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### ► To cite this version:

Caroline Passaes, Antoine Millet, Vincent Madelain, Valerie Monceaux, Annie David, et al.. Optimal maturation of the SIV-specific CD8 + T-cell response after primary infection is associated with natural control of SIV. ANRS SIC study.. 2020. pasteur-02865346

**HAL Id: pasteur-02865346**

**<https://pasteur.hal.science/pasteur-02865346v1>**

Preprint submitted on 11 Jun 2020

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1 **Optimal maturation of the SIV-specific CD8<sup>+</sup> T-cell response after primary infection**  
2 **is associated with natural control of SIV.**

3 **ANRS SIC study**

4  
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26

27 **ABSTRACT**

28 Highly efficient virus-specific CD8<sup>+</sup> T-cells are associated with immune control of HIV  
29 infection, but it remains unclear how these cells are generated and maintained over time.  
30 We used a macaque model of spontaneous control of SIVmac251 infection to monitor the  
31 development and evolution of potent antiviral CD8<sup>+</sup> T-cell responses. SIV-specific CD8<sup>+</sup> T-  
32 cells emerged during primary infection in all animals. However, the ability of CD8<sup>+</sup> T cells to  
33 suppress SIV replication was low in early stages but increased after a period of maturation,  
34 temporally linked with the establishment of sustained low-level viremia in controller  
35 macaques. SIV-specific CD8<sup>+</sup> T-cells with a central memory phenotype expressed higher  
36 levels of survival markers in controllers *versus* non-controllers. In contrast, a persistently  
37 skewed differentiation phenotype was observed among central memory SIV-specific CD8<sup>+</sup> T-  
38 cells in non-controllers since primary infection, typified by relatively high expression levels of  
39 T-bet.

40 Collectively, these data show that the phenotype of SIV-specific CD8<sup>+</sup> T-cells defined early  
41 after SIV infection favor the gain of antiviral potency as a function of time in controllers,  
42 whereas SIV-specific CD8<sup>+</sup> T-cell responses in non-controllers fail to gain antiviral potency  
43 due to early defects imprinted in the central memory pool.

## 44 INTRODUCTION

45 The ability of CD8<sup>+</sup> T-cells to control viral replication has been extensively documented in the  
46 setting of HIV/SIV infection (McBrien et al., 2018; Walker and McMichael, 2012). Primary  
47 infection is characterized by massive viremia, which subsides following the expansion of  
48 HIV/SIV-specific CD8<sup>+</sup> T-cells (Borrow et al., 1994; Koup et al., 1994). However, the virus is  
49 not eradicated, leading to the emergence of immune escape variants (Allen et al., 2000;  
50 Borrow et al., 1997; O'Connor et al., 2002; Price et al., 1997) and to CD8<sup>+</sup> T-cell exhaustion  
51 during the chronic phase of infection (Day et al., 2006; Petrovas et al., 2006; Petrovas et al.,  
52 2007; Trautmann et al., 2006). These observations suggest that naturally generated HIV/SIV-  
53 specific CD8<sup>+</sup> T-cells are frequently suboptimal in terms of antiviral efficacy, potentially  
54 reflecting limited cross-reactivity and/or intrinsic defects in the arsenal of effector functions  
55 required to eliminate infected CD4<sup>+</sup> T-cells (Du et al., 2016; Lecuroux et al., 2013). The latter  
56 possibility is especially intriguing in light of *ex vivo* experiments showing that effective  
57 suppression of viral replication is a particular feature of CD8<sup>+</sup> T-cells isolated from HIV  
58 controllers (HICs) (Angin et al., 2016; Saez-Cirion et al., 2007; Saez-Cirion et al., 2009; Tansiri  
59 et al., 2015).

60

61 HICs are a rare group of individuals who control viremia to very low levels without  
62 antiretroviral therapy (Saez-Cirion and Pancino, 2013). Understanding the mechanisms  
63 associated with such spontaneous control of HIV infection seems crucial for the  
64 development of new strategies designed to achieve remission. Efficient CD8<sup>+</sup> T-cell  
65 responses are almost universally present in HICs (Betts et al., 2006; Chowdhury et al., 2015;  
66 Hersperger et al., 2011a; Hersperger et al., 2010; Migueles et al., 2002; Migueles et al., 2008;  
67 Saez-Cirion et al., 2007; Saez-Cirion et al., 2009; Zimmerli et al., 2005). These individuals also

68 frequently express the protective human leukocyte antigen (HLA) allotypes HLA-B\*27 and  
69 HLA-B\*57, further supporting a key role for CD8<sup>+</sup> T-cells in the natural control of HIV  
70 (Lecuroux et al., 2014; Migueles et al., 2000; Pereyra et al., 2008). However, the presence of  
71 protective HLA alleles is neither sufficient nor necessary for natural control of infection, and  
72 HICs carrying non-protective HLA class I alleles also carry CD8<sup>+</sup> T-cells with strong HIV  
73 suppressive capacity (Lecuroux et al., 2014). Although the qualitative properties of CD8<sup>+</sup> T-  
74 cells from HICs have been extensively characterized, these analyses have been essentially  
75 performed during chronic infection, when viremia was already under control, often several  
76 years after the acquisition of HIV. It therefore remains unclear how these high-quality CD8<sup>+</sup>  
77 T-cell responses develop from the early stages of infection and evolve over time.

78  
79 Cynomolgus macaques (CyMs, *Macaca fascicularis*) infected with SIVmac251 closely  
80 recapitulate the dynamics and key features of HIV infection, including similar levels of viral  
81 replication in the acute and chronic phases of infection, memory CD4<sup>+</sup> T-cell depletion, rapid  
82 seeding of the viral reservoir, and eventually progression to AIDS with diarrhea, weight loss,  
83 high incidence of lymphoblastic lymphomas and marked decrease of CD4<sup>+</sup> T cells within 145  
84 to 464 days post-infection (Antony and MacDonald, 2015; Feichtinger et al., 1990; Karlsson  
85 et al., 2007; Mannioui et al., 2009; Putkonen et al., 1989). As in humans, some individuals  
86 control infection naturally in the absence of treatment. CyMs from Mauritius offer the  
87 additional advantage of limited MHC diversity, making them particularly attractive for the  
88 study of CD8<sup>+</sup> T-cell responses. Indeed, natural SIV control in Mauritius CyMs is favored by  
89 the presence of the MHC haplotype M6 (Aarnink et al., 2011; Mee et al., 2009). Natural SIV  
90 control can be also achieved in CyMs inoculated with a relatively low virus dose exposure  
91 through the intra rectal route (*i.r.*), independent of their MHC haplotype (Bruel et al., 2015).

92 We therefore took advantage of these validated CyM models spreading from natural SIV  
93 control to progression to AIDS to study the dynamics of SIV-specific CD8<sup>+</sup> T-cell responses in  
94 blood and tissues from the onset of infection in both SIV controllers and viremic macaques.  
95 Using this approach, we identified an optimal maturation pathway that enabled SIV-specific  
96 CD8<sup>+</sup> T-cells to acquire potent antiviral functions, control viremia, and survive in SICs.  
97

98 **RESULTS**

99 ***SIV controllers are characterized by partial restoration of CD4<sup>+</sup> T-cell counts and***  
100 ***progressive decline in the frequency of SIV-carrying cells in blood***

101 We monitored prospectively the outcome of infection in 12 SIV controllers (SICs) and 4  
102 viremic CyMs (VIRs) inoculated *i.r.* with SIVmac251. These animals carried or not the  
103 protective M6 haplotype and were inoculated with 5 or 50 animal infectious dose<sub>50</sub> (AID<sub>50</sub>) of  
104 SIVmac251 (Supplemental Table 1). SIV controllers decreased plasma viral load (VL) to levels  
105 below 400 SIV-RNA copies/mL, at least twice, over a follow up period of 18 months, while  
106 VIRs consistently maintained VL above 400 SIV-RNA copies/mL. The threshold of 400 RNA  
107 copies/mL was chosen in coherence with our studies in human cohorts of natural HIV control  
108 (Angin et al., 2016; Noel et al., 2016; Saez-Cirion et al., 2013; Saez-Cirion et al., 2007; Saez-  
109 Cirion et al., 2009). Ten SICs achieved control of viremia within 3 months. The other two SICs  
110 (BL669 and BO413) achieved VL below 400 SIV-RNA copies/mL for the first time 14 months  
111 after inoculation. One VIR CyM (AV979) developed a tonsillar lymphoma, an AIDS related  
112 event reported at high frequency in this species upon SIV infection (Feichtinger et al., 1990).

113  
114 Some differences in peak viremia were observed between SICs and VIRs (Figure 1A, Table 1).  
115 These differences became more pronounced over time (Figure 1A), because plasma viremia  
116 was suppressed more rapidly in SICs versus VIRs (Table 1). Levels of cell-associated SIV-DNA  
117 in blood from SICs and VIRs were comparable before peak viremia, but differences became  
118 apparent as plasma VLs declined and were maintained throughout chronic infection (Figure  
119 1B, Table 1). In addition, CD4<sup>+</sup> T-cell counts declined markedly in blood from both SICs and  
120 VIRs during primary infection (Figure 1C, Table 1). Subsequently, a degree of recovery was  
121 observed in SICs, whereas further gradual decline was observed in VIRs (Figure 1C, Table 1).

122

123 These results evidenced the distinctive dynamics of SIV infection in SICs and VIRs,  
124 characterized by very modest differences during the early weeks following inoculation that  
125 were progressively exacerbated during transition to chronic infection. The differences  
126 between SICs and VIRs during the chronic phase of SIV infection were consistent with the  
127 observations in human cohorts of HIV controllers.

128

### 129 ***SIV control is associated with early preservation of lymph nodes***

130 To characterize the extent of SIV control in greater depth, we monitored CD4<sup>+</sup> T-cells and  
131 total SIV-DNA longitudinally in peripheral lymph nodes (PLNs) and rectal biopsies (RBs). At  
132 the end of the study, we conducted similar evaluations in bone marrow, spleen, mesenteric  
133 lymph nodes (MLNs), and colonic mucosa, comparing SICs versus VIRs. The frequency of  
134 CD4<sup>+</sup> T-cells similarly declined in RBs from both SICs and VIRs during the acute stage of  
135 primary infection. While the frequency of CD4<sup>+</sup> T-cells was later partially restored in SICs, it  
136 continued to decline in VIRs (Figure 2A). These results matched the observations in blood  
137 samples (Figure 1B). In contrast, the frequency of CD4<sup>+</sup> T-cells was maintained close to  
138 baseline in PLNs from SICs, even during primary infection (day 14 post-infection [*p.i.*]), but  
139 steadily declined over time in VIRs (Figure 2B). At the time of euthanasia, CD4<sup>+</sup> T-cell  
140 frequencies were substantially higher in blood (Figure 1C), bone marrow, spleen, PLNs,  
141 MLNs, and colonic mucosa (Figure 2C) from SICs versus VIRs.

142

143 Cell-associated SIV-DNA levels closely mirrored the dynamics of CD4<sup>+</sup> T-cells. Similarly high  
144 levels of cell-associated SIV-DNA were observed in RBs from SICs and VIRs during primary  
145 infection, but lower levels were observed in RBs from SICs *versus* VIRs during chronic



146 infection (Figure 2D). Of note, SIV-DNA levels were already approximately 1 log lower in  
147 PLNs *versus* RBs from SICs during primary infection, and accordingly, lower levels were  
148 observed in PLNs from SICs *versus* VIRs since day 14 *p.i.* (Figure 2E). This finding suggests  
149 that early viral replication may be contained more efficiently in lymphoid nodes in SICs  
150 compared with other explored anatomical compartments. Moreover, SIV-DNA was also  
151 detected in alveolar macrophages from all CyMs throughout the course of infection, again at  
152 lower levels in SICs *versus* VIRs during chronic infection (Figure S1A, bottom panel). In  
153 addition, SIV-DNA levels trended to decline progressively over time in SICs in all tissues  
154 analyzed, whereas SIV-DNA levels remained stable after primary infection in VIRs (Figure 1B,  
155 2D-F). At the time of euthanasia, SIV-DNA levels were substantially lower in blood (Figure  
156 1B), bone marrow, PLNs, MLNs, and gut mucosa (Figure 2F and Figure S1B) from SICs *versus*  
157 VIRs.

158

159 Collectively, these data indicate that progressive systemic control of viral replication is  
160 achieved in SICs with CD4<sup>+</sup> T-cell preservation and lower pan-anatomical reservoirs of SIV-  
161 DNA. Our results also underline the early preservation of PLNs in these animals.

162

### 163 ***The dynamics of CD8<sup>+</sup> T-cells expansion and activation do not predict control of SIV***

164 To understand the mechanisms that contribute to immune control of SIV, we first monitored  
165 the proliferation and activation dynamics of total CD8<sup>+</sup> T-cells in blood and lymphoid tissues  
166 from SICs and VIRs. Recent studies in cohorts of hyperacute HIV infected individuals indicate  
167 that the changes observed in the total CD8<sup>+</sup> T-cell activation during acute infection may be  
168 largely related to changes in the HIV-specific CD8<sup>+</sup> T-cell pool (Ndhlovu et al., 2015; Takata et  
169 al., 2017). The frequencies of CD8<sup>+</sup> T-cells expressing Ki-67 in blood increased to maximum

170 levels during primary infection (measured peak at day 15 *p.i.*), coinciding with the measured  
171 peak of viremia, then declined steadily to baseline levels during chronic infection (Figure 3A).  
172 Similar dynamics were observed in PLNs (Figure 3B) and gut mucosa (Figure 3C). In general,  
173 there were no significant differences between SICs and VIRs with respect to the dynamics of  
174 Ki-67 expression within the CD8<sup>+</sup> T-cell pool, although lower frequencies of CD8<sup>+</sup> T-cells  
175 expressing Ki-67 were observed during chronic infection in PLNs from SICs *versus* VIRs  
176 (Figure 3B).

177  
178 The frequencies of CD8<sup>+</sup> T-cells expressing the activation markers CD38 and HLA-DR in blood,  
179 PLNs, and gut mucosa increased similarly during primary infection (measured peak at day 28  
180 *p.i.*), following the dynamics of Ki-67 expression in the same compartments (Figure 3D–F).  
181 Again, there were no significant differences between SICs and VIRs with respect to the early  
182 dynamics of total CD8<sup>+</sup> T-cell activation, but lower frequencies of CD8<sup>+</sup> T-cells expressing  
183 CD38 and HLA-DR were observed during chronic infection in PLNs from SICs *versus* VIRs  
184 (Figure 3E).

185  
186 Overall, our findings indicate that although lower activation and proliferation is observed in  
187 of CD8<sup>+</sup> T-cells from SICs than VIRs in the chronic stage of infection, the early proliferation  
188 and activation dynamics of the total pool of CD8<sup>+</sup> T-cells do not distinguish subsequent  
189 progression rates.

190

### 191 ***SIV-specific CD8<sup>+</sup> T-cell frequencies do not predict control of SIV***

192 In parallel experiments, we analyzed CD8<sup>+</sup> T-cell responses to a pool of optimal SIVmac251  
193 peptides, which included peptides from different SIV proteins recognized by the most

194 frequent MHC haplotypes in CyMs (M1, M2 and M3) and by the MHC haplotype M6  
195 (Supplemental Table 2). All the animals carried at least one haplotype matching some  
196 peptide, and overall there was not difference in the number of peptides tested theoretically  
197 recognized in controllers and non-controllers ( $p=0.35$ ). SIV-specific CD8<sup>+</sup> T-cells producing  
198 TNF $\alpha$  (cytokine showing the lowest background in the absence of peptide or in presence of  
199 peptide during the baseline and hence used as reference) emerged in all CyMs during  
200 primary infection, coinciding with the peak of viremia, and no significant differences were  
201 observed between SICs and VIRs with respect to the frequencies of these cells in any  
202 anatomical compartment at any stage of infection (Figure 4A). Similarly, no consistent  
203 differences were observed between SICs and VIRs with respect to the frequencies of SIV-  
204 specific CD8<sup>+</sup> T-cells that produced other cytokines, including IFN $\gamma$  (Figure S2A and S3A) and  
205 IL-2 (Figure S2B and S3B), or mobilized CD107a (Figure S2C and S3C). The overall SIV-specific  
206 CD8<sup>+</sup> T-cell response, determined in each CyM as the frequency of cells displaying at least  
207 one function (TNF $\alpha$ , IFN $\gamma$ , IL-2, or CD107a), was also equivalent between SICs and VIRs  
208 across anatomical compartments during primary and chronic infection (Figure S2D and S3D).  
209 In addition, no clear differences between SICs and VIRs were observed with respect to the  
210 frequencies of SIV-specific CD8<sup>+</sup> T-cells displaying at least three functions simultaneously in  
211 blood or PLNs during acute infection, but higher frequencies of polyfunctional SIV-specific  
212 CD8<sup>+</sup> T-cells were present during chronic infection in lymphoid tissues from SICs *versus* VIRs  
213 (Figure 4B and S4). Of note, no differences in the magnitude (Figure S5A) and polyfunction  
214 (Figure S5B) of SIV-specific CD8<sup>+</sup> T-cells from SICs and VIRs were observed either when a pool  
215 of overlapping peptides spanning SIV Gag was used instead of the optimal peptide pool to  
216 stimulate the cells.

217

218 These data suggest that natural control of SIV is not associated with acutely generated,  
219 functionally superior SIV-specific CD8<sup>+</sup> T-cell responses, defined on the basis of cytokine  
220 production and degranulation.

221

222 ***Progressive acquisition of CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity is associated with***  
223 ***control of SIV***

224 CD8<sup>+</sup> T-cells from HICs typically suppress *ex vivo* infection of autologous CD4<sup>+</sup> T-cells (Angin  
225 et al., 2016; Buckheit et al., 2012; Julg et al., 2010; Saez-Cirion et al., 2007; Saez-Cirion et al.,  
226 2009; Tansiri et al., 2015). We therefore investigated this property as a potential  
227 discriminant between SICs and VIRs. The capacity of CD8<sup>+</sup> T-cells in blood and PLNs to  
228 suppress infection of autologous CD4<sup>+</sup> T-cells was relatively weak in all CyMs during acute  
229 infection (Figure 5A), but remarkably, this activity correlated negatively with viremia on day  
230 15 *p.i.* (Figure 5B, upper panel) suggesting its contribution to control viremia since early time  
231 points. Interestingly, the CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity increased  
232 substantially over time in SICs (Figure 5A and S6), either in blood or tissues. No such  
233 acquisition of SIV-suppressive activity was observed in VIRs (Figure 5A and S6). Moreover,  
234 CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity on day 70 *p.i.* correlated negatively (or trended  
235 to correlate) with all subsequent determinations of plasma VL (Figure S7) and there was a  
236 negative correlation between the CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity at  
237 euthanasia and the viremia at this time (Figure 5C, upper panel). In contrast, no significant  
238 correlations were identified at any time point between SIV-specific CD8<sup>+</sup> T-cell frequencies,  
239 categorized according to TNF $\alpha$  production in response to SIV peptides, and measurements  
240 of plasma VL (Figure 5B-C, bottom panels). Moreover, CD8<sup>+</sup> T-cell-mediated SIV-suppressive  
241 activity across the entire follow-up period, quantified as area under the curve, trended to

242 correlate negatively with plasma VL ( $r_s = -0.47$ ,  $p = 0.07$ ), whereas no such association was  
243 identified for the frequency of SIV-specific CD8<sup>+</sup> T-cells ( $r_s = -0.01$ ,  $p = 0.97$ ) (Figure 5D).

244

245 In a complementary study, Madelain et al (submitted) developed a mathematical model to  
246 fit the longitudinal SIV RNA data in this cohort of animals. The best fit to the data was  
247 obtained by using a model including an immune-response-mediated infected-cell elimination  
248 compartment where the loss rate of productively infected cells increased over time.  
249 Interestingly, the pattern of increase in cell loss rates (based on the analysis of SIV RNA only)  
250 nicely matched in most animals the changes in the capacity of CD8<sup>+</sup> T-cells to suppress  
251 infection that were obtained experimentally. Moreover, a *post hoc* positive correlation was  
252 found between the theoretical immune-response-mediated infected-cell elimination rate  
253 and the experimental CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity, but not with the  
254 frequency of SIV-specific CD8<sup>+</sup> T-cells.

255

256 Therefore, our results exposed a disconnection between the development of SIV-specific  
257 CD8<sup>+</sup> T-cells producing cytokines and cytolytic molecules and the ability of these cells to  
258 suppress SIV infection, as measured *ex vivo* (Figure 5E, Figure S6). SIV-specific CD8<sup>+</sup> T-cell  
259 frequencies increased sharply as the initial viremia began to fall and remained high for the  
260 duration of the study in CyMs irrespectively of their level of viremia. However, the  
261 substantial decline in viremia to levels below 400 copies/mL in SICs coincided with the raise  
262 of SIV-suppressive activity *ex vivo*. The increase of CD8<sup>+</sup> T-cell-mediated SIV-suppressive  
263 activity was delayed in the late controller #BL669 and #BO413, but nonetheless preceded  
264 optimal control of viremia in these CyMs. A very strong capacity of CD8<sup>+</sup> T-cells to suppress  
265 SIV was observed at day 36 in the LN from two animals (BA209 and BC657) that did not show

266 such capacity in the blood (Figure 5A). Only one animal (29925) did not develop any  
267 detectable SIV suppressive activity during our follow up. This animal had the weakest peak of  
268 viremia (1 log lower than any other) and achieved the fastest control of viremia. Whether a  
269 very rapid or local development of the CD8<sup>+</sup> T-cell suppressive capacity may have occurred  
270 or other mechanisms were associated with control of viremia in this animal remains  
271 unknown (Figure S6). At the time of euthanasia, superior CD8<sup>+</sup> T-cell-mediated SIV-  
272 suppressive activity was detected in a vast majority of SICs across all anatomical  
273 compartments, with the exception of bone marrow (Figure 5A), which nonetheless harbored  
274 SIV-specific CD8<sup>+</sup> T-cells at frequencies comparable to other tissues (Figure 4A). Thus,  
275 although abundant, SIV-specific CD8<sup>+</sup> T-cells induced during primary SIV infection had limited  
276 SIV suppressive capacity when compared to cells found at later time points in SICs (Figure  
277 5E).

278

279 To confirm that the capacity of CD8<sup>+</sup> T-cells to suppress *ex vivo* SIV infection did not increase  
280 in VIRs, we analyzed this activity in an additional group of 14 non-M6 CyMs infected  
281 intravenously (*i.v.*) with 1,000AID<sub>50</sub> of SIVmac251 and characterized by high setpoint viremia  
282 (ANRS pVISCNTI study). In these animals, the CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity  
283 also remained modest throughout the follow-up (Figure S8A). The combined analysis of the  
284 CD8<sup>+</sup> T-cells from all VIR CyMs (n=4 50AID<sub>50</sub> + n=14 1,000AID<sub>50</sub>) exposed early significant  
285 differences in the CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity when compared to the SICs  
286 (Figure S8B). Moreover, early initiation (day 28 post-infection) of antiretroviral treatment in  
287 another group of CyM inoculated with 1,000AID<sub>50</sub> of SIVmac251 sharply decreased viremia  
288 and CD8<sup>+</sup> T-cell activation levels (Figure S8C) but did not change the capacity of CD8<sup>+</sup> T-cells  
289 from these animals to suppress infection *ex vivo*, which remained extremely weak (Figure

290 S8C). These results, which are in agreement with our previous observations in early treated  
291 HIV-infected individuals (Lecuroux et al., 2013), show that low SIV suppressive capacity  
292 during acute infection was neither a consequence of strong activation of these cells *in vivo*  
293 nor of high antigen burden.

294

295 Collectively, our results show that the capacity of SIV-specific CD8<sup>+</sup> T-cells to suppress  
296 infection *ex vivo* was a genuine quality that progressively amplifies in SICs. Our results  
297 further uncover a temporal link between the acquisition by CD8<sup>+</sup> T-cells of potent capacity to  
298 suppress infection and sustained control of SIV.

299

300 ***Acquisition of CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity in SICs occurs independently of***  
301 ***MHC haplotype***

302 Our primary intention in this study was to explore the mechanisms underlying natural  
303 control of SIV infection, independently of MHC background or infectious dose. However, as  
304 expected (Bruel et al., 2015), inoculation with low-dose virus and carriage of the protective  
305 M6 haplotype independently favored spontaneous control of viremia below 400 copies/mL  
306 in CyMs in our study (Supplemental Table 3). We therefore evaluated whether these  
307 parameters influenced the dynamics of control and the development of the CD8<sup>+</sup> T-cell  
308 response upon infection. We found that CD4<sup>+</sup> T-cells and the levels of cell-associated SIV  
309 DNA similarly evolved in the blood and PLNs from M6 and non-M6 controllers (Figure S9A).  
310 There was just a tendency for M6 controllers vs non-M6 controllers to better recovery of  
311 CD4<sup>+</sup> T-cells in blood at the end of the study (p=0.07). Similarly, we did not find important  
312 differences between M6 and non-M6 controllers in their development of SIV-specific CD8<sup>+</sup> T-  
313 cell responses (Figure S9B). M6 and non-M6 controllers developed similar frequencies of SIV-

314 responding cells during acute infection that were maintained during the follow up. Of note,  
315 the capacity of CD8<sup>+</sup> T-cells to suppress *ex vivo* SIV infection of CD4<sup>+</sup> T-cells progressively  
316 increased in both M6 and non-M6 controllers. The only difference that we could appreciate  
317 was a faster acquisition (day 36 *p.i.*) of CD8<sup>+</sup> T-cell mediated SIV suppressive activity in the  
318 PLN from M6 SICs versus non-M6 SICs (Figure S9B). Intriguingly, non-M6 SICs had higher  
319 frequencies of SIV responding CD8<sup>+</sup> T-cells in this tissue at the same time point. Overall these  
320 results show that while the M6 background gave a selective advantage to CyMs to control  
321 infection in conditions of higher viral inoculum, this MHC haplotype was not indispensable  
322 for the acquisition of SIV suppressive capacity by CD8<sup>+</sup> T-cells, which occurred both in M6  
323 and non M6 SICs. The results are in agreement with the observations in HIV controllers.  
324 Although cohorts of HICs are enriched in individuals carrying protective HLA class I alleles  
325 (mainly HLA-B\*57, B\*27), many HICs do not carry protective HLA class I alleles but have CD8<sup>+</sup>  
326 T-cells with strong HIV suppressive capacity *ex vivo* (Lecuroux et al., 2014). Therefore, the  
327 development of efficient CD8<sup>+</sup> T-cell responses with antiviral activity is a characteristic of  
328 most HICs/SICs, independently of their MHC background.

329

330 ***Skewed maturation of central memory SIV-specific CD8<sup>+</sup> T-cells is associated with defective***  
331 ***acquisition of SIV-suppressive activity***

332 To dissect the phenotypic correlates of *ex vivo* measured antiviral potency, we analyzed the  
333 differentiation status of SIV-specific CD8<sup>+</sup> T-cells using selected markers in conjunction with  
334 MHC class I tetramers (Figure S10, S11). Tetramer-binding SIV-specific CD8<sup>+</sup> T-cells were  
335 detected in all CyMs, displayed early similar differentiation profiles in SICs and VIRs, but  
336 evolved differently, such that higher frequencies of central memory (CM) SIV-specific CD8<sup>+</sup> T-



337 cells were present in SICs versus VIRs on day 105 *p.i.* ( $p = 0.018$ ) and day 535 *p.i.* ( $p = 0.013$ )  
338 (Figure 6A, B).

339

340 In further analyses, we found that higher frequencies of SIV-specific CD8<sup>+</sup> T-cells from SICs  
341 expressed the IL-7 receptor CD127, which is associated with cell survival and memory  
342 responses (Schluns et al., 2000), whereas higher frequencies of SIV-specific CD8<sup>+</sup> T-cells from  
343 VIRs expressed the transcription factor T-bet, which is associated with cellular differentiation  
344 and effector functionality (Sullivan et al., 2003; Szabo et al., 2002) (Figure 7A). These  
345 differences appeared since primary infection and became statistically significant at later time  
346 points (Figure 7A). Expression levels of CD127 and T-bet also varied as a function of  
347 differentiation among SIV-specific CD8<sup>+</sup> T-cells from SICs and VIRs (Figure 7B). In particular,  
348 CM and transitional memory (TM) SIV-specific CD8<sup>+</sup> T-cells expressed lower levels of T-bet  
349 throughout the course of infection in SICs *versus* VIRs, whereas CM SIV-specific CD8<sup>+</sup> T-cells  
350 tended to express higher levels of CD127 during chronic infection in SICs *versus* VIRs.

351

352 Accordingly, negative correlations were observed during primary infection and at euthanasia  
353 between the expression levels of CD127 on SIV-specific CD8<sup>+</sup> T cells and plasma viral loads  
354 (Figure 8A). Of note, the levels of CD127 correlated positively with CD8<sup>+</sup> T-cell-mediated SIV-  
355 suppressive activity at the same time points (Figure 8B). On the contrary, negative  
356 correlations were observed during primary infection and at euthanasia between CD8<sup>+</sup> T-cell-  
357 mediated SIV-suppressive activity and the contemporaneous frequencies of T-bet<sup>+</sup>CD127<sup>-</sup>  
358 SIV-specific CD8<sup>+</sup> T-cells (Figure 8C) and between CD8<sup>+</sup> T-cell-mediated SIV-suppressive  
359 activity and expression levels of T-bet among CM SIV-specific CD8<sup>+</sup> T-cells (Figure 8D).

360

361 Collectively, these results suggest that SIV-specific CM CD8<sup>+</sup> T-cells are primed for survival in  
362 SICs, enabling long-term memory, sustained antiviral activity and viral control, whereas the  
363 corresponding SIV-specific CD8<sup>+</sup> T-cells in VIRs adopt a skewed phenotype associated with  
364 cellular differentiation and suboptimal antiviral activity.

365 **DISCUSSION**

366 The data presented in this study provide new insights into the immune correlates of natural  
367 control of SIV. Although SIV-specific CD8<sup>+</sup> T-cells were generated during acute infection with  
368 equivalent dynamics and global frequencies in all CyMs, preventing discrimination between  
369 SICs and VIRs, antiviral efficacy *ex vivo* developed progressively over time and was associated  
370 with spontaneous SIV control. This dichotomy was underpinned by distinct early memory  
371 programs within the SIV-specific CD8<sup>+</sup> T-cell pool. Collectively, these findings identify a  
372 cohesive set of immunological parameters that associate with effective and sustained  
373 control of SIV.

374

375 To monitor the establishment of natural control prospectively, we took advantage of  
376 previous reports showing that carriage of the MHC haplotype M6 and *i.r.* inoculation with  
377 low-dose (5AID<sub>50</sub>) virus independently favor spontaneous control of SIVmac251 infection in  
378 CyMs (Aarnink et al., 2011; Bruel et al., 2015; Mee et al., 2009). Our results corroborate  
379 previous reports. In particular, although the presence M6 haplotype favored more frequent  
380 and more rapid control of infection among animals receiving a high dose of the virus  
381 (50AID<sub>50</sub>) (Supplemental Table 3), no significant differences were observed in the dynamics  
382 of SIV control in M6 and non-M6 controllers. At the time of euthanasia, a higher proportion  
383 of CD4<sup>+</sup> T-cells and lower cell-associated SIV-DNA levels were found in multiple tissues from  
384 SICs *versus* VIRs, demonstrating systemic control of SIV. These differences were much more  
385 subtle during primary infection. However, PLNs from SICs harbored approximately 10-fold  
386 less SIV-DNA in the acute phase than PLNs from VIRs. In addition, the frequency of CD4<sup>+</sup> T-  
387 cells were maintained close to baseline throughout the course of the study in PLNs, but not  
388 in blood or RBs, from SICs. These observations suggest that early containment of viral

389 replication in lymph nodes (Buggert et al., 2018; Reuter et al., 2017) may be a key event for  
390 subsequent immune control of SIV.

391

392 In line with previous studies in humans (Lecuroux et al., 2013; Ndhlovu et al., 2015;  
393 Trautmann et al., 2012) and non-human primates we observed early and robust expansions  
394 of SIV-specific CD8<sup>+</sup> T-cells in all CyMs. However, the functional profiles and overall  
395 frequencies of SIV-specific CD8<sup>+</sup> T-cells (as determined by intra cellular cytokine staining  
396 upon SIV antigen stimulation) during the acute phase of infection were largely equivalent in  
397 SICs and VIRs, and neither parameter correlated with subsequent determinations of plasma  
398 VL. Similarly, the functional profiles and overall frequencies of SIV-specific CD8<sup>+</sup> T-cells  
399 during the chronic phase of infection were largely equivalent in SICs and VIRs, although  
400 polyfunctionality (defined as the capacity to produce simultaneously several cytokine and/or  
401 degranulate) was impaired at the time of euthanasia in VIRs. These results suggest that  
402 differences in polyfunctionality found during chronic infection are a surrogate marker of viral  
403 replication rather than an accurate determinant of antiviral efficacy, although low number of  
404 animals in the VIR group may limit statistical power.

405

406 The capacity of CD8<sup>+</sup> T-cells to suppress infection of autologous CD4<sup>+</sup> T-cells directly *ex vivo*  
407 is a particular feature of HICs (Almeida et al., 2009; Angin et al., 2016; Buckheit et al., 2012;  
408 Julg et al., 2010; Saez-Cirion et al., 2007; Saez-Cirion et al., 2009; Tansiri et al., 2015) that is  
409 mediated by the rapid elimination of infected CD4<sup>+</sup> T-cells (Saez-Cirion et al., 2007).  
410 Irrespective of subsequent outcome, we detected relatively weak CD8<sup>+</sup> T-cell-mediated SIV-  
411 suppressive activity during primary infection, despite the vigorous mobilization of SIV-  
412 specific CD8<sup>+</sup> T-cells. This observation parallels our previous findings in the setting of HIV

413 (Lecuroux et al., 2013) and point to limited antiviral potential of CD8<sup>+</sup> T-cell responses  
414 generated during primary infection. However, a remarkable negative correlation was already  
415 observed between the CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity and viremia at this early  
416 time point, showing early temporal association of this antiviral activity and reduction of  
417 viremia. Of note, this SIV-suppressive capacity of CD8<sup>+</sup> T-cells increased progressively over a  
418 period of weeks in some animals, carrying or not the protective MHC haplotype M6, and  
419 correlated temporally with the establishment of viral control. At the time of euthanasia,  
420 these highly potent antiviral CD8<sup>+</sup> T-cells were present in all tissues, with the exception of  
421 bone marrow. It is important to notice that CD8<sup>+</sup> T-cell-mediated SIV suppression was very  
422 weak also in LN during the first weeks following infection but increased over time in SICs.  
423 Therefore, the increase in the capacity of CD8<sup>+</sup> T-cells to suppress infection that we observed  
424 in this study was not the result of the recirculation of CD8<sup>+</sup> T-cells from lymph nodes once  
425 control was established but a genuine progressive augmentation of the antiviral potential of  
426 the cells. The development of potent antiviral CD8<sup>+</sup> T-cells is therefore a *bone fide* correlate  
427 of sustained control of SIV.

428

429 The divergent antiviral properties of SIV-specific CD8<sup>+</sup> T-cells in SICs *versus* VIRs were  
430 associated with early differences in the expression of CD127 and T-bet, especially within the  
431 less differentiated memory pools (CM and TM). In particular, higher frequencies of SIV-  
432 specific CM CD8<sup>+</sup> T-cells expressed CD127 in SICs, whereas higher frequencies of SIV-specific  
433 CM and TM CD8<sup>+</sup> T-cells expressed T-bet in VIRs. These differences became more  
434 pronounced throughout the course of infection. Studies in mice have shown that decreased  
435 expression of T-bet among memory CD8<sup>+</sup> T-cells allows the establishment of long-lived  
436 CD127<sup>hi</sup> cells, which maintain the capacity to proliferate and control successive infections

437 (Joshi et al., 2007; Joshi et al., 2011). Accordingly, our data suggest that SICs develop true  
438 memory-like SIV-specific CD8<sup>+</sup> T-cell responses, which is key for the acquisition of antiviral  
439 ability, whereas VIRs develop SIV-specific memory CD8<sup>+</sup> T-cell responses skewed towards  
440 more effector-like characteristics. In line with this supposition, the proportion of CD127<sup>+</sup> SIV-  
441 specific CD8<sup>+</sup> T cells during acute infection (day 15 *p.i.*) and at euthanasia correlated  
442 positively with CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity at the corresponding time  
443 points, while the frequencies of T-bet<sup>+</sup> CD127<sup>-</sup> SIV-specific CD8<sup>+</sup> T-cells and the expression  
444 levels of T-bet among CM SIV-specific CD8<sup>+</sup> T-cells during acute infection correlated  
445 negatively with CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity. These findings are broadly  
446 consistent with several previous reports describing immune profiles that associate with the  
447 control of viremia in HICs during chronic infection. Favorable characteristics include high  
448 frequencies of CD57<sup>+</sup> eomesodermin<sup>hi</sup> HIV-specific CD8<sup>+</sup> T-cells with superior proliferative  
449 capacity, increased expression levels of CD127, and intermediate expression levels of T-bet  
450 (Simonetta et al., 2014), and high frequencies of HIV-specific CD8<sup>+</sup> T-cells with the capacity  
451 to upregulate T-bet, granzyme B, and perforin in response to antigen encounter (Hersperger  
452 et al., 2011b; Migueles et al., 2008).

453

454 In a recent single cell study (Angin et al., 2019b), we also found differences in the program of  
455 HIV-specific CM CD8<sup>+</sup> T-cells from HIV controllers and non-controllers on cART: whereas HIV-  
456 specific CM CD8<sup>+</sup> T-cells from HIC upregulated the expression of effectors genes linked with  
457 mTORC2 activation and cell survival (including CD127), central memory cells from non-  
458 controllers had a skewed profile associated with mTORC1 activation (including T-bet) and  
459 glycolysis. This was traduced in a dependency on glucose of HIV-specific CD8<sup>+</sup> T-cells from  
460 non-controllers to react to HIV antigens, while HIV-specific CD8<sup>+</sup> T-cells from controllers

461 were characterized by metabolic plasticity and being able to exert their function even in  
462 conditions of glucose deprivation. Of note, these differences in the metabolic program of  
463 cells from controllers and non-controllers could also be recapitulated with SIV-specific CD8+  
464 T-cells from SICs and VIR CyMs from the present study (Angin et al., 2019b), further  
465 corroborating the validity of our CyM model to study the development of the protective  
466 CD8+ T-cell responses characteristics of HIV/SIV controllers. The present results extend these  
467 observations and support a key role for long-lived memory responses in the control of SIV.  
468 Importantly, our data also show that distinct memory responses are formed early after  
469 infection, potentially reflecting different priming conditions. Interestingly, although the  
470 antiviral activity of CD8+ T-cells increased over time in SICs, we already found a negative  
471 correlation between this activity and the plasma viremia at day 15. On this basis, we propose  
472 that the amplification of potent antiviral activity matters in long term control and is the result  
473 of a maturation process, the trajectory of which is linked to early optimal programming of  
474 the CD8+ T-cell memory compartment.

475

476 It remains unclear which factors are required to encourage the development of memory  
477 CD8+ T-cell responses that provide optimal protection against HIV/SIV. In some viral  
478 infections, expression of T-bet is tightly regulated by cytokines, such as IL-12 (Rao et al.,  
479 2012; Takemoto et al., 2006). Low levels of inflammation may therefore favor the  
480 emergence of long-lived memory CD8+ T-cells. It is also interesting to note that maturation  
481 through persistent or repeated exposure to antigen can drive the selection of specific  
482 clonotypes bearing high-affinity T-cell receptors (TCRs) (Busch and Pamer, 1999; Ozga et al.,  
483 2016; Price et al., 2005) which have been shown to suppress HIV replication more efficiently  
484 than clonotypes targeting the same antigen via low-affinity TCRs (Almeida et al., 2007;

485 Almeida et al., 2009; Ladell et al., 2013). Increase in antigen sensitivity over time would be  
486 compatible with the progressive increase in antiviral potency that we observed for the CD8<sup>+</sup>  
487 T-cells from controllers in our study.

488

489 A recent study in the LCMV murine model of infection has shown that memory CD8<sup>+</sup> T-cell  
490 responses expressing the transcription factor TCF1 developed during chronic infection (in an  
491 immunosuppressive environment) have a distinct molecular program, resist contraction, had  
492 increased long-term functionality, are less prone to exhaustion and are thus critical for  
493 controlling ongoing viral replication; in contrast, memory cells that are developed at the  
494 onset of infection (in a pro-inflammatory environment) become short-term effectors and are  
495 rapidly exhausted (Snell et al., 2018). Accordingly, we suggest that balanced inflammatory  
496 responses (Barouch et al., 2016) arising as a consequence of lower viral burdens in lymph  
497 nodes during acute infection in SICs might facilitate antigen-specific priming events  
498 associated with optimal memory programs (Ozga et al., 2016) and minimize the loss of CD4<sup>+</sup>  
499 T-cells, which provide helper functions that are critical for the development of long-lived  
500 memory CD8<sup>+</sup> T-cells (Khanolkar et al., 2004).

501

502 Collectively, the data presented here underscore the importance of early host-pathogen  
503 interactions in the development of adaptive immunity and reveal an optimal maturation  
504 pathway associated with the generation and maintenance of potent and sustained antiviral  
505 CD8<sup>+</sup> T-cell responses, which in turn dictate the outcome of infection with SIV.



506 **METHODS**

507 ***Ethical statement***

508 Cynomolgus macaques (CyMs, *Macaca fascicularis*) were imported from Mauritius and  
509 housed in facilities at the *Commissariat à l'Energie Atomique et aux Energies Alternatives*  
510 (CEA, Fontenay-aux-Roses, France). All non-human primate studies at the CEA are conducted  
511 in accordance with French National Regulations under the supervision of National Veterinary  
512 Inspectors (CEA Permit Number A 92-03-02). The CEA complies with the Standards for  
513 Human Care and Use of Laboratory Animals of the Office for Laboratory Animal Welfare  
514 under Assurance Number #A5826-01. All experimental procedures were conducted  
515 according to European Directive 2010/63 (Recommendation Number 9). The SIC and  
516 pVISCONTI studies were approved and accredited under statement number A13-005 and  
517 A15-035 by the ethics committee "*Comité d'Ethique en Expérimentation Animale du CEA*",  
518 registered and authorized under Number 44 and Number 2453-2015102713323361v2 by the  
519 French Ministry of Education and Research. CyMs were studied with veterinary guidance,  
520 housed in adjoining individual cages allowing social interactions, and maintained under  
521 controlled conditions with respect to humidity, temperature, and light (12 hour light/12  
522 hour dark cycles). Water was available *ad libitum*. Animals were monitored and fed once or  
523 twice daily commercial monkey chow and fruit by trained personnel. Environmental  
524 enrichment was provided including toys, novel foodstuffs, and music under the supervision  
525 of the CEA Animal Welfare Body. Experimental procedures (animal handling, viral  
526 inoculations, and samplings) were conducted after sedation with ketamine chorhydrate  
527 (Rhone-Merieux, Lyon, France, 10 mg/kg). Tissues were collected at necropsy: animals were  
528 sedated with ketamine chlorhydrate 10 mg/kg) then humanely euthanized by intravenous  
529 injection of 180 mg/kg sodium pentobarbital.

530

531 ***Animals and SIV infection***

532 A total of 16 healthy adult male CyMs (median age = 6.8 years at inclusion, IQR = 5.8–7.2)  
533 were selected for this study on the basis of MHC haplotype (M6<sup>+</sup>, n = 6; M6<sup>-</sup>, n = 10) (34).  
534 CyMs were inoculated *i.r.* with either 5AID<sub>50</sub> or 50AID<sub>50</sub> of uncloned SIVmac251 (A.M.  
535 Aubertin, Université Louis Pasteur, Strasbourg, France). The following experimental groups  
536 were studied: (i) M6<sup>-</sup> CyMs inoculated *i.r.* with 5AID<sub>50</sub> (non-M6 5AID<sub>50</sub>, n = 4); (ii) M6<sup>+</sup> CyMs  
537 inoculated *i.r.* with 50AID<sub>50</sub> (M6 50AID<sub>50</sub>, n = 6); and (iii) M6<sup>-</sup> CyMs inoculated *i.r.* with  
538 50AID<sub>50</sub> (non-M6 50AID<sub>50</sub>, n = 6). Animals were monitored for 18 months post-infection.

539

540 The outcome of infection generally matched expectations based on previous studies for each  
541 experimental group (Figure S12, Supplemental Table 1). Only one M6<sup>+</sup> CyM (31041) was  
542 unable to control viremia below 400 copies/mL. This animal was homozygous for MHC class I  
543 (Supplemental Table 1), which intrinsically limits immune control of HIV/SIV (Carrington et  
544 al., 1999; O'Connor et al., 2010). The dynamics of viral replication during acute infection  
545 were very similar in the three experimental groups, with peak VLs of 5.9, 6.4, and 6.3 log SIV-  
546 RNA copies/mL of plasma on day 14 *p.i.* for non-M6 5AID<sub>50</sub>, M6 50AID<sub>50</sub>, and non-M6  
547 50AID<sub>50</sub> CyMs, respectively (Supplemental Table 1).

548

549 CyMs in the pVISCNTI study (median age = 5 years at inclusion, IQR = 4.1–5.3) were  
550 inoculated with 1000 AID<sub>50</sub> of uncloned SIVmac251 through the intravenous route. None of  
551 these animals carried the M6 haplotype. An antiretroviral regimen containing emtricitabine  
552 (FTC), dolutegravir (DTG), and the tenofovir prodrug tenofovir-disoproxil-fumarate (TDF), co-

553 formulated as a once daily subcutaneous injection, was initiated at day 28 post-inoculation  
554 in 6 animals. TDF was administered at 5.1 mg/kg, FTC at 40 mg/kg and DTG at 2.5 mg/kg.

555

#### 556 ***Blood collection and processing***

557 Peripheral blood was collected by venous puncture into Vacutainer Plus Plastic K3EDTA  
558 Tubes or Vacutainer CPT Mononuclear Cell Preparation Tubes with Sodium Heparin (BD  
559 Biosciences). Complete blood counts were monitored at all time points from the Vacutainer  
560 Plus Plastic K3EDTA Tubes. Plasma was isolated from Vacutainer Plus Plastic K3EDTA Tubes  
561 by centrifugation for 10 min at 1,500 g and stored at  $-80^{\circ}\text{C}$ . Peripheral blood mononuclear  
562 cells (PBMCs) were isolated from Vacutainer CPT Mononuclear Cell Preparation Tubes with  
563 Sodium Heparin according to manufacturer's instructions (BD Biosciences), and red blood  
564 cells were lysed in ACK ( $\text{NH}_4\text{Cl}$  0.15 M,  $\text{KHCO}_3$  10 mM, EDTA 0.1 mM, pH 7.4).

565

#### 566 ***Tissue collection and processing***

567 Axillary or inguinal lymph nodes (PLNs), rectal biopsies (RBs) and broncho-alveolar lavages  
568 (BAL) were collected longitudinally from each animal at the indicated time points. In  
569 addition, bone marrow, spleen, mesenteric lymph nodes (MLNs), duodenum, jejunum, ileum  
570 and colon were collected at necropsy. Tissue samples were snap-frozen in liquid nitrogen for  
571 storage at  $-80^{\circ}\text{C}$  or collected in RPMI medium at  $2-8^{\circ}\text{C}$ . At each time point a complete PLN  
572 group was collected. LNs were washed and cells were freshly isolated in RPMI medium upon  
573 mechanical disruption with a GentleMACS dissociator as recommended by the manufacturer  
574 (Miltenyi Biotec). Cell suspension was filtered ( $70\mu\text{m}$ ), then red blood cells were lysed in  
575 ACK. RB lymphocytes were obtained from approximately  $4\text{ mm}^2$  of rectal mucosa. Colonic  
576 lymphocytes were obtained from mucosa taken from approximately 10 cm of tissue. RBs and

577 colonic tissue were washed extensively in R10 medium (RPMI medium supplemented with  
578 10% fetal calf serum and penicillin/neomycin/streptomycin), then digested for 45 minutes  
579 with collagenase II prior to mechanical disruption. Lymphocytes were isolated over a Percoll  
580 67/44 gradient (Sigma-Aldrich). Bone marrow cells were purified using Lymphocyte  
581 Separation Medium (Lonza Bioscience) diluted to 90% in DPBS, centrifuged for 20 minutes at  
582 350 g, and separated from red cells in ACK. Spleen cells were processed by mechanical  
583 disruption in RPMI medium using a GentleMACS™ Dissociator (Miltenyi Biotec), purified as  
584 described for bone marrow cells, and separated from red cells in ACK. Total cells were  
585 immediately designated to T-cell activation and proliferation analyses by flow cytometry,  
586 CD4<sup>+</sup> and CD8<sup>+</sup> T-cells separation with magnetic beads for antiviral activity assay and the  
587 remaining cells were frozen for further assessment of cytokine production by ICS or tetramer  
588 analyses.

589

#### 590 ***Quantification of plasma viral load***

591 Plasma viremia was monitored longitudinally in all animals using quantitative real-time  
592 RTqPCR with a limit of detection of 12.3 copies/mL (Angin et al., 2019a). Viral RNA was  
593 prepared from 100 µl of cell-free plasma. Quantitative RT-PCR was performed with a  
594 SuperScript III Platinum One-Step qRT-PCR Kit (Thermofisher) in a CFX96 Touch Real-Time  
595 PCR Detection System (BioRad) under the following conditions: 12.5 µl of 2X reaction  
596 mixture, 0.5 µl of RNaseOUT (40U/µl), 0.5 µl of Superscript III reverse transcriptase/Platinum  
597 Taq DNA Polymerase, 1 µl of each primer (125 µM), 0.5 µl of the fluorogenic probe (135 µM),  
598 and 10-µl of RNA elution samples. The probe and primers were designed to amplify a region  
599 of SIVmac251 *gag*. Forward primer was 5'-GCAGAGGAGGAAATTACCCAGTAC-3' (24 bp) and  
600 reverse primer was 5'-CAATTTTACCCAGGCATTTAATGTT-3' (25 bp). The TaqMan probe

601 sequence was 5'-FAM-TGTCCACCTGCCATTAAGCCCGA-BHQ1-3' (23 bp). This probe had a  
602 fluorescent reporter dye, FAM (6-carboxyfluorescein), attached to its 5' end and the  
603 quencher BHQ1 (Black Hole Quencher 1) attached to its 3' end. Samples were heated for 30  
604 min at 56°C and 5 min at 95°C, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min.

605

### 606 **Quantification of SIV-DNA**

607 Total DNA was extracted from purified CD14<sup>+</sup> alveolar macrophages, buffy coats and snap-  
608 frozen tissues. CD14<sup>+</sup> alveolar macrophages were purified by positive selection using  
609 antibody-coated magnetic beads following manufacturer's instructions (Miltenyi Biotec).  
610 Purity was checked by flow cytometry (Figure S1A, upper panel). Snap-frozen tissues were  
611 mechanically disrupted with a MagNA Lyser (Roche Diagnostics). DNA extraction was  
612 performed using a QIAamp DNA Blood Mini Kit (Qiagen) following manufacturer's  
613 instructions. SIV-DNA was quantified using an ultrasensitive quantitative real-time PCR. For  
614 blood samples, 150,000 cells were analyzed for each SIV-DNA PCR. Due to sample size  
615 limitations, for rectal biopsies and bronchoalveolar lavages 50,000 and 20,000 cells per PCR  
616 were tested, respectively. All amplifications were performed on 2–4 replicates. The cell line  
617 SIV1C, which contains 1 copy of SIV integrated/cell, was used as a standard for  
618 quantification. 1 µg of DNA was considered to be equivalent to 150,000 cells. Amplification  
619 was performed using primers and a probe located in the *gag* region. The *CCR5* gene was  
620 used to normalize results per million cells. Results were then adjusted by the frequencies of  
621 CD4<sup>+</sup> T-cells in blood and tissues, when available. The limit of quantification was 2  
622 copies/PCR. Primers and probes were: SIV *gag* F: 5'-GCAGAGGAGGAAATTACCCAGTAC-3'; SIV  
623 *gag* R: 5'-CAATTTTACCCAGGCATTTAATGTT-3'; SIV *gag* probe: 5'-FAM-  
624 TGTCCACCTGCCATTAAGCCCGA-BHQ1-3'; *CCR5* F: an equimolar mix of 5'-

625 CAACATGCTGGTCgATCCTCAT-3' and 5'-CAACATACTGGTCGTCCTCATCC-3'; CCR5 R: 5'-  
626 CAGCATAGTGAGCCCAGAAG-3'; and CCR5 probe: 5'-HEX-CTGACATCTACCTGCTCAACCTG-  
627 BHQ1-3'.

628

### 629 ***Measurement of T-cell activation and proliferation***

630 T-cell activation and proliferation were assessed using fresh PBMCs and tissue cell  
631 suspensions. Blood samples were treated with FACS Lysing Solution (BD Biosciences). Cells  
632 were surface stained for CD3, CD4, CD8, CD38, CD45, CCR5, and HLA-DR,  
633 fixed/permeabilized using a Cytofix/CytoPerm Kit (BD Biosciences), and stained  
634 intracellularly for Ki-67. The following antibodies used were: anti-CD3-PE (clone SP34-2, BD  
635 Biosciences), anti-CD4-PerCP-Cy5.5 (clone L200, BD Biosciences), anti-CD8-BV650 (clone  
636 RPA-T8, BioLegend), anti-CD38-FITC (clone AT-1, StemCell Technologies), anti-CD45-V500  
637 (clone D058-1283, BD Biosciences), anti-CCR5-APC (clone 3A9, BD Biosciences), anti-HLA-  
638 DR-APC-H7 (clone G46-6, BD Biosciences), and anti-Ki-67-AF700 (clone B56, BD  
639 Biosciences). Data were acquired using an LSRII flow cytometer (BD Biosciences) and  
640 analyzed with FlowJo software version 10 (TreeStar Inc.).

641

### 642 ***Intracellular cytokine staining***

643 Frozen PBMCs, PLN cells, bone marrow cells, splenocytes and MLN cells were thawed,  
644 resuspended at  $1 \times 10^6$ /mL in R20 medium, and stored overnight at 37 °C. Cells were then  
645 stimulated with a pool of 24 optimal SIV peptides (8-10 amino acids long) (2 µg/mL each,  
646 Supplemental Table 2) or with a pool of 125 overlapping SIV Gag 15-mer peptides (2 µg/mL  
647 each, NIH AIDS Reagent Program, SIVmac239 Gag Peptide Set #12364) in the presence of  
648 anti-CD28 (1 µg/mL, clone L293, BD Biosciences) and anti-CD49d (1 µg/mL, clone 9F10, BD

649 Biosciences) and stained with anti-CD107a (clone H4A3, BD Biosciences) for 30 minutes prior  
650 to the addition of GolgiStop (1  $\mu$ L/mL, BD Biosciences) and brefeldin A (BFA, 5  $\mu$ g/mL, Sigma-  
651 Aldrich). Costimulatory antibodies alone were used as a negative control, and concanavalin A  
652 (5  $\mu$ g/mL, Sigma-Aldrich) was used as a positive control. Cells were incubated for a total of 6  
653 hours. After washing, cells were surface stained for CD3, CD4, and CD8, fixed/permeabilized  
654 using a Cytotfix/CytoPerm Kit (BD Biosciences), and stained intracellularly for IFN $\gamma$ , TNF $\alpha$ , and  
655 IL-2. The following antibodies were used: anti-CD107a–V450 (clone H4A3, BD Biosciences),  
656 anti-CD3–AF700 (clone SP34-2, BD Biosciences), anti-CD4–PerCP-Cy5.5 (clone L200, BD  
657 Biosciences), anti-CD8–APC-Cy7 (clone RPA-T8, BD Biosciences), anti-IFN $\gamma$ –PE-Cy7 (clone  
658 B27, BD Biosciences), anti-IL-2–PE (clone MQ1-17H12, BD Biosciences), and anti-TNF $\alpha$ –PE-  
659 CF594 (clone Mab11, BD Biosciences). Data were acquired using an LSRII flow cytometer (BD  
660 Biosciences) and analyzed with FlowJo software version 10 (TreeStar Inc.). Results were  
661 corrected for background by subtracting the peptide stimulated response from the negative  
662 (no peptide) control. Negative responses were given an arbitrary value of 0.001. All data are  
663 represented. A representative flow cytometry gating strategy used to analyze cytokine  
664 production via intracellular staining after peptide stimulation is shown in Figure S13.

665

#### 666 ***MHC class I tetramer staining***

667 Biotinylated complexes of Nef RM9 (RPKVPLRTM)–Mafa A1\*063:02, Gag GW9  
668 (GPRKPIKCW)–Mafa A1\*063:02, and Vpx GR9 (GEAFEWLNR)–Mafa B\*095:01 were produced  
669 as described previously(53). The corresponding tetramers were generated via stepwise  
670 addition of APC-conjugated streptavidin (Thermo Fisher Scientific). Frozen PBMCs were  
671 stained with the pool of these tetramers for 30 minutes at 37 °C, washed, and surface  
672 stained for CD3, CD4, CD8, CD14, CD20, CD27, CD45RA, CCR7, HLA-DR, and CD127. Cells

673 were then fixed/permeabilized using a Cytofix/CytoPerm Kit (BD Biosciences) and stained for  
674 T-bet. The following antibodies were used: anti-CD3–AF700 (clone SP34-2, BD Biosciences),  
675 anti-CD4–PerCP-Cy5.5 (clone L200, BD Biosciences), anti-CD8–APC-Cy7 (clone RPA-T8, BD  
676 Biosciences), anti-CD14–BV786 (clone M5E2, BD Biosciences), anti-CD20–BV786 (clone L27,  
677 BD Biosciences), anti-CD27–PE (clone M-T271, BD Biosciences), anti-CD45RA–PE-Cy7 (clone  
678 5H9, BD Biosciences), anti-CCR7–PE-Dazzle594 (clone G043H7, BioLegend), anti-HLA-DR–  
679 Pacific Blue (clone G46-6, BD Biosciences), anti-CD127–FITC (clone MB15-18C9, Miltenyi  
680 Biotec), and anti-T-bet–BV711 (clone 4B10, BioLegend). Data were acquired using an AriaIII  
681 flow cytometer (BD Biosciences) and analyzed with FlowJo software version 10 (TreeStar  
682 Inc.). A representative flow cytometry gating strategy used to analyze T-cell differentiation  
683 and tetramer staining are shown in Figure S10, S11.

684

#### 685 ***Measurement of SIV-suppressive activity***

686 Autologous CD4<sup>+</sup> and CD8<sup>+</sup> T-cells were purified from freshly isolated PBMCs or tissue cell  
687 suspensions by positive and negative selection, respectively, using antibody-coated magnetic  
688 beads with a RoboSep instrument (StemCell Technologies). Purified CD4<sup>+</sup> T-cells were  
689 stimulated for 3 days with concanavalin A (5µg/mL, Sigma-Aldrich) in the presence of IL-2  
690 (100 IU/mL, Miltenyi Biotec). Purified CD8<sup>+</sup> T-cells were cultured in the absence of mitogens  
691 and cytokines (*ex vivo* CD8<sup>+</sup> T-cells). Stimulated CD4<sup>+</sup> T-cells (10<sup>5</sup>) were superinfected in U-  
692 bottom 96-well plates with SIVmac251 (MOI = 10<sup>-3</sup>) in the presence (1:1 effector to target  
693 cell ratio) or absence of *ex vivo* CD8<sup>+</sup> T-cells (10<sup>5</sup>) from the same tissue via spinoculation for 1  
694 hour (1,200 g at room temperature) followed by incubation for 1 hour at 37 °C. Cells were  
695 then washed and cultured in R10 medium containing IL-2 (100 IU/mL, Miltenyi Biotec).  
696 Culture supernatants were assayed on day 7 using an SIV p27 Antigen ELISA Kit



697 (Zeptomatrix). Antiviral activity was calculated as  $\log_{10}$  (mean p27 ng/mL in SIV-infected  
698 CD4<sup>+</sup> T-cell cultures without CD8<sup>+</sup> T-cells) / (mean p27 ng/mL in SIV-infected CD4<sup>+</sup> T-cell  
699 cultures + *ex vivo* CD8<sup>+</sup> T-cells) (Saez-Cirion et al., 2010).

700

#### 701 **Data visualization and statistical analyses**

702 Data visualization was performed using Tableau version 2018.1.4 (Tableau Software).  
703 Statistical analyses were performed using GraphPad version 8.1.2 (Prism Software) and  
704 SigmaPlot version 12.5 (SYSTAT Software). Results are given as median with interquartile  
705 range. The non-parametric Mann-Whitney U-test was used to compare data sets between  
706 groups. Correlations were assessed by Spearman-rank analyses. Given the exploratory  
707 nature of the analyses, p values were not adjusted for multiple comparisons. All p values less  
708 than 0.05 were defined as significant.

709 **ACKNOWLEDGMENTS**

710 This study was funded by the French National Agency of AIDS and Viral Hepatitis Research  
711 (ANRS) and by MSDAvenir. Additional support was provided by the Programme  
712 Investissements d’Avenir (PIA), managed by the ANR under reference ANR-11-INBS-0008,  
713 funding the Infectious Disease Models and Innovative Therapies (IDMIT, Fontenay-aux-  
714 Roses, France) infrastructure, and ANR-10-EQPX-02-01, funding the FlowCyTech Facility  
715 (IDMIT, Fontenay-aux-Roses, France). C.P. was supported by an ANRS Postdoctoral  
716 Fellowship Grant, A.M. was supported by MSDAvenir, and D.A.P. was supported by a  
717 Wellcome Trust Senior Investigator Award. We thank Benoit Delache, Brice Targat, Claire  
718 Torres, Christelle Cassan, Jean-Marie Robert, Julie Morin, Patricia Brochard, Sabrina  
719 Guenounou, Sebastien Langlois, and Virgile Monnet for expert technical assistance, Antonio  
720 Cosma for helpful discussion, Lev Stimmer for anatomopathology expertise, Delphine  
721 Desjardins and Isabelle Mangeot-Méderlé for helpful project management at IDMIT, and  
722 Christophe Joubert for veterinarian assistance at the animal facility at CEA. The SIV1C cell  
723 line was kindly provided by François Villinger. The SIVmac239 Gag Peptide Set was obtained  
724 through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH. FTC, DTG and TDF were  
725 obtained from Gilead and ViiV healthcare through the “IAS Towards an HIV Cure” common  
726 Material Transfer Agreement for preclinical studies in HIV cure research.

727

728 **AUTHOR CONTRIBUTIONS**

729 C.P. and A.M. designed and performed experiments, analyzed data, and interpreted results.  
730 V.Ma., J.G., and V.A.F. analyzed data and interpreted results. V.Mo., A.D., P.V., and N.S.  
731 performed experiments and analyzed data. E.G. and D.A.P. produced bespoke reagents.  
732 N.D.B. designed experiments, analyzed data and interpreted results. D.A.P., A.B., G.P., R.L.G.,

733 O.L., M.M.T., and C.R. interpreted results. B.V. and A.S.C. designed experiments, analyzed  
734 data, interpreted results, and supervised the study. C.P., B.V., and A.S.C. wrote the paper  
735 with assistance from A.M., V.Ma., V.Mo., A.D., P.V., N.S., D.A.P., N.D.B., R.L.G., O.L., M.M.T.,  
736 C.R., J.G., and V.A.F.

737

738 **DECLARATION OF INTERESTS**

739 The authors declare no competing interests.

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980  
981



982 **FIGURE LEGENDS**

983 **Figure 1. SIV controllers are characterized by partial restoration of CD4<sup>+</sup> T-cell counts and**  
984 **progressive decline in the frequency of SIV-carrying cells in blood. (A)** Plasma VL kinetics,  
985 **(B)** kinetics of SIV-DNA levels in blood and **(C)** longitudinal evolution of CD4<sup>+</sup> T-cell counts  
986 (results are shown as fold-change in absolute CD4<sup>+</sup> T-cell counts relative to baseline) in blood  
987 in SIV controllers (SICs, black) and viremic CyMs (VIRs, red). Median and interquartile range  
988 are shown. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001; Mann-Whitney U-test.

989  
990 **Figure 2. SIV control is associated with early preservation of lymph nodes. (A–B)**  
991 Longitudinal evolution of CD4<sup>+</sup> T-cells in rectal mucosa **(A)**, and peripheral lymph nodes **(B)** in  
992 SIV controllers (SICs, black) and viremic CyMs (VIRs, red). Results in rectal mucosa and  
993 peripheral lymph nodes are shown as fold-change in percent frequencies of CD3<sup>+</sup> CD4<sup>+</sup> T-  
994 cells among CD3<sup>+</sup> lymphocytes relative to baseline. **(C)** Percent frequencies of CD3<sup>+</sup> CD4<sup>+</sup> T-  
995 cells among CD3<sup>+</sup> lymphocytes in bone marrow, spleen, peripheral and mesenteric lymph  
996 nodes, and colon mucosa at euthanasia. **(D–E)** Kinetics of SIV-DNA levels in rectal mucosa  
997 **(D)**, and peripheral lymph nodes **(E)** in SIV controllers (black) and viremic CyMs (red). **(F)**  
998 Levels of SIV-DNA in bone marrow, spleen, peripheral and mesenteric lymph nodes, and  
999 colon at euthanasia. Results are expressed as copies SIV-DNA/million CD4<sup>+</sup> T-cells. Median  
1000 and interquartile range are shown. \*p < 0.05, \*\*p < 0.01; Mann-Whitney U-test.

1001

1002 **Figure 3. The dynamics of CD8<sup>+</sup> T-cells expansion and activation do not predict control of**  
1003 **SIV. (A–C)** Evolution of Ki-67<sup>+</sup> CD8<sup>+</sup> T-cells in blood **(A)**, peripheral lymph nodes **(B)**, and  
1004 rectal mucosa **(C)** in SIV controllers (black) and viremic CyMs (red). **(D–F)** Evolution of CD38<sup>+</sup>  
1005 HLA-DR<sup>+</sup> CD8<sup>+</sup> T-cells in blood **(D)**, peripheral lymph nodes **(E)**, and rectal mucosa **(F)** in SIV

1006 controllers (black) and viremic CyMs (red). Median and interquartile range are shown.  
1007 Vertical dashed lines indicate peak VLs. \* $p < 0.05$ , \*\* $p < 0.01$ ; Mann-Whitney U-test.

1008

1009 **Figure 4. SIV-specific CD8<sup>+</sup> T-cell frequencies do not predict control of SIV. (A)** TNF $\alpha$   
1010 production by SIV-specific CD8<sup>+</sup> T-cells in blood and peripheral lymph nodes over the course  
1011 of infection and in bone marrow, spleen, and mesenteric lymph nodes at euthanasia in SIV  
1012 controllers (black) and viremic CyMs (red). Results are shown as percent frequencies among  
1013 CD8<sup>+</sup> T-cells. Median and interquartile range are shown. **(B)** Functional profiles of SIV-specific  
1014 CD8<sup>+</sup> T-cells in blood and peripheral lymph nodes over the course of infection and in bone  
1015 marrow, spleen, and mesenteric lymph nodes at euthanasia in SIV controllers (black) and  
1016 viremic CyMs (red). Doughnut charts show median percent frequencies of SIV-specific CD8<sup>+</sup>  
1017 T-cells expressing IFN $\gamma$ , TNF $\alpha$ , IL-2, and/or CD107a. Colors indicate number of simultaneous  
1018 functions (blue, 1; green, 2; yellow, 3; red, 4). \* $p < 0.05$ ; Mann-Whitney U-test.

1019

1020 **Figure 5. Progressive acquisition of CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity is**  
1021 **associated with control of SIV. (A)** CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity in blood  
1022 and peripheral lymph nodes over the course of infection and in bone marrow, spleen, and  
1023 mesenteric lymph nodes at euthanasia in SIV controllers (black) and viremic CyMs (red).  
1024 Results are shown as log p27 decrease in the presence of CD8<sup>+</sup> T-cells. \* $p < 0.05$ , \*\* $p < 0.01$ ;  
1025 Mann-Whitney U-test. **(B)** Spearman correlations between CD8<sup>+</sup> T-cell-mediated SIV-  
1026 suppressive activity (upper panel) or TNF $\alpha$  production by SIV-specific CD8<sup>+</sup> T-cells (bottom  
1027 panel) on day 15 *p.i.* with plasma VL on day 15 *p.i.* Grey symbols, SIV controllers; red  
1028 symbols, viremic CyMs. **(C)** Spearman correlations between CD8<sup>+</sup> T-cell-mediated SIV-  
1029 suppressive activity (upper panel) or TNF $\alpha$  production by SIV-specific CD8<sup>+</sup> T-cells (bottom

1030 panel) at euthanasia with plasma VL at euthanasia. Grey symbols, SIV controllers; red  
1031 symbols, viremic CyMs (D) Spearman correlations between area under the curve (AUC) for  
1032 plasma VL and AUC for CD8<sup>+</sup> T-cell-mediated SIV-suppressive activity (orange) and between  
1033 AUC for plasma VL and AUC for TNF $\alpha$  production by SIV-specific CD8<sup>+</sup> T-cells (blue). AUC for  
1034 plasma VL, TNF $\alpha$  production and CD8<sup>+</sup> T-cell antiviral activities were calculated using the  
1035 sequential values obtained throughout the duration of our study in the blood of the infected  
1036 animals (Figure S6). (E) Side by side comparison of the longitudinal kinetics of TNF $\alpha$   
1037 production by SIV-specific CD8<sup>+</sup> T-cells in blood shown in figure 4A (blue) and CD8<sup>+</sup> T-cell-  
1038 mediated SIV-suppressive activity in blood shown in figure 5A(orange) in SIV controllers (left  
1039 panel) and viremic CyMs (right panel). Median and interquartile range are shown.

1040

1041 **Figure 6. SIV controllers maintain higher frequencies of SIV-specific central memory CD8<sup>+</sup>**  
1042 **T-cells during chronic infection than viremic CyMs. (A)** Doughnut charts showing median  
1043 percent frequencies of SIV-specific CD8<sup>+</sup> T-cells in each phenotypically-defined subset in SIV  
1044 controllers (upper panels) and viremic CyMs (lower panels). Light blue, central memory  
1045 (CM); green, transitional memory (TM); yellow, effector memory (EM); red, effector (Eff). (B)  
1046 Evolution of CM, TM, EM, and Eff SIV-specific CD8<sup>+</sup> T-cells in SIV controllers (black) and  
1047 viremic CyMs (red). Results are shown as percent frequencies of tetramer-binding CD8<sup>+</sup> T-  
1048 cells. \*p < 0.05; Mann-Whitney U-test.

1049

1050 **Figure 7. Altered maturation of central memory SIV-specific CD8<sup>+</sup> T-cells in viremic CyMs.**  
1051 (A) Dynamics of T-bet (left panels) and CD127 expression (right panels) among SIV-specific  
1052 CD8<sup>+</sup> T-cells in SIV controllers (black) and viremic CyMs (red). (B) Dynamics of T-bet (left) and  
1053 CD127 expression (right) among central memory (CM), transitional memory (TM), effector

1054 memory (EM), and effector (Eff) SIV-specific CD8<sup>+</sup> T-cells in SIV controllers (black) and  
1055 viremic CyMs (red). \*p < 0.05, \*\*p < 0.01; Mann-Whitney U-test.

1056

1057 **Figure 8. Skewed maturation of central memory SIV-specific CD8<sup>+</sup> T-cells is associated with**  
1058 **defective acquisition of SIV-suppressive activity.** Spearman correlations between CD127<sup>+</sup>  
1059 SIV specific CD8<sup>+</sup> T cell frequencies and viral loads (**A**) and CD8<sup>+</sup> T-cell-mediated SIV-  
1060 suppressive activity (**B**) during acute (left panel) and chronic infection (right panel).  
1061 Spearman correlations between T-bet<sup>+</sup> CD127<sup>-</sup> SIV-specific CD8<sup>+</sup> T-cell frequencies (**C**) or T-  
1062 bet expression levels in central memory SIV-specific CD8<sup>+</sup> T-cells (**D**) and CD8<sup>+</sup> T-cell-  
1063 mediated SIV-suppressive activity during acute (left panel) and chronic infection (right  
1064 panel). Grey symbols, SIV controllers; red symbols, viremic CyMs.

1065 **Table 1.** Virologic and immunologic characteristics from SICs *versus* VIRs.

	Controllers	Viremics	p
<b>RNA viral load</b>			
Peak (Log SIV-RNA copies/mL)	6.1 [5.1 – 7.1]	6.6 [6.3 – 6.9]	0.058
Time to peak (days <i>p.i.</i> )	12.5 [11 – 17]	14 [14 – 17]	0.239
Set-point <sup>#</sup> (Log SIV-RNA copies/mL)	1.5 [1.1 – 3.6]	4.0 [3.2 – 5.2]	<b>0.014</b>
Slope after peak viremia (1/slope peak – set-point)	-1.3x10 <sup>-4</sup> [-1.5x10 <sup>-3</sup> to -2.1x10 <sup>-5</sup> ]	-5.5x10 <sup>-5</sup> [-1.2x10 <sup>-4</sup> to -2.3x10 <sup>-5</sup> ]	0.058
<b>DNA viral load</b>			
Peak (Log SIV-DNA copies/million CD4)	4.0 [3.5 – 4.7]	4.6 [4.1 – 5.3]	0.133
Time to peak (days <i>p.i.</i> )	15 [15 – 36]	15 [15 – 36]	1
Set-point <sup>#</sup> (Log SIV-DNA copies/million CD4)	2.7 [2.1 – 3.5]	4.0 [3.8 – 4.5]	<b>0.002</b>
Descending slope (1/slope peak – set-point)	-9.0x10 <sup>-3</sup> [-4.8x10 <sup>-2</sup> to -1.0x10 <sup>-3</sup> ]	-4.9x10 <sup>-3</sup> [-1.2x10 <sup>-2</sup> to -4.4x10 <sup>-4</sup> ]	0.262
<b>CD4<sup>+</sup> T-cell counts</b>			
Nadir CD4 <sup>+</sup> T-cells (cells/μL blood)	238 [71 – 910]	179 [73 – 276]	0.379
Time to nadir CD4 <sup>+</sup> T-cells (days)	15 [9 – 36]	19 [15 – 28]	0.122
Set-point <sup>#</sup> CD4 <sup>+</sup> T-cells (cells/μL blood)	602 [257 – 1468]	154 [80 – 363]	<b>0.008</b>

1066 **Median and range are indicated. p, Mann-Whitney U-test. #Set point defined as Month 3 post-infection**

Figure 1

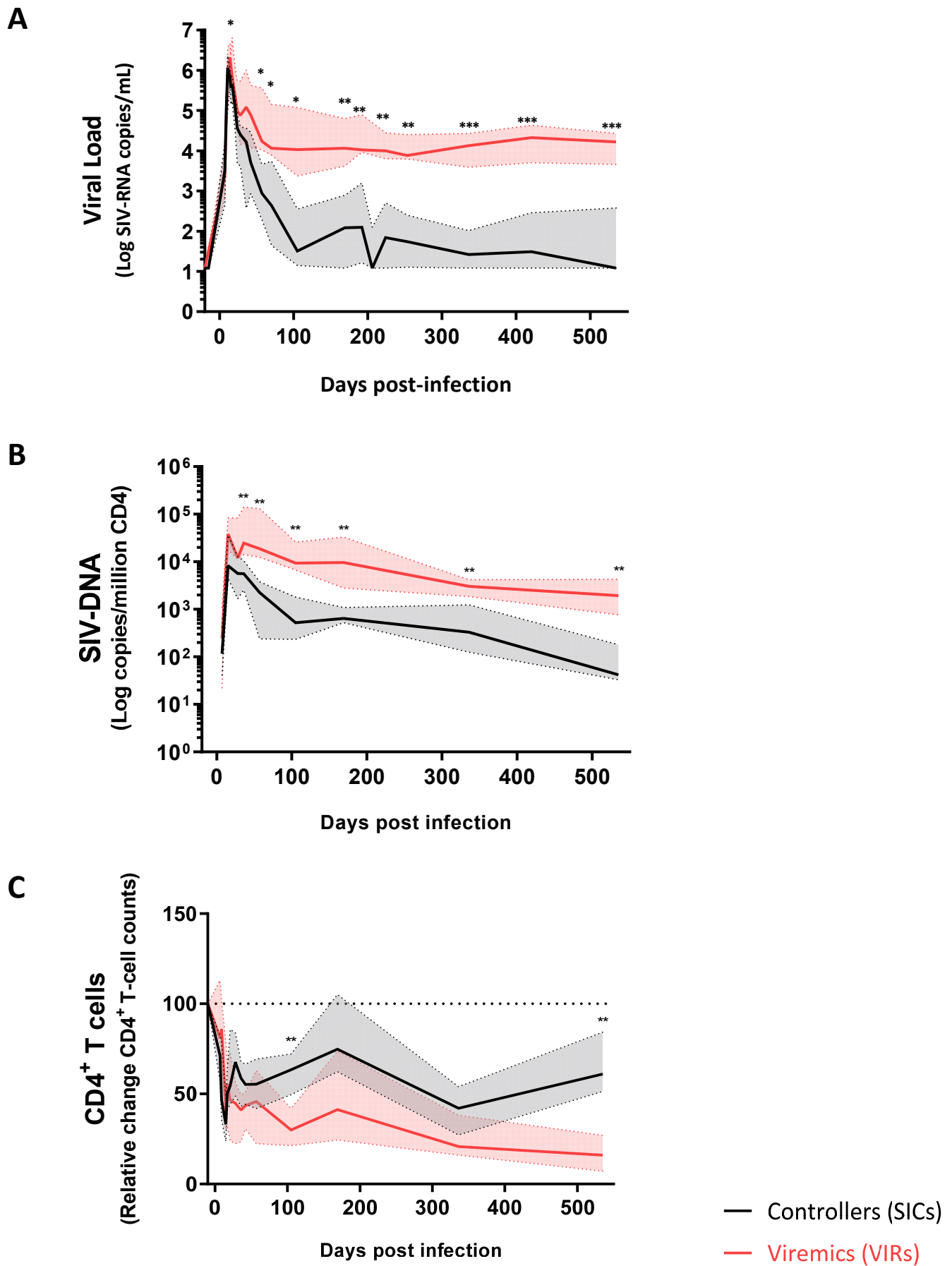


Figure 2

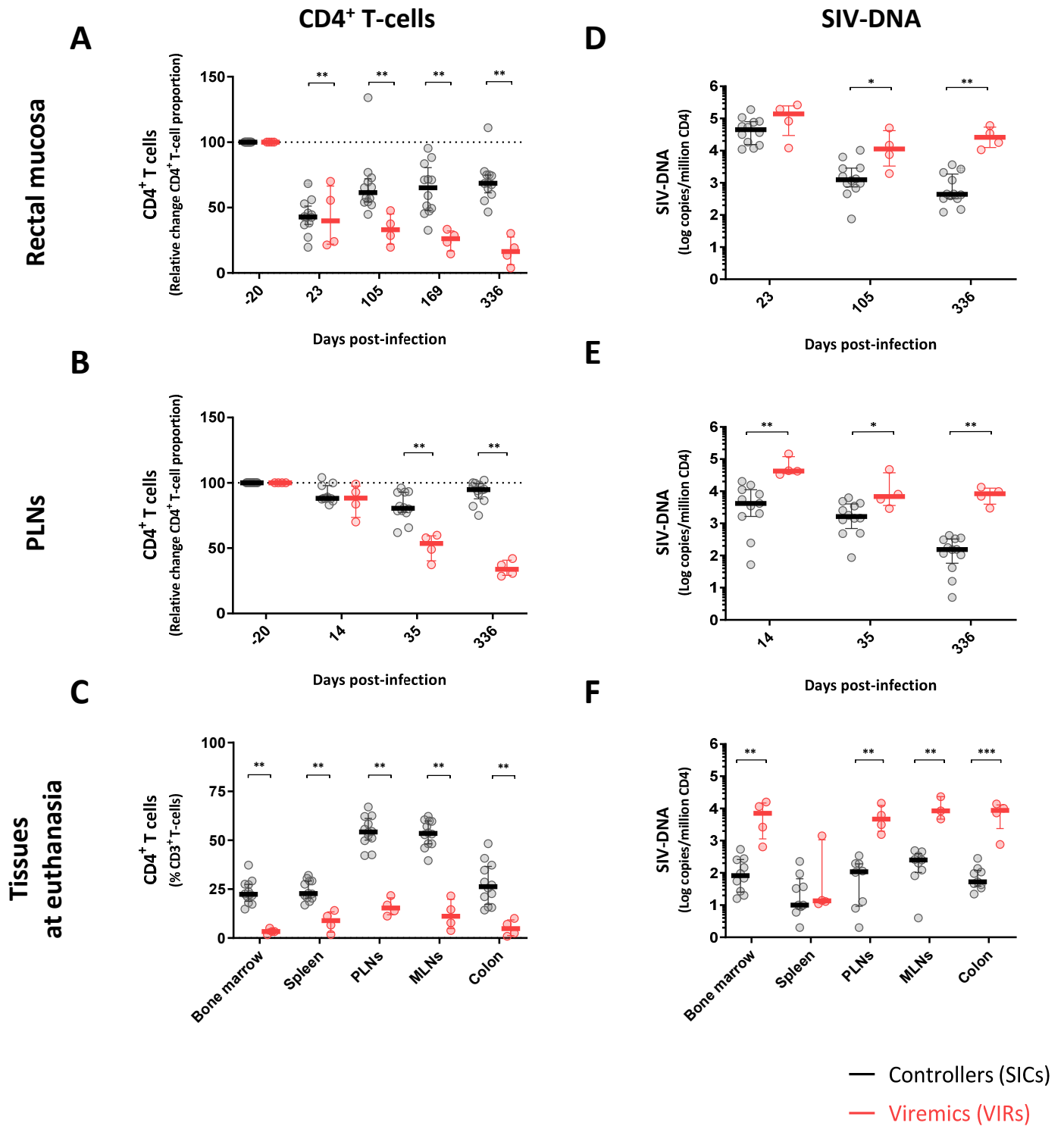
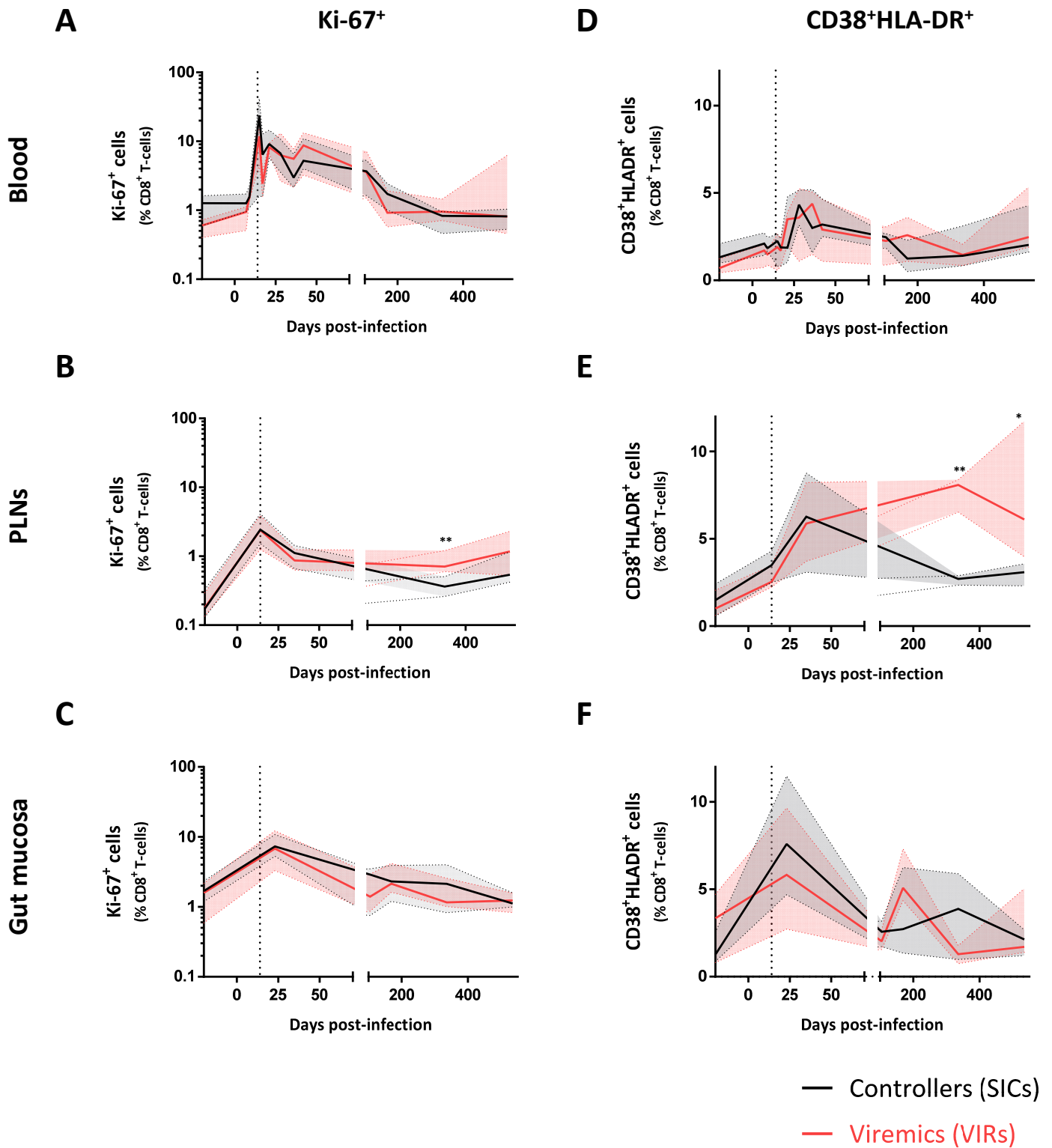
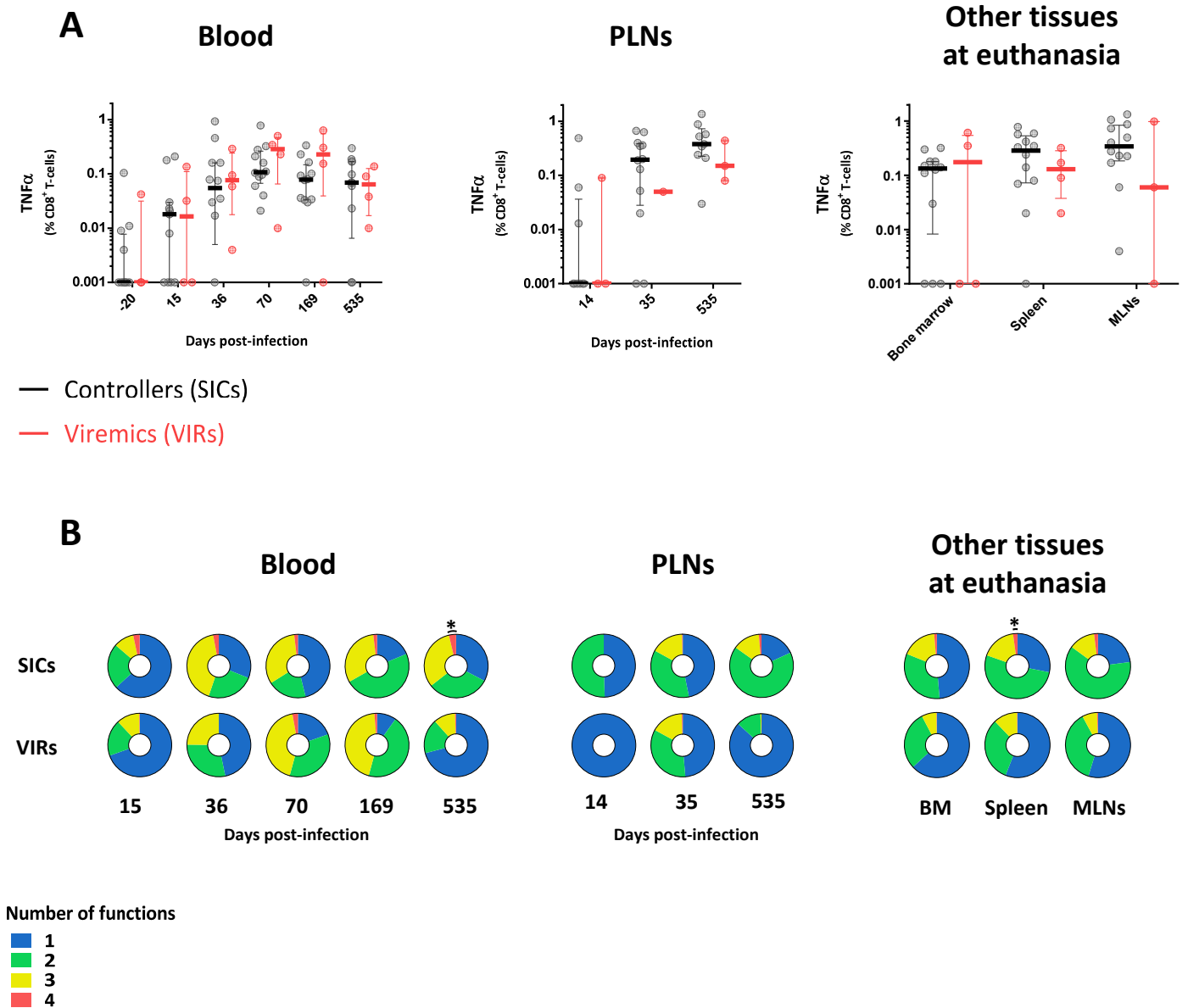


Figure 3

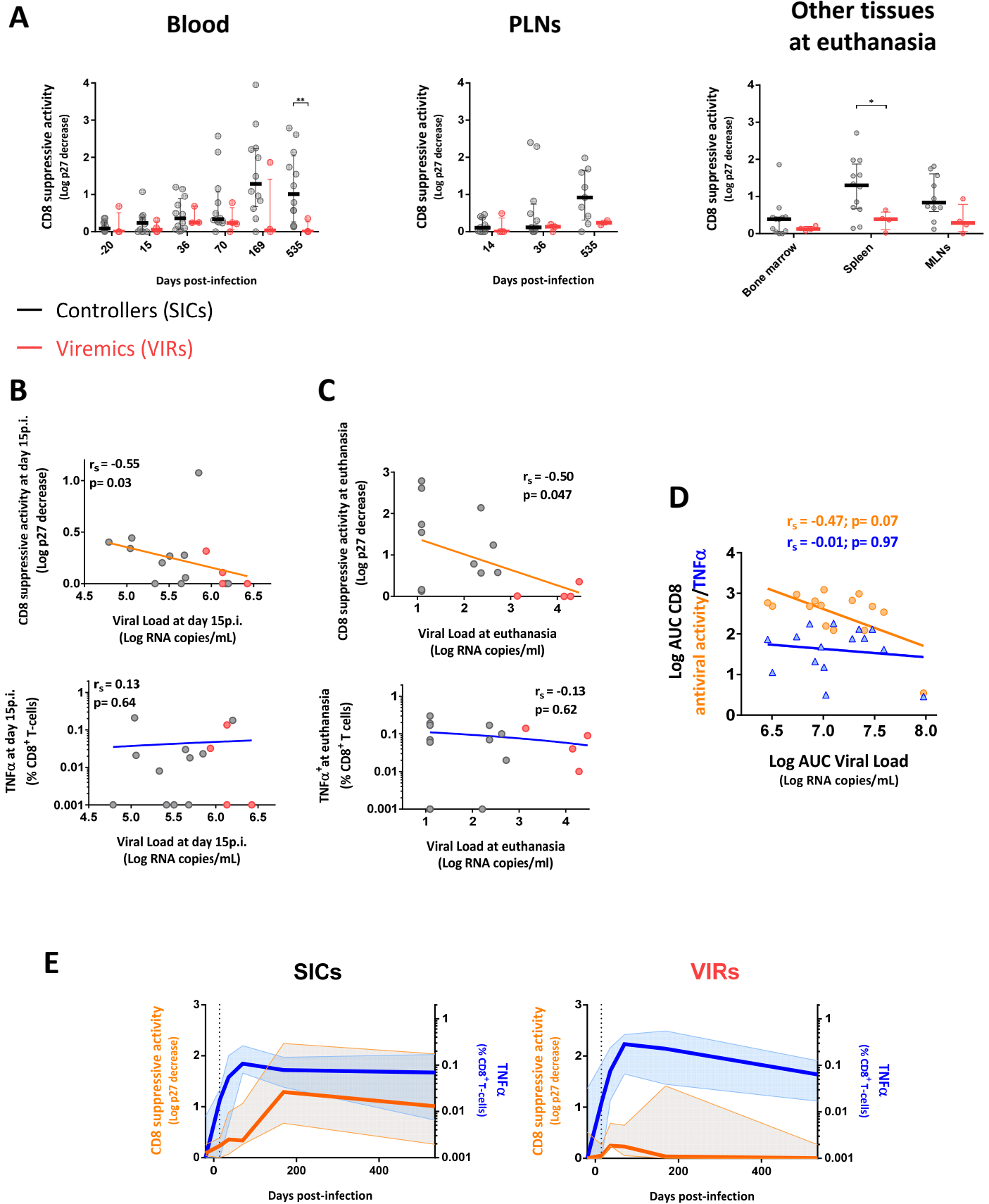




## Figure 4

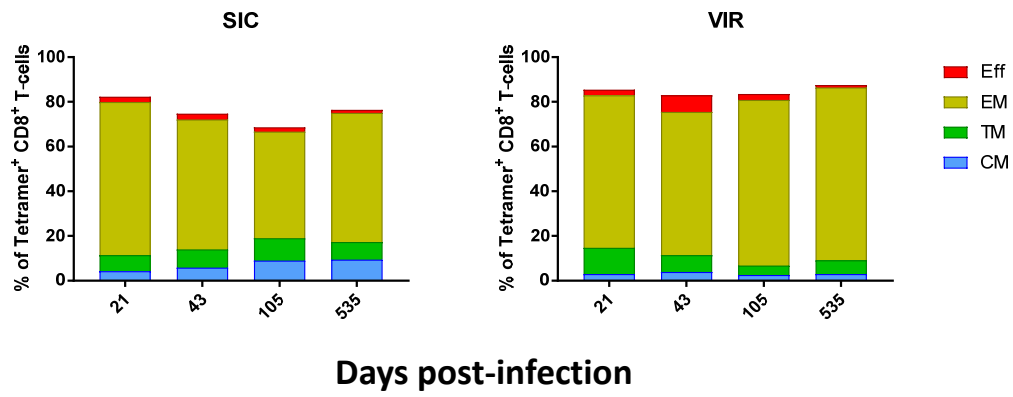


## Figure 5

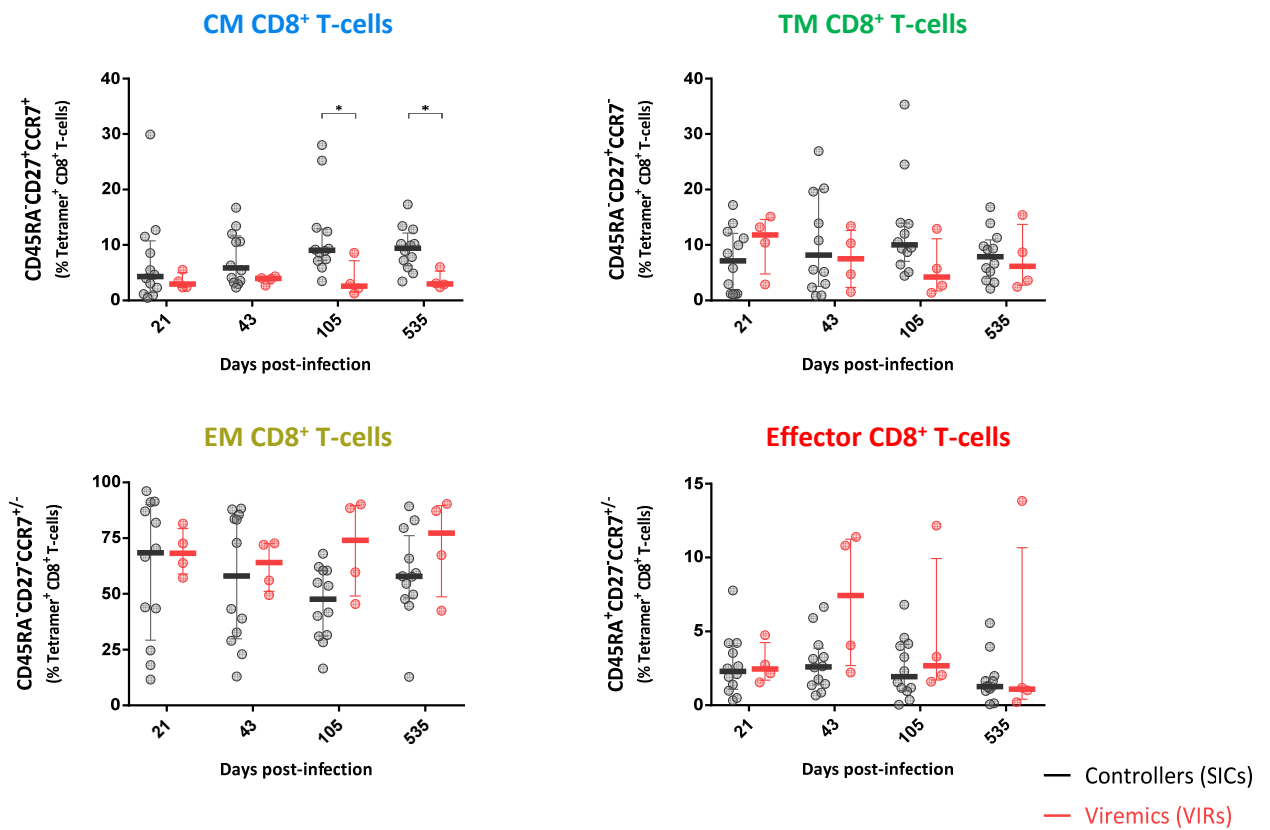


## Figure 6

A

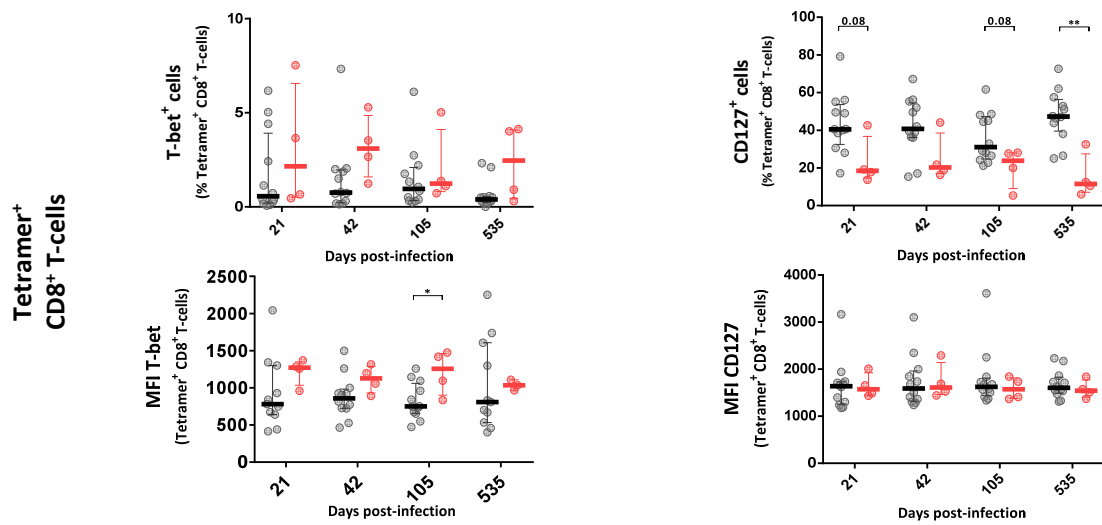


B

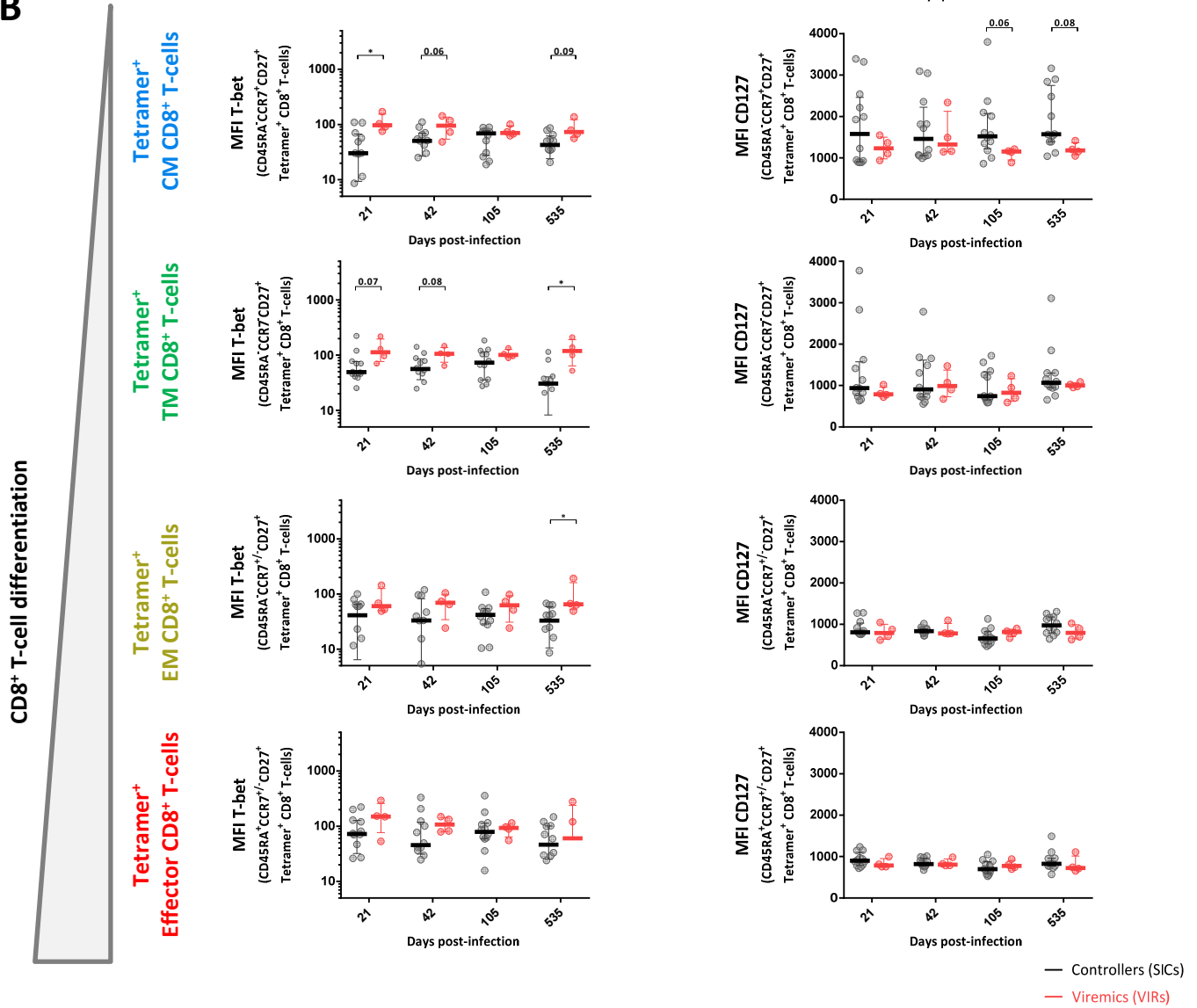


## Figure 7

A



B



## Figure 8

