Supplementary methods

Soluble markers and T cell phenotypes

EDTA plasma and peripheral blood mononuclear cells (PBMC) were sampled at inclusion. Soluble markers of inflammation were analyzed in snap-frozen plasma by ELISA (IP-10, sCD14 and hsIL-6) and multiplex (IL-1β, IL-6, IL-7, IL-10, IL-22, Macrophage Chemoattractant Protein 1, MIP-1β and TNF), and LPS by Limulus Amebocyte Lysate colorimetric assay [1]. Plasma concentrations of tryptophan, kynurenine and neopterin were investigated by liquid chromatography-tandem mass spectrometry [2]. Cryopreserved PBMC were analyzed by 8-color flow cytometry and the fractions of the following $CD4^+$ and $CD8^+$ T cell subsets were determined; activated ($CD38^+HLA\text{-}DR^+$) and differentiated (naïve; $CD45RA^+CD27^+$, effector memory (EM); $CD45RO^+CD27^-$), and activated and resting regulatory T cells [aTregs; $\%CD147^{high}CD25^{high}$ of $CD4^+$ [3] and rTregs; $\%CD45RA^+Foxp3^+$ of $CD4^+$ (both gated from lymphocytes, singlets, $CD3^+CD4^+$ and then the respective Treg subset markers [4])]. More details and plots illustrating the gating strategy can be found in [4].

References

1. Troseid M, Nowak P, Nystrom J, Lindkvist A, Abdurahman S, Sonnerborg, **A. Elevated plasma levels of lipopolysaccharide and high mobility group box-1 protein are associated with high viral load in HIV-1 infection: reduction by 2-year antiretroviral therapy.** *AIDS.* 2010; **24**:1733-1737.
2. Midttun O, Hustad S, Ueland PM. **Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry.** *Rapid Commun Mass Spectrom.* 2009; **23**:1371-1379.
3. Solstad T, Bains SJ, Landskron J, Aandahl EM, Thiede B, Tasken K, *et al.* **CD147 (Basigin/Emmprin) identifies FoxP3+CD45RO+CTLA4+-activated human regulatory T cells.** *Blood* 2011; **118**:5141-5151.
4. Stiksrud B, Lorvik KB, Kvale D, Mollnes TE, Ueland PM, Troseid M, *et al.* **Plasma IP-10 is increased in immunological nonresponders and associated with activated regulatory T cells and persisting low CD4 counts.** *J Acquir Immune Defic Syndr* 2016; **73**:138-148.

Figure S1

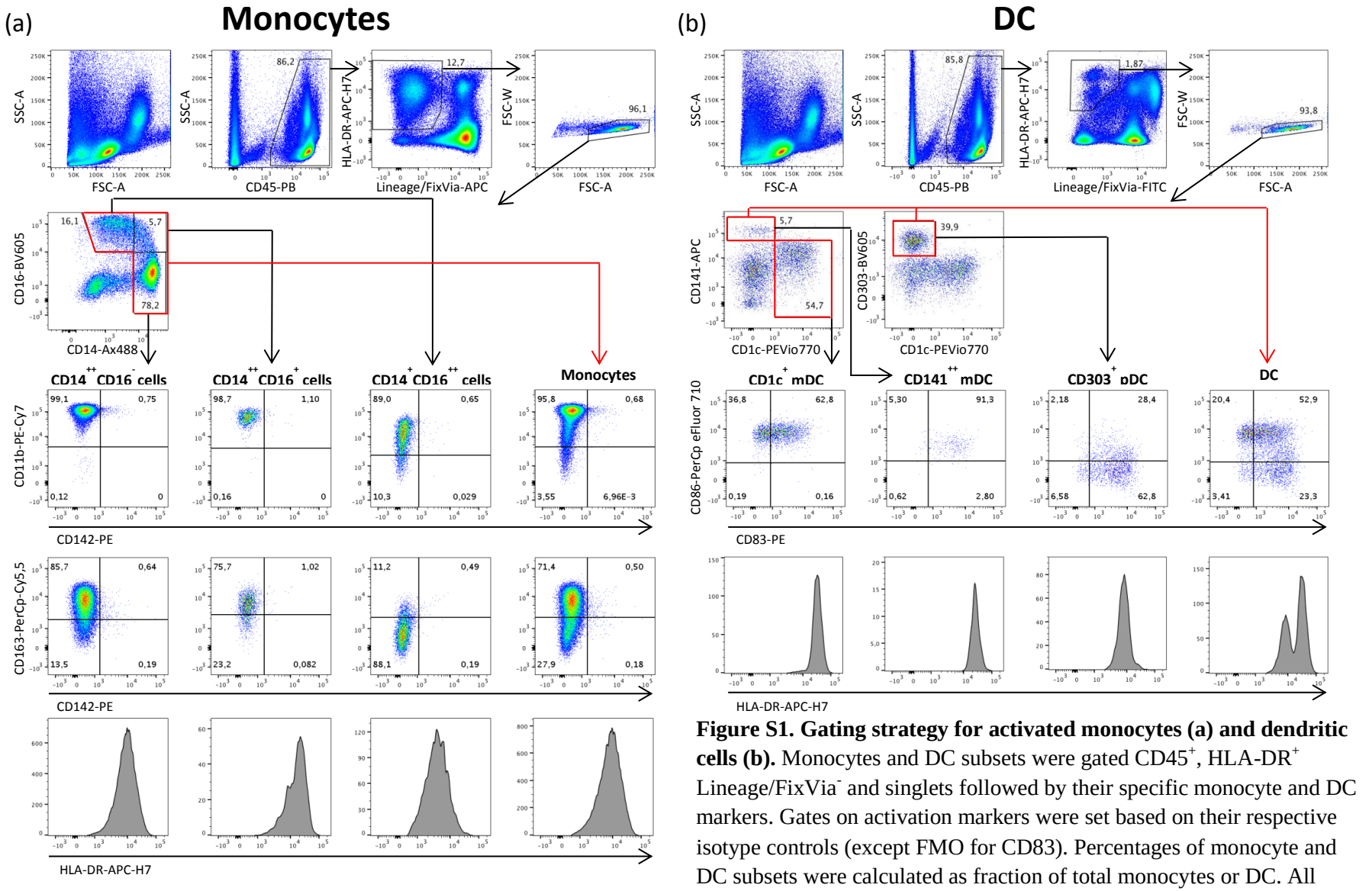

Figure S1. Gating strategy for activated monocytes (a) and dendritic cells (b). Monocytes and DC subsets were gated $CD45^{+}$, $HLA\text{-}DR^{+}$ $Lineage/FixVia^{-}$ and singlets followed by their specific monocyte and DC markers. Gates on activation markers were set based on their respective isotype controls (except FMO for CD83). Percentages of monocyte and DC subsets were calculated as fraction of total monocytes or DC. All figures are from one representative HIV immunological non-responder patient. DC, dendritic cells. Lineage, monocyte panel: anti-CD3, CD19, CD20, CD56. Lineage, DC panel; anti-CD3, CD14, CD19, CD20. FixVia, fixable viability stain.

Figure S2

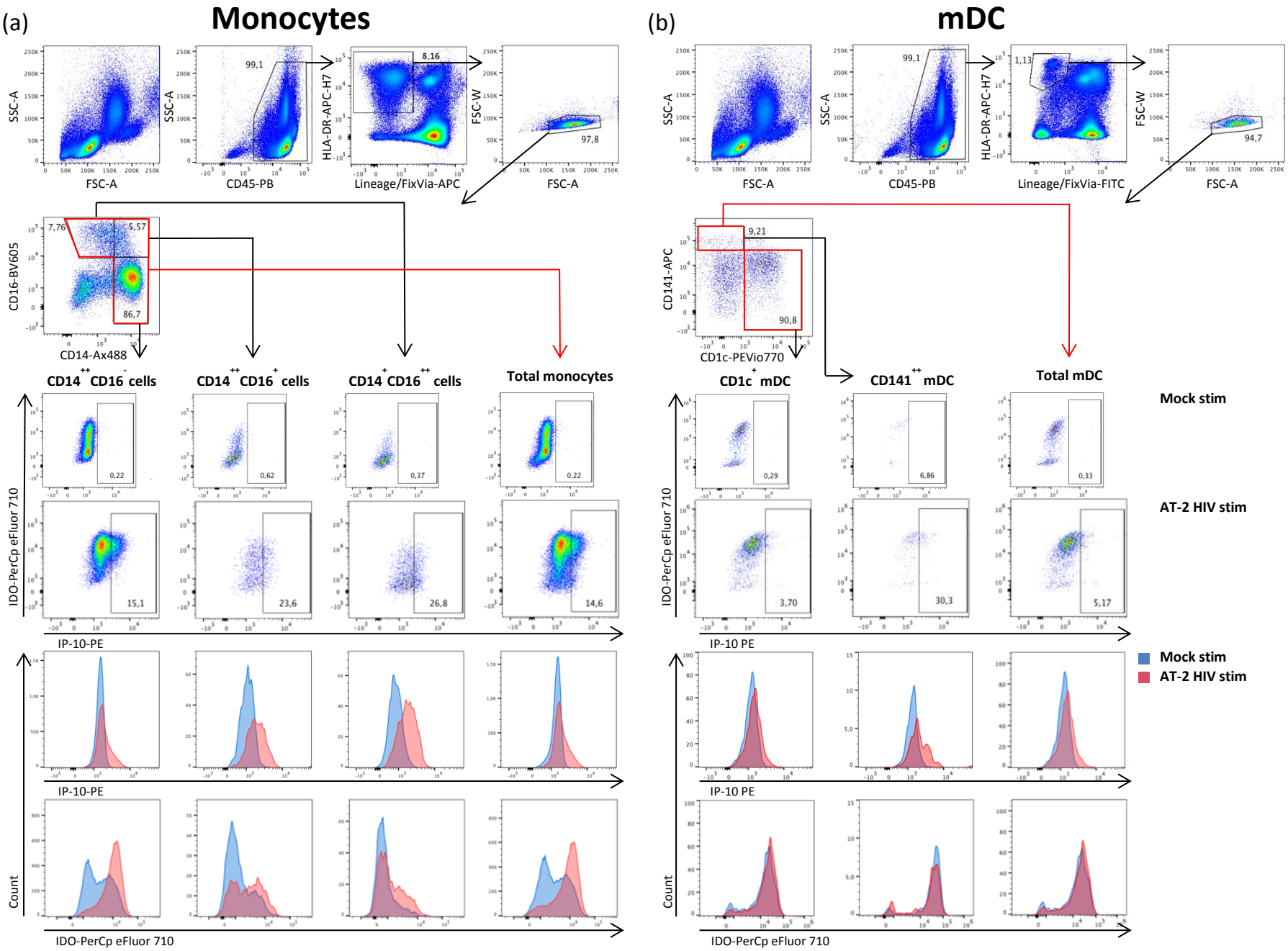
(a)
Monocytes
(b)
mDC
SSC-A
FSC-A
99,1
CD45-PB
8,16
HLA-DR-APC-H7
Lineage/FixVia-APC
FSC-W
97,8
1,13
Lineage/FixVia-FITC
94,7
7,76
5,57
86,7
CD16-BV605
CD14-Ax488
9,21
90,8
CD141-APC
CD1c-PEVio770
CD14++CD16- cells
CD14++CD16+ cells
CD14+CD16++ cells
Total monocytes
CD1c+ mDC
CD141++ mDC
Total mDC
Mock stim
AT-2 HIV stim
0,22
0,62
0,37
0,22
0,29
6,86
0,33
15,1
23,6
26,8
14,6
3,70
30,3
5,17
IDO-PerCp eFluor 710
IP-10-PE
IP-10 PE
Count

Figure S2. Gating strategy for IP-10 and IDO expression in monocytes (a) and mDC (b) after exposure to mock and AT-2 HIV *in vitro*. Monocytes and mDC subsets were gated from $CD45^{+}$, $HLA\text{-}DR^{+}$ Lineage/$FixVia^{-}$ and singlets followed by IP-10 and IDO expression. Due to reduction of the CD303 expression in this assay, IP-10 and IDO upregulation were not analyzed in pDC. Gates on IP-10 and IDO were based on mock stimulated sample as negative control. Percentages of monocyte and mDC subsets were calculated as fraction of total monocytes or mDC. The histograms display overlay plot of mock and AT-2 HIV stimulated samples for IP-10 and IDO, respectively. All figures are from one representative HIV immunological non-responder patient. mDC, myeloid dendritic cells; AT-2 HIV, aldrithiol-2 inactivated HIV-1. Lineage, monocyte panel: anti-CD3, CD19, CD20, CD56. Lineage, DC panel; anti-CD3, CD14, CD19, CD20. FixVia, fixable viability stain.

Figure S3

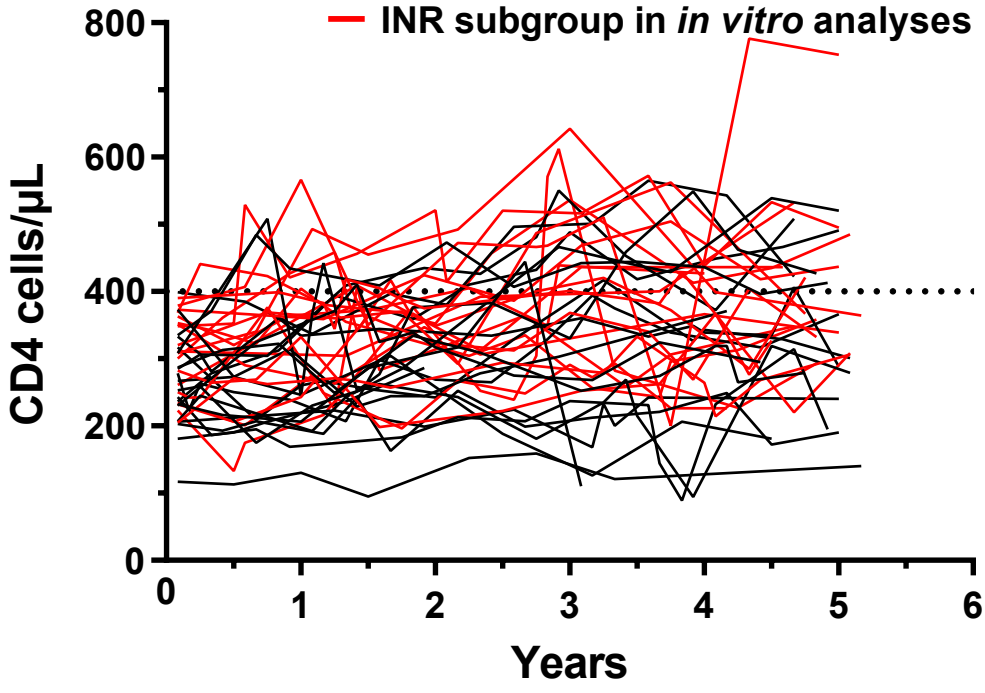

Figure S3. Line-plots showing CD4 counts in INR from inclusion and median 4.7 years prospectively.

Line-plots showing CD4 counts in the INR cohort obtained at inclusion and from all routine clinical visits prospectively for median 4.7 years. INR patients included in *in vitro* AT-2 HIV stimulation assays are shown in red. INR, immunological non-responder; AT-2 HIV, aldrithiol-2 inactivated HIV-1 virus.

Figure S4

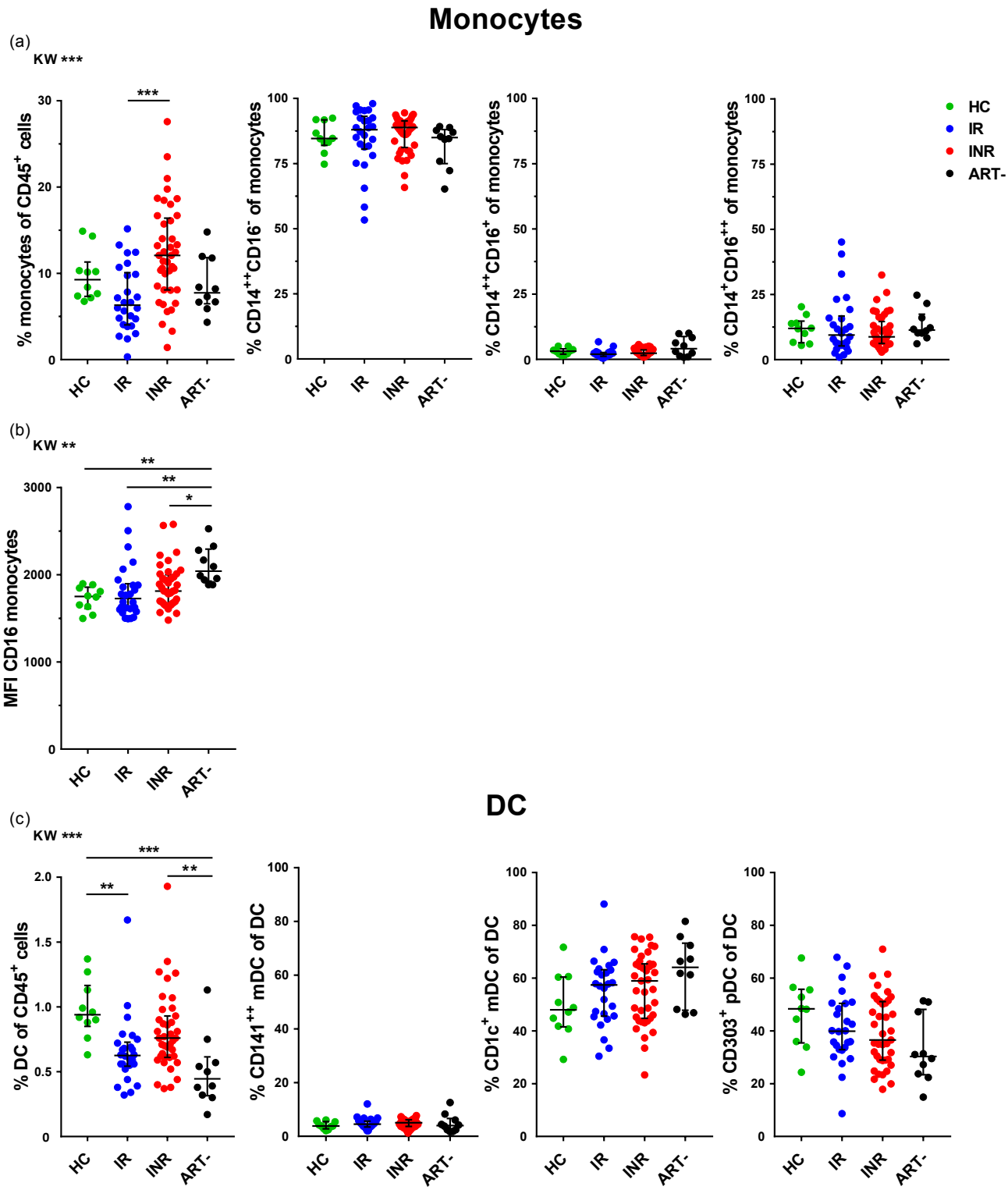

Figure S4. Distribution of monocytes and dendritic cell subsets in different study groups. (a) displays percentages of total monocytes of CD45 $^{+}$ cells and the distribution of specific monocyte subsets in the different study groups and (c) the same for total DC and DC subsets. In (b) MFI values of CD16 in monocytes are shown. Kruskal-Wallis Test (KW) followed by Dunn's posthoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Lines indicate median and interquartile range. DC, dendritic cells; MFI, median fluorescence intensity; HC, healthy control; IR, immune responder; INR, immunological non-responder; ART-, antiretroviral therapy naive.

Figure S5

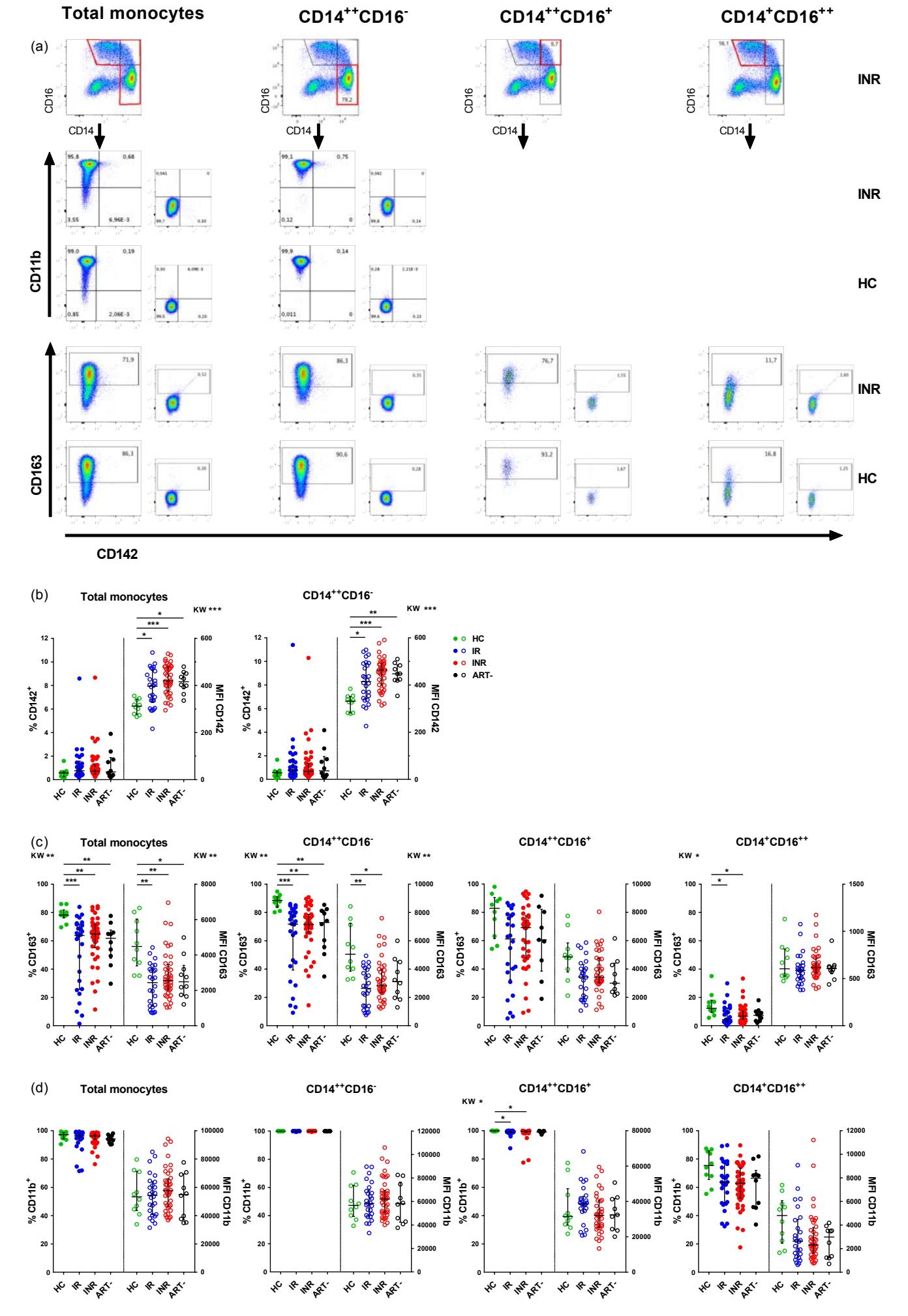
Total monocytes
CD14++CD16-
CD14++CD16+
CD14+CD16++
(a)
CD16
CD14
CD11b
CD163
CD142
INR
INR
HC
INR
HC
(b)
Total monocytes
CD14++CD16-
KW ***
% CD142+
MFI CD142
HC
IR
INR
ART-
(c)
Total monocytes
CD14++CD16-
CD14++CD16+
CD14+CD16++
KW **
KW *
% CD163+
MFI CD163
(d)
Total monocytes
CD14++CD16-
CD14++CD16+
CD14+CD16++
KW *
% CD11b+
MFI CD11b

Figure S5. Monocyte activation markers in the different cohorts. (a) illustrate gating strategy of monocyte activation markers based on isotype as negative control in total monocytes and different monocyte subsets. The plots show representative examples from one INR and one HC. In (b), (c), and (d) the graphs display both percentages (solid dots) and MFI (open dots) values of the activation markers CD142, CD163 and CD11b in the specific monocyte subsets for each group. Kruskal-Wallis Test (KW) followed by Dunn's posthoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Lines indicate median and interquartile range. INR, immunological non-responder; HC, healthy control; IR, immune responder; ART-, antiretroviral therapy naïve HIV-infected; MFI, Median fluorescence intensity.

Figure S6

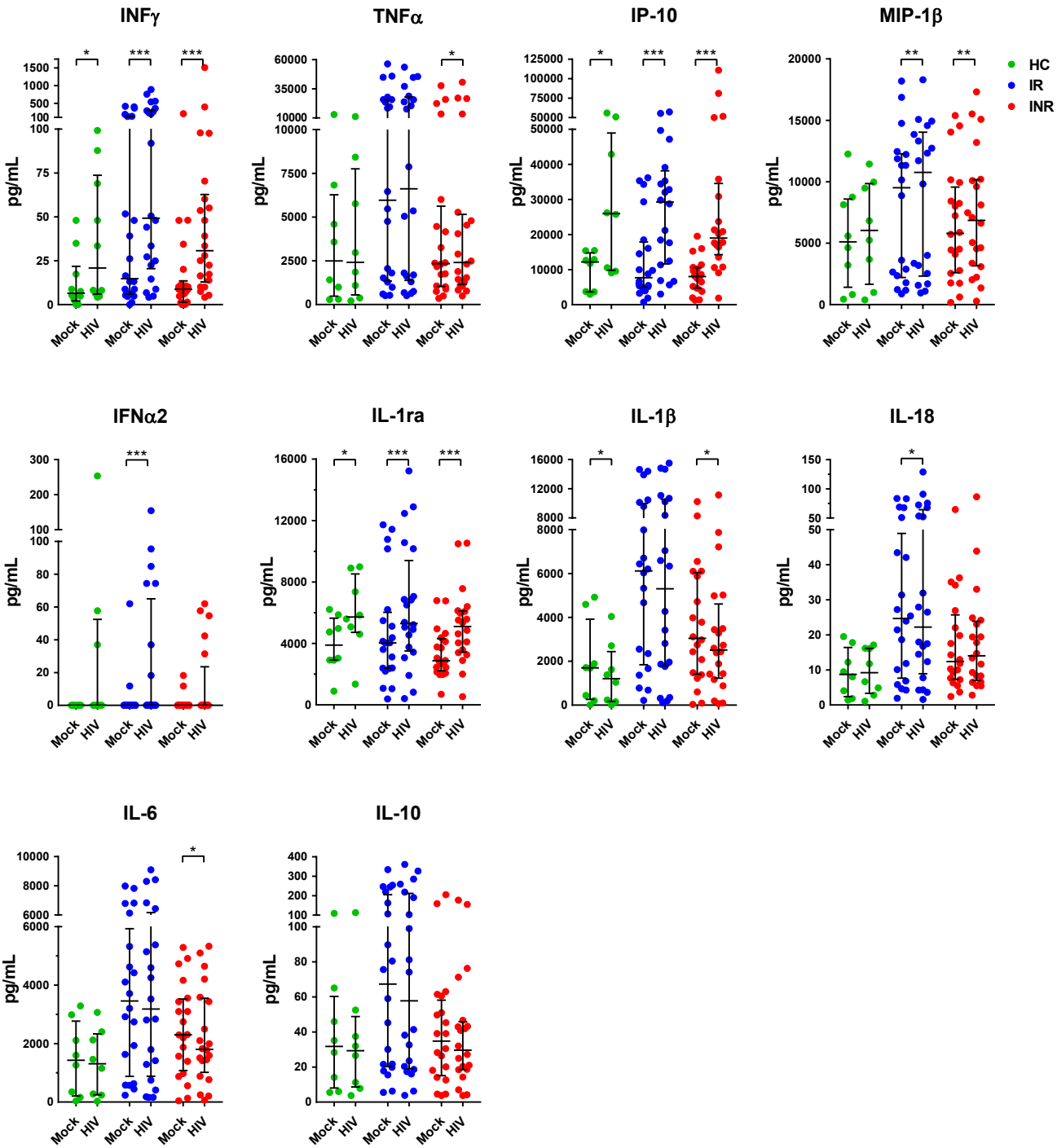

Figure S6. Cytokine responses in supernatants after exposure to AT-2 HIV *in vitro*.

This figure shows various cytokine responses in supernatants after *in vitro* stimulation with either mock or AT-2 HIV in the HC, IR and INR subgroups. There were no significant differences in the cytokine responses between the groups (Kruskal-Wallis Test). Wilcoxon Test for pairwise comparisons between mock and AT-2 HIV stimulation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Lines indicate median and interquartile range. AT-2 HIV, aldrithiol-2 inactivated HIV-1 virus; HC, healthy control; IR, immune responder; INR, immunological non-responder.

TABLE S1. Antibodies and dyes used to determine monocyte and DC frequencies and activation and IP-10 and IDO upregulation after exposure to AT-2 HIV *in vitro*

	Flurochrome	Clone	Company	Location
Monocyte activation				
CD45	PB	HI30	BioLegend	San Diego, CA, USA
HLA-DR	APC-H7	G46-6	BD Biosciences	San Jose, CA, USA
Fixable Viability	eFluor 660		eBioscience, Thermo Fischer Scientific	Carlsbad, CA, USA
Lineage, anti CD3, CD19, CD20, CD56	APC	UCHT1, HIB19, 2H7, 5.1H11	BioLegend	San Diego, CA, USA
CD16	BV605	3G8	BD Biosciences	San Jose, CA, USA
CD14	Ax488	M5E2	BD Biosciences	San Jose, CA, USA
CD142	PE	HTF-1	BD Biosciences	San Jose, CA, USA
CD11b	PECy-7	ICRF44	BioLegend	San Diego, CA, USA
CD163	PerCp-Cy 5.5	GHI/61	BD Biosciences	San Jose, CA, USA
IgG1 κ isotype, mouse	PE		BD Biosciences	San Jose, CA, USA
IgG1 κ isotype, mouse	PECy-7		BioLegend	San Diego, CA, USA
IgG1 κ isotype, mouse	PerCp-Cy 5.5		BD Biosciences	San Jose, CA, USA
DC activation				
CD45	PB	HI30	BioLegend	San Diego, CA, USA
HLA-DR	APC-H7	G46-6	BD Biosciences	San Jose, CA, USA
Fixable Viability	eFluor 520		eBioscience, Thermo Fischer Scientific	Carlsbad, CA, USA
Lineage 3, anti CD3, CD14, CD19, CD20,	FITC	SK7, MϕP9, SJ25C1, L27	BD Biosciences	San Jose, CA, USA
CD1c	PE-Vio770	AD5-8E7	Miltenyi Biotec GmbH	Bergisch Gladbach, Germany
CD141	APC	AD5-14H12	Miltenyi Biotec GmbH	Bergisch Gladbach, Germany
CD303	BV605	201A	BioLegend	San Diego, CA, USA
CD83	PE	HB15e	BioLegend	San Diego, CA, USA

CD86	PerCp eFluor 710	IT2.2	eBioscience, Thermo Fischer Scientific	Carlsbad, CA, USA
IgG2b κ isotype, mouse	PerCp eFluor 710		eBioscience, Thermo Fischer Scientific	Carlsbad, CA, USA
Tandem Signal Enhancer			Miltenyi Biotec GmbH	Bergisch Gladbach, Germany
IDO and IP-10 upregulation in monocytes after exposure to AT-2 HIV *in vitro*				
CD45	PB	HI30	BioLegend	San Diego, CA, USA
HLA-DR	APC-H7	G46-6	BD Biosciences	San Jose, CA, USA
Fixable Viability	eFluor 660		eBioscience, Thermo Fischer Scientific	Carlsbad, CA, USA
Lineage, anti CD3, CD19, CD20, CD56	APC	UCHT1, HIB19, 2H7, 5.1H11	BioLegend	San Diego, CA, USA
CD16	BV605	3G8	BioLegend	San Diego, CA, USA
CD14	Ax488	M5E2	BD Biosciences	San Jose, CA, USA
IP-10	PE	6D4/D6/G2	BD Biosciences	San Jose, CA, USA
IDO	PerCp-eFluor 710	eyedio	eBioscience, Thermo Fischer Scientific	Carlsbad, CA, USA
IDO and IP-10 upregulation in DC after exposure to AT-2 HIV *in vitro*				
CD45	PB	HI30	BioLegend	San Diego, CA, USA
HLA-DR	APC-H7	G46-6	BD Biosciences	San Jose, CA, USA
Fixable Viability	eFluor 520		eBioscience, Thermo Fischer Scientific	Carlsbad, CA, USA
Lineage 3, anti CD3, CD14, CD19, CD20,	FITC	SK7, MϕP9, SJ25C1, L27	BD Biosciences	San Jose, CA, USA
CD1c	PE-Vio770	AD5-8E7	Miltenyi Biotec GmbH	Bergisch Gladbach, Germany
CD141	APC	AD5-14H12	Miltenyi Biotec GmbH	Bergisch Gladbach, Germany
CD303	BV605	201A	BioLegend	San Diego, CA, USA

IP-10	PE	6D4/D6/G2	BD Biosciences	San Jose, CA, USA
IDO	PerCp-eFluor 710	eyedio	eBioscience, Thermo Fischer Scientific	Carlsbad, CA, USA
Tandem Signal Enhancer			Miltenyi Biotec GmbH	Bergisch Gladbach, Germany

DC, dendritic cells; AT-2 HIV, aldrithiol-2 inactivated HIV-1

TABLE S2. Characteristics of the Subgroups for *in vitro* AT-2 HIV stimulation at Inclusion

Total study population	**INR (n=20)**	**IR (n=20)**	**HC (n=8)**	**P-value**[a]
Age, (IQR)	47.4 (41.0-56.5)	45 (40.2-55.3)	46.4 (40.4-57.3)	NS
Male gender, n (%)	18 (90)	15 (75)	6 (75)	NS
Ethnicity, n (%)				
Caucasian	14 (70)	13 (65)	8 (100)	NS
Risk group, n (%)				
MSM	9 (45)	10 (50)		NS
Other[1]	11 (55)	10 (50)		NS
Comorbid diseases, n (%)				
Cardiovascular	2 (10)	0 (0)		NS
Any comorbidity[2]	9 (45)	5 (25)		NS
CMV IgG pos	20 (100)	20 (100)		NS
HIV characteristics, (IQR)				
Years since HIV diagnosis	8.1 (5.4-13.5)	9.5 (7.5-14.3)		NS
Years of continuous ART	5.5 (3.5-6.1)	6.7 (5.9-8.6)		**0.01**
Viral load at ART initiation, cop/mL	41000 (25600-71000)	160000 (65400-460000)		**0.02**
Duration of viral suppression, years	3.8 (2.6-5.5)	6.2 (4.4-7.6)		**0.01**
Viral load at inclusion, cop/mL	≤20	≤20		NS
CD4 count nadir, cells/µL	151.5 (95-165)	148.5 (94-190)		NS
CD4 count at inclusion, cells/µL	309.5 (242.5-358)	795.5 (732-847.5)		**<0.001**
CD8 count at inclusion, cells/µL	665.5 (544-921)	989.5 (796-1575.5)		**0.002**
CD4/CD8 at inclusion	0.44 (0.35-0.55)	0.79 (0.55-0.99)		**<0.001**

Data are presented as no. (%) of study participants or median (interquartile range (IQR)) values.

[a]P-values for Kruskal-Wallis Test or Pearson Chi-Square test for comparison between multiple groups, Mann-Whitney U Test or Fischer's Exact Test for comparison INR vs IR.

[1]Other. Heterosexual or unknown. There were no intravenous drug abusers.

[2]One or more of the following comorbidities; cardiovascular disease, hypertension, diabetes, renal disease, osteoporosis, chronic obstructive pulmonary disease, neurodegenerative disease, previous cancer or Mycobacterium tuberculosis infection.

AT-2 HIV, aldrithiol-2 inactivated HIV-1; INR, immunological non-responders; IR, immunological responders; HC, healthy control; MSM, men who have sex with men; CMV, cytomegalovirus; ART, antiretroviral therapy.

TABLE S3. INR group (n=38): Correlation between HIV baseline characteristics and CD4 count and CD4/CD8 after four years and the increase in CD4 and CD4/CD8 from inclusion

	CD4 (cells/µL) (rho)	**CD4 increase (rho)**	**CD4/CD8 (rho)**	**CD4/CD8 increase (rho)**
Duration of continuous ART	**-0.40***	**-0.55*****	**-0.63*****	**-0.47****
Duration of continuous viral suppression	-0.26	**-0.37***	**-0.48****	**-0.42***
Duration of HIV infection	**-0.33***	**-0.33***	**-0.46****	**-0.35***
Nadir CD4	**0.46****	**0.35***	-0.31	0.15
Age	**-0.38***	-0.32	**-0.37***	-0.25

Spearman rank order correlation. * P < 0.05, ** P < 0.01, *** P < 0.001

INR, immunological non-responder; ART, antiretroviral therapy

TABLE S4. Correlation between expression of activation markers on monocytes or DC subsets and T cell activation and differentiation, aTregs/rTregs, and CD4 count at inclusion, in INR and IR

Cell population		Act. $CD4^+$ T cells (rho)	Act. $CD8^+$ T cells (rho)	EM $CD4^+$ T cells (rho)	Naïve/ EM $CD8^+$ T cells (rho)	aTregs/ rTregs (rho)	CD4 count (cells/µL) (rho)
Total monocytes	**MFI HLA-DR**	**0.62****	**0.48***	**0.42****	**-0.37***	0.05	**-0.32****
$CD141^{++}$ mDC	**$\%CD83^+$**	0.43	0.47	0.18	-0.20	0.16	**-0.42****
	$\%CD86^+$	**0.57***	**0.51***	0.30	-0.34	**0.40***	**-0.54*****
	MFI CD86	0.38	0.12	0.20	-0.18	0.31	**-0.45*****
	$\%CD83^+CD86^+$	0.43	**0.51***	0.20	-0.28	0.25	**-0.49*****
$CD1c^+$ mDC	**MFI HLA-DR**	**0.62****	0.38	**0.33***	**-0.33***	0.09	**-0.36****
	MFI CD86	**0.44***	0.27	0.27	-0.23	**0.31***	**-0.56*****
$CD303^+$ pDC	**$\%CD83^+$**	**0.60****	**0.47***	0.19	-0.28	**0.31***	**-0.43****
	MFI CD83	**0.65****	**0.52***	0.18	-0.24	**0.40****	**-0.37****

Spearman rank order correlation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

DC, dendritic cells; mDC, myeloid dendritic cells; pDC, plasmacytoid dendritic cells; Act, activated cells ($CD38^+HLA\text{-}DR^+$); EM, effector memory cells ($CD45RO^+CD27^-$); naïve ($CD45RA^+CD27^+$), aTregs; activated regulatory T cells ($\%CD147^{high}CD25^{high}$ of $CD4^+$); rTregs, resting Tregs ($\%CD45RA^+Foxp3^+$ of $CD4^+$); MFI, median fluorescence intensity.

TABLE S5. Correlation between cytokines responses in supernatants and IP-10 and IDO expression in monocytes and mDC after exposure to AT-2 HIV *in vitro* in INR and IR

	INR (n=20)				**IR (n=20)**
	Monocytes			**mDC**	**mDC**
Supernatants (pg/mL)	**MFI IP-10 (rho)**	**% IP-10 (rho)**	**MFI IDO (rho)**	**MFI IP-10 (rho)**	**MFI IP-10 (rho)**
MIP-1β	**0.45***	0.35	**0.53***	**0.56***	**0.55***
IFNα2	**0.63****	0.37	0.28	**0.58****	-0.22
IL-1ra	**0.59****	**0.45***	0.14	**0.65****	0.26
IL-18	**0.63****	0.17	0.35	**0.47***	-0.03
IL-1β	0.41	-0.12	0.04	0.06	-0.11
IFNγ	0.41	0.15	0.43	**0.47***	-0.04
TNFα	0.39	0.14	0.31	0.42	0.01
IP-10	**0.67****	**0.53***	0.20	**0.63****	0.44
IL-6	0.30	-0.16	0.12	0.08	-0.13

Correlation between delta values (mock stimulation subtracted).

Spearman rank order correlation. * $P < 0.05$, ** $P < 0.01$

IDO, indoleamine 2, 3 dioxygenase; mDC, myeloid dendritic cells; AT-2 HIV, aldrithiol-2-inactivated HIV-1; INR, immunological non-responder; IR, immune responder; MFI, median fluorescence intensity.