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## **Inhibitors of the Interferon Response Increase the Replication of Gorilla Simian Foamy Viruses**

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1 **Brief report**

2 **Inhibitors of the Interferon Response Increase the**  
3 **Replication of Gorilla Simian Foamy Viruses**

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12

## 13        **Abstract**

14            Simian foamy viruses (SFVs) are complex retroviruses widespread throughout nonhuman  
15 primates. SFVs can also be transmitted to humans, mostly through bites. We previously observed  
16 that the primary zoonotic gorilla SFV strains much more slowly than laboratory-adapted chimpanzee  
17 strains. Here, we tested the hypothesis that SFV growth is limited by interferon (IFN)-induced  
18 restriction factors using inhibitors of cellular signaling pathways involved in type I IFN induction or  
19 action. Inhibitors of JAK1/2 (Ruxolitinib) and TBK-1 (BMX795) led to a 2 to > 20-fold higher  
20 percentage of infected BHK-1 and HT1080 cells. However, replication of the laboratory-adapted  
21 prototype foamy virus was not sensitive to these molecules, and IKK2 inhibitors had no effect on  
22 any of the SFV strains. In conclusion, the addition of small molecules that inhibit type I IFN response  
23 to the culture medium can be used as a simple and efficient method to enhance the replication of  
24 zoonotic gorilla SFVs.

## 25        **Keywords**

26            foamy virus; spumaretroviruses; zoonosis; immune response; interferon; virus replication

27

## 28 Introduction

29 Simian foamy viruses (SFVs) are complex retroviruses widespread throughout nonhuman  
30 primates (NHPs) (reviewed in [1]). SFVs can also be transmitted to humans through bites,  
31 establishing a persistent infection [1-5]. To date, neither pathogenic effects nor secondary  
32 transmission between humans have been reported [5, 6]. Replication-competent SFVs have been  
33 isolated from blood cells years or even decades after infection [7-9] and cell-associated SFV DNA has  
34 been detected in human blood and buccal samples [2, 8, 10-14]. An understanding of the persistence  
35 of SFV in humans and its consequences requires *in vitro* experiments performed with primary  
36 strains. Indeed, strains may differ in cell tropism and susceptibility to inhibition by innate and  
37 adaptive immune effector molecules and cells [7, 9, 15, 16]. However, repeated cycles of *in vitro*  
38 production may lead to the selection of the fittest viral variants in specific cell-culture conditions  
39 and/or the expansion of strains that would have been efficiently repressed *in vivo* by immune  
40 responses. Thus, tropism and susceptibility to IFNs or antibodies frequently differ between  
41 laboratory-adapted and primary strains of retroviruses [17-20].

42 Central African villagers from East and South Cameroon and North Gabon are at high risk for  
43 SFV acquisition and approximately 75% of infected individuals carry strains from gorillas [2, 6, 10,  
44 21]. The only gorilla SFV isolated directly from an animal has limited *in vitro* replication capacity [22].  
45 We previously isolated two zoonotic gorilla SFV strains and demonstrated their high genetic  
46 similarity with their NHP counterparts [9, 23]. The sequences of these primary viral strains are  
47 identical to those of gene fragments amplified from human PBMC-associated viral DNA [9]. Next,  
48 we performed *in vitro* infections, using viral stocks produced after inoculation with original or first  
49 passage cell lysates which are referred to as primary strains [15, 16, 24]. We observed that primary

50 gorilla SFV grow more slowly than laboratory-adapted chimpanzee SFV on hamster kidney (BHK-21)  
51 cells, which are used for their isolation and production [24]. Furthermore, primary gorilla SFV stocks  
52 collected from cell cultures displaying an extensive cytopathic effect have 100-fold lower cell-free  
53 titers on the GFAB indicator cells in which their autologous LTR directs the expression of a reporter  
54 molecule [24].

55       Among host factors that affect replication efficacy, the induction and action of IFN are of  
56 utmost importance [25, 26]. SFVs are sensitive to the action of type I IFNs [15, 27, 28]. Tripartite  
57 motif protein 5 $\alpha$ , APOBEC3 cytidine deaminase, N-myc interactor, IFN-induced protein 35, and  
58 tetherin all inhibit SFV replication [3, 29-31]. Systematic screening of IFN-stimulated genes (ISGs)  
59 has identified additional SFV inhibitors [32]. In plasmacytoid dendritic cells, SFVs trigger type I IFN  
60 production after sensing through TLR7 [15]. Cytosolic pattern-recognition receptors recognize viral  
61 nucleic acids and initiate a molecular cascade, resulting in IRF3, IRF7 and/or NF- $\kappa$ B translocation to  
62 the nucleus and the triggering of IFN gene and ISG transcription [25]. Cytoplasmic sensing of SFV  
63 occurs in monocytic cells [33]. However, SFV sensing has not yet been demonstrated in  
64 nonhematopoietic cells [34, 35]. Secreted IFN binds to its cellular receptor and initiates a signaling  
65 cascade through the Janus kinase signal transducer and activator of transcription (JAK-STAT)  
66 pathway [25, 26].

67       The titers of primary gorilla SFV stocks produced on BHK-21 cells are usually low and  
68 sometimes insufficient to carry out *in vitro* infections. Therefore, we tested three strategies to  
69 enhance the level and/or speed of primary gorilla SFV replication: the search for sensitive human  
70 cell lines, improvement of viral entry, and blockade of the type I IFN response. The third approach

71 was successful, as small molecules that inhibit IFN signaling or the IFN response enhanced the

72 replication of zoonotic gorilla SFVs.

73

## 74 Results

75 Primary gorilla SFV strains replicate more slowly than laboratory-adapted chimpanzee  
76 SFV strains

77 BHK-21 and HT1080 cells were infected at the same moi with primary zoonotic gorilla SFV  
78 strains GI-D468 and GII-K74 and laboratory-adapted chimpanzee SFV strains CI-PFV and CII-SFV7 [9,  
79 24, 36]. Infection was monitored by flow cytometry. GI-D468 and GII-K74 replicated at the same rate  
80 in both BHK-21 and HT1080 cells and massive cell lysis was observed at day 25 (Figures 1a and 1b).  
81 CI-PFV and CII-SFV-7 spread rapidly in BHK-21 cells reaching 90% of infected cells by day 14, before  
82 the destruction of the cell monolayer (Figure 1a). The growth kinetics of the two chimpanzee strains  
83 were even more rapid in HT-1080 cells, infecting approximately 80% of the cells by day 6 (Figure 1b).

84 We then tested the infection of several human hematopoietic cell lines. In preliminary  
85 experiments, gorilla SFV appeared to grow at a very slow rate: only 11% of K562 cells infected with  
86 undiluted gorilla SFV expressed Env after 41 days in culture. We therefore tested the susceptibility  
87 of four additional cell lines using the fast replicating CI-PFV. K562 cells were productively infected  
88 using a moi of 0.6, with an infection level of 75% at day 23, before cell lysis (Figure 1c). Raji, Jurkat,  
89 THP-1, and HEL 92.1.7 cells did not express SFV Env during a 41 day-long culture. We used a moi of  
90 0.6 which is 10-fold higher than the maximum reachable with our primary SFV stocks. Unfortunately,  
91 concentration of gorilla SFV particles by *iodixanol*-gradient ultracentrifugation or centrifugal filters  
92 resulted in reduced infectivity and lower infectious titers on GFAB cells (data not shown). Therefore,  
93 we did not further investigate the susceptibility of various cell lines to gorilla SFV. We monitored the  
94 appearance of CPE in human fibroblasts (MRC5), U-87MG, murine *mus dunni*, and simian IFN-  
95 deficient VERO cells before constructing the GFAB indicator cells and receiving the anti-Env

96 monoclonal antibody. All showed similar or even lower susceptibility to gorilla SFV than BHK-21 cells  
97 (Lambert, Gouzil and Buseyne, data not shown). In conclusion, human adherent HT1080 cells were  
98 susceptible to infection with primary gorilla SFV strains which spread more slowly than the  
99 chimpanzee SFV strains.

#### 100        Procedures targeting the early steps of the replication cycle do not enhance infection 101 with gorilla SFV

102        When expressed at the cell surface, SFV Env-mediated fusion is inducible by short exposure  
103 to acidic pH [37] and the fusion process is much slower for macaque SFV Env than CI-PFV Env [38].  
104 We therefore tested whether changing the pH could enhance infection with viral particles. GFAB  
105 cells were infected for 2 h, exposed to pH 7 or pH 5.5 for 1 or 15 min, and cultured for 72 h. Exposure  
106 to acidic pH did not significantly increase the number of cells infected with any of the four SFV strains  
107 (Figure 2a), whereas it increased cell fusion by PFV Env [37] and gorilla SFV Env (data not shown)  
108 after transfection with plasmids expressing *env* gene.

109        Cationic polymers can increase the adsorption of virions on target cell membranes, leading to  
110 the enhancement of infection of some retroviruses [39]. We infected GFAB cells with CI-PFV or GI-  
111 D468 in the presence of polyethylenimine, polybrene, or DEAE dextran at concentrations ranging  
112 from 0.5 to 8 µg/mL. Cationic polymers did not increase infection (Figure 2b).

#### 113        IFN inhibitors enhance infection with gorilla SFVs

114        We addressed whether SFV replication is sensitive to type I IFN-mediated restriction by adding  
115 small molecules that inhibit either kinases involved in IFN induction (TBK-1 and IKK2) or the JAK-STAT  
116 pathway [40-42]. Inhibitors of JAK1/2 (Ruxolitinib), TBK-1 (BX795), or IKK2 (TPCA-1 and BMS345541)  
117 were added for 4 h before GFAB cell infection and during the 72 h-long culture. Ruxolitinib and



118 BX795 increased the number of GFAB cells infected with GII-BAK74 by a factor of two (Figure 3a).  
119 Neither molecule increased infection with CI-PFV. No additive effect was observed if both molecules  
120 were mixed. At the highest concentrations tested, we observed a cytotoxic effect that was more  
121 obvious for BX795.

122         Neither of two IKK2 inhibitors affected GFAB-cell infection with either CI-PFV or GII-K74  
123 (Figure 3b). Thus, blocking IFN signaling through JAK1/2 or the TBK-1-dependent IFN response  
124 increased GFAB-cell infection with GII-K74, whereas it had no effect on infection with CI-PFV.

125         We further characterized the effect of ruxolitinib on several cycles of SFV replication in GFAB  
126 and HT1080 cells and followed Env expression by flow cytometry. GI-D468 and GII-K74 efficiently  
127 spread in GFAB and HT1080 cells treated with ruxolitinib (Figures 4a and 4b). At day 8 post-infection,  
128 23% of the ruxolitinib-treated GFAB cells were infected with GI-D468 *versus* 0.6% of the untreated  
129 cells (Figure 4a). In addition, ruxolitinib increased GI-D468 infection of HT1080 cells (16.2% vs. 3.4%  
130 infected cells, Figure 4b). We observed a similar effect for GII-K74, although it was less efficient than  
131 for HT1080 cells (Figure 4a-b). Ruxolitinib did not affect the intensity of envelope protein staining.  
132 It also had no effect on infection of HT1080 cells with CI-PFV (Figure 4c), in accordance with the  
133 results on GFAB cells (Figure 3a). These data show that treatment with ruxolitinib significantly  
134 enhanced the number of cells infected with primary gorilla SFVs but not those infected with CI-PFV.

135

## 136 Discussion

137 Here, we sought to increase the *in vitro* replication of primary gorilla SFV strains and found  
138 that inhibition of the type I IFN response is an efficient way to achieve such a goal. Indeed, the  
139 supplementation of culture medium with JAK1/2 or TBK-1 inhibitors is a simple and broadly  
140 applicable method, efficient for a variety of viruses [41-43], including retroviruses [44]. While the  
141 growth of primary gorilla SFV was sensitive to inhibition of the IFN response, the growth of  
142 laboratory-adapted CI-PFV was not.

143 The blockade of IFN signaling by JAK1/2 inhibitors and the TBK-1-mediated IFN response  
144 increased gorilla SFV replication. In contrast, blocking IKK2, which is another component of the IFN  
145 response, had no effect on *in vitro* SFV growth. Our data indirectly suggest that gorilla SFV activates  
146 the IRF3 but not the classical NF- $\kappa$ B pathway. Indeed, CI-PFV induces IRF3-dependent ISGs in human  
147 monocytes [33]. However, its replication was not enhanced by TBK-1 inhibition in our hands. This  
148 discrepancy may be explained by differences in innate sensing between hematopoietic and  
149 nonhematopoietic cells, as CI-PFV is unable to induce IFN production in nonhematopoietic cells [34,  
150 35]. Alternatively, the rapid and high level of PFV replication may saturate antiviral molecules  
151 targeted by the drugs we used.

152 We tested the susceptibility of several human cell lines to primary gorilla SFVs. No gorilla cell  
153 lines or samples are available to test the replication kinetics of gorilla SFV in their natural host cells.  
154 Here, we focused on human cells because SFV infection in humans is our major research theme and  
155 because more cell biology and immunology reagents are available for their study. We observed that  
156 CI-PFV replicates faster in HT1080 than in BHK-21 cells, as reported by others [45]. HT1080 cells  
157 were susceptible to both GI-D468 and GII-K74, but both gorilla SFVs had similar replication kinetics

158 in HT1080 and BHK-21 cells. Overall, we observed the slow/low replication kinetics of gorilla SFV in  
159 both the hamster cells used for their isolation and human fibroblasts, described to have the highest  
160 susceptibility to CI-PFV [45].

161 CI-PFV infects a wide range of adherent and nonadherent cells [46-48]. Here, we tested the  
162 infection of five myeloid and lymphoid human cell lines with the fast-growing CI-PFV. We used a low  
163 moi for our study, *i.e.* a screen of cells that could be infected at an moi achievable with primary  
164 gorilla SFV stocks. Under these conditions, only K562 cells were susceptible to PFV infection. The  
165 low moi is a likely explanation for the low cell susceptibility to CI-PFV in this study relative to that  
166 reported in previous publications [47, 49, 50].

167 We tested several classical methods to enhance the early steps of SFV infection. The use of  
168 polycations to reduce surface charge had no impact on gorilla SFV, as reported by others for PFV  
169 [48, 51]. The process regulating SFV Env fusogenic activity and variation across various viral species  
170 is not fully defined [37]. Although exposure to a pulse of acidic pH induced fusion of gorilla SFV Env  
171 expressed at the cell surface, as described in [37] we observed no effect on SFV Env exposed at the  
172 surface of viral particles. We hypothesized that gorilla SFV Env fusion is a slow process, as described  
173 for macaque SFV Env [38], and exposed cells to low pH after incubation with the viral inoculum. This  
174 timing might be suboptimal if most of the particles were already internalized and unaffected by the  
175 change in the pH of the culture medium. Of note, we recently produced chimeric foamy virus vectors  
176 expressing gorilla SFV Env and CI-PFV Gag and Pol, for which the titers were equal to or marginally  
177 lower (< 5-fold) than those of vectors expressing CI-PFV Env [16]. Overall, our data argue against a  
178 restriction of gorilla SFV replication at the Env-mediated early steps of the viral cycle.

179           We aimed to avoid the selection of fast-growing laboratory-adapted gorilla SFV strains for the  
180 realization of *in vitro* infections. We show here that the presence of ruxolitinib during the production  
181 of gorilla SFV stocks is a simple way to overcome the slow/low *in vitro* replication of these strains.  
182 At first glance, blocking restriction mechanisms to enhance SFV growth does not appear to fit our  
183 initial goal, because viral particles produced in ruxolitinib-treated cells may differ from those  
184 produced in untreated cells, with active innate sensing and an IFN response. It will thus be critical  
185 to consider the final experimental outcome before producing viral stocks with IFN blockade. In  
186 addition, we propose the use of ruxolitinib for a single round of virus amplification, starting from  
187 original material for each new round of production to limit the selection of fitter viral variants over  
188 serial passages.

189           Although we focused on the production of primary SFV stocks, our results may be relevant for  
190 the isolation of new replicating SFV strains. Our current procedure relies on the stimulation of  
191 human peripheral blood cells to induce viral replication and coculture with susceptible cells from a  
192 nonhuman species to avoid inhibition by human IFN- $\gamma$  and other soluble mediators produced by  
193 activated PBMCs [9, 10, 52]. The use of small-molecule inhibitors of the type I IFN response may be  
194 useful for improving the isolation of new primary SFV, as demonstrated by the inhibition of IFN- $\gamma$   
195 [52].

196           In conclusion, we show that the addition of JAK1/2 or TBK-1 inhibitors to culture medium is a  
197 simple and efficient procedure to enhance the growth of slow/low primary gorilla SFV isolates. The  
198 same inhibitors did not enhance replication of the laboratory-adapted CI-PFV, showing the utility of  
199 primary SFV strains for the study of innate sensing. Our results should be useful for researchers in  
200 the field of foamy viruses.

## 201 **Materials and Methods**

### 202 *Cells*

203 K562 (ECACC 89121407, human erythroleukemia cells) and BHK-21 (ATCC-CLL-10, hamster  
204 kidney fibroblasts) cells were obtained from colleagues at the Institut Pasteur. BHK-21-derived  
205 gorilla foamy virus-activated  $\beta$ -galactosidase (GFAB) cells were generated in our laboratory [24].  
206 Other cell lines were purchased from LGC standards or Sigma-Aldrich and stocks were produced  
207 after less than 10 passages. The cells were screened for mycoplasma infection every 10 passages  
208 with a bioluminescent assay (#LT07-418, Lonza). The HT1080 cells (ECACC 85111505, human  
209 fibrosarcoma) were cultivated in Eagle's Minimum Essential Medium with Earle's Balanced Salts and  
210 L-Glutamine (EMEM-EBSS, Lonza) supplemented with 10% fetal bovine serum (FBS, PAA  
211 Laboratories) and 1% nonessential amino acids (NEAA, Invitrogen). K562, HEL 92.1.7 (ATCC-TIB-180,  
212 human erythroleukemia cells), THP-1 (ECACC 88081201, human monocytic leukemia cells), Raji  
213 (ATCC-CCL-86, human B lymphoma cells), and Jurkat (ATCC-TIB-152, human T leukemia cells) were  
214 grown in RPMI medium containing GlutaMAX I (Invitrogen) supplemented with 10% FBS. BHK-21  
215 and GFAB cells were cultivated in Dulbecco's modified Eagle's medium (DMEM-GlutaMAX I,  
216 Invitrogen) supplemented with 5% FBS. G418 (300  $\mu$ g/ml, Sigma-Aldrich) was added to the GFAB  
217 cultures.

### 218 *Viruses*

219 SFV strains consisted of the primary zoonotic gorilla SFVs, SFVggo\_huBAD468 (GI-D468) and  
220 SFVggo\_huBAK74 (GII-K74) [9], and the laboratory-adapted chimpanzee SFVs, SFVpsc\_huPFV (CI-  
221 PFV) and SFVpve\_Pan2 (CII-SFV7) [33]. GI-D468 and GII-K74 viral stocks were produced by infecting  
222 BHK-21 cells with original or first passage cell lysates. CI-PFV and CII-SFV7 viral stocks were obtained

223 by a single round of infection with aliquots obtained from A. Saib and A. Rethwilm, respectively.  
224 Infected cultures were passaged twice a week and uninfected cells added after the appearance of  
225 the first syncytia to amplify the virus. Once the cytopathic effect (CPE) had destroyed > 70% of the  
226 cell layer, infected cells and supernatants were treated by three cycles of freezing and thawing (-  
227 80°C; +37°C) to enable the release of viral particles. The lysate was cleared by centrifugation (1500  
228 x g for 10 min), filtered through a 0.45 µm pore-size filter, and stored as single-use aliquots at -80°C.  
229 Both chimpanzee and gorilla SFV are efficiently detected by GFAB cells [24]. Virus titers were  
230 determined by infecting GFAB cells at 30 to 40% confluence in flat-bottom 96-well plates with 30  
231 µl/well of serially-diluted viral solutions prepared in DMEM. After a 2-h incubation, 170 µl DMEM-  
232 5% FBS was added to each well. Tests were performed in triplicate. The cells were fixed after 72 h  
233 with 0.5% glutaraldehyde in a phosphate-buffered saline solution (PBS) for 10 min at room  
234 temperature (RT). Cells were washed with PBS and incubated 1 h at 37°C with an X-Gal staining  
235 solution (2 mM MgCl<sub>2</sub>; 10 mM Potassium ferricyanide, 10 mM Potassium ferrocyanide; and 0.5  
236 mg/mL 5-Bromo-4-chloro-3-indolyl-B-D-galactopyranoside in PBS). An Ultimate UV Image analyzer  
237 (CTL Europe, Bonn, Germany) was used to count X-Gal stained cells. One infectious unit was defined  
238 as a blue cell or syncytia.

### 239 SFV infections

240 Adherent cells were infected at 30 to 40% confluence. On the day before the infection, BHK-  
241 21 and GFAB cells were seeded at 5 10<sup>3</sup> cells/P96-well and HT1080 were seeded at 2.5 10<sup>3</sup> cells/P96  
242 well. Adherent cells were infected at 30 to 40% confluence with 25µl of undiluted GI-D468 and GII-  
243 K74 (5 10<sup>3</sup> infectious unit (IU)/ml). CI-PFV (2 10<sup>6</sup> IU/ml) and CII-SFV7 (3 10<sup>5</sup> IU/ml) stocks were  
244 diluted to achieve the same moi as the gorilla SFV strains in experiments comparing the four strains.

245 The corresponding moi were 0.01 for BHK-21 and GFAB cells, 0.02 for HT1080. Certain CI-PFV  
246 infections were carried out at a 10-fold higher moi to reduce the length of the experiments. Non-  
247 adherent cells ( $10^6$  cells) were centrifuged at 500 x g before the addition of the 500  $\mu$ l of CI-PFV to  
248 the cell pellet. Infected cultures were passaged twice a week by dilution into fresh culture medium  
249 at the same cell density as for the propagation of uninfected cells and maintained up to the  
250 occurrence of a massive CPE. GFAB cells were infected in triplicate using the titration protocol and  
251 stained after 72 h. The corresponding means and standard deviations (SD) were calculated.

252 Exposure to acidic pH was performed 2 h post-infection by removing the medium, adding PBS  
253 at pH 7 or 5.5, and incubation for 1 or 15 min. The PBS was discarded and DMEM with 5% FBS added  
254 to the wells. Polyethyleneimine (jetPEI, #101-10, Polypplus), 1,5-dimethyl-1,5-diazaundecamethylene  
255 polymethobromide (Polybrene, # TR-1003, Sigma-Aldrich), and diethylaminoethyl dextran (DEAE  
256 Dextran, wt 500.000, #D9885 Sigma-Aldrich) were added to the viral inoculum immediately before  
257 the infection of GFAB cells. Inhibitors of JAK1/2 (Ruxolitinib, #S1378, Euromedex), TBK-1 (BX795,  
258 #S1274, Euromedex), and IKK2 (TPCA-1, #T1452 and BMS345541, #B9935, Sigma-Aldrich) were  
259 added to the culture medium after a 2-h incubation with the viral inoculum.

## 260 Flow cytometry

261 A murine monoclonal antibody specific for the leader peptide of the SFV envelope (clone  
262 P6G11G11 generated by M.L. Linial) was conjugated to Alexa Fluor 647 dye (anti-Env-AF647).  
263 Antibody production, coupling, and purification were performed by RD Biotech, Besançon, France.  
264 Cells were fixed with 2% paraformaldehyde (PFA) diluted in PBS for 10 min at RT, washed in PBS  
265 supplemented with 0.1% bovine serum albumin (BSA), permeabilized with 0.5% Triton X-100 in PBS-  
266 0.1% BSA for 10 min at RT, washed before addition of anti-Env-AF647 at 20 ng/mL and viability dye

267 (0.5  $\mu$ l/tube, Live Dead Aqua, #L34957, Life Technologies), and incubated for 30 min at RT. Cells were  
268 washed with PBS-0.1%BSA and resuspended in 300  $\mu$ L PBS-2%PFA. Data was acquired on a Gallios  
269 cytometer (Beckman Coulter) and analyzed with Kaluza software. Results are expressed as the  
270 percentage among viable cells.

271



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## 280 **Conflicts of Interest**

281 The authors had no conflicting interests relevant to the study.

282

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395

## 396 **Figure legends**

397 **Figure 1. Replication kinetics of SFVs in several cell lines.** (a) BHK-21 and (b) HT1080 cells  
398 were infected with CI-PFV (black), CII-SFV7 (green), GI-D468 (blue), or GII-K74 (red) at a moi of 0.01  
399 for BHK-21 cells and 0.02 for HT1080. (c) K562, Jurkat, Raji, THP-1, and HEL 92.1.7 cells were infected  
400 with CI-PFV at a moi of 0.6. Infected cultures were split twice a week and a fraction of the cells  
401 stained with a viability marker and an anti-Env-AF647 antibody. Cultures were maintained until their  
402 death due to a massive cytopathogenic effect. The percentage of Env<sup>+</sup> cells among live cells is  
403 presented as a function of time. The lack of symbols at day 25 in panels a and b indicate that most  
404 cells expressed Env but that their accurate quantification was not possible because most were  
405 already dead. The data correspond to a representative test of two (panel c) or three independent  
406 experiments (panel a and b).

407 **Figure 2. Factors affecting early steps of the replication cycle have no effect on infection**  
408 **with SFV.** (a) Effect of reducing pH treatment on SFV replication. GFAB cells were infected with CI-  
409 PFV, CII-SFV7, GI-D468, or GII-K74 at a moi of 0.01. Two hours post-infection, medium was removed  
410 and PBS at pH 7 or 5.5 added and the cultures incubated for 1 or 15 min. After 72 h of infection,  $\beta$ -  
411 galactosidase expression was detected by X-gal staining. The number of infected cells/well is shown  
412 as the mean and SD of triplicates. (b) Effect of cationic polymers on SFV replication. GFAB cells were  
413 infected with CI-PFV (black) or GI-D468 (blue) viruses at a moi of 0.01 in the presence of  
414 polyethyleneimine (PEI), polybrene (PB), or DEAE Dextran (DEXT) at various concentrations. After 72  
415 h of infection,  $\beta$ -galactosidase expression was detected by X-gal staining. The number of infected  
416 cells/well is shown as the mean and SD of triplicates. All the data correspond to a representative  
417 experiment of three independent experiments.

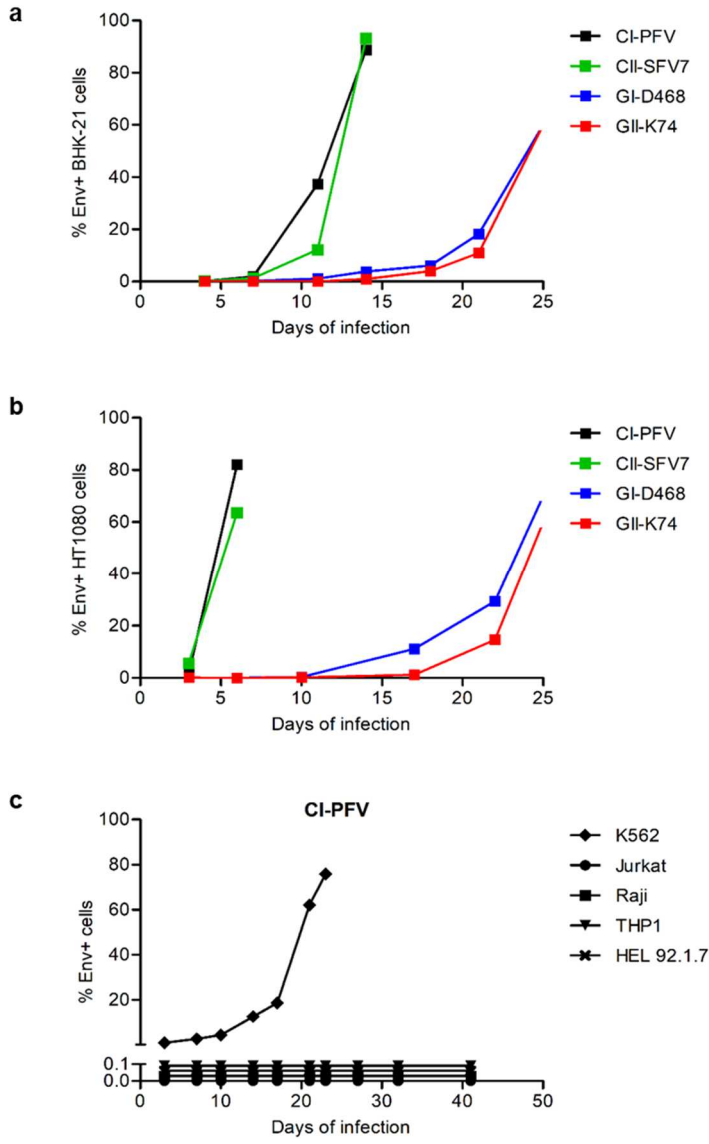


418 **Figure 3. Effect of inhibitors of the IFN response on SFV replication.** GFAB cells were treated  
419 with IFN inhibitors for 4 h at various concentrations prior to infection. After 72 h of infection,  $\beta$ -  
420 galactosidase expression was detected by X-gal staining. The number of infected cells/well is shown  
421 as the mean and SD of triplicates. **(a)** GFAB cells were treated with ruxolitinib, BX795, or a mix of the  
422 two molecules and infected with CI-PFV (black, moi 0.1) or GII-K74 (red, moi 0.02). **(b)** GFAB cells  
423 were treated with TPCA-1 or BMS345541 and infected with CI-PFV (black, moi 0.1) or GII-K74 (red,  
424 moi 0.02). All the data correspond to a representative experiment of three independent  
425 experiments.

426 **Figure 4. Ruxolitinib enhances replication of gorilla SFV but has no effect on CI-PFV.** GFAB  
427 **(a)** and HT1080 **(b and c)** cells were treated with ruxolitinib at 10  $\mu$ M for 4 h and infected with SFV  
428 strains at a moi of 0.02. Infected cultures were stained twice a week with a viability marker and an  
429 anti-Env-AF647 antibody. Data are presented at the peak of infection on a FSC/anti-Env dot-plot of  
430 viable cells. Percentages of Env+ cells are indicated. **(a)** GI-D468, day 8 post-infection; **(b)** GII-K74,  
431 day 8 post-infection; **(c)** CI-PFV, day 5 post-infection.  
432

433 Figure 1

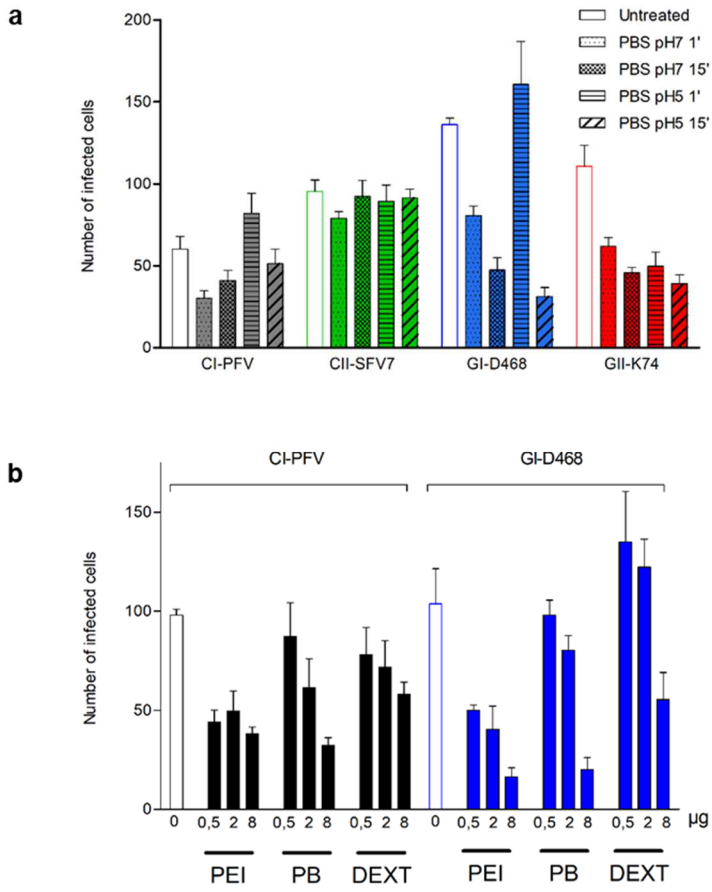
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Figure 2

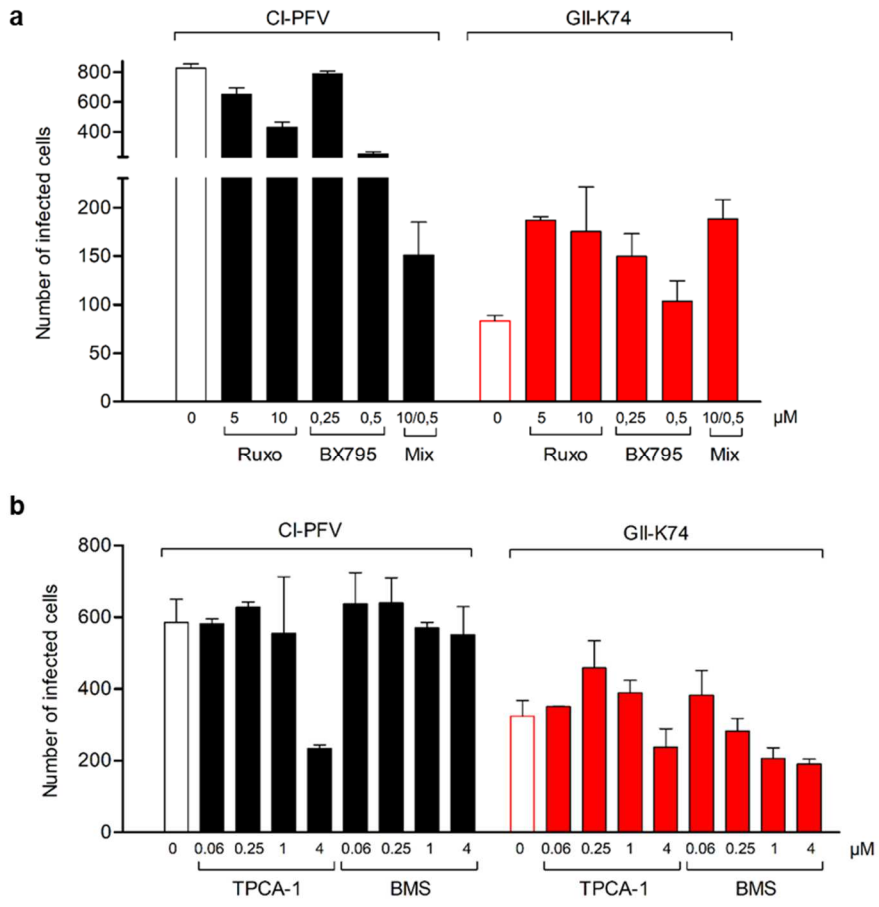


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Figure 3

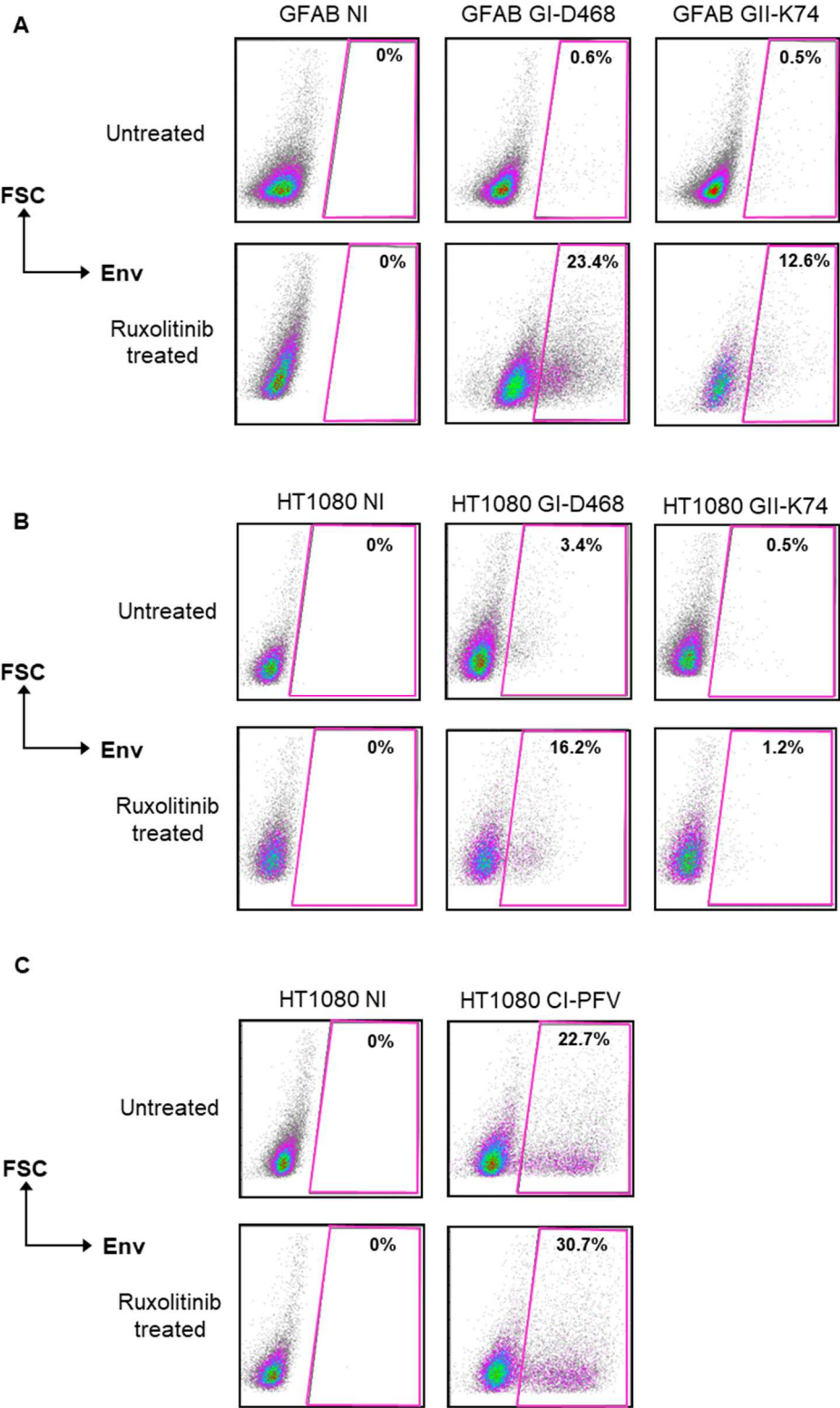


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Figure 4



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