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1 **Preservation of lymphopoietic potential and virus suppressive** 2 **capacity by CD8⁺ T-cells in HIV-2 infected controllers**

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32 **Running title:** Effective CD8+ T cells in HIV-2 infected controllers

33

34 **Abstract**

35 Compared to Human immunodeficiency virus 1 (HIV-1), HIV-2 infection is
36 characterized by a larger proportion of slow or non-progressors. A better understanding of
37 HIV-2 pathogenesis should open new therapeutic avenues to establish control of HIV-1
38 replication in infected patients. Here, we studied the production of CD8⁺ T cells and their
39 capacity for viral control in HIV-2 controllers from the French ANRS CO5 HIV-2 cohort. HIV-2
40 controllers display a robust capacity to support long-term renewal of the CD8⁺ T-cell
41 compartment by preserving immune resources, including hematopoietic progenitors and
42 thymic activity, which could contribute to the long-term maintenance of the CD8⁺ T-cell
43 response and the avoidance of premature immune aging. Our data support the presence of
44 HIV-2 Gag-specific CD8⁺ T-cells that display an early memory differentiation phenotype and
45 robust effector potential in HIV-2 controllers. Accordingly, we show for the first time that
46 HIV-2 controllers possess CD8⁺ T-cells that show an unusually strong capacity to suppress
47 HIV-2 infection in autologous CD4⁺ T- cells *ex vivo*, an ability that likely depends on the
48 preservation of host immune resources. This effective and durable antiviral response
49 probably participates in a virtuous circle, during which controlled viral replication permits
50 the preservation of potent immune functions, thus preventing HIV-2 disease progression.

51

52 **Introduction**

53 In the absence of an effective vaccine that prevents the HIV-1 acquisition, the long-
54 term suppression of viral replication in infected individuals remains a major objective to
55 prevent further disease progression and dissemination. Although progression to AIDS can
56 now be significantly delayed by effective combinatorial antiretroviral therapy (cART), HIV
57 remission, which is observed in HIV-1 controllers (HIC1) (1) and rare patients who gain
58 independence from ART after early initiation (2), remains a priority. Much effort, therefore,
59 has been given to the identification of immune correlates of natural HIV control in an
60 attempt to decipher the immunological mechanisms that underlie effective control of HIV-1
61 replication in the absence of ART. A better understanding of these correlates would provide
62 insights into therapeutic approaches that would modulate the immune system to mimic the
63 immune parameters of HIV-1 controllers, ultimately leading to a functional cure of HIV-1
64 infection.

65 Although HIV-2 shares the same modes of transmission and intracellular mechanisms
66 of replication as HIV-1, it provides a unique model of attenuated infection by a human
67 immunodeficiency virus. Compared to HIV-1 infection, HIV-2 infection is predominantly
68 characterized by a slower decline of CD4+ T-cells and lower levels of immune activation.(1-
69 3). Whilst HIV-2 infected patients who progress towards disease present similar clinical
70 manifestations and AIDS severity as those infected with HIV-1, they remain a minority (~15-
71 20% of those infected (4)). Many HIV-2 infected individuals spontaneously control their
72 infection and remain asymptomatic while maintaining undetectable viral loads, signifying a
73 high prevalence of HIV-2 controllers (HIC2) (9.1% of HIC2 in the ANRS CO5 HIV-2 cohort (5)
74 vs 0.22% HIC1 in the ANRS CO4 FHDH (French Hospital Database on HIV)(6)). Deciphering the

75 immune correlates of HIV-2 control and contrasting these findings with those from HIV-1
76 controllers may illuminate the key features of HIV pathogenesis and provide critical
77 information on what is needed to establish natural control of HIV.

78 It is well established that CD8⁺ T-cell mediated immunity is critical in the control of
79 HIV-1 replication from the earliest days of acute HIV-1 infection. However, the HIV-specific T-
80 cell response declines over time and is often lost in the later phases of chronic infection (7).
81 Moreover, HIV-1 infected individuals with protracted infection usually demonstrate reduced
82 thymic output and lower naïve T-cell numbers (8-10). This situation, which is reminiscent of
83 immune aging, likely accounts for the loss of CD8⁺ T-cell regenerative capacity over time, and
84 the failure of the T-cell response to adapt to the emergence of escape variants. Only rare
85 HIC1 (often possessing specific “protective” HLA-class I alleles (11)), are able to maintain
86 viremia spontaneously and durably at extremely low levels (12). Furthermore, this level of
87 control has been associated with their capacity to develop and sustain the production of
88 HIV-specific CD8⁺ T-cells that are endowed with the capacity to efficiently suppress HIV-1
89 infection of autologous CD4⁺ T-cells (13).

90 In the present work, we focus on the potential role of cellular immunity as a key
91 player in controlling HIV-2 infection. Strong Gag-specific CD8⁺ T-cell responses have been
92 previously observed in HIV-2 infected patients, and their magnitude was inversely associated
93 with viremia (14, 15). Of note, these HIV-2 Gag-specific CD8⁺ T-cells were described as
94 polyfunctional, although they did not contain high levels of cytolytic markers (16). Persistent
95 CD8⁺ T-cell renewal is therefore likely to be essential for maintaining long term immune
96 efficacy against HIV. We embarked here on the fine characterization of HIV-2-specific CD8⁺ T-
97 cells in HIC2 from the French ANRS CO5 HIV-2 cohort, with particular focus on the

98 production of HIV-specific CD8⁺ T-cells and their HIV-2 suppressive capacity. We describe for
99 the first time that CD8⁺ T-cells from HIC2 demonstrate strong capacity to suppress HIV-2
100 infection in autologous CD4⁺ T-cells *ex vivo*. Importantly, HIC2 retain robust lymphopoiesis in
101 general and CD8⁺ T-cell production in particular, suggesting that the marked premature
102 immune aging phenotype seen in HIV-1 infection is not present in these individuals, which is
103 likely to be key to the maintenance of a durable and efficient CD8⁺ T-cell response. Overall,
104 our data suggest that the immune correlates of effective control in HIV-2 infected individuals
105 may derive from the capacity of the immune system to maintain the potent HIV suppression
106 capacity that is mediated by CD8⁺ T-cells.

107

108 **Material and Methods**

109 **Study subjects**

110 HIV-2 controllers (HIC2) were part of the ANRS CO5 VIH-2 cohort and included in the ANRS
111 IMMUNOVIR 2 study, which focuses on the study of patients with non-progressive infection.
112 All patients in the present study had characteristic features of HIV controllers i.e.
113 asymptomatic treatment naïve individuals, infected for at least 5 years, with a CD4⁺ T cell
114 count > 400 cells/ μ l and a viral load < 400 RNA copies/ml.

115 HIC2 were compared to HIV-1-infected individuals: HIV-1 controllers (HIC1) from the ANRS
116 CO21 Codex cohort and two groups of HIV-1 viremic individuals (one with CD4⁺ T cell count
117 below 200 cells/ml and another with CD4⁺ T cell count above 500 cells/ml) from the Pitié
118 Salpêtrière Hospital (France). Mononuclear cells were isolated over a Lymphoprep gradient
119 and then either used directly or cryopreserved. PBMC from uninfected healthy adults from
120 the French Blood Bank (Etablissement Français du Sang) were also analyzed. A summary of
121 clinical attributes of the patients studied is displayed in Table II.

122 All participants gave their written informed consent. The study was approved by the
123 institutional ethics committee (i.e. Comité de Protection des Personnes of Ile de France XI).

124

125 **Flow cytometry and reagents**

126 In order to generate tetramers, immunodominant HIV-2 epitopes were identified by
127 screening HIV-2 p27 overlapping peptides in IFN γ elispot assays using HLA typed HIV-2
128 infected patient PBMCs, going on to define the optimal epitope sequences and length (15).

129 HLA-B*5301 p27 TPYDINQML (TL9) and p27 DRFYKSLRA (DA9) tetramers were synthesized as
130 previously described (17). PBMC were stained with pre-titrated concentrations of
131 pentamer/tetramer (conjugated to PE) followed by a panel of antibodies as previously
132 described (18). Directly conjugated and unconjugated antibodies were obtained from the
133 following vendors: BD Biosciences (BD Biosciences): CD34 (PE), lineage cocktail (CD3, CD14,
134 CD16, CD19, CD20, CD56 / FITC), CD3 (PerCP-Cyanine 5.5] or Alexa Fluor 700), CD45RA
135 (FITC), CCR7 (PE-Cyanine 7), CD107 α (PE-Cyanine 5), IFN γ (Alexa Fluor 700), and TNF α (PE-
136 Cyanine 7); Beckman Coulter (Beckman Coulter): CD28 (PE-TexasRed), CD45RA (PE-
137 TexasRed); Caltag (Thermo Fisher): CD8 (Alexa Fluor 405), granzyme B (PE-TexasRed); R&D
138 Systems (R&D Systems Europe): MIP-1 β (FITC); BioLegend (Ozyme): CD127 (BV-650), CD27
139 (Alexa Fluor 700). Cell surface marker stainings were performed using standard
140 methodologies. CD34+ cell phenotyping was performed on enriched populations. The
141 immunomagnetic enrichment of CD34+ cells was carried out on PBMCs using MACS
142 technology, according to the provider's recommendations (Miltenyi Biotech). Stainings were
143 analyzed on an LSR Fortessa flow cytometer (BD Biosciences) and data analyzed using FlowJo
144 v8.2 (FlowJo, LLC) and DIVA (BD Biosciences) softwares.

145

146 **Intracellular cytokine staining assay**

147 Purified PBMC were thawed and rested overnight at 37°C in complete RPMI media (RPMI
148 1640 supplemented with 10% heat-inactivated fetal calf serum, L-glutamine and antibiotics);
149 viability was then examined by trypan blue exclusion (typically \geq 70% viable). For
150 stimulation, cells were then incubated in the presence of 15-mer overlapping peptides

151 covering the HIV-2 p27 protein (10 μ M) or an overlapping peptide pool encompassing clade
152 B HIV-1 Gag (2 μ g/ml). p27 stimulated cells were incubated in the presence of anti-CD107 α
153 antibodies for 1h at 37 °C in a 5% CO₂ incubator, followed by an additional 5h in the
154 presence of the secretion inhibitors monensin (2.5 μ g/mL; Sigma-Aldrich) and Brefeldin A (5
155 μ g/mL; Sigma-Aldrich). BD Cytofix/Cytoperm™ (BD Biosciences) was used for
156 permeabilization of the cells prior to staining for intracellular markers.

157

158 ***In vitro* HIV-2 suppression assay**

159 To assess the capacity of CD8⁺ T-cells to suppress HIV-2 infection of autologous CD4⁺ T-cells
160 *in vitro*, we adapted our previously published HIV-1 suppression assay (19). Briefly, PBMC
161 were isolated from peripheral blood by density centrifugation. CD4⁺ and CD8⁺ T-cells were
162 then isolated by, respectively, positive and negative magnetic-bead sorting (STEMCELL
163 Technologies). CD4⁺ T-cells were activated in complete RPMI media supplemented with
164 phytohemagglutinin (1 μ g/ml) and interleukin-2 (IL-2) (100 IU/ml) for 3 days. CD8⁺ T-cells
165 were cultured in non-supplemented culture media. Activated CD4⁺ T-cells were infected with
166 HIV-2 SBL (20) using a spinoculation protocol (21) and cultured alone or with autologous
167 CD8⁺ T-cells at a 1:1 ratio during 14 days in IL-2 (100 IU/ml) supplemented culture media.
168 Viral replication was measured by p27 production in culture supernatants every 3-4 days as
169 determined by enzyme-linked immunosorbent assay (Gentaur). The capacity of CD8⁺ T-cells
170 to suppress HIV infection was calculated at the peak of viral replication as the log decrease in
171 p27 production when superinfected CD4⁺ T-cells were cultured in the presence of CD8⁺ T-
172 cells (log[p27 in CD4 T-cell culture/p27 in CD8:CD4 1:1 co-cultures]).

173

174 **TREC quantification**

175 Thymic function was estimated by quantification of signal joint T-cell reception excision
176 circles (sjTREC) as previously described (22). Briefly, PBMC lysates were subjected to
177 multiplex polymerase chain reaction (PCR) for 22 cycles using sjTREC and CD3 γ chain outer
178 primer pairs. Each of the amplicons was then quantified using Light Cycler technology (Roche
179 Diagnostics), performed on 1/100th of the initial PCR, in independent experiments, but on
180 the same first-round, serially diluted standard curve. This highly sensitive, nested
181 quantitative PCR assay allows detecting one copy of sjTREC in 10⁵ cells. The sjTRECs were
182 quantified in triplicate.

183

184 **Statistical analysis**

185 Statistical analyses were performed using Prism software (GraphPad). Non-parametric tests
186 of significance were performed throughout all analyses, using Kruskal-Wallis and Mann-
187 Whitney testing for intergroup comparisons and Spearman rank test to determine
188 correlations. *P* values >0.05 were considered not significant.

189

190 **Results**

191 **HIV-2-positive population characteristics**

192 We studied 20 HIV-2-positive individuals with controlled viremia in the absence of cART. The
193 detailed characteristics of the patients are reported in Table I. The proportion of female
194 patients was 60% (n=12); the median age was 49 (Interquartile range (IQR): 42 - 52). At
195 inclusion, the median time since HIV-2 infection diagnosis was 13.6 years (IQR: 9.7 - 19.3)
196 and the median CD4⁺ T-cell counts were 893 cells/ μ l of blood (IQR: 707 - 1170 cells/ μ l). Two
197 individuals had detectable, albeit low, HIV-2 viral load (54 and 117 RNA copies/ml). Three
198 individuals were born in France, 1 in Colombia and 16 in west-African countries (Table I).

199 Only two of the HIV-2 infected patients studied carried the "HIV-1 protective" B*57 allele
200 and just one the B*27 allele. The most common class I HLA allele was C*04, which was
201 carried by nine individuals (45%), of whom five were homozygotes (Table I). All five
202 C*04/C*04 individuals were born in Ivory Coast. C*04 is a relatively common gene in France
203 and West Africa. Overall, 11 (55%) individuals expressed homozygous alleles, suggesting that
204 these individuals came from bottleneck populations with limited genetic diversity.

205

206 **HIV-2 controllers show preserved CD8⁺ T-cell production capacities**

207 We previously showed that exhaustion of lymphopoiesis is a major correlate of
208 disease progression in HIV-1 infection (9, 23). Our analyses highlighted a marked decline of
209 primary immune resources in HIV-1 progressors, including CD34⁺ hematopoietic progenitor
210 cells (HPC) and naïve CD4⁺ and CD8⁺ T-cell numbers. These alterations, which are the

211 hallmark features of advanced immune aging, were not observed in HIC1. We therefore
212 performed similar analyses in HIC2, directly analyzing the frequency and phenotype of CD34⁺
213 hematopoietic progenitor cells (Fig. 1A, 1B), as well as the frequency of naïve CD8⁺ T-cells
214 from blood (Fig. 1C). The frequency of circulating HPCs (CD34⁺Lin⁻) in HIC2 was high
215 compared to levels found in untreated HIV-1 infected patients with low CD4⁺ T-cell counts,
216 and equivalent to those observed in HIC1 (Fig. 1A). In a similar manner, HIC2 displayed a high
217 proportion of lymphoid precursor progenitor cells that were identified by the CD117⁺
218 CD45RA⁺ phenotype among CD34⁺ cells (Fig. 1B). Moreover, we found high levels of naïve
219 lymphocytes within the CD8⁺ T-cell compartment of these individuals (Fig. 1C). Our results
220 demonstrate the maintenance of effective lymphopoiesis in HIV-2 infection, which is likely to
221 be linked with a capacity to sustain the long-term production of naïve CD8⁺ T-cells.

222 In HIV-1 infection, the progressive decrease in naïve T-cell proportions in HIV-infected
223 adults likely results from the antigen-driven maturation of these cells in the absence of
224 adequate T-cell renewal capacity due to the declining thymic output of new cells (8, 10, 24).
225 We and others previously reported that thymic function is preserved in HIV-2-infected
226 individuals with low viral load (25). It was also recently shown that HIV-2 infection in
227 thymocytes is impaired (26, 27), which may contribute to the maintenance of high CD4⁺ T-
228 cell counts in HIV-2 infected patients. Here, we measured signal joint T-cell receptor (sjTCR)
229 excision circles (TREC) levels in total PBMCs to estimate thymic function in HIC2. Naïve CD8⁺
230 T-cell counts directly correlated with the amount of sjTREC/ml (Fig. 1D), suggesting that the
231 maintenance of these cells was related to sustained thymic activity. This is in line with the
232 correlation between naïve CD8⁺ and naïve CD4⁺ T-cell counts in blood taken from the same
233 individuals (Supplementary Fig. 1).

234 In addition, we assessed the levels of recent thymic emigrants using CD31 expression
235 (28), which primarily provides information on the thymic history of naïve CD4⁺ T-cells. In line
236 with the TREC data, a strong correlation was observed between the frequency of recent
237 thymic emigrants (i.e. CD31⁺ naïve CD4⁺ T-cells) and the naïve CD8⁺ and CD4⁺ T-cell counts
238 (Fig. 1E and Supplementary Fig. 1). Taken together, these data support the maintenance of a
239 robust lymphopoietic capacity and thymic output in HIC2 and signifies robust preservation of
240 the naïve CD8⁺ T-cell compartment. The latter is central to the mounting of *de novo*
241 responses, as recently shown in elderly subjects (29) and old vaccinated primates (30), and
242 the ability to sustain effective T-cell responses against a virus with high evolutionary rates, as
243 is the case with HIV.

244

245 **HIV-2 Gag-specific CD8⁺ T-cells display early memory differentiation and robust effector**
246 **potential**

247 We next performed a comprehensive characterization of HIV-2-specific CD8⁺ T-cell
248 responses. Since the IFN γ response of Gag-specific CD8⁺ T-cells was found to correlate
249 inversely with viremia in HIV-2 infected individuals, suggesting an important role of these
250 cells in controlling HIV-2 infection (15), we decided to focus on phenotyping CD8⁺ T-cells that
251 were specific for p27, which is the major component of HIV-2 Gag. To assess the overall
252 magnitude of HIV-2 Gag-specific CD8⁺ T-cells in our patients, we performed intracellular IFN γ
253 staining on CD8⁺ T-cells from PBMCs that were stimulated with 15 mer overlapping peptides
254 that spanned the proteome of the HIV-2 p27 protein. Robust p27-specific CD8⁺ T-cell
255 responses were detected in most patients (Fig. 2A). These responses were of similar

256 magnitude to those observed in HIC1, although a direct comparison cannot be made,
257 considering that a different antigen would be used to stimulate HIV-1-specific responses.
258 These cells also exhibited other effector functions, as revealed by co-staining for TNF α , MIP-
259 1 β and CD107 α (Supplementary Fig. 2), highlighting a polyfunctional profile that has been
260 previously described (31). We furthered our investigation by using recombinant MHC class I-
261 peptide tetrameric complexes, which more precisely capture the memory differentiation
262 phenotype of p27-specific CD8⁺ T-cells (Fig. 2B). We analyzed in particular CD8⁺ T-cells that
263 were restricted by HLA-B*5301, since B*53 was the most common allele in our group of
264 patients. We were able to generate HLA-B*5301 tetramers with the immunodominant p27
265 epitope TPYDINQML (TL9). All of the seven HLA-B*53 patients tested positive for TL9-specific
266 CD8⁺ T-cells. We also detected HLA-B*1401 restricted p27 DA9 (DRFYKSLRA)-specific CD8⁺ T-
267 cells in two out of two HLA-B*14 patients.

268 The differentiation phenotype of HIV-2-specific CD8⁺ T-cells was assessed based on
269 the surface expression of the receptors CD27, CD28, CCR7, CD45RA and CD127 (Fig. 2C), and
270 compared to the phenotype of HLA-B*2705 restricted p24 KK10-specific CD8⁺ T-cells, the
271 latter selected as the prototype of primary effector cells in HIV-1 infection (18, 32). Overall,
272 both groups of HIV-specific CD8⁺ T-cells displayed similar phenotypes, being mainly CD27⁺
273 CCR7⁻ CD127⁻, with slight but noticeable differences. For example, HIV-2-specific CD8⁺ T-cells
274 trended towards higher CD45RA expression. In HIV-1 infected patients, CD45RA expression
275 was the hallmark of a rare population of resting cells that resemble long-lived memory cells
276 and were particularly noticeable shortly after viral replication was curtailed by cART (33).
277 Furthermore, the expression of CD28 was significantly higher on HIV-2-specific CD8⁺ T-cells
278 (Fig. 2D), in line with previous observations made with HLA-B*3501 restricted p27

279 NPVPVGNIY (NY9)-specific CD8⁺ T-cells (34). This indicates that HIV-2-specific CD8⁺ T-cells are
280 in general less differentiated (35, 36), displaying an even younger phenotype than highly
281 effective HIV-1-specific CD8⁺ T-cells. Accordingly, HIV-2 specific CD8⁺ T-cells are likely to
282 retain proliferative potential, as previously suggested (34). Nevertheless, despite their early
283 memory differentiation state, HIV-2-specific CD8⁺ T-cells express high levels of the
284 transcription factors T-BET and EOMES (Supplementary Fig. 3), which are key regulators of
285 memory differentiation and the acquisition of effector functions (37). In accordance with the
286 expression of these transcription factors, HIV-2-specific CD8⁺ T-cells possessed high
287 intracellular levels of granzyme B, at least relative to what is seen in effective HIV-1-specific
288 CD8⁺ T-cells (Fig. 2D). Taken together, these data support the presence of robust Gag-specific
289 CD8⁺ T-cell responses in HIC2. These cells are characterized by an early or young
290 differentiation phenotype and display strong effector potential.

291

292 **Unstimulated CD8⁺ T-cells from HIV-2 controllers suppress HIV-2 infection *ex vivo***

293 Lastly, we aimed to show that this strong phenotypic functional potential translated
294 into an effective capacity of these cells to suppress HIV-2 infection. We previously showed
295 that HIC1 often possess HIV-specific CD8⁺ T-cells with a striking capacity to suppress infection
296 by killing HIV-infected autologous CD4⁺ T-cells (38, 39). This capacity was not found in cART
297 treated or untreated viremic HIV-1 patients, either during the acute or chronic phase of
298 infection (38-41). HIV-1 suppression assays provide the most robust method to distinguish
299 between the functional capacities of CD8⁺ T-cells from HIV-1 controllers and non-controllers

300 (40, 42, 43). We thus sought to adapt this *in vitro* assay to the study of HIV-2 using an SBL
301 virus strain (20), with supernatant p27 concentration as a read-out.

302 We found that CD8⁺ T-cells from HIC2 have a strong capacity *ex vivo* to suppress HIV-
303 2 infection of autologous CD4⁺ T-cells (Fig. 3A), with a median reduction in p27 production of
304 2.7 logs [IQR: 1.3-3.3] (Fig. 3B). The viral suppressive capacity shown by the CD8⁺ T-cells
305 from HIC2 was remarkably high, comparable to the levels observed with cells from HIC1
306 from the ANRS CO21 cohort (Fig. 3B) or even among our previous studies (38, 39) . Similarly
307 to what we found in HIV-1 controllers (39), the capacity of CD8⁺ T-cells from HIV-2
308 controllers to suppress infection was relatively stable over time (Fig. 3C). There was however
309 some heterogeneity in HIV-2 suppressive capacity among patients, with logp27 decrease
310 values ranging from 0.02 to 3.7 (Fig. 3B). Interestingly, in two patients who had the steepest
311 decline in CD4⁺ T-cell counts over the 3 years preceding study inclusion (92.5 and 215.7
312 cells/mm³/year), their CD8⁺ T-cells showed no *ex vivo* capacity to suppress HIV-2 infection in
313 our assay (Fig. 4A). Accordingly, we found a positive correlation between capacity of CD8⁺ T-
314 cells to mediate HIV-2 suppression *ex vivo* and the patients' CD4 T-cell counts (Fig. 4B),
315 which supports the key role of these CD8⁺ T-cells in suppressing HIV-2 infection and
316 preventing disease progression. These results are in line with previous studies showing that
317 strong CD8⁺ T-cell mediated HIV-1 suppression *ex vivo* correlates with better clinical outcome
318 (38, 44, 45), and strengthen the value of the HIV-1/2 suppression assay as a correlate of HIV
319 control.

320

321 **Discussion**

322 Although the immune dynamics responsible for the slow progression of HIV-2
323 infection has remained puzzling for years, we are gaining momentum in piecing together the
324 precise picture of events that are responsible for this largely non-progressive HIV infection.
325 At equivalent plasma viral loads, proviral DNA loads are very similar between HIV-1 and HIV-
326 2 infections (1, 46-48). When left unchecked by cART, the survival of HIV-2 infected patients
327 is strongly related to both CD4⁺ T-cell counts and plasma viral load. However, in contrast to
328 HIV-1, HIV-2 is often controlled to undetectable plasma viral loads in the absence of cART,
329 which most likely involves a complex immune-related mechanism.

330 Owing to the maintenance of a strong lymphopoietic capacity, T-cell mediated
331 immune responses appear to be better preserved in HIV-2 infection and HIC2 can sustain the
332 robust suppression of viremia over several years or even decades of chronic infection. HIV-2-
333 specific CD8⁺ T-cells display an early or young differentiated phenotype, potentially reflecting
334 their potential for T-cell renewal. They also harbor particularly potent effector functions,
335 highlighted by their exceptional capacity to suppress the virus in autologous CD4⁺ T-cells.
336 These CD8⁺ T-cell characteristics are similar to those observed in HIC1 in many aspects, and
337 are likely to be part of the cause or consequence of effective control over the virus. This
338 robust lymphopoietic potential is not preserved or restored in the CD8⁺ T-cells of HIV-
339 infected patients undergoing antiretroviral treatment even when initiated during primary
340 HIV-1 infection (41), which argues more in favor of causality.

341 Although overall, strong CD8⁺ T cell-mediated HIV-2 suppression is observed in HIC2
342 patients, some heterogeneity, as in HIC1, is observed in these patients (39, 49-51). The

343 absent or low CD8⁺ T cell-mediated HIV-2 suppression in four patients may be due to several
344 factors. First, we cannot exclude the possibility that strong CD8⁺ T cell viral suppression was
345 not elicited in these individuals due to other immunodominant epitopes that were not
346 encoded by the HIV-2 SBL virus strain used in our experiments. However, we have previously
347 shown that suppressive CD8 T-cell activity in HIV-1 controllers remained low in some donors
348 even when autologous virus was used (39). Alternatively, the absence of suppressive activity
349 may be a consequence of the stringent control of viremia, resulting in the contraction of the
350 HIV-2 specific CD8⁺ T cell response to a small pool of high quality memory cells that fall
351 below the detection threshold of our assay (49, 52, 53). Moreover, we were only able to
352 analyse the CD8⁺ T cell response in blood, and would not be able to measure more robust
353 responses that were active locally in patient tissues (54). Finally, other mechanisms such as
354 innate or humoral responses, or infection with unfit virus, may exert a greater role than the
355 activity of CD8⁺ T cells in controlling viremia in these patients.

356 The effective suppression of viral replication in HIC2 will prevent the development of
357 chronic immune activation and further damage to the lymphopoietic system, thus allowing
358 the maintenance of an effective CD8⁺ T-cell immune response which provides long-term
359 control of the virus. This equilibrium between HIV-2 and host immunity may be referred to
360 as a virtuous cycle, in contrast to the vicious cycle in HIV-1 infection, where apart from
361 exceptional HIC1 cases, poorly controlled viral replication results in elevated chronic immune
362 activation. The intensity of prolonged HIV-1 replication contributes to a process of
363 premature immune aging that exerts its toll and further weakens cellular immunity in
364 infected individuals.

365

366 We do not yet have a complete picture to explain the benign virtuous cycle of HIV-2
367 infection; whilst many epidemiological studies have found an association between specific
368 HLA class I alleles and HIV-1 disease outcome, i.e. rapid or delayed progression to AIDS (11),
369 the role of HLA polymorphism in HIV-2 is still unclear. Evidence exists that HLA-B*35 (55) and
370 B*1503 (56) alleles are associated with HIV-2 disease progression; nevertheless several
371 individuals bore HLA-B*35 alleles in our study, yet controlled their infection. Similarly, in the
372 context of HIV-1 infection, HLA class I homozygosity is associated with rapid progression to
373 AIDS (57, 58); however most of the HIC2 in our study are homozygotes. Interestingly, no HLA
374 allele associated with control of infection has been found in HIV-2 infection. It was suggested
375 by Yindom et al. (56) that this might be due to the fact that HIV-2 infection is easier to
376 control compared to HIV-1, and therefore that less stringent requirements for control may
377 not have led to the selection of “protective” epitopes that are restricted by specific HLA
378 molecules during HIV-2 infection. Additionally, unlike the partial control exerted by CD8⁺ T
379 cells in HIV-1 infection (59), the robust CD8⁺ T cell control of HIV-2 could limit the emergence
380 of escape variants, and therefore abrogate the requirement for HLA Class I molecules that
381 possess sufficient flexibility to adapt to new variants.

382 While still hypothetical at this stage, a greater sensitivity of HIV-2, compared with
383 HIV-1, to intracellular restriction factors may play a crucial role in constraining the initial
384 replication of HIV-2 in CD4⁺ T-cells. It has been shown that HIV-2 is more susceptible to
385 restriction by tripartite motif protein isoform 5 alpha (TRIM5a) than HIV-1 (60).
386 Subsequently, it was found that tetherin interacts differentially with the two viruses
387 depending on whether it is counteracted by Vpu or Env, as is the case in HIV-1 and HIV-2
388 respectively (61, 62). This difference might reflect a different sensitivity of HIV-1 and HIV-2

389 to tetherin control at the cell membrane. cART-naïve HIV-2 infected patients from the ANRS
390 CO5 HIV-2 Cohort also demonstrated high level of APOBEC3F/G editing activity in a previous
391 study (63); however, immunovirological parameters were not found to be associated with
392 the latter.

393 Finally, it has been proposed that the presence of the HIV-2/SIVsmm-specific Vpx, an
394 accessory protein that inhibits the action of SAMHD1, enhances the sensing of HIV-2 by
395 dendritic cells, which may promote the induction of more potent CD8⁺ T-cell responses (64).
396 This requires further investigation, as other groups have reported that dendritic cells are
397 refractory to HIV-2 infection, which could in turn, restrict HIV-2 replication (65, 66). Overall,
398 an enhanced sensitivity of HIV-2 to host restriction factors may be crucial in limiting its
399 replication initially, preventing further damage to the host immune system, providing the
400 necessary window of opportunity for the subsequent induction and enactment of an
401 effective T-cell response that establishes and maintains control of the virus. Once activated,
402 this timely bottleneck would prevent the over-exertion and subsequent exhaustion of
403 immune resources - usually related to the maintenance of the CD4 T-cell pool and the
404 consumption of HIV-specific immune cells – together with the presence of the associated
405 hyper inflammatory status often seen in HIV-1 infection; ultimately preserving the immune
406 functions of the infected person. Combined together, this synergy in innate and adaptive
407 factors may provide the necessary conditions for to establish the HIV-2 virtuous circle, and
408 therefore exemplify a holistic mechanism for natural control of the virus.

409

410

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417

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650 derived dendritic cells. *Retrovirology* 12: 2.

651

652

653 **Footnotes**

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663 5. Abbreviations:

664 *APOBEC 3F/G*: Apolipoprotein B mRNA Editing Enzyme Catalytic Subunit 3F/G; *cART*:
665 combination Antiretroviral Therapy; *HIC1/2*: HIV-1/2 Controller; *HPC*: Hematopoietic
666 Progenitor Cell; *IQR*: Interquartile Range; *SAMHD1*: SAM domain and HD domain-containing
667 protein 1; *sjTREC*: signal joint T-cell Receptor Excision Circle; *TRIM5α*: Tripartite Interaction
668 Motif 5a.

669

670 **Figure Legends**

671 **Figure 1. Lymphopoietic capacity of HIV-2 infected individuals.** (a) Representative example
672 of CD34 and lineage staining to identify HPC within total PBMCs from a HIV-2 infected
673 patient (left panel) and absolute counts (right panel) of CD34⁺ Lin⁻ cells in middle aged
674 healthy adults (HIV neg), treatment naïve viremic HIV-1 infected patients with high (HIV-1
675 CD4>500 CD4⁺ T-cells/ μ l) or low (HIV-1 CD4<200 CD4⁺ T-cells/ μ l) CD4⁺ T cell count, HIV-1
676 controllers (HIC1) and HIV-2 controllers (HIC2). (b) Representative example of CD117 and
677 CD45RA staining on CD34⁺ enriched cells to identify circulating lymphoid precursors in a HIV-
678 2 infected patient (left panel) and absolute counts (right panel) of CD117⁻ CD45RA⁺ CD34⁺
679 cells in middle aged healthy adults (HIV neg), treatment naïve viremic HIV-1 infected patients
680 with high (HIV-1 CD4>500 CD4⁺ T-cells/ μ l) or low (HIV-1 CD4<200 CD4⁺ T-cells/ μ l) CD4⁺ T cell
681 count, HIV-1 controllers (HIC1) and HIV-2 controllers (HIC2). (c) Representative example of
682 CCR7 and CD45RA staining on CD8⁺ T-cells to identify naïve cells in a HIV-2 infected patient
683 (left panel) and frequency (right panel) of naïve cells in middle aged healthy adults (HIV neg),
684 treatment naïve viremic HIV-1 infected patients with high (HIV-1 CD4>500 CD4⁺ T-cells/ μ l) or
685 low (HIV-1 CD4<200 CD4⁺ T-cells/ μ l), HIV-1 controllers (HIC1) and HIV-2 controllers (HIC2).
686 (d) Correlation between sj TREC levels (sjTREC/mL) and naïve CD8⁺ T-cell counts in HIV-2
687 controllers. (e) Correlation between the counts of recent thymic emigrants and naïve CD8⁺ T-
688 cells in HIV-2 controllers. The Mann-Whitney test was used for comparing groups. The
689 Spearman's rank test was used to determine correlations. Bars indicate the median.

690

691 **Figure 2. Characterization of p27-specific CD8⁺ T-cells in HIV-2 infected individuals.** (a)
692 Representative example of IFN γ secretion in CD8⁺ T-cells from a HIV-2 infected patient

693 unstimulated or upon stimulation with p27 overlapping peptides (left panel) and frequencies
694 of IFN γ ⁺ CD8⁺ T-cells in HIV-1 controllers (HIC1) and HIV-2 controllers (HIC2) upon stimulation
695 with HIV-1 and HIV-2 peptides respectively (right panel). **(b)** Representative stainings of HLA-
696 B*5301 restricted p27 TPYDINQML (TL9) (left panel) or HLA-B*1401 restricted p27
697 DRFYKSLRA (DA9) (right panel) specific CD8⁺ T-cells from HIV-2 infected patients. **(c)**
698 Representative stainings for the expression of the cell surface markers CD27, CD28, CD45RA,
699 CCR7, CD127 and intracellular granzyme B in TL9-specific CD8⁺ T-cells. **(d)** Comparative
700 expression of the cell surface markers CD27, CD28, CD45RA, CCR7, CD127 and intracellular
701 granzyme B between B27 KK10-specific CD8⁺ T-cells from HIV-1 infected patients (open
702 diamonds) and B53 TL9 or B14 DA9-specific CD8⁺ T-cells from HIV-2 infected patients (full
703 circles). The Mann-Whitney test was used for comparing groups. Bars indicate the median.

704

705 **Figure 3. *Ex vivo* viral suppressive capacity of HIV-2 infected individuals CD8⁺ T-cells.** **(a)**
706 Examples of p27 concentration in supernatant of CD4⁺ T-cells isolated from an HIV-2
707 controller (top) or an healthy donor (bottom), alone (red) or co-cultured with autologous
708 CD8⁺ T-cells (blue) at different days post infection. Data are represented as Means and
709 Standard Deviations. **(b)** Log p27 decrease (p27 concentration in supernatant of infected
710 CD4⁺ T-cells alone divided by p27 concentration in supernatant of infected CD4⁺ T-cells co-
711 cultured in presence of autologous CD8 T-cells) in HIV-1 (open diamonds) or HIV-2
712 controllers (black circles). Bars indicate the median. **(c)** p27 concentration in supernatant of
713 CD4⁺ T-cells isolated from 3 different HIV-2-infected individuals, alone (red columns) or co-
714 cultured with autologous CD8 T-cells (blue columns) at time of inclusion (Month 0, M0) or 12
715 months later (M12). Histograms are represented as Means and Standard Deviations.

716

717 **Figure 4. HIV-2 viral suppressive function and markers of disease progression. (a)** p27
718 concentrations in supernatant of CD4⁺ T-cells isolated from HIV-2 infected individuals with
719 rapidly decreasing CD4⁺ T-cells during at least 3 years (92.5 cells/mm³/year (left) and 215.7
720 cells/mm³/year (right)), alone (red) or co-cultured with autologous CD8 T-cells (blue) at
721 different days post infection. Data are represented as Means and Standard Deviations. **(b)**
722 Correlation of CD4⁺ T-cell counts (cells/mm³) and log p27 decrease (p27 concentration in
723 supernatant of infected CD4⁺ T-cells alone divided by p27 concentration in supernatant of
724 infected CD4⁺ T-cells co-cultured in presence of autologous CD8 T-cells) in HIV-2-infected
725 individuals. The Spearman's rank test was used to determine correlations.

726

Fig. 1

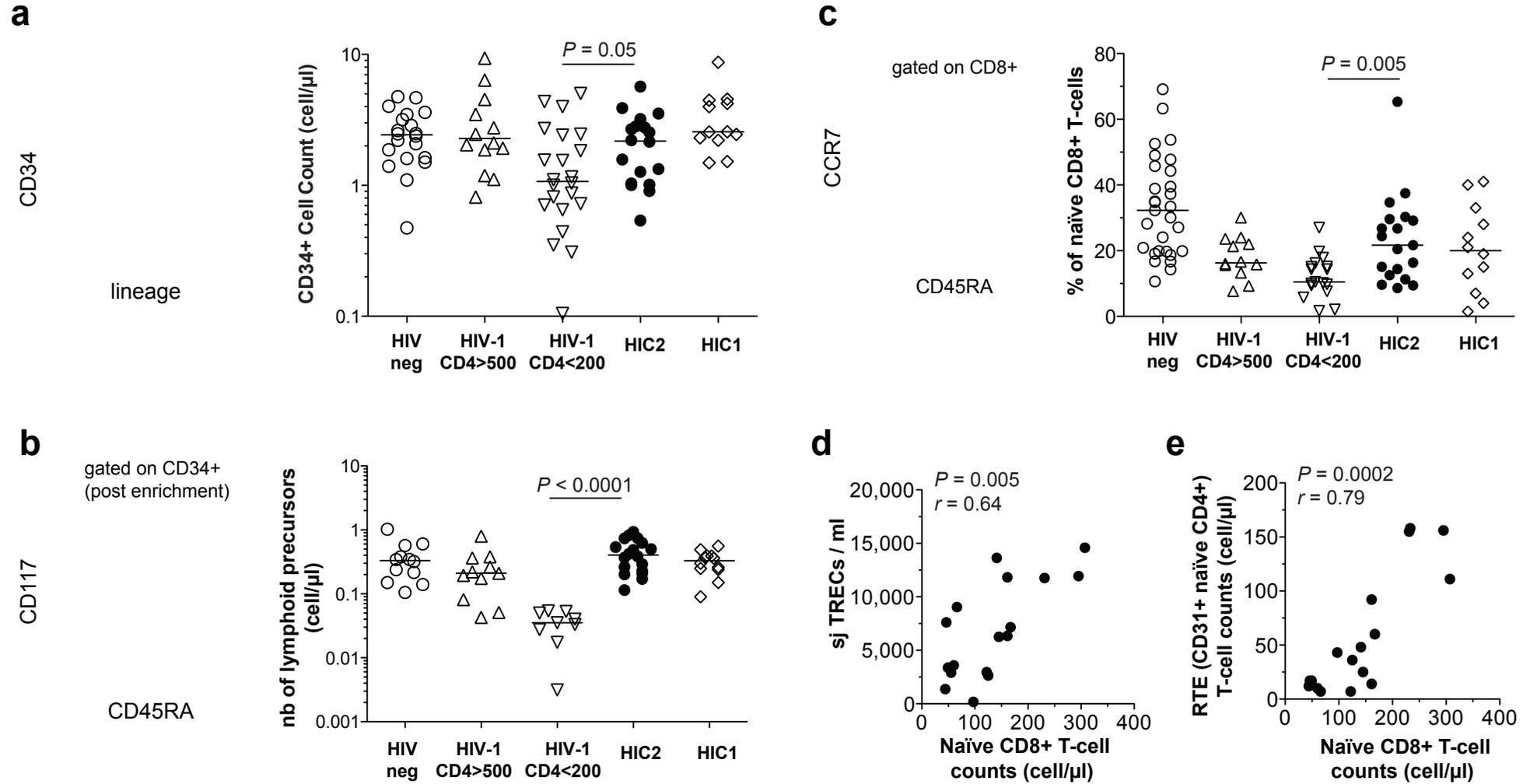


Fig. 2

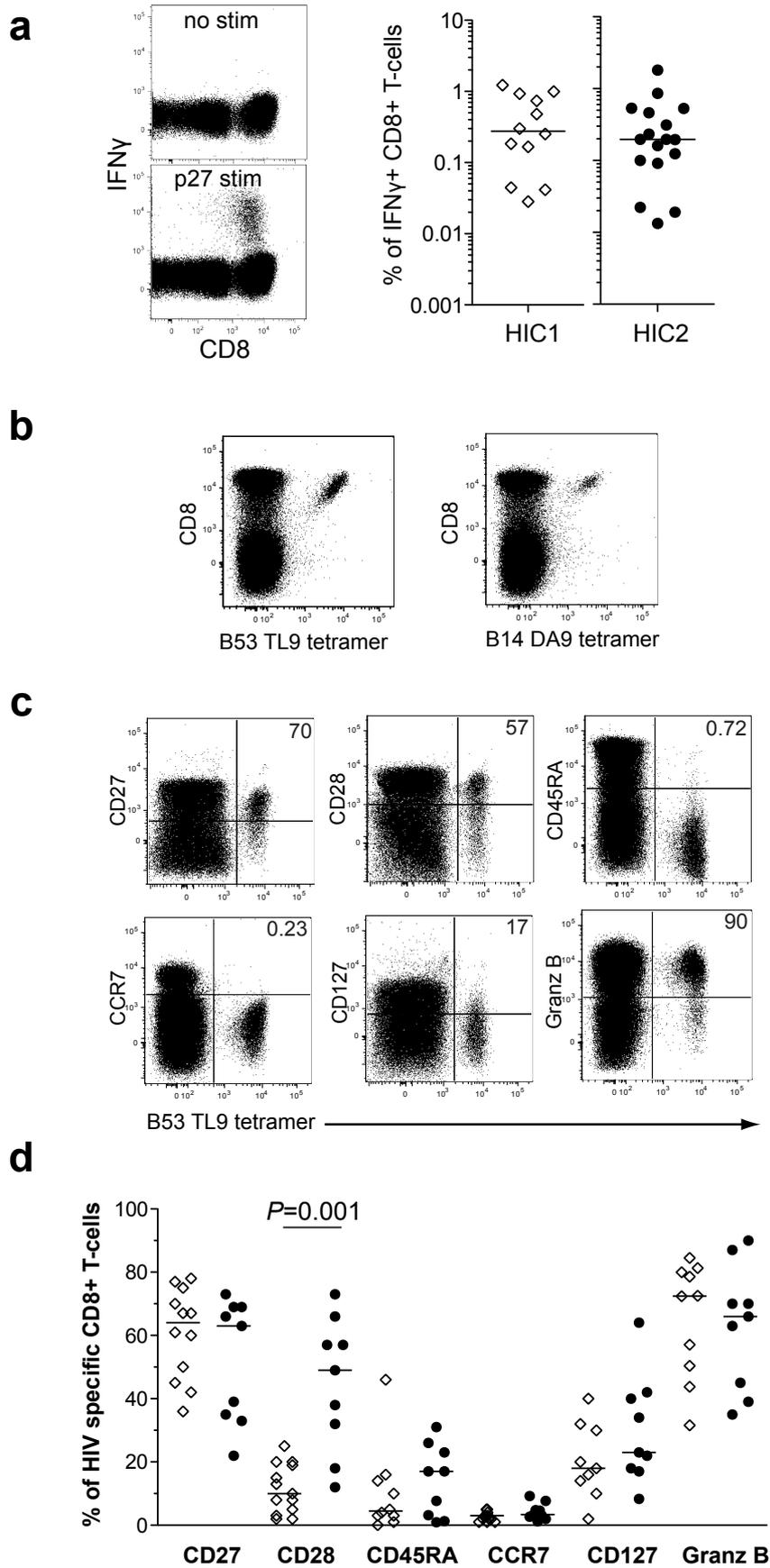


Fig. 3

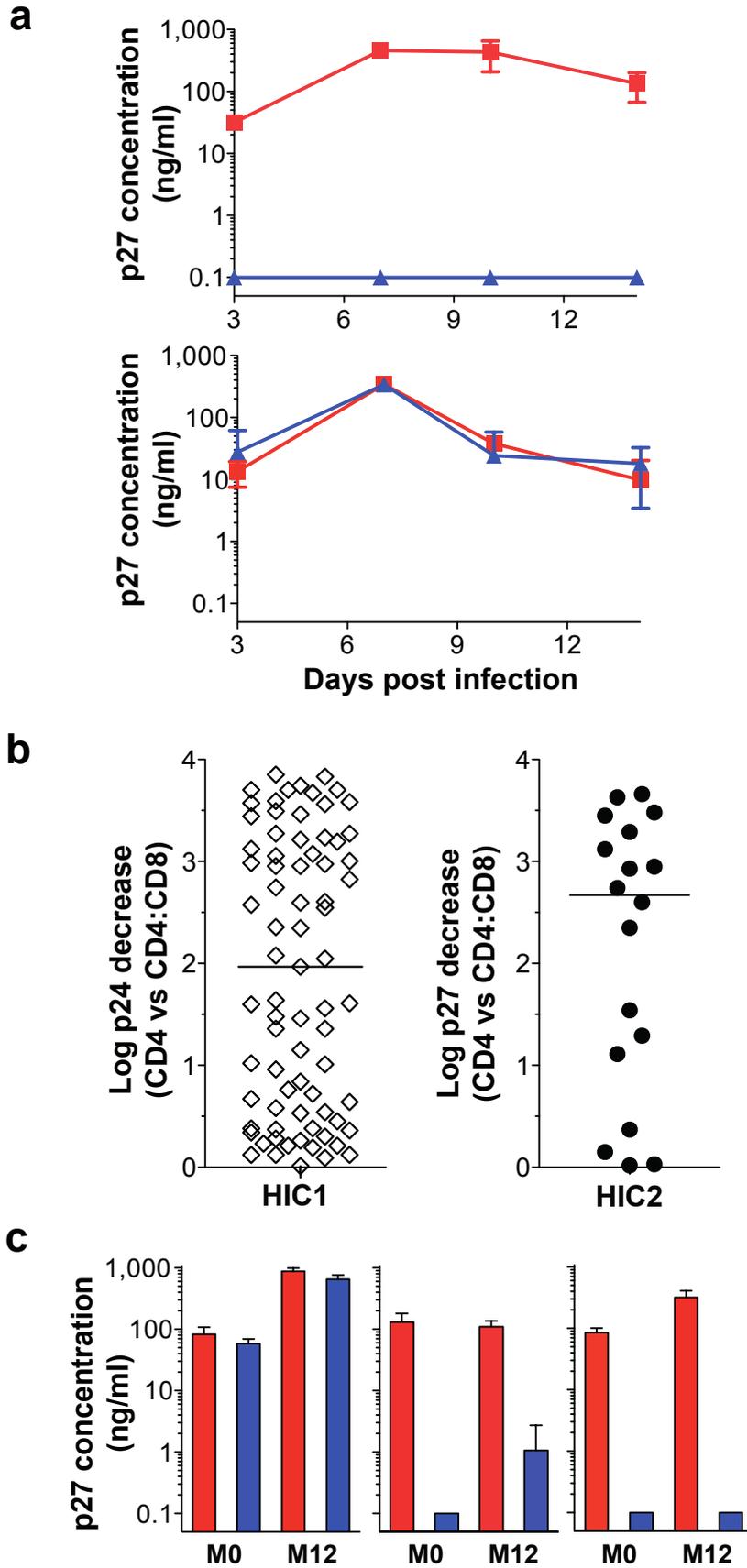
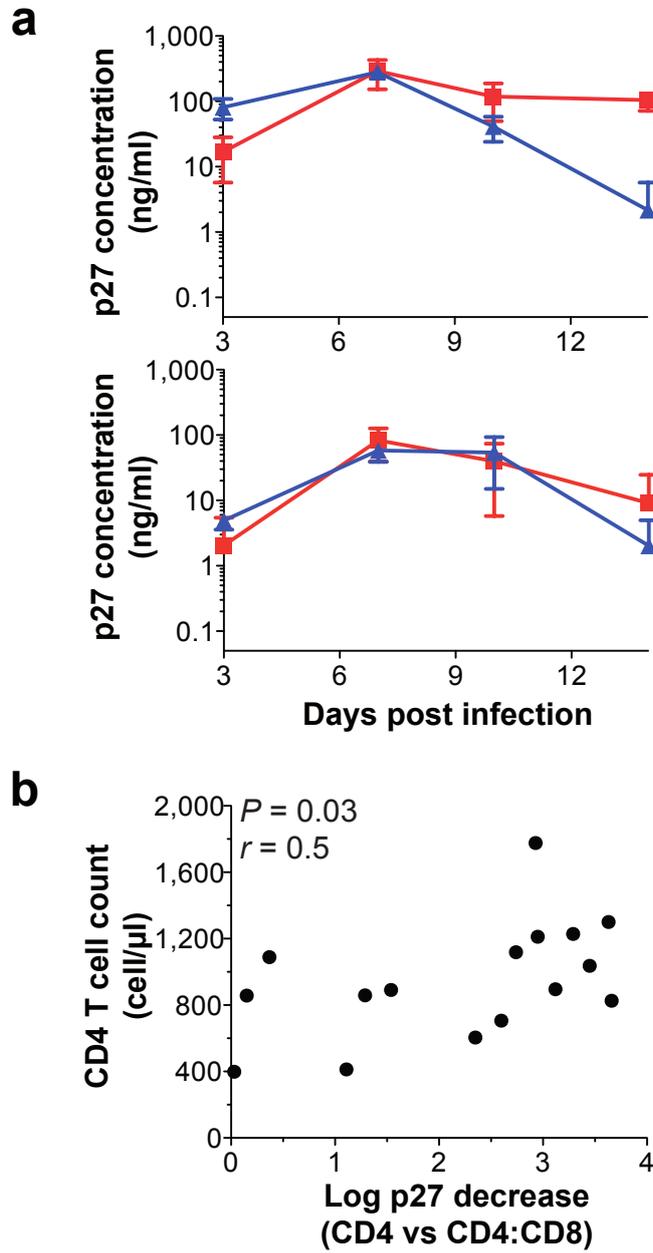


Fig. 4



1 **Table I. Clinical attributes of the HIV-2-infected patients studied**

ID	Gender	Age (years)	CD4 count (cells/ μ l)	Time since diagnosis (years)	Viral load (copies/mL)	HLA A	HLA B	HLA C	Country of Birth
012-006	M	44	707	20.5	<40	03/23	35/53	04/04	Ivory Coast
012-009	F	54	1,118	27.3	<100	03/74	14/15	07/08	Guinea Conakry
012-045	F	43	858	22.7	<40	1/33	15/35	04/14	France
012-073	M	52	891	11.3	<100	02/03	49/57	07/18	Ivory Coast
012-084	F	50	1,776	8.8	<40	68/68	07/52	-	Ghana
012-088	M	70	502	7.4	117	02/02	27/53	02/04	Senegal
012-101	F	34	1,212	12.5	<40	03/26	58/58	03/07	The Gambia
013-035	F	48	859	9.2	<40	34/34	15/53	02/04	Guinea Conakry
013-037	M	59	1,300	8.8	<40	01/29	44/57	06/16	Colombia
013-049	F	52	604	25.6	<40	02/23	15/52	02/16	Guinea Conakry
019-010	F	40	1,170	15.4	-	33/68	53/53	04/04	Ivory Coast
023-008	F	41	-	5.0	<40	34/34	7/53	04/07	Ivory Coast
028-012	F	28	827	10.2	<40	-	18/78	05/16	Guinea Conakry
028-016	F	49	895	11.3	<40	02/68	15/51	14/16	Ivory Coast
036-018	F	39	1,036	12.7	<100	03/03	35/53	04/04	Ivory Coast
036-019	M	53	1,228	17.6	<40	23/23	07/14	07/08	Guinea Bissau
045-007	F	50	-	16.2	40	33/33	53/53	04/04	Ivory Coast
051-007	M	48	399	14.4	<40	02/29	39/44	07/16	France
075-001	M	52	413	18.2	54	24/25	35/44	05/12	France
082-005	M	57	1,090	23.0	<100	23/34	53/53	04/04	Ivory Coast

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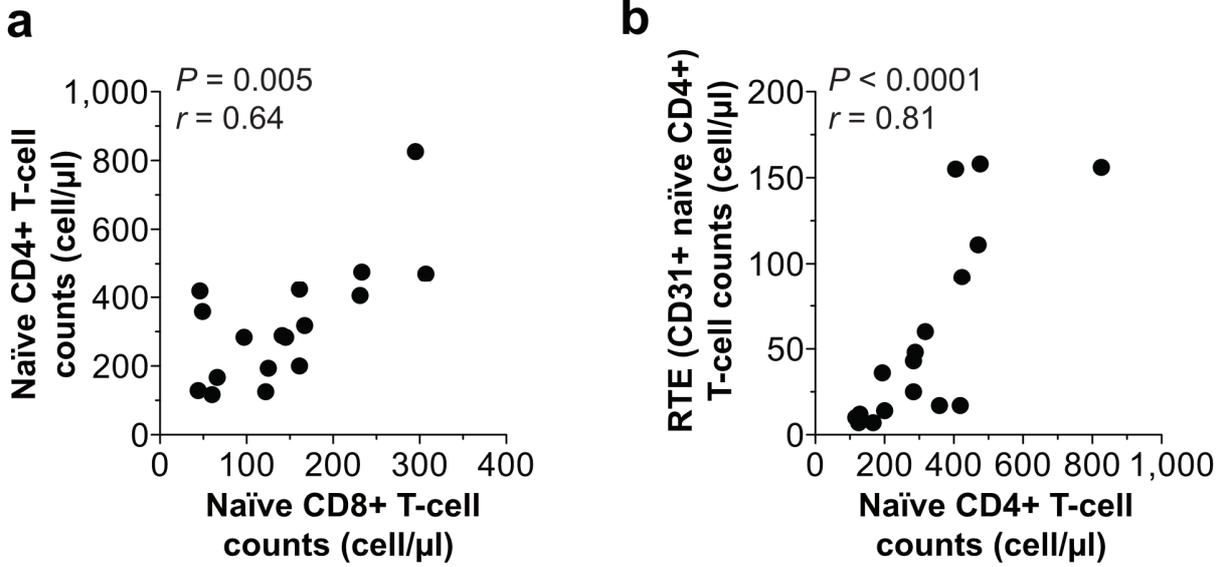
1 **Table II. Summary of clinical attributes of the patients studied**

Group	Gender (%female)	Age (years)	CD4 count (cells/ μ l)	Viral load (copies/mL)
HIV1-negative	51%	39 [33-47]	860 [610-1,110]	NA
HIV1+ CD4<200	30%	40 [32-48]	82 [42-136]	127,330 [3,040-543,000]
HIV1+ CD4>500	11%	39 [31-48]	690 [560-910]	16,540 [800-6,300]
HIV1+-controllers	53%	48 [42-51]	751 [519-953]	<40 [<40-57]
HIV2+-controllers	60%	49 [42-52]	893 [707-1,170]	<40 [<40-88.5]

2 Values are expressed as: median [interquartile range]

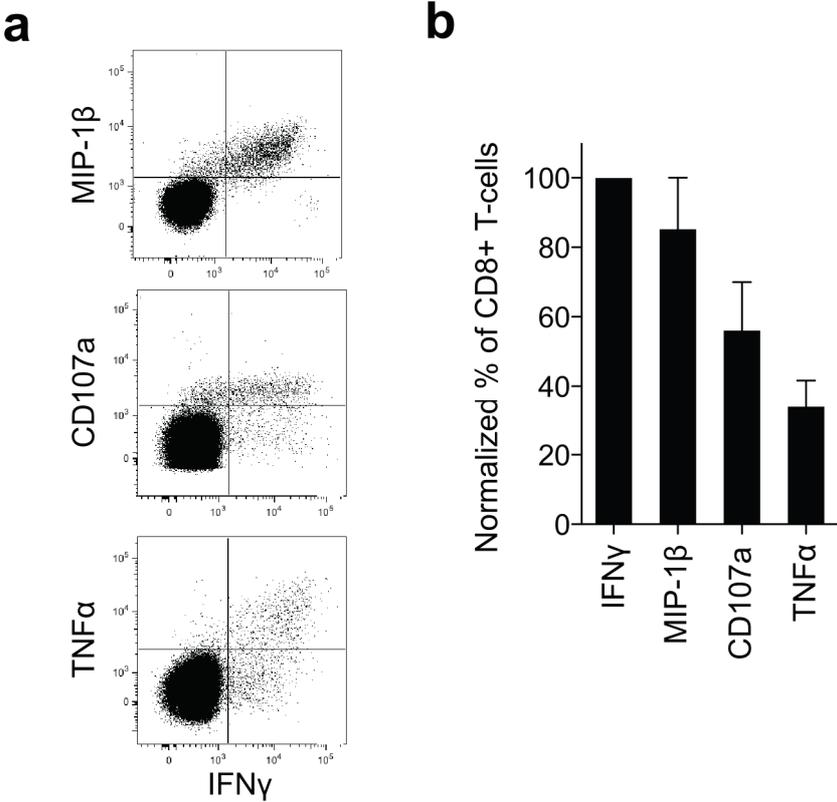
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Supplementary Figure 1. Naïve T-cell count levels in HIV-2 infected individuals.



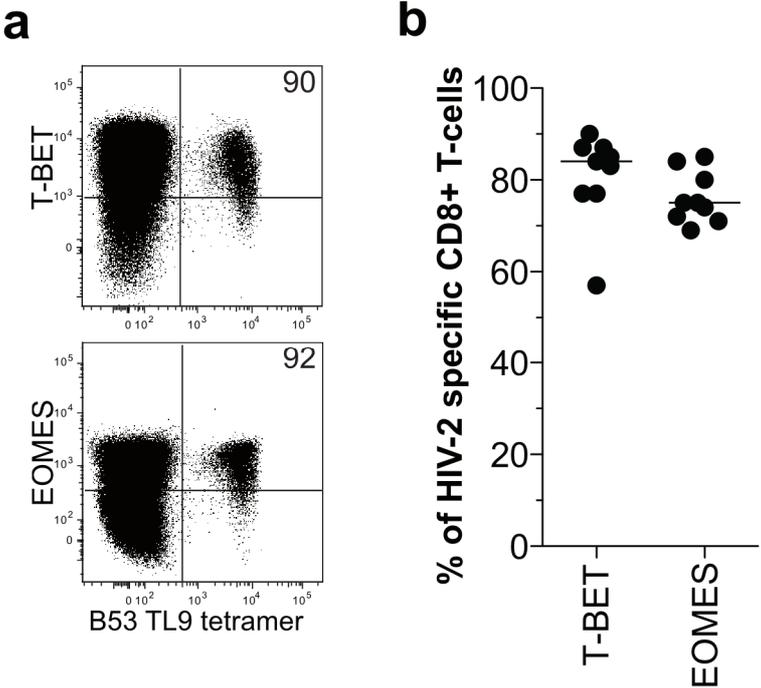
(a) Correlation between naïve CD8⁺ and naïve CD4⁺ T-cell counts in HIV-2 controllers. (b) Correlation between the counts of recent thymic emigrants (RTE) and naïve CD4⁺ T-cells in HIV-2 controllers. The Spearman’s rank test was used to determine correlations.

Supplementary Figure 2. Polyfunctional potential of HIV-2 p27-specific CD8⁺ T-cells.



(a) Representative examples of MIP-1β, CD107a, TNFα and IFNγ secretion in CD8⁺ T-cells from a HIV-2 infected patient upon stimulation with p27 overlapping peptides. (b) Frequencies of CD8⁺ T-cells secreting IFNγ, MIP-1β, CD107a or TNFα in HIV-2 infected patients upon stimulation with p27 overlapping peptides respectively. Data are normalized mean percentages from nine patients

Supplementary Figure 3. Expression of T-BET and EOMES in HIV-2 p27-specific CD8⁺ T-cells.



(a) Representative stainings for the intracellular expression of the transcription factors T-BET and EOMES in B53-TL9-specific CD8⁺ T-cells. (b) Expression of the transcription factors T-BET and EOMES in B53 TL9 or B14 DA9-specific CD8⁺ T-cells from HIV-2 infected patients.