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1 **Early antiretroviral therapy favors post-treatment SIV control associated with the**
2 **expansion of enhanced memory CD8⁺ T-cells**

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25 **Abstract**

26 HIV remission can be achieved in some people, called post-treatment HIV controllers, after
27 antiretroviral treatment discontinuation. Treatment initiation close to the time of infection
28 was suggested to favor post-treatment control, but the circumstances and mechanisms
29 leading to this outcome remain unclear. Here we evaluate the impact of early (week 4) vs. late
30 (week 24 post-infection) treatment initiation in SIVmac₂₅₁-infected male *cynomolgus*
31 *macaques* receiving 2 years of therapy before analytical treatment interruption. We show that
32 early treatment strongly promotes post-treatment control, which is not related to a lower
33 frequency of infected cells at treatment interruption. Rather, early treatment favors the
34 development of long-term memory CD8⁺ T cells with enhanced proliferative and SIV
35 suppressive capacity that are able to mediate a robust secondary-like response upon viral
36 rebound. Our model allows us to formally demonstrate a link between treatment initiation
37 during primary infection and the promotion of post-treatment control and provides results
38 that may guide the development of new immunotherapies for HIV remission.

39 **INTRODUCTION**

40 Despite its unquestionable success, antiretroviral therapy (ART) cannot completely eliminate
41 HIV reservoirs, which remains a major barrier to HIV eradication. As a consequence, a rapid
42 viral rebound is observed in most persons living with HIV (PLWH) when ART is interrupted ¹⁻³.
43 Nevertheless, in a very limited number of individuals designated post-treatment HIV
44 controllers (PTCs), viremia remains suppressed for prolonged periods after ART withdrawal ^{1,4-}
45 ¹⁵.

46

47 Identifying immunological or virological signatures that could predict the time to viral rebound
48 after stopping ART and understanding the mechanisms leading to post-treatment control is a
49 priority to guide the development of innovative strategies for sustained HIV remission. Several
50 markers, such as low cell-associated HIV DNA levels, high CD4/CD8 ratios, loss of functional
51 plasmacytoid dendritic cells or low expression of immune checkpoint molecules, have been
52 associated with delayed viral rebound after ART discontinuation ^{4,13,16-19}, and distinctive
53 transcriptomic and metabolomic signatures have been observed in post-treatment controllers
54 ^{20,21}. However, the mechanisms underlying durable HIV control after ART interruption remain
55 elusive.

56

57 The VISCONTI study provided the first comprehensive description of the virological and
58 immunological features of PTCs and suggested that the immunological mechanisms driving
59 post-treatment control might differ from those observed in natural HIV controllers ¹³. The
60 VISCONTI study indicated that ART initiation during the first weeks following infection and
61 then maintenance for several years might favor post-treatment HIV control. Indeed, ART was
62 initiated in a higher frequency of PTCs during primary HIV infection (PHI) ^{5,7,8,10}, although ART

63 was initiated in some PTCs during the chronic phase ^{4,15}. The CHAMP study confirmed a higher
64 frequency of post-treatment control among individuals who were treated during early
65 infection compared to those treated during chronic infection ¹¹. Early ART initiation limits the
66 seeding of viral reservoirs and viral evolution, diminishes residual inflammation, protects
67 against tissue damage and preserves immune responses ²²⁻²⁶. However, viremia control upon
68 treatment discontinuation is observed in only a fraction of PLWH with early ART
69 administration, and ART initiation during the earliest stages of infection does not impede viral
70 rebound after treatment interruption ²⁷. It remains unclear whether there is a window of
71 opportunity for ART initiation that may limit the extent of seeding of HIV reservoirs without
72 restraining the maturation of the developing HIV-specific immune response that may be later
73 important for post-treatment control. The retrospective observational nature of cohort
74 studies, the relatively low number of individuals with long-term post-treatment control status,
75 an important level of heterogeneity regarding the time to ART initiation and ART regimens,
76 the duration of antiretroviral treatment prior to treatment interruption, and the criteria for
77 defining PTCs among the different cohorts have hampered the evaluation of the role of early
78 ART and the factors that lead to post-treatment control ²⁸.

79
80 Nonhuman primate (NHP) models of SIV infection closely recapitulate events occurring after
81 HIV infection in humans, including the dynamics of viral reservoir seeding, development of
82 immune responses and evolution upon antiretroviral treatment initiation ²⁹⁻³³. NHP studies
83 have provided valuable insights into the factors associated with HIV pathogenesis and control
84 and potential strategies aimed at delaying viral rebound after analytical treatment
85 interruption (ATI) ^{26,34-37}. NHP models offer a unique opportunity to study in homogeneous
86 experimental conditions the impact of early initiation of multiyear ART, which was

87 documented among the PTCs of the VISCONTI study ¹³. We therefore explored here
88 (pVISCONTI study) how early (week 4 post-infection, roughly the median delay to ART
89 initiation that we had observed in the VISCONTI study) vs. late (week 24 post-infection)
90 initiation of ART impacted the outcome after treatment discontinuation in *cynomolgus*
91 *macaques* (CyMs, *Macaca fascicularis*) infected with SIVmaC₂₅₁, and we performed extensive
92 characterization of the virological and immunological features before and after ART. We
93 showed that early ART initiation strongly favored durable post-treatment SIV control in this
94 model, which was associated with mobilization of memory CD8⁺ T cells with enhanced antiviral
95 capacities against the rebounding virus.

96

97 **RESULTS**

98 ***Early ART initiation favored delayed viral rebound and a high frequency of post-treatment***

99 ***SIV controllers***

100 To assess the impact of early versus late ART initiation on viral control after treatment
101 interruption, we monitored the kinetics of SIVmac₂₅₁ replication in CyMs in which ART was
102 initiated either at 4 weeks p.i. (n=2x6) or at 24 weeks p.i. (n=2x6). After 24 months of ART,
103 treatment was interrupted, and animals were monitored over 24 to 48 weeks (Figure S7). As
104 expected, all animals exhibited a primary SIV infection with a high plasma viral load (pVL) that
105 peaked at 10 days p.i. (6.8 [6.5-7.0] log SIV-RNA copies/mL) (Figure 1A). No differences in peak
106 viremia were observed between the animals in the early- and late-treatment groups (p=0.11)
107 (Figure 1A and 1C). Similar early viral dynamics were observed in CyMs (n=17) infected under
108 the same experimental conditions and that never received ART (Figure S7C). In the W4 group,
109 ART was initiated during the declining phase of initial viremia, while in animals in the W24
110 group, pVL was already stable at the time of ART initiation (Figure 1A, Figure S7). Nevertheless,
111 pVL did not differ overall between early- (5.1 [4.5-5.4] log SIV-RNA copies/mL) and late- (4.4
112 [3.7-5.7] log SIV-RNA copies/mL) treated macaques (p=0.4) at the time of ART initiation (Figure
113 1C). One animal (CB296A) from the early-treated group that was euthanized before ART
114 following severe acute infection and one animal (BA979I) from the late-treated group with
115 controlled viremia below 400 copies/mL before ART initiation (Figure S7) were excluded from
116 further analyses. The initiation of ART efficiently suppressed viremia, and the time to achieve
117 undetectable pVL levels (defined as at least 3 consecutive pVL measurements <10 SIV RNA
118 copies/ml) was comparable between groups (112 days [48-167] for W4-treated CyMs; 64 days
119 [28-169] for W24-treated CyMs, p=0.47). In one of the animals treated at W24 (BB425F), the
120 pVL remained low but detectable despite ART (<100 SIV RNA copies/ml of plasma). pVL was

121 undetectable during ART in all the other animals with some sporadic low-level blips (Figure
122 S7). The pVL at the time of ATI was similar between early- and late-treated animals ($p=1$)
123 (Figure 1C). No differences were observed between the W4- and W24-treated macaques in
124 the levels of cell-associated SIV RNA in blood or peripheral lymph nodes (PLNs) at this time
125 (Figure S8).

126

127 Following treatment interruption, significant differences in pVL dynamics were observed
128 between the W4- and W24-treated groups. Overall, viral rebound with a rapid increase in pVL,
129 reaching levels higher than 1,000 copies/mL, was observed in all animals, except for two early-
130 treated CyMs exhibiting maintenance of low viremia (CCB065 and BB9I) (Figure 1B and 1D).
131 The time to rebound (defined as the first pVL measurement higher than 400 copies/mL) was
132 delayed by two weeks in the W4-treated animals (median time to viral rebound 28 days post-
133 ATI) when compared with the W24-treated group (median 14 days post-ATI) (Figure 1B,D and
134 G) ($p=0.0003$). Moreover, significant differences in the magnitude of pVL were observed
135 between the early- and late-treatment groups at all time points following viral rebound post-
136 ATI (Figure 1D). After ART interruption, late-treated CyMs exhibited a higher peak of pVL (all
137 reaching $pVL > 10,000$ copies/mL) ($p=0.0066$) (Figure 1E) and were exposed to higher levels of
138 cumulative viremia ($p=0.004$) (Figure 1F) than early-treated CyMs. The levels of pVL
139 progressively declined in most early-treated macaques but remained relatively stable and high
140 for most late-treated animals (Figure 1D). Before the end of the study, 9 out of 11 (82%) W4-
141 treated animals exhibited controlled pVL levels below 400 SIV-RNA copies/mL, indicating the
142 status of post-treatment controllers (PTC), whereas this was the case for only 2/11 (18%) W24-
143 treated animals (Figure 1G) ($p=0.008$). All PTCs had at least two pVL measurements of <400
144 copies RNA/ml after viral rebound control, and all, except BA736J (last $pVL=514$ RNA/ml),

145 exhibited pVL maintenance of <400 copies until the end of the study. Of note, among the 17
146 untreated CyMs that were studied in parallel, only 2 (12%) naturally exhibited a pVL of < 400
147 copies/ml, while the 15 others stably maintained high-level viremia ($>10^4$ copies/ml) (Figure
148 1G, Figure S7). Although animals carrying the M6 MHC haplotype, strongly favoring natural
149 SIV control in this model (e.g., ³⁶), were not included in the pVISCNTI study, we cannot
150 exclude that other immunogenetic factors may have influenced the outcome of treatment
151 interruption. However, the early- and late-treated groups of this study were matched based
152 on their MHC haplotypes, and therefore, the overall differences in the outcomes of the groups
153 were predominantly related to the delay in treatment initiation. Our results showed that 2
154 years of ART initiated at W4 p.i. strongly enhanced the probability of post-treatment SIV
155 control when compared to ART initiated at W24 or to the rates of spontaneous SIV control in
156 this model.

157

158 ***Early treatment leads to lower SIV reservoir levels and preservation of CD4⁺ T cells after***
159 ***treatment interruption***

160 We explored whether the time of ART initiation influenced CD4⁺ T-cell counts or cell-
161 associated SIV-DNA levels before ART interruption, which could explain differences in viral
162 rebound.

163

164 CD4⁺ T-cell-associated SIV-DNA levels closely mirrored the dynamics of plasma viremia. High
165 levels of CD4⁺ T-cell-associated SIV-DNA were observed during primary SIV infection in the
166 blood of W4- and W24-treated CyMs (Figure 2A). As expected, SIV-DNA levels in CD4⁺ T cells
167 were high at treatment initiation in the W4 macaques since these animals were still in the
168 transition phase between primary and chronic infection. Consequently, lower levels were

169 found at the time of ART initiation in the blood and peripheral lymph nodes (PLNs) of W24
170 macaques (Figure 2A,B). However, no difference was observed in SIV-DNA levels between
171 early- and late-treated macaques at the time of ART interruption (Figure 2A,B). Following ART
172 discontinuation, an increase in SIV-DNA levels was observed, particularly in W24-treated
173 animals, which, at the end of the study, tended to have higher levels of SIV-DNA in blood,
174 mesenteric and inguinal lymph node cells than W4-treated animals (Figure 2A-C). To take into
175 account the potential influence of the animals with a divergent outcome within the W4- and
176 W24-treated groups, we compared the PTC and non-PTC animals after ART discontinuation
177 irrespective of the time of ART initiation (Figure S9A-D). No significant differences were
178 observed in the levels of CD4⁺ T-cell-associated SIV-DNA between PTCs or non-PTCs during
179 primary infection or before ART interruption in blood or lymph nodes (Figure S9E,F). SIV-DNA
180 levels strongly increased in non-PTCs after ART interruption, reaching levels similar to those
181 found before ART initiation, while the levels in PTCs remained close to those found during ART
182 (Figure S9E-G).

183

184 Similarly, no differences were found in the dynamics of CD4⁺ T-cell counts at primary SIV
185 infection between animals in the W4- and W24-treated groups (Figure 2D,E). In blood, the
186 nadir CD4⁺ T-cell counts were observed at 7 days p.i. A trend toward lower CD4⁺ T-cell counts
187 in blood and a lower proportion of CD4⁺ T cells in PLNs were observed in the late-treated group
188 at the time of ART initiation. However, no difference was observed between the groups at the
189 time of ATI in blood and in PLNs (Figure 2D,E). Following ATI, CD4⁺ T-cell counts did not
190 decrease within the first weeks; rather, a gradual decline was observed in late-treated
191 animals, resulting in significant differences between groups at week 24 post-ATI in blood
192 (Figure 2D), in PLNs (Figure 2E) and in other lymphoid and nonlymphoid tissues analyzed at

193 the time of euthanasia (BM, MLN, spleen, colonic mucosa, and BAL) (Figure 2F). Overall, higher
194 CD4⁺ T-cell levels in blood and tissues were maintained in PTCs at the end of the study,
195 irrespective of the time of treatment initiation. This was related neither to differences
196 occurring during primary infection nor at the time of ART interruption in PTC versus non-PTC
197 macaques (Figure S9H-J).

198

199 We further characterized the impact of early vs. late ART initiation on the T-cell compartment
200 by analyzing the CD4/CD8 ratio and the frequency of central memory (CM) CD4⁺ T cells in
201 blood and tissues, two parameters that have been suggested to be associated with post-
202 treatment control in human cohorts and NHPs in previous studies ^{13,17,38}. As expected, an
203 inversion of the CD4/CD8 ratio was observed upon SIV infection in all animals (Figure 2G). The
204 ratio was normalized after two years on ART independent of the time of ART initiation, and no
205 differences were found between the W4- and W24-treated groups at the time of ATI.
206 However, a significant decrease was noted in the W24-treated group upon ART
207 discontinuation, resulting in a lower CD4/CD8 ratio in W24- vs. W4-treated animals at the end
208 of the study in the lymph nodes, spleen and colon (Figure 2H). Likewise, higher frequencies of
209 CM CD4⁺ T cells were detected in early-treated animals in blood and tissues at the end of the
210 study (Figure 2I).

211

212 Collectively, our results show that PTCs, which significantly predominated in macaques with
213 ART initiation early during SIV infection (W4), were characterized by durable maintenance of
214 low SIV-DNA levels and pananatomical preservation of the CD4⁺ T-cell compartment when
215 compared to non-PTCs, which predominated in the group of macaques with ART initiation in

216 established chronic infection (W24). These differences were absent at the time of ART
217 interruption.

218

219 ***Magnitude of the anti-gp140 antibody response after exposure to viremia***

220 We next explored whether early ART initiation in CyMs may have influenced the maturation
221 of adaptive immune responses developing during primary infection, which may have later
222 facilitated immune control of viral rebound. Hence, we first determined the levels of anti-SIV
223 Env antibodies over time in the plasma of W4- and W24-treated macaques (Figure 3A). Weak
224 anti-gp140 IgG titers were comparably detected in early- and late-treated macaques at week
225 4 p.i. (Figure 3A,B). The production of anti-SIV Env IgGs was halted by ART initiation in the
226 animals treated at this time, while the titers continued to increase in the W24-treated animals
227 (Figure 3A). ART initiation led to a drop in anti-gp140 IgG titers in all animals, but higher IgG
228 titers were detected among the W24-treated animals prior to treatment interruption
229 ($p=0.0032$) (Figure 3B). IgG titers sharply increased following viral rebound after ART
230 interruption in all animals, except for CCB065 (W4-treated group), which did not experience
231 strong viral relapse (Figure 3A). IgG titers increased faster after ATI than following infection in
232 both the W4- and W24-treated macaques (Figure S10). The overall magnitude of the anti-SIV
233 IgG response during the 24 weeks following ATI was significantly higher among the W24-
234 treated macaques than among the W4-treated macaques ($p=0.0018$) (Figure 3C) and higher
235 among non-PTCs than among PTCs irrespective of the time of ART initiation (Figure S9K).
236 Moreover, when all animals were considered together, the IgG titers post-ATI positively
237 correlated with the magnitude of viral rebound ($r=0.6552$; $p=0.0009$ – Figure 3D).

238

239 We did not find significant anti-SIV gp140 IgA levels at week 4 p.i. in either early- or late-
240 treated macaques (Figure 3E). In contrast, anti-gp140 IgA antibodies were detected by week
241 24 p.i. in the late-treated animals (Figure 3F). After two years of ART, anti-SIV Env IgA titers
242 did not differ from baseline levels, and no differences were observed between W4- and W24-
243 treated macaques prior to treatment interruption (Figure 3F). Similar to the anti-gp140 IgG
244 titers, IgA titers increased post-ATI faster than during primary infection (Figure S10), but no
245 difference in the global levels of anti-SIV Env IgA antibodies post-ATI was observed between
246 W4- and W24-treated animals (Figure 3G). As opposed to IgG titers, no correlation was found
247 between the magnitude of anti-gp140 IgA titers post-ATI and the magnitude of viral rebound
248 (Figure 3H).

249

250 Altogether, these results indicate that SIV Env-specific memory B cells persisted during
251 prolonged treatment and were reactivated by viral rebound following treatment interruption,
252 favoring a prompt antibody response per se. Although anti-Env IgG and IgA antibodies were
253 not sufficient to limit viral rebound, further functional analyses are warranted to evaluate the
254 impact of ART initiation on the quality of SIV-specific IgA/IgG responses.

255

256 ***Early treatment enhanced the capacity of CD8⁺ T cells to suppress SIV after ART interruption***

257 We next longitudinally evaluated the functional activity of SIV-specific CD8⁺ T cells by analyzing
258 the *ex vivo* capacity of CD8⁺ T cells to suppress SIV infection in autologous CD4⁺ T cells (Figure
259 4A, Figure S11)³⁹, an approach that has allowed us to reveal the presence of highly efficient
260 CD8⁺ T cells in spontaneous HIV and SIV controllers^{36,40-42}. The SIV suppressive activity of CD8⁺
261 T cells was weak in the blood (Figures 4A and 4B) and in PLNs (Figure 4E) during primary
262 infection. At week 4 p.i., the CD8⁺ T-cell-mediated SIV-suppressive activity in blood did not

263 differ from baseline in either the early- or late-treated groups. These results reflect a limited
264 antiviral capacity for SIV-specific CD8⁺ T cells induced during primary infection, confirming our
265 previous observations in PLWH⁴³ and CyMs infected with SIVmac₂₅₁³⁶. A modest but
266 significant increase in CD8⁺ T-cell-mediated SIV-suppressive activity was observed over
267 months in the W24-treated group (p=0.02) (Figure 4A,B). ART initiation did not immediately
268 impact the capacity of CD8⁺ T cells to suppress SIV infection, but a tendency toward higher
269 CD8⁺ T-cell-mediated SIV-suppressive activity was observed in early-treated macaques before
270 treatment interruption (blood p=0.056), and the difference was more marked in PLNs
271 (p=0.005) (Figure 4B).

272

273 Following treatment interruption, a strong increase in CD8⁺ T-cell-mediated SIV suppressive
274 activity was observed in the W4- and W24-treated macaque groups, either in the blood
275 (Figures 4A,C; Figure S11) or PLNs (Figure 4E). The overall magnitude of the CD8⁺ T-cell-
276 mediated SIV-suppressive activity during the 24 weeks that followed ATI tended to be higher
277 in the early-treated group (p=0.09) (Figure 4D), and the maximum levels of SIV suppressive
278 activity achieved by CD8⁺ T cells after ART interruption were higher during this period for the
279 W4-treated vs. the W24-treated CyMs (Figure 4C). The CD8⁺ T-cell-mediated SIV-suppressive
280 capacity was also higher in PLNs of W4-treated macaques at the end of the study (Figure 4E).
281 A stronger capacity of CD8⁺ T cells to suppress SIV infection was observed in the blood, PLNs
282 and spleen of PTCs when compared to non-PTCs irrespective of time to ART initiation (Figure
283 S9L-N). Finally, when all animals were considered together, the overall magnitude of the CD8⁺
284 T-cell-mediated SIV-suppressive activity post-ATI in blood tended to negatively correlate with
285 the magnitude of viral rebound (r=-0.4; p=0.097 – Figure 4F) or the frequency of CD4 carrying
286 SIV-DNA (r=-0.39; p=0.075 – Figure 4H). A negative correlation was observed between the

287 capacity of CD8⁺ T cells of PLNs to suppress SIV infection at the end of the study and the pVL
288 ($r=-0.55$; $p=0.015$ – Figure 4G) or the frequency of infected cells ($r=-0.56$; $p=0.016$ – Figure 4I)
289 at this timepoint.

290

291 Our results indicate that prolonged ART favored the enhancement of the antiviral potential of
292 CD8⁺ T cells, which were able to exert stronger activities upon viral rebound than during
293 primary infection. Our results indicate that this effect was stronger in PTC macaques, in which
294 CD8⁺ T cells were able to produce superior SIV suppressive activity in blood and lymphoid
295 tissues and were favored by early ART initiation. These results suggest a role for CD8⁺ T cells
296 in limiting viral rebound and the number of infected cells and a potential association with the
297 establishment of post-treatment SIV control.

298

299 ***Interruption of early-initiated ART was followed by the expansion of weakly activated***
300 ***memory CD8⁺ T cells***

301 To better understand the striking differences in the antiviral capacity of CD8⁺ T cells observed
302 during primary infection and those observed in the same animals after ART interruption, we
303 compared the phenotypic characteristics of CD8⁺ T cells during these periods. We first
304 evaluated cell activation as the proportion of CD8⁺ T cells coexpressing CD38 and HLA-DR. In
305 agreement with previous reports in human cohorts and NHP models^{30,36,44,45}, we found very
306 strong CD8⁺ T-cell activation during primary SIV infection in all animals (Figure 5A). The
307 frequency of activated cells remained high and relatively stable in the late-treated macaques,
308 but ART initiation rapidly reduced CD8⁺ T-cell activation in both W4- and W24-treated animals.
309 Upon ART interruption, a rapid increase in CD8⁺ T-cell activation was observed in the W24-
310 treated group, reaching levels similar to those observed during primary infection (Figure 5A,B).

311 In contrast, no or little CD8⁺ T-cell activation was observed for the animals in the W4-treated
312 group (Figure 5A). Lower overall CD8⁺ T-cell activation levels were observed between primary
313 infection (AUC weeks 0-4 p.i.) and post-ATI for early-treated macaques (AUC weeks 0-4 post-
314 ATI), whereas no differences were noticed for the late-treated group (Figure 5B). A trend
315 toward higher CD8⁺ T-cell activation levels was observed in PLNs of W24-treated animals at
316 the end of the study ($p=0.12$) (Figure 5C). No correlation was found between CD8⁺ T-cell
317 activation levels and their capacity to suppress SIV infection post-ATI (Figure 5D).

318
319 High frequencies of proliferating (Ki67⁺) CD8⁺ T cells were also observed during primary SIV
320 infection (Figure 5E). The frequency of these cells decreased with the transition to chronic
321 infection in the animals treated at week 24 p.i., then stayed stable until ART initiation and was
322 drastically reduced following ART initiation in all cases. Ki-67 expression was lower post-ATI
323 than during primary infection, both in W4- and W24-treated animals (Figure 5E,F), but overall
324 higher levels of proliferating CD8⁺ T cells were found early post-ATI in the W4-treated
325 macaques than in the W24-treated macaques in blood ($p=0.004$) (Figure 5F) and PLNs
326 ($p=0.009$) (Figure 5G). Of note, a positive correlation ($r=0.76$, $p=0.006$) was found between the
327 rate of proliferating CD8⁺ T cells after ART interruption and their capacity to suppress SIV
328 infection during the same period (Figure 5H).

329
330 We then investigated differences between primary infection and post-ATI in the CD8⁺ T-cell
331 subpopulations. Primary infection was characterized by the predominant expansion of CD8⁺ T
332 cells with an effector memory (EM) or effector phenotype (Figure 6A,B). In contrast, CD8⁺ T
333 cells with a central memory (CM) phenotype were expanded after treatment interruption in
334 W4-treated macaques, while the proportion of EM and effector CD8⁺ T cells remained low

335 (Figure 6A). A higher proportion of CM CD8⁺ T cells was also found after ART interruption than
336 during primary infection in PLNs of early-treated macaques (Figure 6C). For the animals
337 treated at week 24 p.i., an expansion of EM and effector CD8⁺ T cells was again observed in
338 blood and PLNs after ART interruption (Figure 6B,D). No differences were observed in the
339 frequency of CM CD8⁺ T cells between W4- and W24-treated macaques at the time of ART
340 interruption ($p=0.8$, Figure 6A,B), but CM CD8⁺ T cells were more frequently found in the
341 blood and PLNs of W4-treated macaques 14 days after ATI ($p=0.013$ and $p=0.002$, for CD8⁺ T
342 cells with the CM phenotype in W4- vs. W24-treated macaques in blood and PLNs,
343 respectively). Moreover, higher levels of proliferating cells were found among CM CD8⁺ T cells
344 in the blood and PLNs of W4-treated macaques after ART interruption than in W24-treated
345 macaques (Figure 6E). A positive correlation was found between the proportion of CM CD8⁺ T
346 cells post-ATI in blood (day 28 post-ATI) and PNLs (day 14 post-ATI) and the capacity of CD8⁺
347 T cells to suppress SIV infection at the same time points ($r=0.58$, $p=0.02$; $r=0.72$, $p=0.01$ –
348 Figure S12) or the cumulative SIV suppressive capacity post-treatment interruption ($r=0.49$,
349 $p=0.05$ in blood; $r=0.78$, $p=0.004$ in PNL – Figure 6F). No correlation was found between the
350 capacity of CD8⁺ T cells to suppress SIV infection and the proportion of naïve, CM, transitional
351 memory (TM) or EM CD8⁺ T cells (Figure S12), but a trend toward a negative correlation was
352 found with the proportion of effector CD8⁺ T cells ($r=-0.43$, $p=0.086$).

353

354 Therefore, while strongly activated effector CD8⁺ T cells with limited antiviral potential were
355 expanded during primary infection, early and prolonged ART appeared to promote the
356 preservation or development of CD8⁺ T cells with central memory characteristics that were
357 able to rapidly expand after ART interruption and to exert potent antiviral activities with
358 limited activation.

359

360 **SIV-specific CD8⁺ T cells with enhanced survival and proliferative capacities were mobilized**
361 **after ART interruption in early-treated macaques**

362 We next explored the characteristics of CD8⁺ T cells that specifically reacted to stimulation
363 with pools of SIV peptides *in vitro* (Figure S3). SIV-specific CD8⁺ T cells producing IFN γ , TNF α
364 or IL-2 or expressing CD107 in response to 6 hours of SIV peptide stimulation were detected
365 after primary SIV infection in all animals analyzed (Figure 7A). The frequency of SIV-specific
366 CD8⁺ T cells decreased as expected during ART but progressively increased after viral rebound,
367 and no differences in the magnitude or the polyfunctionality of these cells were observed
368 between W4- and W24-treated animals before ART initiation or after ART interruption (Figure
369 7A,B). No difference was observed either in the magnitude or polyfunctionality of SIV-specific
370 CD8⁺ T cells during primary infection vs. after ART interruption, which could explain the
371 striking increase in the SIV-suppressive activity of CD8⁺ T cells observed for all macaques after
372 ATI (Figure 7A-C). In contrast, we found higher expression levels of the IL-7 receptor (CD127)
373 on SIV-specific CD8⁺ T cells from W4-treated macaques than on W24-treated macaques after
374 ART interruption, suggesting the presence of long-living memory cells in the former (Figure
375 7C). Similarly, no difference was observed in the magnitude of the SIV-specific CD8⁺ T-cell
376 response between PTCs and non-PTCs; however, SIV-specific CD8⁺ T cells from the former
377 expressed higher levels of CD127 at the end of the study (Figure S9O,P).

378

379 Further analysis of the memory phenotype of the SIV-specific CD8⁺ T cells at this time (Figure
380 S4) showed that memory (CD45RA^{neg}) SIV-specific CD8⁺ T cells from the W4-treated
381 macaques were distinguished by higher expression of CCR7 and lower expression of CD39 than
382 cells from W24-treated macaques. We also found a trend toward higher expression of the

383 transcription factor TCF-1, which has been associated with stemness potential, in the cells
384 from the W4-treated macaques (Figure 7D). A higher expression of CCR7 and TCF1 on SIV-
385 specific memory CD8⁺ T cells was even more evident in animals classified as PTCs vs. non-PTCs
386 (Figure S9Q). The cells from non-PTCs were, in contrast, characterized by higher levels of PD-
387 1. These results corroborated the presence after ART interruption of a higher fraction of
388 memory SIV-specific CD8⁺ T cells with long-lived and stem-like traits in the W4-treated
389 animals, while a more differentiated, prone-to-exhaustion profile was present in the cells from
390 the late-treated animals.

391

392 To more precisely evaluate the memory potential of SIV-specific CD8⁺ T cells, we sequentially
393 stimulated circulating CD8⁺ T cells collected from early- and late-treated macaques with SIV
394 peptides *in vitro* at different timepoints in our study and evaluated the proliferative capacity
395 of responding cells in a 6-day-interval experiment (Figure S5). We found that in general, SIV-
396 specific CD8⁺ T cells present after ART interruption in W4-treated macaques showed a higher
397 division and proliferation index than cells from W24-treated macaques (Figure 7E). Moreover,
398 we found that, for the early-treated macaques, the proliferative capacity of SIV-specific CD8⁺
399 T cells was stronger after ART interruption than during primary infection (Figure 7E). We then
400 used Phenograph unsupervised clustering analysis to explore the characteristics of these SIV-
401 specific CD8⁺ T cells. Phenograph analyses identified 7 potential clusters of SIV-specific CD8⁺ T
402 cells (Figure 7F). For W4-treated macaques, the frequency of Cluster 1 cells was decreased,
403 and those of Clusters 2 and 3 were increased post-ATI when compared to pre-ART (Figure 7G).
404 In contrast, no differences in cluster frequencies were observed over time for the W24-treated
405 animals (Figure S6). Cells in Clusters 1 and 3 shared relatively similar expression levels of the
406 CD45RA, CCR7 and CD27 differentiation markers. However, Cluster 1, which was more

407 represented during primary infection in W4-treated macaques, was characterized by higher
408 expression of IFN γ and pS6 (a marker of activation of the mTORC1 pathway), while Cluster 3,
409 which was more frequently present after ART interruption, was characterized by higher levels
410 of CD127 and TNF α (Figure 7H). Cells in Cluster 2 also differed from those in Cluster 1 in terms
411 of high expression of TNF α and low expression of IFN γ , IL-2 or pS6 but expressed higher levels
412 of CD45RA. All three clusters were characterized by the presence of cells with high levels of
413 pAKT (a marker for the activation of the mTORC2 pathway) (Figure 7H). Of note, we have
414 recently associated the capacity to produce TNF α sustained by activation of mTORC2 without
415 upregulation of mTORC1 as a hallmark of CD8⁺ T cells with stemness and memory potential
416 that are frequently found in people with natural control of HIV infection ^{46,47}.

417

418 Collectively, our results indicate that early treatment initiation in SIV-infected CyMs favored
419 the establishment of memory-like SIV-specific CD8⁺ T cells with enhanced proliferative and
420 survival capacity that could mediate an efficient secondary response to rebounding virus after
421 ART interruption. These observations suggest an association between the memory potential
422 of SIV-specific CD8⁺ T cells during ART and the development of post-treatment SIV control.

423

424 **DISCUSSION**

425 In this study, we show that 2 years of ART initiated at week 4 post-infection strongly promoted
426 durable post-treatment SIV control in CyMs infected with SIVmac₂₅₁. This beneficial effect of
427 ART was largely lost when treatment initiation was deferred to 6 months post-infection. This
428 study was specifically designed to assess the impact of early vs. late ART initiation on post-
429 treatment control, and the experimental conditions were informed by our previous
430 observations based on cohorts of PLWH^{13,23,48}.

431

432 The underlying hypothesis of the study was that in the context of early ART introduction,
433 obtaining an equilibrium between limiting the extent and diversity of the viral reservoir and
434 allowing the maturation of immune responses might be critical to achieving viral control after
435 treatment interruption. Previous studies have shown that initiation of treatment during the
436 eclipse phase of infection in macaques (2-4 days p.i.) or hyperacute infection (Fiebig I-II) in
437 PWH drastically limits the extent of viral spread, but it does not preclude viral rebound after
438 treatment interruption^{26,27}. Indeed, an extremely early treatment (before peak viremia is
439 reached) most likely prevents the development of antiviral responses. Therefore, treatment
440 initiation at this point might merely halt the virus and delay primary infection. In contrast, we
441 hypothesized that starting treatment a little later, once immune responses have been primed,
442 might allow an optimal maturation of the responses, in a context of rapidly decreasing viremia,
443 and their preservation, while still impacting the formation of the viral reservoir if treatment is
444 maintained long enough. The delay to treatment initiation (not too soon, not too late) and the
445 length of the treatment would therefore be critical to achieve this balance⁴⁹. We selected 4
446 weeks post-infection for the initiation of early treatment because this period roughly
447 corresponded to the median time to treatment initiation that we had estimated for the post-

448 treatment controllers in the ANRS VISCONTI study ¹³. We planned to treat animals for 24
449 months, as we had also reported that maintaining ART over several years was important to
450 successfully reduce viral reservoirs and achieve immune reconstitution in people in whom
451 treatment was initiated during primary infection ⁴⁸, and we had observed a steady decrease
452 in the frequency of cells carrying HIV-DNA over two years after initiation of early ART ²³. A
453 recent study also reported a continuous decay in the frequency of intact SIV genomes during
454 the first two years of ART in SIV-infected macaques ³¹.

455

456 Our results showed an unprecedented frequency of post-treatment controllers among the
457 animals in which treatment was started at week 4 post-infection. The rate of post-treatment
458 control in our model was higher than previously anticipated from estimations calculated
459 retrospectively in early-treated PLWH ^{10,11,13,50}. However, the incidence of post-treatment
460 control was much higher than the rate of spontaneous SIV control without ART observed in
461 this model under the same experimental conditions. Moreover, the advantage of early ART
462 initiation was evidenced by the striking difference observed in the probability of post-
463 treatment control between groups of macaques differing only by the delay to treatment
464 initiation. The replication of the comparison between W4- and W24-treated animals in two
465 different experimental phases with similar results makes our observation highly robust. Our
466 study therefore formally establishes a role of early ART initiation in posttreatment control.

467

468 Although no predictive marker of post-treatment control has been validated thus far, some
469 studies have suggested that various markers, such as lower viral loads pre-ART, lower HIV-
470 DNA levels or higher CD4/CD8 ratios before ART interruption ^{17,19,38}, might be associated with
471 lower viremia or delayed rebound after ART discontinuation. As expected, all controls and

472 early- and late-treated animals experienced similar primary infection, with equivalent viral
473 load peaks and cell-associated SIV-DNA and CD4⁺ T-cell levels in blood and PLNs. Late-treated
474 animals were exposed longer to uncontrolled viremia and tended to have lower CD4⁺ T cells
475 at the time of ART initiation. However, after two years of ART, no difference was observed in
476 residual viremia, SIV-DNA levels, cell-associated SIV RNA levels, CD4⁺ T-cell counts or the
477 CD4/CD8 ratio between early- and late-treated animals, which could explain their different
478 outcomes. Notably, treatment initiation at 24 weeks post-infection is still relatively early,
479 particularly when considering that ART is initiated years after infection in most PLWH, which
480 may contribute to explaining the normalization of these parameters during ART in both groups
481 of animals. No difference was observed at the time of ART interruption when SIV PTCs were
482 compared to non-PTCs, independent of the delay in ART initiation. Our study nevertheless
483 showed a favorable evolution of post-treatment control, illustrated by low levels of cell-
484 associated SIV-DNA durably maintained after ATI and the preservation of the CD4⁺ T-cell
485 compartment, including a higher frequency of CM CD4⁺ T cells, in blood and lymphoid tissues.
486 These features recapitulate some characteristics of PLWH with durable remission of infection.
487 In contrast, most non-PTCs showed symptomatic progression within months of ART
488 interruption.

489

490 Differences in the outcome of early- and late-treated macaques could be related to changes
491 in the immune responses that were mobilized to counteract viral rebound. Regarding the
492 humoral immune response, we found that anti-Env antibody levels during primary infection
493 were linked to the degree and duration of viral exposure as described in human PTCs⁵¹ but
494 that all rebounding animals exhibited potently increased anti-gp140 IgG/IgA titers within days
495 of viral rebound. The overall magnitude of the IgG response was more pronounced for the

496 animals treated at week 24 p.i., and this could be the result of a combination of a higher
497 frequency of persisting memory B cells primed before ART and superior antigenic stimulation.
498 In contrast, no differences were observed for anti-gp140 IgA titers, globally lower than for
499 IgGs, between early- and late-treated animals after ATI, which may be related to a lower
500 frequency of IgA-memory B cells triggered upon primary infection or with a shorter half-life
501 compared to IgG-memory cells. The quantitative changes in anti-SIV antibody levels observed
502 here are likely linked to the different antigenic stimulation between the groups and may not
503 be directly associated with a protective role. Nonetheless, the neutralizing and Fc-dependent
504 antiviral activities of these antibodies will need to be evaluated to better understand their
505 functional role. Indeed, control of viral rebound has been recently related to the presence of
506 antibodies with neutralizing and Fc-dependent effector activities in some early-treated post-
507 treatment controllers ⁵¹⁻⁵³.

508

509 Finally, our study suggests that CD8⁺ T cells with enhanced antiviral potential may participate
510 in controlling viremia after ART interruption. Although early-induced CD8⁺ T-cell responses
511 contribute to partially decreased viremia and their pressure is reflected by the emergence of
512 escaping viral variants, numerous reports have pointed to the skewed nature and limited
513 cytotoxic capacity of these cells ^{45,54-56}. We reported the lack of viral suppressive capacity *ex*
514 *vivo* of CD8⁺ T cells isolated during primary infection from PWH or SIV-infected macaques ^{36,43}.
515 We previously found that this activity gradually increased over time only in macaques in which
516 viremia was efficiently controlled, namely, those becoming SIV controllers ³⁶. Moreover, a
517 strong capacity of CD8⁺ T cells *ex vivo* to suppress viral infection of autologous CD4⁺ T cells is
518 a characteristic that we have consistently found in HIV-1 controllers and in HIV-2 controllers
519 ^{40,41}. The more frequent occurrence of CD8⁺ T cells with a strong capacity to suppress infection

520 in the context of SIV and HIV-2 infection suggests that viral factors, for instance, allowing
521 better sensing and T-cell priming of SIV/HIV-2, may favor their development ⁵⁷⁻⁵⁹. Here, we
522 show that CD8⁺ T cells from PTC CyMs also showed such a viral suppressive capacity upon ART
523 interruption, enhancing their capacity to counteract viral rebound. The mechanisms
524 underlying this acquisition of the capacity of CD8⁺ T cells to suppress infection remain to be
525 determined but appear to be favored by early ART initiation and could be related to an
526 enhancement of the cytotoxic potential of the cells, an increase in TCR avidity and/or better
527 capacity of CD8⁺ T cells to survive and proliferate in the presence of infected cells ⁶⁰⁻⁶².

528

529 Of note, we found that CD8⁺ T cells expanding in response to primary infection mainly had an
530 effector memory or effector phenotype, while CD8⁺ T cells expanding after treatment
531 interruption in the early-treatment group had a central memory phenotype. These results
532 suggest that early and prolonged treatment favored the establishment of central memory
533 CD8⁺ T cells, which mediated a more efficient, secondary-like response against viral rebound.
534 In contrast, cells expanding in the W24-treated animals after ART interruption still carried
535 mostly an effector profile. We and others have recently associated efficient CD8⁺ T-cell
536 responses in HIV and SIV controllers with the development of memory-like CD8⁺ T cells with
537 stem cell properties ^{36,63,64}. While virus-specific CD8⁺ T cells developed during primary
538 infection do not upregulate molecules associated with long-term memory ^{36,44}, our results in
539 the present pVISCONTI study indicate that early ART introduction may favor the
540 generation/preservation of such cells. These results are consistent with a recent report on
541 early-treated PWH ²⁵. Here, CD8⁺ T cells with long-term memory traits and enhanced
542 proliferative and SIV suppressive capacity mediated a robust response to viral rebound while
543 restraining T-cell activation. These results suggest that immunotherapies aimed at the

544 induction of HIV-specific CD8+ T cells with stem cell-like memory properties might be a
545 promising opportunity to achieve HIV remission.

546

547 Our study has some limitations. This study was not designed to evaluate the effectiveness of
548 the 2-year duration of ART that we used or to precisely identify a window of opportunity for
549 ART initiation that would favor post-treatment control of infection. Previous studies in SIV-
550 infected macaques suggest that this period might start with the first signs of immune
551 activation ³⁵ and last at least several weeks ³⁸. Although the frequency of cells carrying SIV-
552 DNA did not differ between W4- and W24-treated animals at the time of ART interruption in
553 this study, we cannot exclude that early ART initiation impacted the quality, distribution or
554 composition of the viral reservoir, particularly in tissues that were not analyzed here. Many
555 human PTCs appear to consistently maintain undetectable viral loads after ART interruption¹³,
556 while others experience transient episodes of viremia ^{6,11,13,51,65}. In our model, all SIV PTCs
557 except one experienced viral rebound after ATI and may better recapitulate an active control
558 of infection after ART interruption. Some determinants of viral control may differ between
559 macaques and human PTCs. For instance, some immunogenetic traits that have been
560 proposed to favor post-treatment HIV control in PWH ⁶⁶ may be absent in CyMs ⁶⁷. We did not
561 study the impact of innate immune responses (IFN-I, dendritic cells, monocytes, NK cells, etc.)
562 in PTCs. It is not excluded that they play a complementary role in posttreatment control, either
563 by impacting CD8⁺ T-cell function and/or through their antiviral activities. Additional analyses
564 are ongoing to study the impact of early- and late-ART initiation on the innate immune
565 response. Nevertheless, our study showed that post-treatment control, as reported for PWH
566 ^{13,16}, was not related to the presence of protective MHC traits associated with natural control
567 of infection (e.g., HLA-B*27 or B*57 alleles in PWH or MHC-M6 haplotype in CyMs).

568

569 In summary, we formally demonstrate in our model a direct role of ART initiation during
570 primary infection in the promotion of post-treatment SIV control. While the CyM model might
571 not recapitulate all traits present in humans, further studies using this model should provide
572 new insights into the virological and immunological events leading to post-treatment control
573 of infection. Of note, our analyses identified key features in SIV-specific CD8⁺ T cells shaped
574 under early- vs. late-ART that may underlie a different capacity to respond to viral rebound.
575 These results thus pave the way for improving CD8⁺ T-cell-based immunotherapies toward
576 remission of HIV.

577

578 **METHODS**

579 ***Ethical statement***

580 CyMs were imported from Mauritius and housed in facilities at the Infectious Disease Models
581 and Innovative Therapies (IDMIT) center (CEA site in Fontenay-aux-Roses, France). All
582 nonhuman primate studies at IDMIT were conducted in accordance with French national
583 regulations under the supervision of national veterinary inspectors (CEA Permit Number D92-
584 032-02). IDMIT complies with the standards for Human Care and Use of Laboratory Animals
585 of the Office for Laboratory Animal Welfare under Assurance Numbers #A5826-01 and F20-
586 00448. All experimental procedures were conducted according to European Directive 2010/63
587 (Recommendation Number 9). The pVISCONTI study was approved and accredited under the
588 statement A15 035 from the “Comité d’Ethique en Expérimentation Animale du CEA” and was
589 registered and authorized under Number 2453-2015102713323361v3 by the French Ministry
590 of Education and Research. Experimental procedures (animal handling, viral inoculations, and
591 samplings) were conducted after sedation with ketamine chlorhydrate (Imalgene 1000®, 10
592 mg/kg, intravenously (i.v.), Merial). Tissues were collected during follow-up and at necropsy.
593 Animals were euthanized after ketamine chlorhydrate sedation followed by a bolus of sodium
594 pentobarbital (Doléthal, 180 mg/kg, i.v., Laboratoire Vetoquinol).

595

596 ***Animals and experimental conditions***

597 This stage of the pVISCONTI study included a total of 41 male CyMs (median age = 4.8 years
598 at inclusion, IQR = 3.9-7.2). CyMs were inoculated intravenously (i.v.) with uncloned SIVmac₂₅₁
599 at an animal infectious dose (AID)₅₀ of 1000. SIVmac251 stock was kindly provided by Anne-
600 Marie Aubertin (Université Louis Pasteur, Strasbourg, France) ⁶⁸. Briefly, this stock was
601 obtained from the cell-free supernatant of infected rhesus PBMCs, which had been infected

602 *in vitro* with a culture supernatant obtained from a coculture of rhesus PBMCs and a spleen
603 homogenate from a rhesus macaque infected with SIVmac251 (provided by R. C. Desrosiers,
604 New England Regional Primate Center, Southborough, Mass.)

605
606 These experimental conditions ensure homogenous high plasma viremia during primary
607 infection and do not favor spontaneous SIV control⁶⁹. ART containing emtricitabine (FTC, 40
608 mg/kg, Gilead), dolutegravir (DTG, 2.5 mg/kg, ViiV Healthcare), and the tenofovir prodrug
609 tenofovir-disoproxil-fumarate (TDF, 5.1 mg/kg, Gilead) coformulated as a once daily
610 subcutaneous injection was initiated either at week 4 (12 animals) or at week 24 (12 animals)
611 post-infection (p.i.). CyMs received ART for 24 months, which was further interrupted. Animals
612 were then monitored for viral rebound (first viral load >400 SIV RNA copies after ART
613 interruption) and/or post-treatment SIV control. Animals were classified as PTCs if achieved
614 at least one pVL of <400 SIV RNA copies/ml after viral rebound (or if they never experienced
615 viral rebound). The 400 RNA copies/ml threshold mirrored that of HIV PTCs in the VISCONTI
616 study¹³. The prescheduled end of the study was 48 weeks post-treatment interruption.
617 However, most animals without post-treatment control progressed to clinical scores requiring
618 euthanasia before this time. Nevertheless, all animals were monitored for at least 6 months
619 after treatment interruption (Figure S1, Tables S1).

620
621 Of note, this stage of the pVISCONTI study was performed in two independent experimental
622 phases, each including 6 early- and 6 late-treated macaques that were infected, treated, and
623 monitored in parallel (for detailed study protocol, see Figure S1). In addition, a total of 17
624 untreated CyMs in three experimental phases (n=5, 6 and 6) were analyzed throughout the
625 study and were used as a reference for natural evolution under our experimental conditions

626 (Table S1). The different groups of animals studied were matched for age and MHC
627 haplotypes. CyMs harboring the M6 haplotype, previously described to favor natural SIV
628 control^{36,70,71}, were not included in the study. The characteristics of the CyMs in this study are
629 detailed in Table S1. One animal (CB296A) presented an extremely severe acute infection and
630 reached the endpoint requiring euthanasia before ART initiation and was excluded from
631 further analyses. Some analyses of the CD8+ T-cell response were limited to the animals from
632 the pVISCANTI-1 experiment (n=6 animals in each group), in which samples were specifically
633 destined for deeper immunological characterization.

634

635 ***Blood collection and processing***

636 Peripheral blood was collected by venous puncture into Vacutainer Plus Plastic K3EDTA Tubes
637 or Vacutainer CPT Mononuclear Cell Preparation Tubes with Sodium Heparin (BD Biosciences).
638 Complete blood counts were monitored at all time points from the Vacutainer Plus Plastic
639 K3EDTA Tubes. Plasma was isolated either from Vacutainer Plus Plastic K3EDTA Tubes by
640 centrifugation for 10 min at 480 g or from Vacutainer CPT Mononuclear Cell Preparation Tubes
641 with Sodium Heparin after centrifugation for 40 min at 1900 g, and the samples were stored
642 at -80°C. Peripheral blood mononuclear cells (PBMCs) were isolated from Vacutainer CPT
643 Mononuclear Cell Preparation Tubes with Sodium Heparin according to the manufacturer's
644 instructions (BD Biosciences), and red blood cells were lysed in ammonium-chloride-
645 potassium (ACK) buffer (0.15 M NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA, pH 7.4).

646

647 ***Tissue collection and processing***

648 Axillary or inguinal lymph node (peripheral PLN), bone marrow (BM), and bronchoalveolar
649 lavage (BAL) samples were collected longitudinally (Figure S1). In addition, the spleen,

650 mesenteric lymph nodes (MLNs), colon and liver were collected at necropsy. Tissue samples
651 were collected in RPMI medium at 2–8°C. Lymph node cells were isolated into RPMI medium
652 via mechanical disruption using a gentleMACS Dissociator (Miltenyi Biotec). The cell
653 suspension was filtered (70 µm), and red blood cells were lysed in ACK. Bone marrow cells
654 were purified using Lymphocyte Separation Medium (Eurobio Scientific) diluted to 90% in
655 DPBS, centrifuged for 20 min at 350 g, and separated from red cells in ACK. Spleen cells were
656 processed via mechanical disruption in RPMI medium using a gentleMACS Dissociator
657 (Miltenyi Biotec), purified as described for BM cells, and separated from red cells in ACK.
658 Colonic lymphocytes were obtained from mucosa taken from approximately 10 cm of tissue.
659 Colonic tissue was washed extensively in PBS and R10 medium (RPMI medium supplemented
660 with 10% fetal calf serum and penicillin/streptomycin) and then digested for 45 min with
661 collagenase II prior to mechanical disruption. Lymphocytes were isolated over a Percoll 67/44
662 gradient (Sigma-Aldrich). Liver tissue was mechanically disrupted using a gentleMACS
663 Dissociator (Miltenyi Biotec), and the cell suspension was sequentially filtered (300 µm > 100
664 µm > 70 µm). Then, lymphocytes were obtained over an OptiPrep gradient (Sigma-Aldrich). T-
665 cell activation, proliferation and exhaustion phenotyping and measurements of SIV-
666 suppressive activity *ex vivo* were performed using freshly isolated cells. T-cell CFSE
667 proliferation assays and cytokine intracellular staining were performed using viable cells
668 frozen at –196°C in DMSO/FCS.

669

670 ***Quantification of plasma viral load***

671 Plasma viremia was monitored longitudinally in all animals using quantitative RT-PCR with a
672 limit of detection of 12.3 copies/mL. Viral RNA was prepared from 100 µL of cell-free plasma.
673 Quantitative RT-PCR was performed using a SuperScript III Platinum One-Step qRT-PCR Kit

674 (Thermo Fisher Scientific) with a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). Each
675 tube contained 12.5 μL of 2X reaction mixture, 0.5 μL of RNaseOUT (40 U/ μL), 0.5 μL of
676 Superscript III Reverse Transcriptase/Platinum Taq DNA Polymerase, 1 μL of each primer (125
677 μM), 0.5 μL of the fluorogenic probe (135 μM), and 10 μL of eluted RNA. Primer/probe
678 sequences were designed to amplify a region of the SIVmac₂₅₁ *gag* gene. The forward (F)
679 primer sequence was 5'-GCAGAGGAGGAAATTACCCAGTAC-3' (24 bp), and the reverse (R)
680 primer sequence was 5'-CAATTTTACCCAGGCATTTAATGTT-3' (25 bp). The probe sequence was
681 5'-FAM-TGTCCACCTGCCATTAAGCCCGA-BHQ1-3' (23 bp). This probe had a fluorescent
682 reporter dye, FAM (6-carboxyfluorescein), attached to its 5' end and a quencher, BHQ1 (black
683 hole quencher 1), attached to its 3' end (TaqMan, Applied Biosystems). Samples were heated
684 for 30 min at 56°C and 5 min at 95°C, followed by 50 thermocycles, each comprising 15 sec at
685 95°C and 1 min at 60°C.

686

687 ***Quantification of SIV-DNA and cell-associated SIV-RNA***

688 Total DNA and caRNA were extracted from PBMCs with an AllPrep DNA/RNA Mini Kit (Qiagen).
689 For tissue, to control for differences in viral distribution within a single organ, two or three
690 tissue samples were first mechanically disrupted separately with a MagNA Lyser (GmbH,
691 Roche Diagnostics, Brussels, Belgium). Nucleic acids were extracted separately, and the lysate
692 was divided into two parts for DNA (DNA Mini Kit, Qiagen) and RNA extraction (RNeasy Plus
693 Mini Kit, Qiagen). caRNA was treated during extraction by DNase I (Qiagen). Then, viral
694 measurements were performed on each extract.

695 To determine the infection burden, total SIV-DNA was quantified by ultrasensitive real-time
696 PCR targeting the *gag* region⁷². The cycling conditions were 2 minutes at 50°C and 10 minutes
697 at 95°C followed by cycles at 95°C for 15 seconds and 60°C for 1 minute. The limit of detection

698 was 1 copy/PCR. To normalize SIV-DNA per million cells, the CCR5 gene was quantified by real-
699 time PCR with the same cycling conditions described above (forward primers: an equimolar
700 mix of the primers 5'-CAACATGCTGGTCGATCCTCAT-3' and 5'-CAACATACTGGTCGTCCTCA TCC-
701 3', reverse primer: 5'-CAGCATAGTGAGCCCAGAAG-3', probe: 5'-HEX-CTGACA
702 TCTACCTGCTCAACCTG-BHQ1-3'). For both PCRs, the reaction volumes were 50 μ L, containing
703 25 μ L of 2 \times qPCR Mastermix Plus (Eurogentec, Seraing, Belgium), 0.4 μ M each primer and 0.2
704 μ M probe. SIV-DNA levels were reported as copies per 10^6 cells or CD4⁺ T cells or as threshold
705 values when the SIV-DNA level was under the detection threshold or SIV-DNA was not
706 detected.

707 SIV caRNA was quantified by one-step real-time PCR targeting the gag⁷². Two replicates of all
708 amplifications were performed. The cycling conditions were 10 minutes at 50°C and 5 minutes
709 at 95°C followed by cycles at 95°C for 15 seconds and 60°C for 1 minute. The limit of detection
710 was 5 copies/PCR. Normalization of SIV caRNA was performed with ribosomal RNA (one-step
711 real-time PCR using the Ribosomal RNA Control Reagents Kit (Applied Biosystems)). The
712 reaction volume was 20 μ L, containing 5 μ L of 4 \times Master Mix Fast Virus 1-Step Taqman
713 (Applied Biosystems), 0.4 μ M each primer and 0.2 μ M probe.

714 A standard using SIV1C cells containing a single integrated copy of SIVmac₂₅₁ proviral DNA per
715 cell was used (<https://www.resourcenhpir.com/data-sheet-siv-1c-cell-line>)⁷³. The DNA and
716 RNA thresholds varied according to the number of cells and the quantity of total RNA available
717 and were calculated for each assay.

718

719 ***Titration of SIVmac₂₅₁ gp140 IgG and IgA***

720 SIVmac₂₅₁ g140-foldon-type glycoproteins were produced by transient transfection of
721 FreeStyle™ 293-F cells and purified by affinity chromatography using Ni Sepharose® Excel

722 beads (GE Healthcare) ⁷⁴. High-binding 96-well ELISA plates (Costar, Corning) were coated
723 overnight with 250 ng/well of purified recombinant SIV gp140 protein. After washing with
724 0.05% Tween 20-PBS (washing buffer), plates were blocked for 2 h with 2% BSA, 1 mM EDTA,
725 and 0.05% Tween 20-PBS (blocking buffer), washed, and incubated with serially diluted NHP
726 sera in duplicate at 1:250 or 1:50 followed by 7 consecutive 1:4 or 1:3 dilutions in PBS for IgG
727 or IgA detection, respectively. After washing, the plates were revealed by incubation for 1 h
728 with goat HRP-conjugated anti-human IgG or IgA antibodies (Jackson ImmunoResearch, 0.8
729 µg/ml final) and by adding 100 µl of HRP chromogenic substrate (ABTS solution, Euromedex).
730 Optical densities were measured at 405 nm (OD_{405 nm}), and background values based on
731 incubation of PBS alone in coated wells were subtracted. Experiments were performed using
732 a HydroSpeed™ microplate washer and Sunrise™ microplate absorbance reader (Tecan
733 Männedorf, Switzerland).

734

735 ***Measurement of SIV-suppressive activity***

736 Autologous CD4⁺ and CD8⁺ T cells were purified from freshly isolated PBMCs or tissue cell
737 suspensions by positive and negative selection, respectively, using nonhuman primate CD4⁺
738 MicroBeads and the CD8⁺ T-Cell Isolation Kit, with the MultiMACS™ Cell24 Separator (Miltenyi
739 Biotec). Purified CD4⁺ T cells were stimulated for 3 days with concanavalin A (5 µg/mL, Sigma-
740 Aldrich) in the presence of IL-2 (100 IU/mL, Miltenyi Biotec). Purified CD8⁺ T cells were cultured
741 in the absence of mitogens and cytokines (*ex vivo* CD8⁺ T cells). Stimulated CD4⁺ T cells (10⁵)
742 were superinfected in U-bottom 96-well plates with SIV_{mac251} (MOI = 10⁻³) in the presence
743 (1:1 effector-to-target-cell ratio) or absence of *ex vivo* CD8⁺ T cells (10⁵) from the same tissue
744 via spinoculation ⁷⁵ for 1 hour (1,200 g at room temperature) and incubated for 1 hour at 37°C.
745 Cells were then washed and cultured in R10 medium containing IL-2 (100 IU/mL, Miltenyi

746 Biotec). Culture supernatants were assayed on day 7 using an SIV p27 Antigen ELISA Kit
747 (XpressBio). Antiviral activity was calculated as \log_{10} (mean p27 ng/mL in SIV-infected CD4⁺
748 T-cell cultures without *ex vivo* CD8⁺ T cells)/(mean p27 ng/mL in SIV-infected CD4⁺ T-cell
749 cultures with *ex vivo* CD8⁺ T cells)³⁹.

750

751 ***Analyses of T-cell activation and proliferation markers***

752 Total T-cell activation and proliferation were assessed using fresh PBMCs and tissue cell
753 suspensions. Cells were stained with the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo
754 Fisher Scientific) and then surface stained for CD3, CD4, CD8, CD38, and HLA-DR. For
755 differentiation phenotype analyses, cells were additionally stained with CD45RA, CCR7 and
756 CD27. Then, the cells were fixed/permeabilized using a Cytofix/CytoPerm Kit (BD Biosciences)
757 and stained for intracellular Ki-67. The following antibodies were used: anti-CD3–AF700 (clone
758 SP34-2, BD Biosciences), anti-CD4–PerCP-Cy5.5 (clone L200, BD Biosciences), anti-CD8–APC-
759 Cy7 (clone RPA-T8, BD Biosciences), anti-CD38–FITC (clone AT-1, StemCell Technologies), anti-
760 HLA-DR–V450 (clone G46-6, BD Biosciences), anti-CD45RA–PE Cy7 (clone 5H9, BD
761 Biosciences), anti-CCR7–PE-Dazzle594 (clone G043H7, Biolegend), anti-CD27–PE (clone M-
762 T271, BD Biosciences), and anti-Ki-67–AF647 (clone B56, BD Biosciences). Data were acquired
763 using an LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo software version 10
764 (Tree Star Inc.). Cells were classified as follows: naïve—CD45RA⁺CD27⁺CCR7⁺; central memory
765 (CM)—CD45RA⁻CD27⁺CCR7⁺; transitional memory (TM)—CD45RA⁻CD27⁺CCR7⁻; effector
766 memory (EM)—CD45RA⁻CD27⁻CCR7⁻; and effector—CD45RA⁺CD27⁻CCR7⁻ (Figure S2).

767

768 ***Cytokine production, memory and proliferative capacity of SIV-specific T cells***

769 Frozen PBMCs were thawed, resuspended at 1×10^6 /mL in R20 medium, and incubated
770 overnight at 37°C. Cells were then stimulated with a pool of 24 optimal SIV peptides (8–10
771 amino acids, 2 µg/mL each, Table S2³⁶) in the presence of anti-CD28 (1 µg/mL, clone L293, BD
772 Biosciences) and anti-CD49d (1 µg/mL, clone 9F10, BD Biosciences) and stained with anti-
773 CD107a (clone H4A3, BD Biosciences) for 30 min prior to the addition of GolgiStop (1 µL/mL,
774 BD Biosciences) and brefeldin A (BFA, 5 µg/mL, Sigma-Aldrich). Costimulatory antibodies alone
775 were used as a negative control, and concanavalin A (5 µg/mL, Sigma-Aldrich) was used as a
776 positive control. Cells were incubated for a total of 6 hr. To evaluate the differentiation profile,
777 cells were stained with the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher
778 Scientific) and then surface stained for CD3, CD4, CD8, CD45RA, CCR7, CD27 and CD127. Cells
779 were then fixed/permeabilized using the Cytotfix/CytoPerm Kit (BD Biosciences) and stained
780 intracellularly for IFN γ , TNF α , and IL-2 (Figure S3). The results were corrected for background
781 by subtracting the negative (no peptide) control from the peptide-stimulated response. To
782 evaluate the memory profile, cells were stained with the LIVE/DEAD Fixable Aqua Dead Cell
783 Stain Kit (Thermo Fisher Scientific) and then surface stained for CD3, CD8, CD45RA, CCR7,
784 CD27, CD39, and PD-1 in brilliant stain buffer (BD Bioscience). Cells were then
785 fixed/permeabilized using Phosflow buffer (BD Biosciences) and stained intracellularly for TCF-
786 1, IFN γ and TNF α (Figure S4).

787 CD8⁺ T-cell proliferation upon stimulation was evaluated by CFSE staining (1 µM, Thermo
788 Fisher Scientific)(Figure S5). PBMCs were CFSE stained, stimulated with a pool of 24 optimal
789 SIV peptides (2 µg/mL each), and then cultured for 6 days. Twelve hours before completing
790 the total incubation time, an additional dose of the peptides was added as well as GolgiStop
791 (1 µL/mL, BD Biosciences), brefeldin A (BFA, 5 µg/mL, Sigma-Aldrich), and an anti-CD107a
792 antibody. Unstimulated cells were used as a negative control. Cells were stained with the

793 LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific) and then surface stained
794 for CD3, CD4, CD8, CD45RA, CCR7, CD27 and CD127. Cells were then fixed/permeabilized with
795 PhosFlow fix/perm buffers (BD Biosciences) and stained intracellularly for IFN γ , TNF α , IL-2,
796 pS6 Ser235/236 and anti-pAKT Ser473.

797 The following antibodies were used: anti-CD3–AF700 (clone SP34-2, BD Biosciences), anti-
798 CD4–PerCP-Cy5.5 (clone L200, BD Biosciences), anti-CD8–APC-Cy7 (clone RPA-T8, BD
799 Biosciences), anti-CD45RA–PE Cy7 (clone 5H9, BD Biosciences), anti-CCR7–PE-Dazzle594
800 (clone G043H7, Biolegend), anti-CD27–PE (clone M-T271, BD Biosciences), anti-CD127–
801 BUV496 (clone HIL-7R-M21, BD Biosciences), anti-CD107a–BV786 (clone H4A3, BD
802 Biosciences), anti-TNF α –BUV395 (clone MAb11, BD Biosciences), anti-IFN γ –BV605 (clone B27,
803 BD Biosciences), anti-IL2–BUV737 (clone MQ1-17H12, BD Biosciences), anti-phospho S6
804 S235/236–Pacific blue (clone D57.2.2E, Cell Signaling), anti-phospho Akt Ser473–AF647 (clone
805 D9E, Cell Signaling), anti-CD279 (PD1)-BV 421 (clone EH12.2H7, Biolegend), anti-CD39-BV785
806 (clone A1), and anti-TCF-7/TCF-1-FITC (clone S33-96, BD Bioscience). Data were acquired using
807 a Fortessa flow cytometer (BD Biosciences) and analyzed with FlowJo software version 10
808 (Tree Star Inc.). The results were corrected for background by subtracting the negative (no
809 peptide) control from the peptide-stimulated response. Negative responses were given an
810 arbitrary value of 0.01. Division and proliferation index calculations were performed using the
811 proliferation modeling function available in FlowJo. The division index is the average number
812 of cell divisions that a cell in the original population has undergone. The proliferation index is
813 the total number of divisions divided by the number of cells that went into division. Clustering
814 analysis of the SIV-specific CD8⁺ T-cell dataset was performed on concatenated files from
815 early- and late-treated CyMs at the time of ART initiation and post-ATI. Dimensionality
816 reduction was performed using the uniform manifold approximation and projection (UMAP)

817 algorithm (FlowJo plugin v3.1). UMAP was performed using the following parameters:
818 metric—Euclidian, nearest neighbors—15, minimum distance—0.5, and number of
819 components—3. Clustering analysis was performed using the unsupervised PhenoGraph
820 algorithm (FlowJo plugin v2.4). PhenoGraph was used for unsupervised clustering using
821 default parameters (K=30). All markers were considered for UMAP and PhenoGraph analyses,
822 except live/dead and CD4. The median fluorescence intensity (MFI) for each cluster was
823 normalized through the min–max scaling method and can be visualized in the heatmap (R
824 version 4.2.2)(Figure S6).

825

826 ***Data visualization and statistical analyses***

827 Data storage was ensured with the BaTLab laboratory management system of IDMIT, and
828 database interrogation and data visualization were performed using Tableau version 2021.3
829 (Tableau Software). Graphs and statistical analyses were performed using Prism version 9.2.0
830 (GraphPad Software) or R version 4.2.2 (<http://www.R-project.org>). The results are presented
831 as individual data with the median. Groups were compared using the Mann–Whitney U test.
832 The Friedman test and Wilcoxon matched-pairs rank test were used to compare paired
833 samples. **Tests were two-sided.** Correlations were assessed using Spearman rank analyses. No
834 adjustments were made for multiple comparisons, given the exploratory nature of the
835 analyses. All p values less than 0.05 were defined as significant.

836

837 **Data Availability**

838 The data that support the findings of this study are presented in the main figures and
839 supplemental material of this article. **Data are provided as a Source Data file.** Further
840 information and requests for resources and reagents should be directed to the lead contact.

841 Request for biological resources will be fulfilled based on availability and upon the
842 establishment of an MTA.

843

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1079

1080 **AUTHOR CONTRIBUTIONS**

1081 C.P. designed and carried out the experiments, analyzed the data, and interpreted the results.
1082 A.C., V.M., J.L., F.P.C., A.M., C.P., M.G., A.D., N.D., H.G., C.G. and F.R. carried out experiments,
1083 analyzed data, and interpreted results. D.D. and N.D.B. designed the experiments, analyzed
1084 the data, and interpreted the results. J.G., H.M. and V.A.F. analyzed the data and interpreted

1085 the results. A.B.T., O.L., M.M.T., and C.R. contributed to the study design and interpreted the
1086 results. C.R., R.L.G. and A.S.C. obtained funding. R.L.G. and A.S.C. designed the experiments,
1087 analyzed the data, interpreted the results, and supervised the study. C.P. and A.S.C. wrote the
1088 manuscript. All authors critically reviewed the manuscript and contributed to the final version.

1089

1090 **COMPETING INTERESTS**

1091 A.S.C. has received speaker fees from MSD, ViiV Healthcare, Gilead, Janssen. V.A.-F. has
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1094 conferences. The other authors declare no competing interests.

1095 **FIGURE LEGENDS**

1096 **Figure 1. Early therapy is associated with a higher rate of post-treatment controllers.** Plasma
1097 viral load kinetics (A) prior to ART initiation and during ART; and (B) post-ATI in W4-treated
1098 (n=11) and W24-treated CyMs (n=11). Plasma viral load kinetics during the initial six months
1099 following infection in animals not receiving ART (n=17) shown as reference. Medians and IQR
1100 are shown. C) Comparison of plasma viral load levels between W4- and W24-treated CyMs at
1101 the peak (acute infection), at the time of ART initiation and prior to treatment interruption.
1102 D) Comparison of plasma viral load levels between W4- and W24-treated CyMs in the early
1103 days following ATI. The magnitude of the plasma viral load post-ATI in W4- and W24-treated
1104 CyMs is indicated by (E) the viral load peak and by (F) the cumulative pVL post-ATI (area under
1105 the curve - AUC, considering all pVL measurements until 6 months post-ATI). (C-F) Values for
1106 individual animals (W4 n=11, W24 n=11) and medians are shown. *p < 0.05, **p < 0.01; ***p
1107 < 0.001; ns non-significant; 2-sided Mann-Whitney U test. G) Kaplan-Meier analyses of
1108 maintaining no viral rebound (time without pVL > 400 copies/ml) following ART interruption
1109 (left) and achievement of post-treatment control (time to durable pVL < 400 copies/ml after
1110 viral rebound) (right) in W4- and W24-treated macaques (n=11 for each). Frequency of CyMs
1111 spontaneously controlling plasma viremia (<400 copies/mL) in untreated SIV infection and
1112 after antiretroviral treatment interruption (middle and right panels). The frequencies of
1113 posttreatment controllers among W4- and W24-treated CyMs are shown. Mantel Cox log-rank
1114 test was used to compare the two groups. Source data are provided as a Source Data file.

1115

1116 **Figure 2. Post-treatment controllers maintain low number of infected cells and high CD4+ T**
1117 **cell frequencies.** Kinetics of SIV-DNA levels in (A) blood and in (B) PLNs in W4-treated and
1118 W24-treated CyMs. The results are expressed as copies of SIV-DNA/million CD4+ T cells. C)

1119 Levels of SIV-DNA in mesenteric and inguinal lymph nodes at euthanasia. The results are
1120 expressed as copies SIV-DNA/million cells. Longitudinal evolution of CD4⁺ T cells in (D) blood
1121 and in (E) PLNs in W4- and W24-treated CyMs. The results are shown as absolute CD4⁺ T-cell
1122 counts in blood and as the proportion of CD4⁺ T cells among CD3⁺ lymphocytes in PLNs. (F)
1123 Proportion of CD4⁺ T cells among CD3⁺ lymphocytes in BM, MLN, spleen, colon mucosa and
1124 BAL at euthanasia. Longitudinal evolution of the CD4/CD8 ratio in (G) blood. (H) The ratio of
1125 CD4/CD8 in PBMCs, BM, PLNs, MLNs, spleen, colon mucosa and BAL at euthanasia. The dashed
1126 line indicates ratio=1. I) Proportion of central memory (CD45RA⁻CD27⁺CCR7⁺) CD4⁺ T cells in
1127 PBMCs, BM, PLNs, MLNs, spleen, colon mucosa and BAL at euthanasia. (A-I) Individual values
1128 (n=6 or 11 for each group depending on sampling during the pVISCONTI-1 and pVISCONTI-2
1129 study phase). Medians are shown. *p < 0.05, **p < 0.01; ***p < 0.001; ns non-significant; Two-
1130 sided Mann–Whitney U test. Source data are provided as a Source Data file.

1131

1132 **Figure 3. The anti-SIV antibody levels after treatment interruption are associated with the**
1133 **magnitude of antigen stimulation.** A) Kinetics of plasma anti-gp140 SIV IgG (A) and IgA (E) in
1134 W4-treated and W24-treated CyMs. Comparison of anti-gp140 SIV IgG (B) and IgA (F) levels
1135 between W4- and W24-treated CyMs at baseline, on day 28 p.i.; at 6 months p.i. for the W24-
1136 treated group, and prior to ART interruption. C) The magnitude of humoral response post-ATI
1137 is indicated by the cumulative plasma anti-gp140 SIV IgG (C) and IgA (G) measurements (area
1138 under the curve - AUC until 6 months post-ATI). D) Spearman correlation between pVL AUC
1139 post-ATI and anti-gp140 SIV IgG (D) and IgA (H) AUC post-ATI. (A-H) Individual values (n=11
1140 animals per group are shown. (B,C,F,G) Medians are shown, *p < 0.05, **p < 0.01; ***p <
1141 0.001;ns non-significant; Two sided Mann–Whitney U test. Source data are provided as a
1142 Source Data file.

1143

1144 **Figure 4. CD8+ T cells mobilized after interruption of early treatment have enhanced SIV**

1145 **suppressive capacity.** **A)** Kinetics of CD8⁺ T-cell-mediated SIV-suppressive activity in the blood

1146 of W4-treated and W24-treated CyMs at primary SIV infection and post-ATI. **B)** Comparison of

1147 CD8⁺ T-cell-mediated SIV-suppressive activity between W4- and W24-treated CyMs at

1148 baseline, on day 28 p.i.; at 6 months p.i. for the W24-treated group, and prior to ART

1149 interruption. **C)** Comparison of the maximal CD8⁺ T-cell-mediated SIV-suppressive activity

1150 during the period following treatment interruption in W4- and W24-treated CyMs. **D)** The

1151 magnitude of CD8⁺ T-cell-mediated SIV-suppressive activity is indicated by the cumulative

1152 measurements post-ATI (area under the curve – AUC of all measurements until 6 months post-

1153 ATI). **E)** Kinetics of CD8⁺ T-cell-mediated SIV-suppressive activity in PLNs of W4- and W24-

1154 treated CyMs at the time of ART initiation, 14 days post-ATI and at euthanasia. The results are

1155 shown as log p27 decrease in the presence of CD8⁺ T cells. Spearman correlation between **(F)**

1156 pVL AUC post-ATI and blood CD8⁺ T-cell-mediated SIV-suppressive activity AUC post-ATI, **(G)**

1157 pVL and PLN CD8⁺ T-cell-mediated SIV-suppressive activity at euthanasia, **(H)** SIV-DNA levels

1158 in blood CD4 at euthanasia and blood CD8⁺ T-cell-mediated SIV-suppressive activity AUC post-

1159 ATI, **(I)** SIV-DNA levels at euthanasia and PLN CD8⁺ T-cell-mediated SIV-suppressive activity at

1160 euthanasia. **(A-I)** Individual values (n=11 animals per group, except for (E), where n=5-10 per

1161 group were analyzed depending on sample availability) are shown. (B-E) Medians are shown;

1162 *p < 0.05, **p < 0.01; ***p < 0.001; ns non-significant; Two sided Mann–Whitney U test.

1163 Source data are provided as a Source Data file.

1164

1165 **Figure 5. CD8+ T cells expanding after interruption of early treatment express low levels of**

1166 **activation markers.** Expression levels of CD38 and HLA-DR activation markers **(A)** and

1167 intracellular levels of Ki67 proliferation markers (E) in blood CD8⁺ T cells during primary SIV
1168 infection and post-ATI in W4-treated (n=11) and W24-treated CyMs (n=11). Cumulative CD38,
1169 HLA-DR (B) and Ki67 (F) expression levels in blood CD8⁺ T cells during the first 4 weeks
1170 following SIV infection and post-ATI. Comparison of CD38 and HLA-DR (C) and Ki67 (G)
1171 expression levels in PLN CD8⁺ T cells between W4- and W24-treated CyMs at the time of cART
1172 initiation, 14 days post-ATI and at euthanasia. The results are shown as percent frequencies
1173 of CD8⁺ T cells or AUC; Spearman correlation between cumulative (AUC) CD38 and HLA-DR (D)
1174 and Ki67 (H) expression levels post-ATI and blood CD8⁺ T-cell-mediated SIV-suppressive
1175 activity AUC post-ATI. (A-H) Individual data are shown (n=6 animals per group); (B-D,F,G)
1176 Medians are shown; *p < 0.05, **p < 0.01; ***p < 0.001; ns non-significant; Two sided Mann-
1177 Whitney U test. Source data are provided as a Source Data file.

1178

1179 **Figure 6. Expansion of central memory CD8⁺ T cells is observed in blood and lymph nodes**
1180 **after interruption of early treatment.** Evolution of central memory (CM) (CD45RA⁻

1181 CD27⁺CCR7⁺), effector memory (EM) (CD45RA⁻CD27⁻CCR7⁻) and effector (CD45RA⁺CD27⁻CCR7⁻
1182) CD8⁺ T-cell subsets in blood at primary SIV infection (top) and early post-ATI (bottom) in W4-
1183 (A) and W24-treated (B) CyMs. Frequency of CM, EM and effector CD8⁺ T cells in PLNs of W4-
1184 (C) and W24-treated (D) CyMs prior to and 14 days post-ATI. E) Comparison of intracellular
1185 levels of Ki67 in blood (left) and PLN (right) CM CD8⁺ T cells at baseline, at the time of ART
1186 initiation, prior to and post-ATI in W4- and W24-treated CyMs. F) Spearman correlation
1187 between blood CD8⁺ T-cell-mediated SIV-suppressive activity AUC post-ATI and the frequency
1188 of CM CD8⁺ T cells 28 days post-ATI in blood (left) and 14 days post-ATI in PLNs (right). (A-F)

1189 Individual data are shown (n=6 animals per group); (A-E) Medians are shown; *p < 0.05, **p
1190 < 0.01; ***p < 0.001; ns non-significant; (A,B) Friedman test with Dunn's correction; (C,D) Two

1191 sided Wilcoxon matched-pairs rank test. (E) Two sided Mann–Whitney U test. Source data are
1192 provided as a Source Data file.

1193

1194 **Figure 7. SIV-specific CD8⁺ T cells with memory-like characteristics are mobilized after**

1195 **interruption of early treatment.** A) IFN γ , TNF α , IL-2, and CD107a production by SIV-specific

1196 CD8⁺ T cells and the total SIV-specific response in the blood of W4-treated and W24-treated

1197 CyMs at the time of ART initiation and post-ATI. The results are shown as proportions among

1198 CD8⁺ T cells. Negative responses were given an arbitrary value of 0.01. B) Frequencies of SIV-

1199 specific CD8⁺ T cells with 1-to-4 functions based on the expression of IFN γ , TNF α , IL-2, and/or

1200 CD107a in W4- and W24-treated CyMs at the time of ART initiation and post-ATI. Negative

1201 polyfunctional responses are indicated as 0.001. C) CD127 expression levels in SIV-specific

1202 CD8⁺ T cells at the time of ART initiation and post-ATI. The results are shown as the median

1203 fluorescence intensity in SIV-specific CD8⁺ T cells. D) Proportion of SIV-specific memory CD8⁺

1204 T cells expressing PD-1, CD39, CCR7 or TCF1 in the spleen at the end of the study. E) Division

1205 index (left) and proliferation index (right) of CD8⁺ T cells at the time of ART initiation and post-

1206 ATI. F) Left panel: UMAP plot of n=2979 SIV-specific CD8⁺ T cells from the blood of W4- and

1207 W24-treated CyMs at the time of ART initiation and post-ATI. Phenotypically distinct clusters

1208 defined by Phenograph are represented with different colors. Right panel: Heatmap showing

1209 the relative expression of differentiation markers, cytokine secretion and markers of mTORC1

1210 (pS6) and mTORC2 (pAKT) pathway activation for each phenotypically distinct cluster shown

1211 in F. G) Dynamics of Clusters 1, 2 and 3 at the time of ART initiation and post-ATI in W4-treated

1212 CyMs. H) Violin plots comparing the median fluorescence intensity (MFI) of the differentiation

1213 markers, cytokine secretion and markers of mTORC1 and mTORC2 pathway activation among

1214 Clusters 1, 2 and 3 in W4-treated CyMs; one-way ANOVA, *p < 0.05, **p < 0.01; ****p <

1215 0.0001. **(A-E,G,H)** Individual data are shown. **(A-C,E,G,H)** n=6 animals per group. (D) n=12
1216 animals per group. **(A-E)** Medians are shown; *p < 0.05, Two-sided Mann–Whitney U test. **G.**
1217 Two-sided Wilcoxon matched-pairs rank test. Source data are provided as a Source Data file.
1218

Figure 1

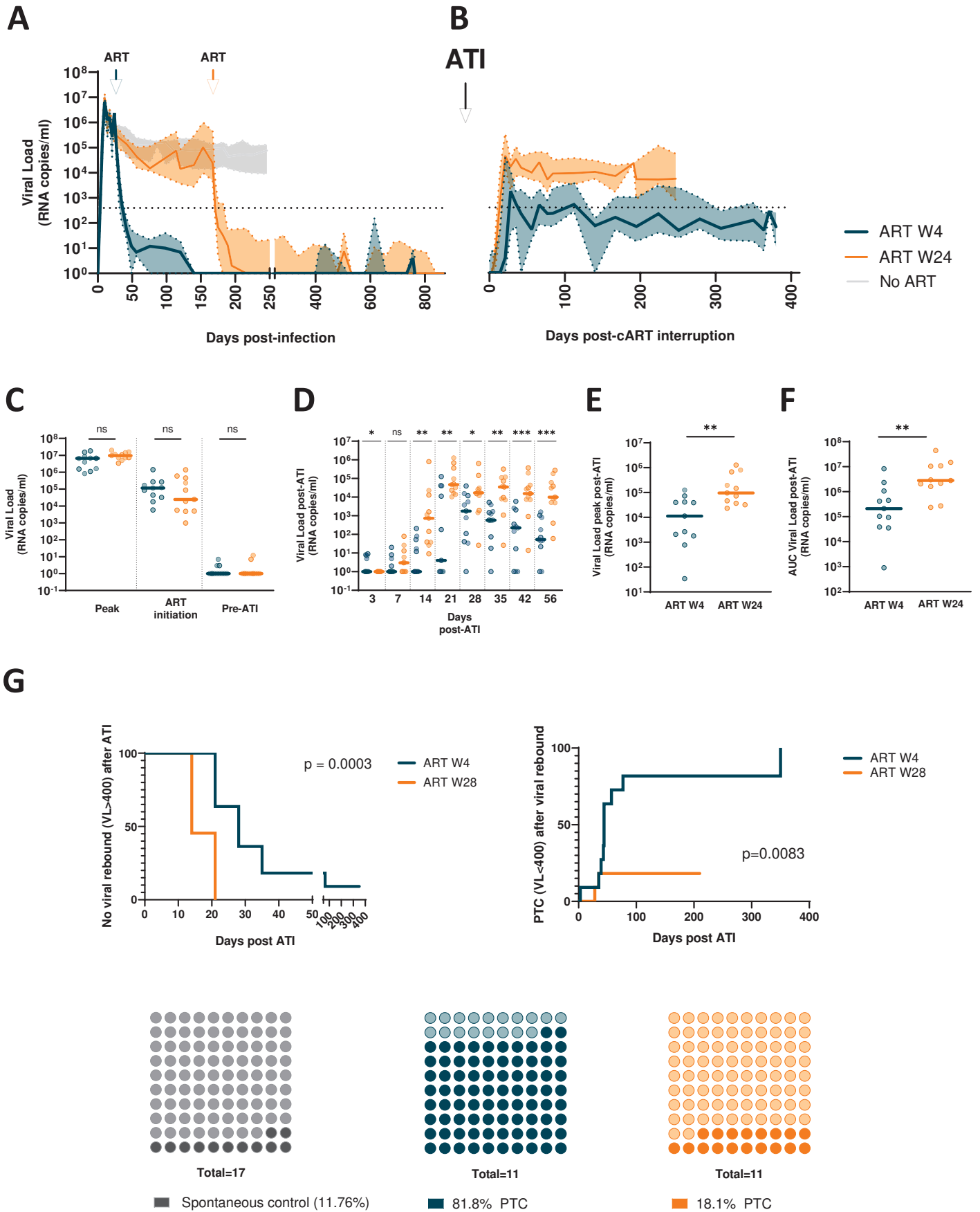


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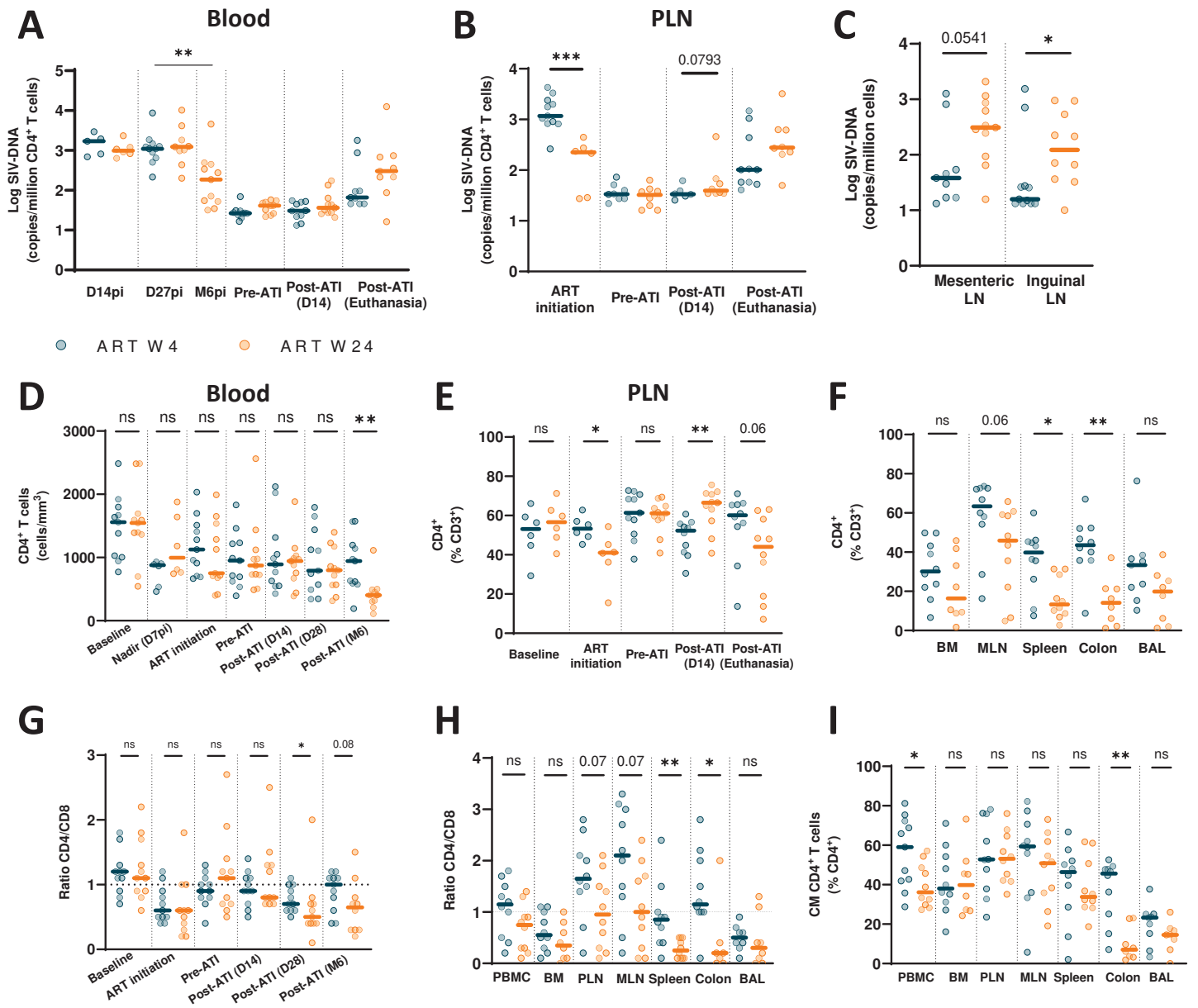


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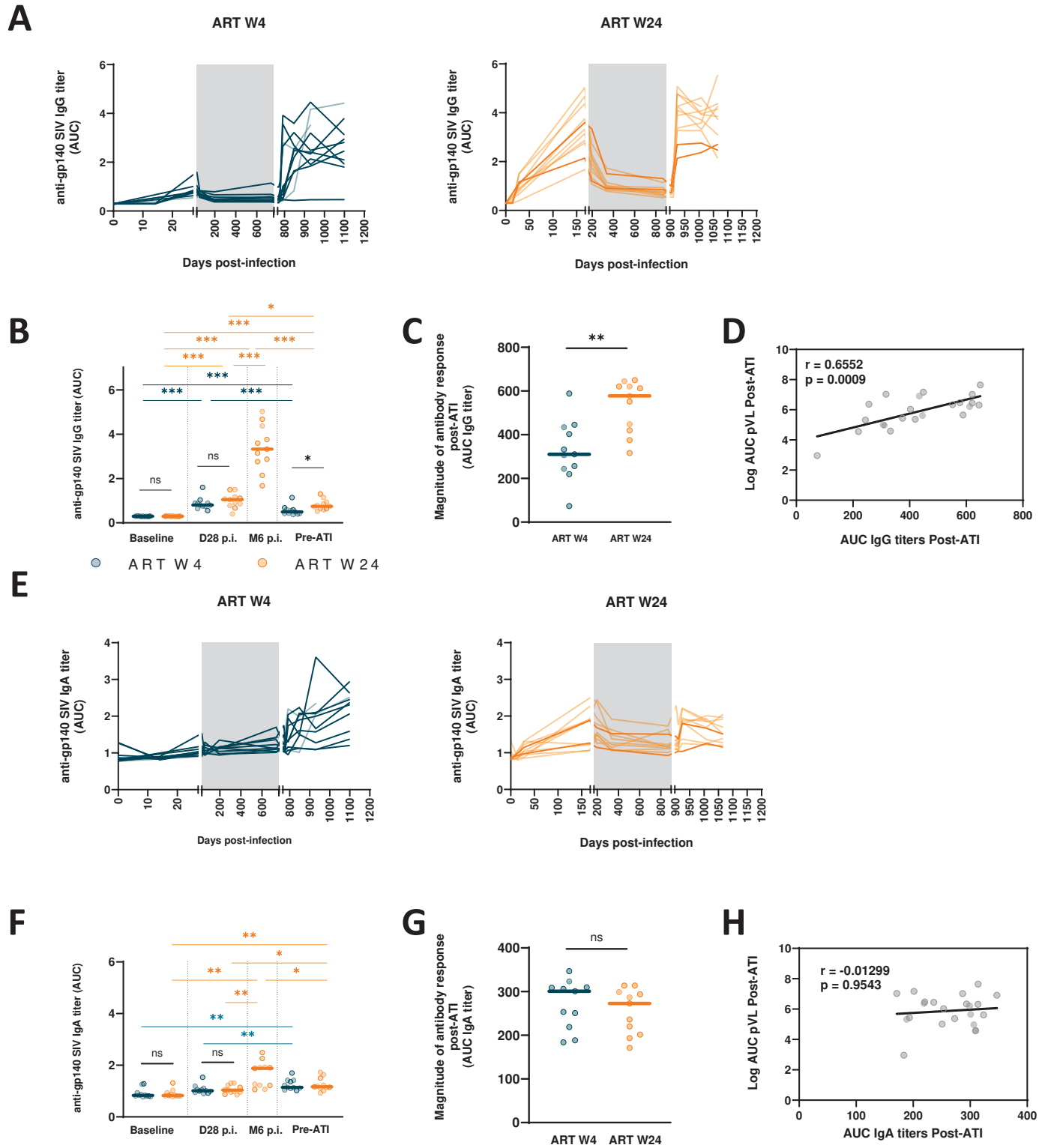


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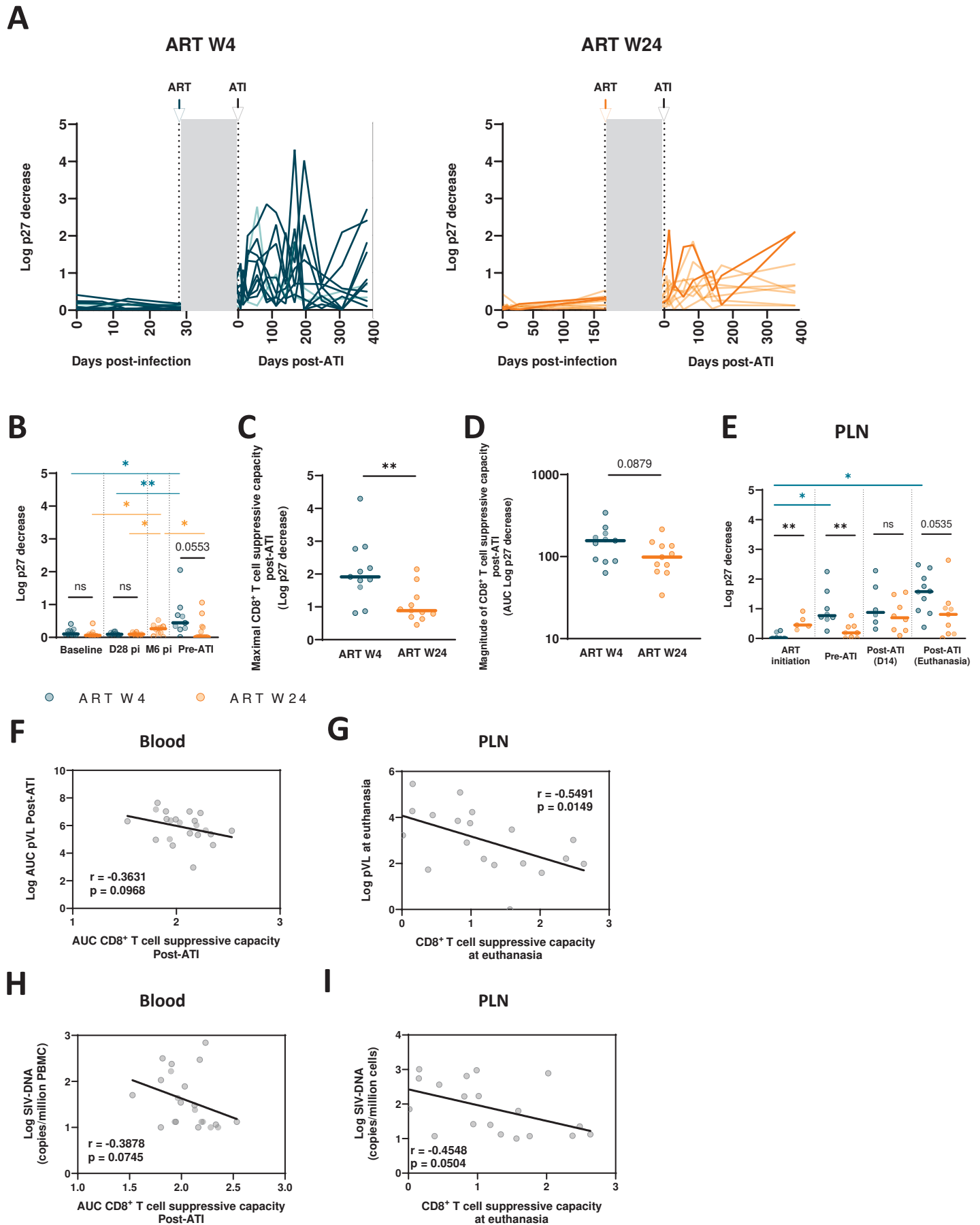


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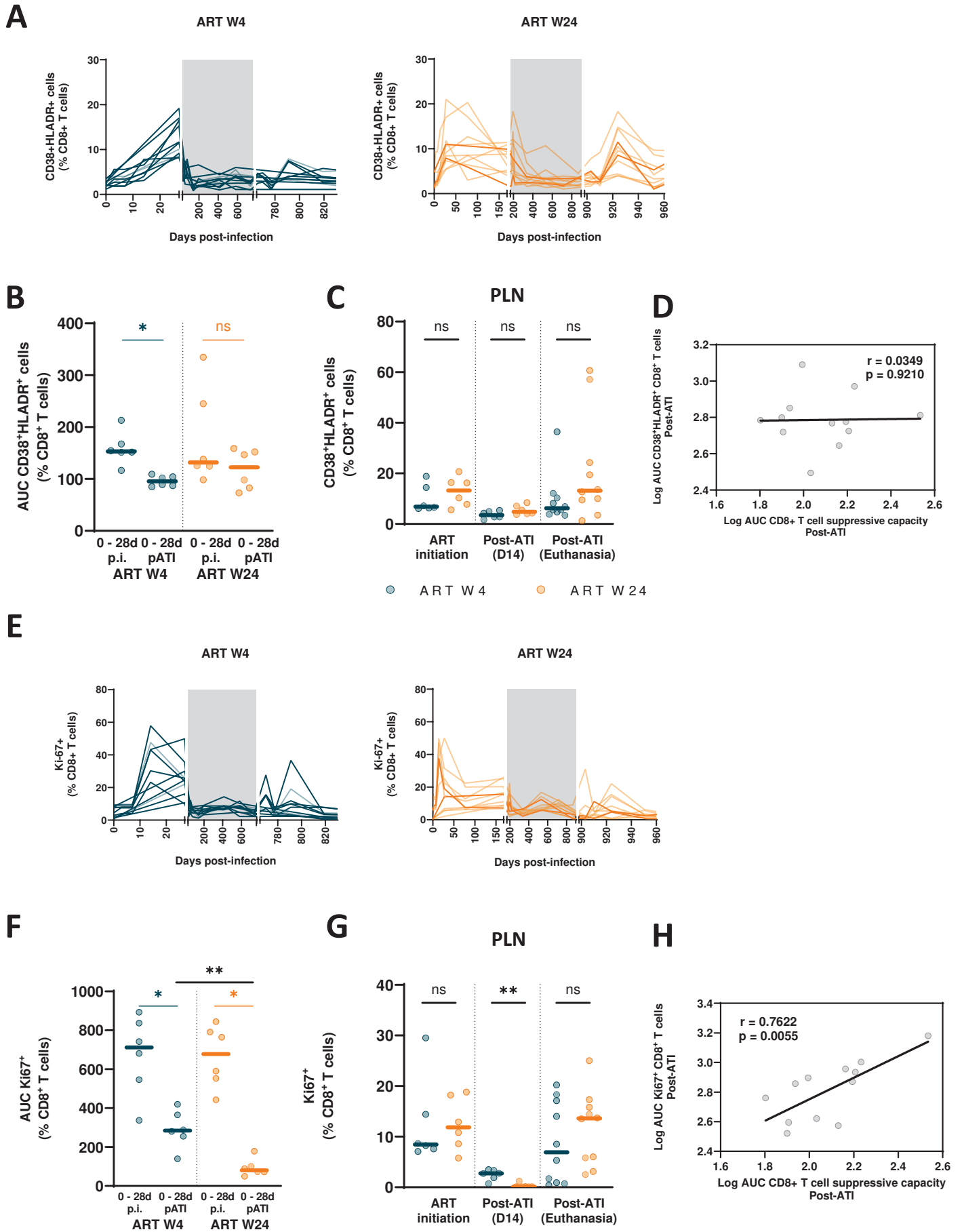


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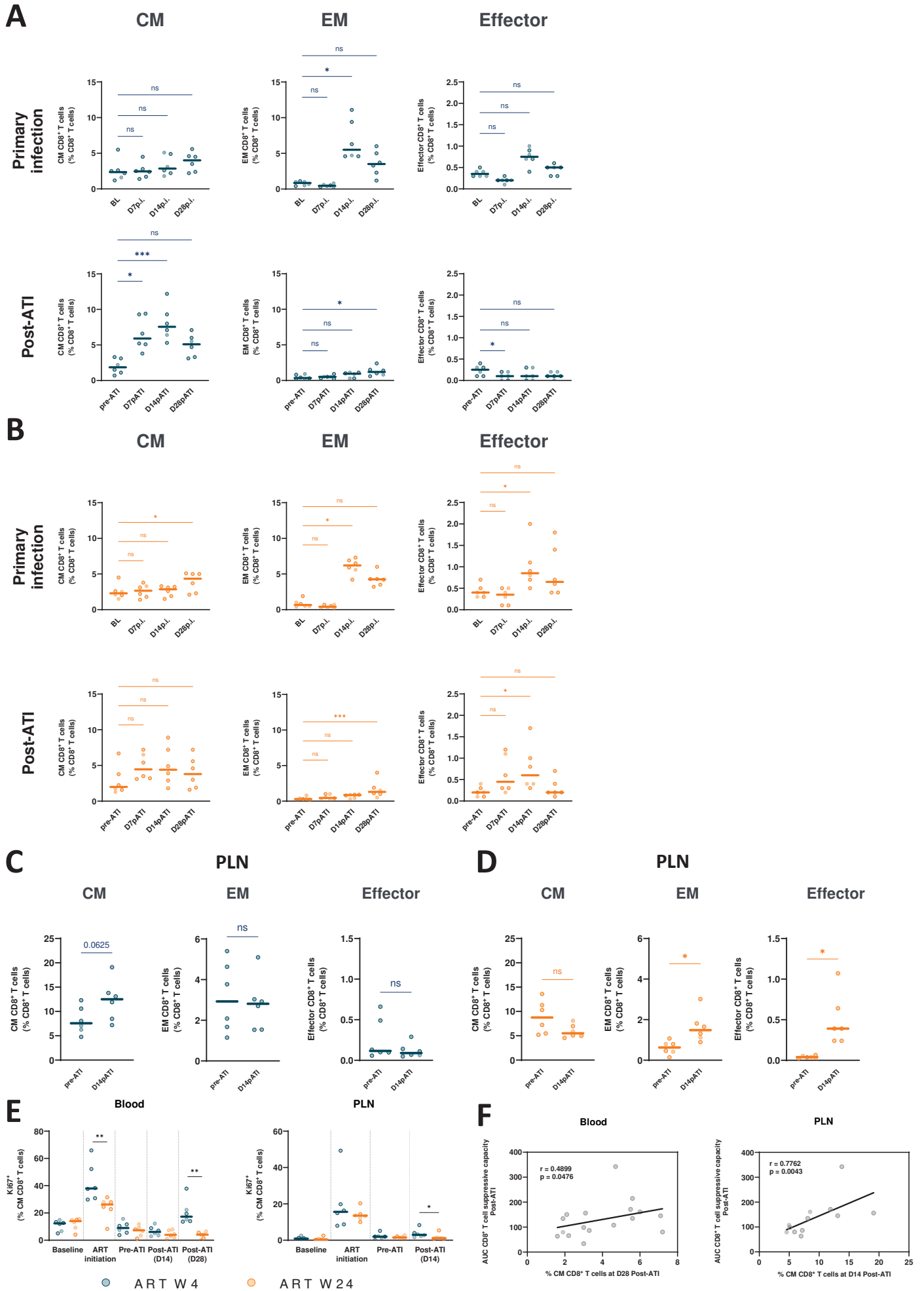
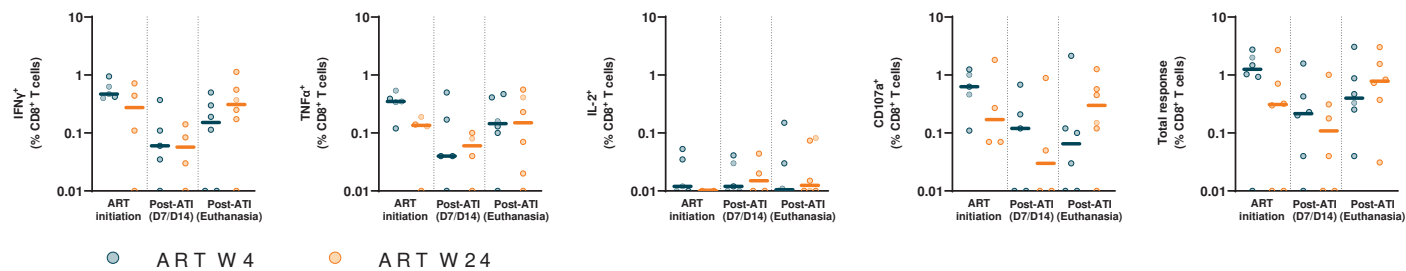
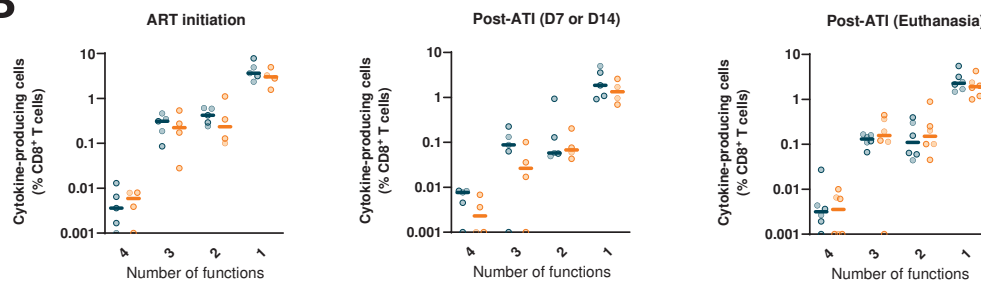


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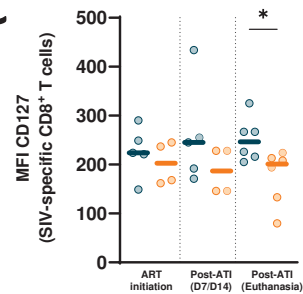
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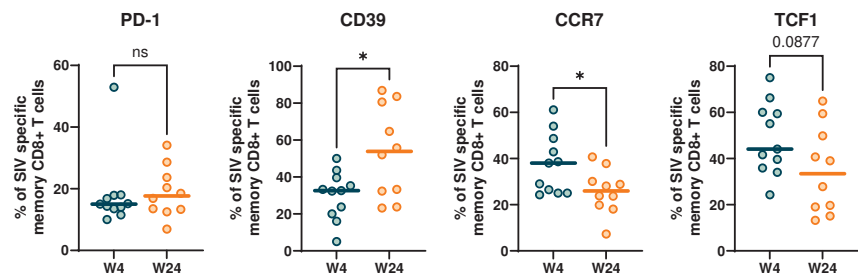
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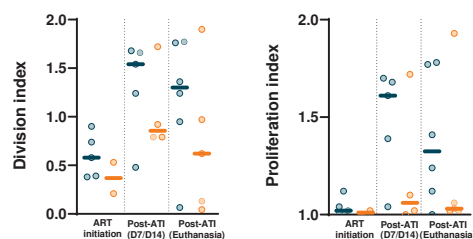
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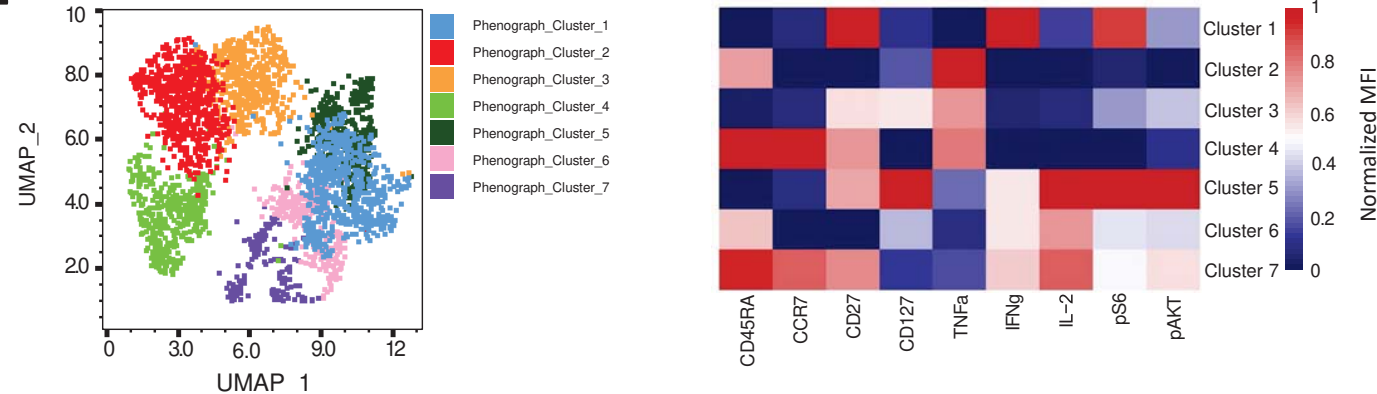
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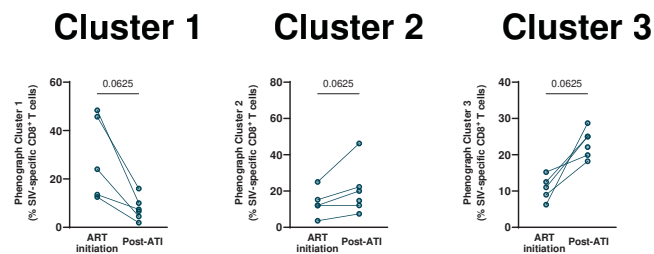
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