6 A)





Supplementary Figure 1. A) CD11b, CD163 and TLR4 expression before and after DAAs treatment and in the HIV control group. No differences were observed in the expression of these markers neither after DAAs nor compared with the HIV control group. CD163 expression in the intermediate subsets present higher levels in HIV control group respect the HIV/HCV

14 coinfected group (p=0.045). The Wilcoxon test was used to compare HIV/HCV coinfected patients at baseline and end treatment. The Man-Whitney U was used to compare HIV/HCV-15 coinfected patients and HIV-monoinfected patients control group. \*p<0.05 and \*\*p<0.001. 16 Abbreviation: NS, not significant; BL, HIV/HCV Baseline; ET, HIV/HCV End of treatment; 17 Ctrl, HIV control group. B) Schematic diagram of the gating strategy for monocyte subset in a 18 19 HIV/HCV coinfected subject before and after DAAs treatment. Histogram representation of IL8 expression on classical, intermediate and nonclassical monocytes stimulated with LPS before 20 21 and after DAAs treatment as a representative example.



### 23 Supplementary Table 1. Characteristics of the confirmatory cohort.

Characteristic	HCV/HIV BL (n=18) Confirmatory Cohort	HCV/HIV BL (n=22)	P VALUE	
Male sex, %	83	55	0.053	
Age, y	51 [46-54]	47 [44-51]	0.186	
CD4 Nadir counts, cells/µL	64 [19-121] 172 [50-393]		0.009	
CD4 <sup>+</sup> T-cells counts, cells/µL	637 [325-781] 671 [446-957]		0.196	
CD8 <sup>+</sup> T-cells counts, cells/µL	870 [575-1537]	1038 [607-1310]	0.701	
Ratio CD4+ to CD8+ T Cells	0.69 [0.36-0.94]	0.72 [0.45-0.99]	0.540	
HIV RNA (Log <sub>10</sub> copies/mL)	1.3 [1.3-1.3]	1.3 [1.3 - 1.3]	1	
HCV RNA (Log <sub>10</sub> copies/mL)	6.4 [5.7 – 6.8]	6.8 [6.2 – 7.2]	0.020	
HCV genotype-1, %	72.2	77.8	0.714	
Fibrosis stage <b>, %</b>				
0-1	38.9	44.4	0.735	
2	11.1	44.4	0.026	
3	16.7	5.5	0.289	
4	33.3	5.5	0.035	
Cirrhosis, %	33.3	5.5	0.035	

N.A.: Not applicable. Categorical variables were expressed as percentage and
continuous variables as median, interquartile range [IQR]. Chi-square test and MannWhitney U test were used to compare categorical and continuous variables,
respectively.

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#### 31 Supplementary Figure 2.



34 Supplementary Figure 2. Results of the confirmatory cohort. After treatment with DAAs the percentage of classical monocytes increased (p=0.02) and the intermediate and nonclassical 35 36 subset diminished (p=0.005; p=0.03; respectively). In addition, a decrease in the levels of 37 CCR5, CD49d and TLR4 expression after treatment with DAAs was observed in classical an intermediate monocytes (p=0.002; p=0.02; p=0.005; p=0.005; p=0.0004; p=0.01; respectively). 38 No differences were observed in CD11b, CX3CR1, CD40 and TLR2 expression. . The 39 40 Wilcoxon test was used to compare HIV/HCV coinfected patients at baseline and at the end treatment. The Man-Whitney U was used to compare HIV/HCV-coinfected patients and HIV-41 monoinfected patients control group. \*p<0.05 and \*\*p<0.001. Abbreviation: NS, not 42 significant; BL, HIV/HCV Baseline; ET, HIV/HCV End treatment; Ctrl, HIV control group. 43

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#### 45 Supplementary Figure 3



Supplementary Figure 3. Effect of DAAs in the levels of IL6, TNF-α and hsCRP levels
changes after DAAs therapy. The Wilcoxon test was used to compare HIV/HCV-coinfected
patients at baseline and end treatment. The Man-Whitney U was used to compare HIV/HCVcoinfected patients and HIV-monoinfected patients control group. Abbreviation: hsCRP, high
sensitivity C reactive protein; NS, not significant; BL, HIV/HCV Baseline; ET, HIV/HCV End
treatment; Ctrl, HIV control group.

# 54 Supplementary Table 2. Multiple cytokine combinations in the different monocyte

## 55 subsets after LPS estimulation.

	Classical		Intermediate		Nonclassical	
	Monocytes		Monocytes		Monocytes	
IL1α IL1β IL6 IL8 IL10 TNF $\alpha^a$	BL vs ET <sup>b</sup>	ET vs Ctrl <sup>c</sup>	BL vs ET	ET vs Ctrl	BL vs ET	ET vs Ctrl
+++++	0.024	0.564	0.671	0.802	1.000	1.000
-++++	0.023	0.020	0.315	0.725	0.755	0.802
+-+++	0.229	0.451	0.932	0.802	1.000	1.000
++-++	0.005	0.108	0.193	0.802	0.887	0.802
+++-++	0.734	0.328	0.051	0.841	0.470	1.000
++++-+	0.054	0.269	0.552	0.802	1.000	1.000
++++-	0.671	0.280	0.810	1.000	1.000	1.000
+++	0.193	0.006	0.479	0.451	0.630	1.000
-+-++	0.016	0.004	0.030	1.000	0.755	0.802
+++	0.044	0.132	0.630	1.000	0.865	0.880
-++-++	0.218	0.012	0.218	0.422	0.315	0.980
+ - + - + +	0.229	1.000	0.887	0.802	0.630	1.000
++++	0.095	0.063	0.041	0.651	0.315	0.132
- + + + - +	0.066	0.016	0.506	0.280	1.000	1.000
+ - + + - +	0.119	0.315	0.810	1.000	0.810	1.000
++-+-+	0.004	0.688	0.229	1.000	0.810	1.000
+ + + +	0.955	0.482	0.470	1.000	0.630	1.000
-+++-	0.497	0.001	0.229	1.000	1.000	1.000
+ - + + + -	0.350	0.802	0.810	1.000	0.755	0.802
++-+-	0.282	0.160	0.630	1.000	1.000	1.000
+++-	0.734	0.315	1.000	1.000	1.000	1.000
+++	0.188	0.380	0.470	1.000	1.000	1.000
++	0.002	0.001	0.174	0.616	0.336	1.000
++	0.745	0.002	0.821	0.292	0.955	0.315
-+++	0.257	0.000	0.149	0.292	0.799	0.145
+++	0.034	0.014	0.084	0.616	0.039	0.292
++-+	0.100	0.007	0.372	0.315	0.723	0.616
-+-+-+	0.022	0.025	0.051	0.900	0.810	1.000
++-	0.000	0.530	0.149	0.670	0.713	0.880
-+++	0.350	0.209	0.013	0.725	0.552	0.802
+-+-+	0.257	0.841	0.470	1.000	0.810	1.000
+++	0.015	0.436	0.229	1.000	0.174	0.802
++-	0.843	0.002	0.202	0.802	1.000	1.000
-+-+-	0.084	0.000	0.084	0.802	1.000	1.000
+++-	0.017	0.315	0.149	1.000	0.810	1.000
-++-+-	0.444	0.009	0.269	0.763	0.766	0.880
+-+-	0.017	0.451	0.692	0.802	1.000	1.000
+++-	0.977	0.050	0.092	1.000	0.630	1.000

- + + +	0.506	0.002	0.039	0.564	0.932	0.802
+ - + +	0.021	0.422	0.462	0.616	1.000	1.000
++-+	0.041	0.192	0.336	1.000	1.000	1.000
+++	0.497	0.802	0.810	0.670	1.000	1.000
++	0.444	0.000	0.745	0.002	0.179	0.008
+-+	0.002	0.005	0.003	0.960	0.213	0.547
++	0.552	0.018	0.777	0.688	0.110	0.616
-++	0.060	0.393	0.029	0.547	0.229	0.530
++	0.004	0.633	0.149	0.564	0.002	0.407
++-	0.054	0.003	0.030	0.219	0.692	0.960
+ - + -	0.671	0.001	0.223	0.145	0.133	0.145
- + + -	0.234	0.001	0.005	0.960	0.411	0.616
++-	0.016	0.120	0.008	1.000	0.470	1.000
+ +	0.590	0.003	0.403	0.006	0.515	0.315
- + - +	0.257	0.012	0.329	0.366	0.396	0.802
+ +	0.000	0.228	0.004	1.000	0.157	0.802
- + +	0.799	0.011	0.079	0.514	0.350	0.451
+ - +	0.218	0.025	0.282	0.393	0.087	0.616
++	0.004	0.763	0.229	0.393	0.810	1.000
+	0.070	0.016	0.022	0.063	0.295	0.160
+-	0.524	0.132	0.041	0.120	0.506	0.422
+	0.028	0.145	0.098	0.063	0.006	0.547
+	0.865	0.002	0.899	0.035	0.571	0.025
-+	0.357	0.467	0.000	0.192	0.034	0.841
+	0.002	0.228	0.003	0.744	0.008	0.802

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<sup>57</sup> <sup>a</sup>Monocyte subset showing the presence (+, symbol) or absence (-,symbol) of cytokine <sup>58</sup> combinations in the order represented in the column heading. <sup>b</sup>p value of Wilcoxon test <sup>59</sup> was used to compared variables before (BL) and after treatment (ET). <sup>c</sup>p value of Chi-<sup>60</sup> square test and Mann-Whitney U test were used to compare categorical and continuous <sup>61</sup> variables respectively, between ET and HIV monoinfected control group. Significant <sup>62</sup> values are shadowed.



Supplementary Figure 4. Association of cell-associated HIV-1 DNA levels with activation
of monocytes at the end of treatment. Graphical representation of the relationship between
HIV-1 DNA Log<sub>10</sub> copies/10<sup>6</sup> PBMCs and the percentage of monocytes expressing CD40,
CCR5 and CD11b after DAAs treatment. Spearman correlation coefficient test was used.

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