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1 **Modulation of Type I interferon-associated viral sensing during acute simian immunodeficiency**
2 **virus (SIV) infection in African green monkeys**

3 **Running title: Very early effects of SIV infection on viral sensing**

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21 **Abstract**

22 Natural hosts of simian immunodeficiency virus (SIV), such as African green monkeys (AGMs),
23 do not progress to AIDS. This associates with an absence of a chronic type I interferon (IFN-I)
24 signature. It is unclear how the IFN-I response is downmodulated in AGMs. We longitudinally
25 assessed the capacity of AGM blood cells to produce IFN-I in response to SIV and Herpes
26 Simplex virus (HSV). Phenotype and function of plasmacytoid dendritic cells (pDCs) and other
27 mononuclear blood cells were assessed by flow cytometry and expression of viral sensors
28 measured by RT-PCR. pDC displayed low BDCA-2, CD40 and HLA-DR expression during acute
29 SIVagm infection. BDCA-2 was required for sensing SIV, but not HSV, by pDCs. In acute infection,
30 AGM PBMCs produced less IFN-I upon SIV stimulation. In chronic phase the production was
31 normal, confirming that the lack of chronic inflammation is not due to a sensing defect of pDCs.
32 In contrast to stimulation by SIV, more IFN-I was produced upon HSV stimulation of PBMC
33 isolated during acute infection, while the frequency of AGM pDCs producing IFN-I upon in vitro
34 stimulation with HSV was diminished. Indeed, other cells started producing IFN-I. This increased
35 viral sensing by non-pDCs was associated with an upregulation of TLR3 and IFI16 caused by IFN-I
36 in acute SIVagm infection. Our results suggest that, as in pathogenic SIVmac infection, SIVagm
37 infection mobilizes bone marrow precursor pDC. Moreover, we show that SIV infection modifies
38 the capacity of viral sensing in cells other than pDCs which could drive IFN-I production in
39 specific settings.

40

41

42 **Importance**

43 The effect of HIV/SIV infections on the capacity of plasmacytoid dendritic cells (pDC) to produce
44 IFN-I *in vivo* is still incompletely defined. As IFN-I can restrict viral replication, contribute to
45 inflammation and influence immune responses, alteration of this capacity could impact viral
46 reservoir size. We observed that even in non-pathogenic SIV infection, the frequency of pDC
47 capable to efficiently sense SIV and produce IFN-I is reduced during acute infection. We
48 discovered that, concomitantly, cells other than pDCs increased their ability of viral sensing. Our
49 results suggest that pDC-produced IFN-I upregulate viral sensors in bystander cells, the latter
50 gaining the capacity to produce IFN-I. These results indicate that in certain settings, cells other
51 than pDCs can drive IFN-I-associated inflammation in SIV infection. This has important
52 implications for the understanding of persistent inflammation and the development of anti-
53 inflammatory treatment strategies in HIV infection.

54 **Introduction**

55 Type I Interferons (IFN-I) play an important role in Human immunodeficiency virus (HIV) and
56 simian immunodeficiency virus (SIV) infection in humans and macaques (MACs). IFN-I can limit
57 viral replication through several mechanisms: upregulation of restriction factors (23),
58 maturation of antigen-presenting cells (13), activation of NK cells (42) and induction of
59 apoptosis in infected cells (22). Indeed, exogenous administration of IFN α in chronically infected
60 HIV patients decreased viremia by seventy-five percent (2). Similarly, IFN α administration in the
61 chronic phase of SIVagm or SIVsmm infection reduced viral loads (20, 50). In one study, it could

62 suppress viral rebound in HIV patients upon antiretroviral treatment interruption (3). Finally,
63 IFN-I blockage in acute SIVmac infection increased the viral reservoir and accelerated
64 progression to AIDS (45). However, IFN-I production can also be detrimental: IFN-I can
65 contribute to chronic immune activation (IA) (18), induce apoptosis of uninfected bystander
66 cells (17) and drive cells to a short-lived effector cell differentiation program (46). Interferon-
67 stimulated gene (ISG) expression associates with disease progression in HIV infection (30, 35,
68 43, 46). Evidence for the detrimental role of chronic IFN-I responses has come from the natural
69 hosts of SIV, such as African green monkeys (AGMs) and sooty mangabeys (SMs) (47). These
70 natural hosts have asymptomatic SIV infections, despite viremia similar to those found in
71 HIV/SIVmac infections. Non-pathogenic SIV infection in natural hosts associates with a lack of
72 chronic inflammation (29). This is despite a robust inflammatory response, exemplified by an
73 increase in ISG expression and production of cytokines, such as IL-15, during acute infection (6,
74 19, 20). However, this inflammatory response is downmodulated by the end of acute infection.
75 Peak IFN-I levels have been found to be lower in the plasma and lymph node cells of SIV-
76 infected AGMs than in MACs, indicating differences in IFN-I production between the two species
77 *in vivo* (9, 19). Nonetheless, exogenous injection of IFN-I at the peak of IFN-I production did not
78 alter the ISG profile observed in AGMs (20). Moreover, AGM and SM plasmacytoid dendritic
79 cells (pDCs) produce high IFN-I levels upon *in vitro* stimulation with synthetic TLR7/9 ligands, SIV
80 or Herpes simplex virus 1 (HSV) (5, 9, 11).

81 pDCs are responsible for the majority of IFN-I production in blood upon *in vitro* stimulation with
82 HIV and HSV (12, 49). In chronically infected HIV patients, pDCs express increased levels of IFN-I
83 mRNA and protein compared to healthy individuals (26, 27). pDCs sense HIV mainly through the

84 endosomal Toll-like receptor (TLR)7, although a role for TLR9 has not been excluded (4, 28).
85 Blocking endosomal signaling decreased IFN-I levels and IA in treated immunological non-
86 responders (39). In acute SIV infections, pDCs are the main IFN-I-producing cells in lymph nodes
87 (9, 16).

88 HIV/SIV infections have an impact on pDC distribution, phenotype and function. It has been
89 shown in SIVmac and SIVagm models that activated pDCs are rapidly recruited from the
90 circulation to lymph nodes (7, 11, 20). Simultaneously, precursor pDCs rapidly egress from the
91 bone marrow during acute SIVmac infection (7, 8). Precursor pDCs have a diminished capacity to
92 produce IFN-I upon viral stimulation (8, 33). In line with this, human pDCs were found to
93 produce less IFN-I upon HSV stimulation during late acute HIV infection, i.e. Fiebig stage III-IV
94 (21, 32). This could also be explained by pDCs becoming refractory after HIV encounter (49).
95 However, another study using purified pDCs reported an increased capacity to produce IFN-I
96 upon AT2-inactivated HIV stimulation during post-acute HIV infection (Fiebig stage V-VI) (44). *In*
97 *vitro*, HIV-1 is contained in an early endosomal compartment of pDCs, preventing pDC
98 maturation and associating with a continued IFN-I producing capacity (31, 37). AGM peripheral
99 blood mononuclear cells (PBMC) and lymph node cells collected during acute SIV infection
100 mounted a stronger IFN-I response upon HSV stimulation than cells from uninfected animals
101 (11). Thus, contradictory data exists on the IFN-I producing capacity of pDCs during acute
102 HIV/SIV infections. The capacity of natural host pDCs to produce IFN-I in response to SIV during
103 acute infection has not been investigated.

104 In this study, we investigated the effect of SIV infection on AGM pDC function to identify events
105 that could explain the different inflammatory profiles found in asymptomatic SIVagm infection

106 compared to pathogenic HIV/SIV infections. We assessed the IFN-I producing capacity of pDCs
107 collected during SIVagm infection in response to *in vitro* stimulation with SIV and HSV. Our data
108 suggest that precursor pDCs, with a decreased IFN-I producing capacity, circulate during acute
109 SIVagm infection. We found that low BDCA-2 expression on pDCs could specifically block the
110 sensing of SIV. Furthermore, we discovered that cells other than pDCs increase their capacity to
111 sense virus during acute infection.

112 **Methods**

113 **Ethics statement**

114 All animals were housed in the facilities of the Commissariat à l'Energie Atomique (CEA),
115 Fontenay-aux-Roses, France, permit number: A 92-032-02) or of Institut Pasteur (Paris, France,
116 permit number: A 78-100-3). All experimental procedures were conducted in strict accordance
117 with the international European guidelines 2010/63/UE on protection of animals used for
118 experimentation and other scientific purposes (French decree 2013-118). The CEA complies with
119 Standards for Human Care and Use of Laboratory of the Office for Laboratory Animal Welfare
120 (OLAW, USA) under OLAW Assurance number #A5826-01. All animal experimental protocols
121 were approved by the Ethical Committee of Animal Experimentation (CETEA-DSV, IDF, France)
122 (Notification numbers: 10-051b and 12-006).

123

124 **Animals and sample collection**

125 Twenty-four African green monkeys (*Chlorocebus sabaeus*) from Caribbean origin and ten
126 Chinese rhesus macaques (*Macaca mulatta*) were used. Blood was collected by venipuncture on
127 sodium heparin tubes that were shipped to Institut Pasteur. Fifteen AGMs were infected via

128 intravenous inoculation with 250 TCID₅₀ of SIVagm.sab92018 and displayed high viremia levels,
129 as described previously (10, 20). For eight AGMs, blood was drawn at days 2, 4, 7, 9, 11, 14, 25,
130 31, 59, 85, 122, 183, 241 and 354 post infection (p.i.). Three more AGMs were sampled at days
131 7, 14, 31 and 65 p.i. Another four AGMs were sampled at days 2, 4, 7, 9, 11, 14, 28, 43 and 58
132 p.i.

133

134 **Stimulations**

135 PBMCs were isolated from whole blood after 1:1 dilution in PBS (Life Technologies), using
136 Lymphocytes separation medium (Eurobio). Cells were spun down at 1100xg for 10' in 50mL
137 Leucosep tubes (Greiner Bio-one) and buffy coats were collected. Residual red blood cells were
138 lysed for 6' at room temperature in a sterile filtered buffer consisting of 1g/L potassium
139 bicarbonate, 8.3 g/L ammonium chloride (both from Sigma) and 1 mM EDTA pH 8 (Life
140 technologies). Cells were cultured at 0.5x10⁶ cells/well in 24-wells plates (Costar) at 37° C, 5%
141 CO₂ and at 10⁶ cells/mL in RPMI 1640 + glutamax medium (Life technologies) with 10% heat-
142 inactivated FCS (PAA or Eurobio) and penicillin-streptomycin (Life technologies).
143 SIVagm.sab92018 or SIVmac251 was added at a concentration of 1500 ng/mL p27, HSV-1 was
144 added at a 10⁶ TCID₅₀. A151, a gift from Pr. Olivier Schwartz (Institut Pasteur, Paris, France), and
145 G-ODN were added to the cells simultaneously with the virus to block the TLR7 and TLR9
146 pathway, respectively. Sodium azide was removed from antibodies against IFNαR2 (MMHAR-2,
147 PBL) and BDCA-2 (AC144, Miltenyi) using PD Minitrap G-25 (GE Healthcare Life Sciences),
148 followed by pre-incubation with these antibodies for an hour at 37° C before stimulation with
149 virus. Previously frozen plasma on EDTA from five AGMs per timepoint was pooled for

150 incubations (four hour at 37° C) with PBMC. PBMC were mock-stimulated in medium for each of
151 the experiments to assess spontaneous production of IFN-I.

152

153 **Functional Interferon alpha assay**

154 Bioactive Type I interferon was quantified as described earlier (1, 11). In short, Mardin-Darby
155 Bovine Kidney (MDBK) cells were incubated with UV-inactivated supernatants for 18 hours, after
156 which the cytopathic effect of vesicular stomatic virus was determined using the CellTiter 96®
157 AQueous Non-Radioactive Cell Proliferation Assay (Promega). IC₅₀ levels were calculated by
158 normalization to a standard using R version 2.15.3 and the “drc” package (40, 41).

159

160 **Flow cytometry**

161 Antibodies used were: CD3 (SP34-2), HLA-DR (L243), CD123 (7G3), CD45 (D058-1283) (all BD
162 Biosciences), CD20 (2H7, ebioscience), BDCA-2 (AC144) and IFN- α (LT27:295), (both Miltenyi).
163 FcR Blocking Reagent (Miltenyi) was used to block unspecific antibody binding. For intracellular
164 staining, cells were stimulated for two hours, after which brefeldin A was added and cells were
165 incubated for another four hours at 37°C. Then, cells were labeled with surface-binding
166 antibodies for ten minutes at room temperature. After fixation with 4% paraformaldehyde for
167 six minutes at 37°C, cells were permeabilized using 10% saponin and incubated with IFN α
168 antibody for fifteen minutes at room temperature. Events were collected on a LSR-II flow
169 cytometer (BD), running FACS Diva 6.0 software (BD), and analyzed with FlowJo 9.4.10
170 (TreeStar). Anti-mouse compensation beads (BD Biosciences) were used to define compensation
171 levels.

172

173 **Quantification of relative gene expression**

174 mRNA levels were quantified using RT-PCR as described previously on a 7500 real time PCR
175 machine (Applied Biosystems) (25). Briefly, mRNA was reverse transcribed with the high
176 capacity cDNA reverse transcription kit (Life technologies), followed by qPCR in triplicate with
177 Taqman gene expression assays (Life technologies). The expression of each gene was
178 normalized against that of 18S rRNA and relative expression levels were calculated using the
179 $\Delta\Delta_{ct}$ method.

180

181 **Statistical analyses**

182 Statistical inference analyses were performed using Prism 5.0 (GraphPad). For paired testing of
183 multiple groups without missing data, a Friedman test, followed by Dunn's multiple comparison
184 test, was employed. In case of missing data, the non-parametric Wilcoxon matched-pairs signed
185 rank test (Wilcoxon) was used to test paired observations with no multiple-testing correction.
186 The correlation between two continuous variables was assessed with the non-parametric
187 Spearman test.

188

189 **Results**

190 **SIVagm infection impacts the IFN-I producing capacity during the acute phase**

191 To determine the effect of SIV infection on AGM pDC function *in vivo*, we collected PBMC at
192 different stages before and after infection and stimulated them with SIVagm *in vitro*. We

193 compared IFN-I production to stimulation with another virus, HSV. PBMC were collected at
194 three timepoints before infection to get a stable baseline. To be able to get information on the
195 earliest and later events after infection, cells were collected and stimulated at seven timepoints
196 in acute infection and seven timepoints in chronic infection. Spontaneous IFN-I production by
197 mock-stimulated PBMC was never observed, even during day two and nine p.i., when high IFN-I
198 levels were present in the plasma. The AGM pDC response to *in vitro* SIV stimulation diminished
199 during acute SIV infection starting at day four p.i. (Figure 1A). This diminished response
200 persisted throughout acute infection until day thirty-one p.i. The median IFN-I level upon
201 stimulation with SIVagm before infection was 78 IU/mL, which decreased to 10 IU/mL at the
202 nadir, on days eleven, fourteen and twenty-five p.i. Statistically significant decreases were found
203 at days four and nine p.i. (Wilcoxon, $p = 0.03$ and $p = 0.02$, respectively), compared to the pre-
204 infection values. As pDCs have been reported to be depleted from the blood in acute HIV-1,
205 SIVmac and SIVagm infections (7, 38), we corrected for pDC counts (Figure 1B). Correction for
206 pDC numbers did not change the observed decreased responsiveness to SIV during acute
207 infection. The median of 0.057 IU/mL IFN-I per pDC before infection decreased to 0.022 IU/mL
208 at day four p.i. (Wilcoxon, $p = 0.04$) and 0.014 IU/mL at day nine p.i. (Wilcoxon, $p = 0.008$). The
209 decrease observed in the acute phase was temporary as the median IFN-I level in the chronic
210 phase (after day twenty-five p.i.) returned close to pre-infection levels: 0.044 IU/mL per pDC.

211 Upon *in vitro* stimulation with HSV of PBMC collected in acute SIVagm infection, we observed an
212 increase in the levels of IFN-I produced compared to before infection (Figure 1C). At days two
213 and nine p.i., there was a significant increase in IFN-I production, with levels of 1350 IU/mL
214 (Wilcoxon, $p = 0.02$) and 1200 IU/mL IFN-I (Wilcoxon, $p = 0.008$) respectively, compared to the

215 median pre-infection level of 239 IU/mL IFN-I. The response to HSV-1 stimulation was not
216 increased at any of the other days. When correcting for pDC numbers, we observed a more
217 sustained increase in IFN-I producing capacity throughout acute and early chronic infection
218 (Figure 1D). The levels of IFN-I produced upon HSV stimulation increased from 0.28 IU/mL per
219 pDC before infection to a zenith of 1.11 IU/mL and 1.12 IU/mL on days nine and thirty-one p.i.,
220 respectively. This increase was statistically significant (Wilcoxon, $p < 0.05$) at days seven, nine,
221 fifty-nine and eighty-five. So, while the IFN-I response to SIV was decreased, the responsiveness
222 to HSV was increased during acute SIVagm infection.

223 **Altered capacity to sense virus during acute SIVagm infection**

224 In order to understand the mechanisms underlying this differential response to SIV and HSV, we
225 investigated the hypothesis that the SIV-sensing machinery of AGM pDCs is specifically altered
226 by SIV infection, leading to a decreased capacity to respond to SIV, but not HSV. HIV and HSV
227 are mainly sensed by two distinct receptors in human pDCs, TLR7 and TLR9, respectively (4, 28).
228 We investigated whether the sensing of SIV by AGM pDCs also occurs through endosomal TLRs,
229 by stimulating PBMC of healthy AGMs with SIV in the presence of antagonists to TLR7 (A151)
230 and/or to TLR9 (G-ODN) (Figure 2A). Blocking TLR7 and/or TLR9 decreased the response to SIV
231 by AGM PBMC. IFN-I levels decreased from 60 IU/mL to 3 IU/mL and 10 IU/mL (Wilcoxon, $p =$
232 0.002 and $p = 0.004$), after blocking TLR7 and TLR9, respectively. Given the stronger than
233 expected inhibition upon TLR9 blockage, we repeated the experiment using MAC PBMC, which
234 were stimulated with either SIVagm or SIVmac. For MAC PBMC, the response to both viruses
235 (median of 147 IU/mL IFN-I) was lost upon blocking TLR7 (median of 14 IU/mL IFN-I), while it
236 was not significantly inhibited upon blocking TLR9 (median of 89 IU/mL, Figure 2A). Since MAC

237 pDCs reacted the same to TLR9 inhibition irrespectively of the viral isolate used, it is unlikely
238 that SIVagm is sensed more broadly than SIVmac. Therefore, AGM pDCs sense SIV through
239 endosomal TLRs as reported for MAC and human before.

240 BDCA-2 has been reported to be involved in attachment of HIV virions to the pDC surface (34). It
241 has been shown the ratio of precursor pCD expressing low levels of BDCA-2 is increased during
242 acute SIVmac infection (8, 33). If BDCA-2 is involved in the efficacy of endocytosis and sensing,
243 lowered expression would specifically affect SIV but not HSV. We therefore evaluated the
244 expression and function of BDCA-2 with respect to SIV- and HSV sensing. BDCA-2 levels on AGM
245 pDC were lower during acute infection than before infection (mean fluorescent intensity (MFI)
246 of 1486 pre-infection to a MFI of 660 at day 14 p.i., Wilcoxon, $p < 0.01$) (Figure 2B). This lower
247 BDCA-2 MFI in the acute phase probably reflected the circulation of precursor pDCs, expressing
248 low BDCA-2 levels, as BDCA-2 MFI closely correlated with the percentage of CD40⁺ pDCs
249 (Spearman, $r = 0.49$, $p = 0.0001$) and HLA-DR expression on pDCs (Spearman, $r = 0.75$, $p <$
250 0.0001) (Figure 2C). These molecules are expressed at lower levels on precursor pDC (33). To
251 assess the role of BDCA-2 in SIV sensing by pDCs, we stimulated AGM PBMC with SIV and HSV in
252 the presence of BDCA-2-blocking antibodies (Figure 2D). Blocking BDCA-2 prevented the
253 response to SIVagm, but not to HSV, in a dose-dependent manner. An isotype control did not
254 alter the IFN-I response to either SIV or HSV. At the end of the acute phase, BDCA-2 expression
255 was similar to pre-infection levels, concomitant with the recovery of IFN-I production upon SIV
256 stimulation in the chronic phase of SIVagm infection. Thus, the decreased response to SIV
257 correlated with a decrease in BDCA-2 on pDCs and an increase in the frequency of precursor
258 pDC in blood.

259 **Non pDCs produce IFN-I upon HSV stimulation due to a positive IFN-I feedback loop in vivo**

260 A higher ratio of precursor pDC should impact both SIV and HSV responses. In order to further
261 investigate the contrasting observations of an increased IFN-I response upon HSV stimulation
262 and an increased circulation of precursor pDCs during acute SIVagm infection, PBMC collected
263 during acute SIV infection were stimulated *in vitro* with HSV and analyzed by intracellular flow
264 cytometric staining for pDC-associated IFN α production (Figure 2E). A median of 91% IFN α_2^+
265 pDCs upon stimulation with HSV was observed in healthy animals. During infection, the
266 percentage of IFN α_2^+ pDCs in response to HSV stimulation fell to a nadir of 65% at day fourteen
267 p.i. After the transition from the acute to the chronic phase of infection, AGM pDCs regained
268 their capacity to produce IFN-I upon HSV stimulation. A non-parametric multiple comparison
269 test showed this was significant (Friedman, $p = 0.001$).

270 Although a decreased number of pDCs were producing IFN-I, a greater amount of IFN-I was
271 detected in the supernatant of HSV-stimulated cells collected in acute infection (Figure 1C). To
272 better understand this increased IFN-I production upon HSV stimulation, we searched for other
273 cellular sources of IFN-I. After stimulation with HSV, up to three percent of CD123 $^-$ cells, thus
274 non-pDCs, were producing IFN α_2 at days two, seven, eleven and fourteen p.i. (Figure 3A). The
275 majority of these IFN α_2^+ cells were CD4 $^-$ T cells, but other HLA-DR $^+$ cells also produced IFN α_2
276 (Figure 4). In the absence of stimulation, no IFN-I production was observed at any time before
277 and during infection (Figure 3B).

278 This substantial non-pDC associated IFN-I production was surprising. A positive association
279 between the levels of IFN-I in the plasma and the amount of IFN-I produced after stimulation

280 with HSV-1 existed during the acute phase (Spearman, $r = 0.58$, $p < 0.001$, Figure 5A and 5B). We
281 therefore hypothesized that IFN-I induced a positive auto-feedback loop. To test this hypothesis,
282 PBMC from healthy AGMs were incubated with plasma collected at various timepoints of
283 SIVagm infection and then stimulated with HSV (Figure 5C). Pre-incubation with plasma
284 collected during acute SIVagm infection increased the IFN-I response to HSV four-fold
285 (Wilcoxon, $p = 0.008$) compared to pre-incubation with plasma from healthy animals. In contrast
286 to acute phase plasma, plasma from animals in the chronic phase of infection did not increase
287 the IFN-I response to HSV. To confirm that IFN-I mediated this increased response to HSV, we
288 blocked the IFN α receptor 2 (IFN α R2) on AGM PBMC, incubated these cells with acute phase
289 plasma and subsequently stimulated with HSV. Blocking with α -IFN α R2, but not with an isotype
290 antibody, prevented the increase in IFN-I production (Wilcoxon, $p = 0.008$). Therefore, IFN-I was
291 required for this increased response to HSV.

292 As IFN α R signaling increased the response to HSV, we studied whether molecules involved in
293 HSV sensing were increased during acute SIV infection. Expression levels of four known HSV
294 sensors (TLR2, TLR3, TLR9 and IFI16) and three molecules associated with IFN-I production (IRF3,
295 IRF5 and IRF7) were measured in PBMC during acute SIVagm infection. Expression levels of
296 TLR3, TLR7, IFI16 and IRF7 were increased during acute SIVagm infection (Figure 6). In contrast
297 expression levels of TLR2, TLR9, IRF3 and IRF5 were not increased, or even decreased, during
298 acute SIVagm infection. TLR3 and IFI16 mRNA levels correlated with the amount of IFN-I
299 produced upon HSV stimulation (Figure 5D, Spearman, $r = 0.37$, $p = 0.01$ and $r = 0.39$, $p = 0.01$,
300 respectively). In contrast, IRF3, IRF5, IRF7, TLR2 and TLR9 expression levels did not associate
301 with the IFN-I response upon HSV stimulation (Figure 5D and not shown). These data show that

302 SIV infection induces an upregulation of at least two sensors in PBMC via IFN-I, which could
303 cause other cells than pDCs to increase their sensitivity for pathogen-associated molecular
304 patterns and to produce IFN-I upon viral encounter.

305 **Discussion**

306 During acute SIV infection, AGM PBMC produced less IFN-I upon *in vitro* stimulation with SIV,
307 but more upon stimulation with HSV, compared to before infection. Blocking BDCA-2 reduced
308 the *in vitro* IFN-I response to SIV, but not to HSV. During acute infection, we observed a lower
309 BDCA-2 expression on pDCs, which could thus specifically impact SIV sensing. Moreover, our
310 data suggest an egress of precursor pDCs during acute SIVagm infection, similar to what has
311 been reported for SIVmac infection (7, 8). As precursor pDC are less efficient in producing IFN-I
312 (33), this could also explain the decreased response to SIV.

313 While the IFN-I production increased upon stimulation of PBMC with HSV during the acute
314 phase, the percentage of pDCs that was producing IFN-I in response to HSV decreased.
315 Surprisingly, cells other than pDCs, notably T cells and also HLA-DR+CD3- cells, started producing
316 IFN-I upon *in vitro* stimulation with HSV during acute SIVagm infection. These data are in
317 agreement with immunohistochemistry data from the spleen of late-stage HIV-infected patients
318 where IFN α colocalized only with few pDCs, but rather with other cells, including T and B
319 lymphocytes, mDCs, and macrophages (36). It is possible that in immunocompromised patients,
320 reactivated latent viruses, such as cytomegalovirus, a member of the *herpesviridae* family, were
321 triggering this non-pDC associated IFN α -production, as these are abundant during late-stage HIV
322 infection (14). Moreover, the expanded gut virome and microbial translocation observed in

323 SIVmac, but not SIVagm, infection could contribute to chronic IA by triggering IFN-I responses in
324 cells normally not associated with these responses (15, 24).

325 The increased sensing of HSV by non-pDCs was due to a positive auto-feedback loop of IFN-I,
326 which is transiently present at high levels in the plasma during the acute phase of HIV/SIV
327 infections and also in late stage patients. While our data do not exclude that other factors might
328 be required in addition to IFN-I for this enhanced viral sensing, it is unlikely that SIV particles in
329 the plasma induced the increased IFN-I response as plasma from chronically infected animals
330 did not increase responsiveness. The increased response to HSV was associated with increased
331 expression levels of the viral sensors TLR3 and IFI16. These data are in line with a study showing
332 that TLR3-mediated sensing dominates the IFN-I response during *in vivo* HSV infection in mice
333 after the initial pDC-associated IFN-I responses (48). Such increased sensitivity might also occur
334 in HIV and SIVmac infections, which could have gone unnoticed as most studies using viral
335 stimuli other than SIV did not analyze such early and numerous timepoints as done here (11, 21,
336 32).

337 Collectively, our data show that SIVagm infection strongly impacts AGM pDC function and
338 dynamics during acute SIV infection, similar to what happens in HIV and SIVmac infections in
339 blood (7, 8). These modifications normalize however after acute SIVagm infection. The
340 previously reported low level of ISG expression during the chronic phase of SIVagm infection is
341 therefore, as suggested earlier, not a reflection of lack of IFN-I producing capacity by AGM pDCs
342 (5, 19). Furthermore, these results suggest that cells that are not classically involved in IFN-I
343 associated inflammation might be contributing to this during chronic HIV infection due to
344 upregulation of viral sensors in response to the existing inflammatory environment. These data

345 suggest that specifically targeting pDC-driven IFN-I responses is not sufficient to reduce
346 inflammation and that cells other than pDCs could be crucial in maintaining persistent
347 inflammation in HIV infection.

348

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362

363 **Conflict of interest**

364 The author(s) declare that they have do not have a commercial or other association that might
365 pose a conflict of interest.

366

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569

570 **Figure legends**

571

572 **Figure 1. Dynamics of type I interferon producing capacity during SIVagm infection.** We
573 stimulated AGM PBMC collected before and during the course of SIVagm infection and
574 measured IFN-I production. The x-axis displays the day post infection at which cells were
575 isolated and stimulated with (A, B) SIV or (C, D) HSV. The y-axis shows (A, C) the total IFN-I
576 produced after 18 hours of stimulation or (B,D) the same IFN-I production corrected for the
577 number of plasmacytoid dendritic cells among the PBMC. Grey circles represent pre-infection,
578 upward triangles acute infection and downward triangles post-acute and chronic infection
579 timepoints. The dotted line shows the median for all pre-infection timepoint stimulations.
580 Symbols represent individual animals (n = 5-11 for each time point). * Wilcoxon, $p < 0.05$, **
581 Wilcoxon, $p < 0.01$. The p-values indicate a significant difference compared to the pre-infection
582 baseline.

583 **Figure 2. Molecules involved in SIV sensing by AGM plasmacytoid dendritic cells.** (A) PBMC
584 from ten non-infected AGMs (open circles) and seven to nine MACs (closed circles) were
585 stimulated with mock (grey) or SIVagm or SIVmac (black). A151 [5µg/mL] and G-ODN [0.5 µM]
586 were added at the same time as SIV to inhibit the TLR7 and TLR9 pathway respectively. (B)
587 Longitudinal follow-up of MFI levels of BDCA-2 on pDCs during early SIVagm infection. BDCA-2
588 expression was followed for four AGMs. (C) Expression of BDCA-2, CD40 and HLA-DR was
589 measured simultaneously on AGM pDCs. pDCs were defined as CD20⁻HLA-DR⁺CD123⁺ BDCA2⁺
590 cells. MFI = mean fluorescent intensity, r = Spearman's correlation. (D) α-BDCA2 or irrelevant
591 IgG1 antibodies were added to PBMC of six uninfected AGM for 1 hour at concentrations of 1
592 µg/mL and 10 µg/mL. After stimulation with SIVagm (S, light grey) or HSV-1 (H, dark grey) for 18
593 hours, we measured IFN-I levels in the supernatant. IFN-I levels were normalized to IFN-I
594 produced after no incubation with antibodies. The dotted line represents no difference
595 compared to stimulation without antibodies. (E) Percentage of IFNα₂⁺ pDCs upon *in vitro* HSV
596 stimulation at different timepoints of SIVagm infection. PBMC were collected before and after
597 SIVagm infection and pDCs analyzed for IFNα₂ production after *in vitro* stimulation. pDCs were
598 defined as CD3⁻HLA-DR⁺CD123⁺ cells. Symbols represent individual animals. * Wilcoxon, p <
599 0.05, ** Wilcoxon, p < 0.01, *** Wilcoxon, p < 0.001

600 **Figure 3. Non pDC-associated IFNα production.** Dot plots showing CD123 and IFNα₂ expression
601 on whole PBMC collected throughout SIVagm infection. One representative AGM (SV048) out of
602 four analyzed is shown. (A) Cells after stimulation with HSV. (B) Mock-stimulated cells.

603 **Figure 4. Identification of cell types producing IFN-I upon HSV stimulation.** PBMC from one
604 representative AGM (SV061) out of four are shown. The chosen timepoint day two post

605 infection is representative of days two, nine and eleven post infection. PBMC were gated for
606 distinct cell populations to identify cell types that produce IFN α_2 . 5.5% of the CD4⁺ T cells, 85.7%
607 of the pDCs and 1% of non-T, non-pDC cells were IFN α ⁺ after HSV stimulation. All IFN α -
608 producing cells expressed high levels of HLA-DR.

609 **Figure 5. Auto-feedback loop of IFN-I during acute SIV infection of AGMs.** (A) IFN-I levels
610 present in the plasma of eight acutely SIV infected AGMs. (B) Correlation between IFN-I levels in
611 the plasma of AGMs during acute SIVagm infection *in vivo* and the amount of IFN-I produced
612 upon *in vitro* HSV stimulation. (C) Plasma collected from healthy AGMs, acutely infected AGMs
613 and chronically infected AGMs was diluted to 5% in RPMI and added to PBMC of eight healthy
614 AGMs. After four hours of incubation, cells were stimulated with herpes simplex virus 1 (HSV)
615 and after another eighteen hours, supernatants were collected for IFN-I quantification. PBMC
616 were incubated with anti-IFN α Receptor 2 (α -IFN α R2) or with an irrelevant antibody as a
617 negative control at 10 μ g/mL for one hour to block IFN α signaling before adding plasma.
618 Symbols represent individual animals. (D) Correlation between mRNA levels of known HSV
619 sensors in PBMC of AGMs during acute SIVagm infection *in vivo* and the amount of IFN-I
620 produced upon *in vitro* HSV stimulation of the same PBMC. ** Wilcoxon, $p < 0.01$. IFI16 =
621 Interferon, Gamma-Inducible Protein 16. TLR = Toll-like receptor. r = Spearman's correlation.

622 **Figure 6. Gene expression profiles of genes involved in HSV sensing and IFN-I responses.**
623 mRNA levels were measured in PBMC from six animals (same animals as in Figure 5) during
624 SIVagm infection. The grey lines indicate individual animals and the black line the median of all
625 animals * Wilcoxon, $p < 0.05$, ** Wilcoxon, $p < 0.01$, *** Wilcoxon, $p < 0.001$. The p-values
626 indicate a significant difference compared to the pre-infection baseline.

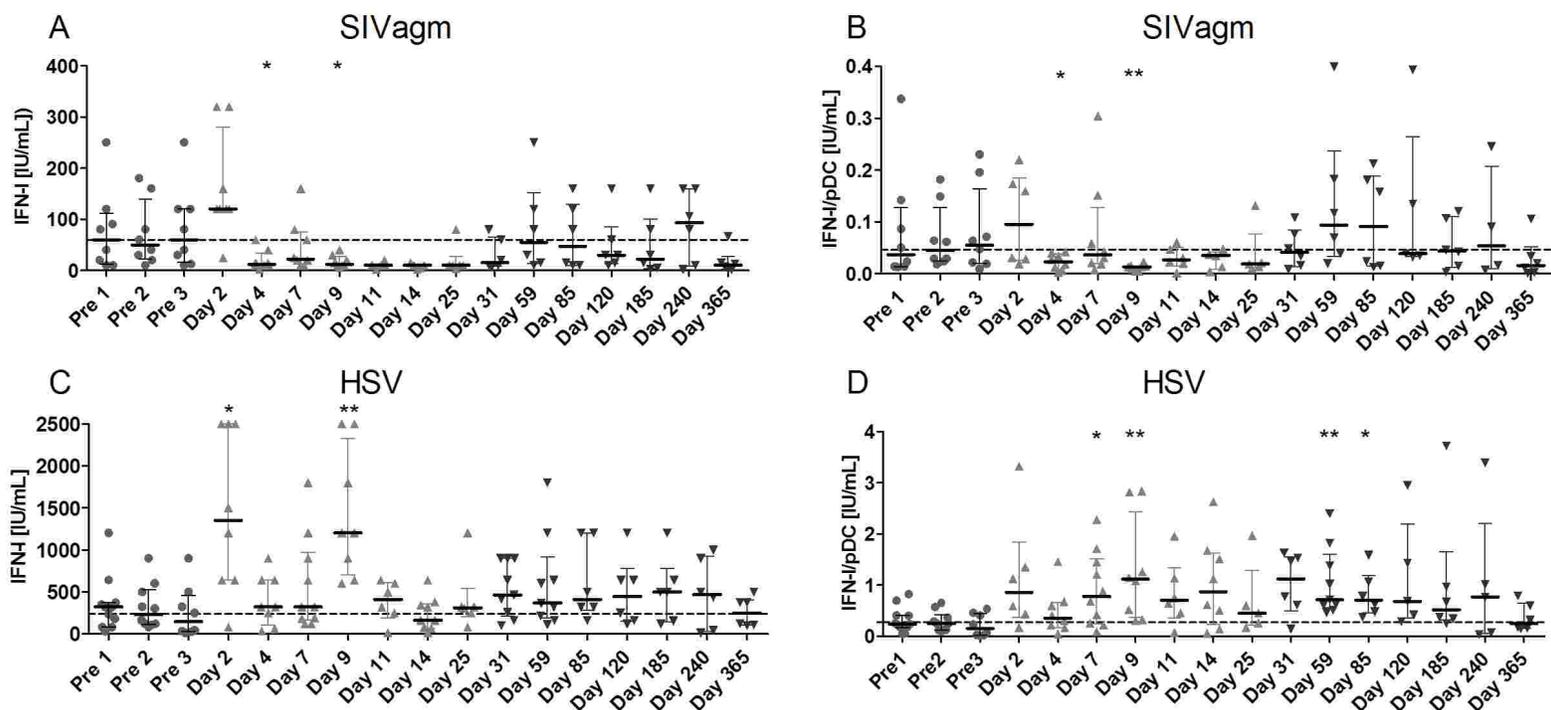


Figure 1. Dynamics of type I interferon producing capacity during SIVagm infection. We stimulated AGM PBMC collected before and during the course of SIVagm infection and measured IFN-I production. The x-axis displays the day post infection at which cells were isolated and stimulated with (A, B) SIV or (C, D) HSV. The y-axis shows (A, C) the total IFN-I produced after 18 hours of stimulation or (B, D) the same IFN-I production corrected for the number of plasmacytoid dendritic cells among the PBMC. Grey circles represent pre-infection, upward triangles acute infection and downward triangles post-acute and chronic infection timepoints. The dotted line shows the median for all pre-infection timepoint stimulations. Symbols represent individual animals (n = 5-11 for each time point). * Wilcoxon, $p < 0.05$, ** Wilcoxon, $p < 0.01$. The p-values indicate a significant difference compared to the pre-infection baseline.

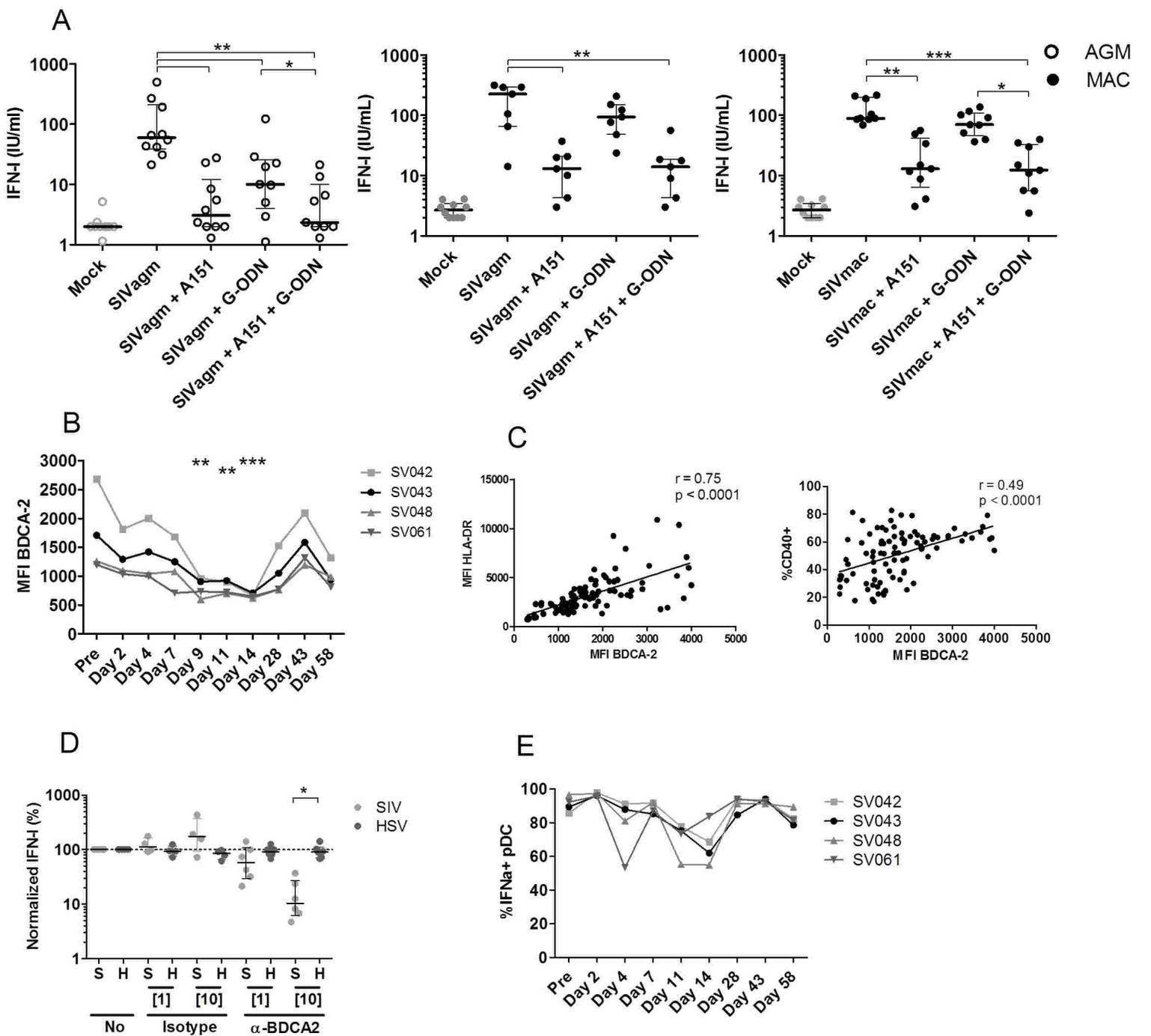


Figure 2. Molecules involved in SIV sensing by AGM plasmacytoid dendritic cells. (A) PBMC from ten non-infected AGMs (open circles) and seven to nine MACs (closed circles) were stimulated with mock (grey) or SIVagm or SIVmac (black). A151 [5ug/mL] and G-ODN [0.5 uM] were added at the same time as SIV to inhibit the TLR7 and TLR9 pathway respectively. (B) Longitudinal follow-up of MFI levels of BDCA-2 on pDCs during early SIVagm infection. BDCA-2 expression was followed for four AGMs. (C) Expression of BDCA-2, CD40 and HLA-DR was measured simultaneously on AGM pDCs. pDCs were defined as CD20-HLA-DR+CD123+ BDCA2+ cells. MFI = mean fluorescent intensity, r = Spearman's correlation. (D) α -BDCA2 or irrelevant IgG1 antibodies were added to PBMC of six uninfected AGM for 1 hour at concentrations of 1 ug/mL and 10 ug/mL. After stimulation with SIVagm (S, light grey) or HSV-1 (H, dark grey) for 18 hours, we measured IFN-I levels in the supernatant. IFN-I levels were normalized to IFN-I produced after no incubation with antibodies. The dotted line represents no difference compared to stimulation without antibodies. (E) Percentage of IFN α 2+ pDCs upon in vitro HSV stimulation at different timepoints of SIVagm infection. PBMC were collected before and after SIVagm infection and pDCs analyzed for IFN α 2 production after in vitro stimulation. pDCs were defined as CD3-HLA-DR+CD123+ cells. Symbols represent individual animals. * Wilcoxon, $p < 0.05$, ** Wilcoxon, $p < 0.01$, *** Wilcoxon, $p < 0.001$

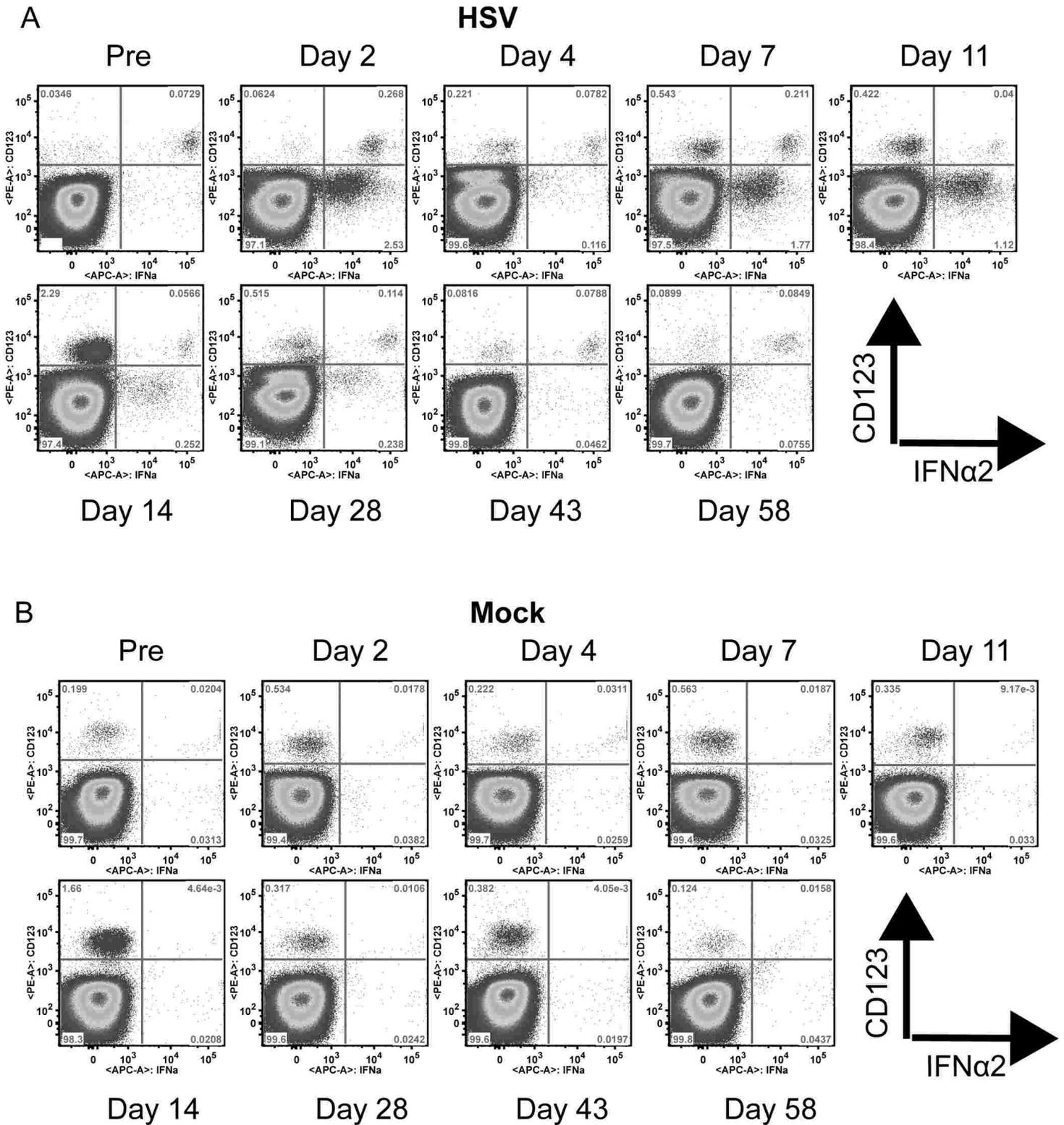


Figure 3. Non pDC-associated IFN α production. Dot plots showing CD123 and IFN α 2 expression on whole PBMC collected throughout SIVagm infection. One representative AGM (SV048) out of four analyzed is shown. (A) Cells after stimulation with HSV. (B) Mock-stimulated cells.

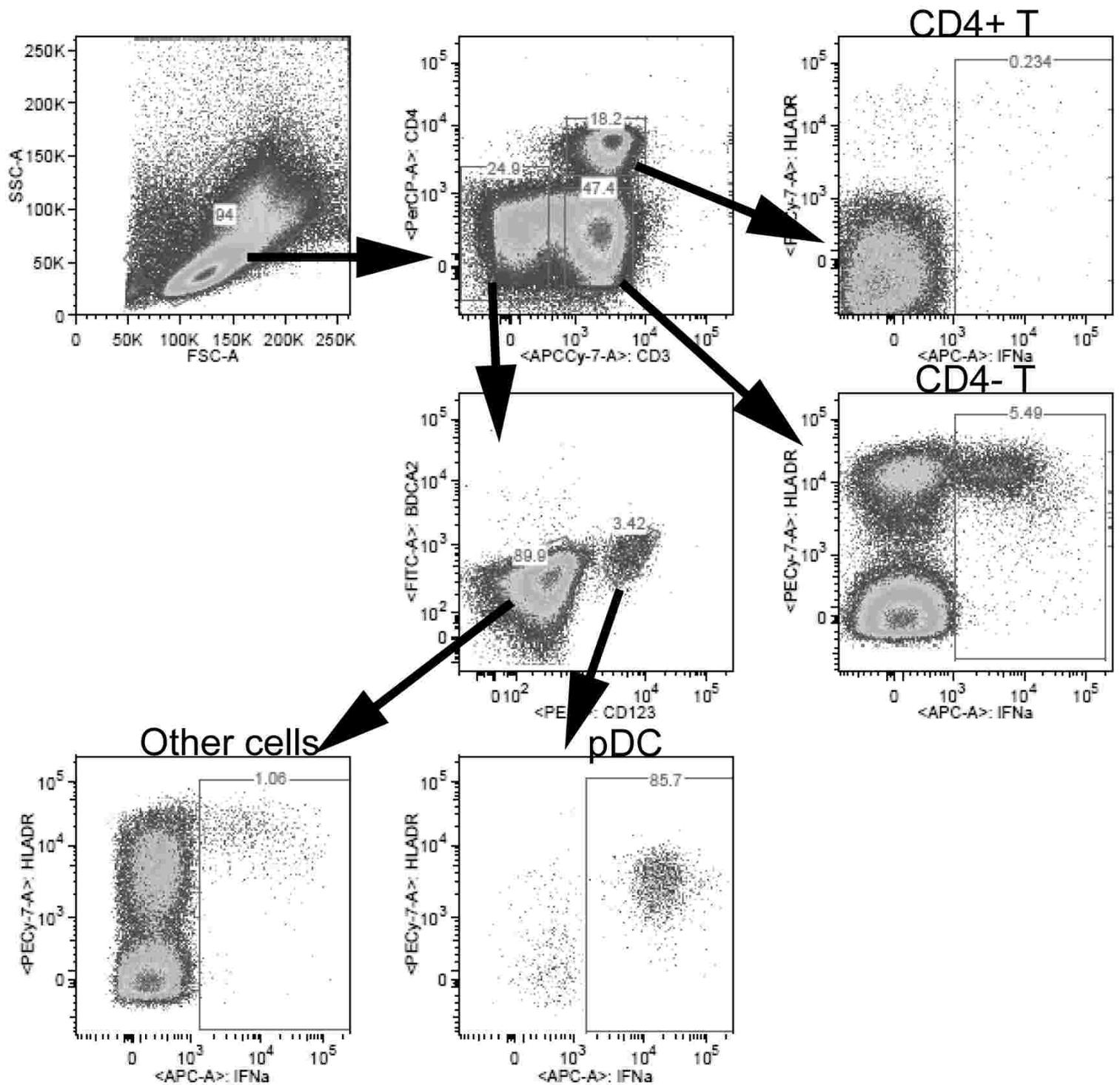


Figure 4. Identification of cell types producing IFN-I upon HSV stimulation. PBMC from one representative AGM (SV061) out of four are shown. The chosen timepoint day two post infection is representative of days two, nine and eleven post infection. PBMC were gated for distinct cell populations to identify cell types that produce IFN α 2. 5.5% of the CD4- T cells, 85.7% of the pDCs and 1% of non-T, non-pDC cells were IFN α + after HSV stimulation. All IFN α -producing cells expressed high levels of HLA-DR.

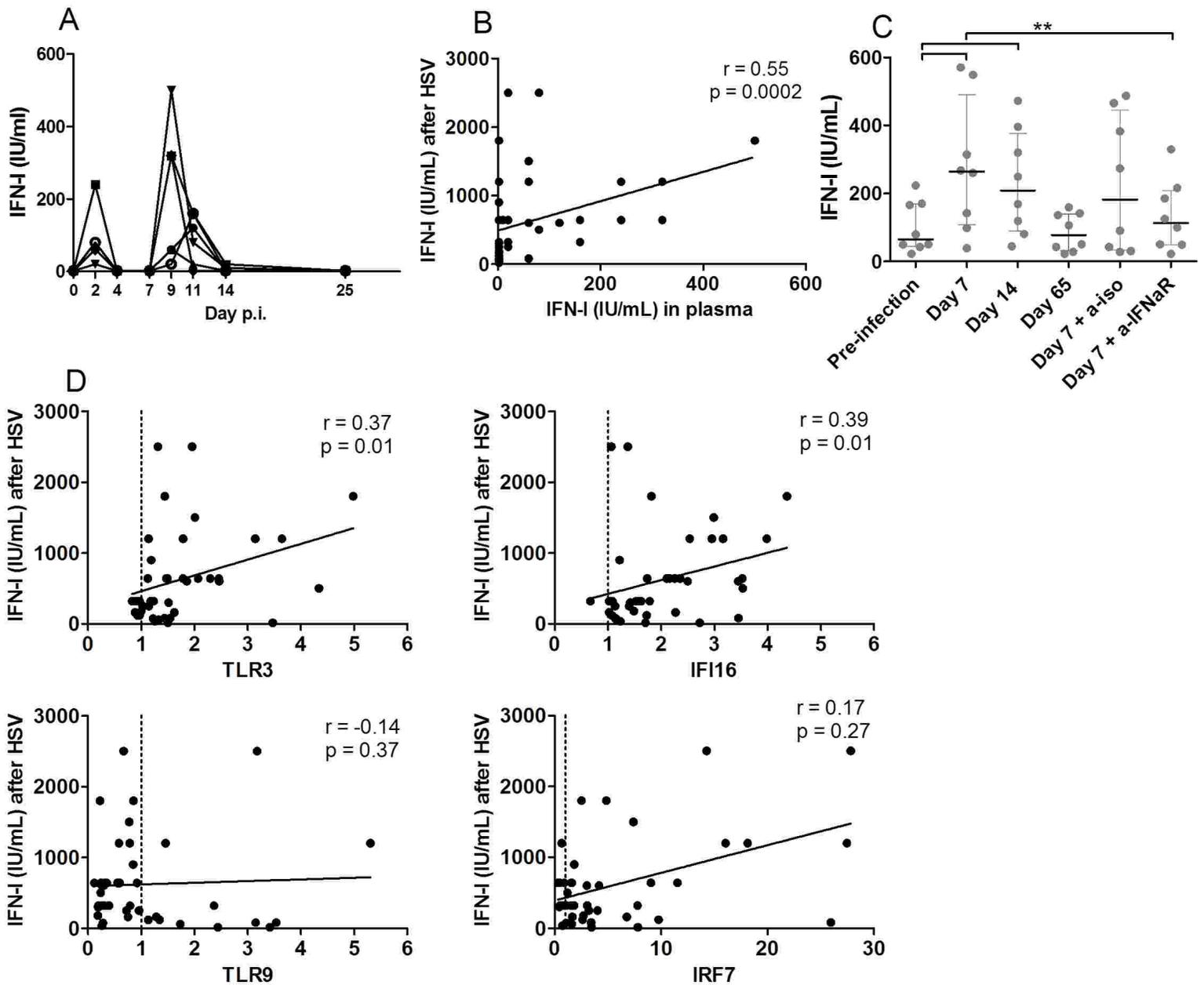


Figure 5. Auto-feedback loop of IFN-I during acute SIV infection of AGMs. (A) IFN-I levels present in the plasma of eight acutely SIV infected AGMs. (B) Correlation between IFN-I levels in the plasma of AGMs during acute SIVagm infection in vivo and the amount of IFN-I produced upon in vitro HSV stimulation. (C) Plasma collected from healthy AGMs, acutely infected AGMs and chronically infected AGMs was diluted to 5% in RPMI and added to PBMC of eight healthy AGMs. After four hours of incubation, cells were stimulated with herpes simplex virus 1 (HSV) and after another eighteen hours, supernatants were collected for IFN-I quantification. PBMC were incubated with anti-IFN γ Receptor 2 (a-IFN γ R2) or with an irrelevant antibody as a negative control at 10 μ g/mL for one hour to block IFN-I signaling before adding plasma. Symbols represent individual animals. (D) Correlation between mRNA levels of known HSV sensors in PBMC of AGMs during acute SIVagm infection in vivo and the amount of IFN-I produced upon in vitro HSV stimulation of the same PBMC. ** Wilcoxon, $p < 0.01$. IFI16 = Interferon, Gamma-Inducible Protein 16. TLR = Toll-like receptor. r = Spearman's correlation.

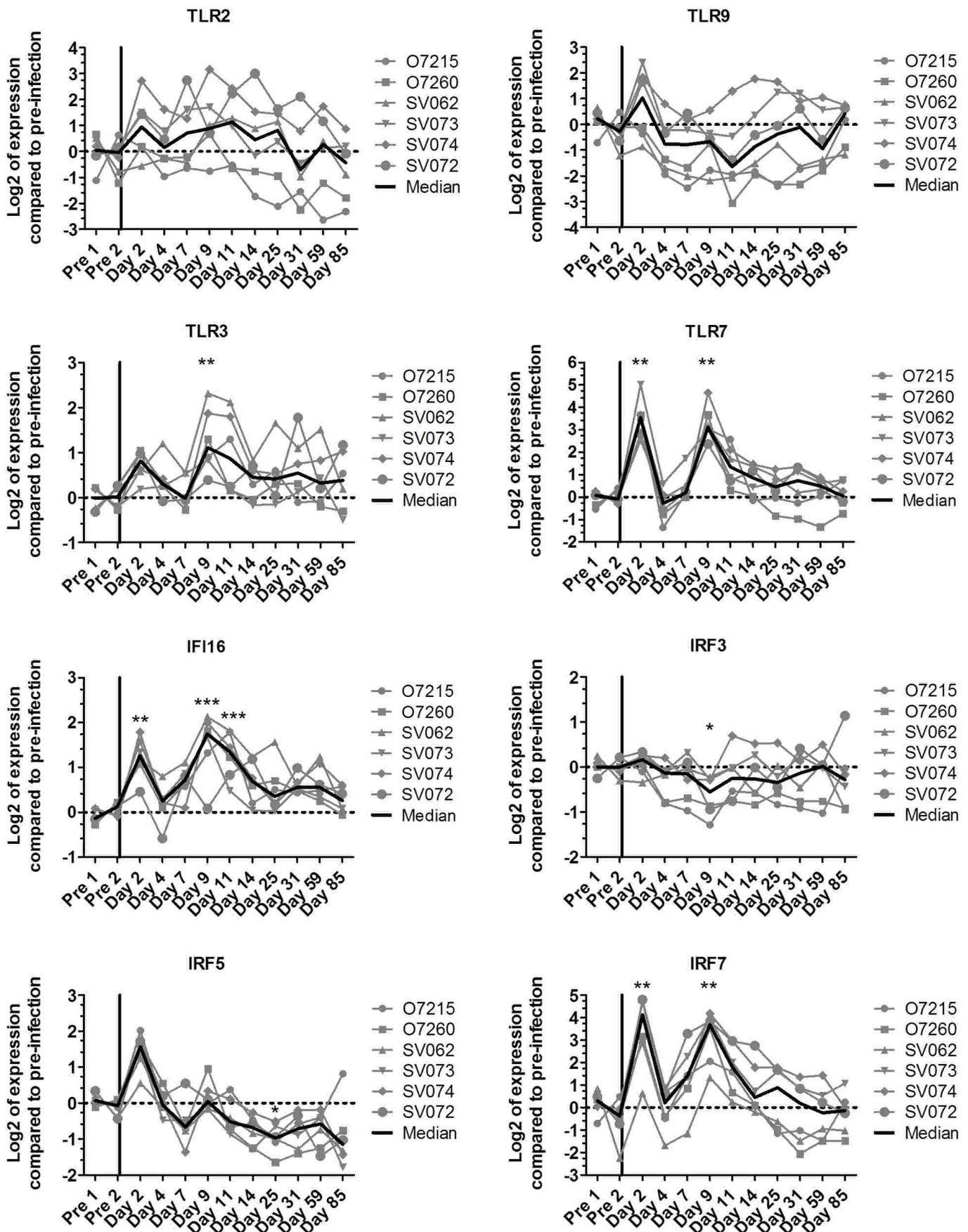


Figure 6. Gene expression profiles of genes involved in HSV sensing and IFN-I responses. mRNA levels were measured in PBMC from six animals (same animals as in Figure 5) during SIVagm infection. The grey lines indicate individual animals and the black line the median of all animals * Wilcoxon, $p < 0.05$, ** Wilcoxon, $p < 0.01$, *** Wilcoxon, $p < 0.001$. The p-values indicate a significant difference compared to the pre-infection baseline.