidigm
Fas/CD95 (DX2)	162Dy	Pre-conjugated	Fluidigm
CD56/NCAM (NCAM16.2)	163Dy	MaxPar Assay	Fluidigm
TCRgd (B1)	164Dy	MaxPar Assay	Fluidigm
α 4 β 7 integrin/LPAM-1 (Hu117)	165Ho	In-house	R&D
CD294 (BM16)	166Er	MaxPar Assay	Fluidigm
CD197/CCR7 (G043H7)	167Er	MaxPar Assay	Fluidigm
CD14 (63D3)	168Er	MaxPar Assay	Fluidigm
CD278/ICOS (C398.4A)	169Tm	Pre-conjugated	Fluidigm
CD3 (UCHT1)	170Er	MaxPar Assay	Fluidigm
CD20 (2H7)	171Yb	MaxPar Assay	Fluidigm
CD66b (G10F5)	172Yb	MaxPar Assay	Fluidigm
HLA-DR (LN3)	173Yb	MaxPar Assay	Fluidigm
IgD (IA6-2)	174Yb	MaxPar Assay	Fluidigm
CD279/PD-1 (EH12.2H7)	175Lu	Pre-conjugated	Fluidigm
CD127 (A019D5)	176Yb	MaxPar Assay	Fluidigm
Cell-ID Intercalator-103Rh	103Rh	MaxPar Assay	Fluidigm

Supplementary Table 2. Inflammatory markers according to CD4/CD8 ratio classification.

Cytokine	Cross-sectional comparisons						Longitudinal comparisons	
	Baseline			Follow-up			<i>p</i> -value (Wilcoxon test)	
	R<0.8	R>1.2	<i>p</i> -value	R<0.8	R>1.2	<i>p</i> -value	R<0.8	R>1.2
β2-microglobulin (mg/L)	3.0[2.7-3.9]	2.3[2.1-3.1]	0.009	2.4[2-2.8]	2[1.8-2.3]	0.015	<0.0001	0.003
D-dimers (ng/mL)	460[280-590]	280[177-385]	0.036	235[183-330]	210[150-265]	0.162	0.014	0.009
Ferritin (ng/L)	192[129-244]	147[92-249]	0.421	158[98-231]	130[67-172]	0.121	0.181	0.017
Homocysteine (mg/L)	2.8[2.1-3.5]	2.8[2.3-3.9]	0.872	3.1[2.4-4.3]	3.3[2.2-4.4]	0.977	0.355	0.074
hsCRP (mg/L)	1.8[0.9-3.6]	1.1[0.7-2.9]	0.484	1.4[0.7-2.6]	0.8[0.5-1.9]	0.097	0.088	0.428
Lactate dehydrogenase (U/L)	213[165-277]	212[168-295]	0.842	221[174-339]	219[177-258]	0.527	0.144	0.923
GM-CSF (pg/mL)	42.1[9.1-62.2]	35.3[0-70]	0.623	23.4[0-55.7]	26.6[6.6-64.8]	0.673	0.650	0.314
IFNα (pg/mL)	4.4[1-6.3]	3.3[0.7-8.1]	0.737	2.5[0.3-5.2]	2.4[1.2-4.1]	0.588	0.112	0.073
IFNγ (pg/mL)	23.7[17.3-28.5]	22.4[11.4-31.7]	0.538	18[10.4-23.9]	18.3[12-27.8]	0.734	0.322	0.302
IL-1α (pg/mL)	2.5[1.4-4.3]	2.7[1.1-4.9]	0.865	1.8[1-4]	1.7[0.8-3.3]	0.681	0.414	0.191
IL-1β (pg/mL)	18.4[5.6-29.7]	15.6[6.6-31.4]	0.755	12.4[4.7-26.7]	11.3[6.5-24]	0.515	0.201	0.219
IL-6 (pg/mL)	37.5[14.8-54.5]	25.8[14.2-56.9]	0.949	20.8[13.5-50.2]	19.4[9.3-41.5]	0.377	0.131	0.037
IL-8 (pg/mL)	3.3[2.9-4.8]	2.9[1.4-5.9]	0.597	2.9[2.2-4.5]	2.7[1.6-5.6]	0.932	0.852	0.493
IL-12 (pg/mL)	45.1[19.1-60.7]	35.7[19-57]	0.806	27.3[15-41]	31.8[19.8-48.4]	0.446	0.316	0.139
IL-17 (pg/mL)	12.6[7.1-19.1]	14[7-22]	0.835	10.5[6.2-17.1]	13.8[7.5-18.3]	0.515	0.715	0.399
IP-10 (pg/mL)	32.1[20.6-55.3]	18.4[14.7-38.3]	0.036	19.6[13.3-29.9]	18.4[10.7-22.1]	0.152	<0.0001	0.001
MCP1 (pg/mL)	47.3[31.1-67.7]	40.9[22.6-75.8]	0.460	49.9[27.5-78.3]	41.9[26.7-82.9]	0.883	0.670	0.111
TNFα (pg/mL)	68.9[38.4-96.4]	65.8[34.7-126.8]	0.865	57.5[31.5-122.3]	64.4[32.8-89]	0.883	0.287	0.387
IL-4 (pg/mL)	17.6[10.5-21.8]	16.8[9.9-24.2]	0.977	15.9[11.4-22.9]	14.2[8.4-20.2]	0.388	0.648	0.333
IL-10 (pg/mL)	9.1[3.2-12]	7.5[2.6-13.8]	0.846	6.5[2.9-10.6]	5.3[2.3-9.3]	0.482	0.181	0.072
IL-13 (pg/mL)	9.8[5.8-14.4]	9.1[5.2-16.9]	0.925	7.1[4.8-14]	8.9[2.3-12.5]	0.588	0.144	0.038
MIP1α (pg/mL)	11[7.8-15.6]	12.1[6.7-19.1]	0.545	11.3[8.6-16.7]	11.6[7-20.5]	0.854	0.605	0.909
MIP1β (pg/mL)	23.9[17.8-31.1]	22.7[17-34.6]	0.794	31.1[20.7-39]	27.2[17.2-40.3]	0.546	0.123	0.248
ICAM-1 (ng/mL)	43.2[31-73.8]	57.6[36.8-77.8]	0.570	63.3[45.5-107.9]	61.8[45-90.5]	0.607	0.052	0.316
E-selectin (ng/mL)	11.5[9-15.3]	9.6[7.8-12.8]	0.061	12.9[9.8-19.5]	12.9[8.8-16.5]	0.279	0.015	0.026
P-selectin (ng/mL)	65.1[41.8-128.5]	70.1[43.2-117.2]	0.762	92[40.1-140.3]	76.2[51-117.3]	0.769	0.301	0.172

Note: Non-parametric U Mann-Whitney test was applied when R<0.8 vs. R>1.2 were compared, while non-parametric Wilcoxon test was applied when baseline vs. follow-up were compared. Sample size: R<0.8, n=24 and R>1.2, n=28.

Supplementary Table 3. Inflammatory markers according to nadir CD4 classification.

Cytokine	Cross-sectional comparisons						Longitudinal comparisons	
	Baseline			Follow-up			<i>p</i> -value (Wilcoxon test)	
	N ≤350	N>350	<i>p</i> -value	N ≤350	N>350	<i>p</i> -value	N ≤350	N>350
β2-microglobulin (mg/L)	3[2.1-3.6]	2.5[2.8-3.1]	0.211	2.1[1.9-2.6]	2.1[1.9-2.4]	0.474	<0.0001	0.001
D-dimers (ng/mL)	360[240-580]	300[213-518]	0.563	195[150-330]	235[185-268]	0.397	0.008	0.016
Ferritin (ng/mL)	192[123-250]	147[92-222]	0.438	175[91-248]	125[78-159]	0.065	0.484	0.002
Homocysteine (mg/L)	2.7[2.4-3.1]	3[2.2-4.1]	0.449	3.1[2.5-4.3]	3.5[2.2-4.4]	0.776	0.178	0.190
hsCRP (mg/L)	1.3[0.8-3.5]	2[0.8-4.1]	0.400	1[0.6-1.6]	0.9[0.5-2.7]	0.949	0.066	0.449
Lactate dehydrogenase (U/L)	203[158-263]	225[171-316]	0.158	214[172-318]	225[180-283]	0.862	0.114	0.895
GM-CSF (pg/mL)	26.5[4.7-59.3]	38.9[0-70.8]	0.595	24[3.4-48.9]	29.3[0-69.8]	0.638	0.569	0.349
IFNα (pg/mL)	3.9[0.7-6.3]	3.6[0.9-8.1]	0.690	2.5[1.4-5.1]	2.4[0-4.7]	0.649	0.140	0.068
IFNγ (pg/mL)	20.4[11.3-32.3]	23.7[15.5-31.3]	0.525	17.1[10.9-19.8]	22.2[12-28.5]	0.385	0.615	0.136
IL-1α (pg/mL)	2.6[1-4]	2.6[1.4-5.4]	0.416	1.5[1-3.8]	2.3[0.9-4]	0.733	0.520	0.131
IL-1β (pg/mL)	16.5[5.3-27.1]	18.2[7.1-36.2]	0.432	12.4[5.3-27.6]	11.7[5.8-24.2]	0.862	0.563	0.056
IL-6 (pg/mL)	37.6[15.6-51.8]	35.8[14.1-59.4]	0.863	14.4[12.3-39]	25[9.3-44.3]	0.741	0.057	0.065
IL-8 (pg/mL)	2.5[1.5-3.8]	3.2[2.1-6.1]	0.144	2.6[1.6-3.5]	3.5[2.3-6.7]	0.032	0.526	0.317
IL-12 (pg/mL)	31[18.8-59]	43[19.4-59.9]	0.719	28.4[20.5-46.2]	29.9[16-46.8]	0.876	0.503	0.053
IL-17 (pg/mL)	11.1[6.6-18.7]	16[7.7-21.3]	0.281	10.5[7.5-16.8]	13.8[7.1-19.2]	0.614	0.927	0.236
IP-10 (pg/mL)	30.1[10.7-43.7]	23.8[14.8-39.8]	0.596	17.9[11.5-23.1]	20[11-23.2]	0.985	<0.0001	0.001
MCP1 (pg/mL)	34.2[27.6-71.7]	46.7[24.8-74.7]	0.791	43.2[25.9-77.3]	52.9[29.3-86]	0.474	0.465	0.172
TNFα (pg/mL)	59.2[33.6-96.4]	67.9[39-121.1]	0.427	57.5[33-116.2]	69.9[31.4-110]	0.883	0.484	0.554
IL-4 (pg/mL)	16.8[10.3-21.1]	16.5[10.7-29]	0.320	14.8[10.8-20.6]	15.7[8.9-23.1]	0.869	0.693	0.279
IL-10 (pg/mL)	6.4[2.8-11.5]	9.1[3.5-13.9]	0.392	6.1[2.7-9.6]	6.3[2.4-9.7]	0.726	0.189	0.052
IL-13 (pg/mL)	7.7[5.6-15]	9.6[6-16.7]	0.334	7.3[5-12]	8.9[4.9-14.4]	0.468	0.091	0.019
MIP1α (pg/mL)	10.8[7.8-16.7]	12.1[7.1-18.1]	0.677	11.2[7.3-16.7]	12.1[7.6-21.3]	0.435	0.715	0.838
MIP1β (pg/mL)	21.1[14.9-28.5]	26[18.9-34.9]	0.091	25.4[19.4-33.3]	32.5[17.7-41.7]	0.485	0.062	0.409
ICAM-1 (ng/mL)	41.3[30.9-73.8]	57.6[38.2-80.3]	0.307	62.6[46.8-102.3]	62.3[43.3-101.9]	0.826	0.042	0.255
E-selectin (ng/mL)	11.2[8.2-13.9]	10[8-13.4]	0.520	13.3[10.1-17.5]	11.9[8.9-17.3]	0.452	0.008	0.026
P-selectin (ng/mL)	61.8[35.6-89]	71.9[51.3-133.7]	0.185	69[40.1-121.1]	94.1[51-157.8]	0.219	0.903	0.048

Note: Non-parametric U Mann-Whitney test was applied when N ≤350 vs. N>350 were compared, while non-parametric Wilcoxon test was applied when baseline vs. follow-up were compared. Sample size: N ≤ 350, n=24 and N>350, n=28.

Supplementary Table 4. Immune cell populations and functional markers according to CD4/CD8 ratio classification

Subsets* (%)	R<0.8 (n=13)	R>1.2 (n=12)	p-value
Total CD4 ⁺ T-cells	35.5[27.8-45.9]	57.8[49.7-63.8]	0.001
Naïve CD4 ⁺ T-cells	48.7[41.5-59.9]	56.6[51.3-64.3]	0.110
Central Memory CD4 ⁺ T-cells	35.9[28.6-42.9]	29.8[24.8-43.0]	0.110
Total CD8 ⁺ T-cells	48.2[44.2-60.7]	31.1[27.1-37.7]	<0.0001
Naïve CD8 ⁺ T-cells	41.0[28.8-50.5]	55.3[46.0-76.9]	0.014
Effector Memory CD8 ⁺ T-cells	6.5[3.2-10.8]	3.0[1.5-9.4]	0.055
Double positive CD4 ⁺ CD8 ⁺ T-cells	2.2[1.9-3.1]	1.5[0.7-2.1]	0.007
Th1	5.0[3.8-5.4]	3.8[3.2-4.6]	0.079
Early NK	34.8[22.7-43.3]	22.8[16.3-37.3]	0.152
Late NK	64.9[54.0-76.6]	76.3[62.0-83.5]	0.110
DC	15.1[10.2-18.4]	10.3[8.0-13.3]	0.026
mDC	36.8[27.8-46.7]	48.0[40.5-50.0]	0.030
MDSC	41.2[25.9-53.0]	32.3[16.7-42.8]	0.042
MAIT/iNKT cells	1.2[1.0-2.2]	0.6[0.5-2.2]	0.074
α4β7 ⁺ Total CD4 ⁺ T-cells	23.1[21.6-25.9]	19.8[16.1-24.9]	0.110
α4β7 ⁺ Naïve CD4 ⁺ T-cells	32.4[29.7-46.1]	24.1[18.4-35.0]	0.011
α4β7 ⁺ Central Memory CD4 ⁺ T-cells	13.0[11.7-18.6]	11.4[10.4-16.8]	0.186
α4β7 ⁺ Treg	4.9[3.8-6.0]	2.8[2.1-3.4]	0.008
Glut1 ⁺ Th2	7.6[4.5-11.1]	4.1[2.8-3.4]	0.029
CD57 ⁺ Effector Memory CD4 ⁺ T-cells	24.5[13.1-35.9]	12.7[4.2-33.7]	0.137
CD57 ⁺ TemRA CD4 ⁺ T-cells	76.4[67.5-90.9]	58.4[27.9-85.5]	0.152
CD57 ⁺ Treg	8.7[5.7-15.9]	4.9[3.5-6.3]	0.015
CD57 ⁺ Th2	0.8[0.5-2.0]	0.4[0.2-1.5]	0.027
CD57 ⁺ Th17	1.4[0.4-3.3]	0.7[0.2-1.5]	0.152
CD98 ⁺ Naïve CD4 ⁺ T-cells	0.4[0.2-0.7]	0.6[0.2-1.8]	0.168
CD98 ⁺ Central Memory CD4 ⁺ T-cells	5.4[0.1-43.5]	45.2[26.9-68.7]	0.010
CD98 ⁺ TemRA CD4 ⁺ T-cells	1.7[0.3-3.7]	3.2[1.9-8.6]	0.137
PD1 ⁺ Total CD4 ⁺ T-cells	13.6[11.1-17.6]	11.1[9.6-12.8]	0.152
CD95 ⁺ CD8 ⁺ T-cells	41.2[35.4-52.4]	30.2[25.8-42.5]	0.040
CD95 ⁺ Naïve CD8 ⁺ T-cells	25.1[20.3-43.5]	18.9[11.1-26.9]	0.060
CD57 ⁺ Total CD8 ⁺ T-cells	43.5[36.1-54.9]	36.5[26.5-42.7]	0.152
CD57 ⁺ Naïve CD8 ⁺ T-cells	14.1[11.1-18.5]	11.6[6.9-13.0]	0.018
CD57 ⁺ Central Memory CD8 ⁺ T-cells	13.8[7.7-16.9]	18.0[9.4-23.3]	0.123
PD1 ⁺ Effector Memory CD8 ⁺ T-cells	35.6[23.6-48.8]	48.9[36.5-54.6]	0.077
PD1 ⁺ Central Memory CD8 ⁺ T-cells	49.5[36.2-68.0]	61.8[58.7-71.2]	0.046
CD38 ⁺ HLA-DR ⁺ Central Memory CD8 ⁺ T-cells	2.3[1.7-2.9]	4.9[3.9-8.32]	0.022
Glut1 ⁺ Central Memory CD8 ⁺ T-cells	74.2[68.6-81.5]	82.1[73.2-85.3]	0.152
Glut1 ⁺ Effector Memory CD8 ⁺ T-cells	70.7[62.2-75.8]	73.8[69.5-79.9]	0.186
Glut1 ⁺ TemRA CD8 ⁺ T-cells	67.2[60.1-74.5]	74.8[67.1-83.2]	0.087

*Only subsets showing significant and trend to significant differences ($p<0.2$) are presented. Quantitative variables expressed as median [IQR]; statistical comparisons between groups were performed using a non-parametric Mann-Whitney test.

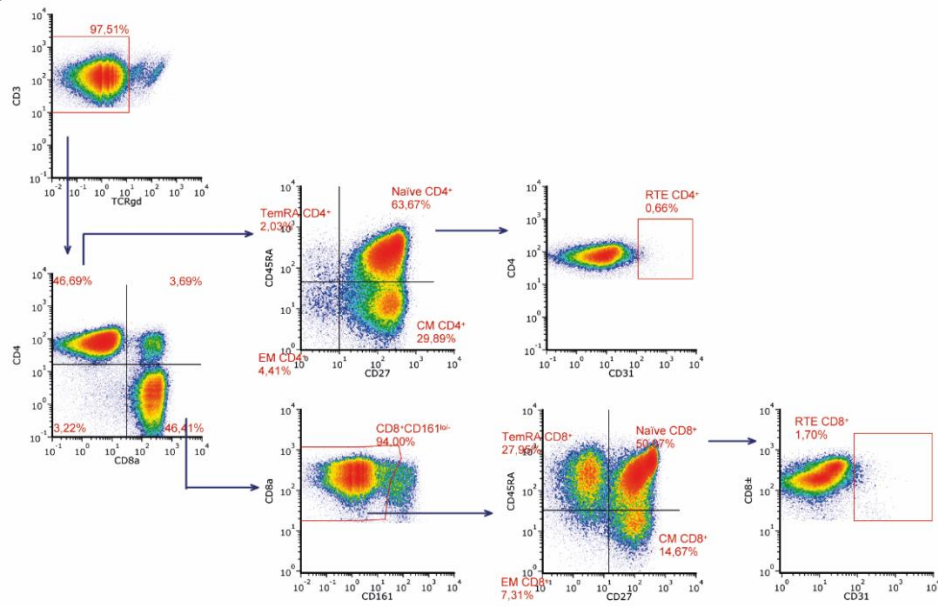
Supplementary Table 5. Immune cell populations and functional markers according to nadir CD4 T-cell count classification

Subsets* (%)	N ≤350 (n=13)	N >350 (n=12)	p-value
Total CD4 ⁺ T-cells	42.3[27.8-49.5]	57.4[36.0-63.8]	0.040
Th1	5.0[3.5-5.5]	3.9[3.3-4.1]	0.137
Total CD8 ⁺ T-cells	45.1[41.5-58.2]	32.4[28.8-46.1]	0.030
DCs	14.9[9.6-18.4]	11.8[8.0-11.9]	0.168
mDCs	56.3[53.1-69.8]	71.2[56.0-82.8]	0.110
pDC	27.7[23.6-38.9]	20.5[14.8-27.7]	0.035
MAIT/iNKT cells	1.5[1.0-2.8]	0.7[0.5-1.5]	0.046
α4β7 ⁺ Central Memory CD4 ⁺ T-cells	15.2[11.5-18.6]	11.7[10.5-14.5]	0.137
α4β7 ⁺ TemRA CD4 ⁺ T-cells	0.7[0.6-1.5]	0.4[0.2-0.5]	<0.0001
Glut1 ⁺ CD4 ⁺ T-cells	8.8[6.3-11.0]	5.9[3.0-9.6]	0.087
Glut1 ⁺ Effector Memory CD4 ⁺ T-cells	13.0[10.5-18.3]	8.7[4.7-21.1]	0.093
Glut1 ⁺ TemRA CD4 ⁺ T-cells	14.3[9.8-22.6]	7.8[6.0-14.8]	0.047
Glut1 ⁺ Th2	6.4[3.9-11.1]	4.4[2.6-8.0]	0.168
CD57 ⁺ Central Memory CD4 ⁺ T-cells	0.2[0.2-0.7]	0.1[0.1-0.2]	0.013
CD57 ⁺ Th2	0.8[0.5-2.6]	0.4[0.1-1.3]	0.018
CD57 ⁺ Treg	8.7[5.3-15.9]	5.1[3.0-6.3]	0.014
CD95 ⁺ Central Memory CD4 ⁺ T-cells	85.9[70.9-91.5]	89.8[87.5-94.2]	0.110
CD95 ⁺ TemRA CD4 ⁺ T-cells	74.1[67.0-82.9]	83.0[78.0-85.2]	0.098
CD95 ⁺ Th2	90.8[87.7-94.8]	96.3[92.9-98.1]	0.016
OX40 ⁺ Naïve CD4 ⁺ T-cells	0.7[0.2-3.0]	0.3[0.2-0.5]	0.152
HLA-DR ⁺ Central Memory CD4 ⁺ T-cells	3.4[3.0-7.4]	6.3[4.0-10.0]	0.093
CD98 ⁺ Central Memory CD4 ⁺ T-cells	28.7[1.3-43.5]	45.2[6.6-67.1]	0.030
Glut1 ⁺ CD8 ⁺ Central Memory CD8 ⁺ T-cells	74.4[67.1-81.8]	81.9[73.7-85.2]	0.137
Glut1 ⁺ Effector Memory CD8 ⁺ T-cells	69.3[62.4-75.8]	74.3[70.7-79.9]	0.098
CD38 ⁺ HLA-DR ⁺ Central Memory CD8 ⁺ T-cells	2.3[1.5-3.9]	4.9[2.7-8.3]	0.035
CD95 ⁺ Central Memory CD8 ⁺ T-cells	67.4[63.1-80.4]	81.0[76.8-87.6]	0.040
CD95 ⁺ Effector Memory CD8 ⁺ T-cells	44.6[34.7-52.6]	57.6[51.9-67.5]	0.014

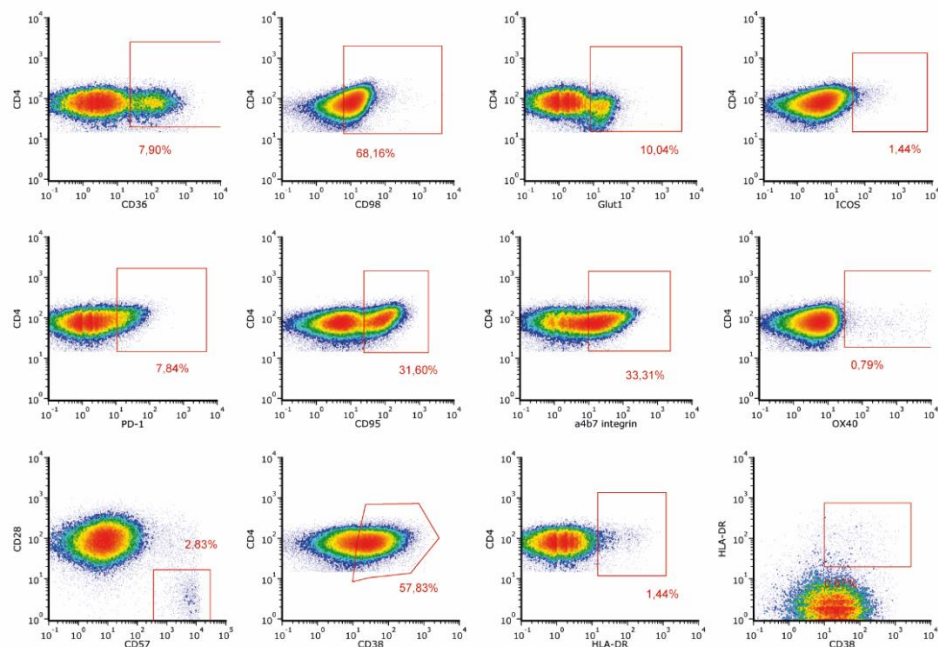
*Only subsets showing significant and trend to significant differences ($p < 0.2$) are presented. Quantitative variables expressed as median [IQR]; statistical comparisons between groups were performed using a non-parametric Mann-Whitney test.

Supplementary Figures

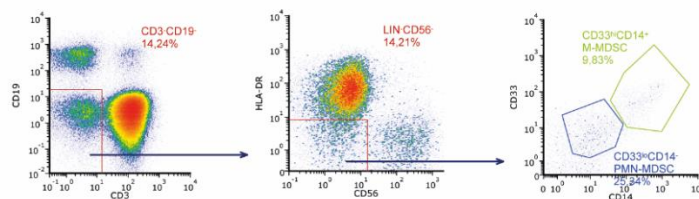
A)



B)

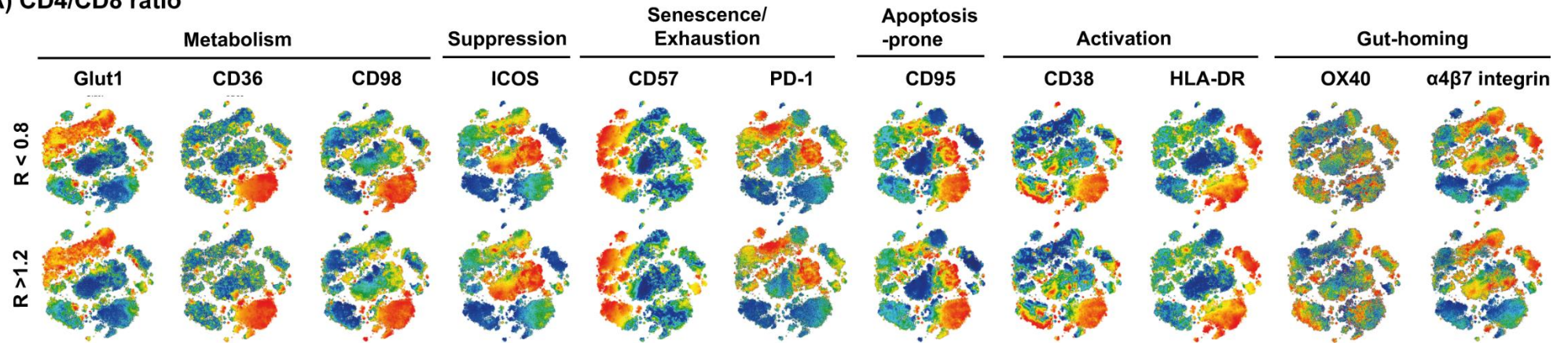


C)

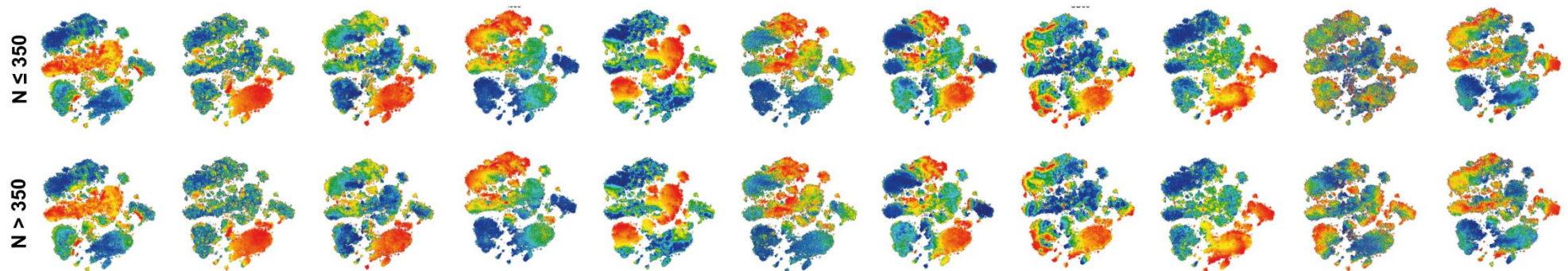


Supplementary Figure 1. Gating strategy for additional markers. A) Modified gating strategy for CD4 and CD8 T-cell subsets and definition of RTE as CD31⁺ naïve CD4 or CD8 T-cells; B) Functional markers were gated in each maturational subset of CD4 and CD8 T-cell (including total, naïve, EM, CM and TemRA); C) M- and PMN-MDSC were defined as LIN⁻CD33^{hi}CD14⁺ and LIN⁻CD33^{lo/int}CD14⁻. Abbreviations: EM, Effector Memory; CM, Central Memory; TemRA, Terminally Differentiated CD45RA⁺; RTE, Recent Thymic Emigrants; LIN, Lineage; M-MDSC, Mononuclear-Myeloid Derived Suppressor Cells; PMN-MDSC, Polymorphonuclear-Myeloid Derived Suppressor Cells.

A) CD4/CD8 ratio



B) Nadir CD4

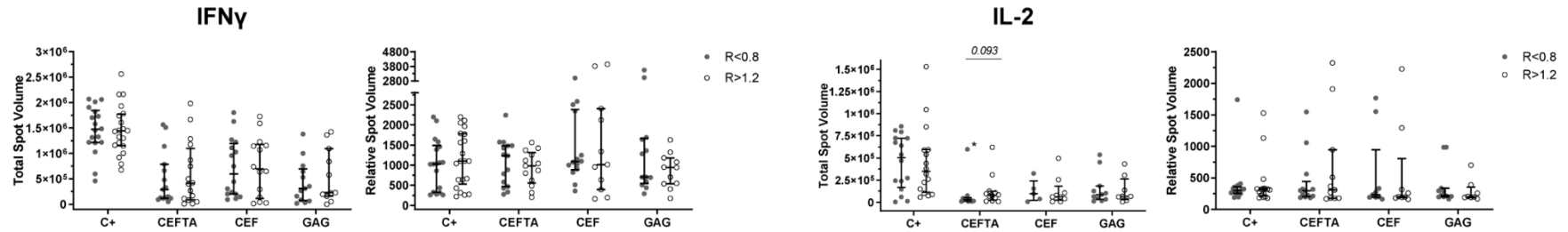


Supplementary Figure 2. Distribution of the relative expression of functional markers among immune cell populations in subjects classified by A) CD4/CD8 ratio and B) Nadir CD4 T-cell counts. Dimensionality reduction (tSNE) analysis was performed with the default configuration (Iteration: 1000, Perplexity: 80, Amount of approximation: 0.30) and a minimum of 100,000 cells and 42 markers were used to generate representative tSNE maps. Populations were then annotated according to the expression of lineage-specific differentiation markers. Sample size: A) R<0.8, n=13 and R>1.2, n=12; B) N≤350, n=13 and N>350, n=12.

A) Spot Forming Units



B) Cytokine secretion

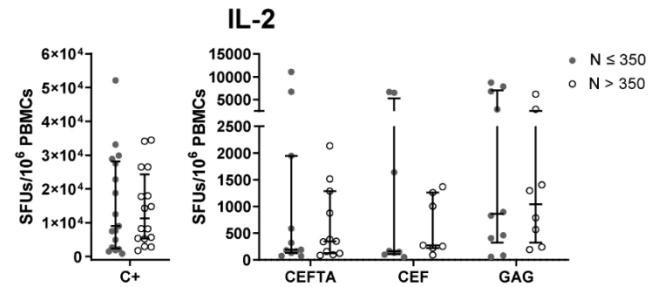
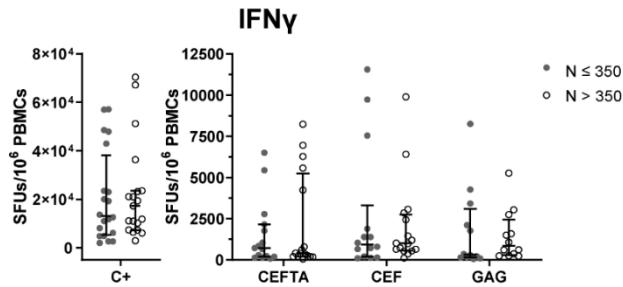


C) Spot Forming Units per CD4 T-cell

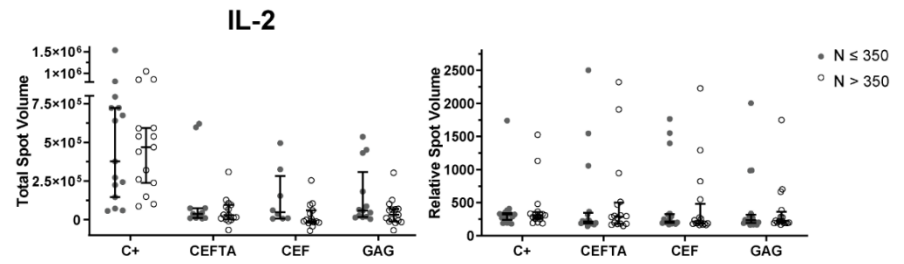
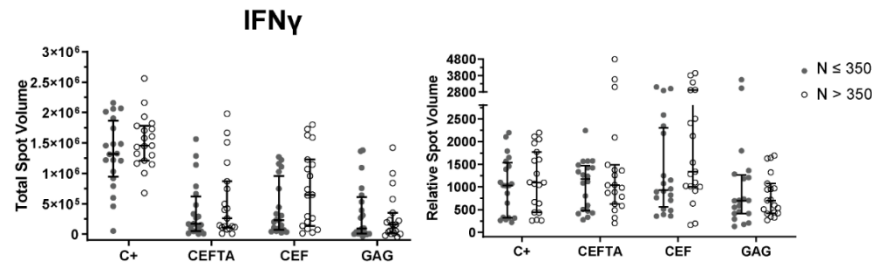


Supplementary Figure 3. Cytokine secretion pattern in response to specific stimulation in subjects with $R < 0.8$ and $R > 1.2$. A) IFN γ (left) and IL-2 (right) Spot Forming Units (SFU), respectively, in response to anti-CD3 (positive control), CEFTA, CEF, or GAG; B) Total production per well and per number of Spot Forming Unit of IFN γ (left) and IL-2 (right), respectively, in response to anti-CD3 (positive control), CEFTA, CEF, or GAG; C) Normalization of the amount of Spot Forming Units per CD4 T-cell count of IFN γ (left) and IL-2 (right) production, respectively. Sample size: $R < 0.8$, $n = 18$ and $R > 1.2$, $n = 22$.

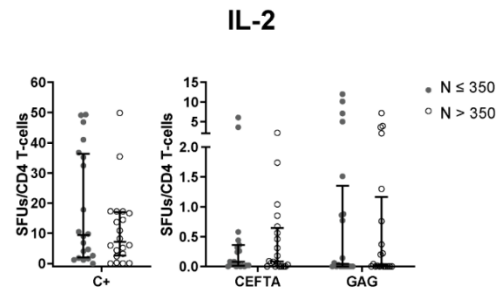
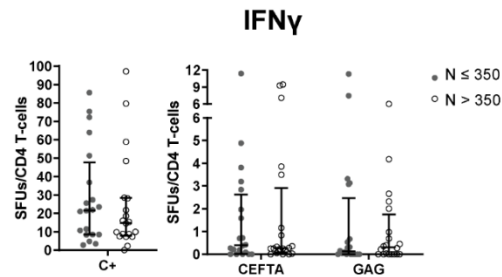
A) Spot Forming Units



B) Cytokine secretion



C) Spot Forming Units per CD4 T-cell



Supplementary Figure 4. Cytokine secretion pattern in response to specific stimulation in subjects with N \leq 350 or N >350. A) IFN γ (left) and IL-2 (right) Spot Forming Units (SFU), respectively, in response to anti-CD3 (positive control), CEFTA, CEF, or GAG; B) Total production per well and per number of Spot Forming Units of IFN γ (left) and IL-2 (right), respectively, in response to anti-CD3 (positive control), CEFTA, CEF, or GAG; C) Normalization of the amount of Spot Forming Units per CD4 T-cell count of IFN γ (left) and IL-2 (right) production, respectively. Sample size: N \leq 350, n=20 and N >350, n=20.

Annex

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