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1 **Brief report**

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

4 Mathilde Couteaudier ^{1,2}, Diego Calzada-Fraile ^{1,2}, Thomas Montange ^{1,2}, Antoine Gessain ^{1,2},
5 and Florence Buseyne ^{1,2*}

6 ¹ Institut Pasteur, Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Institut
7 Pasteur, 28 rue du Dr Roux, 75015 Paris, France;

8 ² UMR CNRS 3569, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, Paris, France

9 * Correspondence: florence.buseyne@pasteur.fr, + 33 1 45 68 88 99

10 Co-authors emails: mathilde.couteaudier@hotmail.fr, diego.calzadafraile@gmail.com,
11 thomas.montange@pasteur.fr, antoine.gessain@pasteur.fr

12

13 **Abstract**

14 Simian foamy viruses (SFVs) are complex retroviruses that are widespread throughout
15 nonhuman primates. SFVs can also be transmitted to humans, mostly through bites. We previously
16 observed that primary zoonotic gorilla SFV strains grow much more slowly than laboratory-adapted
17 chimpanzee strains. Here, we tested the hypothesis that the growth of SFV is limited by interferon
18 (IFN) using inhibitors of cellular pathways involved in the induction or action of type I IFN. Inhibitors
19 of JAK1/2 (Ruxolitinib) and TBK-1 (BX795) led to a 2- to 4-fold higher percentage of cells infected
20 with zoonotic gorilla SFVs but did not affect the replication of laboratory-adapted chimpanzee SFVs.
21 IKK2 inhibitors (TPCA-1 and BMS345541) had no effect on any of the SFV strains. In conclusion, the
22 addition of molecules that inhibit the type I IFN response to the culture medium can be used as a
23 simple and efficient method to enhance the replication of zoonotic gorilla SFVs.

24 **Keywords**

25 foamy virus, spumaretroviruses, zoonosis, immune response, interferon, virus replication

26

27 Introduction

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

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

49 gorilla SFV grow more slowly than laboratory-adapted chimpanzee SFV on hamster kidney (BHK-21)
50 cells, which are used for their isolation and production [23]. Furthermore, primary gorilla SFV stocks
51 collected from cell cultures displaying an extensive cytopathic effect have 100-fold lower cell-free
52 titers on the GFAB indicator cells in which their autologous LTR directs the expression of a reporter
53 molecule [23].

54 Among host factors that affect replication efficacy, the induction and action of IFNs are of
55 utmost importance [24, 25]. Pattern-recognition receptors recognize viral nucleic acids and initiate
56 a molecular cascade, resulting in IFN regulatory factor (IRF)3, IRF7, and/or nuclear factor-kappa B
57 (NF- κ B) translocation to the nucleus and the triggering of IFN gene transcription [24]. In
58 plasmacytoid dendritic cells, SFVs trigger type I IFN production after being sensed by Toll-like
59 receptor 7 [15]. Secreted IFN binds to its cellular receptor and initiates a signaling cascade through
60 the Janus kinase signal transducer and activator of transcription (JAK-STAT) pathway [24, 25]. Type
61 I IFNs restrict SFV replication [15, 26, 27]. Among IFN-stimulated genes (ISGs), tripartite motif
62 protein 5 α , apolipoprotein B mRNA editing catalytic 3G, N-myc interactor, IFN-induced protein 35,
63 and tetherin inhibit the replication of the laboratory-adapted chimpanzee prototype FV strain (CI-
64 PFV) [3, 28-30]. Systematic screening of ISGs has identified additional CI-PFV inhibitors [31].

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

71 **Results**

72 Primary gorilla SFV strains replicate more slowly than laboratory-adapted chimpanzee
73 SFV strains in adherent cell lines

74 Undiluted stocks of primary zoonotic gorilla SFV strains GI-D468 and GII-K74 were used to
75 infect BHK-21 cells. Infection was monitored by flow cytometry at each cell passage, twice per week.
76 Infected cell frequencies > 1% were first detected at day 10-11 or day 13-14 post-infection (pi)
77 (Figure 1A). The infected cell frequencies increased sharply at the following passage. Then, rapid,
78 massive cell lysis occurred. The kinetics of infection varied between experiments and strains: GI-
79 D468 spread more rapidly than GII-K74 in the first experiment and more slowly in the second (Figure
80 1A). We monitored the appearance of CPE in human fibroblasts (MRC5), U-87MG, murine *mus*
81 *dunni*, and simian IFN-deficient VERO cells before constructing the GFAB indicator cells and using
82 the anti-Env monoclonal antibody. All showed similar or lower susceptibility to gorilla SFV than BHK-
83 21 cells (Lambert, Gouzil and Buseyne, data not shown).

84 The human HT1080 cell line has been reported to be more susceptible to CI-PFV than BHK-21
85 cells [32]. We thus infected HT1080 cells with GI-D468 and GII-K74. Infection frequencies > 1% were
86 first detected at day 18 (Figure 1A). The infection of HT1080 with gorilla SFV was reproducible (data
87 not shown), but the replication kinetics were variable, with infection frequencies > 1% detected
88 between the second and third week of infection.

89 We used the laboratory-adapted CI-PFV and CII-SFV7 strains as positive controls of infection.
90 We observed infected cell frequencies > 1% at between 3 and 7 days pi, depending on the
91 experiment and cells, using the same multiplicity of infection (MOI) as for primary gorilla SFV (Figure
92 1A). In conclusion, primary gorilla SFV strains spread more slowly than the chimpanzee SFV strains

93 in adherent cell lines, even when infections were carried out at the same MOI. In addition,
94 frequencies of infected cells varied between independent experiments at a given timepoint pi.

95 Human hematopoietic cell lines are poorly susceptible to infection with SFVs

96 We then tested whether human hematopoietic cell lines are susceptible to gorilla SFV, as we
97 previously reported that human blood lymphoid cells contain SFV DNA [14]. We first tested the
98 susceptibility of four cell lines using the rapidly replicating CI-PFV strain. Cell pellets were infected
99 with either undiluted or 10-fold-diluted stocks that had a high infectious titer (2×10^6 IU/ml). The
100 corresponding MOI were 0.6 and 0.06. K562 cells were productively infected using a MOI of 0.6,
101 with a frequency of infected cells of 75% at day 23 (Figure 1B). We observed a delayed infection at
102 a MOI of 0.06. HEL 92.1.7, Raji, and Jurkat cells did not express SFV Env. Low frequencies of infection
103 in THP-1 cells were not sustained. We infected K562 cells with undiluted GI-468 (MOI 0.0015),
104 undiluted CI-PFV (MOI 0.6), and CI-PFV diluted to a MOI of 0.0015. At a MOI of 0.0015, we detected
105 > 10% GI-D468-infected cells at day 41 (Figure 1C). After infection with CI-PFV at the high MOI,
106 infected K562 cells were detected at day 7 and massive cell lysis occurred at day 28; no infected cells
107 were detectable upon infection with CI-PFV at a MOI of 0.0015. We attempted to concentrate viral
108 stocks to reach a higher MOI. However, the concentration of SFV particles by *iodixanol*-gradient
109 ultracentrifugation or centrifugal filters resulted in reduced infectivity and lower infectious titers on
110 GFAB cells (data not shown). In conclusion, although hematopoietic K562 cells can support primary
111 gorilla SFV, several weeks of culture were required before the detection of infected cells due to the
112 low infectious titers of the viral stocks. Thus, we did not further investigate the susceptibility of
113 various cell lines to gorilla SFV, but sought treatment of the cells that could improve the efficacy of
114 viral entry and spread.

115 Procedures targeting the early steps of the replication cycle do not enhance infection
116 with gorilla SFV

117 SFV Env-mediated fusion is inducible by short exposure to acidic pH when expressed at the
118 cell surface [33]. The fusion process is much slower for macaque SFV Env than CI-PFV Env [34]. We
119 thus tested whether changing the pH could enhance infection with viral particles. GFAB cells were
120 infected for 2 h, exposed to pH 7 or pH 5.5 for 1 or 15 min, and cultured for 72 h. Exposure to acidic
121 pH had no effect on the number of infected GI-D468 cells and decreased the number of those
122 infected with CI-PFV (Figure 2A). Cationic polymers can also increase the adsorption of virions on
123 target cell membranes, leading to the enhancement of infection of certain retroviruses [35]. We
124 infected GFAB cells with GI-D468 or CI-PFV in the presence of polyethylenimine, polybrene, or DEAE
125 dextran at concentrations ranging from 0.5 to 8 $\mu\text{g}/\text{mL}$. Cationic polymers did not increase the
126 frequency of infection with GI-D468 or CI-PFV, and even decreased infectivity at the highest
127 concentrations (Figure 2C).

128 IFN inhibitors enhance infection with gorilla SFVs

129 We addressed whether SFV replication is sensitive to type I IFN-mediated restriction by adding
130 small molecules that inhibit kinases involved in IFN induction (TBK-1 and IKK2) or signaling (JAK1/2)
131 [36-38]. Inhibitors of JAK1/2 (Ruxolitinib), TBK-1 (BX795), or IKK2 (TPCA-1 and BMS345541) were
132 added to cultures 4 h before GFAB cell infection and maintained during the 72 h-long culture.
133 Ruxolitinib and BX795 increased the number of GFAB cells infected with GI-D468 and GII-BAK74 (t
134 test, $P < 0.05$, Figure 3A-B). There was no additive effect if both molecules were mixed (data not
135 shown). At the highest concentrations tested, there was a cytotoxic effect that was more evident
136 for BX795. Neither of the two IKK2 inhibitors affected GFAB cell infection (Figure 3C-D). The CI-PFV

137 strain, used as positive control of infection, was not affected by any of these four molecules (Figure
138 3A-D). Thus, blocking IFN signaling through JAK1/2 or the TBK-1-dependent IFN response increased
139 GFAB cell infection with zoonotic gorilla SFVs.

140 We then tested the effect of ruxolitinib on several cycles of SFV replication and followed Env
141 expression by flow cytometry. Cells were stained after the appearance of syncytia. Addition of
142 ruxolitinib increased the number of GFAB cells infected with GI-D468 and GII-K74 ($P = 0.04$ and $P =$
143 0.03 , respectively, Figure 4). The percentage of Env⁺ HT1080 increased by at least 2 fold with
144 ruxolitinib treatment (data not shown). However, the percentage of infected cells was unrelated to
145 the observed cytopathic effect. The loss of infected HT1080 cells during cell harvest and staining is
146 likely and probably resulted in an underestimation of the SFV replication. There was no effect of
147 ruxolitinib on CI-PFV replication in GFAB (Figure 4) or HT1080 cells (data not shown) under the same
148 conditions of infection and culture (data not shown). In conclusion, we confirmed the enhancing
149 effect of JAK1/2 inhibition on the spread of zoonotic gorilla SFV in infected cultures.

150

151 Discussion

152 We sought to increase the *in vitro* replication of primary gorilla SFV strains and found that
153 inhibition of the type I IFN response is an efficient way to achieve this goal. Indeed, the
154 supplementation of culture medium with JAK1/2 or TBK-1 inhibitors is a simple and broadly
155 applicable method that works well for a variety of viruses [37-39], including retroviruses [40].

156 The blockade of IFN signaling by JAK1/2 and the TBK-1-mediated IFN response increased
157 gorilla SFV replication. In contrast, the blockade of another component of the IFN response, IKK2,
158 had no effect on *in vitro* SFV growth. Conclusions on the cellular pathways that affect the spread of
159 SFV based on the use of pharmacological inhibitors must be made with caution. First, the pleiotropic
160 JAK/STAT signaling pathway regulates the response to not only IFN, but also to cytokines, and
161 several cellular processes, including cell proliferation [41]. Indeed, two opposite effects could be
162 expected from JAK1/2 inhibition: the promotion of viral replication through the inhibition of IFN
163 signaling and the reduction of viral spread through the blockade of mitosis, which is required for
164 SFV provirus integration [42]. Furthermore, some of the inhibitors we used target several molecules.
165 BX795 blocks TBK-1 and Akt [43], whereas TPCA-1 inhibits both IKKs and STAT3 [44].

166 Our primary aim was to enhance the *in vitro* growth of primary gorilla SFV. We thus included
167 the laboratory-adapted chimpanzee CI-PFV strain as a positive control of infection in every
168 experiment. Its susceptibility to IFN blockade differed from that of gorilla SFV strains. Indeed, CI-PFV
169 infection was not enhanced by ruxolitinib or BX795. Several nonexclusive mechanisms could account
170 for such a strain-specific effect. As discussed above, pharmacological inhibitors may block several
171 pathways and/or have several targets with opposite effects. For example, JAK/STAT inhibitors
172 promote the early steps of replication of HIV-1 Env mutant strains in T-cell lines but have no effect

173 on wildtype virus [45]. However, the same molecules decrease wildtype HIV replication in blood
174 primary T lymphocytes [46]. Similarly, certain strain-specific properties may underlie SFV
175 susceptibility to IFN blockade. Few side-by-side comparisons of SFV strains have been carried out.
176 One showed that CI-PFV entry is pH independent, in contrast to several other SFV strains [33]; this
177 step of viral replication is affected by the IFN response [47]. Likewise, the rapid and high-level
178 replication of CI-PFV may saturate antiviral molecules targeted by the drugs we used and explain
179 the strain-specific effect of IFN blockade. In addition, CI-PFV stocks may contain fewer noninfectious
180 virions that can induce the IFN response than the GI-D468 and GII-K74 stocks. Finally, the effect of
181 IFN blockade may depend on the cell type. Indeed, CI-PFV is unable to induce IFN production in
182 nonhematopoietic human epithelial cells [48, 49], whereas it efficiently induces IFN- α production
183 by human plasmacytoid dendritic cells [15].

184 We tested the susceptibility of several human cell lines to primary gorilla SFVs. No gorilla cell
185 lines or samples are available to test the replication kinetics of gorilla SFV in their natural host cells.
186 Here, we focused on human cells because SFV infection in humans is our major research theme and
187 because more cell biology and immunology reagents are available for their study. We observed that
188 CI-PFV replicates faster in HT1080 than BHK-21 cells, as reported by others [32]. HT1080 cells were
189 susceptible to both GI-D468 and GII-K74, but both gorilla SFVs had similar replication kinetics in
190 HT1080 and BHK-21 cells. Overall, we observed the slow/low replication kinetics of gorilla SFV in
191 both the hamster cells used for their isolation and human fibroblasts, described to have the highest
192 susceptibility to CI-PFV [32].

193 We tested several classical methods to enhance the early steps of SFV infection. The use of
194 polycations to reduce surface charge had no impact on gorilla SFV, as reported by others for PFV

195 [50, 51]. The processes that regulate the fusogenic activity of SFV Env and Env in various other viral
196 species are not fully understood [33]. Although exposure to a pulse of acidic pH induced fusion of
197 gorilla SFV Env expressed at the cell surface, as described in [33], we observed no effect on SFV Env
198 exposed at the surface of viral particles. We hypothesized that gorilla SFV Env fusion is a slow
199 process, as described for macaque SFV Env [34], and exposed cells to low pH after incubation with
200 the viral inoculum. This timing might be suboptimal if most of the particles have already been
201 internalized and are unaffected by the change in pH of the culture medium. Overall, our data argue
202 against a restriction of gorilla SFV replication at the Env-mediated early steps of the viral cycle.

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

213 Although we focused on the production of primary SFV stocks, our results may be relevant for
214 the isolation of new replicating SFV strains. Our current procedure relies on the stimulation of
215 human peripheral blood cells to induce viral replication and coculture with susceptible cells from a
216 nonhuman species to avoid inhibition by human IFN- γ and other soluble mediators produced by

217 activated PBMCs [9, 10, 52]. The use of small molecule inhibitors of the type I IFN response may be
218 useful for improving the isolation of new primary SFV, as demonstrated by the inhibition of IFN- γ
219 [52].

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

225 **Materials and Methods**

226 *Cells*

227 K562 (ECACC 89121407, human erythroleukemia cells) and BHK-21 (ATCC-CLL-10, hamster
228 kidney fibroblasts) cells were obtained from colleagues at the Institut Pasteur. BHK-21-derived
229 gorilla foamy virus-activated β -galactosidase (GFAB) cells were generated in our laboratory [23].
230 Other cell lines were purchased from LGC standards or Sigma-Aldrich and stocks were produced
231 after less than 10 passages. The cells were screened for mycoplasma infection every 10 passages
232 with a bioluminescent assay (#LT07-418, Lonza). The HT1080 cells (ECACC 85111505, human
233 fibrosarcoma) were cultivated in Eagle's Minimum Essential Medium with Earle's Balanced Salts and
234 L-Glutamine (EMEM-EBSS, Lonza) supplemented with 10% fetal bovine serum (FBS, PAA
235 Laboratories) and 1% nonessential amino acids (NEAA, Invitrogen). The K562, HEL 92.1.7 (ATCC-TIB-
236 180, human erythroleukemia cells), THP-1 (ECACC 88081201, human monocytic leukemia cells), Raji
237 (ATCC-CCL-86, human B lymphoma cells), and Jurkat (ATCC-TIB-152, human T leukemia cells) cell

238 lines were grown in RPMI medium containing GlutaMAX I (Invitrogen) supplemented with 10% FBS.
239 BHK-21 and GFAB cells were cultivated in Dulbecco's modified Eagle's medium (DMEM-GlutaMAX I,
240 invitrogen) supplemented with 5% FBS. G418 (300 µg/ml, Sigma-Aldrich) was added to the GFAB
241 cultures.

242 Viruses

243 SFV strains consisted of the primary zoonotic gorilla SFVs, SFVggo_huBAD468 (GI-D468) and
244 SFVggo_huBAK74 (GII-K74) [9], and the laboratory-adapted chimpanzee SFVs, SFVpsc_huPFV (CI-
245 PFV) and SFVpve_Pan2 (CII-SFV7) [53]. GI-D468 and GII-K74 viral stocks were produced by infecting
246 BHK-21 cells with original or first-passage cell lysates. CI-PFV and CII-SFV7 viral stocks were obtained
247 by a single round of infection with aliquots obtained from A. Saib and A. Rethwilm, respectively.
248 Infected cultures were passaged twice a week and uninfected cells added after the appearance of
249 syncytia to amplify the virus. Once the cytopathic effect (CPE) had destroyed > 70% of the cell layer,
250 infected cells and supernatants were treated by three cycles of freezing and thawing (-80°C; +37°C)
251 to enable the release of viral particles. The lysate was cleared by centrifugation (1500 x g for 10
252 min), filtered through a 0.45 µm pore-size filter, and stored as single-use aliquots at -80°C. Both
253 chimpanzee and gorilla SFV are efficiently detected by GFAB cells [23]. Virus titers were determined
254 by infecting GFAB cells at 30 to 40% confluence in flat-bottom 96-well plates with 30 µl/well of
255 serially-diluted viral solutions prepared in DMEM. After a 2-h incubation, 170 µl DMEM-5% FBS was
256 added to each well. Tests were performed in triplicate. The cells were fixed after 72 h with 0.5%
257 glutaraldehyde in a phosphate-buffered saline solution (PBS) for 10 min at room temperature (RT).
258 Cells were washed with PBS and incubated 1 h at 37°C with an X-Gal staining solution (2 mM MgCl₂;
259 10 mM Potassium ferricyanide, 10 mM Potassium ferrocyanide; and 0.5 mg/mL 5-Bromo-4-chloro-

260 3-indolyl-B-D-galactopyranoside in PBS). An Ultimate UV Image analyzer (CTL Europe, Bonn,
261 Germany) was used to count X-Gal stained cells. One infectious unit was defined as a blue cell or
262 syncytia.

263 SFV infections

264 For microtitration in P96 plates, BHK-21 and GFAB cells were seeded at 5×10^3 cells/P96-well
265 and HT1080 were seeded at 2.5×10^3 cells/P96 well on the day before the infection. Adherent cells
266 were infected at 30 to 40% confluence with 25 μ l of undiluted GI-D468 and GII-K74 (5×10^3 infectious
267 unit (IU)/ml). CI-PFV (2×10^6 IU/ml) and CII-SFV7 (3×10^5 IU/ml) stocks were diluted to achieve the same
268 MOI as the gorilla SFV strains in experiments comparing the four strains. The MOIs were calculated
269 as the [inoculum volume x infectious titer]/number of cells seeded per well and were 0.01 for BHK-
270 21 and GFAB cells, and 0.02 for HT1080 cells. We used the same MOI as for microtitration for
271 infections carried out in flasks or six-well plates. Non-adherent cells (10^6 cells) were centrifuged at
272 500 x g before the addition of the 300 μ l of viral stocks to the cell pellet. The MOI were 0.6 for
273 undiluted CI-PFV and 0.0015 for undiluted GI-D468. Infected cultures were passaged twice a week
274 by dilution into fresh culture medium at the same cell density as for the propagation of uninfected
275 cells and maintained up to the occurrence of massive CPE. Or microtitration, GFAB cells were
276 infected in triplicate using the titration protocol and stained after 72 h.

277 Exposure to acidic pH was performed 2 h post-infection by removing the medium, adding PBS
278 at pH 7 or 5.5, and incubation for 1 or 15 min. The PBS was discarded and DMEM with 5% FBS added
279 to the wells. Polyethyleneimine (jetPEI, #101-10, Polyplus), 1,5-dimethyl-1,5-diazaundecamethylene
280 polymethobromide (Polybrene, # TR-1003, Sigma-Aldrich), and diethylaminoethyl dextran (DEAE
281 Dextran, wt 500.000, #D9885 Sigma-Aldrich) were added to the viral inoculum immediately before

282 the infection of GFAB cells. Inhibitors of JAK1/2 (Ruxolitinib, #S1378, Euromedex), TBK-1 (BX795,
283 #S1274, Euromedex), and IKK2 (TPCA-1, #T1452 and BMS345541, #B9935, Sigma-Aldrich) were
284 added to the culture medium 2 h before infection and during the 72h following incubation with the
285 viral inoculum.

286 Flow cytometry

287 A murine monoclonal antibody specific for the leader peptide of the SFV envelope (clone
288 P6G11G11 generated by M.L. Linial) was conjugated to Alexa Fluor 647 dye (anti-Env-AF647).
289 Antibody production, coupling, and purification were performed by RD Biotech, Besançon, France.
290 Cells were fixed with 2% paraformaldehyde (PFA) diluted in PBS for 10 min at RT, washed in PBS
291 supplemented with 0.1% bovine serum albumin (BSA), permeabilized with 0.5% Triton X-100 in PBS-
292 0.1% BSA for 10 min at RT, washed before addition of anti-Env-AF647 at 20 ng/mL and viability dye
293 (0.5 µl/tube, Live Dead Aqua, #L34957, Life Technologies), and incubated for 30 min at RT. Cells were
294 washed with PBS-0.1% BSA and resuspended in 300 µL PBS-2% PFA. Data were acquired on a Gallios
295 cytometer (Beckman Coulter) and analyzed with Kaluza software. Results are expressed as the
296 percentage among viable cells.

297 Statistics

298 We used the Student's t test to compare infection levels between cells treated with acidic or
299 neutral pH, cation polymers, or IFN inhibitors.

300

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309 **Conflicts of Interest**

310 The authors have no conflicting interests relevant to the study.

311

312

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460

461 **Figure legends**

462 **Figure 1. Replication kinetics of SFVs in several cell lines.** Infected cultures were split twice a
463 week and a fraction of the cells stained with a viability marker and an anti-Env-AF647 antibody.
464 Cultures were maintained until their death due to a massive cytopathogenic effect. The percentage
465 of Env⁺ cells among live cells is presented as a function of time. The gray zone indicates that most
466 cells expressed Env but that their accurate quantification was not possible because most were dead.
467 **(A)** BHK-21 and HT1080 cells were infected with GI-D468, GII-K74, CI-PFV, or CII-SFV7, at a MOI of
468 0.01 for BHK-21 cells and 0.02 for HT1080 cells. **(B)** K562, Jurkat, Raji, THP-1, and HEL 92.1.7 cells
469 were infected with CI-PFV at a MOI of 0.6 and 0.06. **(C)** K562 cells were infected with GI-D468 and
470 CI-PFV. The MOIs of 0.0015 and 0.6 correspond to the use of undiluted GI-D468 and CI-PFV stocks,
471 respectively.

472 **Figure 2. Factors affecting early steps of the replication cycle have no effect on infection**
473 **with SFV.** **(A)** GFAB cells were infected with GI-D468, or CI-PFV at a MOI of 0.01. Two hours post-
474 infection, the medium was removed, PBS at pH 7 or 5.5 added, and the cells incubated for 1 or 15
475 min before washing and continued culture in medium. After 72 h of infection, β -galactosidase
476 expression was detected by X-gal staining. The number of infected cells/well was determined. The
477 ratios of infected cells after exposure to pH5 and pH7 were calculated. The means and SD from three
478 independent experiments are shown. The t test was used to compare the frequency of infected cells
479 after treatment at pH5 and pH7. GFAB cells were infected with GI-D468 **(B)** or CI-PFV **(C)** at a MOI of
480 0.01 in the presence of polyethyleneimine (PEI), polybrene (PB), or DEAE Dextran (DEX) at various
481 concentrations. After 72 h of infection, β -galactosidase expression was detected by X-gal staining.
482 The number of infected cells/well was determined and the ratio of treated/mock-treated wells

483 calculated. The means and SD from three independent experiments are shown. The t test was used
484 to compare the number of infected cells after polycation exposure *versus* that after mock treatment.

485 **Figure 3. Effect of IFN response inhibitors on SFV replication.** GFAB cells were treated with
486 various concentrations of IFN inhibitors for 4 h prior to infection with GI-D468, GII-K74, or CI-PFV at
487 a MOI of 0.01. After 72 h of infection, β -galactosidase expression was detected by X-gal staining.
488 The number of infected cells/well was determined and the ratio of treated/mock-treated wells
489 calculated. The means \pm SD from three independent experiments are shown. The t test was used to
490 compare the frequency of infected cells after culture in the presence or absence of IFN-response
491 inhibitors: **(A)** Ruxolitinib, **(B)** BX795, **(C)** TPCA-1, **(D)** BMS345541.

492 **Figure 4. Ruxolitinib enhances replication of gorilla SFV but has no effect on CI-PFV.** GFAB
493 cells were treated with 10 μ M ruxolitinib for 4 h and infected with GI-D468 (blue), GII-K74 (red), or
494 CI-PFV (black) at a MOI of 0.01. Cultures were passed twice a week until the appearance of syncytia,
495 which occurred on days 8 to 9 in GI-D468 and GII-K74-infected cultures and days 5 to 6 for those
496 infected with CI-PFV. Cells were stained with a viability marker and an anti-Env-AF647 antibody to
497 quantify the percentage of Env⁺ cells. The ratio of Env⁺ cells in ruxolitinib-treated/mock-treated
498 cultures was calculated. The means and SD from three independent experiments are shown. The t
499 test was used to compare the percentage of Env⁺ cells among infected cells after culture in the
500 presence or absence of ruxolitinib.

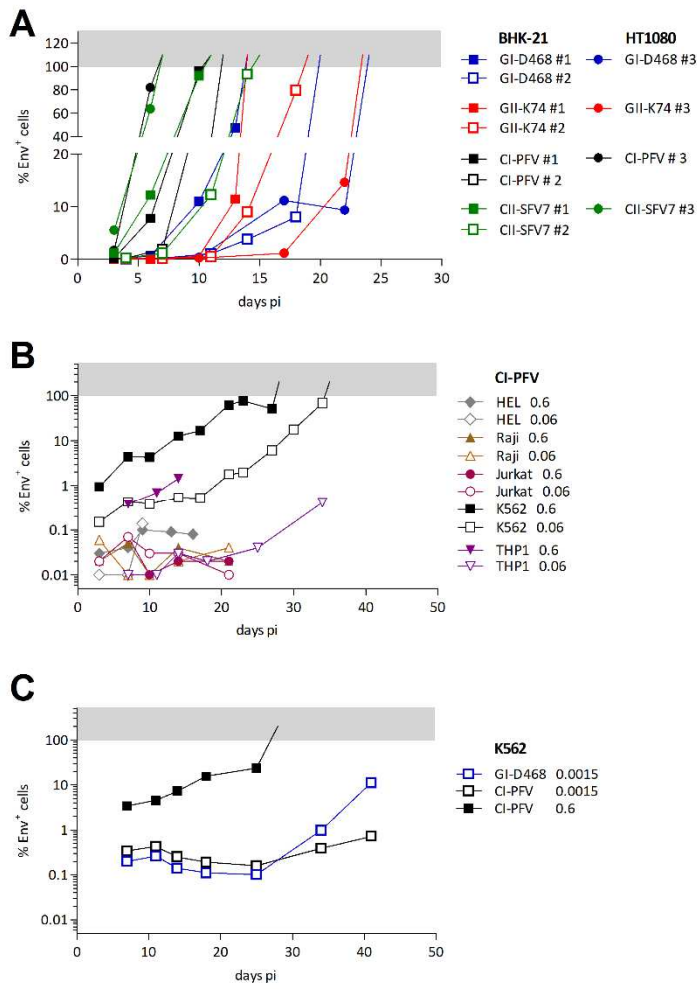
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Figure 1

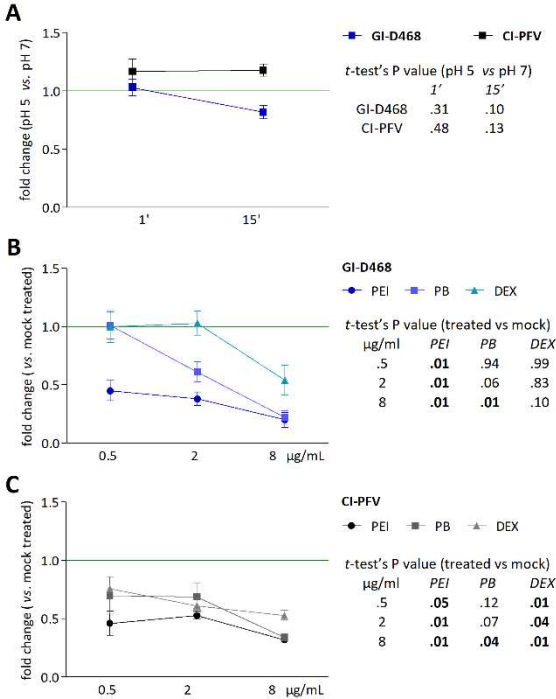


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507 Figure 2

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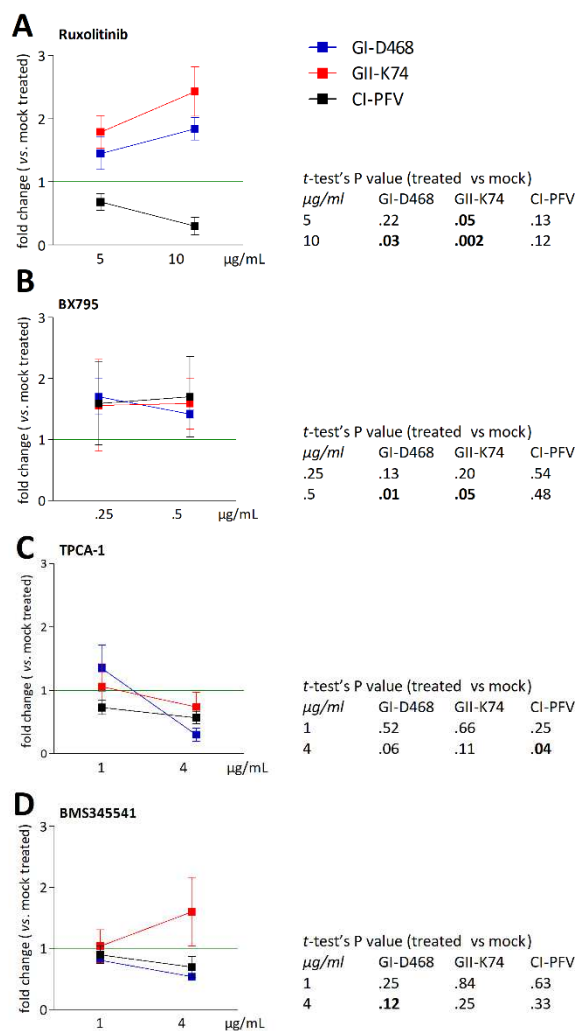
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512 Figure 3

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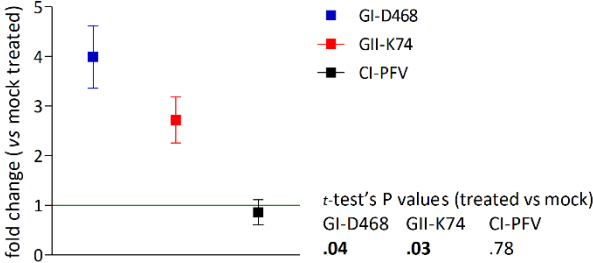
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518 Figure 4

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