



Plasmacytoid Dendritic Cell Infection and Sensing Capacity during Pathogenic and Nonpathogenic Simian Immunodeficiency Virus Infection.

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1 **Plasmacytoid dendritic cell infection and sensing capacity during pathogenic and non-**
2 **pathogenic SIV infection**

3 **Running title: Low AGM pDC CD4 and CCR5 levels allow SIV infection**

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21 **Abstract**

22 HIV in humans and SIV in macaques (MAC) lead to chronic inflammation and AIDS. Natural
23 hosts, such as African green monkeys (AGM) and sooty mangabeys (SM), are protected against
24 SIV-induced chronic inflammation and AIDS. Here, we report that AGM plasmacytoid dendritic
25 cells (pDC) express extremely low levels of CD4, unlike MAC and human pDC. Despite this, AGM
26 pDC efficiently sensed SIVagm, but not heterologous HIV/SIV isolates, indicating a virus-host
27 adaptation. Moreover, both AGM and SM pDC were found to be, in contrast to MAC pDC,
28 predominantly negative for CCR5. Despite such limited CD4 and CCR5 expression, lymphoid
29 tissue pDC were infected to a similar degree as CD4⁺ T cells, both in MAC and AGM. Altogether,
30 our finding of efficient pDC infection by SIV *in vivo* identifies pDC as a potential viral reservoir in
31 lymphoid tissues. We discovered low expression of CD4 on AGM pDC, which did not preclude
32 efficient sensing of host-adapted viruses. Therefore, pDC infection and efficient sensing are not
33 prerequisites for chronic inflammation. The high level of pDC infection by SIVagm suggests that
34 if CCR5 paucity on immune cells is important for non-pathogenesis of natural hosts, it is possibly
35 not due to its role as a co-receptor.

36 **Importance**

37 The ability of certain key immune cell subsets to resist infection might contribute to the
38 asymptomatic nature of simian immunodeficiency virus (SIV) infection in its natural hosts, such
39 as African green monkeys (AGM) and sooty mangabeys (SM). This relative resistance to
40 infection has been correlated with reduced expression of CD4 and/or CCR5. We show that
41 plasmacytoid dendritic cells (pDC) of natural hosts display a reduced CD4 and/or CCR5
42 expression, unlike macaque pDC. Surprisingly, this did not protect AGM pDC, as infection levels

43 were similar to those found in MAC pDC. Furthermore, we show that AGM pDC did not
44 consistently produce IFN-I upon heterologous SIVmac/HIV-1 encounter, while they sensed
45 autologous SIVagm isolates. Pseudotyping SIVmac/HIV-1 overcame this deficiency, suggesting
46 that reduced uptake of heterologous viral strains underlay this lack of sensing. The distinct IFN-I
47 responses depending on host species and HIV/SIV isolates reveal host/virus species-specificity of
48 pDC sensing.

49 **Introduction**

50 Chronic inflammation and immune activation in HIV-infected individuals and in SIV-infected
51 macaques (MAC) lead to depletion of CD4⁺ T cells and progression to AIDS. Natural hosts of SIV,
52 such as African green monkeys (AGM) and sooty mangabeys (SM), do not display chronic
53 inflammation or AIDS (1). This is due to resolution of inflammation before the end of acute
54 infection, rather than to a lack of SIV recognition by the innate immune system (2). Natural
55 hosts further differ from pathogenic HIV/SIV infections by exhibiting reduced infection rates in
56 certain cell subsets, such as central memory CD4⁺ T cells (T_{cm}) (3, 4). This relative resistance has
57 been linked to a reduced expression of the HIV/SIV co-receptor CCR5 on natural host CD4⁺ T
58 cells and to a downmodulation of CD4 on activated CD4⁺ T cells in AGM (3-5).

59 Plasmacytoid dendritic cells (pDC) form a rare cell population that is responsible for the vast
60 majority of IFN-I production after HIV encounter (6). This is also true for AGM pDC as the
61 depletion of pDC from AGM PBMC completely abrogates the IFN-I response to SIVagm
62 stimulation (7). HIV/SIV sensing by pDC is mediated through endocytosis followed by TLR7/9
63 engagement. It requires CD4, but is independent of co-receptor expression (6). Data on the
64 infection rates of pDC *in vivo* are scarce. One study reported the presence of HIV DNA in

65 circulating pDC of chronically HIV-infected patients (8). Another study reported high infection
66 levels in lymph node (LN) pDC during acute SIVmac infection (9).

67 Here, we discovered a restricted CD4 and/or CCR5 expression on pDC in natural hosts. We
68 evaluated the effect of low CD4 expression on the capacity of AGM pDC to efficiently sense
69 distinct forms of SIVagm (free virus, non-infectious particles and SIVagm-infected cells).
70 Furthermore, we examined the infection frequency of pDC during pathogenic and non-
71 pathogenic SIV infection.

72 **Materials and Methods**

73 **Study Approval**

74 All animal experimental protocols were approved by either the Ethical Committee of Animal
75 Experimentation (CETEA-DSV, IDF, France) (Notification numbers: 10-051b and 12-006) or by the
76 Institutional Animal Care and Use Committees (IACUC) of Emory University (IACUC protocol
77 #2000793, entitled “Comparative AIDS Program”). Animals were housed in the facilities of the
78 CEA (“Commissariat à l’Energie Atomique”, Fontenay-aux-Roses, France, permit number: A 92-
79 032-02), Institut Pasteur (Paris, France, permit number: A 78-100-3) or Yerkes National Primate
80 Research Center (Atlanta, GA, USA). All experimental procedures were conducted in strict
81 accordance with the international European guidelines 2010/63/UE on protection of animals
82 used for experimentation and other scientific purposes (French decree 2013-118) and with the
83 recommendations of the Weatherall report or in strict accordance with USDA regulations and
84 the recommendations in the Guide for the Care and Use of Laboratory Animals of the National
85 Institutes of Health. The CEA complies with Standards for Human Care and Use of Laboratory of

86 the Office for Laboratory Animal Welfare (OLAW, USA) under OLAW Assurance number #A5826-
87 01. The monitoring of the animals was under the supervision of the veterinarians in charge of
88 the animal facilities.

89 **In vivo infections and sample collection**

90 Twenty-six African green monkeys (*Chlorocebus sabaenus*) of the sabaenus species with a
91 Caribbean origin, eighttteen Chinese rhesus macaques (*Macaca mulatta*), two cynomolgous
92 macaques (*Macaca fascularis*), sixteen Indian rhesus macaques (*Macaca mulatta*) and sixteen
93 sooty mangabeys (*Cercocebus atys*) were used in this study. Eleven AGMs were infected via
94 intravenous inoculation with 250 TCID₅₀ of SIVagm.sab92018, as described previously (10, 11).
95 Four Chinese rhesus macaques were i.v. infected with 50 AID₅₀ SIVmac251 and two others were
96 infected with 5000 AID₅₀ SIVmac251, as described previously (10, 12). Eight Indian rhesus
97 macaques had been previously infected with SIVmac239 or SIVmac251 and eight sooty
98 mangabeys were either naturally infected or infected experimentally with SIVsmE041 (13, 14).
99 Blood was collected by venipuncture on sodium heparin tubes and shipped to Institut Pasteur or
100 used on site at Yerkes National Primate Research Center. Bone marrow mononuclear cells were
101 isolated on Ficoll and tissue cells were put in suspension before staining. LNs and spleens were
102 disrupted mechanically and rectal tissues were enzymatically degraded as described previously
103 (12, 15).

104 **Viral stimulations**

105 Freshly isolated PBMCs were cultured at 0.5x10⁶ cells/well in 24-well plates (Costar) at 37° C, 5%
106 CO₂ for eighteen hours with or without virus. Cell viability was measured using trypan blue and a
107 Countess (Life Technologies) cell counter. The SIVagm strains used, have been previously

108 described: SIVagm.sab92018 (11), SIVagm.sabD46 and SIVagm.tanB14 (16), SIVagm.sab1c (11,
109 17) and SIVgri1 (18, 19). Free SIVagm was added to PBMCs at a concentration of 1500 ng/mL
110 p27, unless indicated otherwise. HSV-1 was added at a TCID₅₀ of 2x10⁵. HIV-1.Bal-VSV, which is
111 endocytosed independently of CD4 (20), was kindly provided by A. David and AT2-inactivated
112 SIVagm as well as control microvesicles by Dr. Jeff Lifson (National Cancer Institute, Frederick,
113 MD). SIVmac251-VSV was produced by co-transfecting 293T cells with SIVmac251Δenv and VSV-
114 G expression vector using SuperFect (Qiagen), as described previously (21). SIVagm isolates
115 were grown on SupT1 cells, as these cells express Bonzo and are susceptible to SIVagm while
116 SIVmac isolates were grown on CEMx174 as these cells express Bob and are susceptible to
117 SIVmac (22).

118 **Production of infected cells**

119 Cells were infected as previously described (23). Briefly, SupT1 cells were exposed for one hour
120 at 37° C to 3.3x10⁴ TCID₅₀ per 10⁶ cells under constant agitation. Infection levels were assessed
121 by measuring SIV Gag⁺ cells using flow cytometry (see below).

122 **Functional Interferon alpha assay**

123 Bioactive IFN-I levels were quantified as described earlier (7). In short, Mardin-Darby Bovine
124 Kidney (MDBK) cells were incubated with UV-inactivated supernatants for 18 hours, after which
125 the cytopathic effect of vesicular stomatic virus was determined using the CellTiter 96®
126 AQueous Non-Radioactive Cell Proliferation Assay (Promega). Alternatively, a cell line stably
127 transfected with a luciferase gene under an IFN-I inducible promoter was used to measure IFN-I
128 levels, as described previously (23). The limit of detection threshold was set at 2 IU/mL.

129 **Flow cytometry**

130 SM flow cytometric data were acquired at Yerkes National Primate Center and data for AGM
131 were acquired at Institut Pasteur. MAC data were acquired at both Yerkes and Pasteur, with
132 similar results. The following antibodies were used to identify and characterize pDC phenotype
133 and function in whole blood or isolated tissue cells: CD3 (SP34-2), CD4 (L200), HLA-DR (L243),
134 CCR5 (3A9), CD123 (7G3) (all BD Biosciences), CD20 (2H7, ebioscience), CD4 (M-T466), BDCA-2
135 (AC144) (all Miltenyi) and CD4 (SFC112T4D11, Beckman Coulter). FcR Blocking Reagent (Miltenyi)
136 was used to block unspecific antibody binding and in experiments using tissues other than
137 blood, Live/Dead cell viability (Invitrogen) was used to exclude dead cells. For intracellular
138 staining, cells were labeled with surface-binding antibodies and fixed with 4%
139 paraformaldehyde and permeabilized using saponin prior to incubation with anti-CD4. For
140 intracellular SIVagm detection, anti-p24 staining (KC57, Beckman Coulter) was used after cell
141 permeabilization with the IntraPrep Permeabilization Reagent Kit (Beckman Coulter) according
142 to manufacturer's instructions (23). Events ranging from one hundred to one thousand pDC
143 were collected on a BD LSR-II flow cytometer, running BD FACS Diva 6.0 software, and analyzed
144 with FlowJo 9.4.10 (TreeStar). Anti-mouse compensation beads (BD Biosciences) and Arc Amine
145 Reactive Compensation Bead Kit (Life Technologies) were used to define compensation levels.
146 An isotype control antibody was used to define CCR5⁺ cells.

147 **Cell sorting**

148 Splenocytes were frozen in 10% DMSO in liquid nitrogen until use. Cells were thawed in the
149 presence of DNase I (10 IU/mL, Roche) and washed in FCS. Cells were labeled with antibodies
150 against CD3, CD20, HLA-DR, CD4, CD123 and with Live/Dead reagent in the presence of FcR
151 Blocking Reagent. Cells were sorted using a FACSAria II sorter (BD), running on BD FACS Diva 6.0

152 software. Sorted pDC and CD4⁺ T cells were purified a second time to increase purity of the two
153 fractions. CD4⁺ T cells were also isolated from LNs cells using anti-CD4 beads and magnetic
154 stands (Miltenyi) following manufacturer's protocol, after which purity was verified by flow
155 cytometry.

156 **SIV DNA quantification**

157 DNA was extracted as follows: samples were lysed in NaCl (3M), EDTA (0.5M, pH 8), SDS (10%,
158 Bio-Rad) and proteinase K (1mg/mL, Qiagen) in a 45 min incubation at 55 ° C. Then, NaCl (5M)
159 was added and incubated at 4 ° C between 15 and 60 min, followed by centrifugation for 15
160 min at 3000 rpm at 4 ° C. DNA was then precipitated from the supernatant in
161 phenol:chloroform:isoamyl alcohol 25:24:1 (pH = 8, Sigma Aldrich). After DNA extraction, viral
162 DNA was measured by qPCR in duplicate, using primers and probes designed specifically for
163 SIVagm.sab and SIVmac (11, 15). SIVagm and SIVmac plasmids were used as standards to
164 calculate SIV DNA copy numbers. CCR5 DNA quantification was used to normalize the viral levels
165 to the number of cells (24). Sample preparation, enzyme mix preparation and PCR set-up were
166 performed in three separate rooms to avoid PCR contamination. Positive and negative controls
167 were used to exclude sample contamination.

168 **Fluorescence microscopy**

169 Fluorescence microscopy was done as follows with markers against NKp30 (AF29-4D12,
170 Miltenyi), CD123 (5B11, Biolegend), DAPI and SIVagm *env* RNA (made from the region amplified
171 by 5'-GAG GCT TGT GAT AAA ACT TAT TGG GAT-3' and 5'-AGA GCA GTG ACG CGG GCA TTG
172 AGG-3' primers and labeled with fluorochrome Alexa 488 (Life Technologies)). Briefly,
173 cryopreserved sections were permeabilized by incubating in 0.5% (v/v) Triton X-100. This was

174 followed by hybridization of probe and mounted antibodies on tissue. Secondary antibodies
175 were used with to visualize the bound antibodies. Donkey anti-mouse IgG-CFL 594 (Santa Cruz)
176 was used to detect CD123 and Zenon Alexa Fluor 647 Mouse IgG1 Labeling Kit (Life
177 technologies) to reveal the NKP30 antibody. As negative controls for SIV RNA in situ
178 hybridization, we used a RNase degraded probe, taken up in hybridization buffer, as well as
179 lymph nodes of uninfected animals. Images were acquired on a Confocal Laser Scanning
180 Microscope Leica TCS SP8, running LAS AF 3 (Leica Application Suite Advanced Fluorescence).

181 **Statistics**

182 Statistical inference analyses were performed using Prism 5.0 (GraphPad). The non-parametric
183 Wilcoxon signed rank test and Mann-Whitney test were used to test paired and non-paired
184 observations, respectively. In case of multiple testing of unpaired data, a non-parametric
185 Kruskal-Wallis test, followed by a Dunn's multiple comparison test, was used. Multiple testing of
186 paired data without missing values was done by a Friedman test, followed by a Dunn's multiple
187 comparison test.

188 **Results**

189 **Low CD4 expression on AGM pDC**

190 Given the importance of CD4 for HIV/SIV sensing by pDC, we measured CD4 on pDC from
191 uninfected AGM, SM and MAC (Figure 1). PDC were defined as CD3⁻CD20⁻HLA-DR⁺CD123^{hi} cells
192 (Figure 2A) (2). MAC and SM pDC displayed similar CD4 levels as on CD4⁺ T cells (Figure 1A and
193 B). Surprisingly, AGM pDC expressed CD4 at >1 log lower levels than CD4⁺ T cells ($p < 0.001$).

194 As (i) CD4 MFI on CD4⁺ T cells was similar between AGM and MAC and (ii) two additional CD4
195 antibody clones confirmed low CD4 expression on AGM pDC (Figure 2B), it is unlikely that low
196 CD4 on AGM pDC was due to species-specific antibody issues. The absence of intracellular CD4
197 in AGM pDC indicated that recycling from the cell surface was not the underlying mechanism of
198 this low expression (Figure 2C). AGM pDC from primary, secondary and tertiary lymphoid tissues
199 all expressed low levels of CD4 (Figure 2D).

200 **Efficient SIV-sensing by AGM pDC**

201 The low CD4 expression on AGM pDC is paradoxical, since CD4 is essential for HIV/SIV sensing by
202 pDC and AGM pDC have been shown to efficiently sense SIVagm (7, 12). Nonetheless, a lower
203 production of IFN-I has been described during SIVagm infection *in vivo* (12, 25). We wondered
204 whether low CD4 on pDC could have subtle effects on SIV sensing *in vivo*. For instance, only
205 sensing of infectious SIVagm particles has been investigated (12, 25), while (i) most virions
206 produced *in vivo* are non-infectious and (ii) sensing of virus-infected cells is more efficient in
207 human and MAC (23). We stimulated peripheral blood mononuclear cells (PBMC) of a large
208 number of uninfected AGM and MAC with infectious SIV, using HSV as a control for CD4-
209 independent sensing. No quantitative differences in IFN-I production were observed between
210 AGM and MAC (Figure 3A). Both SIV-infected cells and AT2-inactivated SIVagm also induced
211 normal IFN-I responses (Figures 3B and 3C). Longitudinal measurement showed that CD4 levels
212 on pDC did not further decrease during SIV infection (Figure 3D). Altogether, these data
213 demonstrate that low CD4 on AGM pDC did not impair their capacity to sense SIVagm.

214 **SIV isolate-dependent sensing of AGM pDC**

215 We wondered how SIVagm is sensed by pDC despite low CD4 expression. CD4 is a highly
216 polymorphic molecule among primates. We raised the hypothesis that given the long circulation
217 of SIVagm in the AGM population, SIVagm is well-adapted to AGM CD4 (26). This could entail
218 that SIVagm, but not heterologous viruses, can elicit robust IFN-I responses by AGM pDC. We
219 tested this hypothesis by stimulating AGM (sabaesus) and MAC PBMC with nine SIV/HIV isolates
220 (Figure 4A, B). The three SIVagm.sab isolates and the SIVagm.tan isolate induced robust IFN-I
221 production by AGM pDC. SIVagm.gri did not induce an efficient response, which corresponds
222 with the observation that the *CD4* genes of the sabaesus and tantalus AGM species are more
223 closely related to each other than to the *CD4* gene of the grivet AGM species (26).

224 In contrast, HIV-1 and two out of three SIVmac (SIVmac.251 and SIVmac.STM) isolates did not
225 induce robust IFN-I production by AGM pDC ($p < 0.05$ or $p < 0.01$), even at a high viral dose. Only
226 SIVmac239 induced IFN-I production from AGM PBMC, already at a low viral dose. Low dose of
227 SIVmac239 also induced high levels of IFN-I in by MAC PBMC. At the highest dose, SIVmac239
228 was more cytotoxic for AGM PBMC than SIVmac251 ($p < 0.05$, Figure 4C). However, at a dose of
229 150 ng/mL p27, SIVmac239 did not influence viability, so it is unlikely that the strong response
230 to SIVmac239 was related to dying cells, which are known to induce IFN-I responses (Figure 4C).
231 It should be noted that SIVmac239 has been shown to be highly virulent *in vivo* compared to
232 SIVmac251 (27). In contrast to AGM pDC, MAC pDC produced IFN-I upon stimulation with all
233 SIV/HIV isolates, including SIVagm (Figure 4B).

234 If the lack of SIVmac/HIV sensing by AGM pDC is indeed due to low CD4, then forcing viral
235 endocytosis should overcome this sensing deficiency. In line with this, HIV-1, or SIVmac251,
236 pseudotyped with VSV-G were sensed similar to SIVagm (Figure 4D). Indeed, pseudotyped HIV-1

237 and SIVmac251 induced higher levels of IFN-I than their wild-type isolates ($p < 0.05$ and $p < 0.01$,
238 respectively, Figure 4D). Altogether, these findings indicate a virus-host co-adaptation in AGM
239 for viral sensing by pDC.

240 **Predominance of CCR5 negative pDC in natural hosts**

241 We next examined CCR5 expression on AGM pDC, since co-receptor expression, in addition to
242 CD4, is essential for infection. MAC pDC were predominantly CCR5⁺ (median 92.3%), while the
243 majority of AGM and SM pDC did not express detectable levels of CCR5 (7.7% and 40% CCR5⁺
244 pDC, respectively; Figure 1A and 1C). In SMs, the percentage of CCR5⁺ pDC was lower after SIV
245 infection (18.4%, $p = 0.02$; Figure 1C).

246 **pDC are highly infected during pathogenic and non-pathogenic SIV infection**

247 We then addressed the question if these reduced CD4 and CCR5 expressions associate with low
248 infection of AGM pDC. To test this, we purified splenic pDC and CD4⁺ T cells of chronically SIV-
249 infected AGM and MAC and measured cell-associated viral DNA. Cells from uninfected animals
250 were never positive for viral DNA (data not shown). Spleen pDC and CD4⁺ T cells from MAC
251 harbored a median of 8.1×10^4 and 9.2×10^3 copies per million cells, respectively ($p = 0.37$,
252 Figure 5A). AGM harbored 3.2×10^4 and 4.1×10^3 copies per million splenic pDC and CD4⁺ T
253 cells, respectively ($p = 0.44$, Figure 5A). AGM and MAC pDC were thus infected to a similar
254 extent *in vivo*. As infection levels were similar between pDC and CD4⁺ T cells, potentially
255 contaminating CD4⁺ T cells in the pDC fraction cannot explain the levels of detected SIV DNA in
256 pDC. As pDC have limited phagocytic capacities, the high levels of viral DNA in these cells are
257 also unlikely to be associated with engulfed infected T cells.

258 To further demonstrate the infection status of AGM pDC, we immunohistochemically examined
259 a LN of a chronically infected AGM (Figure 5B). CD123 and SIVagm RNA signals overlapped,
260 while NKp30, which is not expressed on pDC, did not co-stain with CD123 or SIVagm RNA.

261 We then measured CD4 and CCR5 expression on splenic pDC of chronically infected AGM and
262 MAC to exclude phenotypic differences compared to pDC from blood or from lymphoid tissues
263 of uninfected animals. Splenic pDC of SIV-infected AGM, but not MAC, expressed low levels of
264 CD4 (Figure 6). Only 4.7% of AGM splenic pDC had detectable CCR5 expression, while 92.9% of
265 MAC splenic pDC were CCR5⁺ ($p = 0.0079$). In conclusion, pDC of AGM and MAC were infected
266 at high levels despite a restricted CD4 and CCR5 expression on AGM pDC.

267 **Discussion**

268 Such low CD4 expression as the one we discovered on AGM pDC is remarkable given the
269 evolutionary conserved high expression of CD4 on mammalian pDC, including primate, murine,
270 cattle and swine pDC (6, 28, 29). The role of CD4 in pDC biology is currently unknown, but this
271 low expression raises questions on the physiological impact for AGM. The low CD4 levels on pDC
272 were sufficient to allow SIVagm endocytosis and subsequent sensing and we demonstrated for
273 the first time that natural host pDC can sense SIV-infected cells. In contrast, most SIVmac and
274 HIV-1 isolates tested were not sensed by AGM pDC. Pseudotyping increased the efficiency of
275 HIV-1/SIVmac251 sensing, which indicates that inefficient sensing of heterologous virus was due
276 to restricted viral uptake. Altogether, this suggests an adaptation between SIVagm and its host-
277 specific CD4. Similar to our findings, it has been shown that HIV-1 poorly interacts with MAC
278 CD4 compared to human CD4 and that HIV-1 could thus infect cells expressing low levels of
279 human CD4, but not cells expressing low levels of MAC CD4 (30). Low CD4 expression also did

280 not prevent AGM pDC from being infected as they were infected at high levels *in vivo*. Since the
281 pDC sensing capacity and infection levels were similar between AGM and MAC, these factors do
282 not determine the level of chronic inflammation.

283 Our study reveals a high infection rate of pDC in secondary lymphoid tissue during chronic SIV
284 infection. These data resemble those of acute SIVmac infection, where pDC were found to be
285 infected to similar levels as CD4⁺ T cells in LN (9). Since only very few data on pDC infection *in*
286 *vivo* are available, this is important and underlines the potential role of pDC as a viral reservoir.
287 Of note, the ratio of pDC to CD4⁺ T cells is low, approximately 1:300 in lymph nodes (2).
288 Therefore, the majority of viral burden in SIV infection is still associated with CD4⁺ T cells.
289 However the contribution of pDC should not be underestimated, given their presence in
290 mucosae, their capacity to migrate to lymph nodes and their ability to efficiently transmit
291 HIV/SIV to CD4⁺ T cells.

292 The lack of correlation between the frequency of CCR5⁺ pDC and SIV infection status does not
293 support the hypothesis that absence of CCR5 expression protects against target cell infection
294 (4). In line with this, a mutation in the SM CCR5 allele, disrupting functional CCR5 expression,
295 does not diminish SIVsm infection prevalence (31). This can be explained by the fact that SIVs,
296 including SIVagm, efficiently use alternative co-receptors, such as CXCR6 (Bonzo) and GPR15
297 (BOB) (22, 31). It is however possible that the low percentage of CCR5 expression in natural
298 hosts is related indirectly to their resistance to disease. Indeed, while CCR5 is not a specific gut-
299 homing receptor, it can induce migration to inflamed tissues (14). Such extremely low levels of
300 CCR5⁺ pDC in natural hosts could therefore be related to the lack of pDC accumulation in the gut
301 after infection (32). Our results therefore suggest that evaluating the function of CCR5 in

302 seeding of viral reservoirs outside its role as a HIV co-receptor is warranted. Teasing out such
303 mechanisms could be helpful for curative approaches.

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443 **Figure 1. CD4 and CCR5 expression on blood plasmacytoid dendritic cells.** (A) CD4 and CCR5
 444 expression on pDC (blue) and Lineage⁺ cells (grey) of one representative Chinese cynomolgous
 445 macaque, African green monkey, sooty mangabey and Indian rhesus macaque. (B) CD4
 446 expression on pDC from SIV-negative Chinese rhesus macaques (n=8, open squares), Indian
 447 rhesus macaques (n=1, open upward triangles), AGM (n=13, open circles) and SM (n=11, filled

448 grey circles). CD4 mean fluorescent intensity (MFI) on pDC was normalized (%) to the CD4 MFI
449 on CD4⁺ T cells. The horizontal, dashed line designates equal expression compared to CD4⁺ T
450 cells. (C) The percentage of CCR5⁺ pDC was determined for SIV-negative Indian rhesus macaques
451 (n = 11, open upward triangles), Chinese cynomolgous macaques (n = 2, open downward
452 triangles), AGM (n = 7, open circles) and SM (n = 8, grey filled circles) and chronically SIV-
453 infected Indian rhesus macaques (n = 8, open upward triangles), AGM (n=3, open circles) and
454 SM (n = 8, grey filled circles). Symbols represent individual animals and lines and bars represent
455 medians and interquartile ranges. * Kruskal-Wallis, p < 0.05, *** Kruskal-Wallis, p < 0.001.

456 **Figure 2. Low CD4 expression on AGM pDCs.** (A) Dot plots showing the gating strategy used to
457 identify pDCs, which are FSC/SSC, singlets, Lineage (CD3/CD20)⁻ cells and HLA-DR⁺ CD123^{hi} cells,
458 for one representative animal. (B) Low CD4 expression on AGM pDCs (n = 3) was confirmed
459 using two additional monoclonal anti-CD4 antibodies (M-T466 and SFC112T4D11). Histograms of
460 representative animals are shown, depicting CD4 expression on CD4⁻ cells (grey, solid), pDCs
461 (blue, solid with contour) and Lineage⁺CD4⁺ cells (red, solid with contour). MFI values of CD4 for
462 the three cell populations are shown in the table. (C) Intracellular and extracellular staining of
463 AGM pDCs (n = 3). Histograms of a representative animal are shown, cell surface expression
464 CD4 expression is depicted for CD4⁻ cells (grey, solid) and for pDCs, intracellular (blue, solid with
465 contour) and extracellular (green, solid with contour) expression is shown, indicating an absence
466 of an intracellular CD4 pool in AGM pDCs. (D) Low CD4 expression was observed on pDCs from
467 bone marrow (n = 1 AGM), LN (n = 1 AGM) and rectal biopsy (n = 1 AGM). Histograms depict
468 CD4 expression on CD4⁻ cells (grey, solid), pDCs (blue, solid with contour) and Lineage⁺CD4⁺ cells
469 (red, solid with contour). MFI values of CD4 are listed in the tables.

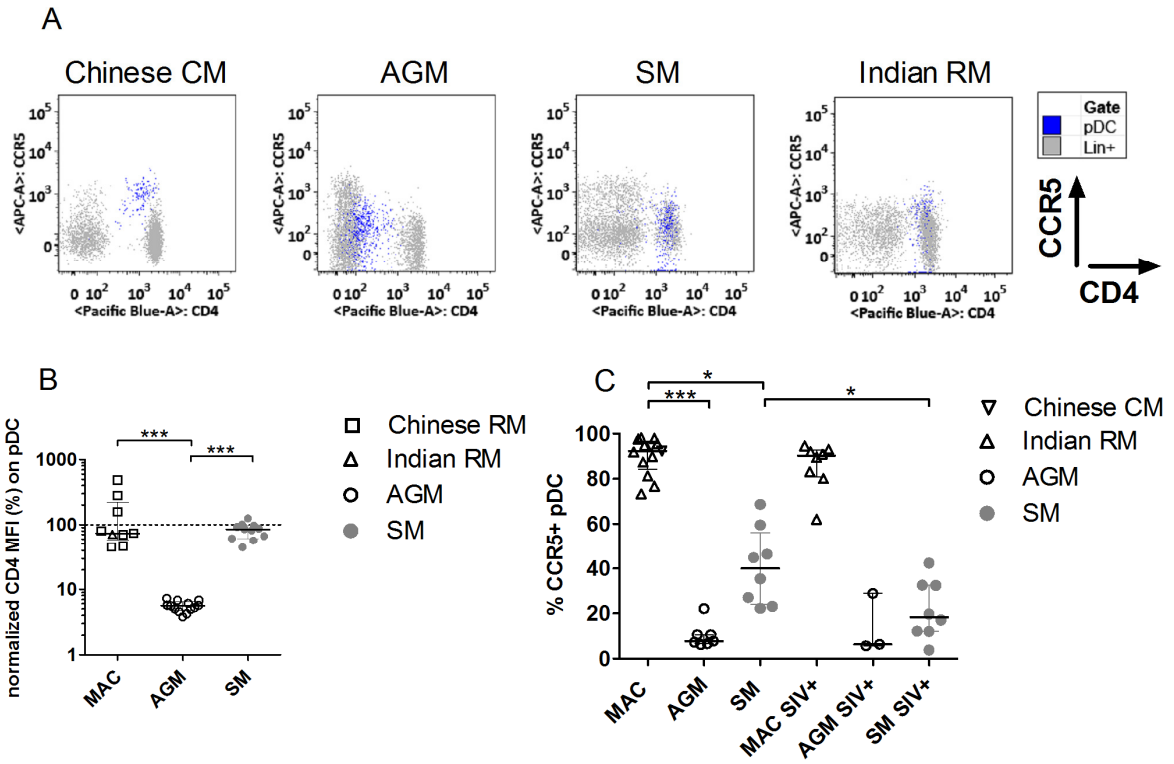
470 **Figure 3. SIV sensing capacity of AGM pDCs.** (A) SIVagm.sab92018 was used to stimulate AGM
471 (n = 22) and SIVmac251 for Chinese rhesus MAC (n = 14) PBMCs. Alternatively, PBMCs were
472 stimulated with HSV-1. (B) CD4 MFI was followed throughout SIVagm infection on four AGMs.
473 Median and interquartile ranges are shown for CD4⁺ Lineage⁺ cells (red), pDCs (blue) and CD4⁻
474 Lineage⁺ cells (grey). (C) SIVagm-infected SupT1 cells and free virions were used to stimulate
475 AGM PBMCs (n = 5). (D) SIVagm or AT2-inactivated SIVagm was used to stimulate AGM PBMCs
476 (n = 8). Individual symbols represent distinct animals. Median and interquartile range are
477 shown. Vertical dashes separate the different viral preparations used to test pDC sensing *
478 Friedman, p < 0.05.

479 **Figure 4. Viral and host determinants of SIV sensing by pDC.** PBMC from (A) AGM (n = 5-23)
480 and (B) Chinese rhesus MAC (n = 2-15) were stimulated with medium (mock), five SIVagm, three
481 SIVmac or HIV-1 (BAL) at three concentrations: 1500 ng/mL, 150 ng/mL and 15 ng/mL p24/p27.
482 Median values are represented by a horizontal line (blue and red for SIVagm and HIV/SIVmac,
483 respectively). * Wilcoxon, p < 0.05, ** Wilcoxon, p < 0.01, compared to equal dose
484 SIVagm.sab92018 stimulation. nd = not determined. Tox = cytotoxic in 18 hour culture. (C) AGM
485 PBMC (n=10) were stimulated with medium (grey circle), 15, 150 or 1500 ng/mL p27 SIVmac251
486 (red square) or SIVmac239 (blue triangle) for 18 hours after which viability was measured.
487 Median and interquartile ranges are depicted. * Wilcoxon, p < 0.05. (D) AGM PBMC were
488 stimulated with HIV (n = 14), VSV-G pseudotyped HIV (n = 10), SIVmac251 (n = 10), VSV-G
489 pseudotyped SIVmac251 (n=9) or SIVagm.sab92018 (n = 23) at 150 ng/mL p24 or p27. Median
490 values and interquartile ranges are presented by a line and bars, respectively. * Kruskal-Wallis,

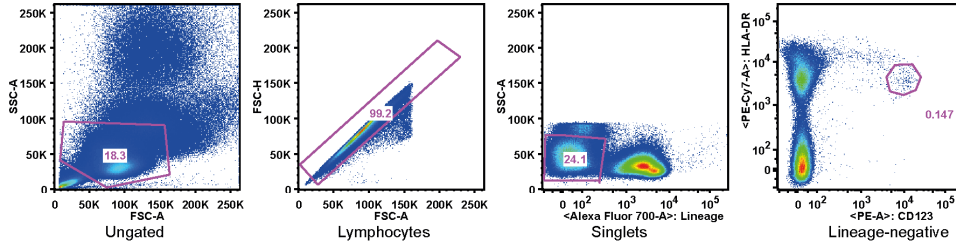
491 $p < 0.05$, ** Kruskal-Wallis, $p < 0.01$, *** Kruskal-Wallis, $p < 0.001$. Symbols represent individual
492 animals, horizontal dashed lines represent the limit of detection.

493 **Figure 5. In vivo pDC infection.** (A) PDC and CD4⁺ T cells of chronically SIV-infected AGM (open
494 circles, $n = 7$) and Chinese rhesus MAC (black circles, $n = 5$) were sorted from 2×10^8 to 4×10^8
495 splenocytes, yielding a median of six thousand pDC after two subsequent sorts (purity 91%).
496 CD4⁺ T cells were also purified from lymph node (LN) cells (purity 97%). SIV DNA was normalized
497 to CCR5 and represented as copies per million cells. Symbols represent individual animals. CD4⁺
498 T cells in LN were infected to a higher extent in MAC than AGM (* Mann-Whitney, $p = 0.016$).
499 SIV DNA copy numbers were similar between spleen and LN CD4⁺ T in both species. (B)
500 Fluorescence microscopy was performed on a LN of one chronically infected AGM. DAPI (blue)
501 shows nuclei, CD123 (yellow) is expressed on pDC, SIV RNA (red) shows infected cells and NKp30
502 (green) is a marker not expressed on pDC. The merge shows an overlap from CD123 and SIV
503 signals.

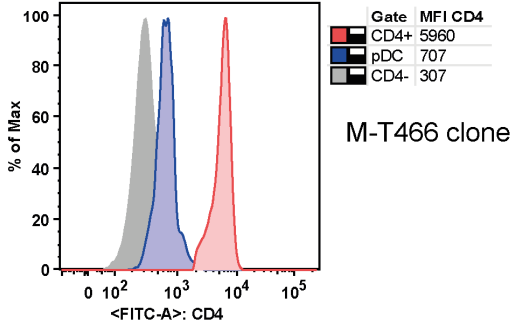
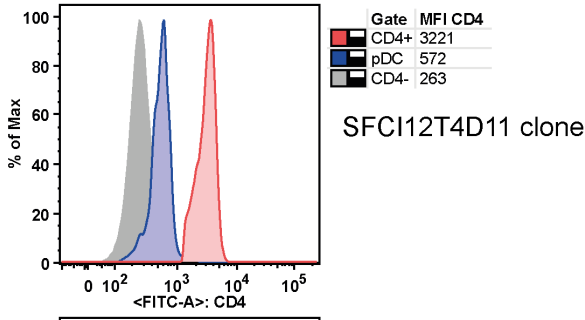
504 **Figure 6. CD4 and CCR5 expression on splenic AGM and MAC pDCs.** (A) CD4 expression was
505 measured on pDCs from spleen of chronically SIV infected Chinese rhesus MACs ($n=5$) and
506 AGMs ($n=5$). The mean fluorescent intensity (MFI) of CD4 on splenic pDCs was normalized (%) to
507 the CD4 MFI on splenic CD4⁺ T cells of the same animal. The horizontal, dashed line designates
508 equal CD4 expression to CD4⁺ Lineage⁺ cells. Symbols represent individual animals and line and
509 bars represent the median and interquartile range, respectively. (B) The percentage of CCR5⁺
510 splenic pDCs was determined for SIV-infected Chinese rhesus MACs ($n = 5$) and AGMs ($n = 5$).
511 Symbols represent individual animals and the line and bars represent the median and
512 interquartile range. ** Mann-Whitney, $p < 0.01$.



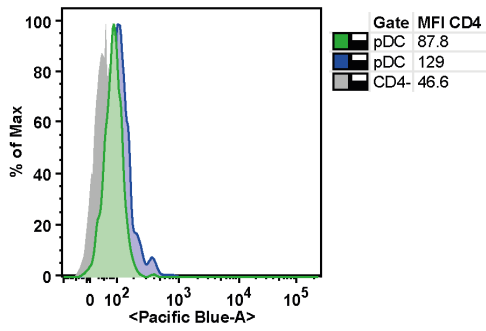
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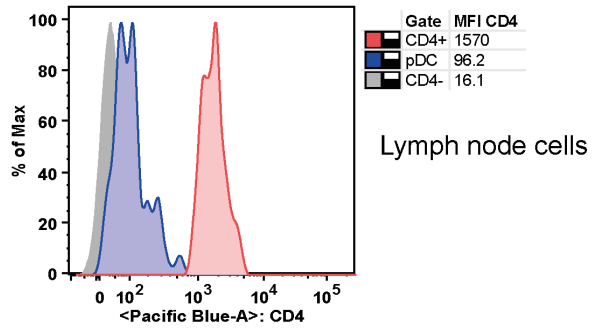
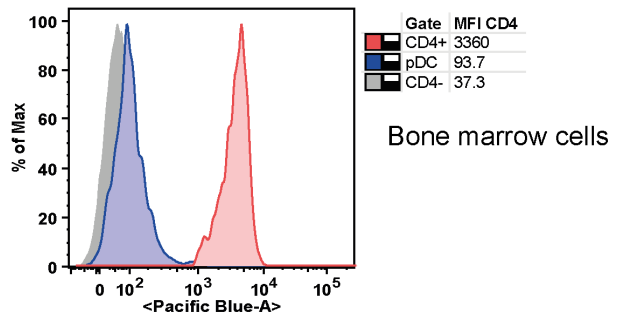
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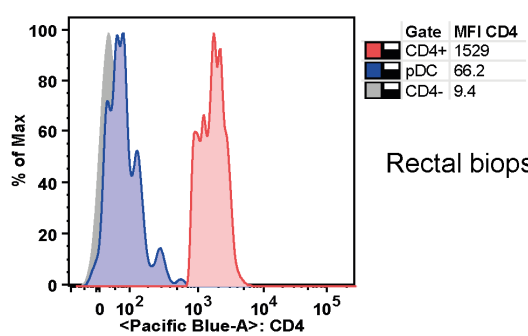
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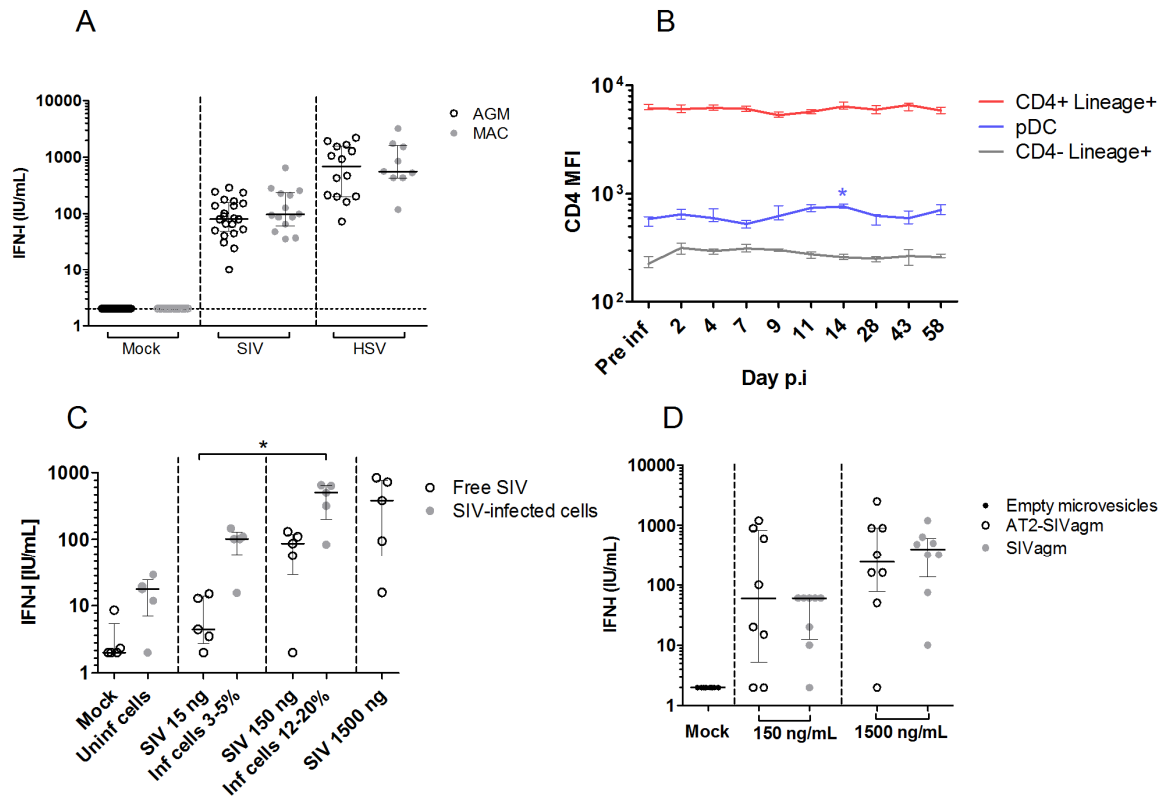


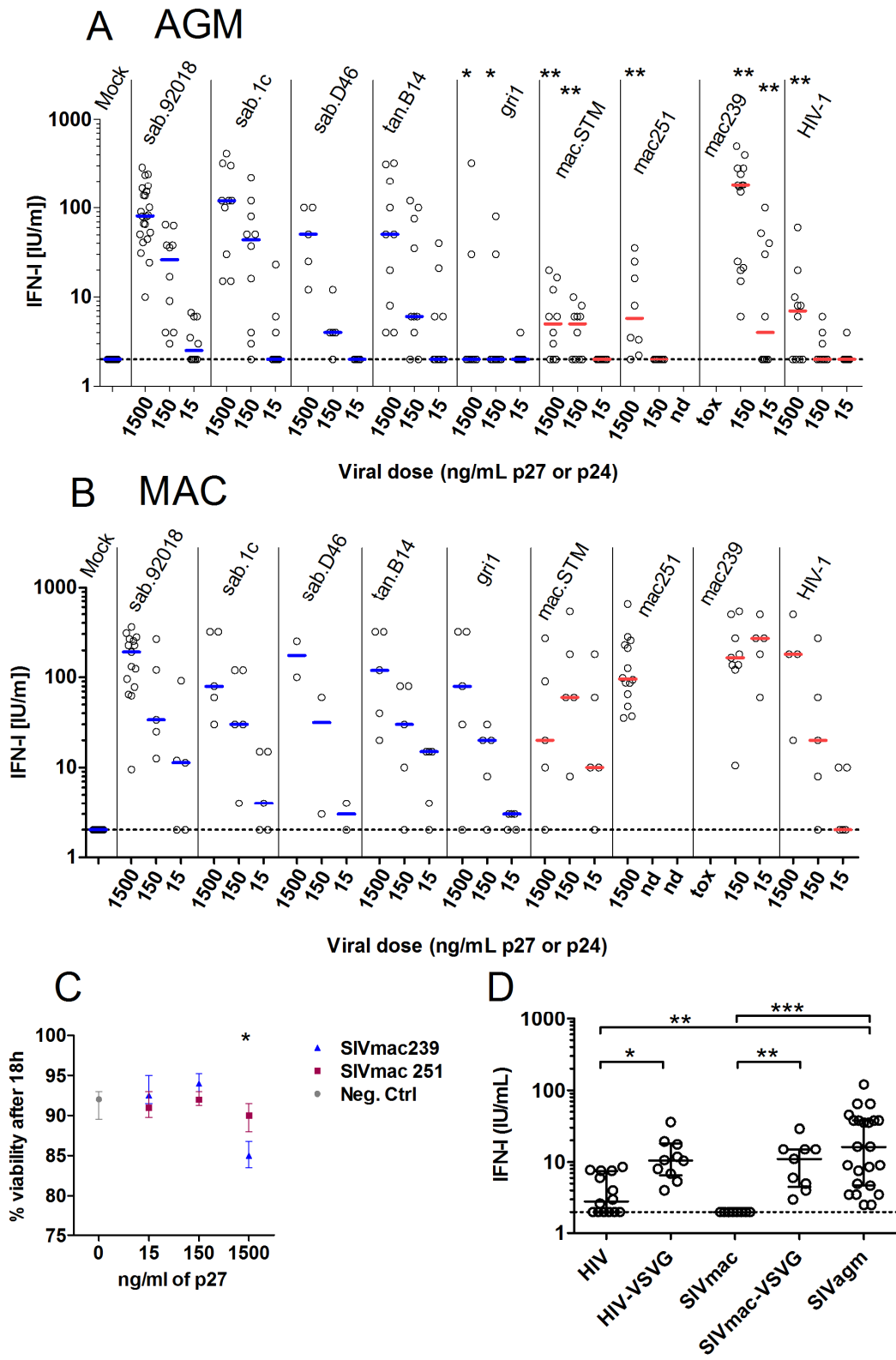
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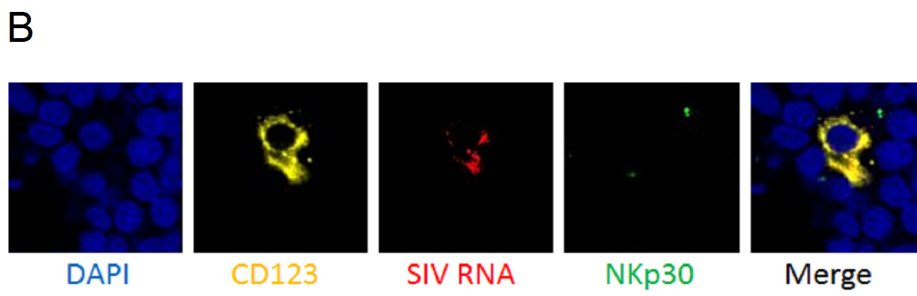
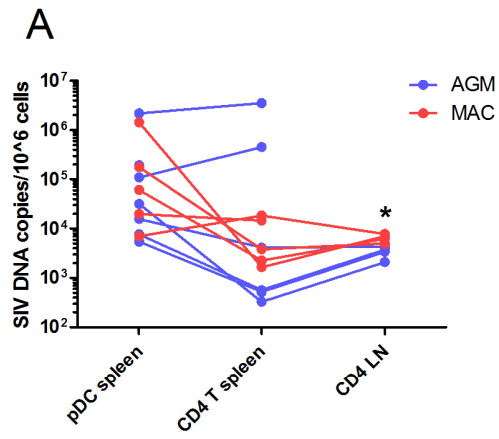


Rectal biopsy cells

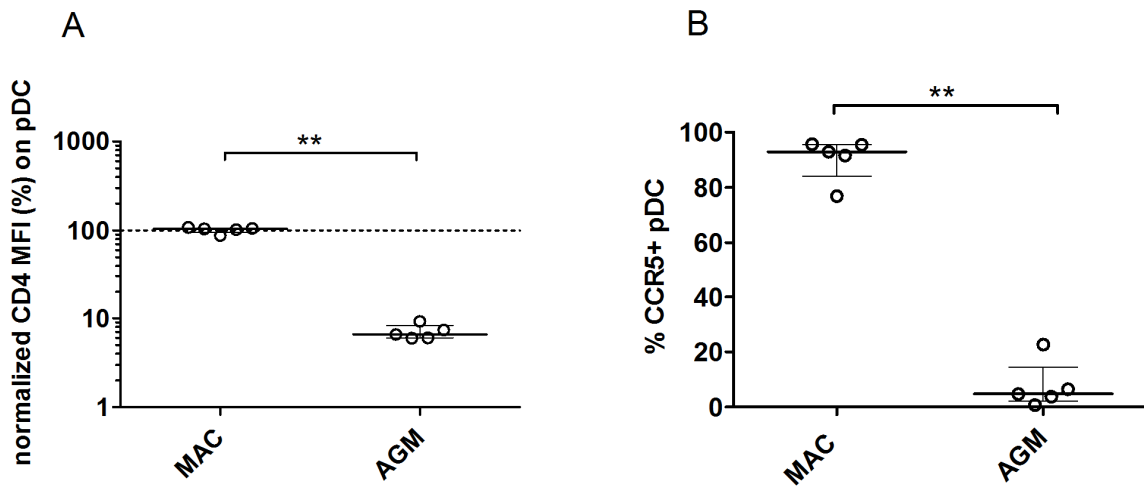








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