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## Case-control study of the immune status of humans infected with zoonotic gorilla simian foamy viruses

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2 Case-control study of the immune status of humans infected  
3 with zoonotic gorilla simian foamy viruses

4

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7

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15 40 word summary: Zoonotic simian foamy viruses (SFVs) establish persistent infections in humans, for  
16 whom the long-term consequences for health are poorly described. We show, for the first time, that  
17 chronic infection with SFV is associated with T lymphocyte differentiation and monocyte activation.

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19

## 20 Abstract

21 Background: Zoonotic simian foamy viruses (SFVs) establish persistent infections in humans, for whom  
22 the long-term consequences for health are poorly described. Here, we aimed to characterize blood-  
23 cell phenotypes and plasma biomarkers associated with gorilla SFV infection in humans.

24 Methods: We used a case-control design to compare 15 Cameroonian hunters infected with gorilla SFV  
25 (cases) to 15 controls matched for age and ethnicity. A flow cytometry-based phenotypic study and  
26 quantification of plasma immune biomarkers were carried out on blood samples from all participants.  
27 Wilcoxon signed rank tests were used to compare cases and controls.

28 Results: Cases had a significantly higher percentage of CD8 T lymphocytes than controls (median:  
29 17.6% vs. 13.7%,  $P = 0.03$ ), but similar levels of B, NK, and CD4 T lymphocytes. Cases also had a lower  
30 proportion of recent CD4 thymic emigrants (10.9% vs. 18.6%,  $P = 0.05$ ), a higher proportion of  
31 programmed death receptor 1 (PD-1) expressing memory CD4 T lymphocytes (31.7% vs. 24.7%,  $P =$   
32 0.01), and higher plasma levels of the soluble CD163 scavenger receptor (0.84 vs 0.59  $\mu\text{g}/\text{mL}$ ,  $P = 0.003$ )  
33 than controls.

34 Conclusion: We show, for the first time, that chronic infection with SFV is associated with T lymphocyte  
35 differentiation and monocyte activation.

36

## 37 Keywords

38 zoonosis, emergence, foamy virus, retrovirus, T lymphocyte, monocyte, immune activation, check-  
39 point inhibitor.

40

41 **Footnotes**

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51 This work was presented at the 26<sup>th</sup> conference on retroviruses and opportunistic infections (CROI)  
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54

## 55 Introduction

56 Foamy viruses (FV) are complex retroviruses that infect several mammal species, including nonhuman  
57 primates (NHPs) [1, 2]. Humans are not natural hosts of FV. However, they are susceptible to infection  
58 by zoonotic simian FV (SFV) [2, 3]. Such cross-species transmission occurs mainly through bites [4, 5].  
59 Viral exposure to several SFV species originating from apes and African and Asian monkeys leads to  
60 lifelong infection, demonstrated by the persistent detection of viral DNA in blood, isolation of  
61 replication-competent virus from blood or saliva, and high SFV-specific antibodies levels [4, 6-15].

62 Thus far, FV are considered to be apathogenic in natural, experimental, and accidental hosts, including  
63 humans [1, 2, 16]. However, we recently demonstrated hematological and biochemical alterations in  
64 SFV-infected humans relative to matched uninfected controls, including an increased prevalence of  
65 mild anemia and increased urea and creatinine blood levels [16]. Experimental inoculation of feline FV  
66 (FFV) to young, healthy, and specific pathogen-free cats leads to persistent infection, without clinical  
67 signs, over the first six months. FFV infection induces mild to moderate blood urea levels, an increased  
68 protein:creatinine ratio in urine, and histopathological and ultrastructural changes in the kidneys [17].  
69 Overall, these data support a subclinical impact of FV on host physiology, which is still largely  
70 unexplored.

71 As any chronic viral infection, either actively replicating or latent/reactivating, SFV infection may affect  
72 the immune system, including pro- and anti-inflammatory processes and innate and adaptive immune  
73 cells [25]. Indeed, we have documented higher IgG levels in SFV-infected humans than in matched  
74 controls [16]. In addition, blood lymphocytes are infected *in vivo* by SFV [13, 26, 27]. Thus, SFV may  
75 affect immunity by inducing immune responses and replicating in immune cells.

76 We set-up a case-control study, including 15 gorilla SFV-infected men living in Cameroon and 15  
77 controls, to define the impact of chronic SFV infection on the human immune system. We performed  
78 a detailed phenotypic study of their blood T, B, and NK lymphocytes. In addition, we quantified immune  
79 biomarkers in their plasma samples to gain information on the lymphoid and myeloid activation state.

80 For the first time, we report differences between SFV-infected and uninfected controls that provide  
81 information on the physiological consequences of zoonotic SFV infection for humans.

## 82 Materials and methods

83 **Study design and participants.** The research was conducted in accordance with the Helsinki  
84 declaration. Ethics approval was obtained from the relevant authorities in Cameroon (National Ethics  
85 Committee and Ministry of Health) and France (*Commission Nationale de l'Informatique et des*  
86 *Libertés*, and *Comité de protection des personnes Ile de France IV*). This study was registered at  
87 [www.clinicaltrials.gov](https://clinicaltrials.gov), <https://clinicaltrials.gov/ct2/show/NCT03225794/>. All participants gave  
88 written informed consent.

89 Participants were Cameroonian men who had been injured by an NHP during hunting. Cases consisted  
90 of individuals infected with a gorilla SFV recruited from participants of our former survey [4, 16]. SFV  
91 infection was defined by positive results on both western blots (presence of the p70-p74 Gag doublet)  
92 and PCR assays (*integrase* gene and/or LTR) [4]. Each case was matched individually for age ( $\pm$  10 years)  
93 and ethnicity with one non-SFV-infected control, recruited from hunters who participated in the same  
94 survey and who lived in the same or neighboring villages as the cases [16]. All participants tested  
95 seronegative for HIV-1 infection (LAV Blot1, Cat No. 72251, Biorad). Six cases and three controls tested  
96 seropositive for human T-cell leukemia virus type 1 (HTLV-1) infection (HTLV Blot 2.4, MP Diagnostics),  
97 no participants tested seropositive for HTLV-2. All participants of the study were apparently healthy at  
98 the time of the analysis.

99 **Biological evaluations.** Blood of the 30 participants was collected into tubes containing EDTA.  
100 Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation and stored in  
101 liquid nitrogen. Plasma samples were stored at  $-80^{\circ}\text{C}$ . Blood tests were carried out by the medical  
102 analysis laboratory at the Centre Pasteur du Cameroun (CPC), in Yaoundé [16]. PBMCs were stained  
103 with Live-Dead-Aqua (Life Technologies, Saint-Aubin, France) and the following antibodies were used  
104 for phenotypic characterization of lymphocyte subsets: CD3-FITC, CD8-Alexa700, CD16-V450, CD19-

105 PerCP-Cy5.5, CD27-V450, CD45RA-V450, CD56-PC7, CD57-APC, CD183(CXCR3)-Alexa 488,  
106 CD194(CCR4)-Alexa 647, CD196(CCR6)-PE, CD197(CCR7)-PC7, TCR  $\gamma\delta$ 1-FITC, TCR V $\delta$ 2-PE (BD, Le Pont  
107 de Claix, France), CD3-ECD, CD8 $\beta$ -ECD, CD10-PE, CD20-ECD, CD21-FITC, CD24-APC, CD27-PE, CD28-  
108 FITC, CD28-PC5, CD31-FITC, CD38-PC5, CD127-PE, HLA-DR-FITC (Beckman Coulter, Villepinte, France),  
109 CD4-APC-eFluor780, CD8 $\alpha$ -A700, CD25-APC, CD38-PC5, CD161-APC, CD279(PD-1)-PE, and integrin  $\beta$ 7-  
110 FITC, (e-bioscience, Paris, France). Data were collected on an LSR II cytometer (BD Biosciences) and  
111 analyzed with Flow-Jo software (Treestar, Ashland, USA). Lymphocyte subset definitions are indicated  
112 in the figure legends and tables. Measurements of plasma analytes were performed using the human  
113 high-sensitivity T-cell 21-plex magnetic milliplex assay (HSTCMAG28PMX21BK, Merck Millipore, saint-  
114 Quentin en Yvelines, France), Human Magnetic Luminex Assay (for BAFF, CXCL9, CXCL10, CXCL13 and  
115 TRAIL, Biotechne, Lille, France), and the ELISA kits DY383 (sCD14) and DY1607 (sCD163) from  
116 Biotechne. The SFV DNA level in buffy coat was quantified by PCR at the time of inclusion in the survey  
117 [4].

118 **Statistics.** Wilcoxon signed-rank tests were used to compare the quantitative variables between cases  
119 and controls. Fisher's exact test was used to analyze qualitative variables. The Mann-Whitney test was  
120 used to compare HTLV-1 seronegative (HTLV-1<sup>neg</sup>) and seropositive (HTLV-1<sup>pos</sup>) individuals. The power  
121 of statistical analyses was calculated for the variables that differed significantly between cases and  
122 controls with an  $\alpha$  of 0.05 for a two-sided test. Spearman's rank test was used to assess correlations  
123 between quantitative parameters.

## 124 Results

### 125 Study participants

126 Participants were men living in rural areas of East and South Cameroon who reported injuries, mostly  
127 bites inflicted during hunting activities [16], and from whom cryopreserved mononuclear cells were  
128 available. The ages ranged from 22 to 75 years, with median values of 45 and 50 years for the cases

129 and controls, respectively (Table 1). The duration of SFV infection ranged from 1 to 45 years, and the  
130 median value was 14 years.

### 131 **SFV-infected individuals have a higher percentage of CD8 T lymphocytes than controls**

132 We used flow cytometry to quantify T, B, and NK lymphocytes and their major subsets (Figure 1). The  
133 cases had a significantly higher percentage of CD8 T lymphocytes and lower CD4/CD8 ratios than  
134 controls (median: 17.6% vs. 13.7%,  $P = 0.03$  and 2.5 vs. 3.5,  $P = 0.04$ , respectively, Figure 1A). The  
135 statistical power of the test for the percentage of CD8 T lymphocytes and CD4/CD8 ratios were  $> 0.80$   
136 and  $> 0.60$ , respectively. The percentage of CD4 T lymphocytes was similar for cases and controls  
137 (46.6% vs. 46.9%,  $P = 0.73$ ). The percentage of gamma-delta T ( $T\gamma\delta$ ), B, and NK lymphocytes also did  
138 not differ between cases and controls (2.5% vs. 2.8%,  $P = 0.86$ , 12.3% vs. 10.5%,  $P = 0.55$ , and 9.6% vs.  
139 6.5%,  $P = 0.30$ , respectively, Figure 1B). Furthermore, the repartition of the major  $T\gamma\delta$ , B, and NK  
140 lymphocyte subsets were similar in the two groups (Supplementary Table 1). In conclusion, SFV-  
141 infected men had an expanded CD8 T lymphocyte population relative to matched controls.

### 142 **SFV-infected individuals have more highly differentiated CD4 and CD8 T lymphocytes than controls**

143 We then used CD45RA, CCR7, CD31, CD27, CD28 molecules to define the phenotype of CD4 and CD8 T  
144 lymphocytes. CD4 and CD8 T lymphocytes were more highly differentiated in cases than in controls.  
145 Indeed, the percentage of naive ( $T_N$ ) and recent thymic emigrants ( $T_{RTE}$ ) among CD4 T lymphocytes  
146 were lower in cases than controls (Figure 2A). Conversely, differentiated CD27<sup>-</sup> effector memory ( $T_{EM}$ )  
147 cells represented a higher proportion of CD4 T lymphocytes in cases than controls. The differences  
148 were statistically significant for CD4  $T_{RTE}$  (10.9% vs. 18.6%,  $P = 0.05$ , Power  $> .70$ ). Among CD8  
149 lymphocytes, we observed a significantly higher percentage of the two  $T_{EM}$  subsets lacking CD28  
150 expression in the cases than controls ( $T_{EM}27^+28^-$ : 2.5 vs. 0.9,  $P = 0.02$ ;  $T_{EM}27^-28^-$ : 16.1 vs. 10.8,  $P = 0.02$ ,  
151 Figure 2B). CD8  $T_{EM}$  subset levels showed high interindividual variation and the power of these analyses  
152 was  $< 0.50$ .

153 We further defined the T-cell phenotype by the quantification of the CXCR3, CCR4, and CCR6  
154 chemokine receptors, which reflect their polarization. We also assessed the expression of CD161 and  
155  $\alpha 4\beta 7$  molecules which are markers of gut homing capacity. Cases had significantly higher levels of  
156 CD161<sup>+</sup> CD4 T lymphocytes and a higher proportion of CXCR3<sup>+</sup>CCR4<sup>+</sup>CCR6<sup>+</sup> (Th1/Th17) cells among their  
157 CD8 T<sub>EM</sub> lymphocytes (supplementary Table 2). While the differences in levels of polarized T  
158 lymphocytes were modest, they were consistent with a more differentiated T-cell compartment in  
159 cases than in controls.

160 **SFV-infected individuals express higher levels of check-point inhibitor, PD-1, on memory CD4 T**  
161 **lymphocytes than controls.**

162 Then, molecules defining functional capacity (PD-1, CD57), homeostatic proliferation potential (CD127,  
163 the high affinity IL-7 receptor) and activation (HLA-DR, CD38) were quantified on memory CD4 and CD8  
164 T lymphocytes. The percentage of PD-1<sup>+</sup> memory CD4 T lymphocytes (PD-1<sup>+</sup>CD4T<sub>M</sub>) was significantly  
165 higher in cases than controls (31.7% vs. 24.7%,  $P = 0.01$ , Figure 3A). The statistical power of the test  
166 was  $> 0.85$ . The differences were observed in both CD4 T<sub>CM</sub> and T<sub>EM</sub> subsets (Supplementary Table 2).  
167 In contrast, the percentage of PD-1<sup>+</sup>CD8T<sub>M</sub> lymphocytes was similar for cases and controls (Figure 3B).  
168 The proportion of CD57<sup>+</sup> cells among total memory CD4 and CD8 T lymphocytes was similar in cases  
169 and controls (Figure 3). However, CD57 was expressed by the most highly differentiated CD28<sup>-</sup>T<sub>EM</sub> and  
170 T<sub>E</sub> lymphocytes. Indeed, the percentage of CD57<sup>+</sup>CD8 T<sub>EM</sub> among all CD8 T lymphocytes was higher in  
171 cases than controls (Supplementary Table 2), a finding consistent with the higher level of total CD28<sup>-</sup>  
172 CD8T<sub>EM</sub> in cases (Figure 2). Cases and controls expressed similar levels of CD127, HLA-DR, and CD38.  
173 We also quantified CD25<sup>hi</sup>CD127<sup>+</sup> CD4 regulatory (T<sub>REG</sub>) lymphocytes, which dampen immune  
174 activation. Their percentage (Figure 3A) and differentiation status (Supplementary Table 2) were  
175 similar in cases and controls. In conclusion, increased expression of PD-1 molecule, a check-point  
176 inhibitor, on CD4 T<sub>M</sub> lymphocytes was the most striking difference between cases and controls.

177 **SFV-infected individuals have higher plasma sCD163 levels than controls**

178 We then quantified 27 cytokines, chemokines, and soluble immune mediators in plasma samples using  
179 multiplex or ELISA assays (Supplementary Table 3). Cases had significantly higher levels of GM-CSF than  
180 controls (64 vs. 46 pg/mL,  $P = 0.04$ ) and tended to have lower IL-8 levels (11 vs. 18 pg/mL,  $P = 0.06$ ).  
181 The statistical power was  $< 0.60$  for these analyses. Cases and controls had similar levels of CCL3, CCL4,  
182 CCL20, IFN- $\gamma$ , IL-2, IL-7, IL-10, IL-12, IL-17, IL-21, IL-23, and TNF- $\alpha$ . Cytokines and chemokines associated  
183 with B-lymphocyte function and activation, namely CXCL13, BAFF, IL-4, IL-5 and IL-13, were present at  
184 comparable levels in cases and controls.

185 Among the plasma molecules associated with inflammation and myeloid cell activation, sCD163 levels  
186 were significantly higher in cases than controls (0.84 vs. 0.59  $\mu\text{g/mL}$ ,  $P = 0.003$ , Figure 4A); the  
187 statistical power of this analysis was  $> 0.95$ . Plasma levels of sCD14, IL-1 $\beta$ , and IL-6 were similar in the  
188 two groups (Figure 4A), as were those of the chemokines and death factors induced by type I and type  
189 II IFNs, namely CXCL9/MIG, CXCL10/IP-10, CXCL11/ITAC and TRAIL (Figure 4B). In conclusion, sCD163  
190 was the only plasma molecule present at a significantly different level in cases and controls.

#### 191 **Lack of an association between immune parameters, infection status, and haematological variables**

192 Five immune parameters were expressed at significantly different levels between cases and controls,  
193 with statistical power of the analyses  $> 0.70$ : percentage of CD8 cells, CD4/CD8 ratio, CD4T<sub>RTE</sub>, PD-  
194 1<sup>+</sup>CD4T<sub>M</sub>, and sCD163 levels. The percentage of CD8 cells strongly correlated with the CD4/CD8 ratio  
195 in both cases (Spearman's  $\rho = -0.835$ ,  $P = 0.0001$ ) and controls ( $\rho = -0.693$ ,  $P = 0.004$ ). We observed  
196 no other correlation between these five parameters (Table 2).

197 Among cases, the five immune parameters were not associated with the duration of infection or SFV  
198 DNA load in blood cells. No immune parameter was associated with age in cases or controls. Complete  
199 blood counts and biochemistry were available for 13 cases and 15 controls [16]. The immune  
200 parameters were not associated with the haematological parameters that differ between the two  
201 groups, *i.e.* haemoglobin, urea, creatinine, and lactate dehydrogenase levels. In conclusion, cases and

202 controls had significantly different immune and haematological profiles, without significant  
203 correlations between the immune and haematological variables in either group.

#### 204 **Immune parameters and SFV infection: no obvious effect of age and viral coinfections**

205 HTLV-1 is a possible confounder, as this infection is found more frequently in SFV-infected individuals  
206 than in matched controls [5]. In our study, 40% of cases and 20% of controls were seropositive for  
207 HTLV-1, and none were seropositive for HTLV-2. The percentage of PD-1<sup>+</sup>CD4T<sub>M</sub> was significantly higher  
208 in HTLV-1<sup>POS</sup> than in HTLV-1<sup>NEG</sup> participants, whereas the percentage of CD8 cells, the CD4/CD8 ratio,  
209 and CD4T<sub>RTE</sub> and sCD163 levels were similar in both groups (Table 3). Among HTLV-1<sup>NEG</sup> participants,  
210 we found trends or significant differences between cases and controls for the percentage of CD8 cells,  
211 the CD4/CD8 ratio, and CD4T<sub>RTE</sub>, and sCD163 levels. The percentage of PD-1<sup>+</sup>CD4T<sub>M</sub> was higher in cases  
212 than in controls, but the difference was not significant. Both the case and control were HTLV-1<sup>POS</sup> for a  
213 single pair of participants, precluding further analysis. Overall, HTLV-1 coinfection cannot account for  
214 all the differences observed between cases and controls.

215 The immune parameters that differ between cases and controls can be modified by several other  
216 chronic viral infections and ageing. After exclusion of participants older than 65 years, differences in  
217 the percentage of CD8 cells and the CD4/CD8 ratio between cases and controls were observed but  
218 were not statistically significant. Differences in CD4T<sub>RTE</sub>, PD-1<sup>+</sup>CD4T<sub>M</sub>, and sCD163 levels remained  
219 significant or closed to significance. CMV and HIV-1 are major drivers of immune activation and ageing.  
220 CMV seroprevalence is close to 100% in the Sub-Saharan African population [28] and all participants  
221 were seronegative for HIV-1 infection. All cases and controls were infected with HBV and two cases  
222 tested positive for HBs antigen [16]. Our recent nationwide survey showed an HCV prevalence of  
223 approximately 5% in men from the southern and eastern regions of Cameroon [29]. The HCV  
224 seroprevalence was 0.6% in Pygmies included in our epidemiological survey [30]. Among the eight  
225 Bantus included in the study, only one control was born before 1960 and thus at high risk for HCV  
226 infection through the iatrogenic route [31]. Furthermore, no liver function anomaly was shown by

227 blood analysis [16]. Overall, CMV, HIV-1, HBV, and HCV coinfection should not account for differences  
228 observed between cases and controls.

## 229 Discussion

230 In a case-control study, we show that humans infected with a zoonotic gorilla SFV have a more highly  
231 differentiated T-lymphocyte phenotype and elevated plasma levels of sCD163, a biomarker of  
232 monocyte activation. These data are the first description of the blood immune parameters of infected  
233 humans and support that SFVs induce an immune reaction common to several chronic viral infections.

234 Here, we show that SFV infection is associated with an increased percentage of blood CD8 T  
235 lymphocytes, a decreased CD4/CD8 ratio, a reduced proportion of naive CD4 T lymphocytes, and  
236 increased expression of the PD-1 molecule on memory CD4 T lymphocytes. These phenotypic changes  
237 are consistent with the T lymphocytes responding to a viral infection and overlap those induced by  
238 HIV-1, CMV, and ageing [32]. We also observed increased IgG levels, another marker of viral infection  
239 [16], in the same population of SFV-infected individuals. Experimental SFVmfa infection of macaques  
240 through transfusion leads to a sequential drop in the number of peripheral blood CD4 and CD8 T  
241 lymphocytes, followed by their restoration, with an elevated proportion of CD8 T lymphocytes [33].  
242 Overall, these data indicate that chronic SFV infection is associated with phenotypic changes in T  
243 lymphocytes.

244 Our data need to be interpreted with caution, because biological markers that differ between SFV-  
245 infected and noninfected individuals may result from SFV infection or other health conditions. Here,  
246 we observed that differences in the percentage of CD8 cells and the CD4/CD8 ratio between cases and  
247 controls were less marked in the youngest participants than in the entire group (Table 3). Similarly, the  
248 differences in the percentage of PD-1<sup>+</sup>CD4T<sub>M</sub> were not significant in participants not infected by HTLV-  
249 1. Age and HTLV-1 infection may be true confounders or stratified analysis may lead to spurious results  
250 due to a small sample size. Importantly, neither age nor HTLV-1 infection modified the five immune  
251 parameters. HTLV-1 induces total CD4 and T<sub>REG</sub>-cell expansion [34]. Here, we only observed CD8 T-cell

252 expansion. As we previously discussed [16], the similarity of clinical signs and leucocyte subset  
253 distributions between SFV-infected individuals and controls support adequate matching for health  
254 factors.

255 The observed T-lymphocyte phenotype could result from multiple causes, such as ongoing viral  
256 replication, infection of immune cells, and coinfections. Direct proof of SFV replication *in vivo* (*i.e.* the  
257 presence of SFV RNA, viral proteins, or virions) is currently lacking in humans. Two studies used  
258 quantitative RT-PCR to detect SFV RNA in human blood and buccal samples and obtained negative  
259 results [12, 35]. These data do not rule out active SFV replication in humans, which may occur at levels  
260 below the detection threshold of conventional PCR-based assays or in tissues. Indeed, among patients  
261 infected with HIV, HBV, and HCV, some have undetectable viral RNA in blood, despite replication in  
262 tissues [36-38]. These situations of HIV control and occult HBV/HCV infection are associated with  
263 potent virus-specific responses and a mild disease course relative to infections with detectable viral  
264 RNA in blood. Despite apparent viral control, infected individuals show elevated inflammation and  
265 some ultimately experience clinical disease or loss of viral control [36-38]. The present data and those  
266 obtained on hematological parameters and specific antibodies challenge the proposed SFV latency in  
267 humans, simians and felines [2, 14-17, 39].

268 FV establishes wide tissue tropism after natural or experimental infection in NHPs, cats, and cattle,  
269 with consistent detection of viral DNA in blood and lymphoid tissues [17, 21, 40-42]. The separation of  
270 lymphocyte subsets from infected individuals showed infection of B and T lymphocytes [13, 26, 27].  
271 No human tissue samples have yet been analyzed. SFVs encode a viral transactivator, Tas, which acts  
272 on cellular genes [43], and microRNAs that suppress innate immunity [44]. Therefore, SFV can infect  
273 immune cells and directly alter their function, similarly to the two human pathogenic retroviruses, HIV-  
274 1 and HTLV-1. Immune changes in SFV-infected individuals may be induced by coinfecting pathogens.  
275 Indeed, interactions with other retroviruses have been demonstrated in animals [18-22]. Microbial  
276 coinfections are a hallmark of infection with the potent immunosuppressive virus HIV-1 [45] and are

277 also common in HTLV-1-infected patients [34]. Whether direct or indirect consequences of SFV  
278 infection are related to the immune changes reported here is an open question.

279 We found significantly elevated sCD163 levels in SFV-infected individuals relative to matched controls.  
280 The differences were particularly robust, with high statistical power, despite the small size of the study  
281 population. CD163 is a transmembrane protein that acts as the hemoglobin scavenger receptor [46].  
282 It is expressed by monocytes and alternatively-activated M2 macrophages. These cells are involved in  
283 tissue repair and mediate mostly anti-inflammatory functions [47]. Upon inflammatory macrophage  
284 activation, the ectodomain is cleaved by matrix metalloproteinases and a soluble form, sCD163, is shed  
285 into the plasma [48]. sCD163 is not involved in hemoglobin or iron metabolism.

286 Of eight plasma molecules related to myeloid-cell activation, sCD163 was the only one for which the  
287 levels differed between SFV-infected individuals and controls. This may result from the activation of a  
288 specific pathway or direct infection, as described above. Indeed, one regulatory FFV protein, Bet, was  
289 detected by immunochemistry in the cytoplasm of macrophage-like cells from several lymphoid organs  
290 (lymph nodes, thymus, tonsil, and spleens) from infected cats [49]. Overall, we document a novel  
291 finding in SFV-infected humans, supporting monocyte/macrophage activation.

292 One limit of our study is that statistically significant associations do not prove causality. Although we  
293 observed immune differences between SFV-infected individuals and carefully matched controls, we  
294 cannot conclude that they are a direct consequence of SFV infection. However, the experimental  
295 infection of cats supports the causal relationship between FV infection, persistent lymphocytic  
296 infiltration in peripheral organs, and histological features in kidneys [17]. Importantly, the biological  
297 findings in SFV-infected hunters are plausible consequences of viral infection.

298 Obtaining biological samples from hunters living in remote areas of the Cameroonian forest is  
299 challenging. The number of participants and quantity of blood drawn are two limiting factors. For  
300 example, persisting T-lymphocyte activation drives other conditions, such as anemia or chronic kidney  
301 diseases. Correlations between viral parameters and host biomarkers were expected, but not

302 observed; this may result from insufficient power for statistical analyses or from the impact of other  
303 health conditions. Importantly, despite the modest size of our study population, we had sufficient  
304 statistical power to perform the analyses of several important parameters.

305 The demonstration of frequent transmission of SFV from NHPs to humans raises the question of  
306 whether SFV infection has consequences for human health, as zoonotic agents, and as vectors for gene  
307 therapy. This first exploration of blood mononuclear cell phenotypes and plasma biomarkers shows  
308 statistically significant differences between SFV-infected individuals and matched controls, supporting  
309 a response of T lymphocytes and monocytes. Whether a causal relationship exists between immune  
310 activation, anemia, and renal alterations is yet to be established.

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314 speaker.

315

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424

425 Figure legends

426 **Figure 1. Major lymphocyte subsets among cases and controls.** Frozen PBMCs from cases and controls  
427 were stained with a viability marker and antibodies defining major lymphocyte subsets. Results are  
428 expressed as the percentage of viable lymphocytes. Wilcoxon signed-rank tests were used to compare  
429 cases and controls and *P* values are indicated above the graphs. A: T lymphocytes (CD3<sup>+</sup>), CD4 T  
430 lymphocytes (CD3<sup>+</sup>CD4<sup>+</sup>), CD8 T lymphocytes (CD3<sup>+</sup>CD8<sup>+</sup>), and CD4/CD8 ratio; B:  $\gamma\delta$  T lymphocytes  
431 (CD3<sup>+</sup>TCR $\gamma\delta$ <sup>+</sup>), B lymphocytes (CD19<sup>+</sup>), and NK lymphocytes (CD3<sup>-</sup>CD16<sup>+</sup>and/orCD56<sup>+</sup>).

432 **Figure 2. T-lymphocyte differentiation among cases and controls.** Frozen PBMCs from cases and  
433 controls were stained with a viability marker and antibodies defining T lymphocytes (CD3, CD4, and  
434 CD8) and their differentiation phenotype. Results are expressed as the percentage among viable CD4  
435 or CD8 T lymphocytes. Wilcoxon signed-rank tests were used to compare cases and controls and *P*  
436 values are indicated above the graphs. Naive T cells (T<sub>N</sub>) were defined as CD45RA<sup>+</sup>CCR7<sup>+</sup>, CD4 recent  
437 thymic emigrant (T<sub>RTE</sub>) as CD45RA<sup>+</sup>CCR7<sup>+</sup>CD31<sup>+</sup>, central memory (T<sub>CM</sub>) as CD45RA<sup>-</sup>CCR7<sup>+</sup>, effector  
438 memory (T<sub>EM</sub>) as CD45RA<sup>-</sup>CCR7<sup>-</sup>, and effector (T<sub>E</sub>) as CD45RA<sup>+</sup>CCR7<sup>-</sup>. CD4 T<sub>EM</sub> were further defined by  
439 expression of the CD27 molecule, and CD8 T<sub>EM</sub> by the expression of the CD27 and CD28 molecules. A:  
440 CD4 T lymphocytes, B: CD8 T lymphocytes.

441 **Figure 3. T-lymphocyte phenotype and regulatory T cells among cases and controls.** Frozen PBMCs  
442 from cases and controls were stained with a viability marker and antibodies defining T lymphocytes  
443 (CD3, CD4 and CD8) and their phenotype: PD-1 (CD279), CD57, CD127, HLA-DR, and CD38. Results are  
444 expressed as the percentage of viable memory (non CD45RA<sup>+</sup>CCR7<sup>+</sup>) CD4 or CD8 T lymphocytes. Results  
445 for CD4 T<sub>REG</sub> (CD25<sup>+</sup>CD127<sup>-</sup>) are expressed as the percentage among viable CD4 T lymphocytes.  
446 Wilcoxon signed-rank tests were used to compare cases and controls and *P* values are indicated above  
447 the graphs. A: CD4 T lymphocytes, B: CD8 T lymphocytes.

448 **Figure 4. Plasma molecules associated with myeloid cells among cases and controls.** Frozen plasma  
449 samples were used to quantify plasma analytes using multiplex bead-based assays or ELISA. Results

450 are presented as pg/mL, except for sCD14 and sCD163, for which the levels are expressed as  $\mu\text{g/mL}$ .

451 Wilcoxon signed-rank tests were used to compare cases and controls and *P* values are indicated above

452 the graphs.

453

1 **Table 1. Characteristics of study participants**

	Cases	Controls	<i>P</i> <sup>a</sup>
Ethnicity: Bantus/Pygmies	4/11	4/11	1.00
Age at sampling, years	45 [40-68]	50 [39-58]	0.02
Duration of infection, years <sup>b</sup>	14 [12-37]	--	--
HTLV-1 infection, Yes/No	6/9	3/12	0.43

2 Abbreviations: Interquartile range, IQR; Human T lymphotropic virus type 1, HTLV-1.

3 <sup>a</sup>Counts or median [IQR] are indicated; Fisher's exact test and Wilcoxon signed-rank tests were used to  
 4 compare cases and controls. <sup>b</sup>The duration of infection in the case group was estimated as the time  
 5 between the reported date of the wound inflicted by the gorilla and the sampling date.

6

1 **Table 2. Immune parameters that differ between cases and controls are not correlated with**  
 2 **parameters of SFV infection and hematological variables.**

	Group	CD8% <sup>a</sup>	CD4/CD8	CD4 T <sub>RTE</sub>	PD-1 <sup>+</sup> CD4 T <sub>M</sub>	sCD163
CD8%	Cases	-.835***				
	Controls	-.693**				
CD4/CD8	Cases	-.111	.233			
	Controls	.111	.104			
CD4 TRTE	Cases	.027	-.283	-.036		
	Controls	.196	.007	-.139		
PD-1+CD4 TM	Cases	-.297	.207	.130	.182	
	Controls	.039	-.050	.269	.000	
Duration of infection	Cases	.139	-.142	-.360	.320	.009
SFV DNA	Cases	.050	-.316	-.168	-.417	.218
Age	Cases	.150	-.288	-.154	.091	.037
	Controls	-.307	.041	-.200	-.433	.160
Hemoglobin	Cases	.108	-.008	.083	-.400	.523
	Controls	.307	-.222	.222	-.031	.052
Urea	Cases	.054	-.169	.204	.633	.137
	Controls	-.366	.470	.045	-.375	.061
Creatinine	Cases	.327	-.252	.128	.092	-.231
	Controls	.044	.013	.118	.179	.387
LDH	Cases	-.041	.146	-.017	.383	-.105
	Controls	.250	-.286	.243	.103	-.214

3

4 <sup>a</sup>Five immune parameters differed significantly between cases and controls. CD8%: percentage of CD8  
 5 T lymphocytes among lymphocytes; CD4/CD8: ratio of CD4 and CD8 T lymphocytes; CD4 T<sub>RTE</sub>:  
 6 percentage of CD31<sup>+</sup>CD45RA<sup>+</sup>CCR7<sup>+</sup> recent thymic emigrants among CD4 T lymphocytes; PD-1<sup>+</sup>CD4T<sub>M</sub>:  
 7 percentage of PD-1<sup>+</sup> cells among memory (nonCD45RA<sup>+</sup>CCR7<sup>+</sup>) CD4 T lymphocytes; sCD163: plasma  
 8 level of soluble CD163 (µg/mL). <sup>b</sup>Spearman's rank test was used to assess correlations between  
 9 parameters. Spearman's correlation coefficients are indicated. \*\*  $P < .01$ ; \*\*\*  $P < .001$ .

10

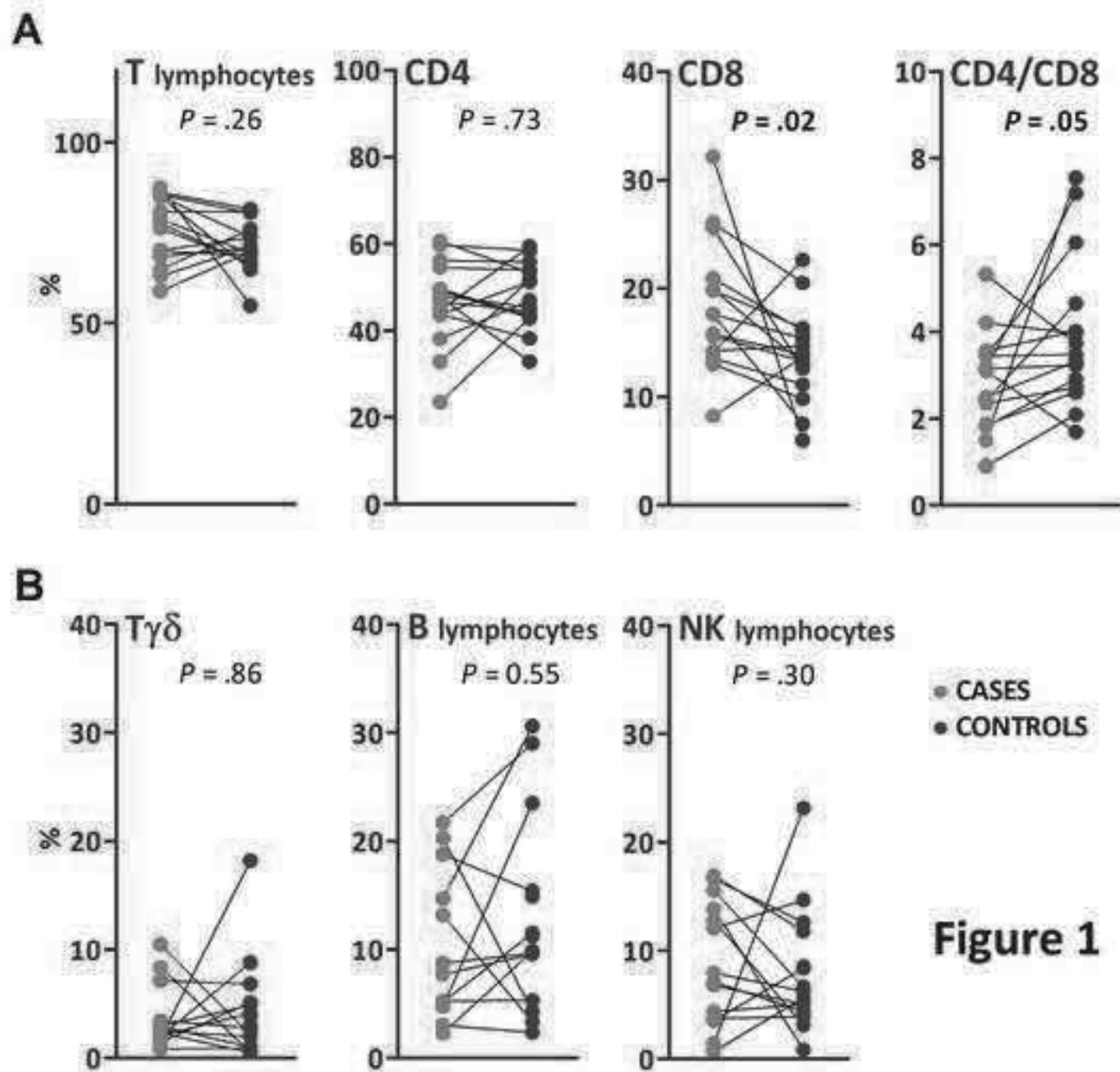
1 **Table 3. Analyses stratified on the basis of HTLV-1 infection and age**

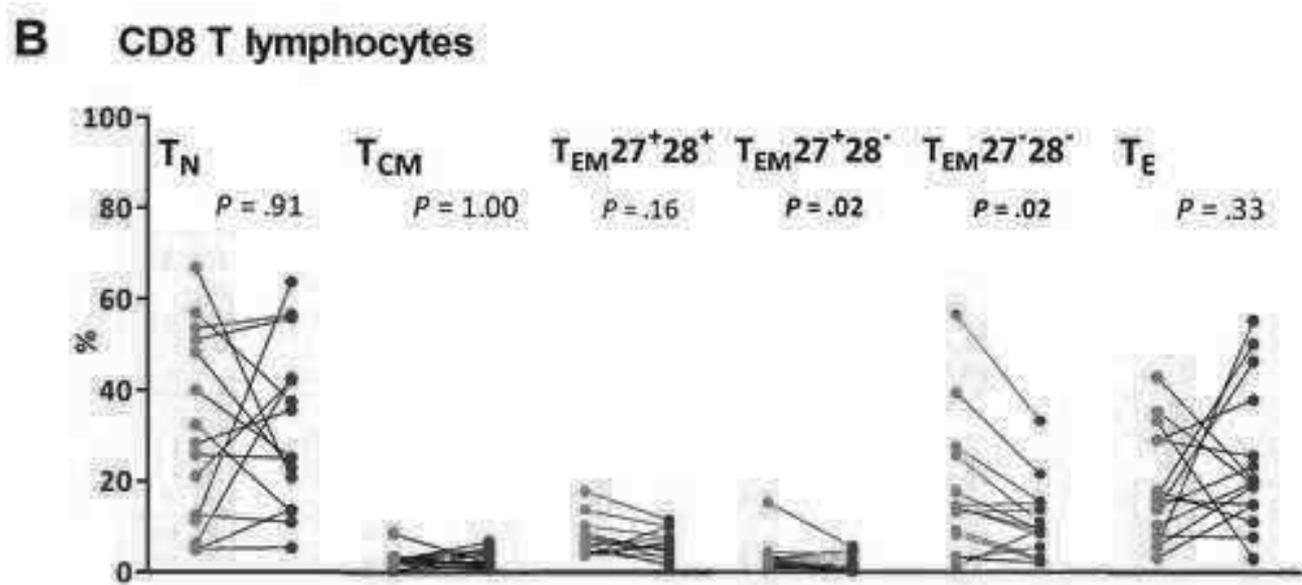
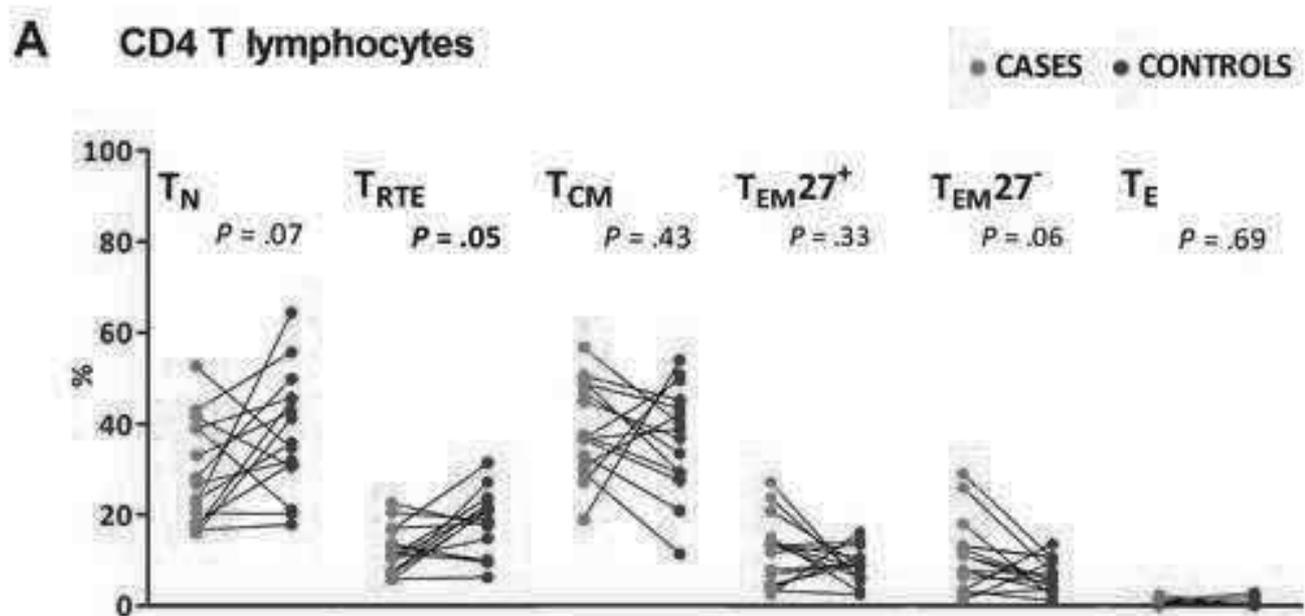
	All <sup>a</sup>			HTLV-1 <sup>neg</sup> <sup>b</sup>			< 65 yrs <sup>c</sup>		
	HTLV-1 <sup>neg</sup>	HTLV-1 <sup>pos</sup>	<i>P</i>	Cases	Controls	<i>P</i>	Cases	Controls	<i>P</i>
CD8 T lymphocytes	15.6 [13.1;18.3]	14.1 [13;15.4]	0.56	18.3 [17.6;25.6]	13.5 [9.4;15.4]	0.09	16.6 [14.1;18.3]	13.8 [11.3;15.4]	0.20
CD4/CD8 ratio	3.2 [2.4;4.0]	3.1 [2.5;3.5]	0.94	2.4 [1.8;3.2]	3.5 [3.1;5.6]	0.09	3.1 [2.4;3.4]	3.6 [3.2;4.0]	0.33
CD4 T <sub>RTE</sub>	16.6 [10.3;21.3]	10.4 [6.4;17.0]	0.15	10.9 [7.5;13.1]	19.9 [16.1;23.1]	0.06	12.0 [7.5;17.0]	19.7 [17.5;21.3]	0.07
CD4 T <sub>M</sub> PD1 <sup>+</sup>	<b>24.8 [21.2;27.2]</b>	<b>32.2 [27.3;40.0]</b>	<b>0.03</b>	27.5 [25.6;30.7]	22.1 [14.4;3]	0.28	29.1 [25.6;34.7]	23.0 [14.4;27.2]	<b>0.05</b>
sCD163, µg/mL	0.71 [0.58;0.85]	0.72 [0.53;0.82]	0.94	<b>0.82 [0.70;0.97]</b>	<b>0.59 [0.52;0.74]</b>	<b>0.04</b>	0.80 [0.70;1.05]	0.55 [0.42;0.67]	<b>0.02</b>

2

3 Abbreviations: Interquartile range: IQR, human T lymphotropic virus type 1: HTLV-1, HTLV-1<sup>neg</sup>: participants who tested seronegative for HTLV-1 infection,4 HTLV-1<sup>pos</sup>: participants who tested seropositive for HTLV-1 infection. <sup>a</sup>n = 21 HTLV-1<sup>neg</sup> and n = 9 HTLV-1<sup>pos</sup>: participants were compared using the Mann-5 Whitney test, <sup>b</sup>cases and controls were compared using the Wilcoxon signed-rank test for the seven pairs in which both participants were seronegative for6 HTLV-1 infection, <sup>c</sup>cases and controls were compared using the Wilcoxon signed-rank test for the ten pairs in which both participants were less than 65 years7 old. Median [IQR] and *P* values are presented.

8



**Figure 2**

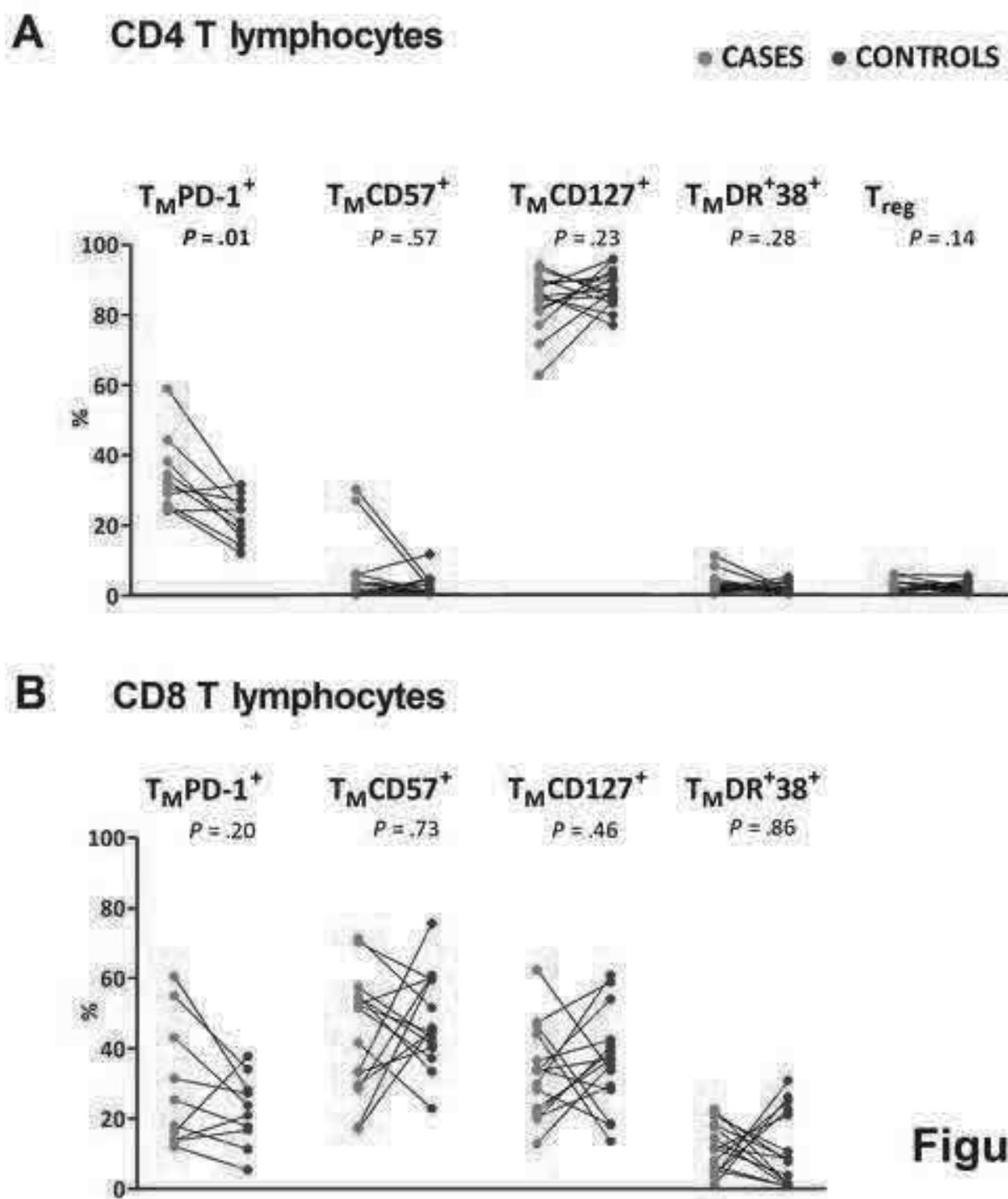
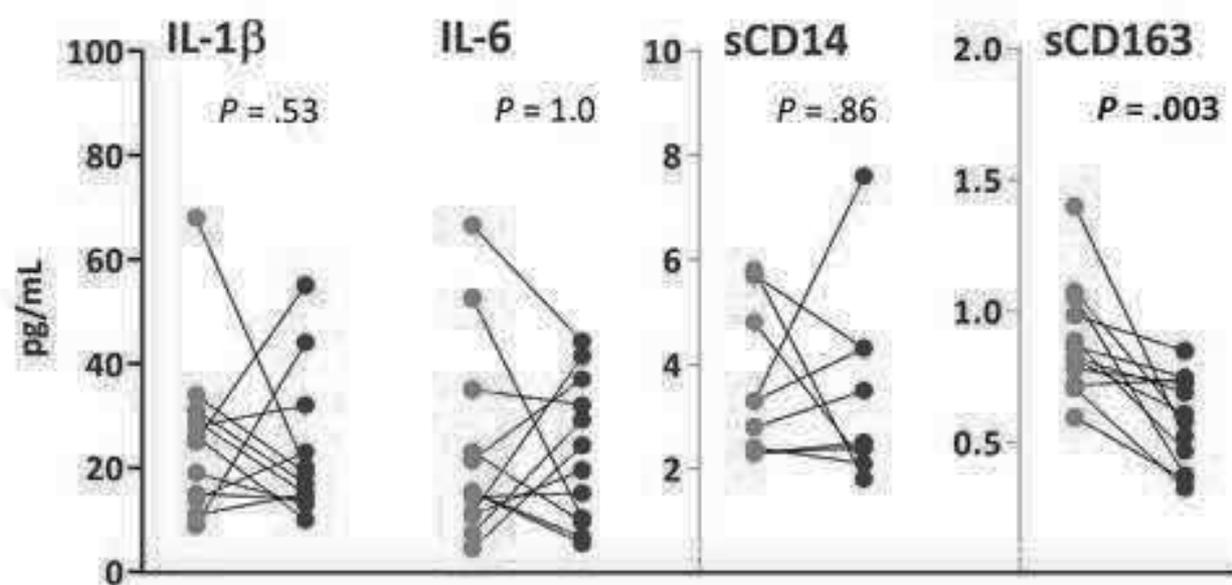
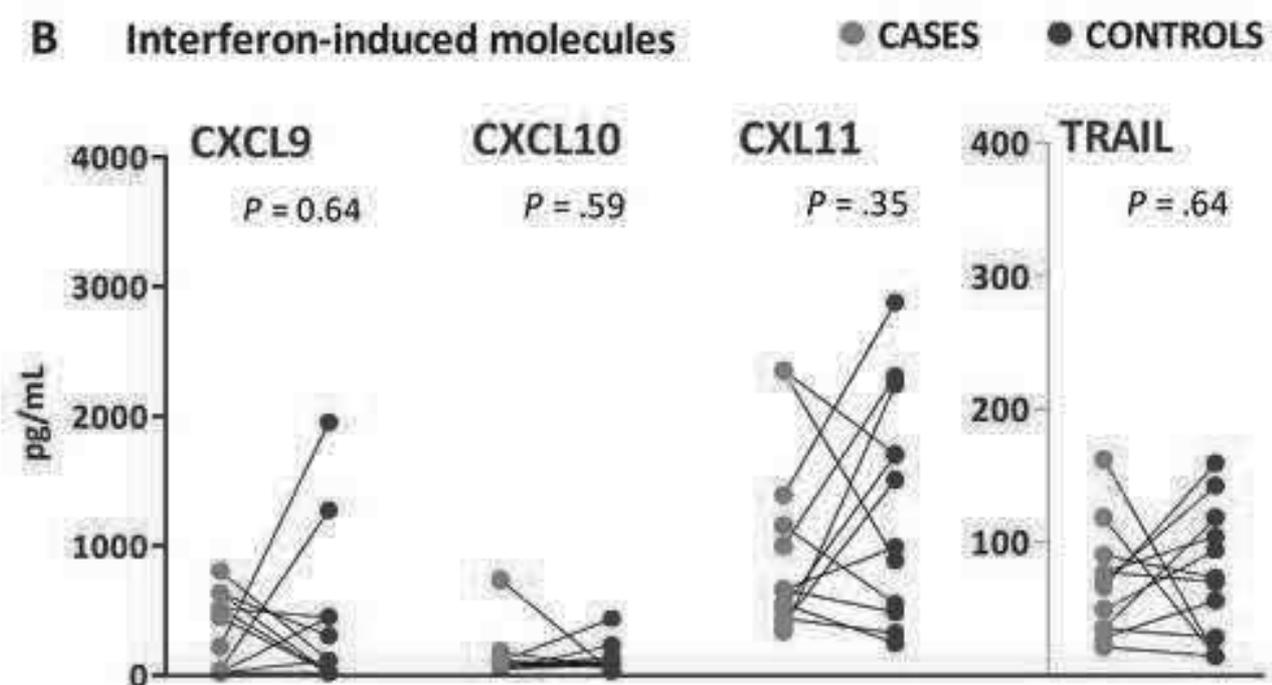


Figure 3

**A Inflammation and monocyte activation****B Interferon-induced molecules****Figure 4**

## Supplementary Tables 1 to 3.

1

2

3 **Supplementary table 1. T, B, and NK lymphocytes subsets among cases and controls**

Lymphocyte subsets	Cases <sup>a</sup>	Controls <sup>a</sup>	<i>P</i> <sup>a</sup>
T lymphocytes <sup>b</sup>	76.2 [66.4;85.1]	69.4 [66.2;75.9]	0.26
CD4 T lymphocytes <sup>b</sup>	46.6 [43.6;49.5]	46.9 [42.9;53.4]	0.73
CD8 T lymphocytes <sup>b</sup>	<b>17.6 [14.1;20.9]</b>	<b>13.3 [11.1;15.4]</b>	<b>0.02</b>
CD4/CD8 ratio	<b>2.5 [1.9;3.4]</b>	<b>3.5 [2.9;4.7]</b>	<b>0.05</b>
γδ T lymphocytes <sup>b</sup>	2.5 [2.0;3.4]	2.8 [1.0;5.1]	0.86
Vδ1 <sup>+</sup> T lymphocytes <sup>c</sup>	63.6 [53.0;73.6]	67.2 [40.2;85.7]	0.75
Vδ2 <sup>+</sup> T lymphocytes <sup>c</sup>	24.1 [9.8;35.1]	30.4 [11.8;39.8]	0.70
NK lymphocytes <sup>b</sup>	9.6 [3.7;15.6]	6.5 [3.9;12.6]	0.30
CD56 <sup>brigh</sup> NK lymphocytes <sup>c</sup>	1.3 [0.6;3.3]	1.4 [0.5;2.4]	0.55
CD16 <sup>+</sup> CD56 <sup>+</sup> NK lymphocytes <sup>c</sup>	70.1 [61.3;77.4]	57.5 [36.1;77.9]	0.27
CD16 <sup>+</sup> CD56 <sup>-</sup> NK lymphocytes <sup>c</sup>	26.6 [19.7;37.9]	40.1 [21.0;61.7]	0.20
B lymphocytes <sup>b</sup>	12.3 [5.2;18.8]	10.5 [9.6;23.5]	0.55
CD21 <sup>+</sup> CD27 <sup>-</sup> naive B lymphocytes <sup>c</sup>	29.7 [22.9;39.8]	39.1 [24.3;51.1]	0.55
CD10 <sup>+</sup> immature B lymphocytes <sup>c</sup>	1.3 [0.9;2.1]	1.6 [1.0;2.4]	0.86
CD21 <sup>+</sup> CD27 <sup>+</sup> resting memory B lymphocytes <sup>c</sup>	11.0 [8.9;15.3]	10.0 [9.4;12.8]	0.65
CD21 <sup>lo</sup> CD27 <sup>+</sup> activated mature memory B lymphocytes <sup>c</sup>	8.8 [3.3;11.6]	5.0 [1.5;9.8]	0.65
CD21 <sup>lo</sup> CD20 <sup>hi</sup> CD27 <sup>-</sup> tissue like memory B lymphocytes <sup>c</sup>	50.4 [32.7;54.3]	43.1 [34.0;56.8]	0.75
CD20 <sup>-</sup> CD38 <sup>+</sup> plasmablasts <sup>c</sup>	16.6 [6.2;22.9]	20.2 [11.5;25.9]	0.25

4 <sup>a</sup>Medians [interquartile range] are indicated; the Wilcoxon signed rank test was used to compare cases  
5 and controls. <sup>b</sup>expressed as the percentage among viable lymphocytes. <sup>c</sup>expressed as the percentage  
6 among γδ, B, or NK lymphocytes.

7

8 Supplementary table 2. T-lymphocyte subsets among cases and controls

Variable <sup>a</sup>	Phenotype	Expressed as % among	n	Cases <sup>b</sup>	Controls <sup>b</sup>	P <sup>b</sup>
CD4 T <sub>N</sub>	CD45RA+CCR7+	CD4	15	26.8 [18.4;39.1]	35.8 [30.6;45.7]	0.07
<b>CD4 T<sub>RTE</sub></b>	<b>CD45RA+CCR7+CD31+</b>	<b>CD4</b>	<b>15</b>	<b>10.9 [7.0;16.6]</b>	<b>18.6 [10.0;22.5]</b>	<b>0.05</b>
CD4 T <sub>CM</sub>	CD45RA-CCR7+	CD4	15	34.1 [27.9;41.5]	34.9 [27.2;42.5]	0.36
CD4 T <sub>TM</sub>	CD45RA-CCR7-CD27+	CD4	15	14.1 [5.0;16.1]	9.7 [6.3;11.5]	0.33
CD4 T <sub>EM</sub> CD28+	CD45RA-CCR7-CD27-CD28+	CD4	15	6.7 [3.4;9.6]	4.4 [3.2;9.0]	0.26
CD4 T <sub>EM</sub> CD28-	CD45RA-CCR7-CD27-CD28-	CD4	15	2.22 [0.30;4.37]	1.42 [0.54;2.47]	0.26
CD4 T <sub>EM</sub> CD28 <sup>+</sup> CD57+	CD45RA-CCR7-CD27-CD28-CD57+	CD4	15	0.93 [0.21;3.11]	0.53 [0.33;1.64]	0.31
CD4 T <sub>E</sub>	CD45RA+CCR7-CD27-CD28-	CD4	15	0.47 [0.17;0.99]	0.49 [0.20;0.91]	0.69
CD8 T <sub>N</sub>	CD45RA+CCR7+	CD8	15	28.3 [11.3;51.0]	25.1 [13.7;42.6]	0.91
CD8 T <sub>CM</sub>	CD45RA-CCR7+	CD8	15	2.8 [2.0;3.3]	2.2 [1.5;4.4]	1.00
CD8 T <sub>TM</sub> CD28+	CD45RA-CCR7-CD27+CD28+	CD8	15	6.9 [4.7;9.8]	6.6 [4.1;8.8]	0.16
<b>CD8 T<sub>TM</sub>CD28-</b>	<b>CD45RA-CCR7-CD27+CD28-</b>	<b>CD8</b>	<b>15</b>	<b>2.45 [1.21;3.42]</b>	<b>0.89 [0.39;3.12]</b>	<b>0.02</b>
CD8 T <sub>TM</sub> CD28-CD57+	CD45RA-CCR7-CD27+CD28-CD57+	CD8	15	1.37 [0.54;1.94]	0.30 [0.05;1.05]	0.10
<b>CD8 T<sub>EM</sub>CD28-</b>	<b>CD45RA-CCR7-CD27-CD28-</b>	<b>CD8</b>	<b>15</b>	<b>16.1 [4.0;29.2]</b>	<b>10.8 [3.6;17.4]</b>	<b>0.02</b>
<b>CD8 T<sub>EM</sub>CD28-CD57+</b>	<b>CD45RA-CCR7-CD27-CD28-CD57+</b>	<b>CD8</b>	<b>15</b>	<b>13.4 [3.5;23.6]</b>	<b>8.9 [2.9;14.3]</b>	<b>0.01</b>
CD8 T <sub>E</sub>	CD45RA+CCR7-CD27-CD28-	CD8	15	16.1 [8.3;29.2]	19.8 [14.9;37.9]	0.33
CD8 T <sub>E</sub> CD57+	CD45RA+CCR7-CD27-CD28-CD57+	CD8	15	13.0 [5.5;22.8]	15.3 [9.7;21.8]	0.50
CD4 T <sub>M</sub> DR+38+	Non(CD45RA+CCR7+)HLA-DR+CD38+	CD4 T <sub>M</sub>	15	2.5 [1.2;3.6]	1.4 [0.6;2.4]	0.28
CD8 T <sub>M</sub> DR+38+	Non(CD45RA+CCR7+)HLA-DR+CD38+	CD8 T <sub>M</sub>	15	11.5 [5.3;18.2]	8.6 [1.3;22.5]	0.86
CD4 T <sub>M</sub> CD127+	Non(CD45RA+CCR7+)CD127+	CD4 T <sub>M</sub>	15	85.0 [80.8;89.1]	86.6 [83.6;90.9]	0.23
CD8 T <sub>M</sub> CD127+	Non(CD45RA+CCR7+)CD127+	CD8 T <sub>M</sub>	15	33.5 [21.5;44.0]	36.7 [28.1;42.3]	0.46
CD4 T <sub>REG</sub>	CD25+CD127-	CD4	15	2.5 [1.5;3.7]	1.9 [0.9;3.1]	0.14
T <sub>N</sub>	CD45RA+CCR7+	CD4 T <sub>REG</sub>	15	33.0 [26.2;53.7]	45.4 [39.9;59.4]	0.13
T <sub>CM</sub>	CD45RA-CCR7+	CD4 T <sub>REG</sub>	15	12.3 [8.5;25.0]	9.1 [5.5;13.7]	0.17
T <sub>EM</sub>	CD45RA-CCR7-	CD4 T <sub>REG</sub>	15	34.4 [25.7;39.4]	27.4 [19.8;34.7]	0.19
T <sub>E</sub>	CD45RA+CCR7-	CD4 T <sub>REG</sub>	15	0.7 [0.4;2.1]	1.4 [0.5;2.5]	0.36
T <sub>REG</sub> DR+38+	HLA-DR+CD38+	CD4 T <sub>REG</sub>	15	5.4 [2.2;10.9]	3.1 [1.9;5.5]	0.09
<b>CD4 T<sub>M</sub> PD1+</b>	<b>Non(CD45RA+CCR7+)CD279+</b>	<b>CD4 T<sub>M</sub></b>	<b>10</b>	<b>31.7 [25.9;38.2]</b>	<b>23.0 [16.8;27.2]</b>	<b>0.01</b>
<b>CD4 T<sub>CM</sub> PD1+</b>	<b>CD45RA-CCR7+CD279+</b>	<b>CD4 T<sub>CM</sub></b>	<b>10</b>	<b>19.0 [14.6;23.2]</b>	<b>12.7 [7.9;17.7]</b>	<b>0.04</b>
CD4 T <sub>EM</sub> PD1+	CD45RA-CCR7-CD279+	CD4 T <sub>EM</sub>	10	38.8 [33.6;44.9]	31.7 [28.6;33.8]	0.06
CD8 T <sub>M</sub> PD1+	Non(CD45RA+CCR7+)CD279+	CD8 T <sub>M</sub>	10	21.8 [14.1;43.1]	22.4 [16.8;28.1]	0.17
CD8 T <sub>CM</sub> CD28+PD1+	CD45RA-CCR7+CD28+CD279+	CD8 T <sub>CM</sub>	10	9.0 [7.1;17.2]	11.6 [11.1;12.3]	0.58
CD8 T <sub>EM</sub> CD28+PD1+	CD45RA-CCR7-CD28+CD279+	CD8 T <sub>EM</sub>	10	14.7 [11.2;18.8]	10.8 [8.0;17.2]	0.24
CD8 T <sub>EM</sub> CD28-PD1+	CD45RA-CCR7-CD28-CD279+	CD8 T <sub>EM</sub>	10	16.1 [10.1;21.1]	17.9 [9.6;28.1]	0.51
CD8 T <sub>E</sub> PD1+	CD45RA-CCR7-CD28-CD279+	CD8 T <sub>E</sub>	10	11.2 [6.4;30.3]	16.9 [9.7;23.1]	0.80
CD4 T <sub>M</sub> CD57+	Non(CD45RA+CCR7+)CD57+	CD4 T <sub>M</sub>	15	1.8 [0.4;5.8]	1.2 [0.9;4.0]	0.57
CD8 T <sub>M</sub> CD57+	Non(CD45RA+CCR7+)CD57+	CD8 T <sub>M</sub>	15	51.5 [29.6;54.9]	44.8 [40.1;60.2]	0.73
CD4 Th1	Non(CD45RA+CCR7+)CXCR3+CCR4-CCR6-	CD4 T <sub>M</sub>	13	1.5 [0.6;3.7]	1.3 [0.5;1.9]	0.55
CD4 Th2	Non(CD45RA+CCR7+)CXCR3-CCR4+CCR6-	CD4 T <sub>M</sub>	13	5.4 [1.2;12.0]	2.8 [1.0;7.4]	0.20
CD4 Th17	Non(CD45RA+CCR7+)CXCR3-CCR4+CCR6-	CD4 T <sub>M</sub>	13	21.3 [16.9;23.8]	20.8 [15.7;24.2]	0.81
CD4 Th1/2	Non(CD45RA+CCR7+)CXCR3+CCR4+CCR6-	CD4 T <sub>M</sub>	13	0.1 [0.0;0.2]	0.0 [0.0;0.1]	0.35
CD4 Th1/17	Non(CD45RA+CCR7+)CXCR3+CCR4-CCR6+	CD4 T <sub>M</sub>	13	0.3 [0.1;0.9]	0.1 [0.1;0.2]	0.46

CD4 Th2/17	Non(CD45RA+CCR7+)CXCR3-CCR4+CCR6+	CD4 T <sub>M</sub>	13	2.5 [0.7;6.3]	1.4 [0.5;4.6]	0.25
CD8 T <sub>CM</sub> Tc1	CD45RA-CCR7+CXCR3+CCR4-CCR6-	CD8 T <sub>CM</sub>	13	11.7 [1.4;18.0]	5.1 [4.4;16.4]	0.42
CD8 T <sub>EM</sub> Tc1	CD45RA-CCR7-CXCR3+CCR4-CCR6-	CD8 T <sub>EM</sub>	13	5.2 [0.8;8.1]	2.2 [1.0;2.9]	0.55
CD8 T <sub>E</sub> Tc1	CD45RA+CCR7-CXCR3+CCR4-CCR6-	CD8 T <sub>E</sub>	13	3.0 [1.3;8.7]	2.0 [0.8;4.0]	0.35
CD8 T <sub>EM</sub> Tc17	CD45RA-CCR7-CXCR3-CCR4-CCR6+	CD8 T <sub>EM</sub>	13	7.2 [4.4;11.0]	5.6 [4.2;9.1]	0.51
CD8 T <sub>CM</sub> Tc1/17	CD45RA-CCR7+CXCR3+CCR4-CCR6+	CD8 T <sub>CM</sub>	13	2.5 [1.9;3.6]	1.4 [0.7;1.8]	0.11
<b>CD8 T<sub>EM</sub>Tc1/17</b>	<b>CD45RA-CCR7-CXCR3+CCR4-CCR6+</b>	<b>CD8 T<sub>EM</sub></b>	<b>13</b>	<b>0.7 [0.5;1.2]</b>	<b>0.4 [0.1;0.6]</b>	<b>0.02</b>
<b>CD4 CD161+</b>	<b>CD161+</b>	<b>CD4</b>	<b>14</b>	<b>12.9 [10.9;18.0]</b>	<b>10.4 [8.8;13.2]</b>	<b>0.05</b>
CD8αβ CD161+	CD8α+CD8β+CD161+	CD8	14	6.0 [2.2;9.0]	3.1 [2.2;7.3]	0.73
CD8αα CD161+	CD8α+CD8β-CD161+	CD8	14	31.3 [12.6;39.6]	19.8 [4.4;54.2]	0.88
CD4 T <sub>M</sub> α4β7+	Non(CD45RA+CCR7+)CD49d+integrinβ7+	CD4 T <sub>M</sub>	11	19.0 [12.2;22.7]	15.1 [12.5;20.4]	0.42
CD8 T <sub>M</sub> α4β7+	Non(CD45RA+CCR7+)CD49d+integrinβ7+	CD8 T <sub>M</sub>	11	31.0 [21.7;38.7]	30.6 [19.6;48.0]	0.66

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10 Abbreviations: CM, central memory; EM, effector memory; E, effector; M, memory, N, naive; Tc, T  
 11 cytotoxic; Th, T helper; T<sub>M</sub>, memory.

12 <sup>a</sup>Frozen PBMCs from cases and controls were stained with a viability marker and antibodies defining T  
 13 lymphocyte phenotypes. The distinction between CD27<sup>+</sup> (T<sub>TM</sub>) and CD27<sup>-</sup> (T<sub>EM</sub>) CD45RA<sup>-</sup>CCR7<sup>-</sup> T  
 14 lymphocytes was made when anti-CD27 antibodies were present in the mix, otherwise T<sub>EM</sub> refers to  
 15 CD45RA<sup>-</sup>CCR7<sup>-</sup> T lymphocytes. When a molecule was expressed at comparable levels on T<sub>CM</sub>, T<sub>EM</sub>, and  
 16 T<sub>E</sub>, we merged the 3 subsets into a single one (T<sub>M</sub>) for data presentation and analysis.

17 <sup>b</sup>Medians [interquartile ranges] are indicated; the Wilcoxon signed rank test was used to compare  
 18 cases and controls.

19

20 **Supplementary table 3. Plasma analytes among cases and controls**

Plasma analyte	Cases <sup>a</sup>	Controls <sup>a</sup>	<i>P</i> <sup>a</sup>
IFN- $\gamma$ , pg/mL	122 [83;157]	123 [76;181]	0.78
IL-10, pg/mL	164 [60;290]	163 [66;179]	0.69
IL-12, pg/mL	68 [44;92]	47 [28;90]	0.39
IL-17, pg/mL	63 [53;103]	80 [52;95]	0.94
IL-2, pg/mL	17 [12;22]	15 [11;18]	0.18
IL-21, pg/mL	388 [172;606]	344 [267;578]	0.91
IL-23, ng/mL	1.9 [1.8;9.9]	2.3 [1.1;3.3]	0.24
IL-7, pg/mL	64 [39;83]	70 [41;94]	0.53
IL-8, pg/mL	11 [7;20]	18 [10;26]	0.06
<b>GM-CSF, pg/mL</b>	<b>64 [47;101]</b>	<b>46 [38;62]</b>	<b>0.04</b>
TNF- $\alpha$ , pg/mL	42 [34;66]	45 [32;56]	0.22
CCL3, pg/mL	68 [45;102]	65 [46;70]	0.11
CCL4, pg/mL	35 [29;44]	46 [38;61]	0.21
CCL20, pg/mL	691 [101;1 276]	451 [44;795]	0.08
IL-4, pg/mL	415 [342;488]	453 [294;600]	0.53
IL-5, pg/mL	16 [8;45]	23 [13;50]	0.69
IL-13, pg/mL	30 [20;51]	40 [27;52]	0.69
CXCL13, pg/mL	29 [9;74]	33 [23;52]	0.91
BAFF, ng/mL	1.5 [1.2;1.7]	1.6 [1.2;1.9]	0.41
IL-1 $\beta$ , pg/mL	26 [15;30]	18 [15;28]	0.53
IL-6, pg/mL	15 [11;29]	22 [10;34]	1.00
sCD14, $\mu$ g/mL	3.3 [2.4;4.8]	2.5 [2.1;4.3]	0.86
<b>sCD163, <math>\mu</math>g/mL</b>	<b>0.84 [0.75;1.02]</b>	<b>0.59 [0.42;0.73]</b>	<b>0.003</b>
CXCL9, pg/mL	449 [32;577]	115 [21;451]	0.64
CXCL10, pg/mL	76 [50;110]	83 [60;133]	0.59
CXCL11, pg/mL	651 [474;1 244]	1222 [513;1 928]	0.35
TRAIL, pg/mL	68 [35;84]	72 [21;111]	0.64

21

22 <sup>a</sup>Medians [interquartile range] are indicated; the Wilcoxon signed rank test was used to compare cases  
 23 and controls.

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