

**Supplemental Table 1.** Clinical and epidemiological characteristics of the therapy naïve and treated HIV-2 infected individuals.

	Seronegatives	Untreated HIV-2	ART-HIV-2 <sup>a</sup>
<b>Number</b> [male/female]	16 [6/10]	28 [9/19]	10 [5/5]
<b>Age</b> , years	44 (27-57)	54 (19-78)	57 (34-62)**
<b>Caucasian/black</b>	15/1	15/13	6/4
<b>CD4<sup>+</sup> T-cells</b> , %	59 (40-77)	40 (10-66)**	26 (4-40)***/##
<b>CD4<sup>+</sup> T-cells</b> /μl	818 (518-1312)	553 (52-1511)*	288 (84-554)***/##
<b>Viremia</b> , HIV RNA cp/ml	-	200 (200-26263)	200 (200-34314)
<b>Proviral DNA</b> , cp/10 <sup>6</sup> PBMC	-	5 (5-1033)	130 (5-726)
<b>B-cells</b> , %	6 (3-13)	6 (3-20)	10 (4-17)*
<b>B-cells</b> /μl	120 (63-369)	131 (30-369)	135 (63-313)

Data are expressed as median, with limits in brackets. Statistical analysis was performed with Mann-Whitney tests.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$  in comparison with seronegatives. ##  $P < 0.01$  for comparisons between infected cohorts.

<sup>a</sup>ART regimens were as follows (number of ART-HIV-2 patients): 3TC+AZT+LPV/r (3); 3TC+d4T+SQV/r (2); 3TC/AZT+IDV/r (1); 3TC+AZT+IDV (1); ABC+3TC+AZT (3). Nucleoside analog reverse transcriptase inhibitors (NRTIs): AZT – Zidovudine, 3TC- Lamivudine, ABC- abacavir, d4T- Stavudine, r- ribavirine. Protease inhibitors (PIs)- LPV- Lopinavir, IDV- Indinavir, SQV- Saquinavir.

**Supplemental Table 2.** Relationship between absolute counts of circulating B-cell populations and markers of disease progression in the HIV-2 and HIV-1 cohorts.

	Total B-cells/ $\mu$ l	B-cells/ $\mu$ l			
		CD27 <sup>neg</sup> IgD <sup>+</sup>	CD27 <sup>+</sup>	CD27 <sup>+</sup> IgD <sup>+</sup>	CD27 <sup>+</sup> IgD <sup>neg</sup>
<b>HIV-2 (n=38)</b>					
CD4 <sup>+</sup> T-cells/ $\mu$ l	<b>0.3522;0.0301</b>	0.1322;0.4288	<b>0.7561;&lt;0.0001</b>	<b>0.7830;&lt;0.0001</b>	<b>0.7137;&lt;0.0001</b>
Viremia, HIV RNA cp/ml	-0.0754;0.6526	0.0557;0.7396	<b>-0.4280;0.0074</b>	<b>-0.3842;0.0172</b>	<b>-0.4273;0.0074</b>
%HLA-DR <sup>+</sup> CD38 <sup>+</sup> within CD4 <sup>a</sup>	0.0153;0.9275	0.1899;0.2535	<b>-0.5002;0.0014</b>	<b>-0.5512;0.0003</b>	<b>-0.4611;0.0036</b>
%HLA-DR <sup>+</sup> CD38 <sup>+</sup> within CD8 <sup>a</sup>	-0.0390;0.8164	0.1324;0.4281	<b>-0.4466;0.0049</b>	<b>-0.4964;0.0015</b>	<b>-0.4166;0.0093</b>
$\beta$ 2-microglobulin (mg/l) <sup>b</sup>	-0.2110;0.2631	-0.0039;0.9837	<b>-0.6758;&lt;0.0001</b>	<b>-0.7367;&lt;0.0001</b>	<b>-0.6326;0.0002</b>
<b>HIV-1 (n=20)</b>					
CD4 <sup>+</sup> T-cells/ $\mu$ l	0.2144;0.3639	0.2023;0.3923	0.2866;0.2206	<b>0.5957;0.0056</b>	0.2153;0.3620
Viremia, HIV RNA cp/ml	0.0098;0.9673	-0.1340;0.5732	0.0919;0.7001	-0.4339;0.0559	0.2027;0.3914
%HLA-DR <sup>+</sup> CD38 <sup>+</sup> within CD4 <sup>a</sup>	-0.2506;0.2866	-0.3106;0.1825	-0.1971;0.4050	-0.5794;0.0074	-0.0941;0.6932
%HLA-DR <sup>+</sup> CD38 <sup>+</sup> within CD8 <sup>a</sup>	-0.0669;0.7792	-0.2857;0.2220	0.1985;0.4015	-0.3350;0.1488	0.3168;0.1736
$\beta$ 2-microglobulin (mg/l) <sup>b</sup>	-0.3740;0.1877	-0.4462;0.1098	-0.1209;0.6806	<b>-0.5433;0.0447</b>	-0.0264;0.9286

Spearman's correlation coefficient was used and results are expressed as R;*P*, with significant correlations in bold.

<sup>a</sup>Frequency of cells co-expressing the activation markers HLA-DR and CD38 within CD4<sup>+</sup> and CD8<sup>+</sup> T-cells (median, range) – HIV-2: 3.4%, 0.4-23.5% (CD4), and 15.1%, 0.6-69.5% (CD8); untreated HIV-1: 5.5%, 0.3-34.8% (CD4), and 23.7%, 1.4-62.2% (CD8). No significant differences were found between infected cohorts, which exhibited significantly higher levels than seronegative controls (CD4: 1.1%, 0.7-2.0%; CD8: 2.7%, 1.3-22.7%).

<sup>b</sup> $\beta$ 2-microglobulin serum levels were assessed for 30 HIV-2 (median: 2.6 mg/l, range: 1.1-7.8 mg/l) and 14 HIV-1 (median: 2.4 mg/l, range: 1.3-6.0 mg/l) infected individuals, with no statistically significant differences being observed between cohorts.

**Supplemental Table 3.** Clinical and epidemiological characteristics of the HIV-2 and HIV-1 cohorts stratified according to degree of CD4<sup>+</sup> T-cell depletion.

	Seronegatives	HIV-2		HIV-1	
		>350 CD4 <sup>+</sup> /μl	<350 CD4 <sup>+</sup> /μl	>350 CD4 <sup>+</sup> /μl	<350 CD4 <sup>+</sup> /μl
<b>Number</b> [male/female]	16 [6/10]	22 [7/15]	16 [7/9]	10 [7/3]	10 [8/2]
<b>Age</b> , years	44 (27-57)	56 (28-78) <sup>*</sup>	51 (19-63)	41 (27-61)	35 (23-49) <sup>#</sup>
<b>Caucasian/black</b>	15/1	13/9	8/8	10/0	5/5
<b>CD4<sup>+</sup> T-cells</b> , %	59 (40-77)	45 (22-66) <sup>**/+</sup>	21 (4-32) <sup>***</sup>	46 (17-74) <sup>*</sup>	14 (2-32) <sup>***/+</sup>
<b>CD4<sup>+</sup> T-cells</b> /μl	818 (518-1312)	661 (406-1511) <sup>++</sup>	179 (52-344) <sup>***</sup>	885 (372-1848)	298 (18-344) <sup>***/+</sup>
<b>Viremia</b> (HIV RNA cp/ml)	-	200 (200-13627)	200 (200-34314)	5x10 <sup>3</sup> (40-19x10 <sup>3</sup> )	5x10 <sup>5</sup> (71-4470x10 <sup>3</sup> ) <sup>++/##</sup>
<b>Proviral DNA</b> (cp/10 <sup>6</sup> PBMC)	-	78 (5-1033)	57 (5-1002)	81 (5-573)	41 (5-975)
<b>B-cells</b> , %	6 (3-13)	7 (3-17)	6 (3-20)	5 (2-11)	6 (2-16)
<b>B-cells</b> /μl	120 (63-369)	141 (52-357)	100 (30-369)	118 (56-406)	117 (33-267)

Data are expressed as median, with limits in brackets. Statistical analysis was performed with Mann-Whitney tests.

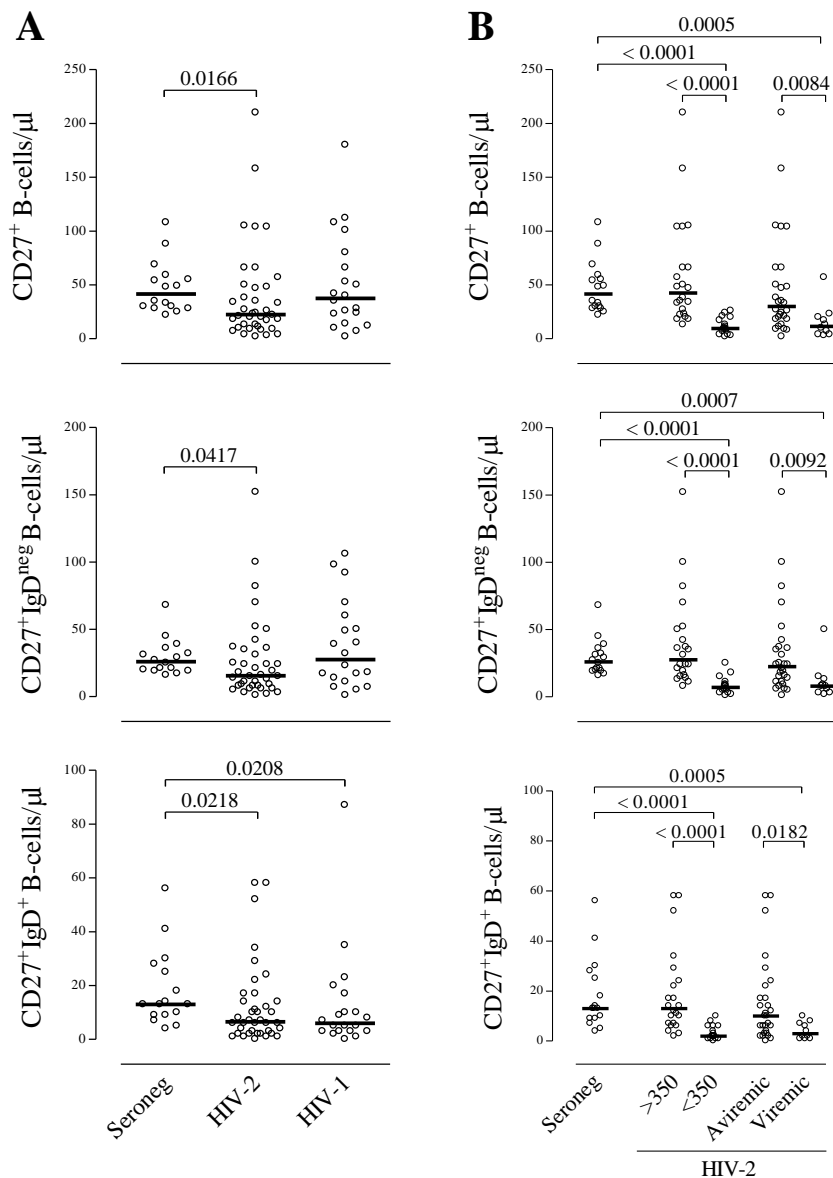
<sup>\*</sup>  $P < 0.05$ ; <sup>\*\*</sup>  $P < 0.01$ ; <sup>\*\*\*</sup>  $P < 0.001$  in comparison with seronegatives. <sup>++</sup>  $P < 0.01$ ; <sup>+++</sup>  $P < 0.001$  in comparison with the reciprocal subgroup (>350 versus <350). <sup>#</sup>  $P < 0.05$ ; <sup>##</sup>  $P < 0.01$  for comparisons between infected cohorts.

**Supplemental Table 4.** Clinical and epidemiological characteristics of the HIV-2 and HIV-1 cohorts stratified according to viremia status.

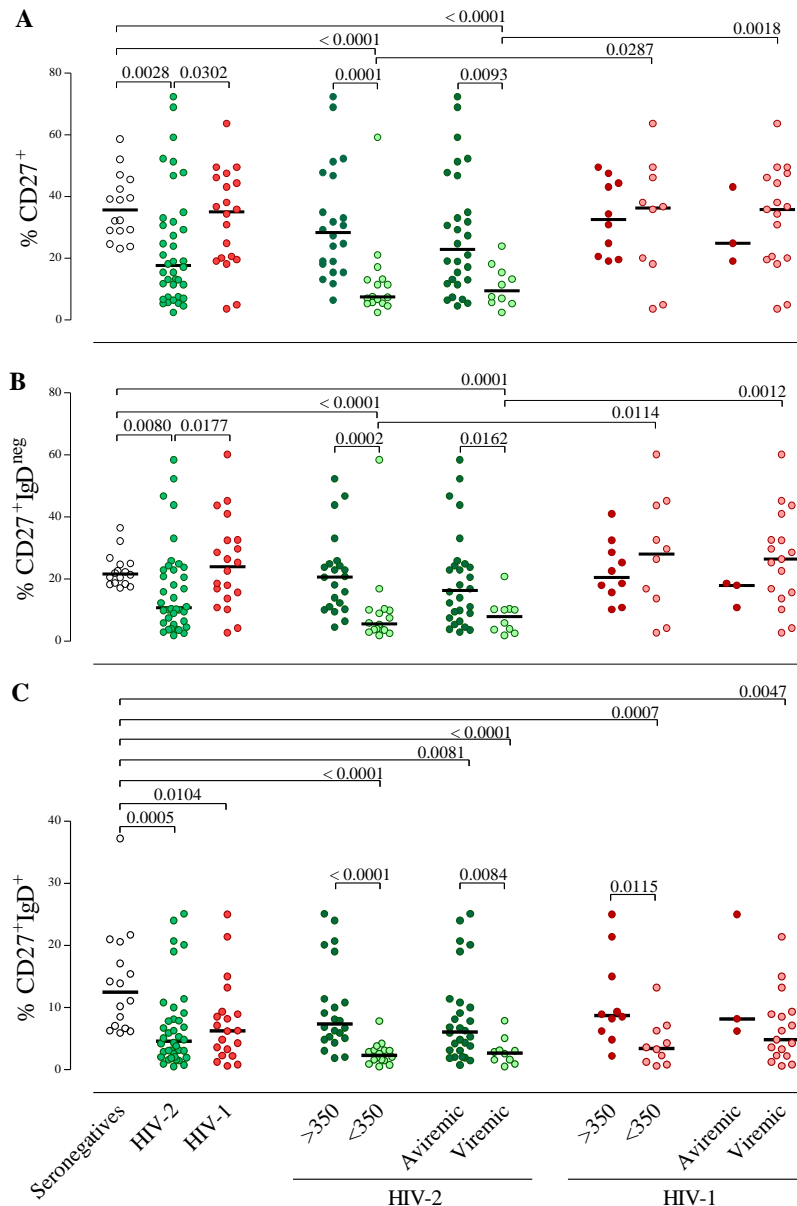
	Seronegatives	HIV-2		HIV-1	
		Aviremic	Viremic	Aviremic	Viremic
<b>Number</b> [male/female]	16 [6/10]	28 [9/19]	10 [5/5]	3 [2/1]	17 [13/4]
<b>Age</b> , years	44 (27-57)	56 (28-78)	52 (19-62)	47 (30-61)	38 (23-59)
<b>Caucasian/black</b>	15/1	17/11	4/6	3/0	12/5
<b>CD4<sup>+</sup> T-cells</b> , %	59 (40-77)	37 (13-66) <sup>***/+</sup>	24 (4-53) <sup>***</sup>	60 (59-74) <sup>#</sup>	27 (2-56) <sup>***/++</sup>
<b>CD4<sup>+</sup></b>	818	546	249	965	341
<b>T-cells</b> /μl	(518-1312)	(52-1511) <sup>*/+</sup>	(84-596) <sup>***</sup>	(521-1425)	(18-1848) <sup>**</sup>
<b>Viremia</b> (HIV RNA cp/ml)	-	<200	7.6x10 <sup>3</sup> (742-34314)	<40	1.9x10 <sup>4</sup> (71-4470x10 <sup>3</sup> )
<b>Proviral DNA</b> (cp/10 <sup>6</sup> PBMC)	-	5 (5-1033) <sup>+</sup>	187 (5-1002)	104 (5-324)	53 (5-975)
<b>B-cells</b> , %	6 (3-13)	7 (3-17)	7 (3-20)	10 (4-11)	6 (2-16)
<b>B-cells</b> /μl	120 (63-369)	135 (37-357)	131 (30-369)	125 (92-324)	111 (33-406)

Data are expressed as median, with limits in brackets. Statistical analysis was performed with Mann-Whitney tests.

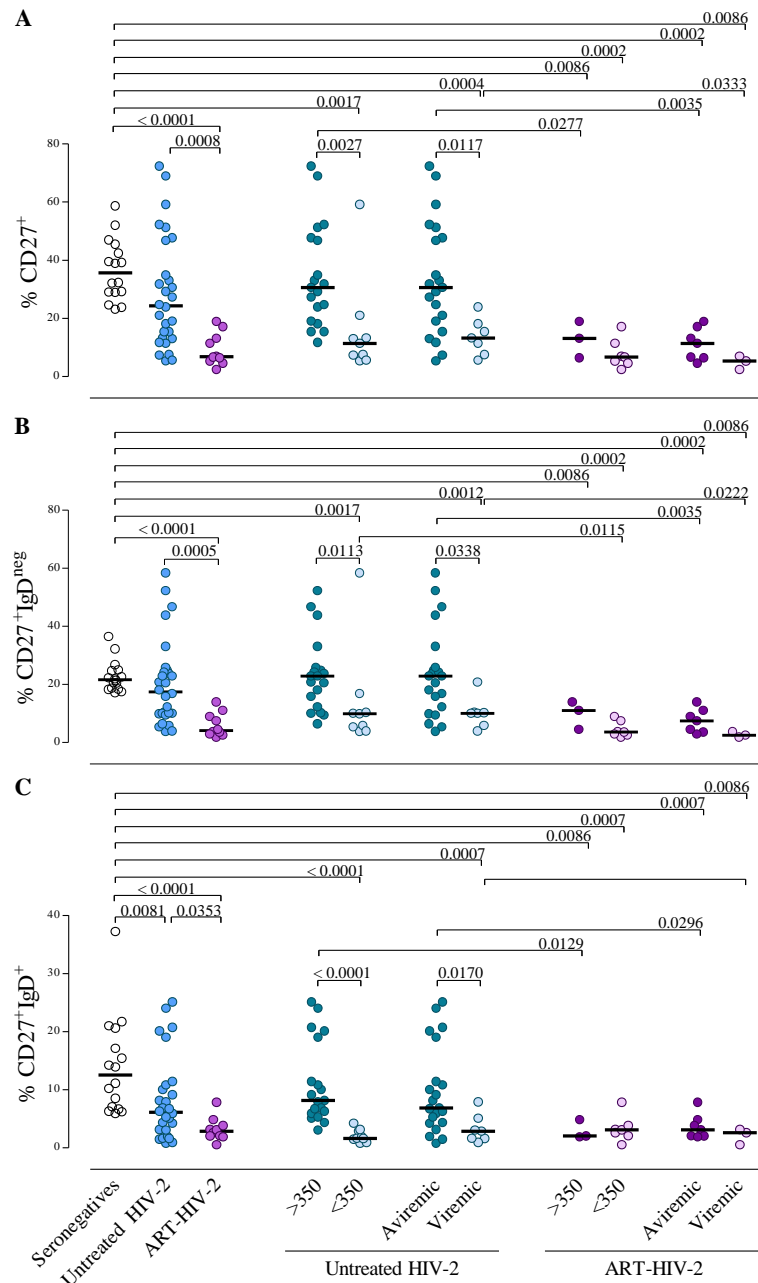
\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$  in comparison with seronegatives. <sup>+</sup> $P < 0.05$ ; <sup>++</sup> $P < 0.01$  in comparison with the reciprocal subgroup (Aviremic versus Viremic). <sup>#</sup> $P < 0.05$  for comparisons between infected cohorts.



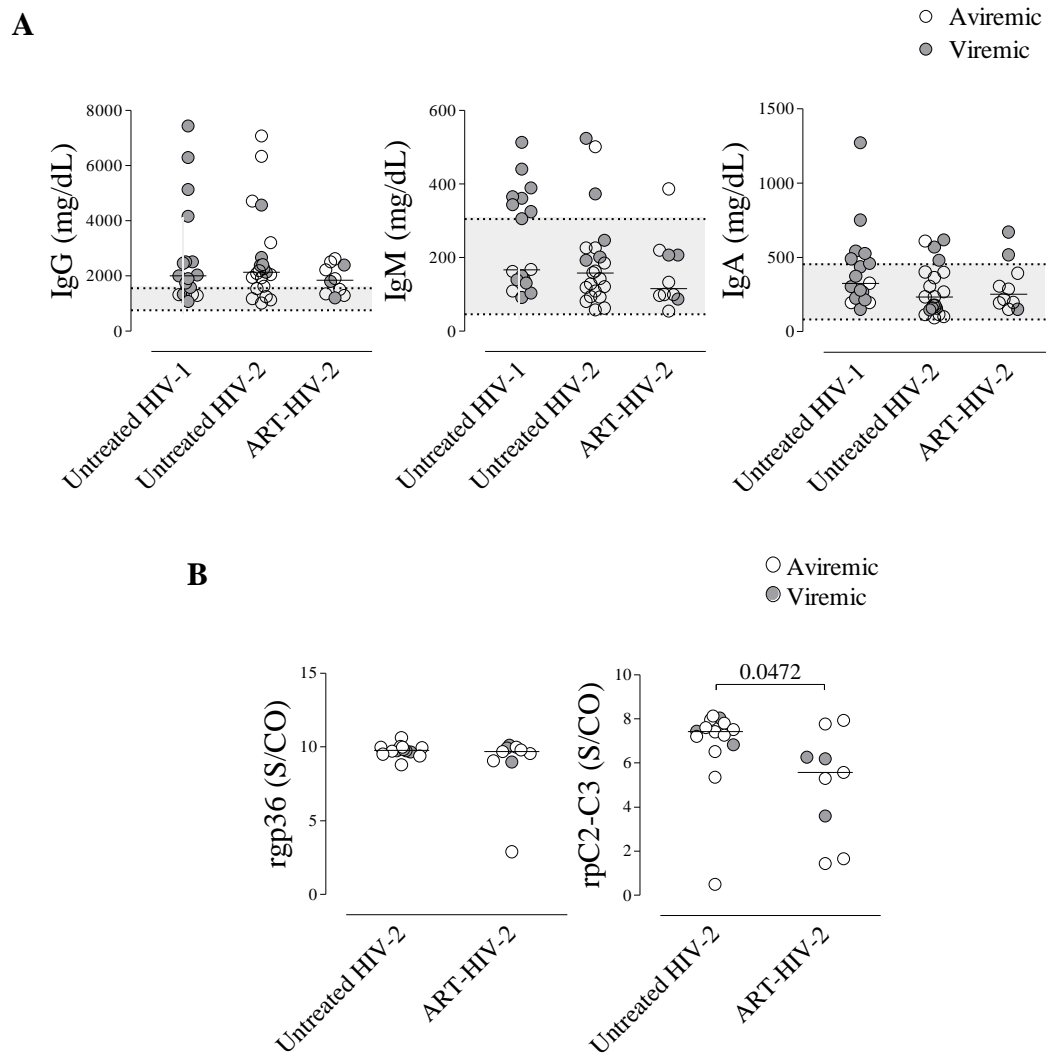
**Supplemental Figure 1. Absolute numbers of memory B-cell subsets in HIV-2 infected individuals.** (A) Circulating numbers of total memory cells (CD27<sup>+</sup>), switched memory cells (CD27<sup>+</sup>IgD<sup>neg</sup>) and unswitched memory cells (CD27<sup>+</sup>IgD<sup>+</sup>) in HIV-2 infected, as well as seronegative (Seroneg) and HIV-1+ individuals. (B) HIV-2+ patients were further subdivided according to disease stage (early: >350 CD4<sup>+</sup> T-cells/ $\mu$ l; late: <350 CD4<sup>+</sup> T-cells/ $\mu$ l) and levels of plasma viral load (aviremic: undetectable plasma viral load; viremic: detectable) and the absolute counts of memory B cell populations are shown. Each dot represents one individual and bars indicate median. Statistical analysis was performed using the Mann-Whitney test and significant *P* values are shown.



**Supplemental Figure 2. B-cell disturbances in HIV-2 as compared to HIV-1 groups of infected patients stratified to disease stage and viremia status.** Patients were divided according to disease stage (early: >350 CD4<sup>+</sup> T-cells/ $\mu$ l; late: <350 CD4<sup>+</sup> T-cells/ $\mu$ l) and levels of plasma viral load (aviremic: undetectable plasma viral load; viremic: detectable). Relative frequencies of (A) total memory cells (CD27<sup>+</sup>), (B) switched memory cells (CD27<sup>+</sup>IgD<sup>neg</sup>) and (C) unswitched memory cells (CD27<sup>+</sup>IgD<sup>+</sup>) within total B-cells. Comparisons were made between seronegatives and all groups of infected individuals. Each dot represents one individual and bars indicate median. Statistical analysis was performed using the Mann-Whitney test and significant *P* values are shown.

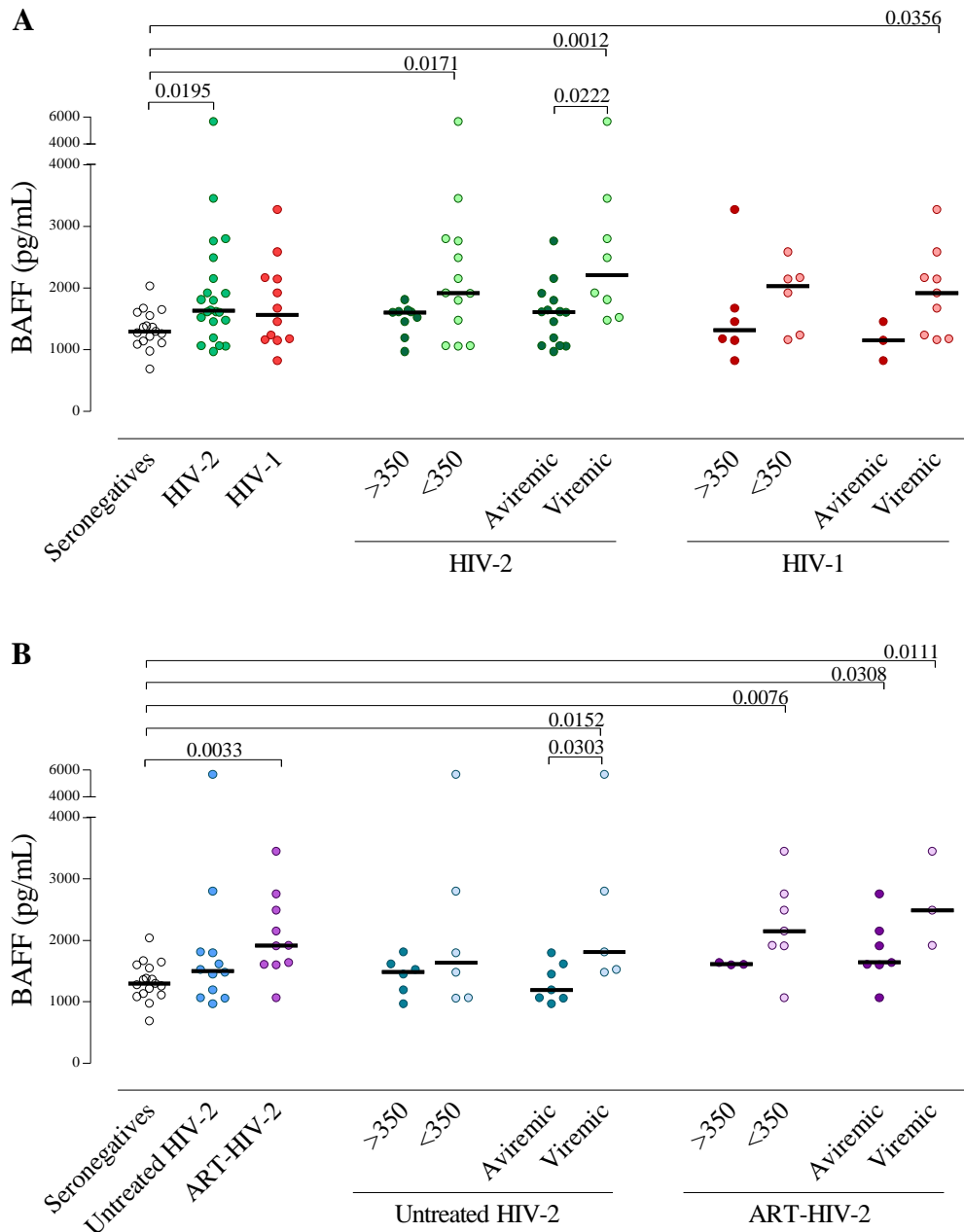


**Supplemental Figure 3. B-cell disturbances in treated as compared to untreated HIV-2 infected patients stratified to disease stage and viremia status.** Patients were divided according to disease stage (early: >350 CD4<sup>+</sup> T-cells/ $\mu$ l; late: <350 CD4<sup>+</sup> T-cells/ $\mu$ l) and levels of plasma viral load (aviremic: undetectable plasma viral load; viremic: detectable). Relative frequencies of (A) total memory cells (CD27<sup>+</sup>), (B) switched memory cells (CD27<sup>+</sup>IgD<sup>neg</sup>) and (C) unswitched memory cells (CD27<sup>+</sup>IgD<sup>+</sup>) within total B-cells. Comparisons were made between seronegatives and all groups of infected individuals. Each dot represents one individual and bars indicate median. Statistical analysis was performed using the Mann-Whitney test and significant *P* values are shown.



**Supplemental Figure 4. Serum levels of immunoglobulin and of specific antibodies against HIV-2 envelope glycoproteins gp125 and gp36.** (A) Serum levels of total IgG, total IgM, and total IgA in untreated HIV+ and in ART-treated HIV-2+ patients. (B) Serum levels of specific antibodies against gp36 and gp125 expressed as OD<sub>clinical sample</sub>/OD<sub>cut-off</sub> (S/CO) ratios in HIV-2+ individuals. Each dot represents one individual. Patients with viremia below cut-off are shown as open dots and viremic patients as filled dots. Normal range of immunoglobulin values is represented by grey shading. Statistical analysis was performed using the Mann-Whitney test and significant *P* values are shown. Bars represent median.





**Supplemental Figure 5. Serum BAFF levels in HIV infected individuals stratified to disease stage and viremia status.** Patients were divided according to disease stage (early:  $>350$   $CD4^+$  T-cells/ $\mu$ l; late:  $<350$   $CD4^+$  T-cells/ $\mu$ l) and levels of plasma viral load (aviremic: undetectable plasma viral load; viremic: detectable). Comparisons were made between seronegatives and (A) groups of HIV-2 and HIV-1 infected individuals; (B) groups of untreated and treated HIV-2 infected individuals. Each dot represents one individual and bars indicate median. Statistical analysis was performed using the Mann-Whitney test and significant  $P$  values are shown.