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Optimal maturation of the SIV-specific CD8⁺ T-cell response after primary infection is associated with natural control of SIV. ANRS SIC study

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28 **ABSTRACT**

29 Highly efficient CD8⁺ T-cells are associated with natural HIV control, but it has remained
30 unclear how these cells are generated and maintained. We have used a macaque model of
31 spontaneous SIVmac251 control to monitor the development of efficient CD8⁺ T-cell
32 responses. SIV-specific CD8⁺ T-cells emerge during primary infection in all animals. The
33 ability of CD8⁺ T-cells to suppress SIV is suboptimal in the acute phase but increases
34 progressively in controller macaques before the establishment of sustained low-level
35 viremia. Controller macaques develop optimal memory-like SIV-specific CD8⁺ T-cells early
36 after infection. In contrast, a persistently skewed differentiation phenotype is observed
37 among memory SIV-specific CD8⁺ T-cells in non-controller macaques. The phenotype of SIV-
38 specific CD8⁺ T-cells defined early after infection appears to favor the development of
39 protective immunity in controllers, whereas SIV-specific CD8⁺ T-cells in non-controllers fail to
40 gain antiviral potency, feasibly as a consequence of early defects imprinted in the memory
41 pool.

INTRODUCTION

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

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

frequently express the protective human leukocyte antigen (HLA) class I allotypes HLA-B*27 and HLA-B*57, further supporting a key role for CD8⁺ T-cells in the natural control of HIV (Lecuroux et al., 2014; Migueles et al., 2000; Pereyra et al., 2008). However, the presence of these allotypes is not sufficient to confer protection, and potent CD8⁺ T-cell responses with the ability to suppress HIV replication directly *ex vivo* also occur in HICs expressing non-protective allotypes (Lecuroux et al., 2014). In addition, the qualitative properties of CD8⁺ T-cells from HICs have only been characterized extensively during chronic infection, when viremia was already under control, often several years after the acquisition of HIV. It has therefore remained unclear how these high-quality CD8⁺ T-cell responses develop from the early stages of infection and evolve over time.

Cynomolgus macaques (CyMs, *Macaca fascicularis*) infected with SIVmac251 closely recapitulate the dynamics and key features of HIV infection, including similar levels of viral replication in the acute and chronic phases of infection, memory CD4⁺ T-cell depletion, rapid seeding of the viral reservoir, and eventual progression to AIDS (Antony and MacDonald, 2015; Feichtinger et al., 1990; Karlsson et al., 2007; Mannioui et al., 2009; Putkonen et al., 1989). Moreover, some individuals control infection naturally, as in humans. CyMs from Mauritius offer the additional advantage of limited major histocompatibility complex (MHC) diversity, making them particularly attractive for the study of CD8⁺ T-cell responses. Natural control of SIV in Mauritian CyMs is favored by the MHC haplotype M6 (Aarnink et al., 2011; Mee et al., 2009). Inoculation with low-dose SIV via the intrarectal (*i.r.*) route is also associated with natural control, irrespective of MHC haplotype (Bruel et al., 2015). We took advantage of these validated models to study the dynamics of SIV-specific CD8⁺ T-cell responses in blood and tissues from the onset of infection in controllers and viremic CyMs.

90 Using this approach, we identified an optimal maturation pathway in SICs that enabled SIV-
91 specific CD8⁺ T-cells to acquire potent antiviral functions, which contributed to control
92 infection.

93

RESULTS

SIV controllers are characterized by partial restoration of CD4⁺ T-cell counts and progressive decline in the frequency of SIV-infected cells in blood

We monitored prospectively the outcome of infection in 12 SIV controllers (SICs) and 4 viremic CyMs (VIRs) inoculated *i.r.* with SIVmac251. These animals carried or not the protective M6 haplotype and were inoculated with 5 or 50 animal infectious dose₅₀ (AID₅₀) of SIVmac251 (Table S1). SIV controllers decreased plasma viral load (VL) to levels below 400 SIV-RNA copies/mL, at least twice, over a follow up period of 18 months, while VIRs consistently maintained VL above 400 SIV-RNA copies/mL. The threshold of 400 RNA copies/mL was chosen in coherence with our studies in human cohorts of natural HIV control (Angin et al., 2016; Noel et al., 2016; Saez-Cirion et al., 2013; Saez-Cirion et al., 2007; Saez-Cirion et al., 2009). Ten SICs achieved control of viremia within 3 months. The other two SICs (BL669 and BO413) achieved VL below 400 SIV-RNA copies/mL for the first time 14 months after inoculation. One VIR (AV979) developed a tonsilar lymphoma, an AIDS related event reported at high frequency in this species upon SIV infection (Feichtinger et al., 1990).

Some differences in peak viremia were observed between SICs and VIRs (Figure 1A, Table S2). These differences became more pronounced over time (Figure 1A), because plasma viremia was suppressed more rapidly in SICs versus VIRs (Table S2). Levels of cell-associated SIV-DNA in blood from SICs and VIRs were comparable before peak viremia, but differences became apparent as plasma VLs declined and were maintained throughout chronic infection (Figure 1C, Table S2). Likewise, CD4⁺ T-cells obtained during early infection from SICs and VIRs produced similar levels of p27 upon stimulation *ex vivo* with ConA and IL-2 (Figure S1A). However, while p27 production remained relatively stable in VIRs, these levels strongly

dropped overtime in SICs. We found a significant correlation between the levels of p27-production by CD4⁺ T-cells and the levels of SIV-DNA ($r=0.775$; $p=0.0002$, 6 months post-infection). Therefore, our results show that SICs, not only progressively reduced the frequency of total infected cells, but also the translation-competent reservoir. In addition, CD4⁺ T-cell counts declined markedly in blood from both SICs and VIRs during primary infection (Figure 1B, Table S2). Subsequently, a degree of recovery was observed in SICs, whereas further gradual decline was observed in VIRs (Figure 1B, Table S2).

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

SIV control is associated with early preservation of lymph nodes

To characterize the extent of SIV control in greater depth, we monitored CD4⁺ T-cells and total SIV-DNA longitudinally in peripheral lymph nodes (PLNs) and rectal biopsies (RBs). At the end of the study, we conducted similar evaluations in bone marrow, spleen, mesenteric lymph nodes (MLNs), and colonic mucosa, comparing SICs versus VIRs. The frequency of CD4⁺ T-cells similarly declined in RBs from both SICs and VIRs during the acute stage of primary infection. While the frequency of CD4⁺ T-cells was later partially restored in SICs, it continued to decline in VIRs (Figure 1D). These results matched the observations in blood samples (Figure 1B). In contrast, the frequency of CD4⁺ T-cells was maintained close to baseline in PLNs from SICs, even during primary infection (day 14 post-infection [*p.i.*]), but

steadily declined over time in VIRs (Figure 1E). At the time of euthanasia, CD4⁺ T-cell frequencies were substantially higher in blood (Figure 1B), bone marrow, spleen, PLNs, MLNs, and colonic mucosa (Figure 1F) from SICs versus VIRs.

Cell-associated SIV-DNA levels closely mirrored the dynamics of CD4⁺ T-cells. Similarly high levels of cell-associated SIV-DNA were observed in RBs from SICs and VIRs during primary infection, but lower levels were observed in RBs from SICs *versus* VIRs during chronic infection (Figure 1G). Of note, SIV-DNA levels were already approximately 1 log lower in PLNs *versus* RBs from SICs during primary infection, and accordingly, lower levels were observed in PLNs from SICs *versus* VIRs since day 14 *p.i.* (Figure 1H). This finding suggests that early viral replication may be contained more efficiently in lymphoid nodes in SICs compared with other explored anatomical compartments. SIV-DNA was also detected in alveolar macrophages from all CyMs throughout the course of infection, again at lower levels in SICs *versus* VIRs during chronic infection (Figure S1B). In addition, SIV-DNA levels tended to decline progressively over time in SICs in all tissues analyzed, whereas SIV-DNA levels remained stable after primary infection in VIRs (Figure 1C, G, H). At the time of euthanasia, SIV-DNA levels were substantially lower in blood (Figure 1C), bone marrow, PLNs, MLNs, and gut mucosa (Figure 1I and Figure S1C) from SICs *versus* VIRs.

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

The dynamics of CD8⁺ T-cell expansion and activation do not predict control of SIV

To understand the mechanisms that contribute to immune control of SIV, we first monitored the proliferation and activation dynamics of total CD8⁺ T-cells in blood and lymphoid tissues from SICs and VIRs. Recent studies in hyperacute HIV infected individuals indicate that the changes observed in the total CD8⁺ T-cell activation during acute infection may be largely related to changes in the HIV-specific CD8⁺ T-cell pool (Ndhlovu et al., 2015; Takata et al., 2017). The frequencies of CD8⁺ T-cells expressing Ki-67 in blood increased to maximum levels during primary infection (measured peak at day 15 *p.i.*), coinciding with the measured peak of viremia, then declined steadily to baseline levels during chronic infection (Figure 2A). Similar dynamics were observed in PLNs (Figure 2B) and gut mucosa (Figure 2C). In general, there were no significant differences between SICs and VIRs with respect to the dynamics of Ki-67 expression within the CD8⁺ T-cell pool, although lower frequencies of CD8⁺ T-cells expressing Ki-67 were observed during chronic infection in PLNs from SICs *versus* VIRs (Figure 2B).

The frequencies of CD8⁺ T-cells expressing the activation markers CD38 and HLA-DR in blood, PLNs, and gut mucosa increased similarly during primary infection (measured peak at day 28 *p.i.*), following the dynamics of Ki-67 expression in the same compartments (Figure 2D–F). Again, there were no significant differences between SICs and VIRs with respect to the early dynamics of total CD8⁺ T-cell activation, but lower frequencies of CD8⁺ T-cells expressing CD38 and HLA-DR were observed during chronic infection in PLNs from SICs *versus* VIRs (Figure 2E).

Overall, our findings indicate that although lower activation and proliferation is observed in CD8⁺ T-cells from SICs than VIRs in the chronic stage of infection, the early proliferation

and activation dynamics of the total pool of CD8⁺ T-cells do not distinguish subsequent progression rates.

SIV-specific CD8⁺ T-cell frequencies do not predict control of SIV

We analyzed CD8⁺ T-cell responses to a pool of optimal SIVmac251 peptides, which included peptides from different SIV proteins recognized by the most frequent MHC haplotypes in CyMs (M1, M2 and M3) and by the MHC haplotype M6 (Table S3). All the animals carried at least one haplotype matching some peptide, and overall there was no difference in the number of peptides tested theoretically recognized in SICs and VIRs ($p=0.35$). SIV-specific CD8⁺ T-cells producing TNF α (cytokine showing the lowest background in the absence of peptide or in presence of peptide during the baseline and hence used as reference) emerged in all CyMs during primary infection, coinciding with the peak of viremia, and no significant differences were observed between SICs and VIRs with respect to the frequencies of these cells in any anatomical compartment at any stage of infection (Figure 3A). Similarly, no consistent differences were observed between SICs and VIRs with respect to the frequencies of SIV-specific CD8⁺ T-cells that produced other cytokines, including IFN γ (Figure S2A) and IL-2 (Figure S2B), or mobilized CD107a (Figure S2C). The overall SIV-specific CD8⁺ T-cell response, determined in each CyM as the frequency of cells displaying at least one function (TNF α , IFN γ , IL-2, or CD107a), was also equivalent between SICs and VIRs across anatomical compartments throughout infection (Figure S2D). In addition, no clear differences between SICs and VIRs were observed with respect to the frequencies of SIV-specific CD8⁺ T-cells displaying at least three functions simultaneously in blood or PLNs during acute infection, but higher frequencies of polyfunctional SIV-specific CD8⁺ T-cells were present during chronic infection in lymphoid tissues from SICs *versus* VIRs (Figure 3B and S2E). Of note, no

differences in the magnitude and polyfunction of SIV-specific CD8⁺ T-cells from SICs and VIRs were observed either when a pool of overlapping peptides spanning SIV Gag was used instead of the optimal peptide pool to stimulate the cells (Figure S3).

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

Progressive acquisition of CD8⁺ T-cell-mediated SIV-suppressive activity is associated with control of SIV

CD8⁺ T-cells from HICs typically suppress *ex vivo* infection of autologous CD4⁺ T-cells (Angin et al., 2016; Buckheit et al., 2012; Julg et al., 2010; Saez-Cirion et al., 2007; Saez-Cirion et al., 2009; Tansiri et al., 2015). We therefore investigated this property as a potential discriminant between SICs and VIRs. The capacity of CD8⁺ T-cells in blood and PLNs to suppress infection of autologous CD4⁺ T-cells was relatively weak in all CyMs during acute infection (Figure 4A), but this activity correlated negatively with VL on day 15 *p.i.* (Figure 4B, upper panel) suggesting its contribution to control viremia since early time points. Interestingly, the CD8⁺ T-cell-mediated SIV-suppressive activity increased substantially over time in SICs (Figure 4A and S4A), either in blood or tissues. No such acquisition of SIV-suppressive activity was observed in VIRs (Figure 4A and S4A). Moreover, CD8⁺ T-cell-mediated SIV-suppressive activity on day 70 *p.i.* correlated negatively (or tended to correlate) with subsequent determinations of plasma VL (Figure S4B) and there was a negative correlation between the CD8⁺ T-cell-mediated SIV-suppressive activity at euthanasia and the VL at this time (Figure 4C, upper panels). In contrast, we never found

negative correlations between VL (at any given time) and the suppressive capacity of CD8⁺ T-cells measured at subsequent time points. No significant correlations were identified at any time point between SIV-specific CD8⁺ T-cell frequencies, categorized according to TNF α production in response to SIV peptides, and measurements of plasma VL (Figure 4B-C, S4B bottom panels). Moreover, CD8⁺ T-cell-mediated SIV-suppressive activity across the entire follow-up period, quantified as area under the curve, tended to correlate negatively with plasma VL ($r_s = -0.47$, $p = 0.07$), whereas no such association was identified for the frequency of SIV-specific CD8⁺ T-cells ($r_s = -0.01$, $p = 0.97$) (Figure 4D). All these results indicate that changes in the suppressive capacity of CD8⁺ T-cell, but not in the frequency of SIV-specific CD8⁺ T-cells were associated with SIV control. We have previously shown that strong HIV-suppressive capacity of CD8⁺ T-cells from HIV-1 controllers was linked to their ability to rapidly eliminate *ex vivo* autologous infected cells through cytotoxic mechanisms (Saez-Cirion et al., 2007). Although we could not repeat here all the analyses we did in our studies in cohorts of HIV controllers, we could confirm that the capacity of CD8⁺ T-cells to suppress SIV infection *ex vivo* required contact with infected CD4⁺ T-cells, and it was lost when CD8⁺ and CD4⁺ T-cells were separated by transwell membranes (Figure S5A).

Our results exposed a disconnection between the development of SIV-specific CD8⁺ T-cells responding to SIV peptides and the ability of these cells to suppress SIV infection, as measured in our viral inhibition assay *ex vivo* (Figure 4E, Figure S4A). SIV-specific CD8⁺ T-cell frequencies increased sharply as the initial viremia began to fall and remained high for the duration of the study in CyMs irrespectively of their level of viremia. However, the substantial decline in viremia to levels below 400 copies/mL in SICs coincided with the raise of SIV-suppressive activity. The increase of CD8⁺ T-cell-mediated SIV-suppressive activity was

262 delayed in the late controller #BL669 and #BO413, but nonetheless preceded optimal control
263 of VL in these CyMs (Figure S4A). A very strong capacity of CD8⁺ T-cells to suppress SIV was
264 observed at day 36 in the LN from two animals (BA209 and BC657) that did not show such
265 capacity in the blood (Figure 4A). Only one animal (29925) did not develop any detectable
266 SIV suppressive activity during our follow up. This animal had the weakest peak of viremia (1
267 log lower than any other) and achieved the fastest control (Figure S4A). Whether a very
268 rapid or local development of the CD8⁺ T-cell suppressive capacity may have occurred or
269 other mechanisms were associated with control of viremia in this animal remains unknown.
270 At the time of euthanasia, superior CD8⁺ T-cell-mediated SIV-suppressive activity was
271 detected in a vast majority of SICs across all anatomical compartments, with the exception
272 of bone marrow (Figure 4A), which nonetheless harbored SIV-specific CD8⁺ T-cells at
273 frequencies comparable to other tissues (Figure 3A). Thus, although abundant, SIV-specific
274 CD8⁺ T-cells induced during primary SIV infection had limited SIV suppressive capacity when
275 compared to cells found at later time points in SICs.

276
277 One limitation of our study was the reduced number of viremic animals we could compare in
278 parallel to SICs. To confirm that the capacity of CD8⁺ T-cells to suppress *ex vivo* SIV infection
279 did not increase in VIRs, we analyzed this activity in an additional group of 14 non-M6 CyMs
280 infected intravenously (*i.v.*) with 1,000AID₅₀ of SIVmac251 and characterized by high
281 setpoint viremia (ANRS pVISCONTI study). In these animals, the CD8⁺ T-cell-mediated SIV-
282 suppressive activity also remained modest throughout the follow-up (Figure S6A). The
283 combined analysis of the CD8⁺ T-cells from all VIRs (n=4 50AID₅₀ + n=14 1,000AID₅₀) exposed
284 early significant differences in the CD8⁺ T-cell-mediated SIV-suppressive activity when
285 compared to the SICs (p=0.052 at day 36 *p.i.* and p<0.05 at days 70 and 169 *p.i.*, Figure S6B),

and significantly higher capacity to suppress SIV infection of CD8⁺ T-cells from SICs than cells from VIRs in all tissues analyzed at the end of the study (Figure S6B). Moreover, early initiation (day 28 post-infection) of antiretroviral treatment in another group of CyM inoculated with 1,000AID₅₀ of SIVmac251 sharply decreased VL and CD8⁺ T-cell activation levels (Figure S6C) but did not change the capacity of CD8⁺ T-cells from these animals to suppress infection *ex vivo*, which remained extremely weak (Figure S6C). These results, which are in agreement with our previous observations in early treated HIV-infected individuals (Lecuroux et al., 2013), show that low SIV suppressive capacity during acute infection was neither a consequence of strong activation of these cells *in vivo* nor of high antigen burden.

Finally, one possible explanation for the weak SIV suppressive capacity of CD8⁺ T-cells from VIRs was that they had a limited capacity to recognize the SIVmac251 we used in our viral inhibition assay, due to potential adaptation of the CD8⁺ T-cell response to evolving viruses in these CyMs. However, CD8⁺ T-cells from VIRs were not able to eliminate either the CD4⁺ T-cells that produced “autologous” SIV upon activation *in vitro*. In contrast, although CD8⁺ T-cells from SICs were not able to suppress autologous virus during early infection, they acquired this capacity progressively at the same rate we found in our classical protocol (Figure S5B). These results confirmed that CD8⁺ T-cells from SICs and VIRs had functional differences that were unlinked to the evolution of the infecting virus.

Collectively, our results show that the capacity of SIV-specific CD8⁺ T-cells to suppress infection *ex vivo* was a genuine quality that progressively amplified in SICs. Our results

further uncover a temporal link between the acquisition by CD8⁺ T-cells of potent capacity to suppress infection and sustained control of SIV.

Acquisition of CD8⁺ T-cell-mediated SIV-suppressive activity in SICs occurs independently of MHC haplotype

Our primary intention in this study was to explore the mechanisms underlying natural control of SIV infection, independently of MHC background or infectious dose. However, as expected (Bruel et al., 2015), inoculation with low-dose virus and carriage of the protective M6 haplotype independently favored spontaneous control of viremia below 400 copies/mL in CyMs in our study (Table S4, Figure S7A). We therefore evaluated whether these parameters influenced the dynamics of control and the development of the CD8⁺ T-cell response upon infection. We found that CD4⁺ T-cells and the levels of cell-associated SIV-DNA similarly evolved in the blood and PLNs from M6 and non-M6 controllers (Figure S7B). There was just a tendency for M6 controllers *versus* non-M6 controllers to a better recovery of CD4⁺ T-cells in blood at the end of the study (p=0.07). Similarly, we did not find important differences between M6 and non-M6 controllers in their development of SIV-specific CD8⁺ T-cell responses (Figure S7C). M6 and non-M6 controllers developed similar frequencies of SIV-responding cells during acute infection that were maintained during the follow up. Of note, the capacity of CD8⁺ T-cells to suppress *ex vivo* SIV infection of CD4⁺ T-cells progressively increased in both M6 and non-M6 SICs. The only difference that we could appreciate was a faster acquisition (day 36 *p.i.*) of CD8⁺ T-cell mediated SIV suppressive activity in the PLN from M6 SICs *versus* non-M6 SICs (Figure S7C). Intriguingly, non-M6 SICs had higher frequencies of SIV responding CD8⁺ T-cells in this tissue at the same time point. Overall these results show that while the M6 background gave a selective advantage to CyMs to control

infection in conditions of higher viral inoculum, this MHC haplotype was not indispensable for the acquisition of SIV suppressive capacity by CD8⁺ T-cells, which occurred both in M6 and non-M6 SICs. The results are in agreement with the observations in HIV controllers. Although cohorts of HICs are enriched in individuals carrying protective HLA class I alleles (mainly HLA-B*57, B*27), many HICs do not carry protective HLA class I alleles but have CD8⁺ T-cells with strong HIV suppressive capacity *ex vivo* (Lecuroux et al., 2014). Therefore, the development of efficient CD8⁺ T-cell responses with antiviral activity is a characteristic of most HICs/SICs, independently of their MHC background.

Skewed maturation of central memory SIV-specific CD8⁺ T-cells is associated with defective acquisition of SIV-suppressive activity

To dissect the phenotypic correlates of *ex vivo* measured antiviral potency, we analyzed the differentiation status of SIV-specific CD8⁺ T-cells using selected markers in conjunction with MHC class I tetramers. Tetramer-binding SIV-specific CD8⁺ T-cells were detected in all CyMs, displayed early similar differentiation profiles in SICs and VIRs, but evolved differently, such that higher frequencies of central memory (CM) SIV-specific CD8⁺ T-cells were present in SICs versus VIRs on day 105 *p.i.* ($p = 0.018$) and day 535 *p.i.* ($p = 0.013$) (Figure 5A, B).

In further analyses, we found that higher frequencies of SIV-specific CD8⁺ T-cells from SICs expressed the IL-7 receptor CD127, which is associated with cell survival and memory responses (Schluns et al., 2000), whereas higher frequencies of SIV-specific CD8⁺ T-cells from VIRs expressed the transcription factor T-bet, which is associated with cellular differentiation and effector functionality (Sullivan et al., 2003; Szabo et al., 2002) (Figure 6A). These differences seemed to appear since primary infection, but due to the limited number of VIRs

analyzed, the differences only reached statistical significance at later time points (Figure 6A). Expression levels of CD127 and T-bet also varied as a function of differentiation among SIV-specific CD8⁺ T-cells from SICs and VIRs (Figure 6B). In particular, CM and transitional memory (TM) SIV-specific CD8⁺ T-cells expressed lower levels of T-bet throughout the course of infection in SICs *versus* VIRs, whereas CM SIV-specific CD8⁺ T-cells tended to express higher levels of CD127 during chronic infection in SICs *versus* VIRs.

To palliate the limited statistical power, we analyzed all animals together, taking into account the spectrum of outcomes rather than group classifications. We found negative correlations during primary infection and at euthanasia between the expression levels of CD127 on SIV-specific CD8⁺ T-cells and plasma VLs (Figure 7A). Of note, the levels of CD127 correlated positively with CD8⁺ T-cell-mediated SIV-suppressive activity at the same time points (Figure 7B). On the contrary, negative correlations were observed during primary infection and at euthanasia between CD8⁺ T-cell-mediated SIV-suppressive activity and the contemporaneous frequencies of T-bet⁺CD127⁺-SIV-specific CD8⁺ T-cells (Figure 7C) and between CD8⁺ T-cell-mediated SIV-suppressive activity and expression levels of T-bet among CM SIV-specific CD8⁺ T-cells (Figure 7D).

Altogether, these results point to the importance of the early establishment and maintenance of optimal memory CD8⁺ T-cell responses for achieving viral control. Recently, the presence of a population of memory-like CD8⁺ T-cells with stem cell-like properties, characterized by the expression of the transcription factor 1 (TCF-1) (Zhou et al., 2010), was shown to be critical to sustain the CD8⁺ T-cell response against chronic viral infections (Snell et al., 2018; Utzschneider et al., 2016). We found that SIV-specific CM CD8⁺ T-cells from SICs

expressed higher levels of TCF-1 than cells from VIRs (Figure 7E). Among TCF-1 expressing cells, a higher frequency of cells from SICs expressed in addition CXCR5 and CCR7, revealing the presence of memory-like responses with the potential capacity to migrate to the lymph node follicles (Leong et al., 2016).

Collectively, these results suggest that SICs developed during early infection SIV-specific CD8⁺ T-cells that enabled long-term memory potential, sustained antiviral activity and viral control, whereas the corresponding SIV-specific CD8⁺ T-cells in VIRs adopt a skewed phenotype associated with cellular differentiation, suboptimal antiviral activity and more prone to exhaustion (Zhou et al., 2010).

DISCUSSION

This study provides insights into the immune correlates of natural SIV control. Although SIV-specific CD8⁺ T-cells were generated during acute infection with equivalent dynamics and global frequencies in all CyMs, preventing discrimination between SICs and VIRs, antiviral efficacy *ex vivo* developed progressively over time and was associated with spontaneous SIV control. This dichotomy was underpinned by distinct early memory programs within the SIV-specific CD8⁺ T-cell pool. Collectively, these findings identify a cohesive set of immunological parameters that associate with effective and sustained control of SIV.

To monitor the establishment of natural control prospectively, we took advantage of previous reports showing that carriage of the MHC haplotype M6 and *i.r.* inoculation with low-dose (5AID₅₀) virus independently favor spontaneous control of SIVmac251 infection in CyMs (Aarnink et al., 2011; Bruel et al., 2015; Mee et al., 2009). Our results corroborate previous reports. In particular, although the presence M6 haplotype favored more frequent and more rapid control of infection among animals receiving a high dose of the virus (50AID₅₀) (Table S4), no significant differences were observed in the dynamics of SIV control in M6 and non-M6 SICs. At the time of euthanasia, a higher proportion of CD4⁺ T-cells and lower cell-associated SIV-DNA levels were found in multiple tissues from SICs *versus* VIRs, demonstrating systemic control of SIV. These differences were much more subtle during primary infection. However, PLNs from SICs harbored approximately 10-fold less SIV-DNA in the acute phase than PLNs from VIRs. In addition, the frequency of CD4⁺ T-cells were maintained close to baseline throughout the course of the study in PLNs, but not in blood or RBs, from SICs. These observations suggest that early containment of viral replication in LNs

(Buggert et al., 2018; Reuter et al., 2017) may be a key event for subsequent immune control of SIV.

In line with previous studies in humans (Lecuroux et al., 2013; Ndhlovu et al., 2015; Trautmann et al., 2012) and non-human primates (Veazey et al., 2001) we observed early and robust expansions of SIV-specific CD8⁺ T-cells in all CyMs. However, the functional profiles and overall frequencies of SIV-specific CD8⁺ T-cells (as determined by intra cellular cytokine staining upon SIV antigen stimulation) during the acute phase of infection were largely equivalent in SICs and VIRs, and neither parameter correlated with subsequent determinations of plasma VL. Similarly, the functional profiles and overall frequencies of SIV-specific CD8⁺ T-cells during the chronic phase of infection were largely equivalent in SICs and VIRs, although polyfunctionality (defined as the capacity to produce simultaneously several cytokine and/or degranulate) was impaired at the time of euthanasia in VIRs. These results suggest that differences in polyfunctionality found during chronic infection are a surrogate marker of viral replication rather than an accurate determinant of antiviral efficacy, although low number of animals in the VIR group may limit statistical power.

The capacity of CD8⁺ T-cells to suppress infection of autologous CD4⁺ T-cells directly *ex vivo* is a particular feature of HICs (Almeida et al., 2009; Angin et al., 2016; Buckheit et al., 2012; Julg et al., 2010; Saez-Cirion et al., 2007; Saez-Cirion et al., 2009; Tansiri et al., 2015) that is mediated by the rapid elimination of infected CD4⁺ T-cells through MHC-restricted mechanisms (Saez-Cirion et al., 2007). Irrespective of subsequent outcome, we detected relatively weak CD8⁺ T-cell-mediated SIV-suppressive activity during primary infection, despite the vigorous mobilization of SIV-specific CD8⁺ T-cells. This observation parallels our

previous findings in the setting of HIV (Lecuroux et al., 2013) and point to limited antiviral potential of CD8⁺ T-cell responses generated during primary infection. However, a negative correlation was already observed between the CD8⁺ T-cell-mediated SIV-suppressive activity and viremia at this early time point, showing early temporal association of this antiviral activity and reduction of viremia. Of note, this SIV-suppressive capacity of CD8⁺ T-cells increased progressively over a period of weeks in some animals, carrying or not the protective MHC haplotype M6. At the time of euthanasia, these highly potent antiviral CD8⁺ T-cells were present in all tissues in SICs, with the exception of bone marrow, where this activity tended to be higher in SICs but overall weaker than in other tissues. It is important to notice that CD8⁺ T-cell-mediated SIV suppression was very weak also in PLNs during the first weeks following infection but increased over time in SICs. Therefore, the increase in the capacity of CD8⁺ T-cells to suppress infection was not the result of the recirculation of CD8⁺ T-cells from LNs once control was established, but a genuine progressive augmentation of the antiviral potential of the cells. The development of potent antiviral CD8⁺ T-cells is therefore a *bone fide* correlate of sustained control of SIV.

We provide several arguments supporting that the progressive enhancement of the capacity of CD8⁺ T-cells to suppress SIV infection led to the establishment of controlled viremia, rather than evolving as a consequence of viral control. As the dynamics of control were not homogeneous, we established a mathematical model to predict the development of effective immune-mediated control of SIV in our study (Madelain et al., 2020). Importantly, the model was constructed using exclusively SIV-RNA and SIV-DNA data from VIRs and SICs. SIV-RNA kinetics were best fitted using a model where the cytotoxic immune response progressively mounted up. The model predicted that faster decay in VL in SICs was

associated with a progressive development of a cytotoxic CD8⁺ T-cell response that decreased over ten times the half-life of infected cells (from 5.5 days to 0.3 days). The model also predicted that in SICs the maximal level of cytotoxic response was reached within 100 days of infection. Interestingly, the predictions of the kinetics of development of cytotoxic CD8⁺ T-cell responses matched, in *post-hoc* analyses, with the values of the capacity of CD8⁺ T-cells to suppress infection that we obtained experimentally, but not with the frequency of CD8⁺ T-cells that responded to SIV peptides. Moreover, we found that SIV suppressive activity of CD8⁺ T-cells 70 days after infection correlated negatively with ulterior VLs, while the suppressive activity never correlated negatively with preceding VLs. Control of viremia driven by antiretroviral treatment, did not lead to the development of SIV suppressive activity by CD8⁺ T-cells. Therefore, the enhancement of SIV suppressive capacity does not occur in response to a decrease in antigen levels.

Due to limited longitudinal sampling in lymphoid tissues, we cannot exclude, however, that SIV control or emergence of SIV suppressive capacity by CD8⁺ T-cells in these tissues, preceded our observations. Moreover, the progressive establishment of control of infection and maturation of the CD8⁺ T-cell response might be the result of a virtuous circle, in which initial reduced dynamics of viral replication in LNs allowed the development of more effective CD8⁺ T-cell responses, which in turn drove a steeper reduction in viremia. “Goldilocks” levels of viral replication might then allow CD8⁺ T-cells to attain their maximal antiviral potential and sustain viral control, as has been proposed to occur in CMV infection (Picker, 2014).

Interestingly, CD8⁺ T-cells that can control certain “latent” chronic viral infections, such as CMV or EBV, do not show features of functional exhaustion when compared to CD8⁺ T-cells against other chronic viral infections such as SIV/HIV, HCV or HBV (Chatterjee et al., 2019; Fenwick et al., 2019; Luxenburger et al., 2018; van den Berg et al., 2019; Ye et al., 2015). This appears to be related to the development of CD8⁺ T-cells with distinct molecular programs that allow long-term memory and the replenishment of the effector compartment in response to antigenic stimulation (Paley et al., 2012). Recent studies have identified a subset of memory-like CD8⁺ T-cells that express the transcription factor TCF-1 as responsible for sustaining the immune response against chronic viral infections (Utzschneider et al., 2016). CD8⁺ T-cells expressing TCF-1 have characteristics of central memory cells, possess strong proliferative potential and are able to produce more differentiated cell subset, while being less prone to exhaustion after repeated antigenic stimulation (Snell et al., 2018; Utzschneider et al., 2016; Zhou et al., 2010). We found that SIV-specific CD8⁺ T-cells from SICs expressed higher levels of TCF-1 than cells from VIRs, and these differences were more visible among less differentiated subsets, and coupled with an enhanced expression of CXCR5, which may reflect the potential of these cells to migrate to germinal centers. During the revision of this manuscript, a preprint became available describing similar findings in cells from both HIV and SIV controllers in an independent study (Rutishauser et al., 2020). In that study, the authors linked the expression of TCF-1 with the capacity of HIV-specific CD8⁺ T-cells to expand.

One decisive characteristic for the establishment of long-term memory is the capacity to upregulate the IL-7 receptor α -subunit (CD127) (Huster et al., 2004). In addition, studies in mice have shown that decreased expression of T-bet among memory CD8⁺ T-cells allows the

establishment of long-lived CD127^{hi} cells, which maintain the capacity to proliferate and control successive infections (Joshi et al., 2007; Joshi et al., 2011). In our study, the divergent outcome of SICs *versus* VIRs was preceded by early differences in the expression of CD127 and T-bet, especially within the less differentiated memory pool (CM and TM). In particular, at day 21 post-infection, higher frequencies of SIV-specific CM CD8⁺ T-cells expressed CD127 in SICs, whereas higher frequencies of SIV-specific CM and TM CD8⁺ T-cells expressed T-bet in VIRs. It is important to note that plasma viremia was uncontrolled at this time point and largely equivalent in animals that became SICs (median 5.19 log SIV RNA) or VIRs (median 5.36, p=0.65). The phenotypic differences remained consistent, or even appeared to become more pronounced throughout the course of infection, although the statistical power of the analyses was affected by the limited number of VIRs studied. Overall, our data suggest that SICs develop very early memory-like SIV-specific CD8⁺ T-cell responses, whereas VIRs develop SIV-specific memory CD8⁺ T-cell responses skewed towards more effector-like characteristics and that these differences may be determinant to achieving and sustaining viral control. These findings are broadly consistent with several previous reports describing immune profiles that associate with the control of viremia in HICs during chronic infection. Favorable characteristics include high frequencies of CD57⁺ eomesodermin^{hi} HIV-specific CD8⁺ T-cells with superior proliferative capacity, increased expression levels of CD127, and intermediate expression levels of T-bet (Simonetta et al., 2014), and high frequencies of HIV-specific CD8⁺ T-cells with the capacity to upregulate T-bet, granzyme B, and perforin in response to antigen encounter (Hersperger et al., 2011b; Migueles et al., 2008).

In a recent single cell study (Angin et al., 2019), we also found differences in the program of HIV-specific CM CD8⁺ T-cells from HICs and non-controllers: whereas HIV-specific CM CD8⁺ T-cells from HICs upregulated the expression of effectors genes linked with mTORC2 activation and cell survival (including CD127), CM cells from non-controllers had a skewed profile associated with mTORC1 activation (including T-bet) and glycolysis. This was traduced in a dependency on glucose of HIV-specific CD8⁺ T-cells from non-controllers to react to HIV antigens, while HIV-specific CD8⁺ T-cells from HICs were characterized by metabolic plasticity and being able to exert their function even in conditions of glucose deprivation. Of note, these differences in the metabolic program of cells from controllers and non-controllers could also be recapitulated with SIV-specific CD8⁺ T-cells from SICs and VIR macaques from the present study (Angin et al., 2019), further corroborating the validity of our CyM model to study the development of the protective CD8⁺ T-cell responses characteristics of HIV/SIV controllers. The present results extend these observations and support a key role for long-lived memory responses in the control of SIV. Importantly, our data also show that distinct memory responses are formed early after infection, potentially reflecting different priming conditions.

Several important questions remain unresolved. It remains unclear which factors are required to encourage the development of memory CD8⁺ T-cell responses that provide optimal protection against HIV/SIV. In some viral infections, expression of T-bet is tightly regulated by cytokines, such as IL-12 (Rao et al., 2012; Takemoto et al., 2006). A recent study in the LCMV murine model of infection suggests that memory-like TCF-1 CD8⁺ T-cell responses with stemness potential and enhanced capacity to react upon secondary challenge, are developed during early chronic infection (in an immunosuppressive

environment), while memory cells that are developed at the onset of infection (in a pro-inflammatory environment) become short-term effectors and are rapidly exhausted (Snell et al., 2018). Based on these observations we propose that balanced inflammatory responses (Barouch et al., 2016) arising as a consequence of lower viral burdens in lymph nodes during acute infection in SICs might facilitate antigen-specific priming events associated with optimal memory programs (Ozga et al., 2016) and minimize the loss of CD4⁺ T-cells, which provide helper functions that are critical for the development of long-lived memory-like CD8⁺ T-cells (Khanolkar et al., 2004; Utzschneider et al., 2016). It is unclear, however, which mechanisms were responsible for the reduced viral dynamics in the lymph nodes of SICs during acute infection. In the case of animals carrying the M6 haplotype, this could be related to more effective NK cell responses, as has been shown for humans carrying some protective HLA alleles (Martin et al., 2002). For animals inoculated with a lower dose of the virus this could be due to a lower diversity of infecting viruses (Liu et al., 2010), which could limit the inflammatory response and be more efficiently controlled by initial CD8⁺ T-cell responses. Although we have shown that the changes in the suppressive capacity of CD8⁺ T-cells were related to intrinsic functional properties of the cells and not to different capacities to react against evolving viruses, a better understanding of the evolution of viral diversity and the quality of immune responses appears important in sight of our results. Finally, we found that the proportion of CD127⁺ SIV-specific CD8⁺ T-cells during acute and chronic infection correlated positively with CD8⁺ T-cell-mediated SIV-suppressive activity and we propose that the amplification of potent antiviral activity is the result of a maturation process, the trajectory of which is linked to the early optimal programming of the CD8⁺ T-cell memory compartment. However, we do not have any direct evidence at the molecular level to explain why/how the capacity to suppress infection increased progressively among CD8⁺

T-cells. It is interesting to note that maturation through persistent or repeated exposure to antigen can drive the selection of specific clonotypes bearing high-affinity T-cell receptors (TCRs) (Busch and Pamer, 1999; Ozga et al., 2016; Price et al., 2005) which have been shown to suppress HIV replication more efficiently than clonotypes targeting the same antigen via low-affinity TCRs (Almeida et al., 2007; Almeida et al., 2009; Ladell et al., 2013; Lima et al., 2020). Increase in antigen sensitivity over time would be compatible with the progressive increase in antiviral potency that we observed for the CD8⁺ T-cells from controllers in our study.

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

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AUTHOR CONTRIBUTIONS

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

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

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621 **DECLARATION OF INTERESTS**

622 The authors declare no competing interests.

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FIGURE LEGENDS

Figure 1. SIV controllers are characterized by early preservation of lymph nodes and progressive decline in the frequency of SIV-carrying cells. Plasma VL kinetics (A), longitudinal evolution of CD4⁺ T-cell counts in blood (B), in rectal mucosa (D), and peripheral lymph nodes (E) in SIV controllers (SICs, grey) and viremic CyMs (VIRs, red). Results are shown as fold-change in absolute CD4⁺ T-cell counts relative to baseline in blood and as fold-change in percent frequencies of CD4⁺ T-cells among CD3⁺ lymphocytes relative to baseline in RBs and pLNs. (F) Percent frequencies of CD4⁺ T-cells among CD3⁺ lymphocytes in bone marrow, spleen, peripheral and mesenteric LNs, and colon mucosa at euthanasia. Kinetics of SIV-DNA levels in blood (C), in RBs (G), and pLNs (H). (I) Levels of SIV-DNA in bone marrow, spleen, peripheral and mesenteric lymph nodes, and colon at euthanasia. Results are expressed as copies SIV-DNA/million CD4⁺ T-cells. Median and interquartile range are shown (n=12 SICs; n=4 VIRs). *p < 0.05, **p < 0.01; Mann-Whitney U-test.

Figure 2. The dynamics of CD8⁺ T-cell expansion and activation do not predict control of SIV. (A–C) Evolution of Ki-67⁺ CD8⁺ T cells in blood (A), peripheral lymph nodes (B), and rectal mucosa (C) in SIV controllers (grey) and vireemics (red). (D–F) Evolution of CD38⁺ HLA-DR⁺ CD8⁺ T cells in blood (D), pLNs (E), and RB (F). Median and interquartile range are shown. Vertical dashed lines indicate peak VLs. *p < 0.05, **p < 0.01; Mann-Whitney U-test.

Figure 3. SIV-specific CD8⁺ T-cell frequencies do not predict control of SIV. (A) TNFα production by SIV-specific CD8⁺ T-cells in blood and pLNs over the course of infection and in bone marrow, spleen, and MLNs at euthanasia in SICs (grey) and VIRs (red). Results are shown as percent frequencies among CD8⁺ T-cells. Median and interquartile range are

shown (n=12 SICs; n=4 VIRs). **(B)** Functional profiles of SIV-specific CD8⁺ T-cells in blood and pLNs over the course of infection and in bone marrow, spleen, and MLNs at euthanasia. Doughnut charts show median percent frequencies of SIV-specific CD8⁺ T-cells expressing IFN γ , TNF α , IL-2, and/or CD107a (n=12 SICs; n=4 VIRs). Colors indicate number of simultaneous functions (blue, 1; green, 2; yellow, 3; red, 4). *p < 0.05; Mann-Whitney U-test.

Figure 4. Progressive acquisition of CD8⁺ T-cell-mediated SIV-suppressive activity is associated with control of SIV. **(A)** CD8⁺ T-cell-mediated SIV-suppressive activity in blood and pLNs over the course of infection and in bone marrow, spleen, and MLNs at euthanasia in SICs (grey) and VIRs (red). Results are shown as log p27 decrease in the presence of CD8⁺ T-cells. *p < 0.05, **p < 0.01; Mann-Whitney U-test. **(B)** Spearman correlations between CD8⁺ T-cell-mediated SIV-suppressive activity (upper panel) or TNF α production by SIV-specific CD8⁺ T-cells (bottom panel) on day 15 *p.i.* with plasma VL on day 15 *p.i.* **(C)** Spearman correlations between CD8⁺ T-cell-mediated SIV-suppressive activity (upper panel) or TNF α production by SIV-specific CD8⁺ T-cells (bottom panel) at euthanasia with plasma VL at euthanasia. **(D)** Spearman correlations between area under the curve (AUC) for plasma VL and AUC for CD8⁺ T-cell-mediated SIV-suppressive activity (orange) and between AUC for plasma VL and AUC for TNF α production by SIV-specific CD8⁺ T-cells (blue). AUC for plasma VL, TNF α production and CD8⁺ T-cell antiviral activities were calculated using the sequential values obtained throughout the duration of our study in the blood of the infected animals (Figure S7). **(E)** Side by side comparison of the longitudinal kinetics of TNF α production by SIV-specific CD8⁺ T-cells in blood shown in figure 2A (blue) and CD8⁺ T-cell-mediated SIV-suppressive activity in blood shown in figure 3A (orange) in SICs (left panel) and VIRs (right panel). Median and interquartile range are shown (n=12 SICs; n=4 VIRs).

959

960 **Figure 5. SIV controllers maintain higher frequencies of SIV-specific central memory CD8⁺**
961 **T-cells during chronic infection than viremic CyMs. (A)** Bar charts showing median percent
962 frequencies of SIV-specific CD8⁺ T-cells in each phenotypically-defined subset in SICs and
963 VIRs. Light blue, central memory (CM); green, transitional memory (TM); yellow, effector
964 memory (EM); red, effector (Eff). **(B)** Evolution of CM, TM, EM, and Eff SIV-specific CD8⁺ T-
965 cells. Results are shown as percent frequencies of tetramer-binding CD8⁺ T-cells. Median and
966 interquartile range are shown (n=12 SICs; n=4 VIRs). *p < 0.05; Mann-Whitney U-test.

967

968 **Figure 6. Altered maturation of central memory SIV-specific CD8⁺ T-cells in viremic CyMs.**
969 **(A)** Dynamics of T-bet (left panels) and CD127 expression (right panels) among SIV-specific
970 CD8⁺ T-cells in SICs (n=12) (grey) and VIRs (n=4) (red). **(B)** Dynamics of T-bet (left) and CD127
971 expression among central memory, transitional memory, effector memory, and effector SIV-
972 specific CD8⁺ T-cells. *p < 0.05, **p < 0.01; Mann-Whitney U-test.

973

974 **Figure 7. Skewed maturation of central memory SIV-specific CD8⁺ T-cells is associated with**
975 **defective acquisition of SIV-suppressive activity.** Spearman correlations between CD127⁺
976 SIV-specific CD8⁺ T-cell frequencies and viral loads **(A)** and CD8⁺ T-cell-mediated SIV-
977 suppressive activity **(B)** during acute (left panel) and chronic infection (right panel).
978 Spearman correlations between T-bet⁺ CD127⁻ SIV-specific CD8⁺ T-cell frequencies **(C)** or T-
979 bet expression levels in central memory SIV-specific CD8⁺ T-cells **(D)** and CD8⁺ T-cell-
980 mediated SIV-suppressive activity during acute (left panel) and chronic infection (right
981 panel). Grey symbols, SICs (n=12); red symbols, VIRs (n=4). **(E)** Expression levels of TCF-1
982 among total, central memory, transitional memory, effector memory, and effector SIV-

983 specific CD8⁺ T-cells (left panel); and proportion of SIV-specific CD8⁺ T-cells coexpressing TCF-
984 1, CCR7 and CXCR5 (right panel). **p < 0.01; Mann-Whitney U-test.

STAR METHODS

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to the Lead Contact, Asier Saez-Cirion (asier.saez-cirion@pasteur.fr). Request for biological resources will be fulfilled based on availability and upon the establishment of a MTA.

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

This study did not generate/analyze datasets/code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethical statement

CyMs were imported from Mauritius and housed in facilities at the *Commissariat à l'Energie Atomique et aux Energies Alternatives* (CEA, Fontenay-aux-Roses, France). All non-human primate studies at the CEA are conducted in accordance with French National Regulations under the supervision of National Veterinary Inspectors (CEA Permit Number A 92-03-02). The CEA complies with the Standards for Human Care and Use of Laboratory Animals of the Office for Laboratory Animal Welfare under Assurance Number #A5826-01. All experimental procedures were conducted according to European Directive 2010/63 (Recommendation Number 9). The SIC and pVISCONTI studies were approved and accredited under statements A13-005 and A15-035 from the “*Comité d’Ethique en Expérimentation Animale du CEA*”, registered and authorized under Number 44 and Number 2453-2015102713323361v2 by the French Ministry of Education and Research. CyMs were studied with veterinary guidance, housed in adjoining individual cages allowing social interactions, and maintained under controlled conditions with respect to humidity, temperature, and light (12 hr light/12 hr dark cycles). Water was available *ad libitum*. Animals were monitored and fed with commercial

monkey chow and fruit once or twice daily by trained personnel. Environmental enrichment was provided in the form of toys, novel foodstuffs, and music under the supervision of the CEA Animal Welfare Body. Experimental procedures (animal handling, viral inoculations, and samplings) were conducted after sedation with ketamine chlorhydrate (Imalgene 1000®, 10 mg/kg, *i.v.*, Merial). Tissues were collected at necropsy. Animals were sedated with ketamine chlorhydrate and euthanized humanely with sodium pentobarbital (Doléthal, 180 mg/kg, *i.v.*, Laboratoire Vetoquinol).

Animals and SIV infection

A total of 16 healthy adult male CyMs (median age = 6.8 years at inclusion, IQR = 5.8–7.2) were selected for this study on the basis of MHC haplotype (M6⁺, n = 6; M6⁻, n = 10) (34). CyMs were inoculated *i.r.* with either 5AID₅₀ or 50AID₅₀ of uncloned SIVmac251 (A.M. Aubertin, Université Louis Pasteur, Strasbourg, France). The following experimental groups were studied: (i) M6⁻ CyMs inoculated *i.r.* with 5AID₅₀ (non-M6 5AID₅₀, n = 4); (ii) M6⁺ CyMs inoculated *i.r.* with 50AID₅₀ (M6 50AID₅₀, n = 6); and (iii) M6⁻ CyMs inoculated *i.r.* with 50AID₅₀ (non-M6 50AID₅₀, n = 6). Animals were monitored for 18 months after infection.

The outcome of infection generally matched expectations based on previous studies for each experimental group (Figure S7A, Table S4). Only one M6⁺ CyM (31041) was unable to control viremia below 400 copies/mL. This animal was homozygous for MHC class I (Table S1), which intrinsically limits immune control of HIV/SIV (Carrington et al., 1999; O'Connor et al., 2010). The dynamics of viral replication during acute infection were very similar in the three experimental groups, with peak VLs of 5.9, 6.4, and 6.3 log SIV-RNA copies/mL of plasma on

day 14 *p.i.* for non-M6 5AID₅₀, M6 50AID₅₀, and non-M6 50AID₅₀ CyMs, respectively (Table S4).

CyMs in the pVISCNTI study (median age = 5 years at inclusion, IQR = 4.1–5.3) were inoculated *i.v.* with 1000 AID₅₀ of uncloned SIVmac251. None of these animals carried the M6 haplotype. An antiretroviral regimen containing emtricitabine (FTC, 40 mg/kg, Gilead), dolutegravir (DTG, 2.5 mg/kg, ViiV Healthcare), and the tenofovir prodrug tenofovir-disoproxil-fumarate (TDF, 5.1 mg/kg, Gilead), coformulated as a once daily subcutaneous injection, was initiated on day 28 *p.i.* in 6 animals.

METHOD DETAILS

Blood collection and processing

Peripheral blood was collected by venous puncture into Vacutainer Plus Plastic K3EDTA Tubes or Vacutainer CPT Mononuclear Cell Preparation Tubes with Sodium Heparin (BD Biosciences). Complete blood counts were monitored at all time points from the Vacutainer Plus Plastic K3EDTA Tubes. Plasma was isolated from Vacutainer Plus Plastic K3EDTA Tubes by centrifugation for 10 min at 1,500 g and stored at –80°C. Peripheral blood mononuclear cells (PBMCs) were isolated from Vacutainer CPT Mononuclear Cell Preparation Tubes with Sodium Heparin according to manufacturer’s instructions (BD Biosciences), and red blood cells were lysed in ACK (0.15 M NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA, pH 7.4).

Tissue collection and processing

Axillary or inguinal LNs (PLNs), RBs, and broncho-alveolar lavages (BALs) were collected longitudinally from each animal at the indicated time points. Bone marrow, spleen,

mesenteric lymph nodes (MLNs), duodenum, jejunum, ileum, and colon were collected at necropsy. Tissue samples were snap-frozen in liquid nitrogen for storage at -80°C or collected in RPMI medium at $2-8^{\circ}\text{C}$. A complete PLN group was collected at each time point. LN cells were isolated into RPMI medium via mechanical disruption using a gentleMACS Dissociator (Miltenyi Biotec). The cell suspension was filtered ($70\text{ }\mu\text{m}$), and red blood cells were lysed in ACK. RB lymphocytes were obtained from approximately 4 mm^2 of rectal mucosa. Colonic lymphocytes were obtained from mucosa taken from approximately 10 cm of tissue. RBs and colonic tissue were washed extensively in R10 medium (RPMI medium supplemented with 10% fetal calf serum and penicillin/neomycin/streptomycin), and then digested for 45 min with collagenase II prior to mechanical disruption. Lymphocytes were isolated over a Percoll 67/44 gradient (Sigma-Aldrich). Bone marrow cells were purified using Lymphocyte Separation Medium (Lonza Bioscience) diluted to 90% in DPBS, centrifuged for 20 min at 350 g, and separated from red cells in ACK. Spleen cells were processed via mechanical disruption in RPMI medium using a gentleMACS Dissociator (Miltenyi Biotec), purified as described for bone marrow cells, and separated from red cells in ACK. T-cell activation and proliferation assays and measurements of SIV-suppressive activity were performed using freshly isolated cells, and intracellular cytokine assays and tetramer stains were performed using cells frozen viably at -80°C .

Quantification of plasma viral load

Plasma viremia was monitored longitudinally in all animals using quantitative RT-PCR with a limit of detection of 12.3 copies/mL. Viral RNA was prepared from 100 μL of cell-free plasma. Quantitative RT-PCR was performed using a SuperScript III Platinum One-Step qRT-PCR Kit (Thermo Fisher Scientific) with a CFX96 Touch Real-Time PCR Detection System (Bio-Rad).

Each tube contained 12.5 µL of 2X reaction mixture, 0.5 µL of RNaseOUT (40 U/µL), 0.5 µL of Superscript III Reverse Transcriptase/Platinum Taq DNA Polymerase, 1 µL of each primer (125 µM), 0.5 µL of the fluorogenic probe (135 µM), and 10 µL of eluted RNA. Primer/probe sequences were designed to amplify a region of SIVmac251 *gag*. The forward (F) primer sequence was 5'-GCAGAGGAGGAAATTACCCAGTAC-3' (24 bp), and the reverse (R) primer sequence was 5'-CAATTTTACCCAGGCATTTAATGTT-3' (25 bp). The probe sequence was 5'-FAM-TGTCCACCTGCCATTAAGCCCGA-BHQ1-3' (23 bp). This probe had a fluorescent reporter dye, FAM (6-carboxyfluorescein), attached to its 5' end and a quencher, BHQ1 (Black Hole Quencher 1), attached to its 3' end (TaqMan, Applied Biosystems). Samples were heated for 30 min at 56°C and 5 min at 95°C, followed by 50 thermocycles, each comprising 15 sec at 95°C and 1 min at 60°C.

Quantification of SIV-DNA

Total DNA was extracted from purified CD14⁺ alveolar macrophages, buffy coats, and snap-frozen tissues. CD14⁺ alveolar macrophages were purified magnetically via positive selection using CD14 MicroBeads (Miltenyi Biotec). Purity was checked using flow cytometry (Figure S1B). Snap-frozen tissues were mechanically disrupted using a MagNA Lyser (Roche Diagnostics). DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen). SIV-DNA was quantified using an ultrasensitive quantitative real-time PCR. For blood samples, 150,000 cells were analyzed in each PCR. Sample limitations restricted input numbers to 20,000 cells for BALs and 50,000 cells for RBs. All amplifications were performed over 2–4 replicates. The cell line SIV1C, which contains 1 copy of SIV integrated/cell, was used as a standard for quantification. A total of 1 µg of DNA was considered to be equivalent to 150,000 cells. Amplification was performed using primers and a probe located in the *gag* region. The *CCR5*

gene was used to normalize results per million cells. Results were adjusted by the frequencies of CD4⁺ T-cells in blood and tissues, according to availability. The limit of quantification was 2 copies/PCR. Primer and probe sequences were: SIV *gag* F: 5'-GCAGAGGAGGAAATTACCCAGTAC-3'; SIV *gag* R: 5'-CAATTTTACCCAGGCATTTAATGTT-3'; SIV *gag* probe: 5'-FAM-TGTCCACCTGCCATTAAGCCCGA-BHQ1-3'; CCR5 F: an equimolar mix of 5'-CAACATGCTGGTCGATCCTCAT-3' and 5'-CAACATACTGGTCGTCCTCATCC-3'; CCR5 R: 5'-CAGCATAGTGAGCCCAGAAG-3'; and CCR5 probe: 5'-HEX-CTGACATCTACCTGCTCAACCTG-BHQ1-3'.

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Viral reactivation in autologous CD4⁺ T-cells

Autologous CD4⁺ T-cells were purified magnetically from freshly isolated PBMCs using an EasySep CD4 Positive Selection Kit with an automated RoboSep (StemCell Technologies). Purified CD4⁺ T-cells were stimulated for 3 days with concanavalin A (5µg/mL, Sigma-Aldrich) in the presence of IL-2 (100 IU/mL, Miltenyi Biotec). Stimulated CD4⁺ T-cells (10⁵) were cultured in R10 medium containing IL-2 (100 IU/mL, Miltenyi Biotec). The production of SIV p27 was measured in culture supernatants on day 7 using an SIV p27 Antigen ELISA Kit (Zeptometrix).

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Measurement of T-cell activation and proliferation

T-cell activation and proliferation were assessed using fresh PBMCs and tissue cell suspensions. Blood samples were treated with FACS Lysing Solution (BD Biosciences). Cells were surface stained for CD3, CD4, CD8, CD38, CD45, CCR5, and HLA-DR, fixed/permeabilized using a Cytofix/CytoPerm Kit (BD Biosciences), and stained intracellularly for Ki-67. The following antibodies used were: anti-CD3-PE (clone SP34-2, BD

1131 Biosciences), anti-CD4–PerCP-Cy5.5 (clone L200, BD Biosciences), anti-CD8–BV650 (clone
1132 RPA-T8, BioLegend), anti-CD38–FITC (clone AT-1, StemCell Technologies), anti-CD45–V500
1133 (clone D058-1283, BD Biosciences), anti-CCR5–APC (clone 3A9, BD Biosciences), anti-HLA-
1134 DR–APC-H7 (clone G46-6, BD Biosciences), and anti-Ki-67–AF700 (clone B56, BD
1135 Biosciences). Data were acquired using an LSRII flow cytometer (BD Biosciences) and
1136 analyzed with FlowJo software version 10 (Tree Star Inc.).

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1138 ***Intracellular cytokine staining***

1139 Frozen PBMCs, PLN cells, bone marrow cells, splenocytes, and MLN cells were thawed,
1140 resuspended at 1×10^6 /mL in R20 medium, and stored overnight at 37°C. Cells were then
1141 stimulated with a pool of 24 optimal SIV peptides (8–10 amino acids, 2 µg/mL each, Table
1142 S3) or with a pool of 125 overlapping SIV Gag 15mer peptides (SIVmac239 Gag Peptide Set
1143 #12364, 2 µg/mL each, NIH AIDS Reagent Program) in the presence of anti-CD28 (1 µg/mL,
1144 clone L293, BD Biosciences) and anti-CD49d (1 µg/mL, clone 9F10, BD Biosciences) and
1145 stained with anti-CD107a (clone H4A3, BD Biosciences) for 30 min prior to the addition of
1146 GolgiStop (1 µL/mL, BD Biosciences) and brefeldin A (BFA, 5 µg/mL, Sigma-Aldrich).
1147 Costimulatory antibodies alone were used as a negative control, and concanavalin A (5
1148 µg/mL, Sigma-Aldrich) was used as a positive control. Cells were incubated for a total of 6 hr.
1149 After washing, cells were surface stained for CD3, CD4, and CD8, fixed/permeabilized using a
1150 Cytofix/CytoPerm Kit (BD Biosciences), and stained intracellularly for IFN γ , TNF α , and IL-2.
1151 The following antibodies were used: anti-CD107a–V450 (clone H4A3, BD Biosciences), anti-
1152 CD3–AF700 (clone SP34-2, BD Biosciences), anti-CD4–PerCP-Cy5.5 (clone L200, BD
1153 Biosciences), anti-CD8–APC-Cy7 (clone RPA-T8, BD Biosciences), anti-IFN γ –PE-Cy7 (clone
1154 B27, BD Biosciences), anti-IL-2–PE (clone MQ1-17H12, BD Biosciences), and anti-TNF α –PE-

1155 CF594 (clone Mab11, BD Biosciences). Data were acquired using an LSRII flow cytometer (BD
1156 Biosciences) and analyzed with FlowJo software version 10 (Tree Star Inc.). Results were
1157 corrected for background by subtracting the negative (no peptide) control from the peptide
1158 stimulated response. Negative responses were given an arbitrary value of 0.001.

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1160 ***MHC class I tetramer staining***

1161 Biotinylated complexes of Nef RM9 (RPKVPLRTM)–Mafa A1*063:02, Gag GW9
1162 (GPRKPIKCW)–Mafa A1*063:02, and Vpx GR9 (GEAFEWLNR)–Mafa B*095:01 were produced
1163 as described previously (Price et al., 2005). The corresponding tetramers were generated via
1164 the stepwise addition of APC-conjugated streptavidin (Thermo Fisher Scientific). Frozen
1165 PBMCs were stained with all tetramers simultaneously for 30 min at 37°C, washed, and
1166 surface stained for CD3, CD4, CD8, CD14, CD20, CD27, CD45RA, CCR7, HLA-DR, and CD127.
1167 Cells were then fixed/permeabilized using a Cytofix/CytoPerm Kit (BD Biosciences) and
1168 stained for T-bet. The following antibodies were used: anti-CD3–AF700 (clone SP34-2, BD
1169 Biosciences), anti-CD4–PerCP-Cy5.5 (clone L200, BD Biosciences), anti-CD8–APC-Cy7 (clone
1170 RPA-T8, BD Biosciences), anti-CD14–BV786 (clone M5E2, BD Biosciences), anti-CD20–BV786
1171 (clone L27, BD Biosciences), anti-CD27–PE (clone M-T271, BD Biosciences), anti-CD45RA–PE-
1172 Cy7 (clone 5H9, BD Biosciences), anti-CCR7–PE-Dazzle594 (clone G043H7, BioLegend), anti-
1173 HLA-DR–Pacific Blue (clone G46-6, BD Biosciences), anti-CD127–FITC (clone MB15-18C9,
1174 Miltenyi Biotec), and anti-T-bet–BV711 (clone 4B10, BioLegend). Data were acquired using
1175 an ArianIII flow cytometer (BD Biosciences) and analyzed with FlowJo software version 10
1176 (Tree Star Inc.). MFI stands for median fluorescence intensity.

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1178 ***TCF-1 staining***

1179 Frozen splenocytes were stained with tetramers as described above, washed, and surface
1180 stained for CD3, CD4, CD8, CD14, CD20, CD27, CD45RA, CCR7, and CXCR5. Cells were then
1181 fixed/permeabilized using a Transcription Factor Buffer Set (BD Biosciences) and stained for
1182 TCF-1. The following antibodies were used: anti-CD3–AF700 (clone SP34-2, BD Biosciences),
1183 anti-CD4–PerCP-Cy5.5 (clone L200, BD Biosciences), anti-CD8–APC-Cy7 (clone RPA-T8, BD
1184 Biosciences), anti-CD14–BV786 (clone M5E2, BD Biosciences), anti-CD20–BV786 (clone L27,
1185 BD Biosciences), anti-CD27–BUV395 (clone M-T271, BD Biosciences), anti-CD45RA–PE-Cy7
1186 (clone 5H9, BD Biosciences), anti-CCR7–PE-Dazzle594 (clone G043H7, BioLegend), anti-
1187 CXCR5–BV710 (clone RF8B2, BD Biosciences), and anti-TCF-1–PE (clone S33-966, BD
1188 Biosciences). Data were acquired using an ArianII flow cytometer (BD Biosciences) and
1189 analyzed with FlowJo software version 10 (Tree Star Inc.).

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1191 ***Measurement of SIV-suppressive activity***

1192 Autologous CD4⁺ and CD8⁺ T-cells were purified from freshly isolated PBMCs or tissue cell
1193 suspensions by positive and negative selection, respectively, using the relevant EasySep Kits
1194 with an automated RoboSep (StemCell Technologies). Purified CD4⁺ T-cells were stimulated
1195 for 3 days with concanavalin A (5μg/mL, Sigma-Aldrich) in the presence of IL-2 (100 IU/mL,
1196 Miltenyi Biotec). Purified CD8⁺ T-cells were cultured in the absence of mitogens and
1197 cytokines (*ex vivo* CD8⁺ T-cells). Stimulated CD4⁺ T-cells (10⁵) were superinfected in U-
1198 bottom 96-well plates with SIVmac251 (MOI = 10⁻³) in the presence (1:1 effector-to-target-
1199 cell ratio) or absence of *ex vivo* CD8⁺ T-cells (10⁵) from the same tissue via spinoculation for 1
1200 hr (1,200 g at room temperature) and incubated for 1 hr at 37°C. Purified CD4⁺ and CD8⁺ T-
1201 cells were separated in some assays using HTS Transwell-96 Permeable Supports with 0.4-
1202 μm Pore Polycarbonate Membranes (Corning). Cells were then washed and cultured in R10

1203 medium containing IL-2 (100 IU/mL, Miltenyi Biotec). Culture supernatants were assayed on
1204 day 7 using an SIV p27 Antigen ELISA Kit (Zeptometrix). Suppression of autologous virus was
1205 assessed similarly without superinfection. Antiviral activity was calculated as log₁₀ (mean
1206 p27 ng/mL in SIV-infected CD4⁺ T-cell cultures without *ex vivo* CD8⁺ T-cells) / (mean p27
1207 ng/mL in SIV-infected CD4⁺ T-cell cultures with *ex vivo* CD8⁺ T-cells) (Saez-Cirion et al., 2010).

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1209 **QUANTIFICATION AND STATISTICAL ANALYSIS**

1210 Data visualization was performed using Tableau version 2018.1.4 (Tableau Software).
1211 Statistical analyses were performed using Prism version 8.1.2 (GraphPad Software) and
1212 SigmaPlot version 12.5 (SYSTAT Software). Results are presented as median ± IQR. Groups
1213 were compared using the Mann-Whitney U-test. Correlations were assessed using Spearman
1214 rank analyses. No adjustments were made for multiple comparisons, given the exploratory
1215 nature of the analyses. All p values less than 0.05 were defined as significant.

1216

Figure 1

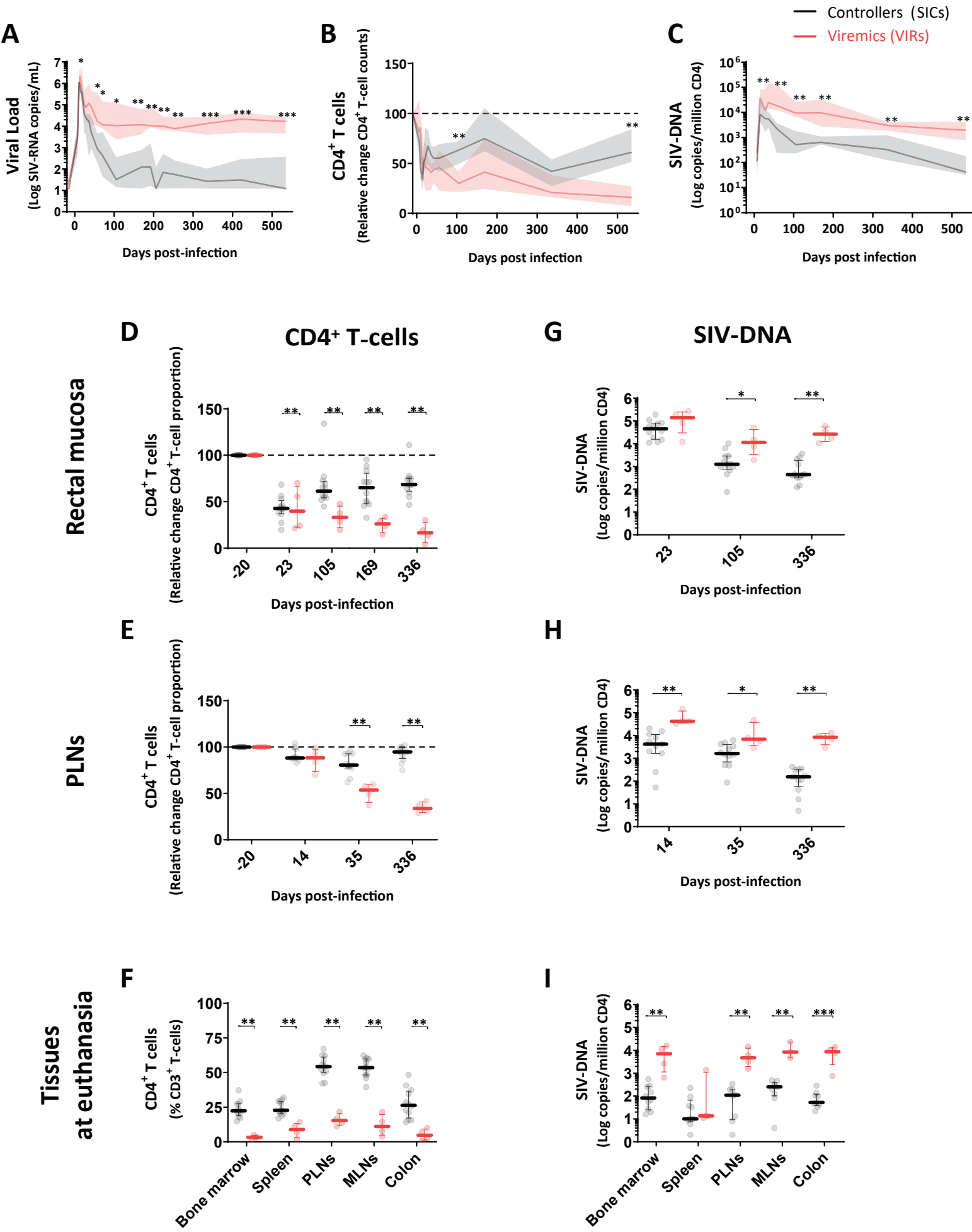


Figure 2

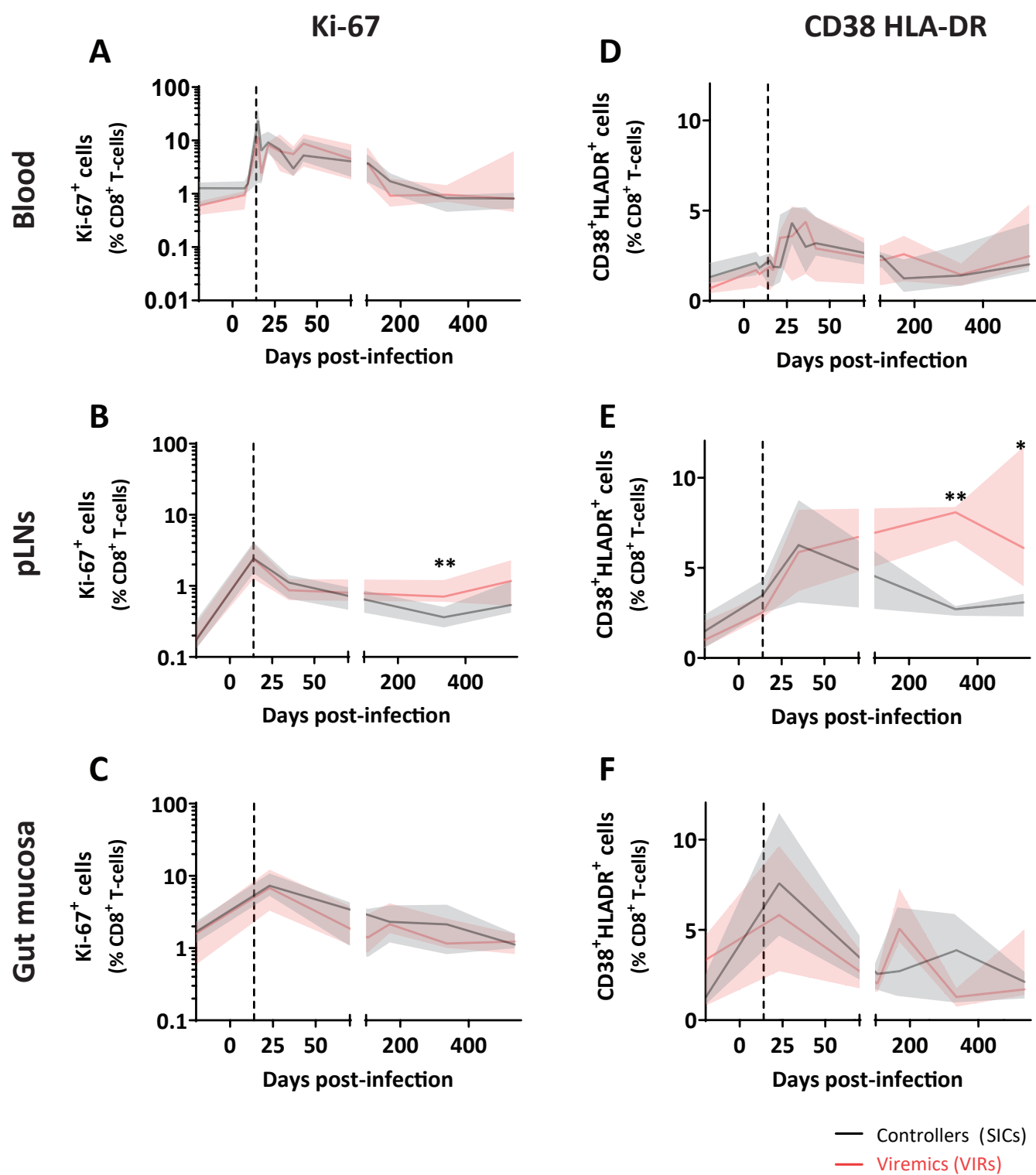


Figure 3

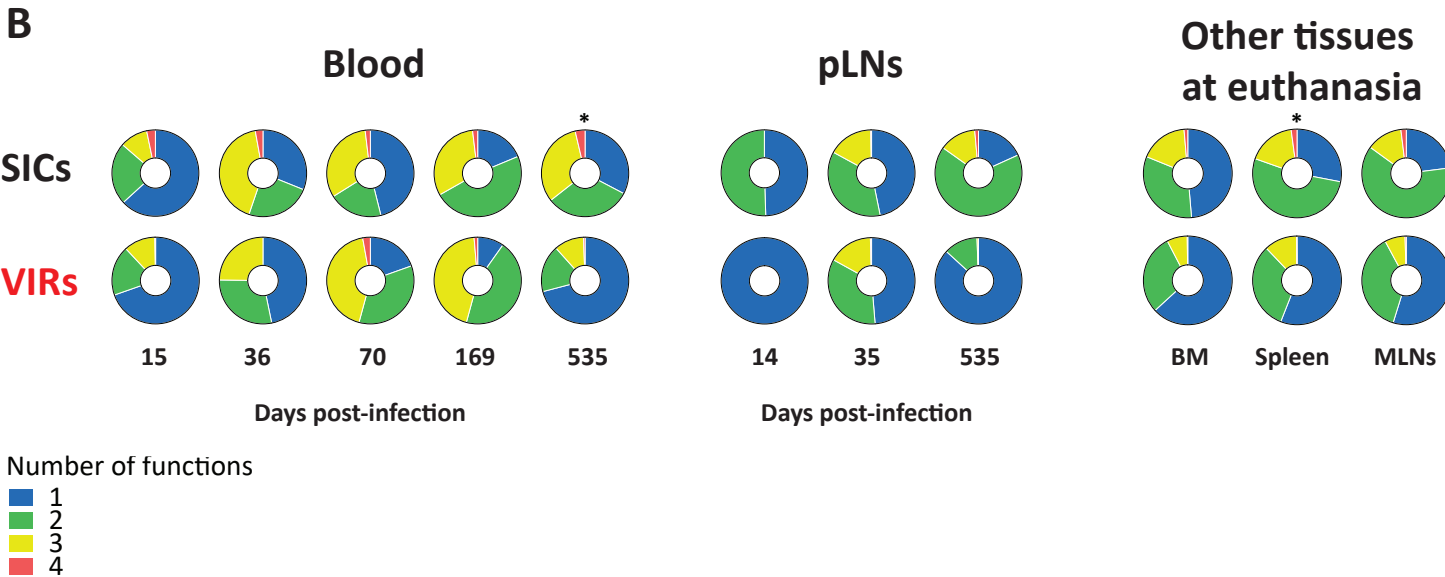
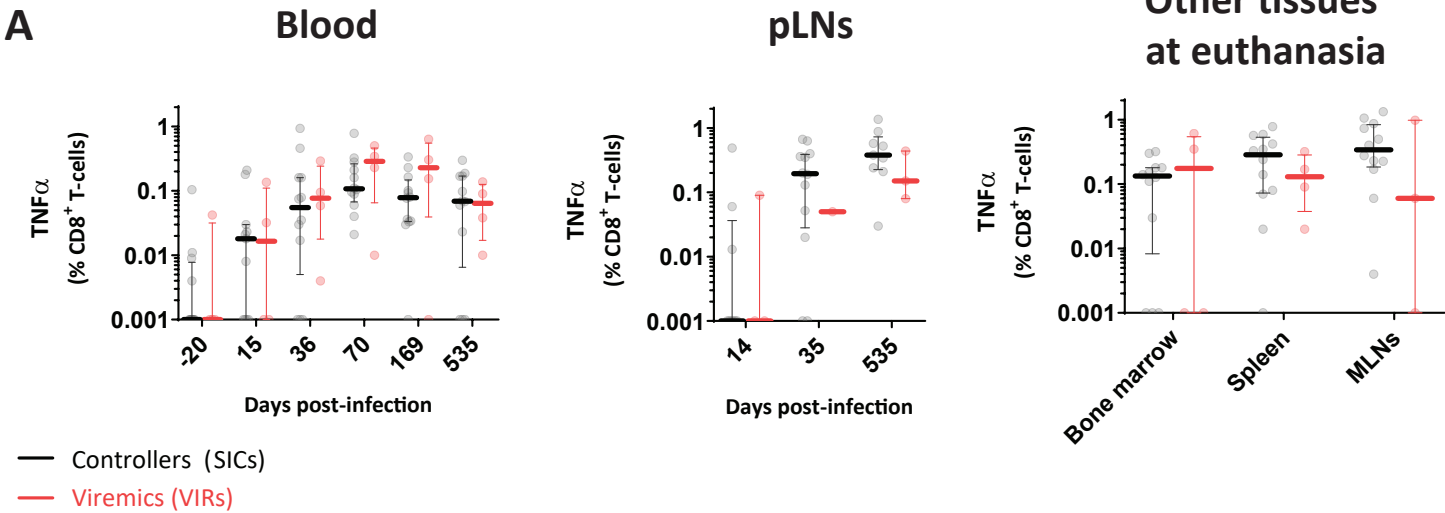


Figure 4

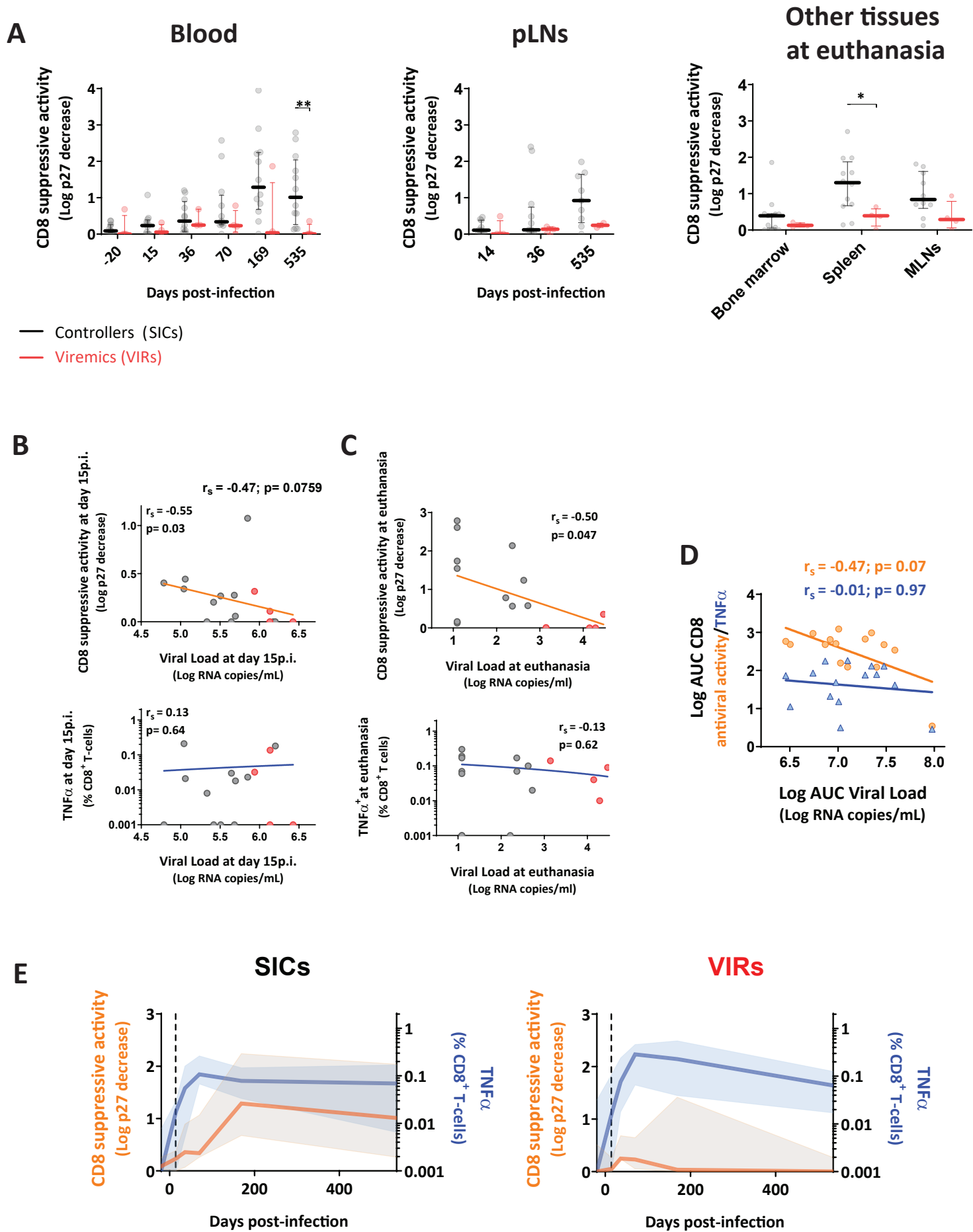


Figure 5

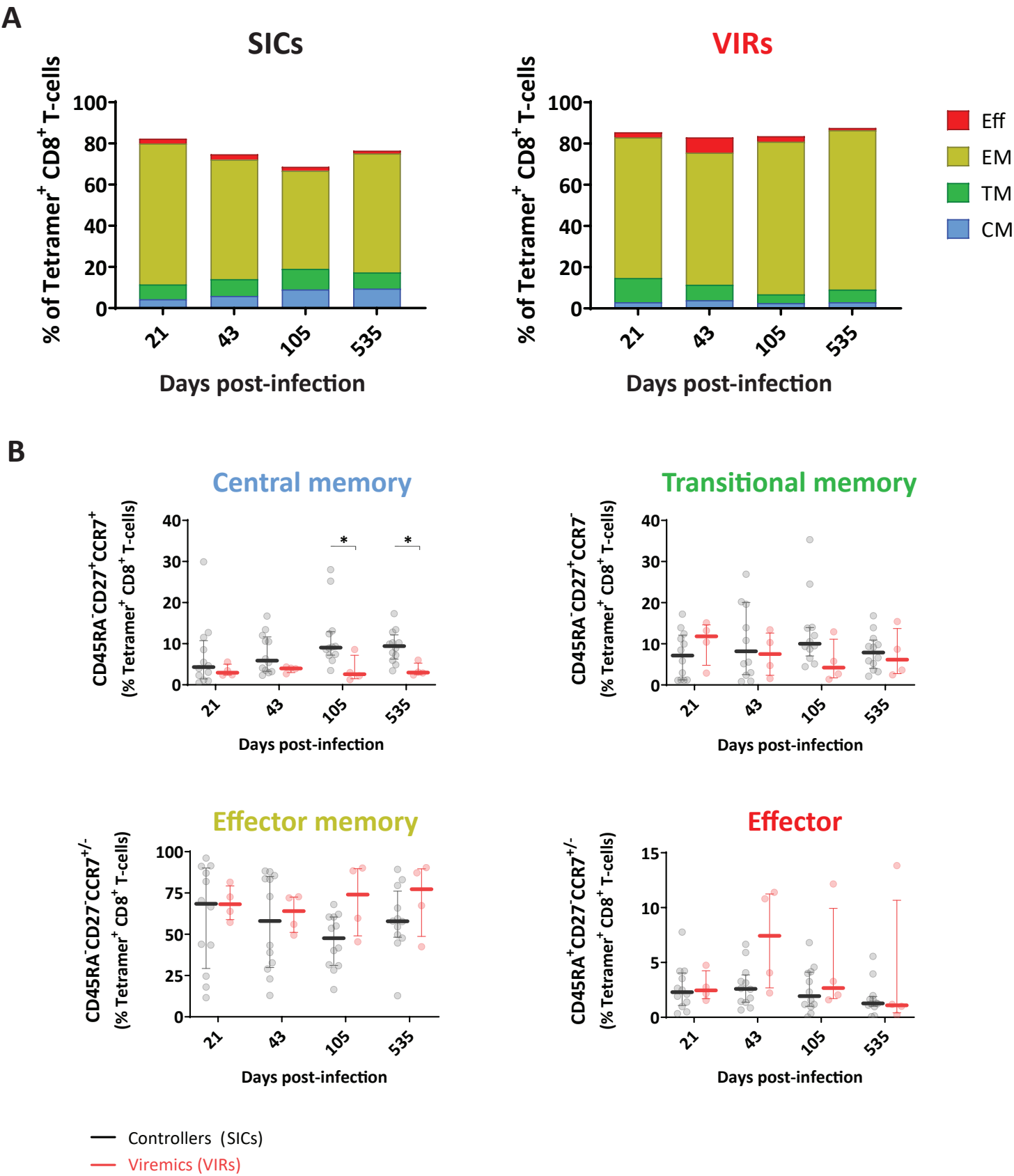


Figure 6

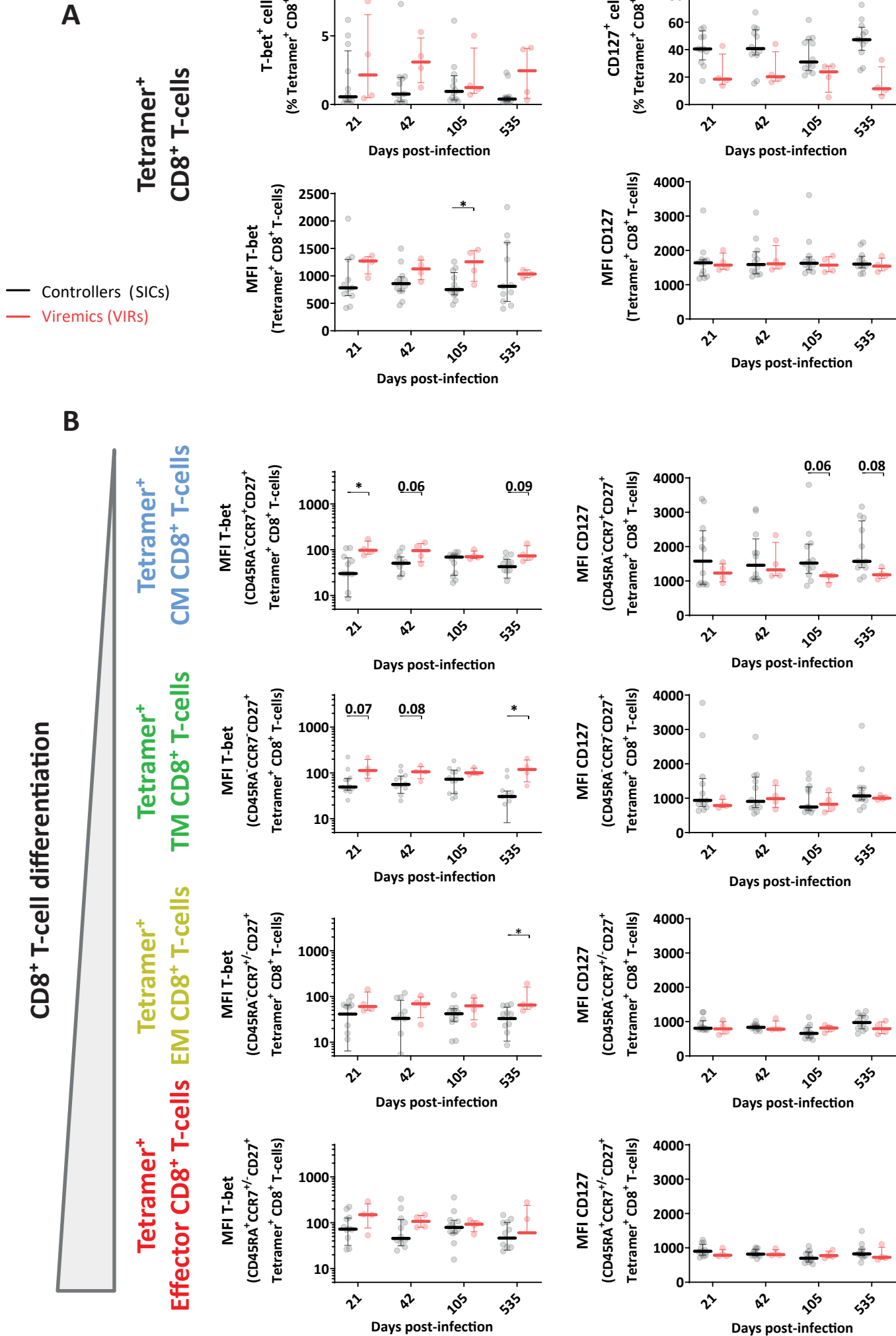


Figure 7

